Electronic Supplementary Information (ESI)

for

Synergistic Approaches for Combating Pathogen *Acinetobacter baumannii*: Dynamic Constitutional Frameworks and Pillararene-based Self-assembled Drug Delivery Systems

*Dmytro Strilets[a], Steven Van Meerbeek[bc], Anke Breine[bc], Charles Van der Henst[bc], Tom Coenye[d], Stéphane P. Vincent[*a]*

[a]CBO, Unité de Chimie Organique, Université de Namur ASBL, Rue de Bruxelles 61 - 5000 Namur, Belgium.

[b]Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, Flanders Institute for Biotechnology (VIB), Pleinlaan 2, Building E-3, 1050 Brussels, Belgium.

[c]Vrije Universiteit Brussel (VUB), Pleinlaan 2, Building E-3, 1050 Brussels, Belgium.

[d]Laboratory of Pharmaceutical Microbiology, Universiteit Gent, Ottergemsesteenweg 460 B-9000 Gent, Belgium.

E-mail: stephane.vincent@unamur.be

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Materials

All reagents and chemicals used in this study were purchased from Sigma-Aldrich, Across, or TCI Europe at ACS grade and used without purification. Deuterated solvents ($CDCl_3$, D_2O, CD_3OD, and $(CD_3)_2SO$) for NMR analysis were purchased from Cambridge Isotopes Laboratories (Eurisotop). All reactions were carried out in standard glassware. Benzene-1,3,5-tricarboxaldehyde 1, poly-(ethylene glycol)-bis(3-aminopropyl) terminated (Mn ~1500 Da) 2 were purchased from TCI Europe.

DCF Preparation

To a solution of 1,3,5-Benzenetryaldehyde (BTA) in acetonitrile, was added poly(ethylene glycol) bis(3-aminopropyl) (1:1 molar ratios), and the mixture was stirred for 2-3 days at room temperature under an inert atmosphere (N_2). Then, the solvent was removed under vacuum, and the product was immediately dissolved in MilliQ water at a concentration of 2 mg of BTA as a Stock Solution (DCF0). To a volume of DCF0 containing 5 mg BTA (0,031mmol), a third building block (functional module) was added (DCF0:Component=1:1 or 1:2 by molar). After adding MilliQ water to obtain a final volume of 5 mL, the mixture was stirred at room temperature for at least 48-72 hours to obtain the final solution of DCFs.

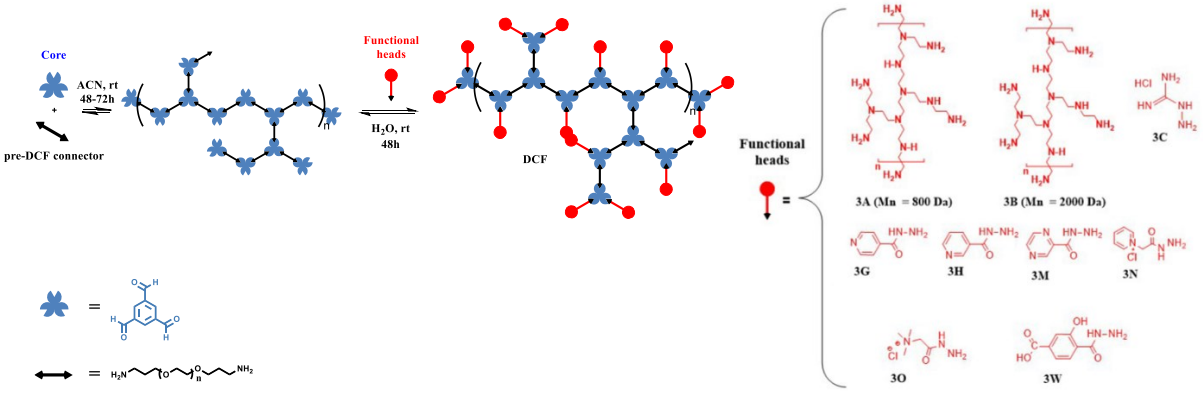

Figure S1. Representation of DCF Components and Preparation Procedure

List of functional heads (**FH 3)**: branched polyethylene imine 800 Da - **3A**, branched polyethylene imine Mw ~ 2000 Da solution 50% in water- **3B**, aminoguanidine hydrochloride - **3C**, Isonicotinic acid hydrazide - **3G**, Nicotinic acid hydrazide - **3H**, nicotinic Pyrazine-2-carbohydrazide-**3M**, Girard's Reagent P - **3N**, Girard's reagent T - **3O**, 2-Furoic hydrazide - **3U**, 4-(hydrazinocarbonil) benzoic acid - **3W**.

Pillar[5]arene Synthesis

Synthesis of APA1

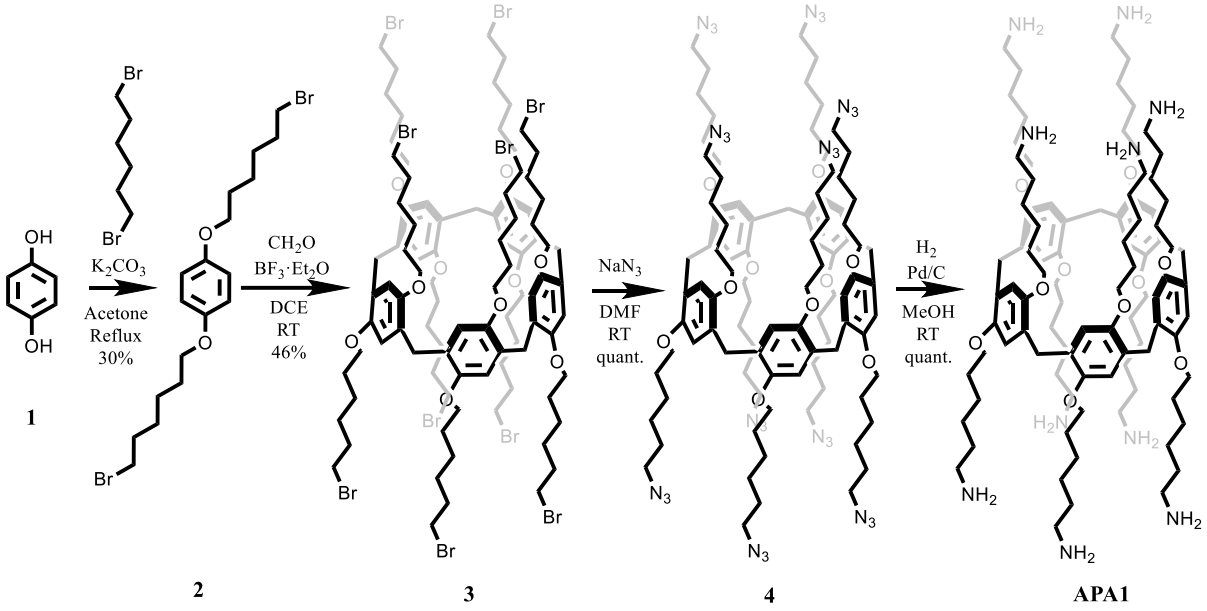

*Figure S2. Synthetic pathway for **APA1***

Synthesis of 2

16g of K_2CO_3 (115 mmol) was added to the solution of 1.6-dibromohexane (8.2 mL, 50.6 mmol) in acetone (80 mL). The mixture was refluxed (60-65 ^oC) for 30 min under inert atmosphere (argon). 40 mL of Hydroquinone solution in acetone (2.53 g, 23 mmol) was added dropwise, and the resulting reaction mixture was refluxed for 48 hours. The reaction mixture was cooled down to room temperature, filtrated, and evaporated. The crude product was dissolved in DCM (50 mL), washed with water and brine, and dried over $MgSO_4$. Purification was done by column chromatography on Silica with Cy:DCM=2:1 eluent system. The final

product was obtained as a white powder (3 g, 30%). Analytical data corroborated with those of the literature.[S1]

Synthesis of 3

The mixture of 2 (3.15 g, 7.22 mmol) and paraformaldehyde (0.685 g, 22.81 mmol) in DCE (50 mL) was stirred for 10 min at room temperature. 1 ml of $BF_3 \cdot Et_2O$ (1.15 g, 14.44 mmol) was added dropwise under argon atmosphere. The reaction mixture was stirred for 2 h at room temperature. Water (50 mL) was added to quench the reaction. After that, the mixture was filtrated, the organic layer was separated, washed with K_2CO_3, water, and brine, and evaporated with rotavap. The crude product was purified with column chromatography (silica, Cy:DCM=1:1), and white powder was obtained (1.49 g, 46%). Analytical data corroborated with those of the literature.[S1]

Synthesis of 4

2 g of 3 (0.9 mmol) was dissolved in dry DMF (50 mL). Sodium azide (1.16 g, 18 mmol) was added under argon, and the reaction mixture was stirred for 24 hours at room temperature. After that, the solvent was evaporated under vacuum and dissolved in DCM (25 mL). The solution was washed with water (3x25mL) and brine and dried with $MgSO_4$. Evaporation of solvent under vacuum gave 1.68 g of 4 as a white solid (quant. yield). Analytical data corroborated with those of the literature.[S1]

Synthesis of APA1

500 mg of 4 (0.27 mmol) was dissolved in dry MeOH (20 mL). Palladium on Carbon (50 mg, 10%) was added under argon atmosphere. Then, hydrogen gas was added to the system, and the reaction mixture was stirred for 2 days. The reaction mixture was filtered and evaporated, giving of APA1 as a white solid (428 mg, quant. yield).

^{1}H NMR (400 MHz, DMSO-D_6): δ 6.76 (H-9/H-10, s, ArH, 10H), 3.91-3.66 (H-6, br, OCH_2, 20H), 3.60 (H-7, s, $ArCH_2$ Ar, 10H), 2.44 (H-1, br, CH_2-NH_2, 20H), 1.81-1.60 (H-2, br, CH_2, 20H), 1.46-1.18 (H-3, H-4, H-5, br, CH_2, 60H) ppm (Fig. S3 SI).

^{1}H NMR (500 MHz, $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1)): δ 6.84 (H-8, s, ArH, 10H), 3.91-3.81 (H-6, br, OCH_2, 20H), 3.70 (H-7, s, $ArCH_2$ Ar, 10H), 2.44 (H-1, br, CH_2-NH_2, 20H), 1.81-1.60 (H-2, br, CH_2, 20H), 1.44-1.28 (H-3, H-4, H-5, br, CH_2, 60H) ppm (Fig. S4 SI).

^{13}C NMR (126 MHz, $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1)): δ 149.65 (C-8), 128.28 (C-9), 114.38 (C-10), 68.20 (C-6), 41.29 (C-1), 32.53 (C-2), 29.61 (C-5), 29.20 (C-7), 26.53 (C-3), 26.31 (C-4) ppm (Fig. S5 SI).

HRMS: $C_{95}H_{160}N_{10}O_{10}$ Calculated 1601.2318; Found 1603.2395

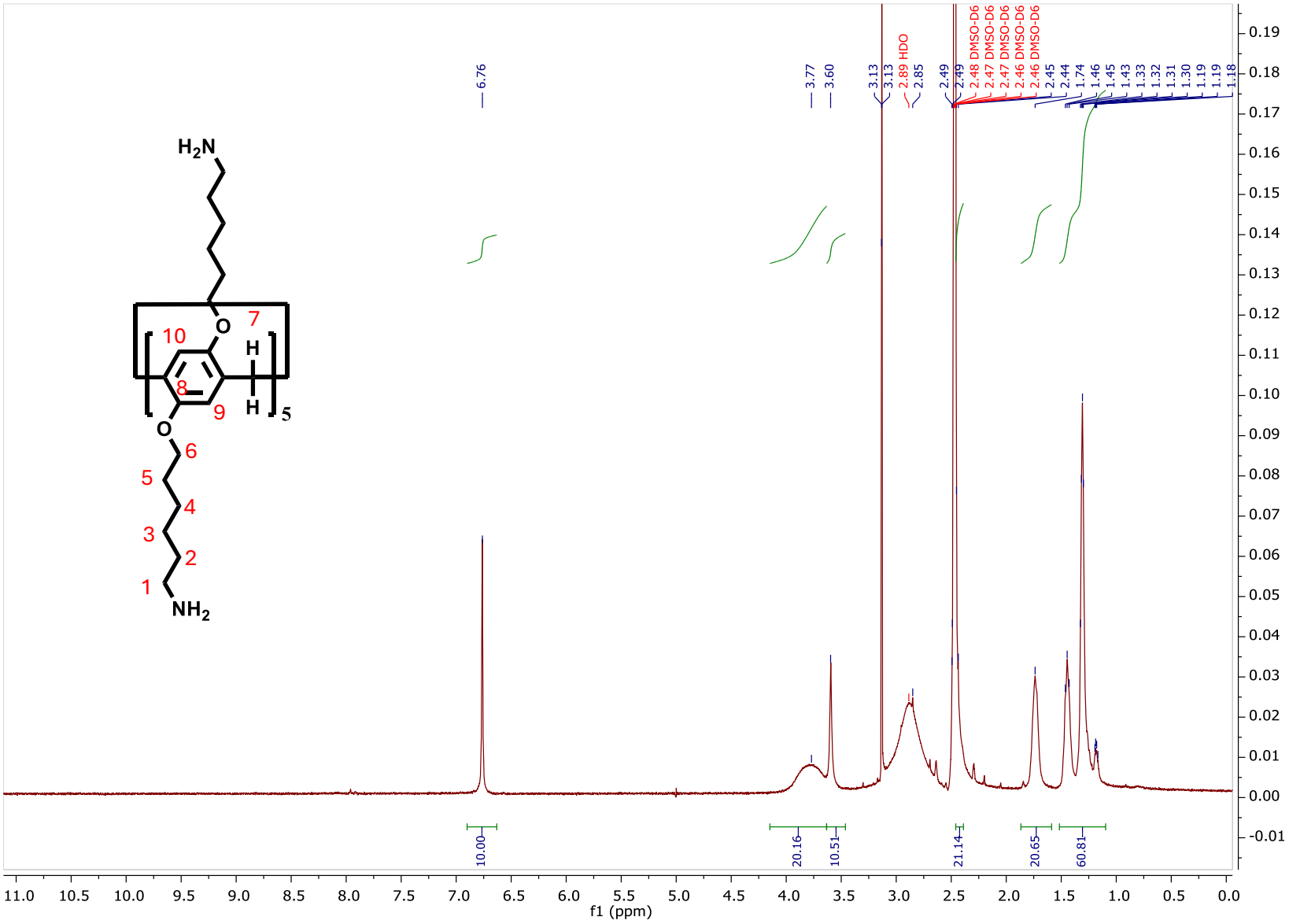

*Figure S3. [1]H NMR of **APA1** in DMSO-D6.*

[1]H NMR (400 MHz, DMSO-D_6): δ 6.76 (H-9/H-10, s, ArH, 10H), 3.91-3.66 (H-6, br, OCH_2, 20H), 3.60 (H-7, s, $ArCH_2$ Ar, 10H), 2.44 (H-1, br, CH_2-NH_2, 20H), 1.81-1.60 (H-2, br, CH_2, 20H), 1.46-1.18 (H-3, H-4, H-5, br, CH_2, 60H) ppm.

