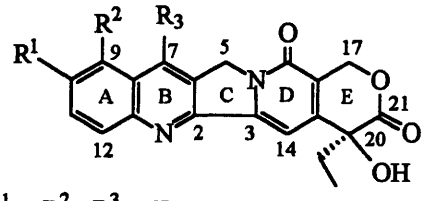
Preparation of 14-nitrocamptothecin derivatives by reactions of camptothecin with nitronium tetrafluoroborate in acidic solvents

Zhisong Cao

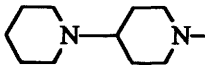
The Stehlin Foundation for Cancer Research and St. Joseph Hospital Cancer Laboratory, 1918 Chenevert, Houston, TX 77003, USA

The new camptothecin derivatives (20*S*)-20-*O*-acetyl-14-nitrocamptothecin 7 and 5-hydroxy-14-nitrocamptothecin 8 are prepared by the reactions of camptothecin 1 with nitronium tetrafluoroborate 9 in acetic anhydride and trifluoroacetic acid, respectively. When acetic acid is used as reaction solvent, the esterification product (20*S*)-20-*O*-acetylcamptothecin 6 is obtained.

The potent antitumour agent (20*S*)-camptothecin **1**, a pentacyclic alkaloid first isolated from the wood and bark of *Camptotheca acuminata* (*nyssaceae*) by Wall *et al.* in 1966, was shown to have antitumour activity against the mouse leukaemia L1210 system.[1] The molecule contains a novel pentacyclic ring



1 $R^1 = R^2 = R^3 = H$

2 $R^1 =$  $R^2 = H$, $R^3 = Et$

3 $R^1 = OH$, $R^2 = CH_2N(CH_3)_2$, $R^3 = H$

4 $R^1 = R^3 = H$, $R^2 = NO_2$

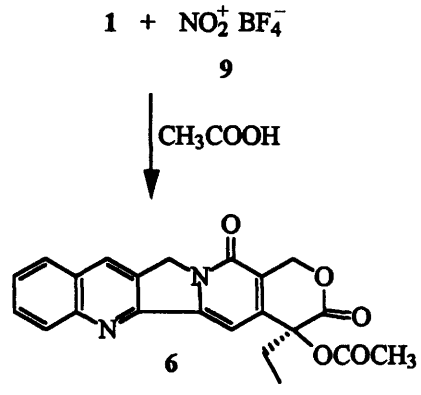
5 $R^1 = R^3 = H$, $R^2 = NH_2$

system that includes a pyrrolo[3,4-*b*]quinoline moiety (rings A, B and C), a lactam (ring D) and an α-hydroxy lactone with one chiral centre (ring E). Natural camptothecin was evaluated clinically in the early 1970s as the water-soluble sodium carboxylate salt form. Unfortunately, the camptothecin molecule produced severe toxicity and seemed devoid of anticancer activity,[2–6] which resulted in the discontinuation of phase II trials. However, the interest in camptothecin and its derivatives suddenly increased after it was discovered that camptothecin inhibits topoisomerase I.[7–10] By using native camptothecin with the closed lactone ring intact, it has been shown in our laboratory that the oral and intramuscular routes of drug administration are superior to intravenous (i.v.) injections of the water-soluble sodium salt.[11] Thus, the anticancer activity of camptothecin could be maximized and much of the severe toxicity observed with the water-soluble carboxylate salt could be eliminated. Many camptothecin derivatives have been synthesized over the years.[12–29] Among these molecules, irinotecan **2** (CPT-11), topotecan **3**, 9-nitrocamptothecin **4** (9-NC), and 9-aminocamptothecin **5** (9-AC) have received much attention in cancer research in recent years because of their higher cell antiproliferative and antitumour activity than the mother compound.[30]

We now report the preparation of 14-nitrocamptothecin derivatives **7** and **8** by the reactions of camptothecin **1** with nitronium tetrafluoroborate **9**.

Results and discussion

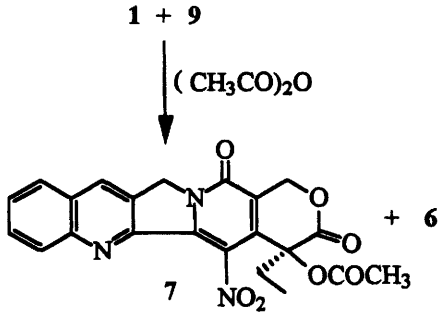
The reaction of camptothecin with nitronium tetrafluoroborate in acetic acid was carried out in an oil bath at 80 °C under an N_2 atmosphere. Nitronium tetrafluoroborate (6 mmol) was added to the stirred solution of camptothecin (1.0 g, 2.9 mmol) in acetic acid. The reaction mixture was stirred at 80 °C for 24 h. An esterification product, (20*S*)-20-*O*-acetylcamptothecin **6** was found to be the only product after separation and purification [eqn. (1)]. No nitration product was observed.



