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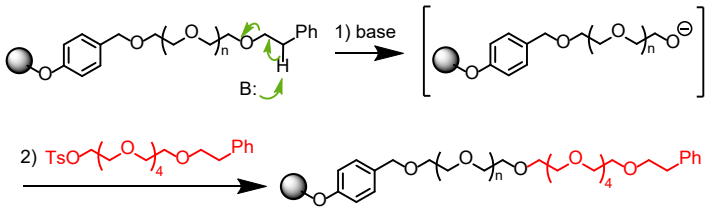
Graphical Abstract:



Automated Stepwise PEG Synthesis Using a Base-labile Protecting Group

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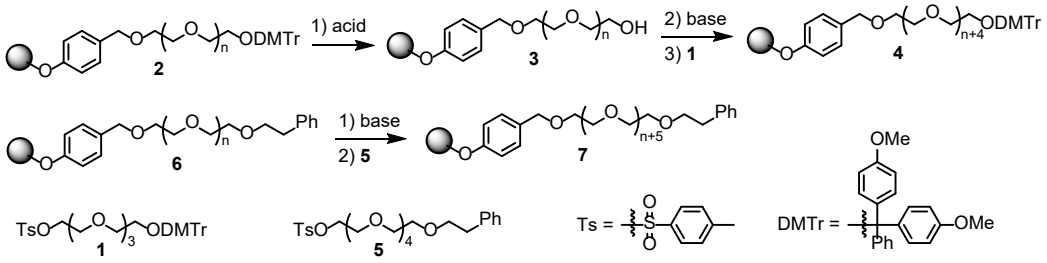
Abstract: Automated stepwise synthesis of polyethylene glycols (PEGs) was achieved using a custom modified peptide synthesizer and a monomer having a base-labile protecting group. The Wang resin, which contains an acid-labile para-methoxybenzyl linker, was used as the solid support. The PEG_5 derivative $TsOPEG_5O(CH_2)_2Ph$, which contains a tosyl leaving group and a base-labile phenylethyl protecting group, was used as the monomer. Automated assembly of PEGs was carried out by deprotonation of the para-methoxybenzyl alcohol on the Wang resin followed by reaction with the monomer in the first cycle. Subsequent cycles consisted of deprotection of the phenylethyl group under basic conditions, and direct coupling with the monomer under less basic conditions. The deprotection gave the PEG as an alkoxide, which made direct coupling with the monomer possible. A separate step for deprotonation was not needed. Purification of intermediates and products was simply achieved by washing the resin. In all the steps, materials were added into and removed from the reaction vessel controlled by the software of the synthesizer. At the end of synthesis, the $PEG_nO(CH_2)_2Ph$ product was cleaved from the resin with TFA. Using the automated method, high quality monodisperse $PEG_{10}O(CH_2)_2Ph$ and $PEG_{15}O(CH_2)_2Ph$ derivatives were synthesized. The $PEG_{20}O(CH_2)_2Ph$ derivative was also synthesized but small amount of impurity was observed. The yields of the syntheses should be close to 100% because the product would otherwise not be monodisperse. In addition for PEG synthesis, the automated method could be readily adapted for the synthesis of a wide range of sequence-defined oligomers and polymers.

Keywords: Automation, base-labile, PEG, protecting group, sequence-defined polymer, solid phase synthesis

