

Development of a robust supramolecular method to prepare well-defined nanofibrils from conjugated molecules

Electronic Supplementary Information (ESI)

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1. Additional Figures (Figures S1–S4)

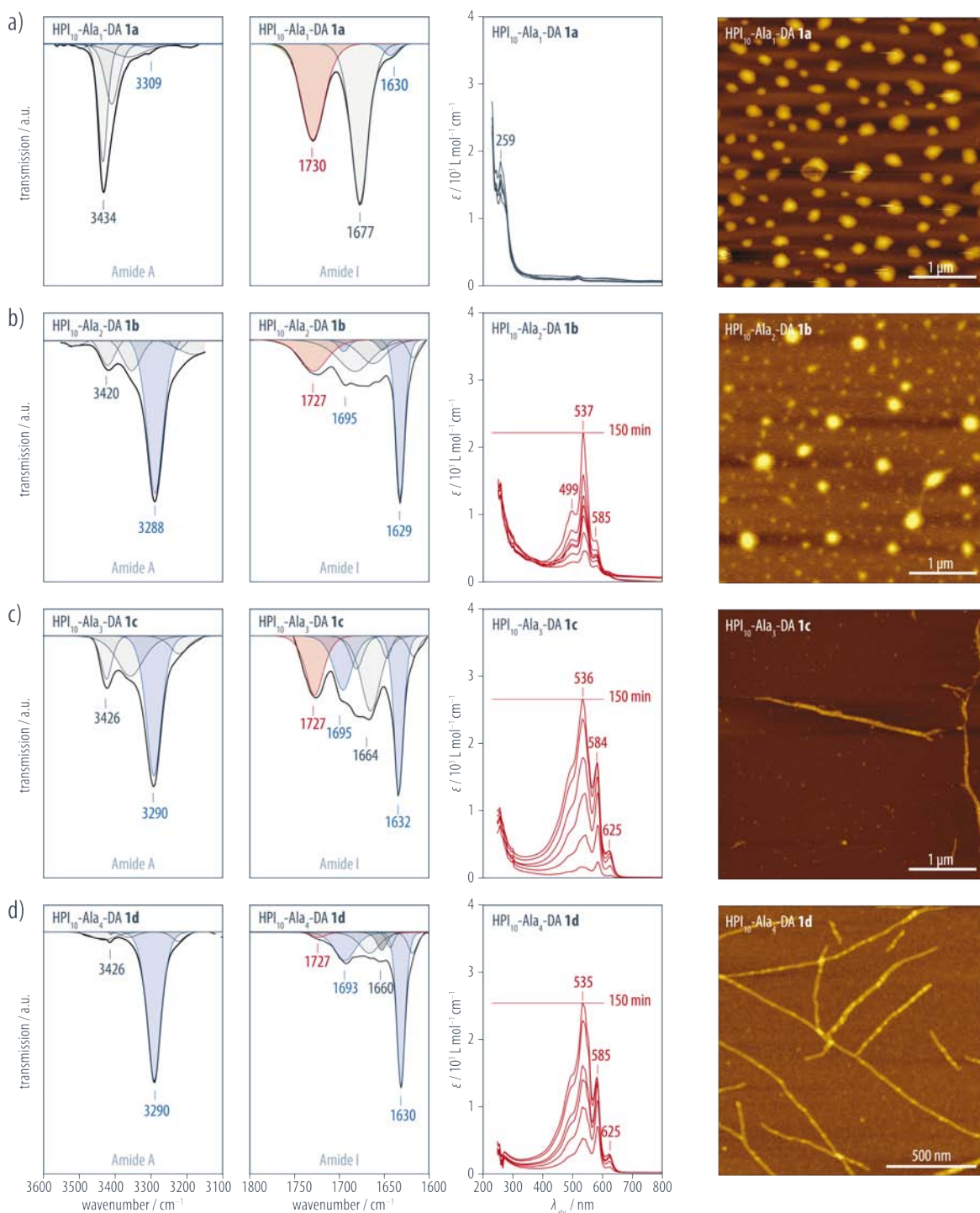


Figure S1. Solution-phase IR spectra (first two columns), time-dependent UV spectra upon UV irradiation (third column), as well as representative AFM images (last column; *z* scale 25 nm for *a*); 15 nm for *b*) and *c*); 10 nm for *d*) of HPI₁₀Ala_n-DA_n (*n* = 1, 2, 3, 4).

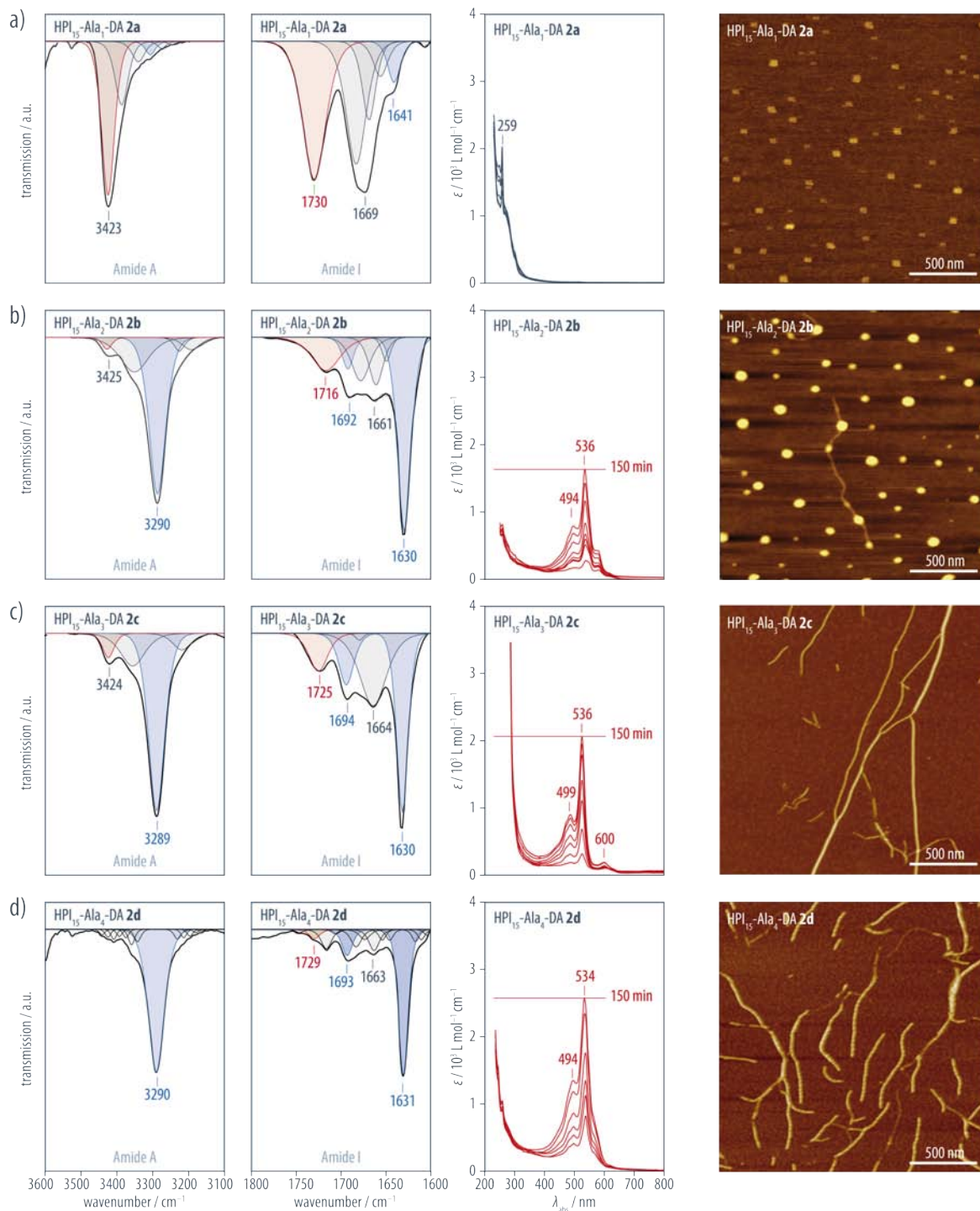


Figure S2. Solution-phase IR spectra (first two columns), time-dependent UV spectra upon UV irradiation (third column), as well as representative AFM images (last column; z scale 8 nm for *a* and *b*); 15 nm for *c* and *d*) of HPI₁₅Ala_n **2a–d** (*n* = 1, 2, 3, 4).

