Electronic Supplementary Material (ESI) for Polymer Chemistry.

Chemoenzymatic synthesis of a peptide containing nylon monomer units for thermally processable peptide material application

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Experimental procedure

Materials. L-Leucine ethyl ester hydrochloride (LeuEt), methyl 4-aminobutyrate hydrochloride (nylon4Me), ethyl 4-aminobutyrate hydrochloride (nylon4Et), beta-alanine methyl ester hydrochloride (nylon3Me), beta-alanine ethyl ester hydrochloride (nylon3Et), and methyl 6-aminohexanoate hydrochloride (nylon6Me) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Papain was purchased from Wako Pure Chemical Industries (Osaka, Japan). All the chemicals and enzymes were used without any purification. The activity of papain was 0.0015 U/mg, and one unit was defined to hydrolyze N_{α}-benzoyl-L-arginine-*p*-nitroanilide hydrochloride and release 1 μmol of *p*-nitroaniline per minute at pH of 7.5 and 25°C. The other chemicals were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and used as received without any purification.

Chemoenzymatic peptide synthesis. Nylon monomer (5.4 M) and LeuEt (0.6 M) were mixed with 50 mg/mL papain in 5 mL of 1 M phosphate buffer at a pH of 8.0. The chemoenzymatic reactions were conducted at 40°C for 12 h and stirred at 1000 rpm using an EYELA ChemiStation (Tokyo, Japan). The resultant white precipitate was obtained by centrifugation at 12,000 x *g* for 5 min and was washed with diluted HCl (pH 2.0) twice and with Milli-Q water. The resultant products were lyophilized.

^{1}H-NMR. ^{1}H-NMR was recorded on a Varian NMR System 500 (500 MHz) spectrometer (Varian Medical Systems, Palo Alto, CA, USA) at 25°C controlled with VnmrJ software (Agilent

Technologies, Santa Clara, CA, USA). The lyophilized samples were dissolved in dimethyl sulfoxide-d_6 (DMSO-d_6) at a concentration of 10 mg/mL. One hundred and twenty-eight scans were recorded during each ^{1}H-NMR measurement. Data were processed and analyzed using ACD/NMR Processor Academic Edition, version 12.01 (Advanced Chemistry Development, Toronto, ON, Canada).

MALDI-TOF MS. The MALDI-TOF MS spectra were obtained using an Autoflex Speed MALDI-TOF-MS system spectrophotometer (Bruker, Bremen, Germany) operating in linear positive mode. The lyophilized product (0.4 mg/mL) was dissolved in TA solution, consisting of 0.1% trifluoroacetic acid, 80% acetonitrile, and 20% water. Then, the sample solution was mixed with 20 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA), which was used as the matrix. The prepared sample (1 µL) was spotted on the target plate and dried at an ambient temperature. The acquired data were analyzed with FLEX analysis software (Bruker, Bremen, Germany).

Infrared spectra. An attenuated total reflectance Fourier transform infrared spectroscope (ATR-FTIR; IR Prestige-21; Shimadzu Corporation, Kyoto, Japan) equipped with a multiple-reflection, horizontal MIRacle ATR attachment (using a Ge crystal, from Pike Tech, Madison, WI) was used to evaluate the N-H stretching of the amide group in the oligo(LeuEt-*co*-nylon). For each measurement, 64 scans were accumulated with a resolution 4 cm^{-1}, and the wavenumber ranged from 3100 cm^{-1} to 4000 cm^{-1}.

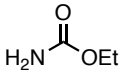
Molecular docking simulations. All molecular docking studies were performed using the docking program AutoDock Vina version 1.1.2.[1] For the protein molecule, the crystallographic structure of papain (PDB ID: 1ppn) solved at 1.6 Å resolution was used.[2] Before docking, all ligands and water molecules were removed from the PDB file. Polar hydrogens were added to ligands and receptor by using the Hydrogen module in AutoDock Tools version 1.5.6, then Gasteiger united atom partial charges and atom types were assigned and PDBQT files were generated for Autodock Vina docking. The protein molecule was kept rigid, while all the torsional bonds in substrates were set free to rotate. A 15 Å docking box around sulfur atom of Cys25 was defined. Images were prepared using PyMOL 1.8.5.

Thermal analysis. TGA and DSC measurements were conducted by using a TGA/DSC2 (Mettler Toledo, Greifensee, Switzerland). The sample synthesized by chemoenzymatic polymerization (3–5 mg) was weighed and sealed in an aluminum pan. The lid of the aluminum pan had a pinhole to prevent the pan from bursting due to an increase in the inner pressure during the heating process. The product was heated at 10°C/min from 30°C to 500°C under a nitrogen atmosphere in triplicate. The device was calibrated with an empty cell to enter a baseline and with indium to characterize the heat flow and temperature of the system.

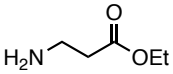
Wide angle X-ray scattering (WAXS) and temperature-dependent WAXS. The synchrotron WAXS and temperature dependent-WAXS measurements of the powdery samples were performed at BL45XU beamline at SPring-8, Harima, Japan, using an X-ray energy of 12.4 keV (wavelength: 0.1 nm). The scanning rate of the DSC for temperature dependent-WAXS

measurement was 10°C/min from 50°C to 300°C. The sample of 3 mg was used for the measurement.

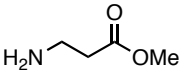
(a) nylon1Et



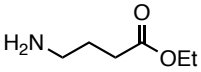
(b) nylon3Et



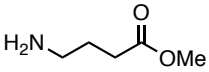
(c) nylon3Me



(d) nylon4Et



(e) nylon4Me



(f) nylon6Me

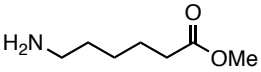


Fig. S1 Nylon monomers used in this study.

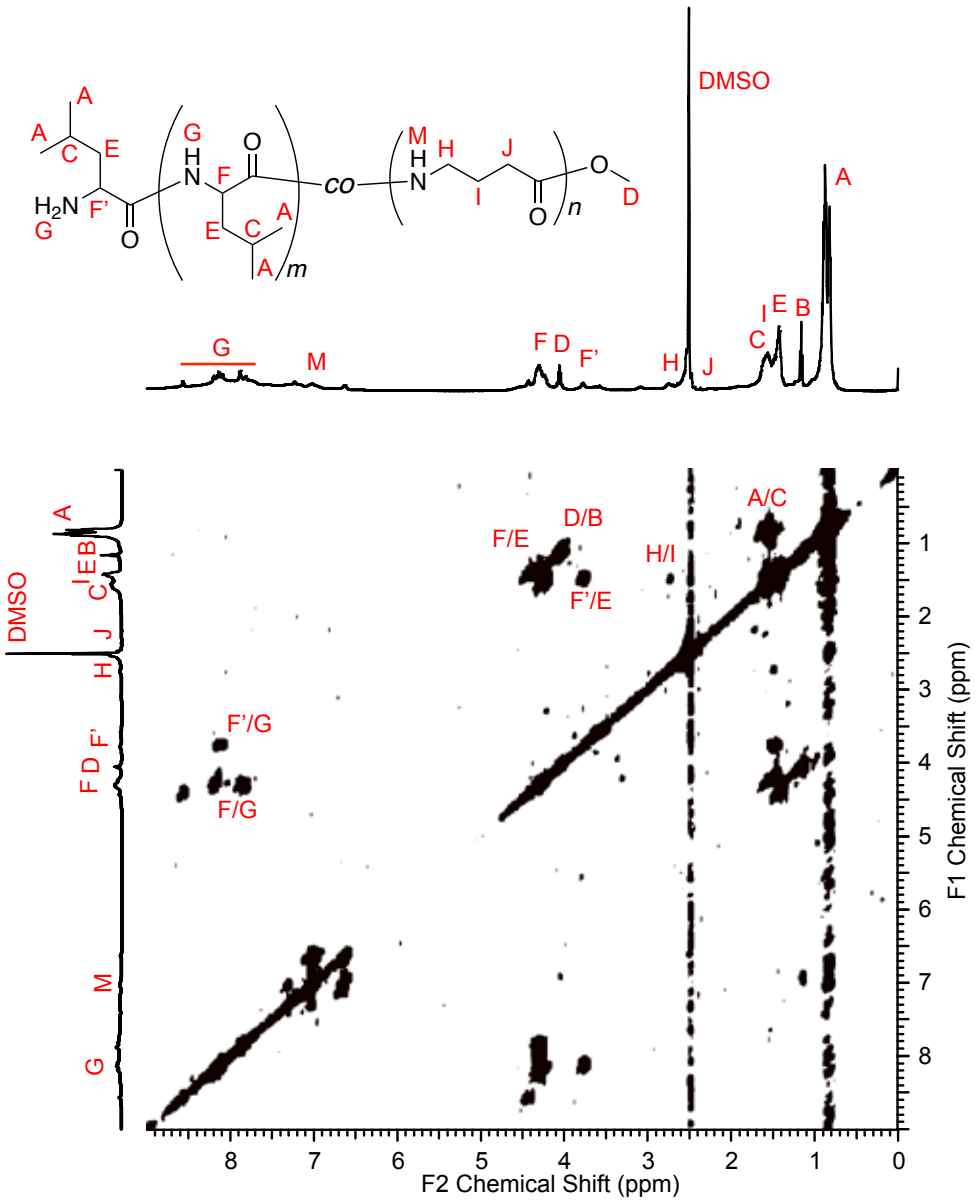


Fig. S2 g-COSY NMR study of oligo(LeuEt-*co*-nylon4Me) depicting peak assignment.

