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α-1-Tributyltin-O-2,3-bisacetyl-4,6-ethylidene-glucose as a Convenient Glycosidation Reagent: An Efficient Synthesis of Etoposide.

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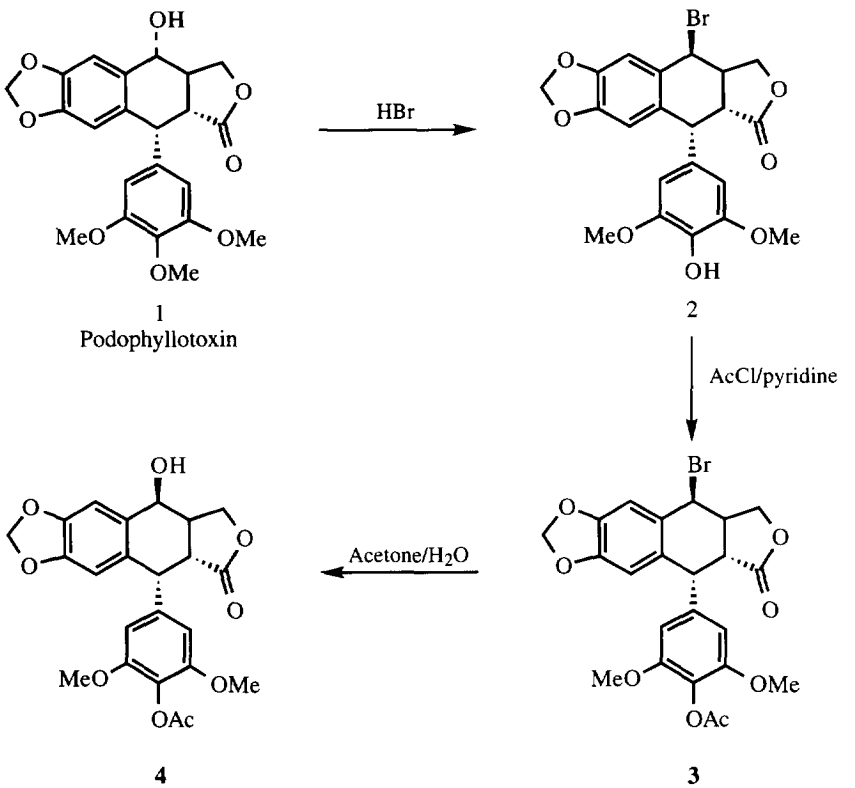
Abstract: The antineoplastic drug etoposide has been prepared by a chemically and operationally simple process. The salient reaction is the BF_3·etherate promoted, room temperature condensation of 4'-demethyl-4'-acetyl-epipodophyllotoxin **4**, with α-1-Bu_3Sn-O-2,3-bisacetyl-4,6-ethylidene-glucose **6**. The latter compound was prepared from 4,6-β-ethylidene glucose triacetate and Bu_3Sn-OMe obtained *in situ* from $(Bu_3Sn)_2O$ and dimethyl carbonate. A readily separable mixture of α and β–etoposide triacetate epimers was obtained where the desired β–epimer predominated. In contrast, 4,6-α-ethylidene glucose diacetate and **4**, even at 0°C, gave an equimolar mixture of epimers. It is proposed that the stereochemical outcome may be attributed to electronic effects in the activated tin-glucose reagent.

Various synthetic methods [1a,b,c] for the preparation of etoposide have been reported. Condensation of 1-OH-glucose tetracetate with 4'-carbobenzoxy epipodophyllotoxin in the presence of BF_3.etherate, was used by Kuhn et al. [1a] An improved procedure was described by Japanese investigators [2] who used 1-hydroxy-2,3-bis-chloroacetyl-4,6-ethylidene glucose and 4'-chloroacetyl- epipodophyllotoxin, where the chloroacetyl groups could be easily removed. Allevi [3] synthesized etoposide at -20°C from unprotected 4'-demethyl-epipodophyllotoxin. At higher temperature the product was mainly the α-epimer.

