

Introduction

DNA-based supramolecular hydrogels are 3D polymeric networks that swell when in water. They utilize DNA binding modes with non-covalent interactions that provide them with dynamic properties; i.e. viscoelasticity, self-healing, stimuli-responsiveness.¹ As DNA binders can exhibit both enthalpic and entropic behaviors, we can strategically tune thermal and viscoelastic response within our materials by using both intercalators and minor groove binders (mGBs) as cross-linkers.¹

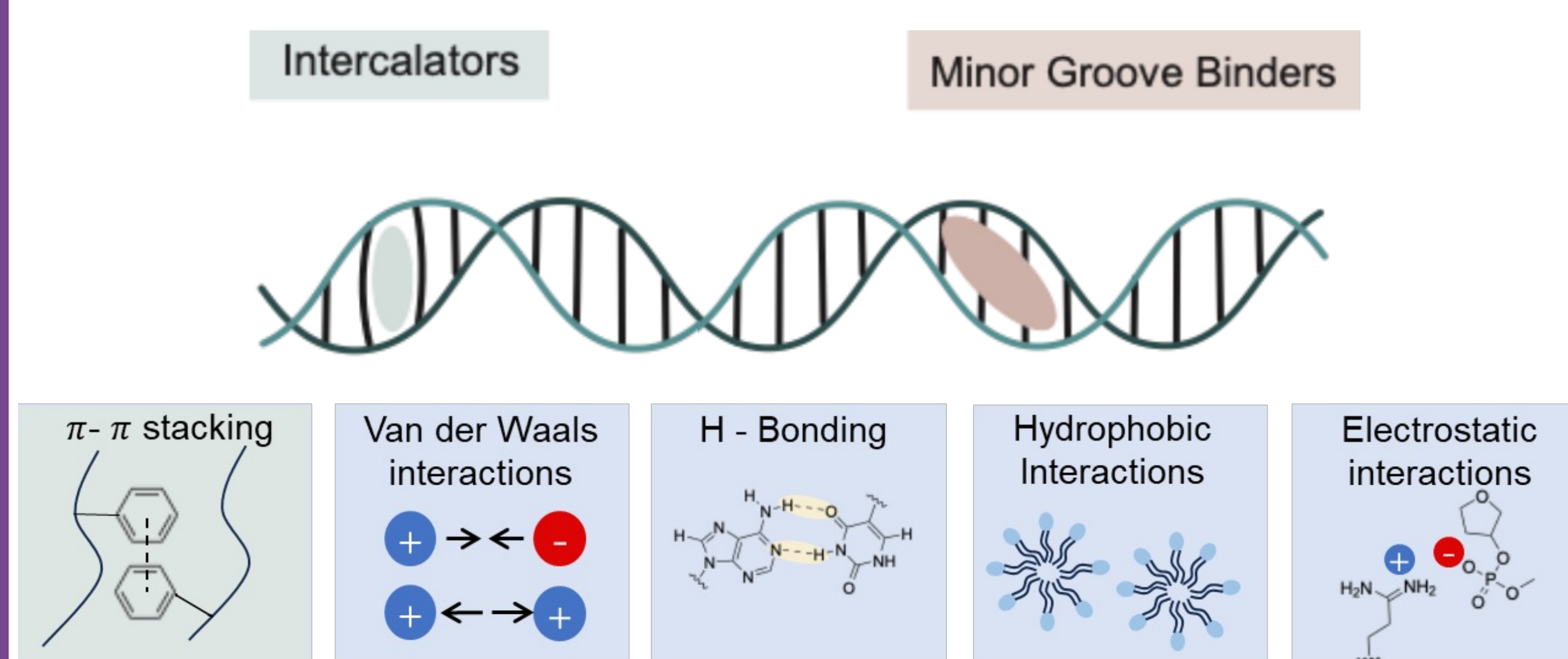


Figure 1. DNA binders and their non-covalent interactions with DNA. Intercalators are planar aromatic molecules that insert in between the base pairs of DNA. Minor groove binders are crescent shaped molecules that insert into the minor groove of DNA, displacing water.

