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Supporting Information

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Supporting Information

for

Identification of Physiological and Toxic Conformations in Aβ42 Aggregates

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	1 10 20 30 42
Wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 21-24	DAEFRHDSGY EVHHQKLVFF **AEDV**GSNKGA IIGLMVGGVVIA
Wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 25-27	DAEFRHDSGY EVHHQKLVFF AEDV**GSN**KGA IIGLMVGGVVIA
E22K-Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 21-24	DAEFRHDSGY EVHHQKLVFF **AKDV**GSNKGA IIGLMVGGVVIA
E22K-Aβ42 where ring-C_6 in Phe-19 were labeled with ^{13}C, and Gly-25, Ser-26 and Asn-27 were uniformly labeled with ^{13}C and ^{15}N	DAEFRHDSGY EVHHQKLV**F**F AKDV**GSN**KGA IIGLMVGGVVIA
Aβ42-lactam(22K-23E) where C_α and C=O in Gly-25 were labeled with ^{13}C	DAEFRHDSGY EVHHQKLVFF AK⌐EV**G**SNKGA IIGLMVGGVVIA

Figure S1. Selective labeling of Aβ42 derivatives with ^{13}C and ^{15}N. Labeling scheme: red letter, uniformly labeled with ^{13}C and ^{15}N; blue letter, only ring-C_6 are labeled with ^{13}C; green letter, C_α and C=O are labeled with ^{13}C. In Aβ42-lactam(22K-23E), the side chains of Lys-22 and Glu-23 are linked with an amide bond. E22K-Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 21-24 was synthesized and analyzed in the previous work.[1]

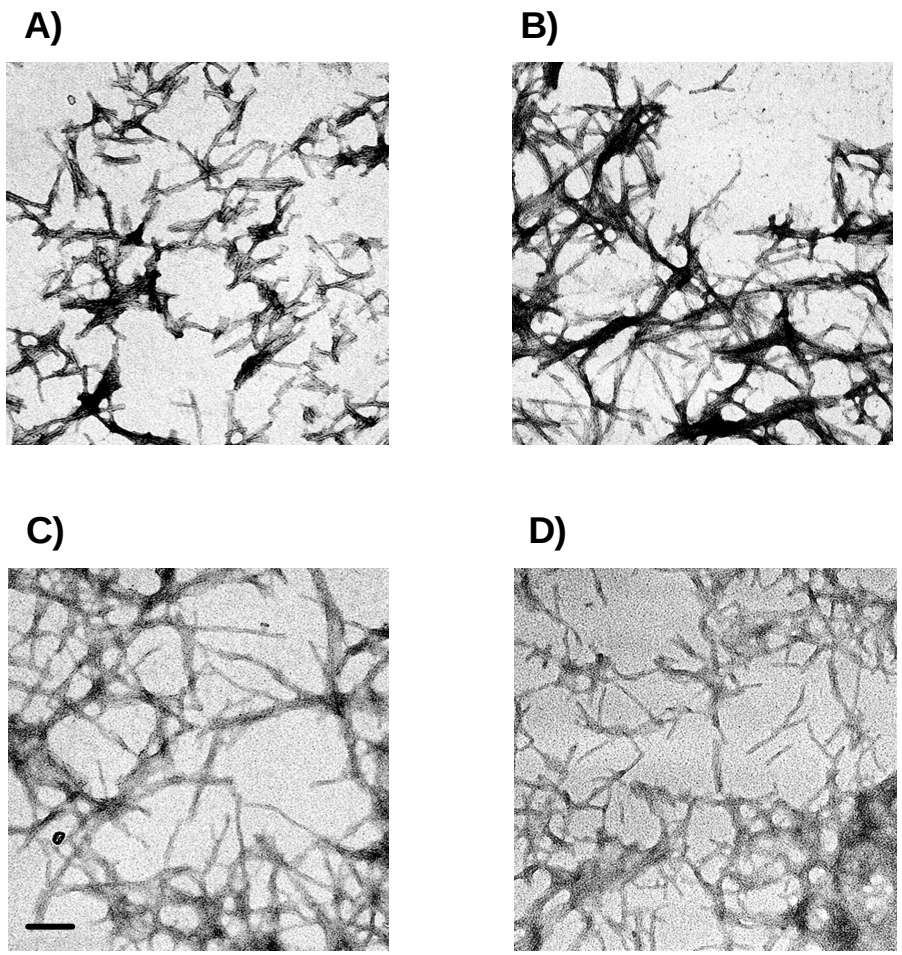


Figure S2. Transmission electron micrographs of negatively stained preparations of fibrils formed by the Aβ42 labeled with ^{13}C and ^{15}N. (A) Wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 21-24. (B) Wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 25-27. (C) E22K-Aβ42 where ring-C_6 in Phe-19 are labeled with ^{13}C, and Gly-25, Ser-26 and Asn-27 are uniformly labeled with ^{13}C and ^{15}N. (D) Aβ42-lactam(22K-23E) where C_α and C=O in Gly-25 are labeled with ^{13}C. *Scale bar* = 100 nm.

