Experimental Section:

Materials. Boc-Ala-OSu, glycine ethyl ester hydrochloride, trifluoroacetic acid (TFA), dichloromethane (DCM), acetonitrile (ACN), dimethyl sulfoxide (DMSO), triethylamine (TEA), hexafluoro-2-propanol (HFIP), deuterated trifluoroacetic acid (CF_3COOD), α-cyano hydroxycinnamic acid (CHCA, MALDI-TOF matrix) were all purchased from Sigma-Aldrich and were used as received. Deionized water (DI, 18.2 MΩ·cm purity) was obtained from a RIOS 16/MILLQ synthesis Millipore water purification system. Crude papain (EC # 3.4.22.2; source-Carica papaya; 30 000 USP units/mg of solid; molecular weight 21K) was purchased from CalBioChem.

Synthetic Methods

General route to synthesize AG-OEt: The method used was adapted from published literature methods.[27] Boc-A-OSu (858.8 mg, 3 mmol) and 3.3 mmol (418.8 mg) G-OEt.HCl were dissolved in 10 mL DMSO at room temperature. Subsequently, 0.8 mL TEA was added and the reaction mixture was stirred at room temperature for one day. Boc-AG-OEt was obtained as a precipitate by addition of the reaction solution into 20 volumes of cold water. Deprotection was performed in 2:1 DCM/TFA (v/v) at room temperature for 18 h.

General procedure followed for protease-catalyzed synthesis of $(AG)_x$. AG-OEt (348.4 mg, 2 mmol), and 4 mL 0.9M phosphate buffer solution were transferred to a 50 mL Falcon tube. A Tiamo automatic titration system and a Metrohm CH9101 dosing unit were used to control reaction medium pH within ± 0.05 units of the set value during oligomerizations. The dosing solution (3M NaOH) was added at 0.05-0.1 μL/min, and the frequency at which the probe checked the pH was 1.0 s. After manual pre-titration or using the pH-stat to set the monomer

solution to the desired pH, 80 mg papain was added. The reaction flask was placed in an external water bath set at 40 ^{o}C, the flask contents were gently mixed by magnetic stirring, the pH was held constant by the pH stat and reactions were continued for predetermined times. Subsequently, the reaction mixture was centrifuged at 8000 rpm, the precipitated fraction was washed with 5 mL portions of water twice before it was lyophilized for two days to give a solid.

Instrumental Methods.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Proton (^{1}H) NMR and 2D Correlated ^{1}H-^{1}H NMR (COSY) spectra were recorded on a Bruker *DP*X 300 spectrometer at 300 MHz. NMR experiments were performed in TFA-*d* solvent at 10 mg/mL with a data acquisition delay of 1 s. Data were collected and analyzed by MestRe-C software. Proton chemical shifts were referenced to tetramethylsilane (TMS) at 0.00 pm. The Inverse-gated-decoupling carbon (^{13}CIG) NMR spectrum and the edited Heteronuclear Single Quantum Coherence (HSQC) spectrum were both recorded on a Bruker Avance III 500 spectrometer using deuterated trifluoroacetic acid (TFA-*d*) as solvent.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF). MALDI-TOF spectra were obtained on an OmniFlex MALDI-TOF mass spectrometer (Bruker Daltonics, Inc.). The instrument was operated in a positive ion reflection mode with an accelerating potential of +20 kV. The TOF mass analyzer had pulsed ion extraction. Omni-FLEX TOF control software was used for hardware control and calibration while X-mass OminFLEX 6.0.0 was used for data processing. Spectra were acquired by averaging at least 400 laser shots. The pulsed ion extraction delay time was set at 200 ns. To generate the matrix solution, a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) was prepared in trifluoroacetic acid/acetonitrile (TA) 1-to-10 v/v. Oligopeptide samples were dissolved in hexafluoroisopropanol (HFIP), diluted to 1-5 pmol/μL

with TA solution and then mixed with an equal volume of a saturated matrix solution. Then, 1 µL of this mixture was applied onto the clean target which was subsequently dried with a dryer using a stream of cold air. The relative intensity threshold was set so that, peaks with intensity values less than 1% of the highest peak were considered as noise and removed from the database.