Introduction

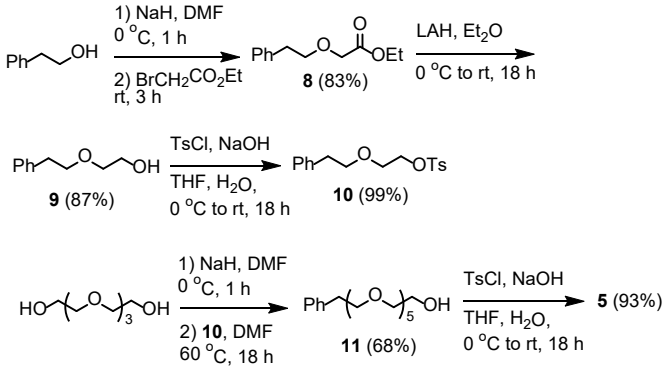
Due to their unique properties such as simple, neutral, and flexible backbone, high chemical, enzymatic and physical stability, high solubility in water and many organic solvents, non-toxicity, and non-immunogenicity, polyethylene glycol (PEG) and their derivatives have found wide applications.[1-7] The most efficient method for their synthesis is to polymerize ethylene oxide under basic or acidic conditions.[8] The method is inexpensive, but the products are polydisperse. For many applications including as linkers in organic synthesis and bioconjugation,[9] as surfactants in nanomedicine to stabilize nanoparticles and to enhance nanoparticle cell entry,[10-12] and PEGylation agents to stabilize drugs based on biomolecules,[1, 13] monodisperse PEGs are required or highly desired. To meet the demand, many efforts have been made to develop methods for the synthesis of monodisperse PEGs and their analogs. These methods involve solution phase stepwise organic synthesis, which has drawbacks such as high labor demand, slow reaction and the need of chromatographic purification of products after almost each of the many steps.[14-26]



Scheme 1. A comparison of base-labile and acid-labile protecting groups for solid phase stepwise PEG synthesis.

Recently, we reported a solid phase method for monodisperse PEG synthesis.[27] Compound **1**, which contains the tosyl (Ts) leaving group at one end and the 4,4'-dimethoxytrityl (DMTr) group at the other, was used as the monomer (Scheme 1). The intermediates of the synthesis can be represented with **2**. In each synthesis cycle, PEG elongation was achieved in three steps – deprotection of the DMTr group with an acid to give **3**, deprotonation of **3** with a base followed by coupling with monomer **1** to give **4**. Purification of intermediates and the final product were achieved by washing with solvents. In this paper, we report the use of the monomer **5** for solid phase synthesis of monodisperse PEGs and the automation of the process using a custom modified peptide synthesizer. Monomer **5**, unlike **1**, which contains the acid-labile DMTr protecting group, contains the base-labile phenethyl protecting group (Scheme 1).[14] The intermediate of solid phase PEG synthesis can be represented with **6**. In each synthesis cycle, PEG elongation was achieved in two steps – deprotection of **6** and coupling with **5** to give **7**. There was no need of the

deprotonation step because deprotection of **6** gave directly the alkoxide intermediate needed for the coupling step. Using the method, we were able to synthesize monodisperse $PEG_{10}O(CH_2)_2Ph$ and $PEG_{15}O(CH_2)_2Ph$ automatically on a peptide synthesizer. The longer PEG derivative $PEG_{20}O(CH_2)_2Ph$ was also synthesized but small amount of impurity was observed.



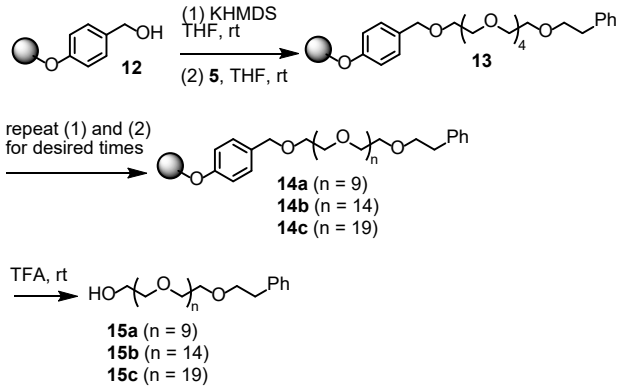
Scheme 2. Synthesis of monomer **5**.

