Supporting Information

Investigation of the triazolinedione (TAD) reaction with tryptophan as a direct route to copolypeptide conjugation and cross-linking

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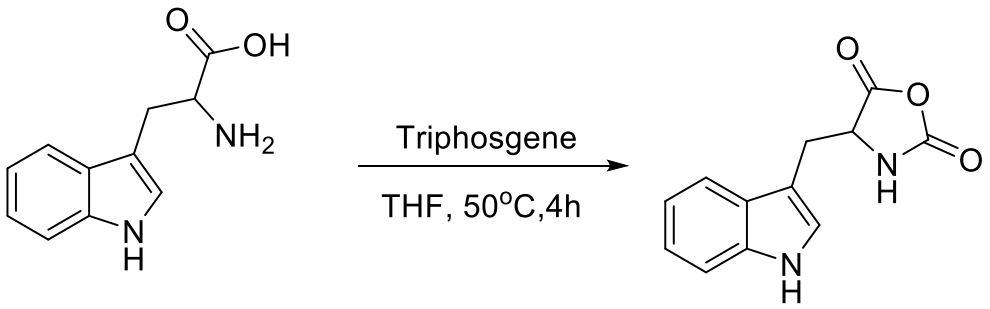
Chemicals and Reagents: All reagents were purchased from Sigma-Aldrich unless otherwise noted. Protected amino acids were purchased from BACHEM. Triphosgene was purchased from Fluorochem. Snake skin dialysis tubing (3.5K MWCO) was obtained from VWR. Silica nitric acid was prepared as described in literature.[1] Tris buffer solution was prepared using Trizma® base. O-Benzyl-L-tyrosine and Z-L-Lysine were converted to their corresponding NCAs using triphosgene as described in literature.[2] Hexamethylene bis-TAD was synthesized following our previously reported procedure.[3]

Methods: Nuclear Magnetic Resonance (NMR) analysis was completed using a Bruker Avance 400 (400 MHz) spectrometer at room temperature. Attenuated total reflection (ATR) FT-IR spectra were recorded using a Perkin-Elmer Spectrum 100 in the region of 4000-650 cm^{-1}. Four scans were completed with a resolution of 2 cm^{-1}. A background measurement was performed prior to loading the sample onto the ATR for measurement. Size exclusion chromatography (SEC) was performed on a system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector (35 °C), a Waters 2707 auto sampler, and a PSS PFG guard column followed by two PFG-linear-XL (7 µm, 8 × 300 mm) columns in series at 40 °C. Hexafluoroisopropanol (HFIP) with potassium trifluoroacetate (3 $g \cdot L^{-1}$) was used as eluent at a flow rate of 0.8 $mL \cdot min^{-1}$. The molecular weights were calculated against poly(methyl methacrylate) standards (Polymer Laboratories, M_p = 580 Da up to $M_p = 7.1 \times 10^6$ Da). SEC of P1 and its modification products was performed on an Agilent 1200 system in combination with two PSS GRAM analytical (8 × 300 and 8 × 100, 10 µ) columns, a Wyatt Dawn Heleos 8 multi angle light scattering detector (MALS) and a Wyatt Optilab rEX differential refractive index detector (DRI) with a 658 nm light source. The eluent was DMF

containing 0.1 M LiBr at a flow rate of 1 mL/min. The column temperature was set to 40 °C with the MALS detector at 35 °C and the DRI detector at 40 °C. Molar masses (M_w) and polydispersities were calculated from the MALS signal by the Astra software (Wyatt) using the refractive index increment (dn/dc) of 1.599. ATR-FTIR spectra were collected on a Perkin-Elmer Spectrum 100 in the spectral region of 650−4000 cm^{-1} and were obtained from 16 scans with a resolution of 2 cm^{-1}. Background measurement was taken before the sample was loaded for measurements.

1. Synthesis of Tryptophan NCA



Tryptophan (10 g, 48.97 mmol) was placed into 250 mL three neck round bottom flask and dried in vacuum oven for 2 days at 40 °C. Then, 100 mL of anhydrous THF was added together with α-pinene (19.8 g, 17 mL, 145 mmol) under an inert atmosphere. The mixture was heated to 50 °C. Triphosgene (7g, 23.6 mmol) was dissolved in anhydrous THF and added to this mixture dropwise using dropping funnel. The reaction mixture became a clear yellow solution after 2-3 hours. Then, half of the solvent was evaporated and the solution precipitated into cold heptane and kept in -20 °C overnight. The semi crystalline powder precipitate was collected by vacuum filtration and recrystallized using 1:1 ethyl acetate/heptane mixture without stopping the stirring while cooling during the period of crystallization. A yellowish white powder was collected by vacuum filtration and dried under vacuum. Yield: 7.47 g, 66.2%. ^{1}H-NMR (400 MHz, DMSO-d^{6},ppm): 3.13-3.19 (dd, 2H), 4.76 (t, 1H), 6.98 (m, 1H), 7.07 (m, 1H), 7.13 (d, 1H), 7.35 (d, 1H), 7.52 (d, 1H), 9.05 (s, 1H), 10.57 (s, 1H). ^{13}C-NMR (400 MHz, DMSO-d6): 26.42, 58.18, 106.98, 111.39, 118.39, 118.57, 121.05, 124.44, 127.15, 135.93, 151.83, 171.23. FTIR (neat, cm^{-1}): 3355, 3002, 1842, 1773, 1618, 1491, 1457, 1432, 1432, 1359, 1337, 1289, 1232, 1177, 1133, 1104, 1081, 1007, 977, 933, 899, 814, 783, 764, 750, 674, 650, 633, 609.

