Electronic Supplementary Material (ESI) for MedChemComm.

A Zwitterionic Near-infrared Dye Linked TrkC Targeting Agent For Imaging Metastatic Breast Cancer

Zhen Yang[1,3], Syed Muhammad Usama[2,3], Feng Li[1], Kevin Burgess*[2] and Zheng Li[1]*

[1] Center for Bioenergetics, Houston Methodist Research Institute, Houston, TX 77030, USA

[2] Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842, USA

[3] Both authors contributed equally to this work

E-mail: burgess@tamu.edu; zli@houstonmethodist.org

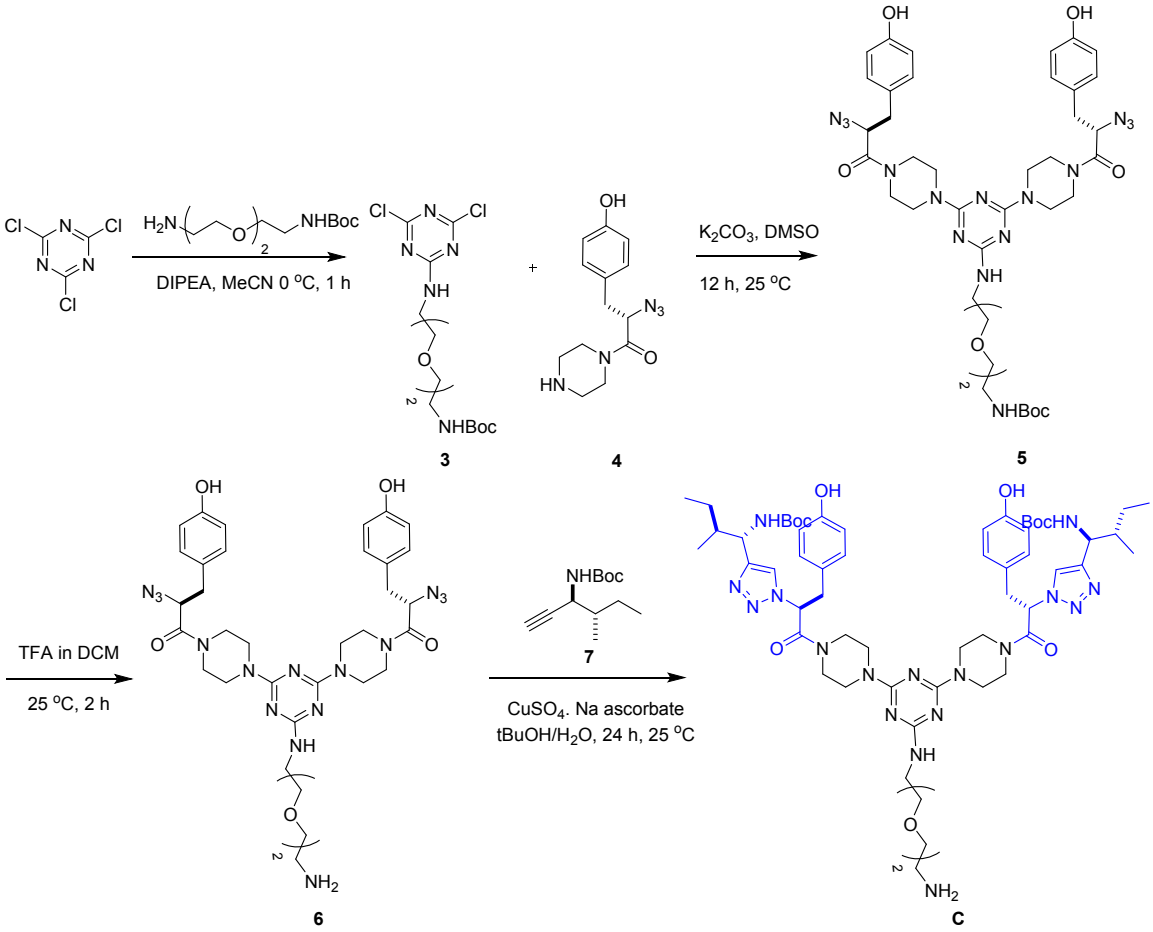
Table of Contents

A. General Experimental Procedures

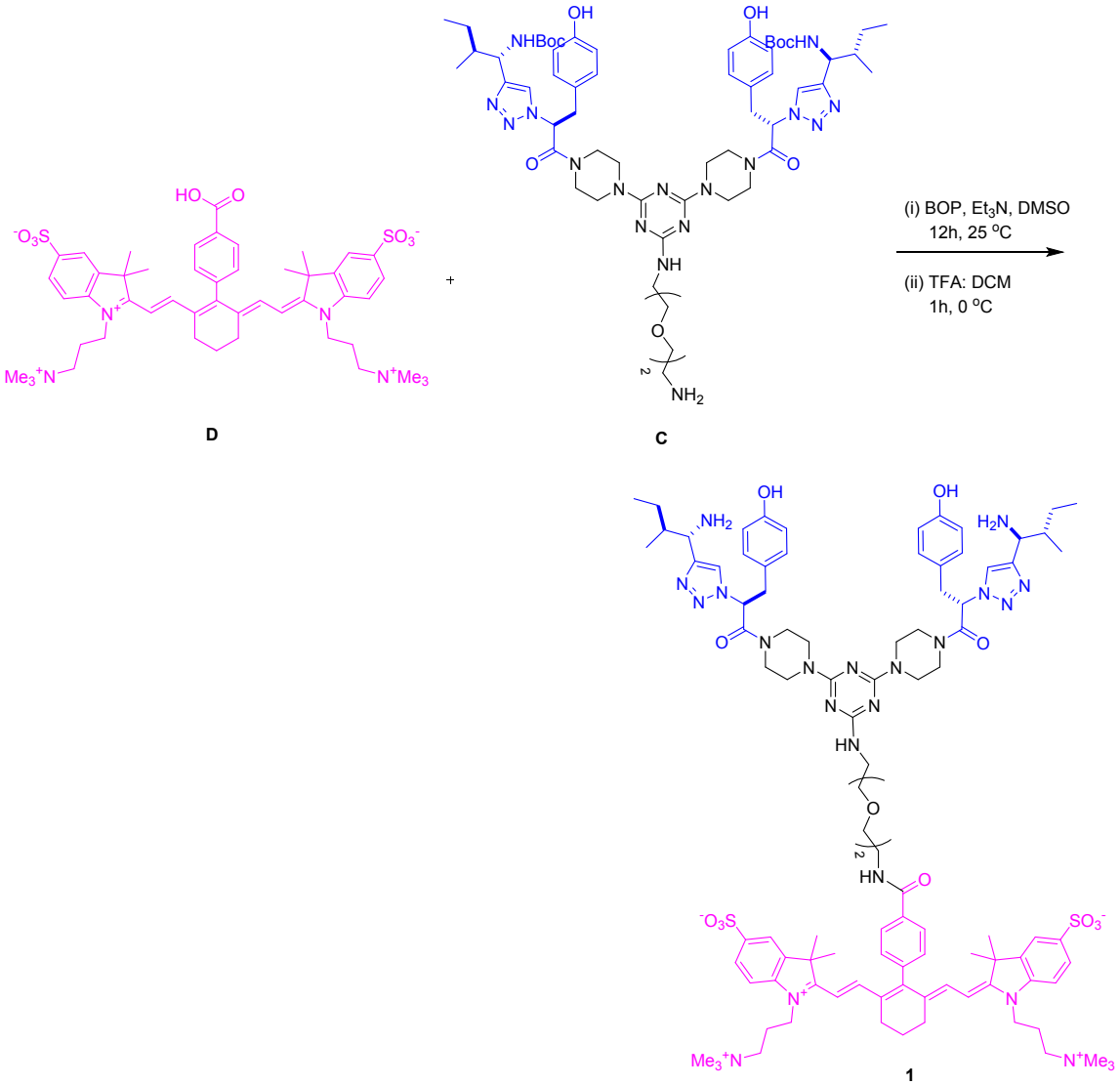
All reactions were carried out under an argon atmosphere. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. High field NMR spectra were recorded with Bruker Avance III at 400 MHz for ^{1}H, and 100 MHz for ^{13}C and were calibrated using residual non-deuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.24, ^{13}C NMR = 77.0, MeOD: ^{1}H NMR = 3.30, ^{13}C NMR = 49.0, DMSO-d_6: ^{1}H NMR = 2.50, ^{13}C NMR = 39.5). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double doublet, dt = double triplet, dq = double quartet, m = multiplet, br = broad. Electrospray ionization mass spectrometry (ESI-MS) data were collected on triple-stage quadrupole instrument in a positive mode. Flash chromatography was performed using silica gel (230-400 mesh). LC-MS analyses were collected from Agilent 1260 Infinity Quaternary LC and Agilent 6120 Quadrupole LC/MS modules using Poroshell 120 EC-C18 2.7 µM (4.6 x 50 mm) column in 5-95% CH_3CN/water gradient with 0.1% formic acid over 10 minutes. Prep HPLC was performed on Agilent 1260 Infinity in 50-90 CH_3CN/water gradient with 0.1% TFA over 20 mins. All statistical analyses were carried out by Graphpad Prism version 6.0 (Graphpad Software