Results and Discussion

Monomer **5** was synthesized using a procedure shown in Scheme 2. The inexpensive 2-phenylethanol was deprotonated with sodium hydride and reacted with ethyl bromoacetate to give **8**.[28] The ester was reduced with lithium aluminum hydride to give the alcohol **9**,[29] which was tosylated to give **10**. The inexpensive PEG_4 was deprotonated with NaH, and reacted with **10** to give **11**. Excess PEG_4 was used, and the monoalkylated **11** could be formed almost exclusively. The excess PEG_4 was conveniently removed from the product via partition between saturated sodium chloride and ethyl acetate as PEG_4 is soluble in water, and **11** is more soluble in ethyl acetate than PEG_4. Tosylation of **11** gave the needed monomer **5** in excellent yield. With only limited efforts for optimization of the reaction conditions, we were able to synthesize large quantities of the target compound at scales as high as 50 grams.

As shown in Scheme 3, when **5**, which contains a base-labile protecting group, is used as the monomer, the procedure for solid phase PEG synthesis is impressively simple. In the first cycle, the Wang resin (**12**) was deprotonated with potassium hexamethyldisilazide (KHDMS) or potassium tertiary butoxide in THF, and coupled with monomer **5** to give **13** in DMF or THF. In the next synthesis cycles, the conditions were essentially the same as the first cycle. Treating **13** with a base in THF caused β-elimination of the phenethyl group,[14] which gave the deprotected PEG intermediate in the form of alkoxide. The excess base along with the deprotection side product styrene were removed by washing with DMF. The alkoxide intermediate was reacted with **5** in

DMF directly to give **14a**. Repeating the synthesis cycle for one and two times gave **14b** and **14c**, respectively. The base used for deprotonating **12** and deprotecting **13** could be either KHMDS or potassium tertiary butoxide. However for deprotecting **14a** and **14b**, KHMDS was found significantly more effective than potassium tertiary butoxide. All the reactions were carried out at room temperature. Excess reagents including the base and monomer were used to drive the reaction to completion. The delivery and removal of reagents and solvents were controlled by the software of the peptide synthesizer. After each step, the intermediates were purified by washing with solvents (see details in the experimental section). Thus the entire process was fully automated.



Scheme 3. Automated solid phase synthesis of PEG derivatives **15a-c**.

At the end of the automated synthesis, to cleave the PEG product from **14**, the resin was soaked in pure TFA in a centrifuge tube at room temperature for about two hours (Scheme 3). After removing the supernatant, the resin was rinsed with additional TFA for two times. The resin was further washed with THF. The TFA and THF solution were combined, and volatiles were evaporated under vacuum. The residue was mixed with water extensively by vortexing, and the mixture was centrifuged to bring down insoluble materials. The supernatant, which contained the PEG product, was transferred to a different centrifuge tube. This process removed all organic impurities that were insoluble in water. Water was evaporated under vacuum, and to the residue THF was added. The materials were mixed extensively by vortexing, and then centrifuged to bring down insoluble materials. The supernatant, which contained the PEG product, was transferred to a different centrifuge tube. This process removed all inorganic impurities that were insoluble in THF. To the supernatant, of which the volume was sometimes reduced as needed, diethyl ether was added, mixed and centrifuged. This precipitated the PEG product because it is insoluble in diethyl ether. The additional purification by diethyl ether precipitation is optional, and was not always performed.

