Studies of Phthalazine-5,8-quinone, A Ring Contraction, and Some Novel and Potentially Useful Fluorescent Phthalimides

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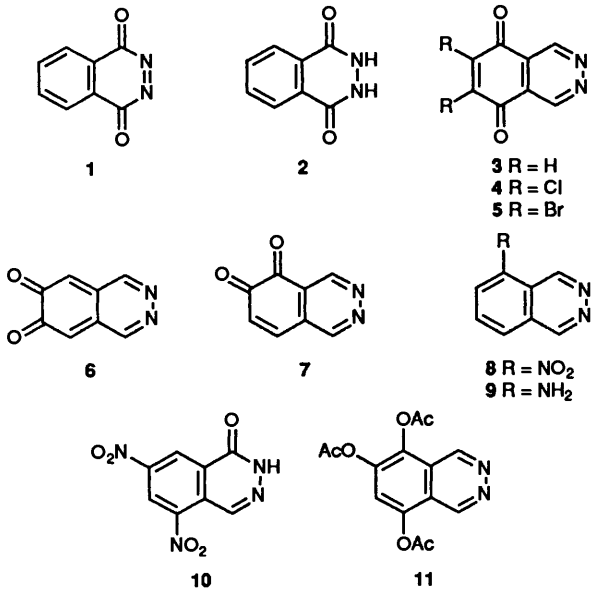
Phthalazine-5,8-quinone **3** has been obtained and some derivatives prepared. Treatment of 5,8-dimethoxy **14** and 5-hydroxy-8-methoxy-2,3-dihydrophthalazin-1,4-dione **16** with ceric ammonium nitrate produces an efficient ring contraction reaction to yield the highly fluorescent 3,6-dimethoxyphthalimide **18** and *N*-amino-3-hydroxy-6-methoxyphthalimide **19**, respectively. The phthalimide **18** has been converted into potentially useful fluorescent labels for use in amine and peptide chemistry and investigations of peptidase activity, while **19** has yielded a potentially useful thiol probe **29**.

The phthalazinequinone **1** is a reactive intermediate useful as a powerful dienophile in Diels–Alder reactions and is formed by mild oxidation of the 2,3-dihydrophthalazine-1,4-dione **2**.[1] However, none of the parent phthalazinequinones of the type **3**, **6** or **7** are known[2,3] though the 6,7-dichloro derivative **4** of **3** has been reported.[4] We now describe a preparation of **3**, its



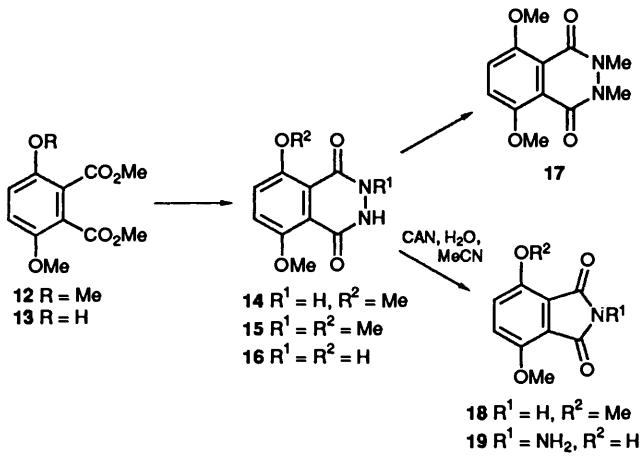
conversion into some derivatives, the uncovering of a novel ring contraction reaction found in unsuccessful attempts to prepare phthalazinequinones, and studies of novel and potentially useful, highly fluorescent phthalimides.

The oxidation of bicyclic heterocycles containing a benzene ring fused to a heterocycle and having one or more electron releasing substituents on the carbocycle has been a useful route to heterocyclic quinones.[2,3] In the case of phthalazine, the 5-amino derivative seemed worthy of exploration as a precursor of the quinone **3** since nitration of phthalazine was known to give the 5-nitro derivative **8**, though only in 17% yield.[4] We found that an increase in the proportion of the potassium nitrate to 4.8 molar equivalents (from the 1.5 molar equivalents used in the literature procedure) produced a significant increase in yield to 50% of **8**. A concomitant disadvantage was that a second product was then formed, though in low yield (3%). This byproduct was readily removed from the reaction mixture and was thought to be 5,7-dinitrophthalazin-1(2*H*)-one **10** because the presence of the two nitro groups and a carbonyl group was clear from the evidence of the mass and IR spectra. The ^{1}H NMR spectrum showed that the nitro groups were *meta* related and that the low field signal (9.15 ppm) for the 4-H was consistent with the presence of a 5-nitro substituent. Both 5- and 8-nitrophthalazin-1(2*H*)-one are known[5] and are formed by nitration of phthalazin-1(2*H*)-one, when the former is the major product. We repeated the preparation and separated the isomers by TLC and found that 5-nitrophthalazine showed a signal at 8.6 ppm for 4-H in its NMR spectrum while the 8-isomer, showed the corresponding signal at higher field, 8.21 ppm. The evidence therefore supported the structure **10** and indicated that the compound was probably formed by nitration of phthalazinone which had been produced by oxidation of phthalazine.

