Synthetic study of peptide aldehyde via acetal/thioacetal transformation: Application for Lys/Ser-containing peptides

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Abstract-----The preparation of peptide aldehydes via efficient transformation of acetal/thioacetal structures followed by treatment of *N*-bromo succinimide is described. Peptide acetals derived from the corresponding Fmoc-amino acids and hexane-1,2,6-triol were prepared by the conventional Fmoc-SPPS. Subsequently, the peptide acetals were efficiently converted to thioacetal structures followed by treatment of *N*-bromo succinimide to give peptide aldehydes. Since alkyl and phenyl-containing peptides were applied to give reasonable results in a previous report, we attempted the synthesis of three variant peptide aldehydes in the present study. A C-terminal Phe-containing peptide aldehyde, Ac-Leu-Ala-Phe-H (**3**), to compare the reactivity of C-terminal amino acids was designed and synthesized. In this case, the reactivity was guided by steric hindrance between acetal structures and side chains of selected amino acids. In contrast, the Lys-containing peptide aldehyde, Ac-Lys-Ala-Leu-H (**4**), and the Ser-containing peptide, Ac-Ser-Phe-Leu-H (**5**), were applicable. These results suggest that this methodology has good potential.

Abbreviations: SPPS, solid phase peptide synthesis; NBS, *N*-bromo succinimide; SARS, severe acute respiratory syndrome; DMF, dimethyl formamide; DIPEA, diisopropylethylamine; DIPC, diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Fmoc, 9-fluorenylmethyloxycarbonyl; BOP, (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate; Boc, *t*-butyloxycarbonyl; HFIP, 1,1,1,3,3,3-hexafluoropropan-2-ol.

1. Introduction

Peptide aldehydes have been attractive targets for drug discovery since leupeptin, which inhibited a variety of proteases potently, was isolated from *actinomycetes* by Umezawa *et al.*¹ Peptide C-terminal aldehydes have properties as transition-state analogues toward numerous classes of cysteine proteases.^{2,3} To date, synthetic protocols of solution and/or solid phases have been reported. Reduction of Weinreb amide,^{4,5} oxidation of alcohol and ozonolysis of the corresponding olefin^{6,7,8} were disclosed by the Martinez group and Hall *et al.*, independently. On the other hand, oxazoline,⁹ threonine-type,^{10,11,12} and semicarbazone¹³ linkers were developed for the attachment of aldehydes. Particularly, an acetal linker attached peptide C-terminal aldehyde with alkyl-1,2-diol is stable connector between peptide aldehyde and resin. However, cleavage reaction of the peptide aldehydes from the linkers frequently caused serious problems. Yao *et al.* reported synthesis of peptide aldehydes contained with the aliphatic amino acid residues using acetal linkers.¹⁴ Barany developed a synthetic protocol for peptide aldehydes which was protected as the acetal and anchored by a backbone amide linkage.¹⁵ Designed peptide aldehydes reported by Barany were also constructed with amino acid residues with aliphatic and carboxylic acid side chains. Therefore, these methods should be applicable to aldehydes with other amino acids. In contrast, we reported preparation of peptide aldehydes using a very simple and cheap alkyl triol linker to attach Fmoc-aminals (**A**) for SPPS. We found that the peptide acetals (**B**) were efficiently converted to thioacetal structures (**C**) followed by treatment of NBS to give peptide aldehydes (**D**). In this case, alkyl and phenyl-containing peptides were applied to give reasonable results. This method has an advantage following: (i) commercially available linker; (ii) stable and useful for the SPPS; (iii) transform the aldehyde without strong acid conditions (Scheme 1).¹⁶

Scheme 1. Synthetic protocol for peptide aldehyde

In addition, designed His/Thr-containing peptides were also synthesized on solid support and consequently, we discovered a tetrapeptide aldehyde, Ac-Thr-Val-Phe(Hexahydro)-His-H (**1**), with an IC₅₀ value of 98 nM against SARS 3CL protease.^{17,18} Moreover, we have completed the synthesis of tokaramide A (**2**), p-HBz-Val-Val-Arg-H, as a cyclic hemi-aminal structure on Weinreb AM resin using the acetal/thioacetal methodology.^{16,19} This strategy could be applicable to Arg-containing peptides. In our research program on peptide aldehyde preparation by this methodology, we herein report synthesis of three variant peptide aldehydes (**3**), (**4**) and (**5**) (Fig. 1).

