Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Synthesis of the Src SH2 domain protein and its application in bioassays for mirror-image screening

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Experimental procedures

D-Src(145-187) (D-3a). By the identical procedure described for the synthesis of peptide **L-3a**, thioester **D-3a** was synthesized (7.8 mg, 2.1% yield) from H-Rink Amide-ChemMatrix resin (40 mg × 3 portions, 0.048-0.072 mmol). MS(ESI): Calcd for $C_{231}H_{357}N_{64}O_{71}S$: 5198.83 ($MH^+$); observed: $[M+6H]^{6+}$ *m/z* = 867.4, $[M+5H]^{5+}$ *m/z* = 1040.7, $[M+4H]^{4+}$ *m/z* = 1300.4, $[M+3H]^{3+}$ *m/z* = 1733.4.

L-Src(145-187)^{TMR} (L-3b). By the identical procedure described for the synthesis of peptide **L-3a**, thioester **L-3b** was synthesized (3.5 mg, 2.7% yield) from H-Rink Amide-ChemMatrix resin (40 mg, 0.016-0.024 mmol). TMR (43 mg, 0.1 mmol) was coupled by using *N*,*N*'-diisopropylcarbodiimide (DIC) (15.5 mL, 0.1 mmol)/HOBt·H_2O (15.3 mg, 0.1 mmol) in DMF for 4 h. MS(ESI): Calcd for $C_{259}H_{382}N_{67}O_{76}S$: 5682.36 ($MH^+$); observed: $[M+7H]^{7+}$ *m/z* = 812.7 $[M+6H]^{6+}$ *m/z* = 948.0, $[M+5H]^{5+}$ *m/z* = 1137.4, $[M+4H]^{4+}$ *m/z* = 1421.3.

D-Src(145-187)^{TMR} (D-3b). By the identical procedure described for the synthesis of peptide **L-3b**, thioester **D-3b** was synthesized (3.8 mg, 2.9% yield) from H-Rink Amide-ChemMatrix resin (40 mg, 0.016-0.024 mmol). MS(ESI): Calcd for $C_{259}H_{382}N_{67}O_{76}S$: 5682.36 ($MH^+$); observed: $[M+7H]^{7+}$ *m/z* = 812.6, $[M+6H]^{6+}$ *m/z* = 947.9, $[M+5H]^{5+}$ *m/z* = 1137.3, $[M+4H]^{4+}$ *m/z* = 1421.4.

D-Src(188-251) (D-4). By the standard protocol for peptide synthesis, peptide **D-4** was synthesized (5.5 mg, 1.6% yield) from H-Rink Amide-ChemMatrix resin (40 mg × 2 portions, 0.032-0.048 mmol). MS(ESI): Calcd for $C_{318}H_{500}N_{91}O_{94}S_3$: 7198.22 ($MH^+$); observed: $[M+8H]^{8+}$ *m/z* = 900.7, $[M+7H]^{7+}$ *m/z* = 1029.1, $[M+6H]^{6+}$ *m/z* = 1200.1, $[M+5H]^{5+}$ *m/z* = 1440.2, $[M+4H]^{4+}$ *m/z* = 1799.7.

D-Src(145-251) (D-5a). By the identical procedure described for the synthesis of peptide **L-5a**, peptide **D-4a** was synthesized (1.1 mg, 18% yield). MS(ESI): Calcd for $C_{541}H_{848}N_{155}O_{163}S_3$: 12227.84 ($MH^+$); observed: $[M+15H]^{15+}$ *m/z* = 813.9, $[M+14H]^{14+}$ *m/z* = 874.5, $[M+13H]^{13+}$ *m/z* = 941.6, $[M+12H]^{12+}$ *m/z* = 1020.0, $[M+11H]^{11+}$ *m/z* = 1112.8, $[M+10H]^{10+}$ *m/z* = 1223.9, $[M+9H]^{9+}$ *m/z* = 1359.8, $[M+8H]^{8+}$ *m/z* = 1529.5, $[M+7H]^{7+}$ *m/z* = 1747.5.

L-Src(145-251)^{TMR} (L-5b). By the identical procedure described for the synthesis of peptide **L-5a**, peptide **L-4b** was synthesized (0.45 mg, 13% yield). MS(ESI): Calcd for $C_{569}H_{873}N_{158}O_{168}S_3$: 12711.36 ($MH^+$); observed: $[M+15H]^{15+}$ *m/z* = 848.5, $[M+14H]^{14+}$ *m/z* = 909.1, $[M+13H]^{13+}$ *m/z* = 978.9, $[M+12H]^{12+}$ *m/z* = 1060.5, $[M+11H]^{11+}$ *m/z* = 1156.7, $[M+10H]^{10+}$ *m/z* = 1272.3, $[M+9H]^{9+}$ *m/z* = 1413.3, $[M+8H]^{8+}$ *m/z* = 1590.2, $[M+7H]^{7+}$ *m/z* = 1760.0.

D-Src(145-251)^{TMR} (D-5b). By the identical procedure described for the synthesis of peptide **L-5a**, peptide **D-5b** was synthesized (0.25 mg, 14% yield). MS(ESI): Calcd for $C_{569}H_{873}N_{158}O_{168}S_3$: 12711.36 ($MH^+$); observed: $[M+15H]^{15+}$ *m/z* = 848.6, $[M+14H]^{14+}$ *m/z* = 909.0, $[M+13H]^{13+}$ *m/z* = 978.9, $[M+12H]^{12+}$ *m/z* = 1060.4, $[M+11H]^{11+}$ *m/z* = 1156.7, $[M+10H]^{10+}$ *m/z* = 1272.2, $[M+9H]^{9+}$ *m/z* = 1413.4, $[M+8H]^{8+}$ *m/z* = 1588.1.

D-hmT pY324 (D-6): By the identical procedure described for the synthesis of peptide **L-6**, peptide **D-6** was synthesized (14.5 mg, 30% yield). MS(ESI): Calcd for $C_{66}H_{99}N_{13}O_{23}P$: 1473.56 ($MH^+$); observed: $[M+H]^+$ *m/z* = 1473.0.

L-hmT pY324^{biotin} (L-7, biotin-Acp-EPQpYEEIPIYL-NH$_2$): By the identical procedure described for the synthesis of peptide **L-6**, peptide **L-7** was synthesized (38 mg, 35% yield). D-Biotin was coupled with HBTU (73.3 mg, 0.3 mmol), HOBt·H_2O (45.9 mg, 0.3 mmol) and (^{i}Pr)$_2$NEt (0.104 mL, 0.6 mmol) in DMF. MS(ESI): Calcd for $C_{82}H_{124}N_{16}O_{26}PS$: 1813.01 ($MH^+$); observed: $[M+H]^+$ *m/z* = 1812.7.

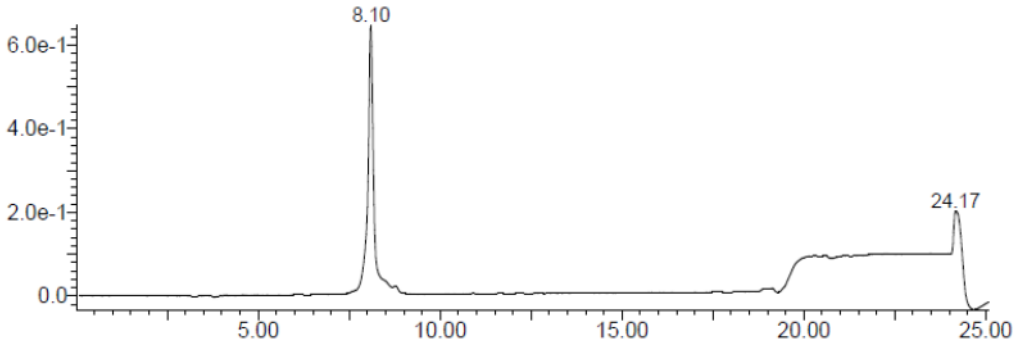
D-hmT pY324^{biotin} (D-7): By the identical procedure described for the synthesis of peptide **L-7**, peptide **D-7** was synthesized using D-biotin (24.2 mg, 44% yield). MS(ESI): Calcd for $C_{82}H_{124}N_{16}O_{26}PS$: 1813.01 ($MH^+$); observed: $[M+H]^+$ *m/z* = 1813.0.

