Synthesis of 2,5-Dioxygenated Pyrazine 4-Oxides: Total Synthesis of a New Inhibitor of Superoxide Anion Generation, OPC-15161

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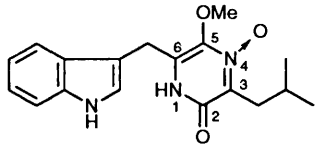
The total synthesis of OPC-15161 **1**, a new inhibitor of superoxide anion generation, is described in full. Three approaches, routes A–C, have been investigated focusing on the pivotal structure 2,5-dioxygenated pyrazine 4-oxide. Among them, route A led to the total synthesis of **1**. That is, the key precursor, 5-hydroxypyrazin-2(1*H*)-one 4-oxide **7** has been prepared from tryptophan methyl ester **2** in three steps, and direct methylation of the 5-hydroxy group of **7** or three-step methylation *via* the 2-*O*-Boc derivative **10** afforded **1** in 9.9–10.6% overall yields.

Very recently OPC-15161 **1** was isolated as a main degradation product of OPC-15160 which was obtained from the culture broth of fungus *Thielavia minor* OFR-1561.[1] The agent **1**



1 OPC-15161

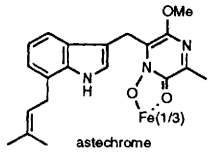
showed potent inhibitory activity (IC_{50} 2.8 × 10^{-5} mol dm^{-3}) on superoxide anion generation by guinea pig peritoneal macrophages. Since it has recently been suggested that superoxide anion released by macrophages or neutrophiles contributes to tissue damage in ischemic or inflammatory processes and that inhibitors of superoxide anion generation are effective in protecting against tissue damage in *in vitro* and *in vivo* models of ischemia and inflammation,[2] **1** has promising chemotherapeutic potential in the above-mentioned disease. However, its supply by fermentation is insufficient for further studies (up to 5 g of **1** was obtained from 1 ton of fermentation medium). Therefore, a practical preparation of **1** is an important goal. The exact structure of **1** was elucidated by X-ray analysis and shown to have a unique and highly oxygenated pyrazine ring with an indole-side chain. Its strong therapeutic potency and the urgent demand for larger supplies of it have prompted us to study the total synthesis of **1**. Furthermore, its highly oxygenated unsymmetrical pyrazine ring is quite rare, and an effective construction of such structures has been a challenging subject.†

We have set up three approaches for the synthesis of **1** focusing on the pivotal structure of 2,5-dioxygenated pyrazine 4-oxides: site-selective methylation of the 5-hydroxy group of 5-hydroxypyrazin-2(1*H*)-one 4-oxide **I** (route A), site-selective oxidation at the N-4 position of 2,5-dioxygenated pyrazines **II** or **III** (route B) and *ipso*-substitution by alkoxy anion on 2- or 5-halogenated pyrazine 4-oxides **IV** or **V** (route C) (Scheme 1). Here we report a full account of our studies on the synthesis of 2,5-dioxygenated pyrazine 4-oxides and the total synthesis of **1**.[6,7]

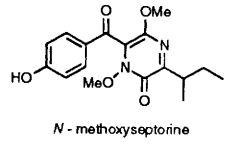
Results and Discussions

The pivotal precursor in route A, 5-hydroxy-6-(1*H*-indol-3-ylmethyl)-3-(2-methylpropyl)pyrazin-2(1*H*)-one 4-oxide **7** was prepared as follows. Condensation of L-tryptophan methyl ester (*S*)-**2**‡ and α-hydroxyimino carboxylic acid **3** with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) in dioxane gave the amide **4** in 95% yield. Direct cyclization of **4** would be expected to be the best method for the preparation of **7**, but the reaction did not occur even under forcing conditions such as refluxing acetic acid or an excess toluene-*p*-sulfonic acid or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in refluxing dioxane. When **4** was treated with refluxing formic acid or NaH in refluxing dioxane, the amide bond was cleaved without producing any cyclized product. Next, activation of the carboxylic acid **5** (obtained quantitatively by alkaline hydrolysis of **4**) and the subsequent cyclization were studied. Initial attempts employing some of the usual dehydrative condensation methods for macrolide synthesis [2,4,6-trichlorobenzoyl chloride–triethylamine–*N*,*N*-dimethylaminopyridine (DMAP), *N*,*N*′-carbonyldiimidazole, trifluoroacetic anhydride and cyanuric chloride–triethylamine] gave complex mixtures not containing any **7**. Treatment of **5** by Mukaiyama's method[8] [triphenylphosphine (2 equiv.) and 2,2′-dipyridyl disulfide (2 equiv.)] in tetrahydrofuran (THF) under carefully controlled reaction conditions (at room temperature for 1 h) gave the desired **7** in up to 12% yield. The reaction conditions, however, were crucial and trials for improving the yield of **7** by increasing the amount of the reagents used, addition of DMAP or acetic acid, higher

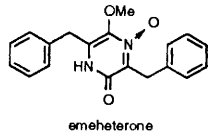
† Recently related natural products having highly oxygenated pyrazine rings, astechrome,[3] *N*-methoxyseptorine[4] and emeheterone,[5] have been isolated. Among them, only emeheterone has been synthesized.[5b]



astechrome

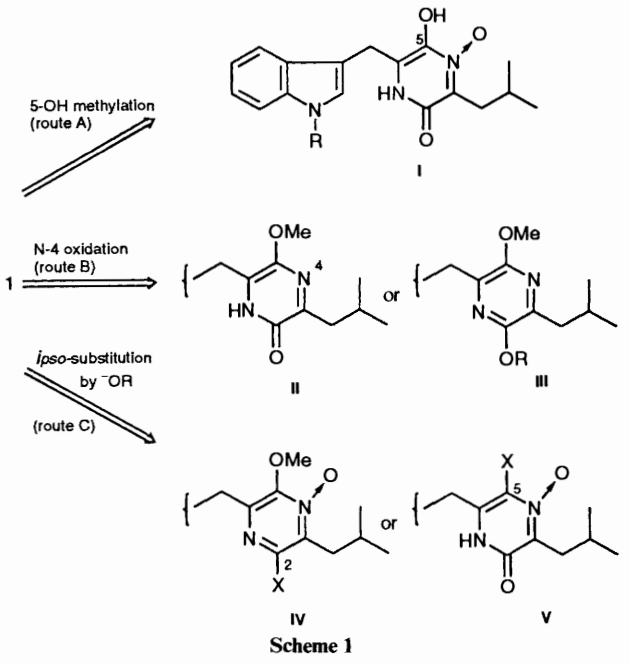


N-methoxyseptorine



emeheterone

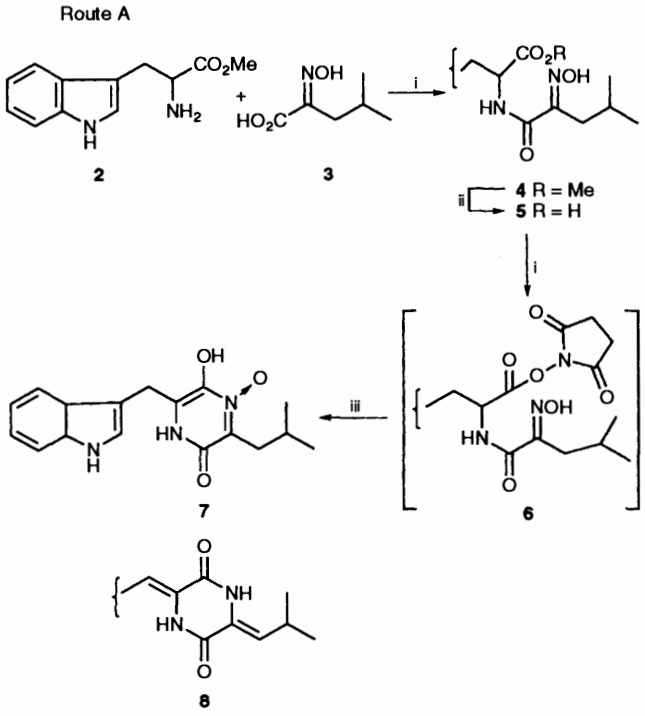
‡ Because of the inexpensive availability of L-tryptophan methyl ester (*S*)-**2**, optically active compound was used in this route. Racemic **2** gave almost the same yields of the products.



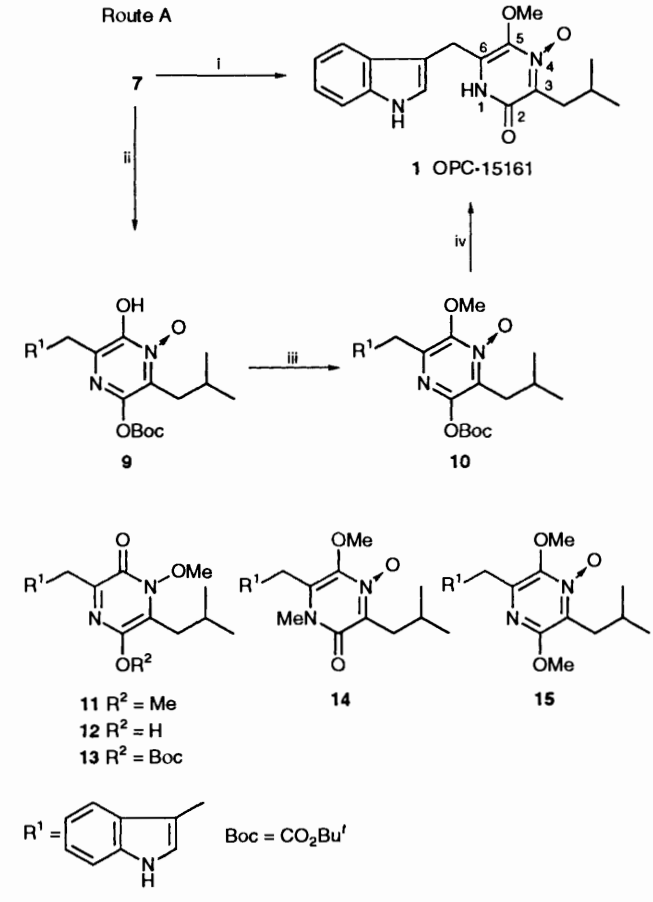
Scheme 1

temperature and longer reaction time resulted in complex mixtures. Under these conditions, **7** was very unstable due to further reaction of the reagents at the N-4 oxide followed by base-induced elimination to provide **8** as a major by-product. The use of a powerful condensation reagent (trimethylsilyl)-ethoxyacetylene[9] in acetonitrile at 45 °C for 5 h, provided **7** in 15–20% yield accompanied by 10–20% yield of unchanged **5**. Finally, treatment of **5** with DCC and HOSu in dioxane at room temperature yielded the *N*-carboxyimide **6**, which was cyclized with NaOAc to give a 52% yield of **7** (Scheme 2).

