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Photocleavable Antimicrobial Peptide Mimics for Precluding Antibiotic Resistance

Yang Feng,[ab] Yang-Yang Zhang,[ab] Ke Li,[ab] Na Tian,[ab] Wei-Bo Wang,[a] Qian-Xiong Zhou,*[a] and Xue-Song Wang*[a]

[a] *Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China. E-mail: xswang@mail.ipc.ac.cn, zhouqianxiong@mail.ipc.ac.cn*

[b] *University of Chinese Academy of Sciences, Beijing 100049, P. R. China.*

Experimental Section

Material. 4-(Bromomethyl)-3-nitrobenzoic acid, 1-butanol, 1-heptanol, 1-decanol, 1-tridecanol, 1-hexadecanol, *N,N'*-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), di-*tert*-butyl dicarbonate (Boc_2O), L-lysine monohydrochloride, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), trifluoroacetic acid were purchased from Acros and Alfa Aesar, and used without further purification, unless otherwise mentioned. Dulbecco's modification of Eagle's medium (DMEM), penicillin, streptomycin, fetal bovine serum, and 10 mM PBS (pH = 7.4) were purchased from Corning.

Instruments. ^{1}H NMR spectra were recorded on a Bruker DMX-400 MHz spectrophotometer, using $SiMe_4$ as the standard. High-resolution ESI mass spectrometry (HR ESI-MS) was determined on a Bruker APEX IV (7.0T) FT_MS. UV-vis absorption spectra were taken on a Shimadzu UV-1601PC spectrophotometer.

Synthesis. Boc-protected lysine (Lys(Boc)-OH) was synthesized following a reported method.[1] A_n, B_n, and C_n (n denotes the carbon number of the alkyl chain) were also prepared following a published method.[2]

Synthesis of Boc-Lys(Boc)-OH. L-Lysine hydrochloride (5 g, 27.3 mmol) was dissolved in H_2O (50 mL), and to it $NaHCO_3$ (6.9 g, 82.1 mmol) was added and stirred. Then Boc_2O (7.16 g, 65.5 mmol) in 50 mL of THF was added at 0 °C. The reaction mixture was stirred at room temperature for 12 h. After that, another aliquot of Boc_2O (7.16 g, 65.5 mmol) was added at 0 °C and the mixture was stirred for additional 12 h at room temperature. THF was removed under reduced pressure and the aqueous layer was washed with diethyl ether three times. Then the aqueous layer was acidified to pH 4- 5 using citric acid solution. The aqueous layer was extracted with CH_2Cl_2. The organic layer was then washed with brine and dried over anhydrous Na_2SO_4. After removal of organic solvent under reduced pressure, Boc-Lys(Boc)-OH was obtained in 90% yield.

Synthesis of A_n. DCC (396 mg, 1.92 mmol) in CH_2Cl_2 (2.5 mL) was added to a mixture of 3-nitro-4-(bromomethyl) benzoic acid (500 mg, 1.92 mmol), alkyl alcohol (1.93 mmol), and DMAP (20 mg, cat.) in CH_2Cl_2 (10 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 1 h at 0 °C and 24 h at room temperature. The precipitate was separated by filtration and the crude product was recovered by evaporation of the solvent. The product was further purified by silica gel column chromatography (hexane/EtOAc, 80/10) to yield a yellow oily liquid or a pale yellow solid (80%-90%).

A_4: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.65 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 4.38 (t, *J* = 6.6 Hz, 2H), 1.77 (dd, *J* = 14.4, 7.1 Hz, 2H), 1.48 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H). GC/HR-TOF MS: m/z calcd 315.0106 for $C_{12}H_{14}NO_4Br$, found 315.0104.

