Supporting Information for

A Peptoid with Extended Shape in Water

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General remarks. Chemicals and solvents used in this study were purchased from commercial suppliers and used without further purification. Synthesis under microwave conditions was performed on Initiator+ (Biotage). Preparative HPLC was performed on a Prominence HPLC system (Shimazu) with a 5C18-MS-II column (Nacalai tesque, 10 mm I.D.×150 mm, 34355-91). Analytical HPLC was performed on a Prominence HPLC system with a 5C18-AR-II column (Nacalai tesque, 4.6 mm I.D.×150 mm, 38144-31). HRMS data was obtained using micrOTOF II (Bruker Daltonics). MALDI-TOF MS analysis was performed on autoflex speed (Bruker Daltonics) using super-DHB (Santa Cruz Biotechnology) as matrix. All quantum calculations were carried out with the Gaussian16 package[1] and all molecular dynamics simulations were carried out with the Gromacs 2018 package and the CHARMM36m/CGenFF force field.

Generation of Ramachandran-type energy diagrams of NSG and NSA. Ramachandran-type energy diagrams[2] of acetyl-*N*-methylglycine dimethylamide and acetyl-*N*-methylalanine dimethylamide were generated by combinatorially fixing φ and ψ at every 15° from –180° to 180°. Each conformer was optimized at the B3LYP/6-31G* level. The ω angle was fixed to 180° through the calculation. Ramachandran-type energy landscapes of acetyl-*N*-ethylalanine dimethylamide was generated by combinatorially fixing φ and ψ at every 15° from –180° to 180°. Each conformer was optimized at the B3LYP/6-31G* level. The ω angle was fixed to 180° through the calculation. Calculations were performed for two molecules with a fixed χ angle of either 100° or –100°.

χ scan of acetyl-*N*-ethylalanine dimethylamide. χ scan was performed by optimizing a conformer with χ angle of –180° to 180° with 10° increment. The calculation started with an initial conformation of (χ, φ, ψ) = (0°, –120°, 90°).

Computationally optimized conformations of a model NSA pentamer. Conformations of acetyl-*N*-ethylalanine pentamer dimethylamide with dihedral angles (χ, φ, ψ, ω) of either (100°, –105°, 105°, 180°) or (–100°, –120°, 90°, 180°) were optimized by quantum mechanical calculations. The two sets of the angles are the ones that were calculated to be the lowest energy angles in the Ramachandran-type diagrams of acetyl-*N*-ethylalanine dimethylamide. Each conformer was optimized at the B3LYP/6-31G* level using a self-consistent reaction field (SCRF) model with water as the solvent. Single point energy of the optimized conformer was also calculated at the B3LYP/6-31G* level using a SCRF model with water as the solvent.

Synthesis of NSA-containing peptide using bis(trichloromethyl)carbonate (BTC) as a coupling reagent. Rink amide ChemMatrix resin was swelled with minimal volume of *N*,*N*-dimethylformamide (DMF) in a syringe for 30 min. DMF was filtered off and the resin was treated with DMF solution of Fmoc-Trp(Boc)-OH (4 equiv., 0.2 M),

1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU) (4 equiv., 0.2 M) and DIPEA (*N*,*N*-diisopropylethylamine) (8 equiv., 0.4 M) with continuous shaking for 2 h. After removing solution, the resin was washed with DMF three times. The resin was treated with 20% piperidine/DMF (3 min and 12 min) and then washed with DMF and tetrahydrofuran (THF) three times each. The resin was incubated with 27 equiv. of DIPEA in anhydrous THF for 15 min. A solution of BTC (4 equiv., 0.1 M) and (*R*)-2-bromopropionic acid or (*S*)-2-bromopropionic acid (0.1 M, 12 equiv.) in anhydrous THF was cooled at –20 °C for 15 min and mixed with 36 equiv. of 2,4,6-trimethylpyridine. The solution was immediately applied to the resin and the reaction mixture was shaken for 2 h. After the reaction, the resin was washed with THF and DMF three times each. The resin was incubated with 2 M (80 equiv.) of an isobutylamine solution in anhydrous DMF for 18 h at 60 °C. The solution was filtered off and the resin was washed with DMF and dichloromethane (DCM) three times each. The peptides were cleaved by treating the resin with 95/5 TFA (trifluoroacetic acid)/H_2O for 2 h. The solution was transferred to a recovery flask and TFA solution was removed under reduced pressure. The crude product was dissolved in 10/90 acetonitrile/water and analyzed by a reversed phase column on HPLC.

Investigation about coupling conditions and evaluation of racemization during synthesis. Rink amide ChemMatrix resin was swelled with minimal volume of DMF in a syringe for 30 min. DMF was filtered off and the resin was treated with DMF solution of Fmoc-Trp(Boc)-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) with continuous shaking for 40 min. After removing solution, the resin was washed with DMF three times. The resin was treated with 20% piperidine/DMF (3 min and 12 min) and then washed with DMF three times. After deprotection, the resin was treated with DMF solution of Fmoc-Ala-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M). After 40 min, the resin was washed with DMF and Fmoc group was removed by treatment with 20% piperidine/DMF. The resin was incubated with 1 M (20 equiv.) of an isobutylaldehyde solution in anhydrous DMF. Aldehyde solution was filtered off and the resin was quickly washed with DMF and DCM. A freshly prepared suspension of $NaBH_4$ (10 equiv.) in 75/25 DCM/Methanol was added to the resin and shaken for 30 min. The cap of the syringe was occasionally detached for degassing to prevent inner pressure is increased too much. After reduction, the resin was washed with methanol five times then with DCM, DMF and dioxane three times each. When using COMU or 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) as a coupling reagent, a solution of Fmoc-L-Ala-OH (4 equiv., 0.2 M), coupling reagent (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) in anhydrous DMF was added to the resin and shaken under the condition shown in Figure S3. After the reaction, the resin was washed with DMF three times. When BTC was used as the coupling reagent, the resin was washed with THF three times and incubated with 8 equiv. of DIPEA in anhydrous THF for 15 min.

A solution of Fmoc-L-Ala-OH (3.5 equiv., 0.1 M) and BTC (1.17 equiv., 0.1 M) in anhydrous THF was cooled at –20 °C for 15 min and mixed with 10 equiv. of 2,4,6-trimethylpyridine. The solution was immediately applied to the resin and the reaction mixture was shaken under the condition shown in Figure S3. After the reaction, the resin was washed with THF and DMF three times each. When using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), the resin was washed with dioxane three times and a solution of Fmoc-L-Ala-OH or Fmoc-D-Ala-OH (4 equiv., 0.2 M) and EEDQ (4 equiv., 0.2 M) in dioxane was added to the resin and shaken under the condition shown in Figure S3. After the reaction, the resin was washed with dioxane and DMF three times each. After the coupling reaction to *N*-substituted alanine terminus with each coupling reagent, Fmoc protecting group was removed with 20% piperidine/DMF. The peptides were cleaved by treating the resin with 95/5 TFA/H_2O for 2 h. The solution was transferred to a flask and TFA solution was removed under reduced pressure. The crude product was dissolved in acetonitrile and water and analyzed by a reversed phase column on HPLC.

General procedure for oligo-NSA synthesis. Trityl resin was swelled with minimal volume of THF in a flask for 10 min. THF solution of piperazine (4 equiv., 0.2 M) and piperidine (16 equiv., 0.8 M) was added to the resin. Piperidine was added in order to reduce the reactive points on the resin. After stirring for 2 h, resin was moved to syringe and washed with THF and DCM three times each. The resin was treated with 85/10/5 DCM/methanol/DIPEA solution for 15 min and washed with DCM and DMF three times each. The resin was treated with DMF solution of Fmoc-Ala-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) with continuous shaking for 2 h. After removing solution, the resin was washed with DMF and DCM three times each and dried. A small part of the resin was treated with 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/DMF solution and then the load amount of Fmoc-alanine was determined from the UV absorbance of dibenzofulvene at 304 nm as described in reference.[3] In the following procedures, the amount of reagents was calculated based on the determined loading amount. After the quantification, the resin was swelled with minimal volume of DMF for 30 min. The resin was treated with 20% piperidine/DMF (3 min and 12 min) to remove Fmoc protecting group and washed with DMF three times. The resin was incubated with 1 M (20 equiv.) of an aldehyde solution in DMF. Aldehyde solution was filtered off and the resin was quickly washed with DMF and DCM. A freshly prepared suspension of $NaBH_4$ (10 equiv.) in 75/25 DCM/methanol was added to the resin and shaken for 30 min. The cap of the syringe was occasionally detached for degassing to prevent inner pressure is increased too much. After reduction, the resin was washed with methanol five times then with DCM, DMF and dioxane three times each. Fmoc-Ala-OH (4 equiv.) and EEDQ (4 equiv.) were dissolved in dioxane to prepare 0.2 M solution and the mixture was shaken. After 30 min, the mixture was added to the resin and shaken for 3 h at 60 °C. This coupling reaction was repeated once more. After double coupling, Fmoc protecting group was removed with 20% piperidine/DMF and substituents

were introduced by reductive amination. The coupling, deprotection and reductive amination procedures were repeated to afford objective oligomers on resin. The oligomers were cleaved by treating the resin with 30% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)/DCM for 20min five times. The solution was transferred to a glass vial. The resin was washed with methanol three times and the solutions were collected in the same glass vial. The collected solution was removed under reduced pressure. The crude product was dissolved in acetonitrile and water and purified by a reversed phase column on HPLC using acetonitrile and water containing 0.1% TFA as solvents. The yields were calculated by comparing molecular weight of each obtained compound as TFA salt and the determined loading amount of the first Fmoc-alanine. The crude and purified products were analyzed on a reversed phase column by HPLC and ESI-TOF MS.

***N*-Isobutylalanine piperazineamide (1).** 42 mg of trityl resin (1.96 mmol/g, 83 μmol) was used for synthesis. Fmoc-alanine and isobutylaldehyde were used as submonomers. After loading first Fmoc-alanine, load amount was determined as 0.48 mmol/g. Yield was 7.2 mg (80%). ^{1}H NMR (CD_3CN, 400 MHz): *d* 1.00–1.04 (q, 6H, *J* = 6.9 Hz), 1.55 (d, 3H, *J* = 6.9), 1.97–2.10 (m, 1H), 2.74–2.82 (dd, 1H, *J* = 7.3, 12.4), 2.74–2.99 (dd, 1H, *J* = 6.9, 12.4), 3.33-3.43 (m, 4H), 3.83–3.97 (m, 4H), 4.48 (q, 1H, *J* = 6.9 Hz). ^{13}C NMR (CD_3CN, 400 MHz): δ 14.7, 19.1, 19.2, 25.9, 39.1, 42.0, 42.8, 42.8, 53.9, 54.4, 168.3. The ^{1}H NMR is shown in Figure S7 and ^{13}C NMR spectra is shown in Figure S8**.** HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{11}H_{24}N_3O^+$ 214.1914; Found 214.1929.

***N*-Isobutylalanine dimer piperazineamide (2).** 42 mg of trityl resin (1.96 mmol/g, 83 μmol) was used for synthesis. Fmoc-alanine and isobutylaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.48 mmol/g. Yield was 8.2 mg (71%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{18}H_{37}N_4O_2^+$ 341.2911; Found 341.2889.

***N*-Isobutylalanine trimer piperazineamide (3).** 41 mg of trityl resin (1.96 mmol/g, 80 μmol) was used for synthesis. Fmoc-alanine and isobutylaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.33 mmol/g. Yield was 5.1 mg (54%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{50}N_5O_3^+$ 468.3908; Found 468.3916.

***N*-Isobutylalanine tetramer piperazineamide (4).** 39 mg of trityl resin (1.96 mmol/g, 76 μmol) was used for synthesis. Fmoc-alanine and isobutylaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.46 mmol/g. Yield was 8.1 mg (55%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{32}H_{63}N_6O_4^+$ 595.4905; Found 595.4905.

***N*-Isobutylalanine pentamer piperazineamide (5).** 42 mg of trityl resin (1.96 mmol/g, 83 μmol) was used for synthesis. Fmoc-alanine and isobutylaldehyde were used as submonomers. After

loading first Fmoc-alanine, the loading amount was determined as 0.48 mmol/g. Yield was 9.2 mg (47%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{39}H_{76}N_7O_5^+$ 722.5902; Found 722.5890.

***N*-Benzylalanine pentamer piperazineamide (6).** 38 mg of trityl resin (1.96 mmol/g, 75 μmol) was used for synthesis. Fmoc-alanine and benzaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.56 mmol/g. Yield was 6.4 mg (27%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{54}H_{66}N_7O_5^+$ 892.5120; Found 892.5132.

NSA heteropentamer (7). 32 mg of trityl resin (1.96 mmol/g, 63 μmol) was used for synthesis. Fmoc-alanine, benzaldehyde, isobutylaldehyde and acetaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.51 mmol/g. Yield was 6.2 mg (41%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{38}H_{66}N_7O_5^+$ 700.5120; Found 700.5120.

NSA heteropentamer (8). 40 mg of trityl resin (1.96 mmol/g, 78 μmol) was used for synthesis. Fmoc-alanine, acetaldehyde, isobutylaldehyde, cyclohexanecarboxaldehyde, n-butylaldehyde and cyclopropanecarboxaldehyde were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.38 mmol/g. Yield was 4.9 mg (35%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{40}H_{74}N_7O_5^+$ 732.5746; Found 732.5742.

NSA heteropentamer (S6). 24 mg of trityl resin (1.96 mmol/g, 47 μmol) was used for synthesis. Fmoc-alanine, isobutylaldehyde, tert-butyl formylacetate and tert-butyl *N*-(2-oxoethyl)carbamate were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 1.28 mmol/g. The procedures for elongation of oligomers on resin are almost the same as '**General procedure for oligo-NSA synthesis**', but the conditions for reductive amination were as follows. After the removal of Fmoc group, the resin was washed with 1,2-dichloroethane (DCE) three times. The resin was incubated with 1 M (20 equiv.) of an aldehyde solution in DCE. Aldehyde solution was filtered off and the resin was quickly washed with DCE three times. A freshly prepared suspension of $NaBH(OAc)_3$ (10 equiv.) in DCE was added to the resin and shaken. After 30 min, the resin was washed with methanol five times then with DCE and DMF three times each. Yield was 0.3 mg (1%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{45}H_{85}N_8O_9^+$ 881.6434; Found 881.6409.

NSA heteropentamer (S7 and S8). 42 mg of trityl resin (1.96 mmol/g, 82 μmol) was used for synthesis. Fmoc-alanine, isobutylaldehyde, allyl formylacetate and allyl-*N*-(3-oxopropyl)carbamate were used as submonomers. After loading first Fmoc-alanine, the loading amount was determined as 0.91 mmol/g. The procedures are almost the same as the procedures for NSA heteropentamer **S6**, but the conditions for reductive amination using allyl formylacetate were as follows. After the removal of Fmoc group, the resin was washed with DCE three times. The resin was incubated with 0.1 M

(1.2 equiv.) of an aldehyde solution in DCE. Aldehyde solution was filtered off and the resin was quickly washed with DCE three times. A freshly prepared suspension of $NaBH(OAc)_3$ (10 equiv.) in DCE was added to the resin and shaken. After 30 min, the resin was washed with methanol five times then with DCE, DMF three times each.

After the solid-phase synthesis, resin was split into two. The protected compound was cleaved from the half of the resin to yield heteropentamer **S7**. Yield was 0.6 mg (3%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{44}H_{79}N_8O_9^+$ 863.5965; Found 863.5993.

The other half was treated with tetrakis(triphenylphosphine)palladium(0) (6 equiv.) and phenylsilane (24 equiv.) in anhydrous DCM for 30 min. The resin was washed with DCM and DMF three times each followed by the treatment with 0.5% DIPEA in DMF for 10 min and washed with 0.5% DIPEA in DMF twice. The solution of 0.5% sodium diethyldithiocarbamate in DMF was added and the reaction vessel was shaken for 10 min. The solution was filtered off and the resin was washed with the same solution twice. The resin was again washed with DMF and DCM three times each. The unprotected compound was cleaved from the resin to yield heteropentamer **S8**. Yield was 0.3 mg (1%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{37}H_{71}N_8O_7^+$ 739.5440; Found 739.5468.

Synthesis of *N*-isobutylglycine pentamer with C-terminal piperazine amide (5-NSG). 56 mg of trityl resin (1.96 mmol/g, 110 μmol) was used for synthesis. Fmoc-Gly-OH and isobutylaldehyde were used as submonomers. The procedures are almost the same as '**General procedure for oligo-NSA synthesis**', but Fmoc-Gly-OH was used instead of Fmoc-Ala-OH and coupling reactions are conducted by the treatment of resin with DMF solution of Fmoc-Gly-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) for 3 h. Yield was 2.3 mg (4%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{34}H_{66}N_7O_5^+$ 652.5120; Found 652.5117.

Synthesis of *N*-isobutylalanine pentamer with C-terminal amide (5-amide). 98 mg of Sieber Amide resin (0.54 mmol/g, 53 μmol) was swelled with minimal volume of DMF in a syringe for 30 min. The resin was treated with 20% piperidine/DMF (3 min and 12 min) to remove Fmoc protecting group and washed with DMF three times. DMF solution of Fmoc-Ala-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) was added to the resin and shaken for 1 h. The resin was washed with DMF three times. After the removal of Fmoc protecting group, isobutyl group was introduced on nitrogen by reductive amination. The coupling reaction with EEDQ, deprotection and introduction of substituents by reductive amination were conducted and repeated as described in '**General procedure for oligo-NSA synthesis**' to afford objective oligomers on resin. The oligomers were cleaved by treating the resin with 1% TFA/DCM for 2 min. The solution was transferred to a glass vial containing pyridine/methanol to quench TFA. This cleavage process was repeated five times. The resin was washed with methanol three times and the washing solutions were

collected in the glass vial. The solution was removed under reduced pressure. The crude product was dissolved in acetonitrile and water and purified by a reversed phase column on HPLC using acetonitrile and water containing 0.1% TFA as solvents. The yield was calculated by comparing the amount of the obtained product with the amount of the first Fmoc-alanine loaded on resin. The purified products were analyzed on a reversed phase column by HPLC and ESI-TOF MS. Yield was 13.6 mg (34%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{35}H_{69}N_6O_5^+$ 653.5324; Found 653.5343.

Synthesis of tert-butyl formylacetate. The compound was synthesized according to the previous reports.[4,5]

First, formyl Meldrum's acid was synthesized. Meldrum's acid (7.21 g, 50.0 mmol) in a recovery flask was mixed with triethyl orthoformate (20.8 mL, 125 mol). The mixture was heated in an oil bath at 85°C with continuous stirring. The reaction was performed for 2 h. The excess triethyl orthoformate was removed under reduced pressure. 100 mL of 2 M HCl aq. was added to the product with continuous stirring. The mixture was stirred at room temperature for 30 min and then filtered. The solid was dissolved in 60 mL of DCM and washed with brine twice (30 mL each). The filterate was extrated with DCM three times (30 mL each). The all organic phases were combined, dried, filtered and evaporated to give a bright yellow solid. Yield was 6.66 g (77%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.77 (s, 6H), 8.56 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.4, 95.6, 107.2, 160.7, 168.2, 177.1. HRMS (ESI-TOF MS) *m/z*: $[M - H]^-$ Calcd for $C_7H_7O_5^-$ 171.0293; Found 171.0299.

The desired aldehyde was synthesized using the formyl Meldrum's acid. Under nitrogen atmosphere, a solution of 3.05 g of formyl Meldrum's acid (17.7 mmol) and 1.99 mL of tert-butyl alcohol (20.9 mmol) in 40 mL of toluene was stirred at 80 °C for 3 h. The solvent was removed under reduced pressure at room temperature, and the residue was purified by Kugelrohr destillation at 70 °C under reduced pressure to yield colorless liquid. According to NMR spectroscopy, the compound is a mixture of an aldehyde and an enol tautomer (aldehyde/enol = 77/23). Yield was 0.41 g (16%). ^{1}H NMR ($CDCl_3$, 400 MHz): aldehyde, δ 1.50 (s, 9H), 3.31 (d, J = 2.4 Hz, 2H), 9.79 (t, J = 2.4 Hz, 1H); enol, δ 1.50 (s, 9H), 4.98 (d, J = 6.1 Hz, 1H), 7.06 (dd, J = 12.5, 6.1 Hz, 1H), 11.57 (d, J = 12.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): aldehyde, δ 28.2, 49.9, 82.7, 166.1, 195.8; enol, δ 28.4, 81.4, 95.0, 163.2. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_7H_{12}O_3Na^+$ 167.0679; Found 167.0676.

Synthesis of allyl formylacetate. The compound was synthesized according to the previous report.[5] Under nitrogen atmosphere, a solution of 2.97 g of formyl Meldrum's acid (17.3 mmol) and 1.43 mL of allyl alcohol (20.9 mmol) in 40 mL of toluene was stirred at 80 °C for 3 h. The solvent was removed under reduced pressure at room temperature, and the residue was purified by Kugelrohr

destillation at 70 °C under reduced pressure to yield colorless liquid. According to NMR spectroscopy, the compound is a mixture of an aldehyde and two enol tautomers (aldehyde/enol 1/enol 2 = 53/28/19). Yield was 0.40 g (18%). ^{1}H NMR ($CDCl_3$, 400 MHz): aldehyde, δ 3.44 (d, J = 2.4 Hz, 2H), 4.68 (dt, J = 5.8, 1.2 Hz, 2H), 5.20–5.40 (m, 2H), 5.84–6.07 (m, 1H), 9.83 (t, J = 2.4, 1H); enol 1, δ 4.64–4.67 (m, 2H), 5.13 (d, J = 6.1, 1H), 5.20–5.40 (m, 2H), 5.84–6.07 (m, 1H), 7.13 (dd, J = 12.8, 6.1 Hz, 1H), 11.34 (d, J = 13.1 Hz, 1H); enol 2, δ 4.81 (dt, J = 5.8, 1.4 Hz, 2H), 5.22–5.40 (m, 2H), 5.84–6.07 (m, 1H), 7.66 (d, J = 12.8, 1H), 12.61 (d, J = 12.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): aldehyde, δ 48.5, 66.2, 119.3, 131.5, 166.6, 194.9; enol 1, 64.9, 93.3, 118.6, 132.0, 164.1, 171.8; enol 2, 65.2, 104.6, 119.7, 131.3, 169.6, 170.9. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_6H_8O_3Na^+$ 151.0366; Found 151.0372.

Synthesis of allyl-*N*-(3-oxopropyl)carbamate. The compound was synthesized according to the previous report.[5,6]

First, allyl 3-hydroxypropylcarbamate was synthesized. A solution of 3-aminopropanol (1.52 mL, 20.0 mmol) and Na_2CO_3 (2.22 g, 20.9 mmol) in 30 mL of 2/1 H_2O/ACN was cooled to 0 ºC. 2.32 mL of allyl chloroformate (21.9 mmol) was added to the solution over 20 min with continuous stirring and then the mixture was stirred at room temperature overnight. After the evaporation of acetonitrile, the solution was acidified to pH 2 with HCl aq. and extracted with ethyl acetate three times. The organic phase was dried, filtered and evaporated to give colorless oil. Yield was 2.91 g (92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.66–1.78 (m, 2H), 2.84 (bs, 1H), 3.34 (dd, J = 10.4, 6.0 Hz, 2H), 3.68 (t, J = 6.0 Hz, 2H), 4.57 (d, J = 5.5, 2H), 5.20 (bs, 1H), 5.22 (dd, J = 10.3, 1.4 Hz, 1H), 5.30 (dd, J = 17.4, 1.4 Hz, 1H), 5.85–5.99 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 32.6, 37.8, 59.6, 65.8, 117.8, 132.9, 157.3. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_7H_{13}NO_3Na^+$ 182.0788; Found 182.0793.

The desired aldehyde was synthesized using allyl 3-hydroxypropylcarbamate. Under nitrogen atmosphere, DMSO (2.13 mL, 30.0 mmol) solution in anhydrous DCM (30 mL) was added to a stirred solution of oxalyl chloride (1.71 mL, 20.0 mmol) in anhydrous DCM (30 mL) at –78 ºC and the mixture was stirred for 30 min at the same temperature. To the stirred reaction mixture, 40 mL of allyl 3-hydroxypropylcarbamate (1.59 g, 10.0 mmol) solution in DCM was added at –78 ºC. After 1 h, triethylamine (6.9 mL, 50.0 mmol) was added and the mixture was stirred at 0 ºC for 1 h. The reaction mixture was allowed to gradually warm to room temperature and stirred for another 1.5 h. After adding saturated aqueous $NaHCO_3$, the organic phase was separated. The aqueous phase was extracted with chloroform three times. The all organic phases were combined and washed with brine, dried, filtered and evaporated. The residue was purified with automated flash column chromatography (Isolera One, Biotage). The product was a yellow liquid. Yield was 0.47 g (30%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.74 (t, J = 6.1, 2H), 3.49 (q, J = 6.1 Hz, 2H), 4.55 (d, J = 5.5 Hz, 2H), 5.15 (bs, 1H), 5.21 (d, J = 10.4, 1H), 5.29 (dd, J = 17.9, 1.1 Hz, 1H), 5.83–5.99 (m, 1H), 9.81 (s,

1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 34.6, 44.2, 65.7, 117.9, 132.9, 156.3, 201.3. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_7H_{11}NO_3Na^+$ 180.0631; Found 180.0640.