The next site-selective methylation at the 5-hydroxy group of **7** was difficult, since **7** has four reactive centres on the pyrazine ring: N-1, 2-carbonyl, 4-oxide and 5-hydroxy.* We studied methylation of **7** and also its 2-*O*-Boc derivative **9**. Preliminary methylation of **7** by the use of excess iodomethane–K_2CO_3 in *N*,*N*-dimethylformamide (DMF) gave a mixture (3–4:1) of dimethylated products **11** and **14** without formation of **1**. Treatment of **7** with an excess of diazomethane, both with and without BF_3·OEt_2 as catalyst, in dichloromethane–methanol (3:1) gave a mixture (*ca.* 1:1:1) of **11**, **14** and **15**; formation of **1** was not observed. Mono-methylation by decreasing the amount of the reagents used was unsuccessful owing to slow and/or non-selective reaction, while treatment of the DBU salt of **7** (prepared by treatment of **7** with 1 equiv. DBU in DMF at 0 °C in 90% yield) with methyl trifluoromethanesulfonate (MeOTf) (3 equiv.) in 1,2-dichloroethane at room temperature provided the desired **1** and its regioisomer **12** in a ratio of 1:5 in about 50% yield.† After intensive study employing various methylating reagents such as methyl sulfonate derivatives, methyloxonium salts and methylsulfonium salts, treatment of the DBU salt of **7** with $Me_3O^+BF_4^-$ in dichloromethane provided **1** in 22% yield accompanied by **12** in 45% yield (Scheme 3).



Scheme 2 *Reagents:* i, DCC, HOSu; ii, NaOH; iii, AcONa



Scheme 3 *Reagents:* i, DBU then $Me_3O^+BF_4^-$; ii, $(Boc)_2O$, Et_3N, DMAP; iii, CH_2N_2, BF_3·OEt_2; iv, CF_3CO_2H

Alternatively **7** was selectively converted into the 2-*O*-*tert*-butoxycarbonyl (Boc) derivative **9** by treatment with $(Boc)_2O$, triethylamine and DMAP in DMF at −5 °C in 63% yield.

* Although the indole ring is also reactive, we studied methylation without protection of its NH group aiming at a simple and practical synthesis. Another comparative approach *via* the indole *N*-Boc derivative of **7** has also been carried out. However, no remarkable advantages of the protection have been observed so far.

† 4-Methoxy derivatives **11**, **12** and **13** are unstable at room temperature even after purification, gradually decomposing to give complex mixtures. Therefore, some of these compounds decomposed during the reaction and purification.

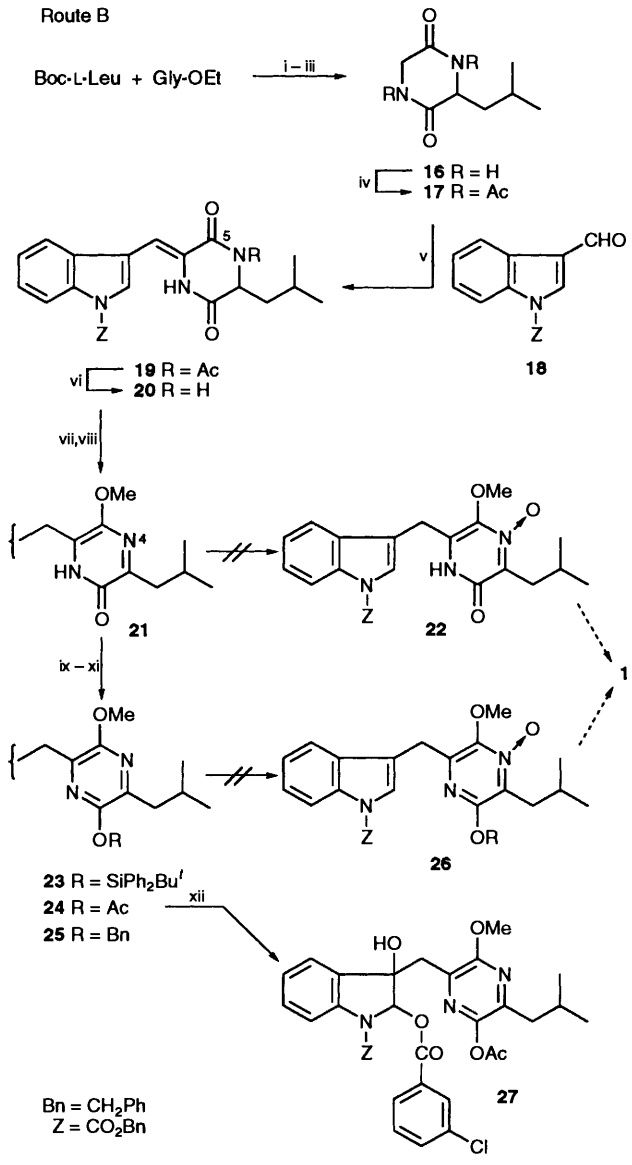
Treatment of **9** by the method effective for **7** ($Me_3O^+BF_4^-$ and DBU in dichloromethane at 0 °C for 2 h) resulted in formation of the desired compound **10** and its regioisomer **13** in a ratio of 1:9 in about 60% yield; also with an excess of iodomethane–Ag_2CO_3 in acetonitrile and sonication, **13** was again obtained preferentially. On the other hand, methylation of **9** with an excess of diazomethane in the presence of a catalytic amount of BF_3·OEt_2 in dichloromethane–methanol (3:1) resulted in an improved ratio (1:1.6) of **10** and **13** in a quantitative yield. Deprotection of **10** (separated by preparative TLC on silica gel) with trifluoroacetic acid led to **1** in 34% yield from **9**. The thus obtained **1** was in all respects identical with the natural OPC-15161.

Alternatively, we have investigated the synthesis of the 2,5-dioxygenated pyrazine 4-oxide by two other routes, B and C, and report here the results concisely, although these routes did not lead to the total synthesis of **1**.

Route B was examined on compounds **21** and **23–25**, prepared as follows. The 2,5-dioxopiperazine **16**, readily obtained from Boc-L-leucine and glycine ethyl ester in 92% overall yield, was acetylated to give **17** (96% yield), which was condensed with the *N*-protected 3-formylindole **18** and then hydrolysed to give the dioxopiperazine **20** (60% yield). Methylation of its 5-carbonyl group by MeOTf and the subsequent alkene migration provided the key compound **21** (63% yield). The silyl **23**, acetyl **24** and benzyl derivatives **25** were prepared by standard methods in 87–100% yields. Oxidation of **21** and **23–25** at their N-4 positions was studied under various oxidation conditions [*m*-chloroperbenzoic acid (MCPBA)–phosphate buffer (pH 7)–dichloromethane, room temperature; peracetic acid–acetic acid or 1,2-dichloroethane, room temperature; 30% H_2O_2–methanol, room temperature and *tert*-butyl hydroperoxide–$VO(acac)_2$–*tert*-butyl alcohol, reflux]. However, each reaction resulted in the formation of a complex mixture or deprotection giving **21**, and the desired fully functionalized pyrazines **22** and **26** were not obtained at all. The only identified product was the 2-(3-chlorobenzoyloxy)-3-hydroxy-2,3-dihydroindole **27** (33% yield) obtained from the reaction of **24** and MCPBA. In this case, oxidation occurred at the indole ring and the pyrazine ring was left intact. These results have shown the difficulty of oxidation of 2,5-dioxygenated pyrazines bearing indole rings susceptible to oxidation (Scheme 4).

Route C was tried on two types of halogenated pyrazine 4-oxides, the 2-halogenated one **35** (route C-i) and the 5-halogenated one **48** (route C-ii). Condensation of tryptophan methyl ester **2** and the acid chloride **28** gave the amide **29** (98% yield), which was cyclized with hydroxylamine to give the 3,6-*trans*-4-hydroxy-2,5-dioxopiperazine **30a** (12% yield) and its *cis*-isomer **30b** (28% yield). Protection of the 4-hydroxy group and the indole-NH group of **30b** followed by treatment with PCl_5 and $POCl_3$ gave the 2-chloropyrazin-5(4*H*)-one **33** (12% yield). Hydrogenolysis of **33** provided the 2-chloro-5-hydroxypyrazine 4-oxide **34** quantitatively, which was treated with diazomethane to give the key compound **35** (40% yield) accompanied by its regioisomer **38** (49% yield). We envisioned that *ipso*-substitution of **35** would occur by treatment with 3 equiv. of NaOBn in THF at room temperature for 1 h, however, the reaction occurred not at the C-2 position but at the C-5 position giving 5-benzyloxy derivative **36** in 12% yield along with several unidentified products. In spite of intensive studies on changing reaction temperature, solvents and additives such as 18-crown-6, no method was found which could provide the desired 2-benzyloxy derivative **37** (Scheme 5).

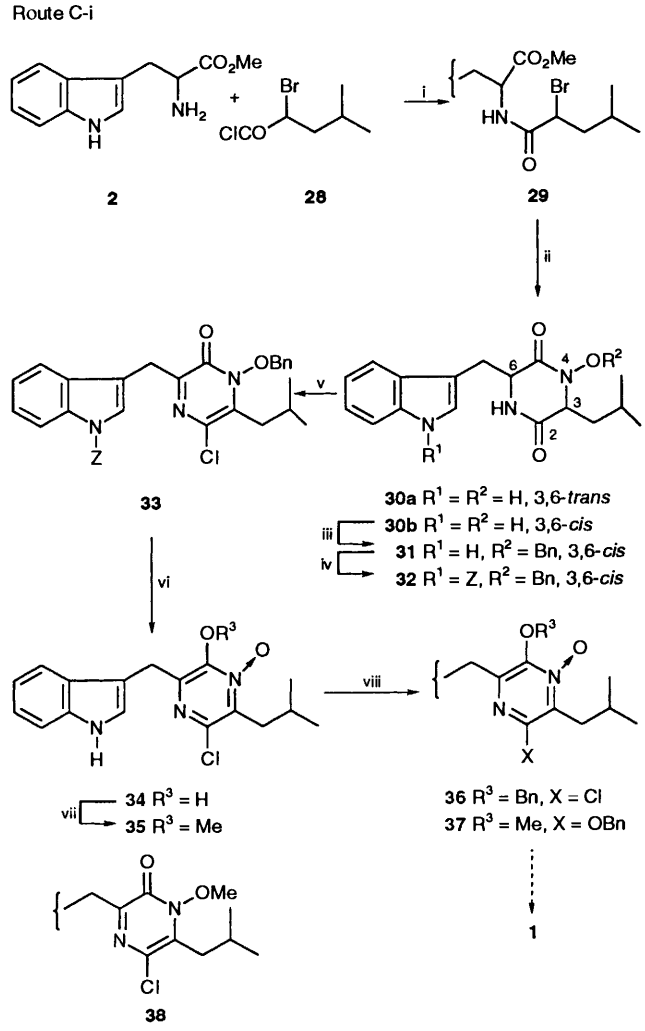
In route C-ii, we have devised a route for the preparation of 5-halogenated pyrazin-2(1*H*)-one 4-oxide **48** from the 5-amino precursor **45**. Gramine **39** was converted into the α-amino nitrile **43** in three steps in 72% overall yield, which was condensed with the α-hydroxyimino carboxylic acid **3** followed