This paper describes a novel synthetic approach using 4'-acetyl-epipodophyllotoxin **4**, obtained from podophyllotoxin (Scheme I), and α-1-Bu_3Sn-O-2,3-bisacetyl-4,6-ethylidene-glucose **6** as reagents (Scheme II). Advantages of this procedure over reported methods [1] include better stereoselectivity at convenient room temperature conditions. In our experience, Kuhn's glycosidation method had to be conducted at ice temperature or below, giving approximately equal amounts of α- and β-anomeric etoposide triacetates. Kuhn reported that at lower temperatures (ca. -20°C) an improved β:α ratio of epimers was obtained. Allevi [1c] reported that starting with a 70:30 α:β mixture of anomeric 2,3,4,6-glucose tetraacetates at -30°C a 1:1 ratio of anomeric O-glucosides was obtained, thus indicating a slow anomerization rate. In the present process, using **6**, at room temperature the epimeric ratio of the products was ca. 4:1 in favor of the desired β-anomer.

Scheme I



Compound **6**, was obtained from β-ethylidene glucose triacetate **5** and Bu_3SnOMe (Scheme I). Bu_3SnOMe was prepared *in situ* from $(n\text{-}Bu_3Sn)_2O$ and an excess of dimethyl carbonate [4], and without isolation was treated with ethylideneglucose triacetate **5** [5]. The stereochemistry of **6** either in its crude form or when crystallized from hexane, was found to be α, as indicated by NMR and X-ray diffraction (Fig. 1). In agreement with Beyon et al., [6] we found that the α and β anomers of **6** were thermally stable and did not equilibrate even at 100°C/5h. Furthermore, since the coordination sphere of the Sn in α-**6** is tetrahedral, the oxygen of the SnO group is a relatively poor nucleophile. When the coordination sphere of the tin is increased to a trigonal bipyramid, the oxygen atom assumes an apical position where it has enhanced nucleophilicity [7]. This was accomplished by treating a CH_2Cl_2 or $CHCl_3$ solution of α-**6** with BF_3.etherate which caused rapid anomerization to give an approximately equimolar ratio of anomers, as indicated by NMR, whereby the H-1 doublet at 5.39 ppm of the α anomer decreased and a new doublet at 4.83 ppm for the β-anomer appeared. The BF_3 caused a slight general downfield shift in the NMR spectrum. In the presence of the BF_3 it is assumed that epimerization involves an open ring sugar moiety **11** in equilibrium with a polar pentacoordinated intermediate **12.** Other complexes wherein the tin atom is bound to two oxygens have been described [8].

Although **12** can equilibrate between the α and β-forms, positive charge delocalization on the 2-OAc group stabilizes the β-conformation. (Scheme III). BF_3 plays a dual role in the reaction, it activates the tin complex as described, and converts **4** into its corresponding carbonium ion. It is well documented [9] that regioselectivity in carbohydrate alkylation and acylation reactions, pentacoordination of the tin derivative is of prime significance. Thus, subsequent reaction of **12**, the pentacoordinated tin complex in its predominant β-form, with **4** in the presence of BF_3 gave predominantly the β-etoposide-triacetate **7.** A small amount of the α-anomer **8** was also produced. This room temperature selectivity is unique to the 1-$OSnBu_3$ derivative, whereas analogous predominant formation of the β-anomer when using the 1-OH [1a] or 1-$OSiMe_3$ [3] sugar derivatives could be accomplished only at -20°C. The best isolated yields of **7** (ca. 40%) were obtained with a 1:2:3 ratio of **4**:α-**6**:BF_3. With smaller amounts of BF_3 the isolated yield of **7** was considerably diminished.

