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Thio-Bromo "Click" Reaction Derived Polymer–Peptide Conjugates for Their Self-Assembled Fibrillar Nanostructures

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The synthesis and self-assembly of peptide–polymer conjugates into fibrillar nanostructures are reported, based on the amyloidogenic peptide KLVFF. A strategy for rational synthesis of polymer–peptide conjugates is documented via tethering of the amyloidogenic peptide segment LVFF ($A\beta_{17-20}$) and its modified derivative FFFF to the hydrophilic poly(ethylene glycol) monomethyl ether (mPEG) polymer via thio-bromo based "click" chemistry. The resultant conjugates mPEG-LVFF-OMe and mPEG-FFFF-OMe are purified via preparative gel permeation chromatography technique (with a yield of 61% and 64%, respectively), and are successfully characterized via combination of spectroscopic and chromatographic methods, including electrospray ionization time-of-flight mass spectrometry. The peptide-guided self-assembling behavior of the as-constructed amphiphilic supramolecular materials is further investigated via transmission electron microscopic and circular dichroism spectroscopic analysis, exhibiting fibrillar nanostructure formation in binary aqueous solution mixture.

1. Introduction

Over the past decades fundamental challenges persist in the design of synthetic hybrid materials as biomimics of natural peptides or proteins, resembling their highly ordered structures and functions.[1,2] Although there has been considerable development in the area,[3] significant attention has been paved recently by the material chemists toward the rational designing of polymer–peptide conjugates inspired by natural peptides self-assembling to form β-sheet directed fibrillar nanostructures,[4,5] such as amyloid-β (Aβ) peptide based fibrils, being responsible for neurodegenerative diseases (Alzheimer's, Parkinson's, and type II diabetes),[6,7] or silk protein based fibrils.[8] Such supramolecular hybrid-materials, could provide increased bio-functionality and enhanced bio-interaction in natural processes.[9,10] Therefore the optimization of artificial supramolecular conjugates to generate 1D fibrous structure can lead to promising advanced materials for a deeper understanding of the dynamic properties and to overcome the limitations in natural systems,[11,12] particularly in case of pathogenic proteins such as $A\beta_{40/42}$ peptide, α-synuclein, and prion protein, all proven to form fibrillar structures.[13] We here describe a different strategy (in comparison to Hamley et al.[14] and Tzokova et al.[15]) to synthesize amphiphilic polymeric conjugates based on the hydrophobic tetrapeptide derivatives of LVFF and FFFF, linked to the hydrophilic poly(ethylene glycol) (PEG) polymer derivative via a thio-bromo "click" reaction, in-turn self-assembling into fibrillar nanostructures. PEGylation of peptides (or proteins) is advantageous for biomedicinal application, offering not only improved water solubility and stability, but also enhanced circulation time,[16,17] as reviewed extensively.[18,19] Although there are many coupling chemistries documented in the literatures to prepare PEG-peptide conjugates,[20,21] such as 1) on-resin coupling via *N*-terminal peptide resins to PEG-COOH derivatives by Borner et al.,[22] and Hamley et al.,[23,24] 2) copper-catalyzed azide–alkyne cycloaddition (CuAAC) of alkynylated peptides to azido PEG polymers by Tzokova et al.,[15,25] 3) coupling thiol-containing peptides to maleimide-PEG derivatives as reported by van Hest et al.,[26] and so on,[27,28] the use of the thio-bromo based "click" reaction has not been reported yet for this and such endeavors, to the best of our knowledge. This coupling reaction, less explored reaction among other "click" chemistry approaches,[29,30] represents a very efficient method, performed under metal-free reaction condition,[31,32] especially the latter being desirable to prepare pharmaceutically applicable polymers. In this article, this new coupling strategy has been adopted to enable the direct ligation of brominated peptide

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with a thiol-derivative of PEG polymer, followed by the exclusive purification of the resultant conjugates via preparative gel permeation chromatography (GPC). The so generated synthetic conjugates were further studied for the peptide-guided folding of their PEGylated conjugates to form well-defined hierarchical fibrillar morphology.[33]

2. Experimental Section

2.1. Materials

L-Valine methyl ester hydrochloride (HCl.H_2N-V-OMe, 98.5%), and L-phenylalanine methyl ester hydrochloride (HCl.H_2N-F-OMe, 98%) were purchased from Carbolution chemicals. Boc-L-leucine monohydrate (Boc-L-OH.H_2O, 99%), and Boc-L-phenylalanine monohydrate (Boc-F-OH.H_2O, 99%) were received from TCI chemicals. 1-Hydroxybenzotriazole hydrate (HOBt, 97%), trifluoroacetic acid (TFA, 99%), poly(ethylene glycol) monomethyl ether (mPEG-OH, M_n = 750 g mol^{-1}), potassium thioacetate (KSOAc, 98%), triethylamine (Et_3N, 99%), sodium methoxide solution (NaOMe, 25 wt% in methanol), and 2-bromopropionyl bromide (97%) were purchased from Sigma-Aldrich and used as received. *N,N*-Dicyclohexylcarbodiimide (DCC, 99%) and mesylchloride (MsCl, 99%) were received from Merck and Alfa Aesar, respectively. The solvents dichloromethane (CH_2Cl_2), methanol (MeOH), and tetrahydrofuran (THF) were dried and distilled according to the standard procedures.

2.2. Instrumentation

2.2.1. Gel Permeation Chromatography

GPC spectra for polymers were recorded in solution of 10 mM lithium *bis*(trifluoromethanesulfonyl)imide ($LiNTf_2$) in DMF at 60 °C (flow rate 1.0 mL min^{-1}) using a Viscotek GPCmax VE 2001 from Viscotek equipped with a column set of a H_{HR}-H-Guard-17369 and a GMH_{HR}-N-18055 column. Polystyrene (PS) standards were used as external calibration. OmniSEC software (V 4.5.6) was used for data analysis.

2.2.2. Preparative GPC

Preparative GPC studies were carried in a solution of THF solvent at 30 °C with a flow rate of 1 mg mL^{-1} using a VWR HITACHI Chromaster equipped with a KD-2002.5 column from Shodex. The sample solutions were injected with a concentration of 20 mg mL^{-1} and the detection was performed by using refractive index (RI) detector from VWR at 30 °C. The obtained data was analyzed in EZChrom Elite (version 3.3.2 SP2) software.

