Supporting Information

Mild boroxazolidone formation and dissociation: Application toward target identification of bioactive molecules

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General Procedures:

All reactions for the synthesis of compounds was monitored by TLC silica gel $60F_{254}$ (Merck). All reagents were purchased commercially and used without further purification, unless otherwise indicated. Especially, TentaGel S-NH_2 (130 μm, 0.25 mmol/g) was purchased from Peptide Institute Inc. Avidin, Neutralized, from Egg White was obtained from Fujifilm Wako Pure Chemical Corporation. PBS was prepared by using PBS Tablet (Takara Bio Inc.). Crude materials were purified by column chromatography on silica gel 60N (63–210 μm, Kanto Chemical). NMR spectra were measured on JEOL JMN-ECA600 and JMN-ECS400 spectrometers with tetramethylsilane as an internal standard, and chemical shifts are stated as δ values. For ^{13}C-NMR, the solvent peak of acetone-d_6 or methanol-d_4 was used as a reference at 29.8 or 49.0 ppm, respectively. HRMS data were recorded on a JEOL JMS-700 MStation mass spectrometer. Purity of synthetic compounds was determined as >95% by ^{1}H NMR.

Experimental Procedures:

4-Carboxyphenyl-4'-fluorophenylborinic acid (1)

To a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (100 mg, 0.40 mmol) in THF (1.5 mL) was slowly added (4-fluorophenyl)magnesium bromide (1.6 mL, 1.6 mmol, 1.0 M in THF) under Ar at 0°C. After stirring for 2 h, the reaction mixture was poured into 1 M HCl aq. and extracted with Et_2O (3×10 mL). The organic layer was washed with sat. $NaHCO_3$ aq., 1 M HCl aq., and brine, dried over Na_2SO_4, and evaporated. The residue was purified by reversed-phased medium pressure liquid chromatography (MPLC) on C18 reversed-phased silica gel, eluting with H_2O-CH_3CN (3:1, 1:1) to afford **1** (81 mg, 83%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, J = 8.3 Hz, 2H), 7.86–7.80 (m, 4H), 7.15 (t, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ 167.7, 165.7 (d, J = 246.9 Hz), 138.4 (d, J = 7.7 Hz), 135.0, 132.7, 129.4, 115.4 (d, J = 20.0 Hz); ^{11}B NMR (128 MHz, acetone-d_6): δ 43.6; HRMS-FAB (m/z): $[M–H]^-$ calcd for $C_{13}H_9BFO_3$, 243.0629; found, 243.0634.

4-Ethylcarbamoyl-4'-fluorophenylborinic acid (2)

To a solution of **1** (106 mg, 0.40 mmol) and HOBt (116 mg, 0.90 mmol) in DCM (6.0 ml) were added DIC (0.13 ml, 0.90 mmol) and 70% Ethylamine Solution (0.069 ml, 0.90 mmol) under Ar at room temperature. After stirring for 4 h, the mixture was poured into H_2O and extracted with DCM (3×10 mL). The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The residue was purified by reversed-phased medium pressure liquid chromatography (MPLC) on C18 reversed-phased silica gel, eluting with H_2O-CH_3CN (3:2) to afford **2** (80 mg, 69%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, acetone-d_6): δ 9.33 (brs, 1H), 7.93 (d, J = 7.8 Hz, 2H), 7.86 (t, J = 7.1 Hz, 2H), 7.81 (d, J = 7.8 Hz, 2H), 7.20 (t, J = 8.9 Hz, 2H), 3.43 (quint, J = 7.1 Hz, 2H), 1.20

(t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, acetone-d_6): δ 167.0, 165.6 (d, J = 246.9 Hz), 138.4 (d, J = 8.6 Hz), 137.6, 135.1, 127.0, 115.4 (d, J = 20.0 Hz), 35.2, 15.2; ^{11}B NMR (128 MHz, acetone-d_6): δ 43.6; ^{19}F NMR (376 MHz, acetone-d_6): δ -110.9; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{16}FNO_2B$, 272.1258; found, 272.1254.

***N*-Ethyl-4-[2-(4-fluorophenyl)-4-isobutyl-5-oxo-1,3,2λ^4-oxazaborolidin-2-yl]benzamide (3)**

To a solution of **2** (66 mg, 0.20 mmol) in EtOH (0.50 ml) was added L-leucine (31 mg, 0.20 mmol) in H_2O (0.50 ml). After stirring for 4 h at 80°C, the mixture was evaporated. The solid was washed with hexane, Et_2O and acetone to afford **3** (72 mg, 79%) as a diastereomer mixture. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.75–7.72 (m, 2H), 7.62 (brs, 1H), 7.55–7.52 (m, 2H), 7.50–7.46 (m, 2H), 7.00–6.95 (m, 2H), 6.15 (brs, 1H), 3.89–3.79 (m, 1H), 3.42–3.35 (m, 2H), 1.99–1.89 (m, 1H), 1.86–1.69 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H), 0.92–0.90 (m, 6H); ^{13}C NMR (150 MHz, acetone-d_6): δ 174.2, 167.5, 163.1 (d, J = 247.5 Hz), 134.3, 134.1, 133.9 (d, J = 7.2 Hz; two different peaks are overlapped), 131.9, 131.8, 126.8, 126.7, 114.7 (d, J = 19.1 Hz), 114.6 (d, J = 19.1 Hz), 54.9 (d, J = 15.2 Hz), 54.7 (d, J = 15.2 Hz), 40.3, 40.2, 35.0, 25.4, 23.2, 21.2, 15.3; ^{11}B NMR (128 MHz, acetone-d_6); δ 3.7; ^{19}F NMR (564 MHz, acetone-d_6): δ -118.1; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{27}FNO_3B$, 385.2099; found, 385.2089.

***N*-Ethyl-4-[(4*S*)-2-(4-fluorophenyl)-5-oxo-4-(4-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamido}butyl)-1,3,2λ^4-oxazaborolidin-2-yl]benzamide (4)**

To a solution of **2** (9.7 mg, 0.036 mmol) in DMF (2.3 ml) was added biocytin [13 mg, 0.75 mL, 0.036 mmol, 48 mM in phosphate buffer (0.1 M, pH 7.4)] at room temperature. After stirring for 1 h at 37°C, the mixture was poured into H_2O and extracted with EtOAc (3×10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by C18 reversed-phase preparative TLC, eluting with MeOH-H_2O (3:1) to afford **4** (18 mg, 80%) as a diastereomer mixture. ^{1}H NMR (400 MHz, CD_3OD): δ 7.71–7.69 (m, 2H), 7.55–7.53 (m, 2H), 7.45–7.41 (m, 2H), 6.97–6.94 (m, 2H), 4.48–4.45 (m, 1H), 4.29–4.26 (m, 1H), 3.66–3.60 (m, 1H), 3.41–3.37 (m, 2H), 3.20–3.12 (m, 3H), 2.90–2.85 (m, 1H), 2.69–2.66 (m, 1H), 2.18–2.15 (m, 2H), 1.98–1.91 (m, 1H), 1.74–1.75 (m, 5H), 1.51–1.39 (m, 6H), 1.22–1.19 (m, 3H); ^{13}C NMR (150 MHz, CD_3OD): δ 177.24, 177.22, 176.1, 170.7, 170.6, 166.1, 163.8 (d, J = 247.2 Hz), 134.12 (d, J = 6.7 Hz), 134.08 (d, J = 6.7 Hz), 133.9, 133.8, 132.4, 132.3, 127.2, 127.1, 115.1 (d, J = 20.1 Hz), 115.0 (d, J = 20.1 Hz), 63.4, 61.6, 57.0, 56.9, 56.7, 41.0, 39.8, 36.8, 35.7, 30.9, 30.8, 30.0, 29.7, 29.5, 26.8, 24.32, 24.30, 15.0; ^{11}B NMR (128 MHz, CD_3OD); δ 3.7; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{42}FN_5O_5SB$, 626.2984; found, 626.2978.

Borinic acid beads (5)

To a solution of **1** (9.2 mg, 0.038 mmol), HOBt (10 mg, 0.080 mmol) and DMAP (9.2 mg, 0.080

mmol) in DMF (1.0 ml) were added TentaGel S-NH_2 resin (100 mg, 0.030 mmol of -NH_2 group) and EDCI (0.014 ml, 0.08 mmol). After stirring for 7h at room temperature, the resulting gel resins were washed with MeOH, DCM, and DMF (three times each) and stored in DMF. Complete coupling was confirmed by the Kaiser ninhydrin test.

Boroxazolidone beads (6)

To a solution of **5** (25 mg, 6.3 μmol) in DMF (0.37 ml) was added biocytin [2.3 mg, 0.12 mL, 6.3 μmol, 53 mM in phosphate buffer (0.1 M, pH 7.4)] at room temperature. After stirring for 1 h at 37°C, the resulting gel resins were washed with DMF three times at 0°C and stored in DMF. The boroxazolidone formation was confirmed by the Kaiser ninhydrin test.

4-[(4*S*)-2-(4-Fluorophenyl)-5-oxo-4-(4-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamido}butyl)-1,3,2λ^4-oxazaborolidin-2-yl]benzoic acid (7)

To a solution of 3 (6.5 mg, 0.027 mmol) in DMF (1.6 ml) was added biocytin [10 mg, 0.53 mL, 0.027 mmol, 50 mM in phosphate buffer (0.1 M, pH 7.4)] at room temperature. After stirring for 1 h at 37°C, the mixture was evaporated. The residue was purified by C18 reversed-phase preparative TLC, eluting with CH_3CN-H_2O (1:1) to afford **7** (7.1 mg, 44%) as a diastereomer mixture. ^{1}H NMR (400 MHz, CD_3OD): δ 7.97–7.94 (bm, 1H), 7.92–7.90 (m, 2H), 7.58–7.55 (m, 2H), 7.46–7.41 (m, 2H), 6.99–6.94 (m, 2H), 4.48–4.45 (m, 1H), 4.30–4.26 (m, 1H), 3.68–3.60 (m, 1H), 3.20–3.14 (m, 3H), 2.91–2.85 (m, 1H), 2.69–2.65 (m, 1H), 2.19–2.14 (m, 2H), 1.98–1.90 (m, 1H), 1.76–1.55 (m, 5H), 1.51–1.37 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 177.2, 176.1, 170.8, 170.7, 166.1, 163.7 (d, J = 242.5 Hz), 134.11 (d, J =6.7 Hz), 134.07 (d, J =6.7 Hz), 132.25, 132.20, 130.1, 130.0, 129.69, 129.66, 115.1 (d, J = 20.1 Hz), 115.0 (d, J = 20.1 Hz), 63.4, 61.6, 57.0, 56.8, 56.7, 41.0, 39.82, 39.79, 36.8, 36.7, 30.9, 30.8, 30.0, 29.7, 29.5, 26.8, 24.31, 24.26; ^{11}B NMR (128 MHz, CD_3OD); δ 4.9; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{37}FN_4O_6SB$, 599.2511; found, 599.2504

***N*-(2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethyl)-4-[(fluorophenyl)(hydroxy)boraneyl] benzamide (8)**

To a solution of **1** (4.5 mg, 0.020 mmol), HOBt (5.4 mg, 0.040 mmol) and DMAP (4.9 mg, 0.040 mmol) in DMF (1.0 ml) were added EDCI (8.5 μl, 0.040 mmol), 11-Azido-3,6,9-trioxaundecan-1-amine (7.9 μl, 0.040 mmol) under Ar. After stirring for overnight at rt, the mixture was poured into H_2O and extracted with EtOAc (3×10 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The mixture was purified by reversed-phase preparative TLC on Silica gel eluting with MeOH/ H_2O (3:1) to afford **7** (7.2 mg, 80%) as a colorless oil. ^{1}H NMR (600 MHz, acetone-d_6): δ 9.30 (brs, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.87 (dd, J = 8.4, 6.3 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.79 (brs, 1H), 7.20 (t, J = 9.0 Hz, 2H), 3.67–3.64 (m, 4H), 3.63–3.62 (m, 8H), 3.60–3.57 (m,

2H), 3.36 (t, J = 4.9 Hz, 2H); ^{13}C NMR (150 MHz, acetone-d_6): δ 167.3, 165.6 (d, J = 248.5 Hz), 138.4 (d, J = 8.3 Hz), 137.4, 135.1, 127.1, 115.4 (d, J = 20.2 Hz), 71.23, 71.22, 71.1, 70.9, 70.6, 70.2, 51.3, 43.0; ^{11}B NMR (128 MHz, acetone-d_6): δ 43.0; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{27}FN_4O_5B$, 445.2059; found, 445.2062.

_N_-(2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}ethyl)-4-[(4_S_)-2-(4-fluorophenyl)-5-oxo-4-(4-{5-[(3a_S_,4_S_,6a_R_)-2-oxohexahydro-1_H_-thieno[3,4-_d_]imidazol-4-yl]pentanamido}butyl)-1,3,2λ^4-oxazaborolidin-2-yl]benzamide (9)

To a solution of **8** (4.5 mg, 9.8 μmol) in DMF (0.58 ml) was added biocytin [0.19 mL, 9.8 μmol, 50 mM in phosphate buffer (0.1 M, pH 7.4)] at room temperature. After stirring for 1 h at 37°C, the mixture was evaporated. The residue was purified by C18 reversed-phase preparative TLC, eluting with CH_3CN-H_2O (1:1) to afford **9** (5.1 mg, 66%) as a diastereomer mixture. ^{1}H NMR (400 MHz, CD_3OD) : δ 7.73–7.72 (m, 2H), 7.57–7.54 (m, 2H), 7.46–7.41 (m, 2H), 6.98–6.94 (m, 2H), 4.49–4.45 (m, 1H), 4.30–4.26 (m, 1H), 3.66–3.60 (m, 10H), 3.58–3.52 (m, 7H), 3.19–3.13 (m, 3H), 2.91–2.84 (m, 1H), 2.69–2.65 (m, 1H), 2.18–2.15 (m, 2H), 2.00–1.89 (m, 1H), 1.74–1.56 (m, 5H), 1.52–1.40 (m, 6H); ^{13}C NMR (150 MHz, CD_3OD): δ 177.24, 177.21, 176.1, 170.9, 170.8, 166.1, 163.75 (d, J = 243.1 Hz), 163.73 (d, J = 243.1 Hz), 134.11 (d, J = 6.8 Hz), 134.07 (d, J = 6.8 Hz), 133.7, 133.6, 127.3, 127.4, 115.1 (d, J = 19.8 Hz), 115.0 (d, J = 19.8 Hz), 71.6, 71.5, 71.3, 71.1, 70.6, 63.4, 61.6, 57.0, 56.9, 56.7, 51.7, 41.03, 41.01, 40.9, 39.82, 39.81, 36.7, 30.9, 30.8, 30.0, 29.7, 29.5, 26.8, 24.33, 24.30; ^{11}B NMR (128 MHz, CD_3OD): δ 4.9; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{37}H_{53}FN_8O_8SB$, 799.3784; found, 799.3802.