(1)

(20*S*)-20-*O*-Acetylcamptothecin **6** was originally prepared from the reaction of camptothecin with acetic anhydride by Wall *et al.*[1] Only mp, UV, IR and the molecular ion peak data were reported. The ^1H and ^{13}C NMR data were not available.

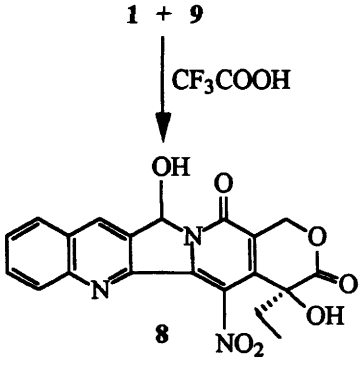
The reaction of camptothecin with $NO_2^+BF_4^-$ in acetic anhydride under the same conditions gave both product **6** in 35% yield as obtained above and the nitration product, (20*S*)-20-*O*-acetyl-14-nitrocamptothecin **7** in 28% yield [eqn. (2)].



(2)

The reaction probably consists of two steps, the esterification of camptothecin with solvent acetic anhydride to form **6** and the subsequent nitration with nitronium salt to give **7**.

On reacting camptothecin **1** with nitronium tetrafluoroborate **9** in trifluoroacetic acid (TFA) under the same conditions, the product 5-hydroxy-14-nitrocamptothecin **8** was found to be the only major product (55%) as shown in eqn. (3). The proton NMR



(3)

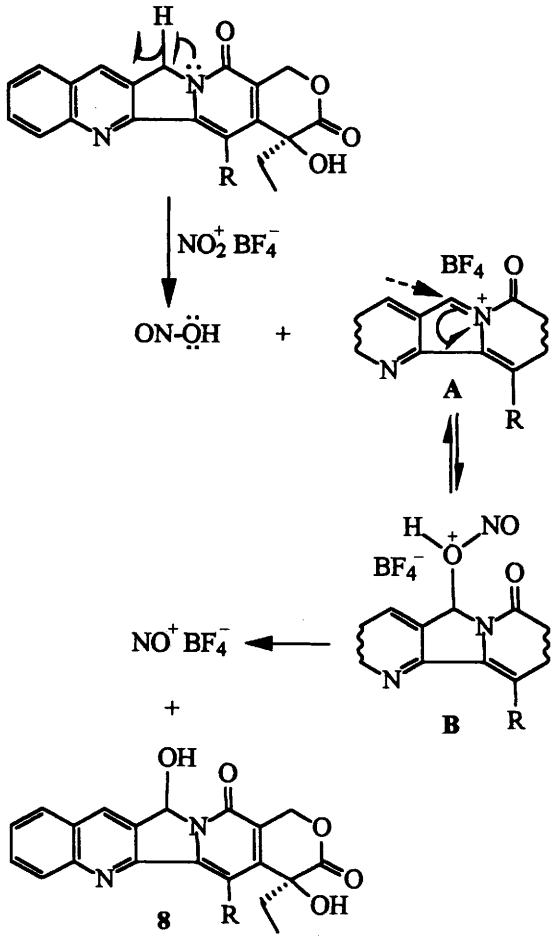
spectrum of **8** showed that the C5-H signal showed a downfield shift of about 1 ppm due to the presence of the 5-hydroxy group when compared with the ^{1}H NMR spectra of **6** and **7**. A similar effect was also observed in the ^{13}C NMR spectrum of product **8**. C5 in **8** absorbed at 87.4 ppm, a downfield shift from 54.5 ppm (in **6**) and 53.9 ppm (in **7**). No significant changes in chemical shift values for the remaining protons and carbons of **8** were observed when compared with products **6** and **7**.

