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Enantioselective Entry into Benzoxabicyclo[2.2.1]heptyl Systems via Enzymatic Desymmetrization: Toward Chiral Building Blocks for Lignan Synthesis

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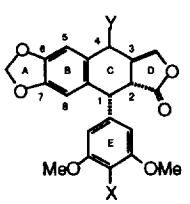
Abstract: The *meso* diacetate, **9**, available in seven steps from piperonal, is efficiently desymmetrized with preferential cleavage of the *R*-arm-acetate under catalysis by porcine pancreatic lipase in 10% DMSO-phosphate buffer, pH 8. The resulting monoacetate **12**, a potentially useful chiral building block for the synthesis of derivatives of the *Podophyllum* lignans, is obtained in good chemical yield (66-83%) and in high optical yield (95% ee) on a multigram scale.

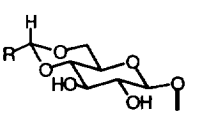
Semisynthetic derivatives of the aryl tetralin lignan (-)-podophyllotoxin (**1**), such as etoposide (**2**)[1] and teniposide (**3**), are important chemotherapeutic agents. Etoposide has displayed remarkable efficacy as a single agent in the treatment of small cell lung cancer, testicular cancer, Hodgkin's and non-Hodgkin's lymphoma, several leukemias and Kaposi's sarcoma (the tumor most closely associated with AIDS).[2-4] More recently, etoposide has been identified as a very good candidate for the treatment of life-threatening cytomegalovirus (CMV) infections.[5]



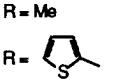
1 (-)-Podophyllotoxin X = OMe Y = 

2 Etoposide X = OH

3 Teniposide X = OH

Y = 

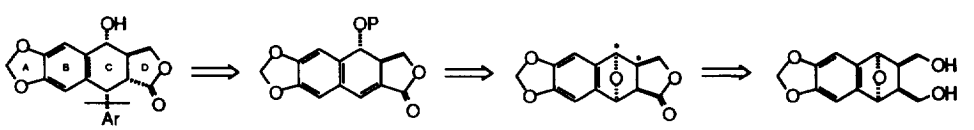
R = Me

R = 

Against the backdrop of etoposide's superlative clinical performance, podophyllotoxin has become an important synthetic target.[6] In recent years, the synthetic focus has been upon asymmetric approaches to the podophyllotoxin skeleton. Meyers and coworkers recorded the first enantioselective synthesis of (-)-podophyllotoxin wherein all stereochemical information was elegantly derived from a chiral oxazoline.[7a] Bush and Jones recently described a second auxiliary-mediated approach to (-)-**1**.[7b,c] Vandewalle's group has reported an asymmetric approach to (-)-epipodophyllotoxin, the C_4-epimer of **1**.[7d]

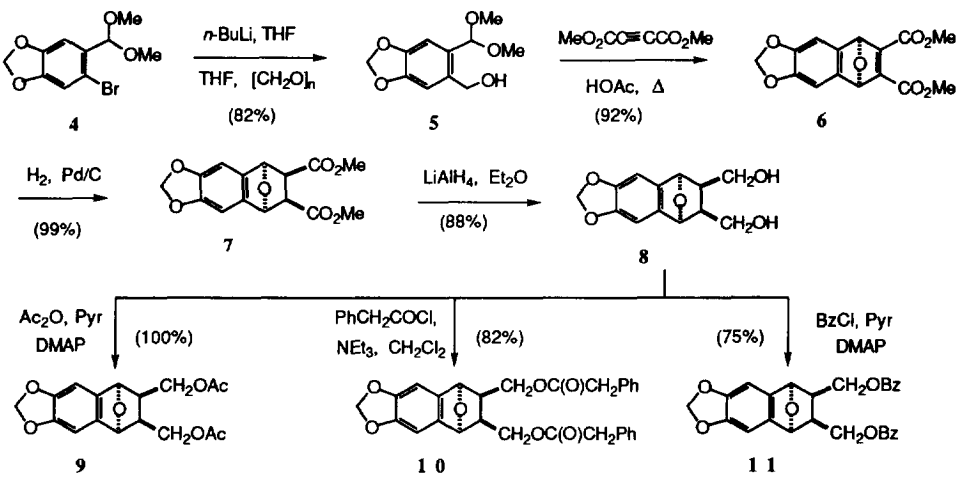
We have been particularly interested in developing an enantioselective approach to the podophyllotoxin skeleton which is modular in ring E, so as to provide access to heretofore unavailable ring E-modified analogs of the natural product. Hence, we envisioned the introduction of ring E late in the synthesis (Scheme 1). We also wished to introduce asymmetry catalytically, via an enzyme-mediated desymmetrization of an advanced *meso* synthetic intermediate.[8] We report herein our progress toward achieving this latter goal.

