Electronic Supplementary Information

for

Differential Calixarene Receptors Create Patterns that Discriminating Glycosaminoglycans

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1. General methods and materials

All the reagents and solvents were commercially available and used as received unless otherwise specified purification. Eosin Y (EY), heparin (HEP), hyaluronic acid (HA) and dextran sulfate (DS) were purchased from Sigma-Aldrich. Chondroitin sulfate A (CSA), chondroitin sulfate B (CSB) and chondroitin sulfate C (CSC) were purchased from Macklin.

5,11,17,23-Tetraguanidinium-25,26,27,28-tetrabutoxycalix[4]arene (GC4A),[1] 5,11,17,23,29-pentaguanidinium-31,32,33,34,35-pentamethoxycalix[5]arene (GC5A-CH_3) and 5,11,17,23,29-pentaguanidinium-31,32,33,34,35-penta (4-methylpentloxy)calix[5]arene (GC5A) were synthesized according to the previous literature.[2]

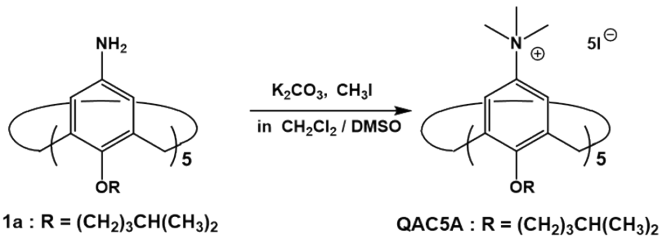
The HEPES buffer solution of pH 7.4 was prepared by dissolving 2.38 g of 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) in approximate 900 mL double-distilled water. Titrate to pH 7.4 at the lab temperature of 25 °C with NaOH and make up volume to 1000 mL with double-distilled water. The MES buffer solution of pH 5.0 was prepared by dissolving 1.95 g of 4-Morpholineethanesulfonic acid in approximate 900 mL double-distilled water, then titrate to pH 5.0 at the lab temperature of 25 °C with NaOH and make up volume to 1000 mL with double-distilled water. The pH value of the buffer solution was then verified on a pH-meter calibrated with three standard buffer solutions.

The fluorescence competition assays were carried out by first adding reporter pairs, and then incubating 10 minutes to obtain the initial intensities. After adding glycosaminoglycans and incubating 10 minutes, the final intensities were obtained. All mean values from fluorescence titrations and limit of detection were measured from at least three experiments.

^1H, ^{13}C and were recorded on a Bruker AV400 spectrometer. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Cary Eclipse equipped with a Cary single-cell peltier accessory.

Principal component analysis (PCA) was performed using the PLS toolbox Solo 4.0 (Eigenvector Research, USA), linear discriminant analysis (LDA) and hierarchical cluster analysis (HCA) were carried out using PAST 3.20.[3]

2. Synthesis of QAC5A



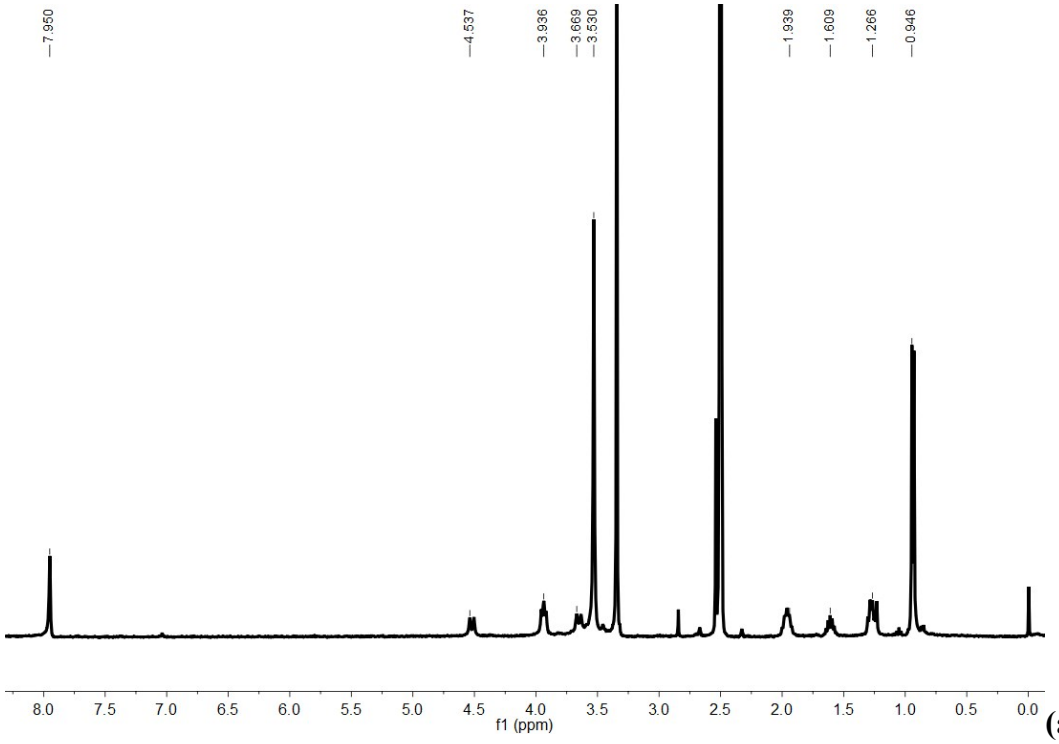
1a : R = $(CH_2)_3CH(CH_3)_2$ QAC5A : R = $(CH_2)_3CH(CH_3)_2$

Scheme S1. Synthetic route of **QAC5A**.

