Supplementary Information for

2-Mercaptophenylboronic Acid: A Superior Alternative to 2-Mercaptoethanol for Thioester Hydrolysis

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General information

All commercially available reagents and solvents were used as received. Trityl-OH ChemMatrix resin, HMPB ChemMatrix resin was purchased from Biotage Japan Ltd. Dry dichloromethane (CH_2Cl_2), thionyl chloride, dry *N,N*-dimethylformamide (DMF), methanol (MeOH), diethyl ether (Et_2O), sodium nitrite ($NaNO_2$), sodium hydrogen carbonate ($NaHCO_3$), and disodium hydrogenphosphate (Na_2HPO_4) were purchased from KANTO CHEMICAL CO., INC. To the resin was added9-Fluorenylmethyl carbazate, trifluoroacetic acid (TFA), triisopropylsilane (TIS), *m*-cresol, sodium 2-mercaptoethanesulfonate (MESNa), 2-mercaptoethanol, piperidine, 1.6 M butyllithium in hexane, 2-bromothiophenol, and trimethyl borate, 3-mercaptophenyl boronic acid, and 4-mercaptophenyl boronic acid were purchased from Tokyo Chemical Industry Co., Ltd. DMF, Et_2O, acetonitrile (CH_3CN), dimethyl sulfoxide (DMSO), tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl), and iodine were purchased from FUJIFILM Wako Pure Chemical Corporation. Thioanisole, guanidine hydrochloride (Gn·HCl) were purchased from NACALAI TESQUE, INC. *N,N'*-Diisopropylcarbodiimide (DIPCI) and DMF was purchased from WATANABE CHEMICAL INDUSTRIES, LTD. *N,N*-Diisopropylethylamine (DIPEA), ethyl cyanohydroxyiminoacetate (Oxyma pure), 4-mercaptophenylacetic acid (MPAA), and NovaSyn®TGT alcohol resin were purchased from Merck KGaA. Sodium hydroxide (NaOH) and sodium sulfate (Na_2SO_4) were purchased from Kishida Chemical Co., Ltd. Triphenylmethanol was purchased from Combi-Blocks, Inc. Fmoc-Ala-OH·H_2O was purchased from Bachem AG. Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Val-OH, Fmoc-Tyr(*t*-Bu)-OH were purchased from CEM corporation.

For thin-layer chromatography, Merck 60 F_{254} silica gel plates were used, and compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid in ethanol followed by heating. Flash column chromatography was performed using KANTO silica gel 60N (particle size 63–210 μm). Elemental analysis was performed using a Thermo Electron Flash EA elemental analyzer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE III HD spectrometer at ambient temperature. Chemical shifts were given in δ relative to tetramethylsilane (TMS) as an internal standard (δ = 0 ppm) for ^{1}H NMR, and $CDCl_3$ and acetone-d_6 were used as an internal standard (δ = 77.1 for $CDCl_3$, 39.5 for DMSO-d_6, 29.8 for acetone-d_6) for ^{13}C NMR. Coupling constants (*J*) are reported in Hz. High-resolution mass spectra (HRMS) were recorded on a Bruker Compact mass spectrometer (ESI-Q-TOF) or a UPLC-MS (Agilent Technologies, America, 1290 Infinity II, 6230 LC/TOF). Melting points were measured using a CORNES Technologies MPA 100. IR spectra were recorded on a JASCO FT/IR 6300 with ATR and were reported in wavenumbers (cm^{-1}). LC analyses were carried out on a system using JASCO PU-2089 Plus, UV-2070 Plus, AS-2057 Plus, and CO-2060 Plus (set temperature: 30 °C). For HPLC separations, a Cosmosil 5C_{18}-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min), a Cosmosil 5C_{18}-AR-II preparative column (Nacalai Tesque, 20 × 250 mm,

flow rate 10 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% (v/v) TFA aq (solvent A) and 0.1% (v/v) TFA in MeCN (solvent B) was used for HPLC elution.

Synthesis of model peptide thioesters (H-LYRAX-$SCH_2CH_2SO_3H$)

Hydrazine-incorporated trityl (Trt) resin was prepared using previously reported methods.[S1] Trt-OH ChemMatrix resin (437 mg, 0.16 mmol) or NovaSyn®TGT alcohol resin (250 mg, 0.050 mmol) were swollen in dry CH_2Cl_2 and treated with thionyl chloride (10 equiv.) at room temperature (rt) for 2 h. After being washed with dry CH_2Cl_2, the resulting resin was swollen in dry CH_2Cl_2. A solution of 9-fluorenylmethyl carbazate (4.0 equiv.) and DIPEA (10 equiv.) in dry DMF was added dropwise to the resin at 0 °C. The reaction mixture was shaken at rt overnight, then MeOH was added. After 10 min, the resin was filtered and successively washed with DMF, water, DMF, MeOH, and Et_2O, and then dried under reduced pressure. The loading of the resins was confirmed by quantification of the Fmoc group after piperidine treatment [S2] (0.34 mmol/g from Trt-OH ChemMatrix resin, 0.17 mmol/g or 0.14 mmol/g from NovaSyn®TGT alcohol resin).

Model peptides H-**LYRAX**-$NHNH_2$ (X = Thr, Val, Gly, Ala, Ser, Lys, Pro) were elongated on the hydrazine incorporated resin (from Trt-OH ChemMatrix resin (TCM resin) or NovaSyn®TGT alcohol resin (NST resin)) by a manual synthesis (coupling: Fmoc-protected amino acid, Oxyma pure, and DIPCI (4.0 equiv. each) in DMF (0.3 M), 60 min, rt; Fmoc removal: 20% (v/v) piperidine in DMF, 10 min, rt). The resulting resin was treated with TFA-thioanisole-*m*-cresol-TIS-H_2O (80:10:5:2.5:2.5, (v/v)) at rt for 2 h. 14% $NaNO_2$ aq (10 equiv.) was added to the mixture at -10 °C.[S3] After 30 min reaction at -10 °C, chilled Et_2O was added to form a precipitate, which was successively washed with Et_2O. The resulting crude peptides were dissolved in 6 M Gn·HCl, 100 mM Na phosphate buffer (pH 7.3) containing MESNa (50 equiv.), after which the mixture allowed to react for 30 min to form the thioester. The crude peptides were purified by preparative HPLC.

H-LYRAT-$SCH_2CH_2SO_3H$ (1a)

Isolated yield: 40% (11.7 mg, 12.0 μmol from NovaSyn®TGT alcohol resin (0.14 mmol/g, 214 mg, 29.9 μmol)).

HPLC purification conditions: A Cosmosil $5C_{18}$-AR-II preparative column with a linear gradient of solvent B in solvent A, 8% to 18% over 60 min.

HPLC analytical conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

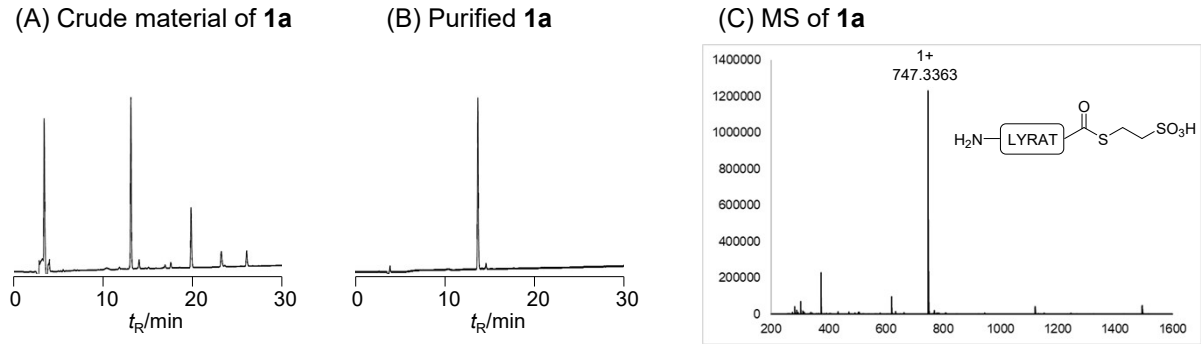


Figure S1. (A) HPLC trace of the crude material of peptide thioester **1a**. (B) HPLC trace of the purified peptide thioester **1a**. (C) MS spectrum of **1a**. **1a**: Retention time = 13.7 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{30}H_{51}N_8O_{10}S_2$ 747.3164, found 747.3363.

H-LYRAV-SCH$_2$CH$_2$SO$_3$H (1b)

Isolated yield: 23% (11.1 mg, 11.4 μmol from NovaSyn®TGT alcohol resin (0.17 mmol/g, 294 mg, 50.0 μmol)).

HPLC purification conditions: A Cosmosil 5C_{18}-AR-II preparative column with a linear gradient of solvent B in solvent A, 13% to 23% over 60 min.

HPLC analytical conditions: A Cosmosil 5C_{18}-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

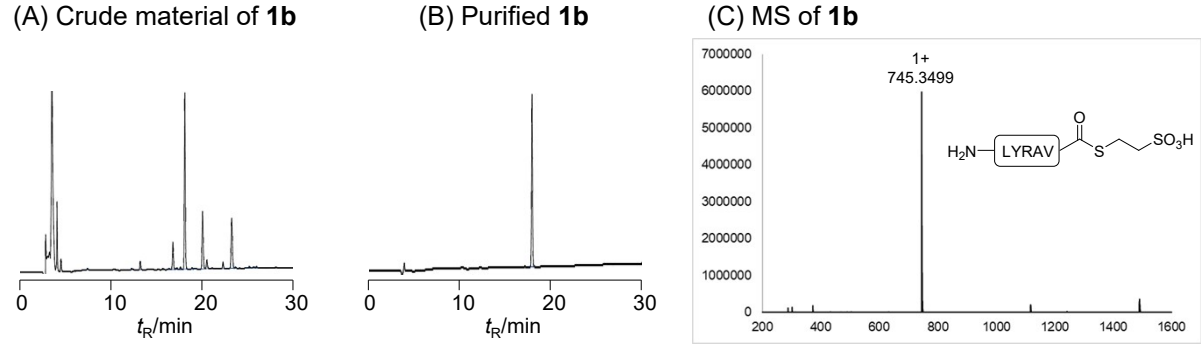


Figure S2. (A) HPLC trace of the crude material of peptide thioester **1b**. (B) HPLC trace of the purified peptide thioester **1b**. (C) MS spectrum of **1b**. **1b**: Retention time = 17.8 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3499.

H-LYRAG-SCH$_2$CH$_2$SO$_3$H (1c)

Isolated yield: 52% (18.6 mg, 20.0 μmol from Trt-OH ChemMatrix resin (0.34 mmol/g, 13.9 mg, 38.7 μmol)).

HPLC purification conditions: A Cosmosil 5C_{18}-AR-II preparative column with a linear gradient of solvent B in solvent A, 6% to 16% over 60 min.

HPLC analytical conditions: A Cosmosil 5C_{18}-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

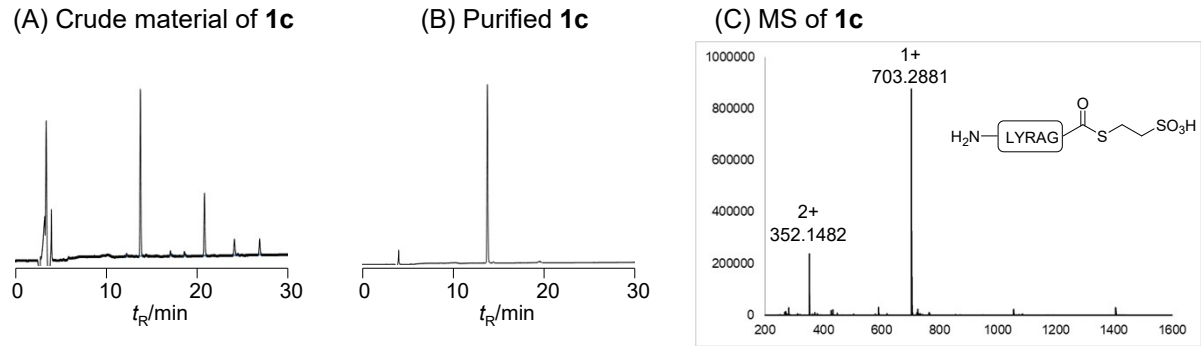


Figure S3. (A) HPLC trace of the crude material of peptide thioester **1c**. (B) HPLC trace of the purified peptide thioester **1c**. (C) MS spectrum of **1c**. **1c**: Retention time = 13.8 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{28}H_{47}N_8O_9S_2$ 703.2902, found 703.2881.

H-LYRAA-SCH$_2$CH$_2$SO$_3$H (1d)

Isolated yield: 3% (0.7 mg, 0.72 μmol from NovaSyn®TGT alcohol resin (0.17 mmol/g, 132 mg, 22.4 μmol)).

HPLC purification conditions: A Cosmosil 5C_{18}-AR-II preparative column with a linear gradient of solvent B in solvent A, 10% to 18% over 60 min.

HPLC analytical conditions: A Cosmosil 5C_{18}-AR-II column with a gradient of solvent B in solvent A, from 10% to 14% over 15 min then 14% to 55% over another 15 min.