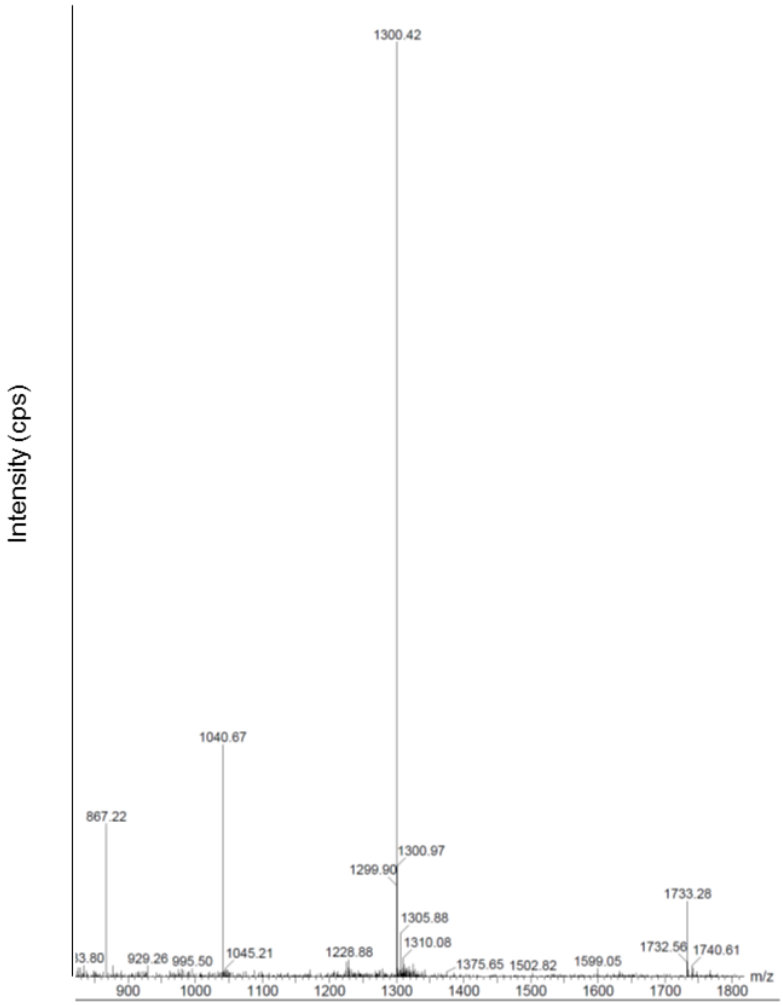
L-FMT1^{FAM} (L-8, 5-FAM-GpYEEIA-NH$_2$): By the identical procedure described for the synthesis of peptide **L-6**, peptide **L-8** was synthesized (7.9 mg, 23% yield). Labeling of 5-carboxyfluorecein (33.9 mg, 0.090 mmol) was carried out with DIC (0.0139 mL, 0.090 mmol) and HOBt·H_2O (13.8 mg, 0.090 mmol) in DMF. MS(MALDI-TOF): Calcd for $C_{51}H_{57}N_7O_{20}P$: 1119.02 (MH^+); observed: $[M+H]^+$ *m/z* = 1118.7, $[M+Na]^+$ *m/z* = 1141.7, $[M+K]^+$ *m/z* = 1156.6.

D-FMT1^{FAM} (D-8): By the identical procedure described for the synthesis of peptide **L-8**, peptide **D-8** was synthesized (11.6 mg, 33% yield). MS(MALDI-TOF): Calcd for $C_{51}H_{57}N_7O_{20}P$: 1119.02 (MH^+); observed: $[M+H]^+$ *m/z* = 1118.7, $[M+Na]^+$ *m/z* = 1141.7, $[M+K]^+$ *m/z* = 1156.7.

Analytical HPLC trace and mass spectrometry data of synthetic proteins and peptides

L-Src(145-187) (L-3a)

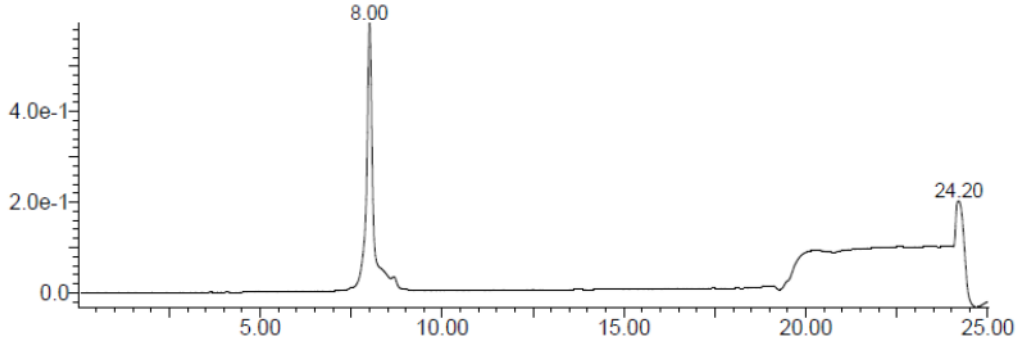


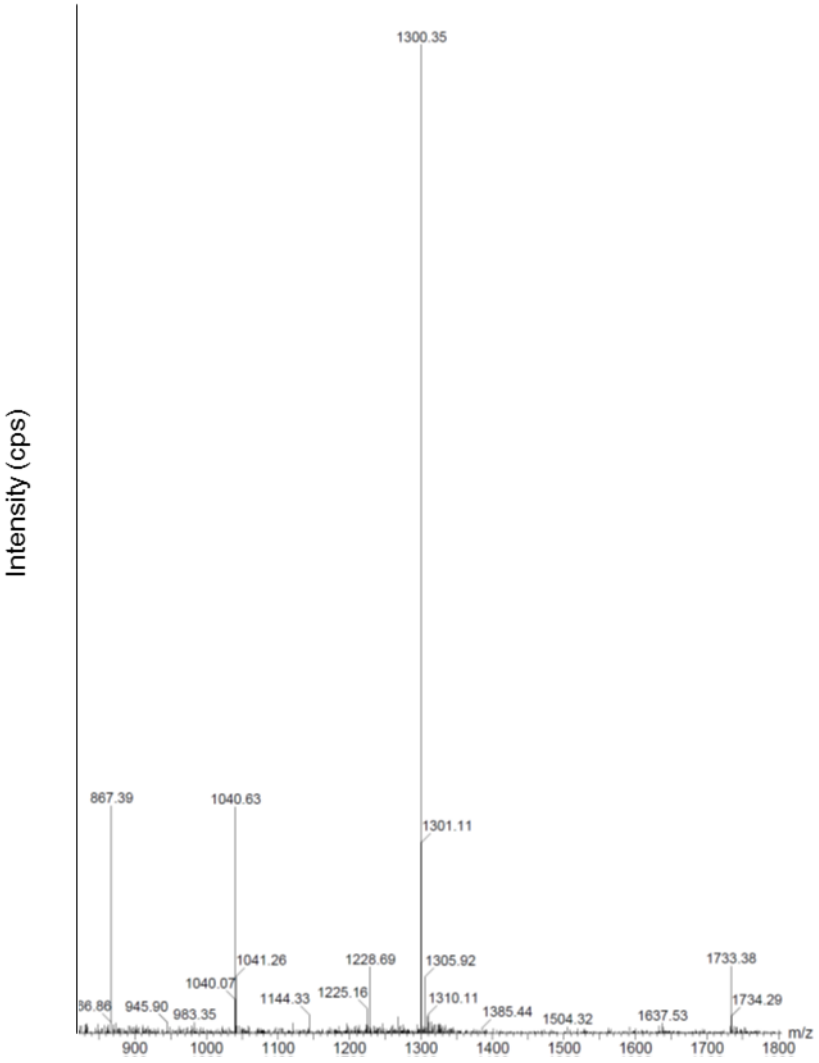


Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 5198.83 (MH^+); Major observed ions: 867.2 (+6), 1040.7 (+5), 1300.4 (+4), 1733.3 (+3).

D-Src(145-187) (D-3a)

