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Efficient Synthesis of the Anticancer Drug Etoposide 4′-Phosphate: Use of Benzylic Ether-Protecting Groups on the Carbohydrate Segment[1]

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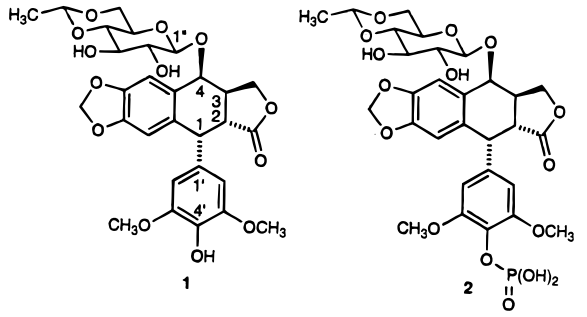
Abstract:

The prodrug etoposide phosphate 2 is synthesized efficiently in three steps in 54.6% overall yield from 4′-demethylepipodophyllotoxin 3. The strategy pursued in the synthesis of 2 places the phosphate on 3 prior to coupling with the sugar and employs benzyl ether-protecting groups on both the phosphate and the sugar, allowing easy removal in one step. The importance of solvent, steric effects, and electronic effects in the coupling reaction is demonstrated. Two features of the synthesis are an unusual thermal anomerization of the carbohydrate component 5a and completely diastereoselective, one-pot crystallization of the coupled product 6a-*β*. The process has been demonstrated on multi-kilogram scale.

Introduction

Etoposide **1**, a synthetic podophyllotoxin derivative, is an important drug in the treatment of leukemia, testicular cancer, and small cell lung cancer.[2] However, formulation of this drug is problematic because of poor water solubility.[2a] It was reasoned that attaching a phosphate group to the phenolic hydroxyl would significantly increase the water solubility. The resulting prodrug, etoposide 4′-phosphate **2**,[3] has excellent water solubility and activity comparable to **1** in vivo.[3a]



Scientists at Sandoz were the first to synthesize **1**,[4] and most subsequent efforts[5] have basically followed the same sequence: (1) protection of the phenolic hydroxyl group of 4′-demethylepipodophyllotoxin **3**, (2) $BF_3{\cdot}OEt_2$ promoted coupling with a 2,3,4,6-protected glucose derivative,[5c] (3) deprotection of the sugar, and in some cases ethylidination at the 4,6 position, and (4) deprotection of the phenol to give **1**. The key step in the sequence is the glycosylation reaction, which occurs by stereoselective attack of the anomeric hydroxyl on the carbocation formed at C-4 of the lignan.

Recently, it has been shown by several groups that protection of the phenol is not necessary.[5c,m,n] Other groups have made the phenol- and hydroxyl-protecting groups the

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(2) Reviews: (a) *Etoposide (VP-16) Current Status and Developments*; Issell, B. F., Muggia, F. M., Carter, S. K., Eds.; Academic Press: New York, 1987. (b) Stahelin, H. F.; von Wartburg, A. *Cancer Res.* **1991**, *51*, 5. (c) Stahelin, H.; von Wartburg, A. *Prog. Drug Res.* **1989**, *33*, 169. (d) Arnold, A. M.; Whitehouse, J. M. A. *Lancet* **1981**, *2*, 912. (e) Jardine, I. *Podophyllotoxin in anticancer Agents Based on Natural Products Model*; Academic Press: New York, 1980; pp 319−351. Leukemia: (f) Stadtmauser, E. A.; Cassileth, P. A.; Gale, R. P. *Leuk. Res.* **1989**, *13*, 639. (g) Bostrom, B.; Weisdorf, D. J.; Kim, T.; Kersey, J. H.; Ramsay, N. K. C. *Bone Marrow Transplant.* **1990**, *5*, 83−89. Testicular cancer: (h) Price, B. A.; Peters, N. H. *Eur. J. Cancer* **1992**, *28*, 615. Small cell lung cancer: (i) Aisner, J.; Whitacre, M. Y.; Budman, D. R.; Propert, K.; Staruss, G.; Vanecho, D. A.; Perry, M. *Cancer Chemother. Pharmacol.* **1992**, *29*, 435.

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(5) (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) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Bigatti, E.; Macdonald, P. *J. Org. Chem.* **1993**, *58*, 4175. (d) Ohnuma, T.; Hoshi, C. U.S. Patent 4,997,931, 1991. (Allopyranose, not etoposide): (e) Robin, J.-P.; Houlbert, N.; Lenain, V. Eur. Patent 435709-A1, 1991. (f) Robin, J.-P.; Lenain, V. Eur. Patent 445021-A2, 1991. (g) Saito, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. *Chem. Lett.* **1987**, 799. (h) Kolar, C. Eur. Patent 394907-A1, 1990. (i) Kolar, C.; Moldenhauer, H.; Kneissl, G. *J. Carbohydr. Chem.* **1990**, *9*, 571. (j) Allevi, P.; Anastasia, M.; Bigatti, E.; Macdonald, P. WO 9302094, 1993. (k) Sterling, J.; Nudelman, A.; Herzig, J.; Keinan, E.; Weiner, B. Z. U.S. Patent 4,900,814, 1990. (l) Nudelman, A.; Herzig, J.; Keinan, E.; Weiner, B. Z.; Sterling, J. Eur. Patent 226202, 1987. (m) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Sanvito, A. M.; Macdonald, P. *Tetrahedron Lett.* **1992**, *33*, 4831. (n) Wang, Z.; Ma, W.; Zhang, C. U.S. Patent 5,206,350, 1993. (o) Hashimoto, S.; Honda, T.; Ikegami, S. *Tetrahedron Lett.* **1991**, *32*, 1653. (p) Kurabayash, K.; Kinoshita, H.; Saito, H.; Takahashi, T. Eur. Patent 111058-A1, 1984. (q) Fujii, T.; Chikui, Y. *U. S. Pat. 4, 757, 138*, 1988. (r) Japanese Kokai J58-225-096. (s) Miyazawa, Y.; Sato, H.; Yoshikawa, H.; Kouichi, O.; Noriko, T. Eur. Patent 0567089-A1. (t) Vogel, C. U.S. Patent 5463040. (u) Vogel, K.; Sterling, J.; Herzig, Y.; Nudelman, A. *Tetrahedron* **1996**, *52*, 3049. (v) Fusauchi, Y. M.; Yoshikawa, H. F. Eur. Patent Appl. 0778282-A1.

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same so that both could be removed in one step.[5h,i,k,l,t,u] One of the difficulties in the syntheses is removal of the sugar-protecting groups, because the lignan is very sensitive to both acid and base.[6] In all but a few of the efforts, the 2,3-protecting groups have been esters or carbonates, and degradation during their removal is common. Efforts to mediate this problem have been mostly aimed at more labile ester/carbonate groups, such as dichloroacetate.[5q] The reason ester groups have generally been used is because the coupling step requires the use of essentially pure β-sugar. Technology exists for making these sugars with esters in the 2,3-positions, but the literature suggests that it is still not reliable in regard to anomeric purity, except in the case of 2,3,4,6-tetraacetyl-β-D-glucopyranose.[5c] Our own experience with acyl-protected sugars agreed with this observation. Because anomerically pure sugars are difficult to obtain, usually some of the undesired anomer is produced in the coupling reaction. This can be problematic, since chromatography is undesirable on an industrial scale.

