Half-curcumin analogues as PET imaging probes for Amyloid beta species

Jian Yang[a,b,‡], Ran Cheng[c, ‡], Hualong Fu[d], Jing Yang[a,e], Mohanraja Kumar[f], Jie Lu[g], Yungen Xu[b], Steven H Liang[c], Mengchao Cui[d]*, and Chongzhao Ran[a]*

[a]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, MA, 02129; [b]School of Pharmacy, China Pharmaceutical University, Nanjing, 210009; [c]Nuclear Medicine and Molecular Imaging, Massachusetts General Hospital and Department of Radiology, Harvard Medical School, Boston, MA, 02114; [d]Key Laboratory of Radiopharmaceuticals, College of Chemistry, Beijing Normal University, Beijing 100875, China; [e]School of Pharmacy, Soochow University, Suzhou, 215006, China; [f]Department of Chemistry, Massachusetts Institute of Technology; [g]XuanWu Hospital, Capital Medical University, Beijing, 100053, China; [‡]These authors contribute equally to this work.

Contents

1. Materials and Methods

Materials:

All the chemicals employed in the syntheses were purchased from commercial vendors and used without further purification. The pH of the PBS buffer was 7.4. Column chromatography was performed on silica gel (SiliCycle Inc., 60 Å, 40−63 mm) slurry packed into glass columns. Synthetic Aβ peptides (1−40/42) were purchased from rPeptide (Bogart, GA, 30622). Aggregates for in vitro studies were generated by the slow stirring of Aβ40 in PBS buffer for 3 days at room temperature. F-CRANAD-101 was dissolved in DMSO to prepare a 25.0μM stock solution. ^{1}H and ^{13}C NMR spectra were recorded at 300 and125 MHz, respectively, and reported in ppm downfield from tetramethylsilane. Fluorescence measurements were carried out using an F-4500 fluorescence spectrophotometer (Hitachi). Transgenic female APP-PS1 mice and age matched wild-type female mice were purchased from Jackson Laboratory. All animal experiments were approved by the Institutional Animal Use and Care Committee at Massachusetts General Hospital.

Chemical synthesis：

Intermidiate compound 1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde: To a solution of 1-(4-methoxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde (1 g, 4.6 mmol) in 10 mL anhydrous DCM, BBr_3 (1 M, 9.2 mmol, 2.0 eq) was added drop-wisely at -78°C under N_2 protection. The resulting mixture was stirred at -78°C for 30 min., then warmed to room temperature and stirred for another 2 h. After the completion of the reaction, the mixture was quenched with water, and the resulting solution was adjusted with Na_2CO_3 to pH=8. For the mixture, the organic layer was separated, and the aqueous phase was extracted with EtOAc for twice, and the organic layers were combined and dried over anhydrous Na_2SO_4. After removing the solvent, the residue was purified with a silica column (Hexanes/ethyl acetate =1/1) to afford compound ***3*** as a gray solid (740 mg, yield: 79.1%). ^{1}H NMR ($CDCl_3$) δ (ppm) 2.54 (s, 3H), 6.84 (d, *J*=8.7Hz, 2H), 7.19 (d, *J*=8.7Hz, 2H), 8.06 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 11.46, 116.28, 121.35, 127.01, 130.28, 141.96,143.91, 156.97, 185.01; ESI-MS (M+H) m/z = 203.0

Intermediate compound 1-(4-(3-hydroxypropoxy) phenyl)-3-methyl-1H-pyrazole-4-carbaldehyde: A mixture of 1-(4-hydroxyphenyl)-3-methyl-1H-pyrazole-4-carbaldehyde (214.0 mg, 1.1 mmol,

1.0 eq), 3-iodopropan-1-ol (1.27g, 1.3 mmol, 1.2 eq), K_2CO_3 (292.0 mg, 2.1 mmol, 2.0 eq) in 5 mL CH_3CN was heated to reflux for 24h. After the completion the reaction, the mixture was filtered, and the solid was washed with EtOAc for twice (2 x 10mL), the organic layers were concentrated and purified on a silica column to afford compound ***4*** as a yellow solid (190 mg, yield: 69%). ^{1}H NMR ($CDCl_3$) δ (ppm) 2.04-2.12 (m, 2H), 2.55 (s, 3H), 3.86-3.90 (t, 2H), 4.15-4.19 (t, 2H), 7.03 (d, *J*=9.0Hz, 2H), 7.34 (d, *J*=9.0Hz, 2H), 8.03 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 11.55, 31.88, 59.94, 65.77, 114.97, 121.48, 126.74, 131.18, 142.13,143.41, 159.13, 184.94; ESI-MS (M+H) m/z = 261.0

*3-(4-(4-formyl-3-methyl-1H-pyrazol-1-yl) phenoxy) propyl 4-methylbenzenesulfonate (**1**)*

To a solution of 1-(4-(3-hydroxypropoxy) phenyl)-3-methyl-1H-pyrazole-4-carbaldehyde (190.0 mg, 0.73 mmol, 1.0 eq) in 5 mL anhydrous DCM, Et_3N (305 µL, 2.0 eq) was added, followed by the addition of DMAP (90 mg, 0.93 mmol, 1.0 eq). To the resulting mixture, 4-methylbenzene-1-sulfonyl chloride (167 mg, 0.87 mmol, 1.2 eq) was added in portion-wise at 0°C, and the reaction was warmed to room temperature and stirred for 4h. After the reaction was completed, the solution was concentrated, and the residue was purified on a silica column (Hexanes/ethyl acetate =1/1) to afford compound ***1*** as a yellow solid (210 mg, yield: 69.4%). ^{1}H NMR ($CDCl_3$) δ (ppm) 2.11-2.19 (m, 2H), 2.39 (s, 3H), 2.54 (s, 3H), 4.0-4.04 (t, 2H), 4.23-4.27 (t, 2H), 6.87 (d, *J*=9Hz, 2H), 7.27 (m, 2H), 7.75 (d, *J*=8.1Hz, 2H), 8.02 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 11.54, 21.60, 28.76, 63.53, 66.77, 114.88, 121.52, 126.67, 127.82, 129.83, 131.34, 132.75, 142.11, 143.37, 144.87, 158.73, 184.91; ESI-MS (M+H) m/z =415.0

*1-(4-(3-fluoropropoxy) phenyl)-3-methyl-1H-pyrazole-4-carbaldehyde (**2**):* To a solution of compound ***7*** (210 mg, 0.507 mmol, 1 eq) in 5 mL anhydrous THF, TEAB (1M in toluene, 1.2 eq) was added, and the resulting mixture was heated to reflux for 4h. After the reaction was over, the solvent was removed, and the residue was purified with a silica column (Hexanes/EtOAc =1/1) to afford compound ***2*** as a yellow solid (69.0 mg, yield: 51.9%). ^{1}H NMR ($CDCl_3$) δ (ppm) 2.12-2.29 (m, 2H), 2.54 (s, 3H),4.13-4.17 (t, 2H), 4.57-4.61 (t, 1H), 4.72-4.76 (t, 1H) 6.98 (m, 2H), 7.31 (m, 2H), 8.02 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 11.54, 30.14, 30.40, 63.85, 63.91, 79.37, 81.56, 114.96, 121.49, 126.74, 131.27, 142.13, 143.37, 159.04, 184.92; ^{19}F NMR ($CDCl_3$) δ (ppm) 218.26, 218.35, 218.43, 218.52, 218.59, 218.69. ESI-MS (M+H) m/z = 223.0

