Electronic Supplementary Information for

Molecularly Pure Miktoarm Spherical Nucleic Acids: Preparation and Usage as Scaffold for Abiotic Intracellular Catalysis

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Materials and Instrumentation

HPLC grade acetonitrile (ACN) was purchased from TEDIA (USA). All other reagents and solvents were purchased from Adamas Reagent Co. (China), Sigma-Aldrich Co. (USA), Aladdin Reagent Co. (China), or Sangon Biotech Co. (China), and used as received. Bio-reagents were purchased from Beyotime Ltd. (Beijing, China) unless noted otherwise. Ultrapure water (18.2 MΩ·cm resistivity) was obtained from a Milipore system (Milli-Q, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz NMR spectrometer (Germany) and chemical shifts (δ) were reported in ppm. HPLC purification was carried out on a Waters Breeze 2 HPLC system (USA) equipped with a Waters 2998 photodiode array detector. MALDI-ToF MS measurements were performed on a Bruker AutoFlex-III mass spectrometer (Bruker Daltonics Inc., USA). Temperature control and mixing during the coupling of DNA to azide-functionalized cores were achieved using an Eppendorf Thermomixer C instrument (Germany). Gels were imaged on a FluochemQ imaging system (ProteinSimple Inc., USA). Confocal images were taken on a Nikon TI-E+A1 microscope (Nikon, Japan). Flow cytometry studies were performed on an Accuri C6 Plus instrument (BD, USA). UV-vis measurements were performed on a Cary 5000 UV-vis spectrophotometer (Agilent, USA).

All DNA synthesis reagents were obtained from Glen Research (USA) and used as received. Oligonucleotide strands were synthesized using an ABI DNA synthesizer on controlled pore glass (CPG) beads (1000 Å). After synthesis, DNA strands were cleaved from the CPG support using aqueous ammonium hydroxide (28-30% NH_3 basis) at 55 °C for 17 h, and purified by reverse-phase HPLC (RP-HPLC) equipped with a Waters SunFire C18 column (5 μm, 4.6 × 250 mm), using triethylammonium acetate (TEAA) buffer (0.05 M) and HPLC-grade ACN as mobile phases.

MALDI-TOF MS was used to verify the successful syntheses of the desired DNA sequences, using 3-hydroxypicolinic acid (HPA), 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (α-CHCA), 2,5-dihydroxyacetophenone (DHAP) or sinapinic acid (SA) as the matrix. DNA concentrations were determined by measuring the corresponding aqueous solution's absorbance at 260 nm, and calculating using the corresponding extinction

coefficients predicted by the OligoAnalyzer tool (Integrated DNA Technologies). A complete list of the oligonucleotides used in this work can be found in **Table S1**.

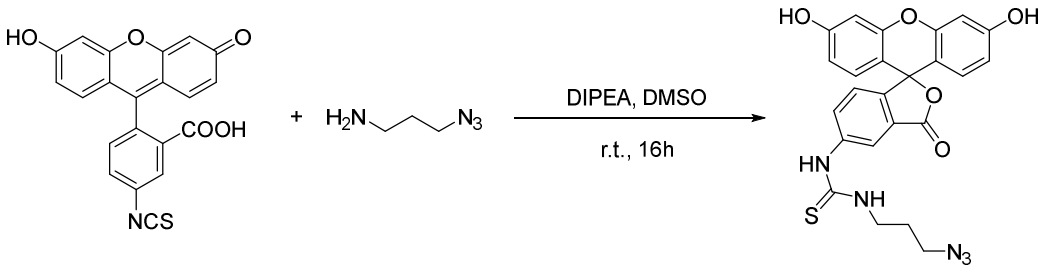
Table S1. All sequences used for materials preparation in this study.

Abbreviation	DNA sequence	Source
DBCO-6M	5'-DBCO-$(CH_2CH_2O)_4$-TTC CTT-3’	Synthetic
FAM-T12-NH_2	5'-FAM-TTT TTT TTT TTT-NH_2-3’	Synthetic
FAM-T12-N_3	5'-FAM-TTT TTT TTT TTT-NH-$COCH_2$-N_3-3’	Synthetic
18M-DBCO	5'-TTC CTT CCT TTC CTT TTT-DBCO-3’	Commercial
DBCO-19M	5'-DBCO-$(CH_2CH_2O)_4$-T TTT CTC CAT GGT GCT CAC-3'	Commercial
DBCO-19M-FAM	5'-DBCO-$(CH_2CH_2O)_4$-T TTT CTC CAT GGT GCT CAC-FAM-3'	Synthetic
TTA-19M-FAM	5’-TTA-$(CH_2CH_2O)_4$-T TTT CTC CAT GGT GCT CAC-FAM-3'	Synthetic

Synthetic Procedures

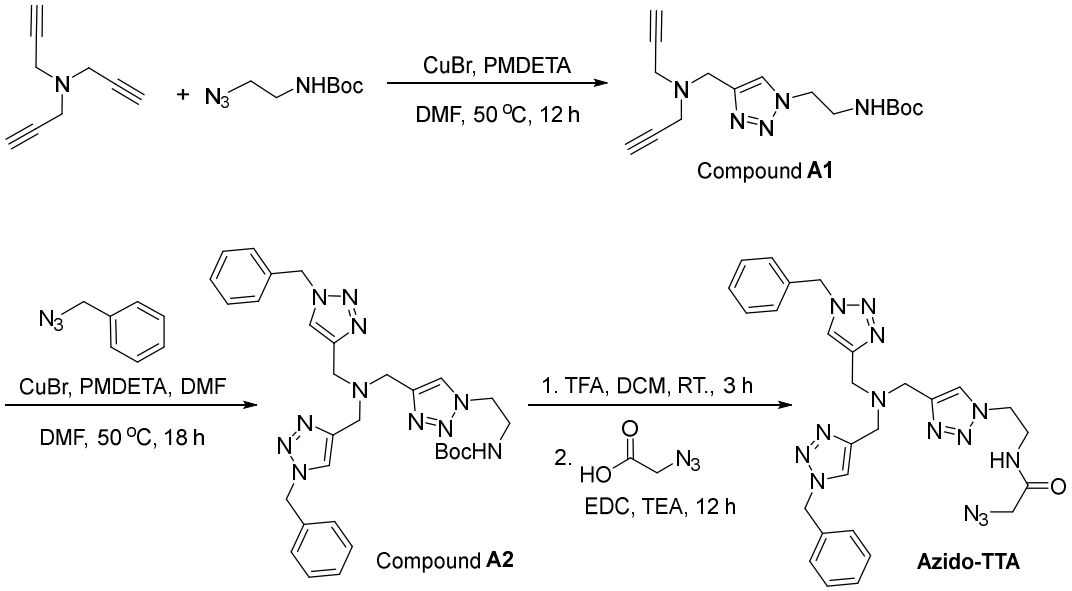
Synthesis of functional molecules

Synthesis of azido-FITC. This fluorescein derivative was synthesized as shown below:



Preparation of this compound was achieved using a reported protocol.[1] Characterization results were consistent with the reported values.

Synthesis of azido-TTA. The azido-TTA compound was prepared in multiple steps as shown below:



Synthesis of Compound A1. N^1-Boc-2-azidoethylamine (2.13 g, 11.44 mmol, 1.5 eq.) and tripropargylamine (1.0 g, 7.62 mmol, 1 eq.) were added into a two-neck round bottom flask containing DMF (20 mL). The mixture was added CuBr (109 mg, 0.76 mmol, 0.10 eq.), PMDETA (175 μL, 0.84 mmol, 0.11 eq.), and the mixture was stirred overnight at 50˚C under nitrogen. Water (100 mL) was added to the reaction mixture, and the resulting mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were washed with brine three times, dried with anhydrous Na_2SO_4 and concentrated to dryness *in vacuo* to give a crude product as a yellow oil, which was further purified by column chromatography (CH_2Cl_2/MeOH, 30:1 to 20:1) to afford the pure product as a light-yellow oil (1.8 g, 75%). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 7.54 (s, 1H), 4.87 (broad *s*, 1H), 4.46 (*t*, *J* = 5.8 Hz, 2H), 3.87 (*s*, 2H), 3.63 (*q*, *J* = 5.9 Hz, 2H), 3.48 (*s*, 4H), 2.27 (*s*, 2H), 1.43 (*s*, 9H).

Synthesis of Compound A2. Compound **A1** (1.0 g, 3.15 mmol, 1 eq.) and benzyl azide (932 mg, 7.0 mmol, 2.2 eq.) were added into a two-neck round bottom flask containing DMF (20 mL). The mixture was added CuBr (90 mg, 0.63 mmol, 0.20 eq.), PMDETA (146 mg, 0.70 mmol, 0.22 eq.), and the mixture under nitrogen was stirred overnight at 50˚C. Water (100 mL) was added to the reaction mixture, and the resulting mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were washed with brine three times, dried with anhydrous $MgSO_4$ and concentrated to dryness *in vacuo* to give a crude product as a yellow solid, which was further purified by column chromatography (CH_2Cl_2/MeOH, 15:1) to afford the pure product

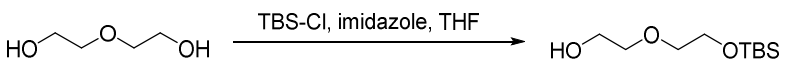
as a light-yellow solid (1.59 mg, 86%). ^{1}H NMR: (400 MHz, DMSO-d_6): δ 8.08 (*s*, 2H), 7.96 (*s*, 1H), 7.43 – 7.27 (*m*, 10H), 6.97 (*t*, *J* = 5.8 Hz, 1H), 5.60 (*s*, 4H), 3.61 (*d*, *J* = 8.1 Hz, 6H), 3.36 (*q*, *J* = 6.0 Hz, 2H), 3.29 (*s*, 2H), 1.29 (s, 9H).

Synthesis of azido-TTA. Compound **A2** (0.44 g, 0.75 mmol, 1 eq.) was deprotected with 50% trifluoroacetic acid in 5 mL DCM for 3 h at room temperature, then the reaction mixture was concentrated to dryness *in vacuo* to give the pure trifluoroacetate salt, which was directly used in the next step without purification. The TFA salt was transferred into a round-bottom flask and dissolved in 10 mL of anhydrous DMF. To this solution was sequentially added azidoacetic acid (156 mg, 1.5 mmol, 2 eq.), HBTU (744 mg, 1.96 mmol, 2.6 eq.), HOBt (184 mg, 1.36 mmol, 1.8 eq.), and triethylamine (1.6 mL, 11.5 mmol, large excess). This solution was heated to 50 ^{o}C for 12 h. The resulting solution was poured into a mixture of ethyl acetate (40 mL) and water (50 mL) and was vigorously shaken in a separation funnel. The organic layer was collected and washed with 0.1 M NaOH (50 mL) twice. The solution was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH, 20:1) to give the pure product as a light-yellow solid (270 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (*broad*, 1H), 8.10 (*s*, 2H), 8.01 (*s*, 1H), 7.26-7.40 (*m*, 10H), 5.61 (*s*, 4H), 4.43 (*broad s*, *i*2H), 3.79 (*s*, 2H), 3.63 (*s*, 4H), 3.61 (*s*, 2H), 3.56 (*m*, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 167.68, 143.75, 143.29, 136.21, 128.71, 128.01, 127.70, 124.26, 124.17, 52.70, 50.67, 48.59, 46.99, 46.87, 38.98. High-res ESI: calculated for $C_{27}H_{31}N_{14}O^+$ ([M+H]$^+$): 567.2800, found 567.2805.

Synthesis of functional alcohols

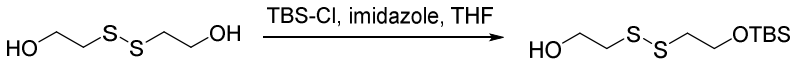
General protocol for TBS protection. *tert*-Butyl chlorodimethylsilane (TBS-Cl, 1.2 eq), imidazole (1.5 eq.), and alcohol (1 eq.) were added to THF, stirred and kept at room temperature overnight. The solvent was then removed, and the residue was purified on a silica column (petroleum ether / ethyl acetate, 8:1 to 4:1).

Synthesis of 2-(2-((*tert*-butyldimethylsilyl)oxy)ethoxy)ethan-1-ol



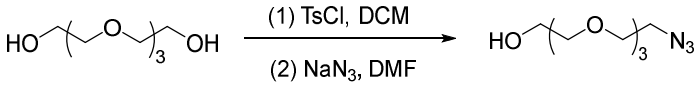
TBS-Cl (340 mg, 2.3 mmol, 1.2 eq.), imidazole (192 mg, 2.8 mmol, 1.5 eq.), and 2,2'-oxybis(ethan-1-ol) (200 mg, 1.9 mmol, 1 eq.) were used. Product was a colorless viscous oil. Yield: 215.8 mg (52%). Characterization of the product was consistent with the reported values in literature.[2]

Synthesis of 2-((2-((tert-butyldimethylsilyl)oxy)ethyl)disulfanyl)ethan-1-ol



TBS-Cl (6.80 g, 45.1 mmol, 1.2 eq.), imidazole (3.84 g, 56.5 mmol, 1.5 eq.), and 2,2'-disulfanediylbis(ethan-1-ol) (4.88 g, 31.6 mmol, 1 eq.) were used. Product was a pale-yellow viscous oil. Yield: 3.65 g (43%). Characterization of the product was consistent with the reported values in literature.[3]

Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol

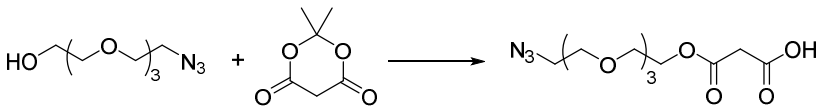


This compound was prepared following a reported procedure.[1] The final product was obtained as a light-yellow oil (5 g, 42% yield over two steps). Characterization of the product was consistent with the reported values in literature.[4]

<u>General protocol for the synthesis of mono-malonates</u>

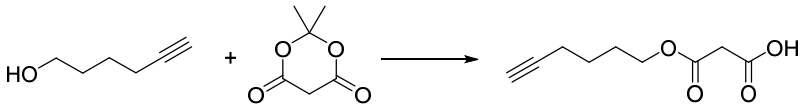
A mixture of the alcohol of interest (1 eq.) and Meldrum's acid (1 eq.) were added in a round-bottom flask equipped with a reflux condenser. The system was stirred at 90-110 ˚C for 4-16 h with or without solvent (DMF). After the reaction, the mixture was cooled and added water before it was extracted with DCM for 3 times. The organic layers were combined, dried over Na_2SO_4, evaporated and purified through flash chromatography to obtain the product as clear oils (40%~80% yield).