The 5-nitrophthalazine **8** was reduced with sodium dithionite to the corresponding aminophthalazine **9** and this was oxidised with potassium dichromate in sulfuric acid to give the phthalazinequinone **3** in 58% yield. The quinone was converted into the known[4] 6,7-dichloro derivative **4** by the action of chlorine in chloroform and to the novel dibromo derivative **5** by reaction with bromine. Treatment of the quinone **3** with acetic acid in the presence of a catalytic quantity of boron trifluoride–diethyl ether gave the Thiele reaction product, 5,6,8-triacetoxyphthalazine **11**, but only in 12% yield.

We now turned our attention to the synthesis of 5,8-quinones derived from 2,3-dihydrophthalazine-1,4-diones and it appeared that an approach using oxidation in a strongly acidic medium was unlikely to be successful. A method offering more promise seemed to be the oxidation of *p*-dimethoxy compounds with ceric ammonium nitrate (CAN). The first requirement was the preparation of suitably substituted phthalazinediones. The known dimethyl 3,6-dimethoxyphthalate **12**[6] yielded 5,8-dimethoxy-2,3-dihydrophthalazine-1,4-dione **14** on treatment with hydrazine hydrate (Scheme 1) and the *N*-methyl derivative **15** when heated with *N*-methylhydrazine. The dimethyl derivative **17** was most conveniently obtained by methylation of **15**. 5-Hydroxy-8-methoxy-2,3-dihydrophthalazine-1,4-dione **16** was obtained from dimethyl 3-hydroxy-6-methoxyphthalate **13**,[7] previously prepared as a precursor of **12**.

Attempts to oxidise the *p*-dimethoxyphthalazinediones with CAN gave unexpected results. No quinones were obtained and nor was a nitration product isolated; the latter type of reaction sometimes has been observed, *e.g.* the formation of 5-nitro-4,7-dimethoxyisatin from dimethoxyisatin on treatment with CAN in aqueous acetonitrile.[8] Interestingly, the dimethoxyphthalazinediones **14** and **15** on treatment with CAN gave 3,6-dimethoxyphthalimide **18** consistently and in good yield. The



Scheme 1

Table 1 Absorption and fluorescent properties of phthalimides

	UV absorption maximum		Fluorescence		
Compound	λ nm	log ε	$\lambda_{ex.}$nm	$\lambda_{em.}$nm	φ
18	375	3.65	375	455	0.49
20	380	3.89	380	440	0.48
21	392	3.91	392	442	0.48
29	360	3.98	360	468	0.12
30	386	4.16	386	492	0.51

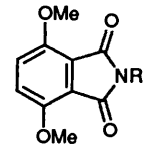
later finding that a similar reaction with 5-hydroxy-8-methoxyphthalazinedione **16** gave *N*-amino-3-hydroxy-6-methoxyphthalimide **19** in good yield was therefore unexpected but useful.

The literature concerning the reactions of phthalic anhydride and hydrazine and its methyl derivatives is confused until the careful work of Drew and his colleagues.[9] Their work and later investigations have shown that *N*-aminophthalimide is an intermediate in one of the pathways by which the phthalazinedione system is formed from phthalic anhydride and hydrazine, and that phthalimides react with hydrazine to give phthalazinedione.[10] However the reverse reaction, *i.e.* the contraction of the six-membered heterocycle in phthalazinedione to give phthalimide does not occur readily and the difficulty has been commented upon.[11] A photochemical ring contraction reaction has been reported but the yields of the resultant phthalimides are low (*ca.* 30%).[11] Thus, the ring contraction in the presence of CAN is potentially useful since it is simple to perform and, in the cases investigated, gives a good yield. In the absence of CAN and either with or without the addition of acid, the formation of the ring contraction product is detectable by TLC but the yield is very small. The details of the mechanism of the reaction are not known but it seems likely that the metal ion plays some part.

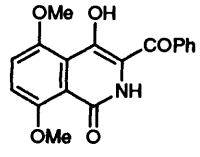
3,6-Dimethoxyphthalimide is the third member of the four possible dimethoxyphthalimides to be obtained because both 3,4-dimethoxy-[12] and 4,5-dimethoxyphthalimide[13] are known. Although phthalic acid derivatives have been known for a long time, their chemistry is still able to excite academic interest[14,15] and to prove useful in medicinal chemistry.[16] In addition, their chemistry has other industrial applications.[17] The striking property of both the novel 3,6-dimethoxyphthalimide **18** and its *N*-amino derivative **19** is their fluorescence which is in marked contrast to the non-fluorescence of phthalimide, though 3- and 4-aminophthalimide and their *N*,*N*-dimethyl derivatives are fluorescent. The fluorescence emission maximum for **18** is 455 nm with a quantum yield of 0.49 in methanolic solution (Table 1). The potential advantage of **18** as a fluorophore for use in quantitative analytical applications is that, unlike the *C*-aminophthalimides, **18** is not basic and the fluorescence is relatively insensitive to changes in pH. We looked at the possibilities for the development of reagents of use in two types of application: the first type of reagent for use as fluorescent protecting groups in amine and peptide synthesis and the second type of reagent for use in the estimation of thiols.

