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Solid Phase Stepwise Synthesis of Polyethylene Glycol

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Abstract: Polyethylene glycol (PEG) and derivatives with eight and twelve ethylene glycol units were synthesized by stepwise addition of tetraethylene glycol monomers on a polystyrene solid support. The monomer contains a tosyl group at one end and a dimethoxytrityl group at the other. The Wang resin, which contains the 4-benzyloxy benzyl alcohol function, was used as the support. The synthetic cycle consists of deprotonation, Williamson ether formation (coupling), and detritylation. Cleavage of PEGs from solid support was achieved with trifluoroacetic acid. The synthesis including monomer synthesis was entirely chromatography-free. PEG products including those with different functionalities at the two terminals were obtained in high yields. The products were analyzed with ESI and MALDI-TOF MS and were found close to monodispersity.

Introduction

Polyethylene glycol (PEG) has several attracting structural, physical and biophysical properties, which include stable, flexible and neutral backbone, and good solubility in water and many other solvents. It is biocompatible, nonimmunogenic and nonantigenic. For these reasons, it has been widely used in the pharmaceutical industry and biomedical research for PEGylating biomacromolecules to improve drug solubility and stability, and reduce immunogenicity and dosing frequency.[1] The PEG used for such applications requires a size of 4K Da to have the desired biophysical effects, and the most commonly used size is 40K Da.[2] Besides applications as PEGylating agents in pharmaceutics, PEG and its derivatives are also used frequently in other areas including surface science,[3] nanotechnology,[4] carbon nanotube functionalization,[5] organic-inorganic hybrid materials[6] and bioconjugation.[7] In some of these cases, the sizes can be smaller than 1K Da and the compounds are more suitable to be called oligoethylene glycol (OEG), even though in some articles including this one they are still called PEG for convenience.

PEGs are typically made by polymerization of ethylene oxide. Due to the randomness of the process, the products are polydisperse admixtures of many different molecules of varying length and molecular weight.[8] Although admixtures are currently used for PEGylation of pharmaceuticals, they are not ideal for reasons such as difficulty to achieve consistent composition for products from different batches, difficulty to characterize the products, losses of intended biological activities of pharmaceutical ingredient due to heterogeneity of physical properties caused by the different sizes of the PEG tags, and challenges to obtain FDA approval.[2c,2d,8c,9] As a result, significant efforts have been made to synthesize monodisperse PEGs via stepwise organic synthesis.[2b-d,8-9,10] Currently, the longest PEGs that can be synthesized using stepwise organic synthesis are those with about 45 ethylene glycol units, which corresponds to molecular weights of about 2K Da.[2c,8b,8c] Challenges for the synthesis of longer PEGs include low efficiency of Williamson ether formation reaction when applied to long PEG substrates, depolymerization of PEG during the ether formation process,[8a,10i] and the lack of technology to separate PEGs of different lengths.[2b,2c,8b,8c] For short PEG (e.g. 8 – 40 ethylene glycol units) synthesis, one of the major problems is the need of multiple column chromatography to purify monomers, intermediates and the final product. This problem has severely

impeded the practical use of stepwise synthesis for inexpensive large scale monodisperse PEG production.[2c,8b,10b,10c,10e]

In this paper, we report our results on using solid phase technology for stepwise synthesis of monodisperse PEGs and their derivatives. We envisioned that using solid phase technology, we would be able to avoid all chromatographic purifications. In addition, due to the ease of purification of intermediates and product by washing, we could use excess reactants to overcome the low efficiency of the key Williamson ether formation reaction. Further, because excess reactants could be used and the ether formation reaction could be performed at lower temperature and in a shorter time, the anionic PEG depolymerization reaction, which generates shorter PEGs that are impossible to remove from product,[8a,10i] could be completely suppressed. Our results are encouraging. Significantly, the monomers used for the synthesis did not require chromatographic purification and impure ones could be used directly, the Williamson ether formation reaction could proceed at room temperature with useful rates, the anionic PEG depolymerization might be completely suppressed, all intermediates in the solid phase synthesis were purified by washing, and pure final products were obtained without chromatography. Using the technology, we were able to synthesize close-to-monodisperse $(PEG)_8$ and $(PEG)_{12}$ and their derivatives with high yields.

Results and Discussion

The solid phase PEG synthesis design

The solid phase synthesis route is illustrated in Scheme 1. The $(PEG)_4$ (tetraethylene glycol) derivative **1**, which can be synthesized from the inexpensive $(PEG)_4$, was chosen as the monomer for the stepwise synthesis. The Wang resin (**2**), which is 1% divinylbenzene cross-linked polystyrene dotted with 4-benzyloxy benzyl alcohols, was chosen as the solid support. The loading of the resin can be as high as 1.0 mmol/g and the resin can be purchased at remarkably low prices. A typical synthesis cycle consists of three steps, which are deprotonation, coupling and detritylation. In the first step, the resin is deprotonated with a base such as *t*BuOK to convert **2** to **3**. In the second step, the alkoxide **3** is coupled with monomer **1** via the Williamson ether formation reaction to give **4**. In the third step, the 4,4'-dimethoxytrityl (DMTr) protecting group in **4** is removed with a dilute acid to give **5**. The cycle is then repeated to give **6**. At this stage, the PEG is asymmetric, which is highly desirable in most applications but more challenging to make using other methods.[2c,8b,10c] However, if it were cleaved from the solid support, it would become symmetric. As a result, before cleavage, **6** should be functionalized to give **7**, in which the R group must be stable under the acidic conditions needed for the cleavage. After functionalization, the product is cleaved from the resin with a strong acid such as TFA to give asymmetric PEG **8** (Scheme 1).