In contrast to the reaction in acetic acid, the esterification product was not isolated from the reaction in CF_3CO_2H. However, nitration at C14 and electrophilic oxygenation at C5 occurred. Previously, Olah and Ramaiah found a similar electrophilic oxygenation indicative of the ambient reactivity of NO_2^+ upon treatment of bicyclo[2.2.1]heptane and bicyclo[2.2.2]octane with $NO_2^+BF_4^-$ in pure nitroethane.[31] Surprisingly, we also observed this type of behaviour of NO_2^+ with camptothecin in CF_3CO_2H. To account for the result it is suggested according to Olah's description [31] that initial hydride abstraction with NO_2^+ takes place to give intermediate **A**. Reaction of **A** with the by-product HNO_2 gives intermediate **B** which readily cleaves to give product **8** (Scheme 1). Direct hydroxylation at C5 was previously reported. For example, 5-hydroxycamptothecin was prepared under radical reaction conditions by Miyasaka *et al.*[32]

A mechanism for the esterification of camptothecin **1** with acetic acid catalysed by nitronium tetrafluoroborate **9** is depicted in Scheme 2. In trifluoroacetic acid, no esterification product was obtained, probably due to hydrolysis of the labile TFA ester on work-up.

In order to show the role of nitronium tetrafluoroborate in the esterification reaction, camptothecin was stirred in either acetic acid or trifluoroacetic acid without $NO_2^+BF_4^-$ at 80 °C for 24 h. No reaction occurred and camptothecin was recovered in 100% yield.

Thus, it has been shown that the direct substitution at C14 with a nitro group may be achieved readily from the reaction of camptothecin **1** with nitronium tetrafluoroborate **9** in acidic solvents.



Scheme 1

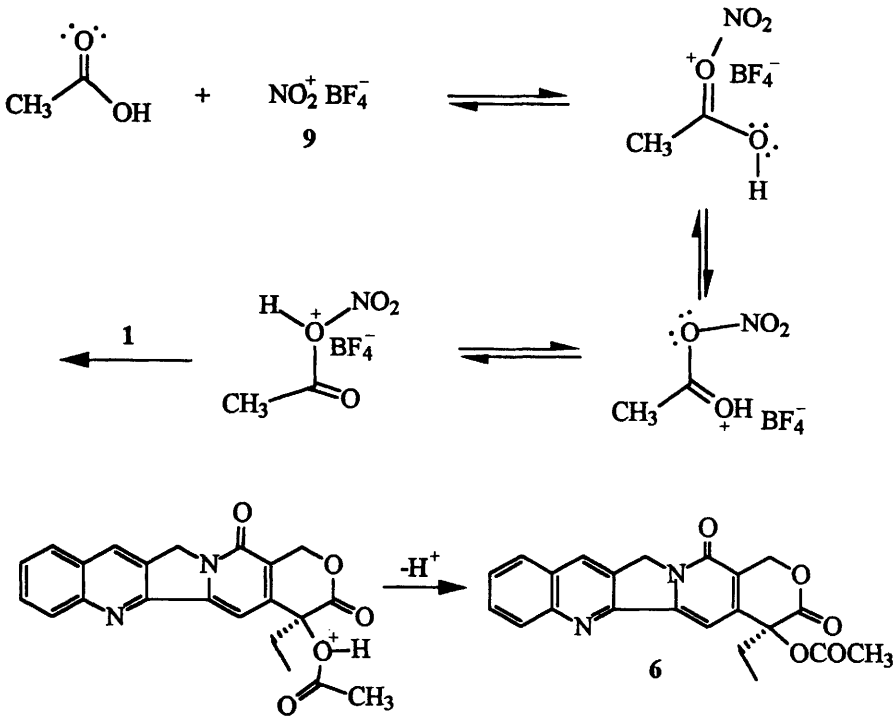
Experimental

General

Dry nitrogen was routinely used as the reaction atmosphere in all reactions. All glassware was baked at 80–100 °C for a minimum of 2 h before being used. Melting points were obtained with a Mel-Temp melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra of approximately 10% (w/v) solution in CF_3CO_2D, unless otherwise indicated, were obtained at 270.05 MHz with a JEOL GX-270 WB NMR spectrometer. Chemical shifts are reported in parts per million (δ), employing tetramethylsilane as an internal standard. In reporting the NMR data, we have used the following abbreviations: coupling constants in Hz (J), singlet (s), doublet (d), triplet (t), broad singlet (bs), multiplet (m). Mass spectra were recorded using a VG ZAB-SEQ mass spectrometer (VG Analytical Co., UK) with a resolution of 10 000. Routinely used solvents such as chloroform and dichloromethane were dried and freshly distilled. Silica gel (230–400 mesh, Aldrich) for column chromatography was used for all product separations. Eastman chromagram (silica gel with fluorescent indicator on polyethylene) sheets were employed in thin-layer chromatography (TLC) operations. The numbering system used in reporting NMR data is shown in structure **1**.

