

THE ELECTRONIC EFFECTS ON THE FORMATION OF *N*-ARYL-MALEIMIDES AND ISOMALEIMIDES

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Abstract– The synthesis of a range of aromatic maleimides and isomaleimides is reported. The effect of different substituents on the aromatic system and the products formed is also discussed.

N-Maleimide derivatives and especially *N*-alkyl/arylmaleimides have numerous applications in organic synthesis,¹ polymer chemistry^{2,3} and biochemistry.⁴ For example different *N*-substituted maleimide derivatives are used as precursors in the synthesis of terpenes and prostacyclin analogues. It was also reported that the 5-yildenpyrrol-2(5*H*)-one structural unit can be easily synthesised in high yield from a range of *N*-maleimides therefore enabling the production of biologically important natural products including holomycin and pukeleimide.⁵ In biochemistry *N*-maleimides react rapidly with thiols forming a stable thioether *via* Michael addition and are frequently used to react with the thiol group of cysteine in protein labelling.⁶ In a similar approach, reaction of thiolated DNA with fluorescent *N*-maleimides has also been reported as a method for labelling of oligonucleotide probes for use in bioanalysis.⁷

Recently we have reported the use of Diels-Alder cycloaddition as a method of labelling oligonucleotides using diene tagged oligonucleotides and a variety of *N*-maleimides.^{8,9} The *N*-maleimides used have been commercially available fluorophores and also specifically designed and synthesised dyes for use in surface enhanced resonance Raman scattering (SERRS) studies. The method chosen for the synthesis of all the *N*-maleimides used in our previous studies involved the addition of maleic anhydride to a primary amine followed by ring closure. During these studies it was noticed that two products were formed and the dominance of one particular product could be affected by the conditions and the nature of the amine initially used. The two products were either the desired maleimide or the undesired product, isomaleimide (Figure 1). To be useful in further reaction either by Diels-Alder cycloaddition or Michael addition the maleimide must be used as the isomaleimide was found to be unreactive. The study reported

here shows how different substituents on the aromatic ring of the amine used affect the production of the desired maleimide and how a hypothesis can now be drawn that relates the nature of the amine to the product formed.

Although synthesis of *N*-arylmaleimides by cyclisation of the substituted *N*-maleamic acid generally proceeds well, synthesis of *N*-alkylmaleimides is less successful. Thus, in order to investigate the effect of different amines, the starting material chosen was that of aromatic amines with a variety of substituents. The first step in the procedure was the same for all compounds examined and involved the addition of maleic anhydride in DCM to the aromatic amine in DCM. The *N*-maleamic acid formed was easily isolated by filtration and then used in the cyclisation experiments.

A range of methods for maleamic acid cyclisation were investigated and the subsequent products identified. The synthesis of *N*-(4-aminophenyl)maleimide was the first attempted using acetic anhydride and sodium acetate as described by Cava¹⁰ and the maleamic acid (**1**) produced from *p*-phenylenediamine. However, compound (**2**) was formed as expected and the subsequent cleavage of the acetyl-amide was unsuccessful leading instead to the undesired opening of maleimide ring. To avoid the use of the large excess of acetic anhydride a range of other methods were used (Table 1) which all lead to the formation of isomaleimide (**3**) in 63 to 75 % yield depending on the procedure.