Sample preparation for SDS-PAGE analysis (Figure 2)

All manipulations were performed on ice. Beads **6** (prepared by method A, 5 mg) was washed with NaPB (10 mM, pH 7.4, 50 μL) five times with centrifugation (15,000 g, 30 sec) at -5°C. To the beads was added avidin (1 mg/mL, 20 μL) and incubated for 5 min at 0°C. After the addition of PBS containing 1 mM EDTA and 5% Glycerol (PBS buffer, 80 μL), the beads suspension was divided into two aliquots. The supernatant was removed, and to the beads was added PBS buffer (50 μL). The resultant supernatant was collected immediately (Figure 2b, lane 1) or after 1 h incubation at 37°C (Figure 2b, lane 2). Biocytin treated avidin (Figure 2c, lane 1, 10 μL) was prepared by adding a biocytin (10 mg/mL, 10 μL) to an avidin (1 mg/mL, 100 μL). The typical acetone precipitation method was performed to concentrate each sample before SDS-PAGE analysis, however this process can be omitted.

Sample preparation for SDS-PAGE analysis (Figure S35)

All manipulations were performed same as described above. The different parts were explained below.

Centrifugation was carried out at 0°C. Beads **6** (prepared by method B, 5 mg) was washed with NaPB (10mM, pH 7.4, 50 μL) three times. To the beads was added avidin (1 mg/mL, 10 μL) and incubated for 5 min at 0°C. After the addition of PBS buffer (40 μL), the supernatant was collected immediately (lane 2) or after 1 h incubation at 37°C (lane 3).

SDS-PAGE analysis

All reagents and devices were purchased from Bio-Rad Laboratories, Inc. SDS-PAGE was performed with Mini-PROTEAN Tetra Cell connected to PowerPac HC in constant current mode, 0.02 A. The gel was prepared using TGX FastCast Acrylamide Kit, 12%. The sample was mixed with 2x Laemmli Sample Buffer in the presence of 5% 2-mercaptoethanol and boiled at 95°C for 5 min. Precision Plus Protein All Blue Prestained Protein Standards was used as a marker. After electrophoresis, the gel was stained with Bio-Safe CBB G-250 Stain in accordance with manufacturer's protocol. The stained gel images were captured with image analyzer LAS-4000 (Fujifilm).

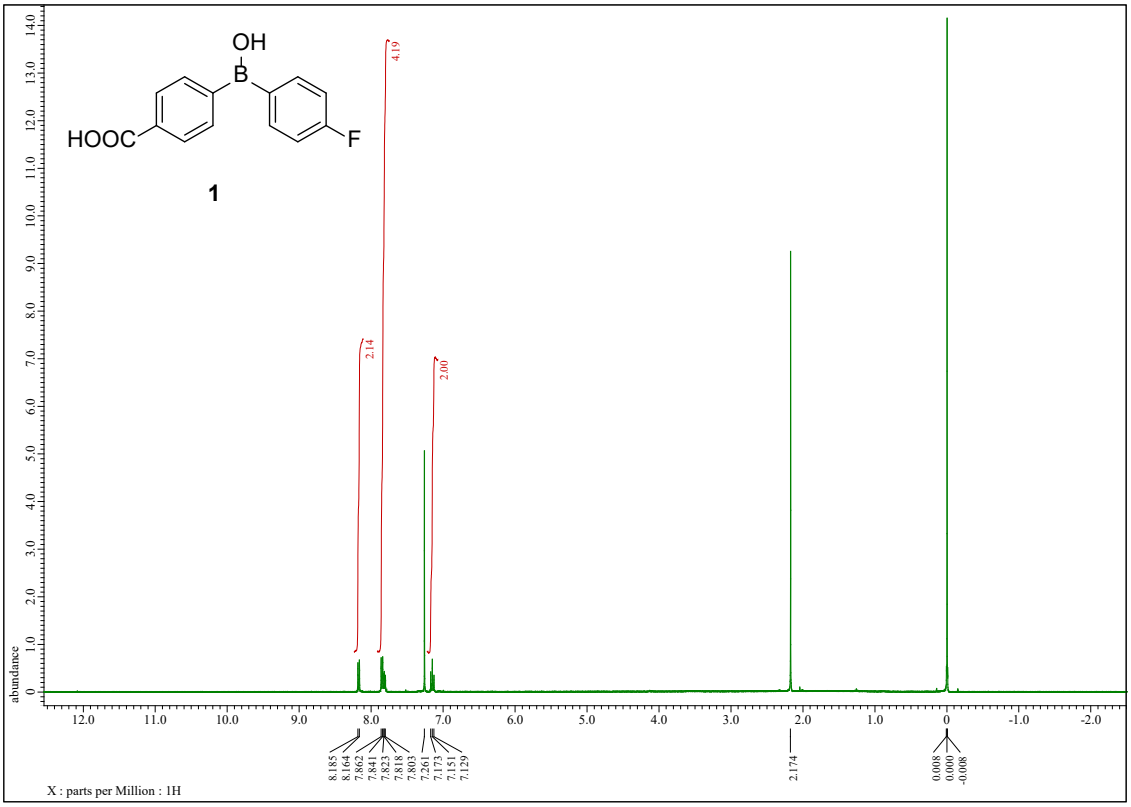


Figure S1. ^{1}H NMR spectrum of compound **1** (400 MHz, in $CDCl_3$).

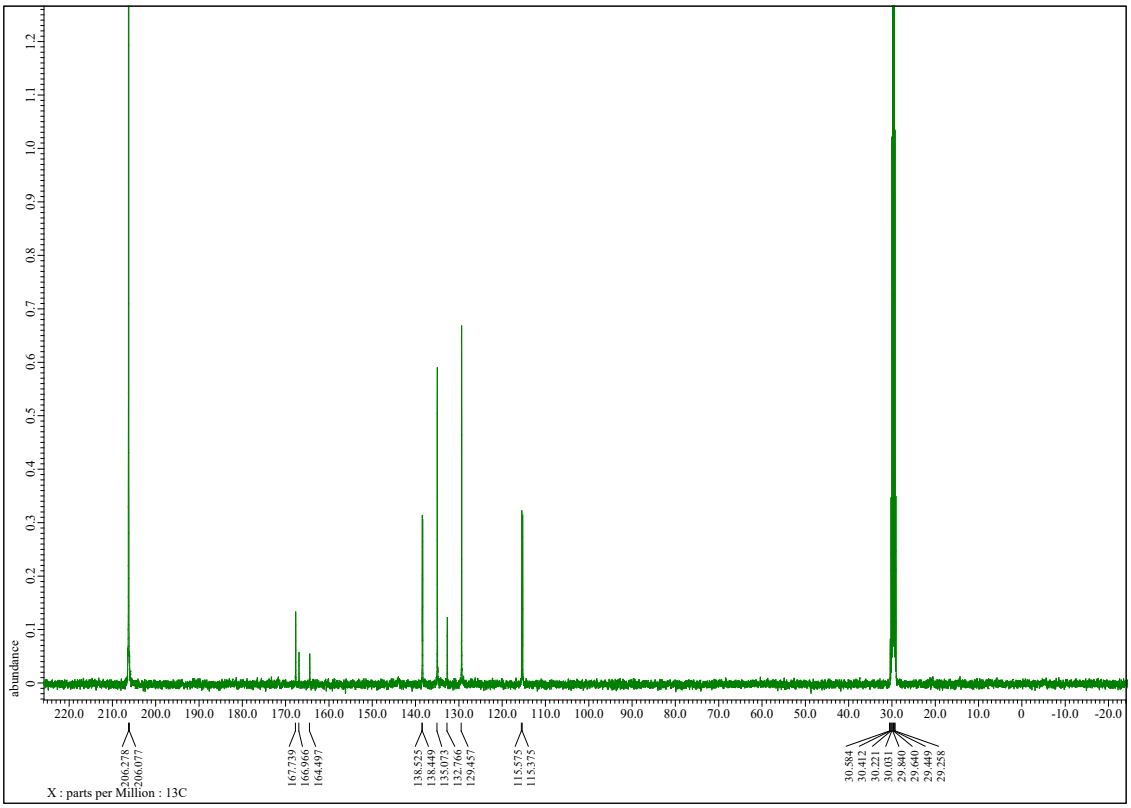


Figure S2. ^{13}C NMR spectrum of compound **1** (100 MHz, in acetone-d_6).

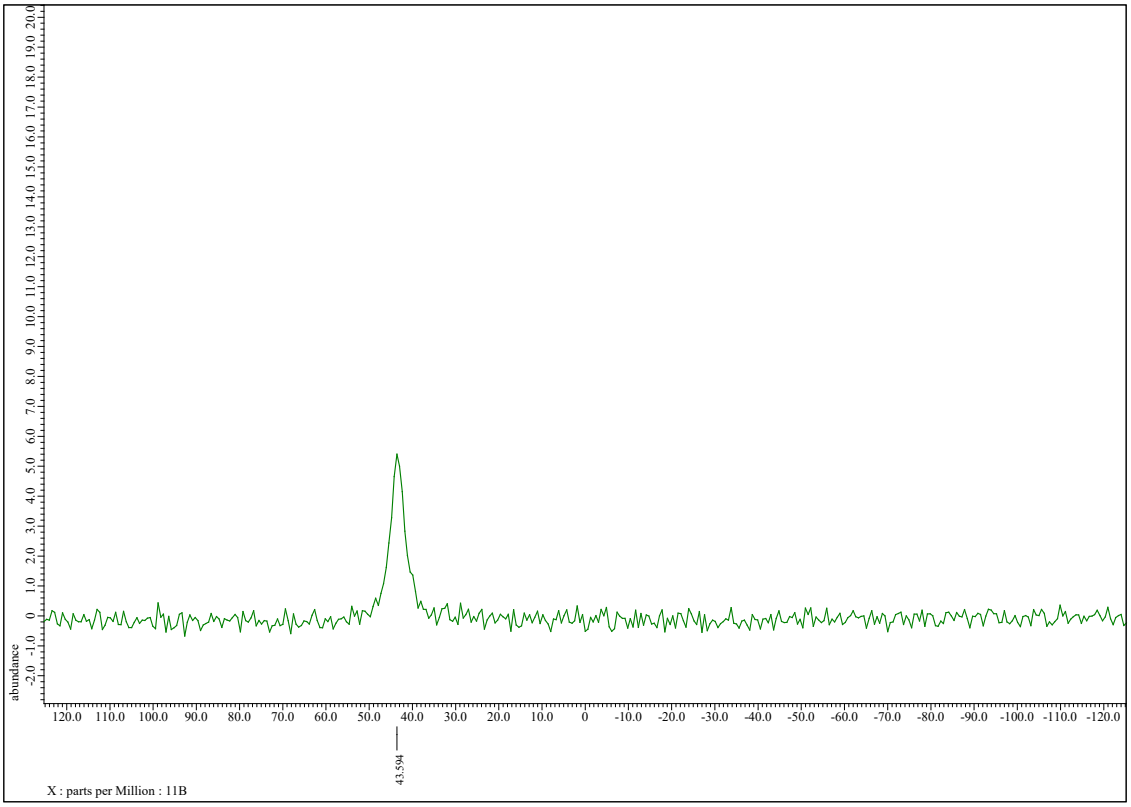


Figure S3. ^{11}B NMR spectrum of compound **1** (128 MHz, in acetone-d_6).

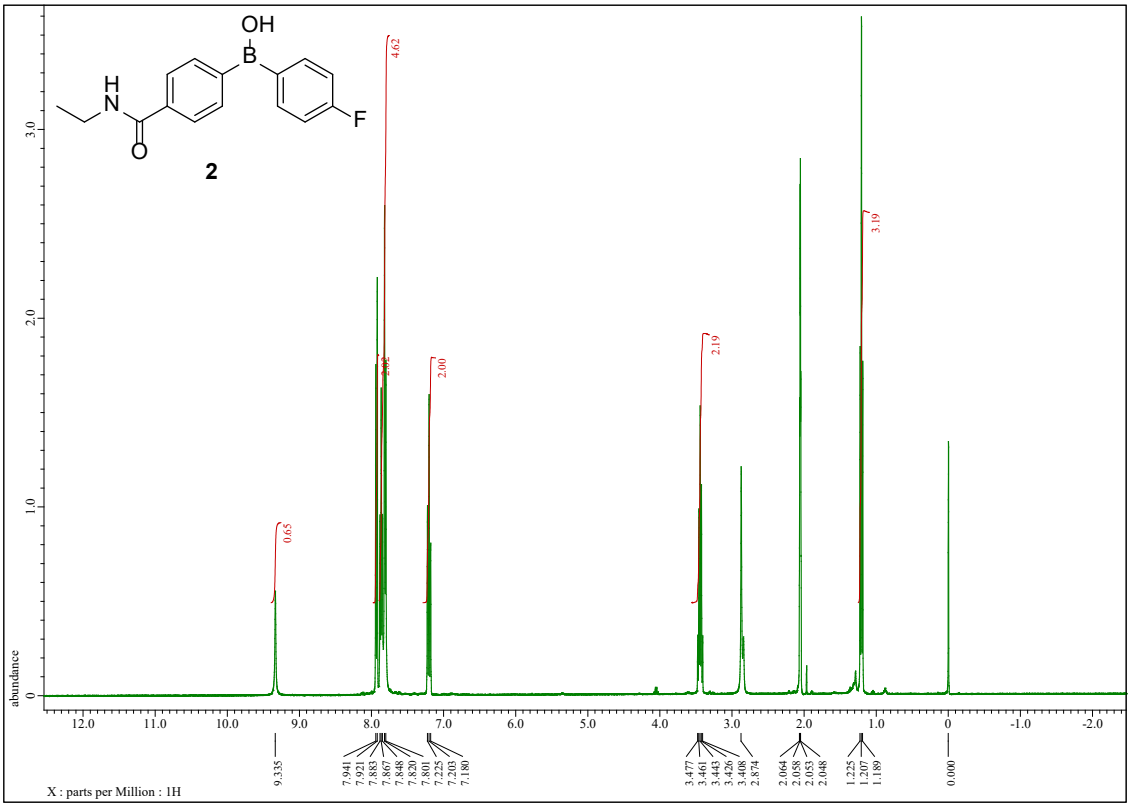


Figure S4. ^{1}H NMR spectrum of compound **2** (400 MHz, in acetone-d_6).