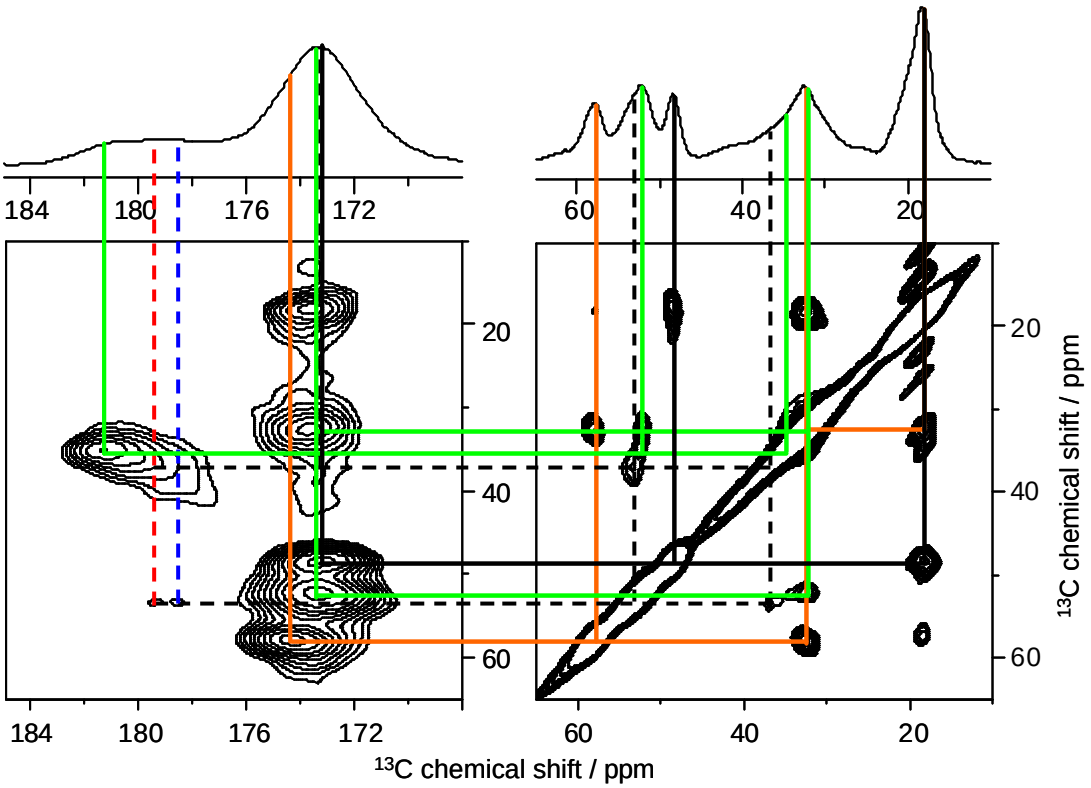


Figure S3. 2D DARR spectrum (mixing time: 20 ms) of wild-type Aβ42 aggregates uniformly labeled with ^{13}C and ^{15}N at positions 21-24 in the carbonyl and aliphatic region. An assignment path for each amino acid residue is shown on the spectrum: black line, Ala-21; green line, Glu-22; black dotted line, Asp-23; blue dotted line, the major conformer; red dotted line, the minor conformer; orange line, Val-24.

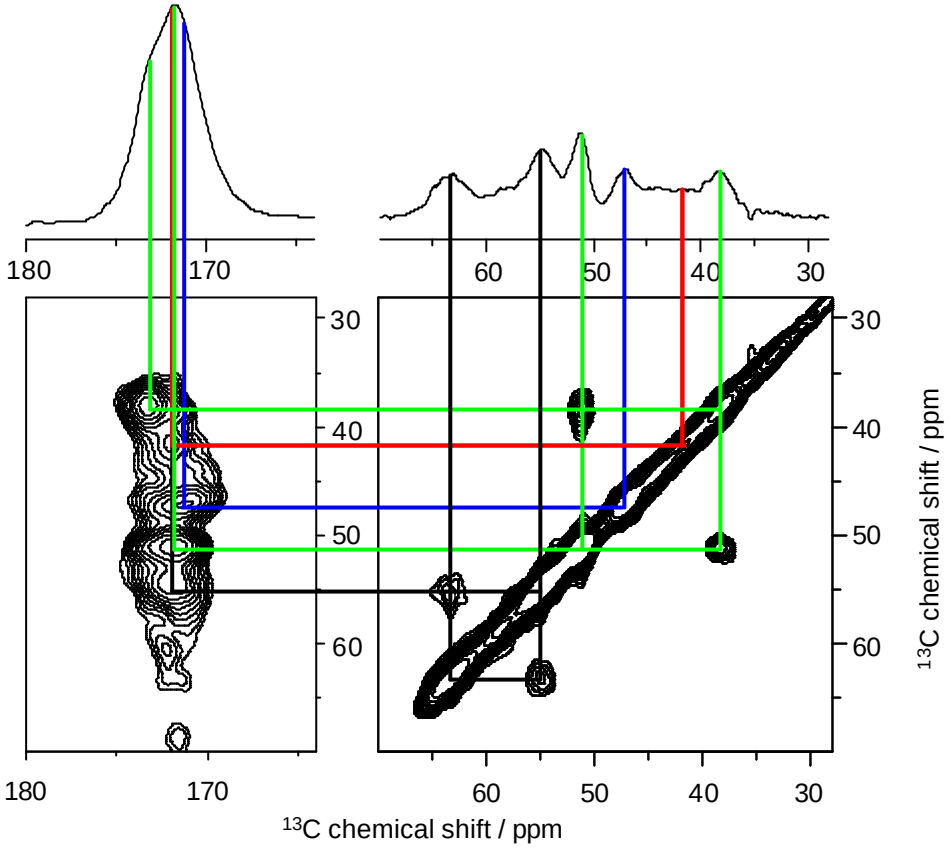


Figure S4. 2D DARR spectrum (mixing time: 20 ms) of the wild-type Aβ42 aggregate uniformly labeled with ^{13}C and ^{15}N at positions 25-27 in the carbonyl and aliphatic region. An assignment path for each amino acid residue is shown on the spectrum: blue line, the major conformer in Gly-25; red line, the minor conformer in Gly-25; black line, Ser-26; green line, Asn-27.

