Chemoenzymatic and Ring E-Modular Approach to the (−)-Podophyllotoxin Skeleton. Synthesis of 3′,4′,5′-Tridemethoxy-(−)-podophyllotoxin

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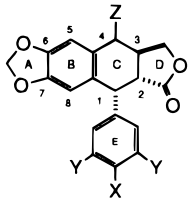
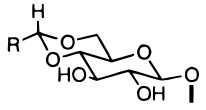
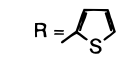
(−)-Podophyllotoxin (**1**) acts as an antimitotic, inhibiting tubulin assembly. Its semisynthetic derivatives, etoposide (**3**) and teniposide (**4**), though not antimitotics, are important clinical chemotherapeutic agents.[1] Several *in vitro* studies assign functional roles to ring E in etoposide. For example, etoposide promotes topoisomerase II-mediated DNA scission,[2] and ring E oxygenation may be required for this activity.[3] On the other hand, etoposide can be "activated" *in vitro* by dealkylative oxidation of ring E to produce derivatives (e.g. the semiquinone or the *o*-quinone) capable of cleaving DNA[4a] or of covalently binding to proteins[4b,c] and DNA.[4d]

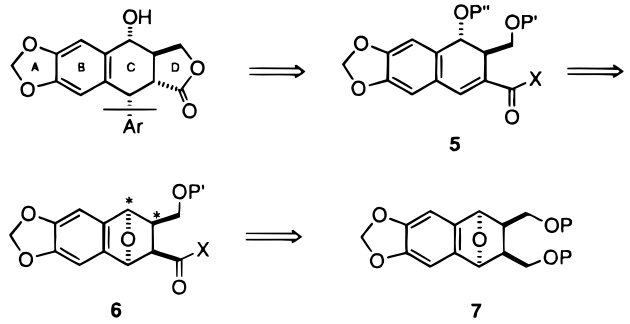


1 (-)-Podophyllotoxin
2 3',4',5'-Demethoxy-(-)-podophyllotoxin
3 Etoposide
4 Teniposide

1 X, Y = OMe Z = 
2 X, Y = H Z = 
3 X = OH; Y = OMe; Z =  R = Me
4 X = OH; Y = OMe; R = 

However, it remains uncertain whether the degree of oxygenation or the oxidation state of ring E is related to the oncolytic properties of podophyllotoxin or etoposide, *in vivo*. Herein we describe the a synthetic approach to the (−)-podophyllotoxin skeleton that is modular in ring E, as a tool for the study of its functional role. As proof of principle, we report

Scheme 1



the first synthesis and biological characterization of 3′,4′,5′-tridemethoxy-(−)-podophyllotoxin (**2**), the ring E deoxygenated analogue of (−)-podophyllotoxin.

Podophyllotoxin has captured the attention of organic chemists for some time.[5] Yet only recently have enantioselective approaches to the natural product appeared.[6,7] Philosophically, our approach differs from these syntheses in two fundamental ways: (1) absolute stereochemistry is introduced catalytically, by means of an enzyme-catalyzed transformation upon an unnatural substrate,[8,9] and (2) ring E is introduced as late as possible in the synthesis (Scheme 1).

Among several meso intermediates of the general structure **7**, **10** proved to be the most useful as an enzyme substrate. Diacetate **10** is readily constructed in seven steps (45% yield; Scheme 2).[10] The key step is an isobenzofuran Diels−Alder reaction in which DMAD serves as both solvent and dienophile.[11] PPL selectively deacetylates the (*R*)-acetoxymethyl arm of **10** to furnish (+)-**11** (95% ee) in 83% yield.[12]

Aldehyde **12** is available from **11** via silylation, deacetylation, and Swern oxidation (Scheme 3). Acetyl migration is not

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‡ Eli Lilly and Company; inquiries specifically regarding the cytotoxicity assays should be directed to these authors.

(1) Doyle, T. W. In *Etoposide (VP-16). Current Status and New Developments*; Academic Press: New York, 1984.

(2) (a) Robinson, M. J.; Osherhoff, N. *Biochemistry* **1991**, *30*, 1807−1813. (b) Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *259*, 13560−13566 and references therein.

(3) It has been suggested that a free 4′-OH is essential for DNA breakage activity: (a) Long, B. H.; Musial, S. T.; Brattain, M. G. *Biochemistry* **1984**, *23*, 1183−1188. (b) Loike, J. D.; Horwitz, S. B. *Biochemistry* **1976**, *15*, 5443−5448. However, a related, "ring E"-deoxygenated lignan displays potent topoisomerase II inhibition activity: (c) Kamal, A.; Atchinson, K.; Daneshtalab, M.; Micetich, R. G. *Anti-Cancer Drug Des.* **1995**, *10*, 545−554.

