Electronic Supplementary Material (ESI) for ChemComm.

Structural Characterization of An Unusual Non-Covalent Complex Of An Electrophilic Small Molecule and Streptavidin

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Supplementary Information

Experimental Procedures

All chemicals and solvents were purchased from commercial sources and used without further purification unless otherwise noted. All oligonucleotides were purchased from Integrated DNA Technologies (IDT) as desalted lyophilate and used without further purification.

Buffers:

All buffers were prepared in ≥18 ma MilliQ water.

BTPLB-10x=10× Bis-Tris propane ligation buffer- 500 mM NaCl, 100 mM MgCl2, 10 mM ATP, 0.2% Tween 20, 100 mM Bis-Tris, pH 7.6

BTPWB= Bis-Tris propane wash buffer- 50 mM NaCl, 0.04% Tween 20, 10 mM Bis-Tris, pH 7.6

BTPBB= Bis-Tris propane breaking buffer - 100 mM NaCl, 10 mM EDTA, 1% SDS, 1% Tween 20, 10 mM Bis-Tris, pH 7.6

CRB= click reaction buffer- 50% DMSO, 30 mM TEAA, 0.04% Tween 20, pH 7.5

10X PCR buffer - 2 mM each dNTP, 15 mM MgCl2, 500 mM KCl, 100 mM Tris, pH 8.3

1× GC-PCR buffer-1× PCR buffer, 8% DMSO, 1 M betaine

GLB=Denaturing polyacrylamide gel electrophoresis loading buffer (GLB)- 6 M urea, 0.5 mg/mL bromophenol blue, 12% w/v Ficoll 400, 1× TBE buffer, pH 8.5

C&S= Crush and soak buffer- 500 mM NaCl, 1 mM EDTA, 10 mM Tris pH 7.6,

Phosphate buffer- 1M KH_2PO_4, pH 8.0

PBS-T=Phosphate buffered saline with Tween– 10 mM PO_4^{-3}, 137 mM NaCl, 2.7 mM KCl, 0.1% Tween (v/v)

Maldi Matrix- 10 mg/mL HCCA in 1:1 CAN 0.1% TFA

Standard Solid Phase Coupling Procedure:

All amino acid coupling procedures carried out utilizing standard Fmoc solid phase peptide synthesis techniques: (1) Fmoc deprotection (20% piperidine in DMF, 5 min RT, 15 min RT); (2) N-α-Fmoc-amino acid (80 mM, 500 µL, DMF) pre-activation (2 min, RT) with DIC/Oxyma/TMP (125 mM/80 mM/180 mM); (3) N-α-Fmoc-amino acid coupling to resin by addition of activated acid (500 µL) to resin and incubating while shaking (3 h, 37 °C). After each deprotection and coupling step, the resin washed (3x DMF, 500 µL; 3x DCM, 500 µL; 3x DMF, 500 µL).

Standard Peptoid and Peptoid-Like Synthesis Coupling Procedure:

The deprotected N- terminus was acylated by preparing a solution of halide displaying backbone (80 mM) and coupling reagents (DIC/Oxyma/TMP 125 mM, 80 mM, 80 mM) preactivated for 2 min, added to resin and incubated (3 hr, 37 °C). The resin was washed as above and amine containing solution (1M, DMF) added and incubated shaking (3 hr, 37 °C).