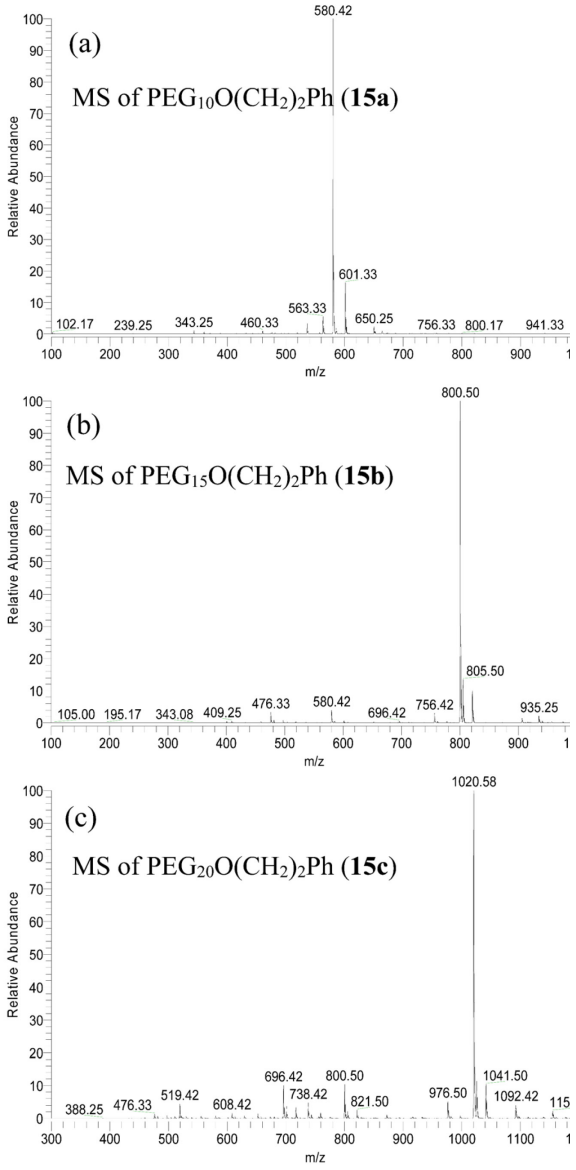


Figure 1. ESI MS of PEG derivatives **15a-c**. (a) MS of **15a**. Calcd for [**15a** + NH_4]$^+$ 580.37, found 580.42 at 100%; calcd for [**15a** + H]$^+$ 527.35, found 563.33 at ~6%; calcd for [**15a** + K]$^+$ 601.30, found 601.33 at ~16%. (b) MS of **15b**. Calcd for [**15b** + NH_4]$^+$ 800.50, found 800.50 at 100%; calcd for [**15b** + Na]$^+$ 805.46, found 805.50 at ~12%; calcd for [**15b** + K]$^+$ 821.43, found 821.42 at ~10%; calcd for [**15b** – $(CH_2)_2O$ + NH_4]$^+$ 756.47, found 756.42 at ~2%; calcd for [**15a** + NH_4]$^+$ 580.37, found 580.42 at ~2%. (c) MS of **15c**. calcd for [**15c** + NH_4]$^+$ 1020.63, found 1020.58 at 100%; calcd for [**15c** + K]$^+$ 1041.56, found 1041.50 at ~10%; calcd for [**15c** – $(CH_2)_2O$ + NH_4]$^+$ 976.60, found 976.50 at ~2%; calcd for [**15b** + NH_4]$^+$ 800.50, found 800.50 at ~10%; calcd for [PEG_{15} + NH_4]$^+$ 696.43, found 696.42 at ~10%.

Using the automated procedure, the PEG_{10} and PEG_{15} derivatives **15a-b** were obtained readily. Their MS spectra are shown in Figure 1, and their NMR spectra are in supporting information. According to their MS, the steps for the deprotection and coupling were close to 100% complete because no significant amount of shorter PEGs could be observed in the spectra. In addition, the peaks corresponding to the molecular peaks with one ethylene glycol unit lost were minimal, which indicated that PEG depolymerization under basic conditions did not occur or only occurred to a neglectable extent as the small quantities of shorter PEGs might be originated from minute quantities of PEG_3 in the PEG_4 starting material used for the synthesis of monomer **5**.[22, 25, 30] Using the same procedure, we made efforts to synthesize the longer PEG derivative **15c**. However, the reaction was incomplete. As shown in Figure 1, a small amount of **15b** (~10%) and PEG_{15} (~10%) appeared in the MS, which indicated that the protecting group was not completely removed and the conversion of the coupling reaction was not 100%. To make these reactions complete, additional time for deprotection and coupling may be able to solve the problem.