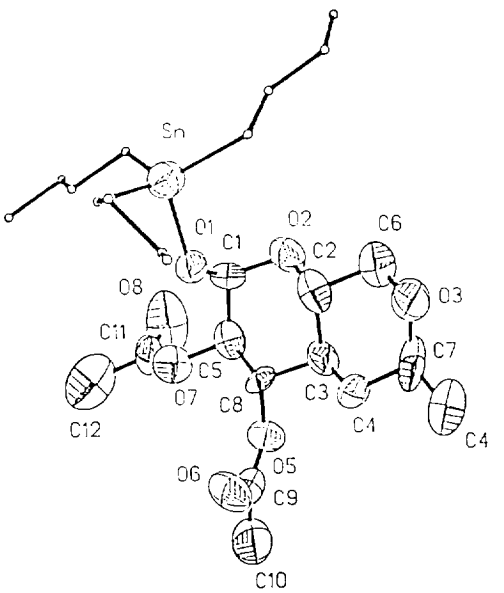
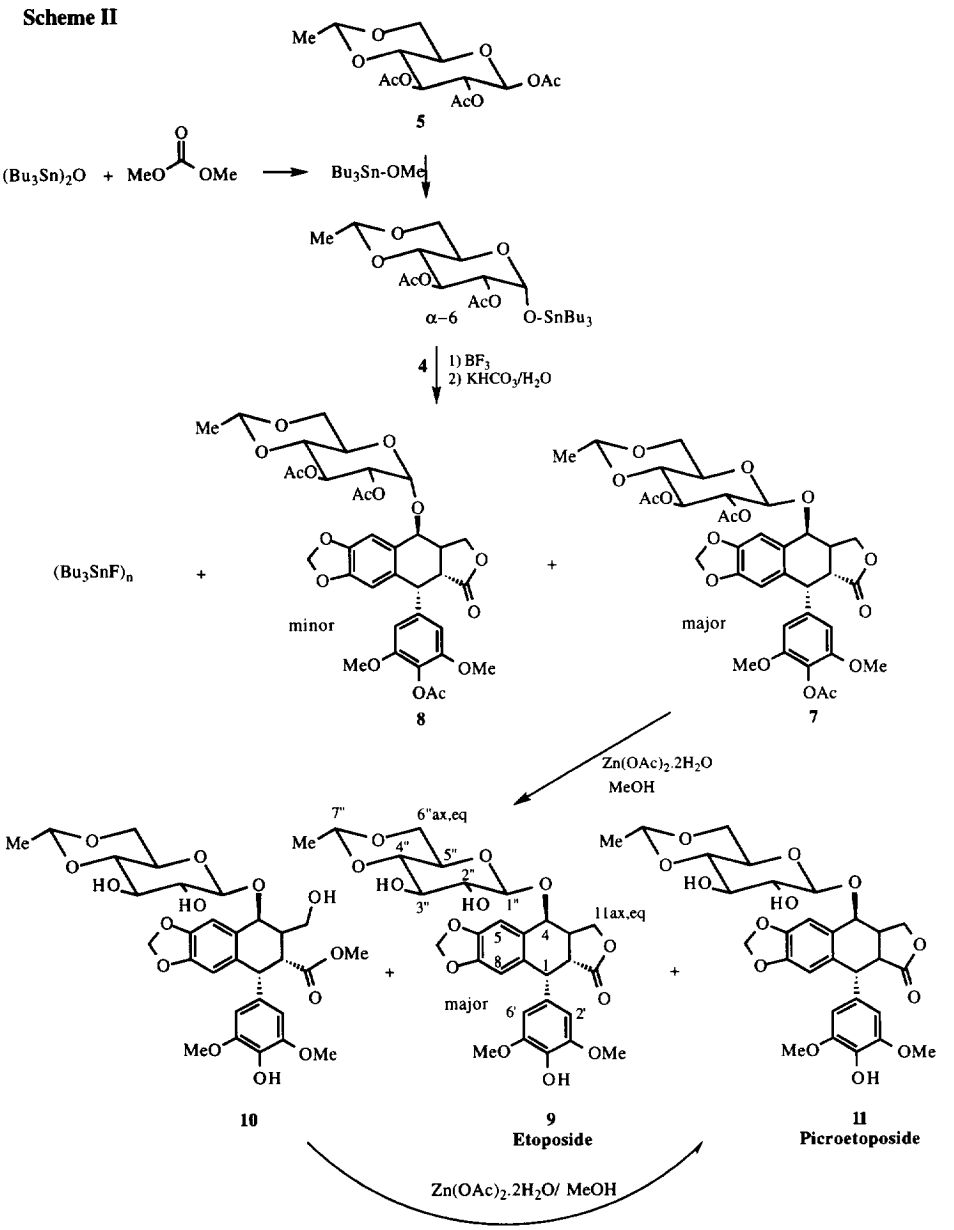


