Supporting information for

Cysteine-free Intramolecular Ligation of *N*-Sulfanylethylanilide Peptide Using 4-Mercaptobenzylphosphonic Acid: Synthesis of Cyclic Peptide, Trichamide

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General methods

All reactions were carried out under an atmosphere of argon. All commercial reagents were used without further purification. Column chromatography was performed using silica gel (spherical, 63−210 μm; KANTO CHEMICAL Co, Inc.). Preparative thin layer chromatography (PTLC) was carried out on silica gel precoated plates (TLC Silica gel 60G F_{254}; Merck Millipore). Mass spectra were recorded on Waters MICROMASS® LCT PREMIER™ by electrospray ionization time-of-flight (ESI-TOF) reflectron experiments. NMR spectra were recorded on Bruker AV400N at 400 MHz frequency for ^{1}H, and on JEOL JNM-AL300 at 75 MHz frequency for ^{13}C. Chemical shifts were calibrated using solvent signals. The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Optical rotations were recorded on JASCO P-2200 polarimeter. For HPLC separation, Cosmosil $5C_{18}$-AR-II analytical column (4.6 × 250 mm, flow rate 1.0 mL/min) or Cosmosil $5C_{18}$-AR-II preparative column (20 × 250 mm, flow rate 10.0 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA aqueous solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) was used for HPLC elution. For chiral HPLC analysis, CHIRALPAK IA (DAICEL, 4.6 × 250 mm) was employed and eluting products were detected by UV at 254 nm.

Trichamide **3** was further analyzed by ^{1}H, ^{13}C, and two-dimensional NMR using a Varian INOVA 500 (^{1}H 500 MHz, ^{13}C 125 MHz) NMR spectrometer with a 3 mm Nalorac MDBG probe. HPLC-MS was performed using a Micromass Quattro-II (Waters) instrument in positive ion mode. UPLC-MS was done so on an Agilent 6530 Accurate Mass Q-TOF dual ESI mass spectrometer equipped with a Phenomenex Luna C18 250 x 4.6 mm column. Mobile phase A: water (0.1% formic acid), mobile phase B: ACN (0.1% FA). Gradient: 0-1 min, hold 5% B, 1-10 min, linear increase to 40% B, then 8 min wash at 99% B followed by a 7 min equilibriation at 5% B. Flow 1 mL/min, 10 μL injection volume. MS/MS was conducted at collision energies of 50, 100 and 200 V. Fourier-transform (FT) MS/MS was performed by isolating ion *m/z* 550.23 Da using an LTQ-FT hybrid mass spectrometer (ThermoElectron Corp). Primary mass spectra were acquired with the FT-ICR part of the instrument; MS/MS fragmentation spectra were acquired in the ion trap part of the instrument. Fragmentation was performed by collision-induced dissociation (CID) in the linear ion trap, using a 3.00 Da isolation width and 23 normalized collision energy.

Synthesis of peptides

Fmoc solid-phase peptide synthesis (SPPS)

Peptides **4**, **7a-d** and **19** were synthesized manually on NovaSyn® TGR resin (0.25 mmol amine/g). Fmoc SPPS was performed according to the following protocol.

1. Removal of Fmoc groups was carried out using 20% (v/v) piperidine in DMF for 10 min at room temperature.
2. The resin was washed with DMF (× 5).
3. A standard Fmoc-protected amino acid (3.0 equiv.) was coupled with the aid of *N*,*N*-diisopropylcarbodiimide (DIPCDI) (3.0 equiv.) and 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) (3.3 equiv.) in DMF for 1.5 h at room temperature. Thiazole derivatives **17** and **18** (3.0 equiv.) were coupled with the aid of *N*,*N*-diisopropylethylamine (DIPEA) (3.0 equiv.) and *N*,*N*,*N*',*N*'-tetramethyl-*O*-(benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 2.9 equiv.). Completion of the coupling reaction was checked by the Kaiser ninhydrin test. The coupling reaction was repeated until the Kaiser test became negative.
4. The resin was washed with DMF (× 3).
5. A cycle of step 1 to 4 was repeated.

Deprotection of acid-labile protecting groups with concomitant release of peptides from a resin was achieved using a cocktail of TFA/*m*-cresol/thioanisole/1,2-ethanedithiol/H_2O (80/5/5/5/5 (v/v), 50 μL/1 mg resin) at room temperature for 2 h. After the resin was filtered off, cooled diethyl ether (Et_2O) was added to the filtrate and the precipitate was collected by centrifugation. The obtained precipitate was washed with cooled Et_2O and purified by preparative HPLC. Characterization data of peptides is listed in Table S1.

Boc SPPS

Peptide **6** was manually prepared on a methylbenzhydrylamine (MBHA) resin (0.70 mmol amine/g) using the following protocol.

1. Removal of Boc groups was performed with a solution of TFA/triethylsilane/toluene (50/5/45 (v/v)) for 10 min at room temperature.
2. The resin was washed with toluene (× 5) followed by DMF (× 5).
3. A solution of Boc-protected amino acid (3.0 eq.), DIPCDI (3.0 eq.), and HOBt·H_2O (3.3 eq.) in DMF was added to the reaction vessel, and the N-terminal amine was then neutralized with DIPEA (2.0 eq.) *in situ*[S1]. The reaction mixture was stirred for 1.5 h at room temperature.

Completion of the coupling reaction was checked by the Kaiser ninhydrin test.

4. The resin was washed with DMF (× 5) followed by toluene (× 5).
5. A cycle of step 1 to 4 was repeated.

The resulting completed resin was treated with 1 M trimethylsilyl trifluoromethanesulfonate (TMSOTf)–thioanisole in TFA (50 μL/1 mg resin)/*m*-cresol (100/5 (v/v)) at 4 °C for 2 h. The resin in the reaction mixture was filtered off and the obtained filtrate was poured into cooled diethyl ether (Et_2O) to give a precipitate. The precipitate was collected by centrifugation and washed with cooled Et_2O and purified by preparative HPLC. Characterization data is listed in Table S1.

Table S1. Characterization data of synthetic peptides.

Peptide	Synthetic Method	Analytical HPLC[a]		Preparative HPLC[b]	*m/z*		Yield (%)
		Retention time (min)	Gradient (%)	Gradient (%)	Calcd	Found	
4	Fmoc	20.3	1–40	7–22	567.3 $[M + 2H]^{2+}$	567.4	50
7a	Fmoc	20.2	5–45	16–26	574.4 $[M + 2H]^{2+}$	574.3	37
7b	Fmoc	22.6	5–45	20–30	583.3 $[M + 2H]^{2+}$	583.3	38
7c	Fmoc	20.5	5–45	17–27	574.3 $[M + 2H]^{2+}$	574.2	39
7d	Fmoc	20.3	5–45	17–27	545.3 $[M + 2H]^{2+}$	545.3	40
6	Boc	22.6	5–45	14–24	606.3 $[M + 2H]^{2+}$	606.3	26

[a]Cosmosil $5C_{18}$-AR-II analytical column was employed with a linear gradient of solvent B in solvent A over 30 min. [b]Cosmosil $5C_{18}$-AR-II preparative column was employed.

Typical procedure for intramolecular ligation using SEAlide peptide

Peptide **4** (5.0 μmol) was dissolved in 10 mL of degassed ligation buffer [NMP/H_2O = 4/1 (v/v) containing 20 mM 4-mercaptobenzylphosphonic acid (MBPA) and 15 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl), final pH 7.6]. Progress of the reaction was monitored by HPLC (Figure S1). After incubation for 72 h at 37 ºC, the ligation buffer was diluted with H_2O containing 0.1% TFA (v/v), and the products were purified by preparative HPLC. Cyclic pepetide **5** was obtained as a white powder after lyophilization (3.3 mg, 3.3 μmol, 82% HPLC conversion, 65% isolated yield).

Conversion of the reaction was calculated as follows. A: peak area detected by HPLC at 220 nm.

Conversion (%) = $100\times (A_{\text{cyclized product}}) / [(A_{\text{cyclized product}}) + (A_{\text{substrate}}) + (A_{\text{thioester intermediate}}) + (A_{\text{hydrolized product}})]$

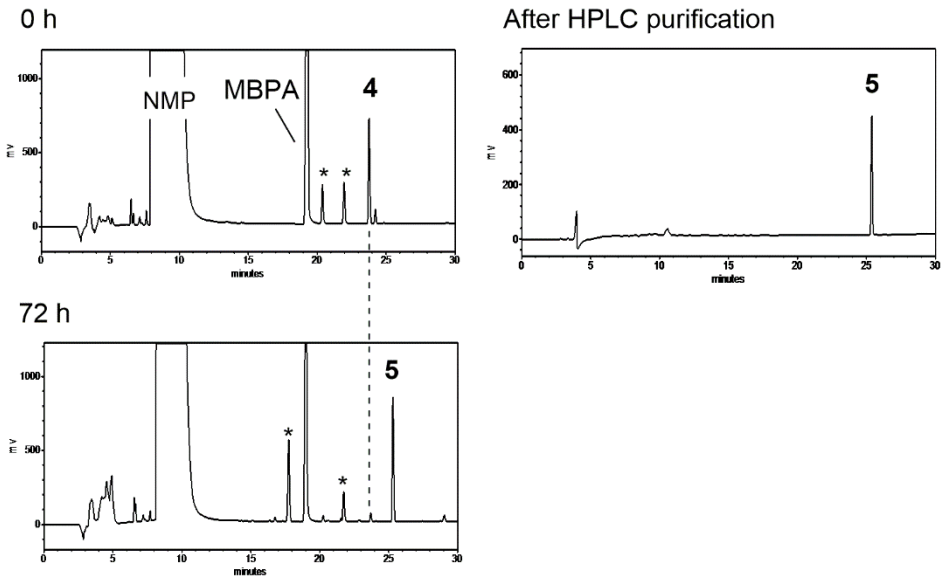
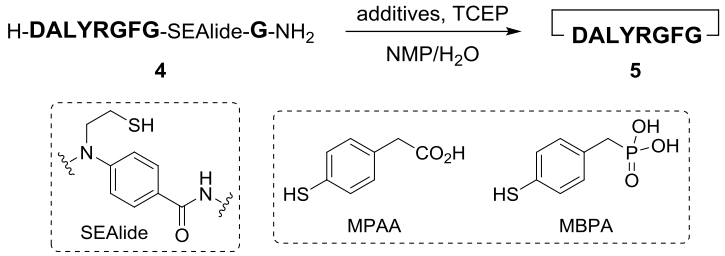


Figure S1. HPLC monitoring of intramolecular ligation. HPLC conditions: linear gradient of solvent B/solvent A (1/99–40/60 over 30 min). *non-peptidic compounds.

Intramolecular ligation using SEAlide peptide **4** in the presence of various additives

Peptide **4** (0.05 μmol) was dissolved in 100 μL of degassed ligation buffer [NMP/H_2O = 4/1 (v/v) containing 20 mM each additive and 15 mM TCEP·HCl, final pH 7.6] and the obtained reaction mixture was incubated at 37 °C. Reactions were monitored by HPLC, and conversion was calculated according to an equation described in the previous section (Figure S2).



	Entry	Additive
■	1	MPAA
▲	2	MPAA, Na_2HPO_4
◆	3	MPAA, benzylphosphonic
○	4	MBPA

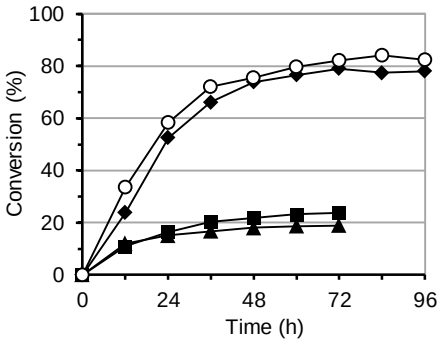
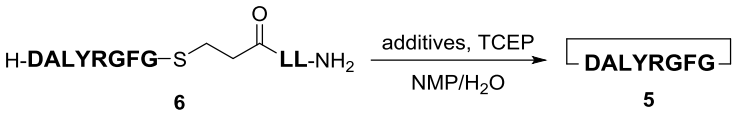


Figure S2. Reaction profile of intramolecular ligation using SEAlide peptide **4** as described in Table 1.

Intramolecular ligation using peptide thioester **6**

Peptide thioester **6** (0.05 μmol) was dissolved in 100 μL of degassed ligation buffer [NMP/H_2O = 4/1 (v/v) containing 20 mM each additive and 15 mM TCEP·HCl, final pH 7.6] and incubated at 37 °C. Reactions were monitored by HPLC, and the conversion was calculated according to an equation shown in page S5 (Figure S3).



	Entry	Additive
■	1	MPAA
▲	2	MPAA, Na_2HPO_4
◆	3	MPAA, benzylphosphonic acid
○	4	MBPA

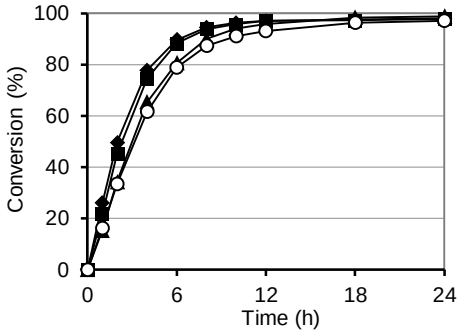
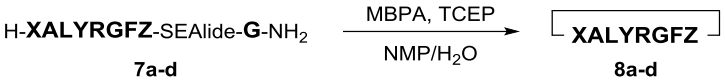


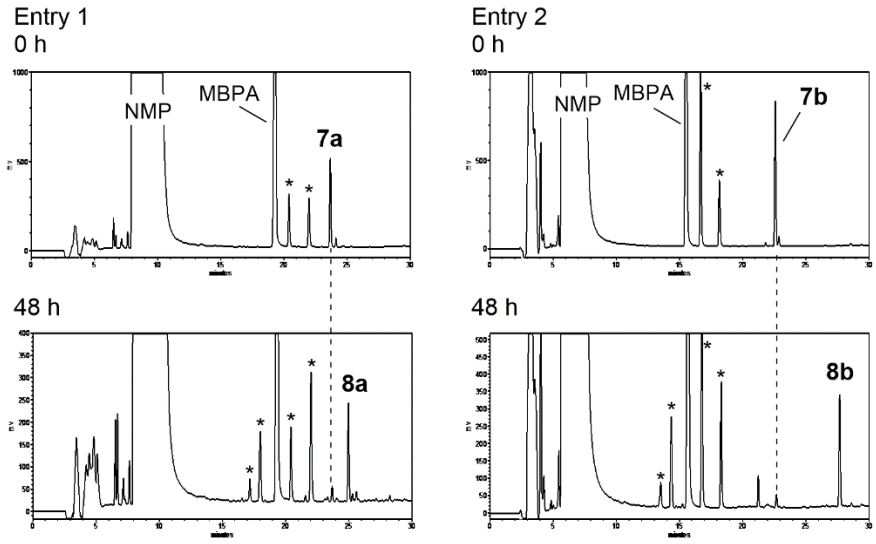
Figure S3. Reaction profile of intramolecular ligation using peptide thioester **6**.

