Supporting Information

In Vivo Targeting Using Arylboronate/Nopoldiol Click Conjugation

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General procedures:

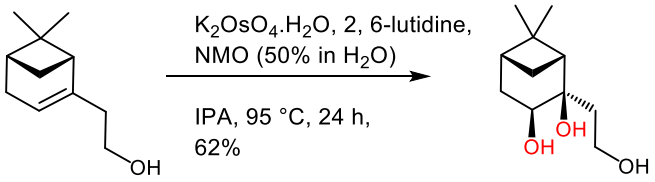
Unless otherwise indicated, all reactions were performed under a nitrogen atmosphere using glassware that was washed thoroughly prior to use. All reagents were purchased from Sigma-Aldrich, Combi-Blocks or Alfa Aesar and used as received. DMF, DCM and THF were used directly from an MBraun Solvent Purification System. Thin layer chromatography (TLC) was performed on Merck Silica Gel 60 F254 plates and visualized with UV light and curcumin stain. Flash chromatography was performed on ultra-pure silica gel 230-400 mesh. Nuclear magnetic resonance (NMR) spectra were recorded on Agilent/Varian INOVA-400 and INOVA-500 MHz instruments. The residual solvent protons (^{1}H) of $CDCl_3$ (7.26 ppm), acetone-d_6 (2.05 ppm), DMSO-d_6 (2.50 ppm), and D_2O (4.79 ppm) were used as internal standards, and the carbons signal (^{13}C) of $CDCl_3$ (77.06 ppm), DMSO-d_6 (39.51) and acetone-d6 (29.84 and 206.26 ppm) were used as an internal standard. Abbreviations used for multiplet signals are: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet), app (apparent). MestReNova software was used to analyze all the NMR data. IVIS experiments were carried out using Xenogen in vivo imaging system.

Crystallization of Compound 4:
Crystallization was performed using vapor diffusion technique. Pure compound **4** (10 mg) was dissolved in EtOAc (1 mL) in a 4 mL vial, which was placed in a 20 mL vial containing hexanes (4 mL). Finally, while the 4 mL vial was uncapped, 20 mL vial was sealed and this set up was kept at room temperature for 3 days, after which crystal formation was observed.

***In vivo* assessment of fluorophore capture:** 8-week-old female CD1 mice were injected intradermally with 50 µL of 25 mM boronate-NHS in PBS (n=3) or PBS as control (n=3) and imaged on the IVIS imager to obtain a background fluorescence signal. ICG/ICG excitation and emission filters were used for all IVIS images presented with no image math in the Living Image software performed. All mice were then retro-orbitally injected with 100 µL of 50 mg/L of diol-NIR followed by IVIS imaging at 0h, 3h, 24h and 1 week. Diol-NIR fluorescent intensity at the boronate-NHS site as well as PBS control was quantified by drawing a region of interest (ROI) that covers the injection sites and expressed as total radiant efficiency ([p/sec/cm^2/sr]/Mm/cm]). The increase in radiant efficiency at 24 h and 1 week was measured by subtracting the ROI values obtained with ICG background/ICG excitation and emission filters (autofluorescence) and subtracting the ROI before the diol-NIR injection.

Synthetic procedures:
Synthetic procedures for synthesis of early intermediates were adapted from prior publication[1].

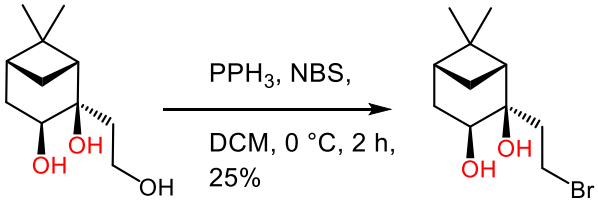
Compound S1: (1R,2R,3S,5R)-2-(2-Hydroxyethyl)-6,6-dimethylbicyclo[3.1.1]heptane-2,3-diol



8 **S1**

NMO (50%) (1.3 equiv, 2.4 mL, 12 mmol) and 2,6-lutidine (1.2 equiv, 1.2 mL, 11 mmol) were added to commercially available (–)-nopol (**8**) (1.0 equiv, 1.5 g, 8.9 mmol) in isopropanol (20 mL). $K_2OsO_4•H_2O$ (Strem chemicals, 1.7 mol%, 55 mg, 0.15 mmol) was added to the reaction mixture, which was then stirred and heated to reflux at 95 °C for 24 hours under ambient atmosphere. The resulting mixture was concentrated in vacuo and diluted with EtOAc (100 mL). The organic phase was washed with distilled water (1 × 10 mL), 1 M HCl (1 × 10 mL), and brine (1 × 10 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue, which contained ca. 10% of the minor diol diastereomer, was separated by flash chromatography (1:3 acetone:hexanes) to afford the diastereomerically pure compound **S1** as a colorless oil (1.14 g, 62% yield). All spectral data matched the literature data.[2]

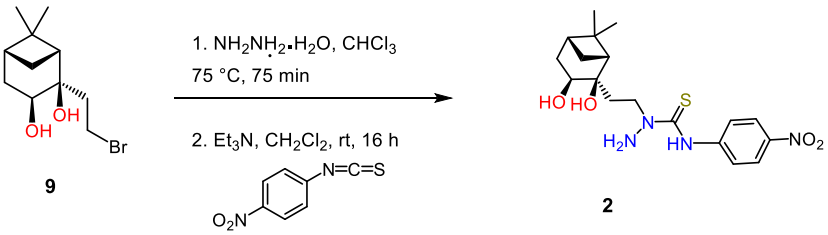
Compound 9: (1R,2R,3S,5R)-2-(2-Bromoethyl)-6,6-dimethylbicyclo[3.1.1]heptane-2,3-diol



S1 **9**

To a mixture of compound **S1** (1.0 equiv, 300 mg, 1.50 mmol) and triphenylphosphine (1.0 equiv, 433 mg, 1.65 mmol) in DCM (10 mL) at 0 ºC was added NBS (1.0 equiv, 293 mg, 1.65 mmol) at once (to get improved yield, NBS needs to be added portion-wise to the reaction mixture). The reaction mixture was then brought to room temperature and stirred for 2 hours after which time it was concentrated in vacuo. The crude residue was purified by flash chromatography (15:85 acetone:hexanes) to provide compound **9** as white solid (98.5 mg, 25% yield). All spectral data matched the literature data.[2]