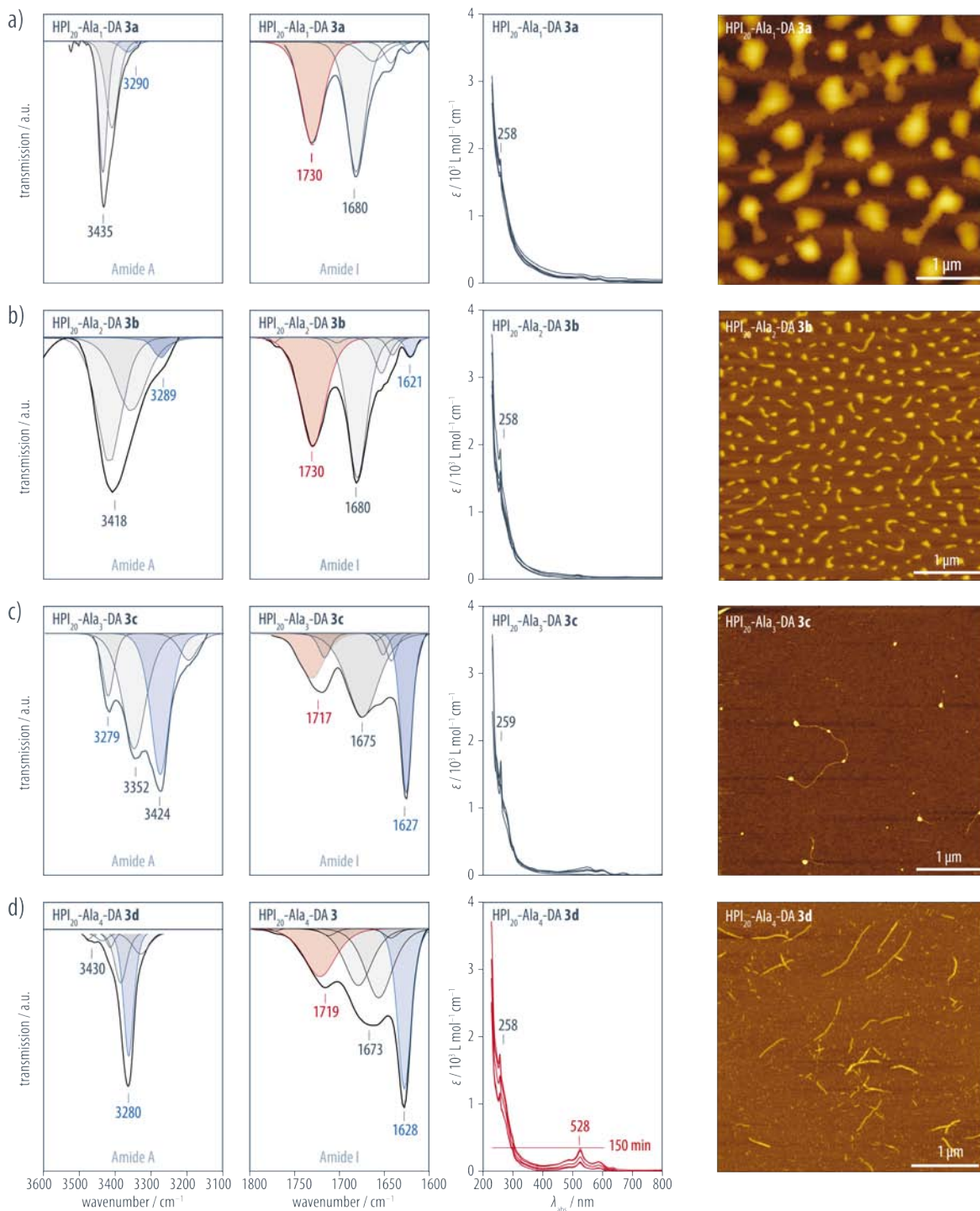


Figure S3. Solution-phase IR spectra (first two columns), time-dependent UV spectra upon UV irradiation (third column), as well as representative AFM images (last column; z scale 18 nm for a); 15 nm for b); 5 nm for c); 8 nm for d)) of HPI₂₀Ala_n-DA_n (n = 1, 2, 3, 4).

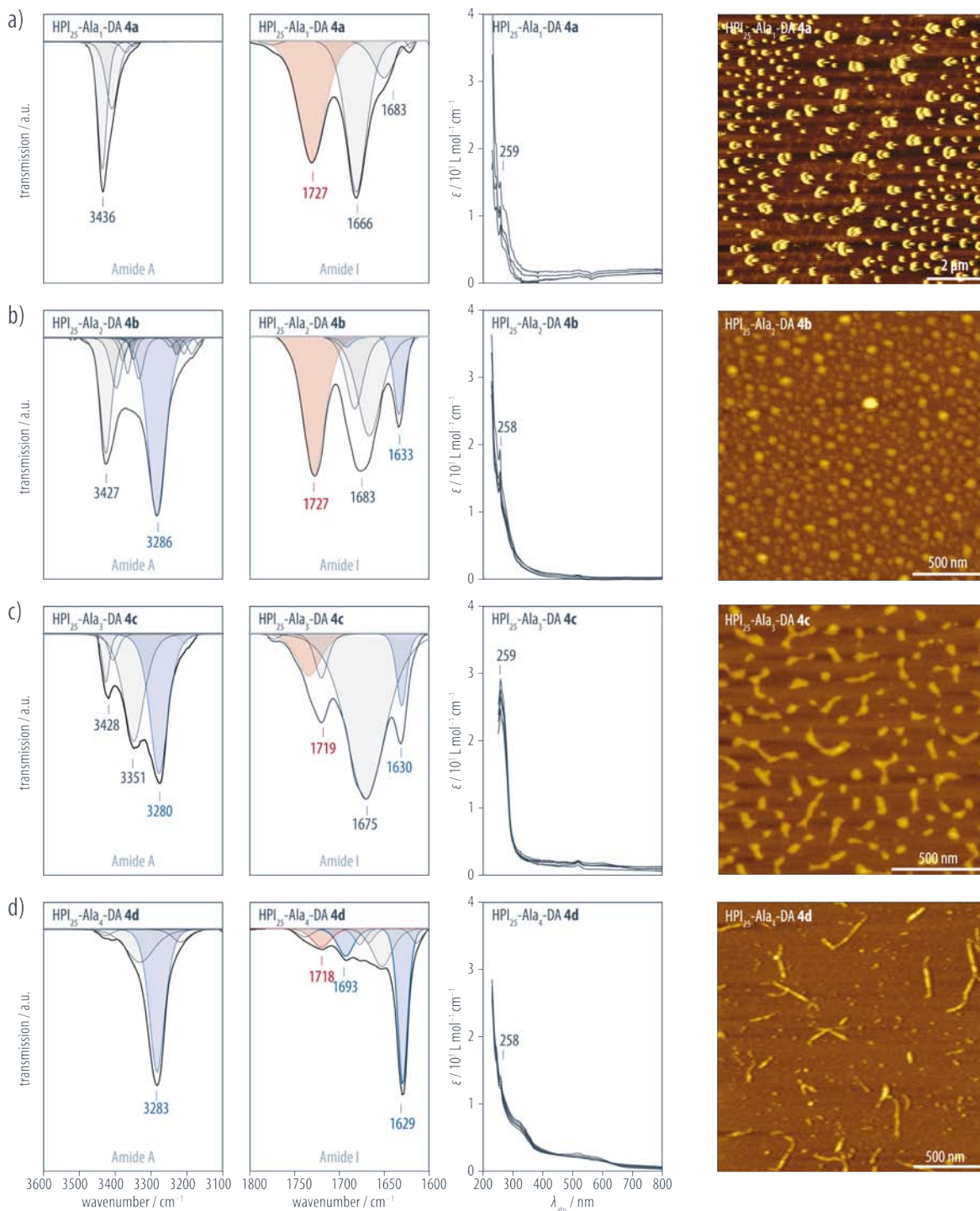
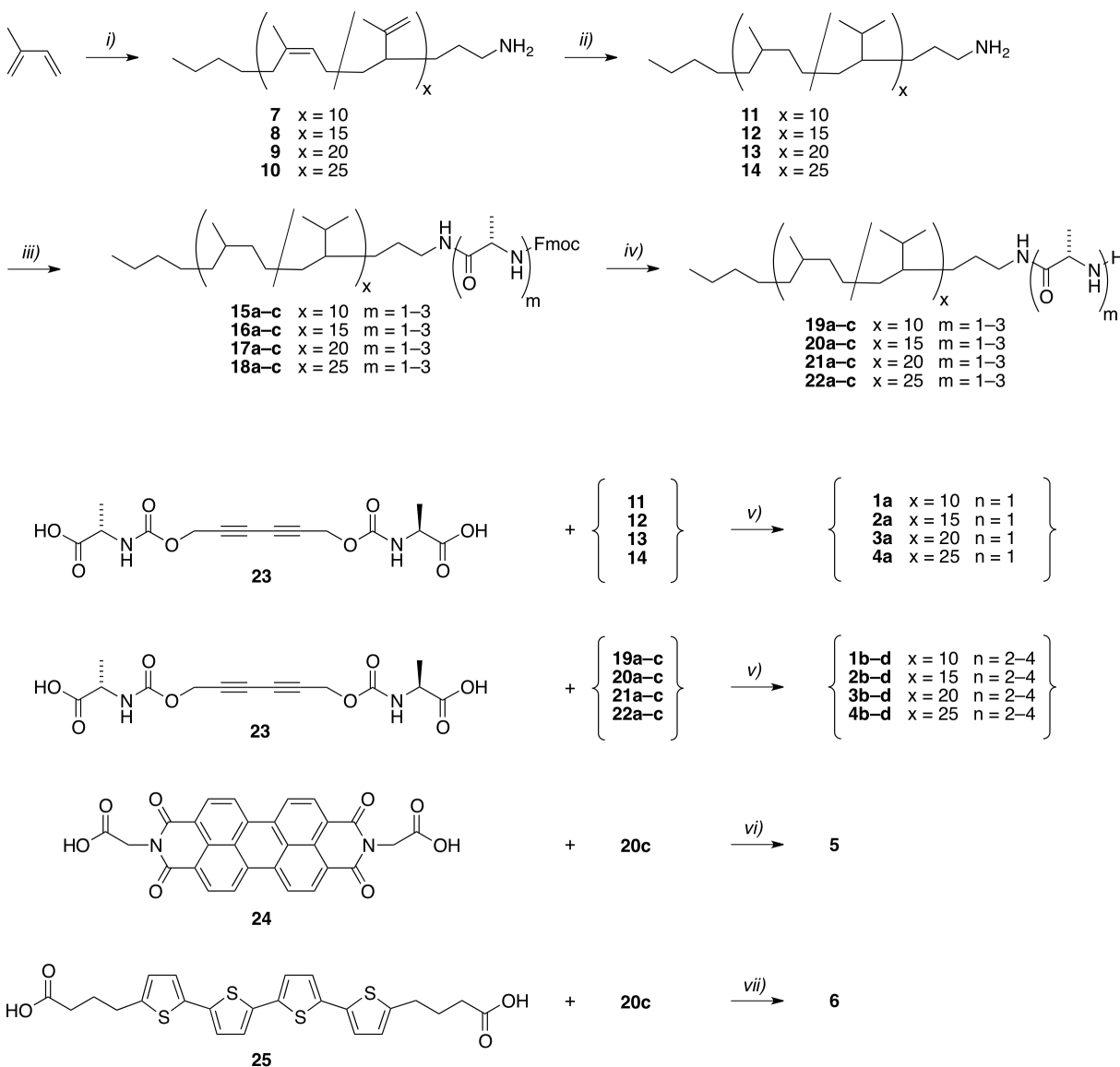


Figure S4. Solution-phase IR spectra (first two columns), time-dependent UV spectra upon UV irradiation (third column), as well as representative AFM images (last column; z scale 45 nm for *a*); 15 nm for *b*) and *c*); 10 nm for *d*) of HPI₂₅Ala_n-DA **4a-d** ($n = 1, 2, 3, 4$).

