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Facile and Efficient One-Pot Synthesis of 4β-Arylamino-podophyllotoxins: Synthesis of DNA Topoisomerase II Inhibitors (NPF and W-68)†

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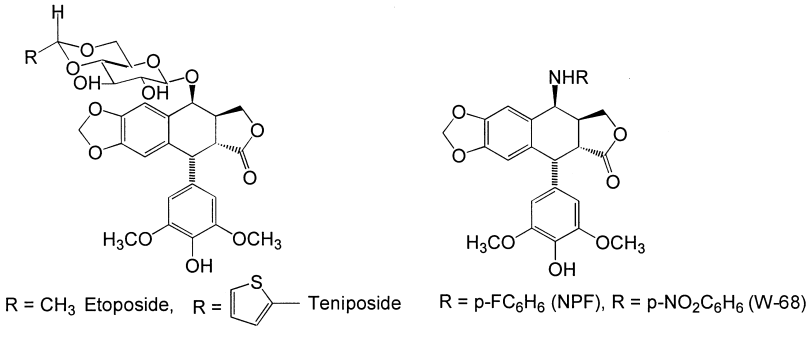
Abstract—A series of 4β-arylamino-4′-*O*-demethylepipodophyllotoxins and 4β-arylaminoepipodophyllotoxins have been synthesized with significant stereoselectivity and improved yields by employing the methanesulphonic acid/sodium iodide reagent system. Compounds NPF, W-68 and other DNA topoisomerase II inhibitors are prepared in good to excellent yields by this method and these are active or more active than etoposide in their inhibition of the human DNA topoisomerase II.

Etoposide and teniposide are semisynthetic drugs derived from podophyllotoxin and are in clinical use for the treatment of many cancers, particularly small cell lung cancer and testicular cancer.[1] These compounds block the catalytic activity of DNA topoisomerase II and concurrent enzyme-mediated production of lethal DNA strands leading to DNA damage and cytotoxicity.[2] Etoposide is widely used in clinical cancer therapy and this has stimulated a renewed interest by the researchers in the chemical and biological studies of podophyllotoxin derived antitumour agents.[3–7] The recent synthetic studies on podophyllotoxin have been focussed on the synthesis of the C-4 non-sugar substituted analogues which show improved topoisomerase II inhibition and cytotoxicity. In this endeavour, Lee and co-workers[8] have synthesized a large number of 4β-anilino derivatives of podophyllotoxin, in particular, they have identified 4′-*O*-demethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (NPF) and 4′-*O*-demethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin (W-68) as potential derivatives.

This new anticancer agent NPF has been found to exhibit 10-fold more potent topoisomerase II inhibition and 100-fold more cytotoxic activity against various human tumour cells and etoposide-KB resistant cells. The synthetic route for the 4β-anilino substituted 4′-*O*-demethylepipodophyllotoxin compounds reported[8] in the literature comprises of three steps, i.e. C-4 epimerization, C-4 bromination (modified Kuhn's method) and nucleophilic displacement with anilino compounds giving the overall yields ranging from 14 to 30%. In our recent studies,[9] we have improved the selectivity and yields in