Fig. 1. His and Arg-containing peptide aldehydes (**1**) and (**2**) and model tripeptide aldehydes (**3**), (**4**) and (**5**)

2. Result and discussion

Prior to the synthesis of peptide aldehydes, we investigated the potential of acetal/thioacetal transformation using each Fmoc-amino acid; Leu, Ala, Phe, Ser, Gln, Glu, His, Arg, Trp and Met. Acetal/thioacetal transformation of the selected Fmoc-amino acids was attempted; (i) attachment of Fmoc-aminals with alkyl triol linker, (ii) conversion of acetals to thioacetals, (iii) treatment with NBS to give Fmoc-aminals. Non-polar amino acids (Leu, Ala, Phe), polar amino acid (Ser) and basic amino acids (His and Arg) were applicable without any problems. In an attempt to achieve thioacetal formation of Fmoc-Trp(Boc) or -Gln(Trt) type acetals, the corresponding Fmoc-Trp-(SEt)₂ or Fmoc-Gln-(SEt)₂ were given in 24 % and ~5% yields, respectively. Unfortunately, treatment of these thioacetals with eternal conditions yielded poor results. Preparation of Glu-containing compound was planned in the general scope of this methodology, acetal/thioacetal conversions had hardly proceeded to give complexed mixtures. Barany's method,¹⁵ which is a favorite for preparation of acidic peptides, was helpful in these sequences. In contrast, the treatment of Fmoc-Met type acetal with EtSH/BF₃-Et₂O for the thioacetal formation directly afforded Fmoc-Met-H in 95% yield. We guessed that thiomethyl group of Met participated the transformation. For the reason, preparation of Phe, Ser, Lys-containing peptides was attempted since we have already reported the synthesis of Leu, Ala, His and Arg-containing peptides in previous report.¹⁶

In order to evaluate the utility of this methodology, we firstly designed a C-terminal Phe-containing peptide aldehyde, Ac-Leu-Ala-Phe-H (**3**), to compare the reactivity of C-terminal amino acids since preparation of peptide aldehydes with Ala, Leu, Arg, and His as a C-terminus have already been investigated. The designed tripeptide aldehyde, Ac-Leu-Ala-Phe-H (**3**) was synthesized using our reported strategy according to the route shown in Scheme 1. At first, Fmoc-Phe-H (**6**) was prudently prepared from Fmoc-Phe-OH by two steps; Weinreb amidation and reduction with LiAlH₄ in 46% yield. The acetalization of Fmoc-Phe-H (**6**) with 1.5 equiv. hexane-1,2,6-triol and 10 mol% BF₃-Et₂O in dioxane at room temperature for 26 h obtained the hydroxyl acetal (**7**) in 54% yield. After oxidation of the hydroxyl acetal (**7**) by Jones condition, carboxylic acid (**8**) was loaded on 2-chlorotriyl chloride resin in the presence of DIPEA to give **9**. The conventional Fmoc-SPPS for **9** was performed. The Fmoc group of the resulting resin was removed with 20% piperidine/DMF and Fmoc-Ala-OH was condensed by

DIPC/HOBt in the presence of DIPEA in DMF. The Fmoc deprotection and coupling with Fmoc-Leu-OH by the same procedure were carried out. Subsequently the Fmoc deprotection followed by acetylation of the resulting *N*-terminal amine led to the desired sequence. All processes on resin were monitored by Kaiser tests, which were applicable to these peptides synthesis. The treatment of resin with 20% HFIP/CH₂Cl₂ for 2 h at room temperature gave the tripeptide derivative (**10**), after purification, in 32% yield from the resin loading. Similar results were given using Rink amide resin. The acetal derivative (**10**) was smoothly converted to the thioacetal (**11**) in 84% yield by treatment with 15 equiv. of EtSH and catalytic BF₃·Et₂O for 18.5 h at room temperature. The conversion of acetal to thioacetal is generally rapid from our previous experience but it takes 18.5 h to complete consumption of the starting material. We assume this is due to steric hindrance between acetal structures and side chains of selected amino acids. It was possible to give tripeptide acetal with trimethylolethane selected as a linker, but the acetal/thioacetal conversion of the corresponding peptide was extremely slow to afford thioacetal (**11**) in 3% yield. Finally, peptide aldehyde (**3**) was quickly (~10 min) obtained from thioacetal (**11**) using NBS in 90% THF aq. at a yield of 44% and ~80% after the purification. In this case, the purification by silica gel column chromatography was immediately needed to quench the reaction mixtures because the products caused some epimerization of α -proton bearing the aldehyde and then decomposed. Nevertheless, some epimerization of α -proton of aldehyde proceeded within 30 min after the purification from the analysis by ¹H NMR (neighboring δ 9.6 ppm). This phenomenon was reported by Barany¹⁵ and Martinez⁵ and was not surprising. We achieved synthesis of the model tripeptide aldehyde, Ac-Leu-Ala-Phe-H (**3**) in 14 steps (Scheme 2).