Evaluation of water solubility of oligo-NSA and oligo-NSG. 400 μL of 10 mM solution of oligo-NSA **5**, **5-amide** or oligo-NSG **5-NSG** in water was transferred to a microtube and lyophilized. 10 μL of water was added to the lyophilized oligomer and the suspension was sonicated for 30 min. Then the sample was centrifuged at 20,000g for 30 min. 1 μL of the supernatant was aliquot and diluted to 1 mL with water. One eighth of each sample was injected to HPLC and analyzed on a reversed phase column. To determine the amount of dissolved compound, 1, 5, 10, 20 or 50 nmol of oligo-NSA **5**, **5-amide** or oligo-NSG **5-NSG** were also analyzed by HPLC and calibration curves were prepared based on the peak area of each sample.

Crystallization. A lyophilized product of *N*-benzylalanine pentamer piperazineamide (**6**) was dissolved in a mixture of hexane and dichloromethane. The solution was left in a glass vial capped with a plastic cap with small holes to let solvent slowly evaporate until crystals appeared in solution.

X-ray crystallography. A single crystal was mounted with mineral oil on a loop-type mount and set on VariMax Dual (Rigaku). The X-ray diffraction data was measured at –180 °C using Mo *Kα* radiation (λ = 0.7107 Å). Data was processed using the CrystalClear software (Rigaku). The structure was solved by a direct method using SHELXT[7] and refined using SHELXL. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed on ideal positions.

Molecular dynamics simulations. MD simulations of the oligo-NSA, the oligo-NSG and the oligomer of alternate NSA and NSG residues were performed using GROMACS 2018.1[8] with the CHARMM36m force field and the TIP3P water model.[9] The force field for the peptoid molecules was derived from the CGenFF.[10] The initial structure of each simulation was derived from the crystal structure of NSA and was solvated with TIP3P water in a rectangular box such that the minimum distance to the edge of the box was 15 Å under periodic boundary conditions through the CHARMM-GUI.[11] Na and Cl ions were added to imitate a salt solution of concentration 0.14 M. The system was energy-minimized for 10,000 steps and equilibrated with the NVT ensemble (298 K) for 1 ns. Further simulations were performed with the NPT ensemble at 298 K for 500 ns. For each system, the simulation was repeated 5 times with different initial velocities (i.e. 2.5 μs in total for each peptoid). The time step was set to 2 fs throughout the simulations. A cutoff distance of 12 Å was used for Coulomb and van der Waals interactions. Long-range electrostatic interactions were evaluated by means of the particle mesh Ewald method.[12] Covalent bonds involving hydrogen atoms were constrained by the LINCS algorithm.[13] A snapshot was saved every 10 ps. For the analysis of each trajectory, we employed the last 400 ns.

One-dimensional dihedral scanning. The QM and MM dihedral scans were performed using the Force Field Toolkit through the VMD interface.[14,15] The QM profile was constructed with Gaussian16 package at the B3LYP/6-31G* level. The MM profile was obtained with the NAMD energy plugin in VMD,[16] based on the CGenFF potential used in our MD simulations. The parameter files generated by CGenFF were provided as Table S1.

Synthesis of oligo-NSA with terminal spin labels (7). 20 mg of trityl resin (1.96 mmol/g, 39 μmol) was used for synthesis. NSA hexamer was synthesized on resin as described in **'General procedure for oligo-NSA synthesis'** using Fmoc-alanine, isobutylaldehyde, and acetaldehyde as submonomers. After condensation of Fmoc-alanine at *N*-terminus and removal of Fmoc protecting group, the oligomer was cleaved and purified on HPLC. 1.23 μmol of the oligomer was used for further reaction. Terminal spin labeling was conducted by incubating a DMF solution of the oligomer (45 μL), 2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl-3-carboxylic acid (2.7 equiv., 0.07 M), (Benzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1.3 equiv., 0.03 M), and DIPEA (2.3 equiv., 0.06 M) at room temperature overnight. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_{59}H_{101}N_{11}O_{11}Na^+$ 1162.7574; Found 1162.7575.

Synthesis of NSA oligomer with a terminal spin label at C-termini (7-monolabel). When the oligo-NSA **7** was labeled with the nitroxide spin radical as described in **'Synthesis of NSA oligomer with terminal spin labels (7)'**, an oligomer that is monolabeled at either N-terminus or C-terminus was isolated during the HPLC purification process. The oligomer that is monolabeled at C-terminus was stored and used as a control for EPR measurement. HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{51}H_{89}N_{10}O_9^+$ 974.6887; Found 974.6885.

Synthesis of oligo-NSG with terminal spin labels (7-NSG). 20 mg of trityl resin (1.96 mmol/g, 39 μmol) was swelled with minimal volume of THF in a flask for 10 min. THF solution of piperazine (4 equiv., 0.2 M) and piperidine (16 equiv., 0.8 M) was added to the resin. After stirring for 2 h, resin was moved to syringe and washed with THF and DCM three times each. The resin was treated with 85/10/5 DCM/methanol/DIPEA solution for 15 min and washed with DCM and DMF three times each. DMF solution of bromoacetic acid (20 equiv., 1 M) and *N*,*N*-diisopropylcarbodiimide (10 equiv., 0.5 M) were incubated for 10 min and mixed with 20 equiv. of TMP. The mixture was added to the resin and the reaction mixture was shaken for 30 min. The resin was washed with DMF three times and incubated with DMF solution of 1 M ethylamine. After 2 h, the resin was washed with DMF three times. Acylation and amination procedures were repeated until NSG hexamer was synthesized on resin using bromoacetic acid, ethylamine and isobutylamine as submonomers. After condensation of Fmoc-alanine at N-terminus and removal of Fmoc group, the oligomer was cleaved

and purified on HPLC. 0.63 μmol of the oligomer was labeled as described in **'Synthesis of NSA oligomers with terminal spin labels (7).'** HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_{53}H_{89}N_{11}O_{11}Na^+$ 1078.6635; Found 1078.6633.

Synthesis of oligomer consisting of alternate NSA/NSG residues with terminal spin labels (7-NSA/NSG). 20 mg of trityl resin (1.96 mmol/g, 39 μmol) was used for synthesis. Oligomer with alternate NSA/NSG residues with C-terminal piperazine amide was synthesized on resin as described in **'General procedure for oligo-NSA synthesis'** using Fmoc-alanine, Fmoc-glycine, isobutylaldehyde, and acetaldehyde as submonomers. For coupling reaction, the resin was treated with Fmoc-amino acid (4 equiv., 0.2 M) and EEDQ (4 equiv., 0.2 M) in dioxane for 3 h. After condensation of Fmoc-alanine at N-terminus and removal of Fmoc protecting group, the oligomer was cleaved and purified for further labeling. 1.63 μmol of the oligomer was labeled as described in **'Synthesis of NSA oligomers with terminal spin labels (7).'** HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_{56}H_{95}N_{11}O_{11}Na^+$ 1120.7105; Found 1120.7106.

DEER measurements. EPR experiments were performed by Bruker E680 spectrometer with an Oxford Instruments CF935 liquid helium cryostat. The spin-labeled samples were loaded into EPR tubes at room temperature, then rapidly frozen in liquid nitrogen bath. All the EPR spectra were measured at 20 K. For electron spin echo (ESE) field sweep measurement, a $\pi/2$-τ_1-π sequence with time interval τ_1 of 200 ns was used. Figure S20 shows the ESE field swept spectrum of the oligo-NSA, which closely resembles spectra of the other labeled oligomer samples (data not shown). Experimental conditions: microwave frequency, 9.69 GHz; pulse lengths, 16 and 24 ns; repetition time, 500 μs. For DEER measurement, a four-pulse $\pi/2$-τ_1-π-τ_1-τ_2-π sequence with time interval τ_1 of 200 ns and τ_2 of 1200 ns were used. An 8 step τ_1 averaging with $\Delta\tau_1$ = 8 ns was utilized to remove artifacts arising from proton nuclear modulation in DEER signals.[17] Pulse lengths of the observation $\pi/2$ and π pulse were 16 and 24 ns, respectively, and length of the pumping pulse was 20 ns. Repetition time was 350 μs. The observation and pumping microwave frequencies with a difference of 72 MHz were set to resonate the magnetic fields shown by the filled (observation) and blank (pumping) arrows in Figure S20. The distance distributions were computed with DeerAnalysis2016 software.[18] Figure S21 represents DEER signals after background correction using a homogeneous distribution model with a dimension fitting to DEER decay trace of monolabeled oligo-NSA.

NMR spectroscopic studies. NMR spectra of NSA pentamer **8** were recorded at 5 mM in D_2O on a JEOL ECS-400. 1H NMR and 13C NMR spectrum are shown in Figure S23 and Figure S25. COSY spectrum was recorded with relaxation delay of 1.5 s and receiver gain of 42 (Figure S24). HMBC spectrum was recorded with x points of 2048, y points of 2048, relaxation delay of 1.5 s and receiver gain of 90 (Figure S26). NOESY spectrum was recorded with relaxation delay of 1.5 s, mixing time

of 0.8 s and receiver gain of 42 (Figure S27 and S28). Chemical shifts of 1H NMR, HMBC, COSY and NOESY spectrum are reported in p.p.m relative to solvent peaks as internal standards (δH, H_2O 4.79 ppm). Assignment of 1H NMR was assisted by COSY and HMBC spectrum. Sequential assignment of main chain alpha protons was completed from their intra-residual and inter-residual cross peaks with carbonyl carbon in HMBC spectrum. Sequential assignment of alpha protons on *N*-substituents was completed from their inter-residual cross peaks with carbonyl carbon in HMBC spectrum. All assignment was shown in Figure S23. The obtained cross peaks were interpreted based on QM calculations of a model NSA dimer. More specifically, acetyl-*N*-ethylalanine dimer dimethylamide with dihedral angles (χ, φ, ψ, ω) of (–100°, –120°, 90°, 180°) was systematically rotated at the φ angle of C-terminal residue or ψ angle of *N*-terminal residue with 15° increment at a time and optimized at the B3LYP/6-31G* level using a SCRF model with water as the solvent. The distances between α or β protons and N_α protons on each conformer were listed as tables on Figure S29. NMR spectroscopic studies were also conducted in acetonitrile-d_3 and methanol-d_4. 1H NMR, COSY, 13C NMR, HMBC and NOESY in acetonitrile-d_3 are shown in Figure S30–S35. COSY spectrum was recorded with relaxation delay of 1.5 s and receiver gain of 42. HMBC spectrum was recorded with x points of 2048, y points of 2048, relaxation delay of 1.5 s and receiver gain of 84. NOESY spectrum was recorded with relaxation delay of 1.5 s, mixing time of 0.8 s and receiver gain of 42. Chemical shifts are reported in p.p.m relative to solvent peaks as internal standards (δH, CH_3CN 1.94 ppm; δC, CH_3CN 1.32 ppm). 1H NMR, COSY, 13C NMR, HMBC and NOESY in methanol-d_4 are shown in Figure S36–S41. COSY spectrum was recorded with relaxation delay of 1.5 s and receiver gain of 48. HMBC spectrum was recorded with x points of 2048, y points of 2048, relaxation delay of 1.5 s and receiver gain of 86. NOESY spectrum was recorded with relaxation delay of 1.5 s, mixing time of 0.8 s and receiver gain of 48. Chemical shifts are reported in p.p.m relative to solvent peaks as internal standards (δH, methanol 3.31 ppm; δC, methanol 49.00 ppm).

Circular dichroism studies. Solution of oligo-NSA was prepared by dissolving lyophilized compounds in water, ~~or~~ acetonitrile or methanol followed by diluting to the desired concentration solution in 10 mM phosphate buffer, acetonitrile or methanol. CD spectra were acquired at 25 °C with a CD spectrometer (JASCO, J-1500) using 1 mm path length quartz cell (JASCO, 209J). For measurement of temperature dependence, temperature of the sample holder was set to measurement temperature (5–85 ºC) 1 min prior to the measurement. Data pitch was set to 0.1 mm. The scanning speed was set to 100 nm/min and spectra were averaged from three scans. Spectral baseline was recorded using 10 mM phosphate buffer, acetonitrile or methanol. All data points were baseline subtracted, converted to a uniform scale of molar ellipticity per residue and plotted.

Synthesis of NSA heteropentamer (10) for MDM2-binding. 34 mg of trityl resin (1.96 mmol/g,

67 μmol) was swelled with minimal volume of THF in a syringe for 10 min. A suspension of piperazine (20 equiv.) in THF was added to the resin. After stirring for 2 h, the resin was washed with THF and DCM three times each. The resin was treated with 85/10/5 DCM/methanol/DIPEA solution for 15 min and washed with DCM and DMF three times each. The resin was treated with DMF solution of Fmoc-Ala-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) with continuous shaking for 2 h. After removing the solution, the resin was washed with DMF and DCM three times each and dried. A small part of the resin was treated with 2% DBU/DMF solution and then the amount of Fmoc-alanine loaded on resin was determined from the UV absorbance of dibenzofulvene at 304 nm. In the following procedures, the amount of reagents was calculated based on the amount of Fmoc-alanine loaded on resin. The resin was swelled with minimal volume of DMF for 30 min. The resin was treated with 20% piperidine/DMF (3 min and 12 min) to remove Fmoc protecting group and washed with DMF three times. The resin was incubated with 1 M (20 equiv.) of isovaleraldehyde solution in DMF for 1 h. DMF solution was filtered off and the resin was quickly washed with DMF and DCM. A freshly prepared suspension of $NaBH_4$ (10 equiv.) in 75/25 DCM/methanol was added to the resin and shaken for 30 min. The cap of the syringe was occasionally removed for degassing to prevent inner pressure is increased too much. After reduction, the resin was washed with methanol five times then with DCM, DMF and dioxane three times each. Fmoc-*N*-Me-Ala-OH (4 equiv.) and EEDQ (4 equiv.) were dissolved in dioxane to prepare 0.2 M solution and the mixture was shaken for 30 min. The mixture was then added to the resin and the reaction vessel was shaken for 3 h at 60 °C. This coupling reaction was repeated once more. After the double coupling, the resin was washed with dioxane and DMF three times each and Fmoc protecting group was removed with 20% piperidine/DMF. Fmoc-Ala-OH (4 equiv.) and EEDQ (4 equiv.) were dissolved in dioxane to prepare 0.2 M solution and the mixture was shaken for 30 min. The mixture was added to the resin and shaken for 3 h at 60 °C. After washing the resin with dioxane and DMF three times each, Fmoc protecting group was removed with 20% piperidine/DMF. The resin was washed with trimethyl orthoformate (TMOF) three times. The resin was incubated with a suspension of indole-3-carboxaldehyde (20 equiv.) in TMOF containing 1% acetic acid for 1 h then 10 equiv. of $NaBH(OAc)_3$ was added to the mixture. After 30 min, the resin was washed with methanol five times and with DMF and dioxane three times each. Coupling of Fmoc-*N*-Me-Ala-OH, deprotection, coupling of Fmoc-Ala-OH and deprotection were conducted as described above. Benzyl group was introduced on nitrogen by reductive amination. Imine formation was conducted in DMF and reductive reaction was conducted in 75/25 DCM/methanol with $NaBH_4$ as described above. After the wash of the resin, the resin was treated with the solution of 0.5 M (10 equiv.) acetic anhydride and 1 M (20 equiv.) DIPEA in DMF for 1.5 h. This acetylation reaction was repeated once more. The oligomers were cleaved by treating the resin with 30% HFIP/DCM for 20 min five times and the resin was washed with methanol three times. The HFIP solutions and the washing solutions were collected in a glass vial and the solution was removed under reduced

pressure. The crude product was dissolved in acetonitrile and water and purified by a reversed phase column on HPLC using acetonitrile and 10 mM triethylammonium acetate (TEAA)/water as solvents. After lyophilization, the purified product was dissolved in PBS and the amount was determined based on the UV absorbance at 280 nm. The yield was calculated by comparing the amount of the obtained product with the amount of the first Fmoc-alanine loaded on resin. The purified product was analyzed on a reversed phase column by HPLC and ESI-TOF MS. Yield was 0.86 μmol (2%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{44}H_{65}N_8O_6^+$ 801.5022; Found 801.5007.

Synthesis of NSG heteropentamer (10-NSG) for MDM2-binding. 33 mg of trityl resin (1.96 mmol/g, 65 μmol) was used for synthesis. Fmoc-Gly-OH, Fmoc-Sar-OH, isovaleraldehyde, indole-3-carboxaldehyde and benzaldehyde were used as submonomers. The procedures are the same as the procedures of '**Synthesis of NSA heteropentamer (10) for MDM2-binding**', except that Fmoc-Gly-OH and Fmoc-Sar-OH were used instead of Fmoc-Ala-OH and Fmoc-*N*-Me-Ala-OH, respectively. Coupling of Fmoc-Sar-OH to *N*-substituted glycine terminus was conducted by the treatment of resin with DMF solution of Fmoc-Sar-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) for 3 h. Coupling of Fmoc-Gly-OH to sarcosine terminus was conducted by the treatment with DMF solution of Fmoc-Gly-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) for 3 h. Yield was 0.61 μmol (2%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{39}H_{55}N_8O_6^+$ 731.4239; Found 731.4252.

Synthesis of heteropentamer composed of alternating NSA and NSG residues (10-NSA/NSG) for MDM2-binding. 34 mg of trityl resin (1.96 mmol/g, 67 μmol) was used for synthesis. Fmoc-Ala-OH, Fmoc-Sar-OH, isovaleraldehyde, indole-3-carboxaldehyde and benzaldehyde were used as submonomers. The procedures are the same as the procedures of '**Synthesis of NSA heteropentamer (10) for MDM2-binding**', except that Fmoc-Sar-OH was used instead of Fmoc-*N*-Me-Ala-OH. Coupling of Fmoc-Sar-OH to *N*-substituted alanine terminus was conducted by the treatment of resin with dioxane solution of Fmoc-Sar-OH (4 equiv., 0.2 M) and EEDQ (4 equiv., 0.2 M) at 60 ºC for 3 h. Coupling of Fmoc-Ala-OH to sarcosine terminus was conducted by the treatment with DMF solution of Fmoc-Ala-OH (4 equiv., 0.2 M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv., 0.4 M) for 3 h. Yield was 0.76 μmol (2%). HRMS (ESI-TOF MS) *m/z*: $[M + H]^+$ Calcd for $C_{42}H_{61}N_8O_6^+$ 773.4709; Found 773.4698.

Synthesis of fluorescently labeled p53-TAD peptide (Ac–SQETFSDLWK(Fluorescein)LLPE –NH_2) for fluorescence polarization assay. Peptide was synthesized by standard Fmoc solid phase peptide synthetic method using Rink Amide resin and Fmoc-amino acids. For coupling reaction, DMF solution of Fmoc-amino acid (4 equiv., 0.2M), COMU (4 equiv., 0.2 M) and DIPEA (8 equiv.,

0.4 M) was used and the reaction was conducted for 40 min with continuous shaking. To orthogonally remove the protecting group of lysine side-chain, Fmoc-Lys(Mtt)-OH was used as a building block. The N-terminus of the peptide was acetylated by treating the peptide on resin with DMF solutions of acetic anhydride (6 equiv., 0.2 M) and pyridine (12 equiv., 0.4 M) for 30 min. Mtt protecting group was removed by treating the resin with 1/5/94 TFA/TIPS/DCM solution for 3 min five times. The resin was washed with DCM, 20% piperidine/DMF, DMF three times each. The DMF solution of 5(6)-carboxyfluorescein (2.5 equiv., 0.1 M), DIC (2.5 equiv., 0.1 M) and HOBt (2.5 equiv., 0.1 M) was added to the resin with continuous shaking overnight. After removing the solution, the resin was washed with DMF three times and incubated with 20% piperidine/DMF for 45 min to remove any side products bearing additional carboxyfluoresceins. After removing the solution, the resin was washed with DMF, methanol and DCM three times each. Peptide was cleaved and deprotected by treating the resin with 95/2.5/2.5 TFA/TIPS/H_2O for 2 h. The solution was transferred to a recovery flask and TFA solution was removed under reduced pressure. Peptide was precipitated by adding diethylether to the flask. The precipitated crude product was dissolved in acetonitrile and water and purified by a reversed phase column on HPLC using acetonitrile and water containing 0.1% TFA as solvents. The purified product was analyzed on a reversed phase column by HPLC and ESI-TOF MS. HRMS (ESI-TOF MS) *m/z*: $[M + Na]^+$ Calcd for $C_{105}H_{136}N_{20}O_{33}Na^+$ 2227.9471; Found 2227.9466.

Recombinant expression and purification of MDM2. Human MDM2, corresponding to residues 17-125, was expressed using pGEX-6P-2 vectors in BL21 (DE3) cells grown in LB medium. The pGEX-6P-2-MDM2 (17-125) was a gift from Gary Daughdrill (Addgene plasmid # 62063).[19] Expression of MDM2 was induced by adding IPTG to the final concentration of 0.5 mM when the OD_{600} reached to 0.5. The induction was performed at 25 °C overnight with continuous shaking and cultures were centrifuged at 7,000g for 10 min at 4 °C. Pellets were stored at –80 °C. For purification, pellets were suspended in 50 mM Tris, 300 mM NaCl, 2.5 mM EDTA, 2 mM DTT (pH 7.4) and sonicated for 20 min with the repeat of 3 sec sonications and 10 sec intervals at the output level of 80 on an ultrasonic disruptor (TOMY, UD-100). The lysate was centrifuged at 12,000g for 30 min. The supernatant was applied to glutathione sepharose 4 fast flow resin. GST tag was cleaved from GST-MDM2 on the resin by incubating the resin with a solution containing HRV 3C protease (TaKaRa) in 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 8.0) at 4 °C overnight. HRV 3C protease was removed by applying the supernatant to Ni-NTA resin. The flow through was collected and the buffer was exchanged to PBS (pH 7.4) using a dialysis membrane with 3.5 K molecular weight cut filter.

Isothermal calorimetry measurement of oligo-NSA and MDM2. Binding affinities of **10**, **10-NSG**, and **10-NSA/NSG** were evaluated in PBS at 25 ºC on an Auto-iTC200 (GE Healthcare).

The ligand solutions of 200 μM **10**, **10-NSG**, and **10-NSA/NSG** in PBS were titrated into 20 μM MDM2 solutions in PBS. As a control experiment, each ligand solution was titrated into PBS. The measured heat flow was recorded as a function of time and each peak was integrated to determine the total heat generated from each injection. The recorded heat was plotted against the molar ratio of MDM2 and the plot was fitted to generate a binding curve by applying one-site model using the software Origin7 (GE healthcare). ΔH and K_D were determined from the fitting of the plot then ΔG and ΔS were determined using the following two equations: (1) $\Delta G = RT\ln\Delta K_D$ and (2) $\Delta S = (\Delta H - \Delta G)/T$.

Fluorescence polarization binding assay of fluorescently labeled p53-TAD. Serially diluted MDM2 solutions were mixed with fluorescently labeled p53-TAD (Flu-p53) solution in 384-well plate to prepare 15 μL of 37 nM–80 μM MDM2 and 50 nM Flu-p53 in PBS (pH 7.4) containing 0.01% Tween 20. To determine the baseline, solutions of 50 nM Flu-p53 not containing MDM2 were also prepared. The plate was spun down at 1,000g for 5 min and the solutions were incubated for 1 h. Fluorescence polarization (FP) was measured on a plate reader (TECAN, infinite M1000Pro) at 25 ºC. Excitation wavelength and emission wavelength were set to 470 nm and 521 nm, respectively. ΔFP values were determined from the difference between the average of three measurements and baseline, and plotted as a function of the concentration of MDM2. Dissociation constants (K_D) were determined by fitting the plot to the Hill equation on ORIGIN (LightStone): $y = y_{min} + (y_{max} - y_{min})/(1 + (K_D/x)^n)$. x, y, and n denote MDM2 concentration, ΔFP, and hill coefficient, respectively.