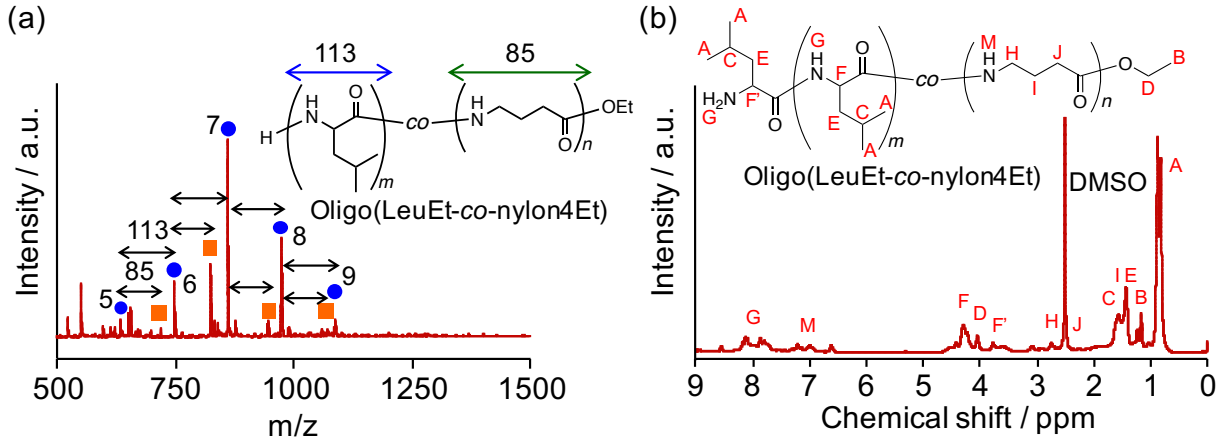


Fig. S3 MALDI-TOF MS (**a**) and ^{1}H–NMR (**b**) spectra of the papain-catalyzed reaction product using LeuEt with nylon4Et as a substrate. Mass spectrum was obtained in the linear positive mode using CHCA as the matrix. Peaks with blue circles denote oligo(LeuEt) with various DP, whereas peaks with orange squares denote oligo(LeuEt-*co*-nylon4Et). DMSO-d_6 was used as the solvent in NMR measurements.

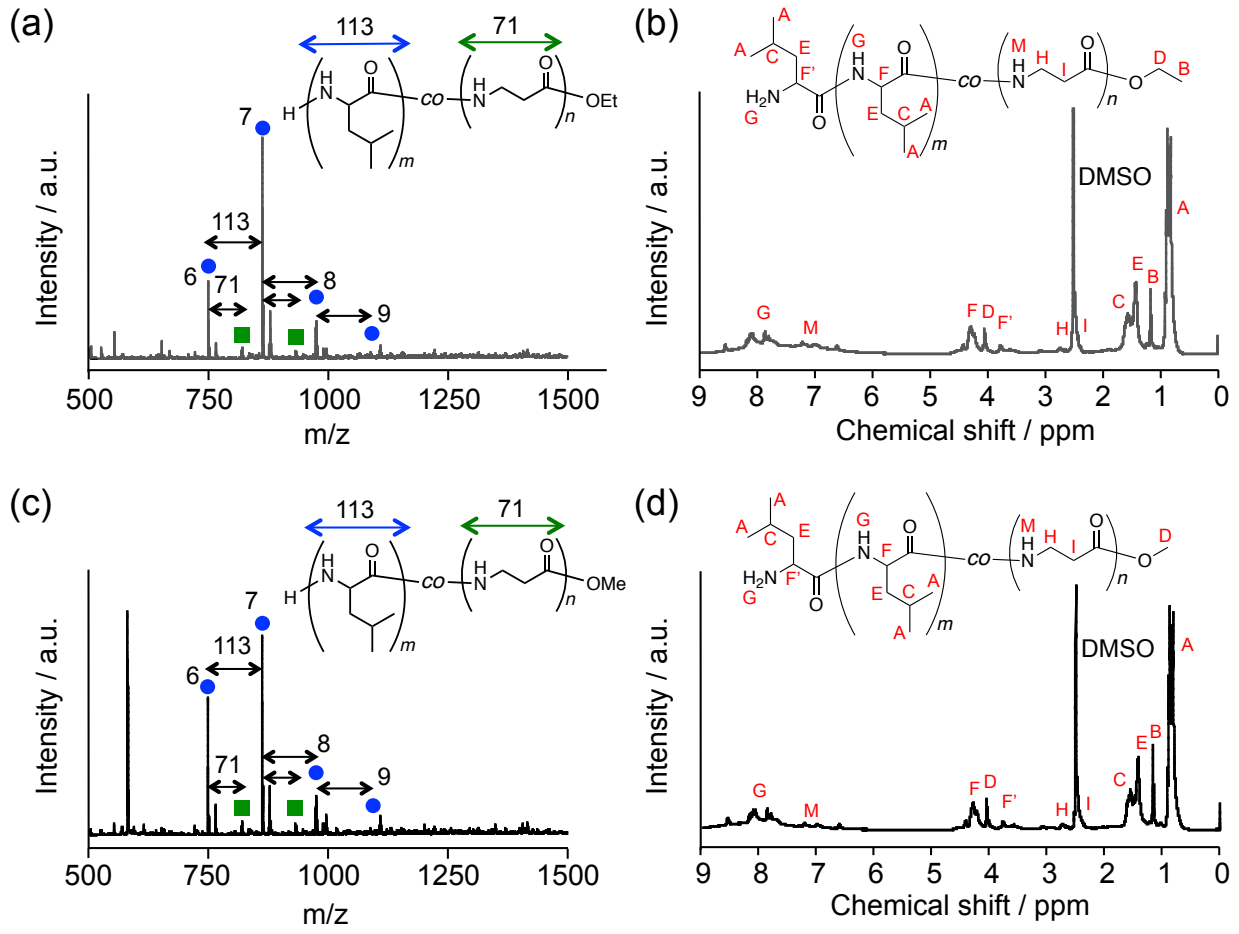


Fig. S4 MALDI-TOF MS (**a**,**c**) and ^{1}H–NMR (**b**,**d**) spectra of the papain-catalyzed reaction product using LeuEt with nylon3Et (**a**,**b**) or nylon3Me (**c**,**d**) as substrates. Mass spectra were obtained in the linear positive mode using CHCA as the matrix. Peaks with blue circles denote oligo(LeuEt) with various DP, whereas peaks with green squares denote oligo(LeuEt-*co*-nylon3). DMSO-d_6 was used as the solvent in NMR measurements.

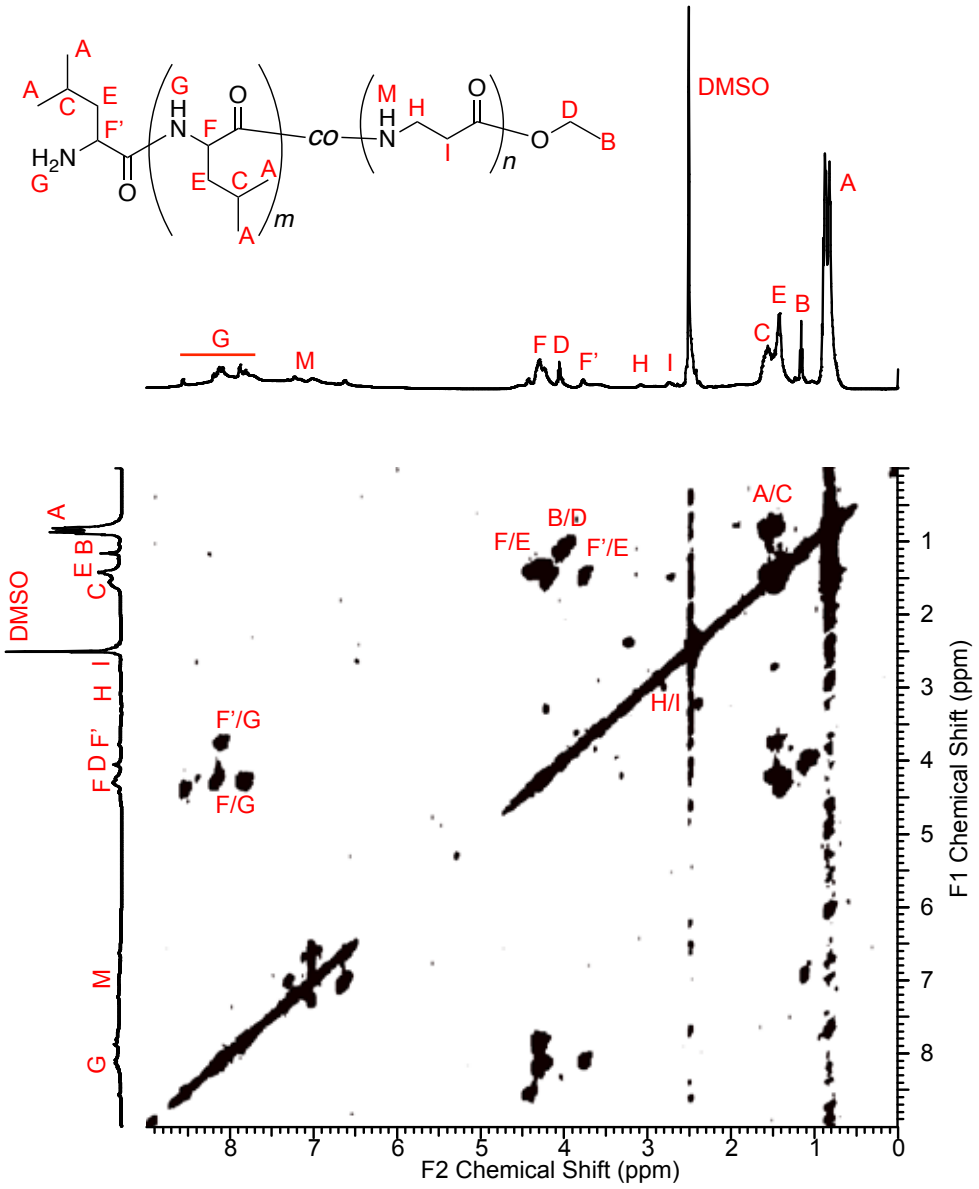


Fig. S5 g-COSY NMR study of oligo(LeuEt-*co*-nylon3Et) depicting peak assignment.

