Supplementary Information (SI) for Analyst.

Supporting information

Photo-Switchable Supramolecular Glycochips for Capturing Suspension Tumor Cells and Real-Time Profiling of Cell Surface Glycosylation

Biqing Chen,[‡a] Xian Chen,[‡a] Lan Chen,[c] Huanyu Liu,[a] Juan Liu,[b] Yuxin Pei,[a] Teodor Aastrup,[d] Yanbin He,[b] Changping Hu,[b] Zhichao Pei[a*] and Yuchao Lu,[*b]

[a] Chemical Biology, College of Chemistry and Pharmacy, Northwest A&F University, Yangling, Shaanxi 712100, China

[b] School of Pharmacy, Changzhi Medical College, Shanxi Provincial Department-Municipal Key Laboratory Cultivation Base for Quality Enhancement and Utilization of Shangdang Chinese Medicinal Materials, Changzhi, Shanxi 046000, P. R. China

[c] School of Public Health, Changzhi Medical College, Changzhi, Shanxi 046000, P. R. China

[d] Attana, Stockholm SE-11419, Sweden

‡These authors contributed equally to this work.

Corresponding Authors

* Email: peizc@nwsuaf.edu.cn (Zhichao Pei).

* Email: luyuchao@czmc.edu.cn (Yuchao Lu).

Table of Contents

1.Materials and Instruments

11-mercaptoundecanoic acid (MUA), dopamine hydrochloride, and sodium ascorbate were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Di-tert-butyl dicarbonate, p-diaminoazobenzene, hydrobromic acid, N-hydroxysuccinimide (NHS), N-acetylglucosamine, and L-fucose were purchased from Sarn Chemical Technology (Shanghai) Co., Ltd. Trifluoroacetic acid was purchased from Tianjin Xiensi Biochemical Technology Co., Ltd. Lactose and iodine were purchased from Shanghai Xianding Biotechnology Co., Ltd. Anhydrous sodium sulfate was purchased from Tianjin Zhiyuan Chemical Reagent Company. D-galactose, 732 strong acid styrene cation exchange resin, and sodium methoxide were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Copper sulfate pentahydrate was purchased from Chengdu Kelong Chemicals Co., Ltd. 1- (3-dimethylaminopropyl) -3-ethylcarbodiimide (EDC) was purchased from TCI (Shanghai) Chemical Industry Development Co., Ltd. Hoechst 33258, glycine, D-mannose, and concanavalin A (Con A) were purchased from Sigma-Aldrich. Wheat germ agglutinin (WGA), ulex europaeus agglutinin I (UEA-1), and soybean agglutinin (SBA) were purchased from Vector Laboratories. A penicillin/streptomycin mixture was purchased from Nanjing Kaiji Co., Ltd. RPMI 1640 medium was purchased from Gibco. Human chronic myeloid leukemia cells (K562) were a gift from the Institute of Microbiology, Chinese Academy of Sciences (Prof. Bin Gao); mouse hepatoma ascites tumor cells (H22) were a gift from Hubei Medical University (Prof. Liu Hongtao).

Attana Cell A200 QCM Biosensor (Attana AB, Stockholm, Sweden) was used. Bare gold chips were obtained from Attana AB (Sweden). PB - 10 pH Meter (Sartorius AG, Göttingen, Germany) was used for pH measurement. BX53 Upright Fluorescence Microscope (Olympus Corporation, Tokyo, Japan) was used for fluorescence microscopy observation. Type 3111 CO_2 Incubator (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for cell culture. DMi8 Inverted Microscope (Leica Microsystems AG, Wetzlar, Germany) was used for inverted microscopy observation.

Fourier Transform Infrared Spectrometer (Bruker Corporation, Billerica, MA, USA) was used for infrared spectral analysis. Field Emission Scanning Electron Microscope (FEI Company, Hillsboro, OR, USA; Model: Quanta FEG 250) was used for scanning electron microscopy observation.

1.1 Preparation of MUA-Azo-P[5] chips

The gold sensor chips were washed with 1% NaClO, piranha solution (98% H_2SO_4:30% H_2O_2, 3:1, v/v), and ultrapure water, respectively. Gold chips were cleaned and dried, then immersed in a 10 mM solution of MUA in ethanol and incubated under dark conditions for 24 h. After gentle washing with ultrapure water (2-3 times), 50 μL of 20 mM EDC solution was added and reacted for 10 min, followed by addition of 50 μL of 15 mM NHS solution with 30 min reaction. The chip was then washed again with ultrapure water (2-3 times) to yield a surface-activated MUA-modified gold chip. Then, 50 μL of Azo molecules (10 mM) were added to the surface of the MUA chip. The azobenzene guest molecules were covalently linked to the chip surface through an amide reaction between the terminal amino group of the guest molecule and the carboxyl group of the activated MUA. After incubating at room temperature for 3 hours, the chip was washed with ddH_2O and dried to obtain the MUA-Azo chip. Then, 50 μL of Gal-P[5] host molecules (10 mM) were added to the MUA-Azo-P[5] chip surface. After incubating at room temperature for 2 hours, the chip was washed with ddH_2O 2-3 times and dried to obtain the MUA-Azo-P[5] chip through host-guest interaction.

1.2 Surface characterization

In order to investigate the chip surface modification, the chip surface morphology and formed compounds were systematically characterized by scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) in this study.

1.3 Preparation of living suspension cancer cell sensor chips

Following our established protocols[1], H22 and K562 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin.

Logarithmic-phase cells were harvested, washed, and resuspended in PBS (pH 7.4) at 1.4×10^6 cells/mL. The MUA-Azo-P[5] chips were incubated with 0.5 mL cell suspension (7×10^5 cells/well) in a 24-well plate for 1.5 h at 37°C/5% CO_2. Cell immobilization occurred via specific lectin-glycan interactions between surface Gal moieties on the chip and glycoprotein receptors on suspension cells. After PBS washing (2-3 times) to remove non-adherent cells, immobilized cells were stained with Hoechst 33258 (5 μg/mL, 15 min). Cell morphology and surface coverage were analyzed using fluorescence microscopy (Olympus BX53).

1.4 QCM detection

The functionalized chip was mounted in an Attana QCM biosensor. After stabilizing with PBS (pH 7.4, 20 μL/min) until frequency drift <5 Hz over 600 s, surface non-specific sites were blocked by sequential injection of 100 μg/mL BSA (3-5 cycles) until stable baseline. For binding studies, 50 μg/mL lectin was injected (105 s association/295 s dissociation; total cycle 400 s). Kinetic experiments employed serial lectin concentrations (12.5-100 μg/mL in PBS), each followed by surface regeneration via 300 mM cognate monosaccharide injection (15 s). Subsequent lectin injections quantified specific glycan expressions on H22 and control cells. All measurements were performed in triplicate.

