A Crystallization-Induced Stereoselective Glycosidation Reaction in the Synthesis of the Anticancer Drug Etoposide[†]

Lee J. Silverberg,*[‡] Sean Kelly,[§] Purushotham Vemishetti,[⊥] David H. Vipond,[§] Frank S. Gibson,[⊥] Brian Harrison,[§] Richard Spector,[⊥] and John L. Dillon*[⊥, ‖]

Bristol-Myers Squibb Co., Technical Operations Development, Chemical Development Laboratories, P.O. Box 4755, Syracuse, New York 13221-4755, and Watery Lane, County Dublin, Ireland

john.dillon7@honeywell.com

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ABSTRACT
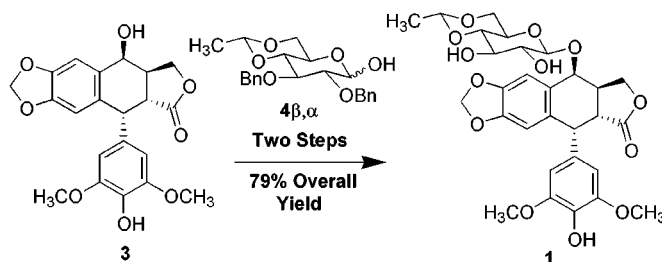
The anticancer drug etoposide, 1, is prepared in 79% overall yield from readily available 4′-demethyl-4-epipodophyllotoxin, 3, and 4,6-*O*-ethylidene-2,3-*O*-dibenzyl-D-glucose, 4, via a crystallization-induced stereoselective glycosidation reaction followed by catalytic hydrogenation.

Podophyllotoxin is a lignan derivative of the May apple, the most notable analogue of which is etoposide (Vepesid), **1**, which continues to serve as an important drug for the treatment of leukemia, testicular cancer, and small-cell lung cancer.[1] The key step in the synthesis of etoposide involves the glycosidation of podophyllotoxin derivative **3**,[2] protected at the phenolic postion, with a suitably protected 4,6-*O*-ethylidene glucose. During the course of our studies directed at developing a low cost, commercial synthesis of this anticancer agent, we discovered a stereoselective glycosidation reaction of sugar **4** with 4′-demethyl-4-epipodophyllotoxin, **3**, which is reported herein.

Of particular importance in the design of a synthetic route to etoposide is the proper selection of protecting groups for the sugar. Conditions for protecting group removal must be mild in order to proceed in high yield and avoid base-catalyzed cleavage of the *cis*-fused lactone or acid-catalyzed cleavage of the ethylidene linkage. We were interested in examining use of the benzyl group for protection of the 2- and 3-positions of 4,6-*O*-ethylidene-glucose since it is removed by catalytic hydrogenation, a process that generally proceeds in high yield under neutral conditions and is readily scaled commercially. We were concerned, however, that stereoselectivity in the glycosidation reaction would be

(1) Reviews: (a) Damayanthi, Yalamati; Lown, W. J. *Curr. Med. Chem.* **1998**, 5(3). (b) von Wartburg, A.; Stahelin, H. *Chronicles of Drug Discovery*; Lednicer, D., Ed.; American Chemical Society: Washington, DC, 1993; Vol. 3, p 349. (c) *Etoposide (VP-16) Current Status and Developments*; Issel, B. F., Carter, S. K., Eds. Academic Press: New York, 1987. (d) Stehelin, H. F.; von Wartburg, A. *Cancer Res.* **1991**, *51*, 5. (e) Stehelin, H. F.; von Wartburg, A. *Prog. Drug Res.* **1989**, *33*, 169. (f) Arnold, A. M.; Whitehouse, J. M. A. *The Lancet* **1981**, 912. (g) Stadtmauser, E. A.; Cassileth, P. A.; Gale, R. P. *Leuk. Res.* **1989**, *13*, 639. (h) Bostrom, B.; Weisdorf, D. J.; Kim, T.; Kersey, J. H.; Ramsay, N. K. C. *Bone Marrow Transplant* **1990**, *5*, 83. (i) Price, B. A.; Peters, N. H. *Eur. J. Cancer* **1992**, 28, 615. (j) Aisner, J.; Whitacre, M. Y.; Budman, D. R.; Propert, K.; Staruss, G.; Vanecho, D. A.; Perry, M. *Cancer Chem. Pharmacol.* **1992**, *29*, 435.

