Efficient Synthesis and Characterization of Monoprotected Symmetrical Poly(Ethylene Glycol) Diamine

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Solid-phase synthesis of monoprotected poly(ethylene glycol) (PEG) diamine is demonstrated. 2-Chlorotrityl chloride (2-CTC) resin was used as a solid support for inducing monofunctionalization using excess PEG diamine. Fmoc-monoprotected PEG was prepared from 2-CTC resin and fully characterized by high-performance liquid chromatography and electrospray ionization mass spectrometry. We envision that this strategy will provide an efficient method of preparing various kinds of heterobifunctional PEG molecules without a need for further purification steps.

Keywords: Monofunctionalization, Solid-phase synthesis, 2-Chlorotrityl chloride resin, Poly(ethylene glycol), Fmoc group

Introduction

Poly(ethylene glycol) (PEG)-derived polymers have been widely used in a variety of research fields including tissue engineering and drug delivery systems.[1–3] It has been reported that PEGylation improves the quality of various kinds of materials used for biomedical application in such way of increasing protein/peptide stability, promoting efficient drug delivery, and increasing biocompatibility toward nonfouling medical devices.[4–7] Versatile functional groups at the terminal sites of PEG polymers enable the attachment of various biomolecules such as proteins, nucleic acids, and carbohydrates.[8–11] Of the numerous functional PEG polymers, heterobifunctional PEG derivatives are often used as cross-linkers or spacers between two biomolecules.[12–14] The monofunctionalization of symmetric bifunctional PEG derivatives by solution-phase method results in a mixture of the desired monofunctional product as well as bifunctional substituted side product.[15,16] This leads to a low purity synthesis and the resulting mixture needs to be purified by chromatography. Solid-phase synthesis (SPS) can be used to overcome the inefficiency of the solution-phase-based monofunctionalization of the symmetrical molecules. Low density of functional groups on the solid matrices can induce monofunctionalization by using excess bifunctional reagents. After a single functional group of the symmetric bifunctional molecule is attached to the solid matrix, the remaining functional group at another terminal can be modified using various chemical reagents. The modification would be more versatile if the cleavage condition from the solid matrix is mild. The cleavage condition of peptide products from the 2-chlorotritylchloride functionalized polymer support (2-CTC resin) is known to be mild (1% trifluoroacetic acid [TFA], 10% acetic acid, and 10% trifluoroethanol).[17–19] Hence, the 2-CTC resin can be considered as an ideal polymer matrix for the monofunctionalization of symmetric bifunctional PEG polymers. Oliver *et al*. overcame the problems of multifunctionalized by-products and difficult purification steps by utilizing solid-phase assisted strategy.[20] In their work, they reported monofunctionalization of cyclic polyamines including cyclen, cyclam, and piperazine. However, diamines of flexible polymer, which are prone to be bridged within polymer matrices, have not been tried for monofunctionalization. Therefore, we suggest a method for the monofunctionalization of Jeffamine, a widely used PEG diamine derived polymer, using 2-CTC resin as the solid matrix.

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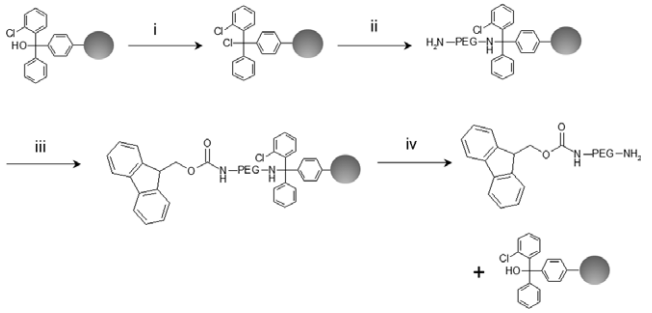
Experimental

Materials. 2-Chlorotritylalcohol (2-CTA) resin was purchased from BeadTech (Seoul, Korea). 9-Fluorenylmethyloxycarbonyl-glycine (Fmoc-Gly-OH) and Fmoc-chloride (Fmoc-Cl) were purchased from GL Biochem (Shanghai, China). All other chemicals were obtained from Sigma (St. Louis, MO, USA) or Aldrich Chemicals (Milwaukee, WI, USA). All solid-phase reactions were performed in LibraTube with filters purchased from HiPep Laboratories (Kyoto, Japan) and with a programmable rotator (Multi Bio RS-24) purchased from Biosan (Riga, Latvia).