The use of the phthaloyl protecting group for amines was introduced by King and Kidd[18] and independently by Sheehan[19] but the experimental procedure has been developed over the years mainly in order to avoid the possibilities of racemisation in the protection and deprotection stages.[20] In addition *N*-hydroxyphthalimide has been shown to be a useful reagent for the *C*-protection of amino acids or peptides.[21] The possibilities for the development of this chemistry but utilising a neutral phthaloyl derivative giving fluorescence labelled products seemed to be potentially useful in facilitating the identification of the required species in reaction mixtures and also in the synthesis of fluorescence labelled peptides having a terminal quenching moiety for use in investigations of sequence specific proteases.[22] The first step in developing these ideas was to prepare the necessary reagents.

N-Alkylation of the dimethoxyphthalimide **18** was explored by ethylation to give **20** with ethanol in the Mitsunobu reaction and, in better yield, with ethyl bromide and base. Thus, fluorescence labelled and *N*-protected aliphatic amines were obtained in one step by this route. *N*-Hydroxymethyl-3,6-dimethoxyphthalimide **21** was readily available by a standard



20 R = Et
21 R = CH_2OH
22 R = CO_2Et
23 R = OH
24 R = CH_2COPh
25 R = NH_2



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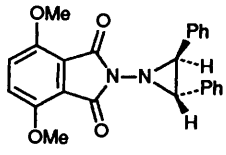

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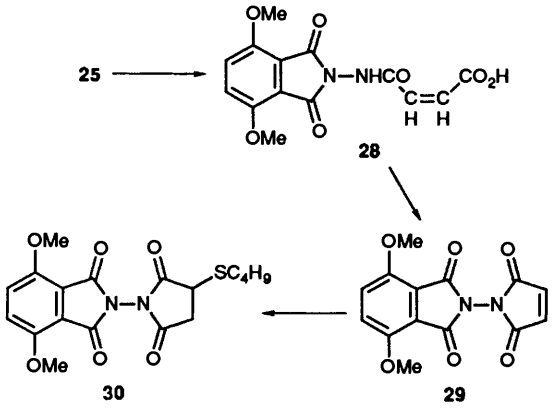
route from **18** as were the potentially useful fluorescent *N*- and *C*-terminus amino acid protecting agents, *N*-ethoxycarbonyl-**22** and *N*-hydroxy-3,6-dimethoxyphthalimide **23**. Phthalimides are of potential use as intermediates for the formation of more complex molecules and the preparation of the isoquinoline derivative **26** from 3,6-dimethoxy-*N*-phenacylphthalimide **24** by the Gabriel–Colman rearrangement was demonstrated.

We now turned our attention to the development of potentially useful reagents from *N*-amino-3,6-dimethoxyphthalimide **19**. Selective *O*-methylation of **19** to yield the dimethyl ether **25** was achieved using methyl trichloroacetate.[23] The amine **25** was then used to form the labelled *trans*-2,3-diphenylaziridine **27** by trapping with *trans*-stilbene of the nitrene formed by oxidation of **25**.[24] However, the main importance of **25** was as an intermediate in the formation of a potential thiol probe.

It is known that thiols readily add to *N*-substituted maleimides, and *N*-ethylmaleimide (NEM) is a common selective thiol trapping reagent when used in solution at pH 8 or below.[25] When a fluorophore is directly bonded to the nitrogen of maleimide, the fluorescence is often markedly decreased but saturation of the olefinic bond (as occurs on

addition of a thiol to the maleimide nucleus) produces a molecule which exhibits strong fluorescence. This principle is utilised in several thiol probes which are used in the quantitative estimation of thiols.

It seemed likely that a similar reagent based on 3,6-dimethoxyphthalimide as the fluorophore might possess similar useful properties. The reaction of **25** with maleic anhydride in acetonitrile at reflux temperature gave the maleamide **28** and this was cyclised in acetic anhydride to the required maleimide **29** (Scheme 2). This maleimide was tested as a potential thiol probe by investigating its reaction with butanethiol: reaction occurred at room temperature in aqueous medium at pH 5 to give the fluorescent adduct **30**.



Scheme 2

Studies of the fluorescence properties of the dimethoxyphthalimides were made (Table 1). With the exception of **29** all had a fluorescence quantum yield (φ) of between 0.4 and 0.5 with fluorescence maxima [$\lambda_{em.}$ (max.)] between 420 and 455 nm when measured in methanolic solution. The maleimide **29** showed fluorescence with $\lambda_{em.}$ (max.) 468 nm and φ = 0.12. The latter was a disappointingly high value and may be due to the steric and charge interactions of the four carbonyl groups causing some twisting about the N–N bond so that the nuclei were not coplanar with consequent partial loss of the quenching effect of the maleimide nucleus. However, the thiol adduct **30** showed a significant increase in quantum yield, φ = 0.51, and, importantly, a shift of $\lambda_{em.}$ (max.) to 492 nm.

Thus, the maleimide **29** is a potentially useful thiol probe as the fluorescence at 492 nm may be monitored. In addition, the UV absorption maxima of the probe **29** and the adduct **30** are different so that the formation of the adduct is observable.