Scheme 2. Synthesis of Ac-Leu-Ala-Phe-H (**3**)

In contrast, synthesis of non-protected amine containing peptide aldehydes has never been attempted because of N-terminal acetamide, a component of the previous peptides. Synthesis of the Lys-containing tripeptide aldehyde, Ac-Lys-Ala-Leu-H (**4**), was conducted using similar strategy shown in Scheme 2. Fmoc-Leu-H (**12**) derived from Fmoc-Leu-OH in 2 steps was treated with 1.5 equiv. hexane-1,2,6-triol and 75 mol% $\text{BF}_3\cdot\text{Et}_2\text{O}$ in THF at room temperature for 30 h to give the hydroxyl acetal (**13**) in 54% yield. The hydroxyl acetal (**13**) was oxidized to the corresponding carboxylic acid (**14**) by Jones condition and subsequently loading on rink amide resin in the condition of BOP/HOBt/DIPEA was performed successively to yield **15**. The resin (**15**) was coupled with Fmoc-Ala-OH and Fmoc-Lys(Boc)-OH by Fmoc-SPPS without any problems. Continuous treatment of resin with 95% TFA/ H_2O for 2 h at room temperature gave an acetal amide derivative (**16**), which was smoothly converted to thioacetal (**17**) by the treatment with EtSH and catalytic $\text{BF}_3\cdot\text{Et}_2\text{O}$ at a yield of 33% from the resin loading. It is noted that the peptide with a free amine of the Lys side chain is applicable. The Lys-containing peptide aldehyde, Ac-Lys-Ala-Leu-H (**4**) as a form of hemiaminal structure was obtained from thioacetal (**17**) under the above-mentioned conditions in 27% yield because we assigned no aldehyde proton peak (~ 9.6 ppm) by ^1H NMR and $[\text{M}+\text{Na}]^+$ of **4** was detected by ESIMS (Scheme 3).

In the depicted in Scheme 4, a Ser-containing peptide, Ac-Ser-Phe-Leu-H (**5**), was designed and synthesized. Using the resin-bounded Leu-type derivative (**15**), elongation of the peptide chain with Fmoc-Phe-OH and Fmoc-Ser(^tBu)-OH and cleavage from the resin with 95% TFA/H₂O afforded the acetal-containing amide derivative (**18**). Crude **18** was then transformed to peptidyl thioacetal (**19**) in 15% overall yield from resin loading after the purification by column chromatography. The peptidyl thioacetal (**19**) was treated with NBS in CH₂Cl₂/H₂O for 1 h, followed by the purification with silica gel column chromatography to afford Ac-Ser-Phe-Leu-H (**5**) in 25% yield (75%de) (Scheme 4).

In conclusion, we prepared the three peptide aldehydes, Ac-Leu-Ala-Phe-H (**3**), Ac-Lys-Ala-Leu-H (**4**) and Ac-Ser-Phe-Leu-H (**5**), using a very simple and cheap alkyl

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2
3 triol linker. This methodology, acetal/thioacetal conversion followed by production of
4 aldehyde with NBS/H₂O, is applicable for Lys/Ser-containing peptides. This method has
5 good potential for optimization of Lewis acids or bromonium species and should be
6 applicable to aldehydes with more complex sequences.
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10 11 **3. Experimental**

12 13 **3.1. General**

14 Amino acid derivatives and resins were purchased from Watanabe Chemical Industries.
15 Amino acids were generally *N*^α-Fmoc protected and the following side-chain protecting
16 groups were employed; t-Bu for Glu, Ser; Boc for Lys, Trp. Other chemicals were
17 purchased from Aldrich, Wako chemical and Nacalai tesque. Optical rotations were
18 determined with a JASCO DIP-371 polarimeter at the sodium D line. IR spectra of
19 sample were obtained as films with a HORIBA FT-720 spectrometer. ¹H (400 and 500
20 MHz) and ¹³C NMR (100 and 125 MHz) were determined on a JNM-ECX400 and
21 JNM-ECX500. Chemical shifts are reported in ppm with reference to tetramethylsilane
22 [¹H NMR: TMS (0.00)], or solvent signals [¹H NMR: CDCl₃ (7.26), MeOH-d₄ (3.30);
23 ¹³C NMR: CDCl₃ (77.16), MeOH-d₄ (50.05)]. Mass spectra were recorded on a JEOL
24 AccuTOF JMS-T100LC (ESI) and a Bruker Autoflex-III (MALDI-TOF). Analytical
25 TLC was performed on Merck Silica gel 60F₂₅₄. Crude products were purified by
26 column chromatography on Silica Gel 60N [Kanto, particle size, (spherical, neutral)
27 63-210 μm or 100-200 μm. HPLC system (monitored at 220 nm) equipped with
28 Cosmosil 5C18 AR-II column (4.6×150 mm or 10×250 mm) using MeCN in 0.1%
29 aqueous TFA.
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45 46 **3.2. Fmoc-L-Phe-type hydroxyl acetal (7)**