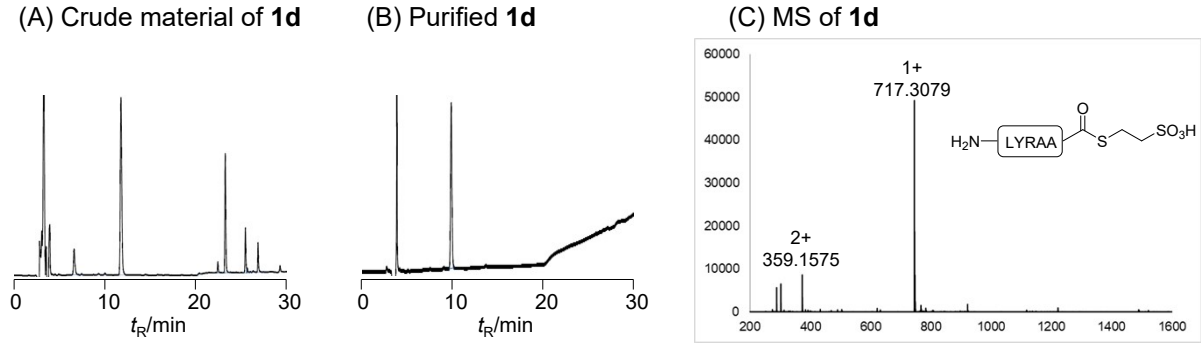


Figure S4. (A) HPLC trace of the crude material of peptide thioester **1d**. (B) HPLC trace of the purified peptide thioester **1d**. (C) MS spectrum of **1d**. **1d**: Retention time = 9.8 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{29}H_{49}N_8O_9S_2$ 717.3058, found 717.3079.

H-LYRAS-SCH$_2$CH$_2$SO$_3$H (1e)

Isolated yield: 48% (18.0 mg, 18.7 μmol from Trt-OH ChemMatrix resin (0.34 mmol/g, 115 mg, 39.0 μmol)).

HPLC purification conditions: A Cosmosil 5C_{18}-AR-II preparative column with a linear gradient of solvent B in solvent A, 6% to 16% over 60 min.

HPLC analytical conditions: A Cosmosil 5C_{18}-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

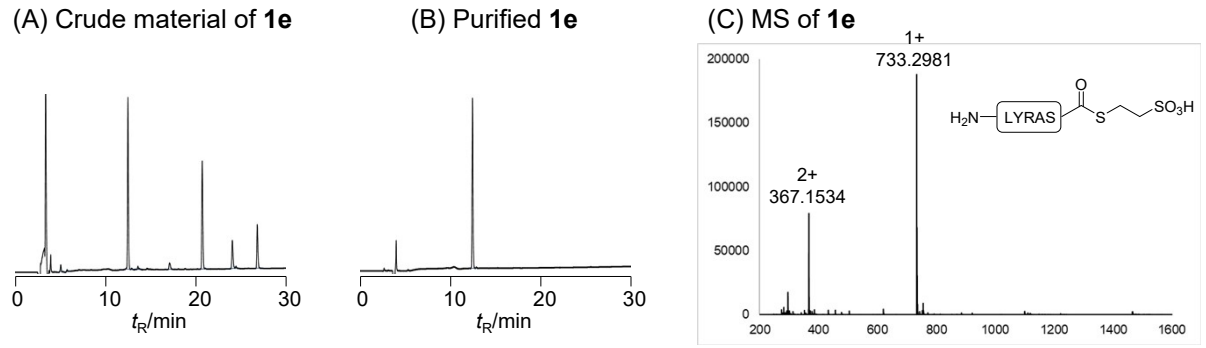


Figure S5. (A) HPLC trace of the crude material of peptide thioester **1e**. (B) HPLC trace of the purified peptide thioester **1e**. (C) MS spectrum of **1e**. **1e**: Retention time = 12.5 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{29}H_{49}N_8O_{10}S_2$ 733.3008, found 733.2981.

H-LYRAK-SCH$_2$CH$_2$SO$_3$H (1f)

Isolated yield: 41% (18.0 mg, 16.1 μmol from Trt-OH ChemMatrix resin (0.34 mmol/g, 114 mg, 38.9 μmol)).

HPLC purification conditions: A Cosmosil 5C$_{18}$-AR-II preparative column with a linear gradient of solvent B in solvent A, 6% to 16% over 60 min.

HPLC analytical conditions: A Cosmosil 5C$_{18}$-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

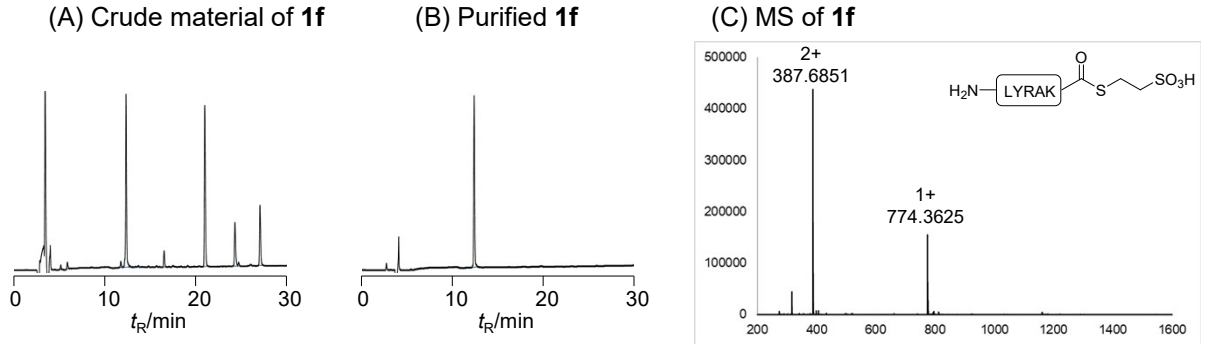


Figure S6. (A) HPLC trace of the crude material of peptide thioester **1f**. (B) HPLC trace of the purified peptide thioester **1f**. (C) MS spectrum of **1f**. **1f**: Retention time = 12.3 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{32}H_{56}N_9O_9S_2$ 774.3637, found 774.3625.

H-LYRAP-SCH$_2$CH$_2$SO$_3$H (1g)

Isolated yield: 41% (15.6 mg, 16.1 μmol from Trt-OH ChemMatrix resin (0.34 mmol/g, 114 mg, 38.9 μmol)).

HPLC purification conditions: A Cosmosil 5C$_{18}$-AR-II preparative column with a linear gradient of solvent B in solvent A, 10% to 30% over 60 min.

HPLC analytical conditions: A Cosmosil 5C$_{18}$-AR-II column with a linear gradient of solvent B in solvent A, from 5% to 35% over 30 min.

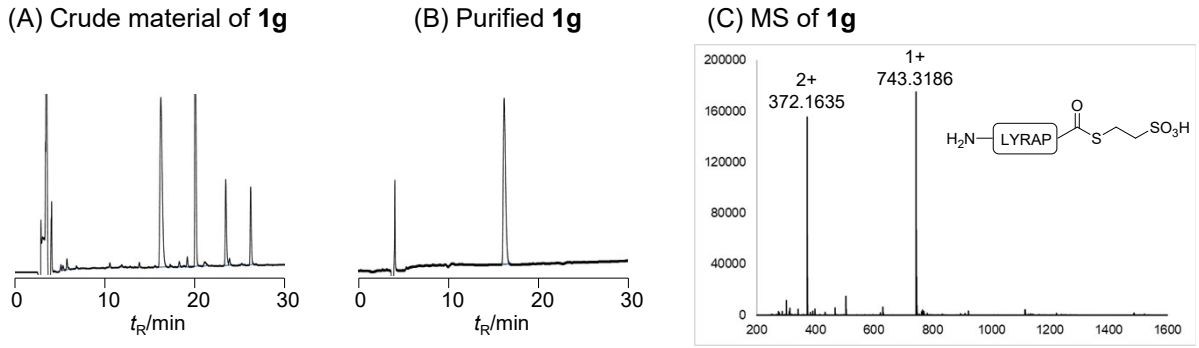


Figure S7. (A) HPLC trace of the crude material of peptide thioester **1g**. (B) HPLC trace of the purified peptide thioester **1g**. (C) MS spectrum of **1g**. **1g**: Retention time = 16.2 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{31}H_{51}N_8O_9S_2$ 743.3215, found 743.3186.

Thioester hydrolysis under boric acid-containing solution

Boric acid (0.62 mg, 10 μmol) and TCEP·HCl (2.87 mg, 10 μmol) were dissolved in 50 mM HEPES buffer (95 μL), and the pH was then adjusted to 7.5. To the solution was added 2.4% benzamide solution (H_2O-MeCN (9:1, (v/v), 5 μL) as an internal standard. Each peptide thioester (H-**LYRAX**-$SCH_2CH_2SO_3H$, **X** = T, V, or S, 0.1 μmol) was dissolved in the solution, then the reaction mixture was incubated at 37 °C for 7 h. The reactions were monitored by HPLC using a Cosmosil 5C_{18}-AR-II column.

For H-**LYRAT**-$SCH_2CH_2SO_3H$ (**1a**):

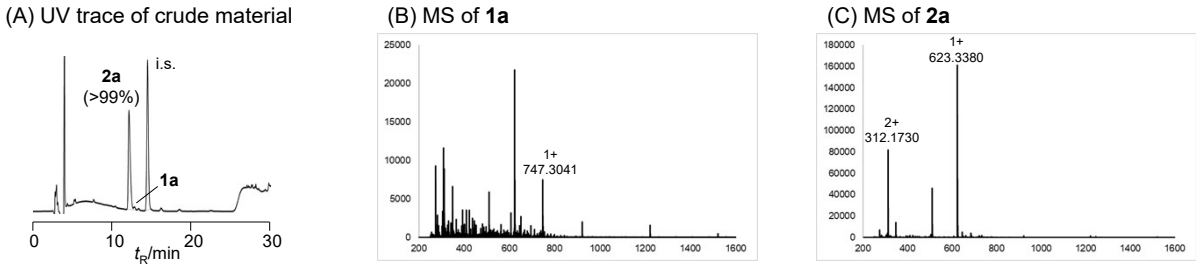


Figure S8. (A) HPLC trace of the reaction mixture after 7 h. HPLC conditions: a linear gradient of solvent B in solvent A, 10% to 11% over 20 min then 11% to 80% over 10 min. (B) MS spectrum of **1a**. **1a**: Retention time = 12.7 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{30}H_{51}N_8O_{10}S_2$ 747.3164, found 747.3041. (C) MS spectrum of **2a**. **2a**: Retention time = 12.0 min, MS (ESI-Q-TOF) *m/z*: ([M+H]$^+$) calcd for $C_{28}H_{47}N_8O_8$ 623.3511, found 623.3380.

For H-**LYRAS**-$SCH_2CH_2SO_3H$ (**1e**):

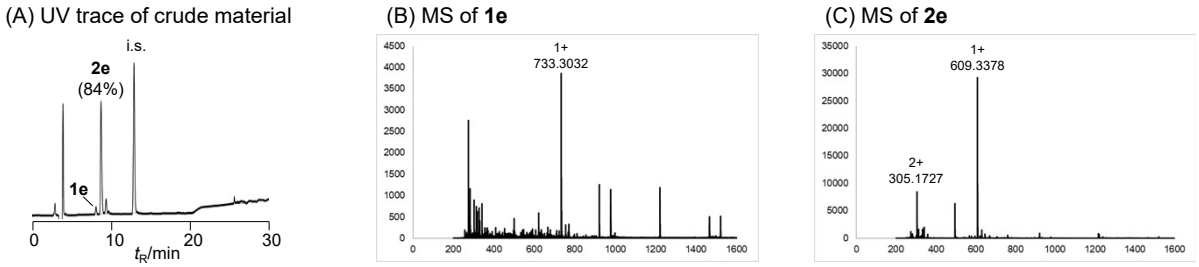


Figure S9. (A) HPLC trace of the reaction mixture after 7 h. HPLC conditions: a linear gradient of solvent B in solvent A, 10% to 14% over 15 min then 14% to 55% over 15 min. (B) MS spectrum of **1e**. **1e**: Retention time = 8.0 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{29}H_{49}N_8O_{10}S_2$ 733.3008, found 733. 3032. (C) MS spectrum of **2e**. **2e**: Retention time = 8.7 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{27}H_{45}N_8O_8$ 609.3355., found 609.3378.

For H-**LYRAV**-$SCH_2CH_2SO_3H$ (**1b**):

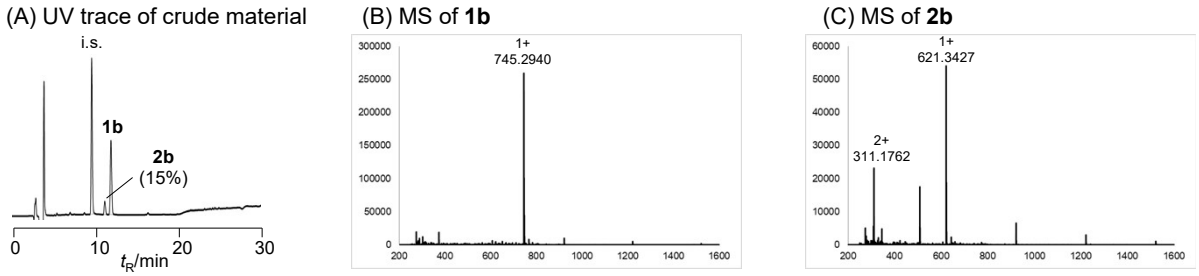
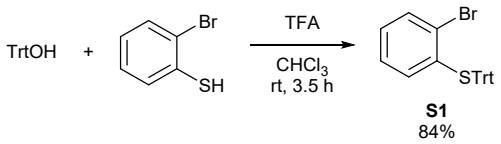


Figure S10. (A) HPLC trace of the reaction mixture after 7 h. HPLC conditions: a linear gradient of solvent B in solvent A, 14% to 18% over 15 min then 18% to 55% over 15 min. (B) MS spectrum of **1b**. **1b**: Retention time = 11.9 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.2940. (C) MS spectrum of **2b**. **2b**: Retention time = 11.1 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719, found 621.3427.