Synthesis of azido-functionalized mono-malonate

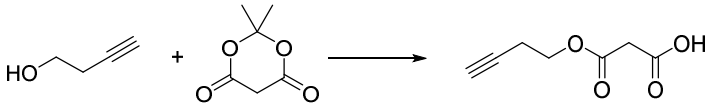


The compound was synthesized using 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (6.57 g, 30 mmol, 1 eq.) and Meldrum's acid (4.32 g, 30 mmol, 1 eq.). The product was purified on a silica column chromatography using petroleum ether / ethyl acetate (1/1, v/v). Product was a clear yellow oil. Yield: 7.3 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.34-4.32 (*t*, *J* = 5.7 Hz, 2H), 3.75 – 3.72 (*m*, 2H), 3.70 – 3.67 (*m*, 10H), 3.45 (*s*, 2H), 3.42-3.39 (*t*, *J* = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.7, 167.2, 70.8, 70.7, 70.6, 70.5, 70.0, 68.6, 64.8, 50.6, 40.8; Hi-res ESI-MS: calculated for $C_{11}H_{19}N_3O_7Na^+$ ($[M+Na]^+$): 328.1115; found 328.1125.

Synthesis of alkyne-functionalized mono-malonates

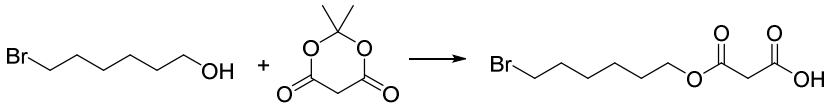


The compound was synthesized using hex-5-yn-1-ol (981.5 mg, 10 mmol, 1 eq.) and Meldrum's acid (1.44 g, 10 mmol, 1 eq.). Yield: 732 mg (40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (*t*, *J* = 6.5 Hz, 2H), 3.45 (*s*, 2H), 2.26-2.22 (*m*, 2H), 1.97 (*t*, *J* = 2.6 Hz, 1H), 1.84-1.77 (*m*, 2H), 1.65-1.57 (*m*, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.1, 166.9, 83.7, 68.9, 65.4, 40.9, 27.4, 24.7, 18.0; Hi-res ESI-MS: calculated for $C_9H_{13}O_4^+$ ($[M+H]^+$): 185.0808, found 185.0821.



The compound was synthesized using but-3-yn-1-ol (701 mg, 10 mmol, 1 eq.) and Meldrum's acid (1.44 g, 10 mmol, 1 eq.). Yield: 1.3 g (83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.25 (*t*, *J* = 7.0 Hz, 2H), 3.45 (s, 2H), 2.55 (*t*, *J* = 6.9 Hz, 2H), 2.02 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.05, 166.46, 79.53, 70.26, 63.30, 40.87, 18.73; Hi-res ESI-MS: calculated for $C_7H_8O_4Na^+$ ($[M+Na]^+$): 179.0315, found 179.0320.

Synthesis of bromide-functionalized mono-malonate



The compound was synthesized using 6-bromo-1-hexanol (1.81 g, 10 mmol, 1 eq.) and Meldrum's acid (1.44 g, 10 mmol, 1 eq.). Yield: 934 mg (35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.87 (*broad*, 1H), 4.16 (*t*, *J* = 6.6 Hz, 2H), 3.43 (*s*, 2H), 3.39 (*t*, *J* = 6.8 Hz, 2H), 1.81-1.90 (*m*, 2H), 1.62-1.72 (*m*, 2H), 1.33-1.51 (*m*, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.63, 166.75, 65.74, 40.99, 33.68, 32.53, 28.20, 27.67, 24.96; Hi-res ESI-MS: calculated for $C_9H_{16}BrO_4^+$ ($[M+H]^+$):

267.0227, 269.0207; found 269.0237, 269.0217.

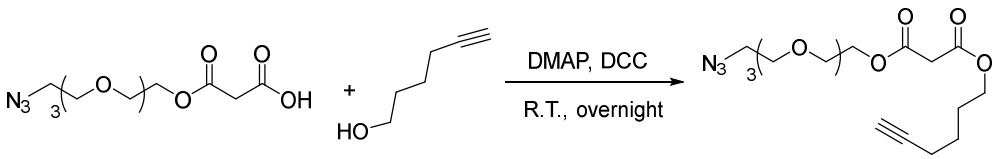
General protocol for the synthesis of dual-functional malonates

The corresponding mono-malonate (R_1OCOCH_2COOH, 1 eq.), alcohol of interest (R_2-OH, 1.5 eq.), and 4-dimethylamino-pyridine (DMAP, 2 eq.) were added to dry dichloromethane at room temperature in a round-bottom flask, followed by the addition of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 2.3 eq.). The mixture was stirred vigorously for 16 h before it was filtered to remove the insoluble byproducts. Additional dichloromethane was added to dilute the solution, which was washed with water (40 mL, 3 times) and brine (40 mL), dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (silica, petroleum ether / ethyl acetate, 2:1 v/v) to give the product as a colorless or pale-yellow oil. Yield: 30-67%. A list of difunctional malonates synthesized in this work can be find in **Table S2** below.

Table S2. Difunctional malonates and fullerene cores synthesized in this work.

Name	Malonate Arms		Theoretical m/z	Conjugation Sites Provided After Unmasking	
	Type 1	Type 2		Type 1	Type 2
Mal-AzAk	Arm-1	Arm-2	385	-N_3	-C≡CH
Mal-AzOBn	Arm-1	Arm-3	483	-N_3	-OH
Mal-AzOSi	Arm-1	Arm-4	507	-N_3	-OH
Mal-AzSS	Arm-1	Arm-6	556	-N_3	-SH or -OH
Mal-SSAk'	Arm-6	Arm-7	406	-SH or -OH	- C≡CH
Mal-BrAk'	Arm-5	Arm-7	318	-Br	-C≡CH
Core-AzAk	Arm-1	Arm-2	3020	-N_3	-C≡CH
Core-AzOBn	Arm-1	Arm-3	3609	-N_3	-OH
Core-AzOSi	Arm-1	Arm-4	3754	-N_3	-OH
Core-SSAk'	Arm-6	Arm-7	3146	-SH or -OH	-C≡CH

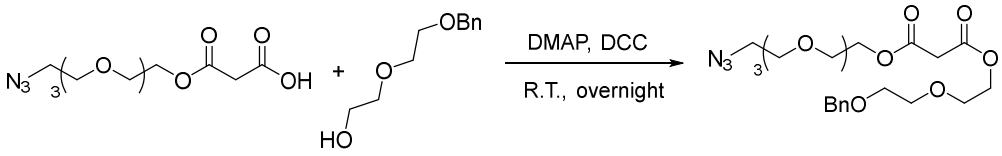
Synthesis of the difunctional malonate with Arm-1 and Arm-2 (Mal-AzAk)



This compound was prepared using 1-azido-13-oxo-3,6,9,12-tetraoxapentadecan-15-oic acid (611 mg, 2.0 mmol, 1 eq.), hex-5-yn-1-ol (295 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol,

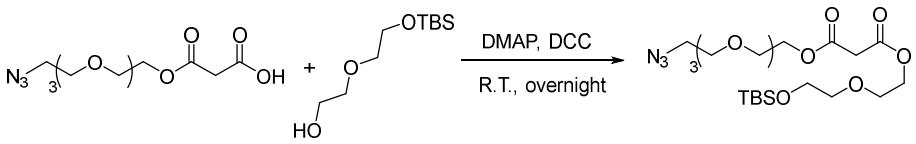
2.0 eq.), and DCC (949 mg, 4.0 mmol, 2.0 eq.). Yield: 439 mg (57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.30 (*t*, *J* = 8.0 Hz, 2H), 4.18 (*t*, *J* = 6.5 Hz, 2H), 3.72 (*t*, *J* = 4.0 Hz, 2H), 3.70 - 3.61 (*m*, 10H), 3.41 (*s*, 2H), 3.39 (*t*, *J* = 4.0 Hz, 2H), 2.23 (*t*, *J* = 7.0 Hz, 2H), 1.97 (*s*, 1H), 1.79 (*m*, 2H), 1.60 (*m*, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.54, 166.44, 83.72, 70.70, 70.67, 70.62, 70.04, 68.85, 64.97, 64.57, 50.68, 41.41, 27.46, 24.74, 17.99. Two peaks in the polyglycol region were overlapping with other peaks. Hi-res ESI-MS: calculated for $C_{17}H_{26}N_3O_7^-$ ([M-H]$^-$): 384.1776, found 384.1768.

Synthesis of the difunctional malonate with Arm-1 and Arm-3 (Mal-AzOBn)



This compound was prepared using 1-azido-13-oxo-3,6,9,12-tetraoxapentadecan-15-oic acid (611 mg, 2.0 mmol, 1 eq.), 2-(2-(benzyloxy)ethoxy)ethan-1-ol (589 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol, 2.0 eq.), and DCC (949 mg, 4 mmol, 2 eq.). Yield: 405 mg (42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (*m*, *J* = 4.2 Hz, 4 H), 7.29 (*m*, 1H), 4.57 (*s*, 2H), 3.76 – 3.60 (*m*, 12H), 3.44 (*s*, 2H), 3.39 (*t*, *J* = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.45, 138.18, 128.38, 127.72, 127.63, 73.26, 70.69, 70.66, 70.47, 70.03, 69.47, 69.40, 68.86, 64.60, 64.57, 50.67, 41.27, 33.95, 24.97. The peaks from the two carbonyl carbons were overlapping. ESI-MS: calculated for $C_{22}H_{34}N_3O_9^+$ ([M+H]$^+$): 484.2290, found 484.2295.

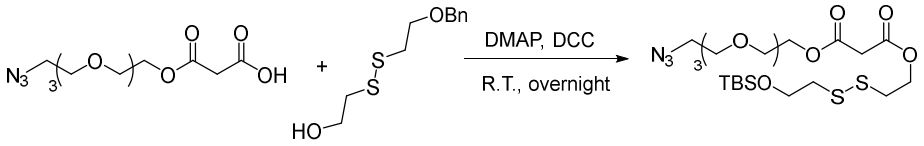
Synthesis of the difunctional malonate with Arm-1 and Arm-4 (Mal-AzOSi)



This compound was prepared using 1-azido-13-oxo-3,6,9,12-tetraoxapentadecan-15-oic acid (611 mg, 2.0 mmol, 1 eq.), 2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethan-1-ol (661 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol, 2.0 eq.) and DCC (949 mg, 4.0 mmol, 2.0 eq.) in 50 mL of dry DCM. Yield: 476 mg (47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (*m*, 4H), 3.79 – 3.61 (*m*, 16H), 3.54 (*t*, *J* = 5.1 Hz, 2H), 3.42 (*s*, 2H), 3.36 (*t*, *J* = 5.0 Hz, 2H), 0.88 (*s*, 9H), 0.06 (*s*, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.48, 166.46, 72.71, 70.73, 70.69, 70.64, 70.06, 68.93, 68.86, 64.70, 64.58, 62.76, 50.70, 41.27, 25.92, 18.36, -5.26. One peak in the polyglycol region

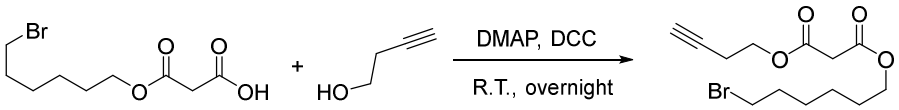
was overlapping with another peak. MALDI-TOF-MS: calculated for $C_{21}H_{41}N_3O_9SiNa^+$ ($[M+Na]^+$): 530.2505, found 530.2513 ($[M+Na]^+$).

Synthesis of the difunctional malonate with Arm-1 and Arm-6 (Mal-AzSS)



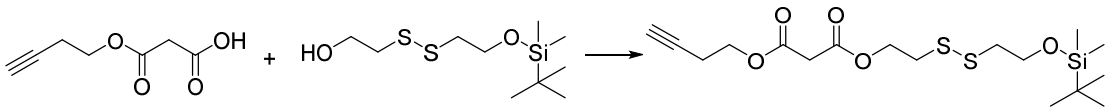
This compound was prepared using 1-azido-13-oxo-3,6,9,12-tetraoxapentadecan-15-oic acid (611 mg, 2.0 mmol, 1 eq.), 2-((2-((tert-butyldimethylsilyl)oxy)ethyl)disulfanyl)ethan-1-ol (806 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol, 2.0 eq.) and DCC (949 mg, 4.0 mmol, 2.0 eq.). Yield: 433 mg (39 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.41 (*t*, *J* = 6.6 Hz, 2H), 4.31 (*s*, 2H), 3.85 (*t*, *J* = 6.5 Hz, 2H), 3.69 (*m*, 12H), 3.43 (*s*, 2H), 3.40 (*t*, *J* = 4.1 Hz, 2H), 2.92 (*t*, *J* = 6.6 Hz, 2H), 2.83 (*t*, *J* = 6.5 Hz, 2H), 0.90 (*s*, 9H), 0.08 (*s*, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.35, 166.22, 70.73, 70.70, 70.65, 70.07, 68.86, 64.65, 63.69, 61.72, 50.70, 41.62, 41.29, 36.75, 25.89, 18.34, -5.25. One peak in the polyglycol region was overlapping with another peak. MALDI-TOF-MS: calculated for $C_{21}H_{41}N_3O_8S_2SiNa^+$ ($[M+Na]^+$): 578.1997, found 578.2001.