2.2.3. Nuclear Magnetic Resonance

The solution state ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy were carried out on a Varian Gemini 2000 (400 MHz) or on a Varian Unity INOVA 500 (500 MHz) NMR spectrometer using $CDCl_3$ or DMSO-d_6 as solvent at 27 °C. MestRec-C software (version 4.9.9.6) was used for interpretation of NMR data.

2.2.4. Electrospray Ionization Time-of-Flight Mass Spectroscopy

Electrospray ionization time-of-flight mass spectroscopy (ESI-TOF MS) measurements were performed on a Focus Micro TOF of Bruker Daltonics. Spectra were recorded in a positive mode by applying an accelerator voltage of 4.5 kV, a transfer line with 190 °C at the spectral rate of 1 Hz. The obtained spectra were processed on Brucker Daltonic ESI compass 1.3 for microTOF (Data Analysis 4.0).

2.2.5. Transmission Electron Microscopy

The self-assembled morphology of the polymer in solution was investigated by an EM 900 transmission electron microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). The micrographs were taken from the sample solution droplet placed on a Cu grid coated with a carbon film, stained with uranyl acetate solution (2%), and air dried.

2.2.6. Circular Dichroism

Circular dichroism (CD) spectra for polymer solutions were recorded on a JASCO Corp., J-810, Rev.1.00 system at 20 °C using a cuvette with a space length of 1 mm. The measurements were performed in the wavelength ranging from 250 to 190 nm with a scan rate of 1 nm s^{-1} and absorbance $A < 2$ at any measured point. The final CD spectra were reported after subtraction of the blank solvent measurement from the sample spectra.

2.3. Synthesis of Bromo-Group Modified Tetrapeptide Br-FFFF-OMe

To a solution of the 1.8 g of the tetrapeptide H_2N-FFFF-OMe (**11**, 2.89 mmol) in 100 mL anhydrous THF, 0.61 mL Et_3N (0.44 g, 4.35 mmol) was added in ice-water bath condition and was left for stirring for 20 min. Then, 0.46 mL of 2-bromopropionyl bromide (0.94 g, 4.35 mmol) was added dropwise and the mixture was allowed to come at room temperature and was stirred for 18 h. The reaction mixture was filtered, dried, and was further diluted with 150 mL of $CHCl_3$ which was successively washed once with 100 mL of 1 N HCl, saturated $NaHCO_3$ solution, and brine solution. The organic layer was dried over anhydrous Na_2SO_4 followed by evaporation in vacuo. The product was further purified by silica gel column chromatography using CH_2Cl_2:ethyl acetate (3:1) as eluent, resulting in the tetrapeptide Br-FFFF-OMe (**7**) with a yield of 72 %, further characterized by ^{1}H and ^{13}C NMR, and ESI-TOF MS (**Figure 1**; Figure S15, Supporting Information).

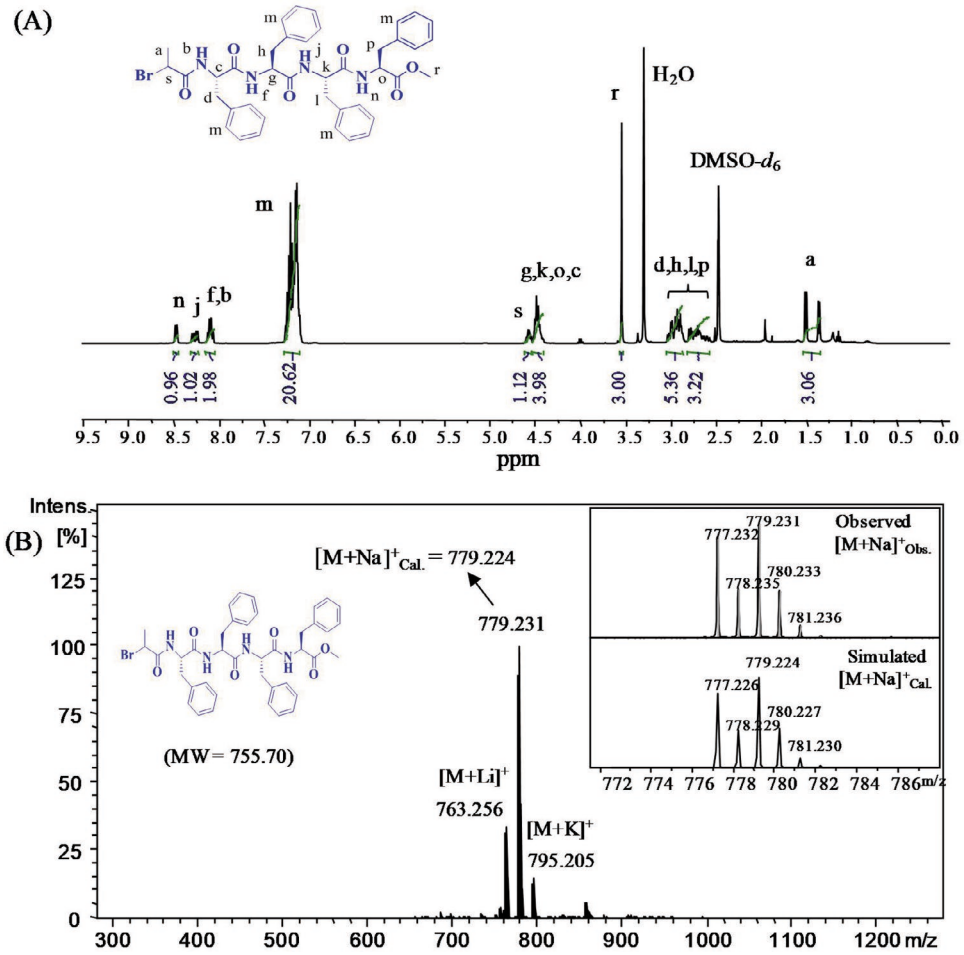


Figure 1. A) ^{1}H NMR spectrum and B) ESI-TOF MS spectrum of FFFF-based brominated peptide **7**. Inset in (B) shows the observed and simulated isotopic signals.