*(2,2-difluoro-6-methyl-2H-1λ^3,3,2λ^4-dioxaborinin-4-yl)-cyclobutane (**3**):* To a solution of 1-

cyclobutylbutane-1,3-dione (1g, 7.1 mmol) in 10 mL DCM, $BF_3.Et_2O$ (2.0 eq) was added drop-wisely at room temperature. The mixture was stirred for 12h, and TLC showed no starting material was remained. The above reaction mixture was concentrated and purified with a silica column (Hexanes/ethyl acetate =2/1) to afford compound ***3*** as a pale-yellow liquid (740 mg, yield: 90 %). ^{1}H NMR ($CDCl_3$) δ (ppm) 1.89-2.12 (m, 2H), 2.28 (s, 3H), 2.30-2.41 (m, 4H), 3.30-3.36 (m, 1H), 5.92 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 17.97, 24.14, 25.71, 40.85, 99.58, 192.17, 196.42; ^{19}F NMR ($CDCl_3$) δ (ppm) 138.75, 138.69; ESI-MS ($M+H_2O$) m/z = 206.1, ($M+Na^+$) =211.0

F-CRANAD-101: A mixture of compound ***2*** (69.0 mg, 0.26 mmol, 1.0 eq), compound ***3*** (49.5mg, 0.26 mmol, 1.0 eq), 1,2,3,4-tetrahydroisoquinoline (8 μL), and AcOH (40 μL) in 3 mL CH_3CN was heated to reflux for 15mins, and the reaction mixture was cooled to room temperature. The reaction mixture was concentrated, and the residue was purified with a silica gel column (Hexanes/EtOAc =1/1) to afford F-CRANAD-101 as a yellow solid (30mg, yield: 26.4%). ^{1}H NMR ($CDCl_3$) δ (ppm) 2.12-2.44 (m, 10H), 2.39(s, 3H), 3.33-3.39 (m, 1H), 4.14-4.18 (t, 2H), 4.57-4.61 (t, 1H), 4.73-4.77 (t, 1H), 5.87 (s, 1H), 6.39 (d, *J*=15.3Hz, 1H), 6.99 (d, *J*=9.0Hz, 1H), 7.31 (d, *J*=9.0Hz, 2H), 7.94 (s, 1H),7.97 (d, *J*=15.3Hz, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 10.94,18.05, 25.94, 30.15, 30.42, 41.12, 63.86, 63.93, 79.38, 81.56, 98.69, 114.97, 116.61,117.34, 126.49, 131.75, 138.74,138.98, 142.01, 158.93, 180.52, 194.19; ^{19}F NMR ($CDCl_3$) δ (ppm) 136.29, 136.35,218.45, 218.52, 218.62, 218.69, 218.78. ESI-MS (M+H) m/z = 433.0

Radiolabeling of [^{18}F]-CRANAD-101:

Step 1: The [^{18}F] fluoride was trapped on an ion exchange cartridge and subsequently released with a solution of TEAB (2.0 mg) in 1 mL of MeCN/H_2O (v/v 7:3) into a 5 mL V-shaped vial sealed with a Teflon septum. The solution was evaporated at 110 °C under a stream of nitrogen. The evaporation was repeated three times with addition of dry acetonitrile (1 mL) each time. After that, the dried [^{18}F] fluoride was re-dissolved in 0.2 mL dry acetonitrile. To an oven-dried vial containing compound ***1*** (4.15 mg) and a magnetic stirrer bar, anhydrous acetonitrile (0.3 mL) was added, followed by the addition of 0.1 mL of [^{18}F] TEAF in acetonitrile. The vial was sealed and heated at 80 °C for 10 min. After that, a mixture aliquot (2-5 μL) was taken for analysis by radioTLC (eluent: EtOAc) by using a Bioscan AR-2000 radio-TLC imaging scanner and WinScan software for radiochemical conversion (RCC).

Step 2: 0.015 mL tetrahydroisoquinoline, 0.05 mL AcOH and 0.01 mL compound ***3*** were added to the above mixture, and the resulting mixture was continued to be heated at 80 °C for 10 min. Then the mixture was cooled down, diluted with mobile phase (4 mL). The radiolabeled [^{18}F]-*CRANAD-101* was purified from a semi-prep radio-HPLC. Product identity was determined via co-injection with nonradioactive standard. Analytical radio-HPLC conditions: Column: luna 5u C18 100 Å 250 × 4.6 mm. Mobile phase: 60% CH_3CN, 40% 0.1 M $NH_4{\cdot}HCO_2$ (aq); Flow rate: 1.0 mL/min, RT = 18 min. Semi-prep radio-HPLC conditions: Column: luna 5u C18 (2) 100 Å 250 × 10.00 mm. Mobile phase: 60% CH_3CN, 40% 0.1 M $NH_4{\cdot}HCO_2$ (aq); Flow rate: 5.0 mL/min, RT = 18 min.

Fluorescence Spectral Testing of F-CRANAD-101 with Aβs: To record the fluorescence response of F-CRANAD-101 with Aβs, we utilized the following procedures. Step 1: 1.0 mL of PBS buffer was added to a quartz cuvette as a blank control and its fluorescence was recorded with the same parameters as for F-CRANAD-101. Step 2: The fluorescence emission spectrum of a F-CRANAD-101 solution (1.0 mL, 250 nM) was recorded with excitation at 420 nm and emission from 450 to 800nm. Step 3: To the above F-CRANAD-101 solution, 10 μL Aβs (25 μM) was added (final concentration 250nM). The emission spectra were recorded (Ex=420nm, Em=450-800nm). The final spectra from steps 2 and 3 were corrected using the blank control from Step 1.

Brain slice testing: A brain slice of 11-month old APP/PS1 mouse was washed with double-distilled water for 5 minutes, and then fixed with 4% formalin for 5minutes, and washed with double-distilled water. First, a background image of a brain slice was taken using a fluorescence microscope before treatment. Second, an image was taken after the slice was incubated with F-CRANAD-101(25 μM) for 30 minutes.

[^{18}F]-CRANAD-101 autoradiograph test: Brain slices from an APP/PS1 mouse and WT mice were washed with double-distilled water for 5 minutes, and then fixed with 4% formalin for 5minutes, and washed with double-distilled water. The slices were subsequently dried, then incubated with [^{18}F]-CRANAD-101 (5 μCi) for 30mins. The slices were then washed with 20% ethanol double-distilled water and dried. The dried slices were exposed to an imaging plate for 1 hour, and the plate was scanned with autoradiography system Cyclone (Perkin Elmer).