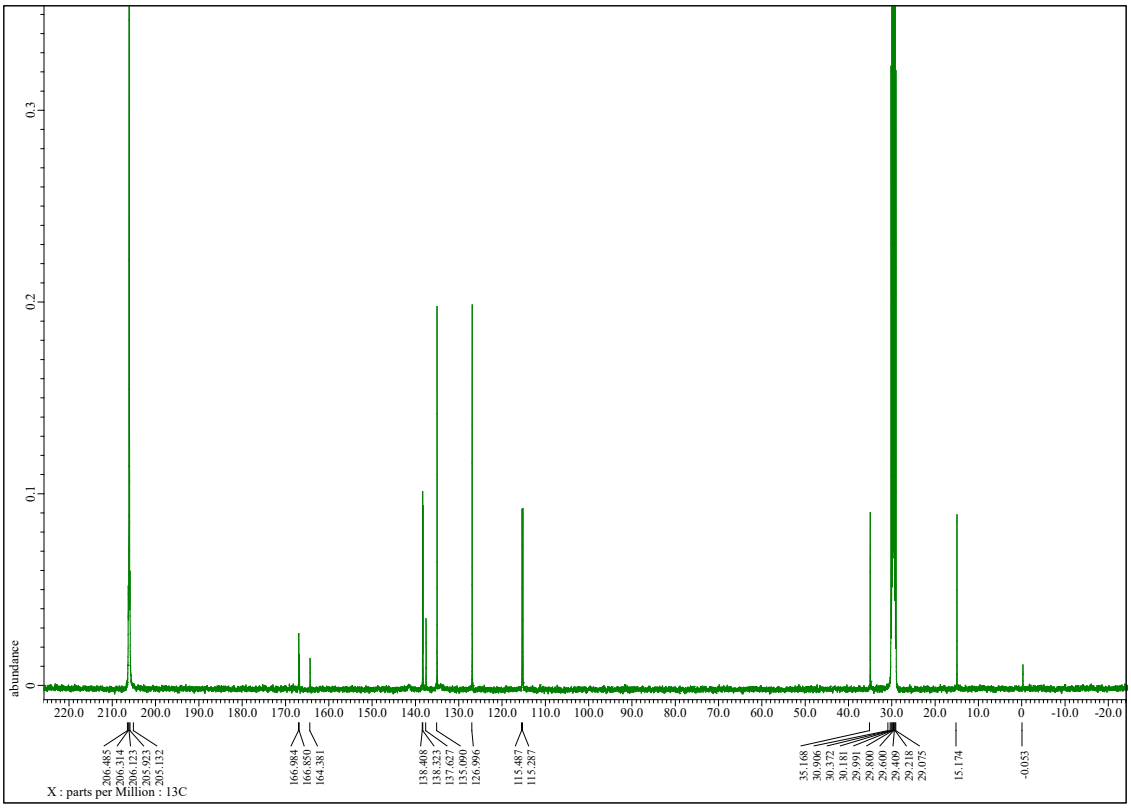


Figure S5. ^{13}C NMR spectrum of compound **2** (100 MHz, in acetone-d_6).

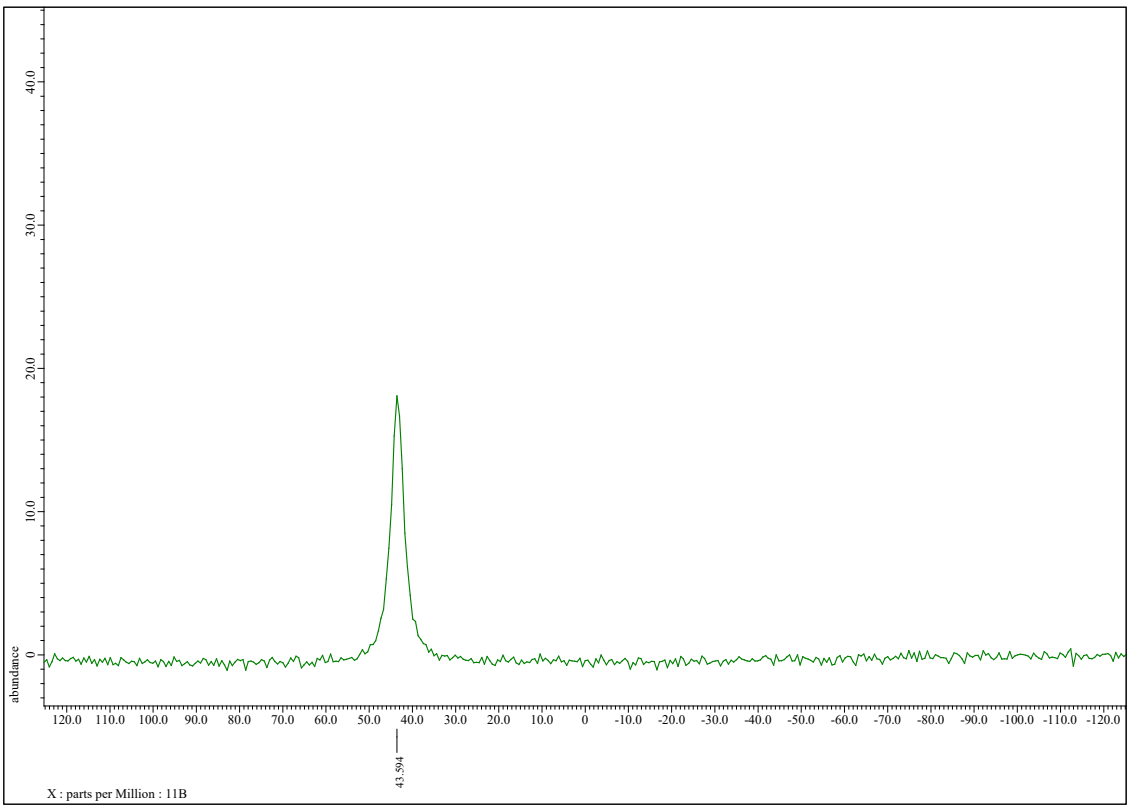


Figure S6. ^{11}B NMR spectrum of compound **2** (128 MHz, in acetone-d_6).

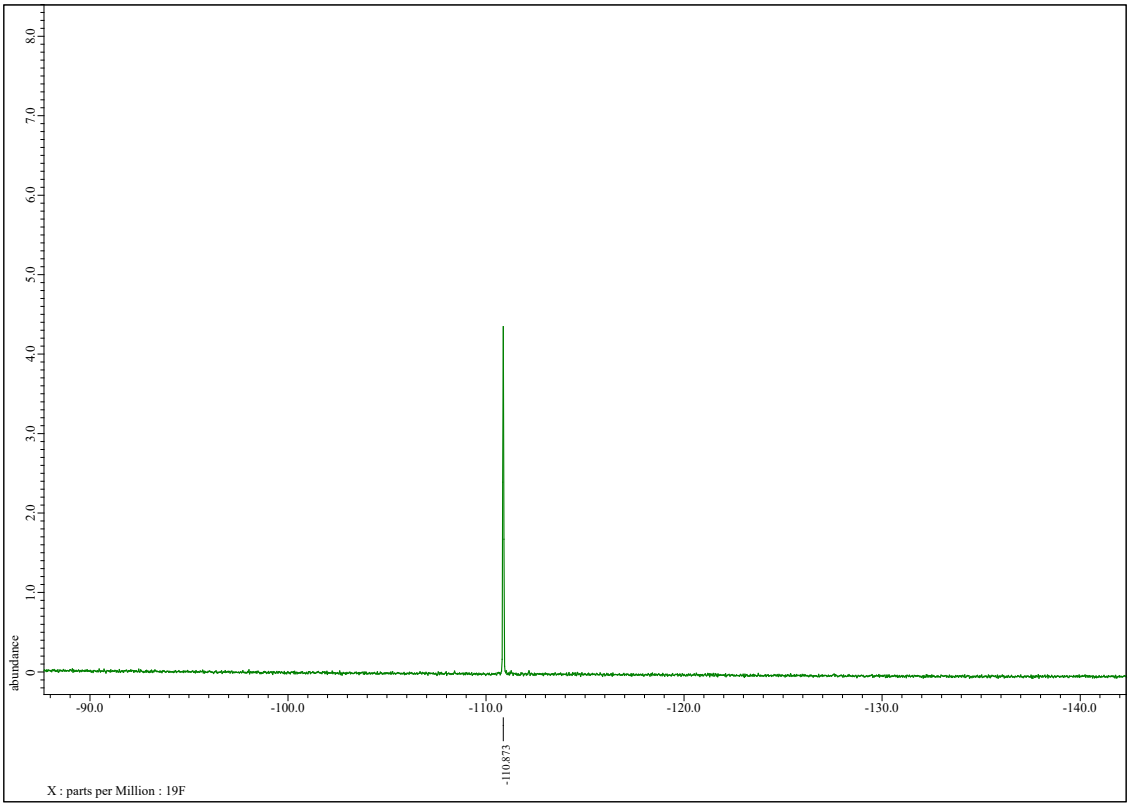


Figure S7. ^{19}F NMR spectrum of compound **2** (376 MHz, in acetone-d_6).

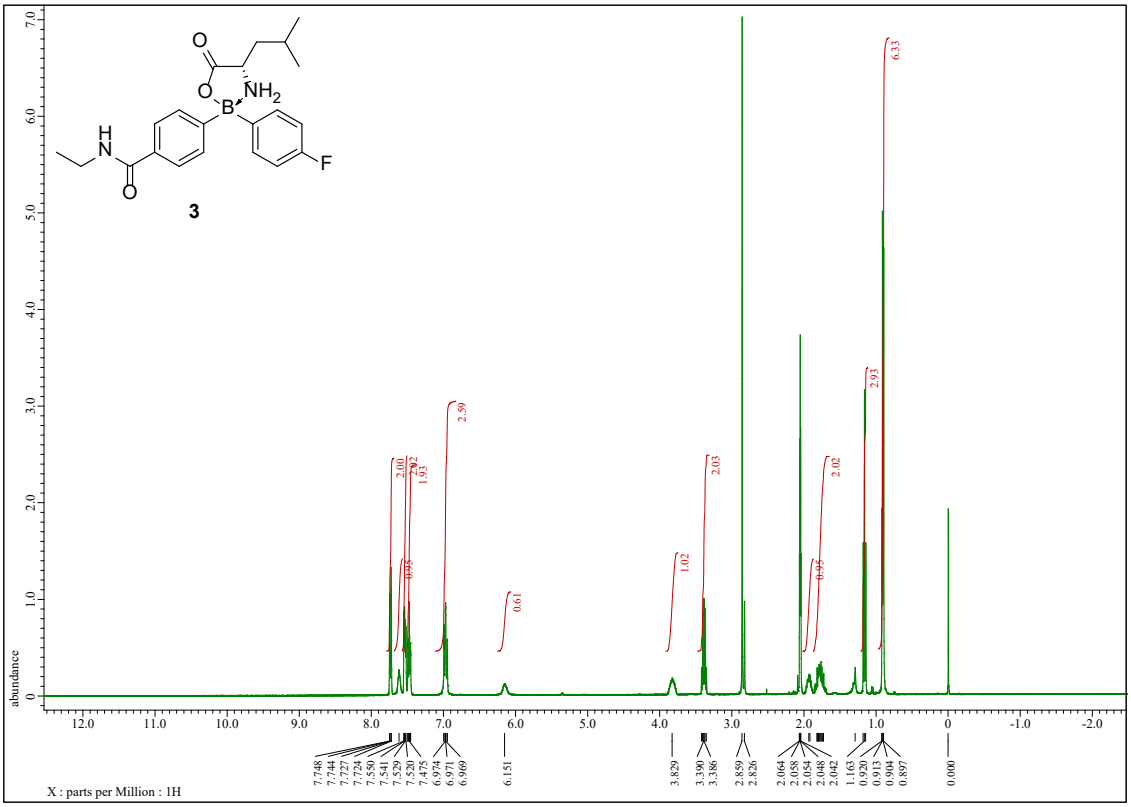


Figure S8. ^{1}H NMR spectrum of compound **3** (400 MHz, in acetone-d_6).

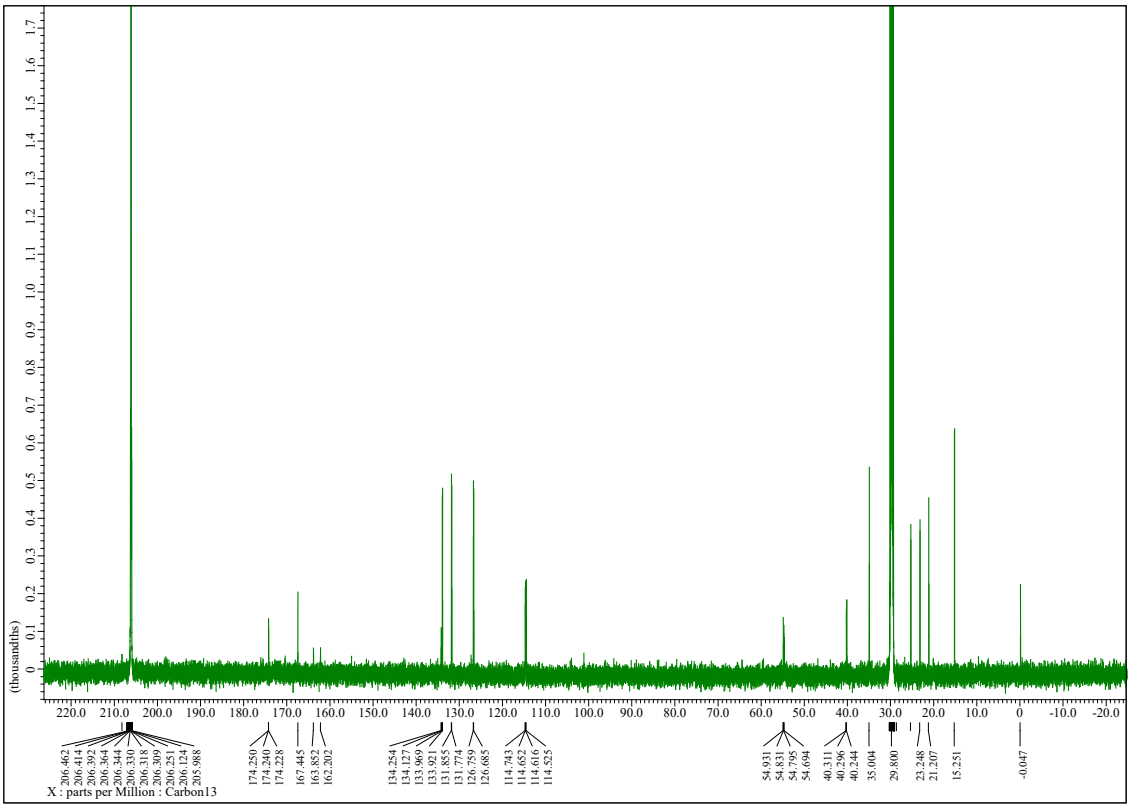


Figure S9. ^{13}C NMR spectrum of compound **3** (150 MHz, in acetone-d_6).