2. Complete Synthesis and Numbering Scheme (Scheme S1)

Scheme S1. Synthesis of the symmetric diacetylene derivatives HPI_x-Ala_n-DAs **1–4** carrying oligopeptide-polymer substituents with $x = 10, 15, 20, 25$; $n = 1, 2, 3, 4$ (see Table 1), as well as the corresponding perylene bisimide HPI₁₅-Ala₃-PBI **5** and the quaterthiophene HPI₁₅-Ala₃-T_{4 **6**.}



Reaction conditions: *i)* BuLi, THF, $-78^{\circ}\text{C} \rightarrow \text{r.t.}$; then 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane; *ii)* H_2 , 100 bar, Pd/C, toluene, 80°C , 5 d; *iii)* PyBOP, DIEA, THF; *iv)* piperidine, CHCl_3 ; *v)* HO-Ala-C(O)O- CH_2 - $\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{CH}_2$ -OC(O)-Ala-OH **23**, PyBOP, DIEA, THF; *vi)* HOOC- CH_2 -PBI- CH_2 -COOH **24**, DCC, chlorendic hydroxyimide, DMF/THF; *vii)* HOOC(CH_2)₃-($\text{C}_4\text{H}_2\text{S}$)₄-(CH_2)₃COOH **25**, PyBOP, DIEA, THF.

3. Molecular Weight Determination of Products and Intermediates (Tables S1 and S2)

Table S1. Nominal degrees of polymerization x determined from the monomer/initiator ratio in the anionic polymerization yielding **7–10**, number of alanine residues n , as well as number-average molecular weights M_n , number-average degrees of polymerization P_n , and polydispersity indexes PDI (M_w/M_n) of all intermediates, as calculated from ^1H NMR spectroscopy and MALDI mass spectrometry.

Compound	x	n	^1H NMR		Mass Spectrometry		
			M_n	P_n	M_n	P_n	PDI
7	10	n. a.	820	10	820	10	1.050
8	15	n. a.	1200	16	1000	13	1.030
9	20	n. a.	1300	18	1400	21	1.027
10	25	n. a.	1800	25	1570	23	1.021
11	10	n. a.	880	11	860	10	1.019
12	15	n. a.	1200	16	960	12	1.020
13	20	n. a.	1400	19	1340	17	1.022
14	25	n. a.	1900	26	1980	26	1.010
15a	10	1	1200	12	1140	10	1.020
15b	10	2	1300	12	1210	10	1.020
15c	10	3	1400	12	1280	10	1.010
16a	15	1	1500	16	1280	12	1.010
16b	15	2	1600	15	1420	13	1.030
16c	15	3	1700	16	1480	13	1.010
17a	20	1	1700	18	1610	17	1.020
17b	20	2	1800	18	1660	17	1.011
17c	20	3	1900	20	1730	17	1.009
18a	25	1	2200	26	2320	27	1.011
18b	25	2	2400	28	2400	27	1.011
18c	25	3	2400	27	2480	28	1.011
19a	10	1	940	11	960	11	1.033
19b	10	2	1100	12	1070	12	1.040
19c	10	3	1200	13	1080	10	1.016
20a	15	1	1300	16	1290	16	1.024
20b	15	2	1300	14	1250	14	1.020
20c	15	3	1400	15	1460	16	1.020
21a	20	1	1500	19	1610	17	1.020
21b	20	2	1700	21	1440	17	1.014
21c	20	3	1700	20	1600	18	1.013
22a	25	1	2100	28	2110	28	1.018
22b	25	2	2300	29	2200	28	1.009
22c	25	3	2200	27	2250	27	1.014

Table S2. Nominal degrees of polymerization x determined from the monomer/initiator ratio in the anionic polymerization yielding **7–10**, number of alanine residues n , as well as number-average molecular weights M_n , number-average degrees of polymerization P_n , and polydispersity indexes PDI (M_w/M_n) of all diacetylene derivatives HPI $_x$ Ala $_n$ DA **1–4**, the perylene bisimide HPI $_{15}$ Ala $_3$ PBI **5**, and the quaterthiophene HPI $_{15}$ Ala $_3$ T $_4$ **6**, as calculated from ^1H NMR spectroscopy and MALDI mass spectrometry.

Compound	x	n	^1H NMR		Mass Spectrometry		
			M_n	P_n	M_n	P_n	PDI
1a	10	1	2000	22 ^{a)}	1950	20 ^{a)}	1.010
1b	10	2	2100	21 ^{a)}	2250	22 ^{a)}	1.010
1c	10	3	2600	26 ^{a)}	2580	25 ^{a)}	1.010
1d	10	4	2500	22 ^{a)}	2720	23 ^{a)}	1.001
2a	15	1	2600	29 ^{a)}	2280	25 ^{a)}	1.018
2b	15	2	3000	33 ^{a)}	2900	31 ^{a)}	1.006
2c	15	3	3300	31 ^{a)}	2900	29 ^{a)}	1.005
2d	15	4	3000	30 ^{a)}	3040	30 ^{a)}	1.004
3a	20	1	4000	43 ^{a)}	3340	40 ^{a)}	1.009
3b	20	2	3400	34 ^{a)}	3030	33 ^{a)}	1.005
3c	20	3	3500	38 ^{a)}	3320	36 ^{a)}	1.005
3d	20	4	3700	40 ^{a)}	3520	37 ^{a)}	1.007
4a	25	1	4200	53 ^{a)}	4300	54 ^{a)}	1.009
4b	25	2	4300	52 ^{a)}	4400	53 ^{a)}	1.006
4c	25	3	4600	54 ^{a)}	4500	52 ^{a)}	1.003
4d	25	4	5200	60 ^{a)}	5020	57 ^{a)}	1.001
5	15	3	n. d. ^{b)}	–	2700	23	1.030
6	15	3	3400	30 ^{a)}	2800	24 ^{a)}	1.012

a) The degrees of polymerization P_n given for compounds **1–6** are calculated for the two terminally attached polymer chains together and, hence, correspond to $2x$; *b)* Due to strong aggregation in solution, the signals of the protons of both the oligopeptide segments and the perylene core in the ^1H NMR were broad and unspecific, even at high temperatures or upon the addition of hydrogen-bond-breaking agents such as trifluoroacetic acid (TFA) to the NMR samples.

4. Experimental Part

4.1 Instrumentation, Materials, and Methods

Spectroscopy. NMR spectroscopy was carried out on either a Bruker Avance 300 spectrometer or a Bruker Avance 500 spectrometer, operating at frequencies of 300.23 MHz and 500.134 MHz for ^1H nuclei, respectively, as well as 75.49 MHz and 125.04 MHz for ^{13}C nuclei, respectively. IR spectra were recorded on a Perkin-Elmer Spectrum One IR spectrometer using a solution phase cuvette with CaF_2 windows. UV/vis spectra were recorded on a Perkin-Elmer UV-20 UV spectrometer.

AFM Imaging. Solutions of the derivatives in DCM were prepared as described above and then diluted to a concentration of about 0.005 g/L and then spin-coated at 3000 rpm onto silicon substrates that had been pretreated with ethanol and ultrapure water. The obtained samples were then analyzed in tapping mode using a Nanoscope IIIa (Veeco Instruments Inc., Santa Barbara, USA) instrument at room temperature in air. Cantilevers with a resonance frequency on average of $f_0 = 315$ kHz and $k = 14$ N/m were used. Scan rates between 0.5 and 2 Hz were applied, the image resolution was 512×512 pixels. For the AFM images on Au substrates, solutions of the diacetylene derivatives in tetrachloroethane were prepared as described above, diluted to 0.005 g/L, and then spin-coated onto Au-coated Si-wafers. For a deposition on Si wafers covered with a native oxide layer, a DCM solution with a concentration of 0.05 g/L was used instead. All samples were imaged under ambient conditions within a few days after deposition, using a MultiMode™ AFM (Veeco Instruments Inc., Santa Barbara, USA) with a Nanoscope IV controller and silicon cantilevers (Pointprobe® NCH from NanoWorld AG, Neuchâtel, Switzerland), typically with $f_0 = 280$ kHz, and $k = 9.5$ N/m. AFM height images were rendered as three-dimensional surfaces with the corresponding AFM phase image used as surface texture. Fibrils (displayed in color) were manually segmented from the surrounding substrate (displayed in grey scale).