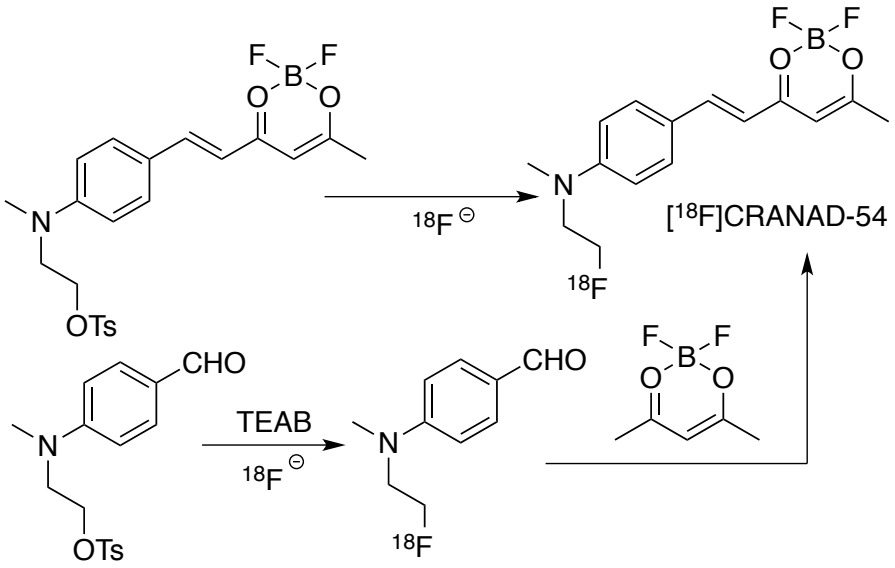
Two-photon imaging. A mouse (19-months old) was anesthetized with Ketamine/xylazine (70 mg/kg) and a cranial imaging window was surgically prepared as described. A detailed procedure

and a video can be found in ref. [1]. F-CRANAD-101 (2.0 mg/kg in a fresh solution containing 15% cremorphor, 15% DMSO and 70% PBS) was injected intravenously at time 0 minute by a bolus injection during image acquisition. Two-photon fluorescence excitation was accomplished with a 900-nm laser (Prairie Ultima). Imaging was performed using a two-photon microscope (Prairie Technologies) equipped with a 20x water immersion objective (N.A. 1.0, Zeiss). Images were collected for 15 seconds per frame 512×512 μm matrix for 45 min with a green (500-550 nm) channel.

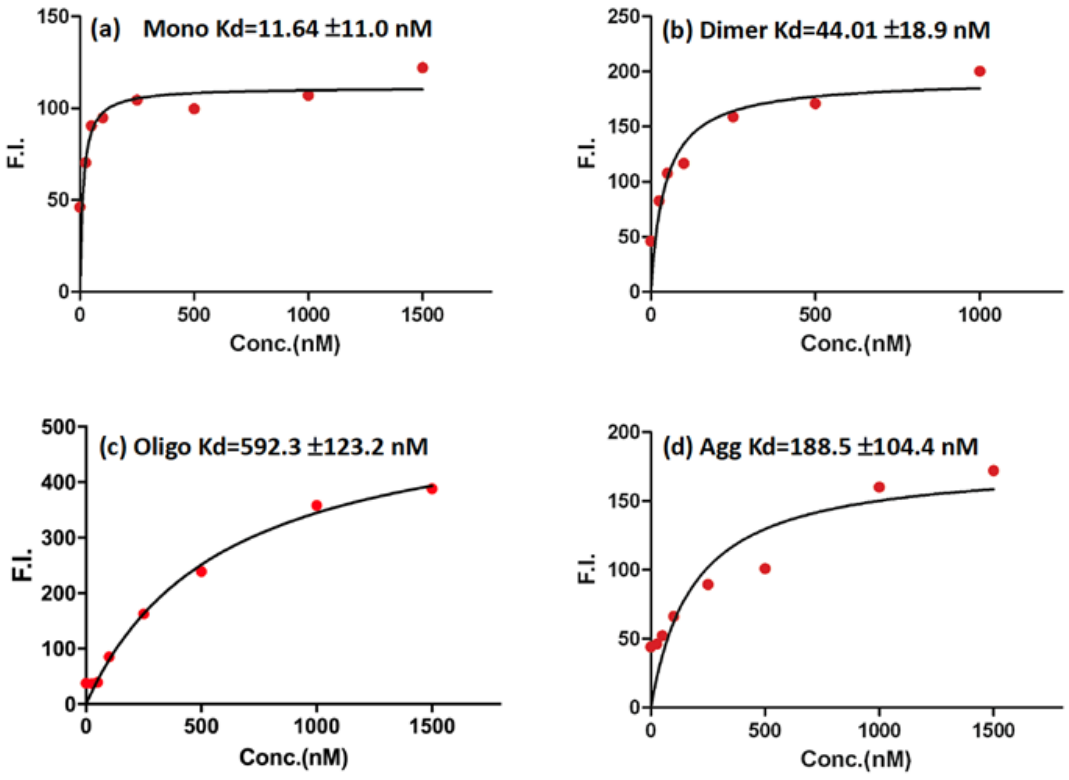
***In vivo* PET imaging.** All animal experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC). In vivo PET imaging was conducted with 14-month (n = 4) and 5-month (n = 3) old APP/PS1 mice (Charles River Laboratories, MA) and age-matched WT mice. Mice were injected via tail-vein with 100 μL of 20-30 μCi of [^{18}F]-CRANAD-101 in 10% ethanol saline. MicroPET imaging experiments were conducted on a Sophie Biosciences microPET G4 scanner (Culver City, CA, USA), 60-minutes dynamic imaging was performed under general anesthesia (isoflurane/O_2). Imaging analysis was conducted with Amide.

[1] R. Mostany, E. M. Valdizan and A. Pazos, *Neuropharmacology*, 2008, **55**, 18-26.

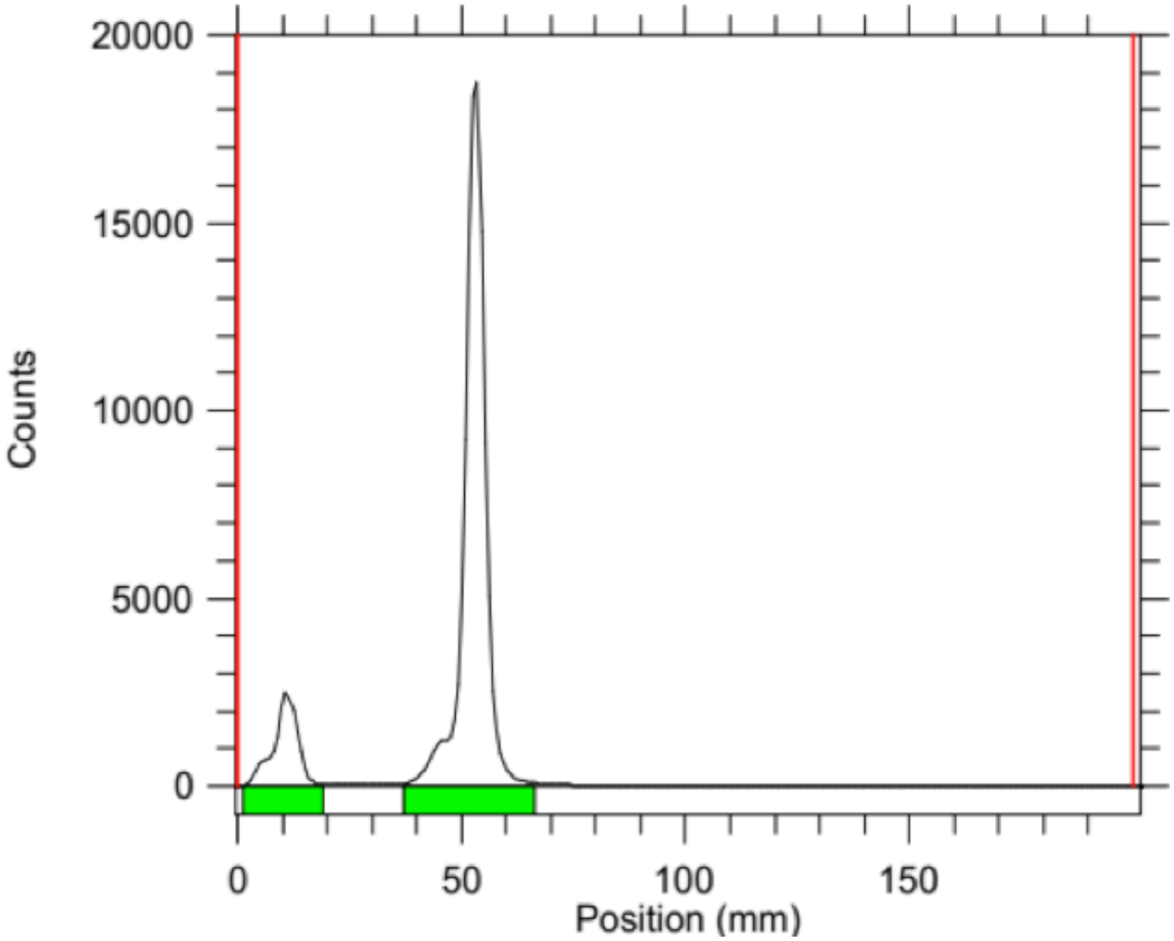
2. Supplemental Figures



SI Fig.1 Synthetic routes for ^{18}F-CRANAD-54.