Results and Discussion:

NMR Spectra and Their Analysis

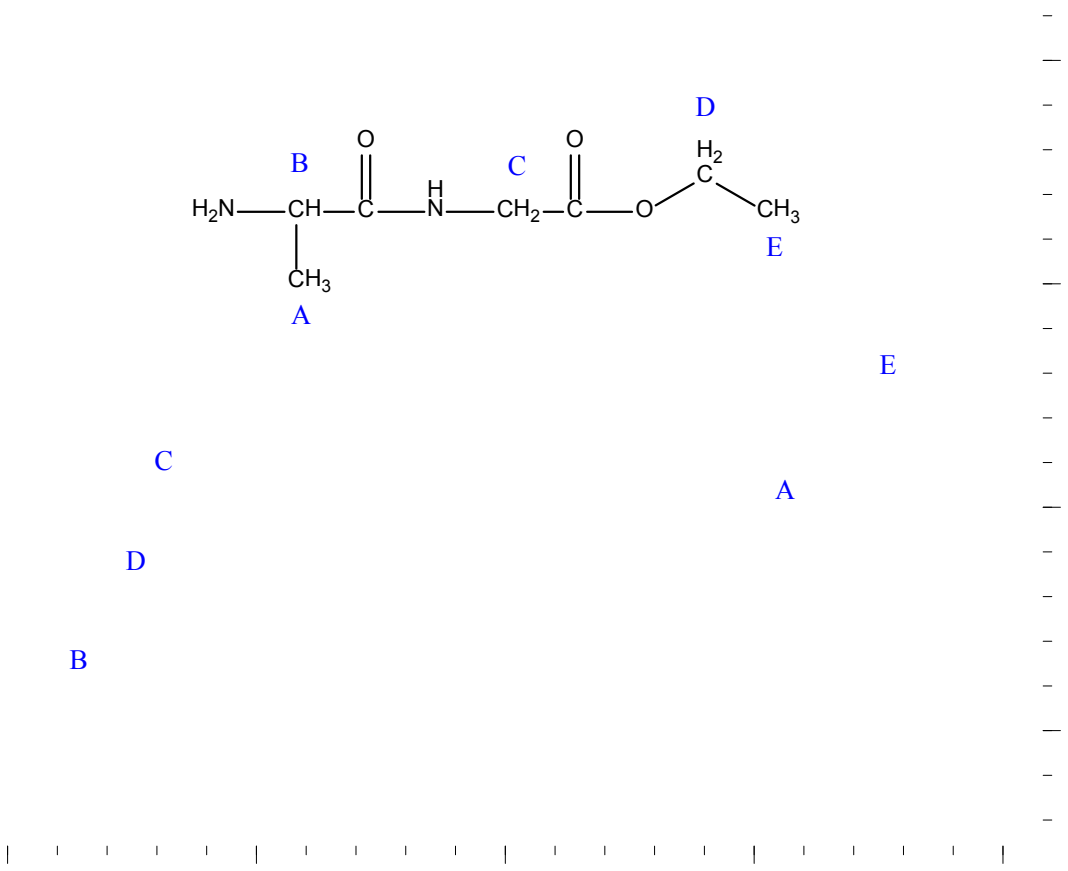

Figure S-1. ^{1}H-NMR (300 MHz, TFA-*d*) spectra of AG-OEt.