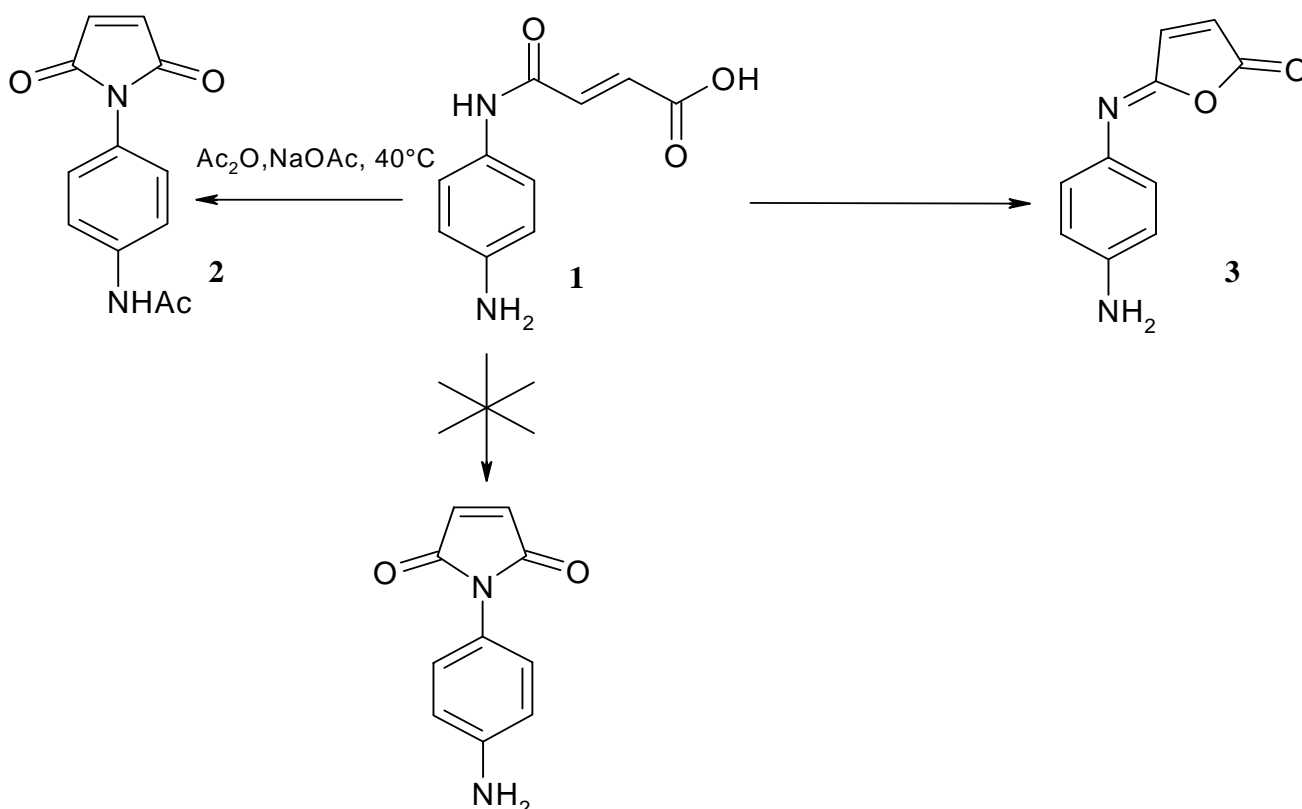


Figure 1

Table 1. Reagents and products of different cyclisation methods used.

Reagents and conditions	Product
acetic anhydride, sodium acetate, 40°C, reflux, 40 min	2
Co (II) naphthenate, acetic anhydride, 70°C, reflux, 1 h	3
DCC (1.2 and 3 eqv.), HOBt, DCM, 12 h	3
DCC, HOBt, dry DMF, rt, 12 h	3
DCC, HOBt, DMA, 40°C, 1 h	3
morpho CDI, HOBt, DCM, rt and 50°C, 12 h	3

Most of the reagents used were standard activating agents for carboxylic acids except for the cobalt naphthenate. Cobalt naphthenate was used by Corrie in the preparation of fluorescent *N*-maleimides.¹¹ The role of the cobalt is not fully understood but is presumed to act as a coordination template to assist cyclisation. Previously it was reported that reactions conducted at low temperatures tended to favour isomaleimide formation¹² but although the temperature was increased in some of the reactions only the isomaleimide (**3**) was isolated.

