Supporting Information

Acetal-Functionalized Pillar[5]arene: a pH-Responsive and Versatile Nanomaterial for the Delivery of Chemotherapeutic Agents

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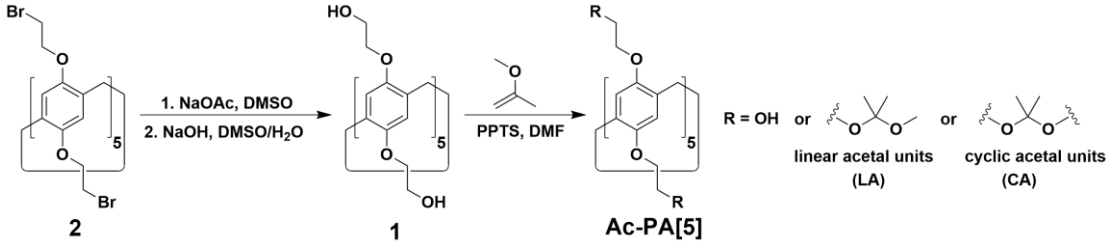
1. Experimental procedures



1.1 Synthesis of Ac-PA[5].

Synthesis of host 1: Host **2** was first synthesized according to reported literature procedures.[1] NaOAc (1.23 g, 15 mmol) was added into a solution of host **2** (1.68 g, 1 mmol) in DMSO (50 mL). The mixture was stirred at 90 °C for 3 h. Aqueous NaOH (15 mL, 15 mmol) was added to the reaction mixture and stirred for 30 min. The reaction mixture was poured into H_2O, the product was collected by centrifugation at 10 000 rpm, then rinsed with H_2O (3 × 30 mL). The product was recrystallized from methanol to obtain host **1** as a white solid (540 mg, 51%). ^{1}H NMR (400 MHz, MeOD) δ (ppm): 6.95 (s, 10H), 3.95 (t, J = 4.7 Hz, 20H), 3.88 (t, J = 4.8 Hz, 20H), 3.82 (s, 10H). ^{13}C NMR (100 MHz, MeOD) δ (ppm): 151.16, 130.33, 116.05, 71.35, 62.19, 30.17.

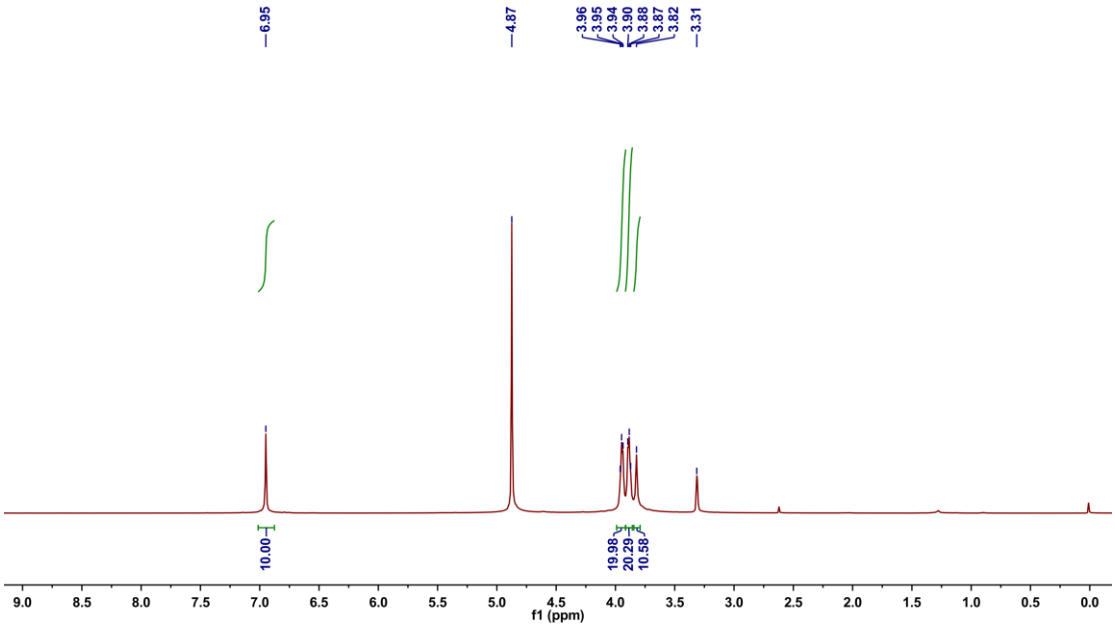


Figure S1. ^{1}H NMR spectrum (400 MHz, MeOD) of host **1**.

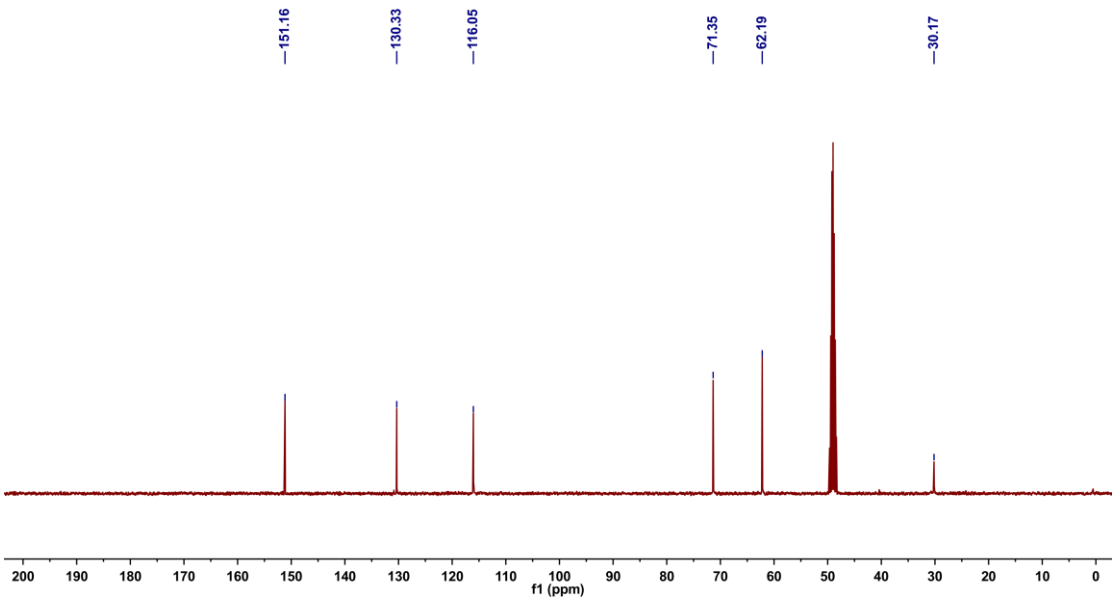


Figure S2. ^{13}C NMR spectrum (100 MHz, MeOD) of host **1**.

Synthesis of Ac-PA[5][2]: A flame-dried flask was charged with host **1** (1.05 g, 1 mmol) and purged with N_2. Anhydrous DMF (50 mL) was added and the resulting mixture was stirred until host **1** was completely dissolved. Pyridium *p*-toluenesulfonate (25 mg, 0.1 mmol) was added followed by 2-methoxypropene (1.9 mL, 20 mmol). The system was maintained under positive N_2 pressure and then sealed. After 3 h, the reaction was quenched with trimethylamine (0.5 mL). The product was precipitated with H_2O and isolated by centrifugation. The resulting product was washed thoroughly with H_2O and lyophilized to obtain **Ac-PA[5]** as a white powder (0.73 g).

