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A One-Pot, Efficient Synthesis of the Potent Cytotoxic Podophyllotoxin Derivative NPF

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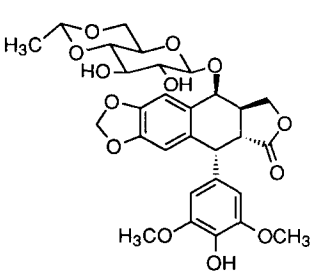
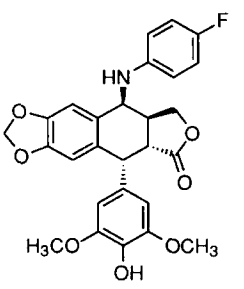
Abstract: One-pot syntheses of 4'-demethylepipodophyllotoxin **7** and NPF **2** (4'-*O*-demethyl-4β-(4"-fluoroanilino)-4-deoxypodophyllotoxin) are described from podophyllotoxin **3** *via* a protocol using trimethylsilyl iodide in 72% and 52% overall yields, respectively.

Etoposide (VP16-213) **1** is an important antineoplastic agent commonly used for the treatment of many human malignancies,[1] either alone or in combination chemotherapy. Etoposide's major side effect is neutropenia which is the dose limiting-effect. Topoisomerase II is the intracellular target for this drug that stabilizes covalent enzyme-DNA cleavage complexes at specific sites, thereby leading to DNA double strand breaks and cell death.[2] Recently, Osheroff *et al.*[3] reported a combination of kinetic and binding studies to gain further insight into the mechanism of action of **1**. Consequently, a pathway of etoposide-induced DNA cleavage complex formation has been proposed, based on the resulting important data and the positional poison model[3] regarding the mechanistic basis of DNA cleavage enhancement by topoisomerase II poisons: the key pathway for the formation of the non covalent enzyme-drug-DNA ternary complex at specific sites which is in equilibrium with a covalent cleavage complex, proceeds through etoposide-topoisomerase II interactions. The shift of this equilibrium to the covalent complex is determined essentially by the ability of **1** to inhibit DNA religation at those sequences.



1

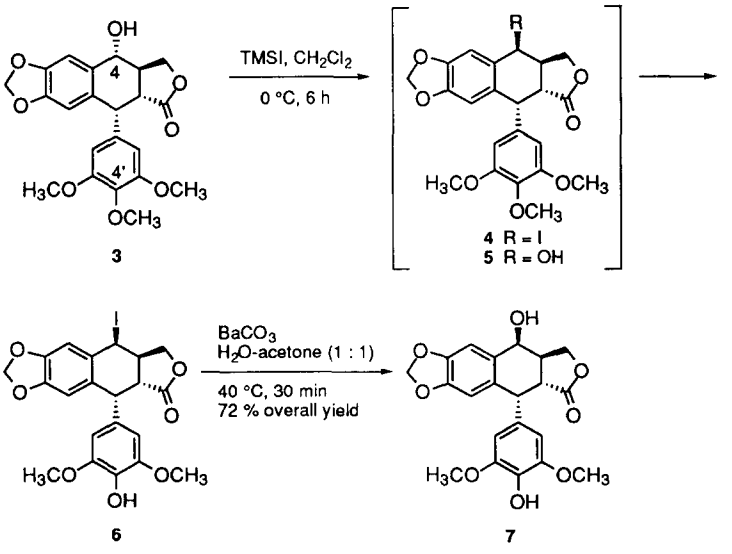


2

Much of the recent synthetic work on podophyllotoxin **3** has been focused on the synthesis of C-4 nonsugar-substituted analogues[4] which may show improved topoisomerase II inhibition and cytotoxicity. In particular, efforts in this area have resulted in the identification of 4'-*O*-demethyl-4β-(4"-fluoroanilino)-4-deoxypodophyllotoxin (NPF) **2** by Lee and co-workers.[5] It was found[6] to be 10-fold more potent as inhibitor of topoisomerase II and 100-fold more cytotoxic against various human tumor cells and etoposide-KB resistant cells. This compound is a promising source of new anticancer agents. Lee's synthetic route to **2** involves the following reactions from podophyllotoxin **3**: one-pot C-4 epimerization/4'-O-demethylation (52%), then C-4 bromination (100% crude yield) *via* a modified Kuhn's method,[7] and nucleophilic displacement with 4-fluoroaniline (45%). The overall yield of the synthesis is 23%. C-4α substitution was also observed but in low yield. It is interesting to note that addition of $Bu_4N^+I^-$ in the last step resulted in better yield (72%) with much improved selectivity as reported by Indian authors[8]. This result was ascribed to a dynamic kinetic resolution (DKR) process.[9] The origin of this highly stereoselective nucleophilic addition is the steric interaction of the nucleophile with the bulky E-ring and $Bu_4N^+I^-$.