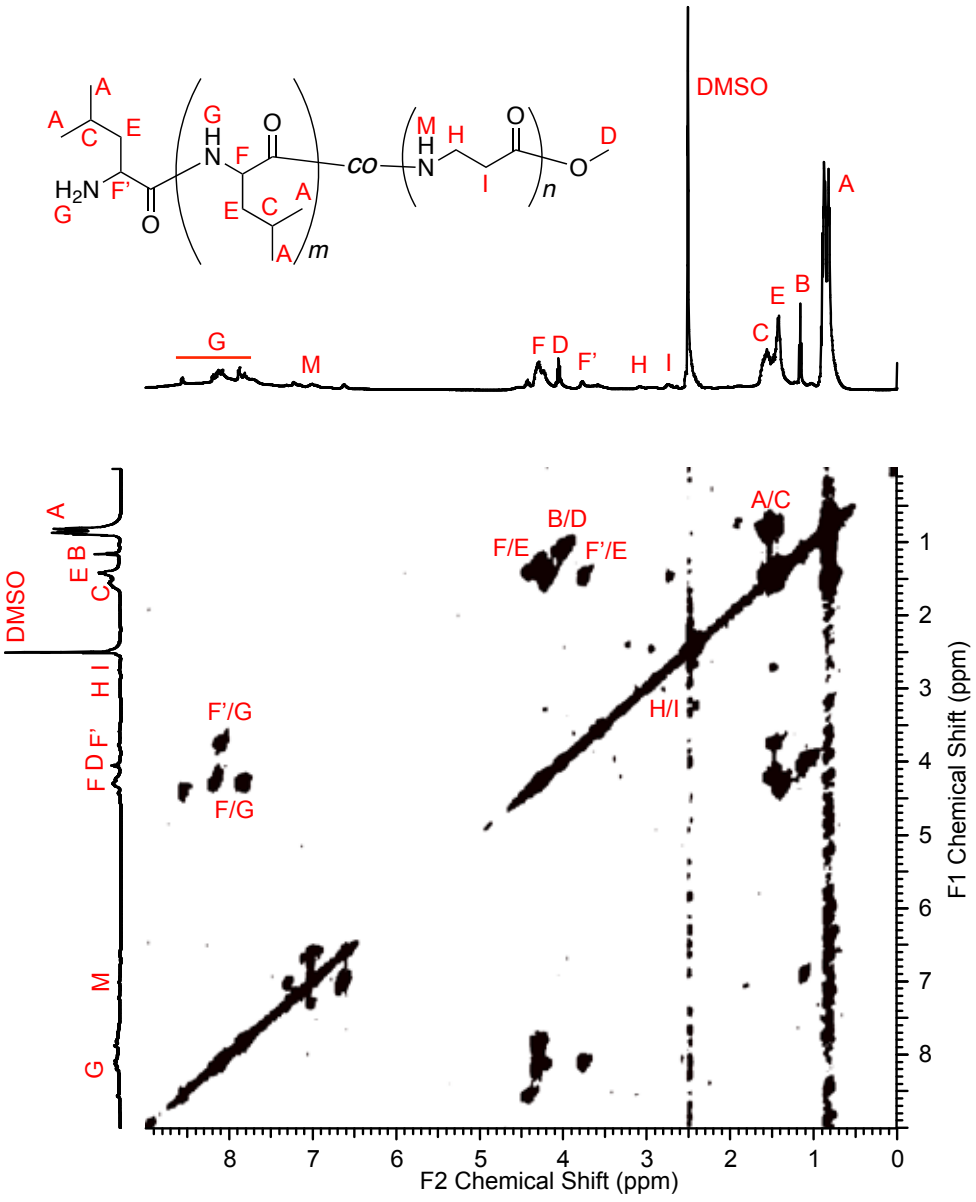


Fig. S6 g-COSY NMR study of oligo(LeuEt-*co*-nylon3Me) depicting peak assignment.

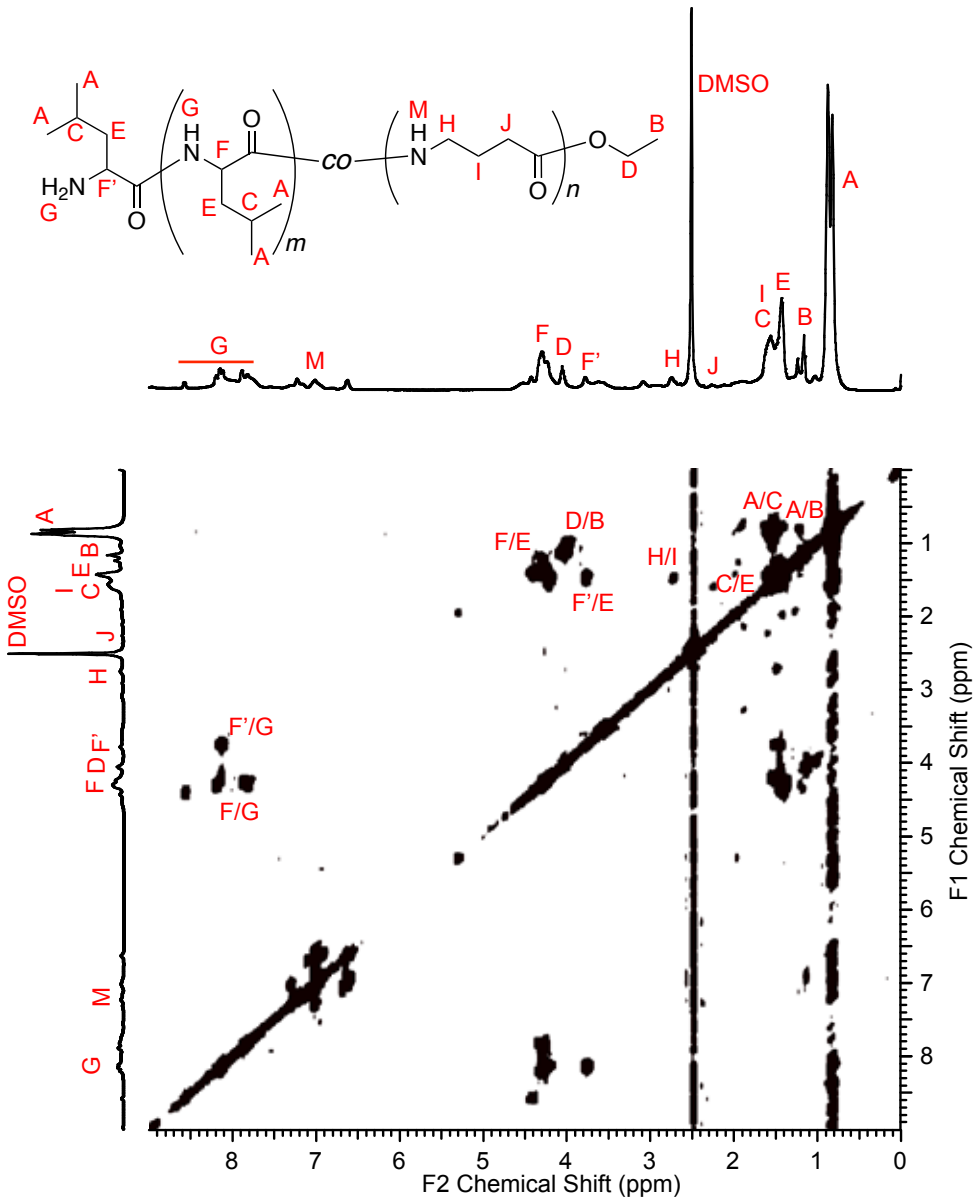


Fig. S7 g-COSY NMR study of oligo(LeuEt-*co*-nylon4Et) depicting peak assignment.