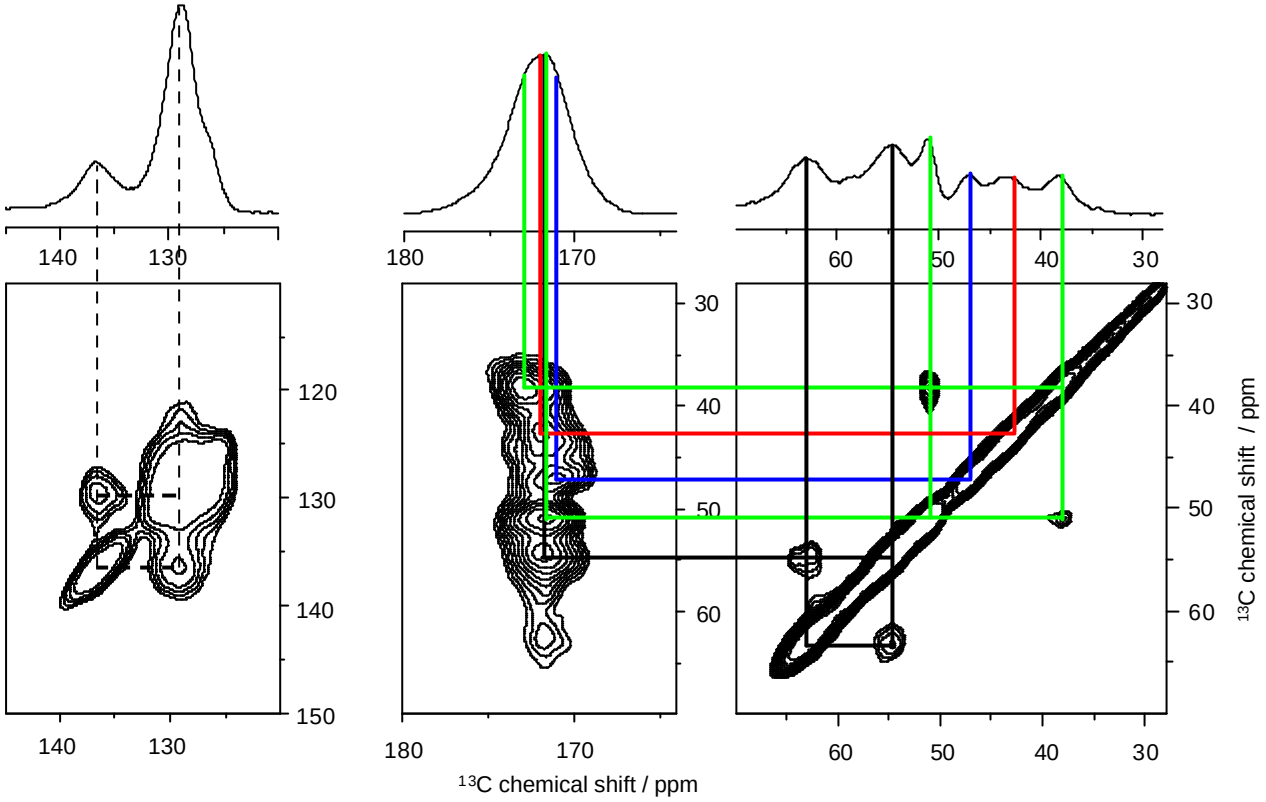


Figure S5. 2D DARR spectrum (mixing time: 20 ms) of the E22K-Aβ42 aggregates where ring-C_6 in Phe-19 were labeled with ^{13}C, and Gly-25, Ser-26 and Asn-27 were uniformly labeled with ^{13}C and ^{15}N. Aromatic, carbonyl, and aliphatic regions of the spectrum were shown above. An assignment path for each amino acid residue is shown on the spectrum: dotted line, Phe-19; blue line, the major conformer in Gly-25; red line, the minor conformer in Gly-25; black line, Ser-26; green line, Asn-27. We labeled ring-$^{13}C_6$ in Phe-19 for the purpose of measuring the distance between Phe-19 and the residues at positions 25-27, which might be close to each other in the structural model of the minor conformer of E22K-Aβ42 (Figure 3D). However, no cross-peaks were observed in the DARR experiment at a mixing time of 500 ms (data not shown).

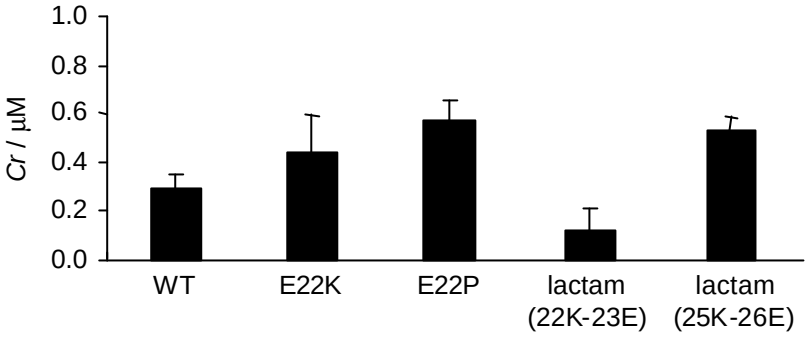


Figure S6. Thermodynamic stability of Aβ42 derivatives. The molar concentration of soluble peptides present in equilibrium with aggregates (critical concentration, *Cr*) was measured after 120-h incubation of each Aβ solution (25 μM) at 37°C.

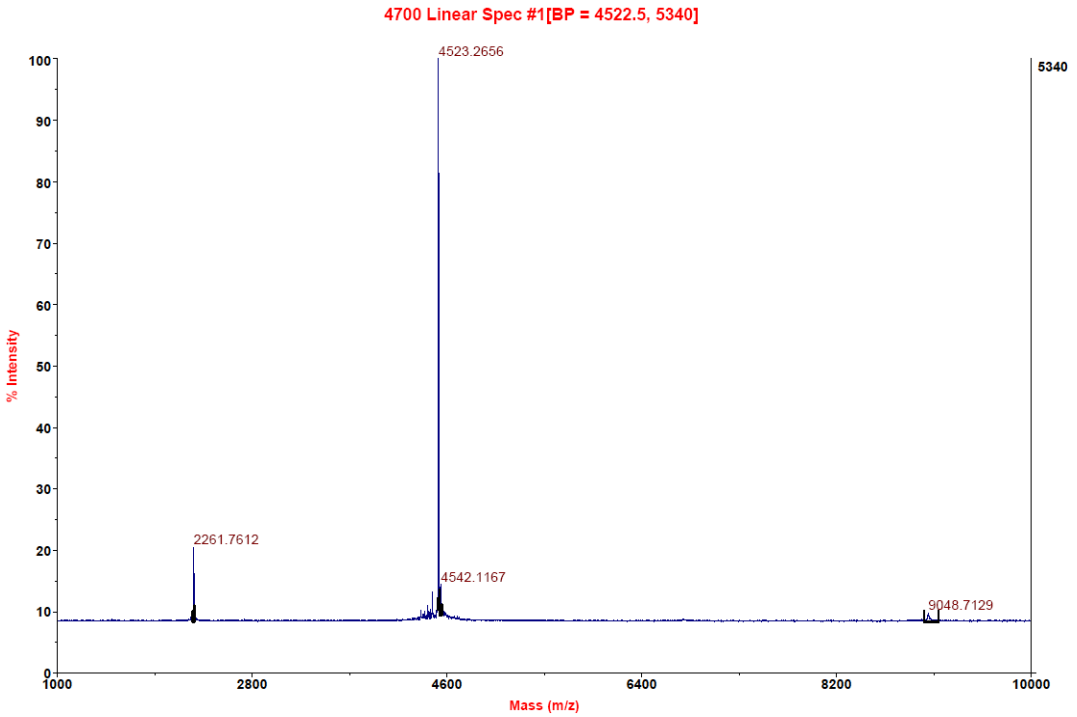


Figure S7. MALDI-TOF-MS data of E22P,G25P-Aβ42 (MH^+, average molecular mass; observed 4523.12, calculated 4523.18).