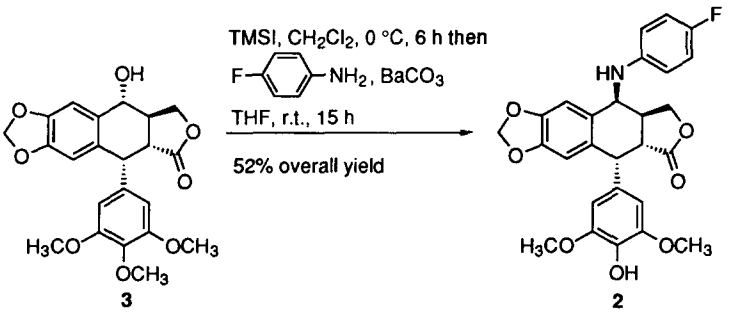
As part of an ongoing medicinal chemistry program[10] in the podophyllotoxin area, we report an improved synthesis of 4'-demethylepipodophyllotoxin **7**, precursor of etoposide **1**, and the one-pot synthesis of NPF **2** based upon the application of TMSI (trimethylsilyl iodide) to podophyllotoxin **3**.

Studies[11] have demonstrated the effectiveness of TMSI in attacking hindered methoxy groups in a regiospecific fashion. Furthermore, it is an excellent reagent for iodination of alcohols.[12] So far, to our knowledge, use of TMSI for both 4'-O-demethylation and C-4 epimerization has not been described for podophyllotoxin **3** itself. However, there has been one report[13] on the synthesis of structural analogues of the epipodophyllotoxins exemplifying the potential application of TMSI for the two reactions afore-mentioned. Therefore, we considered that TMSI could exhibit appropriate properties for transformation of **3** into the desired key intermediate **6**. Fortunately, the iodination-demethylation sequence of **3** occurred to give **6** without γ-lactone opening[14] detected (Scheme 1).



Scheme 1

Thus, 4'-demethylepipodophyllotoxin **7** was prepared in 72% overall yield by treatment[15] of podophyllotoxin **3** with TMSI (3 equiv.) in methylene chloride at 0 °C for 6 h followed by weak basic hydrolysis (H_2O-acetone, then $BaCO_3$). Additionally, epipodophyllotoxin **5**[16] could be isolated by addition of both water and $BaCO_3$ to quench the reaction mixture as soon as **3** disappeared as judged by TLC control, establishing the order of steps, i.e. **3** ->**4** -> **6**. On the other hand, NPF **2** was obtained in 52% overall yield[15] when 4-fluoroaniline (1.3 equiv.) was used instead of water in the presence of $BaCO_3$ (2 equiv.) in THF at room temperature (Scheme 2).



Scheme 2

In summary, the synthetic route outlined above provides a one-pot efficient method for the preparation of 4'-demethylepipodophyllotoxin **7** and NPF **2**. The extension of this methodology to the synthesis of 4β-alkylamino derivatives of podophyllotoxin is underway.

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15. Typical procedure for the preparation of **7** and **2**:

Compound **7**:

To a solution of podophyllotoxin **3** (3.22 g, 7.77 mmol) in dry methylene chloride (100 mL) was added at 0 °C a solution of TMSI [3.3 mL, 23.3 mmol in methylene chloride (10 mL)]. The reaction mixture was stirred for 5 h at 0 °C then a mixture of H_2O/acetone (50 mL/50 mL) and $BaCO_3$ (1.55 g, 7.85 mmol) were added successively. After 30 min at 40 °C, the resultant mixture was diluted with methylene chloride (100 mL), then poured into 10% $Na_2S_2O_3$ solution (500 mL). The organic layer was dried over $MgSO_4$, concentrated *in vacuo*. Flash chromatography on silica gel with methylene chloride/acetone : 92/8 as eluent gave 2.23 g of **7** (72%).

Spectral data, specific rotation, and mp agree with those in the literature.[7]

Compound **2**:

To a solution of podophyllotoxin **3** (504 mg, 1.24 mmol) in dry methylene chloride (15 mL) was added at 0 °C a solution of TMSI [620 µL, 4.36 mmol in methylene chloride (2 mL)]. The reaction mixture was stirred for 6 h at 0 °C then concentrated *in vacuo* at room temperature to give a brown residue. This crude product was dissolved in dry THF (12 mL) then $BaCO_3$ (490 mg, 2.48 mmol) and 4-fluoroaniline (141 µL, 1.49 mmol) were added successively. The mixture was stirred overnight at room temperature, then filtered and concentrated under reduced pressure. Flash chromatography on silica gel with cyclohexane/ethyl acetate : 3/1 as eluent gave 322 mg of **2** (52%).

Spectral data, specific rotation, and mp agree with those in the literature.[5]

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