Experimental

M.p.s were determined in open capillary tubes and are uncorrected. Thin-layer chromatography was performed on silica gel (Camlab, 0.25 mm layer) or reverse phase plates (Whatman, KC_{18}). Column chromatography used silica gel MPD 60 Å. IR spectra were recorded for potassium bromide discs on a Perkin-Elmer 1420 spectrometer. NMR spectra were obtained with solutions containing tetramethylsilane as an internal standard using either a Varian CFT-20 or JEOL FX200 spectrometer. *J* values are given in Hz. Low resolution mass spectra were determined at an ionisation potential of 70 eV with an MS 902 spectrometer. UV spectra were recorded for methanolic solutions in quartz cells using a Perkin-Elmer 9 spectrometer and fluorescence spectra were recorded for methanolic solutions with an absorbance of 0.01 at the excitation wavelength using a Perkin-Elmer LS 50 spectrometer. A solution of quinine sulfate in perchloric acid (0.1 mol dm^{-3}) with an absorbance of 0.01 was used as the standard for quantum yield (φ) measurements and was taken to have φ = 0.59. Elemental analyses were performed by Butterworth Laboratories, Teddington, and Medac Ltd., Brunel University.

Solvents for use in UV and fluorescence spectroscopy or chromatography were dried and distilled before use.

5,7-Dinitrophthalazin-1(2H)-one **10** *and 5-Nitrophthalazine* **8**.—The method used was similar to that described by Johnson *et al.*[4] but a larger proportion of potassium nitrate was employed. Potassium nitrate (7.32 g, 72 mmol) was added in small portions to a solution of phthalazine (2 g, 15 mmol) in conc. sulfuric acid (96%; 15 cm^3). After the addition was complete, the mixture was heated on a steam bath for 12 h and then diluted with water (250 cm^3). The precipitate was filtered off and crystallised from water to give deep yellow, *5,7-dinitrophthalazin-1(2H)-one* (110 mg, 3%), m.p. 288–289 °C (Found: C, 41.0; H, 1.55; N, 23.7. $C_8H_4N_4O_5$ requires C, 40.7; H, 1.7; N, 23.7%); ν_{max}/cm^{-1} 3100 (NH), 1660 (CO) ,1520 (NO_2) and 1340 (NO_2); δ[$(CD_3)_2SO$] 5.9–6.1 (1 H, br s, exchanges with D_2O, NH), 9.15 (1 H, s, 4H), 9.7 (1 H, d, *J* 1.8, 8-H) and 10.28 (1 H, d, *J* 1.8, 6-H).

The filtrate was neutralised with sodium hydroxide solution to give pale yellow 5-nitrophthalazine (1.34 g, 50%), m.p. 188–189 °C (lit.,[4] 188–189 °C).

Phthalazine-5,8-dione **3**.—Sulfuric acid (6 mol dm^{-3}; 0.5 cm^3) was added to 5-aminophthalazine[4] (300 mg) in water (10 cm^3) and cooled to 5 °C. After the addition of ice (5 g), the mixture was stirred vigorously while an ice-cold solution of potassium dichromate (0.61 g) and conc. sulfuric acid (10 cm^3) in water (100 cm^3) was added rapidly. The mixture was stored in an ice bath for 2 h and then extracted with chloroform. The organic extract was washed with dilute sulfuric acid and with saturated aqueous chloride. Evaporation of the extract under reduced pressure gave a solid which was crystallised from water to give the red–brown *phthalazine-5,8-dione* (0.19 g, 58%), m.p. > 300 °C but with decomp. from 173 °C (Found: 58.1; H, 2.7; N, 16.8. $C_8H_4N_2O_2 \cdot \frac{1}{4}H_2O$ requires C, 58.35; H, 2.7; N, 17.0%); ν_{max}/cm^{-1} 1670 (CO); $\delta_H(CDCl_3)$ 7.08 (2 H, s, 6- and 7-H) and 9.75 (2 H, s, 1- and 4-H); *m/z* 160 (M^+, 65%), 132 (12) and 69 (100).

6,7-Dichlorophthalazine-5,8-dione **4** *and 6,7-Dibromophthalazine-5,8-dione* **5**.—*General procedure.* An excess of a solution of the halogen in carbon tetrachloride was added to a solution of phthalazine-5,8-dione (100 mg) in chloroform (10 cm^3) and the mixture stirred at room temperature for 1 h. The volatile material was removed and light petroleum (b.p. 60–80 °C) added. The solid was then crystallised.

6,7-Dichlorophthalazine-5,8-dione (from ethanol) was obtained in 87% yield and had m.p. 223–225 °C (lit.,[4] m.p. 225 °C).

6,7-Dibromophthalazine-5,8-dione (from water) (120 mg, 63%), m.p. 176–180 °C (decomp.) (Found: C, 30.2; H, 4.0; N, 8.8, $C_8H_2Br_2O_2 \cdot 4H_2O$ requires C, 29.9; H, 3.9; N, 8.5%); ν_{max}/cm^{-1} 1690 (CO); δ_H[$(CD_3)_2SO$] 9.79 (1 H, s, 1- and 4-H).