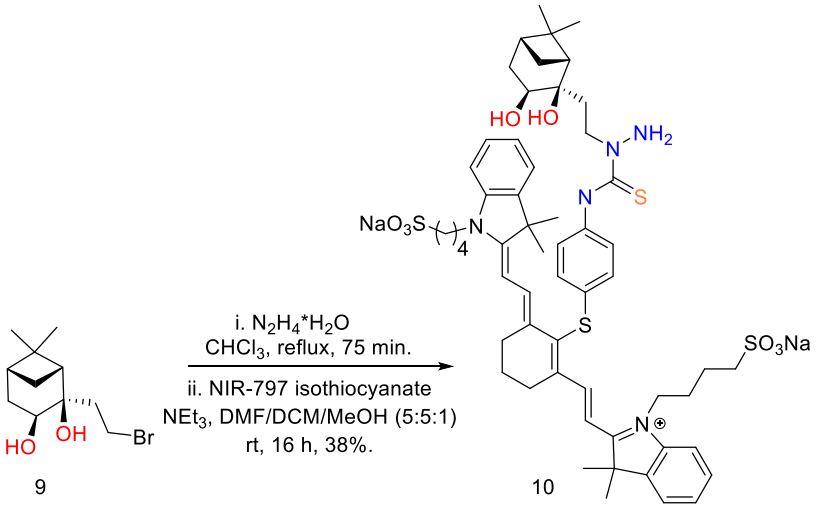
Compound 2: 1-(2-((1R,2R,3S,5R)-2,3-dihydroxy-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)-N-(4-nitrophenyl)hydrazine-1-carbothioamide



9 **2**

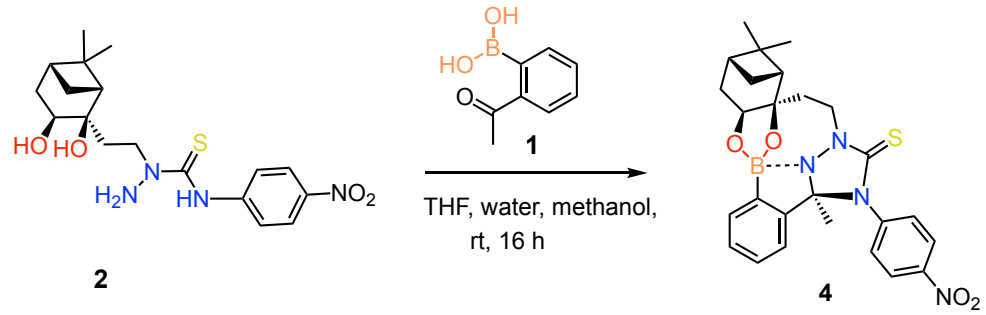
To a solution of compound **9** (1.0 equiv, 60 mg, 0.22 mmol) in $CHCl_3$ (2.0 mL) was added $NH_2NH_2•H_2O$ (20 equiv, 220 µL, 4.6 mmol) under inert atmosphere at room temperature. The reaction mixture was refluxed for 75 min at 75 °C and then concentrated in vacuo. The crude residue was kept under vacuum for 2 hours and used in the next step without further purification. Then, Et_3N (1.2 equiv, 42 µL, 0.30 mmol) was added to the crude residue in CH_2Cl_2 (2.00 mL) at room temperature under inert atmosphere. Finally, 4-nitrophenyl isothiocyanate (0.9 equiv, 28 mg, 0.20 mmol) was added to the reaction mixture, which was then stirred for 16hoursat room temperature. Then, the reaction mixture was concentrated in vacuo and passed through a short silica plug using 5% MeOH/DCM solvent system to yield compound **2**. The crude residue obtained was used directly in the next step, which was boronic ester formation. All spectral data matched the literature data.[2]

Compound 10: 4-((E)-2-((E)-2-(2-((4-(1-(2-((1R,2R,3S,5R)-2,3-dihydroxy-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)hydrazine-1-carbothioamido)phenyl)thio)-3-((E)-2-(3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-2-yl)vinyl)cyclohex-2-en-1-ylidene)ethylidene)-3,3-dimethylindolin-1-yl)butane-1-sulfonate:



To a solution of compound **9** (1.0 equiv 3.00 mg, 0.011 mmol) in $CHCl_3$ (1.0 mL) was added NH_2NH_2•H_2O (20 equiv 11.0 µL, 0.228 mmol) under inert atmosphere at room temperature. The reaction mixture was stirred under reflux for 75 min at 75 °C and then concentrated in vacuo. The crude residue was kept under vacuum for 2 hours and used in the next step without further purification. To the crude residue was added NIR-797 isothiocyanate (1.0 equiv, 10.0 mg, 0.011 mmol) and the reagents dissolved in DMF/DCM/MeOH (0.45 mL/0.45 mL/0.090 mL) at room temperature under inert atmosphere. NEt_3 (1.2 equiv, 21 µL, 0.15 mmol) was added via syringe and the reaction was stirred for 16 hours at room temperature. The reaction mixture was concentrated in vacuo and purified using reverse phase HPLC using a Agilent Eclipse XDB-C18, 5um, 9.4X250 mm column with 10 mM ammonium acetate in both water (A) and acetonitrile (B). Elution (3 mL/min) was with a linear gradient from 0 to 20% B in 2 min, 20% to 95% B for 12 min and a post run elution from 95% to 20% B over 2 min to provide the compound **10** as a greenish blue powder (4.2 mg, 38% yield, over all yield: 5.9%). See **Figure S3** for LC/MS analysis and **Figure S4** for MS.

Compound 4: (10aR,11R,13R,14aS)-5,12,12-Trimethyl-6-(4-nitrophenyl)-5,6,9,10,12,13,14,14a-octahydro-5,8-epimino-10a,16-epoxy-11,13 methanodibenzo[c,k][1,6,8,2]oxadiazaboracyclododecin-7(11H)-one)



To a solution of crude intermediate (**2**) in THF/MeOH/water (4.00/0.50/0.50 mL) was added 2-acetylphenylboronic acid (**1**) (1.0 equiv, 36 mg, 0.22 mmol) at room temperature. Reaction mixture was stirred for 16 h, after which it was concentrated in vacuo and purified by flash chromatography (1:1, EtOAc/hexanes) to provide compound **4** (Fig. 1) as a yellow solid (18 mg, 16% yield over 3 steps).

