Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Short polyglutamine peptide forms a high-affinity binding site for thioflavine-T at the *N*-terminus

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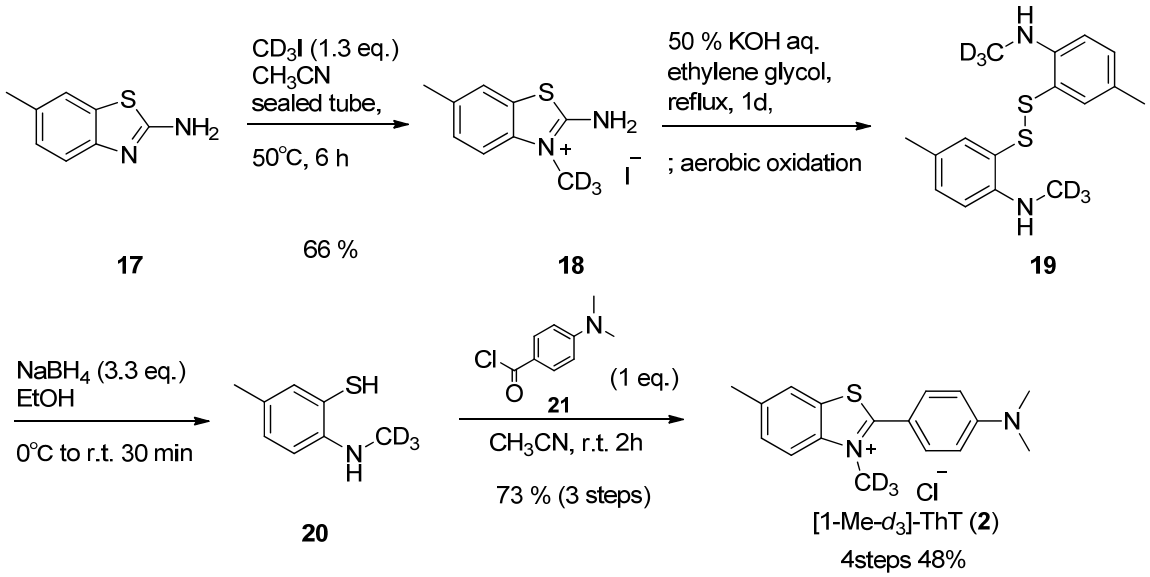
26 pages

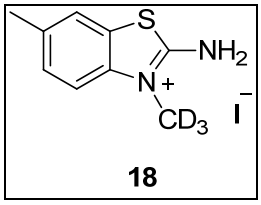
Syntheses of stable isotope labeled compounds

General methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. All manually performed reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm E. Merck silica gel (60 F_{254}) plates, using ultraviolet light (254 nm), 10% ethanolic phosphomolybdic acid or 37% *n*-buthanolic ninhydrin acid (with 1% acetic acid) and heat as developing agents. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. HPLC was performed on a JASCO HPLC system equipped with a PU-2089 Plus intelligent pump, a UV-2070 Plus UV detector and GL sciences Inertsil® SIL 100A (4.6 × 250 mm) or Inertsil® SIL 100A (10 × 250 mm) at a flow rate of 1.0 mL min^{-1}, 1.5mL min^{-1}, or 4.0 mL min^{-1}. The eluate was monitored by UV absorption at 265 nm. Solution NMR spectra were recorded on a JEOL JNM-ECA500, JNM-ECX500, JNM-ECS400 instruments, and data processing was performed with ALICE or ALICE2 software (JEOL). Data are presented as follows: chemical shifts, integration, multiplicities (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants in hertz (Hz) and assignment. Chemical shifts are reported in δ (ppm) using internal standards; δ C*H*Cl_3 of 7.26 and 77.00 for ^{1}H- and ^{13}C-NMR, C*H*D_2OD of δ 3.31 and 49.00 for ^{1}H- and ^{13}C-NMR, C_6*H*D_5 of δ 7.16 and 128.06 for ^{1}H- and ^{13}C-NMR, δ *H*D_2C-SO-CD_3 (dimethylsulfoxide) of δ 2.50 and 39.52 for ^{1}H- and ^{13}C-NMR, respectively. MALDI-TOF MS analyses were performed with a Shimadzu Biotec Axima-TOF^2 mass spectrometer using α-cyano-4-hydroxycinnamic acid as a matrix. ESI-TOF MS spectra were recorded on a Bruker Daltonics BioTOF-Q mass spectrometer. Optical rotations were determined by measurements on a JASCO DIP-1000 polarimeter. Fluorescence spectra were recorded by using Molecular Devices SpectraMax Gemini EM.

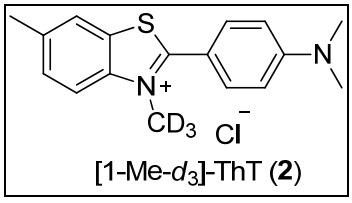
Synthesis of [3-Me-d_3]ThT





[3-Me-d_3]2-Amino-3,6-dimethylbenzothiazolium iodide (18). To a solution of 2-amino-6-methylbenzo-thiazole (**17**, 0.985 g, 0.6 mmol) in MeCN (12 mL) in a sealed tube was added iodomethane-d_3 (0.52 mL, ISOTEC, >99.5 atom%) at room temperature, and the resultant solution was stirred at 50 °C for 12 hours. After being cooled to room temperature, the reaction mixture was diluted with Et_2O (10 mL), and then the white suspension was filtered. The collected precipitates were washed with cold Et_2O and dried under vacuum to afford **18** (1.23 g, 66%).

18: white solid; m.p. > 260 °C; FT-IR (film) ν 1490.7, 1563.0, 1633.4, 3025.8 cm^{-1}; ^1H NMR (400 MHz, DMSO) δ 9.91 (br, 2H), 7.78 (s,1H), 7.55 (d, J = 8.6 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 2.39 (s, 3 H); ^{13}C NMR (100 MHz, DMSO) δ 20.7, 31.7 (Sep, J_{CD} = 22.0 Hz) 113.1, 122.1, 123.2, 128.5, 134.9, 136.9, 167.7; ^2H NMR (400 MHz, $CDCl_3$) δ 4.25.

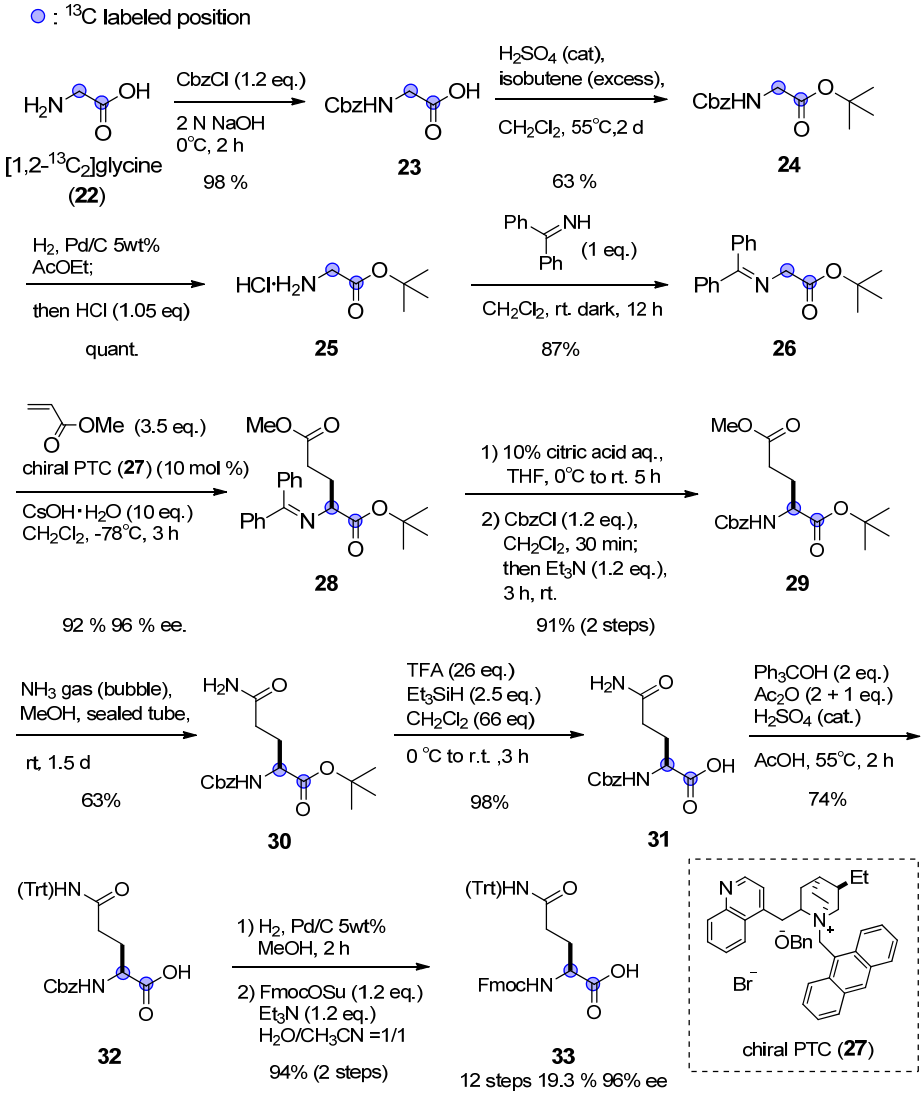


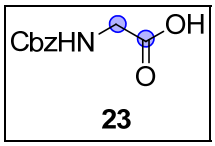
[3-Me-d_3]ThT (2). To a solution of 50% aqueous KOH (v/v, 10 mL) and ethylene glycol (10 mL), **18** (155 mg, 0.5 mmol) was added, and the resultant solution was refluxed for 24 h under argon atmosphere, and further stirred under air for 8 h. The reaction mixture was cooled to room temperature, diluted with saturated aqueous NH_4Cl, and extracted with $CHCl_3$. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated to provide crude **19**.

To a solution of crude **19** in ethanol (4 mL), $NaBH_4$ (50 mg, 1.5 mmol) was added at 0 °C. The reaction mixture was stirred for 30 min at room temperature, diluted with saturated aqueous NH_4Cl, and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, and concentrated to afford crude **20**.