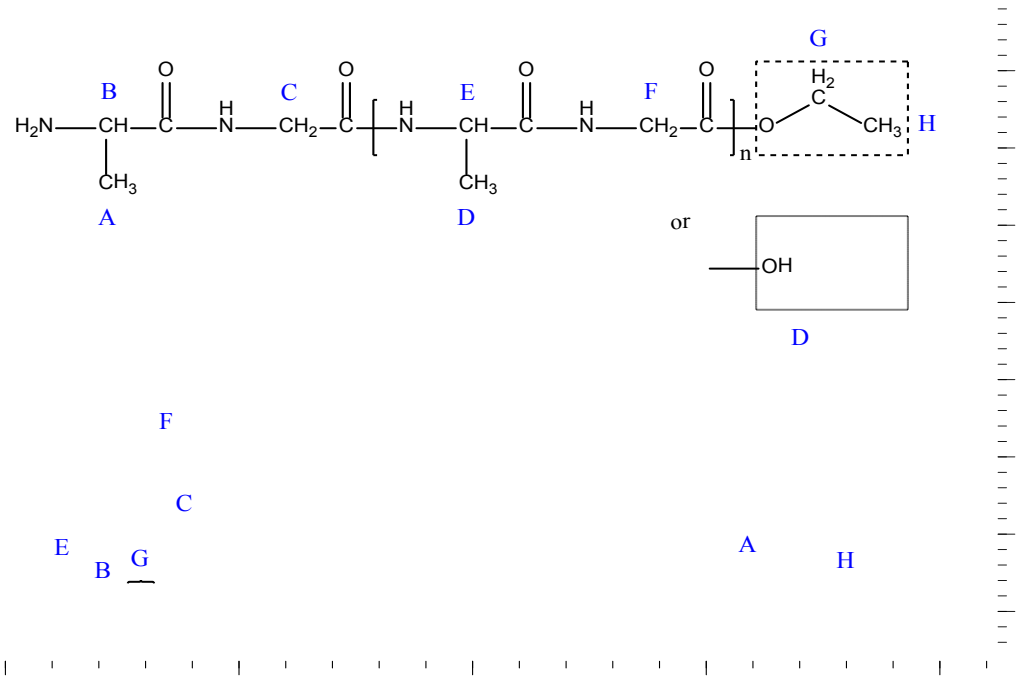

Figure S-2. ^{1}H-NMR (300 MHz, TFA-*d*) spectrum of $(AG)_x$.

The ^{1}H-NMR spectrum of the product analyzed by MALDI-TOF (Figure 1) is shown in Figure S-2. To confirm assignments given on the 1-D ^{1}H-NMR spectrum, a 2D Correlated ^{1}H-^{1}H NMR (COSY) spectrum was recorded and the spectrum is displayed in supplemental materials (Figure S-3). Crosspeaks observed between protons A and B, D and E, and G and H corroborate our assignments on 1-D ^{1}H-NMR spectrum. Signals with peaks labeled A and D are assigned to methyl groups of A-units at the N-terminal AG residue and other oligopeptide AG-units (internal and C-terminus), respectively. Since the quantity of N-terminal AG-units equals the number of peptide chains, DP_{avg} values of $(AG)_x$ is determined from peak integration values by the following relationship: $DP_{avg} = (A+D)/A$ (*). Similarly, signals with peaks labeled B and E are assigned to the methine proton of A-units at the N-terminal AG residue and A-units of internal and C-terminal AG oligopeptide residues, respectively. Following the same logic as above, an

alternative means to calculate DP_{avg} of $(AG)_x$ is by the relationship $DP_{avg} = (B+E)/B$ (**). DP_{avg} values determined by equations (*) and (**) are in excellent agreement. Since signals with peaks at A and D were baseline resolved, equation (*) was selected to calculate $(AG)_x$ DP_{avg} values. Signals with peaks at 1.4 and 1.8 ppm correspond to methyl group protons H of C-terminal ester moieties and N-terminal A-units, respectively. Since the quantity of N-terminal AG-units equals the number of C-terminal ester moieties in the absence of ester hydrolysis, %-ester hydrolysis is determined from peak integration values by the relationship (H)/(A) x 100.