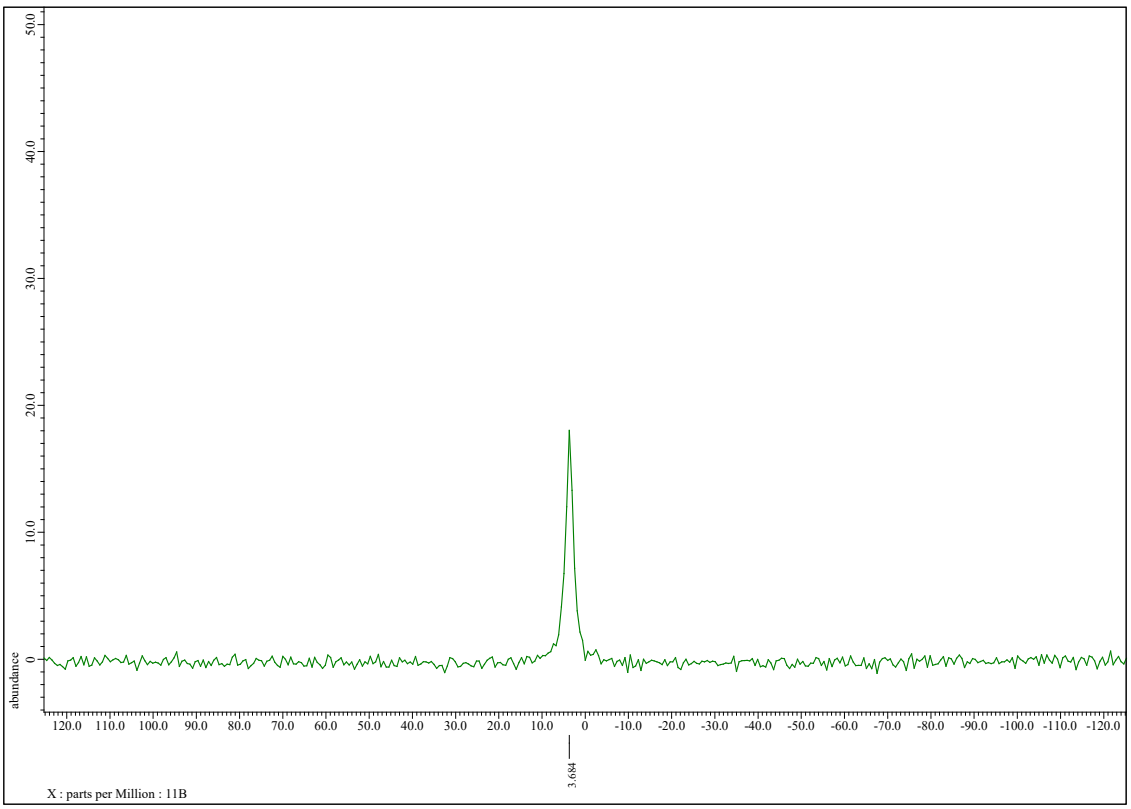


Figure S10. ^{11}B NMR spectrum of compound **3** (128 MHz, in acetone-d_6).

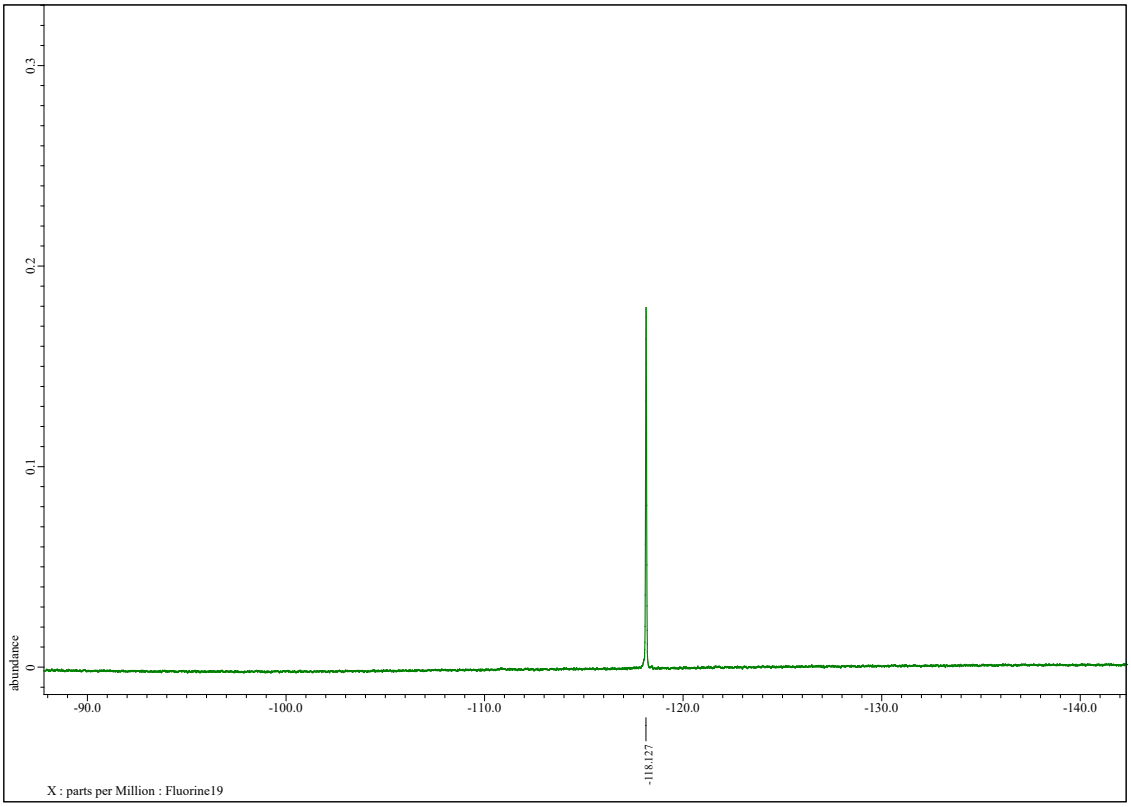


Figure S11. ^{19}F NMR spectrum of compound **3** (564 MHz, in acetone-d_6).

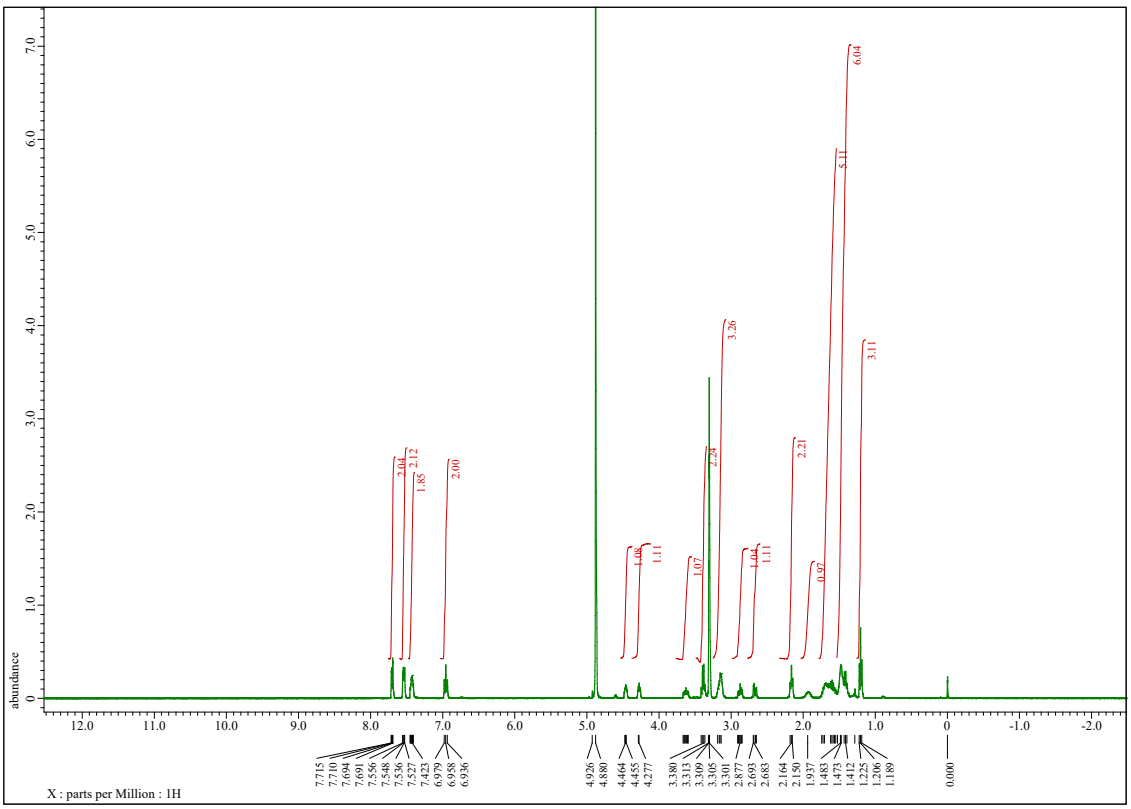


Figure S12. ^{1}H NMR spectrum of compound **4** (400 MHz, in CD_3OD).

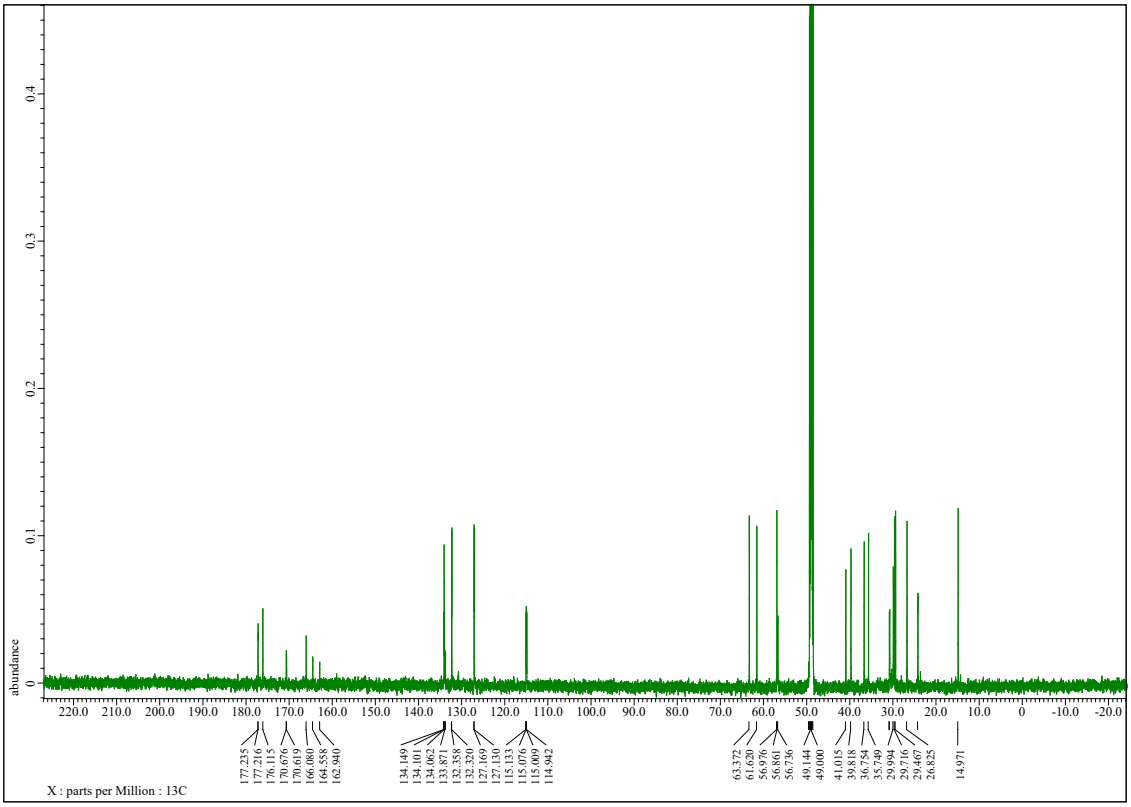


Figure S13. ^{13}C NMR spectrum of compound **4** (150 MHz, in CD_3OD).

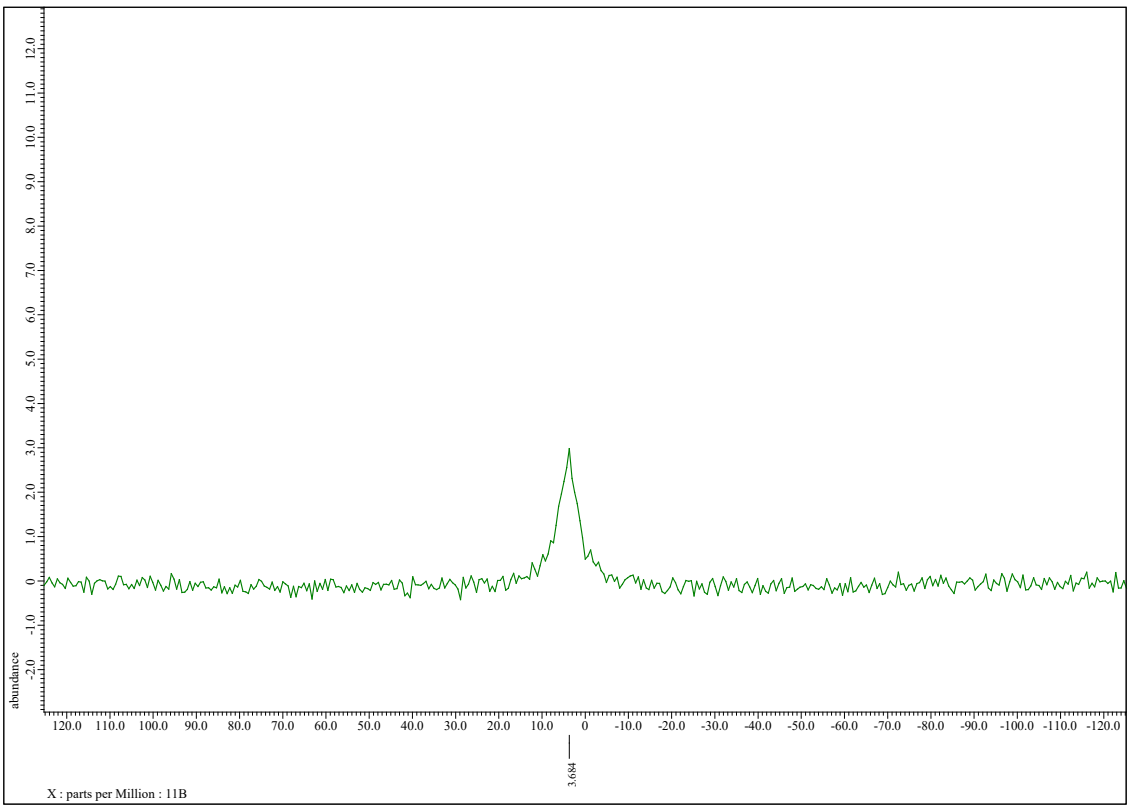


Figure S14. ^{11}B NMR spectrum of compound **4** (128 MHz, in CD_3OD).