B. General Experimental Procedures

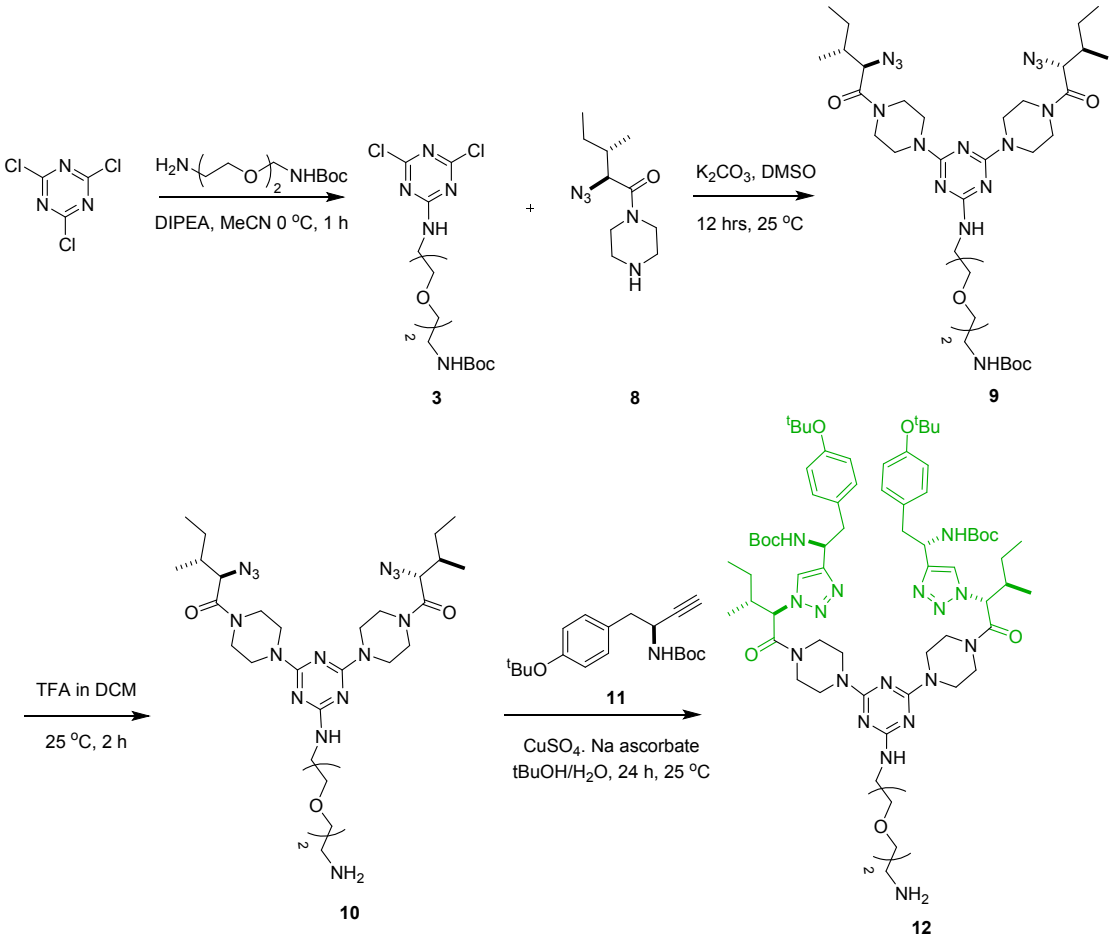
Synthesis of 1 and 2



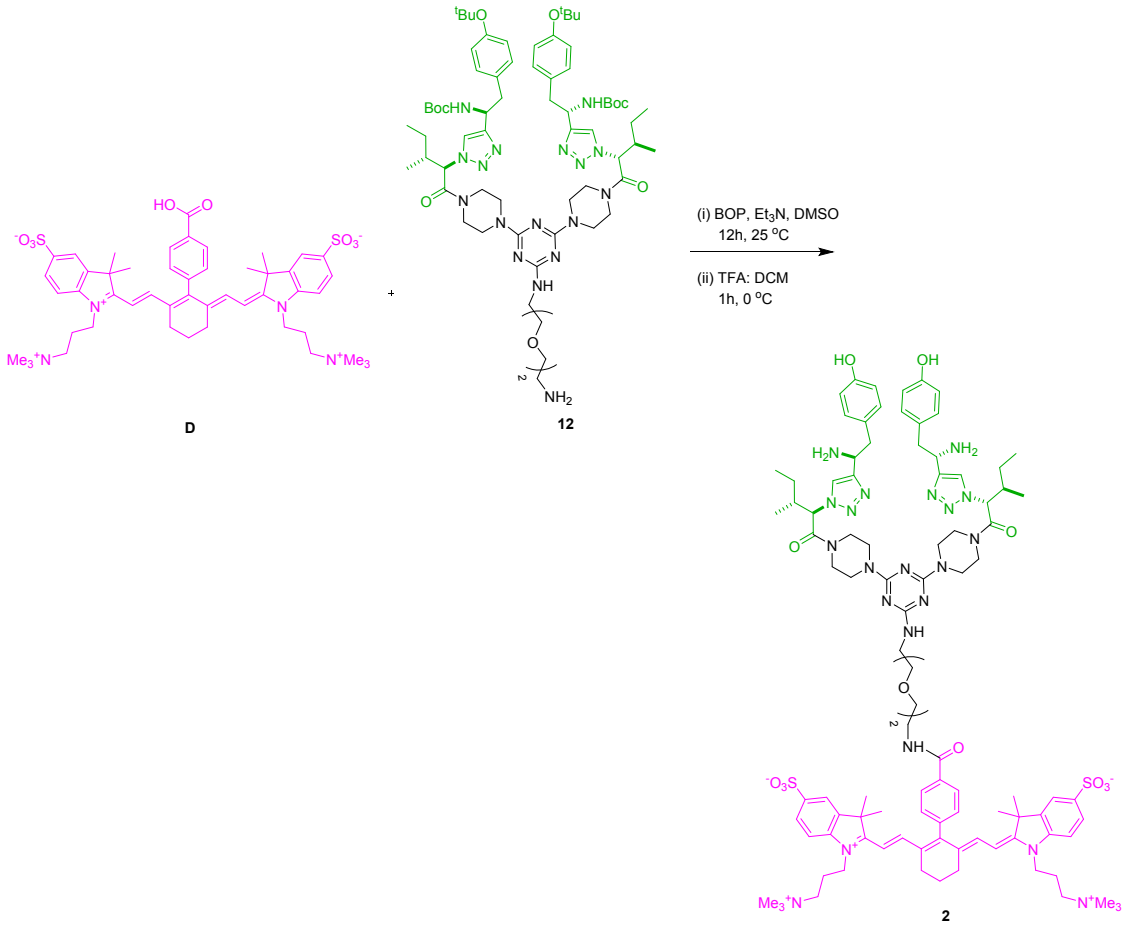
Scheme S1. Synthesis of IY-IY Fragment (**C**)



Scheme S2. Synthesis of **1**



Scheme S3. Synthesis of YI-YI Fragment (**12**)



Scheme S4. Synthesis of **2**

C. Synthesis Procedure

Synthesis of C:

Compound **4, 7 8, 11** were synthesized according to previous procedure[1].

Synthesis of *tert-butyl (2-(2-(2-((4,6-dichloro-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamate* (**3**)

At 0 ^{o}C, N-Boc- 2,2′-(ethylenedioxy)diethylamine (218.5 mg) and cynuric chloride (162.1 mg) were added together followed by DIPEA (306.5 uL) and stirred for 1 hr. Progress of reaction was monitored by TLC. After completion of reaction, the solvent was revomed under vacuum. The crude was taken to next step without further purification.

LRMS (ESI-) m/z calcd for $C_{14}H_{23}Cl_2N_5O_4$ $(M+H)^+$ 396.1; found 396.7

Synthesis of *tert-butyl (2-(2-(2-((4,6-bis(4-((S)-2-azido-3-(4-hydroxyphenyl)propanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamate* (**5**)

3 (0.88, 348.48), **4** (586.1 mg) and K_2CO_3 (364.8 mg) were add together in DMSO (10mL) and stirred at room temperature for 24 h. . Progress of reaction was monitored by TLC. After completion of reaction, the solvent was removed. The crude was taken to next step without further purification.

LRMS (ESI+) m/z calcd for $C_{40}H_{55}N_{15}O_8$ $(M+H)^+$ 874.4; found 874.4

Synthesis of (2S,2'S)-1,1'-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-azido-3-(4-hydroxyphenyl)propan-1-one) (**6**)

TFA:DCM (1:1, 10 mL) was added to crude **5** and stirred for 1.5 h. Progress of reaction was monitored by TLC. After completion of reaction, the solvent was removed.

LRMS (ESI+) m/z calcd for $C_{35}H_{47}N_{15}O_6$ $(M+H)^+$ 774.3; found 774.4.

Synthesis of di-tert-butyl ((1S,1'S,2S,2'S)-(((2S,2'S)-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(3-(4-hydroxyphenyl)-1-oxopropane-1,2-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(2-methylbutane-1,1-diyl))dicarbamate (**C**)

Under inert atmosphere **6** (680.24 mg) and **7** (223.1 mg) were dissolved in tBuOH:H_2O (1:1, 5 mL), followed by $CuSO_4$ (44 mg) and Na ascorbate (139.46 mg). The reaction was stirred for 24 hrs at room temperature. The solvent was removed and the crude was purified by prep HPLC (280 mg, 32%).

^{1}H NMR (400 MHz, MeOD) δ 8.00 (s, 2H), 7.03 (d, J = 8.4 Hz, 4H), 6.70 (dd, J = 8.4, 3.7 Hz, 4H), 6.07 (t, J = 7.7 Hz, 2H), 3.69 (qd, J = 10.5, 4.5 Hz, 18H), 3.56 – 3.38 (m, 7H),

3.14 (d, *J* = 4.9 Hz, 2H), 1.90 – 1.77 (m, 2H), 1.44 (d, *J* = 4.3 Hz, 18H), 1.10 (ddd, *J* = 35.2, 14.8, 7.4 Hz, 2H), 0.96 – 0.85 (m, 7H), 0.81 (d, *J* = 6.8 Hz, 5H).

^{13}C NMR (101 MHz, MeOD) δ 167.03, 156.74, 156.52, 156.39, 148.46, 130.19, 125.67, 121.69, 117.68, 115.15, 114.79, 78.99, 69.96, 69.95, 68.54, 66.50, 60.55, 51.88, 44.90, 43.30, 41.69, 40.38, 39.26, 39.08, 37.61, 27.79, 27.38, 25.72, 24.87, 14.57, 13.75, 10.40, 10.23.

HRMS (ESI+) m/z calcd for $C_{59}H_{89}N_{17}O_{10}$ $(M+H)^+$ 1196.6978; found 1196.6959.

Synthesis of 10

Synthesis of *tert-butyl (2-(2-(2-((4,6-bis(4-((2R,3R)-2-azido-3-methylpentanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamate* (**9**)

1 (645.48 mg), **8** (881.35 mg) and K_2CO_3 (675.79 mg) were add together in DMSO (10 mL)and stirred at room temperature for 24h. . Progress of reaction was monitored by TLC. After completion of reaction, the solvent was removed. The crude was taken to next step without further purification.

LRMS (ESI+) m/z calcd for $C_{34}H_{59}N_{15}O_6$ $(M+H)^+$ 774.4; found 774.4

Synthesis of (2R,2'R,3R,3'R)-1,1'-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(2-azido-3-methylpentan-1-one) (**10**)

TFA:DCM (1:1, 10 mL) was added to crude **9** and stirred for 1.5 h. Progress of reaction was monitored by TLC. After completion of reaction, the solvent was removed.

LRMS (ESI+) m/z calcd for $C_{29}H_{51}N_{15}O_4$ $(M+H)^+$ 674.4; found 674.4.

Synthesis of di-tert-butyl ((1S,1'S)-(((2R,2'R,3R,3'R)-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(3-methyl-1-oxopentane-1,2-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(2-(4-(tert-butoxy)phenyl)ethane-1,1-diyl))dicarbamate (**12**)

Under inert atmosphere **10** (771.73 mg) and **11** (452.3 mg) were dissolved in tBuOH:H_2O (1:1, 5 mL), followed by $CuSO_4$ (57.5 mg) and Na ascorbate (184.24 mg). The reaction was stirred for 24 hrs at room temperature. The solvent was removed and the crude was purified by prep HPLC (560 mg, 55%).