Synthesis of Hoechst

Previous work has mGBs can exhibit entropically-driven behavior through the displacement of water. Hoechst is a mGB of interest for the formation of DNA cross-linked hydrogels to create networks with high thermal stability.

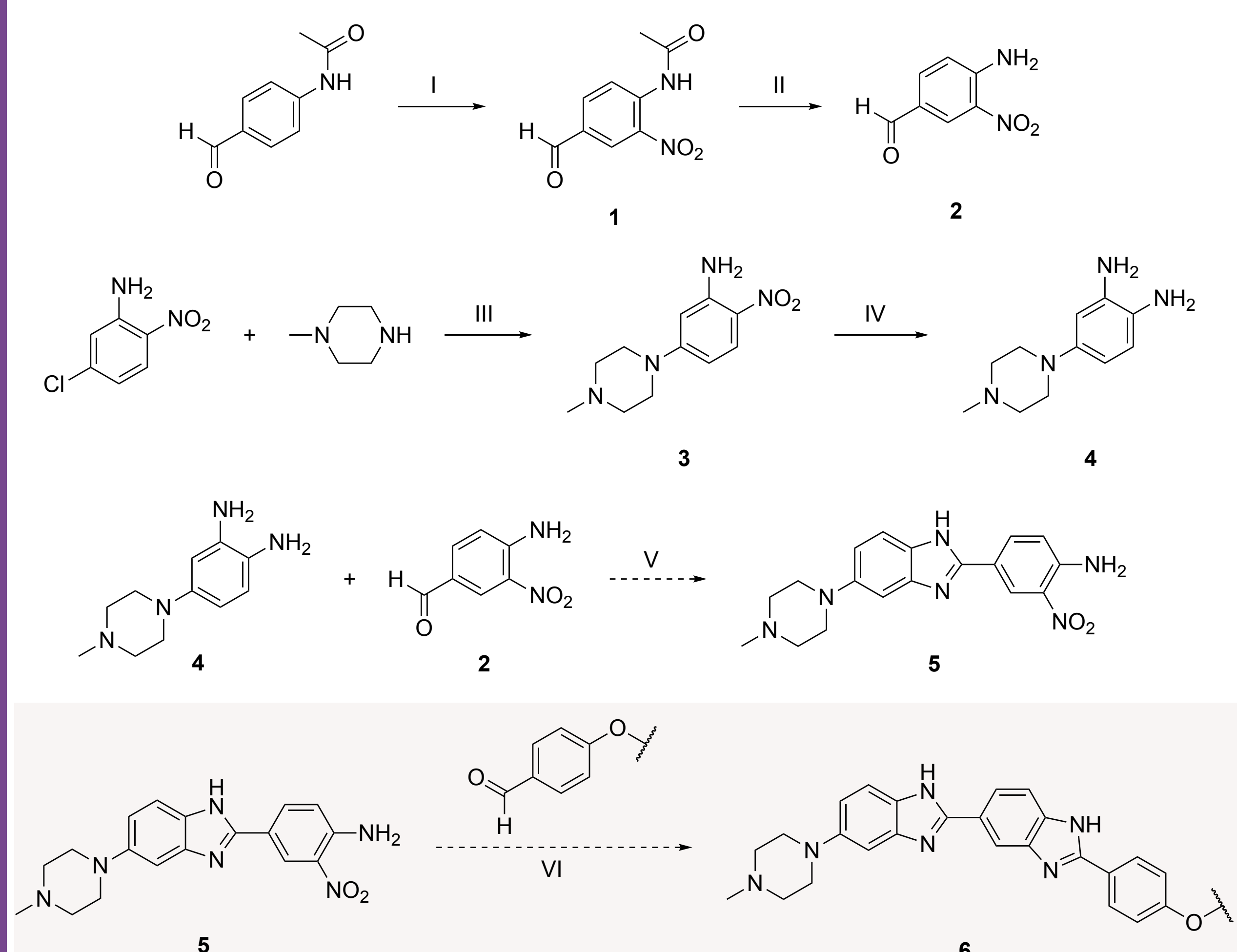


Figure 2. Stepwise-Synthesis for mGB Hoechst (**6**): **I.** $\text{HNO}_3/\text{H}_2\text{SO}_4$ (1/4), rt, 2 hr. **II.** 10% H_2SO_4 , 100 °C, o/n. **III.** K_2CO_3 , anhydrous DMF, 120 °C, o/n. **IV.** 10% Pd/C, MeOH/EtOAc (1/4), H_2 , rt, 48 hr. **V.** $\text{Na}_2\text{S}_2\text{O}_5$, DMF, 80 °C, o/n. **VI.** Benzaldehyde-PEG_{2k}, $\text{Na}_2\text{S}_2\text{O}_5$, anhydrous DMF, 90 °C, 24 hr.³

Synthesis of Psoralen Derivatives

Psoralen is an intercalator with the ability to undergo a [2+2] cycloaddition with thymine when exposed to UV-A light, transitioning from a dynamic to a covalent network.^{1,4} We have previously investigated Psoralen-PEG properties within our supramolecular hydrogels.⁴ To fully understand the impact of molecular structure on the thermodynamic and physical properties of cross-linked hydrogels, we have developed derivatives.¹