Method for Screening the Fig. 1 DEL against A647 SA. Into a mobicol spin column was added approximately 1 million beads (approximately six copies of the library). To this was added Streptavidin-Alexafluor647 (100 nM) in 1:1 PBS-TBST (500 µl total volume). The mobicol spin column was rotated (12 hrs, 37 °C) and washed with PBS-T (3x). To strip non-covalently bound protein the sample was washed (3x 1:1 DMF:PBS-T, 3x DMF) and incubated (12 hrs, RT, rotating) in DMF. The sample was washed 3x with DCM, and incubated (3 hrs, RT, rotating) in DCM, followed by washes with DMF(3x), equilibrated in DMF (3 hrs, RT, rotating) and then washed PBS-T (3x) and equilibrated (12 hrs, RT, rotating). The sample was washed with PBS-T (3x) and run on the calibrated FACSJazz instrument (see below). Hit beads were prepared as described above, sequenced, analyzed and hits resynthesized using standard SPPS techniques. Note that this harsh washing protocol using organic solvents is designed to allow for the isolation of covalent ligand-protein complexes while dissociating most reversible complexes. However, Streptavidin is unusually stable when a ligand occupies its biotin binding pocket, thus allowing the recovery of reversible ligands.

Bead Cytometry

BD FACSJazz 3 laser configuration with BD FACS Sortware (version 1.2.0.142), lasers and filter channels: 488 nm excitation, emission filters (wavelength/bandpass) 530/40, 585/30, 670/LP. 640 nm excitation, emission filters 660/20, 750/LP. 405 nm excitation, emission filter 450/50. Calibration done per manufacturers recommended protocols. Briefly, system was set up and allowed to run for 1 hour prior to calibration and use. For fluorescent calibration Sphero Ultra Rainbow 3.1 µm beads (Spherotech, cat. URFP01-30-10K) were run to align the laser paths and stream focus to maximize signal and minimize population distribution. Once aligned the gain settings for all fluorescent channels (set to linear) listed above were normalized to 45,000 rfu. To calibrate sorting the piezo amplitude for droplet generation was adjusted to align droplet breakoff within the FACSJazz camera view. Per manufacturer SOP the charge plates were activated and the deflection amplitude for the side streams generated were adjusted to properly align with the collection vessel used. The drop delay was determined by engaging the accudrop filter and BD FACS Accudrop beads (Cat #345249). Accudrop beads were visualized, sorted and the drop-delay time adjusted to achieve highest accurate sort. These calibration procedures were performed at the beginning of each day to ensure normalized results. To set forward and side scatter gain settings an aliquot of 10 µm TentaGel beads were run (~10K events) and a gate created for single bead events (excluding all doublet and triplet aggregated beads).

General sample preparation for FACS

After incubation with the appropriate proteins, detection reagents and washing the 10 µm bead samples were resuspended in a volume of 400 µL of PBS-T, vortexed (30 s) and the sample tube was loaded onto the FACSJazz. To generate gates and adjust event rates for library screening a 10,000 bead aliquot from the sample was run. Based on the events recorded appropriate fluorescent gates were set to differentiate hit from non-hit in the single bead population. To ensure sorting accuracy the off-set pressure was adjusted to achieve an event rate no greater than 4000 events/sec. The 1-drop yield sort settings were used for sorting events. If necessary during a run the sample tube could be removed, the sample line backflushed and additional buffer added to ensure the samples run to completion. The desired bead populations were sorted into 5 mL culture tubes (BD Falcon, cat. 60819).

Fluorescence Polarization Assay

The probe concentrations of the molecules were determined using the absorbance of fluorescein at absorbance max (495 nm, ε495 =78,000 $M^{-1}cm^{-1}$) using a Nanodrop UV-vis spectrophotometer (Thermo Scientific). FP binding saturation were performed in 384-well, medium bind black microtiter plates (Greiner Bio-One). Serial dilutions of streptavidin (4.9 nM to 50 μM) were titrated into PBS containing the fluorescent probe (10 nM final concentration, 15 μL final volume). Plates were incubated (5 min, RT, shaking) and read on a infinite M1000 Pro Plate Reader (Tecan) using 470 nm excitation and 530 nm emission filters. Inhibition experiments streptavidin (25 μM) and biotin (100 μM) was pre-incubated (5 min, RT) to this was added fluorescent probe molecules (10 nM final concentration). The K_D values were obtained using Prism (GraphPad Software, Inc.) with a nonlinear regression with one site total. The data shown are averages of technical triplicates, with the standard deviation.