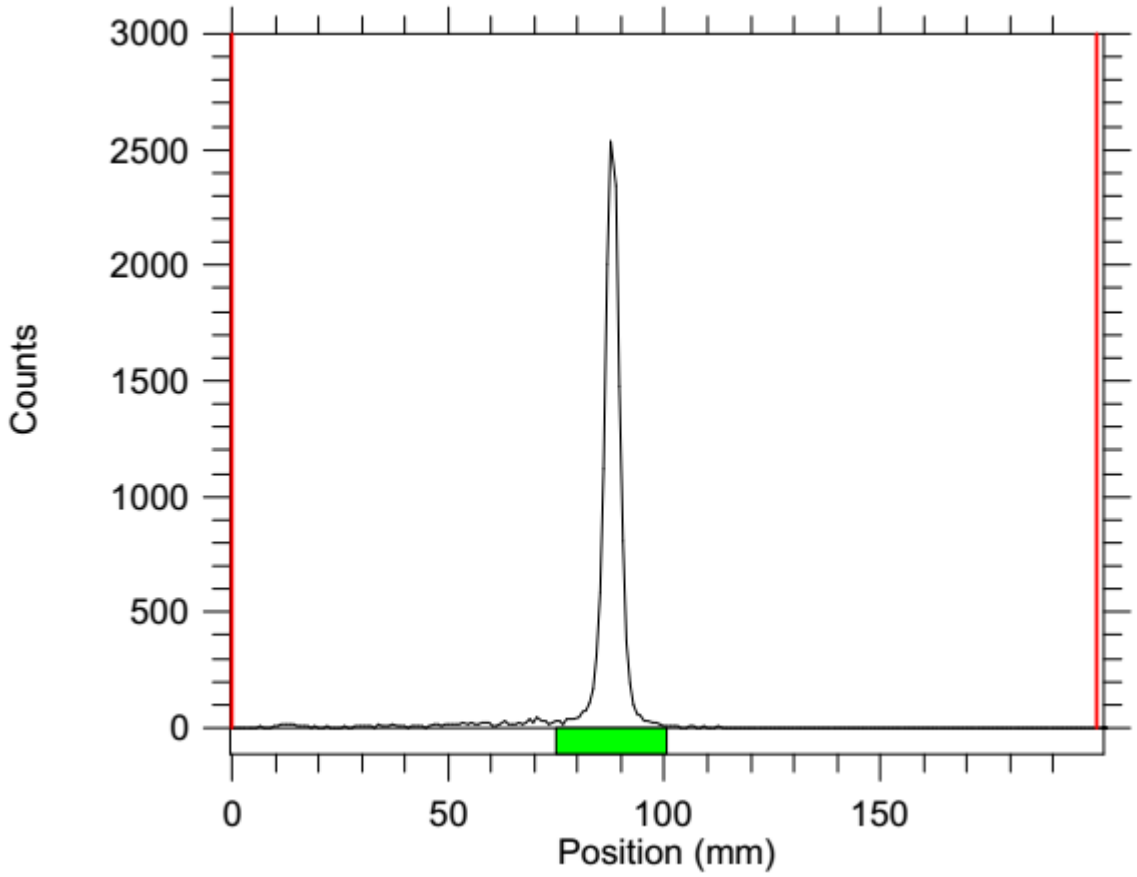


SI Fig.2 Titration of F-CRANAD-101 with various Aβ species for determining K_d.

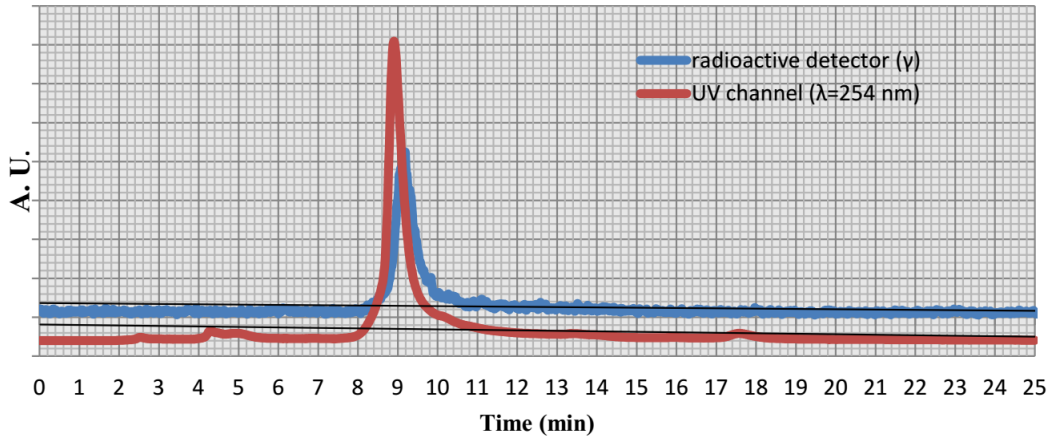


Run	1	2	3	mean	standard deviation
radioTLC yield (%)	82	85	77	81	4

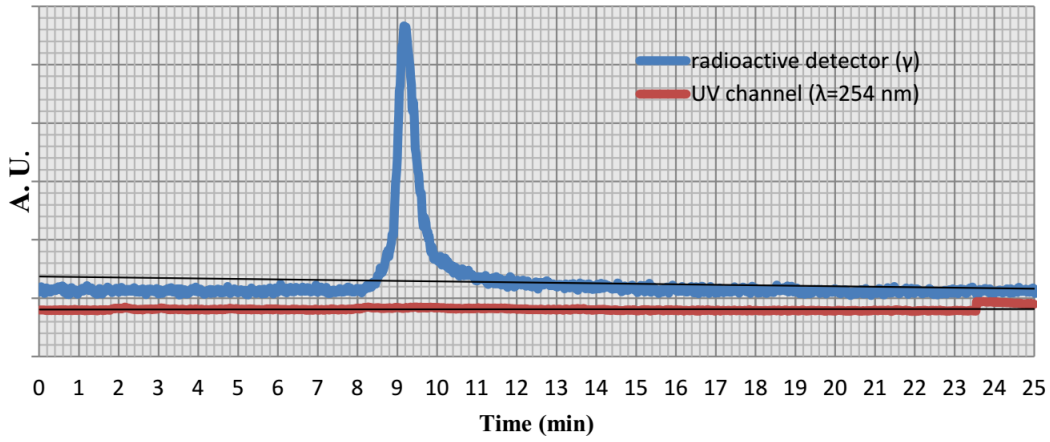
SI Fig. 3a Radio-TLC of the reaction mixtures of the first step (compound [^{18}F]-**1**) and RCC analysis.