(4) (a) Sinha, B. K.; Eliot, H. M.; Kalayanaraman, B. *FEBS Lett.* **1988**, *227*, 240−244. (b) Haim, N.; Nemec, J.; Roman, J.; Sinha, B. K. *Biochem. Pharm.* **1987**, *36*, 527−536. (c) Van Maanen, J. M. S.; de Ruiter, C.; de Vries, J.; Kootstra, P. R.; Gobas, F.; Pinedo, H. M. *Eur. J. Cancer Clin. Oncol.* **1985**, *21*, 1099−1106. (d) Van Maanen, J. M. S.; de Vries, J.; Pappie, D.; van der Akker, E.; Vincent, M.; Lafleur, M.; Retel, J.; van der Greef, J.; Pinedo, H. M. *Cancer Res.* **1987**, *47*, 4658−4662.

(5) For a review of synthetic approaches to the *Podophyllum* lignans, see: (a) Ward, R. S. *Synthesis* **1992**, 719−730. For syntheses of (±)-podophyllotoxin, see: (b) Gensler, W. J.; Gastonis, C. G. *J. Org. Chem.* **1966**, *31*, 4004−4008. (c) Kende, A. S.; Liebeskind, L. S.; Mills, J. E.; Rutledge, P. S.; Curran, D. P. *J. Am. Chem. Soc.* **1977**, *99*, 7082−7083. (d) Murphy, W. S.; Wattanasin, S. *J. Chem. Soc., Chem. Commun.* **1980**, 262−263. (e) Kende, A. S.; King, M. L.; Curran, D. P. *J. Org. Chem.* **1981**, *46*, 2826−2828. (f) Rajapaksa, D.; Rodrigo, R. *J. Am. Chem. Soc.* **1981**, *103*, 6208−6209. (g) Jung, M. E.; Lam, P. Y.; Mansuri, M. M.; Speltz, L. M. *J. Org. Chem.* **1985**, *50*, 1087−1105. (h) Jung, M. E.; Lowen, G. T. *Tetrahedron Lett.* **1986**, *27*, 5319−5322. (i) Van der Eycken, J.; De Clerq, P.; Vandewalle, M. *Tetrahedron Lett.* **1985**, *26*, 3871−3874. (j) Macdonald, D. I.; Durst, T. *J. Org. Chem.* **1986**, *51*, 4749−4750. (k) Vyas, D. M.; Skonezny, P. M.; Jenks, T. A.; Doyle, T. W. *Tetrahedron Lett.* **1986**, *27*, 3099−3102. (l) Kaneko, T.; Wong, H. *Tetrahedron Lett.* **1987**, *28*, 517−520. (m) Peterson, J. R.; Hoang, D. D.; Rogers, R. D. *Synthesis* **1991**, 275−277. (n) Jones, D. W.; Thompson, A. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1797−1798. (o) Kraus, G. A.; Wu, Y. *J. Org. Chem.* **1992**, *57*, 2922−2925.

(6) (a) Andrews, R. C.; Teague, S. J.; Meyers, A. I. *J. Am. Chem. Soc.* **1988**, *110*, 7854−7858. (b) Bush, E. J.; Jones, D. W. *J. Chem. Soc., Perkin Trans. 1* **1996**, 151−155.

(7) Two formal syntheses of **1** have also been reported. (a) (−)-Epipodophyllotoxin: Van Speybroeck, R.; Guo, H.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron* **1991**, *47*, 4675−4682. (b) (−)-Neopodophyllotoxin: Charlton, J. L.; Koh, K. *J. Org. Chem.* **1992**, *57*, 1514−1516.

(8) For recent reviews of chemoenzymatic natural product synthesis, see: (a) Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769−3826. (b) Mori, K. *Synlett* **1995**, 1097−1109.

(9) Kutney has described an ambitious biotechnological approach to these lignans. Thus, the combination of H_2O_2 and crude plant cell extracts (e.g., from *P. peltatum* or *N. sylvestris*) has been reported to effect a ring C-forming cyclization reaction on appropriately substituted dibenzylbutanolides. However, in these cyclizations the unnatural stereochemistry at C_1 (*S*) apparently predominates (i.e., for C_2-H: dd, $J = 11$, 14 Hz) providing epiisopodophyllotoxins: (a) Kutney, J. P.; Du, X.; Naidu, R.; Stoynov, N. M.; Takemoto, M. *Heterocycles* **1996**, *42*, 479−484. (b) Kutney, J. P.; Chen, Y. P.; Gao, S.; Hewitt, G. M.; Kuri-Brena, F.; Milanova, R. K.; Stoynov, N. M. *Ibid.* **1993**, *36*, 13−20.

