Tetrahedron Letters 55 (2014) 2110–2113

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fluorous synthesis of mono-dispersed poly(ethylene glycols)

Yu Li [a,†], Qi Guo [a,†], Xuefei Li [a], Hua Zhang [a], Fanghua Yu [a], Weijiang Yu [a], Guiquan Xia [a], Mingyang Fu [a], Zhigang Yang [a,b,*], Zhong-Xing Jiang [a,b,c,*]

[a] Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, PR China
[b] Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, Wuhan 430071, PR China
[c] Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, PR China

ARTICLE INFO

Article history:
Received 6 December 2013
Revised 24 January 2014
Accepted 13 February 2014
Available online 26 February 2014

Keywords:
Fluorous chemistry
Solid-phase extraction
Fluorinated surfactant
Mono-dispersed PEGs

ABSTRACT

Mono-dispersed poly(ethylene glycols) (PEGs) are of great value in the development of biopharmaceuticals. However, tedious synthesis limits the availability of mono-dispersed PEGs. To address this issue, a fluorous synthesis of mono-dispersed PEGs, discretely PEGylated surfactants and ^{19}F magnetic resonance imaging (MRI) agents has been developed. During the synthesis, both fluorous and normal phase silica gel-based solid-phase extractions were successfully employed to simplify the purifications. This synthesis provided an easy access to valuable mono-dispersed PEGs and related molecules for biomedical application on multi-gram scales.

PEGs are a class of flexible, water soluble, and biocompatible polymers which play important roles in biopharmaceutical formulations.[1] The history of PEGylation can be dated back to the 1970's when Davis et al., first conjugated PEGs to bioactive proteins and found PEGylated proteins exhibited dramatically longer in vivo half-time and lower immunogenicity than their parent proteins.[2] Since this pioneering work, PEGylation has been extensively explored in biomedical research and PEGs have set the 'golden standard' for polymers used in pharmaceutical research. Now, the so-called 'stealth effect' of PEGs has been routinely employed in drug development to increase solubility and stability, reduce immunogenicity and dosing frequency, and optimize the pharmacokinetics profiles of therapeutics.[1] Till 2012, there are already a dozen of PEGylated drugs approved by the FDA. For example, Neulastim, a PEGylated filgrastim protein, and Pegasys, a PEGylated interferon alfa, have a combined annual sale of over $5 billion in 2011.

As a class of widely used polymers, the heterogeneity of PEGs has brought a range of problems in their biomedical applications. Since the 1970's, PEGs with polydispersity indexes (PDI) within 1.09, such as PEG_{400}, PEG_{2000}, PEG_{4000} etc., are routinely used in biomedical research. However, commercially available PEGs with 6 and more ethylene glycol units are usually a mixture of homologs. For example, over 30 components were detected by MALDI-TOF from a commercially available PEG_{3400} with a PDI of 1.01.[3a] Such heterogeneity complicates many stages of PEGs' biomedical applications, such as PEGylation,[3e] purification, characterization, clinical application, and drug regulatory approval.[3a] To avoid such heterogeneity, mono-dispersed PEGs are highly preferred for biomedical applications. Unfortunately, mono-dispersed PEGs are either not commercially available, or very expensive. Therefore, it is of great importance to synthesize mono-dispersed PEGs on preparative scales.

Recently, a few methods for preparing mono-dispersed PEGs from commercially available oligo(ethylene glycols) of defined length have been developed.[3] However, due to the high polarity of PEGs, normal phase chromatography can hardly purify the desired products. Therefore, reverse phase chromatography[3e] or gel-permeation chromatography[3c] is usually required. Such tedious purification dramatically limited the availability of mono-dispersed PEGs. To this end, fluorous technology provides a number of convenient purification methods, such as fluorous liquid-phase extraction, fluorous solid-phase extraction (FSPE), and fluorous HPLC.[4] As a separation technology that is based not on the polarity but on the fluorous interaction,[5] fluorous separation would be a good choice for the rapid purification of fluorous-tagged PEGs. In this way, it can dramatically improve synthesis efficiency. Herein, we describe a fluorous synthesis of mono-dispersed PEGs with up to 20 ethylene glycol units on multi-gram scales.

* Corresponding authors. Tel.: +86 27 68759220; fax: +86 27 68759850.
E-mail addresses: zgyang@whu.edu.cn (Z. Yang), zxjiang@whu.edu.cn (Z.-X. Jiang).
† These two authors contributed equally to this work.

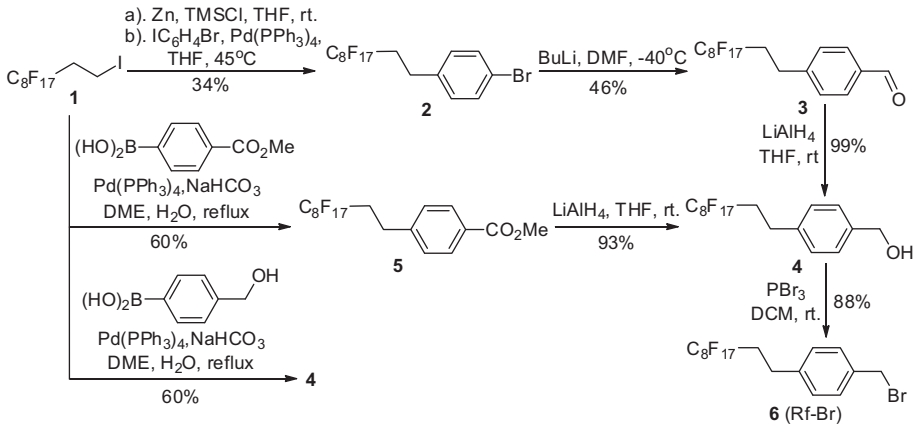
http://dx.doi.org/10.1016/j.tetlet.2014.02.047
0040-4039/

