Supporting Information

The Chiral Interfaces Fabricated by D/L-Alanine-Pillar[5]arenes for Selectively Adsorbing ctDNA

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I. Supplementary Methods

Materials.

Triphenylphosphine (CP), 4-dimethylaminopyridine (DMAP) (CP), acetonitrile (AR), paraformaldehyde (CP), 1,2-dichloroethane (AR), boron trifluoride etherate (AR), dimethylformamide (DMF) (AR), ethyl acetate (AR), trifluoroacetic (TFA) (AR), dichloromethane(DCM) (AR), 98% sulfuric acid (H_2SO_4) (AR), 30% hydrogen peroxide (H_2O_2) (AR), potassium hexacyanoferrate (II) ($K_4[Fe^{-}(CN)_6]\cdot 3H_2O$), potassium hexacyanoferrate (III) ($K_3[Fe^{-}(CN)_6]$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Copper sulfate pentahydrate, sodium ascorbate (AR) were acquired from Shanghai Chemical Reagent Co., Ltd. 1,4-Bis(2-hydroxyethoxy)benzene, carbon tetrabromide (AR), sodium azide (CP), were supplied by Aladdin. ctDNA (BR, 99.5%) was obtained from Sigma company. Alumina Powder (0.05 μm, 0.3 μm, 1.0 μm) was purchased from Gaoss Union company. All chemicals were used as procured without further purification. Aqueous solutions were prepared from deionized water. All ctDNA which was used in this paper was resolved in PBS buffer solution (pH = 7.4).

Instruments and Methods.

Nuclear magnetic resonance hydrogen spectrums (^{1}H-NMR) and nuclear magnetic resonance carbon spectrums (^{13}C-NMR) were measured on Varian Mercury Plus 600 spectrometer (USA). ALL mass spectrums were obtained on MALDI-TOF-MS spectrometer (USA). Circular dichroism spectrum (CD) was taken by CHIRASCAN. X-ray photoelectron spectra (XPS) data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300 W Al Kα radiation. All peaks were referenced to C 1s (CHx) at 284.8 eV in the deconvoluted high resolution C 1s spectra. Contact angle (CA) was measured on an OCA 20 contact angle meter (Germany). Atomic force microscope (AFM) images were recorded on a Bruker Multimode 8 AFM. All electrochemical measurements were performed on a CHI660C with software EC MFC Application. The gold electrode used for modification was consisted of a working electrode, a calomel reference electrode and a platinum auxiliary electrode. All EIS experiments were performed in 0.1 mol/L PBS solution (PH = 7.4) containing equimolar (5 mmol/L) $[Fe^{-}(CN)_6]^{3-/4-}$, and the experimental conditions were as follows: open-circuit potential, 0.023 V; frequency range, 1-105 Hz. The surface wettability of the electrode was evaluated by the detection of WCAs (3 μL of water droplets as the probe liquid) using an OCA 20 contact angle system. For measuring the underwater oil contact angle (UOCA), D/L-AP5 modified gold surfaces were first placed into a transparent glass container filled with ultrapure water. An oil droplet of 1,2-dichloroethane (3 μL) was then dropped onto the surface of D/L-AP5 modified electrodes using a 5 μL micro injector. Gaussian Calculation was carried out at the density functional theory b3lyp/6-31G (d) levels using Gaussian 03.

Synthesis process of Boc-D/L-propargylalanine.

Based on the literature,[1] Boc-L-alanine (Boc-D-alanine) (2.0 g, 10.6 mmol) and propargyl alcohol (1.19 g, 21.2 mmol) were added in anhydrous dichloromethane (75 mL) under protection of nitrogen atmosphere. The mixture was stirred at 0 °C for 0.5 h. Then, the DMAP and EDC were added in the solution and stirred for another 0.5 h. At last, the reaction mixture was stirred at room temperature overnight to give white precipitation. The mixture was filtered, collected, and washed with saturated brines (2 × 25 mL). The organic phase was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure. Purification via flash chromatography afforded colorless oily liquid (2.2 g, yeild: 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.07 (s, 1H, CCHC), 4.85-4.62 (m, 2H, OCH_2C), 2.58-2.40 (m, 1H, CCH), 1.62-1.23 (m, 12H, CCH_3) ppm.

Synthesis process of 1,4-Bis(2-bromoethoxy)benzene Monomer (4).

According to the literature,[2] 1,4-bis(2-hydroxyethoxy)benzene (5.0 g, 25.2 mmol) and triphenylphosphine (16 g, 60 mmol) were added in acetonitrile (150 mL) and cooled with an ice bath. Under vigorous stirring, carbon tetra bromide (20.0 g, 60 mmol) was slowly added. The mixture was stirred at room temperature for 4 hours. Then, cold water (100 mL) was added to the reaction mixture to give white precipitation. The precipitate was filtered, collected, and washed with water and little methanol. At last, the precipitate was recrystallized from methanol, and dried under vacuum to afford Br-M as white solid (1.9 g, yeild: 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (s, 4H, ArH), 4.23 (t, J = 5.9 Hz, 4H, OCH_2C), 3.61 (t, J = 6.1 Hz, 4H, CCH_2Br) ppm.

Synthesis process of 1,4-Bis(2-bromoethoxy)pillar[5]arene (2).

1,4-Bis(2-bromoethoxy)pillar[5]arene (Br-P5) was synthesized according to the method described by Li group.[3] Br-M (3.37 g, 11.5 mmol) and paraformaldehyde (0.349 g, 11.5 mmol) were dissolved in 1,2-dichloroethane (50 mL) and cooled with ice bath. Boron trifluoride etherate (3.26 g, 23.0 mmol) was added to the solution and the mixture was stirred at room temperature for 1 hour. The reaction mixture was then washed with water (2 × 50 mL) and dried with anhydrous Na_2SO_4. The solvent was evaporated to provide a crude product, which was purified by column chromatography (eluent: petroleum ether/dichloromethane = 4/1) to afford a white solid (1.8 g, yeild: 54%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.93 (s, 10H, ArH), 4.24 (t, J = 6.0 Hz, 20H, OCH_2C), 3.86 (s, 10H, $ArCH_2Ar$), 3.66 (d, J = 6.0 Hz, 20H, CCH_2Br) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ 148.54, 127.92, 114.96, 67.84, 29.75, 28.30 ppm.

Synthesis process of Nitrine-Pillar[5]arene (1).

Nitrine-pillar[5]arene (N_3-P5) was synthesized according to the previous procedure.[4] Sodium azide (1.93 g, 30 mmol) and Br-P5 (1.0 g, 0.6 mmol) were added in 50 mL DMF. After stirring at 100 °C for 12 h under protection of nitrogen atmosphere, the mixture was cooled to room temperature and poured into water (100 mL). The precipitate was collected by filtration, and washed with water to yield N_3-P5 as white solid (1.2 g, yeild: 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (s, 10H, ArH), 4.12-3.93 (m, 20H, OCH_2C), 3.83 (s, 10H, $ArCH_2Ar$), 3.64-3.44 (m, 20H, CCH_2Br) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): 148.75, 127.75, 114.51, 66.35, 49.86, 28.51 ppm.

Synthesis process of Boc-D/L-Alanine-Pillar[5]arene (Boc-D/L-AP5).

Based on the previous literature,[5] N_3-P5 (130 mg, 0.1 mmol), Boc-L-propargylalanine (Boc-D-propargylalanine) (272 mg, 1.2 mmol), copper sulphate pentahydrate (25 mg, 0.1 mmol) and sodium ascorbate (40 mg, 0.2 mmol) were added in anhydrous DMF (5 mL). The mixture was heated to 80 °C for 12 h under protection of nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (20 mL) and washed with saturated brines (4 × 25 mL). The organic phase was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure. Purification via flash chromatography afforded target molecules as a faint yellow solid (325 mg, yeild: 91%). ^{1}H NMR (600 MHz, DMSO): δ 8.31 (d, J = 6.0 Hz, 10H, CNHC), 7.29 (d, J = 7.2 Hz, 10H, CCHN), 6.73 (s, 10H, ArH), 5.18 (dd, J = 37.8, 12.9 Hz, 20H, OCH_2C), 4.85 (s, 20H, OCH_2C), 4.48 (s, 10H, CCH_2N), 4.12 (s, 10H, CCH_2N), 4.02-3.92 (m, 10H, $ArCH_2Ar$), 3.13 (s, 10H, CCHC), 1.32 (d, J = 18.0 Hz, 90H, t-Bu), 1.14 (t, J = 6.0 Hz, 30H, CCH_3) ppm; ^{13}C NMR (150 MHz, DMSO): δ 170.73, 160.01, 152.63, 146.84, 140.08, 126.20, 121.91,

113.48, 77.27, 64.84, 55.69, 47.70, 46.62, 25.64, 15.58 ppm; MS Calcd.for m/z = 3571.8 found: m/z = 3595.2 [M+Na^+].

Synthesis process of D/L-Alanine-Pillar[5]arene (D/L-AP5).