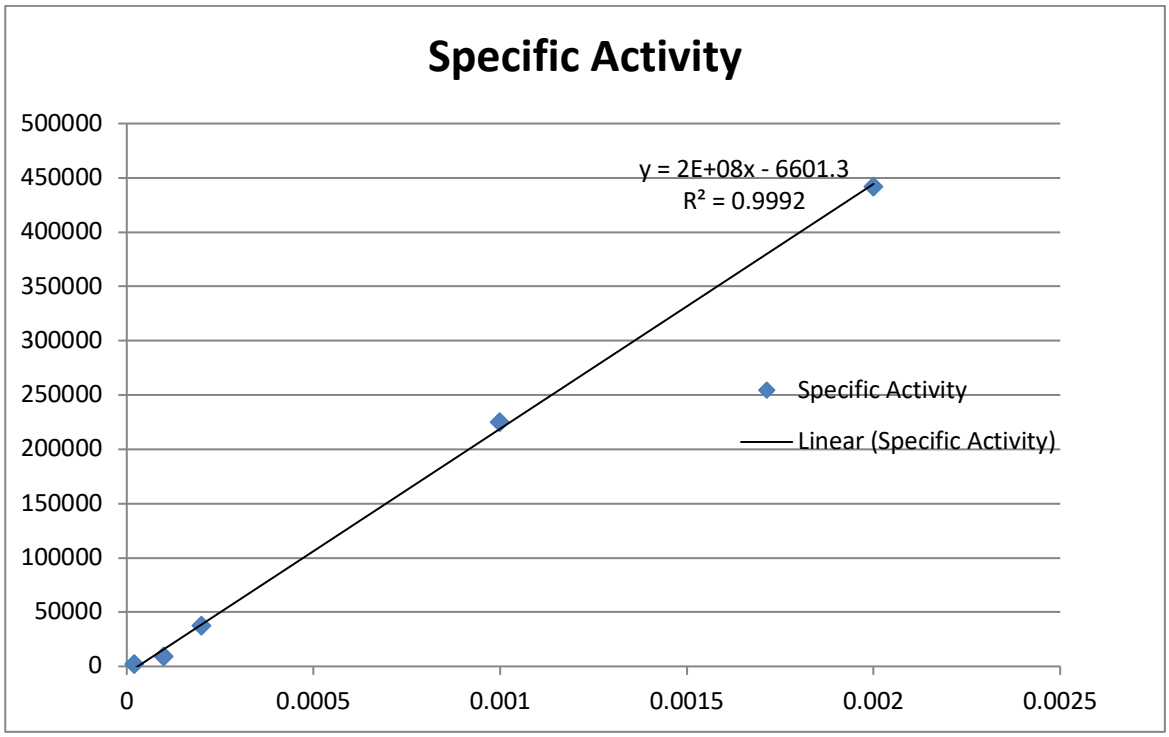
SI Fig.3b Radio-purity of separated [^{18}F]**-CRANAD-101** on radio-TLC



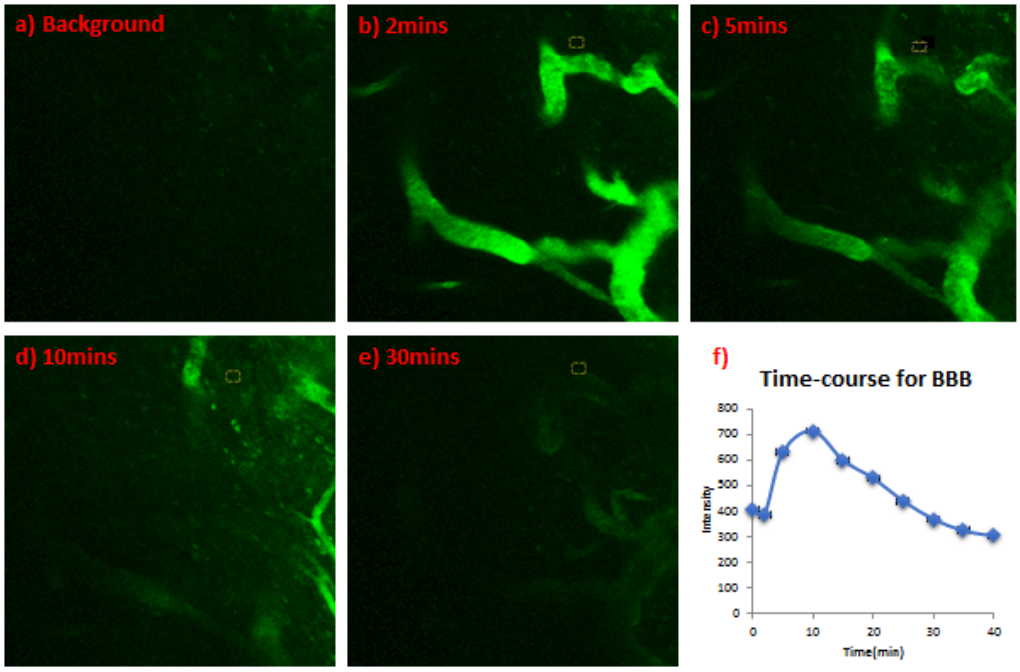
SI Fig.3c Co-injection of ^{18}F-**CRANAD-101** and cold F-**CRANAD-101.**



SI Fig.3d Radiochemical and chemical purity of separated ^{18}F-**CRANAD-101**.



SI Fig.3e Standard curve for the specific activity determination of [^{18}F]**-CRANAD-101**. The specific activity of [^{18}F]**-CRANAD-101** was determined to be 1.19 Ci/μmol before injection to the mice.



SI Fig.4 Time-lapse two-photon imaging of a WT mouse with F-CRANAD-101 (a-e), and the accumulation time-course of F-CRANAD-101 (f).

