Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Synthesis of Amino Acid-derived Vinyl Polymers with Precisely Controlled Hydropathy and Their Thermoresponsive Behavior in Water

Atsushi Sawamoto, Shin-nosuke Nishimura, Nobuyuki Higashi and Tomoyuki Koga

Department of Molecular Chemistry and Biochemistry, Faculty of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, JAPAN

Corresponding Author:

tkoga@mail.doshisha.ac.jp (TK)

Experimental Procedure

Synthesis of *N*-acryloyl leucine methyl ester (NALMe).

H-Leu-OMe hydrochloride 5.00 g (27.5 mmol) and TEA 8.4 mL (60.6 mmol) were dissolved in DCM (200 mL) and cooled in an ice-bath. Acryloyl chloride 2.67 mL (33.0 mmol) was diluted with DCM (15 mL) and added dropwise to the amino acid solution over 90 min. After addition, the reaction solution was stirred overnight in an ice-bath. The solution was washed repeatedly with 1.5 M $MgSO_4$ *aq.* (100 mL × 5). The organic phase was collected and dried over anhydrous Na_2SO_4. After the solution was filtered and concentrated *in vacuo*, the obtained monomer was purified by column chromatography using a silica gel column (Wakogel FC-40) with hexane/ethyl acetate (v/v = 2/1) mixed solution as the eluent. The resultant solution was concentrated *in vacuo* and the obtained monomer was recrystallized from diethyl ether.

Yield: 2.6 g (53 %).

^{1}H-NMR (DMSO-d_6, TMS) (Fig. S1): 0.8-1.0 ppm (6H, -$CH_2CH(CH_3)_2$: side chain of Leu), 1.4-1.7 ppm (2H, -$CH_2CH(CH_3)_2$, 1H, -$CH_2CH(CH_3)_2$: side chain of Leu), 3.6 ppm (3H, - $COOCH_3$: methyl ester), 4.3-4.4 ppm (1H, -COCHNH-: Leu), 5.6-5.7 ppm (1H, CH_2CH-: vinyl (*cis*)), 6.0-6.1 ppm (1H, CH_2CH-: vinyl (*trans*)), 6.2-6.3 ppm (1H, CH_2CH-: vinyl), 8.4-8.6 ppm (1H, -CHNHCO-: amide).

Synthesis of *N*-acryloyl phenylalanine methyl ester (NAFMe).

H-Phe-OMe hydrochloride 5.00 g (23.2 mmol) and TEA 6.62 mL (47.5 mmol) were dissolved in DCM (200 mL) and the mixture was stirred for 20 min. Acryloyl chloride 1.97 mL (24.3 mmol) was diluted in DCM (15 mL) and added dropwise to the amino acid solution in an ice-bath for 90 min. The reaction solution was stirred overnight in an ice-bath. The solution was washed repeatedly with 1.5 M $MgSO_4$ *aq.* (100 mL × 5). The organic phase was collected and dried over anhydrous Na_2SO_4. After filtration and evaporation, the obtained monomer was recrystallized from a methanol system to produce pure NAFMe as a white solid.

Yield: 2.9 g (54 %).

^{1}H-NMR (DMSO-d_6, TMS) (Fig. S1): 2.8-3.1 ppm (2H, -$CH_2C_6H_5$: side chain of Phe), 3.6 ppm (3H, - $COOCH_3$: methyl ester), 4.4-4.8 ppm (1H, -COCHNH-: Phe), 5.6-5.7 ppm (1H, CH_2CH-: vinyl (*cis*)), 6.0-6.1 ppm (1H, CH_2CH-: vinyl (*trans*)), 6.2-6.4 ppm (1H, CH_2CH-: vinyl), 7.1-7.4 ppm (5H, -$CH_2C_6H_5$: side chain of Phe), 8.5-8.7 ppm (1H, - CHNHCO-: amide).

Synthesis of *N*-acryloyl valine methyl ester (NAVMe).

