

Electronic Supplementary Information

Light-Responsive Arylazopyrazole-Based Hydrogels:

Their Applications as Shape-Memory Materials,

Self-Healing Matrices and Controlled Drug Release Systems.

*Gilad Davidson-Rozenfeld^a, Lucas Stricker^b, Julian Simke^b, Michael Fadeev^a,
Margarita Vázquez-González^a, Bart Jan Ravoo^{*b} and Itamar Willner^{*a}*

a. Institute of Chemistry, The Minerva Center for Biohybrid Complex Systems, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

b. Organic Chemistry Institute and Center for Soft Nanoscience, Westfälische Wilhelms-Universität Münster, Corrensstrasse 40 48149 Münster, Germany

e-mail: B.J.Ravoo@uni-muenster.de, willnea@vms.huji.ac.il

Table of content:

- 1. Characterization of β -CD functionalized CMC polymer**
- 2. β -CD loading ratio effect on rheometric properties**
- 3. Characterization of *trans*-AAP/ β -CD/self-complementary (2) - functionalized CMC polymer**
- 4. Characterization of *trans*-AAP/ β -CD/half-G-quadruplex (4) - functionalized CMC polymer**
- 5. DNA loading ratio effect on shape-memory properties**
- 6. Doxorubicin calibration curve**
- 7. Synthesis of bis-AAP**
- 8. Synthesis of AAP-C2-NH₂, (amino AAP)**

1. Characterization of β -CD functionalized CMC polymer:

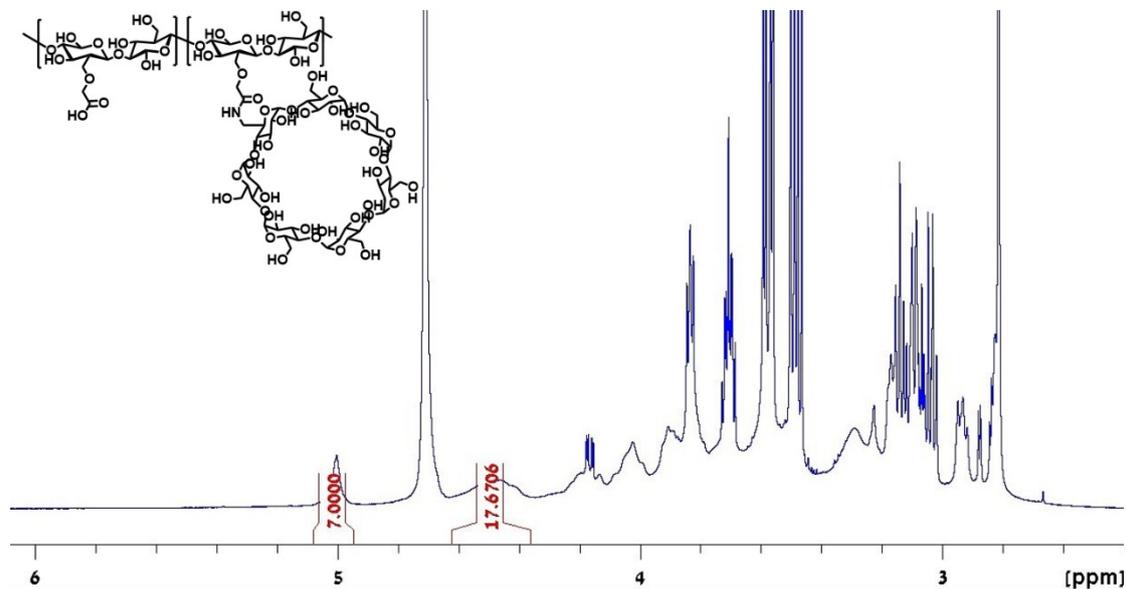


Figure S1. $^1\text{H-NMR}$ of carboxymethylcellulose (CMC), modified with β -cyclodextrin (β -CD). While the signal at 5 ppm corresponds to the α -anomeric C-H of β -CD, the signal at 4.5 ppm corresponds to the β -anomeric C-H of the CMC carbohydrate backbone. The signal of the α -anomeric C-H is set to an integration value of 7, corresponding to 1 molecule of β -CD. Using relative integration, the loading ratio of the polymer is 1 (β -CD) on a cellulose backbone unit composed of 15 carboxymethyl-anhydroglucose and 2 anhydroglucose.

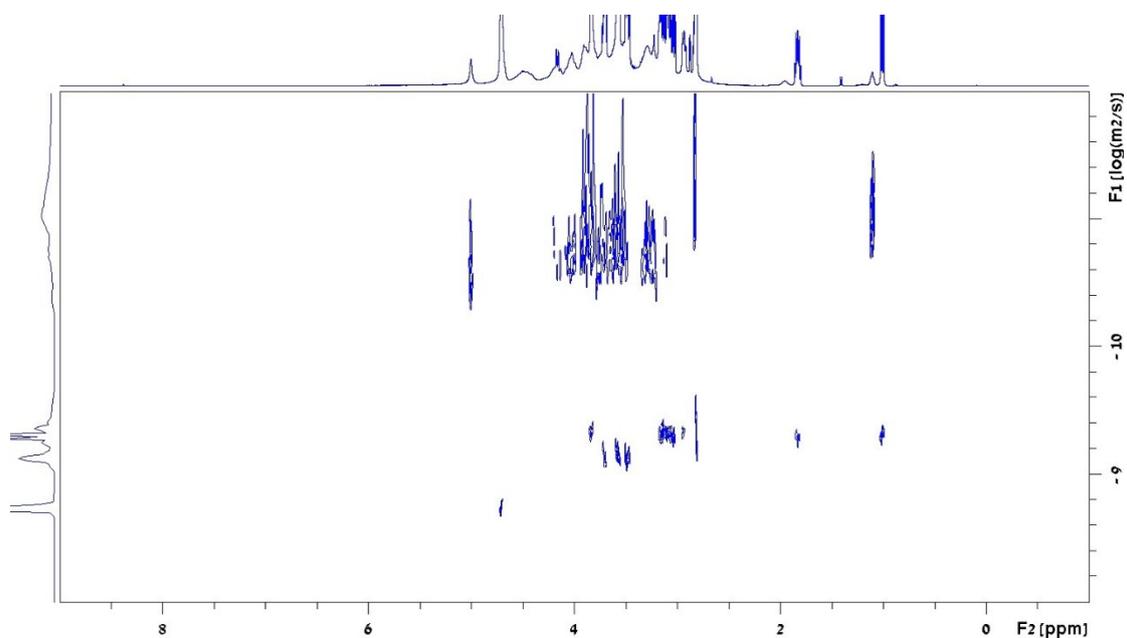


Figure S2. DOSY $^1\text{H-NMR}$ of CMC polymer modified with β -CD.

2. β -CD loading ratio effect on rheometric properties:

The loading of the CMC scaffold with the β -CD units controls the stiffness of the hydrogel. While the loading ratio of β -CD on the polymer shown in Figure 1 corresponds to 1:17, a polymer of lower loading degree that corresponds to 1:41 is shown in figure S3.