2.4. Synthesis of Mesyl Protected Polymer mPEG-OMs

To 6 g (8.0 mmol) poly(ethylene glycol) monomethyl ether (mPEG-OH, **1**, M_n = 750 g mol^{-1}) and 3.35 mL Et_3N (2.43 g, 24.0 mmol) in 200 mL dry CH_2Cl_2 solution, 1.86 mL mesyl chloride (2.75 g, 24.0 mmol) were added dropwise at ice-water bath condition under N_2 atmosphere. The reaction mixture was allowed to come at room temperature and was further stirred for 18 h. Then the reaction mixture was filtered and organic layer was washed with 100 mL of 1 N HCl (3×), saturated $NaHCO_3$ (3×), and brine solution (3×), and was dried over anhydrous Na_2SO_4 followed by evaporation in vacuum. The crude product was further purified by silica gel column chromatography using CH_2Cl_2:methanol (11:1) as eluent, resulting in the mesyl protected polymer mPEG-OMs (**2**) as liquid in a yield of 82%. The obtained pure polymer **2** was characterized by ^{1}H and ^{13}C NMR (Figures S17 and S18, respectively, Supporting Information).

2.5. Synthesis of Thioacetylated Polymer mPEG-SAc

To a stirring solution of 3 g (2.61 mmol) of polymer **2** ($M_{n,GPC}$ = 1150 g mol^{-1}, $Đ$ = 1.04) in 100 mL dry DMF solvent, 2.49 g potassium thioacetate (21.8 mmol) were added portion wise at 75 °C under N_2 atmosphere. The reaction mixture was allowed to stir further for 48 h. Then the reaction mixture was filtered while hot solution and the filtrate were dried via a rotary evaporator. 200 mL of CH_2Cl_2 were added to this crude product and the organic layer was washed with 100 mL of water and brine solution, dried over anhydrous Na_2SO_4, followed by evaporation in vacuum. The crude product was further purified by silica gel column chromatography using CH_2Cl_2:MeOH (19:1) as eluent, resulting in the thioacetylated polymer mPEG-SAc (**3**) in a yield of 80%. The obtained pure polymer **3** was characterized by ^{1}H and ^{13}C NMR, and ESI-TOF MS (Figures S19, S20, and S23, Supporting Information).

2.6. Synthesis of Thiolated Polymer mPEG-SH

1 g of the as-synthesized thioacetylated polymer **3** (0.83 mmol, $M_{n,GPC}$ = 1200 g mol^{-1}, $Đ$ = 1.05) was dissolved in 20 mL of dry methanol solvent and the solution was purged thoroughly under N_2 atmosphere for 20 min followed by addition of 0.24 mL of NaOMe solution (4.17 mmol).[34,35] The reaction mixture was allowed to stir at room temperature for 10 h and

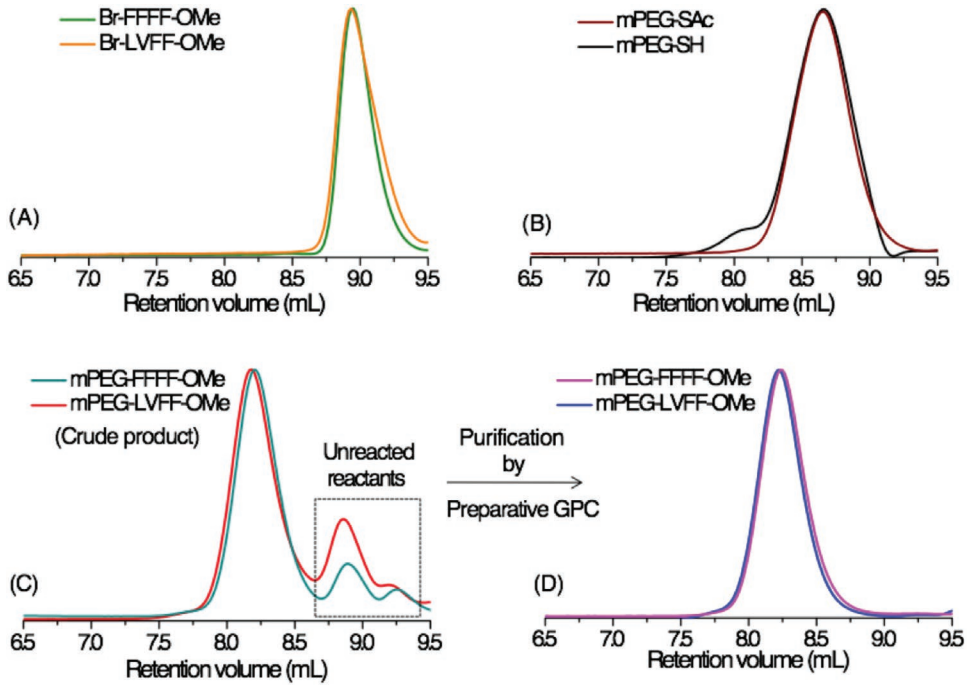


Figure 2. Normalized RI traces by the analytical GPC studies of A) brominated peptide **7** and **8**, B) polymer **3** and **4**, C) the peptide–polymer conjugate based crude products just after the reaction, and D) the corresponding conjugated products **9** and **10** after purification via preparative GPC.

then was dried under rotary evaporator. 50 mL of dry CH_2Cl_2 were added to this crude and the organic layer was thoroughly washed with 50 mL of degassed aqueous solution of 1 N HCl (3×), and was finally dried over anhydrous Na_2SO_4 followed by evaporation in rotary and vacuum pump. The obtained thiolated polymer mPEG-SH (**4**) in a yield of 85% was characterized by ^1H and ^{13}C NMR and ESI-TOF MS (Figures S21, S22, and S24, respectively, Supporting Information).