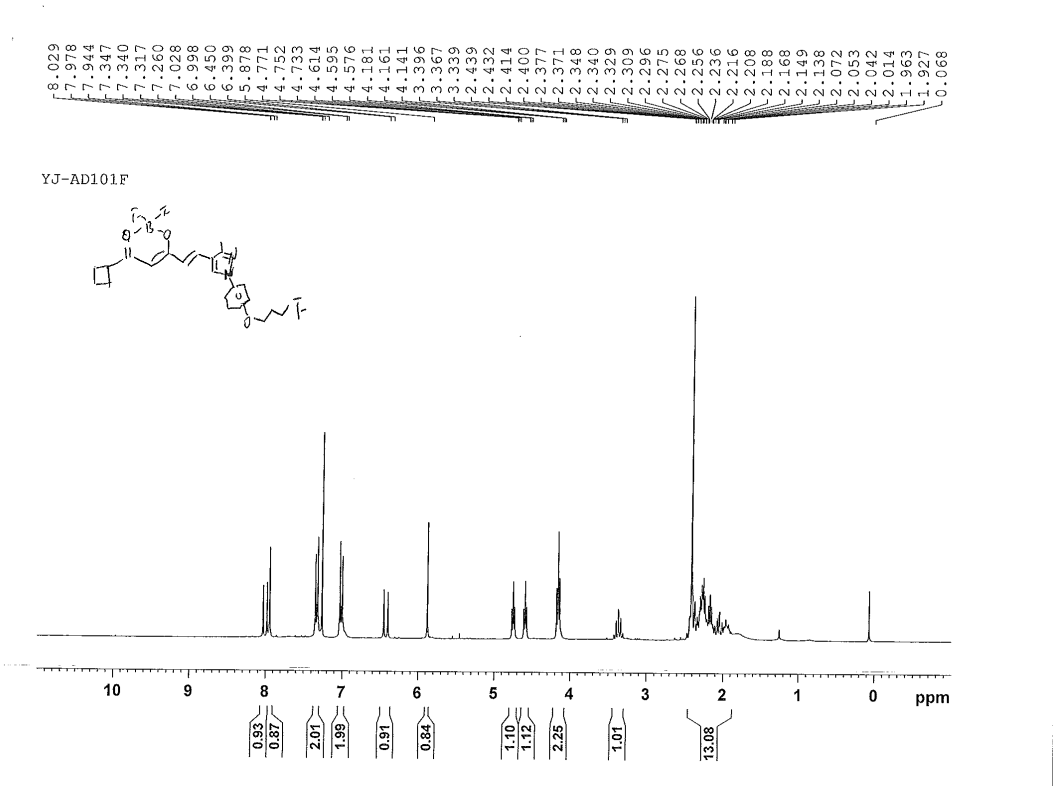


Fig 5a: ^{1}H NMR spectrum of F-CRANAD-101.

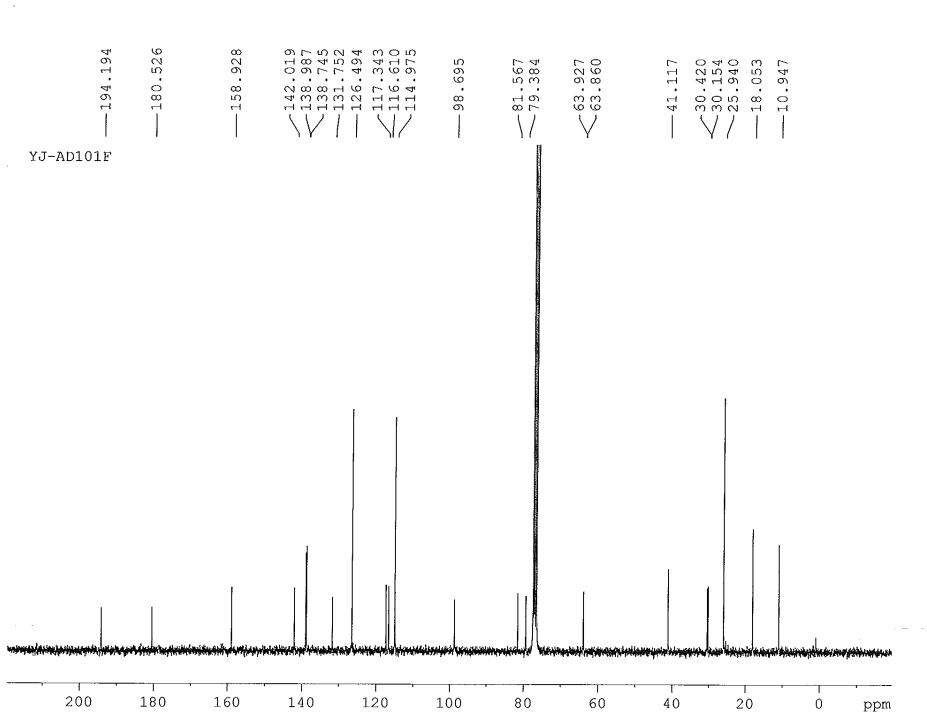


Fig 5b: ^{13}C NMR spectrum of F-CRANAD-101.

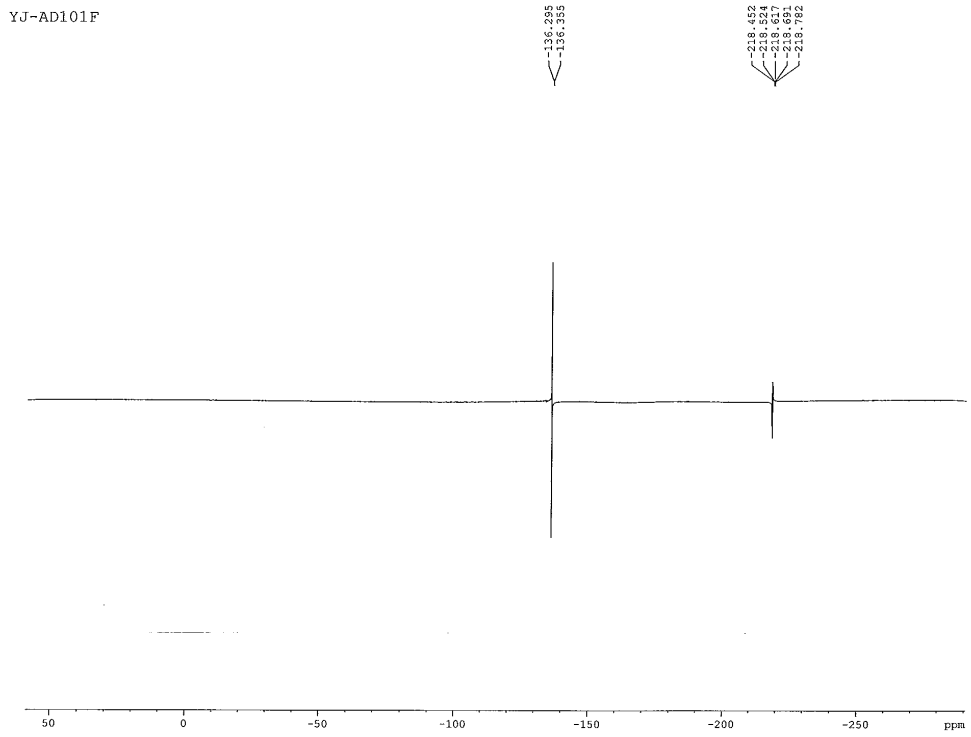


Fig 5C: ^{19}F NMR spectrum of F-CRANAD-101.