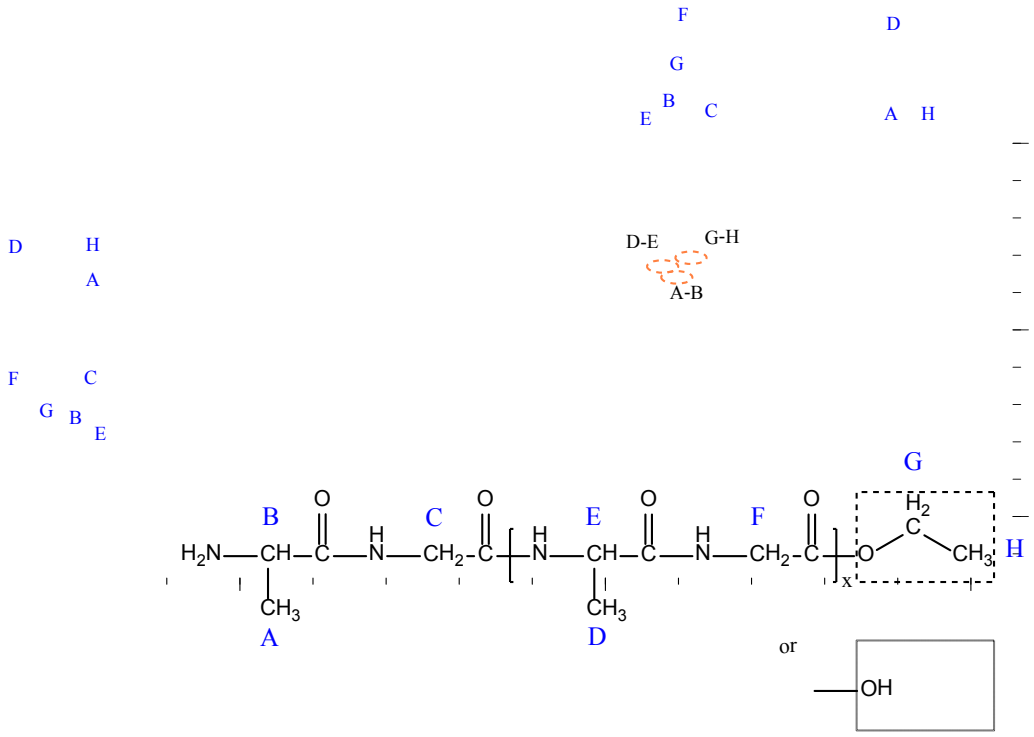

Figure S-3: 2D Correlated ^1H-^1H NMR **(**COSY**)** spectrum of oligopeptide $(AG)_x$.

The ^{13}C-NMR spectrum of alternating oligopeptide $(AG)_x$ is shown in Figure S-4.

