Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 21, pp. 2567-2572, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00382-3

SYNTHESIS OF ETOPOSIDE PHOSPHATE, BMY-40481: A WATER-SOLUBLE CLINICALLY ACTIVE PRODRUG OF ETOPOSIDE

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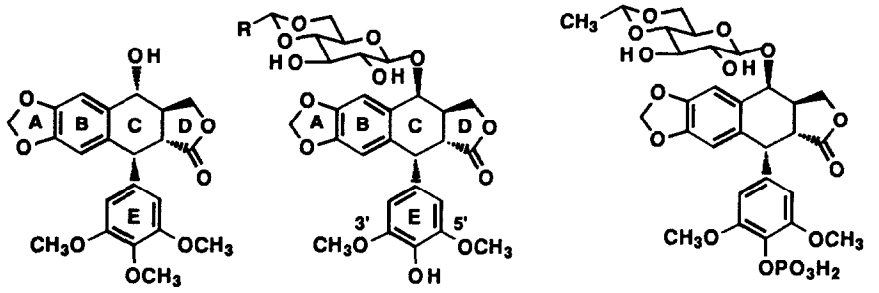
ABSTRACT: *Etoposide Phosphate (BMY-40481) is synthesized semisynthetically from the clinically approved anticancer parent drug, etoposide (VP-16-213), and also from natural (-)-epipodophyllotoxin. Etoposide phosphate functions as a clinically active, water soluble prodrug of etoposide. The NDA for etoposide phosphate was filed in June, 1994.*

(-)-Podophyllotoxin (**1**), a mitotic spindle poison, is isolated from the roots and rhizomes of the May Apple (*Podophyllum peltatum*) and was used as a medicinal agent hundreds of years ago by the American Indians. Human clinical trials were abandoned due to the toxicity of **1**, but a rather extensive semi-synthetic program at Sandoz, led by H. Stahelin, resulted in the development of etoposide (VP-16-213, **2**, Vepesid®) and teniposide (VM-26, **3**) by 1971.[1] Since its entry into clinical trials, etoposide has been found to be active in the treatment of a wide range of malignancies.[2] Response rates of 20% or more are shown with etoposide as a single agent in a variety of tumor types. Moreover, when used in combination chemotherapy, etoposide can contribute to the prolongation of survival, and, in small cell lung cancer and testicular cancer, cures sometimes result (greater than 5 year disease free survival)[3,4].

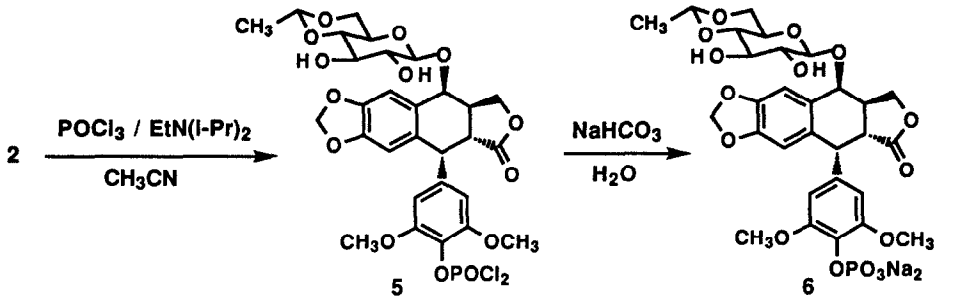
The clinical use of etoposide is adversely affected by its very poor water solubility. It is formulated with Tween® 80, polyethylene glycol, and ethanol, which themselves are considered toxic.[5,6]. In mice, vehicle alone, at levels equivalent to that given with doses of etoposide of 100 mg / kg or 75 mg / kg, results in acute mortality. Converting mg / kg in mice to mg / m^2 in man, the dose at which vehicle alone is toxic in mice is equivalent to a dose of 150 mg / m^2. Because of its poor solubility, etoposide should be diluted to a concentration not exceeding 0.4 mg / mL to avoid precipitation. A dose of 100 mg / m^2 given to a person of 2 m^2 would have to be diluted in 500 mL. At a rapid flow rate of 250 mL / h, treatment would take 2 hours. This type of administration requires prolonged nursing supervision, higher expenses, and some patient inconvenience and discomfort.

Thus, the ability to deliver a prodrug[7] of etoposide in a more concentrated form, over a much shorter period of time using a safe vehicle, is of clear benefit to the patient. In 1986, our antitumor group began a program to discover such an appropriate prodrug of etoposide. From the results of these extensive studies, we now describe the synthesis of etoposide phosphate (**4**, "etopofos"), the water soluble 4'-phosphate ester of etoposide.