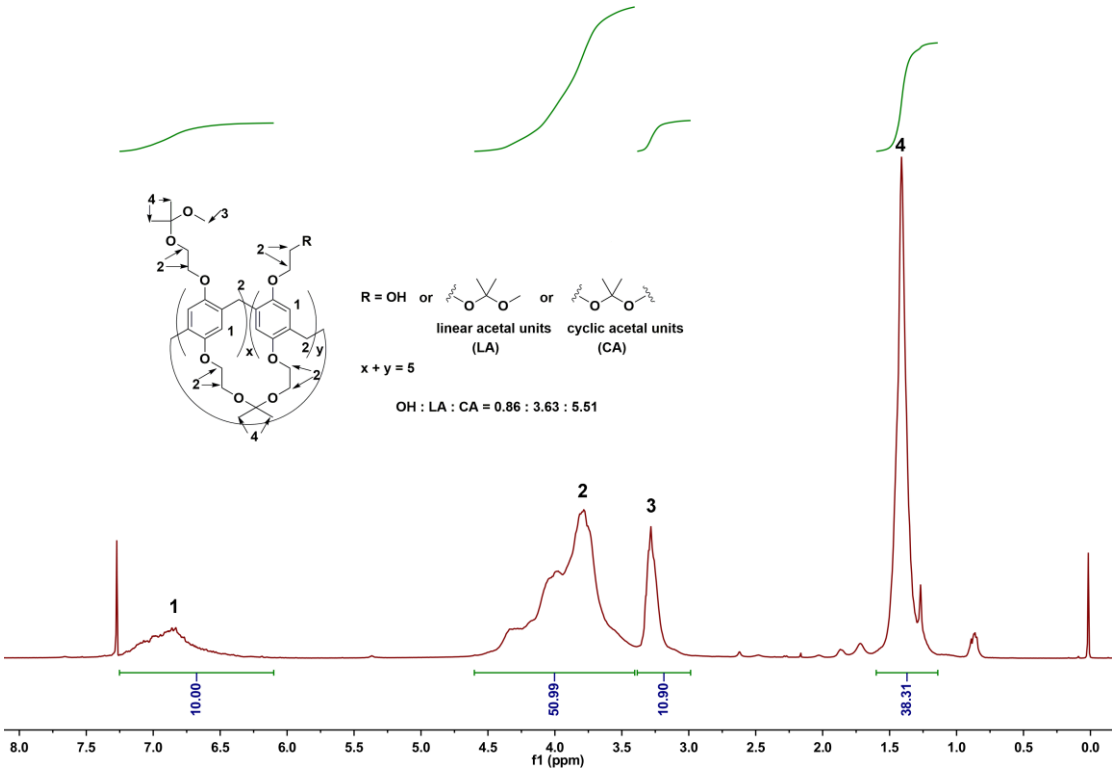


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) recorded for **Ac-PA[5]**.

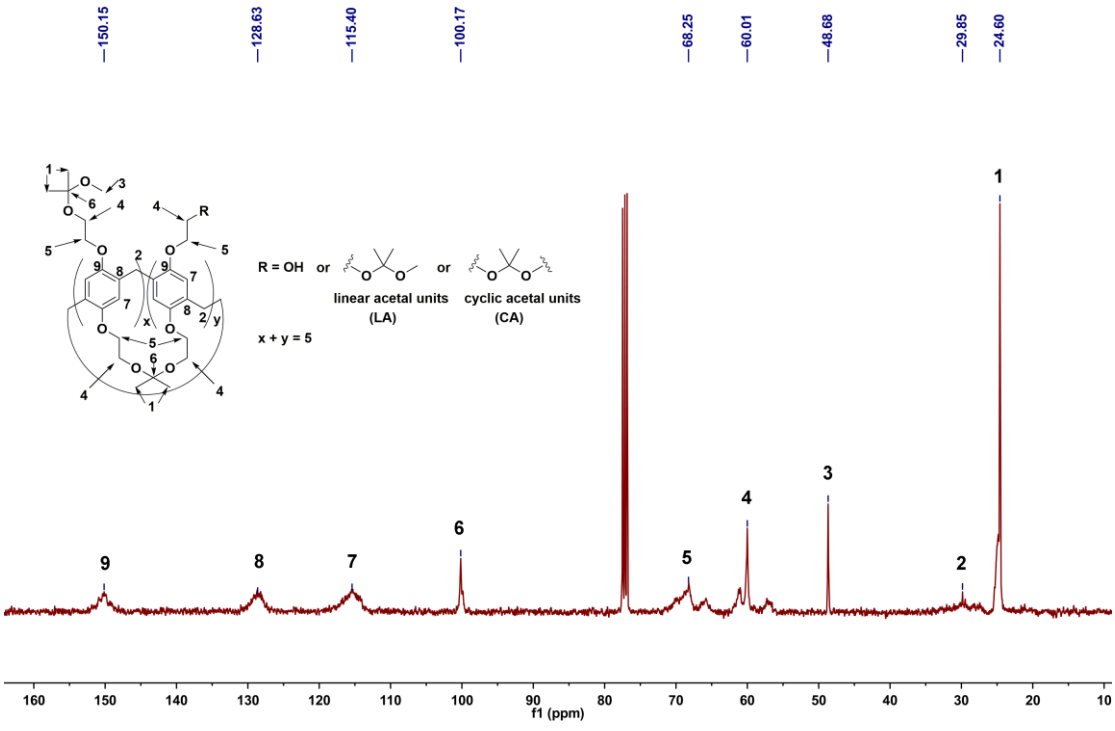
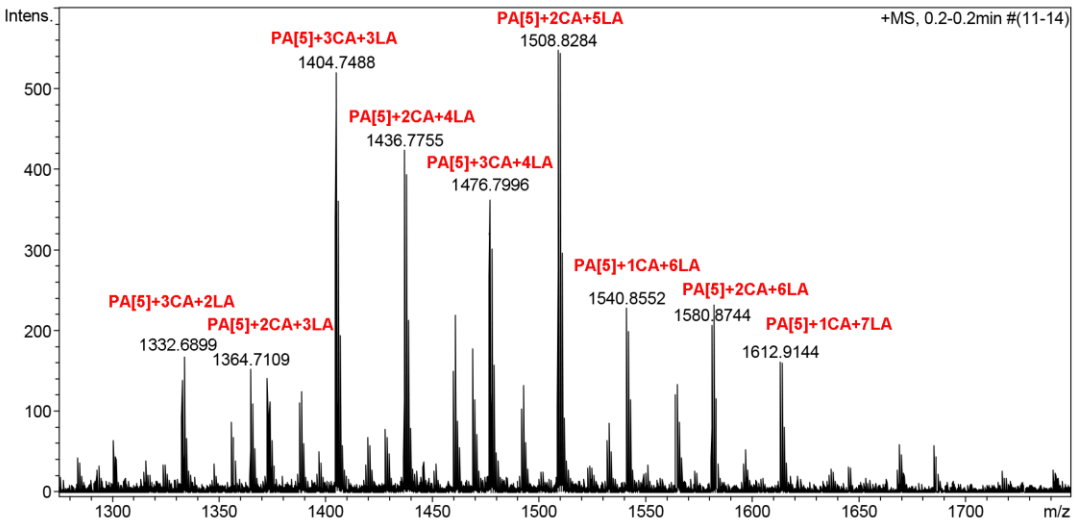


Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) recorded for **Ac-PA[5]**.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1404.7488
PA[5]+2CA+4LA
1436.7755
PA[5]+3CA+4LA
1476.7996
PA[5]+2CA+5LA
1508.8284
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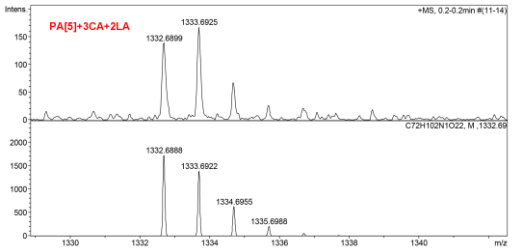
PA[5]+1CA+7LA
1612.9144
m/z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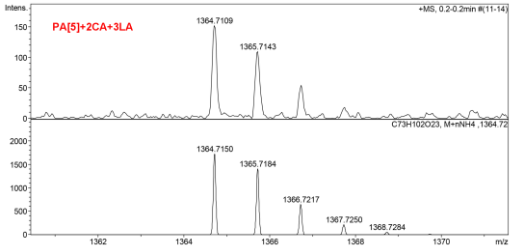
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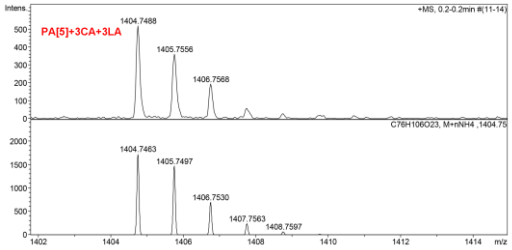
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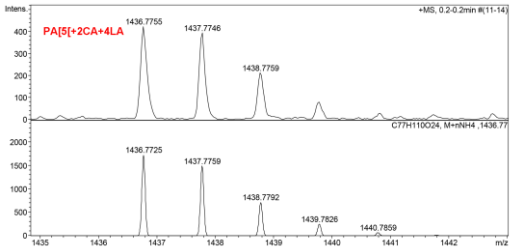
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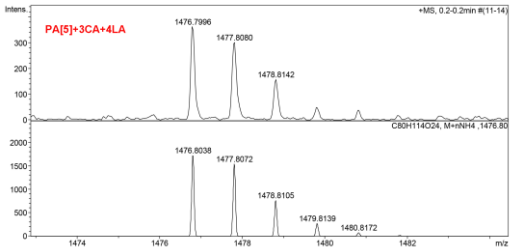
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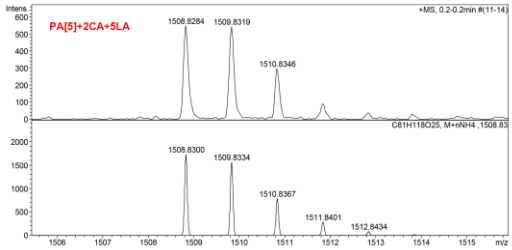
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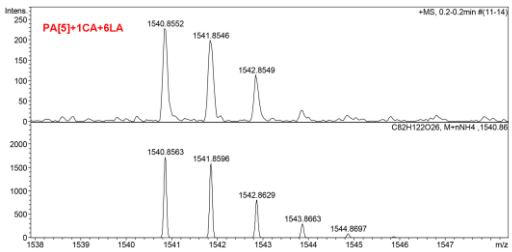
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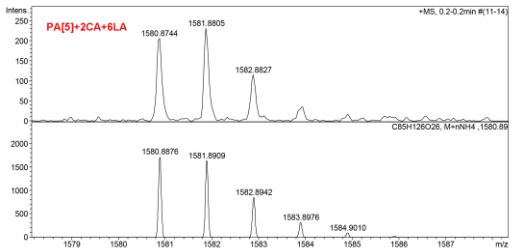
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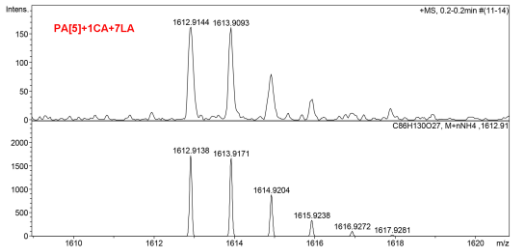