Synthesis of the difunctional malonate with Arm-2 and Arm-7 (Mal-BrAk')



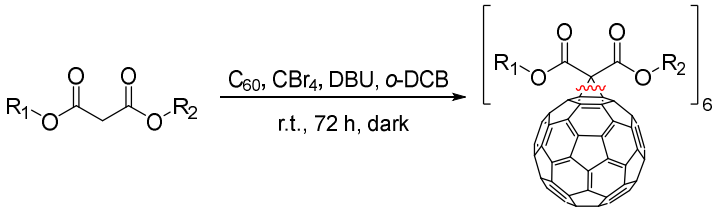
This compound was prepared using 3-((6-bromohexyl)oxy)-3-oxopropanoic acid (532 mg, 2.0 mmol, 1 eq.), 3-butyn-1-ol (210 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol, 2.0 eq.), and DCC (949 mg, 4.0 mmol, 2.0 eq.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (*t*, *J* = 6.6 Hz, 2H), 4.16 (*t*, *J* = 6.7 Hz, 2H), 3.50 – 3.36 (*m*, 4H), 2.57 (*t*, *J* = 6.7 Hz, 2H), 2.02 (*s*, 1H), 1.88 (*t*, *J* = 7.3 Hz, 2H), 1.69 (*d*, *J* = 7.2 Hz, 2H), 1.44 (*dt*, *J* = 35.3, 7.5 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.38, 166.32, 79.62, 70.11, 65.46, 63.05, 41.41, 33.65, 32.55, 28.28, 27.71, 25.01, 18.84. Hi-res ESI-MS: calculated for $C_{13}H_{20}BrO_4^+$ ($[M+H]^+$): 319.0540, 321.0520; found 319.0546, 321.0522.

Synthesis of the difunctional malonate with Arm-6 and Arm-7 (Mal-SSAk')



This compound was prepared using 3-(but-3-yn-1-yloxy)-3-oxopropanoic acid (312 mg, 2.0 mmol, 1 eq.), 2-((2-((tert-butyldimethylsilyl)oxy)ethyl)disulfanyl)ethan-1-ol (806 mg, 3.0 mmol, 1.5 eq.), DMAP (489 mg, 4.0 mmol, 2.0 eq.), and DCC (949 mg, 4.0 mmol, 2.0 eq.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.40 (*t*, *J* = 6.7 Hz, 2H), 4.25 (*t*, *J* = 6.8 Hz, 2H), 3.85 (*t*, *J* = 6.6 Hz, 2H), 3.42 (*s*, 2H), 2.92 (*t*, *J* = 6.7 Hz, 2H), 2.82 (*t*, *J* = 6.6 Hz, 2H), 2.55 (*td*, *J* = 6.9, 2.6 Hz, 2H), 2.02 (*s*, 1H), 0.89 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.08, 166.04, 79.62, 70.17, 63.40, 63.10, 61.68, 41.60, 41.24, 36.74, 25.87, 18.82, 18.31, -5.25. Hi-res ESI-MS: calculated for $C_{17}H_{30}O_5S_2SiNa^+$ ([M+Na]$^+$): 429.1197; found 429.1201.

General protocol for the synthesis of dual functional fullerene cores



Fullerene C_{60} (1 eq.) and molecular sieve dried *o*-dichlorobenzene (50-150 mL) were added into a flame-dried flask. The malonate of interest (12 eq.), carbon tetrabromide (100 eq.), and dried 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 20 eq.) were added immediately into the system with a vigorous stirring under nitrogen atmosphere. The reaction was stirred in dark at the room temperature for 72 h under N_2. The reaction mixture was first purified via a short silica column (100% DCM) to remove dichlorobenzene, then the crude product was purified using column chromatography (silica, MeOH in DCM or hexanes in DCM). Multiple times of column purification might be needed to obtain pure product. Alternatively, column-purified product might be dissolved in a small amount of DCM, and the solution was carefully layered below hexanes. The precipitated sticky matter formed (as the purified product) was collected by decanting the DCM/hexanes mixture. The fullerene cores were characterized by NMR (Figure S1-S8), normal phase HPLC (Figure S11), and MALDI-TOF MS (Figure S12).

Note: All integrals of proton NMR of the fullerene cores were based on one malonate arm, and

the final total number of hydrogen atoms should be multiplied by 6.

Core-AzAk. 925 mg of the corresponding malonate **Mal-AzAk** (with **Arm-1** and **Arm-2**, 2.0 mmol, 12 eq.), 144 mg of fullerene C_{60} (0.2 mmol, 1 eq.), 6.64 g of CBr_4 (20 mmol, 100 eq.), 609 mg of DBU (4.0 mmol, 20 eq.), and 150 mL of *o*-DCB. Yield: 468 mg (31 %, dark orange oil). ^1H and ^{13}C NMR characterization data are provided on page S16 (**Figure S1**/**S2**). MALDI-TOF-MS: calculated for $C_{162}H_{151}N_{18}O_{42}^+$ ($[M+H]^+$): 3020, found 3021 (**Figure 1c**, **Figure S12**). Note: azide and alkyne moieties can undergo Huisgen cycloaddition very slowly, especially when they are put in close proximity. Thus, the prepared **Core-AzAk** should be stored at -20 ^oC, and have its purity checked after long-term storage before use.

Core-AzOBn. 1.04 g of the corresponding malonate **Mal-AzOBn** (with **Arm-1** and **Arm-3**, 2.0 mmol, 12 eq.), 144 mg of fullerene C_{60} (0.2 mmol, 1 eq.), 6.64 g of CBr_4 (20 mmol, 100 eq.), 609 mg of DBU (4.0 mmol, 20 eq.), and 150 mL of *o*-DCB. Yield: 166 mg (23%, light brown oil). ^1H and ^{13}C NMR characterization data are provided on page S17 (**Figure S3**/**S4**). MALDI-TOF-MS: calculated for $C_{192}H_{186}N_{18}O_{54}^+$ (M^+): 3609, found 3608 (**Figure S12**).

Core-AzOSi. 1.22 g of the corresponding malonate **Mal-AzOSi** (with **Arm-1** and **Arm-4**, 2.0 mmol, 12 eq.), 144 mg of fullerene C_{60} (0.2 mmol, 1 eq.), 6.64 g of CBr_4 (20 mmol, 100 eq.), 609 mg of DBU (4.0 mmol, 20 eq.), and 150 mL of *o*-DCB. Yield: 203 mg (27%, dark orange oil). ^1H and ^{13}C NMR characterization data are provided on page S18 (**Figure S5**/**S6**). MALDI-TOF-MS: calculated for $C_{186}H_{234}N_{18}O_{54}Si_6Na^+$ ($[M+Na]^+$): 3776, found 3777 (**Figure S12**).

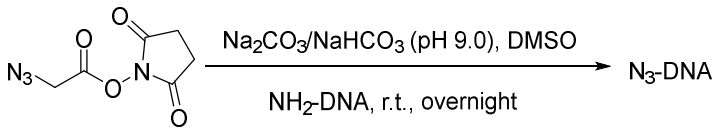
Core-SSAk'. 812 mg of the corresponding malonate **Mal-SSAk'** (with **Arm-6** and **Arm-7**, 2.0 mmol, 12 eq.), 144 mg of fullerene C_{60} (0.2 mmol, 1 eq.), 6.64 g of CBr_4 (20 mmol, 100 eq.), 609 mg of DBU (4.0 mmol, 20 eq.), and 150 mL of *o*-DCB. Yield: 296 mg (47%, dark orange oil). ^1H and ^{13}C NMR characterization data are provided on page S18 (**Figure S7**/**S8**). MALDI-TOF-MS: calculated for $C_{162}H_{168}O_{30}S_{12}Si_6^+$ (M^+): 3146, found 3145 (**Figure S12**).

Functionalization of ssDNAs

Synthesis of DBCO-DNA. 6-fold of DBCO-sulfo-NHS ester was reacted with the chosen DNA-NH_2 following the reagent provider's recommended method, in pH 8.6 Na_2CO_3/$NaHCO_3$ buffer

for 16 h to get the DBCO-modified DNA. Then the reaction mixture was purified via RP-HPLC to get the pure 5'-DNA-DBCO-3'. Note: DNA strands used in this paper with 5'-end DBCO were prepared using SPPS (not through post-functionalization).

Synthesis of azido-modified DNA. Azide-modified DNA was synthesized using the corresponding amino-functionalized DNA and *N*-hydroxysuccinimidyl azidoacetate (see scheme below). The reaction mixture was purified by using RP-HPLC after desalting. Identity and purity of the products were characterized MALDI-TOF and HPLC.



Synthesis of TTA-DNA. The ligand-bearing DNA was synthesized from the corresponding DBCO-DNA and azido-TTA via cooper-free "Click" reaction. Azido-TTA (100 μM, final concentration, same for all the following) in 50 μL of DMSO was carefully added into the aqueous solution of DBCO-DNA (50 μM, 1 mL). The mixture was stirred at 40 ˚C for 24 h. Pure TTA-DNA was obtained through RP-HPLC purification. The purity and identity of the product was verified by HPLC and MALDI-TOF characterizations.

Derivatization of Core-AzAk

Reaction of Core-AzAk with DBCO-NH_2 using copper-free click chemistry. Core-AzAk and 1.2 equivalent of DBCO-NH_2 were dissolved in DMSO, and the solution was stirred at room temperature for 48 h. Then the reaction mixture was separated by RP-HPLC to remove the byproduct (showing as a shoulder of the product peak, presumably the penta-substituted product). The purified product **Core-NH_2Ak** was analyzed by MALDI-TOF mass spectrometry. The m/z measured was consistent with theoretical value.

Reaction of Core-NH_2Ak with azidocoumarin using CuAAC. A solution of 3-azido-7-hydroxycoumarin (5 eq.) in DMSO, and a solution of 0.2% (w/w) of $CuSO_4$ and 0.2% (w/w) of sodium ascorbate in water were successively added into a DMSO solution of **Core-NH_2Ak**. The volume ratios of the solutions should be adjusted to make sure that no precipitation took place

during the mixing. The solution was vigorously stirred at room temperature for 24 h. The crude reaction mixture was diluted using water and filtered on a centrifugal unit (MWCO = 1.5 kDa), re-dissolved in water/DMSO, and was purified by RP-HPLC. Condition used: MeOH/H_2O, 0.75/0.25 to 1/0 over 15min, *v*/*v*, 1 mL/min.

Reaction of Core-NH_2Ak with FAM-T12-N_3 DNA using CuAAC. A solution of FAM-T12-N_3 DNA (12 eq.) in water, a mixture of $CuSO_4$ (0.2%, w/w), TBTA (1 eq. of Cu) and 0.2% (w/w) of sodium ascorbate in water were successively added into a DMSO solution of **Core-NH_2Ak**. The volume ratios of the solutions should be adjusted to make sure that no precipitation took place during the mixing. The solution was stirred at room temperature for 24 h. The crude reaction mixture was passed through a GE Illustra NAP-5 column, and was purified by RP-HPLC. Condition used: 0.05 M TEAA/ ACN, 0.95 / 0.05 to 0 / 1 in 40 min, v/v, 1 mL/min.

Synthesis of SNAs

Synthesis of the 6-arm SNA. The protocol of for the synthesis of 6-arm SNA was analogous to our previously reported procedure,[5] using the corresponding DNA and core. The pure 6-arm SNA was purified by RP-HPLC.

Functionalization of the 6-arm SNA with FITC. The 6-arm SNA with 6 alkyne anchors (5 μM, final concentration, same for all the following), azido-FITC (150 μM), TBTA (45 μM) and $CuSO_4$ (45 μM) were mixed together in a 1 mL glass vial. Concentrated sodium ascorbate solution was added to the solution (final concentration = 1 mM, pH 7.0-7.4) to start the reaction. After 24 h, the reaction mixture was purified by RP-HPLC. Ratio of DNA and FITC moiety was verified using spectrometric analysis.

Functionalization of the 6-arm SNA with 6 TTA arms. The 6-arm SNA with 6 alkyne anchors (10 μM, final concentration, same for all the following), azido-TTA (150 μM), and $CuSO_4$ (300 μM) were mixed together in a 1 mL glass vial. Concentrated sodium ascorbate solution was added to the solution (final concentration = 1 mM, pH 7.0-7.4) to start the reaction. The solution was stirred at 40 ˚C for 48 h under N_2. The resulting mixture was purified by RP-HPLC (0.05 M TEAA / CH_3CN, 0.95 / 0.5 to 0.05 / 0.95 in 50 min, v/v, 1 mL/min). *Note: TEAA* =

triethylammonium acetate.