2.7. Synthesis of Peptide–Polymer Conjugates by Thio-Bromo "Click" Reaction

To a typical example, 50 mg (0.045 mmol) of the thiolated polymer **4** ($M_{n,GPC}$ = 1100 g mol^{-1}, $Đ$ = 1.06) was dissolved in 3 mL of dry THF followed by the addition of 23.0 mg of Et_3N (0.227 mmol, 31.68 µL). Then this reaction mixture was thoroughly degassed by applying five consecutive cycles of freeze-pump-thaw, and was allowed to stir in room temperature for 1 h. Further, 33.7 mg of peptide **7** (0.05 mmol) was added to this reaction mixture and was stirred under N_2 atmosphere for 2 h. The crude reaction product was then transferred to a dialysis membrane bag (molecular weight cut off of 1 kDa) and was extensively dialyzed against a solvent mixture of THF/MeOH (1:1) for 48 h to remove the unreacted residues. The conjugate mPEG-FFFF-OMe (**9**) was obtained by evaporating the dialyzed solution and was further subjected for preparative GPC based purification for achieving the pure product with 64% yield (where the low yield could be attributed to the loss of compound during such purification processes). Similarly, the synthesis of LVFF-based conjugate mPEG-LVFF-OMe (**10**) was accomplished and purified followed via the above procedure (with 61% yield), and finally both the conjugates were dried in high vacuum pump and characterized via GPC, ^1H NMR, and ESI-TOF MS (see **Figures 2**D, **3**, and **4**, respectively).

2.8. Fibril Formation from Peptide–Polymer Conjugates

To a typical example, a particular amount of LVFF-polymer conjugate **10** was dissolved in THF solvent followed by the dropwise addition of distilled water to reach the concentration of 8 mg mL^{-1} in the solvent mixture of H_2O/THF (8/2, v/v) and was allowed to self-assemble at room temperature for 24 h. Then the prepared sample was further diluted with the above binary solvent mixture to reach the final concentration of 0.4 mg mL^{-1} and was subsequently utilized to probe self-assembly via transmission electron microscopy (TEM) measurements.

3. Results and Discussion

To prepare the polymer–peptide conjugate, first α-bromo-acyl derivatives of the peptides were synthesized via a solution phase peptide synthesis approach.[36] As a typical example, initially tetrapeptide Boc derivative of FFFF-OMe was synthesized (Scheme S1, Supporting Information) with the *N*-terminus protected by the Boc-group and the *C*-terminus protected by the methyl group via classical peptide-synthesis, followed by methylester deprotection, where all the intermediate compounds were fully characterized (Figures S1–S10, Supporting Information). The complete structural confirmation of the synthesized peptide Boc-FFFF-OMe (**5**) was made by NMR and ESI-TOF

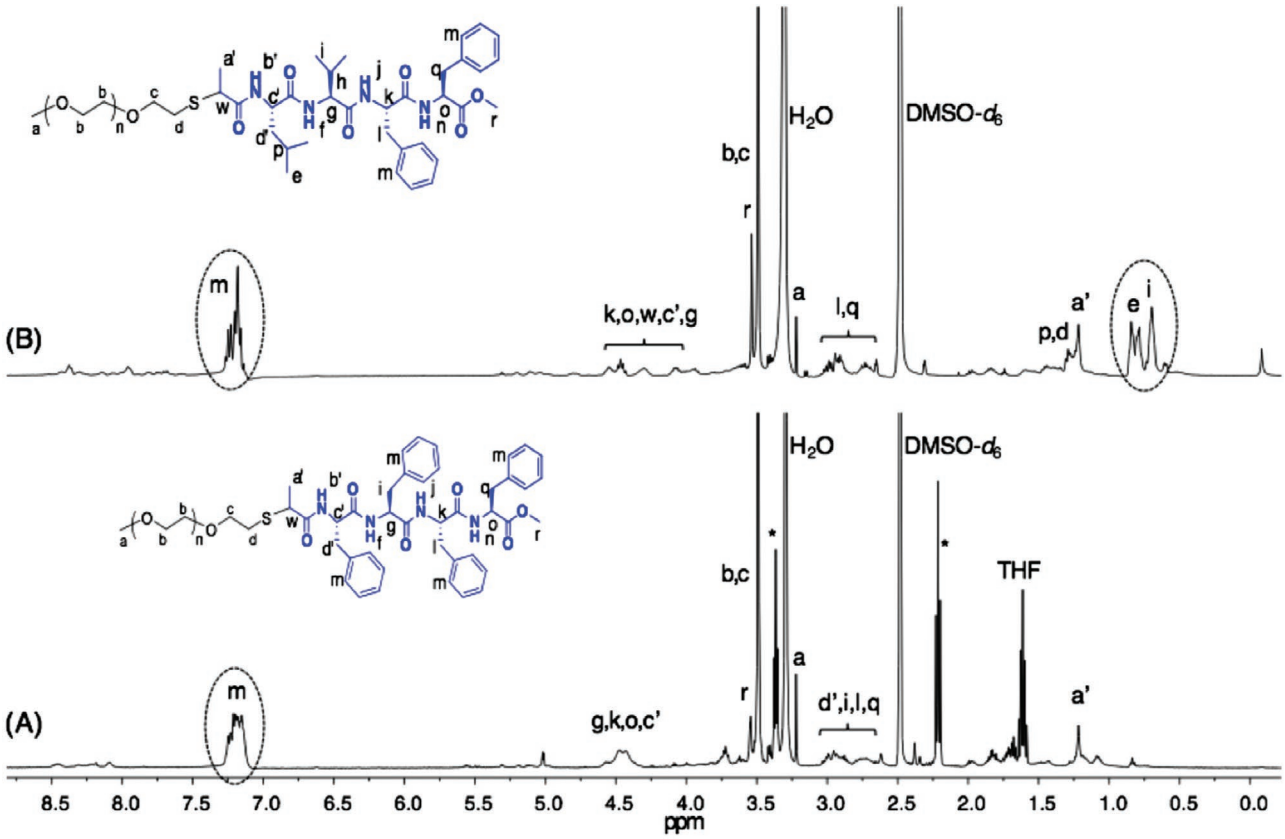


Figure 3. 1H. NMR spectra of PEGylated peptide based A) conjugate **9**, and B) conjugate **10**. Encircled sections in (A) and (B) represent major peaks from peptidic constituent of the conjugate.