47 To a solution of aldehyde (6) (502 mg, 1.35 mmol) in 1,4-dioxane was added
48 hexane-1,2,6-triol (376 mg, 2.80 mmol) and BF₃·Et₂O (17 μl, 10 mol%) at room
49 temperature. After being stirred for 26 h, DIPEA was added to the solution. The organic
50 phase was washed with H₂O and brine, dried over MgSO₄ and evaporated in vacuo. The
51 residue was purified by column chromatography (hexane:AcOEt = 3:1) to give acetal (7)
52 (356 mg, 0.730 mmol, 54 %) as a colorless oil. IR (film): ν 1034, 1132, 1248, 1450, 1516,
53 1709, 2868, 2939, 3022, 3064, 3338, 3423 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 1.33-1.67
54 (6H, m), 1.96 (1H, brs), 2.75-2.93 (2H, m), 3.40-3.62 (3H, m), 3.88-4.40 (6H, m),
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4.80-5.08 (2H, m), 7.13-7.28 (7H, m), 7.37 (2H, t, $J = 7.5$ Hz), 7.48-7.53 (2H, m), 7.73 (2H, d, $J = 8.5$ Hz). ^{13}C NMR (125 MHz, CDCl_3): δ 14.3, 21.2, 22.0, 22.08, 22.14, 32.47, 32.52, 32.8, 33.0, 36.6, 47.3, 53.7, 60.5, 62.4, 62.5, 62.6, 66.6, 66.9, 70.2, 70.7, 77.1, 103.4, 120.1, 125.2, 125.3, 126.6, 127.1, 127.7, 128.6, 129.4, 137.6, 141.4, 144.0, 144.1, 156.3. MS-ESI $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{33}\text{NO}_5$ Na: 510.22; found: 510.22. $[\alpha]_{\text{D}}^{25}$ -13.8 (c 1.0, CHCl_3).

3.3. Fmoc-L-Phe-type acetal (8)

To a solution of acetal (7) (327 mg, 671 μmol) in acetone at 0°C was added Jones reagent (600 μl) dropwise. After being stirred for 3 h, 2-propanol was added to the mixture. The mixture was filtered on Celite pad and filtrate was dried over MgSO_4 and concentrated in vacuo. The crude carboxylic acid (8) (318 mg, 634 μmol , 95%) was used for the next reaction without further purification. IR (film): ν 741, 758, 1246, 1450, 1714, 1948, 2877, 2945, 3024, 3064, 3329 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ 1.48-1.76 (4H, m), 2.31-2.38 (2H, m), 2.59-2.89 (2H, m), 3.40-3.50 (1H, m), 3.90-4.36 (7H, m), 4.80-4.95 (1H, m), 5.12 (1H, d, $J = 11.0$ Hz), 7.16-7.35 (9H, m), 7.47-7.50 (2H, m), 7.71 (2H, d, $J = 7.5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 21.0, 21.2, 29.3, 31.1, 31.9, 32.4, 32.5, 33.8, 36.5, 37.1, 47.3, 53.7, 53.85, 53.94, 55.0, 66.8, 67.0, 67.4, 70.0, 70.1, 70.8, 76.6, 76.8, 103.5, 120.1, 125.2, 126.7, 127.2, 127.8, 128.6, 129.5, 137.6, 137.9, 144.0, 144.1, 156.5, 178.4, 207.8. MS-ESI $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{31}\text{N}_1\text{O}_6$ Na: 524.20; found: 524.21.

3.4. Ac-Leu-Ala-Phe-acetal (10)

2-Chlorotrityl chloride resin (408 mg, 640 μmol , 100-200 mesh, 1% DVB, 1.60 mmol/g) in DMF was agitated for 20 min. Carboxylic acid (8) (320 mg, 0.64 mmol) and DIPEA (330 μl , 1.92 mmol) in DMF were added to the resin and the mixture was agitated for 3 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Fmoc-Ala-OH (632 mg, 1.92 mmol), DIPCI (300 μl , 1.92 mmol), HOBt (294 mg, 1.92 mmol) and DIPEA (440 μl , 2.56 mmol) in DMF were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was