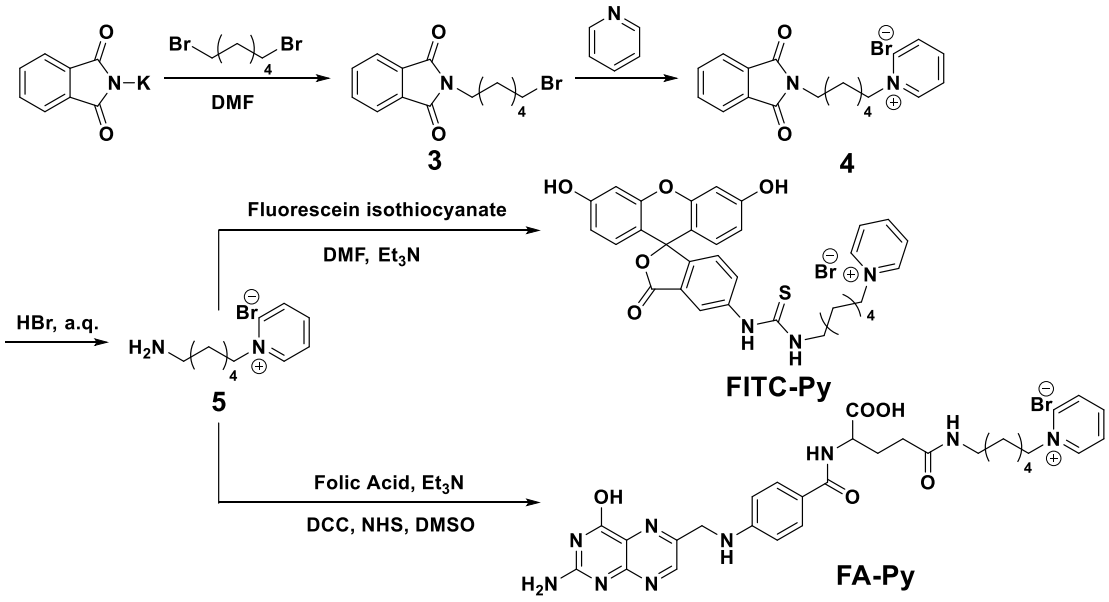
Figure S5. Electrospray ionization mass spectra of **Ac-PA[5]**.

Note: In the mass spectra of **Ac-PA[5]**, LA = linear acetal units, CA = cyclic acetal units, PA[5]+*m*CA+*n*LA represents the number of CA and LA unit in acetalated PA[5] molecule is *m* and *n*, respectively.

molecules	Experimental$[M+NH_4]^+$	Calculated $[M+NH_4]^+$	Error (ppm)
PA[5] + 1CA + 6LA	1540.8552	1540.8563	0.7
PA[5] + 1CA + 7LA	1612.9144	1612.9138	0.4
PA[5] + 2CA + 3LA	1364.7109	1364.7150	3.0
PA[5] + 2CA + 4LA	1436.7755	1436.7725	2.1
PA[5] + 2CA + 5LA	1508.8284	1508.8300	1.1
PA[5] + 2CA + 6LA	1580.8744	1580.8876	8.3
PA[5] + 3CA + 2LA	1332.6899	1332.6888	0.8
PA[5] + 3CA + 3LA	1404.7488	1404.7463	1.8
PA[5] + 3CA + 4LA	1476.7996	1476.8038	2.8

Table S1 Experimental and calculated E/Z values found for **Ac-PA[5]** and corresponding error (ppm).

1.2 Synthesis of FITC-Py and FA-Py.



Synthesis of compound 3[3]**:** A solution of 1,6-dibromohexane (12.25 g, 50 mmol) and potassium phthalimide (1.86 g, 10 mmol) in DMF (10 mL) was stirred at 90 ℃ for 24 h. After the mixture was cooled to room temperature, the solvent was removed by reduced pressure. The crude mixture was purified by column chromatography on silica gel to giva a white solid (2.76 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.71 (dd, J = 5.5, 3.0 Hz, 2H), 3.68 (t, J = 7.2 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 1.89 - 1.82 (m, 2H), 1.73 - 1.66 (m, 2H), 1.52 - 1.45 (m, 2H), 1.41 - 1.33 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 168.58, 134.03, 132.29, 123.33, 37.98, 33.79, 32.74, 28.54, 27.84, 26.16.

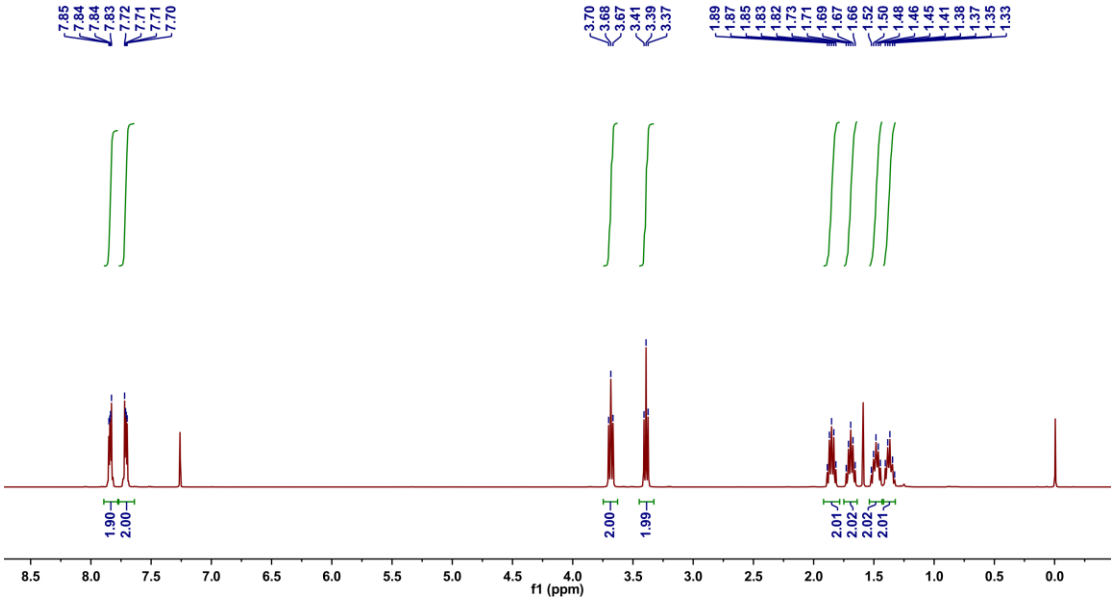


Figure S6. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) recorded for compound **3**.