Analytical methods for monitoring the completeness of solid phase PEG synthesis reactions

To successfully implement the solid phase PEG synthesis design to make monodisperse PEGs without any chromatographic purifications, each of the three steps in all synthesis cycles must be 100% complete and devoid of any side reactions that alter the length of the growing PEG. Therefore, analytical methods that could accurately monitor the progress of the reactions were needed. During the course of our studies, we successfully evolved such methods. For steps 1-2, in the first cycle (converting **2** to **4**, Scheme 1), because the reactions were found easy to complete, we did not develop any method to check their completeness. For subsequent cycles, since the Williamson coupling reaction is not highly efficient and the efficiency of the reaction drops with increasing length of PEG substrates as demonstrated in solution phase synthesis,[2b,8b] monitoring the progress of the reaction to ensure complete reaction before carrying out step 3 was crucial. Otherwise, the final PEG product would not be monodisperse. Therefore, before next detritylation, a small portion of resin (e.g. 4 mg) was treated with TFA, and the cleaved PEG was analyzed with ESI-MS (Method A). If only peaks corresponding to expected PEG product appeared, the reaction was complete. If peaks corresponding to PEG that did not undergo the coupling reaction appeared, the reaction

was incomplete. If the latter happens, the resin was subjected to deprotonation and coupling again. This was repeated until un-coupled PEG peaks completely disappear in MS.

For step 3 (e.g. converting **4** to **5**, Scheme 1), complete detritylation before going to the next deprotonation and coupling reactions was also required for obtaining monodisperse PEG product. Otherwise, the un-detritylated PEG would fail to couple in the current cycle and gave PEGs shorter than the final PEG product at the end of the solid phase synthesis. Since the trityl cation side product of the detritylation reaction had an intense red to light yellow color depending on concentration, it was possible to visualize the progress of the reaction. However, this was not enough to ensure complete detritylation to the degree necessary for obtaining monodisperse PEG at the end of the synthesis. For this reason, we evolved two methods to monitor detritylation more accurately. One was to analyze the last batch of detritylation solution with ESI-MS (Method B). Specifically, a portion of the last batch of detritylation solution was concentrated to dryness and dissolved in methanol (e.g. 2 ml), and the methanol solution was analyzed with ESI-MS. If DMTr cation was observed, the detritylation reaction was incomplete. In this case, detritylation was repeated. In our studies, we tested the sensitivity of ESI-MS to detect DMTr cation using standard DMTrCl solutions, and found that the cation could be reliably observed when the concentration of DMTrCl solution was above 0.001 ng/µl. If DMTr cation was not observable in ESI-MS, to further ensure complete detritylation, we intended to analyze the resin using an additional method (Method C). This method involved treating a small portion of the resin (e.g. 6 mg) with a mixture of excess *t*BuOK and BnBr, which alkylated the detritylated PEGs but not un-detritylated ones. After cleaving with TFA, the products were analyzed with ESI-MS. If MS showed that all PEGs were benzylated, the detritylation reaction was complete. If un-benzylated PEG appeared and treating the resin with *t*BuOK and BnBr further did not reduce un-benzylated PEG, detritylation was incomplete (Method C). In the latter case, detritylation should be executed more times.

Synthesis of $(PEG)_{12}$ on a DNA/RNA synthesizer

To find out the feasibility of the solid phase technology and quickly identify the conditions for each step of the synthesis cycle, we started our studies using an ABI-394 DNA/RNA synthesizer on a small scale (275 mg resin, 0.9 mmol/g loading, 0.25 mmol PEG product) using a similar procedure we reported previously for the synthesis of peptides and peptide nucleic acids using the same synthesizer.[11] A homemade synthesis column was used as the reaction vessel, which was similar to the commercially available 10 µmol DNA synthesis column for the synthesizer except that it had a larger capacity (10 ml, Figure S1). To agitate the reaction mixture, the column was connected to the synthesizer with relatively long lines so that it could be placed on an orbital shaker. Using the setup, the reagents and solvents including 0.1 M *t*BuOK THF solution, 0.31 M THF solution of monomer **1**, TCA solutions, DCM and THF could be delivered to the synthesis column conveniently from the bottles on the synthesizer.

The required monomer **1** is a known compound, and was conveniently synthesized from $(PEG)_4$ in two steps by tritylation with DMTrCl and tosylation with tosyl chloride (TsCl).[8b,12] The products were purified with flash column chromatography. Using the procedure, we were able to prepare **1** at scales of 10 grams with reasonable efforts. The solid phase synthesis started with swelling the resin in THF for about 20 minutes, which was then removed using the synthesizer's reverse flush function. Deprotonation was achieved using 0.1 M *t*BuOK THF solution at room temperature for 30 minutes. This converted **2** to **3** (Scheme 1). The excess base and solvent were removed by reverse flush and rinsing the resin with dry THF. Coupling was achieved by delivering a 0.31 M solution of monomer **1** in THF to the column and shaking the column at room temperature for seven hours. This converted **3** to **4**. After coupling, the excess monomer **1** was conveniently recovered by delivering to a bottle on the synthesizer, which contains a basic solution (e.g. 5% Na_2CO_3). Under these conditions, the recovered **1** had good purity with only slight contamination by the side product resulted from β-elimination of **1** as indicated by TLC (Figure S2). As stated earlier, we did not design a procedure for monitoring the completeness of the deprotonation and coupling steps for the first synthesis cycle. However, to obtain monodisperse PEG at the end of solid phase

synthesis, complete conversion of **2** to **4** must be achieved. Otherwise, the remaining hydroxyl group in **2** would participate in the coupling reaction in the subsequent cycles, and gave shorter PEGs. With these considerations, we repeated the deprotonation and coupling steps two more times under similar conditions. The resin was then washed thoroughly and subjected to detritylation (converting **4** to **5**), which was achieved using 3% trichloroacetic acid (TCA) in DCM and 3% TCA in toluene. The acid solutions were delivered to the column and then removed by reverse flush. We monitored the progress of the reaction with ESI-MS using Methods B and C, and found that complete detritylation could be achieved with 3% TCA in DCM for 10 times followed by 3% TCA in toluene for five times. This concluded the first synthesis cycle and the resin-$(PEG)_4$ (**5**) was obtained.