In view of published results on the cyclisation of *N*-maleamic acids,^{5,13,14} the formation of isomaleimide under all of the above conditions was not expected. Gill *et al.*⁵ prepared a series of simple maleimides in high yields by reaction of the appropriate maleic anhydride with either ammonium acetate or methylammonium acetate in boiling acetic acid. Chaurasia and coworkers¹³ synthesised 3-maleimidocoumarin in 40% yield using morpho CDI but did not mention any side products. *N*-(4-Aminophenyl)- and *N*-(4-isothiocyanophenyl)maleimides were also prepared in other research labs by DCC-HOBt procedure, which gave isomaleimide as the main product in our hands.¹⁴ However, it should be noted that many authors investigating the synthesis of imides did not give a proper account of possible side products.

Further consideration of the reactions indicated that there was likely to be an electronic basis behind the observed results. Brady and Hegarthy described the imide–isoimide rearrangements (Figure 2).¹⁵ They studied the kinetics of the rearrangement for a range of isoimides and determined the inductive effect of different substituents. They observed that electron donating R or X groups shift the equilibrium towards isoimide and explained this result with the steric effects that occur in the highly restricted transition state. Therefore the formation of the isomaleimide (**3**) as the only product can be attributed to the electron donating characteristic of the amino group on the aromatic ring. When the donating ability was decreased by acetylation, maleimide (**2**) was obtained.

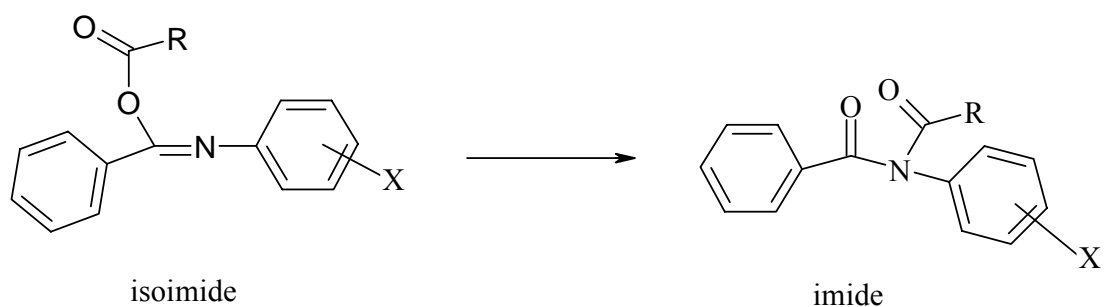


Figure 2

On the basis of this observation, it was concluded that the presence of the electron withdrawing nitro group on the aromatic ring would favour the formation of maleimide. Thus, *N*-(4-nitrophenyl)maleimide (**5**) was easily formed using acetic anhydride and sodium acetate (Figure 3).

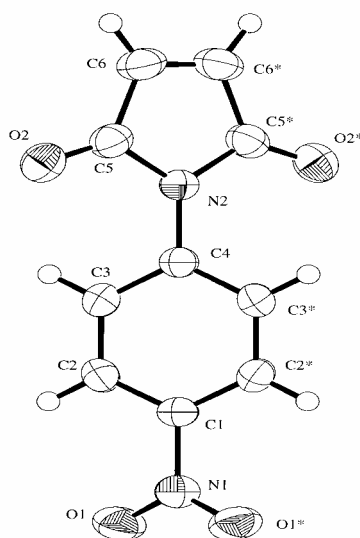


Figure 3. ORTEP diagram of compound (**5**)

However, reduction of the nitro group under a variety of conditions (hydrazine on graphite, Fe in acetic acid under inert atmosphere at different temperatures) failed to produce the desired amine. Although the products were not isolated, it can be assumed that the reduction of the maleimide double bond is favoured.¹⁴

m- and *p*-Anisidine were also used as reagents to make the corresponding maleamic acids which were then used in cyclisation reactions. The presence of the electron donating methoxy substituents was expected to give the isomaleimides (**7**) and (**9**) and was indeed the case.