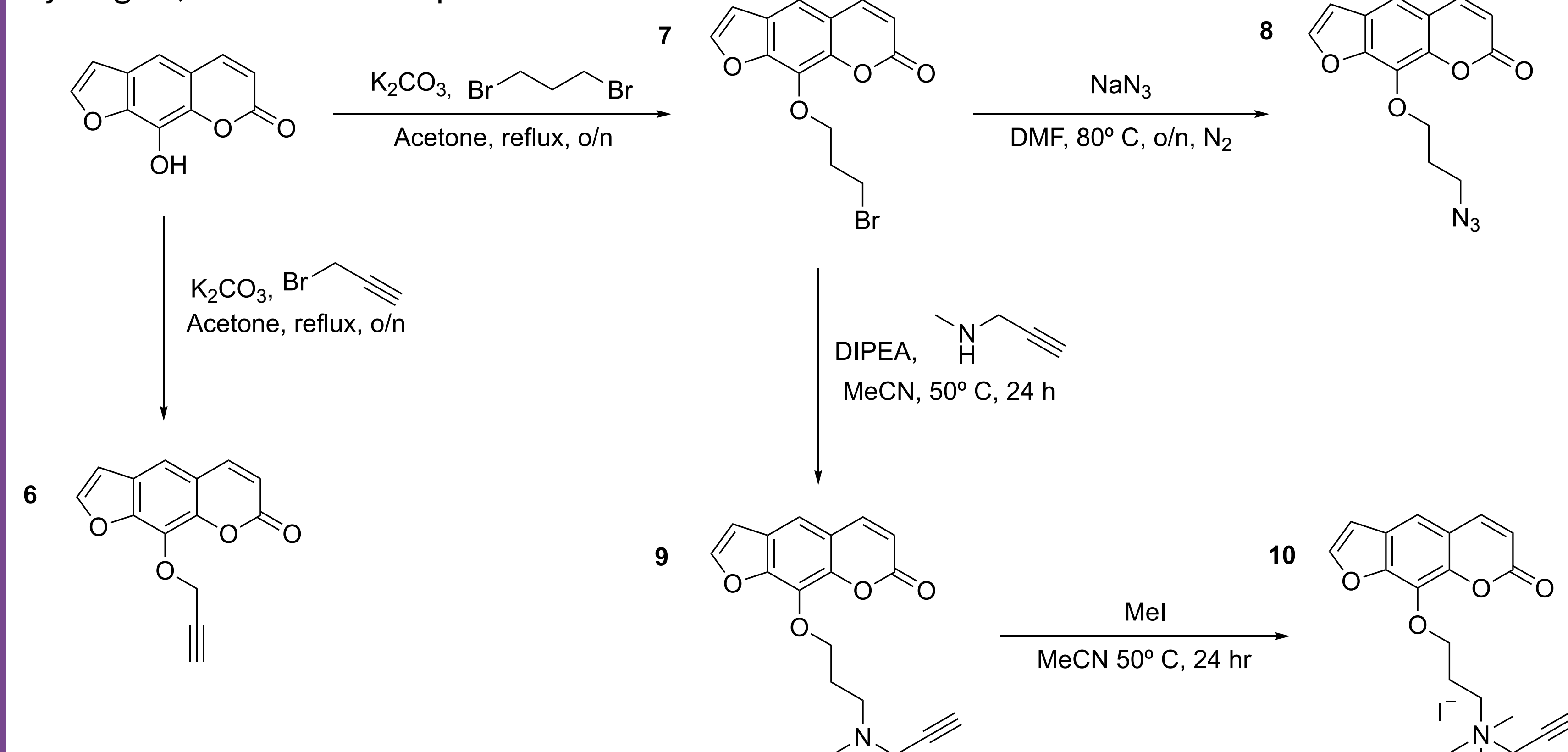


Figure 3. Synthesis of psoralen derivatives: 8-(prop-2-ynoxy)psoralen (**6**)⁵, 8-(3-azopropoxy)psoralen (**8**)^{5,6}, and 8-(3-N-dimethyl-2-propyn-1-ylamino)propoxy-Psoralen (**10**)^{5,7,8}