Intramolecular ligation of SEAlide peptides with different N-/C-terminal amino acids.

Peptide **7a–d** (0.05 μmol) were dissolved in 100 μL of degassed ligation buffer [NMP/H_2O = 4/1 (v/v) containing 20 mM MBPA and 15 mM TCEP·HCl, final pH = 7.6] and the obtained mixture was incubated at 37 °C for 48 h. Reactions were monitored by HPLC, and the conversion was calculated according to an equation shown in page S5 (Figure S4).



entry	peptide			conversion (%)
		X	**Z**	
1	**7a**	Glu	Gly	81
2	**7b**	Phe	Gly	74
3	**7c**	Asp	Ala	82
4	**7d**	Gly	Ala	86



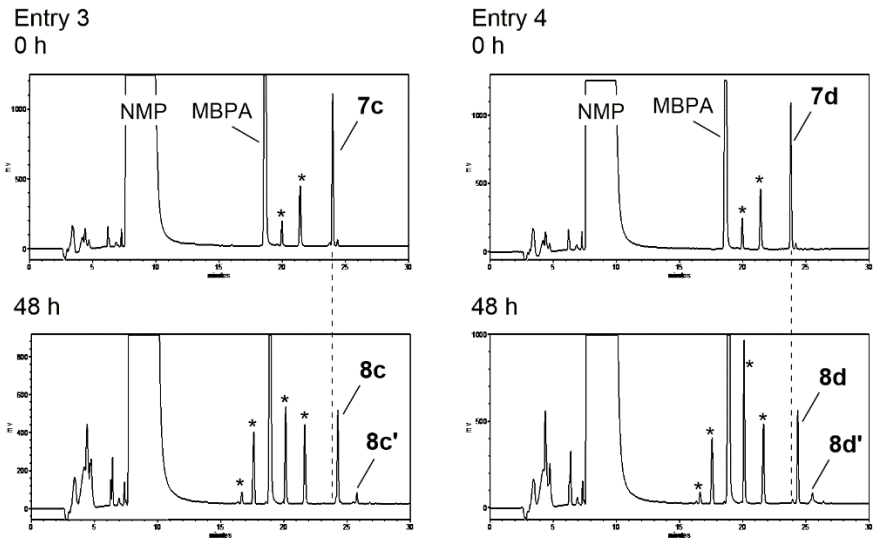
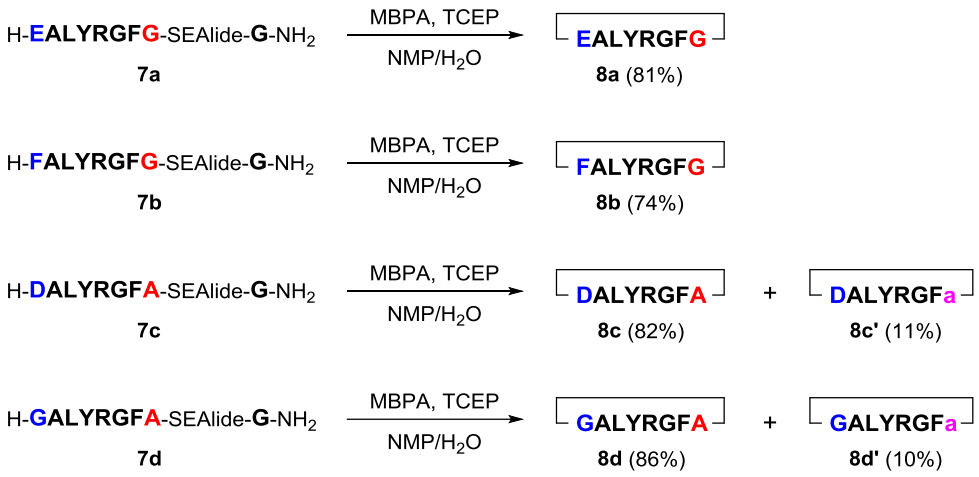


Figure S4. HPLC monitoring of intramolecular ligation using peptide **7a**–**d**. HPLC conditions: linear gradient of solvent B/solvent A (entries 1, 2, and 3: 1/99–40/60 over 30 min; entry 4: 5/95–45/55 over 30 min). *non-peptidic compounds. **8c'** and **8d'** were byproducts resulting from epimerization of the C-terminal alanine.

Table S2. Characterization data of cyclic peptides.



Peptide			Analytical HPLC[a]		*m/z*		Conversion[b]
	X	Z	Retention time (min)	Gradient (%)	Calcd	Found	(%)
8a	Glu	Gly	24.9	1–40	894.5 $[M + H]^+$	894.4	81
8b	Phe	Gly	27.6	5–45	912.5 $[M + H]^+$	912.1	74
8c	Asp	Ala	24.2	1–40	894.5 $[M + H]^+$	894.1	82
8c'	Asp	D-Ala	25.6	1–40	894.5 $[M + H]^+$	894.1	11
8d	Gly	Ala	24.3	1–40	836.4 $[M + H]^+$	836.1	86
8d'	Gly	D-Ala	28.5	1–40	836.4 $[M + H]^+$	836.2	10

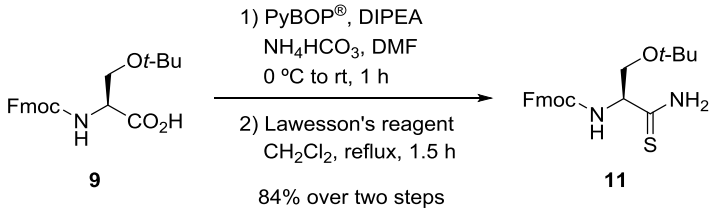
[a]Cosmosil $5C_{18}$-AR-II analytical column was employed with a linear gradient of solvent B in solvent A over 30 min. [b]Conversion of the reaction was calculated based on the peak areas (= A) of desired cyclized peptide ($A_{\text{cyclized product}}$) or byproduct ($A_{\text{cyclized byproduct}}$) derived from epimerization was detected by HPLC at 220 nm.

Conversion (%, **8a–d**) = 100× ($A_{\text{cyclized }\underline{\textbf{product}}}$) / [($A_{\text{cyclized product}}$) + ($A_{\text{cyclized byproduct}}$) + ($A_{\text{substrate}}$) + ($A_{\text{thioester intermediate}}$) + ($A_{\text{hydrolized product}}$)]

Conversion (%, **8c'** and **8d'**) = 100× ($A_{\text{cyclized }\underline{\textbf{byproduct}}}$) / [($A_{\text{cyclized product}}$) + ($A_{\text{cyclized byproduct}}$) + ($A_{\text{substrate}}$) + ($A_{\text{thioester intermediate}}$) + ($A_{\text{hydrolized product}}$)]

Synthetic procedures for thiazole derivatives.

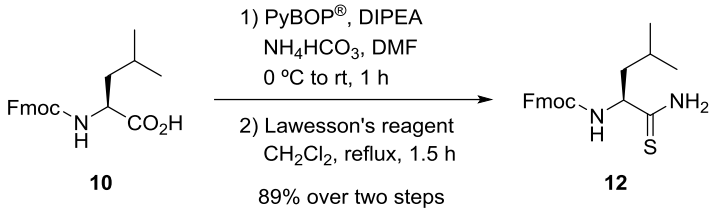
Compound **11**:



To a solution of **9** (1.50 g, 3.91 mmol) in DMF (20 mL), DIPEA (804 μL, 4.70 mmol) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP®, 2.44 g, 4.70 mmol) were added at 0 °C. After stirring at 0 ºC for 20 min, NH_4HCO_3 (372 mg, 4.70 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for additional 1 h, and then concentrated *in vacuo* and diluted with EtOAc and 5% (w/v) aq. $KHSO_4$. The solution was extracted with EtOAc, and the organic phase was washed with 5% (w/v) aq. $KHSO_4$ followed by saturated aq. $NaHCO_3$ and subsequent brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting mixture was passed through a short silica gel pad and concentrated *in vacuo*. The obtained white powder (1.54 g) was subjected to the following reaction without further purification. The crude material (2.70 g) was dissolved in CH_2Cl_2 (35 mL) and Lawesson's reagent (1.57 g, 3.88 mmol) was added to the solution at room temperature. The obtained mixture was refluxed for 1.5 h, and the reaction was then quenched by the addition of saturated aq. $NaHCO_3$. The mixture was extracted with EtOAc and the extract was washed with 5% (w/v) aq. $KHSO_4$ followed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **11** as a white powder (2.37 g, 5.95 mmol, 84% over two steps).

^{1}H NMR (CD_3OD, 400 MHz): δ = 1.16 (9H, s), 3.64 (1H, dd, J = 5.4 and 9.2 Hz), 3.70 (1H, dd, J = 5.4 and 9.2 Hz), 4.17 (1H, t, J = 6.8 Hz), 4.29–4.39 (2H, br m), 4.54 (1H, t, J = 5.4 Hz), 7.27 (2H, t, J = 7.5 Hz), 7.35 (2H, t, J = 7.5 Hz), 7.62 (2H, dd, J = 6.8 and 7.5 Hz), 7.74 (2H, d, J = 7.5 Hz).
^{13}C NMR (CD_3OD, 75 MHz): δ = 27.7, 48.2, 62.5, 65.2, 68.0, 74.6, 120.9, 126.1, 128.1, 128.7, 142.4, 145.0, 157.8, 207.3.
HRMS (ESI-TOF): *m/z* calcd for $C_{22}H_{26}N_2O_3SNa$ ($[M + Na]^+$): 421.1562, found: 421.1558.
Optical rotation: $[\alpha]^{20}_D$ = 17.9 (*c* 1.01, CH_3OH)

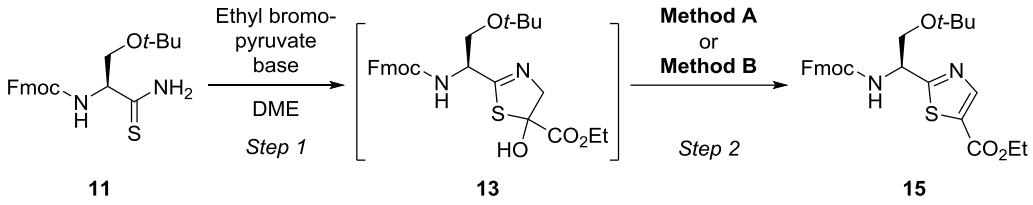
Compound **12**:



Thioamide **12** was prepared from **10** as similar to that of **11**.
A white powder was isolated (1.13 g, 3.07 mmol, 89% over 2 steps).

^{1}H NMR ($CDCl_3$, 400 MHz): 0.95 (6H, br s), 1.55–1.80 (3H, m), 4.21 (1H, t, *J* = 6.7 Hz), 4.38 (2H, d, *J* = 6.7 Hz), 4.43–4.52 (1H, m), 7.30 (2H, t, *J* = 7.6 Hz), 7.38 (2H, t, *J* = 7.6 Hz), 7.66 (2H, dd, *J* = 6.7 and 7.6 Hz), 7.78 (2H, d, *J* = 7.6 Hz).
^{13}C NMR (CD_3OD, 75 MHz): δ = 21.9, 23.5, 26.1, 45.3, 48.4, 60.5, 67.8, 120.9, 126.2, 128.1, 128.7, 142.5, 145.0, 158.2, 211.6.
HRMS (ESI-TOF): *m/z* calcd for $C_{21}H_{24}N_2O_2SNa$ ($[M + Na]^+$): 391.1456, found: 391.1439.
Optical rotation: $[\alpha]^{21}_D$ = –31.3 (*c* 1.03, $CDCl_3$)

Compound **15**:



Method A (Table 3, entry 1): $KHCO_3$ (550 mg, 5.49 mmol) was added to a solution of **11** (199 mg, 0.500 mmol) in 1,2-dimethoxyethane (DME, 5 mL) at –40 °C. After stirring for 30 min, ethyl bromopyruvate (187 µL, 1.50 mmol) was added dropwise to the reaction mixture, and the temperature of the mixture was allowed to gradually reach 0 °C over 12 h. The solution was then filtered through a short pad of Celite®535, and the filtrate was concentrated *in vacuo*. The obtained residue was dissolved in DME (8 mL), and 2,6-lutidine (581 µL, 4.99 mmol) was added to the solution at –40 °C. After a few min, the solution of trifluoroacetic anhydride (TFAA, 172 µL, 1.25 mmol) in DME (2 mL) was added dropwise to the reaction mixture, and the obtained mixture was stirred at –40 °C for 12 h. After completion of the reaction, EtOAc and saturated aq. $NaHCO_3$ were added slowly. The mixture

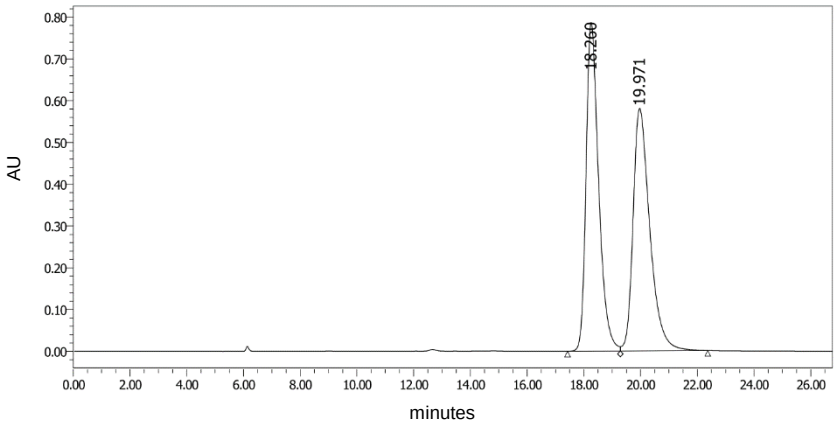
was extracted with EtOAc, and the organic phase was washed with saturated aq. $NaHCO_3$, 5% (w/v) aq. $KHSO_4$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (hexanes/EtOAc = 3/1–2/1 (v/v)) to yield thiazole **15** (194 mg, 0.392 mmol, 79%, 80% ee) as a yellow oil.

Method B (Table 3, entry 5): To a solution of **11** (20.0 mg, 50.2 μmol) in DME (0.5 mL), 2,6-lutidine (17.6 μL, 151 μmol) and ethyl bromopyruvate (18.8 μL, 151 μmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 30 min. 2,6-lutidine (17.6 μL, 151 μmol) and a solution of TFAA (17.3 μL, 126 μmol) in DME (500 μL) were then added to the reaction mixture at 0 ºC. Following to stirring at room temperature for 30 min, the mixture was then diluted with EtOAc. The organic phase was washed with saturated aq. $NaHCO_3$ followed by 5% aq. $KHSO_4$ and subsequent brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by PTLC (hexanes/EtOAc = 2/1 (v/v)) to yield thiazole **15** (23.3 mg, 46.9 μmol, 93%, >99% ee) as a yellow oil.