Mass Spectrometry. MALDI-TOF mass spectrometry was performed on an Ultraflex II system (Bruker Daltonics, Germany), with a SmartBeam Laser II. Positive ion mass spectra were acquired using the ToF in reflecting mode with an accelerating voltage of 25.0 kV, reflecting lens voltage 26.3 kV, pulsed ion-extraction of 50 ns, mass range of m/z 500–10000, ion-suppress of 500 Da, laser attenuator of 75%, laser focus offset of 0%, laser range of 20%, value of 2%, laser frequency of 50–100 Hz. The system was externally calibrated in positive mode using peptide calibration mix II (Bruker Daltonics, Germany) in the highly precision polynomial calibration mode. A total of 500–2000 shots were accumulated for each mass spectrum (15 different regions on the same sample spot). For comparison the results were also calculated to the experimentally obtained data using the *Flex Analysis* software (Bruker Daltonics, Germany). Advanced calculations of average molecular weights M_n and M_w , as well as polydispersity indexes were calculated using the *PolyTools* software (Bruker Daltonics, Germany).

Sample Preparation for MALDI-TOF with DCTB as the Matrix. A solution of *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB; Aldrich, >99%) was prepared in dichloromethane (Aldrich, >99%) at a concentration of 40 g/L. Sodium acetate (Aldrich, >98%) at typical concentrations of 5 g/L in methanol (Aldrich, >99%) was used as the ionization agent in order to produce more stable sodium pseudo-molecular ions $[M+Na]^+$. The sample compound was dissolved in dichloromethane or chloroform (Aldrich, >99%) at a concentration of approximately 1 g/L. For the MALDI-TOF experiment, the matrix, the sample compound, and the salt solution were mixed in a ratio of 10:1:1. The solution (1 μ l) was pre-spotted onto the ground steel MALDI target and dried at room temperature.

Sample Preparation for MALDI-TOF with DHB as the Matrix for Compounds 6, 7, 9, 10, and 12. A saturated solution of 2,5-dihydroxybenzoic acid (DHB; Fluka, >99%) was prepared in a mixture of acetonitrile/ethanol/water 50:45:5 (Aldrich, >99%). The solution (1 μ l) was pre-spotted onto the MTP 384 ground steel MALDI target and dried at room temperature. The sample compound was dissolved in dichloromethane or chloroform (Aldrich, >99%) at a concentration of approximately 1 g/L. The obtained solution (1 μ l) was then deposited on the pre-spotted DHB target and dried at room temperature.

Preparation of Sample Solutions for IR Spectroscopy, UV Polymerization, and AFM Imaging. The oligopeptide-polymer derivatives were dissolved in DCM (5 g/L). The solutions were thoroughly degassed in three freeze-pump-thaw cycles. The degassed solutions were ultrasonicated for 1 h at 60°C, then for another 1 h without heating, and finally left to slowly cooling to room temperature overnight.

UV Polymerization. The sample solutions were prepared as described above and then transferred into a thermostated Schlenk flask purged with nitrogen and equipped with a septum. UV Polymerizations were then performed at 25°C using a 250 W Ga-doped high-pressure Hg lamp from UV-Light Technology, Birmingham, UK placed 20 cm away from the reaction flask.

Materials. Chromatography solvents were purchased as reagent grade and distilled once prior to use. THF, acetonitrile and dichloromethane used for synthetic operations were purchased as HPLC grade and dried using a solvent purification system from Innovative Technologies. All reagents were commercially obtained and used without further purification. Hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23**, 3,4,9,10-perylenetetracarboxylimide-*N,N'*-bis(methylcarboxylic acid) **24**, quaterthiophene-2,5'''-bis(propylcarboxylic acid) **25**, and chlorendic acid hydroxylimide were prepared as will be reported elsewhere.

4.2 General Synthesis Procedures

General Procedure for Peptide Coupling (Procedure A). The carboxylic acid derivative and the amine (1 equiv) were dissolved in dry THF, and then an excess of *N*-ethyl-diisopropylamine (DIEA) and 1.05 equivalents of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) were added. The reaction mixture was stirred for 4 h at room temperature. The crude product was typically purified by precipitation in water (see General Procedure E).

General Procedure for the Anionic Polymerization of Isoprene (Procedure B). Isoprene was freshly distilled from CaH₂ prior to use. A pre-dried Schlenk flask was filled with THF, and then *n*-butyl lithium was slowly added at -78°C. Isoprene was added to the mixture as fast as possible, leading to a color change from yellow to orange. The temperature of the mixture was adjusted to 0°C. After its color had turned to yellow again, 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane was transferred to the flask at -78°C. After stirring the reaction mixture for 2 h at room temperature, an aqueous solution of HCl was added. After stirring overnight, the mixture was diluted with CHCl₃, washed twice with KOH and once with saturated aqueous NaCl solution, and then dried over MgSO₄. The crude product was purified by column chromatography.

General Procedure for Hydrogenation of Poly(isoprene) (Procedure C). The amine-terminated poly(isoprene) was dissolved in toluene, and Pd/C was added. The mixture was then transferred to a high-pressure autoclave and stirred at a hydrogen pressure of 100 bar at 80°C for 7 d. The amine-terminated hydrogenated poly(isoprene) was obtained after filtration and removal of the solvent.

General Procedure for Fmoc Deprotection (Procedure D). The Fmoc-protected amine derivatives were dissolved in CHCl₃. Then, a large excess of piperidine was added, and the solution was stirred over night. The reaction was monitored by thin layer chromatography. After completion of the reaction, the solvents were removed in vacuum. The crude product was purified by column chromatography.

General Procedure for the Precipitation of Polymer-Substituted Products (Procedure E). After completion of the reaction affording the desired compound, the reaction mixture was concentrated to half of its original volume. A large excess of a mixture of aqueous 1 M HCl and ice-cold deionized water was added, which led to a precipitation of the compound. The filtered precipitate was then re-dissolved in THF and precipitated again, following the same procedure as described above. After three repetitive precipitations, the crude product was finally dissolved in chloroform. The solution was dried over MgSO₄ and concentrated *in vacuo*.

4.3 Synthesis Procedures and Analytical Data for Compounds 1–22

Synthesis of (HPI₁₀-NH-Ala-C(O)O-CH₂-C≡C-)₂ 1a. Following General Procedure A, HPI₁₀-NH₂ **11** (0.20 g, 0.25 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (0.04 g, 0.12 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.13 g, 0.25 mmol, 2.1 equiv) and DIEA (0.06 g, 0.48 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.20 g, 86%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 256H, aliphatic *H*), 3.2 (m, 4 h, CH₂NHR), 4.2 (m, 2H, CHCH₃), 4.7 (6H, CH₂CO, residual olefin *H*), 5.6 (s, 1H, NH), 6.0 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₁₂₈H₂₄₆N₄O₆Na ([M+Na]⁺) 1958.50; found 1958.69.

Synthesis of (HPI₁₀-NH-Ala₂-C(O)O-CH₂-C≡C-)₂ 1b. Following General Procedure A, HPI₁₀-Ala-H **19a** (200.0 mg, 0.23 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (37.4 mg, 0.11 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (120.1 mg, 0.23 mmol, 2.1 equiv) and DIEA (56.8 mg, 0.44 mmol, 4eq) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (181.5 mg, 87%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 247H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.7 (m, 10H, CHCH₃, CH₂CO) ppm. MS (MALDI): calcd mass for C₁₃₄H₂₅₆N₆O₈Na ([M+Na]⁺) 2100.97; found 2100.89.

Synthesis of (HPI₁₀-NH-Ala₃-C(O)O-CH₂-C≡C-)₂ 1c. Following General Procedure A, HPI₁₀-Ala₂-H **19b** (200 mg, 0.21 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (34.6 mg, 0.10 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (111.2 mg, 0.21 mmol, 2.1 equiv) and DIEA (52.6 mg, 0.41 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (178.2 mg, 86%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 309H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.7 (m, 12H, CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₁₄₀H₂₆₆N₈O₁₀Na ([M+Na]⁺) 2244.05; found 2244.22.

Synthesis of (HPI₁₀-NH-Ala₄-C(O)O-CH₂-C≡C-)₂ 1d. Following General Procedure A, HPI₁₀-Ala₃-H **19c** (200.0 mg, 0.19 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (32.2 mg, 0.09 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (103.5 mg, 199.0 mmol, 2.1 equiv) and DIEA (49.0 mg, 0.38 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (150.0 mg, 75%).

^1H NMR (300 MHz, CDCl_3): δ = 0.8–1.7 (m, 267H, aliphatic *H*), 3.2 (m, 4H, CH_2NHR), 4.2–4.7 (m, 14H, CHCH_3 , CH_2CO , residual olefin *H*) ppm. MS (MALDI): calcd mass for $\text{C}_{146}\text{H}_{276}\text{N}_{10}\text{O}_{12}\text{Na}$ ($[\text{M}+\text{Na}]^+$) 2387.13; found 2387.92.

Synthesis of $(\text{HPI}_{15}\text{-NH-Ala-C(O)O-CH}_2\text{-C}\equiv\text{C-})_2$ **2a.** Following General Procedure A, $\text{HPI}_{15}\text{-NH}_2$ **12** (0.20 g, 0.19 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (0.03 g, 0.09 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.10 g, 0.20 mmol, 2.1 equiv) and DIEA (0.05 g, 0.38 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.18 g, 80%). ^1H NMR (300 MHz, CDCl_3): δ = 0.8–1.8 (m, 324H, aliphatic *H*), 3.2 (m, 4H, CH_2NHR), 4.1 (m, 2H, CHCH_3), 4.7 (6H, CH_2CO , residual olefin *H*), 5.4 (s, 2H, *NH*), 5.8 (s, 2H, *NH*) ppm. MS (MALDI): calcd mass for $\text{C}_{178}\text{H}_{346}\text{N}_4\text{O}_6\text{Na}$ ($[\text{M}+\text{Na}]^+$) 2659.67; found 2659.09.