^{1}H-NMR (400 MHz, D_2O:CD_3CN (7:3)) δ 7.88 (s, 2H), 7.08 (d, *J* = 8.4 Hz, 4H), 6.85 (d, *J* = 8.4 Hz, 4H), 5.62 (d, *J* = 10.2 Hz, 2H), 5.02 (t, *J* = 7.3 Hz, 3H), 4.06 (s, 3H), 3.89 (s, 7H), 3.72 – 3.59 (m, 10H), 3.56 (s, 4H), 3.33 (dt, *J* = 3.3, 1.6 Hz, 4H), 3.21 – 3.09 (m, 4H), 3.08 – 2.98 (m, 1H), 2.40 (d, *J* = 3.6 Hz, 2H), 1.38 (s, 20H), 1.29 (s, 18H), 1.04 (d, *J* = 6.6 Hz, 10H), 0.88 (d, *J* = 7.0 Hz, 5H).

^{13}C-NMR (101 MHz, D_2O:CD_3CN (7:3)) 7.88, 7.09, 7.07, 6.86, 6.84, 5.63, 5.60, 5.04, 5.02, 5.00, 4.06, 3.89, 3.74, 3.73, 3.71, 3.70, 3.68, 3.56, 3.34, 3.33, 3.33, 3.33, 3.32, 3.20, 3.19, 3.17, 3.15, 3.15, 3.13, 3.09, 3.07, 3.06, 3.04, 1.38, 1.29, 1.05, 1.03, 0.89, 0.87.

HRMS (ESI+) m/z calcd for $C_{67}H_{105}N_{17}O_{10}$ $(M+H)^+$ 1308.8230; found 1308.8259

Synthesis of 1 and 2:

Synthesis of **D** was done as reported in literature[2].

^{1}H NMR (400 MHz, D_2O) δ 8.52 (d, *J* = 7.8 Hz, 2H), 8.30 (d, *J* = 8.2 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 3H), 7.94 (s, 2H), 7.49 (s, 1H), 7.35 (s, 3H), 6.43 (s, 1H), 4.19 (s, 4H), 3.71 – 3.62 (m, 4H), 3.35 (s, 21H), 2.99 (d, *J* = 19.6 Hz, 3H), 2.50 – 2.33 (m, 6H), 1.32 (s, 12H)

^{13}C NMR (101 MHz, D_2O) δ 172.78, 169.04, 149.06, 143.47, 140.99, 134.31, 133.45, 130.90, 130.42, 129.89, 129.11, 127.29, 120.08, 119.28, 110.87, 101.17, 63.35, 53.40, 48.78, 40.84, 27.22, 24.61, 21.08

LRMS (ESI+) m/z calcd for $C_{49}H_{63}N_4O_8S_2^+$ 899.4; found 900.6.

Synthesis of 2-((E)-2-((E)-4'-((2-(2-(2-((4,6-bis(4-((S)-2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamoyl)-6-(2-((E)-3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate (**1**)

BOP (22 mg), **C** (38 mg)**, D** (30 mg) Et_3N were added in 1 mL DMSO and stirred for 12 hr. After removal of DMSO. The crude was cooled on ice for 10 mins. TFA:DCM (1:1, 1 mL) was added to the mixture and stirred for 1 hr. Solvent was removed on vacuum and purified by reverse phase prep HPLC. (40 mg, 60%).

^{1}H-NMR (400 MHz, D_2O:CD_3CN (7:3)) ^{1}H NMR (400 MHz, D_2O) δ 8.43 (d, *J* = 15.4 Hz, 3H), 8.28 (d, *J* = 8.7 Hz, 2H), 8.15 – 8.03 (m, 3H), 7.90 – 7.82 (m, 3H), 7.62 – 7.39 (m, 6H), 7.29 (d, *J* = 8.2 Hz, 6H), 6.97 (s, 5H), 6.37 (d, *J* = 44.0 Hz, 5H), 3.72 (tdd, *J* = 31.9, 20.7, 11.9 Hz, 34H), 3.31 (s, 18H), 2.91 (s, 3H), 2.66 (s, 2H), 2.42 (s, 4H), 2.28 – 2.17 (m, 7H), 2.05 – 1.80 (m, 6H), 1.68 (s, 3H), 1.54 (dt, *J* = 19.4, 7.8 Hz, 10H), 1.34 (s, 12H), 1.13 (s, 7H), 1.04 (d, *J* = 6.2 Hz, 10H).

^{13}C-NMR (101 MHz, D_2O:CD_3CN (7:3)) ^{13}C NMR (101 MHz, D_2O) δ 185.11, 168.45, 167.65, 162.80, 162.46, 162.11, 161.76, 155.88, 155.46, 153.79, 146.04, 144.08, 142.16, 141.98, 130.93, 129.71, 127.81, 127.19, 126.14, 124.39, 121.18, 118.27, 115.86, 115.36, 114.84, 112.78, 112.46, 110.79, 63.41, 62.75, 61.21, 53.51, 53.34, 51.76, 48.80, 43.33, 40.86, 39.91, 39.20, 37.53, 37.39, 28.88, 27.22, 26.12, 25.81, 25.45, 24.67, 22.33, 21.06, 14.60, 13.65, 10.59, 10.32.

HRMS (ESI+) m/z calcd for $C_{67}H_{105}N_{17}O_{10}$ $(M+H)^+$ 1876.9906; found (M/2+H)+ 938.9998

Synthesis of 2-((E)-2-((E)-4'-((2-(2-(2-((4,6-bis(4-((2R,3R)-2-(4-((S)-1-amino-2-(4-hydroxyphenyl)ethyl)-1H-1,2,3-triazol-1-yl)-3-methylpentanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamoyl)-6-(2-((E)-3,3-dimethyl-5-sulfonato-

1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate (**2**)

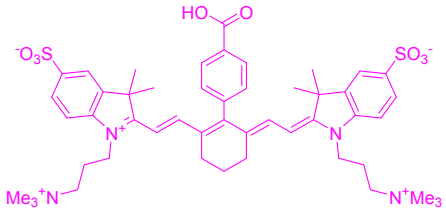
BOP (21.67 mg) , **12** (86.32 mg)**, D** (30 mg) Et_3N (6.83 uL) were added in 1 mL DMSO and stirred for 12 hr. After removal of DMSO. The crude was cooled on ice for 10 mins. TFA:DCM (1:1, 1 mL) was added to the mixture and stirred for 1 hr. Solvent was removed on vacuum and purified by reverse phase prep HPLC (35 mg, 56%).

^{1}H NMR (400 MHz, D_2O:CD_3CN (7:3)) δ 8.51 – 8.32 (m, 5H), 8.15 (dd, *J* = 33.1, 12.0 Hz, 3H), 8.03 (d, *J* = 16.6 Hz, 2H), 7.87 (s, 1H), 7.81 – 7.71 (m, 2H), 7.67 (s, 1H), 7.50 (s, 2H), 6.98 (d, *J* = 8.2 Hz, 5H), 6.72 (d, *J* = 14.7 Hz, 1H), 6.52 (d, *J* = 13.8 Hz, 1H), 5.89 (s, 2H), 5.12 (dd, *J* = 10.2, 5.5 Hz, 3H), 4.45 (d, *J* = 7.1 Hz, 2H), 4.25 (s, 3H), 4.14 – 3.81 (m, 30H), 3.83 – 3.68 (m, 6H), 3.66 (dd, *J* = 13.6, 5.4 Hz, 4H), 3.49 (dd, *J* = 25.1, 12.0 Hz, 5H), 3.47 – 3.37 (m, 18H), 3.35 – 3.25 (m, 4H), 3.28 (d, *J* = 6.3 Hz, 2H), 3.14 (s, 1H), 3.02 (s, 2H), 2.74 – 2.43 (m, 7H), 2.39 – 2.27 (m, 6H), 1.67 (d, *J* = 19.9 Hz, 2H), 1.64 – 1.57 (m, 3H), 1.54 (dd, *J* = 20.1, 10.0 Hz, 3H), 1.46 (d, *J* = 5.9 Hz, 6H), 1.36 (d, *J* = 17.5 Hz, 2H), 1.24 (dd, *J* = 19.6, 6.9 Hz, 13H), 1.11 – 1.01 (m, 5H).

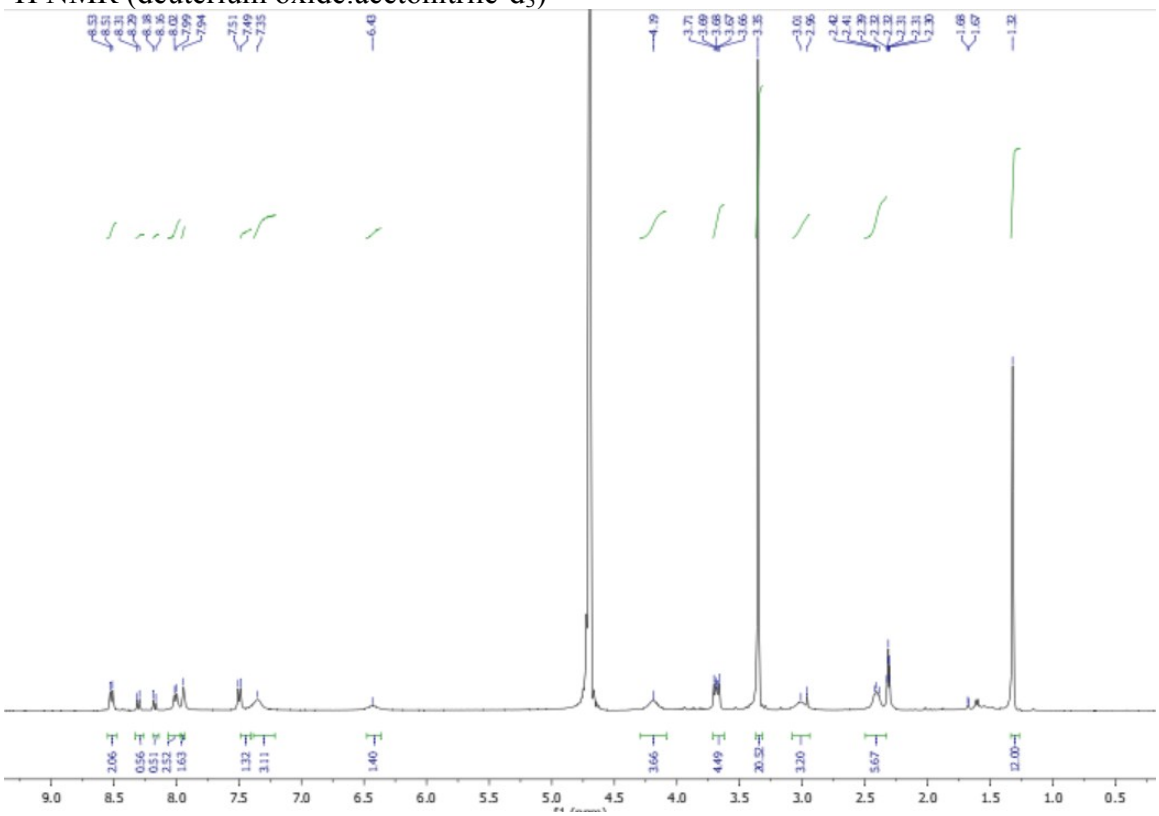
^{13}C NMR (101 MHz, D_2O:CD_3CN (7:3)) δ 168.84, 167.56, 162.53, 162.18, 161.83, 161.48, 155.98, 155.66, 143.41, 141.92, 130.92, 129.60, 129.39, 128.02, 127.31, 126.56, 124.12, 121.21, 120.22, 115.75, 115.40, 112.49, 107.09, 70.10, 69.46, 68.78, 64.09, 63.50, 53.57, 53.44, 50.12, 49.43, 48.73, 44.04, 43.56, 40.60, 40.05, 37.92, 37.76, 27.37, 27.27, 27.03, 26.38, 24.44, 22.75, 21.19, 17.50, 14.95, 12.37, 10.26.
HRMS (ESI+) m/z calcd for $C_{67}H_{105}N_{17}O_{10}$ $(M+H)^+$ 1876.9906; found (M/2+H)+ 938.9980