1.5 Regeneration of chips

Post-assay, the MUA-Azo-P[5] chip was irradiated (365 nm UV, 4 h) to induce azobenzene photoisomerization (E→Z transition). This structural change disrupted host-guest binding, releasing surface-immobilized cells. The chip was then washed 2-3 times with PBS (pH 7.4) to remove residual cellular debris. Complete cell detachment was verified by fluorescence microscopy (Olympus BX53) after Hoechst 33258 staining (5 μg/mL, 15 min), with absence of fluorescent signal confirming removal efficacy.

1.6 Data process

The changes of resonance frequency of the suspension-cell sensors were measured and recorded in real-time by Attana AB and its Attester software. The experimental data of the competitive experiment and the kinetic study were fitted with the evaluation software (Attana AB) and ClamXP.

2. Fitting equation

The curve fitting was performed by Graphpad Prism 5 software using the following equation:

$$\Delta f = \Delta f_{min} + \left(\frac{\Delta f_{max} - \Delta f_{min}}{1 + 10^{(\log C - \log IC50)}}\right) \quad \text{Equation (2-1)}$$

Where: Δf mean response frequency Hz; Δf_{max} intended to be the maximum Hz of the response frequency; Δf_{min} mean response frequency nadir Hz; C representative inhibitor concentration M; IC_{50} mean half inhibitory concentration M.

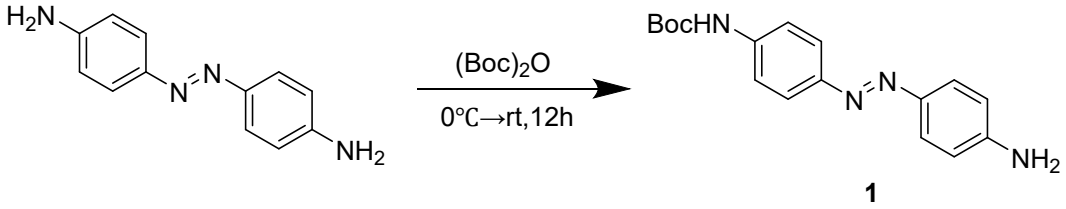
3. Synthesis and characterizations of compound

3.1 Synthesis of azobenzene derivative guest molecules

(1) Compound **1**

106 mg of p-diaminoazobenzene (0.5 mmol) was dissolved in 3 mL of dry THF, and 380 mg of $(Boc)_2O$ was weighed and dissolved in 1 mL of THF (dry), and the $(Boc)_2O$ solution was slowly added dropwise to the reaction solution at 0 ℃. After the dropwise addition, the reaction was transferred to room temperature for 12 h. After the reaction was monitored by TLC, the solvent was discarded under reduced pressure, 10 mL of ethyl acetate was added, and the reaction solution was washed with 10 mL of saturated NaCl solution for three times, and the organic phases were combined and dried with anhydrous Na_2SO_4. After filtration, evaporation and concentration and column separation (eluent: ethyl acetate: petroleum ether = 1:4), yellow solid compound 1 was

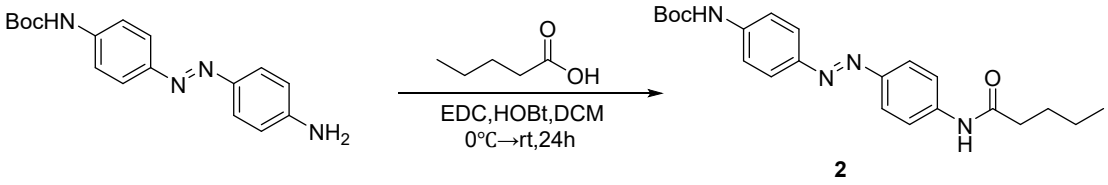
obtained in 80% yield.[1]H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 17.7, 8.8 Hz, 4H), 7.47 (d, *J* = 8.8 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 1.57 (s, 9H) ◦



1

(2) Compound 2

6.7 mg HOBt was added to 2 mL of DCM solution dissolved in 33 μL of valeric acid (0.3 mmol) at 0 ℃, then 58 mg EDC was added in batches, and 78 mg of compound 1 (0.25 mmol) was added after stirring for 10 min. The reaction was carried out at room temperature for 3 h. After monitoring the complete reaction by TLC, the reaction solution was transferred into a dispensing funnel, and the reaction solution was washed three times with 5 mL of water, then washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, evaporated, and concentrated, and then separated on a column (eluent: ethyl acetate: petroleum ether = 1:6) to give the yellow solid compound 2 in 86% yield. [1]H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 8.8, 4.3 Hz, 4H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.52 (s, 2H), 2.43 – 2.37 (m, 2H), 1.77 – 1.70 (m, 2H), 1.52 (d, *J* = 16.1 Hz, 9H), 1.43 (d, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.5 Hz, 3H) ◦

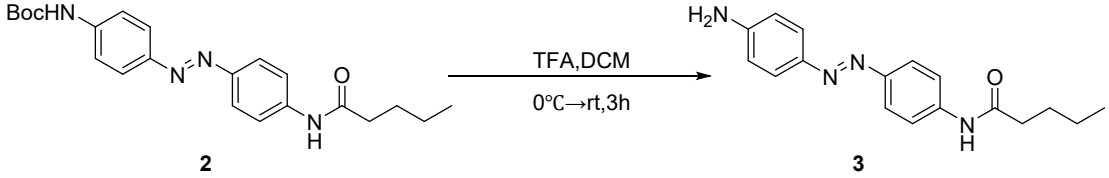


2

(3) Compound 3

60 mg of compound 2 was weighed and dissolved in 2 mL of dichloromethane, 1 mL of TFA was slowly added dropwise to the reaction solution at 0 ℃, and the reaction was carried out at room temperature for 3 h. After the reaction was completely detected by TLC, the solvent was removed under reduced pressure, and the yellow solid

compound azobenzene guest molecule was obtained, which was recorded as compound 3, and the yield was 100%.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J = 8.8, 4.4 Hz, 4H), 7.67 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 5.30 (s, 2H), 2.40 (t, J = 7.6 Hz, 2H), 1.75 (d, J = 7.7 Hz, 2H), 1.46 – 1.41 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H)。