Scheme 1 *Retrosynthetic Analysis: Exploiting Cσ Symmetry*



An efficient route to appropriate *meso* benzoxobicyclo[2.2.1]heptyl derivatives beginning from piperonal has been developed (Scheme 2). The first two steps, the regiospecific bromination and the acetalization of piperonal to give **4** are known transformations, and proceed in very good yield, as reported (Scheme 1).[9] The hydroxymethylation of **4** was examined under a variety of conditions. Halogen-metal exchange (*n*-butyllithium) was followed by addition of the electrophile at low temperature. Although **5** could be obtained with DMF (followed by $NaBH_4$) and formaldehyde gas as electrophiles, solid paraformaldehyde proved to be the electophile of choice, in terms of both yield and reproducibility.

Scheme 2



The key step involves the in situ generation of 5,6-(methylenedioxy)isobenzofuran[10-12] from **5** and its trapping with a suitable Diels-Alder dienophile. By employing dimethyl acetylenedicarboxylate (DMAD) as both dienophile and solvent, excellent yields of Diels-Alder product **6** are reproducibly obtained, even on a 20 g scale (90%). Excess DMAD is reclaimed by distillation and reused. Catalytic hydrogenation gives the desired *cis*-vicinal diester **7** quantitatively. The dimethyl ester **7** may be transformed into several related *meso* compounds, **8-11**, chosen as potential substrates for enzymatic desymmetrizations.

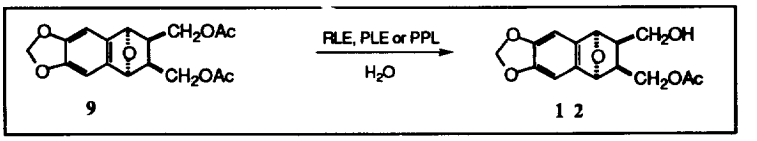
Our choice of enzymes/*meso* substrates was guided by available information in the literature. Several enzymes, including horse liver alcohol dehydrogenase (HLADH),[13] lipase GC (*Geotrichum candidum*)[14] and pig liver esterase (PLE)[15] are known to accept simpler *meso* compounds of the bicyclo[2.2.1]heptyl variety as substrates. *Bis*-(2-phenyl)acetate **10** was chosen with penicillin acylase (PA) in mind, as this hydrolase cleaves phenacyl esters or amides of great structural variety.[16] Dibenzoate **11** was regarded as a potential chymotrypsin A (CA) substrate.[17] Diol **8** and diesters **7**, **9-11** were all candidates for lipase-mediated desymmetrization.

We have now screened the five substrate candidates **7-11**, along the lines suggested above. Several variables have been examined including: buffer, pH, temperature, wgt. equivalents of enzyme, enzyme stabilizers, percent and nature of organic cosolvents (see Table for several examples). Unfortunately, HLADH fails to accept diol **8** as substrate under a large variety of conditions, including those of Jones[13a] and of Klibanov.[13b] Indeed, in several cases, the enzymes examined (e.g. HLADH, PA, CA) did not accept these relatively large, bridged, tetracyclic compounds as substrates at all. On the other hand, matches were found for both diol **8** and diacetate **9**. Both lipase P and lipase GC slowly acetylate diol **8** in vinyl acetate. However, the enantioselectivity [29% ee favoring the (R)-arm for both enzymes] is not synthetically useful.