washed sequentially with DMF (x5). Fmoc-Leu-OH (679 mg, 1.92 mmol), DIPCI (300 μ l, 1.92 mmol), HOBt (294 mg, 1.92 mmol) and DIPEA (440 μ l, 2.56 mmol) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Acetic anhydride (605 μ l, 6.40 mmol), pyridine (516 μ l, 6.40 mmol) and DMAP (2 mg) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5) and Et₂O (x5). The resultant resin was treated with 20% HFIP/CH₂Cl₂ for 3 h. The solvent was removed by filtration and cooled Et₂O was added to the filtrate. To the residue in Et₂O was centrifuged and decanted. The crude product was purified by column chromatography (CHCl₃:MeOH = 75:25) to give peptide acetal (**10**) (102 mg, 0.200 mmol, 32% for 8 steps) as a colorless oil. IR (film): ν 665, 754, 1146, 1246, 1385, 1549, 1635, 1712, 2874, 2956, 3086, 3280 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 0.86 (6H, s), 1.02-1.72 (9H, m), 1.96 (3H, d, *J* = 6.8 Hz), 2.35-2.52 (2H, m), 2.73-2.94 (2H, m), 3.39-3.50 (1H, m), 3.88-4.13 (2H, m), 4.34-4.43 (1H, m), 4.64-4.69 (2H, m), 4.82-4.96 (1H, m), 7.15-7.20 (5H, m). ¹³C NMR (125 MHz, CDCl₃): δ 18.8, 19.0, 21.1, 21.3, 21.5, 22.2, 22.3, 23.0, 24.7, 29.8, 32.3, 32.4, 33.0, 33.6, 33.8, 36.6, 36.7, 42.3, 54.7, 48.9, 51.5, 51.7, 69.9, 70.4, 76.4, 76.6, 103.2, 126.5, 128.5, 129.3, 137.5, 170.9, 172.6, 172.7, 176.8. MS-ESI [M+Na]⁺ calcd for C₂₆H₃₉N₃O₇Na: 528.27; found: 528.27.

3.5. Ac-Leu-Ala-Phe-(SEt)₂ (**11**)

To a solution of peptide acetal (**10**) (102 mg, 0.200 mmol) in CH₂Cl₂ at room temperature was added EtSH (225 μ l, 3.03 mmol), BF₃-Et₂O (100 μ l, 9.31 μ mol) and stirred for 18.5 h. DIPEA (200 μ l) was added to the solution. The organic layer was washed with H₂O and brine, dried over MgSO₄, and evaporated in vacuo. The mixture was purified with column chromatography (CHCl₃:MeOH = 99:1) to give thioacetal (**11**) (81.9 mg, 170 μ mol, 84 %) as a colorless oil. IR (film): ν 667, 700, 1246, 1454, 1545, 1628, 1684, 1722, 2870, 2927, 2956, 3074, 3267, 3305 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 0.85 (6H, d, *J* = 5.6 Hz), 1.05-1.34 (10H, m), 1.41-1.56 (3H, m), 1.97 (3H, s), 2.54-2.86 (5H, m), 3.13-3.22 (1H, m), 3.60-3.92 (1H, m), 4.41-4.48 (1H, m), 4.70-4.80 (2H, m), 7.12-7.24 (5H, m). ¹³C NMR (100 MHz, CDCl₃): δ 14.6, 14.7, 18.5, 18.9, 19.2,

22.4, 22.5, 22.9, 23.0, 23.2, 23.8, 24.8, 25.9, 26.0, 26.3, 26.6, 29.7, 36.5, 37.0, 42.4, 42.5, 48.6, 48.7, 51.4, 53.9, 54.3, 54.7, 55.1, 64.8, 64.9, 81.29, 81.35, 126.6, 128.4, 128.5, 129.2, 129.3, 138.0, 138.3, 170.3, 171.8, 172.26, 172.31, 172.4, 172.5. MS-ESI $[M+Na]^+$ calcd for $C_{24}H_{39}N_3O_3S_2Na$: 504.23; found: 504.24. $[\alpha]_D^{25}$ -53.3 (*c* 0.31, $CHCl_3$).

3.6. Ac-Leu-Ala-Phe-H (3)

To a solution of thioacetal (**11**) (23.3 mg, 48.4 μ mol) in THF (1.8 ml) was added NBS (86.1 mg, 484 μ mol) and H_2O (0.20 ml) at room temperature and stirred for 10 min. The mixture was purified with silica gel column chromatography ($CHCl_3:MeOH = 97:3$) to give Ac-Leu-Ala-Phe-H (**3**) (7.9 mg, 21.0 μ mol, 44 %, ~80%de) as a colorless oil. Diastereomeric excess was calculated by the aldehyde proton signal on 1H NMR. IR (film): ν 665, 754, 1373, 1456, 1541, 1645, 1712, 2871, 2933, 2958, 3032, 3072, 3290 cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): δ 0.85 (6H, d, $J = 5.6$ Hz), 1.05-1.34 (6H, m), 1.97 (3H, s), 3.13-3.22 (2H, m), 4.41-4.48 (2H, m), 4.70-4.80 (1H, m), 6.33 (1H, brs), 7.00 (1H, brs), 7.12-7.24 (5H, m), 8.54 (1H, brs), 9.52 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3, 18.4, 22.1, 22.2, 22.6, 22.8, 23.0, 23.1, 24.8, 29.7, 34.9, 35.0, 41.7, 48.8, 48.9, 51.7, 51.8, 58.5, 59.9, 77.3, 127.2, 128.5, 128.77, 128.79, 129.3, 129.4, 135.89, 135.94, 170.8, 172.7, 172.8, 178.3, 198.9. MS-ESI $[M+Na]^+$ calcd for $C_{20}H_{29}N_3O_4Na$: 398.21; found: 398.21. $[\alpha]_D^{25}$ -42.6 (*c* 0.22, $CHCl_3$).

