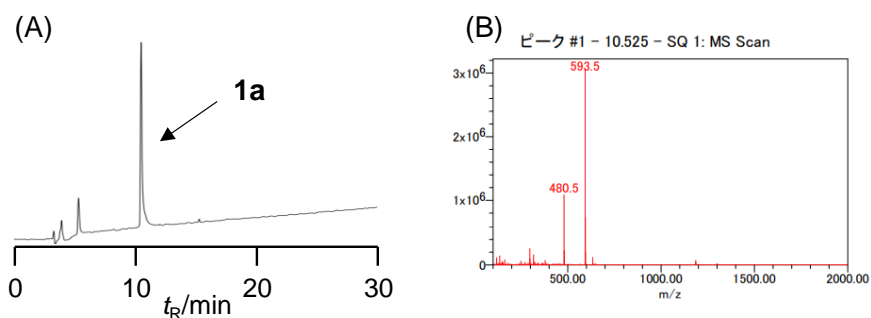


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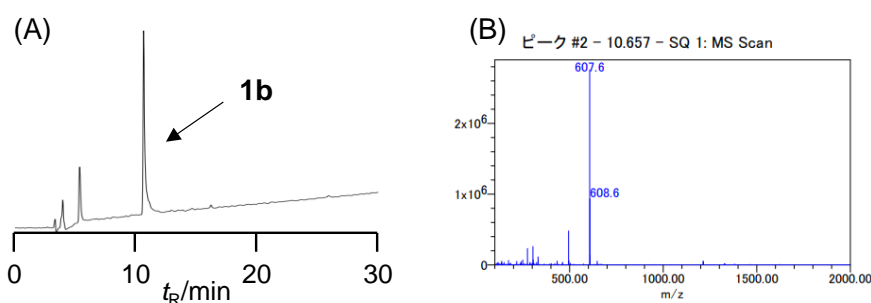
<i>LCMS analyses of peptide hydrazides H-LYRA-Xaa-NHNH<sub>2</sub></i> .....	2
<i>LCMS analyses of oxidants screening</i> .....	8
<i>LCMS analyses of hydrazide conversion to carboxylic acid using oxone</i> .....	9
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<i>LCMS analyses of ammonolysis of peptidyl azides derived from hydrazides</i> .....	22
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LCMS analyses of peptide hydrazides *H-LYRA-Xaa-NHNH<sub>2</sub>*

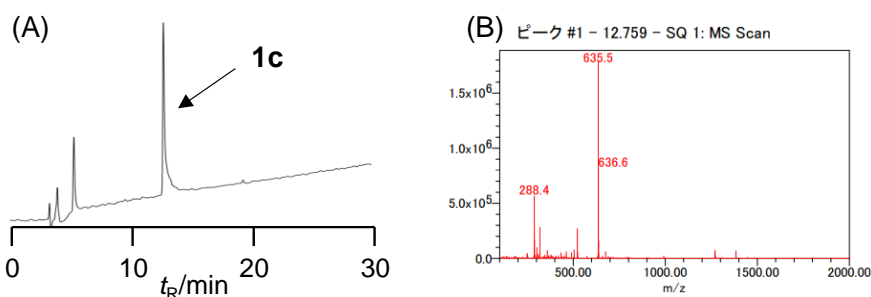
LCMS conditions for Figures S1–S18 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min.



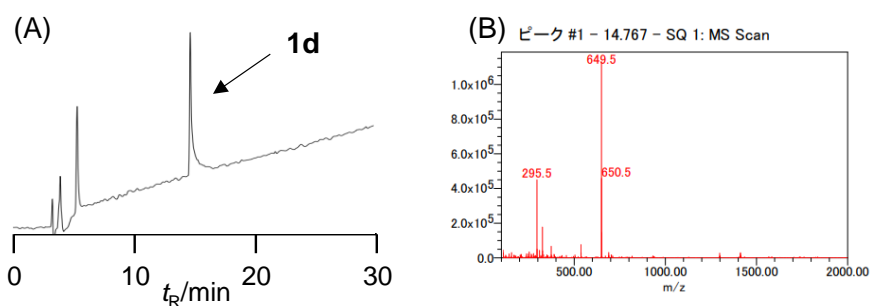
**Figure S1** (A) UV trace of crude material of **1a**. (B) MS spectrum of **1a**. Xaa = Gly (**1a**): Retention time = 10.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>26</sub>H<sub>45</sub>N<sub>10</sub>O<sub>6</sub> 593.4, found 593.5.



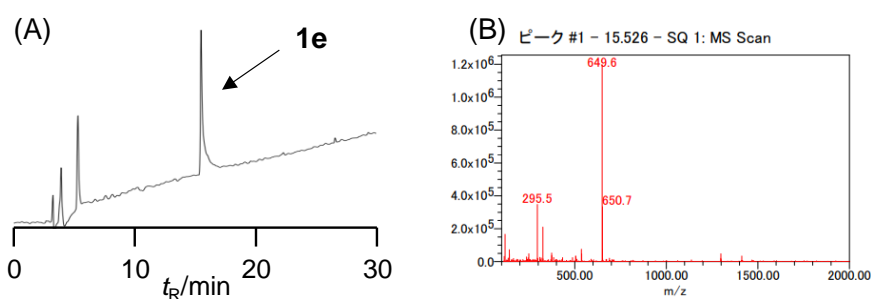
**Figure S2** (A) UV trace of crude material of **1b**. (B) MS spectrum of **1b**. Xaa = Ala (**1b**): Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>27</sub>H<sub>47</sub>N<sub>10</sub>O<sub>6</sub> 607.4, found 607.6.



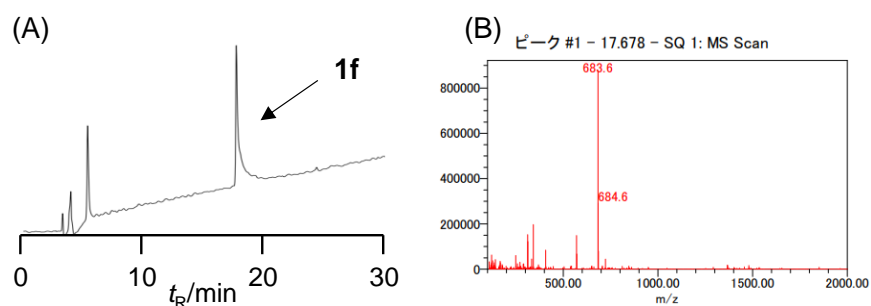
**Figure S3** (A) UV trace of crude material of **1c**. (B) MS spectrum of **1c**. Xaa = Val (**1c**): Retention time = 12.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>29</sub>H<sub>51</sub>N<sub>10</sub>O<sub>6</sub> 635.4, found 635.5.



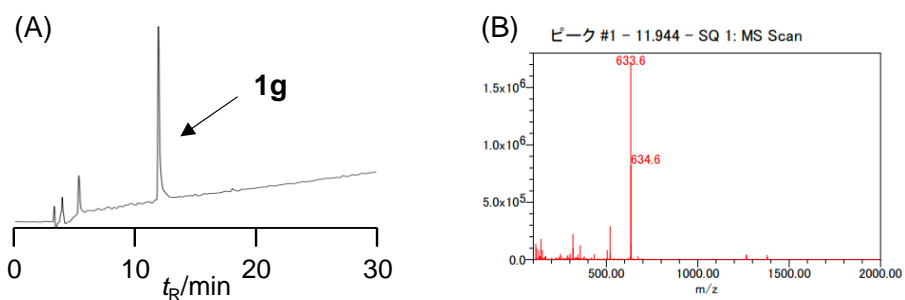
**Figure S4** (A) UV trace of crude material of **1d**. (B) MS spectrum of **1d**. Xaa = Ile (**1d**): Retention time = 14.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{10}O_6$  649.4, found 649.5.



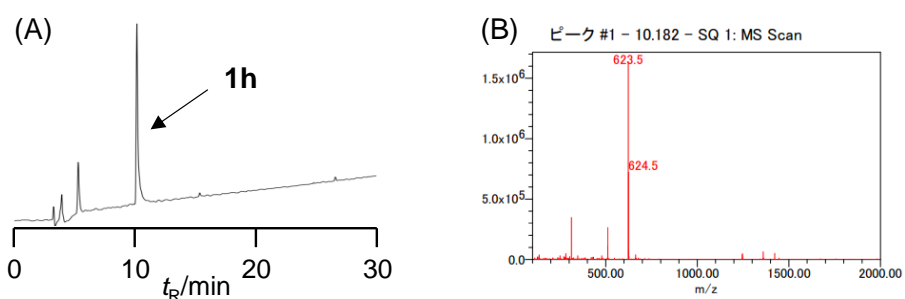
**Figure S5** (A) UV trace of crude material of **1e**. (B) MS spectrum of **1e**. Xaa = Leu (**1e**): Retention time = 15.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{10}O_6$  649.4, found 649.6.



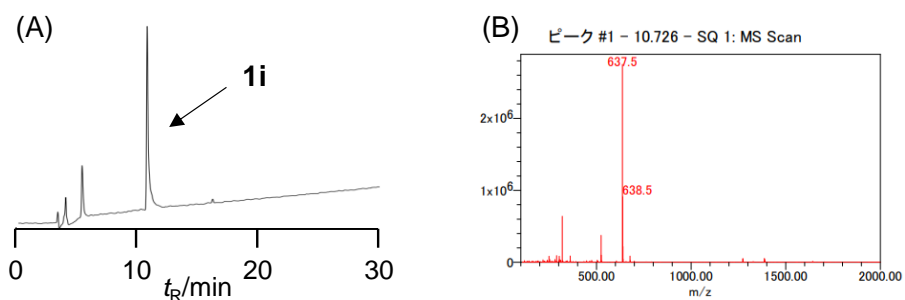
**Figure S6** (A) UV trace of crude material of **1f**. (B) MS spectrum of **1f**. Xaa = Phe (**1f**): Retention time = 17.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{51}N_{10}O_6$  683.4, found 683.6.



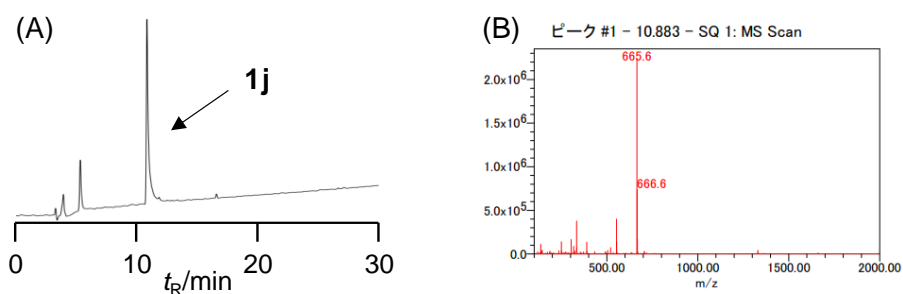
**Figure S7** (A) UV trace of crude material of **1g**. (B) MS spectrum of **1g**. Xaa = Pro (**1g**): Retention time = 11.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_{10}O_6$  633.4, found 633.6.



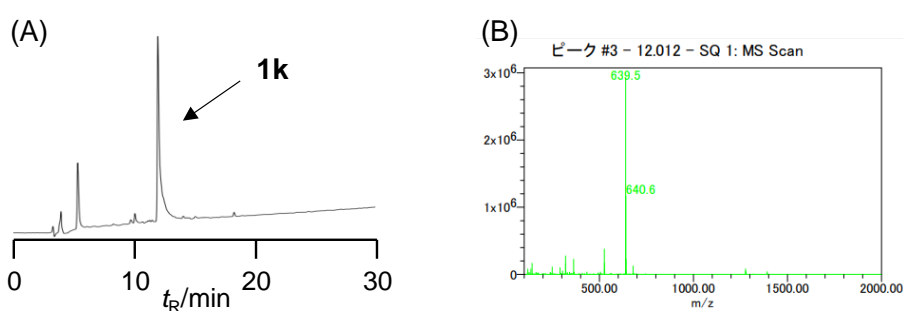
**Figure S8** (A) UV trace of crude material of **1h**. (B) MS spectrum of **1h**. Xaa = Ser (**1h**): Retention time = 10.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{47}N_{10}O_7$  623.4, found 623.5.



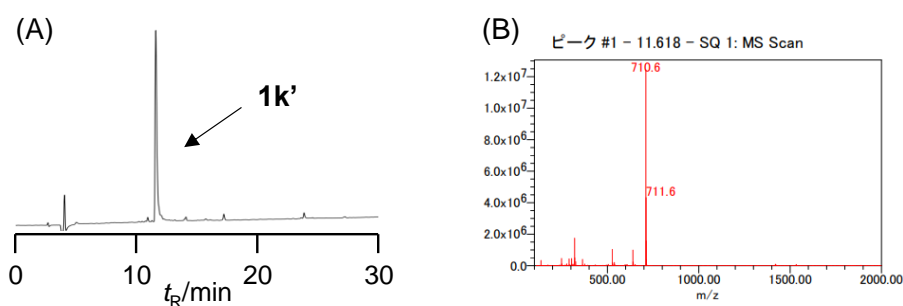
**Figure S9** (A) UV trace of crude material of **1i**. (B) MS spectrum of **1i**. Xaa = Thr (**1i**): Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{49}N_{10}O_7$  637.4, found 637.5.



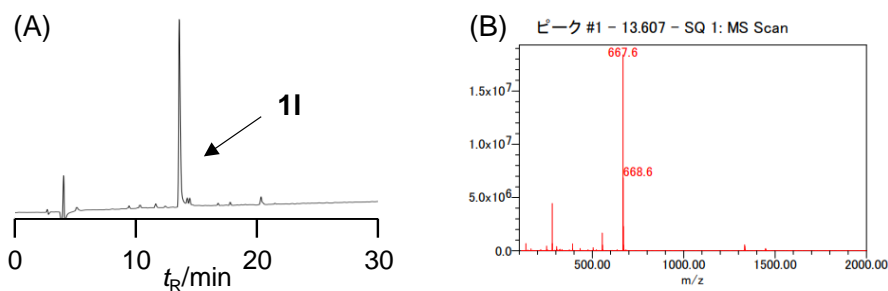
**Figure S10** (A) UV trace of crude material of **1j**. (B) MS spectrum of **1j**. Xaa = Glu (**1j**): Retention time = 10.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_{10}O_8$  665.4, found 665.6.



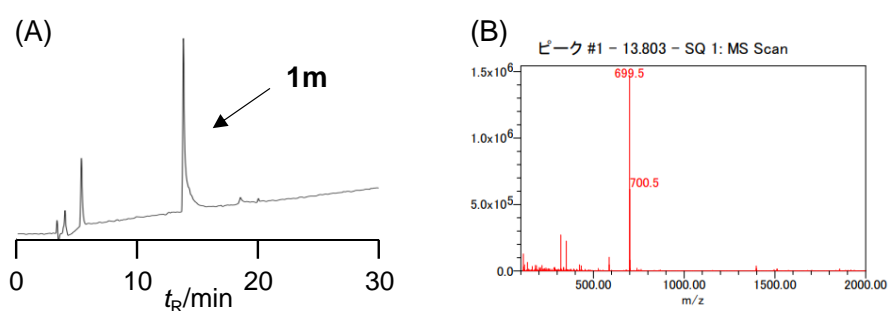
**Figure S11** (A) UV trace of crude material of **1k**. (B) MS spectrum of **1k**. Xaa = Cys (**1k**): Retention time = 12.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{47}N_{10}O_6S$  639.3, found 639.5.



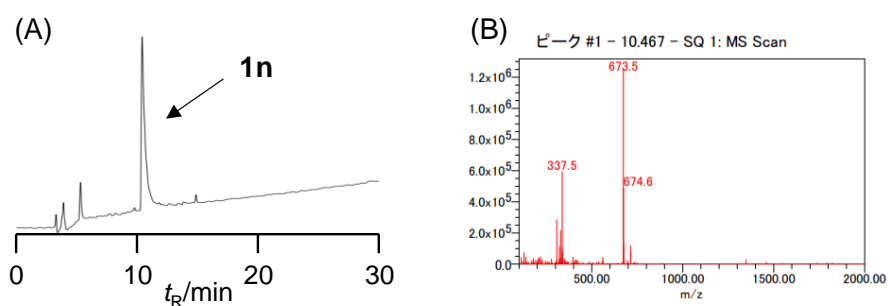
**Figure S12** (A) UV trace of crude material of **1k'**. (B) MS spectrum of **1k'**. Xaa = Cys(Acm) (**1k'**): Retention time = 11.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_{11}O_7S$  710.4, found 710.6.



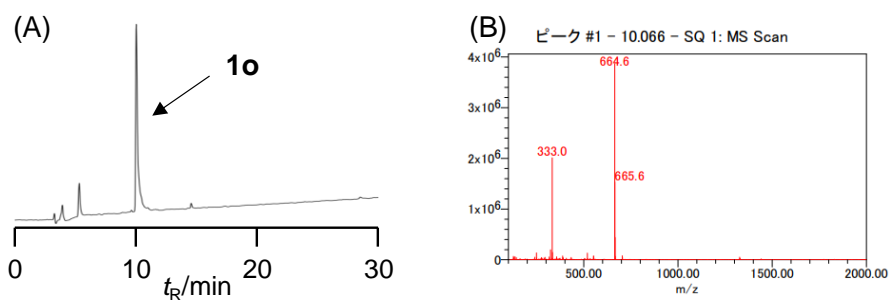
**Figure S13** (A) UV trace of crude material of **1l**. (B) MS spectrum of **1l**. Xaa = Met (**1l**): Retention time = 13.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{51}N_{10}O_6S$  667.4, found 667.6.



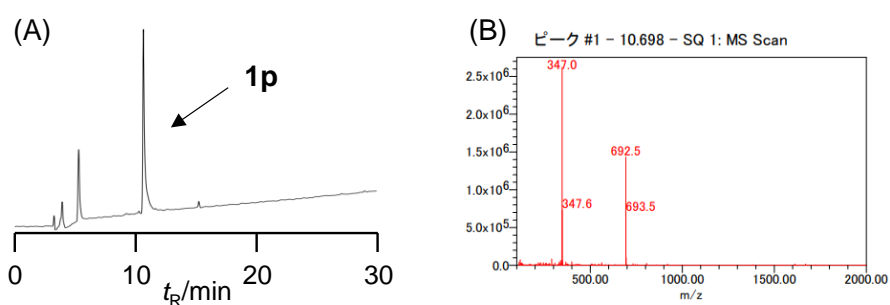
**Figure S14** (A) UV trace of crude material of **1m**. (B) MS spectrum of **1m**. Xaa = Tyr (**1m**): Retention time = 13.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{51}N_{10}O_7$  699.4, found 699.5.



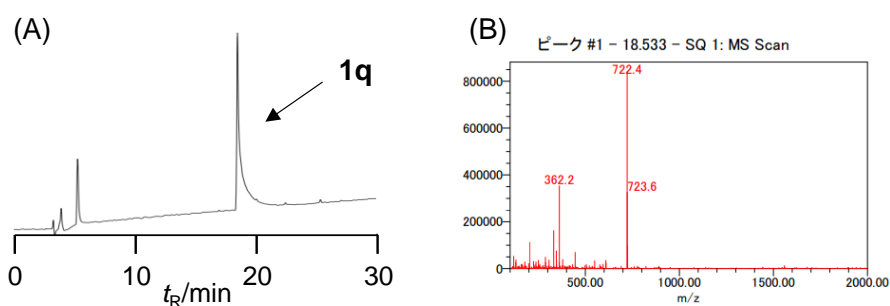
**Figure S15** (A) UV trace of crude material of **1n**. (B) MS spectrum of **1n**. Xaa = His (**1n**): Retention time = 10.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{49}N_{12}O_6$  673.4, found 673.5.



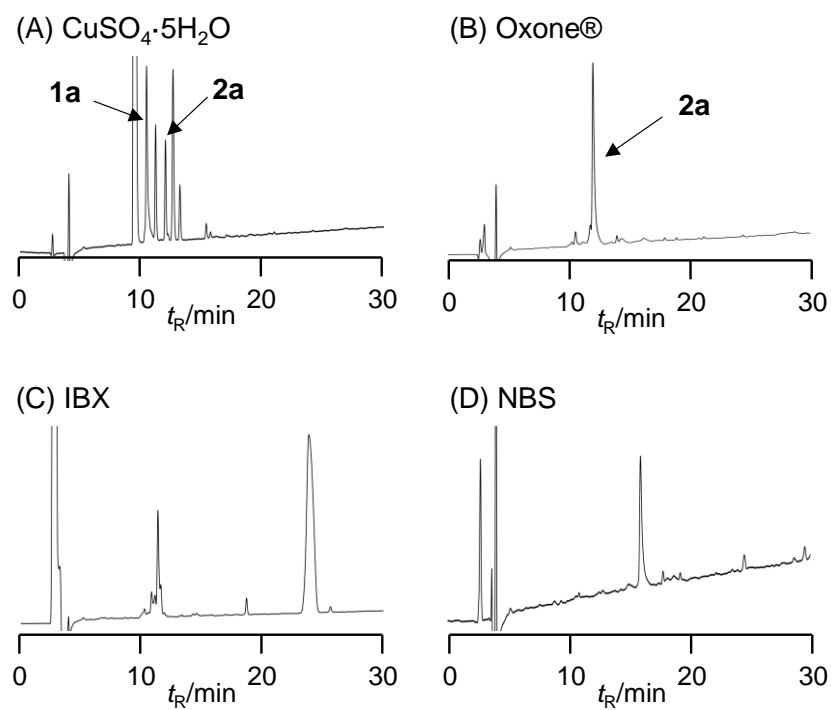
**Figure S16** (A) UV trace of crude material of **1o**. (B) MS spectrum of **1o**. Xaa = Lys (**1o**): Retention time = 10.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{54}N_{11}O_6$  664.4, found 664.6.