Fluorescence polarization competition assay of 10, 10-NSG and 10-NSA/NSG. Serially diluted inhibitor oligomers were mixed with MDM2 solution and Flu-p53 solution in 384-well plate to prepare 15 μL of 0.13–300 μM inhibitor oligomer, 2 μM MDM2 and 50 nM Flu-p53 in PBS (pH 7.4) containing 0.01% Tween 20. To determine the baseline, solutions of 50 nM Flu-p53 not containing MDM2 were also prepared. The plate was spun down at 1,000g for 5 min and the solution was incubated for 1 h. Fluorescence polarization (FP) was measured on a plate reader (TECAN, infinite M1000Pro) at 25 ºC. Excitation wavelength and emission wavelength were set to 470 nm and 521 nm, respectively. ΔFP values were determined from the difference between the average of three measurements and baseline, and plotted as a function of the concentration of inhibitor oligomers. IC_{50} values were determined by fitting the plot to the Hill equation on ORIGIN (LightStone): $y = y_{min} + (y_{max} - y_{min})/(1 + (IC_{50}/x)^n)$. x, y, and n denote inhibitor oligomer concentration, ΔFP and hill coefficient, respectively. Inhibition constants (K_i) were calculated as previously reported.[20]

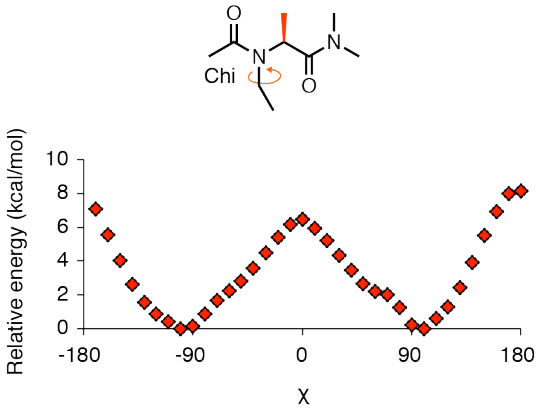


Figure S1. χ scan of acetyl-*N*-ethylalanine dimethylamide. χ angle was scanned from an initial conformation with dihedral angles of (χ, φ, ψ) = (0°, –120°, 90°).

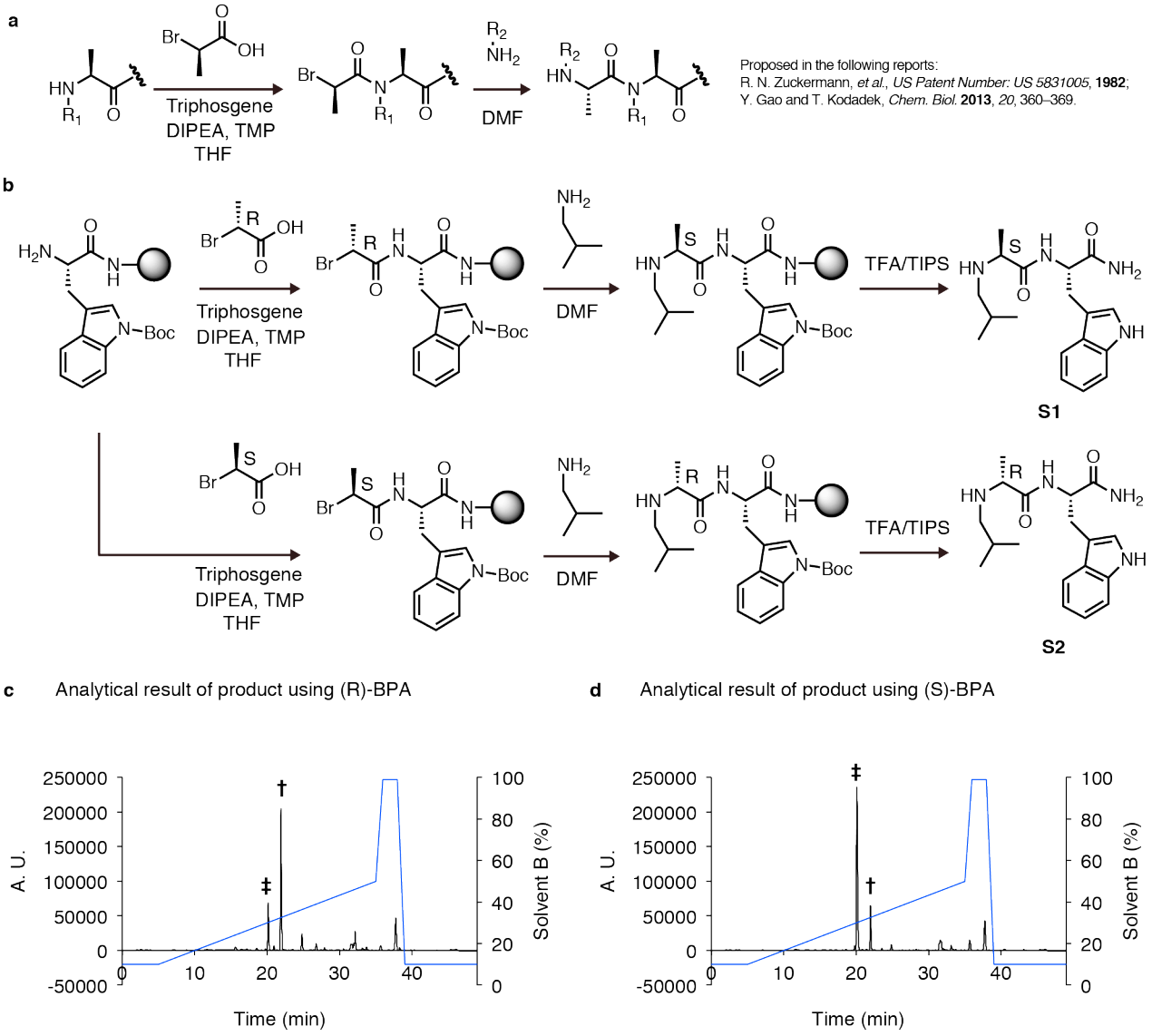
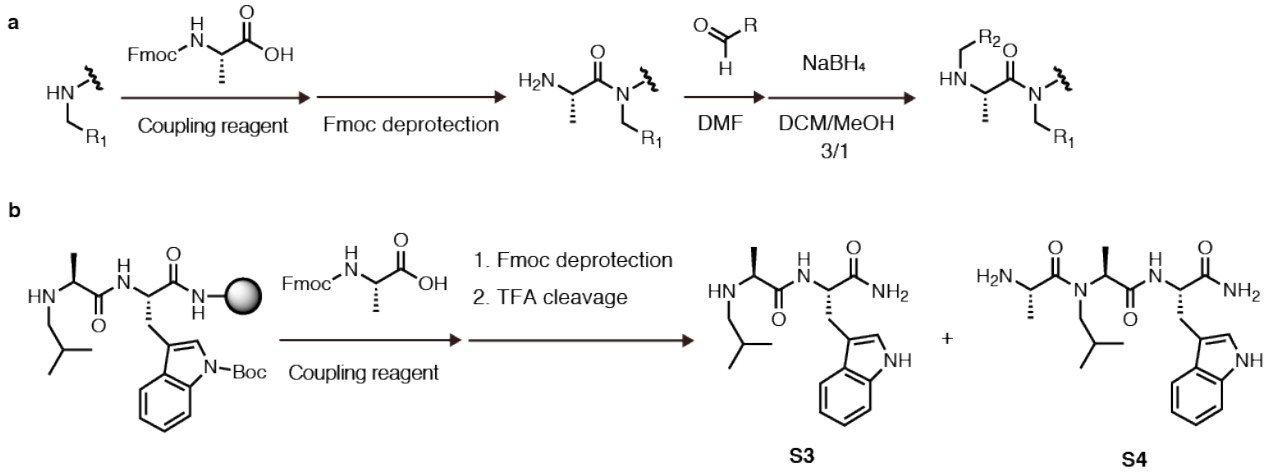


Figure S2. Submonomeric synthesis of oligo-NSA using chiral 2-bromopropionic acid and primary amine. (**a**) Submonomer synthetic method of oligo-NSA proposed by Zuckermann and Kodadek. (**b**) The Synthetic scheme of model peptides containing an NSA residue for evaluating racemization during synthesis using the submonomer synthetic method. (**c**) HPLC chromatogram of crude product of **S1**. † denotes the fraction containing desired product **S1** and ‡ denotes the fraction containing the epimerized product **S2**. (**d**) HPLC chromatogram of crude product of **S2**. ‡ denotes the fraction containing desired product **S2** and † denotes the fraction containing the epimerized product **S1**. For **(c)** and **(d)**, HPLC analysis was performed using a linear gradient of solvent A (water containing 0.1% TFA) and solvent B (acetonitrile containing 0.1% TFA). Blue line denotes the percentage of solvent B. Products were monitored at 280 nm.



c

Coupling reagent	Solvent	Time	Temperature (°C)	Yield (%)
HATU	DMF	3 h	RT	44
COMU	DMF	3 h	RT	20
BTC	THF	3 h	RT	28
EEDQ	DMF	3 h	RT	25
EEDQ	THF	3 h	RT	63
EEDQ	Dioxane	3 h	RT	76
EEDQ	Dioxane	3 h	60	82
EEDQ	Dioxane	3 h x 2	60	quant.
EEDQ	Dioxane	1 h	100 (MW)	88
EEDQ	Dioxane	1 h x 2	100 (MW)	quant.

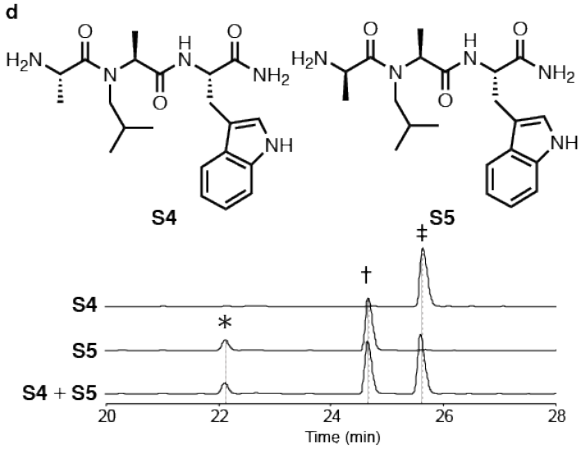


Figure S3. Submonomeric synthesis of oligo-NSA using Fmoc-Ala-OH and aldehyde. **(a)** Submonomer synthetic method of oligo-NSAs proposed by Pels and Kodadek. **(b)** Synthetic scheme of model peptides containing an NSA residue for evaluating coupling efficiency and racemization. **(c)** A table summarizing the conditions and yields of coupling reaction of Fmoc-Ala-OH on *N*-substituted alanine terminus under the conditions described in the table. The HPLC chromatogram of the crude product using each condition is listed in **Figure S4**. Yield of each reaction was determined from the area of HPLC chromatogram of crude product monitored at 280 nm using the following equation: Yield (%) = **S4** / (**S3** + **S4**) × 100. MW denotes microwave heating. **(d)** HPLC chromatograms of crude products of peptides **S4** and **S5**. **S4** and **S5** were synthesized using the scheme described in **Figure S3b.** In the step of coupling of Fmoc-Ala-OH on *N*-substituted alanine terminus, double coupling using EEDQ was conducted at 60 °C for 3 h each. Fmoc-D-Ala-OH was used instead of Fmoc-Ala-OH for the synthesis of **S5**. Fractions containing **S4** and **S5** are labeled with † and ‡, respectively. The fraction containing the non-acylated peptide **S3** is labeled with *. No detectable epimerized products were observed on both the chromatograms. Products were monitored at 280 nm.

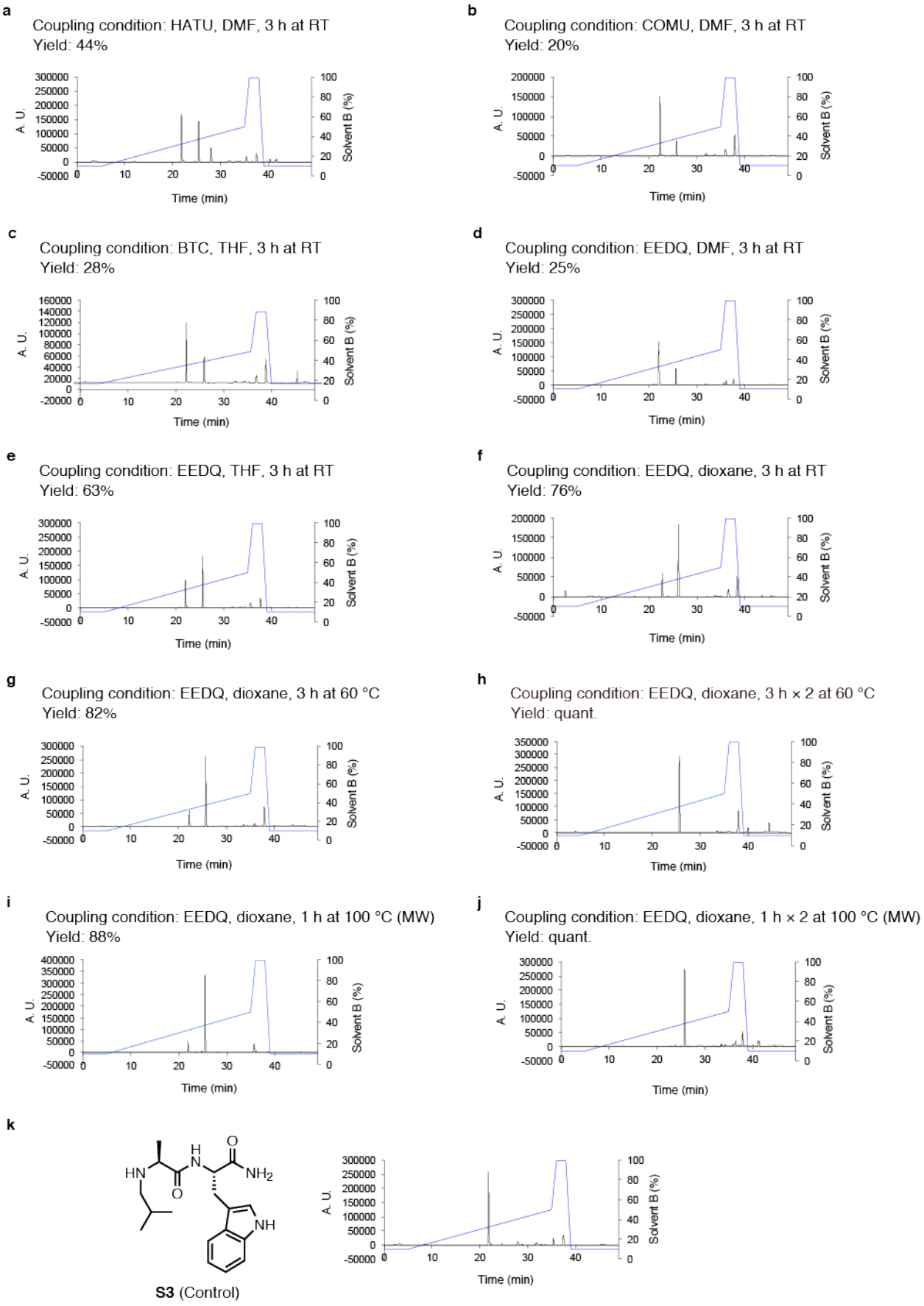


Figure S4. HPLC chromatograms of crude products of S4 synthesized with different coupling conditions. **(a)–(j)** HPLC chromatograms of **S4** synthesized by the scheme shown in **Figure S3b** using various coupling conditions listed on **Figure S3c**. The coupling condition and the yield are described above each

chromatogram. **(k)** HPLC chromatogram of **S3** as a reference. Products were monitored at 220 nm.

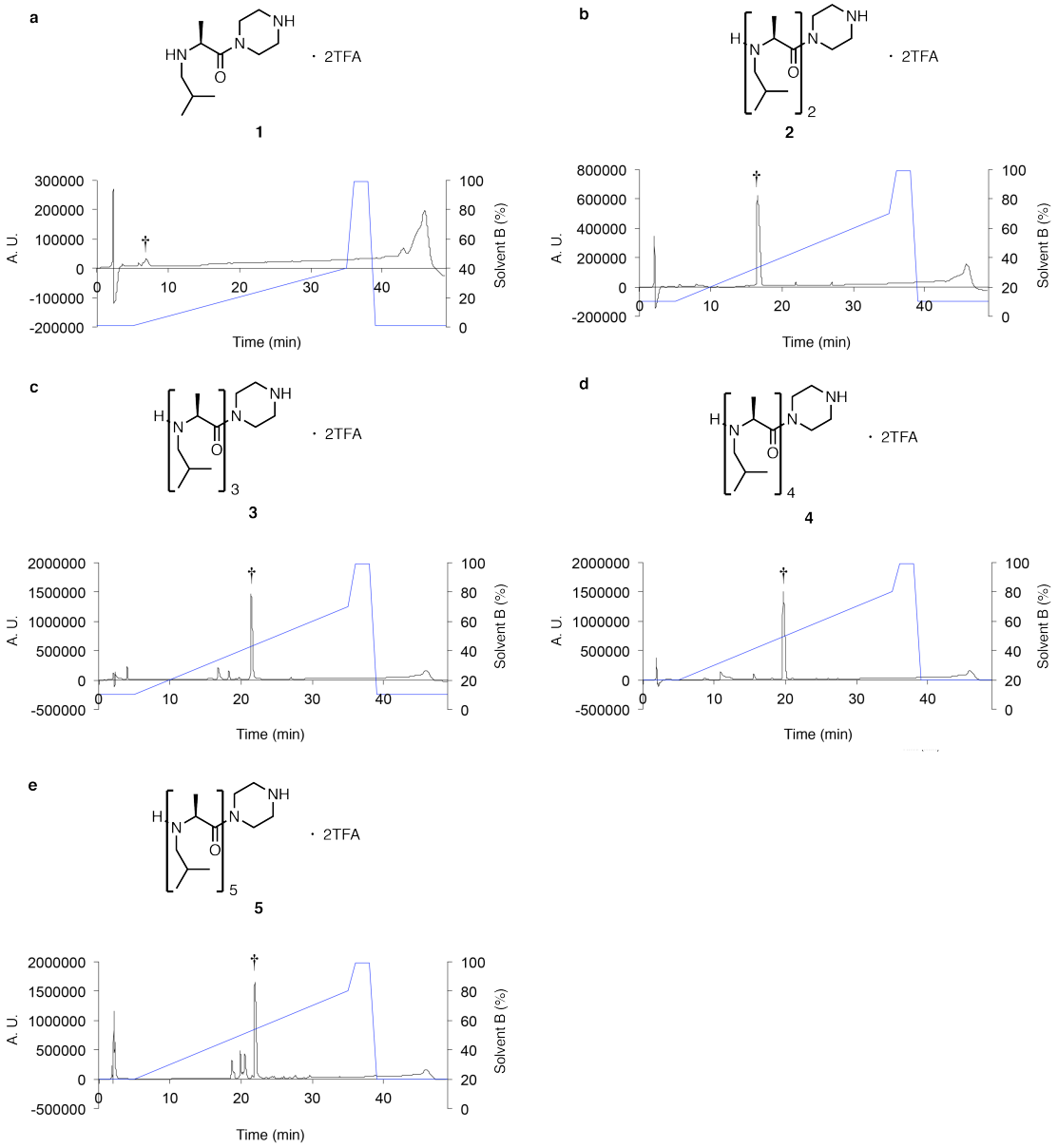


Figure S5. HPLC chromatograms of crude products of oligo-NSAs **1–5**. HPLC chromatogram of **(a) 1**, **(b) 2**, **(c) 3**, **(d) 4**, **(e) 5**. Products were monitored at 220 nm.

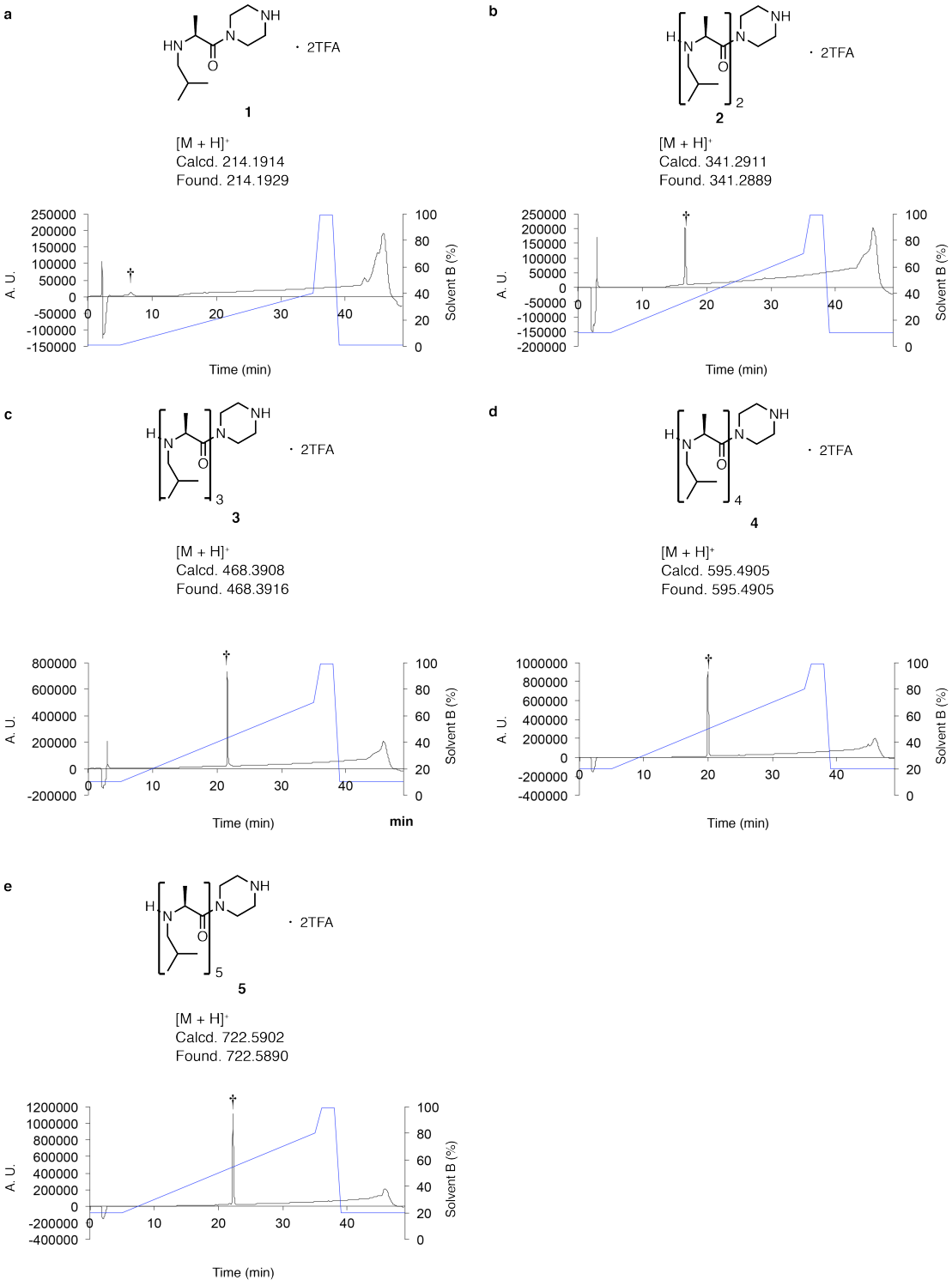


Figure S6. HPLC chromatograms of products of oligo-NSAs **1–5** after HPLC purifications. HPLC chromatogram of **(a) 1**, **(b) 2**, **(c) 3**, **(d) 4**, **(e) 5** after purification. Products were monitored at 220 nm. † denotes the fraction containing desired product. The calculated and found mass values of the desired compound are labeled under the chemical structure of each compound.