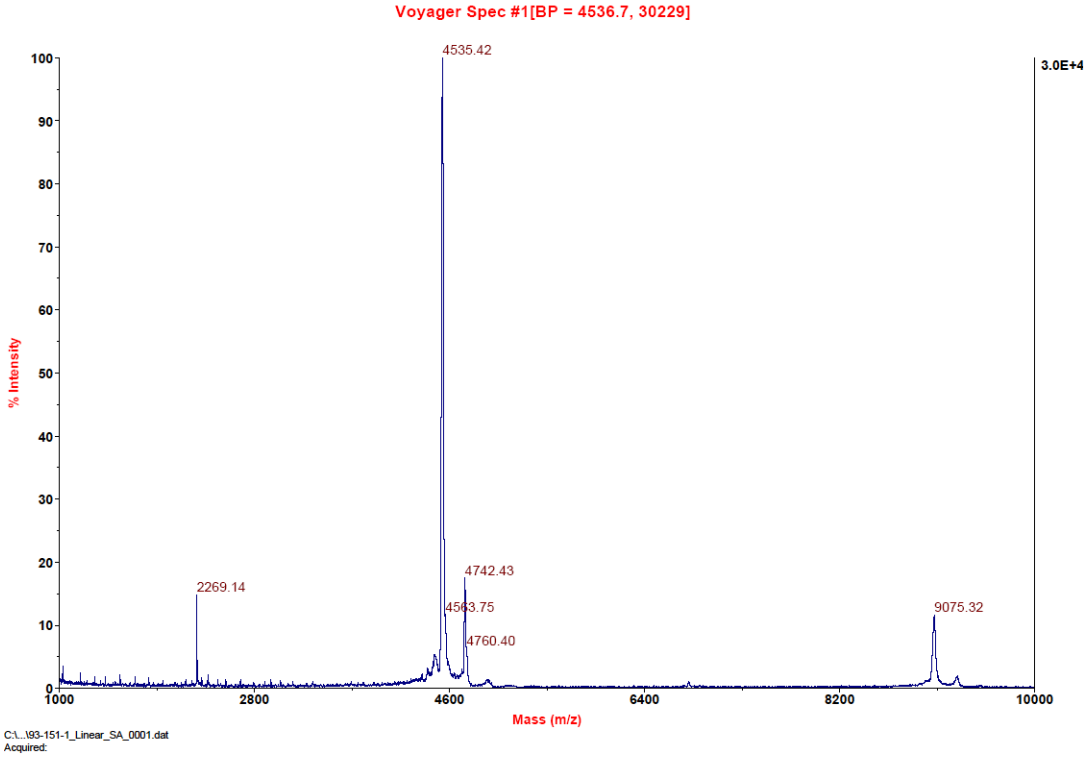


Figure S8. MALDI-TOF-MS data of wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 21-24 (MH^+, average molecular mass; observed 4535.45, calculated 4535.99).

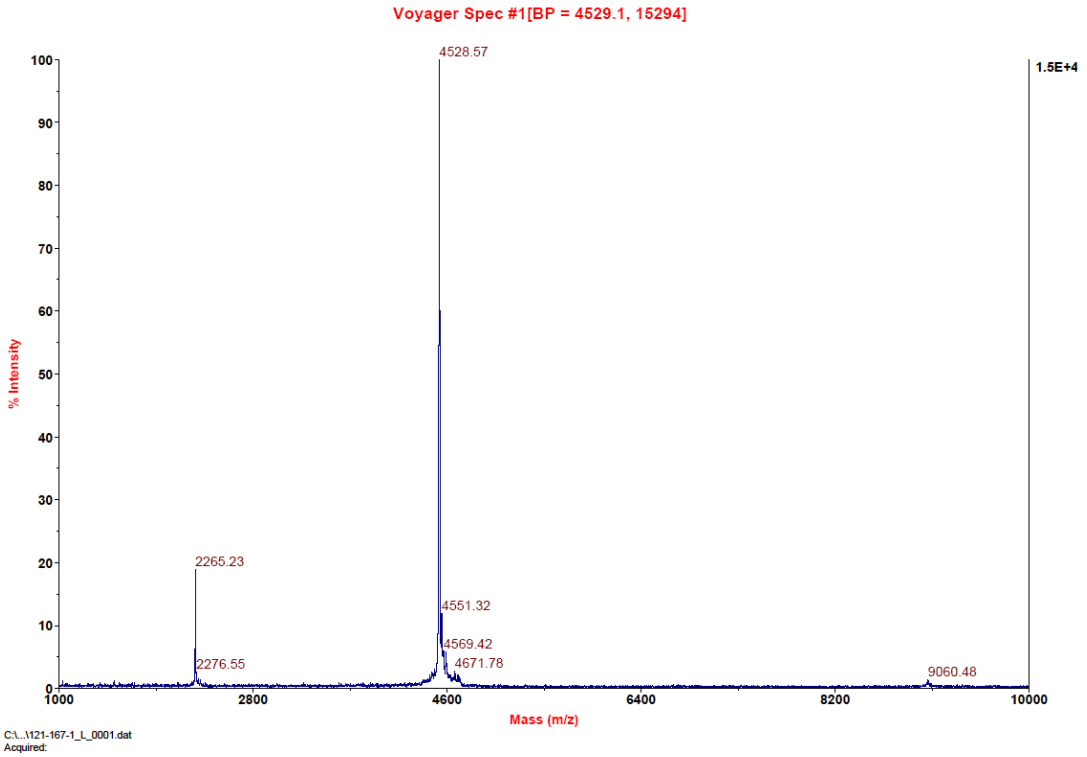


Figure S9. MALDI-TOF-MS data of wild-type Aβ42 uniformly labeled with ^{13}C and ^{15}N at positions 25-27 (MH^+, average molecular mass; observed 4528.09, calculated 4527.99).

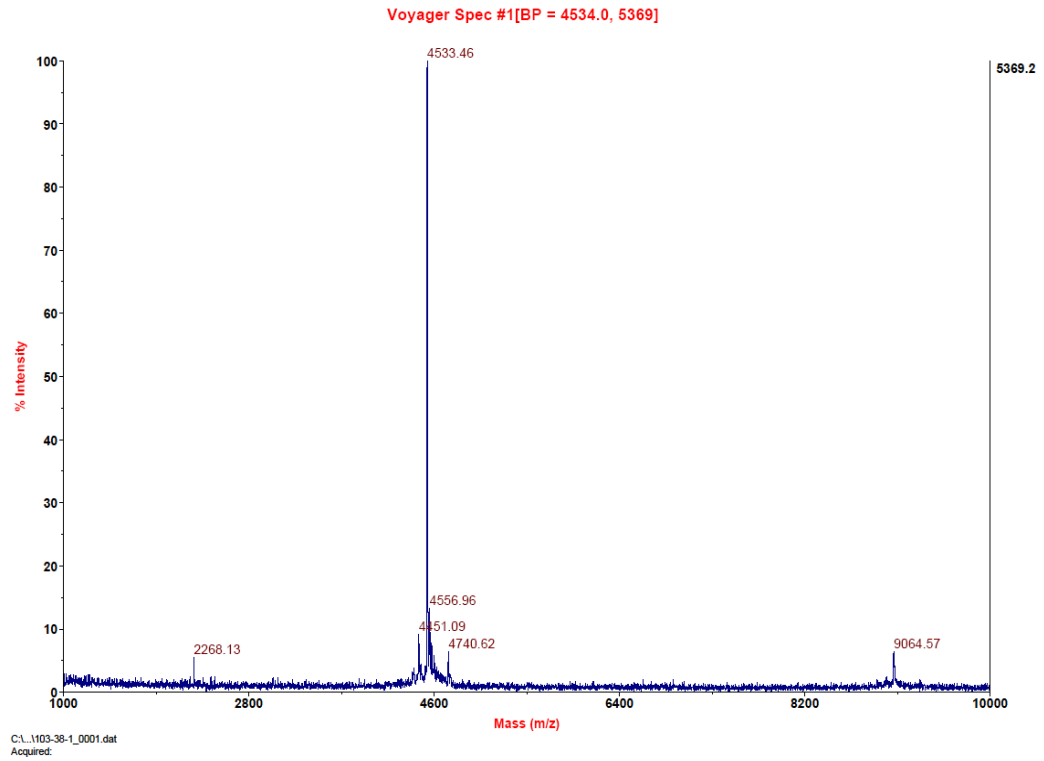


Figure S10. E22K-Aβ42 where ring-C_6 were labeled with ^{13}C, and Gly-25, Ser-26 and Asn-27 were uniformly labeled with ^{13}C and ^{15}N (MH^+, average molecular mass; observed 4533.09, calculated 4533.08).