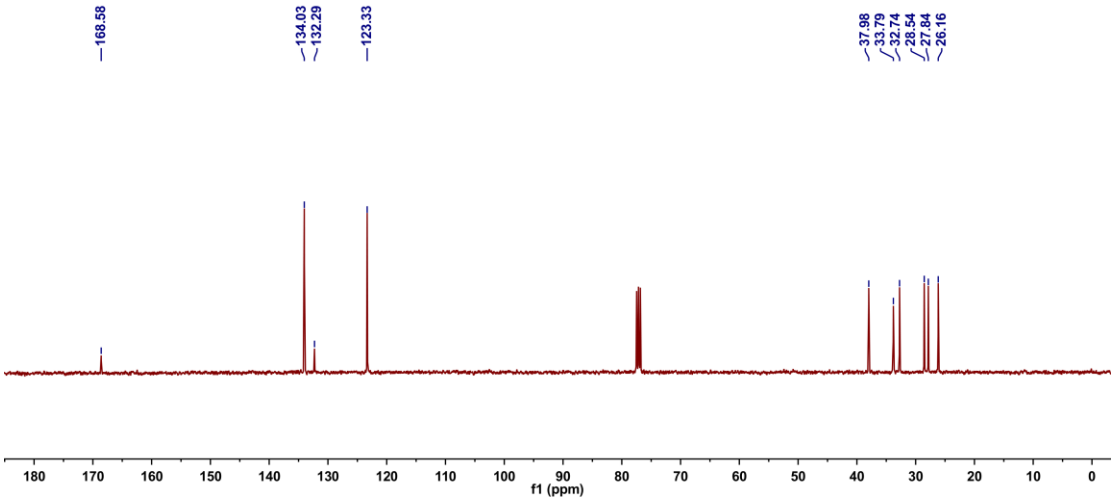


Figure S7. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) recorded for compound **3**.

Synthesis of compound 4[3]**:** Compound **3** (3.11 g, 10 mmol) was dissolved in dry pyridine (10 mL), and the resulting solution was heated to reflux for 3 h. After cooling to room temperature, the mixture was poured into diethyl ether (30 mL). The product was collected by centrifugation and washed with diethyl ether (3 × 30 mL) to give a white solid (3.7 g, 95%). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.87 (d, J = 6.0 Hz, 2H), 8.55 (t, J = 7.9 Hz, 1H), 8.08 (t, J = 7.0 Hz, 2H), 7.74 (dd, J = 5.6, 3.1 Hz, 2H), 7.67 (dd, J = 5.8, 2.9 Hz, 2H), 4.63 (t, J = 7.3 Hz, 2H), 3.52 (t, J = 7.0 Hz, 2H), 2.06 - 1.98 (m, 2H), 1.61 - 1.54 (m, 2H), 1.41 - 1.28 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 170.39, 145.38, 144.01, 134.55, 130.92, 128.04, 123.05, 61.68, 37.41, 30.19, 27.18, 25.28, 24.61.

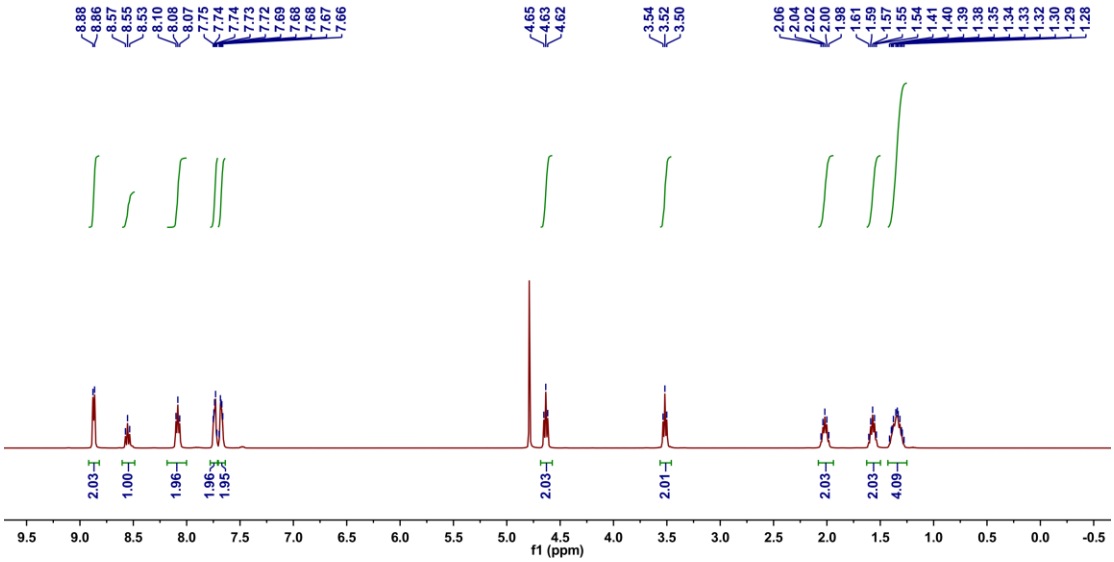


Figure S8. ^{1}H NMR spectrum (400 MHz, D_2O) recorded for compound **4**.

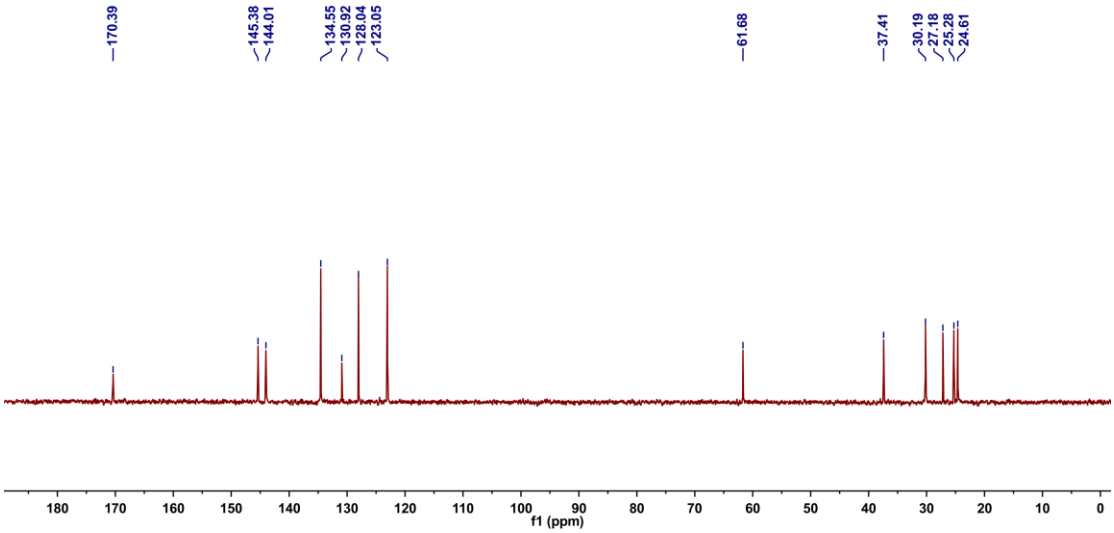


Figure S9. ^{13}C NMR spectrum (100 MHz, D_2O) recorded for compound **4**.

Synthesis of compound 5[3]**:** Compound **4** (3.89 g, 10 mmol) was dissolved in 40% HBr solution (30 mL) and heated at reflux for 3 h. The mixture was cooled in an ice bath, and the precipitate was removed by filtration. The aqueous filtrate was poured into acetone (30 mL). The product was collected by centrifugation and washed with acetone (3 × 30 mL) to give a white solid (2.9 g, 85 %). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.83 (d, J = 6.0 Hz, 2H), 8.53 (t, J = 7.8 Hz, 1H), 8.05 (t, J = 7.0 Hz, 2H), 4.59 (d, J = 7.4 Hz, 2H), 2.96 (t, J = 7.6 Hz, 2H), 2.06 - 1.99 (m, 2H), 1.67 - 1.60 (m, 2H), 1.45 - 1.35 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 145.30, 143.91, 127.98, 61.49, 39.10, 29.99, 26.14, 24.74, 24.47.