Synthesis of 12-arm SNA with 6+6 type DNA arms. The 6-arm SNA with 6 alkyne anchors (20 μM, final concentration, same for all the following), azido-DNA (300 μM), TBTA (60 μM) and $CuSO_4$ (60 μM) were mixed together in a 1 mL glass vial. Concentrated sodium ascorbate solution was added to the solution (final concentration = 1 mM, pH 7.0-7.4) to start the reaction. The solution was stirred at 40 ˚C for 48 h under N_2. The resulting mixture was purified by RP-HPLC (0.05 M TEAA/ CH_3CN, 0.95 / 0.5 to 0.05 / 0.95 in 50 min, v/v, 1 mL/min).

Miscellaneous Experimental Procedures

PAGE characterization. Polyacrylamide gel was cast by pouring a gel stock solution between two pre-assembled glass plates and adding a comb at the top before the gel fully polymerized. Gel stock solution was prepared at 1× Tris / Borate / EDTA (TBE) and diluted to the desired acrylamide concentration (from 5% to 20 %). 2.5 mg of ammonium persulfate (APS, fresh) and 25 μL of tetramethylethylenediamine (TEMED) was added for every 15 mL of gel stock solution just before casting. The samples were loaded into the wells in amounts that corresponded to ~ 20 optical density (OD). Glycerol and the loading buffer (e.g., xylene cyanol and bromophenol blue) was added to sample solution for non-denaturing conditions to create a dense solution suitable for loading and for tracking the gel. Submerged in 1× TBE buffer, the gel was first run at 12 V/cm for 1 h without sample loading to purify the background of the gel, then was run at 4 V/cm to 8 V/cm gradually in order to increase the resolution of the gel for better separation of samples. For analytical gels, the gel was visualized using GelRed stain and imaged.

Verification of 1:1 ratio of the two functional moieties on $SNA_{TEG\text{-}6M/FITC}$. An absorption-based calibration curve was first established to quantify the FITC moieties on $SNA_{6M/FITC}$, using FITC-N_3 as the standard molecule. Solutions of FITC-N_3 of different concentrations had their absorption at 490 nm measured, and the values were plotted against concentration to establish the standard curve (**Figure S20**). The molar absorptivity at 260 nm of the DNA sequence was then calculated using the OligoAnalyzer tool (IDT). Finally, the absorptivity of a $SNA_{TEG\text{-}6M/FITC}$

solution (at both 260 nm and 490 nm) was measured, and the concentrations of DNA strands and FITC moieties were determined using the above calibration curve and molar absorptivity value. The concentrations were then compared to verify the 1:1 ratio of the two functionalities on SNA.

Verification of 1:1 ratio of the two types of DNA strands on $SNA_{FAM\text{-}T12/18M}$. A fluorescence-based calibration curve was first established to quantify the FAM moieties on $SNA_{FAM\text{-}T12/18M}$, using the corresponding free DNA (FAM-T12-N_3) as the standard. Using an excitation wavelength of 490 nm, solutions of FAM-T12-N_3 of different concentrations had their emission at 525 nm measured, and the values were plotted against concentration to establish the standard curve as $I = Kc + N$, where K and N are constants (**Figure S21**). The molar absorptivity (ε) at 260 nm of the two DNA sequence was then calculated using the OligoAnalyzer tool (IDT). Finally, the absorptivity (A) of a $SNA_{FAM\text{-}T12/18M}$ solution (at 260 nm) was measured, and fluorescence intensity (I) of the same solution (at 525 nm, excited at 490 nm) was recorded. Set the concentration of FAM-T12 as x, and the concentration of 18M as y, there is:

$$A = x * \varepsilon(\text{FAM-T12-N}_3) + y * \varepsilon(18\text{M})$$

$$I = x * K + N$$

Thus x and y could be calculated based on these two equations, and the values could be compared to verify the ratio of the two types of strands.

Cell uptake study. 500,000 MCF-7 cells were seeded into wells of a cell culture dish. For each well, the final volume was 1.5 mL. Cells were incubated 16 h to attach to the plate. The cells were incubated with FAM-labeled DNA or SNA (TTA-19M-FAM, DBCO-19M-FAM, 12-arm $SNA_{18M/FAM\text{-}T12}$, 6-arm $SNA_{19M\text{-}FAM}$ and miktoarm $SNA_{19M\text{-}FAM/TTA}$, concentration of DNA strand/FAM = 500 nM) for 6 h at 37 °C in an incubator with a 5% CO_2 atmosphere. After incubation, cells were lifted off using Typsin-EDTA, washed 3 times using PBS, and resuspended into PBS (1 mL). Cells were analyzed by a flow cytometer equipped with a 488 nm laser. FAM fluorescence emitted by SNA or DNA was detected on FL1. In all cases, 10,000 events were counted.

Enzyme digestion experiment. The enzyme digestion experiment of SNA materials and ssDNAs were carried out under conditions recommended by the manufacturer. The digestion processes were monitored by RP-HPLC, using FAM or rhodamine dye as the internal standard. The extent of digestion was calculated using the normalized integration values of the corresponding material at different time points. HPLC condition used: 0.05 M TEAA / ACN, 0.95 / 0.05 to 0.05 / 0.95 in 50 min, v/v, 1 mL/min.

Intracellular catalysis experiment. 300,000 RAW 264.7 or MCF-7 cells were seeded into wells of a glass-bottom cell culture dish. For each well, the final volume was 1.5 mL. Cells were incubated for 16 h to be attached to the plate. Medium was removed, and the cells were washed 3 times with PBS (1 mL). The cells were then incubated with different catalytic moieties (TTA-19M, $SNA_{19M/TTA}$ or TBTA in DMEM, tris-triazole moiety's final concentration = 500 nM) for 6 h at 37 ˚C in an incubator with a 5% CO_2 atmosphere. DMEM was removed, and the cells were washed again with PBS for 4-5 times. The cells were then incubated with the two substrates (150 μM) and sodium *L*-ascorbate (1 mM) in new DMEM solutions for 2 h at 37 °C in an incubator with 5% CO_2 atmosphere. After incubation, substrate- and ascorbate-containing DMEM was removed, the cells were washed 3 times with PBS. Finally, 500 μL of PBS was added to cells, which were taken to a confocal microscope for imaging. *Note: during the imaging, settings (laser strength, gain, etc.) were kept consistent for all samples so that the brightness of the images could reflect the efficiency of the intracellular reaction.*

MTT Cytotoxicity Assay. MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS at 37 °C in a humidified atmosphere with 5% CO_2. The cells were seeded into 96-well plates at 1 × 10^4 cells per well and incubated for 12 h at 37 °C in 5% CO_2 to ensure full confluency. Cells were treated with SNA in DMEM ranging from 0.75 to 4 μM (final concentration) and further incubated for 24 h. Cells without the added SNA served as controls. After incubation, the media were removed and the cells were washed with PBS, then the cell media were changed to 120 μL of fresh MTT solution (0.5 mg/mL in DMEM). The cells were incubated for another 1.5 h at 37 °C. The media were replaced with 100 μL of DMSO and the solutions' absorbances were measured at 595 nm using a microplate reader. Cell viability was calculated by comparing the absorbance to the control.

CuAAC catalysis in live zebrafish. This experiment was conducted with the approval from the relevant national and institutional authority. Approval number: SYXK (Hunan) 2018-0006. Fish were divided into four groups. For the experiment group, $SNA_{19M/TTA}$ (0.083 μM, 0.5 μM of TTA moiety, 5 μL) was first injected intraperitoneally, followed by an intraperitoneal injection of a solution (5 μL) containing azidocoumarin (5 μM), ethynylanisole (5 μM), sodium ascorbate (0.43 mM) and $CuSO_4$ (0.5 μM) in 12 h. For the control groups, the first injection was skipped, and the second intraperitoneally injection (at 12 h) contained:

(1) TBTA-$CuSO_4$ (0.5 μM) plus sodium ascorbate (0.43 mM) and substrates (5 μM each) in 5 μL of solution;

(2) $CuSO_4$ (0.5 μM) plus ascorbate (0.43 mM) and substrates (5 μM each);

(3) azidocoumarin only (5 μM). (This was to verify if azidocoumarin could be metabolically transformed into a fluorescent product without artificial CuAAC catalysis)

The fish were transferred to fresh water, and their feces at day 2 were collected for visualization under a fluorescent confocal microscope. *Note: during the imaging, settings (laser strength, gain, etc.) were kept consistent for all samples so that the brightness of the images could reflect the efficiency of the intracellular reaction.*

NMR Characterization

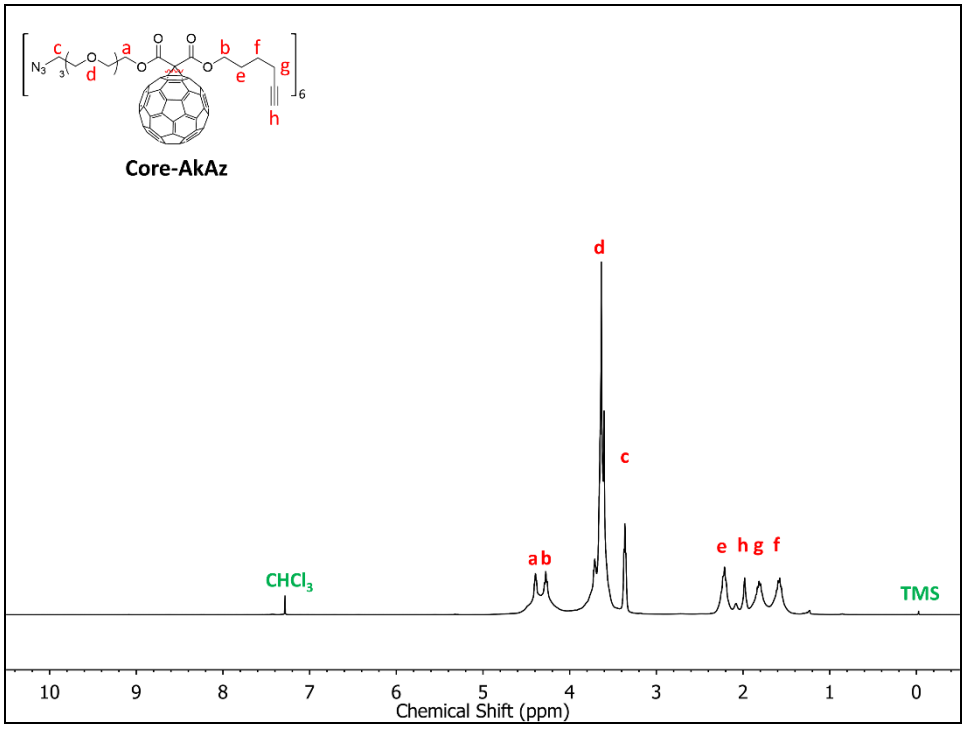


Figure S1. ^{1}H NMR spectrum of **Core-AkAz**.

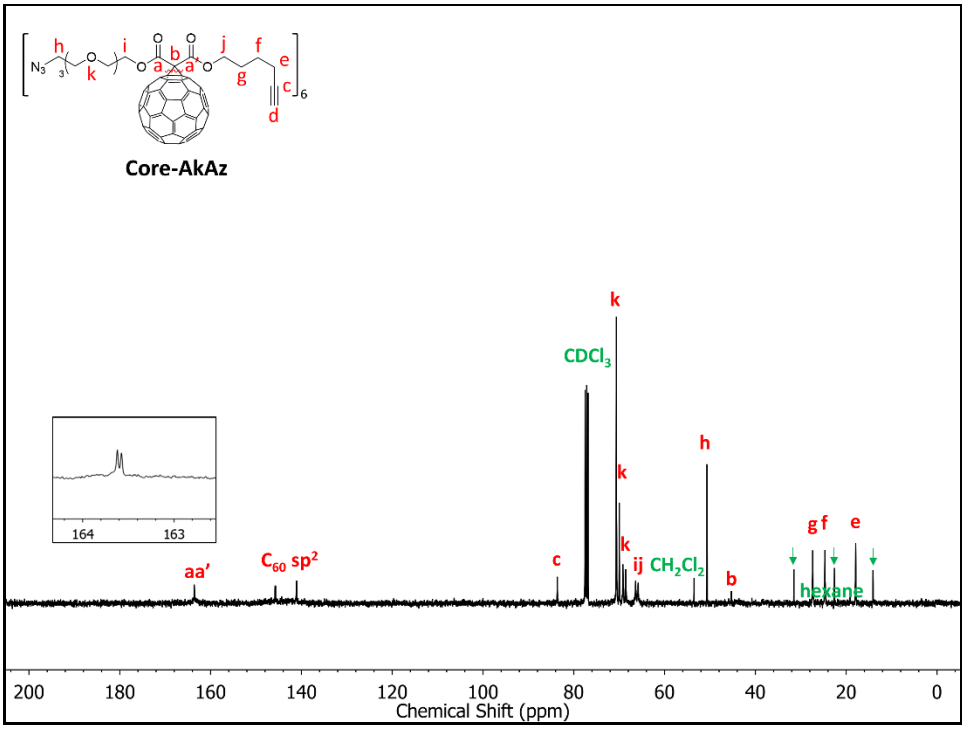


Figure S2. ^{13}C NMR spectrum of **Core-AkAz**. Only two types of fullerene sp^2 carbons are visible, indicating that the scaffold is symmetrically substituted with 6 groups.