^{1}H NMR (CD_3OD, 400 MHz): δ = 1.12 (9H, s), 1.35 (3H, t, J = 7.1 Hz), 3.70–3.84 (2H, m), 4.18 (1H, t, J = 6.6 Hz), 4.34 (1H, dd, J = 7.1 and 10.6 Hz), 4.42 (1H, dd, J = 7.1 and 10.6 Hz), 5.10 (1H, br t, J = 5.1 Hz), 7.26 (2H, t, J = 7.5 Hz), 7.35 (2H, t, J = 7.5 Hz), 7.63 (2H, d, J = 7.5 Hz), 7.73 (2H, d, J = 7.5 Hz), 8.23 (1H, s).
^{13}C NMR (CD_3OD, 75 MHz): δ = 14.6, 27.6, 48.3, 55.6, 62.4, 64.6, 67.9, 74.9, 120.9, 126.2, 128.1, 128.7, 129.3, 142.5, 145.1, 147.5, 158.2, 162.6, 174.0
HRMS (ESI-TOF): *m/z* calcd for $C_{27}H_{30}N_2O_5SNa$ ($[M + Na]^+$): 517.1773, found: 517.1775.
Optical rotation: $[\alpha]^{21}_D$ = –8.9 (*c* 1.01, CH_3OH)

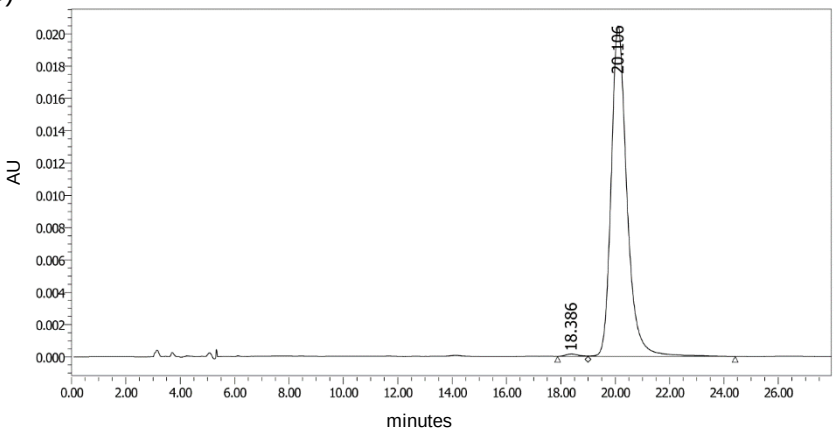
Methods for determining the enantioselectivity of the thiazole synthesis, using (±)-**15** and (–)-**15**; Column: CHIRALPAK IA; dimensions: 4.6 × 250 mm; eluent: *n*-hexane/2-propanol = 90/10; flow rate; 1.0 mL/min; wavelength: λ = 254 nm; retention times: (minor) 18.4 min, (major) 20.1 min.

(A)



Peak #	Retention time (min)	Area (μV/min)	% Area
1	18.3	24167524	50.45
2	20.0	23737525	49.55

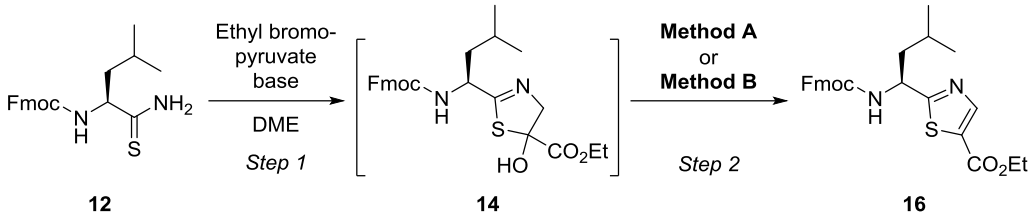
(B)



Peak #	Retention time (min)	Area (μV/min)	% Area
1	18.4	4641	0.56
2	20.1	821194	99.44

Figure S5. (A) Chromatogram of (±)-**15** on a chiral column, showing ~0% ee. (B) Chromatogram and reports of (–)-**15** on a chiral column, showing >99% ee.

Compound **16**:



12 **14** **16**

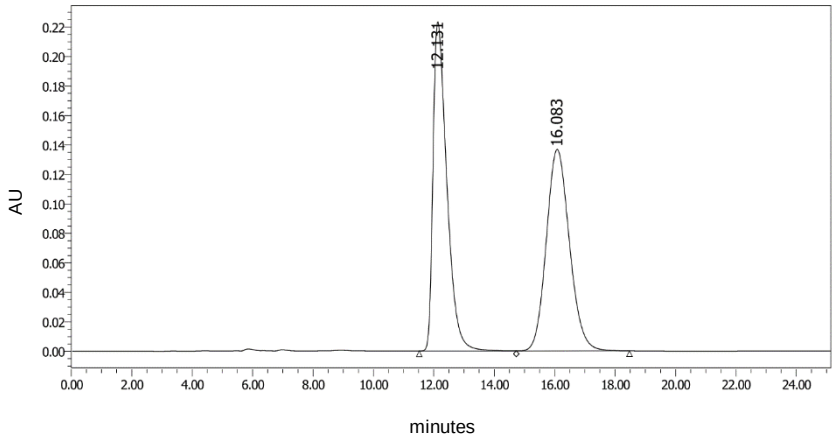
Thiazole **16** was prepared from **12** as similar to that of **15**.

Method A (Table 3, entry 2): a white powder (754 mg, 1.62 mmol, 80%, 95% ee).
Method B (Table 3, entry 6): a white powder (123 mg, 266 μmol, 98%, 99% ee).

^{1}H NMR (CD_3OD, 400 MHz): δ = 0.95 (6H, br d, J = 5.7 Hz), 1.38 (3H, t, J = 7.1 Hz), 1.58–1.72 (1H, m), 1.72–1.85 (1H, m), 1.85–1.98 (1H, m), 4.19 (1H, br t, J = 6.4 Hz), 4.40 (2H, q, J = 7.1 Hz), 4.44 (2H, t, J = 6.4 Hz), 5.12 (1H, br q, J = 8.1 Hz), 5.66 (2H, br d, J = 8.1 Hz), 7.28 (2H, t, J = 6.3 Hz), 7.37 (2H, t, J = 6.3 Hz), 7.57 (2H, br d, J = 6.3 Hz), 7.73 (2H, br d, J = 6.3 Hz), 8.04 (1H, s).
^{13}C NMR ($CDCl_3$, 75 MHz): δ = 14.2, 21.6, 22.8, 24.7, 44.3, 47.1, 51.5, 61.3, 66.6, 119.8, 124.8, 126.9, 127.6, 141.2, 143.5, 147.2, 155.6, 161.2, 173.5.
HRMS (ESI-TOF): *m/z* calcd for $C_{26}H_{28}N_2O_4SNa$ ($[M + Na]^+$): 487.1667, found: 487.1662.
Optical rotation: $[\alpha]^{21}_D$ = –27.2 (*c* 1.00, $CHCl_3$)

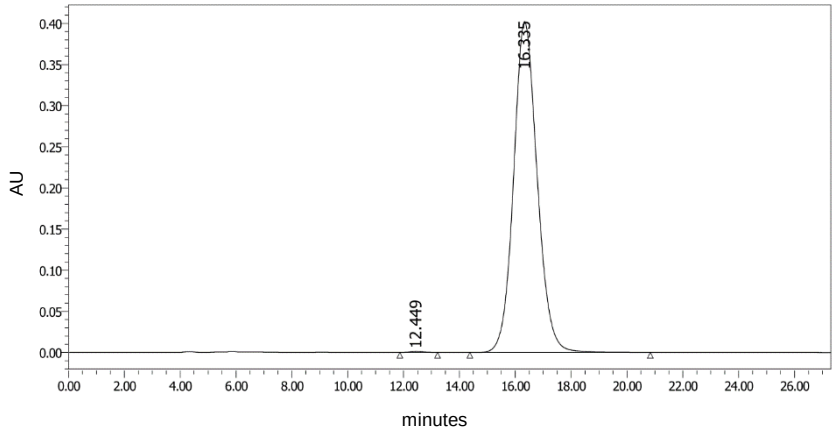
Methods for determining the enantioselectivity of the thiazole synthesis, using (±)-**16** and (-)-**16**; Column: CHIRALPAK IA; dimensions: 4.6 × 250 mm; eluent: *n*-hexane/2-propanol = 85/15; flow rate; 1.0 mL/min; wavelength: λ = 254 nm; retention times: (minor) 12.5 min, (major) 16.3 min.

(A)



Peak #	Retention time (min)	Area (μV/min)	% Area
1	12.1	7089004	49.33
2	16.1	7282158	50.67

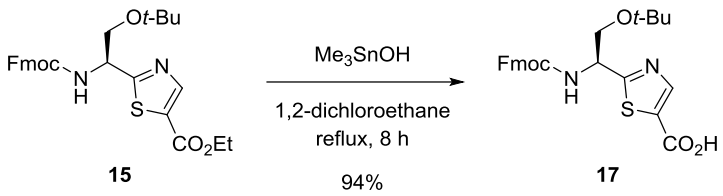
(B)



Peak #	Retention time (min)	Area (μV/min)	% Area
1	12.5	42339	0.18
2	16.3	23590463	99.82

Figure S6. (A) Chromatogram of (±)-**16** on a chiral column, showing ~0% ee. (B) Chromatogram and reports of (–)-**16** on a chiral column, showing >99% ee.

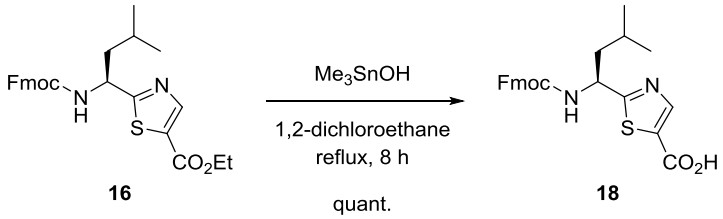
Compound **17**:



To a solution of **15** (50.0 mg, 101 μmol) in 1,2-dichloroethane (2 mL), Me_3SnOH (183 mg, 1.01 mmol) was added at room temperature[S2]. The reaction mixture was then refluxed for 8 h and concentrated *in vacuo*. The obtained product was purified by column chromatography ($CHCl_3$/MeOH = 20/1) to yield **17** (43.8 mg, 93.8 μmol, 93%) as a white amorphous powder.

^{1}H NMR (CD_3OD, 400 MHz): δ = 1.04 (9H, s), 3.61–3.77 (2H, m), 4.13 (1H, t, J = 6.3 Hz), 4.26–4.42 (2H, m), 4.80 (1H, br t, J = 5.1 Hz), 7.20 (2H, t, J = 7.5 Hz), 7.28 (2H, t, J = 7.5 Hz), 7.57 (2H, d, J = 7.5 Hz), 7.69 (2H, d, J = 7.5 Hz), 8.17 (1H, s)
^{13}C NMR (CD_3OD, 75 MHz): δ = 27.6, 48.4, 55.7, 64.6, 68.0, 74.9, 120.9, 126.2, 128.1, 128.8, 129.3, 142.6, 145.1, 148.2, 158.3, 164.1, 173.9
HRMS (ESI-TOF): *m/z* calcd for $C_{25}H_{26}N_2O_5SNa$ ($[M + Na]^+$): 489.1460, found: 489.1442.
Optical rotation: $[\alpha]^{21}_D$ = –8.2 (*c* 1.01, CH_3OH)

Compound **18**:



Carboxylic acid **18** was prepared from **16** as a white amorphous powder (29.1 mg, 66.7 μmol, quant) following the same procedure for the synthesis of **17**.

^{1}H NMR (CD_3OD, 400 MHz): δ = 0.97 (3H, d, J = 6.5 Hz), 0.98 (3H, d, J = 6.5 Hz), 1.60–1.90 (3H, m), 4.21 (1H, t, J = 6.4 Hz), 4.45 (1H, dd, J = 6.4 and 10.6 Hz), 4.50 (1H, dd, J = 6.4 and 10.6 Hz), 5.02 (1H, dd, J = 4.8 and 10.6 Hz), 7.30 (1H, t, J = 7.6 Hz), 7.31 (1H, t, J = 7.6 Hz), 7.39 (2H, t, J = 7.6 Hz), 7.66 (2H, d, J = 7.6 Hz), 7.79 (2H, d, J = 7.6 Hz), 8.24 (1H, s)

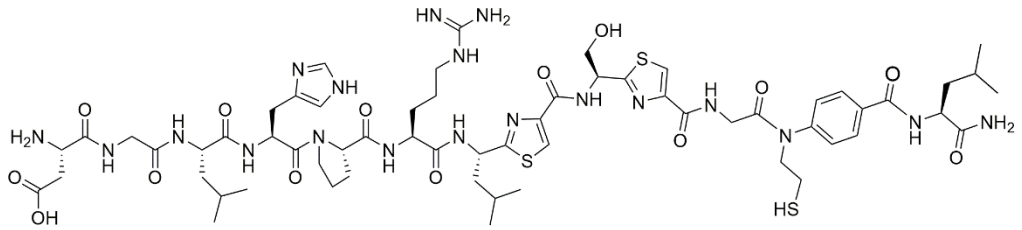
^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.8, 23.4, 26.0, 44.4, 48.3, 52.7, 67.8, 120.9, 124.3, 126.1, 128.1, 128.7, 142.5, 145.0, 154.8, 158.5, 168.8, 175.6

HRMS (ESI-TOF): *m/z* calcd for $C_{24}H_{24}N_2O_4SNa$ ($[M + Na]^+$): 459.1354, found: 459.1355.

Optical rotation: $[\alpha]^{21}_D = -20.4$ (*c* 1.02, $CHCl_3$)

Synthesis of trichamide

Compound **19**

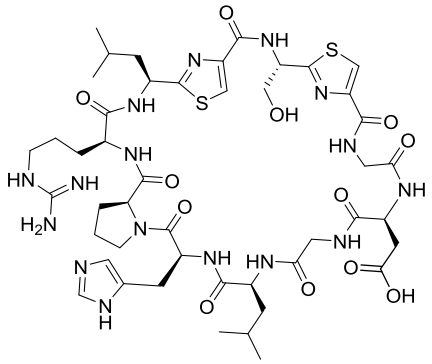


19

SEAlide peptide **19** was synthesized by standard Fmoc SPPS. The crude product was purified by preparative HPLC to give **19** as a white lyophilized powder (9.03 mg, 5.16 μmol, 34%)

Preparative HPLC conditions: Cosmosil 5C_{18}-AR-II preparative column (20 × 250 mm, flow rate 10.0 mL/min) with a linear gradient of solvent B/solvent A (20/80–30/70 over 30 min).
Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm, flow rate 1.0 mL/min) with a linear gradient of solvent B/solvent A (10/90–55/45 over 30 min (curve 7 of the Waters 600E)), retention time: 18.2 min.
HRMS (ESI-TOF): *m/z* calcd for $C_{61}H_{89}N_{19}O_{14}S_3$ ($[M + H]^+$): 1408.6077, found for 1408.6084.