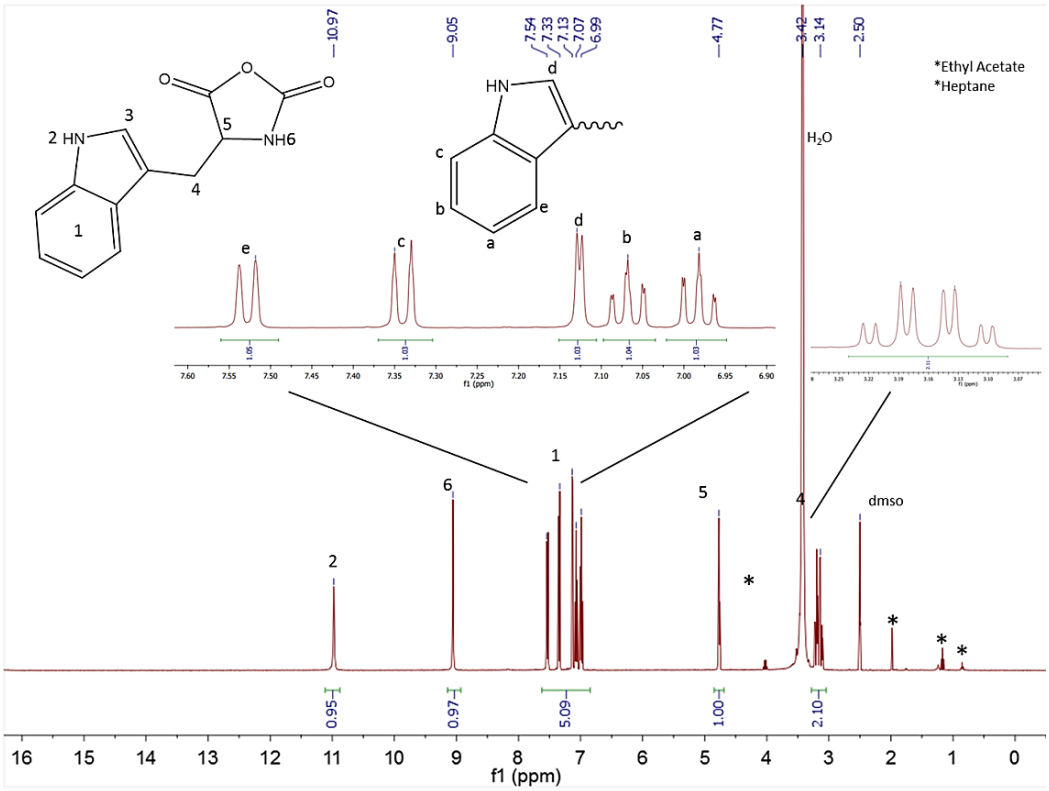


Figure S1. ^{1}H-NMR of tryptophan NCA.

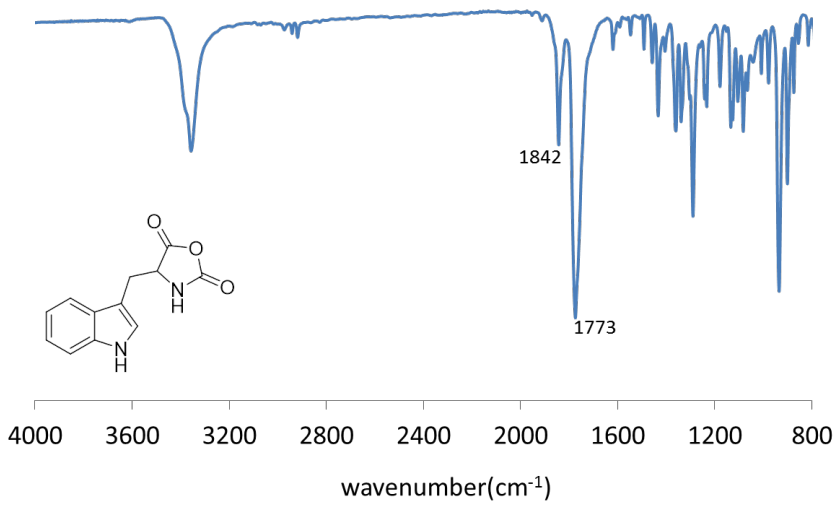


Figure S2. FTIR spectrum of tryptophan NCA.

