Supplemental Information

Tunable Supramolecular Assemblies from Amphiphilic Nucleoside Phosphoramidate Nanofibers by Enzyme Activation

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General Procedures:

A. General Procedure for Synthesis of (1):

Standard Fmoc-based solid phase chemistry was utilized to obtain the NAP-FF peptide. Example Synthesis: Fmoc-Phenylalanine pre-loaded Wang Resin (1.0 mmol) was swelled in dichloromethane for 15 minutes and washed three times with DMF. The resin was suspended in 20% Piperidine in DMF with agitation provided N_2 bubbling for three minutes. The resin was washed with 20% Piperidine in DMF and again agitated for 18 additional minutes to ensure complete removal of Fmoc protecting group. After three washings with DMF, the resin was suspended in DMF and to the reaction vessel was charged Fmoc-Phenylalanine (3 eq., 1.16 g), HATU (3 eq., 1.9 g), and DIEA (5 eq., 870 uL). The coupling solution was agitated with N_2 bubbling for 45 minutes. Following the coupling reaction, the resin was again washed three times with DMF and deprotected with the same procedure as the pre-loaded resin. Following Fmoc deprotection, naphthyl acetic acid (3 eq., 558 mg), HATU (3 eq., 1.14 g), and DIEA (5 eq., 870 uL) were charged to the reaction vessel and agitated with N_2 for 45 minutes. Following the coupling reaction, the resin was washed 3 times with DMF and 3 additional times with DCM. The resin was dried *in vacuo* overnight

Following resin drying, the resin was treated with 95:5 TFA/H_2O for 2 hours to effect peptide cleavage. The cleavage cocktail was agitated by shaking during this time. Resin particulates were filtered away and the filtrate was concentrated to a clear gum. The crude peptide residue was purified via flash chromatography to provide NAP-FF in good yield (Yields: 62-79%). ^{1}H NMR spectrum (DMSO-d_6): 2.73 (q, 1H), 2.93 (m, 1H), 3.00 (dd, 1H), 3.07 (dd, 1H), 3.53 (q, 2H), 4.46 (q, 1H), 4.58 (m, 1H) 7.85 (8.28 (d,1H), 8.34 (d, 1H), 12.77 (s, 1H).

Purified NAP-FF-OH peptide was charged to a round bottomed flask containing a solution of 3,6,9-trioxa-1-azidoundecamine (1.2 eq.) in anhydrous DCM. HBTU (2 eq.) and DIEA (1.5 eq.) were added to the solution and allowed to stir overnight at room temperature. The reaction solution was diluted in DCM and washed with 0.1N HCl (100 mL) and brine (2 x 100 mL). The organic layer was dried over magnesium sulfate and concentrated to a clear gum which was purified with normal phase chromatography (DCM/MeOH 0-15%) (70-98%). ^{1}H NMR spectrum (DMSO-d_6): 2.69 (q, 1H), 2.81 (q, 1H), 2.97 (dd, 2H), 3.16 (m, 1H), 3.22 (m, 1H), 3.34 (d, 1H), 3.37 (t, 2H), 3.48-3.49 (m, 14H), 4.47-4.55 (m, 2H), 7.15-7.25 (m, 10 H), 7.47 (m, 2H), 7.61 (s, 1H), 7.75 (d, 1H), 7.79 (d, 1H), 7.85 (d, 1H), 7.96 (t, 1H), 8.13 (d, 1H), 8.27 (d, 1H)

B. General Synthesis of Phosphoramidate Pro-Gelators (2-5)

5'-Nucleoside-monophosphate (2.5 mmol) was charged to a round-bottomed flask and suspended in minimal de-ionized water (≈ 500 uL). To the suspension was added propargyl amine (5 eq.) and the pH was adjusted to ≈ 7 with 6N HCl. EDCI (4 eq.) was added to the red solution which was magnetically stirred. Reaction progress was monitored with ^{31}P NMR which indicated reaction completion within 30 minutes. The reaction solution was concentrated to a red gum and the crude material was partially purified through reverse phase chromatography. The resulting nucleotide propargyl phosphoramidate (2.0 eq) in H_2O (2 mL) was added to a suspension of **1** (0.325-0.366 mmol) in 4 mL of t-BuOH. To this mixture was added 0.1 eq. of $CuSO_4$ x $5H_2O$ and 0.2 eq. of Sodium ascorbate. The reaction vessel was purged with Argon and allowed to stir at room temperature overnight in the dark. The suspensions were then purified with reverse phase chromatography to obtain the TEA salts of the phosphoramidate pro-gelators. Sodium salts were obtained of final compounds through cation exchange chromatography with Dowex 50wx8 resin (Na^+ form).

NAP-FF-AMP (2): Yield 33.2%, ^{31}P NMR (DMSO-d_6): 6.296 ppm, HRMS (ESI-): Calculated 1063.4191, Found 1063.4174. ((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((1-((4S,7S)-4,7-

dibenzyl-1-(naphthalen-2-yl)-2,5,8-trioxo-12,15,18-trioxa-3,6,9-triazaicosan-20-yl)-1H-1,2,3-triazol-4-yl)methyl)phosphoramidate