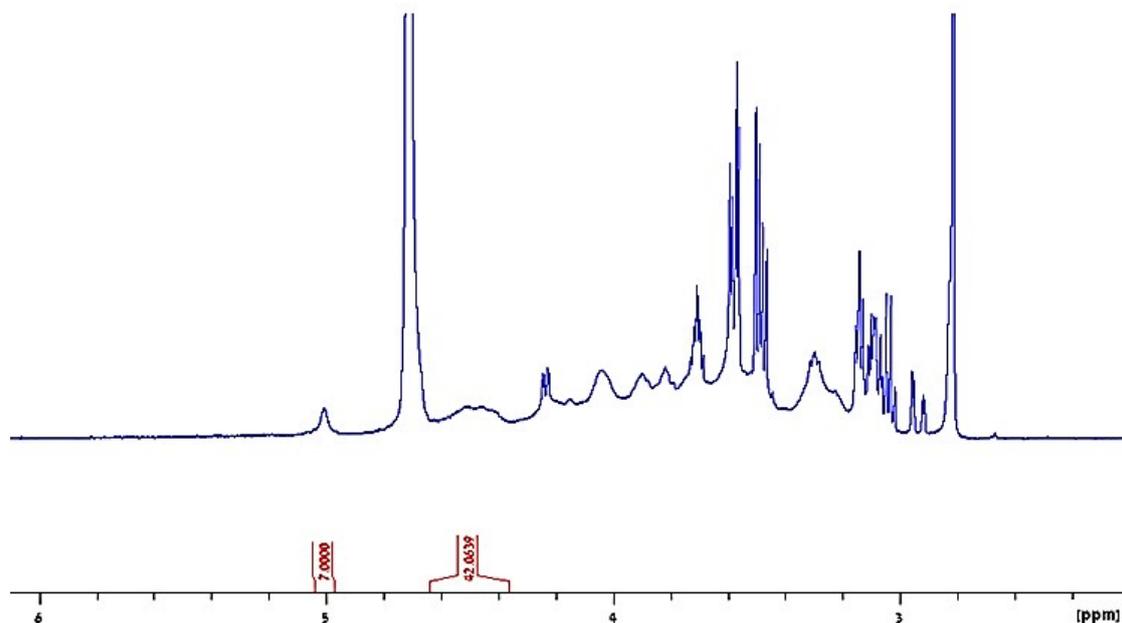


Figure S3. ¹H-NMR of CMC, modified with β -CD of a lower loading ratio corresponding to 1 β -CD on a cellulose backbone unit composed of 37 carboxymethyl-anhydroglucose and 4 anhydroglucose.

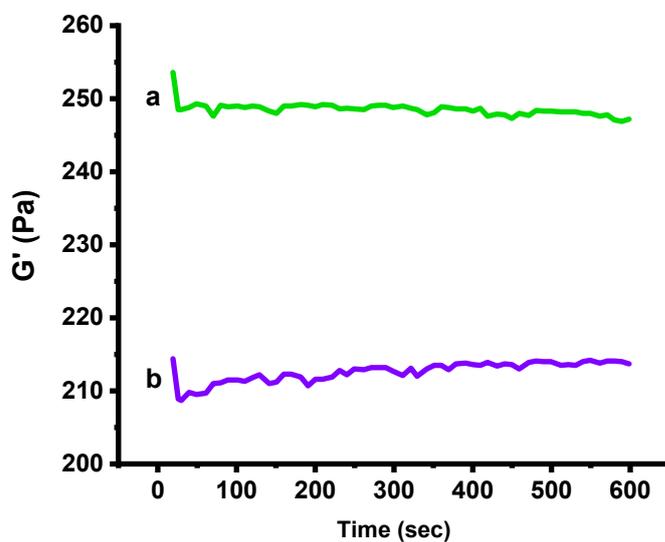


Figure S4. Comparison of the G' values of the hydrogels formed by the 1:17 loaded polymer (a), and the 1:41 loaded polymer (b).

Evidently the G' values of the hydrogel formed by the lower-loaded polymer decreased from 250 Pa to 210 Pa.

3. Characterization of *trans*-AAP/ β -CD/self-complementary (2) - functionalized CMC polymer:

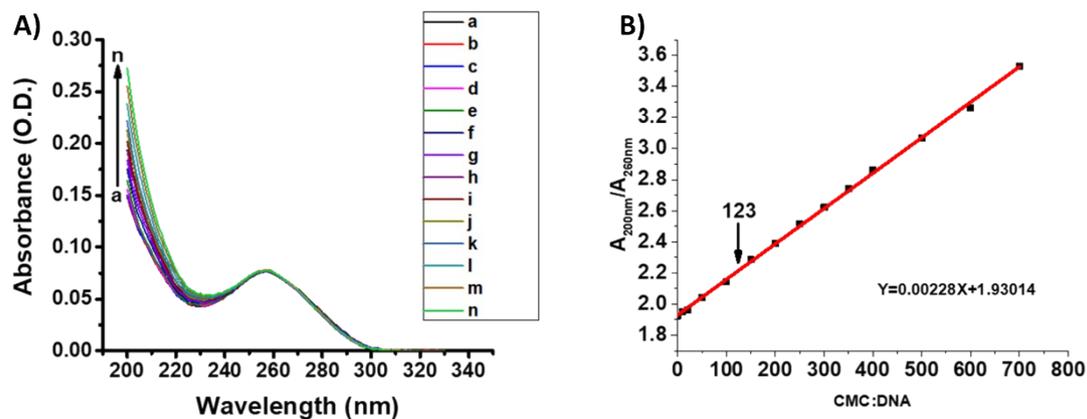


Figure S5: A) UV/Vis absorbance spectra of: (a) 0.5 μ M NH_2 -self complementary nucleic acid strand (**2**), (b-n) 0.5 μ M NH_2 -self complementary nucleic acid strand (**2**) and CMC polymers in calculated monomeric ratio from 10:1 up to 700:1. B) Calibration curve corresponding to the ratio of absorbance at $\lambda=200$ nm to $\lambda=260$ nm. The absorbance of the synthesized polymer was measured and the ratio of CMC monomers to nucleic acid strands was calculated to be 123:1.