3.7. Fmoc-L-Leu-type hydroxyl acetal (13)

To a solution of aldehyde (**12**) (725 mg, 2.15 mmol) in THF was added hexane-1,2,6-triol (433 mg, 3.23 mmol) and $BF_3 \cdot Et_2O$ (200 μ l, 5 mol%) at room temperature. After being stirred for 30 h, DIPEA was added to the solution. The organic phase was washed with H_2O and brine, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (hexane:AcOEt = 3:1) to give acetal (**13**) (552 mg, 1.15 mmol, 54 %) as a colorless oil. IR (film): ν 621, 665, 741, 1022, 1115, 1223, 1250, 1327, 1450, 1508, 1522, 1541, 1558, 1716, 2868, 2952, 3421 cm^{-1} . 1H NMR (500 MHz, $CDCl_3$): δ 0.79-0.94 (6H, m), 1.27-1.67 (9H, m), 1.80 (1H, brs), 3.35-3.63 (3H, m), 3.88-4.06 (3H, m), 4.21 (1H, t, $J = 7.5$ Hz), 4.32-4.51 (2H, m), 4.63-4.93 (2H, m), 7.30 (2H, t, $J = 7.3$ Hz), 7.38 (2H, t, $J = 7.3$ Hz), 7.58-7.64 (2H, m), 7.75 (2H, d, $J = 8.0$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.9, 21.97, 22.04, 22.1, 23.6, 24.6, 32.4, 32.5, 32.7, 32.8, 32.9, 33.0, 39.2, 39.3, 47.4, 47.5, 50.8, 50.9, 62.4, 62.5, 62.6, 66.3, 66.7, 70.1, 104.6, 104.7, 120.0, 125.09,

125.13, 125.3, 127.1, 127.7, 141.4, 144.1, 144.2, 156.6. MS-ESI $[M+Na]^+$ calcd for $C_{27}H_{35}N_1O_5Na$: 476.24; found: 476.24. $[\alpha]_D^{18}$ -19.4 (*c* 1.3, $CHCl_3$).

3.8. Fmoc-L-Leu-type acetal (14)

To a solution of acetal (13) (662 mg, 1.46 mmol) in acetone at 0°C was added Jones reagent (700 μ l) dropwisely. After being stirred for 1 h, 2-propanol was added to the solution. The mixture was filtered on Celite pad and filtrate was dried over $MgSO_4$ and concentrated in vacuo. The crude carboxylic acid (14) (629 mg, 1.35 mmol, 98%) was used for the next reaction without further purification. IR (film): ν 621, 665, 741, 760, 1022, 1115, 1171, 1221, 1250, 1338, 1369, 1387, 1415, 1450, 1469, 1522, 1541, 1709, 2870, 2956, 3068, 3406 cm^{-1} . 1H NMR (400 MHz, $CDCl_3$): δ 0.92 (6H, d, J = 6.8 Hz), 1.21-1.74 (7H, m), 2.23-2.41 (2H, m), 3.33-3.48 (1H, m), 3.77-4.07 (3H, m), 4.20 (1H, t, J = 6.8 Hz), 4.31-4.50 (2H, m), 4.74-5.63 (2H, m), 7.28 (2H, t, J = 7.4 Hz), 7.36 (2H, t, J = 7.6 Hz), 7.58 (1H, d, J = 7.2 Hz), 7.72 (2H, t, J = 8.0 Hz), 9.94 (1H, brs). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 21.2, 21.9, 23.5, 23.6, 24.7, 32.3, 32.5, 39.0, 39.2, 47.3, 47.4, 47.5, 50.80, 50.82, 66.5, 66.8, 69.9, 70.0, 70.6, 104.8, 120.1, 125.1, 127.1, 127.8, 141.4, 144.1, 144.2, 156.7, 178.5. MS-ESI $[M+Na]^+$ calcd for $C_{23}H_{25}N_1O_5Na$: 490.22; found: 490.22.