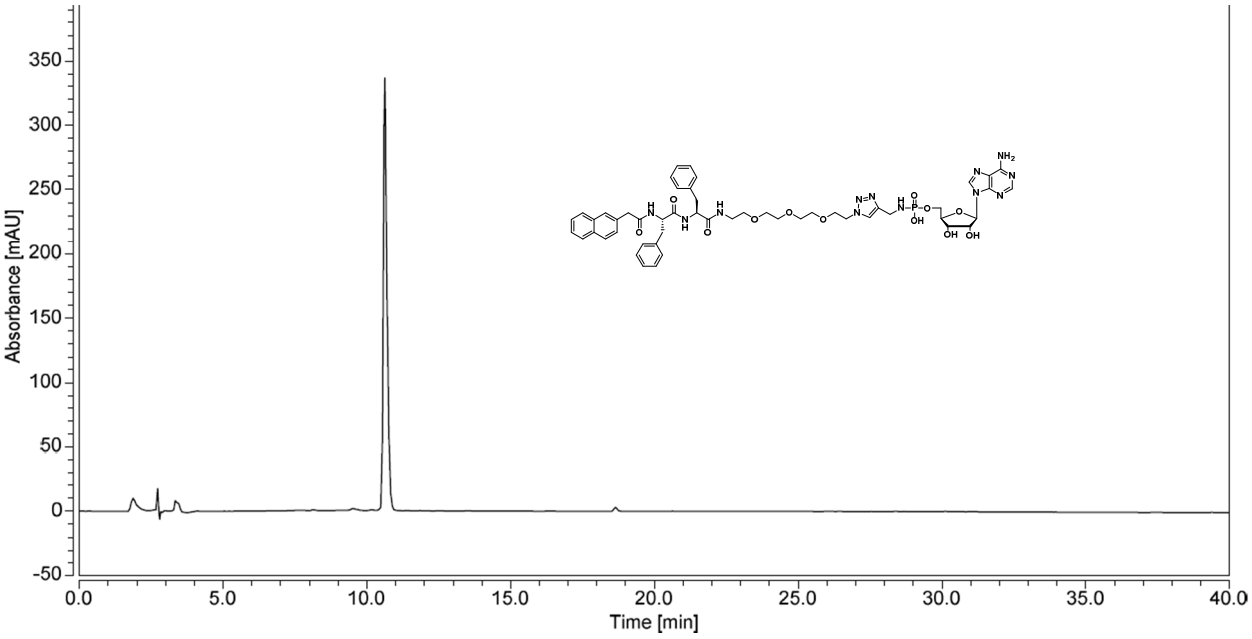
NAP-FF-UMP; (3): Yield 25.8%, ^{31}P NMR (DMSO-d_6): 6.226 ppm, HRMS (ESI-): Calculated 1040.3919, Found 1040.3905. ((2R,3S,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((1-((4S,7S)-4,7-dibenzyl-1-(naphthalen-2-yl)-2,5,8-trioxo-12,15,18-trioxa-3,6,9-triazaicosan-20-yl)-1H-1,2,3-triazol-4-yl)methyl)phosphoramidate

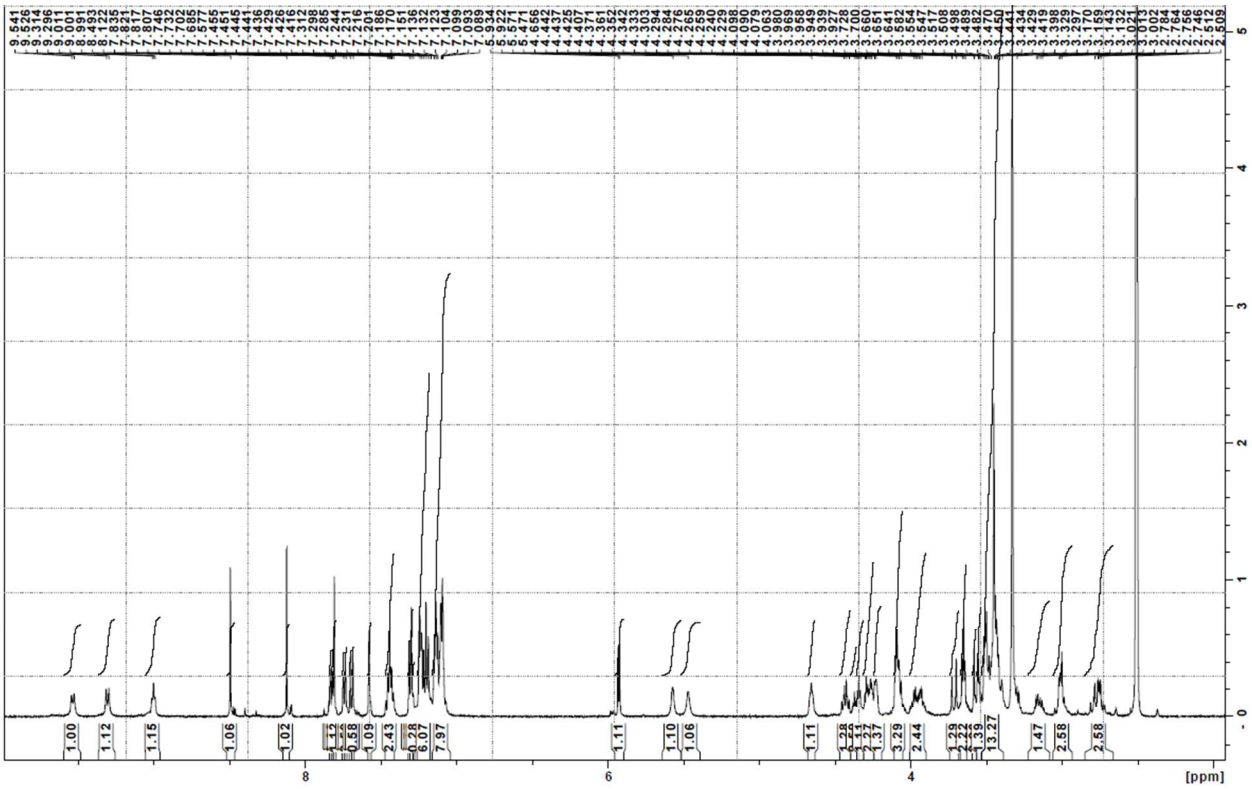
NAP-FF-GMP (4): Yield 62.7%, ^{31}P NMR (DMSO-d_6): 6.314 ppm, HRMS (ESI-): Calculated 1079.4140, Found 1079.4125.((2R,3S,4R,5R)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((1-((4S,7S)-4,7-dibenzyl-1-(naphthalen-2-yl)-2,5,8-trioxo-12,15,18-trioxa-3,6,9-triazaicosan-20-yl)-1H-1,2,3-triazol-4-yl)methyl)phosphoramidate