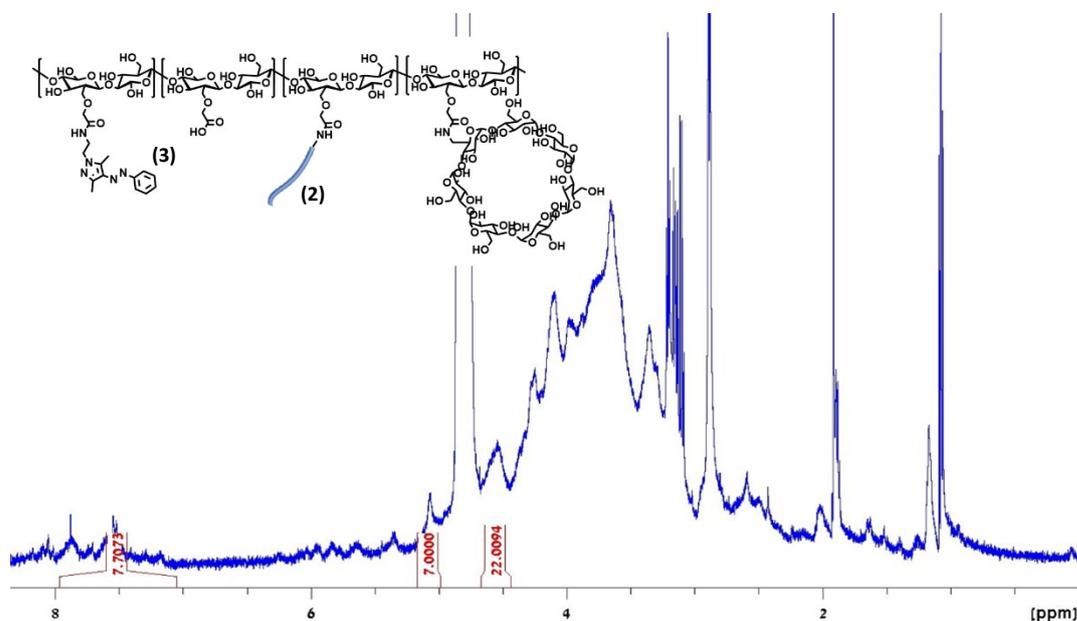


Figure S6. $^1\text{H-NMR}$ of CMC modified with self-complementary nucleic acid (**2**), β -CD and arylazopyrazole (AAP) (**3**). The signals between the chemical shifts of 6.2-7.9 ppm corresponds to the AAP aromatic C-H bonds. Here, the relative integration yields a ratio of 19.5 (CMC); 1.5 (**3**); 1 (β -CD). Adding the nucleic acid modification (123; 1), the loading ratio of the three functionalities is 8.5 (**3**); 5.5 (β -CD); 1 (**2**); on a CMC scaffold unit composed of 111 carboxymethyl- anhydroglucose and 12 anhydroglucose units.

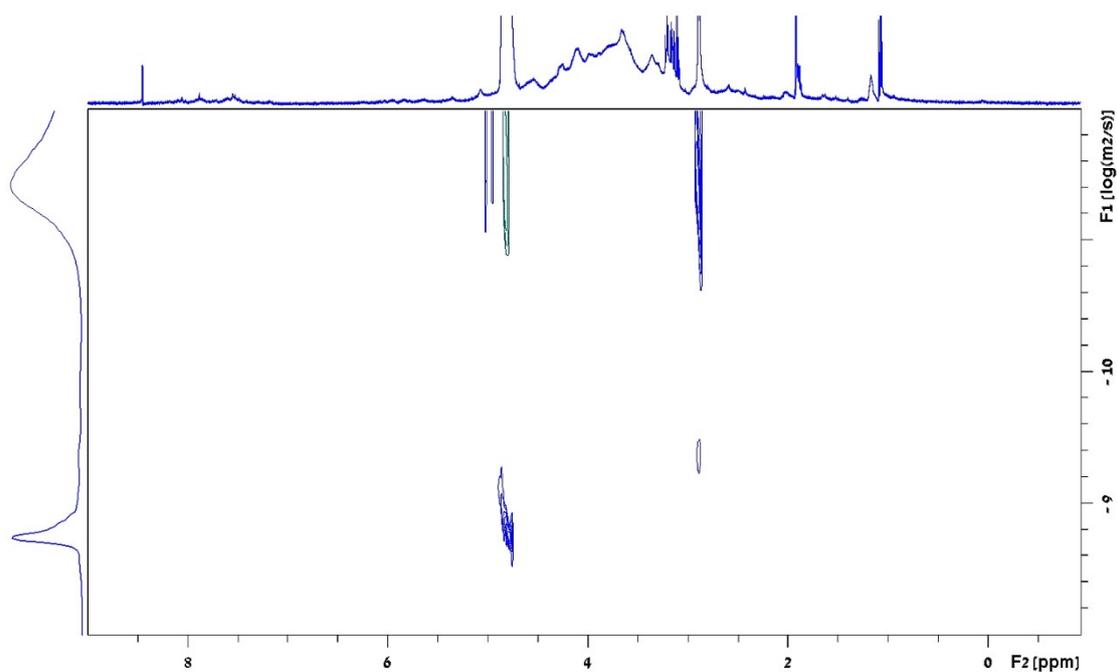


Figure S7. DOSY $^1\text{H-NMR}$ of CMC modified with self-complementary nucleic acid, $\beta\text{-CD}$ and arylazopyrazole (AAP).

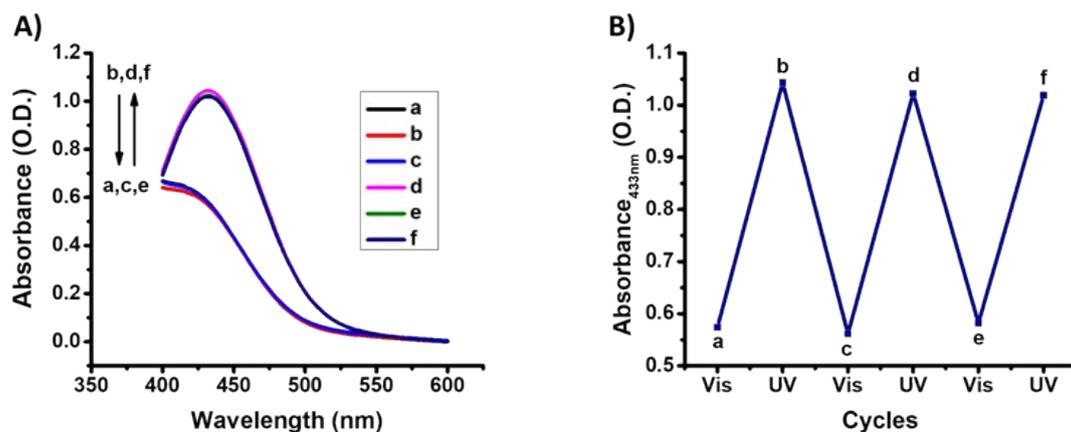
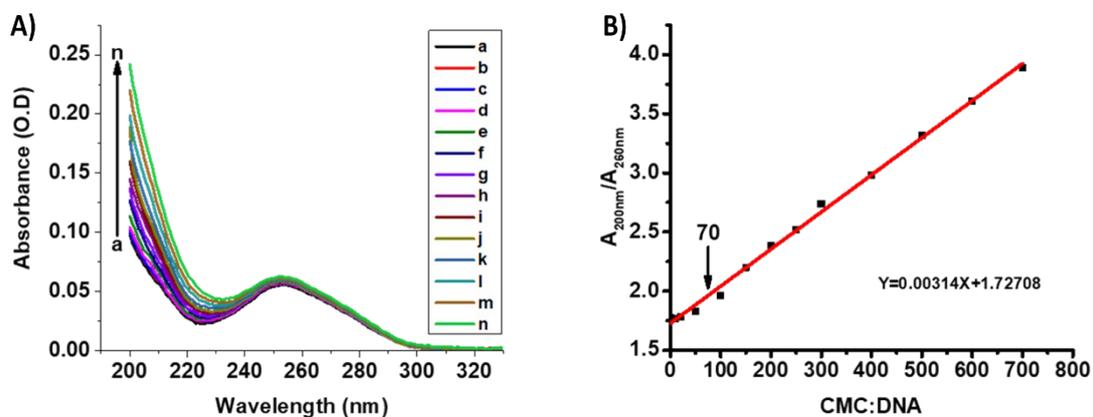