Besides the features such as full automation, and the need of only two steps in each synthetic cycle instead of three steps as in the case of our previous method,[27] several additional features of the current PEG synthesis method are notable. In the course of the study, we found that the base-labile phenethyl group is much easier to remove than the acid-labile DMTr group. For removing the DMTr group, the resin needed to be washed with dilute acid for about five times before the red color of the trityl cation to disappear. To ensure 100% completion of the deprotection, we usually flushed the resin for about five more times.[27] For removing the phenethyl group during the synthesis of **14a-b**, washing the resin with the KHMDS one time was believed to be able to complete the reaction, although to ensure complete deprotection, we usually washed the resin with the base solution for an additional time. The reason for the more efficient removal of phenethyl group than the DMTr group is that the former reaction is irreversible while the latter one is reversible. For the synthesis of **14a-b**, we also found that using monomer **5**, for each synthetic cycle, we only performed the coupling reaction one time, and close to 100% conversion was achieved according to MS analysis of the end products. While for similar synthesis using monomer **1**, two or more couplings were performed to drive the reaction to completion.[27] Our rationale for the difference is the different hydrophobicity of the DMTr and phenethyl groups. The higher hydrophobicity of the DMTr group may be more likely for the PEGs bearing it to adopt higher order structures that can make the coupling reaction less efficient. The phenethyl group is less hydrophobic and the adverse effect may be less.

Before using KHMDS as the base for deprotecting the phenethyl group, we tested the weaker base potassium tertiary butoxide for the purpose. We found that for converting **13** to **14a**, potassium tertiary butoxide worked well. However, for converting **14a** to **14b**, using potassium tertiary butoxide, the deprotection did not go to completion. We thus used the stronger base KHMDS for the synthesis of **15b** and **15c**. Because attaching the bottle of the base solution to the synthesizer required a brief exposure of the solution to air, we carefully tested the safety of

KHMDS solution in air. We first exposed a few micro liters of the solution in air, and found no observable reactions. We then did the test tested using larger volumes of the solution, the same phenomena were observed. We also found that dripping the solution onto ice or water did not result in violent reactions. Therefore, we felt safe to use KHMDS for the experiments. However, despite our safety test results, we recommend that the safety of KHMDS should still be taken seriously by the community.

In recent years, significant efforts have been made for the synthesis of sequence-defined oligomers and polymers.[31-33] In these materials, the location of functional groups are precisely defined, and the macromolecules are intended to be perfectly homogenous. Such materials are useful for applications including data storage[34-36] and medicine.[37] Reported methods for their synthesis include stepwise solution phase synthesis, stepwise solid phase synthesis, and fluorous- and polymer-tethered approaches.[33] In addition to the synthesis of monodisperse PEGs and their derivatives, our stepwise solid phase synthesis method, which is impressively simple and convenient because of automation and minimized number of synthetic steps due to the use of a base-labile protecting group, could be readily adapted for the synthesis of a wide variety of sequence-defined oligomers and polymers.

Conclusion

In summary, the use of the base-labile phenethyl protecting group for solid phase monodisperse PEG synthesis was investigated. In addition, the process was fully automated using a peptide synthesizer. The base-labile protecting group showed significant advantages over the acid-labile protecting group reported by us earlier,[27] which include shortening the synthesis cycle from three steps to two steps, and higher efficiency for both deprotection and coupling steps. In addition to the synthesis of monodisperse PEGs and PEG derivatives, the simple and automated method could be readily adapted for the synthesis of a wide range of sequence-defined oligomers and polymers.

Experimental Section

General information: All compounds from commercial sources were used as received unless noted otherwise. Anhydrous DMF, DMSO and NMP were dried over molecular sieves. Et_2O was distilled over CaH_2. THF was dried using the Innovative Technology Pure-Solv™ system. All reactions were carried out under nitrogen using oven-dried glassware. Thin layer chromatography (TLC) was performed using Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 250 μm thickness. ^1H and ^{13}C NMR spectra were obtained on a Varian spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts (δ) were reported in reference to residue solvent peaks ($CHCl_3$ at δ 7.24 ppm for ^1H and $CDCl_3$ at δ 77.00 ppm for ^{13}C). HRMS was obtained on a Thermo HR-Orbitrap Elite Mass Spectrometer. LRMS was obtained on a Thermo Finnigan LCQ Advantage ion trap mass spectrometer.

