Synthesis of 2,4-Diaza-4-deoxypodophyllotoxin, a New Analogue of Podophyllotoxin possessing Antitumour Activity

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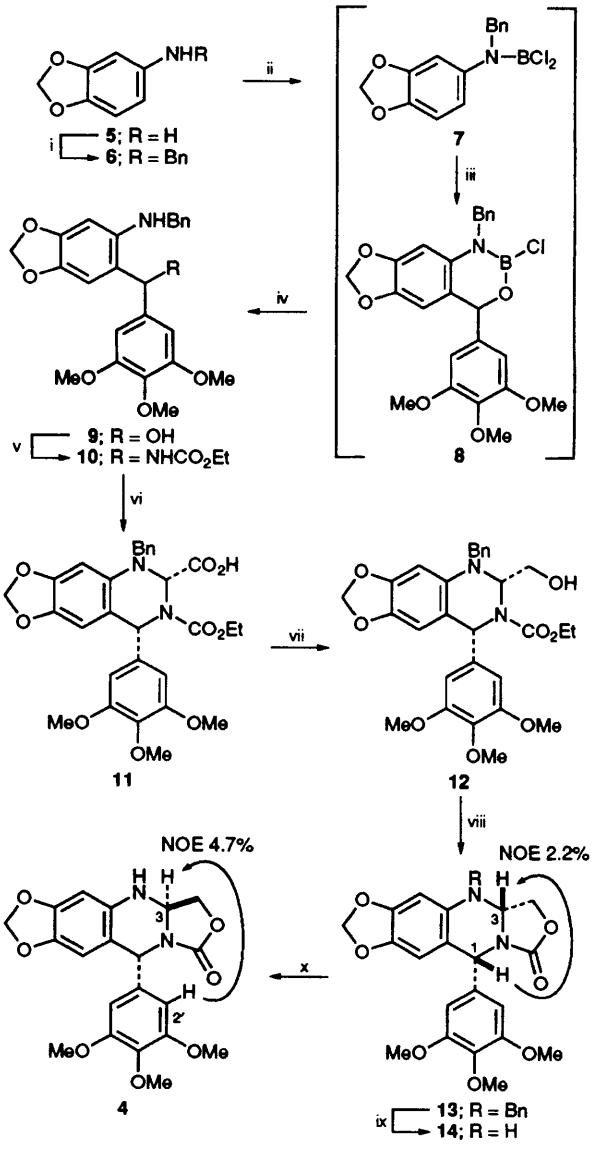
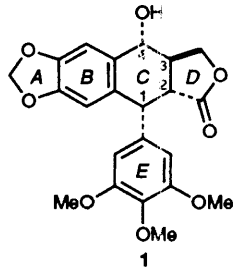
2,4-Diaza-4-deoxypodophyllotoxin **4** and its *cis* analogue **14** are synthesised stereoselectively from 3,4-methylenedioxyaniline **5**, and show significant activity against vincristine-resistant P-388 leukaemia *in vivo*.

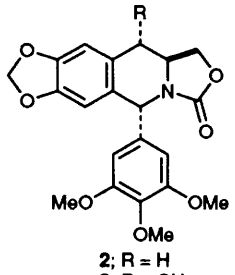
Podophyllotoxin **1** has been known to possess antitumour activity and is still considered an important lead for antineoplastic agents.[1] Previous efforts to find a less toxic analogue of **1** have been conducted mainly through derivatization from **1**. Recently 2-azapodophyllotoxin analogues, such as **2** and **3**, have been synthesised by several groups[2] and attract much interest since some of them showed pronounced *in vivo* antitumour activity comparable to that of **1**.[2b] However no 2-aza analogue incorporating an additional heteroatom at position 4 or other positions of ring *C* has been reported thus far due to the probable chemical instability and accordingly due to less accessibility. Thus, we designed and synthesised 2,4-diaza-4-deoxypodophyllotoxin **4** since it was expected that introduction of a nitrogen atom at this position should little alter the whole stereostructure, but might pose significant influence upon its biological properties through increasing the electron density on ring *B*.