**Figure S17** (A) UV trace of crude material of **1p**. (B) MS spectrum of **1p**. Xaa = Arg (**1p**): Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{54}N_{13}O_6$  692.4, found 692.5.



**Figure S18** (A) UV trace of crude material of **1q**. (B) MS spectrum of **1q**. Xaa = Trp (**1q**): Retention time = 18.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{35}H_{52}N_{11}O_6$  722.4, found 722.4.

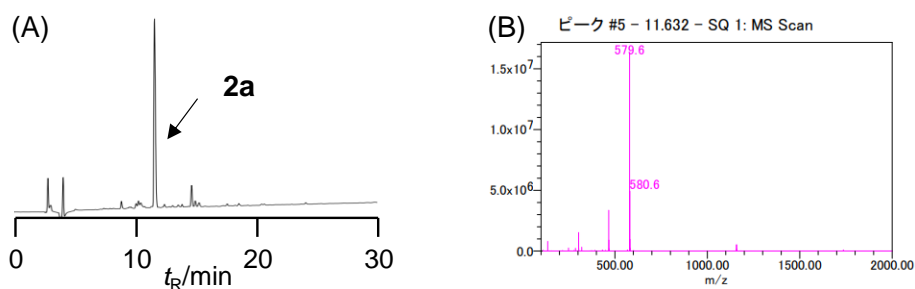


**Figure S19** HPLC analyses of oxidants screening for oxidative hydrazide conversion. Analytical HPLC conditions: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min.

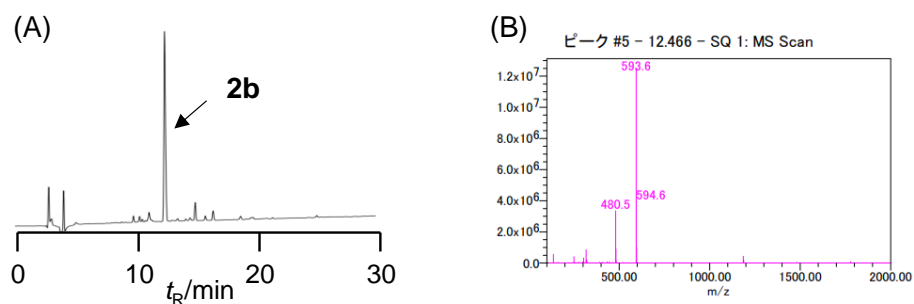


LCMS analyses of hydrazide conversion to carboxylic acid using oxone

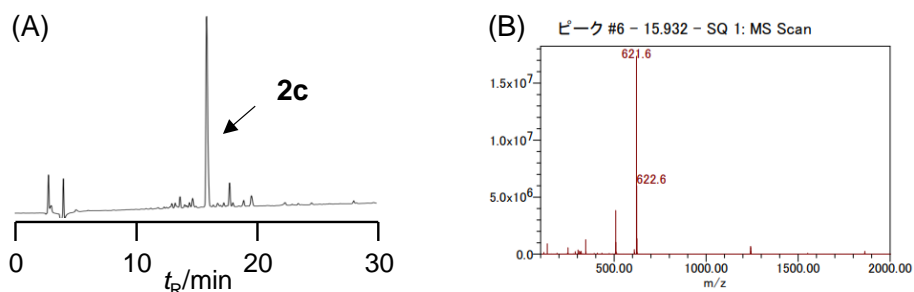
LCMS conditions for Figures S20–S37 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min.



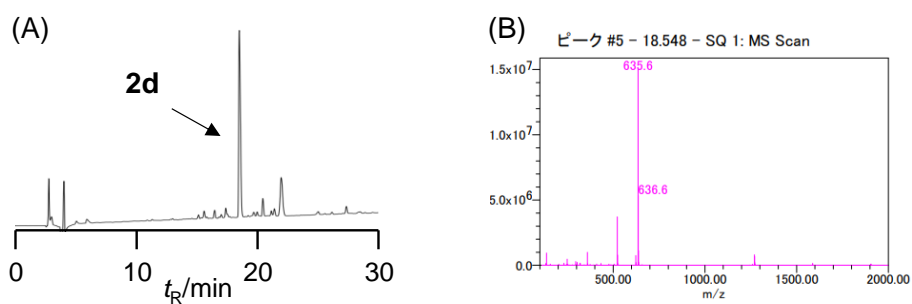
**Figure S20** (A) UV trace of oxidation (Xaa = Gly;  $t = 30$  min). (B) MS spectrum of **2a**. **2a**: Retention time = 11.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>26</sub>H<sub>43</sub>N<sub>8</sub>O<sub>7</sub> 579.3, found 579.6.



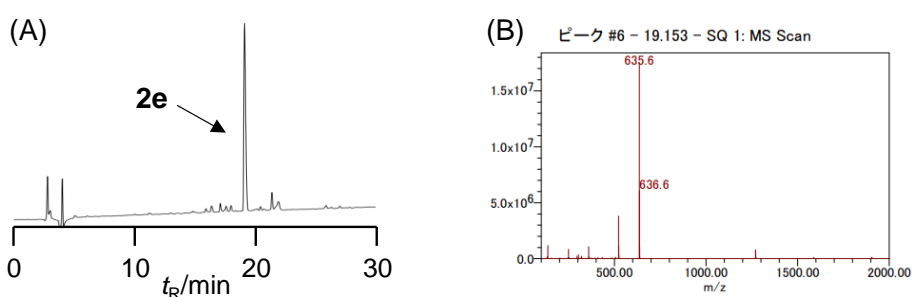
**Figure S21** (A) UV trace of oxidation (Xaa = Ala;  $t = 30$  min). (B) MS spectrum of **2b**. **2b**: Retention time = 12.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>27</sub>H<sub>45</sub>N<sub>8</sub>O<sub>7</sub> 593.3, found 593.6.



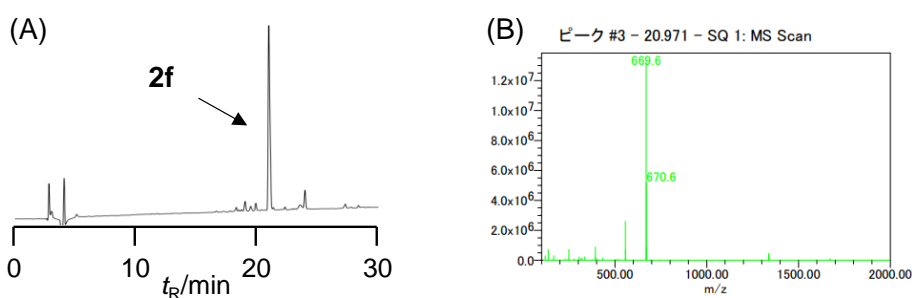
**Figure S22** (A) UV trace of oxidation (Xaa = Val;  $t = 30$  min). (B) MS spectrum of **2c**. **2c**: Retention time = 15.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>29</sub>H<sub>49</sub>N<sub>8</sub>O<sub>7</sub> 621.4, found 621.6.



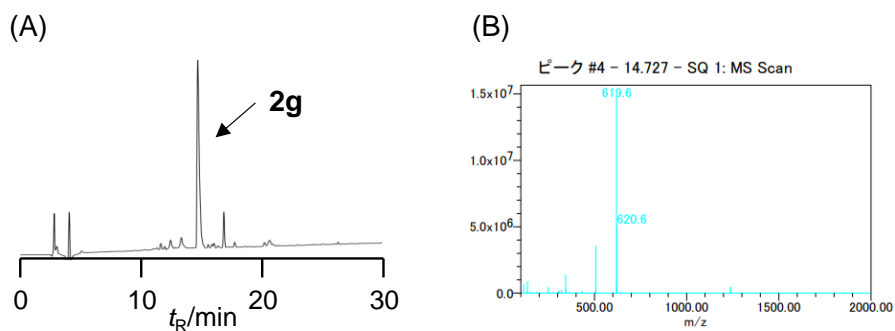
**Figure S23** (A) UV trace of oxidation (Xaa = Ile;  $t = 30$  min). (B) MS spectrum of **2d**. **2d**: Retention time = 18.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_8O_7$  635.4, found 635.6.



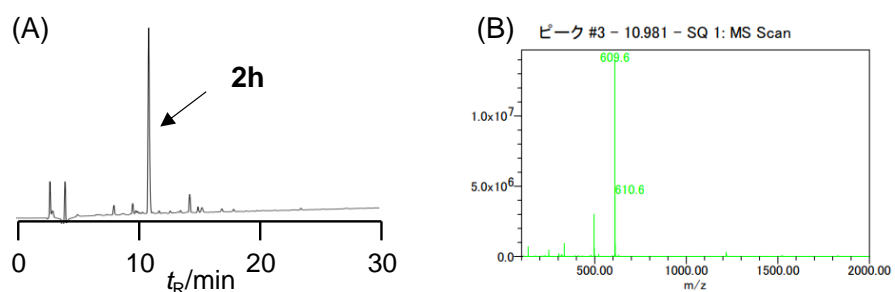
**Figure S24** (A) UV trace of oxidation (Xaa = Leu;  $t = 30$  min). (B) MS spectrum of **2e**. **2e**: Retention time = 19.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_8O_7$  635.4, found 635.6.



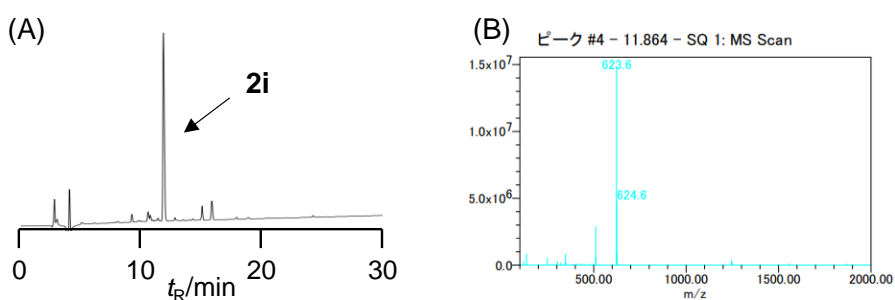
**Figure S25** (A) UV trace of oxidation (Xaa = Phe;  $t = 30$  min). (B) MS spectrum of **2f**. **2f**: Retention time = 21.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_7$  669.4, found 669.6.



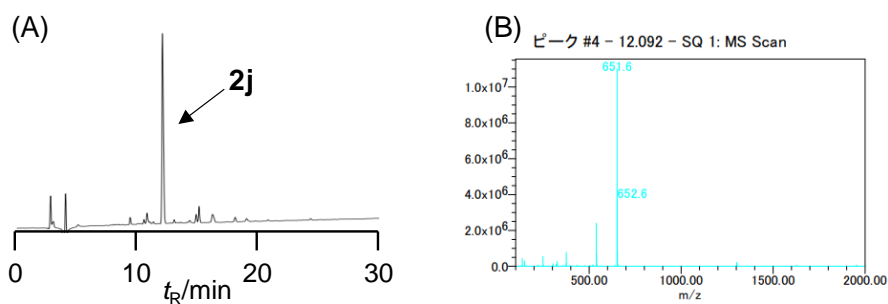
**Figure S26** (A) UV trace of oxidation (Xaa = Pro;  $t = 30$  min). (B) MS spectrum of **2g**. **2g**: Retention time = 14.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_7$  619.4, found 619.6.



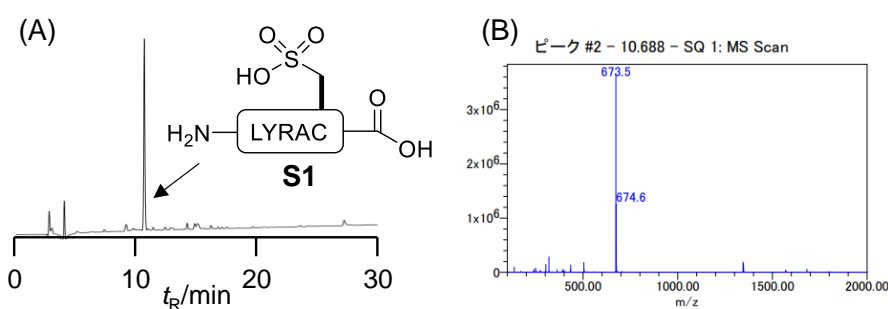
**Figure S27** (A) UV trace of oxidation (Xaa = Ser;  $t = 30$  min). (B) MS spectrum of **2h**. **2h**: Retention time = 11.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_8$  609.3, found 609.6.



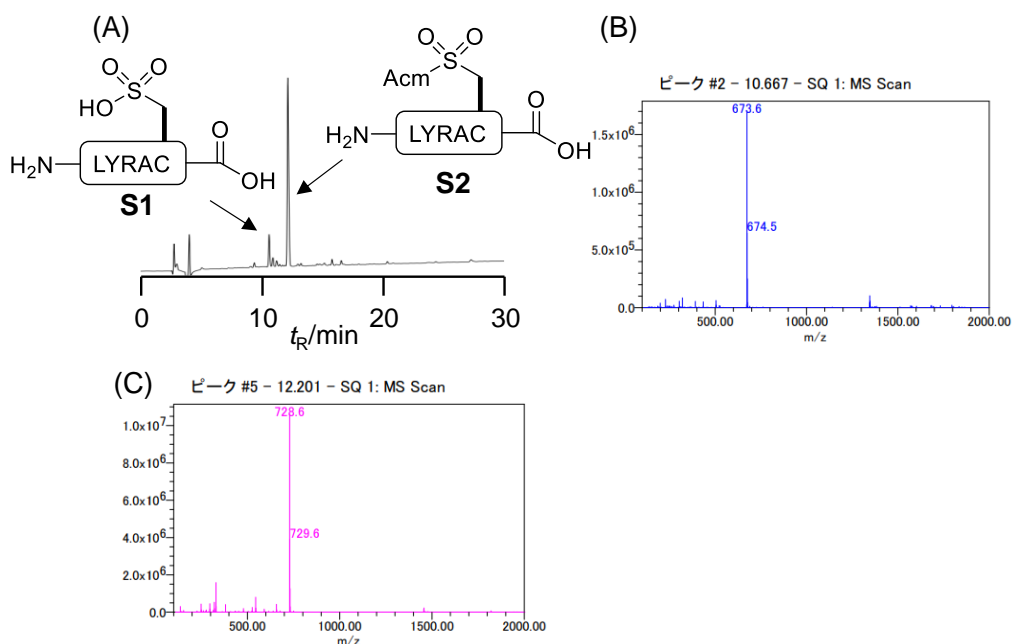
**Figure S28** (A) UV trace of oxidation (Xaa = Thr;  $t = 30$  min). (B) MS spectrum of **2i**. **2i**: Retention time = 11.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{47}N_8O_8$  623.4, found 623.6.



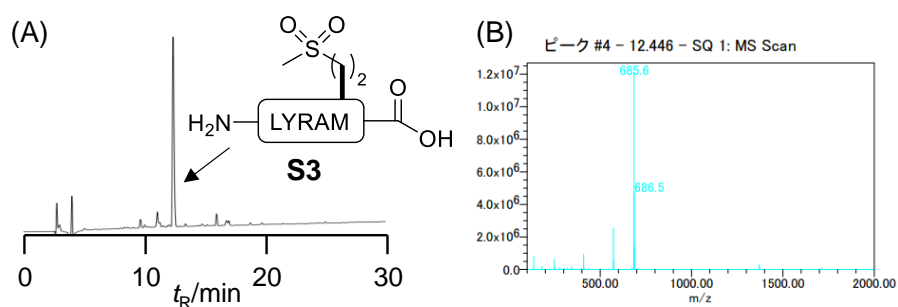
**Figure S29** (A) UV trace of oxidation (Xaa = Glu;  $t = 30$  min). (B) MS spectrum of **2j**. **2j**: Retention time = 12.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_9$  651.3, found 651.6.



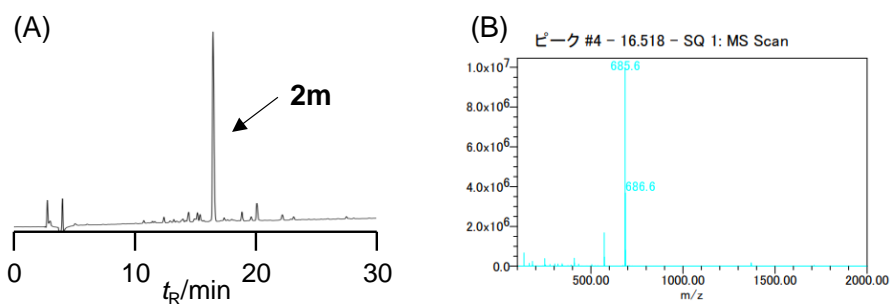
**Figure S30** (A) UV trace of oxidation (Xaa = Cys;  $t = 30$  min). (B) MS spectrum of **S1**. **S1**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_{10}S$  673.3, found 673.5.



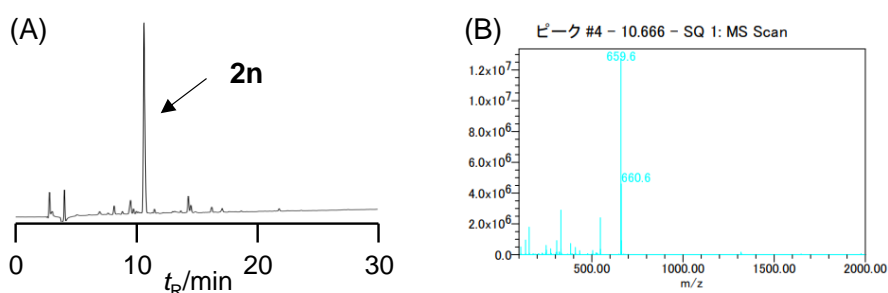
**Figure S31** (A) UV trace of oxidation (Xaa = Cys(Acm);  $t = 30$  min). (B) MS spectrum of **S1**. (C) MS spectrum of **S2**. **S1**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_{10}S$  673.3, found 673.6. **S2**: Retention time = 12.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{50}N_9O_{10}S$  728.3, found 728.6.



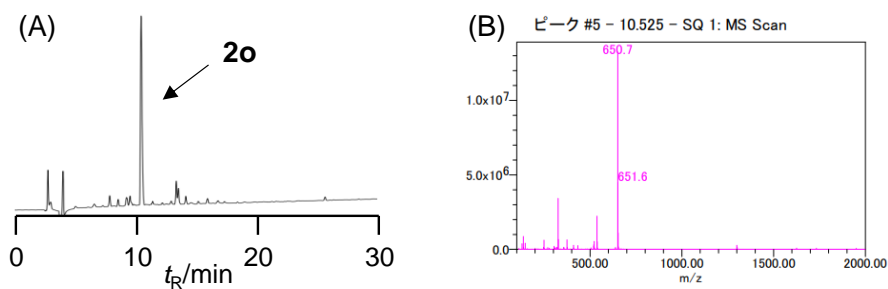
**Figure S32** (A) UV trace of oxidation (Xaa = Met;  $t = 30$  min). (B) MS spectrum of **S3**. **S3**: Retention time = 12.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_9S$  685.3, found 685.6.



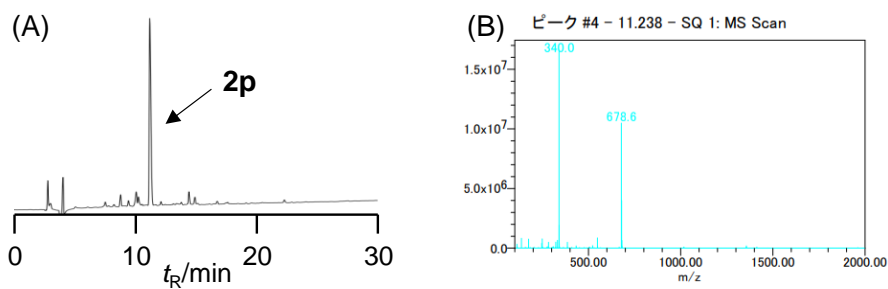
**Figure S33** (A) UV trace of oxidation (Xaa = Tyr;  $t = 30$  min). (B) MS spectrum of **2m**. **2m**: Retention time = 16.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_8$  685.4, found 685.6.



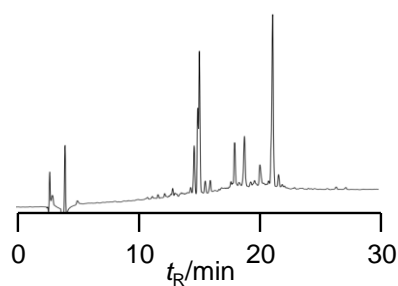
**Figure S34** (A) UV trace of oxidation (Xaa = His;  $t = 30$  min). (B) MS spectrum of **2n**. **2n**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{47}N_{10}O_7$  659.4, found 659.6.