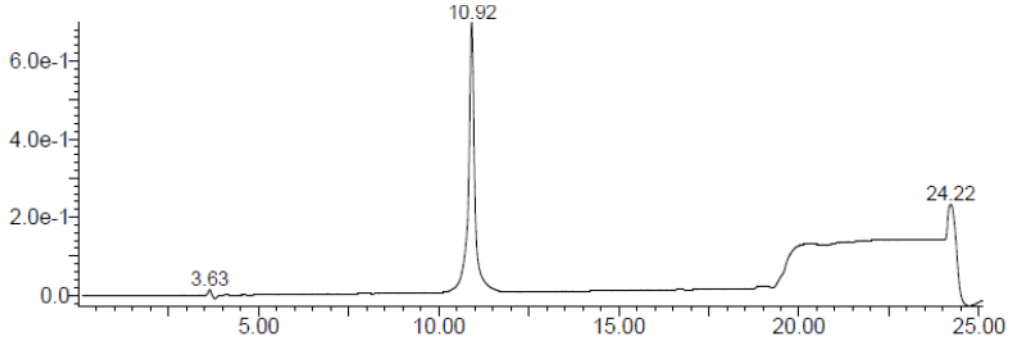


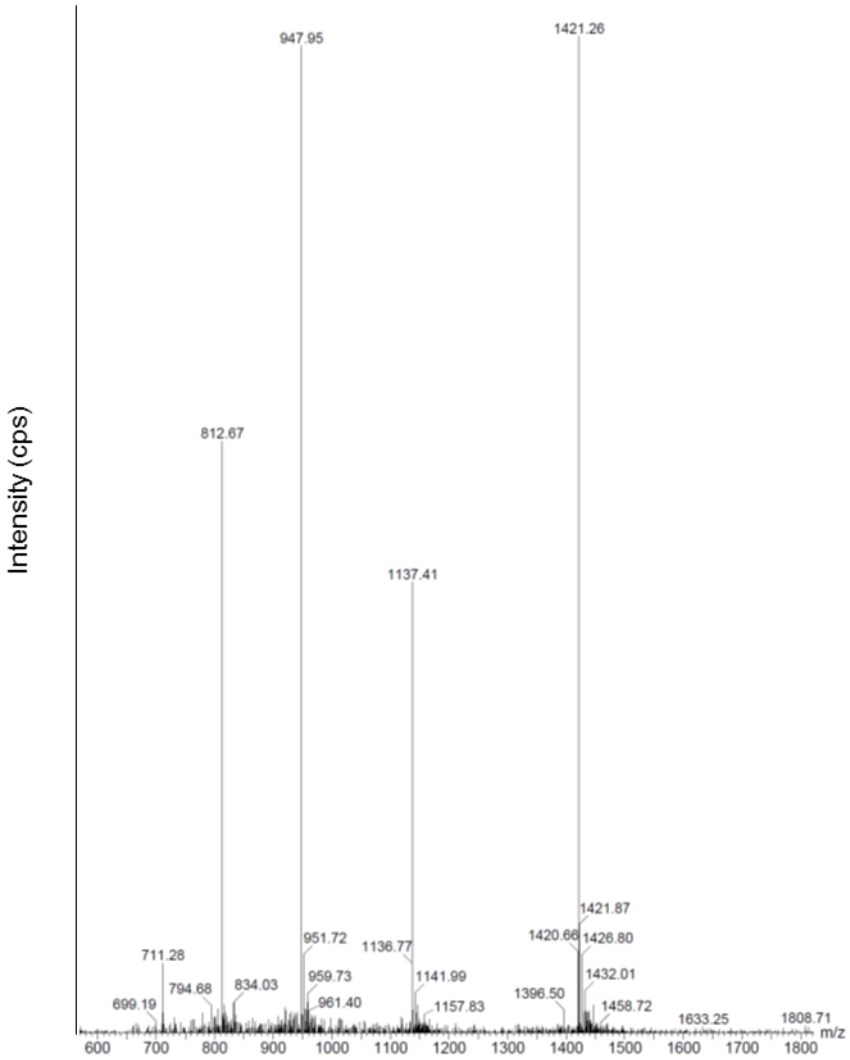


Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 5198.83 (MH^+); Major observed ions: 867.4 (+6), 1040.7 (+5), 1300.4 (+4), 1733.4 (+3).

L-Src(145-187)^{TMR} (L-3b)

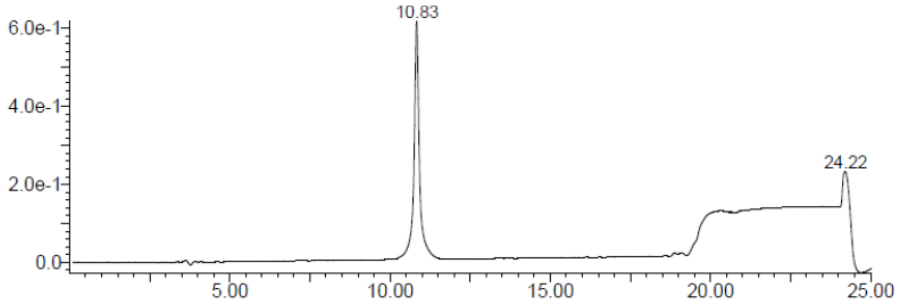


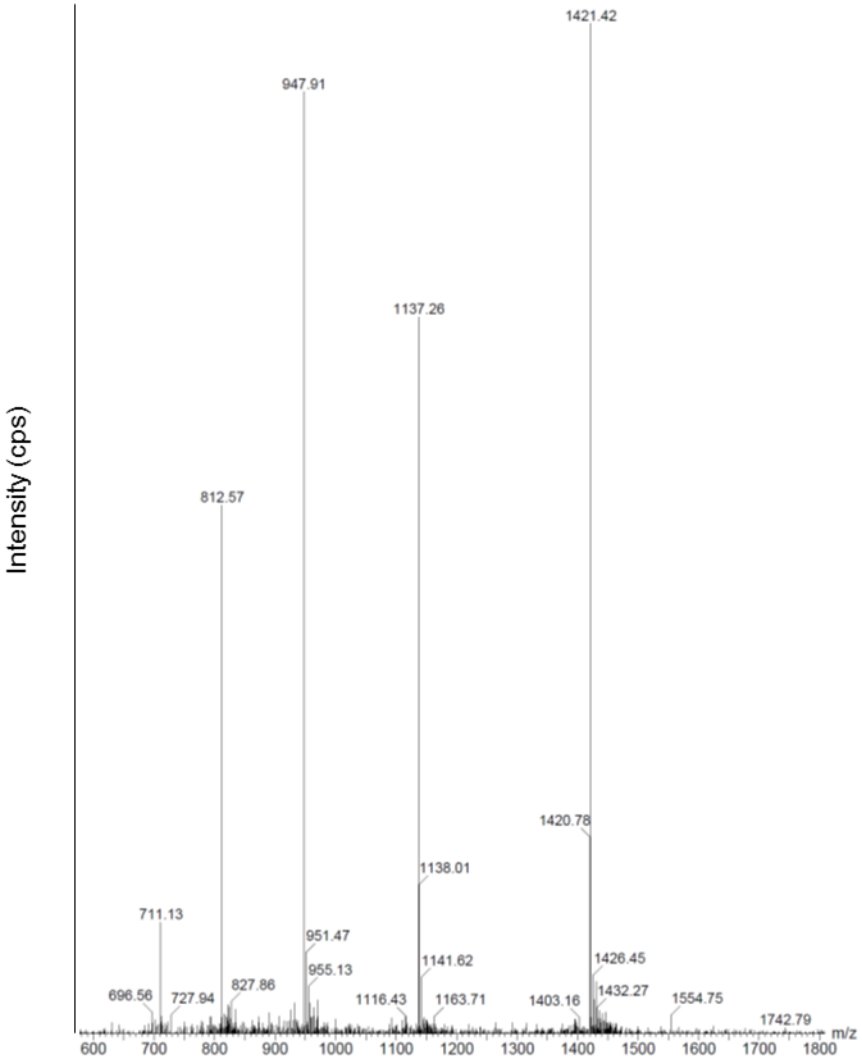


Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 5682.36 (MH^+); Major observed ions: 812.7 (+7), 948.0 (+6), 1137.4 (+5), 1421.3 (+4).

D-Src(145-187)^{TMR} (D-3b)

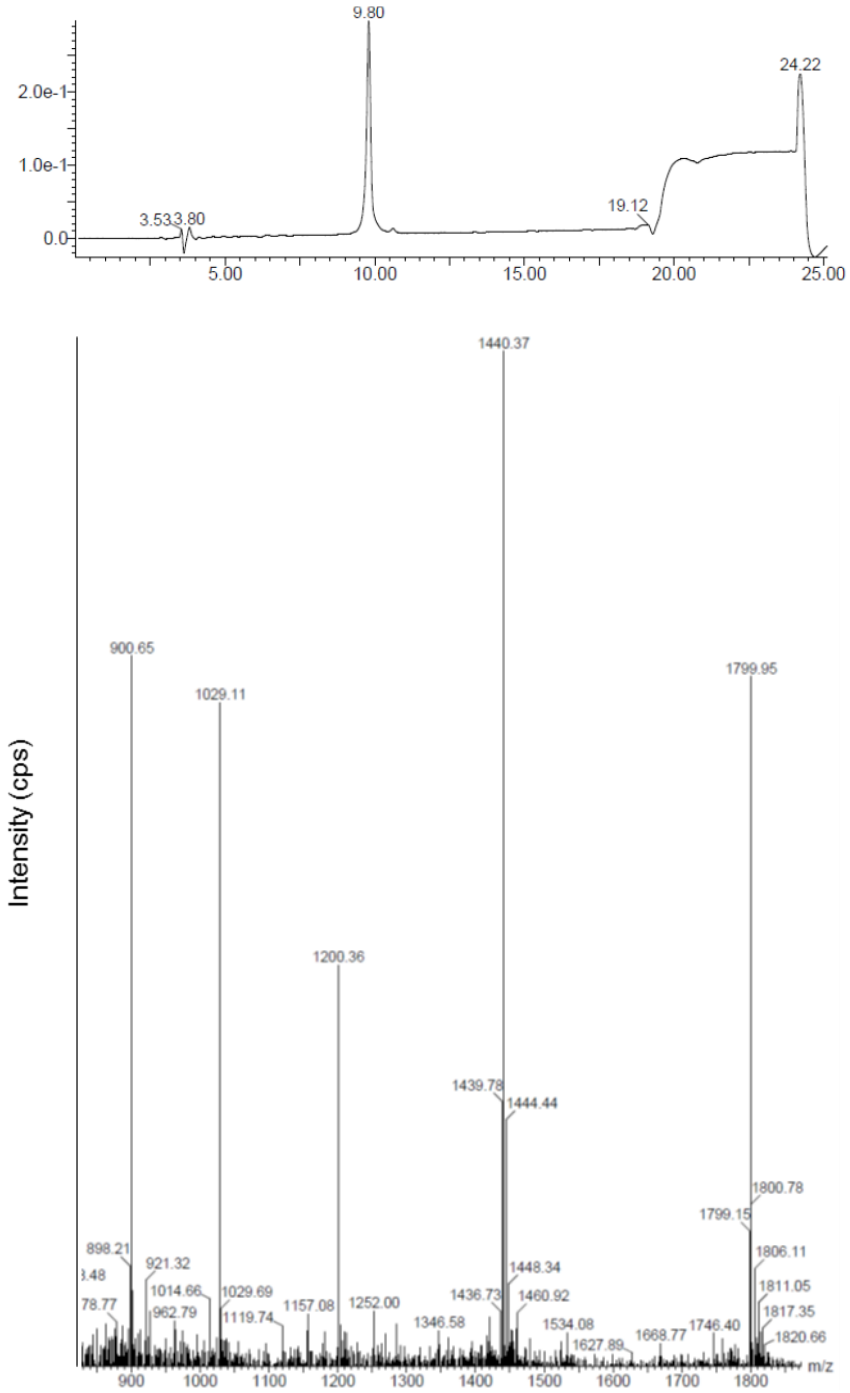




Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 5682.36 (MH^+); Major observed ions: 812.6 (+7), 947.9 (+6), 1137.3 (+5), 1421.4 (+4).