MS spectroscopy (Figures S11–S13, Supporting Information). ^{1}H NMR spectrum depicted the resonance signals of the peptidic segment at δ 7.30–7.10 and 3.08–2.66 ppm correspond to the phenyl group and benzyl protons, respectively, of the four phenylalanine units, along with the presence of methyl protons of the corresponding *N*-terminus and *C*-terminus protected Boc and methylester groups, at δ 1.25 and 3.56 ppm, respectively. ESI-TOF MS spectra displayed the major peak at m/z = 743.34 g mol^{-1} as the observed molecular mass for $[M+Na]^+$ which matches well with the simulated isotopic peaks for their theoretical molecular mass. Further, trifluoroacetic acid was added for *N*-terminal deprotection by removal of the Boc group to afford the free primary amino group carrying peptide **11**, which was confirmed by the disappearance of the above Boc signal in ^{1}H NMR spectrum in Figure S14, Supporting Information. Finally, the achieved peptide **11** was reacted with 2-bromopropionyl bromide in presence of Et_3N to achieve their brominated tetrapeptide derivative **7**. The full structural characterization of the as-synthesized peptide **7** was performed by ^{1}H and ^{13}C NMR, and ESI-TOF MS spectroscopic studies. The ^{1}H NMR spectrum in Figure 1A demonstrated the appearance of new methyl proton and chiral proton signals of α-bromo position to the amide moiety at δ 1.54–1.34 and 4.59 ppm, respectively, along with the persistence of the peaks correspond to the other peptidic constituents. ^{13}C NMR further evidenced the absence of racemic mixture with the residual chiral carbon resonances present at δ 54.21–44.19 ppm (Figure S15, Supporting Information). ESI-TOF MS spectrum in Figure 1B illustrated the full agreement between the isotopic peaks of observed molecular mass of $[M+Na]^+$ at m/z = 729.231 g mol^{-1} and their simulated species at m/z = 729.224 g mol^{-1}. Furthermore, a second bromo peptide derivative, Br-LVFF-OMe **(8)**, corresponding to the central hydrophobic core segment peptide LVFF of the amyloid-β protein (A$\beta_{17\text{-}20}$) was further synthesized and characterized similar to previous investigations (Figure S16, Supporting Information).[35]

After successful synthesis of the brominated peptidic counterparts for designing the peptide–polymer conjugate, thiol group carrying PEGylated polymer **4** was synthesized via three successive reactions. First, monohydroxyl group of the commercially available polymer **1** was modified to their mesylated polymer derivative **2** (see **Scheme 1**) followed by further reaction with potassium thioacetate (KSAc) to prepare the thioacetate-capped PEGylated polymer **3**. The chain-end modification from the −OH to −OMS conversion was confirmed by the appearance of the methyl proton peaks of $-OSO_2CH_3$ at δ 2.96 ppm and the carbon peak at δ 37.48 ppm in ^{1}H and ^{13}C NMR spectrum, respectively (Figures S17 and S18, Supporting Information), whereas successful conversion of −OMs to the −SAc group was further demonstrated by the absence of above mentioned characteristic signals of $-OSO_2CH_3$ along with the appearance of methyl proton and carbon signals of $-SCOCH_3$ at δ 2.28 and 28.78 ppm in ^{1}H and ^{13}C NMR spectrum, respectively (Figures S19 and S20, Supporting Information).