**Figure S35** (A) UV trace of oxidation (Xaa = Lys;  $t = 30$  min). (B) MS spectrum of **2o**. **2o**: Retention time = 10.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_7$  650.4, found 650.7.



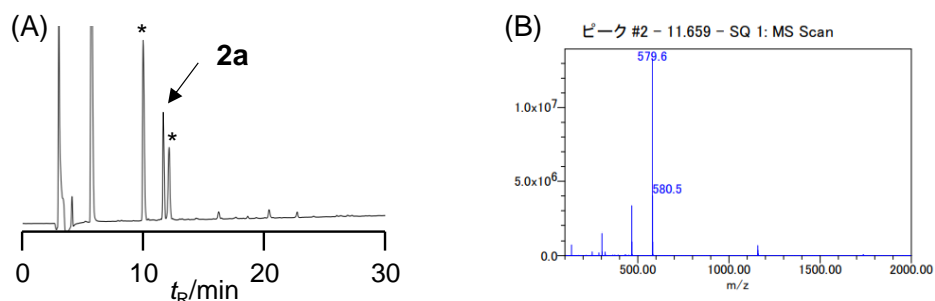
**Figure S36** (A) UV trace of oxidation (Xaa = Arg;  $t = 30$  min). (B) MS spectrum of **2p**. **2p**: Retention time = 11.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_{11}O_7$  678.4, found 678.6.



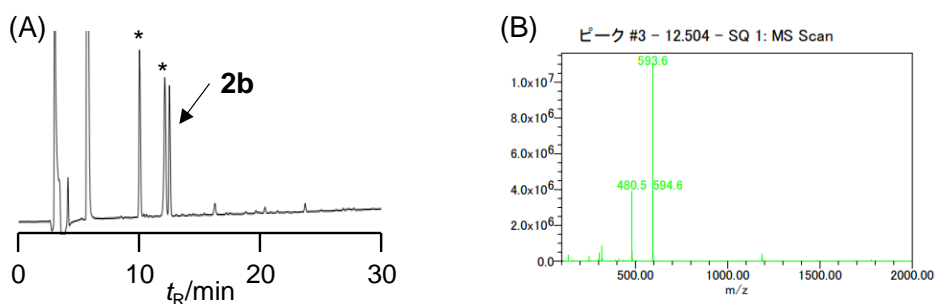
**Figure S37** UV trace of oxidation (Xaa = Trp;  $t = 30$  min). No desired product was observed.

LCMS analyses of BME-mediated conversion of hydrazides to carboxylic acids

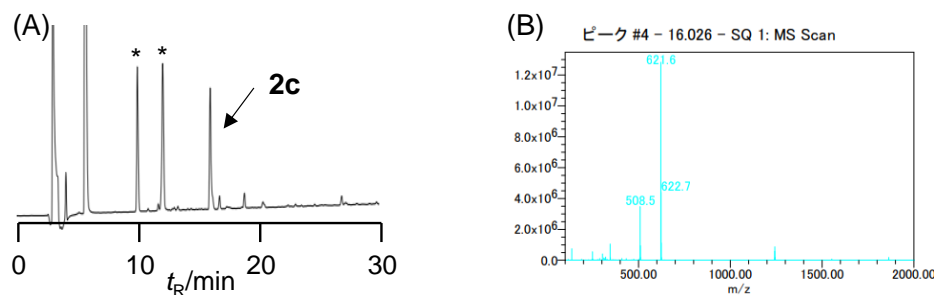
LCMS conditions for Figures S38–S54 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min, 7 to 17% for Glu and Thr. Asterisks indicate non-peptidic compounds derived from additives.



**Figure S38** (A) UV trace of BME-mediated conversion (Xaa = Gly;  $t = 30$  min). (B) MS spectrum of **2a**. **2a**: Retention time = 11.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>26</sub>H<sub>43</sub>N<sub>8</sub>O<sub>7</sub> 579.3, found 579.6.

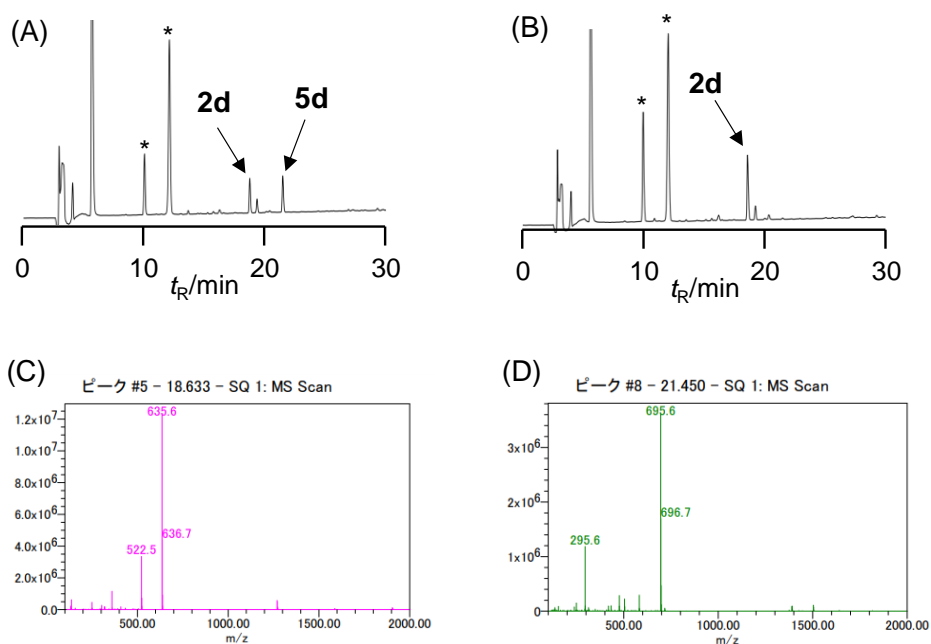


**Figure S39** (A) UV trace of BME-mediated conversion (Xaa = Ala;  $t = 30$  min). (B) MS spectrum of **2b**. **2b**: Retention time = 12.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>27</sub>H<sub>45</sub>N<sub>8</sub>O<sub>7</sub> 593.3, found 593.6.

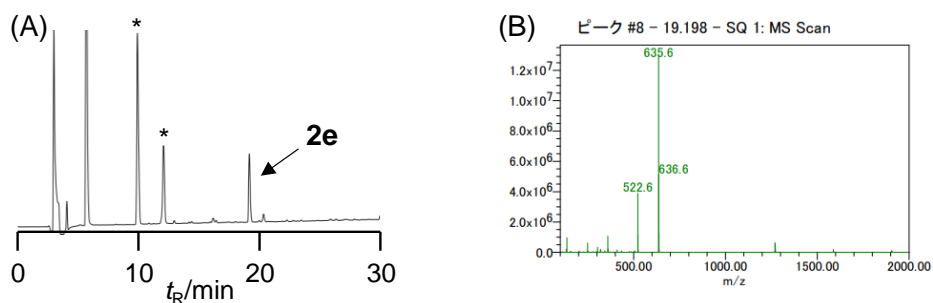


**Figure S40** (A) UV trace of BME-mediated conversion (Xaa = Val;  $t = 30$  min). (B) MS spectrum of **2c**. **2c**: Retention time = 16.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for C<sub>29</sub>H<sub>49</sub>N<sub>8</sub>O<sub>7</sub> 621.4, found 621.6.

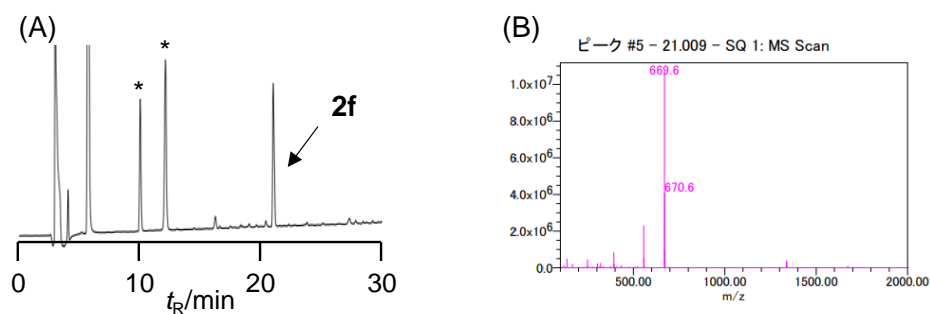




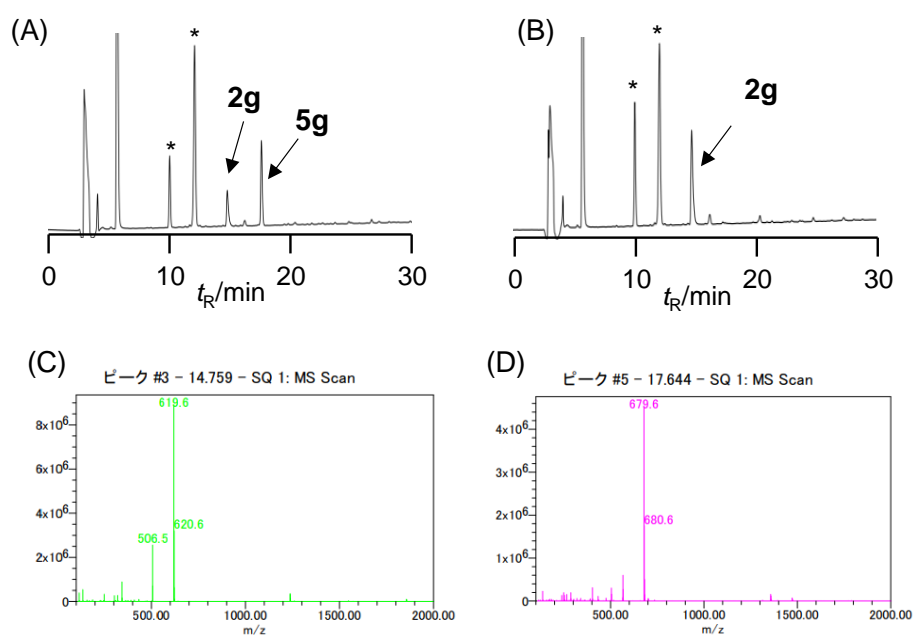
**Figure S41** (A) UV trace of BME-mediated conversion ( $X_{aa} = \text{Ile}$ ;  $t = 30$  min). (B) UV trace of BME-mediated conversion ( $t = 20$  h). (C) MS spectrum of **2d**. (D) MS spectrum of **5d**. **2d**: Retention time = 18.8 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{30}\text{H}_{51}\text{N}_8\text{O}_7$  635.4, found 635.6. **5d**: Retention time = 21.5 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{32}\text{H}_{55}\text{N}_8\text{O}_7\text{S}$  695.4, found 695.6.



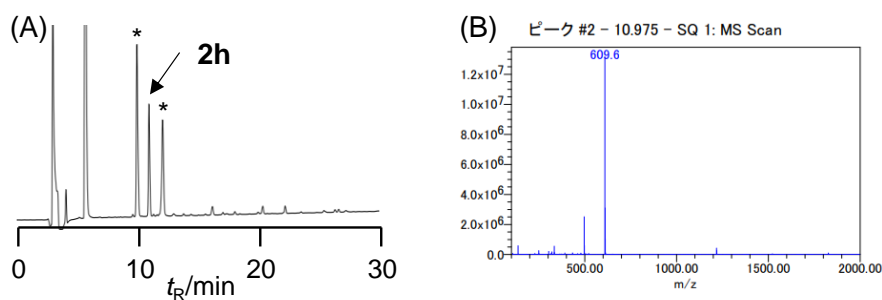
**Figure S42** (A) UV trace of BME-mediated conversion ( $X_{aa} = \text{Leu}$ ;  $t = 30$  min). (B) MS spectrum of **2e**. **2e**: Retention time = 19.2 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{30}\text{H}_{51}\text{N}_8\text{O}_7$  635.4, found 635.6.



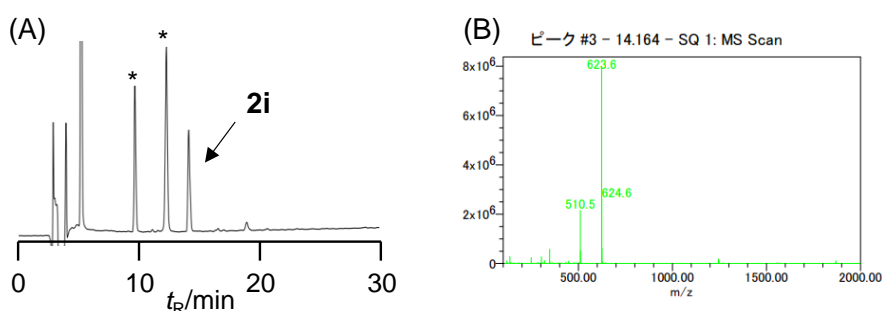
**Figure S43** (A) UV trace of BME-mediated conversion (Xaa = Phe;  $t = 30$  min). (B) MS spectrum of **2f**. **2f**: Retention time = 21.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_7$  669.4, found 669.6.



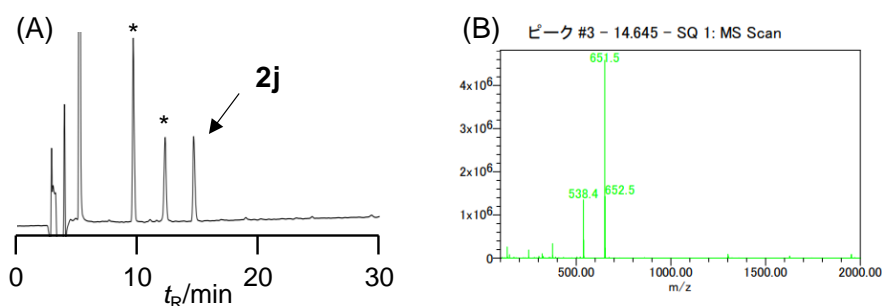
**Figure S44** (A) UV trace of BME-mediated conversion (Xaa = Pro;  $t = 30$  min). (B) UV trace of BME-mediated conversion ( $t = 20$  h). (C) MS spectrum of **2g**. (D) MS spectrum of **5g**. **2g**: Retention time = 14.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_7$  619.4, found 619.6. **5g**: Retention time = 17.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{31}H_{51}N_8O_7S$  679.4, found 679.6.



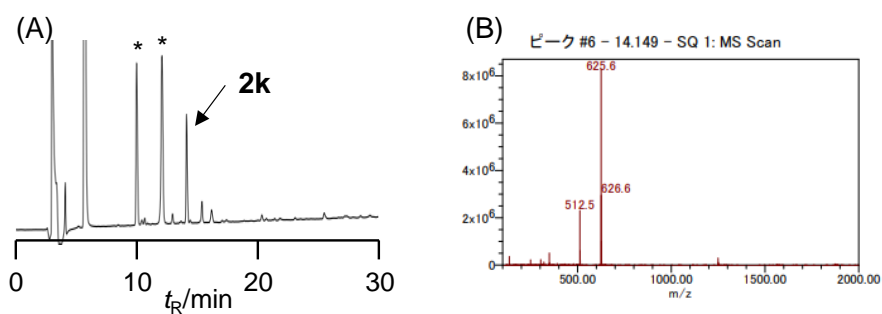
**Figure S45** (A) UV trace of BME-mediated conversion (Xaa = Ser;  $t = 30$  min). (B) MS spectrum of **2h**. **2h**: Retention time = 11.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_8$  609.3, found 609.6.



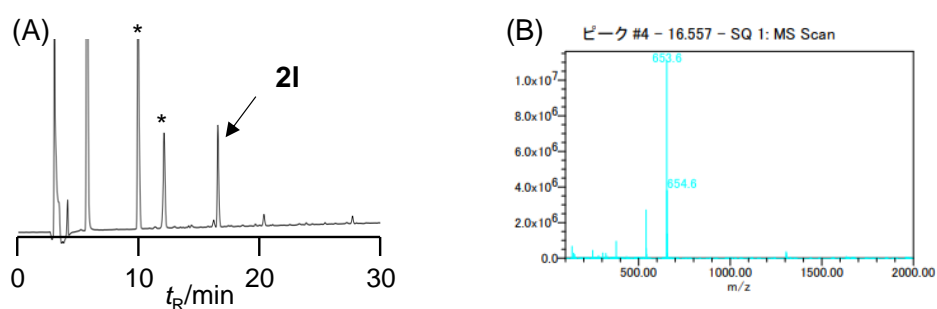
**Figure S46** (A) UV trace of BME-mediated conversion (Xaa = Thr;  $t = 30$  min). (B) MS spectrum of **2i**. **2i**: Retention time = 14.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{47}N_8O_8$  623.4, found 623.6.



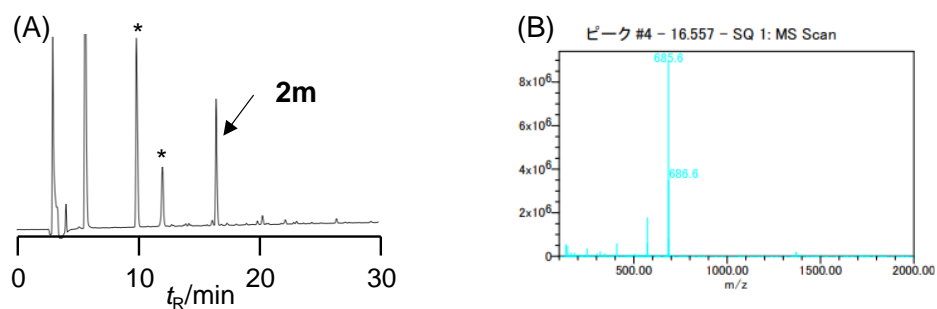
**Figure S47** (A) UV trace of BME-mediated conversion (Xaa = Glu;  $t = 30$  min). (B) MS spectrum of **2j**. **2j**: Retention time = 14.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_9$  651.3, found 651.5.



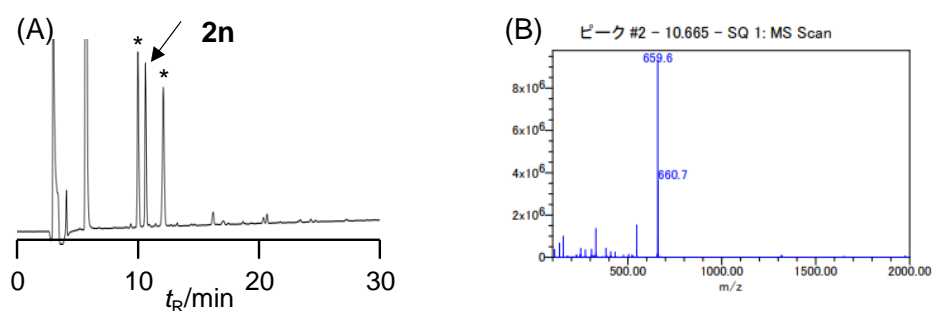
**Figure S48** (A) UV trace of BME-mediated conversion (Xaa = Cys;  $t = 30$  min). (B) MS spectrum of **2k**. **2k**: Retention time = 14.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_7S$  625.3, found 625.6.



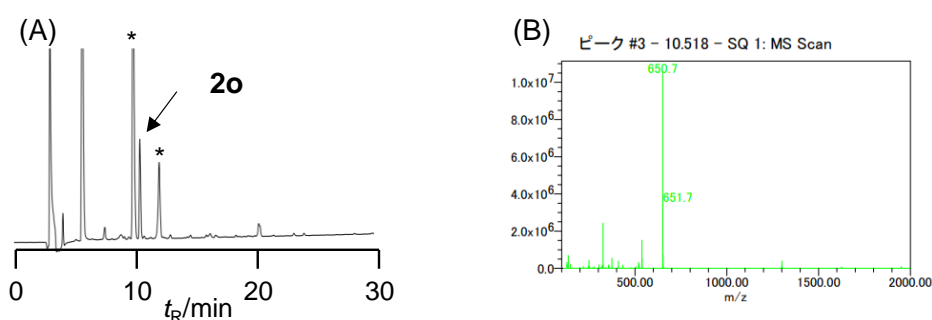
**Figure S49** (A) UV trace of BME-mediated conversion (Xaa = Met;  $t = 30$  min). (B) MS spectrum of **2l**. **2l**: Retention time = 16.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_7S$  653.3, found 653.6.



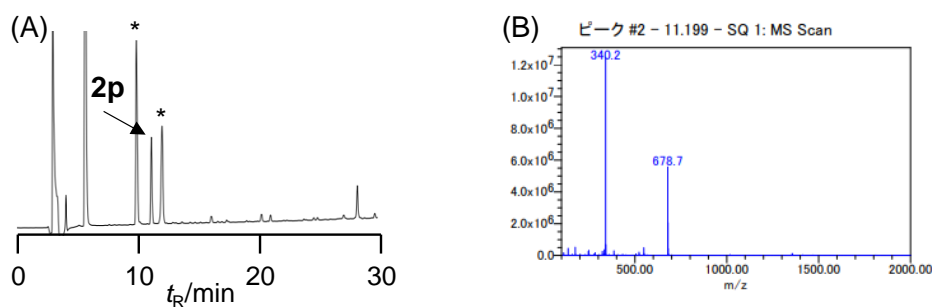
**Figure S50** (A) UV trace of BME-mediated conversion (Xaa = Tyr;  $t = 30$  min). (B) MS spectrum of **2m**. **2m**: Retention time = 16.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_8$  685.4, found 685.6.