D. Compound Characterization

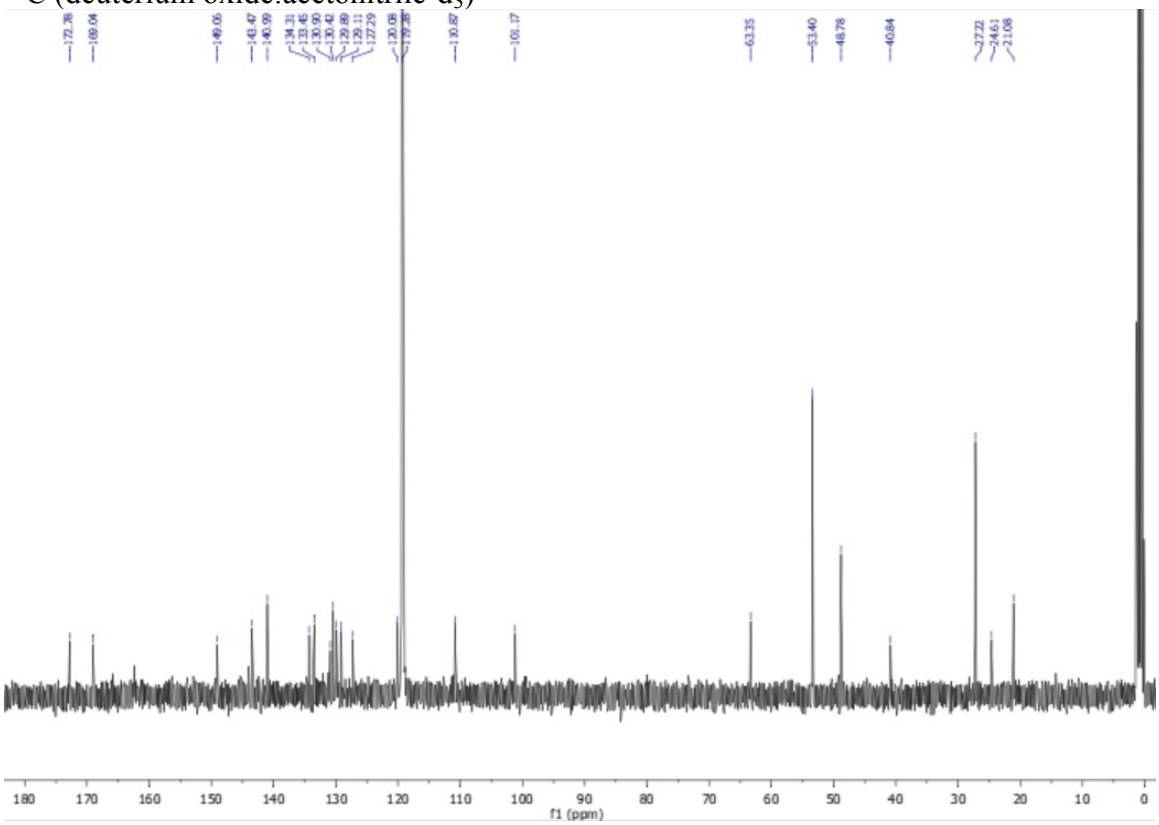
2-((E)-2-((E)-4'-carboxy-6-(2-((E)-3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate (**D**)



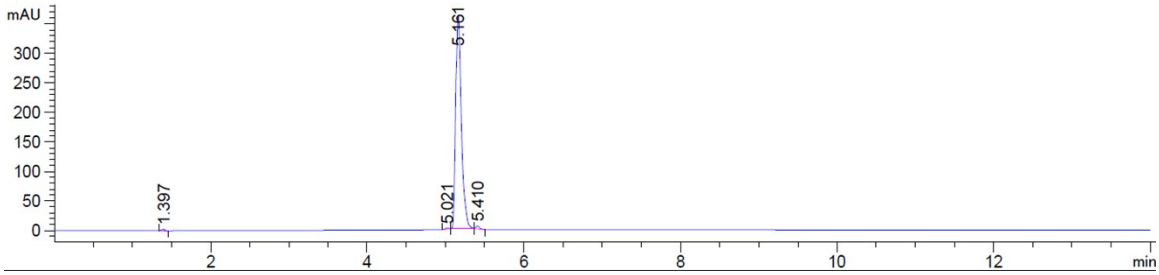
^{1}H NMR (deuterium oxide:acetonitrile-d_3)



^{13}C (deuterium oxide:acetonitrile-d_3)

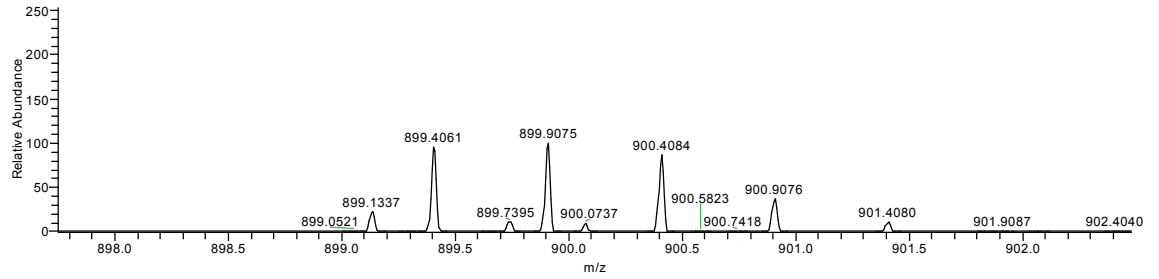


LC/MS at 600 nm

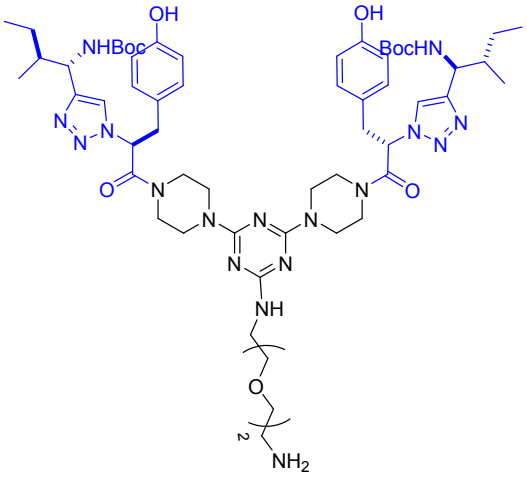


HRMS

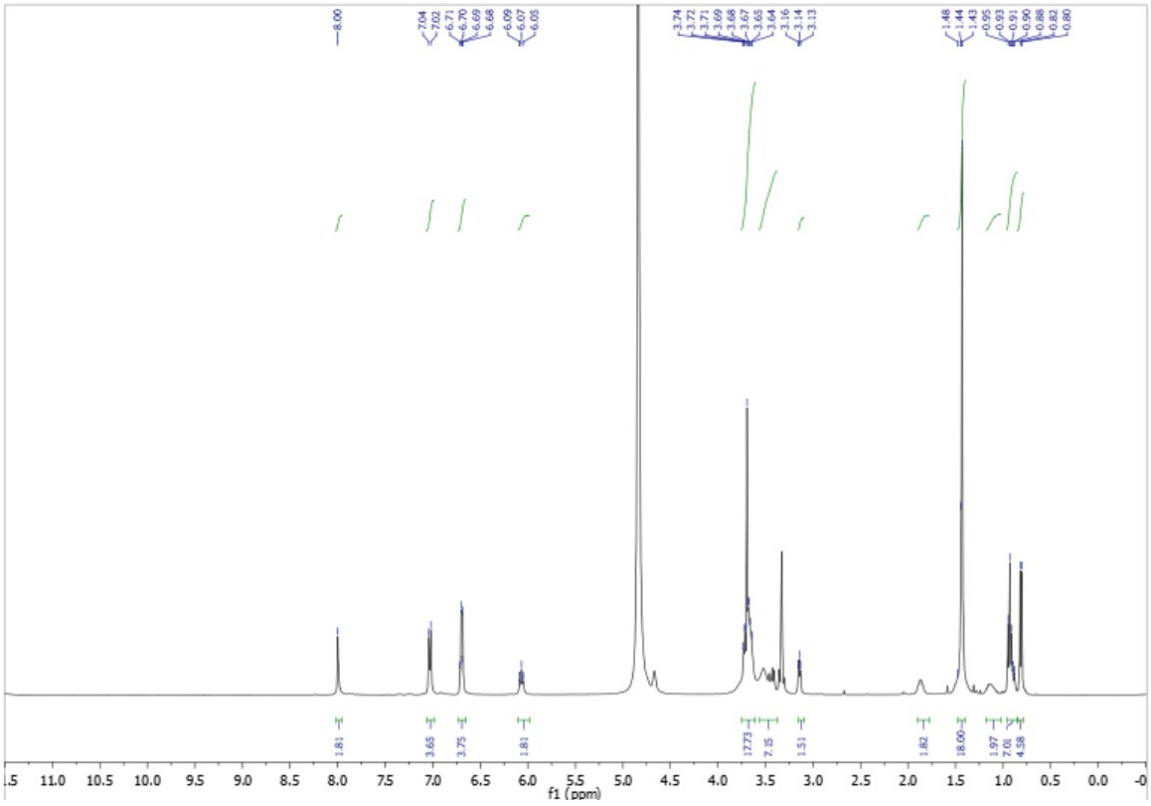
022818-1h #30-77 RT: 0.13-0.34 AV: 48 NL: 1.74E6
T: FTMS + p ESI Full ms [86.7000-1300.0000]