A_7: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.65 (d, *J* = 1.5 Hz, 1H), 8.25 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 4.37 (t, *J* = 6.7 Hz, 2H), 1.85-1.72 (m, 2H), 1.62-1.20 (m, 8H), 0.90 (t, *J* = 6.8 Hz, 3H). GC/HR-TOF MS: m/z calcd 357.0576 for $C_{15}H_{20}NO_4Br$, found 357.0580.
A_{10}: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.65 (d, *J* = 1.3 Hz, 1H), 8.24 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 4.37 (t, *J* = 6.7 Hz, 2H), 1.87- 1.68 (m, 2H), 1.65-1.14 (m, 14H), 0.88 (t, *J* = 6.7 Hz, 3H). GC/HR-TOF MS: m/z calcd 399.1045 for $C_{18}H_{26}NO_4Br$, found 399.1042.
A_{13}: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.65 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 4.38 (t, *J* = 6.6 Hz, 2H), 1.86-1.72 (m, 2H), 1.68-1.14 (m, 20H), 0.88 (t, *J* = 6.7 Hz, 3H). GC/HR-TOF MS: m/z calcd 441.1515 for $C_{21}H_{32}NO_4Br$, found 441.1520.
A_{16}: ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.66 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 4.37 (t, *J* = 6.7 Hz, 2H), 1.82-1.73 (m, 2H), 1.70-1.11 (m, 26H), 0.88 (t, *J* = 6.7 Hz, 3H). GC/HR-TOF MS: m/z calcd 483.1984 for $C_{24}H_{38}NO_4Br$, found 483.1943.

Synthesis of B_n. In a nitrogen atmosphere, DBU (66 mg, 0.43 mmol) in CH_3CN (5 mL) was added dropwise to a mixture of A_n (0.28 mmol) and Boc-Lys(Boc)-OH (135 mg, 0.39 mmol) in CH_3CN (10 mL). The reaction was heated to 50 °C for 15 h. The solvent was then evaporated and the crude product was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 90/10) to yield a yellow oily liquid or a pale yellow solid (70%-80%).
B_4: ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.60 (s, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 5.52 (s, 2H), 4.32 (t, *J* = 6.6 Hz, 2H), 4.12 (dd, *J* = 8.8, 5.0 Hz, 1H), 2.96 (dd, *J* = 11.7, 5.9 Hz, 2H), 1.77-1.19 (m, 28H), 0.94 (t, *J* = 7.4 Hz, 3H). HR ESI-MS: m/z calcd 581.2948 for $C_{28}H_{43}N_3O_{10}$, found 581.2924.
B_7: ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.56 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 5.49 (s,2H), 4.28 (t, *J* = 6.5 Hz, 2H), 4.08 (dd, *J* = 8.8, 4.9 Hz, 1H), 2.92 (dd, *J* = 12.0, 6.0 Hz, 1H), 1.80-1.13 (m, 34H), 0.79 (t, *J* = 6.3 Hz, 3H). HR ESI-MS: m/z calcd 623.3418 for $C_{31}H_{49}N_3O_{10}$, found 623.3413.
B_{10}: ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.56 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 5.49 (s, 2H), 4.28 (t, *J* = 6.5 Hz, 2H), 4.09 (dd, *J* = 8.8, 4.9 Hz, 1H), 2.94 (t, *J* = 6.1 Hz, 2H), 1.90-1.16 (m, 40H), 0.81 (t, *J* = 6.3 Hz,3H). HR ESI-MS: m/z calcd 665.3888 for $C_{34}H_{55}N_3O_{10}$, found 665.3887.
B_{13}: ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.66 (s, 1H), 8.30 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 5.50 (s, 2H), 4.39-4.25 (m, 2H), 4.12 (dd, *J* = 8.9, 4.9 Hz, 1H), 3.05 (t, *J* = 6.1 Hz, 2H), 1.84-1.12 (m, 46H), 0.89 (t, *J* = 6.7 Hz, 3H). HR ESI-MS: m/z calcd 707.4357 for $C_{37}H_{61}N_3O_{10}$, found 707.4360.
B_{16}: ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.60 (s, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 5.52 (s, 2H), 4.30 (t, *J* = 6.2 Hz, 2H), 4.12 (d, *J* = 4.4 Hz, 1H), 2.96 (dd, *J* = 11.6, 6.0 Hz, 2H), 1.78-1.17 (m, 52H), 0.82 (t, *J* = 6.7 Hz, 3H). HR ESI-MS: m/z calcd 749.4826 for $C_{40}H_{67}N_3O_{10}$, found 749.4806.