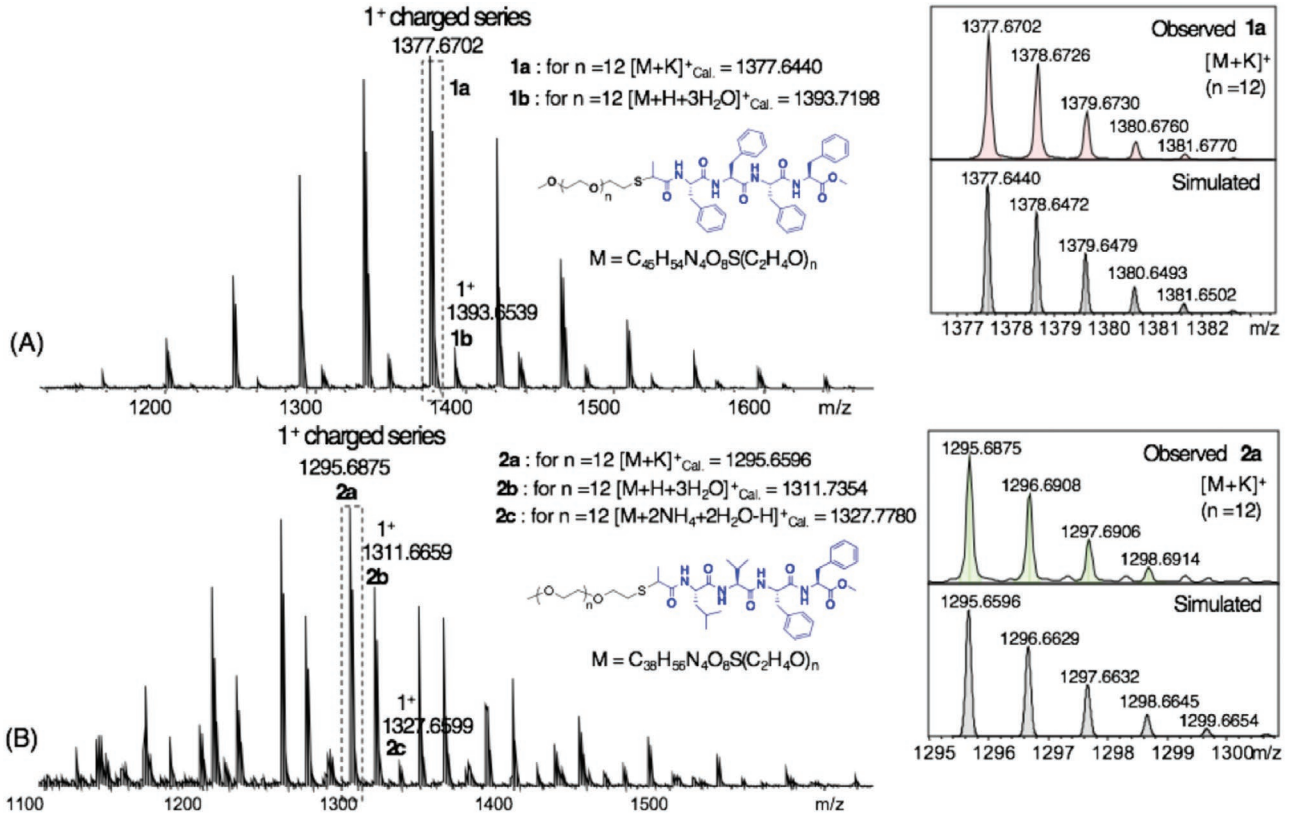


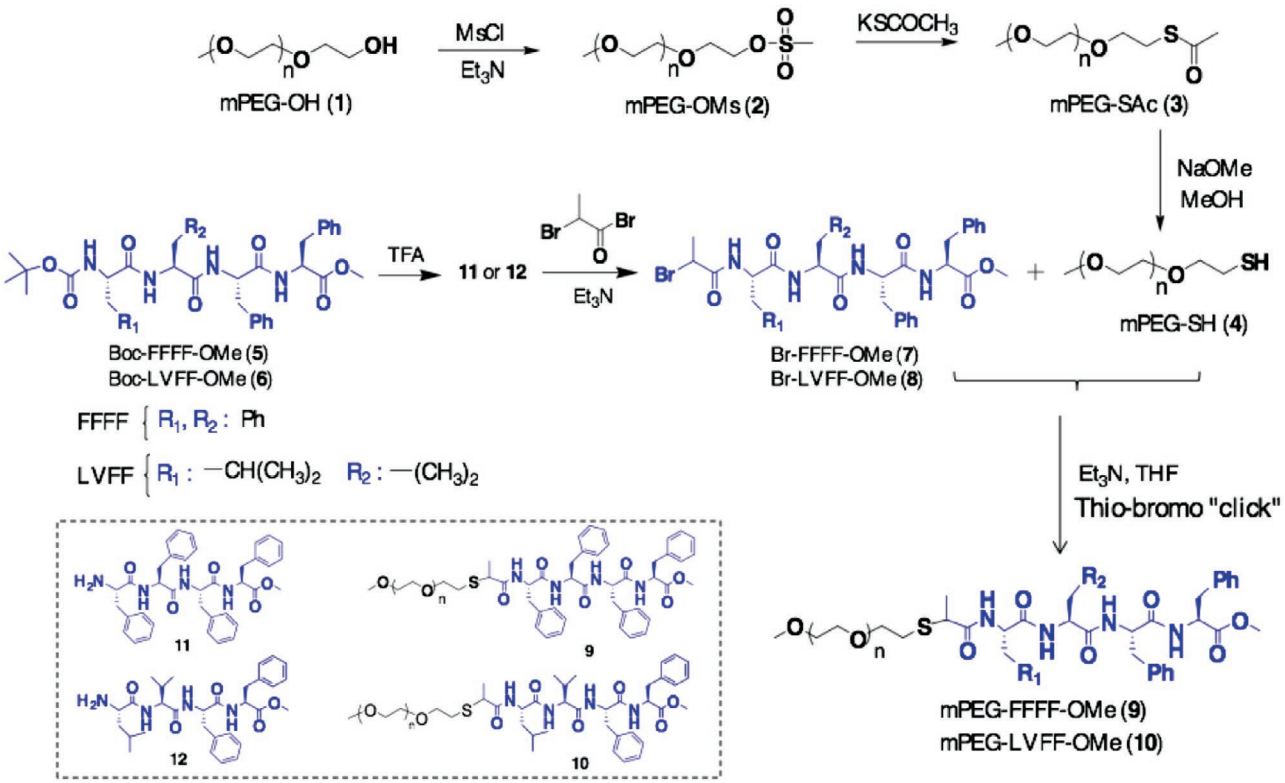
Figure 4. ESI-TOF MS spectra of A) FFFF-based conjugate **9** and B) LVFF-based conjugate **10**. Insets in (A) and (B) illustrate observed and simulated isotopic pattern of a signal corresponding to the framed section.

Finally, deprotection of the acetyl group was effected to generate the free thiol group, polymer **4**, further characterized by ^{1}H and ^{13}C NMR spectroscopic studies. Evidence for SH-group formation was proven by the loss of singlet for the above stated characteristic three proton signals of $-SCOCH_3$ along with the existence of a triplet for the $-SH$ proton peak at δ 1.58 ppm in ^{1}H NMR spectrum (Figure S21, Supporting Information), in line with previous reports for polymer **4**,[37,38] proving the absence of eventual disulfide bridged polymer mPEG-SS-mPEG (see Figure S22, Supporting Information) which were also further supported by absence of a peak at $\delta \approx 39.0$ ppm, corresponding to the α-carbons to $-SS-$ group as reported previously by Millstone et al.[39]

Moreover, the complete chain-end modification of polymer **3** to polymer **4** and their chemical compositions were further confirmed by ESI-TOF MS based spectroscopic investigations. Figures S23 and S24, Supporting Information, for polymer **3** and **4**, respectively, depict each set of ions with 1^+ charged state, separated by m/z = 44.03 g mol^{-1}, corresponding to the ethylene glycol repeating unit (calculated 44.05 g mol^{-1}), and upon transformation of $-SAc$ to $-SH$ there was a lower shift in the molar mass peaks. As a typical example, peak maxima for a degree of polymerization (n) = 12 for the major mass series $[M+Na]^+$ at m/z = 729.3642 g mol^{-1} for polymer **3** were found to be shifted at m/z = 687.3513 g mol^{-1} for polymer **4** (further successfully simulated to match their corresponding isotopic peaks of the theoretical mass at m/z = 729.3702 and 687.3596 g mol^{-1}, respectively).