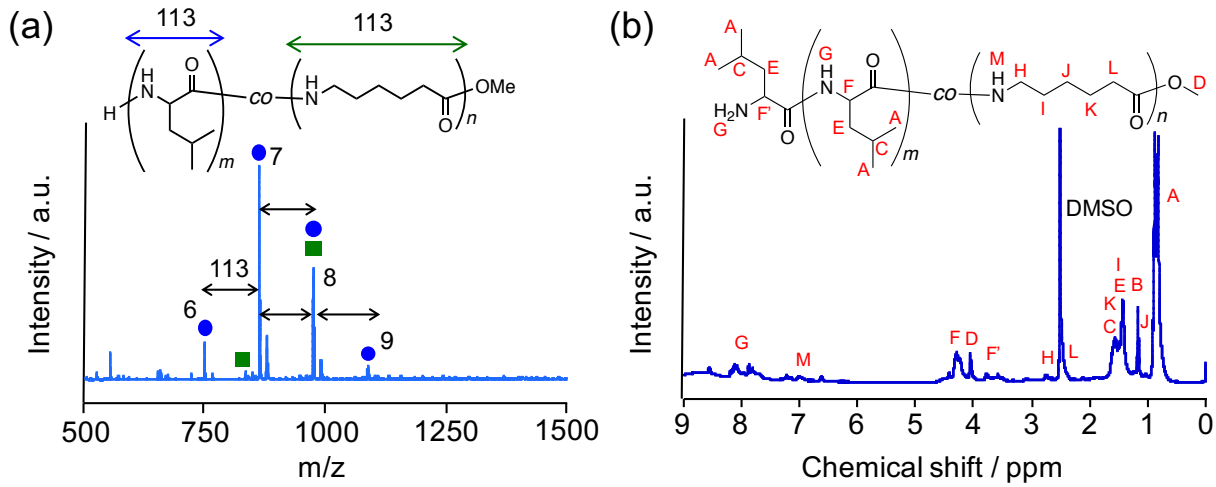


Fig. S8 MALDI-TOF MS (**a**) and ^{1}H–NMR (**b**) spectra of the papain-catalyzed reaction product using LeuEt and nylon6Me as substrates. Mass spectra were obtained in the linear positive mode using CHCA as the matrix. Peaks with blue circles denote oligo(LeuEt) with various DP, whereas peaks with green squares denote oligo(LeuEt-*co*-nylon6Me). DMSO-d_6 was used as the solvent in NMR measurements.

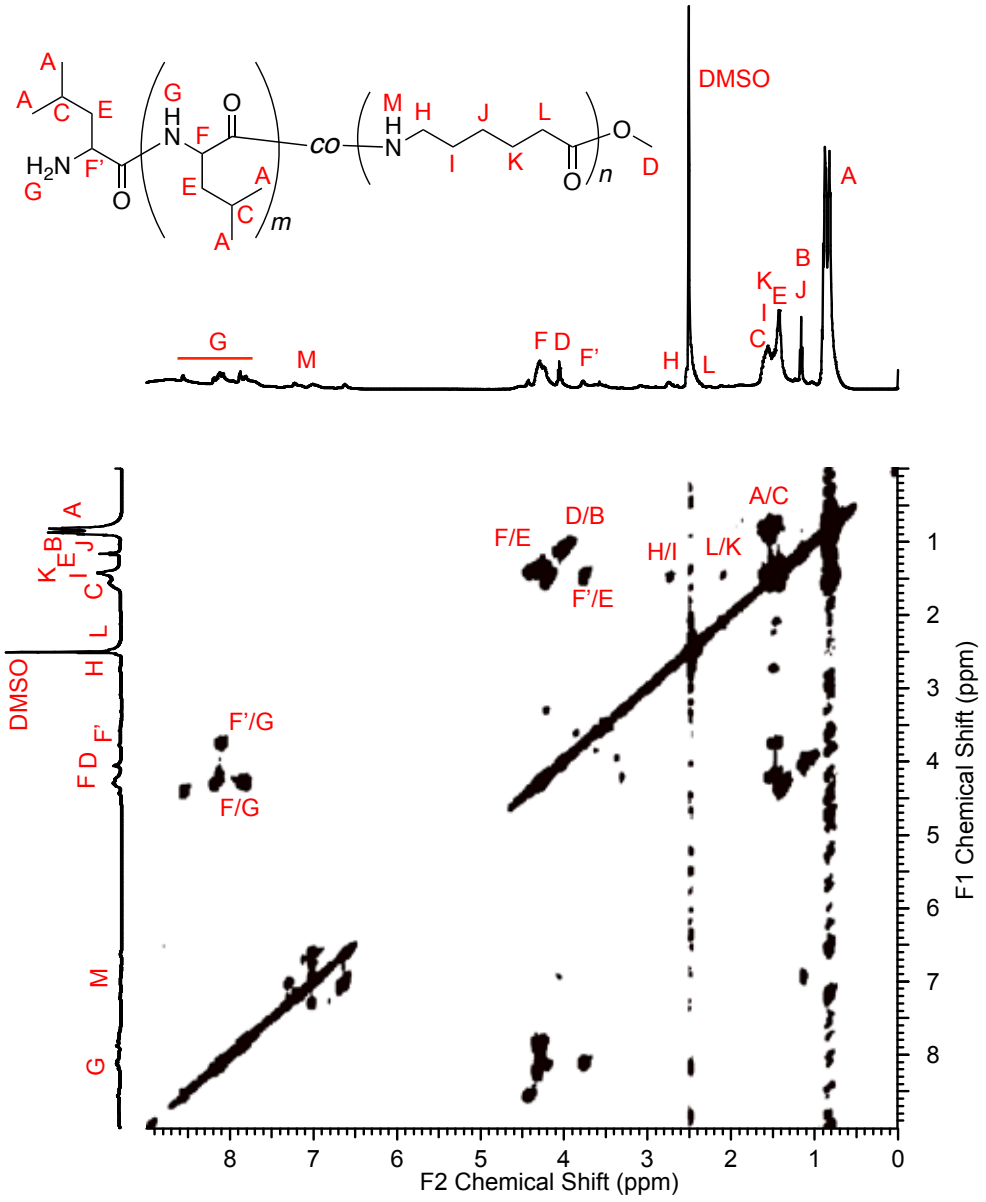


Fig. S9 g-COSY NMR study of oligo(LeuEt-*co*-nylon6Me) depicting peak assignment.

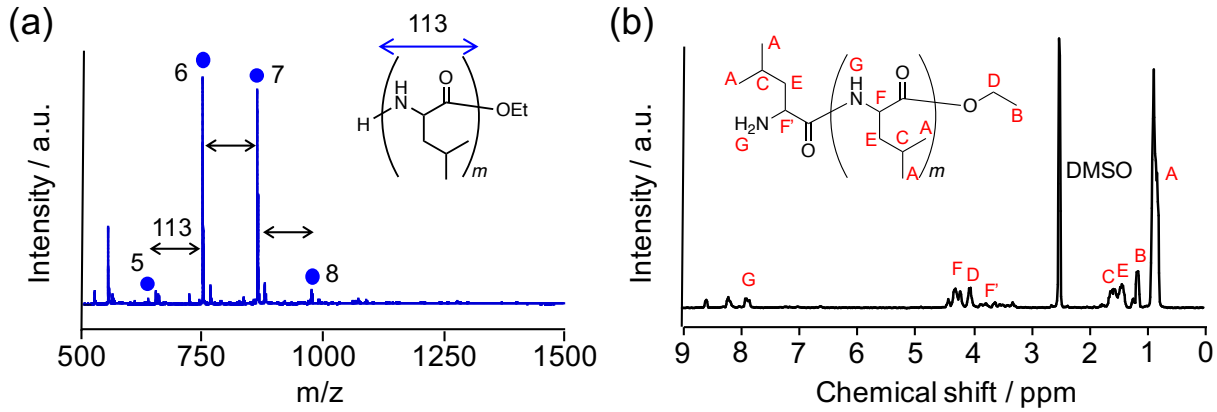


Fig. S10 MALDI-TOF MS (**a**) and ^{1}H–NMR (**b**) spectra of the papain-catalyzed reaction product using LeuEt as a substrate. Mass spectra were obtained in the linear positive mode using CHCA as the matrix. Peaks with blue circles denote oligo(LeuEt) with various DP. DMSO-d_6 was used as the solvent in NMR measurements.