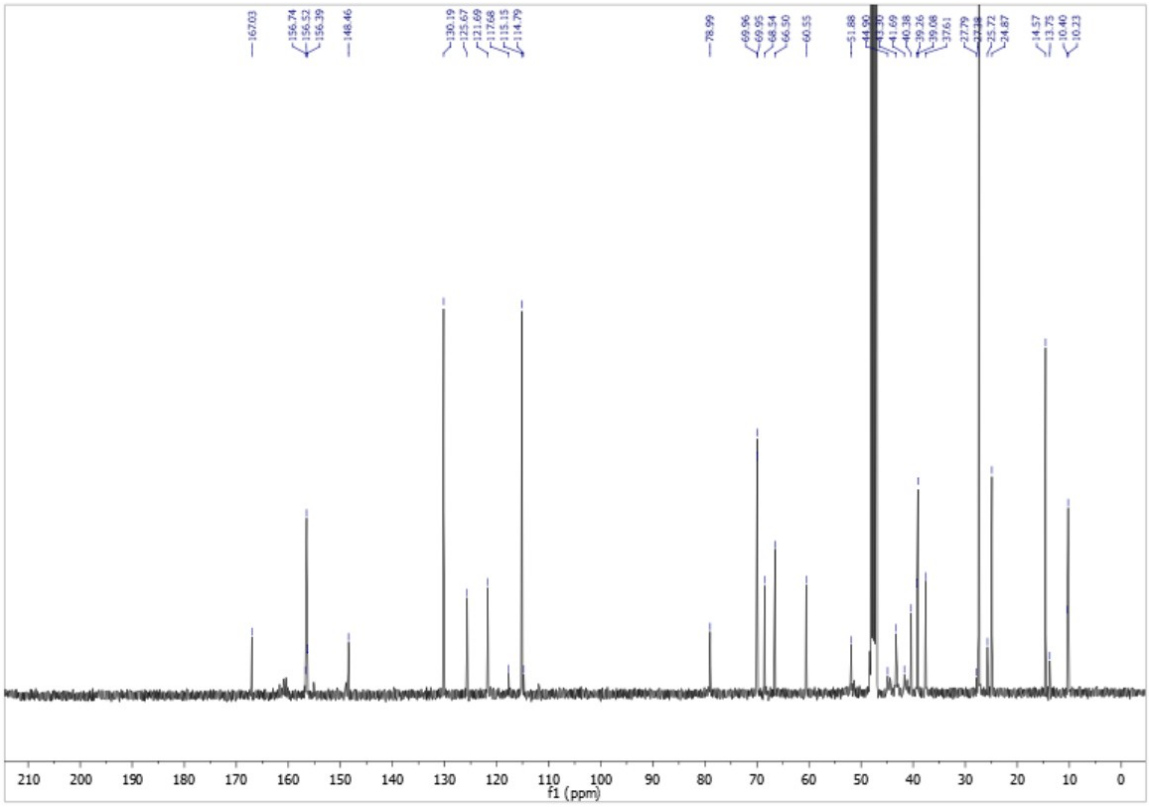
di-tert-butyl ((1S,1'S,2S,2'S)-(((2S,2'S)-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(3-(4-hydroxyphenyl)-1-oxopropane-1,2-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(2-methylbutane-1,1-diyl))dicarbamate (**C**)



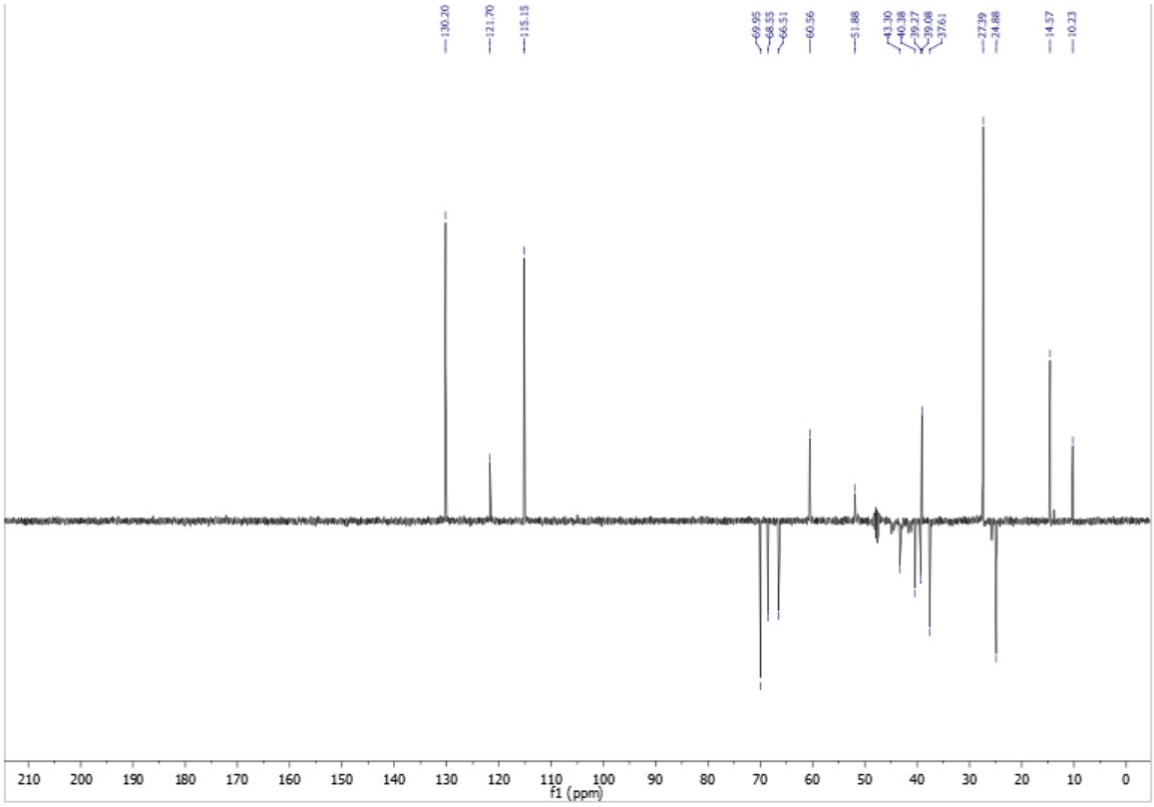
^{1}H NMR (methanol-d_4)



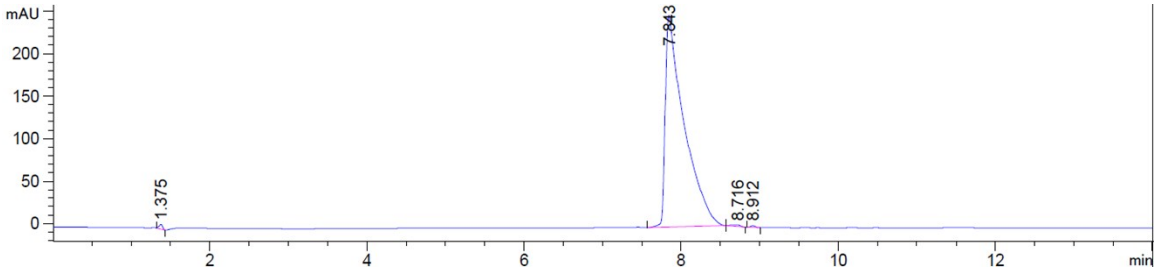
^{13}C NMR (methanol-d_4)



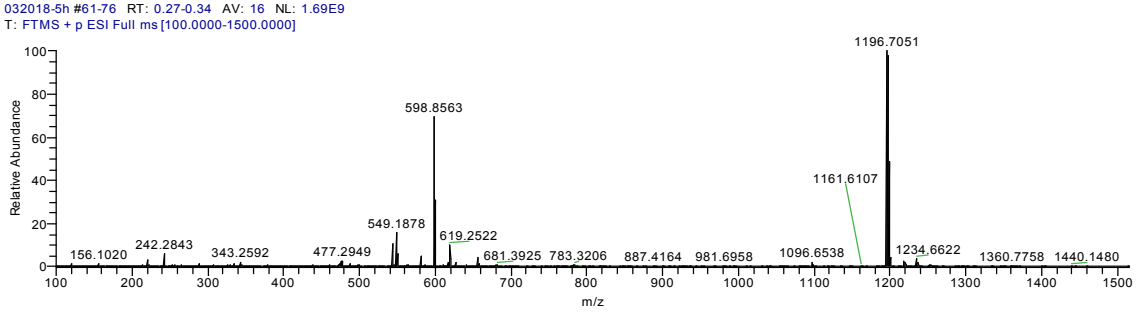
135 DEPT (methanol-d_4)