To a solution of **20** in MeCN (2 mL) was added *p*-dimethylamino benzoylchloride (**21**, 65.3 mg, 0.4 mmol) in MeCN (2 mL) at room temperature, and the resultant solution was stirred for 2 h. The reaction mixture was diluted with H_2O (10 mL), and washed with EtOAc (3 x 10 mL). The aqueous layer was saturated with NaCl, and extracted with CH_3Cl (6 x 10 mL). The combined organic layer was dried over Na_2SO_4. Solvent was removed under vacuum to provide **2**. **2:** yellow solid; m.p. > 260 °C; FT-IR (film) ν 1661.6, 2956.8, 3028.7, 3378.7 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, 1H, J = 8.7 Hz), 7.94 (d, 2H, J = 9.2 Hz), 7.88 (s, 1H), 7.61 (d, 1H, J = 8.7 Hz), 6.88 (d, 2H, J = 9.2 Hz), 3.16 (s, 6H), 2.56 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 21.4, 38.6(Sep, J_{CD} = 23.0 Hz), 40.1, 110.8, 112.2, 116.4, 123.4, 128.1, 131.0, 132.5, 138.8, 140.7, 153.9, 172.4; ^2H NMR (400 MHz, $CDCl_3$) δ 4.57; HRMS (MALDI) $C_{17}H_{16}D_3N_2S^+$ calcd for 286.1452 $(M\text{-}Cl)^+$, found 286.1459.

Synthesis of [1,2-$^{13}C_2$]FmocQ(Trt)OH

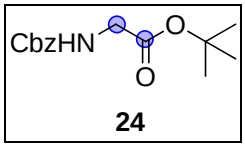




[1,2-$^{13}C_2$]Cbz-Gly-OH 23. To a stirred solution of [1,2-$^{13}C_2$]glycine (**22**, 385 mg, 5.0 mmol, CIL, Andover, MA, 1,2-$^{13}C_2$, 97-99%) in 2 M aqueous NaOH solution (2.5 mL) were added benzyl chloroformate (0.86 mL, 6.0 mmol) and 4 M aqueous NaOH solution (1.5 mL) simultaneously over a period of 90 min at 0°C. The reaction mixture was stirred for 30 min at room temperature. After being washed with Et_2O, the aqueous solution was cooled in an ice bath and acidified with concentrated hydrochloric acid. Protonated form of **23** was extracted with EtOAc (100 mL x 3). Combined organic

solvent was washed with brine, dried over Na_2SO_4, and concentrated. The further purification of product **23** (1.04 g, 98%) was carried out by recrystallization from $CHCl_3$.

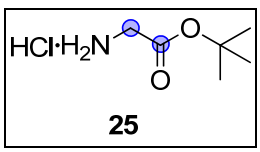
23: white solid; m.p. 115°C ; FT-IR (film) ν 3336.3, 3066.3, 3034.4, 2959.2, 1529.3, 1500.4, 1454.1, 1403.0, 1350.9, 1276.6, 1212.0, 1047.2, 976.8, 777.2, 737.6, 698.105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.67 (ddd, 2H, $^1J_{CH}$ = 138 Hz, $^2J_{CH}$ 5.7 Hz, *J*=5.7 Hz), 5.04 (s, 2H,), 7.35 (m, 5H), 7.56 (dd, 1H, *J* = 5.5, $^2J_{CH}$ = 5.5 Hz), ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.11 (d, J_{CC} = 58 Hz), 65.46, 127.72, 127.82, 128.37, 137.02, 156.42, 171.58 (d, J_{CC} = 58 Hz).



24

[1,2-$^{13}C_2$]Cbz-Gly-O*t*Bu 24. To a stirred CH_2Cl_2 solution (16 mL) of **23** (1.39 g, 6.6 mmol) in a sealed tube was added sulfuric acid (350 μL), and the resultant mixture was cooled to -40°C. Then, isobutylene gas (16 mL) was introduced into the solution. The mixture was stirred at 50°C for two days, and diluted in 0.5 M aqueous NaOH (120 mL). The product was extracted with EtOAc. The obtained organic layer was washed with water (50 mL) and brine (50 mL), and dried over Na_2SO_4. Compound **24** (1.23 g, 70%) was obtained as colorless oil after purification by silica gel chromatography with hexane-EtOAc (80:20 v/v).

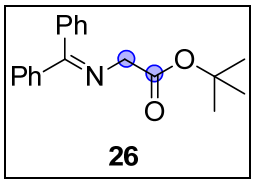
24: colorless oil; FT-IR (film) ν 3359.4, 3033.5, 2978.5, 2934.2, 1727.9, 1700.9, 1521.6, 1455.0, 1406.8, 1393.3, 1369.1 1349.0 1257.4, 1211.1, 1045.2, 984.5, 846.6, 780.07, 751.1, 698.105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 3.88 (ddd, 2H, $^1J_{CH}$ = 141 Hz, $^2J_{CH}$ 5.7 Hz, *J*=5.7 Hz), 5.12 (s, 2H), 5.20 (br, 1H), 7.35 (m, 5H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.11, 43.91(d, J = 61 Hz), 82.3, 128.57, 128.59, 128.63, 128.99, 133.91, 157.2 ,169.51(d, J = 61 Hz).



25

[1,2-$^{13}C_2$]H-Gly-O*t*Bu·HCl 25. To a stirred solution of **24** (0.76 g, 2.78 mmol) in EtOAc (14.3 mL) was added 10% Pd-C (76 mg). The reaction mixture was stirred at room temperature under H_2 atmosphere for 16 h. The catalyst was removed by filtration through a pad of celite. The filtrate was diluted with 3 mL of 1 M aqueous hydrochloric acid/EtOAc, and then concentrated to give **25** (482 mg)**.**

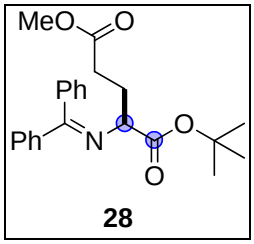
25: white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 3.84 (d, 2H, $^{1}J_{CH}$ = 147 Hz), 8.56 (br, 3H).



26

[1,2-$^{13}C_2$](Ph)$_2$C=Gly-O*t*Bu 26. To a stirred solution of crude **25** in CH_2Cl_2 (11.3 mL) was added benzophenone imine (473 μL, 2.83 mmol), and the resultant solution was stirred at room temperature for 24 h in the dark. After concentration, the obtained residue was dissolved into Et_2O and washed with water. The organic layer was dried over Na_2SO_4, and concentrated. Recrystallization from Et_2O/hexane gave **26** (87%, 735 mg).

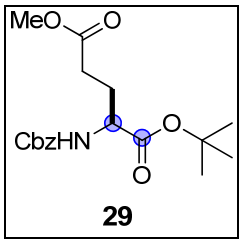
26: white solid; m.p. 112°C; FT-IR (film) ν 3056.6, 2977.6, 2930.3, 2364.3, 1698.0, 1625.7, 1597.7, 1575.6, 1490.7, 1445.4, 1392.4, 1368.3, 1315.2, 1290.1, 1254.5, 1140.7, 1029.8, 846.6, 782.0, 750.2, 696.2, 648.929 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6) δ 1.35 (s, 9H), 4.23 (dd, 2H, $^{1}J_{CH}$ = 136 Hz, $^{2}J_{CH}$ = 7.3 Hz), 6.96-7.09 (m, 8H), 7.93 (m, 2H); ^{13}C NMR (100 MHz, C_6D_6) δ 27.83, 56.7(d, $^{1}J_{CC}$ = 64 Hz), 80.8, 128.47, 129.00, 130.22, 136.5, 169.23 (d, $^{1}J_{CC}$ = 63 Hz), 171.3.



28

[1,2-$^{13}C_2$](Ph)$_2$C=Glu(Me)-O*t*Bu 28. To a stirred solution of **26** (730 mg, 2.46 mmol) in CH_2Cl_2 (6 mL), 149 mg (0.246 mmol) of *O*-allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide (**27**) was added. The reaction mixture was cooled to -78°C in a dry ice-acetone bath. To the well-stirred reaction mixture was added cesium hydroxide monohydrate (4.12 g, 24.6 mmol). After being stirred for 5 min, a solution of methyl acrylate (774 μL, 8.60 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise into the reaction mixture over 10 min. After being stirred for 1.5 h, the reaction was quenched with Et_2O (3 mL) and water (3 mL). After 1 h at room temperature, the reaction mixture was extracted with EtOAc (50 mL x 4). The combined organic layers was washed with brine, dried over Na_2SO_4, and concentrated. Compound **28** (864 mg, 90%, 96%ee) was obtained by silica gel column chromatography with hexane-EtOAc (12:1 v/v).

28: $[\alpha]^{20}_{D}$ -90.8 (c=1.0, $CHCl_3$); colorless oil; FT-IR (film) ν 3058.6, 3022.8, 2977.6, 2933.2, 1738.5, 1690.3, 1623.8, 1597.7, 1576.5, 1445.4, 1368.3, 1317.1, 1278.6, 1253.5, 1142.6, 1075.1, 846.6, 781.0, 766.6, 698.105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.22(m, 2H), 2.37 (m, 2H), 3.59 (s, 3H), 3.97 (ddt, 1H, $^1J_{CH}$ = 135 Hz, $^2J_{CH}$ = 6.8 Hz, J = 5.9 Hz), 7.18 (m, 2H), 7.30-7.47 (m, 6H), 7.64 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.17, 30.66, 51.64, 64.92 (d, J_{CC} = 61 Hz), 81.31, 127.78, 127.98, 128.42, 128.57, 128.77, 130.29, 136.41, 136.47, 139.40, 139.46, 170.73, 170.89 (d, J_{CC} = 62 Hz), 173.87 ;HRMS (MALDI) calcd for $C_{21}{}^{13}C_2H_{27}NO_4$, $(M+Na)^+$ 406.1905, found 406.1873.

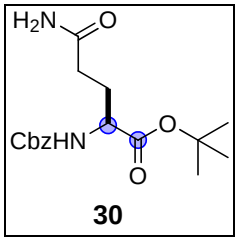


29

[1,2-$^{13}C_2$]Cbz-Glu(Me)-O*t*Bu 29. To a stirred solution of **28** (843 mg, 2.2 mmol) in THF (32 mL), 10% aqueous citric acid (16 mL) was added at 0°C, and stirred at room temperature for 8 h. After removal of THF under reduced pressure, the residue was extracted with 0.05 M aqueous HCl (50 mL x 3). After basification by adding solid $NaHCO_3$, the combined aqueous layer was saturated with NaCl, re-extracted with CH_2Cl_2 (30 mL x 6). The combined organic layer was dried over Na_2SO_4, and concentrated.