Preparation of (20*S*)-20-*O*-acetylcamptothecin 6

Nitronium tetrafluoroborate **9** (0.8 g, 6 mmol) was added to a solution of camptothecin (1.0 g, 2.9 mmol) in acetic acid (50 ml) in a 100 ml round-bottomed flask equipped with a magnetic stirrer, a condenser and a drying tube and immersed in an oil bath. The mixture was stirred at 80 °C (bath temperature) for 24 h. After cooling to room temperature, the reaction mixture was poured into 500 ml cold water while stirring. The yellow suspension in water was extracted with dichloromethane (250 ml × 4). The combined extracts were washed with water (250 ml × 2) and dried over anhydrous sodium sulfate for 5 h. The dried solution was filtered and the dichloromethane was removed by rotary evaporation under vacuum. The residue was chromatographically separated with methanol–chloroform as eluent. The product **6** was obtained as a white powder, mp 275 °C (decomp.) (lit.,[1] 271–274 °C, decomp.), yield 58%; δ_H 1.15 (3 H, bs, 19-CH_3), 2.43 (5 H, bs, 18-CH_2 and $OCOCH_3$), 5.72–5.92 (4 H, m, 5- and 17-CH_2), 7.98 (1 H, s, 14-H), 8.17–



Scheme 2

8.52 (4 H, m, Ar 9-H to 12-H), 9.41 (1 H, s, 7-H); δ_C 8.7 (C19), 22.0 ($OCOCH_3$), 34.3 (C18), 54.5 (C5), 70.1, 79.9 (C17, C20), 107.0, 114.5, 118.7, 127.2, 132.5, 132.5, 133.9, 134.7, 140.6, 141.2, 142.1, 145.9, 150.6 (C2, C3, C6–C16), 160.1 (C16a), 173.7, 177.9 (C21 and $OCOCH_3$); *m/z* (relative intensity) 390 (M^+, 70%), 347 [M − 43 (CH_3CO), 5], 330 [M − 60 (CH_3CO_2H), 100], 315 [M − 75 ($CH_3CO_2H + CH_3$), 55], 302 [M − 88 (CH_3CO_2H + CO), 95], 287 (45), 275 (15), 246 (18), 210 (15), 205 (15), 191 (10), 149 (10), 104 (10) (Found: M^+, 390.1215; lit.,[1] 390.1217. $C_{22}H_{18}N_2O_5$ requires *M*, 390.1216).

Preparation of 20-*O*-acetyl-14-nitrocamptothecin 7

Nitronium tetrafluoroborate **9** (0.8 g) was added to a solution of camptothecin **1** (1 g) in acetic anhydride (50 ml) in a 100 ml round-bottomed flask equipped with a magnetic stirrer, a condenser and a drying tube and immersed in an oil bath. The mixture was stirred at 80 °C for 24 h. After cooling to room temperature, the mixture was poured onto 500 ml ice–water while stirring. The yellow suspension in water was extracted with dichloromethane (250 ml × 4). The combined extracts were washed with water (250 ml × 2) and dried over anhydrous sodium sulfate for 5 h. The dried solution was filtered and the dichloromethane was removed by rotary evaporation under vacuum. The residue was chromatographically separated with methanol–chloroform as eluent. The product **6** was obtained in 35% yield. 20-*O*-Acetyl-14-nitrocamptothecin **7** was obtained as a white powder, mp 303 °C (decomp.), yield 28%; δ_H($CDCl_3$) 1.06 (3 H, t, *J* 7.33, 19-CH_3), 2.17 (3 H, s, $OCOCH_3$), 2.30 (1 H, m, 18-CH_2), 2.50 (1 H, m, 18-CH_2), 5.29 (2 H, s, 5-CH_2), 5.35 (1 H, d, *J* 14.20, 17-CH_2), 5.69 (1 H, d, *J* 14.15, 17-CH_2), 7.70 (1 H, t, *J* 7.25, 10-H), 7.84 (1 H, t, *J* 7.20, 11-H), 7.92 (1 H, d, *J* 8.10, 9-H), 8.20 (1 H, d, *J* 8.12, 12-H), 8.43 (1 H, s, 7-H); δ_C 8.5 (C19), 20.8 ($OCOCH_3$), 31.8 (C18), 53.9 (C5), 68.1, 79.9 (C17, C20), 113.5, 117.7, 123.5, 127.0, 132.0, 132.1, 134.7, 135.4, 138.0, 140.8, 141.4, 144.0, 146.7 (C2, C3, C6–C16), 170.8, 177.2 (C21, $OCOCH_3$); the lactam carbonyl C16a in ring D was not found (buried in TFA area); *m/z* (relative intensity) 435 (M^+, 30%), 389 [M − 46 (NO_2), 90], 347 [M − 88 (NO_2 + CH_2CO), 100], 329 (30), 319 (25), 303 (59), 273 (10), 246 (15), 217 (20), 205 (15), 190 (13), 167 (8), 148 (13) (Found: M^+, 435.1068. $C_{22}H_{17}N_3O_7$ requires *M*, 435.1067).

