Electronic Supplementary Information

of

Coumarin derivative as fluorogenic glycoproteomic probe for biological imaging

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Experimental Section

Materials

The following materials and chemicals were used as received without further purification:

7-hydroxycoumarin-4-acetic acid was purchased from HEOWNS BIOCHEM TECHNOLOGIES (Tianjing, China).

2,2,2-trifluoro ethanol, copper(I) iodide (CuI), copper(I) bromide (CuBr), *N*-phenyl-bis(trifluoromethanesulfonimide), *N*,*N*-diisopropylethylamine, D-biotin, propargylamine, Dichlorobis(triphenylphosphine)-palladium, (trimethylsilyl)acetylene, tetrabutylammonium fluoride (1M in THF), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl), tripropargylamine, *N*,*N*,*N'*,*N''*,*N''*-pentamethyldiethylenetriamine (PMDETA), and 3-chloropropanol, sodium azide, tetrabutylammonium hydrogensulfate were purchased from Sigma-Aldrich (United States).

Sulfuric acid (H_2SO_4), ethyl acetate, sodium bicarbonate ($NaHCO_3$), anhydrous magnesium sulfate ($MgSO_4$), *N*,*N*-dimethylformamide (DMF), cyclohexane, tetrahydrofuran (THF), chloroform, methanol, and sodium sulfate were provided by Shanghai Chemical Co. (China).

Acetonitrile was purchased from Fisher Scientific (United States).

Instrumentation

NMR

^{1}H NMR spectra were recorded on a Varian Unity 300 MHz spectrometer. ^{1}H chemical shifts values, 300 MHz, are reported as δ using the residual solvent signal as internal standard.

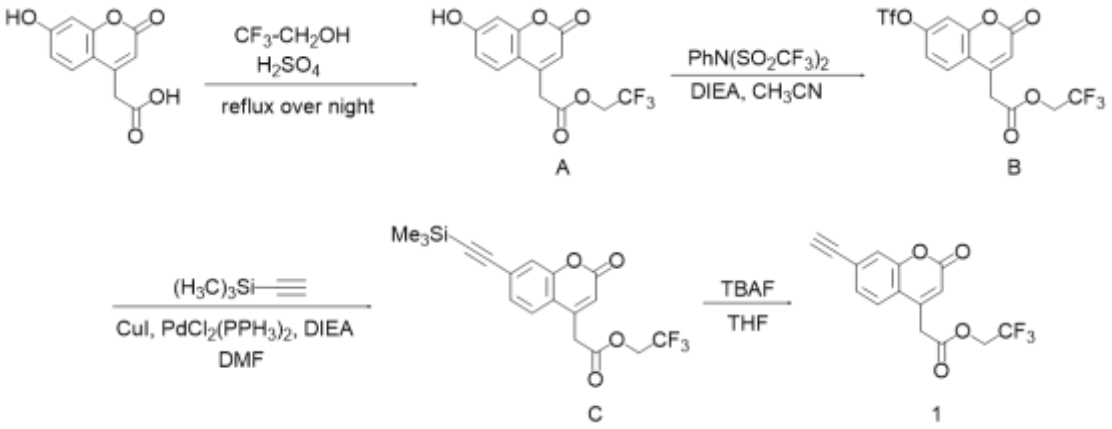
Fluorescence microscopy

Fluorescence microscopy images were taken using a Carl Zeiss fluorescence microscopy, NOL-LSM 710.

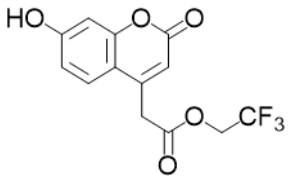
Flow cytometry

Flow cytometry data were collected by a BD Biosciences multicolor flow cytometry, BD FACSAria™ III.