To the CH_2Cl_2 solution of the crude amine (20 mL) was added benzyl chloroformate (383 μL, 2.64 mmol) at 0 °C. After 5 min, Et_3N (368 μL, 2.64 mmol) was added to the reaction mixture and the mixture was stirred for 3 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL) and 0.05 M HCl aqueous solution (5 mL). The reaction mixture was extracted with CH_2Cl_2 (30 mL x 3). The combined organic layers was dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography with hexane-EtOAc (75:25 v/v) gave **29** (708 mg, 91%).

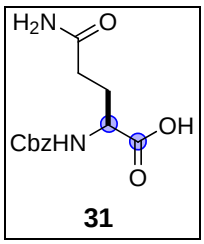
29: $[\alpha]^{20}_{D}$ 6.25 (c=0.47, $CHCl_3$); colorless oil; FT-IR (film) ν 3339.1, 2979.5, 1732.7, 1694.2, 1522.5, 1456.0, 1370.2, 1253.4, 1149.4, 1046.2 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.97 (m, 1H), 2.18 (m, 1H), 2.39 (m, 2H), 3.66 (s, 3H), 4.29 (d, $^1J_{CH}$ = 139 Hz), 5.10 (s, 2H), 5.36 (d, J = 6.8 Hz), 7.36 (m, 5H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8, 28.1, 30.0, 44.3 51.77, 53.7 (d, $^1J_{CC}$ = 58 Hz), 82.5, 127.0, 127.7, 128.1, 128.5, 153.4, 170.8 (d, $^1J_{CC}$ = 58 Hz), 173.4 ;HRMS (MALDI) calcd for $C_{16}{}^{13}C_2H_{25}NO_6$, $(M+Na)^+$ 376.1647, found 376.1616;



30

[1,2-$^{13}C_2$]Cbz-Gln-O*t*Bu 30. In a stirred solution of **29** (836 mg, 2.37 mmol) in MeOH (15 mL) in a sealed tube, NH_3 gas was introduced at 0 °C for 30 min, and the resultant mixture was stirred at room temperature for 2 days. The mixture was cooled at 0°C, diluted with toluene, and concentrated. Silica gel chromatography with $CHCl_3$-MeOH (20:1 v/v) gave **30** (575 mg, 72%).

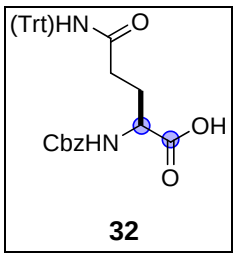
30: $[\alpha]^{20}_D$ -17.7 (c=0.77, CH3OH); colorless oil; FT-IR (film) ν 3725.8, 3629.4, 2360.4, 2341.2, 1078.7, 1507.1, 1148.4 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.45 (s, 9H), 1.90 (m, 1H), 2.10 (m, 1H), 2.32 (m, 2H), 3.31 (s, 3H), 4.06 (d, $^1J_{CH}$ = 139 Hz), 5.09 (d, 2H, *J* = 3.6 Hz), 7.35 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 28.2, 28.6, 32.6, 55.8 (d, $^1J_{CC}$ = 58 Hz), 67.6, 82.9, 126.3, 128.9, 129.5, 138.2, 158.6, 173.2 (d, $^1J_{CC}$ = 58 Hz), 177.6; HRMS (MALDI) calcd for $C_{15}{}^{13}C_2H_{24}N_2O_5$, $(M+Na)^+$ 361.1650, found 361.1659.



31

[1,2-$^{13}C_2$]Cbz-Gln-OH 31. To a stirred solution of **30** (575 mg, 1.70 mmol) in CH_2Cl_2 (6.8 mL) and triethylsilane (679 µL, 4.25 mmol) was added trifluoroacetic acid (3.4 mL) at 0 °C, and the solution was stirred for 3 h at room temperature. The reaction mixture was diluted with toluene, and concentrated. The residue was triturated with 3 mL of Et_2O, filtered, washed with Et_2O (3 mL), and dried to give **31** (472 mg, 98%).

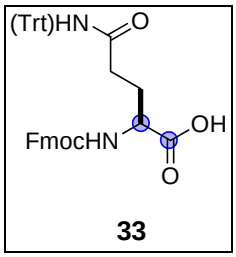
31: $[\alpha]^{20}_D$ -158.3 (c=1.06, CH_3OH); white solid; m.p. 138°C ; FT-IR (film) ν 3318.9, 3032.5, 2358.5, 2340.2, 1679.7, 1529.3, 1454.1, 1237.1, 1209.2, 1082.8, 1048.1, 913.13 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.93 (m, 1H), 2.17 (m, 1H), 2.32 (ddt, J = 3.2, 3.6, 4 Hz), 4.17 (d, $^1J_{CH}$ = 139 Hz), 5.09 (s, 2H), 7.35 (m, 5H), ^{13}C NMR (100 MHz, CD_3OD) δ 28.4, 32.7, 54.4 (d, $^1J_{CC}$ = 58 Hz), 67.7, 128.8, 129.0, 129.0, 129.5, 138.1, 158.7, 175.6 (d, $^1J_{CC}$ = 58 Hz), 177.8; HRMS (MALDI) calcd for $C_{11}{}^{13}C_2H_{16}N_2O_5$, $(M+Na)^+$ 305.1024, found 305.1025.



32

[1,2-$^{13}C_2$]Cbz-Gln(Trt)-OH 32. To a stirred solution of **31** (472 mg, 1.67 mmol) and triphenylmethanol (870 mg, 3.34 mmol) in acetic acid (5 mL) and sulfuric acid (8.9 μL, 0.17 mmol) was added acetic anhydride (314 μL, 3.34 mmol) at room temperature. After 1 h at 55°C, acetic anhydride (314 μL, 3.34 mmol) was added, and the solution was stirred for additional 30 min. The reaction mixture was diluted in precooled water (3 mL), and extracted with EtOAc (30 mL x 4). The combined organic layers was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography with $CHCl_3$-MeOH (20:1 v/v) gave **32** (681 mg, 78%).

32: $[\alpha]^{20}_D$ 2.51 (c=0.3, $CHCl_3$); white solid; m.p. >300°C ; FT-IR (film) ν 3318.9, 3058.6, 3030.6, 2924.5, 1690.3, 1513.9, 1493.6, 1447.3, 1331.6, 1215.9, 1046.2, 903.5, 752.1, 623.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (m, 2H), 2.52 (d, br, 2H, J = 47.6 Hz), 4.16 (d, $^1J_{CH}$ = 143.7 Hz), 5.09 (s, 2H), 5.84 (s, 1H), 7.02 (s, 1H), 7.16-7.34 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 33.8, 54.4(d, $^1J_{CC}$ = 56 Hz), 67.2, 71.3, 127.4, 128.2, 128.3, 128.4, 128.7, 128.8, 136.3, 144.1, 156.3, 173.1 (d, $^1J_{CC}$ = 56 Hz), 171.8 ;HRMS (MALDI) calcd for $C_{30}{}^{13}C_2H_{30}N_2O_5$, $(M+Na)^+$ 547.2120, found 547.2114;



33

[1,2-$^{13}C_2$]Fmoc-Gln(Trt)-OH 33. To a stirred solution of **32** (378 mg, 0.732 mmol) in MeOH (7.3 mL) was added 5% Pd-C (37.8 mg), and the resultant solution was stirred under H_2 atmosphere at room temperature for 2 h. The catalyst was removed by filtration through a pad of celite. The filtrate was subjected to azeotropic drying with toluene. To the crude mixture in H_2O (7.3 mL), MeCN (5 mL) and Et_3N (123 μL, 0.88 mmol), FmocOSu (296 mg, 0.88 mmol) in MeCN (2 mL) was added at 0 °C for 2 h.

The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL). After acidification with 1 mL of 0.1 M aqueous HCl, the solution was extracted with EtOAc (30 mL x 3). The combined organic layer was dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography with $CHCl_3$-MeOH (20:1 v/v) gave **33** (708 mg, 91%).

33: $[\alpha]^{20}_D$ -13.8 (c=1.02, CH_3OH); white solid; m.p. 118°C ; FT-IR (film) ν 3311.12, 3056.6, 2950.6, 1716.3, 1662.3, 1507.1, 1491.7, 1448.3, 1403.9, 1300.8, 1248.7, 1043.3, 742.5, 701,0, 619.04 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.71 (m, 1H), 1.92 (m, 1H), 2.36 (m, 2H), 3.95 (d, $^1J_{CH}$ = 141 Hz), 4.24 (m, 1H), 4.28 (m, 2H), 7.21 (m, 15H), 7.33 (m, 2H), 7.41(t, J = 6.8 Hz), 7.60 (d, J = 6.0 Hz), 7.73 (d, J = 5.0 Hz), 7.89 (dd, J = 5.5, 7.2 Hz), ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 33.2, 47.0, 53.4 (d, $^1J_{CC}$ = 59 Hz), 67.0, 70.9, 120.57, 125.1, 127.0, 127.7, 127.9, 128.5, 140.8, 144.2, 144.2, 144.9, 156.3, 172.6, 173.8 (d, $^1J_{CC}$ = 59 Hz); HRMS (MALDI) calcd for $C_{37}{}^{13}C_2H_{34}N_2O_5$, $(M+Na)^+$ 635.2433, found 635.2412.