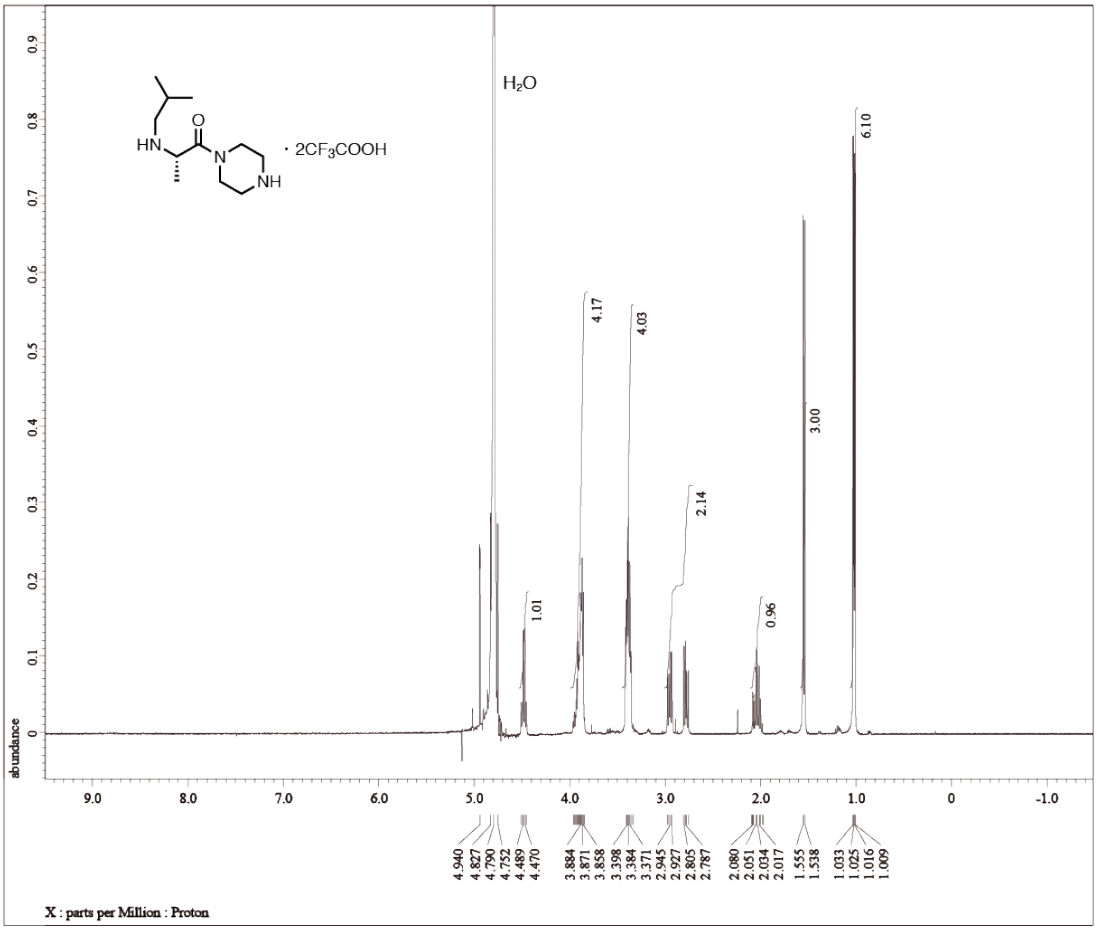


Figure S7. 1H NMR spectrum of *N*-isobutylalanine piperazineamide. Spectrum was recorded in D_2O at 25 °C.

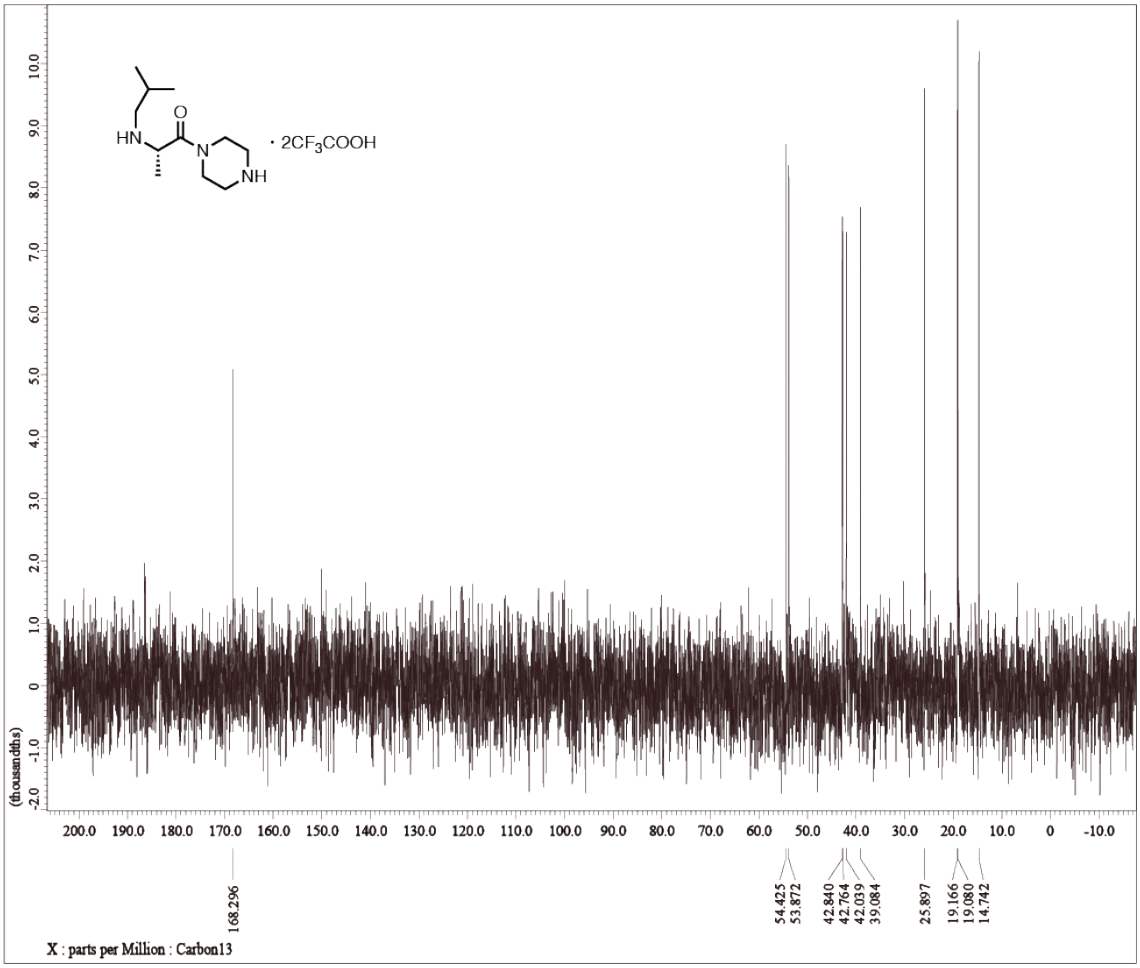


Figure S8. 13C NMR spectrum of *N*-isobutylalanine piperazineamide. Spectrum was recorded in D_2O at 25 °C.

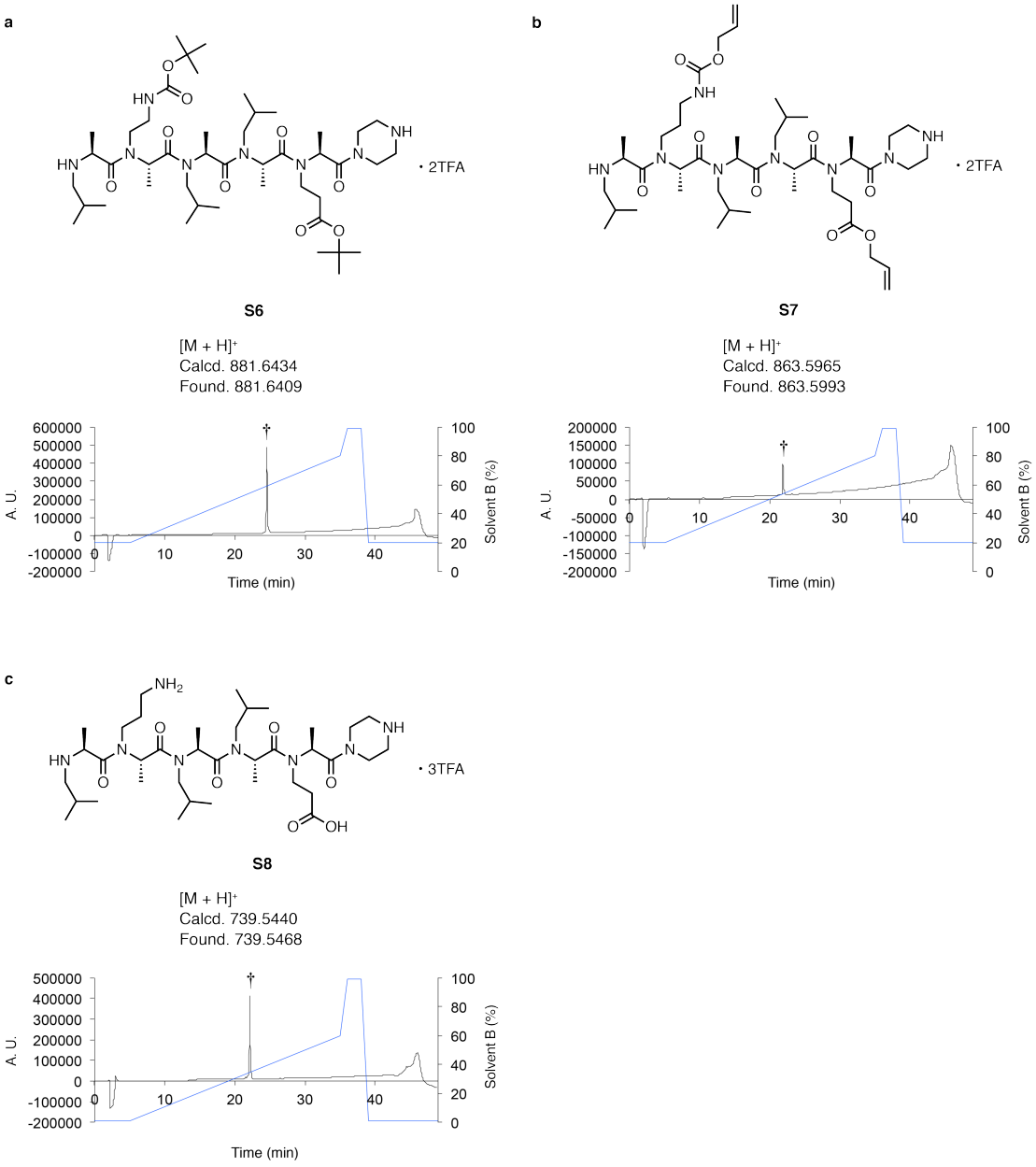
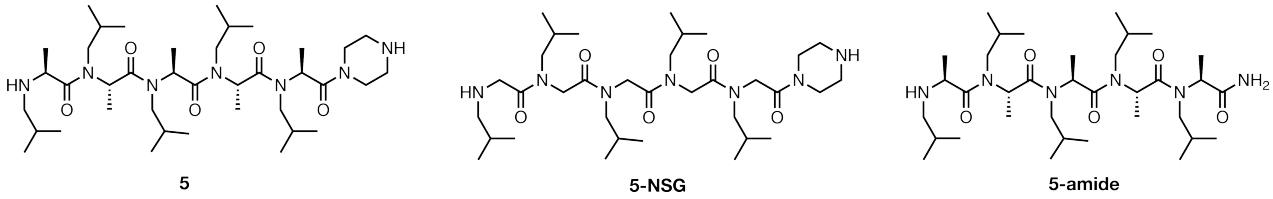


Figure S9. HPLC chromatograms of products of oligo-NSAs **S6–8** after HPLC purifications. HPLC chromatogram of **(a) S6**, **(b) S7**, **(c) S8** after purification. † denotes the fraction containing desired product. Products were monitored at 220 nm. The calculated and found mass values of the desired compound are labeled under the chemical structure of each compound.



5

5-NSG

5-amide

	5	5-NSG	5-amide
Water solubility (mM)	270	320	87

Figure S10. Water solubility of oligo-NSA and oligo-NSG. Chemical structures of **5**, **5-NSG** and **5-amide** are shown. The water solubility of each compound is described in the table.

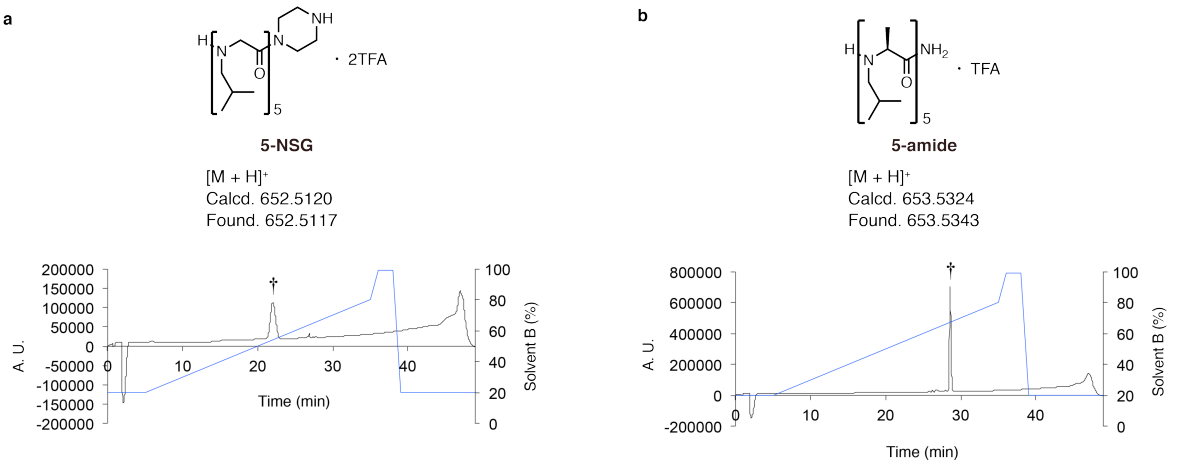


Figure S11. HPLC chromatograms of **5-NSG** and **5-amide** after purification. HPLC chromatograms of **(a) 5-NSG** and **(b) 5-amide** after purification. Products were monitored at 220 nm. Fractions containing the desired products are labeled with †. The calculated and found mass values of the desired compound are labeled under the chemical structure of each compound.

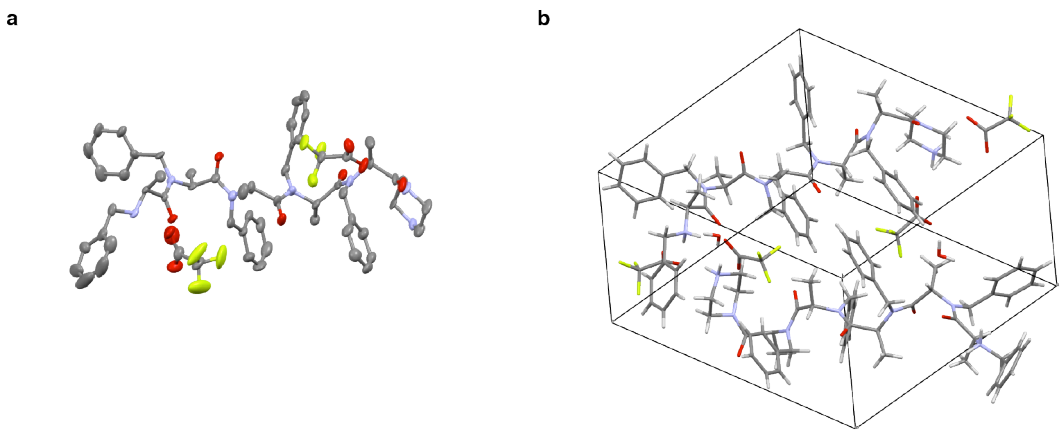


Figure S12. X-ray crystal structure of oligo-NSA **6**. **(a)** The crystal structure is shown as an ORTEP diagram. **(b)** Molecules in one unit of the cell are shown. Two molecules of the oligo-NSA **6**, four molecules of trifluoroacetic acid, and two water molecules were in one unit of the cell.

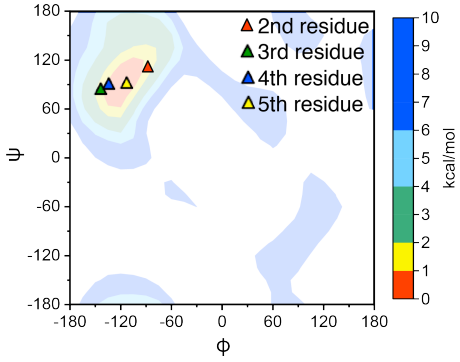


Figure S13. Dihedral angles observed in the crystal structure were spotted on the Ramachandran-type plot of acetyl-*N*-methylalanine dimethylamide (Figure 2b).

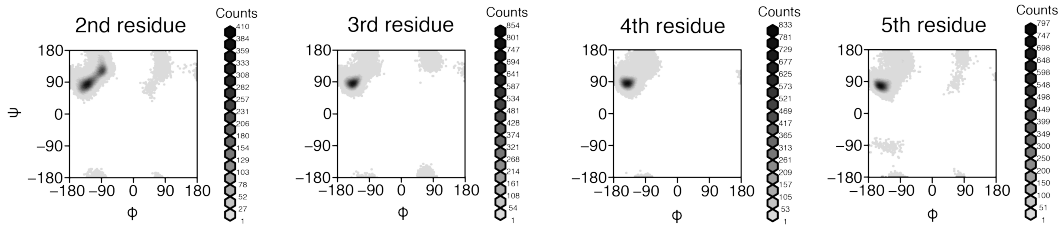


Figure 14. Distribution of dihedral angles of each residue of oligo-NSA **6** during the last 400 ns (out of 500 ns) × 5 of MD calculations. The calculations were conducted using the crystal structure as the initial conformation.

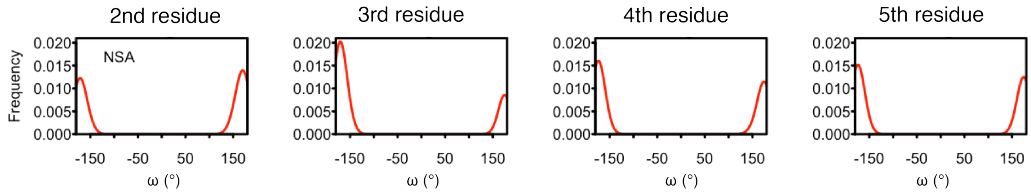


Figure S15. Distribution of ω angles of oligo-NSA **6** during MD simulations. Omega angle of each residue during the MD simulations was plotted with kernel density estimation.

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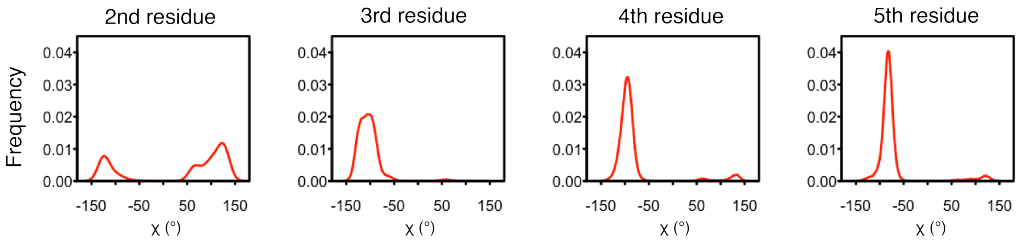


Figure S16. Distribution of χ angles of oligo-NSA **6** during MD simulations. χ angle of each residue during the MD simulations was plotted with kernel density estimation.

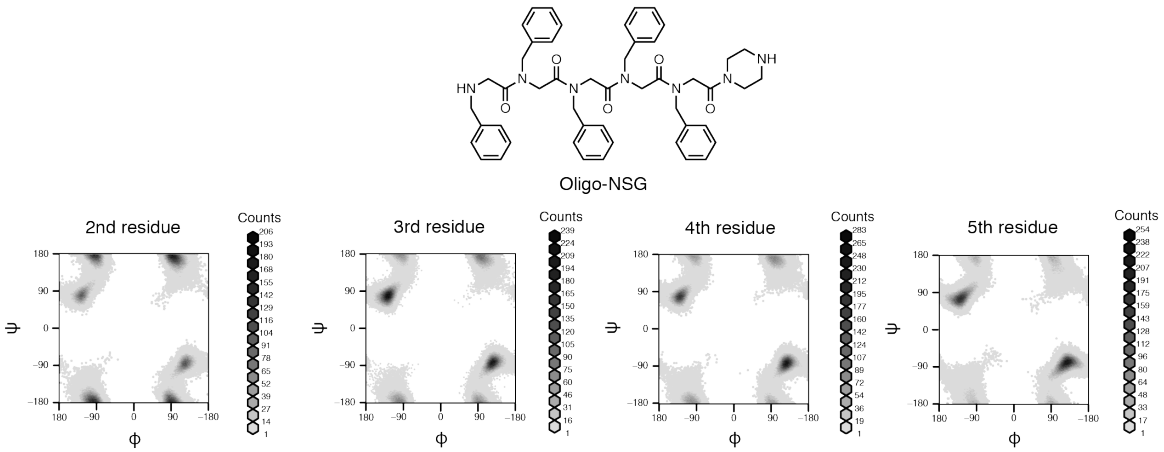


Figure S17. Distribution of φ and ψ angles of oligo-NSG during MD simulations. φ and ψ angles of each residue during the MD simulations were plotted.

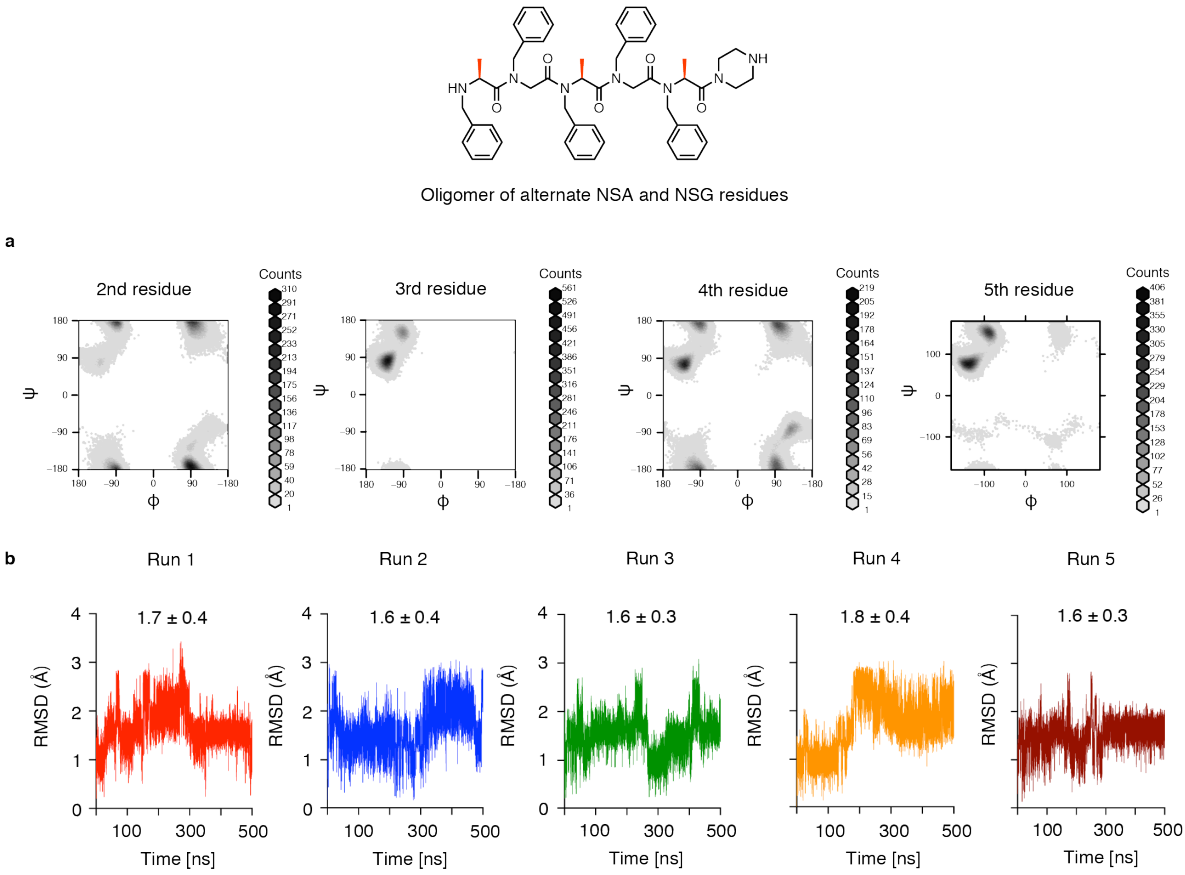


Figure S18. Distribution of φ and ψ angles of oligomer of alternate NSA and NSG residues during MD simulations. **(a)** The same MD calculations for oligo-NSA **6** were conducted for the oligomer with alternate NSA and NSG residue using the same initial backbone conformation with oligo-NSA **6**. φ and ψ angles of each residue during the MD simulations were plotted. **(b)** RMSD values of the oligomer from the initial conformation during each MD run are plotted. RMSD value of the oligomer from the initial conformation is plotted. The average and standard deviations of the RMSD values are shown above each plot.

