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Synthesis of (–)-4-aza-4-deoxypodophyllotoxin from (–)-podophyllotoxin

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Abstract

(-)-4-Aza-4-deoxypodophyllotoxin (4) was synthesized from (-)-podophyllotoxin (1) through C-ring cleavage, Curtius rearrangement and intramolecular N-alkylation. Analogue 4 showed potent cytotoxicity against P388 leukemia cells. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: podophyllotoxin; 4-aza-4-deoxypodophyllotoxin; Curtius rearrangement; cytotoxicity.

Microtubules are an important target in the development of novel anticancer drugs, and *Catharanthus* alkaloids, vinblastine and vincristine, and taxol have already been used clinically. Podophyllotoxin (1) is also known to bind to tubulin, but its binding site differs from that of vinblastine¹ and taxol.² This evidence suggests that podophyllotoxin (1) may become a lead compound having activity against tumors resistant to the above drugs. Recently, podophyllotoxin heterocyclic analogues have attracted much attention since some of them showed more promising antitumor activity and were less toxic than podophyllotoxin (1).³ The findings that 2,4-diaza-4-deoxypodophyllotoxin (2) showed significant antitumor activity against vincristine-resistant P388 leukemia (P388/VCR) cells in vivo,⁴ while podophyllotoxin (1)⁴ and 2-aza-4-deoxypodophyllotoxin (3)⁵ showed only marginal or weak activity, suggested that substitution of the carbon atom at the position 4 by a nitrogen atom would be important to bring about such changes in the biological profile. We describe herein the synthesis of (-)-4-aza-4-deoxypodophyllotoxin (4) which has not been known thus far.



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0040-4039/99/\$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved. *P11:* S0040-4039(99)01932-2 Since the configuration at the C-1 center is known to be critical for expressing the activity,⁶ we planned to synthesize this compound in an optically pure form from natural (–)-podophyllotoxin (1). To avoid loss in chiral integrity at the C-2 position throughout the transformation, which would occur readily under basic conditions, we reduced the lactone D-ring in the initial step. Lithium aluminum hydride reduction of 1 gave triol 5 in 71% yield (Scheme 1).⁷ Compound 5 was then treated with ethyl chloroformate in pyridine to afford tricarbonate 6 quantitatively. Interestingly, when compound 6 was heated in 1-methylnaphthalene at 170°C for 1.75 h, the ethoxycarbonyloxy group at the position



Scheme 1. *Reagents and conditions*: (a) LiAlH₄, THF, room temp., 71%; (b) ClCO₂Et, pyridine, -20° C, quant.; (c) 1-methyl-naphthalene, 170°C; (d) O₃, MeOH–CHCl₃, -78° C, then Me₂S, 56% from 6; (e) BH₃, THF, room temp., 24% (9) and 63% (10); (f) MnO₂, CH₃CN, room temp., 84% for 11, 85% for 14; (g) MsCl, Et₃N, CHCl₃, room temp., 84%; (h) NaClO₂, 2-methyl-2-butene, *t*-BuOH, pH 4 buffer, quant. for 13; (i) DPPA, Et₃N, dioxane, room temp. ~120°C, 69% from 13, 34% from 15; (j) PPh₃, I₂, imidazole, PhMe, 90°C, 68%; (k) BnBr, K₂CO₃, MeOH–H₂O, 65°C, 82%; (l) 4-methylmorpholine *N*-oxide, *n*-Pr₄NRuO₄, MS 4A, CH₂Cl₂, room temp., 26% (18) and 33% (19); (m) H₂, Pd/C, AcOH–EtOH, 89%