5,6,8-Triacetoxyphthalazine **11**.—Boron trifluoride–diethyl-ether (8 cm^3) was added to a stirred mixture of phthalazine-5,8-dione (300 mg, 1.88 mmol) in acetic anhydride (8 cm^3) and maintained at room temperature for 36 h. The mixture was poured into crushed ice and then extracted with chloroform. Evaporation of the solvent gave a brown residue which was purified by flash chromatography (ethyl acetate–light petroleum 9:1) to give *5,6,8-triacetoxyphthalazine* (68 mg, 12%), m.p. 280–282 °C (Found: C, 48.2; H, 3.7; N, 7.6. $C_{14}H_{12}N_2O_6$ requires C, 47.8; H, 3.4; N, 7.95%); ν_{max}/cm^{-1} 2980 (CH), 1770 (CO) and 1740 (CO); δ_H[$(CD_3)_2SO$] 3.82 (3 H, s, CH_3), 3.87 (6 H, s, 2 × CH_3), 7.21 (1 H, s, 7-H) and 9.81 (2 H, s, 1- and 4-H); *m/z* 304 (M^+, 5%), 262 (17), 220 (33) and 178 (100).

2,3-*Dihydro*-5,8-*dimethoxyphthalazine*-1,4-*dione* **14**.—Hydrazine hydrate (98%, 3.9 g) was added to a warmed solution of dimethyl 3,6-dimethoxyphthalate[6] (0.4 g) in ethanol (50 cm^3) and refluxed for 48 h. Evaporation of the volatile material left a solid which was crystallised from methanol to give yellow 2,3-*dihydro*-5,8-*dimethoxyphthalazine*-1,4-*dione* (0.33 g, 77%), m.p. 244–246 °C (Found: C, 53.9; H, 4.4; N, 12.6. $C_{10}H_{10}N_2O_4$ requires C, 54.05; H, 4.5; N, 12.6%); ν_{max}/cm^{-1} 3290 (NH) and 1650 (CO); $\delta_H(CDCl_3)$ 3.66 (6 H, s, 2 × OCH_3), 5.27 (2 H, s, exchanged with D_2O, 2 × NH) and 6.31 (2-H, s, 6- and 7-H); *m/z* 222 (M^+, 100%), 221 (24) and 206 (23).

5,8-*Dimethoxy*-3-*methyl*-(2H)-*phthalazine*-1,4-*dione* **15**.—A mixture of dimethyl 3,6-dimethoxyphthalate (1.5 g, 5.9 mmol) and methylhydrazine (6.8 g, 7.7 mmol) in ethanol (200 cm^3) was heated at reflux until all the ester was consumed (TLC) and the solvent evaporated to half volume. The cold solution deposited a solid which was recrystallised from ethanol to yield 5,8-*dimethoxy*-3-*methyl*-(2H)-*phthalazine*-1,4-*dione* (0.95 g, 68%), m.p. 160–170 °C (Found: C, 55.55; H, 5.1; N, 11.7; $C_{11}H_{12}N_2O_4$ requires C, 55.9; H, 5.1; N, 11.9%); ν_{max}/cm^{-1} 3270 (NH), 1750 (CO) and 1700 (CO); $\delta_H[(CD_3)_2SO]$ 2.54 (3 H, s, NCH_3), 3.72 (2 H, s, OCH_3), 5.82 (1 H, s, exchanged with D_2O, NH) and 7.53 (2 H, s, 6- and 7-H); *m/z* 236 (M^+, 45%), 221 (16) and 209 (100).

2,3-*Dihydro*-5-*hydroxy*-8-*methoxyphthalazine*-1,4-*dione* **16**.—A mixture of dimethyl 3-hydroxy-6-methoxyphthalate[7] (0.5 g), hydrazine hydrate (3.9 g, 98%) and ethanol (50 cm^3) was boiled under reflux for 12 h. Evaporation to small volume gave a solid which was crystallised from water to give the *title compound* (0.42 g, 84%), m.p. 248–250 °C (Found: C, 51.9; H, 3.8; N, 13.5. $C_9H_8N_2O_4$ requires C, 51.9; H, 3.9; N, 13.5%); ν_{max}/cm^{-1} 3370 (OH), 3250 (NH) and 1680 (CO); $\delta_H[(CD_3)_2SO]$ 3.72 (3 H, s, OCH_3), 6.83 (1 H, d, *J* 8, 6-H), 7.02 (1 H, d, *J* 8, 7-H) and 8.61 (2 H, br s, exchanged with D_2O, OH and NH); *m/z* 208 (M^+, 100%), 193 (80), 164 (15) and 135 (22).

2,3-*Dihydro*-5,8-*dimethoxy*-2,3-*dimethylphthalazine*-1,4-*dione* **17**.—A mixture of 5,8-dimethoxy-2-methylphthalazine-1,4-dione (0.3 g, 1.3 mmol), methyl iodide (0.26 g, 1.5 mmol), potassium carbonate (0.2 g) in acetone (15 cm^3) was heated at reflux for 3 h, and the solid removed. Evaporation of the filtrate gave a solid which was crystallised from ethanol to give 2,3-*dihydro*-5,8-*dimethoxy*-2,3-*dimethylphthalazine*-1,4-*dione*, (0.21 g, 67%), m.p. 246–248 °C (Found: C, 57.9; H, 5.5; N, 11.0. $C_{12}H_{14}N_2O_4$ requires C, 57.6; H, 5.6; N, 11.2%); ν_{max}/cm^{-1} 1750 and 1710 (CO); $\delta_H[(CD_3)_2SO]$ 2.54 (6 H, s, 2 × NCH_3), 3.89 (6 H, s, 2 × OCH_3) and 7.46 (2 H, s, 6- and 7-H); *m/z* 250 (M^+, 48%) 236 (14), 221 (12) and 208 (100).

