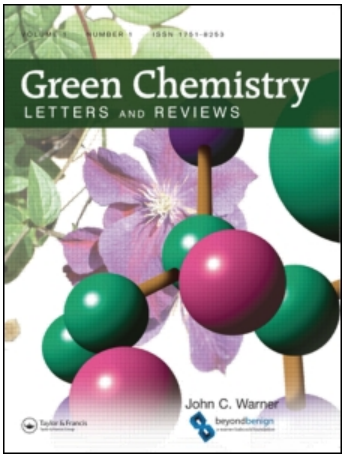

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ORIGINAL ARTICLE

A green synthetic process for the preparation of water-soluble drugs: pegylation of menadiol and podophyllotoxin

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Pegylation of drugs is a frequently employed strategy used to improve the pharmacokinetics and pharmacodynamics of drugs. Despite this, the virtues of pegylation as a green synthetic approach to enhance the water solubility of drugs has not been discussed. Using two well-known active pharmaceutical ingredients, menadiol sodium diphosphate (**1**) and the semisynthetic natural product, etoposide phosphate (**3**), green metrics for the processes of pegylation versus phosphorylation are presented and discussed. Menadiol (**2**) was prepared by an ultrasound mediated reduction of menadione (**9**). In a study done by the National Cancer Institute, PEG podophyllotoxin **12** was screened for activity against 60 different human cancer cell lines and the desired anticancer properties of podophyllotoxin (**5**) were retained. As podophyllotoxin (**5**) is a natural product, the concept of "green drugs" is espoused.

Keywords: pegylation; podophyllotoxin; atom economy; natural products; cancer drugs

Introduction

Economic stimuli, government legislation, and public pressure are all reasons for industrial green chemistry practices. Process yield improvements when they are coupled with a reduction in waste are particularly important as the reaction mass efficiency (RME) is proportional to the yield (ε) and is expressed by equation 1 (*1*). SF is the stoichiometric factor and AE is the atom economy. MRP is the material recovery parameter and this term details the consumption of auxiliary materials such as reaction solvents, work-up materials and purification materials (*2*).

$$RME = \varepsilon AE \frac{1}{SF} MRP \quad (1)$$

The Chemistry and Manufacturing Control (CMC) section of a New Drug Application (NDA) requires that the environmental impact of the process be assessed and the NDA also requires the pollution potential of the drug to be determined. The latter characteristic has been of particular concern because of the detection of pharmaceuticals in drinking water (*3*). Public pressure for green consumables has caused great increase in sales for natural personal care products (*4*).

Process research and development is uniquely situated as the liaison between drug discovery and manufacturing and as such, may be able to spearhead activities for the development of "green drugs" in which all components of a drug product, including the drug substance are either from green sources or made by green chemical processes. As we have shown for chemical processes, solid process development cannot change a process in which the route has fundamental issues associated with the amount and kind of waste that is generated from it. Early intervention is needed to correct such situations. For the specific case of the syntheses of thioamides, new thionating methodology would be required for a truly green chemistry process for the synthesis of a stryrl thiazole, Ro 24-5904 (*5*).

The water solubility is an important characteristic of drugs and for compounds which contain a basic nitrogen, the literature abounds with examples of compounds which have been rendered water-soluble by the formation of salts. For compounds, which do not contain a basic nitrogen but contain a reactive hydroxyl group, these compounds can be rendered water-soluble by the formation of phosphates. Perhaps, the first example of phosphorylation for the purposes of water solubility was described by Fieser in the 1940s for the synthesis of menadiol tetrasodium diphosphate (**1**) (*6*). Commercially, menadiol sodium phosphate (**1**) is used to alleviate a hemorrhagic condition in newborn infants (*7*). Menadione, 2-methyl-1,4-napthoquinone is vitamin K3 and its

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bisulfite adduct is used as a stabilized form in the animal feed industry (*8*).