3.9. Ac-Lys-Ala-Leu-(SEt)₂ (17)

To a suspension of Rink amide resin (200 mg, 130 μ mol, 100-200 mesh, 1% DVB, 0.65 mmol/g) in DMF was treated with 20% piperidine in DMF for 20 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Carboxylic acid (14) (91.2 mg, 195 μ mol), BOP (173 mg, 390 μ mol), HOBT (52.7 mg, 390 μ mol) and DIPEA (226 μ l, 1.30 mmol) were added to the resin and the mixture was agitated for 3 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF and agitated for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Fmoc-Ala-OH (129 mg, 390 μ mol), DIPCI (60.4 μ l, 390 μ mol), HOBT (52.7 mg, 390 μ mol) and DIPEA (136 μ l, 780 μ mol) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Fmoc-Lys(Boc)-OH (183 mg, 390 μ mol), DIPCI

(60.4 μ l, 390 μ mol), HOBt (52.7 mg, 390 μ mol) and DIPEA (136 μ l, 780 μ mol) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Acetic anhydride (123 μ l, 1.30 mmol), pyridine (105 μ l, 1.30 mmol) and DMAP (2 mg) were added to the resin and the mixture was agitated for 3 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5) and Et₂O (x5). The resultant resin was treated with 95% TFA in H₂O for 3 h. The solvent was removed by filtration and cooled Et₂O was added to the filtrate. To the residue in Et₂O was centrifuged and decanted. The crude product was purified by column chromatography (CHCl₃:MeOH = 99:1) to give peptide acetal (**16**). To a solution of peptide acetal (**16**) in CH₂Cl₂ at room temperature was added EtSH (154 μ l, 2.07 mmol), BF₃-Et₂O (100 μ l, 9.31 μ mol) and stirred for 6.5 h. The mixture was purified with column chromatography (CHCl₃:MeOH = 70:30) to give thioacetal (**17**) (19.6 mg, 42.4 μ mol, 33 %, 9 steps) as a colorless oil. IR (film): ν 665, 706, 721, 800, 887, 879, 976, 1138, 1178, 1203, 1261, 1375, 1450, 1468, 1545, 1643, 1778, 1914, 2871, 2931, 2958, 3072, 3282 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 0.74-0.82 (6H, m), 1.14 (6H, m), 1.27-1.71 (12H, m), 1.92-1.93 (3H, m), 2.59 (4H, m), 2.89 (2H, t, *J* = 7.8 Hz), 3.90-4.22 (4H, m). ¹³C NMR (100 MHz, D₂O): δ 13.8, 13.9, 16.7, 20.5, 20.7, 21.7, 22.1, 22.7, 24.6, 25.9, 26.4, 30.3, 30.7, 38.8, 39.2, 47.2, 49.6, 51.4, 51.5, 53.6, 55.1, 173.6, 174.3, 174.5. MALDI-TOFMS [M+H]⁺ calcd for C₂₁H₄₂N₄O₃S₂Na: 463.28; found: 463.46. [α]_D²⁵ -49.0 (*c* 0.98, MeOH).

3.10. Ac-Lys-Ala-Leu-H (**4**)

To a solution of thioacetal (**17**) (12.9 mg, 36.2 μ mol) in THF (2.0 ml) was added NBS (32.2 mg, 181 μ mol) and H₂O (2.0 ml) at room temperature and stirred for 1.5 h. The mixture was purified with preparative RP-HPLC (2 ml/min, 0~30 min 0~50% CH₃CN/H₂O; Rt 18.2 min) to give Ac-Lys-Ala-Leu-H (**4**) (1.1 mg, 3.09 μ mol, 9%) as a colorless oil. IR (film): ν 665, 721, 800, 837, 924, 1043, 1138, 1184, 1201, 1294, 1373, 1432, 1456, 1471, 1541, 1658, 1711, 2873, 2958, 3072, 3282 cm⁻¹. ¹H NMR (500 MHz, D₂O): δ 0.76-0.82 (6H, m), 1.26-1.33 (5H, m), 1.53-1.69 (7H, m), 1.90 (3H, s), 2.87 (2H, t, *J* = 7.8 Hz), 4.12-4.24 (3H, m), 4.70 (1H, m, overlapped) ¹³C NMR (125 MHz, D₂O): δ 16.3, 20.6, 21.7, 22.0, 22.3, 24.4, 26.3, 29.5, 30.6, 39.2, 39.4, 49.5, 51.8, 53.5, 173.7,

174.3, 176.9: MS-ESI $[M+Na]^+$ calcd for $C_{17}H_{32}N_4O_4Na$: 379.23; found: 379.23. $[\alpha]_D^{25}$ -98.2 (*c* 0.06, MeOH).