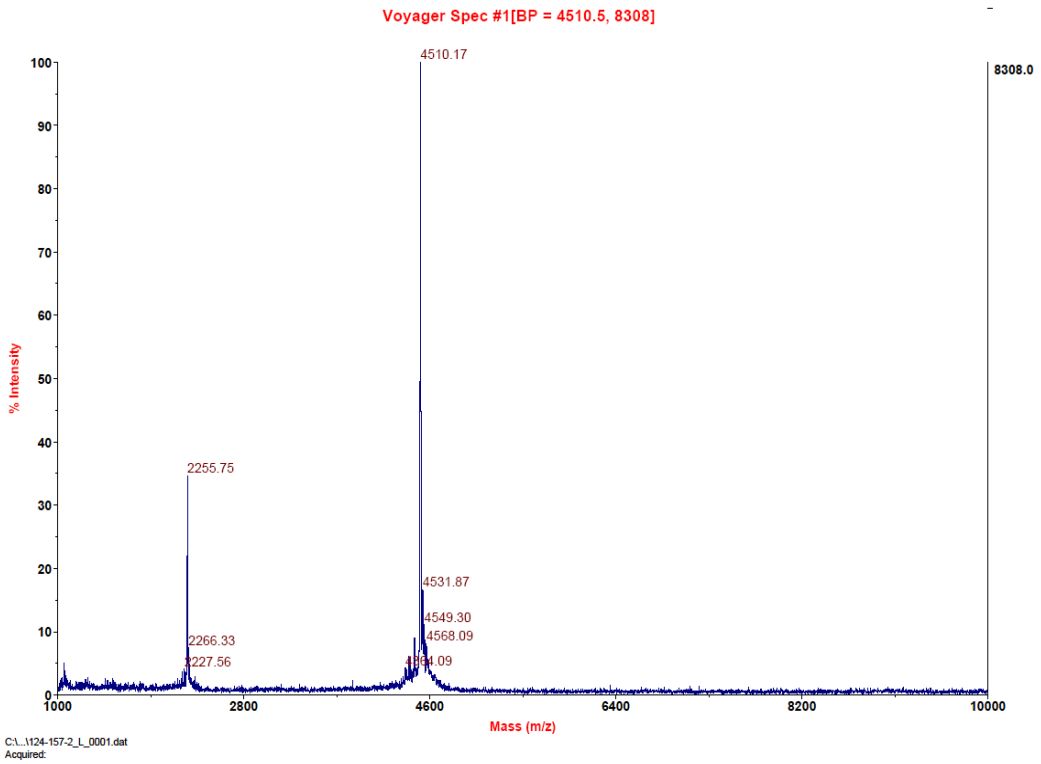


Figure S11. MALDI-TOF-MS data of Aβ42-lactam(22K-23E) (MH^+, average molecular mass; observed 4509.77, calculated 4510.18).

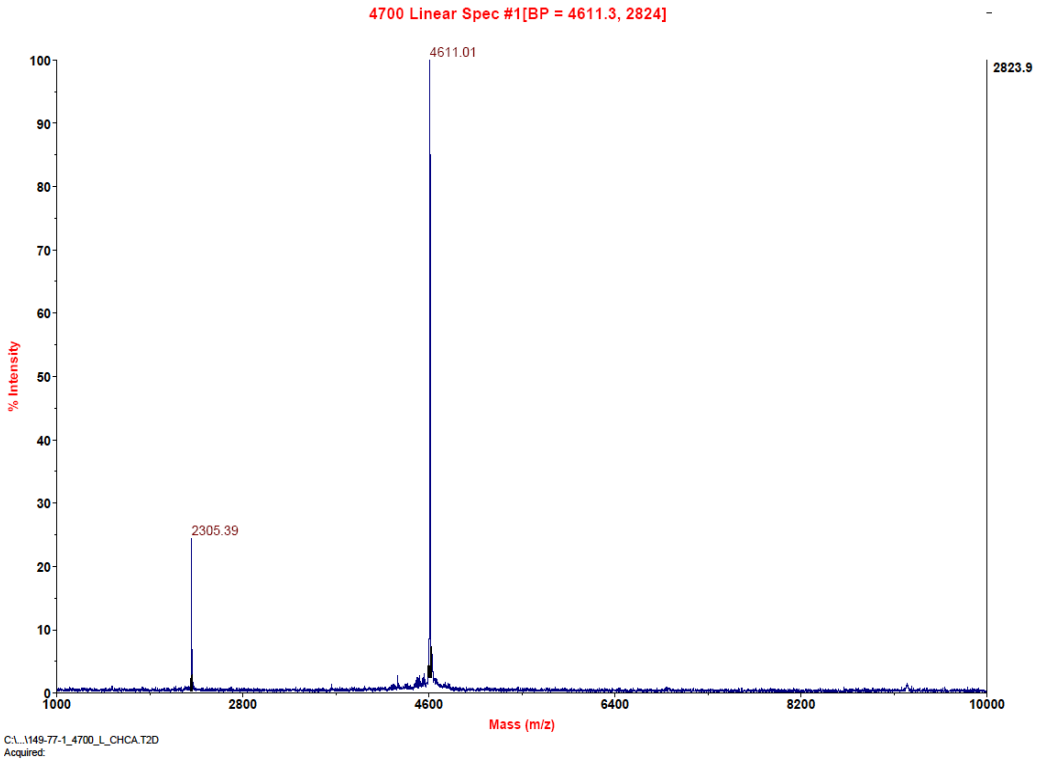


Figure S12. MALDI-TOF-MS data of Aβ42-lactam(25K-26E) (MH^+, average molecular mass; observed 4610.95, calculated 4610.26).