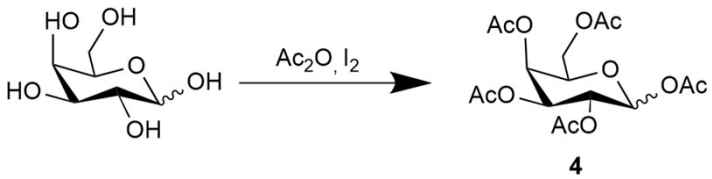
2 3

3.2 Synthesis of galactose-conjugated pillar[5]arene

3.2.1 Synthesis of azido-galactose molecules

(1) Compound 4

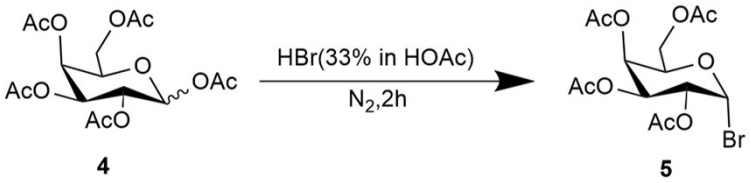
To 100 mL of acetic anhydride were added 10.00 g of galactose (55.6 mmol) and 0.22 g of iodine (0.87 mmol). The reaction mixture was stirred at room temperature for 12 h until the solution was clear. After adding 200 mL of CH_2Cl_2 to dissolve the solution, saturated sodium carbonate solution was added and stirred continuously until no bubbles appeared, then the solution was transferred to a separatory funnel and the lower organic phase was collected, dried with anhydrous Na_2SO_4, and the solvent was removed by spin evaporation to give compound 4 as a light yellow solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, J = 8.3 Hz, 1H), 5.42 (dd, J = 3.4, 1.2 Hz, 1H), 5.33 (dd, J = 10.4, 8.3 Hz, 1H), 5.07 (dd, J = 10.4, 3.4 Hz, 1H), 4.13 (t, J = 6.6 Hz, 2H), 4.05 (s, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.04 (d, J = 0.7 Hz, 6H), 1.99 (s, 3H)。The hydrogen spectrum of compound 4 is in agreement with the literature[2].



4

(2) Compound 5

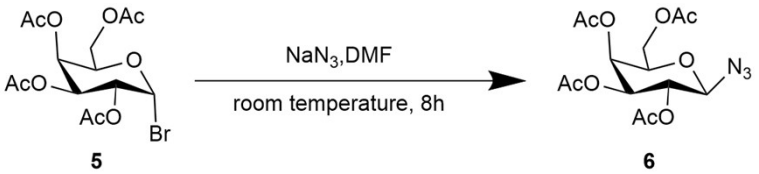
78 mg of compound 4 (0.2 mmol) was weighed and added to 15 mL of CH_2Cl_2 solution under nitrogen protection, followed by HBr (33% in HOAc, 0.7 mL), and the reaction mixture was stirred at room temperature for 2 h. After the reaction was monitored for completeness by TLC, the reaction mixture was partitioned by the addition of 1 mL of CH_2Cl_2 and 1 mL of H_2O and then re-extracted with the CH_2Cl_2 aqueous layer three times. The organic phases were collected and combined, washed sequentially with saturated sodium bicarbonate and saturated sodium chloride solution, and dried with anhydrous sodium sulfate to obtain product 5 in 100% yield. Due to the instability of product 5, the reaction was carried out directly to the next step.



4 5

(3) Compound 6

1.94 g of sodium azide (29.3 mmol) was added to a dry DMSO (50 mL) solution of compound 5 (8.40 g, 19.9 mmol) and stirred at room temperature for 10 min. After the reaction was monitored for completeness by TLC, the reaction solution was diluted by addition of 100 mL of water, and then 100 mL of methylene chloride was added to extract the reaction solution for three times, and the organic phases were collected and combined in the lower layer and washed with The organic phase was collected, and the combined lower layer was washed with saturated sodium chloride solution and dried with anhydrous sodium sulfate. After filtration, evaporation and concentration, and column separation (elution solvent: dichloromethane: methanol = 25:1), the white solid compound 6 was obtained in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 – 5.22 (m, 1H), 5.12–5.04 (m, 1H), 4.78 (d, J = 9.2 Hz, 1H), 4.28–4.11 (m, 2H), 3.91 (q, J = 9.5 Hz, 1H), 3.46 (d, J = 1.9 Hz, 1H), 2.08 (s, 3H), 2.02 (dd, J = 3.2, 1.2 Hz, 6H), 1.96 (d, J = 1.2 Hz, 3H) ◦ The hydrogen spectrum of compound 6 is in agreement with the literature[3].

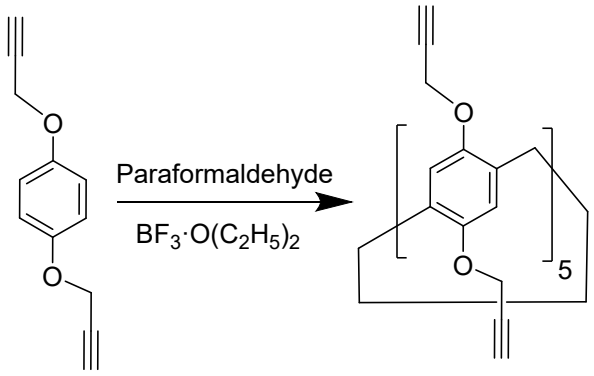


5 **6**

3.2.2 Synthesis of host molecules

(1) Compound 7

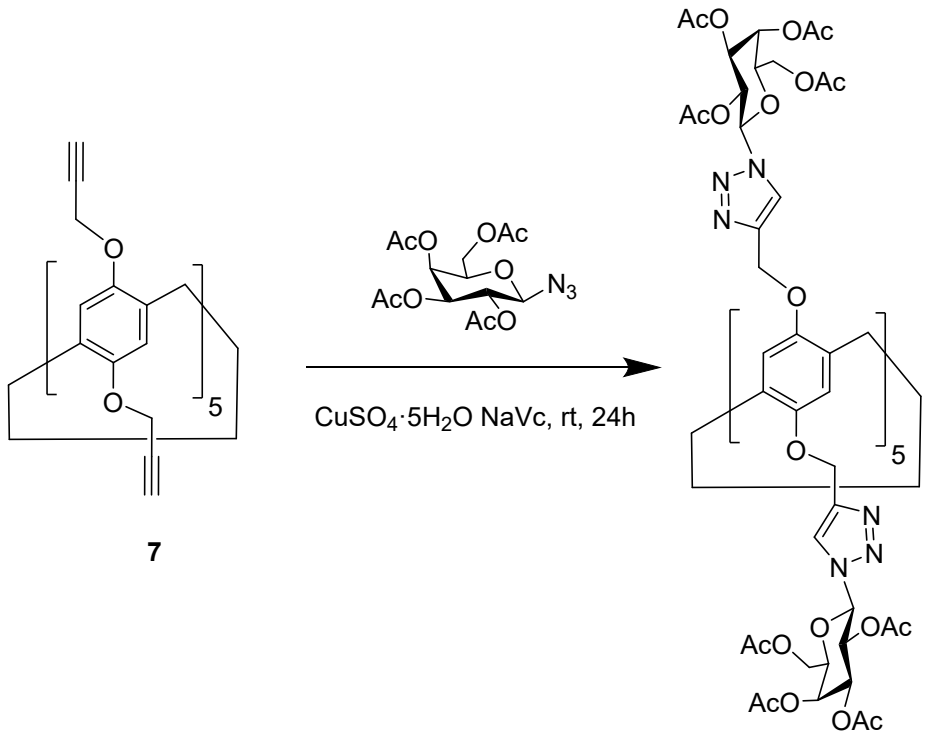
1,4-Bis(2-propynyloxy)benzene (3.72 g, 20 mmol) was dissolved in 1,2-dichloroethane (60 mL), paraformaldehyde (1.40 g, 40 mmol) was added under a nitrogen atmosphere, and then boron trifluoride ethyl ether (4 mL) was taken and injected into the reaction vial and stirred for 1 hr at room temperature. After the reaction was complete, as detected by TLC, the reaction was quenched by the addition of water (20 mL). The mixture was filtered and sequentially extracted once with saturated $NaHCO_3$, deionized water, and saturated NaCl, respectively, and the organic layer below the partition funnel was collected, combined, and dried with anhydrous sodium sulfate. After filtration, the filtrate was spin-dried and separated on a column packed with silica gel (elution solvent: dichloromethane: ethanol = 40:1) to give white solid compound 7 in 80% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 10H), 4.57 (d, J = 2.4 Hz, 20H), 3.82 (s, 10H), 2.41 (t, J = 2.4 Hz, 10H) ◦ The hydrogen spectrum of compound 7 is in agreement with the literature[3].