Synthesis of C_n. Typically, B_n was dissolved in CH_2Cl_2 and subsequently CF_3COOH (50% by volume) was added and stirred at RT. The reactions were monitored by TLC until complete removal of starting material was observed. The solvent was then evaporated to yield a yellow oily liquid (95%).

C_4: ^{1}H NMR (400 MHz, MeOD), δ (ppm): 8.67 (d, *J* = 1.6 Hz, 1H), 8.35 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 5.70 (q, *J* = 14.2 Hz, 2H), 4.40 (t, *J* = 6.6 Hz, 2H), 4.20 (t, *J* = 6.3 Hz, 1H), 2.97-2.93 (m, 2H), 2.02-1.30 (m, 10H), 1.01 (t, *J* = 7.4 Hz, 3H). HR ESI-MS: m/z calcd 382.1956 for $C_{18}H_{28}N_3O_6^+$, found 382.1962.

C_7: ^{1}H NMR (400 MHz, MeOD), δ (ppm): 8.68 (d, *J* = 1.6 Hz, 1H), 8.35 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 5.70 (q, *J* = 14.3 Hz, 2H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.21 (d, *J* = 6.3 Hz, 1H), 2.97-2.93 (m, 2H), 1.83-1.30 (m, 16H), 0.89 (t, *J* = 6.9 Hz, 3H). HR ESI-MS: m/z calcd 424.2435 for $C_{21}H_{34}N_3O_6^+$, found 424.2425.

C_{10}: ^{1}H NMR (400 MHz, MeOD), δ (ppm): 8.68 (d, *J* = 1.6 Hz, 1H), 8.35 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 5.70 (q, *J* = 14.3 Hz, 2H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.20 (t, *J* = 6.3 Hz, 1H), 2.97-2.93 (m, 2H), 2.02-1.30 (m, 22H), 0.89 (t, *J* = 6.9 Hz, 3H). HR ESI-MS: m/z calcd 466.2904 for $C_{24}H_{40}N_3O_6^+$, found 466.2898.

C_{13}: ^{1}H NMR (400 MHz, MeOD)，δ (ppm): 8.68 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 5.70 (q, *J* = 14.3 Hz, 2H), 4.41 (t, *J* = 6.4 Hz, 2H), 4.20 (t, *J* = 6.3 Hz, 1H), 2.98-2.91 (m, 2H), 2.09-1.21 (m, 28H), 0.88 (t, *J* = 6.9 Hz, 3H). HR ESI-MS: m/z calcd 508.3374for $C_{27}H_{46}N_3O_6^+$, found 508.3363.

C_{16}: ^{1}H NMR (400 MHz, MeOD), δ (ppm): 8.68 (d, *J* = 1.6 Hz, 1H), 8.35 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 1H), 5.70 (q, *J* = 14.2 Hz, 2H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.20 (t, *J* = 6.3 Hz, 1H), 2.97-2.93 (m, 2H), 2.02-1.29 (m, 34H), 0.90 (t, *J* = 6.8 Hz, 3H). HR ESI-MS: m/z calcd 550.3843 for $C_{30}H_{52}N_3O_6^+$, found 550.3832.

Photodegradation of C_{10}. A C_{10} (17.33 μg) solution in 2.5 mL of methanol (100 μM) in a quartz cuvet was irradiated at 365 nm (LED, 11.38 mW/cm^2). At each 5-min interval, the cuvet was transferred to a UV-vis spectrophotometer to measure its absorption spectra to follow the photoreaction. Alternatively, a CD_3OD solution of C_{10} (13.1 mM) in an NMR tube was irradiated at 365 nm (LED, 11.38 mW/cm^2). After irradiation for a period of time, the ^{1}H NMR spectra were compared. Additionally, an aqueous solution of C_{10} (2×10^7 μg/L) was irradiated using a solar simulator with Xenon arc lamp (Newport SP94043A-SR3, 103.4 mW/cm^2) as the light source, and TLC was used to monitor the photoreaction.

