Supplementary Information

Terminal Alkyne Substituted O^6-Benzylguanine for Versatile and Effective Syntheses of Fluorescent Labels to Genetically Encoded SNAP Tags

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All chemicals are obtained from commercial suppliers and used without further purification. The 400 (^{1}H) MHz NMR and 100 (^{13}C) MHz NMR spectra are registered at room temperature on a 400 MHz spectrometer using perdeuterated solvents as internal standard. Images are acquired with a confocal microscopy from OLYMPUS.

4-[2-Propynylmethoxy]benzyl alcohol .

It is synthesized according to the literature procedures. [1] 1,4-benzenedimethanol (3 g, 21.71 mmol) is dissolved in 10 mL dry DMF, and NaH (1g) is added in small portions over 10min at 0℃. The mixture is sttired for 15min. Propargyl bromide (2.32 g 19.54 mmol) is added and the mixture is sttired for 1h at 25℃. The mixture is quenched with water, extracted with CH_2Cl_2, and purified by flash column chromatography with CH_2Cl_2 to get 4-[Prop-2-ynyloxymethy]-benzyl alcohol (2.68g, 15.21mmol, 70%).

1-(2-Amino-7H-purin-6-yl)-1-methyl-pyrrolidinium chloride

It is synthesized according to the literature procedures.[2] 6-Chloro-guanine (0.5g , 5.9 mmol)) is dissolved in 15 ml DMF at 50°C. After cooling to room temperature, 1-methyl-pyrrolidin (1.4 ml, 13.2 mmol) is added and the reaction mixture is stirred for 24 h. 2 ml of acetone are added to complete precipitation. The solid is filtered, washed with ether and dried in vacuo, yielding 0.55 g product (2.16 mmol, 71%).

O^6-[4-[2-propynylmethoxy]benzyl]guanine (PYBG).

4-[2-Propynylmethoxy]benzyl alcohol (662 mg, 7.33 mmol) is dissovled in 3 mL dry DMF, and NaH (65mg) is added in small portions over 3 min at 0℃. The mixture is sttired for 15 min. 1-(2-Amino-7H-purin-6-yl)-1-methyl-pyrrolidinium chloride (300 mg) and 4-dimethylaminopyridine (30 mg) are added and the mixture is sttired for 1h at 25℃. The mixture is quenched with 0.5 mL water and purified by flash column chromatography (gradient:CH_2Cl_2/CH_3OH 40:1→15:1)to yield 255 mg **PYBG** (0.83 mmol, 72%). mp 111.1-112.9°C.^{1}H NMR (400 MHz, DMSO) δ 11.60 (s, 1H), 6.99 (s, 1H), 6.64 (d, J = 7.8 Hz, 2H), 6.50 (d, J = 7.8 Hz, 2H), 5.45 (s, 2H), 4.63 (s, 2H), 3.68 (s, 2H), 3.33 (s, 2H), 2.65 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 159.6 , 137.4 , 136.2 , 128.4 , 127.8 , 80.1 , 77.5 , 70.4 , 66.4 , 56.9 . m/z (FTMS+p ESI): Calcd $[M+H]^+$ for $C_{16}H_{16}N_5O_2$: 310.1299, found : 310.1295.

PYBG-TMR.

6-Br-tetramethylrhodamine(6-Br-TMR) is synthesized according to literature procedures. [3]

A mixture of 6-Br-TMR(20 mg, 0.064 mmol), PPh_3 (5 mg, 0.02 mmol), PYBG (20 mg, 0.064mmol), $Pd[P[C_6H_5]_3]_4$(2 mg, 0.002mmol), CuI (1mg, 0.005mmol), and DMF/Triethylamine (1.5 mL/0.5 mL) are placed in a 10 mL round-bottomed flask with a magnetic stirrer bar under a nitrogen atmosphere . The mixture is heated at 75℃ for 4 h. Then the mixture is purified by column chromatography(gradient:CH_2Cl_2/CH_3OH 40:1→8:1) to yield 35mg **PYBG-TMR** (0.05mmol, 78%). mp More than 300°C. m/z (TOF-LD+): Calcd $[M+H]^+$ for $C_{40}H_{36}N_7O_5$: 694.2778, found : 694.2833. The resolutions of ^1H NMR and ^{13}C NMR spectra are not very satifactory due to the extremely poor solubility of **PYBG-TMR** in $CDCl_3$, CD_3OD, and DMSO-*d6* etc. The purity is measured by HPLC is 94.4%.