Formation of **18** *and* **19** *by CAN Oxidation of* **14** *and* **16**, *Respectively. General Method*.—A solution of CAN (2.9 g, 5.3 mmol) in water (10 cm^3) was added dropwise over 10 min to a stirred solution of 2,3-dihydro-5,8-dimethoxyphthalazine-1,4-dione (0.32 g, 1.44 mmol) in acetonitrile–water (2:1, 30 cm^3) and the mixture was stirred for 4 h. Water (15 cm^3) was added and the mixture extracted.

The dichloromethane extract of the product from **14** yielded a solid which was crystallised from water to provide yellow 3,6-*dimethoxyphthalimide* (190 mg, 68%), m.p. 221–223 °C (Found: C, 57.7; H, 4.6; N, 6.4. $C_{10}H_9NO_4$ requires C, 58.0; H, 4.35; N, 6.8%); ν_{max}/cm^{-1} 3200 (NH), 1780 and 1740 (CO); $\delta_H[(CD_3)_2SO]$ 3.85 (6 H, s, 2 × OCH_3) and 7.36 (2 H, s, 5- and 6-H); *m/z* 207 (M^+, 100%), 178 (43), 160 (84) and 133 (88).

The ethyl acetate extract of the reaction mixture from **16** gave a solid which was crystallised from water to give pale yellow N-*amino*-3-*hydroxy*-6-*methoxyphthalimide* (230 mg, 77%), m.p. 240–242 °C (Found: 50.0; H; 4.4; N, 12.8. $C_9H_8N_2O_4{\cdot}H_2O$ requires C, 49.8; H, 4.1; N, 12.9%); ν_{max}/cm^{-1} 3650 and 3530 (NH_2), 3350 (OH) and 1670 (CO); $\delta_H[(CD_3)_2SO]$ 3.85 (3 H, s, OCH_3), 7.16 (1 H, d, *J* 8, 6-H), 7.47 (1 H, d, *J* 8, 5-H), 12.12 (2 H, s, exchanged with D_2O, NH_2) and 12.84 (1 H, s, exchanged with D_2O, OH); *m/z* 208 (100%), 193 (93) and 165 (21).

N-*Ethyl*-3,6-*dimethoxyphthalimide* **20**.—Methyl iodide (9.15 g, 1.05 mmol) was added to a mixture of 3,6-dimethoxyphthalimide (0.2 g, 0.94 mmol), acetone (100 cm^3) and potassium carbonate (0.5 g), and refluxed for 6 h. The solid was removed, the liquid evaporated and the residue crystallised from diisopropyl ether to give light yellow N-*ethyl*-3,6-*dimethoxyphthalimide* (0.13 g, 58%), m.p. 169–170 °C (Found: C, 61.6; H, 5.5; N, 6.0. $C_{12}H_{13}NO_4$ requires C, 61.3; H, 5.6; N, 5.95); ν_{max}/cm^{-1} 1750 (CO); $\delta_H(CDCl_3)$ 1.25 (3 H, t, *J* 3, CH_3), 3.69 (2 H, q, *J* 7, CH_2), 3.93 (6 H, s, 2 × OCH_3) and 7.13 (2 H, s, 5- and 6-H); *m/z* 235 (M^+, 72%) 220 (33) and 206 (100).

N-*Hydroxymethyl*-3,6-*dimethoxyphthalimide* **21**.—A mixture of 3,6-dimethoxyphthalimide (50 mg, 0.242 mmol), water (15 cm^3) and aqueous formaldehyde (0.2 g, 37% w/v) was refluxed for 36 h. The solid was removed from the cold mixture and crystallised from water to yield yellow N-*hydroxymethyl*-3,6-*dimethoxyphthalimide* (70 mg, 64%), m.p. 236–238 °C (Found: C, 54.2; H, 4.9; N, 5.7. $C_{11}H_{11}NO_5{\cdot}\frac{1}{2}H_2O$ requires C, 53.9; H, 4.9; N, 5.7%); ν_{max}/cm^{-1} 3450–3200 (OH) and 1725 (CO); $\delta_H(CDCl_3)$ 3.38, (1 H, s, exchanged with D_2O, OH), 3.97 (6 H, s, 2 × OCH_3), 4.1 (2 H, s, NCH_2) and 7.25 (2 H, s, 5- and 6-H); *m/z* 237 (M^+, 100%), 236 (34), 220 (16) and 206 (70).

N-*Ethoxycarbonyl*-3,6-*dimethoxyphthalimide* **22**.—Triethylamine (2.29 g, 2.9 mmol) was added to a mixture of 3,6-dimethoxyphthalimide (0.3 g, 1.45 mmol), ethyl chloroformate (0.32 g, 2.9 mmol) and dimethylformamide (100 cm^3) and heated at reflux for 24 h. Removal of the volatile material under vacuum gave a solid which was purified by flash chromatography (ethyl acetate–methanol, 8.5:1.5) gave N-*ethoxycarbonyl*-3,6-*dimethoxyphthalimide* (190 mg, 48%), m.p. 278–279 °C (Found: C, 49.6; H, 5.0; N, 4.9. $C_{13}H_{13}NO_6{\cdot}2H_2O$ requires C, 49.5; H, 5.4; N, 4.5%); ν_{max}/cm^{-1} 1730 (CO) and 1700 (CO); $\delta_H(CD_3OD)$ 1.33 (3 H, t, *J* 3, CH_3), 4.21 (2 H, q, *J* 9, OCH_2) and 7.32 (2-H, s, 5- and 6-H); *m/z* 279 (M^+, 13%), 206 (28) and 193 (100).