As described by Fieser, the synthesis of menadiol sodium diphosphate (**1**) involves reaction of menadiol (**2**) with phosphorus oxychloride using pyridine as the solvent (Scheme 1). After the reaction is complete, the reaction is quenched with water and solid sodium carbonate is added. The pyridine layer is separated and is removed. Extensive manipulations are required to separate menadiol phosphate (**1**) from inorganic salts (*6*). Both the use of pyridine as a solvent and the formation of three moles of pyridinium hydrochloride per mole of phosphate group detracts from the greenness of this elegant and seminal approach to the formation of water-soluble drugs.

A more contemporary example of phosphorylation for the purposes of enhancement of the water solubility of drugs would be etoposide phosphate (**3**). Etoposide (**4**) is an antineoplastic agent and is a semisynthetic derivative of the natural product, podophyllotoxin (**5**) (Figure 1) (*9*). Podophyllotoxin (**5**) is found in the roots of the mayapple tree and in the leaves of the Eastern red cedar (*10*). Podophyllotoxin (**5**) disrupts microtubular assembly in miotic cells and etoposide (**4**) modulates the activity of DNA topoisomerase II (*11*).

Although the reports of these phosphorylation processes span a time period of more than 50 years, they share several common features. The Bristol-Myers process for the phosphorylation of 4′-demethylepipodophyllotoxin (**6**) involves the reaction of *in situ*-prepared dibenzyl chlorophosphate in the presence of Hunig's base, N,N-diisopropylethylamine (DIEA) and a catalytic amount of dimethylaminopyridine. Both of these processes utilize chloride as a leaving group and a base to trap the resulting hydrogen chloride. The Bristol-Myers process does not involve a tedious work-up procedure to separate the product from byproducts and an aqueous work-up procedure is used. A second hydrogenation step is required to remove the benzyl groups from the latent phosphate **8** (Scheme 2) (*9*).

Given the array of potential therapeutics, which are essentially neutral and possess a hydroxy group, we wish to present data that pegylation is a green process strategy for the preparation of water-soluble drugs. During the drug discovery stage, routine pegylation of small molecule libraries might identify candidates with superior water solubilities, which might otherwise be overlooked for the same reason. The model drugs for this green chemistry pegylation process are menadiol (**2**) and podophyllotoxin (**5**).

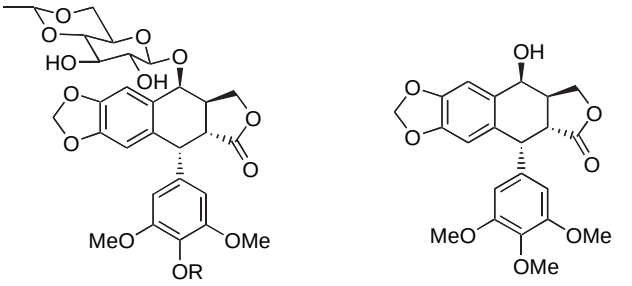
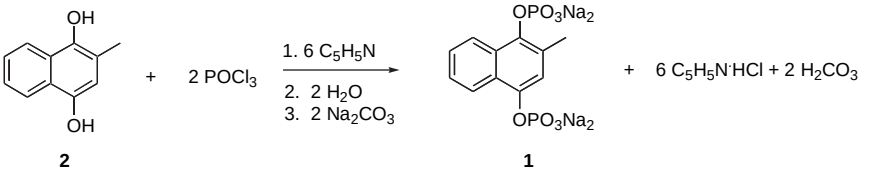


Figure 1. Structures of etoposide phosphate (**3**), etoposide (**4**) and podophyllotoxin (**5**).