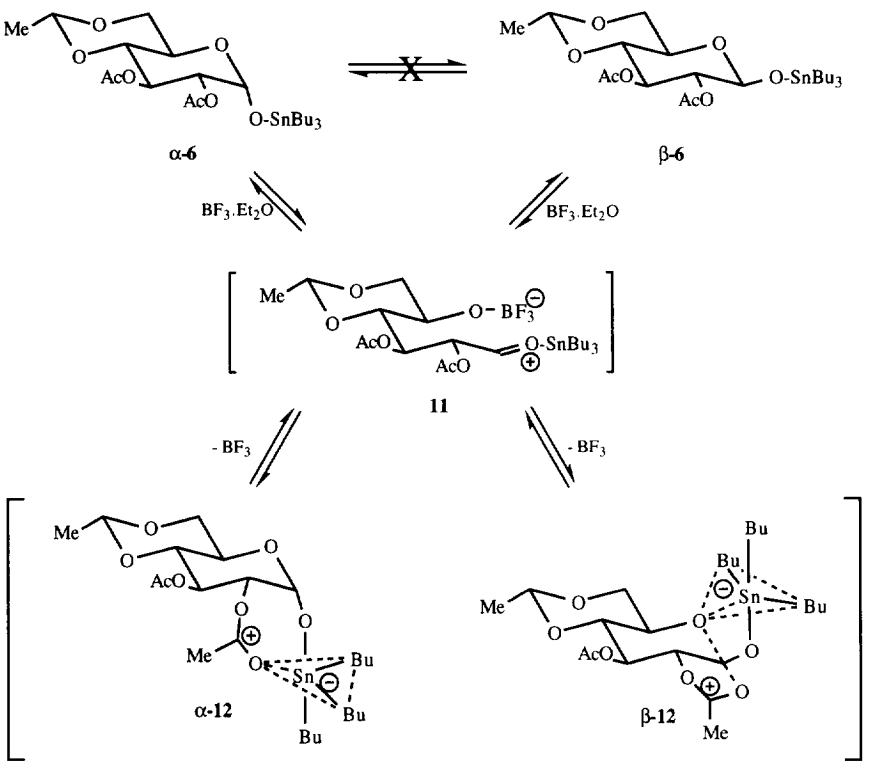
Fig. 1 The ORTEP drawing of the molecular structure of α–**6.**

Compound **7** was deacetylated to etoposide **9** by treatment with $Zn(OAc)_2.2H_2O$ in refluxing MeOH [1a]. In the course of the reaction, about 25% of a by-product, that could be isolated by column chromatography, was formed. Based on FTIR (absence of lactone absorption at 1771 cm^{-1}), MS and NMR analysis, this compound was shown to be the methyl ester **10**, having the same *trans*-stereochemistry of **9** (COSY and spin decoupling data). In addition, a small amount (ca. 7-10%) of picroetoposide **11** was also found in the crude reaction mixture. Both **10** and **11** were readily soluble in CH_2Cl_2 and could be separated from etoposide by simple digestion and filtration. Further treatment of **10** with $Zn(OAc)_2.2H_2O$ in MeOH easily converted it into **9** and **11**. By this procedure an additional 20% of **9** could be isolated.