7

(2) Compound 8

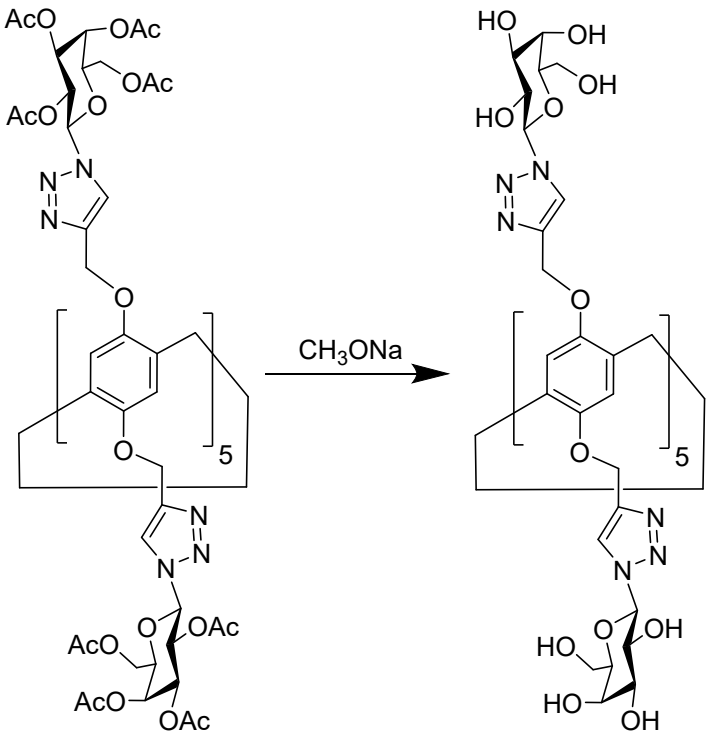
After 0.50 g of compound 6 (1.34 mmol) and 0.11 g of compound 7 (0.11 mmol) were dissolved in 3 mL of dichloromethane, evacuated, and protected by N_2, 0.05 g of copper sulfate pentahydrate (0.20 mmol) and 0.13 g of sodium ascorbate (0.94 mmol) dissolved in 2 mL of H_2O were added to the reaction solution and stirred for 24 h at room temperature. After the complete reaction was monitored by TLC, the reaction solution was diluted with 10 mL of dichloromethane, washed with deionized water (10 mL × 3), and extracted, and the combined organic phases were collected, dried with anhydrous sodium sulfate, and filtered. After evaporation and concentration of the filtrate and column separation (elution solvent: dichloromethane: ethanol = 40:1), yellow solid compound 8 was obtained in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 10H), 6.75 (s, 10H), 5.91 (d, J = 7.5 Hz, 10H), 5.63 (s, 10H), 5.54 (s, 10H), 5.34 (d, J = 9.7 Hz, 10H), 4.86 (d, J = 11.5 Hz, 10H), 4.79 (d, J = 12.5 Hz, 10H), 4.39–4.14 (m, 30H), 3.74 (s, 10H), 2.22 (d, J = 3.1 Hz, 30H), 2.00 (s, 60H), 1.85 (s, 30H) ◦ The hydrogen spectrum of compound 8 is in agreement with the literature[4].



7 **8**

(3) Compound 9

2.36 g of compound 8 (0.5 mmol) was placed into a 100 mL flask, followed by the addition of 35 mL of sodium methanol solution at a concentration of 0.15 M to the flask, and the reaction was carried out at room temperature for 12 h. After the reaction was completely detected by TLC, the 732 strongly acidic styrene cation exchange resin was added and stirred for 30 min until the reaction solution was neutral, the resin was filtered off, and the compound was spun-dried to obtain the white solid compound 9, which had a yield of 71%.^{1}H NMR (400 MHz, D_2O) δ 8.15 (s, 10H), 6.59 (d, J = 42.4 Hz, 10H), 5.62 – 5.54 (m, 10H), 4.65 (d, J = 12.6 Hz, 10H), 4.49 (d, J = 11.3 Hz, 10H), 4.21 (s, 10H), 4.04 (s, 10H), 3.82 (d, J = 11.0 Hz, 20H), 3.65 (s, 30H) ◦ The hydrogen spectrum of compound 9 is in agreement with the literature[4].



