Supporting Information Appendix

Design of a Multivalent Bifunctional Chelator for Diagnostic ^{64}Cu PET Imaging in Alzheimer's Disease

Hong-Jun Cho,[1] Truc T. Huynh,[2,3] Buck E. Rogers,[2,*] and Liviu M. Mirica[1,4,*]

[1] Department of Chemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Avenue, Urbana, Illinois 61801, United States

[2] Department of Radiation Oncology, Washington University School of Medicine, St. Louis, Missouri 63108, United States

[3] Department of Chemistry, Washington University, St. Louis, Missouri 63130, United States

[4] Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO 63110, United States

* e-mail: mirica@illinois.edu, b.rogers@wustl.edu

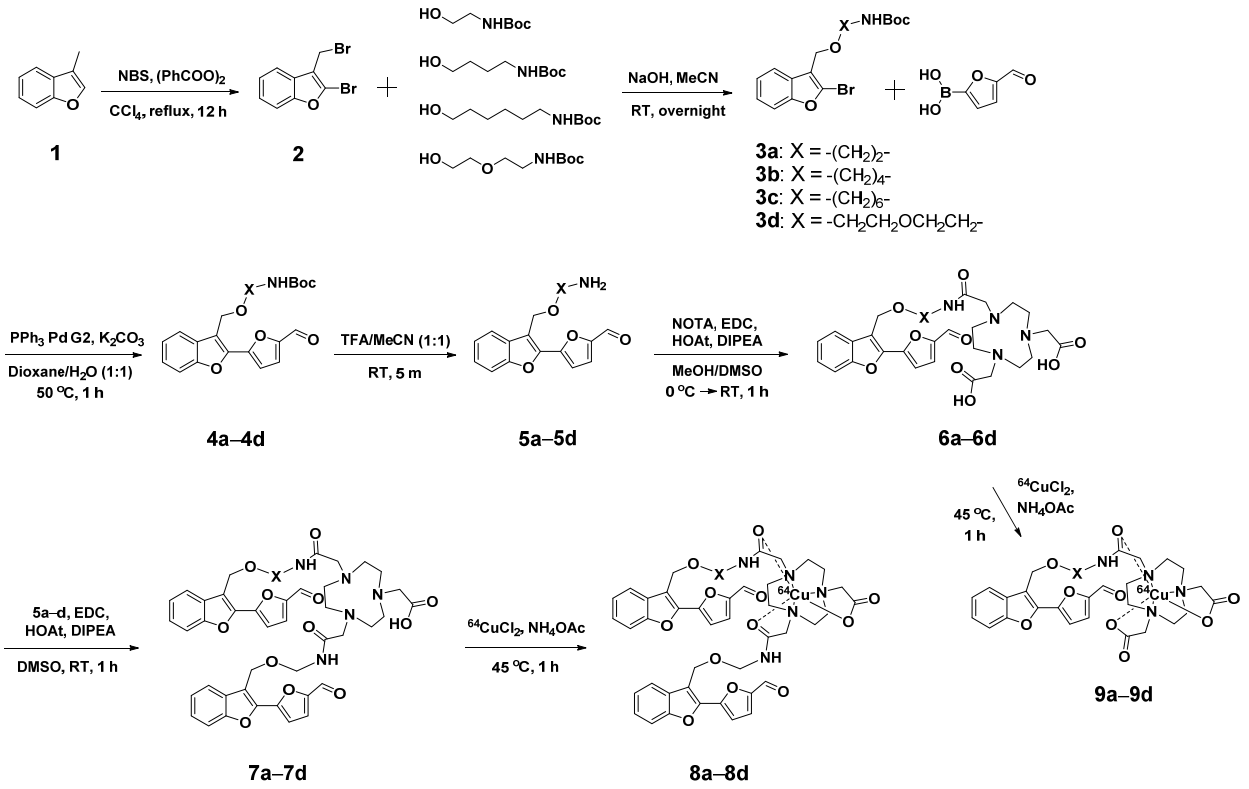
Table of Contents

www.pnas.org/cgi/doi/10.1073/pnas.2014058117

General methods

Unless otherwise noted, all chemical reagents and solvents were purchased from commercial suppliers and used without further purification. The analysis and purification of the compounds were carried out on an Agilent Technologies 1260 Infinity II HPLC system (Santa Clara, CA, USA) equipped with UV-VIS and fluorescence detector using InfinityLab Poroshell 120 EC-C18 columns (4.6 × 100 mm and 9.4 × 150 mm, 4 μm). Mass spectra were acquired on a high-resolution electrospray ionization mass spectrometry (HR-ESI-MS, Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap) (Thermo Scientific, San Jose, CA, USA). UV–vis absorption and fluorescence emission spectra were measured by a SpectraMax M2e plate reader (Molecular Devices, Sunnyvale, CA, USA). The ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) spectra were recorded on a Varian VXR 500 (500 MHz and 126 MHz, respectively) using $CDCl_3$ as a solvent and TMS as an internal standard. The Neuro-2a (N2A) mouse neuroblastoma cell line was purchased from the American Type Culture Collection (ATCC) and cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% antibiotic (penicillin-streptomycin) in a humidified 5% CO_2 incubator at 37 °C. 5xFAD transgenic mice overexpressing mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) were purchased from Jackson Laboratories (Bar Harbor, ME, USA), and wild type (WT) mice were purchased from Jackson Laboratories (B6SJLF1/J) and Charles River Laboratories (CD-1). Monoclonal anti-Aβ-antibody (HJ3.4) was obtained from Dr. David Holtzman, Department of Neurology at Washington University School of Medicine. The antibody was directly labeled with CF™ 594 dye using Mix-n-Stain™ CF™ 594 Antibody Labeling Kit purchased from Millipore Sigma (St. Louis, MO, USA), in accordance with the protocol provided by the manufacturer. Fluorescence images for brain sections were visualized using an Invitrogen EVOS FL Auto 2 Imaging System (ThermoFisher, USA). Colocalization analysis and determination of the Pearson's correlation coefficient was performed with the imaging software Fiji (ImageJ 1.52p). Radioactivity was counted with a Beckman Gamma 8000 counter containing a NaI crystal (Beckman Instruments, Inc., Irvine, CA, USA). High-performance liquid chromatography (HPLC) analysis was performed using Kinetex (Phenomenex) C-18 column (4.6 mm × 150 mm, 5 μm) in Agilent Technologies 1200 series HPLC equipped with a NaI radiotracer detector and a photodiode array detector. Positron emission tomography/computed tomography (PET/CT) images were taken on an Inveon small animal PET/CT scanner (Siemens Medical Solutions, Knoxville, TN, USA). Dynamic images were collected and reconstructed with the Maximum Aposteriory Probability (MAP) algorithm followed by CT co-registration with the Inveon Research Workstation image display software (Siemens Medical Solutions, Knoxville, TN, USA).

Synthesis details



Scheme S1. Synthetic route for the multivalent ^{64}Cu-labeled complexes (**8a–8d** and **9a–9d**)

*Compound **2** (2-bromo-3-bromomethylbenzofuran)*

Compound **2** was synthesized according to a modified procedure previously described.(1) *N*-Bromosuccinimide (NBS, 10 g, 56.19 mmol) and benzoyl peroxide (68 mg, 0.28 mmol) were added to a solution of 3-methylbenzofuran (3.71 g, 28.09 mmol) in carbon tetrachloride (80 mL) under nitrogen atmosphere. The reaction mixture was heated under reflux and monitored by GC-MS. After 4 h, NBS (1 g, 5.62 mmol) was added into the reaction mixture followed by reflux for 2 h. If mono-brominated contents were still over 20%, additional 1 g of NBS was added and the reaction mixture was heated under reflux for 2 h. After cooling to room temperature, the solvent was evaporated and the residue diluted with diethyl ether. The organic solution was washed with brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (eluent: hexane and ethyl acetate, > 100:1) and crystalized in hexane. The compound **2** was obtained in 64% yield (5.23 g) as white crystals. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.66–7.60 (m, 1H), 7.48–7.43 (m, 1H), 7.34–7.29 (m, 2H), 4.56 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 155.60, 129.04, 126.98, 125.20, 123.81, 119.30, 116.98, 111.36, 22.08; MS (m/z, %): 292 (M^++4, 6), 290 (M^++2, 13), 288 (M^+, 6), 212 (10), 211 (97), 210 (11), 209 (100), 105 (8), 104 (9), 103 (7), 102 (76), 101 (23), 76 (15), 75 (19), 74 (12), 63 (8), 51 (17), 50 (11).

*Compounds **3a**–**3d***

N-Boc-ethanolamine (467 mg, 2.90 mmol), 4-(boc-amino)-1-butanol (548 mg, 2.90 mmol), 6-(boc-amino)-1-hexanol (630 mg, 2.90 mmol), or 2-[2-(boc-amino)ethoxy]ethanol (595 mg, 2.90 mmol) was added to the solution of compound **2** (420 mg, 1.45 mmol) in MeCN (2 mL). After adding NaOH (232 mg, 5.79 mmol) into the solution, the reaction mixture was stirred vigorously overnight at room temperature. The reaction mixture was filtered, and the filtrate diluted with ethyl acetate. The organic solution was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (eluent: hexane and ethyl acetate, 5:1). The compounds (**3a**–**3d**) were obtained in 85–91 % yields as colorless (or light yellow) oils (**3a** (91%, 487 mg); **3b** (90%, 520 mg); **3c** (85%, 527 mg); **3d** (90%, 541 mg)).

3a: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.64–7.59 (m, 1H), 7.47–7.41 (m, 1H), 7.32–7.23 (m, 2H), 4.84 (s, 1H), 4.62 (s, 2H), 3.54 (t, *J* = 5.0 Hz, 2H), 3.34–3.30 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.04, 155.59, 128.70, 128.03, 124.80, 123.67, 119.51, 116.43, 111.16, 79.39, 69.19, 63.64, 40.55, 28.51; HRMS: calculated exact mass = 370.0653 for $C_{16}H_{21}BrNO_4$ $[M+H]^+$, found 370.0661.

3b: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.65–7.60 (m, 1H), 7.46–7.41 (m, 1H), 7.31–7.22 (m, 2H), 4.59 (s, 2H), 4.55 (s, 1H), 3.49 (t, *J* = 6.2 Hz, 2H), 3.14–3.08 (m, 2H), 1.66–1.58 (m, 2H), 1.58–1.50 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.11, 155.59, 128.45, 128.17, 124.71, 123.58, 119.69, 116.77, 111.08, 79.19, 69.89, 63.51, 40.48, 28.57, 27.09, 27.00; HRMS: calculated exact mass = 398.0967 for $C_{18}H_{25}BrNO_4$ $[M+H]^+$, found 398.0966.

3c: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.66–7.61 (m, 1H), 7.46–7.41 (m, 1H), 7.31–7.23 (m, 2H), 4.59 (s, 2H), 4.47 (s, 1H), 3.46 (t, *J* = 6.5 Hz, 2H), 3.11–3.03 (m, 2H), 1.59 (quint., *J* = 6.6 Hz, 2H), 1.49–1.39 (m, 2H), 1.44 (s, 9H), 1.39–1.24 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.12, 155.59, 128.38, 128.23, 124.67, 123.53, 119.77, 116.89, 111.05, 79.17, 70.22, 63.48, 40.66, 30.15, 29.70, 28.58, 26.71, 25.99; HRMS: calculated exact mass = 426.1280 for $C_{20}H_{29}BrNO_4$ $[M+H]^+$, found 426.1280.

3d: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.70–7.63 (m, 1H), 7.47–7.39 (m, 1H), 7.32–7.22 (m, 2H), 4.95 (s, 1H), 4.68 (s, 2H), 3.61 (s, 4H), 3.52 (t, *J* = 5.1 Hz, 2H), 3.33–3.28 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 156.10, 155.59, 128.62, 128.10, 124.77, 123.60, 119.77, 116.49, 111.08, 79.35, 70.42, 69.15, 63.84, 40.54, 28.56; HRMS: calculated exact mass = 414.0916 for $C_{18}H_{25}BrNO_5$ $[M+H]^+$, found 414.0904.

*Compounds **4a**–**4d***

Compound **3a** (77 mg, 208 μmol), compound **3b** (83 mg, 208 μmol), compound **3c** (89 mg, 208 μmol), or compound **3d** (86 mg, 208 μmol) was dissolved in dioxane (4 mL). 5-Formyl-2-

furanylboronic acid (35 mg, 250 μmol), K_2CO_3 (58 mg, 417 μmol), and PPh_3 Pd G2 (chloro(triphenylphosphine) [2-(2′-amino-1,1′-biphenyl)]palladium(II), 6 mg, 10 μmol) dissolved in water (4 mL) were added to the compound solutions. The mixtures were stirred at 50 °C for 2 h. The reaction mixture was filtered, and the filtrate diluted with diethyl ether, and the organic solution was washed with water 3 times. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (eluent: hexane and ethyl acetate, 4:1). The compounds (**4a**–**4d**) were obtained in 60–92 % yields as brown powders (**4a** (92%, 74 mg); **4b** (83%, 71 mg); **4c** (60%, 55 mg); **3d** (89%, 80 mg)).

4a: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.73 (s, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.45–7.30 (m, 3H), 7.04 (d, *J* = 3.7 Hz, 1H), 5.07 (s, 2H), 4.92 (s, 1H), 3.67 (t, *J* = 5.1 Hz, 2H), 3.41–3.34 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.21, 156.08, 154.91, 152.78, 151.14, 143.24, 128.73, 126.47, 123.84, 122.65, 121.16, 116.93, 111.57, 111.24, 79.35, 69.60, 62.92, 40.63, 28.51; HRMS: calculated exact mass = 386.1604 for $C_{21}H_{24}NO_6$ $[M+H]^+$, found 386.1609.

4b: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.71 (s, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.41–7.27 (m, 3H), 7.01 (d, *J* = 3.8 Hz, 1H), 5.01 (s, 2H), 4.60 (s, 1H), 3.60 (t, *J* = 6.2 Hz, 2H), 3.17–3.04 (m, 2H), 1.72–1.61 (m, 2H), 1.61–1.50 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.21, 156.14, 154.92, 152.73, 151.27, 143.00, 128.84, 126.39, 123.73, 122.71, 121.36, 117.44, 111.49, 111.18, 79.15, 70.34, 62.80, 40.49, 28.56, 27.14, 26.99; HRMS: calculated exact mass = 414.1917 for $C_{23}H_{28}NO_6$ $[M+H]^+$, found 414.1920.

4c: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.73 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.43–7.29 (m, 3H), 7.03 (d, *J* = 3.7 Hz, 1H), 5.03 (s, 2H), 4.52 (s, 1H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.14–3.03 (m, 2H), 1.65 (quint., *J* = 6.6 Hz, 2H), 1.50–1.42 (m, 2H), 1.45 (s, 9H), 1.42–1.28 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.23, 156.11, 154.91, 152.71, 151.29, 142.94, 128.88, 126.36, 123.68, 122.63, 121.44, 117.61, 111.47, 111.18, 79.14, 70.68, 62.82, 40.66, 30.13, 29.76, 28.57, 26.72, 26.01; HRMS: calculated exact mass = 442.2230 for $C_{25}H_{32}NO_6$ $[M+H]^+$, found 442.2240.

4d: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.71 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.41–7.28 (m, 3H), 7.04 (d, *J* = 3.7 Hz, 1H), 5.10 (s, 2H), 4.97 (s, 1H), 3.77–3.71 (m, 2H), 3.68–3.62 (m, 2H), 3.53 (t, *J* = 5.0 Hz, 2H), 3.34–3.27 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 177.17, 156.12, 154.93, 152.74, 151.20, 143.11, 128.79, 126.44, 123.76, 122.73, 121.48, 117.19, 111.49, 111.26, 79.33, 70.41, 69.65, 63.18, 40.57, 28.55; HRMS: calculated exact mass = 430.1866 for $C_{23}H_{28}NO_7$ $[M+H]^+$, found 430.1862.

*Compounds **6a**–**6d***

Compound **4a** (3.2 mg, 8 μmol), compound **4b** (3.4 mg, 8 μmol), compound **4c** (3.6 mg, 8 μmol), or compound **4d** (3.5 mg, 8 μmol) was dissolved in MeCN (0.5 mL). Trifluoracetic acid (0.5 mL)

was added to the compound solutions. After completion of Boc deprotection confirmed by HPLC, the solvent was completely dried by nitrogen blowing for 10 min. The deprotected amine compounds (**5a**–**5d**) were dissolved in EtOH (1 mL) and treated with the preactivated 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) solution, which was prepared with NOTA (10.0 mg, 33 μmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 9.5 mg, 49 μmol), 1-hydroxy-7-azabenzotriazole (HOAt, 5.7 mg, 49 μmol), and *N*,*N*-diisopropylethylamine (DIPEA, 11.5 μL, 66 μmol) in DMSO (1 mL). The mixtures were stirred at 0 ℃ for 1 h and then allowed to warm up to room temperature. After depletion of the boc-deprotected residues confirmed by HPLC, the mixture was stopped by adding several drops of TFA, and then purified with HPLC. For the compound analysis in HPLC, a flow rate of 1.0 mL/min and a linear gradient of 10–80% solvent B over 20 min, then a linear gradient of 80–100% solvent B over 5 min followed by a 5 min-constant flow of 100% solvent B (solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile) were used with an InfinityLab Poroshell 120 EC-C18 column (4.6 × 150 mm, 4 μm, Agilent, USA). For the compound purification, a flow rate of 4.0 mL/min and a linear gradient of 20–80% solvent B over 20 min, then a linear gradient of 80–100% solvent B over 5 min followed by a 5 min-constant flow of 100% solvent B were used with a custom Poroshell 120 EC-C18 column (9.4 × 150 mm, 4 μm, Agilent, USA). Absorbance was measured at 230 and 260 nm, and fluorescence detection used excitation at 355 nm and emission at 460 nm. The lyophilized compounds (**6a**–**6d**) were obtained in 45–59 % yields from freeze drying of HPLC fraction containing the product (**6a** (53%, 2.5 mg); **6b** (45%, 2.2 mg); **6c** (45%, 2.3 mg); **6d** (59%, 3.0 mg)).

6a: HRMS: calculated exact mass = 571.2404 for $C_{28}H_{35}N_4O_9$ $[M+H]^+$, found 571.2430.

6b: HRMS: calculated exact mass = 599.2717 for $C_{30}H_{39}N_4O_9$ $[M+H]^+$, found 599.2725.

6c: HRMS: calculated exact mass = 627.3030 for $C_{32}H_{43}N_4O_9$ $[M+H]^+$, found 627.3036.

6d: HRMS: calculated exact mass = 615.2666 for $C_{30}H_{39}N_4O_{10}$ $[M+H]^+$, found 615.2678.

*Compounds **7a**–**7d***

Compound **6a** (1.7 mg, 3 μmol), compound **6b** (1.8 mg, 3 μmol), compound **6c** (1.9 mg, 3 μmol), or compound **6d** (1.8 mg, 3 μmol) was dissolved in DMSO (0.5 mL). The corresponding boc-deprotected compounds (**5a**–**5d**, 3.6 μmol), EDC (1.2 mg, 6 μmol), HOAt (0.7 mg, 6 μmol), and DIPEA (2.1 μL, 12 μmol) dissolved in DMSO (0.5 mL) were added to each compound solution. The mixtures were stirred for 1 h at room temperature, and the reaction progress was monitored by HPLC analysis. If the starting materials (**6a**–**6d**) were still observed in HPLC, additional 1 equiv. of coupling reagents (EDC, HOAt, and DIPEA) were added. After depletion of the starting materials (**6a**–**6d**) confirmed by HPLC, the mixture was stopped by adding several drops of TFA, and then purified with HPLC. HPLC conditions for analysis and purification of the compounds were the same as used for **6a**–**6d**. The final lyophilized compounds were obtained in 56–68 % yields from freeze drying of HPLC fraction containing the product (**7a** (68%, 1.7 mg); **7b** (56%,

1.5 mg); **7c** (63%, 1.8 mg); **7d** (61%, 1.7 mg)).