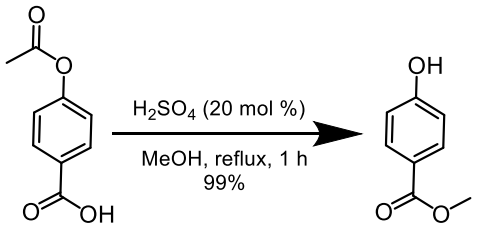
^{1}H NMR d/ppm: See **Figure S5**. (500 MHz, $CDCl_3$) 10.03 (s, 1H), 8.08 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.07 (t, *J* = 7.3 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.82 (t, *J* = 7.5 Hz, 1H), 6.18 (d, *J* = 7.7 Hz, 1H), 4.74 (d, *J* = 15.6 Hz, 1H), 4.55 (d, *J* = 8.3 Hz, 1H), 3.69 (t, *J* = 14.0 Hz, 1H), 2.98 – 2.79 (m, 1H), 2.66 – 2.47 (m, 1H), 2.34 – 2.23 (m, 1H), 2.11 (t, *J* = 5.2 Hz, 1H), 2.00 (app s, 2H), 1.86 (d, *J* = 14.7 Hz, 1H), 1.81 (s, 3H), 1.65 (d, *J* = 10.8 Hz, 1H), 1.32 (s, 3H), 0.96 (s, 3H)

^{13}C NMR d/ppm: See **Figure S6**. (126 MHz, $CDCl_3$) 176.6, 147.6, 141.8, 140.7, 139.2, 132.1, 131.2, 129.5, 128.9, 124.0, 123.6, 85.7, 83.6, 83.5, 72.3, 52.2, 44.9, 40.4, 37.89, 37.87, 35.9, 29.8, 27.2, 26.9, 24.8, 20.9. Note: the spectrum contains traces of residual EtOAc (60, 15 ppm) from the purification.

IR (Microscope, cm^{-1}) 3032, 2979, 2925, 2871, 2820, 2560, 1737, 1608, 1595, 1524, 1496, 1379, 1349.

^{11}B NMR /ppm: See **Figure S1**. (128 MHz, CD3OD) 13.8.

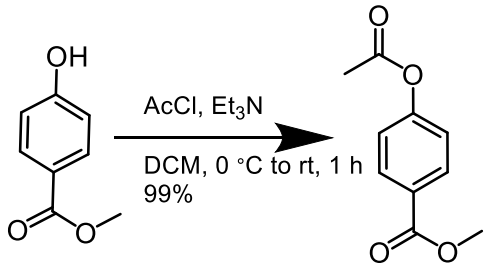
Compound S2: Methyl 4-hydroxybenzoate:



5 **S2**

To a 100 mL round bottom flask was added 4-acetoxybenzoic acid **5** and MeOH (32.0 mL). Concentrated sulfuric acid (8.00 mL) was added dropwise and the reaction refluxed for 2 hours then brought to room temperature. The solution was concentrated in vacuo until 5-10 mL MeOH remained then diluted with 40 mL EtOAc. The phases were separated and the aqueous phase extracted with EtOAc (2 x 15 mL) and the combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to afford the compound **S2** as a pale-yellow solid (3.34 g, 99%) without any further purification. All spectral data matched the literature data[2].

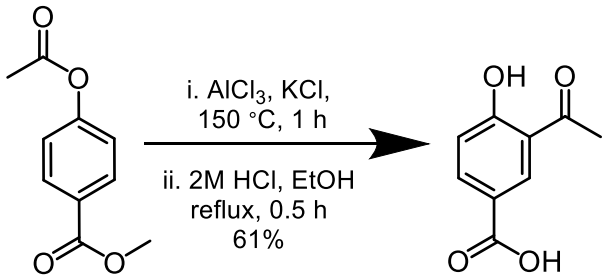
Compound S3: Methyl 4-acetoxybenzoate:



S2 **S3**

To a 100 mL round bottom flask was added **S2** (1.0 equiv, 3.28 g, 21.6 mmol) and dry DCM (50.0 mL) and cooled to 0 ºC. Triethylamine (1.5 equiv 4.52 mL, 32.4 mmol) was added dropwise and the reaction stirred for 1 hour followed by the addition of acetyl chloride (1.70 mL, 23.8 mmol) via syringe. The solution was brought to room temperature and stirred for 2 hours and diluted with water (20 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 x 15 mL) and the combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to afford the compound **S3** as an off-white solid (4.14 g, 99%) without any further purification. All spectral data matched the literature data[3].

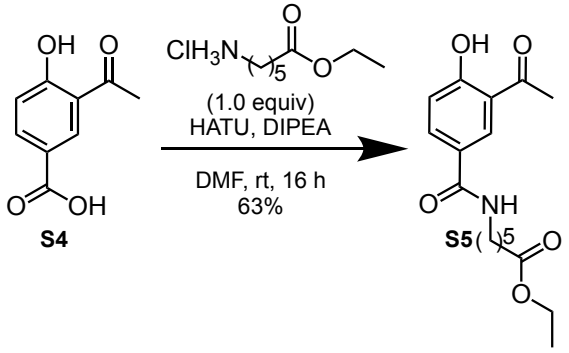
Compound S4: 3-Acetyl-4-hydroxybenzoic acid:



S3 **S4**

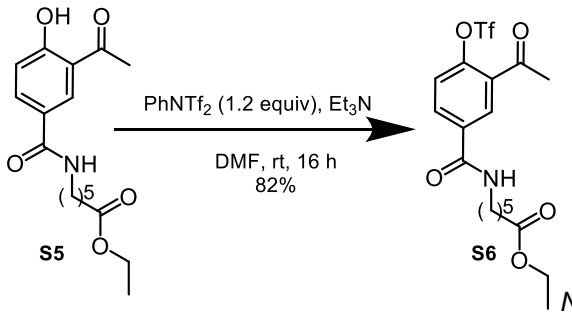
To a 250 mL round bottom flask was added **S3** (1.0 equiv 4.05 g, 20.9 mmol), $AlCl_3$ (3.0 equiv 8.36 g, 62.7 mmol), and KCl (1.0 equiv 1.63 g, 21.9 mmol) equipped with an adapter under vacuum. The mixture was heated at 120 °C for 30 min and then heated at 150 °C for 1 h. After cooling to room temperature, a mixed solvent of 2 N HCl (100 mL) and EtOH (20 mL) was added to the reaction mixture. The resulting suspension was refluxed for 0.5 h. The crude product was filtered using Büchner funnel to afford the compound **S4** as a yellow solid (2.30 g, 61% yield). All spectral data matched the literature data.[4]