Trichamide **3**



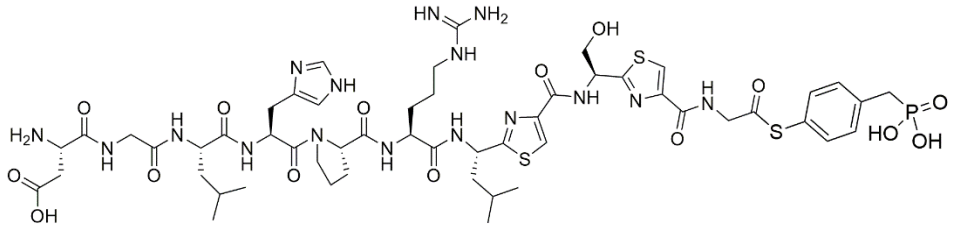
SEAlide peptide **19** (5.2 mg, 3.0 μmol) was dissolved in 6.0 mL of degassed ligation buffer [NMP/H_2O = 4/1 (v/v) containing 20 mM MBPA, 15 mM TCEP·HCl, final pH 7.6], and the reaction mixture was incubated at 37 °C. After 72 h, the mixture was diluted with 0.1% aq. TFA and the product was purified by preparative HPLC to give trichamide **3** as a white lyophilized powder (2.5 mg, 1.9 μmol, 63%).

Preparative HPLC conditions: Cosmosil 5C_{18}-AR-II preparative column (20 × 250 mm, flow rate 10.0 mL/min) with a linear gradient of solvent B/solvent A (20/80–30/70 over 30 min).

Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm, flow rate 1.0 mL/min) with a linear gradient of solvent B/solvent A (10/90–55/45 over 30 min (curve 7 of the Waters 600E)), retention time: 22.4 min.

HRMS (ESI-TOF): *m/z* calcd for $C_{46}H_{66}N_{16}O_{12}S_2$ ($[M + H]^+$): 1099.4566, found for 1099.4569.

Compound **20**



20

Thioester **20** was observed as an intermediate during the intramolecular ligation for the synthesis of trichamide.

Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm, flow rate 1.0 mL/min) with a linear gradient of solvent B/solvent A (10/90–55/45 over 30 min (curve 7 of the Waters 600E)), retention time: 23.3 min.

LRMS (ESI-TOF): *m/z* calcd for $C_{53}H_{75}N_{16}O_{15}PS_3$ ($[M + 2H]^{2+}$): 652.2, found for 652.2.

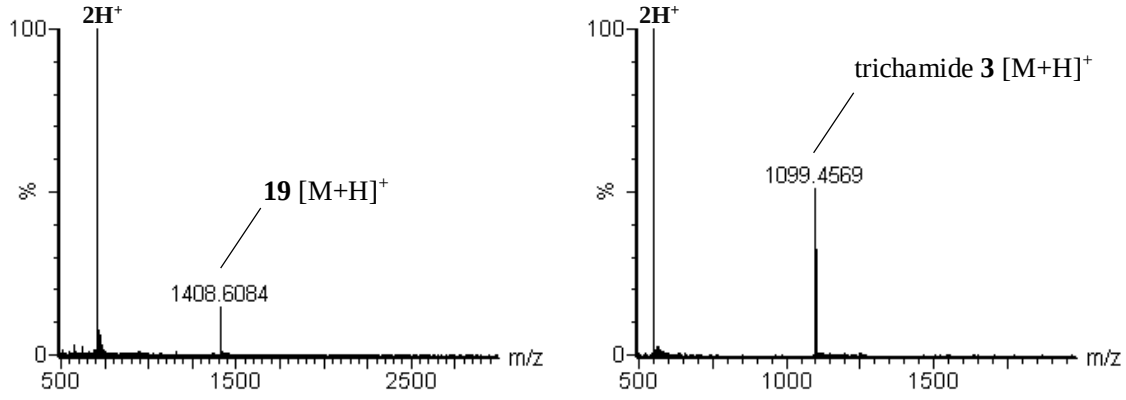


Figure S7. Mass spectra of linear peptide **19** and trichamide **3**.

Production of trichamide by strains *T. erythraeum* IMS101 and GBR

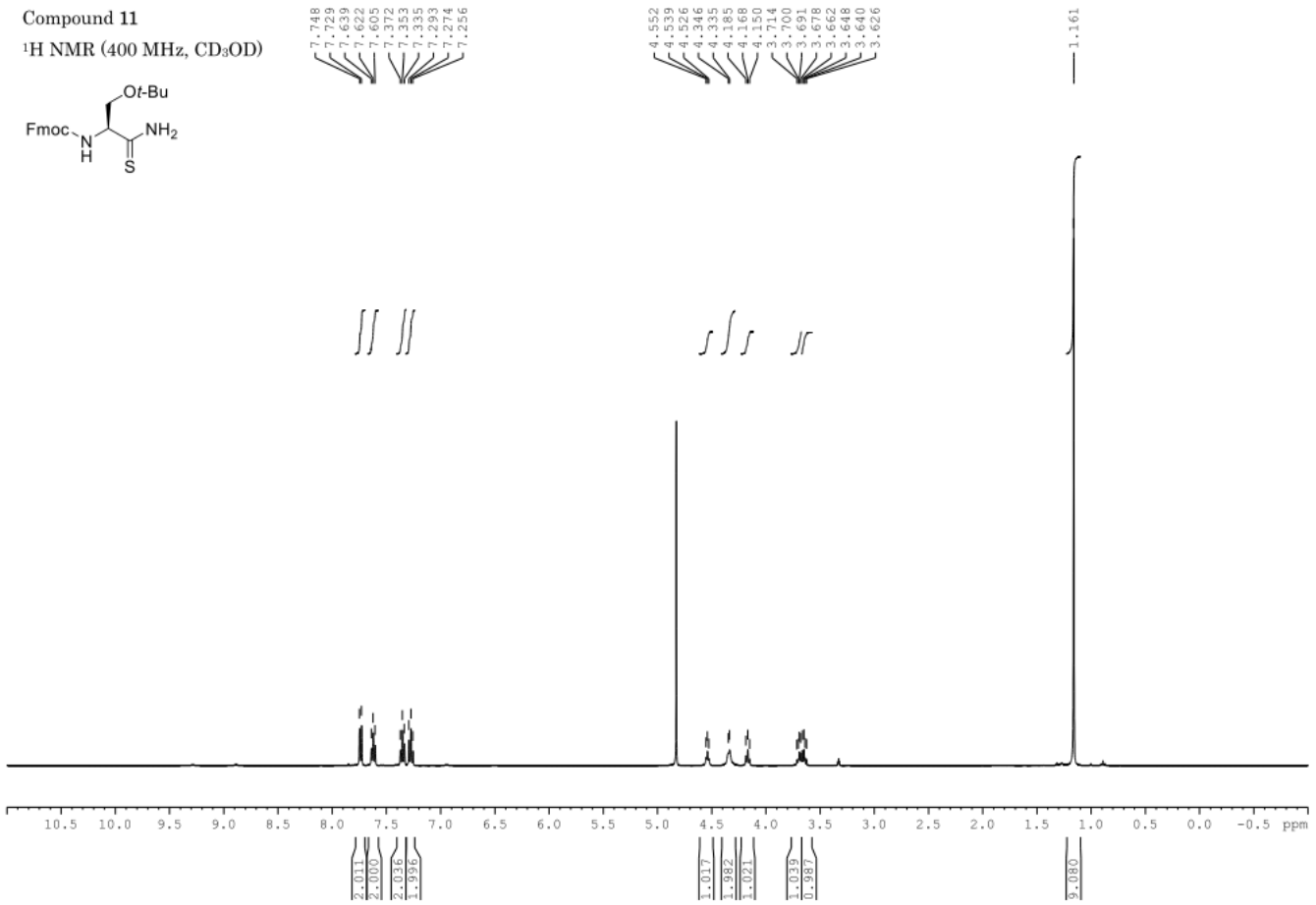
T. erythraeum strains were grown maintained in modified Aquil medium[S3] without combined nitrogen under a cool-white fluorescent light at a light intensity of 120 µmol photons $m^{-2}s^{-1}$ and a light-dark cycle of 12:12 high: dark (LD) in 26 °C incubators. The cultures were kept optically thin to avoid self-shading and nutrient limitation. Semi-continuous dilution culturing methods were practiced in this experiment to allow the cultures to grow in extended steady state exponential growth phase before sampling. Each bottle was diluted individually based on the growth rate calculated for that bottle.[S4] Growth rates were monitored based on *in vivo* chlorophyll fluorescence measurements using a Turner 10 AU fluorometer. Prior to sampling, the growth rates remained constant for at least 5 generations. Cells were grown in the volumes shown in the table below.

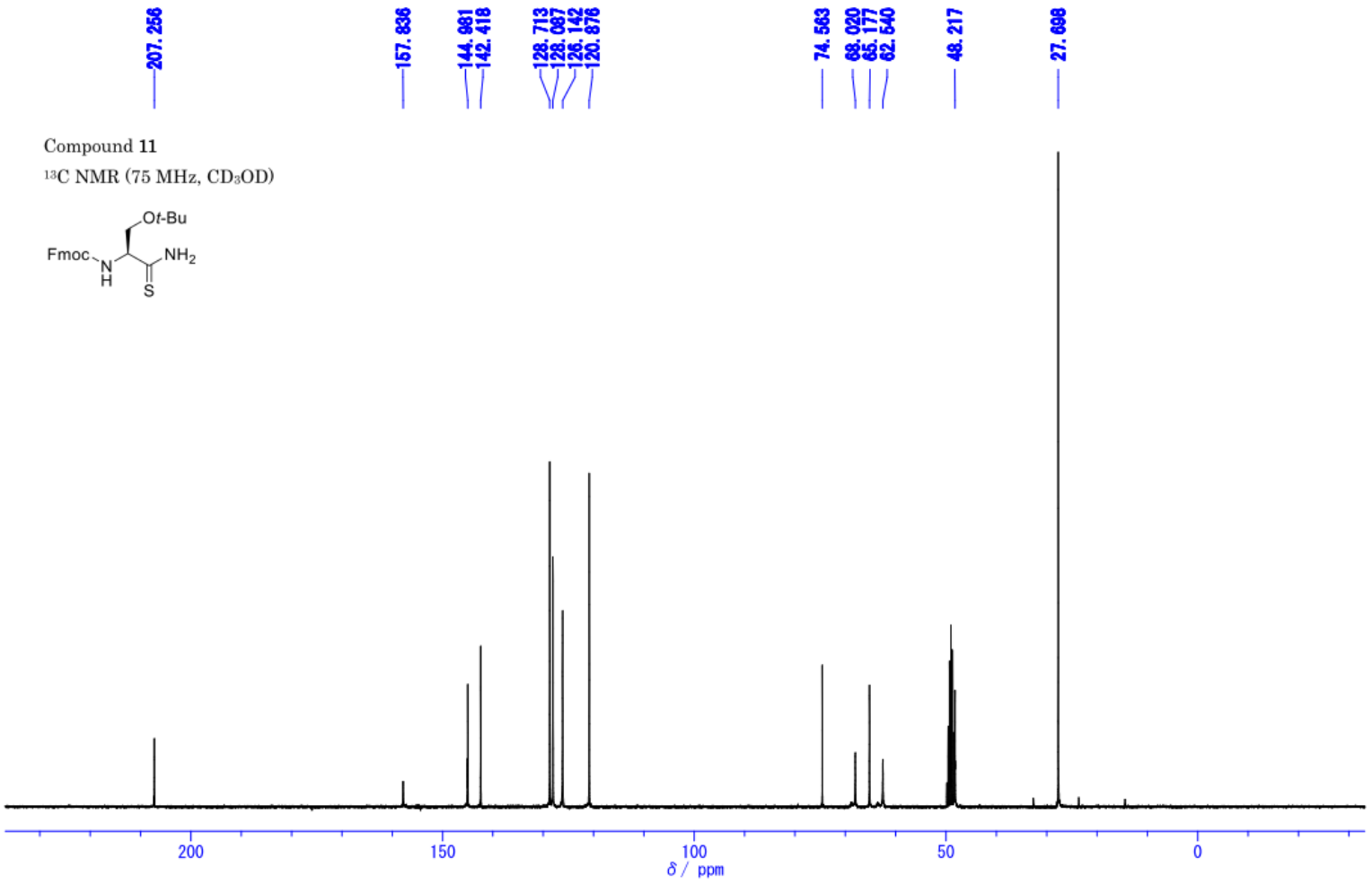
Cells were obtained by filtration and dried. The resulting filtrates were each extracted in methanol (1 mL) by overnight incubation at 4 ºC. From this 1 mL extract, 50 mL was removed and analyzed by HPLC-MS. Extracts, synthetic compounds, and combinations of each were used in independent HPLC runs to confirm identity and co-elution.

strain	phosphate	22 °C	28 °C	32 °C	volume	detectable **3**
IMS	+	√			500 mL	yes[a]
	+	√			200 mL	yes[a]
	+		√		350 mL	yes[b]
	–		√		500 mL	yes[b]
	–		√		380 mL	no
	+		√		350 mL	no
	+			√	360 mL	no
	+			√	400 mL	no
GBR	–	√			390 mL	no
	+	√			260 mL	no
	+	√			250 mL	no
	–	√			400 mL	no
	–		√		500 mL	no
	+		√		200 mL	no
	+		√		290 mL	no
	–		√		410 mL	no

Estimated yield [a] 10 ug/L, [b] 5 ug/L.