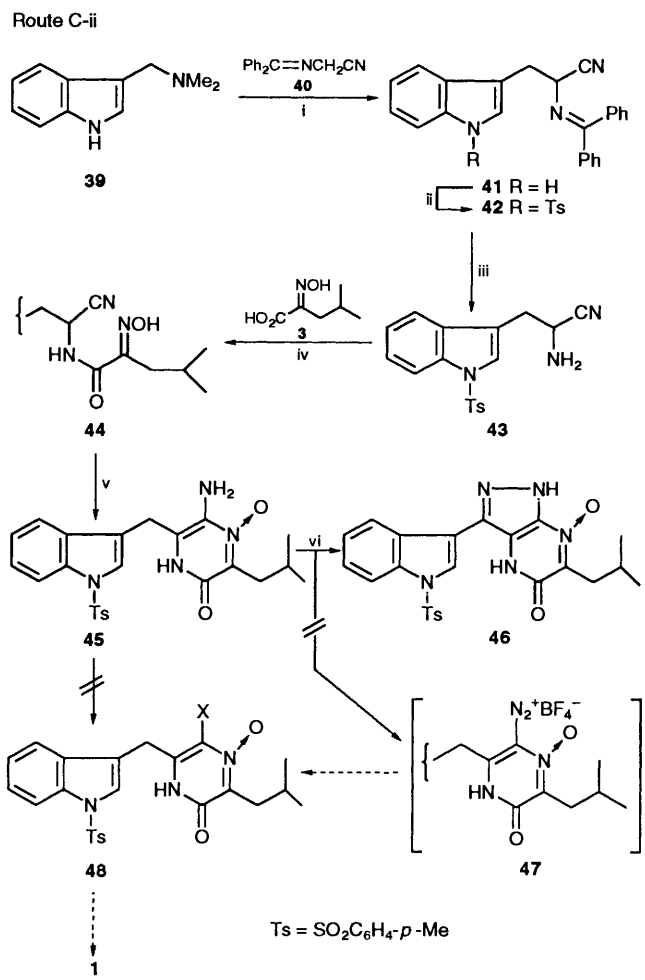
Scheme 4 *Reagents:* i, $(EtO)_2POCN$, Et_3N; ii, HCO_2H; iii, DBU; iv, Ac_2O; v, BuLi, **18** then DBU; vi, 1 mol dm^{-3} HCl; vii, MeOTf; viii, DBU; ix, Ph_2Bu^tSiCl, imidazole; x, Ac_2O, Et_3N, DMAP; xi, BnBr, K_2CO_3; xii, MCPBA, buffer (pH 7)

by cyclization in AcOH to afford **45** (49% yield). Transformation of the amino group of **45** into the halogeno group was studied by employing isopentyl nitrite in various solvent [THF, DMF, acetonitrile, dimethyl sulfoxide (DMSO) and ethanol] with halogen sources ($CuCl_2$, KI and I_2) or in halogenated solvents (carbon tetrachloride and diiodomethane) in the range room temperature to 85 °C. However, we could not obtain the desired 5-halogenated compound **48**. The main product was the 1*H*-pyrazolo[3,4-*b*]pyrazine 7-oxide **46** isolated in up to 35% yield. Another attempt to isolate the diazonium tetrafluoroborate **47** by using isopentyl nitrite and 48% HBF_4 was also unsuccessful. Even at low temperature, **46** was formed in 53% yield (Scheme 6).

In conclusion, preparation of the 2,5-dioxygenated pyrazine 4-oxide having an indole-side chain was very laborious, partly owing to the presence of the reactive indole ring. We, however, have accomplished the total synthesis of **1** by route A in four steps in 9.9% overall yield or in six steps in 10.6% overall yield. Our method features the use of commercially available amino acid **2** and 4-methyl-2-oxovaleric acid (from which **3** was



Scheme 5 *Reagents:* i, Na_2CO_3; ii, H_2NOH·HCl, NaOH; iii, BnBr, Bu^{t}OK; iv, ZCl, NaOH, Bu_4NHSO_4; v, PCl_5, $POCl_3$; vi, 10% Pd–C, H_2; vii, CH_2N_2; viii, NaOBn



Scheme 6 *Reagents:* i, Me_2SO_4, NaOH; ii, TsCl, NaOH, Bu_4NHSO_4; iii, 1 mol dm^{-3} HCl; iv, DCC, HOSu; v, AcOH; vi, isopentyl nitrite, 42% HBF_4

prepared quantitatively) as starting materials, short steps and practical processes, and would also supply an efficient route to related natural products having highly oxygenated pyrazine rings such as astechrome,[3] *N*-methoxyseptorine[4] and emeheterone.[5]

Experimental

All boiling and melting points are uncorrected. IR spectra were determined on a JASCO HPIR-102 or a JASCO IR-810 spectrometer. ^{1}H NMR spectra were recorded with tetramethylsilane as an internal standard on a JEOL JNM FX-90Q, a Bruker AC-200, a Bruker AC-250 and a JEOL JNM-GX500 spectrometer. *J* Values are given in Hz. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Electron ionization (EI) mass spectra were recorded at 70 eV using a Shimadzu GCMS-QP1000 spectrometer. Fast atom bombardment (FAB) mass spectra were recorded using a JEOL JMS-SX102 spectrometer. E. Merck silica gel 60 (0.063–0.200 nm, 70–230 mesh ASTM) and E. Merck pre-coated TLC plates, silica gel 60 F_{254} were used for column chromatography and for preparative TLC, respectively. The known compounds **3**,[10] **18**[11] and **40**[12] were prepared according to the reported methods and other materials are commercially available.

(S)-N-(2-*Hydroxyimino*-4-*methylvaleryl*)*tryptophan Methyl Ester* **4**.—To a mixture of the ester (*S*)-**2** (6.5 g, 30 mmol), acid **3** (4.4 g, 60 mmol) and HOSu (3.6 g, 32 mmol) in dry dioxane (220 cm^3) was added DCC (6.2 g, 30 mmol). The reaction mixture was stirred at room temperature for 1 d and then filtered and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt, 4:1) to give the *title compound* **4** (9.8 g, 95%) as colourless crystals; m.p. 123–124 °C (from hexane–Et_2O); $[\alpha]_D^{27}$ +26.0 (*c* 0.90, $CHCl_3$); $\nu_{max}(CHCl_3)$/cm^{-1} 3575, 3490, 3410, 1740, 1670, 1630 and 1515; δ_H(250 MHz; $CDCl_3$) 0.89 (6 H, d, *J* 6.5), 1.95–2.07 (1 H, m), 2.51 (2 H, d, *J* 7.5), 3.27 (2 H, d, *J* 6), 3.65 (3 H, s), 4.95 (1 H, dt, *J* 8 and 6), 6.91 (1 H, d, *J* 2.5), 7.09 (1 H, td, *J* 8 and 1), 7.17 (1 H, td, *J* 8 and 1), 7.36 (1 H, d, *J* 8), 7.50 (1 H, d, *J* 8) and 8.13 (1 H, br s); *m/z* (EI) 345 (M^+) (Found: C, 62.45; H, 6.6; N, 12.1. $C_{18}H_{23}N_3O_4$ requires C, 62.6; H, 6.7; N, 12.15%).

Racemic **4** was similarly prepared from racemic **2**; colourless prisms; m.p. 100–102 °C (from hexane–Et_2O) (Found: C, 62.55; H, 6.5; N, 12.2. $C_{18}H_{23}N_3O_4$ requires C, 62.6; H, 6.7; N, 12.15%).

(S)-N-(2-*Hydroxyimino*-4-*methylvaleryl*)*tryptophan* **5**.—A mixture of a solution of the ester (*S*)-**4** (0.68 g, 2.0 mmol) in EtOH (20 cm^3) and aqueous NaOH (1 mol dm^{-3}; 6.0 cm^3, 6.0 mmol) was stirred at room temperature for 30 min. HCl (1 mol dm^{-3}; 7.0 cm^3) was added to the stirred reaction mixture, cooled in ice and the whole was extracted with AcOEt twice. The combined extracts were washed with brine, dried ($MgSO_4$) and then concentrated under reduced pressure to give the *title compound* **5** (0.67 g, quant.) as colourless needles. Recrystallization from CH_2Cl_2 gave analytically pure **5** as the mono

hydrate; m.p. 97–98 °C; $[\alpha]_D^{27}$ −1.8 (*c* 1.00, MeOH); ν_{max}(KBr)/cm^{-1} 3500–2600, 3425, 3390, 1735, 1660, 1635 and 1520; δ_H(250 MHz; $CDCl_3$–2% [$^2H_6$]DMSO) 0.91 (6 H, d, *J* 7), 1.95–2.15 (1 H, m), 2.51 (2 H, d, *J* 7), 3.63 (2 H, d, *J* 5), 4.90 (1 H, dt, *J* 8 and 5), 6.97 (1 H, br s) 7.02 (1 H, td, *J* 8 and 1), 7.10 (1 H, td, *J* 8 and 1), 7.28 (1 H, d, *J* 8), 7.37 (1 H, d, *J* 8), 7.56 (1 H, d, *J* 8) and 9.19 (1 H, s); *m/z* (EI) 331 (M^+) (Found: C, 58.35; H, 6.6; N, 12.0. $C_{17}H_{21}N_3O_4$·H_2O requires C, 58.45; H, 6.65; N, 12.05%).

Racemic **5** was similarly prepared from racemic **4**; colourless needles; m.p. 202–204 °C (from AcOEt) (Found: C, 61.25; H, 6.1; N, 12.65. $C_{17}H_{21}N_3O_4$ requires C, 61.6; H, 6.4; N, 12.7%).

Cyclization of Tryptophan **5**.—(*a*) *By the use of* Ph_3P *and* 2,2′-*dipyridyl disulfide*. A mixture of the tryptophan **5** (0.66 g, 2.0 mmol), Ph_3P (1.1 g, 4.0 mmol) and 2,2′-dipyridyl disulfide (0.88 g, 4.0 mmol) in dry THF (20 cm^3) was stirred at room temperature for 1 h. Water (0.20 cm^3) was added to the reaction mixture which was then concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 20:1 ⟶ 4:1) to give 5-*hydroxy*-6-(1*H*-*indol*-3-*ylmethyl*)-3-(2-*methylpropyl*)*pyrazin*-2(1*H*)-*one* 4-*oxide* **7** (75 mg, 12%) as pale brown crystals; m.p. 166–168 °C (decomp.); ν_{max}(KBr)/cm^{-1} 3500–2750 and 1605; δ_H(250 MHz; [$^2H_6$]-DMSO) 0.85 (6 H, d, *J* 7), 1.98–1.28 (1 H, m), 2.60 (2 H, d, *J* 7), 3.99 (2 H, s), 6.93 (1 H, t, *J* 8), 7.04 (1 H, t, *J* 8), 7.16 (1 H, d, *J* 2), 7.31 (1 H, d, *J* 8), 7.57 (1 H, d, *J* 8) and 10.84 (1 H, s); *m/z* (FAB) 314 (MH^+) (High resolution FAB MS found: M, 314.1533, $C_{17}H_{20}N_3O_3$ requires MH^+, 314.1505).