7a: HRMS: calculated exact mass = 838.3300 for $C_{44}H_{48}N_5O_{12}$ $[M+H]^+$, found 838.3297.

7b: HRMS: calculated exact mass = 894.3926 for $C_{48}H_{56}N_5O_{12}$ $[M+H]^+$, found 894.3914.

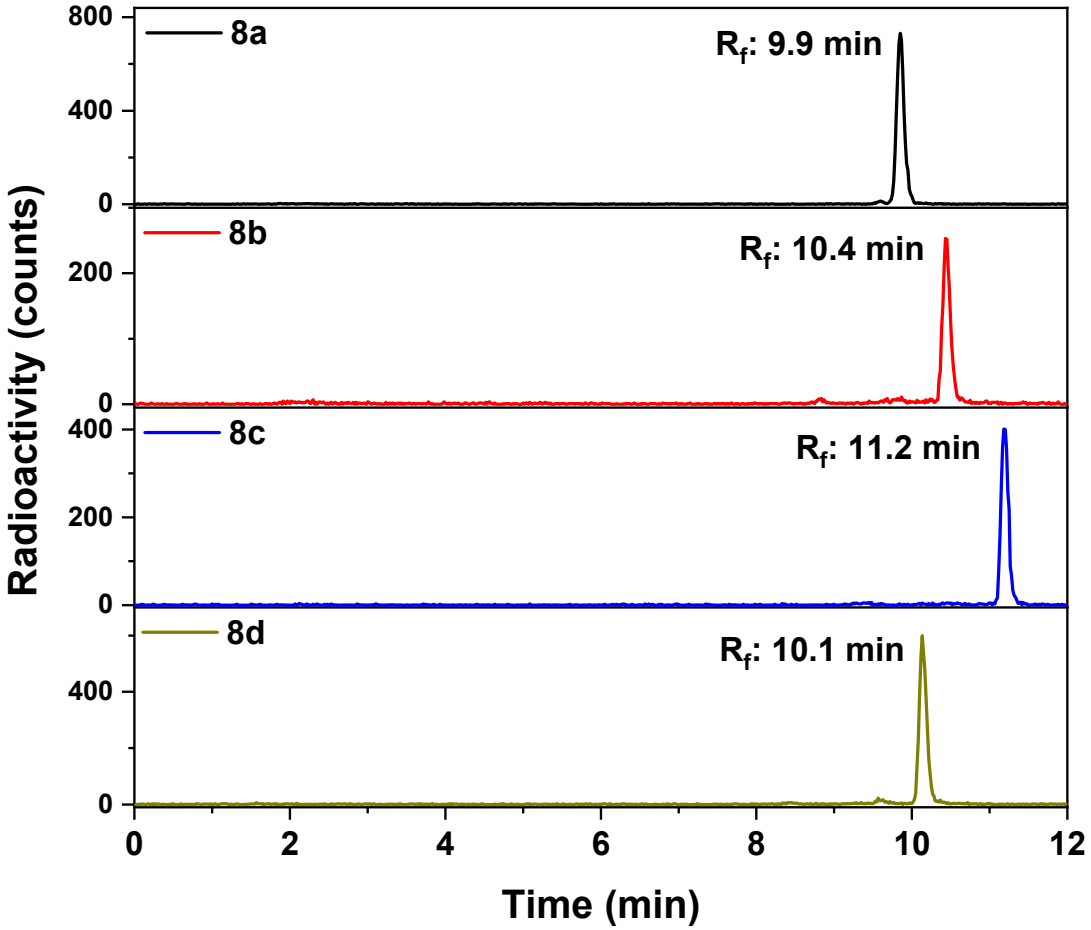
7c: HRMS: calculated exact mass = 950.4552 for $C_{52}H_{64}N_5O_{12}$ $[M+H]^+$, found 950.4523.

7d: HRMS: calculated exact mass = 926.3824 for $C_{48}H_{56}N_5O_{14}$ $[M+H]^+$, found 926.3790.

Cell viability studies

The Neuro-2A (N2A) cells were seeded (2.5×10^4 cells/well) onto 96-well plates containing DMEM with 10% FBS and incubated for 24 h. The media was replaced with serum-free medium containing N2 supplement. After 1 h, the Cu complexes **8a′**–**8d′** (2–20 μM) were added into the different wells, followed by incubation at 37 °C. The final volume in each well was 100 μL with <1% DMSO. After 24 h, each well was treated with 10 μL of the Cell Counting Kit-8 (CCK-8) reagent and the cells were incubated for 1 h. Absorbance was measured at 450 using a SpectraMax M2e plate reader (Molecular Devices, USA).

(a)


8a
R_f: 9.9 min
8b
R_f: 10.4 min
8c
R_f: 11.2 min
8d
R_f: 10.1 min
Radioactivity (counts)
Time (min)
800
400
0
200
0
2
4
6
8
10
12

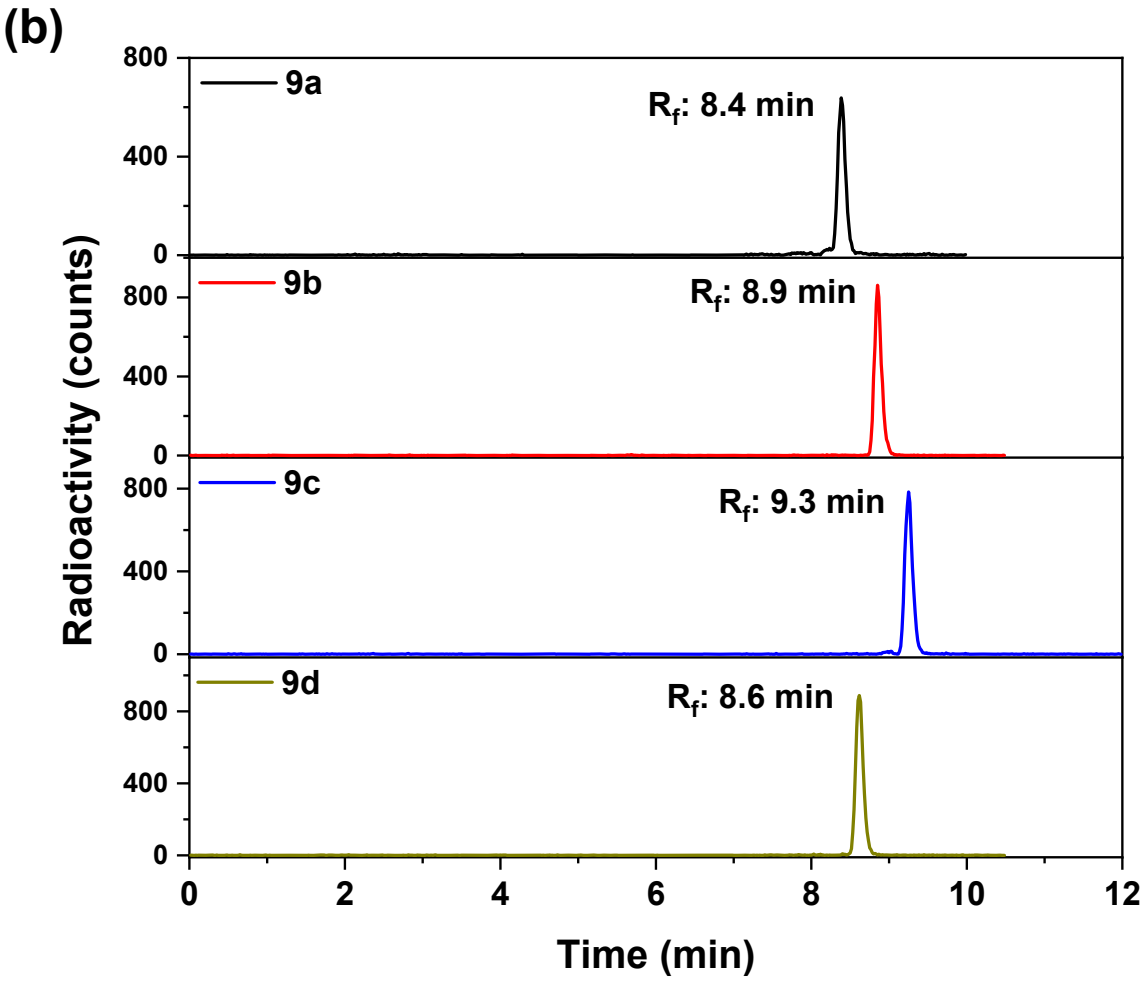


Figure S1. Radio-HPLC profiles of ^{64}Cu-labeled complexes: (a) **8a**–**8d** and (a) **9a**–**9d**, showing quantitative radiolabeling. If present, free ^{64}Cu would appear at 2.1 min.

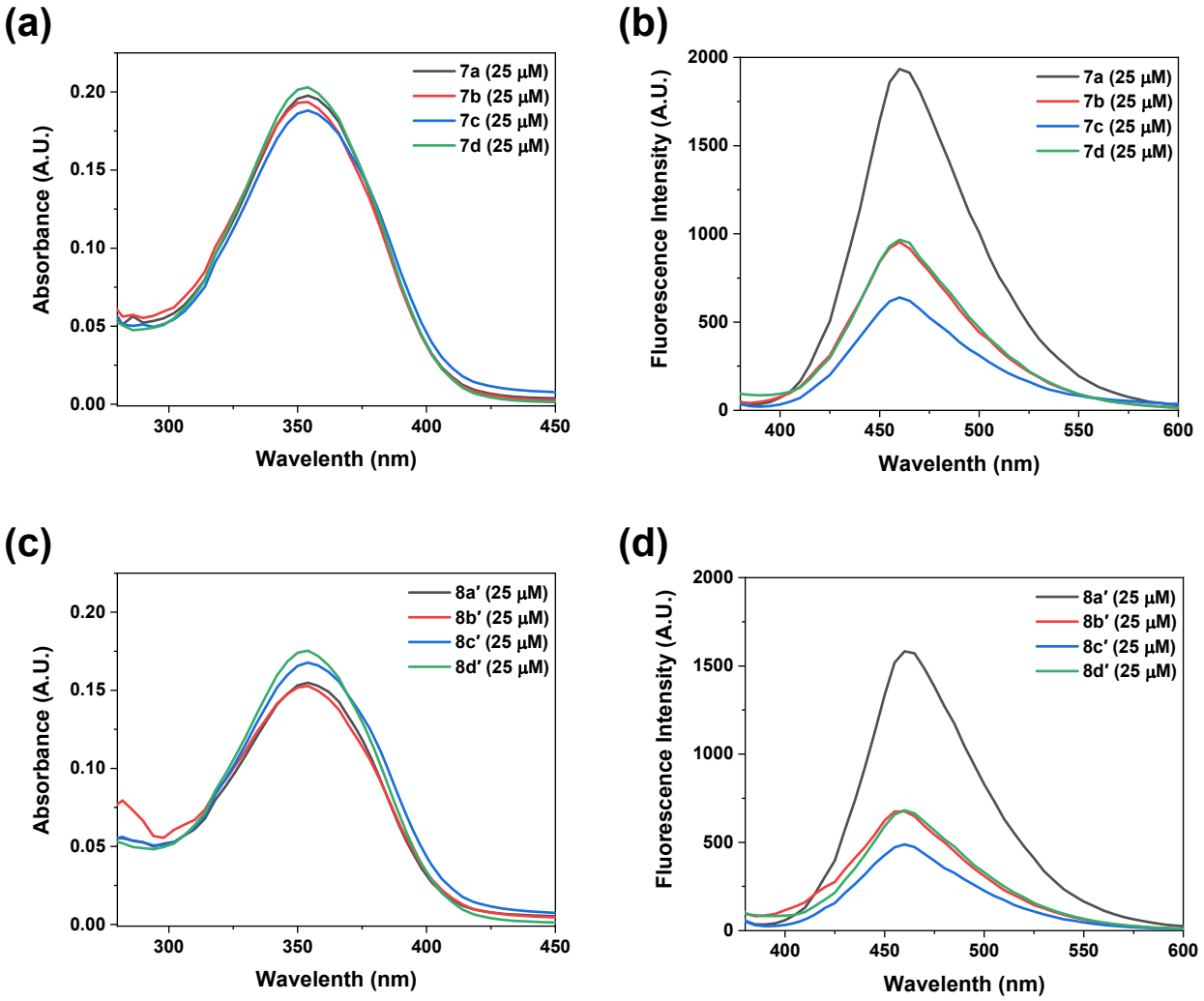
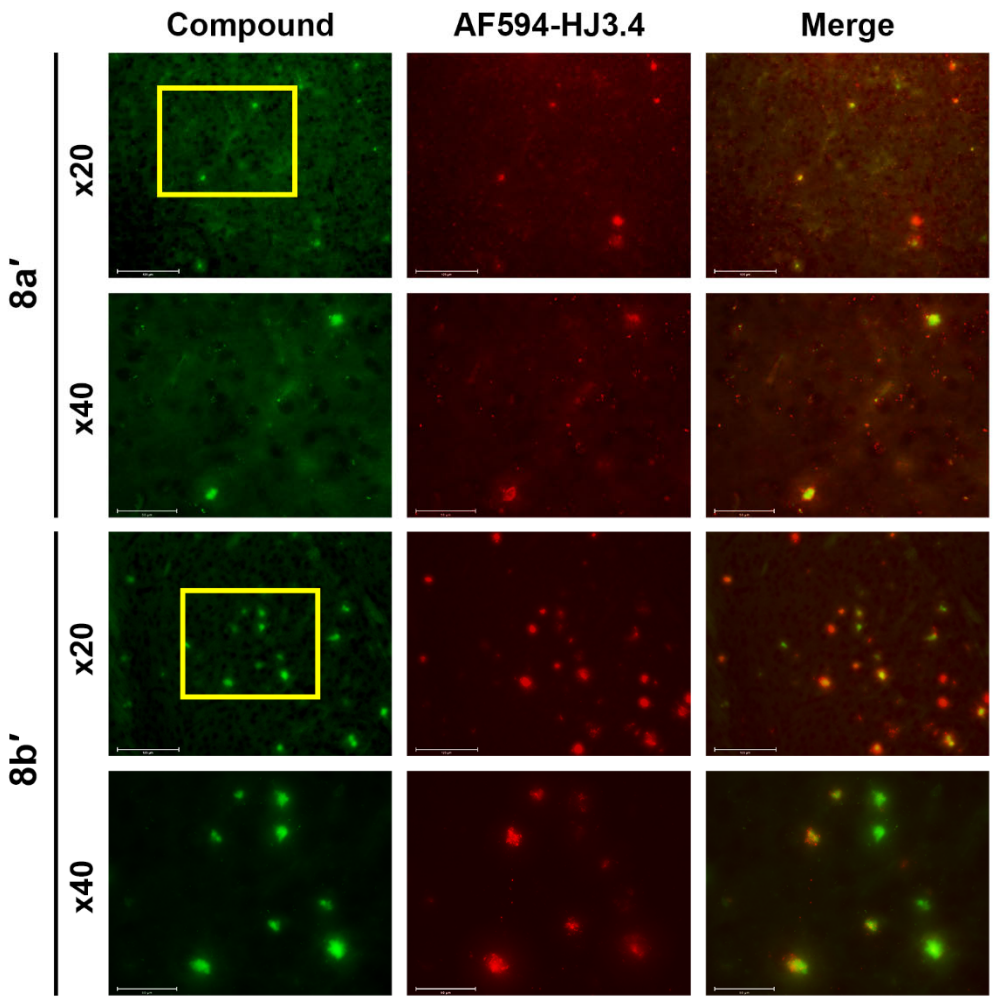


Figure S2. Optical properties of compounds (**7a**–**7d**) and nonradioactive Cu-complexes (**8a′**–**8d′**). (a) UV-VIS spectra of **7a**–**7d**. (b) Fluorescence spectra of **7a**–**7d**. (c) UV-VIS spectra of **8a′**–**8d′**. (d) Fluorescence spectra of **8a′**–**8d′**. Fluorescence emission spectra were obtained under excitation at 355 nm.


Compound
AF594-HJ3.4
Merge
8a′
8b′
x20
x40
x20
x40

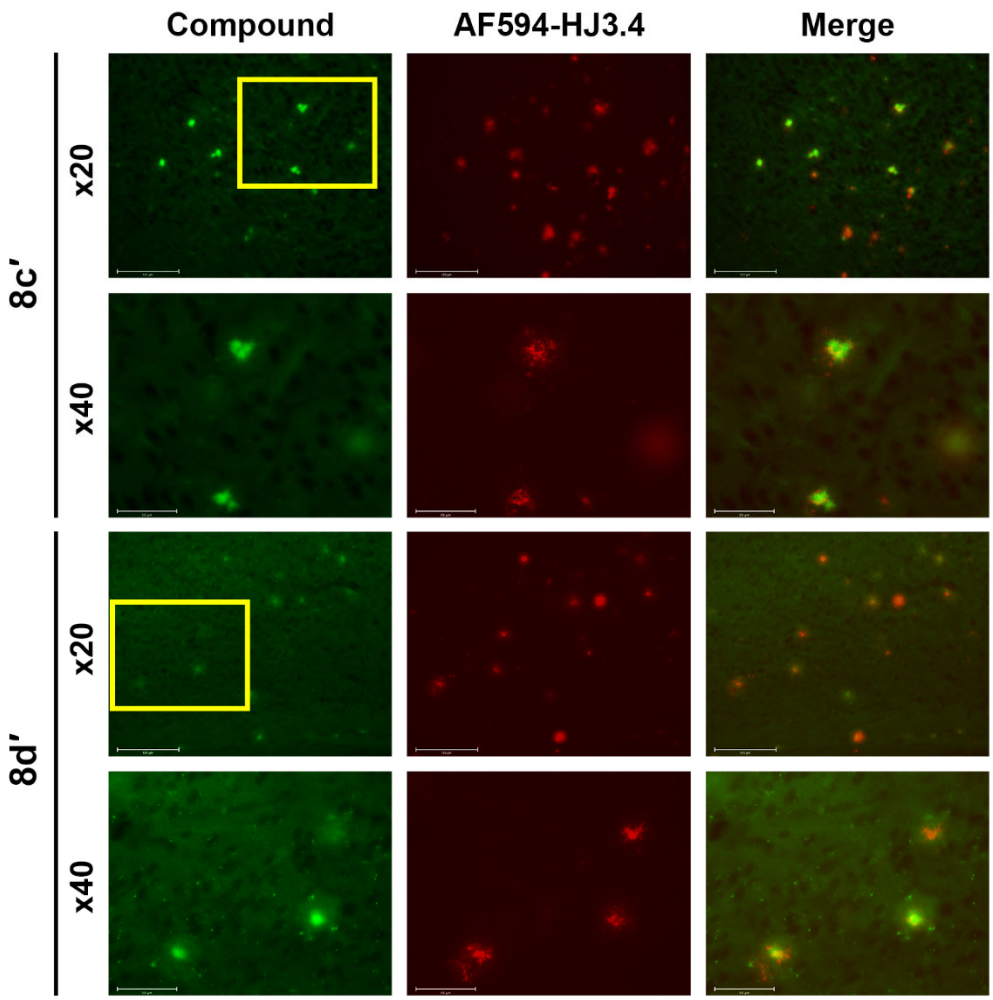


Figure S3. Fluorescence microscopy images of 5xFAD mice brain sections incubated with nonradioactive Cu-complexes (**8a′**–**8d′**). The fluorescence signals from Cu-complexes and AF594-HJ3.4 antibody were monitored at 510/42 nm and 624/40 nm under excitation 470/22 nm and 585/29 nm, respectively. The ×40 images are the zoomed-in regions highlighted by a yellow rectangle in the "×20" images. Scale bar: 125 μm for "×20" and 50 μm for "×40".