H-Val-OMe hydrochloride 5.00 g (29.8 mmol) and TEA 8.4 mL (66.1 mmol) were dissolved in DCM (200 mL) and cooled in an ice-bath. Acryloyl chloride 2.91 mL (36.0 mmol) was diluted in DCM (15 mL) and added dropwise to the amino acid solution for 90 min. After addition, the reaction solution was stirred overnight in an ice-bath. The solution was washed repeatedly with 1.5 M $MgSO_4$ *aq.* (100 mL × 5). The organic phase was collected and dried over anhydrous Na_2SO_4. After the solution was filtered and concentrated *in vacuo*, the obtained monomer was purified by column chromatography using a silica gel column (Wakogel FC-40) with hexane/ethyl acetate (v/v = 2/1) mixed solution as the eluent.

The resultant solution was concentrated *in vacuo* and the obtained monomer was recrystallized from diethyl ether.

Yield: 3.70 g (66 %).

^{1}H-NMR (DMSO-d_6, TMS) (Fig. S1): 0.8-0.9 ppm (6H, -CH(CH_3)$_2$: side chain of Val), 2.0-2.1 ppm (1H, --CH(CH$_3$)$_2$: side chain of Val), 3.6 ppm (3H, -COOCH_3: methyl ester), 4.2-4.3 ppm (1H, -COCHNH-: Val), 5.6-5.7 ppm (1H, CH_2CH-: vinyl (*cis*)), 6.0-6.1 ppm (1H, CH_2CH-: vinyl (*trans*)), 6.3-6.4 ppm (1H, CH$_2$CH-: vinyl), 8.3-8.4 ppm (1H, -CHNHCO-: amide).

Synthesis of *N*-acryloyl serine methyl ester (NASMe).

H-Ser-OMe hydrochloride 4.97 g (31.8 mmol) and DIPEA 11.1 mL (63.2 mmol) were dissolved in DCM (200 mL) and cooled in an ice-bath. Acryloyl chloride 2.56 mL (31.5 mmol) was diluted in DCM (15 mL) and added dropwise to the amino acid solution. After addition, the reaction solution was stirred overnight in an ice-bath. The reaction mixture was washed five times with saturated NaCl *aq.* (100 mL). The organic layer was collected and dried over anhydrous Na_2SO_4. The solution was concentrated *in vacuo* and then passed through a silica gel column using a mixed solution of DCM/acetone/MeOH (v/v/v = 5/1/1) as the eluent. The resulting solution was concentrated *in vacuo*. Subsequently, it was freeze-dried in water to produce pure NASMe as a white solid.

Yield: 3.66 g (67 %).

^{1}H-NMR (DMSO-d_6, TMS) (Fig. S1): 3.6-3.9 ppm (3H, -COOCH_3: methyl ester; 2H, -CH_2OH: side chain of Ser), 4.3-4.5 ppm (1H, -COCHNH-: Ser), 5.1 ppm (1H, -CH$_2$OH: side chain of Ser), 5.6-5.7 ppm (1H, CH_2CH-: vinyl (*cis*)), 6.1-6.2 ppm (1H, CH_2CH-: vinyl (*trans*)), 6.3-6.4 ppm (1H, CH$_2$CH-: vinyl), 8.5-8.6 ppm (1H, -CHNHCO-: amide).

Synthesis of *N*-acryloyl lysine(Z) methyl ester (NAK(Z)Me).

H-Lys(Z)-OMe hydrochloride 3.33 g (10.1 mmol) and TEA 2.8 mL (20.0 mmol) were dissolved in DCM (200 mL) and cooled over an ice-bath. Acryloyl chloride 0.81 mL (10.0 mmol) was diluted in DCM (15 mL) and added dropwise to the amino acid solution. After addition, the reaction solution was stirred overnight in an ice-bath. The reaction mixture was washed five times with saturated NaCl *aq.* (100 mL). The organic layer was collected and dried over anhydrous Na_2SO_4. The solution was concentrated *in vacuo* and then passed through a silica gel column using hexane/ethyl acetate (v/v = 2/1) mixed solution as the eluent. The resulting solution was concentrated *in vacuo*. Subsequently, it was freeze-dried in water to produce pure NAK(Z)Me as a white solid.

