Supporting Information

Functionalization of fluorous thin films via "click" chemistry

Catherine M. Santos, Amit Kumar, Wen Zhang and Chengzhi Cai^{*}

Department of Chemistry & Center for Materials Chemistry, University of Houston, Houston, Texas 77204.

Email: cai@uh.edu; Fax: (713)-743-2710

Synthesis of compounds 1-6

General: Air sensitive reactions were performed under a nitrogen atmosphere using a Schlenk technique. All reagents were purchased from Sigma-Aldrich (St. Louis, MO), Thermo-Scientific (Pittsburgh, PA), Quanta BioDesign, Ltd. (Powell, OH), Matrix Scientific (Columbia, SC) and Alfa Aesar (Ward Hill, MA, and used without purification. Flash chromatography was carried out on silica gel (60Å, Sorbent Technologies). All ¹H- and ¹³C NMR spectra were recorded in CDCl₃ using residual CHCl₃ as an internal standard. Mass spectroscopy (MS) measurements were carried out using electrospray ionization (ESI) technique.

Compound 1



N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-

eptadecafluorononanamide (1) To a solution of the azide S1 (86 mg, 0.38 mmol) and triethylamine (0.14 mL, 1.0 mmol) in dry THF (0.3 mL) at 0 °C under nitrogen was dropwise added $C_8F_{17}COCI$ (S2, 120 mg, 0.25 mmol) dissolved in dry THF (0.1 mL). After being stirred for 1.5 h at 0 °C, the solution was allowed to warm up and stirred overnight at room temperature. Saturated aqueous NH₄Cl (5 mL) was added, and the mixture was extracted three times with Et₂O. The combined organic layers were washed with water, brine, and dried over Na₂SO₄. The solvent was removed under vacuum affording a crude product, which was purified by flash chromatography (ethyl acetate) to give the azide 1 (94 mg, 0.14 mmol, 57%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃: δ 3.37 (t, *J* = 4.5 Hz, 2H), 3.56-3.67 (m, 14H), 7.25-7.13 (br,

1H). ¹³C NMR (75 MHz, CDCl₃): δ 39.9, 50.8, 68.7, 70.0, 70.4, 70.6, 70.7 104.4-115.5, 119.0 (t, *J* = 121 Hz), 157.6 (t, *J* = 95 Hz). ¹⁹F NMR (282 MHz, CDCl₃): δ 132.0, 130.1, 129.8, 129.2, 129.0, 125.6, -81.5, -81.6. MS (ESI) *m/z* calcd for C₁₈H₃₁NO₇: 664.3; found: 687.3 ([M + Na]⁺).





4

708.18



126615
158052
129/186
278.921
130,161
132,000

Compound 3



2-(2-(2-(2-(4-((methylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethanol (3) To a stirred solution of methyl propargylamine (**S5**, 150 mg, 2.17 mmol) in CH₃CN (1.0 mL) was treated sequentially with the azide **S4** (565 mg, 2.60 mmol), 2,6-lutidine (193 mg, 1.81 mmol), and Cu(MeCN)₄PF₆ (7.70 mg, 30.0 µmol). After the mixture was stirred at room temperature for 12 hours, the solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **3** (0.60 g, 0.47 mmol, 96%) as a light brown viscous liquid. ¹H NMR (300 MHz, CDCl₃): δ 7.53 (s, 1 H), 4.21 (t, 2 H, J = 5.0 Hz), 3.77 (s, 2 H), 3.54 (t, 2 H, J = 5.0 Hz), 3.37-3.24 (m, 14 H), 2.12 (s, 3 H); ¹H NMR (300 MHz, CDCl₃ + D₂O): δ 7.87 (s, 1 H), 4.59 (t, 2 H, J = 5.0 Hz), 3.94-3.85 (m, 4 H), 3.80-3.55 (m, 14 H), 2.50 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃): δ 145.6, 123.7, 73.0, 70.7-70.5, 69.8, 61.4, 50.4, 46.1, 35.4; MS (ESI) *m/z* calcd for C₁₂H₂₄N₄O₄: 288.3; found: 311.3 ([M + Na]⁺).





Compound 2



N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-

eptadecafluorononanamide (2) To a solution of alkyne S3⁻¹ (100 mg, 0.43 mmol) and triethylamine (0.14 mL, 1.0 mmol) in dry THF (0.3 mL) at 0 °C under nitrogen was dropwise added C₈F₁₇COCl (S2, 240 mg, 0.50 mmol) dissolved in dry THF (0.1 mL). After being stirred for 1.5 h at 0 °C, the solution was allowed to warm up and stirred overnight at room temperature. Saturated aqueous NH₄Cl (5 mL) was added and the mixture was extracted three times with Et₂O. The combined organic layers were washed with water, brine, and dried over Na₂SO₄. The solvent was removed under vacuum affording a crude product, which was purified by flash chromatography (ethyl acetate) to give amide **2** (224 mg, 0.33 mmol, 77%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃: δ 2.42 (s, 1H), 3.53-3.63 (m, 16H), 4.13 (s, 2H), 7.63 (s, NH, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 39.7, 58.0, 68.5, 68.8, 70.0, 70.1, 70.2, 70.3, 74.3, 79.1, 104.4-115.5, 118.9 (t, *J* = 121 Hz), 157.5 (t, *J* = 95 Hz). MS (ESI) *m/z* calcd for C₂₀H₂₀F₁₇NO₅: 677.3; found: 700.3 ([M + Na]⁺).