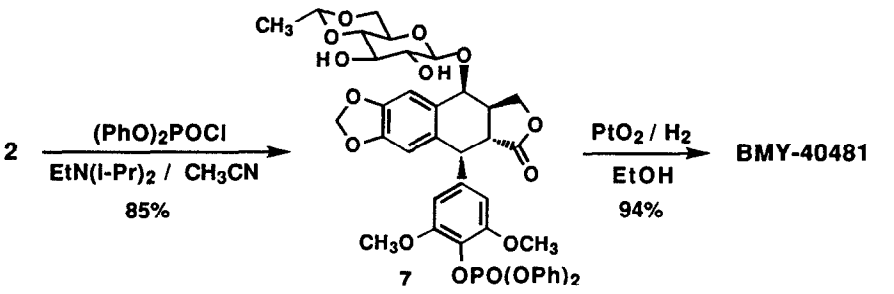


1 2; R = CH_3 3; R = 2-thienyl 4; BMY-40481

Using N,N-diisopropylethylamine (4 equiv) and neat $POCl_3$ (1.1 equiv) in acetonitrile at 0 °C, etoposide **2** is regioselectively converted to its 4'-dichlorophosphate intermediate **5**.[8] Following hydrolysis using aqueous sodium bicarbonate, and reverse phase chromatography over octadecylsilane bonded to silica gel, H_2O : CH_3OH (4:1) elutes the disodium salt of etopofos (**BMY-40481-30**; **6**) in 36% yield following lyophilization.[9] This salt form was used for our initial preclinical biology studies which have been previously reported.[10]

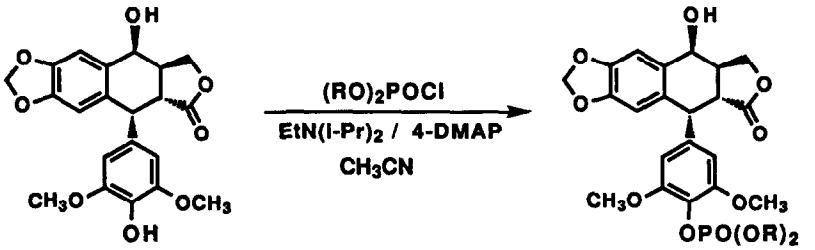


A more efficient and direct method to synthesize etopofos **4**, is via direct phosphorylation of etoposide **2** using diphenyl chlorophosphate ($EtN(i\text{-}Pr)_2$, CH_3CN), to yield the 4'-diphenyl phosphate **7** in 85% yield following flash chromatography[11] on silica gel. Platinum oxide catalyzed hydrogenation of **7** (EtOH, 45-50 psi H_2) gives **BMY-40481** in 94% yield.[8]



We previously reported the synthesis of a 3',5'-didesmethoxyetoposide analog[12] by glycosylation of a 4'-phenol-protected epipodophyllotoxin intermediate. In fact, several patented syntheses of etoposide proceed from natural (-)-podophyllotoxin **1**. Initially, **1** is demethylated[13] and then converted to a 4'-protected epipodophyllotoxin intermediate. This intermediate is condensed with a protected ß-d-glucopyranose, to give etoposide after eventual deprotection of all hydroxyl groups. We reasoned that a shorter synthesis of etopofos would obtain via a 4'-phosphate-protected epipodophyllotoxin, wherein a diphenyl or dibenzyl phosphate moiety would serve as a protecting group, and also, as the water-solubilizing phosphate itself, after eventual unmasking.

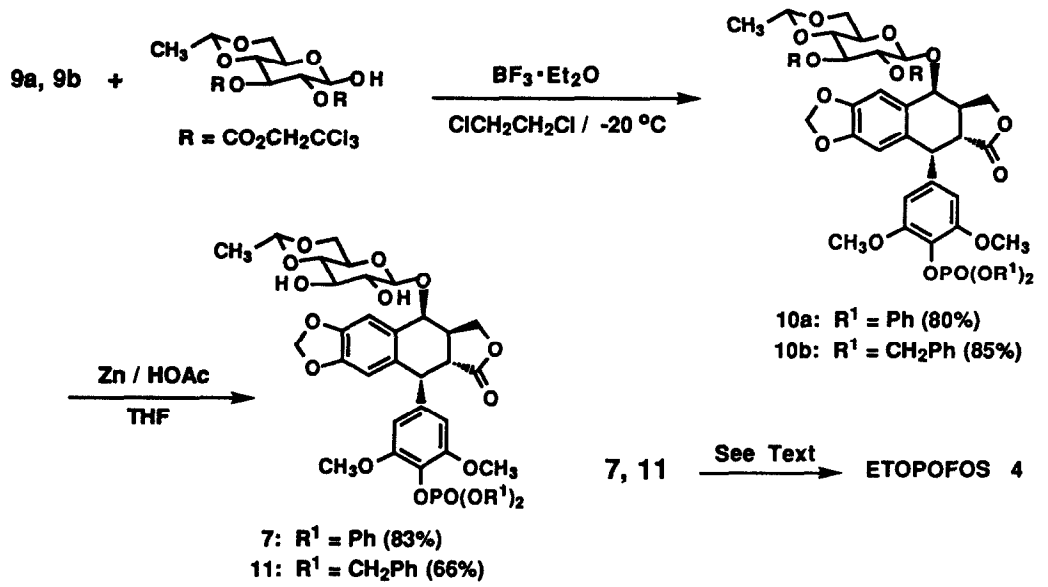
Thus, 4'-demethylepipodophyllotoxin **8**, prepared from (-)-podophyllotoxin[13], is phosphorylated using diphenyl chlorophosphate and dibenzyl chlorophosphate ($EtN(i\text{-}Pr)_2$, 4-DMAP, CH_3CN, room temperature, 16 h) to give the corresponding 4'-demethylepipodophyllotoxin-4'-phosphates **9a** (30%) and **9b** (38%).[14]