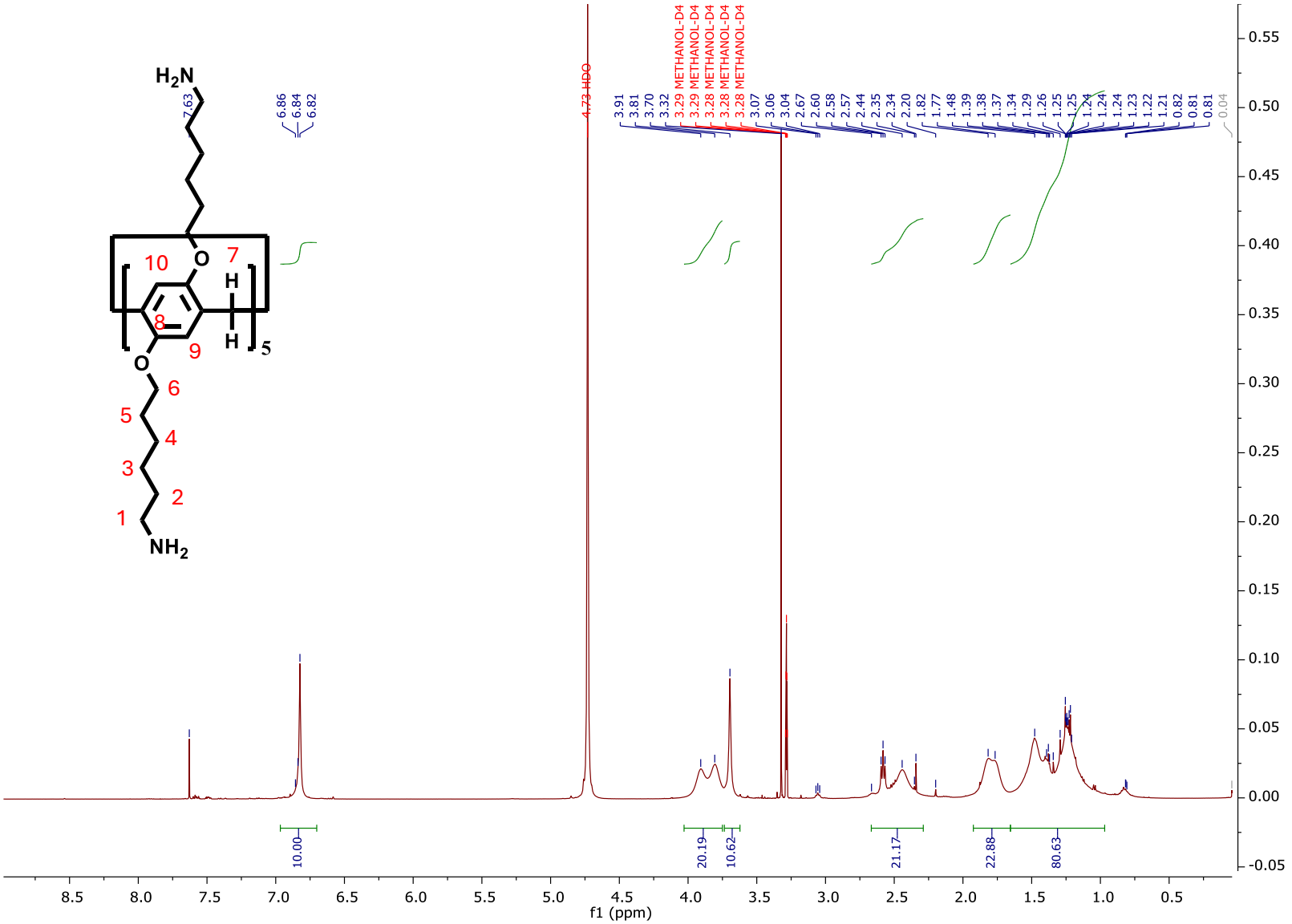

*Figure S4. ^1H NMR of **APA1** in $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1).*

^{1}H NMR (500 MHz, $CDCl_3$ - CD_3OD mixture): δ 6.84 (H-8, s, ArH, 10H), 3.91-3.81 (H-6, br, OCH_2, 20H), 3.70 (H-7, s, $ArCH_2$ Ar, 10H), 2.44 (H-1, br, CH_2-NH_2, 20H), 1.81-1.60 (H-2, br, CH_2, 20H), 1.44-1.28 (H-3, H-4, H-5, br, CH_2, 60H) ppm.

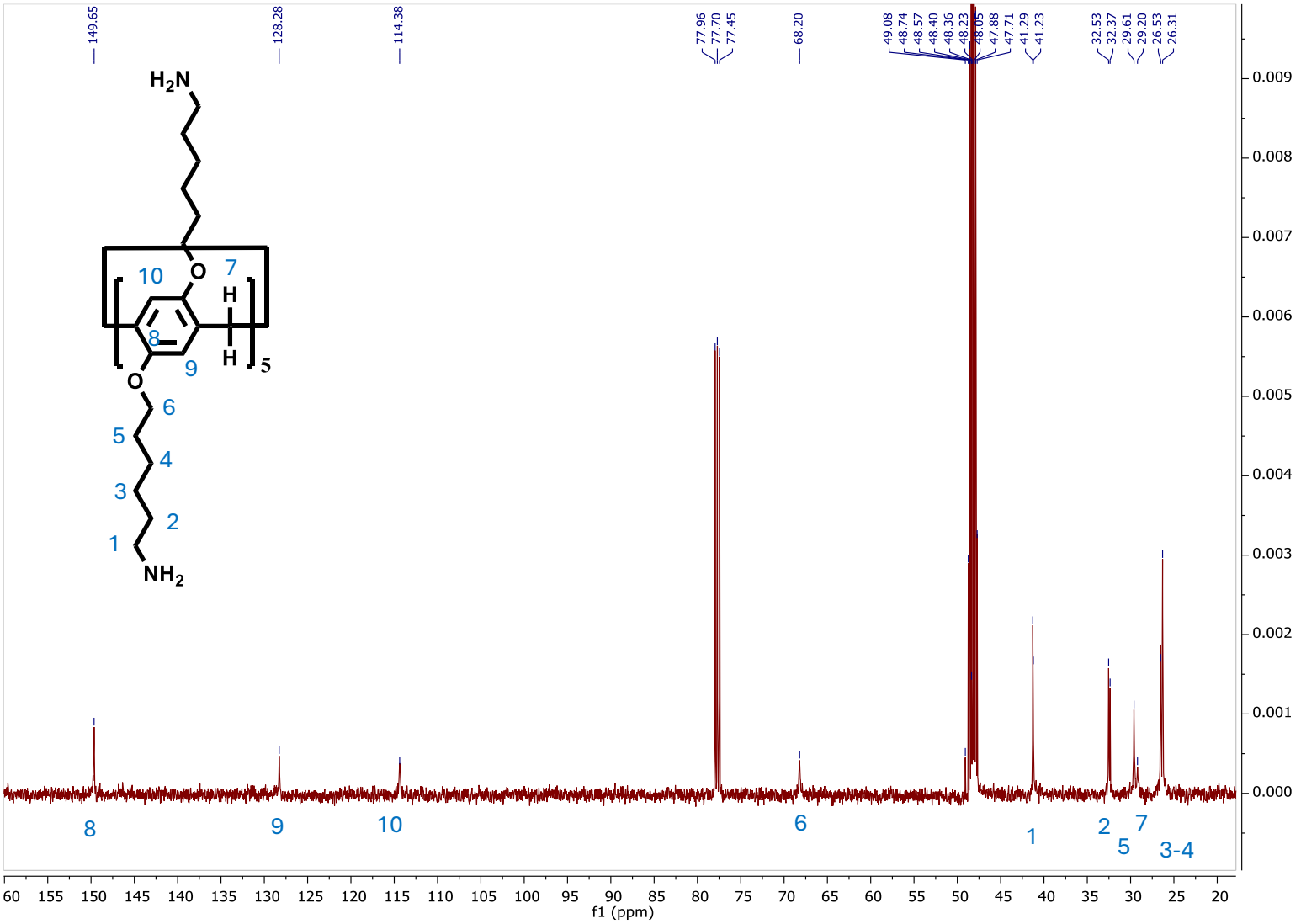

Figure S5. ^{13}C NMR of APA1 in $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1).

^{13}C NMR (126 MHz, $CDCl_3$ – CD_3OD mixture): δ 149.65 (C-8), 128.28 (C-9), 114.38 (C-10), 68.20 (C-6), 41.29 (C-1), 32.53 (C-2), 29.61 (C-5), 29.20 (C-7), 26.53 (C-3), 26.31 (C-4) ppm