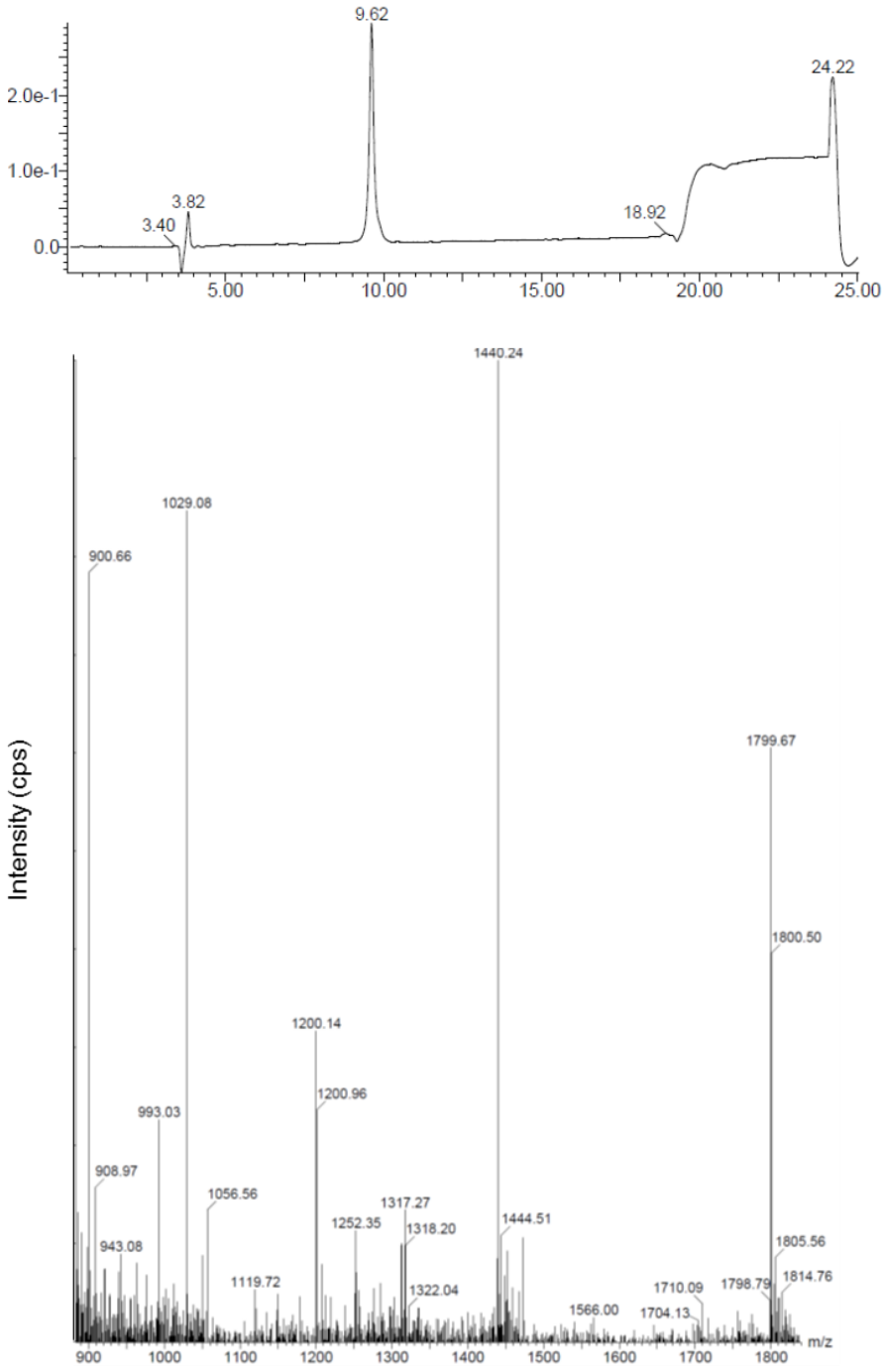
L-Src(188-251) (L-4)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 7198.22 (MH^+); Major observed ions: 900.7 (+8), 1029.1 (+7), 1200.4 (+6), 1440.4 (+5), 1800.0 (+4).

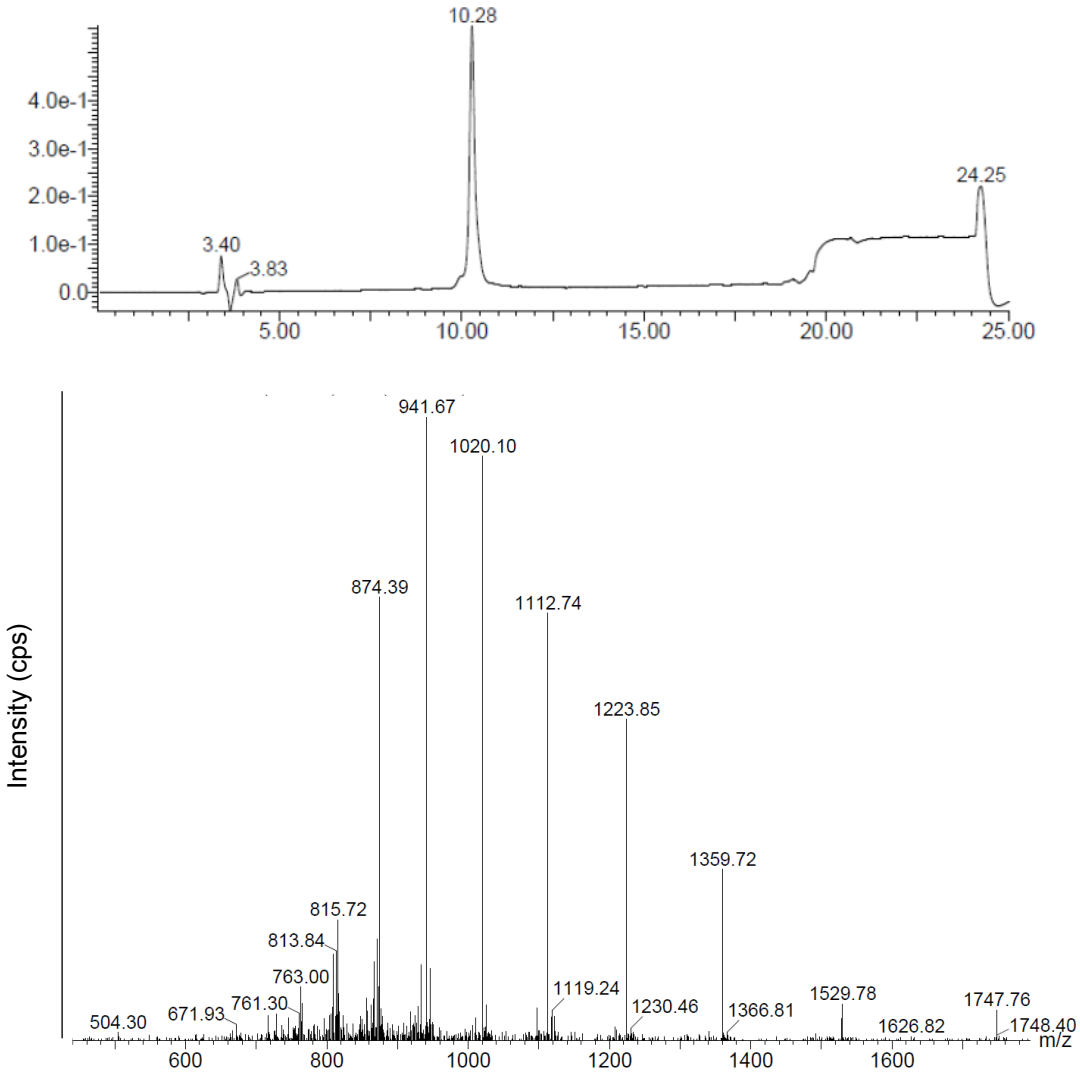
D-Src(188-251) (D-4)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 7198.22 (MH^+); Major observed ions: 900.7 (+8), 1029.1 (+7), 1200.1 (+6), 1440.2 (+5), 1799.7 (+4).