Synthesis of Psoralen-PEG (Pso-PEG) Derivatives

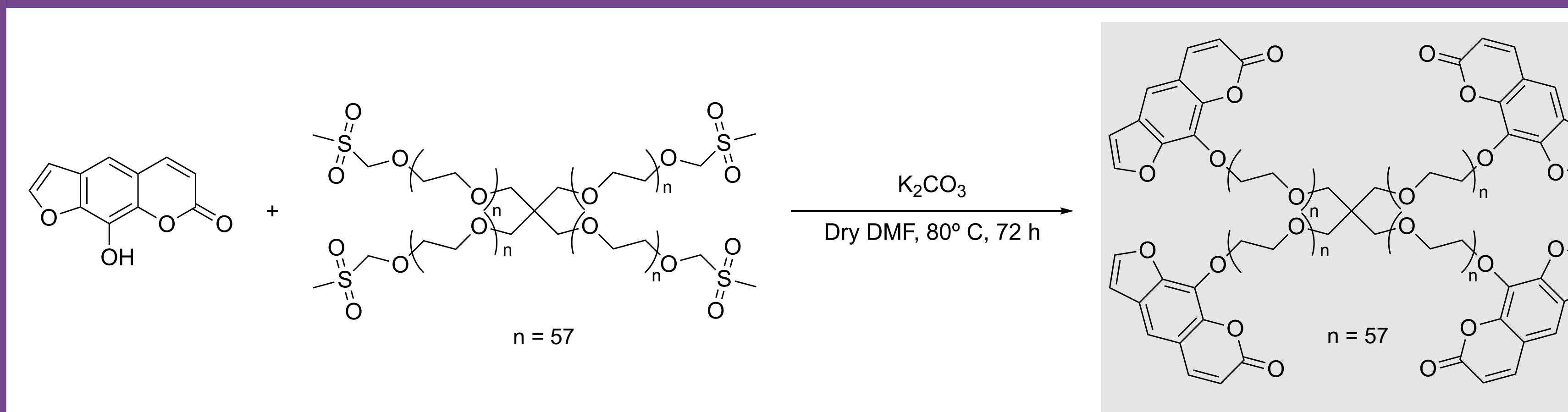


Figure 4. Synthesis of Pso-PEG. 8-hydroxypsoralen, 4-arm mesylated PEG, and potassium carbonate were stirred in DMF at 80 °C for 3 days.⁴