NAP-FF-CMP (5): Yield 28.0%, ^{31}P NMR (DMSO-d_6): 6.289 ppm, HRMS (ESI-): Calculated 1039.4079, Found 1039.4071. ((2R,3S,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((1-((4S,7S)-4,7-dibenzyl-1-(naphthalen-2-yl)-2,5,8-trioxo-12,15,18-trioxa-3,6,9-triazaicosan-20-yl)-1H-1,2,3-triazol-4-yl)methyl)phosphoramidate

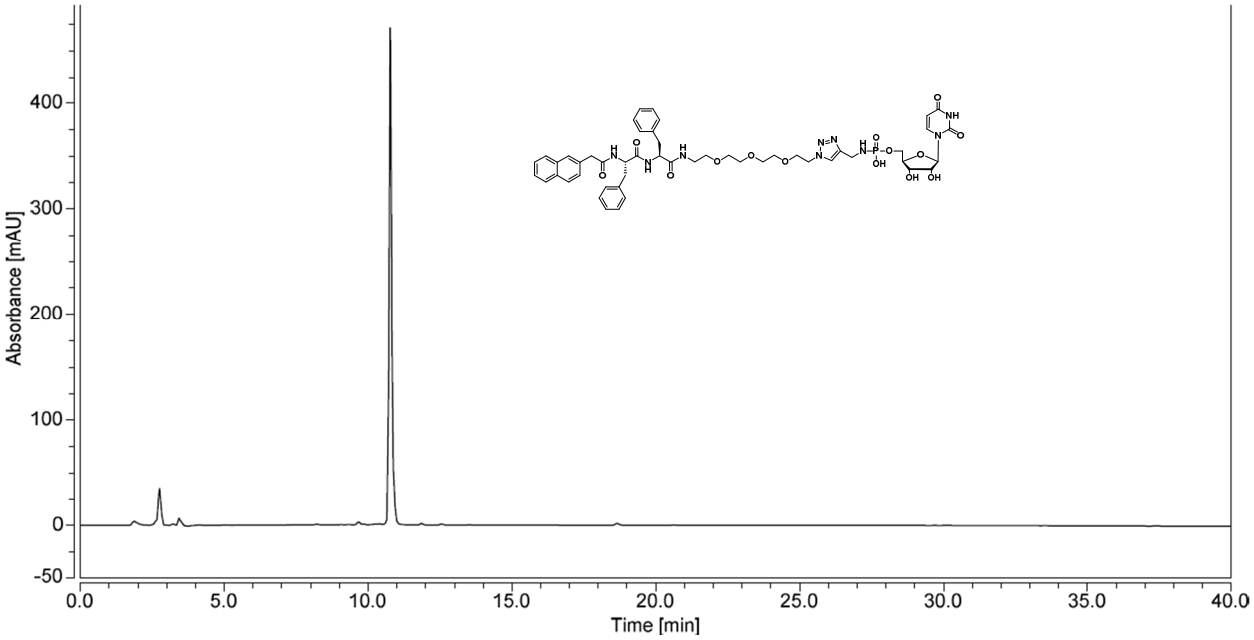
C. [1]HNMR and HPLC Chromatograms of Phosphoramidate Pro-Gelators (2-5)