High-Performance Liquid Chromatography Analysis. UV absorbance data were obtained with a UV–Vis spectrophotometer (UV-1601, Shimazu, Japan). High-performance liquid chromatography (HPLC) was performed on an



Scheme 1. Mono-functionalization of Jeffamine using 2-CTC resin. Reagents and conditions: (i) $SOCl_2$ (3 eq.) in DCM, rt., 3 h; (ii) Jeffamine 600 (5–20 eq.) and DIPEA (5–20 eq.) in DMF, rt., 4 h; (iii) Fmoc-Cl (3 eq.) and DIPEA (6 eq.) in DMF, rt., 3 h; (iv) 5% (v/v) TFA in DCM, rt., 1 h.

Agilent HPLC 1260 infinity system. Jeffamine and Fmoc-Jeffamine were modified with 10 eq. of dansyl chloride and *N*,*N*-diisopropylethylamine (DIPEA) in *N*,*N*-dimethylformamide (DMF) prior to performing HPLC. The general HPLC method was as follows: Agilent C18 column; 4.6 mm × 250 mm; 5 μm; 1.0 mL/min; 0 to 20 min gradient from 100% aqueous H_2O (0.1% TFA) to 70% CH_3CN (0.1% TFA); 20 to 25 min gradient from 70% CH_3CN (0.1% TFA) to 100% aqueous H_2O (0.1% TFA); UV absorbance at 254 nm. Electrospray ionization mass spectrometry (ESI-MS) analyses were performed on an LC/MS system LCQ (Thermo Finnigan, Waltham, MA, USA).

Fmoc Quantitation. A series of concentrations of Fmoc-Gly-OH were treated with 20% piperidine in DMF for 1 h to prepare a calibration curve. The correlation between absorbances recorded using UV–Vis spectrophotometer (λ = 301 nm) vs. concentrations of dibenzofulvene was plotted and the equation of the curve was obtained as below:

Concentration of Fmoc groups (μM) = 135.41 × Absorbance (A.U.) – 13.22.

To calculate the number of Fmoc groups on the resin, the amount of resin was precisely measured (ca. 30 mg). The resin was swollen in DMF (1 mL) for 5 min, prior to the addition of 20% piperidine in DMF (1 mL). The tubes were rotated for 1 h with 20% piperidine in DMF and the supernatant was used for Fmoc quantitation.

Preparation of 2-CTC Resin. The 2-CTA resin (2 g, initial hydroxyl loading level: 1.43 mmol/g) was suspended with dichloromethane (DCM) in a fritted reaction tube. Thionyl chloride (312 μL, 4.29 mmol) in DCM (20 mL) was added to the tube and rotated with a rotator for 3 h. The resin was washed with DCM (×3) and methanol (MeOH) (×3) and dried *in vacuo*. The loading density of the 2-CTC resin was calculated as 0.23 mmol/g using the quantitation of Fmoc-Gly loaded resin.

Synthesis of Fmoc-Jeffamine. Jeffamine and DIPEA (5, 10, 15, 17, and 20 eq., each to the original chloride in the 2-CTC resin) in DCM (3 mL) was added to the 2-CTC resin (50 mg) in each tube and rotated with a rotator for 4 h. Each resin was washed with DCM (×3) and MeOH (×3). Fmoc-Cl (3 eq.) and DIPEA (6 eq.) in DCM were then added to the tubes and rotated for 3 h. Each resin was washed with DCM (×3) and MeOH (×3) and dried *in vacuo*. To calculate the yield of Fmoc-Jeffamine, 5% TFA in DCM (2 mL) was added to the resin which was reacted with 10 eq. of Jeffamine and the reaction tube was rotated for 1 h. The solution was collected and evaporated after the resin was filtered out. The yield of TFA salt of the Fmoc-Jeffamine ED-600 was 82% (5.3 mg) based on the amount of chloride groups in the 2-CTC resin. The cleaved product was used for ^{1}H NMR, HPLC, and ESI-MS analyses.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89(d, 2H), 7.69(d, 2H), 7.41(t, 2H), 7.33(t, 2H), 4.28(d, 2H), 4.21(t, 1H), 3.3~3.6(m, 50H), 1.25(t, 5H), 1.0–1.2(m, 14H).

Results and Discussion

Our synthetic strategy for obtaining monoprotected PEG diamine is summarized in Scheme 1. 2-Chlorotrityl-chloride (2-CTC) resin was prepared from 2-chlorotrityl-alcohol (2-CTA) resin by activation with $SOCl_2$.[21,22] *O*,*O*′-Bis(2-aminopropyl) polypropylene glycol-block-polyethylene glycol-block-polypropylene glycol 500 (Jeffamine ED-600, Sigma-Aldrich, St. Louis, MO, USA) was loaded onto the activated 2-CTC resin by mixing the resin with various amounts of Jeffamine. The excess amount of unreacted Jeffamine was filtered out. 9-Fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) was then coupled to the amino group on the other side of the Jeffamine. Treatment with 5% TFA in DCM cleaved the monoprotected product from the resin and the product was characterized using HPLC and ESI-MS.