N-*Hydroxy*-3,6-*dimethoxyphthalimide* **23**.—A solution of *N*-ethoxycarbonyl-3,6-dimethoxyphthalimide (0.2 g, 0.72 mmol) in ethanol (50 cm^3) was added to a stirred, hot solution of hydroxylamine hydrochloride (0.5 g) and triethylamine (0.071 g) in ethanol (20 cm^3). After 10 min the mixture was poured into dilute hydrochloric acid (50 cm^3) and extracted with chloroform. Removal of the solvent gave a solid which was crystallised from aqueous methanol to yield N-*hydroxy*-3,6-*dimethoxyphthalimide* (90 mg, 56%), m.p. 225–227 °C (Found: C, 49.6; H, 4.7; N, 5.7. $C_{10}H_9NO_5{\cdot}H_2O$ requires C, 49.8; H, 4.6; N, 5.8%); ν_{max}/cm^{-1} 3370 (OH) and 1730 (CO); $\delta_H[(CD_3)_2SO]$ 4.3 (1 H, br s, exchanged with D_2O, OH), 3.82 (6 H, s, OCH_3) and 7.28 (2 H, s, 5- and 6-H); *m/z* 223 (M^+, 7%), 207 (29) and 194 (100).

N-*Phenacyl*-3,6-*dimethoxyphthalimide* **24**.—A mixture of 3,6-dimethoxyphthalimide (50 mg, 0.24 mmol), phenacyl bromide (80 mg, 0.4 mmol), potassium carbonate (0.5 g) and acetone (50 cm^3) was heated at reflux for 12 h, cooled, and the solid filtered off. Evaporation of the filtrate and chromatography (ethyl acetate–light petroleum, 8:2) of the residue yielded a solid which crystallised from ethyl acetate as yellow N-*phenacyl*-3,6-*dimethoxyphthalimide* (63 mg, 57%), m.p. 217–218 °C (Found: C, 66.1; H, 4.8; N, 4.25. $C_{18}H_{15}NO_5$ requires C, 66.5; H, 4.65; N,

4.3%); ν_{max}/cm^{-1} 1750 (CO) and 1700 (CO); δ_H($CDCl_3$) 3.93 (6 H, s, 2 × OCH_3), 5.02 (2 H, s, CH_2) and 7.12–8.03 (7 H, m, 5- and 6-H and C_6H_5); *m/z* 325 (M^+, 21%), 221 (86) and 105 (100).

3-*Benzoyl*-4-*hydroxy*-5,8-*dimethoxyisoquinolin*-1(2H)-*one* **26**.—*N*-Phenacyl-3,6-dimethoxyphthalimide (100 mg) in methanol (20 cm^3) was added to methanolic sodium methoxide (10 cm^3; 2 mol dm^{-3}) and the mixture was heated at reflux under nitrogen until homogeneous (42 h) and for a further 2 h. Neutralisation of the mixture gave a solid which was purified by preparative TLC to give 3-*benzoyl*-4-*hydroxy*-5,8-*dimethoxyisoquinolin*-1(2H)-*one* (38 mg, 38%), m.p. 227–228 °C (Found: C, 66.5; H, 4.7; N, 4.25. $C_{18}H_{15}NO_5$ requires C, 66.5; H, 4.65; N, 4.3%); ν_{max}/cm^{-1} 3370 (OH), 3160 (NH), 1670 (CO) and 1660 (CO); δ_H[$(CD_3)_2SO$] 3.89 (3 H, s, CH_3), 4.12 (1 H, s, exchanged with D_2O, NH), 7.4–8.1 (7 H, m, 5- and 6-H and C_6H_5) and 9.34 (1 H, br s, exchanged with D_2O, OH); *m/z* 325 (M^+, 100%) and 221 (80).

N-*Amino*-3,6-*dimethoxyphthalimide* **25**.—A mixture of *N*-amino-3-hydroxy-6-methoxyphthalimide (0.15 g, 0.72 mmol) methyl iodide (0.1 g, 0.69 mmol), potassium carbonate (0.02 g), methyl trichloroacetate (0.12 g, 0.675) and 18-crown-6 (0.02 g) was warmed in a flask fitted with a distillation head and receiver flask. Carbon dioxide evolution started at 110 °C and the temperature was maintained at 150 °C for 2 h when gas evolution had ceased and chloroform was no longer being collected in the receiver flask cooled with dry ice. Water was added to the reaction mixture, the solid collected and crystallised from diisopropyl ether as N-*amino*-3,6-*dimethoxyphthalimide* (0.13 g, 81%), m.p. 206–207 °C (Found: C, 54.1 H, 4.6; N, 12.4. $C_{10}H_{10}N_2O_4$ requires C, 54.05; H, 4.5; N, 12.6%): ν_{max}/cm^{-1} 3420 and 3380 (NH_2), 1720 (CO) and 1690 (CO); δ_H 3.74 (6 H, s, 2 × CH_3), 4.65 (2 H, br s, exchanged with D_2O, NH_2) and 7.12 (2 H, s, 5- and 6-H); *m/z* 222 (M^+, 100%).