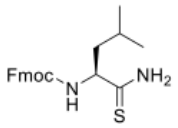
^{1}H NMR and ^{13}C NMR spectra of compounds

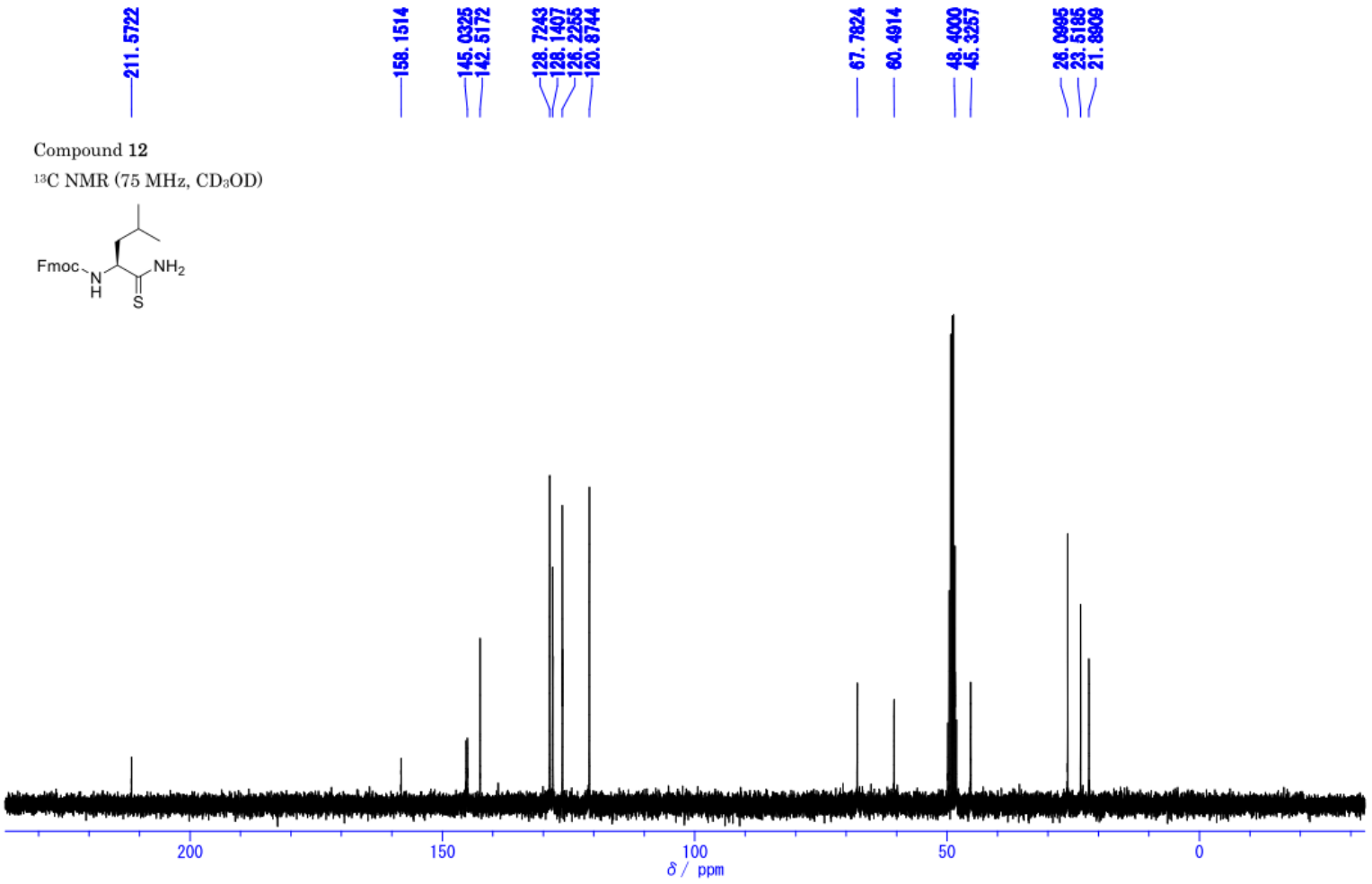


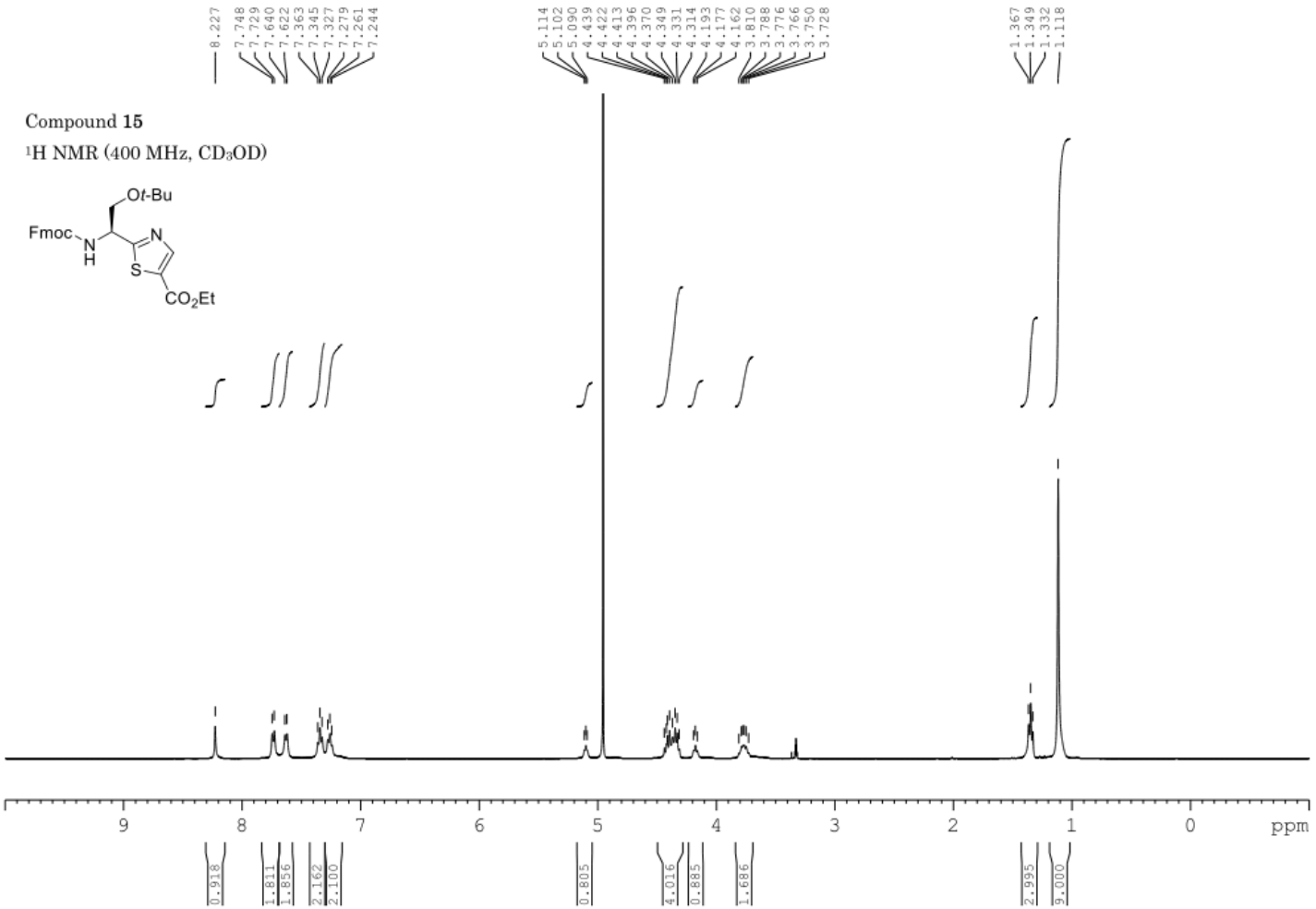

207.256
157.836
144.981
142.418
128.713
128.087
126.142
120.876
74.563
68.020
65.177
62.540
48.217
27.698
Compound 11
13C NMR (75 MHz, CD3OD)
Ot-Bu
Fmoc
N
H
NH2
S
200
150
100
50
0
δ / ppm

Compound **12**

^{1}H NMR (400 MHz, $CDCl_3$)

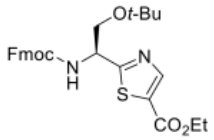


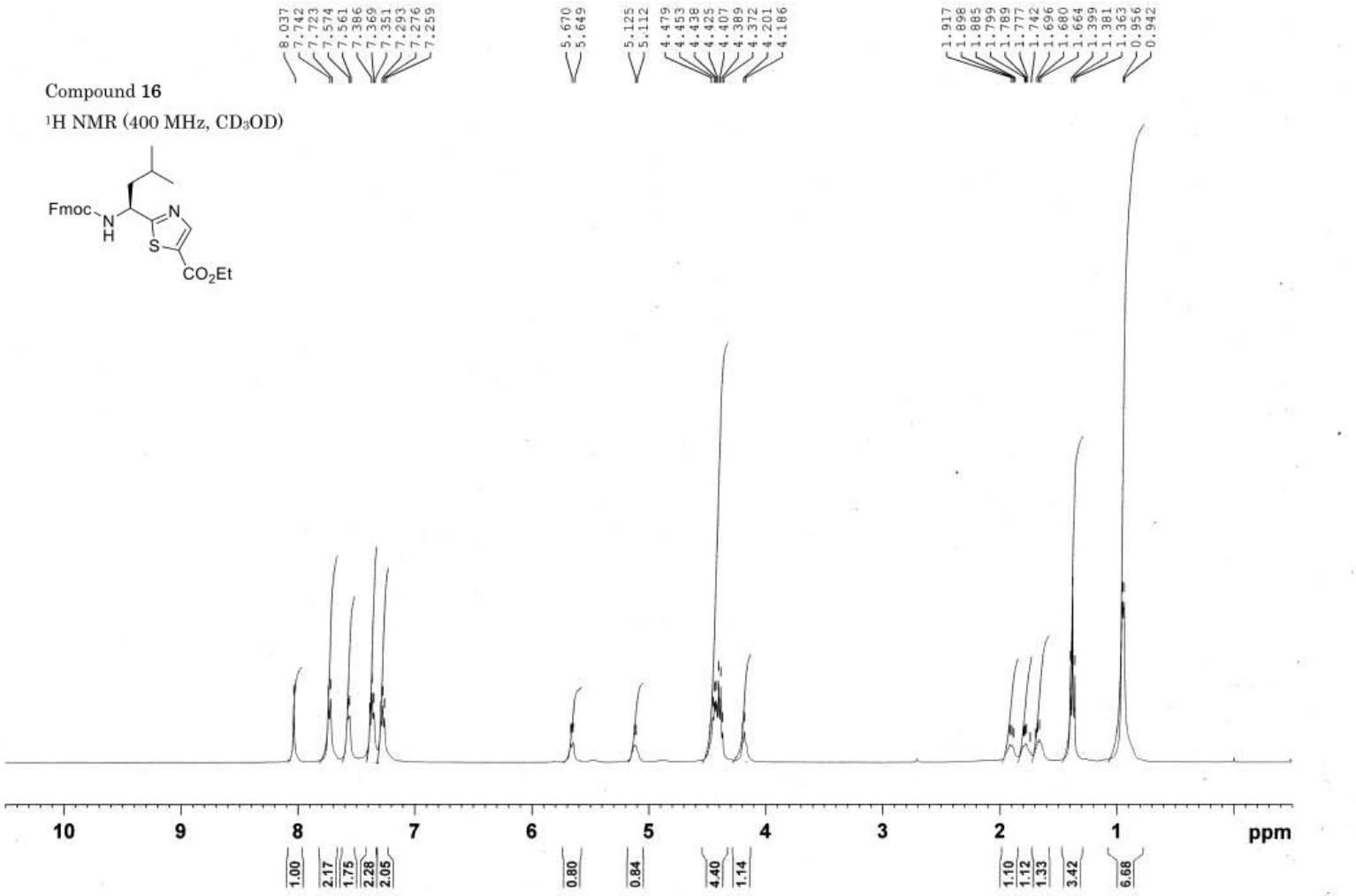

211.5722
158.1514
145.0325
142.5172
128.7243
128.1407
126.2265
120.8744
67.7824
60.4914
48.4000
45.3257
26.0995
23.5185
21.8909
Compound 12
13C NMR (75 MHz, CD3OD)
Fmoc
N
H
NH2
S
200
150
100
50
0
δ / ppm


Compound 15
1H NMR (400 MHz, CD3OD)
Ot-Bu
Fmoc
N
H
S
N
CO2Et
8.227
7.748
7.729
7.640
7.622
7.363
7.345
7.327
7.279
7.261
7.244
5.114
5.102
5.090
4.439
4.422
4.413
4.396
4.370
4.349
4.331
4.314
4.193
4.177
4.162
3.810
3.788
3.776
3.766
3.750
3.728
1.367
1.349
1.332
1.118
9
8
7
6
5
4
3
2
1
0
ppm
0.918
1.811
1.856
2.162
2.100
0.805
4.016
0.885
1.686
2.995
9.000

Compound **15**

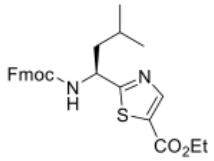
^{13}C NMR (75 MHz, CD_3OD)