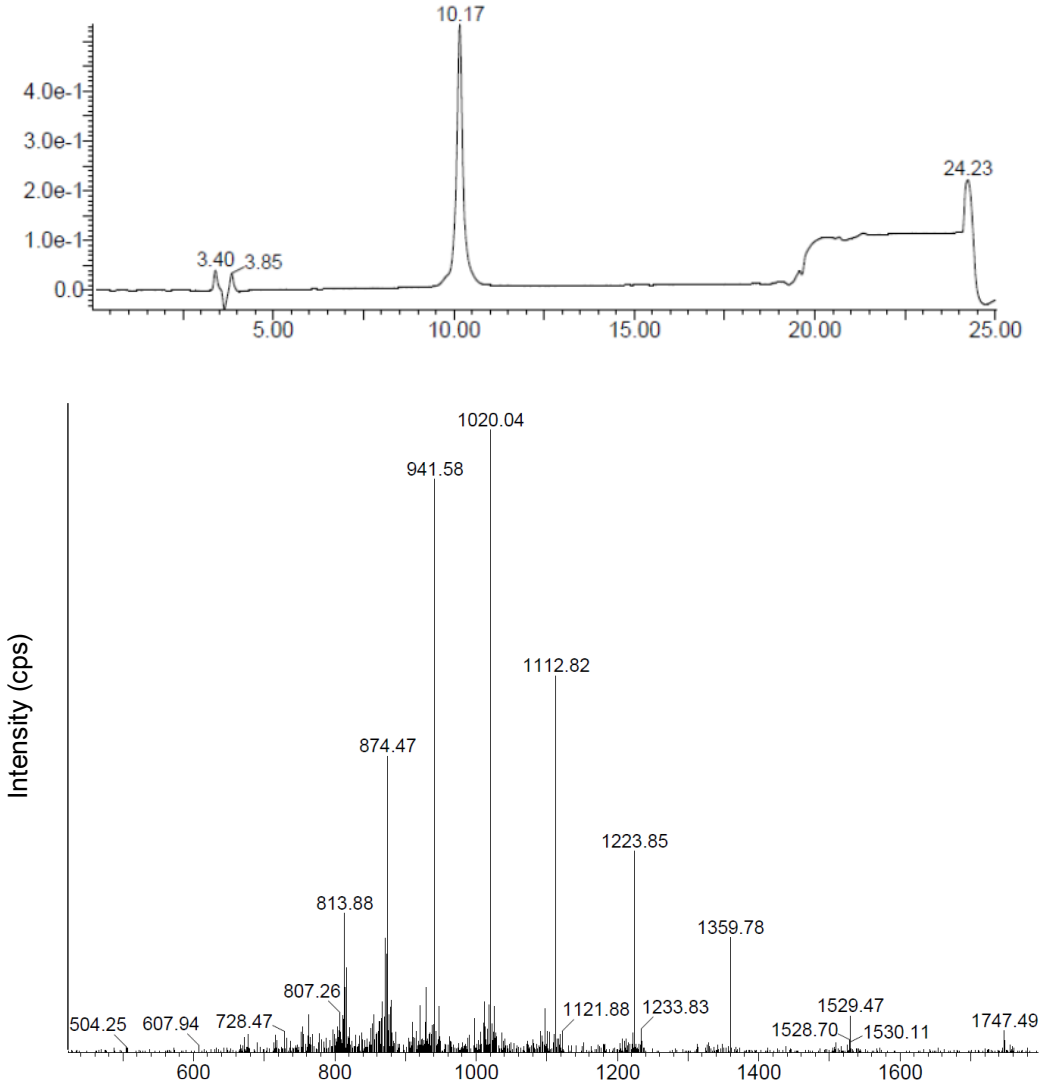
L-Src(145-251) (L-5a)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 12227.84 (MH^+); Major observed ions: 813.9 (+15), 874.4 (+14), 941.7 (+13), 1020.1 (+12), 1112.7 (+11), 1223.9 (+10), 1359.7 (+9), 1529.8 (+8), 1747.8 (+7).

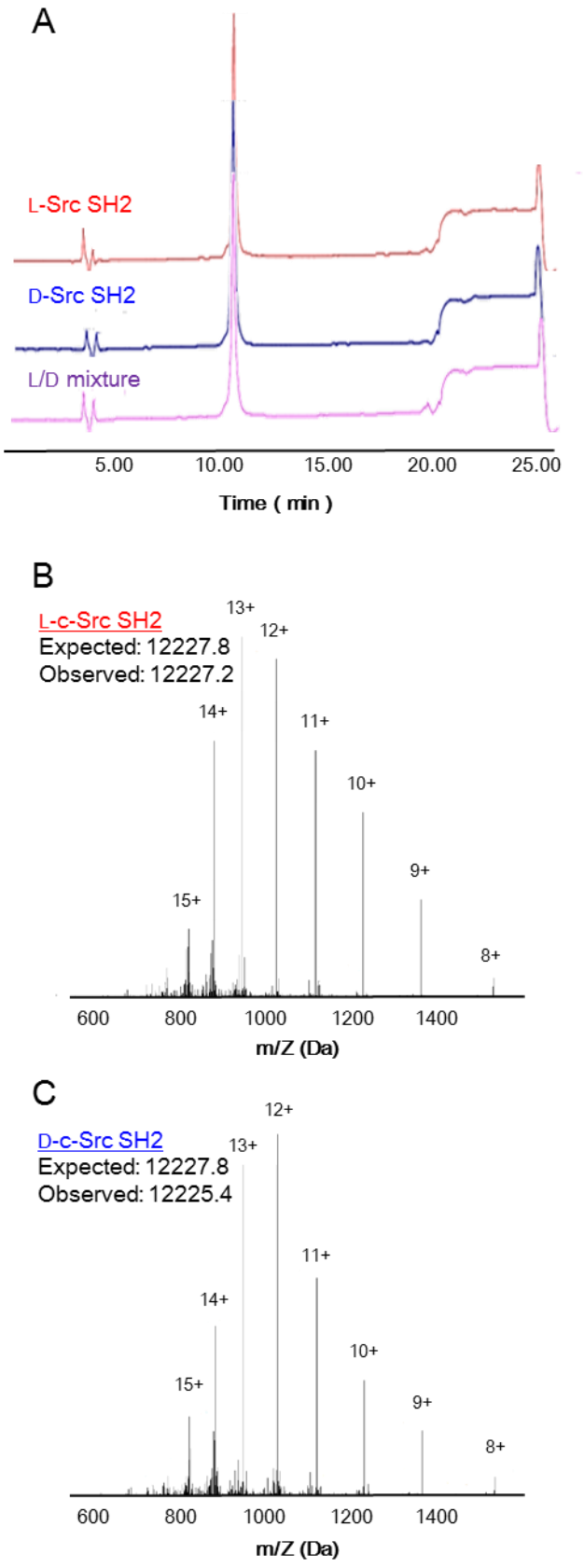
D-Src(145-251) (D-5a)



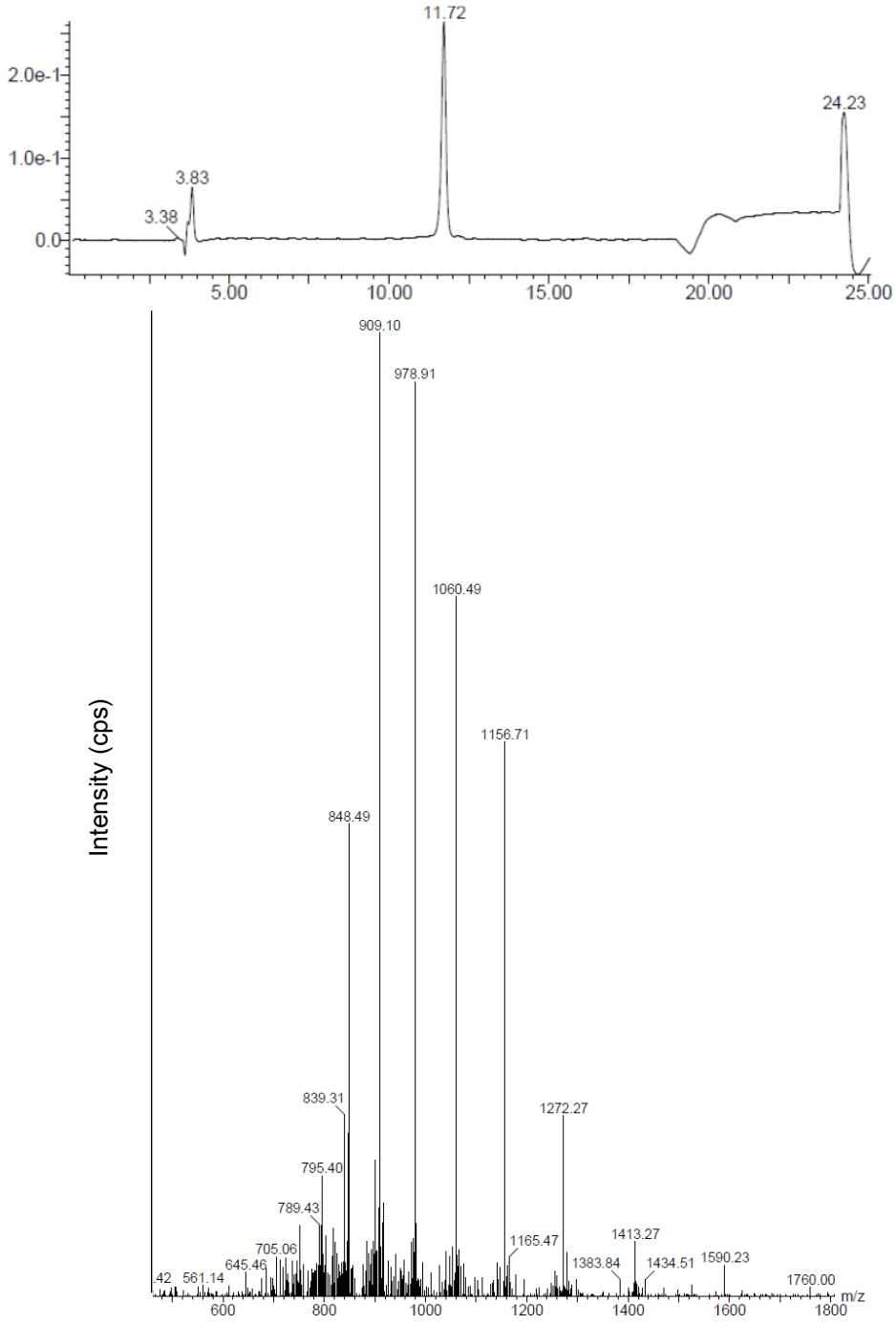
Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 12227.84 (MH^+); Major observed ions: 813.9 (+15), 874.5 (+14), 941.6 (+13), 1020.0 (+12), 1112.8 (+11), 1223.9 (+10), 1359.8 (+9), 1529.5 (+8), 1747.5 (+7).