Yield: 2.51 g (72 %).

^{1}H-NMR (DMSO-d_6, TMS) (Fig. S1): 1.2-1.8 ppm (2H, -CHCH$_2$CH_2CH$_2$CH$_2$-: side chain of Lys; 2H, -CHCH_2CH$_2$-: side chain of Lys; 2H, -NHCH$_2$CH_2CH$_2$-: side chain of Lys), 2.9-3.0 ppm (2H, -NHCH_2CH$_2$-: side chain of Lys), 3.6 ppm (3H, -COOCH_3: methyl ester), 4.2-4.3 ppm (1H, -COCHNH-: Lys), 5.1 ppm (2H, -OCH_2C-: Z protecting group), 5.6-5.7 ppm (1H, CH_2CH-: vinyl (*cis*)), 6.1-6.2 ppm (1H, CH_2CH-: vinyl (*trans*)), 6.3-6.4 ppm (1H, CH$_2$CH-: vinyl), 7.2-7.4 ppm (5H, -CH$_2$C$_6$H$_5$: phenyl of Z protecting group), 8.5-8.6 ppm (1H, -CHNHCO-: amide).

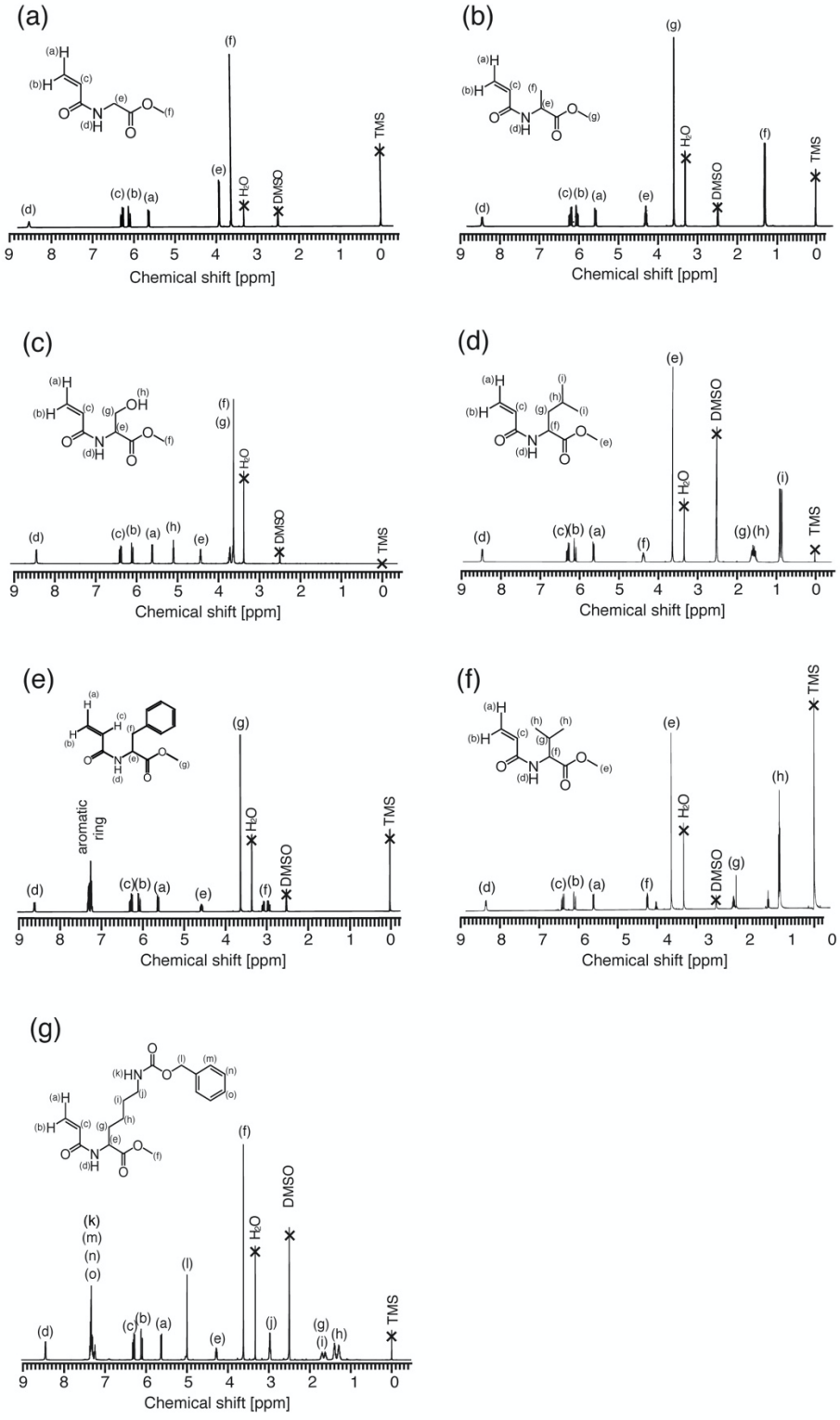

Figure S1. ^{1}H-NMR spectra of NAGMe (a), NAAMe (b), NASMe (c), NALMe (d), NAFMe (e), NAVMe (f), and NAK(Z)Me (g) in DMSO-*d*$_6$ at 25 °C (Internal standard: TMS).