The use of ether-protecting groups, on the other hand, has barely been studied. Ohnuma and Hoshi reported the use of 2,3-di-*O*-benzyl-4,6-*O*-ethylidineallopyranose to make etoposide analogues, and although using an anomeric mixture, they reported isolating only the β-anomer of the coupled product.[5d]

Allevi and co-workers recently reported the use of 1-*O*-trimethylsilyl-2,3,4,6-tetra-*O*-benzylglucopyranose in coupling to lignans, and achieved β:α coupled ratios as high as 97:3.[5c]

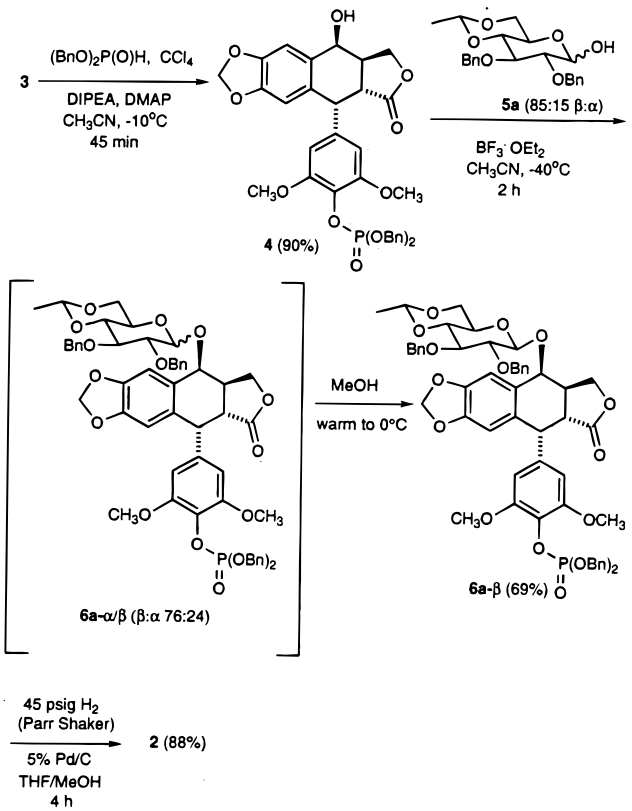
Syntheses of **2** have generally relied on coupling of the sugar and lignan prior to phosphorylation.[3a,5] Saulnier and co-workers conceived of putting the phosphate on first and using it as the phenolic protecting group during the coupling.[8]

Using this strategy, we have developed an efficient and practical synthesis of **2** which was demonstrated on multi-kg scale for commercial manufacture**.** The major feature of the synthesis is the use of benzyl protecting groups on both the phosphate and the sugar, which allows for a single mild, neutral and high yielding deprotection step. The pursuit of this strategy has led to new insight into the chemistry of the coupling reaction, and some interesting discoveries.

Results and Discussion

Phosphorylation. The starting material for the synthesis was 4′-demethylepipodophyllotoxin **3 (**Scheme 1**)**.[9] Saulnier et al. first reported the phosphorylation of **3**, but the yields were low. In the course of this work, we developed a new protocol for phosphorylation of phenols,[10] which works efficiently for this substrate. Steinberg,[11] following Atherton's

Scheme 1. **Synthesis of etoposide phosphate**



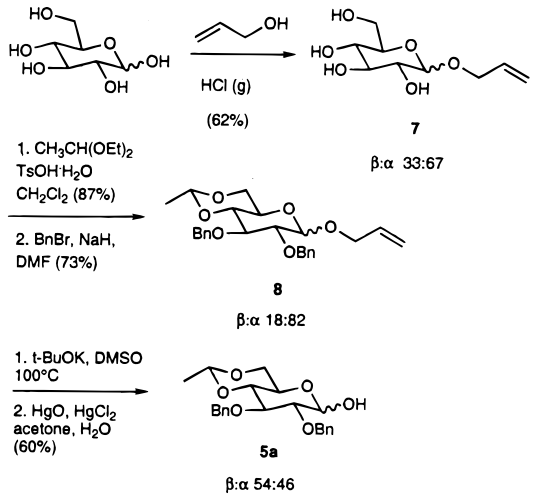
lead,[12] reported that aliphatic alcohols could be phosphorylated by a combination of a dialkyl phosphite, carbon tetrachloride, and a tertiary amine (to make dibenzyl chlorophosphate in situ). Yields were only moderate. However, Kenner and Williams[13] applied this technology to phosphorylation of phenols with diethyl phosphite and achieved excellent yields after stirring at room temperature overnight. We found that **3** can be rapidly phosphorylated in high yield under mild conditions with dibenzyl phosphite, carbon tetrachloride, Hunig's base, and catalytic DMAP in acetonitrile at −10 °C. The reaction is completely selective for the phenol and gives **4** in 90% yield and high purity after recrystallization from isopropyl alcohol.

Coupling Studies. Our synthetic scheme centered around the key step, the coupling reaction. On the basis of the existing literature, we reasoned that *if* we could make 2,3-di-*O*-benzyl-4,6-*O*-ethylidine **5a,** a previously unknown compound, in largely β form, the coupling reaction would proceed with retention of stereochemistry at the anomeric center, giving mostly the desired precursor to **2**.

(A) The Carbohydrate Component. 2,3-Di-*O*-benzyl-4,6-*O*-ethylidene-α,β-D-glucopyranose, **5a**-α/β, was prepared initially in analogy with known procedures (Scheme 2).[5d,14] Fischer glycosylation[15] afforded the allyl glycoside **7** as a 33:67 β:α mixture of anomers after chromatography (Scheme 3). Ethylidenation, benzylation, and deallylation produced

(6) *Int. J. Pharm.* **1988**, *41*, 169.
(7) Japanese Kokai 63/192.793, 1988.
(8) (a) Saulnier, M. G.; Kadow, J. F.; Langley, D. R.; Tun, M. M. Eur. Patent 511563-A1, 1992. (b) Saulnier, M. G.; Langley, D. R.; Kadow, J. F.; Senter, P. D.; Knipe, J. O. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2567.
(9) von Kuhn, M.; Keller-Juslen, C.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 944.
(10) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. *Tetrahedron Lett.* **1996**, *37*, 771.
(11) Steinberg, G. M. *J. Org. Chem.* **1950**, *15*, 637.
(12) (a) Atherton, F. R.; Todd, A. R. *J. Chem. Soc.* **1947**. 674. (b) Atherton, F. R.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 660.
(13) Kenner, G. W.; Williams, N. R. *J. Chem. Soc.* **1955**, 522.
(14) Details of an improved process will be presented in a future paper.
(15) (a) Talley, E. A.; Vale, M. D.; Yanovsky, E. *J. Am. Chem. Soc.* **1945**, *67*, 2037. (b) Fischer, E. *Chem. Ber.* **1893**, *26*, 2400.

Scheme 2. **Synthesis of the sugar component.**



5a, which after chromatography provided a viscous, oily mixture of anomers, in a ratio of 54:46 β:α as determined by ^{1}H NMR.[16] Alternatively, Koenigs−Knorr allylation[17] (69%) of 1-*O*-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose[18] followed by deacetylation (NaOMe/MeOH, quantitative yield) delivered **7** as pure β anomer. However, after further elaboration as above, the anomeric ratio of **5a** was again 54:46 β:α. Fortuitously, it turned out that the sugar **5a** had unusual properties. While in solution, the α-anomer was slightly favored (63:37 α:β in $CDCl_3$ after equilibration over 5 days). Surprisingly, we found that **5a** crystallized preferentially in the β-form. For example, a syrupy 1:1 mixture of anomers, if allowed to stand, neat, for 2−3 months, hardened into a solid and became 85:15 β:α. Alternatively, recrystallization from hexane gave white crystals that were >95% β. Recovery was not high, but the procedure could be repeated as the mother liquor equilibrated. From a practical standpoint, the best method for anomerization has been found to be crystallization from methanol/water, which gives a solid (60% β) that undergoes anomerization upon heating in a drying oven *below the melting point* for several days. This thermal "solid-state" anomerization increases the anomeric content up to 93−100% β. This anomerization has been conducted on multi-kilogram scale repeatably using industrial drying equipment. A possible explanation for this particular phenomenon is that there is some microscopic melting occurring during the heating and that the melted solid anomerizes in the liquid form and then crystallizes as the β anomer. Since the anomeric equilibrium is approximately equal in solution, it appears that **5a** forms a more ordered crystal lattice in the β form than in the α, thus overcoming the anomeric effect. By comparison, 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranose has never been reported.