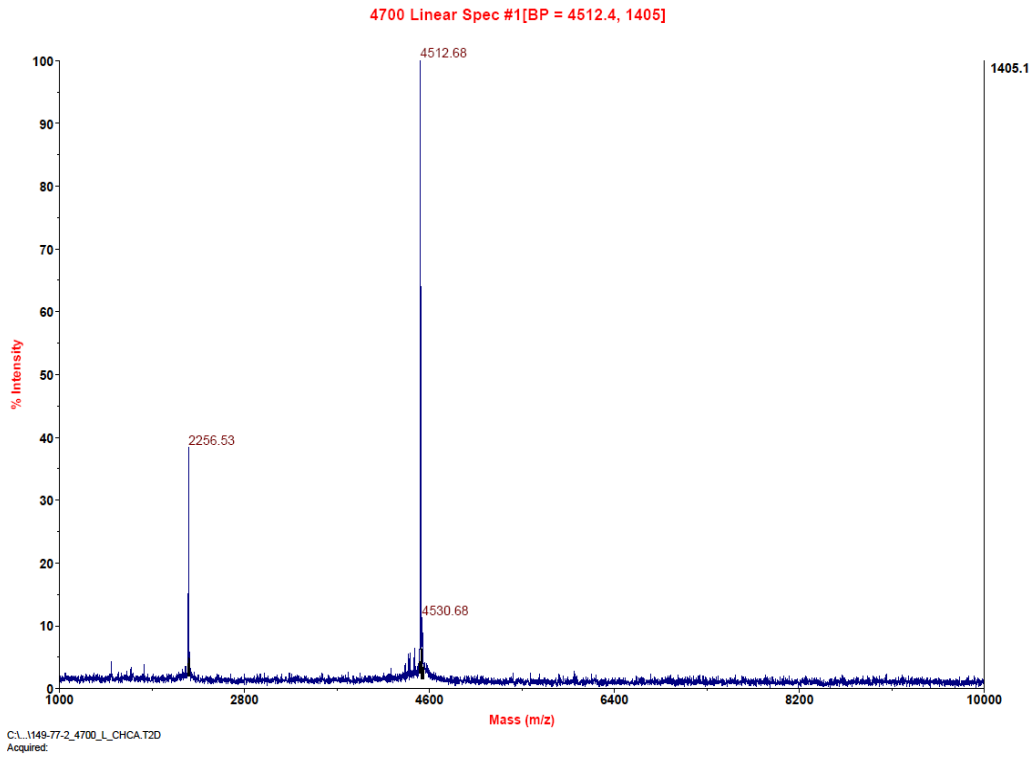


Figure S13. MALDI-TOF-MS data of Aβ42-lactam(22K-23E) where C_α and C=O in Gly-25 were labeled with ^{13}C (MH^+, average molecular mass; observed 4512.62, calculated 4512.16).

Experimental Section

General: The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; $[\alpha]_D$, Jasco DIP-1000; ^1H and ^{13}C NMR in solution, Bruker AVANCE 400 and JEOL ECP 500 (ref. TMS); FAB-MS, JEOL JMS-600H (matrix: glycerol). Wakogel™ C-200 (silica gel, Wako Pure Chemical Industries) and Silica gel 60 (0.040-0.063 mm) (MERCK) were used for column chromatography. L-Alanine ($^{13}C_3$,^{15}N), L-asparagine ($^{13}C_4$,$^{15}N2$), L-aspartic acid ($^{13}C_4$,^{15}N), L-glutamic acid ($^{13}C_5$,^{15}N), glycine ($^{13}C_2$,^{15}N), glycine ($^{13}C_2$), L-phenylalanine (ring-$^{13}C_6$), L-serine ($^{13}C_3$,^{15}N), and L-valine ($^{13}C_5$,^{15}N) were purchased from Taiyo Nippon Sanso Corporation (Tokyo, Japan). *N*-α-Carbobenzoxy-L-lysine (Z-Lys-OH) and *N*-α-tert-butoxycarbonyl-L-glutamic acid α-methyl ester (Boc-Glu-OMe) were obtained from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan).

Preparation of protected amino acids labeled with ^{13}C and ^{15}N: Fmoc derivatives of L-alanine ($^{13}C_3$,^{15}N), glycine ($^{13}C_2$,^{15}N), glycine ($^{13}C_2$), L-phenylalanine (ring-$^{13}C_6$), and L-valine ($^{13}C_5$,^{15}N) were synthesized as reported previously.[2] The crude compounds were purified by column chromatography on Wakogel™ C-200 using hexane and increasing amounts of

EtOAc containing 0.1% acetic acid, followed by recrystallization from hexane-EtOAc. The yields were 71-91%. The structures were confirmed by ^{1}H NMR, ^{13}C NMR, and FAB-MS measurements.

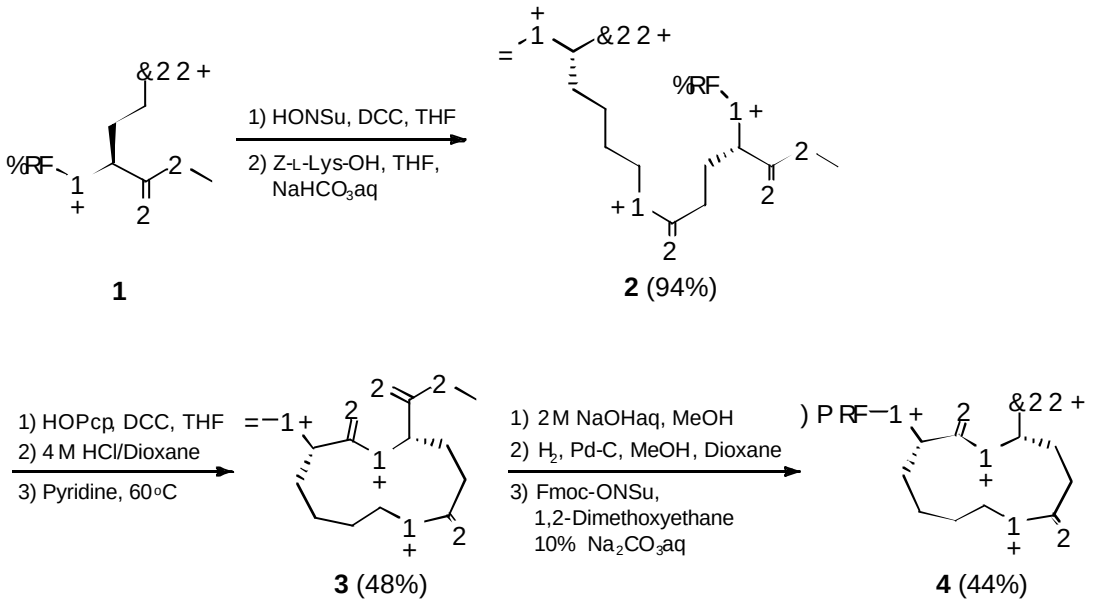
N-α-Fmoc-L-aspartic acid ($^{13}C_4$,^{15}N) β-tert-butyl ester and *N*-α-Fmoc-L-glutamic acid ($^{13}C_5$,^{15}N) γ-tert-butyl ester were synthesized as reported previously.[3] The crude product was purified by column chromatography on Silica gel 60 using hexane and increasing amounts of EtOAc containing 0.15% acetic acid. Recrystallization from hexane-EtOAc gave the final products as colorless needles with a 12-27% yield. Structures were confirmed by ^{1}H NMR, ^{13}C NMR, and FAB-MS measurements.