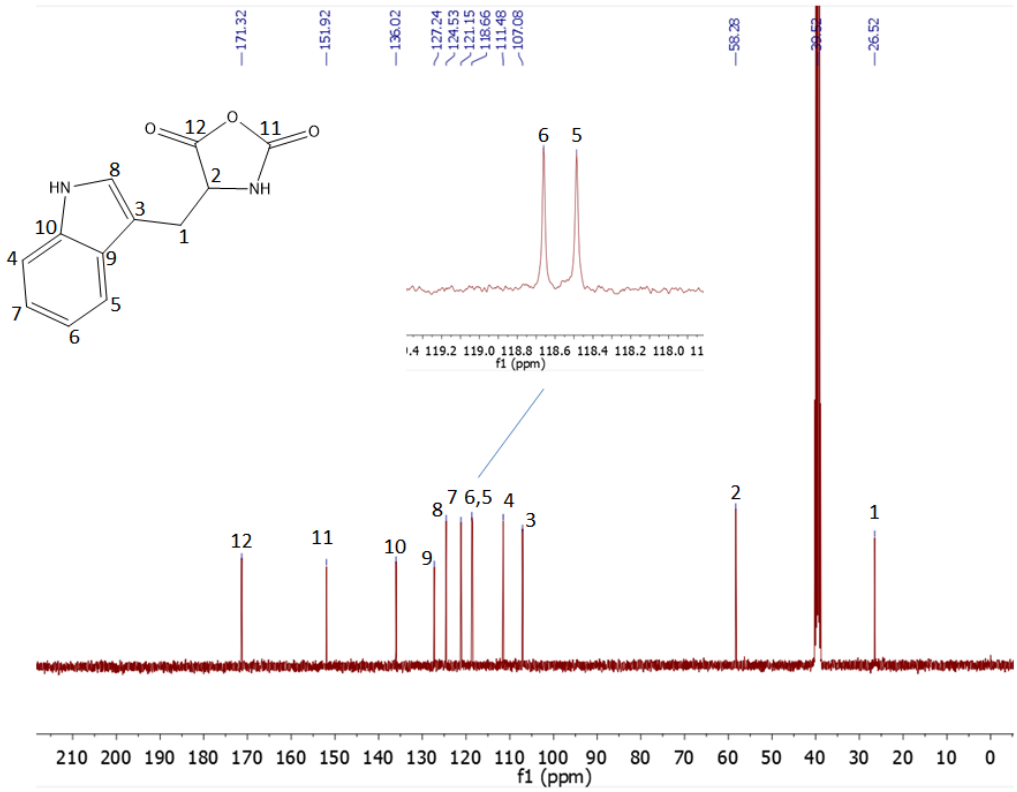


Figure S3. ^{13}C-NMR of tryptophan NCA.

2. Synthesis of P1 (poly(Lys$_{85}$-*st*-Trp$_{15}$))

2.6 g (8.5 mmol) of Z-Lysine NCA and 345 mg (1.5 mmol) of Trp NCA were added to a dried Schlenck flask under inert atmosphere and dissolved in 15 mL DMF. To this solution, 10.7 mg of benzylamine (0.1 mmol) from a stock solution in DMF was added fast and the solution stirred under vacuum. After 5-6 hours the solution was precipitated into diethyl ether. The sticky product was dissolved in chloroform and reprecipitated into diethyl ether. Finally, a non-sticky powder was collected by vacuum filtration and dried under vacuum. Yield: 2.23 g, 91 %.

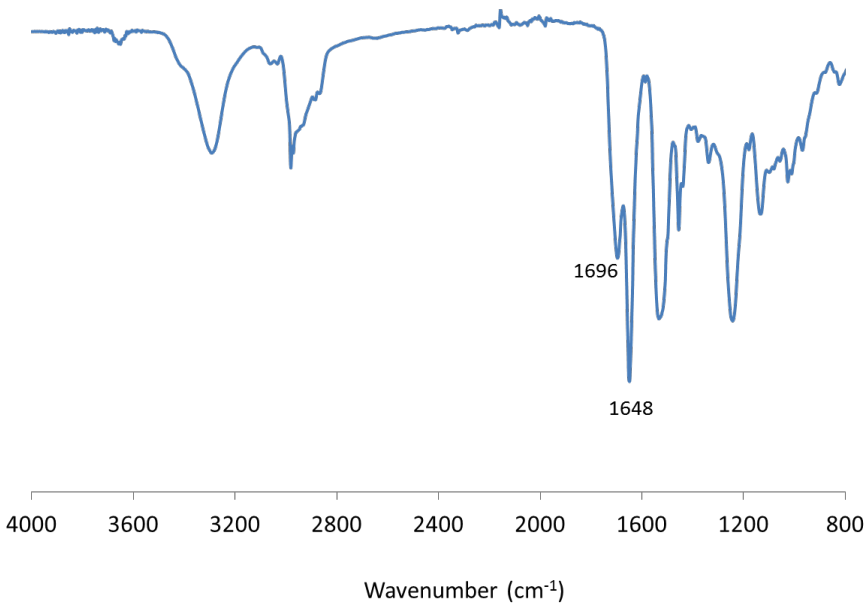


Figure S4. FTIR spectrum of **P1**.