Ethyl 2-phenthoxyacetate (**8**):[28] The suspension of NaH (60% in mineral oil, 3.64 g, 82.8 mmol, 1.0 equiv.) in anhydrous DMF (150 mL) in a 2-neck round bottom flask under nitrogen was cooled on an ice bath. The solution of $Ph(CH_2)_2OH$ (10.0 mL, 82.8 mmol, 1.0 equiv) in anhydrous DMF (250 mL) was added dropwise via a cannula over ~1 h. After addition, the reaction mixture was stirred at 0 °C for ~1 h. This gave the clear solution of $NaO(CH_2)_2Ph$. Ethyl bromoacetate (13.8 g, 82.8 mmol, 1.0 equiv) was dissolved in anhydrous DMF (100 mL). The solution of $NaO(CH_2)_2Ph$ was added dropwise via a cannula. After addition, the mixture was stirred at 0 °C for 4 h, and the reaction was then quenched with EtOH. DMF was removed on a rotary evaporator under vacuum. The residue was partitioned between EtOAc (700 mL) and saturated NaCl (150 mL). The organic phase was washed with saturated NaCl (150 mL × 3), dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was dried under high vacuum, and purified with flash chromatography (SiO_2, EtOAc/hexanes 1:4) to give compound **8** (14.4 g, 83%) as a clear oil: TLC R_f = 0.6 (SiO_2, hexanes/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.17 (m, 5H), 4.17 (d, J = 8.0 Hz, 2H), 4.04 (s, 2H), 3.73 (t, J = 8.0 Hz, 2H), 2.92 (t, J = 8.0 Hz, 2H), 1.24 (t, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.48, 138.58, 129.03, 128.53, 126.45, 72.83, 68.71, 61.03, 36.45, 14.53 ; HRMS (ESI) *m/z*: calcd for $[M + Na]^+$ 231.0997; found, 231.0987.

2-Phenethoxyethan-1-ol (**9**):[29] Lithium aluminum hydride (LAH) (1.98 g, 51.8 mmol, 0.75 equiv.) was placed in a two neck round bottom flask and flushed with nitrogen. The flask was placed on an ice bath. Anhydrous Et_2O (75 mL) in another flask under nitrogen was added dropwise via a cannula. To the mixture, the solution of **8** (14.4 g, 69.1 mmol, 1.0 equiv) in anhydrous Et_2O (300 mL) was added dropwise via a cannula over ~1 h. After addition, the reaction mixture was stirred at rt for 8 h. The reaction was quenched at 0 °C by sequential dropwise addition of water (1.98 mL), 15% NaOH solution (1.98 mL) and water (5.94 mL). The white solid was filtered off, and the filtrate was dried over anhydrous $MgSO_4$. The solution was evaporated to dryness under reduced pressure. The residue was purified with flash chromatography (SiO_2, EtOAc/hexanes 1:5) to give compound **9** (9.96 g, 86%) as a clear oil: TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.18 (m, 5H), 3.67 (t, J = 8.0 Hz, 4H), 3.52 (t, J = 4.0 Hz, 2H), 2.88 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.91, 128.55, 128.50, 126.44, 72.18, 61.96, 36.53; HRMS (ESI) *m/z*: calcd for $[M + Na]^+$, 189.0892; found, 189.0881.

2-Phenethoxyethyl 4-methylbenzenesulfonate (**10**): Compound **9** (5.7 g, 31.1 mmol, 1.0 equiv.) in THF (70 ml) in a round bottom flask was cooled on an ice bath. To the flask was added the solution of NaOH (12.45 g, 311 mmol, 10 equiv.) in water (70 ml). After the mixture was stirred at 0 °C for 1 h, *p*-toluene sulfonyl chloride (8.86 g, 46.6 mmol, 1.5 equiv.) in THF (140 mL) was added dropwise via a cannula over ~1 h. After addition, the mixture was stirred for 18 h while warming to rt gradually. The mixture was partitioned between EtOAc (500 mL) and saturated NaCl (50 mL). The organic phase was washed with saturated NaCl (50 mL × 3), dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue