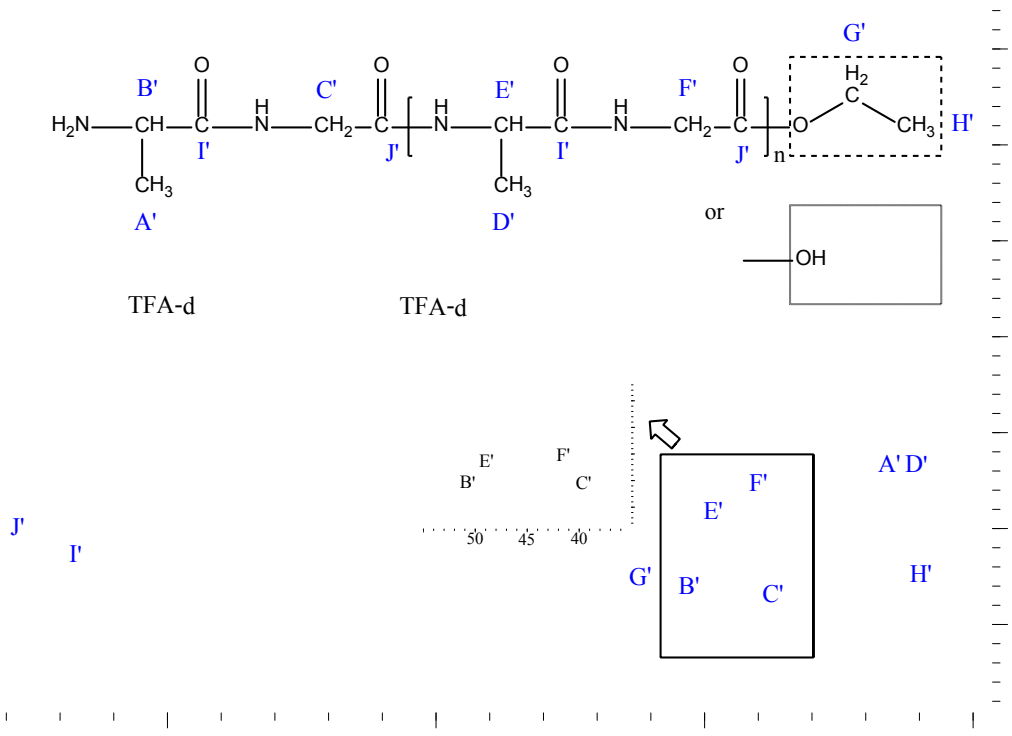

Figure S-4. ^{13}C-NMR (400 MHz, TFA-*d*) spectrum of $(AG)_x$.

To confirm assignments in the 1-D ^{13}C-NMR spectrum, a Heteronuclear Single-Quantum Correlation (HSQC) spectrum was recorded and is displayed in Figure S-5.

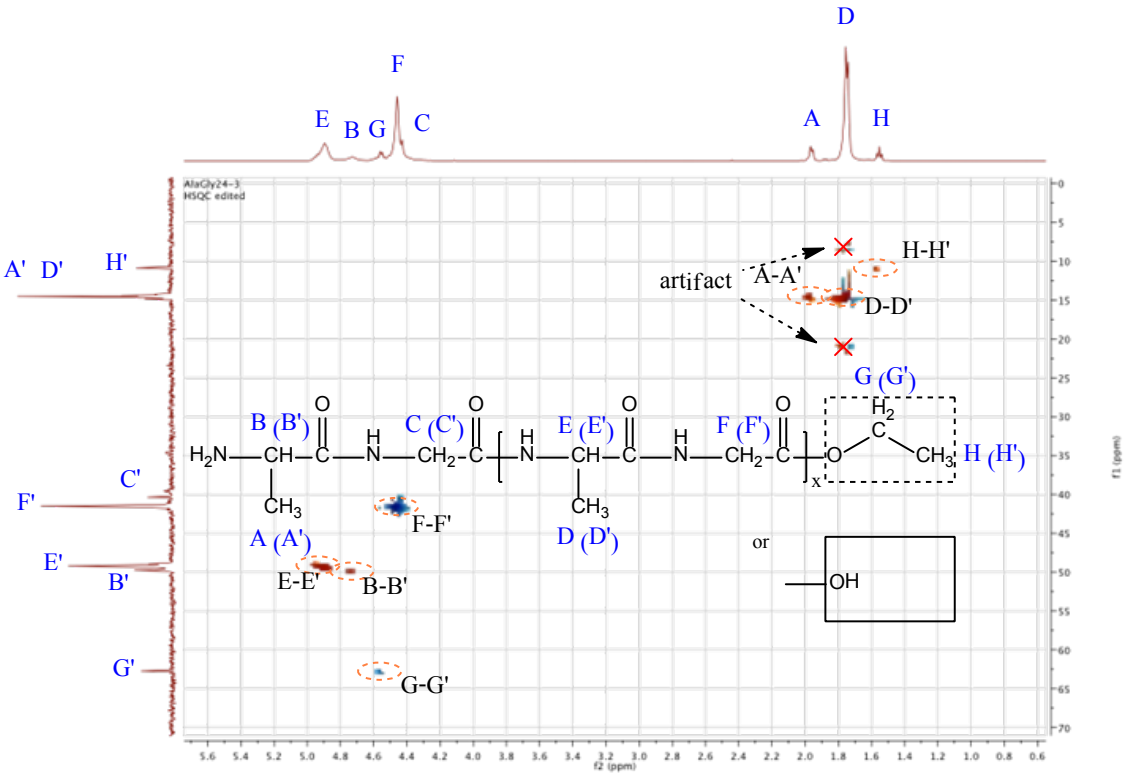

Figure S-5 HSQC 2-D NMR spectra of oligopeptide $(AG)_x$.