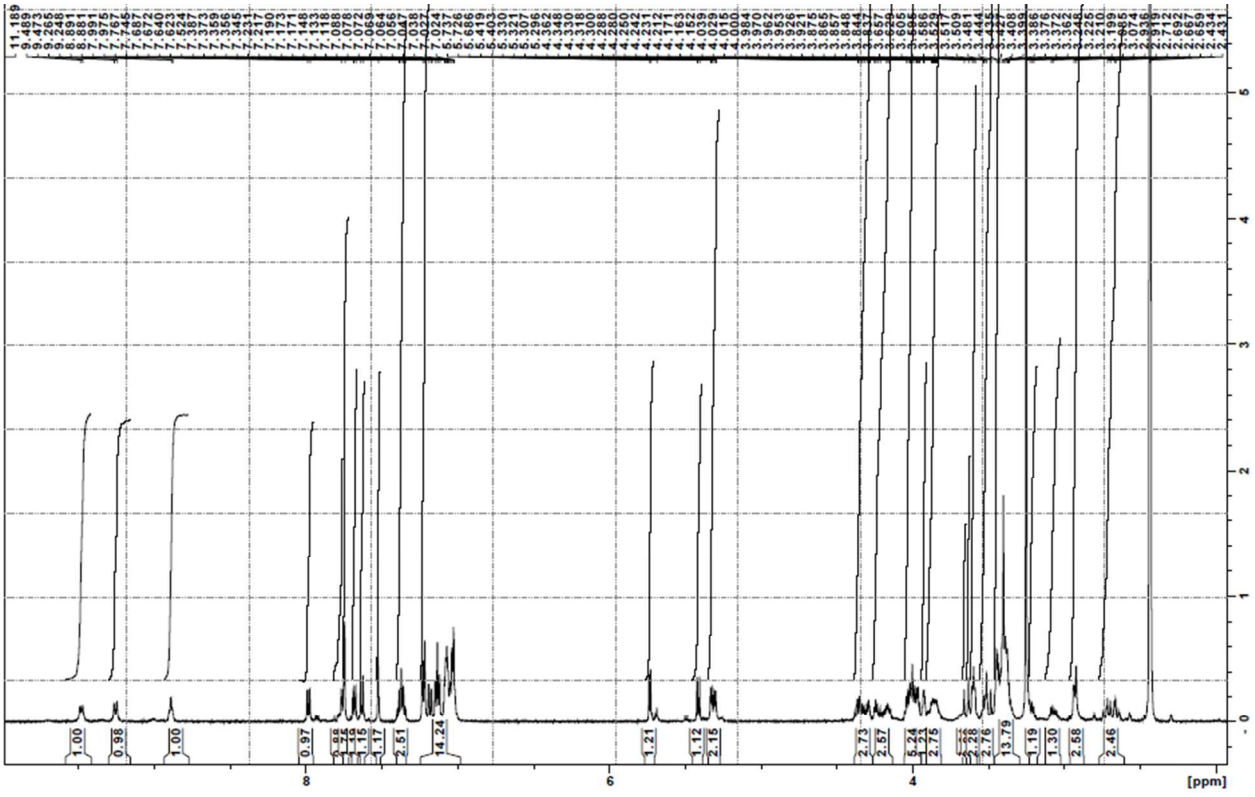
NAP-FF-AMP (**2**)