8 **9**

4. Figure S1-S10

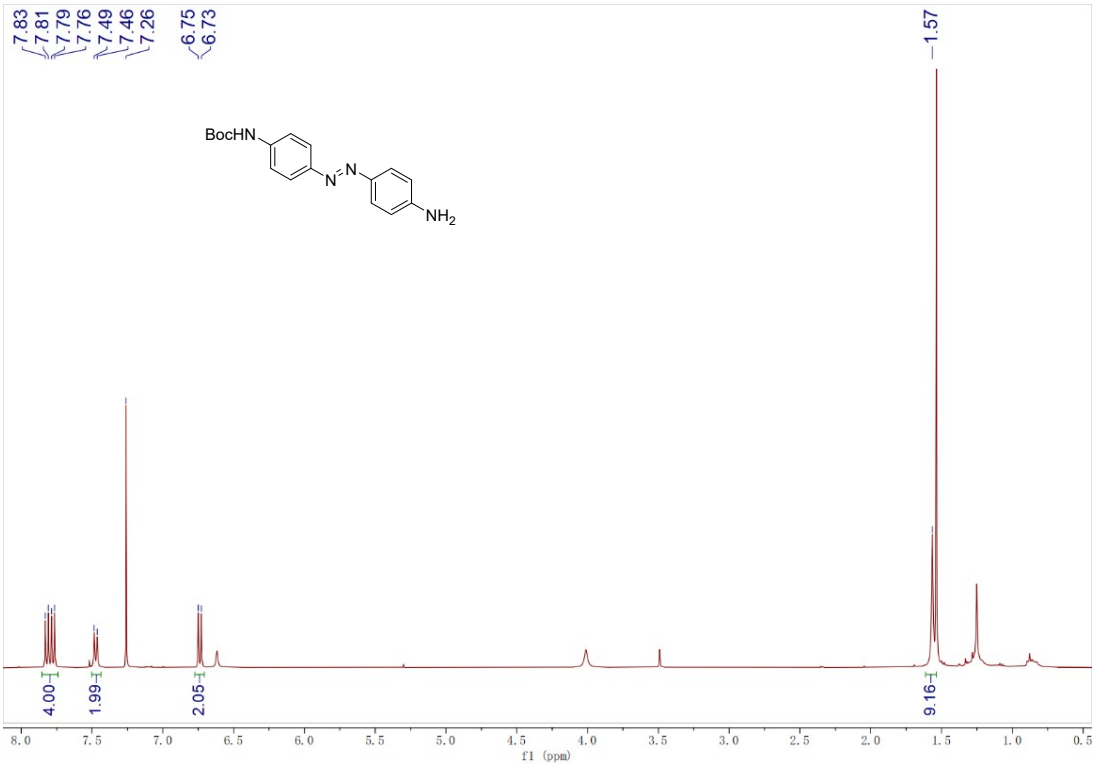


Fig. S1 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **1**.

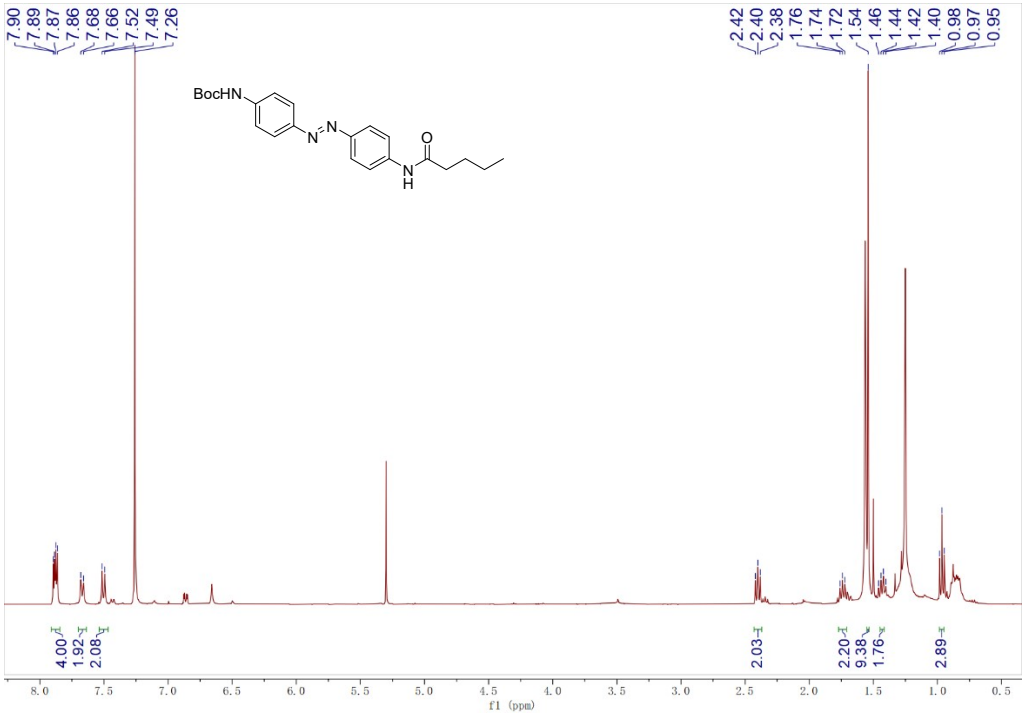


Fig. S2 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **2**.

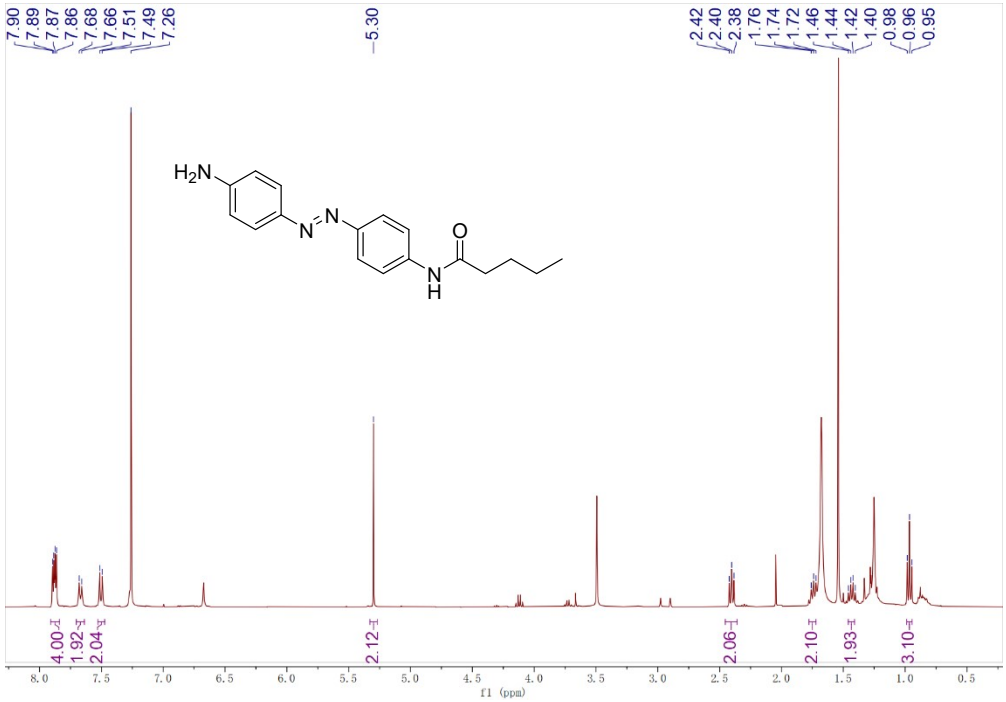


Fig. S3 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **3**.