was purified with flash chromatography (SiO_2, EtOAc/hexanes 1:4) to give compound **10** (7.06 g, 98%) as a clear oil: TLC R_f = 0.6 (SiO_2, hexanes/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.74 (d, 2H) 7.30-7.12 (m, 8H), 4.11 (t, J = 4.0 Hz, 2H), 3.57 (m, 4H), 2.78 (t, J = 8.0 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.89, 138.74, 133.19, 129.94, 128.11, 126.41, 72.47, 69.49, 68.46, 36.41, 21.94; HRMS (ESI) *m/z*: calcd for $[M + Na]^+$, 343.0980; found, 343.0967.

17-Phenyl-3,6,9,12,15-pentaoxaheptadecan-1-ol (**11**): The suspension of NaH (60% in mineral oil, 0.98 g, 24.5 mmol, 1.2 equiv.) in anhydrous DMF (50 mL) in a 2-neck round bottom flask under nitrogen was cooled on an ice bath. The solution of tetraethylene glycol (PEG_4, 19.7 g, 17.5 mL, 204 mmol, 5.0 equiv.) in anhydrous DMF (150 mL) was added dropwise via a cannula over ~1 h. The mixture was stirred at 0 °C for ~1 h giving a clear solution of $NaOPEG_4OH$. The solution was warmed to rt and then heated to 60 °C. Compound **10** (4.7 g, 20.4 mmol, 1.0 equiv.) in anhydrous DMF (50 mL) was added dropwise via a cannula over ~3 h. After addition, the mixture was stirred at 60 °C for 8 h. The reaction was quenched with EtOH, and DMF was removed on a rotary evaporator under vacuum. The residue was partitioned between EtOAc (400 mL) and saturated NaCl (50 mL). The organic phase was washed with saturated NaCl (50 mL × 3), dried over anhydrous $MgSO_4$ and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was purified with flash chromatography (SiO_2, EtOAc/hexanes 2:1) to give compound **11** (4.73 g, 68%) as a clear oil: TLC R_f = 0.3 (SiO_2, hexanes/EtOAc 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17-7.09 (m, 5H), 3.61-3.54 (m, 22H), 2.80 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.96, 129.02, 128.39, 126.23, 72.45, 70.82, 70.75, 70.43, 69.30, 36.47; HRMS (ESI) *m/z*: calcd for $[M + Na]^+$, 365.1940; found, 365.1922.

17-Phenyl-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (**12**): Compound **11** (4.3 g, 12.5 mmol, 1.0 equiv.) in THF (30 mL) in a round bottom flask was cooled on an ice bath. NaOH (5.0 g, 125 mmol, 10 equiv.) in water (30 ml) was added. The mixture was stirred vigorously at 0 °C for 1 h. *p*-Toluene sulfonyl chloride (3.5 g, 18.8 mmol, 1.5 equiv.) in THF (60 mL) was added dropwise via a cannula over ~1 h. After addition, the mixture was stirred for ~18 h while warming to rt gradually. The mixture was partitioned between EtOAc (200 mL) and saturated NaCl (25 mL). The organic phase was washed with saturated NaCl (25 mL × 3), dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was purified with flash chromatography (SiO_2, EtOAc/hexanes 1:1) to give compound **12** (5.23 g, 92%) as a clear oil: TLC R_f = 0.4 (SiO_2, hexanes/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.72 (d, 2H) 7.29-7.15 (m, 7H), 4.09 (t, J = 4.0 Hz, 2H), 3.62-3.51 (m, 20H), 2.84 (t, J = 8.0 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.88, 139.02, 133.15, 129.95, 129.03, 128.08, 126.28, 72.50, 70.94, 70.48, 69.51, 68.88, 36.53, 21.93; HRMS (ESI) *m/z*: calcd for $[M + Na]^+$, 519.2028; found, 519.2007.