Scheme II


5
(Bu3Sn)2O + MeO OMe
Bu3Sn-OMe
α–6
O-SnBu3
4
1) BF3
2) KHCO3/H2O
(Bu3SnF)n
minor
major
8
7
Zn(OAc)2.2H2O
MeOH
10
9
Etoposide
11
Picroetoposide
Zn(OAc)2.2H2O/ MeOH

Scheme III



EXPERIMENTAL

^{1}H-NMR spectra were obtained on a Brucker AM-300 spectrometer. Chemical shifts are expressed in ppm downfield from Me_4Si used as internal standard. $CDCl_3$ was used as solvent, unless otherwise stated. Mass spectra were obtained on a Finnigan TSQ 70 spectrometer (CI=chemical ionization). IR spectra were obtained on a Nicolet 205 instrument. High performance liquid chromatograms (HPLC) were obtained on a Hitachi L6200 A apparatus. Progress of the reactions was monitored by TLC on silica gel (Merck, Art. 5554).

Tributyl-[(2,3-di-O-acetyl-4,6-O-ethylidene-α-D-glucopyranosyl)oxy]tin, 6 [5] - A mixture of (bis-tributyltin)oxide (13.1 g, 22 mmol) in dimethyl carbonate (50 mL) was refluxed (94°C) for 2 h. To the resulting clear solution, was added ethylidene-β-D-glucose triacetate **5** [10] (13.3 g, 40 mmol) and reflux was continued for an additional 2 h. The solvent was evaporated to give an oil 23.2 g, which consisted primarily of **6** contaminated with traces of **5** (tlc silica gel, EtOAc:petroleum ether 1:1, developed with alkaline $KMnO_4$ or by spraying with ethanolic H_2SO_4 and charring at 100°C, (R_f **6** 0.56, **5** 0.75). ^{1}H NMR ($CDCl_3$) δ 5.52 (t, 1H,

H-3), 5.30 (d, 1H, H-1), 4.72 (dd, 1H, H-2), 4.66 (q, 1H, H-7), 4.1-3.83 (m, 2H, H-6eq and H-5), 3.58-3.4 (m, 1H, H-6ax), 3.37 (t, 1H, H-4), the MeCH is buried under the peaks of the Bu_3Sn.

4'-Acetyl-1-bromo-4'-demethyl-epipodophyllotoxin, 3 - To a suspension of crude 1-bromo-4'-demethyl-epipodophyllotoxin **2** (100 g), obtained from podophyllotoxin and HBr [1], in methylene chloride (600 mL), was added pyridine (39 mL) followed by acetyl chloride (32 mL), while maintaining the temperature below 10°C. The temperature was then allowed to rise and was maintained at 25-30°C for 3 h. While keeping the temperature below 30°C, water (250 mL) was added and the mixture was stirred for 15 min. The organic phase was washed with 5% aqueous acetic acid (200 mL), water (200 mL), and was dried (Na_2SO_4). Evaporation of the solvent gave an amorphous solid (115 g). Tlc of the residue (silica gel, EtOAc:petroleum ether:MeCN 1:1:0.1) indicated the presence of a single major spot and a minor spot immediately below it.

4'-Acetyl-4'-demethyl-epipodophyllotoxin, 4 - To crude **3** (115 g) in acetone (300 mL), heated to 45°C until complete dissolution, was added water (300 mL), while maintaining the temperature at 45°C. Within ca. 15 min a precipitate began to form and stirring was continued for a total of 1 h. The mixture, cooled to below 10°C, was further stirred for 3 h, filtered and the collected solid was washed with 50% aqueous acetone (150 mL). The solid, 70-75 g (overall yield based on podophyllotoxin ca. 40%), dried to constant weight at 50°C under reduced pressure, was recrystallized from CH_2Cl_2/MeOH, mp 261-264°C. ^{1}H-NMR ($CDCl_3$) δ 6.87 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.31 (s, 2H, H-2' + H-6'), 5.98 (dd, 2H, OCH_2O), 4.82 (brt, 1H, H-4), 4.64 (d, 1H, H-1), 4.44-3.93 (m, 2H, H-11 + H-11'), 3.68 (s, 6H, 3'-OMe, 5'-OMe), 3.29 (dd, 1H, H-2), 2.98-2.72 (m, 1H, H-3), 2.30 (s, 3H, Ac), 1.86 (brd, 1H, OH). MS (CI-isobutane) m/z 443 (MH^+).