[†] Dedicated to Professor Alfred Hassner on the occasion of his 70th birthday.
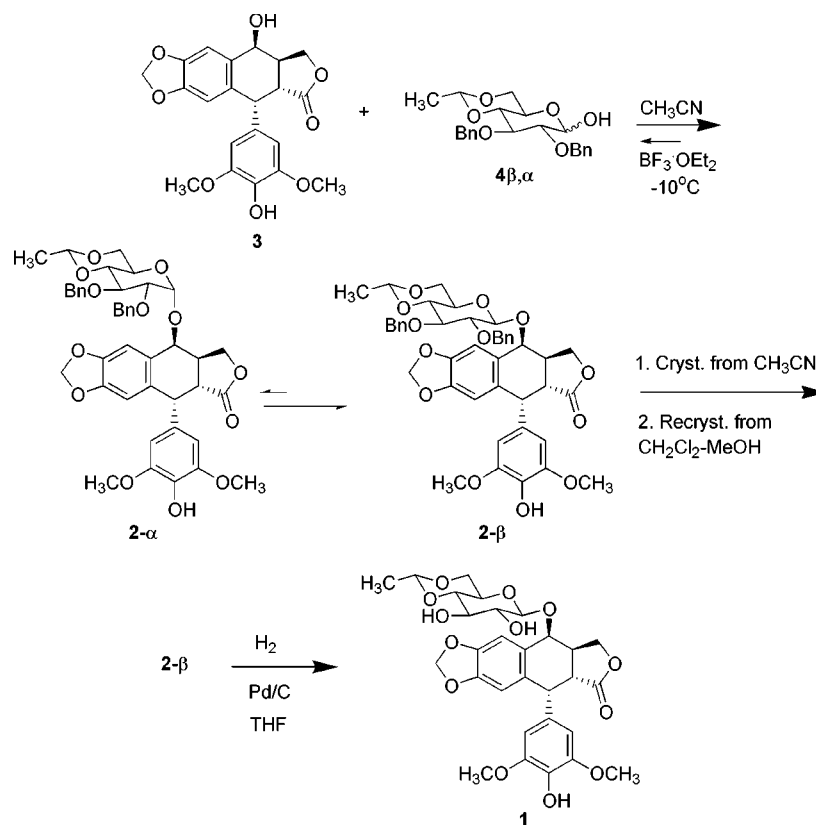[‡] Present address: Johnson Matthey Co., 2003 Nolte Drive, West Deptford, NJ 08066.
[§] Watery Lane, County Dublin, Ireland.
[⊥] P.O. Box 4755, Syracuse, New York.
[‖] Present address: Honeywell Co., Pharmaceutical Fine Chemicals, 20 Peabody St., Buffalo, NY 14210.

Scheme 1

compromised due to use of an ether-type protecting group. In particular, examination of literature precedent and our earlier studies on the synthesis of etoposide phosphate established that the hydrolytic conditions, required for removal of the C-1 oxygen protecting group of **4**, led to the formation of an anomeric mixture. Altough **4** could be converted primarily to the β-isomer by heating below its melting point, we found that **4β** underwent partial anomerization under the reaction conditions (Lewis acid) required for coupling of the sugar to the aglycone.