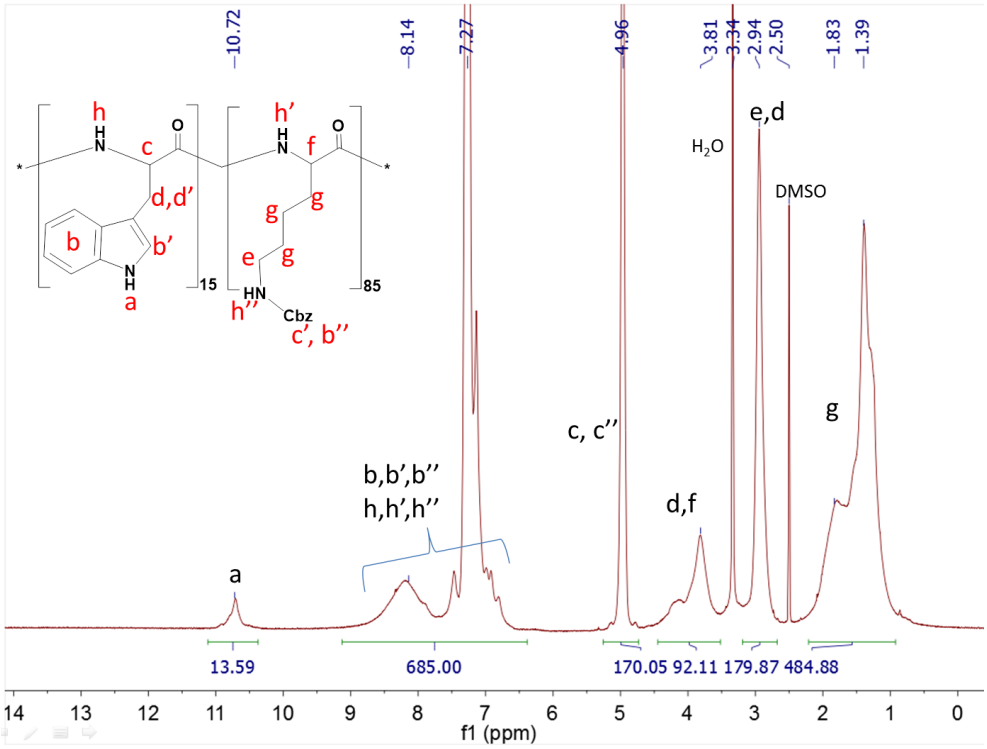
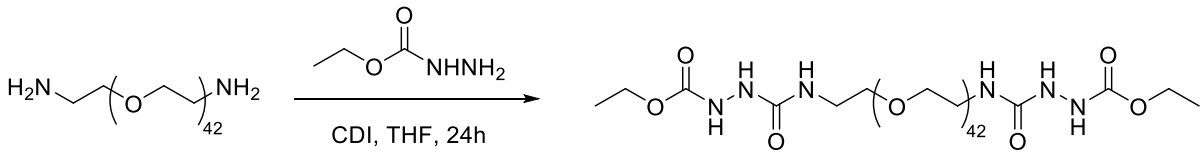


Figure S5. ^{1}H-NMR spectrum of **P1**.

3. Synthesis of PEG2K- bishydrazine carboxylate



44 mg Ethyl carbazate was dissolved in 2 mL anhydrous THF. To this solution, 65 mg of CDI added in portions over 15 min. Then, 400 mg of PEG2K-diamine (dissolved in 2mL) was added to the first solution and stirred for 24 hours. Afterwards, all THF was evaporated and the resulting solid dissolved in DI water, filtered and placed into a dialysis bag (0.1-0.5 kDa). The solution was dialyzed for 2 days and the product collected as a white powder after freeze-drying. Isolated yield: 381mg. M_w: 14,300 g/mol, PDI: 1.1.

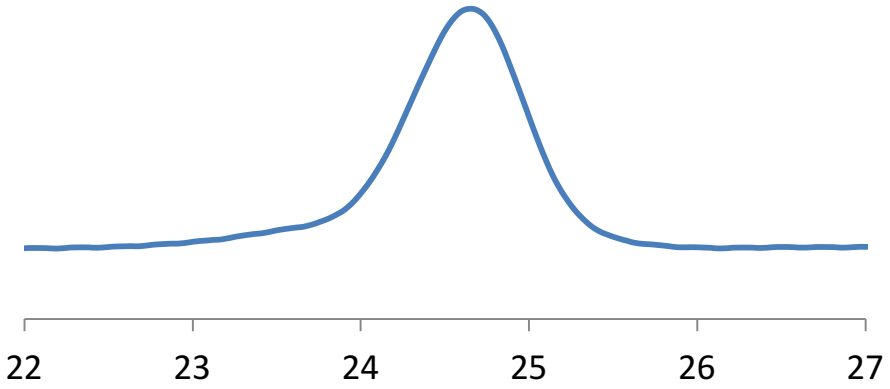
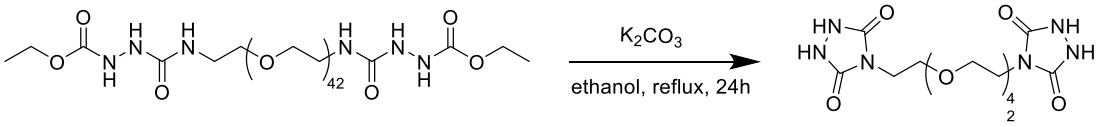