trans-1-(3,6-*Dimethoxyphalimido*)-2,3-*diphenylaziridine* **27**.—Lead tetraacetate (0.09 g) was added to *N*-amino-3,6-dimethoxyphthalimide (0.05 g, 0.225 mmol) and *trans*-stilbene (0.2 g, 1.12 mmol) in dry dichloromethane (15 cm^3) and stirred for 30 min. The solid was filtered off and washed with dichloromethane. The combined filtrate and washings was evaporated, the residue purified by flash column chromatography (ethyl acetate–petroleum spirit, 7:3), and crystallised from ethanol to give trans-(3,6-*dimethoxyphthalimido*)-2,3-*diphenylaziridine* (70 mg, 78%), m.p. 268–270 °C (Found: C, 71.75; H, 5.0; N, 7.0. $C_{24}H_{20}N_2O_4$ requires C, 72.0; H, 5.0; N, 7.0%); ν_{max}/cm^{-1} 1760 (CO) and 1710 (CO); δ_H($CDCl_3$) 3.87 (6 H, s, 2 × CH_3), 3.9 (1 H, d, *J* 7, CH), 4.95 (1 H, d, *J* 7, CH) and 7.07–7.6 (12 H, m, 6- and 7-H and 2 × C_6H_5); *m/z* 400 (M^+, 22%) ,296 (100), and 194 (78).

N-(3,6-*dimethoxyphthalimido*)*maleamic acid* **28**.—A mixture of *N*-amino-3,6-dimethoxyphthalimide (50 mg, 0.22 mmol), maleic anhydride (0.86 g, 0.84 mmol) and acetonitrile (50 cm^3) was refluxed for 8 h and then the solvent was removed. The residue was purified by flash column chromatography (ethyl acetate:methanol, 7:3) and crystallised from water as N-(3,6-*dimethoxyphthalimido*)*maleamic acid* (65 mg, 93%), m.p. 180–192 °C (Found: C, 50.5; H, 3.7; N, 8.9. $C_{14}H_{12}N_2O_7 \cdot \frac{1}{4}H_2O$ requires C, 50.4; H, 3.7; N, 8.5%); ν_{max}/cm^{-1} 3300–2200 (OH), 1740 (CO) and 1700 (CO); δ_H[$(CD_3)_2SO$] 3.80 (6 H, s, 2 × CH_3), 6.16 (1 H, d, *J* 12, NCHOC*H*), 6.53 (1 H, d, *J* 12, C*H*CO_2H), 7.51 (2 H, s, 5- and 6-H) and 10.55 (2 H, br s, s, exchanged with D_2O, NH and CO_2H); *m/z* 302 (M − H_2O, 28%) and 207 (199).

N-(3,6-*Dimethoxyphthalimido*)*maleimide* **29**.—A mixture of *N*-(3,6-dimethoxyphthalimido)maleamic acid (1.5 g, 4.68 mmol), acetic anhydride (1.43 g, 14 mmol) and anhydrous sodium acetate (0.03 g, 0.468 mmol) was heated at 100 °C for 20 min. Water was added to the reaction mixture followed by aqueous sodium hydrogen carbonate to give a basic liquid which was extracted with dichloromethane. The extract yielded a solid which was crystallised from water to afford N-(3,6-*dimethoxyphthalimido*)*maleimide* (1.2 g, 73%), m.p. 216–217 °C (Found: C, 54.8; H, 3.8; N, 9.4. $C_{14}H_{10}N_2O_6 \cdot \frac{1}{4}H_2O$ requires C, 54.8; H, 3.4; N, 9.15%); ν_{max}/cm^{-1} 1730 (CO); δ_H(CD_3OD) 3.98 (6 H, s, 2 × CH_3), 7.21 (2 H, s, CH=CH) and 7.58 (2 H, s, 5- and 6-H); *m/z* 302 (M^+, 88%) and 206 (100).

3-*Butylthio*-N-(3,6-*dimethoxyphthalimido*)*succinimide* **30**.—*N*-(3,6-dimethoxyphthalimido)maleimide (50 mg, 0.16 mmol) in sodium acetate–acetic acid buffer (10 cm^3, pH 5) and butanethiol (14 mg, 0.16 mmol) were stirred together at room temperature for 24 h. Water was added and the mixture extracted with chloroform. The extract yielded pure 3-*butylthio*-N-(3,6-*dimethoxyphthalimido*)*succinimide* (48 mg, 80%), m.p. 268–270 °C (decomp.) (Found: C, 54.8; H, 5.0; N, 6.8. $C_{18}H_{20}N_2O_6S$ requires C, 55.2, H, 4.9; N, 7.2%); ν_{max}/cm^{-1} 1740 (CO) and 1730 (CO); δ_H[$(CD_3)_2SO$] 1.2 (3 H, t, *J* 3, CH_3), 2.81 (4 H, m, 2 × CH_2), 2.96 (2 H, m, SCH_2), 3.87 (1 H, m, 3-H), 3.98 (6 H, s, 2 × CH_3), 4.06 (2 H, m, succin CH_2) and 7.26 (2 H, s, 4- and 5-H); *m/z* 392 (M^+, 1%), 335 (M^+ − Bu, 10) and 206 (100).

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