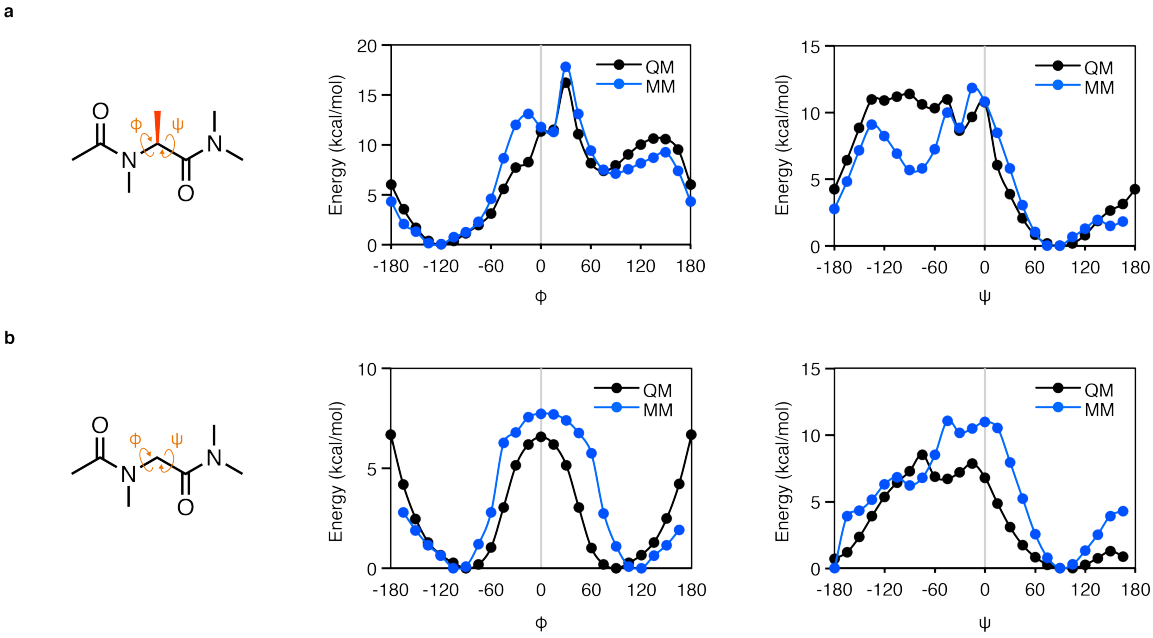


Figure S19. One-dimensional energy profiles of minimal models of oligo-NSA and oligo-NSG as a function of each dihedral angle. **(a)** One-dimensional energy profiles of acetyl-*N*-methylalanine dimethylamide. **(b)** One-dimensional energy profiles of acetyl-*N*-methylglycine dimethylamide. The energy profiles from QM and MM calculations are plotted in black and blue, respectively.

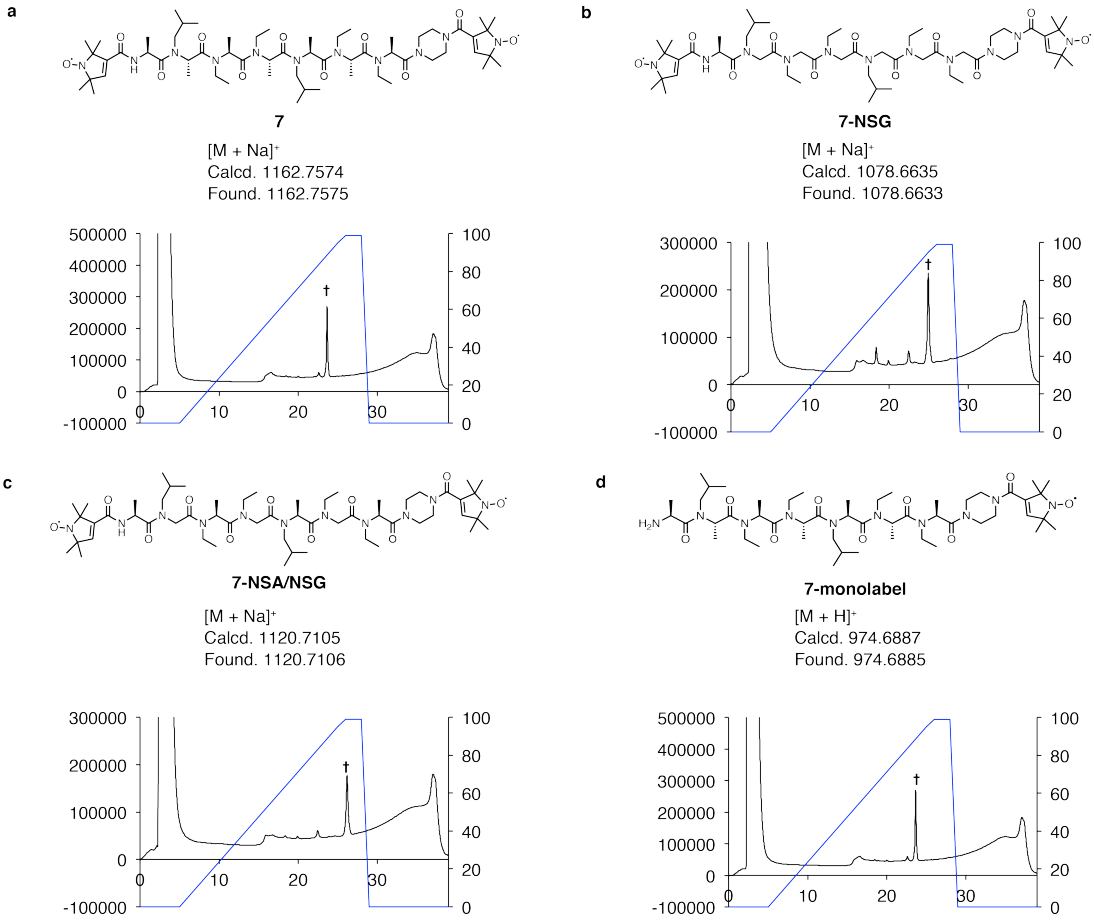


Figure S20. HPLC chromatograms of **(a) 7**, **(b) 7-NSG** and **(c) 7-NSA/NSG** and **(d) 7-monolabel** after the DEER measurement. Products were monitored at 220 nm. Fractions containing the desired products are labeled with †. The calculated and found mass values of the desired compound are labeled under the chemical structure of each compound.

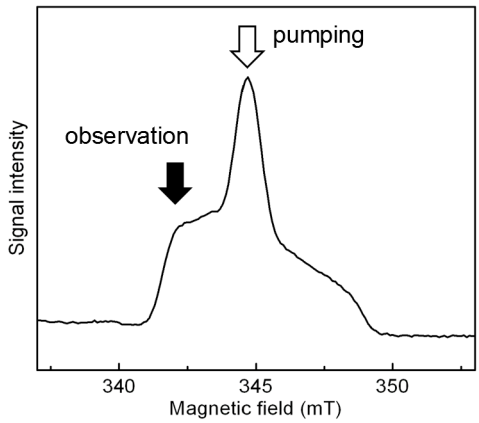


Figure S21. Electron spin echo (ESE) field swept spectrum of the oligo-NSA. The observation and pumping microwave frequencies with a difference of 72 MHz were set to resonate the magnetic fields shown by the filled (observation) and blank (pumping) arrows

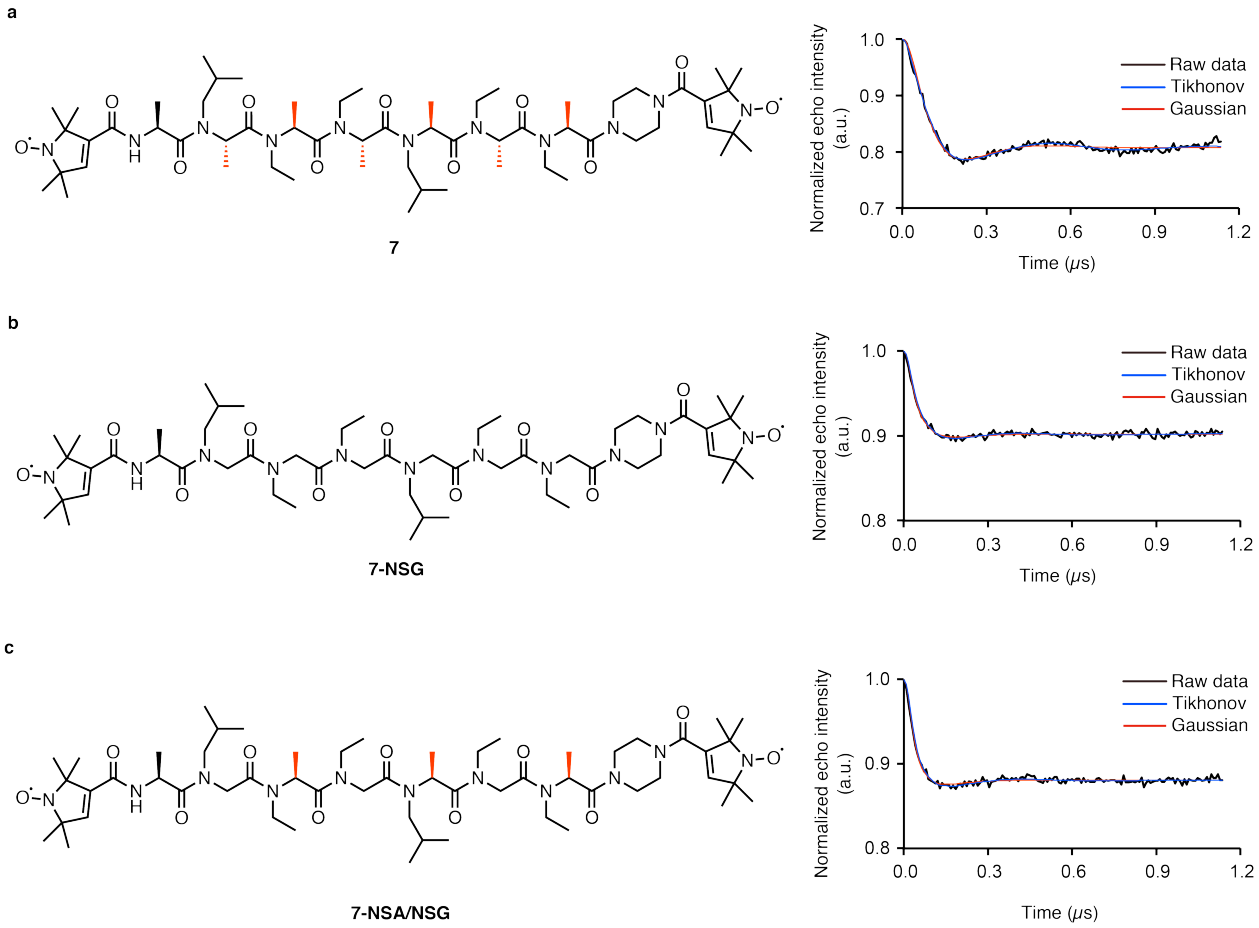


Figure S22. DEER time traces of **(a)** oligo-NSA **7**, **(b) 7-NSG** and **(c) 7-NSA/NSG** measured in water:DMSO = 1:1 solution (black lines). The blue lines indicate Tikhonov regularization and the red lines indicate Gaussian model fitting of the raw data.

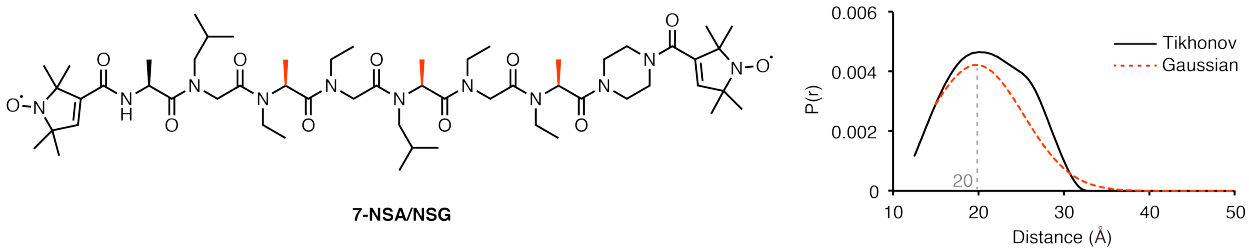


Figure S23. Distribution of the end-to-end distance of an oligomer with alternating NSA and NSG residues measured by DEER. The distribution of distance between the two spins labeled on the both termini of the oligomer was determined from Tikhonov fitting (solid black line) and Gaussian fitting (dashed red line). The average length of each oligomer in the Gaussian model fitting is described in gray in the graph.

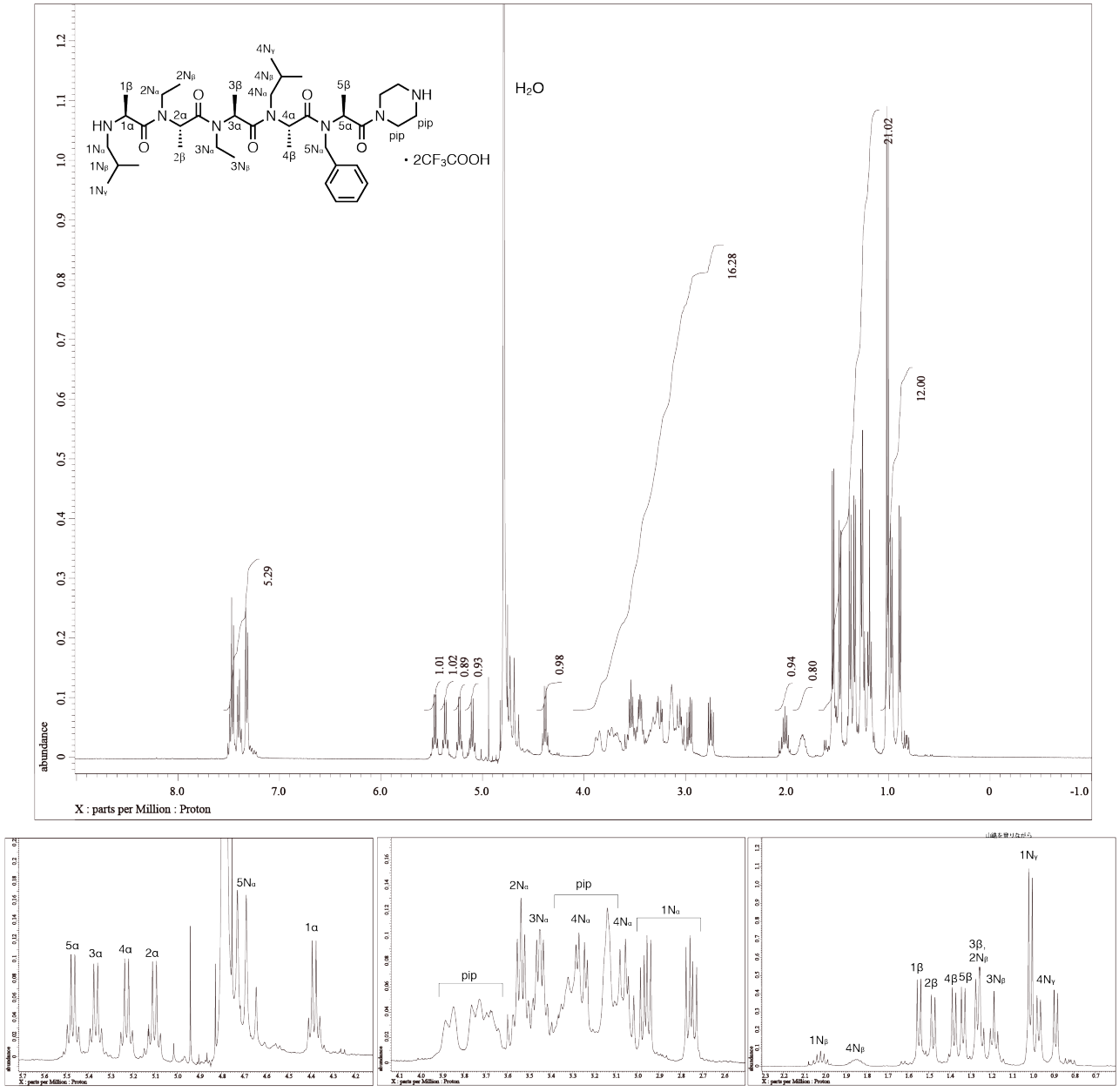


Figure S24. 1H NMR spectrum of oligo-NSA **8** in D_2O. Spectrum was recorded in D_2O at 25 °C.

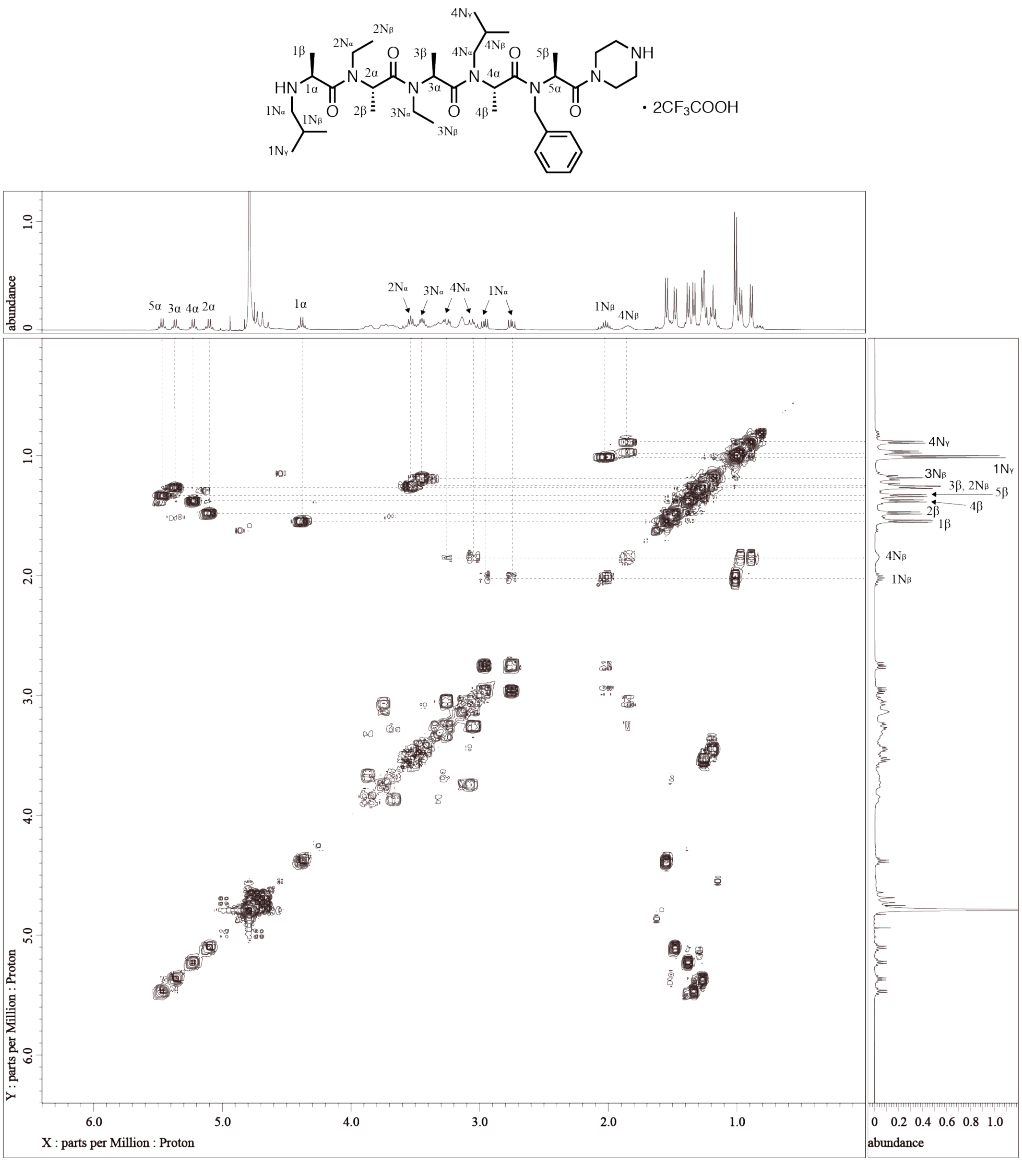


Figure S25. COSY spectrum of oligo-NSA **8** in D_2O. Spectrum was recorded in D_2O at 25°C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

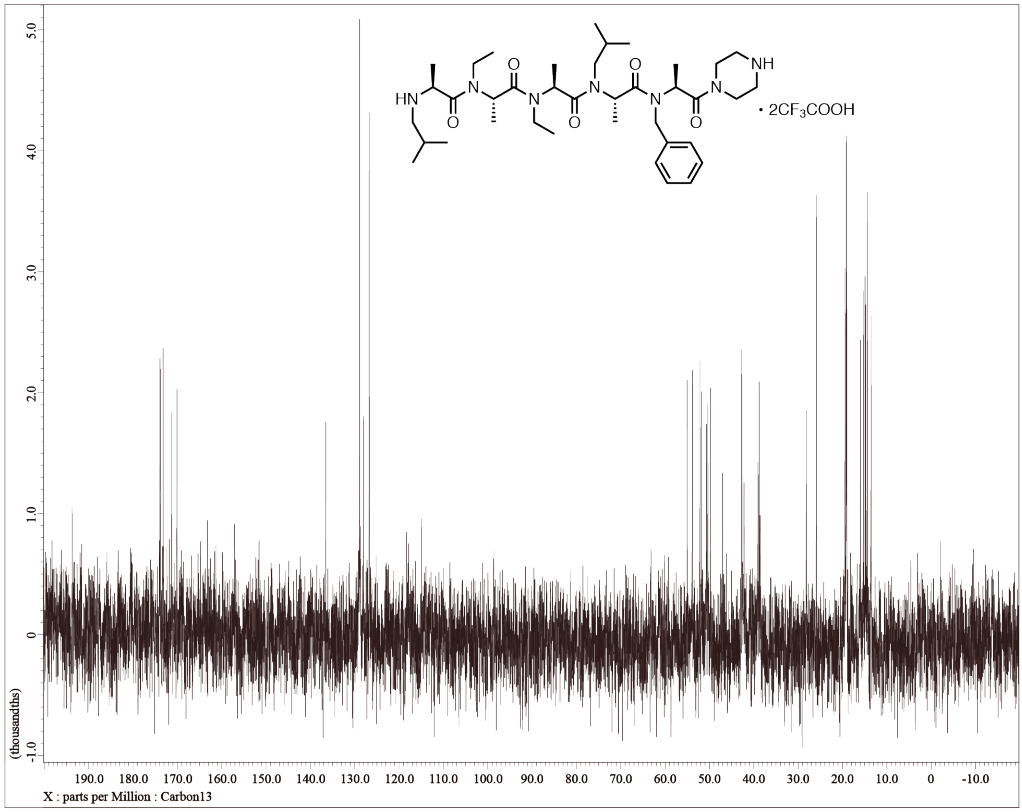


Figure S26. 13C NMR spectrum of oligo-NSA **8** in D_2O. Spectrum was recorded in D_2O at 25°C.