Synthesis of disulfide dimer of 2-mercaptophenylboronic acid

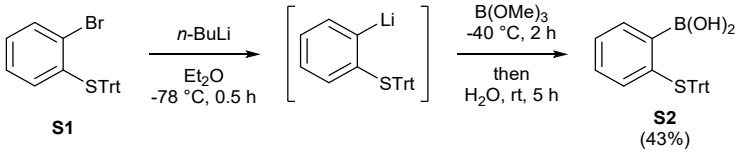
(2-Bromophenyl)(trityl)sulfane (S1)



Triphenylmethanol (6.00 g, 23.0 mmol) and 2-bromothiophenol (3.03 mL, 25.4 mmol, 1.1 eq) were dissolved in dry $CHCl_3$ (80 mL). To the solution was added trifluoroacetic acid (3.51 mL, 46.1 mmol, 2.0 eq) in an ice bath. After removal of the ice bath, the reaction mixture was stirred at rt for 3.5 h. Upon completion, the reaction was quenched by the addition of sat. $NaHCO_3$ aq (30 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (hexane-EtOAc = 10:0 to 9:1, v/v) to afford a white solid (8.36 g, 19.4 mmol, 84%).

The ^{1}H NMR spectrum was identical to previously reported data.[S4] ^{1}H NMR ($CDCl_3$) δ: 6.77–6.94 (3H, m), 7.18–7.36 (9H, m), 7.38–7.50 (7H, m).

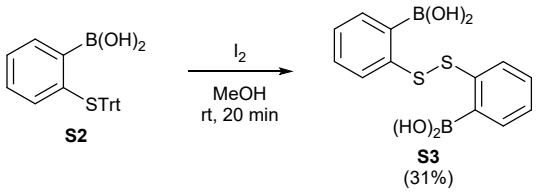
(2-(Tritylthio)phenyl)boronic acid (S2)



Compound **S1** (163 mg, 0.38 mmol) was dissolved in dry-Et_2O (15 mL), and then cooled to -78 °C. To the solution was added dropwise 1.48 M *n*-butyllithium in hexane (0.31 mL, 0.45 mmol), and then the mixture was stirred at -78 °C. After stirring for 30 min, $B(OMe)_3$ (0.21 mL, 1.88 mmol) was added dropwise followed by stirring at -40 °C for 2 h. Then water (10 mL) was added and stirred at rt for 5 h. After completion, the organic layer was separated and dried over Na_2SO_4. After filtration, the resulting solution was concentrated *in vacuo*. The residue was purified by column chromatography (eluent: $CHCl_3$-EtOAc 10:0 to 95:5, v/v) to afford a white solid (64 mg, 0.16 mmol, 43%).

mp: 126 °C; IR (ATR) cm^{-1}: 3335 (OH); elemental analysis Found: C, 75.5; H, 5.3. S, 7.8 $C_{13}H_{13}NO_4$ requires C, 75.8; H, 5.3, S, 8.1%; ^{1}H NMR ($CDCl_3$) δ: 5.66 (2H, s), 7.13 (1H, ddd, J = 8.6, 7.6, 1.6 Hz), 7.20–7.39 (17H, m), 7.80 (1H, dd, J = 7.6, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 72.8, 127.2, 127.9, 128.9, 130.0, 130.1, 130.5, 136.5, 136.9, 137.8, 143.7.

2-Boronophenyldisulfide (S3)



Iodine (465 mg, 1.83 mmol) was dissolved in MeOH (3.6 mL). To the solution was added compound **S2** (363 mg, 0.92 mmol) in MeOH (6.1 mL) dropwise, and the mixture was stirred at rt for 20 min. The reaction was quenched by addition of 10% w/w ascorbic acid aq until the solution became colorless. The solution was extracted with EtOAc three times, and the combined organic phase was dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was dissolved in 0.1 M HCl aq (5 mL) and Et_2O (5 mL) and stirred at rt for 30 min. The aqueous phase was separated and washed with Et_2O. The organic phase was combined and then dried over Na_2SO_4. After filtration and concentration under reduced pressure, the residue was recrystallized from Et_2O-hexane to afford white solid (43 mg, 0.14 mmol, 31%).

The ^1H and ^{13}C NMR spectra indicated that the product contained approximately 25% of anhydride. The anhydride disappeared after addition of D_2O to the NMR sample. Treatment of the NMR sample of the product with Bu_3P gave the ^1H NMR spectrum identical to the previously reported data.[S5] ^1H NMR (D_2O-acetone-d_6) δ: 7.80 (1H, dd, J = 7.6, 1.5 Hz), 7.36 (1H, td, J = 7.6, 1.6 Hz), 7.19-7.14 (2H, m).

mp: 140 °C; IR (ATR) cm^{-1}: 3295 (OH); HRMS (ESI-TOF) m/z: ($[M+Na]^+$) calcd for $C_{12}H_{12}B_2NaO_4S_2$ 329.0255, found 329.0258; ^1H NMR (D_2O-acetone-d_6) δ: 7.62-7.58 (4H, m), 7.28 (2H, td, J = 7.4, 1.6 Hz), 7.16 (2H, td, J = 7.4, 1.0 Hz); ^{13}C NMR (acetone-d_6) δ: 142.2 (2C), 135.2 (2C), 130.9 (2C), 126.7 (2C), 126.5 (2C); ^{11}B NMR (acetone-d_6) δ: 29.0.

Hydrolysis of peptide thioester 1b using various additives

Each additive (100 mM) and TCEP·HCl (100 mM) was dissolved in 50 mM HEPES buffer, after which pH was adjusted to 7.5. The buffer (95 μL) was added to the peptide thioester (H-**LYRAV**-$SCH_2CH_2SO_3H$ (**1b**), 0.1 μmol). Then 2.4% (w/v) benzamide in H_2O-MeCN (9:1, (v/v), 5 μL) was added to the reaction mixture as an internal standard, which was allowed to react at 37 °C.

For 2-MPBA, disulfide dimer of 2-MPBA (**S3**, 1.54 mg, 5 μmol) and TCEP·HCl (2.87 mg, 10 μmol) were dissolved in 50 mM HEPES buffer (95 μL), and then pH of the solution was adjusted to 4. After 1 h at 37 °C, pH was adjusted to 7.5 and then 2.4% (w/v) benzamide in H_2O-MeCN (9:1, (v/v), 5 μL) was added to the solution as an internal standard. Peptide thioester **1b** was dissolved in the solution. The reaction mixture was maintained at 37 °C.

Each reaction was monitored by HPLC, 220 nm at 0 h and 3 h except for entry 2 (0 h and 8 h). HPLC conditions: A Cosmosil 5C_{18}-AR-II column with a linear gradient of solvent B in solvent A, 14% to 18% over 15 min then 18% to 55% over additional 15 min.

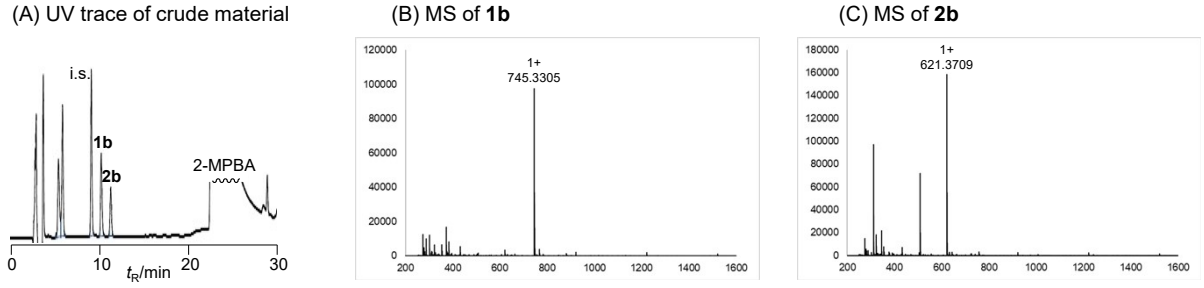


Figure S11. (A) UV trace of table 2 entry 1 (3 h). (B) MS spectrum of **1b**. **1b**: Retention time = 11.3 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3305. (C) MS spectrum of **2b**. **2b**: Retention time = 10.2 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719, found 621.3709.

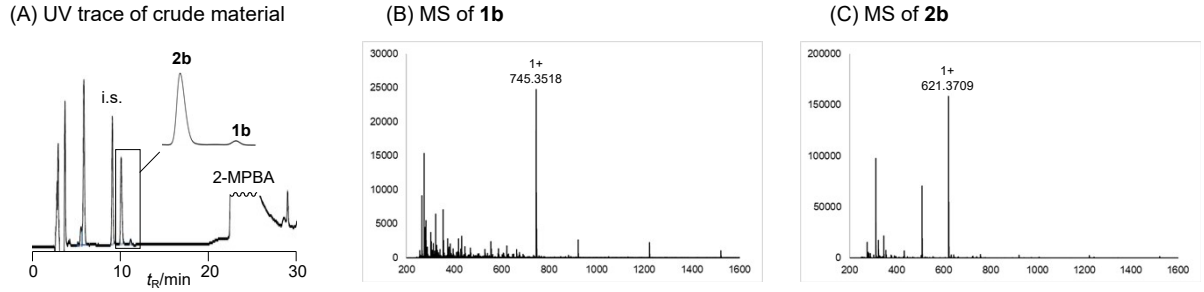


Figure S12. (A) UV trace of table 2 entry 2 (8 h). (B) MS spectrum of **1b**. **1b**: Retention time = 11.1 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3518. (C) MS spectrum of **2b**. **2b**: Retention time = 10.1 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719, found 621.3709.

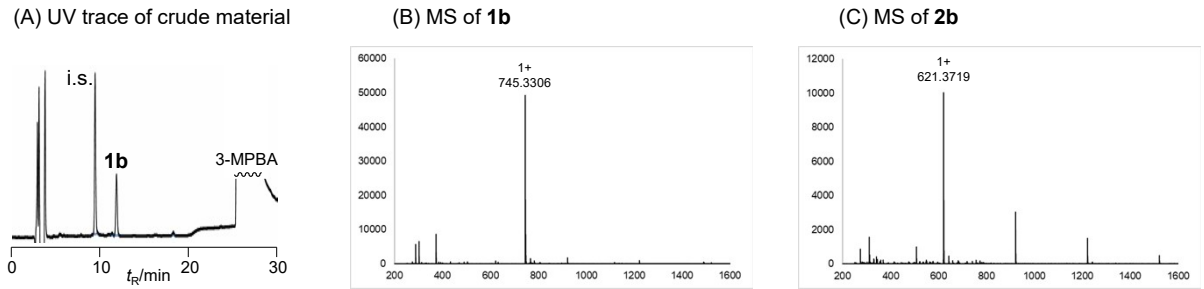


Figure S13. (A) UV trace of table 2 entry 3 (3 h). (B) MS spectrum of **1b**. **1b**: Retention time = 11.8 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3306. (C) MS spectrum of **2b**. **2b**: Retention time = 11.3 min, MS (ESI-Q-TOF) *m*/*z*: ([M+H]$^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719, found 621.3719.

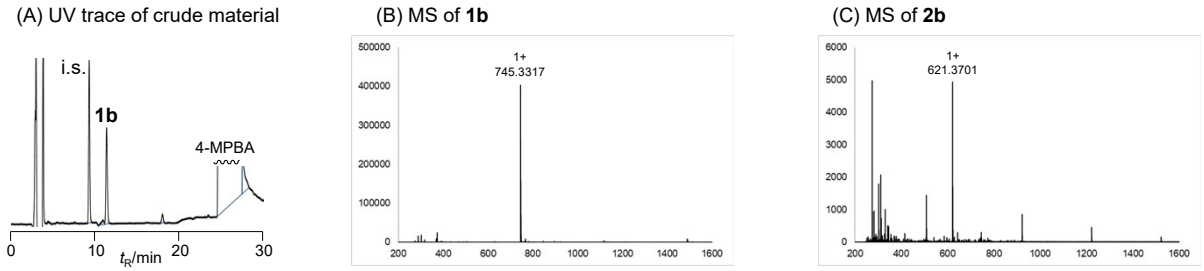


Figure S14. (A) UV trace of table 2 entry 4 (3 h). (B) MS spectrum of **1b**. **1b**: Retention time = 11.3 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3317. (C) MS spectrum of **2b**. **2b**: Retention time = 10.8 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719, found 621.3701.