Compound S5: Ethyl 6-(3-acetyl-4-hydroxybenzamido)hexanoate:



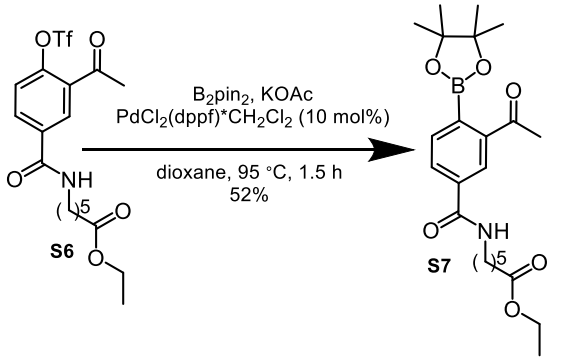
To a 100 mL round bottom flask was added **S4** (1.0 equiv 890 mg, 4.94 mmol) and HATU coupling reagent (1.0 equiv 1.88 g, 4.94) and dry DMF (20 mL) at room temperature under nitrogen. Ethyl 6-aminocaproate hydrochloride (1.0 equiv 967 mg, 4.94 mmol, prepared according to Sinyakov and coworkers.[5]) and N,N-diisopropylethylamine (2.75 mL, 3.2 equiv 15.8 mmol) were added. The reaction mixture was stirred for 16hoursat room temperature, concentrated in vacuo and the crude residue dissolved in DCM (50 mL). The solution was washed with 1 M HCl (1 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (1:9 to 5:5 EtOAc:hexanes) to afford the compound **S5** was a yellow solid (1.00 g, 63% yield). All spectral data matched the literature data.[4]

Compound S6: Ethyl 6-(3-acetyl-4-(((trifluoromethyl)sulfonyl)oxy)benzamido)hexanoate:



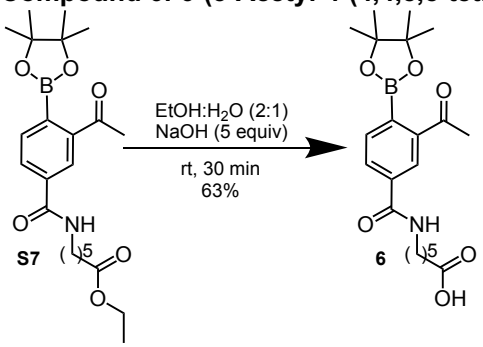
N-Phenyl-bis(trifluoromethanesulfonimide) (1.0 equiv 3.05 g, 8.54 mmol) was added portion wise to a solution of **S5** (2.11 g, 6.57 mmol) and NEt_3 (3.0 equiv 3.67 mL, 26.3 mmol) in dry DMF (20 mL) at room temperature under argon, and the reaction mixture was stirred for 16 h. The solution was concentrated in vacuo and purified by flash chromatography (1:3 to 1:1 EtOAc:hexanes) to afford the compound **S6** as a beige solid (2.44 g, 82% yield). All spectral data matched the literature data.[4]

Compound S7: Ethyl-6-(3-acetyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzamido)hexanoate:



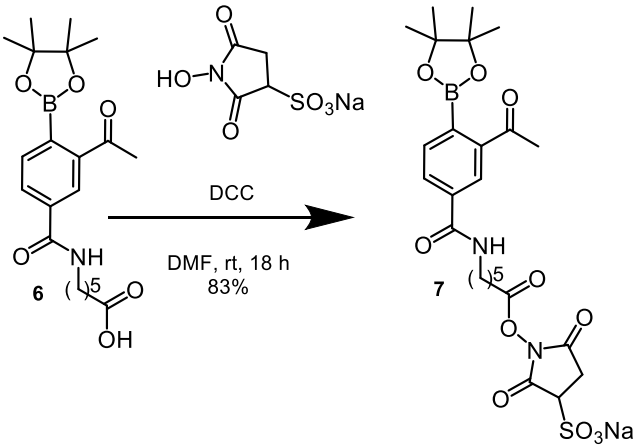
To a flame-dried round bottom flask flushed with argon was added **S6** (1.0 equiv 1.12 g, 4.323 mmol), bis(pinacolato)diboron (2.0 equiv 2.20 g, 8.64 mmol), KOAc (3.0 equiv 1.27 g, 13.0 mmol), $PdCl_2$(dppf) (0.1 equiv, 353 mg, 0.43 mmol) and freshly distilled dioxane (45 mL). The reaction mixture was stirred and heated at 80 °C for 90 min then concentrated in vacuo. The crude material was purified by flash chromatography (3:7 EtOAc:DCM) to afford the compound **S7** as a colorless oil (688 mg, 52% yield). All spectral data matched the literature data.[4]

Compound 6: 6-(3-Acetyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamido)hexanoic acid:



To a solution of **S7** (1.0 equiv 66 mg, 0.153 mmol) in EtOH (500 µL) and water (250 µL) was added NaOH (5.0 equiv 30.6 mg, 0.765 mmol) and the solution stirred for 30 min at room temperature. The solution was acidified with 1 M HCl (2 mL) and diluted with EtOAc (10 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 × 5 mL) and the organic extracts dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (8% MeOH in EtOAc) to afford the compound **6** as an off-white solid (38.8 mg, 63% yield). All spectral data matched the literature data.[4]

Compound 7: 1-((6-(3-acetyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamido)hexanoyl)oxy)-2,5-dioxopyrrolidine-3-sulfonate



To a flame-dried 10 mL round bottom flask was added **6** (1.0 equiv, 53.0 mg, 0.131 mmol), sulfo-NHS (0.8 equiv, 25.7 mg, 0.118 mmol), DCC (1.1 equiv 30.0 mg, 0.144 mmol) and dry DMF (1 mL). The mixture was stirred at room temperature for 18 hours and concentrated in vacuo. The urea by-product DCU was precipitated with EtOAc and filtered using a Büchner funnel three times to provide the compound **7** as a white solid (58.3 mg, 83%, over all yield: 8.4%). Material was analyzed by LC/MS on a Phenomenex Luna Omega C18 Polar column (2.1X50 mm, particle size 1.6 µM). LC/MS conditions: 50 °C. A: 0.1% formic acid in water; B: 0.1% formic acid in ACN; flow rate: 0.5 mL/min.
Gradient:

Time	.1% formic acid in water	1% formic acid ACN
(min)	(%)	(%)
0	98.0	2.0
5	40.0	60.0
5.5	5.0	95.0

UV recorded
214nm
254nm
284nm

See **Figure S7** for LC/MS traces. The resulting LC/MS trace shows the appearance of two major peaks at 3.4 and 5.3 min. As confirmed by the TIC analysis (negative mode), compound **7** occurs at 5.3 min as the intact pinacol ester (m/Z calculated for C25H32BN2O11S = 579.10; found = 579.1). The free boronic acid occurs at 3.4 min. Partial hydrolysis of a pinacol boronic ester is expected under the aqueous acidic conditions of the LC analysis. Indeed, when exposed to dilute solutions in water, pinacol boronic esters are expected to slowly hydrolyze into the corresponding boronic acid, and are often employed as a "pro-drug" in biological experiments. The minor peak at 4.3 min was identified as being a small amount of the protodeboronated side-product. Protodeboronation is also an expected side-reaction under acidic aqueous conditions and is likely to be an artifact of the analytical method.

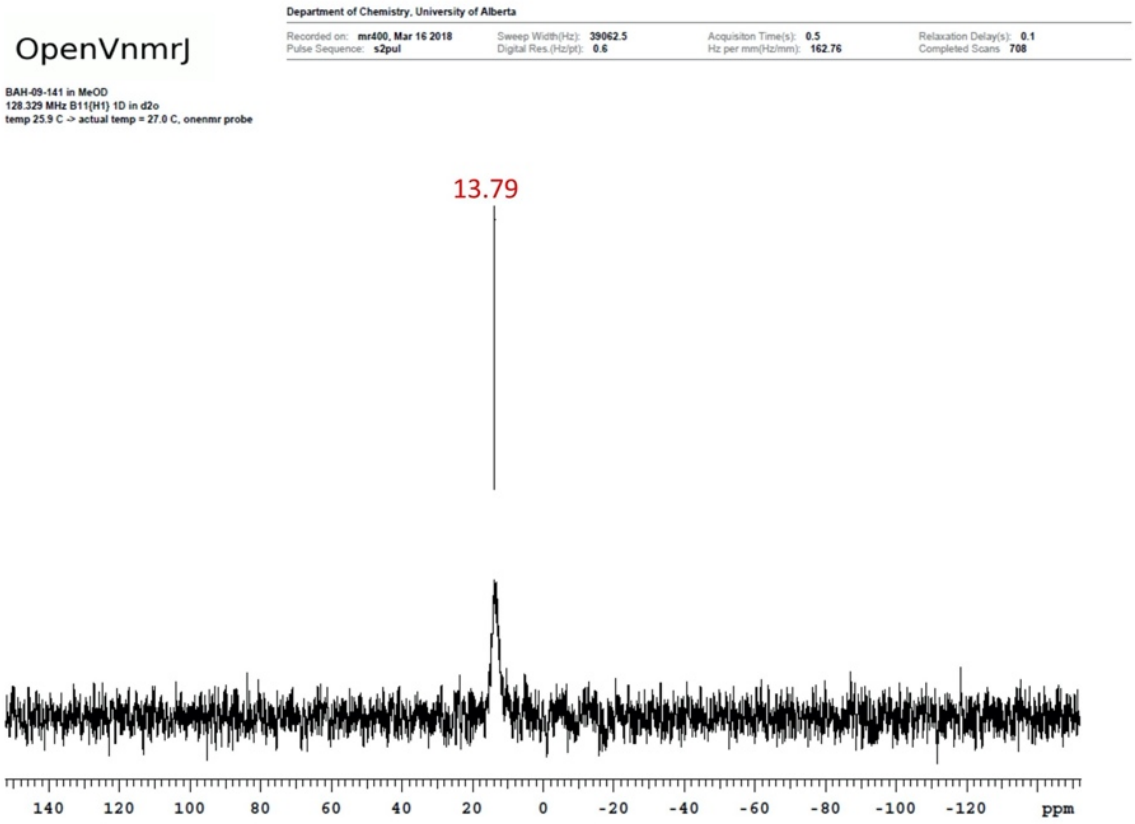


Figure S1: ^{11}B NMR spectrum of compound 4.