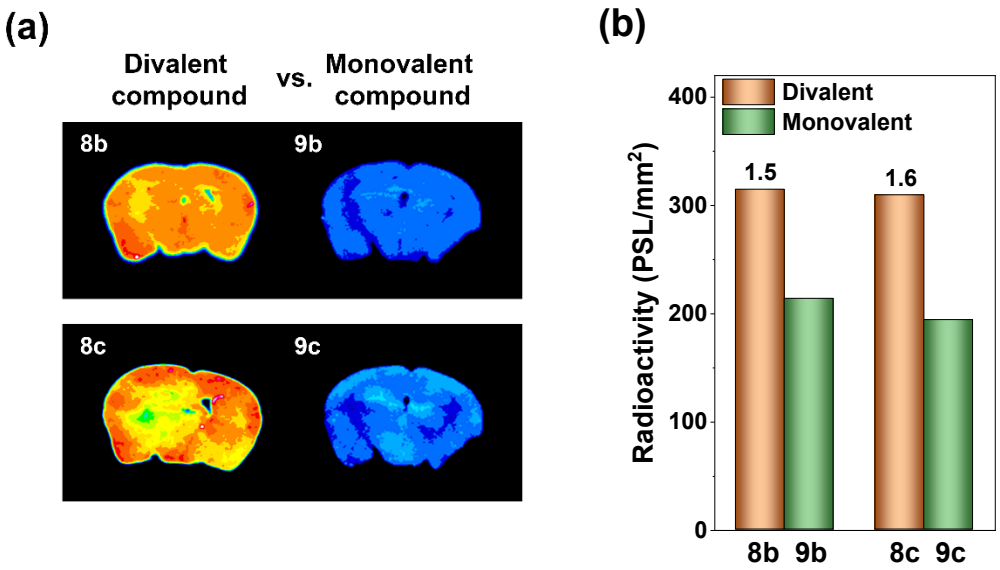


Figure S4. (a) Autoradiography images of the brain sections from 5xFAD mice after treatment of **8b**–**8c** and **9b**–**9c**. (b) Average intensities of the brain sections in the autoradiography images. The numbers in the bar graph are the intensity ratios of divalent to monovalent: 1.5 and 1.6, respectively.

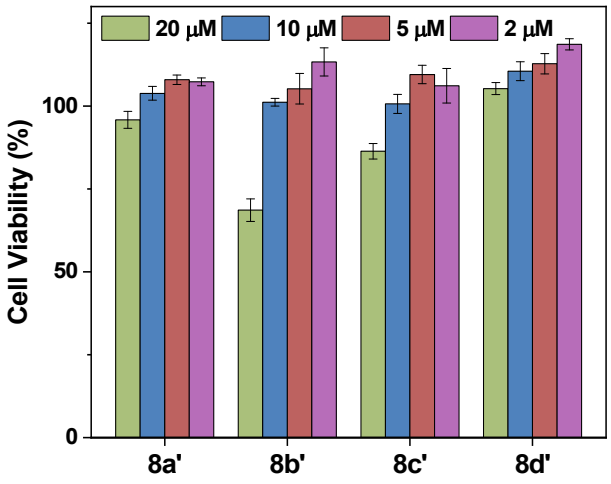


Figure S5. Cell viability of N2A cells (normalized to a 1% DMSO control) after 24 h treatment with **8a′**–**8d′**, as assessed by the CCK-8 assay. Concentration: 2–20 μM.

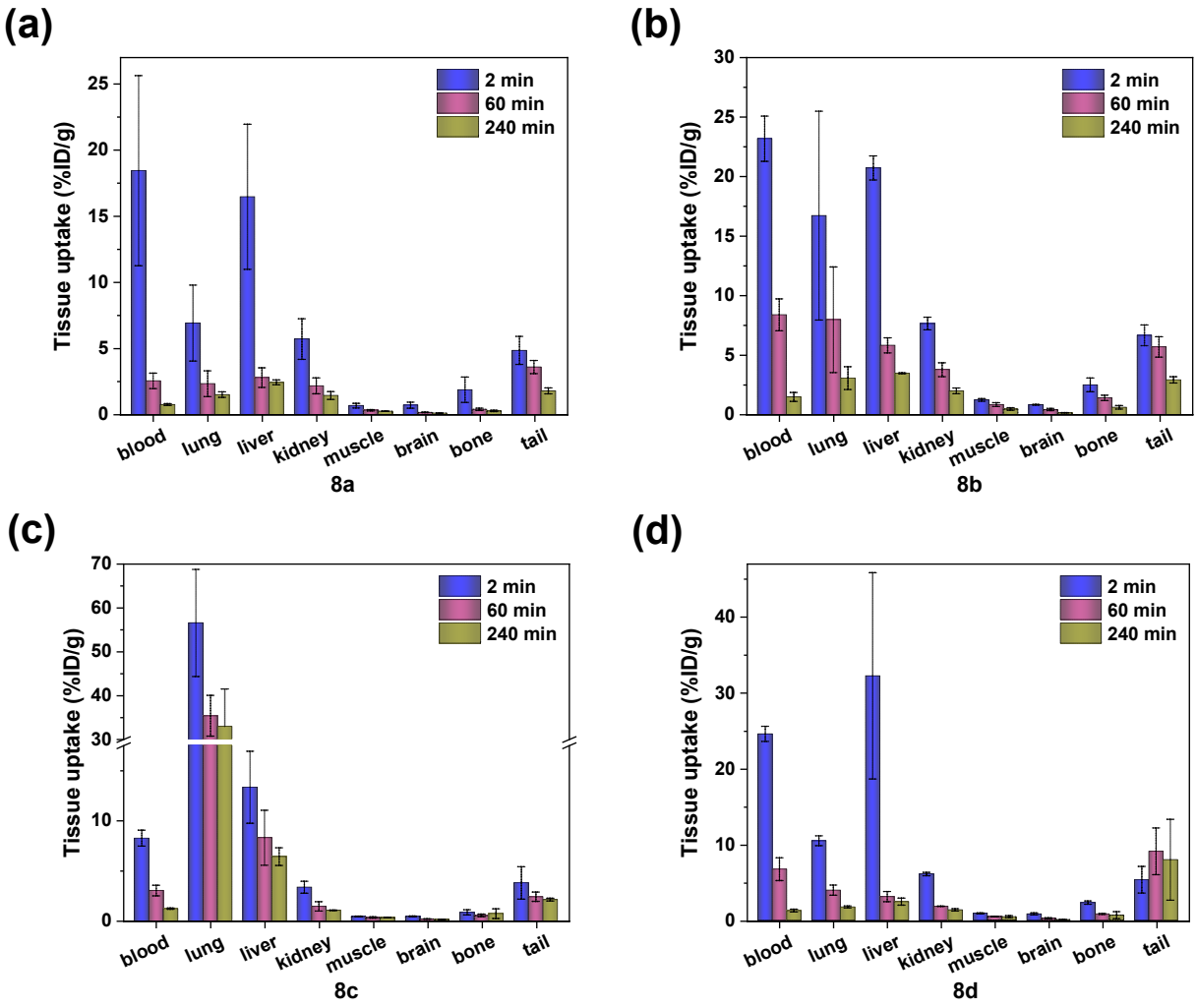


Figure S6. Biodistribution results of **8a**–**8d** in CD-1 mice at 2, 60, and 240 min post-injection.

Table S1. Biodistribution results of **8a**–**8d** in CD-1 mice at 2, 60, and 240 min post-injection.

	8a, 2 min	**8a**, 60 min	**8a**, 240 min	**8b**, 2 min	**8b**, 60 min	**8b**, 240 min
blood	18.43 ± 7.21	2.49 ± 0.58	0.69 ± 0.07	23.17 ± 1.91	8.33 ± 1.35	1.42 ± 0.38
lung	6.88 ± 2.88	2.28 ± 0.98	1.45 ± 0.20	16.69 ± 8.80	7.92 ± 4.44	3.00 ± 0.96
liver	16.45 ± 5.49	2.75 ± 0.74	2.38 ± 0.18	20.70 ± 1.01	5.76 ± 0.63	3.41 ± 0.04
kidney	5.66 ± 1.55	2.11 ± 0.60	1.39 ± 0.30	7.60 ± 0.53	3.72 ± 0.59	1.92 ± 0.24
muscle	0.62 ± 0.17	0.27 ± 0.04	0.19 ± 0.01	1.17 ± 0.11	0.77 ± 0.16	0.40 ± 0.10
brain	**0.65 ± 0.23**	**0.10 ± 0.03**	**0.05 ± 0.00**	**0.76 ± 0.03**	**0.35 ± 0.10**	**0.08 ± 0.00**
bone	1.82 ± 0.96	0.34 ± 0.09	0.22 ± 0.06	2.43 ± 0.58	1.35 ± 0.23	0.55 ± 0.15
tail	4.80 ± 1.06	3.54 ± 0.50	1.73 ± 0.23	6.60 ± 0.88	5.63 ± 0.85	2.85 ± 0.27
	8c, 2 min	**8c**, 60 min	**8c**, 240 min	**8d**, 2 min	**8d**, 60 min	**8d**, 240 min
blood	8.21 ± 0.79	2.97 ± 0.53	1.15 ± 0.07	24.60 ± 0.99	6.74 ± 1.51	1.27 ± 0.18
lung	56.56 ± 12.23	35.33 ± 4.68	32.87 ± 8.56	10.50 ± 0.65	3.97 ± 0.68	1.75 ± 0.13
liver	13.30 ± 3.61	8.26 ± 2.75	6.37 ± 0.89	32.25 ± 13.61	3.12 ± 0.65	2.45 ± 0.47
kidney	3.30 ± 0.60	1.39 ± 0.48	1.00 ± 0.03	6.09 ± 0.23	1.84 ± 0.02	1.37 ± 0.17
muscle	0.37 ± 0.03	0.28 ± 0.07	0.28 ± 0.02	0.91 ± 0.06	0.50 ± 0.01	0.48 ± 0.15
brain	**0.38 ± 0.04**	**0.13 ± 0.02**	**0.08 ± 0.01**	**0.83 ± 0.14**	**0.27 ± 0.05**	**0.09 ± 0.02**
bone	0.79 ± 0.27	0.48 ± 0.13	0.67 ± 0.48	2.34 ± 0.23	0.84 ± 0.08	0.68 ± 0.46
tail	3.73 ± 1.62	2.35 ± 0.47	2.06 ± 0.14	5.35 ± 1.76	9.10 ± 3.09	7.98 ± 5.34

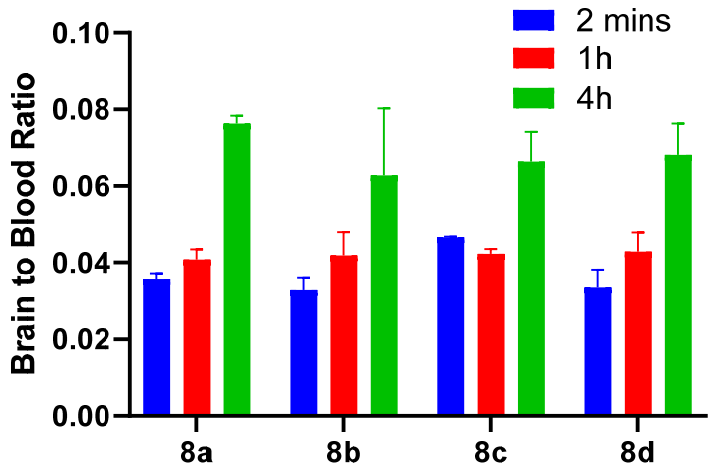


Figure S7. Brain to blood ratios from the biodistribution results of **8a**–**8d** in CD-1 mice at 2, 60, and 240 min post-injection.

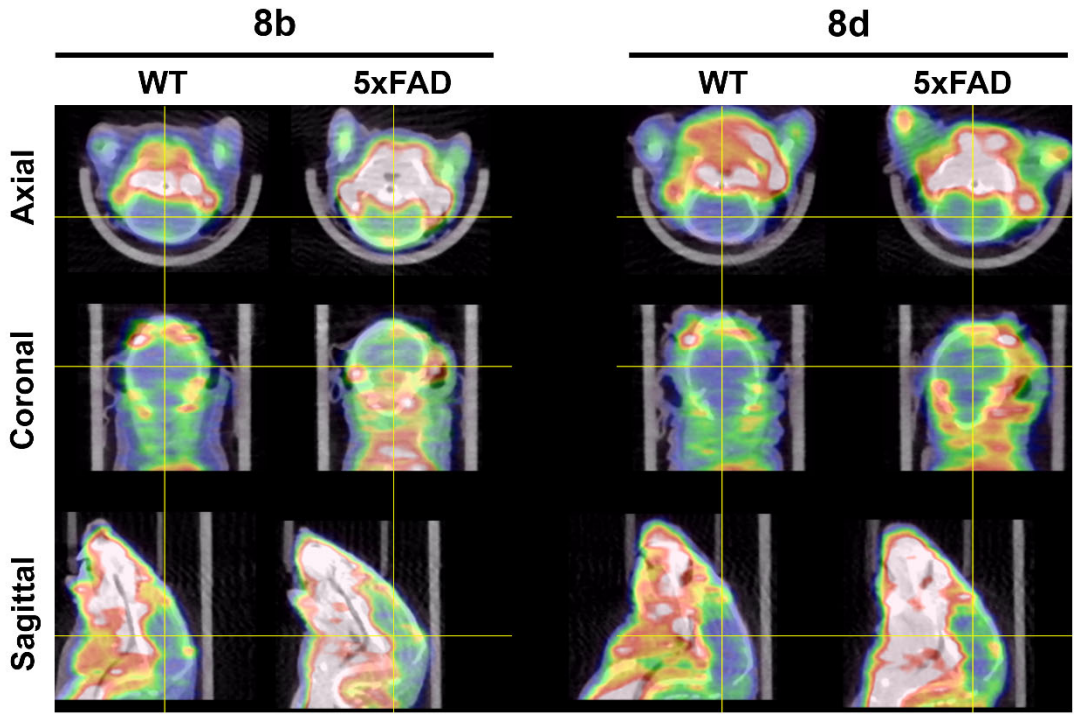


Figure S8. Representative axial, coronal, and sagittal PET/CT images of **8b** and **8d** in WT and 5xFAD mice with dynamic scans summed from 7.5 to 27.5 min post-injection.

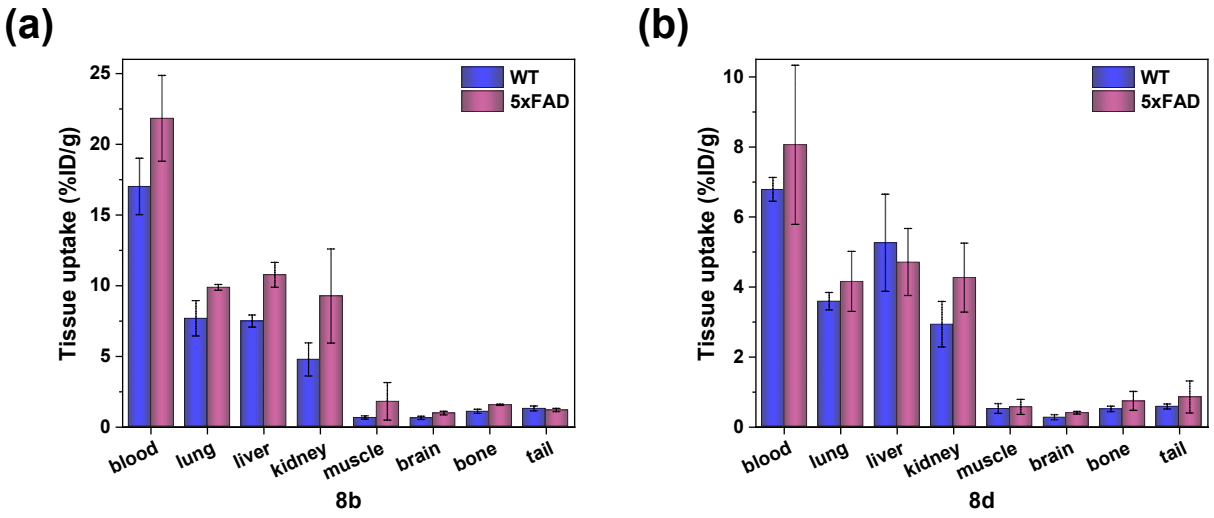


Figure S9. Post-PET biodistribution results of (a) **8b** and (b) **8d** in WT and 5xFAD mice after PET/CT scans.

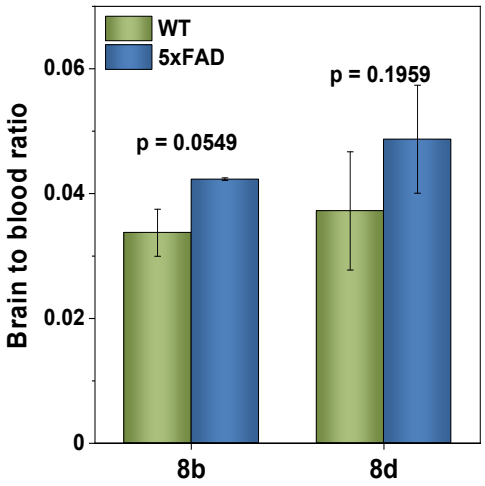


Figure S10. Brain to blood ratios from the post-PET biodistribution results of **8b** and **8d** in WT and 5xFAD mice after PET/CT scans.