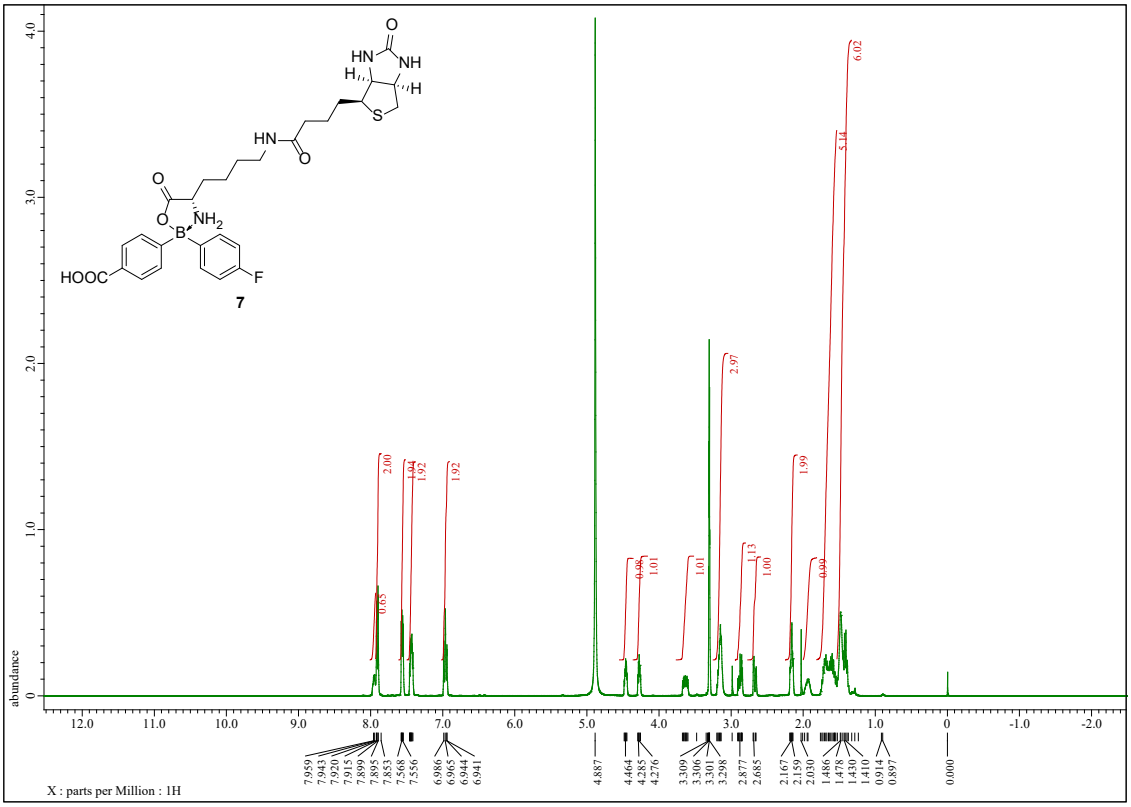


Figure S15. ^{1}H NMR spectrum of compound **7** (400 MHz, in CD_3OD).

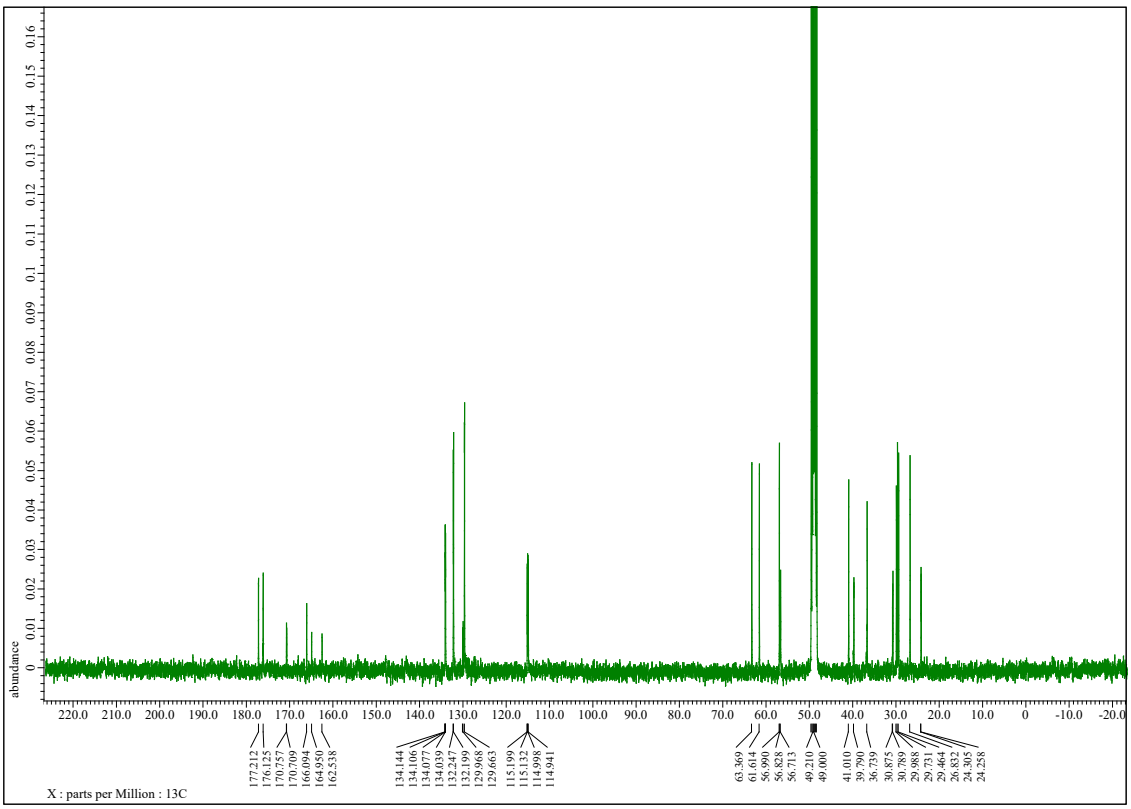


Figure S16. ^{13}C NMR spectrum of compound **7** (100 MHz, in CD_3OD).

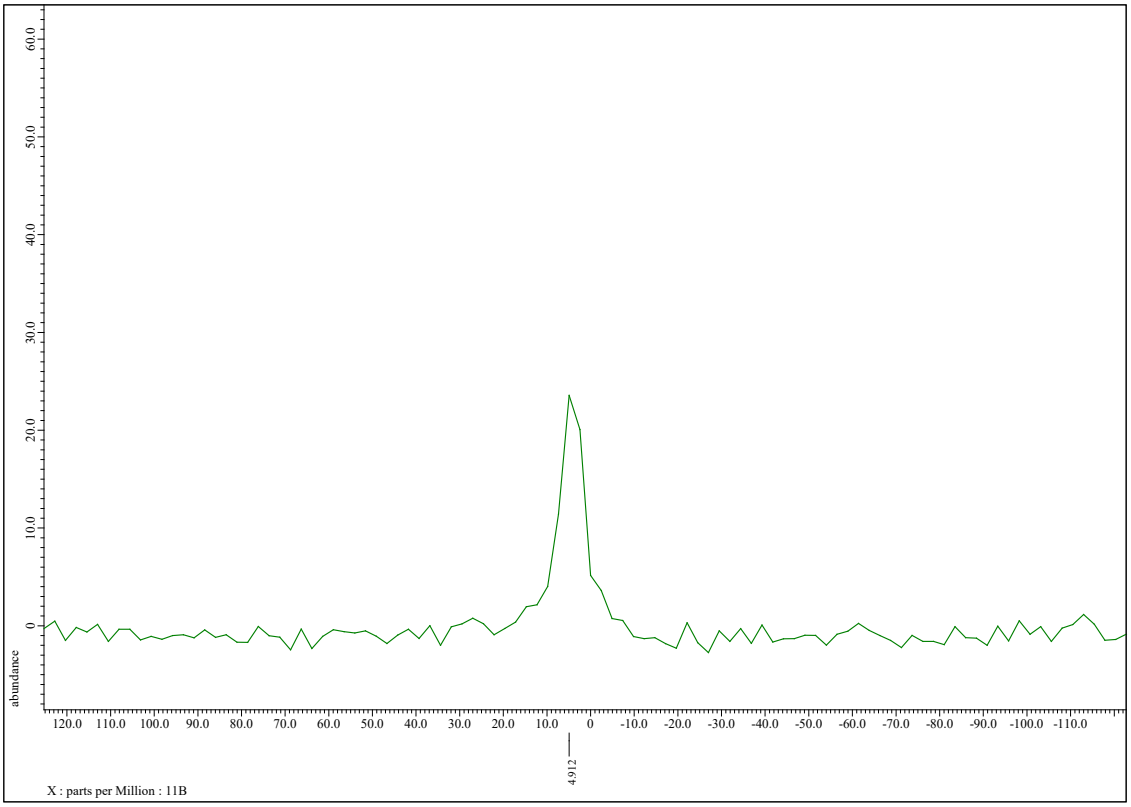


Figure S17. ^{11}B NMR spectrum of compound **7** (128 MHz, in CD_3OD).

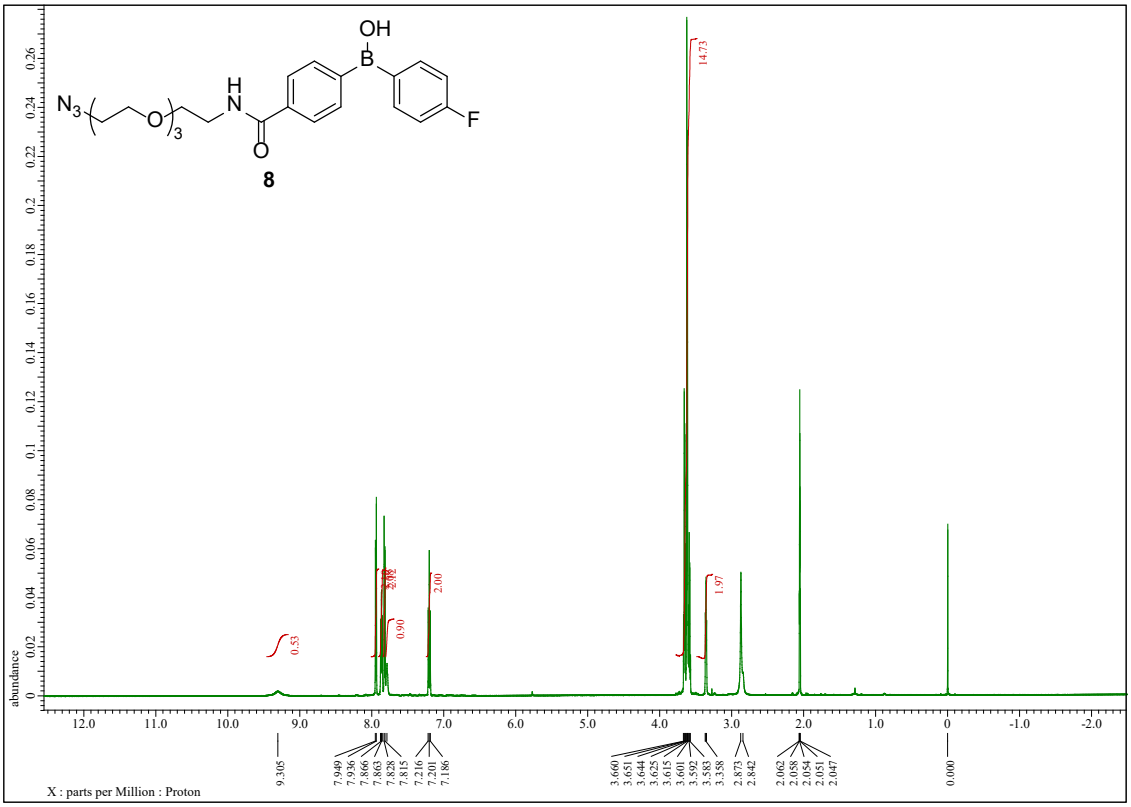


Figure S18. ^{1}H NMR spectrum of compound **8** (600 MHz, in acetone-d_6).

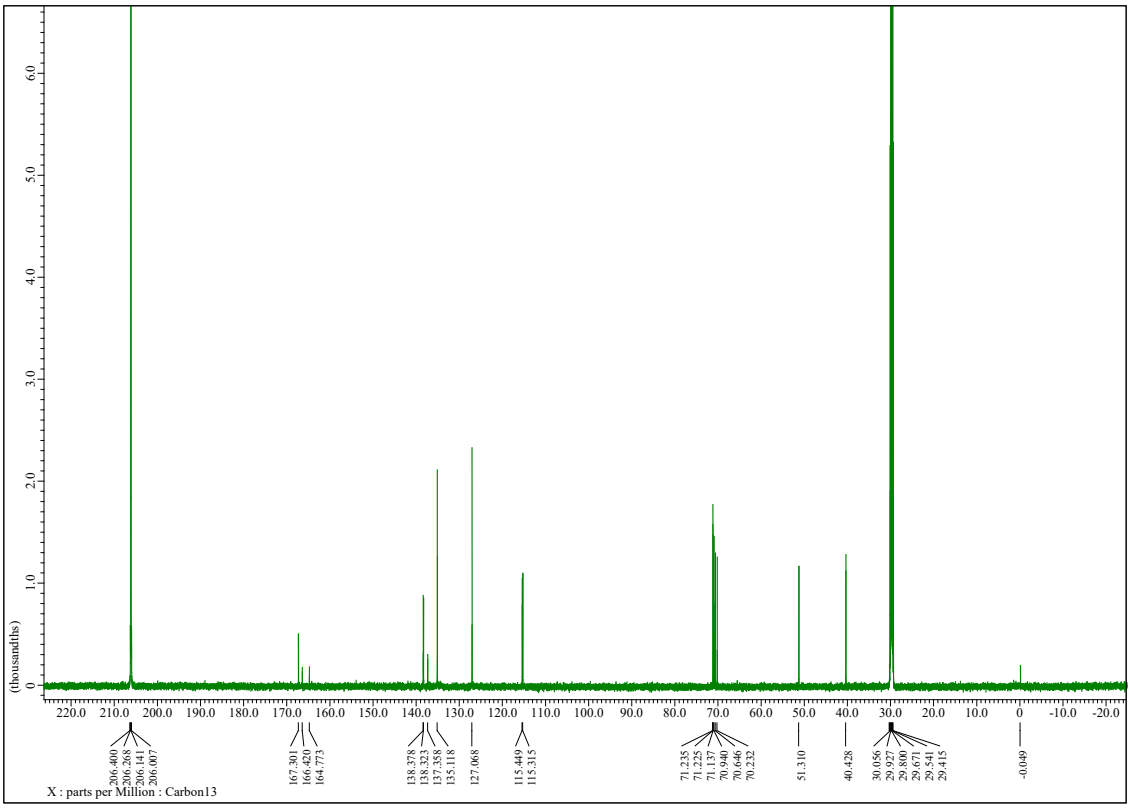


Figure S19. ^{13}C NMR spectrum of compound **8** (150 MHz, in acetone-d_6).