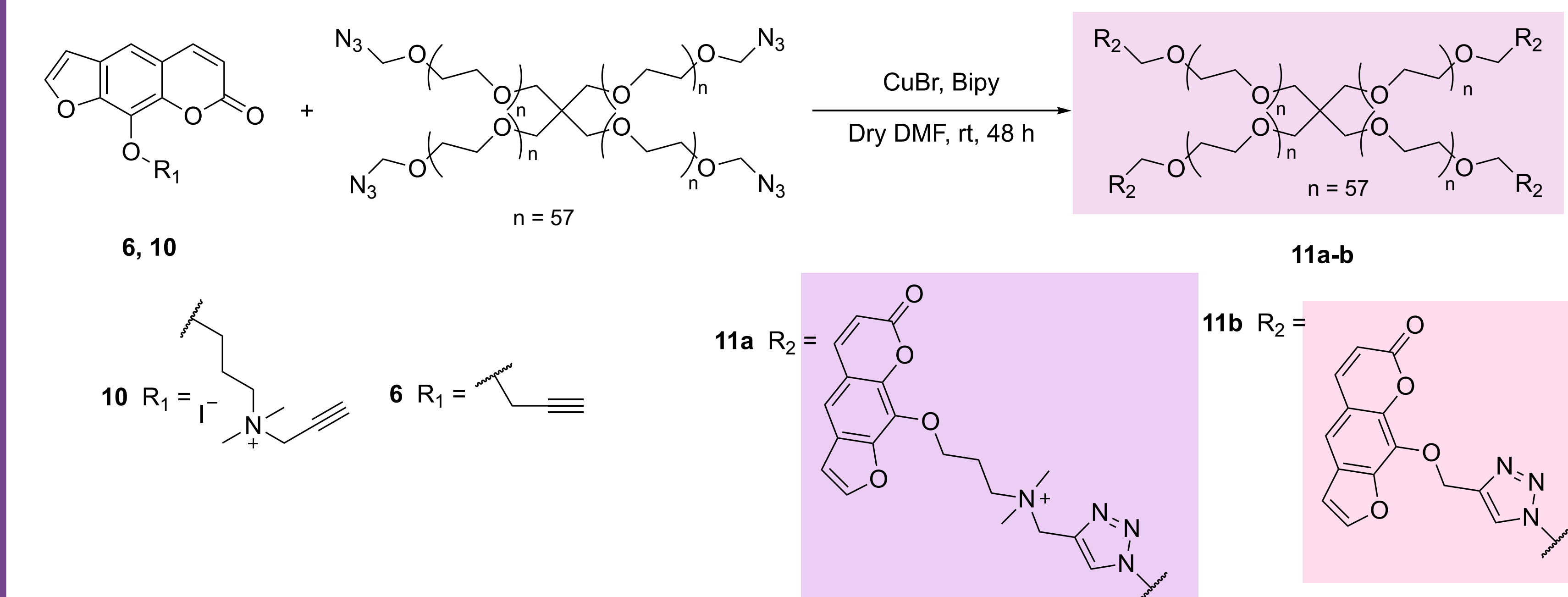


Figure 5. Synthesis of two copper catalyzed azide alkyne Pso-PEG derivatives. Psoralen-alkyne derivatives, 4-arm PEG-azide, copper bromide, and 2,2'-bipyridine were stirred in DMF at room temperature for 2 days.⁹