Synthesis of 1



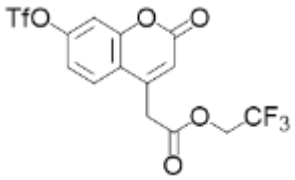
Synthesis of (7-hydroxy-2-oxo-2H-chromen-4-yl)-acetic acid 2,2,2-trifluoroethyl ester (A)



A mixture of 7-hydroxycoumarin-4-acetic acid (1.03 g, 4.7 mmol), 2,2,2-trifluoro ethanol (40 mL) and H_2SO_4 (4.2 mL) was refluxed at 95^{o}C overnight. The reaction mixture was diluted with ethyl acetate and saturated aqueous solution of $NaHCO_3$ was added until the effervescence ceased, then the organic phase was washed with brine thrice, and dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporator to yield the product A as brownish power. Yield: 1.13 g, 80 %.

^{1}H-NMR (DMSO-d_6): δ 7.59 (d, J = 8.7, 1H), 6.86 (dd, J = 2.4, J = 8.7, 1H), 6.78 (d, J = 2.4, 1H), 6.27 (s, 1H), 4.75 (q, J = 9.0, 2 H), 4.09 (s, 2H).

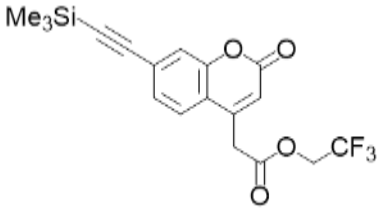
Synthesis of (2-oxo-7-trifluoromethanesulfonyloxy-2H-chromen-4-yl)-acetic acid 2,2,2-trifluoroethyl ester (B)



A mixture of A (1.05 g, 3.5 mmol), N-phenyl-bis(trifluoromethanesulfonimide) (1.38g, 3.8 mmol) and N,N-diisopropylethylamine (0.83 mL, 4.58 mmol) in acetonitrile (50 mL) was stirred at 20^{o}C for about 18 hours. Then, the reaction mixture was diluted with ethyl acetate. The organic layer was washed with weak brine twice and saturated brine once, then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using a 7:3 cyclohexane/ethyl acetate mixture as eluent to obtain the product B as a yellowish solid. Yield: 1.28 g, 84 %.

^{1}H-NMR (DMSO-d_6): δ 7.99 (d, J = 9.0,1H), 7.57 (d, J = 2.4, 1H), 7.48 (dd, J = 2.4, J = 9.0, 1H), 6.65 (s, 1H), 4.76 (q, J = 8.7, 2 H), 4.26 (s, 2H).

Synthesis of (2-oxo-7-trimethylsilanylethynyl-2H-chromen-4-yl)-acetic acid 2,2,2-trifluoroethyl ester (C)

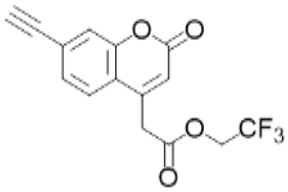


Dichlorobis(triphenylphosphine)-palladium (0.07 g, 0.10 mmol), CuI (19.4 mg, 0.10 mmol), triethylamine (0.60 mL, 4.49 mmol) and (trimethylsilyl)acetylene (0.44 mL, 3.06 mmol) were added to a solution of B (0.89 g, 2.04 mmol) in dry DMF (27 mL) under argon atmosphere. The reaction mixture was degassed by three frezze-thraw cycles and stirred at 30 ºC for 36 h. After that, the mixture was diluted with ethyl acetate. The organic layer was washed with weak brine twice and saturated brine once, then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using a 7:3 hexane/ethyl acetate mixture as eluent to obtain product C as orange solid. Yield:

0.52 g, 67 %.

^{1}H-NMR (DMSO-d_6): δ 7.99 (d, J = 9.0,1H), 7.57 (d, J = 2.4, 1H), 7.48 (dd, J = 2.4, J = 9.0, 1H), 6.65 (s, 1H), 4.76 (q, J = 8.7, 2 H), 4.26 (s, 2H).

Synthesis of (7-ethynyl-2-oxo-2H-chromen-4-yl)-acetic acid 2,2,2-trifluoroethyl ester (1)



Tetrabutylammonium fluoride (2 mL 1M in THF, 2.0 mmol) was added to a solution of C (260 g, 0.68 mmol) in THF (13 mL). The mixture was stirred at 25 ^{o}C for about 14 h. After that, the reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with brine (3 x 25 mL) and dried over anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 3:2 hexane/ethyl acetate mixture as eluent to obtain product 1 as a yellow solid. Yield: 0.50 g, 70 %.