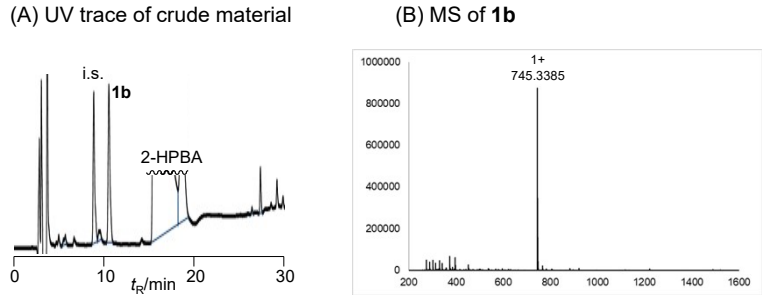


Figure S15. (A) UV trace of table 2 entry 5 (3 h). (B) MS spectrum of **1b**. **1b**: Retention time = 10.5 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3358.

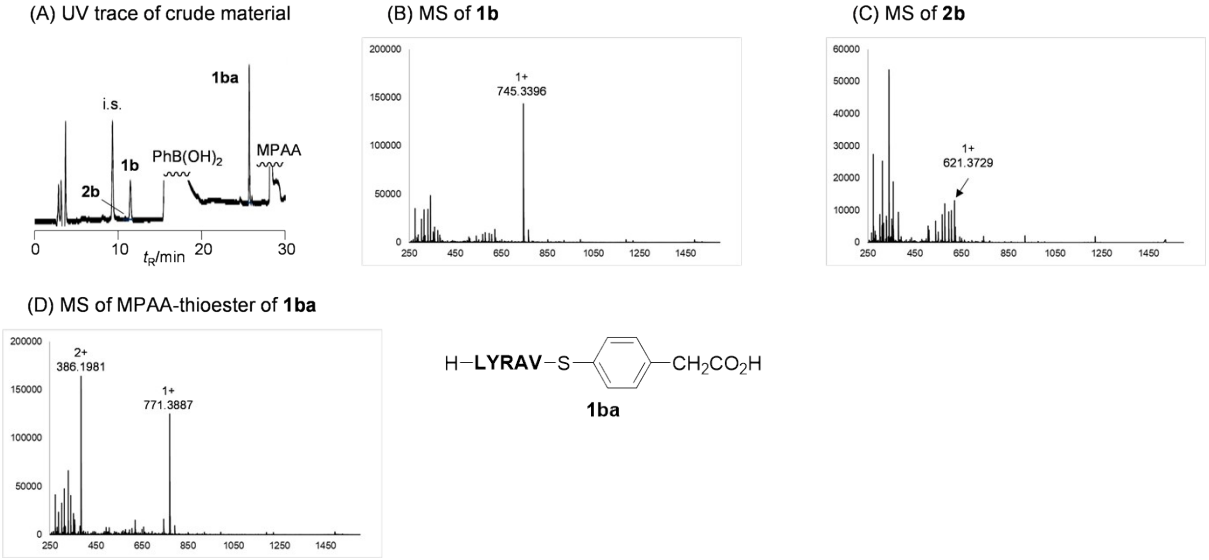


Figure S16. (A) UV trace of table 2 entry 6 (3 h). (B) MS spectrum of **1b**. **1b**: Retention time = 11.4 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{31}H_{53}N_8O_9S_2$ 745.3371, found 745.3396. (C) MS spectrum of **2b**. **2b**: Retention time = 10.8 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{29}H_{49}N_8O_7$ 621.3719 found 621.3729. (D) MS spectrum of **1ba**. **1ba**: Retention time = 25.5 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{37}H_{55}N_8O_8S$ 771.3858, found 771.3887.

Comparison of hydrolysis using 2-ME and 2-MPBA

Disulfide dimer of 2-MPBA (**S3**, 1.54 mg, 5 μmol) and TCEP·HCl (2.87 mg, 10 μmol) were dissolved in 50 mM HEPES buffer (95 μL), and then pH of the solution was adjusted to 4. After 1 h later at 37 °C, to the solution was added 2.4% (w/v) benzamide in H_2O-MeCN (9:1, (v/v), 5 μL) as an internal standard and then pH was adjusted to 6.0, 7.5, or 8.5. Peptide thioester **1b** (0.1 μmol) was dissolved in the solution. The reaction mixture was maintained at 37 °C.

For the reaction using 2-ME, same procedure was used as described above (reaction condition: 100 mM 2-ME (0.70 μL, 10 μmol), 100 mM TCEP·HCl (2.87 mg, 10 μmol) in 50 mM HEPES buffer, 37 °C) under pH 6.0, 7.5, or 8.5, respectively.

HPLC conditions: A Cosmosil 5C_{18}-AR-II column with a linear gradient of solvent B in solvent A, 14% to 18% over 15 min then 18% to 55% over another 15 min.

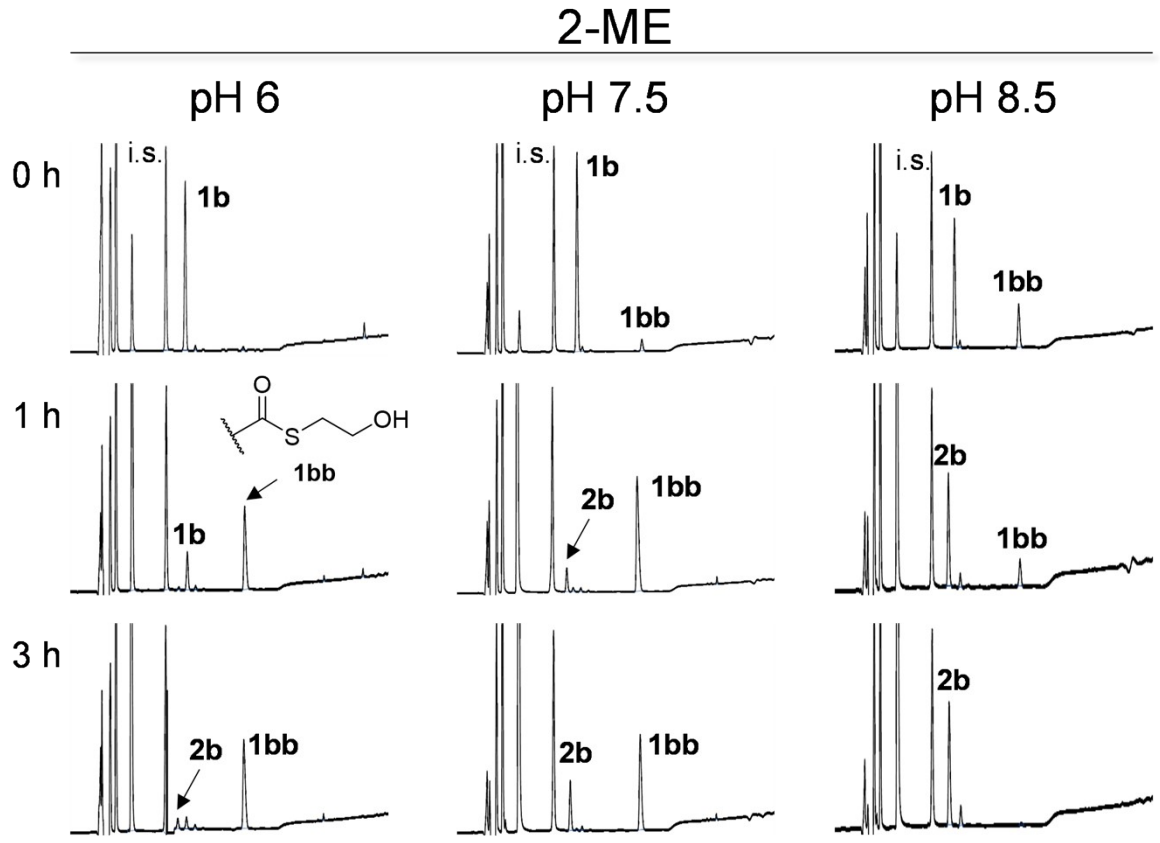


Figure S17. UV trace of hydrolysis of **1b** using 2-ME.

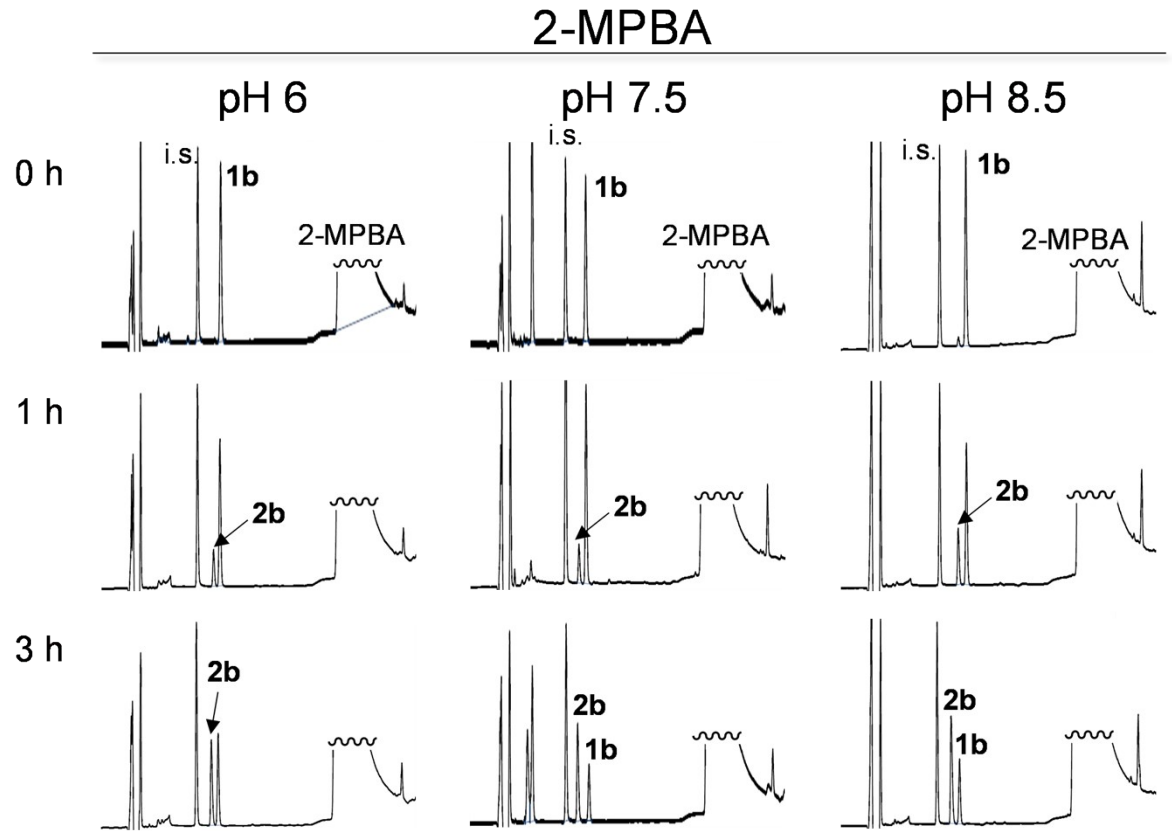


Figure S18. UV trace of hydrolysis of **1b** using 2-MPBA.

Scope of the C-terminal amino acids

Hydrolysis was conducted as described above using peptide thioesters (H-**LYRAX**-$SCH_2CH_2SO_3H$, 0.1 μmol). Each reaction was monitored by HPLC, 220 nm until the reactions completed.

For H-**LYRAG**-$SCH_2CH_2SO_3H$:

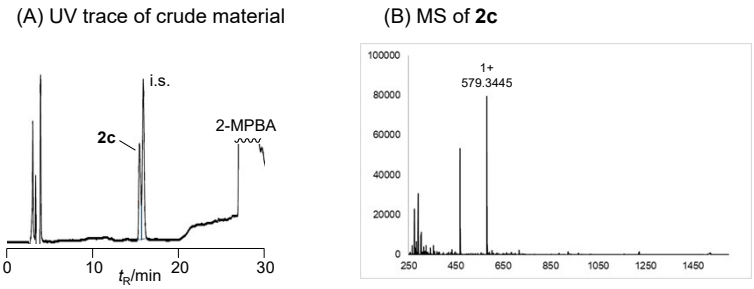


Figure S19. (A) UV trace of the crude material at (1 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 8% to 10% over 15 min then 10% to 60% over another 15 min. (B) MS spectrum of **2c**. **2c**: Retention time = 15.4 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{26}H_{43}N_8O_7$ 579.3249, found 579.3445.

For H-**LYRAA**-$SCH_2CH_2SO_3H$:

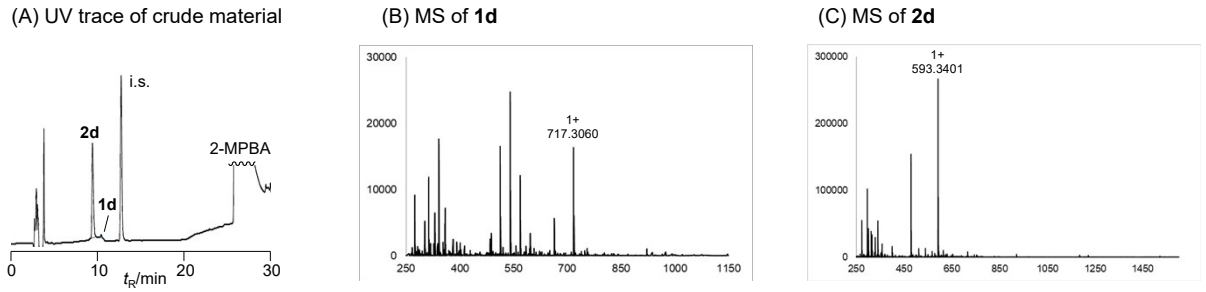


Figure S20. (A) UV trace of the crude material (1 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 10% to 14% over 15 min then 14% to 55% over another 15 min. (B) MS spectrum of **1d**. **1d**: Retention time = 10.4 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{29}H_{49}N_8O_9S_2$ 717.3058, found 717.3060. (C) MS spectrum of **2d**. **2d**: Retention time = 9.4 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{27}H_{45}N_8O_7$ 593.3406, found 593.3401.