Boc-D/L-AP5 (200 mg, 0.047 mmol) was added in anhydrous dichloromethane (20 mL). Then trifluoroacetic acid (3.5 mL, 47 mmol) was added to the solution and the mixture was stirred at room temperature for 4 hours. The solvent was evaporated to provide a crude product, which was purified by recrystallizing from methanol and diethyl ether, and dried under vacuum to afford D/L-AP5 as faint yellow solid (115 mg, yeild: 92%). ^{1}H NMR (600 MHz, DMSO): δ 8.57 (s, 20H, CNH_2), 8.41 (s, 10H, CCHN), 6.77 (s, 10H, ArH), 5.33 (dd, J = 28.8, 11.8 Hz, 20H, OCH_2C), 4.88 (s, 20H, OCH_2C), 4.50 (s, 10H, CCHC), 4.12 (d, J = 26.3 Hz, 20H, CCH_2N), 3.12 (s, 10H, $ArCH_2Ar$), 1.34 (s, 30H, CCH_3) ppm; ^{13}C NMR (150 MHz, DMSO): δ 169.88, 158.21, 148.75, 141.47, 128.35, 114.85, 67.27, 58.70, 55.10, 49.86, 47.86, 15.63 ppm; MS Calcd.for m/z = 2571.2 found: m/z = 2591.4 [M+Na^+].

Synthesis process of D/L-Alanine-Monomer (D/L-AM).

The synthesis process D/L-AM was as same as L/D-AP5. The first step included the preparation of 1,4-Bis(2-nitrineethoxy)benzene Monomer (N_3-M) (**3**) by modification of Br-M. In the following step, Boc-D/L-alanine were introduced in N_3-M to achieve Boc-L/D-AM. At last, L/D-AM were achieved by eliminating the Boc-group from Boc-L/D-AM (yeild: 81%). ^{1}H NMR (600 MHz, DMSO): δ 8.43 (s, 4H, CHN_2), 8.26 (s, 2H, CCHN), 6.82 (s, 4H, ArH), 5.27 (s, 2H, OCH_2C), 4.72 (s, 4H, OCH_2C), 4.30 (s, 4H, CCH_2N), 4.11 (s, 2H, CCHC), 1.35 (d, J = 6.9 Hz, 6H, CCH_3) ppm; ^{13}C NMR (150 MHz, DMSO): δ 172.47, 161.20, 154.89, 143.73, 118.16, 57.64, 51.84, 50.50, 18.29 ppm.

The Fabrication of chiral interfaces.

The beginning step involved the activation of gold electrode (GE) according to references which had been reported.[6] In the following step, transforming GE to GE-COOH was achieved by immersing GE in mercaptoacetic acid solution (10^{-3} mol/L) for 4 h at room temperature. Then, GE-COOH was washed with small amount of deionized water and dried with nitrogen. After this, GE-COOH was active by immersing in aqueous solution (4 mL) of 1-(3-dimethylaminopropyl)-3-ethylenediamine hydrochloride (EDC) (30 mg) and N-hydroxysuccinamide (NHS) (10 mg) for 1 h. In the last step, in order to achieve L-AP5 and D-AP5 modified electrodes, GE-COOH was immersed in L-AP5 and D-AP5 solution (10^{-3} mol/L) for 24 h, then flushed with small amount of deionized water and dried with nitrogen. The approach of fabricating D/L-AM-interfaces was as same as D/L-AP5-interfaces.

Characterization.

XPS data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300 W Al Kα radiation. All peaks were referenced to C 1s (CHx) at 284.8 eV in the deconvoluted high resolution C 1s spectra. The surface composition was investigated using the integrated peak areas. AFM images were recorded on a Bruker Multimode 8 AFM. The images were acquired in acoustic mode using silicon nitride tips (resonance frequency c.a 350 kHz). All electrochemical measurements were performed on a CHI660C with software EC MFC Application. The gold electrode used for modification was consisted of a working electrode, a calomel reference electrode and a platinum auxiliary electrode. All EIS experiments were performed in 0.1 mol/L PBS solution (PH = 7.4) containing equimolar (5 mmol/L) $[Fe^-(CN)_6]^{3-/4-}$, and the experimental conditions were as follows: open-circuit potential, 0.023 v; frequency range, 1-10^5 Hz. For measuring the underwater oil contact angle (UOCA),[7] D/L-AP5 modified gold surfaces were first placed into a transparent glass container filled with ultrapure water.

An oil droplet of 1,2-dichloroethane (3 µL) was then dropped onto the surface of D/L-AP5 modified gold interfaces by using a 5 µL micro injector.

II. Supplementary Figures

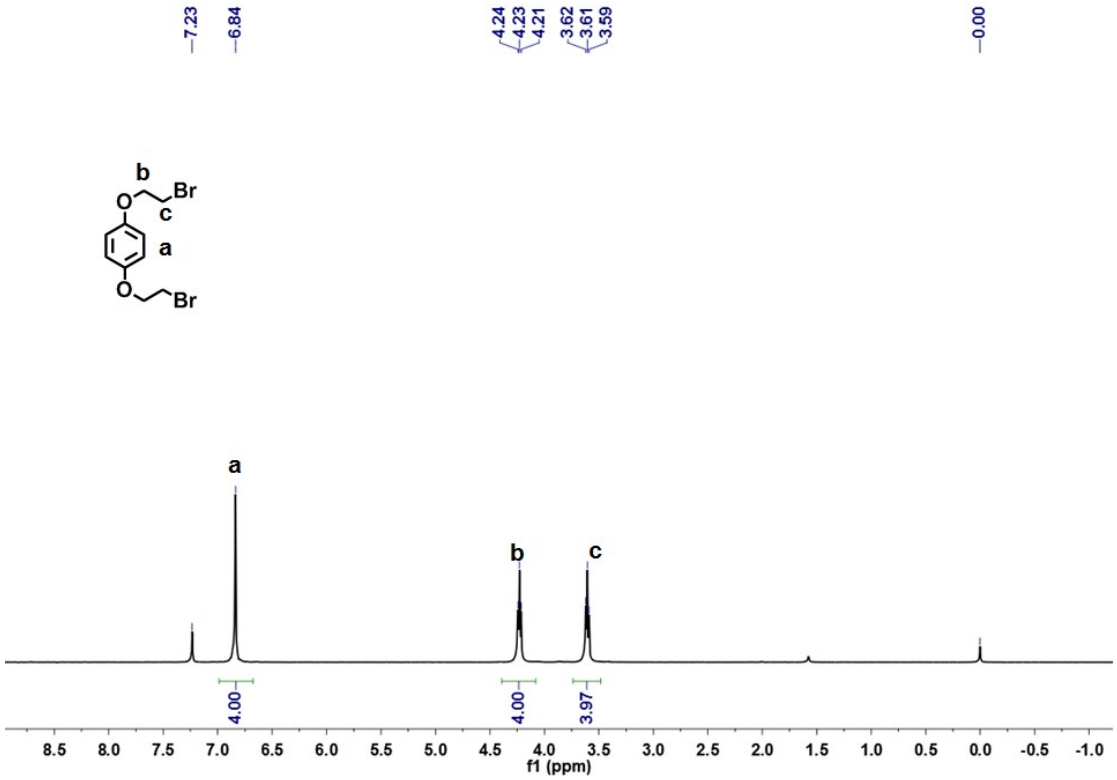

Figure S1. ^{1}H NMR spectrum (600 MHz, $CDCl_3$, 293 K) of Br-M.

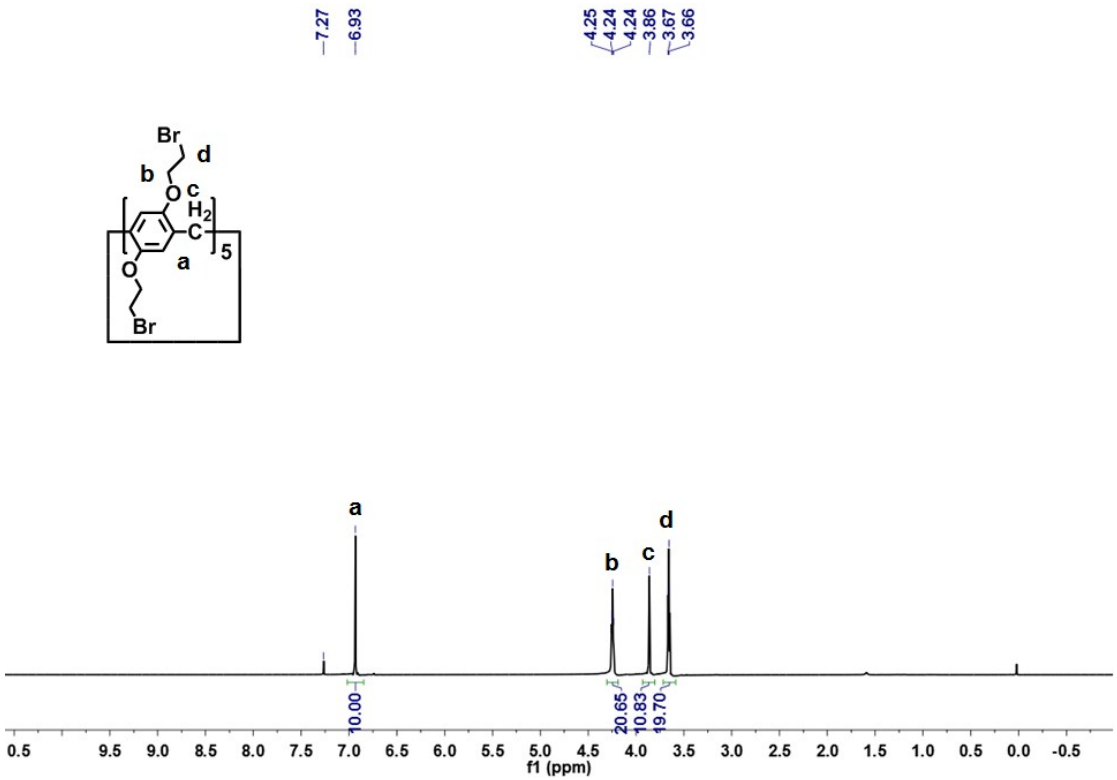

Figure S2. ^{1}H NMR spectrum (600 MHz, $CDCl_3$, 293 K) of Br-P5.