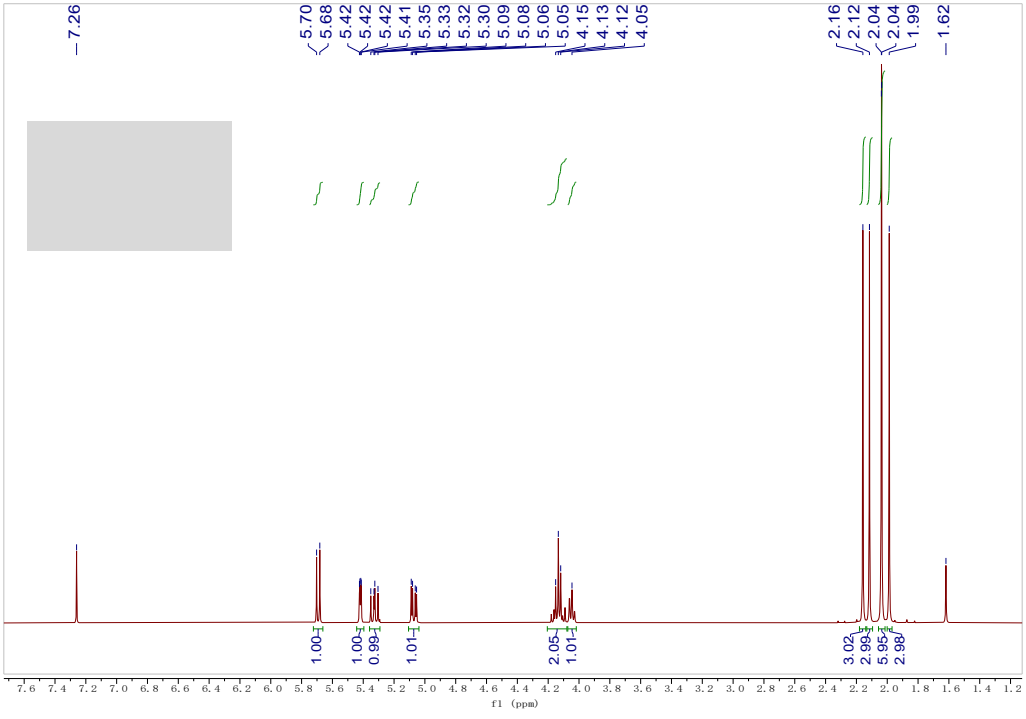


Fig. S4 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **4**.

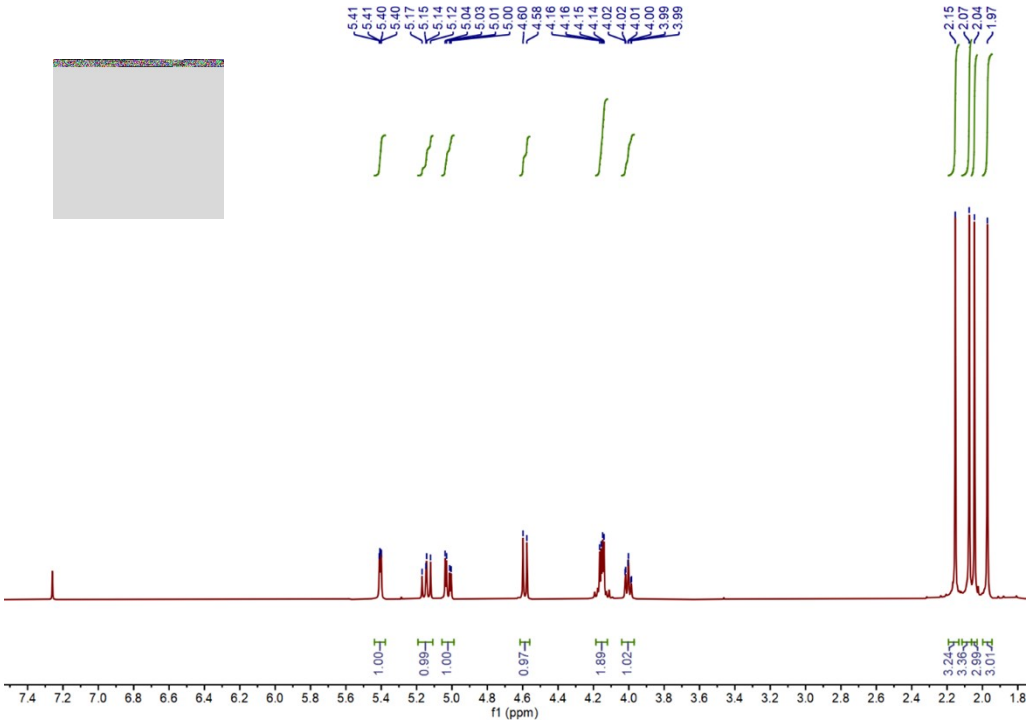


Fig. S5 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **6**.

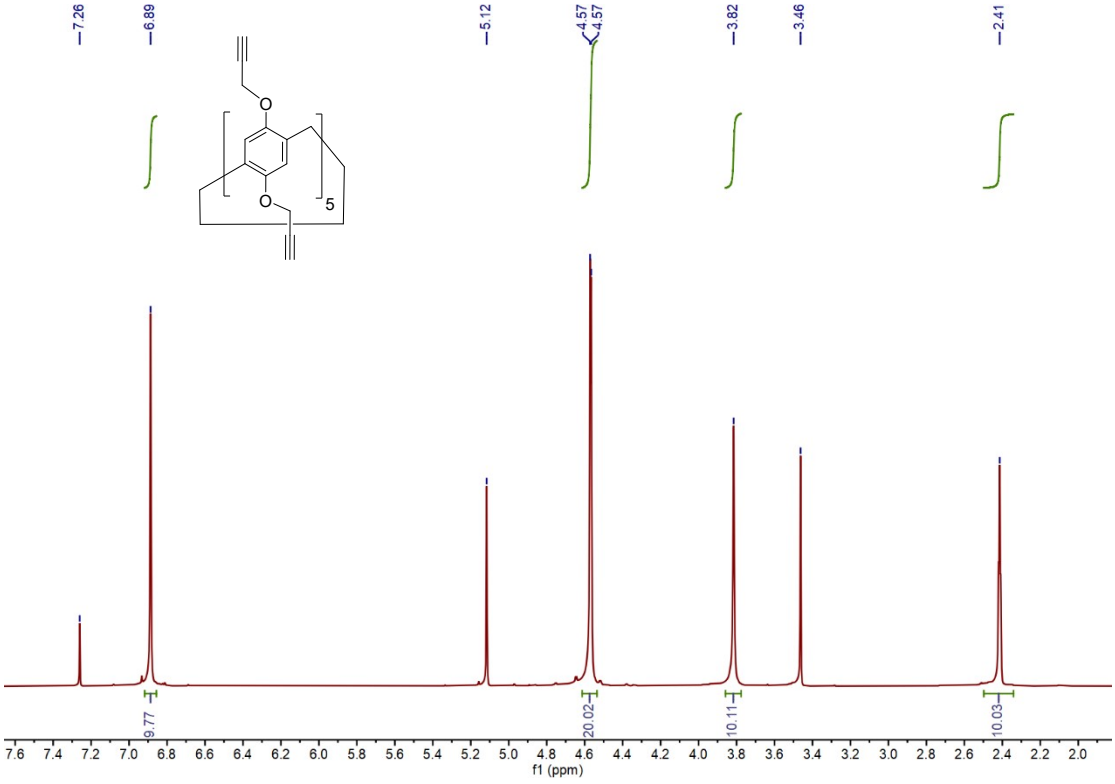


Fig. S6 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **7**.