With the purified products (brominated peptide **7** and **8**, and thiolated polymer **4**) in hand, the synthesis of peptide–polymer conjugates were carried out via the thio-bromo "click" chemistry approach. As illustrated in Scheme 1, the stoichiometric mixture of polymer **4** and peptide **7** or **8** (with ratio of 1:1.1, respectively) was reacted in presence of Et_3N base in dry THF solvent at room temperature. Analytical GPC studies (Figure 2B) of free thiol containing polymer **4** revealed the appearance of peak maxima with similar retention volume (R_v) position at ≈8.65 mL as of the corresponding acetyl protected polymer **3** ($M_{n,GPC}$ = 1200 g mol^{-1}, $Đ$ = 1.05). Note that, the appearance of one minor peak at $R_v \approx 8.0$ mL observed here can be attributed to the formation of a polymer adduct, possibly after exposure during the sampling and running via GPC instrument in DMF solvent, as there is no disulfide polymer formation observed in their ESI-TOF MS or NMR studies as discussed above. Liu et al. had also reported the presence of similar minor peak for the GPC trace of the commercially available polymer **4** of higher molecular weight.[40]

After the reaction was completed, we proceeded for further purification steps of the peptide–polymer conjugates. Initially, it was found that the typical purification method via dialysis was not effective in this case due to the low molecular weight range of the utilized polymer and their formed conjugates. Therefore, we did apply here preparative GPC recently exploited by our group,[41] for the purification of other peptide–polymer conjugates. First, the completion of the reaction was determined by the analytical GPC studies of their



Scheme 1. Synthesis of FFFF- and LVFF-peptide-based polymer conjugates **9** and **10**, respectively, via thio-bromo "click" chemistry approach.

direct crude sample after 2 h of the reaction where the normalized RI chromatograms (Figure 2C) described the shift in the main peak with maxima at R_v = 8.19 mL proving the complete product formation of the peptide–polymer conjugates as compared to R_v = 8.65 mL for **4** ($M_{n,GPC}$ = 1100 g mol^{-1}, $Đ$ = 1.06). The existence of their other minor peaks can be referred to the unreacted reactants for example, peak maxima at R_v = 8.87 mL can be observed due to the residual amount of the bromo peptide moieties as compared to their traces in Figure 2A. Once the reaction completion was confirmed, the crude products were dialyzed and further subjected to their purification via preparative GPC in THF solvent as described in the instrumental section. The effective purification via this technique is shown by analytical GPC (Figure 2D) of the final purified product **9** ($M_{n,GPC}$ = 1900 g mol^{-1}, $Đ$ = 1.07) and **10** ($M_{n,GPC}$ = 2000 g mol^{-1}, $Đ$ = 1.06), where there was no trace of the unreacted reactants observed. The formation of the desired peptide–polymer conjugates (**9** and **10**) were further determined by their structural investigation via NMR spectroscopic studies. ^{1}H NMR spectrum of conjugate **9** (Figure 3A) and conjugate **10** (Figure 3B) exhibited the appearance of characteristic major proton resonances corresponding to the peptide segment FFFF (at δ 7.32–7.10 ppm for four phenyl ring protons and at δ 3.04–2.68 ppm of four benzyl group protons, of tetraphenylalanine), and LVFF (at δ 7.31–7.13 ppm for both phenyl ring protons of diphenylalanine and at δ 0.87–0.65 ppm of dimethyl protons of leucine and valine unit), respectively, along with the existence of characteristic proton signals at δ 3.49 ppm for the repeating unit $-OCH_2CH_2-$ of PEGylated polymer constituent.

The complete structural investigation of the as synthesized peptide–polymer conjugates was further accomplished by ESI-TOF MS studies. As shown in Figure 4, a clear shift toward larger molecular ions was observed in the spectrum of both the conjugates in comparison to the native thiolated polymer. ESI-TOF MS spectrum of conjugate **9** displayed the appearance of a singly charged molecular ion main series with $[M+K]^+$ along with the minor series $[M+H+3H_2O]^+$, where the observed molecular mass, (for e.g., isotopic peak maxima **1a** at m/z = 1377.6702 g mol^{-1} for $[M+K]^+$ for n = 12), was successfully simulated to match with the corresponding species (Figure 4A). In the case of ESI-TOF MS examination of conjugate **9**, the presence of three different molecular ion series with 1^+ charged states (major peak series of $[M+K]^+$ and $[M+H+3H_2O]^+$ along with the minor peak series of $[M+2NH_4+2H_2O-H]^+$) were identified, where the observed molecular mass (for e.g., isotopic peak maxima **2a** at m/z = 1295.6875 g mol^{-1} for $[M+K]^+$ of n = 12) was found to be in full agreement with their corresponding calculated theoretical mass values (Figure 4B).

After performing the successful characterizations, the self-assembly behavior of the readily prepared peptide–polymer based supramolecular conjugates were studied in aqueous media.[42,43] Due to the presence of the highly hydrophobic properties of peptidic segment FFFF or LVFF, their conjugates could not be well dissolved in pure aqueous solution. As a consequence, the self-assembly experiments were carried out in H_2O/THF mixtures with a ratio of 8/2 v/v.[23] To prepare the solutions, first the appropriate amount of each moiety (native peptide and their conjugate) was completely dissolved in THF solvent where water was added then in dropwise manner to

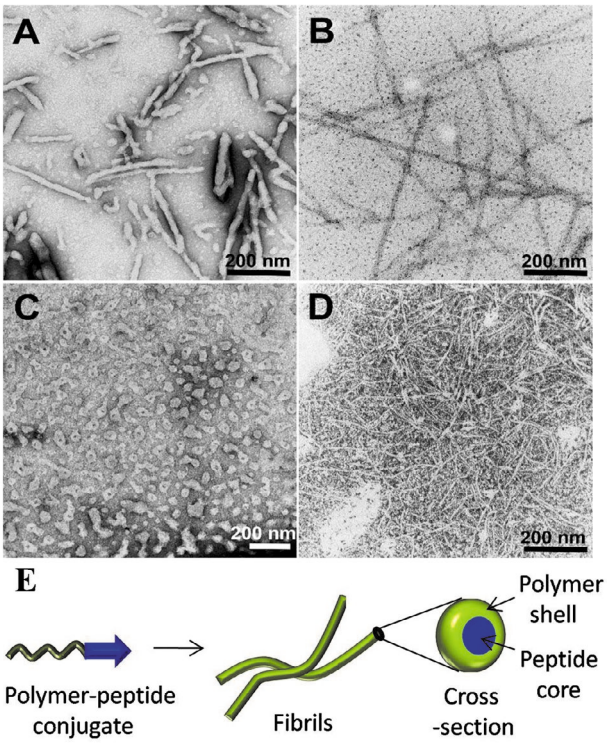


Figure 5. A,C) TEM images (scale bar of 200 nm) of native peptide **12** (c = 0.4 mg mL^{-1}) and **11** (c = 0.7 mg mL^{-1}), B,D) corresponding peptide–polymer conjugate **10** (c = 0.4 mg mL^{-1}) and **9** (c = 0.7 mg mL^{-1}) in H_2O/THF (8/2, v/v) solution. E) A cartoon representation for the possible fibrillar structure formation by the polymer–peptide conjugates.