Crystallization and data collection

The crystals of KPM-6•SA complex was obtained by vapor diffusion at room temperature using 0.2 M potassium iodide and 20% PEG3350 as precipitant. A diffraction dataset of Bragg spacings to 1.4 Å was collected on Pilatus3 6M at the ALS beamline 501. The dataset was processed with HKL2000 (HKL Research, Inc). The dataset was phased using Phaser (Phenix suite)[1] with PDB ID 1MK5 as the serarch model for molecular replacement. Initial phased map showed positive density for KPM-6. Restraints and coordintates for KPM-6 was generated using eLBOW (Phenix suite) and incorporated into SA coordinate. The model was refined using phenix.refine (Phenix suite). The model was manually inspected and adjusted after each refinement cycle using Coot.[2] The refinement was completed after the free and crystallographic R-factors stabilized. Data processing and refinement statistics are given in Table S1. Structural analysis and figure preparations were done with PyMol (Schrodinger, Inc).

Suppl. Table 1. Data collection and refinement statistics

Wavelength (Å)	0.9774
Resolution range (Å)	26.11 - 1.4 (1.45 - 1.4)
Space group	I 2 2 2
Unit cell (Å, °)	47.31, 93.52, 104.45, 90, 90, 90
Total reflections	625,864 (48,629)
Unique reflections	45,968 (4,502)
Multiplicity	13.6 (10.8)
Completeness (%)	99.83 (99.01)
Mean I/sigma(I)	30.73 (3.39)
Wilson B-factor ($Å^2$)	13.96
R-merge	0.045 (0.367)
R-meas	0.049 (0.415)
R-pim	0.020 (0.190)
CC1/2	0.999 (0.861)
Reflections used in refinement	45,939 (4,502)
Reflections used for R-free	2,251 (209)
R-work	0.1619 (0.2111)
R-free	0.1744 (0.2369)
Number of non-hydrogen atoms	2242
macromolecules	1876
ligands	62
solvent	304
Protein residues	242
RMS(bonds) (Å)	0.013
RMS(angles) (°)	1.65
Ramachandran favoured (%)	96.64
Ramachandran allowed (%)	3.36
Ramachandran outliers (%)	0.00

Rotamer outliers (%)	0.00
Clash score	1.62
Average B-factor (Å^2)	20.84
Macromolecules (Å^2)	18.65
Ligands (Å^2)	21.63
Solvent (Å^2)	34.17

Statistics for the highest-resolution shell are shown in parentheses.

Figure S1. Structure of the Figure 1 library and the building blocks employed to make it. In the first position (X_1/NR_1), either an amino acid (see bottom row) or chloroacids followed by an amine were employed.