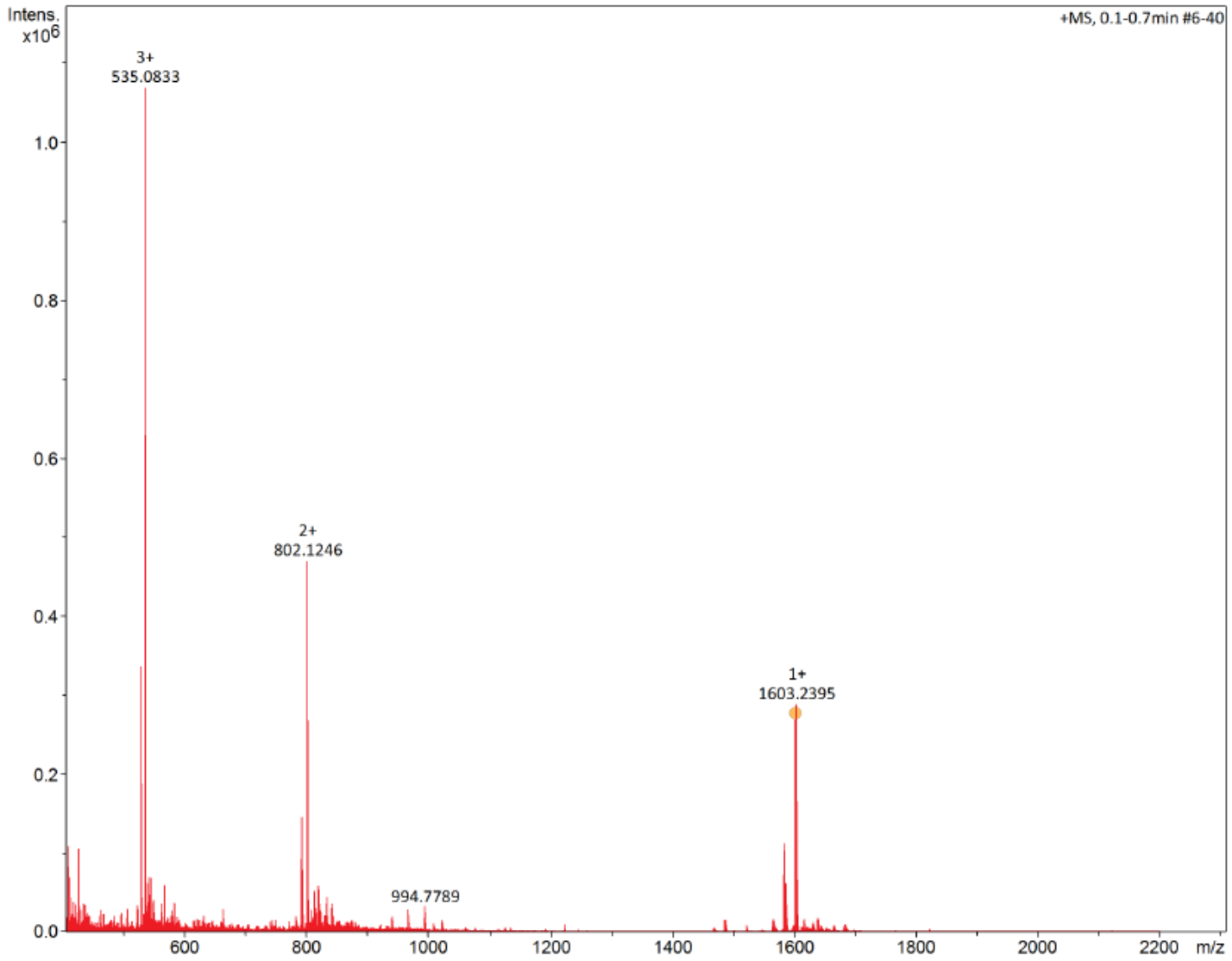

*Figure S6. HRMS of **APA1***

$C_{95}H_{160}N_{10}O_{10}$

Calculated 1601.2318; Found 1603.2395

Synthesis of APA2

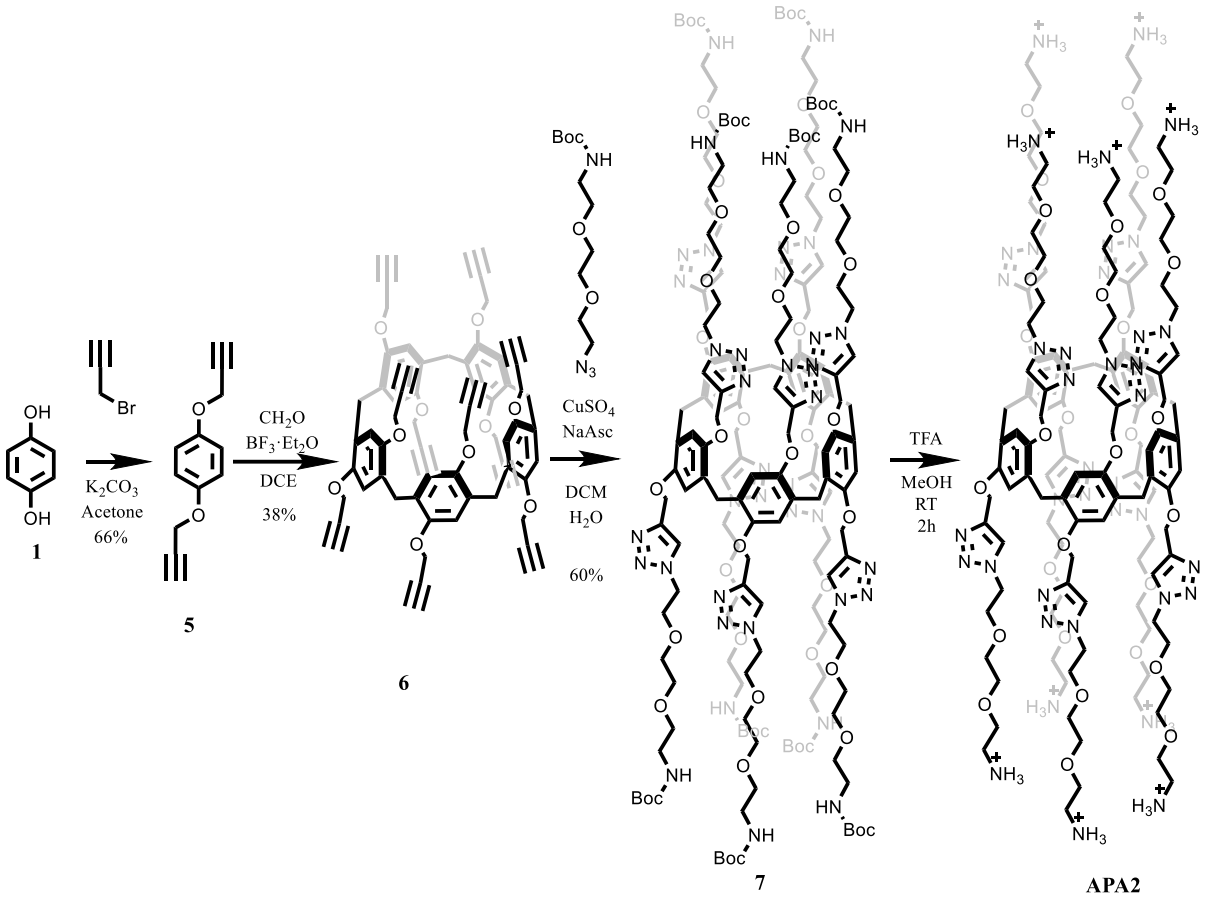

*Figure S7. Synthetic pathway for **APA2***

Synthesis of 5

K_2CO_3 (16g,115 mmol) was added to the solution of propargyl bromide (3.82 mL, 6 g, 50.6 mmol) in acetone (80 mL). The mixture was refluxed for 30 min under an inert atmosphere (argon). Hydroquinone 1 solution in acetone (40 mL, 2.53 g, 23 mmol) was added dropwise, and the resulting reaction mixture was refluxed for 48 hours. The reaction mixture was cooled down to room temperature, filtrated, and evaporated. The crude product was dissolved in DCM (50 ml), washed with water and brine, and dried over $MgSO_4$. Purification was done by column chromatography on Silica with Cy:DCM=2:1 as eluent system. The product 5 was obtained as a white powder (3 g, 66%). Analytical data corroborated with those of the literature.[S2]

Synthesis of 6

A mixture of 5 (1.25 g, 6.713 mmol) and paraformaldehyde (0.6 g, 22.81 mmol) in DCE (25 mL) was stirred for 10 min at room temperature. $BF_3 \cdot Et_2O$ (1.91 ml, 1.66 g, 13.42 mmol) was added dropwise under argon atmosphere. The reaction mixture was stirred for 2 h at room temperature. Methanol (25 mL) was added to quench the reaction. After that, the mixture was filtrated and evaporated under vacuum. The crude product was purified by column chromatography (silica gel, Cy:DCM=1:1), and 6 was obtained as a white powder (505 mg, 38%). (Analytical data corroborated with those of the literature [S3]).

Synthesis of 7

50 mg of 6 (0.05 mmol, 1 eq.) was dissolved together with 274.3 mg of N_3-PEG_2-NH-Boc (1 mmol, 20 eq.) in 1.5 mL of DCM and stirred at room temperature under argon atmosphere. $CuSO_4$ (16 mg, 2 eq.) in water (0.75 mL) and sodium ascorbate (66 mg, 6.6 eq.) in water (0.75 mL) were added to the reaction and stirred overnight. The reaction mixture was diluted in 25 mL of water and 25 mL of DCM, and the organic layer was separated. Then, the solution was dried over $MgSO_4$, filtrated, and evaporated under vacuum. Purification was performed by column chromatography (silica gel, DCM:MeOH=9:1) to give 7 as a pale yellow oil (34 mg, 60%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97(bt, H-4, 10H), 6.94 (m, H-1, 10H), 5.25- 4.83 (m, H-5, 20H), 4.52(bs, H-3, 20H), 3.85 (bs, H-2, 10H), 3.71-3.43-3.24 (m, H-6 – H-10, 100H), 1.40 (s, H-11, 90H) ppm (Fig. S8 SI).

^{13}C NMR (126 MHz, $CDCl_3$) δ 156.09 (C-14), 149.73 (C-1), 144.09 (C-6), 128.77 (C-7), 124.25 (C-3), 116.15 (C-2), 79.28 (C-15), 70.53 (C-5), 70.29, 70.17, 69.49 (C-9 - C-12), 62.08 (C-4), 50.19 (C-8), 40.35 (C-13), 28.52 (C-16) ppm (Fig. S9 SI).

Synthesis of APA2

To a solution of 7 (34 mg, 0.01 mmol) in DCM (1.0 mL) at room temperature, TFA (0.5 mL) was added. The reaction mixture was stirred at RT for 2 h, after which the mixture was co-distilled with DCM 4-5 times in vacuo, which gave the resulting product APA2 as a yellow oil (35 mg, quant. yield).

^{1}H NMR (500 MHz, $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1)) δ 8.06 (s, H-4, 10H), 6.84 (s, H-1, 10H), 4.76 - 4.63(m, H-5, 20H), 4.49 (s, H-3, 20H), 3.86 (s, H-2, 10H), 3.72-3.42 (m, H-6 – H-9, 80H) 3.08(m, H-10, 20H) ppm (Fig. S9 SI).

^{13}C NMR (126 MHz, $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1)) δ 149.39 (C-1), 143.84 (C-6), 128.70 (C-7), 125.02 (C-3), 114.91 (C-2), 69.81(C-9), 69.61(C-5) 68.90(C-12), 66.59(C-10, C-11), 61.29 (C-4), 50.15(C-8), 39.36(C-13) ppm (Fig. S10 SI).

HRMS: $C_{125}H_{190}N_{40}O_{30}$ Exact Mass 2731.4572 (Calculated); 2733.4750 ($[M+H]^+$, Found) (Fig. S11 SI)

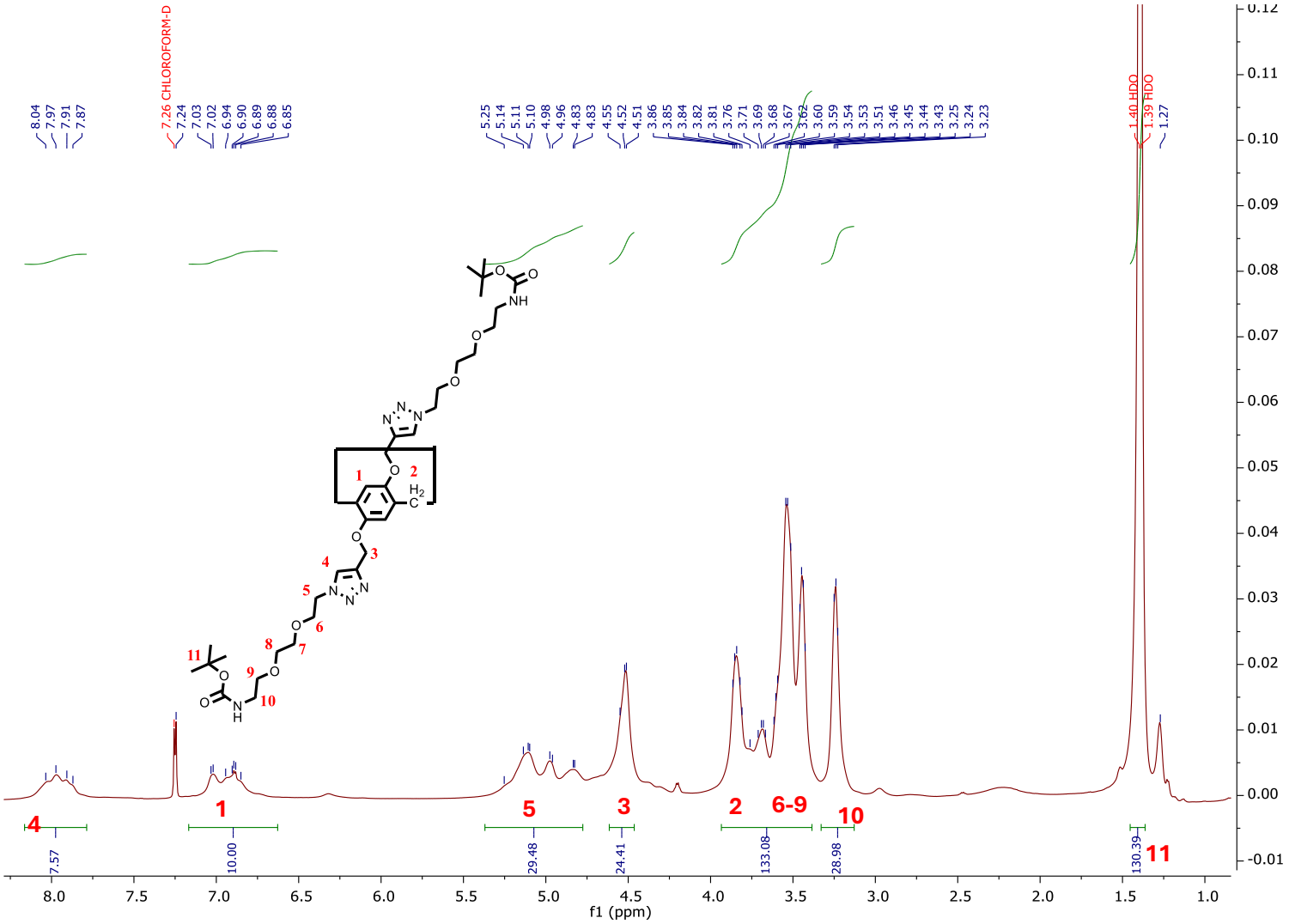

Figure S8. [1]H NMR of 7.

[1]H NMR (500 MHz, $CDCl_3$) δ 7.97(bt, H-4, 10H), 6.94 (m, H-1, 10H), 5.25- 4.83 (m, H-5, 20H), 4.52(bs, H-3, 20H), 3.85 (bs, H-2, 10H), 3.71-3.43-3.24 (m, H-6 – H-10, 100H), 1.40 (s, H-11, 90H).