PYBG-BODIPY 4,4-difluoro-8-[4-[[3-azidopropyloxy]phenyl]]-1,3,5,7-teramethyl-4-bora-3a,4a-diazaindacene is synthesized according to literature procedures.[4]

A mixture of **BODIPY**(20mg, 0.05mmol), **PYBG**(15mg, 0.05mmol) and N,N-Diisopropylethylamine(0.05ml, 0.28mmol) are dissovled in 3mL DMF under a nitrongen atmosphere. $CuSO_4 \cdot 5H_2O$(2mg, 0.008mmol) dissovled in 0.5mL water is added and then sodium ascorbate dissolved in 0.5mL water is added. The mixture is sttired for 7h at 25℃. Then the mixture is purified by column chromatography (gradient:CH_2Cl_2/CH_3OH 40:1→15:1) to yield 29mg **PYBG-BODIPY** (0.04mmol, 85%). mp 50.5-57.1°C. m/z (FTMS+p ESI): Calcd $[M+H]^+$ for $C_{38}H_{40}N_{10}F_2O_3B$: 733.3340, found : 733.3342. The resolutions of ^1H NMR and ^{13}C NMR spectra were not very satifactory due to the extremely poor solubility of PYBG-BODIPY in $CDCl_3$, CD_3OD, and DMSO-*d6* etc. The purity is measured by HPLC is 99.1%.

Plasmid Construction

$pSNAP_f$ vector and $pSNAP_f$-H2B plasmid are obtained from New England Biolabs (NEB). $pSNAP_f$-COX8A plasmid is constructed by insertion of mitochondrial targets sequence of cytochrome c oxidase subunit 8 (COX8A) to the N-terminus of SNAP ($pSNAP_f$ vector). The COX8A sequence is obtained from anneal of oligonucleotides according to literature procedure with minor modification.[4] The oligonucleotides Mito1-1 + Mito1-2 and Mito2-1 + Mito2-2 are annealed respectively to provide Mito1 and Mito2 dsDNAs. The dsDNAs are ligated and insert into pSNAPf vector at EcoR V and EcoR I sites. All the plasmids are amplified in E. coil DH5α competent cells, and $pSNAP_f$-COX8A plasmid is verified by DNA sequencing.

Mito 1-1: ATCATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGC

Mito 1-2: CCGGGCCGAGCCTGTCAAGCCCCGCAGCAGCAGCGGCGTCAGGACGGACATGAT

Mito 2-1: TCGGCCCGGCGGCTCCCAGTGCCGCGCGCCAAGATCCATTCGTTGGGGG

Mito 2-2: AATTCCCCCAACGAATGGATCTTGGCGCGCGGCACTGGGAGCCG

Cell Culture and labeling

COS-7 cells (Cell Bank of Type Culture Collection of Chinese Academy of Sciences) are cultured in Dulbecoo's modified Eagle's medium (DMEM,Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C in an atmosphere of 5% CO2/95% air (CO2 incubator, Thermo Scientific). Cells are transiently transfected with pSNAPf-H2B plasmid or $pSNAP_f$-COX8A plasmid by using Lipofectamine 2000 (Invitrogen) following the standard protocol. And stable transfection is established by G418 (Geneticin, Invitrogen) selection. Cells for imaging are seeded on 35 mm glass

bottom culture dishes (Φ=20 mm) and cultured for 24-48 h. The dyes PYBG-TMR and PYBG-BODIPY are dissolved in DMSO (1 mM) by vortexing for 3 min to used as the stock solution. And when the dyes are submitted for cell imaging study, the stock solution are mixed with preheated (37°C) culture medium (DMEM with 10%FBS) and pipetted up and down more then 10 times to obtain the labeling solution. Cell labelling is achieved by incubating with PYBG conjugated dyes: PYBG-TMR (3 μM) or PYBG-BODIPY (5 μM) for 30 min at 37°C, and washed with PBS (3×1 mL). After incubate for 2 h in DMEM (10% FBS), the medium is replaced with fresh DMEM (10% FBS) 30 min before imaging.

Purified SNAP protein for kinetics of the reaction

The SNAP protein for kinetics research is achieved from purifying by a C-terminus fused HisTag according to literature procedure.[6] The SNAP coding sequence is cut from pSNAPf vector using EcoRI and XhoI restriction endonucleases. And the gene fragment is ligating in PET28a(+) (Novagen) using corresponding restriction sites. The PET28a-SNAP plasmid is transformed into E. coli strain BL-21(DE3), and the bacterial is grown in LB medium with 50 μg/mL Kanamycin at 37 °C to reach the optical density (OD600nm) of 0.8. Then, isopropyl-β-D-thiogalactopyranoside (IPTG, 1 mM) is added for the inducing of the expression of SNAP-HisTag. The culture is grown at 16 °C for 12 h. The subsequent purification of SNAP-HisTag is totally based on the literature method[6] except a HisTrap HP (GE Healthcare) which is used for the purification. Finally, 6 mg purified SNAP-HisTag (80 μM in 3 mL elution buffer) is obtained.