Determination of enantiomeric excess of 33

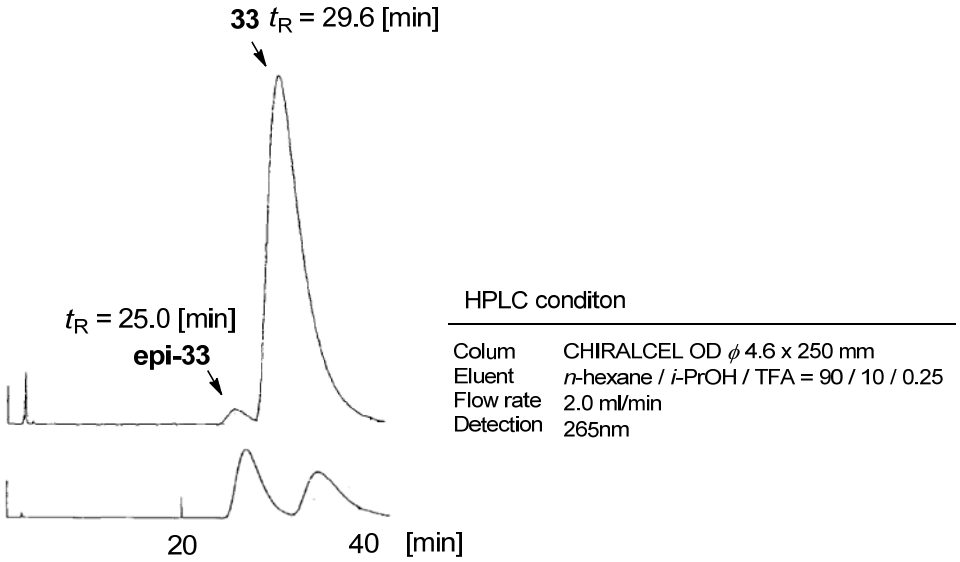
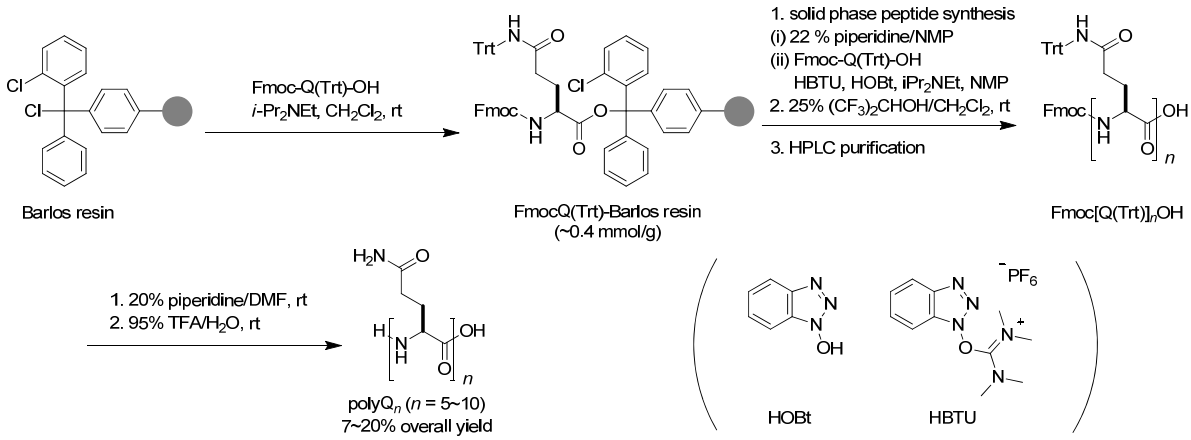


Fig S1. HPLC chart of [1,2-$^{13}C_2$]FmocQ*(Trt)OH (top) and racemic FmocQ(Trt)OH (bottom).

The enantiomeric excess of **33** was determined by normal phase HPLC with a chiral column CHIRALCEL OD ϕ4.6 x 250 mm (eluent A: 0.25% TFA in *n*-hexane, eluent: 0.25% TFA in *i*-PrOH, isocratic elution by eluent A/eluent B at 90%/10%, flow rate: 2.0 mL/min, detection: UV 265 nm, temperature: 27°C) to be 96 %ee.

Solid phase peptide syntheses



Loading the first Fmoc[Q(Trt)] residue onto 2-chlorotrityl resin

Amino acid loading was performed in Libra Tube® (Hipep). The Barlos resin (200 mg) was swollen in CH_2Cl_2 (2 mL) and then excess solvent was removed by filtration. To a stirred solution of Fmoc-Q(Trt)-OH (0.21 mmol, 130 mg) and *i*-Pr_2NEt (0.85 mmol, 109.9 mg) in CH_2Cl_2 (2.5 mL) was added the swollen Barlos resin, and stirred for 25 min. After being washed with CH_2Cl_2 (2 x 2.5 mL), DMF (2 x 2.5 mL), CH_2Cl_2 (2 x 2.5 mL) and MeOH (2.5 mL), the resin was dried for 1 h to give Fmoc-Q(Trt)-Barlos resin (306 mg). Loading rate of Fmoc-Q(Trt) residue was determined by UV spectroscopy as follows. Dried Fmoc-Q(Trt)-Barlos resin (5.1 mg) was suspended into 20% piperidine in DMF (1 mL), and stirred for 30 min. Aliquots of supernatant were diluted 50 times in DMF and subjected to measurement of UV absorption at 301 nm, which corresponds to the characteristic absorbance of 2-methylene-2H-fluorene generated by deprotection of Fmoc group. The loading rate was calculated to be 0.394 mmol/g.

Automatic solid phase peptide synthesis

Polyglutamine peptides were prepared on a peptide synthesizer model 433A (Applied Biosystems, Inc.). The system was operated with Fmoc chemistry files, FastMoc 0.10ΩmonPrevPk (default). Fmoc-[Q(Trt)]-OH was purchased from Novabiochem and used without further purification. 4 eq. of Fmoc-[Q(Trt)]-OH to the peptides loaded on resin was used in every coupling step. NMP was used as a reaction solvent. Condensing agent (0.5 M HBTU/HOBt) was prepared as NMP solution. Default system operation was shown as following.

FastMoc 0.10ΩmonPrevPk

Step 1. Module B: Fmoc group of the solid supported peptide was removed by 22% piperidine/NMP solution (180 s).

Step 2. Module A: Fmoc-[Q(Trt)]-OH (4 eq.) was dissolved in NMP and activated by 0.5 M HBTU/HOBt/DMF solution (10 eq.).

Step 3. Module D: The resin in the reaction vessel was washed by NMP.

Step 4. Module E: 2 M *i*-Pr_2NEt/NMP solution (20 eq.) was added to the activated Fmoc-[Q(Trt)]-OH solution and then the resulting mixture was transferred to the reaction vessel.

Step 5. Module F: Activated Fmoc-[Q(Trt)]-OH was coupled with the peptide on the resin (300 s) and then the reaction vessel was washed by NMP.

Step 1~5 were repeated and glutamines were condensed onto the solid support.

Microwave assisted solid phase peptide synthesis

Polyglutamine peptides were prepared on microwave assisted peptide synthesizer MWS-1000 (EYELA Co.). Fmoc-[Q(Trt)]-OH was purchased from Novabiochem. 3 eq. of Fmoc-[Q(Trt)]-OH to the peptide loaded on resin was used in every coupling step. DMF was used as a reaction solvent. Standard operation was shown as following.

Step 1. Fmoc group of the solid supported peptide was removed by 20% piperidine/DMF solution (3 min, 50°C, assisted with microwave irradiation at 200 W).

Step 2. The resin in the reaction vessel was washed by DMF 5 times.

Step 3. To a solution of *i*-Pr_2NEt (6 eq.) in DMF were added Fmoc-[Q(Trt)]-OH (3 eq.), HBTU (3 eq.), and HOBt (3 eq.). The solution of activated Fmoc-[Q(Trt)]-OH was injected to the reaction vessel, and the resultant mixture was stirred for 10 min at 50°C assisted with microwave irradiation at 200 W

Step 4. The resin in the reaction vessel was washed by DMF 5 times.

Step 1~4 were repeated and glutamines were condensed onto the solid support.

Manual solid phase peptide synthesis

Manual solid phase peptide syntheses were performed in Libra Tube® 3 eq. of Fmoc-Q(Trt)-OH to the first glutamine residue mounted on resin was used in every coupling step except for the step using labeling compound. For the condensation of synthetic [1,2-$^{13}C_2$]Fmoc-Q(Trt)-OH (Fmoc-Q*(Trt)-OH, **33**), the amount of Fmoc amino acid was reduced to 2 eq. of the first glutamine residue mounted on resin. DMF was used as a reaction solvent. Standard operation was shown as following.

Step 1. Fmoc group of the solid supported peptide was removed by 20% piperidine/DMF solution (15 min).
Step 2. The resin placed in Libra Tube® was washed by DMF 4 times.
Step 3. To a solution of *i*-Pr_2NEt (6 eq.) in NMP were added Fmoc-Q(Trt)-OH (3 eq.), HBTU (3 eq.), and HOBt (3 eq.) in DMF. The solution of activated Fmoc-Q(Trt)-OH was injected to Libra Tube®, and stirred for 40 min.
Step 4. The resin was washed by DMF 4 times.
Step 1~4 were repeated and glutamine was condensed onto the solid support.

Cleavage of Fmoc-[Q(Trt)]$_n$-OH from resin

To the peptide-loaded resin in Libra Tube® was added 25% $(CF_3)_2CHOH$ in CH_2Cl_2, and stirred for 30 min. After filtration, the resin was washed with 25% $(CF_3)_2CHOH$ in CH_2Cl_2 three times. The filtrates were combined and concentrated. The concentrated filtrate was dried under vacuum to obtain crude peptide.
The crude peptide was further purified with normal phase HPLC to provide Fmoc-[Q(Trt)]$_n$-OH with yields of 5.9~20% from the first loaded residue on resin.

Fmoc removal from Fmoc-[Q(Trt)]$_n$-OH

To the purified Fmoc-[Q(Trt)]$_n$-OH was added 20% piperidine/DMF solution (1 mL) and then stirred for 10 h at room temperature. The reaction mixture was concentrated, suspended into H_2O (0.5 mL) and Et_2O (0.5 mL), and stirred for 5 min. The suspension was centrifuged at 5000 rpm for 5 min (MRX-150 and TMA-4, TOMY) to be phase separated. Et_2O layer was removed by decantation to remove 2-methylene-2H-fluorene generated from deprotection of Fmoc group. The water layer was further washed with Et_2O (0.5 mL) 3 times, and then lyophilized to give H-[Q(Trt)]$_n$-OH.

Trt removal from H-[Q(Trt)]$_n$-OH

To H-[Q(Trt)]$_n$-OH was added 95% TFA/H_2O, and the resultant solution was stirred for 1 h at room temperature. The mixture was concentrated, and suspended into H_2O (0.5 mL) and Et_2O (0.5 mL). After being stirred for 5 min, the mixture was centrifuged at 5000 rpm for 5 min to be phase separated. Et_2O layer was removed by decantation to remove the trityl derivatives generated from deprotection of Trt groups. The water layer was further washed with Et_2O (0.5 mL) 3 times, and then lyophilized to give H-Q_n-OH with yields of 5.9~20% from the first loaded glutamine residue on resin.