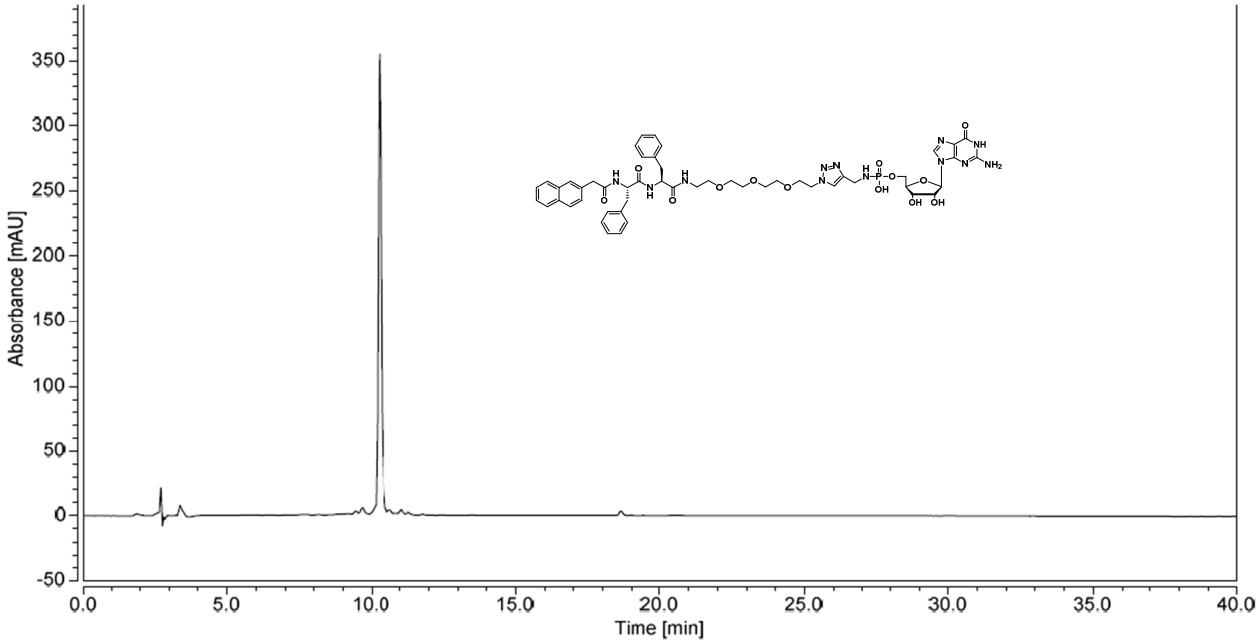


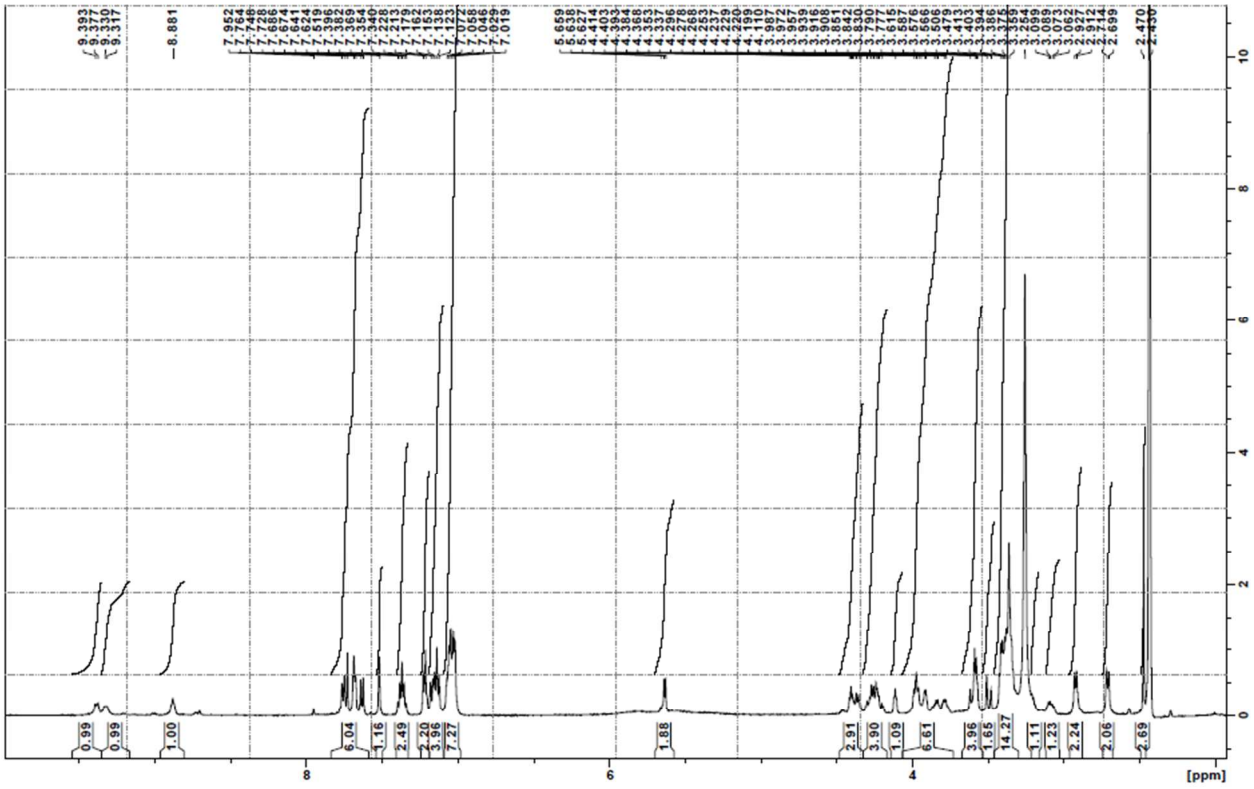
NAP-FF-UMP (**3**)