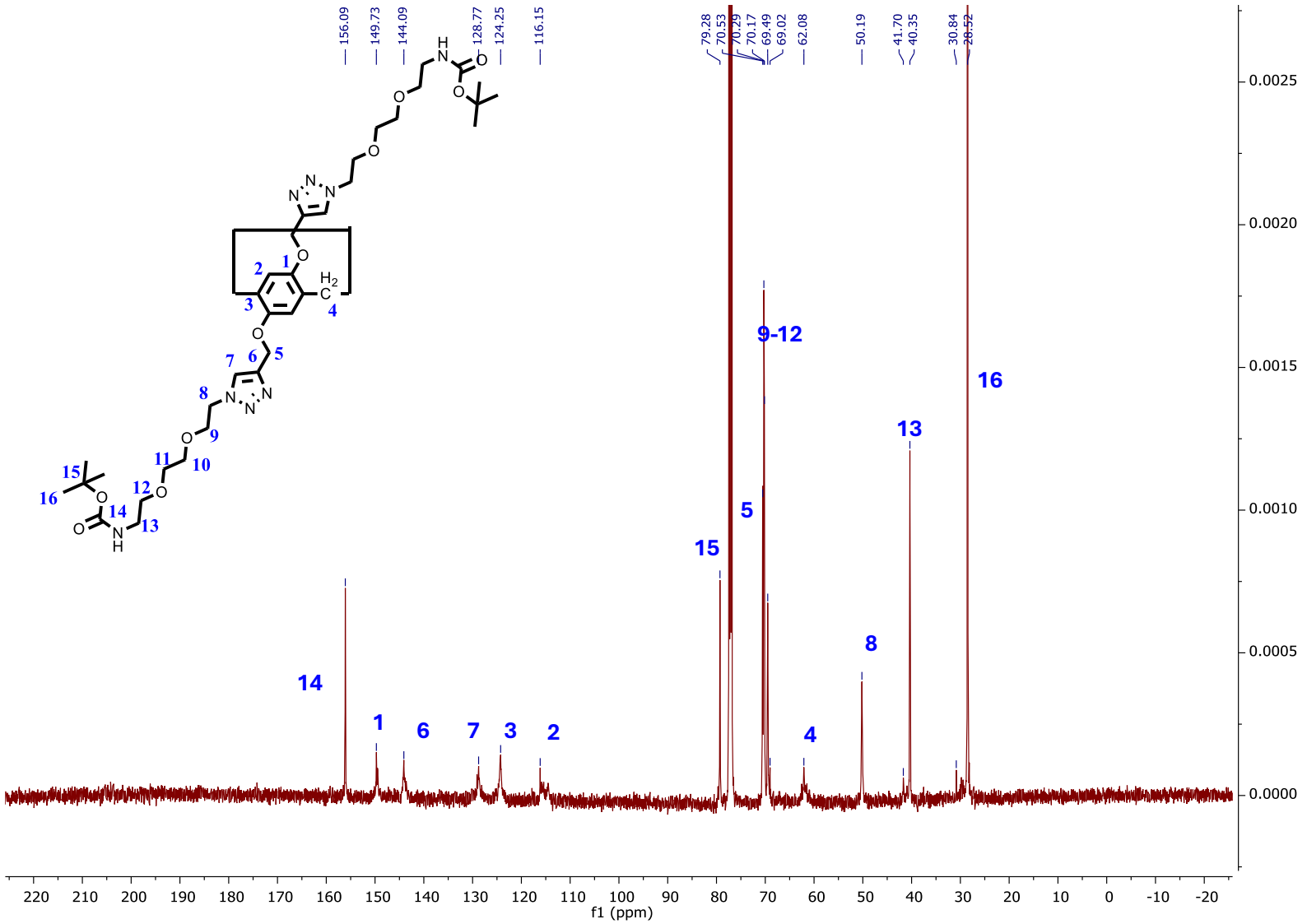

Figure S9. ^{13}C MNR of 7 in $CDCl_3$

^{13}C NMR (126 MHz, $CDCl_3$) δ 156.09 (C-14), 149.73 (C-1), 144.09 (C-6), 128.77 (C-7), 124.25 (C-3), 116.15 (C-2), 79.28 (C-15), 70.53 (C-5), 70.29, 70.17, 69.49 (C-9 - C-12), 62.08 (C-4), 50.19 (C-8), 40.35 (C-13), 28.52 (C-16).

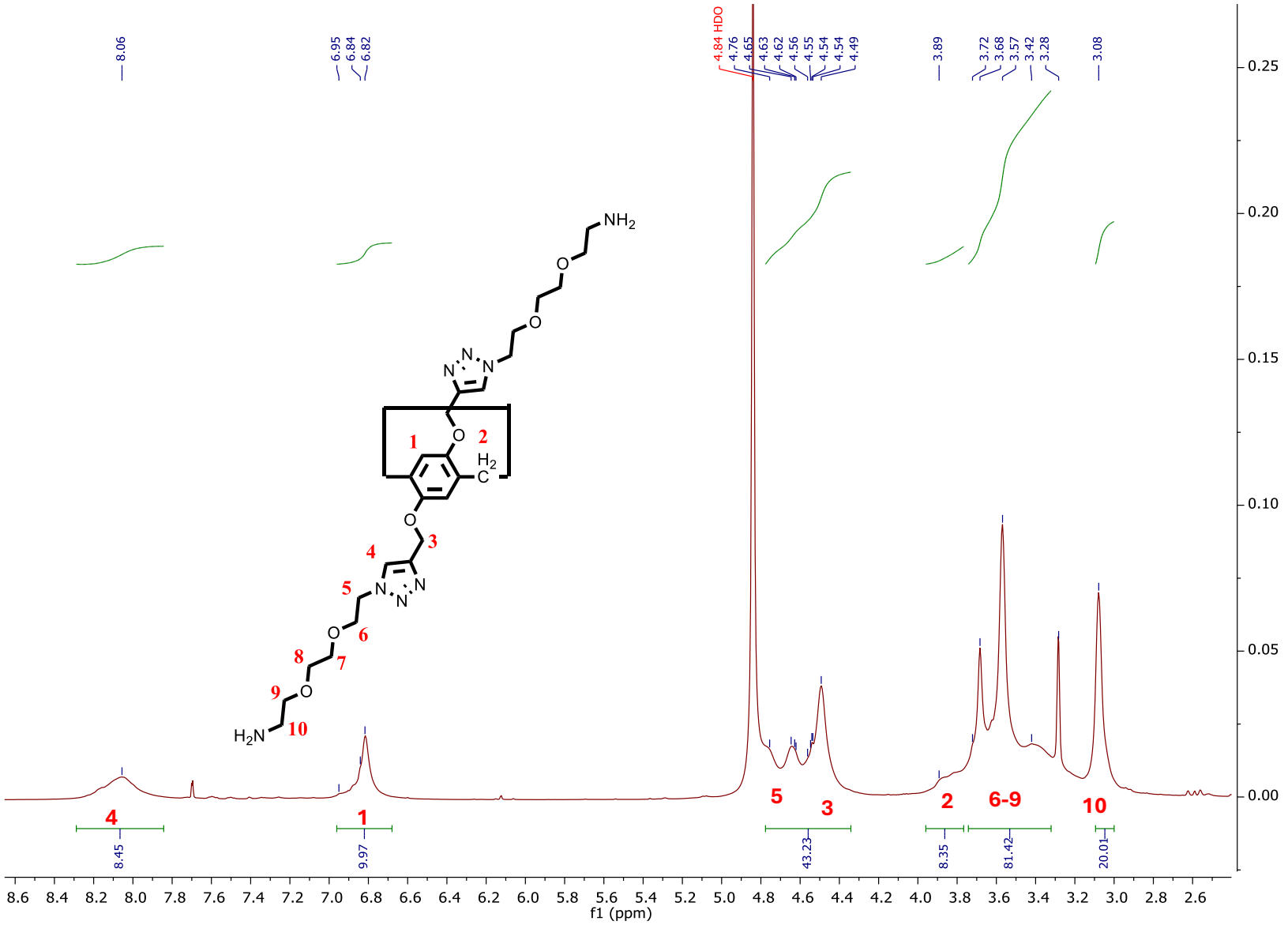

*Figure S10. ^{1}H NMR of **APA2** in $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1).*

^{1}H NMR (500 MHz, $CDCl_3$ – CD_3OD mixture) δ 8.06 (s, H-4, 10H), 6.84 (s, H-1, 10H), 4.76 - 4.63(m, H-5, 20H), 4.49 (s, H-3, 20H), 3.86 (s, H-2, 10H), 3.72-3.42 (m, H-6 – H-9, 80H) 3.08(m, H-10, 20H).

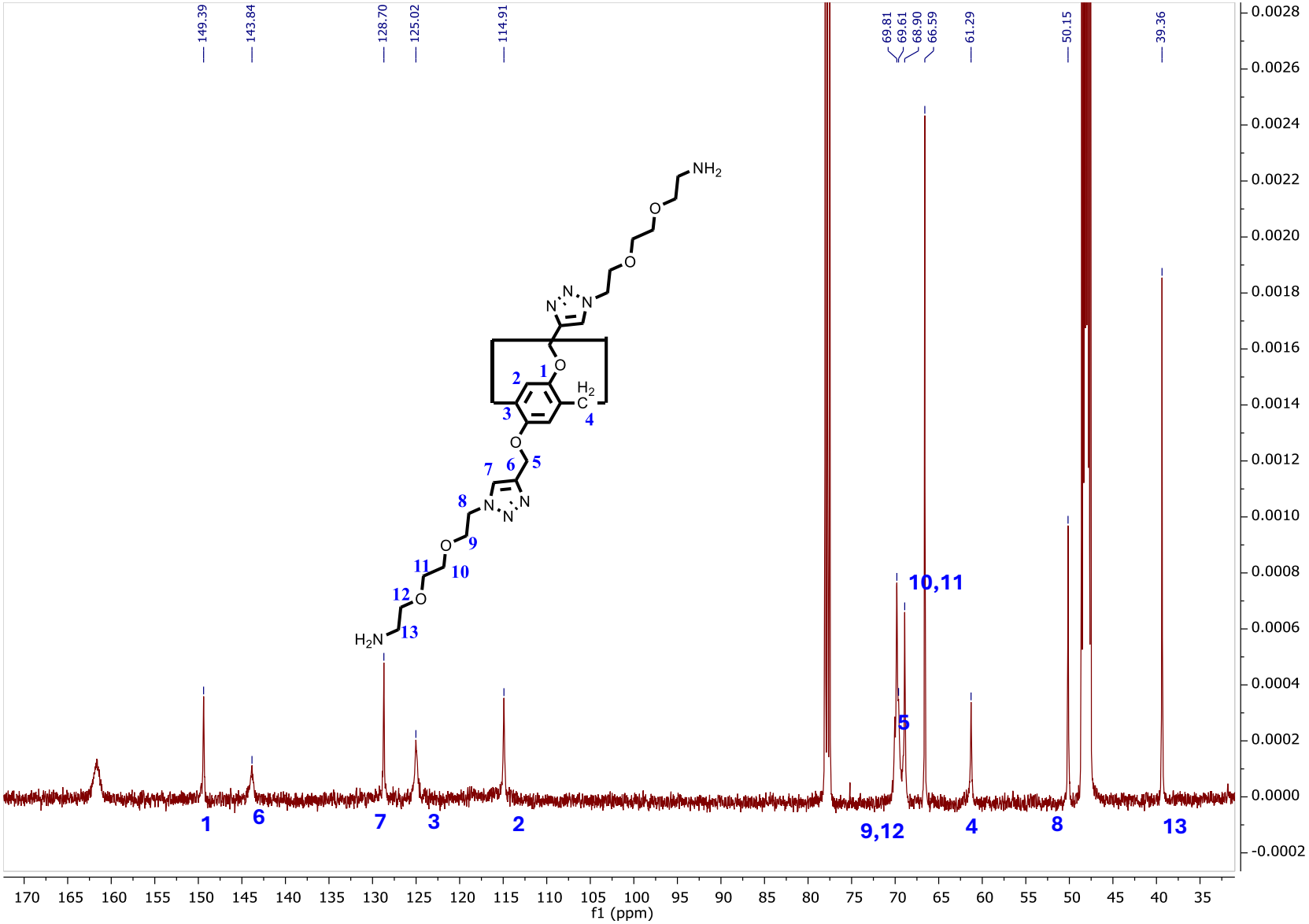

*Figure S11. ^{13}C NMR of **APA2** in $CDCl_3$ – CD_3OD mixture ($CDCl_3$:CD_3OD = 9:1).*

^{13}C NMR (126 MHz, $CDCl_3$ – CD_3OD mixture) δ 149.39 (C-1), 143.84 (C-6), 128.70 (C-7), 125.02 (C-3), 114.91 (C-2), 69.81(C-9), 69.61(C-5) 68.90(C-12), 66.59(C-10, C-11), 61.29 (C-4), 50.15(C-8), 39.36(C-13).