In the second synthesis cycle for converting **5** to **10** (Scheme 2), the deprotonation and coupling steps were carried out under the same conditions used in the first cycle. After coupling four times, ESI-MS analysis using Method A indicated that the reaction was complete. Detritylation of **9** to give **10** was achieved under similar conditions used in the first cycle except that the resin was further treated with a cocktail containing 5% TCA, 1% triisopropylsilane (TIPS), 5% MeOH, 5% thioanisole, and 5% phenol in DCM (Cocktail A) five times. ESI-MS analyses using Methods B and C indicated that the additional treatments were needed for 100% conversion of **9** to **10**. In the third cycle for converting **10** to **12** (Scheme 2), the deprotonation and coupling steps were conducted five times. ESI-MS analysis using Method A indicated complete reaction. Complete detritylation was achieved using 5% TCA in toluene (10 times), Cocktail A (10 times) and Cocktail B (20% TCA, 1% TIPS, 5% MeOH, 5% thioanisole, and 5% phenol in DCM; 10 times). We were aware that at this stage the detritylation conditions were quite harsh, and the PEG could be prematurely cleaved from the resin. We carefully analyzed the detritylation solutions with ESI-MS, and found that no detectable amount of PEG fell off from the resin, which indicated that the 4-benzyloxy benzyl alkyl ether linkage was completely stable under these conditions.

Cleaving the PEG **13a** from the resin **12** was found fairly straightforward even though we were concerned that the 4-benzyloxy benzyl alkyl ether linkage might be shielded by the PEG and the cleavage reaction might be difficult.[8b] A portion of **12** (128 mg out of 384 mg) was treated with pure TFA at room temperature for two hours (Scheme 2). The resin was washed with THF, and the washes and TFA were combined. The volatiles were evaporated, and the residue was co-evaporated with water. Pure PEG **13a** was obtained as a light yellow oil. ESI-MS (Figure 1) and ^1H and ^{13}C NMR (Figures S3-4) analyses indicated that the synthesis was successful. In ESI-MS, we did not see any $(PEG)_4$ and $(PEG)_8$, which indicated that all the reactions from **2** to **12** were 100% complete. One impurity was $(PEG)_{11}$, which was most likely from the starting $(PEG)_4$ used for making monomer **1** because ESI-MS analysis of $(PEG)_4$ also showed small amount of $(PEG)_3$ (Figure S5). This observation was consistent with previous reports, which also noted contamination of commercial $(PEG)_4$ with $(PEG)_3$.[8b] Another possibility for the appearance of $(PEG)_{11}$ was depolymerization of the deprotonated PEG intermediates[8a,10i] but this possibility was low given the mild conditions we used in the coupling step.[8a] Other than containing minute amount of $(PEG)_{11}$, PEG **3a** was highly pure. We also analyzed the sample with MALDI-TOF-MS (image in ESI). The $(PEG)_{11}$ impurity was less obvious. The amount of **13a** obtained was 36 mg, which corresponds to an 81% overall yield based on 33% (128 mg out of 384 mg) of 275 mg resin and 0.9 mmol/g loading of resin **2**. However, in theory, since all reactions from **2** to **12** were 100%, the overall yield should be quantitative (45 mg). The discrepancy between theoretical and experimental yields could be attributed to incomplete cleavage of **13a** from **12**, but we subjected the remaining resin to additional cleavage and no more PEG was obtained. Other reasons for the lower than theoretical yield could be loss of resin during the synthesis, the consumption of portions of resin for ESI-MS analysis, loss of PEG after cleavage and lower than stated loading of the resin.

Synthesis of longer PEGs and PEG derivatives

After successful synthesis of $(PEG)_{12}$, we tested to couple **12** with **1** to synthesize longer PEGs under similar conditions used in the first three cycles. For the synthesis of $(PEG)_{16}$, after five rounds of coupling, ESI-MS analysis using Method A showed 86% $(PEG)_{16}$ and 14% $(PEG)_{12}$ (Figure S6). For $(PEG)_{20}$, the

resin carrying $(PEG)_{16}$ and $(PEG)_{12}$ was coupled with **1** five times. ESI-MS analysis using Method A showed 77% $(PEG)_{20}$ and 23% $(PEG)_{16}$ (Figure S7). Although these results were significant because the longer PEGs could be easily separated from shorter ones using techniques involving tagging followed by chromatography, polymerization or extraction[10c,10e,13] such studies had not been pursued. Instead, it came to our attention that the synthesis of pure $(PEG)_{12}$ and its asymmetric derivatives using a convenient method without any chromatography is highly significant. The compound and some of its derivatives are known[7d,8b,10e,14] and commercially available, but they could have been made using procedures involving multiple column chromatography purifications and therefore are highly expensive. With this consideration, we decided to pursue the synthesis of derivatives of **13a** and the synthesis of one of them on a multiple gram scale.

A significant advantage of the solid phase method is that the difficult-to-access asymmetric PEG derivatives can be easily synthesized.[2c] To demonstrate this, we prepared compounds **13b**-**e** (Scheme 3). The asymmetric $BnO(PEG)_8$ (**13b**) was synthesized by simply soaking **10** in a THF solution of BnBr and *t*BuOK, which gave **14**. Treating **14** with TFA gave the product **13b**. The product was purified by precipitation from THF with Et_2O. Derivatization of **13b** to **13d** was achieved in solution using the excellent reaction conditions for tosylation of alcohols first reported by Ouchi and co-workers.[15] Thus, treating **13b** with slightly excess TsCl in the presence of NaOH in a solvent mixture of THF and water at room temperature quantitatively converted the starting material to **13d**. The product was purified by partition between water and ether, passing through a Celite pad and precipitation from diethyl ether with hexanes. For the synthesis of **13c**, we coupled **10** with the monomer **15** to give **16** directly (Scheme 3) instead of using the route involving benzylation of **12**. The required additional monomer **15** was prepared on small scale according to literature procedure.[16] Cleavage of **13c** from **16** and tosylation of **13c** to give **13e** were achieved as described for **13b** and **13d**. As described below, using the solid phase method, we actually did not need to use pure monomers **1** and **15**, and the monomers did not need any chromatographic purification. If this were considered, preparing **13b**-**e** were all entirely chromatography-free. The products were analyzed with ESI-MS and ^1H and ^{13}C NMR (Figures S8-15). As shown in ESI-MS (Figure 2), compounds **13c** and **13e** were devoid of derivatives of $(PEG)_4$ and $(PEG)_8$, which could be formed from incomplete reactions during solid phase synthesis, were not observable. Derivatives of $(PEG)_{11}$, which could be from the $(PEG)_3$ in the starting $(PEG)_4$ or less likely from depolymerization of PEG under basic conditions,[8a,10i] could be observed, but the amounts were minimal. Similar results were observed for **13b** and **13d** (Figures S16-17). We also analyzed **13b**-**e** with MALDI-TOF-MS (images in ESI). The impurities arisen from PEG depolymerization were less obvious.