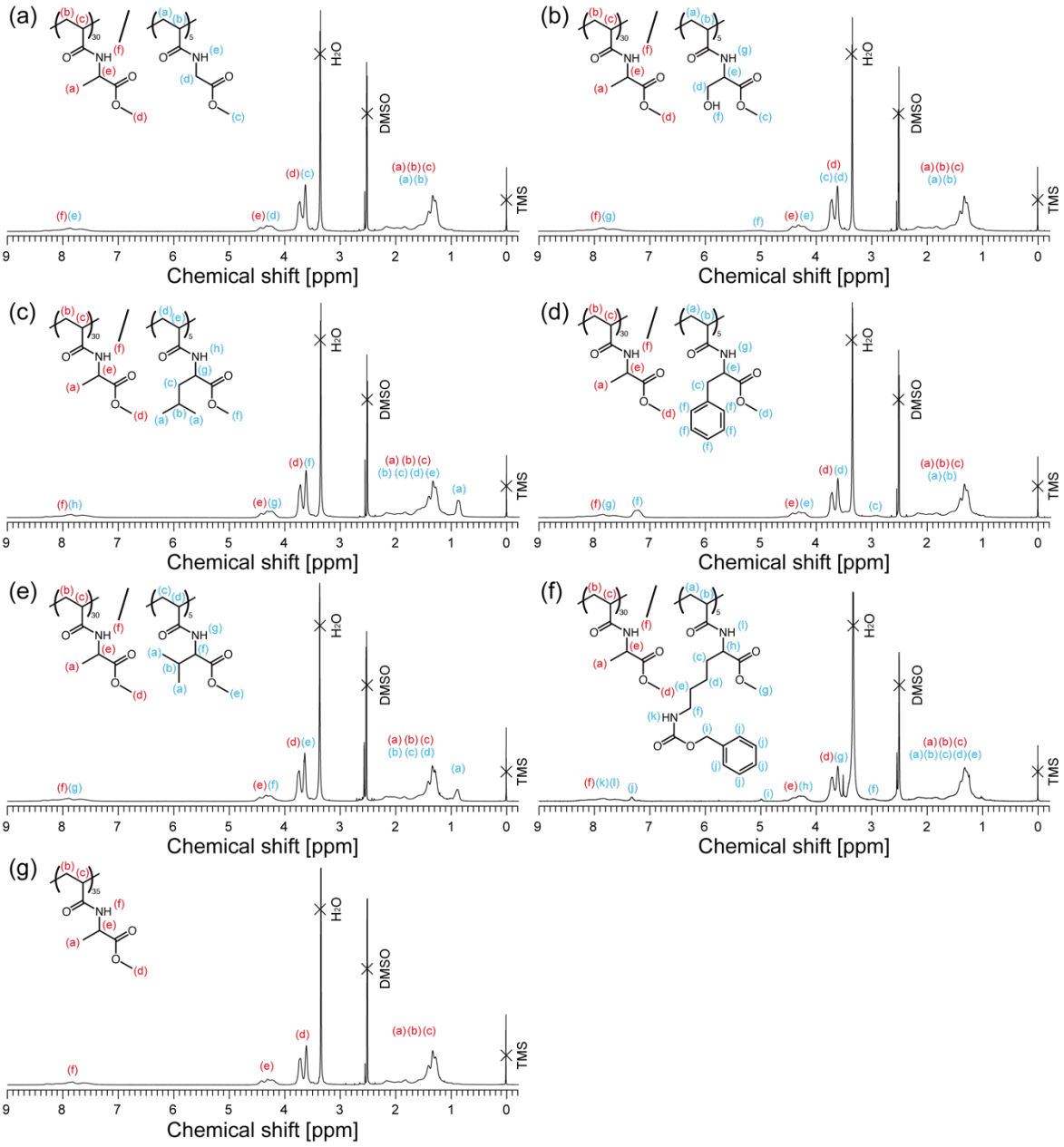

Figure S2. ^{1}H-NMR spectra of A_{15}-*b*-G_5-*b*-A_{15} (a), A_{15}-*b*-S_5-*b*-A_{15} (b), A_{15}-*b*-L_5-*b*-A_{15} (c), A_{15}-*b*-F_5-*b*-A_{15} (d), A_{15}-*b*-V_5-*b*-A_{15} (e), A_{15}-*b*-K(Z)$_5$-*b*-A_{15} (f) and