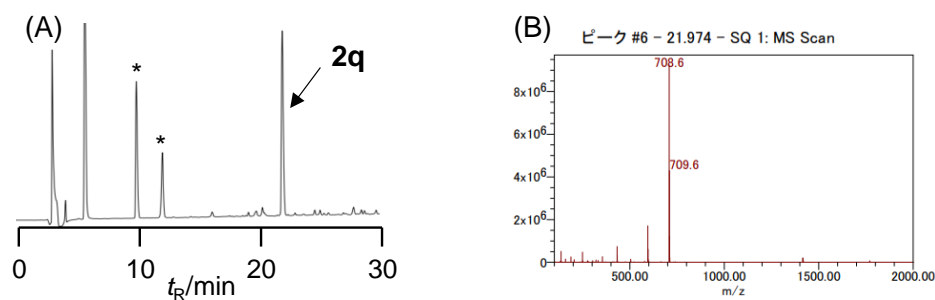
**Figure S51** (A) UV trace of BME-mediated conversion (Xaa = His;  $t = 30$  min). (B) MS spectrum of **2n**. **2n**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{47}N_{10}O_7$  659.4, found 659.6.



**Figure S52** (A) UV trace of BME-mediated conversion (Xaa = Lys;  $t = 30$  min). (B) MS spectrum of **2o**. **2o**: Retention time = 10.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_7$  650.4, found 650.7.



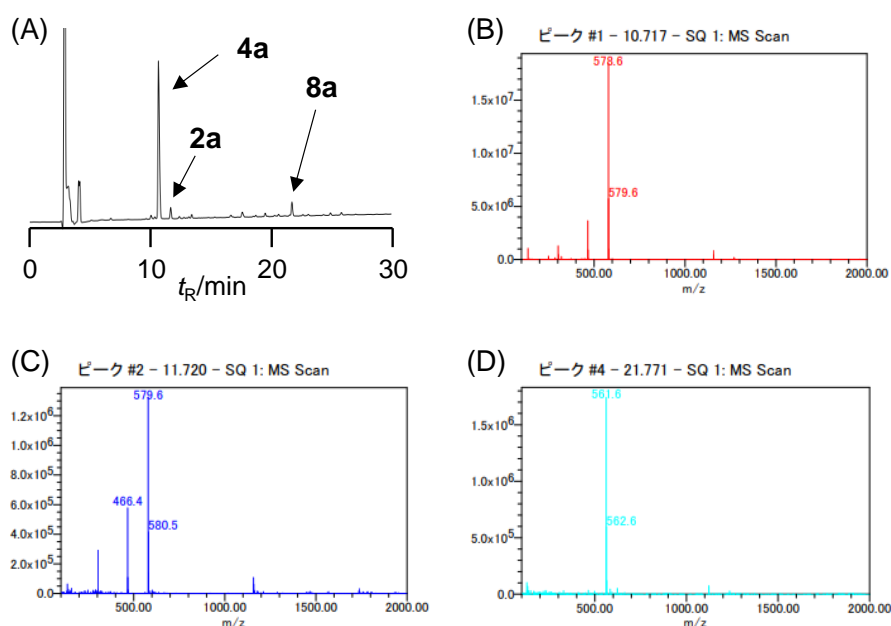
**Figure S53** (A) UV trace of BME-mediated conversion (Xaa = Arg;  $t = 30$  min). (B) MS spectrum of **2p**. **2p**: Retention time = 11.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_{11}O_7$  678.4, found 678.7.



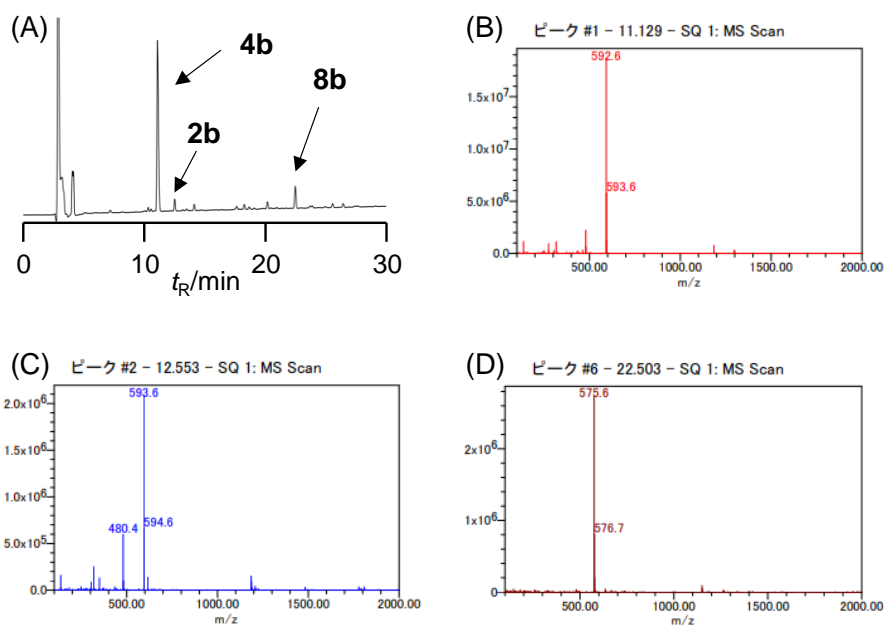
**Figure S54** (A) UV trace of BME-mediated conversion (Xaa = Trp;  $t = 30$  min). (B) MS spectrum of **2q**. **2q**: Retention time = 22.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{35}H_{50}N_9O_7$  708.4, found 708.6.

*LCMS analyses of ammonolysis of peptidyl azides derived from hydrazides*

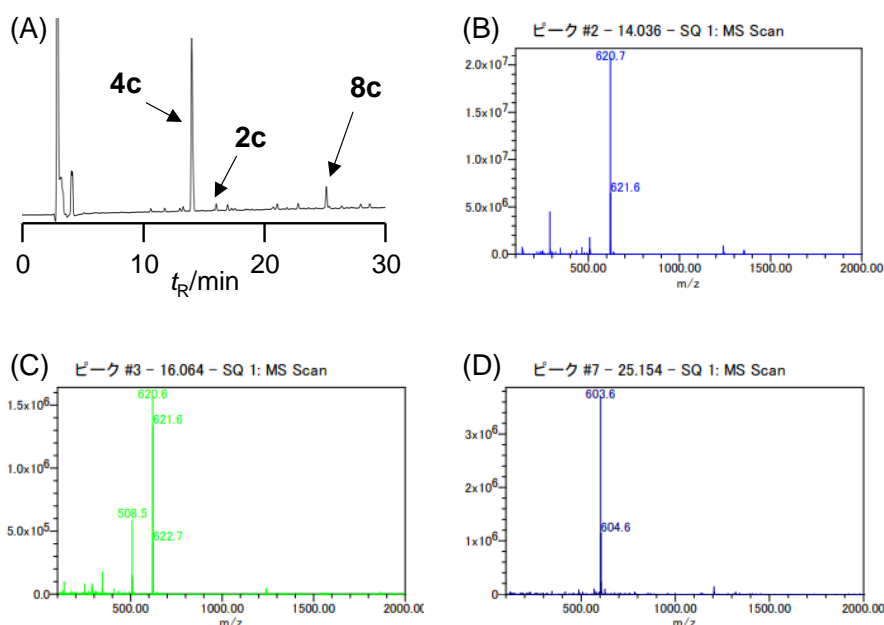
LCMS conditions for Figures S55–S72 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min.



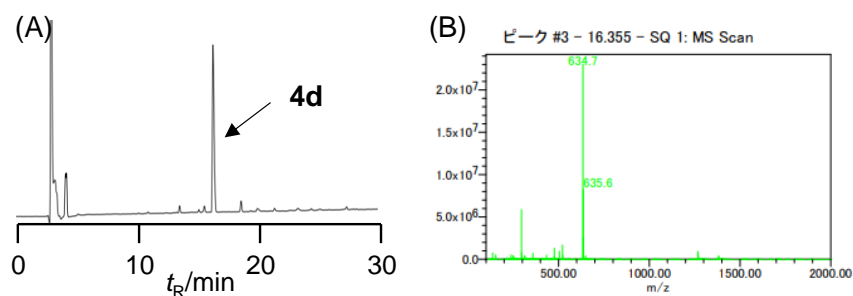
**Figure S55** (A) UV trace of amidation through ammonolysis (Xaa = Gly;  $t = 30$  min). (B) MS spectrum of **4a**. (C) MS spectrum of **2a**. (D) MS spectrum of **8a**. **4a**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{26}H_{44}N_9O_6$  578.3, found 578.6. **2a**: Retention time = 11.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{26}H_{43}N_8O_7$  579.3, found 579.6. **8a**: Retention time = 21.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{26}H_{41}N_8O_6$  561.3, found 561.6.



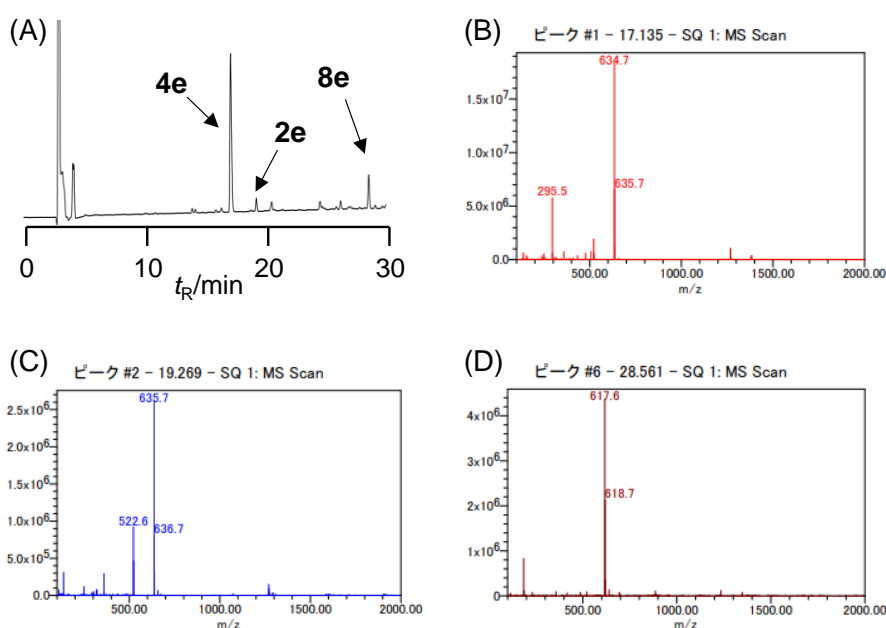
**Figure S56** (A) UV trace of amidation through ammonolysis ( $X_{aa} = \text{Ala}$ ;  $t = 30$  min). (B) MS spectrum of **4b**. (C) MS spectrum of **2b**. (D) MS spectrum of **8b**. **4b**: Retention time = 11.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6$  592.4, found 592.6. **2b**: Retention time = 12.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_7$  593.3, found 593.6. **8b**: Retention time = 22.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{43}N_8O_6$  575.3, found 575.6.



**Figure S57** (A) UV trace of amidation through ammonolysis ( $X_{aa} = \text{Val}$ ;  $t = 30$  min). (B) MS spectrum of **4c**. (C) MS spectrum of **2c**. (D) MS spectrum of **8c**. **4c**: Retention time = 14.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{50}N_9O_6$  620.4, found 620.7. **2c**: Retention time = 16.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_7$  621.4, found 621.6. **8c**: Retention time = 25.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_6$  603.4, found 603.6.

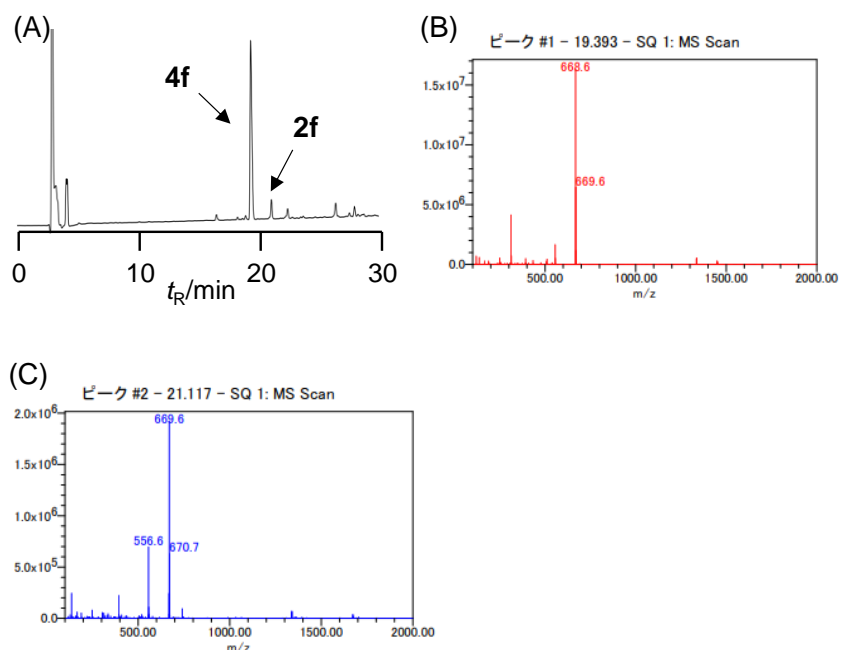


**Figure S58** (A) UV trace of amidation through ammonolysis (Xaa = Ile;  $t = 30$  min). (B) MS spectrum of **4d**. **4d**: Retention time = 16.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7.

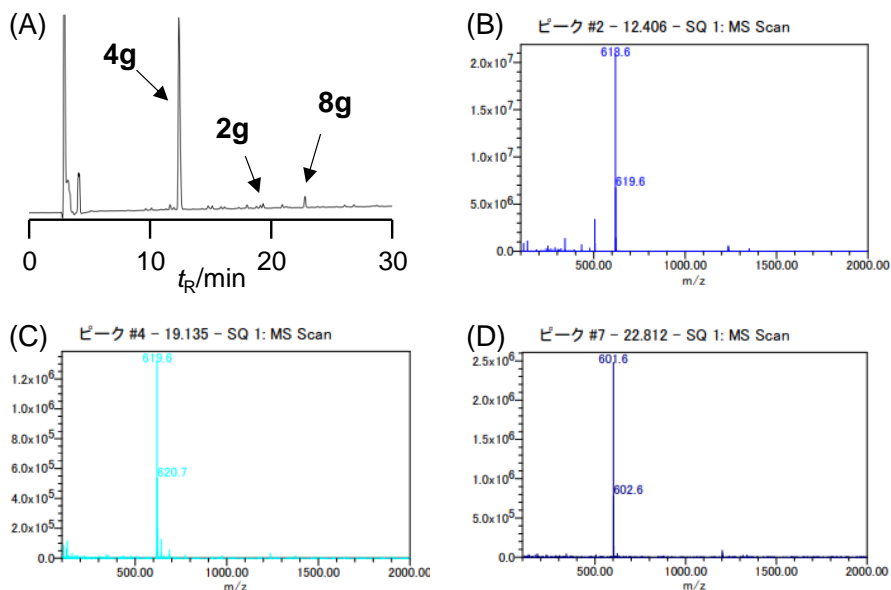


**Figure S59** (A) UV trace of amidation through ammonolysis (Xaa = Leu;  $t = 30$  min). (B) MS spectrum of **4e**. (C) MS spectrum of **2e**. (D) MS spectrum of **8e**. **4e**: Retention time = 17.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7. **2e**: Retention time = 19.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_8O_7$  635.4, found 635.7. **8e**: Retention time = 28.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{49}N_8O_6$  617.4, found 617.6.

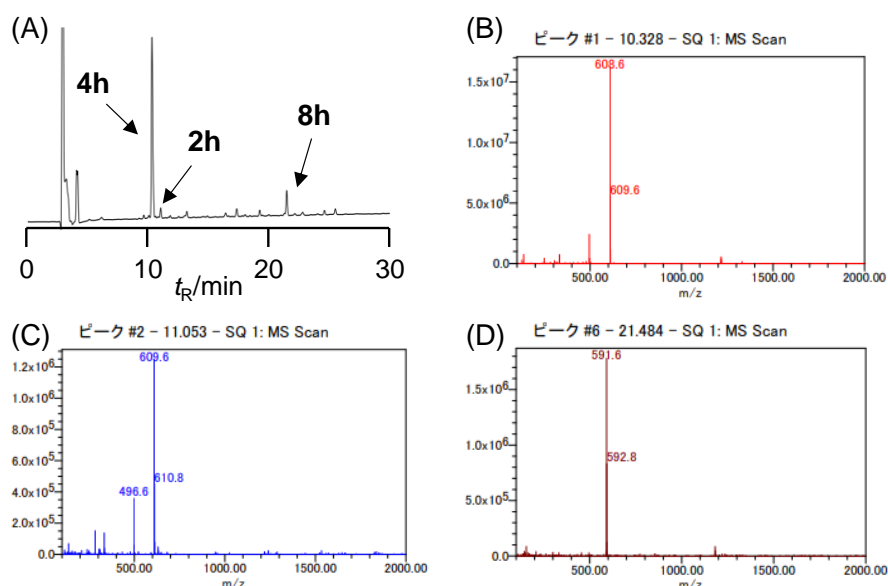




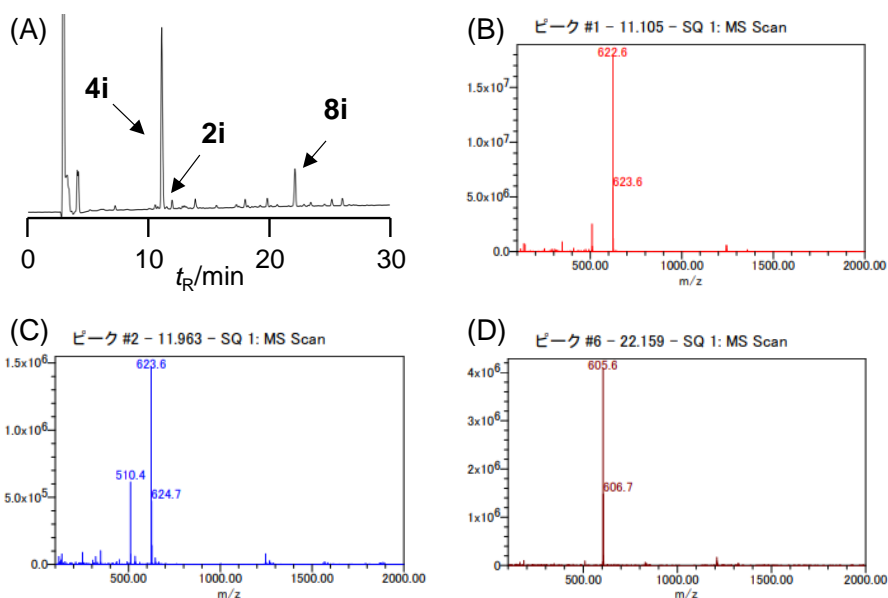
**Figure S60** (A) UV trace of amidation through ammonolysis ( $X_{aa} = \text{Phe}$ ;  $t = 30$  min). (B) MS spectrum of **4f**. (C) MS spectrum of **2f**. **4f**: Retention time = 19.4 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{33}\text{H}_{50}\text{N}_9\text{O}_6$  668.4, found 668.6. **2f**: Retention time = 21.1 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{33}\text{H}_{49}\text{N}_8\text{O}_7$  669.4, found 669.6.



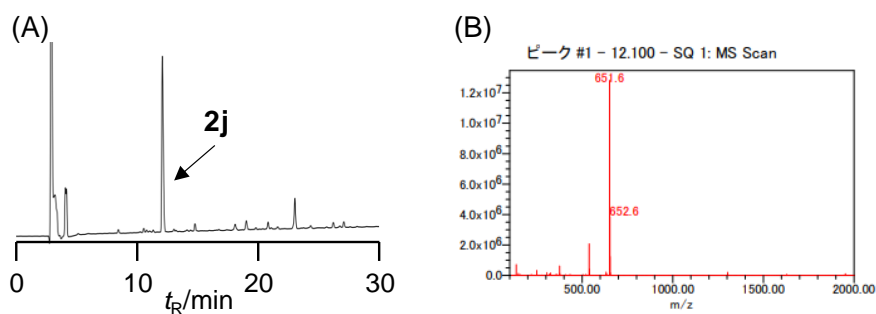
**Figure S61** (A) UV trace of amidation through ammonolysis ( $X_{aa} = \text{Pro}$ ;  $t = 30$  min). (B) MS spectrum of **4g**. (C) MS spectrum of **2g**. (D) MS spectrum of **8g**. **4g**: Retention time = 12.4 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{29}\text{H}_{48}\text{N}_9\text{O}_6$  618.4, found 618.6. **2g**: Retention time = 19.1 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{29}\text{H}_{47}\text{N}_8\text{O}_7$  619.4, found 619.6. **8g**: Retention time = 22.8 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{29}\text{H}_{45}\text{N}_8\text{O}_6$  601.3, found 601.6.