Synthesis of $BnO(PEG)_{12}$ manually on a larger scale in a peptide synthesis vessel

After establishing feasibility of the solid phase PEG synthesis method and successful identification of reaction conditions using small scale synthesis on an automated synthesizer, to demonstrate the practical usefulness of the method, we decided to prepare the asymmetric $BnO(PEG)_{12}$ (**13c**) on a significantly larger scale (theoretically 6.3 mmol, 4.02 grams of product). The synthetic route involving coupling **10** with **15** was used (Scheme 3). Relatively large amount of monomers **1**,[8a,8b,12,16f] and **15**[16] was required for the synthesis and they were prepared using procedures similar to reported ones with slight but important modifications. Importantly, we did not purify any of the compounds with column chromatography, and impure monomers were used directly for the solid phase synthesis.

For the synthesis of **1** (Scheme 4), the scale was at the level of 147 mmol of DMTrCl, which was the limiting starting material for the first step of the synthesis. With this scale, theoretically 96 grams of **1** could be produced at the end of monomer synthesis. Slow addition of a DMTrCl (1 equivalent) solution in pyridine to the solution of $(PEG)_4$ (5 equivalent) in the same solvent gave the desired $DMTrO(PEG)_4$ and the symmetric ditritylated $(PEG)_4$ side product **17**. Other materials in the crude reaction mixture include pyridine, excess $(PEG)_4$, and pyridinium chloride. These impurities were readily removed by evaporation and partition. After these simple manipulations, TLC (Figure S18) and ^1H NMR (Figure S19) indicated that the

desired product DMTrO(PEG)$_4$ was only contaminated with small quantities of **17**. Compound **17** could be removed with flash column chromatography, but at this scale, it was inconvenient and therefore not pursued. Because the next tosylation reaction and later the coupling reaction on solid phase using **1** did not require accurate amount of materials, we did not determine the molar ratio of **1** and **17**, and simply treated the mixture as pure **1** for calculation purpose even though it was possible that about 5 mol% **17** were present. Using the procedure involving TsCl and NaOH,[15] DMTrO(PEG)$_4$ was converted to monomer **1** with quantitative conversion according to TLC. After several simple manipulations including partition and precipitation, monomer **1** was only contaminated with **17** according to TLC (Figure 3) and ^{1}H NMR (Figure S20). Again, **1** and **17** were not separated and the mixture was used directly for solid phase PEG synthesis. For the synthesis of **1**, it is notable that due to the use of the slow addition technique, we were able to keep the ditritylated side product **17** at a minimum while using only five equivalents (PEG)$_4$. In contrast, in some previous reports concerning the synthesis of DMTrO(PEG)$_4$ and similar compounds, 10 equivalents (PEG)$_4$ were used and the amount of symmetric side product could be higher.[8b,10b,10c]

The synthesis of monomer **15** was carried out at the scale of 208 mmol NaH, which was the limiting starting material of the first step of the synthesis (Scheme 4). With this scale, theoretically 73 grams of **15** could be produced at the end of monomer synthesis. Although synthesis of the compound has been reported previously,[16] to minimize the formation of di-benzylated and depolymerized products [e.g. BnO(PEG)$_3$][10i] and avoid chromatography purification, we modified the conditions, which mainly featured the use of a different ratio of (PEG)$_4$, NaH and BnBr, carrying out alkylation at lower temperature and timely quenching the reaction. We treated four equivalents (PEG)$_4$ in THF with one equivalent NaH at 0 °C. The resulting alkoxide was alkylated with 0.8 equivalents BnBr at 40℃. This gave the mono-benzylated BnO(PEG)$_4$ and small quantities of di-benzylated **18** according to TLC (Figure S21) and ESI-MS (Figure S22). After removing the remaining (PEG)$_4$, NaBr, and THF by simple manipulations such as evaporation and partition, The product BnO(PEG)$_4$ and **18** were not separated, and the mixture were subjected to tosylation as described for the synthesis of **1** (Scheme 4). Without chromatography, monomer **15**, which was contaminated with **18** (TLC, Figure 4; ^{1}H NMR, Figure S23), was used directly for the solid phase PEG synthesis.

For the synthesis of BnO(PEG)$_4$, in reported procedures, more equivalents NaH or other bases were generally used. Under those conditions, the yields of mono-benzylated product were usually around 70% or lower[16a-j] although a few papers reported higher yields.[16k] The product was mostly purified with chromatography to remove the di-benzylated **18** except that in one report, a two-step procedure was used, which enabled chromatography-free production of pure BnO(PEG)$_4$.[17] Compared to known methods, we achieved higher yields of mono-benzylated product and potentially lower percentage of di-benzylated product. In addition, our conditions could have also reduced the depolymerized BnO(PEG)$_3$, which is highly important for stepwise monodisperse long PEG synthesis because a small percentage of shorter monomers will rapidly accumulate in the growing PEG in a repetitive synthesis and render the entire synthesis useless.[8a,10i] The above advantages of the modified conditions can be explained using Scheme 5. With four equivalents (PEG)$_4$ and one equivalent NaH, we should get one equivalent **19** and three equivalent (PEG)$_4$ with some **20**. Benzylating **19** should give the desired product **21**, which could form an equilibrium with **22** in the presence of **19**-**20** and (PEG)$_4$ in the course of the reaction. The intermediate **20** could be benzylated to give **22** or **18**. Intermediate **22** could also be benzylated to give **18**. When the reaction was complete, all BnBr was consumed but significant amount of **19**-**20** and **22** remained because less equivalents BnBr than NaH were used. With a higher molar ratio of (PEG)$_4$ over NaH or other bases than in previous reports, in the course of the reaction, the molar ratio of **22** over **19** (**20** as well) was minimized according to the equation [**22**]/[**19**] = K[**21**]/[(PEG)$_4$] where K is the equilibrium constant, and therefore the chance for the formation of **18** was reduced. The use of less equivalents BnBr than NaH was intended to minimize the formation of the depolymerized product BnO(PEG)$_3$.[10i] With less BnBr, at the stage close to the end of the reaction, the last few molecules of BnBr were expected to be easier to find their reaction partners compared with the case in which equal moles of NaH and BnBr were used. This minimized the life time of **19**-**20** that were converted to **21**, and therefore reduced their chance to depolymerize to **23**, which