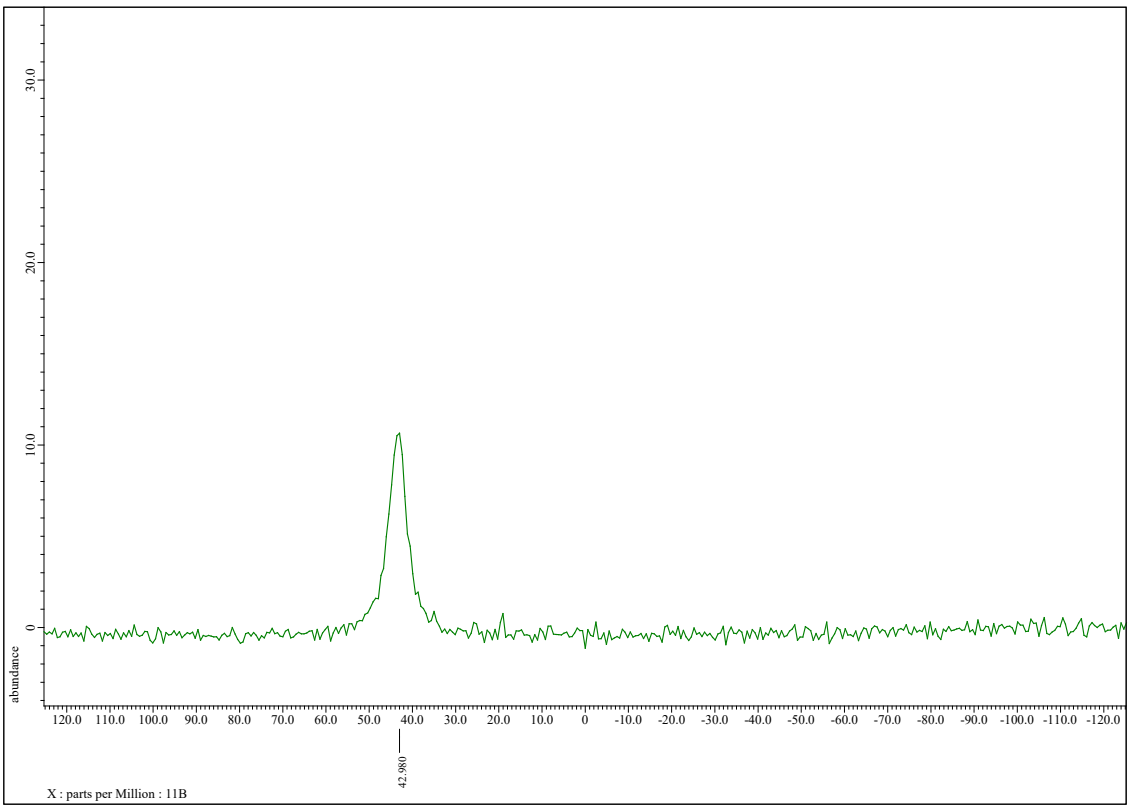


Figure S20. ^{11}B NMR spectrum of compound **8** (128 MHz, in acetone-d_6).

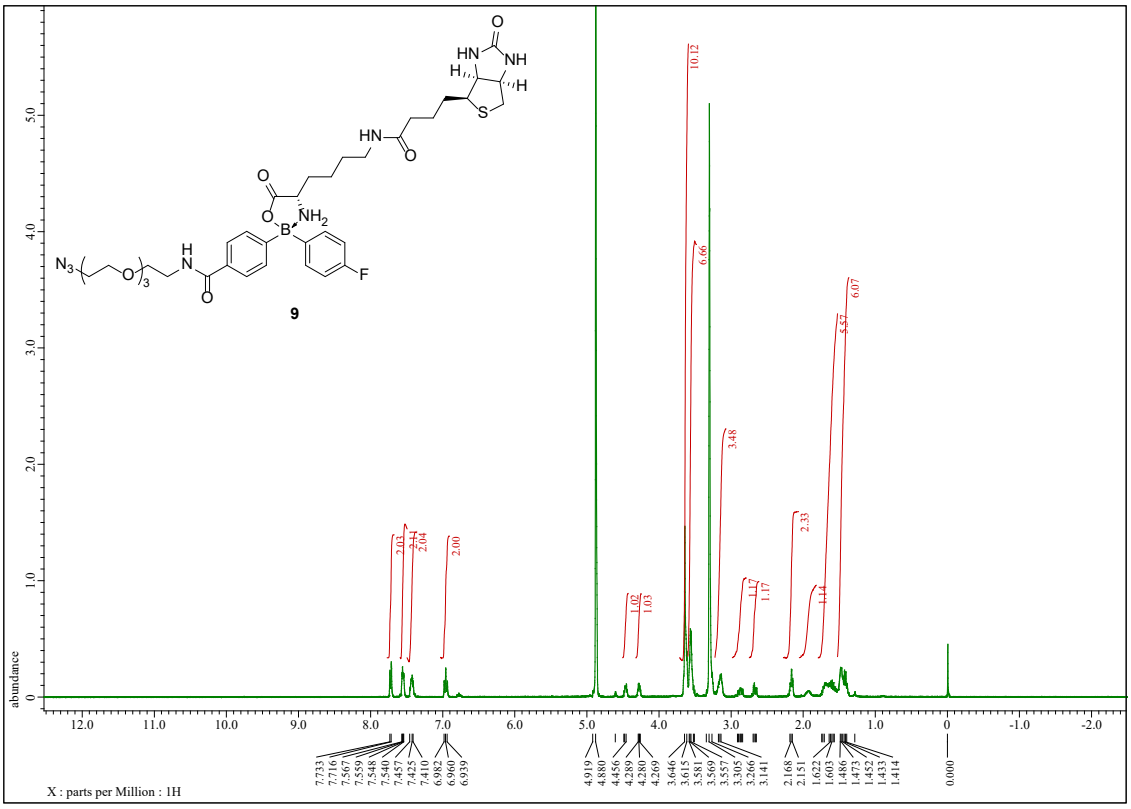


Figure S21. ^{1}H NMR spectrum of compound **9** (400 MHz, in CD_3OD).

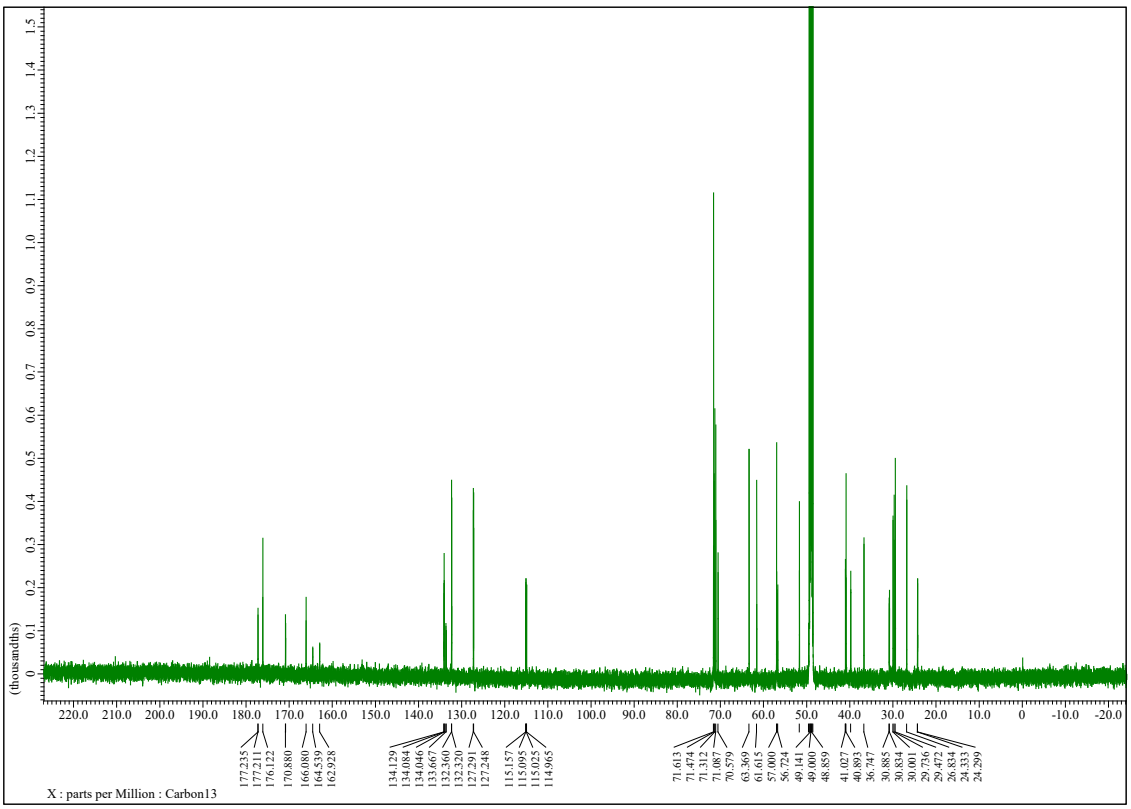


Figure S22. ^{13}C NMR spectrum of compound **9** (100 MHz, in CD_3OD).

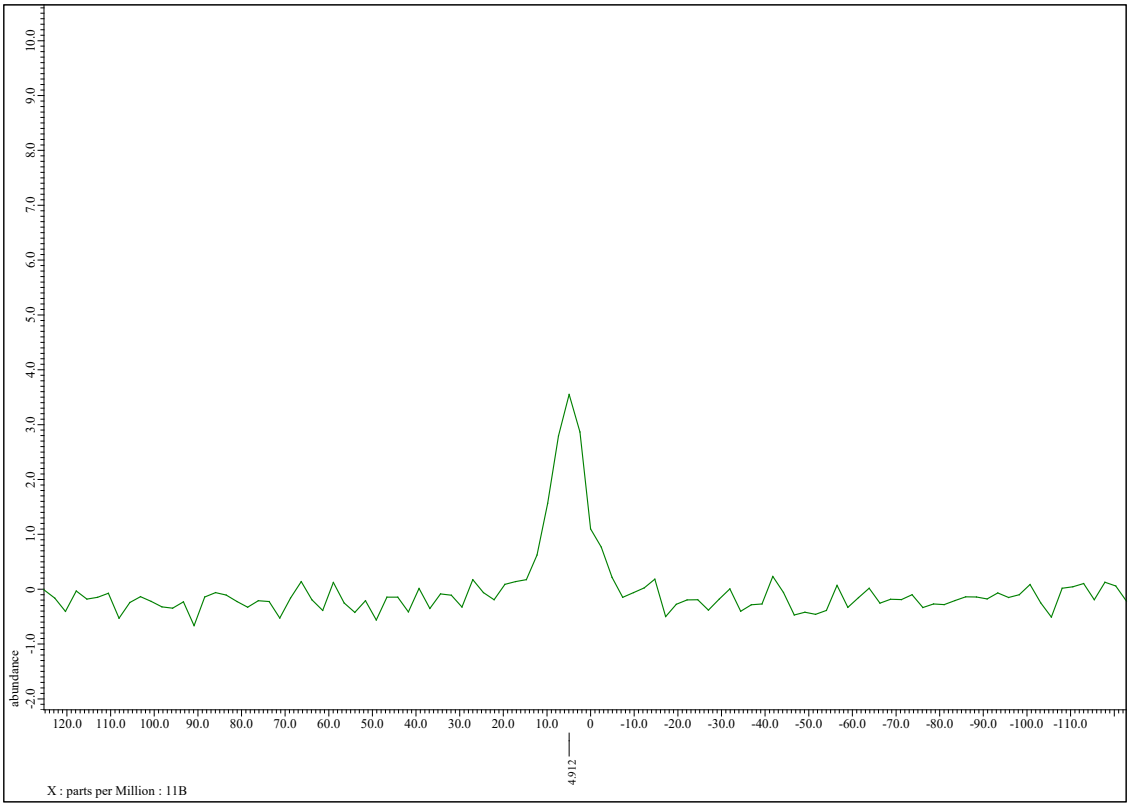
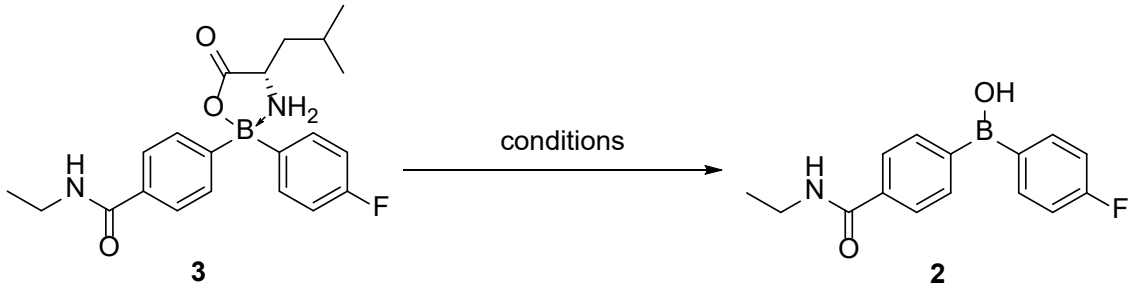


Figure S23. ^{11}B NMR spectrum of compound **9** (128 MHz, in CD_3OD).

Table S1. Dissociation of boroxazolidone **3** in various conditions.



entry	conditions	results[a]
1	12N HCl, MeOH, 2 h	full conversion to **2**[b]
2	*m*CPBA (3.0 eq), CH_2Cl_2, overnight	no reaction
3	TBAF 1.0 M in THF (2.0 eq), DMF, 1 h	full conversion to **2**
4	TBAF 1.0 M in THF (2.0 eq), DMF/H_2O=5:1, 1 h	full conversion to **2**

[a]Conversions were confirmed by ^{19}F-NMR (376 MHz, acetone-d_6). The small amount of reaction mixture was dissolved in acetone-d_6 and then directly monitored by ^{19}F-NMR. [b]Conversion had to be validated by other methods such as TLC because the chemical shift of the reaction mixture provided -115.4 ppm instead of -110.9 ppm, which is a typical chemical shift of **2**.

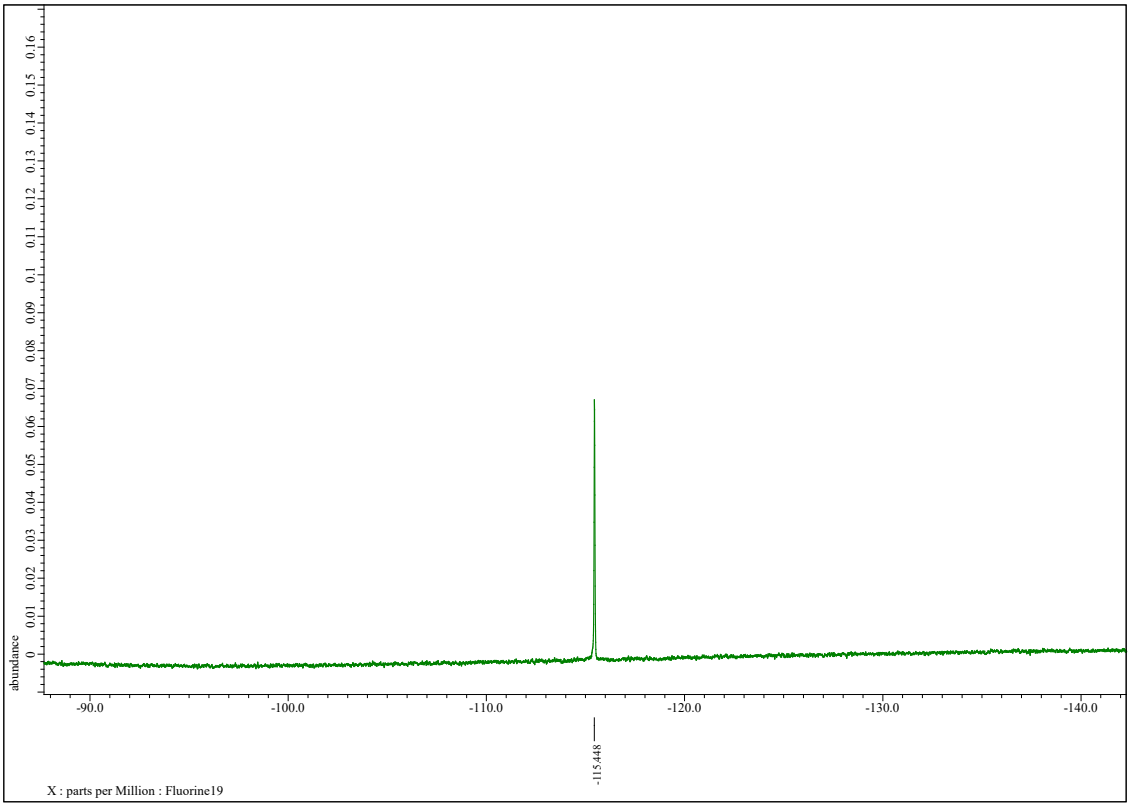


Figure S24. ^{19}F NMR spectrum of entry 1 on Table S1 (564 MHz, in acetone-d_6).