(10) Bromination of piperonal proceeds in 84% yield: (a) Conrad, P. C.; Kwiatkowski, P. L.; Fuchs, P. L. *J. Org. Chem.* **1987**, *52*, 586−591. Acetalization provides **8** in 96% yield: (b) Keay, B. A.; Plaumann, H. P.; Rajapaksa, D., Rodrigo, R. *Can. J. Chem.* **1983**, *61*, 1987−1995.

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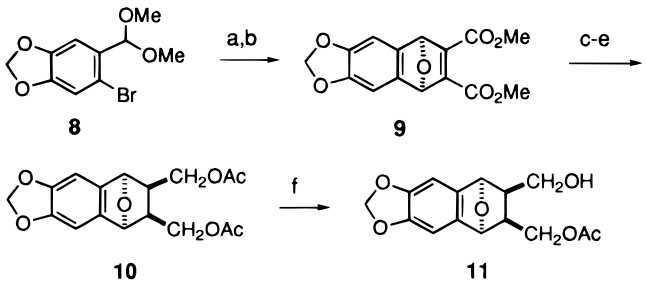
Scheme 2[a]



[a] (a) *n*-BuLi, THF, $(CH_2O)_n$, 82%; (b) HOAc, DMAD, 80 °C, 92%; (c) H_2, Pd/C, 99%; (d) $LiAlH_4$, Et_2O, reflux, 88%; (e) Ac_2O, Pyr, DMAP, −5 °C, 100%; (f) PPL, 10% DMSO 50 mM KPO_4 buffer, pH 8, 66% (83% corr.), 95% ee.

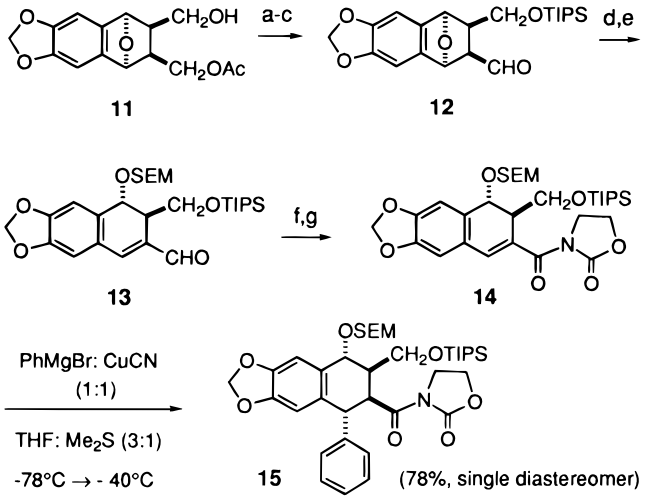
observed under the silylation conditions nor is silyl migration observed under the deacetylation conditions as established by Mosher esterification.[13] Efficient retro-Michael ring opening[14] of **12** unveils the (methylenedioxy)cinnamyl system **5** envisioned as a vehicle for the introduction of ring E. Following protection of the C_4−OH and aldehyde oxidation, the acyl oxazolidinone functionality is installed.

Michael acceptor **14** was designed to bias the system toward conjugate addition from the *re* face with the following considerations: (a) the TIPS ether might sterically block approach from the *si* face, (b) the SEM ether could conceivably coordinate to copper to direct *re* face attack,[15] and (c) the relatively sterically demanding substituents at C_3 and C_4 might be disposed pseudoequatorially in the transition state, thereby enforcing *re* face addition of the cuprate (pseudoaxial trajectory of approach). Pleasingly, Cu^I-mediated conjugate addition of PhMgBr to **14** occurs exclusively from the desired *re* face and is quite efficient (78%, Scheme 3).

Only three steps separate conjugate addition product **15** from the targeted analogue of (−)-podophyllotoxin **2** (Scheme 4). Chemoselective TIPS deprotection is effected by careful heating of **15** with TBAF. Following lactonization, epimerization at C_2 using the conditions of Kende et al.[5e,16] proceeds smoothly to provide largely the *trans*-lactone. Modified Kim conditions (EtSH, $MgBr_2$)[17] convert the previously inseparable *cis*-lactone **16** into the readily separable thioether **17** and effect SEM deprotection of the *trans*-lactone to provide the title compound **2**. The facile and stereocontrolled conversion of **14** to **2** in just four steps attests to the potential of this synthetic route for the generation of other ring E-modified analogues of (−)-podophyllotoxin.