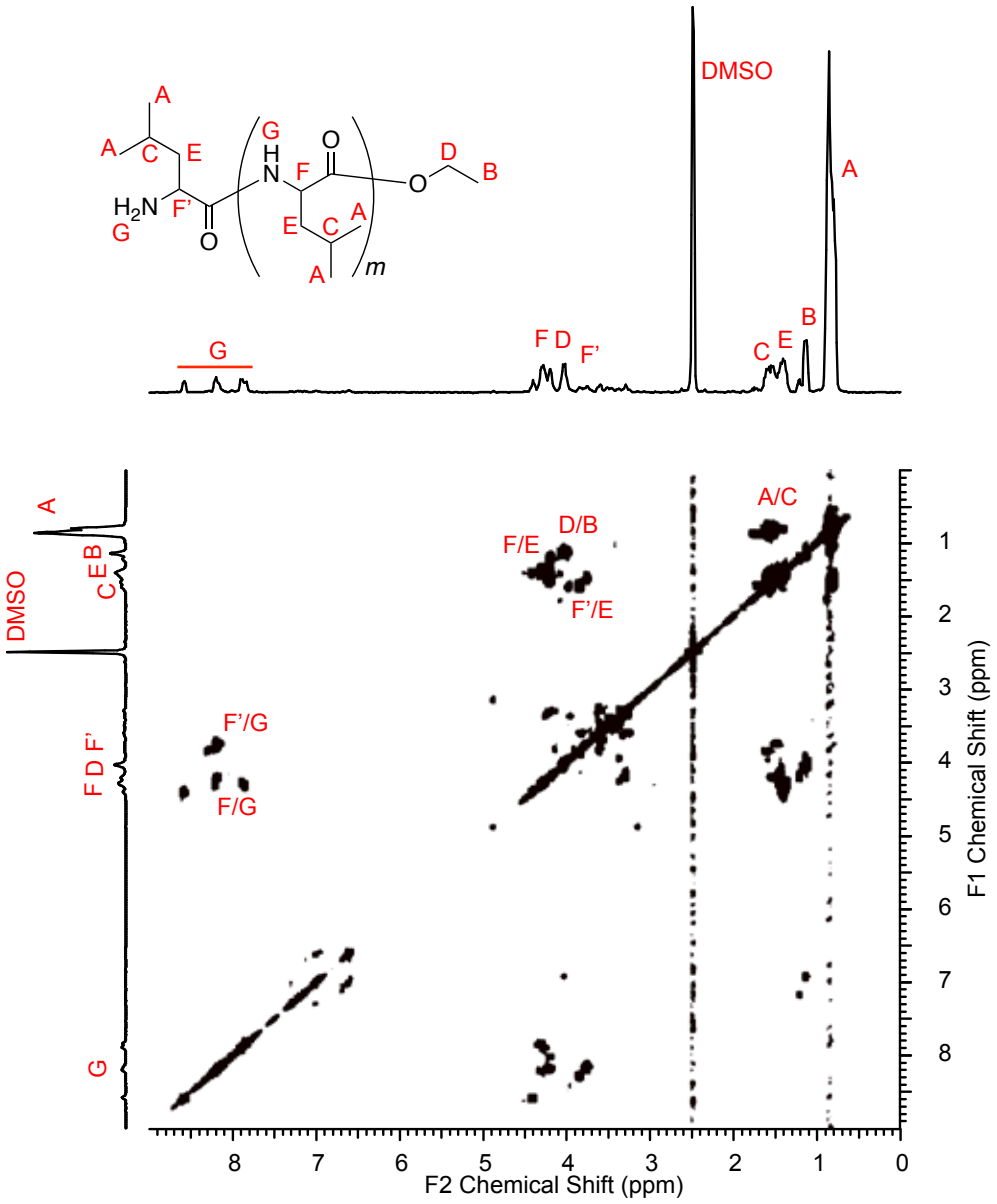


Fig. S11 g-COSY NMR study of oligo(LeuEt) depicting peak assignment.

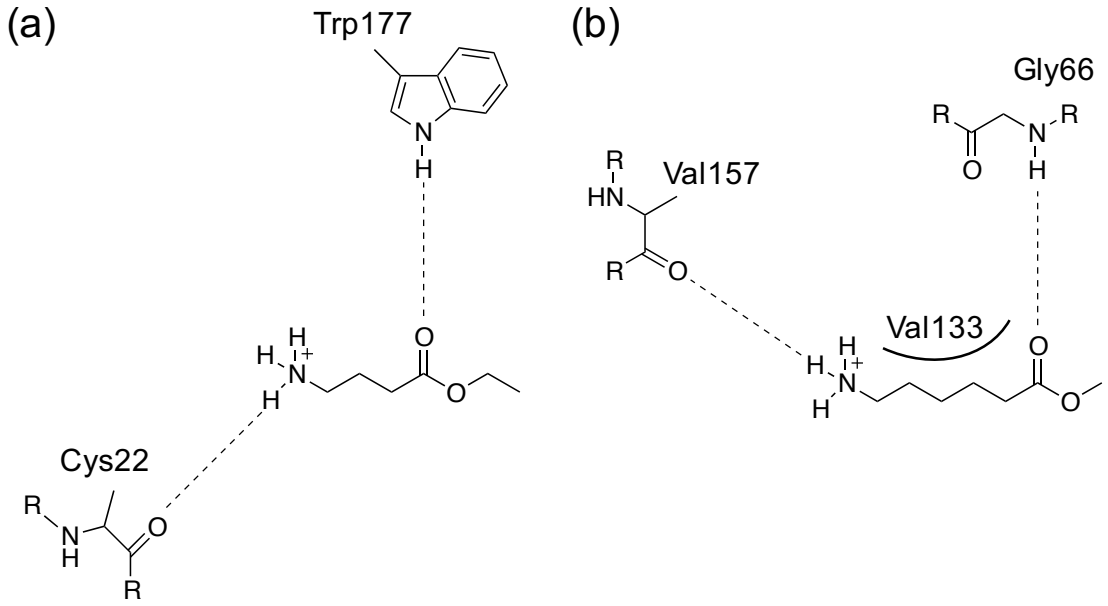


Fig. S12 Best docking interactions between papain and (a) nylon4Et and (b) nylon6Me. Hydrogen bonding between papain and nylon monomers are shown as dashed lines, whereas hydrophobic interactions are shown as spline sections.

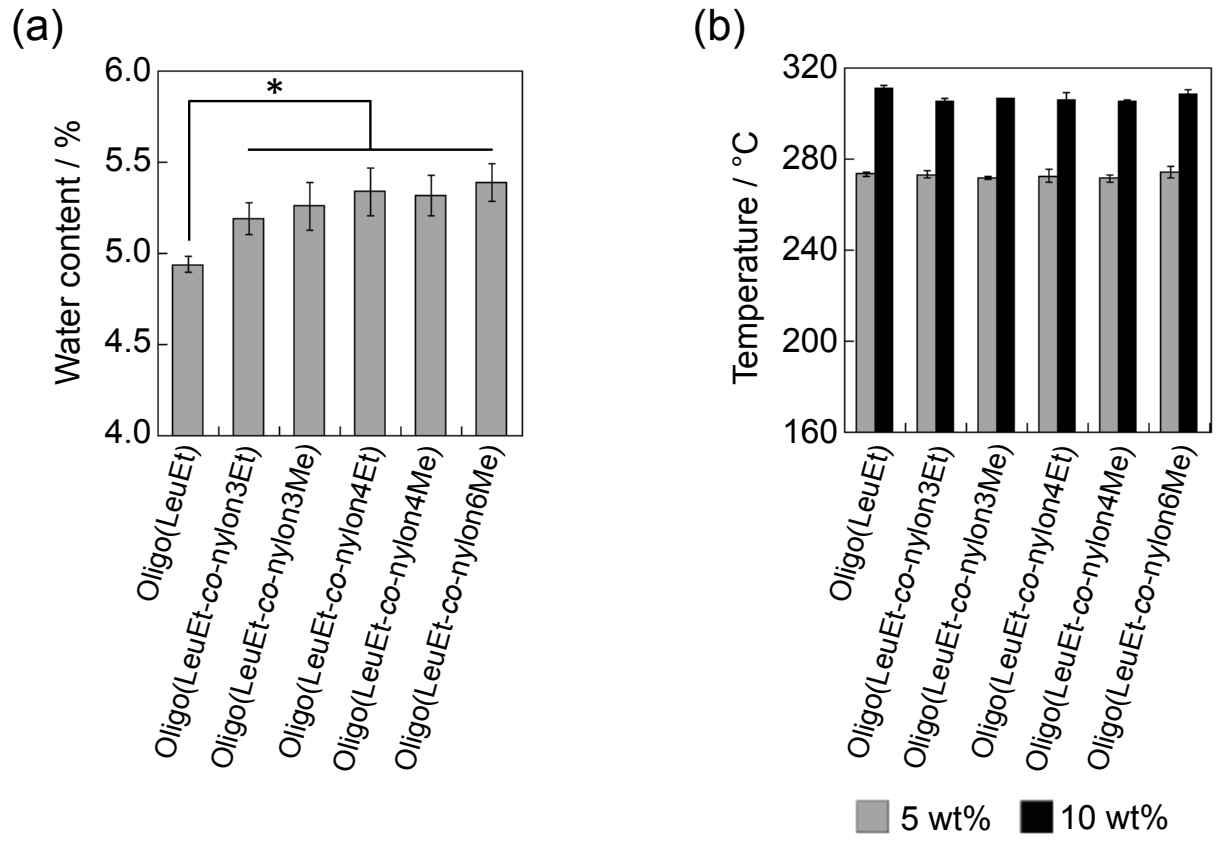


Fig. S13 Effect of nylon monomer units on (a) water content and (b) 5 wt% and 10 wt% thermal degradation temperature of oligo(LeuEt) and oligo(LeuEt-*co*-nylon). Error bars represent the standard deviation of triplicate experiments. *Significant differences between groups at $p < 0.05$.