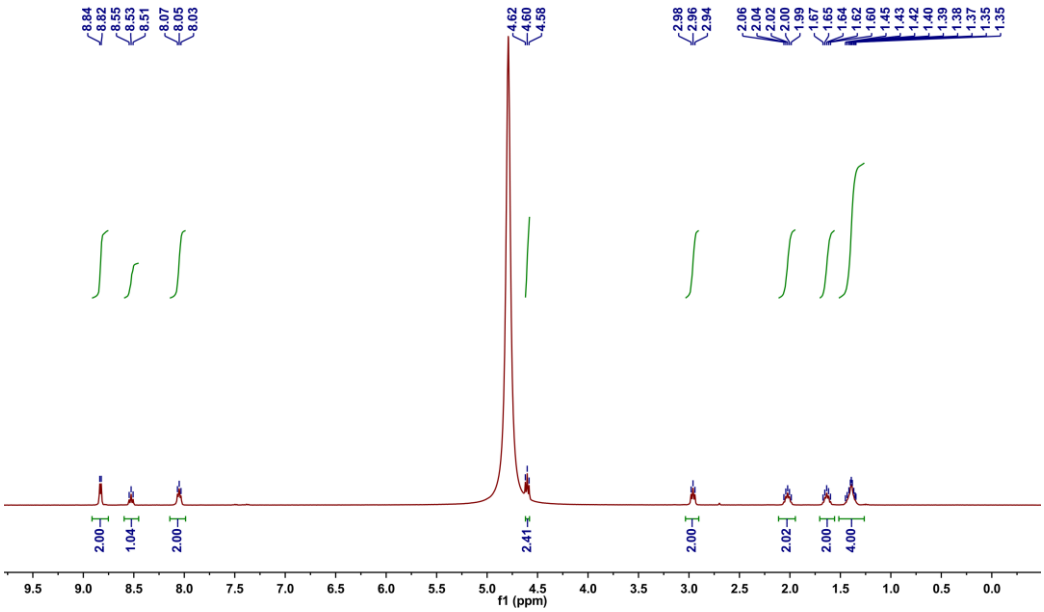


Figure S10. ^{1}H NMR spectrum (400 MHz, D_2O) recorded for compound **5**.

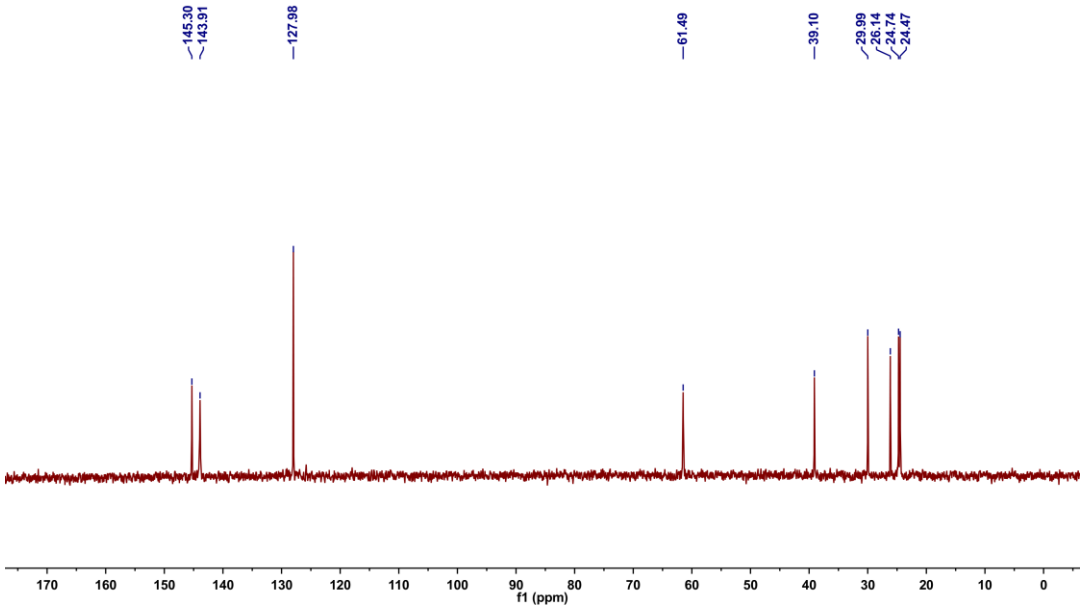


Figure S11. ^{13}C NMR spectrum (100 MHz, D_2O) recorded for compound **5.**

***Synthesis of FITC-Py*[4]:** Fluorescein isothiocyanate (389 mg, 1 mmol) and compound **5** (340 mg, 1 mmol) were dissolved in anhydrous DMF (25 mL). Et_3N (200 µL) was added and the mixture was stirred for 3 h at room temperature in the dark. Then, the mixture was poured into diethyl ether (100 mL) and the orange precipitate was collected by centrifugation and washed with diethyl ether (3 × 30 mL) to give an orange solid (508 mg, 80 %). ^{1}H NMR (400 MHz, MeOD) δ (ppm): 9.03 (d, J = 6.1 Hz, 2H), 8.59 (t, J = 7.8 Hz, 1H), 8.21 - 8.04 (m, 3H), 7.69 (d, J = 8.2 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.68 (s, 2H), 6.56 (d, J = 8.8 Hz, 2H), 4.67 (t, J = 7.6 Hz, 2H), 3.63 (t, J = 7.1 Hz, 2H), 2.12 - 2.05 (m, 2H), 1.73 - 1.67 (m, 2H), 1.54 – 1.45 (m, 2H). ^{13}C NMR (100 MHz, MeOD) δ (ppm): 182.64, 171.57, 164.88, 155.21, 146.85, 145.97, 142.20, 130.84, 130.44, 129.54, 126.97, 115.31, 112.48, 103.62, 63.04, 45.19, 32.34, 29.54, 27.17, 26.72. MS: *m/z* calcd for $[M]^+$ $C_{32}H_{30}N_3O_5S$, 568.1901, found 568.1916, error 2.6 ppm. FT-IR (cm^{-1}): 3053m, 2936s, 2675s, 2490m, 1752s, 1608s, 1541s, 1501s, 1487s, 1460s, 1329s, 1255s, 1207m, 1176s, 1108s, 849m, 680m.

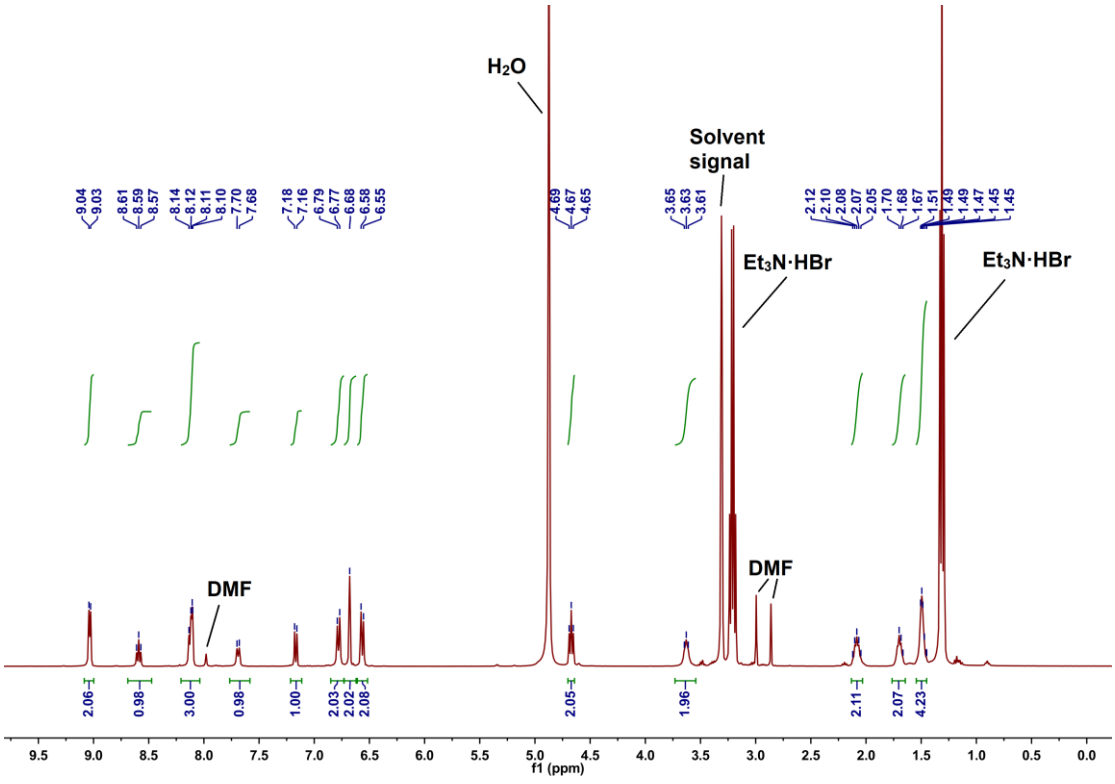


Figure S12. ^{1}H NMR spectrum (400 MHz, MeOD) recorded for **FITC-Py**.