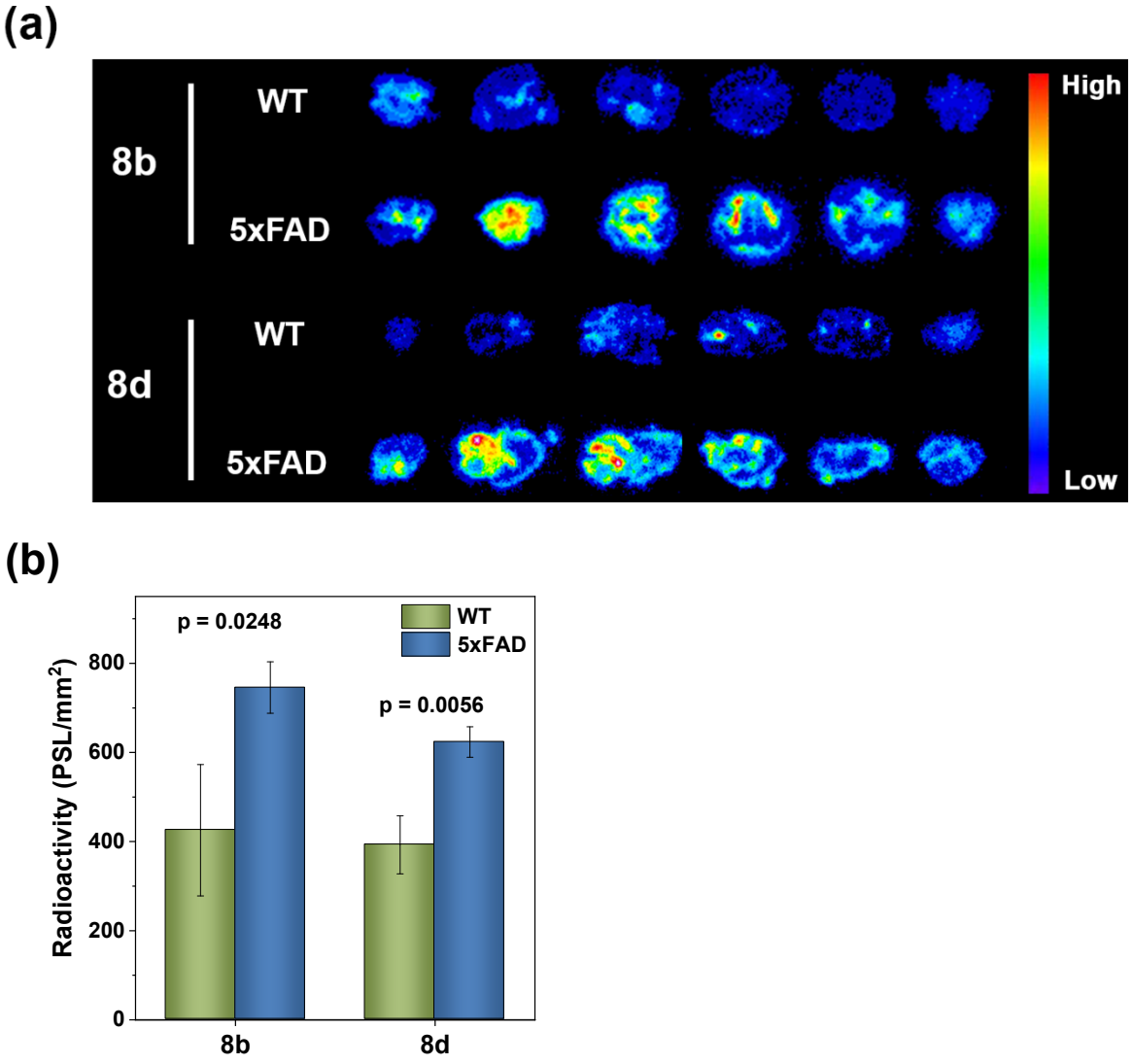
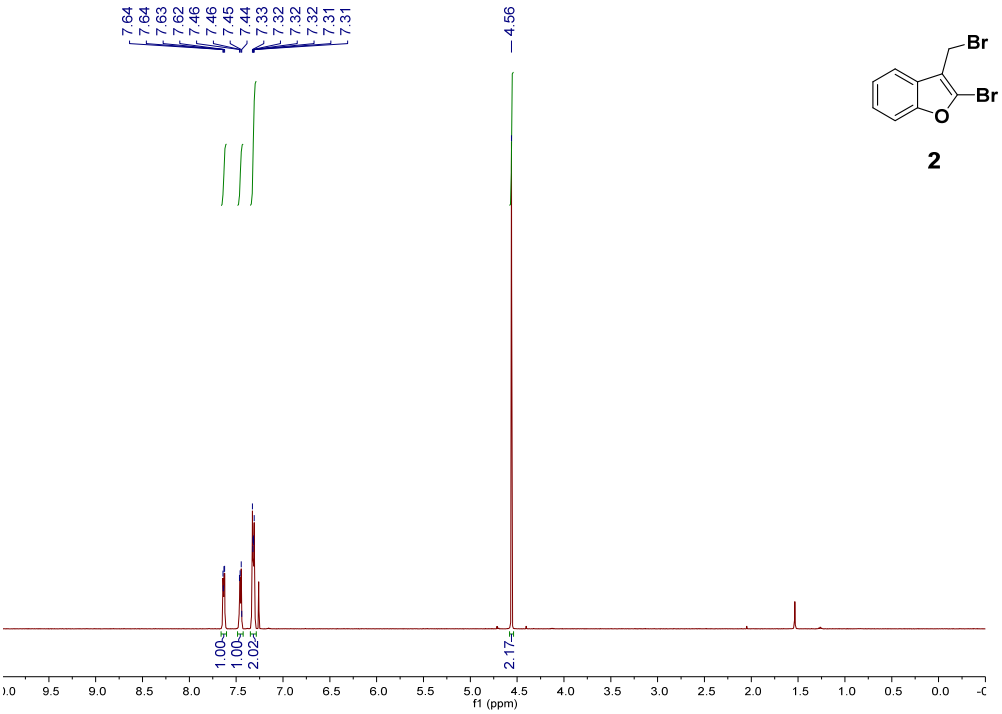
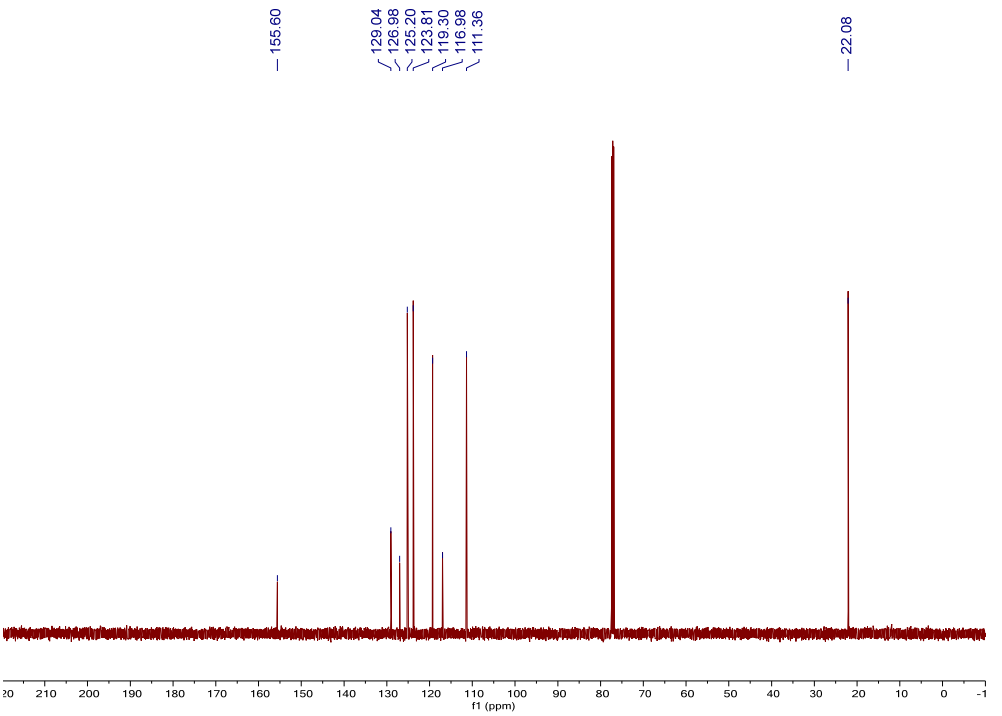


Figure S11. (a) Representative post-PET autoradiography images of **8b** and **8d**. (b) Average intensities of the brain sections treated with **8b** and **8d** in post-autoradiography.

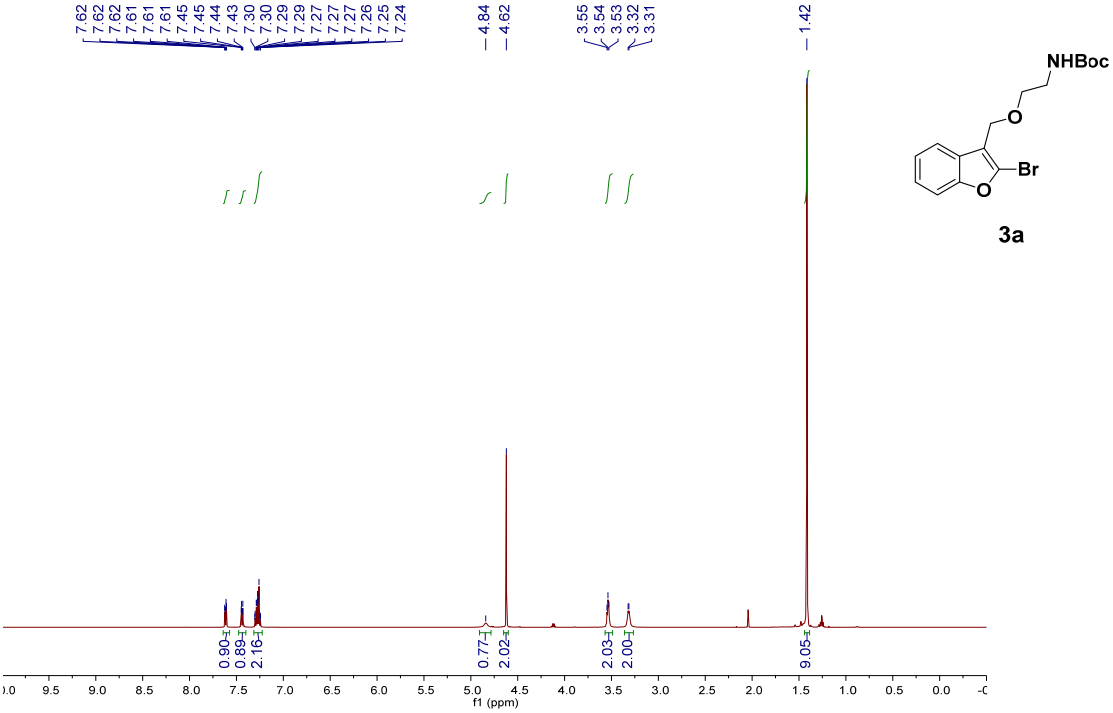
^{1}H-NMR, ^{13}C-NMR, mass spectra, and HPLC profiles

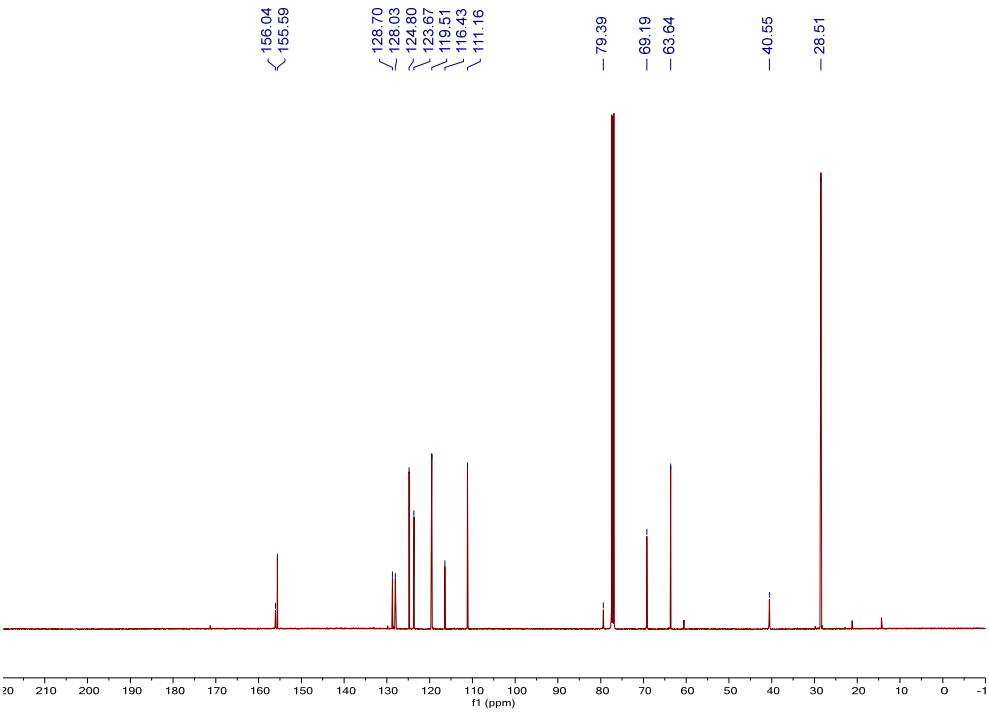
*^{1}H-NMR and ^{13}C-NMR spectra of **2***

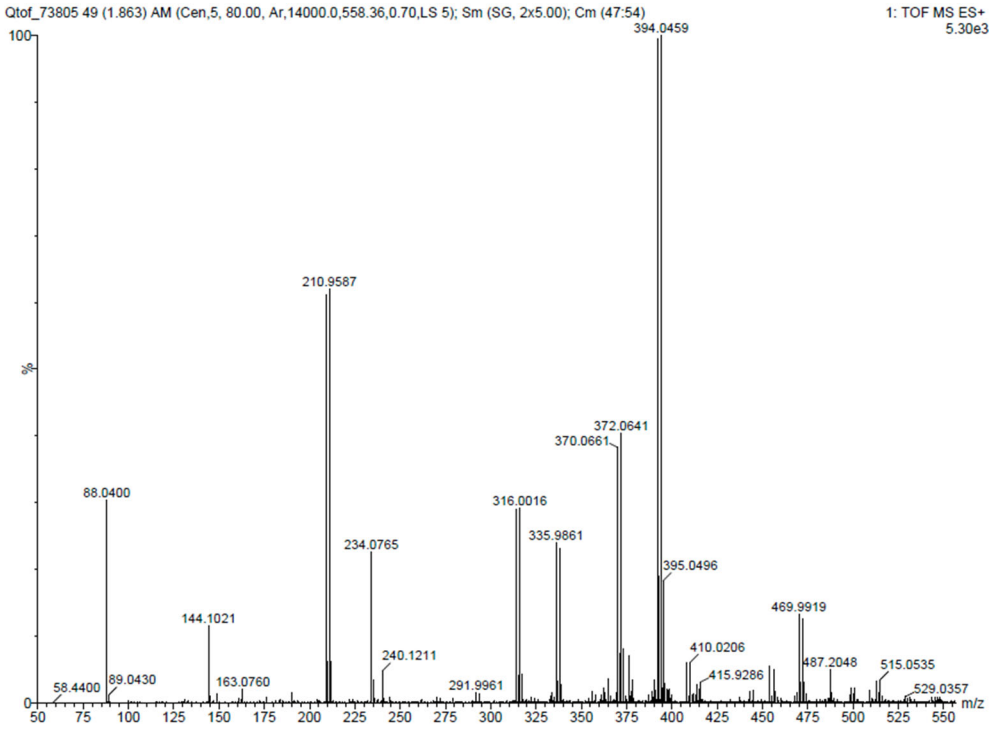




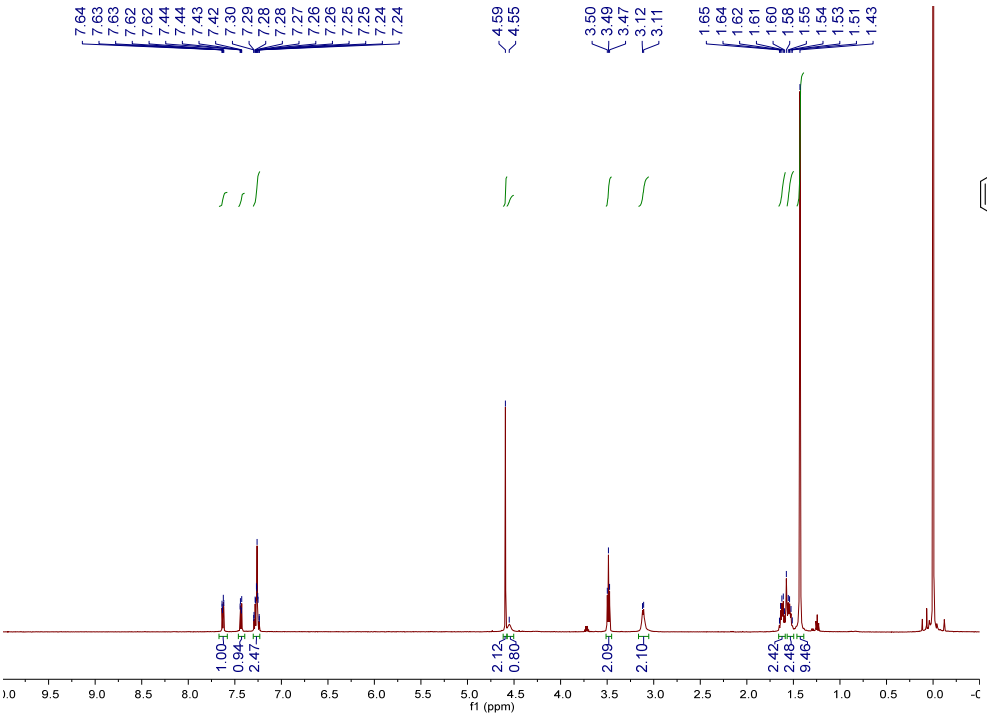
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **3a***

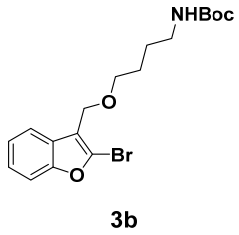




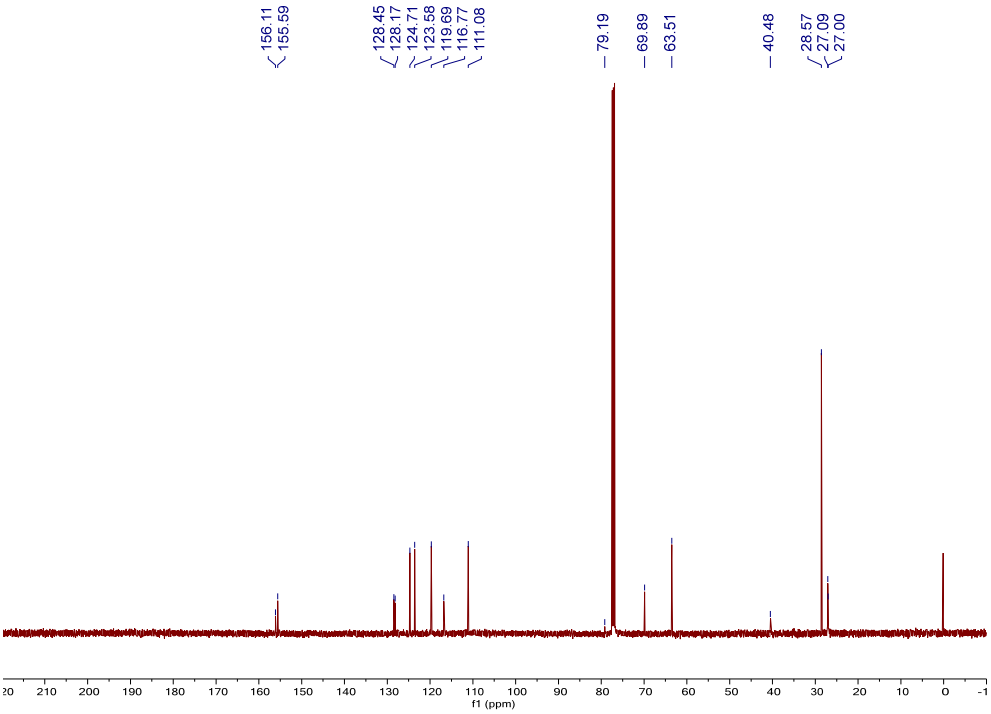

Qtof_73805 49 (1.863) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (47:54)
1: TOF MS ES+
5.30e3
100
%
0
394.0459
210.9587
372.0641
370.0661
88.0400
316.0016
335.9861
234.0765
395.0496
469.9919
144.1021
410.0206
240.1211
487.2048
515.0535
415.9286
58.4400
89.0430
163.0760
291.9961
529.0357
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550
m/z

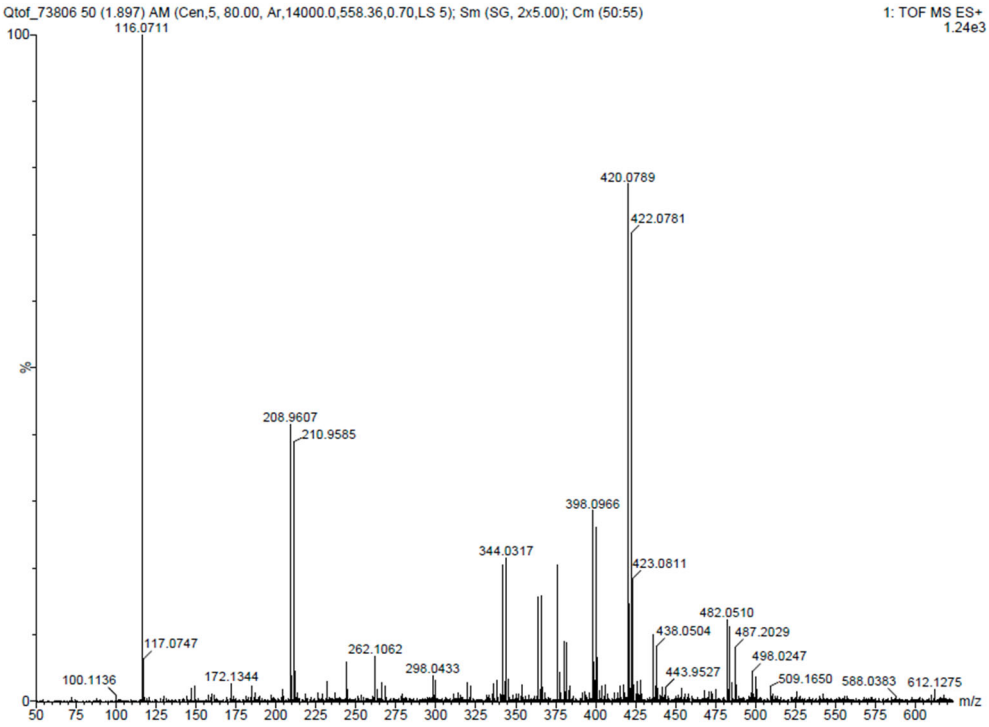
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **3b***