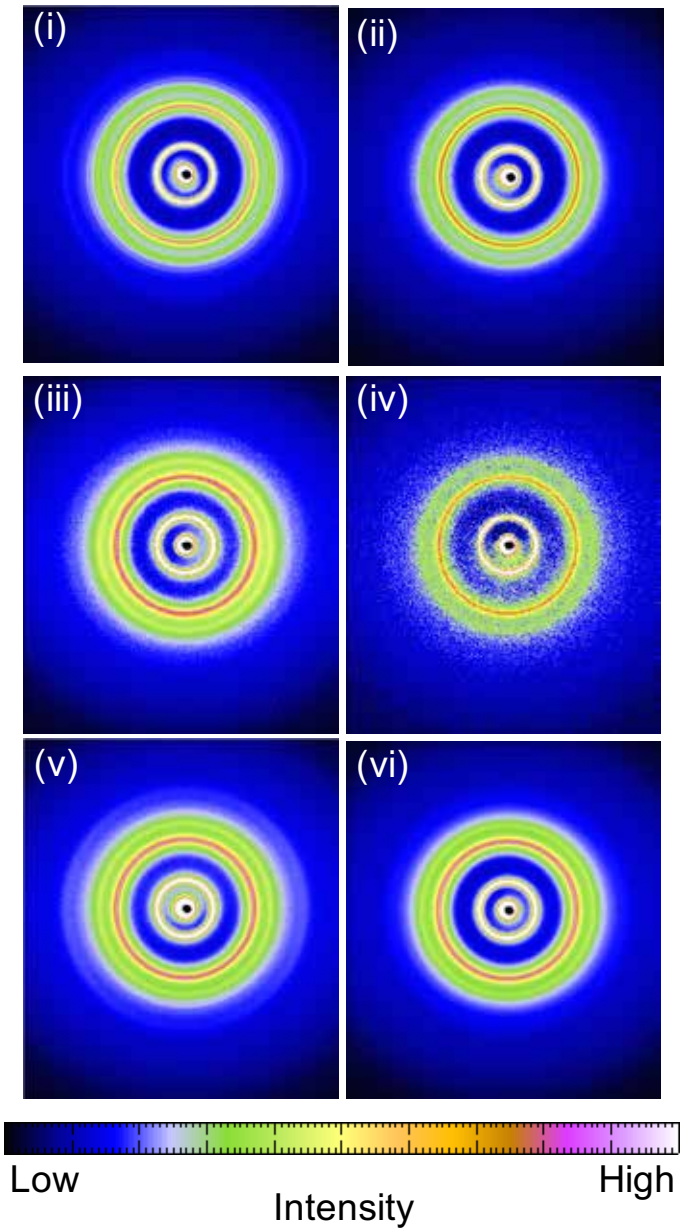


Fig. S14 Two dimensional profiles of wide-angle X-ray scattering of (i) oligo(LeuEt), (ii) oligo(LeuEt-*co*-nylon3Et), (iii) oligo(LeuEt-*co*-nylon3Me), (iv) oligo(LeuEt-*co*-nylon4Et), (v) oligo(LeuEt-*co*-nylon4Me), and (vi) oligo(LeuEt-*co*-nylon6Me).

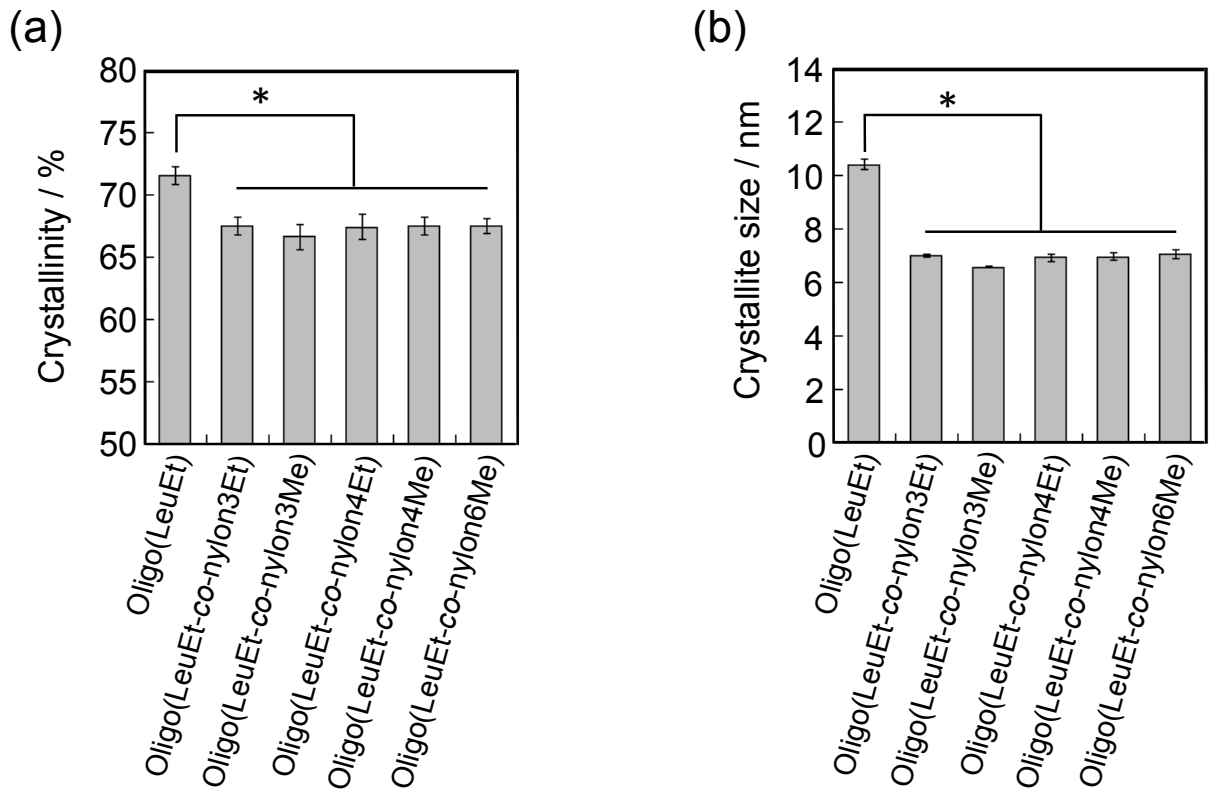


Fig. S15 Effect of nylon monomer unit on (a) crystallinity and (b) crystallite size of the oligo(LeuEt-*co*-nylon). Error bars represent the standard deviation of triplicate experiments. *Significant differences between groups at $p < 0.05$.

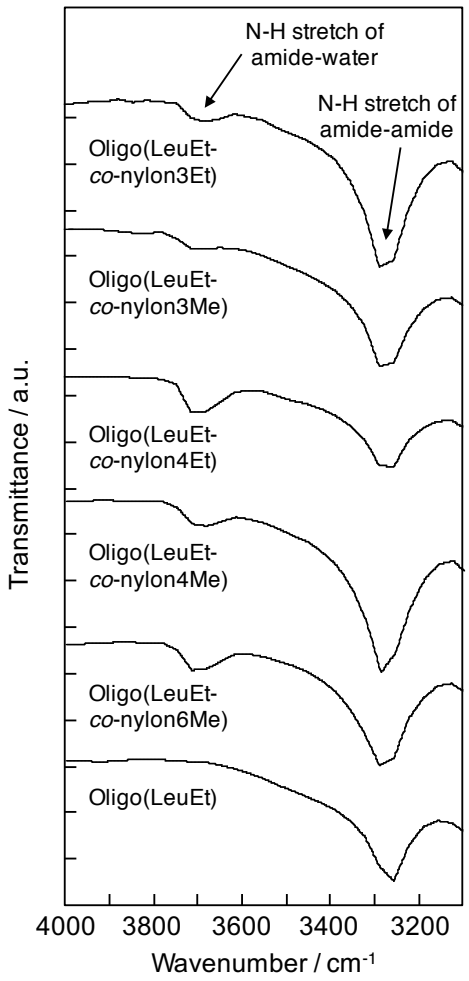


Fig. S16 IR spectra of oligo(LeuEt) and oligo(LeuEt-*co*-nylon).

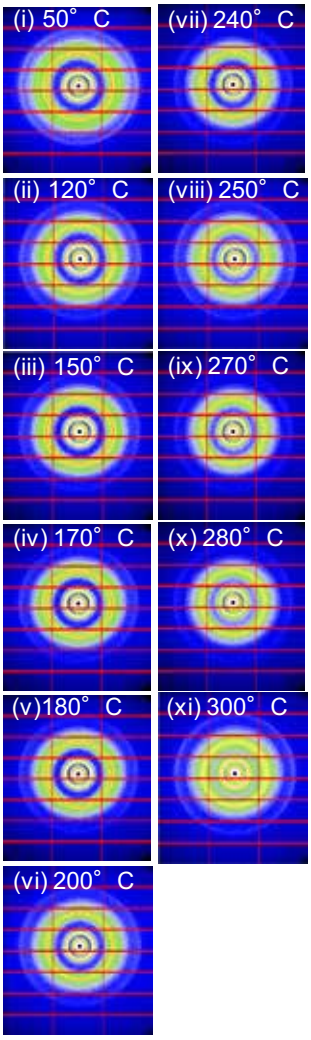


Fig. S17 Two dimensional profiles of temperature-dependent WAXS of oligo(LeuEt-*co*-nylon4Et).