Figure S8. A) Absorbance spectra of a CMC hydrogel crosslinked by self-complementary nucleic acids **(2)/(2)**; $\beta\text{-CD}/\text{trans-AAP}$: (a) initial, dual crosslinked state. (b) After UV irradiation. (c) After visible light irradiation. (d-f) After alternating irradiation of UV, visible light and UV, respectively. B) Corresponding cyclic photoisomerization of the hydrogel.

4. Characterization of *trans*-AAP/ β -CD/half-G-quadruplex (**4**) - functionalized CMC



polymer:

Figure S9: A) Absorbance spectra of: (a) 0.5 μ M NH₂-half G-quadruplex nucleic acid strand (**4**). (b-n) 0.5 μ M NH₂-half G-quadruplex nucleic acid strand (**4**) and CMC polymers in calculated monomeric ratio from 10:1 up to 700:1. B). Calibration curve, corresponding to the ratio of absorbance at $\lambda=200$ nm to $\lambda=260$ nm. The absorbance of the synthesized polymer was measured and the ratio of CMC monomers to nucleic acid strands was calculated to be 70:1.

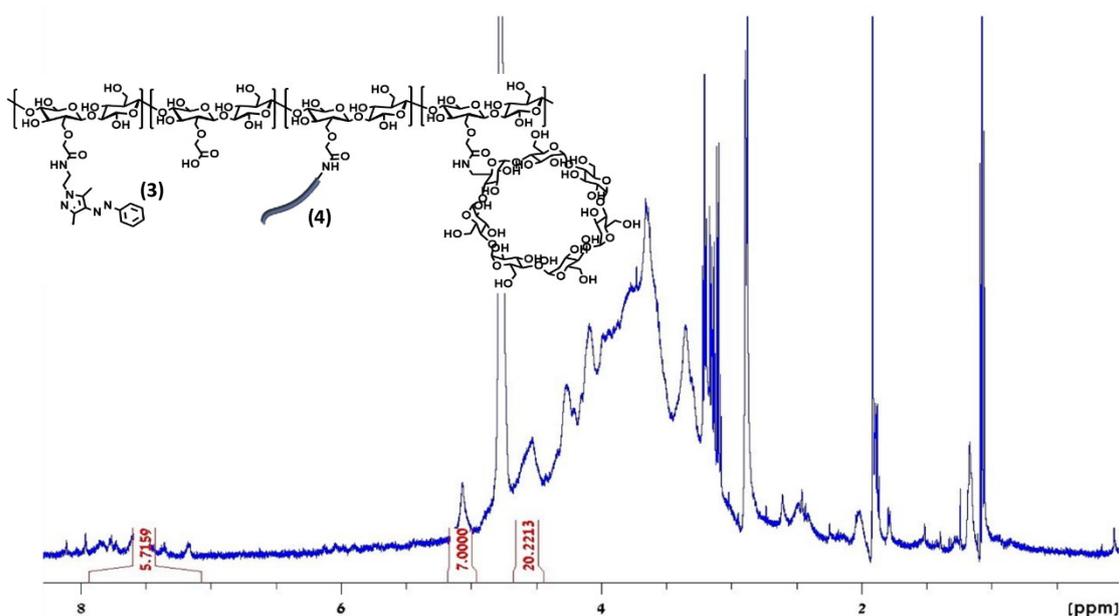


Figure S10. ¹H-NMR of CMC modified with half-G-quadruplex nucleic acid (**4**), β -CD and AAP (**3**). The relative integration yields a ratio of 18 (CMC); 1 (AAP); 1 (β -CD). Adding the nucleic acid modification (70; 1), the overall loading ratio of the three functionalities is 3.5 (β -CD); 3.5 (AAP); 1 (**2**); on a cellulose scaffold unit composed of 63 carboxymethyl-glucose and 7 glucose units.

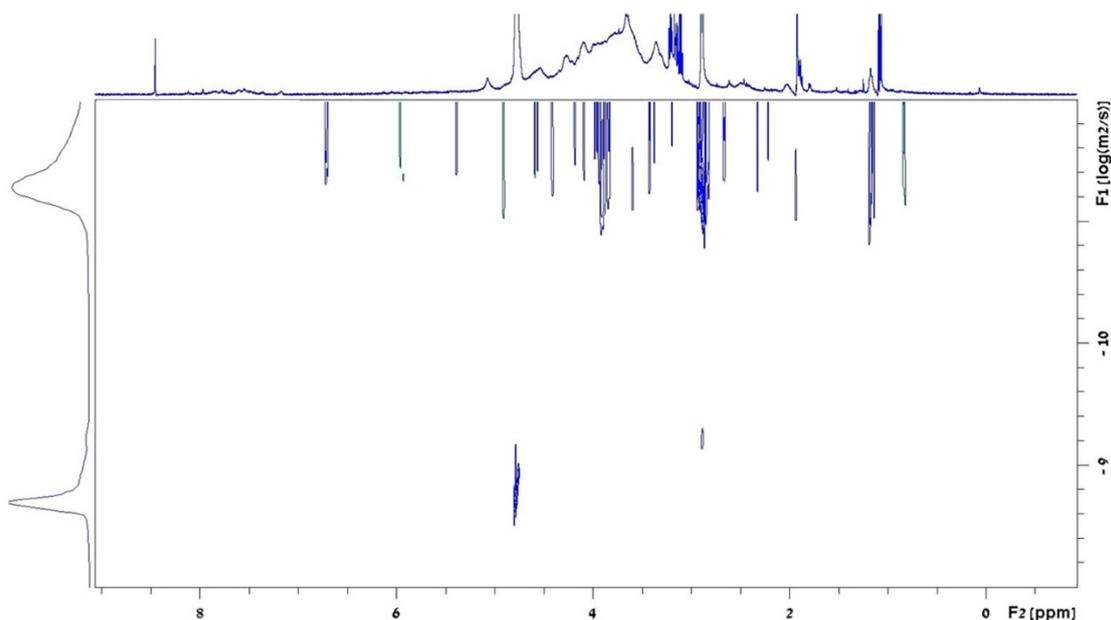


Figure S11. DOSY ^1H -NMR of CMC modified with half G-quadruplex nucleic acid, β -CD and arylazopyrazole (AAP).