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4 was selectively eliminated to give a rather unstable dihydronaphthalene intermediate 7, which was then subjected to ozonolysis to produce ketoaldehyde 8 in 56% yield from 6. Reduction of 8 using a borane-tetrahydrofuran complex gave epimeric diols 9 and 10 in yields of 24 and 63%, respectively.^{8,9} Diols 9 and 10 were converted into the same intermediate 16 separately. Selective oxidation of diol 9 using activated manganese dioxide gave aldehyde 11 in 84% yield, which was then converted to mesylate 12 in 84% yield. Chlorite oxidation of 12 gave carboxylic acid 13 quantitatively.¹⁰ Curtius rearrangement of 13 using diphenylphosphoryl azide (DPPA), and successive intramolecular N-alkylation of the resultant amine intermediate produced tetrahydroquinoline 16 in 69% yield. The epimeric diol 10 was oxidized to aldehyde 14 in 85% yield, and then converted into iodide 15 in 68% yield by using triphenylphosphine, iodine and imidazole.¹¹ Chlorite oxidation of 15 gave an unstable carboxylic acid, which was then subjected to Curtius rearrangement to provide 16 in 34% yield from 15.12 Treatment of 16 with benzyl bromide under basic aqueous conditions resulted in N-benzylation and decarbonylation to afford diol 17 in 82% yield. Catalytic oxidation of 17 using tetrapropylammonium perruthenate, 4-methylmorpholine N-oxide and powdered molecular sieves 4A provided lactones 18 and 19 in yields of 26 and 33%, respectively.¹³ Debenzylation of 18 gave compound 4 in 89% yield. The stereochemistry of this compound was confirmed by the ¹H NMR spectrum observing 3.3% nuclear Overhauser enhancement between H-2' and H-3, and a large coupling constant of 12.8 Hz between H-2 and H-3 having an antiperiplanar relationship.¹⁴

A preliminary biological evaluation of analogue 4 and compound 1 was performed using P388 leukemia cells. Both compounds showed the same IC₅₀ value of 0.0050 μ g/mL. The in vivo antitumor activity of analogue 4 against P388/VCR cells will be reported in due course.¹⁵

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- 8. All attempts to improve the selectivity of this reduction were unsuccessful due to ready migration and/or reductive elimination of the protective ethoxycarbonyl groups.
- 9. The stereochemistry of diols 9 and 10 was determined by chemical evidence. Diol 9 was subjected to hydrogenolysis, and successive dehydration under Mitsunobu conditions, which proceeds through an *anti* E2 mechanism, gave Z-olefin 20. In the same manner, *E*-olefin was obtained from diol 10.



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- 14. Compound 4: Amorphous powder, $[\alpha]_D^{24}$ -168.8 (*c* 0.10, CHCl₃); ¹H NMR (500 MHz, C₅D₅N, ref: (C₅D₄HN)=7.21 ppm, *J*/Hz) δ 6.88 (s, 2H), 6.75 (s, 1H), 6.73 (s, 1H), 6.56 (d, 1H, *J*=1.5), 5.94 (d, 1H, *J*=1.2), 5.91 (d, 1H, *J*=1.2), 4.74 (d, 1H, *J*=5.0), 4.43 (dd, 1H, *J*=7.5, 6.5), 4.17 (m, 1H), 4.07 (dd, 1H, *J*=9.7, 7.5), 3.87 (s, 3H), 3.70 (s, 6H), 3.33 (dd, 1H, *J*=12.8, 5.0); ¹³C NMR (125 MHz, C₅D₅N, ref: 135.5 ppm) δ 173.43 (s), 153.35 (s), 148.20 (s), 141.43 (s), 141.26 (s), 138.71 (s), 137.99 (s), 115.24 (s), 110.84 (d), 109.06 (d), 101.34 (t), 98.27 (d), 71.21 (t), 60.49 (q), 56.10 (q), 49.13 (d), 47.76 (d), 42.84 (d).
- 15. Although one of the referees urged us to add the results of the in vivo biological evaluation of analogue 4 to enhance the value of this manuscript, we decided to disclose the salient points of our study at this stage since the preparation of a sufficient amount of the analogue and such in vivo biological evaluation would involve an extended period of time, inevitably delaying the urgent publication of this synthetic work.