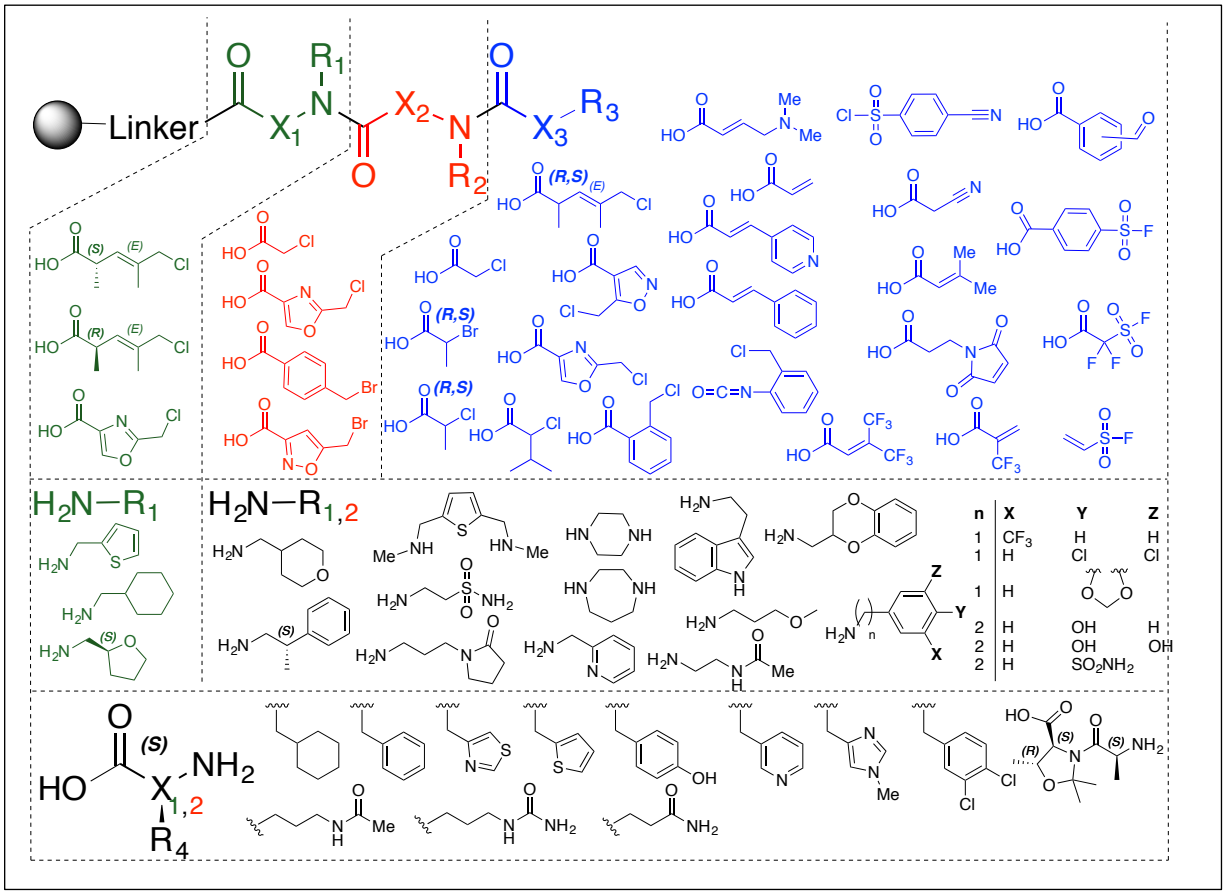


Figure S2. Predicted structures of the redundant hits from screening the library shown in Figure S2. The red numbers indicate the number of different beads on which each compound was found in the hit pool.