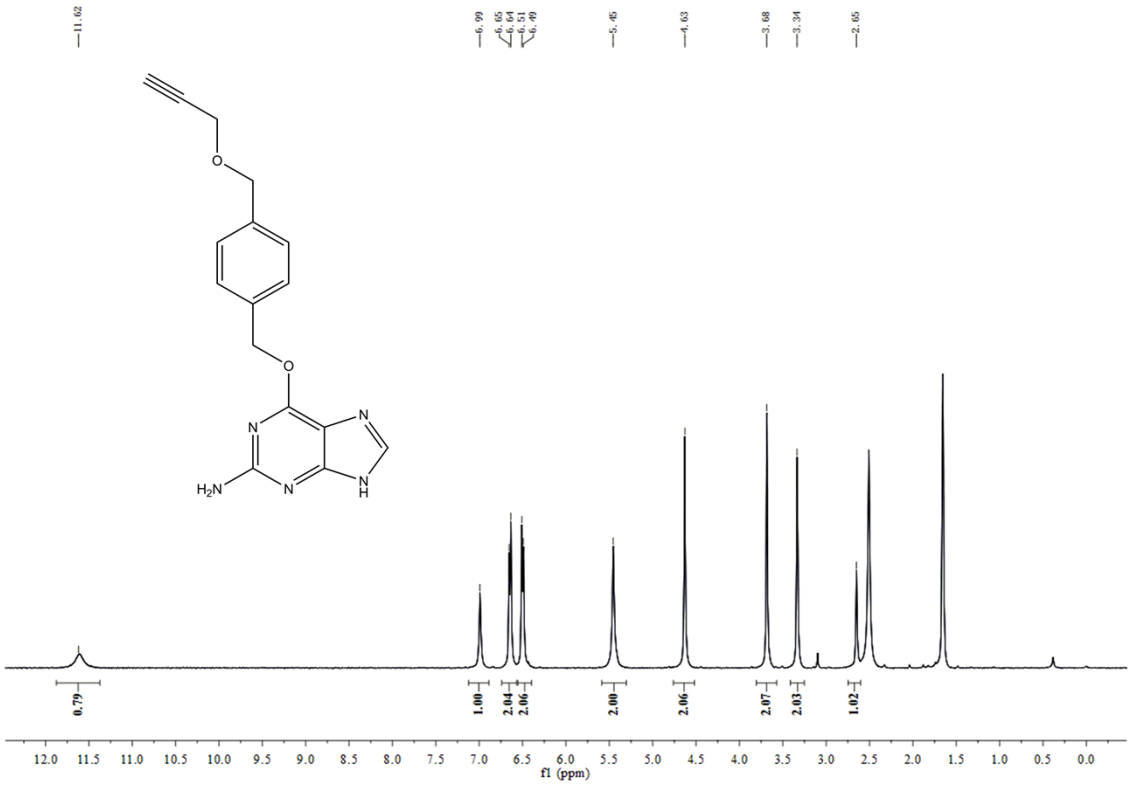


Figure 1.PYBG ^{1}H NMR

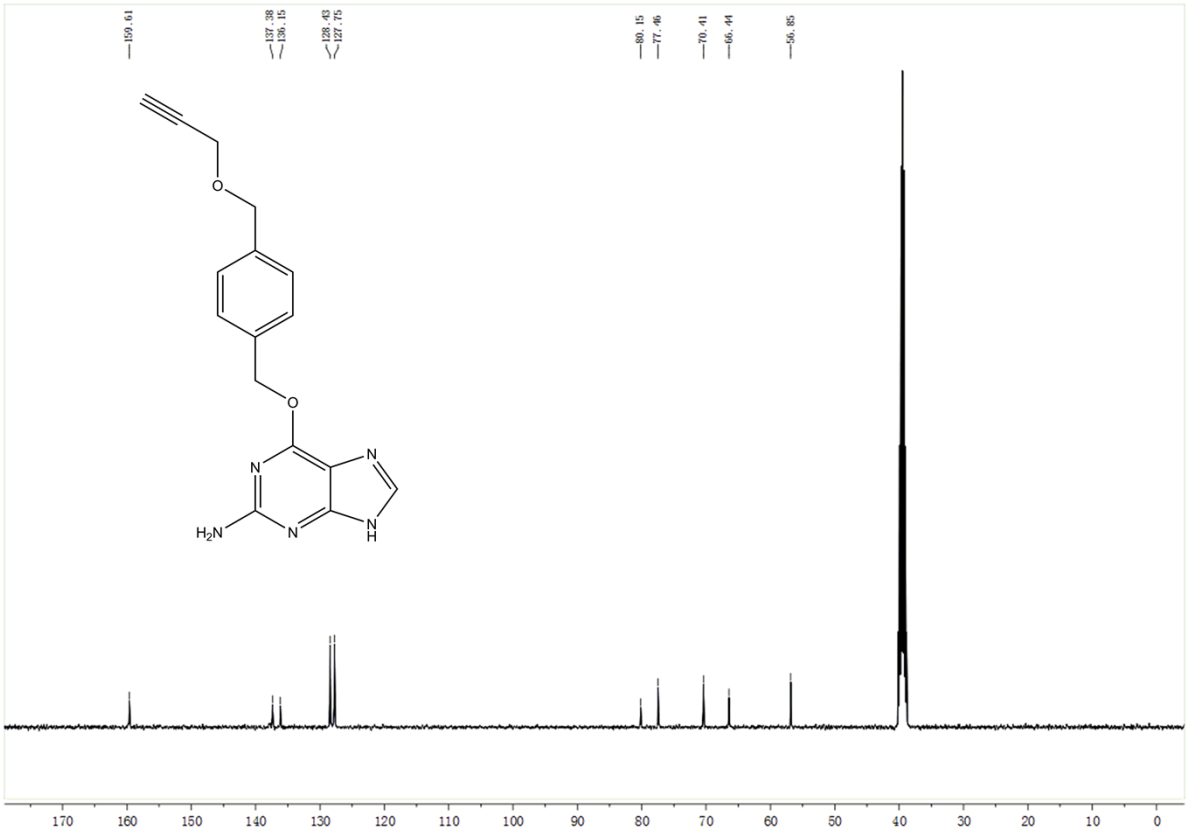


Figure 2.PYBG ^{1}C NMR

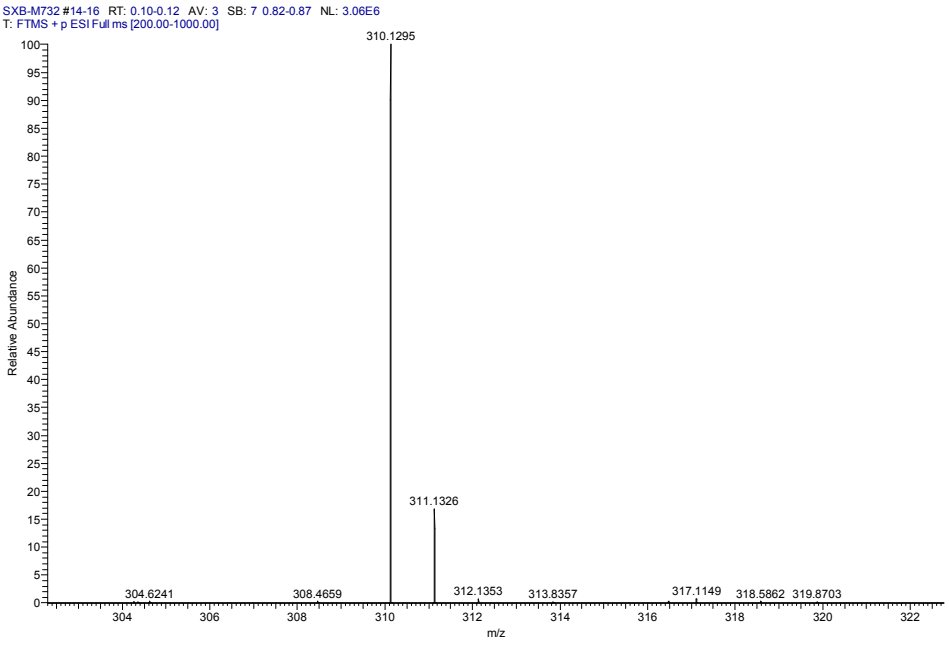


Figure 3.ESI ms Spectrum PYBG

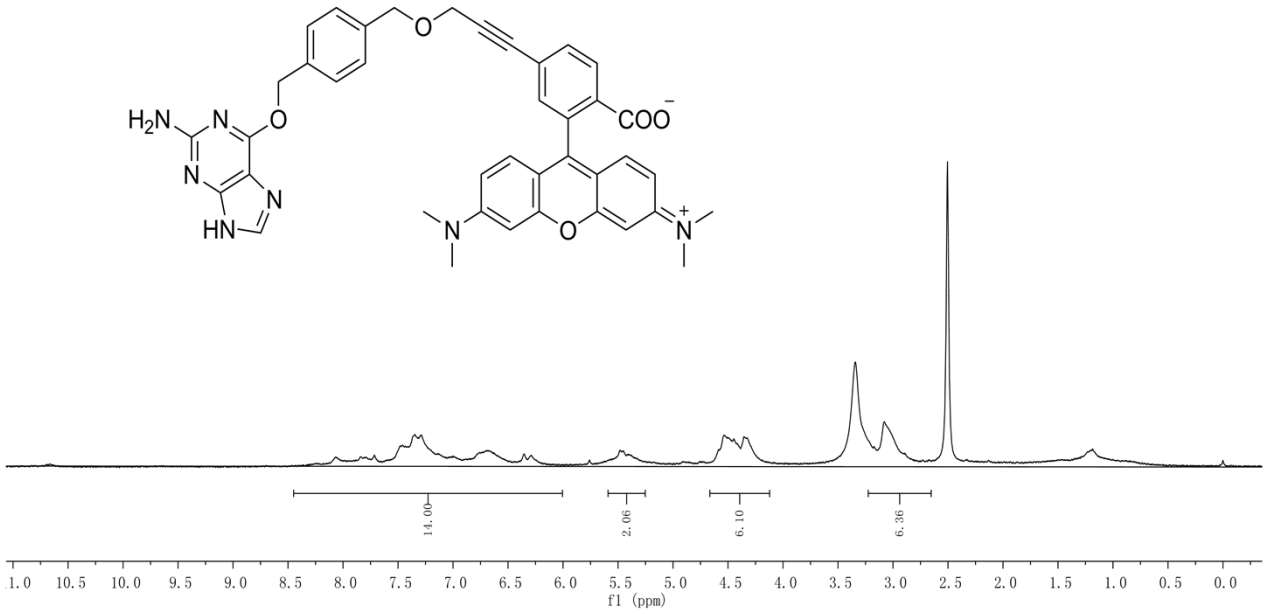