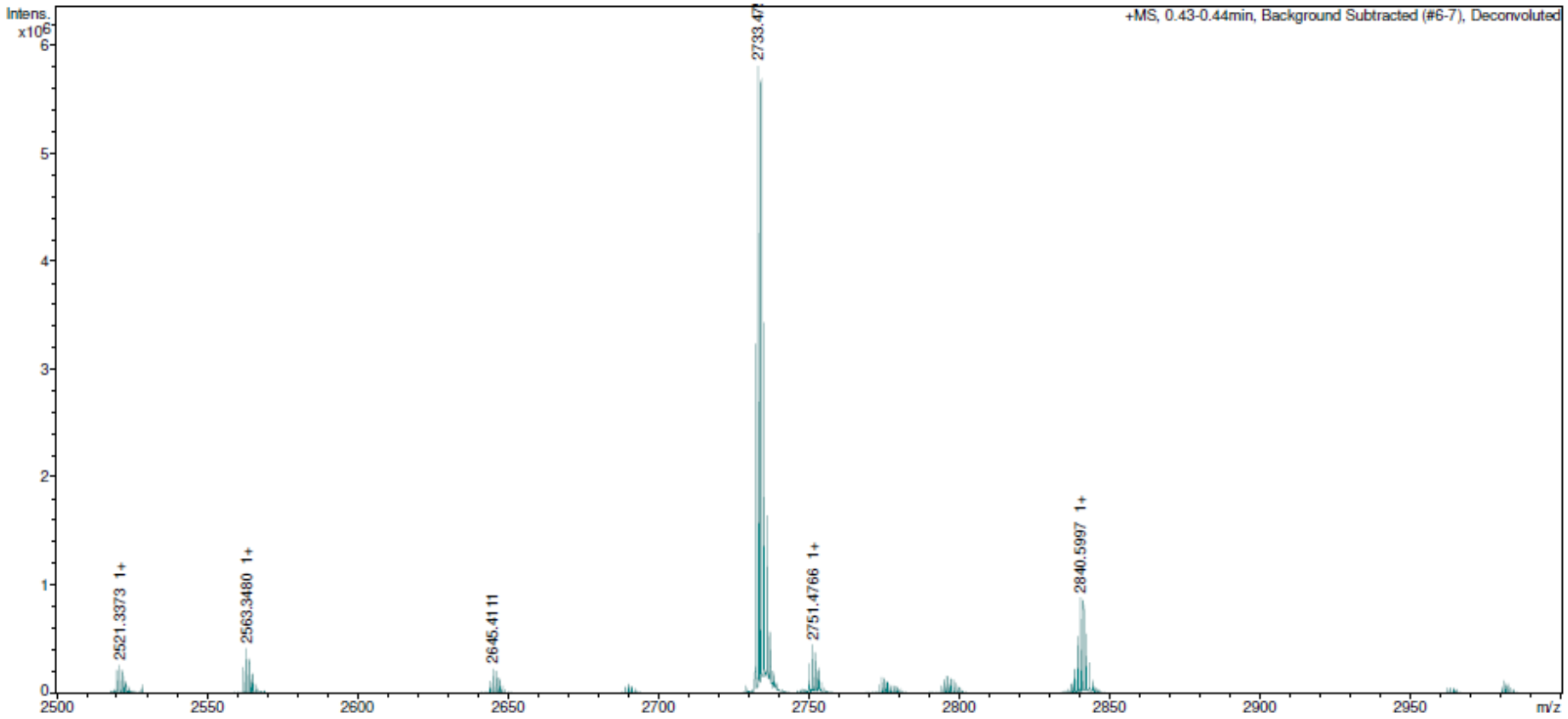

*Figure S12. HRMS of **APA2***

HRMS: $C_{125}H_{190}N_{40}O_{30}$ Exact Mass 2731.4572 (Calculated); 2733.4750 ($[M+H]^+$, Found)

Investigation of Host-Guest Interactions

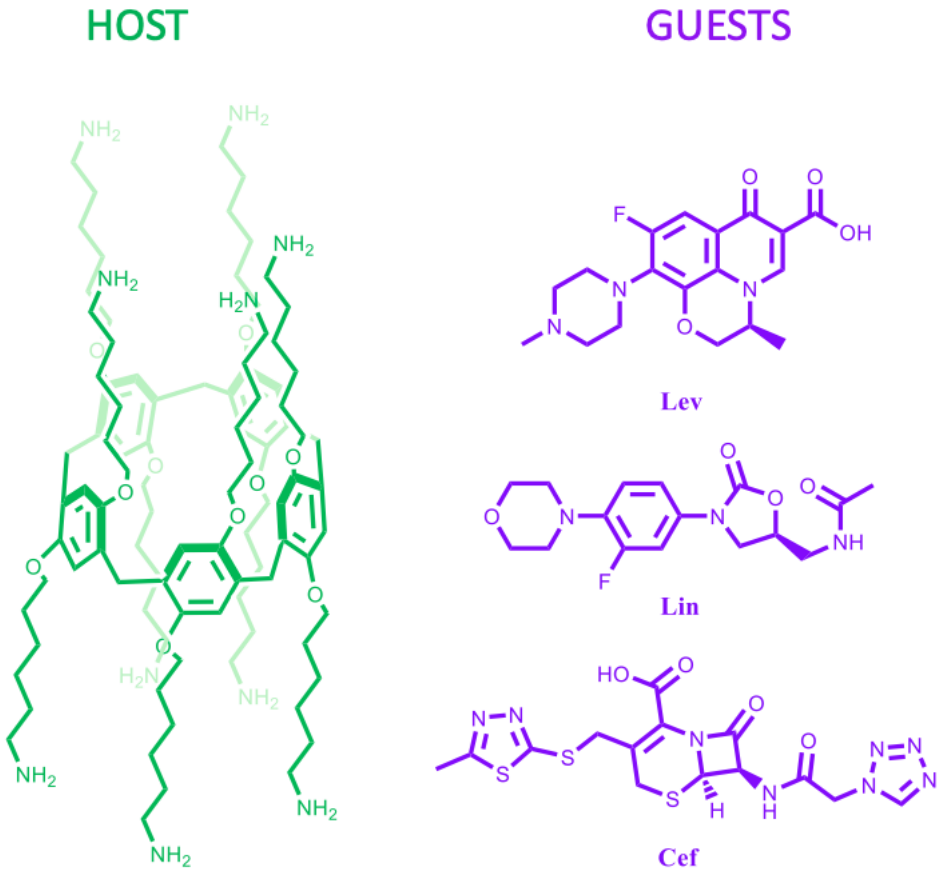

Figure S13. Chosen Compounds for Host-Guest Investigation

Preparation of solutions

A series of solutions with different ratios Host:Guest in DMSO-D_6 were prepared: 2:1, 1:1, 1:2 by molar ratios. The concentration of APA1 was fixed at 4 mM.

For the APA1-Lev complex, NMR titration was performed with a fixed concentration of APA1 at 1 mM. A series of complex solutions with concentrations of 0.125, 0.25, 1.00, 2.00, 4.00, and 8.00 mM Lev was prepared (See Table S1).

NMR analysis

^{1}H NMR spectroscopy was used to investigate Host-Guest interaction between Pillararene APA1 (Host) and Antibiotics Levoflaxacin Lev, Linezolid Lin and Cefazolin Cef (Guests). ^{1}H-NMR spectra were recorded on a JEOL ECX 400 or 500.

For the NMR titration of the APA1-Lev complex, a JEOL ECX 500 spectrometer was used. Due to the low concentration of the complex, the number of scans was increased to 256.

APA1-Lev Host-Guest Binding properties

The APA1-Lev complex was analyzed in detail using NMR titration to determine the association constant K_a. From the NMR spectra (Fig. S14), it was found that the most significant change in Proton shifts was for protons 1', 2', and 3', which are part of the piperazine ring of the Levofloxacin molecule. However, at low content of Lev in the APA1-Lev complex (guest equivalent less than 1), the protons 1' and 2' are hided by solvent peak of DMSO-D6 (2.5 ppm) and the residue of D_2O (~3.33 ppm), thus, it was decided to use the shifts of Lev Proton 3'. The experimental data for binding are presented in Table S1. To calculate the association constant, the observed chemical shifts (δ) of Proton 3' were plotted against the concentration of Levofloxacin (or guest/host equivalents). This titration data was then subjected to non-linear regression analysis, fitting it to a suitable binding isotherm equation (e.g., a 1:1 binding model)[4]. This fitting procedure enables the determination of the binding parameters, including the association constant K_a, which was found to be 1159 M^{-1}. This value quantifies the affinity between APA1 and Levofloxacin under the experimental conditions.

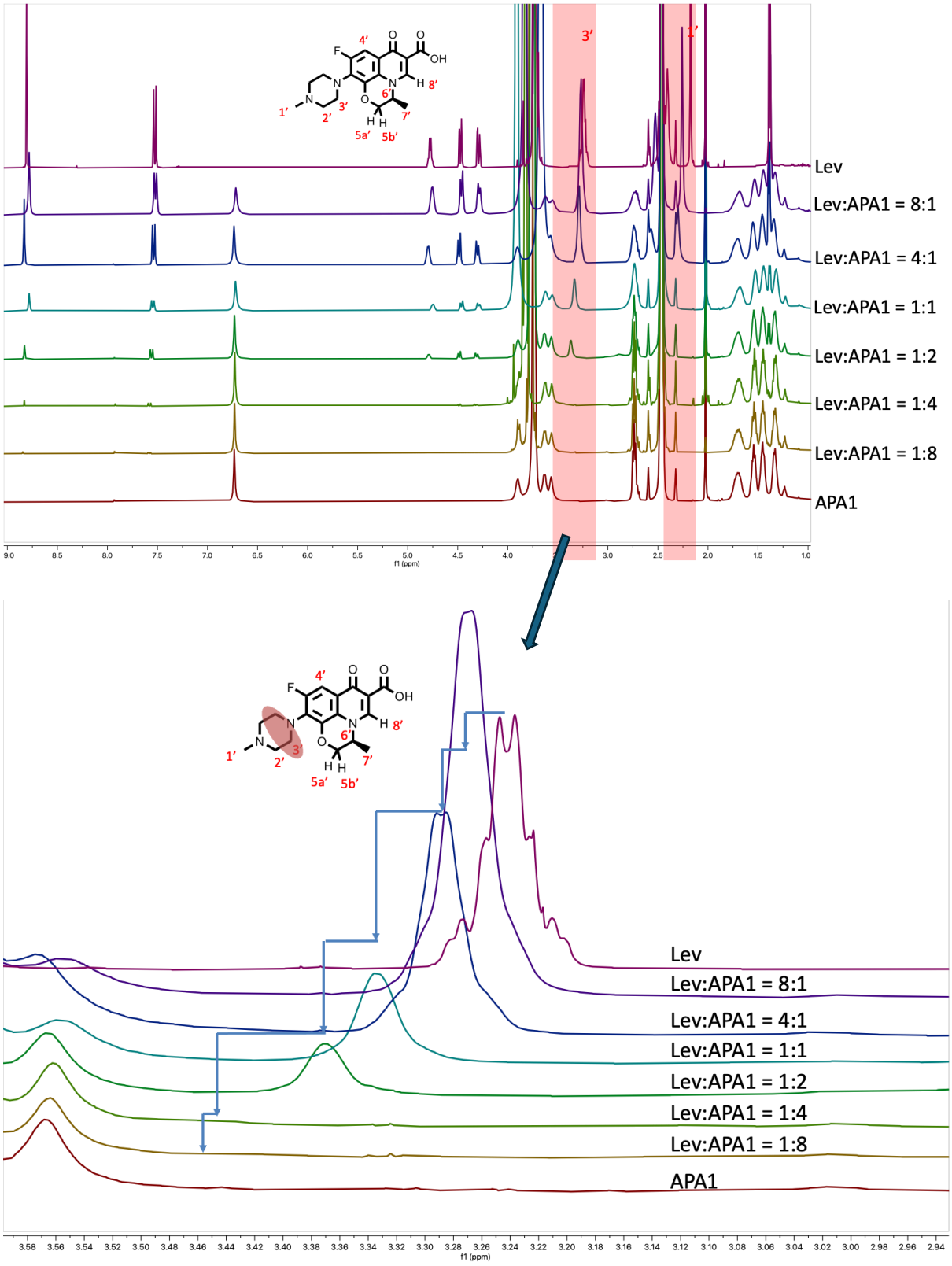

Figure S14. ^{1}H NMR titration for Pillararene:Lev complex in DMSO-D_6

Table S1. Shifts of Proton 1' of Levofloxacin during NMR titration.