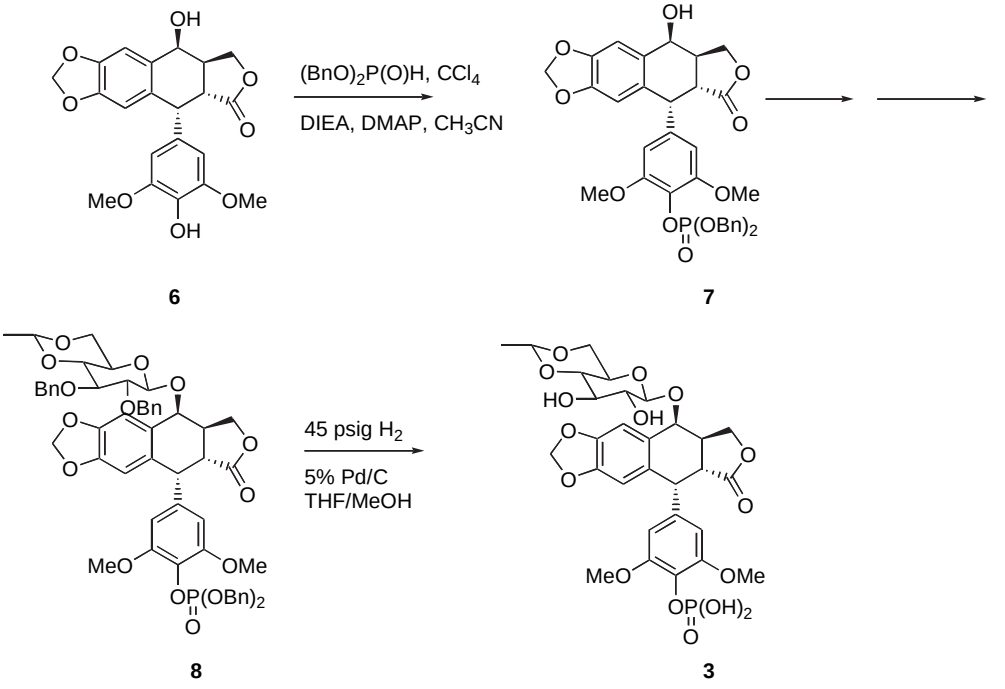
Results and discussion

Menadiol (**2**) was prepared by an ultrasound mediated sodium dithionate reduction of menadione (**9**) (Scheme 3). The corrected isolated yield of menadiol (**2**) was 79%. On an analytical scale, menadiol (**2**) was found to rapidly oxidize back to menadione (**9**) in solvents, which were not degassed and so the yield is quite good given the instability of menadiol (**2**). The menadiol (**2**) ultrasound reduction is particularly well suited for this molecule as the heterogeneous nature of the reaction means that only minute amounts of menadiol (**2**) are in solution at any one time. The elimination of an organic solvent as both a reaction medium and as the product isolation solvent are important green characteristics of this new process.

The balanced chemical equation for the preparation of menadiol diphosphate (**1**) when phosphorus oxychloride is used as the phosphorylating agent requires that six moles of pyridine to be present to serve as a scavenger of hydrogen chloride (Scheme 1). This situation detracts from the atom economy (AE) for this process and the AE is only 0.341 (Table 1). As



Scheme 1. Synthesis of menadiol sodium diphosphate (**1**).



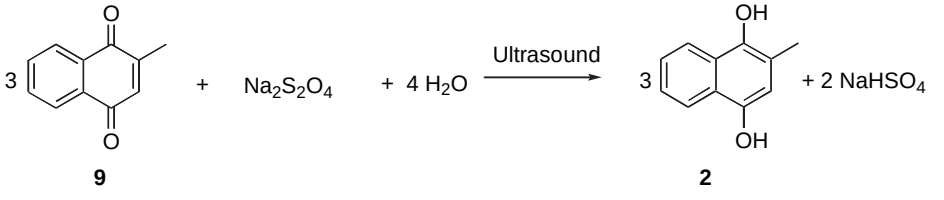
Scheme 2. Synthesis of etoposide phosphate (**3**).

exemplified by the Fieser procedure, a tedious work-up is required in order to separate the water-soluble pyridinium hydrochloride and inorganic phosphate from menadiol sodium diphosphate (**1**).