Diacetate **9** proved to be the best unnatural substrate (see Table). Three enzymes [PLE, rabbit liver esterase (RLE), and porcine pancreatic lipase (PPL)] catalyze its hydrolysis. Enantiomeric excesses range from unacceptable (18%, RLE), to modest (33%, PLE), to outstanding (95%, PPL). In the latter case, a 66% yield

(95% ee) of enzymatically desymmetrized monoacetate can be obtained. This corresponds to an 83% yield based on recovered diacetate. The reaction occurs reproducibly in a matter of 1-3 h in phosphate buffer, pH 8 with 10% DMSO as cosolvent. The reaction is easily performed on a multigram scale (entry 8). Approximately 13 weight equivalents of enzyme are employed, but PPL currently sells for 10¢/gram from Sigma.

Table *Enzymatic Desymmetrizations of Meso Diacetate 9*



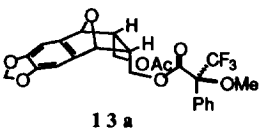
9 12

	Enzyme	Medium	Time	Wgt. Eq. Enz.	Yield (12)	%ee	Recovered Diacetate (9)	Diol (8)
(1)	PLE	10% DMSO, 50 mM KPO_4, pH 8	2 days	2				All diol
(2)	PLE	25% DMSO, 50 mM KPO_4, pH 7	2 days	1	52%	33%	47%	
(3)	PLE	10% acetone, 50 mM KPO_4, pH 8	2 days	2	14%	5%	20%	25%
(4)	RLE	10% DMSO, 50 mM KPO_4, pH 8 (BSA added)	3 days	0.05	23%	18%	20%	
(5)	RLE	10% DMSO, 50 mM KPO_4, pH 8 (Triton X-100 added)	3 days	0.05	11%	10%	15%	
(6)	PPL	10% DMSO, 50 mM KPO_4, (T = 35°C) pH 8	2 days	22	46%	n.d.	0%	47%
(7)	PPL	10% DMSO, 50 mM pyrophosphate, pH 8.5	2 days	22	63%	89%	5%	27%
(8)[A,B]	PPL (20 g scale)	10% DMSO, 50 mM KPO_4, pH 8	2.5 h	13	66%	95%	21%	5%

(A) Other buffers (pH's) giving lower yields (10% DMSO as cosolvent): phosphate, pH 7; borate, pH's 8.5 & 9; pyrophosphate, pH 9; imidazole, pH 7.

(B) Increasing the temperature to 35-50°C also gave lower yields of **12**

For all entries in the Table, enantiomeric excesses were determined by 500 MHz ^{1}H-NMR analysis of the diastereomeric esters derived from *(R)*-Mosher chloride.[18] To determine the absolute stereochemistry of PPL-derived **12**, its Mosher ester **13a**, was recrystallized and its X-ray crystal structure determined:



13a =

The crystal structure reveals that all five enzymes (PPL, PLE, RLE, lipase P and lipase GC) act preferentially on the *R*-arm of the *meso* diacetate **9**. Interestingly, this stereoselectivity is opposite to that

predicted by the most general model for PPL hydrolyses yet developed.[19] However, that model is based upon simple acyclic or monocyclic substrates that had been described by others.[20] Indeed, to the best of our knowledge, no enzymatic transformations of a tetracyclic benzoxabicyclo[2.2.1]heptyl derivatives such as **9** have been heretofore reported. Studies directed at the synthesis of unnatural lignans from chiral building block **12** are in progress and will be reported in due course.

Acknowledgment.