Synthesis of $(\text{HPI}_{15}\text{-NH-Ala}_2\text{-C(O)O-CH}_2\text{-C}\equiv\text{C-})_2$ **2b.** Following General Procedure A, $\text{HPI}_{15}\text{-Ala-H}$ **20a** (200.0 mg, 0.18 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (30.2 mg, 0.09 mmol, 1 equiv) were dissolved in dry THF (15 mL), and then PyBOP (97.1 mg, 0.19 mmol, 2.1 equiv) and DIEA (45.9 mg, 0.36 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (162.3 mg, 81%). ^1H NMR (300 MHz, CDCl_3): δ = 0.8–1.8 (m, 376H, aliphatic *H*), 3.2 (m, 4H, CH_2NHR), 4.1 (m, 10H, CHCH_3 , CHCH_3 , CH_2CO , residual olefin *H*) ppm. MS (MALDI): calcd mass for $\text{C}_{184}\text{H}_{356}\text{N}_6\text{O}_8\text{Na}$ ($[\text{M}+\text{Na}]^+(\text{A}+1)$) 2802.76; found 2802.97.

Synthesis of $(\text{HPI}_{15}\text{-NH-Ala}_3\text{-C(O)O-CH}_2\text{-C}\equiv\text{C-})_2$ **2c.** Following General Procedure A, $\text{HPI}_{15}\text{-Ala}_2\text{-H}$ **20b** (200.0 mg, 0.17 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (28.4 mg, 0.08 mmol, 1 equiv) were dissolved in dry THF (15 mL), and then PyBOP (91.2 mg, 0.18 mmol, 2.1 equiv) and DIEA (43.1 mg, 0.34 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (155.2 mg, 78%). ^1H NMR (300 MHz, CDCl_3): δ = 0.8–1.8 (m, 339H, aliphatic *H*), 3.2 (m, 4H, CH_2NHR), 4.1 (m, 2H, CHCH_3), 4.4–4.7 (11H, CHCH_3 , CH_2CO , residual olefin *H*), 5.8 (s, 2H, *NH*), 6.8 (s, 2H, *NH*), 7.1 (s, 2H, *NH*) ppm. MS (MALDI): calcd mass for $\text{C}_{190}\text{H}_{366}\text{N}_8\text{O}_{10}\text{Na}$ ($[\text{M}+\text{Na}]^+$) 2943.80; found 2943.39.

Synthesis of $(\text{HPI}_{15}\text{-NH-Ala}_4\text{-C(O)O-CH}_2\text{-C}\equiv\text{C-})_2$ **2d.** Following General Procedure A, $\text{HPI}_{15}\text{-Ala}_3\text{-H}$ **20c** (200.0 mg, 0.16 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (26.8 mg, 0.08 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (86.0 mg, 0.17 mmol, 2.1 equiv) and

DIEA (40.7 mg, 0.31 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (151.0 mg, 78%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.8 (m, 335H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.1–4.7 (14H, CHCH₃, CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₁₉₆H₃₇₉N₁₀O₁₃ ([M+H]⁺) 3083.97; found 3083.83.

Synthesis of (HPI₂₀-NH-Ala-C(O)O-CH₂-C≡C-)₂ 3a. Following General Procedure A, HPI₂₀-NH₂ **13** (0.20 g, 0.15 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (24.1 mg, 0.07 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (77.4 mg, 0.15 mmol, 2.1 equiv) and DIEA (36.6 mg, 0.28 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.18 g, 82%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 464H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2 (m, 2H, CHCH₃), 4.8 (6H, CH₂CO, residual olefin *H*), 5.3 (s, 1H, NH), 5.8 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₂₂₈H₄₄₆N₄O₆K ([M+K]⁺) 3376.43; found 3376.52.

Synthesis of (HPI₂₀-NH-Ala₂-C(O)O-CH₂-C≡C-)₂ 3b. Following General Procedure A, HPI₂₀-Ala-H **21a** (200.0 mg, 0.14 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (22.9 mg, 0.07 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (73.6 mg, 0.14 mmol, 2.1 equiv) and DIEA (34.8 mg, 0.27 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (158.2 mg, 84%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.9 (m, 374H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.8 (m, 10H, CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₂₃₄H₄₅₆N₆O₈K ([M+K]⁺ (A+3)) 3520.52; found 3520.69.

Synthesis of (HPI₂₀-NH-Ala₃-C(O)O-CH₂-C≡C-)₂ 3c. Following General Procedure A, HPI₂₀-Ala₂-H **21b** (200.0 mg, 0.13 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (21.8 mg, 64.2 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (70.2 mg, 0.13 mmol, 2.1 equiv) and DIEA (33.2 mg, 0.26 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (156.4 mg, 83%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 426H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.8 (m, 12H, CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₂₄₀H₄₆₆N₈O₁₀K ([M+K]⁺) 3660.58; found 3660.69.

Synthesis of (HPI₂₀-NH-Ala₄-C(O)O-CH₂-C≡C-)₂ 3d. Following General Procedure A, HPI₂₀-Ala₃-H **21c** (200.0 mg, 0.13 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (20.9 mg, 0.61 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (67.02 mg, 0.13 mmol, 2.1 equiv) and DIEA (31.7 mg, 0.25 mmol, 4 equiv) were added. After a reaction time of 1 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (157.0 mg, 82%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 445H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.8 (m, 14H, CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₂₄₆H₄₇₉N₁₀O₁₃ ([M+H]⁺) 3784.72; found 3784.31.

Synthesis of (HPI₂₅-NH-Ala-C(O)O-CH₂-C≡C-)₂ 4a. Following General Procedure A, HPI₂₅-NH₂ **14** (200.0 mg, 0.12 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (19.2 mg, 0.05 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (64.7 mg, 0.12 mmol, 2.1 equiv) and DIEA (30.0 mg, 0.23 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (177.0 mg, 83%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 557H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2 (m, 2H, CHCH₃), 4.8 (4H, CH₂CO), 5.4 (s, 1H, NH), 5.9 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₂₇₈H₅₄₆N₄O₆Na ([M+Na]⁺) 4061.24; found 4061.03.

Synthesis of (HPI₂₅-NH-Ala₂-C(O)O-CH₂-C≡C-)₂ 4b. Following General Procedure A, HPI₂₅-Ala-H **22a** (200.0 mg, 0.11 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (18.5 mg, 0.05 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (59.3 mg, 0.12 mmol, 2.1 equiv) and DIEA (28.3 mg, 0.22 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (151.0 mg, 82%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 560H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.8 (m, 8H, CHCH₃, CH₂CO), 5.4 (s, 1H, NH), 5.9 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₂₈₄H₅₅₆N₆O₈Na ([M+Na]⁺) 4204.32; found 4204.55.

Synthesis of (HPI₂₅-NH-Ala₃-C(O)O-CH₂-C≡C-)₂ 4c. Following General Procedure A, HPI₂₅-Ala₂-H **22b** (200.0 mg, 0.11 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (17.8 mg, 0.05 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (57.0 mg, 0.11 mmol, 2.1 equiv) and DIEA (27.0 mg, 0.21 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (146.1 mg, 80%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.8 (m, 541H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.1–4.7 (10H, CHCH₃,

CHCH₃, CH₂CO, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₂₉₀H₅₆₉N₈O₁₁ ([M+H]⁺) 4341.42; found 4341.02.

Synthesis of (HPI₂₅-NH-Ala₄-C(O)O-CH₂-C≡C-)₂ 4d. Following General Procedure A, HPI₂₅-Ala₃-H **22c** (200.0 mg, 0.10 mmol, 2.05 equiv) and hexa-2,4-diynylene-1,6-bis(oxycarbonyl-L-alanine) **23** (17.1 mg, 50.3 mmol, 1 equiv) were dissolved in dry THF (100 mL), and then PyBOP (54.91 mg, 0.11 mmol, 2.1 equiv) and DIEA (26.0 mg, 0.20 mmol, 4 equiv) were added. After a reaction time of 1 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (144.0 mg, 80%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 563H, aliphatic *H*), 3.2 (m, 4H, CH₂NHR), 4.2–4.8 (m, 12H, CHCH₃, CH₂CO) ppm. MS (MALDI): calcd mass for C₂₉₆H₅₇₆N₁₀O₁₂Na ([M+Na]⁺) 4487.47; found 4488.77.

Synthesis of HPI₁₅-NH-Ala₃-C(O)-CH₂-PBI-CH₂-C(O)-Ala₃-NH-HPI₁₅ 5. Dicyclohexylcarbodiimide (DCC; 17 mg, 79 μmol, 2 equiv) was added into a 10 mL Schlenk tube that was subsequently evacuated and flushed with argon. Dry DMF (3 mL) was then added, followed by HOOC-CH₂-PBI-CH₂-COOH **24** (20 mg, 39 μmol, 1 equiv) and chlorendic hydroxyimide (31 mg, 79 μmol, 2 equiv). The resulting suspension was stirred at 50°C for 18 h which resulted in a dark red solution. The DMF was evaporated, and the residue was redissolved in THF (10 mL) and subsequently added to a solution of HIP₁₅-Ala₃-H **18c** (115 mg, 87 μmol, 2.2 equiv) in THF (30 mL). The solution immediately turned dark red and was stirred for 1 h at room temperature. The reaction mixture was then precipitated into 1 M aqueous HCl, the precipitate was redissolved in CHCl₃ and the resulting solution was precipitated into MeOH. The precipitation into MeOH was repeated two more times. The product was obtained as an amorphous black solid (83 mg, 71%). MS (MALDI) calcd mass for C₂₁₀H₃₇₄N₁₀O₁₂Na ([M+Na]⁺ (A+1)) 3252.89; found 3252.63.