N-α-Fmoc-*O*-tert-butyl-L-serine ($^{13}C_3$,^{15}N) was synthesized as reported previously.[4] The crude product was purified by column chromatography on Wakogel^{TM} C-200 using hexane and increasing amounts of EtOAc containing 0.1% acetic acid. Recrystallization from hexane-EtOAc gave the final product as colorless needles with a 43% yield. Structure was confirmed by ^{1}H NMR, ^{13}C NMR, and FAB-MS measurements.

N-α-Fmoc-*N*-β-trityl-L-asparagine ($^{13}C_4$,$^{15}N_2$) was synthesized as reported previously.[5] The crude product was purified by column chromatography on Wakogel^{TM} C-200 using hexane and increasing amounts of EtOAc containing 0.1% acetic acid. Recrystallization from hexane-EtOAc gave the final product as colorless needles with a 57% yield. Structure was confirmed by ^{1}H NMR, ^{13}C NMR, and FAB-MS measurements.

Synthesis of Fmoc-Lys-Glu(lactam)-OH (4): Fmoc-Lys-Glu(lactam)-OH **(4)** was prepared as shown in Scheme S1. The procedure for cyclization of 12-membered lactam is based on methods described by Manesis *et al.*[6]

Boc-Glu-OMe (995 mg, 3.81 mmol) and *N*-hydroxysuccinimide (879 mg, 7.64 mmol) were dissolved in tetrahydrofuran (6.6 mL) and cooled to 0°C. With stirring, *N,N'*-dicyclohexylcarbodiimide (1.17 g, 5.67 mmol) in tetrahydrofuran (5.0 mL) was added to the mixture. The reaction mixture was stirred at room temperature for 4 h, cooled to 0°C, and *N,N'*-dicyclohexylurea was removed by filtration. The filtrate was used as the activated ester solution in the following reaction.



Scheme S1. Synthesis of Fmoc-Lys-Glu(lactam)-OH (**4**).

To a suspension of Z-Lys-OH (1.61 g, 5.74 mmol) in 3% aqueous $NaHCO_3$ at 0°C was added the above-mentioned activated ester solution. The reaction mixture was stirred at room temperature for 3 h. After evaporation of volatiles, the aqueous residue was acidified to pH 2 with 2 M HCl, extracted with EtOAc, and washed with brine. The EtOAc layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on Wakogel^{TM} C-200 using hexane and increasing amounts of EtOAc containing 0.1% acetic acid to give **2** (1.87 g, 3.57 mmol, 94% in two steps). Compound **2**: $[\alpha]_D$ - 1.20° (*c* 0.41, MeOH, 23.6°C). ^{1}H NMR δ (400 MHz, $CDCl_3$, 0.094 M, 296 K) ppm: 1.38 (2H, m, Lys γ-CH_2), 1.43 (9H, s, -OC(CH_3)$_3$, Boc), 1.52 (2H, m, Lys δ-CH_2), 1.77 (1H, m, Lys β-CH_aH), 1.89 (2H, m, Lys β-CH_bH, Glu β-CH_aH), 2.13 (1H, m, Glu β-CH_bH), 2.25 (2H, t, *J* = 6.7 Hz, Glu γ-CH_2), 3.21 (2H, m, Lys ε-CH_2), 3.71 (3H, s, -COOCH_3), 4.24 (1H, m, Glu α-CH), 4.36 (1H, m, Lys α-CH), 5.09 (2H, s, ArCH_2O-), 5.45 (1H, d, *J* = 7.2 Hz, Glu α-NH), 5.73 (1H, d, *J* = 7.1 Hz, Lys α-NH), 6.55 (1H, s, Lys ζ-NH), 7.27-7.34 (5H, m, Ar). ^{13}C NMR δ (100 MHz, $CDCl_3$, 0.094 M, 296K) ppm: 22.2 (Lys γ-CH_2), 28.4 (-OC(*C*H_3)$_3$, Boc), 28.9 (Lys δ-CH_2), 29.2 (Glu β-CH_2), 31.8 (Lys β-CH_2), 32.7 (Glu γ-CH_2), 39.2 (Lys ε-CH_2), 52.7 (-COO*C*H_3), 53.1 (Glu α-CH), 53.8 (Lys α-CH), 67.1 (Ar*C*H_2O-), 80.6 (-O*C*(CH_3)$_3$, Boc), 128.2, 128.3, 128.6 (2-, 3-, 4-, 5-, and 6-C, Ar), 136.4 (1-C, Ar), 156.2, 156.3 (-OCONH-, Z and -OCONH-, Boc), 172.9 (Glu α-CO and Glu δ-CO), 174.8 (Lys α-CO). HR-FAB-MS *m/z*: 524.2588 (MH^+, calcd for $C_{25}H_{38}N_3O_9$, 524.2608).

To a solution of **2** (1.86 g, 3.55 mmol) and pentachlorophenol (1.23 g, 4.62 mmol) in tetrahydrofuran (7.0 mL) at 0°C was added a solution of *N*,*N'*-dicyclohexylcarbodiimide (959 mg, 4.65 mmol) in tetrahydrofuran (4.0 mL). The reaction mixture was stirred at room temperature for 3 h, cooled to 0°C, and the resultant *N*,*N'*-dicyclohexylurea was removed by filtration. The filtrate was concentrated and purified by column chromatography on Wakogel^{TM} C-200 using hexane and increasing amounts of EtOAc to give the activated ester, which was dissolved in 4 M HCl/dioxane (16 mL) at 0°C. After stirring at room temperature for 30 min, the solvent was removed under reduced pressure to give the hydrochloride salt of the activated ester as an oil.