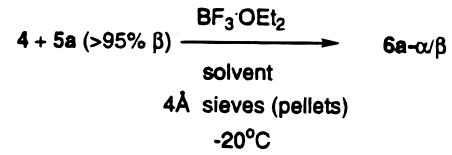
A variety of analogues of **5a** were also prepared, all containing substituted benzyl groups in the 2 and 3 positions (see Table 2).

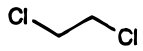
(16) The anomeric ratio was determined by integration of the two AB quartets at δ 4.03 (β) and 3.96 (α) in CD_3CN.

(17) Koenigs, W.; Knorr, E. *Chem Ber.* **1901**, *34*, 957.

(18) (a) Redemann, C. E.; Niemann, C. *Organic Syntheses*; Wiley & Sons: New York, 1955; Collect. Vol. III, p 11. (b) Available commercially from Aldrich Chemical Co.

Table 1. **Solvent effect on coupling: halogenated solvents**



Solvent	6a–β:6a–α[a]
	52:48
CH_2Cl_2	49:51
$CHCl_3$	44:56

a) Determined by HPLC.

[a] Determined by HPLC.

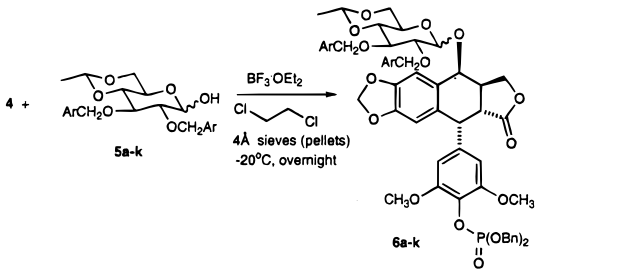
While the synthesis of the sugar component **5a** was satisfactory in affording sufficient quantities for coupling studies (see below), it was clearly not acceptable for industrial-scale application. Accordingly, a significantly more efficient route was developed which requires no chromatography and eliminates environmentally unfriendly reagents such as mercury. This process will be published in due course. Outsourcing of the synthesis of **5a** reduces the in-house synthesis to three steps.

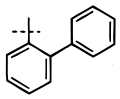
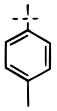
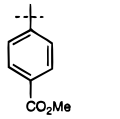
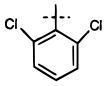
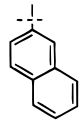
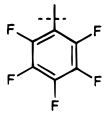
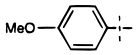
(B) Couplings in Halogenated Solvents. The couplings were first carried out in halogenated solvents based on literature precedent for these reactions. The optimized coupling of **4** with **5a** (1:1 mixture of anomers) in the presence of $BF_3.OEt_2$ in 1,2-dichloroethane (DCE) at −20 °C in the presence of 4 Å molecular sieves gave a 54:46 mixture of **6a**-β**:6a**-α as expected (39.8% yield). Using **5a** that was >95% β gave a similar result. NMR studies revealed that anomerization of **5a** under the reaction conditions (BF_3OEt_2/$ClCD_2CD_2Cl$/−20 °C) was virtually instantaneous. Thus, regardless of the anomeric composition of the input **5a**, the same result was obtained in the coupling reaction. Conducting the reaction in methylene chloride or chloroform gave a similar result, but with the α-product slightly favored (Table 1).

To test the effect of steric and electronic factors on the selectivity of the coupling reaction, a series of analogues of **5a** were prepared (Table 2). We were unable to prepare analogues with substitution at the benzyl methine due to steric hindrance between the adjacent hydroxyls). Sugars with ortho-substituted benzyl groups gave preference to the β-product (examples d and f), and the larger groups gave greater differentiation. Substitution in the meta-position had only a minor effect (examples c and e), while para-substituents made no apparent steric difference (examples b and e).

The methyl series (examples b, c, and d) suggested that electron donation favored the α-product. While the ortho methyl had favored the β-product as expected, the *p*-methyl, which was expected to have no steric effect, favored the

Table 2. **Benzylic substituent effects on coupling**



	Ar	5β:5α[a]	6β:6α[b]		Ar	5β:5α[a]	6β:6α[b]
a)		60:40	54:46	f)		47:53	68:32
b)		40:60	48:52	g)			66:34
c)		45:55	51:49	h)			57:43
d)		58:42	63:37	i)		54:46	62:38
e)		75:25	52:48[b]	j)		55:45	80:20[b]
				k)			no coupled product

(a) Determined by ^{1}H NMR. (b) Determined by HPLC.

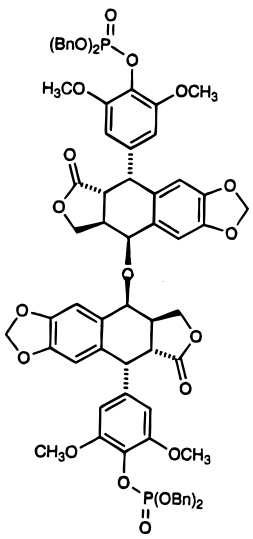
[a] Determined by ^{1}H NMR. [b] Determined by HPLC.

α-product as compared to the unsubstituted case (example a). Further studies supported the notion that electron-withdrawing groups favored the β-product (examples g, h, i, and j), while electron donators favored the α. The most favorable result was with pentafluorobenzyl (example j). The *p*-methoxy compound did not give any coupled product (example k). None of the analogues examined offered a economical alternative to the benzyl group.

Other Lewis acids, including $AlCl_3$, Et_2AlCl, $ZnCl_2$, Zn(OTf)$_2$, TMSOTf, and triflic acid were investigated in the reaction of **4** and **5a**, but all were less satisfactory than BF_3·OEt_2.

Several C-1-*O* derivatives of **5a** were also prepared, including tri-*n*-butyltin,[5k,l,t,u] trichloroacetimidate,[19] and *tert*-butyldimethylsilyl. The tri-*n*-butyltin derivative **9** consisted of 81:19 β:α isomers by NMR, but when coupled to **4**, afforded the same ratio as **5a**. The other two β-derivatives also gave similar ratios, but the reactions did not proceed cleanly. It appears that when the carbocation of **4** is not able to easily couple with the sugars, it dimerized to compound **10**. This also occurred when **4** alone was reacted with BF_3.OEt_2 in DCE at −20 °C. We generally observed only 3% of **10** when **5a** and **4** are reacted under the described conditions.

(19) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212.



10

In addition, Allevi's method[5c] was investigated. Thus, **5a** was silylated with TMSCl and Et_3N in toluene at room temperature and gave a quantitative yield of 1-*O*-TMS derivative of **5a** in a 96:4 β:α ratio. Reaction of this compound with **4** under Allevi's conditions (TMSOTf, CH_2Cl_2, −70 °C) gave an 83.3:16.7 ratio, but with 9.7 area % **10** by HPLC.

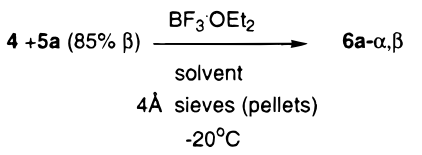
(C) Coupling Reactions in Nonhalogenated Solvents. Along with the halogenated solvents, a number of nonhalogenated, polar solvents were tested (Table 3). Acetonitrile was found to be superior in all respects. When **4** was coupled with **5a** (85:15 β:α) at −20 °C in the presence of BF_3.OEt_2, a ratio of 72:28 **6a**-β**:6a**-α was obtained. This result is in marked contrast to halogenated solvents where the high anomeric ratio of **5a** (85:15 β:α) produced an nearly equal ratio of **6a**-β and **6a**-α in the reaction mixture. In addition, the reaction was much faster than in DCE (2 h vs 18 h). Another advantage was that, while the reaction in DCE was cleaner when 4 Å molecular sieves were added, the reaction in acetonitrile proceeded cleanly in their absence. Although the reaction in acetone gave a high ratio of **6**β**:6**α, the results were poor otherwise. Coupling of **5j** (55:45 β:α) with **4** in CH_3CN gave only a 63:37 **6j**-β:**6j**-α ratio.