G/H Ratio	C(APA1), M	C(Lev), M	δ, ppm	Δδ, ppm
0.125	0.001	0.000125	3.444	0.2058
0.25	0.001	0.00025	3.4439	0.2057
1.00	0.001	0.001	3.3702	0.132
2.00	0.001	0.002	3.3317	0.0935
4.00	0.001	0.004	3.2822	0.044
8.00	0.001	0.008	3.268	0.0298
-	-	0.008	3.2382	-

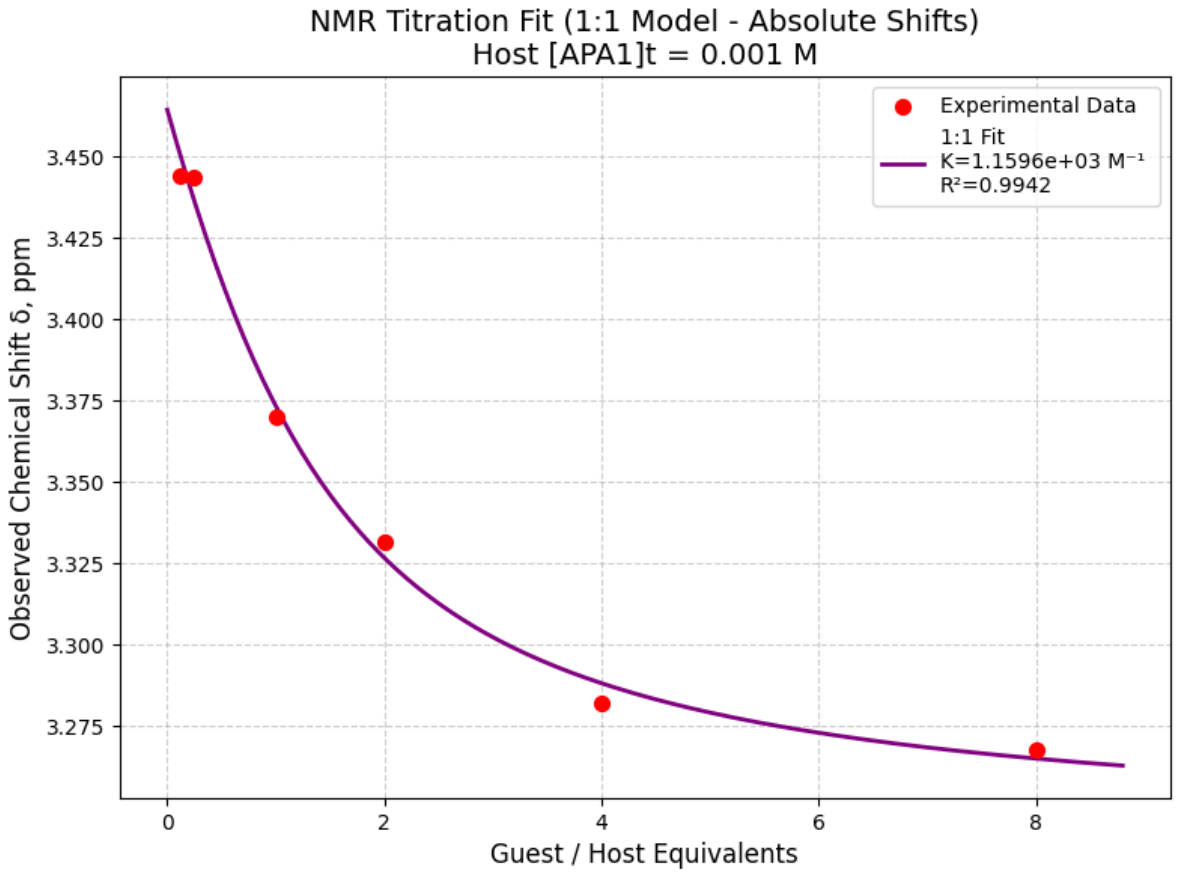

Figure S15. Fitting for NMR titration of Lev-APA1 complex using 1:1 binding model of non-linear regression and Nelder-Mead optimization algorithm

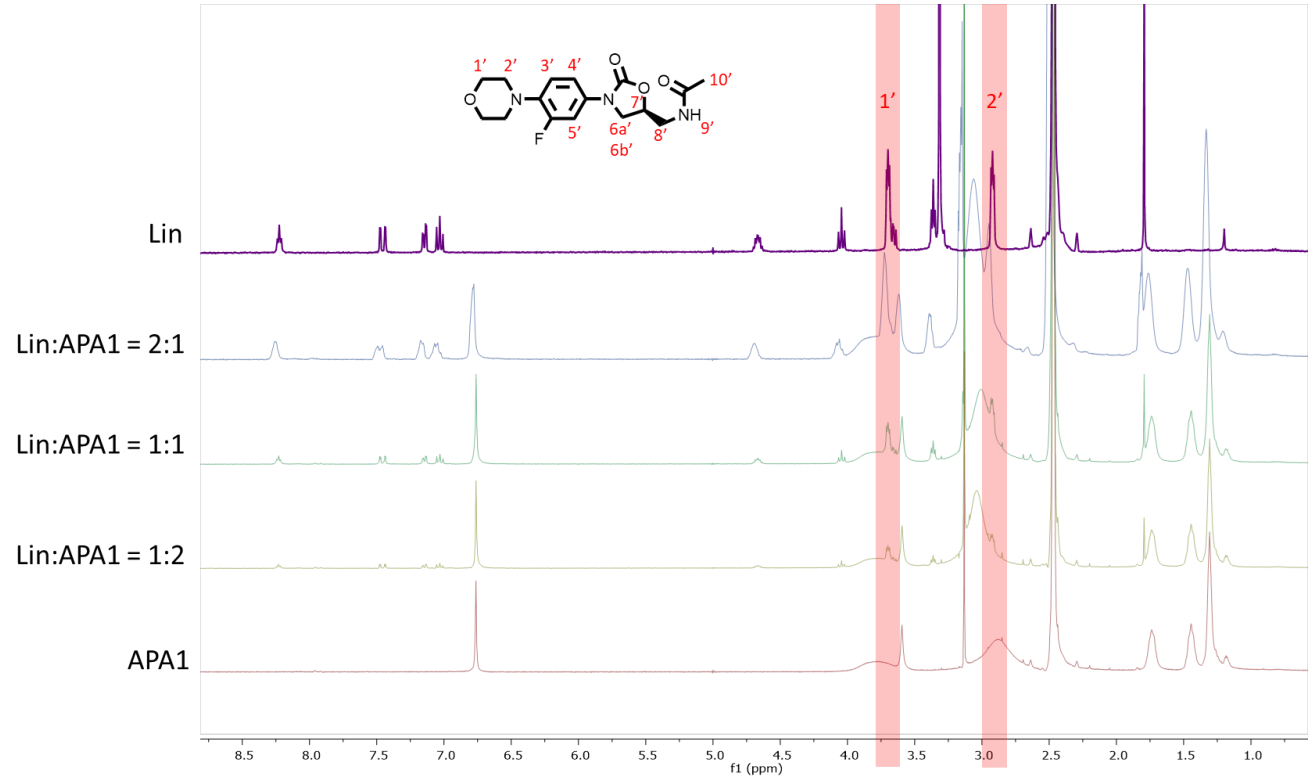

Figure S16. [1]H NMR spectras of Pillararene:Lin solutions with different ratios in DMSO-D_6

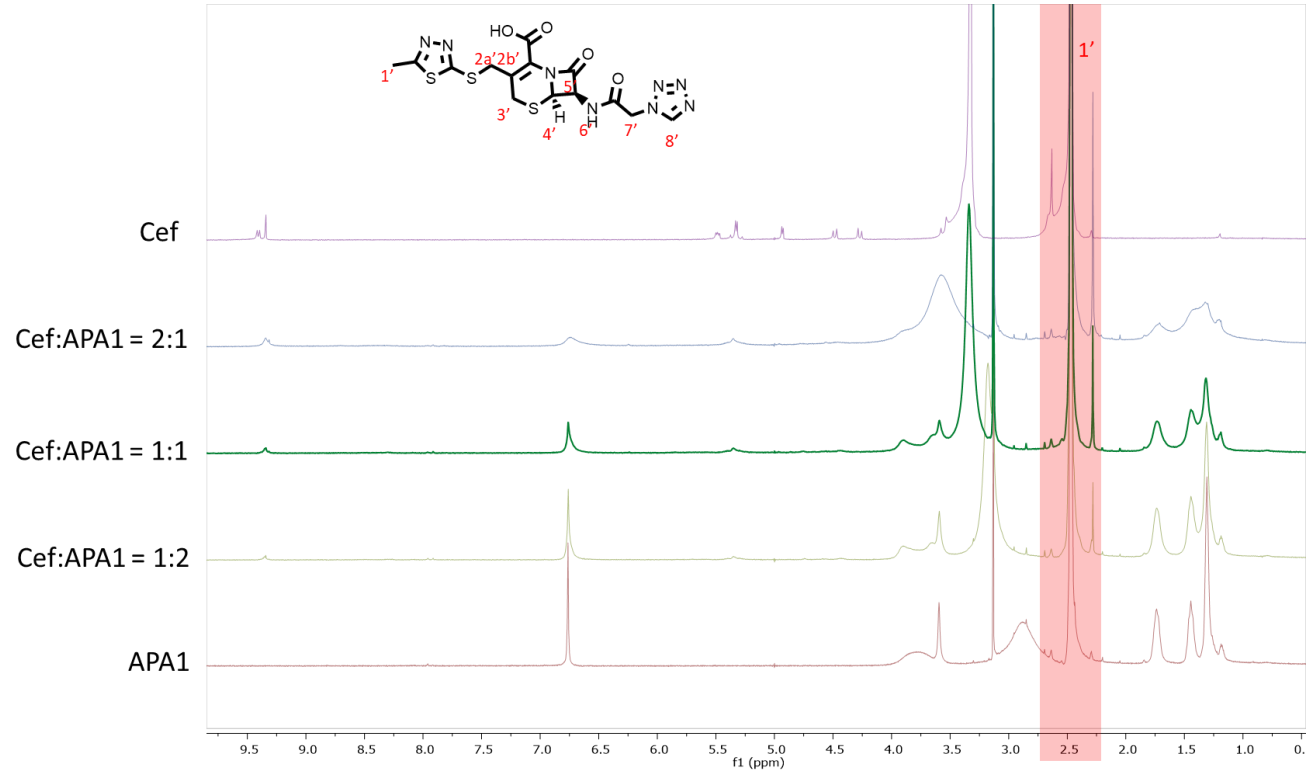

Figure S17. 1H NMR spectras of Pillararene:Cef solutions with different ratios

Material Characterization

DLS Analysis

80 μL solution of sample (DCF0, DCF with Functional Heads, Pillararene, Pillararene/Antibiotic, or their combinations) was mixed with 420 μL MilliQ water. This solution was introduced into a disposal microcuvette (d=10mm) and analyzed using DelsaTM Nano C Particle Analyzer with the following settings: Accumulation time 70s, scattering angle 165°, correlation method D, attenuator 1-100 %, pinhole 100 μm. The reported hydrodynamic diameter values are based on the intensity distribution data.

TEM

Transmission electron microscopy (TEM) images were obtained using a Philips Tecnai 10 microscope operating at 80 kV. Samples were deposited onto a copper grid as an aqueous solution and dried at room temperature.

AFM

The samples were imaged using a Bruker Nanoscope operated in tapping mode under ambient conditions. Silicon cantilever tips with a gold reflecting coating, a resonance frequency of 140–390 kHz, a force constant of 3.1–37.6 N m−1, and a tip curvature radius of 10 nm were used. Sample preparation: A 10-μL aliquot solution was deposited on freshly cleaved mica substrates and dried in air at room temperature prior to imaging.

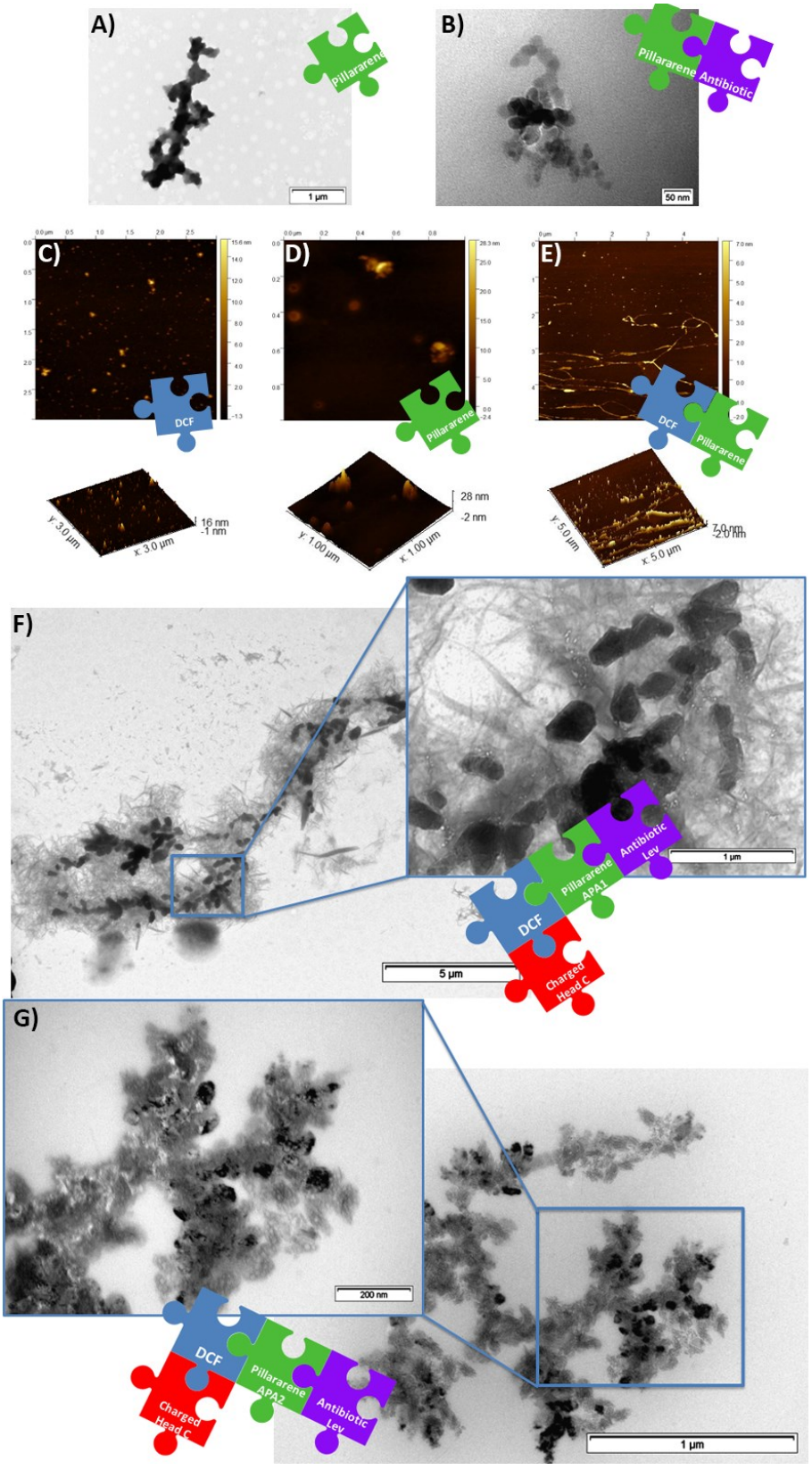

Figure S18. TEM and AFM images of dynamic combinations. A) and B) TEM images of Pillararene APA1 alone and Pillararene-Antibiotic complex APA1-Lev; C-E – AFM images of DCF0 alone, APA1 alone, and DCF-APA1 combination, respectively; F) TEM image of 4-component system DCF-C-APA1-Lev; G) TEM image of 4-component system DCF-C-APA2-Lev

Biological Evaluation

Screening of multicomponent systems for antimicrobial activity against A. baumannii

Sample solutions of individual components and multi-component systems were placed in a 96-well plate (50 µL each) and diluted with MilliQ water (1:2 dilution with 4% DMSO solution) to obtain seven concentrations. A bacterial culture of *A. baumannii* (150 µL) was then added. Bacterial growth monitoring was conducted at 37°C with periodic readings of optical density OD600 over 24 hours.