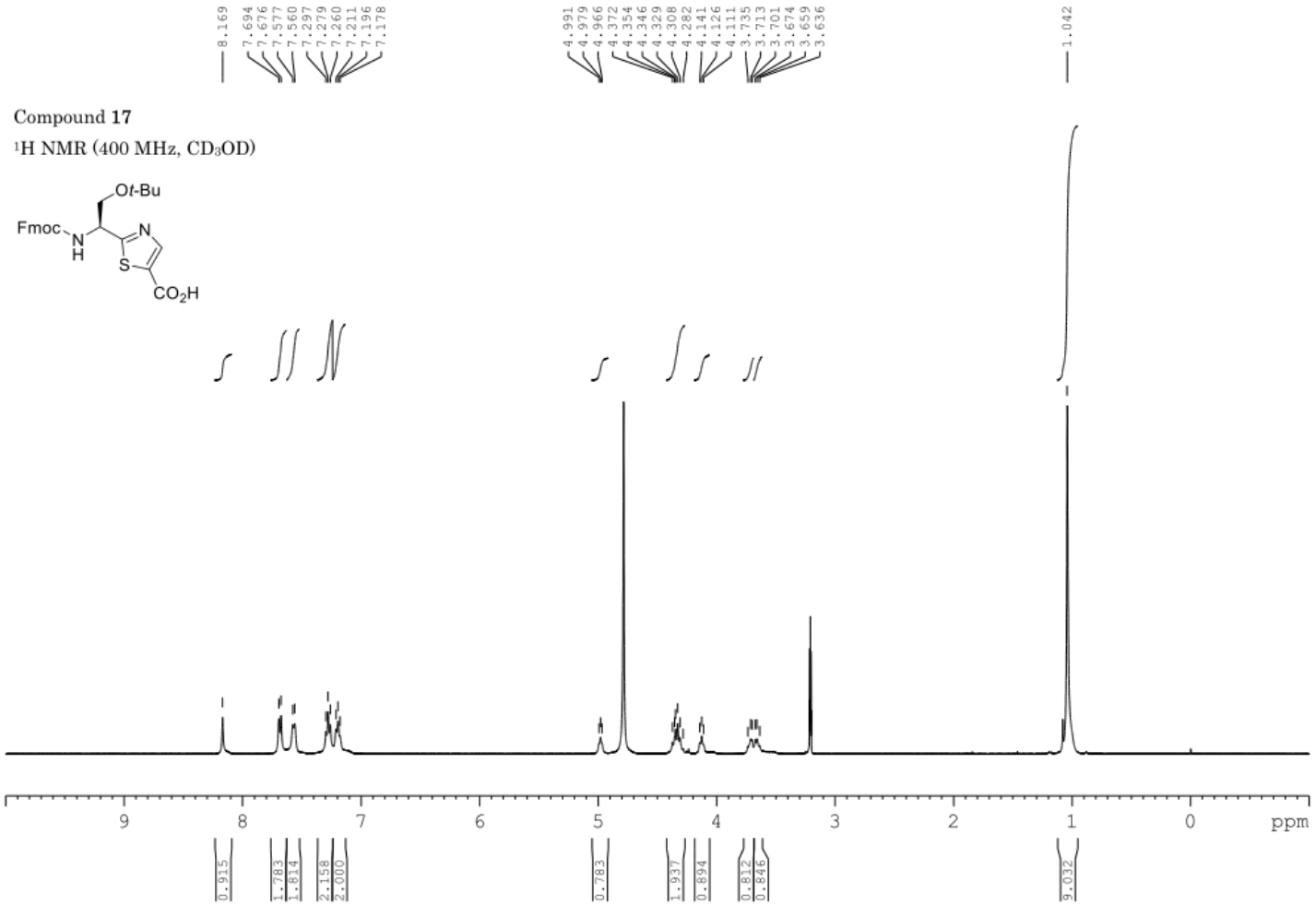



8.037
7.742
7.723
7.574
7.561
7.386
7.369
7.351
7.293
7.276
7.259
5.670
5.649
5.125
5.112
4.479
4.453
4.438
4.425
4.407
4.389
4.372
4.201
4.186
1.917
1.898
1.885
1.799
1.789
1.777
1.742
1.696
1.680
1.664
1.399
1.381
1.363
0.956
0.942
Compound 16
1H NMR (400 MHz, CD3OD)
Fmoc
N
H
S
N
CO2Et
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.17
1.75
2.28
2.05
0.80
0.84
4.40
1.14
1.10
1.12
1.33
3.42
6.68

Compound **16**

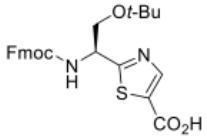
^{13}C NMR (75 MHz, $CDCl_3$)




8.169
7.694
7.676
7.577
7.560
7.297
7.279
7.260
7.211
7.196
7.178
4.991
4.979
4.966
4.372
4.354
4.346
4.329
4.308
4.282
4.141
4.126
4.111
3.735
3.713
3.701
3.674
3.659
3.636
1.042
Compound 17
1H NMR (400 MHz, CD3OD)
Ot-Bu
Fmoc
N
H
N
S
CO2H
9
8
7
6
5
4
3
2
1
0
ppm
0.915
1.783
1.814
2.158
2.000
0.783
1.937
0.894
0.812
0.846
9.032

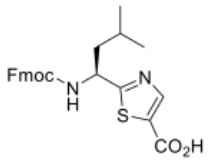
Compound **17**

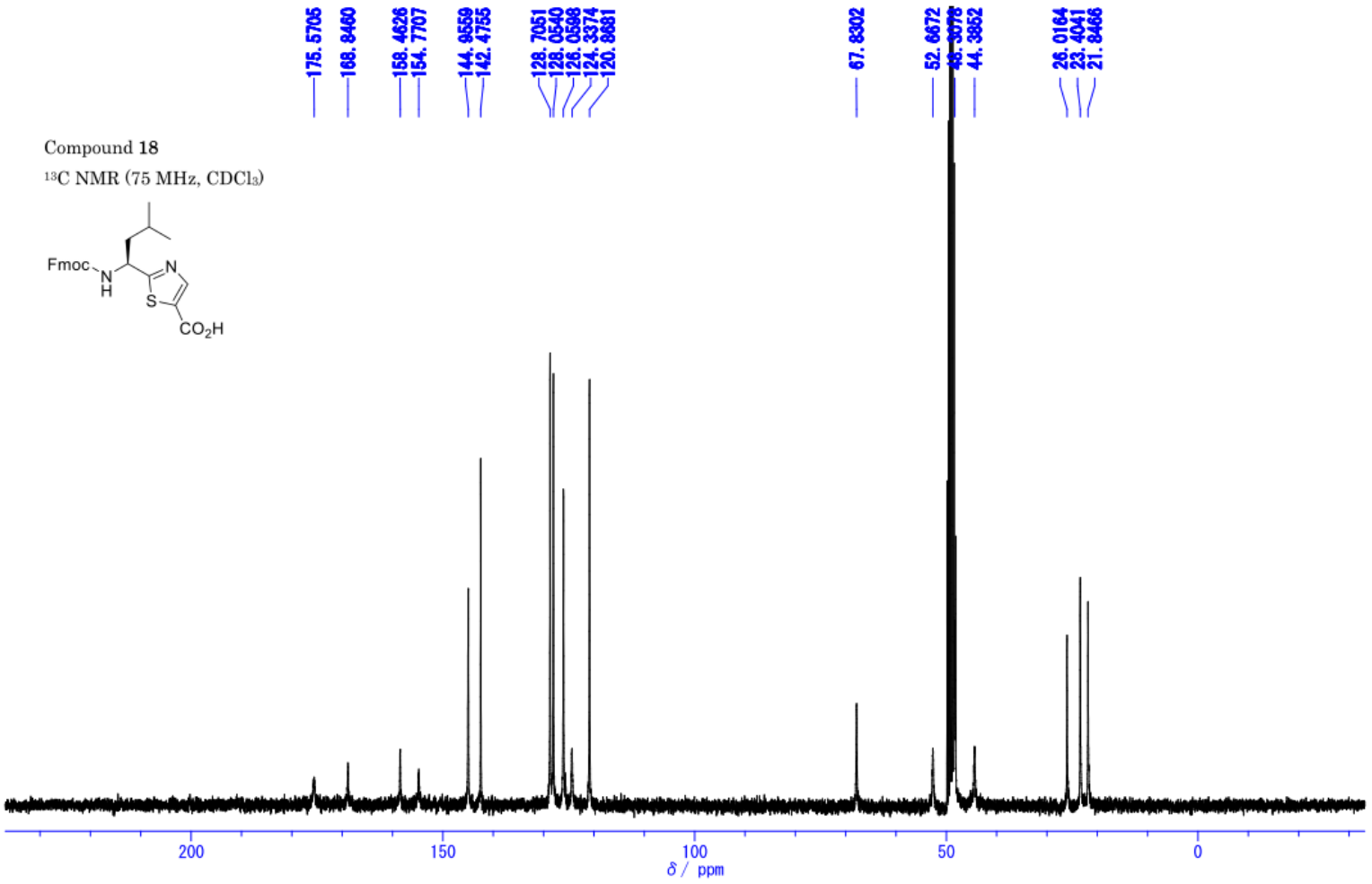
^{13}C NMR (75 MHz, CD_3OD)



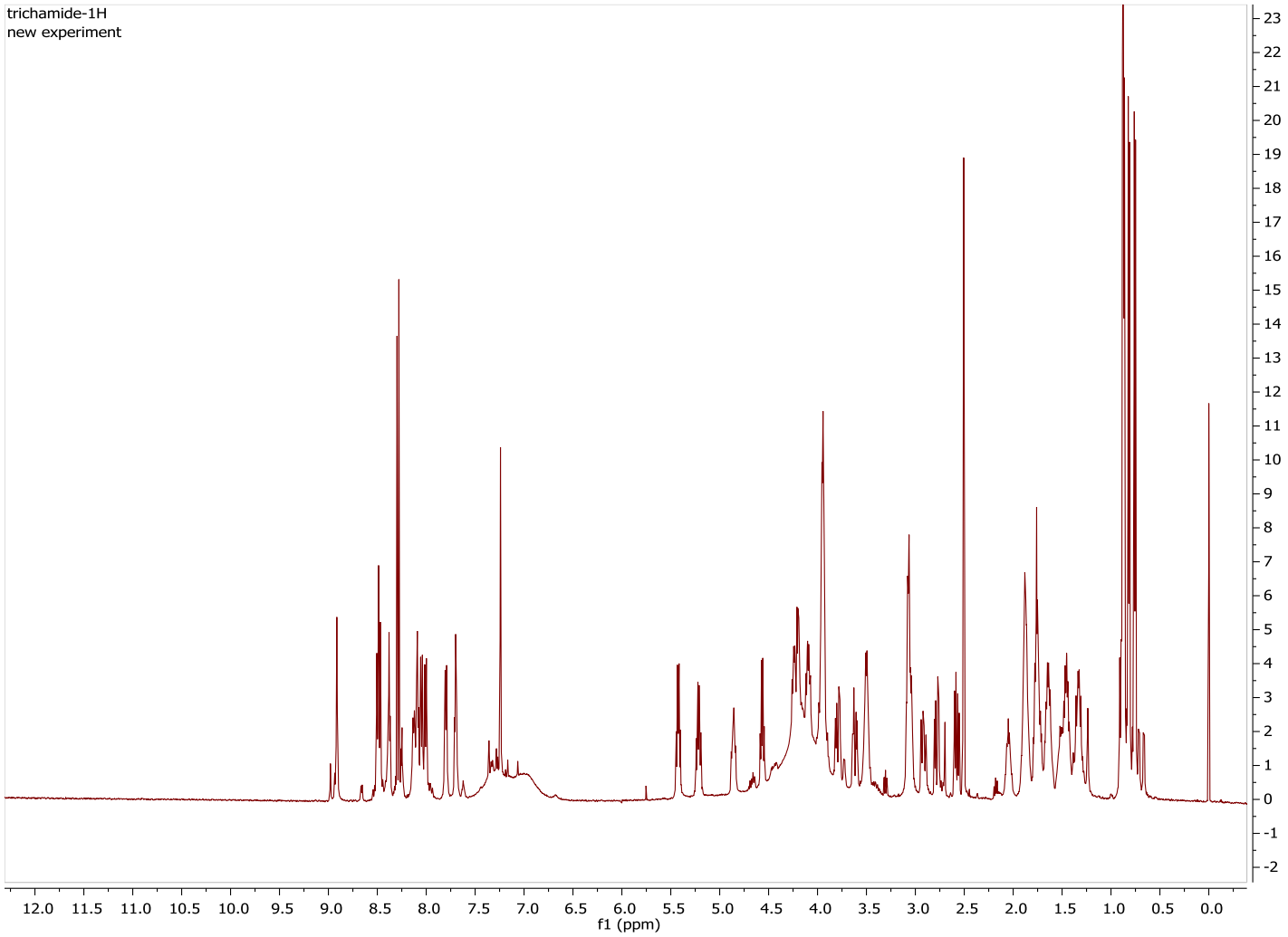
Compound **18**

^{1}H NMR (400 MHz, CD_3OD)

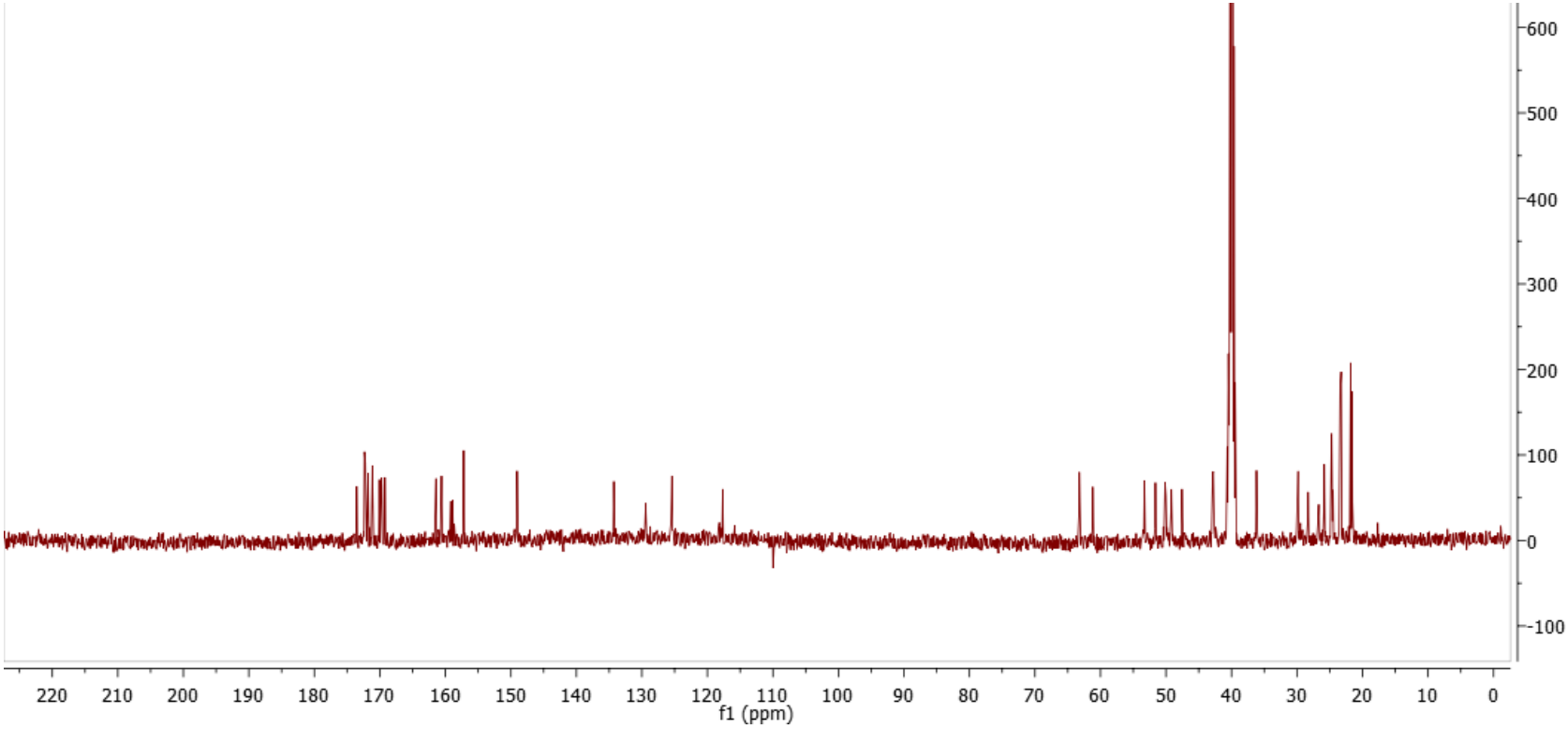



175.5705
168.8460
158.4626
154.7707
144.9559
142.4755
128.7051
128.0540
126.0588
124.3374
120.8681
67.8302
52.6672
48.3078
44.3852
26.0164
23.4041
21.8466
Compound 18
13C NMR (75 MHz, CDCl3)
Fmoc
N
H
N
S
CO2H
200
150
100
50
0
δ / ppm