For H-**LYRAS**-$SCH_2CH_2SO_3H$:

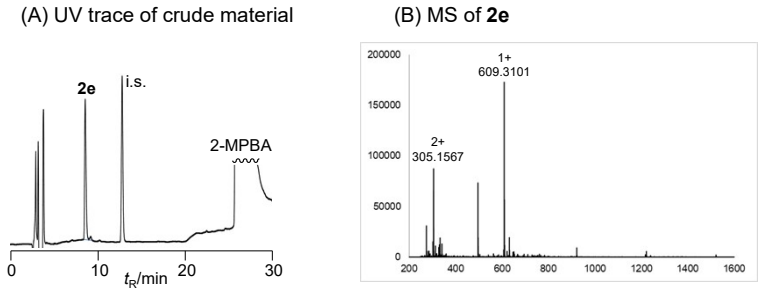


Figure S21. (A) UV trace of the crude material (1 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 10% to 14% over 15 min then 14% to 55% over another 15 min. (B) MS spectrum of **2e**. **2e**: Retention time = 8.6 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{27}H_{45}N_8O_8$ 609.3355, found 609.3101.

For H-**LYRAK**-$SCH_2CH_2SO_3H$:

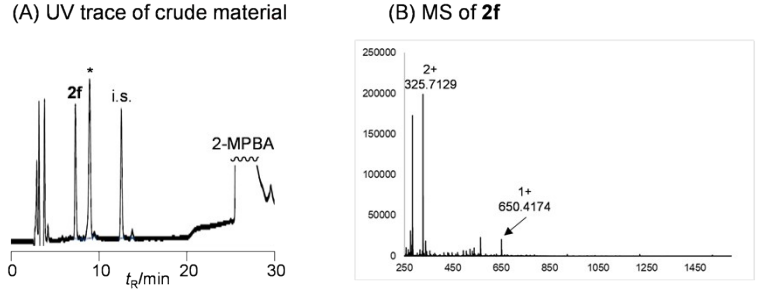


Figure S22. (A) UV trace of the crude material (3 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 10% to 14% over 15 min then 14% to 55% over another 15 min. (B) MS spectrum of **2f**. **2f**: Retention time = 7.3 min, MS (ESI-Q-TOF) *m*/*z*: ($[M+H]^+$) calcd for $C_{30}H_{52}N_9O_7$ 650.3984, found 650.4174.

For H-**LYRAT**-$SCH_2CH_2SO_3H$:

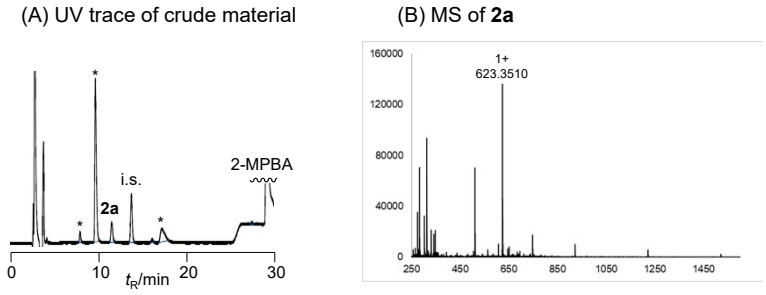


Figure S23. (A) UV trace of the crude material (3 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 10% to 11% over 20 min then 11% to 80% over another 10 min. (B) MS spectrum of **2a**. **2a**: Retention time = 11.5 min, MS (ESI-Q-TOF) *m/z*: ($[M+H]^+$) calcd for $C_{28}H_{47}N_8O_8$ 623.3511, found 623.3510.

For H-**LYRAP**-$SCH_2CH_2SO_3H$:

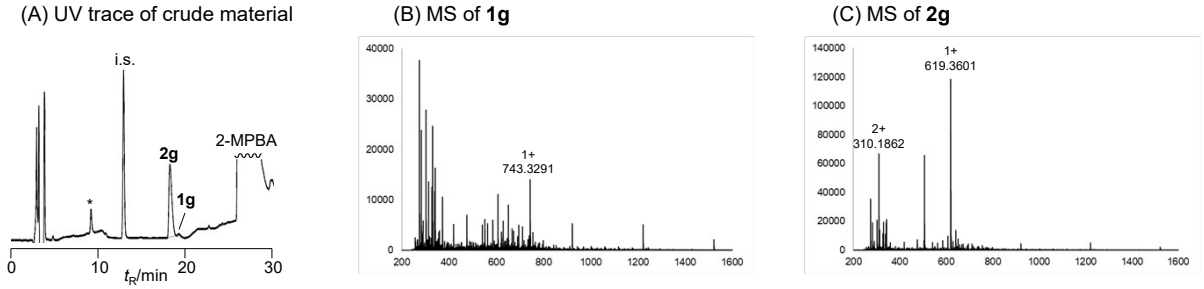


Figure S24. (A) UV trace of the crude material (24 h). HPLC conditions: A Cosmosil $5C_{18}$-AR-II column with a linear gradient of solvent B in solvent A, 10% to 14% over 15 min then 14% to 55% over another 15 min. (B) MS spectrum of **1g**. **1g**: Retention time = 19.1 min, MS (ESI-Q-TOF) *m/z*: ($[M+H]^+$) calcd for $C_{31}H_{51}N_8O_9S_2$ 743.3215, found 743.3291. (C) MS spectrum of **2g**. **2g**: Retention time = 18.1 min, MS (ESI-Q-TOF) *m/z*: ($[M+H]^+$) calcd for $C_{29}H_{47}N_8O_7$ 619.3562, found 619.3601.

References for Supplementary Information

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S2 S. Eissler, M. Kley, D. Bächle, G. Loidl, T. Meier and D. Samson, *J. Pept. Sci.*, 2017, **23**, 757.

S3 K. Sato, S. Tanaka, K. Yamamoto, Y. Tashiro, T. Narumi and N. Mase, *Chem. Commun.*, 2018, **54**, 9127.

S4 Y. Kim, K. Kanemoto, K. Shimomori, T. Hosoya and S. Yoshida, *Chem. Eur. J.*, 2020, **26**, 6136.

S5 J. Krajewska, P. Chyży, K. Durka, P. Wińska, K. A. Krzyśko, S. Luliński and A. E. Laudy, *Molecules*, 2023, **28**, 7362.

Cartesian coordinates

3+4

C	1.618000	-1.222000	-1.324000
S	2.843000	-1.941000	-0.251000
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5 after TS1

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5 before TS2

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TS2

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S	-0.643000	-1.266000	-1.331000
C	-1.248000	0.374000	-0.940000
C	-2.283000	0.551000	0.011000
C	-2.666000	1.863000	0.330000
C	-2.066000	2.973000	-0.274000
C	-1.052000	2.779000	-1.223000
C	-0.649000	1.488000	-1.554000
B	-3.043000	-0.633000	0.722000
O	-3.062000	-0.610000	2.097000
C	1.697000	-2.625000	-0.932000
C	0.930000	0.686000	1.588000

H	3.049000	-0.206000	-0.542000
H	-3.447000	2.028000	1.069000
H	-2.385000	3.980000	-0.008000
H	-0.580000	3.635000	-1.702000
H	0.148000	1.329000	-2.270000
H	-3.586000	-1.307000	2.528000
H	1.694000	-2.794000	-2.012000
H	2.726000	-2.692000	-0.571000
H	1.104000	-3.407000	-0.448000
H	1.518000	0.958000	2.468000
H	1.330000	1.189000	0.699000
H	-0.117000	0.977000	1.720000
O	3.945000	-0.373000	0.050000
H	4.318000	0.538000	0.337000
H	3.578000	-0.799000	0.858000
O	-3.698000	-1.565000	-0.042000
H	-4.155000	-2.276000	0.453000
O	4.750000	2.056000	0.696000
H	4.146000	2.463000	1.342000
H	5.640000	2.130000	1.079000

6+7

C	0.762000	2.088000	-0.214000
O	1.221000	1.519000	0.766000
S	-0.955000	1.984000	-0.711000
C	-1.625000	0.833000	0.500000
C	-2.063000	-0.457000	0.125000
C	-2.529000	-1.296000	1.157000
C	-2.576000	-0.883000	2.489000
C	-2.145000	0.403000	2.830000
C	-1.672000	1.259000	1.834000
B	-2.096000	-1.040000	-1.340000
O	-1.895000	-2.395000	-1.459000
C	1.610000	2.919000	-1.148000
H	-2.859000	-2.300000	0.901000
H	-2.944000	-1.558000	3.256000
H	-2.176000	0.740000	3.862000
H	-1.328000	2.255000	2.094000
H	-1.969000	-2.755000	-2.356000
H	2.288000	3.540000	-0.555000

H	2.215000	2.234000	-1.753000
H	1.016000	3.548000	-1.816000
O	-2.392000	-0.213000	-2.393000
H	-2.398000	-0.631000	-3.268000
S	1.508000	-1.344000	-1.481000
C	1.109000	-1.819000	0.251000
H	1.957000	-2.323000	0.720000
H	0.830000	-0.935000	0.826000
H	0.254000	-2.496000	0.199000
H	2.545000	-0.529000	-1.181000
H	3.058000	0.976000	0.595000
O	3.906000	0.629000	0.254000
H	4.071000	-0.204000	0.745000
O	4.415000	-1.855000	1.533000
H	4.622000	-2.518000	0.853000
H	5.198000	-1.831000	2.107000

3’+4

C	-2.886000	-0.225000	0.141000
S	-4.480000	-0.399000	-0.633000
O	-2.645000	0.691000	0.923000
C	-1.862000	-1.249000	-0.267000
C	-5.347000	1.054000	0.057000
H	-1.146000	-0.748000	-0.934000
H	-1.311000	-1.590000	0.615000
H	-2.302000	-2.103000	-0.790000
H	-6.358000	1.040000	-0.357000
H	-5.389000	0.985000	1.146000
H	-4.836000	1.973000	-0.239000
S	0.729000	0.919000	-2.125000
C	1.651000	-0.268000	-1.178000
C	2.533000	0.151000	-0.144000
C	3.255000	-0.806000	0.586000
C	3.125000	-2.178000	0.332000
C	2.252000	-2.595000	-0.683000
C	1.531000	-1.657000	-1.423000
B	2.692000	1.682000	0.173000
O	1.862000	2.232000	1.137000
H	3.929000	-0.478000	1.377000
H	3.691000	-2.904000	0.911000

H	2.131000	-3.655000	-0.896000
H	0.856000	-1.996000	-2.206000
H	1.977000	3.177000	1.329000
O	3.704000	2.390000	-0.428000
H	3.765000	3.330000	-0.192000
O	-0.231000	0.796000	2.382000
H	0.458000	1.304000	1.905000
H	-1.033000	0.818000	1.815000
O	0.458000	-1.898000	2.380000
H	1.167000	-1.999000	1.721000
H	0.259000	-0.931000	2.386000

TS3

C	-1.377000	-0.331000	-0.583000
S	-2.551000	1.125000	-0.962000
O	-1.105000	-0.536000	0.629000
C	-1.759000	-1.510000	-1.470000
C	-2.015000	2.253000	0.367000
H	-0.975000	-2.271000	-1.408000
H	-2.694000	-1.940000	-1.086000
H	-1.897000	-1.221000	-2.515000
H	-2.657000	3.137000	0.335000
H	-2.110000	1.758000	1.336000
H	-0.971000	2.540000	0.207000
S	0.478000	0.459000	-1.632000
C	1.722000	-0.447000	-0.735000
C	2.433000	0.169000	0.323000
C	3.362000	-0.596000	1.051000
C	3.603000	-1.940000	0.749000
C	2.905000	-2.541000	-0.305000
C	1.976000	-1.800000	-1.039000
B	2.222000	1.690000	0.683000
O	1.505000	1.992000	1.817000
H	3.909000	-0.133000	1.871000
H	4.326000	-2.510000	1.326000
H	3.084000	-3.584000	-0.556000
H	1.437000	-2.269000	-1.858000
H	1.425000	2.937000	2.027000
O	2.848000	2.639000	-0.094000
H	2.700000	3.561000	0.170000

O	-2.788000	-2.050000	2.095000
H	-2.773000	-2.965000	1.773000
H	-2.099000	-1.568000	1.563000
O	-4.863000	-0.511000	0.933000
H	-4.304000	0.019000	0.328000
H	-4.220000	-1.103000	1.387000

5’