General procedure for automated PEG synthesis: The CBS Bio CS136X peptide synthesizer was modified for the automated synthesis. The synthesizer has two measuring vessels called MVA and MVB, which use sensors to determine the volume of solutions or solvents to be delivered to the reaction vessel (RV). MVA is used to measure solutions or solvents that need to be kept anhydrous. MVB is used to measure solutions or solvents that contain water or acids, or to measure solutions or solvents that do not need to be kept anhydrous. To meet the needs of the project, several reagent or solvent bottles connected to MVA were changed to connect to MVB, and the software was modified to accommodate the modification. In addition, the argon going into the synthesizer was dried via molecular sieve in a drying tube, and the gas venting lines of the synthesizer were connected to a drying tube filled with Drierite before reaching to air. An example synthesis is given. To prepare for the synthesis, the Wang resin (**12**, 1.0 g, 0.9 mmol/g loading, 0.9 mmol) was loaded into a 20 ml RV. Dry THF (15 ml) was delivered to the RV, and the resin was allowed to swell at rt for 10 min. Mixing of the resin and solvent was achieved by rotating the RV 180º back and forth, which is the mixing mechanism of the synthesizer. After draining, the resin was washed with anhydrous solvents. The washing scheme of sequential THF, DMF, DMSO and NMP washes with 10 min waiting and five repetitions was used. For converting **12** to **13**, KHMDS (or *t*BuOK) in THF (0.25 M, 15 ml, 3.75 mmol, 4.1 equiv.) was delivered to RV. After mixing at rt for 5 min, the solution was drained. The deprotonation was repeated one time. After draining, the resin was washed with anhydrous DMF two times. The solution of monomer **5** (0.5 M in DMF, 15 ml, 7.5 mmol, 8.33 equiv.) was delivered into RV, and the materials were mixed at rt for 6 h. The solution was drained, and the resin was washed with THF (10 mL × 2), THF/H_2O (v/v 1:1, 15 mL × 5); THF (10 mL × 3); DMF (10 mL × 3); DMSO (10 mL × 3). For converting **13** to **14a**, **14a** to **14b**, and **14b** to **14c**, the same conditions for converting **12** to **13** were used except that for converting **14a** to **14b**, and **14b** to **14c**, *t*BuOK could not serve as an alternative base, and KHMDS was used.

Cleavage of PEG from resin: To the resin (50 mg), extensively washed as described above and dried, in a 1.5 mL centrifuge tube was added TFA (300 µL). The mixture was shaken at rt for 2 h. The tube was spun shortly to bring down liquids to the bottom, and the supernatant was transferred to another 1.5 mL tube. The resin was washed with TFA (50 µL × 2) and THF (50 µL × 3). The supernatant and the washes were combined. Volatiles were evaporated under vacuum. To the residue was added water (100 µL). The tube was vortexed and centrifuged. The supernatant was transferred to another 1.5 mL tube. The volatiles were evaporated under vacuum. The residue was dissolved in THF (100 µL), vortexed and centrifuged. The supernatant was transferred to another 1.5 mL tube, and the PEG product was obtained by evaporating THF, or alternatively, by precipitating from the THF solution with Et_2O (200 µL).

*$PEG_{10}O(CH_2)_2Ph$ (**15a**)*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22-7.14 (m, 5H), 3.58 (m, 42H), 2.86 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.90, 129.19, 128.31, 126.49, 72.69, 70.13, 36.41. HRMS (ESI) *m/z*: calcd for $[M + NH_4]^+$ 580.37, found 580.42.

*$PEG_{15}O(CH_2)_2Ph$ (**15b**)*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.13 (m, 5H), 3.58 (m, 62H), 2.85 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.88, 129.03, 128.47, 126.28, 72.68, 69.90, 61.19, 36.45. HRMS (ESI) *m/z*: calcd for $[M + NH_4]^+$ 800.50, found 800.50.

*$PEG_{20}O(CH_2)_2Ph$ (**15c**)*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.13 (m, 5H), 3.60 (m, 82H), 2.86 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.97, 129.04, 128.46, 126.31, 72.68, 70.67, 61.40, 36.52. HRMS (ESI) *m/z*: calcd for $[M + NH_4]^+$ 1020.63, found 1020.58.

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