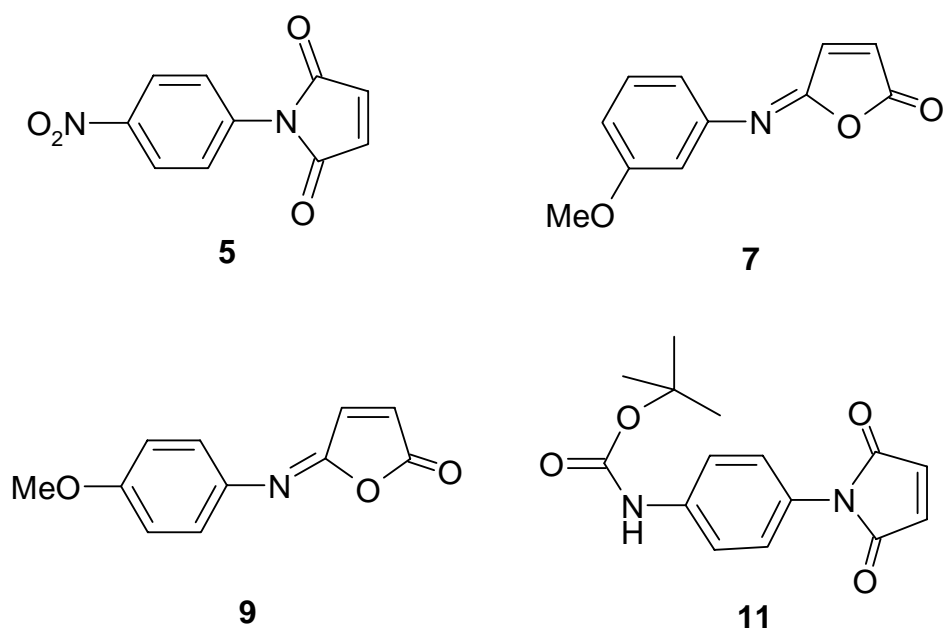


Figure 4.

In order to obtain *N*-(4-aminophenyl)maleimide another approach was adopted. The formation of the maleimide proceeds readily when the amino group was acetylated. Thus it was reasoned that if the amino group was protected with an alternative protecting group that did not utilise an amide linkage then the amine could be regenerated in the presence of the maleimide after the desired ring closure. The protecting group chosen for this was di-*t*-butyl carbonate, BOC which can be removed either thermally or by acidic hydrolysis. Maleimides are stable to acidic conditions making BOC a compatible protecting group. The protection had two functions; firstly it prevented acetylation of the amine during ring closure by acetic anhydride and secondly, it decreased the electron donating character of the amino group therefore favouring the formation of the maleimide. Maleimide (**11**) was obtained in excellent yield using morpho CDI and HOBt as the reagents for cyclisation. Removal of the BOC group was unsuccessful after several attempts with trifluoroacetic acid in different concentrations, hydrochloric acid and thermal deprotection. This means that a route to the *p*-aminophenylmaleimide has not been obtained but significant information on the formation of maleimides as opposed to isomaleimides has been gathered.

In conclusion, a range of aromatic isomaleimides and maleimides were formed to investigate the imide–isoimide formation under a variety of conditions. The use of substituted aromatics allowed the effect of the substituent on maleimide formation to be determined. This indicated that the presence of an electron donating group favours the isomaleimide formation and is attributed to the favouring of the imino form of the amide that can then attack the carboxylic acid. Electron withdrawing groups favour the formation of the maleimide and we propose that this allows the nitrogen of the amide to attack the carboxylic acid. This information is useful in designing the synthesis of new maleimides for use in a range of applications including biological labelling.

EXPERIMENTAL

General. All chemicals were purchased from Aldrich Ltd. ^1H NMR spectra were recorded on a Brücker 400 (400.00 MHz) instrument. MS spectra were recorded on a JEOL AX505 spectrometer using chemical ionization (CI) or fast atom bombardment (FAB) in a 3-nitrobenzyl alcohol matrix. Flash chromatography was carried out using silica gel 60 (Merck). Thin layer chromatography was carried out on aluminium sheets, coated with silica gel 60 F₂₅₄, 0.2 mm layer (Merck) (A) DCM-MeOH (9:1); (B) EtOAc-Hexane 1:1.5. Melting points were measured using a Buchi melting point B-545 apparatus and are uncorrected.