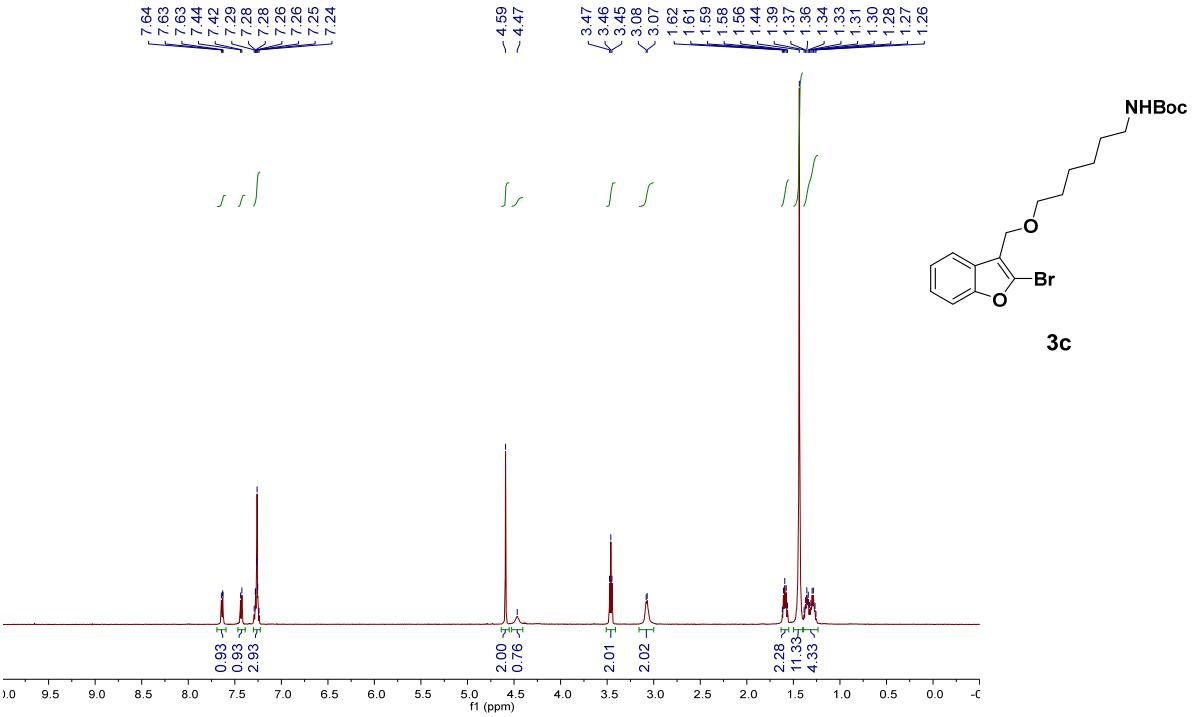


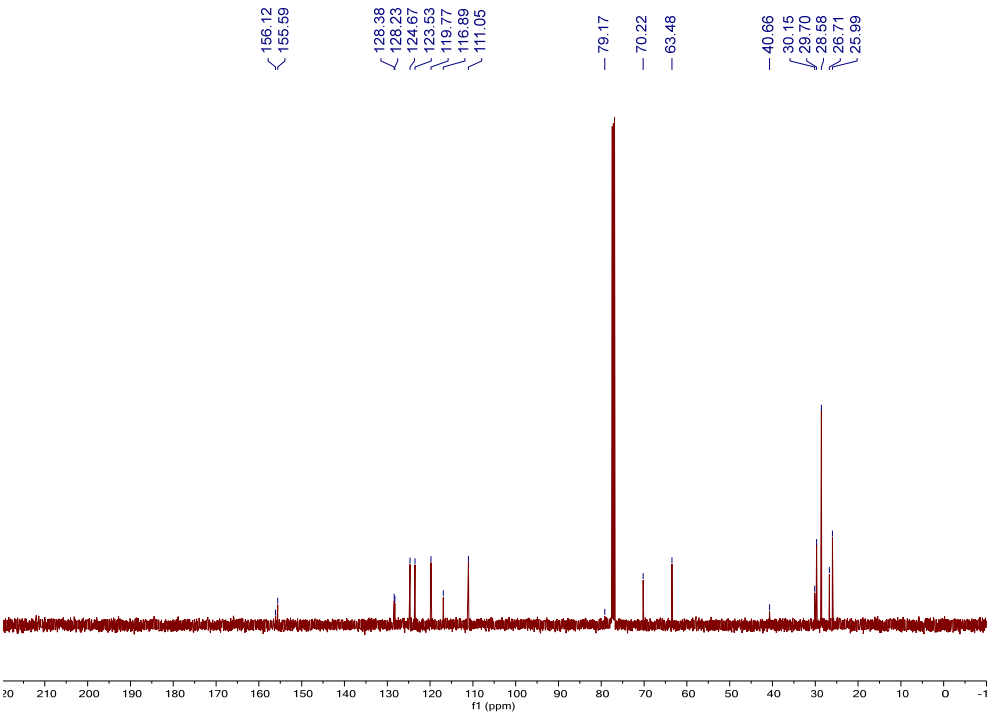
3b

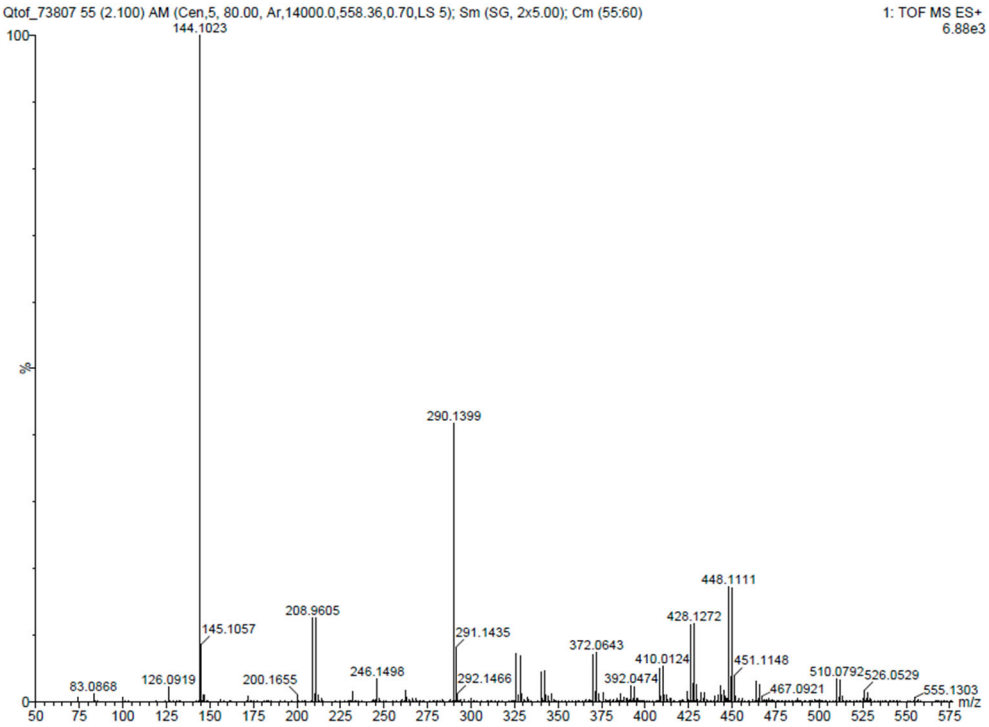



Qtof_73806 50 (1.897) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (50:55)
1: TOF MS ES+
1.24e3
100
%
0
116.0711
420.0789
422.0781
208.9607
210.9585
398.0966
344.0317
423.0811
482.0510
438.0504
487.2029
498.0247
117.0747
262.1062
298.0433
443.9527
509.1650
588.0383
612.1275
100.1136
172.1344
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600
m/z

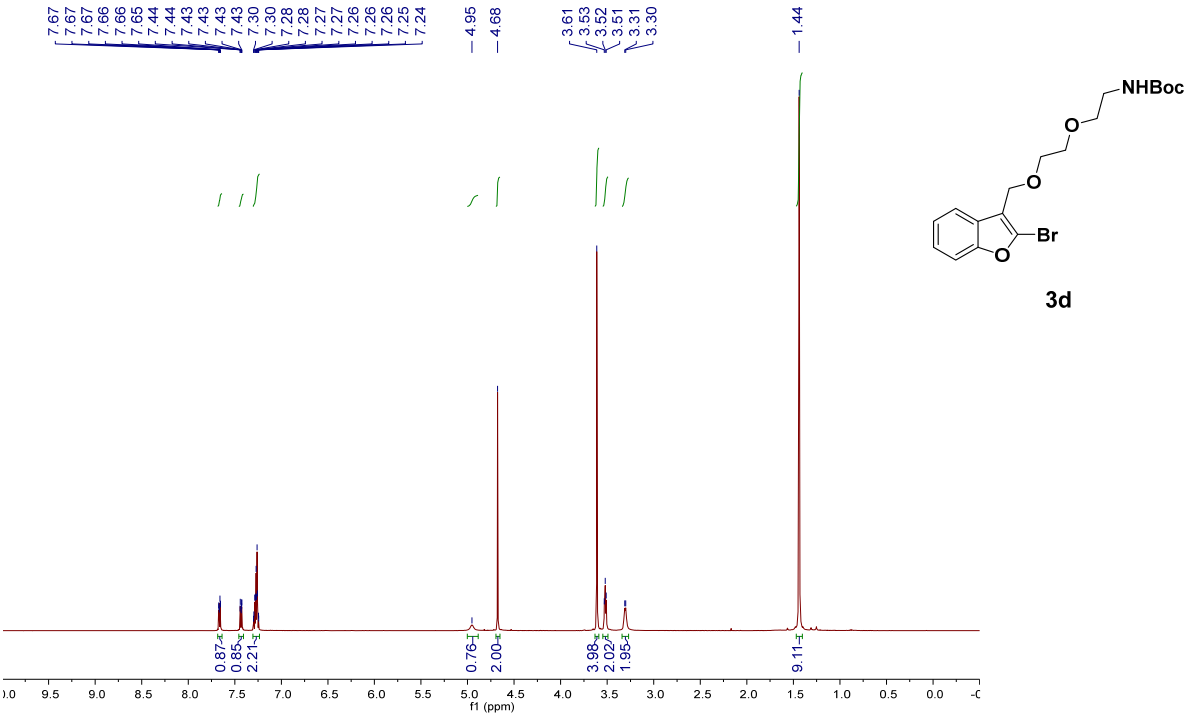
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **3c***

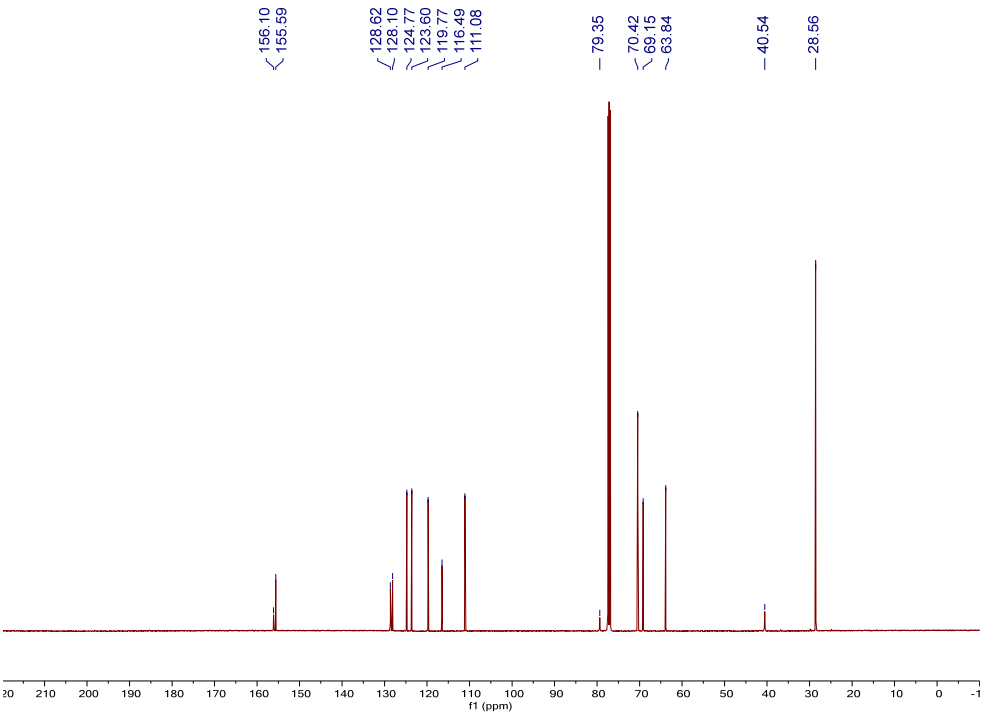


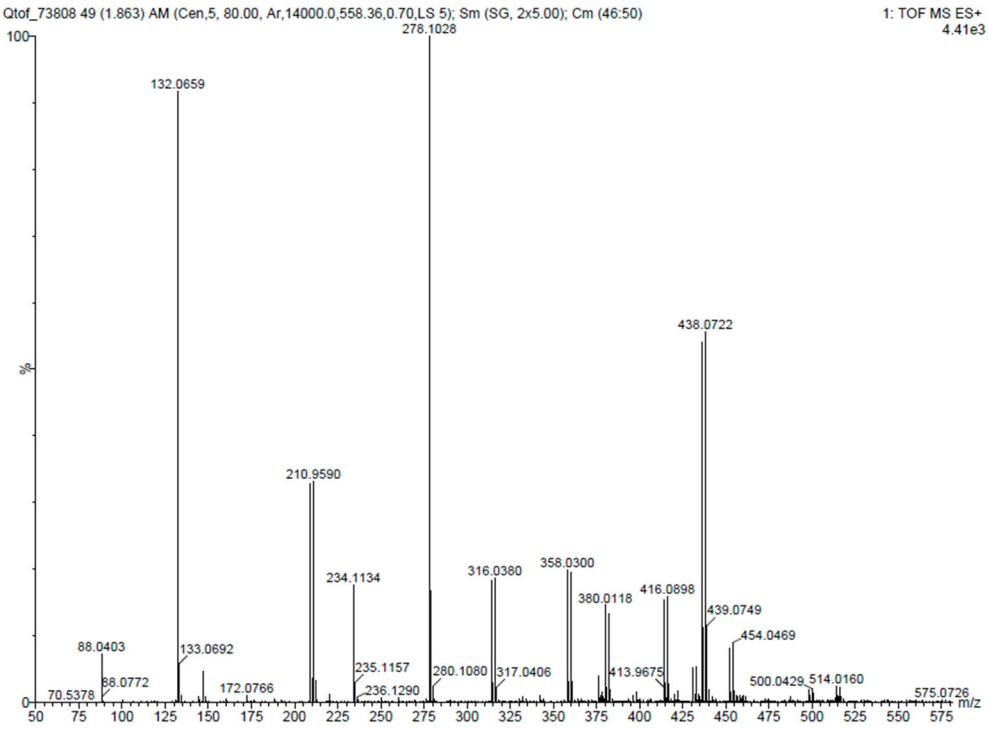



Qtof_73807 55 (2.100) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (55:60)
1: TOF MS ES+
6.88e3
100
%
0
144.1023
290.1399
448.1111
208.9605
428.1272
145.1057
291.1435
372.0643
451.1148
410.0124
510.0792
526.0529
83.0868
126.0919
200.1655
246.1498
292.1466
392.0474
467.0921
555.1303
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575

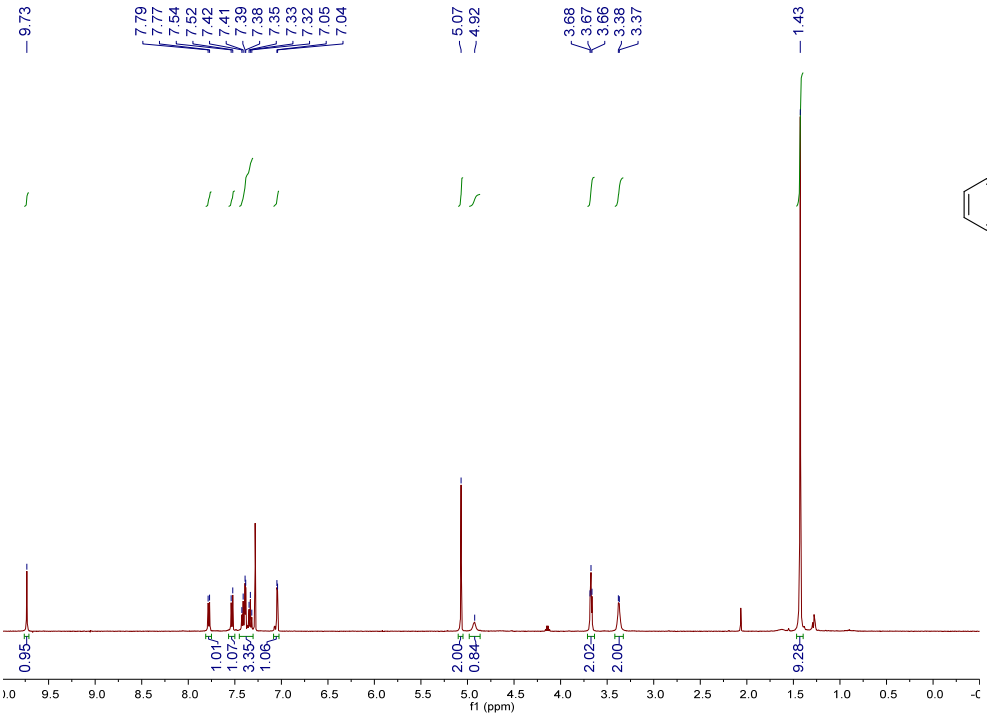
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **3d***