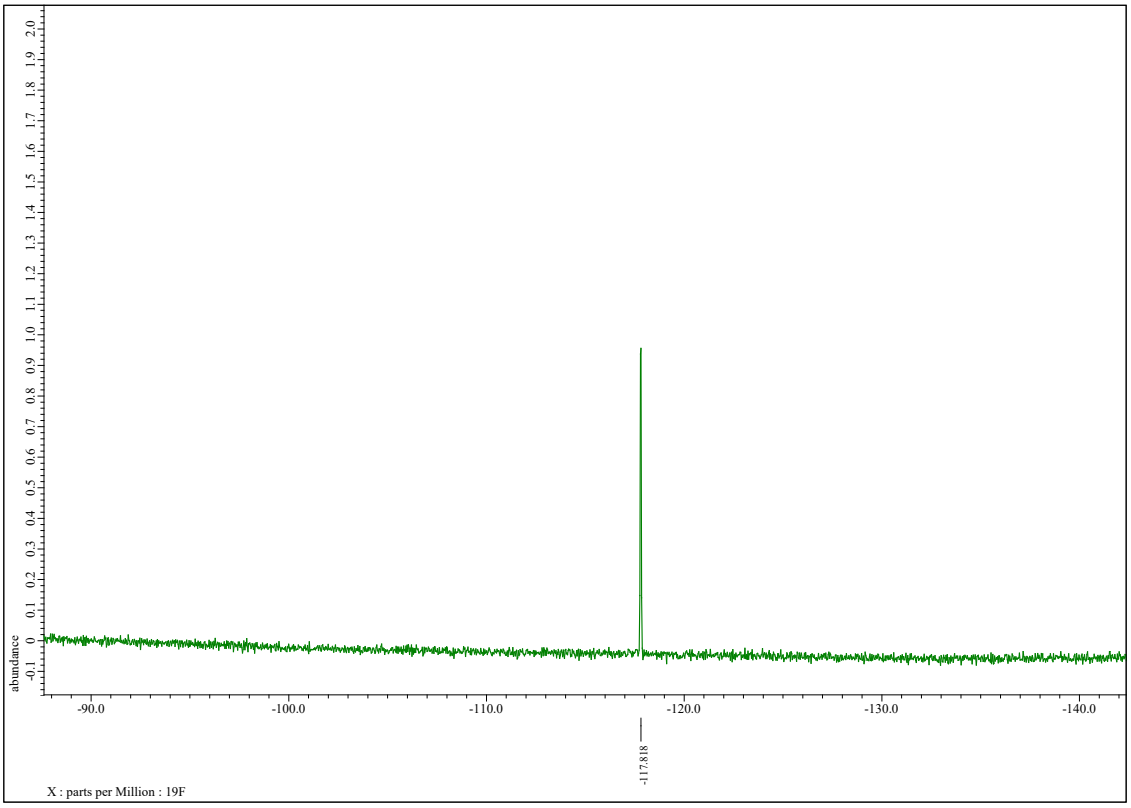


Figure S25. ^{19}F NMR spectrum of entry 2 on Table S1 (376 MHz, in acetone-d_6).

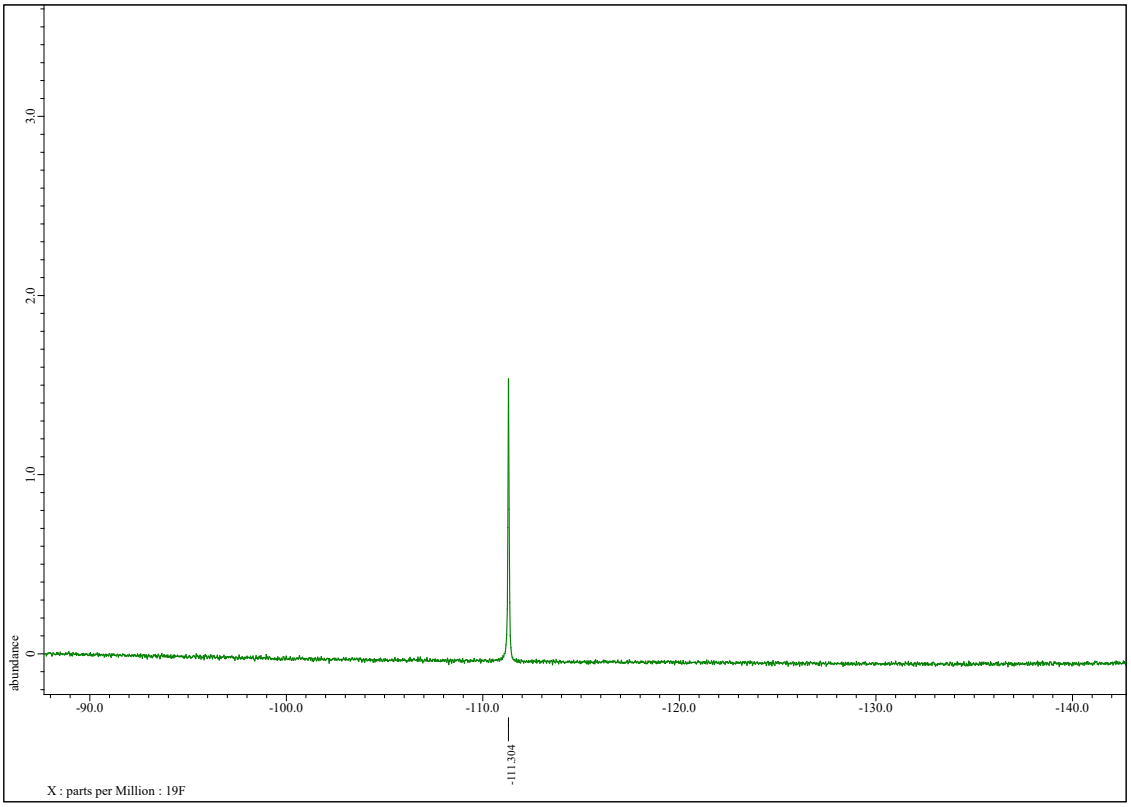


Figure S26. ^{19}F NMR spectrum of entry 3 on Table S1 (376 MHz, in acetone-d_6).

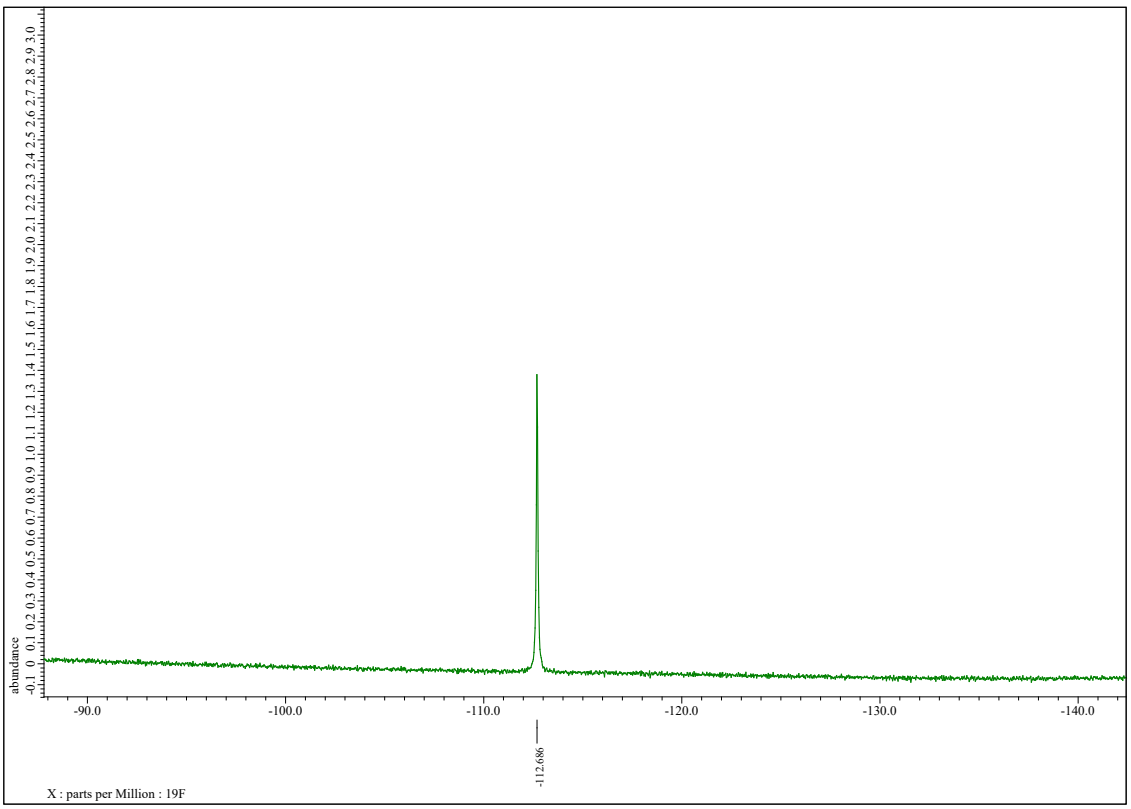
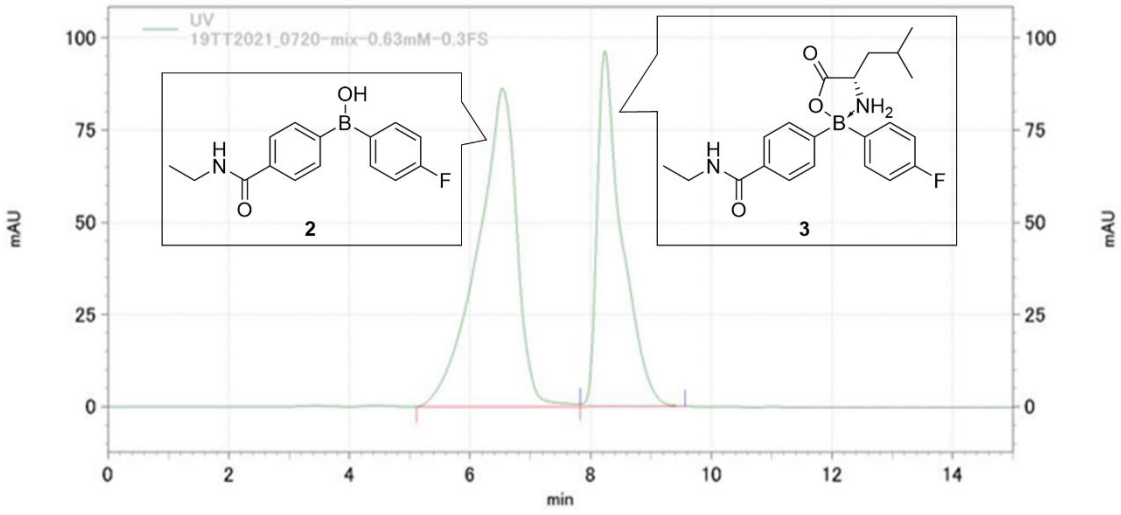


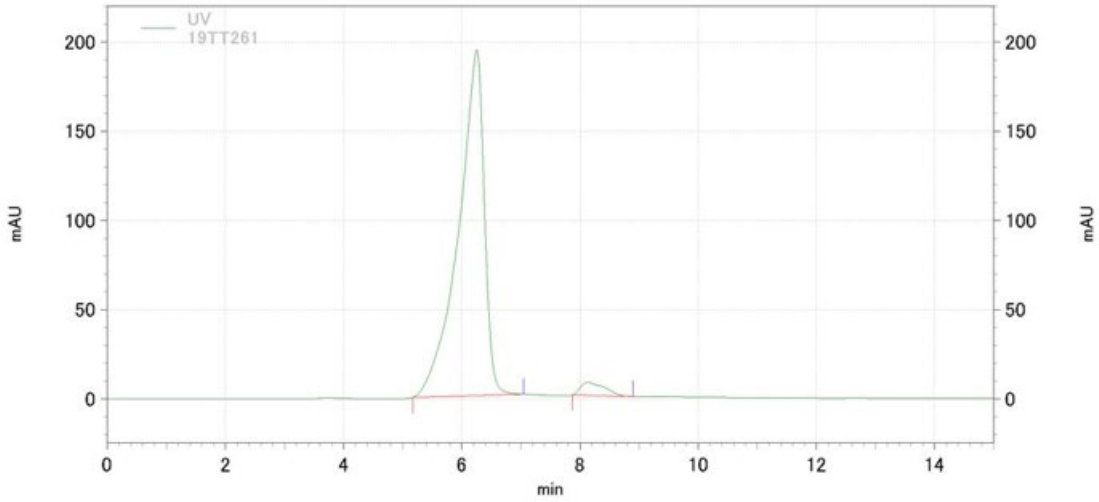
Figure S27. ^{19}F NMR spectrum of entry 4 on Table S1 (376 MHz, in acetone-d_6).