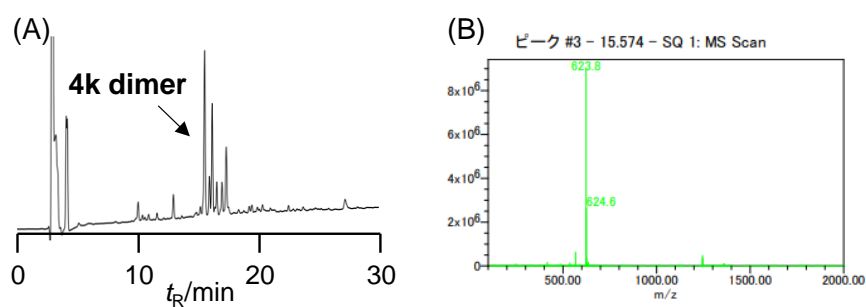
**Figure S62** (A) UV trace of amidation through ammonolysis (Xaa = Ser;  $t = 30$  min). (B) MS spectrum of **4h**. (C) MS spectrum of **2h**. (D) MS spectrum of **8h**. **4h**: Retention time = 10.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_7$  608.4, found 608.6. **2h**: Retention time = 11.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_8$  609.3, found 609.6. **8h**: Retention time = 21.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{43}N_8O_7$  591.3, found 591.6.



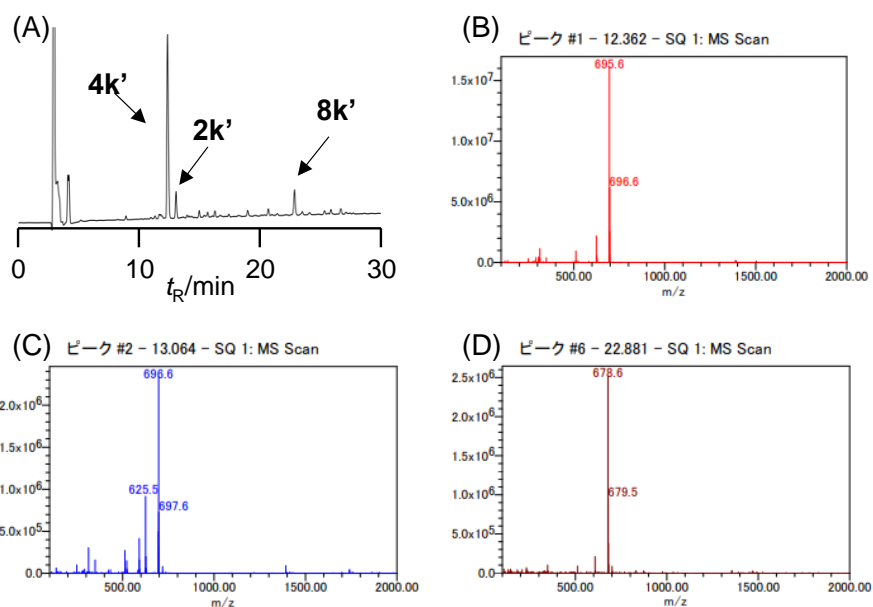
**Figure S63** (A) UV trace of amidation through ammonolysis (Xaa = Thr;  $t = 30$  min). (B) MS spectrum of **4i**. (C) MS spectrum of **2i**. (D) MS spectrum of **8i**. **4i**: Retention time = 11.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{48}N_9O_7$  622.4, found 622.6. **2i**: Retention time = 12.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{47}N_8O_8$  623.4, found 623.6. **8i**: Retention time = 22.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{45}N_8O_7$  605.3, found 605.6.



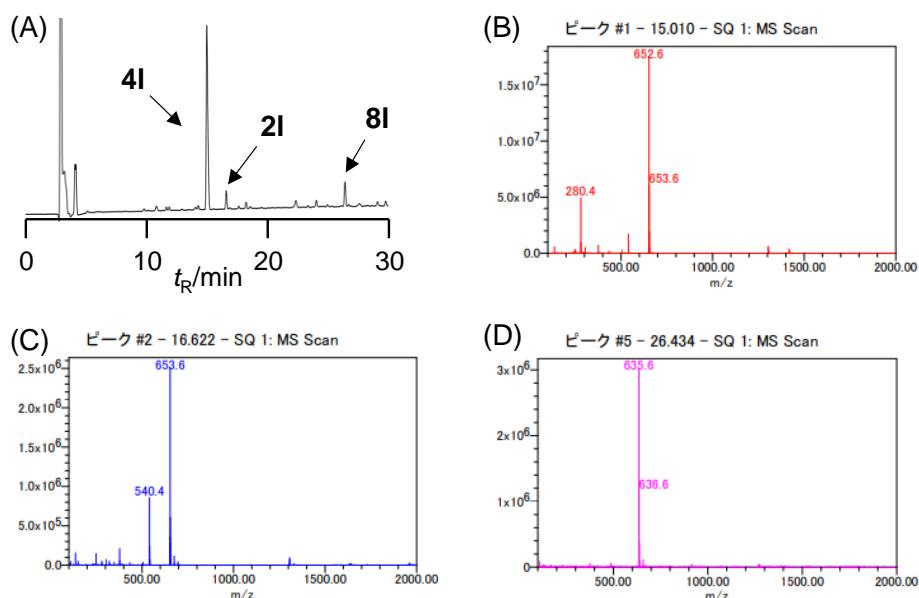
**Figure S64** (A) UV trace of amidation through ammonolysis (Xaa = Glu;  $t = 30$  min). (B) MS spectrum of **2j**. **2j**: Retention time = 12.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_9$  651.3, found 651.6.



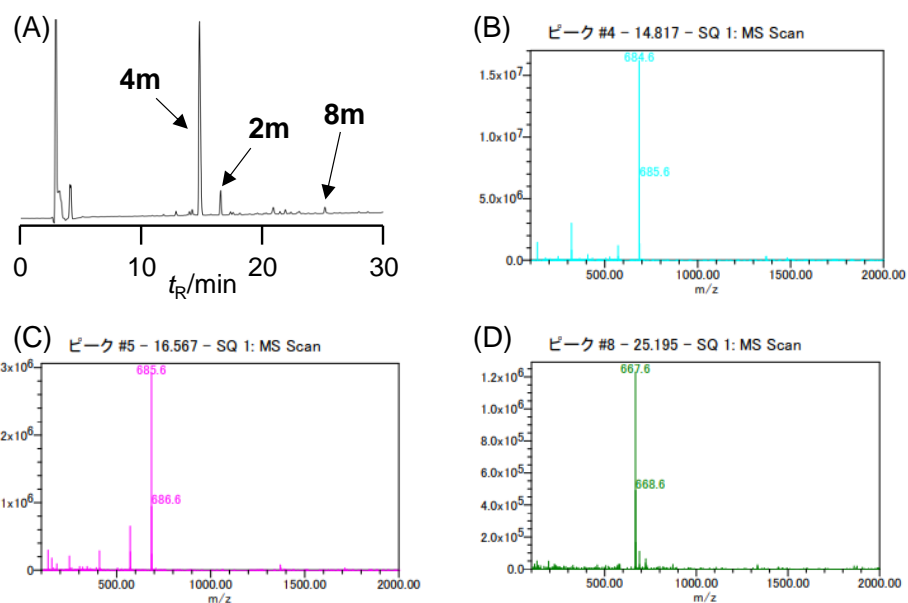
**Figure S65** (A) UV trace of amidation through ammonolysis (Xaa = Cys;  $t = 30$  min). (B) MS spectrum of **4k dimer**. **4k dimer**: Retention time = 15.6 min, MS (ESI-SQ)  $m/z$ :  $[M + 2H]^{2+}$  calcd for  $C_{54}H_{90}N_{18}O_{12}S_2$  623.3, found 623.8.



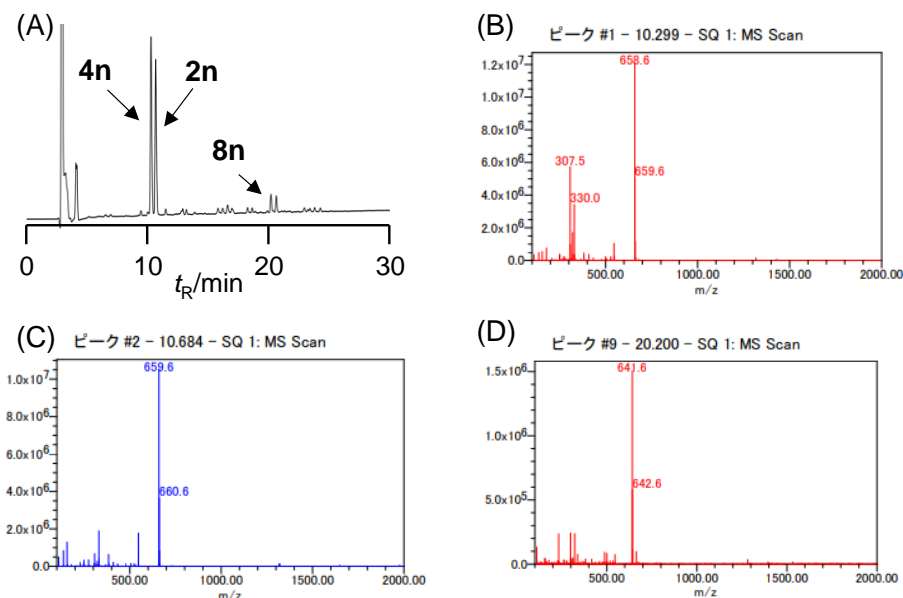
**Figure S66** (A) UV trace of amidation through ammonolysis (Xaa = Cys(Acm);  $t = 30$  min). (B) MS spectrum of **4k'**. (C) MS spectrum of **2k'**. (D) MS spectrum of **8k'**. **4k'**: Retention time = 12.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_{10}O_7S$  695.4, found 695.6. **2k'**: Retention time = 13.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{50}N_9O_8S$  696.3, found 696.6. **8k'**: Retention time = 22.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{48}N_9O_7S$  678.3, found 678.6.



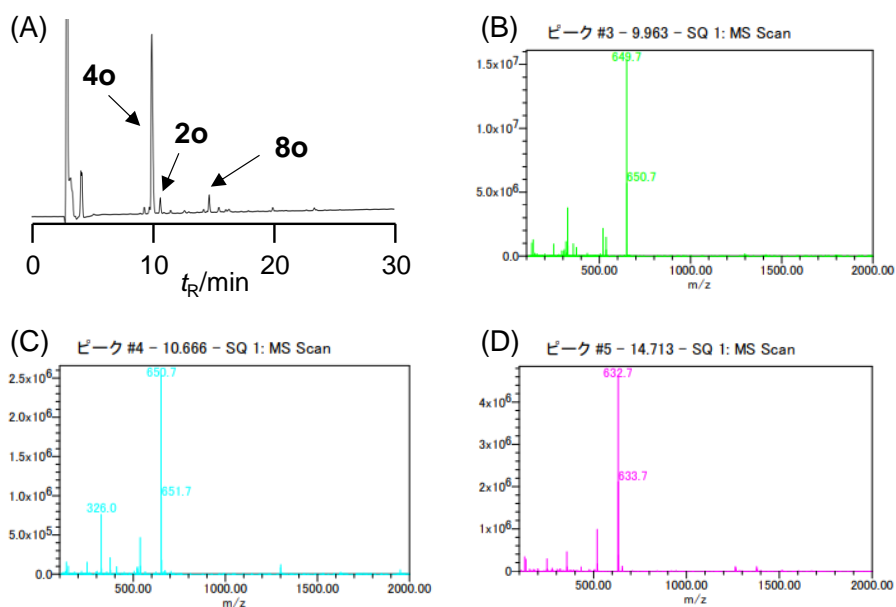
**Figure S67** (A) UV trace of amidation through ammonolysis (Xaa = Met;  $t = 30$  min). (B) MS spectrum of **4l**. (C) MS spectrum of **2l**. (D) MS spectrum of **8l**. **4l**: Retention time = 15.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{50}N_9O_6S$  652.4, found 652.6. **2l**: Retention time = 16.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_7S$  653.3, found 653.6. **8l**: Retention time = 26.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_6S$  635.3, found 635.6.



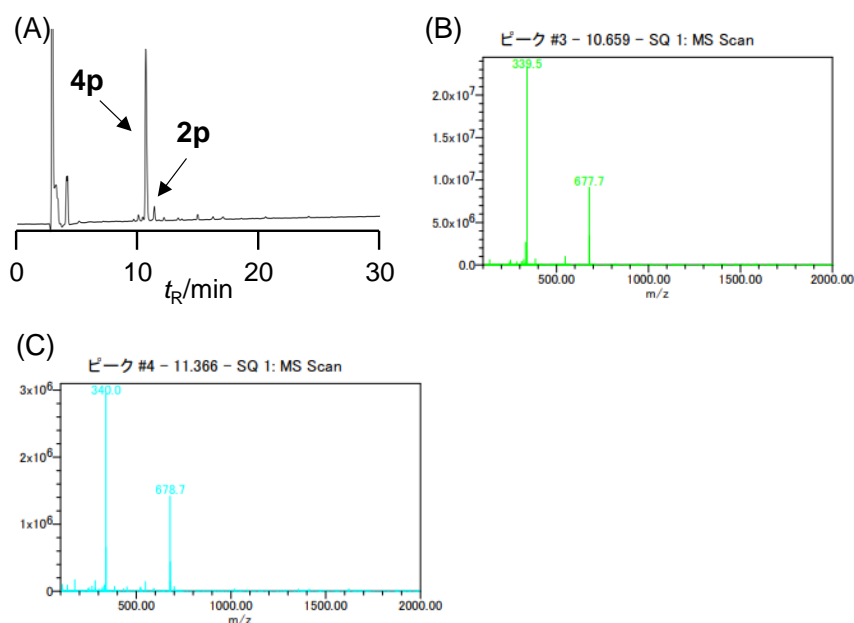
**Figure S68** (A) UV trace of amidation through ammonolysis (Xaa = Tyr;  $t = 30$  min). (B) MS spectrum of **4m**. (C) MS spectrum of **2m**. (D) MS spectrum of **8m**. **4m**: Retention time = 14.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{50}N_9O_7$  684.4, found 684.6. **2m**: Retention time = 16.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_8$  685.4, found 685.6. **8m**: Retention time = 25.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{47}N_8O_7$  667.4, found 667.6.



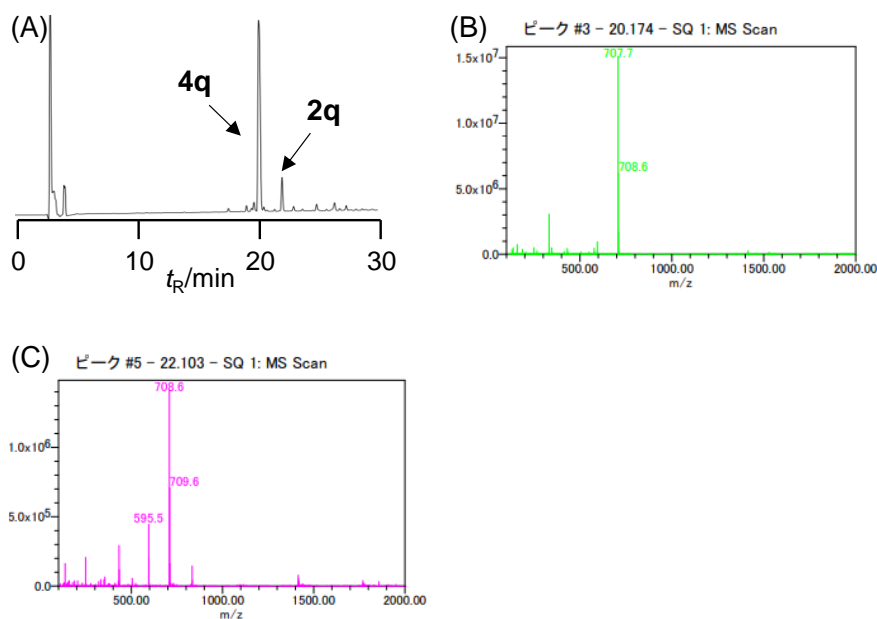
**Figure S69** (A) UV trace of amidation through ammonolysis (Xaa = His;  $t = 30$  min). (B) MS spectrum of **4n**. (C) MS spectrum of **2n**. (D) MS spectrum of **8n**. **4n**: Retention time = 10.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{48}N_{11}O_6$  658.4, found 658.6. **2n**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{47}N_{10}O_7$  659.4, found 659.6. **8n**: Retention time = 20.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{45}N_{10}O_6$  641.4, found 641.6.



**Figure S70** (A) UV trace of amidation through ammonolysis (Xaa = Lys;  $t = 30$  min). (B) MS spectrum of **4o**. (C) MS spectrum of **2o**. (D) MS spectrum of **8o**. **4o**: Retention time = 10.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{10}O_6$  649.4, found 649.7. **2o**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_7$  650.4, found 650.7. **8o**: Retention time = 14.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{50}N_9O_6$  632.4, found 632.7.

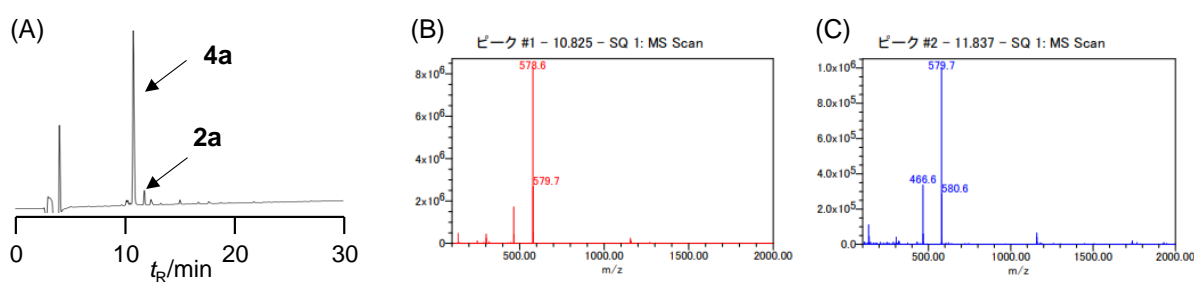


**Figure S71** (A) UV trace of amidation through ammonolysis (Xaa = Arg;  $t = 30$  min). (B) MS spectrum of **4p**. (C) MS spectrum of **2p**. **4p**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{12}O_6$  677.4, found 677.7. **2p**: Retention time = 11.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_{11}O_7$  678.4, found 678.7.

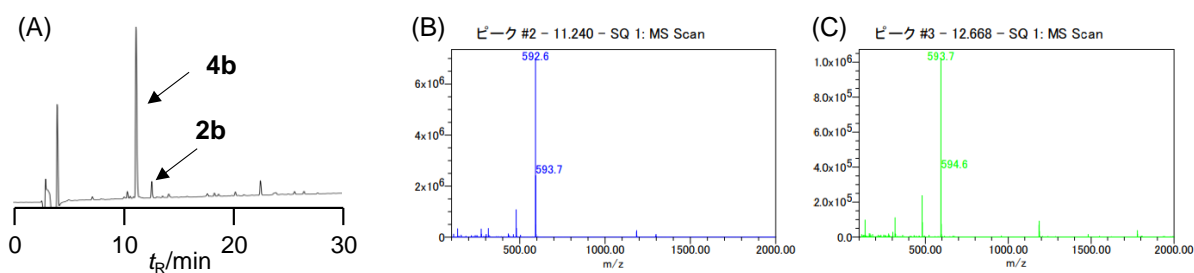


**Figure S72** (A) UV trace of amidation through ammonolysis ( $X_{aa} = \text{Trp}$ ;  $t = 30$  min). (B) MS spectrum of **4q**. (C) MS spectrum of **2q**. **4q**: Retention time = 20.2 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{35}\text{H}_{51}\text{N}_{10}\text{O}_6$  707.4, found 707.7. **2q**: Retention time = 22.1 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{35}\text{H}_{50}\text{N}_9\text{O}_7$  708.4, found 708.6.

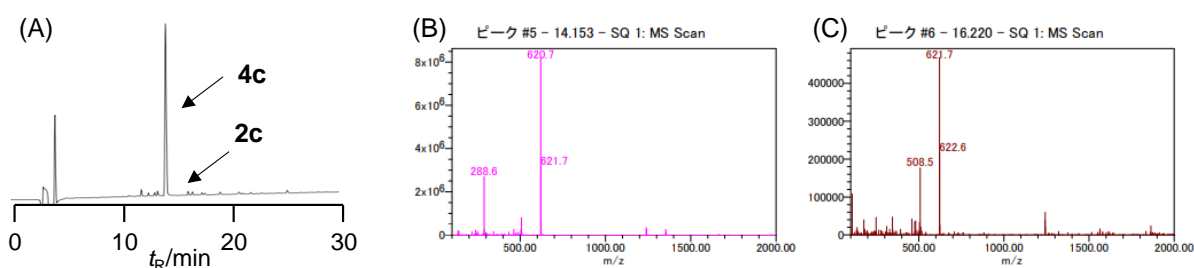
*LCMS analyses of Staudinger reaction after azidation of hydrazides in acidic aqueous solution*  
 LCMS conditions for Figures S73–S90 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min.