Characterization and Rheology of Pso-PEG Derivatives

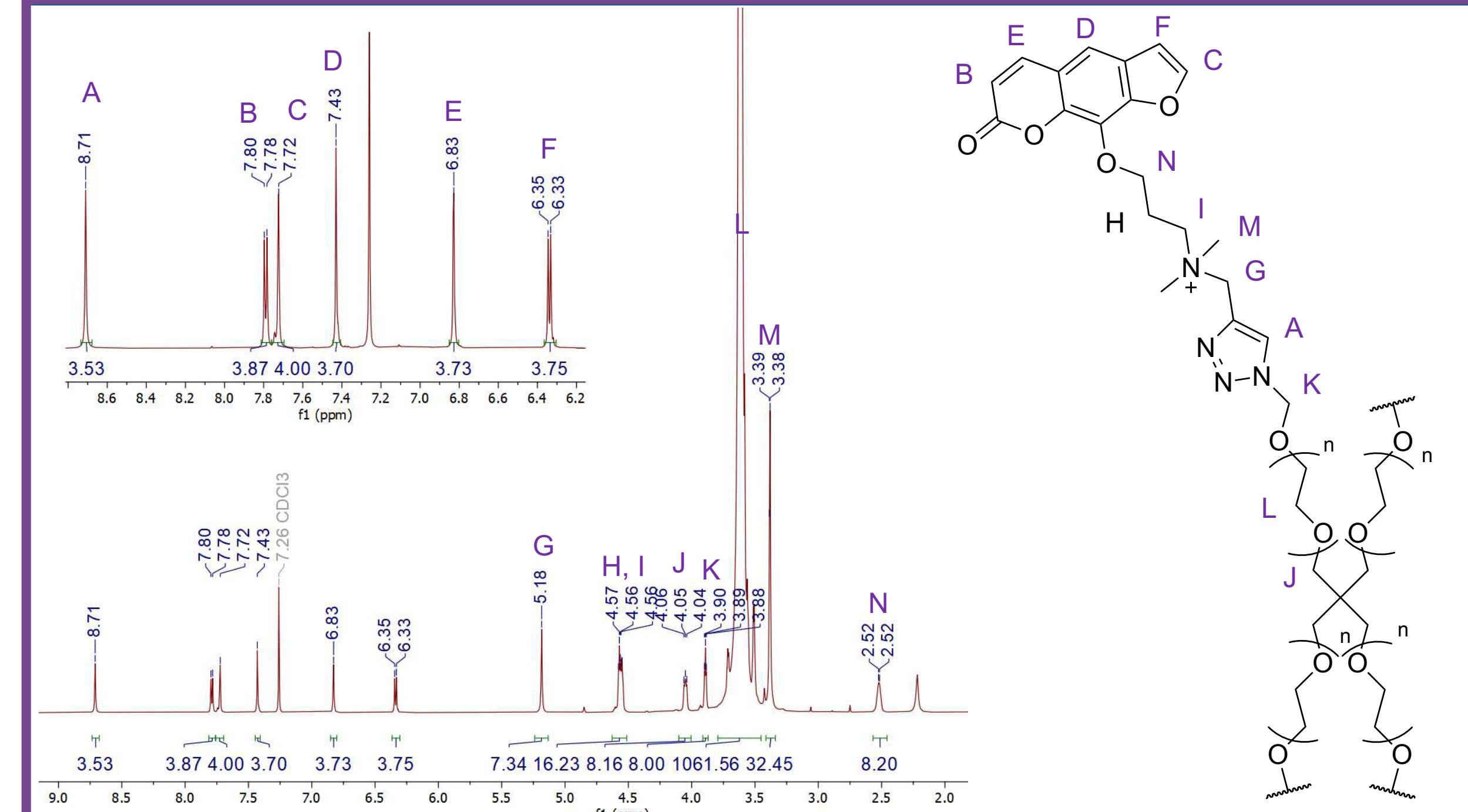


Figure 6. ¹H NMR of Pso(+)-PEG (**11a**) in CDCl_3 . The NMR confirms the degree of functionalization of the PEG-cross-linker.

Typically, hydrogels utilizing non-covalent interactions are reversible systems due to their dynamic networks. However, upon psoralen's photo cross-linking with DNA, the hydrogel network becomes covalent.^{1,4} To study how molecular structure impacts the UV cross-linked psoralen-DNA hydrogel network, we evaluated the thermal and light-responsive behavior of three psoralen-PEG derivatives. Each derivative revealed unique rheological properties; for example, the protonated psoralen showed significant enhanced photo cross-linking, reaching a higher modulus at a shorter timescale than the other derivatives. These results indicate structural changes within the same intercalating molecule can significantly impact hydrogel behavior.