In contrast, an analogous water-soluble drug, PEG menadiol **11** can readily be prepared by pegylation of menadiol (**2**) with monomethoxypoly(ethylene glycol) succinimido carbonate (mPEG-SC) (**10**) (Scheme 4). Although menadiol (**2**) is stable in the solid state, its instability in solution means that an excess of menadiol (**2**) was used as menadione (**9**) was detected by TLC analysis. Improvements in analytical methodology for the detection of menadiol (**2**) in the presence of PEG menadiol **11** should render a more accurate charge of the amount of menadiol (**2**). The Fieser phosphoryation process would have the same issue of competitive rates of phosphorylation versus oxidation.

For the pegylation reaction, the only byproduct in this chemistry is N-hydroxysuccinimide (NHS), which in principle can be recycled to prepare the reagent for the synthesis of mPEG-SC (**10**), N,N'-disuccinimidyl carbonate (DSC). Although a search of the literature revealed that recycling of NHS has not been demonstrated, the synthesis of DSC involves the reaction of NHS with phosgene (*13*). The molecular weight of NHS is similar to the molecular weight of pyridinium hydrochloride, but only two moles of NHS are formed whereas in the Fieser process, six moles of pyridinium hydrochloride are produced. Because of the large increase in molecular weight of the PEG product, the atom economy of the pegylation process skyrockets to 0.977. However, due to the nature of the waste product, NHS and other physical advantages of PEG menadiol **11**, the atom economy might well be a measure of the greenness of the drug substance and the drug product.

There are several noteworthy physical properties of PEG menadiol **11**. When the extinction coefficient of menadione (**9**) in methanol is used, the water solubility of vitamin K3 was estimated at 1.5×10^{-5} g/L. In contrast, PEG menadiol **11** was



Scheme 3. Ultrasound reduction of menadione (**9**).

Table 1. Green metrics for the preparation of water-soluble derivatives of menadiol (**2**).

Step	AE	E_{mw}	Yield (ε)	SF	RME	E_m
Ultrasound reduction	0.685	0.460	80%	8.871	0.0618	15.184
Fieser phosphorylation	0.341	1.417	86%	4.351	0.0660	14.141
PEG Diesterification	0.977	0.0231	63.6%	1.008	0.616	0.622

freely water-soluble and has a solubility of at least 300 g/L. Using a simple nut and bolt, PEG menadiol **11** was directly compressible and readily formed a tablet. No excipient was necessary.

The reaction mass efficiency (RME) for the Fieser phosphorylation is only 0.0660 and this is due to a low atom economy and relatively high stoichiometric factor (SF). The isolated yield for the phosphorylation is good at 86%. Sufficient experimental details were not present in the Fieser procedure to calculate the material recovery parameter (MRP) and so the RME is considering only the SF, ε(yield) and the AE. A high stoichiometric factor could be improved by process development, but a low atom economy is a fundamental characteristic of the chemistry.

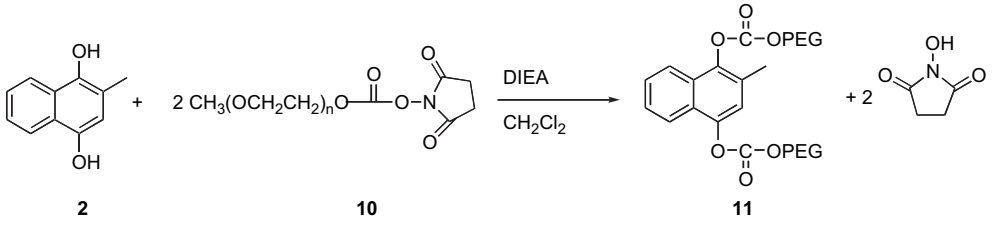
The preparation of a water-soluble derivative of menadiol (**2**) by pegylation is inherently more green than the conventional phosphorylation method. Recovery of the ethyl acetate from the crystallization would be required as the RME plummets to 0.005 when no reclaimation of the ethyl acetate is considered and the E_m soars to 188 g of waste per gram of product .[1] Substitution of ethyl acetate for methylene chloride in the reaction would also mean that a single solvent is used in the process (*14*).