β-Etoposide Triacetate, 7 - To a cooled mixture of **4** (70 g, 0.16 mol) and **6** (195 g, 0.34 mol) in CH_2Cl_2 (500 mL), was added BF_3.etherate (62 mL, 0.5 mol) while maintaining the temperature below 15°C. The temperature was allowed to rise and was maintained at 25-30°C for 2 h. The mixture was then poured into a solution of $KHCO_3$ (84 g, 0.84 mol) in water (500 mL), stirred for 5-10 min, filtered and the collected amorphous solid was discarded. The organic phase in the filtrate was separated, washed twice with water, dried (Na_2SO_4) and evaporated, to give an amorphous solid (161 g). MeOH (225 mL) was added to the solid and the mixture was refluxed for 2 h. It was then cooled in ice, and filtered. The collected solid was washed with cold MeOH, and was dried to constant weight at 80°C under reduced pressure to give 48-50 g (42-44% yield). The product, analyzed by HPLC consisted of essentially pure β-etoposide triacetate **7** with only a trace of the α-epimer **8**. ^{1}H NMR ($CDCl_3$) δ 6.73 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.27 (s, 2H, H-2' and H-6'), 6.01-5.97 (dd, 2H, OCH_2O), 5.22 (t, 1H, H-2"), 4.93 (dd, 1H, H-3"), 4.83 (d, 1H, H-4), 4.79 (d, 1H, H-1"), 4.70 (q, 1H, H-7"), 4.62 (d, 1H, H-1), 4.40 (dd, 1H, H-11ax), 4.27-4.13 (m, 2H, H-11eq + H-6"eq), 3.68 (s, 6H, 3'-OMe, 5'-OMe), 3.58 (t, 1H, H-6"ax), 3.47 (t, 1H, H-4"), 3.42 (ddd, 1H, H-5"), 3.17 (dd, 4H, H-2), 2.85 (ddt, 1H, H-3), 2.30 (s, 3H, AcOAr), 2.05 (s, 3H, Ac), 1.85 (s, 3H, Ac), 1.36 (d, 3H, MeCH). MS (CI-isobutane) m/z 715 (MH^+). FTIR 1772 cm^{-1} (lactone), 1746 cm^{-1} (ester).

Etoposide, 9 (Method I) - A mixture of **7** (50 g, 0.07 mol) and $Zn(OAc)_2.2H_2O$ (50 g) in MeOH (500 mL) was stirred and refluxed for 96 h. The cooled mixture was evaporated, leaving an amorphous solid which was dissolved in a mixture of CH_2Cl_2 (300 mL) and 10% aqueous acetic acid (330 mL). The aqueous phase was washed with CH_2Cl_2 and the combined organic phase was washed with water (5 x 300 mL), while MeOH (50 mL) was added to the first and third washings to prevent precipitation of the product. The organic phase was dried (Na_2SO_4), and was evaporated to yield a solid (39 g). The product, analyzed by HPLC consisted of 4,6-O-ethylidene-β-D-glucopyranosyl-epipodophyllinate methyl ester **10** (20%), **9** (66%), and picroetoposide **11** (ca. 7%), and the remainder, a mixture of etoposide acetylated products. The residual mixture was digested twice with refluxing CH_2Cl_2 (300 mL), cooled and filtered to give etoposide 18-20 g (44-48% yield). The product, analyzed by HPLC was 99% pure **9**.