***N*-(4-Aminophenyl)maleamic acid (1).** 1,4-Phenylenediamine (2.16 g, 20 mmol) was dissolved in DCM (20 mL) and maleic acid anhydride (1.96 g, 20 mmol) in DCM (5 mL) added drop wise over 30 min. After 12 h of stirring at rt, the resulting precipitate was collected, washed well with DCM and dried, giving the title product (3.93 g, 95%) as an orange powder, mp 179-181 °C (decomp). R_f (A) 0.05. ^1H NMR δ_{H} (DMSO- d_6): 6.27 (1H, d, J 12.0, CHCHCO₂H), 6.46 (1H, d, J 12.0, CHCHCO₂H), 6.53 (2H, d, J 8.7, Ar-H), 7.31 (2H, d, J 8.7, Ar-H), 10.49 (1H, br s, -CO₂H). FAB-MS m/z 207.07697 [C₁₀H₁₀N₂O₃ (M+H)⁺ < 1.5 ppm]. Anal. Calcd for C₁₀H₁₀N₂O₃: C, 58.25; H, 4.49; N, 13.60. Found: C, 58.28; H, 5.07; N, 13.13.

***N*-(4-Acetylamidophenyl)maleimide (2).** Compound (1) (0.28 g, 1.4 mmol) was added to a solution of sodium acetate (0.11 g, 1.4 mmol) in acetic anhydride (5 mL). The reaction mixture was refluxed and after 5 min the acid dissolved and colour of the solution changed to pale yellow. Stirring was continued for 1 h until TLC showed the reaction to be complete. Water was added to the cooled solution and the pale yellow precipitate collected, washed with water and dried. The pure product was obtained after recrystallisation from methanol as pale yellow powder (0.24 g, 85 %), mp 190-192 °C (decomp). R_f (A) 0.48. ^1H NMR δ_{H} (DMSO- d_6): 2.80 (3H, s, CH₃), 7.00 (2H, s, CH=CH), 7.27 (2H, d, J 8.8, Ar-H), 7.73 (2H, d, J 8.8, Ar-H), 9.25 (1H, s, NH). FAB-MS m/z 231.0769. [C₁₂H₁₁N₂O₃ (M+H)⁺ < 6.3 ppm]. Anal. Calcd for C₁₁H₁₀N₂O₃: C, 58.54; H, 4.10; N, 11.38. Found: C, 59.46; H, 4.51; N, 11.05.

***N*-(4-Aminophenyl)isomaleimide (3).** *Method 1.* *N*-(4-Aminophenyl)maleamic acid (1) (1.03 g, 5.0 mmol) was dissolved in DCM (20 mL) and cooled to 0°C. 1-Hydroxybenzotriazole (0.70 g, 5.0 mmol) and dicyclohexylcarbodiimide (1.24 g, 6.0 mmol) were added respectively. The colour of the solution changed to red after 2 h of stirring. After 12 h at rt, the precipitate was removed by filtration and the solvent removed under reduced pressure before the crude product was purified by column chromatography eluting with MeOH in DCM (1:9) to give the title compound as an orange powder (0.60 g, 63%). *Method 2.* To a solution of acid derivative (2) (0.26 g, 1.26 mmol) in DMA (2 mL), cobalt naphthenate solution (0.01 mL, 0.06 g in 10 mL DMA) was added under nitrogen and heated to 40° C. Acetic acid anhydride (0.12 mL, 2.52 mmol) was then added and the reaction mixture warmed to 70-80°C

