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Easy Silica Gel Supported Desymmetrization of PEG

Barbara La Ferla,* Cristiano Zona, Francesco Nicotra
Department of Biotechnology and Bioscience, University of Milano Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
Fax +39(02)64483565; E-mail: barbara.laferla@unimib.it
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Abstract: Our work shows how poly(ethylene glycol), differentially functionalized at two ends, can be easily and efficiently synthesized from commercial symmetric dihydroxy PEG 600. First, we linked the polymer onto functionalized silica gel using one of its hydroxy ends. Then, the second hydroxy group was modified, and finally, the desymmetrized PEG was cleaved from the solid support.

Key words: desymmetrization, mono protection, poly(ethylene glycol) polymer, solid phase

Poly(ethylene glycol) (PEG) is a linear polymer that, because of its ideal properties, that is, very low toxicity, excellent water solubility, extremely low immunogenicity, and antigenicity, is used in a wide range of applications. Though it is not biodegradable, PEG is readily excreted after administration into living organisms. Additionally, it has excellent pharmacokinetic and biodistribution characteristics. All of these properties1–9 make PEG particularly useful for biomedical applications as an ideal linker to graft biomolecules.

The main disadvantages for using PEG as a spacer are its symmetry and the difficulty in differentiating between the two terminal hydroxy groups. Many authors have proposed different approaches for solving these problems. Some of them are based on polymerization10–17 of differentially protected monomers and require multiple protection and deprotection steps. Others are based on the monoprotection of dihydroxy-PEG6,18–28 which often leads to a mixture of mono-, bi-, and nonprotected compounds that is very difficult to separate.

During a project with the goal of generating nanoparticles properly linked to antiamyloidogenic compounds, we were interested in differently and orthogonally protected or bifunctionalized PEG linkers.

Here we propose a new, simple, and general method for the desymmetrization of PEG. This method is based on the connection of the polymer to a solid support through one of two terminal hydroxy groups. Then, the unbound hydroxy group can be protected or converted into different functionalities (Br, N3, SSSH, OBN). The cleavage from the solid support, and possible functionalization of the second hydroxy group, generates the bifunctional polymer XCH2CH2(OCH2CH2)n Y.

As a solid support, we used silica gel 60 (mesh 230–400), which is a readily available material generally used for chromatography. Silanization39,40 of a silica gel–toluene suspension was obtained by using (3-aminopropyl)triethoxysilane (Scheme 1) under reflux in a Dean–Stark apparatus. This technique led to a solid support with amino functionalities (1; loading 0.53 mmol/g). The treatment of 1 with succinic anhydride introduced a carboxylic function, suitable for loading PEG, and permitted easy cleavage from the support after proper modifications.

The treatment of a suspension of solid support 1 in anhydrous THF (r.t. for 12 h) with succinic anhydride led to a carboxylic functionalized solid support 2 obtained in a quantitative yield, measured using TNBS test.11 Next, the polyethylene glycol was loaded onto the generated solid support through a simple coupling process: commercial PEG 600 (average MW 546) and 2 (1 g), suspended in anhydrous THF (10 mL), was reacted with N,N′-disopropylcarbodiimide (DIC) and 4-(dimethylamino)pyridine (DMAP). This coupling provided 3 with a loading of 0.1 mmol of PEG per gram of solid support.32 This, in turn, led to a lower loading than was needed for solid support 1 (0.53 mmol/g). This can be explained by considering the steric hindrance of the attached PEG chains. Even if the latter outcome is not very high, for our purposes, it is satisfactory.

Scheme 1 Reagents and conditions: i) (3-aminopropyl)triethoxysilane, toluene, reflux, Dean–Stark apparatus; ii) succinic anhydride, THF dry; iii) commercial PEG 600, DIC, DMAP, dry THF.
The ‘free’ hydroxy group of the silica gel loaded polymer 3 was next functionalized as shown in Scheme 2. We followed two different synthetic strategies: the protection of the free OH as a benzyl ether by direct reaction with activated benzyl alcohols and the conversion of the free OH into a bromide and subsequent displacement by other nucleophilic functional groups. The protection was carried out by suspending 3 (1 g) in benzyl 2,2,2-trichloroacetimidate (20 equiv) in anhydrous CH2 Cl2 (10 mL) and adding trifluoromethanesulfonic acid (10 equiv). At the end of this process we obtained derivative 4. Bromide 5 was generated through the reaction of the suspension of 3 (1 g) in anhydrous CH2 Cl2 (10 mL) containing phosphorus tribromide (10 equiv, 12 h). Bromide displacement with an azide or thiol nucleophile led to the formation of derivatives 6 and 7, respectively. Functionalized silica gel 5 was washed with CH2 Cl2, toluene, and MeCN to remove excess reagent prior to being suspended in N,N-dimethylformamide (DMF; 1 g/10 mL) and reacted with NaN3 (10 equiv) or cesium thioacetate (10 equiv).

All PEG derivatives (compounds 4–7) were cleaved from the silica support and suspended in a solution of ammonia in methanol (7 M, 10 mL per gram of pegylated silica gel) and stirred for 12 hours. Then, all the different suspensions of silica gel were filtered and washed with MeOH and stirred for 12 hours. Then, all the different suspensions of silica gel were filtered and washed with MeOH (Scheme 3). The filtrates were concentrated under reduced pressure, affording the monoderivatized poly(ethylene glycol) compounds.