C	-1.228000	-0.391000	-0.534000
S	-2.634000	0.890000	-1.100000
O	-1.004000	-0.402000	0.725000
S	0.335000	0.371000	-1.588000
C	1.702000	-0.422000	-0.744000
C	2.396000	0.250000	0.289000
C	3.403000	-0.449000	0.981000
C	3.742000	-1.765000	0.654000
C	3.065000	-2.412000	-0.385000
C	2.052000	-1.743000	-1.076000
B	2.117000	1.752000	0.678000
O	1.656000	2.010000	1.948000
C	-1.611000	-1.721000	-1.191000
C	-2.236000	2.233000	0.069000
H	3.937000	0.047000	1.790000
H	4.528000	-2.280000	1.201000
H	3.320000	-3.434000	-0.655000
H	1.522000	-2.247000	-1.880000
H	1.559000	2.948000	2.178000
H	-0.793000	-2.435000	-1.044000
H	-2.508000	-2.113000	-0.697000
H	-1.804000	-1.618000	-2.263000
H	-3.100000	2.901000	0.140000
H	-2.027000	1.793000	1.049000
H	-1.361000	2.797000	-0.268000
O	2.446000	2.736000	-0.224000
H	2.265000	3.645000	0.061000
O	-2.594000	-1.806000	2.326000
H	-2.479000	-2.749000	2.134000
H	-1.951000	-1.326000	1.720000
O	-4.787000	-0.666000	0.971000
H	-4.260000	-0.178000	0.302000

H	-4.102000	-1.128000	1.506000

TS4

C	-0.732000	-0.833000	0.390000
O	-0.642000	0.366000	0.668000
S	0.486000	-1.558000	-0.820000
C	2.012000	-0.801000	-0.221000
C	2.217000	0.597000	-0.176000
C	3.443000	1.055000	0.345000
C	4.452000	0.180000	0.757000
C	4.239000	-1.199000	0.679000
C	3.016000	-1.685000	0.210000
B	1.193000	1.685000	-0.682000
O	1.087000	2.833000	0.075000
C	-1.344000	-1.839000	1.338000
H	3.612000	2.127000	0.413000
H	5.393000	0.570000	1.137000
H	5.009000	-1.896000	1.001000
H	2.832000	-2.755000	0.188000
H	0.521000	3.524000	-0.303000
H	-0.585000	-2.066000	2.103000
H	-2.225000	-1.409000	1.820000
H	-1.614000	-2.767000	0.831000
O	0.616000	1.534000	-1.920000
H	-0.043000	2.204000	-2.157000
S	-2.865000	-0.957000	-1.283000
C	-2.734000	0.841000	-1.643000
H	-3.710000	1.332000	-1.563000
H	-2.056000	1.303000	-0.913000
H	-2.336000	1.025000	-2.647000
O	-2.477000	1.590000	2.315000
H	-2.251000	1.392000	3.238000
H	-1.767000	1.166000	1.774000
O	-4.589000	-0.064000	1.334000
H	-4.123000	-0.427000	0.539000
H	-3.928000	0.540000	1.736000

6+7’

C	-0.400000	-1.300000	-1.043000
O	-0.328000	-1.663000	0.124000

S	0.938000	-0.450000	-1.876000
C	2.168000	-0.358000	-0.563000
C	2.231000	0.741000	0.319000
C	3.189000	0.679000	1.351000
C	4.065000	-0.401000	1.485000
C	3.994000	-1.468000	0.586000
C	3.039000	-1.448000	-0.434000
B	1.349000	2.047000	0.236000
O	0.916000	2.566000	1.431000
C	-1.626000	-1.502000	-1.894000
H	3.249000	1.501000	2.059000
H	4.799000	-0.409000	2.287000
H	4.668000	-2.315000	0.679000
H	2.963000	-2.284000	-1.123000
H	0.427000	3.402000	1.377000
H	-1.953000	-2.543000	-1.802000
H	-2.425000	-0.854000	-1.503000
H	-1.456000	-1.261000	-2.947000
O	1.126000	2.629000	-0.984000
H	0.573000	3.426000	-0.977000
S	-3.942000	1.246000	-0.484000
C	-2.158000	1.586000	-0.106000
H	-2.026000	2.596000	0.300000
H	-1.767000	0.877000	0.633000
H	-1.536000	1.505000	-1.004000
O	-2.427000	-2.774000	1.602000
H	-2.891000	-3.419000	1.044000
H	-1.702000	-2.416000	1.040000
O	-4.152000	-0.582000	2.195000
H	-4.144000	-0.024000	1.374000
H	-3.565000	-1.341000	1.989000

8

S	-0.548000	-1.195000	-0.927000
C	2.172000	-1.433000	-0.989000
O	-0.881000	1.061000	1.452000
B	0.136000	1.670000	0.586000
C	1.333000	0.627000	0.096000
C	1.121000	-0.607000	-0.559000
C	-1.097000	-1.982000	0.584000

C	3.752000	0.153000	-0.110000
C	3.497000	-1.055000	-0.766000
C	2.689000	0.963000	0.304000
O	0.805000	2.769000	1.314000
H	1.951000	-2.372000	-1.491000
H	4.777000	0.466000	0.076000
H	4.312000	-1.694000	-1.096000
H	2.907000	1.899000	0.809000
H	0.104000	3.313000	1.703000
O	-2.274000	-2.326000	0.645000
C	-0.121000	-2.174000	1.713000
H	-0.600000	-2.767000	2.496000
H	0.796000	-2.662000	1.370000
H	0.147000	-1.185000	2.098000
H	-1.715000	0.855000	0.993000
O	-0.584000	2.227000	-0.632000
H	0.047000	2.726000	-1.171000
H	-3.371000	-0.668000	0.339000
O	-3.591000	0.282000	0.359000
H	-3.390000	0.615000	-0.549000
O	-2.687000	1.364000	-2.036000
H	-2.405000	0.608000	-2.575000
H	-1.858000	1.661000	-1.557000

TS5

S	0.328000	-1.622000	0.693000
C	-2.398000	-1.437000	0.809000
O	1.257000	0.525000	-0.912000
B	0.023000	1.398000	-0.615000
C	-1.274000	0.508000	-0.195000
C	-1.230000	-0.771000	0.399000
C	1.363000	-1.242000	-0.857000
C	-3.724000	0.436000	0.064000
C	-3.645000	-0.828000	0.656000
C	-2.554000	1.075000	-0.357000
O	-0.308000	2.192000	-1.789000
H	-2.329000	-2.432000	1.242000
H	-4.690000	0.917000	-0.074000
H	-4.545000	-1.346000	0.982000
H	-2.625000	2.054000	-0.826000

H	0.447000	2.758000	-2.010000
O	2.571000	-1.543000	-0.712000
C	0.642000	-1.566000	-2.155000
H	1.240000	-1.182000	-2.986000
H	0.565000	-2.655000	-2.238000
H	-0.359000	-1.132000	-2.198000
H	2.041000	0.806000	-0.382000
O	0.505000	2.258000	0.514000
H	-0.160000	2.936000	0.707000
H	3.447000	-0.312000	0.380000
O	3.579000	0.611000	0.696000
H	3.042000	0.681000	1.519000
O	1.548000	0.877000	2.618000
H	1.178000	-0.005000	2.416000
H	1.101000	1.455000	1.943000

9+3’

S	2.129000	0.136000	-1.978000
C	-0.105000	-1.452000	-1.731000
B	2.212000	-0.485000	1.283000
C	1.081000	-1.082000	0.370000
C	0.973000	-0.856000	-1.036000
C	-0.936000	-2.491000	0.292000
C	-1.043000	-2.253000	-1.084000
C	0.114000	-1.902000	0.993000
O	2.138000	-0.702000	2.647000
H	-0.199000	-1.274000	-2.799000
H	-1.661000	-3.118000	0.805000
H	-1.861000	-2.690000	-1.654000
H	0.197000	-2.075000	2.063000
H	2.889000	-0.299000	3.111000
O	3.271000	0.251000	0.813000
H	3.215000	0.291000	-0.184000
O	-2.381000	0.961000	1.256000
C	-3.081000	0.615000	0.170000
O	-2.955000	1.181000	-0.912000
C	-4.045000	-0.512000	0.430000
H	-4.847000	-0.157000	1.087000
H	-4.474000	-0.863000	-0.509000
H	-3.534000	-1.334000	0.940000

H	-1.767000	1.722000	1.017000
H	-1.380000	2.824000	-0.575000
O	-0.973000	3.008000	0.291000
H	0.005000	2.895000	0.161000
O	1.715000	2.740000	-0.152000
H	1.810000	2.007000	-0.819000
H	2.218000	2.409000	0.613000

6

C	-1.566000	-1.086000	-0.609000
O	-1.453000	-1.241000	0.600000
S	-0.159000	-0.807000	-1.678000
C	1.180000	-0.735000	-0.478000
C	1.659000	0.494000	0.027000
C	2.756000	0.435000	0.911000
C	3.335000	-0.775000	1.298000
C	2.839000	-1.976000	0.784000
C	1.769000	-1.953000	-0.112000
B	1.098000	1.930000	-0.307000
O	-0.254000	2.147000	-0.455000
C	-2.898000	-1.066000	-1.315000
H	3.159000	1.364000	1.305000
H	4.173000	-0.780000	1.990000
H	3.285000	-2.925000	1.070000
H	1.388000	-2.881000	-0.528000
H	-0.494000	3.055000	-0.701000
H	-3.539000	-1.847000	-0.894000
H	-3.359000	-0.091000	-1.109000
H	-2.808000	-1.196000	-2.396000
O	2.032000	2.931000	-0.391000
H	1.690000	3.829000	-0.527000
H	-2.035000	1.768000	0.387000
O	-2.978000	1.892000	0.596000
H	-3.204000	1.178000	1.232000
O	-3.541000	-0.365000	2.223000
H	-3.254000	-0.285000	3.147000
H	-2.833000	-0.871000	1.768000

TS6

C	-2.223000	-0.067000	-0.021000

O	-2.279000	-1.326000	0.310000
S	-0.974000	0.338000	-1.476000
C	0.486000	-0.462000	-0.816000
C	1.506000	0.269000	-0.160000
C	2.616000	-0.450000	0.328000
C	2.708000	-1.839000	0.205000
C	1.694000	-2.544000	-0.451000
C	0.596000	-1.854000	-0.971000
B	1.514000	1.830000	0.069000
O	0.389000	2.448000	0.577000
C	-3.537000	0.604000	-0.390000
H	-1.049000	1.455000	0.923000
H	3.415000	0.091000	0.828000
H	3.571000	-2.364000	0.606000
H	1.759000	-3.623000	-0.564000
H	-0.194000	-2.394000	-1.482000
H	0.456000	3.395000	0.777000
H	-4.184000	0.601000	0.493000
H	-3.397000	1.636000	-0.726000
H	-4.021000	0.025000	-1.183000
O	-1.641000	0.713000	1.191000
H	-0.983000	-0.189000	1.859000
O	2.691000	2.485000	-0.172000
H	2.701000	3.437000	0.015000
O	-0.674000	-1.266000	2.121000
H	0.267000	-1.407000	1.910000
H	-1.284000	-1.585000	1.260000