Treatment of **3**[3] with boron trifluoride etherate (1.5 equiv) in the presence of dibenzyl-sugar **4**[4] ($>$90% β) in acetonitrile at -10 °C led to the formation of a crystalline slurry within 10 min (Scheme 1). HPLC analysis of the mixture after 20 min revealed that the reaction was essentially complete ($<$1.5% of **3**) and showed the presence of the desired, coupled product **2β** along with **2α** in a ratio of 2.4:1. Surprisingly, analysis of the solid revealed the presence of **2β**:**2α** in a ratio of 95:5. After the crystallization was complete (5 h), pyridine was added to quench the Lewis acid. Extractive workup afforded the coupled product after crystallization from acetonitrile. The isolated solid **2β** contained only a trace of **2α** as determined by HPLC analysis.[5,6] Apparently, once the crystallization commences, the equilibrium is driven toward **2β**, thus leading to a *crystallization-*

(2) (a) von Kuhn, M.; von Wartburg, A. *Helv. Chim. Acta* **1968**, *51*, 1631. (b) Kuhn, M.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 948. (c) Wang, Z.; Ma, W.; Zhang, C. U.S. Pat. 5,206,350, 1993. (d) Kurabayash, K.; Kinoshita, H.; Saito, H.; Takahashi, T. Eur. Pat. 111058-A1, 1984. (e) Saito, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. *Chem. Lett.* **1987**, 799. (f) Fujii, T.; Chikui, Y. U.S. Pat. 4,757,138, 1988. (g) Kolar, C.; Moldenhauer, H.; Kneissl, G. *J. Carbohydr. Chem.* **1990**, *9*, 571. (h) Sterling, J.; Nudelman, A.; Herzig, J.; Keinan, E.; Weiner, B. Z. U.S. Pat 4,900,-814, 1990. (i) Ohnuma, T.; Hoshi, C. U.S. 4,997,931, 1991. (allopyranose, not etoposide) (j) Kolar, C. Eur. Pat. 394907-A1, 1990. (k) Robin, J.-P.; Houlbert, N.; Lenain, V. Eur. Pat. 435709-A1, 1991. (l) Robin, J.-P.; Lenain, V. Eur. Pat. 445021-A2 1991. (m) Hashimoto, S.; Honda, T.; Ikegami, S. *Tetrahedron Lett.* **1991**, *32*, 1653. (n) Nudelman, A.; Herzig, J.; Keinan, E.; Weiner, B. Z.; Sterling, J. Eur. Pat. 226,202, 1987. (o) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Sanvito, A. M.; Macdonald, P. *Tetrahedron Lett.* **1992**, *33*, 4831. (p) Miyazawa, Y.; Sato, H.; Yoshikawa, H.; Kouichi, O.; Noriko, T. Eur. Pat. 0567089-A1. (q) Vogel, C. U.S. Pat. 5,463,040. (r) Allevi, P.; Anastasia, M.; Bigatti, E.; Macdonald, P. WO 9302094 1993. (s) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Bigatti, E.; Macdonald, P. *J. Org. Chem.* **1993**, *58*, 4175. (t) Vogel, K.; Sterling, J.; Herzig, Y.; Nudelman, A. *Terahedron* **1996**, *52*, 3049. (u) Fusauchi, Y. M.; Yoshikawa, H. F. Eur. Pat. Appl. 0778282-A1. (v) Zhao, Y.-H.; Yang, F.-Y.; Gu, K.; Li, R.-Y.; Wang, J. K. *Stud. Plant Sci.* **1999**, 6 (Advances in Plant Glycosides, Chemistry and Biology), 330.

(3) Stehelin, H. F.; Hansen, R. B.; Willumsen, A. M.; Jorslov-Lauritsen, N.; Ebbesen, P.; Nielsen, P. E.; Buchardt, O. *Acta Chem. Scand.* **1993**, *47*, 1190.

(4) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P.; Sleezer, P. D.; Discordia, R. P.; Hartung, K.; Gao, Q. *Org. Process Res. Dev.* **2000**, *4*, 34.

(5) Formation of the α-isomer with respect to the O$-$C bond of the sugar and lignan nucleus in coupled product **2** is not observed presumably due to steric hindrance of the α-face by the pendant aryl ring system.