A similar reaction using Ph_3P (4 equiv.) and 2,2′-dipyridyl disulfide (4 equiv.) in CH_2Cl_2 at room temperature for 2 d gave 6-(1*H*-*indol*-3-*ylmethylene*)-3-(2-*methylpropylidene*)*piperazine*-2,5-*dione* **8** (22%) as pale yellow crystals; m.p. > 300 °C (from DMF); ν_{max}(KBr)/cm^{-1} 3390, 3180, 1685, 1670, 1630 and 1610; δ_H(250 MHz; [$^2H_6$]DMSO) 0.99 (6 H, d, *J* 7), 2.88–3.08 (1 H, m), 5.69 (1 H, d, *J* 10.5), 7.07 (1 H, s), 7.12 (1 H, t, *J* 7.5), 7.19 (1 H, t, *J* 7.5), 7.43 (1 H, d, *J* 7.5), 7.66 (1 H, d, *J* 7.5), 8.05 (1 H, d, *J* 2.5), 9.55 (1 H, s), 10.91 (1 H, s) and 11.71 (1 H, s); *m/z* (EI) 295 (M^+) (Found: C, 69.1; H, 5.7; N, 14.05. $C_{17}H_{17}N_3O_2$ requires C, 69.15; H, 5.8; N, 14.25%).

(*b*) *By the use of* (*trimethylsilyl*)*ethoxyacetylene*. Under a nitrogen atmosphere, a mixture of the tryptophan **5** (2.0 g, 6.0 mmol) and (trimethylsilyl)ethoxyacetylene[9] (5.0 cm^3, 29 mmol) in dry MeCN (200 cm^3) was stirred at 45 °C for 5 h. The reaction mixture was concentrated under reduced pressure (finally by high vacuum pump at 40 °C/0.5 mmHg for 1 h). The residue was purified by column chromatography (CH_2Cl_2–MeOH, 20:1 ⟶ 4:1) to give the pyrazinone **7** (0.32 g, 17%), which was identical with the compound obtained above. After further elution unchanged tryptophan **5** (0.2–0.3 g, 10–15%) was recovered.

(*c*) *By the use of DCC and HOSu*. A solution of DCC (0.75 g, 3.6 mmol) in dioxane (5 cm^3) was added to a solution of the tryptophan **5** (1.0 g, 3.0 mmol) and HOSu (0.38 g, 3.3 mmol) in dioxane (15 cm^3). The mixture was stirred at room temperature for 1 h and then filtered. The filtrate was used for the following step. Concentration of the filtrate under reduced pressure gave the N-*carbonyloxysuccinimide* **6** as a colourless solid; ν_{max}(CHCl$_3$)/cm^{-1} 3560, 3490, 3400, 1815, 1785, 1745, 1675 and 1510; δ_H(250 MHz; $CDCl_3$) 0.88 (6 H, d, *J* 6.5), 1.91–2.09 (1 H, m), 2.48 (2 H, d, *J* 7.5), 2.74 (4 H, s), 3.42 (1 H, dd, *J* 15 and 5.5), 3.51 (1 H, dd, *J* 15 and 5.5), 5.33 (1 H, dt, *J* 8 and 5.5), 7.08–7.33 (4 H, m), 7.56 (1 H, d, *J* 7.5) and 8.37 (1 H, br s).

NaOAc (0.25 g, 3.0 mmol) was added to the above filtrate which was then stirred at room temperature for 2 h. After this it was diluted with water (20 cm^3) and stirring was continued for 30 min. The precipitate formed was filtered off and washed with water. A suspension of the precipitate in MeOH (10 cm^3) was warmed to 50–60 °C for 30 min. After cooling, the precipitate was filtered off to give the pyrazinone **7** (0.49 g, 52%), which was identical with the compound obtained above.

Synthesis of OPC-15161 **1** *by Treatment of the Pyrazinone* **7** *with* $Me_3O^+BF_4^-$.—DBU (1.7 cm^3, 11 mmol) was added to a solution of the pyrazinone **7** (3.5 g, 11 mmol) in DMF (15 cm^3) at 0 °C after which the precipitate formed was filtered off and washed with CH_2Cl_2 to give the DBU salt of **7** (4.7 g, 90%). This salt (4.7 g, 10 mmol) was suspended in CH_2Cl_2 (90 cm^3) and the suspension cooled to −10 °C when $Me_3O^+BF_4^-$ (3.0 g, 20 mmol) was added to it. After being stirred at −10 °C for 30 min, the reaction mixture was quenched with saturated aq. $NaHCO_3$. The organic layer was separated and washed with water, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2–AcOEt, 8:1) to give *OPC-15161* **1** (0.87 g). Recrystallization of the product from EtOH gave pure **1** (0.72 g, 22%) as pale yellow crystals, identical (TLC, IR, ^{1}H NMR and mass spectroscopy) with natural **1**. Further elution of the above column with CH_2Cl_2–AcOEt (3:1) gave 2-*hydroxy*-6-(1H-*indol*-3-*ylmethyl*)-4-*methoxy*-3-(2-*methylpropyl*)*pyrazin*-5(4H)-*one* **12** (1.5 g, 45%) as a pale brown solid.

1, M.p. 225–227 °C (lit.,[1] m.p. 223.5–225.5 °C); ν_{max}(KBr)/cm^{-1} 3250 and 1625; δ_H(250 MHz; [$^2H_6$]DMSO) 0.84 (6 H, d, *J* 6.5), 2.00–2.20 (1 H, m), 2.59 (2 H, d, *J* 7), 3.76 (3 H, s), 3.91 (2 H, s), 6.97 (1 H, td, *J* 7.5 and 1.5), 7.06 (1 H, td, *J* 7.5 and 1.5), 7.24 (1 H, d, *J* 2), 7.33 (1 H, d, *J* 7.5), 7.57 (1 H, d, *J* 7.5), 10.95 (1 H, br s) and 11.96 (1 H, br s); *m/z* (EI) 327 (M^+).

12, M.p. 159 °C (decomp.) (from AcOEt); ν_{max}(KBr)/cm^{-1} 3290, 1650 and 1590; δ_H(250 MHz; [$^2H_6$]DMSO) 0.86 (6 H, d, *J* 6.5), 1.84–2.04 (1 H, m), 2.48 (2 H, d, *J* 7), 3.97 (3 H, s), 4.06 (2 H, s), 6.93 (1 H, t, *J* 7.5), 7.00 (1 H, t, *J* 7.5), 7.15 (1 H, d, *J* 2.5), 7.31 (1 H, d, *J* 7.5), 7.57 (1 H, d, *J* 7.5), 9.80 (1 H, s,) and 10.82 (1 H, s); *m/z* (EI) 327 (M^+) (Found: C, 65.95; H, 6.55; N, 12.8. $C_{18}H_{21}N_3O_3$ requires C, 66.05; H, 6.45; N, 12.85%).

2-(tert-*Butoxycarbonyloxy*)-5-*hydroxy*-6-(1H-*indol*-3-*ylmethyl*)-3-(2-*methylpropyl*)*pyrazine* 4-*Oxide* **9**.—Under a nitrogen atmosphere, $(Boc)_2O$ (51 mg, 0.23 mmol), Et_3N (0.029 cm^3, 0.21 mmol) and DMAP (3 mg, 0.02 mmol) were added to a solution of the pyrazinone **7** (67 mg, 0.21 mmol) in dry DMF (2 cm^3) at −5 °C and the mixture was stirred at −5 °C for 30 min. After this saturated aq. NH_4Cl (5 cm^3) was added to the reaction mixture which was then vigorously stirred at room temperature for 10 min and then thrice extracted with CH_2Cl_2. The combined extracts were washed with brine, dried ($MgSO_4$) and then concentrated under reduced pressure (finally by high vacuum pump at 30 °C/0.5 mmHg for 1 h). The residue was purified by column chromatography (CH_2Cl_2–MeOH, 30:1 ⟶ 15:1) to give the *title compound* **9** (54 mg, 63%) as a pale brown solid; m.p. 230–240 °C (decomp.) (from Pr^i_2O); ν_{max}(CHCl$_3$)/cm^{-1} 3400, 1750 and 1600; δ_H(250 MHz; $CDCl_3$) 0.75 (6 H, br s), 1.61 (9 H, s), 1.90–2.05 (1 H, m), 2.58 (2 H, br s), 4.02 (2 H, br s), 6.95–7.06 (4 H, m) and 7.59 (1 H, br s); *m/z* (EI) 413 (M^+) (Found: C, 63.4; H, 6.6; N, 10.05. $C_{22}H_{27}N_3O_5$ requires C, 63.9; H, 6.6; N, 10.15%. High resolution FABMS found: M, 414.1995. $C_{22}H_{28}N_3O_5$ requires MH^+, 414.2029). All the ^{1}H NMR signals appeared broad by both 90 MHz and 500 MHz instruments and the chemical shifts varied with the concentration. Therefore, typical data are shown at a concentration of 0.01 mol dm^{-3}.

Treatment of Pyrazine Oxide **9** *with Diazomethane*.—A solution of CH_2N_2 in Et_2O (*ca.* 0.3 mol dm^{-3}; 3 cm^3, 1 mmol; prepared from *N*-methyl-*N*-nitrosotoluene-*p*-sulfonamide by the standard method) was added to a solution of the pyrazine oxide (50 mg, 0.12 mmol) in CH_2Cl_2–MeOH (3:1) (3 cm^3) at 0 °C, to which was added BF_3·OEt_2 (*ca.* 0.001 cm^3, *ca.* 0.008

mmol). The vessel was sealed and the reaction mixture was stirred at room temperature overnight. After this, triethylamine (2 drops) was added to the mixture which was then concentrated under reduced pressure to leave a yellow solid (52 mg). A 250 MHz ^{1}H NMR study of the crude product showed a 1:1.6 mixture of 2-(tert-*butoxycarbonyloxy*)-6-(1H-*indol*-3-*ylmethyl*)-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* 4-*oxide* **10** and its regioisomer, 2-(tert-*butoxycarbonyloxy*)-6-(1H-*indol*-3-*ylmethyl*)-4-*methoxy*-3-(2-*methylpropyl*)*pyrazin*-5(4H)-*one* **13** with ⩾95% purity. Purification by preparative TLC (hexane–AcOEt, 2:1) gave **10** (19 mg, 37%) as a pale yellow gum and **13** (31 mg, 60%) as a pale brown gum.

10, $\nu_{max}(CHCl_3)/cm^{-1}$ 3490, 1770 and 1595; δ_H(500 MHz; $CDCl_3$) 0.94 (6 H, d, *J* 6.5), 1.55 (9 H, s), 2.15–2.23 (1 H, m), 2.71 (2 H, d, *J* 6.5), 3.89 (3 H, s), 4.23 (2 H, s), 7.10 (1 H, s), 7.11 (1 H, t, *J* 8), 7.18 (1 H, t, *J* 8), 7.33 (1 H, d, *J* 8), 7.73 (1 H, d, *J* 8) and 8.03 (1 H, br s); *m/z* (FAB) 427 (M^+), 428 (MH^+) (Found: C, 64.5: H, 7.05; N, 9.5. $C_{23}H_{29}N_3O_5$ requires C, 64.6; H, 6.85; N, 9.85%).