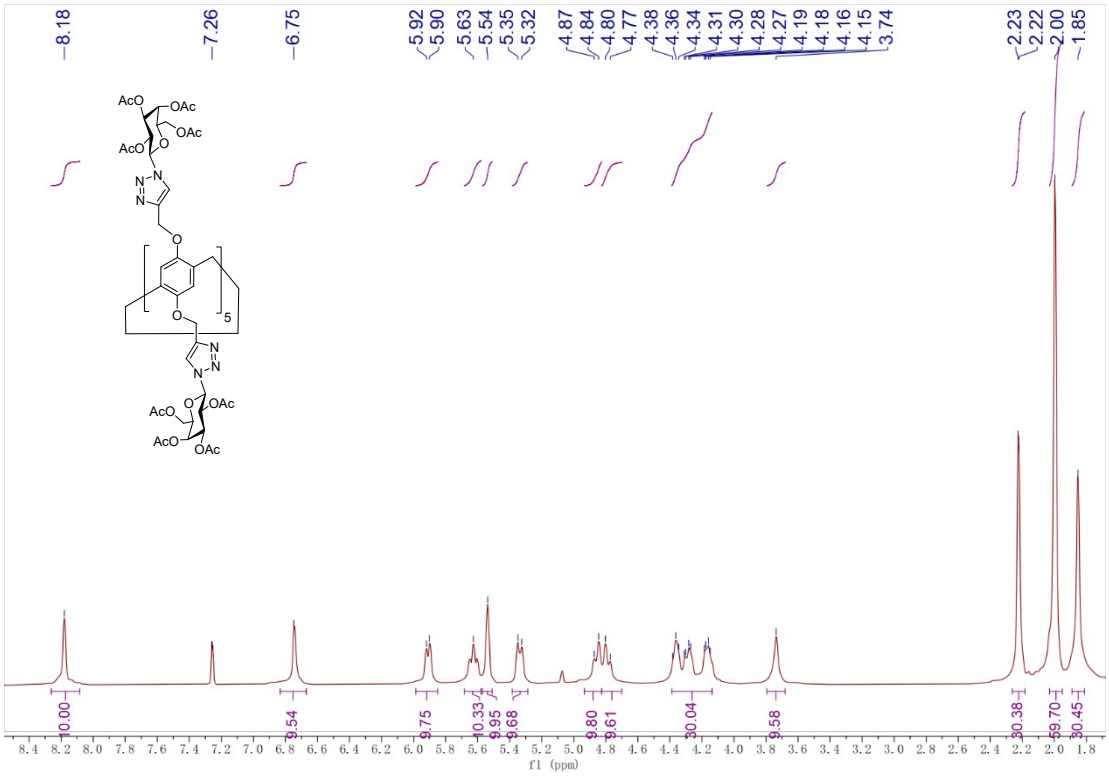


Fig. S7 The ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **8**.

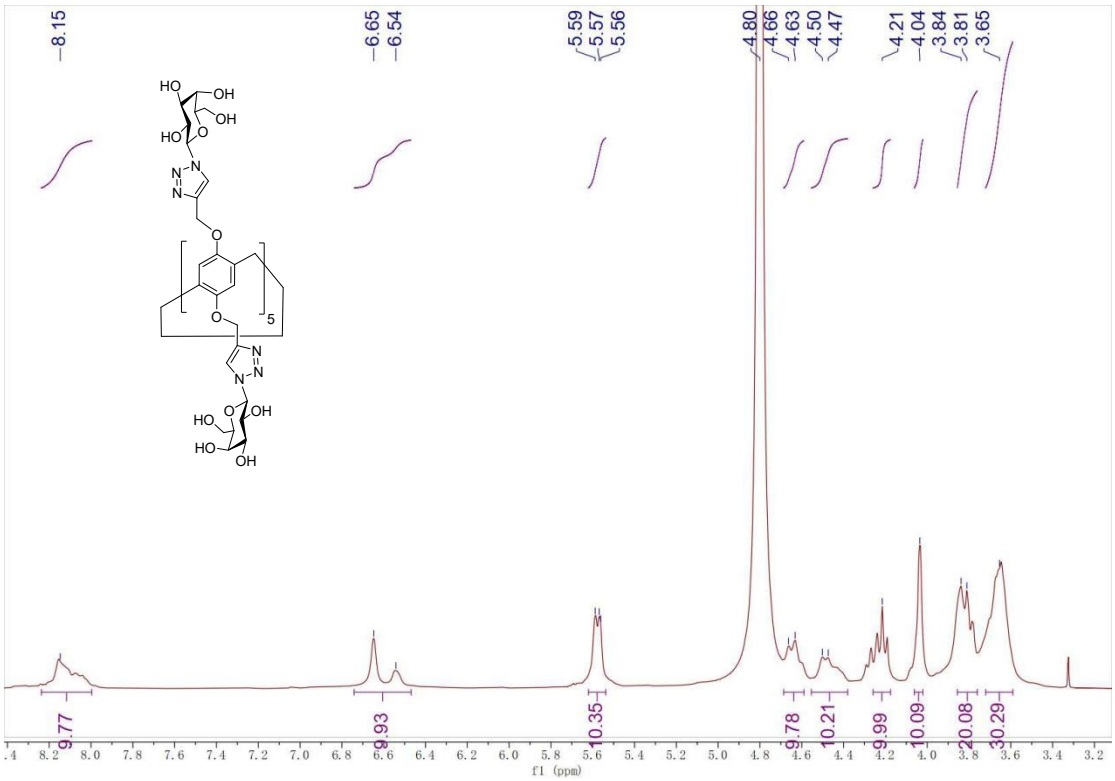


Fig. S8 The ^{1}H-NMR Spectrum (400 MHz, D_2O) of Compound **9**.