Compound 4



5-(2-hex-5-ynamidoethylamino)naphthalene-1-sulfonic acid (4) HOBt (52 mg, 0.37 mmol), Et₃N (77 μ L, 0.56 mmol), and EDC (106 mg, 0.56 mmol) were added to hex-5-ynoic acid (**86**, 61 μ L, 0.56 mmol) in DMF (1 mL). Then, **S5** (100 mg, 0.37 mmol) in DMF (1 mL) was added, and the reaction mixture was stirred for 24 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give 4 (113 mg, 0.31 mmol, 85%) as a yellow solid. ¹H NMR (300 MHz, D₂O): δ 8.03-7.88 (m, 3 H), 7.36 -7.32 (m, 2 H), 6.49 (d, 1 H, *J* = 7.7 Hz), 3.30-3.27 (m, 3 H), 3.08-3.05 (m, 2 H), 2.09 (t, 2 H, *J* = 7.3 Hz), 1.96 (t, 2 H, *J* = 7.3 Hz); ¹³C NMR (75 MHz, D₂O): δ 176.3, 143.7, 138.2, 129.0, 128.3, 126.1, 124.8, 124.2, 123.3, 114.7, 105.9, 84.6, 69.9, 43.1, 38.2, 34.6, 24.0, 16.9.





Compound 5

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4d]imidazol-4 yl)pentanamide (5) was synthesized according to a procedure from the literature.²

Compound 6



1-(2-(2-azidoethoxy)ethyl)-3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-4-

yl)thiourea (6) A stirred solution of Fluorescein isothiocyanate (**S7**, purity: 95 %, 100 mg, 0.243 mmol) in DMF (1.0 mL) was treated with the amine azide **S8** (94 mg, 0.30 mmol) and stirred for 2 hours. The solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **6** (0.146 g, 0.47 mmol, 82%) as a light yellow viscous liquid. ¹H NMR (300 MHz, CDCl₃): δ 8.07-7.4 (m, 4 H), 6.82-6.42 (m, 3 H), 3.36-3.23 (m, 24 H); ¹³C NMR (75 MHz, CDCl₃): δ 30.2, 51.2, 69.9-71.1, 103.5, 111.2, 129.1-129.8, 131.4, 132.4-132.8, 148.4, 153.3, 170.2, 171.6. 695.2; found: 696.3 ([M + H]⁺).





18

HPLC of Compound 6. HPLC was performed using a Shimadzu Prominence series HPLC equipped with a multiwavelength UV detector. HPLC grade ethyl acetate and methanol were acquired from Fisher. Injection volume: 3 μ L; Flow rate: 2.000 mL/min; Column: Alltech column, Econosphere silica 10 μ , 250 \times 10 mm (Alltech) Mobile phase: Ethyl acetate (solvent A) and Methanol (solvent B); and Gradient elution: solvent A 100% to 50% in 30 minutes.



Synthesis of the azido modified FITC-BSA. 25 mg protein (Bovine Serum Albumin, Sigma-Aldrich, St. Louis, MO), 2.0 mg FITC (Thermo-Scientific, Pittsburgh, PA), and 3.0 mg of N₃-EG₁₂-NHS (Quanta BioDesign, Ltd. Powell, OH), were each dissolved in 1.5 ml 0.25 M carbonate-bicarbonate buffer (pH 9.0 measured by a MV RS232 pH Meter; Omega Eng. Inc., Stamford, CT). The solutions were mixed at 4°C and gently stirred overnight at 4°C. Unreacted FITC and azide were removed from the reaction mixture by a Sephadex column (PD-10 G25; GE Healthcare) eluted with 0.01 M PBS.

Preparation of the ethynyl or azido terminated films A or B (Scheme 1) on fluorous slide. A 1 x 1 cm^2 (for surface characterization) or 2.5 x 2 cm^2 (for microarray experiments) fluorous glass slide (Fluorous Technologies Inc., Pittsburgh, PA) was immersed in a 1 mM solution of the ethynyl-terminated perfluorocarbon 1 or azido-terminated perfluorocarbon 2 in methanol for 2 h under nitrogen environment. The slides were removed and rinsed with methanol (~2 mL) for 4 to 6 times, followed by drying in a stream of argon.