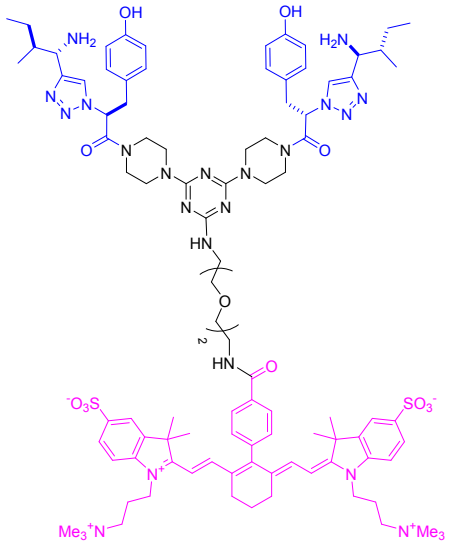
LC/MS at 254 nm



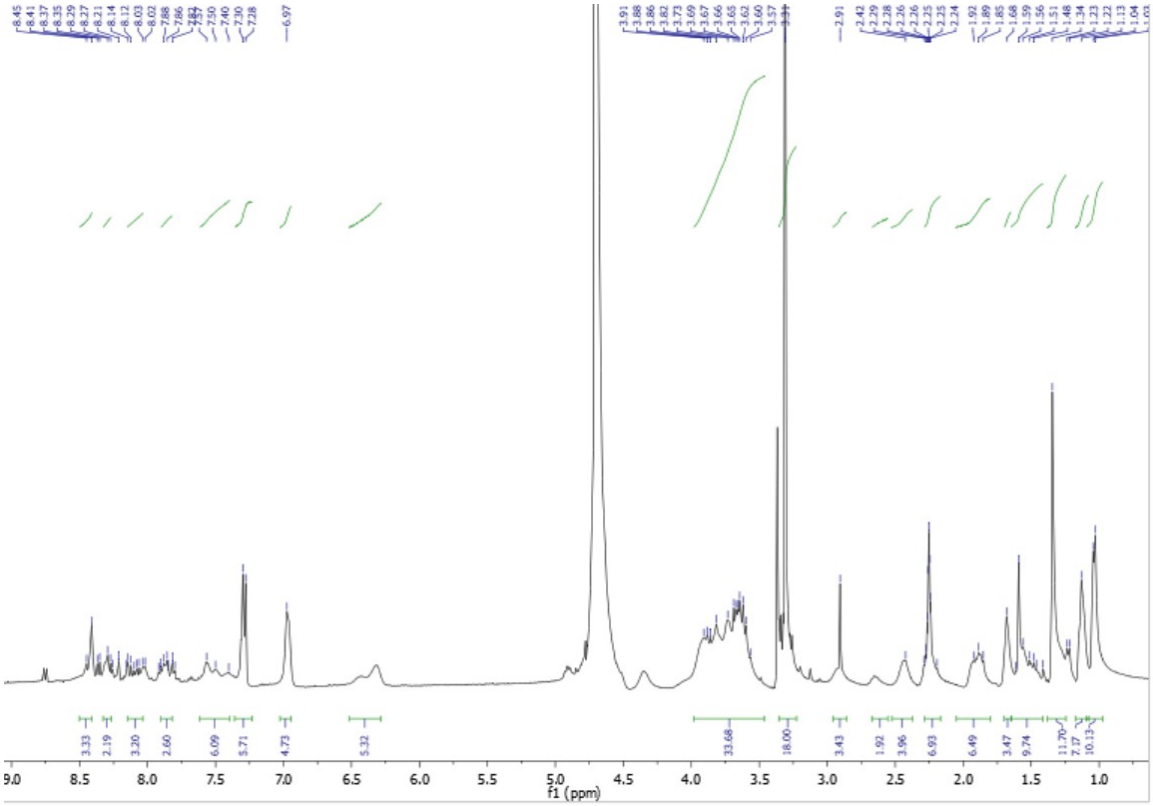
HRMS



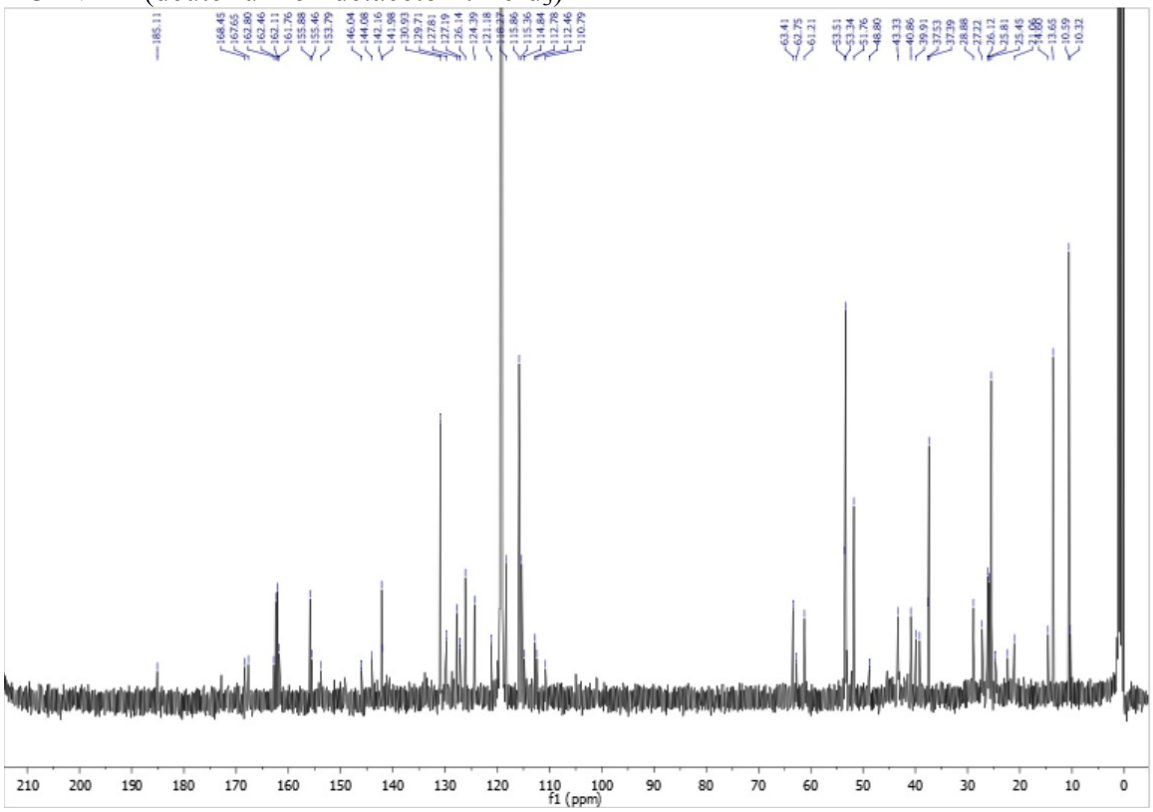
2-((E)-2-((E)-4'-((2-(2-(2-((4,6-bis(4-((S)-2-(4-((1S,2S)-1-amino-2-methylbutyl)-1H-1,2,3-triazol-1-yl)-3-(4-hydroxyphenyl)propanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamoyl)-6-(2-((E)-3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate (**1**)



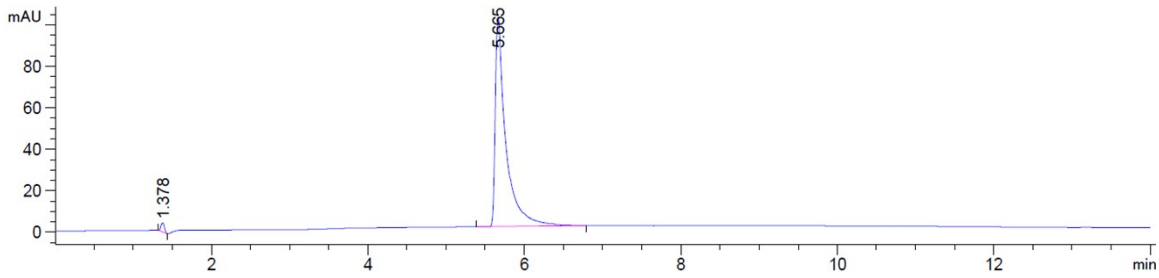
^{1}H NMR – (deuterium oxide:acetonitrile-d_3)



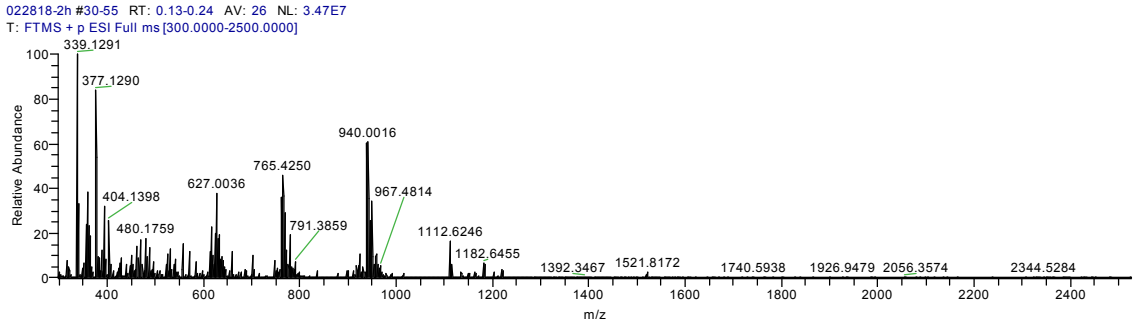
^{13}C NMR (deuterium oxide:acetonitrile-d_3)