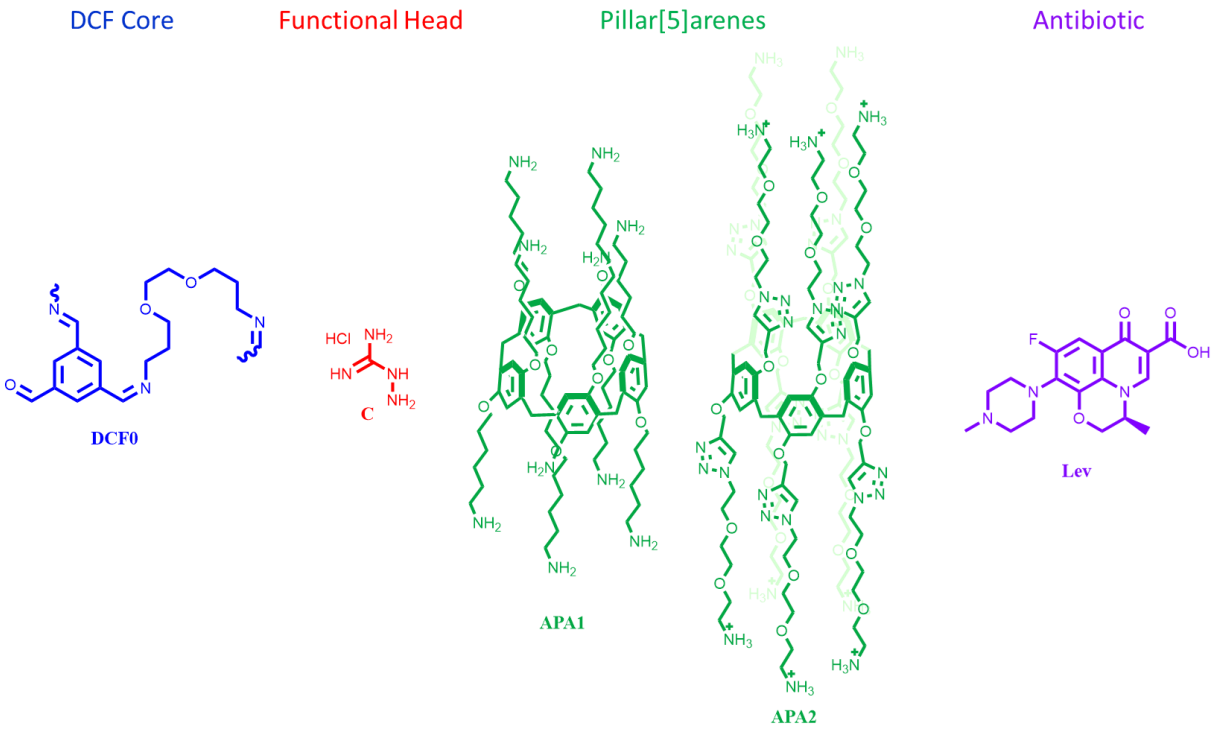

Figure S19 Selection of individual components/compounds for biological evaluations.

Minimum inhibitory concentration and Fractional inhibitory concentration index calculations

The minimum inhibitory concentration (MIC) was determined as the minimum concentration of an antimicrobial agent or its combination that inhibited bacterial growth during 24-hour monitoring time.

The fractional inhibitory concentration index (FICI) was calculated as the ratio of the MIC of the component in the complex system to the MIC of an individual substance. To determine the overall effect of the combination, the sum of FIC for each system was compared with literature data (Table S2).[5]

Table S2. Matching of the Sum of FICI and Combination effect [s5]

Σ FICI	Effect
< 0.5	SYNERGY
0.5-1.0	ADDITIVE
1.0-4.0	INDIFFERENCE
> 4.0	ANTAGONISM

Table S3. Minimal inhibition concentration (μg/mL and mM) of individual components.

Compound	MIC, μg/mL	MIC, mM
DCF0	500	3.12
B	3085	1.54
C	684	6.19
APA1	124	0.08
APA2	1199	0.44
Lev	56	0.15

Table S4. Minimal inhibition concentration MIC (μg/mL and mM) and Fractional inhibition concentration index FICI of combinations.

Combination		MIC (Comp 1), μg/mL	MIC (Comp 1), mM	MIC (Comp 2), μg/mL	MIC (Comp 2), mM	FICI (Comp 1)	FICI (Comp 2)	ΣFICI
DCF	**B**	250	1.54	3085	1.54	0.49	1.00	1.49
DCF	**C**	125	0.77	86	0.78	0.25	0.13	0.37
DCF	**APA1**	500	3.08	248	0.15	0.99	2.00	2.99
DCF	**APA2**	500	3.08	1199	0.44	0.99	1.00	1.99
APA1	**Lev**	31	0.02	14	0.04	0.25	0.25	0.50
APA2	**Lev**	248	0.09	56	0.15	0.21	1.00	1.21

Optimization of molar ratios for 2-component combinations

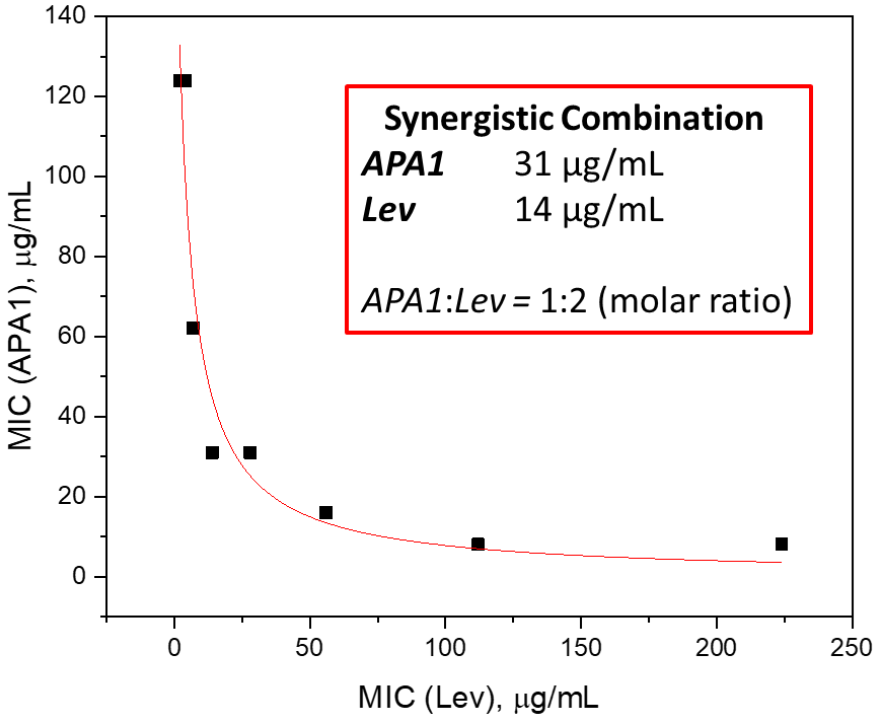

Figure S20. Molar ratio optimization of APA1-Lev combination by checkboard method

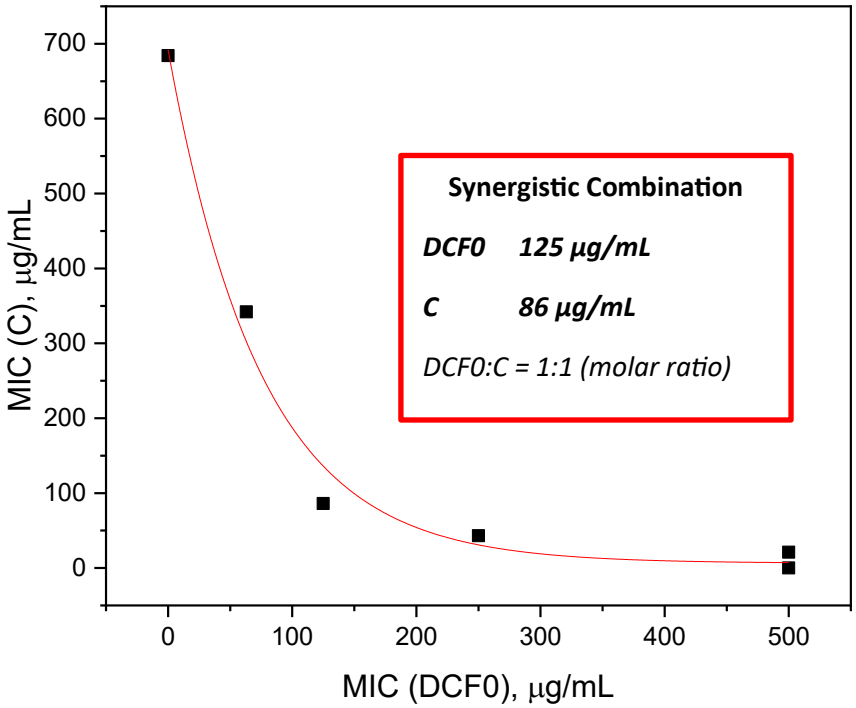

Figure S21. Molar ratio optimization for DCF-C combination by checkboard method

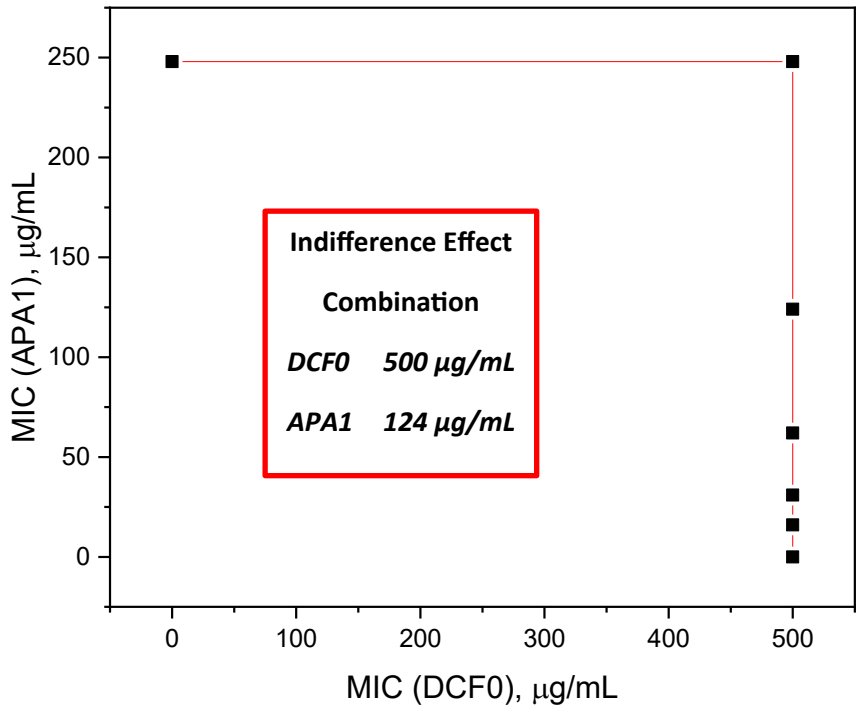

Figure S22. Molar ratio optimization for DCF-APA1 combination by checkboard method