and stirred for 2 h. The solution was cooled, diluted with water and the precipitate removed by filtration before dissolution in DCM. The orange powder obtained after purification by flash chromatography with ethyl acetate/hexane (1:1.5) and recrystallisation from ethyl acetate was identified as the title compound (0.17 g, 72 %), mp 185 °C. R_f (B) 0.30. ^1H NMR δ_{H} (acetone- d_6): 5.10 (2H, br s, NH_2), 6.65 (2H, d, J 8.8, Ar-H), 6.69 (1H, d, J 5.5, $\text{CH}=\text{CH}$), 7.35 (2H, d, J 8.8, Ar-H), 7.47 (1H, d, J 5.5, $\text{CH}=\text{CH}$). FAB-MS m/z 189.1262 [$\text{C}_{10}\text{H}_9\text{N}_2\text{O}_2$ ($\text{M}+\text{H}$) $^+$ < 1.8 ppm]. Anal. Calcd for $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_2$: C, 62.78; H, 4.68; N, 14.07. Found: C, 62.48; H, 5.89; N, 13.55.

***N*-(4-Nitrophenyl)maleamic acid (4)** Maleic acid anhydride (2.17 g, 22 mmol) was dissolved in THF (2 mL) and slowly added to a solution of 4-nitroaniline (1.53 g, 11 mmol) in THF (10 mL) and left to stir overnight at rt. The yellow precipitate was collected, washed well with THF and dried giving the title compound as pale yellow powder (2.16 g, 83%), mp 193 °C. R_f (A) 0.08; ^1H NMR δ_{H} (DMSO- d_6): 6.34 (1H, d, J 12.0, CHCHCO_2H), 6.52 (1H, d, J 12.0, CHCHCO_2H), 7.86 (2H, d, J 9.2, Ar-H), 8.24 (2H, d, J 9.0, Ar-H), 10.87 (1H, s, NHCO), 12.85 (1H, br s, CO_2H). FAB-MS m/z 237.05123 [$\text{C}_{10}\text{H}_8\text{N}_2\text{O}_5$ (M) $^+$ < 0.7 ppm]. Anal. Calcd for $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_5$: C, 50.85; H, 3.41; N, 11.86. Found: C, 50.90; H, 3.40; N, 11.56.

***N*-(4-Nitrophenyl)maleimide (5).** Following the same procedure as for the synthesis of maleimide (3), *N*-(4-nitrophenyl)maleamic acid (4) (2.16 g, 9.1 mmol) and sodium acetate (0.89 g, 10.9 mmol) were stirred in acetic acid anhydride (25 mL) at rt to give the title compound as a pale yellow powder (1.47 g, 74%) which was recrystallised from methanol giving colourless plates, mp 162-164°C; R_f (A) 0.38. ^1H NMR δ_{H} (DMSO- d_6): 7.26 (2H, s, $\text{CH}=\text{CH}$), 7.70 (2H, d, J 5.0, Ar-H), 8.36 (2H, d, J 5.1, Ar-H); CI-MS m/z 219.04058 [$\text{C}_{10}\text{H}_7\text{N}_2\text{O}_4$ ($\text{M}+\text{H}$) $^+$ < 2.5 ppm]. Anal. Calcd for $\text{C}_{10}\text{H}_6\text{N}_2\text{O}_4$: C, 55.10; H, 2.80; N, 12.84. Found: C, 54.80; H, 2.67; N, 12.63. Crystal structure deposited in Cambridge Crystal Database, accession number = 212029.

***N*-(3-Methoxyphenyl)maleamic acid (6)** Maleic acid anhydride (1.60 g, 16 mmol) was dissolved in THF (2 mL) and slowly added to the solution of 3-methoxy aniline (0.91 mL, 0.8 mmol) in THF (10 mL) and left to stir overnight at rt. The precipitate was collected, washed well with THF and dried giving the title compound as a pale yellow powder (0.91 g, 81%), mp 160 °C. R_f (A) 0.1. ^1H NMR δ_{H} (DMSO- d_6): 3.73 (3H, OCH_3), 6.31 (1H, d, J 12, CHCHCO_2H), 6.45 (1H, d, J 12, CHCHCO_2H), 6.66 (1H, d, J 8.2, Ar-H), 7.14 (1H, d, J 8.0, Ar-H), 7.22 (1H, t, J 8.0, Ar-H), 7.31 (1H, s, Ar-H), 10.35 (1H, s, NHCO), 13.05 (1H, br s, CO_2H). FAB-MS m/z 222.07663 [$\text{C}_{11}\text{H}_{12}\text{NO}_4$ ($\text{M}+\text{H}$) $^+$ < -4.2 ppm]. Anal. Calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.75; H, 5.11; N, 6.14.