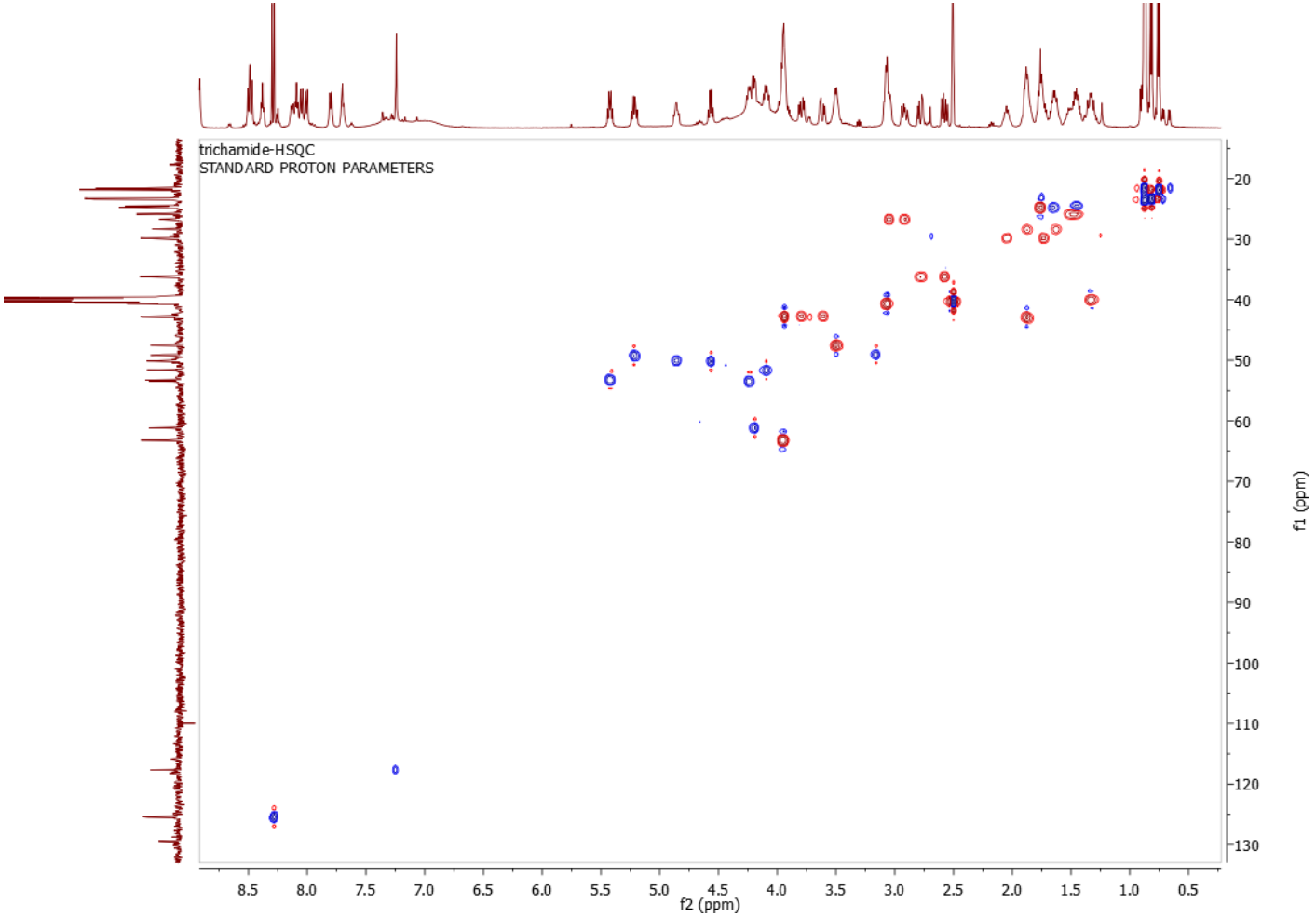
^{1}H NMR of synthetic trichamide



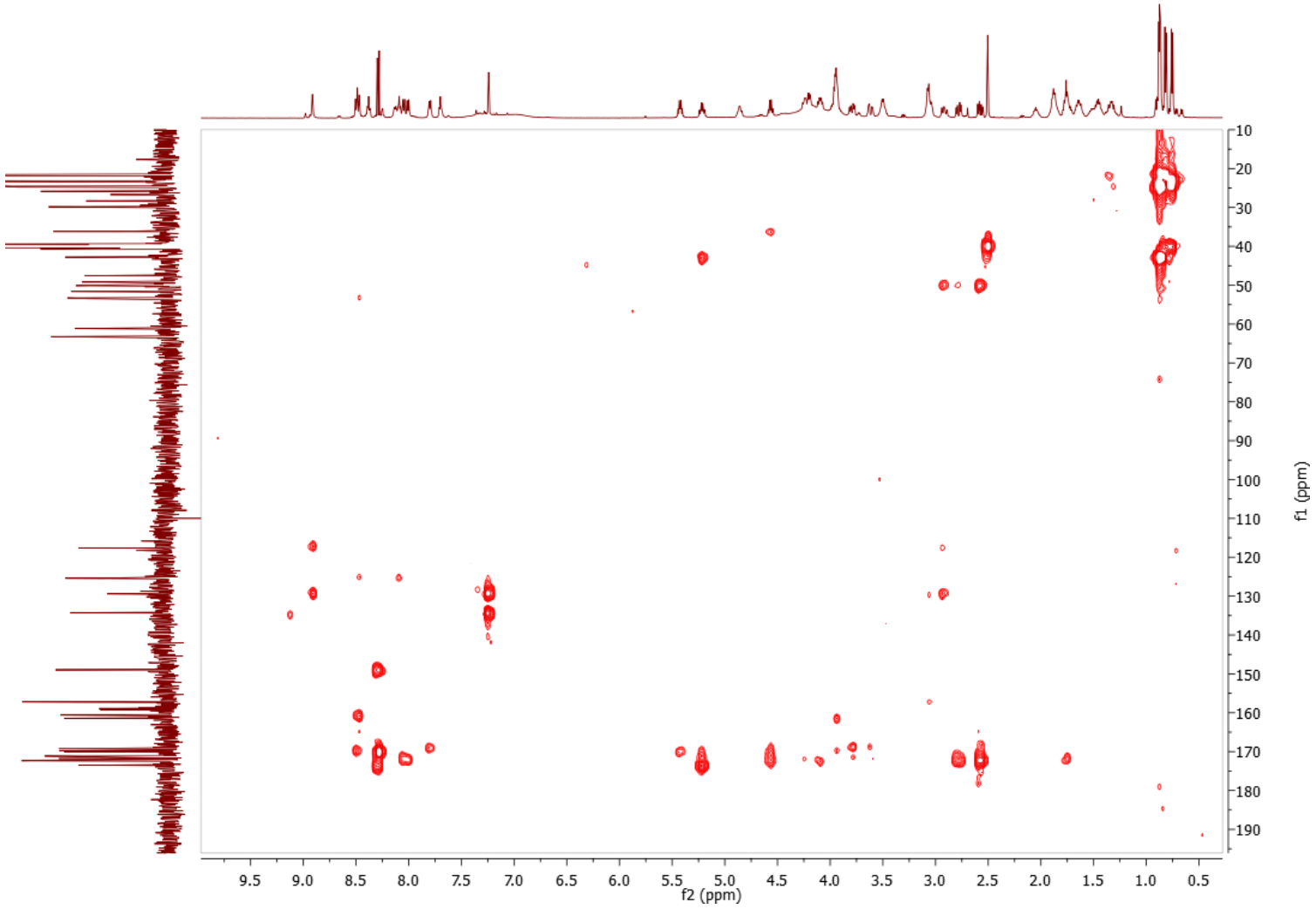
^{13}C NMR of synthetic trichamide



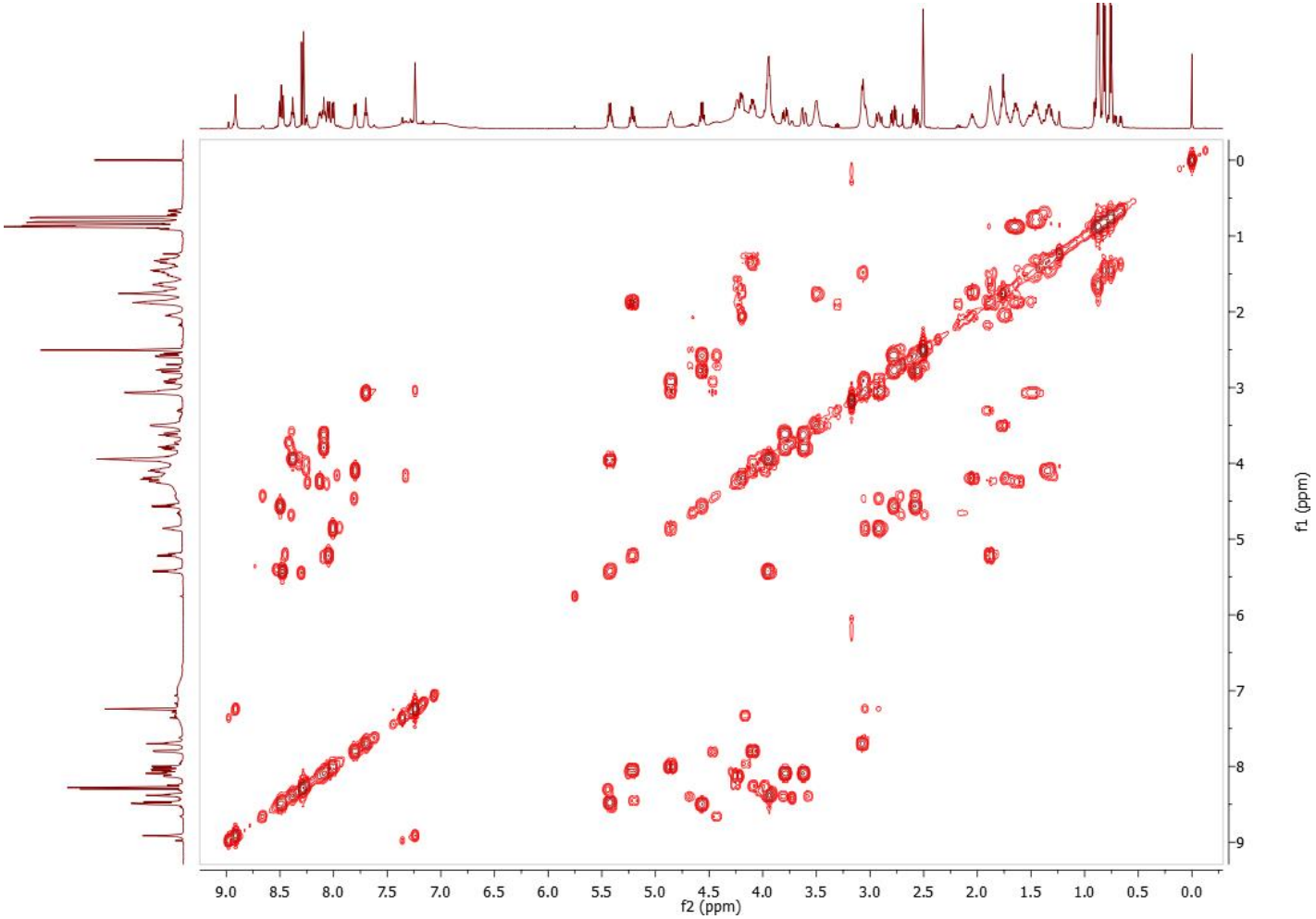
HSQC spectrum of synthetic trichamide



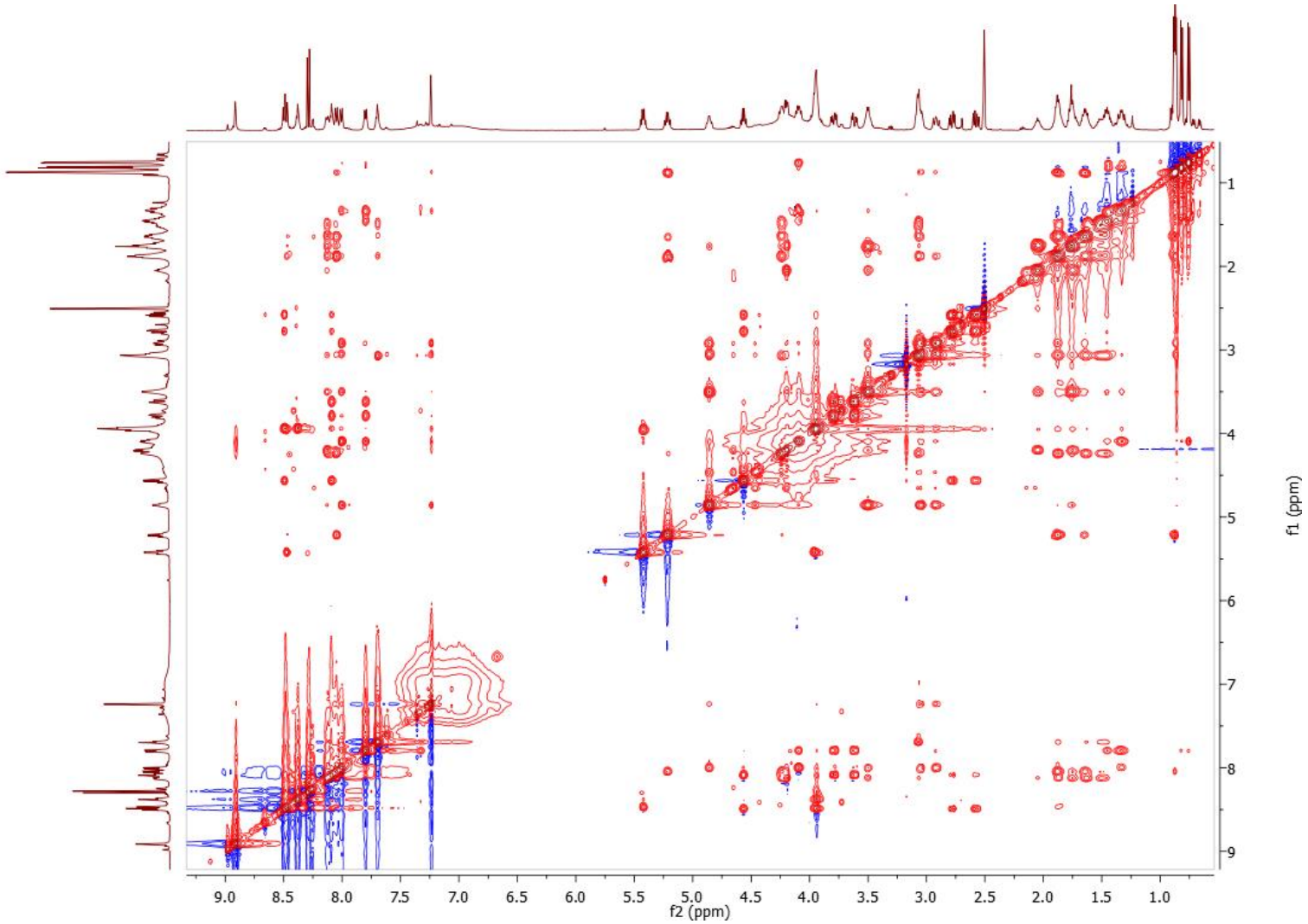
HMBC spectrum of synthetic trichamide



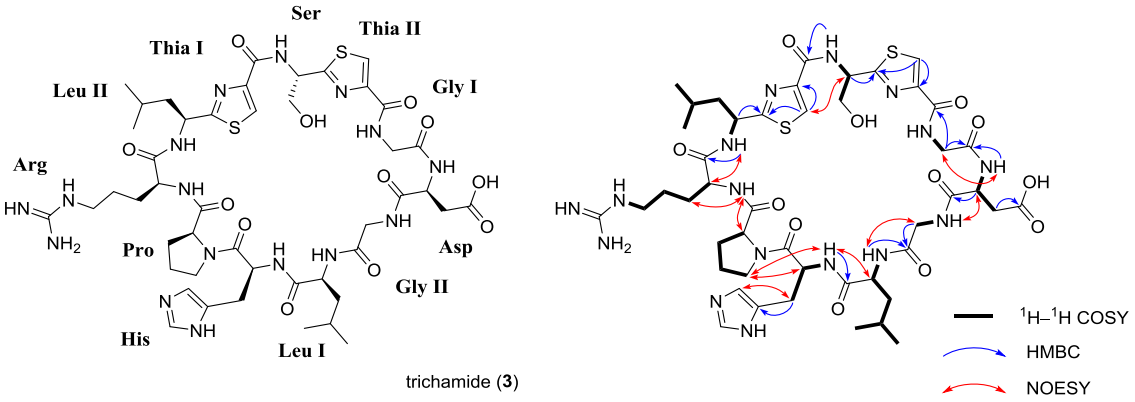
COSY spectrum of synthetic trichamide



NOESY spectrum of synthetic trichamide



Chemical shift assignment of trichamide (**3**)



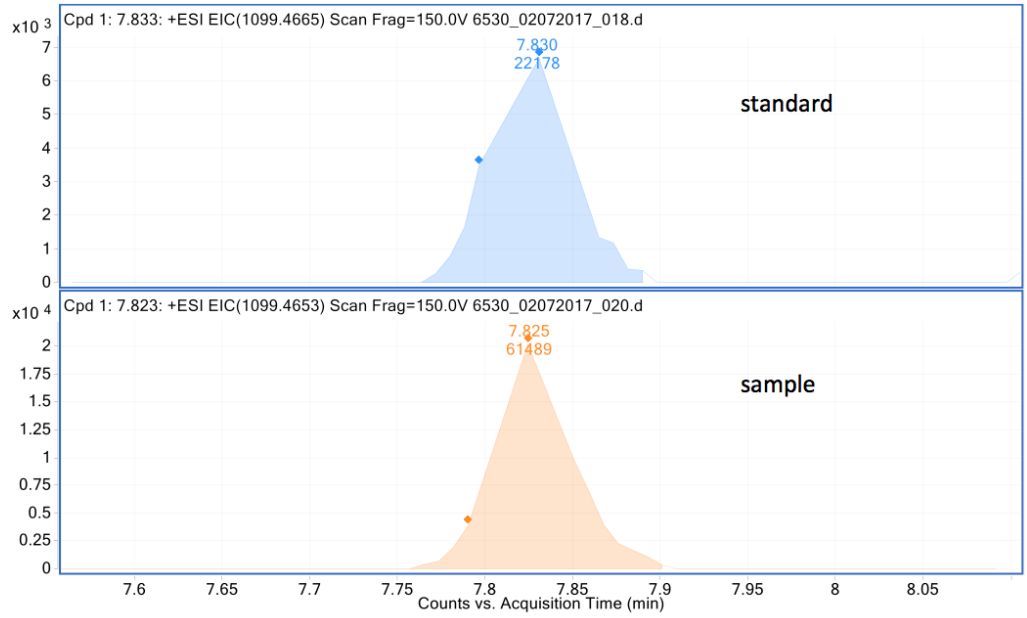
Residue	No.	^{1}H NMR[a]	^{13}C NMR[b]
Gly I	1	–	169.8 C
	2	3.94 (m)	42.8 CH_2
	NH	8.38 (t, 5.7)	–
Asp	1	–	171.8 C
	2	4.56 (q, 7.1)	50.1 CH
	3	2.78 (dd, 16.8, 6.1);	36.2 CH_2
		2.58 (dd, 16.8, 7.8)	
	4	–	172.4 C
	NH	8.50 (d, 7.7)	–
Gly II	1	–	169.2 C
	2	3.79 (dd, 16.8, 5.9);	42.7 CH_2
		3.62 (dd, 16.8, 5.2)	
	NH	8.09 (t, 5.8)	–
Leu I	1	–	172.1 C
	2	4.09 (m)	51.7 CH
	3	1.33 (m)	40.2 CH_2
	4	1.45 (m)	24.5 CH
	5	0.82 (d, 6.7)	23.3 CH_3
	6	0.76 (d, 6.7)	21.8 CH_3
	NH	7.80 (d, 7.8)	–
His	1	-	169.8 C*
	2	4.86 (m)	50.0 CH
	3	3.05 (m);	26.7 CH_2
		2.92 (dd, 15.4, 9.8)	
	4	–	129.5 C
	5	7.24 (s)	117.7 CH
	6	8.92 (s)	134.3 CH
	NH	8.01 (d, 8.7)	–
Pro	1	–	172.0 C*
	2	4.20 (m)	61.2 CH