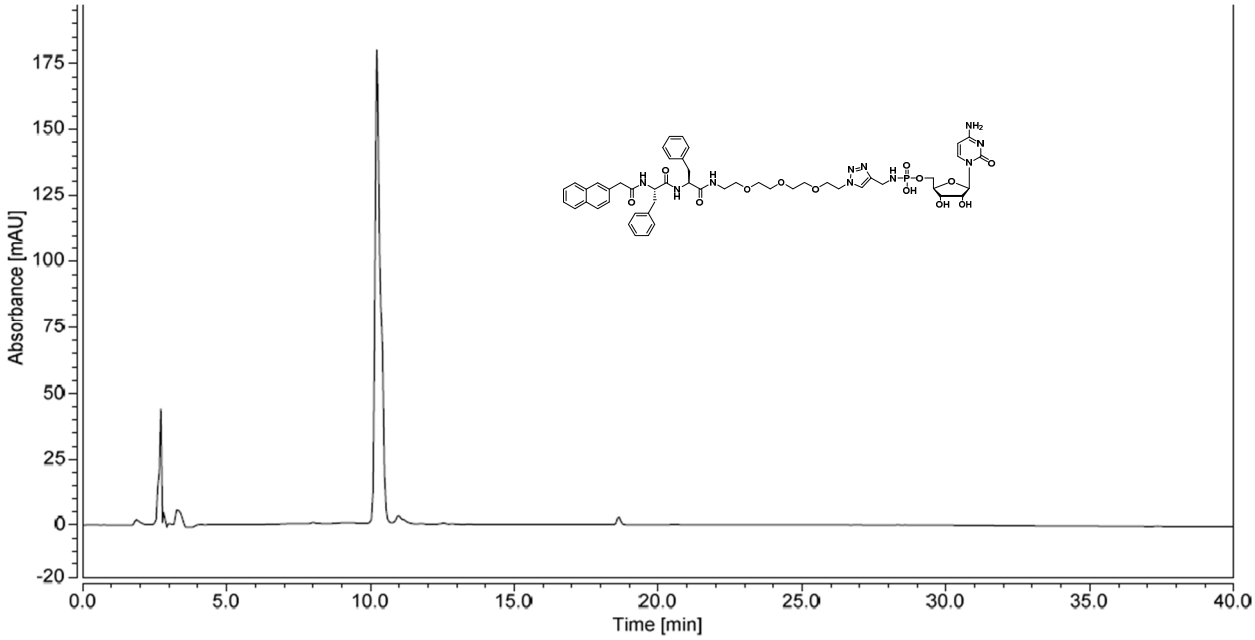


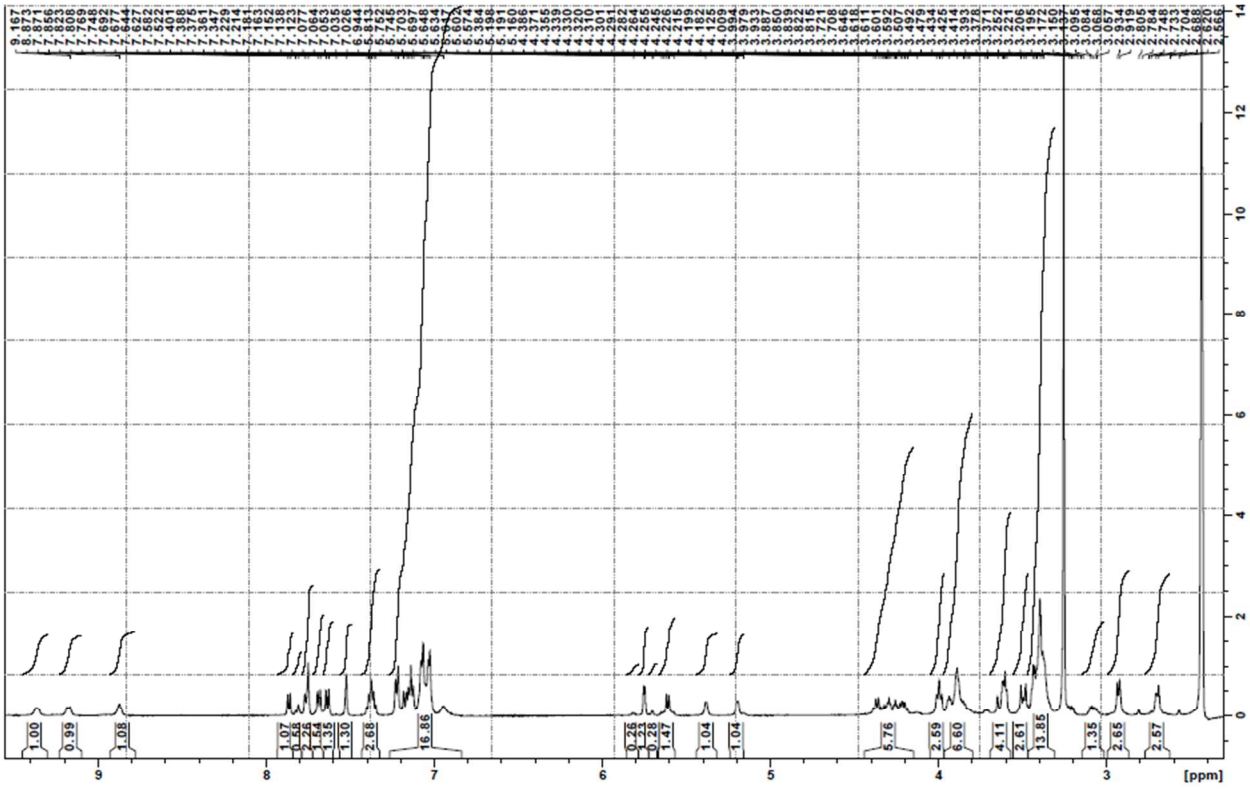
NAP-FF-GMP (**4**)



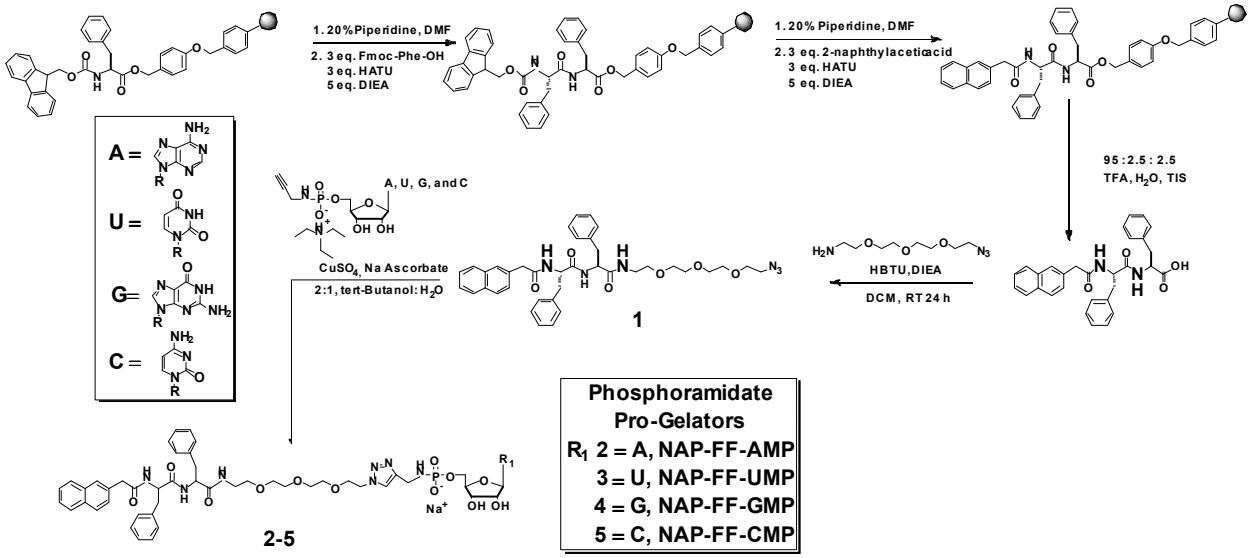


NAP-FF-CMP (**5**)





Supplementary Scheme 1.