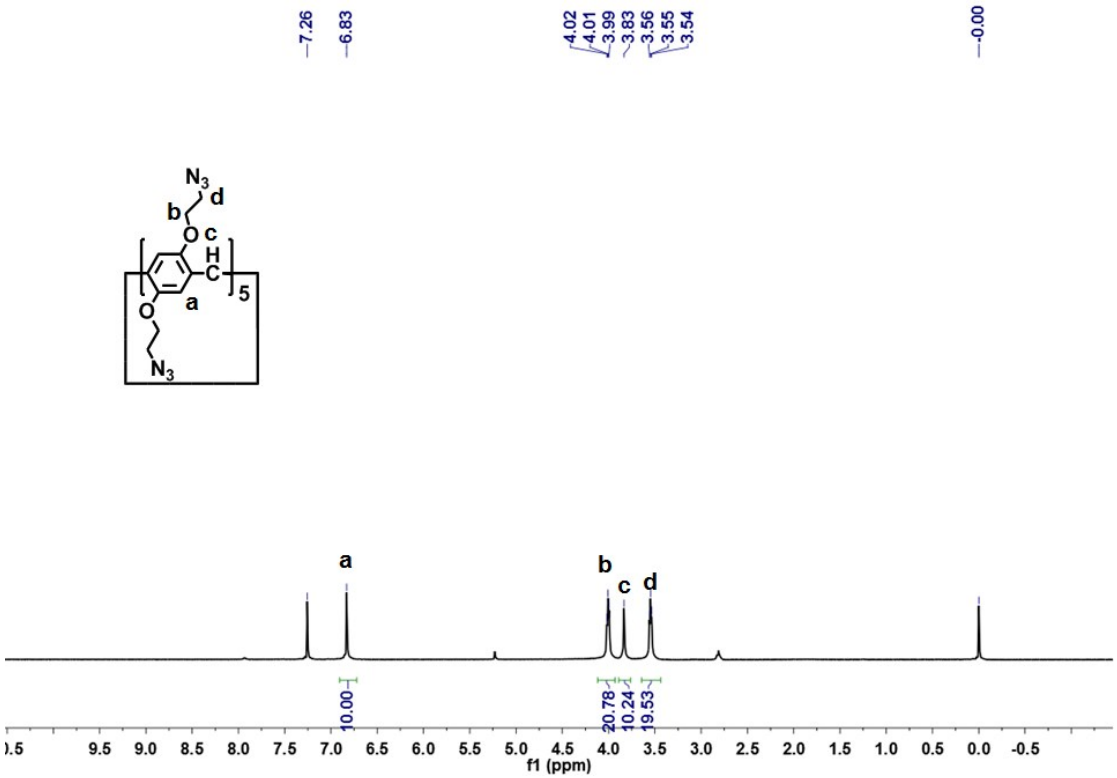

Figure S3. ^{1}H NMR spectrum (600 MHz, $CDCl_3$, 293 K) of N_3-P5.

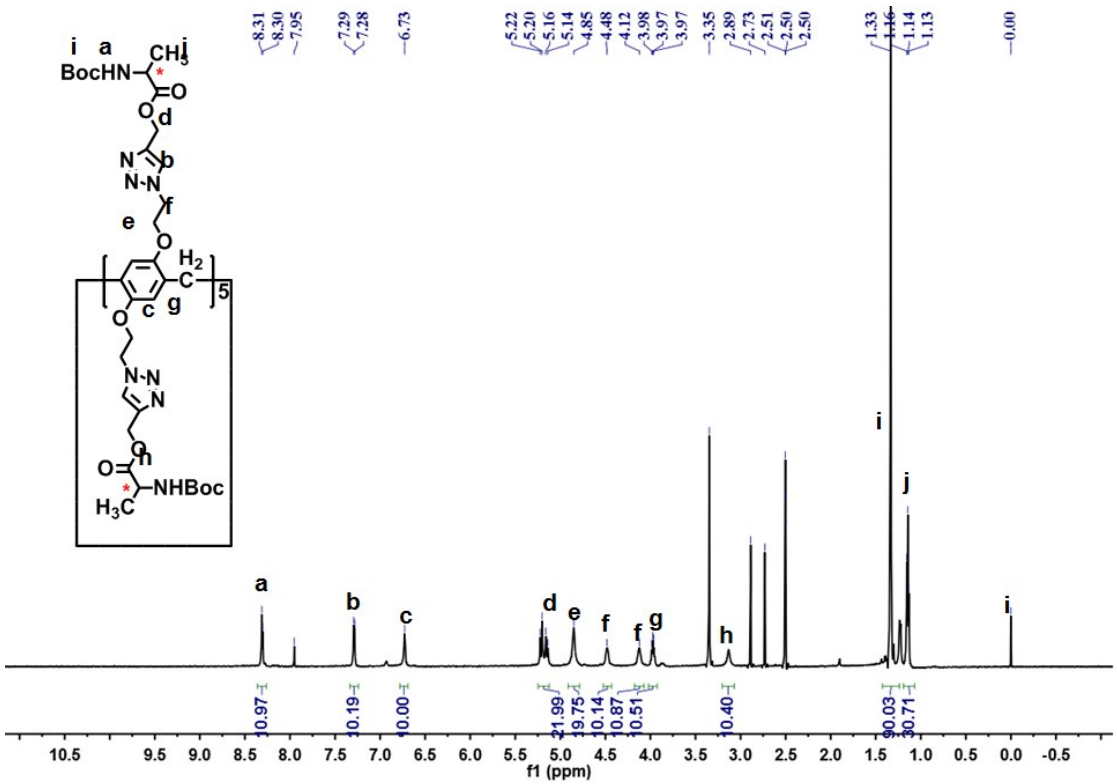

Figure S4. ^{1}H NMR spectrum (600 MHz, DMSO-d6, 293 K) of Boc-L/D-AP5.

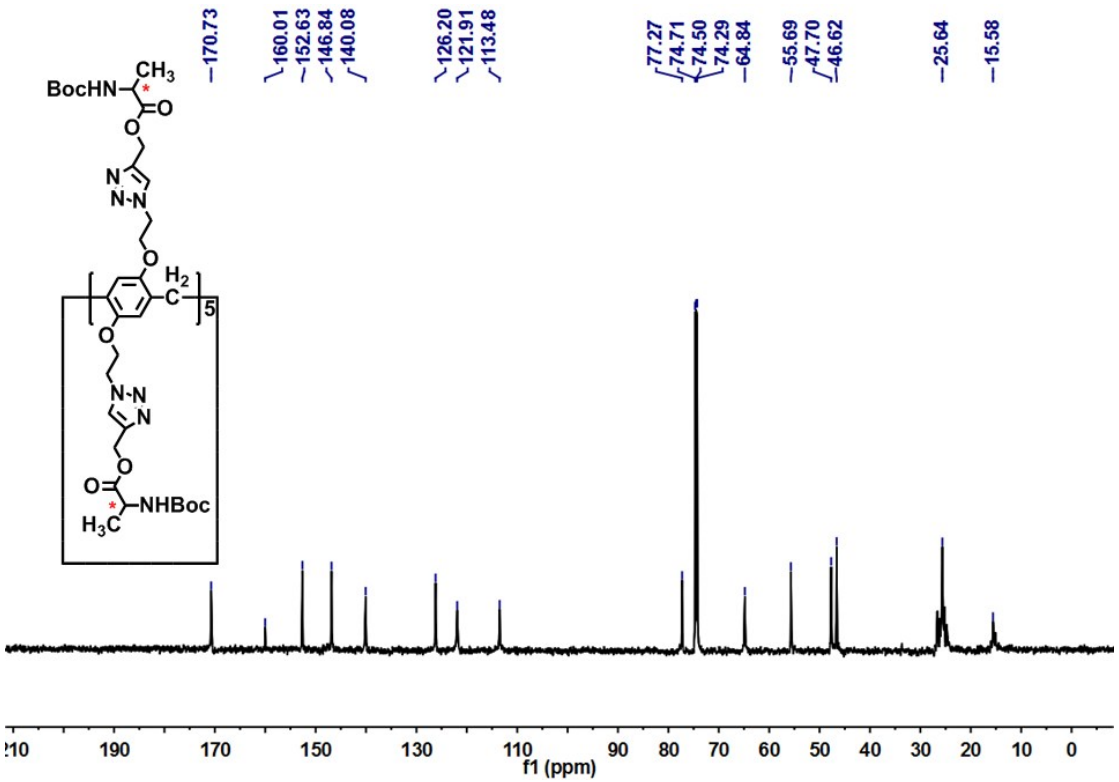

Figure S5. ^{13}C NMR spectrum (150 MHz, DMSO-d6, 293 K) of Boc-L/D-AP5.