Bacterial Growth. Gram-(+) *S. aureus* and *B. subtilis* and Gram-(−) *E. coli* and *P. aeruginosa*, provided by Antibacterial Center of Technical Institute of Physics and Chemistry and China General Microbiological Culture Collection Center, were revived with Luria–Bertani (LB) broth and nutrient agar at 37 °C for 24 hours. The OD_{600} value, the optical density at 600 nm, was monitored to determine the density of bacterial cells.

Antimicrobial Experiments. Bacteria in the exponential growth phase were washed twice with PBS and re-suspended in the media. The antimicrobial properties of C_n

were determined by incubation with bacterial cells (~ 10^8 cells/mL) for 60 min in the dark at 37 °C. And the treated bacterial samples were diluted in PBS and were spread on 3 M Petrifilm Aerobic Count Plate and incubated at 37 °C for 24-48 h. The number of colony-forming units (CFU) was counted by a Shineso G6 Colony Counter. The photodegraded C_{10}, A_n and lysine hydrochloride were also tested under the same condition.

Cell Culture. Human pulmonary carcinoma A549 cells, human uterine carcinoma HeLa cells, human ovary carcinoma SKOV3 cells were cultured with the growth medium (DMEM supplemented with 10% fetal bovine serum (FBS), 100 unit per mL penicillin, 100 μg mL^{-1} streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2. C_n stock solutions in DMSO (5.0 mM) were diluted in the growth medium to the final working concentrations.

MTT Assay. MTT assay was utilized to analyze cell viability of A549, HeLa, and SKOV3 cells in varied conditions. Each cell was plated at 2 × 10^5 per well in a Nunc 96 well plate and allowed to grow for 24 h. The cells were exposed to increasing concentrations (1-100 μM) of C_n and incubated for 4 h at 37 °C. After 20 h of cell incubation, the loading medium was removed and the cells were fed with medium containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The cell viability data were obtained by analysis of the absorbance at 490 nm of each sample using a Thermo MK3 Multiscan microplate reader. The signal was normalized to 100% viable (untreated) cells.

Membrane Integrity Observations. Field emission scanning electronic microscopy (FESEM) was used to evaluate the morphology of bacterial cells before and after treatment by C_{10}. A 2 mL of *E. coli* cells in the exponential phase was harvested, washed twice with PBS (pH 7.4), and 1 mL of which were treated with C_{10} at 25 μM for 4 h at 37 °C. The other 1 mL sample was left untreated as a control. After incubation, the cell pellets were harvested, washed with PBS twice, subjected to fixation with 2.5% glutaraldehyde for 4 h at room temperature, and then fixed with 1% Osmic acid for 2 h after washed with PBS twice again. The cells were dehydrated for 15 min in a graded ethanol series (30%, 50%, 70%, and 90%) followed by 15 min in 100% ethanol twice, and 1 h in isoamyl acetate. Finally, the specimens were dehydrated, dried, coated with gold, and examined using QUANTA FEG 250 FESEM.