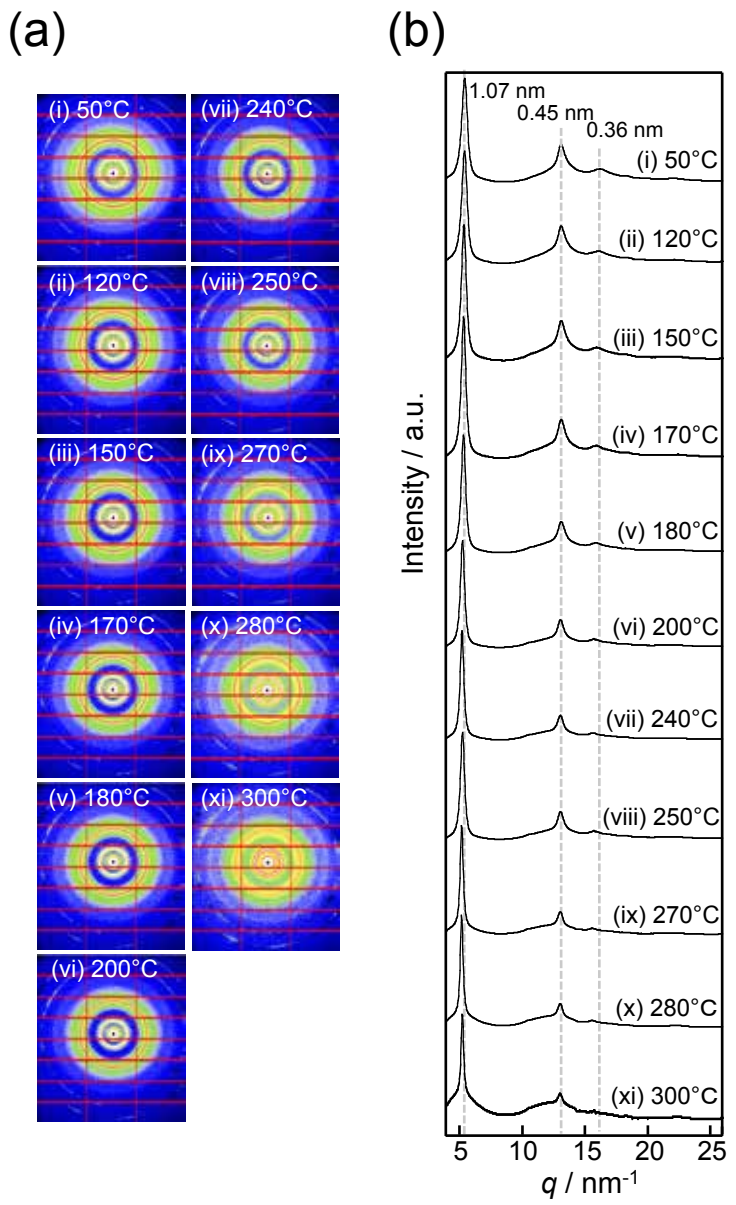


Fig. S18 Temperature-dependent WAXS profiles of oligo(LeuEt-*co*-nylon3Et). (a) Two dimensional profiles and (b) one dimensional profiles for the corresponding profiles in (a).

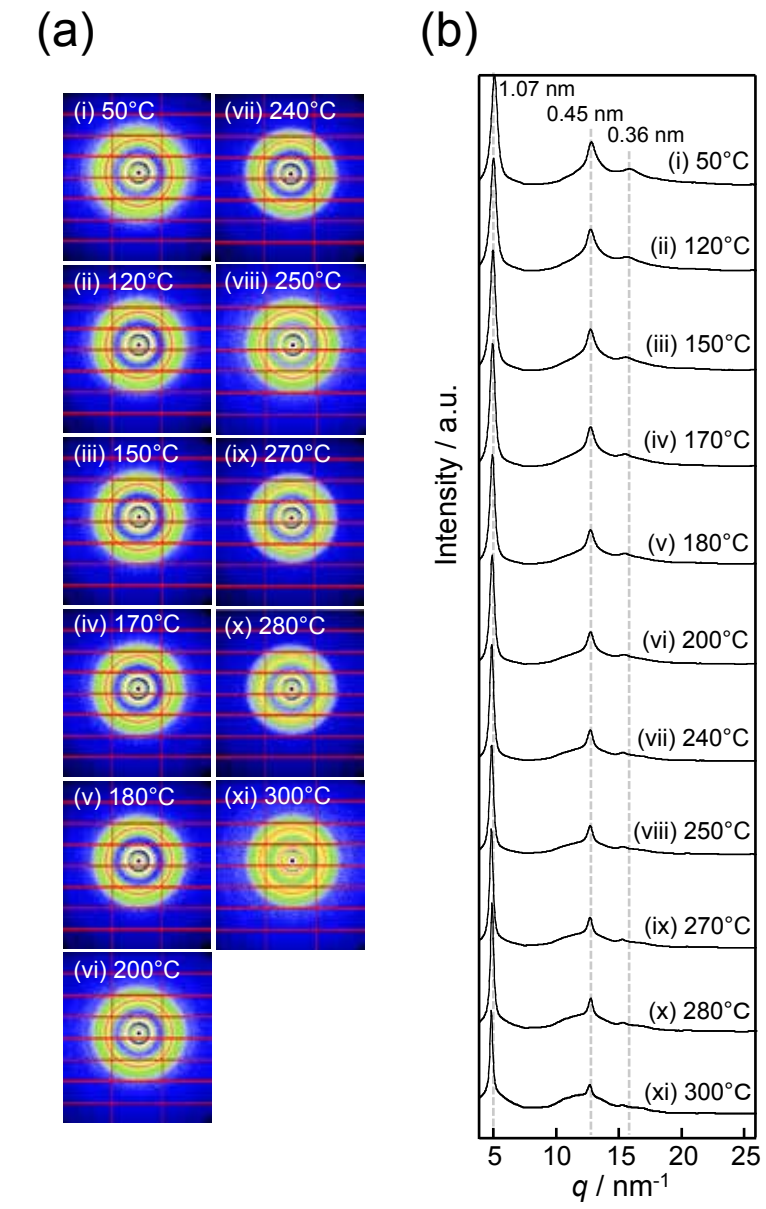


Fig. S19 Temperature-dependent WAXS profiles of oligo(LeuEt-*co*-nylon3Me). (a) Two dimensional profiles and (b) one dimensional profiles for the corresponding profiles in (a).

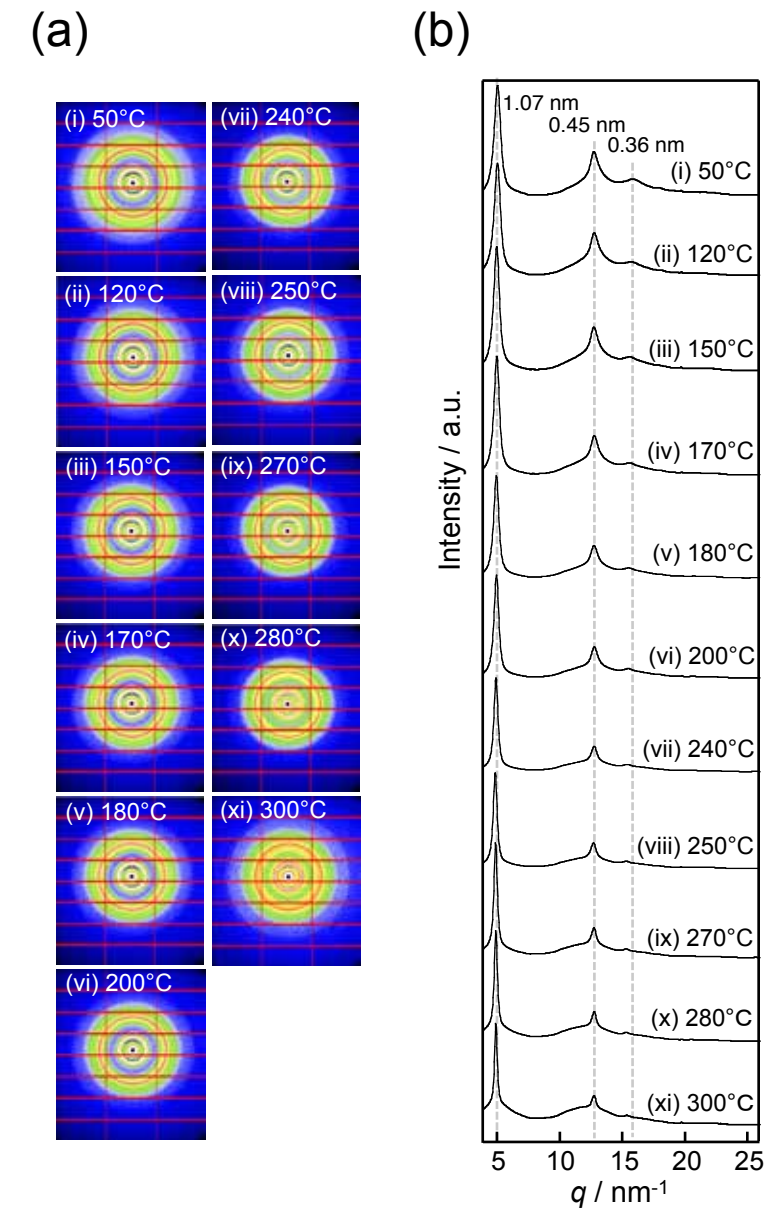


Fig. S20 Temperature-dependent WAXS profiles of oligo(LeuEt-*co*-nylon4Me). (a) Two dimensional profiles and (b) one dimensional profiles for the corresponding profiles in (a).