We speculate that the higher polarity of acetonitrile relative to dichloroethane overcomes the anomeric effect thus, favoring the β-isomer of **5a** and leading to a higher ratio of **6**β**:6**α in the coupling reaction.

As expected, lowering the temperature of the reaction was seen to increase the β:α ratio. In acetonitrile, lowering the temperature to −40 °C (the solvent freezes at −41 °C) raised the content to 74%. A more dramatic effect was seen in propionitrile, where coupling of 85:15 β:α **5a** at −20 °C gave 57% β, but coupling at −78 °C gave a very slow reaction that was 76:24 β:α.

Under optimized conditions, the reaction was run using **5a** as the sugar substrate at −40 °C in acetonitrile (without sieves) with BF_3.OEt_2 as the promoter. With sugar that is ∼95% β, ratios of **6a**-β**:6a**-α are achieved that are approximately 80:20.

Table 3. **Solvent effect on coupling: non-halogenated solvents**



Solvent	6a–β:6a–α[a]
CH_3CN	72:28
CH_3CH_2CN	57:43
DMF	no reaction
Acetone	76:24

a) Determined by HPLC.

[a] Determined by HPLC.

(D) Selective Crystallization. Early on in the project, an important discovery was made. If the crude product mixture from a coupling reaction was recrystallized from methanol, even from a mixture which was 1:1 **6a**-*β*:**6a**-α (and containing other byproducts as well) ***6a**-β is obtained exclusively, in high recovery*. This diastereoselective crystallization is repeatable, and there is no special technique required. This result allowed for simple purification of the product without the need for chromatography. The solid-state structures of **6a**-*β* and **6a**-α were unequivocally determined by single-crystal X-ray analysis.[20] It is interesting to note that the **6a**-α isomer exists in two conformations, while the **6a**-*β* isomer has one conformation and is more ordered than the two conformations found in crystals of **6a**-α.

None of the other analogues tested (**6b**−**j**) crystallized with the same degree of selectivity, and this crystallization was unique to **6a**. It seems to be very particular to both the groups on the sugar and on the phosphate. Coupling of 4′-CBZ-4′-demethylepipodophyllotoxin **12** with **5a** in DCE at −20 °C gave a 54:46 mixture. Two recrystallizations from methanol gave solid that was 97:3 *β*:α. On the other hand, similar coupling of podophyllotoxin **13** with **5a** and recrystallization gave a 1:1 mixture of anomers, with no selectivity to the crystallization.

(20) A crystal of **6a**-*β* obtained as an unstable, colorless plate from EtOAc measuring 0.09 mm × 0.19 mm × 0.40 mm was used for X-ray diffraction measurements. Crystal data: $C_{57}H_{57}PO_{16}{\cdot}C_4H_8O_2$; monoclinic, space group $P2_1$, a = 15.780(1) Å, b = 8.5674(7) Å, c = 22.106(1) Å, α = 90°, β = 107.95(1)°, γ = 90°, V = 2843.1(4) Å^3, Z = 2, d_x = 1.305 g cm^{-3}. A total of 4538 independent reflections were measured of which 3859 were observed with $|I| \geq 2\sigma$. Final agreement factors were $R(F)$ = 0.100 and $\omega R(F)$ = 0.069.

(21) (a) A crystal of **6a**-α obtained as an unstable, colorless plate from MeOH measuring 0.17 mm × 0.40 mm × 0.42 mm was used for X-ray diffraction measurements. Crystal data: $C_{57}H_{57}PO_{16}$; orthorhombic, space group $P2_12_12_1$, a = 14.029(1) Å, b = 17.601(1) Å, c = 43.868(4) Å, α = β = γ = 90°, V = 1.0832(1) Å^3, Z = 8, d_x = 1.262 g cm^{-3}. A total of 8114 independent reflections were measured of which 6602 were observed with $|I| \geq 2\sigma$. Final agreement factors were $R(F)$ = 0.127 and $\omega R(F)$ = 0.089. (b) The authors have deposited atomic coordinates for structures **6a**-*β* and **6a**-α with the Cambridge Crystallographic Data Center. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 IEZ, UK.

Use of acetonitrile in the coupling reaction also allowed for the development of a one-pot process. Thus, after coupling of **4** and **5a** in acetonitrile, simple addition of methanol served to quench the $BF_3{\cdot}OEt_2$ and selectively crystallize the **6a**-*β* in 69% yield with an HPLC purity of 99.9%.

Hydrogenation. Hydrogenation to remove all four benzyl groups from **6a**-*β* was carried out in a Parr Shaker with 5% palladium on carbon catalyst in THF−MeOH at 45 psi. The reaction proceeded smoothly, and recrystallization from ethanol provided the diethanol solvate[2d] of **2**, which matches the compound prepared from **1** in all respects, in 88% yield. Notable observations are that (1) deprotection is under mild, neutral conditions, (2) no soluble metals, which would be difficult to entirely remove from the product, are used, (3) there is no significant degradation of the molecule, and (4) no chromatography is required to obtain **2** of high quality.

6a-α was also hydrogenated to obtain C-1″-α-etoposide phosphate **12**, which was clearly different by HPLC and NMR from genuine **2**.

Conclusions

Etoposide phosphate has been synthesized by a single synthetic sequence in an overall yield of 54.6% from **3**. The synthesis is simple, practical, and involves no chromatography after implementation of the improved synthesis of **5a**. It has been demonstrated in the pilot plant at multi-kilogram scale. This successful strategy and the reports by Allevi et al. and Ohnuma and Hoshi demonstrate clearly the value of benzyl ether-protecting groups in the chemistry of podophyllotoxin derivatives.

Experimental Section

General. All reactions were run under a N_2 atmosphere with oven-dried glassware. Anhydrous solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) TLC plates (silica gel GF, 250 micron, 10 × 20 cm) were purchased from Analtech (Newark, DE). TLC's were visualized under short wave UV with ceric ammonium nitrate/sulfuric acid. Column chromatography was carried out using TLC grade silica gel purchased from Aldrich (cat. no. 28,-850-0). NMR spectra were obtained on a Bruker 360 MHz instrument. HPLC was performed using a Varian Vista 5500. "HI" stands for "homogeneity index" and refers to the uncorrected area percent by HPLC at the stated wavelength.

Allyl α,*β*-D-Glucopyranoside (7α,*β*). An oven-dried three-neck 100 mL round-bottom flask with a stir bar, gas bubbler, two septa, and N_2 inlet was charged with *β*-D-glucose (10.00 g, 55.5 mmol) and allyl alcohol (30.2 mL, 444 mmol). Hydrogen chloride was bubbled in until the mixture became slightly warm and pale brown. The suspension was warmed to reflux. The mixture was refluxed for 2 h, during which time it became a homogeneous red solution. The solution was transferred to a 100 mL round-bottom flask and concentrated in vacuo to a red oil. The oil was chromatographed on TLC mesh silica (100 g). **7**-α,*β* eluted with 20% MeOH/CH_2Cl_2 to yield 7.62 g (62.4%) of a dark yellow oil as an anomeric mixture (3:1 α:*β*). R_f (20% MeOH/

CH_2Cl_2): 0.37. ^{1}H NMR (CD_3OD): δ 5.94−5.83 (m, 1H), 5.25 (d, 1H, J = 17.3 Hz), 5.09 (d, 1H, J = 10.2 Hz), 4.76 (d, 0.75H, buried under MeOH peak), 4.29 (dd, 0.25H, J = 5.2, 12.9 Hz), 4.23 (d, 0.25H, J = 7.7 Hz), 4.14 (dd, 0.75H, J = 5.2, 13.1 Hz), 4.06 (dd, 0.25H, J = 6.0, 12.9 Hz), 3.95 (dd, 0.75H, J = 6.0, 13.0 Hz), 3.80−3.70 (m, 1H), 3.62−3.57 (m, 1.5 H), 3.52−3.47 (m, 0.5H), 3.34 (dd, 1H, J = 3.7, 9.7 Hz), 3.29−3.13 (m, 2H). ^{13}C NMR (CD_3OD): δ 135.57, 135.46, 117.55, 103.14 (b), 98.99 (a), 77.87, 77.71, 74.93, 73.59, 71.58, 71.42, 70.95, 69.16, 62.57, 62.48. Anal. Calcd. for $C_9H_{16}O_6$: C, 49.08; H, 7.32. Found: C, 48.88; H, 7.07.