Preparation of 5-hydroxy-14-nitrocamptothecin 8

Nitronium tetrafluoroborate **9** (0.8 g) was added to a solution of camptothecin **1** (1 g) in trifluoroacetic acid (50 ml) in a 100 ml round-bottomed flask equipped with a magnetic stirrer, a condenser and a drying tube and immersed in an oil bath. The mixture was stirred at 80 °C for 24 h. After cooling to room temperature, the mixture was poured onto 500 ml ice–water while stirring. The yellow suspension in water was extracted with dichloromethane (250 ml × 4). The combined extracts were washed with water (250 ml × 2) and dried over anhydrous sodium sulfate for 5 h. The dried solution was filtered and the dichloromethane was removed by rotary evaporation under vacuum. The residue was chromatographically separated with methanol–chloroform as eluent. The 5-hydroxy-14-nitrocamptothecin **8** was obtained as a white powder, mp 285 °C (decomp.), yield 55%; δ_H([$^2H_6$]DMSO) 0.91 (3 H, m, 19-CH_3), 2.05 (2 H, m, 18-CH_2), 5.40 (2 H, m, 17-CH_2), 6.71 (1 H, s, 5-H), 6.94 (1 H, 20-OH), 7.70–7.90 (3 H, m, 10-H, 11-H and 5-OH), 8.00–8.20 (2 H, m, 9-H and 12-H), 8.74 (1 H, s, 7-H); δ_C 8.5 (C19), 32.5 (C18), 68.4, 77.5 (C17, C20), 87.4 (C5), 114.0, 117.3, 124.2, 130.1, 130.5, 132.5, 133.0, 134.0, 134.5, 135.1, 140.6, 141.2, 147.3 (C2, C3, C6–C16), the amide carbonyl C16a in ring D was not found (buried in TFA area); *m/z* (relative intensity) 409 (M^+, 100%), 393 [M − 16 (O), 40], 380 [M − 28 (CO) + 1 (H), 30], 364 [M − 46 (NO_2) + 1 (H), 15], 347 [M − 62 (NO_2 + O), 10], 330 [M − 79 ($NO_2 + CH_3$ + O), 12], 319 (13), 302 (15), 290 (10), 274 (12), 261 (15), 248 (14), 234 (20), 217 (15), 205 (20), 183 (13), 167 (5), 155 (14), 140 (8), 128 (20), 115 (3), 101 (11) (Found: M^+, 409.0908. $C_{20}H_{15}N_3O_7$ requires *M*, 409.0909).

Copies of ^1H NMR and ^{13}C NMR spectra and mass spectra of compounds **2**, **3** and **4** are available as Supplementary Data, reference no. SUP. 57168 (12 pp.) For details of the Supplementary Publications Scheme see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1996, Issue 1.

Acknowledgements

The author thanks Mr Edward Ezell at the University of Texas Medical Branch for NMR data and Baylor Medical College for mass data. Supporting funds from the Stehlin Foundation for

Cancer Research and The Friends of The Stehlin Foundation are gratefully acknowledged.