Despite the fact that the route for pegylation has distinct advantages over the traditional phosphorylation step, methylene chloride is used as a solvent for the preparation of the PEG derivatives **11** and **12**. Although the process is designed so that the methylene chloride could be recovered, the use of this solvent does detract from its inherent greenness. In other work, we have found that acetonitrile can be used as a substitute for methylene chloride in pegylation chemistry and that for reactive nucleophiles that dry ethyl acetate at 40°C at the 40:1 solvent ratio for the crystallization can also be used.

The green chemical advantages of pegylation versus phosphorylation (Table 2) can also be seen for the preparation of the water-soluble drug, PEG podophyllotoxin **12** (Scheme 5). For this example, pegylation is contrasted with a two step phosphorylation procedure (Table 3). This elegant two step approach, in which the tedious work-up has been eliminated, was accomplished by the use of protecting groups on the phosphate groups. Carbon tetrachloride was also used as the chlorine source to prepare the reagent, dibenzyl chlorophosphate (*9*). The atom economy for the pegylation process is 0.977. As the RME is 0.647 for the pegylation and the RME is 0.266 for the phosphorylation, pegylation as a one step approach for the preparation of a water-soluble drug is superior.

In this analysis, we have compared synthetic processes by which the same characteristic of an active pharmaceutical ingredient can be conferred on a target molecule. In both cases, the RME for the pegylation process for the preparation of water-soluble drug is lower than the RME from phosphorylation chemistry or the RME from a two step protocol using an *in situ* preparation of dibenzylchlorophosphate followed by a reduction. For completeness, the reagent excesses and the isolated yield for the phosphorylation by both methods to the same target molecule, disodium podophyllotoxin-4-phosphate were used to calculate the RME (Table 4). Again the very favorable AE of 0.977 for the preparation of PEG podophyllotoxin **12** causes the RME for the preparation of the pegylated compound to be greater than that for disodium podophyllotoxin-4-phosphate.

Both PEG menadiol **11** and PEG podophyllotoxin **12** were accepted by and submitted to the National Cancer Institute (NCI) for testing in their 60



Scheme 4. Synthesis of PEG menadiol **11**.

Table 2. Green metrics for the preparation of etoposide phosphate (**3**) by a two step phosphorylation method.

Step	AE	E_{mw}	Yield (ε)	SF	RME	E_m
Dibenzyl phosphorylation	0.720	0.431	90%	1.942	0.334	1.993
Deprotection	0.648	0.275	87.4%	1.000	0.566	0.767
Overall	0.586	0.706	78.7%	N/A	0.266	2.760

cell human cancer line screen (*12*). Podophyllotoxin (**5**) and its derivatives are well-known anticancer agents (*9–11*). and menadione (**9**) has been evaluated in a Phase 1 trial of individuals with advanced malignacy (*15*). The screening results from the NCI Developmental Therapeutics Program indicated that PEG menadiol (NSC 743711) **11** did not have a significant growth inhibitory effect against the nine panel 60 cell line screen in a concentration of 1.00×10^{-5} M. PEG podophyllotoxin (NSC 745897) **12**, however, was very active across all nine panels of the 60 cell line screen. The mean growth percent for the 60 cell lines was 8.24 indicating a greater than 91% growth inhibitory effect at the 1.00×10^{-5} M concentration. Based on these results, PEG podophyllotoxin **12** was tested at five dose levels and was found to be very active in a concentration of 1×10^{-6} M across all nine panels in the 60 cell line screen. The results of the multi-dose screen were repeated with the same outcome.