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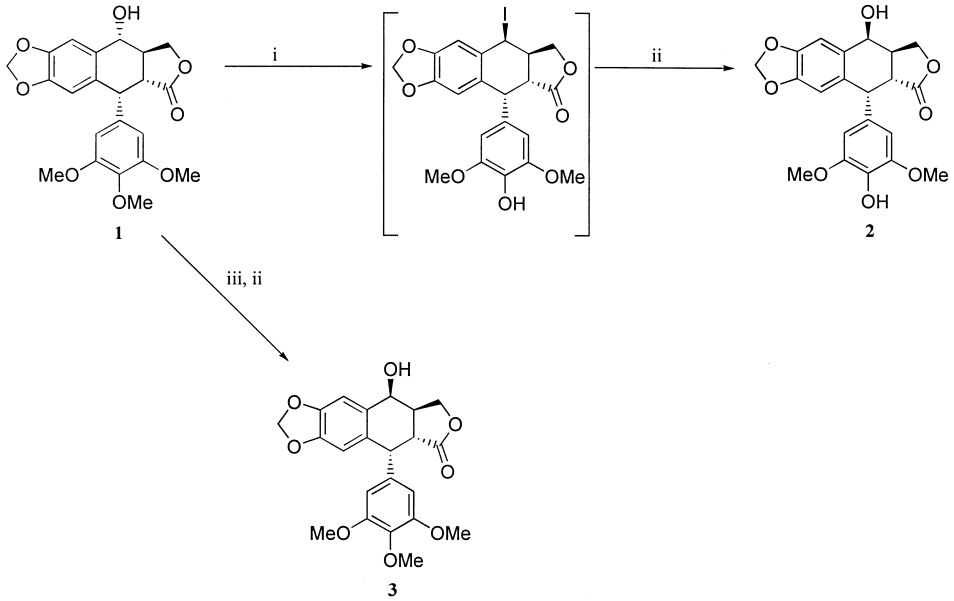
the last step of this nucleophilic displacement by the addition of tetrabutylammonium iodide ($Bu_4N^+I^-$) through the dynamic kinetic resolution process. Recently, a one-pot synthesis of NPF has been reported[10] employing trimethylsilyl iodide (TMSI), wherein 4′-*O*-demethylation and 4β-iodo displacement with *p*-fluoroaniline takes place with improved yield. There are some synthetic routes reported[11] in the literature and as well as developed by us[12–14] for the preparation of 4β-aminopodophyllotoxin and 4β-amino-4′-*O*-demethylepipodophyllotoxin as important precursors towards the synthesis of newer podophyllotoxin congeners.[15,16]

In this paper, we wish to report the synthesis of 4β-arylaminopodophyllotoxin derivatives employing sulphonic acid/sodium iodide as an efficient reagent system. This reagent has not been explored in the literature except for the deoxygenation of sulphoxides to the corresponding sulphides.[17] During a project directed towards the synthesis of new podophyllotoxin congeners, we investigated the methanesulphonic acid and *p*-toluenesulphonic acid/sodium iodide system for iodination of benzylic alcohol and simultaneously attacking the hindered methoxy group in a regiospecific fashion in the podophyllotoxin ring system. This observation clearly indicates that hydrogen iodide is formed in situ from methanesulphonic acid/sodium iodide. With these considerations in mind, this reagent system has been considered for its potential application for both the aspects of 4′-*O*-demethylation and C-4 epimerization in the podophyllotoxin system. Thus, treatment of podophyllotoxin **1** by the above reagent system in CH_2Cl_2 followed by weak basic hydrolysis (water:acetone, $BaCO_3$) gave 4′-*O*-demethylepipodophyllotoxin **2** (yield 65%). In comparison to the literature methods, i.e., reaction of **1** with HBr gas at 0 °C followed by hydrolysis[18,19] and treatment with trimethylsilyl iodide (TMSI),[10] the present route employing the methanesulphonic acid/sodium iodide as a reagent system for the conversion of **1** to **2** is a practical and useful procedure.

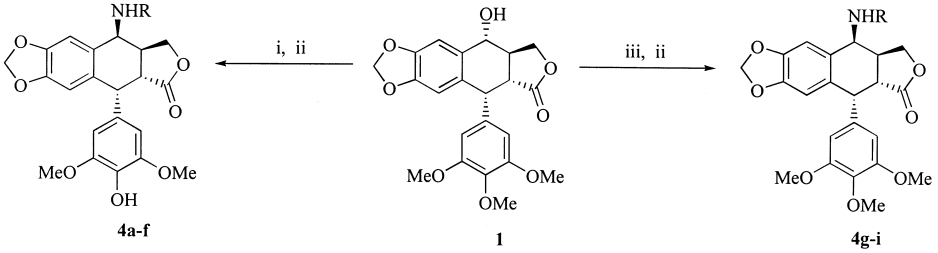
Interestingly, when this reaction has been carried out in MeCN as a solvent, 4′-*O*-demethylation (**3**) product is not observed for 12 h even under reflux conditions by using 3 mmol of the above reagent system, whereas this reaction in chlorinated solvents like CH_2Cl_2 or $(CH_2)_2Cl_2$ in 5 h at room temperature and in 2 h at reflux conditions proceeds to 4′-*O*-demethylation (**2**). Therefore, by using these two different type of solvents, the selectivity of 4′-*O*-demethylation could be manoeuvred by employing this methodology.