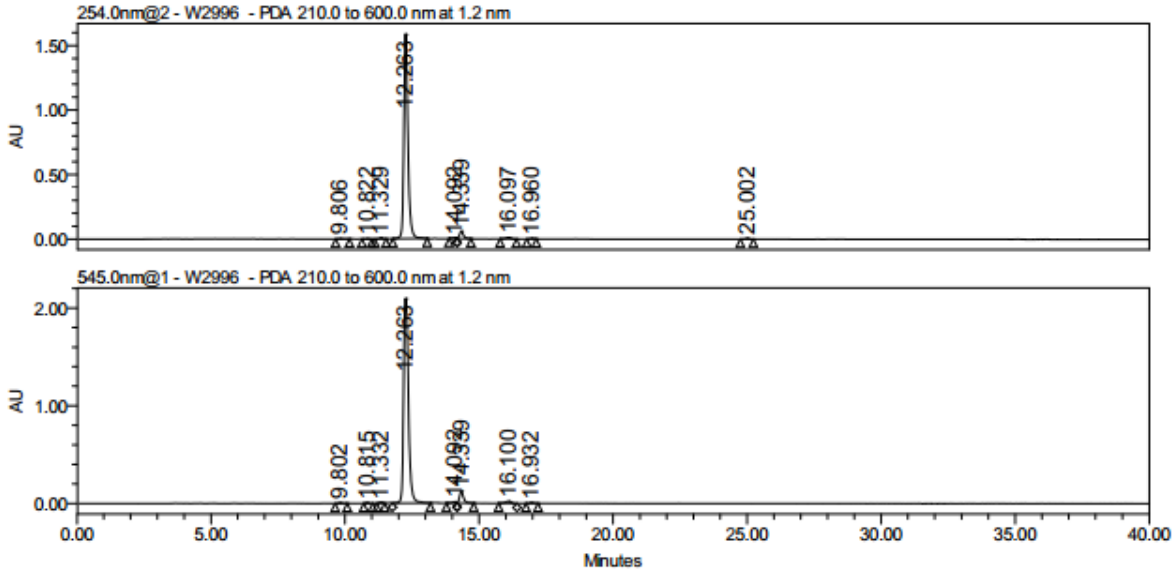


Figure4.PYBG-TMR ^{1}H NMR

SAMPLE INFORMATION

Sample Name:	sxb	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	14070902
Vial:	29	Acq. Method Set:	2A
Injection #:	1	Processing Method:	3
Injection Volume:	2.00 ul	Channel Name:	254.0nm@2, 545.0nm@1
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	PDA 254.0 nm, PDA 545.0 nm

Date Acquired: 7/10/2014 3:33:59 PM CST Project Name: H140707 Channel Id 1262
Date Processed: 7/10/2014 4:25:00 PM CST, 7/10/2014 4:26:00 PM CST