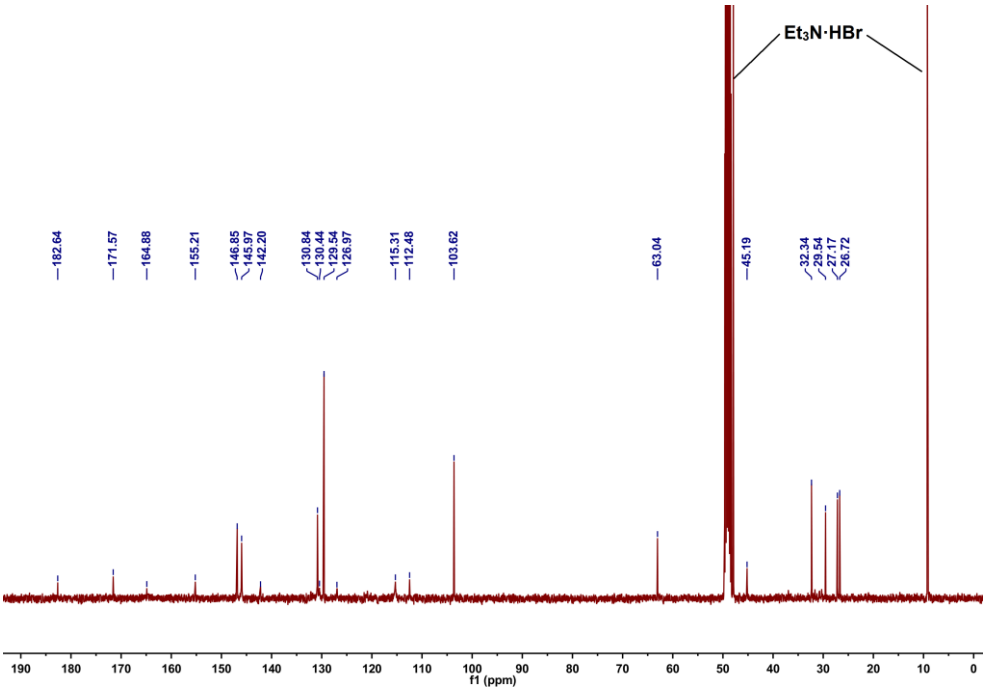


Figure S13. ^{13}C NMR spectrum (100 MHz, MeOD) of **FITC-Py.**

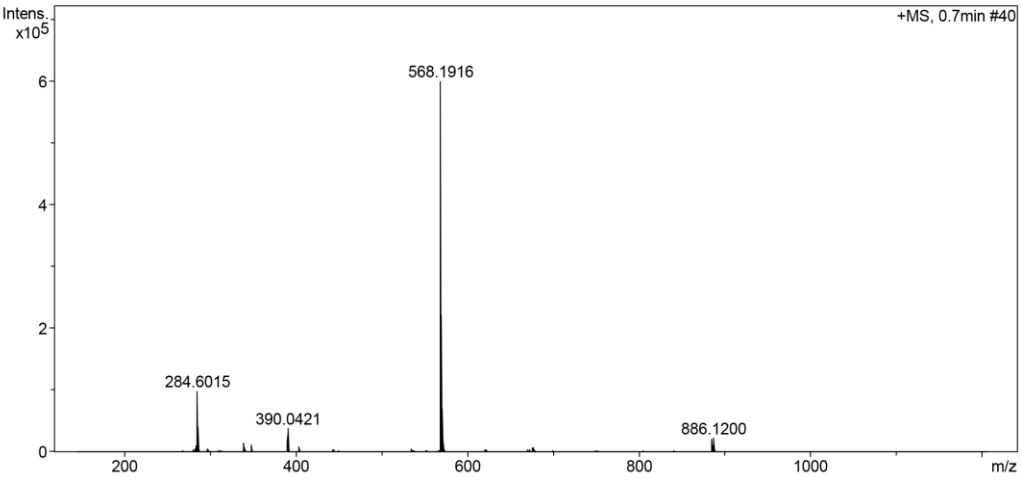


Figure S14. Electrospray ionization mass spectrum of **FITC-Py.**

Synthesis of FA-Py [5]**:** Folic acid (441 mg, 1 mmol) was activated by DCC (247 mg, 1.2 mmol) and NHS (230 mg, 2 mmol) in DMSO (20 mL) at 50 ℃ for 6 h. Compound **5** (340 mg, 1 mmol) and Et_3N (200 μL) was added and the mixture was stirred for another 6 h at room temperature. The precipitate was removed by filtration and the filtrate was poured into acetone (30 mL). The product was collected by centrifugation and washed with acetone (3 × 30 mL) to give a pale yellow solid (566 mg, 83 %). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 9.10 (d, J = 5.6 Hz, 2H), 8.64 (s, 1H), 8.60 (t, J = 8.0 Hz, 1H), 8.15 (t, J = 6.9 Hz, 2H), 8.10 - 7.95 (m, 1H), 7.82 (t, J = 5.8 Hz, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.17 (s, 2H), 6.95 (t, J = 6.2 Hz, 1H), 6.63 (d, J = 9.0 Hz, 2H), 4.63 - 4.53 (m, 2H), 4.53 - 4.40 (m, 2H), 4.34 - 4.20 (m, 1H), 3.04 - 2.97 (m, 2H), 2.34 - 2.21 (m, 1H), 2.18 - 2.14 (m, 1H), 2.06 - 1.75 (m, 4H), 1.39 - 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ (ppm): 174.34, 171.59, 166.12, 161.24, 156.28, 154.13, 150.71, 148.55, 145.46, 144.72, 128.84, 128.09, 127.95, 121.52, 111.21, 60.70, 52.60, 45.93, 38.19, 32.10, 30.61, 28.76, 27.06, 25.65, 25.05. MS: *m/z* calcd for $[M]^+$ $C_{30}H_{36}N_9O_5$, 602.2834, found 602.2814, error 3.3 ppm. FT-IR (cm^{-1}): 3257m, 2933m, 2858m, 1697s, 1636s, 1604s, 1502s, 1374m, 1295m, 1174s, 1125m, 768m, 682m.

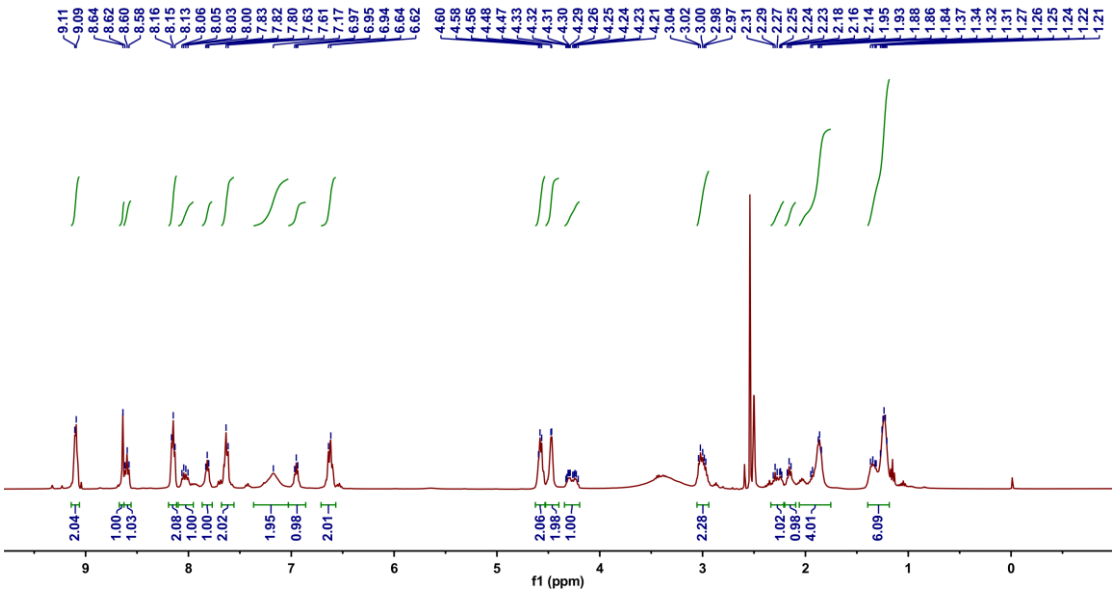


Figure S15. ^{1}H NMR spectrum (400 MHz, DMSO) recorded for **FA-Py**.