reach the above solvent binary mixture. The formation of self-assembled architecture in a particular concentration was further probed by TEM. **Figure 5**A demonstrates the TEM image of the drop casted solution of the native amyloidogenic peptide **12** (A$\beta_{17\text{-}20}$) forming ill-defined fibrils, with large inconsistencies in length and width along with the coexistence of different populations. In contrast, the TEM image of their conjugate **10** revealed the formation of well-defined fibrils with elongated thin (average diameter of 20 nm) and rigid structure (Figure 5B; Figure S25, Supporting Information). In case of other native peptide **11**, TEM image in Figure 5C displayed the existence of irregular spherical aggregates due to the relatively high hydrophobic nature of the used peptides. On the other hand, the appearance of flexible fibrillar assemblies with an average diameter of 17 nm was observed for their corresponding conjugate **9** (Figure 5D), different when compared to the previously reported similar peptidic conjugate with a *C*-terminus linked PEG of higher molecular weight, reported to form short needle-like fibrils by Hamley et al.,[14] and with a triazole ring containing conjugate to form nanotubes by Tzokova et al.[15] Note that the dense fibrillar morphological view has appeared here due to their increased sample concentration as compared to the former conjugate. The flexible nature of such fibrils for conjugate **9** (in comparison to other conjugate **10**) can be attributed to their enhanced π–π interactions, and therefore further reflects the existence of peptide-governed self-assembly behavior of the peptide–polymer conjugates. Moreover, the above results also clearly exemplify the pronounced impact of PEG based polymer block on the self-assembly behavior of the peptides as the obtained peptide–polymer conjugates achieved the appropriate amphiphilic nature with a balanced hydrophilic/hydrophobic ratio required for the formation of well-defined fibrillar architectures. Such assemblies can be comprised of core-shell like structure with the hydrophobic peptide entities to the core of the fibril and hydrophilic PEG polymer in to their shell counterparts as proposed in Figure 5E.

CD spectroscopic investigations were further performed to examine the secondary structure of the above studied materials (native peptide and their conjugate) in H_2O/THF (8/2, v/v) solution. CD spectra (**Figure 6**) of the diluted solution of peptide **11** and the conjugate **9** represents the appearance of positive signal maxima at 197 and ≈217 nm attributed to the significant aromatic stacking between the phenyl rings of the tetraphenylalanine. The broad peak maximum at 217 nm exhibited by n-π^* transitions resulting from above aromatic interactions as documented by Hamley et al.,[14] further suggests the dominance of such π–π stacking as previously reported by Tzokova et al.,[15] and appears to be overlapping over the typical characteristic negative signal usually observed there for β-sheet.[44,45] Furthermore, this peak maximum was found to become more prominent in case of their conjugates (Figure 6B) possibly due to enhanced aromatic stacking interactions in their acquired

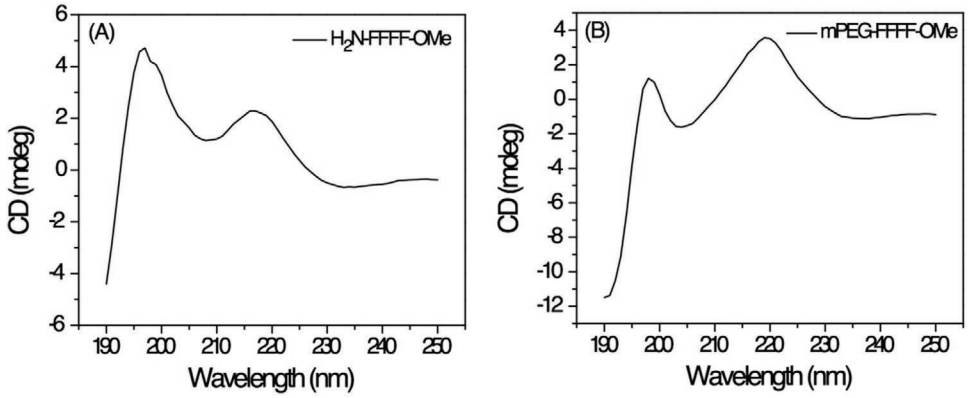


Figure 6. A) CD spectra of native peptide **11** (c = 0.1 mg mL^{-1}) and B) corresponding peptide–polymer conjugate **9** (c = 0.2 mg mL^{-1}) in H_2O/THF (8/2, v/v) solution.

fibrillar structure. The other peptide **12** and its conjugate **10**, (CD, Figure S26, Supporting Information) showed the presence of positive maxima at 197 nm (similar to above moieties), however only the poor quality spectra were obtained due to the unavoidable light scattering problem, caused by the formed fibers, possibly further explained due to the high aggregation behavior of the LVFF-based amyloidogenic peptide constituent.[23]

4. Conclusions

In summary, effective synthetic methodologies have been documented to readily prepare brominated peptide derivates and corresponding PEG-based polymer–peptide conjugates, typically exemplified as mPEG-LVFF-OMe and mPEG-FFFF-OMe by introducing thio-bromo based "click" reaction. The resulting tailor-made supramolecular conjugates were extensively purified utilizing preparative GPC (with yields of 61% and 64%, respectively), and their structural confirmation were further evidenced via different spectrometric studies, suggesting the successful chain-end modification of polymers with ligation of peptides. Self-assembly studies revealed the alteration in morphology of the native peptides upon their PEGylation to form β-sheet enriched fibrillar structures in H_2O/THF (8/2, v/v), with an average diameter of 20 and 17 nm, by placing the peptidic entities to their core with a PEG-based outer shell due to the amphiphilic characteristics of the conjugates. In the endeavor of exploiting the above developed methodology, currently we are synthesizing broad range of functional polymer–peptide conjugates to facilitate cross-linked fibrillar architecture or multi-functional fibrils for various applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

click chemistry, fibrils, functionalization of polymers, peptides, self-assembly

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