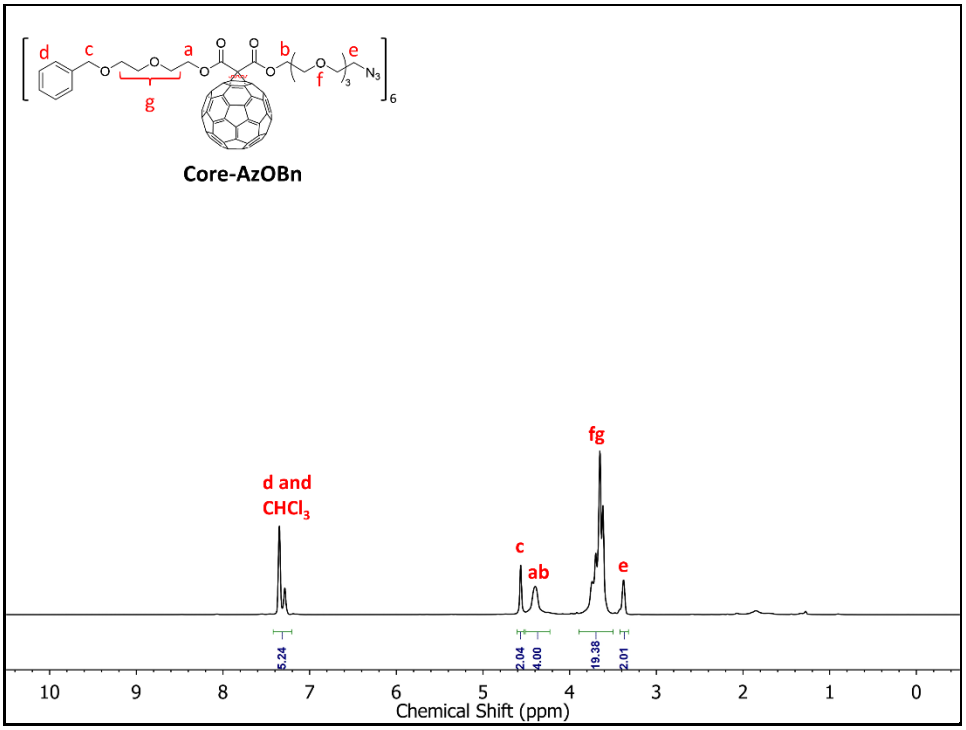


Figure S3. ^{1}H NMR spectrum of **Core-AzOBn**.

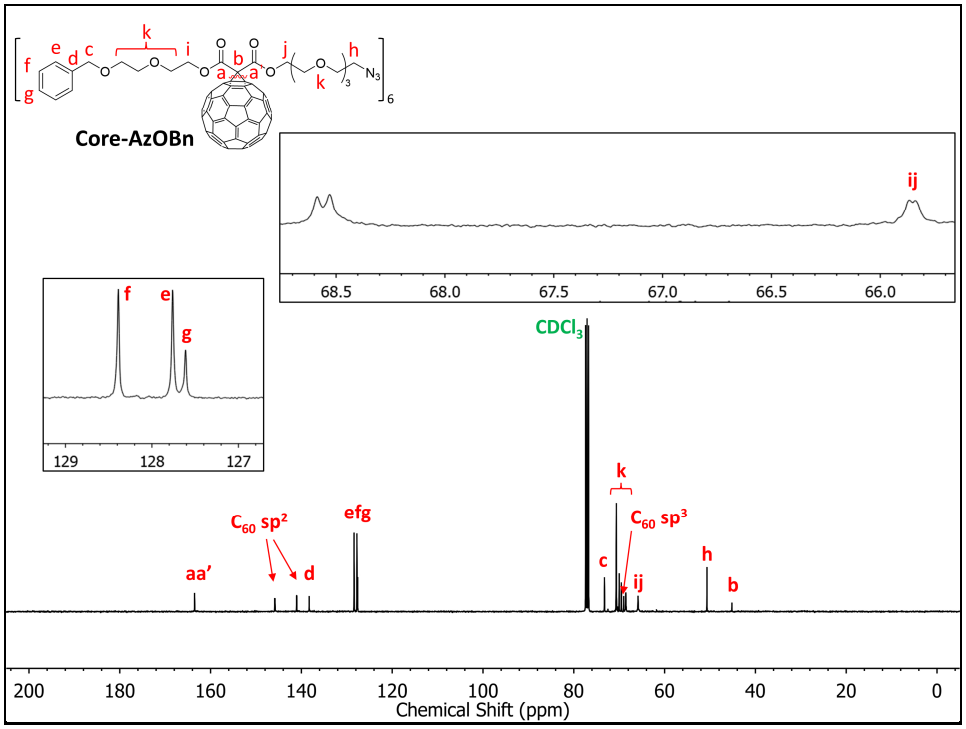


Figure S4. ^{13}C NMR of **Core-AzOBn**. Only two types of fullerene sp^2 carbons are visible, indicating that the scaffold is symmetrically substituted with 6 groups.

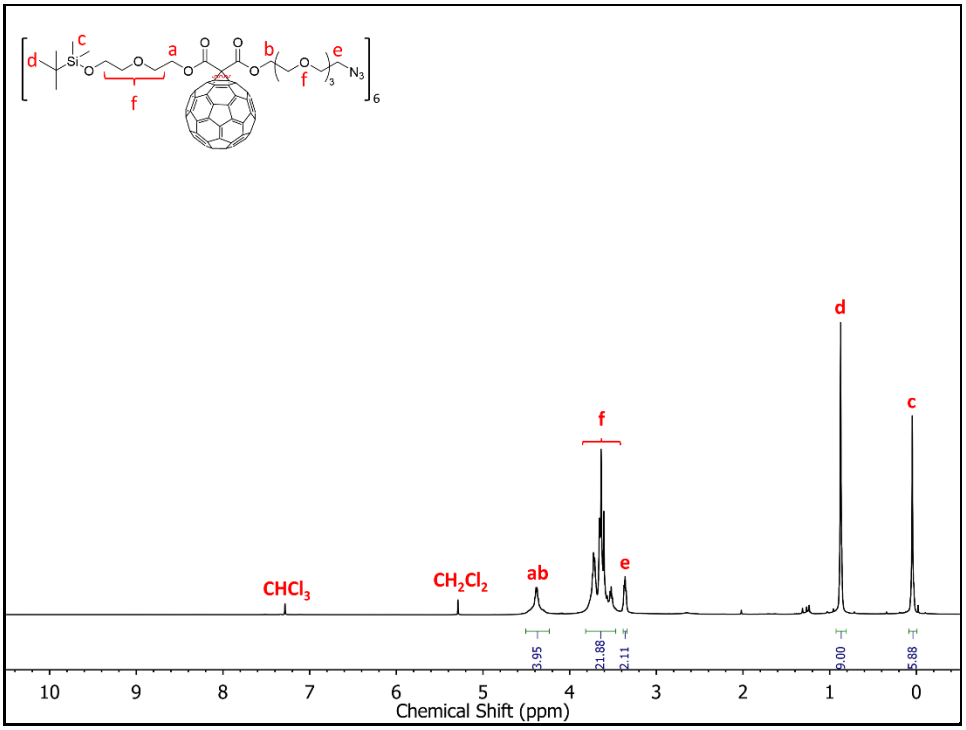


Figure S5. ^{1}H NMR spectrum of **Core-AzOSi**.

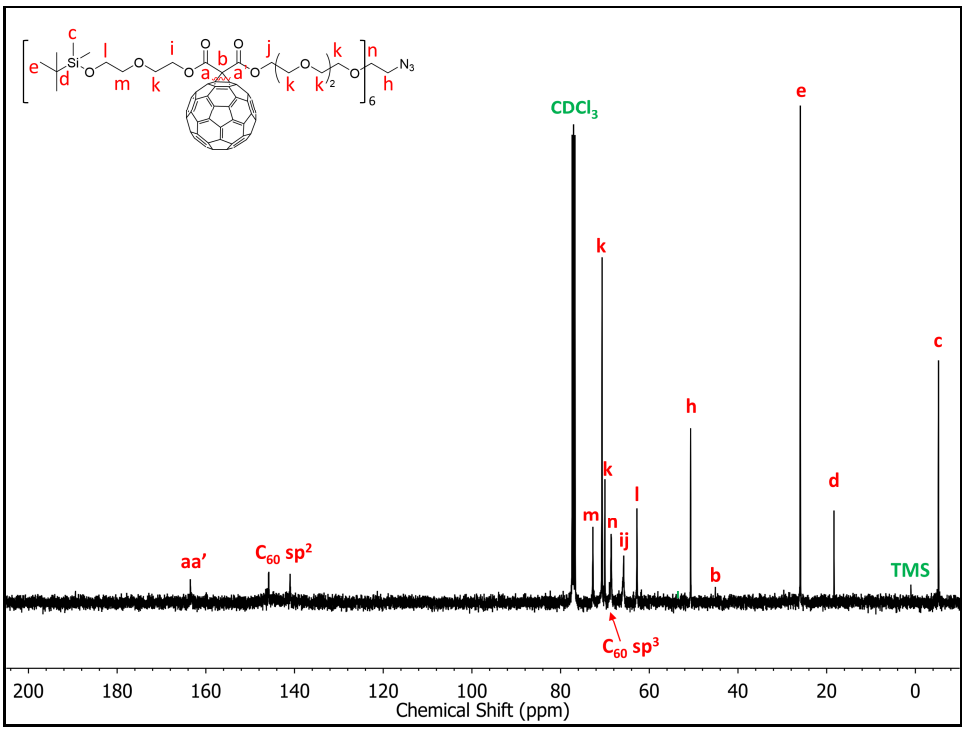


Figure S6. ^{13}C NMR of **Core-AzOSi**. Only two types of fullerene sp^2 carbons are visible, indicating that the scaffold is symmetrically substituted with 6 groups.

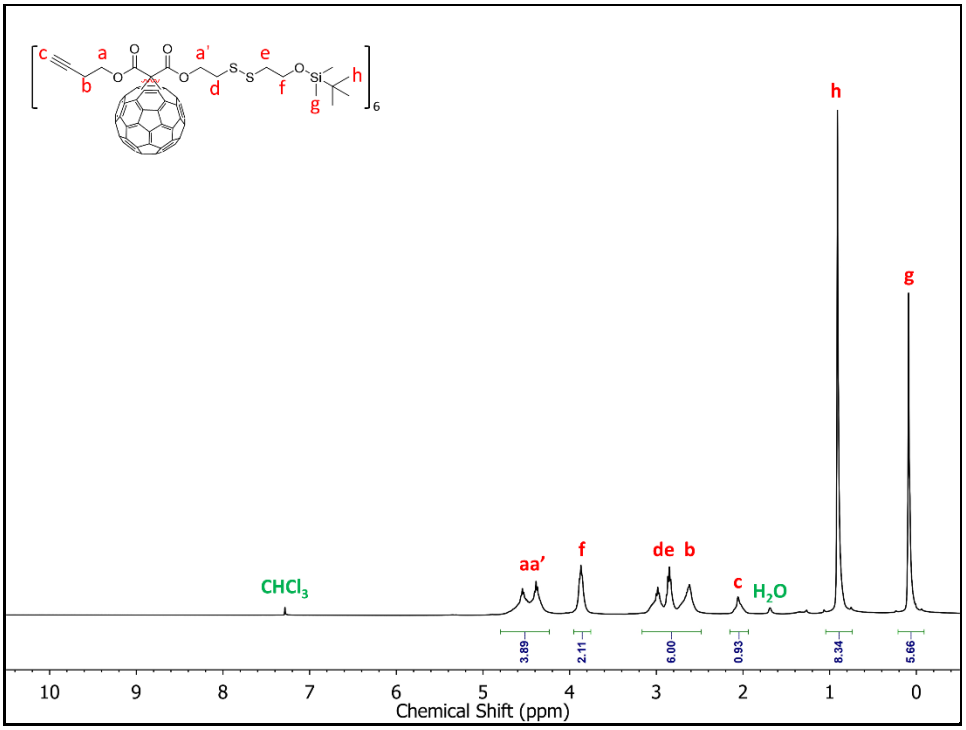


Figure S7. ^{1}H NMR spectrum of **Core-SSAk'**.

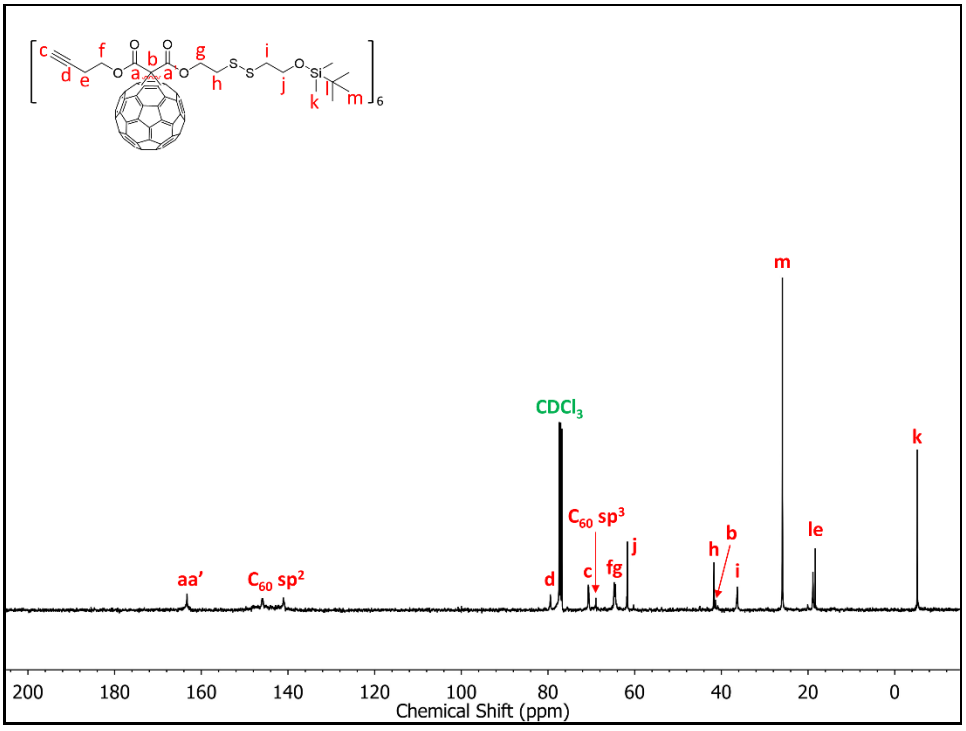


Figure S8. ^{13}C NMR of **Core-SSAk'**. Only two types of fullerene sp^2 carbons are visible, indicating that the scaffold is symmetrically substituted with 6 groups.

