Pergamon

Tetrahedron, Vol. 52, No. 8, pp. 3049-3056, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)01122-6

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

Kalman Vogel*[a], Jeffrey Sterling[a], Yaakov Herzig[a] and Abraham Nudelman*[b]

[a] Teva Pharmaceutical Industries, Ltd., BPC Division, P.O. Box 160 Netanya 42101, Israel

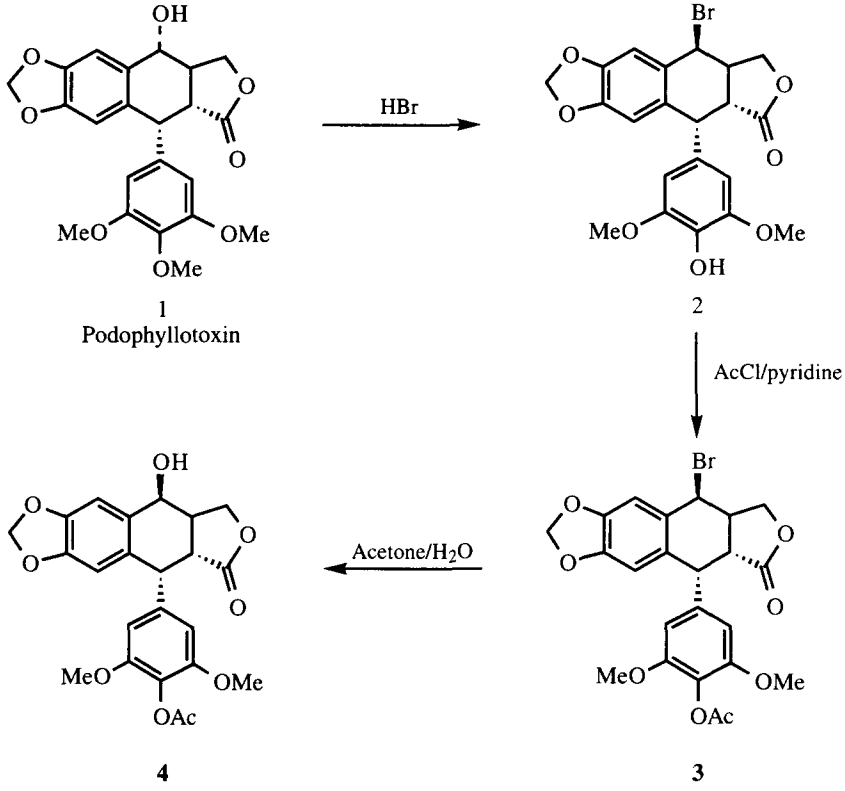
[b] Chemistry Department, Bar Ilan University, Ramat Gan 52900, Israel.

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.

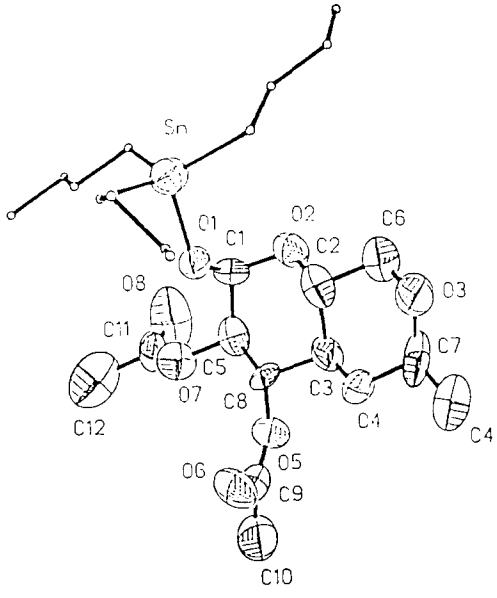
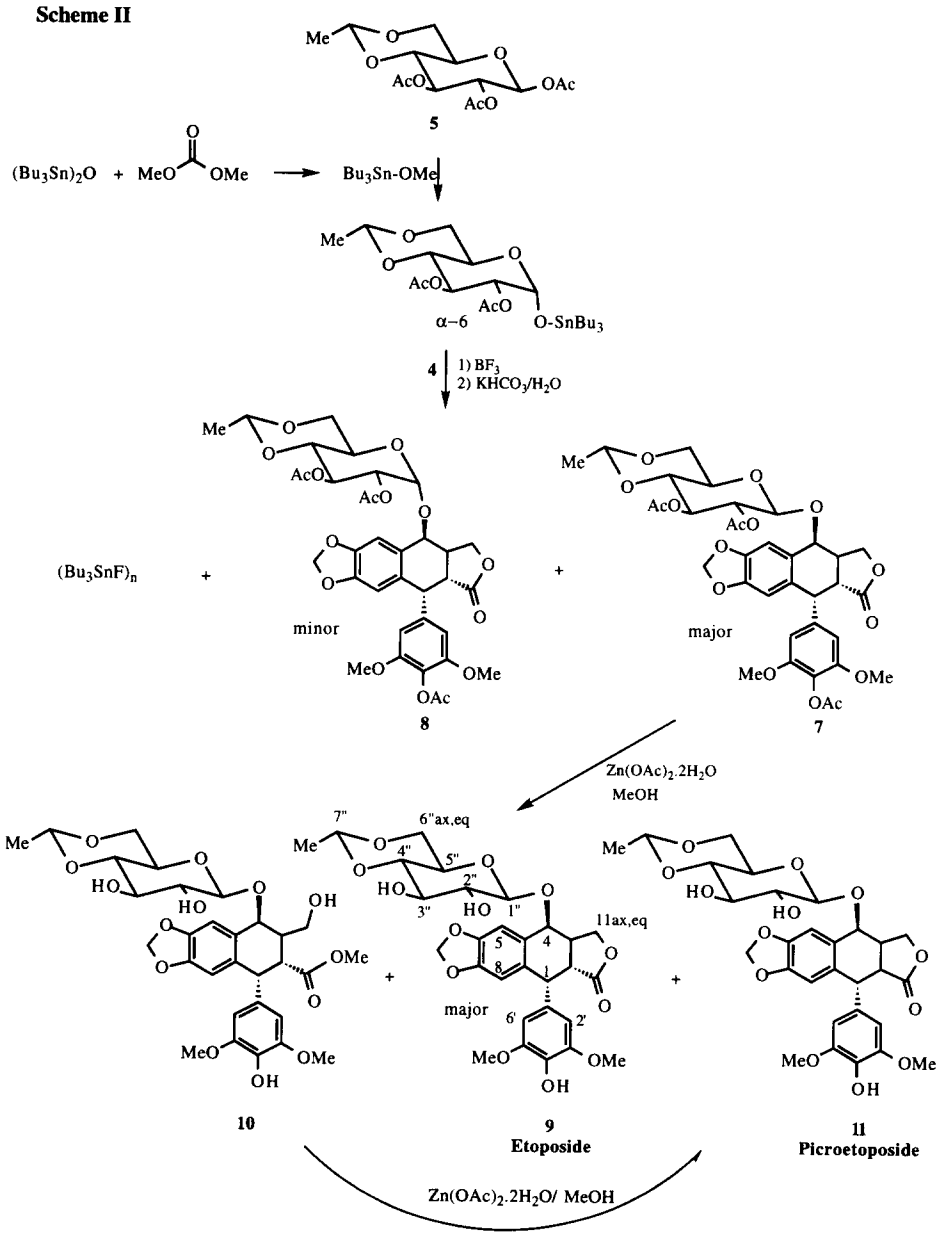