References

1 M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer and G. A. Sim, *J. Am. Chem. Soc.*, 1966, **88**, 3888.
2 J. A. Gottlieb, A. M. Guarino, J. B. Call, V. T. Oliverio and J. B. Block, *Cancer Chemother. Rep., Part 1*, 1970, **54**, 461.
3 J. A. Gottlieb and J. K. Luce, *Cancer Chemother. Rep.*, 1972, **56**, 103.
4 F. M. Muggia, P. J. Creaven, H. H. Hansen, M. H. Cohen and O. S. Selawry, *Cancer Chemother. Rep.*, 1972, **56**, 515.
5 C. G. Moertel, A. J. Schutt, R. J. Reitemeier and R. G. Hahn, *Cancer Chemother. Rep.*, 1972, **56**, 95.
6 U. Schaeppi, R. W. Fleishman and D. A. Cooney, *Cancer Chemother. Rep., Part 3*, 1974, **5**, 25.
7 Y.-H. Hsiang, R. Hertzberg, S. Hecht and L. F. Liu, *J. Biol. Chem.*, 1985, **260**, 14 873.
8 Y.-H. Hsiang and L. F. Liu, *Cancer Res.*, 1988, **49**, 1722.
9 C. Jaxel, K. W. Kohn, M. C. Wani, M. E. Wall and Y. Pommier, *Cancer Res.*, 1989, **49**, 1465.
10 Y.-H. Hsiang, L. F. Liu, M. E. Wall, M. C. Wani, A. W. Nicholas, G. Manikumar, S. Kischenbaum, R. Silber and M. Potmesil, *Cancer Res.*, 1989, **49**, 4385.
11 B. C. Giovanella, H. R. Hinz, A. J. Kozielski, J. S. Stehlin, Jr., R. Silber and M. Potmesil, *Cancer Res.*, 1991, **51**, 3052.
12 J. J. Plattner, R. D. Gless, G. K. Cooper and H. Rapport, *J. Org. Chem.*, 1974, **39**, 303.
13 S. Danishefsky and S. J. Etheredge, *J. Org. Chem.*, 1974, **39**, 3430.
14 J. A. Bristol, D. L. Comins, R. W. Davenport, M. J. Kane, R. E. Lyle, J. R. Maloney and D. E. Portlock, *J. Med. Chem.*, 1975, **18**, 535.
15 T. Sugasaawa, T. Toyoda, N. Uchida and K. Yamaguchi, *J. Med. Chem.*, 1976, **19**, 675.
16 M. C. Wani, P. E. Ronman, L. T. Lindley and M. E. Wall, *J. Med. Chem.*, 1980, **23**, 554.
17 M. C. Wani, A. W. Nicholas and M. E. Wall, *J. Med. Chem.*, 1986, **29**, 2358.
18 M. C. Wani, A. W. Nicholas, G. Manikumar and M. E. Wall, *J. Med. Chem.*, 1987, **30**, 1774.
19 A. W. Nicholas, M. C. Wani, G. Manikumar, M. E. Wall, K. W. Kohn and Y. Pommier, *J. Med. Chem.*, 1990, **33**, 972.
20 W. D. Kingsburg, J. C. Boehm, D. R. Jakas, K. G. Holden, S. M. Hecht, G. Glagher, M. J. Caranfa, F. L. McCabe, L. F. Faucette, R. K. Johnson and R. P. Hertzberg, *J. Med. Chem.*, 1991, **34**, 98.
21 A. Ejima, H. Terasawa, M. Sugimori, S. Ohsuki, K. Ohsuki, Y. Kawato, M. Yasuoka and H. Tagawa, *Chem. Pharm. Bull.*, 1992, **40**, 683.
22 T. Yaegashi, S. Sawada, H. Nagata, T. Furata, T. Yokokura and T. Miyasaka, *Chem. Pharm. Bull.*, 1994, **42**, 2518.
23 M. R. Peel and D. D. Steinback, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2753.
24 H. Wang, S. Liu, K. Hwang, A. T. McPhail and K. Lee, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 77.
25 M. J. Luzzio, J. M. Besterman, D. L. Emerson, M. G. Evans, K. Lackey and P. L. Leitner, *J. Med. Chem.*, 1995, **38**, 395.
26 L. Syder, W. Shen, W. G. Bornmann and S. I. Danishefsky, *J. Org. Chem.*, 1994, **59**, 7033.
27 F. Fang, S. Xie and M. W. Lowery, *J. Org. Chem.*, 1994, **59**, 6142.
28 D. L. Comins, H. Hao and J. Gao, *Tetrahedron Lett.*, 1994, **35**, 5331.
29 A. V. R. Rao, J. S. Yadav and M. Valluri, *Tetrahedron Lett.*, 1994, **35**, 3613.
30 P. Pantazis, N. Harris, J. Mendozo and B. Giovanella, *Eur. J. Haematol.*, 1994, **53**, 246.
31 G. A. Olah and P. Ramaiah, *J. Org. Chem.*, 1993, **58**, 4639.
32 T. Miyasaka, S. Sawada and K. Nokata, *Heterocycles*, 1981, **16**, 1713.

Paper 6/01594H
Received 6th *March* 1996
Accepted 12th *July* 1996