Allyl 4,6-*O*-Ethylidine-α,*β*-D-glucopyranoside (14α,*β*). An oven-dried 250 mL round-bottom flask with a stir bar, septum, and N_2 inlet was charged with allyl α,*β*-D-glucopyranoside (**2**-α,*β*, 7.6 g, 34.5 mmol), anhydrous CH_2Cl_2 (138 mL), and acetal (11.8 mL, 82.8 mmol). The oily starting material remained undissolved. *p*-Toluenesulfonic acid (0.328 g, 0.05 equiv) was added. The mixture was swirled and allowed to stand. The starting material eventually dissolved and the solution was stirred. After 18 h, CH_2Cl_2 (100 mL) was added and the solution was washed with sat. $NaHCO_3$ (30 mL), water (30 mL), and sat. NaCl (20 mL). The organic phase was dried over Na_2SO_4, and concentrated in vacuo to a yellow oil, which slowly solidified on standing. The product **14**α,*β* did not require further purification. Yield 7.37 g (87%). R_f (20% MeOH/CH_2Cl_2): 0.50. ^{1}H NMR ($CDCl_3$): δ 5.91−5.80 (m, 1H), 5.25 (dd, 1H, J = 1.1, 17.2 Hz), 5.15 (d, 1H, J = 10.3 Hz), 4.82 (d, 0.8H, J = 3.3 Hz), 4.65 (q, 1H, J = 5.0 Hz), 4.32 (d, 0.2H, J = 7.7 Hz), 4.15−3.94 (m, 3H), 3.66−3.58 (m, 1H), 3.51−3.39 (m, 2.5H), 3.26−3.18 (m, 1.5H), 1.30 (d, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 133.59, 133.46, 118.11, 102.16 (*β*), 99.60, 97.95 (α), 80.41, 79.92, 74.38, 73.02, 72.72, 71.22, 70.48, 68.68, 68.32, 68.10, 66.26, 62.55, 58.14, 20.26, 18.22. Anal. Calcd. for $C_{11}H_{18}O_6$: C, 53.65; H, 7.37. Found: C, 53.42; H, 7.12.

Allyl 2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-α,*β*-D-glucopyranoside (8α,*β*). An oven-dried three-neck 100 mL round-bottom flask with an addition funnel, stir bar, stopper, two septa, and N_2 inlet was charged with 80% sodium hydride in oil (1.33 g, 44.4 mmol) and anhydrous *N*,*N*-dimethylformamide (DMF). The suspension was cooled in ice. A solution of allyl 4,6-*O*-ethylidene-α,*β*-D-glucopyranoside (**14**α,*β*, 3.635 g, 14.8 mmol) in DMF (30 mL) was added dropwise over 30 min. The ice bath was removed. After 35 min, benzyl bromide (5.28 mL, 44.4 mmol) was added dropwise over 5 min The mixture slowly became orange. After 80 min, methanol was added until H_2 evolution ceased. The solution was diluted with EtOAc (200 mL) and washed with water (1 × 40 mL, 1 × 30 mL). The combined aqueous phase was extracted twice with EtOAc. The combined organic layer was washed once with saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo to a red oil. The oil was chromatographed on TLC mesh silica (50 g). Compound **8**-α,*β* eluted with 5−10% EtOAc/hexane as a light yellow-green oil. The yield was 4.60 g (73%) and the ^{1}H NMR showed an α:*β* ratio of 84:16. R_f (20% EtOAc/hexane): 0.50. ^{1}H NMR ($CDCl_3$): δ 7.41−7.24 (m, 10H), 5.99−5.88 (m, 1H), 5.32 (dd, 1H, J = 1.5, 17.2 Hz), 5.23 (d, 1H, J = 10.3 Hz), 4.91−4.64 (m, 6H), 4.51 (d, 0.16H, J = 7.8 Hz), 4.38 (dd, 0.16H, J = 5.3, 12.8 Hz), 4.19−4.14 (m, 1 H), 4.09−3.95 (m, 3H), 3.78−3.71 (m, 1H), 3.64 (t, 0.16H, J = 8.9 Hz), 3.59−3.42 (m, 2H), 3.37 (t, 0.84H, J = 9.4 Hz), 3.29−3.25 (m, 0.16H), 1.37 (d, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 138.92, 138.21, 133.65, 128.41, 128.27, 128.16, 128.06, 127.91, 127.84, 127.72, 127.52, 118.34 (a), 117.61 (b), 103.11 (*β*), 99.56 (α), 99.44 (*β*), 96.62 (α), 82.11 (*β*), 81.83 (α), 81.01 (*β*), 80.94 (*β*), 79.25 (α), 78.64 (α), 75.37 (*β*), 75.25 (α), 75.01 (*β*), 73.55 (α), 70.67 (*β*), 68.56 (α), 68.40, 66.00 (*β*), 62.49 (*β*), 20.46. Anal. Calcd. for $C_{25}H_{30}O_6$: C, 70.41; H, 7.09. Found: C, 70.22; H, 7.00.

2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-α,*β*-D-glucopyranose (5a-α,*β*). An oven-dried two-neck 25 mL round-bottom flask with a stir bar, two septa, and N_2 inlet was charged with allyl 2,3-di-*O*-benzyl-4,6-*O*-ethylidene-α,*β*-D-glucopyranoside (**8**-α,*β*, 2.00 g, 4.7 mmol) and anhydrous dimethyl sulfoxide (DMSO) (4.7 mL). Potassium *tert*-butoxide (2.64 g, 23.5 mmol) was added, and the solution turned from yellow to black. The solution was heated to 100 °C and held there for 2.5 h. After cooling, the reaction was quenched with water and extracted with ether (3×). The combined organic layer was washed with water (2×) and then saturated NaCl. The solution was dried over Na_2SO_4 and concentrated in vacuo to give crude propenyl ether **15**-α,*β* as a red oil. R_f (20% EtOAc/hexane): 0.50. ^{1}H NMR ($CDCl_3$): δ 7.42−7.24 (m, 10H), 6.21 (dd, 0.2H, J = 1.7, 6.2), 5.97 (dd, 0.8H, J = 1.7, 3.6), 4.93−4.59 (m, 7H), 4.16 (dd, 0.2H, J = 4.9, 10.4), 4.07 (dd, 0.8H, J = 4.9, 9.9), 3.77−3.65 (m, 1H), 3.57−3.50 (m, 1H), 3.48−3.36 (m, 2H), 1.69 (dd, 3H, J = 1.6, 7.0), 1.38 (d, 3H, J = 5.0).