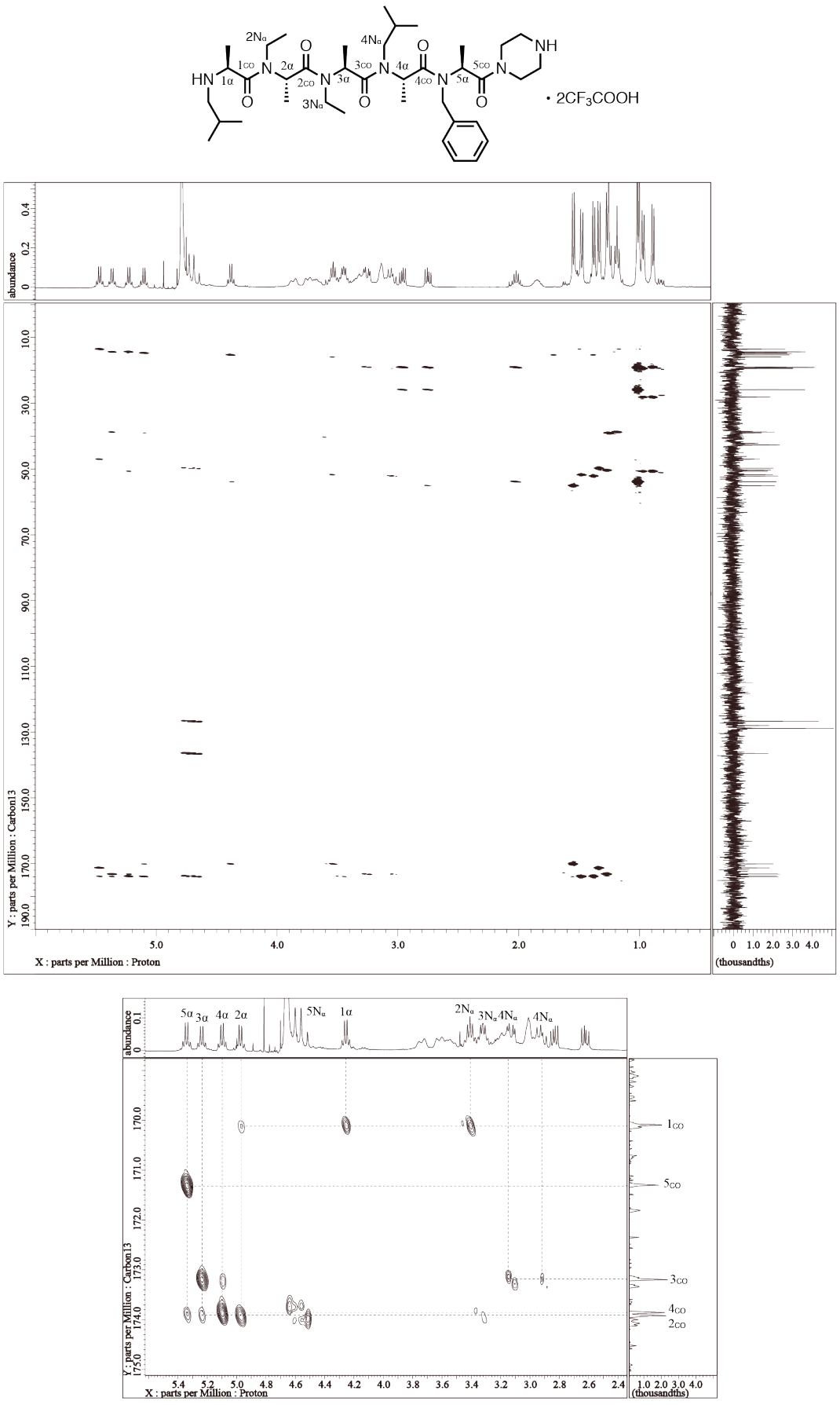


Figure S27. HMBC spectrum of oligo-NSA **8** in D_2O. Spectrum was recorded in D_2O at 25°C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

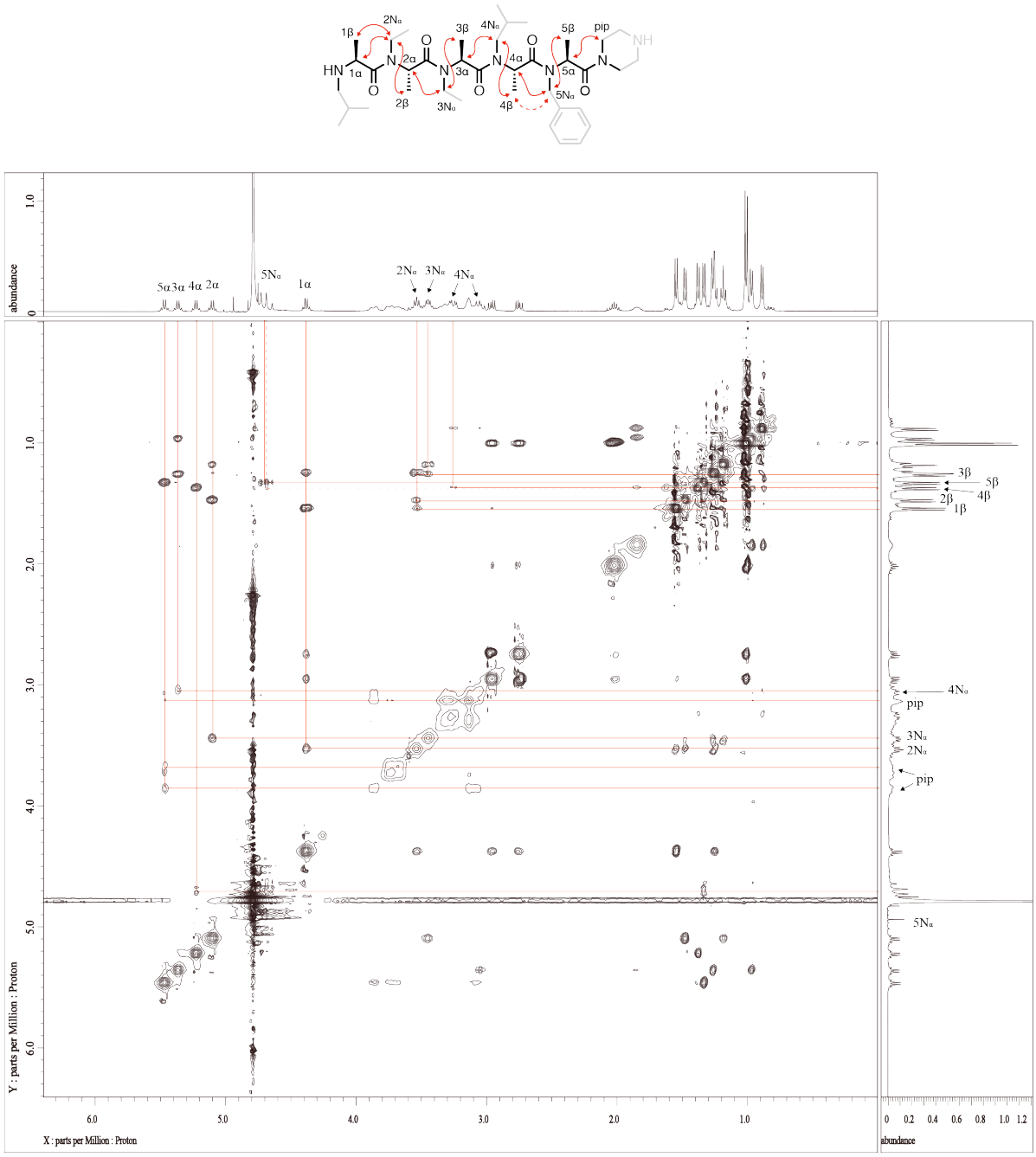


Figure S28. NOESY spectrum of oligo-NSA **8** in D_2O highlighting key cross peaks. Spectrum was recorded in D_2O at 25°C. NOE peaks that support restricted rotations about backbone dihedral angles are shown with red lines. Red lines and arrows indicate the protons that are within 3 Å in the crystal and exhibit strong NOEs. Dashed red lines and arrow indicate protons > 3 Å away from each other but exhibiting NOE. Peaks of piperazine in the two regions (3.0–3.4 ppm and 3.6–3.9 ppm) were not assigned separately, but both piperazine protons have the cross peaks with '5α' proton.

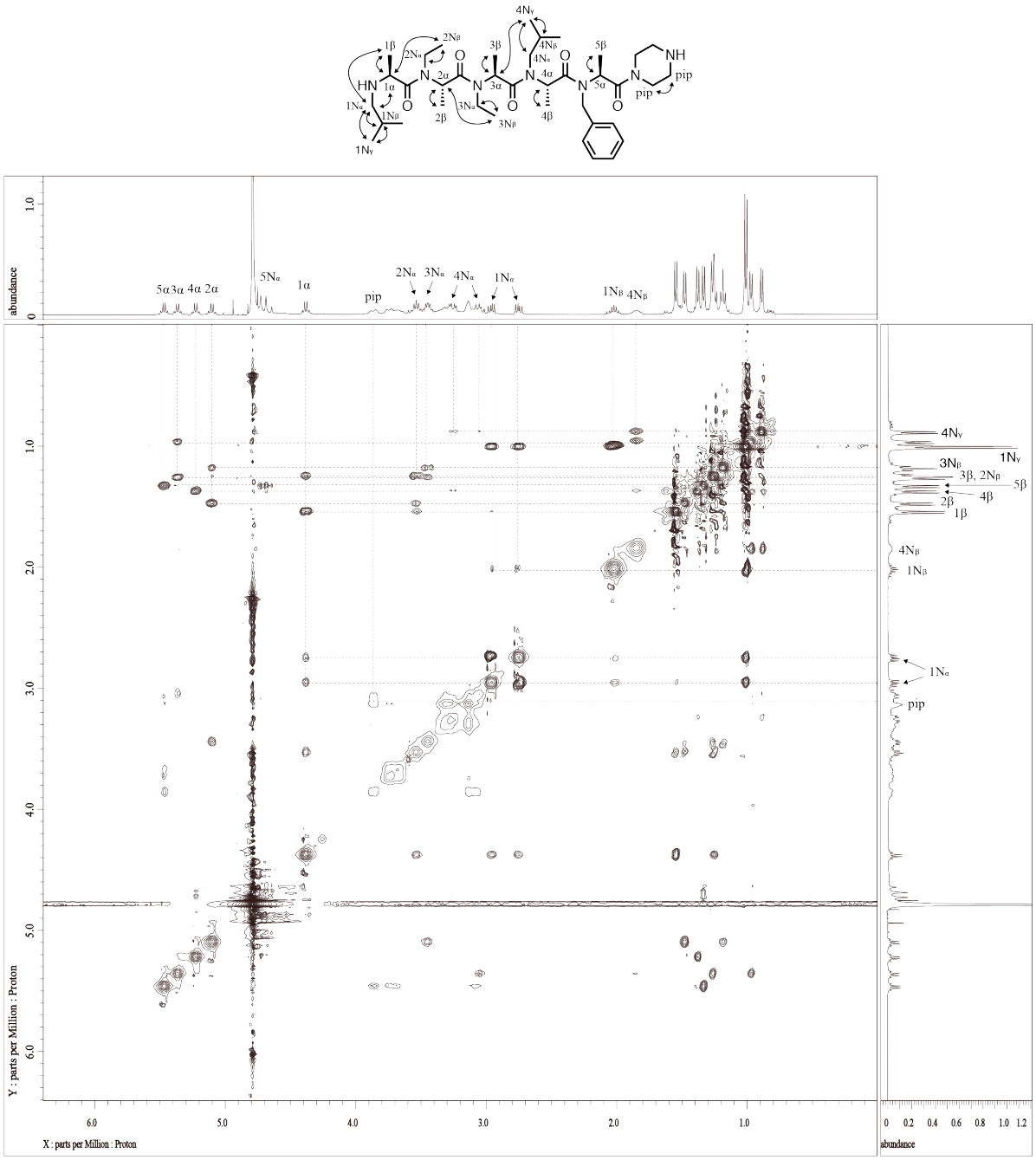


Figure S29. NOESY spectrum of oligo-NSA **8** in D_2O highlighting other NOEs. NOE peaks that are not highlighted in **Figure S28** are highlighted with black lines and arrows.

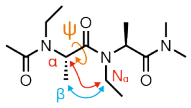
a Distance between N_α proton and α or β protons on a model NSA dimer with 24 different φ angles

φ (°)	α–N_α (Å)	β–N_α (Å)
-120	3.6	2.1
-105	3.6	2.1
-90	3.5	2.1
-75	3.4	2.2
-60	3.3	2.2
-45	3.3	2.1
-30	2.7	2.2
-15	2.4	2.3
0	2.2	2.4
15	2.1	2.7
30	2.0	3.2
45	2.1	3.5
60	2.1	3.6
75	2.1	3.8
90	2.1	3.9
105	2.3	4.4
120	2.4	4.5
135	2.6	4.5
150	2.9	4.4
165	3.1	4.2
180	3.3	4.0
-165	3.4	3.8
-150	3.6	3.1
-135	3.6	2.5

b φ angle and distance between N_α proton and α or β protons observed on the crystal structure

Residue	φ (°)	α–N_α (Å)	β–N_α (Å)
2	-87.7	3.5	2.1
3	-143.4	3.5	2.4
4	-134.3	3.5	2.2
5	-112.8	3.5	2.1

c Distance between N_α proton and α or β protons on a model NSA dimer with 24 different ψ angles



ψ (°)	α–N_α (Å)	β–N_α (Å)
90	2.1	4.4
105	2.0	4.2
120	2.0	4.0
135	2.0	3.8
150	2.1	3.7
165	1.9	2.3
180	2.1	2.1
-165	2.3	2.1
-150	2.7	2.0
-135	3.5	2.0
-120	3.6	2.0
-105	3.7	2.1
-90	4.0	2.1
-75	3.6	2.1
-60	3.6	2.1
-45	3.7	2.4
-30	3.8	2.8
-15	3.8	3.2
0	3.8	3.9
15	3.3	4.5
30	3.1	4.7
45	2.8	4.7
60	2.6	4.6
75	2.4	4.6

d ψ angle and distance between N_α proton and α or β protons observed on the crystal structure

Residue	ψ (°)	α–N_α (Å)	β–N_α (Å)
2	112.7	1.9	3.8
3	85.8	2.2	4.3
4	91.8	2.1	4.3
5	93.3	1.9	3.8

Figure S30. Distance between α or β protons and N_α protons on a model NSA dimer of a series of backbone dihedral angles. **(a)** Distance between α or β protons and N_α protons on the model NSA dimer. Conformations of acetyl-*N*-ethylalanine dimer dimethylamide with a series of φ angle were optimized by quantum mechanical calculations and distance between α or β protons and N_α protons on each conformer were measured. The φ angles that gives the distance between α proton and N_α protons is over 3 Å and the distance between β proton and N_α protons is within 3 Å are highlighted in yellow. **(b)** φ angles and distances between α or β protons and N_α protons on the crystal structure are listed. **(c)** Distance between α or β protons and N_α protons on the model NSA dimer. Conformations of acetyl-*N*-ethylalanine dimer dimethylamide with a series of ψ angle were optimized by quantum mechanical calculations and distance between α or β protons and N_α protons on each conformer were measured. The ψ angles that gives the distance between α proton and N_α

protons is within 3 Å and the distance between β proton and N_α protons is over 3 Å are highlighted in yellow. **(b)** ψ angles and distances between α or β protons and N_α protons on the crystal structure are listed.

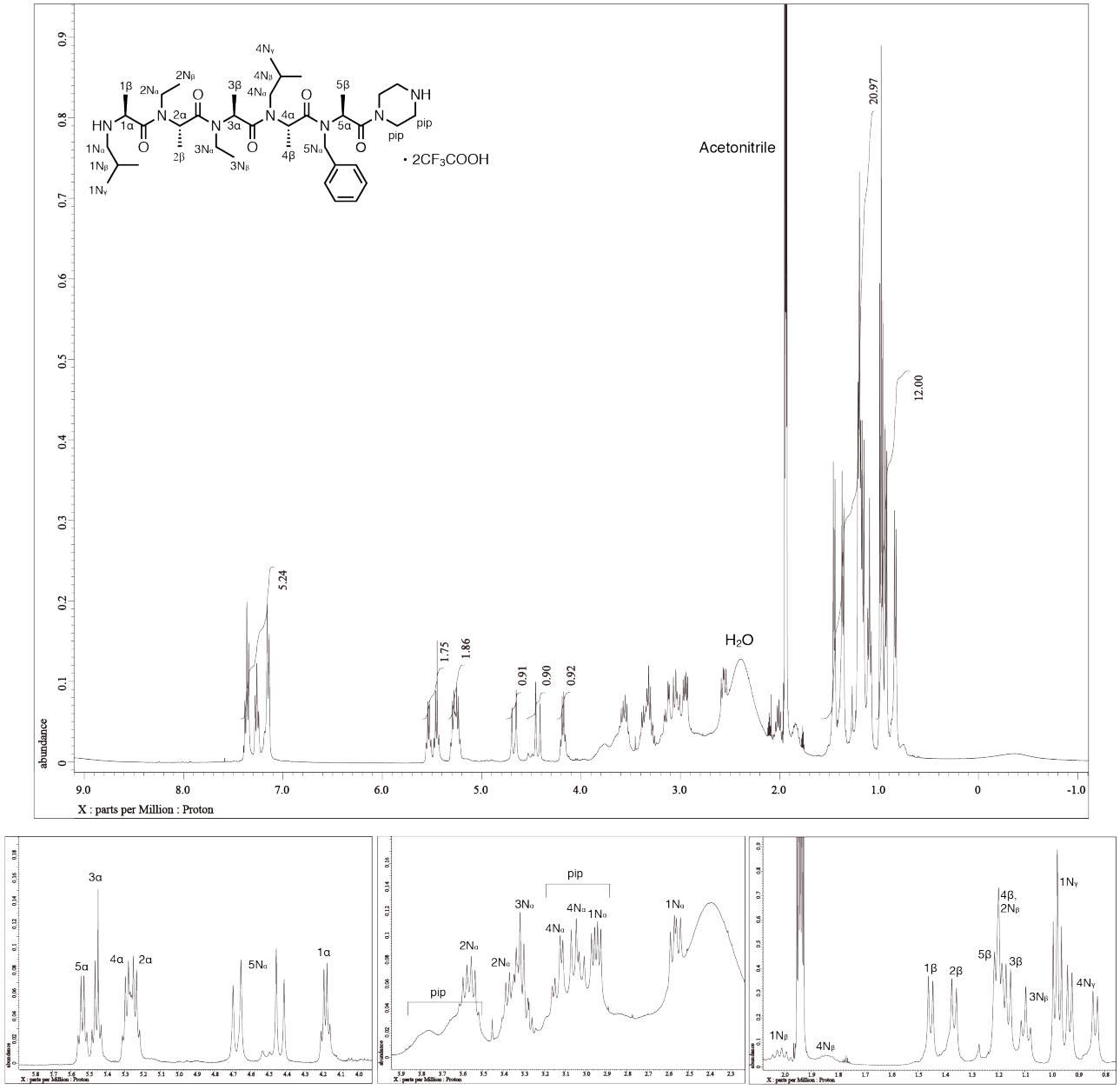


Figure S31. 1H NMR spectrum of oligo-NSA **8** in CD_3CN. Spectrum was recorded at 25 °C.

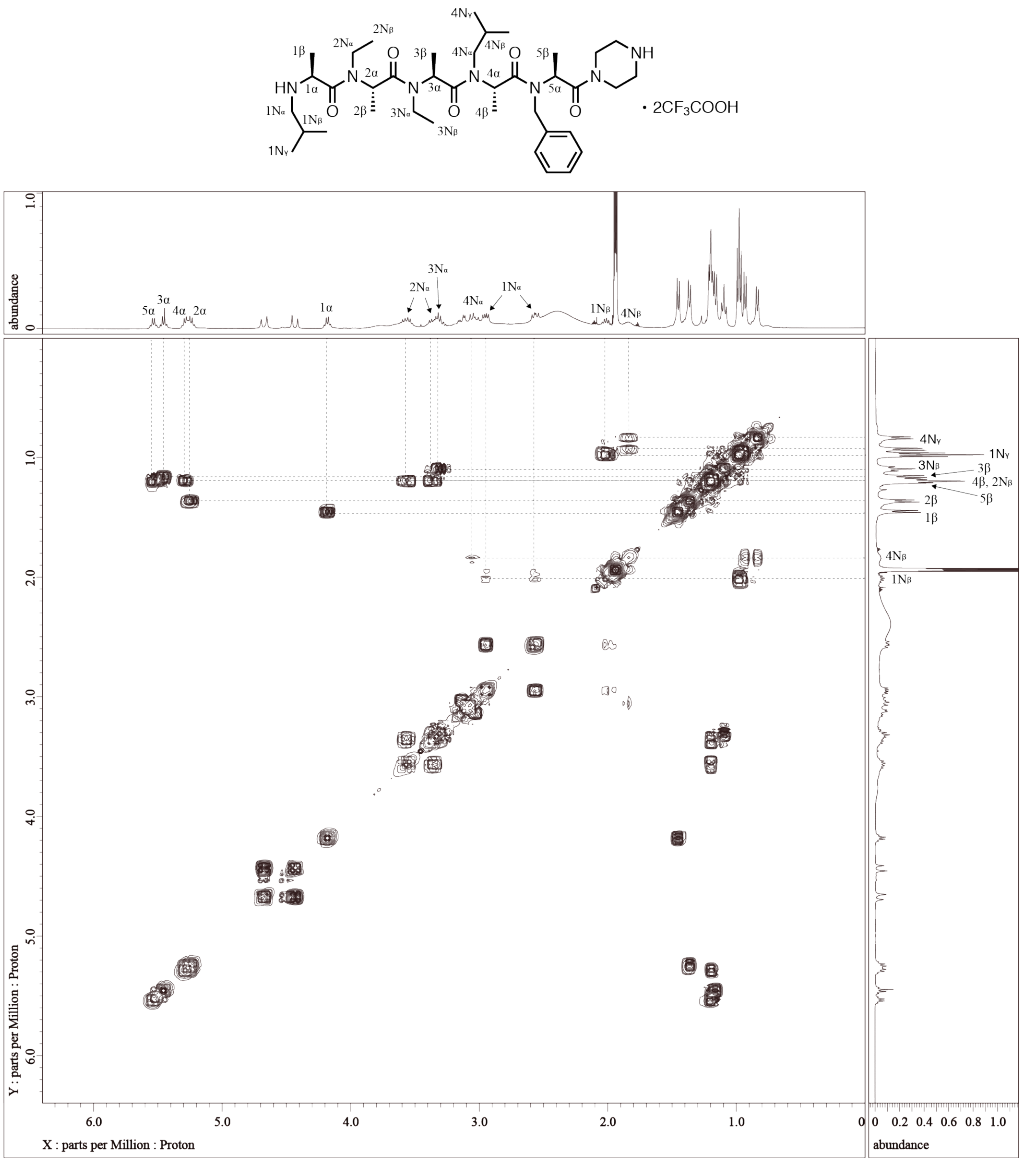


Figure S32. COSY spectrum of oligo-NSA **8** in CD_3CN. Spectrum was recorded at 25 °C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

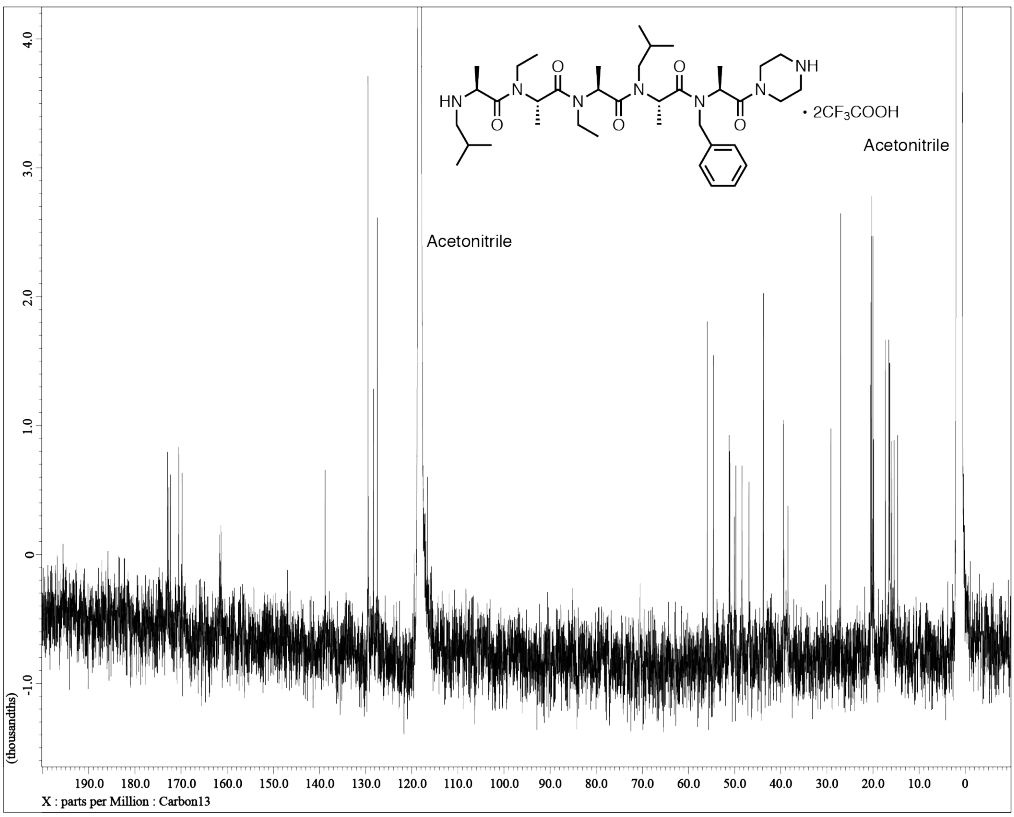


Figure S33. 13C NMR spectrum of oligo-NSA **8** in CD_3CN. Spectrum was recorded at 25 °C.