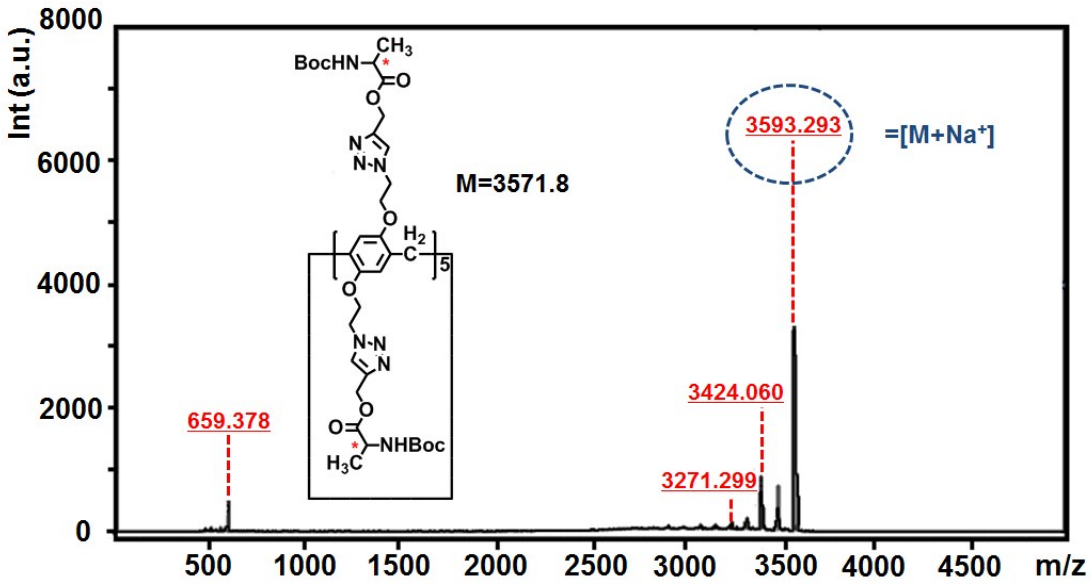

Figure S6. MS spectrum of Boc-L/D-AP5.

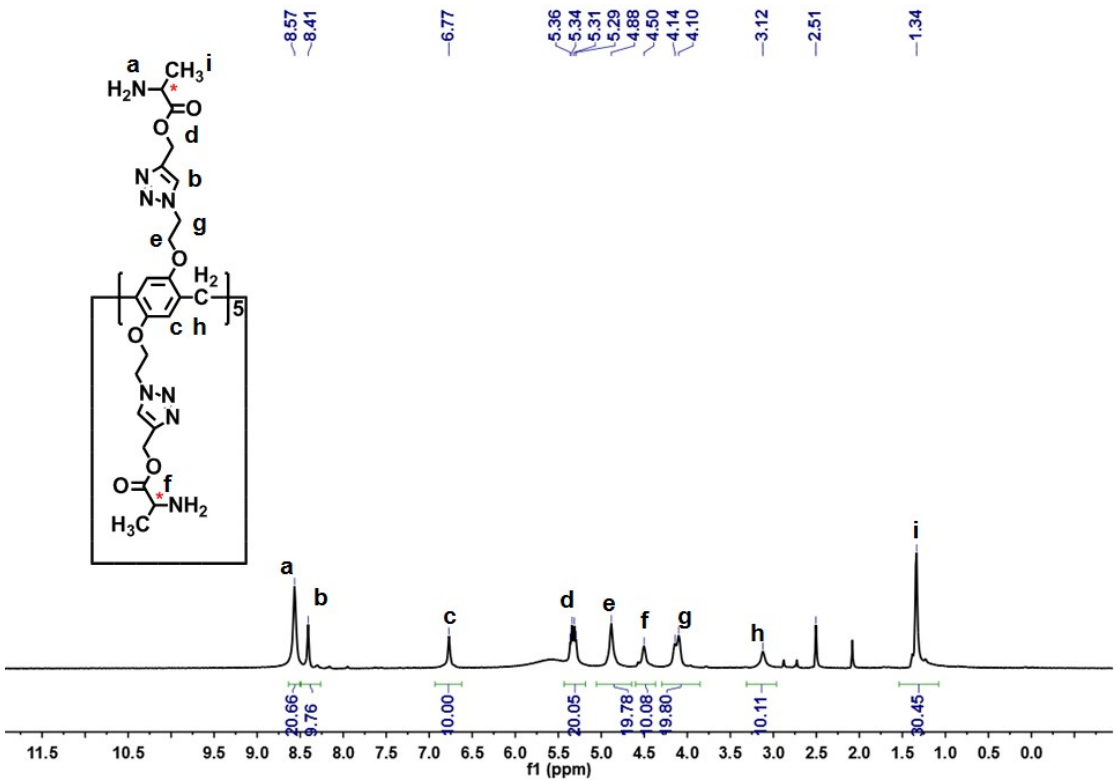

Figure S7. ^{1}H NMR spectrum (600 MHz, DMSO-d6, 293 K) of L/D-AP5.

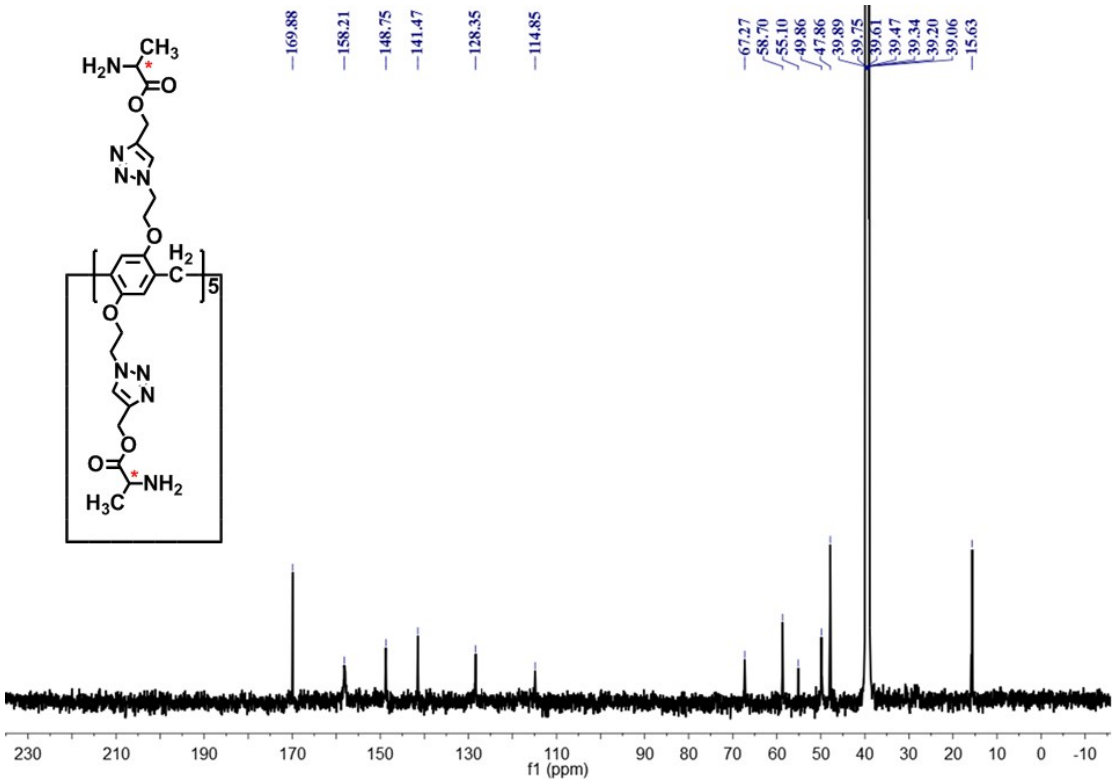

Figure S8. ^{13}C NMR spectrum of L/D-AP5.

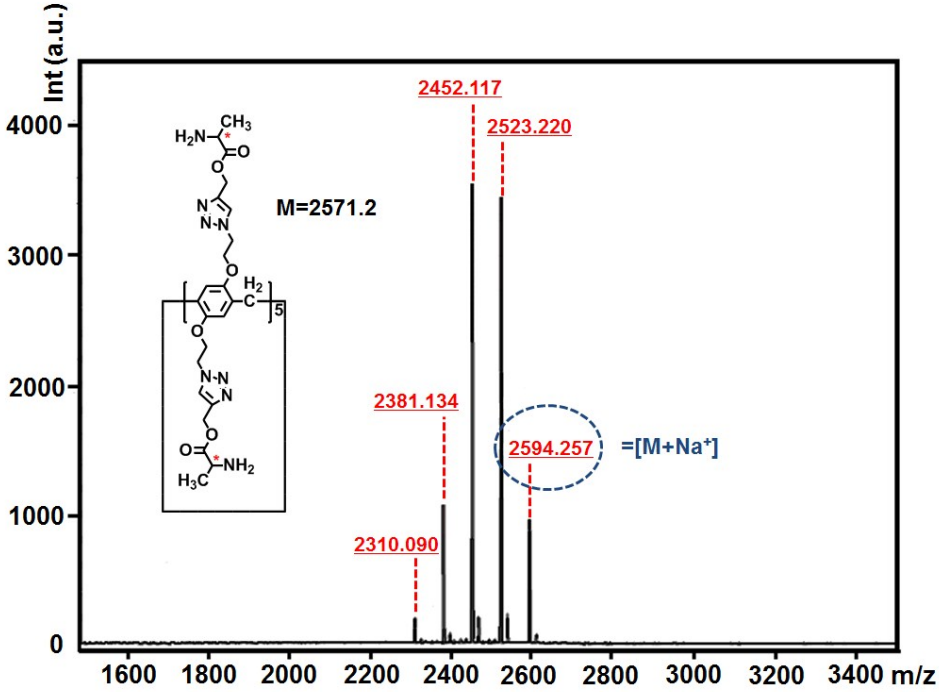

Figure S9. MS spectrum of L/D-AP5.