The initial loading level of 2-CTC resin was calculated as 0.23 mmol/g by the titration of Fmoc-Gly loaded resin. Solution-phase Fmoc-cleavage of Fmoc-Gly-OH was performed to obtain a calibration curve for the UV absorbance at 301 nm vs. the dibenzofulvene-piperidine adduct concentration.[23] In the present work, the loading capacity of the 2-CTC resin was adjusted to a lower level than had been previously reported (0.5–0.8 mmol/g) since a higher loading density might increase the coupling of both the amino groups in Jeffamine to the 2-CTC resin.[21,22]

In order to evaluate the quantity of loaded Jeffamine to the resin, the coupling reaction was performed with various eq. of Jeffamine to the chlorotrityl group in the 2-CTC resin. After the coupling of the Fmoc group to the leftover primary amino group of the loaded Jeffamine, the Fmoc group was released by treating with 20% piperidine in DMF, followed by quantification of the amino group by measuring UV absorbance at 301 nm. As shown in Figure 1, the reaction with more than 10 eq. of Jeffamine resulted in ca. 0.19 mmol/g of loaded Jeffamine, which did

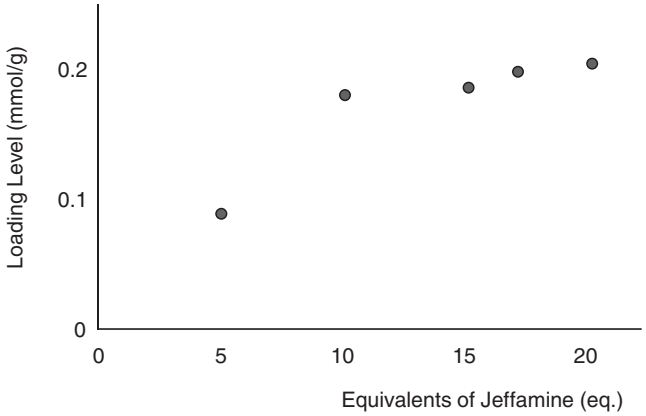


Figure 1. Quantification of loaded Jeffamine on the 2-CTC resin. Jeffamine was used with 5–20 eq. to the functional groups on the 2-CTC resin.

not increase to a significant number with up to 20 eq. of Jeffamine.

Our hypothesis is based on the pseudo-dilution effect[24]: the distance between the 2-CTC groups within the resin matrix is far to prevent both amino groups of the same Jeffamine molecule from bridging, and excess Jeffamine will also accelerate the process of single amino group attachment onto the resin. The cleaved product, by 5% TFA in DCM, from the Fmoc-Jeffamine-loaded 2-CTC resin was analyzed using HPLC. Two chromatograms were obtained from pristine Jeffamine and Fmoc-Jeffamine synthesized by 10 eq. of Jeffamine-reacted 2-CTC resin and compared to each other. As pristine Jeffamine does not possess any group for a UV detector, it was derivatized by dansyl chloride prior to the HPLC analysis. As previously reported, multiple peaks from the oligomeric mixture were identified for Jeffamine-$(dansyl)_2$ (Figure 2, red line).[25] In spite of the complex pattern of the mixed-oligomeric structure, the peaks from monofunctionalized Jeffamine clearly can be observed, as shifted from those of the original dansyl-

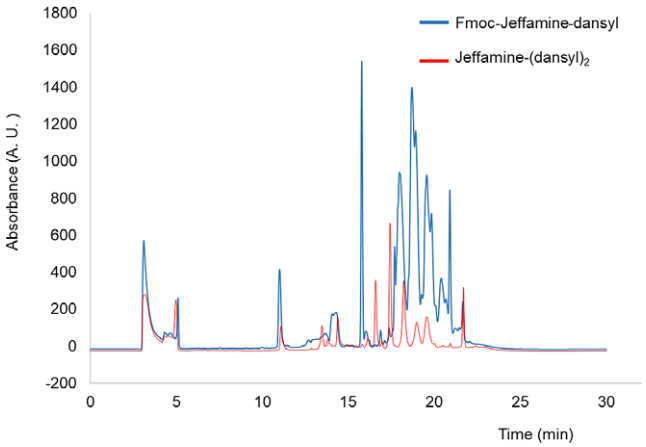


Figure 2. HPLC data of Fmoc-Jeffamine-dansyl (blue line) and Jeffamine ED-600-$(dansyl)_2$ (red line). The analytes were labeled with dancyl chloride for visualization in the spectra.