Synthesis of HPI₁₅-NH-Ala₃-C(O)-(CH₂)₃-(C₄H₂S)₄-(CH₂)₃-C(O)-Ala₃-HPI₁₅ 6. Following General Procedure A, HPI₁₅-Ala₃-H (0.5 g, 0.37 mmol, 2.05 equiv) and quaterthiophene-2,5''-bis(propylcarboxylic acid) **25** (91.1 mg, 0.18 mmol, 1 equiv) were dissolved in dry THF (300 mL), and then DIEA (0.18 g, 1.43 mmol, 8 equiv) and PyBOP (0.21 g, 0.45 mmol, 2.5 equiv) were added. After a reaction time of 1 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl and redissolved in THF. The precipitation was repeated two more times. The final product was obtained as a yellow amorphous solid (0.43 g, 85%). ¹H NMR (400 MHz, CDCl₂CDCl₂): δ = 0.9–1.5 (m, 374H, aliphatic *H*), 1.9 (t 4H, 2 CH₂), 2.1 (t, 4H, 2 C(O)CH₂), 2.9 (t, 4H, 2 CH₂), 3.3 (m, 4H, CH₂NHR), 4.4 (m, 6H, CH), 5.8 (m, 4H, NH), 6.4 (d, 2H, aromatic *H*), 6.5 (d, 2H, aromatic *H*), 6.8 (d, 2H, NH), 7.1 (m, 4H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₂₁₂H₃₉₂N₁₀O₁₀S₄Na ([M+Na]⁺) 3389.92; found 3389.90.

Synthesis of PI₁₀-NH₂ 7. Following General Procedure B, a pre-dried Schlenk flask was filled with THF (40 mL). Then, *n*-butyl lithium (16.15 mmol, 10.09 mL, 1.0 equiv) was slowly added into the Schlenk flask at

-78°C . Freshly distilled isoprene (11.00 g, 161.49 mmol, 10 equiv) was added to the system as fast as possible, leading to a color change from yellow to orange. The temperature of the mixture was adjusted to 0°C . After its color had turned to yellow again, 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane (4.98 g, 17.76 mmol, 1.1 equiv) was transferred to the flask at -78°C . After 2 h of stirring at room temperature, 1 M HCl (2 mL) was added. After continued stirring overnight, the mixture was diluted with CHCl_3 (100 mL). The solution was washed twice with KOH (4 M, 50 mL), and once with saturated aqueous NaCl solution, and dried over MgSO_4 . The crude product was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ 15:1 v/v). The product was obtained as a slightly yellow oil (10.83 g, 84%). ^1H NMR (300 MHz, CDCl_3): $\delta = 0.7\text{--}2.0$ (m, 67H, aliphatic *H*), 2.7 (m, 2H, CH_2NH_2), 4.6–5.0 (m, 15H, olefin *H*), 5.7 (m, 2H, olefin *H*) ppm. MS (MALDI): calcd mass for $\text{C}_{57}\text{H}_{98}\text{N}$ ($[\text{M}+\text{H}]^+$) 796.86; found 797.07.

Synthesis of $\text{PI}_{15}\text{-NH}_2$ 8. Following General Procedure B, a pre-dried Schlenk flask was filled with THF (40 mL). Then, *n*-butyl lithium (10.74 mmol, 6.74 mL, 1.0 equiv) was slowly added into the Schlenk flask at -78°C . Freshly distilled isoprene (11.00 g, 161.49 mmol, 15 equiv) was added to the system as fast as possible, leading to a color change from yellow to orange. The temperature of the mixture was adjusted to 0°C . After its color had turned to yellow again, 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane (4.98 g, 17.76 mmol, 1.1 equiv) was transferred to the flask at -78°C . After 2 h of stirring time at room temperature, 1 M aqueous HCl (2 mL) was added. After continued stirring overnight, the mixture was diluted with CHCl_3 (100 mL). The solution was washed twice with 4 M KOH, and once with saturated aqueous NaCl solution, and dried over MgSO_4 . The crude product was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ 15:1 v/v). The product was obtained as a slightly yellow oil (10.11 g, 94%). ^1H NMR (300 MHz, CDCl_3): $\delta = 0.8\text{--}2.0$ (m, 108H, aliphatic *H*), 2.7 (m, 2H, CH_2NH_2), 4.6–4.9 (m, 28H, olefin *H*), 5.7 (m, 28H, olefin *H*) ppm. MS (MALDI): calcd mass for $\text{C}_{82}\text{H}_{138}\text{N}$ ($[\text{M}+\text{H}]^+$) 1137.08; found 1137.12.

Synthesis of $\text{PI}_{20}\text{-NH}_2$ 9. Following General Procedure B, a pre-dried Schlenk flask was filled with THF (40 mL). Then, *n*-butyl lithium (8.08 mmol, 5.45 mL, 1.0 equiv) was slowly added into the Schlenk flask at -78°C . Freshly distilled isoprene (11.00 g, 161.49 mmol, 20 equiv) was added to the system as fast as possible, leading to a color change from yellow to orange. The temperature of the mixture was adjusted to 0°C . After its color had turned to yellow again, 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane (4.98 g, 17.76 mmol, 1.1 equiv) was transferred to the flask at -78°C . After 2 h of stirring time at room temperature, 1 M aqueous HCl (2 mL) was added. After continued stirring overnight, the mixture was diluted with CHCl_3 (100 mL). The solution was washed twice with KOH (4 M, 50 mL) solution, and once with saturated aqueous NaCl solution, and dried over MgSO_4 . The crude product was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ 15:1 v/v). The product was obtained as a slightly yellow oil (10.02 g, 92%). ^1H NMR (300 MHz, CDCl_3): $\delta = 0.8\text{--}2.3$ (m, 120H, aliphatic *H*), 2.8 (m, 2H, CH_2NH_2), 4.6–4.9 (m, 31H, olefin *H*), 5.7 (m, 3H, olefin *H*) ppm. MS (MALDI): calcd mass for $\text{C}_{107}\text{H}_{178}\text{N}$ ($[\text{M}+\text{H}]^+$) 1477.40; found 1477.49.

Synthesis of PI₂₅-NH₂ 10. Following General Procedure B, a pre-dried Schlenk flask was filled with THF (50 mL), and then *n*-butyl lithium (6.45 mmol, 4.04 mL, 1.0 equiv) was slowly added into the Schlenk flask at -78°C. Freshly distilled isoprene (11.00 g, 161.49 mmol, 25 equiv) was added to the system as fast as possible, leading to a color change from yellow to orange. The temperature of the mixture was adjusted to 0°C. After its color had turned to yellow again, 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane (4.98 g, 17.76 mmol, 1.1 equiv) was transferred to the flask. After 2 h of stirring time at room temperature, 1 M aqueous HCl (2 mL) was added. After continued stirring overnight, the mixture was diluted with CHCl₃ (100 mL). The solution was washed twice with KOH (4 M, 50 mL) solution, and once with saturated aqueous NaCl solution, and dried over MgSO₄. The crude product was purified by silica gel column chromatography (CH₂Cl₂ → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a slightly yellow oil (10.25 g, 87%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.9 (m, 164H, aliphatic *H*), 2.7 (m, 2H, CH₂NH₂), 4.6–4.9 (m, 50H, olefin *H*) ppm. MS (MALDI): calcd mass for C₁₃₂H₂₁₇N: 1816.70 (M⁺); found 1816.38 (the mass spectrum showed additional series which may tentatively be assigned to H₂O and MeOH adducts of **10**).

Synthesis of HPI₁₀-NH₂ 11. Following General Procedure C, PI₁₀-NH₂ **7** (10.69 g, 13.66 mmol) was dissolved in toluene (60 mL), and then Pd/C (4.00 g, 52% H₂O content) was added. The mixture was transferred to a high-pressure autoclave, and then stirred at a H₂ pressure of 100 bar at 80°C for one week. After filtration and removal of the solvent, the amine-terminated hydrogenated poly(isoprene) was obtained as a dark oil (9.52 g, 89%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.4 (m, 123H, aliphatic *H*), 3.2 (m, 2H, CH₂NH₂), 4.7 (m, 1H, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₅₇H₁₁₈N ([M+H]⁺) 816.93; found 816.91.

Synthesis of HPI₁₅-NH₂ 12. Following General Procedure C, PI₁₅-NH₂ **8** (10.06 g, 10.06 mmol) was dissolved in toluene (60 mL), and then Pd/C (4.00 g, 52% H₂O content) was added. The mixture was transferred to a high-pressure autoclave, and then stirred at a H₂ pressure of 100 bar at 80°C for one week. After filtration and removal of the solvent, the amine-terminated hydrogenated poly(isoprene) was obtained as a dark oil (9.3 g, 90%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 169H, aliphatic *H*), 3.2 (m, 2H, CH₂NH₂), 4.7 (m, 1H, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₈₂H₁₆₈N ([M+H]⁺) 1167.31; found 1167.76.

Synthesis of HPI₂₀-NH₂ 13. Following General Procedure C, PI₂₀-NH₂ **9** (9.86 g, 7.35 mmol) was dissolved in toluene (60 mL), and then Pd/C (4.00 g, 52% H₂O content) was added. The mixture was transferred to a high-pressure autoclave, and then stirred at a H₂ pressure of 100 bar at 80°C for one week. After filtration and removal of the solvent, the amine-terminated hydrogenated poly(isoprene) was obtained as a dark oil (9.52 g, 94%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.4 (m, 114H, aliphatic *H*), 3.2 (m, 2H, CH₂NH₂) ppm. MS (MALDI): calcd mass for C₁₀₇H₂₁₇NK ([M+K]⁺(A+1)) 1556.67; found 1556.25.