The above-mentioned activated ester dissolved in DMF (72 mL) was added, over 4 h with vigorous stirring, to pyridine (650 mL) maintained at 60°C. The solution was stirred at 60°C for 2 h. The resulting yellow solution was concentrated under reduced pressure. Traces of DMF and pyridine were removed as a toluene azeotrope. The residual yellow solids were extracted with chloroform and washed successively with 2 M aqueous $NaHSO_4$, 3% aqueous $NaHCO_3$, and brine. The chloroform layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on Wakogel^{TM} C-200 using a mixed solvent (hexane : chloroform = 1 : 1 by volume) and increasing amounts of 2-propanol to give **3** (697 mg, 48% in three steps). Compound **3**: $[\alpha]_D$ - 4.83° (*c* 0.23, MeOH, 22.9°C). ^{1}H NMR δ (400 MHz, $CDCl_3$, 0.050 M, 296 K) ppm: 1.46 (1H, m, Lys γ-C*H*$_a$H), 1.57 (1H, m, Lys δ-C*H*$_a$H), 1.69 (1H, m, Lys γ-C*H*$_b$H), 1.72-1.90 (3H, m, Lys β-CH_2, Lys δ-C*H*$_b$H), 2.11 (2H, m, Glu β-C*H*$_a$H, Glu γ-C*H*$_a$H), 2.30 (1H, m, Glu γ-C*H*$_b$H), 2.45 (1H, m, Glu β-C*H*$_b$H), 3.02 (2H, m, Lys ε-C*H*$_a$H), 3.75 (3H, s, -$COOCH_3$), 3.78 (1H, m, Lys ε-C*H*$_b$H), 4.26 (1H, m, Lys α-CH), 4.33 (1H, m, Glu α-CH), 5.08 (2H, m, ArCH_2O-), 5.56 (1H, d, *J* = 6.3 Hz, Lys α-NH), 6.00 (1H, s, Lys ζ-NH), 6.55 (1H, s, Glu α-NH), 7.27-7.36 (5H, m, Ar). ^{13}C NMR δ (100 MHz, $CDCl_3$, 0.050 M, 296 K) ppm: 18.9 (Lys γ-CH_2), 25.0 (Lys δ-CH_2), 25.6 (Glu β-CH_2), 29.7 (Lys β-CH_2), 32.9 (Glu γ-CH_2), 37.4 (Lys ε-CH_2), 52.4 (-$COOCH_3$), 53.6 (Glu α-CH), 53.8 (Lys α-CH), 66.8 (ArCH_2O-), 128.0, 128.1, 128.5 (2-, 3-, 4-, 5-, and 6-C, Ar), 136.4 (1-C, Ar), 155.6 (-OCONH-, Z), 171.2 (Glu α-CO), 171.4 (Lys α-CO), 173.5 (Glu δ-CO). HR-FAB-MS *m/z*: 406.1974 (MH^+, calcd for $C_{20}H_{28}N_3O_6$, 406.1978).

To a solution of **3** (588 mg, 1.45 mmol) in MeOH (15 mL) and 1,4-dioxane (5 mL) at 0°C was added 2 M aqueous NaOH (20 mL). The reaction mixture was stirred at room temperature for 30 min. The resultant solution was acidified to pH 2 with 2 M HCl, concentrated under reduced pressure to a volume of 5 mL, and extracted with EtOAc. The

EtOAc layer was washed with brine, dried over Na_2SO_4 and concentrated to give white solids, which were dissolved in a mixture of MeOH (20 mL), 1,4-dioxane (4.65 mL), and 4 M HCl/dioxane (0.35 mL). The solution was added to 10% Pd-C (123 mg) suspended in MeOH (5 mL), and the mixture was stirred vigorously under 1 atm of H_2 at room temperature for 30 min. The reaction mixture was filtered and then concentrated to give white solids.

The white solids dissolved in 10 % aqueous Na_2CO_3 (7.2 mL) were pipetted slowly into a solution of Fmoc-succinimide (734 mg, 2.18 mmol) dissolved in 1,2-dimethoxyethane (7.2 mL) at 0°C. After the reaction mixture was stirred at room temperature for 4 h, it was filtered and the volatiles in the filtrate were removed under reduced pressure. The aqueous residue was diluted with distilled water (30 mL) and acidified to pH 2 with 2 M HCl to liberate **4**, which was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel^{TM} C-200 using chloroform and increasing amounts of MeOH containing 0.1% acetic acid. Recrystallization from EtOAc-MeOH gave pure **4** (305 mg, 0.636 mmol, 44% in three steps) as colorless leaflets. The total yield was 20%. Compound **4**: $[\alpha]_D$ - 1.58° (*c* 0.20, DMF, 24.3°C). ^{1}H NMR δ (500 MHz, CD_3OD, 0.044 M, 296 K) ppm: 1.37 (1H, m, Lys γ-C*H*$_a$H), 1.48-1.65 (4H, m, Lys β-CH_2, Lys γ-C*H*$_b$H, Lys δ-C*H*$_a$H), 1.77 (1H, m, Lys δ-C*H*$_b$H), 2.15-2.30 (3H, m, Glu β-CH_2, Glu γ-C*H*$_a$H), 2.38 (1H, m, Glu γ-C*H*$_b$H), 2.95 (1H, dd, *J* = 13.7, 4.4 Hz, Lys ε-C*H*$_a$H), 3.79 (1H, t, *J* = 11.0 Hz, Lys ε-C*H*$_b$H), 3.93 (1H, dd, *J* = 11.2, 4.8 Hz, Lys α-CH), 4.22 (1H, t, *J* = 6.8 Hz, -C*H*CH_2O-, Fmoc), 4.28 (1H, t, *J* = 8.7 Hz, -CHC*H*$_a$HO-, Fmoc), 4.38-4.42 (2H, m, -CHC*H*$_b$HO-, Fmoc and Glu α-CH), 7.31 (2H, t, *J* = 7.5 Hz, 2- and 7-H, Ar, Fmoc), 7.38 (2H, t, *J* = 7.4 Hz, 3- and 6-H, Ar, Fmoc), 7.66 (2H, dd, *J* = 19.4, 7.4 Hz, 1- and 8-H, Ar, Fmoc), 7.78 (2H, d, *J* = 7.6 Hz, 4- and 5-H, Ar, Fmoc). ^{13}C NMR δ (125 MHz, CD_3OD, 0.044 M, 296 K) ppm: 20.6 (Lys γ-CH_2), 26.0 (Lys δ-CH_2), 27.5 (Glu β-CH_2), 29.5 (Lys β-CH_2), 34.5 (Glu γ-CH_2), 36.2 (Lys ε-CH_2), 45.1 (-*C*HCH_2O-, Fmoc), 54.4 (Glu α-CH), 58.3 (Lys α-CH), 68.1 (s, -CH*C*H_2O-, Fmoc), 120.9 (4- and 5-C, Fmoc), 126.2, 126.4 (1- and 8-C, Ar, Fmoc), 128.2, 128.8 (2- and 7-C, Ar, Fmoc), 142.58, 142.65 (4a- and 4b-C, Ar, Fmoc), 145.1, 145.6 (8a- and 9a-C, Ar, Fmoc), 158.0 (-CONH-, Fmoc), 174.2 (Glu α-CO), 174.8 (Lys α-CO), 175.3 (Glu δ-CO). HR-FAB-MS *m/z*: 480.2168 (MH^+, calcd for $C_{26}H_{30}N_3O_6$, 480.2135).

References

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