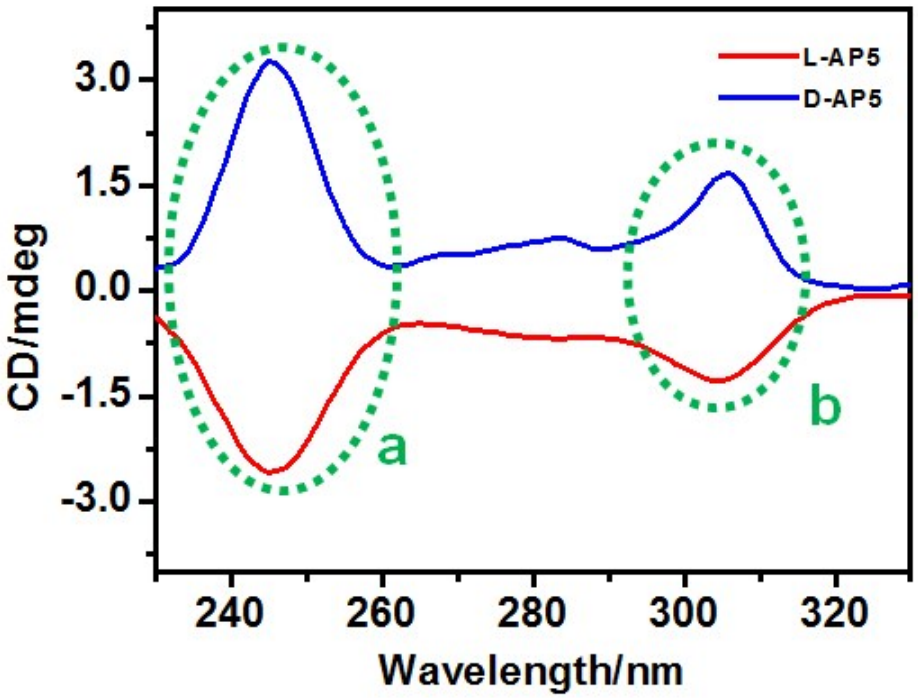

Figure S10. The CD spectrum of L/D-AP5 resolved in water solution under the concentration of 2.0×10^{-5} mol/L. The CD exhibited that the peak at 245 nm was considered to be D/L-alanine group, and the peak at 304 nm was attributed to the planar chirality of D/L-AP5.

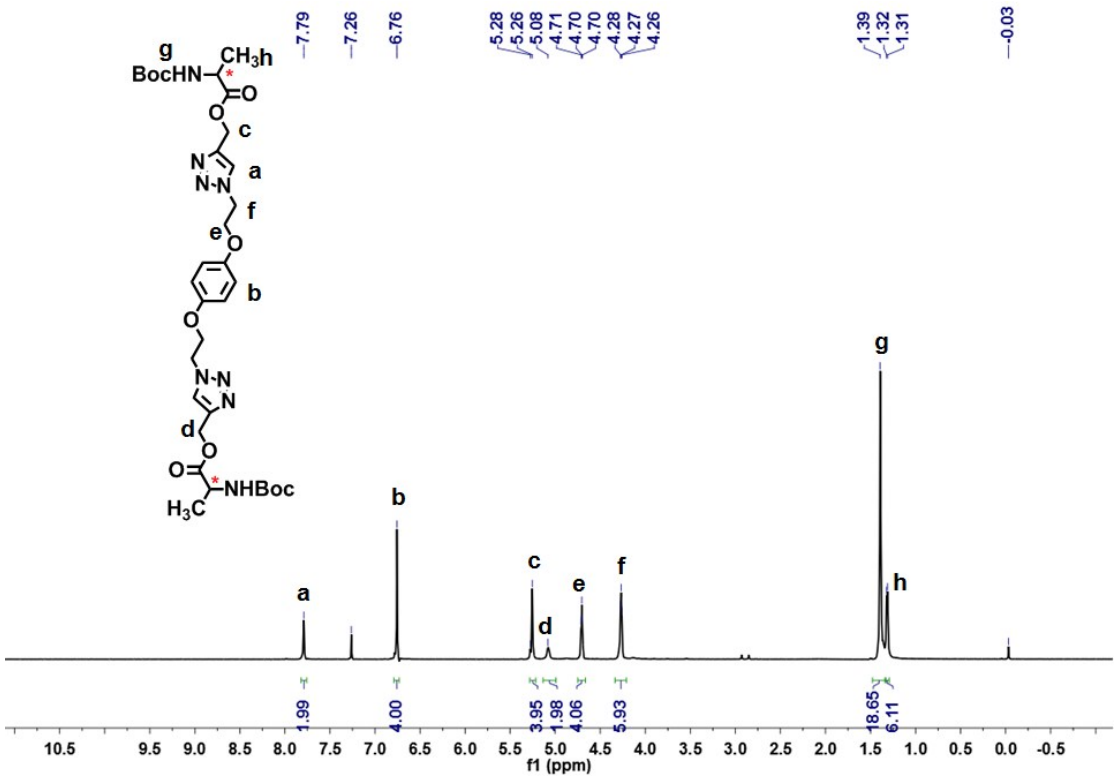

Figure S11. ^{1}H NMR spectrum (600 MHz, $CDCl_3$, 293 K) of Boc-L/D-AM.

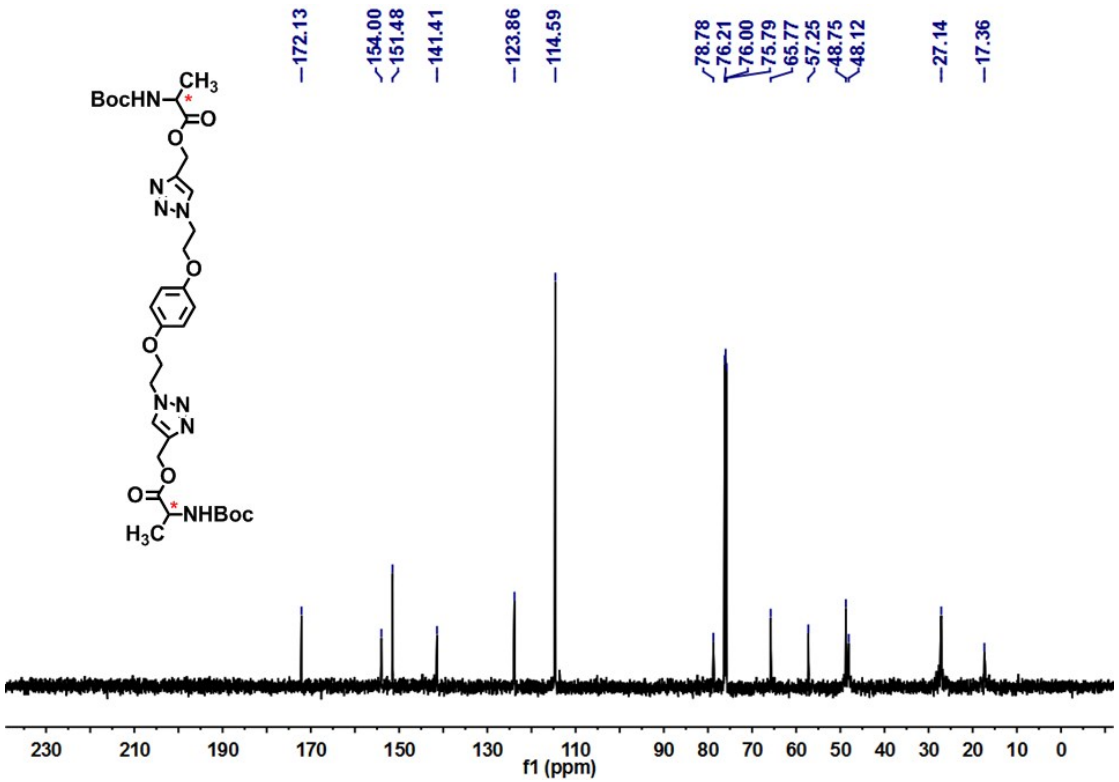

Figure S12. ^{13}C NMR spectrum (150 MHz, $CDCl_3$, 293 K) of Boc-L/D-AM.