Synthesis of HPI₂₅-NH₂ 14. Following General Procedure C, PI₂₅-NH₂ **10** (10.12 g, 6.54 mmol) was dissolved in toluene (60 mL), and then Pd/C (4.00 g, 52% H₂O content) was added. The mixture was transferred to a

high-pressure autoclave, and then stirred at a H₂ pressure of 100 bar at 80°C for one week. After filtration and removal of the solvent, the amine-terminated hydrogenated poly(isoprene) was obtained as a dark oil (9.61 g, 85%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.9 (m, 268H, aliphatic *H*), 2.7 (m, 2H, CH₂NH₂), 4.6–4.9 (m, 50H, olefin *H*) ppm. MS (MALDI): calcd mass for C₁₃₂H₂₆₈N ([M+H]⁺) 1868.10; found 1868.14.

Synthesis of HPI₁₀-Ala-Fmoc 15a. Following General Procedure A, HPI₁₀-NH₂ **11** (0.50 g, 0.61 mmol, 1 equiv) and Fmoc-Ala-OH (0.19 g, 0.61 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.35 g, 1.1 equiv, 0.673 mmol) and DIEA (0.32 g, 2.45 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as a slightly yellow oil (0.59 g, 87%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 134H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2 (m, 2H, CHCH₃, fluorenyl *CH*), 4.4 (m, 2H, Fmoc-CO₂CH₂), 4.7 (1H, residual olefin *H*), 5.5 (s, 1H, *NH*), 6.1 (s, 1H, *NH*), 7.3 (m, 2H, aromatic *H*), 7.4 (m, 2H, aromatic *H*), 7.6 (m, 2H, aromatic *H*), 7.8 (m, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₇₅H₁₃₂N₂O₃Na ([M+Na]⁺) 1132.01; found 1131.94.

Synthesis of HPI₁₀-Ala₂-Fmoc 15b. Following General Procedure A, HPI₁₀-NH₂ **11** (0.50 g, 0.61 mmol, 1 equiv) and Fmoc-Ala₂-OH (0.24 g, 0.61 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.40 mg, 0.77 mmol, 1.25 equiv) and DIEA (0.32g, 2.45 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as a sticky solid (0.65 g, 91%). ¹H NMR (500 MHz, CDCl₃): δ = 0.8–1.7 (m, 134H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2–4.4 (m, 5H, 2CHCH₃, fluorenyl *CH*, Fmoc-CO₂CH₂), 4.7 (1H, residual olefin *H*), 5.3 (s, 1H, *NH*), 6.2 (s, 1H, *NH*), 6.6 (s, 1H, *NH*), 7.3 (m, 2H, aromatic *H*), 7.4 (m, 2H, aromatic *H*), 7.6 (m, 2H, aromatic *H*), 7.8 (m, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₇₈H₁₃₇N₃O₄Na ([M+Na]⁺) 1203.05; found 1202.88.

Synthesis of HPI₁₀-Ala₃-Fmoc 15c. Following General Procedure A, HPI₁₀-NH₂ **11** (0.50 g, 0.61 mmol, 1 equiv) and Fmoc-Ala₃-OH (0.28 g, 0.61 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.40 mg, 0.77 mmol, 1.25 equiv) and DIEA (0.32g, 2.45 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.63 g, 82%). ¹H NMR (500 MHz, CDCl₃ and 5% TFA): δ = 0.8–1.7 (m, 137H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2–4.5 (m, 6H, 3CHCH₃, fluorenyl *CH*, Fmoc-CO₂CH₂), 4.7 (1H, residual olefin *H*), 5.6 (s, 1H, *NH*), 6.9 (s, 1H, *NH*), 7.1 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*) 7.4 (t, 2H, aromatic *H*) 7.6 (d, 2H, aromatic *H*) 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₈₁H₁₄₂N₄O₅Na ([M+Na]⁺) 1274.09; found 1274.15.

Synthesis of HPI₁₅-Ala-Fmoc 16a. Following General Procedure A, HPI₁₅-NH₂ **12** (0.56 g, 0.54 mmol, 1 equiv) and Fmoc-Ala-OH (0.17 g, 0.54 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.35 g, 0.68 mmol, 1.25 equiv) and DIEA (0.29 g, 2.18 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as a sticky solid (0.66 g, 91%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 173H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2 (m, 2H, CHCH₃, fluorenyl *CH*), 4.4 (m, 2H, Fmoc-CO₂CH₂), 4.7 (1H, residual olefin *H*), 5.4 (s, 1H, *NH*), 5.9 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (t, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₁₀₀H₁₈₂N₂O₃Na ([M+Na]⁺ (A+2)) 1484.41; found 1484.31.

Synthesis of HPI₁₅-Ala₂-Fmoc 16b. Following General Procedure A, HPI₁₅-NH₂ **12** (0.55 g, 0.54 mmol, 1 equiv) and Fmoc-Ala₂-OH (0.21 g, 0.54 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.35 g, 0.67 mmol, 1.25 equiv) and DIEA (0.28 g, 2.14 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.66 g, 89%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 186H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2 (m, 2H, CHCH₃, fluorenyl *CH*), 4.4 (m, 3H, CHCH₃, Fmoc-CO₂CH₂), 4.7 (1H, residual olefin *H*), 5.5 (s, 1H, *NH*), 6.2 (s, 1H, *NH*), 6.7 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (t, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₁₀₃H₁₈₇N₃O₄Na ([M+Na]⁺ (A+1)) 1554.44; found 1554.31.

Synthesis of HPI₁₅-Ala₃-Fmoc 16c. Following General Procedure A, HPI₁₅-NH₂ **12** (0.55 g, 0.54 mmol, 1 equiv) and Fmoc-Ala₃-OH (0.24 g, 0.54 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.35 mg, 0.67 mmol, 1.25 equiv) and DIEA (0.28 g, 2.14 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.69 g, 88%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 182H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2–4.7 (m, 7H, 3CHCH₃, fluorenyl *CH*, Fmoc-CO₂CH₂, residual olefin *H*), 5.8 (s, 1H, *NH*), 6.8 (s, 1H, *NH*), 7.1 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (t, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₁₀₆H₁₉₃N₄O₅ ([M+H]⁺) 1602.50; found 1602.50.

Synthesis of HPI₂₀-Ala-Fmoc 17a. Following General Procedure A, HPI₂₀-NH₂ **13** (0.6 g, 0.44 mmol, 1 equiv) and Fmoc-Ala-OH (135.60 mg, 0.44 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (225.16 mg, 0.54 mmol, 1.25 equiv) and DIEA (230.00 mg, 1.74 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (620.00 mg, 85%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 193H, aliphatic

H), 3.2 (m, 2H, CH_2NHR), 4.1 (m, 2H, $CHCH_3$, fluorenyl *CH*), 4.3 (m, 2H, Fmoc- CO_2CH_2), 4.7 (1H, residual olefin *H*), 5.3 (s, 1H, *NH*), 6.6 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (m, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for $C_{125}H_{232}N_2O_3Na$ ($[M+Na]^+$) 1832.79; found 1832.67.

Synthesis of HPI₂₀-Ala₂-Fmoc 17b. Following General Procedure A, HPI₂₀-NH₂ **13** (0.6 g, 0.44 mmol, 1 equiv) and Fmoc-Ala₂-OH (0.17 g, 0.44 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.28 g, 0.54 mmol, 1.25 equiv) and DIEA (0.23 g, 1.74 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.65 g, 86%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 201H, aliphatic *H*), 3.2 (m, 2H, CH_2NHR), 4.2–4.7 (m, 6H, 2 $CHCH_3$, fluorenyl *CH*, Fmoc- CO_2CH_2 , residual olefin *H*), 5.4 (s, 1H, *NH*), 6.3 (s, 1H, *NH*), 6.7 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (m, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for $C_{128}H_{237}N_3O_4Na$ ($[M+Na]^+$) 1903.83; found 1903.63.

Synthesis of HPI₂₀-Ala₃-Fmoc 17c. Following General Procedure A, HPI₂₀-NH₂ **13** (0.60 g, 0.44 mmol, 1 equiv) and Fmoc-Ala₃-OH (0.20 g, 0.44 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.28 g, 0.54 mmol, 1.25 equiv) and DIEA (0.23 g, 2.45 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.69 g, 90%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 218H, aliphatic *H*), 3.2 (m, 2H, CH_2NHR), 4.2–4.7 (m, 7H, $CHCH_3$, fluorenyl *CH*, Fmoc- CO_2CH_2 , residual olefin *H*), 5.4 (s, 1H, *NH*), 6.2 (s, 1H, *NH*), 6.6 (s, 1H, *NH*), 7.3 (t, 2H, aromatic *H*), 7.4 (t, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for $C_{121}H_{222}N_4O_5Na$ ($[M+Na]^+$) 1974.87; found 1974.65.

Synthesis of HPI₂₅-Ala-Fmoc 18a. Following General Procedure A, HPI₂₅-NH₂ **14** (0.5 g, 0.29 mmol, 1 equiv) and Fmoc-Ala-OH (90.10 mg, 0.29 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (188.20 mg, 0.36 mmol, 1.25 equiv) and DIEA (150.00 mg, 1.16 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as an amorphous solid (0.53 mg, 90%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 278H, aliphatic *H*), 3.2 (m, 2H, CH_2NHR), 4.2 (m, 2H, $CHCH_3$, fluorenyl *CH*), 4.4 (m, 2H, Fmoc- CO_2CH_2), 5.3 (s, 1H, *NH*), 5.9 (s, 1H, *NH*), 7.2 (t, 2H, aromatic *H*), 7.3 (t, 2H, aromatic *H*), 7.4 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for $C_{150}H_{282}N_2O_3Na$ ($[M+Na]^+$) 2183.20; found 2183.10.