General procedure for the surface CuAAC reactions in a microarray format. The microarray was prepared using a Spotbot[®]2 Personal Microarray Robot (TeleChem International, Inc., Sunnyvale, CA) in a closed chamber with a relative humidity of 58–60% at 25 °C. To a sample well in the arrayer was added a solution of Cu(MeCN)₄PF₆ (2.5 mM), ascorbic acid (50 mM), **3** (25 mM), and azide **6** (10 mM) or azido FITC-BSA (10 mM) in degassed methanol and water (1:9 v/v) or a solution of CuSO₄ (5 mM), ascorbic acid (50 mM), **3** (25 mM) and azide **6** (10 mM) or azido FITC-BSA (10 mM) in water. Microarray spotting pin (946MP4) was used to spot 1.1 nL droplets of the sample solutions (spot diameter ~100 µm) on the above ethynyl-terminated film **A**. The spotting of 6x4 and 5x3 arrays took 400 and 220 s, respectively. The microarray was incubated in the chamber at the same relative humidity at 25°C for 6-12 h. The film was taken out and thoroughly rinsed with methanol (~2 mL) for 4 to 6 times and water (~2 mL) for 2 to 4 times, followed by drying under a stream of argon.

Xray Photoelectron Spectroscopy (XPS). A PHI 5700 X-ray photoelectron spectrometer was equipped with a monochromatic Al K α X-ray source ($h\nu$ =1486.7 eV) incident at 90° relative to the axis of a hemispherical energy analyzer. The spectrometer was operated both at high and low resolutions with pass energies of 23.5 eV and 187.85 eV, a photoelectron take off angle of 45 ° from the surface, and an analyzer spot diameter of 1.1 mm. The survey spectra were collected from 0 to 1400 eV, and the high resolution spectrum were obtained for photoelectrons emitted from C1s, O1s, S 2p, N1s, and F1s. All spectra were collected at room temperature with a base pressure of 1 x10⁻⁸ torr. Electron binding energies were calibrated with respect to the C1s line at 284.5 eV (C-C).

A PHI Multipak software (version 5.0A) was used for all data processing. The high resolution data were analyzed first by background subtraction using the Shirley routine and a subsequent non-linear fitting to mixed Gaussian-Lorentzian functions. Atomic compositions were derived from the high-resolution scans. Peak areas were obtained after subtraction of the integrated baseline and corrected for sensitivity factors.

Binding of FITC-avidin on the biotinylated surface **D**. A solution of FITC-avidin (0.5 mg/mL) in PBS buffer was spotted on the biotinylated surface **D** using a Spotbot 2 Personal Microarray Robot (TeleChem International, Inc., Sunnyvale, CA) in a closed chamber with a relative humidity of 58–60% at 25 °C. A control experiment with biotin-saturated FITC-avidin solution (1 mg/mL) was also spotted on the same substrate. The biotin-saturated FITC-avidin solution was prepared by adding 270 equivalents of biotin to the solution of 1 mg/mL FITC-avidin. Microarray spotting pin (946MP4) was used to spot 1.1 nL droplets of the sample solutions (spot diameter ~100 μ m) on the biotinylated surface **D**. The spotting of a 5x4 array took 355 s. The spots were allowed to dry at 58-60 % relative humidity for 30 minutes. The film was taken out and thoroughly rinsed with PBS (~2 mL) for 2 to 4 times and dried under a stream of argon. The surface was scanned using a GeneTAC UC 4 Array Scanner with an excitation wavelength at 518 nm.



Fig. S1. XPS spectra of the ethynyl-terminated surface B. (a) Survey (b) C 1s with deconvolution and curve fitting.

Assignment	Binding Energy (eV)	XPS peak ratio	Calculated ratio
CF ₃	294.9	1.0	1*
CF_2	292.5	6.9	7^*
N-C=O	288.1	1.2	1
C-O	286.2	8.0	8
C-N	285.6	1.1	1
C-C	284.8	1.8	2

Table S1. Deconvoluted C 1s Peaks and Assignments for the Ethynyl-terminated Surface B

* Unknown contribution from the fluorous substrate with attenuation.

Assignment	Binding Energy (eV)	XPS peak ratio	Calculated ratio
N=N=N	403.2	1.0	1
N-C=O	401.3	1.1	1
N=N=N	400.1	1.9	2

Table S2. Deconvoluted N 1s Peaks and Assignments for the Initial Azido-terminated Surface A

Table S3. Deconvoluted N 1s Peaks and Assignments for Surfaces C and D

	Surface C			Surface D		
Assignment	Binding Energy (eV)	XPS peak ratio	Calculated ratio [*]	Binding Energy (eV)	XPS peak ratio	Calculated ratio ^{**}
N=N=N	403.2	0.2	0.2			
N-C=O	401.2	2.5	2.6	401.2	3.2	3.1
N=N=N	400.2	0.5	0.4			
N=N	400.0	1.5	1.6	400.0	1.4	1.4
N-N=N	398.7	0.7	0.8	398.7	0.7	0.7

* Calculated based on a reaction yield of 80 %.

** Calculated based on a reaction yield of 70 %.



Fig. S2. Fluorescence images of the control experiments after spotting a solution of FITC-avidin on an (a) ethynyl-surface **B** and a (b) fluorous-coated slide.

References

- 1. L. Polito, D. Monti, E. Caneva, E. Delnevo, G. Russo and D. Prosperi, *Chem. Commun.*, 2008, 5, 621-623.
- 2. X.-L. Sun, C. L. Stabler, C. S. Cazalis and E. L. Chaikof, Bioconjugate Chem., 2006, 17, 52-57.