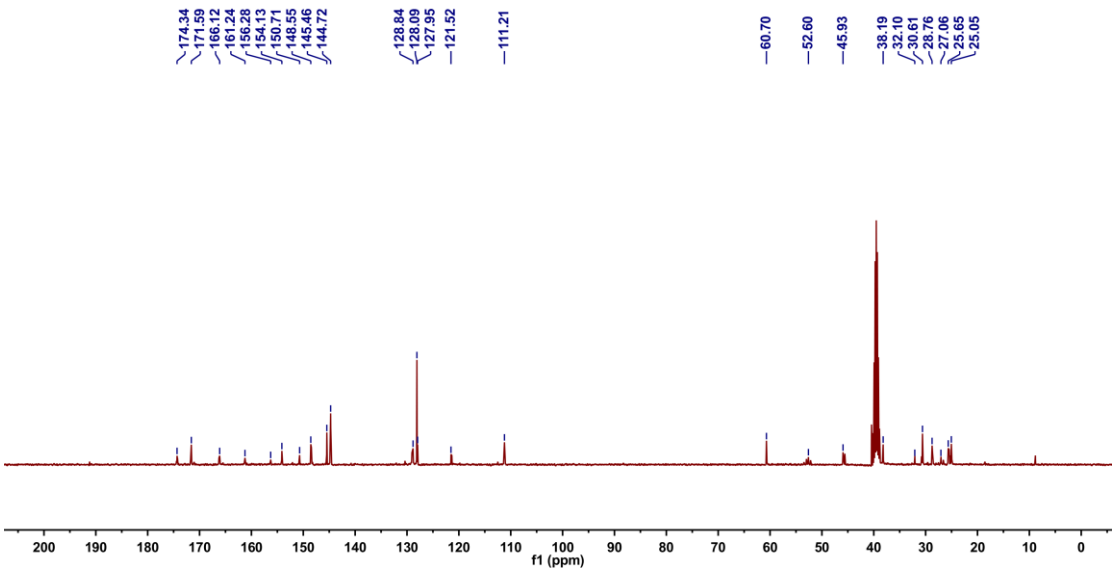


Figure S16. ^{13}C NMR spectrum (100 MHz, DMSO) recorded for **FA-Py**.

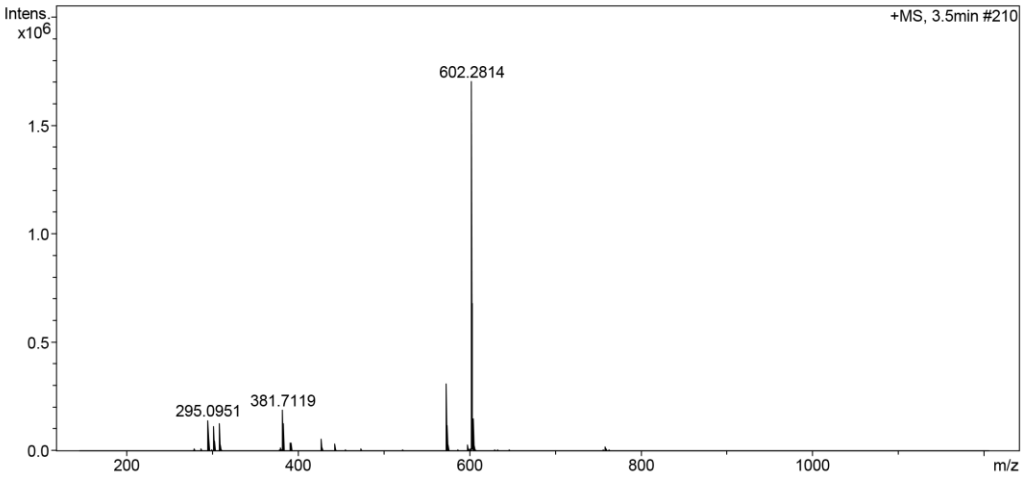


Figure S17. Electrospray ionization mass spectrum of **FA-Py**.

2. Host-Guest interaction

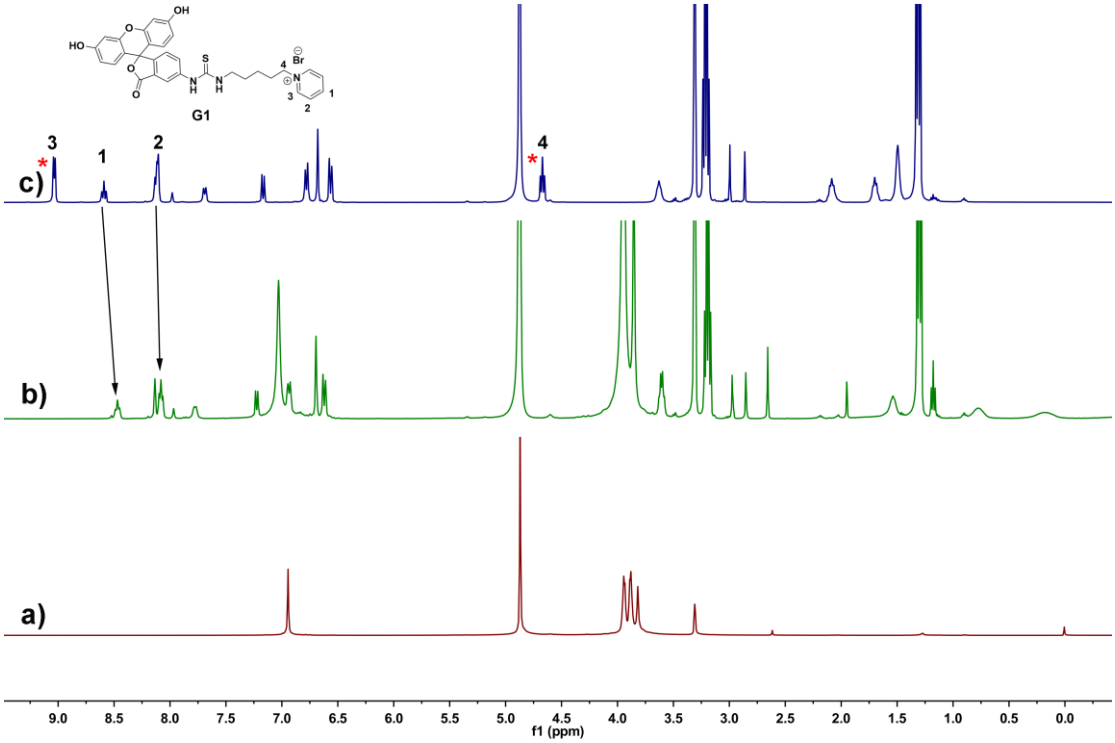


Figure S18. ^{1}H NMR spectra (400 MHz, MeOD) recorded for a) **1**, b) **1** and **FITC-Py** (1:1) and c) **FITC-Py**.

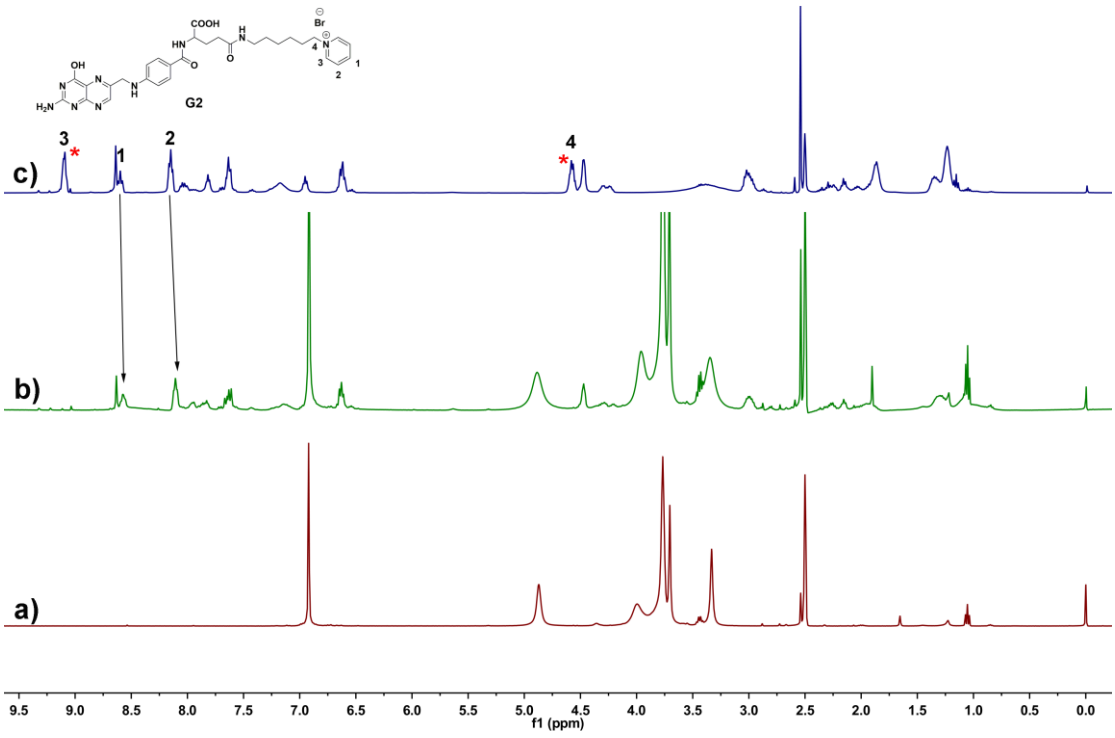


Figure S19. ^{1}H NMR spectra (400 MHz, DMSO) recorded for a) **1**, b) **1** and **FA-Py** (1:1) and c) **FA-Py**.