5. DNA loading ratio effect on the shape-memory properties:

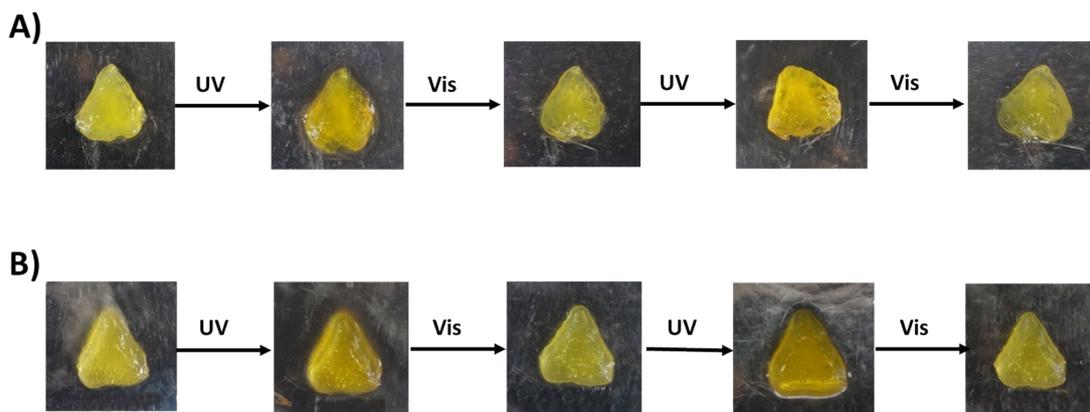


Figure S12. Effect of the DNA-loading ratio on the shape-memory properties of the hydrogels shown in: (A) Figure 2A and, (B) Figure 4A.

A low loading ratio of the self-complementary nucleic acid (**2**) on the CMC backbone (1:180) resulted in a poor recovery (A) of the shapeless hydrogel, while a high loading of G-quadruplex (**4**) units shows insufficient perturb of the shaped hydrogel (B).

6. Doxorubicin calibration curve

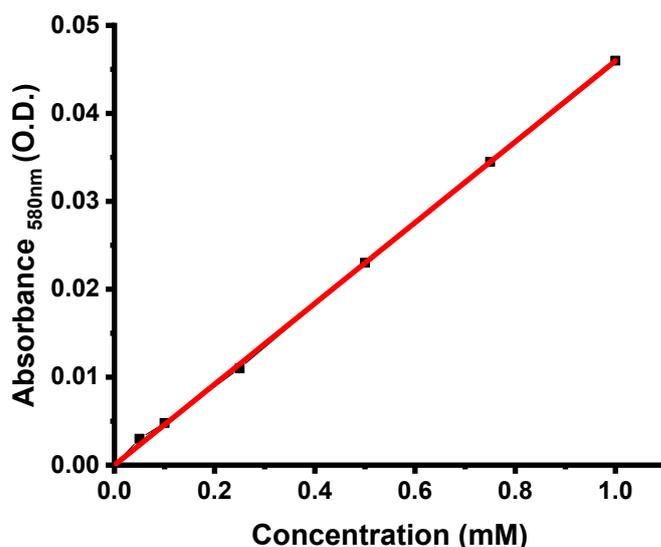


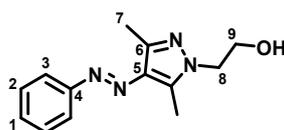
Figure S13. Calibration curve corresponding to different concentrations of DOX at $\lambda=580$ nm.

7. Synthesis of bis-AAP:

The synthesis of the bis-AAP compound was already reported. For details see: L. Stricker, E.-C. Fritz, M. Peterlechner, N. L. Doltsinis, B. J. Ravoo *J. Am. Chem. Soc.*, **2016**, *138*, 4547–4554.

8. Synthesis of AAP-C2-NH₂, (amino AAP):

8.1. Synthesis of AAP-C2-OH



The AAP core (for the synthesis see: L. Stricker, E.-C. Fritz, M. Peterlechner, N. L. Doltsinis, B. J. Ravoo *J. Am. Chem. Soc.*, **2016**, *138*, 4547–4554) (2.30 g, 11.5 mmol, 1.0 eq.) was dissolved in dry acetonitrile (ACN) (100 mL) and 2-bromoethanol (1.63 mL, 23.0 mmol, 2.0 eq.), and K₂CO₃ (8.00 g, 57.5 mmol, 5.0 eq.) were added. The reaction mixture was refluxed for 3 d. K₂CO₃ was filtered off and ACN was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, EtOAc, R_f = 0.3) yielding the desired compound.

Yield: 2.11 g (8.64 mmol, 75%) as orange solid.

¹H-NMR: (400 MHz, CDCl₃) δ = 7.81 – 7.76 (m, 2H, H-3), 7.49 – 7.42 (m, 2H, H-2), 7.40 – 7.34 (m, 1H, H-1), 4.16 – 4.11 (m, 2H, H-8), 4.04 (t, *J* = 4.8 Hz, 2H, H-9), 3.45 (s, 1H, OH), 2.60 (s, 3H, H-7), 2.50 (s, 3H, H-7) ppm.

¹³C-NMR: (101 MHz, CDCl₃) δ = 153.64 (C-4), 142.93 (C-6), 139.61 (C-5), 135.08 (C-6), 129.57 (C-2), 129.03 (C-1), 121.91 (C-3), 61.58 (C-8), 50.29 (C-9), 14.26, 14.16, 10.02, 9.96 (C-7) ppm.

MS (*m/z*): (ESI, MeOH) Calculated for [C₁₃H₁₆N₄OH]⁺: 245.1402; found 245.1400.

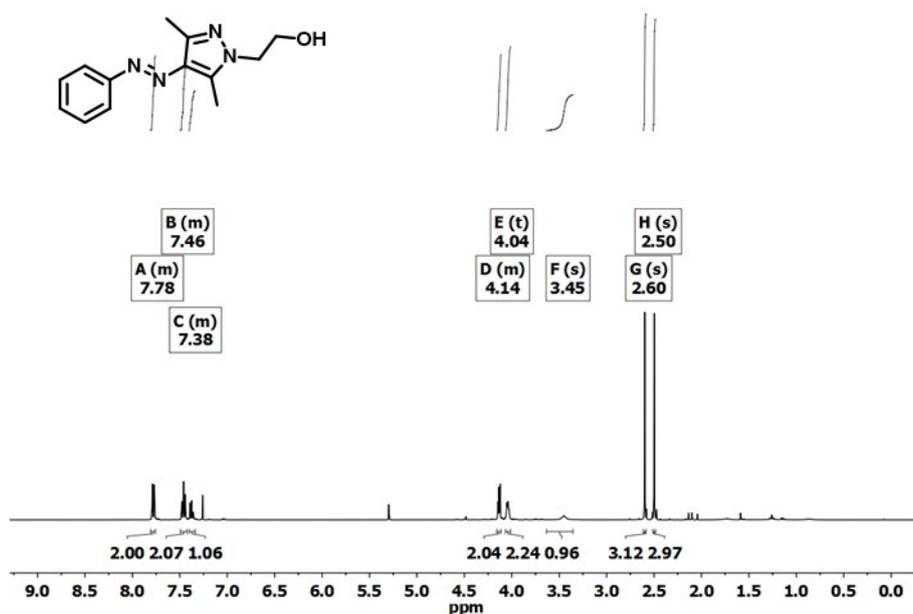


Figure S14. ¹H NMR spectrum of AAP-C2-OH in CDCl₃.