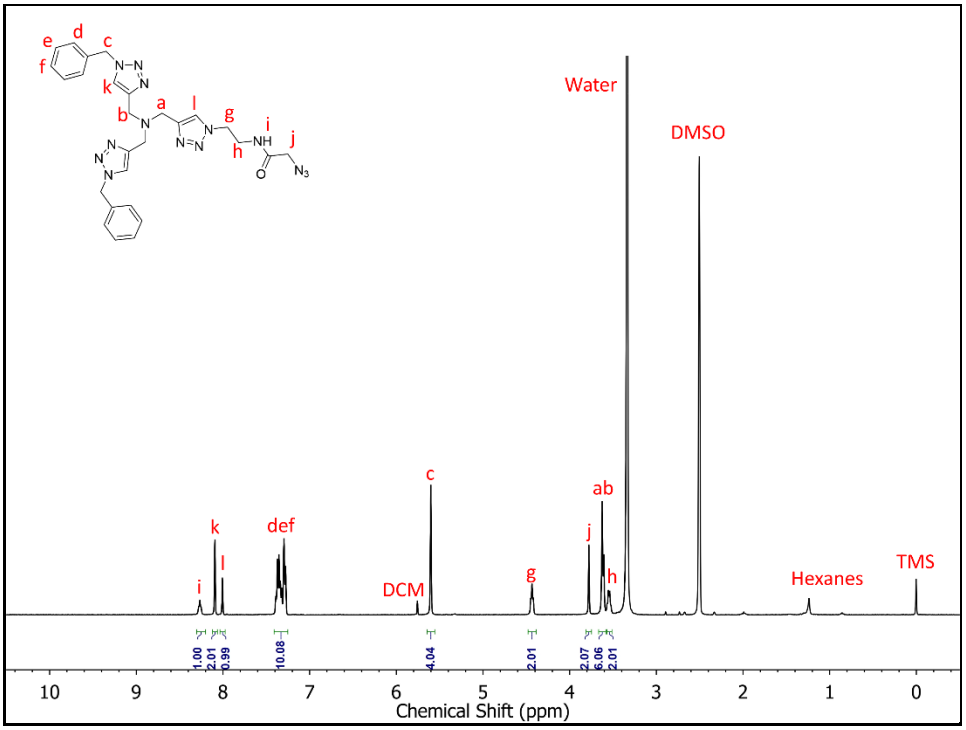


Figure S9. ^{1}H NMR spectrum of **Azido-TTA**.

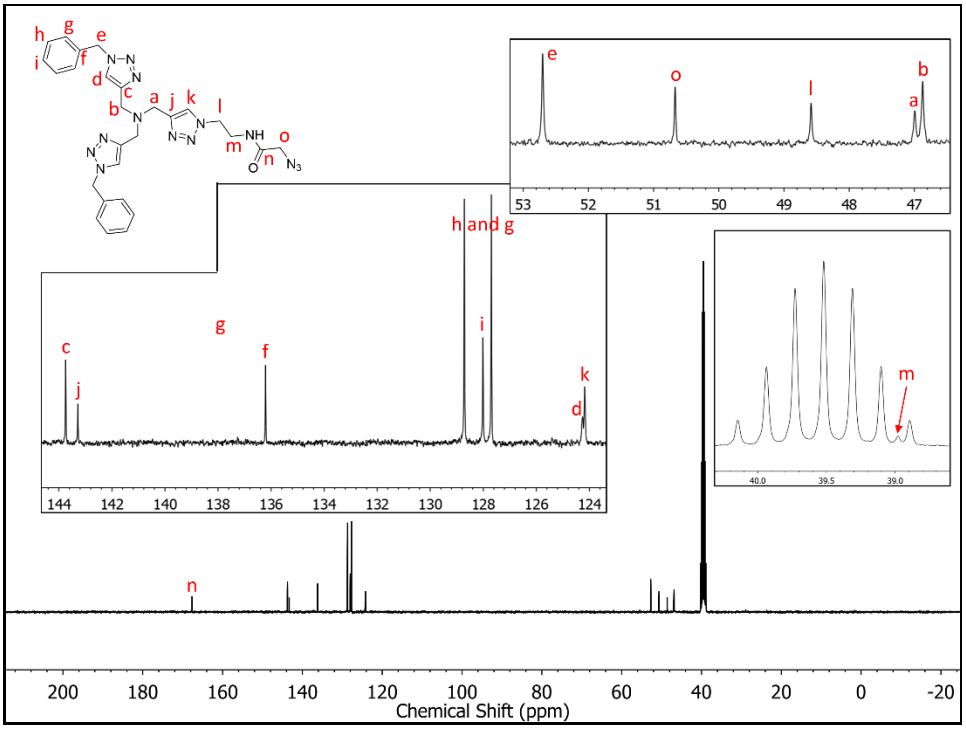


Figure S10. ^{13}C NMR spectrum of **Azido-TTA**.

HPLC and MALDI-TOF Characterization

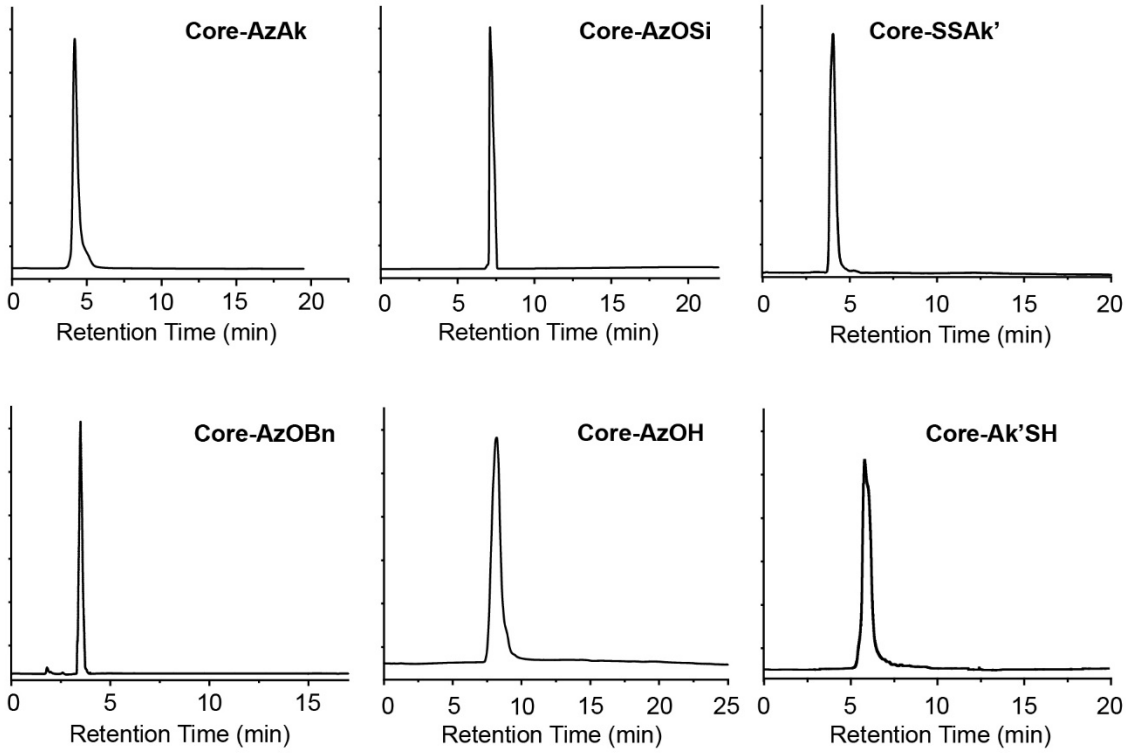


Figure S11. Purity quantification of bifunctional fullerene cores (Table 1 of manuscript) by normal phase HPLC (conditions varied). **Core-AzOH** was the hydroxy-deprotected product (by using TBAF) of **Core-AzOSi**. **Core-Ak'SH** was the disulfide-reduced product (by using dithiothreitol, DTT) of **Core-SSAk'**.

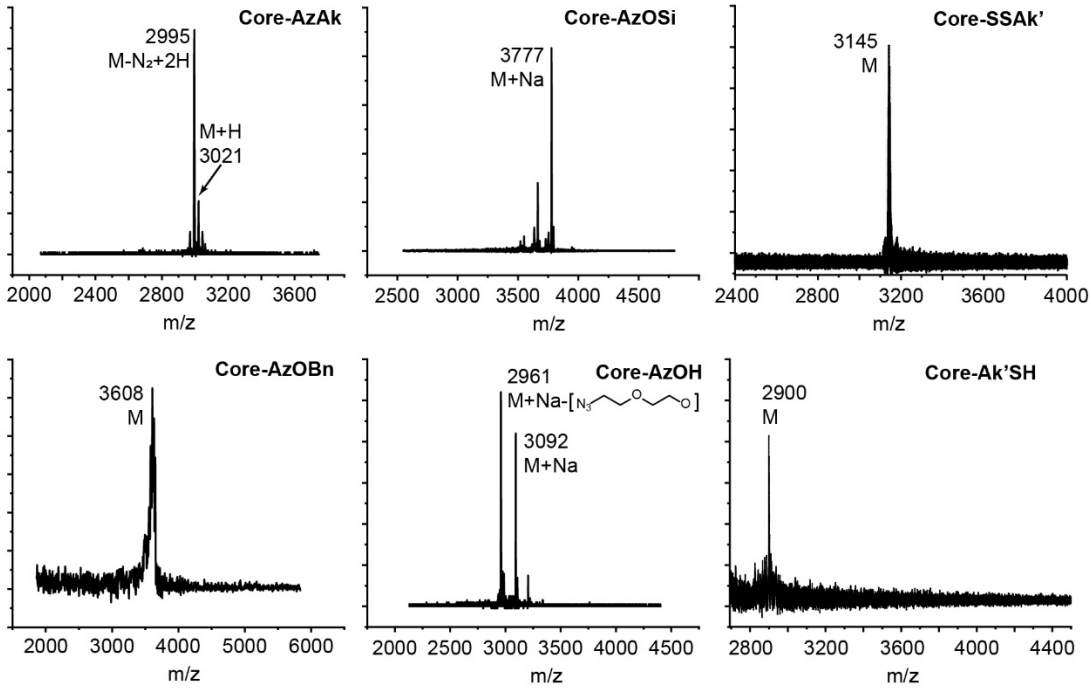


Figure S12. MALDI-TOF mass spectrometric characterization of various bifunctional fullerene cores. **Core-AzOH** was the hydroxy-deprotected product (by using TBAF) of **Core-AzOSi**. **Core-Ak'SH** was the disulfide-reduced product (by using dithiothreitol, DTT) of **Core-SSAk'**.

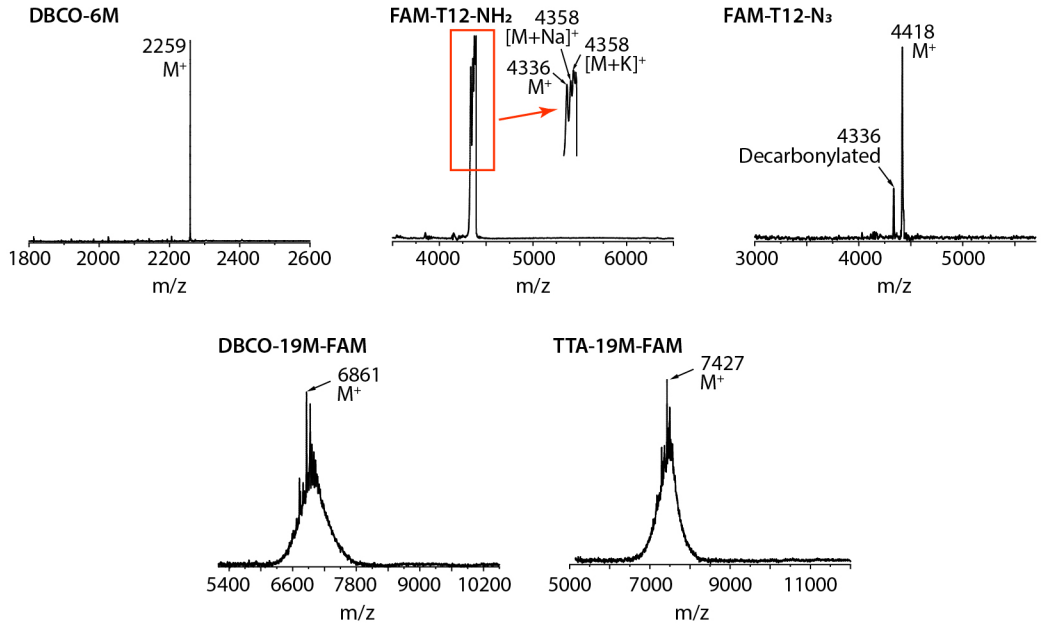


Figure S13. MALDI-TOF mass spectrometric characterization of various functional in-lab-prepared DNAs. DNAs from commercial sources (see Table S1) had their identity and quality confirmed by the manufacturer.