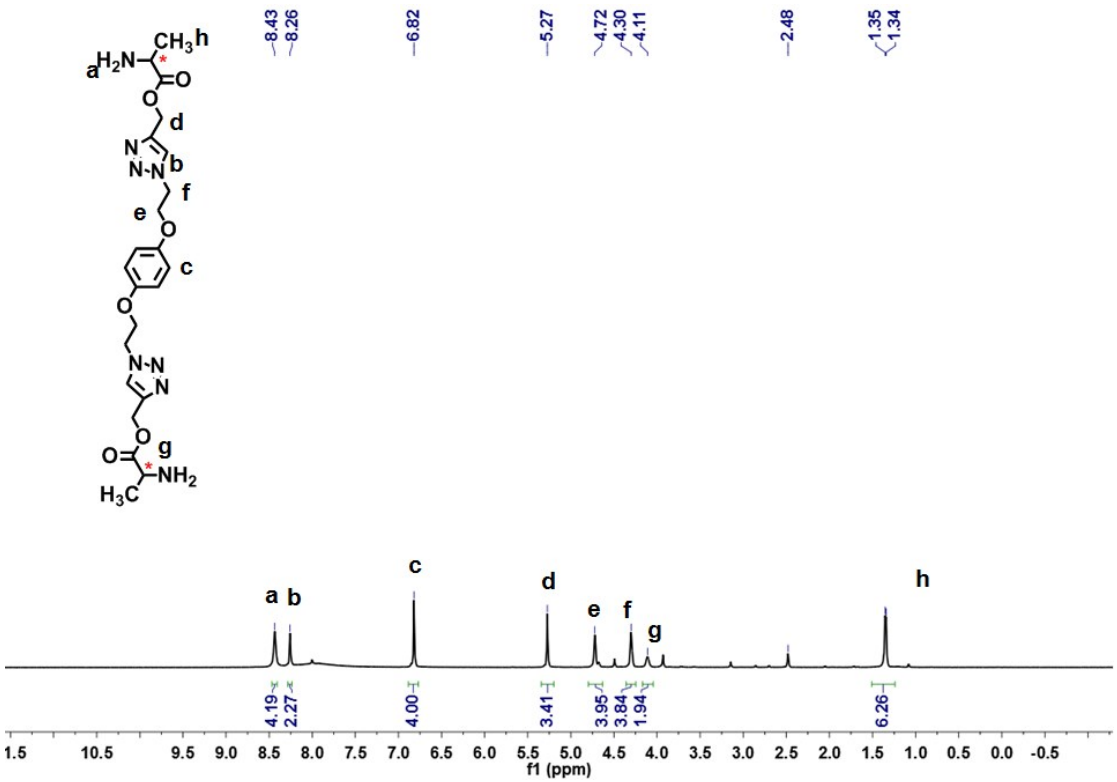

Figure S13. ^{1}H NMR spectrum (600 MHz, DMSO-d6, 293 K) of L/D-AM

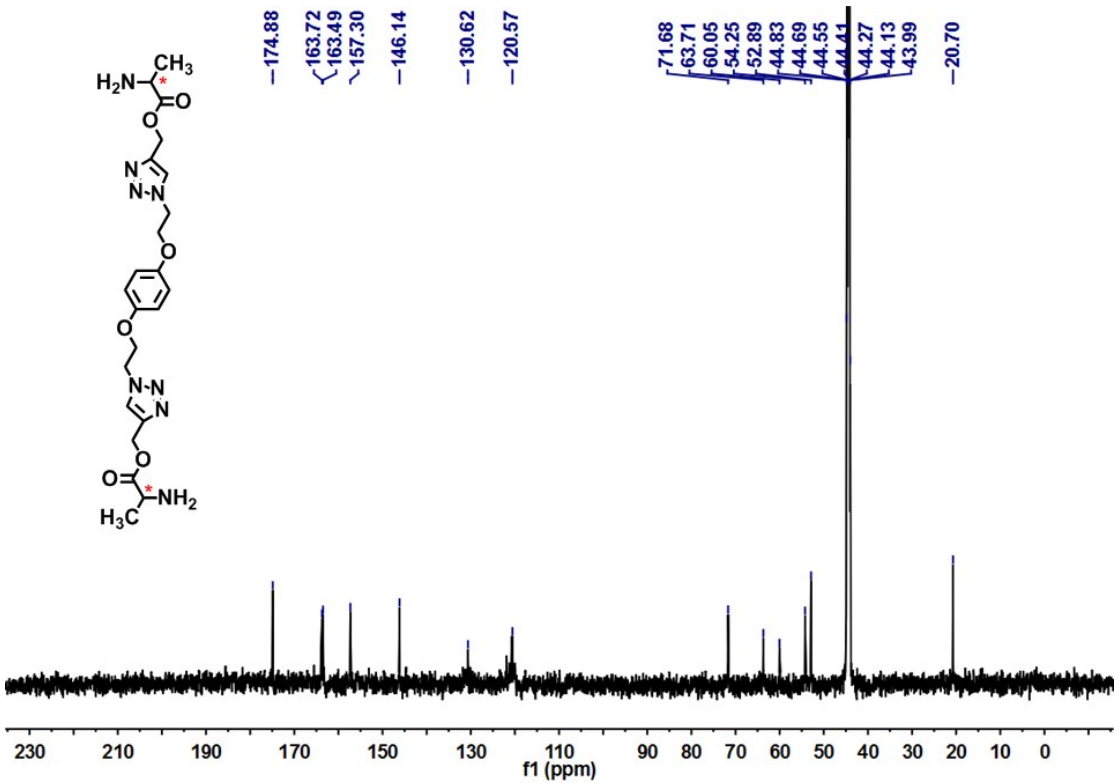

Figure S14. ^{13}C NMR (150 MHz, DMSO-d6, 293 K) of L/D-AM.

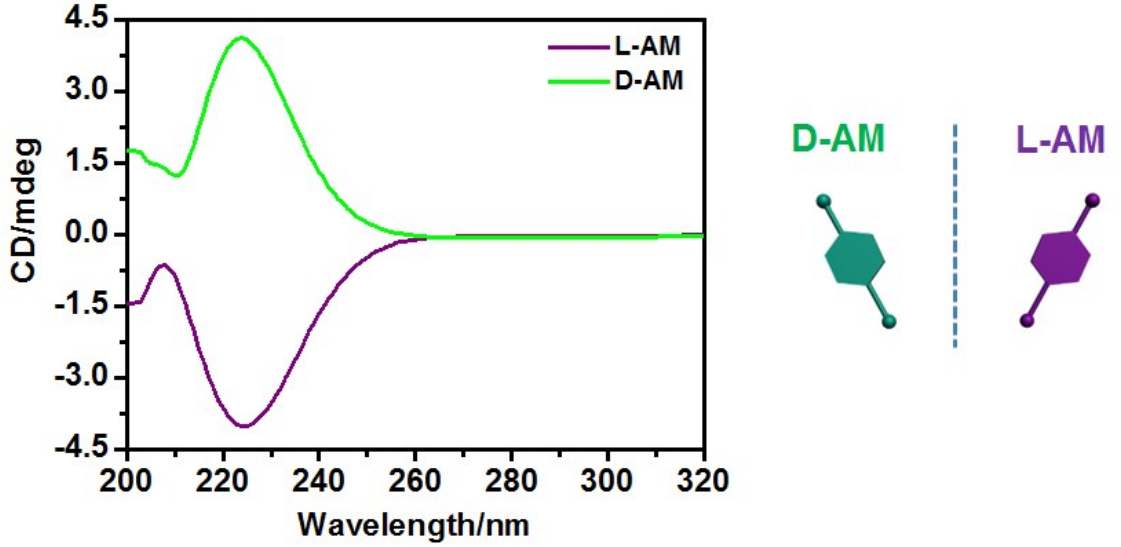

Figure S15. The CD spectrum of L/D-AM resolved in water solution under the concentration of 2.0×10^{-5} mol/L.

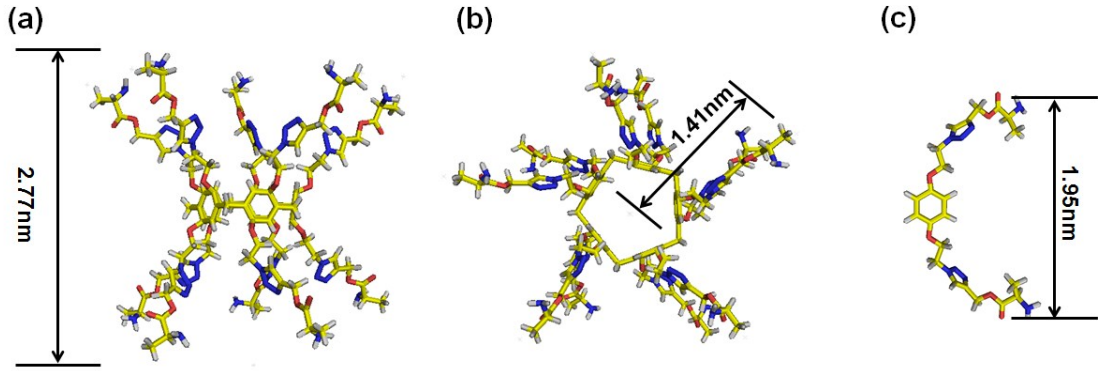

Figure S16. The Gaussian Calculation of three dimensional structures of (a) L-AP5 (front view), (b) L-AP5 (top view), and (c) L-AM optimized at the HF/ 6-31G(d) level.

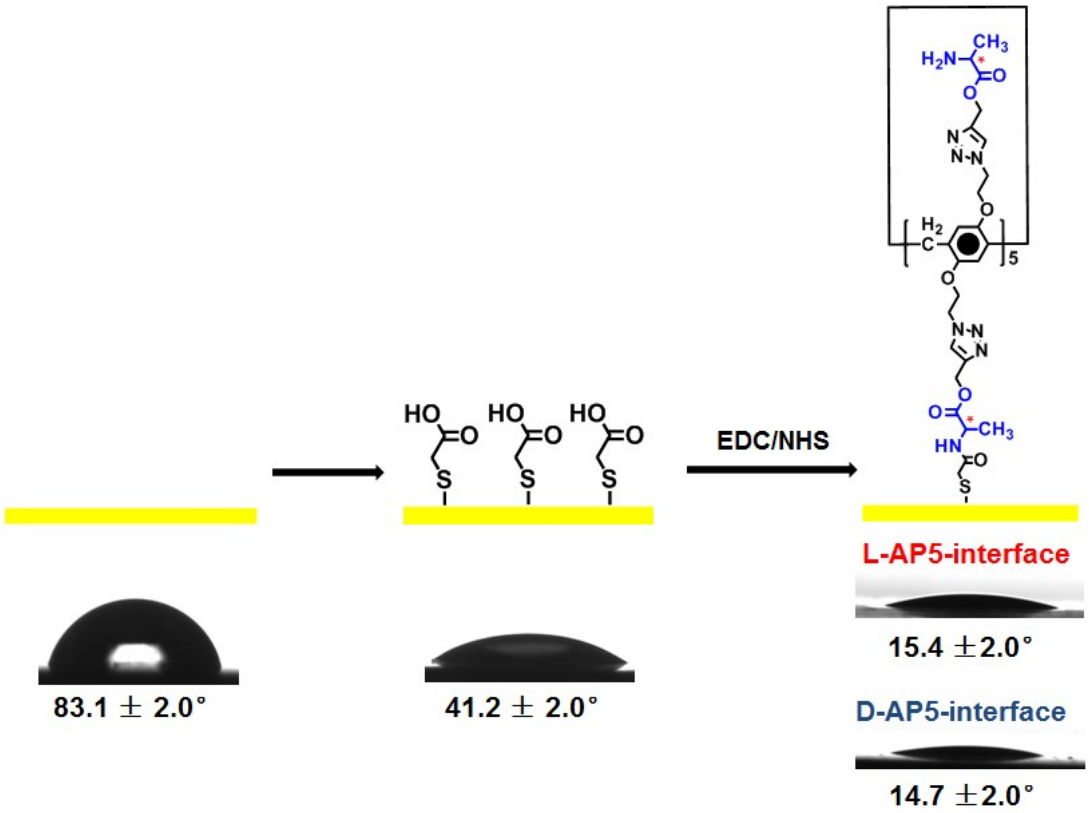

Figure S17. The CA of the gold electrode surface before and after grafting with L/D-AP5.