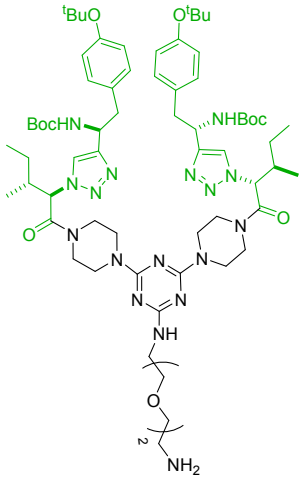
LC/MS at 600 nm



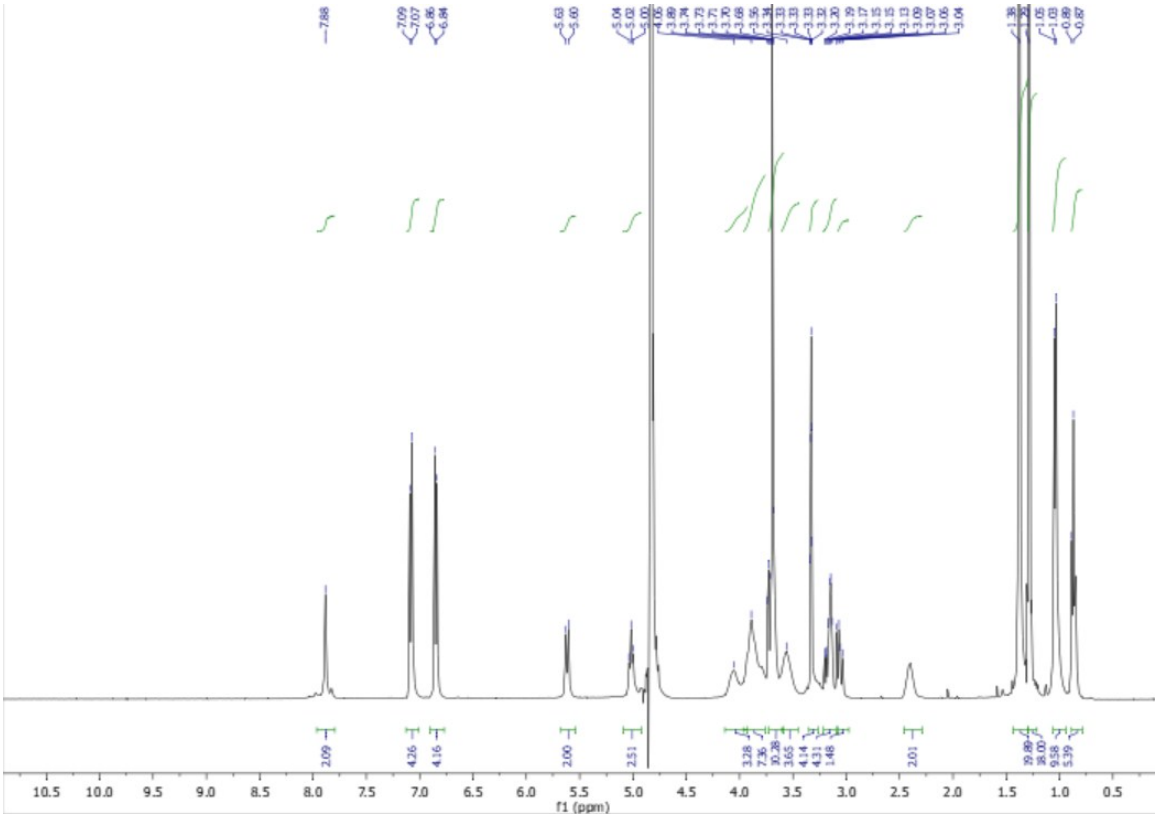
HRMS



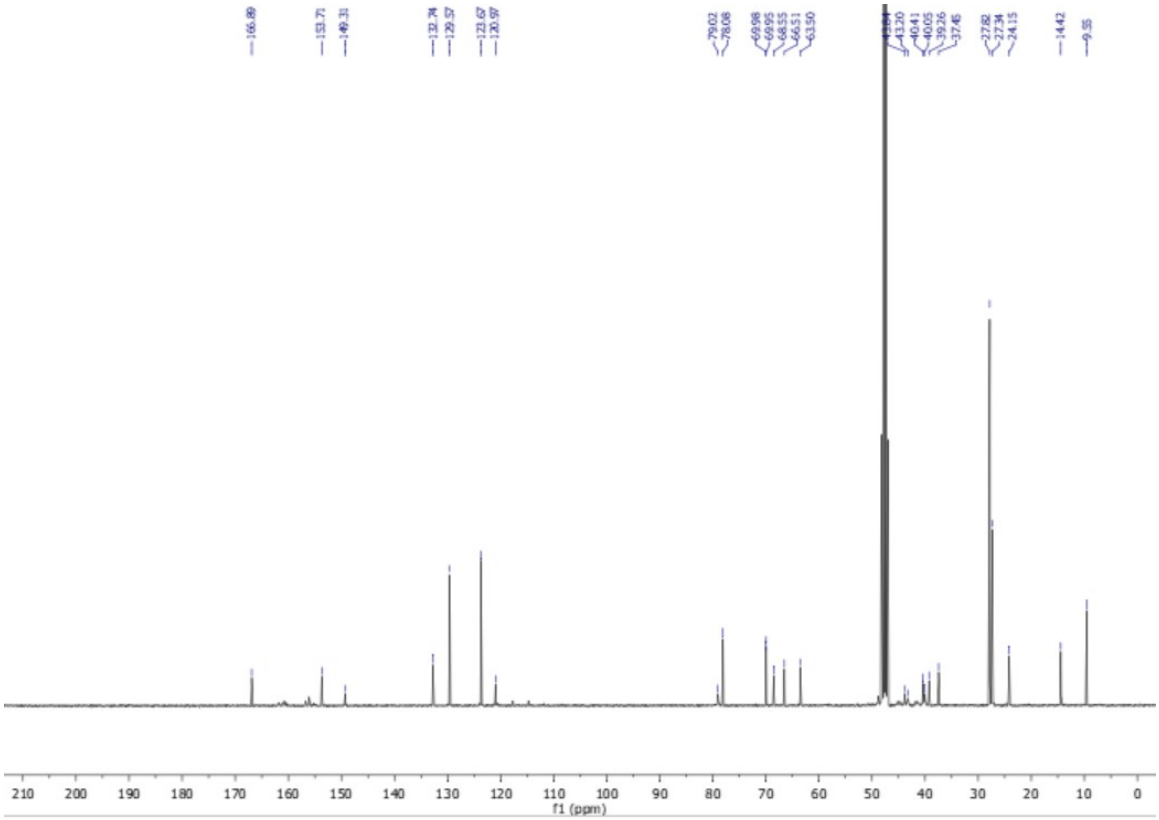
di-tert-butyl ((1S,1'S)-(((2R,2'R,3R,3'R)-((6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(3-methyl-1-oxopentane-1,2-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(2-(4-(tert-butoxy)phenyl)ethane-1,1-diyl))dicarbamate (**12**)



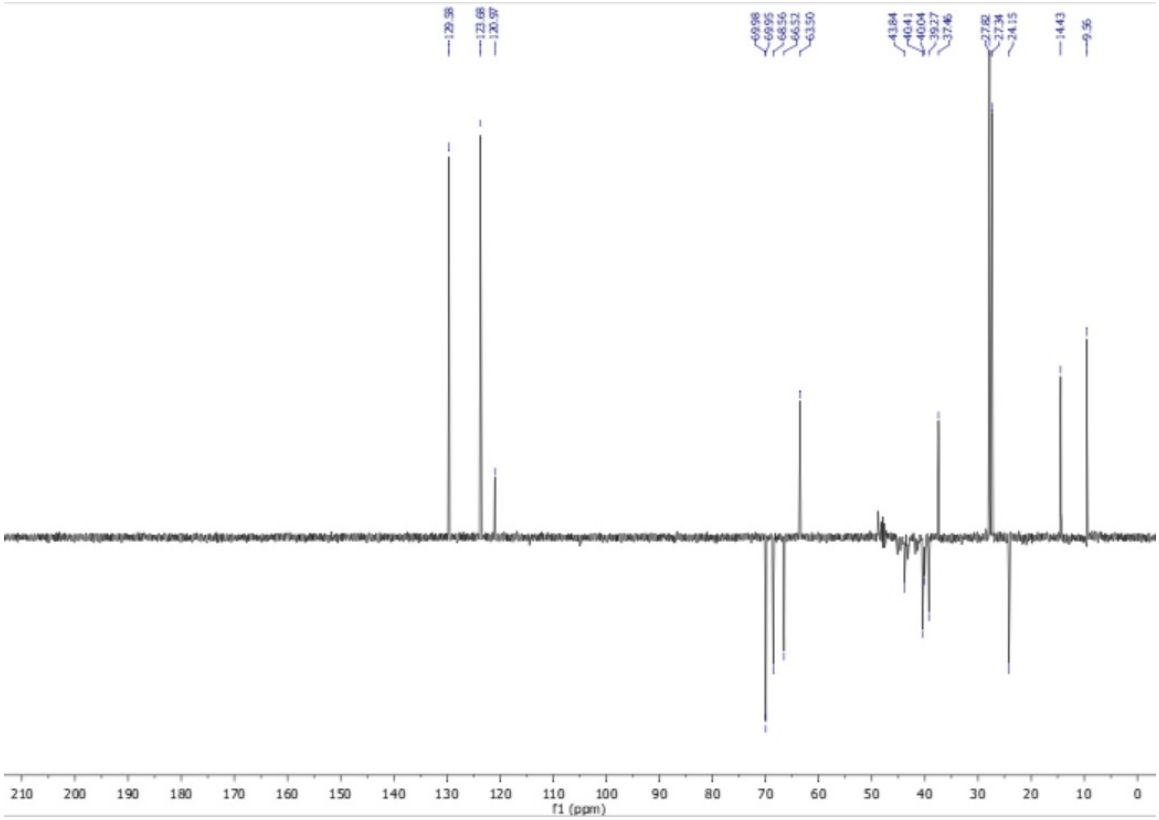
^{1}H (methanol-d_4)



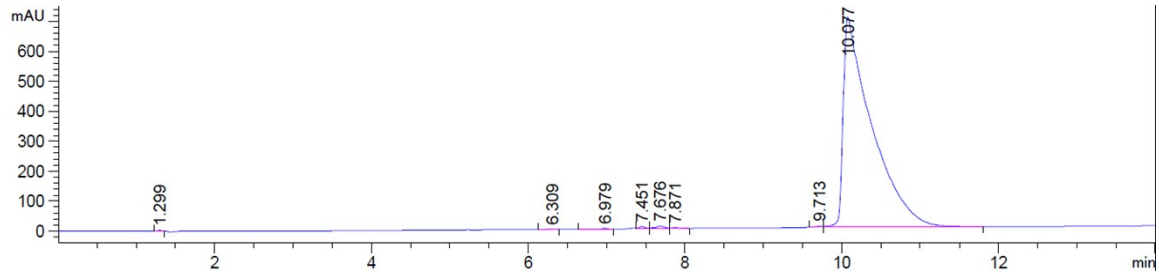
^{13}C (methanol-d_4)



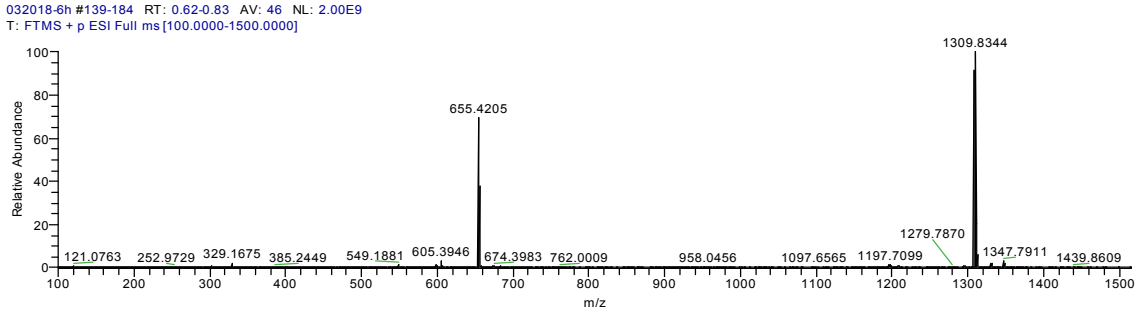
135 DEPT (methanol-d_4)