5,11,17,23,29-Pentaamino-31,32,33,34,35-penta(4-methylpentloxy)calix[5]arene (1a) was synthesized and purified according to procedures reported previously.[2]

Synthesis of 5,11,17,23,29-penta(trimethylammonium)-31,32,33,34,35-penta (4-methylpentloxy)calix[5]arene (QAC5A) :

To a solution of **1a** (0.15 g, 0.13 mmol) in CH_2Cl_2 (2 mL) and DMSO (2 mL), K_2CO_3 (0.45 g, 3.2 mmol) was added gradually and the mixture was stirred at room temperature overnight. The color of the mixture changed from dark green to dark red. Then CH_3I (2 mL) was added into the reaction mixture and the mixture was stirred at 40°C for three days. After removing solid by filtration, the solvent in filtrate was removed in vacuo. The residues was recrystallized from CH_2Cl_2/CH_3OH to obtain pale brown solid **2a** (0.03 g, 16%). ^{1}H NMR (400 MHz, DMSO, δ): 7.95 (s, 10H, ArH), 4.54 (d, J = 13.43 Hz, 5H, Ar-CH_2-Ar), 3.94 (t, J = 8.09 Hz, 10H, CH_2-O-Ar), 3.67 (d, J = 13.43 Hz, 5H, Ar-CH_2-Ar), 3.53 (s, 45H, -N$(CH_3)_3$), 1.94 (m, 10H, -CH_2-CH_2-CH-), 1.61 (m, 5H, -CH-), 1.27 (m, 10H, -CH_2-CH_2-CH-), 0.95 (d, J = 6.58 Hz, 30H, -$(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO, δ): 155.85, 142.35, 134.78, 121.47, 75.13, 55.66, 34.22, 28.72, 27.52, 22.50.



(a)

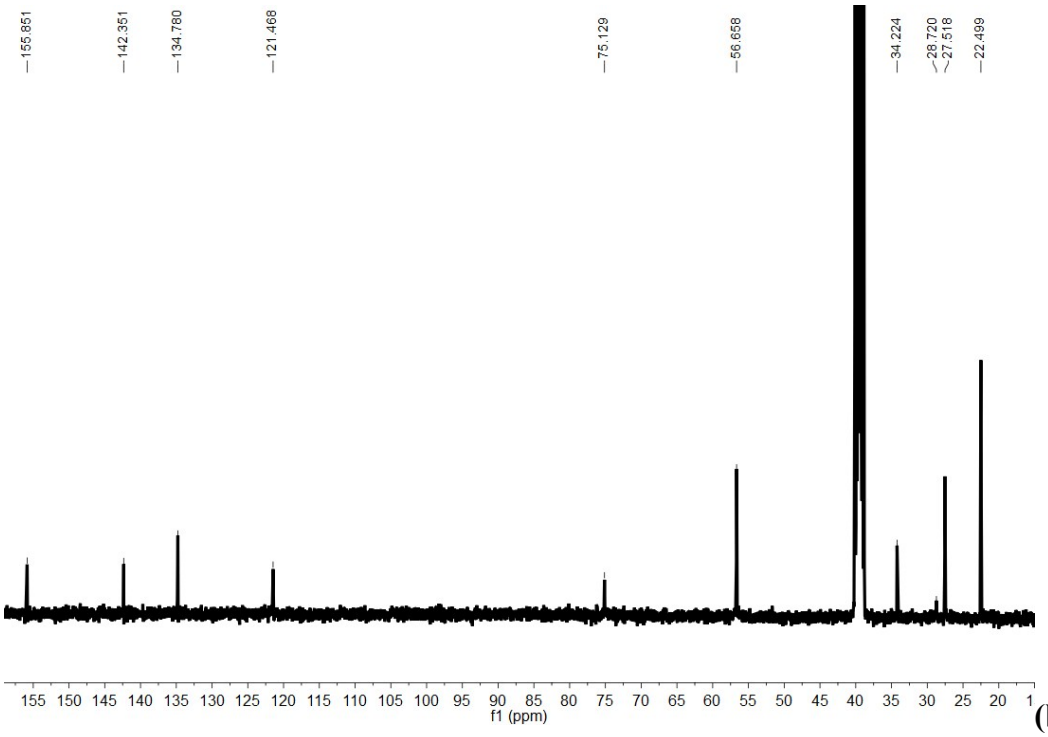


Figure S1. (a) ^{1}H NMR spectrum of **QAC5A** in DMSO, 400 MHz, 25 °C; (b) ^{13}C NMR spectrum of **QAC5A** in DMSO, 100 MHz, 25 °C.

1. F. Sansone, M. Dudic, G. Donofrio, C. Rivetti, L. Baldini, A. Casnati, S. Cellai and R. Ungaro, *J. Am. Chem. Soc.*, 2006, **128**, 14528-14536.
2. Z. Zheng, W.-C. Geng, J. Gao, Y.-Y. Wang, H. Sun and D.-S. Guo, *Chem. Sci.*, 2018, **9**, 2087-2091.
3. (a) Hammer, Ø. & Harper, D.A.T. 2006. Paleontological Data Analysis. Blackwell. (b) Hammer, Ø., Harper, D.A.T., and P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9pp. (c) Harper, D.A.T. (ed.). 1999. Numerical Palaeobiology. John Wiley & Sons.