Crosspeaks generated due to coupling between protons X (A, B, …H), displayed on the 1-D ^{1}H-NMR x-axis, and carbons X′ (A′, B′… H′) displayed on the 1-D ^{13}C-NMR y-axis, corroborate assignments given in the 1-D ^{13}C-NMR spectrum (Figure S-4). An expansion more clearly showing the resolution of signals corresponding to end-group carbons B′ and C′ is displayed within Figure S-4. The dispersity of carbonyl signals observed in the 168 to 178 ppm region in Figure S-4 is small but discernible. The observation of multiple carbonyl signals is consistent with the differences of chemical environments in which they exist (e.g. N- or C-terminus, intrachain, and C-terminal units that may be esterified or exist as free acids).

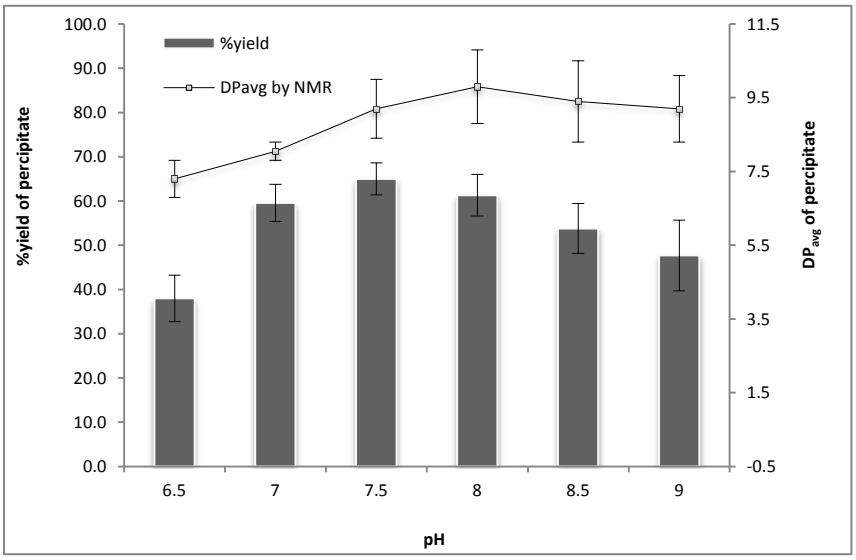

Figure S-6. Activity-pH relationships for papain-catalyzed AG-OEt oligomerizations with pH controlled by a pH stat.

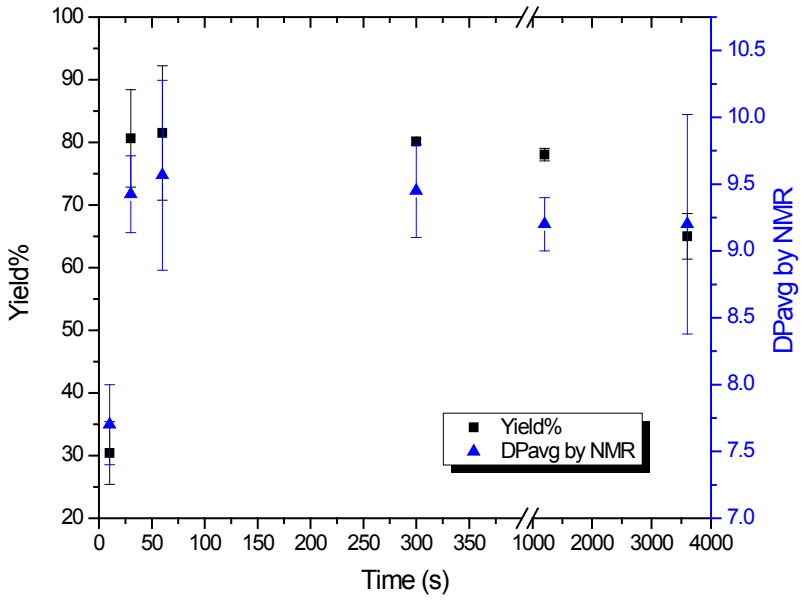

Figure S-7. Percent yield and DP_{avg} values of $(AG)_x$ as a function of reaction time for papain-catalyzed AG-OEt oligomerizations conducted with pH controlled by pH stat at 7.5.