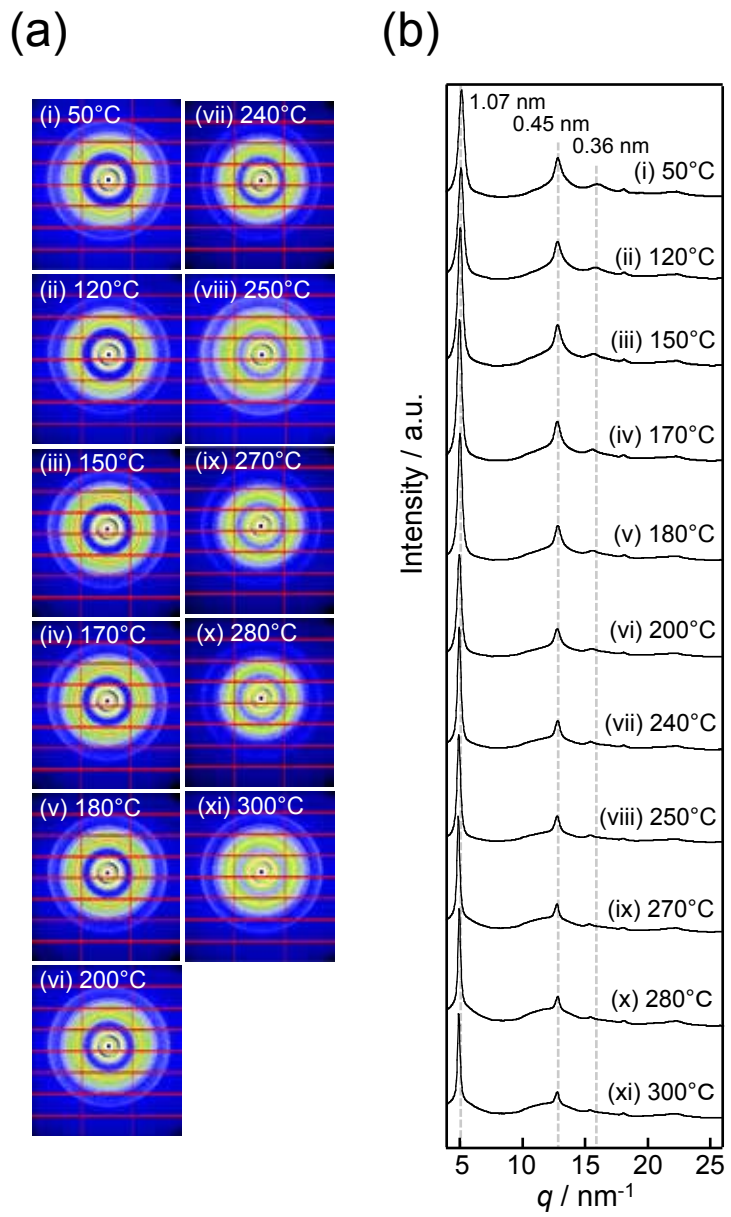


Fig. S21 Temperature-dependent WAXS profiles of oligo(LeuEt-*co*-nylon6Me). (a) Two dimensional profiles and (b) one dimensional profiles for the corresponding profiles in (a).

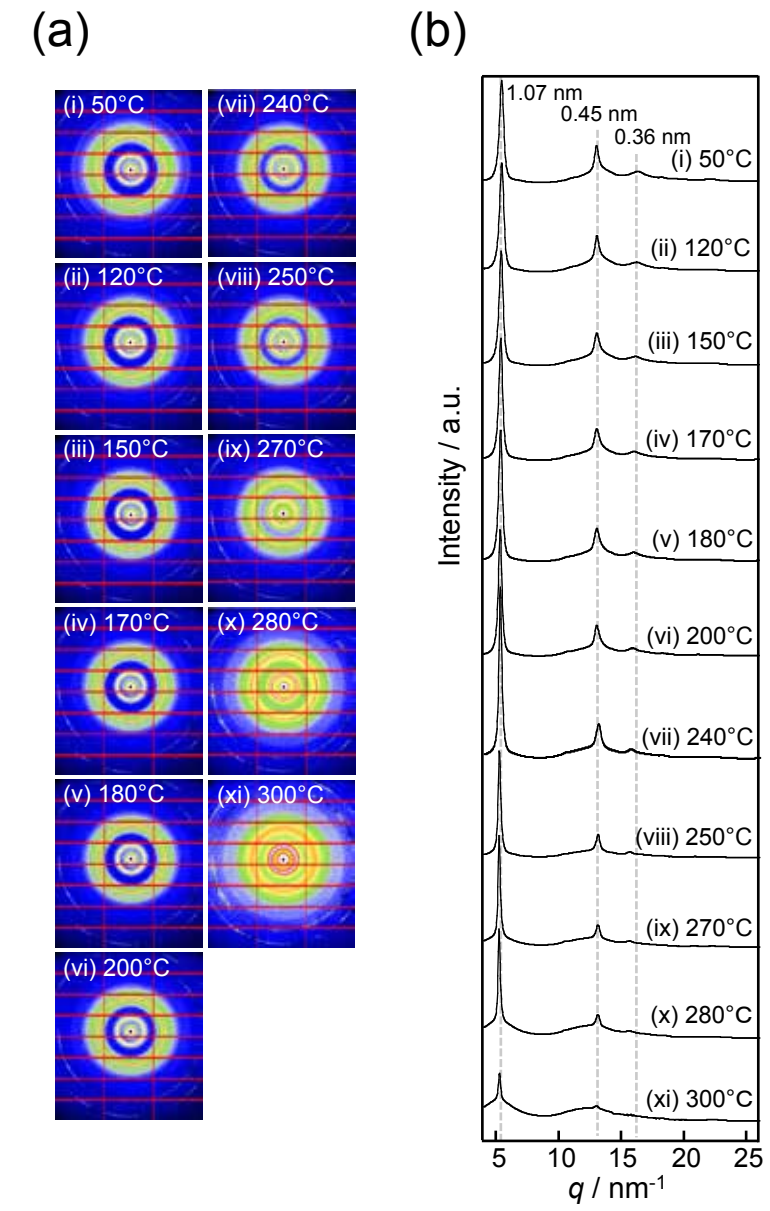


Fig. S22 Temperature-dependent WAXS profiles of oligo(LeuEt). (a) Two dimensional profiles and (b) one dimensional profiles for the corresponding profiles in (a).

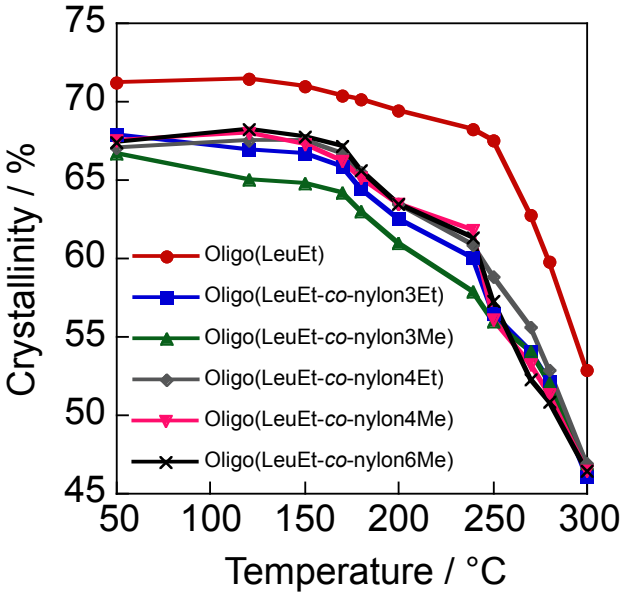


Fig. S23 Temperature-dependent crystallinity change of oligo(LeuEt) and oligo(LeuEt-*co*-nylon) evaluated by WAXS profiles.

Table S1. Estimated free energy of binding of three types of nylon units to papain.

mode	**Estimated $\Delta G_{Binding}$ (kcal/mol)**		
	Nylon 1Et	Nylon 4Et	Nylon 6Me
1	-3.3	-3.9	-4.1
2	-3.2	-3.9	-4.0
3	-3.1	-3.8	-3.9
4	-3.1	-3.8	-3.9
5	-3.1	-3.7	-3.9
6	-3.1	-3.7	-3.9
7	-3.1	-3.7	-3.9
8	-3	-3.7	-3.8
9	-3	-3.7	-3.8

Table S2. Water content and thermal degradation temperature at 5% and 10% weight loss for the oligo(LeuEt) and oligo(LeuEt-*co*-nylon) (*n* = 3).

Product	Water content / %	5% Weight loss / °C	10% Weight loss / °C
Oligo(LeuEt)	4.9 ± 0.1	273.4 ± 1.1	311.1 ± 1.3
Oligo(LeuEt-*co*-nylon3Et)	5.2 ± 0.1	273.3 ± 1.5	305.5 ± 1.0
Oligo(LeuEt-*co*-nylon3Me)	5.4 ± 0.3	271.6 ± 0.6	306.4 ± 0.4
Oligo(LeuEt-*co*-nylon4Et)	5.3 ± 0.1	272.5 ± 2.9	305.8 ± 3.7
Oligo(LeuEt-*co*-nylon4Me)	5.3 ± 0.1	271.6 ± 1.8	305.5 ± 0.8
Oligo(LeuEt-*co*-nylon6Me)	5.4 ± 0.1	274.3 ± 2.3	308.8 ± 1.8

References

1. O. Trott and A. J. Olson. *J. Comput. Chem.*, 2010, **31**, 455.

2. R. W. Pickersgill, G. W. Harris and E. Garman. *Acta Crystallogr. Sect. B.,* 1992, **48**, 59.