13, $\nu_{max}(CHCl_3)/cm^{-1}$ 3490, 1765, 1660 and 1590; δ_H(90 MHz; $CDCl_3$) 0.94 (6 H, d, *J* 6.5), 1.53 (9 H, s), 1.75–2.25 (1 H, m), 2.47 (2 H, d, *J* 7), 4.05 (3 H, s), 4.27 (2 H, s), 7.05–7.35 (4 H, m), 7.75–7.85 (1 H, m) and 8.15–8.2 (1 H, m); *m/z* (FAB) 428 (MH^+) (High resolution FABMS found: M, 428.2166. $C_{23}H_{30}N_3O_5$ requires MH^+, 428.2186).

Synthesis of OPC-15161 **1** *from Pyrazine Oxide* **10**.—To a solution of the pyrazine oxide **10** (11 mg, 0.026 mmol) in CH_2Cl_2 (1 cm^3) was added trifluoroacetic acid (0.1 cm^3) at 0 °C. The mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure. Purification of the residue by preparative TLC (CH_2Cl_2–MeOH, 20:1) gave **1** (7.7 mg, 91%) as a pale yellow solid, which was identical with the compound obtained from **7**.

(3S)-3-(2-*Methylpropyl*)*piperazine*-2,5-*dione* **16**.—To an ice-cooled solution of Boc-L-leucine (16 g, 70 mmol) in dry CH_2Cl_2 (150 cm^3) was added a solution of $(EtO)_2POCN$ (12 g, 74 mmol) in dry CH_2Cl_2 (25 cm^3) over 30 min and the reaction mixture was stirred at the same temperature for 30 min. After this a solution of glycine ethyl ester (7.3 g, 70 mmol), prepared from glycine ethyl ester hydrochloride (9.8 g) and Et_3N (10 cm^3), in dry CH_2Cl_2 (50 cm^3) was added to it. After the mixture had been stirred for 1 h Et_3N (30 cm^3) was added to it and the whole was stirred at room temperature for 2.5 h. The reaction mixture was then successively washed with saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residual pale yellow oil (22 g) was dissolved in 99% HCO_2H (120 cm^3), stirred at room temperature for 21 h and then concentrated under reduced pressure below 30 °C. The residue was dissolved in water (500 cm^3), made alkaline by the addition of $NaHCO_3$ and then extracted with CH_2Cl_2 (300 cm^3 × 3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure and the residual pale yellow oil (14 g) was dissolved in toluene (300 cm^3). DBU (0.2 cm^3) was added to the solution which was refluxed for 4 h and then concentrated under reduced pressure. The residue was purified by recrystallization from EtOH–water to give the *title compound* **16** (11 g, 92%) as colourless needles; m.p. 251–253 °C, $[\alpha]_D^{27}$ +27.3 (*c* 1.00, MeOH) {lit.,[13] m.p. 243 °C; lit.,[14] m.p. 238 °C, $[\alpha]_D^{22}$ +14 (*c* 0.01, MeOH); lit.,[15] m.p. 243–245 °C, $[\alpha]_D^{23}$ +21 (*c* 1, H_2O)}; ν_{max}(KBr)/cm^{-1} 3200, 3055 and 1680; δ_H(250 MHz; [$^2H_6$]DMSO) 0.65 (3 H, d, *J* 6.5), 0.68 (3 H, d, *J* 6.5), 1.51 (2 H, t, *J* 7), 1.63–1.87 (1 H, m), 3.59 (1 H, dd, *J* 17 and 3), 3.64 (1 H, t, *J* 7), 3.82 (1 H, d, *J* 17), 7.97 (1 H, br s) and 8.23 (1 H, br s) (Found: C, 56.1; H, 8.05; N, 16.3. $C_8H_{14}N_2O_2$ requires C, 56.45; H, 8.3; N, 16.45%).

(3S)-1,4-*Diacetyl*-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **17**.—The piperazine **16** (3.0 g, 17.6 mmol) was refluxed in Ac_2O (45 cm^3) for 1.5 h and then concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 100:1) to give the *title compound* **17** (4.3 g, 96%) as white crystals; m.p. 50–52 °C; $[\alpha]_D^{27}$ +66.6 (*c* 1.65, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1725 and 1715; δ_H(250 MHz; $CDCl_3$) 0.99 (3 H, d, *J* 6), 1.05 (3 H, d, *J* 6), 1.52–1.80 (3 H, m), 2.57 (3 H, s), 2.59 (3 H, s), 4.06 (1 H, d, *J* 19), 5.14 (1 H, d, *J* 19) and 5.30 (1 H, t, *J* 7.5); *m/z* (FAB) 255 (MH^+) (Found: C, 56.7; H, 6.85; N, 10.9. $C_{12}H_{18}N_2O_4$ requires C, 56.7; H, 7.15; N, 11.0%).

(3S,Z)-4-*Acetyl*-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethylene*]-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **19**.—Under a nitrogen atmosphere, BuLi (1.6 mol dm^{-3} hexane solution; 12.7 cm^3, 20 mmol) was added to a solution of the piperazinedione **17** (4.5 g, 18 mmol) in dry THF (100 cm^3) at −78 °C and the reaction mixture was stirred for 30 min. A solution of the indole **18** (5.0 g, 18 mmol) in dry THF (30 cm^3) was added to the mixture which was then stirred at −60 °C for 30 min. The mixture was then diluted with water (100 cm^3) and extracted with CH_2Cl_2 (3 × 100 cm^3). The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residual yellow oil (9.0 g) was dissolved in benzene (100 cm^3) and DBU (2 cm^3, 13 mmol) was added to the solution which was then stirred at room temperature for 10 min; it was then concentrated under reduced pressure. The crude product was purified by column chromatography (hexane–AcOEt, 3:1) to give the *title compound* **19** (5.5 g, 66%). Recrystallization from Et_2O gave analytically pure **19** as colourless needles; m.p. 171–173 °C; $[\alpha]_D^{29}$ −102 (*c* 1.14, MeOH); ν_{max}(KBr)/cm^{-1} 1735, 1700, 1685 and 1625; δ_H(250 MHz; $CDCl_3$) 0.97 (3 H, d, *J* 6), 1.03 (3 H, d, *J* 6), 1.60–1.85 (1 H, m), 2.60 (3 H, s), 5.24 (1 H, t, *J* 6.5), 5.49 (1 H, d, *J* 12), 5.55 (1 H, d, *J* 12), 7.31 (1 H, s), 7.36 (1 H, t, *J* 7.5), 7.38–7.49 (4 H, m), 7.49–7.57 (2 H, m), 7.60 (1 H, br s), 7.67 (1 H, d, *J* 8), 7.91 (1 H, s) and 8.21 (1 H, d, *J* 8); *m/z* (EI) 473 (M^+) (Found: C, 68.1; H, 5.7; N, 8.75. $C_{27}H_{27}N_3O_5$ requires C, 68.5; H, 5.75; N, 8.9%).

(3S,Z)-6-[1-(*Benzyloxycarbonyl*)*indol*-3-*ylmethylene*]-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **20**.—To a solution of the piperazinedione **19** (4.0 g, 8.5 mmol) in MeOH (400 cm^3) was added HCl (1 mol dm^{-3}; 80 cm^3) and the mixture was refluxed for 2 h. After ice-cooling, the precipitate was filtered off to give the *title compound* **20** (3.3 g, 91%). Recrystallization from hexane–AcOEt gave analytically pure **20** as colourless needles; m.p. 247–249 °C; $[\alpha]_D^{27}$ −163 (*c* 0.50, DMF); ν_{max}(KBr)/cm^{-1} 3175, 3035, 1740, 1730, 1680 and 1630; δ_H(250 MHz; $CDCl_3$) 0.99 (3 H, d, *J* 6), 1.02 (3 H, d, *J* 6), 1.72–1.97 (3 H, m), 4.16–4.26 (1 H, m), 5.50 (2 H, s), 6.42 (1 H, br s), 7.07 (1 H, s), 7.27 (1 H, t, *J* 7), 7.37–7.57 (6 H, m), 7.64 (1 H, d, *J* 7), 7.82 (1 H, s), 7.88 (1 H, br s) and 8.21 (1 H, d, *J* 7); *m/z* (EI) 431 (M^+) (Found: C, 69.35; H, 5.65; N, 9.55. $C_{25}H_{25}N_3O_4$ requires C, 69.6; H, 5.85; N, 9.75%).

6-[1-(*Benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-5-*methoxy*-3-(2-*methylpropyl*)*pyrazin*-2(1H)-*one* **21**.—A mixture of the piperazinedione **20** (9.5 g, 22 mmol) and MeOTf (12.5 cm^3, 110 mmol) in dry CH_2Cl_2 (500 cm^3) was stirred under reflux for 36 h. After cooling, the reaction mixture was washed with saturated aq. $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane-AcOEt, 5:1→3:1→CH_2Cl_2–MeOH, 3:1) to give (3S,Z)-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethylene*]-5-*methoxy*-3-(2-*methylpropyl*)-3,6-*dihydropyrazin*-2(1H)-*one* (7.0 g, 71%) as colourless needles; m.p. 131–133 °C (from hexane–AcOEt); ν_{max}(KBr)/cm^{-1} 3175, 1750, 1675 and 1635; δ_H(250 MHz; $CDCl_3$) 0.98 (3 H, d, *J* 6.5), 1.01 (3 H, d, *J* 6.5), 1.58–1.72 (1 H, m), 1.78–1.94 (1 H, m), 1.94–2.13 (1 H, m), 3.85 (3 H, s), 4.36 (1 H, dd, *J* 9 and 5), 5.47 (1 H, d, *J* 12.5), 5.52 (1 H, d, *J*

12.5), 6.52 (1 H, s), 7.31 (1 H, td, *J* 7.5 and 1), 7.37–7.48 (4 H, m), 7.48–7.57 (2 H, m), 7.59 (1 H, d, *J* 7.5), 7.61 (1 H, br s), 7.74 (1 H, s) and 8.21 (1 H, d, *J* 7.5); *m/z* (FAB) 446 (MH^+) (High resolution FABMS found: M, 446.2091. $C_{26}H_{28}N_3O_4$ requires MH^+, 446.2079). After further elution, unchanged **20** (1.8 g, 18%) was recovered.

A mixture of the above compound (4.5 g, 10 mmol) and DBU (10 drops) in THF (200 cm^3) was stirred at room temperature for 3 h and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 cm^3) and successively washed with HCl (1 mol dm^{-3}), saturated aq. $NaHCO_3$ and brine, dried with ($MgSO_4$) and concentrated under reduced pressure. Recrystallization of the residual crude solid from hexane–AcOEt gave the *title compound* **21** (4.0 g, 89%) as colourless prisms; m.p. 149–151 °C; ν_{max}(KBr)/cm^{-1} 1735, 1650 and 1620; δ_H(250 MHz; $CDCl_3$) 0.92 (6 H, d, *J* 6.5), 2.08–2.27 (1 H, m), 2.57 (2 H, d, *J* 7), 3.94 (3 H, s), 4.03 (2 H, s), 5.41 (2 H, s), 7.19 (1 H, td, *J* 7.5 and 1), 7.28 (1 H, td, *J* 7.5 and 1), 7.31–7.48 (5 H, m), 7.56 (1 H, s), 7.65 (1 H, dd, *J* 7.5 and 1) and 8.13 (1 H, d, *J* 7.5); *m/z* (EI) 445 (M^+) (Found: C, 69.9; H, 6.05; N, 9.35. $C_{26}H_{27}N_3O_4$ requires C, 70.1; H, 6.1; N, 9.45%).