8

9a: R = Ph
9b: R = CH_2Ph

Glycosylation of **9a** and **9b** with 4,6-O-ethylidene-2,3-O-[(ß, ß, ß-trichloroethoxy)-carbonyl]-ß-d-glucopyranose ($BF_3{\cdot}Et_2O$ / $ClCH_2CH_2Cl$ / -20 °C)[12,14] gives **10a** (80%) and **10b** (85%). Deprotection of the 2" and 3" sugar hydroxyl groups of **10a** and **10b** using zinc dust in 2:1 THF:HOAc, provides **7** (83%) and **11** (66%), respectively. Etoposide 4'-diphenyl phosphate **7** is readily converted to etopofos **4** (*vide supra*). Etoposide 4'-dibenzyl phosphate **11** succumbs to transfer hydrogenolysis (10% Pd/C, 1-methyl-1,4-cyclohexadiene, CH_3OH, 40-45 °C) to provide etopofos **4** (80-90%) following crystallization from absolute ethanol.[14]



We have previously described the preclinical antitumor activity of etoposide phosphate disodium salt (**BMY-40481-30**; **6**).[10] This highly water-soluble prodrug (*vide infra*) of etoposide was administered ip, po, or iv and etoposide **2** was administered ip or po. A comparison of the antitumor activities of drug **2** and prodrug **6** was conducted in several murine and human tumor (xenograft) models.[10] **BMY-40481-30** given iv is more active than etoposide given ip against iv implanted P388 leukemia. Both etoposide and **BMY-40481-30** yield comparable maximum effects against ic P388 leukemia, ic L1210 leukemia, and sc B16 melanoma. In addition, **BMY-40481-30** is more active than the parent drug when both prodrug and drug were administered po versus iv implanted P388 leukemia.

Half-lives at 37 °C for the conversion of **BMY-40481-30** into etoposide were determined in plasma from various sources: (mouse, 39 minutes; monkey, 39 minutes; human, 45 minutes; dog, 124 minutes). The plasma etoposide levels after po administration of **BMY-40481-30** to rats average 2X higher (0-24 h AUC values) than those after an equivalent po dose of etoposide. No **BMY-40481-30** is found in the plasma after its po administration. Rapid and complete *in vivo* conversion of etoposide phosphate disodium salt into etoposide shows similar plasma pharmacokinetic parameters for etoposide after iv administration of either **BMY-40481-30** or etoposide. The $t_{1/2}$ for the conversion of **BMY-40481-30** to etoposide using the enzyme, alkaline phosphatase, (25 °C, pH = 7.2) is less than 2 minutes.

Due to its favorable preclinical antitumor activity and high water solubility, **BMY-40481** (etopofos, **4**) was evaluated in human clinical trials using both iv and po dosing protocols.[15,16] The free phosphate **4** is found to have high water solubility and, due to its somewhat greater chemical stability, was chosen over the disodium salt, **BMY-40481-30**. In humans, etopofos demonstrates high but variable oral absorption and is rapidly converted to etoposide. The

vehicle used for etopofos given iv shows no toxic effects in mice.[17] Etopofos given to patients by iv administration is rapidly and completely converted to etoposide with virtually the same pharmacokinetic profile as the parent drug, etoposide. Therefore, the patient is essentially being given etoposide.

Etopofos provides an advantage over etoposide, in that it can be given to patients in smaller volumes and more rapidly, thus making treatment at standard doses significantly more convenient. Thus, in essence, etopofos can be considered to be an improved formulation of etoposide. Since etopofos can be given at higher doses and more rapidly than etoposide by continuous infusion, it is also possible that it could demonstrate activity in diseases where etoposide is not considered to be effective.[18]

In June 1994, Bristol Myers Squibb filed an NDA for etoposide phosphate.

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18. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their assigned structures. Yields are reported following purification by flash chromatography[11] over silica gel.

(*Received in USA* 11 *July* 1994; *accepted* 22 *September* 1994)