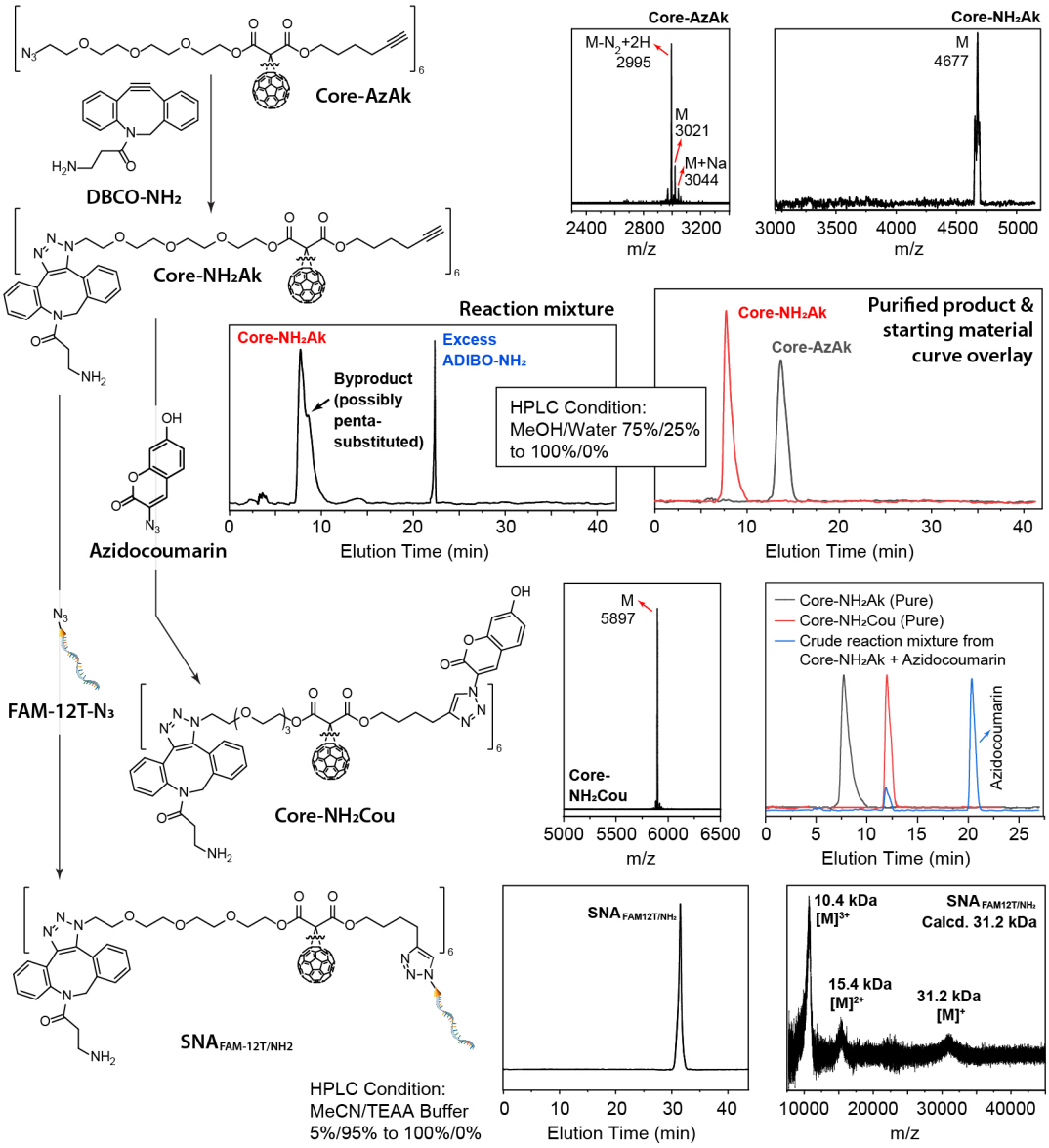


Figure S14. Validation of the structural integrity of **Core-AzAk** using derivatization method. Protocol for these transformations can be found in earlier sections of this document.

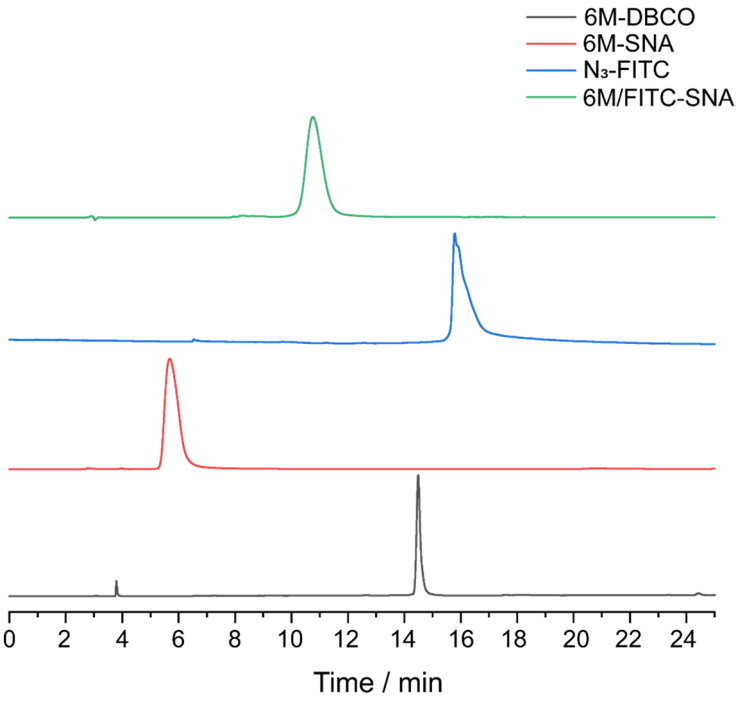


Figure S15. HPLC curves of DBCO-6M DNA (black), N_3-FITC (blue), 6-arm $SNA_{6M/Ak}$ (red) and $SNA_{6M/FITC}$ (green). Condition used: 0.05 M TEAA/ ACN, 0.95 / 0.05 to 0 / 1 in 15 min, v/v, 1 mL/min).

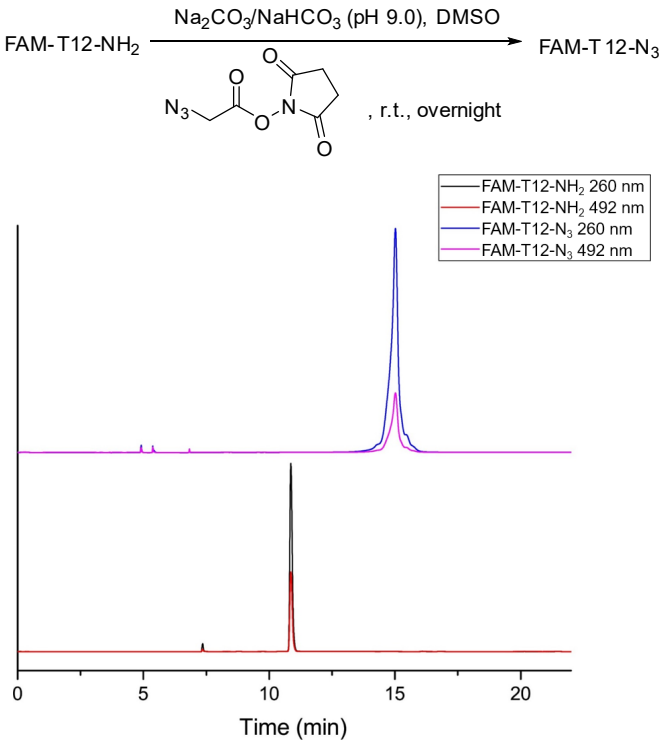


Figure S16. HPLC traces of FAM-T12-NH_2 DNA and FAM-T12-N_3 DNA (0.05 M TEAA / CH_3CN, 0.95 / 0.5 to 0.5 / 0.5 in 60 min, v/v, 1 mL/min).

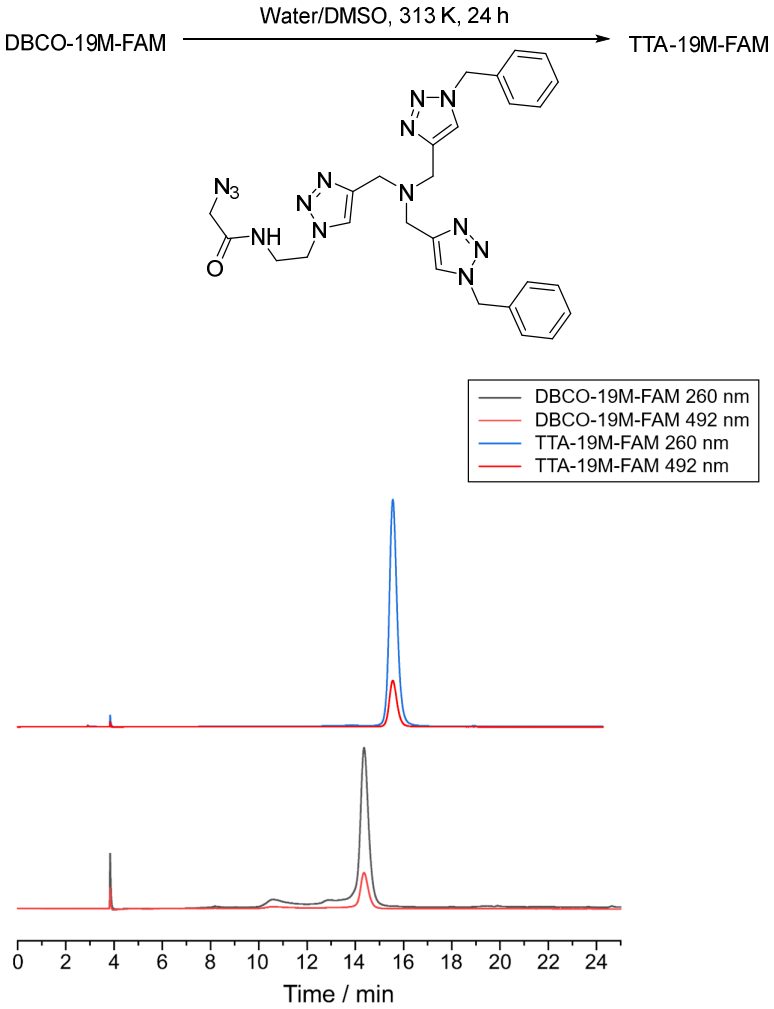


Figure S17. HPLC traces of DBCO-19M-FAM and TTA-19M-FAM. Condition used for analysis: 0.05 M TEAA / CH_3CN, 0.95 / 0.5 to 0.5 / 0.95 in 60 min, v/v, 1 mL/min.

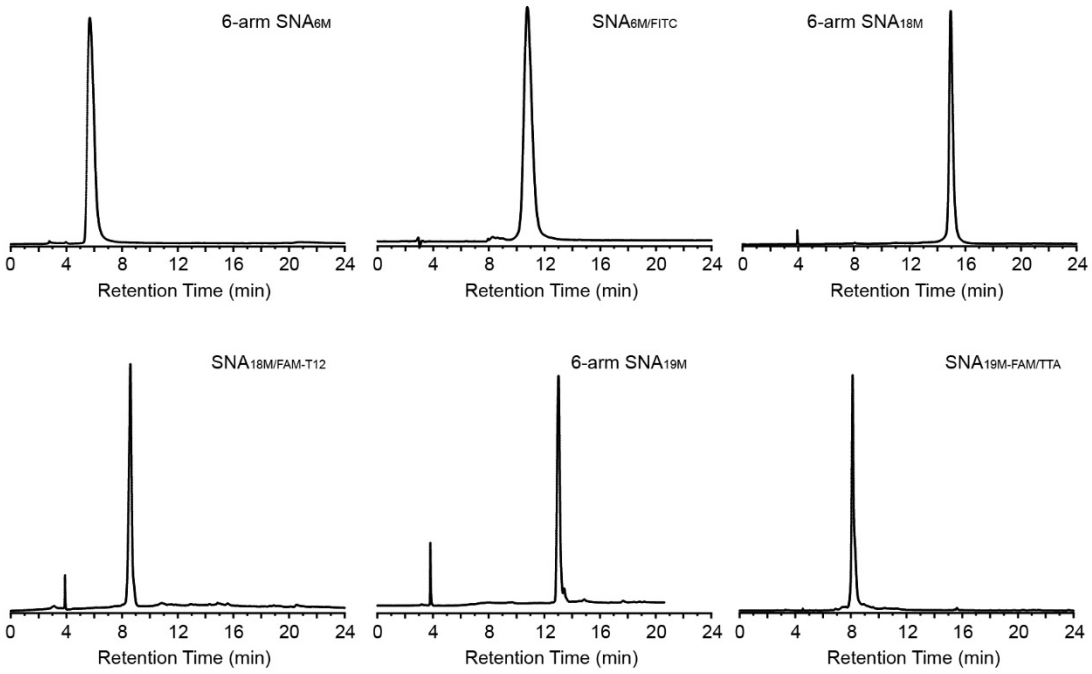


Figure S18. RP-HPLC traces of SNAs prepared in this study.