6-[1-(*Benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-2-(tert-*butyldiphenylsilyloxy*)-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* **23**.—A mixture of the pyrazinone **21** (178 mg, 0.40 mmol), imidazole (82 mg, 1.2 mmol) and *tert*-butyldiphenylsilyl chloride (0.21 cm^3, 0.80 mmol) in dry CH_2Cl_2 (3 cm^3) was stirred at room temperature for 2 h. Ethyl acetate (30 cm^3) was added to the mixture which was then successively washed with saturated aq. $KHSO_4$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt 32:1) to give the *title compound* **23** (262 mg, 96%) as a colourless oil; ν_{max}(neat)/cm^{-1} 1740, 1610, 1590 and 1570; δ_H(250 MHz; $CDCl_3$) 0.98 (6 H, d, *J* 6.5), 1.06 (9 H, s), 2.10–2.30 (1 H, m), 2.68 (2 H, d, *J* 6.5), 3.67 (2 H, s), 3.89 (3 H, s), 5.42 (2 H, s), 6.99 (1 H, t, *J* 8), 7.03 (1 H, s), 7.08–7.33 (8 H, m), 7.33–7.63 (9 H, m) and 8.07 (1 H, d, *J* 7); *m/z* (FAB) 684 (MH^+) (High resolution FABMS found: M, 684.3267. $C_{42}H_{46}N_3O_4Si$ requires MH^+, 684.3257).

2-*Acetoxy*-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* **24**.—A mixture of the pyrazinone **21** (178 mg, 0.40 mmol), Et_3N (0.17 cm^3, 1.2 mmol), DMAP (5 mg, 0.04 mmol) and Ac_2O (0.076 cm^3, 0.80 mmol) in dry CH_2Cl_2 (3 cm^3) was stirred at room temperature for 2.5 h. Ethyl acetate (30 cm^3) was added to the mixture which was then successively washed with saturated aq. $KHSO_4$, saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt, 8:1) to give the *title compound* **24** (195 mg, quant.) as a colourless oil; ν_{max}(neat)/cm^{-1} 1775, 1740, 1615, 1585 and 1555; δ_H(250 MHz; $CDCl_3$) 0.92 (6 H, d, *J* 6.5), 2.07–2.27 (1 H, m), 2.33 (3 H, s), 2.45 (2 H, d, *J* 7), 3.94 (3 H, s), 4.12 (2 H, s), 5.43 (2 H, s), 7.23 (1 H, t, *J* 7), 7.29 (1 H, t, *J* 7), 7.36–7.52 (6 H, m), 7.64 (1 H, d, *J* 7) and 8.12 (1 H, d, *J* 7); *m/z* (EI) 487 (M^+) (Found: M^+, 487.2118. $C_{28}H_{29}N_3O_5$ requires *M*, 487.2107).

2-*Benzyloxy*-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* **25**.—A mixture of the pyrazinone **21** (178 mg, 0.40 mmol), K_2CO_3 (56 mg, 0.40 mmol) and benzyl bromide (0.072 cm^3, 0.60 mmol) in dry DMF (3 cm^3) was stirred at room temperature for 13 h. The reaction mixture was poured into saturated aq. NH_4Cl (30 cm^3) and extracted with Et_2O. The extract was washed thrice with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt, 32:1) to give the *title compound* **25** (187 mg, 87%) as a colourless oil; ν_{max}(neat)/cm^{-1} 1740 and 1610; δ_H(250 MHz; $CDCl_3$) 0.91 (6 H, d, *J* 6.5), 2.07–2.27 (1 H, m), 2.58 (2 H, d, *J* 7), 3.91 (3 H, s), 4.06 (2 H, s), 5.23 (2 H, s), 5.44 (2 H, s), 7.20 (1 H, td, *J* 7 and 1), 7.20–7.53 (11 H, m), 7.48 (1 H, s), 7.66 (1 H, d, *J* 7) and 8.14 (1 H, d, *J* 7); *m/z* (EI) 535 (M^+) (Found: M^+ 535.2463. $C_{33}H_{33}N_3O_4$ requires *M*, 535.2471).

Oxidation of the Pyrazine **24** *by MCPBA*.—MCPBA (80%; 59 mg, 0.27 mmol) was added to a stirred mixture of the pyrazine **24** (88 mg, 0.18 mmol) in CH_2Cl_2 (5 cm^3) and a pH 7 phosphate buffer (3 cm^3) after which the whole was stirred at room temperature for 23 h. After this, a further and similar amount of MCPBA was added to the mixture which was then stirred for an additional 8 h. Dichloromethane (50 cm^3) was added to the mixture which was then successively washed with saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane–AcOEt, 2:1) to give 2-*acetoxy*-6-[1-(*benzyloxycarbonyl*)-2-(3-*chlorobenzoyloxy*)-3-*hydroxy*-2,3-*dihydroindol*-3-*ylmethyl*]-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* **27** (39 mg, 33%) as a colourless oil; ν_{max}(film)/cm^{-1} 3450, 1770, 1730, 1610 and 1580; δ_H(250 MHz; $CDCl_3$) 0.97 (3 H, d, *J* 6.5), 0.98 (3 H, d, *J* 6.5), 2.10–2.37 (1 H, m), 2.28 (3 H, s), 2.54 (2 H, d, *J* 7), 3.17 (1 H, d, *J* 15), 3.32 (1 H, d, *J* 15), 3.79 (3 H, s), 4.71 (1 H, br s), 5.10–5.30 (1 H, m), 5.35 (1 H, d, *J* 12), 6.85 (1 H, s), 7.06 (1 H, t, *J* 7), 7.05–7.60 (8 H, m), 7.47 (1 H, dd, *J* 8 and 2), 7.76 (1 H, d, *J* 8), 7.81 (1 H, d, *J* 2) and 7.83–8.08 (1 H, m); *m/z* (FAB) 682 and 684 [$(M + Na)^+$] [High resolution FABMS found: M^+, 682.1928. $C_{35}H_{34}N_3O_8{}^{35}ClNa$ requires $(M + Na)^+$ 682.1932].

2-*Bromo*-4-*methylvaleryl Chloride* **28**.—4-Methylvaleric acid (24 g, 0.21 mol) and $SOCl_2$ (50 cm^3) were stirred at room temperature for 3 h and then at reflux for 2.5 h. To the refluxing reaction mixture was added bromine (14 cm^3, 0.27 mol) dropwise over 1 h after which the mixture was refluxed for 3 h. Concentration of the reaction mixture under reduced pressure followed by distillation gave the *title compound* **28** (43 g, 96%) as a yellow oil; b.p. 64–66 °C/14 mmHg; ν_{max}(neat)/cm^{-1} 1785; δ_H(250 MHz; $CDCl_3$) 0.95 (3 H, d, *J* 6.5), 1.00 (3 H, d, *J* 6.5), 1.74–2.13 (3 H, m) and 4.54 (1 H, dd, *J* 8 and 6.5); *m/z* (EI) 211, 213 and 215 ($M^+ - H$) [Found: M^+, 210.9503. $C_6H_9O^{35}Cl^{79}Br$ requires $(M^+ - H)$, 210.9488].

N-(2-*Bromo*-4-*methylvaleryl*)*tryptophan Methyl Ester* **29**.—A mixture of (±)-**2**·HCl (48 g, 0.19 mol) and Na_2CO_3 (1 mol dm^{-3}; 95 cm^3) in CH_2Cl_2 (600 cm^3) was stirred at room temperature for 1 h. With ice-cooling, Na_2CO_3 (1 mol dm^{-3}; 200 cm^3) and a solution of the chloride **28** (43 g, 0.20 mol) in CH_2Cl_2 (80 cm^3) were gradually added over 1 h to the mixture. It was then stirred at room temperature for 2 h. The organic layer was separated and successively washed with saturated aq. $KHSO_4$, saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* **29** (73 g, 98%) as a pale yellow oil. Trituration of this with EtOH gave a solid, which was subjected to recrystallization from EtOH to give analytically pure **29** as pale yellow needles; m.p. 137–140 °C; ν_{max}(KBr)/cm^{-1} 3415, 3275, 1750, 1650 and 1570; δ_H(250 MHz; $CDCl_3$) 0.86 and 0.87 (each 1.5 H, d, *J* 6.5), 0.91 and 0.92 (each 1.5 H, d, *J* 6.5), 1.71–1.95 (3 H, m), 3.35 (2 H, d, *J* 5.5), 3.69 (3 H, s), 4.23 (1 H, dd, *J* 7.5 and 7), 4.87–4.98 (1 H, m), 6.76 (1 H, br t, *J* 8), 7.03 and 7.04 (each 0.5 H, d, *J* 2), 7.11 and 7.12 (each 0.5 H, td, *J* 7.5 and 1), 7.20 (1 H, td, *J* 7.5 and 1), 7.36 (1 H, d, *J* 7.5), 7.54 and 7.56 (each 0.5 H, d, *J* 7.5) and 8.17 (1 H, br s); *m/z* (EI) 394 and 396 (M^+) (Found: C, 54.35; H, 5.55; N, 6.9. $C_{18}H_{23}BrN_2O_3$ requires C, 54.7; H, 5.85; N, 7.1%).

3,6-trans-4-*Hydroxy*-6-(1H-*indol*-3-*ylmethyl*)-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **30a** *and its* 3,6-cis-*Isomer* **30b**.—A

solution of hydroxylamine hydrochloride (7.3 g, 104 mmol) and NaOH (4.2 g, 104 mmol) in water (30 cm^3) was added to a solution of the ester **29** (6.9 g, 17 mmol) in EtOH (140 cm^3). The mixture was heated under reflux for 17 h. The same amount of a solution of hydroxylamine hydrochloride and NaOH in water was added again and the mixture was refluxed for 51 h. It was then concentrated under reduced pressure after which AcOEt (200 cm^3) and saturated aq. $KHSO_4$ (200 cm^3) were added to the residue. The organic layer was separated, washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 40:1→8:1). Recovered **29** (0.45 g, 6.5%), **30a** (0.65 g, 12%) and **30b** (1.50 g, 28%) were eluted in this order.

30a, Colourless needles; m.p. 225–226.5 °C (from AcOEt); ν_{max}(KBr)/cm^{-1} 3340, 3075 and 1675; δ_H(250 MHz; [$^2H_6$]-DMSO) 0.79 (3 H, d, *J* 6), 0.80 (3 H, d, *J* 6), 1.53–1.83 (3 H, m), 3.08 (1 H, dd, *J* 14.5 and 4.5), 3.29 (1 H, dd, *J* 14.5 and 4), 3.49 (1 H, dd, *J* 4.5 and 4), 4.31 (1 H, t, *J* 4), 6.94 (1 H, t, *J* 7.5), 7.03 (1 H, t, *J* 7.5), 7.07 (1 H, d, *J* 2.5), 7.29 (1 H, d, *J* 7.5), 7.59 (1 H, d, *J* 7.5), 8.17 (1 H, s), 9.83 (1 H, s) and 10.87 (1 H, br s); *m/z* (EI) 315 (M^+) (Found: C, 64.7; H, 6.4; N, 13.3. $C_{17}H_{21}N_3O_3$ requires C, 64.75; H, 6.7; N, 13.3%).