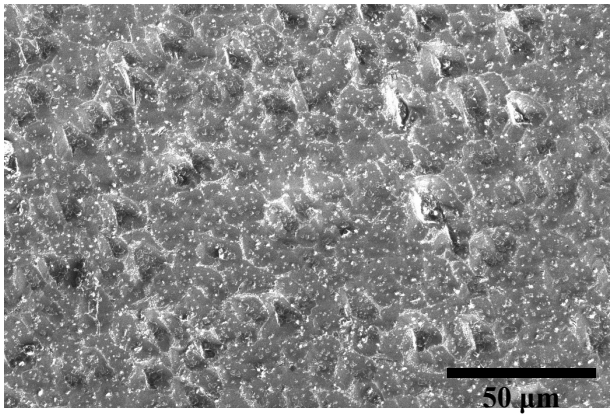


Fig. S9 The SEM image of MUA-Azo-P[5] chip surface. Scale bar: 50 μm.

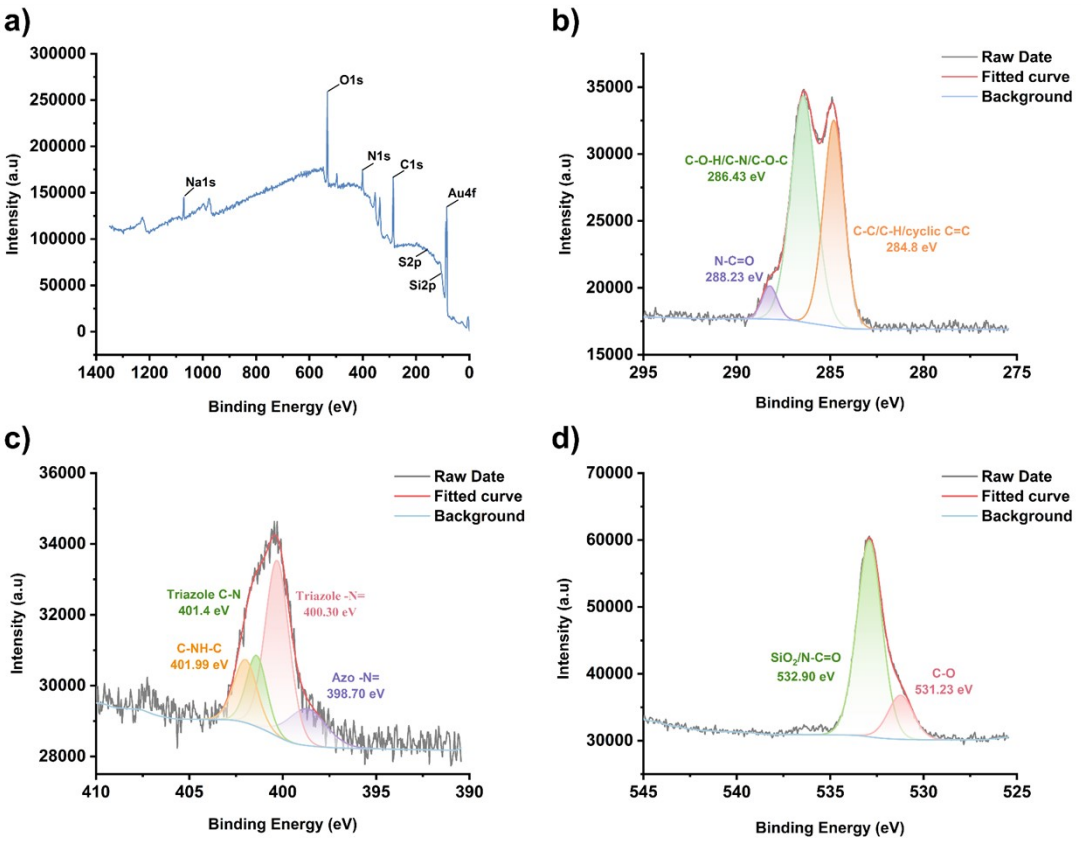


Fig. S10 The XPS characterization of the MUA-Azo-P[5] chip：(a) full spectrum, (b) C1s orbital high-resolution map, (c) N1s, (d) O1s orbital high-resolution map.

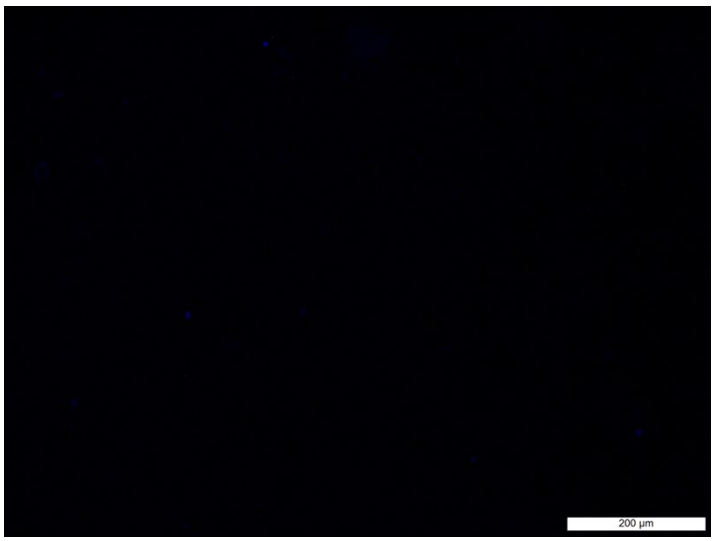


Fig. S11 Fluorescence microscopy image of MUA-Azo chip (without Gal-P[5]) after incubation with H22 cells and Hoechst 33258 staining, showing minimal adhesion. This confirms capture is mediated by specific galactose–protein interactions. Scale bar: 200 μm.

Reference

[1] X. Li, Q. Song, Y. Pei, H. Dong, T. Aastrup, Z. Pei, Direct attachment of suspension cells to PDA surface and its application in suspension-cell QCM biosensor, Sens. Actuators B Chem., 326 (2021) 128823.

[2] J.-d.A.K. Twibanire, R.P. Omran, T.B. Grindley, Facile Synthesis of a Library of Lyme Disease Glycolipid Antigens, Org. Lett., 14 (2012) 3909-3911.

[3] G. Sun, L. Pu, S. Pangannaya, T. Xiao, X.-Y. Hu, J. Jiang, L. Wang, β-D-Galactose-Functionalized Pillar[5]arene With Interesting Planar-Chirality for Constructing Chiral Nanoparticles, Front. Chem., 7(2019) 733.

[4] X. Wu, Y. Zhang, Y. Lu, S. Pang, K. Yang, Z. Tian, Y. Pei, Y. Qu, F. Wang, Z. Pei, Synergistic and targeted drug delivery based on nano-CeO_2 capped with galactose functionalized pillar[5]arene via host–guest interactions, J. Mater. Chem. B, 5 (2017) 3483-3487.