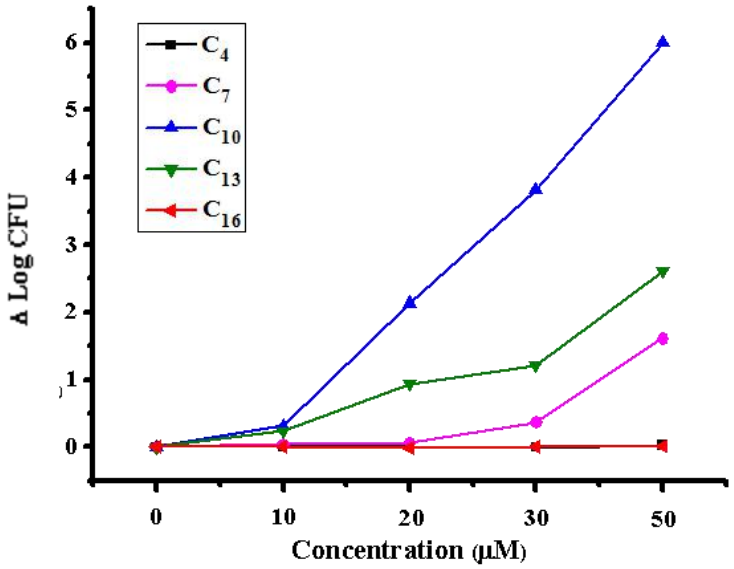

Figure S1. Antibacterial activity of C_n against *E. coli* (n = 4, 7, 10, 13, and 16).

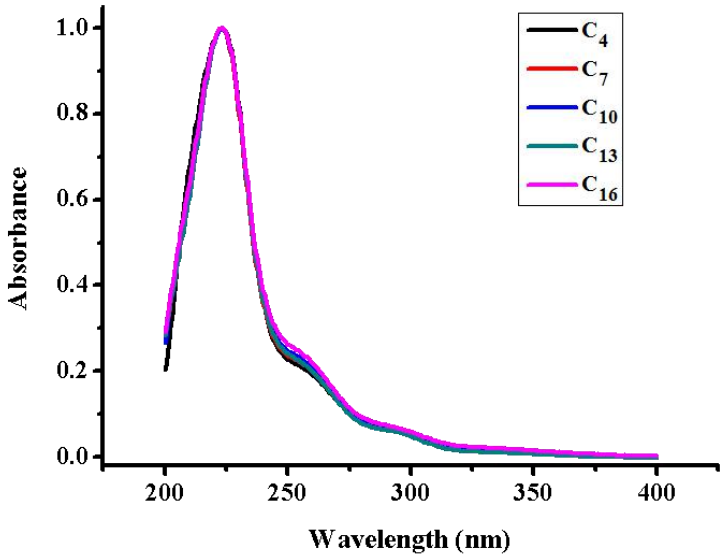

Figure S2. Absorption spectra of C_n (n = 4, 7, 10, 13, and 16).

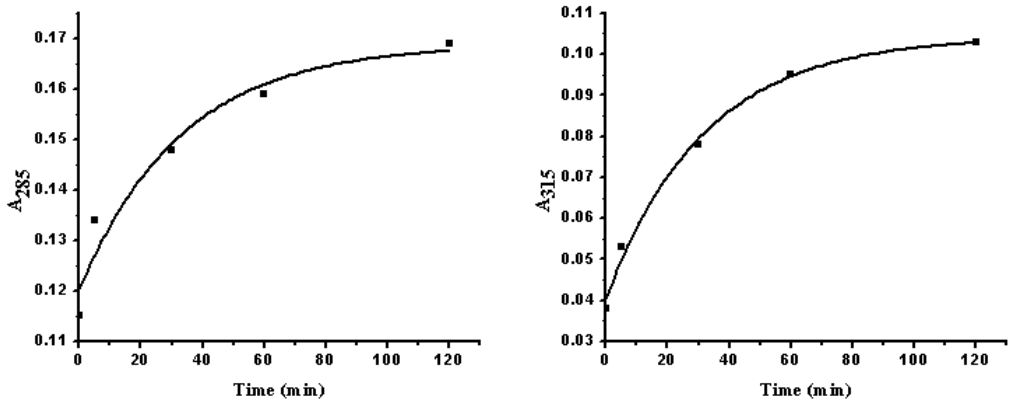

Figure S3. Absorbance changes of C_{10} at 285 nm (A) and 315 nm (B). The solid lines are single exponential fit of the data with rate constants of 0.030 and 0.032 min^{-1} at 285 nm and 315 nm, respectively.