30b, Pale yellow prisms; m.p. 230–232 °C (from hexane–EtOH); ν_{max}(KBr)/cm^{-1} 3345, 3325, 3180 and 1665; δ_H(250 MHz; [$^2H_6$]DMSO) 0.10–0.28 (1 H, m), 0.44 (3 H, d, *J* 6.5), 0.50 (3 H, d, *J* 6.5), 0.50–0.68 (1 H, m), 3.01 (1 H, dd, *J* 14.5 and 4.5), 3.23 (1 H, dd, *J* 14.5 and 3.5), 3.64 (1 H, dd, *J* 7.5 and 5.5), 4.19 (1 H, br s), 6.93 (1 H, t, *J* 7.5), 7.00 (1 H, d, *J* 2.5), 7.02 (1 H, t, *J* 7.5), 7.29 (1 H, d, *J* 7.5), 7.53 (1 H, d, *J* 7.5), 8.26 (1 H, br s), 9.81 (1 H, s) and 10.92 (1 H, br s); *m/z* (EI) 315 (M^+) (Found; C, 64.65; H, 6.8; N, 13.05. $C_{17}H_{21}N_3O_3$ requires C, 64.75; H, 6.7; N, 13.3%).

3,6-cis-4-*Benzyloxy*-6-(1H-*indol*-3-*ylmethyl*)-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **31**.—A mixture of the piperazinedione **30b** (2.7 g, 8.6 mmol), Bu^{t}OK (0.97 g, 8.6 mmol) and dry hexamethyl phosphoramide (HMPA) (7.5 cm^3) in dry THF (30 cm^3) was stirred at room temperature for 1 h. Benzyl bromide (1.3 cm^3, 10 mmol) was added to the mixture which was then stirred at room temperature for 2 h. It was then poured into ice–water and extracted with AcOEt. The organic layer was washed thrice with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residual viscous oil was triturated with Et_2O to give the *title compound* **31** (3.1 g, 88%). Recrystallization from AcOEt gave analytically pure **31** as colourless prisms; m.p. 166–168 °C; ν_{max}(KBr)/cm^{-1} 3265, 3065, 1680 and 1620; δ_H(200 MHz; $CDCl_3$) 0.77 (3 H, d, *J* 6.5), 0.79 (3 H, d, *J* 6.5), 1.08–1.24 (1 H, m), 1.31–1.46 (1 H, m), 1.63–1.92 (1 H, m), 3.16 (1 H, dd, *J* 14.5 and 8.5), 3.49 (1 H, dd, *J* 14.5 and 3.5), 3.86 (1 H, dd, *J* 7 and 5.5), 4.27–4.37 (1 H, m), 5.96 (1 H, br s), 7.07 (1 H, d, *J* 2.5), 7.13 (1 H, t, *J* 7.5), 7.21 (1 H, t, *J* 7.5), 7.32–7.48 (6 H, m), 7.61 (1 H, d, *J* 7.5) and 8.19 (1 H, br s); *m/z* (EI) 405 (M^+) (Found: C, 71.05; H, 6.7; N, 10.35. $C_{24}H_{27}N_3O_3$ requires C, 71.1; H, 6.7; N, 10.35%).

3,6-cis-4-*Benzyloxy*-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-3-(2-*methylpropyl*)*piperazine*-2,5-*dione* **32**.—Benzyl chloroformate (1.7 cm^3, 12 mmol) was added to an ice-cooled mixture of the piperazinedione **31** (2.4 g, 6.0 mmol), pulverized NaOH (0.60 g, 15 mmol) and Bu_4NHSO_4 (0.10 g, 0.30 mmol) in dry CH_2Cl_2 (100 cm^3). The reaction mixture was stirred at room temperature for 28 h and then poured into ice-cooled saturated aq. $KHSO_4$. The organic layer was separated, washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue by column chromatography (hexane–AcOEt, 2:1→1:1) gave the *title compound* **32** (1.7 g, 54%) as a white foam; ν_{max}(KBr)/cm^{-1} 3240, 1735 and 1680; δ_H(250 MHz; $CDCl_3$) 0.76 (3 H, d, *J* 6.5), 0.78 (3 H, d, *J* 6.5), 1.21–1.33 (1 H, m), 1.40–1.53 (1 H, m), 1.69–1.86 (1 H, m), 3.08 (1 H, dd, *J* 14.5 and 8.5), 3.43 (1 H, dd, *J* 14.5 and 2.5), 3.88 (1 H, dd, *J* 7 and 5.5), 4.28–4.37 (1 H, m), 4.96 (2 H, s), 5.41 (1 H, d, *J* 12), 5.47 (1 H, d, *J* 12), 6.04 (1 H, br s), 7.24–7.44 (10 H, m), 7.44–7.52 (2 H, m), 7.53 (1 H, s), 7.57 (1 H, d, *J* 7) and 8.18 (1 H, d, *J* 7.5); *m/z* (EI) 539 (M^+) (Found: C, 70.95; H, 6.2; N, 7.8. $C_{32}H_{33}N_3O_5$ requires C, 71.25; H, 6.2; N, 7.8%). After further elution unchanged **31** (0.39 g, 16%) was recovered.

4-*Benzyloxy*-6-[1-(*benzyloxycarbonyl*)*indol*-3-*ylmethyl*]-2-*chloro*-3-(2-*methylpropyl*)*pyrazin*-5(4H)-*one* **33**.—Phosphorus pentachloride (3.9 g, 19 mmol) was added to a solution of the piperazinedione **32** (6.8 g, 13 mmol) in $POCl_3$ (14 cm^3) in three portions at room temperature over 15 min. The reaction mixture was stirred at room temperature for 4 h and then poured into ice–water and extracted with Et_2O. The organic layer was washed thrice with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2→CH_2Cl_2–MeOH, 160:1→80:1) to give an oil, which was triturated with MeOH to give the *title compound* **33** (1.67 g, 24%) as colourless needles; m.p. 141–142 °C (from MeOH); ν_{max}(KBr)/cm^{-1} 1735, 1665 and 1565; δ_H(250 MHz; $CDCl_3$) 0.90 (6 H, d, *J* 6.5), 1.97–2.18 (1 H, m), 2.54 (2 H, d, *J* 7.5), 4.23 (2 H, s), 5.23 (2 H, s), 5.43 (2 H, s), 7.21–7.44 (10 H, m), 7.44–7.54 (2 H, m), 7.70 (1 H, s), 7.75 (1 H, dd, *J* 7 and 1) and 8.17 (1 H, d, *J* 7.5); *m/z* (EI) 555 and 557 (M^+) (Found: C, 69.15; H, 5.35; N, 7.45. $C_{32}H_{30}N_3O_4Cl$ requires C, 69.1; H, 5.45; N, 7.55%). After further elution unchanged **32** (1.9 g, 28%) was recovered.

2-*Chloro*-5-*hydroxy*-6-(1H-*indol*-3-*ylmethyl*)-3-(2-*methylpropyl*)*pyrazine* 4-*Oxide* **34**.—Under a hydrogen atmosphere, a mixture of the pyrazinone **33** (1.67 g, 3.0 mmol) and 10% Pd–C (0.30 g) in EtOH (90 cm^3) was stirred at 30 °C for 1 h and then was refluxed for 2 h under a nitrogen atmosphere. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give the *title compound* **34** (0.99 g, quant.) as white crystals; m.p. 167–168 °C (from MeOH); ν_{max}(KBr)/cm^{-1} 3330, 3125, 3060, 1625 and 1525; δ_H(200 MHz; $CDCl_3$) 0.99 (6 H, d, *J* 6.5), 2.07–2.34 (1 H, m), 2.79 (2 H, d, *J* 7.5), 4.29 (2 H, s), 7.13 (1 H, td, *J* 7.5 and 1.5), 7.15 (1 H, s), 7.20 (1 H, td, *J* 7.5 and 1.5), 7.33 (1 H, dd, J 7.5 and 1.5), 7.83 (1 H, dd, *J* 7.5 and 1.5) and 8.08 (1 H, br s); *m/z* (FAB) 332 and 334 (MH^+) (High resolution FABMS found: M^+, 332.1143. $C_{17}H_{19}N_3O_2{}^{35}Cl$ requires MH^+, 332.1166).

Methylation of the Pyrazine Oxide **34**.—A large excess of CH_2N_2 (as Et_2O solution) was added to a solution of the pyrazine oxide **34** (0.31 g, 0.94 mmol) in CH_2Cl_2 (30 cm^3). The mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. Purification of the residue by column chromatography (hexane–AcOEt, 4:1) gave 2-*chloro*-6-(1H-*indol*-3-*ylmethyl*)-5-*methoxy*-3-(2-*methylpropyl*)*pyrazine* 4-oxide **35** (0.13 g, 40%) and 2-*chloro*-6-(1H-*indol*-3-*ylmethyl*)-4-*methoxy*-3-(2-*methylpropyl*)*pyrazin*-5(4H)-*one* **38** (0.16 g, 49%).

35, Colourless needles; m.p. 142–143 °C (from EtOH); ν_{max}(KBr)/cm^{-1} 3260, 1625 and 1575; δ_H(250 MHz; $CDCl_3$) 0.98 (6 H, d, *J* 6.5), 2.15–2.36 (1 H, m), 2.89 (2 H, d, *J* 7.5), 3.91 (3 H, s), 4.24 (2 H, s), 7.14 (1 H, td, *J* 7 and 1), 7.16 (1 H, d, *J* 2), 7.20 (1 H, td, *J* 7 and 1), 7.35 (1 H, dd, *J* 7 and 1), 7.75 (1 H, dd, *J* 7 and 1) and 8.07 (1 H, br s); *m/z* (EI) 345 and 347 (M^+) (Found: C, 62.55; H, 5.7; N, 12.1. $C_{18}H_{20}N_3O_2Cl$ requires C, 62.5; H, 5.85; N, 12.15%).

38, Pale brown prisms; m.p. 128–130 °C (from EtOH); ν_{max}(KBr)/cm^{-1} 3310, 1655 and 1570; δ_H(250 MHz; $CDCl_3$) 0.97 (6 H, d, *J* 6.5), 2.00–2.23 (1 H, m), 2.63 (2 H, d, *J* 7.5), 4.04 (3 H, s), 4.27 (2 H, s), 7.12 (1 H, td, *J* 7 and 1.5), 7.17 (1 H, td, *J* 7 and 1.5), 7.30 (1 H, d, *J* 2.5), 7.34 (1 H, dd, *J* 7 and 1.5), 7.84 (1 H, dd,

J 7 and 1.5) and 8.11 (1 H, br s); *m/z* (EI) 345 and 347 (M^+) (Found: C, 62.5; H, 5.7; N, 12.1. $C_{18}H_{20}N_3O_2Cl$ requires C, 62.5; H, 5.85; N, 12.15%).