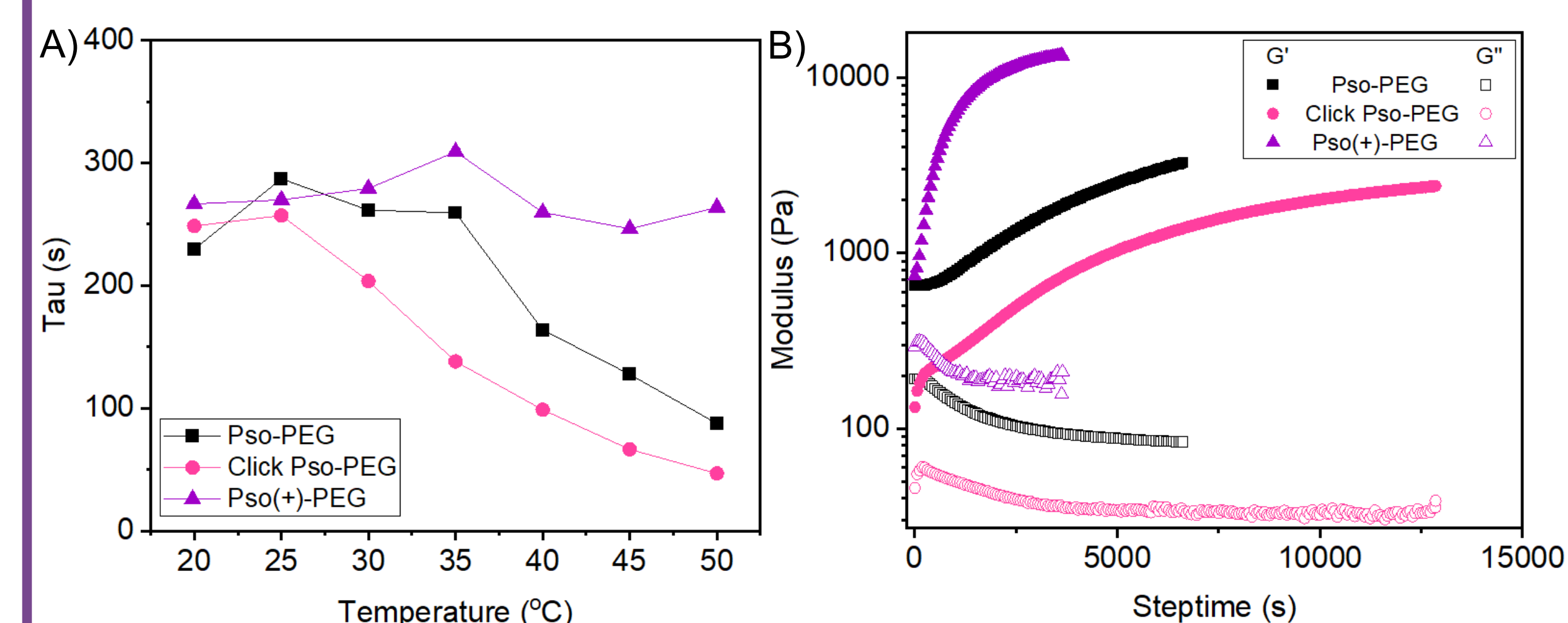


Figure 8. Comparison of thermal and UV response of 3 psoralen-PEG derivatives: Pso-PEG (**12**), Click Pso-PEG (**11b**), and Click Pso(+)-PEG (**11a**). A) Relaxation time (τ) comparison across temperatures 5-35 °C. B) Time sweep with UV exposure

Acknowledgments

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References

- Appel, E. A.; et al. *Chem. Soc. Rev.* **2012**, 41 (18), 6195–6214
- Tuccitto, N.; et al. *Chemistry – A European Journal* **2021**, 27 (55), 13715–13718
- Bronner, H.; et al. *ACS Appl. Mater. Interfaces* **2023**, 15, 11, 14067–14076
- Hughes, S. H.; et al. *Angew. Chem. Int. Ed.* **2024**, 63, e202411116
- Lartillot, V.; et al. *Photochemistry and Photobiology*, **78**(6), 623–32
- Maury, J.; et al. *Tetrahedron* **2012**, 68 (47), 9606–9611.
- Milne, K.; et al. *Bioorganic & Medicinal Chemistry Letters* **2019**, 29 (18), 2626–2631
- Ou, Z.; et al. *Journal of Inorganic Biochemistry* **2016**, 166, 126–134
- Truong, V. X.; et al. *ACS Macro Lett.* **2017**, 6 (7), 657–662