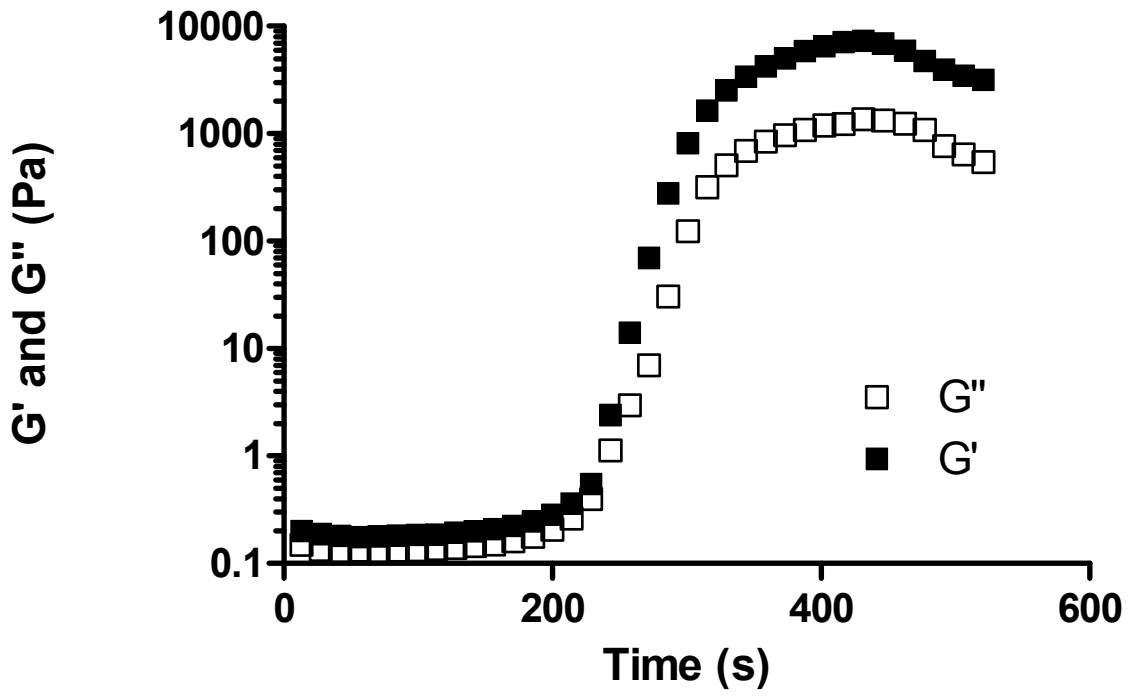
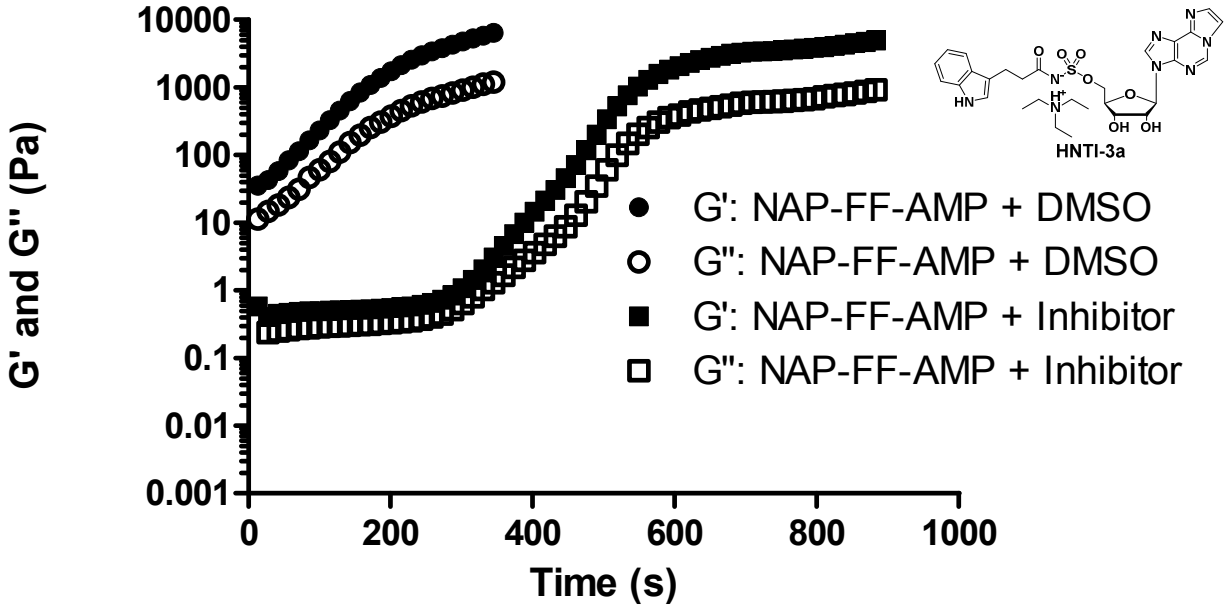


Figure S1.