Figure S6. HFIP-SEC trace of PEG2K-hydrazine carboxylate. M_w: 14,300 g/mol, PDI: 1.1

4.Synthesis of PEG2K-Bisurazole:



PEG2K-bishydrazinecarboxylate (250 mg, approx. 0.24 mmol of hydrazine carboxylate end) was dissolved in 5 mL of absolute ethanol. To this solution, anhydrous potassium carbonate (66 mg, 0.48 mmol) was added and the mixture was refluxed for 24 hours. Then, all ethanol was evaporated and the remaining solid was dissolved in deionized water. The solution was carefully neutralized by HCl and placed into a dialysis bag. Finally, it was dialyzed against DI water for 2 days and the white product was obtained by freeze drying. SEC: M_w: 14,300 g/mol, PDI: 1.1, isolated yield: 195 mg

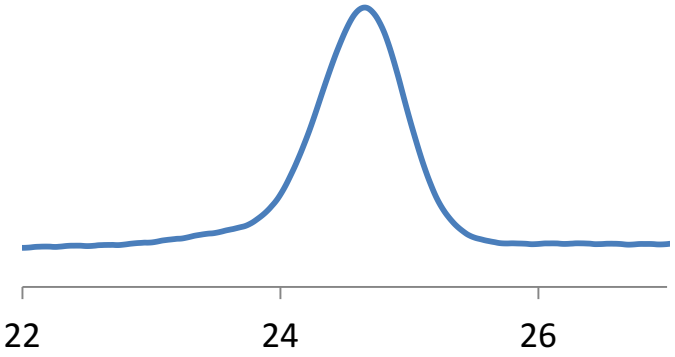
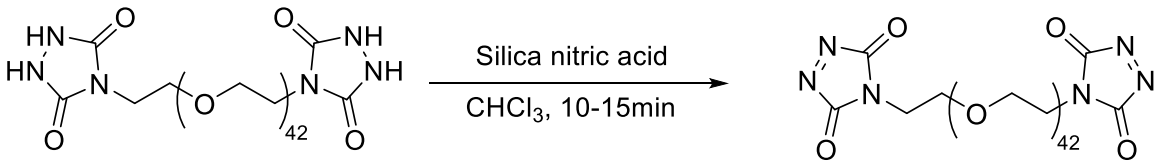


Figure S7. HFIP-SEC trace of PEG2K-bisurazole. M_w: 14,300 g/mol, PDI: 1.1

5. Synthesis of PEG2K-TAD



100 mg of PEG2K-Urazole was dissolved in 1 mL of anhydrous chloroform. To this solution, 15 mg of silica nitric acid was added and shaken for 15 minutes. Then the solution was filtered and precipitated into diethyl ether. The product was collected as a pink powder. Isolated yield: 79 mg, conversion of urazole ends to TADs: 95% from NMR.