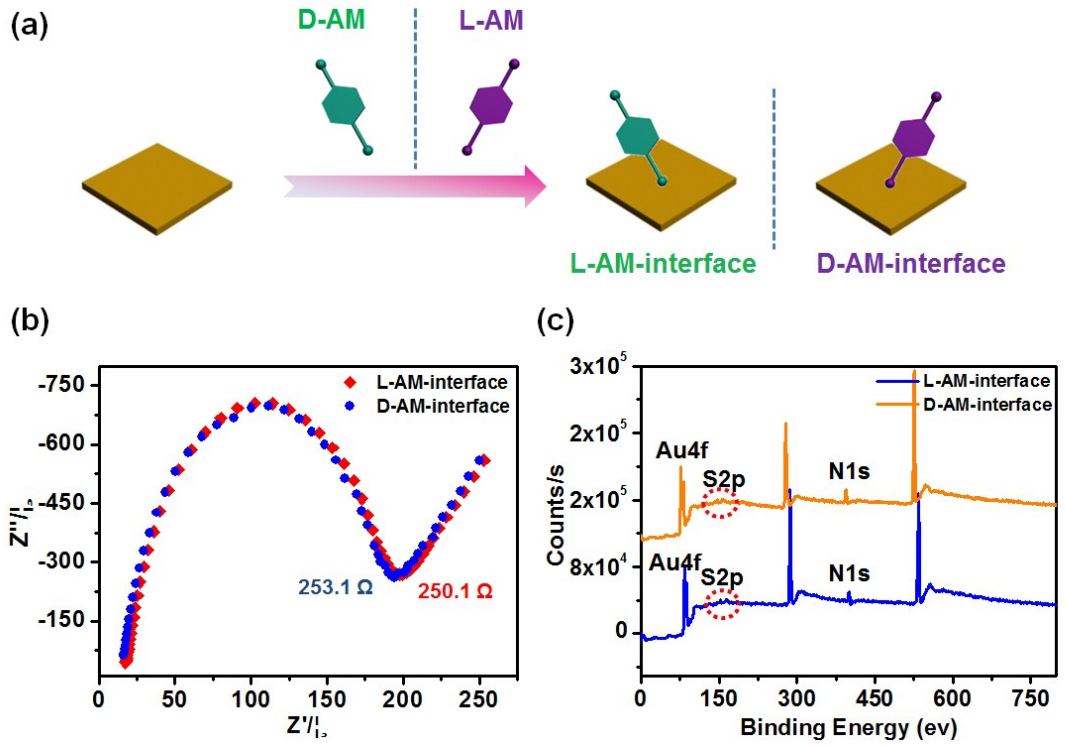

Figure S18. (a) The fabrication of D/L-AM-interfaces. (b) Electrochemical Impedance Spectroscopy (EIS) of D/L-AM-interfaces. (c) X-ray Photoelectron Spectroscopy (XPS) of D/L-AM-interfaces.

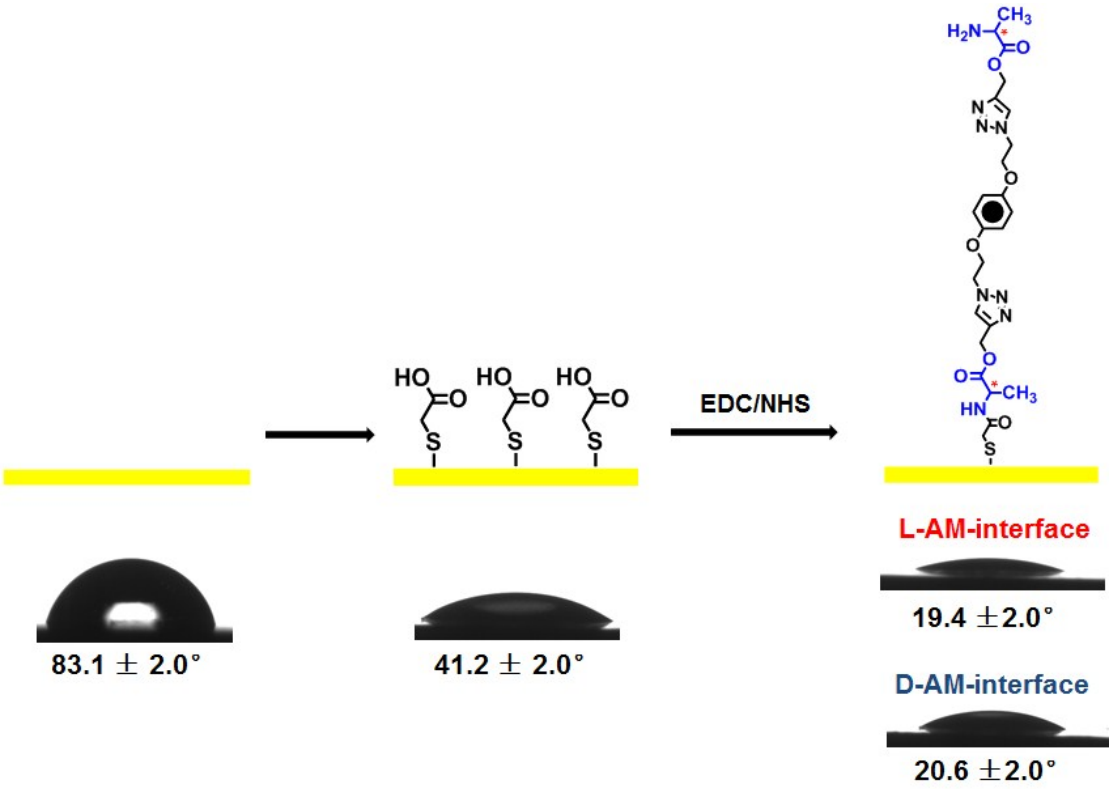

Figure S19. The CA of the gold electrode surface before and after grafting with L/D-AM.

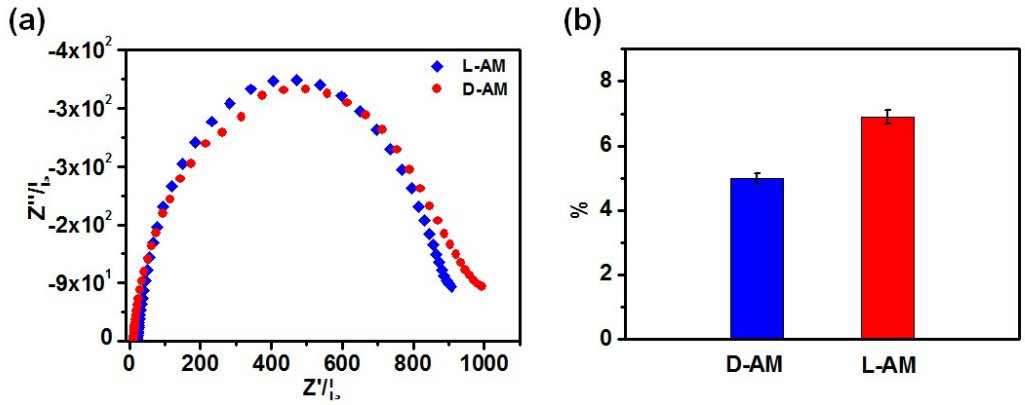

Figure S20. The chiral-response of D/L-AM-interfaces to ctDNA. (a) Electrochemical Impedance Spectroscopy (EIS) of D/L-AM-interfaces after immersing in ctDNA solution. (b) The change rate of EIS.