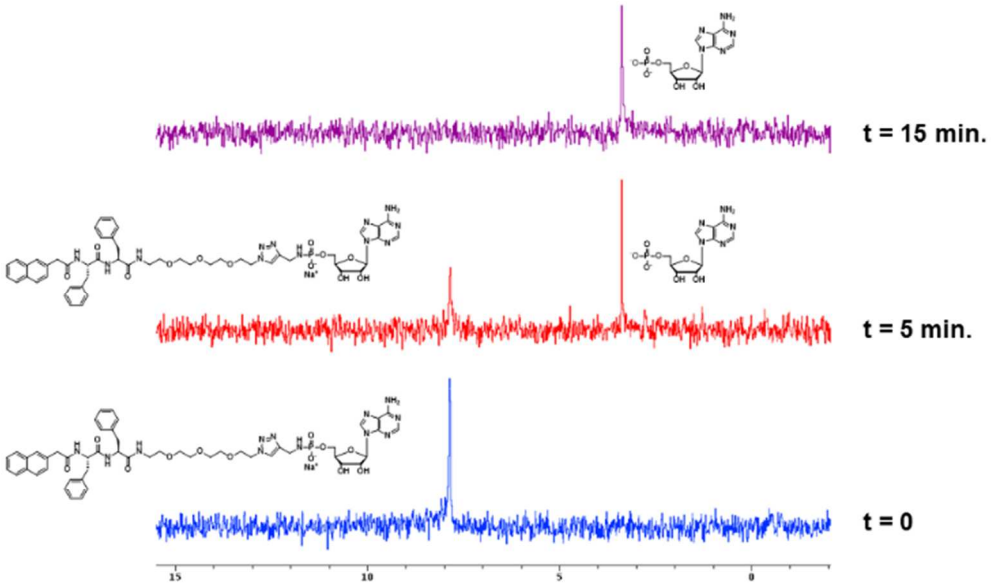
Oscillatory rheometry time course of NAP-FF-AMP (0.9% wt/vol in HINT1 Activity Buffer) gelation in the presence of HINT1 (6μM). A representative curve is shown. Note the decrease in moduli after reaching a maximum. This is attributed to syneresis of the gel and expulsion of water from the hydrogel network. Average gelation times were calculated from three different experiments, all of which exhibited the same syneresis of the gel

Figure S2.



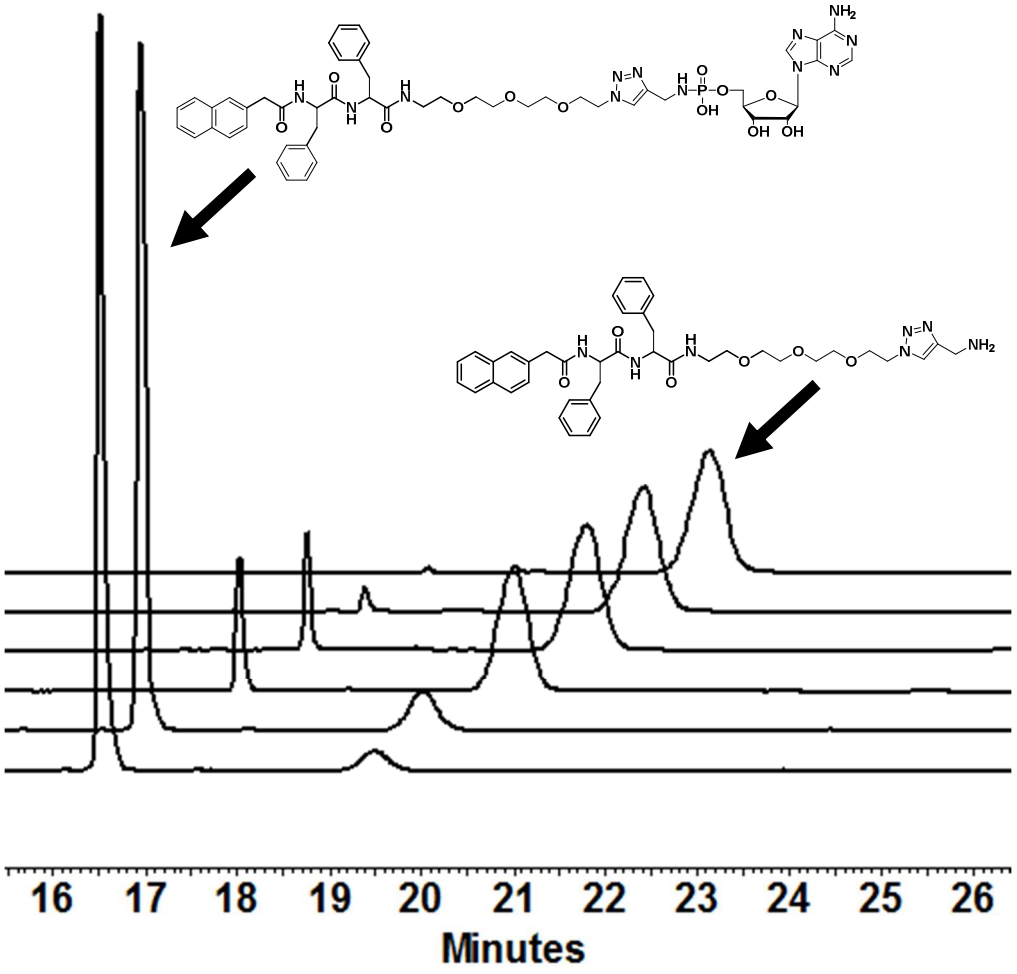
Oscillatory rheometry time sweep of NAP-FF-AMP (0.9% wt/vol in HINT1 Activity Buffer) gelation in the presence of HINT1 and either HNTI-3a (Squares) or DMSO control (Circles). HNTI-3a concentration is 6 mM.

Figure S3.



Enzymatic hydrolysis of NAP-FF-AMP monitored with ^{31}P NMR. Substrate concentration 4.5 mM in HINT1 Activity buffer in the presence of 6 μM HINT1. Qualitative experiments indicate a clear transition from the phosphoramidate pro-gelator to the monophosphate product.

Figure S4.



Catalytic Turnover of NAP-FF-AMP by HINT1: A representative HPLC time course of HINT1 induced degradation of NAP-FF-AMP at time points 10 min, 30 min, 2.5 h, 5 h, 20 h and 24 h. The percent remaining NAP-FF-AMP after 24 h was determined to be 0.8±0.1 % within HINT1 formed hydrogels (average of 4 experiments).