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.54	15650534	59.001	BV
2		8.24	10875152	40.999	VB
ﾄｰﾀﾙ			26525686	100.000	

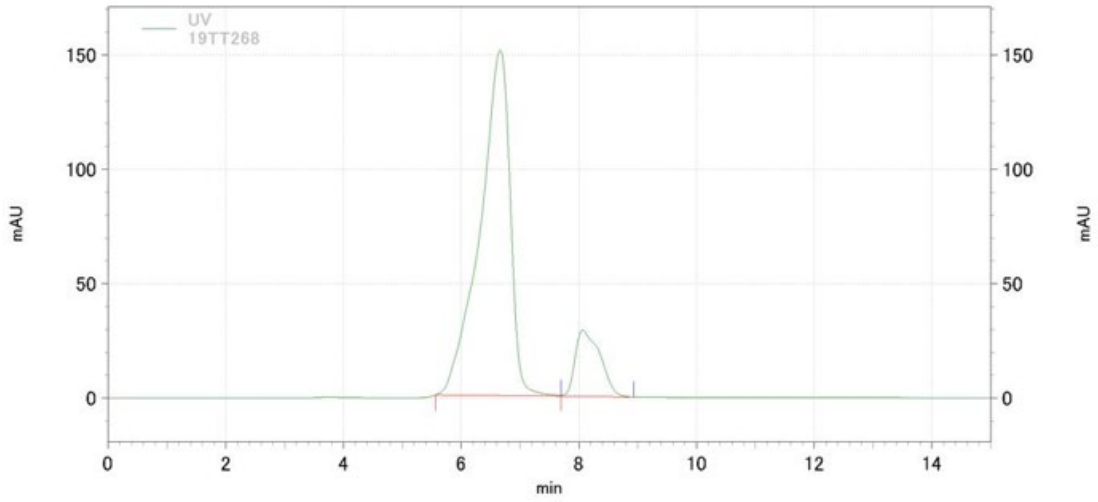
Figure S28. HPLC analysis of the mixture of authentic borinic acid **2** and boroxazolidone **3**.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.25	23424864	96.620	BB
2		8.13	819471	3.380	BB
ﾄｰﾀﾙ			24244335	100.000	

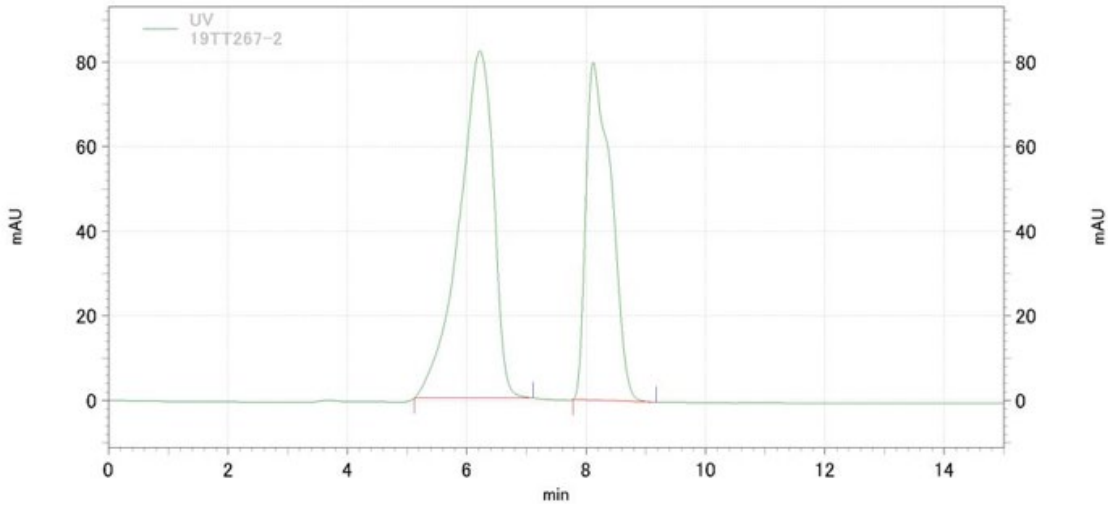
Figure S29. HPLC analysis of boroxazolidone dissociation at 37°C for 1 h.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.66	21430363	86.347	BV
2		8.06	3388555	13.653	VB
ﾄｰﾀﾙ			24818918	100.000	

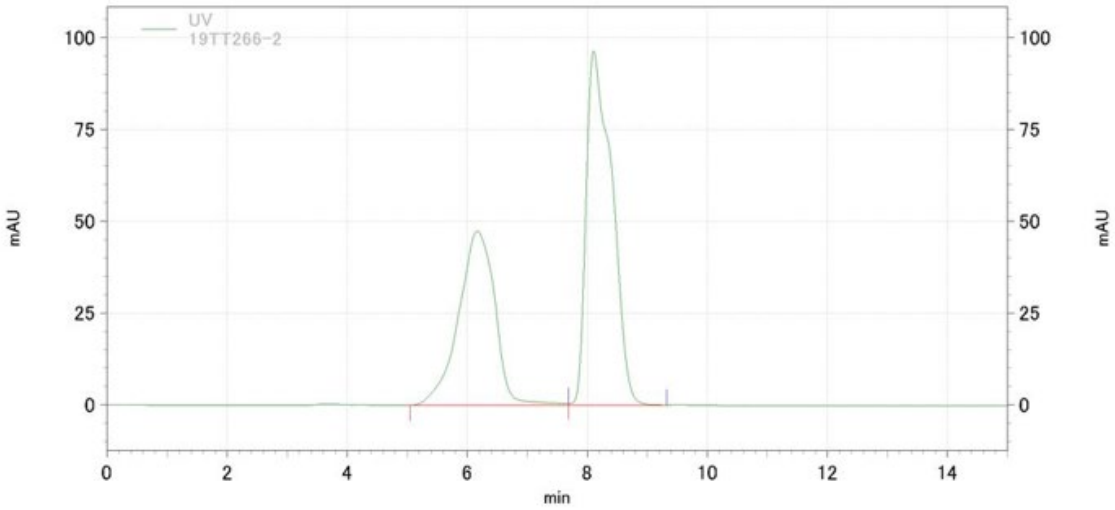
Figure S30. HPLC analysis of boroxazolidone dissociation at 37°C for 30 min.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.22	13149433	58.261	BB
2		8.12	9420563	41.739	BB
ﾄｰﾀﾙ			22569996	100.000	

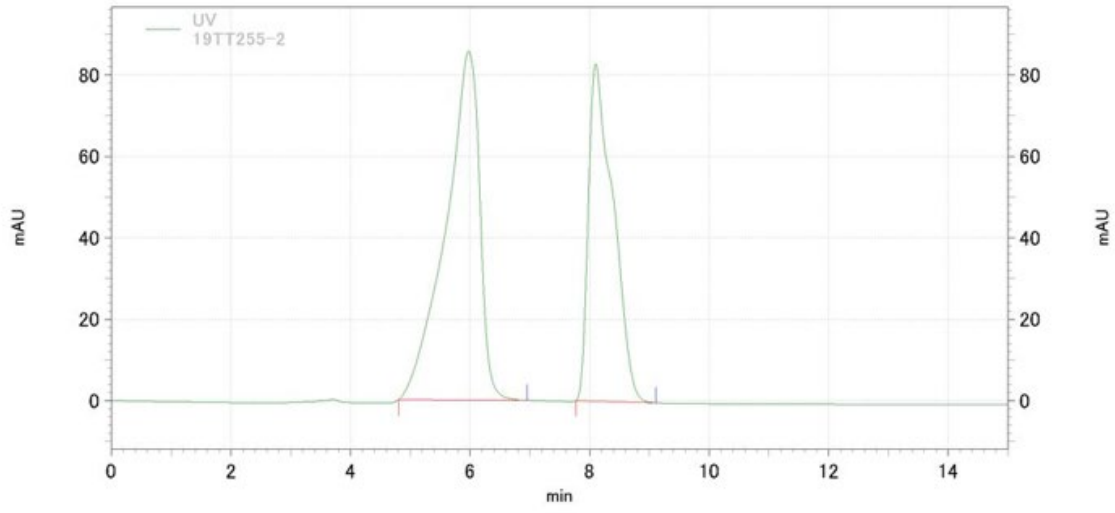
Figure S31. HPLC analysis of boroxazolidone dissociation at 37°C for 15 min.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.17	8002788	41.006	BV
2		8.11	11513401	58.994	VB
ﾄｰﾀﾙ			19516189	100.000	

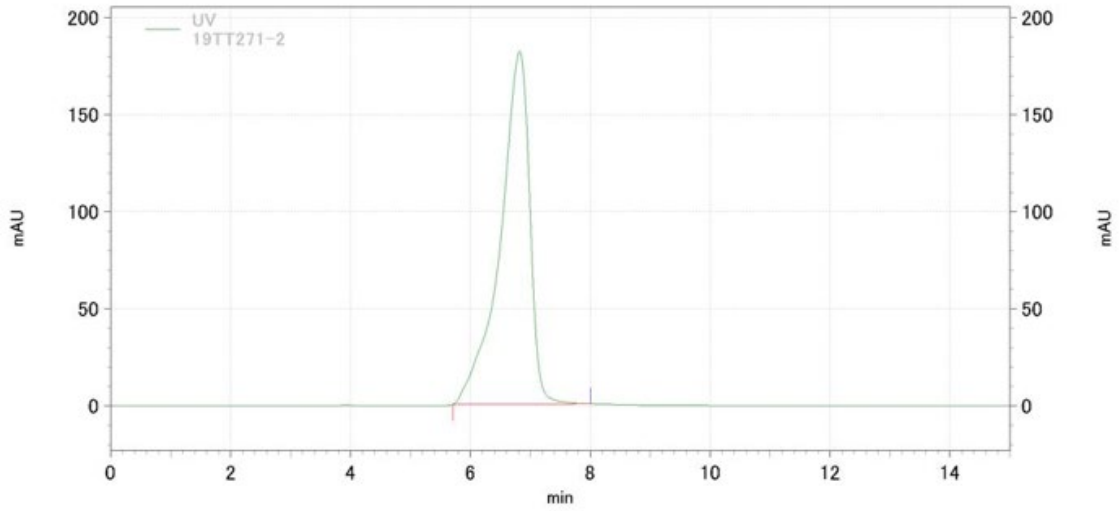
Figure S32. HPLC analysis of boroxazolidone dissociation at 37°C for 5 min.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		5.97	13545417	58.715	BB
2		8.10	9524204	41.285	BB
ﾄｰﾀﾙ			23069621	100.000	

Figure S33. HPLC analysis of boroxazolidone dissociation at room temperature for 1 h.



UV結果

Pk #	名前	保持時間	面積	面積%	ﾍﾞｰｽﾗｲﾝｺｰﾄﾞ
1		6.82	24829188	100.000	BB
ﾄｰﾀﾙ			24829188	100.000	

Figure S34. HPLC analysis of dissociation of boroxazolidone **4** to borinic acid **2** at 37°C for 1 h.

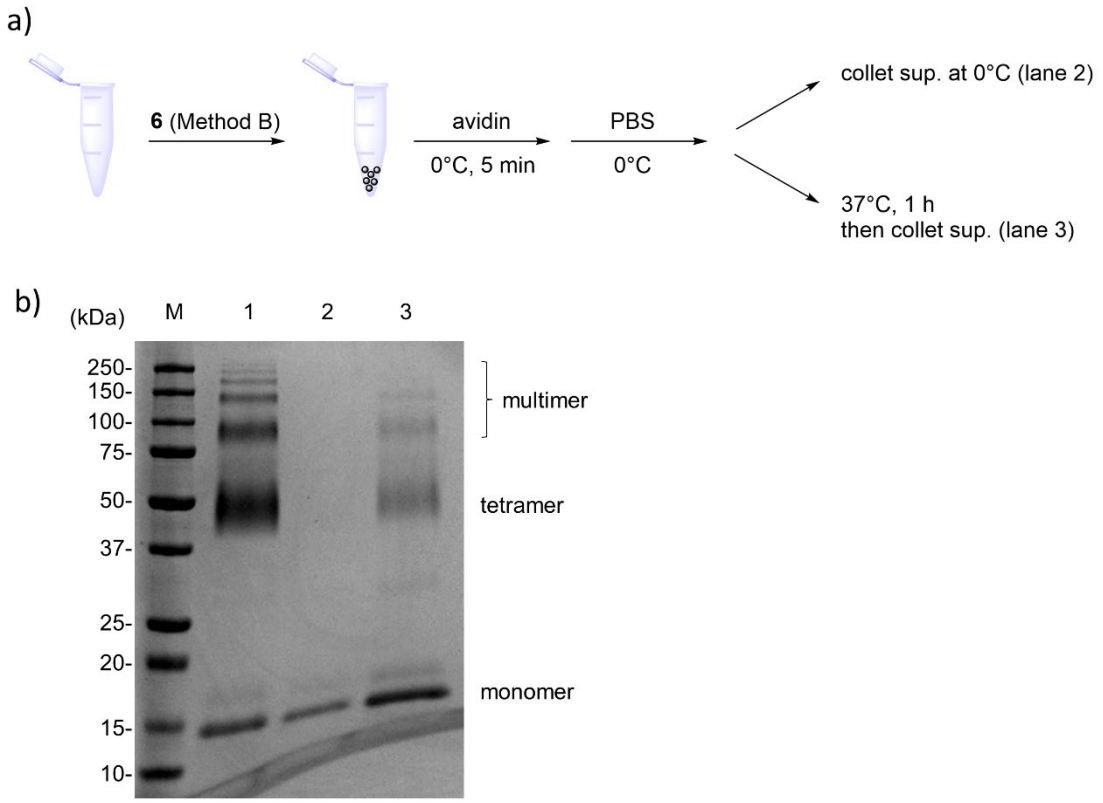


Figure S35. SDS-PAGE analysis of avidin captured by boroxazolidone beads. a) Procedure of sample preparation for SDS-PAGE analysis. b) SDS-PAGE analysis of released protein from beads. M: marker, lane 1: biocytin treated avidin, lane 2: collected supernatant at 0°C, lane 3: after the treatment at 37°C for 1 h.

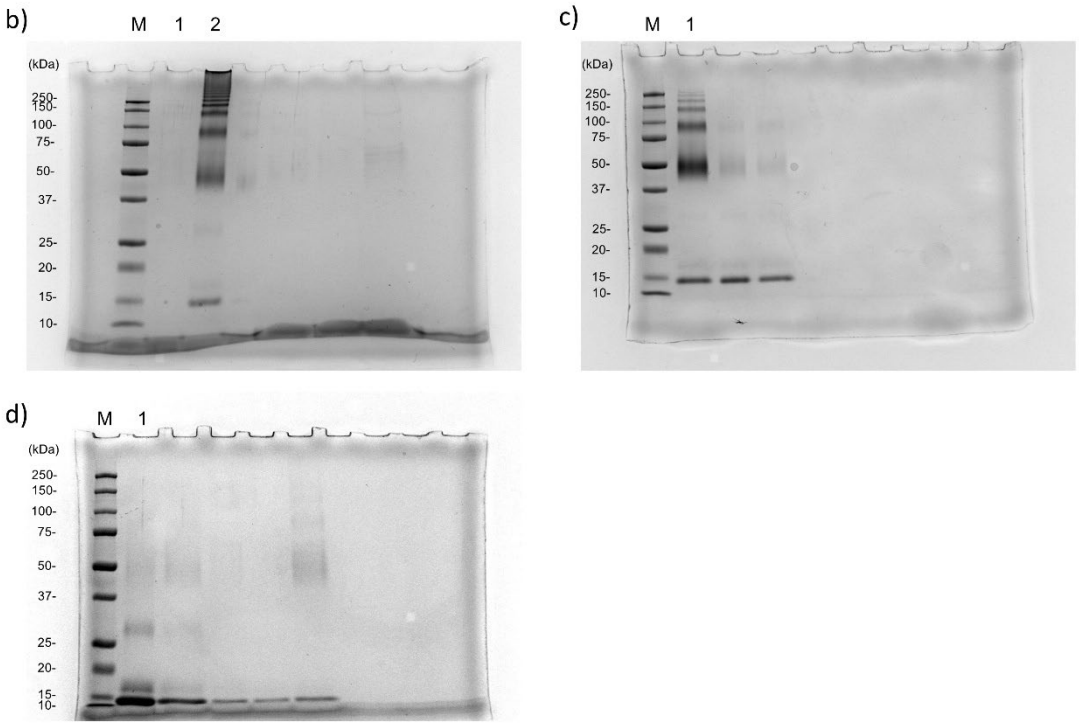


Figure S36. The complete uncropped images for the SDS-PAGE experiments shown in **Fig. 2**.