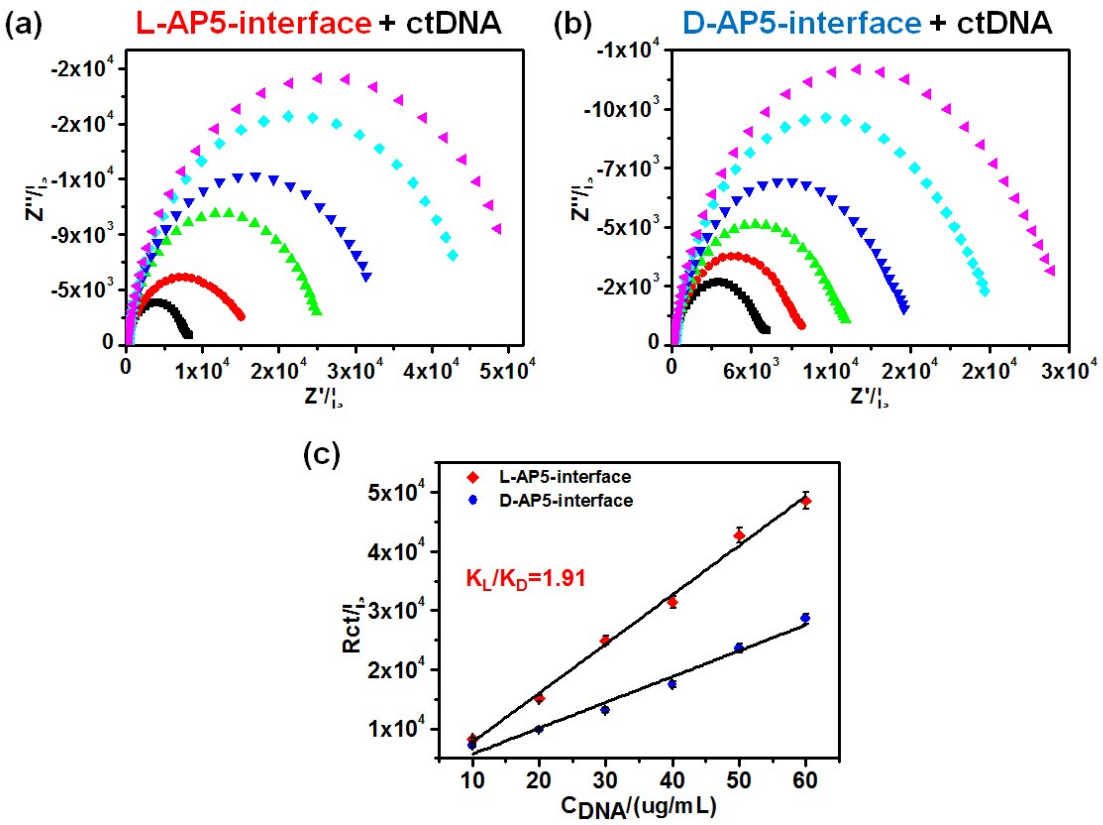

Figure S21. The chiral-response of D/L-AP5-interfaces to ctDNA. (a) EIS of L-AP5-interface adsorbing ctDNA with different concentration. (b) EIS of D-AP5-interface adsorbing ctDNA with different concentration. (c) The selectivity coefficient of D/L-AP5-interfaces to ctDNA. The concentrations of ctDNA were 10, 20, 30, 40, 50 and 60 μg/mL, respectively. D/L-AP5-interfaces were immersed in ctDNA solution for 10 min each time, respectively.

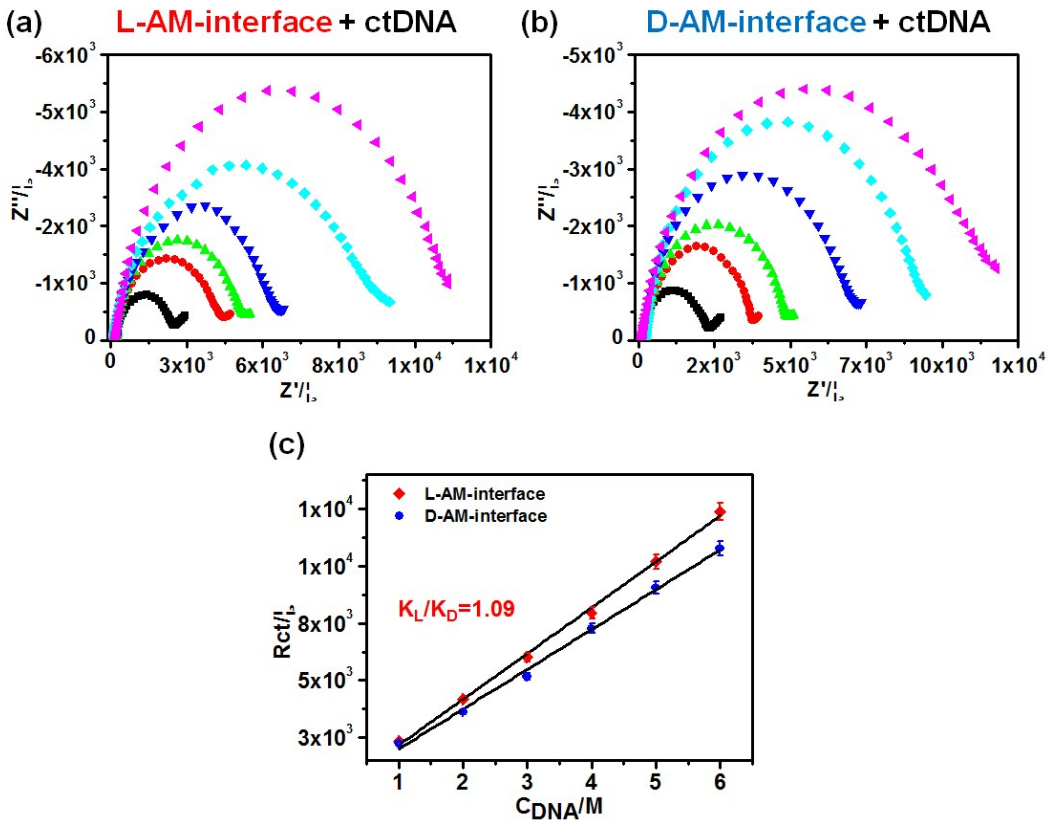

Figure S22. The chiral-response of D/L-AM-interfaces to ctDNA. (a) EIS of L-AM-interface adsorbing ctDNA with different concentration. (b) EIS of D-AM-interfaces adsorbing ctDNA with different concentration. (c) The selectivity coefficient of L/D-AM-interfaces to ctDNA. The concentrations of ctDNA were 10, 20, 30, 40, 50 and 60 μg/mL, respectively. D/L-AM-interfaces were immersed in ctDNA solution for 10 min each time, respectively.

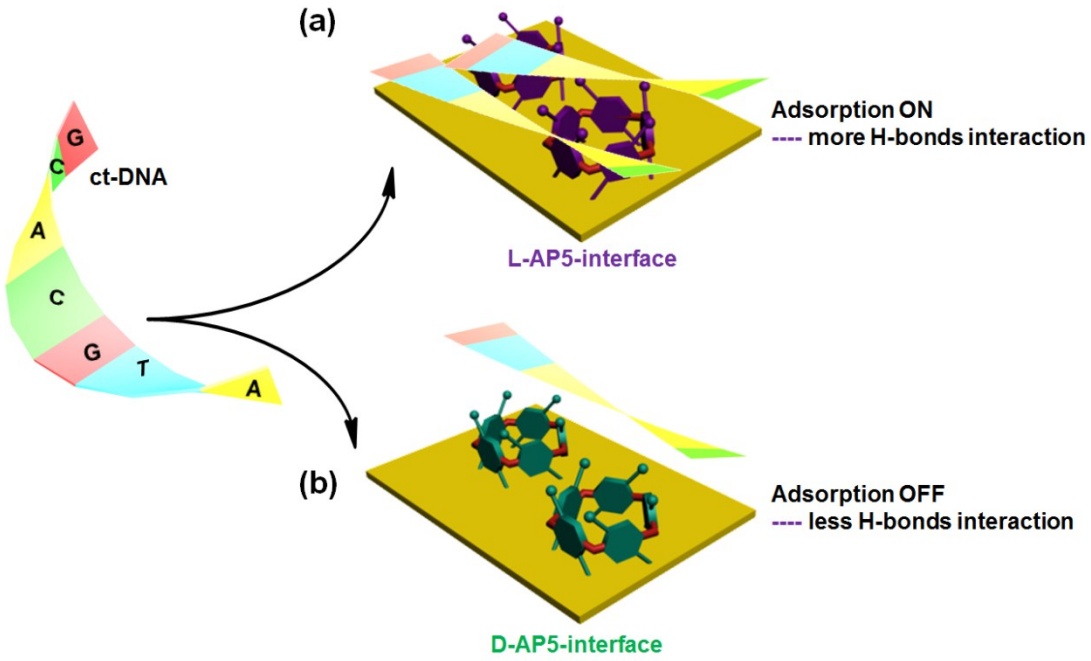

Figure S23. Schematic diagram for the possible stereoselective interaction between ctDNA and D/L-AP5-interfaces. (a) ctDNA adsorb onto L-AP5-interface with a more quantity due to the more H-bonds interaction between L-AP5-interface and the ctDNA. (b) ctDNA adsorb onto D-AP5-interface with a less quantity due to the lack of efficient H-bonds.

Table S1. XPS of the chemical compositions of D/L-AP5-interfaces.

	Name	Start BE	Peak BE	End BE	Height CPS	FWHM eV	Atomic %
L-AP5-surface	Au4f	89.94	83.27	80.98	30069.43	0.79	7.49
	S2p	171.84	163.61	160.11	1064.38	1.18	5.15
	C1s	290.33	284.79	282.43	9461.57	1.55	61.57
	N1s	404.86	399.76	395.93	1210.27	1.58	7.04
	O1s	535.52	532.07	528.37	6736.53	2.13	18.75
D-AP5-surface	Au4f	89.89	84.11	81.19	30062.53	0.77	7.52
	S2p	171.81	161.61	160.18	1067.39	1.16	5.16
	C1s	290.41	284.19	281.13	9464.55	1.51	61.48
	N1s	404.92	398.56	395.79	1212.35	1.52	7.21
	O1s	535.49	531.57	527.56	6732.75	2.03	18.63

III. Supplementary References

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