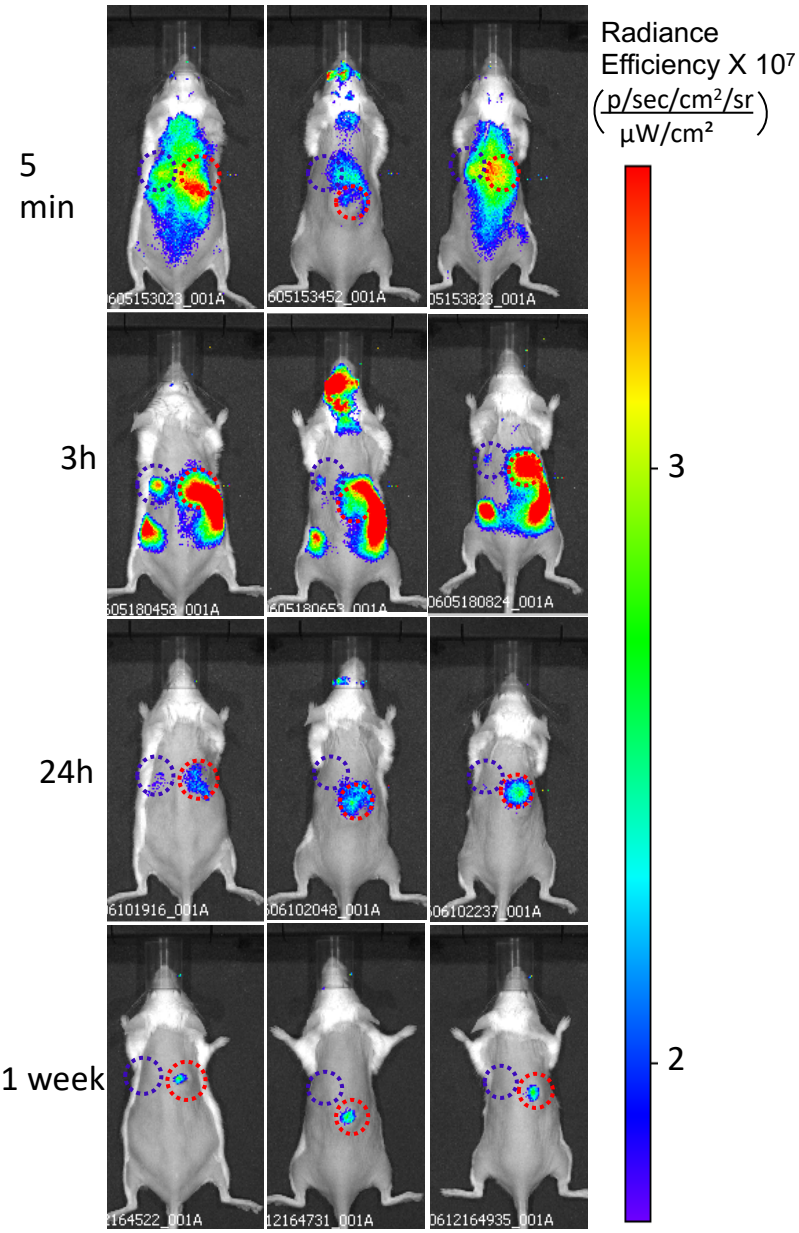


Figure S2: The *in vivo* fluorescence distribution after i.v. administration of Diol-NIR over 1 week period. The dashed circles indicate PBS (blue) and boronate-NHS (red) injection sites. NOTE: The apparent ocular/cranial signal in the middle mouse at 5 min. and 3 h is an artifact associated with retro-orbital injection of fluorophore.

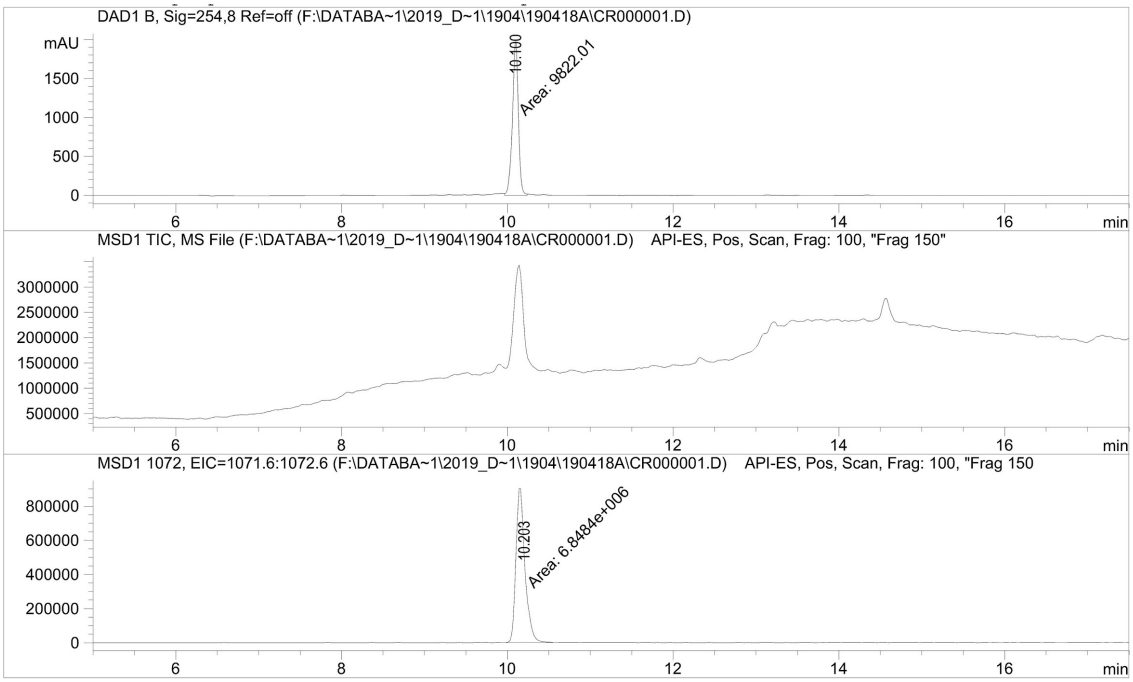


Figure S3: LC-MS Analysis of purified compound **10**. Top: HPLC chromatogram showing UV absorbance at 254 nm. Middle: Chromatogram of total ion count under ESI positive mode. Bottom: Chromatogram of extraction of MS 1072 $(M+H)^+$.

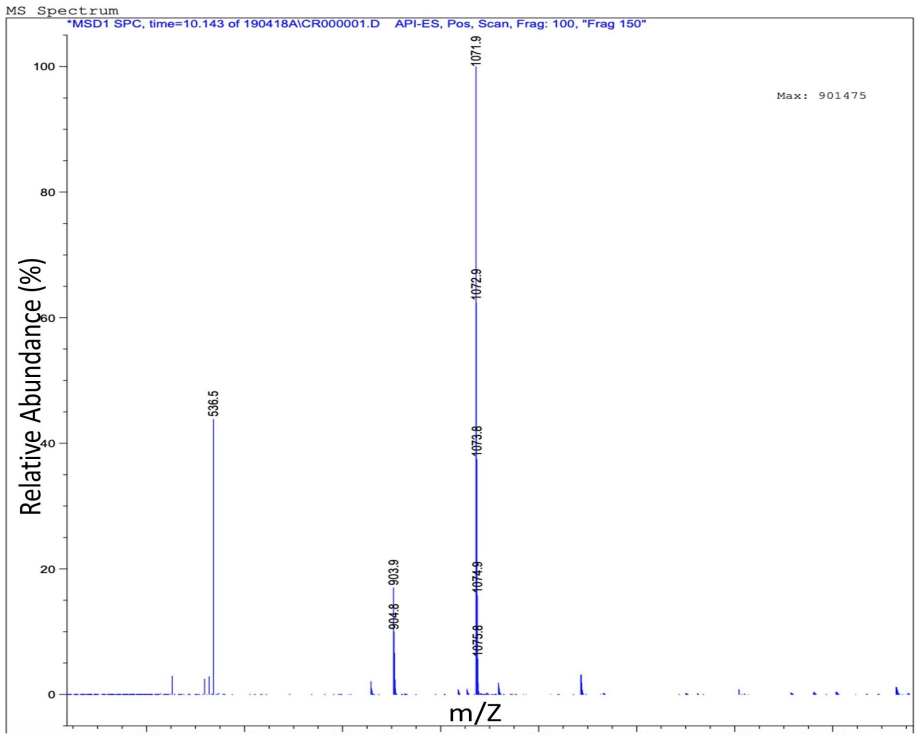


Figure S4: MS Spectrum of compound 10. (M+H).
Calculated for C56H72N2O8S4 = 1070.4269; found = 1070.1