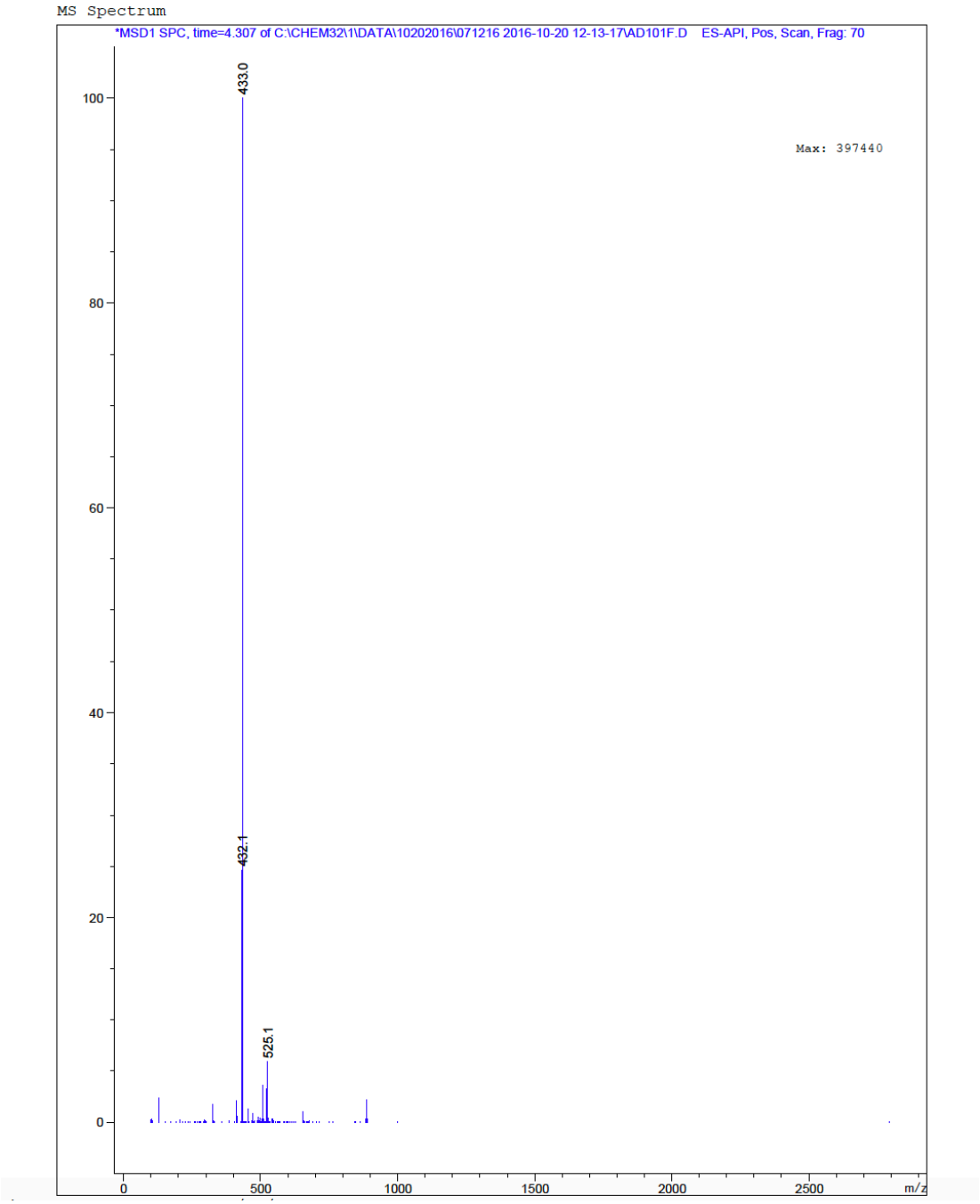


Fig 5d: MS spectrum of F-CRANAD-101.

Qualitative Compound Identification Report

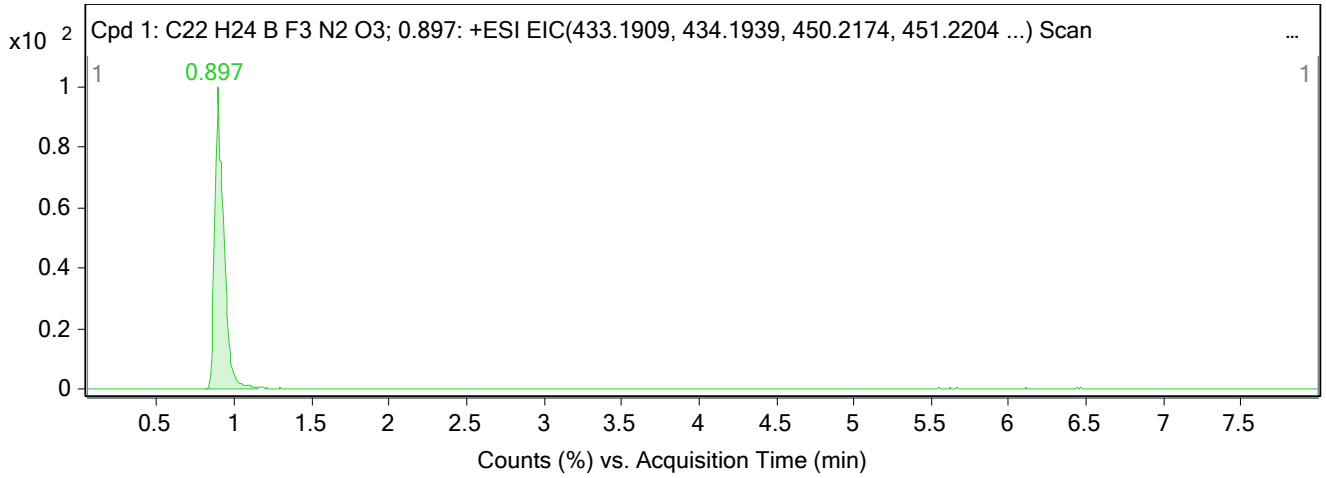
Data File	CRANAD101-F.d	**Sample Name**	blank02122019
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	
Acq Method	Mohan06192018_Chase.m	**Acquired Time**	2/12/2019 11:55:33 AM (UTC-05:00)
IRM Calibration Status	Success	**DA Method**	default.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition Time (Local)**	2/12/2019 11:55:33 AM (UTC-05:00)
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.08.00 (B8058.0)	**QTOF Driver Version**	8.00.00
QTOF Firmware Version	25.712	**Tune Mass Range Max.**	3200

Compound Table

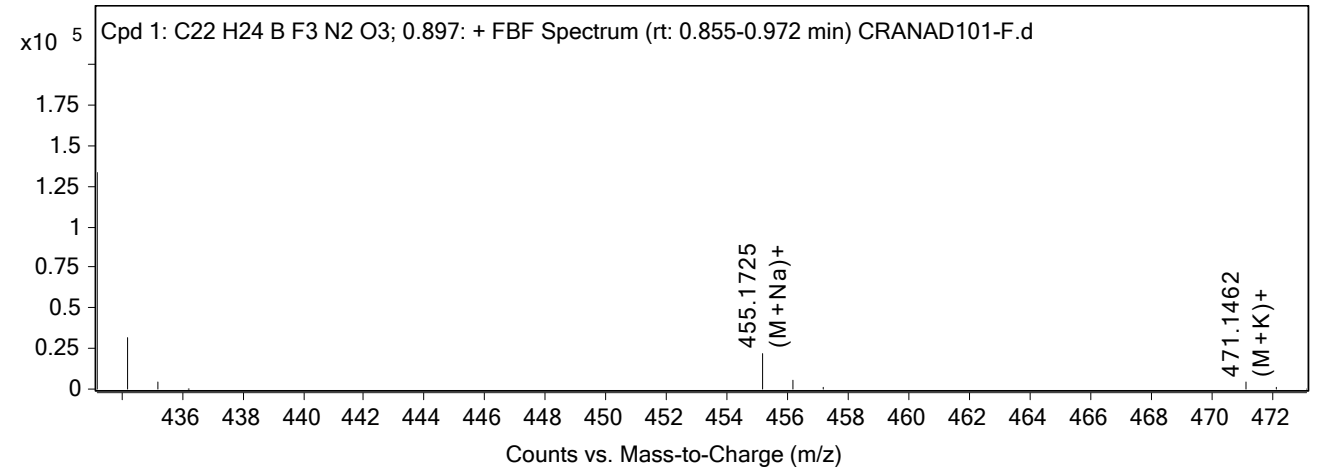
Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	Hits (DB)
Cpd 1: C22 H24 B F3 N2 O3; 0.897	0.897	431.1869	133399	C22 H24 B F3 N2 O3	431.1868	0.03	1

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C22 H24 B F3 N2 O3; 0.897	433.1911	0.897	Find By Formula	431.1869