	3	2.04 (m); 1.73 (m)	29.9 CH_2
	4	1.76 (m)	24.8 CH_2
	5	3.50 (m)	47.6 CH_2

Residue	No.	^{1}H NMR[a]	^{13}C NMR[b]
Arg	1	–	171.8 C
	2	4.24 (m)	53.5 CH
	3	1.87 (m); 1.63 (m)	28.3 CH_2
	4	1.49 (m)	25.9 CH_2
	5	3.07 (m)	40.6 CH_2
	6	–	157.2 C
	α-NH	8.12 (d, 7.5)	–
	δ-NH	7.69 (t, 5.8)	–
Leu II	1	–	173.5 C
	2	5.22 (m)	49.2 CH
	3	1.88 (m)	42.8 CH_2
	4	1.64 (m)	24.7 CH
	5	0.88 (d, 7.5)	21.6 CH_3
	6	0.87 (d, 7.5)	23.4 CH_3
	NH	8.05 (d, 7.6)	–
Thia I	1	–	160.6 C
	2	–	149.1 C
	3	8.30 (s)	125.5 CH
Ser	1	–	170.0 C
	2	5.43 (m)	53.3 CH
	3	3.96 (m)	63.2 CH_2
	NH	8.48 (d, 8.5)	–
Thia II	1	–	161.4 C
	2	–	149.0 C
	3	8.28 (s)	125.4 CH

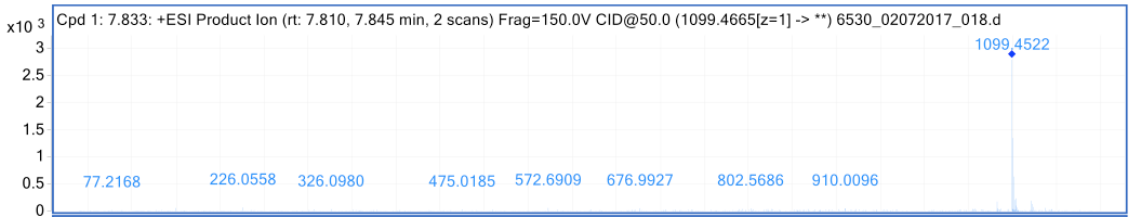
[a] δ ppm (mult., *J* in Hz), 500 MHz. [b] δ ppm, 125 MHz. *Assignments are interchangeable

Comparison of synthetic and natural trichamide by UPLC-MS

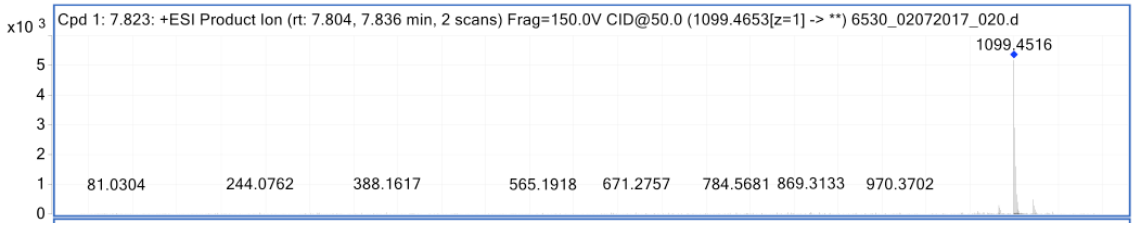
* synthetic trichamide: “standard” ; natural trichamide: “sample”



standard

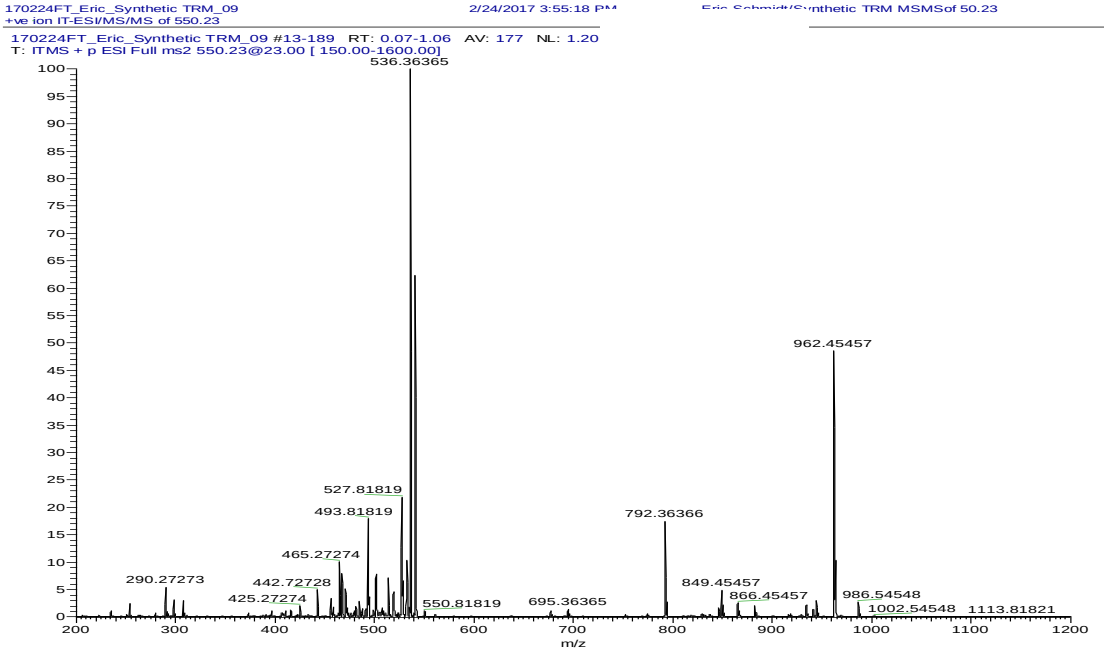


sample

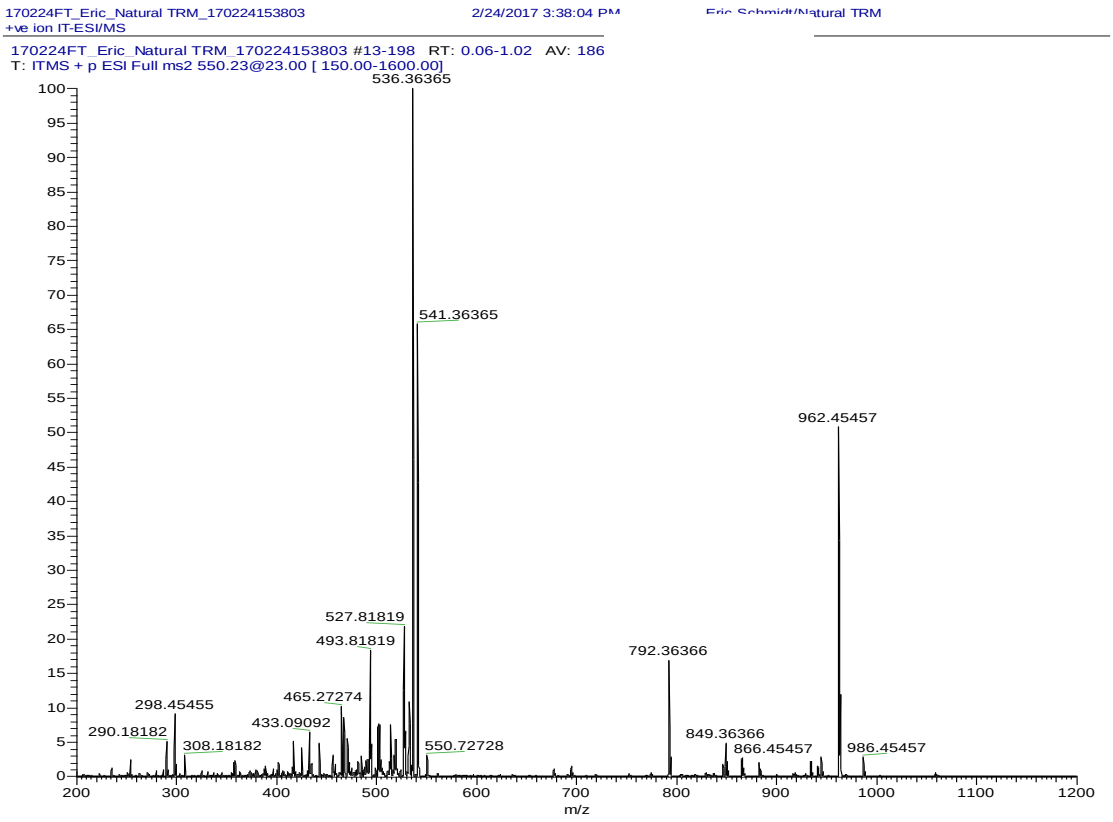


Comparison of synthetic and natural trichamide by FT-MS/MS

(A) Synthetic trichamide



(B) Natural trichamide



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