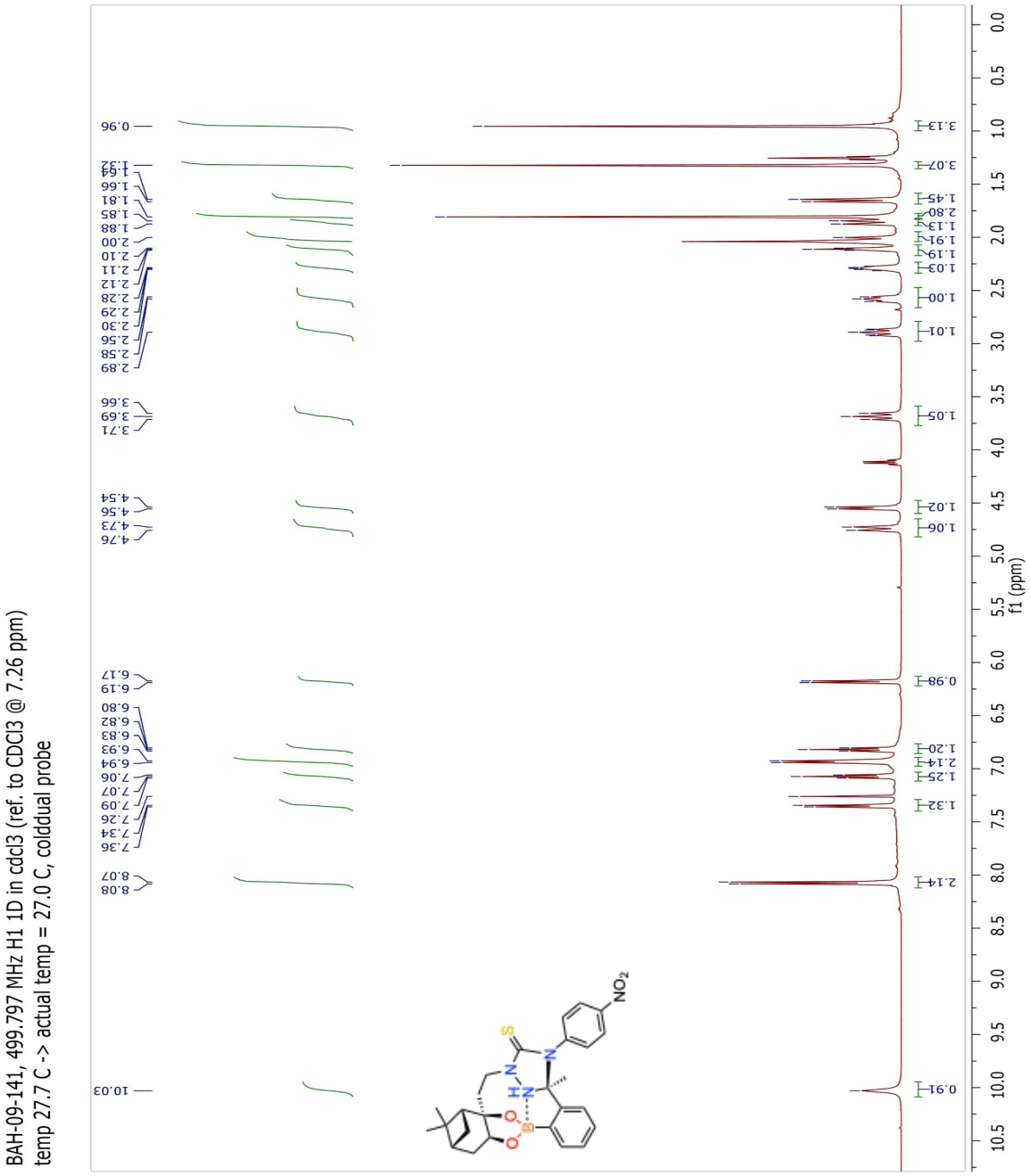


Figure S5: ^{1}H NMR spectrum of compound 4. Note: trace residual EtOAc is present (1.25, 2.05, 4.15 ppm).