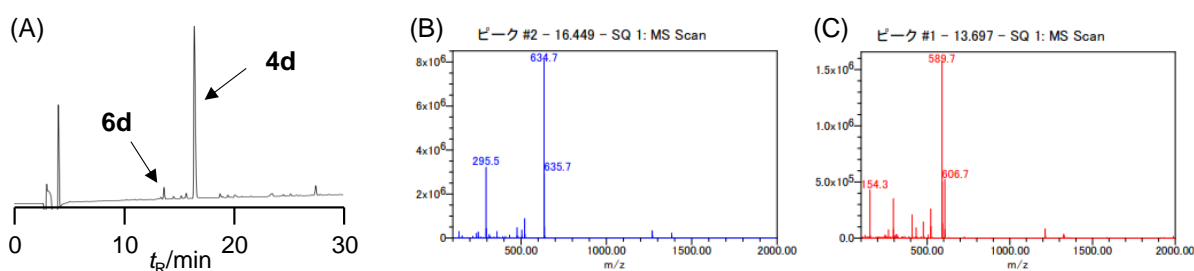
**Figure S73** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Gly}$ ;  $t = 30$  min). (B) MS spectrum of **4a**. (C) MS spectrum of **2a**. **4a**: Retention time = 10.8 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{26}\text{H}_{44}\text{N}_9\text{O}_6$  578.3, found 578.6. **2a**: Retention time = 11.8 min, MS (ESI-SQ)  $m/z$ :  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{26}\text{H}_{43}\text{N}_8\text{O}_7$  579.3, found 579.7.



**Figure S74** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Ala}$ ;  $t = 30$  min). (B) MS spectrum of **4b**. (C) MS spectrum of **2b**. **4b**: Retention time = 11.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6$  592.4, found 592.6. **2b**: Retention time = 12.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_7$  593.3, found 593.7.

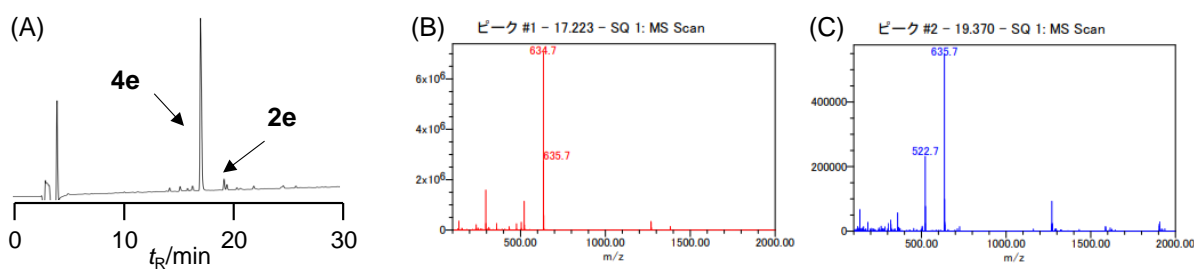


**Figure S75** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Val}$ ;  $t = 30$  min). (B) MS spectrum of **4c**. (C) MS spectrum of **2c**. **4c**: Retention time = 14.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{50}N_9O_6$  620.4, found 620.7. **2c**: Retention time = 16.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_7$  621.4, found 621.7.

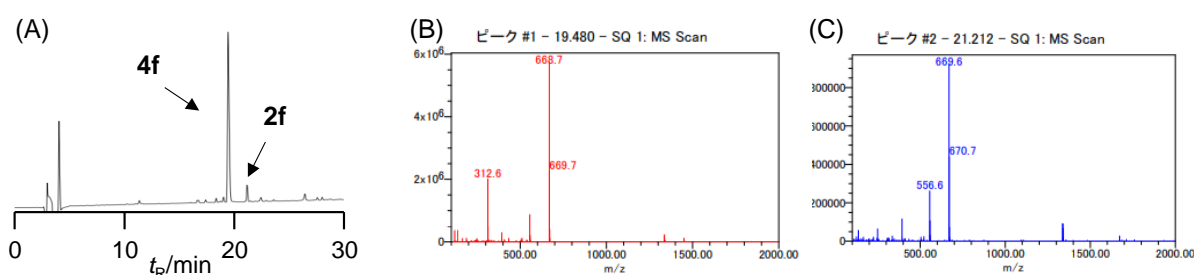


**Figure S76** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Ile}$ ;  $t = 30$  min). (B) MS spectrum of **4d**. (C) MS spectrum of **6d**. **4d**: Retention time = 16.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7. **6d**: Retention time = 13.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{52}N_9O_5$  606.4, found 606.7.

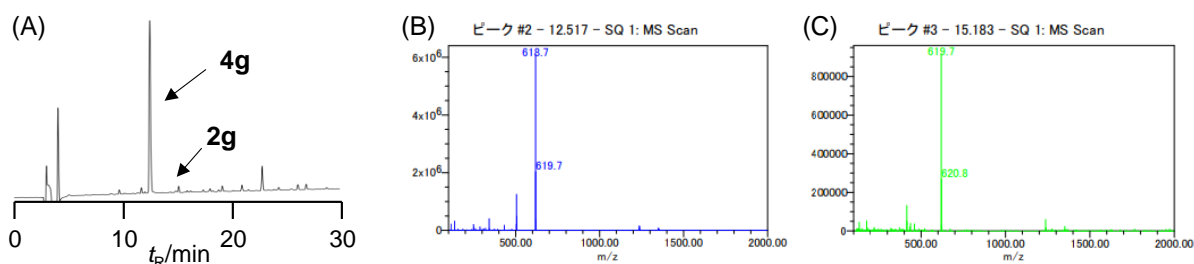




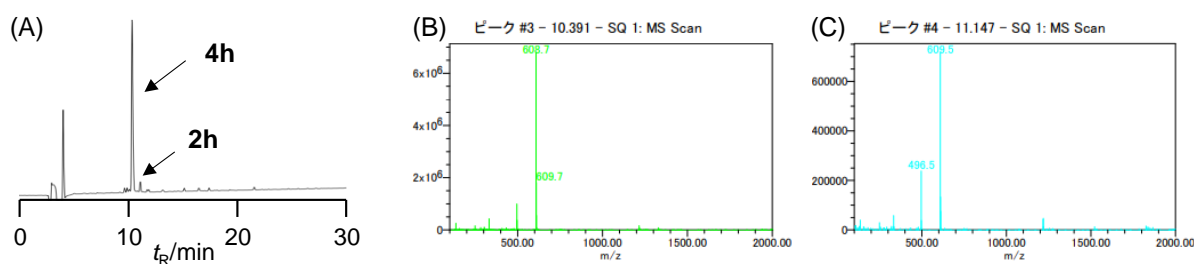
**Figure S77** (A) UV trace of amidation through Staudinger reaction (Xaa = Leu;  $t = 30$  min). (B) MS spectrum of **4e**. (C) MS spectrum of **2e**. **4e**: Retention time = 17.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7. **2e**: Retention time = 19.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_8O_7$  635.4, found 635.7.



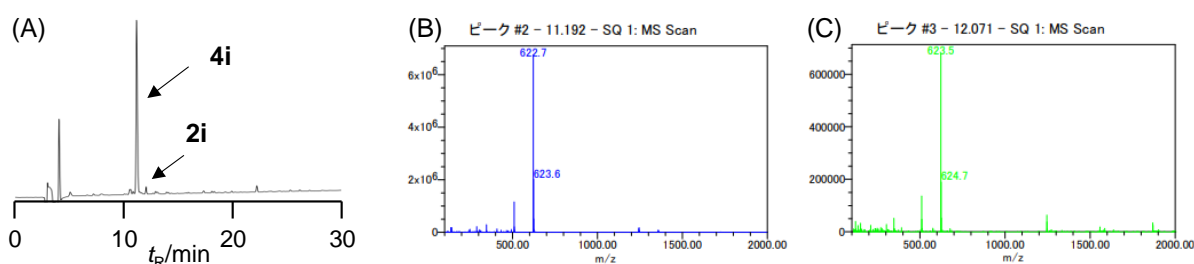
**Figure S78** (A) UV trace of amidation through Staudinger reaction (Xaa = Phe;  $t = 30$  min). (B) MS spectrum of **4f**. (C) MS spectrum of **2f**. **4f**: Retention time = 19.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{50}N_9O_6$  668.4, found 668.7. **2f**: Retention time = 21.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_7$  669.4, found 669.6.



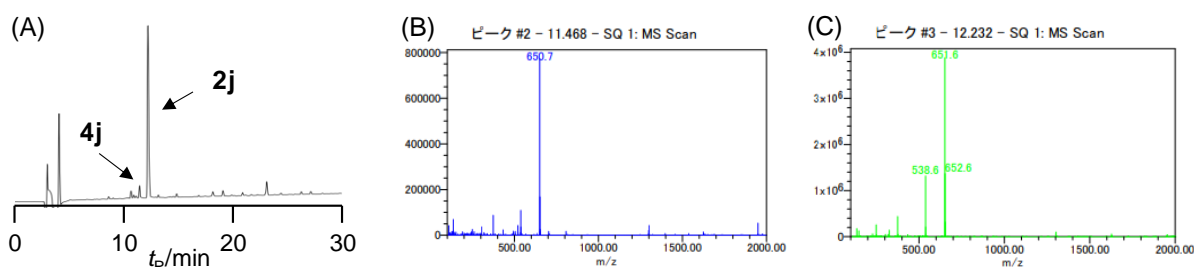
**Figure S79** (A) UV trace of amidation through Staudinger reaction (Xaa = Pro;  $t = 30$  min). (B) MS spectrum of **4g**. (C) MS spectrum of **2g**. **4g**: Retention time = 12.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{48}N_9O_6$  618.4, found 618.7. **2g**: Retention time = 15.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_7$  619.4, found 619.7.



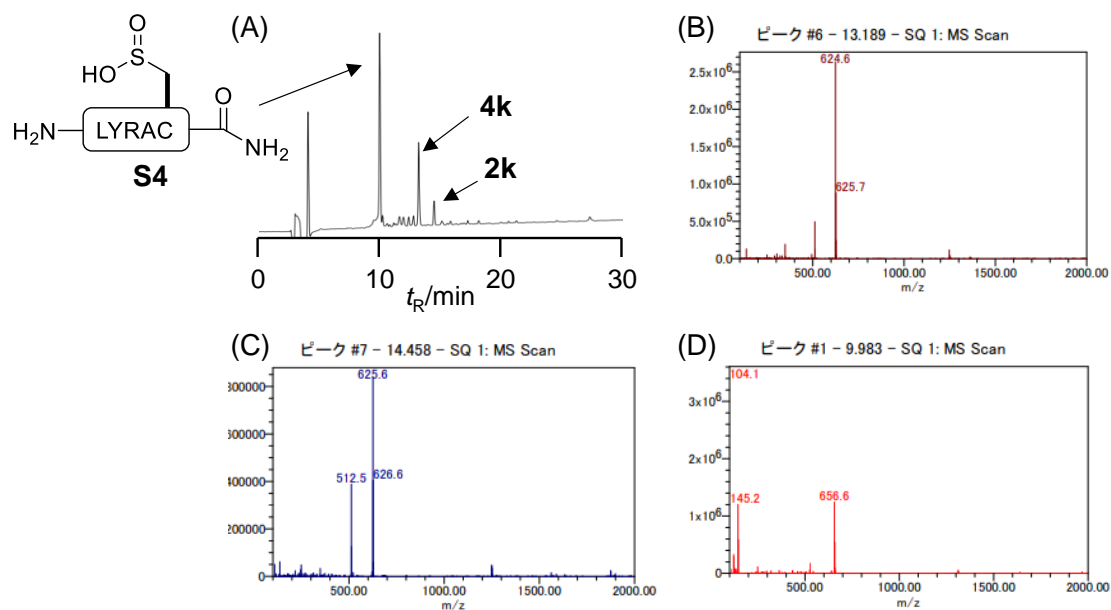
**Figure S80** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Ser}$ ;  $t = 30$  min). (B) MS spectrum of **4h**. (C) MS spectrum of **2h**. **4h**: Retention time = 10.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_7$  608.4, found 608.7. **2h**: Retention time = 11.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_8$  609.3, found 609.5.



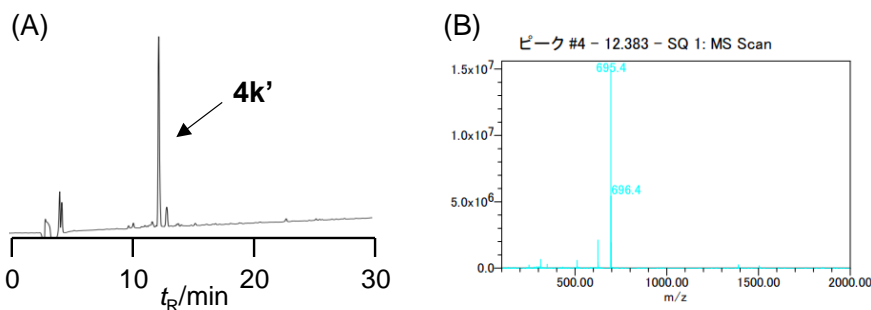
**Figure S81** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Thr}$ ;  $t = 30$  min). (B) MS spectrum of **4i**. (C) MS spectrum of **2i**. **4i**: Retention time = 11.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{48}N_9O_7$  622.4, found 622.7. **2i**: Retention time = 12.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{47}N_8O_8$  623.4, found 623.5.



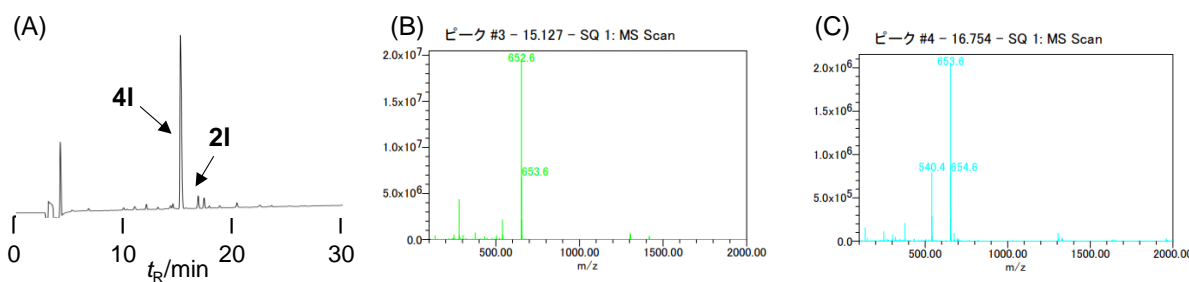
**Figure S82** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Glu}$ ;  $t = 30$  min). (B) MS spectrum of **4j**. (C) MS spectrum of **2j**. **4j**: Retention time = 11.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{48}N_9O_8$  650.4, found 650.7. **2j**: Retention time = 12.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_9$  651.3, found 651.6.



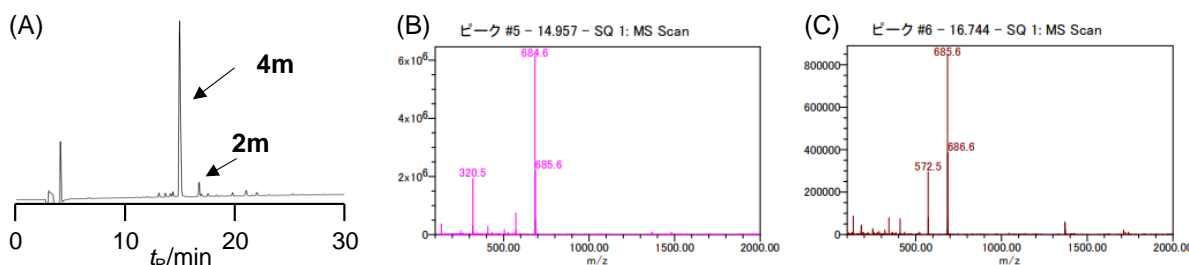
**Figure S83** (A) UV trace of amidation through Staudinger reaction (Xaa = Cys;  $t = 30$  min). (B) MS spectrum of **4k**. (C) MS spectrum of **2k**. (D) MS spectrum of **S4**. **4k**: Retention time = 13.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6S$  624.3, found 624.6. **2k**: Retention time = 14.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{45}N_8O_7S$  625.3, found 625.6. **S4**: Retention time = 10.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_8S$  656.3, found 656.6.



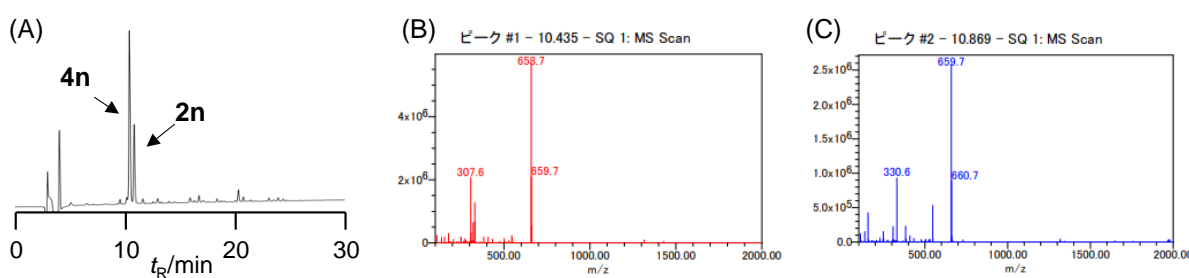
**Figure S84** (A) UV trace of amidation through Staudinger reaction (Xaa = Cys(Acm);  $t = 30$  min). (B) MS spectrum of **4k'**. **4k'**: Retention time = 12.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_{10}O_7S$  695.4, found 695.4.



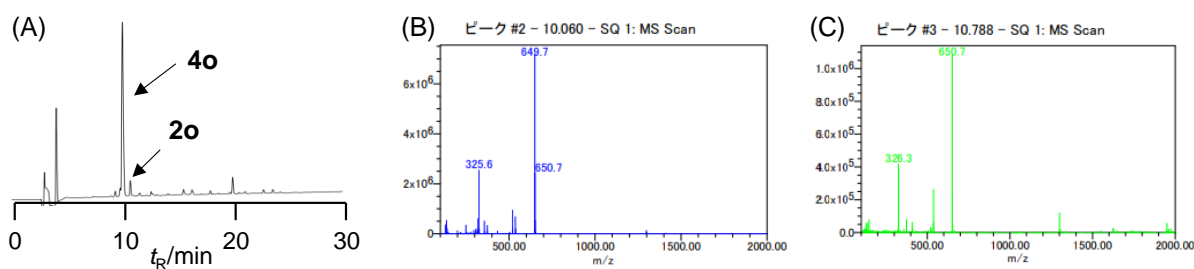
**Figure S85** (A) UV trace of amidation through Staudinger reaction (Xaa = Met;  $t = 30$  min). (B) MS spectrum of **4l**. (C) MS spectrum of **2l**. **4l**: Retention time = 15.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{50}N_9O_6S$  652.4, found 652.6. **2l**: Retention time = 16.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{49}N_8O_7S$  653.3, found 653.6.



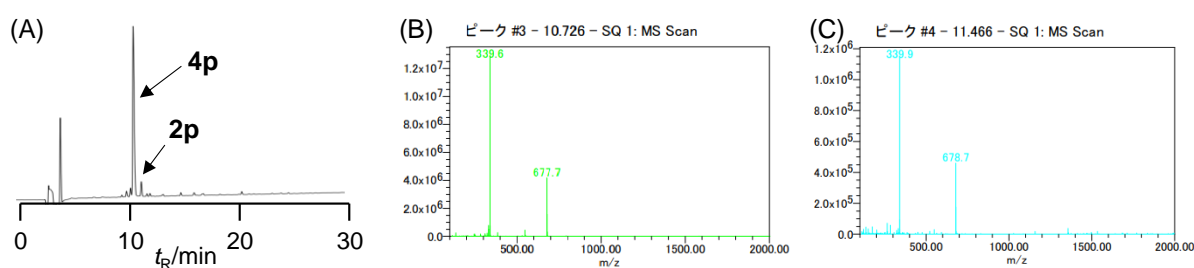
**Figure S86** (A) UV trace of amidation through Staudinger reaction (Xaa = Tyr;  $t = 30$  min). (B) MS spectrum of **4m**. (C) MS spectrum of **2m**. **4m**: Retention time = 15.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{50}N_9O_7$  684.4, found 684.6. **2m**: Retention time = 16.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{49}N_8O_8$  685.4, found 685.6.