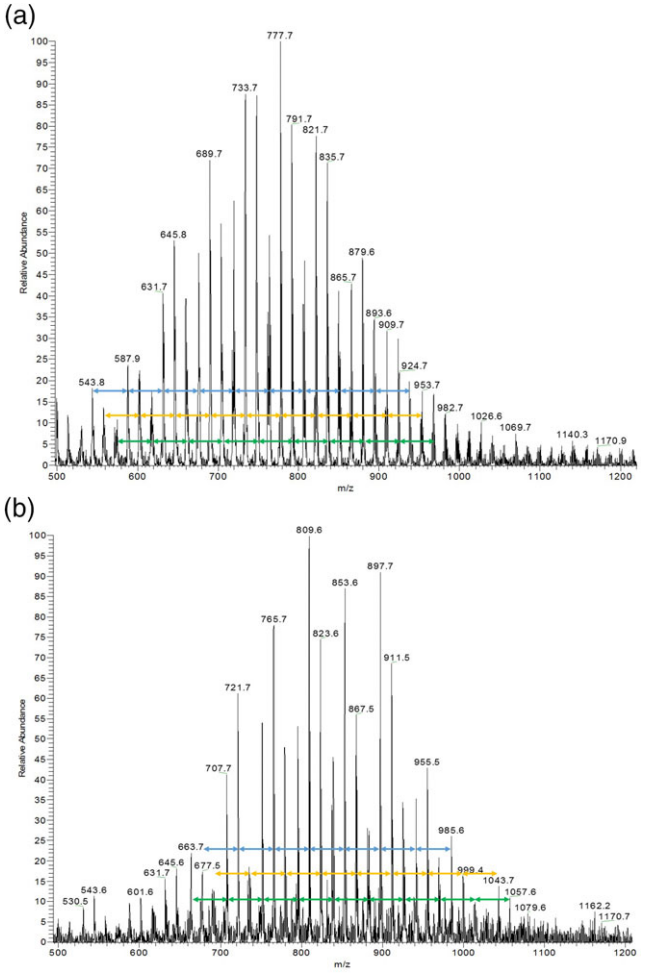


Figure 3. ESI-MS data of (a) Jeffamine ED-600 and (b) Fmoc-Jeffamine ED-600. The mass peaks of Fmoc-Jeffamine shifted from those of Jeffamine increased by 222 m/z corresponding to the molecular weight of Fmoc group. Three colored arrows (blue, orange, and green) represent each corresponding Fmoc functionalization.

coupled Jeffamine (Figure 2, blue line). It was also noted that no peaks for Jeffamine were found in the chromatogram from the Fmoc-Jeffamine. The retention time of the Fmoc-Jeffamine-dansyl was delayed by ca. 1.5 min compared with Jeffamine-$(dansyl)_2$.

The ESI-MS results of the crude cleaved product also showed the mono-Fmoc functionalized Jeffamine (Figure 3). The oligomeric mixture was found in the ESI-MS spectrum of Jeffamine.[26] The cleaved products after Fmoc monoprotection clearly exhibited a mass increase by the exact molecular weight (+222 m/z) of the Fmoc group. The peaks from Jeffamine at 499 + 44n (blue), 513 + 44n (orange), 527 + 44n (green) (Figure 3(a)) were shifted to 721 + 44n (blue), 735 + 44n (orange), 749 + 44n (green) after Fmoc functionalization (Figure 3(b)). Overall, our strategy of monofunctionalization using 2-CTC resin was proven to be valid from these results.

Conclusion

In summary, we suggest a facile method for the mono-functionalization of the symmetric bi-functional chemicals using 2-CTC resin. The reaction between 10 eq. of Jeffamine and 2-CTC resin was the optimal condition for the loading of Jeffamine onto the resin. The Fmoc-mono-protected PEG was prepared from 2-CTC resin and characterized by HPLC and ESI-MS. In the future, this re-use of the reagents will be discussed. We envision that the cleaved trityl resin can be regenerated with the treatment of $SOCl_2$. The majority of the non-reacted Jeffamine is recyclable for the next round of mono-protection reaction as well. We hope that this mono-functionalized PEG molecule will be widely used for the various research areas including tissue engineering and drug delivery system.

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