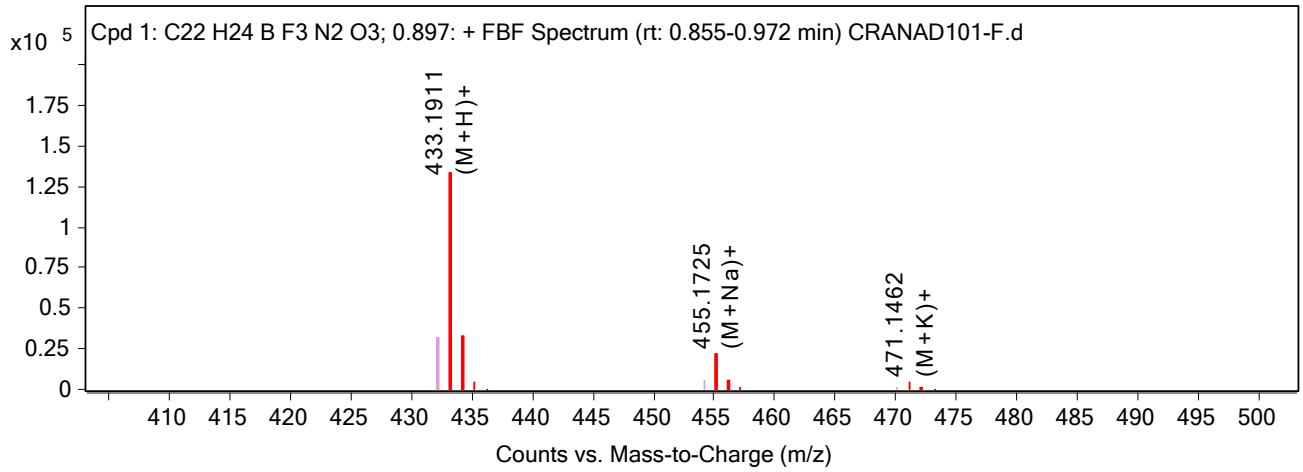
Compound Chromatograms



MS Spectrum



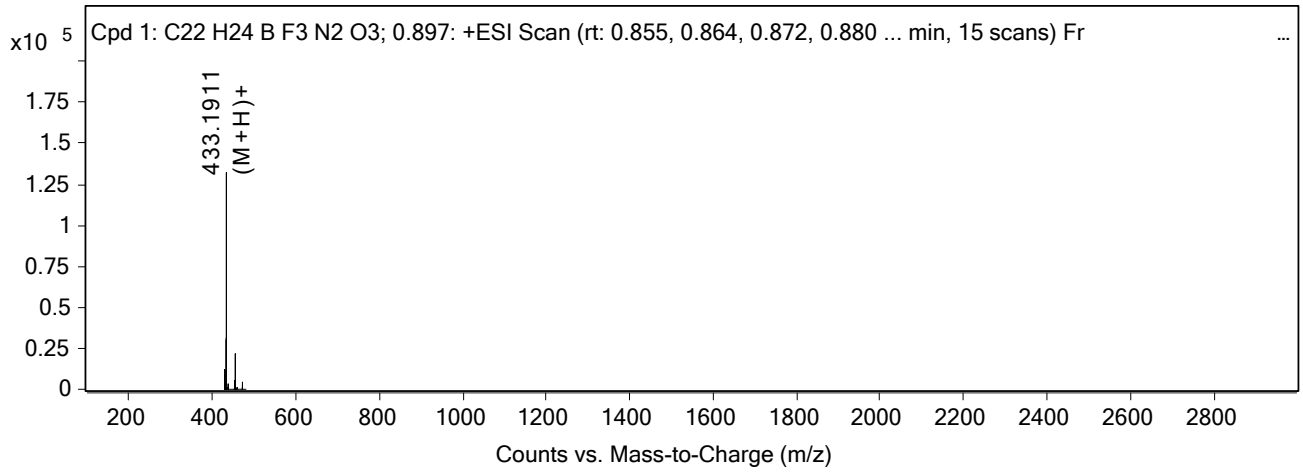
MS Zoomed Spectrum



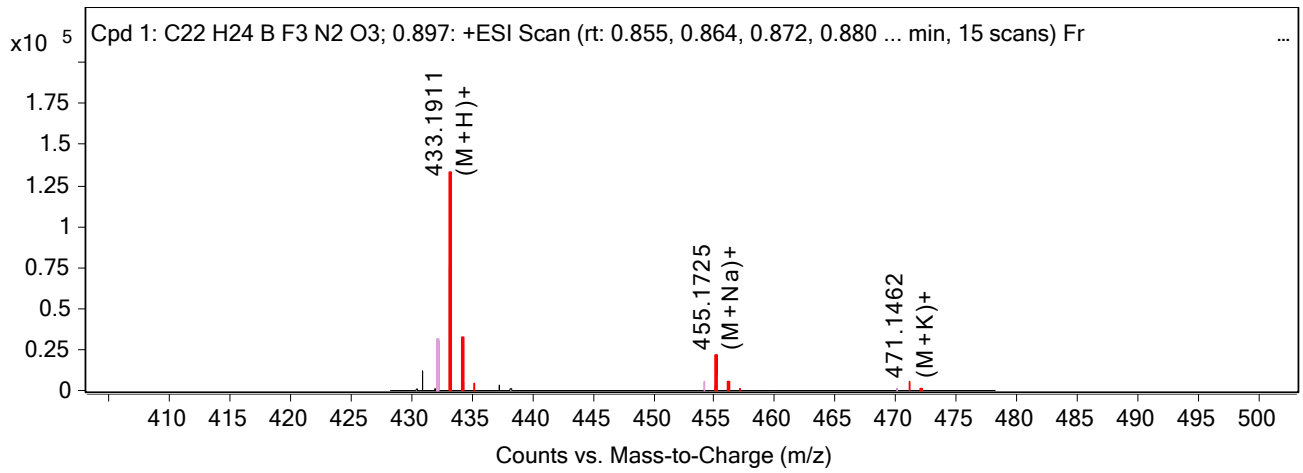
MS Spectrum Peak List

m/z	z	Abund	Ion
433.1911	1	133399.48	(M+H)+
434.1937	1	31770.51	(M+H)+
435.1958	1	4422.6	(M+H)+
436.1984	1	470.91	(M+H)+
455.1725	1	22032.79	(M+Na)+
456.1755	1	5379.56	(M+Na)+
457.179	1	770.9	(M+Na)+
471.1462	1	4890	(M+K)+
472.1483	1	1224.82	(M+K)+
473.1482	1	446.67	(M+K)+

MS Spectrum



MS Zoomed Spectrum



Qualitative Compound Identification Report

MS Spectrum Peak List

m/z	Calc *m/z*	Diff(ppm)	z	Abund	Ion
433.1911	433.1909	-0.44	1	133399.48	(M+H)+
434.1937	434.1939	0.46	1	31770.51	(M+H)+
435.1958	435.1967	1.98	1	4422.6	(M+H)+
436.1984	436.1993	2.27	1	470.91	(M+H)+
455.1725	455.1728	0.67	1	22032.79	(M+Na)+
456.1755	456.1759	0.76	1	5379.56	(M+Na)+
457.179	457.1786	-0.89	1	770.9	(M+Na)+
471.1462	471.1468	1.21	1	4890	(M+K)+
472.1483	472.1497	2.98	1	1224.82	(M+K)+
473.1482	473.1474	-1.81	1	446.67	(M+K)+

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