Isolation and characterization of 4,6-O-ethylidene-β-D-glucopyranosyl-epipodophyllinate methyl ester, 10 - The CH_2Cl_2-filtrate obtained as in Method I from several pooled reactions mixtures was evaporated to give 155 g residue. A 5 g portion of the residue was redissolved in CH_2Cl_2 and was chromatographed on silica gel (Merck 7734) (100g), eluted with a gradient of CH_2Cl_2 and MeOH. A total of 120 fractions, 20 mL each, were collected. Evaporation of fractions 78-105 gave 1.5 g of **10**. ^1H NMR ($CDCl_3$) δ 6.73 (s, 1H, H-5), 6.42 (s, 1H, H-8), 6.05 (s, 2H, H-2' and H-6'), 5.95-5.94 (dd, 2H, OCH_2O), 5.51 (s, 1H, OH), 5.03 (d, 1H, H-4), 4.75 (q, 1H, H-7"), 4.65 (d, 1H, H-1"), 4.41 (d, 1H, H-1), 4.22 (dd, 1H, H-6"eq), 3.88 (dd, 2H, H-11), 3.75 (superimposed s + m, 7H, 3'-OMe, 5'-OMe and H-3"), 3.61 (t, 1H, H-6"ax), 3.48 (s, 3H, COOMe), 3.44-3.38 (m, 4H, H-2, H-5", H-4" and H-2"), 2.56 (ddt, 1H, H-3), 1.38 (d, 3H, MeCH). FTIR 1735 cm^{-1} (ester). MS (CI) m/z 638 ($MH^+ + NH_3$). $C_{30}H_{36}O_{14}$

Etoposide, 9 (Method II) - A mixture of the remaining 150 g of the above residue and $Zn(OAc)_2.2H_2O$ (150 g) in MeOH (1500 mL) was refluxed for 48 h. The reaction was worked up as described above in Method I. The crude residue obtained before digestion with CH_2Cl_2 weighed 51.6 g, consisted (HPLC) of **10** 26%, **9** 57% and picroetoposide 16%. After digestion with CH_2Cl_2, 38g of 99.7% pure **9** was obtained.

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REFERENCES

1. a) Kuhn, M.; Keller-Juslen, G.; Von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 944.; b) Saito, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. *Chem. Lett.*, **1987**, 799. c) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Sanvito, A. M.; Macdonald, P. *Tetrahedron Lett.*, **1992**, *33*, 4831.
2. Nippon Kayaku Kobashiki Kaishu. European Patent application 0111058 (1983).
3. Allevi, P.; Anastasia, M.; Ciuffreda, P.; Bigatti, E.; Macdonald, P. *J. Org. Chem.*, **1993**, *58*, 4175.
4. Davies, A. G.; Kleinschmidt, D. C.; Palan P. R.; Vasishtha, S. C. *J. Chem. Soc. (C)* **1971**, 3972.
5. Herzig, J.; Nudelman, A.; Gottlieb. H.; Keinan E.; Sterling J. *Carbohyd. Res.*, **1987**, *162*, 145.
6. Blunden S. J.; Smith P. J.; Beynon P. J.; Gillies D. G. *Carbohyd. Res.*, **1981**, *88*, 9.
7. David S.; Hanessian, S. *Tetrahedron*, **1985**, *41*, 643.
8. Davies, A. G.; Kleinschmidt, D. C.;. Palan P. R; Vasishtha, S. C. *J. Chem. Soc. (C)* **1971**, 3972.
9. a) Cruzado, C.; Bernabe, M.; Martin-Lumas, M. *J. Org. Chem.*, **1989**, *50*, 465; b) Cruzado, M. C.; Martin-Lumas, M. *Carbohyd. Res.*, **1988**, *175*, 193; c) Alais, J.; Veyrieres, A. *J. Chem. Soc. Perkin I*, **1981**, 377; d) Ogawa, T; Matsui, M. *Carbohyd. Res.*, **1977**, *56*, C1; e) Ogawa, T; Matsui, M. *Tetrahedron*, **1981**, *37*, 2363; Tsuda, Y.; Haque, M. E.; Yoshimoto, K. *Chem Pharm. Bull.*, **1983**, *31*, 1612.
10. Hall, D. M.; Stamm, O. A. *Carbohyd. Res.*, **1970**, *12*, 421.

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