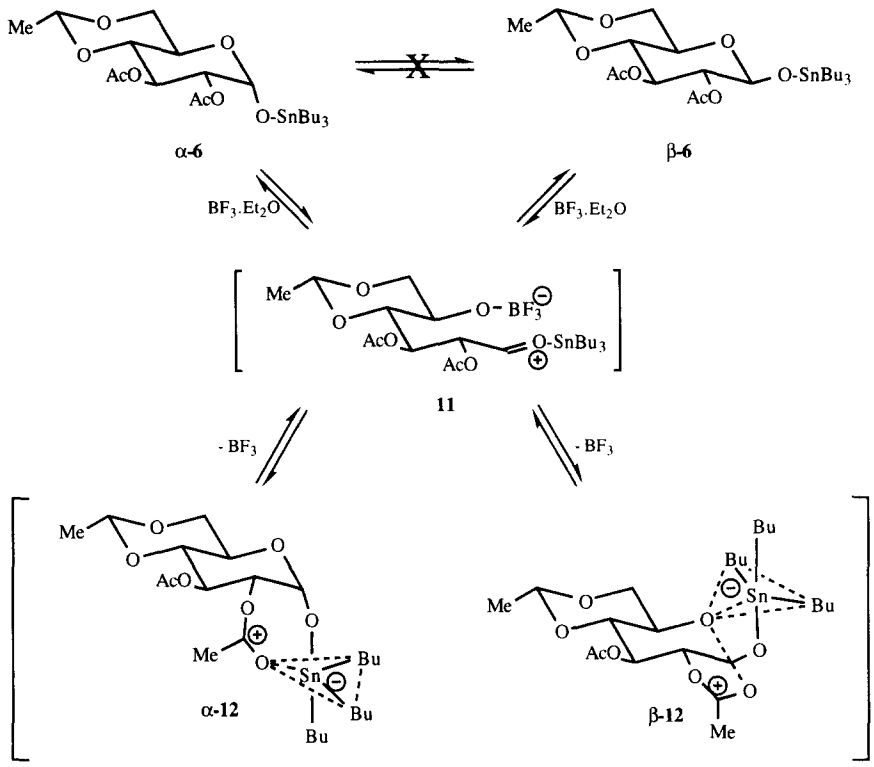
Fig. 1 The ORTEP drawing of the molecular structure of α–**6.**

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
6"ax,eq
11ax,eq
major
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.

Acknowledgment

We would like to thank Prof. Asher Mandelbaum (Haifa Technion) and Dr. Hugo E. Gottlieb (Bar Ilan University) for their respective assistance in obtaining mass spectral and NMR data.

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.

(*Received in UK* 14 *November* 1995; *revised* 8 *December* 1995; *accepted* 20 *December* 1995)