Syntheses of polyglutamine peptides

H-Q_5-OH 3. **3** was synthesized from 253 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol) according to procedures described above with the method of microwave assisted solid phase peptide synthesis. 19.7 mg of Fmoc-[Q(Trt)]$_5$-OH (0.0094 mmol, 7.8 % yield) was obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 4.2 mg of **3** was given as white solid (0.007 mmol, 7% from Fmoc-Q(Trt)-Barlos resin). **3:** HRMS (MALDI) calcd for $C_{25}H_{42}N_{10}NaO_{11}$ $(M+Na)^+$ 681.2932, found 681.2909

HPLC conditions. Column: Inertsil® SIL 100A ϕ4.6 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH, Detection: UV 265 nm, Retention time: 25 min.

Gradient			
$CHCl_3$ *i*-PrOH	100 0	95 5	90 10
Flow rate (mL/min)	1.5	1.5	1.5
Temp.(°C)	25	25	25
Time (min)	0	30	40

H-Q_6-OH 4. **4** was synthesized from 197 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol) according to procedures described above with the method of automatic solid phase peptide synthesis. 19.5 mg of Fmoc-[Q(Trt)]$_6$-OH (0.0079 mmol, 17.9 % yield) was obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 5.7 mg of **4** was given as white solid (0.0075 mmol, 7.5% from Fmoc-Q(Trt)-Barlos resin). **4:** HRMS (MALDI) calcd for $C_{30}H_{50}N_{12}NaO_{13}$ $(M+Na)^+$ 787.3695, found 787.3738.

HPLC conditions. Column: Inertsil® SIL 100A ϕ10 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH Detection: UV 265 nm, Retention time: 20 min.

Gradient				
$CHCl_3$ *i*-PrOH	100 0	95.2 4.8	93.2 6.8	90 10
Flow rate (mL/min)	4.0	4.0	4.0	4.0
Temp.(°C)	25	25	25	25
Time (min)	0	10	30	40

H-Q_7-OH 5. **5** was synthesized from 208 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol)

according to procedures described above with the method of microwave assisted solid phase peptide synthesis. 49.1 mg of Fmoc-[Q(Trt)]$_7$-OH (0.0123 mmol, 12.3 % yield) was obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 2.2 mg of **5** was given as white solid (0.0026 mmol, 12% from Fmoc-Q(Trt)-Barlos resin) from 10.5 mg of Fmoc[Q(Trt)]$_7$OH. **5:** HRMS (MALDI) calcd for $C_{35}H_{58}N_{14}NaO_{15}$ $(M+Na)^+$ 937.4104, found 937.4142.

HPLC conditions. Column: Inertsil® SIL 100A ϕ4.6 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH, Detection: UV 265 nm, Retention time: 37 min.

Gradient			
$CHCl_3$ *i*-PrOH	100 0	95 5	90 10
Flow rate (mL/min)	1.5	1.5	1.5
Temp.(°C)	25	25	25
Time (min)	0	30	40

H-Q$_8$-OH 6. **6** was synthesized from 185 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol) according to procedures described above with the method of automatic solid phase peptide synthesis. 38 mg of Fmoc-[Q(Trt)]$_8$-OH (0.0119 mmol, 20.0 % yield) was obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 4.5 mg of **6** was given as white solid (0.0005 mmol, 8% from Fmoc-Q(Trt)-Barlos resin) from 10.3 mg of Fmoc-[Q(Trt)]$_8$-OH. **6:** HRMS (MALDI) calcd for $C_{40}H_{66}N_{16}NaO_{17}$ $(M+Na)^+$ 1044.0716, found 1044.0689.

HPLC conditions. Column: Inertsil® SIL 100A ϕ10 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH Detection: UV 265 nm, Retention time: 18 min.

Gradient				
$CHCl_3$ *i*-PrOH	100 0	96 4	93 7	90 10
Flow rate (mL/min)	3.0	3.0	3.0	3.0
Temp.(°C)	25	25	25	25
Time (min)	0	10	30	40

H-Q$_9$-OH 7. **7** was synthesized from 238 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol) according to procedures described above with the method of microwave assisted solid phase peptide synthesis. 26 mg of Fmoc-[Q(Trt)]$_9$-OH (0.0073 mmol, 5.9 % yield) was

obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 0.5 mg of **7** was given as white solid (0.0005 mmol, 5.9% from Fmoc-Q(Trt)-Barlos resin) from 2.2 mg of Fmoc[Q(Trt)]$_9$OH. **7:** HRMS (MALDI) calcd for $C_{45}H_{73}N_{18}O_{19}$ $(M+Na)^+$ 1169.5300 , found 1169.5342.

HPLC conditions. Column: Inertsil® SIL 100A ϕ4.6 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH, Detection: UV 265 nm, Retention time: 14 min.

Gradient			
$CHCl_3$ *i*-PrOH	100 0	95 5	90 10
Flow rate (mL/min)	1.5	1.5	1.5
Temp.(°C)	25	25	25
Time (min)	0	30	40

H-Q$_{10}$-OH 8. **8** was synthesized from 320 mg of Fmoc-Q(Trt)-Barlos resin (0.1 mmol) according to procedures described above with the method of microwave assisted solid phase peptide synthesis. 46.8 mg of Fmoc-[Q(Trt)]$_{10}$-OH (0.0126 mmol, 12.6 % yield) was obtained after HPLC purification (conditions were shown in the table below). After the above described deprotection procedures, 8.6 mg of **8** was given as white solid (0.007 mmol, 7% from Fmoc-Q(Trt)-Barlos resin) from 26.1 mg of Fmoc[Q(Trt)]$_{10}$OH. **8:** HRMS (MALDI) calcd for $C_{50}H_{83}N_{20}O_{21}$ $(M+Na)^+$ 1300.3337 , found 1300.3353.

HPLC conditions. Column: Inertsil® SIL 100A ϕ4.6 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH, Detection: UV 265 nm, Retention time: 21 min.

Gradient			
$CHCl_3$ *i*-PrOH	100 0	95 5	90 10
Flow rate (mL/min)	1.5	1.5	1.5
Temp.(°C)	25	25	25
Time (min)	0	30	40

H-Q$_m$Q*Q$_n$-OH (*m* + *n* = 7, *m* = 0–7) 9–16. According to the procedure of manual solid phase peptide synthesis described above, eight H-Q$_m$Q*Q$_n$-OHs (*m* = 0–7, **9**–**16**) were synthesized at a scale of 0.1 mmol. The resulting peptides were cleaved from the resin and purified by normal phase HPLC (conditions were shown in the table below). Fractions containing peptide were combined and concentrated. The residual solvent was further removed under vacuum to give Fmoc-[Q(Trt)]$_m$[Q*(Trt)][Q(Trt)]$_n$-OH.

After the above described deprotection procedures, a series of H-$Q_mQ^*Q_n$-OHs were obtained as follows:

H-Q^*Q_7-OH **9** (24.8 mg, 45 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4772.

H-QQ^*Q_6-OH **10** (8.8 mg, 20 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4762.

H-$Q_2Q^*Q_5$-OH **11** (8.6 mg, 20 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4755.

H-$Q_3Q^*Q_4$-OH **12** (8.7 mg, 20 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4757.

H-$Q_4Q^*Q_3$-OH **13** (18.0 mg, 33 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4777.

H-$Q_5Q^*Q_2$-OH **14** (16.7 mg, 32 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4791.

H-Q_6Q^*Q-OH **15** (17.0 mg, 31 % yield from Fmoc-Q(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4737.

H-Q_7Q^*-OH **16** (18.0 mg, 33 % yield from Fmoc-Q*(Trt)-Barlos resin): white solid; HRMS (MALDI) calcd for $C_{38}{}^{13}C_2H_{66}N_{16}O_{17}$ $(M+Na)^+$ 1067.4757, found 1067.4772.

HPLC conditions. Column: Inertsil® SIL 10 x 250 mm, Eluent: $CHCl_3$/*i*-PrOH Detection: UV 265 nm, Retention time: 18 min.

Gradient				
$CHCl_3$ i-PrOH	100 0	96 4	93 7	90 10
Flow rate (mL/min)	3.0	3.0	3.0	3.0
Temp. (°C)	25	25	25	25
Time (min)	0	10	30	40

X-ray diffraction experiment

X-ray diffraction of Q_n peptides were measured by a Rigaku Ultima IV X-Ray Diffractometer using CuKα radiation at wavelength of 1.5406 nm with voltage of 40 kV and current of 44 mA. 2-3 mg of Q_n peptides were mounted onto a reflection-free silicon sample holder. The scanning angle was range from 10° to 80° with step size of 0.02°. Peaks at 2θ = 18.5° correspond to spacing between the peptide backbones in β-sheet (d = 4.8 Å).

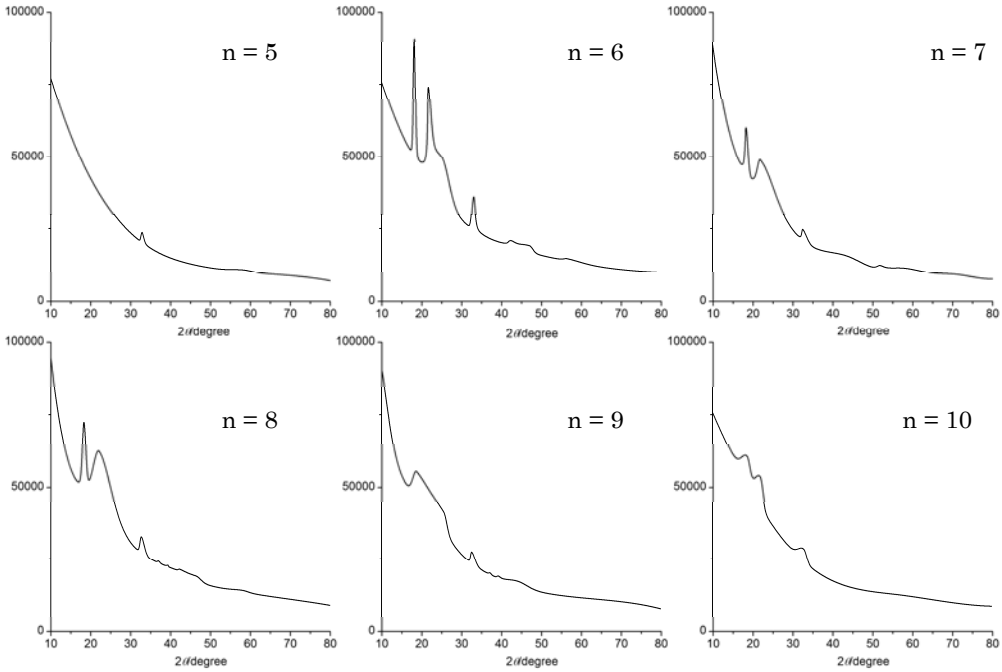


Fig. S2 Powder X-ray diffraction of H-[Q_n]-OH peptides.