3. FT-IR spectra

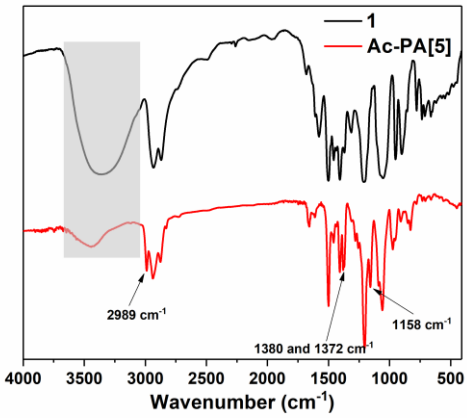


Figure S20. FT-IR spectra of **1** and **Ac-PA[5]**.[6]

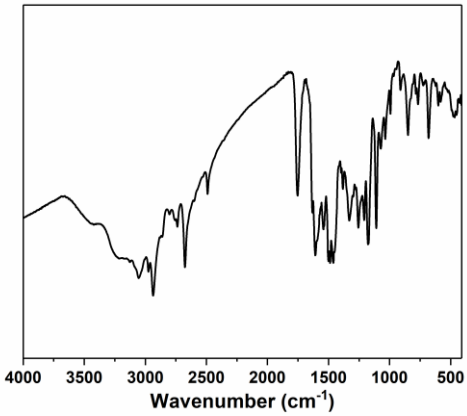


Figure S21. FT-IR spectrum of **FITC-Py**.

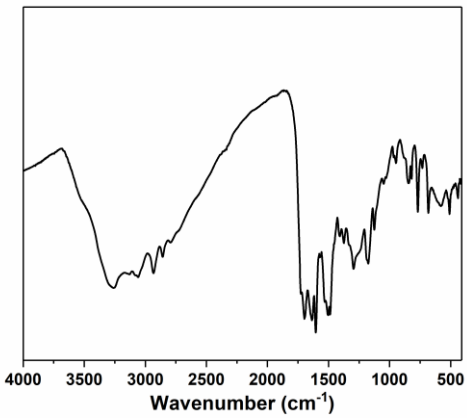


Figure S22. FT-IR spectrum of **FA-Py**.

4. Morphology and size distributions of nanoparticles

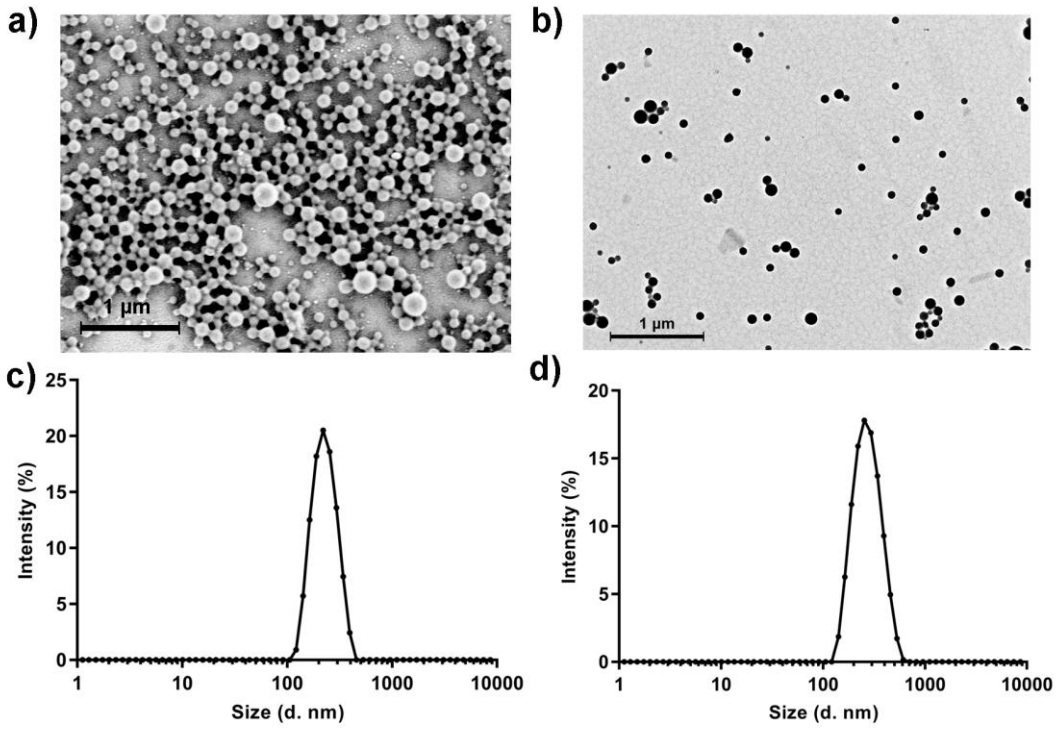


Figure S23. a) SEM, b) TEM images and c) size distribution curve of PTX-NPs (PDI = 0.222). d) Size distribution curve of DOX-NPs (PDI = 0.120).

5. Time-dependent size change of nanoparticles

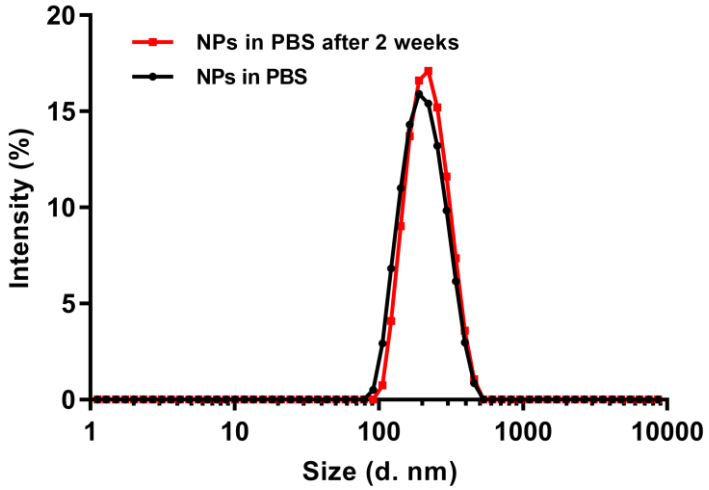


Figure S24. Time-dependent size distribution of blank nanoparticles in phosphate buffered saline (PBS). PDI (red line): 0.093.

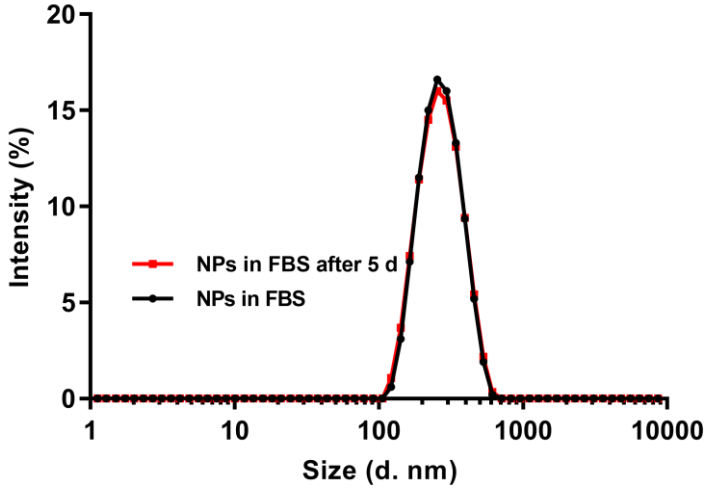


Figure S25. Time-dependent size distribution of blank nanoparticles in fetal bovine serum (FBS). PDI (black line): 0.180. PDI (red line): 0.248.

6. References

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