Synthesis of HPI₂₅-Ala₂-Fmoc 18b. Following General Procedure A, HPI₂₅-NH₂ **14** (0.6 g, 0.35 mmol, 1 equiv) and Fmoc-Ala₂-OH (0.13 g, 0.35 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.18 g,

0.43 mmol, 1.25 equiv) and DIEA (0.22 g, 0.18 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as amorphous solid (0.63 g, 86%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 293H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2–4.4 (m, 5H, 2CHCH₃, fluorenyl CH, Fmoc-CO₂CH₂), 5.5 (s, 1H, NH), 6.3 (s, 1H, NH), 6.8 (s, 1H, NH), 7.3 (t, 2H, aromatic *H*) 7.4 (t, 2H, aromatic *H*), 7.6 (d, 2H, aromatic *H*), 7.7 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₁₅₃H₂₈₇N₃O₄Na ([M+Na]⁺) 2254.22; found 2254.45.

Synthesis of HPI₂₅-Ala₃-Fmoc 18c. Following General Procedure A, HPI₂₅-NH₂ **14** (0.60 g, 0.34 mmol, 1 equiv) and Fmoc-Ala₃-OH (0.15 g, 0.34 mmol, 1 equiv) were dissolved in dry THF (50 mL), and then PyBOP (0.23 g, 0.44 mmol, 1.25 equiv) and DIEA (0.18 g, 1.39 mmol, 4 equiv) were added. After a reaction time of 4 h, the solution was concentrated in vacuum. The reaction mixture was then precipitated into ice-cold 1 M aqueous HCl, and redissolved in THF. The precipitation was repeated two more times. The product was obtained as amorphous solid (0.66 g, 88%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 290H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 4.2–4.5 (m, 6H, 3CHCH₃, fluorenyl CH, Fmoc-CO₂CH₂), 5.6 (s, 1H, NH), 6.6 (s, 1H, NH), 7.2 (t, 2H, aromatic *H*) 7.3 (t, 2H, aromatic *H*), 7.5 (d, 2H, aromatic *H*), 7.8 (d, 2H, aromatic *H*) ppm. MS (MALDI): calcd mass for C₁₅₆H₂₉₂N₄O₅Na ([M+Na]⁺) 2325.26; found 2325.25.

Synthesis of HPI₁₀-Ala-H 19a. Following General Procedure D, HPI₁₀-Ala-Fmoc **15a** (0.30 g, 0.27 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.12 g, 1.35 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 25:1 v/v). The product was obtained as a colorless oil (0.18 g, 78%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.4 (m, 122H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.7 (1H, residual olefin *H*), 6.2 (s, 1H, NH), 6.7 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₆₀H₁₂₃N₂O ([M+H]⁺) 887.96; found 887.86.

Synthesis of HPI₁₀-Ala₂-H 19b. Following General Procedure D, HPI₁₀-Ala₂-Fmoc **15b** (0.30 g, 0.25 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.11 g, 1.25 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a colorless oil (0.21 g, 84%). ¹H NMR (500 MHz, CDCl₃): δ = 0.8–1.7 (m, 136H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 1H, CHCH₃), 4.7 (1H, residual olefin *H*), 6.4 (s, 1H, NH) 7.7 (d, 1H, NH) ppm. MS (MALDI): calcd mass for C₆₃H₁₂₈N₃O₂ ([M+H]⁺) 958.99; found 958.20.

Synthesis of HPI₁₀-Ala₃-H 19c. Following General Procedure D, HPI₁₀-Ala₃-Fmoc **15c** (0.30 g, 0.24 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.10 g, 1.20 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column

chromatography (CHCl₃/CH₃OH 50:1 v/v → CHCl₃/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.20 g, 82%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 149H, aliphatic *H*), 2.5 (s, 2H, NH₂), 3.2 (m, 2H, CH₂NHR), 3.6 (m, 1H, CHCH₃), 4.4 (m, 2H, 2CHCH₃) 4.7 (1H, residual olefin *H*), 6.6 (s, 1H, NH), 7.9 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₆₆H₁₃₂N₄O₃Na ([M+Na]⁺) 1052.02; found 1052.00.

Synthesis of HPI₁₅-Ala-H 20a. Following General Procedure D, HPI₁₅-Ala-Fmoc **16a** (0.30 g, 0.23 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.10 g, 1.17 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 20:1 v/v). The product was obtained as a colorless oil (0.22 g, 85%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 196H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.7 (1H, residual olefin *H*) ppm. MS (MALDI): calcd mass for C₈₅H₁₇₃N₂O ([M+H]⁺) 1238.35; found 1237.93.

Synthesis of HPI₁₅-Ala₂-H 20b. Following General Procedure D, HPI₁₅-Ala₂-Fmoc **16b** (0.30 g, 0.22 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.09 g, 1.10 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.20 g, 80%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 161H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 1H, CHCH₃), 4.7 (1H, residual olefin *H*), 6.3 (s, 1H, NH), 7.7 (d, 1H, NH) ppm. MS (MALDI): calcd mass for C₈₈H₁₇₇N₃O₂Na ([M+Na]⁺ (A+1)) 1332.38; found 1332.09.

Synthesis of HPI₁₅-Ala₃-H 20c. Following General Procedure D, HPI₁₅-Ala₃-Fmoc **16c** (0.30 g, 0.21 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.09 g, 1.03 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.20 g, 80%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 174H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 2H, 2CHCH₃), 4.7 (1H, residual olefin *H*), 6.3 (s, 1H, NH), 6.9 (s, 1H, NH), 7.7 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₈₁H₁₆₂N₄O₃Na ([M+Na]⁺) 1402.41; found 1402.45.

Synthesis of HPI₂₀-Ala-H 21a. Following General Procedure D, HPI₂₀-Ala-Fmoc **17a** (300 mg, 0.18 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (80.00 mg, 0.9 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a white solid (216.00 mg, 84%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.8 (m, 204H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CH₂NHR), 4.7 (1H, residual olefin *H*), 7.3 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₁₁₀H₂₂₂N₂ONa ([M+Na]⁺ (A+1)) 1611.73; found 1611.54.

Synthesis of HPI₂₀-Ala₂-H 21b. Following General Procedure D, HPI₂₀-Ala₂-Fmoc **17b** (0.30 g, 0.17 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.07g, 0.86 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.20 g, 77%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 229H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 1H, CHCH₃), 4.7 (1H, residual olefin *H*), 6.3 (s, 1H, NH), 7.6 (d, 1H, NH) ppm. MS (MALDI): calcd mass for C₁₀₃H₂₀₇N₃O₂Na ([M+Na]⁺) 1682.76; found 1682.53.

Synthesis of HPI₂₀-Ala₃-H 21c. Following General Procedure D, HPI₂₀-Ala₃-Fmoc **17c** (0.30 g, 0.17 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.07 g, 0.83 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CHCl₃/CH₃OH 50:1 v/v → CHCl₃/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.22 g, 83%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 223H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 2H, CHCH₃), 4.7 (1H, residual olefin *H*), 6.3 (s, 1H, NH), 6.9 (s, 1H, NH), 7.8 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₁₁₆H₂₃₂N₄O₃Na ([M+Na]⁺) 1752.80; found 1752.77.

Synthesis of HPI₂₅-Ala-H 22a. Following General Procedure D, HPI₂₅-Ala-Fmoc **18a** (0.30 g, 0.15 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.063 g, 0.74 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 25:1 v/v). The product was obtained as a white solid (0.22 g, 83%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 299H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.7 (m, 1H, CHCH₃) ppm. MS (MALDI): calcd mass for C₁₃₆H₂₇₃N₂O₃ (M⁺ (A+1)) 1984.13; found 1983.97.

Synthesis of HPI₂₅-Ala₂-H 22b. Following General Procedure D, HPI₂₅-Ala₂-Fmoc **18b** (0.30 g, 0.14 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.06 g, 0.72 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CH₂Cl₂/CH₃OH 50:1 v/v → CH₂Cl₂/CH₃OH 25:1 v/v). The product was obtained as a white solid (0.21 g, 79%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 304H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5 (m, 1H, CHCH₃), 4.4 (m, 1H, CHCH₃), 6.5 (s, 1H, NH), 7.7 (s, 1H, NH) ppm. MS (MALDI): calcd mass for C₁₃₈H₂₇₇N₃O₂Li ([M+Li]⁺ (A+1)) 2016.18; found 2016.28.

Synthesis of HPI₂₅-Ala₃-H 22c. Following General Procedure D, HPI₂₅-Ala₃-Fmoc **18c** (0.30 g, 0.14 mmol, 1 equiv) was dissolved in CHCl₃ (50 mL), and then piperidine (0.06 g, 0.70 mmol, 5 equiv) was added. After stirring the mixture overnight, the solvent was evaporated. The crude product was purified by silica gel column chromatography (CHCl₃/CH₃OH 50:1 v/v → CHCl₃/CH₃OH 15:1 v/v). The product was obtained as a white solid (0.23 g, 0.86%). ¹H NMR (300 MHz, CDCl₃): δ = 0.8–1.7 (m, 290H, aliphatic *H*), 3.2 (m, 2H, CH₂NHR), 3.5

(m, 1H, *CHCH*₃), 4.4 (2H, *CHCH*₃), 6.3 (s, 1H, *NH*), 6.9 (s, 1H, *NH*), 7.8 (s, 1H, *NH*) ppm. MS (MALDI): calcd mass for C₁₄₁H₂₈₂N₄O₃Na ([M+Na]⁺) 2103.19; found 2103.27 .