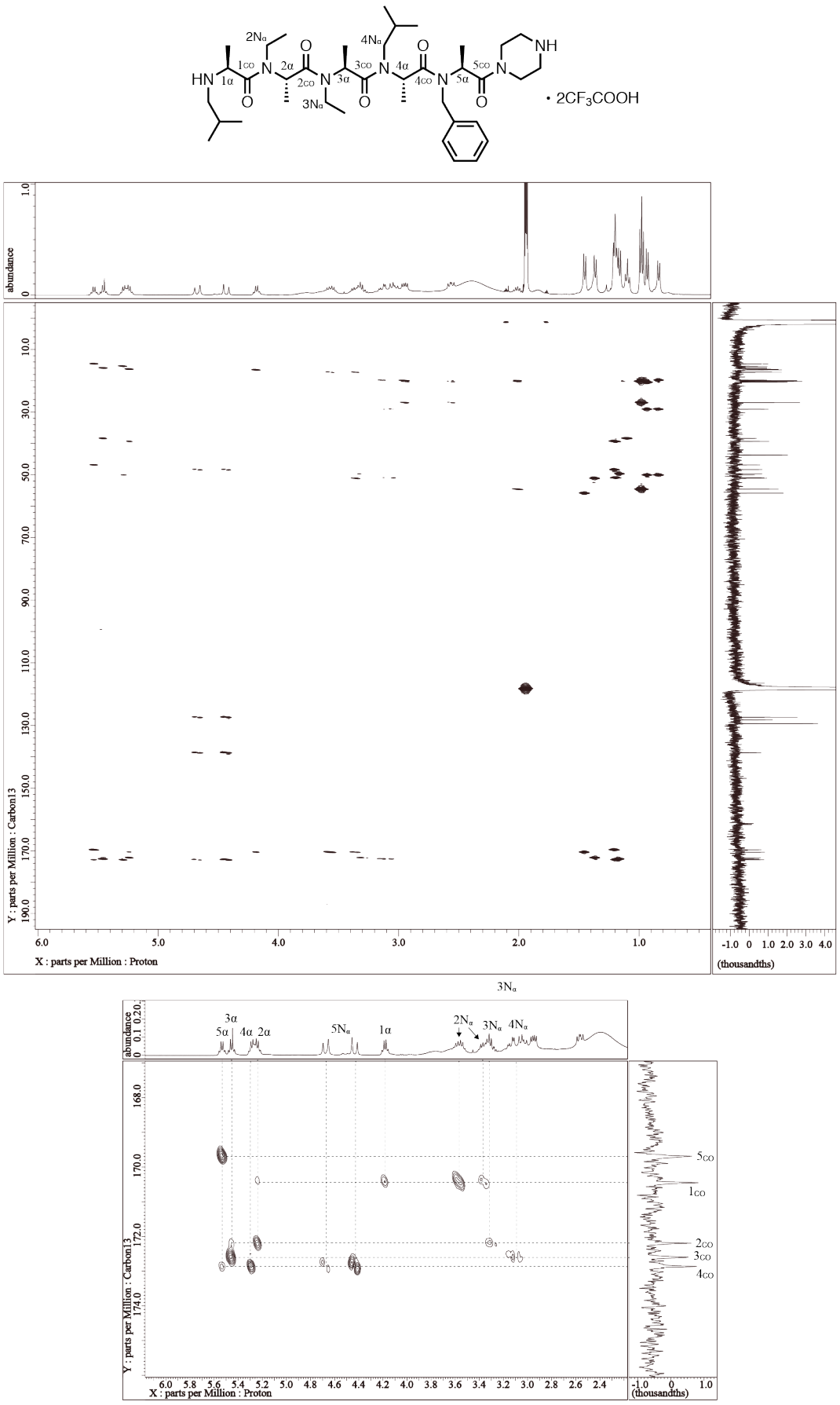


Figure S34. HMBC spectrum of oligo-NSA **8** in CD_3CN. Spectrum was recorded at 25 °C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

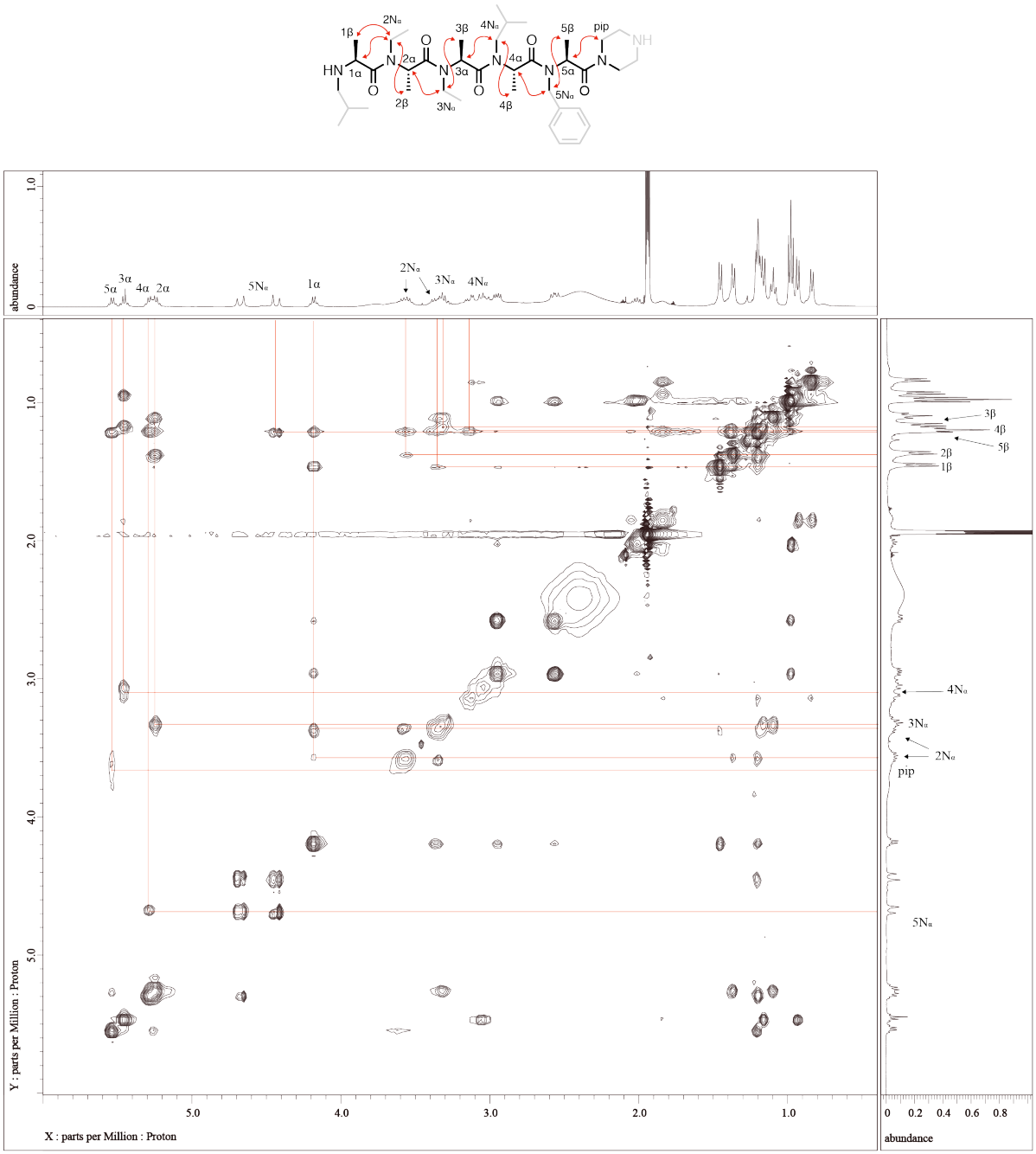


Figure S35. NOESY spectrum of oligo-NSA **8** in CD_3CN. Spectrum was recorded at 25 °C. NOE peaks that support restricted rotations about backbone dihedral angles are shown with red lines. Red lines and arrows indicate the protons that are within 3 Å in the crystal and exhibit strong NOEs.

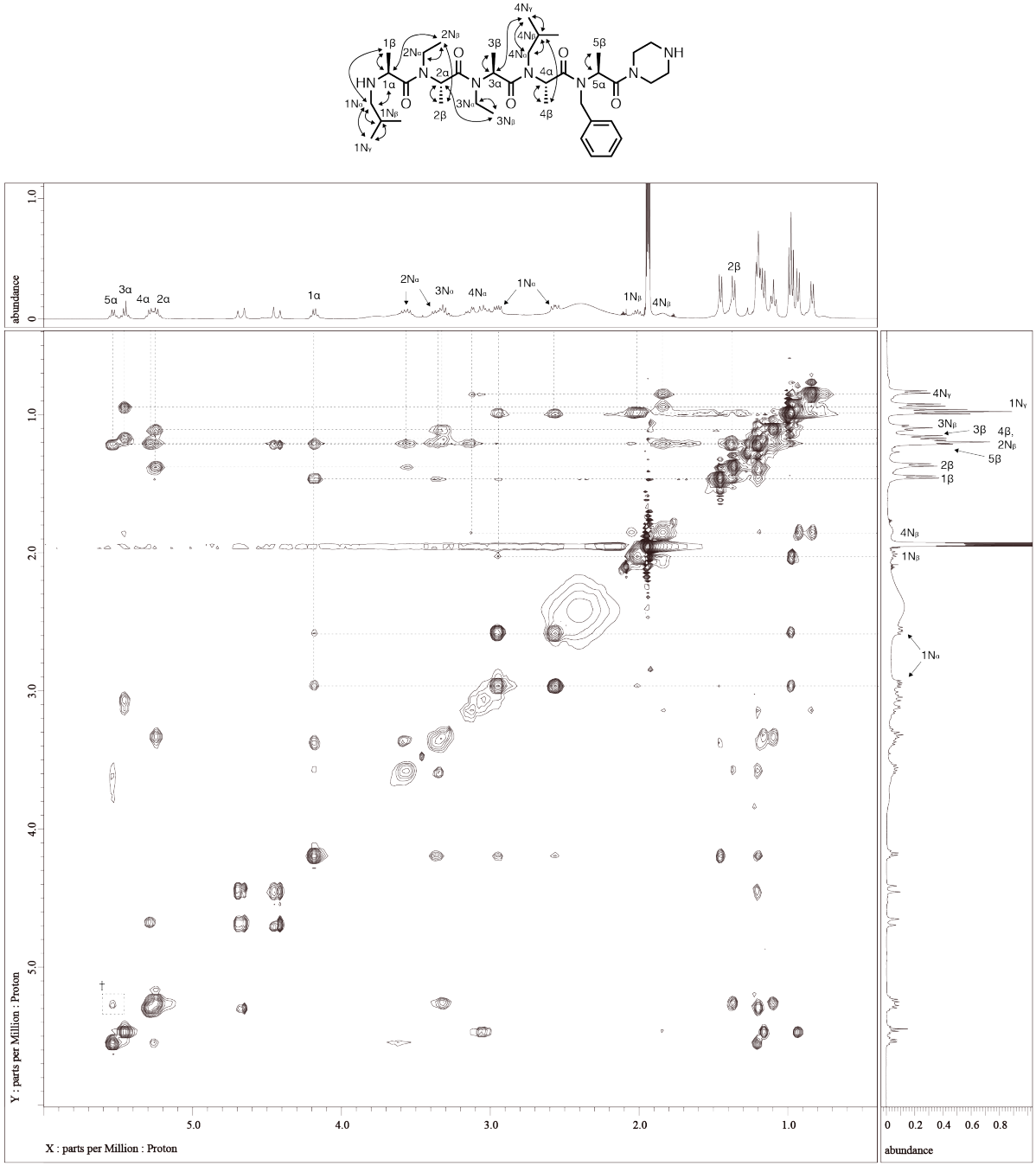


Figure S36. NOESY spectrum of oligo-NSA **8** in CD_3CN highlighting other NOEs. NOE peaks that are not highlighted in **Figure S35** are highlighted with black lines and arrows. The cross peak marked with † was ignored because it is negative NOE in contrast to other cross peaks that are positive NOEs.

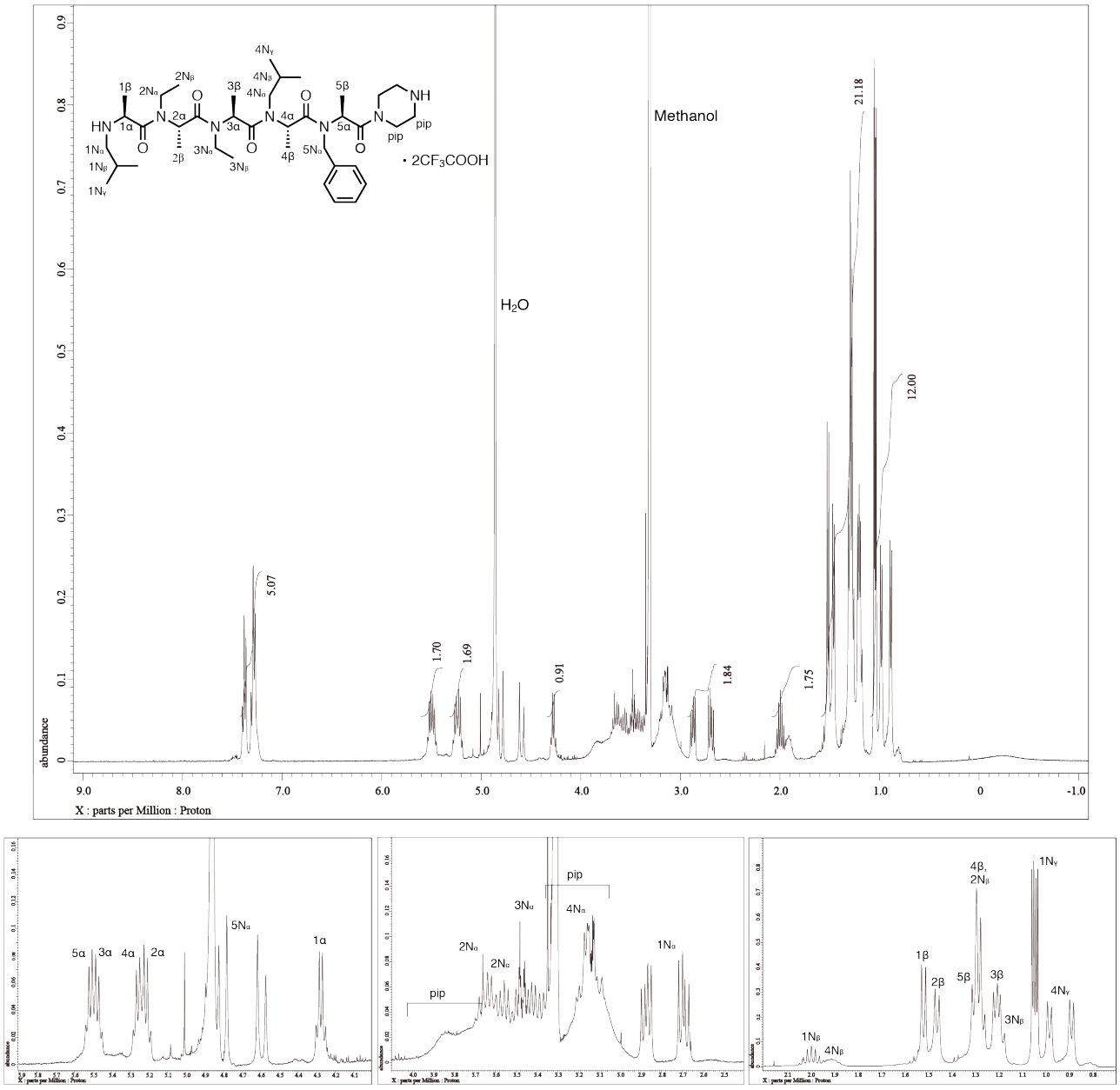


Figure S37. 1H NMR spectrum of oligo-NSA **8** in CD_3OD. Spectrum was recorded at 25 °C.

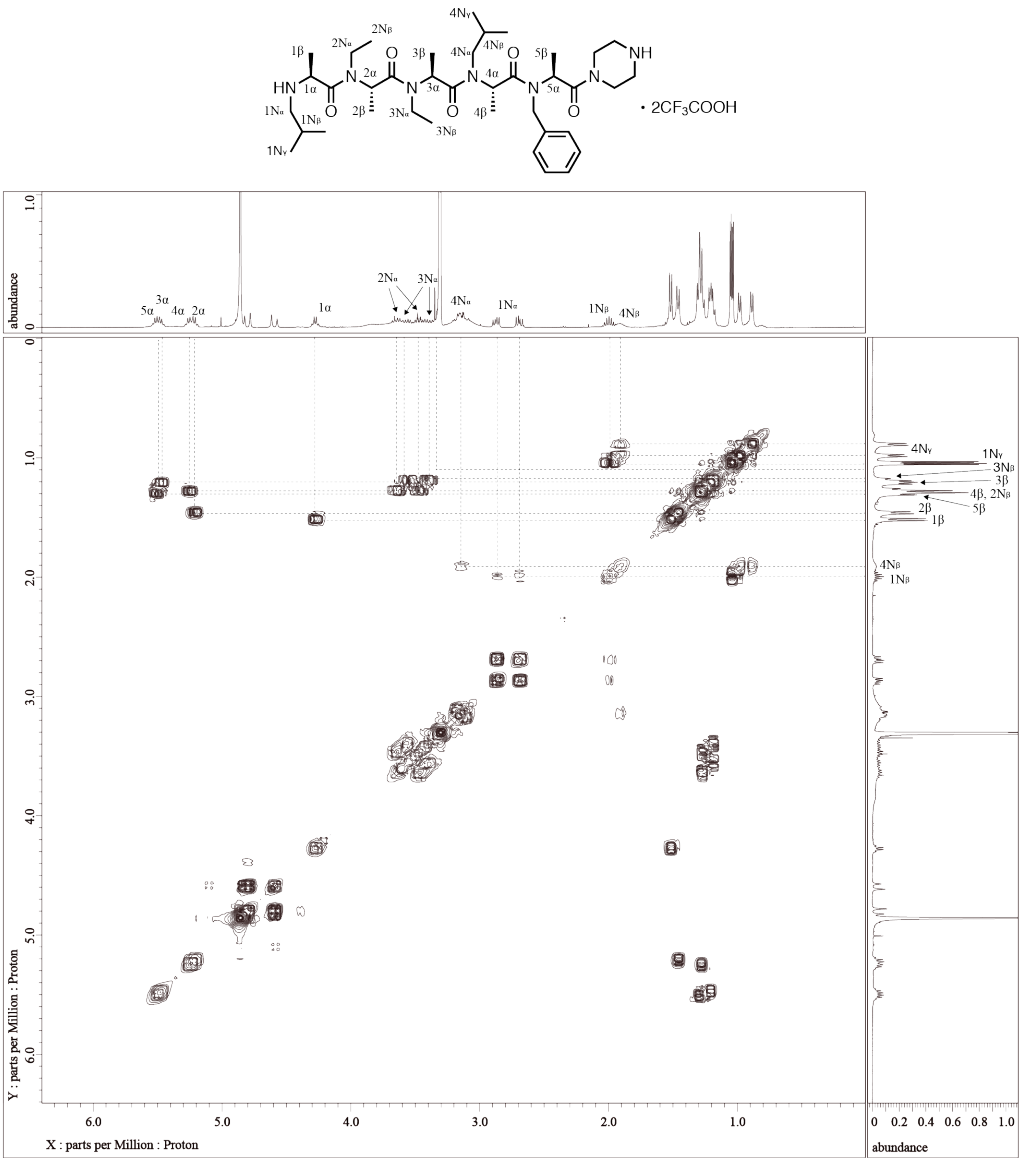


Figure S38. COSY spectrum of oligo-NSA **8** in CD_3OD. Spectrum was recorded at 25°C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

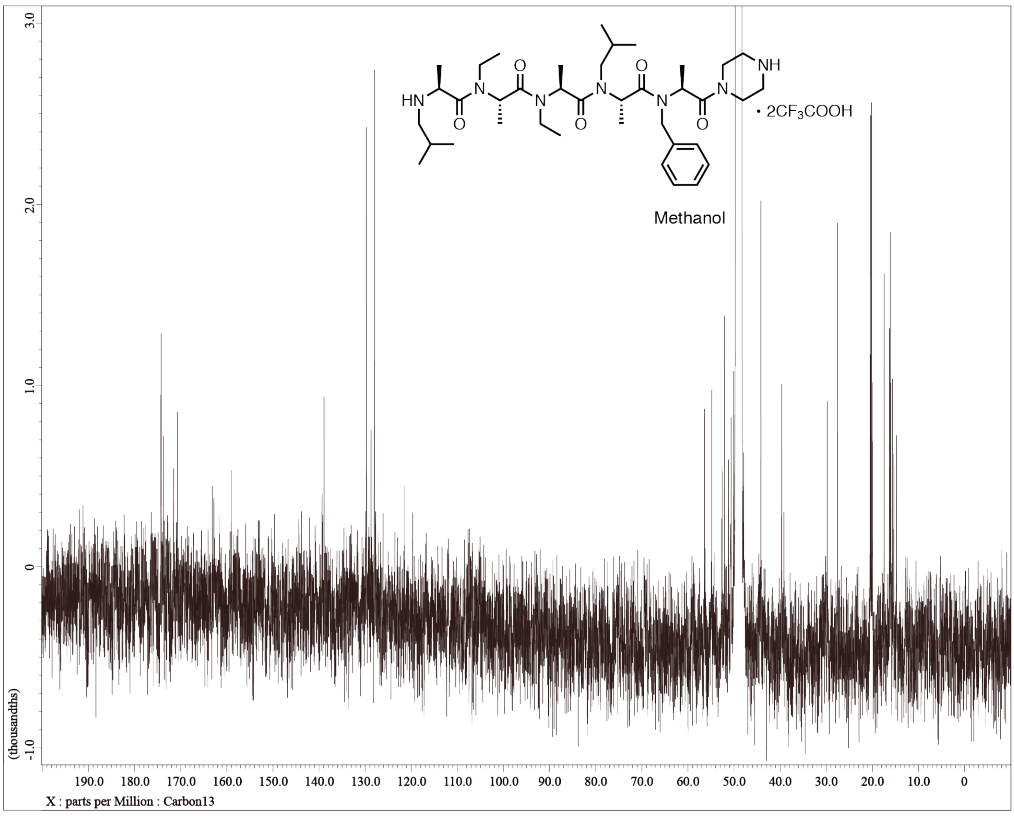


Figure S39. 13C NMR spectrum of oligo-NSA **8** in CD_3OD. Spectrum was recorded at 25 °C.

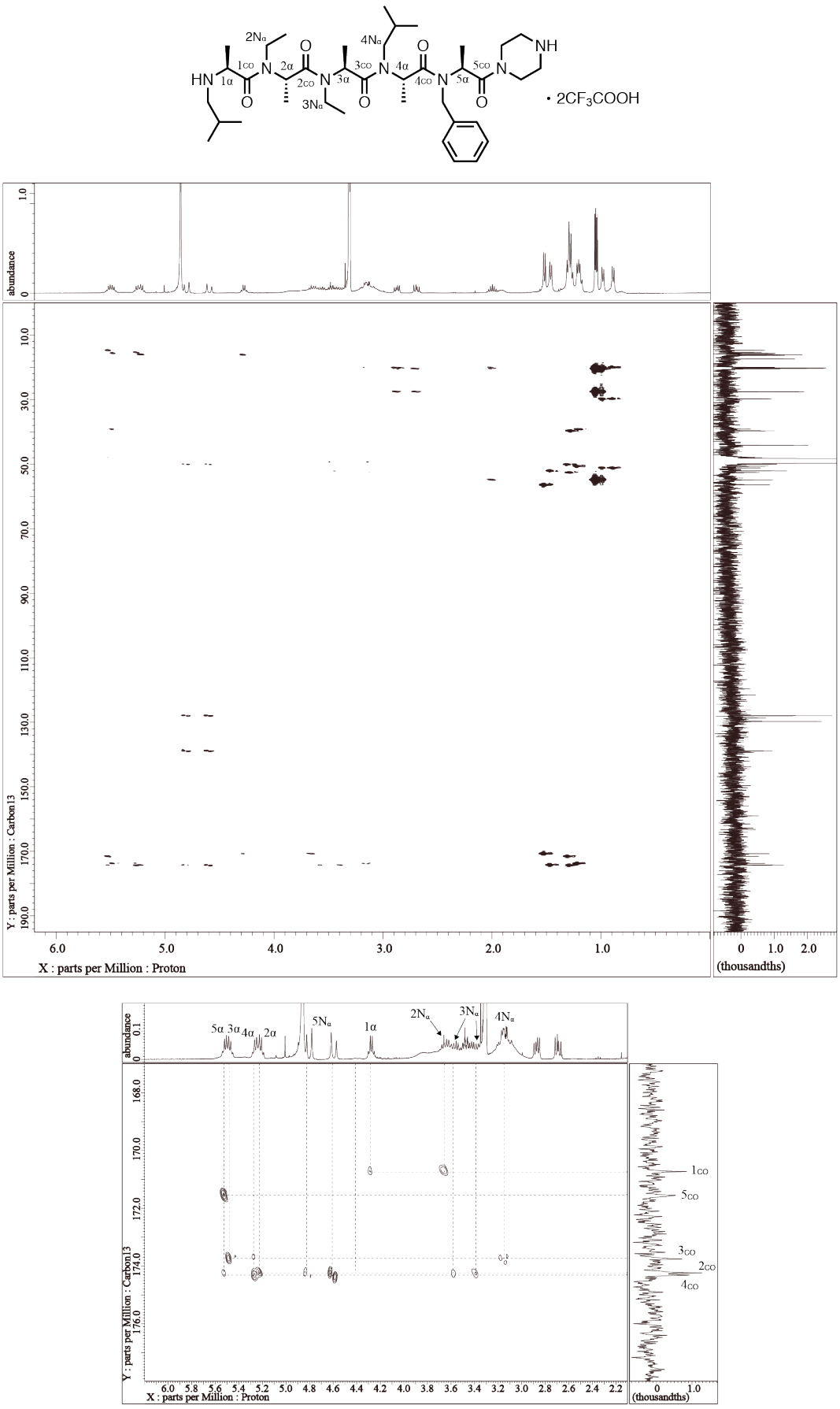


Figure S40. HMBC spectrum of oligo-NSA **8** in CD_3OD. Spectrum was recorded at 25 °C. Correlation peaks that support assignment of 1H NMR are shown with dashed lines.