A_{35} (e) in DMSO-*d*$_6$ at 25 °C (Internal standard: TMS).

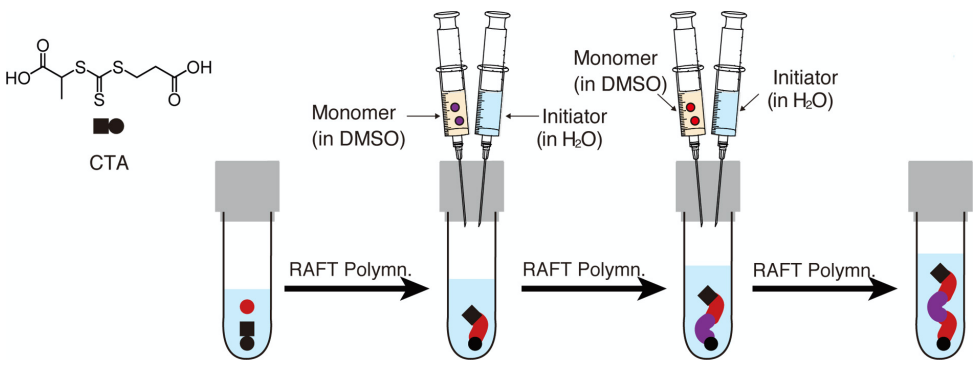

Figure S3. Schematic illustration of the one-pot synthesis of sequence-controlled amino acid-derived block copolymers *via* ultra-rapid RAFT polymerization.