(6) Prior to addition of pyridine, the crystalline slurry could be filtered cold to afford **2β**:**2α** in a ratio of approximately 95:5 in a yield of 78%. In practice it was more suitable to quench the reaction with pyridine and perform an extractive workup to afford a more stable crystalline form of **2β**. One batch which was directly isolated was shown to be unstable, presumably due to the presence of trace amounts of BF$_3$ that were occluded in the crystals. Also, by quenching and workup, **2β** can be isolated from acetonitrile at higher temperature (0 °C), allowing for a nearly complete purge of the **2α** isomer.
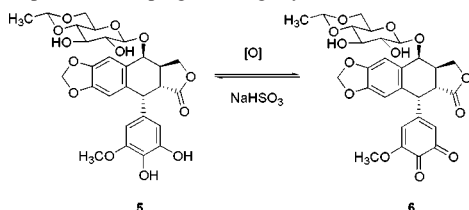
induced stereoselective glycosidation reaction.[7] Recrystallization from methylene chloride−methanol then led to **2β** in 81.8% overall yield from **3** with exceptionally high HPLC purity of 99.7% and no detectable levels of **2α**.

Catalytic hydrogenation of **2β** using Pd on carbon in THF at 50 psi for 4 h afforded, after workup and crystallization from THF−water, etoposide **1** in 96.8% yield with a purity of 100%.[8] The overall yield based on **3** is 79.2%.[9] The sequence has also been accomplished on multikilogram scale.

To support the proposal that this process is a crystallization-induced phenomenon and that there is a dynamic equilbrium in solution between the starting materials **3** and **4** and the coupled isomers **2β** and **2α**, several experiments were conducted. When the coupled product **2α** was isolated from mother liquor, purified, and subjected to the reaction conditions described above, the solution was found to contain an equilibrium mixture of **2β** and **2α** similar to that obtained by reaction of **3** with **4**, and the resulting precipitate was

identified as **2β**. When crystalline product **2β** was subjected to the reaction conditions, it was found to be stable due to its insolubility in acetonitrile. It was also shown to be configurationally stable after quenching and during workup and recrystallization. Finally, when a 60:40 mixture of **4β**:**4α** was used in the process, an isolated yield (80%) similar to that of a reaction utilizing >90% **4β** was obtained. In the latter experiment, significant levels of starting materials **3** and **4** (9.0% and 21.6%, respectively) were detected by HPLC analysis of the solution after 1.5 h of reaction, even though they were not evident after 15 min of reaction.

There are several practical advantages to this protocol. Preparation of anomerically pure sugar **4β** is not necessary.[10] Prior activation of the lignan nucleus with a leaving group at the 4-position is not needed. The addition of molecular sieves normally required in these glycosidation reactions is also obviated. The coupling reaction can be conducted at a higher temperature (−10 °C) than is typically employed for these types of reactions. Finally, protection of the phenol in **3** with the CBz group prior to the coupling reaction, as was done in most prior syntheses of etoposide, is not necessary in this case.

Application of this methodology to the preparation of other lignan derivatives and a more detailed study of the mechanism of this reaction are under investigation in our laboratories.

Supporting Information Available: Experimental procedures for the preparation of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

(7) For an example of a *crystallization-induced asymmetric transformation* in the resolution of two enantiomers of a peripheral CCK antagonist, see: Reider, P. J.; Davis, P.; Hughes, D. L.; Grabowski, E. J. J. *J. Org. Chem.* **1987**, 52, 957.

(8) Initially in this study, formation of a bright orange color was observed upon filtration of the catalyst from the hydrogenation mixture which carried into the isolated product. We traced this color to the formation of the quinone **6** which was derived from oxidation of the catechol **5** from exposure of the hydrogenation mixture to air. The catechol itself was contained as a trace impurity precursor in **3** and coupled with **4** to afford **5** after hydrogenation. This problem was solved by adding a small amount of $NaHSO_3$ as a reducing agent to the mixture following hydrogenation to maintain the reduced compound **5** that purges during crystallization.

(9) For a synthesis of etoposide using 1-β-OTMS-4,6-*O*-ethylidene-glucose with an overall yield of 70%, see ref 2s.

(10) Compound **4** can be prepared in greater than 90% of the β-isomer by heating the anomeric mixture below its melting point, see ref 4. Compound **4β** will undergo anomerization under the reaction conditions, but at a slower rate than the coupling itself.