Further, reaction of podophyllotoxin with this reagent system followed by the addition of arylamines in presence of bases like $BaCO_3$ or K_2CO_3 or $CsCO_3$ gave the 4β-arylaminopodophyllotoxin analogues. As a result, employing CH_2Cl_2 as solvent produced 4′-*O*-demethylation and C-4 epimerization to give 4β-arylamino-4′-*O*-demethylpodophyllotoxin[20] **4a–4f** (40–55% yields), whereas the use of MeCN as a solvent produced C-4 epimerization alone without 4′-*O*-demethylation to afford the corresponding 4β-arylaminopodophyllotoxin analogues[21] **4g–4i** (80–85% yields). This may be attributed to the facile generation of hydrogen iodide in chlorinated solvents as in the case of TMSI, although its role is not clear.[22] However, the use of *p*-toluenesulphonic acid instead of methanesulphonic acid and also the use of other bases like K_2CO_3 or $CsCO_3$ in place of $BaCO_3$ for the preparation of some representative compounds has not shown any noticeable effect on the yields as well as on the rate of the reaction process.

In conclusion, we have developed a highly practical one-pot procedure for the preparation of 4β-arylaminopodophyllotoxin congeners. This methodology also demonstrates the selectivity in 4′-*O*-demethylation by



Scheme 1. Reagents and conditions: (i) $MeSO_3H/NaI$, CH_2Cl_2, rt, 5 h; (ii) H_2O/Me_2CO, $BaCO_3$, rt, 30 min; (iii) $MeSO_3H/NaI$, MeCN, rt, 15 min.



a-f, R=C_6H_5, *p*-FC_6H_4, *p*-ClC_6H_4, *p*-$NO_2C_6H_4$, *o*-OHC_6H_4, *m*-$MeCOC_6H_4$; g-i, R=*P*-FC_6H_4, *o*-OHC_6H_4, *p*-$NO_2C_6H_4$

Scheme 2. Reagents and conditions: (i) $MeSO_3H$/NaI, CH_2Cl_2, rt, 5 h; (ii) THF, RNH_2, $BaCO_3$, rt, 8 h; (iii) $MeSO_3H$/NaI, MeCN, rt, 15 min.

employing the desired type of solvents. This manipulation is useful to prepare selectively 4′-*O*-demethyl or epipodophyllotoxin analogues in good yields. Furthermore, this new methodology has been demonstrated for the facile synthesis of DNA topoisomerase II inhibitors NPF and W-68, and it is extremely cost effective, and this one-pot process could assist other researchers in the preparation of important analogues of this class of podophyllotoxin congeners.

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20. **General procedure for the synthesis of compounds (4a–f)**. To a solution of podophyllotoxin **1** (414 mg, 1 mmol) in dry CH_2Cl_2 (10 mL), NaI (447 mg, 3 mmol) was added and stirred for 5 min. To this stirred suspension, $MeSO_3H$ (288 mg, 3 mmol) was added dropwise with syringe at 0 °C and the stirring was continued for another 5 h at room temperature. Nitrogen was bubbled through the solution to drive off the excess hydrogen iodide. This solution was then evaporated in vacuo and used for the next reaction without further purification. To the above crude product, anhydrous $BaCO_3$ (395 mg, 2 mmol) and the appropriate arylamine (1.2 mmol) in 10 mL of dry THF under nitrogen were added and stirred for 8 h at room temperature. The reaction mixture was filtered, diluted with ethylacetate and washed with water, 10% $Na_2S_2O_4$ solution, dried, and purified via column chromatography (40 g of silica gel with ethyl acetate/hexane as eluent).