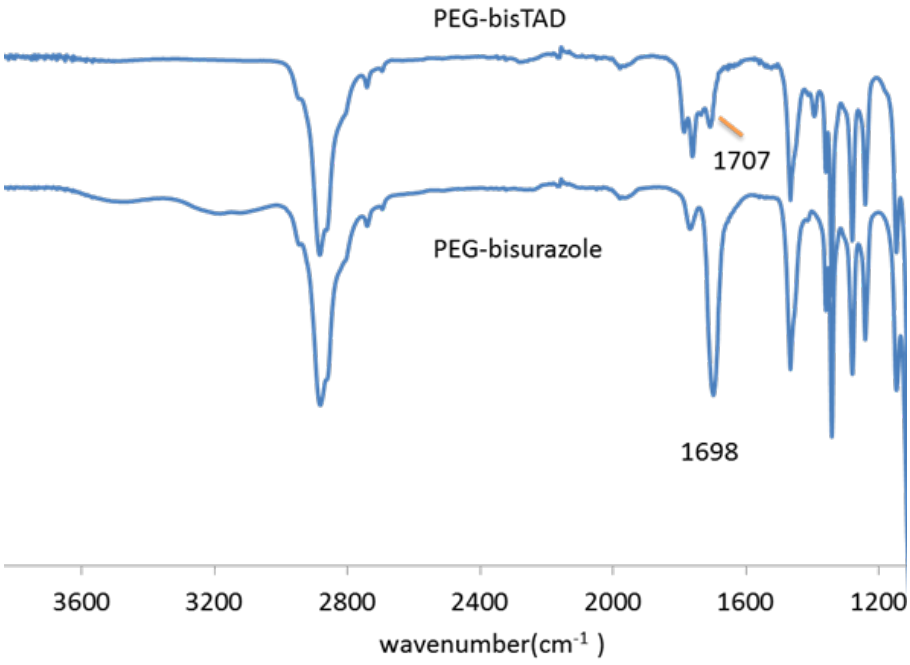
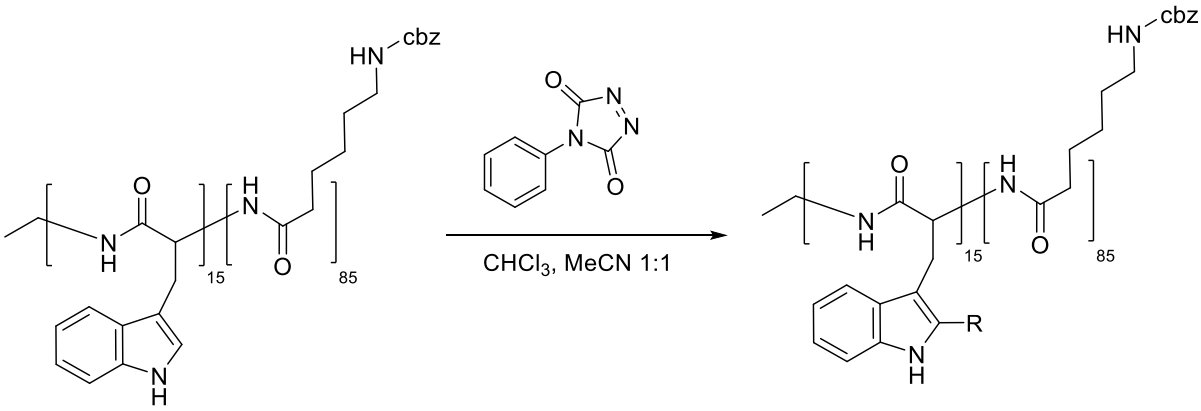


Figure S8. FTIR spectrum of PEG2K-bisurazole and PEG2K-bisTAD.

5. PTAD functionalization of P1



200 mg of P1 was dissolved in 2 mL chloroform/acetonitrile (1:1). Commercially available PTAD (12 mg – theoretically 1 eq. to tryptophan) was dissolved in 0.2 mL acetonitrile and added to the polymer solution. The red colour of PTAD disappeared after 40-60 minutes indicating no more PTAD left in the solution. Then, the solution was precipitated into diethyl ether and collected by vacuum filtration. Isolated yield: 205 mg

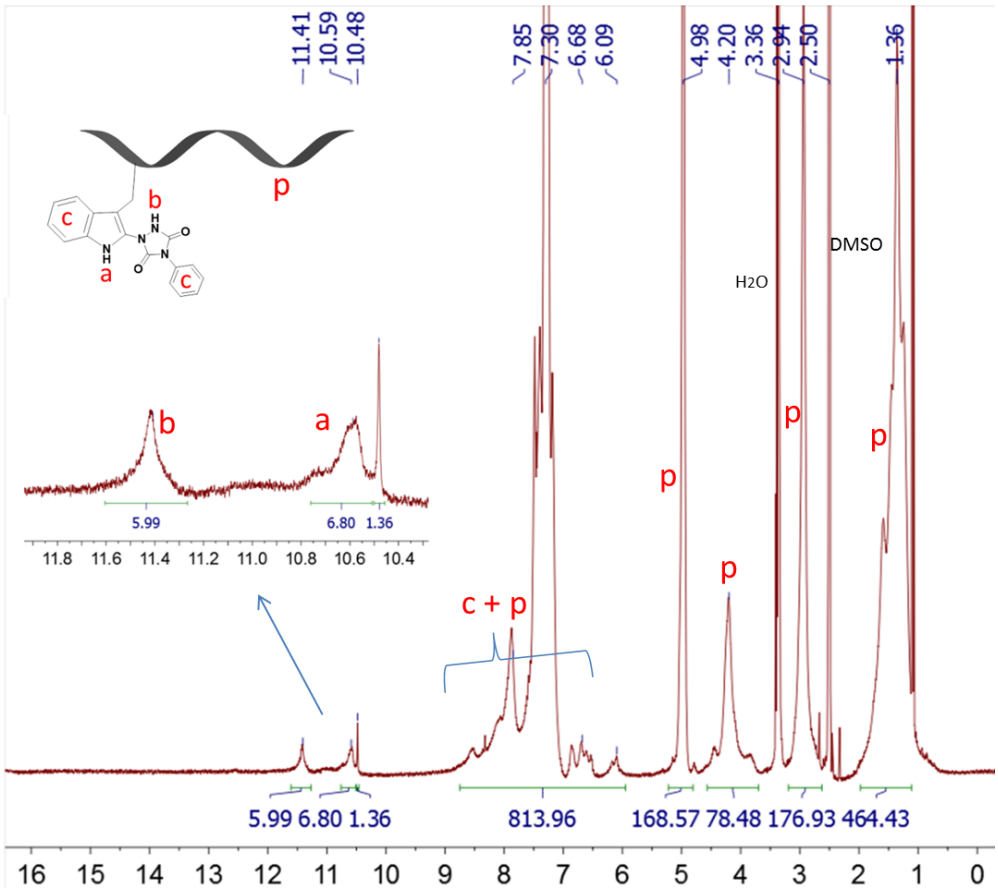


Figure S9. ^{1}H-NMR spectrum of PTAD functionalized P1.