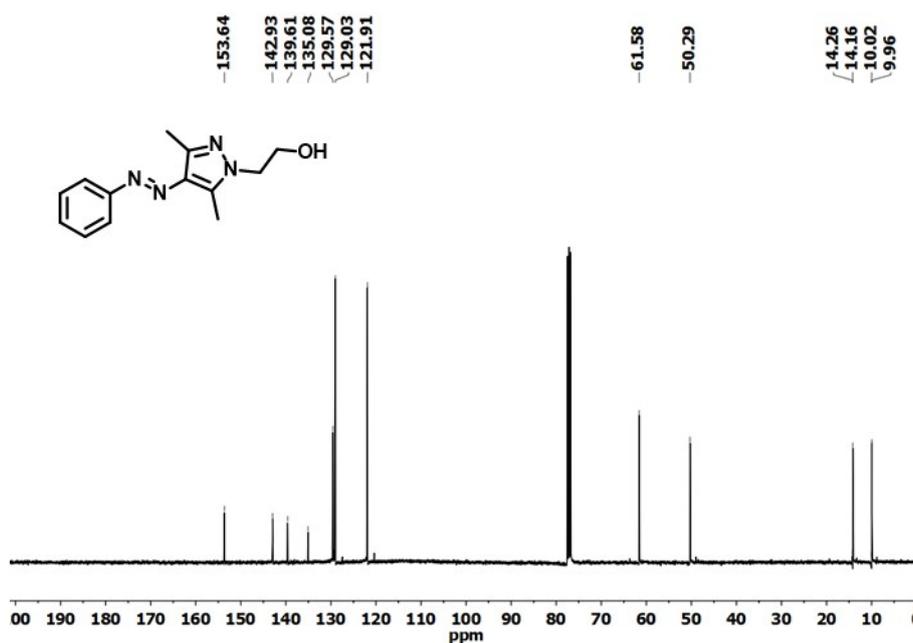
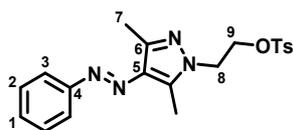


Figure S15. ¹³C-NMR spectrum of AAP-C2-OH in CDCl₃.

8.2. Synthesis of AAP-C2-OTs



Triethylamine (2.37 ml, 17.1 mmol, 2.0 eq.) and DMAP (cat.) were added to a stirred solution of **PAPy-C2-OH** (2.10 g, 8.56 mmol, 1.0 eq.) in dichloromethane (DCM) (100 mL). After that, tosyl chloride (2.45 g, 12.8 mmol, 1.5 eq.) was added portion wise and the reaction mixture was stirred at rt for 18 h. The organic layer was washed with H₂O (50 mL), brine (50 mL), and HCl (1 M, 50 mL), and dried over MgSO₄. The solvent was removed under reduced pressure, followed by purification *via* column chromatography (SiO₂, EtOAc, R_f = 0.64), **AAP-C2-OTs** was obtained.

Yield: 2.96 g (7.36 mmol, 86%) as orange solid.

¹H-NMR: (400 MHz, CDCl₃) δ = 7.82 – 7.76 (m, 2H, H-3), 7.62 – 7.56 (m, 2H, *Tos*), 7.53 – 7.45 (m, 2H, H-2), 7.43 – 7.37 (m, 1H, H-1), 7.22 – 7.16 (m, 2H, *Tos*), 4.43 (dd, *J* = 5.5, 4.5 Hz, 2H, H-8), 4.26 (dd, *J* = 5.4, 4.5 Hz, 2H, H-9), 2.55 (s, 3H, H-7), 2.35 (s, 3H, CH₃-*Tos*), 2.34 (s, 3H, H-7) ppm.

¹³C-NMR: (101 MHz, CDCl₃) δ = 153.58 (C-4), 145.12 (C-6), 143.00 (C-5), 140.47 (*Tos*), 135.04 (C-6), 132.20, 130.05, 129.94, 129.72, 129.68, 129.32, 129.10, 127.95, 127.84, 127.55, 121.92, 120.47 (C-2, C-3, *Tos*), 68.44, 47.72 (C-8), 47.66 (C-9), 21.78 (CH₃-*Tos*), 14.14, 9.85 (C-7) ppm

MS (*m/z*): (ESI, MeOH) Calculated for [C₂₀H₂₂N₄O₃SNa]⁺: 421.1305; found 421.1306.

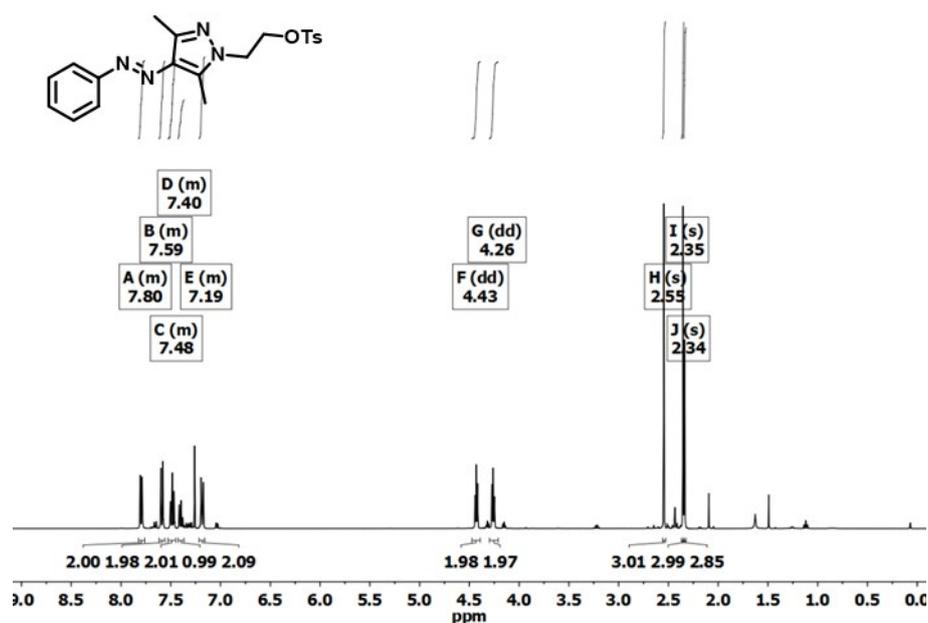


Figure S16. ^1H NMR spectrum of **AAP-C2-OTs** in CDCl_3 .