3.11. Ac-Ser-Phe-Leu-(SEt)₂ (19)

To suspension of Rink amide resin (200 mg, 130 μ mol, 100-200 mesh, 1% DVB, 0.65 mmol/g) in DMF was added 20% piperidine in DMF. The suspension was stirred for 20 min at room temperature and the resin was washed with DMF (x5). Carboxylic acid (**14**) (91.2 mg, 195 μ mol), BOP (173 mg, 390 μ mol), HOBt (52.7 mg, 390 μ mol) and DIPEA (226 μ l, 1.30 mmol) was added to the resin and the mixture was agitated for 3 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Fmoc-Phe-OH (151 mg, 390 μ mol), DIPCI (60.4 μ l, 390 μ mol), HOBt (52.7 mg, 390 μ mol) and DIPEA (136 μ l, 780 μ mol) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was added 20% piperidine in DMF and agitated for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Fmoc-Ser(^tBu)-OH (150 mg, 390 μ mol), DIPCI (60.4 μ l, 390 μ mol), HOBt (52.7 mg, 390 μ mol) and DIPEA (136 μ l, 780 μ mol) were added to the resin and the mixture was agitated for 2 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). The resultant resin in DMF was treated with 20% piperidine in DMF for 30 min. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5). Acetic anhydride (123 μ l, 1.30 mmol), pyridine (105 μ l, 1.30 mmol) and DMAP (2 mg) were added to the resin and the mixture was agitated for 3 h. The solvent was removed by filtration and the resin was washed sequentially with DMF (x5) and Et₂O (x5). The resultant resin was treated with 95% TFA in H₂O for 3 h. The solvent was removed by filtration and cooled Et₂O was added to the filtrate. To the residue in Et₂O was centrifuged and decanted. The crude product was purified by column chromatography (CHCl₃:MeOH = 99:1) to give peptide acetal (**18**). To a solution of peptide acetal (**18**) in CH₂Cl₂ at room temperature was added EtSH (154 μ l, 2.07 mmol), BF₃-Et₂O (60 μ l, 5.59 μ mol) and stirred for 19 h. The organic layer was washed with brine and H₂O, dried over MgSO₄, and evaporated in vacuo. The residue was purified with column chromatography (CHCl₃) to give thioacetal (**19**) (9.4

mg, 18.9 μ mol, 15 %, 9 steps) as a colorless oil. IR (film): ν 665, 700, 752, 879, 918, 974, 1053, 1068, 1155, 1217, 1263, 1282, 1369, 1454, 1541, 1643, 1914, 2731, 2870, 2927, 2958, 2982, 3030, 3064, 3084, 3288 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 0.85-0.94 (6H, m), 1.20-1.62 (9H, m), 1.95 (3H, m), 2.64 (4H, m), 3.13 (2H, m), 3.34 (1H, brs), 3.55 (1H, brs), 3.82-3.84 (1H, m), 4.00 (1H, m), 4.30-4.44 (2H, m), 4.70 (1H, m), 6.31-7.01 (3H, m), 7.20-7.31 (5H, m). ^{13}C NMR (125 MHz, CDCl_3): δ 14.6, 14.9, 21.2, 21.6, 23.1, 23.6, 25.0, 26.5, 26.7, 29.8, 37.5, 37.7, 38.6, 39.2, 51.0, 51.1, 54.1, 54.7, 56.1, 62.6, 62.8, 127.2, 128.8, 129.28, 129.35, 136.3, 170.5, 170.8, 170.9. MALDI-TOFMS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{24}\text{H}_{39}\text{N}_3\text{O}_4\text{S}_2$ Na: 520.23; found: 520.12. $[\alpha]_{\text{D}}^{25}$ -46.8 (c 0.47, CHCl_3).

3.12. Ac-Ser-Phe-Leu-H (5)

To a solution of thioacetal (19) (7.1 mg, 14.3 μ mol) in CH_2Cl_2 (1.5 ml) was added NBS (25.5 mg, 143 μ mol) and H_2O (0.15 ml) at room temperature and stirred for 1 h. The mixture was purified with silica gel column chromatography (CHCl_3 :MeOH = 99:1) to give Ac-Ser-Phe-Leu-H (5) (1.4 mg, 3.58 μ mol, 25%, ~75%de) as a colorless oil. Diastereomeric excess was calculated by the aldehyde proton signal on ^1H NMR. IR (film): ν 665, 698, 744, 1053, 1261, 1373, 1456, 1539, 1645, 1738, 1869, 2856, 2870, 2925, 2956, 3030, 3064, 3332 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ 0.82-0.93 (6H, m), 1.25-1.43 (3H, m), 1.43-1.77 (3H, m), 1.96-1.99 (3H, m), 3.14 (2H, m), 3.66 (1H, m), 4.01 (1H, m), 4.27-4.79 (1H, m), 6.06-7.71 (8H, m), 9.47-9.49 (1H, m). ^{13}C NMR (125 MHz, CDCl_3): δ 21.9, 23.1, 24.7, 31.0, 37.4, 37.7, 54.2, 54.38, 54.42, 54.5, 57.5, 62.5, 127.3, 128.9, 129.3, 136.2, 170.9, 171.0, 199.5, 199.7. MALDI-TOFMS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{29}\text{N}_3\text{O}_5$ Na: 414.20; found: 414.11. $[\alpha]_{\text{D}}^{25}$ -11.4 (c 0.07, CHCl_3).

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Statement of human and animal rights

This article does not contain any studies with human or animal subjects performed by the

any of the authors.

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Synthetic study of peptide aldehyde via acetal/thioacetal transformation: Application for Lys/Ser-containing peptides

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