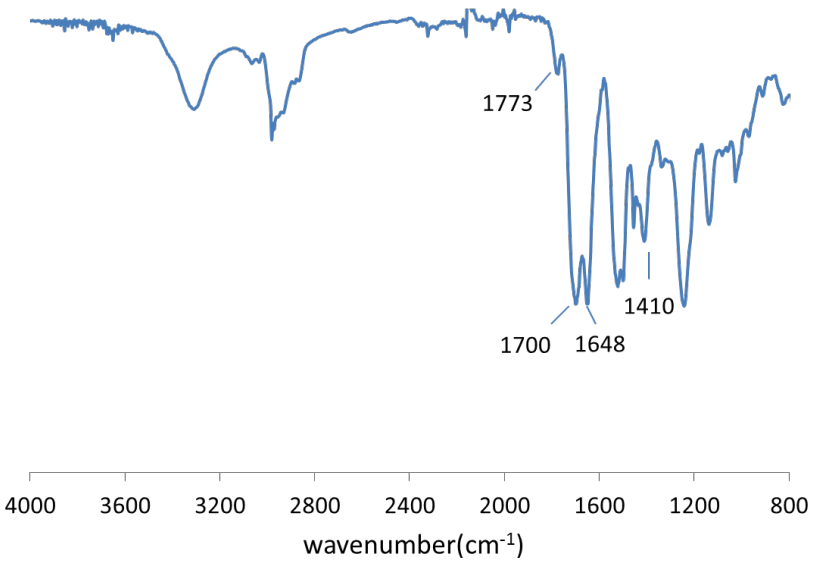


Figure S10. FTIR spectrum of PTAD functionalized P1.

6. Synthesis of P2

161 mg (0.7 mmol) of Tryptophan-NCA was dissolved in 3 mL anhydrous DMF and flushed with N_2 for 5 minutes. In another flask, 200 mg (0.1 mmol) of PEG2K-monoamine was dissolved in 3 mL anhydrous DMF and flushed with N_2 for 5 minutes. The PEG containing solution was injected fast into the Tryptophan-NCA containing solution and stirred for 8 hours under vacuum. Finally, the polymer was precipitated into diethyl ether and dried under vacuum.

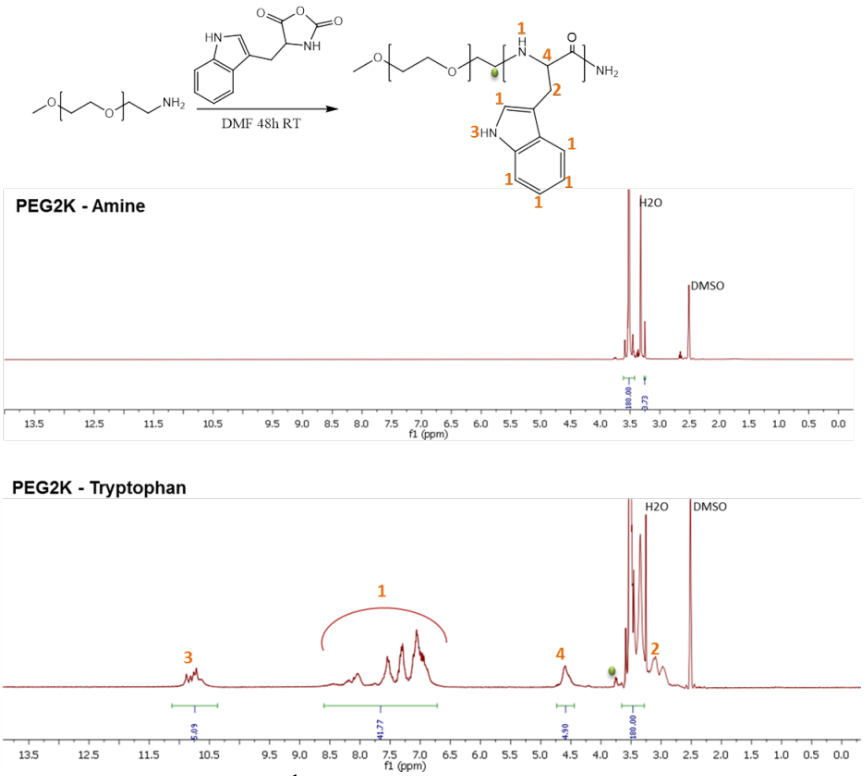


Figure S11. ^{1}H-NMR spectrum of PEG2K-Trp$_6$.