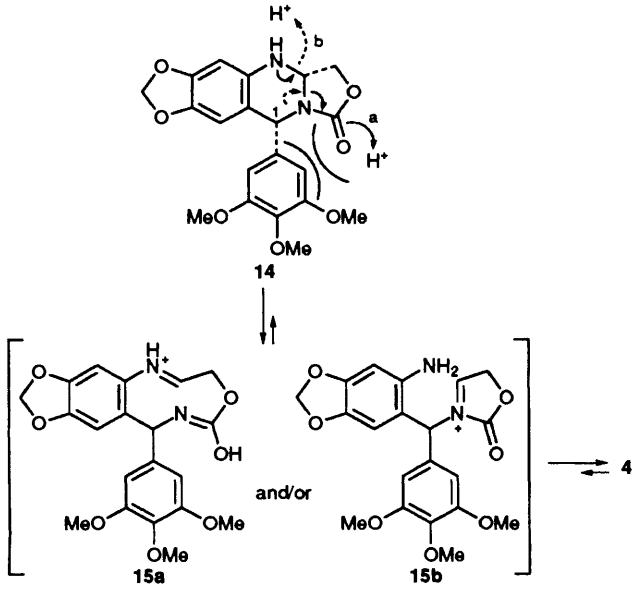
3,4-Methylenedioxyaniline **5** was reacted with benzaldehyde, and successive reduction of the resultant imine with sodium borohydride in a mixture of solvents, EtOH–THF–acetic acid (AcOH), gave *N*-benzyl-3,4-methylenedioxyaniline **6** (bp 186–189 °C/4 mmHg) in 82% yield (Scheme 1). Compound **6** was converted by boron trichloride (1.0 equiv.) and triethylamine (1.15 equiv.) in 1,2-dichloroethane at 0 °C to room temperature for 20 h into the *N*-benzyl-3,4-methylenedioxyanilinodichloroborane **7**,[3] which on treatment with 3,4,5-trimethoxybenzaldehyde (1.0 equiv.) and triethylamine (1.15 equiv.) at room temperature for 3 h gave the benzhydrol **9** in 38% yield from **6** after the hydrolysis of the boracyclic intermediate **8**. The alcohol **9** was reacted with ethyl carbamate (5 equiv.) in polyphosphate ester (PPE)[4]–THF (1 : 4) to give the *N*-benzhydrylurethane **10** (mp 204–205 °C) in 66% yield. The urethane **10** was condensed with glyoxylic acid monohydrate (1.2 equiv.) in refluxing THF† for 1 h to give the tetrahydroquinazolinecarboxylic acid **11**, which, without purification, was reduced with borane prepared *in situ* from sodium borohydride and boron trifluoride etherate in THF to afford the 3-hydroxymethyl derivative **12** (mp 147–149 °C) in 88% yield from **10** as a single stereoisomer. We could not determine its relative stereochemistry at this stage. Sodium methoxide treatment of **12** in refluxing MeOH effected the cyclization to form the oxazolidone **13** (mp 217–218 °C) in 97% yield, and successive debenzylation using palladium on carbon (Pd/C) under H_2 in CH_2Cl_2–MeOH–AcOH (6 : 3 : 1) afforded **14** (mp 249–250 °C)‡ in 95% yield. The relative stereochemistry of **14** between C-1 and C-3 was established to be a *cis* relation by the observation of 2.2% enhancement of NOE between 1-H (δ 5.47) and 3-H (δ 5.09).



Scheme 1 *Reagents and conditions*: i, benzaldehyde (1.1 equiv.), benzene, Dean–Stark, reflux, 1 h; $NaBH_4$ (0.5 equiv.), EtOH–THF–AcOH (60 : 10 : 1), room temp., 1 h (82%); ii, BCl_3 (1.0 equiv.), Et_3N (1.15 equiv.), 1,2-dichloroethane, 0 °C to room temp., 20 h; iii, 3,4,5-trimethoxybenzaldehyde (1.0 equiv.), Et_3N (1.15 equiv.), room temp., 3 h; iv, NH_4OH (38% from **6**); v, ethyl carbamate (5 equiv.), PPE–THF (1 : 4), room temp., 1 h (66%); vi, OHC–CO_2H·H_2O (1.2 equiv.), THF, reflux, 1 h; vii, $NaBH_4$, BF_3·OEt_2, THF, room temp., 72 h (88% from **10**); viii, MeONa, MeOH, reflux, 6 h (97%); ix, H_2, Pd/C, CH_2Cl_2–MeOH–AcOH (6 : 3 : 1), room temp., 9 h (95%); x, $CHCl_3$–CF_3CO_2H (3 : 1), 50 °C, 12 h (99%)