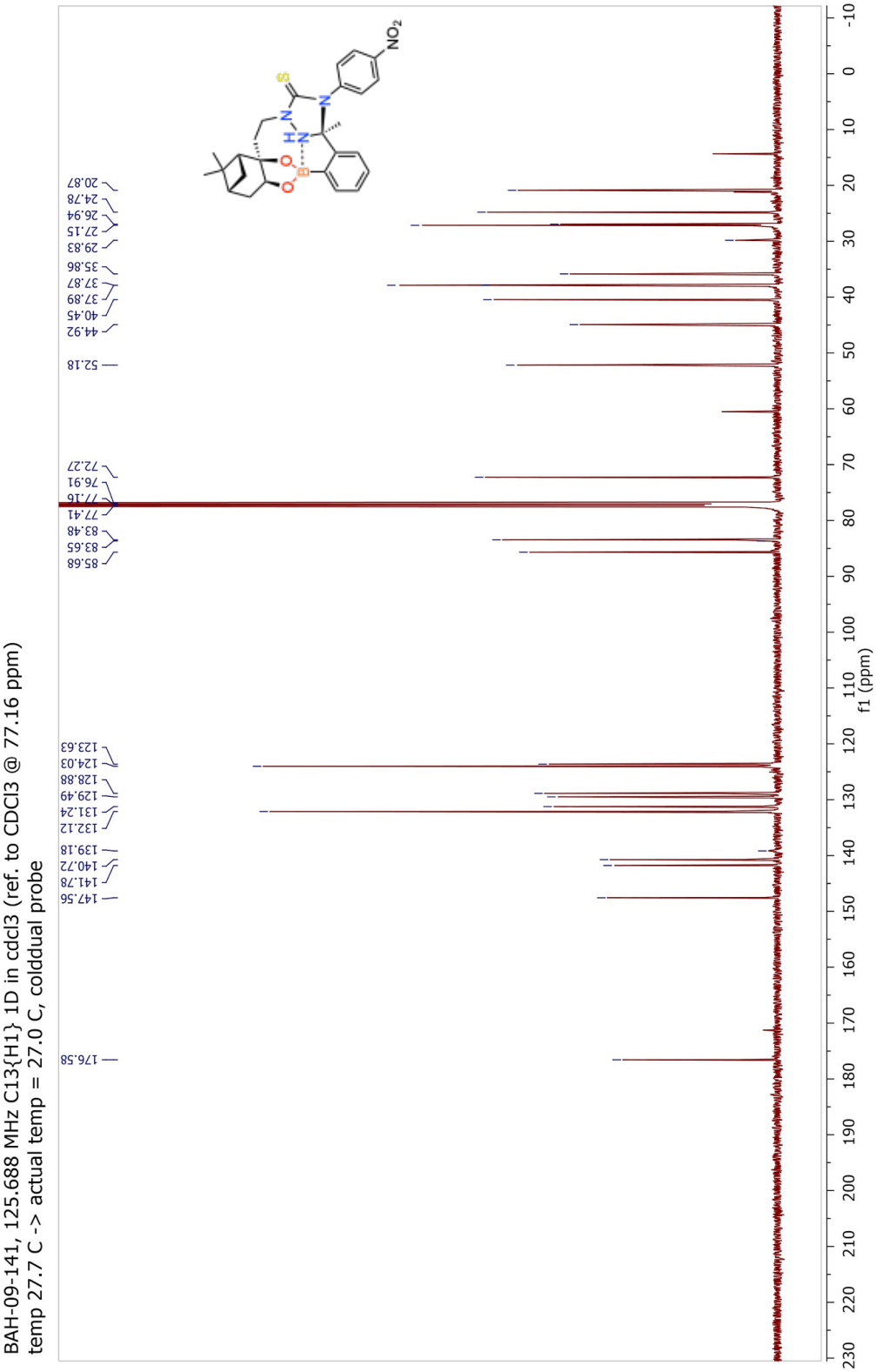


Figure S6: ^{13}C NMR spectrum of compound 4. Note: trace residual EtOAc is present (60, 21, 15 ppm).

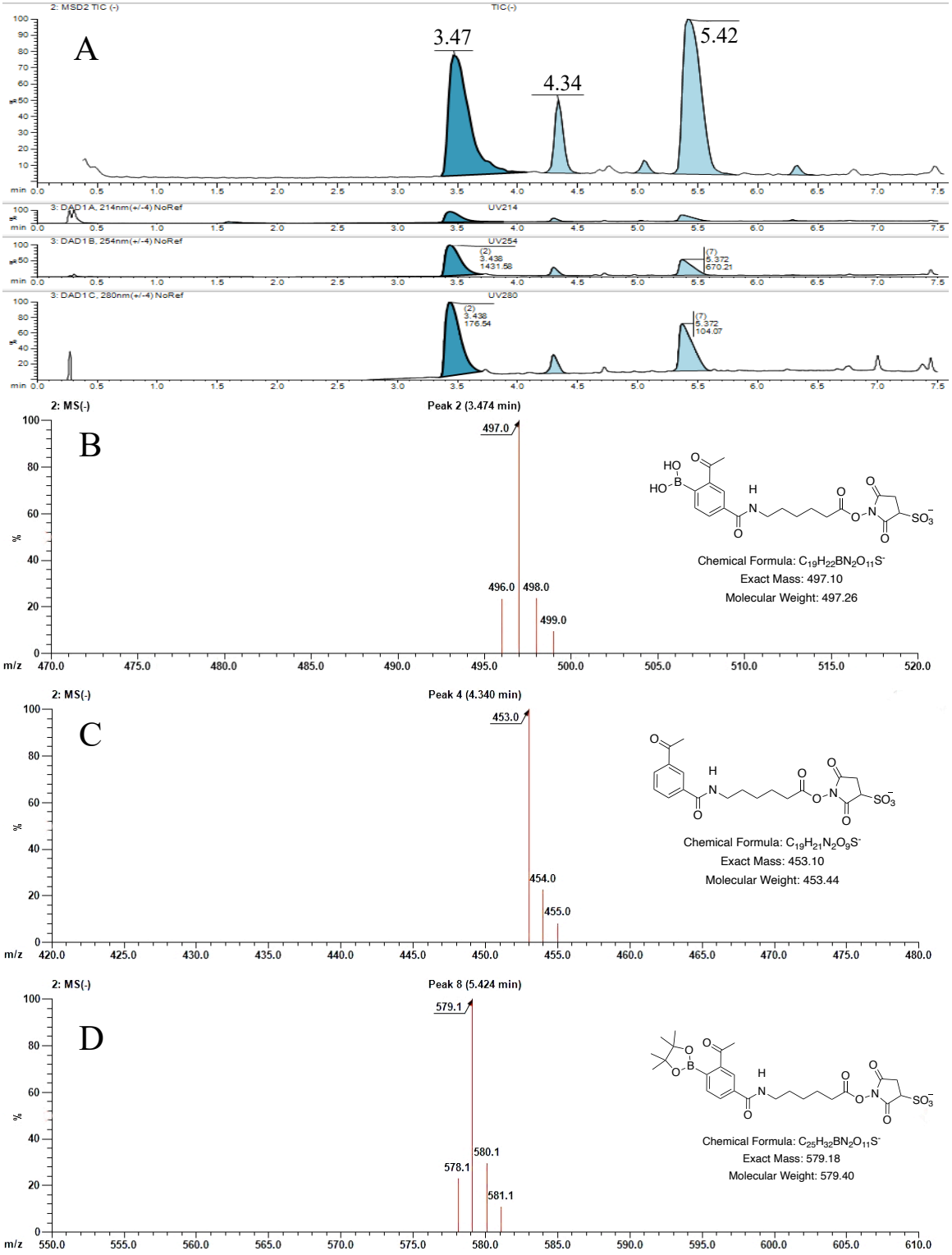


Figure S7: A: LC-MS Spectrum of compound **7**. **B.** TIC of peak at 3.5 min and proposed associated chemical structure for liberated boronic acid. **C.** TIC of peak at 4.3 min and proposed associated chemical structure for deboronated LC/MS artifact **D.** TIC of peak at 5.4 min and proposed associated chemical structure for pinacol boronic ester product. See SI of compound **7** synthesis for chromatographic conditions and a fuller discussion of LC/MS results.