could be benzylated to **24** including the highly undesired $BnO(PEG)_3$. Under our conditions, we believe that depolymerization to give $BnO(PEG)_3$ was minimal. The small amount of $BnO(PEG)_3$ in ESI-MS (Figure S22) was likely from the minute $(PEG)_3$ in $(PEG)_4$ starting material (Figure S5). It is important to note that with less equivalents BnBr than NaH, even after the reaction was complete, if it had not been quenched timely, the product **21** would have had more chances to equilibrate to **22**, which could be depolymerized to $BnO(PEG)_3$.[10i] Therefore, the reaction should be closely monitored and quenched once BnBr was consumed. An alternative is to use more equivalents BnBr than NaH, in which case at the end of the reaction, no anionic species remain, and therefore there is no need to quench the reaction timely. However, a drawback is that the remaining BnBr is difficult to be completely removed from product without chromatography.

With procedures for easy access of large quantities of **1** and **15** in hand, the solid phase PEG synthesis virtually has no limitations in terms of scalability. To execute our plan on synthesizing $BnO(PEG)_{12}$ (**13c**) at 6.3 mmol scale, seven grams of Wang resin (0.9 mmol/g loading) was swelled in a simple 100 ml peptide synthesis vessel (Figure S24) in THF. The volume of the swelled resin was about 50 ml. THF was removed, and the resin was treated with 0.2 M *t*BuOK (1.1 equivalents) for 20 minutes at room temperature. After removing the liquids, the resin was rinsed with dry THF, and the solution of monomer **1** (0.31 M, 2 equivalents), which was contaminated with **17** (at calculation, **1** was assumed pure, so the actual concentration and equivalents were slightly lower), was added to the synthesis vessel. The vessel was rotated gently on a rotatory evaporator at room temperature for 24 hours (Figure S24). This gave **4**. The ditritylated **17** was inert during the coupling reaction, and was conveniently removed with the excess **1** by filtration. The recovered **1** (TLC, Figure S25; ^{1}H NMR, Figure S26), which was in the filtrate and contaminated by **17** and the side product resulted from β-elimination of **1**, could be reused, but since we had synthesized large quantities of **1**, this had not been tested. The resin was washed sequentially with THF, THF/H_2O (v/v 1:1) and dry THF, and dried under vacuum. The deprotonation and coupling steps were repeated two more times using 0.8 equivalents *t*BuOK and 1.5 equivalents **1** to ensure complete alkylation of **3**. As in our small scale studies, the completeness of the reaction was not assessed.

For removing the DMTr group on **4** to give **5**, conditions for the small scale studies as described earlier with slight modifications were used (see details in ESI). Deprotonation of **5** and alkylating with **1** to give **9** in the second synthetic cycle were performed under similar conditions in the first cycle. However, after finishing the first two couplings (first coupling, 1.1 equivalents *t*BuOK, 2 equivalents **1**; second coupling, 0.8 equivalents *t*BuOK, 1.5 equivalents **1**; room temperature, 24 hours for each coupling), we carried out ESI-MS analysis using Method A. The result indicated that the reaction had already reached 100% completion. Therefore, the third coupling was not executed. This informed us that the coupling step in the first cycle could be simplified as well. Detritylation of **9** to give **10** was achieved under the similar conditions described for converting **4** to **5** (see details in ESI). The completeness of the detritylation was determined with ESI-MS using Methods B and C. Coupling of **10** with **15** (Scheme 3), which was contaminated with **18**, was carried out using the same procedure described for converting **5** to **9** in this larger scale synthesis. ESI-MS analysis using Method A after two couplings also indicated that the reaction reached completion. It is noted that the recovered excess **15** after the couplings was also of good quality according to TLC (Figure S27) and could be reused. Cleaving **13c** from the resin (**16**) was achieved using TFA at room temperature as described for small scale synthesis. The product (**13c**) appeared as a light yellow thick oil. The amount was 3.22 grams, which corresponds to an 80% overall yield based on a 0.9 mmol/g loading of resin **2**. The lower than 100% yield could be caused by factors described in small scale synthesis of **13a**.

PEG **13c** was analyzed with ESI-MS (Figure 5), and ^{1}H and ^{13}C NMR (Figures S28-29), which indicated that the synthesis was successful. Like the small scale synthesis, we did not see any $(PEG)_4$ and $(PEG)_8$ in ESI-MS. However, small amount of $BnO(PEG)_{11}$ was observable. Again, this impurity may come from $(PEG)_3$ in the $(PEG)_4$ starting material or less likely depolymerization of the deprotonated PEG in the coupling step.[8a,10i] Other than that minute impurity, the product was highly pure.

Additional discussions

Overall, our study has demonstrated that synthesis of monodisperse PEGs using stepwise solid phase technology is feasible. In addition, we have found that the Williamson ether formation reaction can proceed at room temperature with acceptable rates when one reactant is in excess. Carrying out this reaction at room temperature has several significant advantages, which include less likely to form the difficult-to-remove shorter PEGs resulted from depolymerization of deprotonated PEG reactants (refer to **23** in Scheme 5),[8a,10i] less likely for the tosylate reactant to undergo β-elimination, which consumes reagent and potentially cause problems for product purification,[8b] less capital cost for large scale synthesis and automated synthesis, and as it is almost always true that milder conditions give cleaner products. Other significant findings include that the 4-benzyloxy benzyl alkyl ether linker and the DMTr protecting group of the monomer are compatible for the synthesis even though both are acid-sensitive and the linker has to stay intact completely when the DMTr group is removed under acidic conditions repeatedly. The polar *t*BuOK can penetrate into the relatively hydrophobic polystyrene matrix to efficiently deprotonate hydroxyl groups. Deprotonation of PEGs on the solid support with *t*BuOK prior to adding the tosylate monomers does not cause depolymerization of PEGs at all or to any noticeable degree. With a procedure that separates deprotonation and alkylation (i.e. do these sequentially instead mixing the alcohol and tosylate with the base), the tosylate β-elimination side reaction is minimal. The linker can be readily cleaved using TFA even though it might be wrapped by PEGs, which may slow down the reaction.[8b] In addition, we have developed reliable analytical methods for monitoring the progress of the solid phase reactions (i.e. Methods A, B and C). Developing these methods is highly important because using the methods, we can ensure that every step is 100% complete, which is required for making monodisperse PEG.