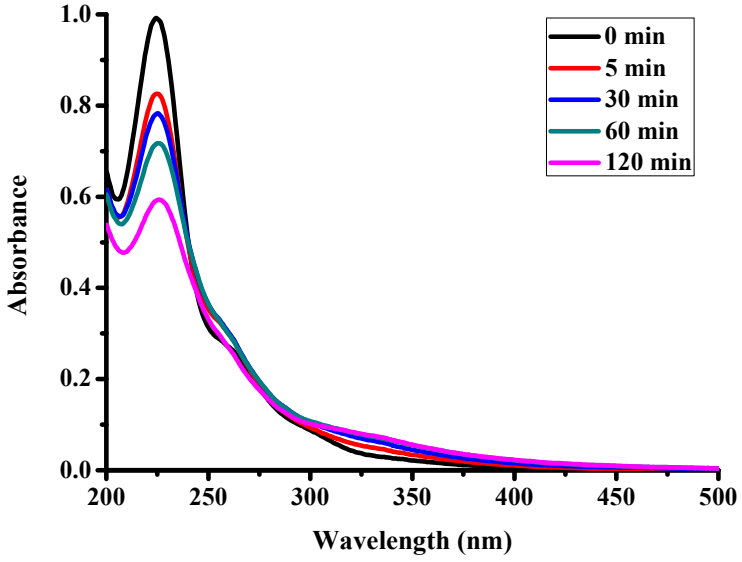

Figure S4. Absorption spectra changes of C_{10} (50 µM) in aqueous solution upon irradiation at 365 nm.

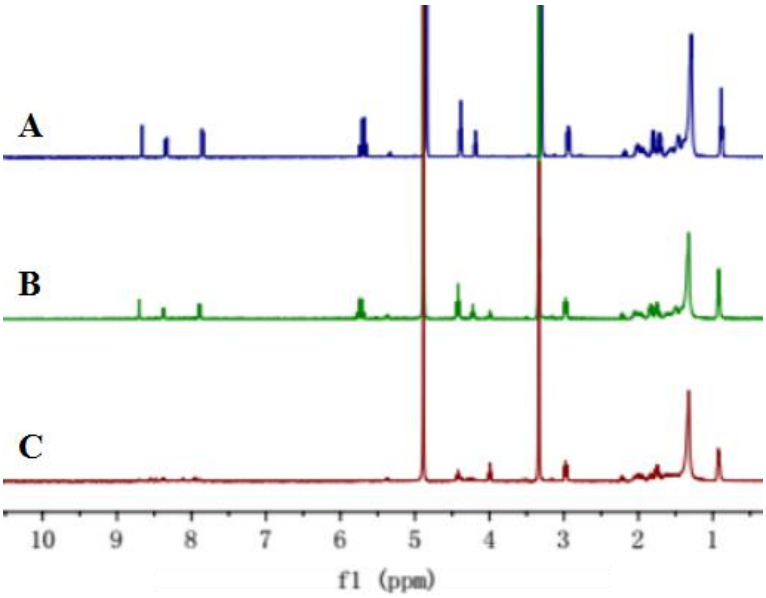

Figure S5. ^{1}H NMR spectra of 13.1 mM C_{10} in methanol-d^4 before (A) and after irradiation at 365 nm for 30 min (B) and 300 min (C).

Figure S6. TLC image of C_7 and C_{10} (14.4 mM, aqueous solution) before and after 1 h of irradiation under simulated sunlight.

Table S1. IC_{50} values of C_n toward A549, HeLa, and SKOV3.

Amphiphiles	IC_{50} (μM)		
	A549	HeLa	SKOV3
C_7	54.6	59.2	64.3
C_{10}	54.8	56.6	78.1
C_{13}	50.9	74.1	60.2

References

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[2] P. Anilkumar, E. Gravel , I. Theodorou , K. Gombert , B. Thézé , F. Ducongé and E. Doris, *Adv. Funct. Mater.*, 2014, **24**, 5246.