With these ideas in mind, our attention was first paid to the preparation of a suitable fluorous tag. Here, the fluorous tag played two roles: (1) as a protective group for the hydroxyl group in PEGs, (2) as a fluorous separative tag for fluorous purification. The benzyl group has been widely used as a protective group in PEG modification because it is stable under basic and acidic conditions and it can be removed under mild condition. Therefore, the *para*-perfluorooctylethyl substituted benzyl bromide **6** was employed in this synthesis (Scheme 1). Initially, the fluorous benzyl bromide **6** was prepared according to Curran's strategy.[6] However, it turned out that the synthesis efficiency was very low (14% yield over 4 steps) and the purification was very tedious. These drawbacks promoted us to develop an alternative synthetic method for this fluorous tag. Then, a Suzuki cross-coupling[7] reaction between 4-(methoxycarbonyl)phenyl boronic acid and β-(perfluorooctyl)-ethyl iodide **1** was explored. This Suzuki cross-coupling reaction is not moisture sensitive and the product **5** can be conveniently prepared on a multi-gram scale with good yield. After reduction of ester **5** with lithium aluminum hydride, the newly formed benzyl alcohol **4** was then transformed into benzyl bromide **6** with phosphorous tribromide. In this way, the fluorous benzyl bromide **6** was prepared on a multi-gram scale with a 49% yield in 3 steps. To further improve the synthesis efficiency, a Suzuki cross-coupling reaction between 4-hydroxymethylphenyl boronic acid and β-(perfluorooctyl)-ethyl iodide **1** was carried out to provide the fluorinated benzyl alcohol **4** in one step with a 60% yield. It is noteworthy that all the intermediates and bromide **6** were conveniently purified by FSPE.

With the fluorous tag **6** in hand, a fluorous synthesis of mono-dispersed PEGs with up to 20 ethylene glycol units was then investigated. The tetra(ethylene glycol) was chosen as the building block for the synthesis because it is the longest commercially available oligo(ethylene glycol) with defined molecular weight and reasonable price. A divergent synthesis of mono-dispersed PEGs with 4n (n = 2, 3, 4, 5) ethylene glycol units was designed by repetitively attaching the modified tetra(ethylene glycol) to the fluorous tag **6** (Scheme 2). In this way, all the synthetic intermediates contain a fluorous tag for rapid fluorous purification. After selectively protecting one of the hydroxyl groups in tetra(ethylene glycol) **7** with triphenylmethyl chloride,[8] the resulting alcohol **8** was then tosylated to give intermediate **9** in 84.2% yield over two steps. In order to simplify fluorous solid-phase extraction (FSPE), fluorous tag **6** was coupled with excess mono-protected tetra(ethylene glycol) **8** in the presence of sodium hydride. As expected, the fluorinated ether **10** was obtained with high yield after convenient FSPE purification.

During the deprotecting-coupling cycle, FSPE and normal phase silica gel based solid phase extraction (SPE) can efficiently purify the intermediates. Removal of the triphenylmethyl group in **10** with catalytic amount of *p*-toluene-sulfonic acid gave alcohol **11**.[9] Quenching the reaction with a base is crucial, otherwise *p*-toluene-sulfonic acid would catalyze the reverse reaction to provide the starting material **10** when evaporating methanol from the reaction mixture. To avoid silica gel-based chromatography, FSPE was initially used to isolate **11** from the reaction mixture. However, the hydrophobic triphenylmethyl-related impurities which have poor solubility in methanol and water can hardly be washed out from fluorous silica gel with a cocktail of methanol/water (8/2). Fortunately, the normal phase silica gel-based SPE successfully purified alcohol **11** by taking advantage of the large polarity difference between alcohol **11** and the impurities. It was also found that most of impurities can be easily removed by filtration of the cold reaction mixture. With fluorous alcohol **11** in hand, fully protected octa(ethylene glycol) **12** was prepared by coupling alcohol **11** with excess amount of tosylate **9** in the presence of sodium hydride.[10] The resulting octa(ethylene glycol) **12** was purified with FSPE. After one cycle of deprotecting-coupling from fluorinated ether **10**, fluoroalkyl-substituted octa(ethylene glycol) **12** was prepared on a scale of over 10 grams with a 74.5% yield in two steps. NMR and mass spectra indicate that mono-dispersed **12** was prepared with high purity after SPE.

With this protocol in hand, a range of procurers for mono-dispersed PEGs can be conveniently prepared by repeating the deprotecting-coupling cycle. Therefore, alcohol **19** with 20 ethylene glycol units was conveniently synthesized by repeating the deprotecting-coupling cycle 5 times from intermediate **10** (Scheme 2). The fluorinated benzyl protective group in **19** was removed by hydrogenolysis under 1 atm of hydrogen atmosphere and the mono-dispersed PEG **20** was prepared on a gram scale. It is noteworthy that the resulting fluorous toluene can be recovered by simple liquid-phase extraction of the reaction mixture with ether. As expected, all the intermediates can be rapidly purified by either FSPE or silica gel-based SPE. It is worth pointing out that some fine tuning is necessary when purifying intermediates with higher molecular weight due to their increased hydrophilicity and decreased fluorophilicity. In these cases, the percentage of water in the FSPE eluant system was increased (from 20% to 50%) to retain the fluorous component on the fluorous silica gel while washing the non-fluorinated impurities out. In this synthesis, silica gel-based solid-phase extraction complements FSPE when low polar impurities are very hydrophobic and insoluble in the methanol/water system.



Scheme 1. Synthesis of fluorous benzyl bromide **6**.