The solid phase PEG synthesis technology has several advantages compared with typical solution phase technologies: (1) There is no need to use column chromatography to purify monomers. This has been demonstrated by our larger scale synthesis in a peptide synthesis vessel. The impurities in the monomers such as **17** and **18** were inert during the coupling reaction and were conveniently removed by simple washings after coupling. In contrast, using solution phase synthesis, the monomers have to be purified with chromatography and impurities such as **17** and **18** have to be removed, because even though they are inert during coupling, they will contaminate the product and may become more difficult to remove. One exception is the method reported by Kinbara group.[10c,10e] The authors elegantly demonstrated the synthesis of monodisperse PEGs without chromatography. However their method is still not ideal because it suffers from drawbacks including limited length of PEGs that can be synthesized, multiple extractions, loss of precious product in each coupling step as symmetric dimers, decreasing purity of product with increasing PEG length, and potential complexity caused by the β-elimination side reaction.[8b] (2) There is no need to handle the highly hydrophilic PEG intermediates and purifying them with column chromatography. Highly polar and water soluble organic compounds are usually difficult to handle and purify with column chromatography by synthetic chemists. Using solution phase methods, after every step, the PEG product has to be isolated and purified with column, which is expensive and imposes significant challenges. The challenge becomes more serious as the length of PEG increases. Using the solid phase technology, these problems do not exist at all. (3) The difficult-to-remove shorter PEGs resulted from depolymerization of deprotonated PEG intermediates[8a,10i] can be avoided or kept minimum. Due to the simplicity to clean up the PEG product anchored to solid support by washing, excess monomers can be used for the coupling reaction to increase reaction rate and lower reaction temperature. Both decrease the chance of PEG depolymerization. In contrast, in solution phase synthesis, if one reactant is used in excess, the product purification process usually becomes complicated. As a result, close to equal molar reactants are usually used and the reaction is typically carried out at elevated temperatures.[8a,8b,10b,10c,10h] (4) The vinyl ether side product from β-elimination of tosylate has no chance to affect PEG product purity. During the coupling reaction, slight β-elimination of tosylate is unavoidable[8b] although this is largely not discussed in previous reports. In solution phase synthesis, if it were not removed by chromatography, it could be hydrolyzed, incorporated into growing PEGs and generate shorter PEGs. Using the solid phase technology, the vinyl ethers were conveniently washed away. (5) The solid phase method does not involve using any

transition metal catalyst or high dilution technique. Some of the solution methods require these and therefore may be less ideal for large scale monodisperse PEG production.[2c,8c] (6) Finally, one important advantage that solid phase synthesis always enjoys is the possibility for automation. Since excess reagents can be removed by simple washing and all the reactions on solid support can be carried out at room temperature, it is easy to envision that the PEG synthesis could be readily achieved on commercially available peptide synthesizers without any modification of the instruments.

To evaluate the practical utility of the solid phase technology for large scale PEG production, a rough estimation of the cost structure for the synthesis of $BnO(PEG)_{12}$ (**13c**) at the scale of theoretical 1 mol (637 g) final PEG product is given. If the synthesis is carried out in one batch, a reaction vessel of 15 L is needed. The required chemicals mainly include resin **2**, $(PEG)_4$, DMTrCl, TsCl, BnBr, NaH, *t*BuOK, TCA, TFA, NaOH and solvents. The amount of resin **2** is 1.1 kg, which is roughly two times the mass of the product. We were able to purchase **2** at a price of \$1.8/g for 100 g. With this price, which could be lower if more were purchased, 1.1 kg costs \$1,980. The cost of other chemicals including solvents is estimated to be around \$5,000 if the procedure described for the synthesis of **13c** in the peptide synthesis vessel is scaled up proportionally. So, the total material cost is around \$7,000. Potentially, the resin can be reused, and the excess monomers in the coupling step and the TCA solution in the detritylation step as well as the TFA for cleavage could be recovered in a cost efficient fashion. If these were considered, the material cost could be lower. For labor, one chemist should be able to finish the synthesis in two weeks without having to work intensely. Considering that compounds similar to **13c** are currently highly expensive (e.g. $Ph_3CO(PEG)_{12}OCPh_3$ \$850-1,300/g, $Ph_3CO(PEG)_{10}$ \$1,800/g) and the high prices may be a result of the need of multiple column chromatography purifications during their production, there is a high chance for the solid phase PEG synthesis method to find practical use. Another way to evaluate the practical utility of the solid phase method is to compare its cost structure with that of typical solution phase methods. The solid phase method needs a resin and excess monomers, but has the advantage of avoiding purification of monomers and intermediates with column chromatography. The overall yield of the final product is high or quantitative. The product is more likely to be devoid of impurities resulted from depolymerization. In contrast, typical solution phase methods do not need a resin and use close to equal molar reactants. These reduce material costs. However, they typically need multiple tedious and expensive chromatographies to purify PEG starting materials, intermediates and product, and when the PEG compounds are long, purification may not always be feasible. In addition, the overall yield of the PEG product will not be quantitative and can be quite low.[2c] Overall, we believe that the additional costs from resin and excess monomers in our solid phase method can be easily offset by the costs of column chromatography purifications in solution phase methods, and there is a high chance for the solid phase method to be widely adopted for PEG synthesis.