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REFERENCES AND NOTES

1. For a review of the chemical, biological and clinical aspects of etoposide see: *Etoposide (VP-16). Current Status and New Developments*; Issell, B. F.; Muggia, F. M.; Carter, S. K. Eds.; Academic Press: New York, 1984.
2. O'Dwyer, P. J.; Leyland-Jones, B.; Alonso, M. T.; Marsoni, S.; Wittes, R. E. *New England J. Med.* **1985**, *312*, 692-700.
3. Issell, B. F.; Crooke, S. T. *Cancer Treatment Reviews* **1979**, *6*, 107-124.
4. Radice, P. A.; Bunn, P. A. Jr.; Ihde, D. C. *Cancer Treatment Reports*, **1979**, *63*, 1231-1239.
5. Huang, E.-S.; Benson, J. D.; Huong, S.; Wilson, B.; van der Horst, C. *Antiviral Research* **1992**, 17, 17-32.
6. For a review of synthetic approaches to the *Podophyllum* lignans, see: Ward, R. S. *Synthesis*, **1992**, 719-730.
7. (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 I* **1996**, 151; (c) Bush, E. J.; Jones, D. W. *J. Chem. Soc. Perkin Chem. Commun.* **1993**, 1200-1201; (d) Van Speybroeck, R.; Guo, H.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron* **1991**, *47*, 4675-4682.
8. For notable recent examples of chemoenzymatic natural product syntheses, see: (a) Tian, X.; Hudlicky, T.; Königsberger, K. *J. Am. Chem. Soc.* **1995**, *117*, 3643-3644; (b) Daieli, B.; Lesma, G.; Mauro, M.; Palmisano, G.; Passarella, D. *J. Org. Chem.* **1995**, *60*, 2506-2513.(c) Johnson, C. R.; Harikrishnan, L. S.; Golebiowski, A. *Tetrahedron Lett.* **1994**, *35*, 7735-7738; (d) For a review, see: Mori, K. *Synlett* **1995**, 1097-1109.
9. (a) Conrad, P. C.; Kwiatkowski, P. L.; Fuchs, P. L. *J. Org. Chem.* **1987**, *52*, 586-591; (b) Keay, B. A.; Plaumann, H. P.; Rajapaksa, D., Rodrigo, R. *Can J. Chem.* **1983**, *61*, 1987-1995.
10. Both acid- and base-catalyzed 1,4-elimination of alkoxydihydroisobenzofurans to the corresponding isobenzofurans are well-known: Tobia, D.; Rickborn, B. *J. Org. Chem.* **1987**, *52*, 2611-2615.
11. Only few isobenzofurans (bearing substitution α to the oxygen) are stable enough to be isolated: Rodrigo, R.; Knabe, S. M. *J. Org. Chem.* **1986**, *51*, 3973-3978.
12. For a review of the use of isobenzofurans in natural product synthesis, see: Rodrigo, R. *Tetrahedron* **1988**, *44*, 2093-2135.
13. (a) Jones, B. J.; Francis, C. J. *Can. J. Chem.* **1984**, *62*, 2578-2582; (b) Grunwald, J.; Wirz, B.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 6732-6734.
14. Murata, M.; Ikoma, S.; Achiwa, K. *Chem. Pharm. Bull.* **1990**, *38*, 2329-2331.
15. Bloch, R.; Guibe-Jampel, E.; Girard, C. *Tetrahedron Lett.* **1985**, *26*, 4087-4090.
16. Holla, E. W. *J. Carbohydrate Chem.* **1990**, *9*, 113-119, and references cited therein.
17. Jones, B. J. *Methods Enzymol.* **1976**, *44*, 831-856.
18. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543-2549.
19. Wimmer, Z. *Tetrahedron* **1992**, *48*, 8431-8436.
20. Hemmerle, H.; Gais, H.-J. *Tetrahedron Lett.* **1987**, *28*, 3471-3474.

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