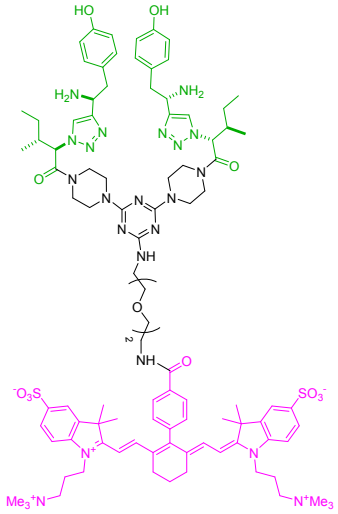
LC/MS at 254 nm



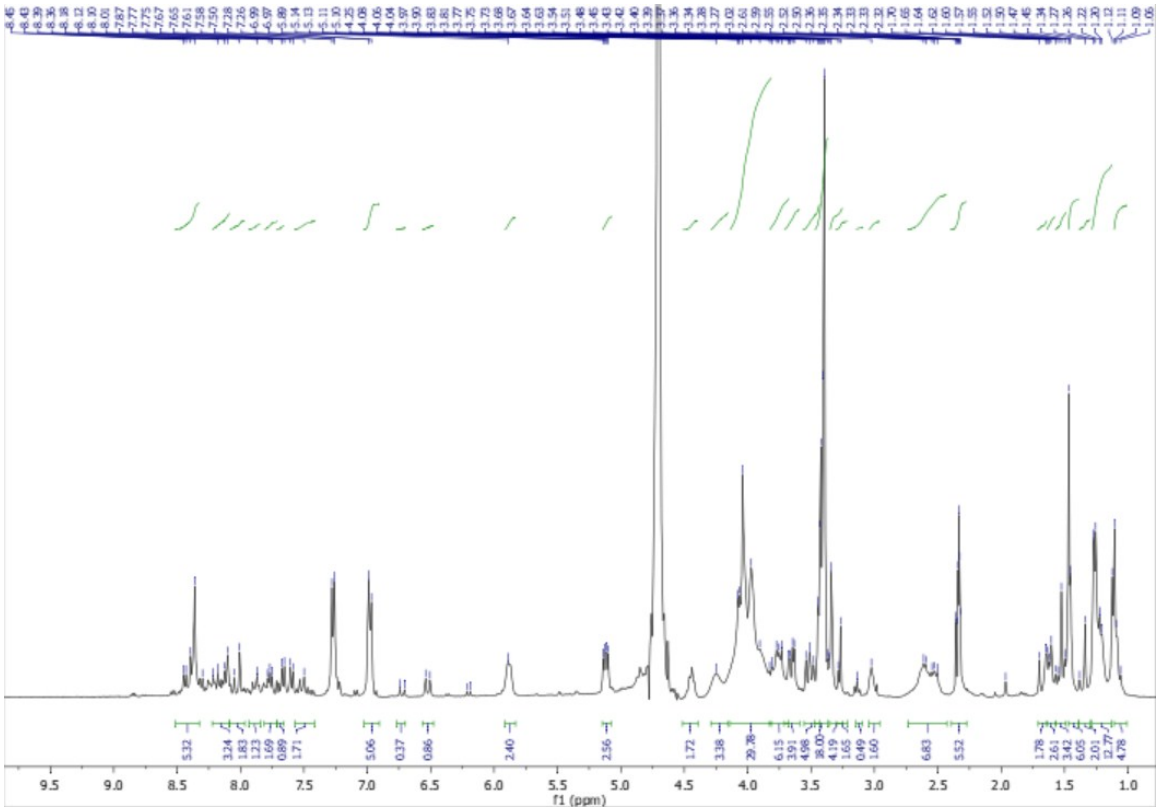
HRMS



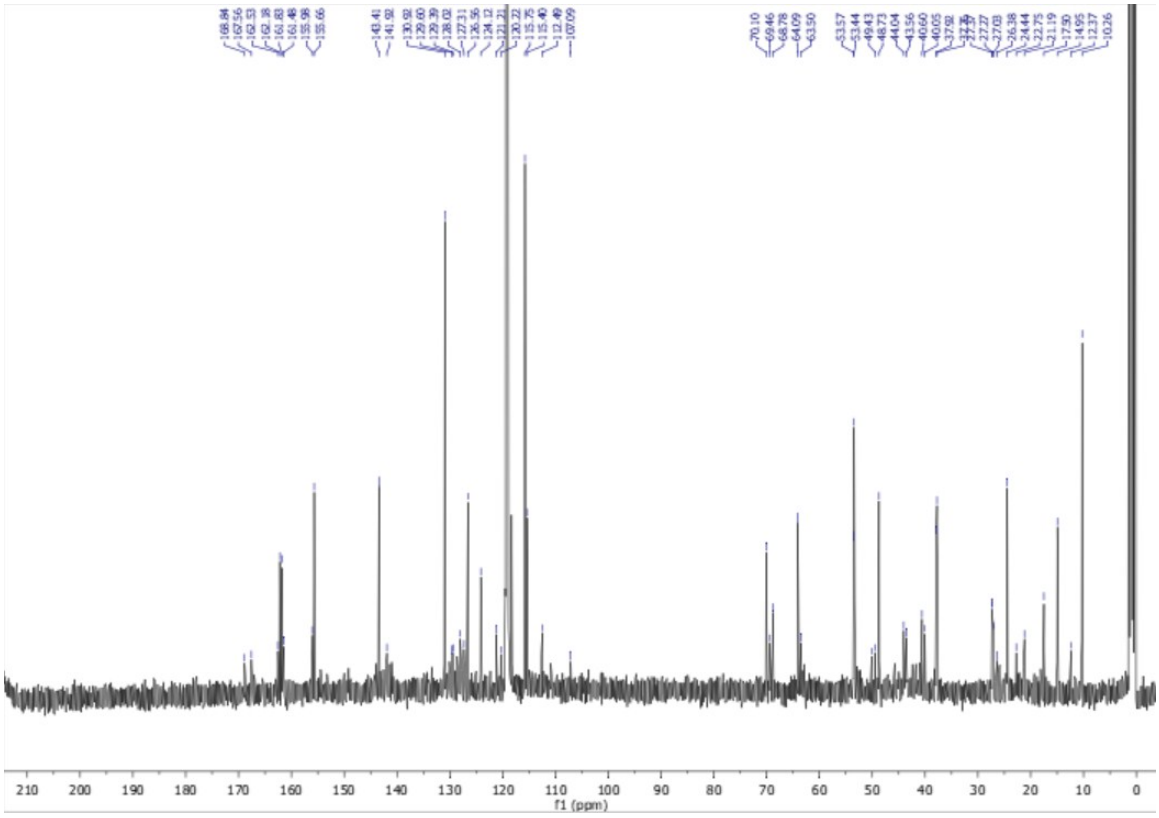
2-((E)-2-((E)-4'-((2-(2-(2-((4,6-bis(4-((2R,3R)-2-(4-((S)-1-amino-2-(4-hydroxyphenyl)ethyl)-1H-1,2,3-triazol-1-yl)-3-methylpentanoyl)piperazin-1-yl)-1,3,5-triazin-2-yl)amino)ethoxy)ethoxy)ethyl)carbamoyl)-6-(2-((E)-3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate **(2)**



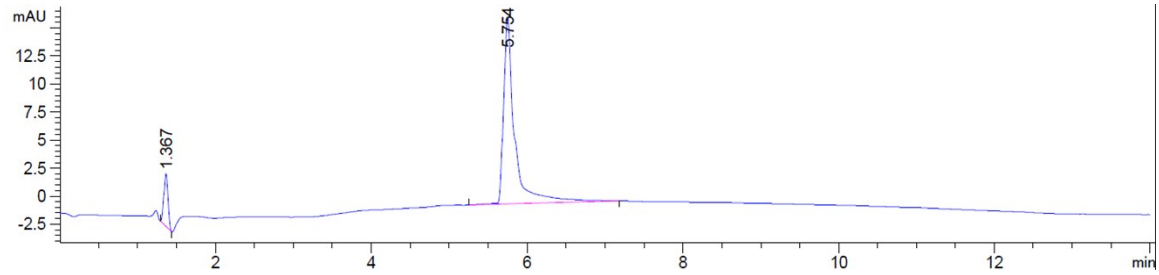
^{1}H NMR – (deuterium oxide:acetonitrile-d_3)



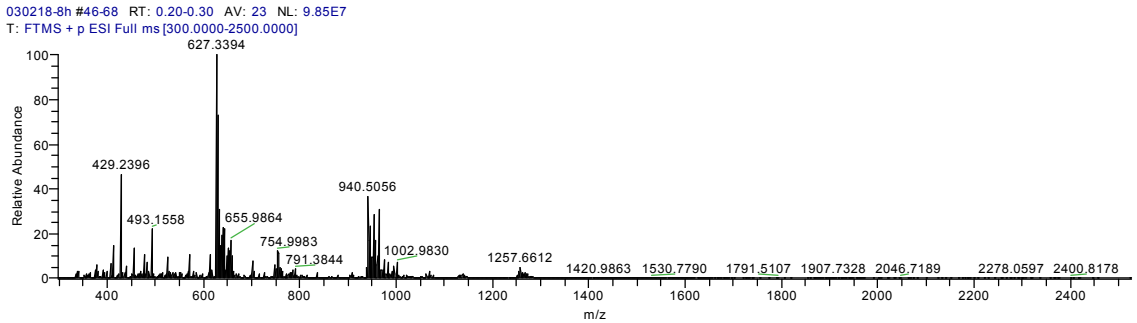
^{13}C NMR – (deuterium oxide:acetonitrile-d_3)



LC/MS at 600 nm



HRMS



E. Supporting Figures

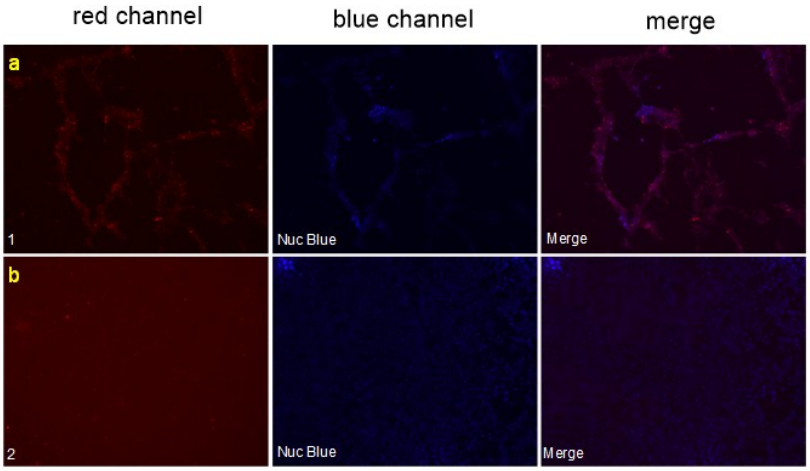


Figure S1: **a**. **1** binds to NIH3T3 TrkC cells stronger than **2** (**b**)

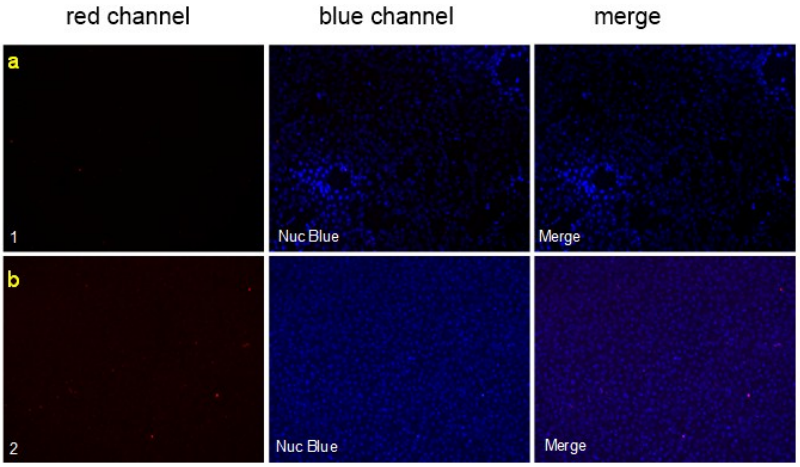


Figure S2: **a**. **1** and **b. 2** does not bind to NIH3T3 WT cells.

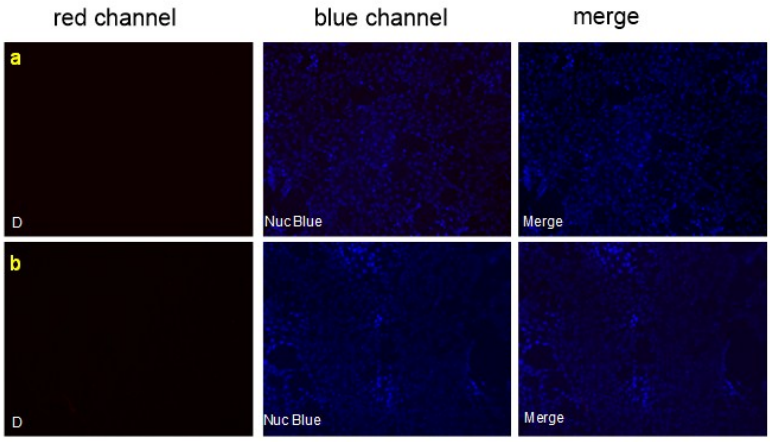


Figure S3. Free zwitterionic cyanine (**D**) does not bind to **a.** NIH3T3 TrkC or **b.** NIH3T3 WT cells.

F. References

(1) Chen, D.; Brahimi, F.; Angell, Y.; Li, Y.-C.; Moscowicz, J.; Saragovi, H. U.; Burgess, K. *ACS Chem. Biol.* **2009**, *4*, 769-781.

(2) Dongdong Su, Chai Lean Teoh, Animesh Samanta, Nam-Young Kang, Sung-Jin Park and Young-Tae Chang *Chem. Commun.,* **2015**, 51, 3989--3992