The crude oil **15**-α,*β* was transferred to a 250 mL round-bottom flask with a stir bar. The oil was dissolved in acetone (40 mL) and water (4 mL). Mercuric oxide yellow (2.0 g) was added and the suspension was stirred. Finally, a mixture of mercuric chloride (1.66 g, 6.1 mmol), acetone (20 mL), and water (2 mL) was added. After stirring for 1h, the mixture was filtered to remove solids, concentrated in vacuo to 10 mL, and then extracted with ether (100 mL). The organic layer was washed with water (2×) and saturated NaCl (1×), dried over Na_2SO_4, and then concentrated in vacuo to a brown sludge. The residue was chromatographed on TLC mesh silica (30 g). Compound **5a**-α,*β* eluted with 30% EtOAc/hexane as an orange oil. The yield was 1.106 g (60%), and the ^{1}H NMR revealed 57:43 *β*:α. R_f (40% EtOAc/hexane): 0.40. ^{1}H NMR ($CDCl_3$): δ 7.39−7.27 (m, 10H), 5.14 (d, 0.5H, J = 3.7 Hz), 4.91−4.66 (m, 5.5H), 4.14 (dd, 0.5H, J = 5.0, 10.5 Hz), 4.09 (dd, 0.5H, J = 5.0, 10.3 Hz), 3.94−3.88 (m, 1H), 3.66 (t, 0.5H, J = 9.0 Hz), 3.56−3.25 (m, 3.5H), 3.10 (bs, 1H, concentrated dependent OH), 1.36 (d, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 128.53, 128.42, 128.31, 128.09, 127.95, 127.83, 127.63, 99.50, 97.72, 92.12, 82.94, 81.44, 81.08, 80.89, 79.31, 78.33, 75.23, 75.12, 74.96, 73.81, 68.53, 68.22, 66.22, 62.48, 20.43. Anal. Calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78 Found: C, 68.34; H, 6.70.

Allyl 2,3,4,6-Tetraacetyl-β-D-glucopyranoside. An oven-dried 3 L round-bottom flask was fitted with a mechanical stirrer, addition funnel, and septa and cooled under N_2. The flask was covered in foil. Acetylbromoglucose (125 g, 0.304 mol) was added. Anhydrous ether (1 L) was added and stirred. Silver oxide (84.6 g, 0.365 mol) was added. Allyl alcohol (207 mL, 3.04 mol) was added from the addition funnel over 15 min. The reaction was stirred overnight, but TLC showed little reaction. Two more charges of silver oxide (82 and 42 g) were added during the day and after stirring overnight again, the reaction was complete. The reaction mixture was filtered through Celite and concentrated in vacuo. The oil was dissolved in ether (500 mL) and treated with hexane (300 mL) while stirring. The solution was filtered to remove some precipitate, and then more hexane (200 mL) was added. Solid crystallized and the mixture was cooled to −20 °C. The white solid was collected and washed with cold 3:2 hexane/ether (2 × 125 mL) and 3:1 hexane/ether (500 mL); 77.25 g (65.8%) was obtained, which also contained some 2,3,4, 6-tetraacetylglucose. A second crop (3.69 g, 3.1%) was also obtained. R_f (50% EtOAc/hexane): 0.52. ^{1}H NMR ($CDCl_3$): δ 5.85−5.74 (m, 1H),5.24 (t, 1H, J = 1.8 Hz), 5.16 (m, 2H), 5.06(t, 1 H, J = 9.75 Hz), 4.97 (1, 1H, J = 8.0), 4.50 9d, 1H, J = 8.0), 4.31−4.19 (m, 2H), 4.10−4.01 (m, 2H), 3.64 (m, 1H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H). ^{13}C NMR ($CCDCl3_3$); δ 170.69, 170.29, 169.40, 169.34, 133.25, 117.64, 99.49, 72.79, 71.70, 71.21, 69.99, 68.33, 61.88, 20.72, 20.66, 20.59. Anal. Calcd. for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.61; H, 6.12.

Allyl 4,6-*O*-Ethylidine-β-D-glucopyranoside (14β). An oven-dried 1 L round-bottom flask was fitted with a stir bar, condenser, and drying tube. Methanol (445 mL) was added. Freshly cut sodium metal (1.2 g, 52.2 mmol) was added carefully and the sirred at room temperature until dissolved. Allyl 2,3,4,6-tetraacetyl-β-D-glucopyranoside (81 g, 0.209 mol) was added. The solution was warmed to reflux. After cooling, the solution was treated with Dowex 50 × 8 resin until mildly acidic (∼16 g). The mixture was filtered through Celite and concentrated to a red oil. Toluene was twice added and then distilled in vacuo to remove water.

In a 2 L round-bottom, CH_2Cl_2 (836 mL) was added and stirred. Diethyl acetal (71.33 mL, 2.4 equiv) and then *p*-toluensulfonic acid monohydrate (1.99 g, 0.05 equiv) were added. The reaction was stirred at room-temperature overnight. More acetal (30 mL) and *p*-toluenesulfonic acid (2 g) were added. After 6 h, the reaction was quenched with a solution of $NaHCO_3$ (4 g) in water (100 mL). The layers were separated, and the aqueous was extracted with CH_2Cl_2. The combined organic phases were washed with water (100 mL) and saturated NaCl (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to a solid. Hexane (300 mL) was added and heated to reflux with stirring. The solution was cooled, and the solid was collected and washed with hexane. After drying, the weight of **14**β was 29.16 g (56.7% for the two steps). ^{1}H NMR ($CDCl_3$): δ 5.95−5.84 (m, 1H), 5.31 (dd, 1H, J = 1.2, 17.2 Hz), 5.20 (d, 1H, J = 10.3 Hz), 4.69 (q, 1H, J = 5.0 Hz), 4.32 (m, 2H), 4.15−4.07 (m, 2H), 3.71 (t, 1H, J = 8.7 Hz), 3.54 (t, 1H, J = 9.7 Hz), 3.33−3.23 (m, 2H), 2.82 (s, 2H, exchangeable protons), 1.34 (d, 3H, J = 4.9 Hz). Anal. Calcd. for $C_{11}H_{18}O_6$: C, 53.65; H, 7.37. Found: C, 53.12; H, 7.12.

Allyl 2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-β-D-glucopyranoside (8β). An oven-dried 500 mL two-neck round-bottom flask with a stir bar, addition funnel two septa, and N_2 inlet was charged with 80% sodium hydride in oil (7.312 g, 0.244 mol). Anhydrous *N*,*N*-dimethylformamide (DMF) (104 mL) was added and the suspension was cooled in ice. A solution of allyl 4,6-*O*-ethylidene-β-D-glucopyranoside (**14**β, 20 g, 81.24 mmol) in DMF (123 mL) was added dropwise over 35 min. The suspension was stirred for 5 min and was allowed to warm to room temperature over 50 min. Benzyl bromide (29.0 mL, 243.75 mmol) was added by syringe. The mixture was stirred for 17 h. Cold water (5 mL) was added dropwise until H_2 evolution ceased. The solution was poured into water (500 mL). The mixture was extracted with ethyl acetate (3 × 150 mL, 1 × 50 mL). The combined organic phase was washed with water (1 × 50 mL) and saturated NaCl (1 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo to a red oil. The oil was chromatographed on TLC mesh silica (100 g). Compound **8**β eluted with 10−20% EtOAc/hexane and was concentrated in vacuo to a yellow solid (31.88 g, 92%). R_f (20% EtOAc/hexane): 0.50. ^{1}H NMR ($CDCl_3$): δ 7.37−7.26 (m, 10H), 5.98−5.88 (m, 1H), 5.33 (dd, 1H, J = 1.5, 17.2 Hz), 5.20 (dd, 1H, J = 1.2, 10.4 Hz), 4.87 (t, 2H, J = 10.7 Hz), 4.80−4.70 (m, 3H), 4.50 (d, 1H, J = 7.7 Hz), 4.38 (dd, 1H, J = 5.3, 12.8 Hz), 4.18−4.11 (m, 2H), 6.63 (t, 1H, J = 9.0 Hz), 3.56 (t, 1H, J = 10.3 Hz), 3.46−3.41 (m, 2H), 3.28−3.22 (m, 1H), 1.36 (d, 3H, J = 5.1 Hz). ^{13}C NMR ($CDCl_3$): δ 138.63, 138.35, 133.73, 128.34, 128.27, 128.16, 127.92, 127.80, 127.72, 127.58, 117.61, 103.12, 99.44, 82.10, 81.00, 80.94, 75.34, 75.02, 70.68, 68.33, 65.99, 20.43. Anal. Calcd. for $C_{25}H_{30}O_6$: C, 70.41; H, 7.09. Found: C, 70.40; H, 7.03.