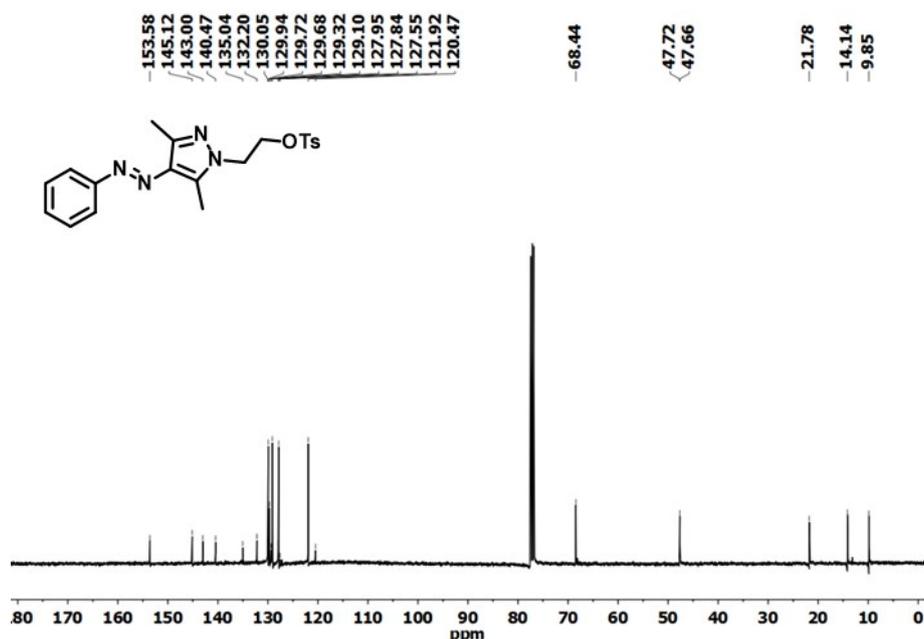
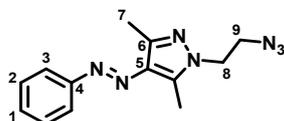


Figure S17. ^{13}C -NMR spectrum of **AAP-C2-OTs** in CDCl_3 .

8.3. Synthesis of **AAP-C2-N₃**



AAP-C2-OTs (2.93 g, 7.35 mmol, 1.0 eq.) was dissolved in dry N, N-dimethylformamide, (DMF) (50 mL) and NaN_3 (2.87 g, 44.1 mmol, 6.0 eq.) was added. The reaction mixture was stirred at 90 °C for 18 h. Solvent was removed under reduced pressure and the residue was dissolved in DCM (30 mL). The organic layer was washed with H_2O (100 mL) and brine (2 × 80 mL), dried over MgSO_4 , and concentrated under reduced pressure yielding the azide.

Yield: 1.96 g (7.28 mmol, 99%) as orange oil.

^1H -NMR: (300 MHz, CDCl_3) δ = 7.84 – 7.75 (m, 2H, H-3), 7.54 – 7.41 (m, 2H, H-2), 7.41 – 7.33 (m, 1H, H-1), 4.15 (dd, J = 6.1, 5.2 Hz, 2H, H-8), 3.76 (dd, J = 6.2, 5.2 Hz, 2H, H-9), 2.62 (s, 3H, H-7), 2.52 (s, 3H, H-7) ppm.

^{13}C -NMR: (75 MHz, CDCl_3) δ = 153.60 (C-4), 143.30 (C-6), 140.01 (C-5), 135.22 (C-6), 129.53, 128.98 (C-3), 121.89 (C-2), 120.47 (C-1), 50.75 (C-8), 47.81 (C-9), 14.22, 9.89 (C-7) ppm.

MS (m/z): (ESI, MeOH) Calculated for $[\text{C}_{13}\text{H}_{15}\text{N}_7\text{H}]^+$: 270.1462; found 270.1474.

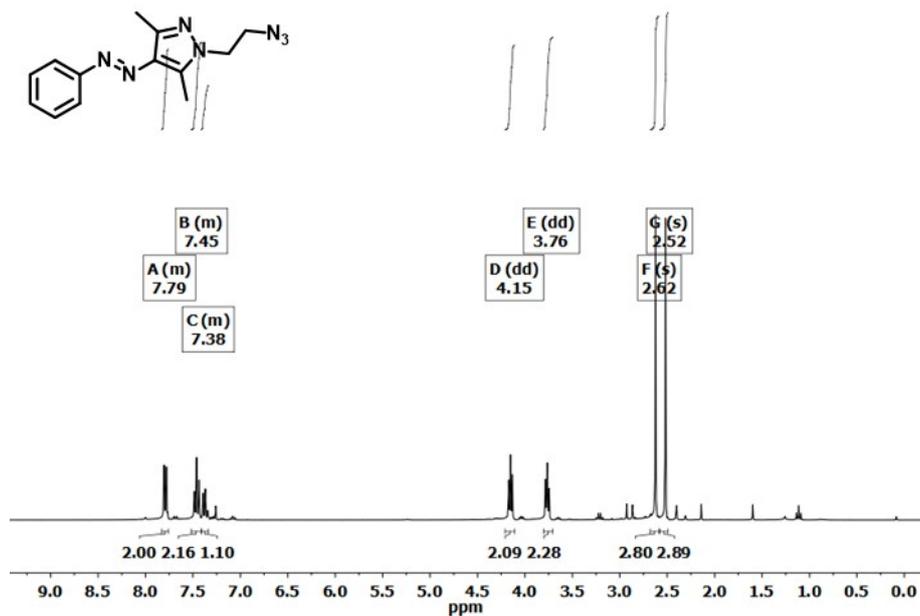


Figure S18. ¹H NMR spectrum of AAP-C2-N₃ in CDCl₃.