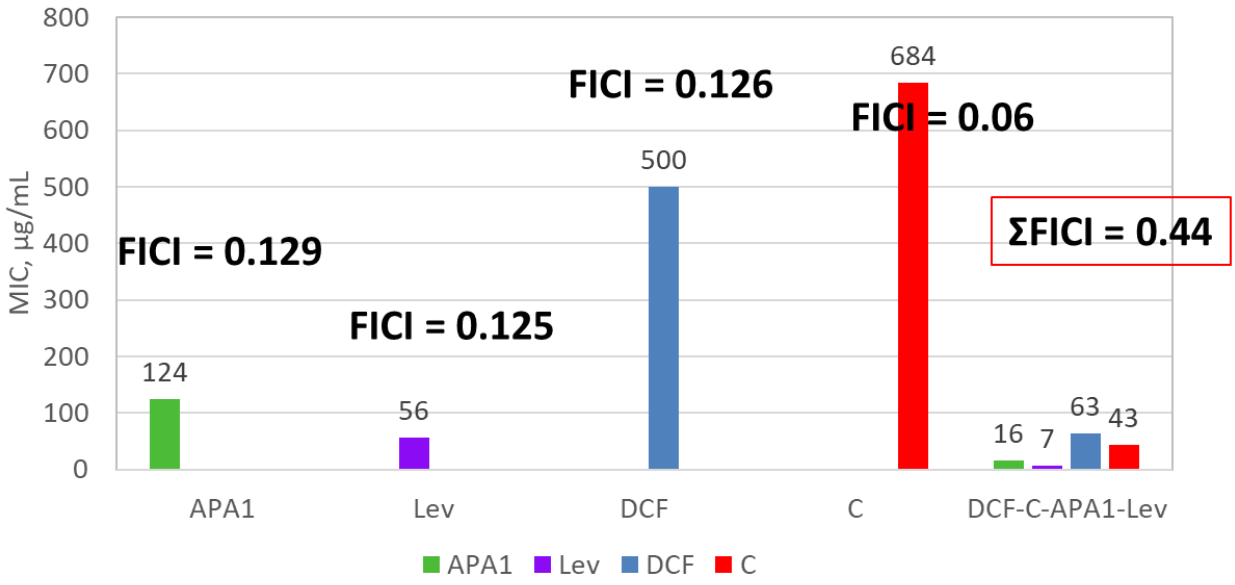

Figure S23. MIC and FICI of 4-component combination DCF-C-APA1-Lev

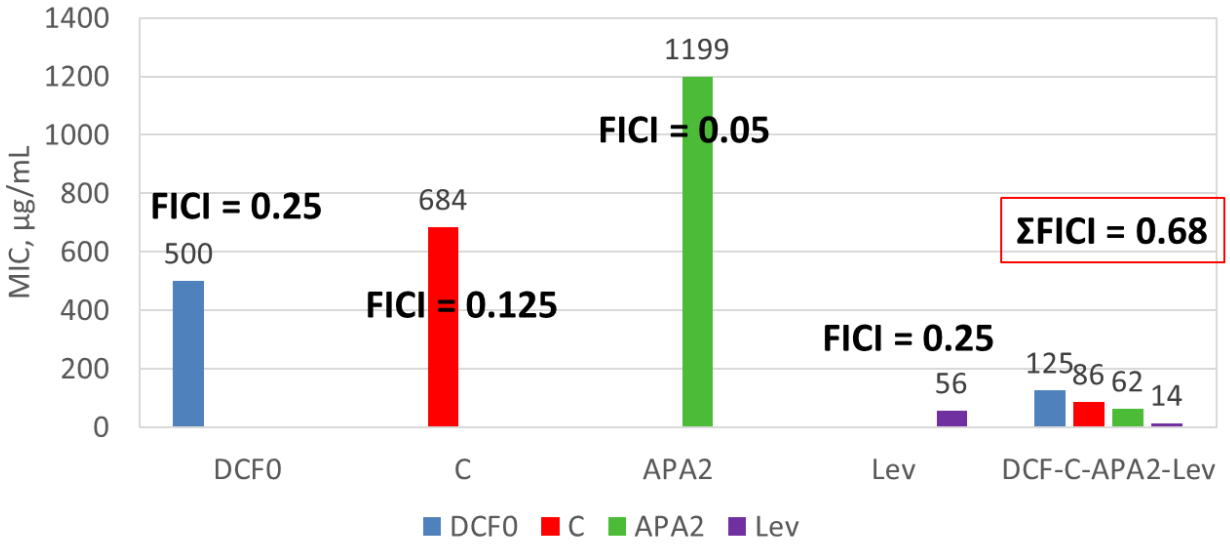

Figure S24. MIC and FICI of 4-component combination DCF-C-APA2-Lev

Biofilm Inhibition

To allow the initial adhesion, a diluted suspension of bacteria (*Acinetobacter baumannii* strain AB5075) was added to a 96-well plate for 4 h. The medium was removed, and a mixture of fresh medium (low salt LB) and compound was added. The 96-well plate was incubated for another 20 h. The medium with compounds was removed, and bacteria were detached to perform a CFU count.

Table S5. Results of antibiofilm assay for individual components and 2-component systems

Compound / Combination	**Concentration, µg/mL**	**CFU (n = 3)**	**σ**	**$\log_{10}$ (CFU)**	**$\Delta\log_{10}$ CFU**	**ΔCFU, %**
Control	-	4.05E+07	3.55E+06	7.61		
DCF0	500	2.05E+06	0.00E+00	6.31	-1.30	-94.93
C	344	1.24E+08	7.62E+07	8.09	0.48	+207
APA1	124	1.11E+08	6.21E+07	8.04	0.43	+174
APA2	248	8.18E+07	7.12E+05	7.91	0.30	+102
Lev	56	1.63E+06	5.83E+02	6.21	-1.40	-95.97
DCF-C	500/344	2.96E+04	3.58E+03	4.47	-3.14	-99.93
DCF-APA1	500/124	2.90E+03	3.69E+04	3.46	-4.15	-99.99
DCF-APA2	500/248	8.07E+04	1.64E+05	4.91	-2.70	-99.80
APA1-Lev	124/56	8.74E+05	4.95E+03	5.94	-1.67	-97.84
APA2-Lev	248/56	8.49E+03	4.17E+07	3.93	-3.68	-99.98

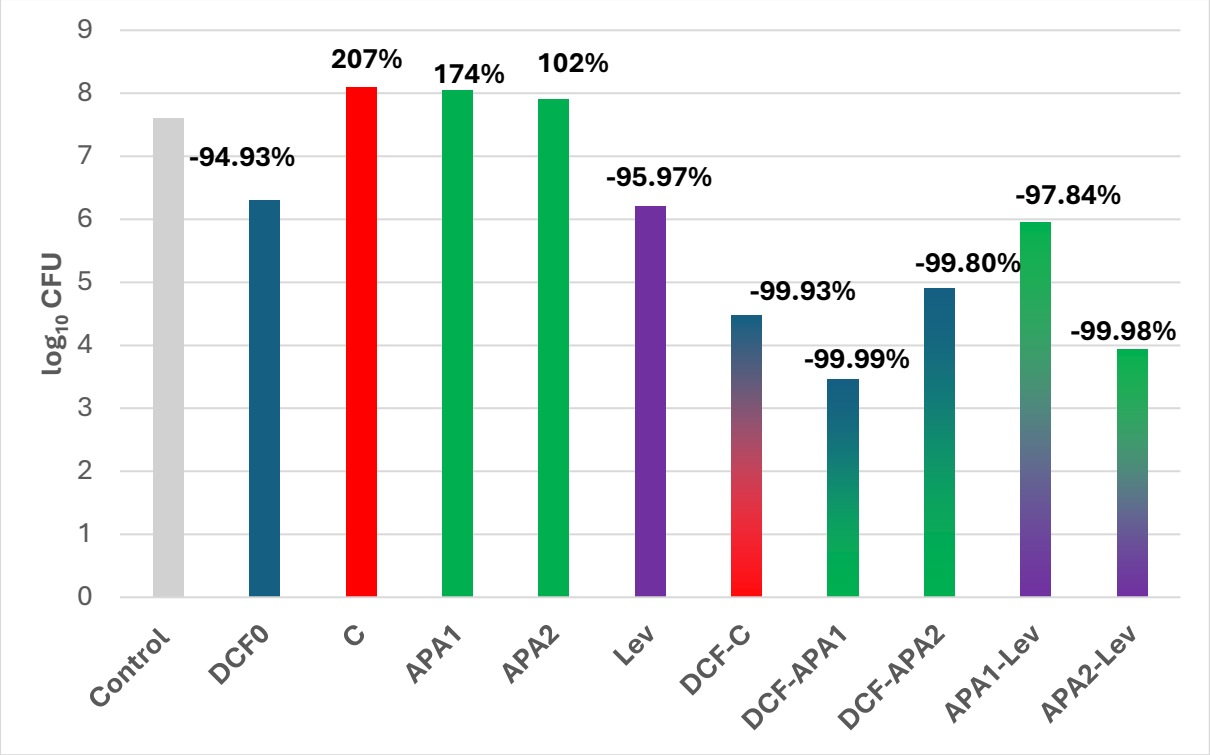

*Figure S25. CFU for individual compounds and their combinations. Color keys: Blue - Dynamic polymer **DCF0**; Red - Charged heads Aminoguanidine **C**; Green – Pillararene **APA1** and **APA2**; Purple – Antibiotic Levofloxacin **Lev**; 2-color gradients – combinations. The percentage represents a decrease in CFU compared to Control.*

Table S6. Results of antibiofilm assay for multi-component systems

Combination	CFU (n = 3)	σ	ΔCFU, %	log10 (CFU)	$\Delta\log_{10}$(CFU)
Untreated control	9.29E+07	2.92E+07	-	7.97	
DCF-C	2.44E+06	3.00E+06	-97.37%	6.39	-1.58
APA1-Lev	1.25E+06	3.86E+05	-98.66%	6.10	-1.87
APA2-Lev	6.48E+03	6.56E+03	-99.99%	3.81	-4.16
DCF-C-APA1-Lev	3.00E+04	5.75E+03	-99.97%	4.48	-3.49
DCF-C-APA2-Lev	5.92E+02	8.75E+02	-100.00%	2.77	-5.20

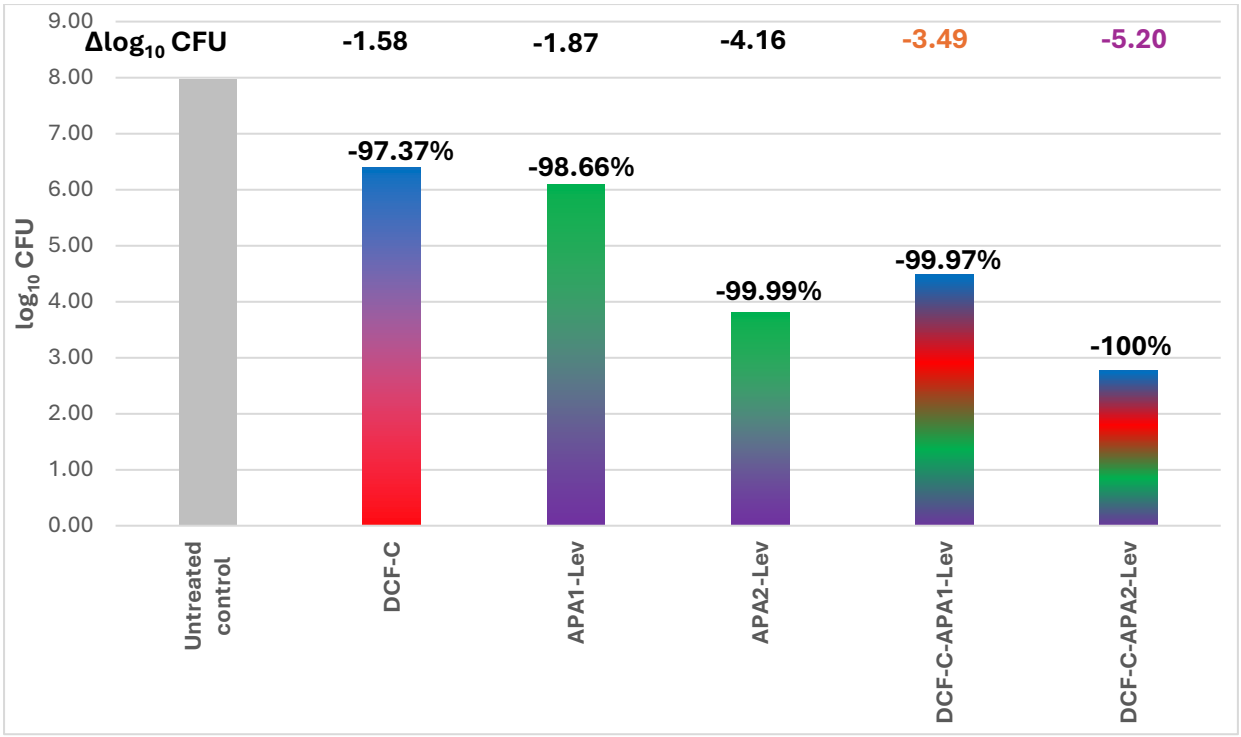

Figure S26. CFU for individual compounds and multicomponent combinations. Color keys: Blue - Dynamic polymer DCF0; Red - Charged heads, Aminoguanidine C; Green – Pillararene APA1 and APA2; Purple – Antibiotic Levofloxacin Lev. The percentage represents a decrease in CFU compared to Control.

References

1. R. Joseph, A. Asok and K. Joseph, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020, **224**, 117390.
2. S. Erbas-Cakmak and E. U. Akkaya, *Angew. Chem.*, 2013, **125**, 11574-11578.
3. I. Nierengarten, M. Nothisen, D. Sigwalt, T. Biellmann, M. Holler, J. S. Remy and J. F. Nierengarten, *Chemistry–A European Journal*, 2013, **19**, 17552-17558.
4. P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305-1323.
5. J. Meletiadis, S. Pournaras, E. Roilides and T. J. Walsh, *Antimicrob. Agents Chemother.*, 2010, **54**, 602-609.