^{1}H-NMR (DMSO-d_6): δ 7.77 (d, J = 8.1, 1H), 7.46 (d, J = 1.5, 1H), 7.43 (d, J = 1.5, 1H), 6.56 (s, 1H), 4.78 (q, J = 9.0, 2 H), 4.21 (s, 2H), 3.95 (s, 1H).

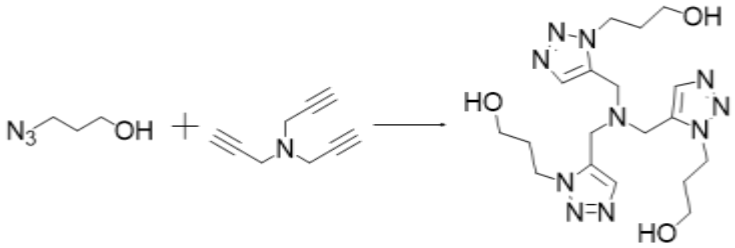
Synthesis of biotinylated alkyne

A mixture of D-biotin (140mg, 0.57mmol), propargylamine (65mg, 1.18mmol) in water (10mL) was stirred at room temperature, the pH of the reaction mixture was adjusted to 5~6 with 2M HCl aqueous solution. Then EDC·HCl (210mg, 1.10mmol) was added, and stirred at room temperature overnight. After that, the reaction mixture was lyophilized and the residue was purified by flash column chromatography on silica gel using 5:1 chloroform/methanol mixture as eluent to obtain biotinylated

alkyne as white powder. Yield: 126.62mg, 78.9%.

^{1}H-NMR (DMSO-d_6) d 1.31-1.39 (m, 2H), 1.51-1.59 (m, 3H), 1.64-1.70 (m, 1H), 2.14 (t, 2H, J = 7.5 Hz), 2.64 (d, 1H, J = 12.0 Hz), 2.88 (dd, 1H, J = 5.0 and 12.0 Hz), 3.13 (t, 1H, J = 2.5 Hz), 3.15-3.18 (m, 1H), 3.89 (q, 1H, J = 2.5 Hz), 4.17-4.21 (m, 1H), 4.35-4.38 (m, 1H), 6.41 (s, 1H), 6.47 (s, 1H), 8.27 (m, 1H).

Synthesis of tris(3-hydroxypropyltriazolylmethyl)amine (THPTA)



Synthesis of 3-Azidopropanol (AzPOH).

3-Chloropropanol (30 mL, 33.93 g, 0.358 mol) was added to a mixture of water (40 mL), sodium azide (47 g, 2 equiv), and tetrabutylammonium hydrogensulfate (1 g). The mixture was stirred at 80 °C for 24 h and then at room temperature for 13-14 h. The product was extracted with ether (3 × 80 mL), the resulting solution was dried over sodium sulfate, the solvent was removed on a rotary evaporator, and after vacuum distillation, 3-azidopropanol was obtained (yield: 30.8 g, 85%).

^{1}H-NMR in $CDCl_3$ (δ, ppm): 3.76 (t, 2H, CH_2O), 3.46 (t, 2H, CH_2N_3), and 1.84 (tt, 2H, CCH_2C).

Synthesis of THPTA

A mixture of tripropargylamine (650mg, 4.9mmol), AzPOH (1.5g, 14.8mmol) PMDETA (200μL, 1mmol), CuBr (10mg, 0.7mmol) in dry DMF (20mL) was stirred at room temperature under a nitrogen atmosphere overnight. After that, the liquid layers were removed by vacuum drying to give a light blue solid. Then the blue solid was purified by flash chromatography on silica gel using methanol as eluent to obtain THPTA as off white solid. Yield: 1.83g, 86%.

^{1}H-NMR in D_2O (δ, ppm): 7.73 (s, 3H, NCHC), 4.29(t, 6H, NCH_2C), 3.62(t, 6H, NCH_2C), 3.36 (t, 6H, CCH_2O), 1.91(t, 6H, CCH_2C).