**Table 1** Summary of the Yields of Desymmetrized PEG 8–12 and Significant Characterization Signals

<table>
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<tr>
<th>Compd</th>
<th>Yield (%)</th>
<th>(^a)</th>
<th>Characterizations</th>
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<tr>
<td>8</td>
<td>74(^b)</td>
<td>1(^b)H NMR (400 MHz, CDCl3, 25 °C): δ = 7.25–7.40 (m, 5 H, Ar), 4.57 (s, 2 H, OCH2Ph), 3.55–3.75 (m).(^c) ESI-MS(^d): (n = 10) 593.5 [M + H]+, 610.6 [M + NH4]+, 615.3 [M + Na]+; (n = 11) 637.5 [M + H]+, 654.6 [M + NH4]+, 659.5 [M + Na]+; (n = 12) 681.5 [M + H]+, 698.6 [M + NH4]+, 703.5 [M + Na]+.</td>
<td>(^a)Calculated from 3. (^b)Oxidation yield from 10. (^c)Typical signals of commercial polymers. (^d)The most intense peaks.</td>
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<tr>
<td>9</td>
<td>78(^a)</td>
<td>1(^a)H NMR (400 MHz, CDCl3, 25 °C): δ = 3.80 (t, J = 6.3 Hz, 2 H, OCH2CH2Br), 3.55–3.75 (m), 3.47 (t, J = 6.3 Hz, 2 H, OCH2CH2Br). ESI-MS(^b): (n = 10) 565.5–567.5 [M + H]+, 582.6–584.6 [M + NH4]+, 587.5–589.5 [M + Na]+; (n = 11) 609.6–611.6 [M + H]+, 626.1–628.3 [M + NH4]+, 631.5–633.7 [M + Na]+; (n = 12) 653.6–655.6 [M + H]+, 670.6–672.6 [M + NH4]+, 675.6–677.6 [M + Na]+.</td>
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<tr>
<td>10</td>
<td>75(^b)</td>
<td>1(^b)H NMR (400 MHz, CDCl3, 25 °C): δ = 3.55–3.75 (m), 3.38 (brt, J = 4.9 Hz, 2 H, CH2N3). IR: ν(_{as}) = 2104.6 (s) cm(^{-1}). ESI-MS(^d): (n = 10) 528.5 [M + H]+, 545.5 [M + NH4]+, 550.5 [M + Na]+; (n = 11) 572.5 [M + H]+, 589.4 [M + NH4]+, 594.5 [M + Na]+; (n = 12) 616.5 [M + H]+, 636.3 [M + NH4]+, 638.5 [M + Na]+.</td>
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<tr>
<td>11</td>
<td>71(^a)</td>
<td>1(^a)H NMR (400 MHz, CDCl3, 25 °C): δ = 3.55–3.75 (m), 2.84 (brt, J = 6.99 Hz, 2 H, OCH2CH2SSH). ESI-MS(^d): (n = 10) 551.4 [M + H]+, 573.4 [M + Na]+; (n = 11) 594.4 [M + H]+, 617.4 [M + Na]+; (n = 12) 639.9 [M + H]+, 661.9 [M + Na]+.</td>
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<tr>
<td>12</td>
<td>73(^b)</td>
<td>1(^b)H NMR (400 MHz, CDCl3, 25 °C): δ = 3.86 (s, 2 H, HOOCCH2O), 3.55–3.75 (m), 3.38 (brt, J = 4.9 Hz, 2 H, CH2N3). ESI-MS(^d): (n = 10) 542.5 [M + H]+, 564.5 [M + Na]+; (n = 11) 586.5 [M + H]+, 608.5 [M + Na]+; (n = 12) 630.5 [M + H]+, 652.5 [M + Na]+.</td>
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\(^a\)Calculated from 3. 
\(^b\)Oxidation yield from 10. 
\(^c\)Typical signals of commercial polymers. 
\(^d\)The most intense peaks.
can be used without further modifications and can be easily converted to a corresponding thiol derivative by treatment with NaBH₄ in MeOH. The obtained compounds were characterized by ¹H NMR, IR, and mass spectroscopy (Table 1). After a meticulous analysis of the mass peaks shown in Table 1, we determined that harsh reaction conditions (PB₃, 7 M NH₄ in MeOH) did not influence the polydispersion of the starting commercial PEG.

In order to verify the possibility of using the functionalized solid support in successive cycles, silica gel 3 was cleaved and, to avoid amide formation (compound 14), we used an aqueous basic solution. Next, 1 g of the commercial PEG was then loaded on the recycled solid support, as presented in Scheme 1. This coupling afforded 3 with a loading of 0.091 mmol of PEG per gram of solid support, a result similar to that achieved for the first coupling on silica 2.

The remaining hydroxy group of the obtained compounds can be further modified to generate other desired heterobifunctional polymers. For example, compound 10 was efficiently oxidized to the corresponding carboxylic acid 12, by treatment in aqueous media at pH >12 with potassium permanganate (2 equiv, Scheme 3).

In conclusion, we reported a successful, efficient, easy, and direct procedure for the monofunctionalization of commercial polyethylene glycol, exploiting inexpensive silica gel as a solid support. The monofunctionalized desymmetrized products can be used after cleavage from the silica support without purification, and eventually, can be directly exploited for additional solid-supported synthesis.

Acknowledgment

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References and Notes


(32) The loading was calculated by treating 1 g of pegylated silica gel 3 with cleavage solution and weighing the recovered PEG.


(34) Analytical sample was treated with NaBH₄ and analyzed with mass spectrometer. ESI-MS (most intense peaks)

\[ n = 10 \] 541.4 [M+N⁺]; \( n = 11 \) 585.4 [M+N⁺]; \( n = 12 \) 629.9 [M+N⁺].