Conclusion

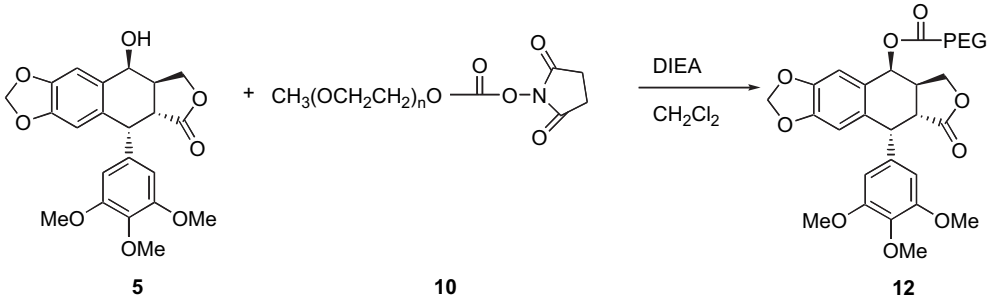
Monomethoxypoly(ethylene glycol) is essentially nontoxic and PEG proteins have been approved for human use (*16*). Pegylation is a commonly used strategy, which is employed to improve the pharmacokinetics and pharmacodynamics of small molecules and the therapeutic dosage is typically much less than that of unpegylated compound (*17,18*). As we have shown, pegylation of small molecules is a green chemistry approach for the preparation of water-soluble drugs as the atom economy for the pegylation step exceeds 0.9. When mPEG-SC (**10**) is used, NHS is the only by-product. Coupled with the fact that pegylated small molecules are directly compressible, this means that these substances can be formulated as such into tablets. With green technology used to prepare both the drug substance and drug product, pegylated small molecules might very well be the first generation of green drugs.

Experimental

mPEG-SC, 5Kda (**10**) was a product of internal manufacture. Menadione (**9**) was used as received from Vanetta Chemical Co and podophylotoxin (**5**) was obtained from Aldrich Chemical Co. HPLC analysis for PEG menadiol **11** was done on a Prosphere HP C18 300Å, 5 μ, 250 mm × 4.6 mm column by Alltech (*19*). Gradient elution was used with a mixture of water and acetonitrile and a flow rate of 1.00 mL/min. The initial conditions were 95% water and 5% acetonitrile. This was changed to 80% water over a 15 minute period. Over an additional 45 minutes, the conditions were changed to 100% acetonitrile. The detection was at 254 nm. Menadiol (**2**) was analyzed on a Luna C8(2), 5 μ, 150 × 4.6 mm column by Phenomenex, operating at 35°C with isocratic elution. A mobile phase composition of 50% aqueous, 0.1% TFA (v/v), and 50% acetonitrile was used. The flow rate was 1.00 mL/min and the detection was done by UV at 230 nm. The HPLC system consisted of modular components from ThermoSeparation Products.

Preparation of 2-methyl-1,4-napthalenediol (2)

Technical grade sodium hydrosulfite (purity equals 85%) (93.5 g; 0.45 mole)and 330 ml of distilled water



5 10 12

Scheme 5. Synthesis of PEG podophyllotoxin **12**.

Table 3. Green metrics for the preparation of water-soluble derivatives of podophyllotoxin (**5**).

Step	AE	E_{mw}	Yield (ε)	SF	RME	E_m
PEG esterification	0.977	0.0216	66.2%	1.00	0.647	0.545
Two step phosphorylation	0.586	0.706	78.7%	N/A	0.266	2.760

were combined. Menadione (**9**) (35.0 g, 0.20 mole) was added and the batch was sonicated. A Bransonic 52 sonicator was used. After three hours, the temperature rose to 38°C. A greenish supernatant with a purple-grey solid was observed. The sonication was continued for a total of 22 hours. The suspension was filtered and the solid was washed twice with 35 mL of water. The product was dried at 40°C under vacuum. There was obtained 30.17 grams of menadiol (**2**) as a purplish-grey powder. The water content by Karl Fischer titration was 6.1% and so the corrected yield is 79%. Menadiol (**2**) had mp 176–178°C (lit (*20*) mp 181°C). In the described HPLC system, menadiol (**2**) elutes at 2.7 minutes whereas menadione (**9**) elutes at 4.6 minutes. The purity by HPLC was 95.7% with the balance being mainly menadione (**9**). A subsequent injection of the same degassed solution taken after 10 minutes had elapsed showed that the purity had decreased to 84.3% with a concomitant increase in the amount of menadione (**9**).