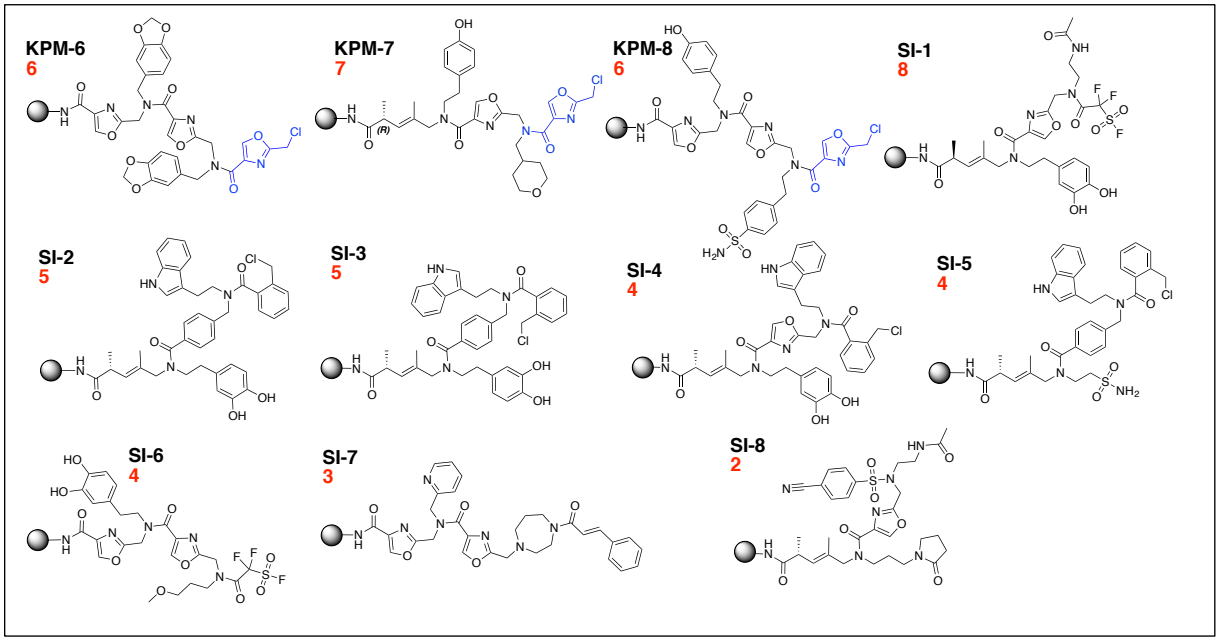
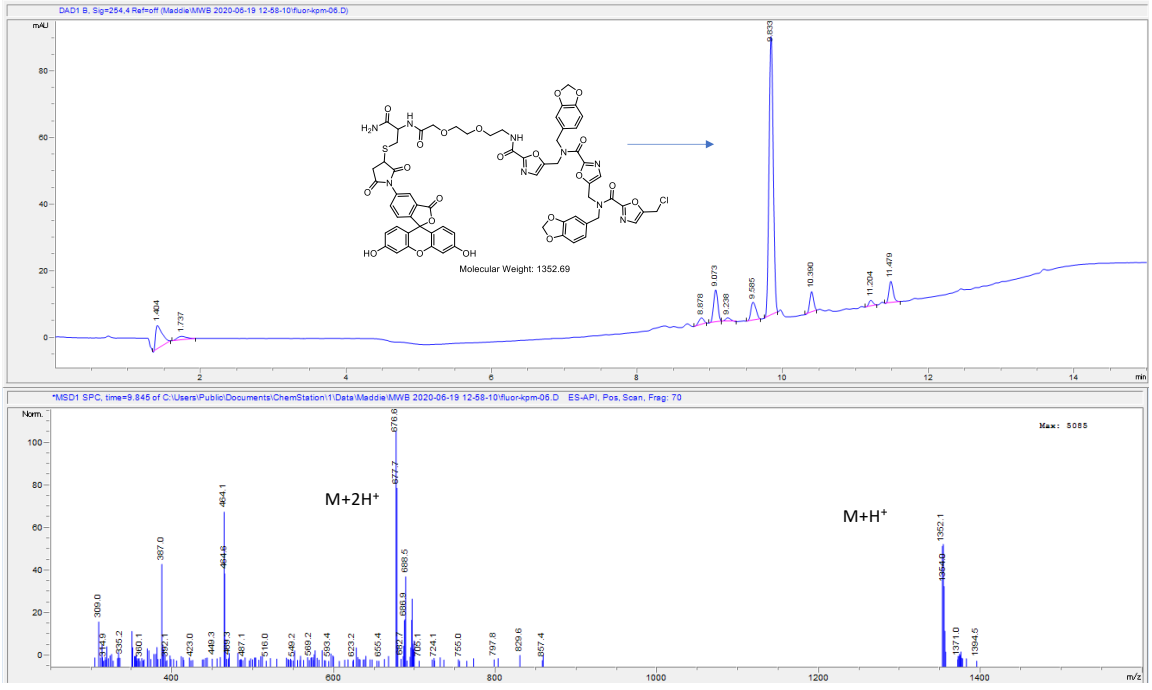