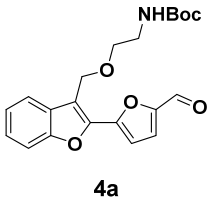




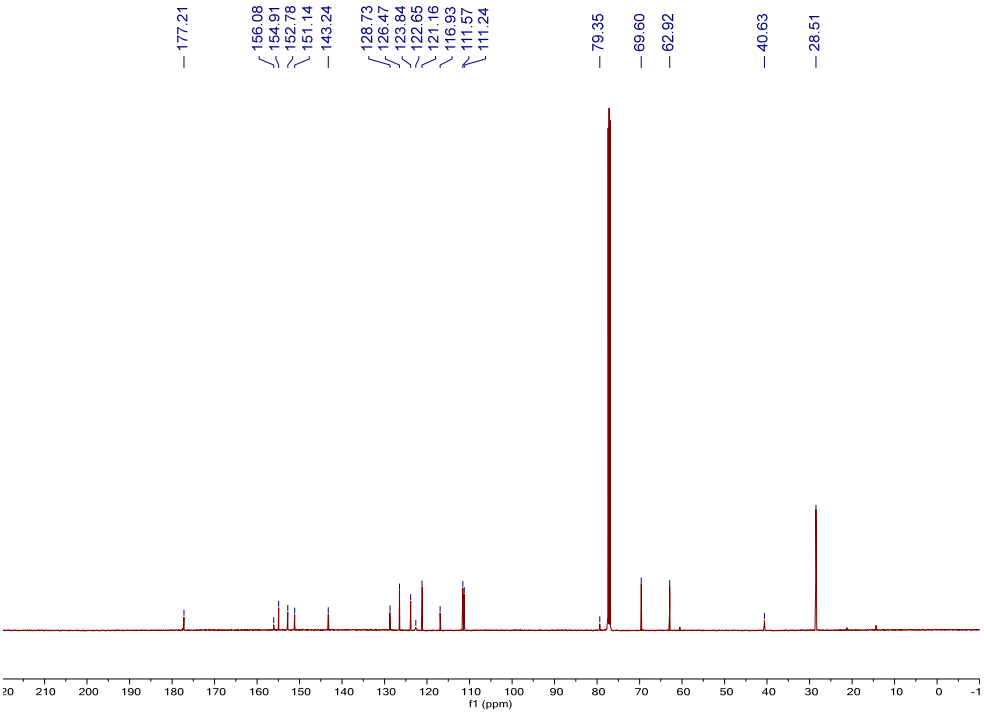

Qtof_73808 49 (1.863) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (46:50)
1: TOF MS ES+
4.41e3
100
%
0
278.1028
132.0659
438.0722
210.9590
234.1134
316.0380
358.0300
380.0118
416.0898
439.0749
454.0469
88.0403
133.0692
88.0772
70.5378
172.0766
235.1157
236.1290
280.1080
317.0406
413.9675
500.0429
514.0160
575.0726
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575

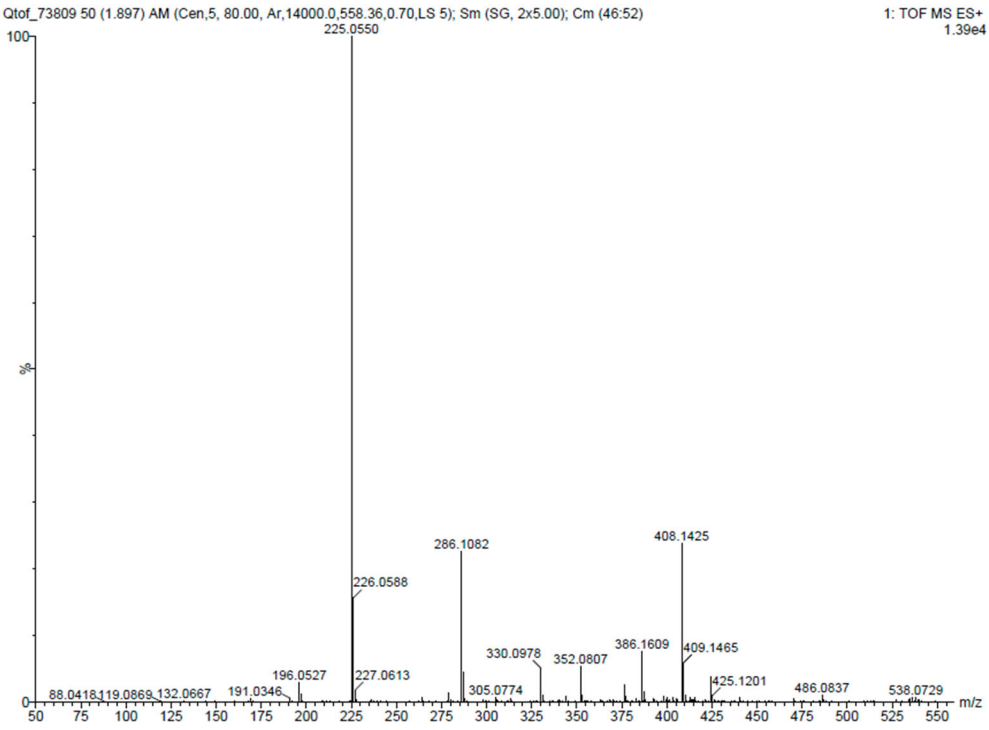
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **4a***



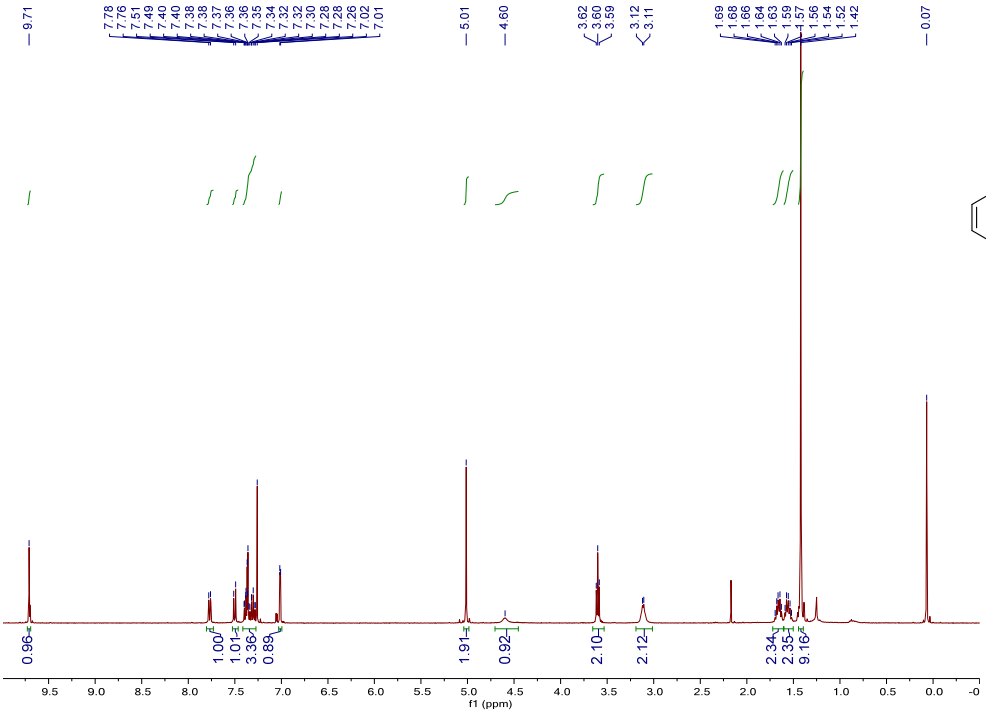


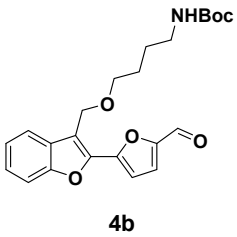
4a



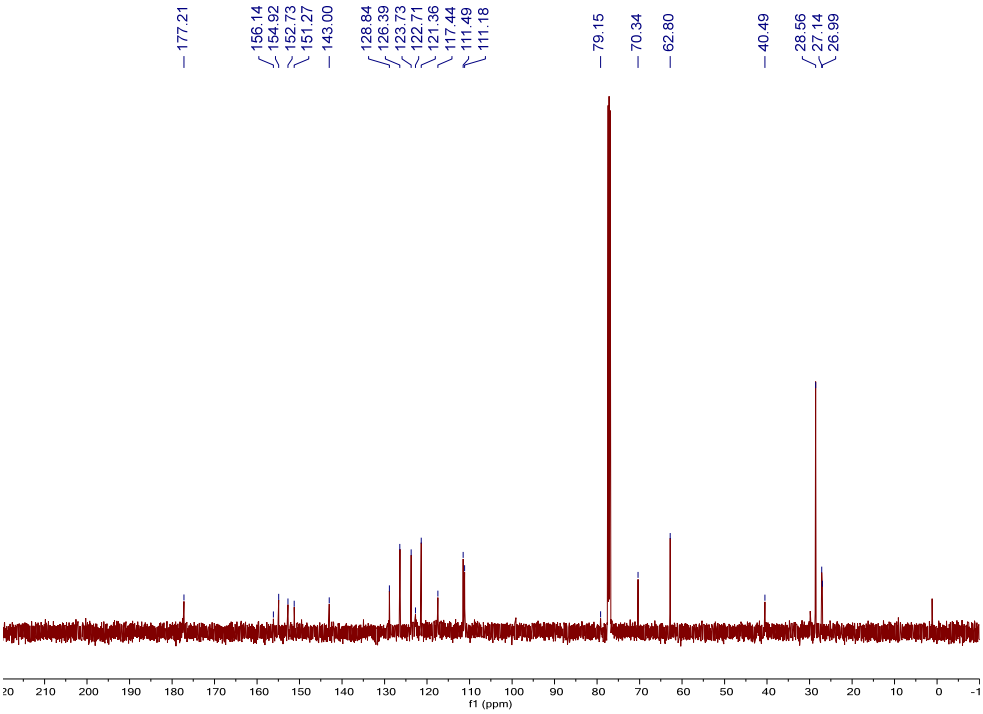

Qtof_73809 50 (1.897) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (46:52)
1: TOF MS ES+
1.39e4
100
%
0
225.0550
286.1082
408.1425
226.0588
330.0978
386.1609
352.0807
409.1465
196.0527
227.0613
425.1201
88.0418
119.0869
132.0667
191.0346
305.0774
486.0837
538.0729
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550

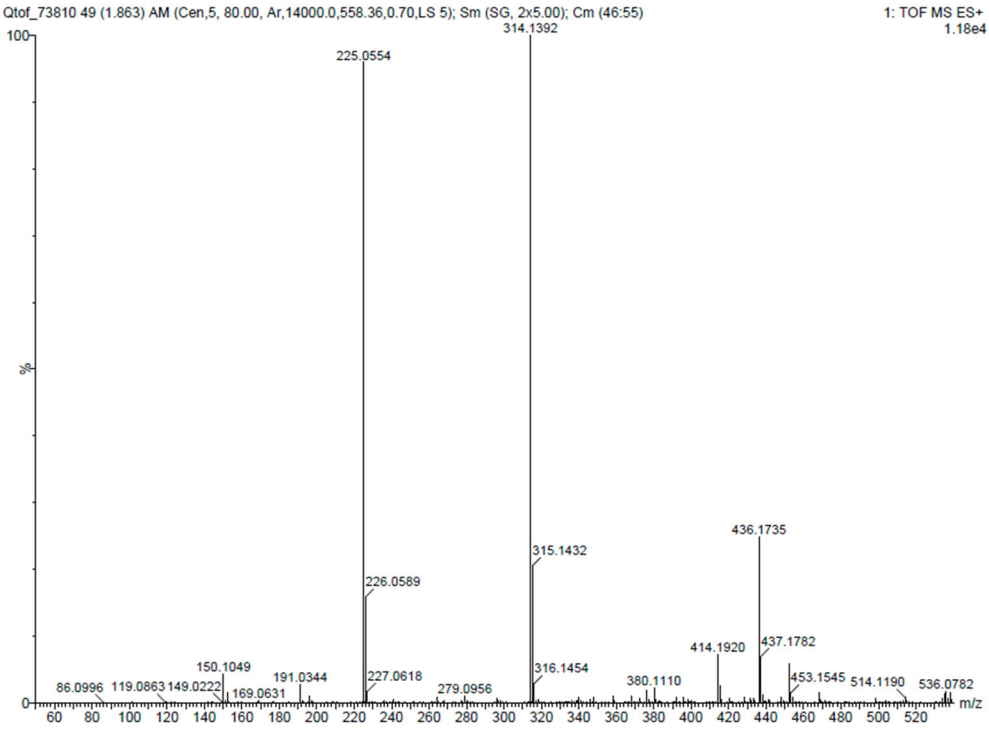
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **4b***



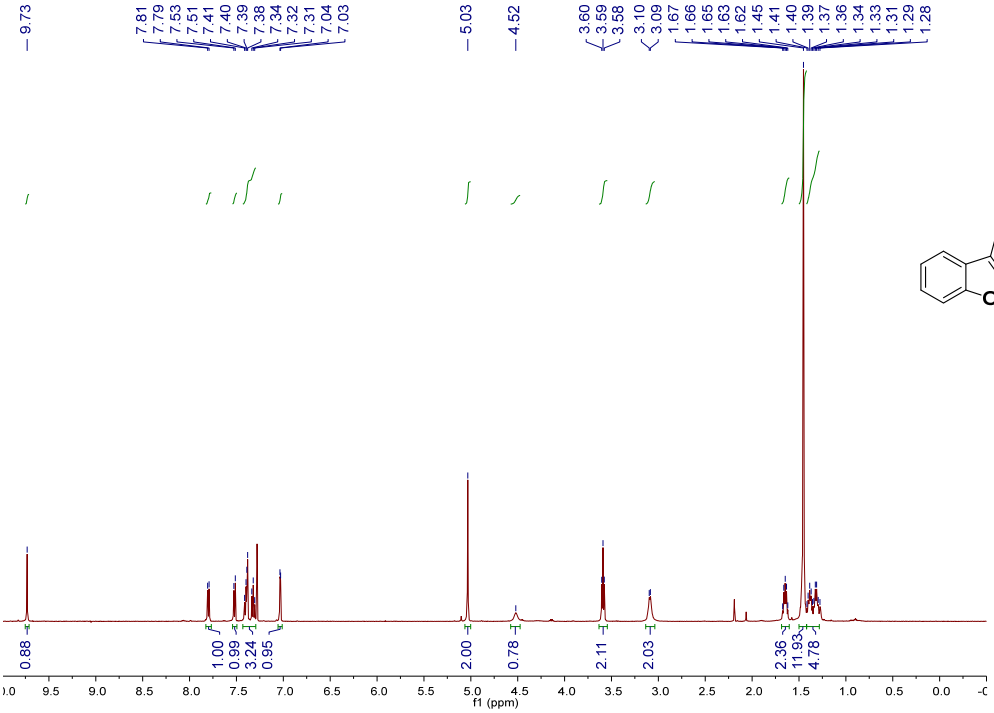


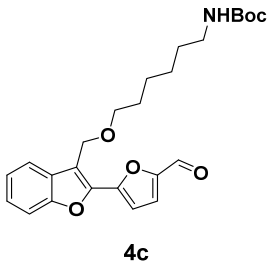
4b



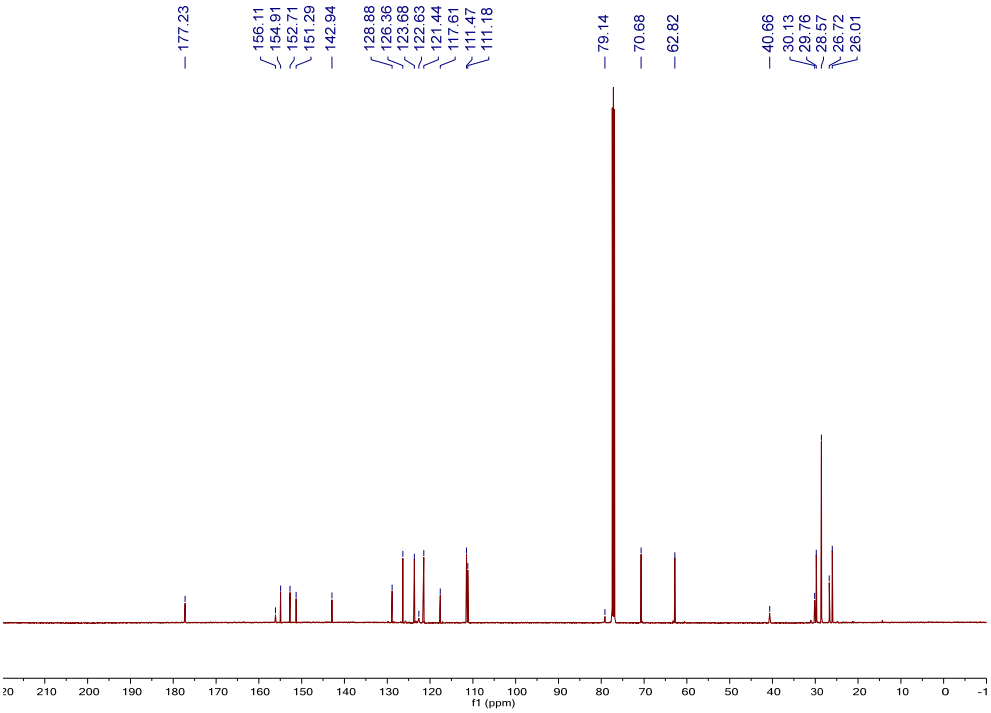

Qtof_73810 49 (1.863) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (46:55)
1: TOF MS ES+
1.18e4
100
%
0
314.1392
225.0554
436.1735
315.1432
226.0589
414.1920
437.1782
150.1049
316.1454
191.0344
227.0618
453.1545
86.0996
119.0863
149.0222
169.0631
279.0956
380.1110
514.1190
536.0782
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520
m/z

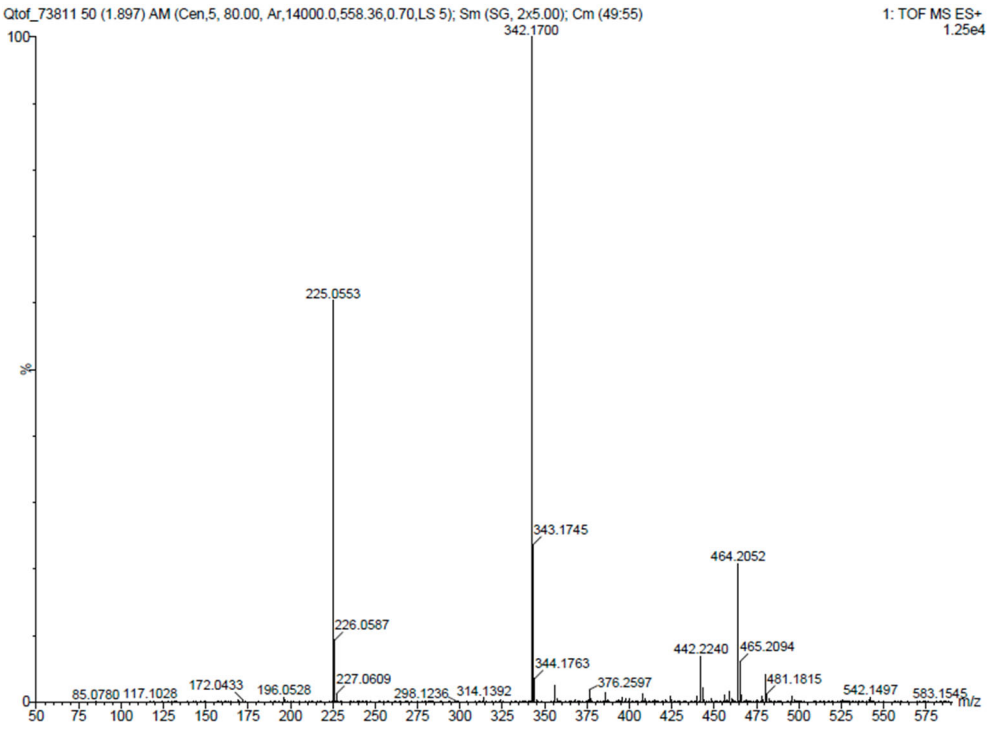
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **4c***