4′ - Demethyl - 4β - (4′ - fluoroanilino) - 4 - desoxypodophyllotoxin (NPF) (4a). Yield 55%; mp 175–177 °C; $[\alpha]_D^{30} = -102°$ ($c = 1$, $CHCl_3$); IR (KBr) 3504 (OH), 3388 (NH), 1772 (lactone), 1619, 1513 and 1473 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (t, $J = 6.90$ Hz, 2H, 3″, 5″-H), 6.75 (s, 1H, 5-H), 6.53 (s, 1 H, 8-H), 6.49 (q, $J = 2.5$, 6.7 Hz, 2H, 2″, 6″-H), 6.29 (s, 2H, 2′, 6′-H), 5.95 (d, $J = 4.6$ Hz, 2H, OCH_2O), 5.36 (s, 1H, exchangeable, 4′-OH), 4.60 (d, 2H, 4-H and 1-H), 4.36 (t, $J = 8.0$ Hz, 1H, 11-H), 3.99 (t, $J = 8.0$ Hz, 1H, 11-H), 3.88 (br, 1H, exchangeable NH), 3.80 (s, 6H, 3′, 5′-OCH_3), 3.15 (dd, $J = 4.8$, 13.7 Hz, 1H, 2-H), 3.05 (m, 1H, 3-H); FAB MS: 493 $(M+H)^+$; FABHRMS calcd for $[C_{27}H_{24}NO_7F + H]^+$ 493.4846, found 493.1536.
21. **General procedure for the synthesis of compounds (4g–i)**. To a solution of podophyllotoxin **1** (414 mg, 1 mmol) in dry MeCN (10 mL), NaI (298 mg, 2 mmol) was added and stirred for 5 min. To this stirred suspension, $MeSO_3H$ (192 mg, 2 mmol) was added dropwise with a syringe at 0 °C and the stirring was continued for another 15 min at room temperature. Nitrogen was bubbled through the solution to drive off the excess hydrogen iodide. This solution was then evaporated in vacuo and used for the next reaction without further purification. To the above crude product, anhydrous $BaCO_3$ (395 mg, 2 mmol), and the appropriate arylamine (1.2 mmol) in 10 mL of dry THF under nitrogen were added and stirred for 8 h at room temperature. The reaction mixture was filtered, diluted with

ethyl acetate and washed with water, 10% $Na_2S_2O_4$ solution, dried, and purified via column chromatography (40 g of silica gel with ethyl acetate/hexane as eluent).

4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (4g). Yield 85%; mp 128–130 °C; $[\alpha]_D^{30} = -78°$ ($c = 1$, $CHCl_3$); IR (KBr) 3388 (NH), 1772 (lactone), 1619, 1513 and 1473 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 (t, $J = 6.90$ Hz, 2H, 3″, 5″-H), 6.75 (s, 1H, 5-H), 6.53 (s, 1H, 8-H), 6.49 (q, $J = 2.5$, 6.7 Hz, 2H, 2″, 6″-H), 6.29 (s, 2H, 2′, 6′-H), 5.95 (d, $J = 4.7$ Hz, 2H, OCH_2O), 4.60 (d, 2H, 4-H and 1-H), 4.36 (t, $J = 8.0$ Hz, 1H, 11-H), 3.99 (t, $J = 8.0$ Hz, 1H, 11-H), 3.88 (br, 1H, exchangeable NH), 3.80 (s, 9H, 3′, 4′, 5′-OCH_3), 3.15 (dd, $J = 4.8$, 13.7 > Hz, 1H, 2-H), 3.05 (m, 1H, 3-H); FAB MS: 507 $(M+H)^+$; FABHRMS calcd for $[C_{28}H_{26}NO_7F + H]^+$ 507.4814, found 507.1693.

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