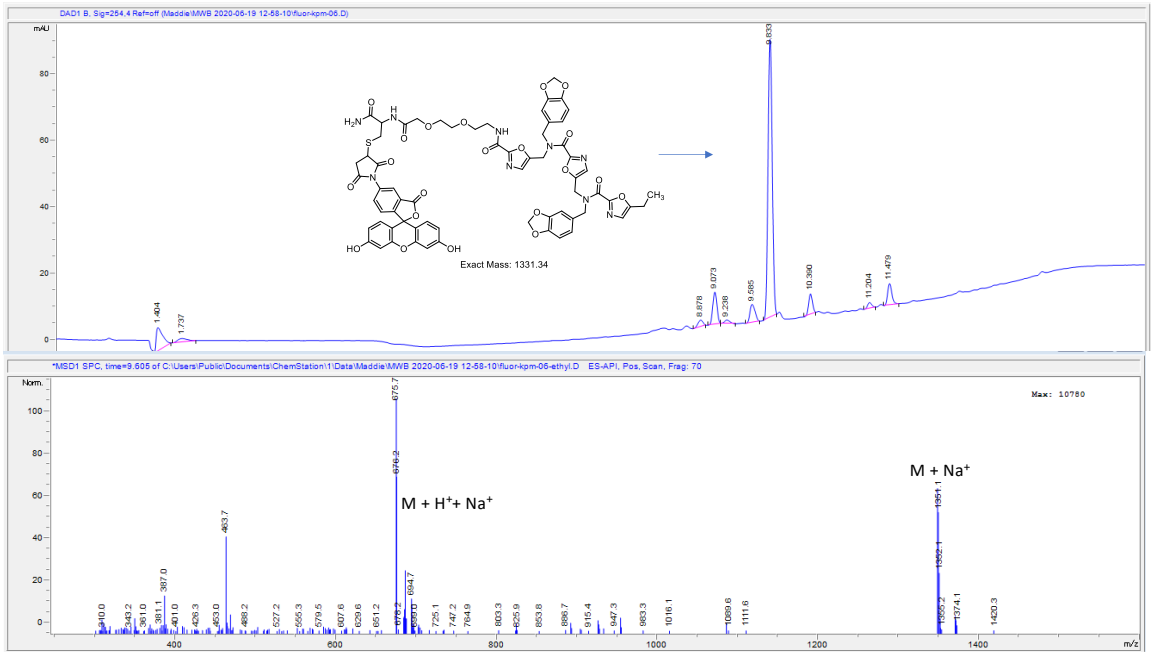