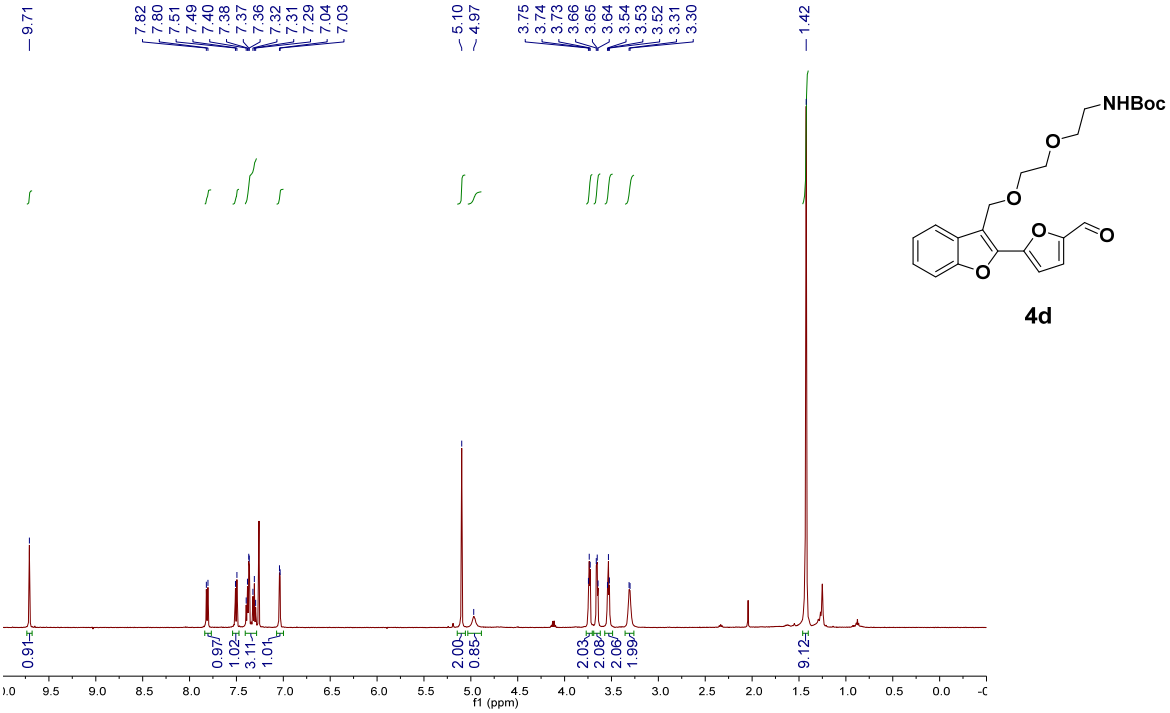


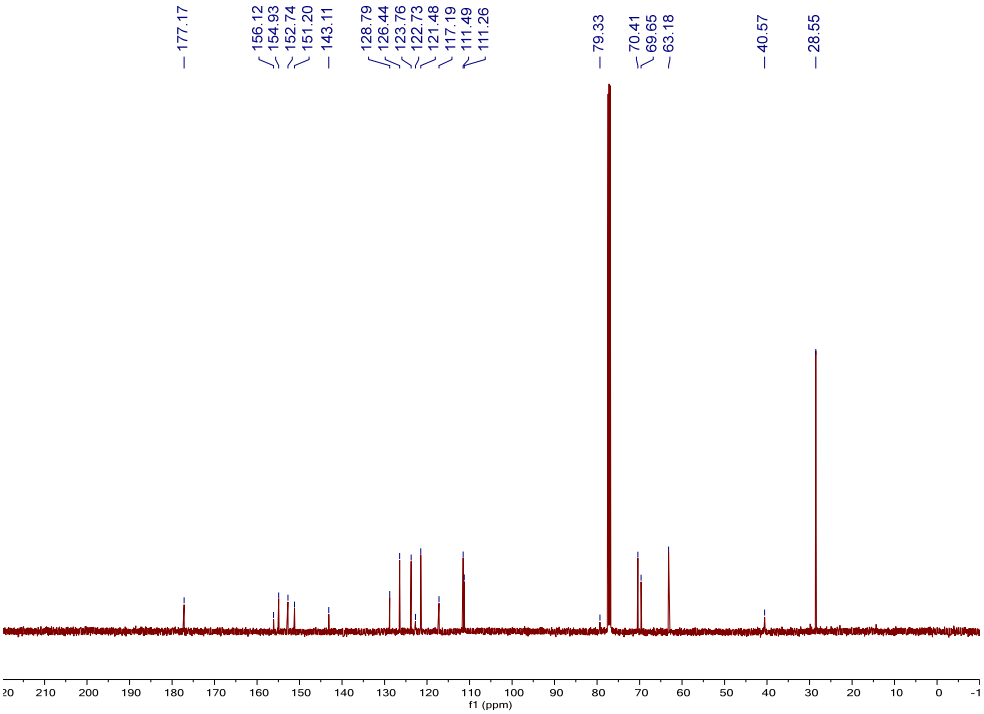
4c

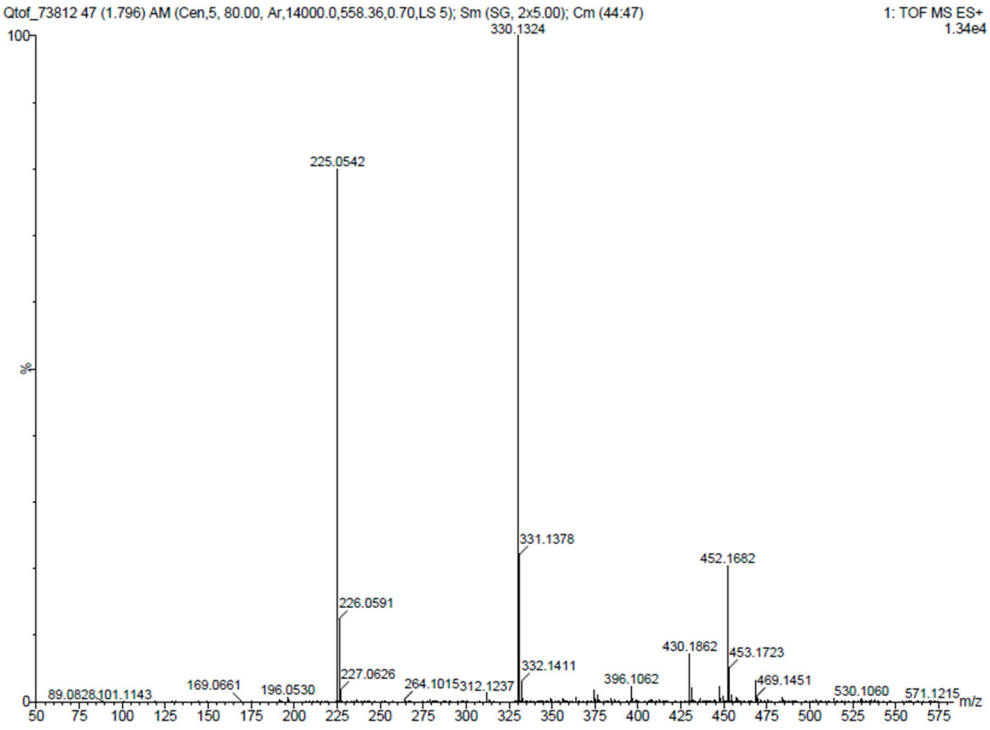



Qtof_73811 50 (1.897) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (49:55)
1: TOF MS ES+
1.25e4
100
%
0
342.1700
225.0553
343.1745
464.2052
226.0587
442.2240
465.2094
344.1763
481.1815
227.0609
376.2597
85.0780
117.1028
172.0433
196.0528
298.1236
314.1392
542.1497
583.1545
m/z
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
450
475
500
525
550
575

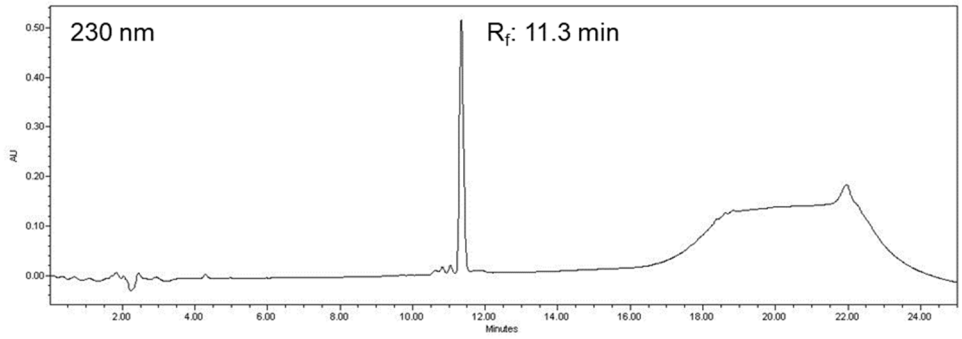
*[1]H-NMR, [13]C-NMR, and HR-MS spectra of **4d***

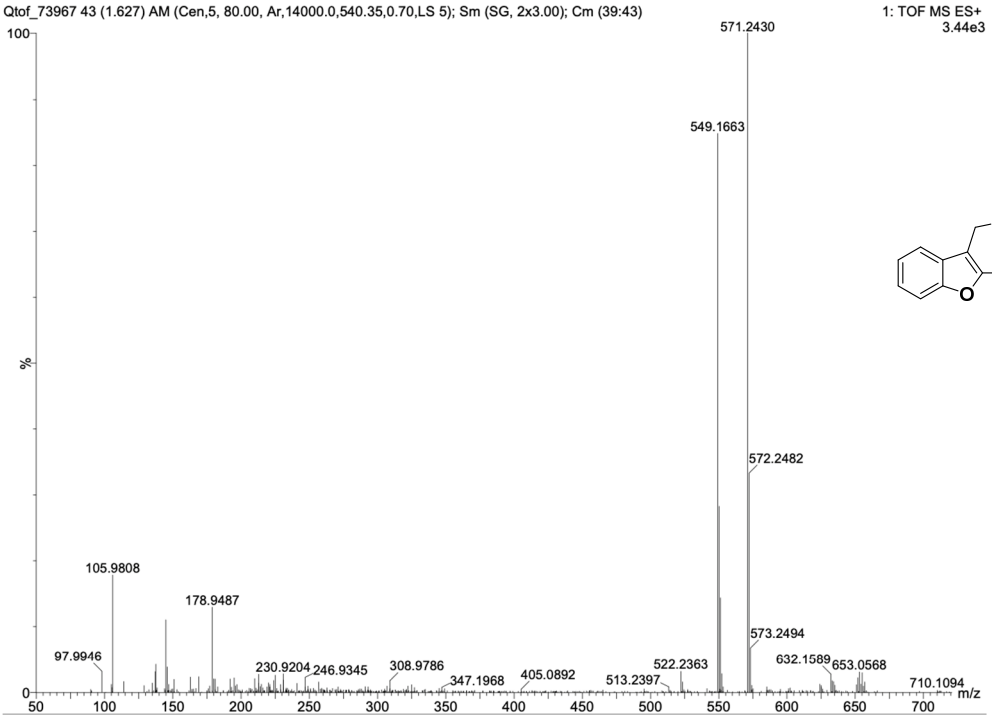


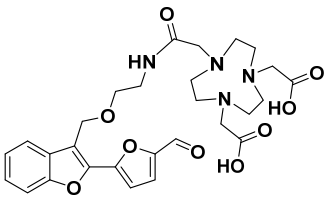



Qtof_73812 47 (1.796) AM (Cen,5, 80.00, Ar,14000.0,558.36,0.70,LS 5); Sm (SG, 2x5.00); Cm (44:47)
1: TOF MS ES+
1.34e4
100
%
0
330.1324
225.0542
331.1378
452.1682
226.0591
430.1862
453.1723
332.1411
227.0626
396.1062
469.1451
89.0828
101.1143
169.0661
196.0530
264.1015
312.1237
530.1060
571.1215
m/z
50 75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575

HPLC profile and HR-MS spectrum of ***6a***

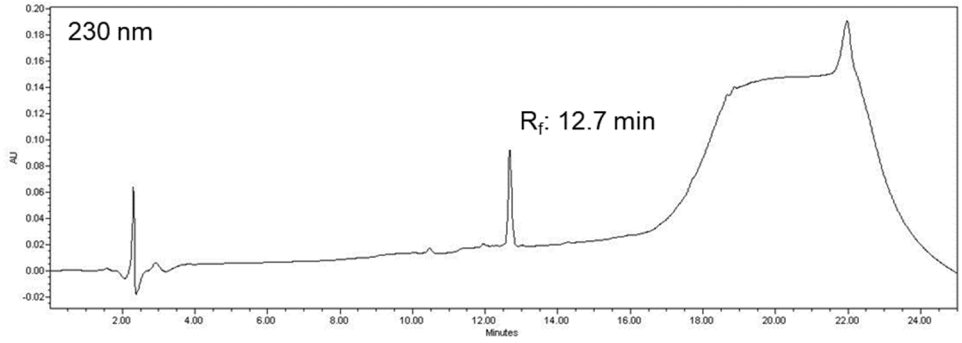


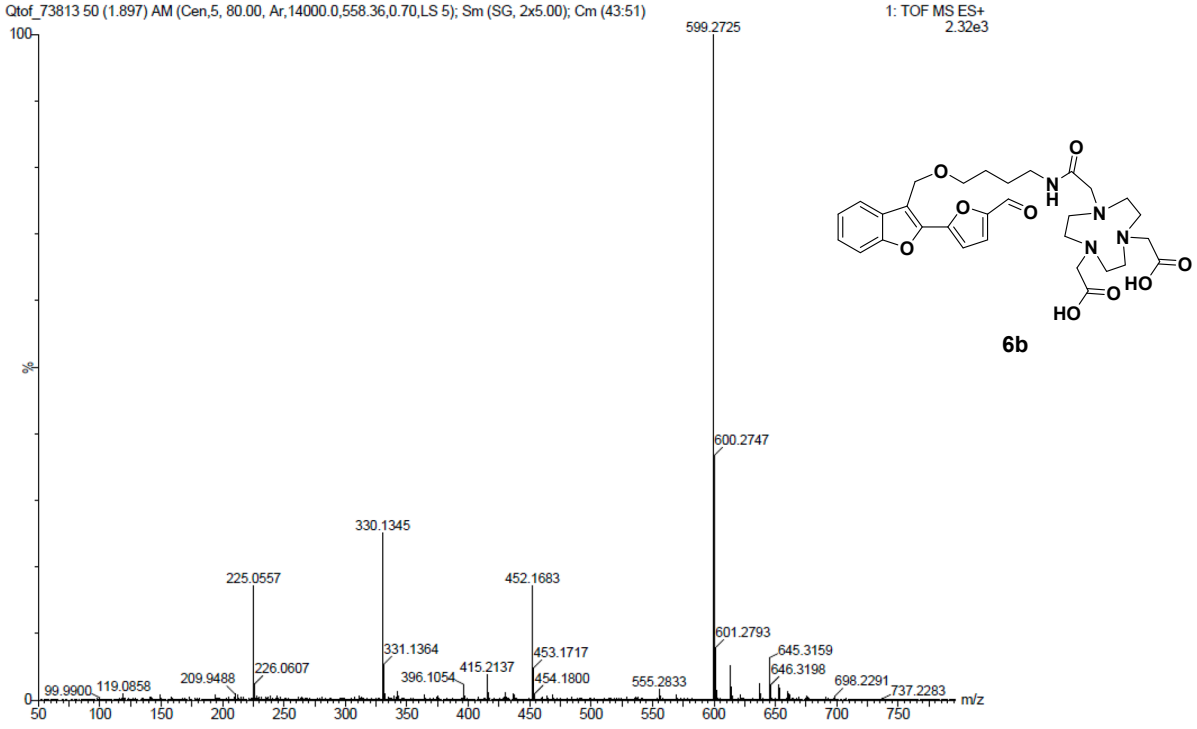




6a

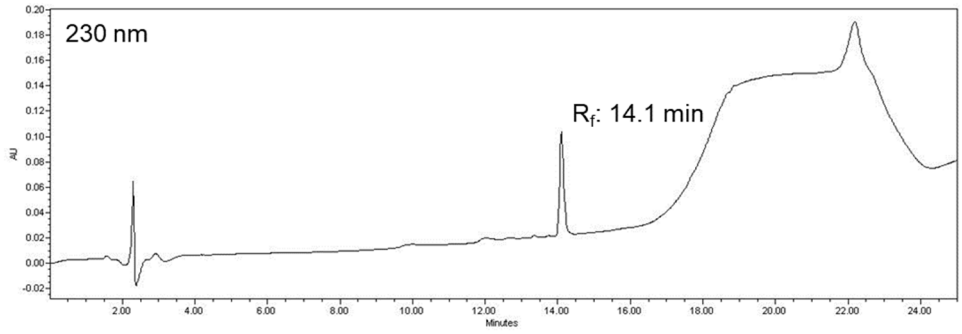
HPLC profile and HR-MS spectrum of ***6b***

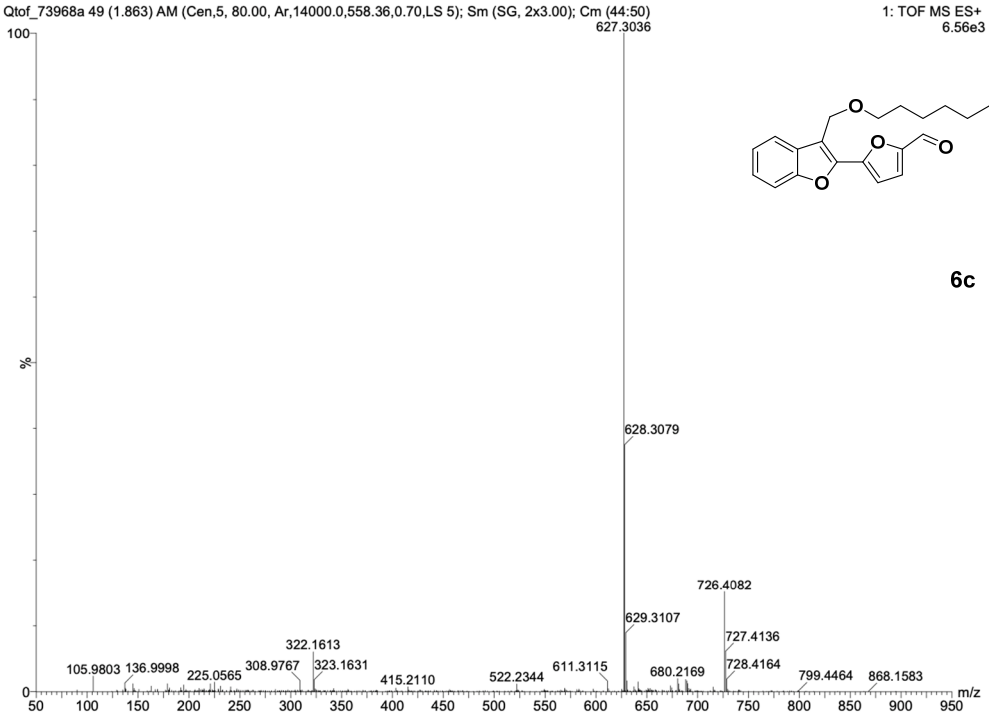




6b

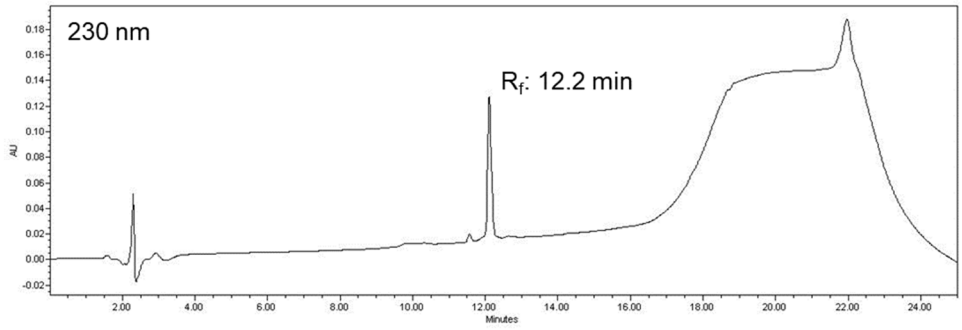
HPLC profile and HR-MS spectrum of ***6c***