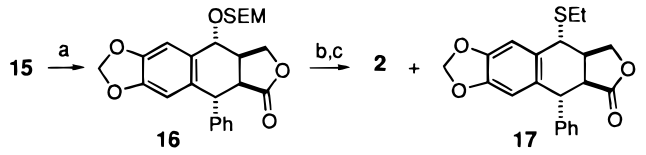
Cytotoxicity Data. **1** and **2** both display potent cytotoxicity against a drug-sensitive human leukemia CCRF-CEM cell line (IC_{50}s of 8 and 34 nM, respectively), in contrast to the less

Scheme 3[a]



[a] (a) TIPSCI, imidazole, DMF, rt, 100%; (b) K_2CO_3, MeOH, rt, 97%; (c) $(COCl)_2$, DMSO, CH_2Cl_2, NEt_3, −78 °C, 100%; (d) NaOMe, MeOH, rt, 90%; (e) SEMCl, *i*-Pr_2NEt, CH_2Cl_2, rt 93%; (f) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl-2-butene, 100%; (g) CDI, THF, 100%; then *n*-BuLi, oxazolidinone, −78 °C, 60%.

Scheme 4[a]



[a] (a) TBAF, THF, 50 °C, 80%; (b) LDA, −78 °C; then pyr-HCl quench, 94%; 2:1 ratio (*trans*-*cis*-lactone); (c) $MgBr_2$, EtSH, Et_2O−PhH (4:1) 0 °C → rt, **17** (32%) and **2** (47%).

cytotoxic oncolytic etoposide (IC_{50} of 1.1 μM). An MDR[18] cell line CEM/VLB100, selected with vinblastine, is 12-fold resistant to etoposide (IC_{50} of 12.9 μM). This resistant cell line is, however, nearly as sensitive to **1** and **2** (IC_{50}s of 11 and 57 nM, respectively) as is the parent cell line.[19] Thus **2** maintains the favorable MDR profile of the natural product. Therefore, the degree of oxygenation in ring E is apparently not a strong determinant of either cytotoxicity or MDR profile in the (−)-podophyllotoxin series. Its effects in the etoposide series remain to be investigated.

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Supporting Information Available: Complete characterization data and copies of the ^1H NMR spectra for all synthetic intermediates; comparison ^1H NMR spectra of **2** and (−)-podophyllotoxin; ^1H NMR spectra of the Mosher esters used to determine the enantiomeric purity of **11**, and a photograph of an SDS-PAGE gel of PPL (29 pages). See any current masthead page for ordering and Internet access instructions.

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(11) For a review of the use of isobenzofurans in natural product synthesis, see: Rodrigo, R. *Tetrahedron* **1988**, *44*, 2093−2135.

(12) Porcine pancreatic lipase (PPL) was purchased from Sigma (10¢/g) and displays largely one band at MW ≈ 50−52 kDa on SDS-PAGE (see Supporting Information). The absolute stereochemistry of **11** was established from the X-ray structure of its Mosher ester derived from (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[13] For details, see: Berkowitz, D. B.; Maeng, J.-H. *Tetrahedron: Asymmetry* **1996**, *7*, 1577−1580.

(13) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549.

(14) Keay, B. A.; Rajapaksa, D.; Rodrigo, R. *Can. J. Chem.* **1984**, *62*, 1093−1098.

(15) (a) Hanessian, S.; Sumi, K. *Synthesis* **1991**, 1083−1089. (b) Hanessian, S.; Thavonekham, B.; DeHoff, B. *J. Org. Chem.* **1989**, *54*, 5831−5833. (c) Dorigo, A. E.; Morokuma, K. *J. Am. Chem. Soc.* **1989**, *111*, 6524--6536.

(16) For an early articulation of this concept, see: Zimmerman, H. E. *J. Org. Chem.* **1955**, *20*, 549−557.

(17) Kim, S.; Kee, I. S.; Park, Y. H.; Park, J. H. *Synlett* **1991**, 183−184.

(18) For a recent review, see: Bellamy, W. T. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 161−183.

(19) (a) Beck, W. T.; Mueller, M. J.; Tanzer, L. R. *Cancer Res.* **1979**, *39*, 2070−2076. (b) Denizot, F.; Lang, R. J. *Immunol. Methods* **1989**, *89*, 271−277.