Analytical HPLC trace and ESI-MS of synthetic Src SH2 domain proteins. (*A*) Analytical HPLC of L-Src(145-187) (**L-5a**, red), synthetic D-Src(145-187) (**D-5a**, blue), and a mixture of **L-5a** and **D-5a** (1:1) (purple) with detection at UV 220 nm. (*B* and *C*) ESI-MS analysis of L-Src(145-187) (**L-5a**) and D-Src(145-187) (**D-5a**), respectively.



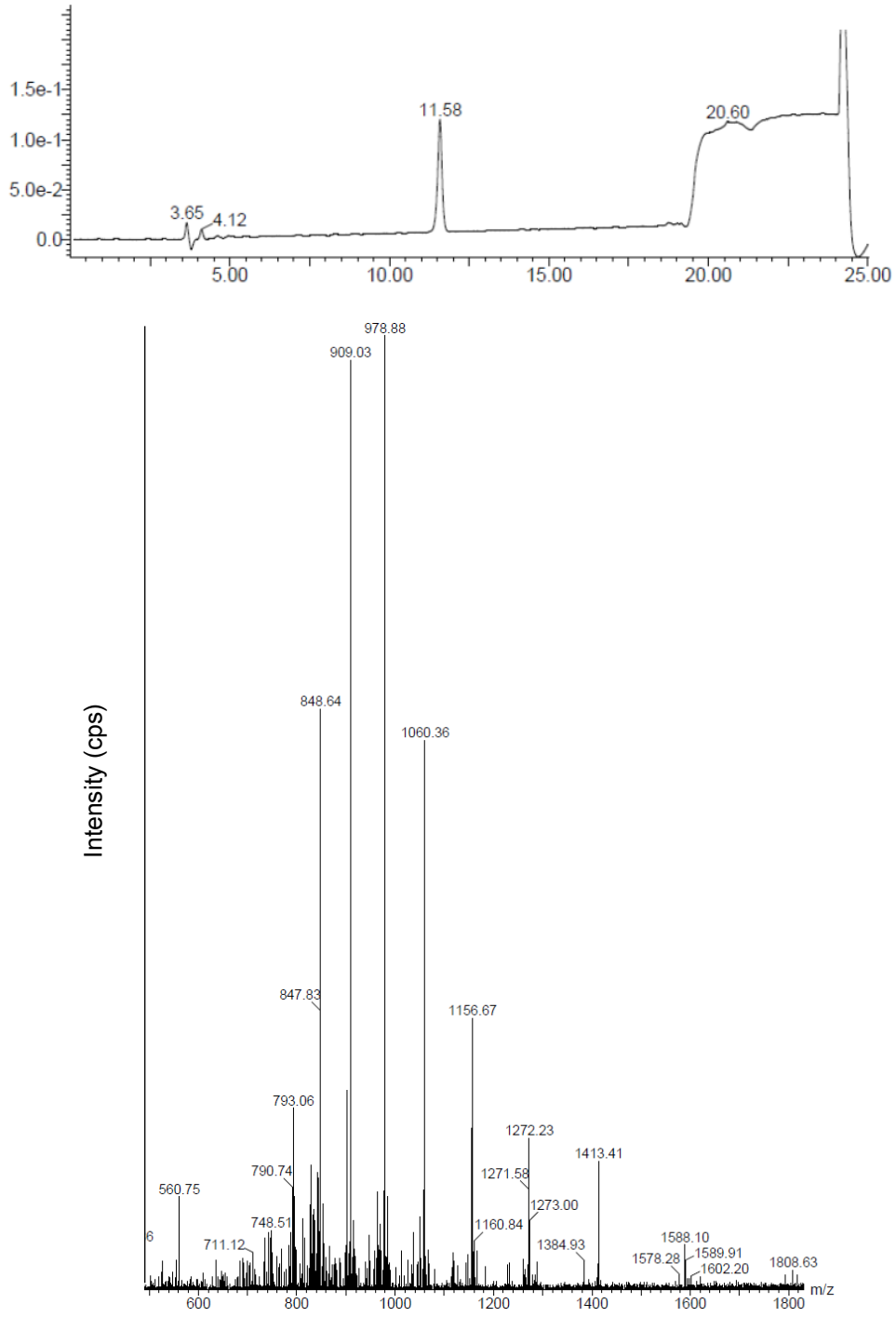
L-Src(145-251)^{TMR} (L-5b)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 12711.36 (MH^+); Major observed ions: 848.5 (+15), 909.1 (+14), 978.9 (+13), 1060.5 (+12), 1156.7 (+11), 1272.3 (+10), 1413.3 (+9), 1590.2 (+8), 1760.0 (+7).

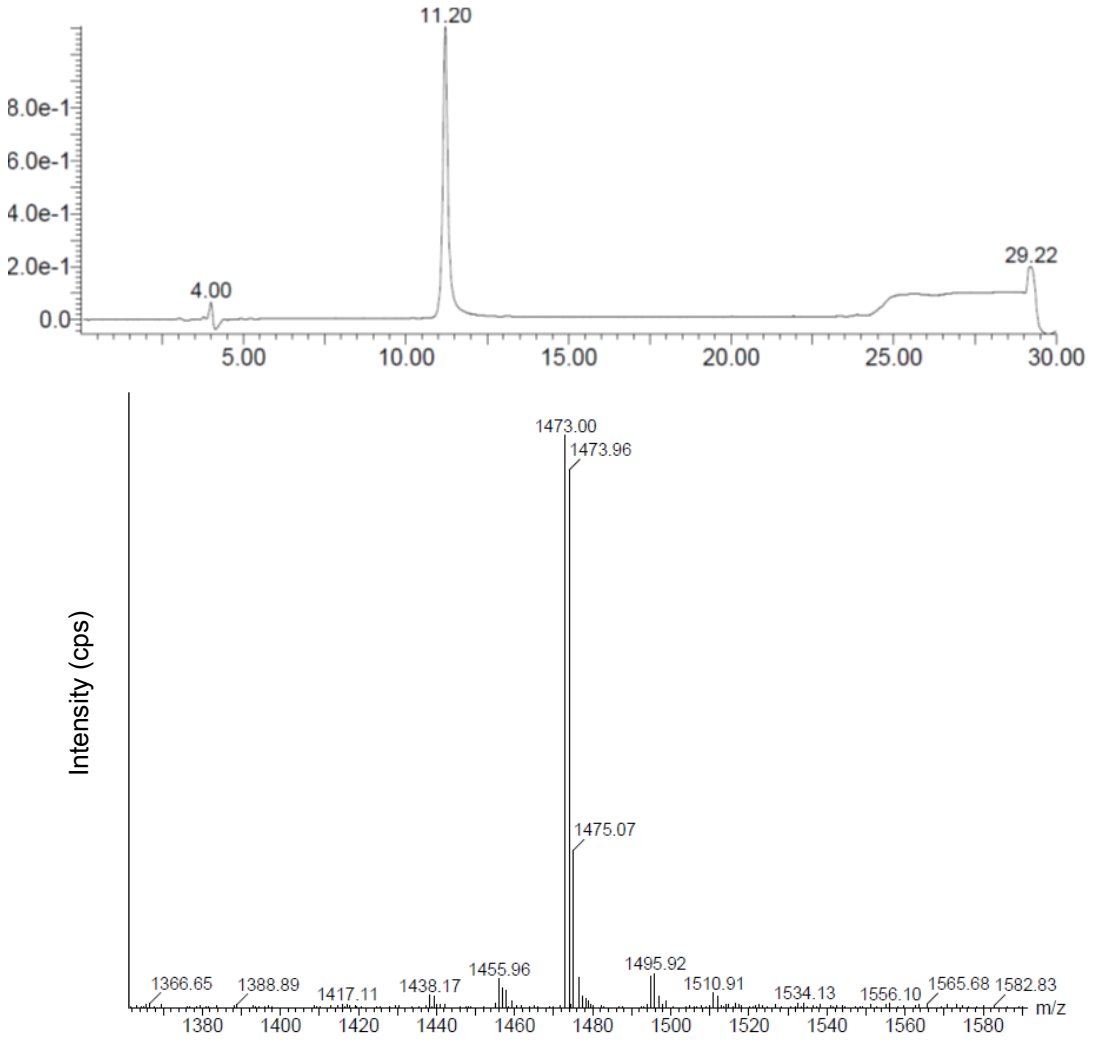
D-Src(145-251)^{TMR} (D-5b)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm)**,** linear gradient of 30-45% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 15 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 12711.36 (MH^+); Major observed ions: 848.6 (+15), 909.0 (+14), 978.9 (+13), 1060.4 (+12), 1156.7 (+11), 1272.2 (+10), 1413.4 (+9), 1588.1 (+8).