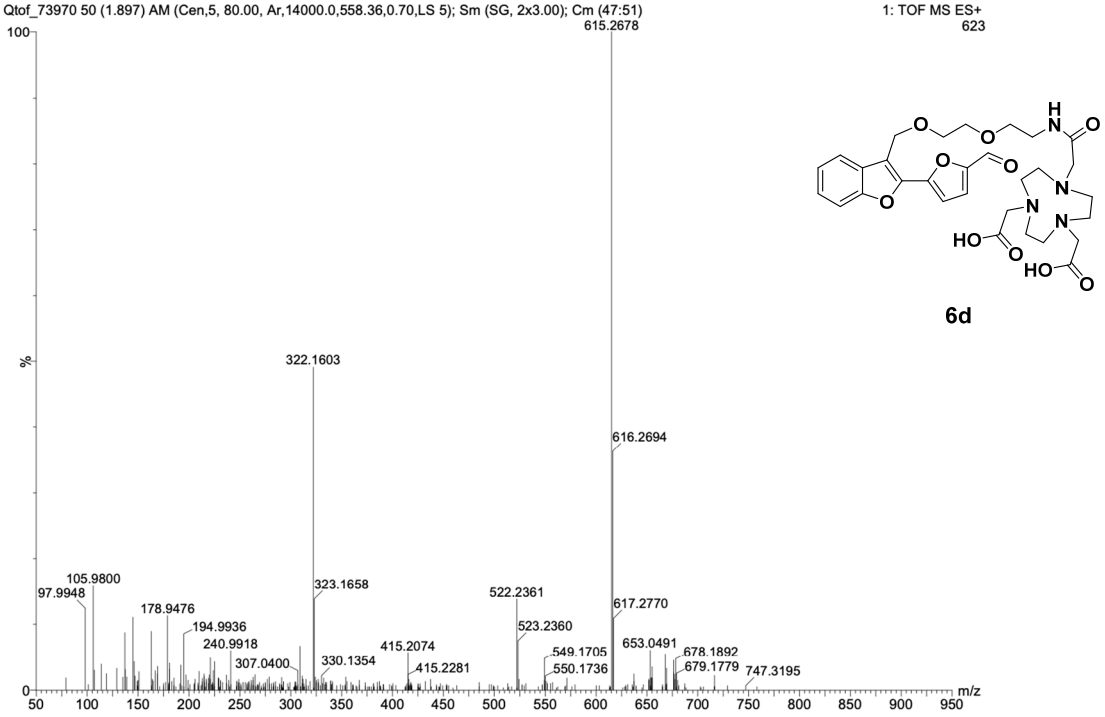




6c

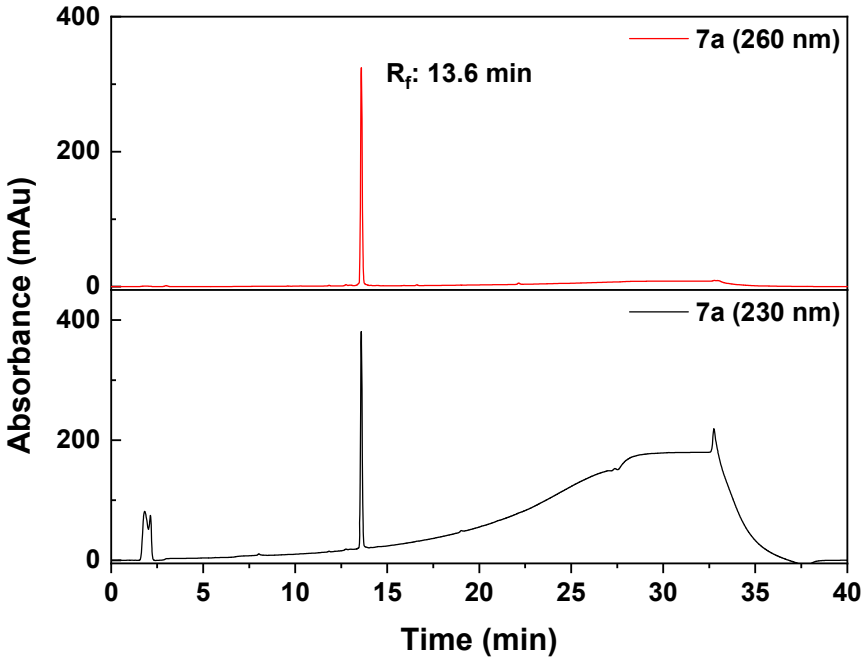
HPLC profile and HR-MS spectrum of ***6d***

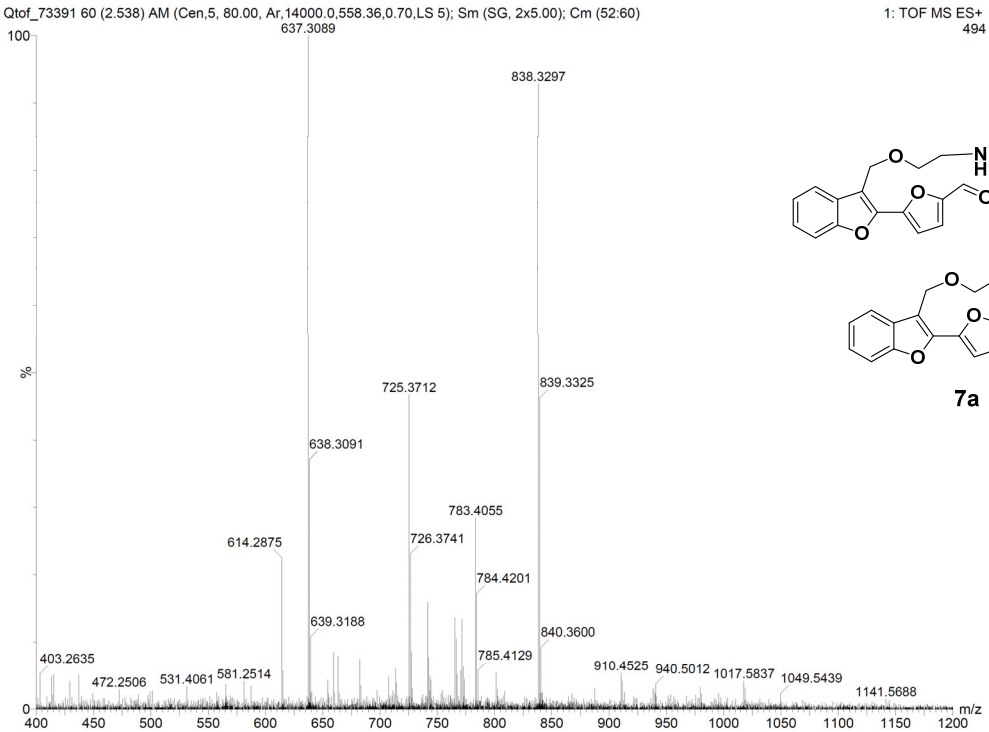




6d

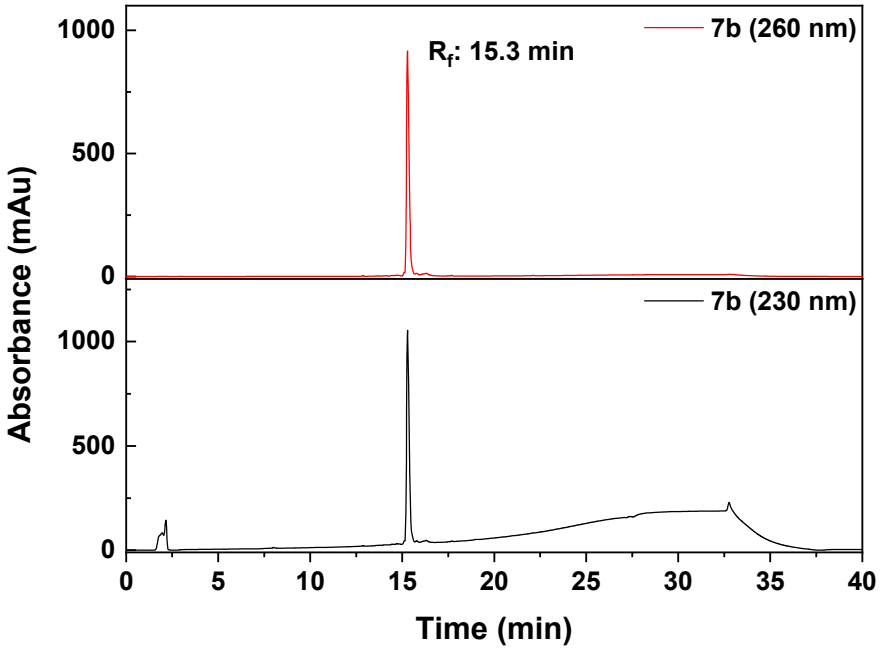
*HPLC profiles and HR-MS spectrum of **7a***

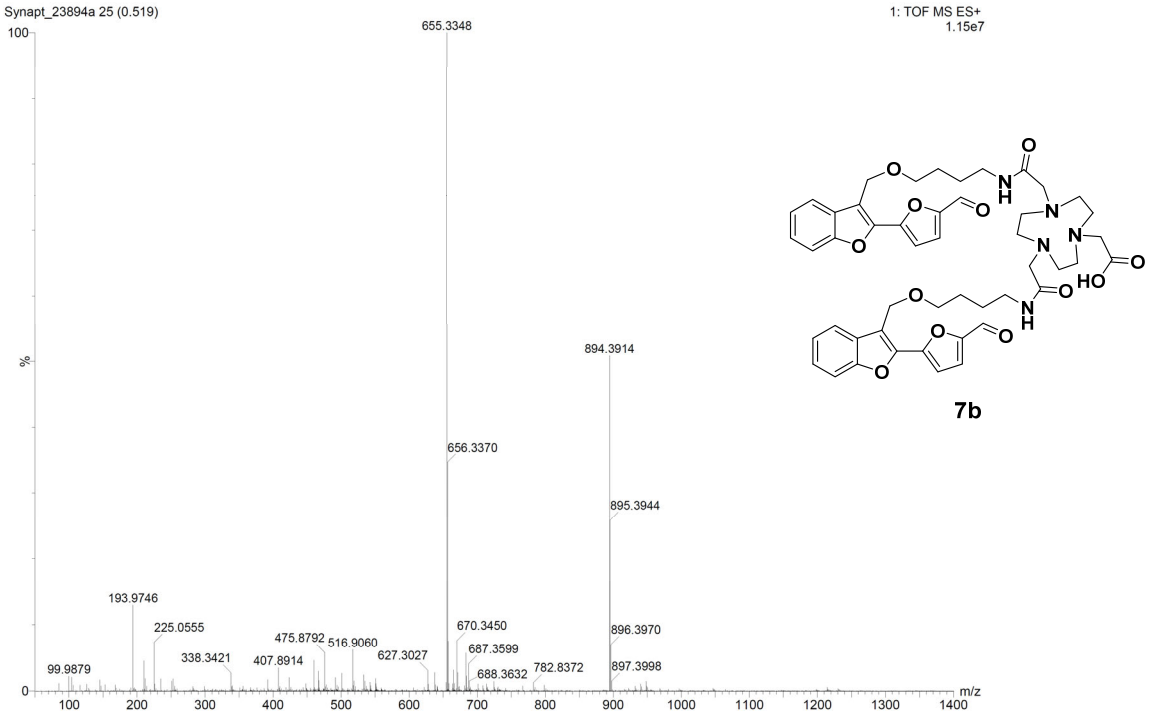




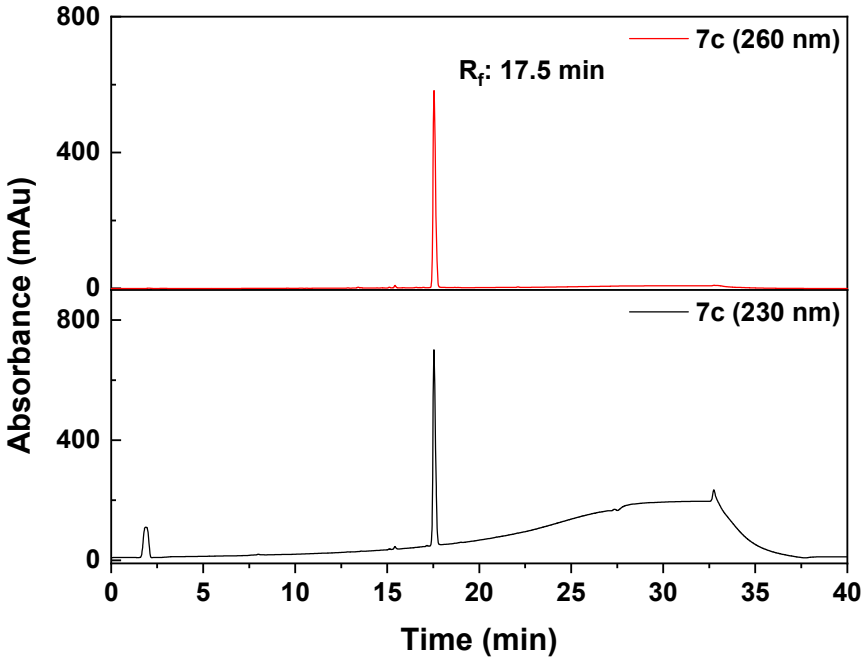
7a

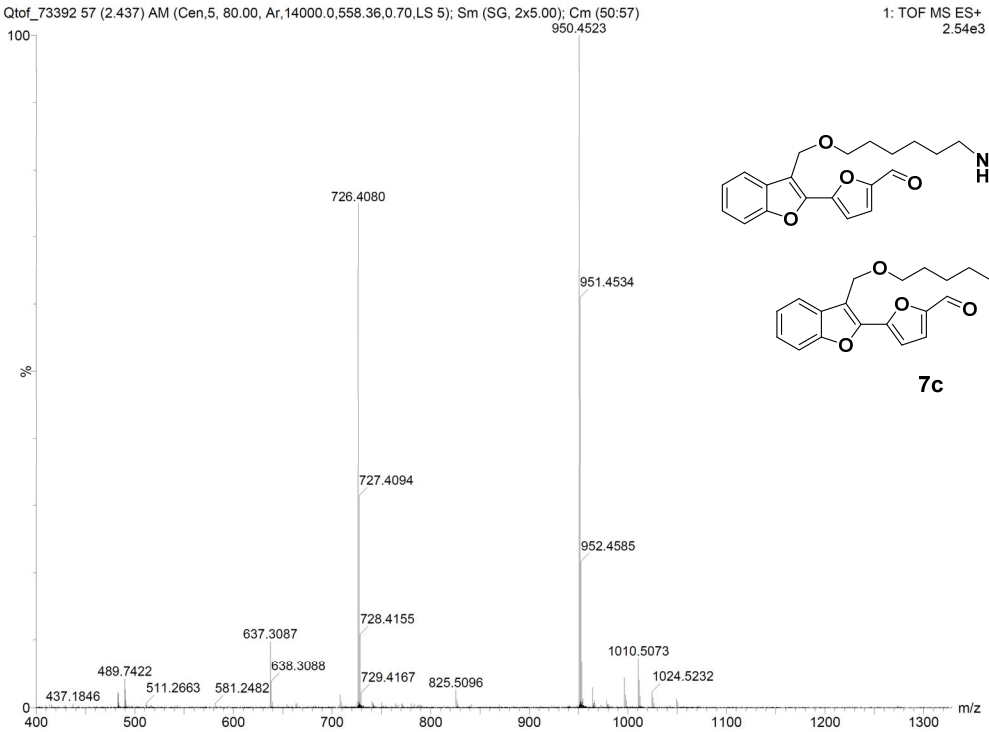
*HPLC profiles and HR-MS spectrum of **7b***





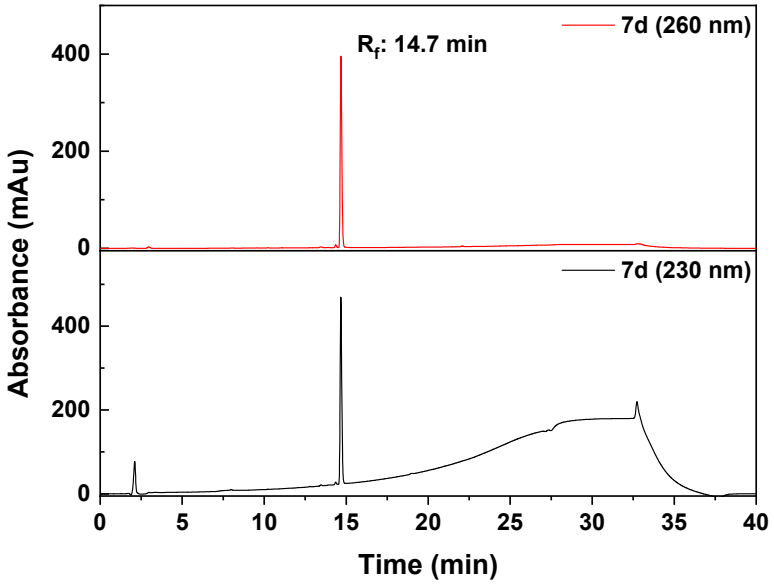
HPLC profiles and HR-MS spectrum of ***7c***

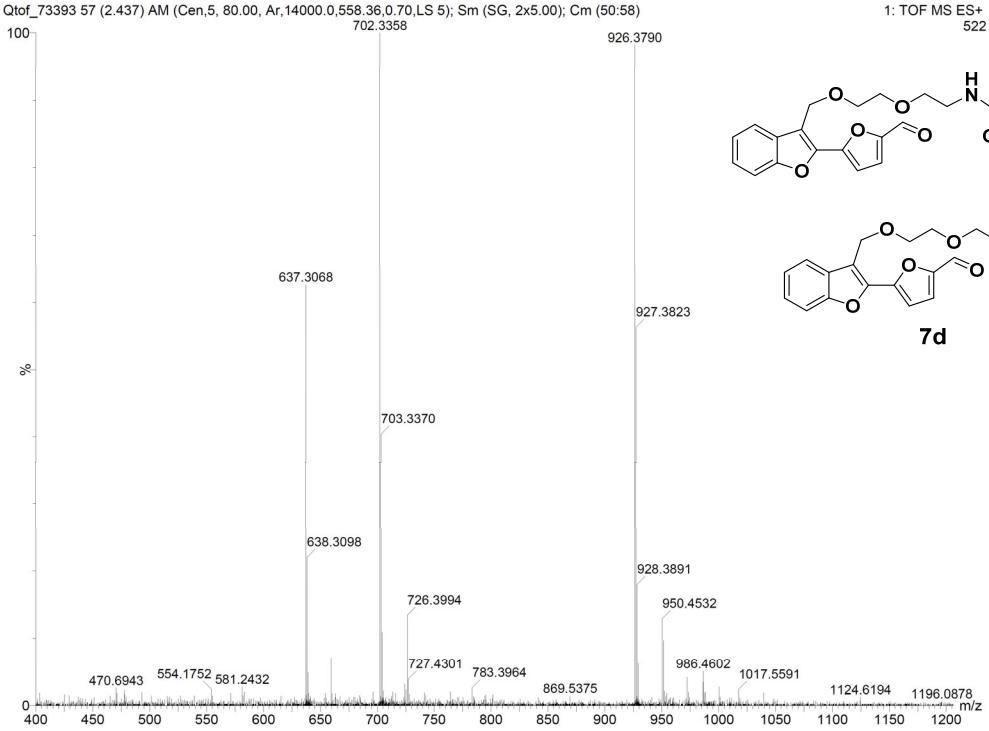




7c

*HPLC profiles and HR-MS spectrum of **7d***





7d

References

1. H. Erlenmeyer, W. Grubenmann, Über die Synthese von zwei mit Tryptophan isosteren Verbindungen: β-[Cumaronyl-(3)]-alanin und β-[Naphtyl-(1)]-alanin. *Helv. Chim. Acta* **30**, 297-304 (1947).