ThT fluorescent staining assay

ThT was purchased from Wako chemicals as chloride salt and used without further purification. To the 50 mM phosphate buffer solution (pH 7.8) in 96-well plate (Coaster 3610) were added ThT as a solution in PBS (100 ~ 1 μM) and Q_n peptides as a suspension in PBS (1 mM). Total volume of sample solution was adjusted to be 100 μL for each well. The final concentrations of Q_n peptides were fixed to be 50 μM. The range of final concentrations of ThT was adjusted to be 0.1 μM ~ 50 μM. After 10 seconds of stirring, samples were subjected to fluorescent measurement by fluorescence microplate reader (Gemini EM, Molecular Devices) observing emission at 485 nm with excitation at 440 nm. All measurements were carried out at 27°C by means of three replicates.

Dissociation constants and numbers of binding sites were estimated by Scatchard analyses. First we estimated those values for low affinity binding sites using data under high concentration of ThT (B > 1.2 μM). For estimation of dissociation constants and numbers of binding sites for high affinity binding sites, data obtained at low concentration were used (B < 1.2 μM).

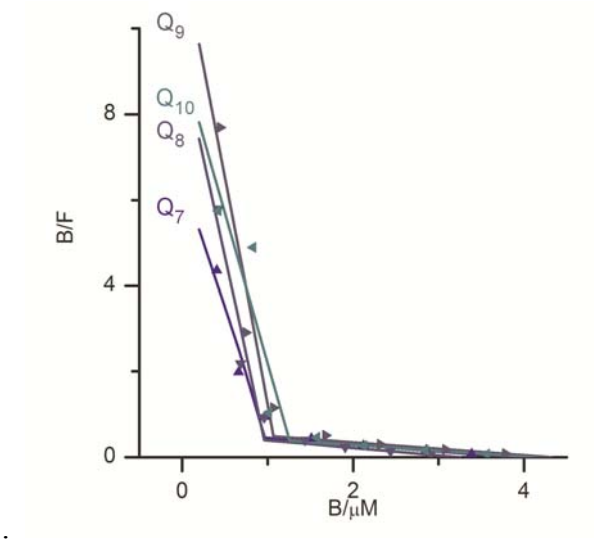


Fig. S3 Scatcherd plot of ThT binding assay for polytglutamine peptides.

Solid state NMR measurement

An aqueous suspension of 50 μM Q_n peptide (~14 mg) with or without 1 μM of ThT (~27 μg) in water was lyophilized and dried under vacuum for 2 days to give a yellow fluffy solid. The obtained solid sample was packed into a magic-angle spinning (MAS) sample rotor with an outer diameter of 4 mm. Six non-labeled Q_ns (n = 5~10) were prepared without ThT and a non-labeled Q_8 and eight [1,2-$^{13}C_2$] labeled Q_8s were prepared with ThT. Weights of sample packed into each rotor were 4-14 mg. Solid-state NMR experiments were performed by using a Chemagnetics CMX-400 infinity spectrometer equipped with a Varian T3 probe set as $^1H/^{13}C$ double resonance or $^1H/^{13}C/^2H$ triple resonance configurations. ^{13}C chemical shift values are described in ppm relative to the high field peak of adamantane as 29.5 ppm. For adjustment of pulse width for ^2H NMR, ND_4Cl molded into resin (Araldite®) was packed into MAS rotor with Q_n sample and used for observation of intense ^2H NMR signal from the inside of sample rotor.

$^{13}C\{^1H\}$ CP-MAS experiments

CP-MAS spectrum of HQ_5OH 3. 7 mg of peptide was used for experiment. 100 kHz and 71.4 kHz of RF fields were applied for ^1H and ^{13}C channels respectively. $^{13}C\{^1H\}$CP was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 2476 scans with 2 s of recycle delay.

CP-MAS spectrum of HQ_6OH 4. 14.2 mg of peptide was used for experiment. 100 kHz and 71.4 kHz of RF fields were applied for ^1H and ^{13}C channels respectively. $^{13}C\{^1H\}$CP was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 1024 scans with 2 s of recycle delay.

CP-MAS spectrum of HQ_7OH 5. 14 mg of peptide was used for experiment. 100 kHz and 71.4 kHz of RF fields were applied for ^1H and ^{13}C channels respectively. $^{13}C\{^1H\}$CP was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 1024 scans with 2 s of recycle delay.

CP-MAS spectrum of HQ_8OH 6. 14 mg of peptide was used for experiment. 100 kHz and 71.4 kHz of RF fields were applied for ^{1}H and ^{13}C channels respectively. $^{13}C\{^{1}H\}CP$ was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 1024 scans with 2 s of recycle delay.

CP-MAS spectrum of HQ_9OH 7. 6 mg of peptide was used for experiment. 100 kHz and 71.4 kHz of RF fields were applied for ^{1}H and ^{13}C channels respectively. $^{13}C\{^{1}H\}CP$ was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 2060 scans with 2 s of recycle delay.

CP-MAS spectrum of $HQ_{10}OH$ 8. 12.4 mg of peptide was used for experiment. 83.3 kHz and 55 kHz of RF fields were applied for ^{1}H and ^{13}C channels respectively. $^{13}C\{^{1}H\}CP$ was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectrum was accumulated 1224 scans with 2 s of recycle delay.

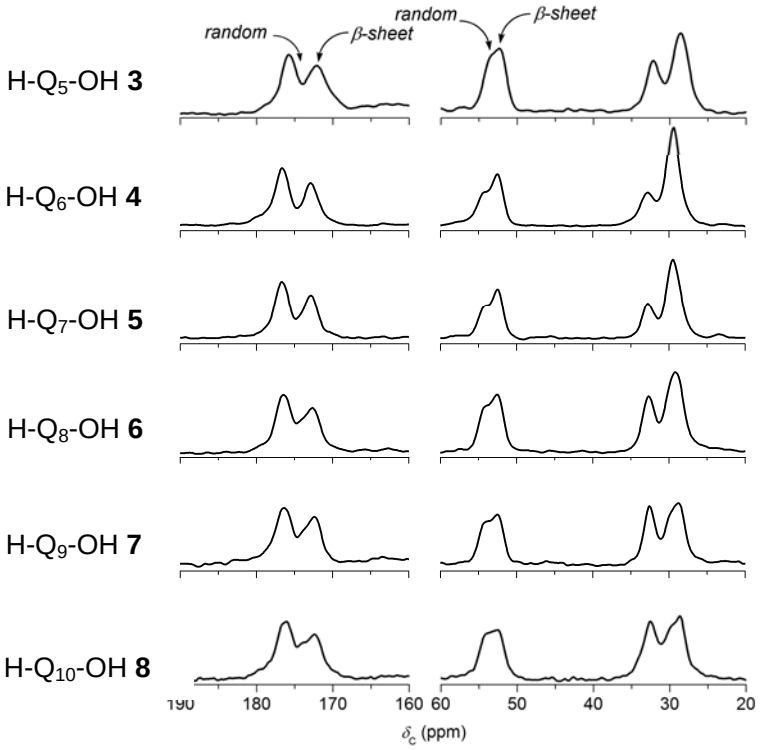


Fig. S4 $^{13}C\{^{1}H\}$ CP MAS spectra of short polyQ$_n$s (n = 5~10).

$^{13}C\{^{2}H\}$REDOR experiments

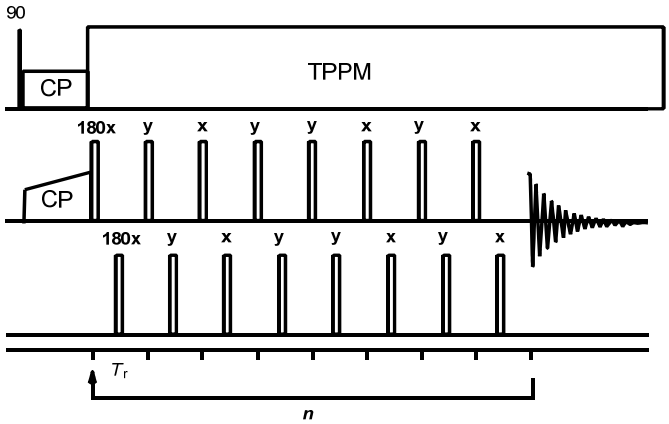


Fig. S5 $^{13}C\{^{2}H\}$REDOR experiments were performed with the pulse sequence shown above. Detail conditions were shown as follows.

$^{13}C\{^{2}H\}$REDOR spectra of [3-Me-d_3]ThT (2)/Q$_8$ (6). 14.2 mg of [3-Me-d_3]ThT (**2**)/Q$_8$ (**6**) (1/50 mol/mol) was used for experiment. 100 kHz, 55 kHz and 45 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. $^{13}C\{^{1}H\}$CP was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected

under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 175,00 scans and 200,000 scans for 4.8 ms (40 T_r) and 9.6 ms (80 T_r) of dephasing times respectively. Experiments were recycled with a delay time of 2 s.

Experimental conditions for $^{13}C\{^2H\}$DQF-REDOR experiments

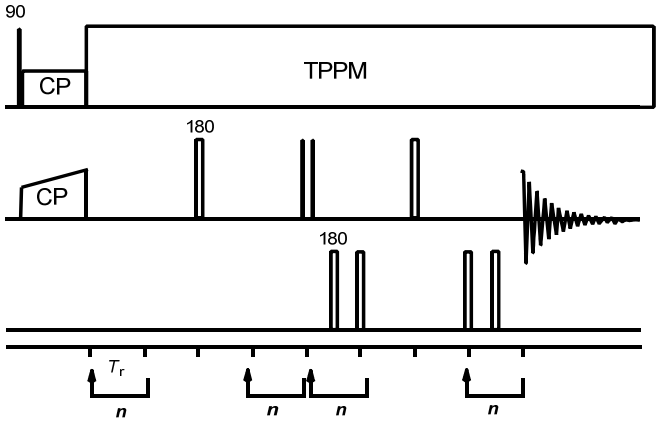


Fig. S6 $^{13}C\{^2H\}$DQF-REDOR experiments were performed with the pulse sequence shown above. Detail conditions were shown for each samples as follows.