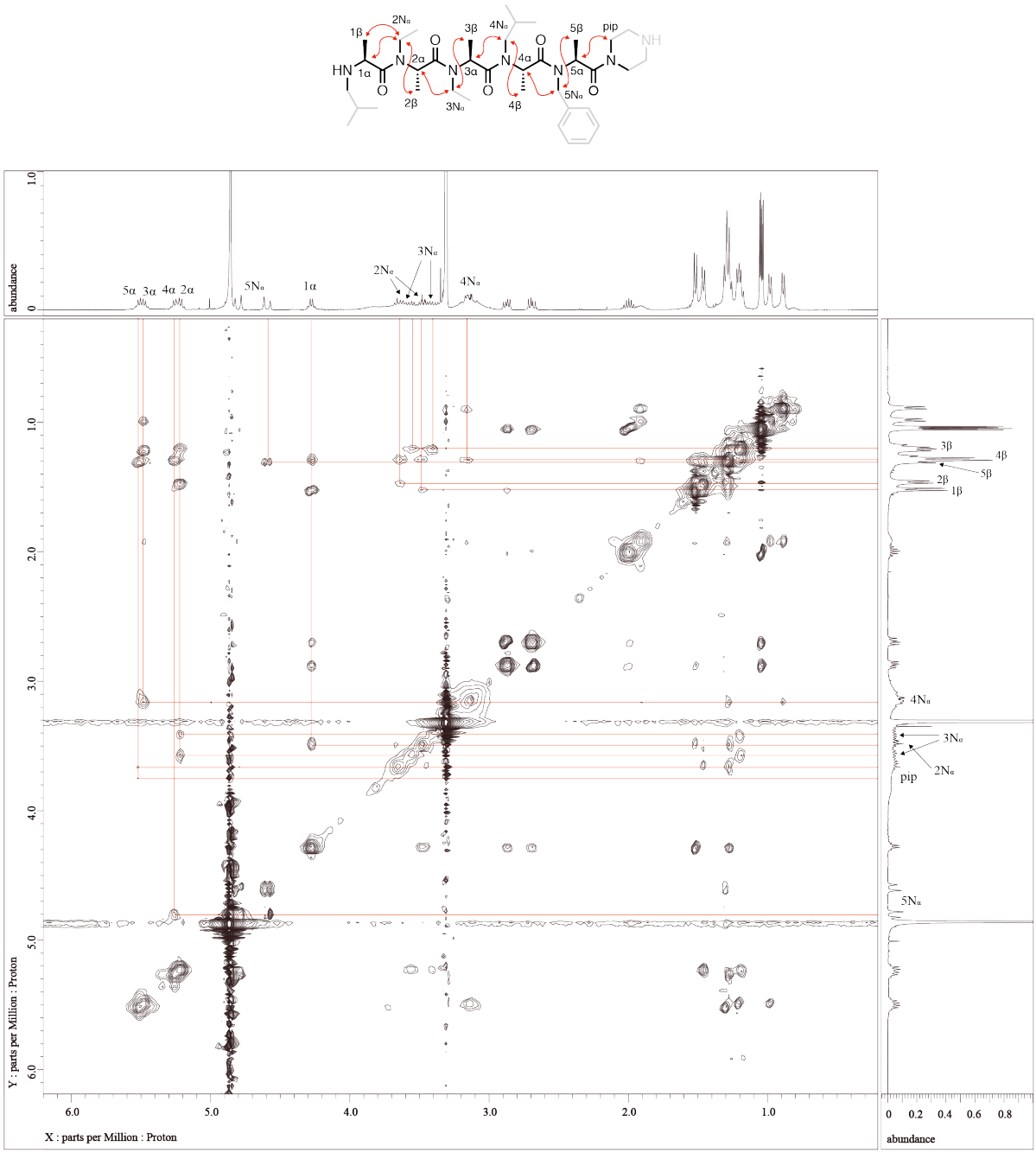


Figure S41. NOESY spectrum of oligo-NSA **8** in CD_3OD. Spectrum was recorded at 25 °C. NOE peaks that support restricted rotations about backbone dihedral angles are shown with red lines. Red lines and arrows indicate the protons that are within 3 Å in the crystal and exhibit strong NOEs.

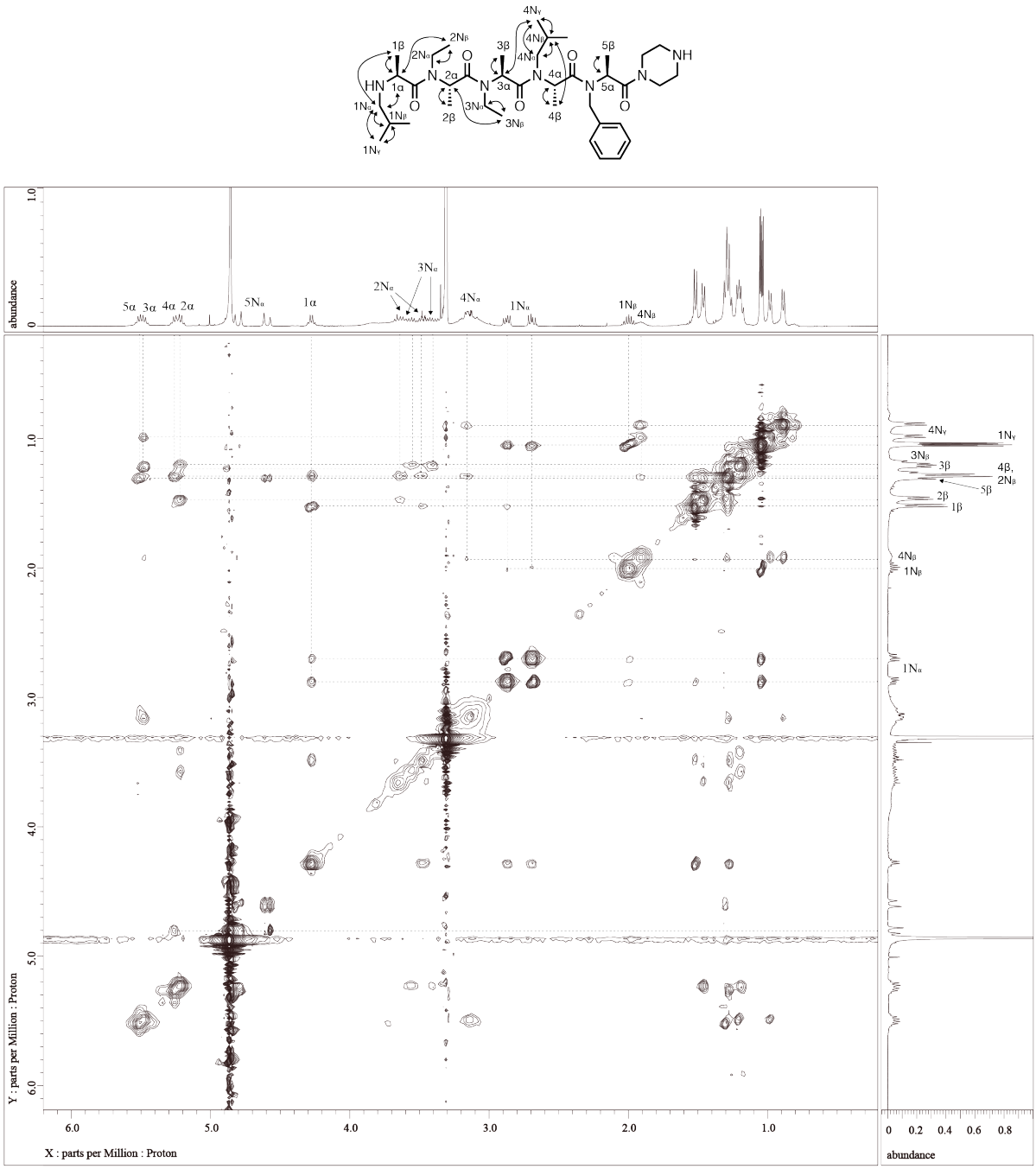


Figure S42. NOESY spectrum of oligo-NSA **8** in CD_3OD highlighting other NOEs. NOE peaks that are not highlighted in **Figure S41** are highlighted with black lines and arrows.

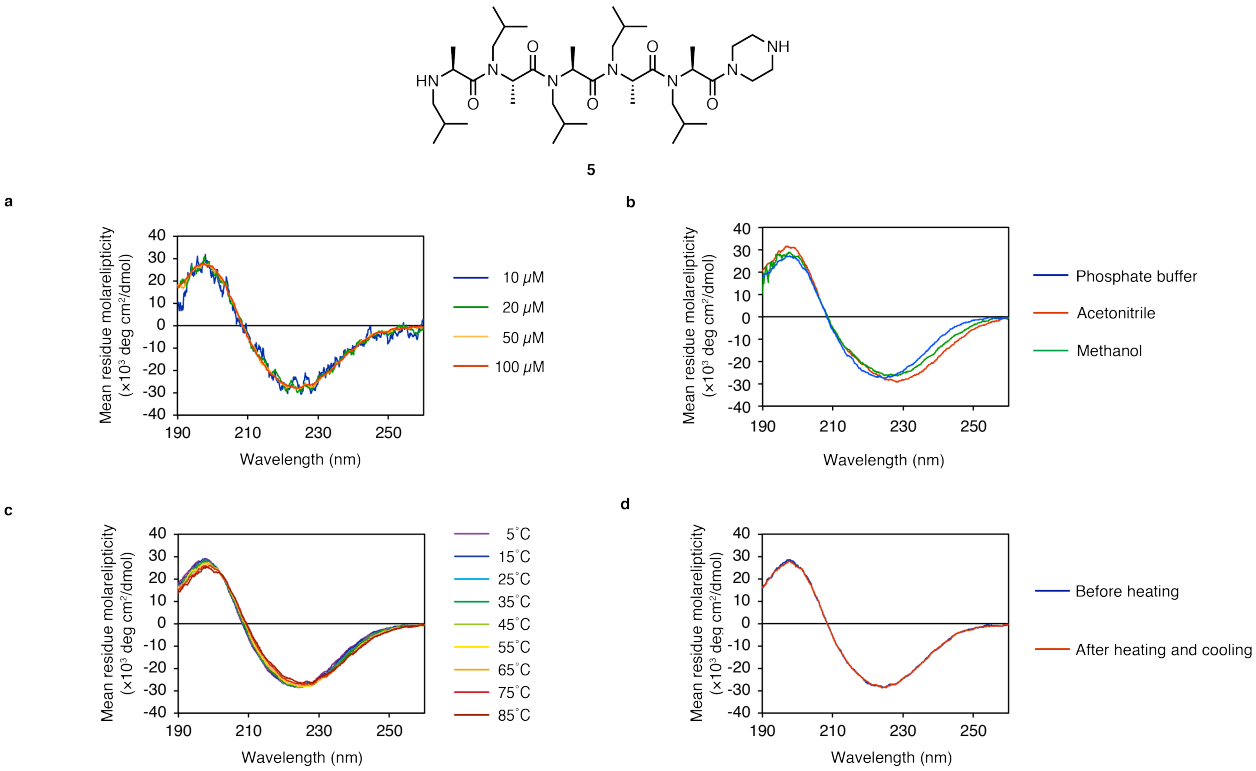


Figure S43. Concentration, solvent and temperature dependence of CD spectrum of *N*-isobutylalanine pentamer piperazine amide **5**. **(a)** CD spectra of oligomer **5** at 10, 20, 50 and 100 μM concentrations in phosphate buffer (pH 7.2). The spectral shape did not change with increased concentrations, indicating that the ordered conformation is not dictated by aggregation of the oligomer. **(b)** CD spectra of oligomer **5** in phosphate buffer, acetonitrile or methanol. All the three spectra were measured with 100 μM oligomer solution. The spectral shape is similar in all the three solvents, supporting the folding is not dictated by remote inter-residue interactions but by local steric effects. **(c)** Temperature dependence of oligo-NSA in phosphate buffer. The spectra were recorded at 5–85 °C with 100 μM oligomer **5**. **(d)** CD spectra of oligo-NSA (**5**) in phosphate buffer before and after heating at 85 °C.

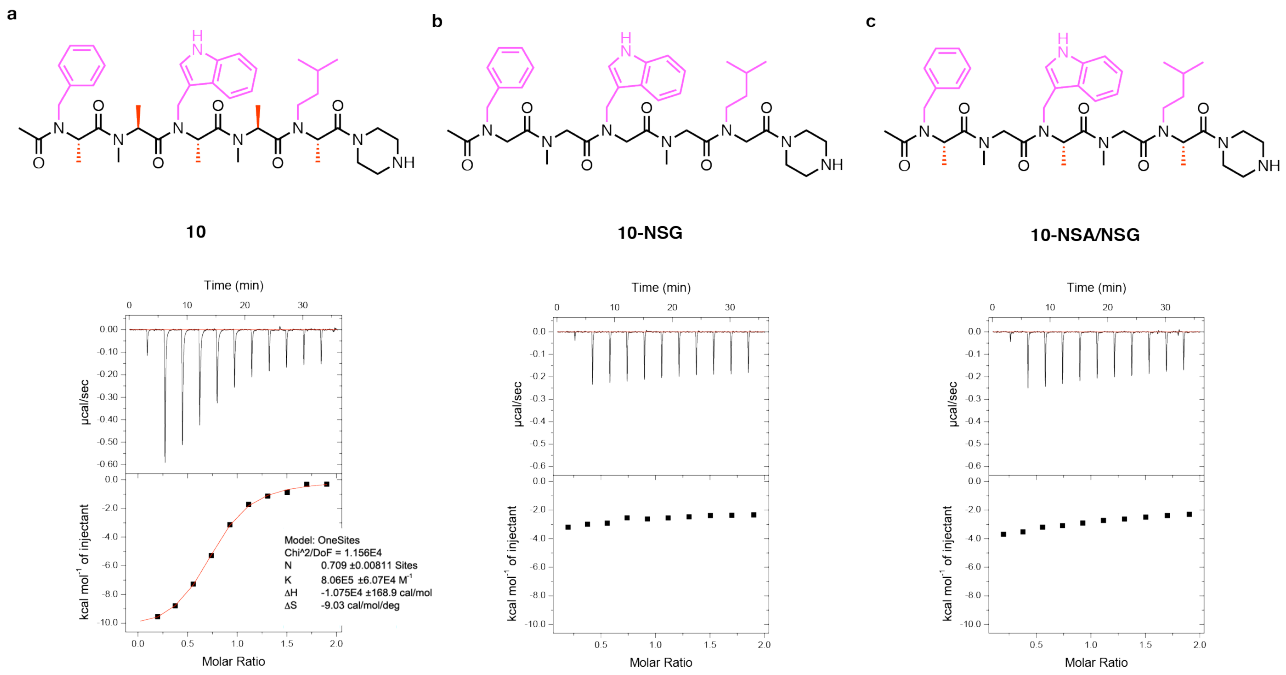


Figure S44. Isothermal calorimetry data of **10**, **10-NSG** and **10-NSA/NSG** binding to MDM2. Isothermal calorimetry data of **(a) 10**, **(b) 10-NSG** and **(c) 10-NSA/NSG**. For **(a)**, full parameters obtained from the fitting are shown. All the binding assays were conducted in PBS.

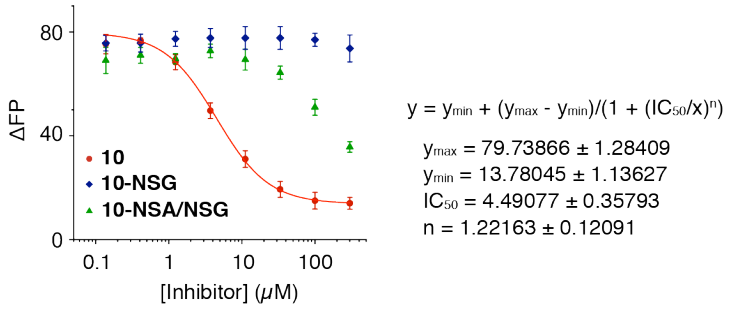


Figure S45. Competitive fluorescence polarization assay of oligo-NSA and derivatives against the interaction between p53-TAD and MDM2. The graph shown in **Figure 6d** is reproduced here with full parameters obtained from the curve fitting of the acquired data for **10** shown on the side.

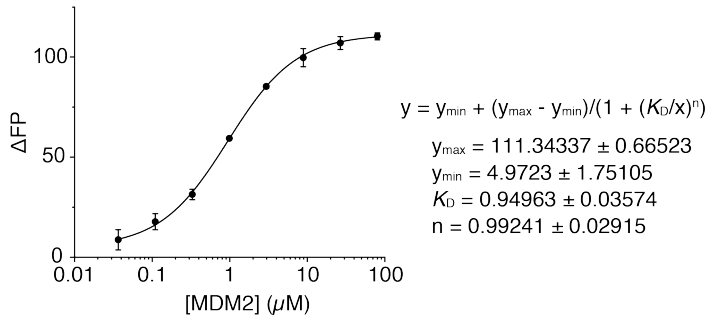
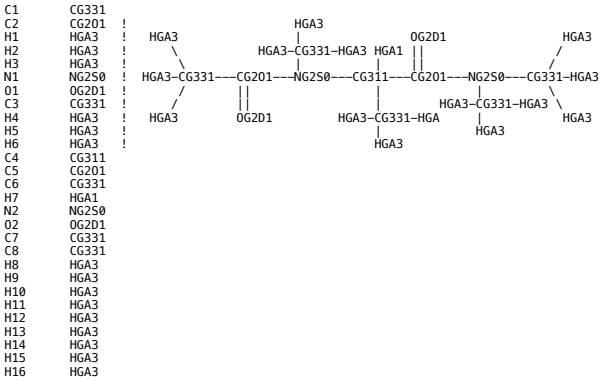


Figure S46. Binding isotherm of fluorescently-labeled p53-TAD and MDM2. Fluorescent polarization assay was conducted using 50 nM fluorescently-labeled p53-TAD with 0.037–80 μM MDM2 in PBS-T (0.01% tween-20) at room temperature. Error bars represent standard deviations of triplicates. Parameters obtained from the curve fitting of the acquired data are shown on the side.

Table S1. The parameter files generated by CGenFF. **(a)** Parameters of acetyl-*N*-methylalanine dimethylamide. **(b)** Parameters of acetyl-*N*-methylglycine dimethylamide.

a



```
C1       CG331
C2       CG2O1  !                        HGA3
H1       HGA3   !   HGA3                  |              OG2D1                HGA3
H2       HGA3   !      \           HGA3–CG331–HGA3 HGA1 ||                   /
H3       HGA3   !       \           |    |   ||                          /
N1       NG2S0  !  HGA3–CG331–––CG2O1–––NG2S0–––CG311–––CG2O1–––NG2S0–––CG331–HGA3
O1       OG2D1  !       /       ||                |          |           \
C3       CG331  !      /        ||                |        HGA3–CG331–HGA3 \
H4       HGA3   !   HGA3        OG2D1          HGA3–CG331–HGA      |          HGA3
H5       HGA3   !                                 |             HGA3
H6       HGA3   !                                HGA3
C4       CG311
C5       CG2O1
C6       CG331
H7       HGA1
N2       NG2S0
O2       OG2D1
C7       CG331
C8       CG331
H8       HGA3
H9       HGA3
H10      HGA3
H11      HGA3
H12      HGA3
H13      HGA3
H14      HGA3
H15      HGA3
H16      HGA3


!Parameters generated by CGenFF
BONDS
CG311  NG2S0   320.00     1.4300

ANGLES
CG2O1  CG311  NG2S0    50.00    107.00
CG331  CG311  NG2S0    70.00    113.50
NG2S0  CG311  HGA1     48.00    108.00
CG2O1  NG2S0  CG311    42.00    119.50
CG311  NG2S0  CG331    45.00    121.00

DIHEDRALS
NG2S0  CG2O1  CG311  NG2S0      0.4000  1     0.00
OG2D1  CG2O1  CG311  NG2S0      0.0000  1     0.00
CG311  CG2O1  NG2S0  CG331      2.6000  2   180.00
CG331  CG2O1  NG2S0  CG311      2.6000  2   180.00
OG2D1  CG2O1  NG2S0  CG311      2.6000  2   180.00
NG2S0  CG311  CG331  HGA3       0.2000  3     0.00
CG2O1  CG311  NG2S0  CG2O1      0.2000  1   180.00
CG2O1  CG311  NG2S0  CG331      0.2000  1   180.00
CG331  CG311  NG2S0  CG2O1      1.8000  1     0.00
CG331  CG311  NG2S0  CG331      1.8000  1     0.00
HGA1   CG311  NG2S0  CG2O1      0.0000  1     0.00
HGA1   CG311  NG2S0  CG331      0.4200  3     0.00
HGA3   CG331  NG2S0  CG311      0.4200  3     0.00
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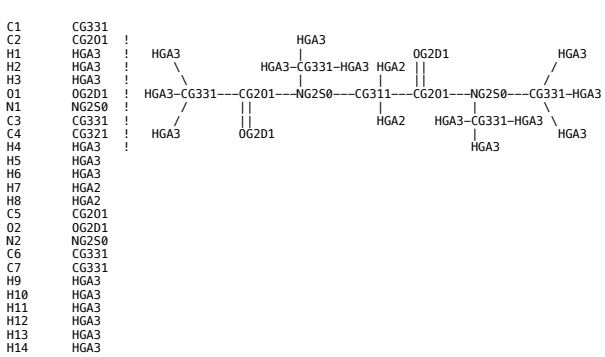
b



```
C1       CG331
C2       CG2O1  !                      HGA3
H1       HGA3   !   HGA3                |              OG2D1                HGA3
H2       HGA3   !      \          HGA3–CG331–HGA3 HGA2 ||                   /
H3       HGA3   !       \          |    |   ||                          /
O1       OG2D1  !  HGA3–CG331–––CG2O1–––NG2S0–––CG311–––CG2O1–––NG2S0–––CG331–HGA3
N1       NG2S0  !       /       ||                |          |           \
C3       CG331  !      /        ||              HGA2    HGA3–CG331–HGA3 \
C4       CG321  !   HGA3        OG2D1                       |             HGA3
H4       HGA3   !                                         HGA3
H5       HGA3
H6       HGA3
H7       HGA2
H8       HGA2
C5       CG2O1
O2       OG2D1
N2       NG2S0
C6       CG331
C7       CG331
H9       HGA3
H10      HGA3
H11      HGA3
H12      HGA3
H13      HGA3
H14      HGA3


!Parameters generated by CGenFF
BONDS
CG321  NG2S0   315.00     1.4340

ANGLES
CG2O1  CG321  NG2S0    50.00    107.00
NG2S0  CG321  HGA2     51.50    109.50
CG2O1  NG2S0  CG321    42.00    119.50
CG321  NG2S0  CG331    45.00    121.00

DIHEDRALS
NG2S0  CG2O1  CG321  NG2S0      0.4000  1     0.00
OG2D1  CG2O1  CG321  NG2S0      0.0000  1     0.00
CG331  CG2O1  NG2S0  CG321      2.6000  2   180.00
OG2D1  CG2O1  NG2S0  CG321      2.6000  2   180.00
CG2O1  CG321  NG2S0  CG2O1      0.2000  1   180.00
CG2O1  CG321  NG2S0  CG331      0.2000  1   180.00
HGA2   CG321  NG2S0  CG2O1      0.0000  3     0.00
HGA2   CG321  NG2S0  CG331      0.4200  3     0.00
HGA3   CG331  NG2S0  CG321      0.4200  3     0.00
```

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