Scheme 2

Surprisingly, treatment of **14** with a 3:1 mixture of $CHCl_3$ and trifluoroacetic acid at 50 °C for 12 h afforded exclusively the desired *trans* analogue **4** (mp 223–224 °C)§ in 99% yield, the stereochemistry of which was determined by the observation of NOE (4.7%) between 2′-H (δ 6.54) and 3-H (δ 4.96), and further supported by the upfield shift [δ 66.9 (**14**) → δ 61.4 (**4**)] of the C-3 resonance in ^{13}C NMR.[5]

This exclusive conversion from the *cis* analogue **14** to the *trans* analogue **4** can be rationalized by considering the intermediates **15a** and/or **15b** generated through path a or b shown in Scheme 2 and the relative thermodynamic stability between **4** and **14**. The *cis* analogue **14** should suffer $A^{(1,2)}$ strain[6] between the carbonyl oxygen atom of the oxazolidone and the pseudo-equatorially oriented trimethoxyphenyl group at C-1, but in the *trans* analogue **4** this strain is relieved by the C-1 substituent adopting a pseudo-axial position.¶

A goal of development of new antineoplastic drugs is to obtain molecules lacking cross resistance with classical agents.[7] Analogues **4** and **14** showed significant activity against vincristine-resistant P-388 leukaemia (P-388/VCR) *in vivo*.‖

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Footnotes

† Upon this condensation, the use of THF as a solvent proved to be important. Other solvents such as MeOH or propan-2-ol resulted in the decrease of diastereoselectivity.

‡ **14**: δ_H ($CDCl_3$; 400 MHz; *J*/Hz) 3.80 (3H, s), 3.82 (6H, s), 3.90 (1H, br s, D_2O exchange), 4.12 (1H, dd, *J* 8.4, 8.4), 4.57 (1H, dd, *J* 8.4, 7.1), 5.09 (1H, br t, *J* 7.7), 5.47 (1H, s), 5.86 (1H, d, *J* 1.3), 5.88 (1H, d, *J* 1.3), 6.34 (1H, s), 6.42 (1H, s) and 6.57 (2H, s). δ_C ($CDCl_3$; 100 MHz) 56.1 (q), 60.5 (d), 60.8 (q), 66.9 (d), 67.4 (t), 99.9 (d), 101.3 (t), 104.6 (d), 107.6 (d), 119.3 (s), 135.6 (s), 137.5 (s), 137.6 (s), 143.4 (s), 147.3 (s), 153.3 (s) and 154.9 (s).

§ **4**: δ_H ($CDCl_3$; 400 MHz) 2.48 (1H, br s, D_2O exchange), 3.81 (6H, s), 3.84 (3H, s), 4.16 (1H, dd, *J* 9.4, 1.5), 4.37 (1H, dd, *J* 9.4, 5.9), 4.96 (1H, dd, *J* 5.9, 1.5), 5.76 (1H, s), 5.87 (1H, d, *J* 1.3), 5.90 (1H, d, *J* 1.3), 6.29 (1H, s) 6.39 (1H, s) and 6.54 (2H, s). δ_C ($CDCl_3$; 100 MHz) 55.5 (d), 56.3 (q), 60.8 (q), 61.4 (d), 68.6 (t), 98.7 (d), 101.1 (t), 105.9 (d), 108.1 (d), 112.5 (s), 136.3 (s), 137.8 (s), 138.0 (s), 142.2 (s), 147.8 (s), 153.4 (s) and 156.8 (s).

¶ Koga *et al.* reported the *cis/trans* isomerization of **2** *via* a benzhydryl cation under strongly acidic conditions.[2b] We cannot completely deny the possibility that this type of reaction might occur in **14** instead of, or concomitantly with, the reaction shown in Scheme 2.

‖ The maximum *T/C* values for **4** and **14** against P-388/VCR *in vivo* (CDF_1 mice, ip-ip, days 1–5) are 152% (at 10 mg kg^{-1} d^{-1} level) and 137% (at 5 mg kg^{-1} d^{-1} level), respectively. Podophyllotoxin **1** showed marginal activity (*T/C* = 125%) upon this experiment.

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