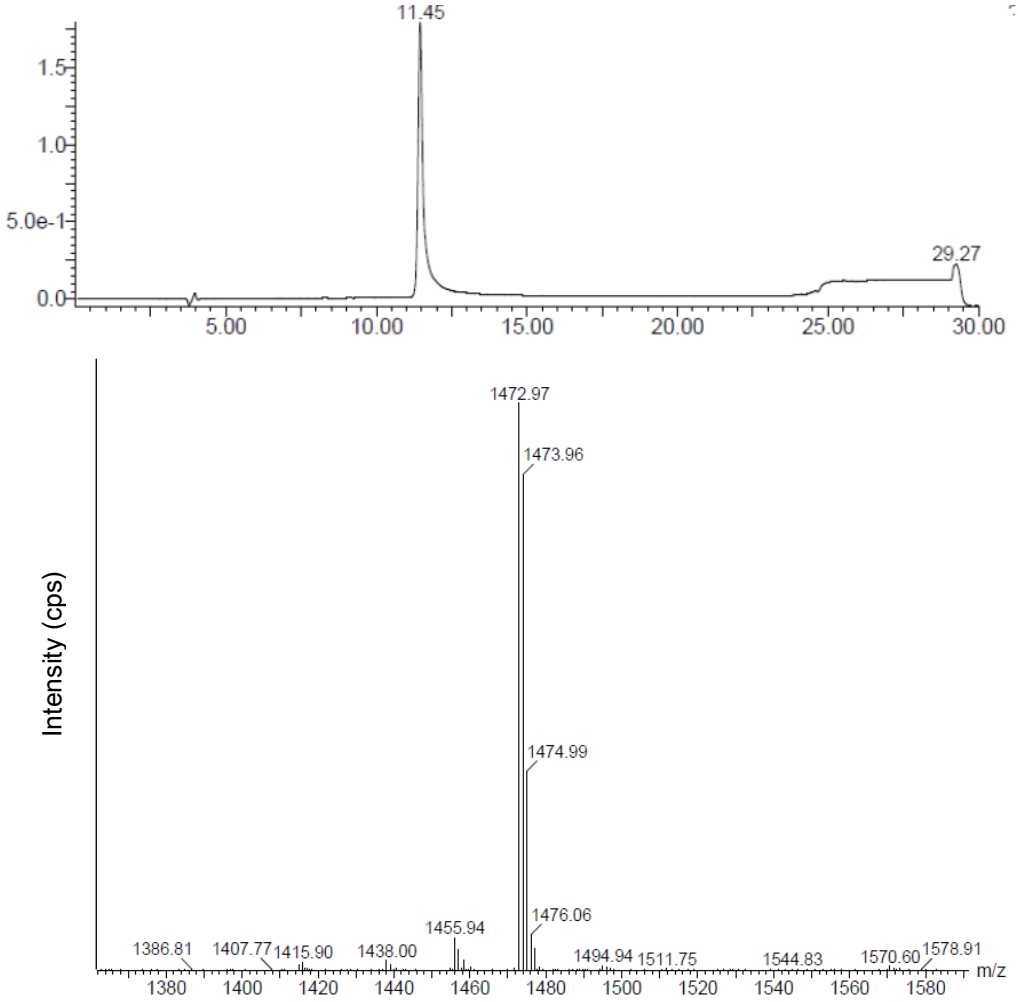
L-hmT pY324 (L-6)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 preparative column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-40% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 20 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1473.56 (MH^+); Major observed ions: 1473.0 (+H).

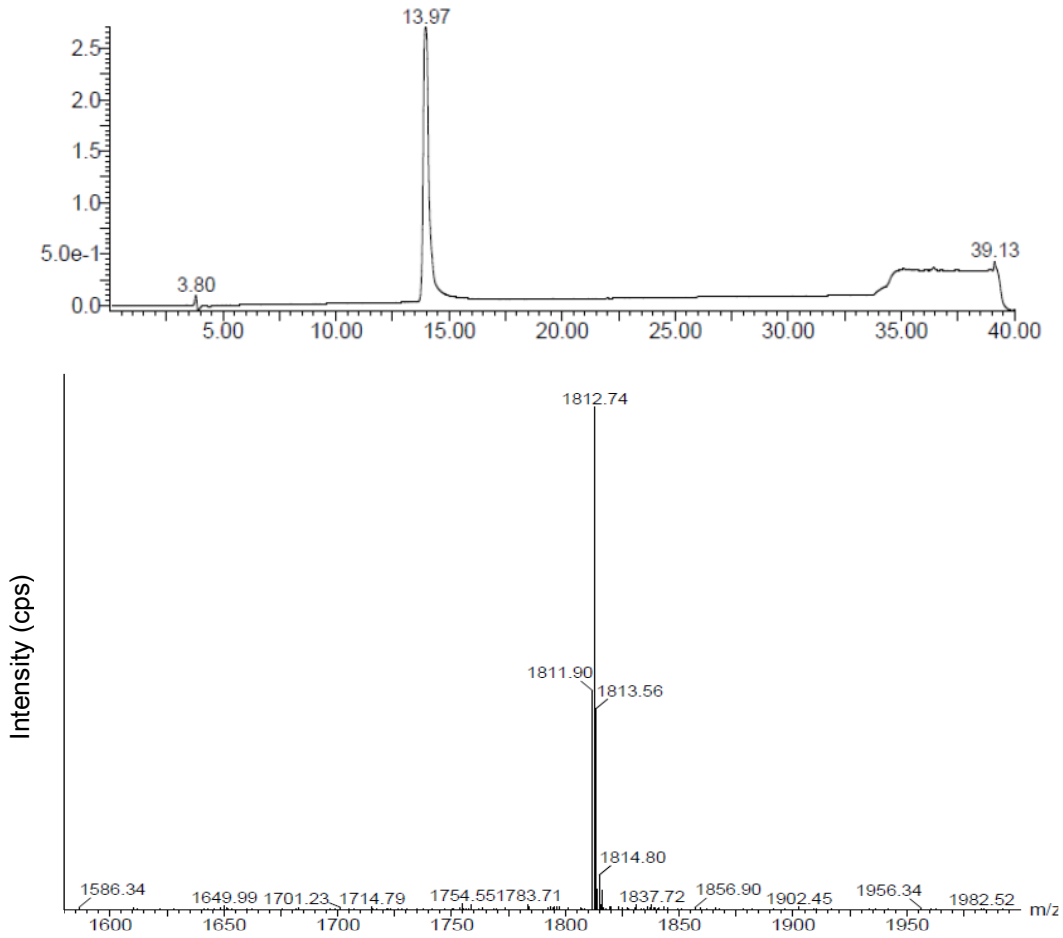
D-hmT pY324 (D-6)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-40% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 20 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1473.56 (MH^+); Major observed ions: 1473.0 (+H).

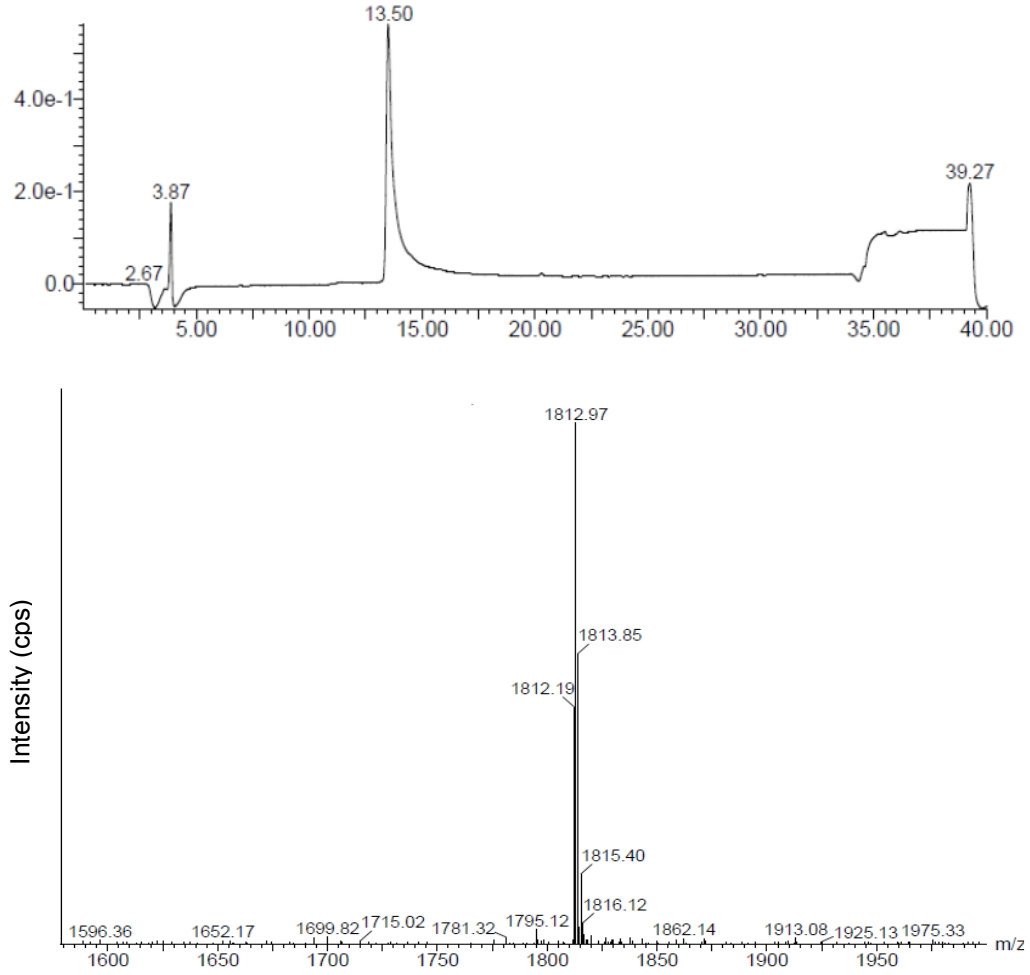
L-hmT pY324^{biotin} (L-7)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-50% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 30 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1813.01 (MH^+); Major observed ions: 1812.7 (+H).