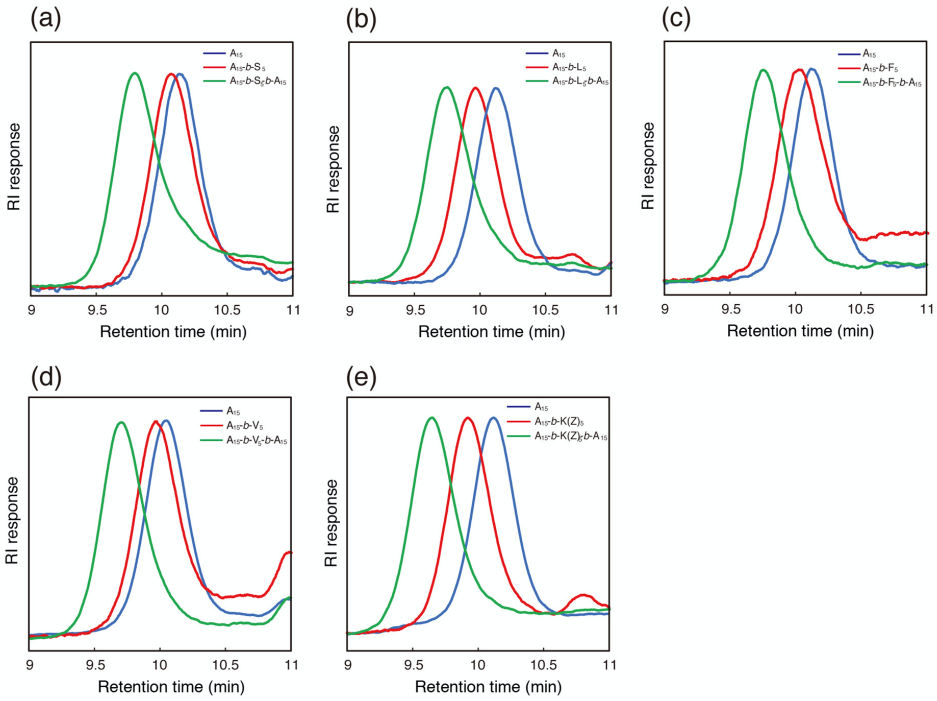

Figure S4. SEC charts for consecutive steps during the synthesis of A_{15}-*b*-S_5-*b*-A_{15} (a), A_{15}-*b*-L_5-*b*-A_{15} (b), A_{15}-*b*-F_5-*b*-A_{15} (c), A_{15}-*b*-V_5-*b*-A_{15} (d), and A_{15}-*b*-$K(Z)_5$-*b*-A_{15} (e). Eluent: THF, temperature: 40 °C.