2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-β-D-glucopyranose (5a-β). The anomeric mixture **5a**-α/β (7 g) was placed in a 250 mL round-bottom flask. Hexane (125 mL) was added, and the suspension was heated to reflux. The sugar became an insoluble oil which sank to the bottom. The suspension was allowed to cool to room temperature, a stir bar was added, and the solution was gently stirred overnight. White, fluffy crystals formed and floated in the hexane above the rest of the impure solid. The crystals were collected by decanting the supernatant into a Buchner funnel. The impure solid was left in the flask (still 1:1 β:α by NMR). The white solid **5a**-β (0.35 g) was dried at room temperature under vacuum (20 mmHg). ^{1}H NMR ($CDCl_3$): δ 7.37−7.27 (m, 10H), 4.90−4.69 (m, 6H), 4.14 (dd, 1H, J = 4.9, 10.4 Hz), 3.66 (t, 1H, J = 9.0 Hz), 3.54 (t, 1H, J = 10.2 Hz), 3.45 (t, 1H, J = 9.3 Hz), 3.37−3.27 (m, 2H), 3.23 (d, 1H, J = 5.5 Hz, concentrated dependent OH), 1.36 (d, 3H, J = 5.1 Hz). ^{13}C NMR ($CDCl_3$): δ_ 128.42, 128.29, 128.11, 127.93, 127.82, 127.63, 99.45, 97.71, 82.93, 81.06, 80.88, 75.22, 74.96, 68.21, 66.21, 20.39. Anal. Calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.46; H, 6.69.

Dibenzyl 4′-Demethyl-4-epipodophyllotoxin-4′-phosphate (4). A three-neck 1L round-bottom flask was fitted

with a dropping funnel, stir bar, thermometer, and two septa. The flask was charged with 4′-demethylepipodophyllotoxin (**3**, 25.00 g, 62.45 mmol) and anhydrous acetonitrile (367 mL, 0.17 *M*). The suspension was cooled to −10°C. Carbon tetrachloride (30.1 mL, 312.25 mmol) was added, keeping the temperature at −10°C. *N*,*N*-Diisopropylethylamine (22.84 mL, 131.15 mmol) was added by syringe over 3 min. *N*,*N*-dimethylaminopyridine (0.763 g, 6.25 mmol) was added all in one portion, followed by the dropwise addition of dibenzyl phosphite (20.00 mL, 90.55 mmol) over a 15 min period. The reaction was somewhat exothermic during the addition, but the internal temperature was kept at −10 °C with additional external cooling. The reaction was stirred at −10 °C for 37 min. During this time, the starting material dissolved. The reaction was followed by HPLC. KH_2PO_4 (0.5 M, 150 mL) was added, and the solution was allowed to warm to room temperature. The mixture was extracted with EtOAc (1 × 350 mL) and then washed with water (2 × 100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to a volume of 150 mL. 2-Propanol (IPA, 500 mL) was added. Solvent (200 mL) was removed in vacuo, and the solid precipitated during this time. IPA (500 mL) was added, and then another 550 mL of solvent was removed in vacuo. Finally, IPA (250 mL) was added, and the mixture was heated to reflux until all solid dissolved. The yellow solution was cooled to room temperature and then to 0°C for 4 h. A white solid was collected, washed twice with cold IPA, and dried in vacuo (40°C, 20 mmHg) to yield 37.15 g (90.1%). HPLC R_t (Waters μ-phenyl bondapak column, 3.9 × 300 mm, (40/30/30 acetonitrile/ methanol/ pH 3.0 0.02 M KH_2PO_4), 1.5 mL/min, 240 nm): 4.0 min. R_f (10% MeOH/CH_2Cl_2): 0.66. ^{1}H NMR ($CDCl_3$): δ 7.37−7.28 (m, 10H), 6.81 (s, 1H), 6.39 (s, 1H), 6.30 (s, 2H), 5.90 (dd, 2H, J = 1.0, 12.7 Hz), 5.28−5.14 (m, 4 H), 4.71 (d, 1H, J = 3.4 Hz), 4.53 (d, 1H, J = 5.1 Hz), 4.25 (dd, 1H, J = 8.7, 10.7 Hz), 3.63 (s, 6H), 3.27 (dd, 1H, J = 5.2, 14.1 Hz), 2.71−2.61 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 175.27, 151.15, 151.11, 148.22, 147.32, 137.28, 136.04, 135.94, 132.19, 131.35, 128.43, 128.30, 128.26, 127.69, 127.64, 110.13, 109.32, 107.66, 101.45, 69.62, 69.53, 69.46, 67.75, 66.17, 56.06, 43.81, 40.39, 38.47. Anal. Calcd. for $C_{35}H_{33}O_{11}$: C, 63.64; H, 5.04. Found: C, 63.84; H, 4.98.

Dibenzyl 4-(2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-*β*-D-glucopyranosyl)-4′-demethyl-4-epipodophyllotoxin-4′-phosphate (6a-*β*) (Coupling in Acetonitrile). A 25 mL two-neck round-bottom flask β with a stir bar, thermometer, and a septum was charged with dibenzyl 4′-demethyl-4-epipodophyllotoxin-4′-phosphate (**4**, 1.00 g, 1.51 mmol), dry 4 Å molecular sieves ($^1/_{16}$ in. pellet) (2.0 g), 2,3-di-*O*-benzyl-4,6-*O*-ethylidene-α,β-D-glucopyranose (**5a**-β/α , 85:15 β:α, 0.702 g, 1.817 mmol), anhydrous acetonitrile (10.0 mL). The solution was stirred until homogeneous and then cooled to −20°C. Boron trifluoride etherate (0.50 mL, 4.08 mmol) was added dropwise over 2 min. The reaction was held at −20°C for 80 min. White solid began precipitating 45 min after addition of BF_3·OEt_2. Pyridine (5.23 mL, 64.7 mmol) was added. The suspension was allowed to warm to room temperature and was diluted with CH_2Cl_2 (10 mL). The white solid dissolved. The solution was filtered to remove remaining solids and then washed with 3% HCl (7 mL). The aqueous phase was back-extracted with CH_2Cl_2 (10 mL). The combined organic phase was washed with water (7 mL), and the aqueous phase was back-extracted with CH_2Cl_2 (10 mL). The combined organic phase was washed finally with saturated NaCl (7 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to an off-white solid. HPLC of the crude product showed a 72:28 ratio of **6a**-β:**6a**-α . The solid was dissolved in CH_2Cl_2 (10 mL) with stirring. Methanol (90 mL) was added, and solid soon precipitated out. The solution was warmed to reflux with stirring, during which time the solid dissolved, and then 20 mL of solvent was distilled off. The solid began crystallizing after 19 mL was collected. The mixture was allowed to cool to room temperature while stirring gently for 5 h. The white solid was collected and rinsed twice with room-temperature methanol. The solid **6a**-β was dried in vacuo (40 °C, 20 mmHg) and yielded 0.830 g (53.3%). HPLC R_t (Waters μ-phenyl bondapak column, 3.9 × 300 mm, (40/30/30 acetonitrile/methanol/ pH 3.0 0.02 *M* KH_2PO_4), 1.5 mL/min, 240 nm): 20.4 min. R_f (50% EtOAc/hexane): 0.36. ^{1}H NMR ($CDCl_3$): δ 7.38−7.18 (m, 18 H), 7.00−6.98 (m, 2H), 6.82 (s, 1H), 6.54 (s, 1H), 6.25 (s, 2H), 5.97−5.89 (dd, 2H, J = 1.0, 26.7 Hz), 5.29−5.18 (m, 4H), 4.89−4.85 (m, 2H), 4.77−4.71 (m, 3H), 4.60−4.49 (m, 3H), 4.39 (t, 1H, J = 10.2 Hz), 4.23 (t, 1H, J = 8.2 Hz), 4.16 (dd, 1H, J = 4.9, 10.4 Hz), 3.63 (s, 6H), 3.55 (t, 1H, J = 10.2 Hz), 3.45−3.34 (m, 2H), 3.32−3.21 (m, 2H), 2.89−2.80 (m, 1H), 1.38 (d, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 174.74, 151.20 148.72, 147.17, 138.48, 137.75, 137.0, 136.3, 136.2, 132.02, 128.62, 128.42, 128.30, 128.21, 128.07, 127.87, 127.70, 127.67, 110.72, 109.18, 107.73, 102.32, 101.60, 99.55, 81.66, 80.95, 75.40, 75.06, 73.45, 69.45, 68.19, 67.87, 65.97, 43.87, 41.22, 37.48, 20.40. Anal. Calcd. for $C_{57}H_{57}O_{16}P$: C, 66.53; H, 5.58. Found: C, 66.79; H, 5.48.