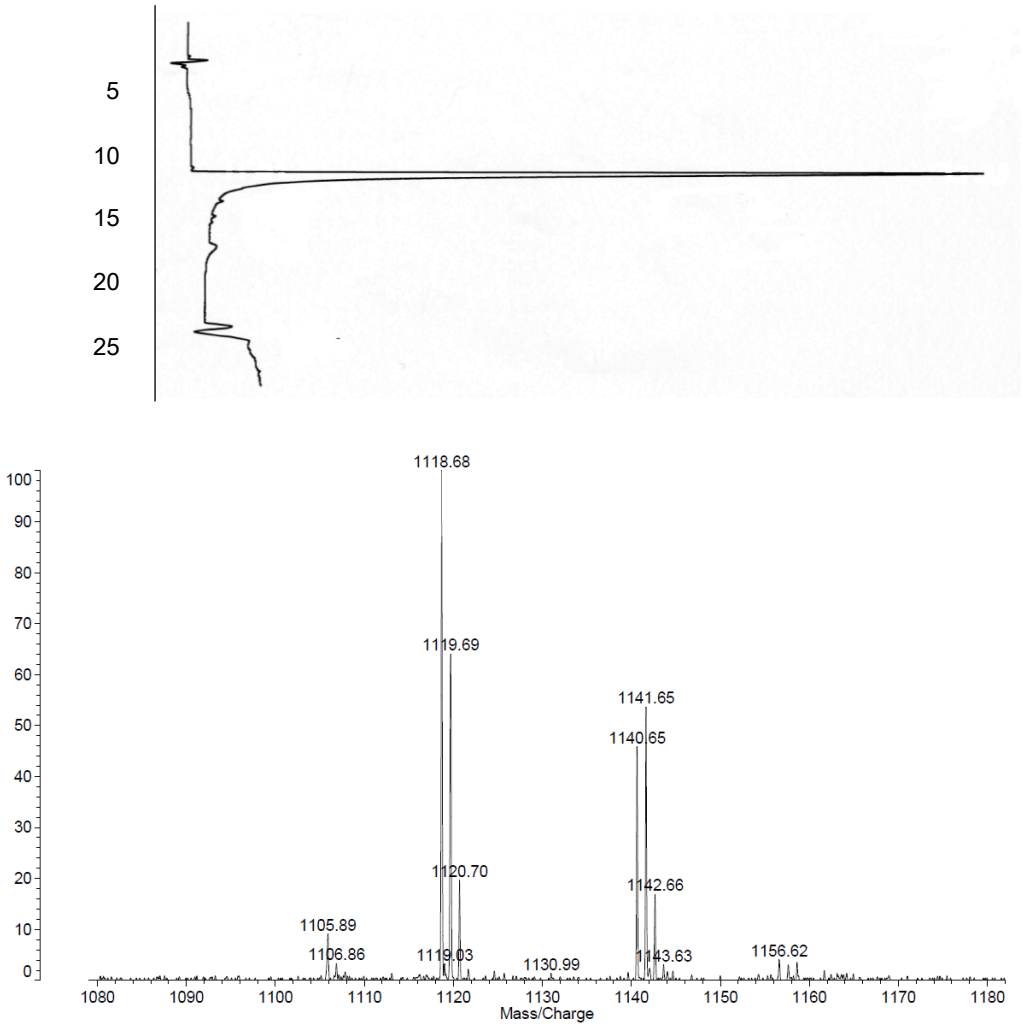
D-hmT pY324^{biotin} (D-7)



Analytical HPLC trace of the purified product: Cosmosil 5C18-AR300 column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-50% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 30 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1813.01 (MH^+); Major observed ions: 1813.0 (+H).

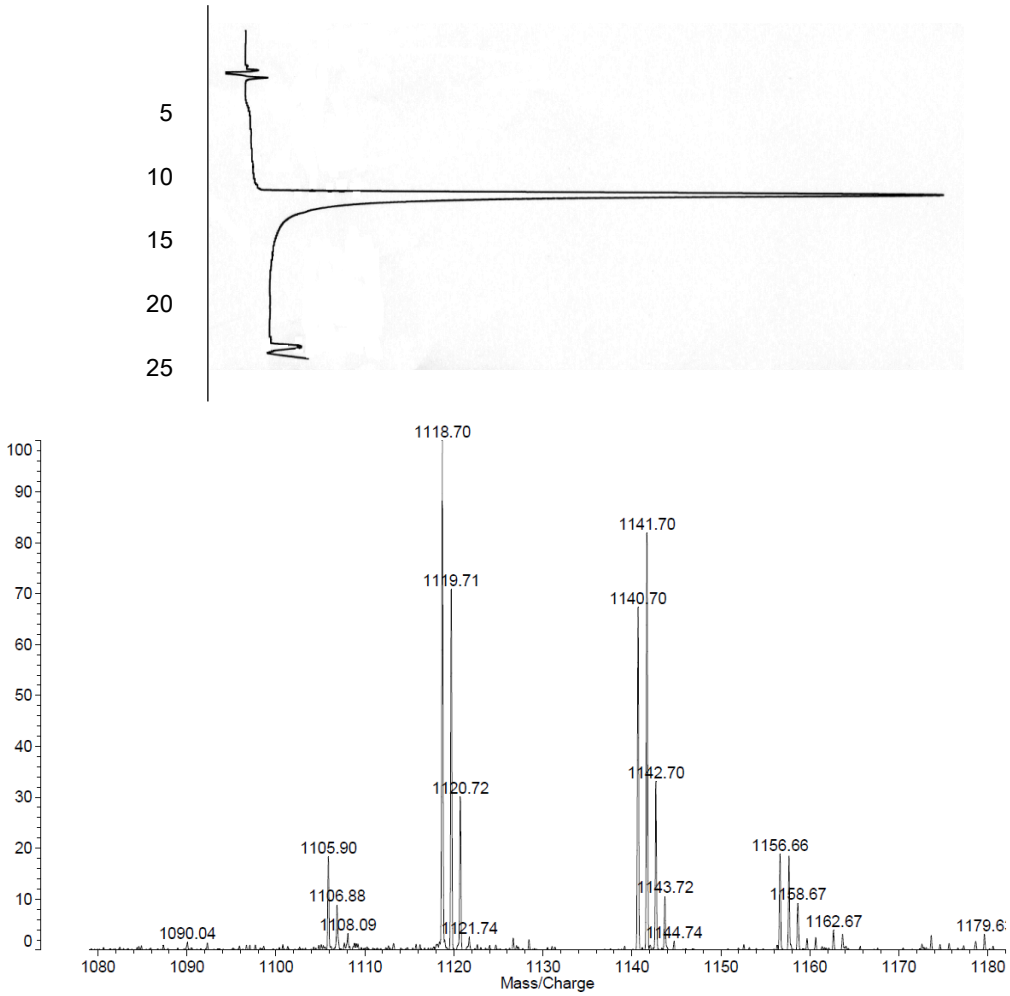
L-FMT1^{FAM} (L-8)



Analytical HPLC trace of the purified product: Cosmosil 5C18-ARII column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-40% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 20 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1119.02 (MH^+); Major observed ions: 1118.7 (+H), 1141.7 (+Na), 1156.6 (+K).

D-FMT1^{FAM} (D-8)



Analytical HPLC trace of the purified product: Cosmosil 5C18-ARII column (Nacalai Tesque, 4.6 × 250 mm), linear gradient of 20-40% CH_3CN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 20 min.

Mass spec analysis of the purified product: Expected mass based on sequence: 1119.02 (MH^+); Major observed ions: 1118.7 (+H), 1141.7 (+Na), 1156.7 (+K).