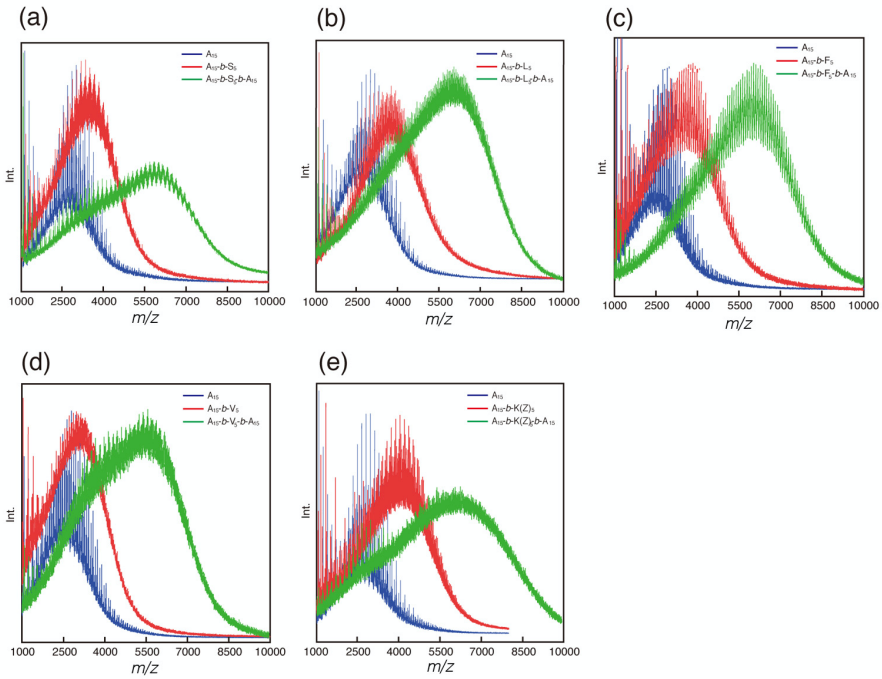

Figure S5. MALDI TOF MS spectra for consecutive steps during the synthesis of A_{15}-*b*-S_5-*b*-A_{15} (a), A_{15}-*b*-L_5-*b*-A_{15} (b), A_{15}-*b*-F_5-*b*-A_{15} (c), A_{15}-*b*-V_5-*b*-A_{15} (d), and A_{15}-*b*-$K(Z)_5$-*b*-A_{15} (e). Matrix: DHBA.

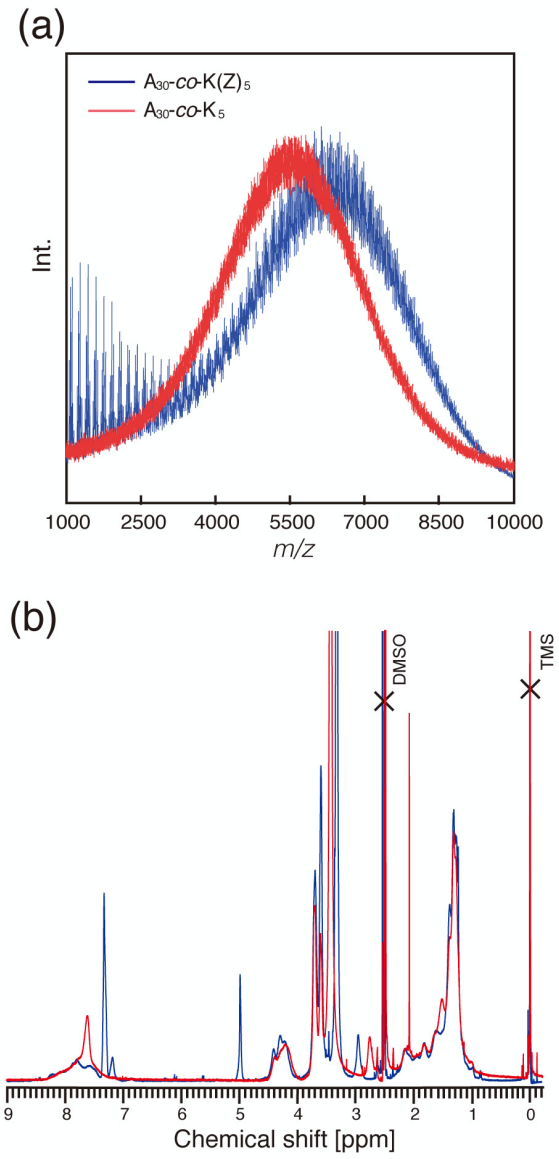

Figure S6. Deprotection of Z groups of A_{30}-*co*-$K(Z)_5$ by treating with 5 % HBr/CH_3COOH. (a) MALDI-TOF MS spectra (matrix: DHBA) and (b) ^{1}H-NMR spectra (in DMSO-d_6 at 25 °C) of A_{30}-*co*-$K(Z)_5$ (blue) and A_{30}-*co*-K_5 (red).

Table S1. Feed composition for preparation of A_{15}-*b*-G_5-*b*-A_{15}.

Step	1	2	3
Monomer	NAAMe	NAGMe	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	42.9 / 0.30	133.5 / 0.85
m_{CTA} (mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	25.0	25.0	25.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S2. Feed composition for preparation of A_{15}-*b*-S_5-*b*-A_{15} .