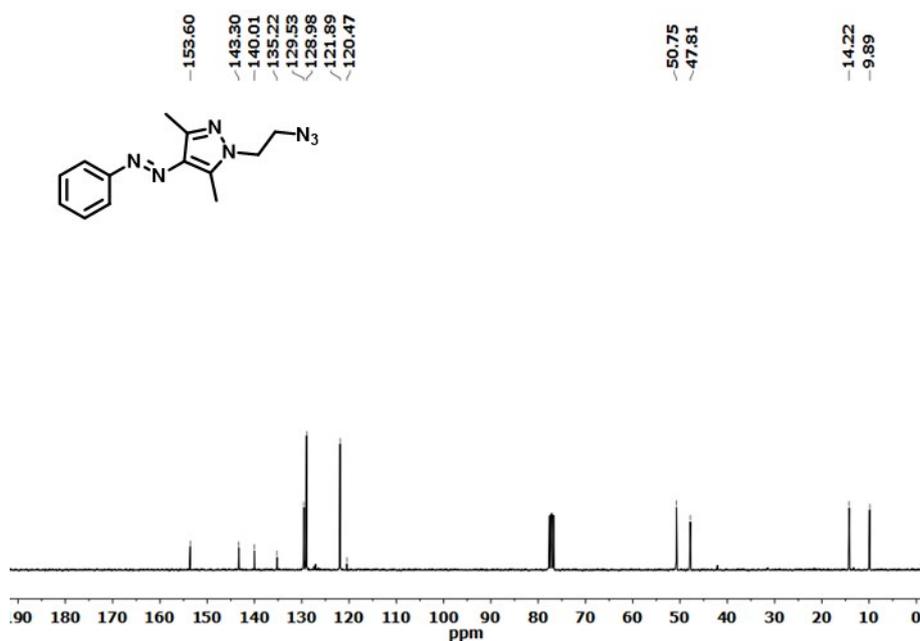
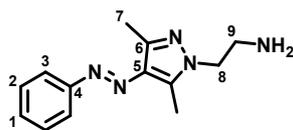


Figure S19. ¹³C-NMR spectrum of AAP-C2-N₃ in CDCl₃.

8.4. Synthesis of AAP-C2-NH₂



To a solution of **AAP-C2-N₃** (536 mg, 2.00 mmol, 1.0 eq.) in dry tetrahydrofuran (THF) (30 mL) at 0 °C, PPh₃ (777 mg, 2.20 mmol, 1.1 eq.) was added. The reaction mixture was slowly warmed to rt and stirred for 18 h. H₂O (100 μL, 4.40 mmol, 2.2 eq.) was added and the mixture was stirred at rt for additional 18 h. The organic layer was extracted with DCM (30 mL), washed with H₂O (30 mL) and brine (30 mL), and dried over MgSO₄. After evaporation of the solvent, the residue was purified by column chromatography (SiO₂, DCM/MeOH 9:1 → DCM/MeOH/NH₄OH 7.5:2.5:0.1, R_f = 0.1 → 0.7). **AAP-C2-NH₂** was isolated.

Yield: 350 mg (1.44 mmol, 72%) as orange sticky oil.

¹H-NMR: (300 MHz, CDCl₃) δ = 7.82 – 7.73 (m, 2H, H-3), 7.50 – 7.40 (m, 2H, H-2), 7.40 – 7.32 (m, 1H, H-1), 4.08 (t, *J* = 5.9 Hz, 2H, H-8), 3.17 (s, 2H, H-9), 2.61 (s, 3H, H-7), 2.51 (s, 3H, H-7), 1.32 (s, 2H, NH₂) ppm.

¹³C-NMR: (75 MHz, CDCl₃) δ = 153.70 (C-4), 142.79 (C-6), 139.38 (C-5), 135.20 (C-6), 129.44, 129.01 (C-3), 121.87 (C-2), 120.40 (C-1), 51.87 (C-8), 41.92 (C-9), 14.20, 10.10 (C-7) ppm.

MS (m/z): (ESI, MeOH) Calculated for [C₁₃H₁₇N₅H]⁺: 244.1557; found 244.1569.

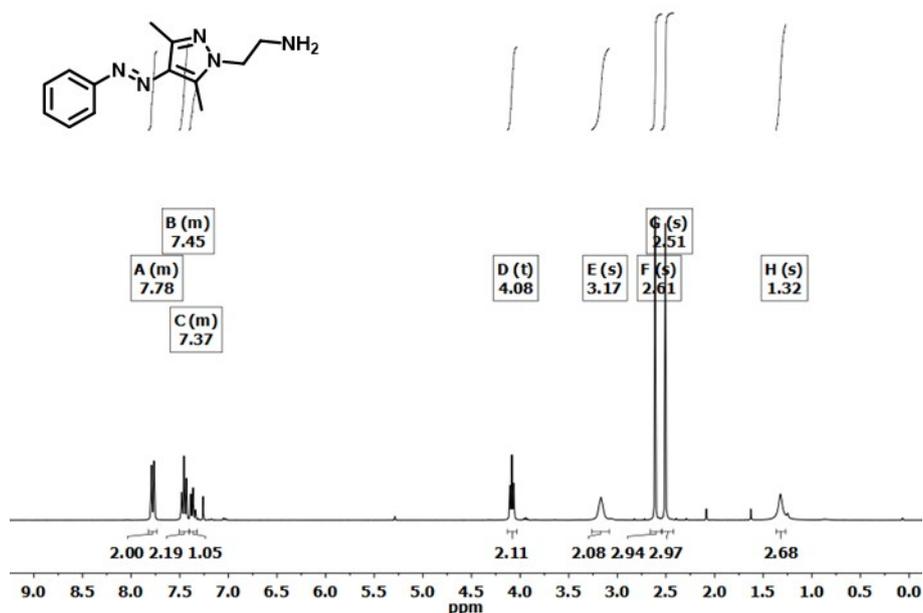


Figure S20. ¹H NMR spectrum of **AAP-C2-NH₂** in CDCl₃.

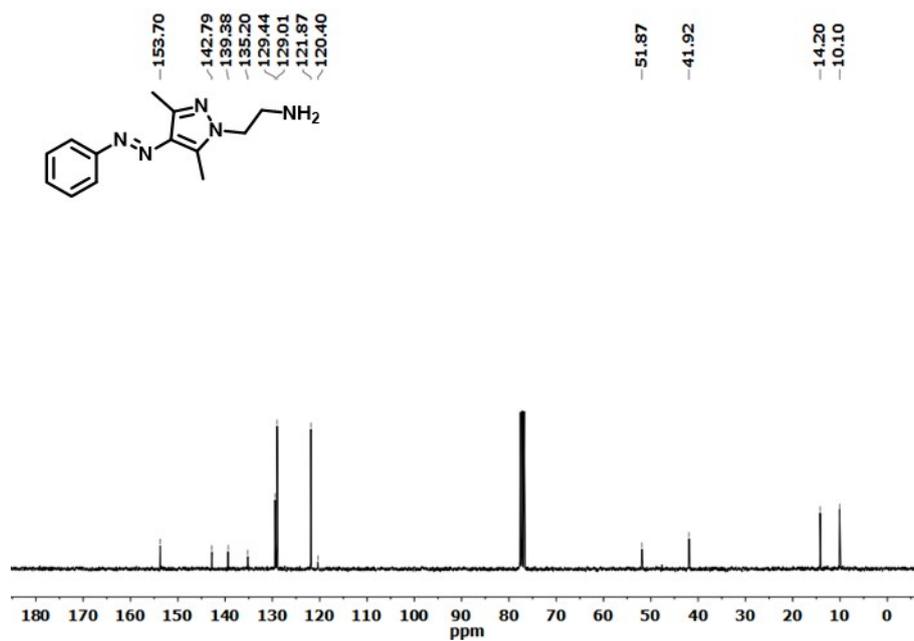


Figure S21. ¹³C-NMR spectrum of AAP-C2-NH₂ in CDCl₃.