$^{13}C\{^2H\}$ DQF-REDOR experiments

$^{13}C\{^2H\}$DQF-REDOR spectra of [3-Me-d_3]ThT (2)/Q*Q$_7$ (9). 5 mg of [3-Me-d_3]ThT (**2**)/Q*Q$_7$ (**9**) (1/50 mol/mol) was used for experiment. 100 kHz, 56.8 kHz and 45.5 kHz of RF fields were applied for ^1H, ^{13}C and ^2H channels respectively. $^{13}C\{^1H\}$CP was performed at RF field of 65 kHz with a contact time of 1.5 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 78,000 scans and 100,000 scans for 4.8 ms (40 T_r) and 9.6 ms (80 T_r) of dephasing times respectively. Experiments were recycled with a delay time of 2 s.

$^{13}C\{^2H\}$DQF-REDOR spectra of [3-Me-d_3]ThT (2)/QQ*Q$_6$ (10). 4.3 mg of [3-Me-d_3]ThT (**2**)/QQ*Q$_6$ (**10**) (1/50 mol/mol) was used for experiment. 100 kHz, 52.1 kHz and 42.4 kHz of RF fields were applied for ^1H, ^{13}C and ^2H channels respectively. $^{13}C\{^1H\}$CP was performed at RF field of 65 kHz with a contact time of 2.0 ms. Data were collected under high power proton decoupling at 100 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 84,000 scans for 9.6 ms (80 T_r) of dephasing time.

Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ $Q_2Q^*Q_5$ (11). 3.5 mg of [3-Me-d_3]ThT (**2**)/$Q_2Q^*Q_5$ (**11**) (1/50 mol/mol) was used for experiment. 108 kHz, 54.3 kHz and 43.9 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 1.5 ms. Data were collected under high power proton decoupling at 108 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 80,000 scans for 9.6 ms (80 T_r) of dephasing time. Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ $Q_3Q^*Q_4$ (12). 3.3 mg of [3-Me-d_3]ThT (**2**)/$Q_3Q^*Q_4$ (**12**) (1/50 mol/mol) was used for experiment. 104 kHz, 54.3 kHz and 44.6 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 1.5 ms. Data were collected under high power proton decoupling at 104 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 80,000 scans for 9.6 ms (80 T_r) of dephasing time. Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ $Q_4Q^*Q_3$ (13). 4.8 mg of [3-Me-d_3]ThT (**2**)/$Q_4Q^*Q_3$ (**13**) (1/50 mol/mol) was used for experiment. 108 kHz, 56.8 kHz and 45.5 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 2.0 ms. Data were collected under high power proton decoupling at 108 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 100,000 scans for 9.6 ms (80 T_r) of dephasing time. Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ $Q_5Q^*Q_2$ (14). 4.0 mg of [3-Me-d_3]ThT (**2**)/$Q_5Q^*Q_2$ (**14**) (1/50 mol/mol) was used for experiment. 104 kHz, 54.3 kHz and 43.9 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 1.8 ms. Data were collected under high power proton decoupling at 104 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 40,000 scans for 9.6 ms (80 T_r) of dephasing time.

Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ Q_6Q^*Q (15). 3.2 mg of [3-Me-d_3]ThT (**2**)/Q_6Q^*Q (**15**) (1/50 mol/mol) was used for experiment. 92.6 kHz, 52.1 kHz and 42.4 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 1.5 ms. Data were collected under high power proton decoupling at 92.6 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 70,000 scans for 9.6 ms (80 T_r) of dephasing time. Experiments were recycled with a delay time of 2 s.

^{13}C{^{2}H}DQF-REDOR spectra of [3-Me-d_3]ThT (2)/ Q_7Q^* (16). 4.7 mg of [3-Me-d_3]ThT (**2**)/Q_7Q^* (**16**) (1/50 mol/mol) was used for experiment. 104 kHz, 56.8 kHz and 42.4 kHz of RF fields were applied for ^{1}H, ^{13}C and ^{2}H channels respectively. ^{13}C{^{1}H}CP was performed at RF field of 65 kHz with a contact time of 1.5 ms. Data were collected under high power proton decoupling at 104 kHz of RF field with two-pulse phase modulation (TPPM). Magic-angle spinning rate was fixed at 8,333 Hz. Spectra were accumulated 100,000 scans for 9.6 ms (80 T_r) of dephasing time. Experiments were recycled with a delay time of 2 s.

Distance estimation based on ^{13}C{^{2}H}DQF-REDOR

Spin system and effective dipolar coupling.

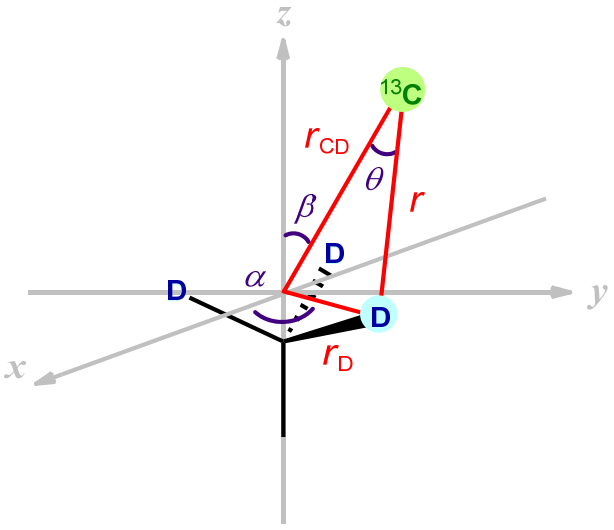


Fig. S7 Spin system for this study. The center of gravity of three ^{2}H spins is taken as the axis origin.

The spin system of CD_3–^{13}C is shown in Fig. S7 with parameters used in the following expressions. The dipolar coupling between ^{13}C and ^{2}H is expressed based on the ^{13}C–^{2}H distance r.

$$\omega_D = \mu_0 \hbar \gamma_C \gamma_D / 8\pi r^3 \qquad (1)$$

The rapid rotation of methyl group attenuates ω_D to give an effective dipolar coupling ω_{Deff}.

$$\omega_{Deff} = a\ \omega_D\ (3\cos^2\theta - 1)/2 \qquad (2)$$

where θ is the angle O–^{13}C–^{2}H; O is the center of the gravity of three ^{2}H spins (Fig. S7). $(3\cos^2\theta - 1)/2$ and a account for dipolar attenuations by the methyl C_3 axis rotation and other molecular motions respectively. 0.8 (20 % reduction) was adopted as attenuation factor a.[1] Distance r and angle θ are expressed as functions of angles α and β, and distances r_D and r_{CD}.

$$r(\alpha, \beta, r_{CD}, r_D) = (r_{CD}^2 + r_D^2 - 2\, r_{CD}\, r_D \sin\beta \cos\alpha)^{1/2} \qquad (3)$$

$$\theta(\alpha, \beta, r_{CD}, r_D) = (r_{CD} - r_D \sin\beta \cos\alpha)/(r_D^2 + r_{CD}^2 - 2 r_{CD}\, r_D \cos\alpha \sin\beta) \qquad (4)$$

The value of rotation radius of the three ^{2}H spins (r_D) was deduced from X-ray structure of L-alanine to be 1.04 Å.[2] Because the time scale of the methyl rotation is far rapider than that of ^{13}C–^{2}H dipolar coupling evolution, ω_{Deff} is observed as an averaged value over 360° rotation of angle α.

$$\bar{\omega}_{Deff}(\beta, r_{CD}) = a\ \mu_0 \hbar\ \gamma_C\ \gamma_D / 8\pi \int_\alpha r(\alpha, \beta, r_{CD})^{-3}\ (3\cos^2\theta(\alpha, \beta, r_{CD}) - 1)/2\ \mathrm{d}\alpha \qquad (5)$$

Thus effective dipolar coupling $\bar{\omega}_{Deff}$ depends angle β and distance r_{CD}.

According to Eq. 5, effective dipolar coupling was calculated and shown as a contour map in Fig. S8. The origin is the center of the gravity of three ^{2}H spins. Because we observed intermolecular C–CD_3 distance in present study, contours are shown only for distances longer than the Van der Waals contacts. At the same size of dipolar coupling, the shortest distance and the longest distance were obtained at β = 0° and 90° respectively.

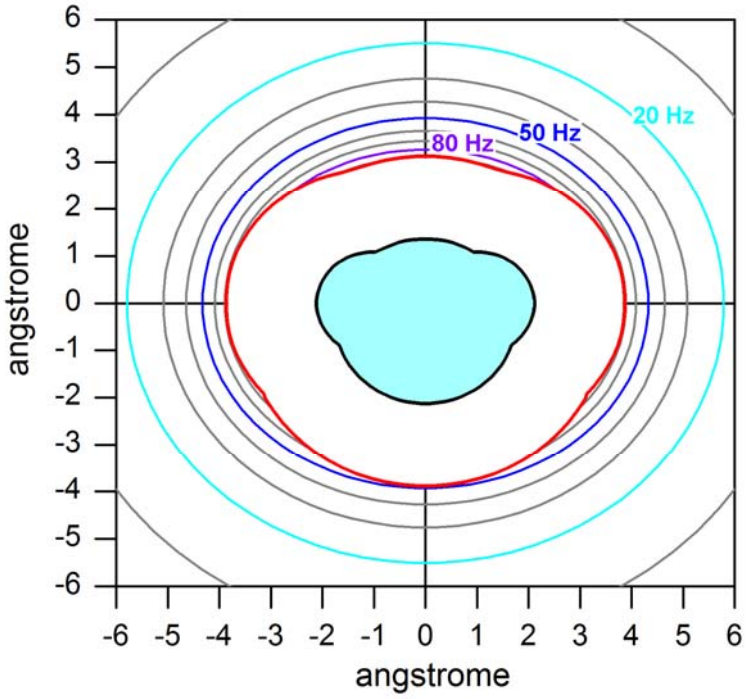


Fig. S8 Contour map of calculated effective dipolar coupling between ^{13}C and ^{2}H for ^{13}C–CD_3 pair system. The axis origin (0,0) is set as the center of the gravity of three ^{2}H spins. The center light blue area shows Van der Waals radius of CD_3 group. The nearest position of the center of the ^{13}C nucleus to the center of the gravity of three ^{2}H spins is shown as red line, where Van der Waals contact occurs between ^{13}C nucleus and CD_3 group.