Conclusions

In conclusion, we have demonstrated that the stepwise solid phase technology is suitable for the synthesis of monodisperse or close to monodisperse PEGs and their derivatives. Advantages of the method include rendering the entire synthesis chromatography-free, milder conditions for the key Williamson ether formation reaction to minimize anionic depolymerization of PEG intermediates and increase monodispersity of products, and high or quantitative overall yield. We also developed analytical methods for monitoring the completeness of the solid phase reactions, which is required for the synthesis of monodisperse PEGs. Using the technology, we successfully synthesized PEG derivatives with eight and twelve ethylene glycol units with close to monodispersity. Currently we are tuning conditions to achieve long PEG synthesis with little purification efforts and searching solutions to further increase monodispersity of PEG products.

Experimental Section

Detailed experimental procedures and full characterization of new compounds along with other materials including images of TLC, NMR and MS are presented in the Supporting Information.

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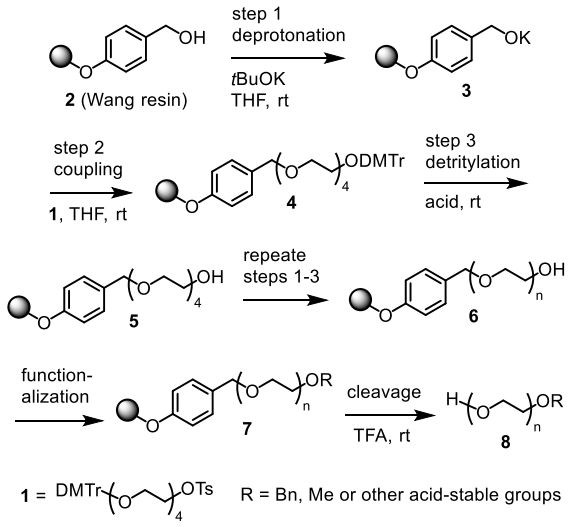
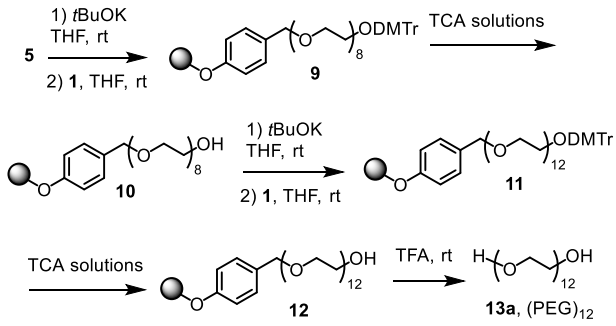
10.1002/chem.201703004

Scheme 1. The solid phase stepwise PEG synthesis design.

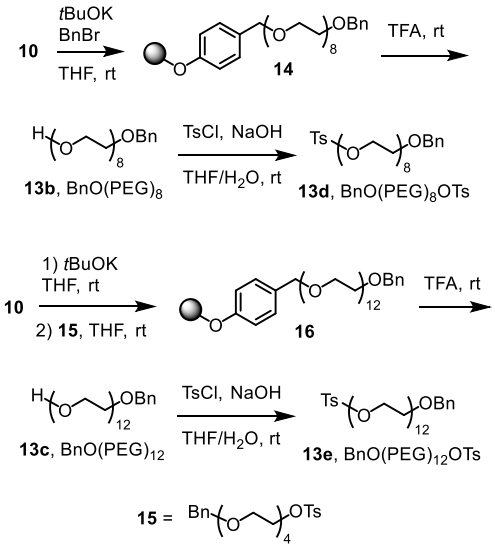


Scheme 2. Solid phase stepwise synthesis of $(PEG)_{12}$ (**13a**)

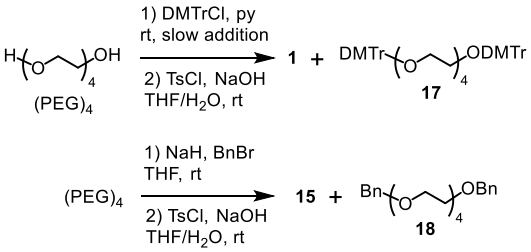


10.1002/chem.201703004

Scheme 3. Synthesis of $(PEG)_8$ and $(PEG)_{12}$ derivatives **13b**-**e**



Scheme 4. Synthesis of monomers $DMTrO(PEG)_4OTs$ (**1**) and $BnO(PEG)_4OTs$ (**15**) at multigram scale without chromatography.



Scheme 5. Explanation of using appropriate equivalents of reactants for the synthesis of $BnO(PEG)_4$ to minimize the formation of $BnO(PEG)_4OBn$ (**18**) and depolymerization product $BnO(PEG)_3$.

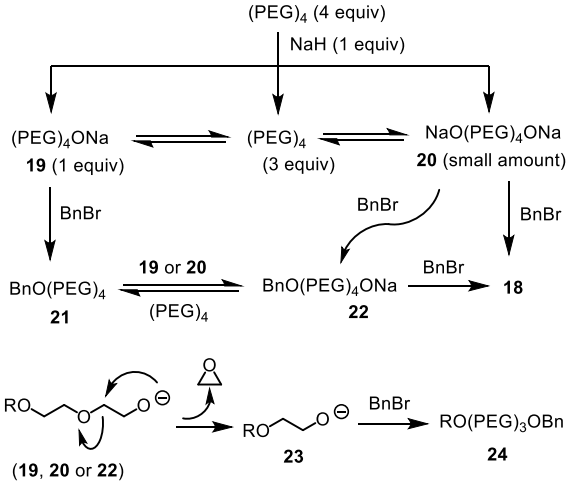


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Figure 1. ESI-MS of $(PEG)_{12}$ (**13a**) from small scale synthesis.

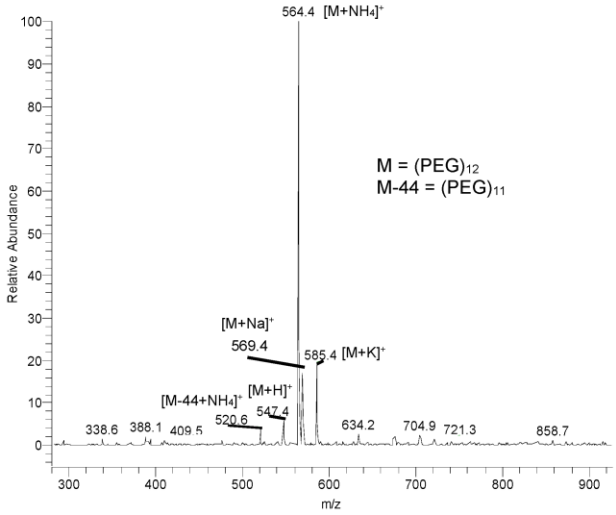

Figure 2. ESI-MS of $BnO(PEG)_{12}$ (**13c**, left) and $BnO(PEG)_{12}OTs$ (**13e**, right) from small scale synthesis.