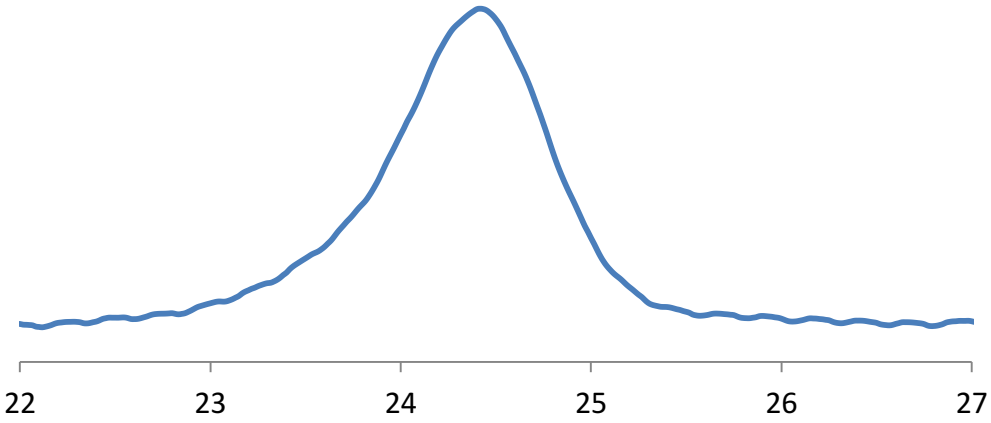


Figure S12. HFIP-GPC trace of PEG2K-Trp_6.

7. Cross-linked films from P1 and hexamethylene bis-TAD

300 mg of P1 was dissolved in 3 mL chloroform-acetonitrile (1:1) mixture. To this solution, 100 mg of HM-TAD from a stock solution (33 mg/mL in chloroform-acetonitrile 1:2) was added and shaken. Then, the solution was slowly poured into a clean glass petri dish. Pink color of TAD was completely disappeared after 10 min. Opaque film become transparent when solvent was evaporated in open air.

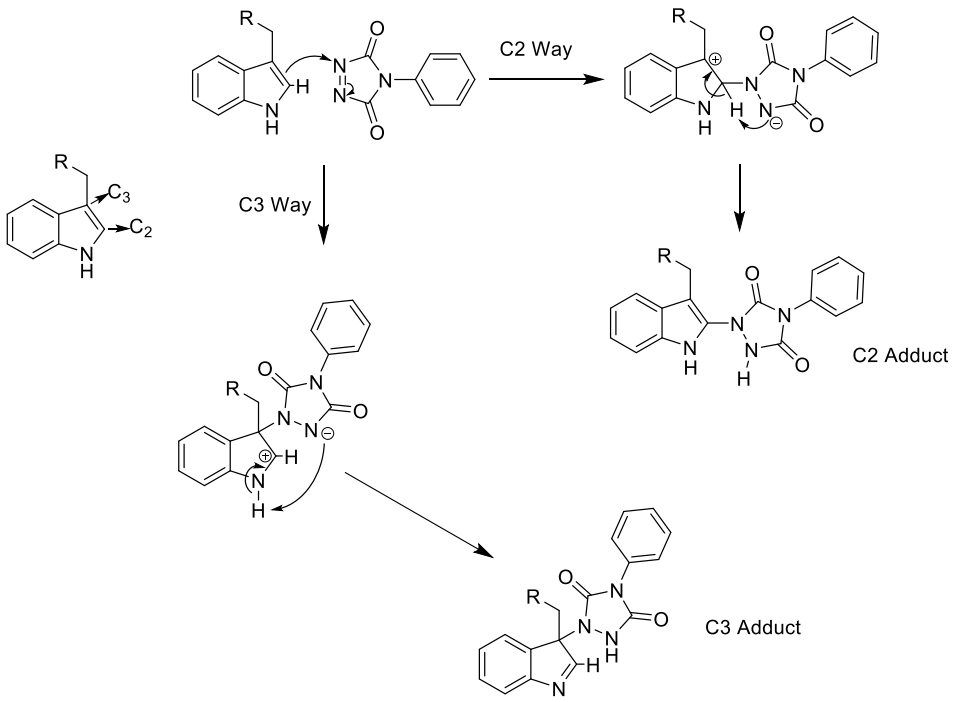


Figure S13. PTAD-Tryptophan reaction products.

[1] A. Ghorbani-Choghamarani, Z. Chenani, S. Mallakpour, *Synth. Commun.* **2009**, *39*, 4264-4270.
[2] J. Huang, C. L. Hastings, G. P. Duffy, H. M. Kelly, J. Raeburn, D. J. Adams, A. Heise, *Biomacromolecules* **2012**, *14*, 200-206.
[3] S. B. Hanay, B. Ritzen, D. Brougham, A. A. Dias, A. Heise, *Macromol. Biosci.* **2017**, DOI: 10.1002/mabi.201700016