Processed Channel Descr.: PDA 254.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 254.0 nm	9.806	30474	0.17	2099
2	PDA 254.0 nm	10.822	22259	0.13	2262
3	PDA 254.0 nm	11.329	56249	0.32	6241
4	PDA 254.0 nm	12.263	16590118	94.42	1586846
5	PDA 254.0 nm	14.092	59885	0.34	6023
6	PDA 254.0 nm	14.339	559655	3.19	56884
7	PDA 254.0 nm	16.097	155446	0.88	10133
8	PDA 254.0 nm	16.960	34151	0.19	3460
9	PDA 254.0 nm	25.002	62350	0.35	5183

Processed Channel Descr.: PDA 545.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 545.0 nm	9.802	65852	0.23	4972

Processed Channel Descr.: PDA 545.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
2	PDA 545.0 nm	10.815	52426	0.19	5465
3	PDA 545.0 nm	11.332	101296	0.36	10807
4	PDA 545.0 nm	12.263	26241190	92.74	2095104
5	PDA 545.0 nm	14.092	146147	0.52	12277
6	PDA 545.0 nm	14.339	1241900	4.39	121647
7	PDA 545.0 nm	16.100	355988	1.26	20274
8	PDA 545.0 nm	16.932	91238	0.32	6796

Figure 5.HPLC spectrum PYBG-TMR

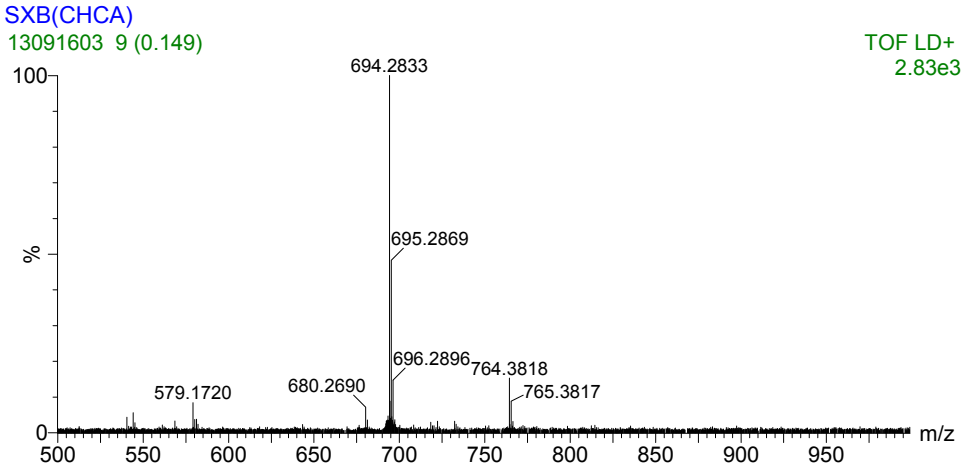


Figure 6.TOF LD+ ms Specturm PYBG-TMR

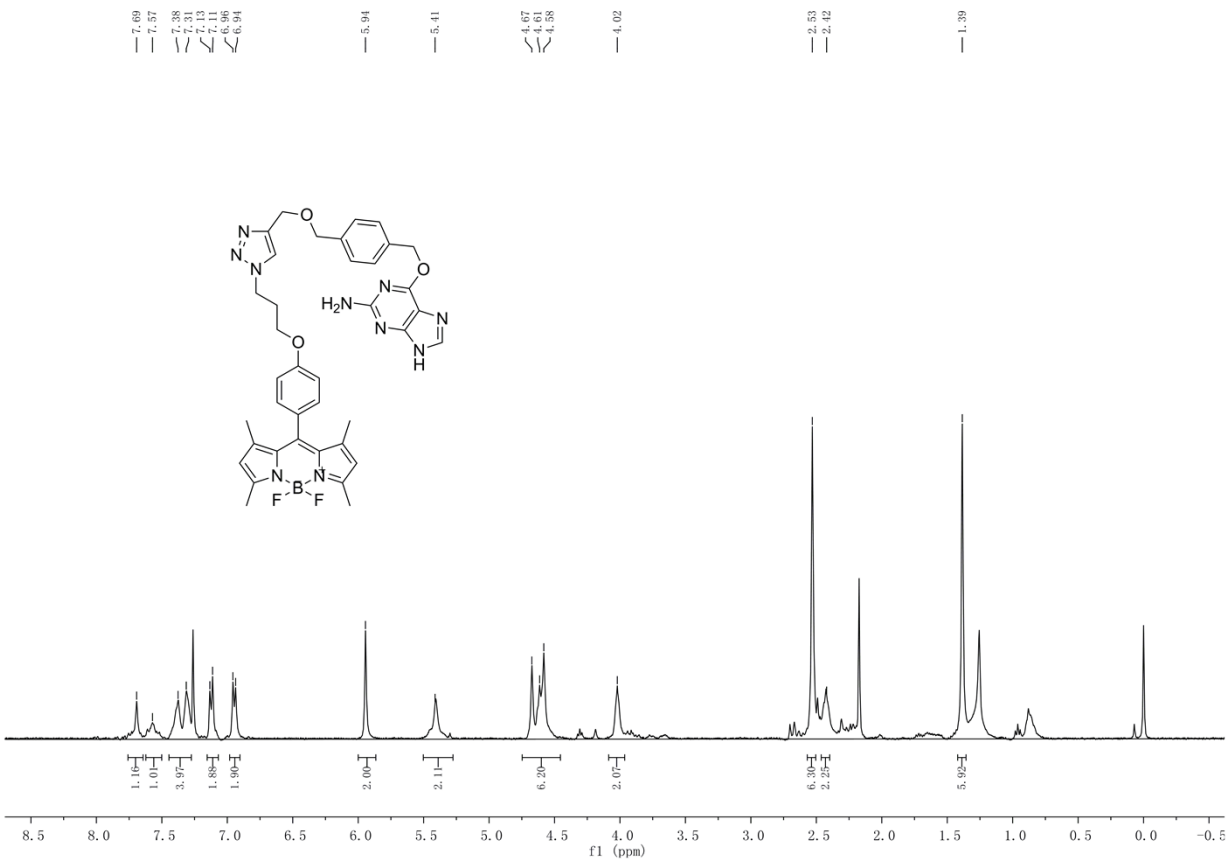


Figure 7.PYBG-BODIPY ^{1}H NMR

SAMPLE INFORMATION

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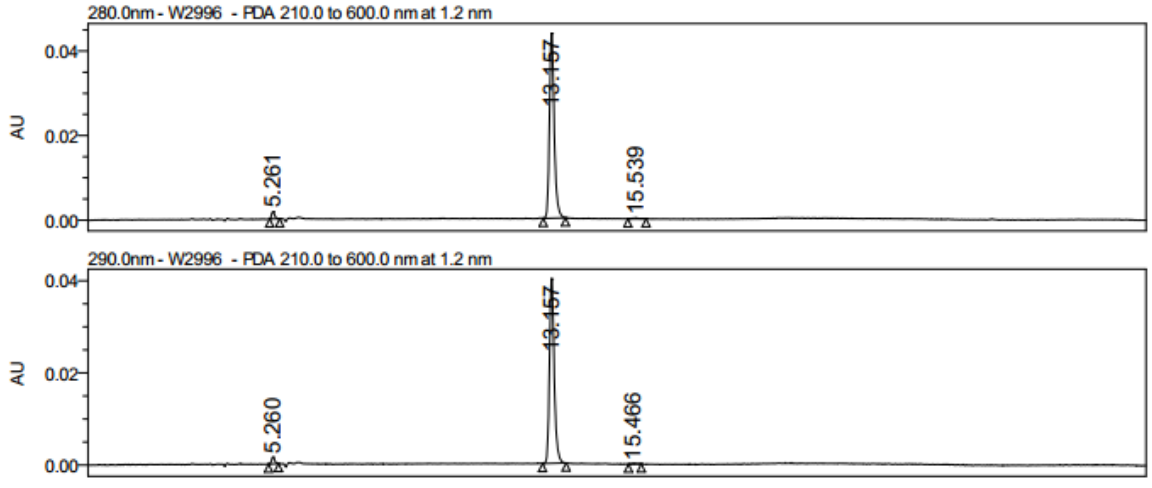
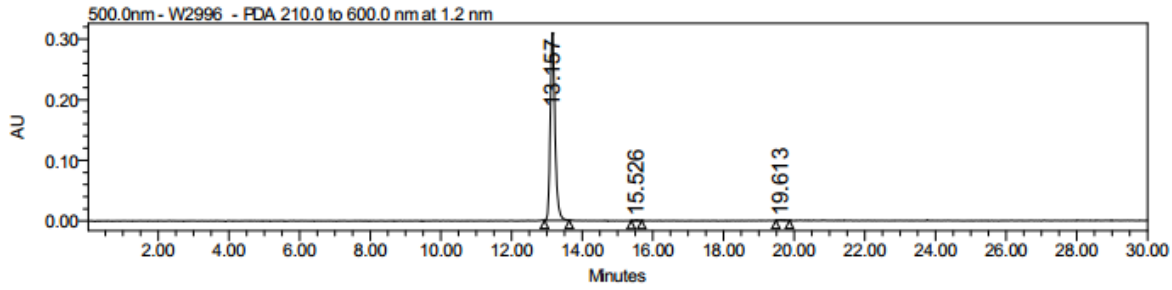
Sample Type:	Unknown	Sample Set Name:	15020401
Vial:	14	Acq. Method Set:	6
Injection #:	1	Processing Method:	2, 3
Injection Volume:	1.00 ul	Channel Name:	280.0nm, 290.0nm, 500.0nm
Run Time:	30.0 Minutes	Proc. Chnl. Descr.:	PDA 280.0 nm, PDA 290.0 nm,