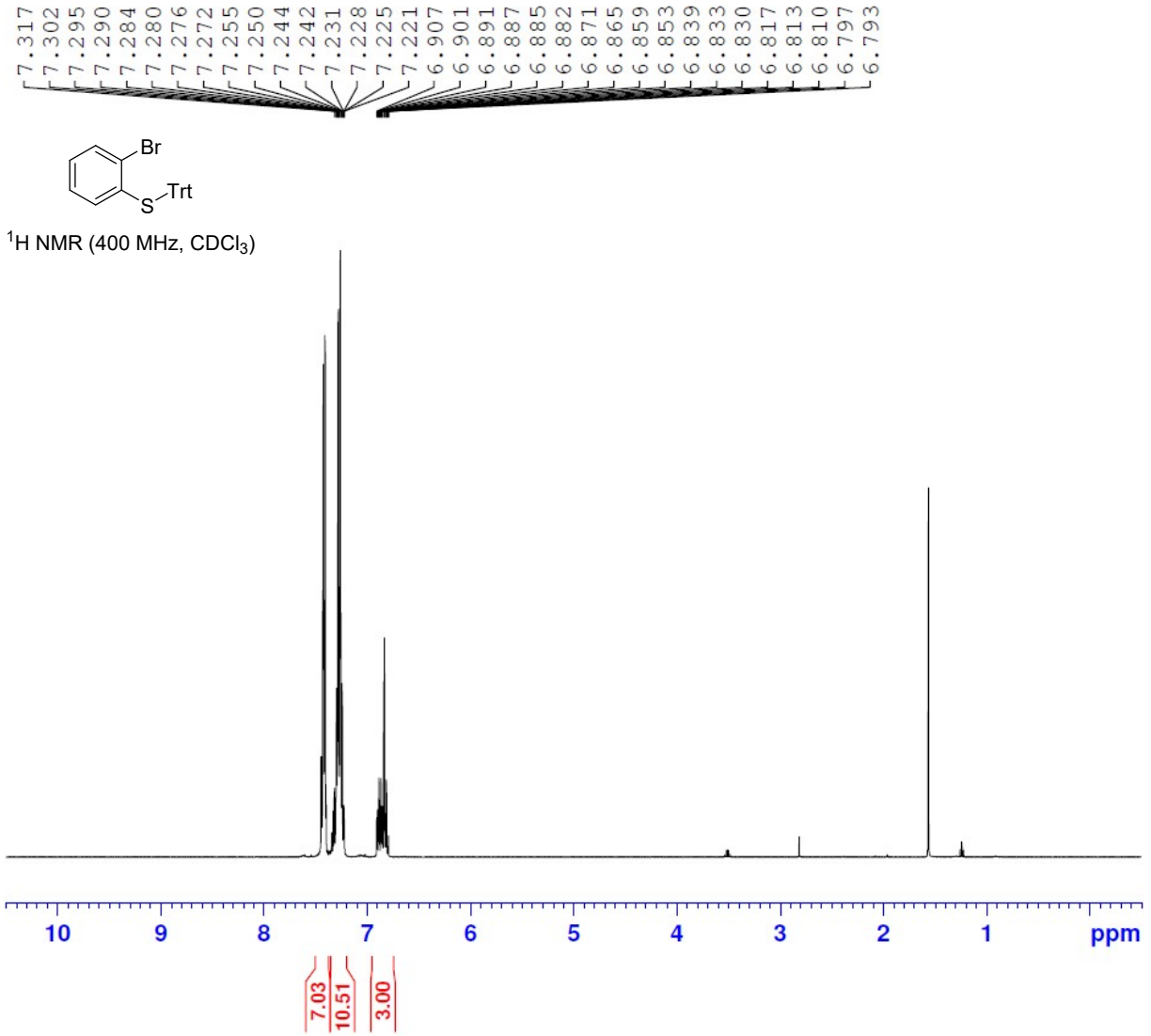
S4

C	-1.896000	0.138000	-0.439000
O	-2.382000	-1.176000	-0.423000
S	-0.433000	0.199000	-1.621000
C	0.850000	-0.602000	-0.648000
C	1.771000	0.158000	0.108000
C	2.814000	-0.534000	0.755000
C	2.926000	-1.925000	0.690000
C	1.997000	-2.660000	-0.052000
C	0.971000	-1.997000	-0.729000
B	1.713000	1.720000	0.313000
O	0.527000	2.304000	0.712000

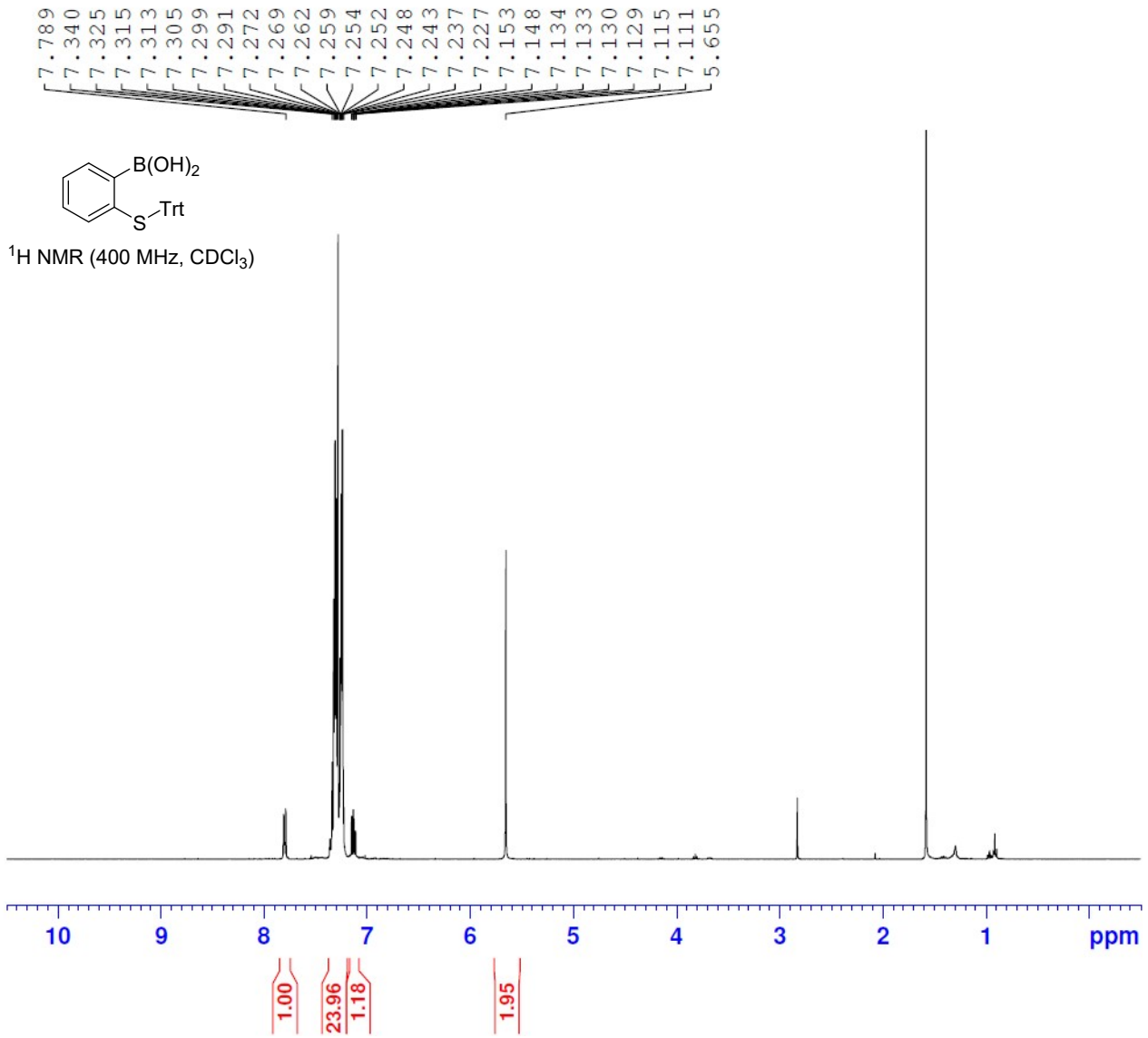
C	-2.939000	1.127000	-0.961000
H	-0.931000	1.249000	0.890000
H	3.547000	0.030000	1.327000
H	3.736000	-2.431000	1.209000
H	2.076000	-3.742000	-0.116000
H	0.260000	-2.559000	-1.327000
H	0.527000	3.267000	0.834000
H	-3.224000	0.882000	-1.989000
H	-3.827000	1.056000	-0.324000
H	-2.558000	2.152000	-0.925000
O	-1.507000	0.453000	0.883000
O	2.882000	2.416000	0.164000
H	2.847000	3.363000	0.372000
H	-2.997000	-1.252000	0.344000
H	-2.933000	-0.202000	2.091000
O	-3.713000	-0.790000	2.032000
H	-3.580000	-1.474000	2.708000