Dibenzyl 4-(2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-*β*-D-glucopyranosyl)-4′-demethyl-4-epipodophyllotoxin-4′-phosphate (6a-*β*) (Coupling in Acetonitrile with Direct Crystallization). A 1 L three-necked round-bottom flask was fitted with a septum, mechanical stirrer and a thermometer. Dibenzyl 4′-demethyl-4-epipodophyllotoxin-4′-phosphate (**4**, 10.00 g, 15.14 mmol) and 2,3-*O*-dibenzyl-4,6-*O*-ethylidene-glucopyranose (**5a**-α/β, 93:7 β:α, 7.02 g, 18.17 mmol) were added. The solids were dissolved in anhydrous acetonitrile (134 mL), and then the solution was cooled to −40 °C. Boron trifluoride etherate (5.00 mL, 40.65 mmol) was added dropwise. The solution was stirred at −40 °C and followed by HPLC. During the reaction some product precipitated. After 2.5 h, methanol (400 mL) was added dropwise. The suspension was allowed to warm to 0 °C with stirring and stirred for 4.5 h. The solid was collected in a Buchner funnel, rinsed twice with 0 °C methanol, and dried in vacuo. This produced 11.016 g of **6a**-β, which contained 2.5% methanol by weight, according to NMR. The weight yield is 69.0% with HI of 99.9%.

Dibenzyl 4-(2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-*β*-D-glucopyranosyl)-4′-demethyl-4-epipodophyllotoxin-4′-phos-

phate (6a-β) (Coupling in Dichloroethane). A 250 mL three-neck round-bottom flask fitted with a stir bar, thermometer, and two septa was charged with dibenzyl 4′-demethyl-4-epipodophyllotoxin-4′-phosphate (**4**, 14.295 g, 21.57 mmol), dry 4 Å molecular sieves ($^1/_{16}$ in. pellet) (28.6 g), 2,3-di-*O*-benzyl-4,6-*O*-ethylidene-α,β-D-glucopyranose (**5a**-α/β, 10.0 g, 25.88 mmol), and anhydrous 1,2-dichloroethane (143 mL). The solution was stirred until homogeneous and then cooled to −20°C. Boron trifluoride etherate (7.15 mL, 58.24 mmol) was added dropwise over 10 min. The reaction was held at −20°C for 18 h. Pyridine (5.23 mL, 64.7 mmol) was added, and the mixture turned from brown to yellow. The cloudy solution was allowed to warm to room temperature and was diluted with CH_2Cl_2 (200 mL) and filtered to remove solids. The solution was washed with 3% HCl (100 mL), water (100 mL), and saturated NaCl (100 mL), dried over Na_2SO_4, and concentrated in vacuo to a yellow oil. Hot methanol (1500 mL) was added while stirring. The mixture was allowed to cool to room temperature and stand overnight. The white solid was collected and rinsed twice with methanol. The solid **6a**-β was dried in vacuo (40 °C, 20 mmHg) and yielded 8.86 g (39.8%).

The C-1″-a isomer **6a**-α remained in the mother liquor, along with some of the desired product **6a**-β . This remaining coupled product was recovered by further crystallizations and/or chromatography. The ratio of β:α of the crude product before crystallization of **6a** was 54:46. The overall yield of coupled product was 81%.

Dibenzyl 4-(2,3-Di-*O*-benzyl-4,6-*O*-ethylidine-α-D-glucopyranosyl)-4′-demethyl-4-epipodophyllotoxin-4′-phosphate (6a-α). HPLC R_t (Waters μ-phenyl bondapak column, 3.9 × 300 mm, (40/30/30 acetonitrile/methanol/ pH 3.0 0.02 M KH_2PO_4), 1.5 mL/min, 240 nm): 18.1 min. R_f (50% EtOAc/hexane): 0.31. ^{1}H NMR ($CDCl_3$): δ 7.38−7.21 (m, 20H), 6.87 (s, 1H), 6.48 (s, 1H), 6.26 (s, 2H), 5.95 (d, 2H, J = 5.8 Hz), 5.29−5.18 (m, 4H), 4.87 (dd, 3H, J = 2.3, 11.1 Hz), 4.79−4.74 (m, 2H), 4.68−4.58 (m, 4 H), 4.11 (t, 1H, J = 7.9 Hz), 3.95 (q, 1H, J = 10.6 Hz), 3.86 (t, 1H. J = 9.2 Hz), 3.63 (s, 6H), 3.51 (dd, 1H, J = 3.6, 9.4 Hz), 3.45 (d, 1H, J = 7.2 Hz), 3.45−3.35 (m, 3H), 2.82−2.75 (m, 1H), 1.32 (d, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 174.91, 151.22, 151.18, 148.44, 147.02, 138.56, 137.83, 137.05, 136.27, 136.18, 132.19, 129.27, 128.59, 128.45, 128.34, 128.24, 128.12, 127.96, 127.89, 127.72, 127.69, 110.44, 109.81, 107.85, 101.61, 101.08, 99.59, 82.07, 79.36, 78.59, 76.76, 75.09, 74.69, 69.52, 69.46, 69.41, 68.18, 67.04, 62.95, 56.15, 43.82, 41.10, 38.41, 20.40. Anal. Calcd. for $C_{57}H_{57}O_{16}P$: C, 66.53; H, 5.58. Found: C, 66.70; H, 5.38.

Etoposide 4′-Phosphate (2). An autoclave apparatus was charged with wet 5% palladium on carbon (2.5 g) under nitrogen. Methanol (100 mL) was added to the reactor, followed by a solution of **6a**-β (10.0 g, 9.72 mmol) in THF (100 mL). The mixture was hydrogenated at ambient temperature and 45 psig hydrogen for 4 h. The mixture was filtered through Celite-521 and a 0.45 μm membrane and rinsed with MeOH. The filtrate was concentrated in vacuo (35 °C, aspirator) to a volume of 100 mL. Absolute ethanol (100 mL) was added, and the solution was again concentrated to 100 mL. The solution was again diluted with ethanol (100 mL) and seeded with crystals of etoposide 4′-phosphate diethanol solvate **2**, and the solution was concentrated to 130 mL. The solution was heated to reflux, and water (0.7 mL) was added. The solution is cooled to room temperature and seeded while cooling. After 2 h, the white crystals were collected by filtration and washed with ethanol (15 mL). The solid was dried under high vacuum at room temperature. There was obtained 6.46 g (87.4% weight yield, 10.5% ethanol by ^{1}H NMR) of etoposide 4′-phosphate diethanol solvate (**2**) which assayed at 99.5 area % purity by HPLC. HPLC R_t (Waters 25 cm phenyl column, (25/75 CH_3CN/ 0.02 M KH_2PO_4), 2.0 mL/min, 240 nm): 2.0 min. Anal. Calcd. for $C_{29}H_{33}O_{16}P$: C, 52.10; H, 4.98. Found: C, 51.96; H, 4.42.

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