Step	1	2	3
Monomer	NAAMe	NASMe	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	52.0 / 0.30	133.5 / 0.85
m_{CTA} (mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	25.0	25.0	25.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S3. Feed composition for preparation of A_{15}-*b*-L_5-*b*-A_{15}.

Step	1	2	3
Monomer	NAAMe	NALMe	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	59.8 / 0.30	133.5 / 0.85
m_{CTA} (mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	25.0	25.0	25.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S4. Feed composition for preparation of A_{15}-*b*-F_5-*b*-A_{15}.

Step	1	2	3
Monomer	NAAMe	NAFMe	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	69.7 / 0.30	133.5 / 0.85
m_{CTA}(mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	25.0	25.0	25.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S5. Feed composition for preparation of A_{15}-*b*-V_5-*b*-A_{15}.

Step	1	2	3
Monomer	NAAMe	NAVMe	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	55.3 / 0.30	133.5 / 0.85
m_{CTA}(mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	25.0	25.0	25.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S6. Feed composition for preparation of A_{15}-*b*-$K(Z)_5$-*b*-A_{15}.

Step	1	2	3
Monomer	NAAMe	NAK(Z)Me	NAAMe
DP_{feed}	15	5	15
$m_{monomer}$ (mg) / (mmol)*	157.7 / 1.00	104.2 / 0.30	133.5 / 0.85
m_{CTA}(mg) / (mmol)	16.9 / 0.066	-	-
$m_{initiator}$ (mg) / (mmol)	4.3 / 0.013	4.3/ 0.013	4.3 / 0.013
$V_{per\ step}$ (mL)	1.0	1.0	1.0
% H_2O	20.0	20.0	20.0
V_{total} (mL)	1.0	1.9	2.8

* After the end of each step, 100 µL of the solution was sampled by using a syringe to further measuremenst of SEC and MALDI TOF MS. The amount of CTA removed from the system was taken into account for the calculations of the next step.

Table S7. Feed composition for preparation of A_{30}-*co*-X_5 (X = A, G, S, L).

Sample	A_{35}	A_{30}-*co*-G_5	A_{30}-*co*-S_5	A_{30}-*co*-L_5
$DP_{feed\ (PNAAMe)}$	35	30	30	30
$DP_{feed\ (PNAXMe)}$	-	5	5	5
m_{NAAMe} (mg) / (mmol)	314.2 / 2.00	268.8 / 1.71	268.8 / 1.71	268.8 / 1.71
m_{NAXMe} (mg) / (mmol)	-	40.9 / 0.29	50.2 / 0.29	57.8 / 0.29
m_{CTA} (mg) / (mmol)	14.5 / 0.057	14.5 / 0.057	14.5 / 0.057	14.5 / 0.057
$m_{initiator}$ (mg) / (mmol)	3.7/ 0.011	3.7 / 0.011	3.7 / 0.011	3.7 / 0.011
V_{total} (mL)	2.0	2.0	2.0	2.0
% H_2O	25.0	25.0	25.0	25.0

Table S8. Feed composition for preparation of A_{30}-*co*-X_5 (X = F, V, K(Z)).

Sample	A_{30}-*co*-F_5	A_{30}-*co*-V_5	A_{30}-*co*-$K(Z)_5$
$DP_{feed\ (PNAAMe)}$	30	30	30
$DP_{feed\ (PNAXMe)}$	5	5	5
m_{NAAMe} (mg) / (mmol)	268.8 / 1.71	268.8 / 1.71	268.8 / 1.71
m_{NAXMe} (mg) / (mmol)	66.5/ 0.29	52.8/ 0.29	99.3 / 0.29
m_{CTA} (mg) / (mmol)	14.5 / 0.057	14.5 / 0.057	14.5 / 0.057
$m_{initiator}$ (mg) / (mmol)	3.7/ 0.011	3.7 / 0.011	3.7 / 0.011
V_{total} (mL)	2.0	2.0	2.0
% H_2O	25.0	25.0	20.0