Preparation of 1,4-bis[monomethoxypoly(ethyleneglycol)carbonyl]oxy-2-methyl-naphthalene (11)

Under an argon atmosphere, 5.14 g (1.00 mmole) of mPEG-SC, 5Kda (**10**), 87.4 mg (0.5 mmol) of menadiol (**2**) and 50 mL of anhydrous methylene chloride were combined. N,N-Diisopropylethylamine (350 μL) was added. The mixture was stirred at room temperature for 24 hours. TLC analysis showed that menadione (**9**) was present and an additional 44.8 mg of menadiol (**2**) was added. After stirring for an additional 18 hours, the solvent was removed under vacuum and the residue was dried. The solid was recrystallized from 200 mL of ethyl acetate. After cooling in wet ice, the suspension was filtered and washed with 2×50 mL of ethyl acetate. The product was dried at 40–45°C until a constant weight was obtained. The recrystallization was repeated to remove color using the same solvent ratio as described above. There was obtained 3.25 g of a peach solid in a 63% yield based on the amount of mPEG-SC (**10**). HPLC showed a main component at 27.8 min for PEG menadiol **11** and menadione (**9**) elutes at 29.5 minutes.

Preparation of [5R-(5α,5aβ,8aα,9α)-5,8,8a,9-tetrahydro-9-[(monomethoxypoly-(ethyleneglycol))-carbonyl]oxy-5-(3,4,5-trimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one (12)

Under an argon atmosphere, 1.03 g (0.2 mmol) of PEG-SC, 5Kda (**10**), 82.9 mg (0.20 mmole) of podophyllotoxin (**5**) and 17 mL of anhydrous methylene chloride were combined. N,N-Diisopropylethylamine (70.0 μL) was added. The mixture was agitated at room temperature for 23 hours. The solvent was removed under vacuum and the residue was dried at 40–45°C on a rotovap. The residue was recrystallized at 40°C from 40 mL of ethyl acetate. After cooling in wet ice, the suspension was filtered and washed with 2×5 mL of ethyl acetate. The solid was dried at 40°C under vacuum. There was obtained 0.72 g of a white solid of PEG podophyllotoxin **12** in a 66.3% yield.

Water Solubility

Menadione (**9**). An estimate of the water solubility of menadione (**9**) was done by using a saturated aqueous solution and measuring the absorbance at 340 nm. The extinction coefficient for menadione (**9**) was determined in methanol and this extinction coefficient was used as an estimate of the extinction coefficient in water. The water solubility was 1.5×10^{-5} g/L.

PEG Menadiol (**11**). PEG menadiol (**11**) (0.5 g) was combined with 1 mL of water and a complete solution was obtained. If 0.5 g of the PEG polymer

Table 4. Comparative green metrics for the preparation of disodium podophyllotoxin-4-phosphate and PEG podophyllotoxin **12**.

Process	AE	E_{mw}	Yield (ε)	SF	RME	E_m
Fieser phosphorylation	0.532	0.567	86%	5.272	0.085	10.740
Bristol-Myers phosphorylation	0.530	0.887	78.7%	N/A	0.280	2.568
Pegylation of Podophyllotoxin (**5**)	0.977	0.0216	66.3%	1.000	0.647	0.545

11 contributes 0.5 mL worth of volume, the estimate of the water solubility of this drug is approximately 300 g/L.

Note

1. We thank the referee for this calculation.

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