PAGE Analysis

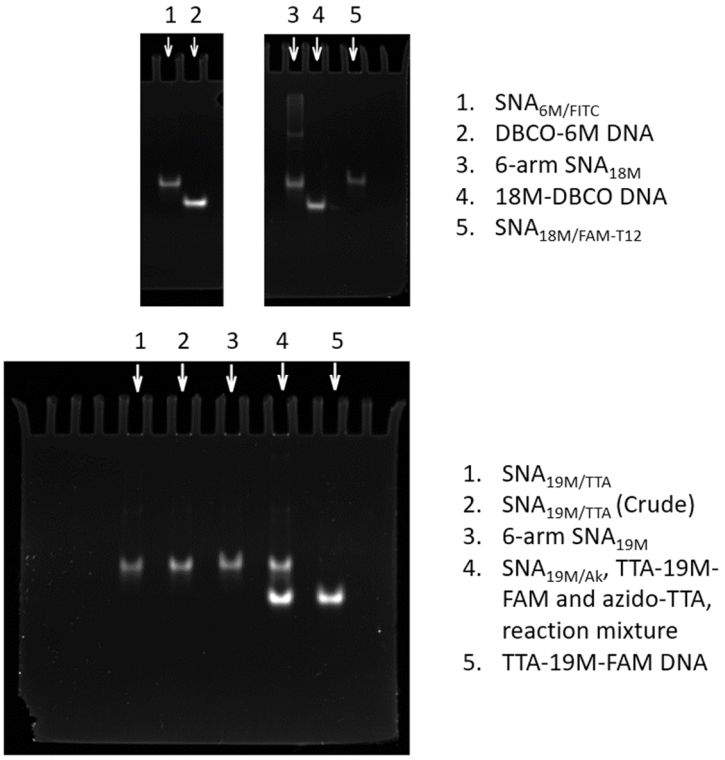


Figure S19. PAGE characterization of various SNAs and DNAs.

Quantification of Functional Moieties on SNAs

$SNA_{6M/FITC}$

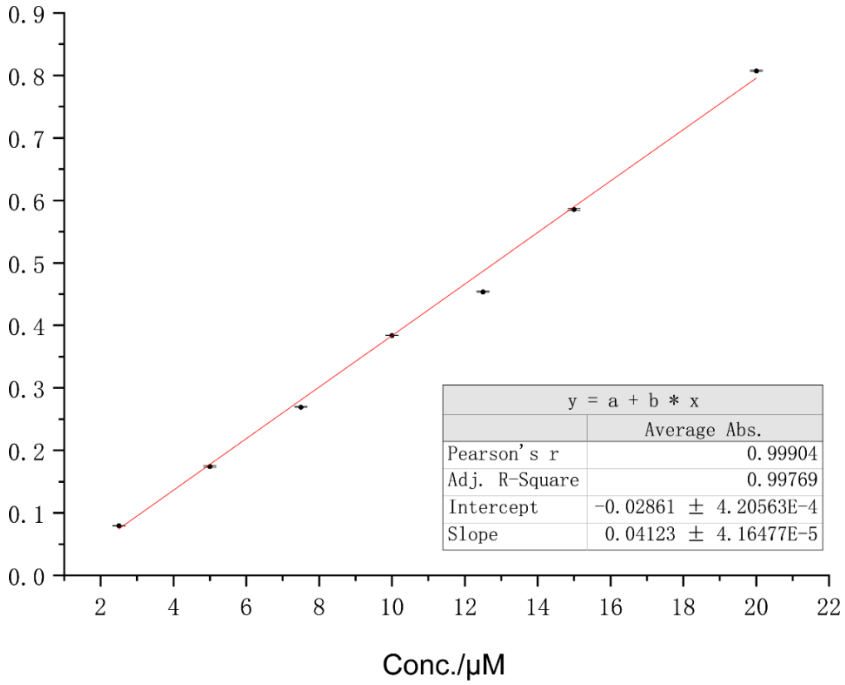


Figure S20. UV-Vis absorbance (490 nm) based calculation curve of FITC-N_3.

For the SNA (**$SNA_{6M/FITC}$**), the 490 nm absorbance was measured to be 0.217, thus the concentration of the FITC moiety was 6.0 μM. The concentration of the DNA strand was measured to be 5.8 μM (using NanoDrop 2000). Thus, the TEG-6M strands and FITC moieties were 1:1 on the fullerene core.

SNA$_{18M/FAM-T12}$

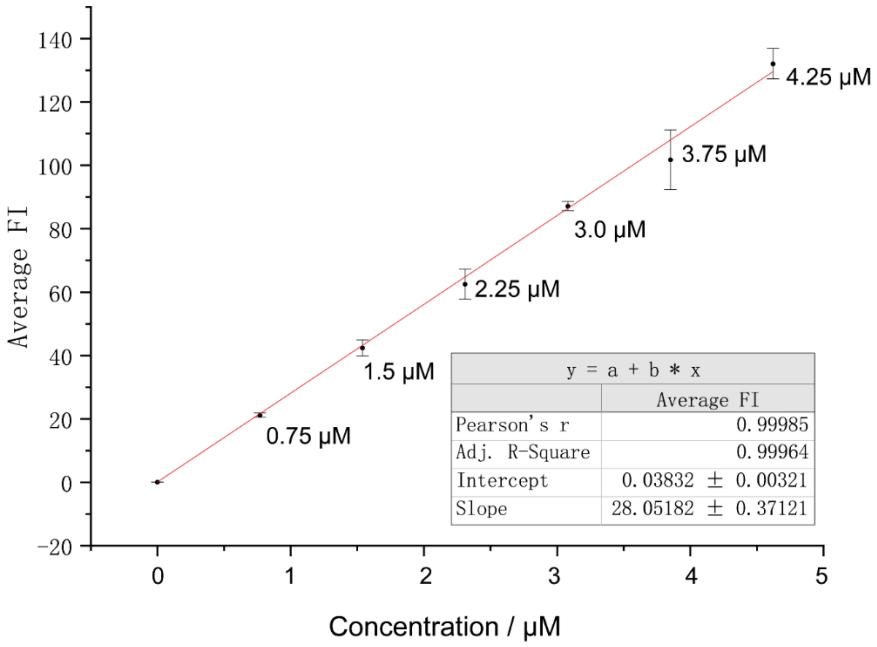


Figure S21. Fluorescence (490/525 nm) based calculation curve of FAM-T12 DNA.

Similar to the calculations for SNA$_{6M/FITC}$, the concentration of the FAM moiety was determined to be 1.2 μM. Using the combined ε of FAM-T12 and 18M-N_3, the concentration of (a hypothetical combination of) FAM-T12 plus 18M-N_3 was determined to be 0.94 μM. Thus, the two kinds of strands were approximately 1:1 on **SNA$_{18M/FAM-T12}$**.

Dynamic Light Scattering

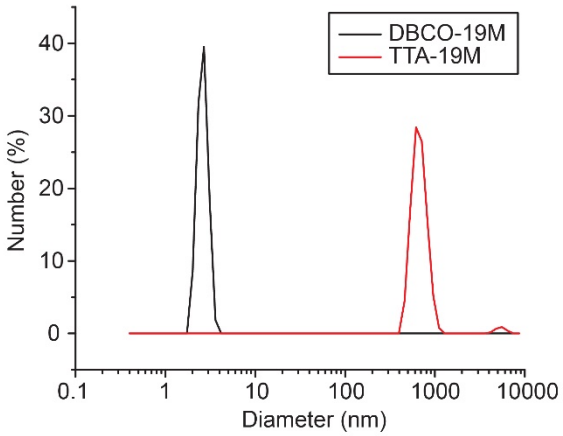


Figure S22. Dynamic light scattering analysis showed that TTA-19M formed large aggregates in PBS, while DBCO-19M showed no such behavior. Presumably, this was because TTA-19M had a larger hydrophobic head.

Additional Fluorescent Imaging Data

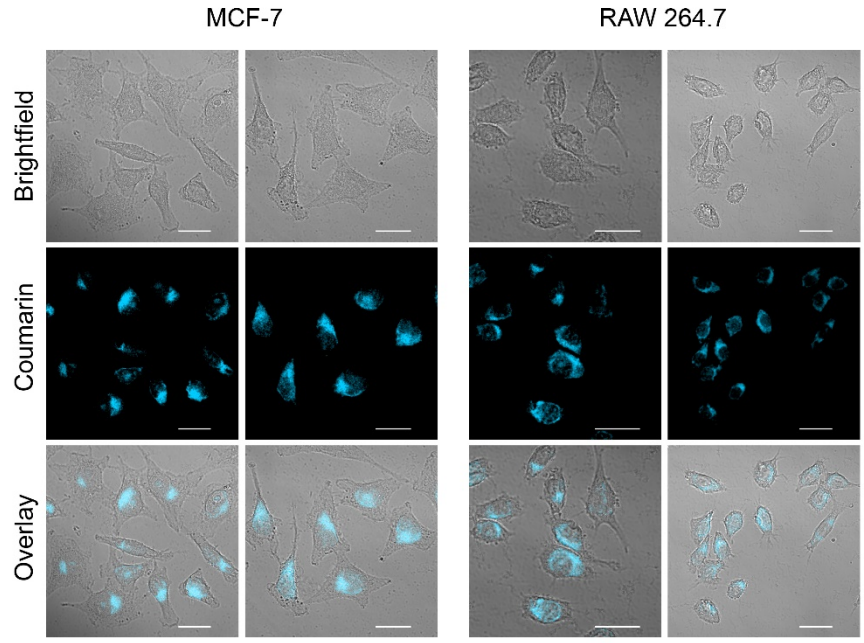


Figure S23. Additional confocal fluorescent images from the intracellular catalysis study. Coumarin derivative's fluorescence could be observed clearly inside cells only when Cu-SNA catalyst was employed.

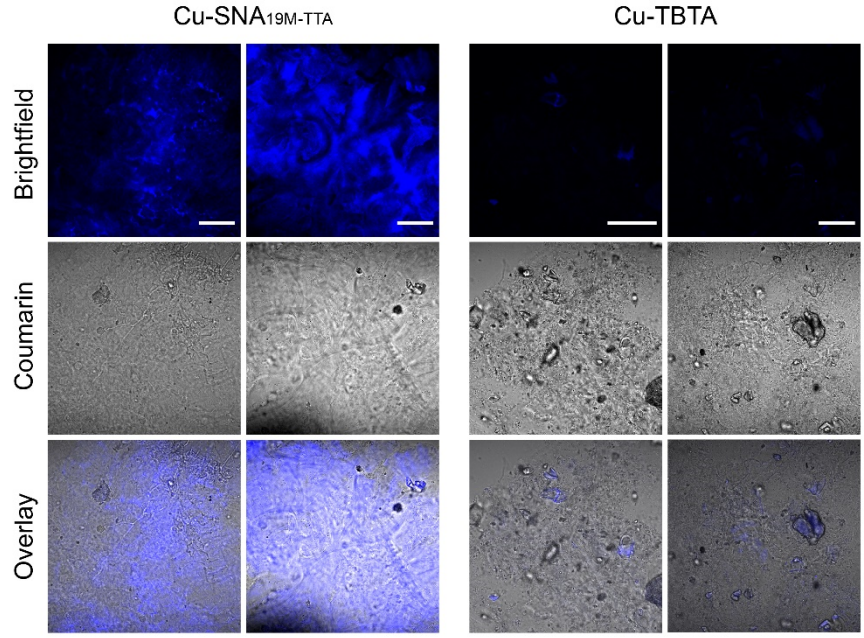


Figure S24. Additional confocal fluorescent images from the *in vivo* catalysis study. Coumarin derivative's fluorescence could be observed clearly in zebrafish feces only when Cu-SNA catalyst was employed.

Toxicity Evaluations

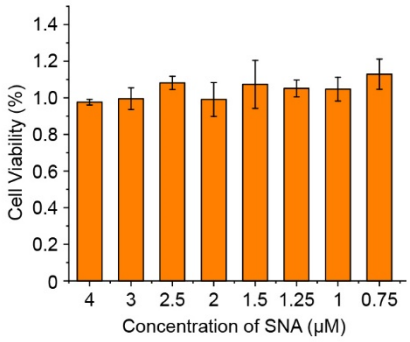


Figure S25. Cytotoxicity evaluation of the $SNA_{19M/TTA}$ using MTT assay with MCF-7 cells. Incubation time of SNA was 24 h.

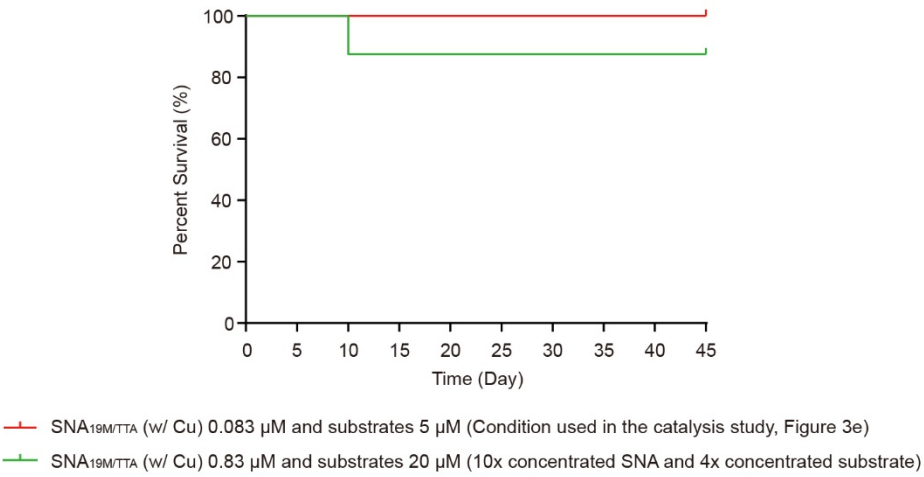


Figure S26. Toxicity evaluation of the combination of $SNA_{19M/TTA}$, $CuSO_4$ and substrates on live zebrafish. The solutions were injected in the same way as in the catalysis study.

References

1 J. H. Kwak, Z. Yang, B. Yoon, Y. He, S. Uhm, H.-C. Shin, B. H. Lee, Y. C. Yoo, K. B. Lee, S.-Y. Han and J. S. Kim, *Biomaterials*, 2015, **61**, 52-60.

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