Calculation of REDOR curves for CD_3 dephaser. Size of REDOR dephasing ($\Delta S/S_0$) by CD_3 was calculated according to the method introduced by Schmidt and Schaefer, as shown in the following expression.[1] We assumed an isolated ^{13}C–CD_3 pair system because of the low concentration of CD_3 dephaser ([3-Me-d_3]ThT/Q*Q_7 = 2 %).

$$S(nT_R) = 1/27\ [7 + 12\cos(2\bar{\omega}_{Deff}nT_R) + 6\cos(4\bar{\omega}_{Deff}nT_R) + 2\cos(6\bar{\omega}_{Deff}nT_R)] \qquad (6)$$

where n is number of rotor cycles of REDOR dephasing, and T_R is a rotor period of magic angle sample spinning. The calculated REDOR curves are shown in Fig. S9. For C–CD_3 distances longer than Van der Waals contact ($r_{CD} \geq 3.12$ Å: D ≤ 89.3 Hz), REDOR curves show monotonically increasing from 4.8 ms to 9.6 ms of dephasing

time. Therefore, we used the ratio of $\Delta S/S_0$ values between data obtained at 4.8 ms and 9.6 ms.

Distance estimation and structural insight. The distances were estimated from the ratio of $\Delta S/S_0$ between diphasing time at 4.8 ms and 9.6 ms ($R_{4.8/9.6}$) for each carbons. By taking ratio between two different dephasing times, we canceled an effect of scaling factor that comes from unkown accurate S_0 value. $R_{4.8/9.6}$ were calculated to be 0.65 and 0.55 for C=O and C^{α} respectively. Ideal $R_{4.8/9.6}$ values were calculated based on the ideal REDOR dephasing curves (Fig. S9) and plotted against $^{13}C–CD_3$ distance for the case of $\beta = 0°$ (the shortest r_{CD}) and $\beta = 90°$ (the longest r_{CD}) in Fig. 6a (main text). The distances were estimated to be 3.6~4.0 Å and 3.9~4.3 Å for C=O and C^{α} respectively. The distance constraints were shown in Fig.S10 (broken and dotted lines for shortest and longest distances respectively) with the Van der Waals radii of ^{13}C spin pairs. The yellow colored area indicates the possible geometry of the center of the three ^{2}H spins of CD_3 dephaser.

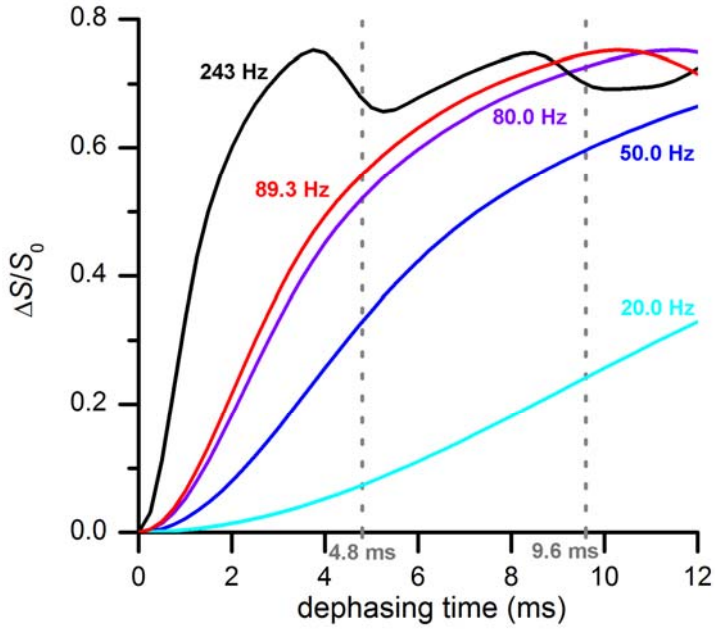


Fig. S9 REDOR dephasing curves for the spin system of ^{13}C-CD_3. The black line shows the REDOR curve for 243 Hz of dipolar coupling which corresponds to ^{13}C-CD_3 distance in zinc [1-^{13}C, 2,2,2-d_3]acetate crystal structure ($\beta = 0°$, $r_{CD} = 1.9$Å) as one of the examples for intramolecular distance.[1] The red line shows the REDOR curve of the nearest distance for an intermolecular ^{13}C-CD_3 pair ($\beta = 0°$, $r_{CD} = 3.12$Å). Color code for other three curves indicates the scale of dipolar coupling shown in Fig. S8.

To gain a detail structural information, we carried out the validation of each position indicated by small circles in Fig. S10. The caluclation was performed by using parameters shown in Fig. 12. Because we obtained 25 sets of four parameters (r_{CD1}, r_{CD2}, β_1, β_2) from $^{13}C\{^2H\}$ DQF-REDOR constraints, we calculated C_{13}-C_{13} distance (r_{CC}) to fit 1.525 Å by changing angle α_2. The best fit was found for r_{CD1} = 3.7 Å, r_{CD2} = 4.1 Å, b_1 = 36.8°, b_2 = 40.7°, and α_1 = 34.6° (red square in Fig. S10).

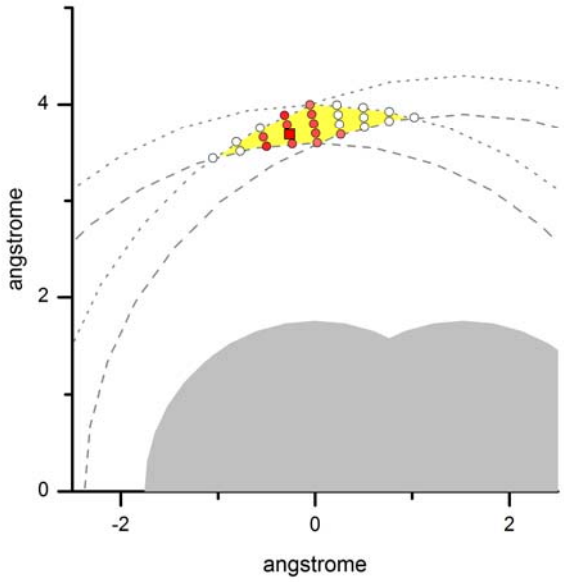


Fig. S10 The distance constraints obtained from $^{13}C\{^2H\}$DQF-REDOR of **2**/**9** complex. ^{13}CO carbon atom of **9** is set as the axis origin (0, 0). ^{13}Cα carbon is placed on the x axis at (1.525, 0). The Cα–CO bond length was taken from that of the alanine crystal structure.[ref] The bottom gray area shows Van der Waals radii of two carbon atoms Cα and CO (1.75 Å for both). Broken lines and dotted lines show the shortest and the longest CD_3–^{13}C distance from each labeled carbon respectively. The yellow filled area shows the possible position of the center of the gravity of three ^{2}H spins estimated from REDOR distance constraints. Validation was carried out for the positions indicated by small circle symbols (0.1Å step in both r_{CD1} and r_{CD2} dimension shown in Fig. S11). Best fit was found for r_{CD1} = 3.7 Å, r_{CD2} = 4.1 Å, b_1 = 36.8°, b_2 = 40.7°, and α_1 = 34.6° (red square).

Finally the most appropriate position of the center of the gravity of three ^{2}H spins was estimated as shown in Fig. S12 indicated as a cyan ball, by including another REDOR distance constraint from the second residue (no dephasing was observed).

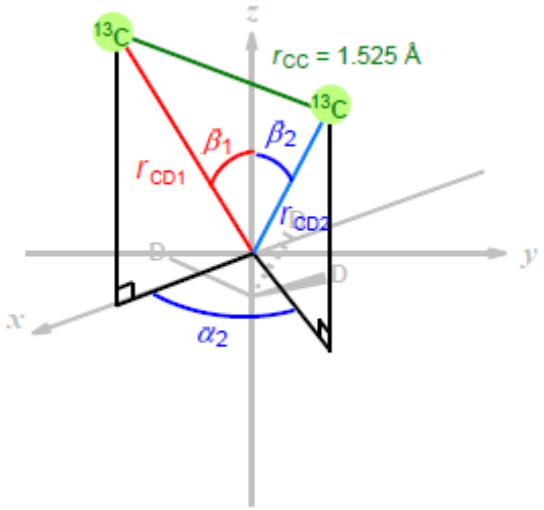


Fig. S11 Spin system for estimation of ^{13}C spin pairs geometry refers to CD_3 group. The center of gravity of three ^{2}H spins is taken as the axis origin.

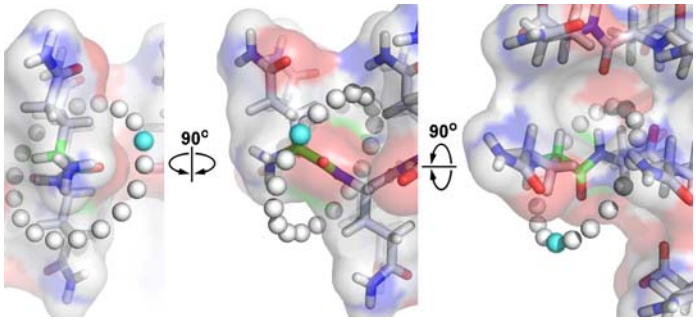


Fig. S12 Schematic diagram of the possible position of CD_3 dephaser deduced from $^{13}C\{^{2}H\}$DQF-REDOR experiments. Based on REDOR distance constraint from data of **2**/**9** complex, the possible positions form a circle around the $^{13}C\alpha$–^{13}CO bond represented by balls. Positions of balls were represented by a step of 20° around the $^{13}C\alpha$–^{13}CO bond. Based on the other REDOR constraint from data of **2**/**10** complex, the farest position from $^{13}C^{\alpha}$ of the second Q residue is estimated to be the most appropriate geometry for CD_3 dephaser (cyan). *N*-terminal of Q_8 peptide was shown as stick model with transparent surface. The molecular graphics were generated by using PyMOL ver 1.0 and ChemBio3D ultra 12.0.

References

1. A. Schmidt, T. Kowalewski and J. Schaefer, *Macromolecules* 1993, **26**, 1729–1733.
2. J. D. Dunitz, and R. R. Ryan, *Acta Cryst.* 1966, **21**, 617–618.