[1]H and [13]C NMR spectra

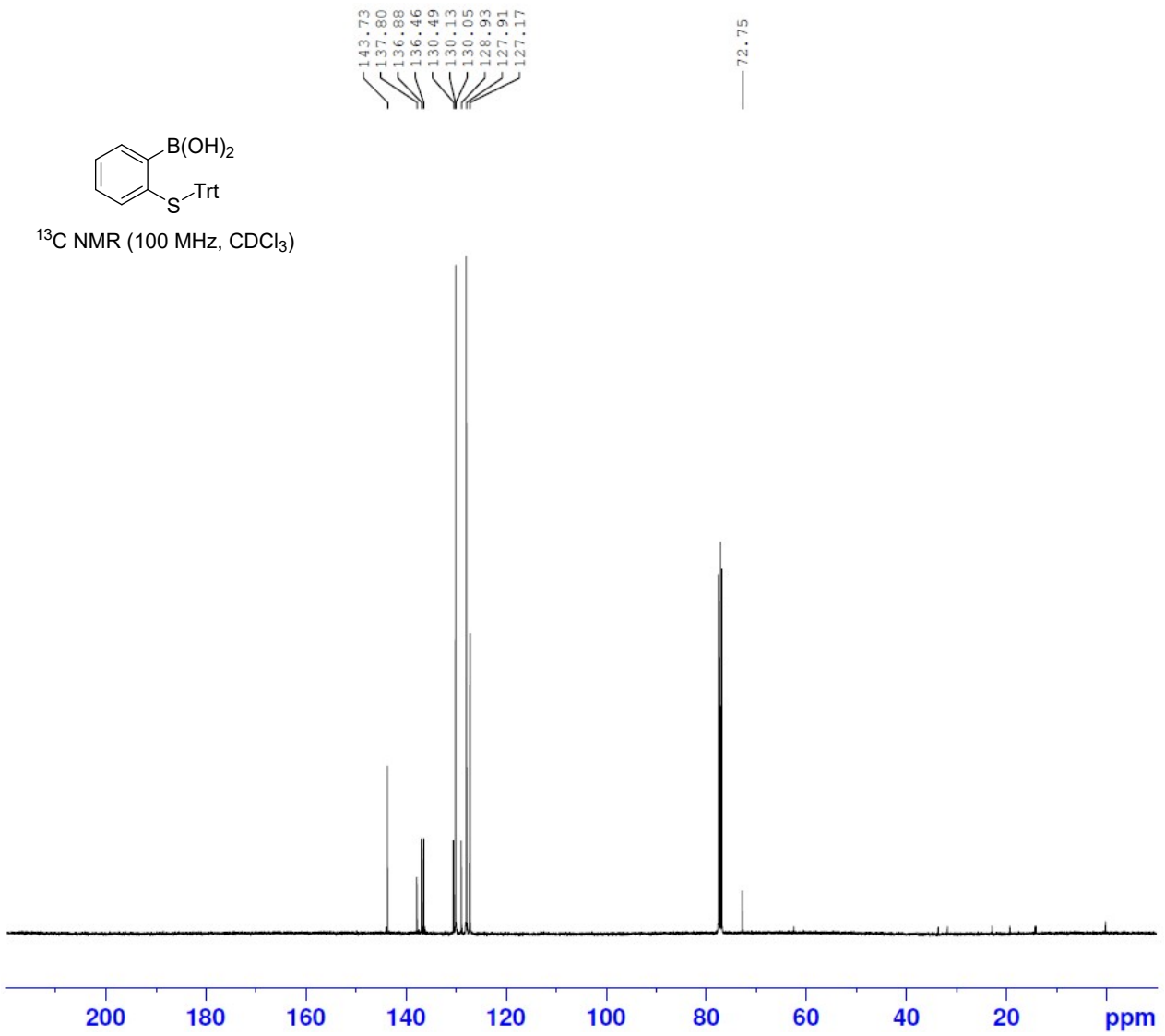
[1]H NMR spectrum of **S1**



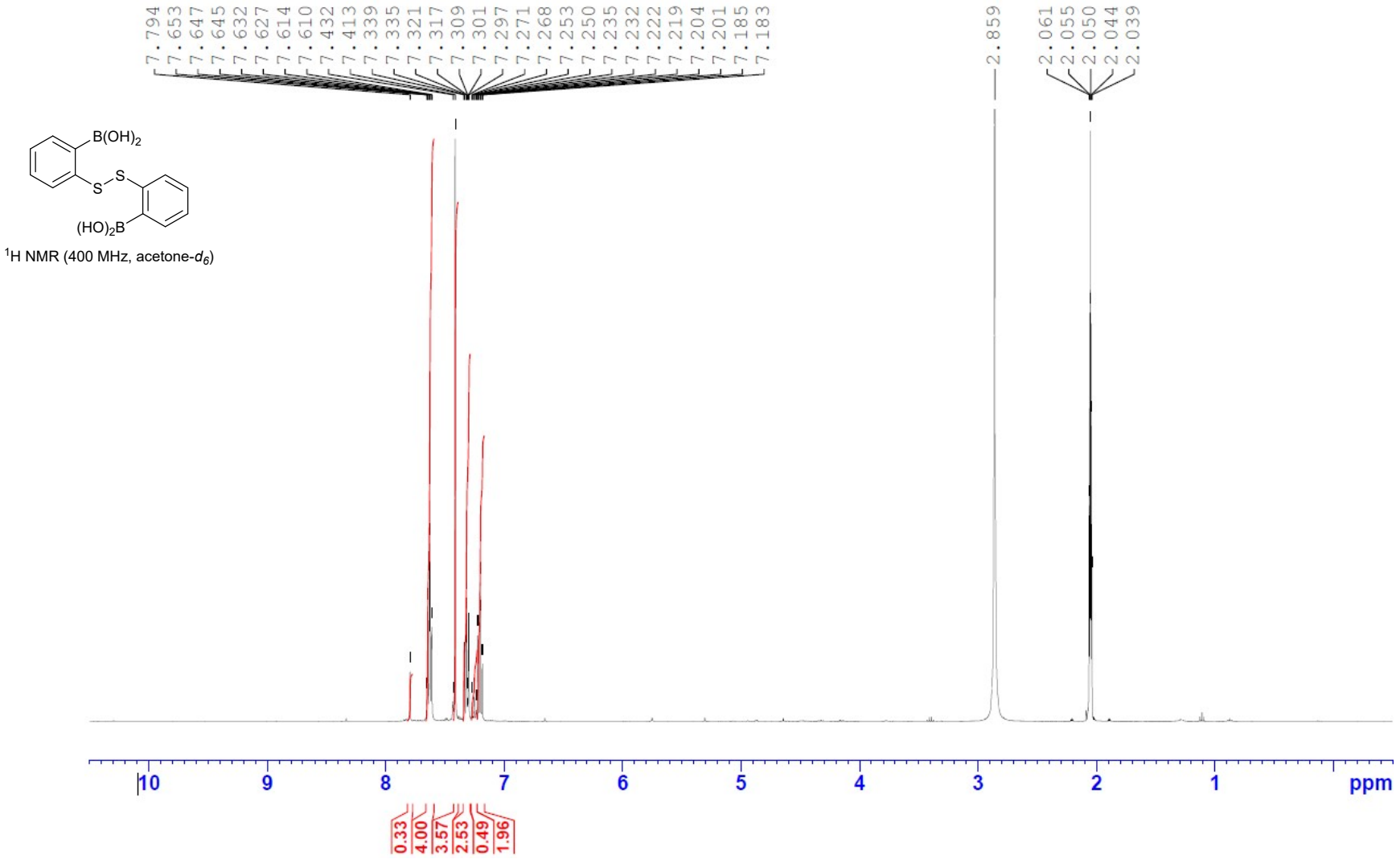
^{1}H NMR spectrum of **S2**



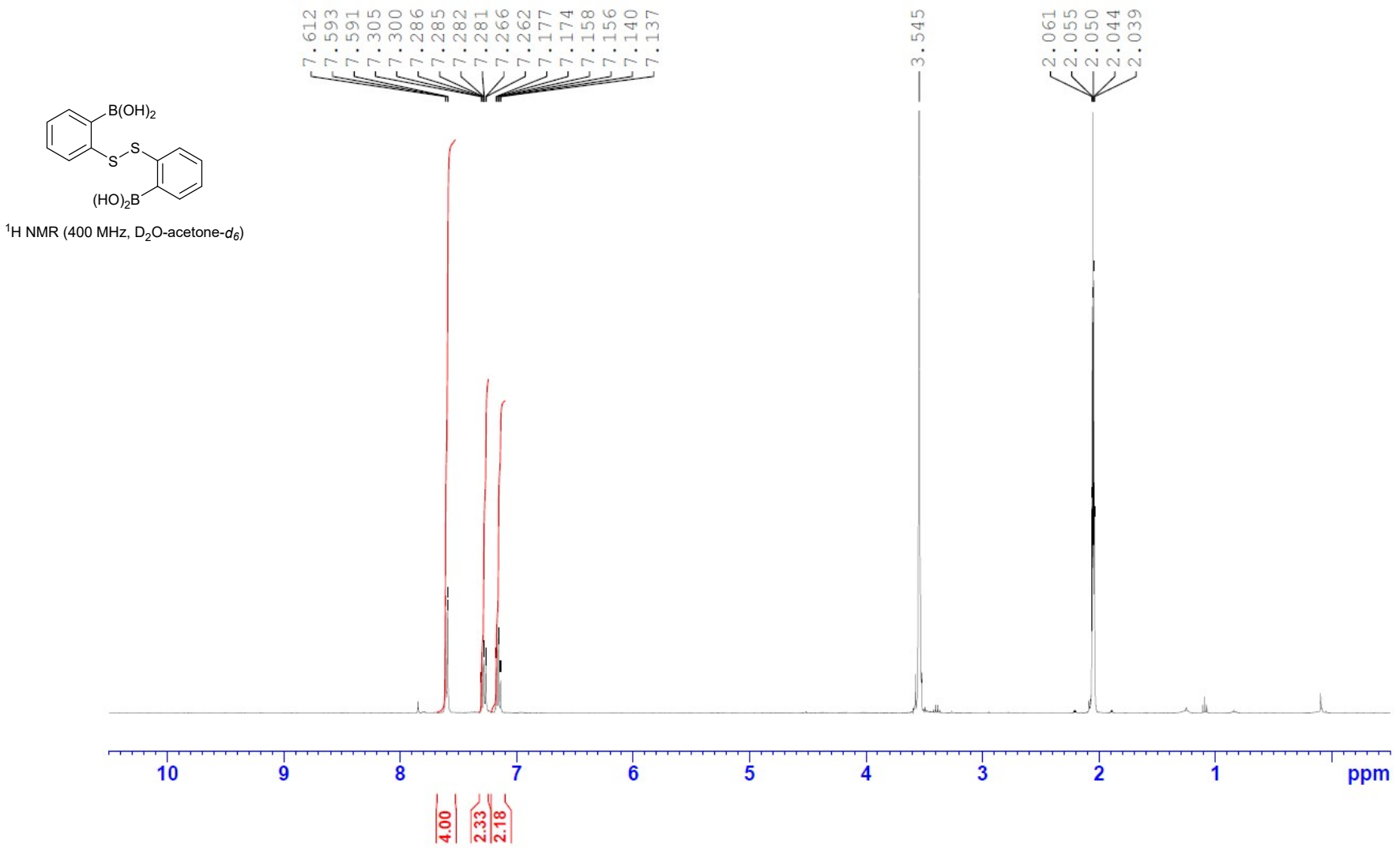
^{13}C NMR spectrum of **S2**



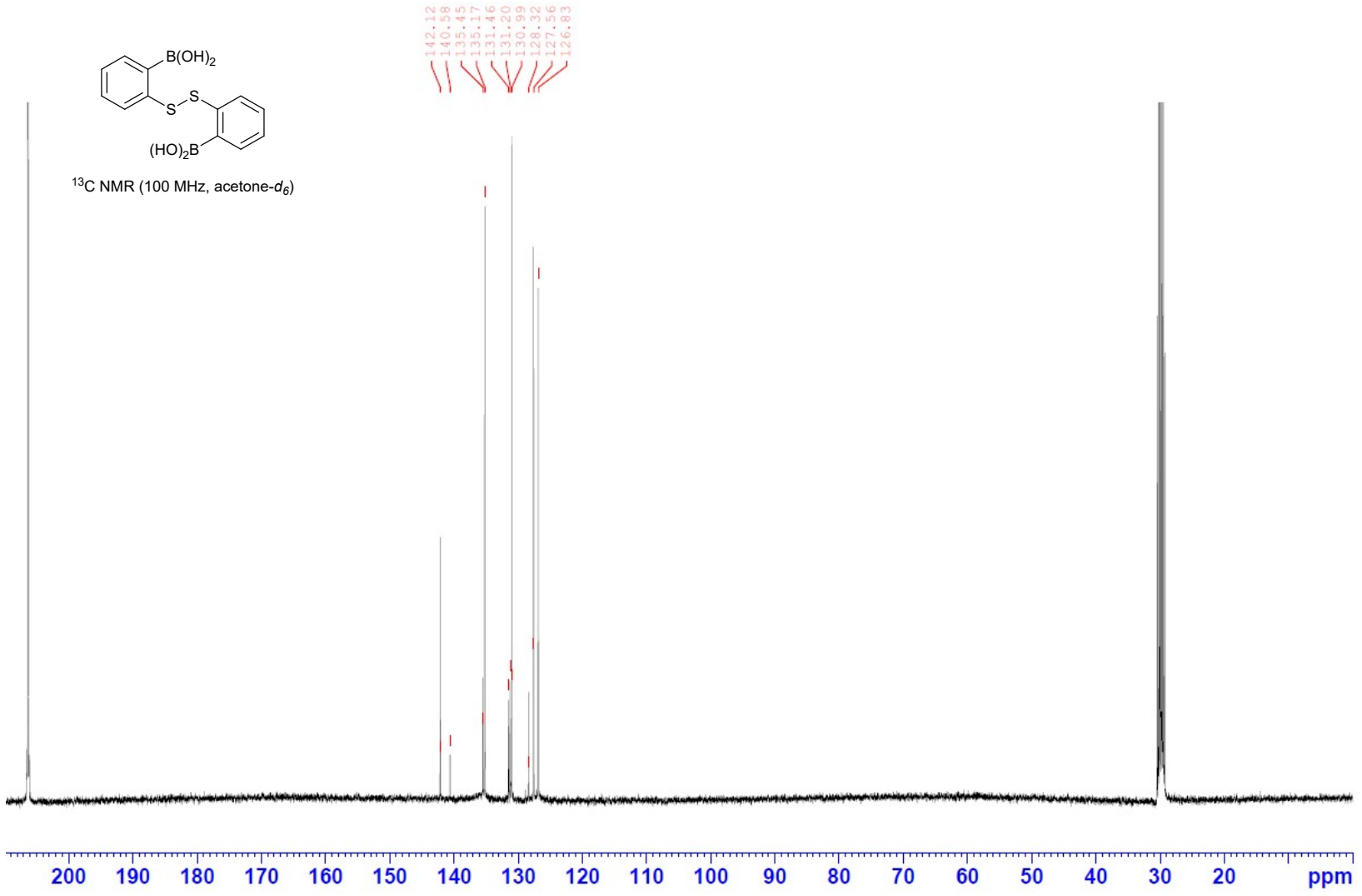
^{1}H NMR spectrum of **S3**



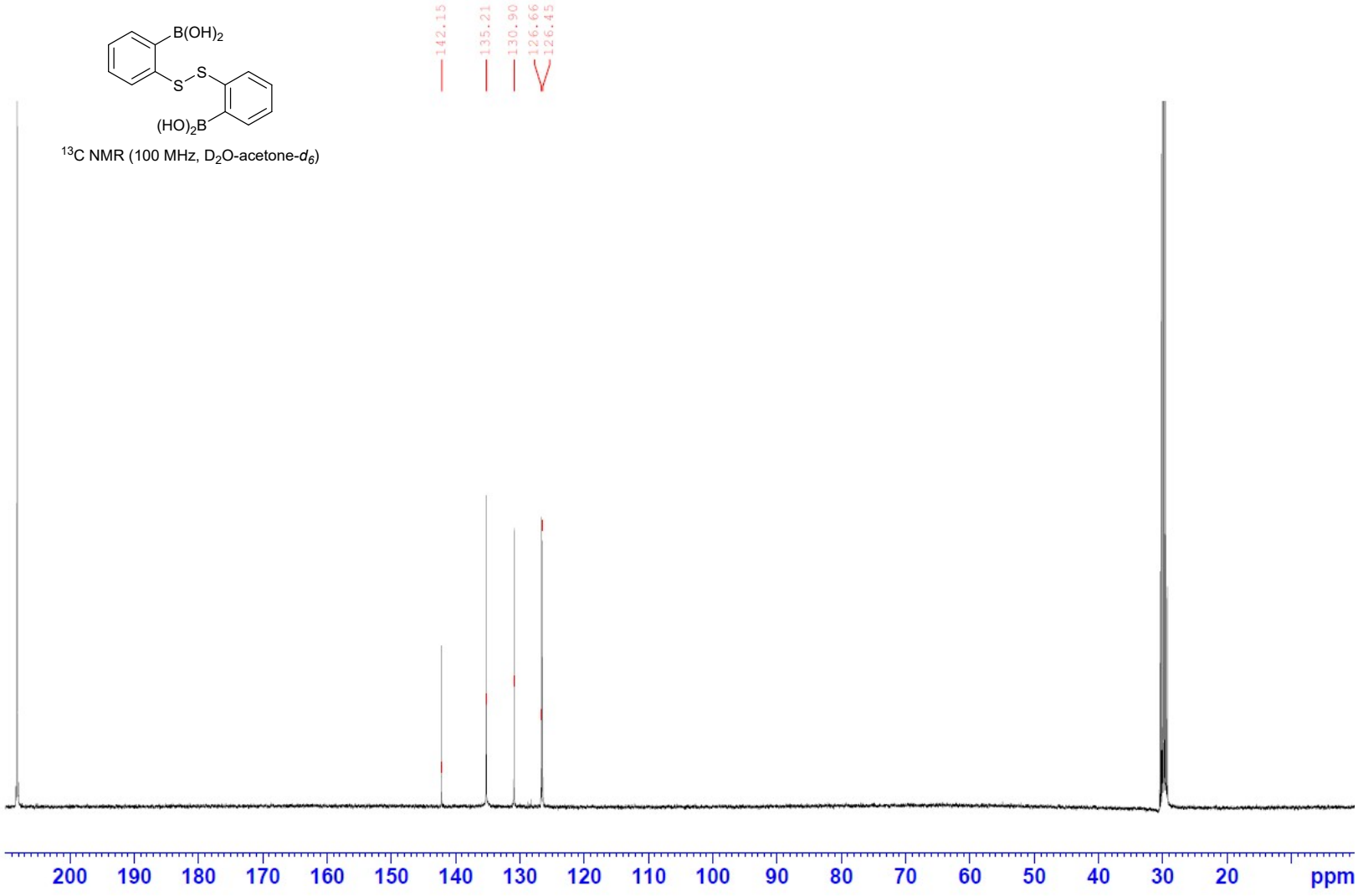
^{1}H NMR spectrum of **S3** (with D_2O)



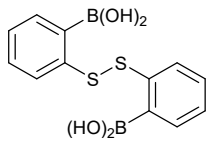
^{13}C NMR spectrum of **S3**



^{13}C NMR spectrum of **S3** (with D_2O)

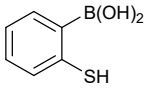


^{11}B NMR spectrum of **S3**



^{11}B NMR (128 MHz, acetone-d_6)

^{1}H NMR spectrum of **S3** after addition of Bu_3P



^{1}H NMR (400 MHz, D_2O-acetone-d_6)

Elemental analysis