Fig. S3. Structures and analytical data for the KPM-6-Fluorescein and analogues. All PICCOs were made by solid-phase synthesis using protocols published previously and discussed above.[3, 4] Fluorescein-Maleimide was employed to label a conserved cysteine residue placed at the C-terminal end of the linker as described.[5] The LC/MS traces and MALDI mass spectra for each compound are shown below.

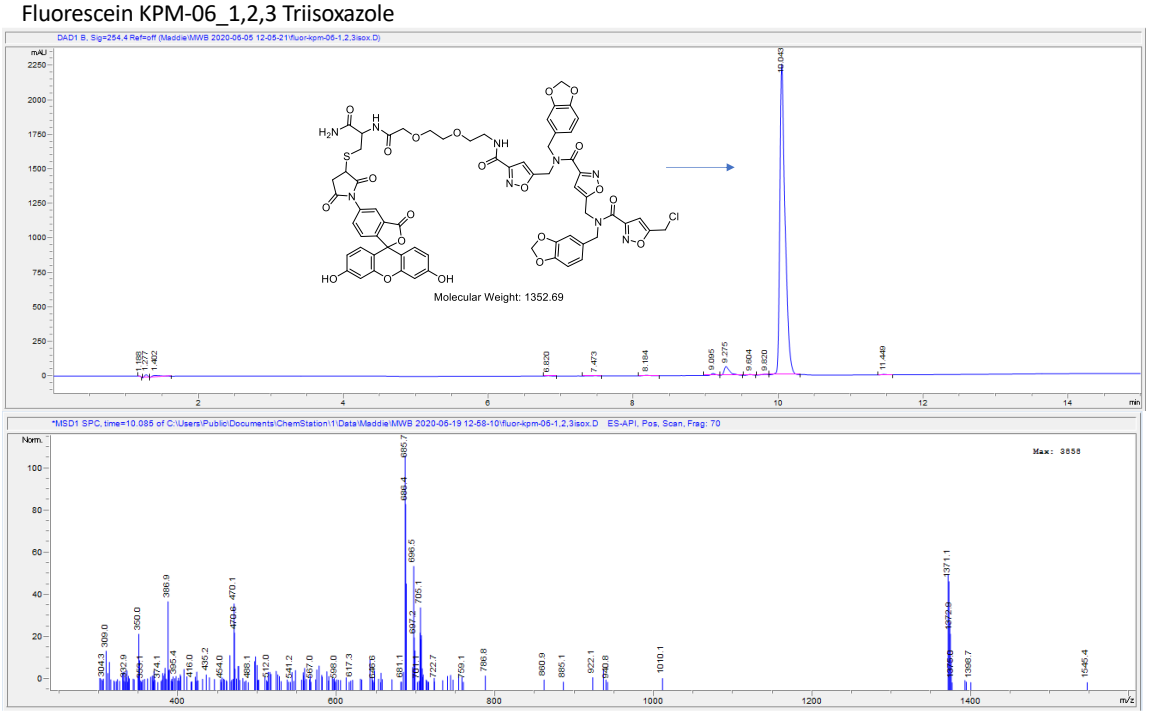
Fluorescein-KPM-06



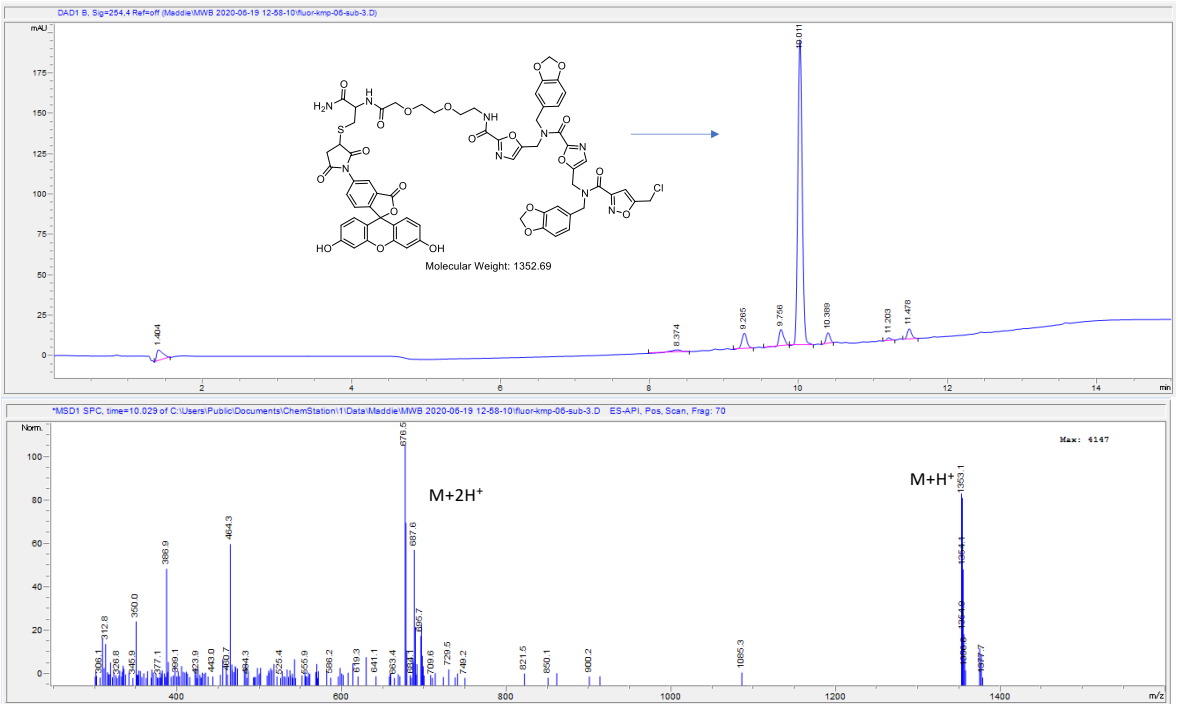
Fluorescein KPM-06_Ethyl



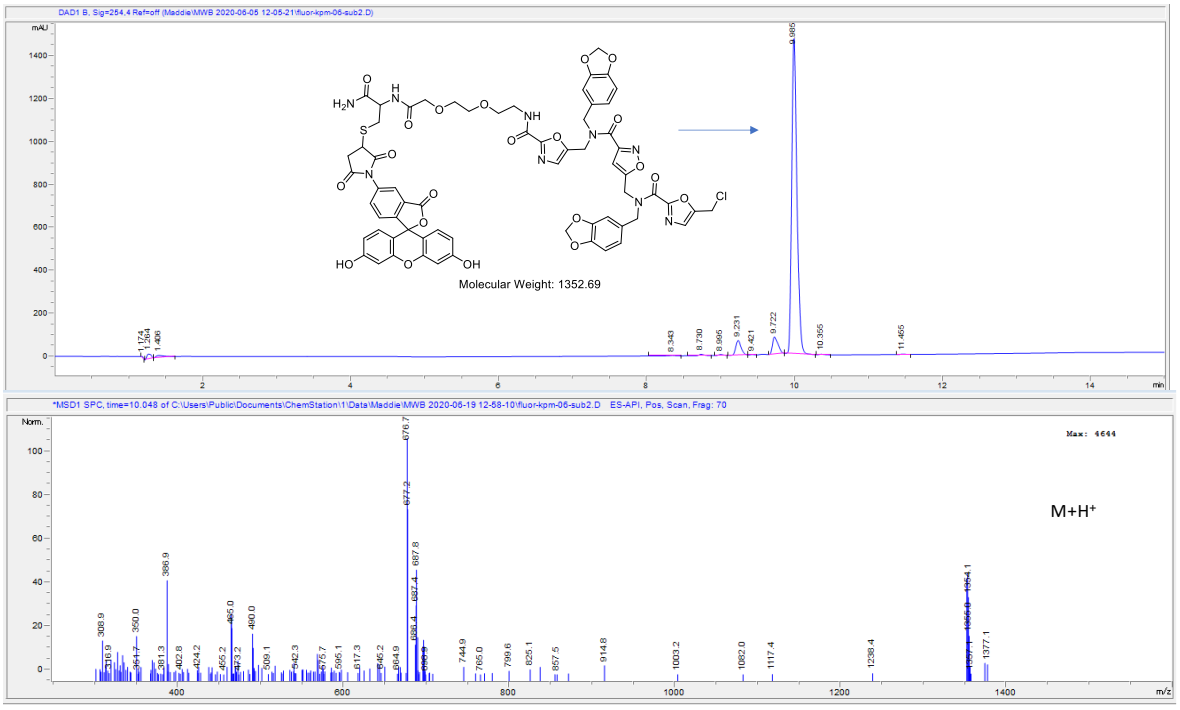
Fluorescein KPM-06_1,2,3 Triisoxazole



Fluorescein KPM-06_3 isoxazole



Fluorescein KPM-06_2 isoxazole



Literature Cited

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