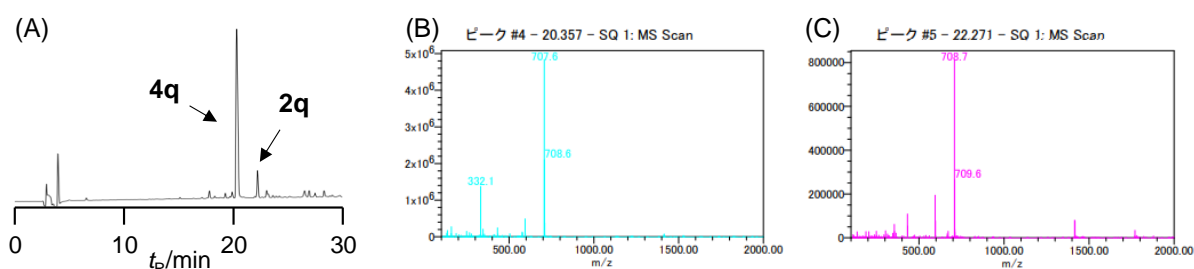
**Figure S87** (A) UV trace of amidation through Staudinger reaction (Xaa = His;  $t = 30$  min). (B) MS spectrum of **4n**. (C) MS spectrum of **2n**. **4n**: Retention time = 10.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{48}N_{11}O_6$  658.4, found 658.7. **2n**: Retention time = 10.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{47}N_{10}O_7$  659.4, found 659.7.



**Figure S88** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Lys}$ ;  $t = 30$  min). (B) MS spectrum of **4o**. (C) MS spectrum of **2o**. **4o**: Retention time = 10.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{30}\text{H}_{53}\text{N}_{10}\text{O}_6$  649.4, found 649.7. **2o**: Retention time = 10.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{30}\text{H}_{52}\text{N}_9\text{O}_7$  650.4, found 650.7.

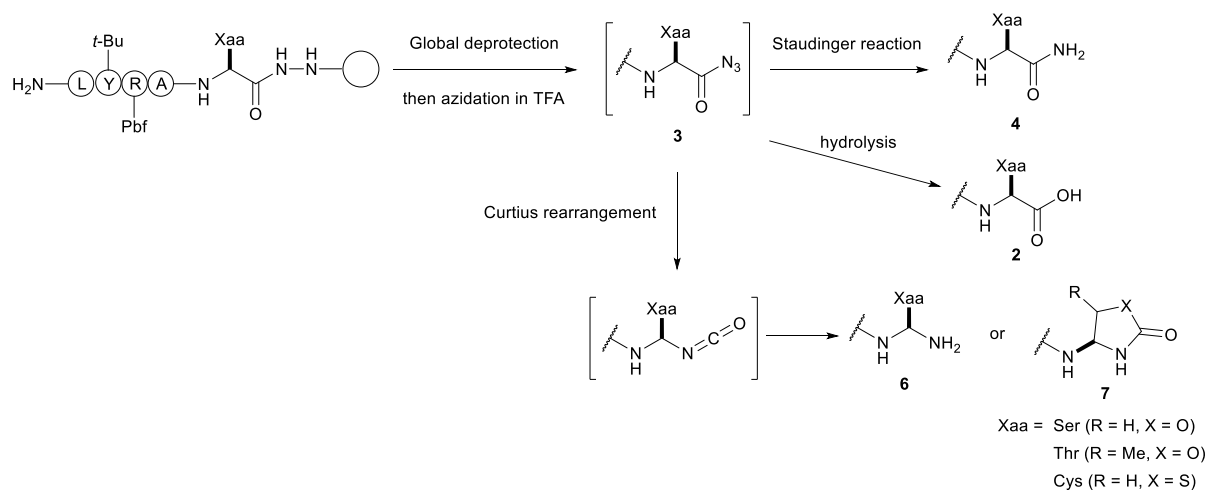


**Figure S89** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Arg}$ ;  $t = 30$  min). (B) MS spectrum of **4p**. (C) MS spectrum of **2p**. **4p**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{30}\text{H}_{53}\text{N}_{12}\text{O}_6$  677.4, found 677.7. **2p**: Retention time = 11.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{30}\text{H}_{52}\text{N}_{11}\text{O}_7$  678.4, found 678.7.



**Figure S90** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Trp}$ ;  $t = 30$  min). (B) MS spectrum of **4q**. (C) MS spectrum of **2q**. **4q**: Retention time = 20.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{35}\text{H}_{51}\text{N}_{10}\text{O}_6$  707.4, found 707.6. **2q**: Retention time = 22.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $\text{C}_{35}\text{H}_{50}\text{N}_9\text{O}_7$  708.4, found 708.7.

*LCMS analyses of Staudinger reaction after azidation of hydrazides in TFA solution*



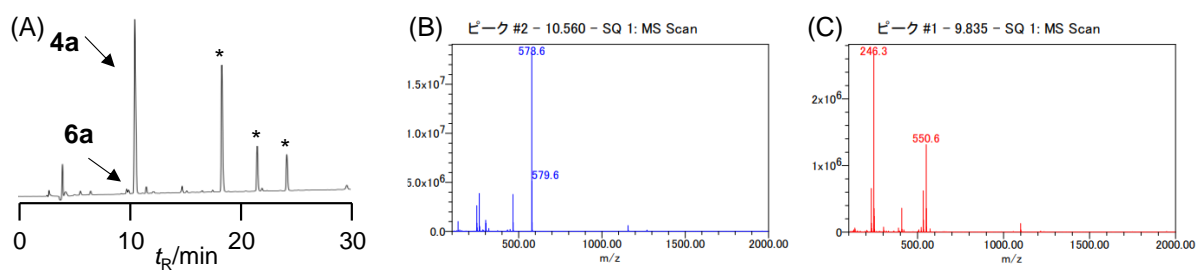
**Figure S91.** Conversion of hydrazides to amides

**Table S1.** Scope of the C-terminal amino acids in conversion to amides

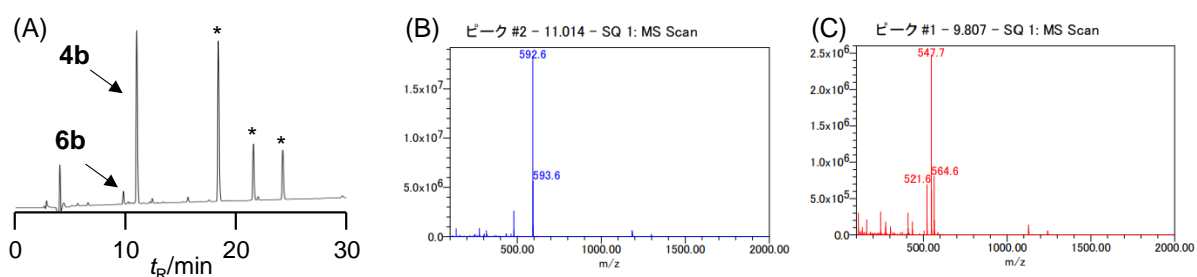
entry	Xaa	product	HPLC purity (%) <sup>a</sup>		
			4	2	6 or 7
1	Gly	<b>2a</b>	91	3	3
2	Ala	<b>2b</b>	90	2	6
3	Val	<b>2c</b>	62	<1	35
4	Ile	<b>2d</b>	53	<1	45
5	Leu	<b>2e</b>	85	<1	13
6	Phe	<b>2f</b>	84	1	11
7	Pro	<b>2g</b>	96	<1	<1
8	Ser	<b>2h</b>	87	<1	10
9	Thr	<b>2i</b>	84	<1	15
10	Glu	<b>2j</b>	74	11	<1
11	Cys	<b>2k</b>	48	<1	7
12	Cys(Acm)	<b>2k'</b>	81	<1	11
13	Met	<b>2l</b>	87	<1	9
14	Tyr	<b>2m</b>	79	<1	17
15	His	<b>2n</b>	83	<1	<1
16	Lys	<b>2o</b>	80	<1	15
17	Arg	<b>2p</b>	81	<1	15
18	Trp	<b>2q</b>	70	<1	22

a: detected at 220 nm.

LCMS conditions for Figures S92–109 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min. Asterisks indicate non-peptidic compounds derived from additives for global deprotection.

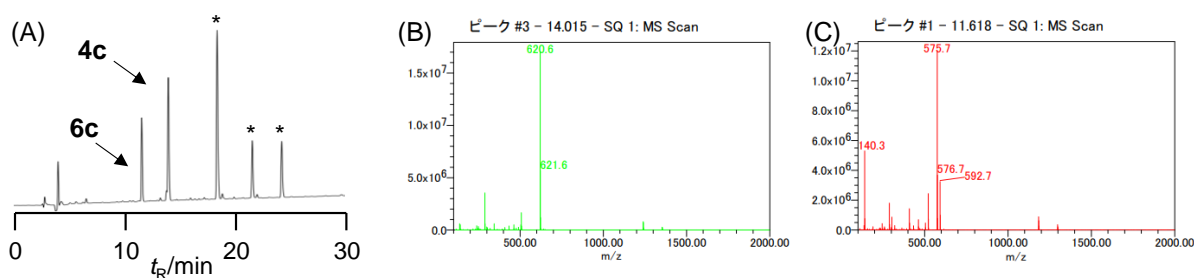


**Figure S92** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Gly}$ ;  $t = 30$  min). (B) MS spectrum of **4a**. (C) MS spectrum of **6a**. **4a**: Retention time = 10.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{26}H_{44}N_9O_6$  578.3, found 578.6. **6a**: Retention time = 9.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{25}H_{44}N_9O_5$  550.3, found 530.6.

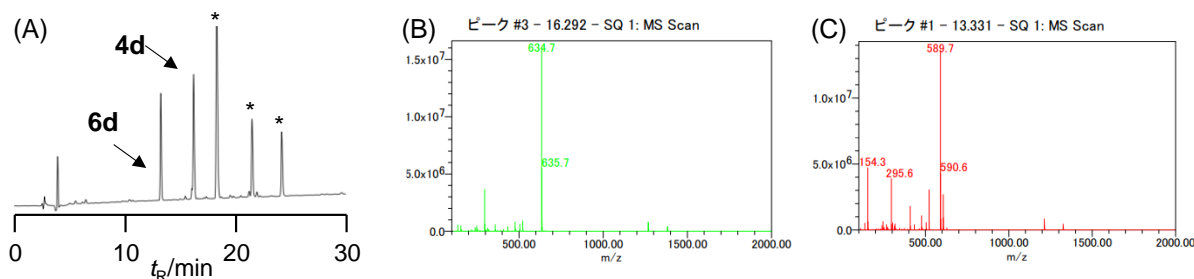


**Figure S93** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Ala}$ ;  $t = 30$  min). (B) MS spectrum of **4b**. (C) MS spectrum of **6b**. **4b**: Retention time = 11.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6$  592.4, found 592.6. **6b**: Retention time = 9.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{26}H_{46}N_6O_5$  564.4, found 564.6.

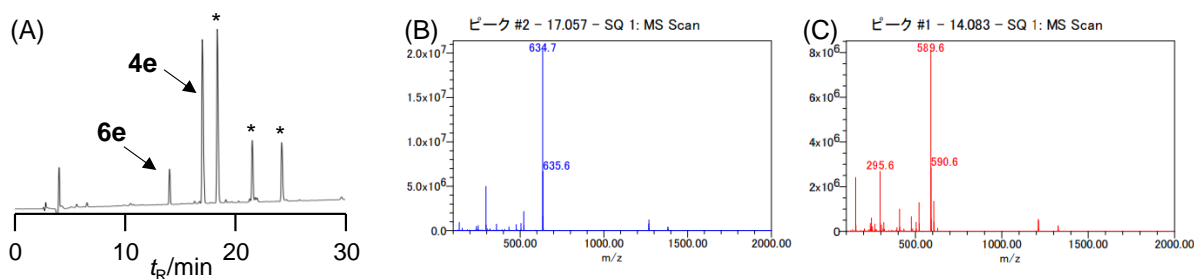




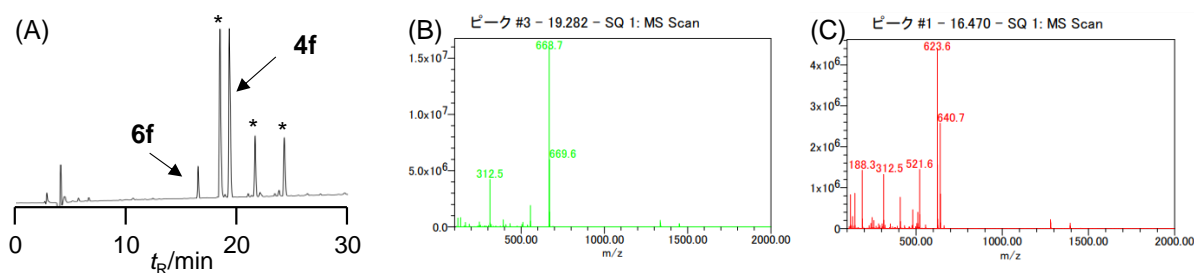
**Figure S94** (A) UV trace of amidation through Staudinger reaction (Xaa = Val;  $t = 30$  min). (B) MS spectrum of **4c**. (C) MS spectrum of **6c**. **4c**: Retention time = 14.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{50}N_9O_6$  620.4, found 620.6. **6c**: Retention time = 11.6 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{50}N_9O_5$  592.4, found 592.7.



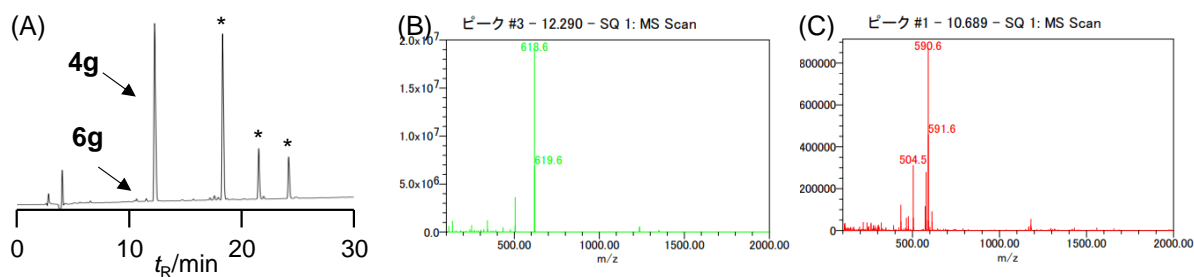
**Figure S95** (A) UV trace of amidation through Staudinger reaction (Xaa = Ile;  $t = 30$  min). (B) MS spectrum of **4d**. (C) MS spectrum of **6d**. **4d**: Retention time = 16.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7. **6d**: Retention time = 13.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{52}N_9O_5$  606.4, found 606.7.



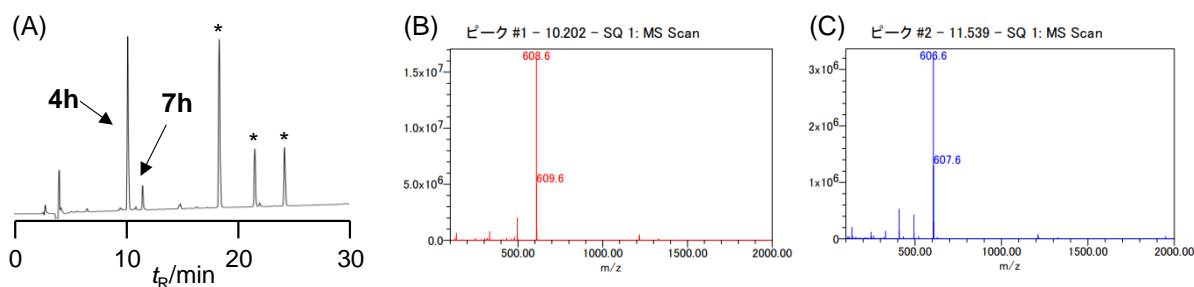
**Figure S96** (A) UV trace of amidation through Staudinger reaction (Xaa = Leu;  $t = 30$  min). (B) MS spectrum of **4e**. (C) MS spectrum of **6e**. **4e**: Retention time = 17.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{52}N_9O_6$  634.4, found 634.7. **6e**: Retention time = 14.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{52}N_9O_5$  606.4, found 606.5.



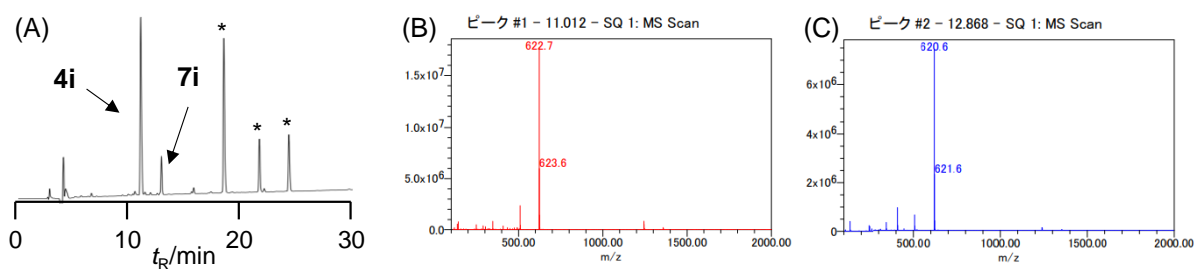
**Figure S97** (A) UV trace of amidation through Staudinger reaction (Xaa = Phe;  $t = 30$  min). (B) MS spectrum of **4f**. (C) MS spectrum of **6f**. **4f**: Retention time = 19.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{33}H_{50}N_9O_6$  668.4, found 668.7. **6f**: Retention time = 16.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{32}H_{50}N_9O_5$  640.4, found 640.7.



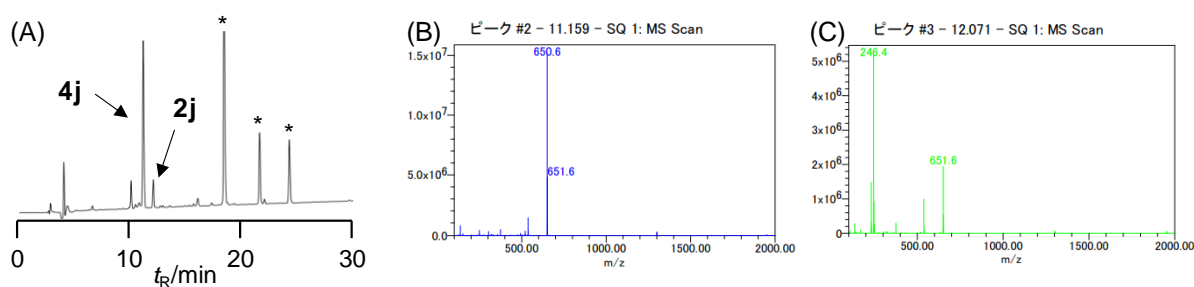
**Figure S98** (A) UV trace of amidation through Staudinger reaction (Xaa = Pro;  $t = 30$  min). (B) MS spectrum of **4g**. (C) MS spectrum of **6g**. **4g**: Retention time = 12.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{48}N_9O_6$  618.4, found 618.6. **6g**: Retention time = 10.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{48}N_9O_5$  590.4, found 590.6.



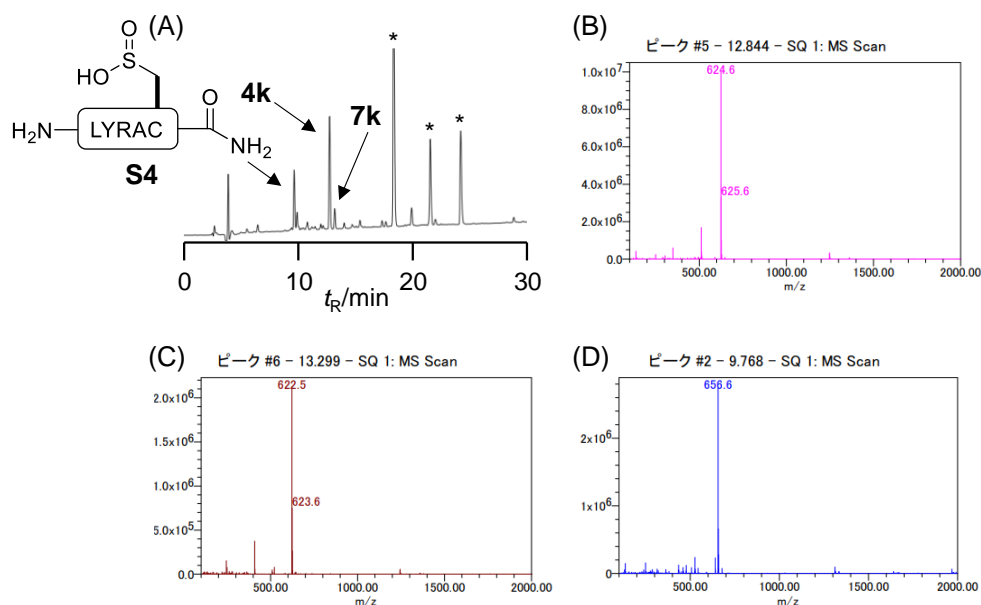
**Figure S99** (A) UV trace of amidation through Staudinger reaction (Xaa = Ser;  $t = 30$  min). (B) MS spectrum of **4h**. (C) MS spectrum of **7h**. **4h**: Retention time = 10.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_7$  608.4, found 608.6. **7h**: Retention time = 11.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{44}N_9O_7$  606.3, found 606.6.