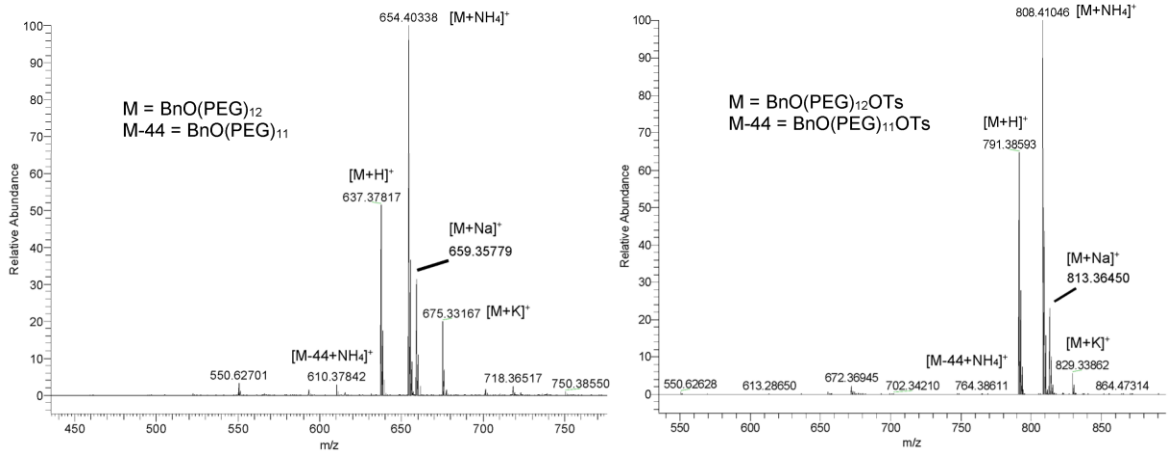

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Figure 3. TLC of DMTrO(PEG)$_4$OTs (**1**) synthesized at large scale without chromatography purification. Eluent: hexanes/EtOAc/Et$_3$N 7:3:0.5. Left lane, DMTrO(PEG)$_4$ contaminated with **17**; middle lane, co-spot of left and right lane samples; right lane, product from the tosylation reaction without chromatography purification.

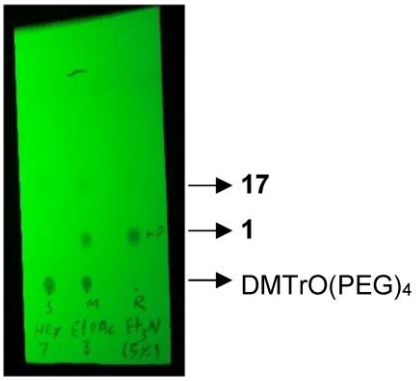

Figure 4. TLC of BnO(PEG)$_4$OTs (**15**) synthesized at large scale without chromatography purification. Eluent: hexanes/EtOAc 1:1. Lane 1 (from left), TsCl; lane 2, co-spot of lanes 1 and 3 samples; lane 3, BnO(PEG)$_4$ contaminated with **18**; lane 4, co-spot of lanes 3 and 5 samples; lane 5, product of the tosylation reaction without chromatography. The amount of **18** is minute and cannot be easily seen but can be seen in ESI-MS (Figure S22).

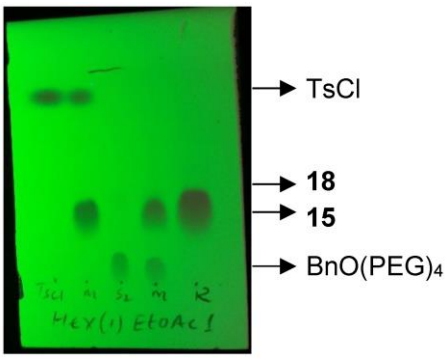

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Figure 5. ESI-MS of BnO(PEG)$_{12}$ (**13c**) from larger scale synthesis using a peptide synthesis vessel.

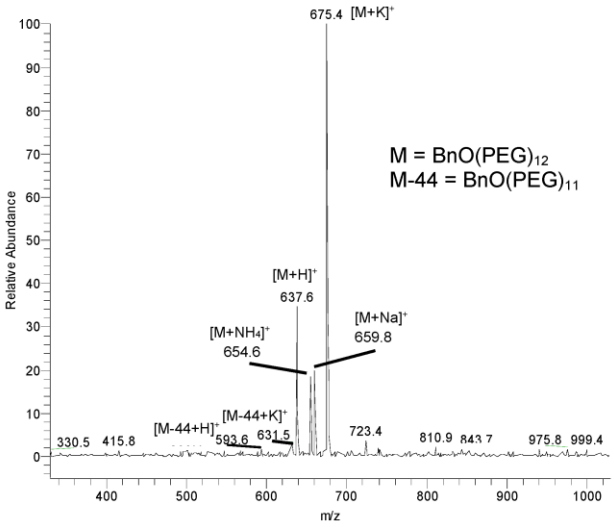
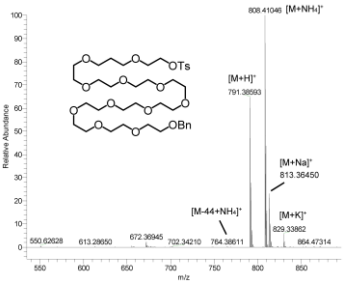


Table of contents:

Pure PEGs and derivatives are readily accessible via stepwise addition of monomers on a polystyrene support. The entire synthesis including monomer synthesis is chromatography-free. Reactions on solid support are quantitative. The depolymerization side reaction can be avoided or kept at minimum.



Keywords: PEG • solid-phase synthesis • monodisperse • synthetic methods • polyethylene glycol