Reaction of the Pyrazine Oxide **35** *with Sodium Benzyl Oxide.*—A mixture of the pyrazine oxide **35** (32 mg, 0.092 mmol) and NaOBn (36 mg, 0.28 mmol) in dry THF (3 cm^3) was stirred at room temperature for 1 h. The reaction mixture was poured into ice-cooled saturated aq. $KHSO_4$ and extracted with AcOEt. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane–AcOEt, 2:1) to give *5-benzyloxy-2-chloro-6-(1H-indol-3-ylmethyl)-3-(2-methylpropyl)pyrazine 4-oxide* **36** (4.8 mg, 12%) as pale brown needles; m.p. 104–106 °C (from EtOH); ν_{max}(film)/cm^{-1} 3420 and 1575; δ_H(250 MHz; $CDCl_3$) 0.99 (6 H, d, *J* 6.5), 2.22–2.42 (1 H, m), 2.92 (2 H, d, *J* 7.5), 4.07 (2 H, s), 5.32 (2 H, s), 6.97 (1 H, d, *J* 2), 7.10 (1 H, t, *J* 8), 7.19 (1 H, t, *J* 8), 7.20–7.47 (6 H, m), 7.64 (1 H, d, *J* 8) and 7.98 (1 H, br s); *m/z* (FAB) 422 and 424 (MH^+) (High resolution FABMS found: M^+, 422.1612. $C_{24}H_{25}N_3O_2{}^{35}Cl$ requires MH^+, 422.1635).

2-(N-Diphenylmethylene)amino-3-(1H-indol-3-yl)propionitrile **41**.—To a water-cooled solution of the indole **39** (13 g, 72 mmol) and nitrile **40** (13 g, 60 mmol) in CH_2Cl_2 (360 cm^3) were added portionwise 35% NaOH (35 cm^3, 0.31 mol) and Me_2SO_4 (10 cm^3, 108 mmol) over 1 h. The mixture was stirred at room temperature for 45 h after which the insoluble material was filtered off through a Celite pad. The organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane–AcOEt, 8:1) to give the *title compound* **41** (17 g, 80%) as colourless prisms; m.p. 121–122 °C (from hexane–AcOEt); ν_{max}(KBr)/cm^{-1} 3445, 2230, 1615, 1600 and 1580; δ_H(250 MHz; $CDCl_3$) 3.26 (1 H, dd, *J* 14 and 7.5), 3.45 (1 H, dd, *J* 14 and 7), 4.56 (1 H, dd, *J* 7.5 and 7), 6.85 (2 H, d, *J* 7), 6.96 (1 H, t, *J* 8), 7.08 (2 H, d, *J* 8), 7.15 (1 H, t, *J* 8), 7.27–7.50 (7 H, m), 7.63 (2 H, dd, *J* 8 and 1) and 8.05 (1 H, br s); *m/z* (EI) 349 (M^+) (Found: C, 82.55; H, 5.65; N, 11.95. $C_{24}H_{19}N_3$ requires C, 82.5; H, 5.5; N, 12.05%).

2-(N-Diphenylmethylene)amino-3-[1-(p-tolylsulfonyl)indol-3-yl]propionitrile **42**.—A mixture of the nitrile **41** (7.0 g, 20 mmol), pulverized NaOH (2.0 g, 50 mmol), Bu_4NHSO_4 (0.34 g, 1.0 mmol) and TsCl (5.7 g, 30 mmol) in dry CH_2Cl_2 (140 cm^3) was stirred at room temperature for 3 h. Water (100 cm^3) was added to the mixture which was then stirred for 1 h. The organic layer was separated, washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure. The residue was triturated with Et_2O to give the *title compound* **42** (10 g, quant.) as a white solid. Analytically pure **42** was obtained by recrystallization from AcOEt as colourless needles; m.p. 201–203 °C; ν_{max}(KBr)/cm^{-1} 2230, 1620, 1600 and 1580; δ_H(250 MHz; $CDCl_3$) 2.22 (3 H, s), 3.25 (1 H, dd, *J* 14.5 and 8), 3.33 (1 H, dd, *J* 14.5 and 6), 4.50 (1 H, dd, *J* 8 and 6), 6.51 (2 H, d, *J* 7), 6.93 (2 H, d, *J* 8.5), 6.99 (1 H, t, *J* 8), 7.05 (1 H, t, *J* 8), 7.17 (2 H, t, *J* 7.5), 7.24–7.57 (5 H, m), 7.45 (1 H, s), 7.57 (1 H, d, *J* 8.5), 7.58 (1 H, d, *J* 8), 7.66 (2 H, d, *J* 8.5) and 7.94 (1 H, d, *J* 8); *m/z* (EI) 503 (M^+) (Found: C, 73.95; H, 5.1; N, 8.35. $C_{31}H_{25}N_3O_2S$ requires C, 73.95; H, 5.0; N, 8.35%).

2-Amino-3-[1-(p-tolylsulfonyl)indol-3-yl]propionitrile **43**.—To a solution of the nitrile **42** (10 g, 20 mmol) in CH_2Cl_2 (120 cm^3) was added HCl (1 mol dm^{-3}; 48 cm^3). The mixture was stirred at room temperature for 47 h and then NaOH (4 mol dm^{-3}; 13 cm^3) was added to it. The organic layer was separated and concentrated under reduced pressure. The residue was dissolved in Et_2O and HCl (1 mol dm^{-3}; 60 cm^3) was added to the solution. The precipitate was filtered off and the acidic layer was separated. The precipitate and the acidic layer were combined and NaOH (4 mol dm^{-3}; 16 cm^3) was added to the mixture. This was then extracted with CH_2Cl_2 and the organic layer was separated, washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* **43** (6.1 g, 90%). Recrystallization from Et_2O gave analytically pure **43** as colourless prisms; m.p. 97.5–98.5 °C; ν_{max}(KBr)/cm^{-1} 3390, 2210 and 1600; δ_H(250 MHz; $CDCl_3$) 1.65 (2 H, br s), 2.34 (3 H, s), 3.13 (2 H, d, *J* 6.5), 4.00 (1 H, br t, *J* 6.5), 7.22 (2 H, d, *J* 8.5), 7.26 (1 H, td, *J* 7.5 and 1), 7.34 (1 H, td, *J* 7.5 and 1), 7.53 (1 H, dd, *J* 7.5 and 1), 7.61 (1 H, s), 7.78 (2 H, d, *J* 8.5) and 7.99 (1 H, dd, *J* 7.5 and 1); *m/z* (EI) 339 (M^+) (Found: C, 63.65; H, 5.1; N, 12.3. $C_{18}H_{17}N_3O_2S$ requires C, 63.7; H, 5.05; N, 12.4%).

N-{1-Cyano-2-[1-(p-tolylsulfonyl)indol-3-yl]ethyl}-2-hydroxyimino-4-methylvaleramide **44**.—In a similar manner to the preparation of compound **4**, the *title compound* **44** (3.9 g, 83%) was obtained from the nitrile **43** (3.4 g, 10 mmol), the acid **3** (1.5 g, 10 mmol), DCC (2.1 g, 10 mmol) and HOSu (1.2 g, 10.5 mmol) as a white solid; m.p. 157–159 °C (from Et_2O); ν_{max}(KBr)/cm^{-1} 3385, 2235, 1680, 1630 and 1595; δ_H(250 MHz; $CDCl_3$) 0.90 (3 H, d, *J* 6.5), 0.91 (3 H, d, *J* 6.5), 1.93–2.12 (1 H, m), 2.34 (3 H, s), 2.51 (2 H, d, *J* 7.5), 3.18 (1 H, dd, *J* 14 and 6.5), 3.27 (1 H, dd, *J* 14 and 6.5), 5.13–5.27 (1 H, m), 7.14 (1 H, br d, *J* 8), 7.22 (2 H, d, *J* 8.5), 7.29 (1 H, t, *J* 7.5), 7.35 (1 H, t, *J* 7.5), 7.55 (1 H, d, *J* 7.5), 7.60 (1 H, s), 7.77 (2 H, d, *J* 8.5), 7.93 (1 H, br s) and 7.97 (1 H, d, *J* 7.5); *m/z* (EI) 466 (M^+) (Found: C, 61.7; H, 5.55; N, 12.0. $C_{24}H_{26}N_4O_4S$ requires C, 61.8; H, 5.6; N, 12.0%).

5-Amino-3-(2-methylpropyl)-6-[1-(p-tolylsulfonyl)indol-3-ylmethyl]pyrazin-2(1H)-one 4-Oxide **45**.—A solution of the valeramide **44** (2.3 g, 5.0 mmol) in AcOH (25 cm^3) was stirred at 65–70 °C for 12 h. After cooling, the precipitate was filtered off and washed with hexane to give the *title compound* **45** (1.4 g, 59%) as a yellow powder; m.p. 188–190 °C (from Et_2O); ν_{max}(KBr)/cm^{-1} 3475, 3350–3320, 1635, 1595 and 1580; δ_H(250 MHz; $CDCl_3$) 0.90 (6 H, d, *J* 6.5), 2.13–2.32 (1 H, m), 2.30 (3 H, s), 2.79 (2 H, d, *J* 7), 4.02 (2 H, s), 4.68 (2 H, br s), 7.18 (2 H, d, *J* 8.5), 7.19 (1 H, t, *J* 8), 7.33 (1 H, t, *J* 8), 7.48 (1 H, s), 7.50 (1 H, d, *J* 8), 7.73 (2 H, d, *J* 8.5) and 7.97 (1 H, d, *J* 8); *m/z* (EI) 466 (M^+) (Found: C, 61.75; H, 5.7; N, 11.7. $C_{24}H_{26}N_4O_4S$ requires C, 61.8; H, 5.6; N, 12.0%).

Reaction of the Pyrazinone Oxide **45** *with Isopentyl Nitrite.*—To a stirred solution of the pyrazinone oxide **45** (0.23 g, 0.50 mmol) in dry THF (15 cm^3) were successively added 42% HBF_4 (0.27 cm^3, 1.3 mmol) and isopentyl nitrite (0.10 cm^3, 0.75 mmol) at −22 °C. The reaction mixture was stirred at the same temperature for 1 h and then diluted with AcOEt (30 cm^3). The mixture was thrice washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residual solid was triturated with CH_2Cl_2 to give *6-(2-methylpropyl)-3-[1-(p-tolylsulfonyl)indol-3-yl]-1H-pyrazolo[3,4-b]pyrazin-5-one 7-oxide* **46** (126 mg, 53%) as a yellow powder; m.p. 256–258 °C; ν_{max}(KBr)/cm^{-1} 3100 and 1645; δ_H(250 MHz; CF_3CO_2D) 1.14 (6 H, d, *J* 6.5), 2.42 (3 H, s), 2.30–2.60 (1 H, m), 3.25 (2 H, d, *J* 6.5), 7.38 (2 H, d, *J* 8.5), 7.43 (1 H, t, *J* 7.5), 7.53 (1 H, t, *J* 7.5), 7.71 (1 H, d, *J* 7.5), 7.99 (2 H, d, *J* 8.5), 8.06 (1 H, d, *J* 7.5) and 8.27 (1 H, s); *m/z* (FAB) 478 (MH^+) (Found: C, 60.1; H, 4.75; N, 14.3. $C_{24}H_{23}N_5O_4S$ requires C, 60.35; H, 4.85; N, 14.65%).

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