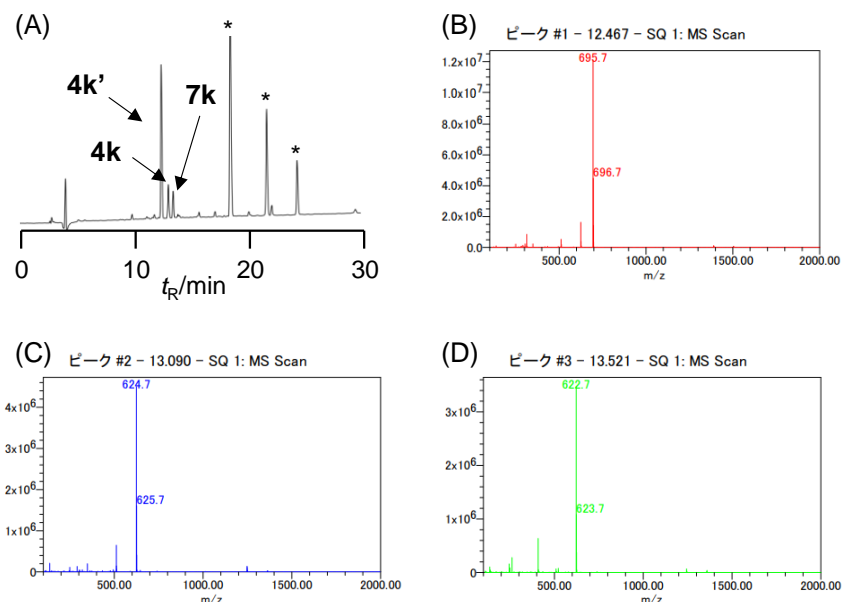
**Figure S100** (A) UV trace of amidation through Staudinger reaction (Xaa = Thr;  $t = 30$  min). (B) MS spectrum of **4i**. (C) MS spectrum of **7i**. **4i**: Retention time = 11.0 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{48}N_9O_7$  622.4, found 622.7. **7i**: Retention time = 12.9 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{28}H_{46}N_9O_7$  620.4, found 620.6.



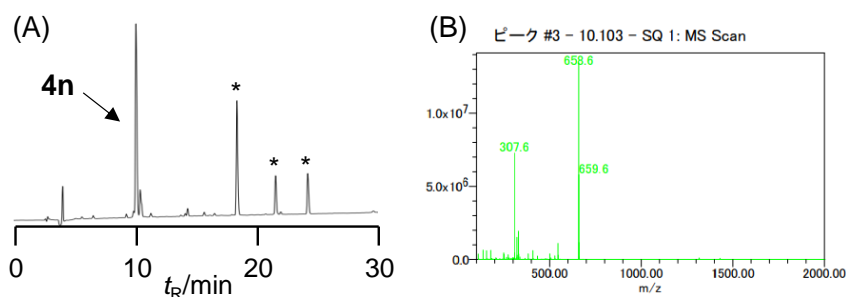
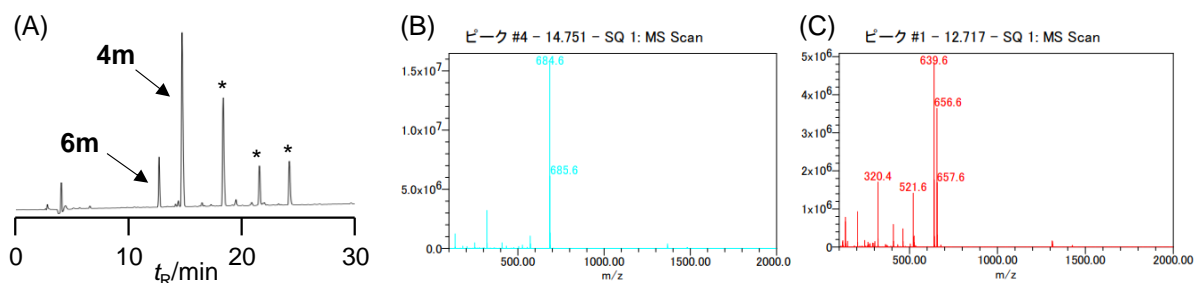
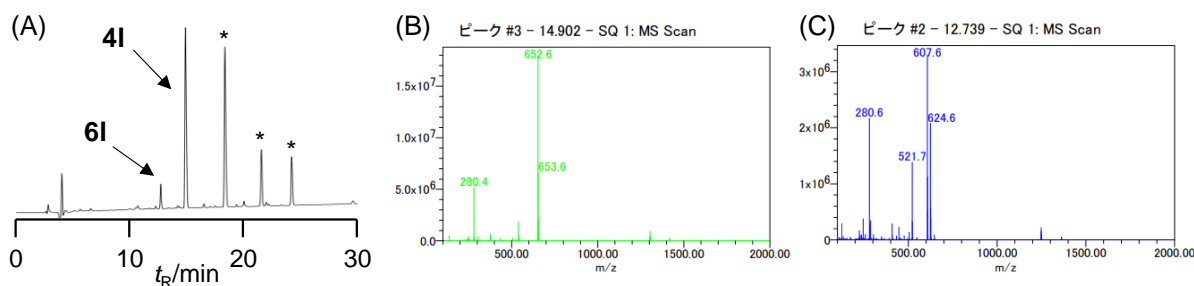
**Figure S101** (A) UV trace of amidation through Staudinger reaction (Xaa = Glu;  $t = 30$  min). (B) MS spectrum of **4j**. (C) MS spectrum of **2j**. **4j**: Retention time = 11.2 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{48}N_9O_8$  650.4, found 650.6. **2j**: Retention time = 12.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{47}N_8O_9$  651.3, found 651.6.



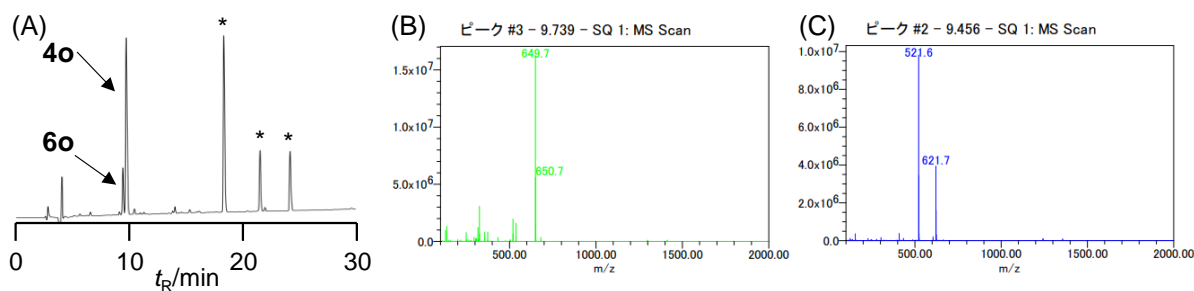
**Figure S102** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Cys}$ ;  $t = 30$  min). (B) MS spectrum of **4k**. (C) MS spectrum of **7k**. (D) MS spectrum of **S4**. **4k**: Retention time = 12.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6S$  624.3, found 624.6. **7k**: Retention time = 13.3 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{44}N_9O_6S$  622.3, found 622.5. **S4**: Retention time = 9.8 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_8S$  656.3, found 656.6.



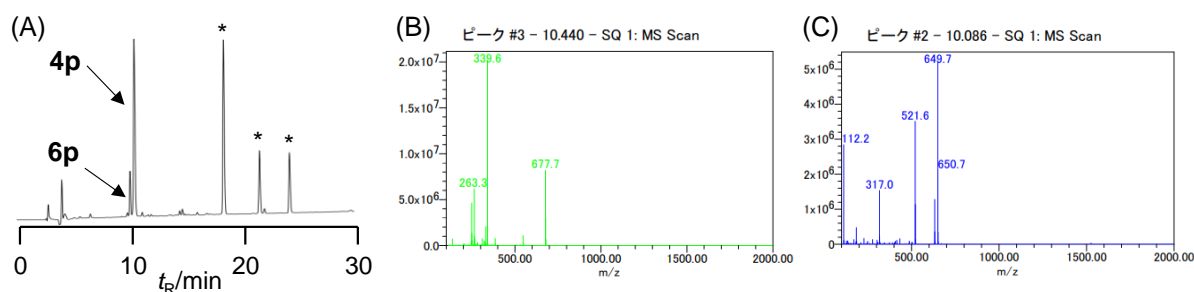
**Figure S103** (A) UV trace of amidation through Staudinger reaction ( $X_{aa} = \text{Cys(Acm)}$ ;  $t = 30$  min). (B) MS spectrum of **4k'**. (C) MS spectrum of **4k**. (D) MS spectrum of **7k**. **4k'**: Retention time = 12.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{51}N_{10}O_7S$  695.4, found 695.7. **4k**: Retention time = 13.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{46}N_9O_6S$  624.3, found 624.7. **7k**: Retention time = 13.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{27}H_{44}N_9O_6S$  622.3, found 622.7.



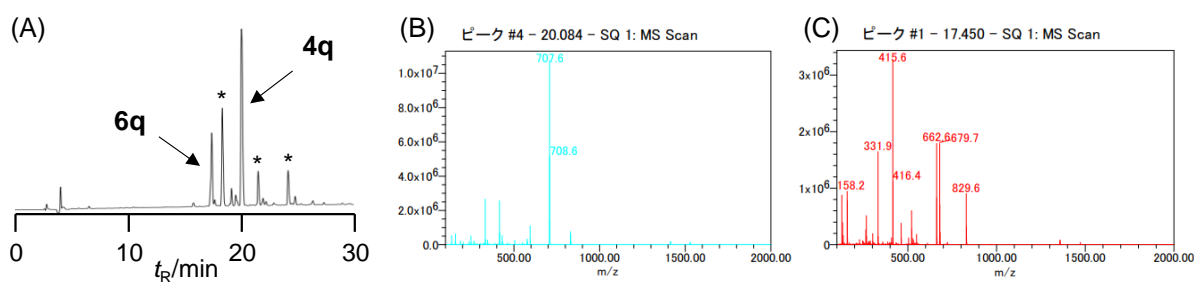
**Figure S106** (A) UV trace of amidation through Staudinger reaction (Xaa = His;  $t = 30$  min). (B) MS spectrum of **4n**. **4n**: Retention time = 10.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{48}N_{11}O_6$  658.4, found 658.6.



**Figure S107** (A) UV trace of amidation through Staudinger reaction (Xaa = Lys;  $t = 30$  min). (B) MS spectrum of **4o**. (C) MS spectrum of **6o**. **4o**: Retention time = 9.7 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{10}O_6$  649.4, found 649.7. **6o**: Retention time = 9.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{53}N_{10}O_5$  621.4, found 621.7.

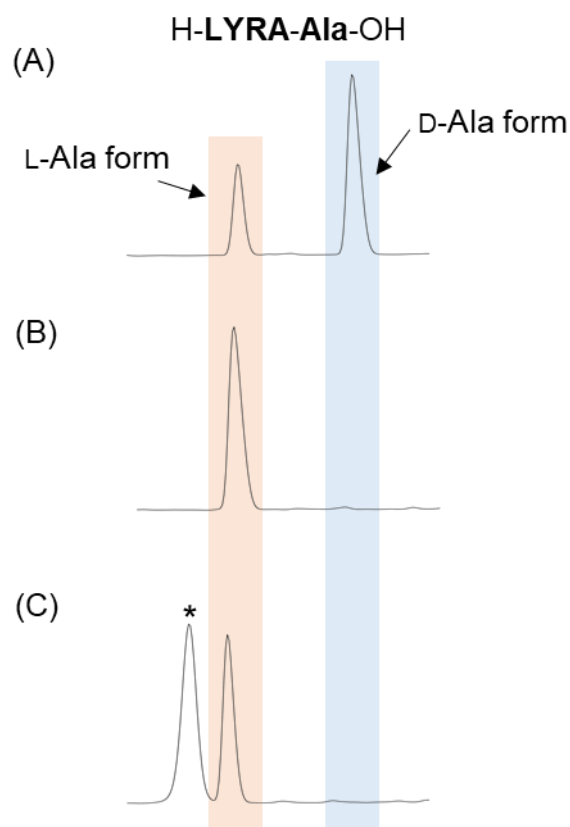


**Figure S108** (A) UV trace of amidation through Staudinger reaction (Xaa = Arg;  $t = 30$  min). (B) MS spectrum of **4p**. (C) MS spectrum of **6p**. **4p**: Retention time = 10.4 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{30}H_{53}N_{12}O_6$  677.4, found 677.7. **6p**: Retention time = 10.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{29}H_{53}N_{12}O_5$  649.4, found 649.7.

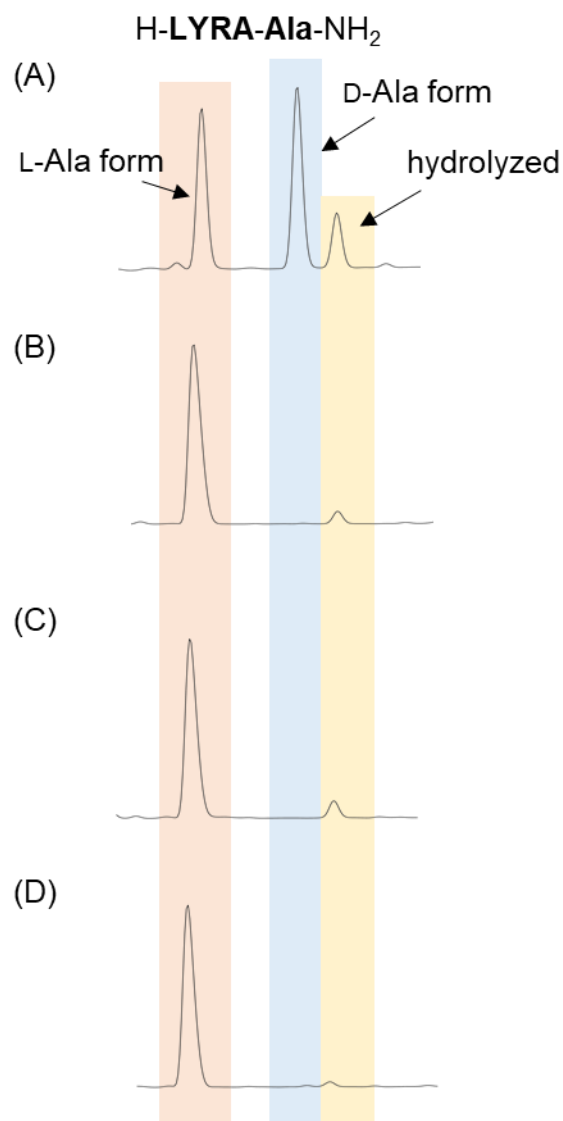


**Figure S109** (A) UV trace of amidation through Staudinger reaction (Xaa = Trp;  $t = 30$  min). (B) MS spectrum of **4q**. (C) MS spectrum of **6q**. **4q**: Retention time = 20.1 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{35}H_{51}N_{10}O_6$  707.4, found 707.6. **6q**: Retention time = 17.5 min, MS (ESI-SQ)  $m/z$ :  $[M + H]^+$  calcd for  $C_{34}H_{51}N_{10}O_5$  679.4, found 679.7.

*Evaluation of C-terminal epimerization*



**Figure S110.** UV trace of C-terminal epimerization during conversion of H-LYRA-Ala-NHNH<sub>2</sub> to carboxylic acids. (A) Co-injection of peptides containing L- or D-Ala. (B) Reaction mixture of Oxone oxidation. (C) Reaction mixture of BME-mediated hydrolysis. Only a critical retention time region of the HPLC charts was enlarged. The asterisk indicates a peak derived from BME. Analytical HPLC conditions: Cosmosil 5C<sub>18</sub>-AR-II analytical column with a linear gradient of solvent in solvent A, 5% to 35% over 30 min.

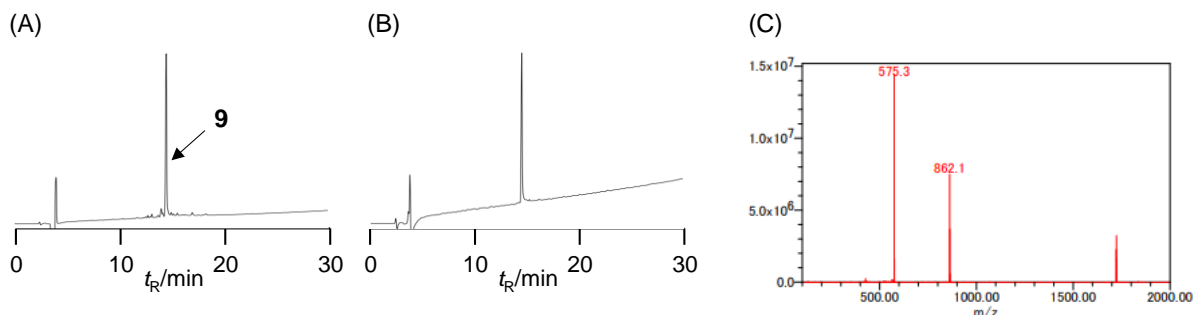


**Figure S111.** UV trace of C-terminal epimerization during conversion of H-LYRA-Ala-NH<sub>2</sub> to amide. (A) Co-injection of peptides containing L- or D-Ala. (B) Reaction mixture of ammonolysis. (C) Reaction mixture of azidation in aqueous solution followed by the Staudinger reaction. (D) Reaction mixture of azidation in TFA followed by the Staudinger reaction. Only a critical retention time region of the HPLC charts was enlarged. Analytical HPLC conditions: Cosmosil 5C<sub>18</sub>-AR-II analytical column with a linear gradient of solvent in solvent A, 5% to 35% over 30 min.

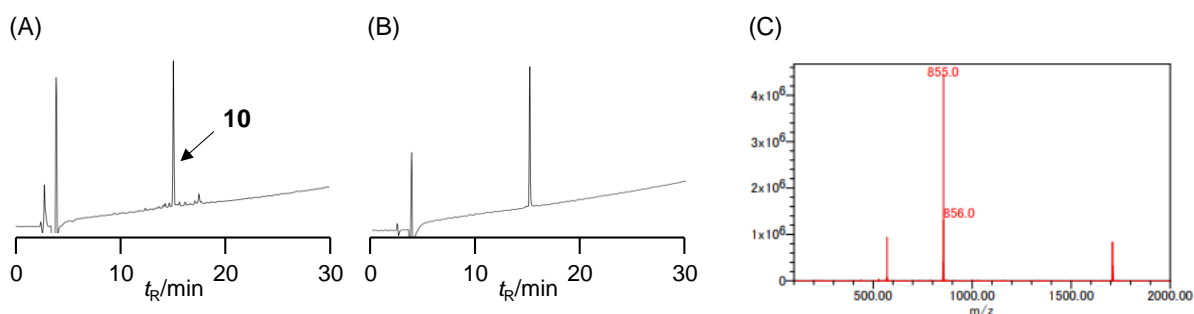


LCMS analyses of modelin-5 derivatives

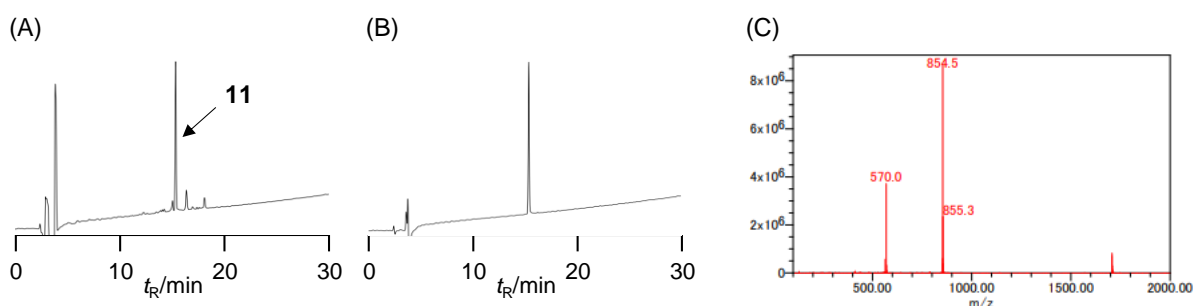
LCMS conditions for Figures S112–114 were as follows: a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min.



**Figure S112** (A) UV trace of crude material of **9**. (B) UV trace of purified **9**. (C) MS spectrum of **9**. Retention time = 14.5 min, MS (ESI-SQ)  $m/z$ :  $[M + 2H]^{2+}$  calcd for C<sub>81</sub>H<sub>158</sub>N<sub>24</sub>O<sub>16</sub> 861.6, found 862.1.



**Figure S113** (A) UV trace of crude material of **10**. (B) UV trace of purified **10**. (C) MS spectrum of **10**. Retention time = 15.1 min, MS (ESI-SQ)  $m/z$ :  $[M + 2H]^{2+}$  calcd for C<sub>81</sub>H<sub>156</sub>N<sub>22</sub>O<sub>17</sub> 854.6, found 855.0.



**Figure S114** (A) UV trace of crude material of **11**. (B) UV trace of purified **11**. (C) MS spectrum of **11**. Retention time = 15.4 min, MS (ESI-SQ)  $m/z$ :  $[M + 2H]^{2+}$  calcd for C<sub>81</sub>H<sub>157</sub>N<sub>23</sub>O<sub>16</sub> 854.1, found 854.5.