Date Acquired: 2/4/2015 9:43:12 PM CST Project Name: H150115 Channel Id 1405
Date Processed: 2/5/2015 8:51:19 AM CST, 2/5/2015 8:51:48 AM CST, 2/5/2015 8:52:09 AM CST





Processed Channel Descr.: PDA 280.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 280.0 nm	5.261	10630	2.52	1710
2	PDA 280.0 nm	13.157	408908	96.80	44082
3	PDA 280.0 nm	15.539	2898	0.69	184

Processed Channel Descr.: PDA 290.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 290.0 nm	5.260	9231	2.40	1485

Processed Channel Descr.: PDA 290.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
2	PDA 290.0 nm	13.157	373756	97.19	40375
3	PDA 290.0 nm	15.466	1579	0.41	127

Processed Channel Descr.: PDA 500.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 500.0 nm	13.157	2887604	99.43	310759
2	PDA 500.0 nm	15.526	7639	0.26	891
3	PDA 500.0 nm	19.613	8799	0.30	823

Figure 8.HPLC spectrum PYBG-BODIPY

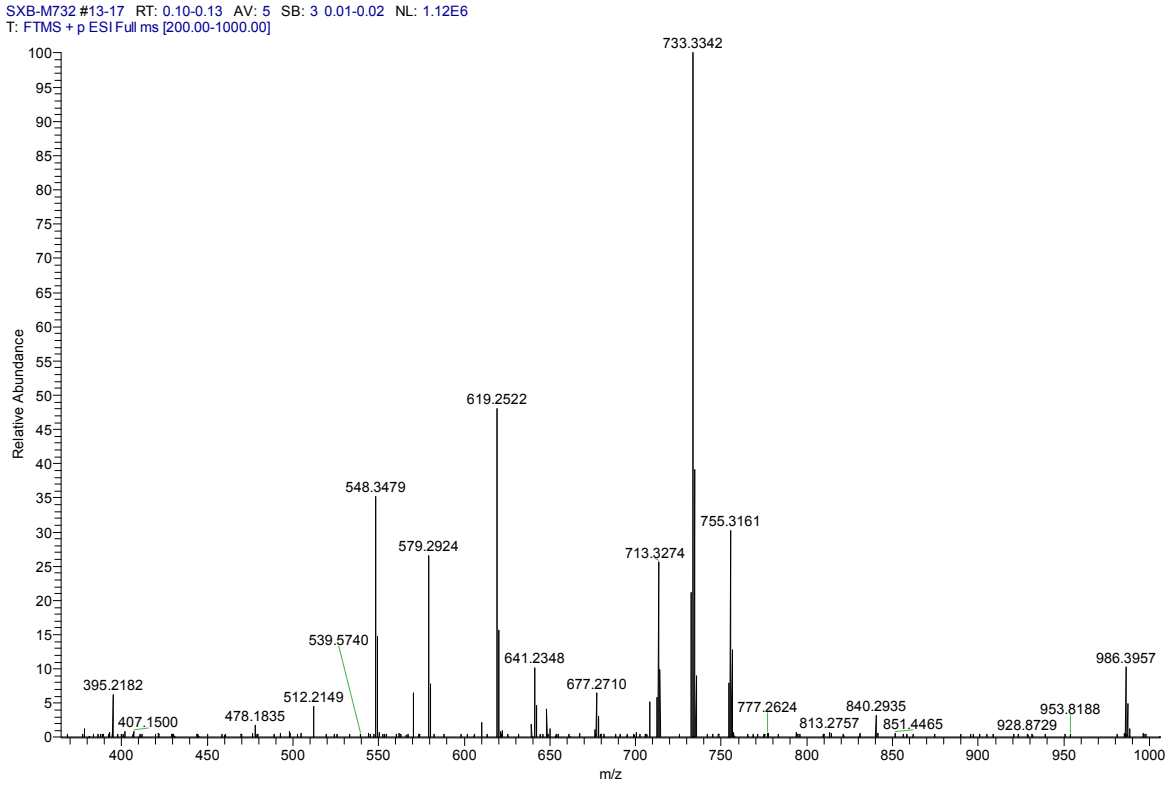


Figure 9.ESI ms Spectrum PYBG-BODIPY

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