***N*-(3-Methoxyphenyl)isomaleimide (7).** Following the same procedure as for the synthesis of maleimide (3), *N*-(3-methoxyphenyl)maleamic acid (0.55 g, 2.5 mmol) and sodium acetate (0.25 g, 3.0 mmol) were stirred for 30 min in acetic acid anhydride (10 mL) at rt and then extracted with DCM. The organic layer

was dried over MgSO_4 and evaporated to give residue. Purification by column chromatography with DCM-methanol (9:1) afforded the title compound as a yellow oil (0.42 g, 82 %). $R_f(\text{A})$ 0.45. ^1H NMR $\delta_{\text{H}}(\text{DMSO}-d_6)$: 3.73 (3H, s, OCH_3), 6.83 (2H, d, J 8.3, Ar-H), 6.90 (1H, s, Ar-H), 7.11 (1H, d, J 5.6, $\text{CH}=\text{CH}$), 7.28 (1H, m, Ar-H), 7.80 (1H, d, J 5.6, $\text{CH}=\text{CH}$). FAB-MS m/z 204.06605 [$\text{C}_{11}\text{H}_9\text{NO}_3(\text{M})^+$ < 0.1 ppm]. Anal. Calcd for $\text{C}_{11}\text{H}_9\text{NO}_3$: C, 64.90 ; H, 4.43; N, 6.90. Found: C, 64.71 ; H, 4.82; N, 6.26.

***N*-(4-Methoxyphenyl)maleamic acid (8).** Maleic acid anhydride (0.80 g, 8 mmol) was dissolved in THF (2 mL) and slowly added to a solution of 4-methoxyaniline (0.50 ml, 4.0 mmol) in THF (10 mL) and left to stir overnight at rt. The precipitate was collected, washed well with THF and dried giving title compound as pale yellow powder (0.90 g, 91 %), mp 142 °C. $R_f(\text{A})$ 0.05. ^1H NMR $\delta_{\text{H}}(\text{DMSO}-d_6)$: 3.81 (3H, OCH_3), 6.33 (1H, d, J 12.8, CHCHCO_2H), 6.67 (1H, d, J 12.8, CHCHCO_2H), 6.97 (2H, d, J 8.5, Ar-H), 7.63 (2H, d, J 8.5, Ar-H), 10.27 (1H, s, NHCO), 14.50 (1H, br s, CO_2H). FAB-MS m/z 222.05815 [$\text{C}_{11}\text{H}_9\text{NO}_3(\text{M})^+$ < -4.0ppm]. Anal. Calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.47; H, 5.06; N, 6.09.

***N*-(4-Methoxyphenyl)isomaleimide (9).** Following the same procedure as for maleimide (3), *N*-(3-methoxyphenyl)maleamic acid (0.40 g, 1.8 mmol) and sodium acetate (0.17 g, 2.0 mmol) were stirred 50 min in acetic acid anhydride (10 mL) at rt. The precipitate was collected, washed well with water and dried. Recrystallisation from acetone afforded the title compound as yellow needles (0.28 g, 71 %). Method 2 using Co naphthenate afforded the same product in 75% yield after purification with column chromatography using DCM-methanol (9:1) and recrystallisation from acetone, mp 115 °C (decomp). $R_f(\text{A})$ 0.50. ^1H NMR $\delta_{\text{H}}(\text{DMSO}-d_6)$: 3.77 (3H, s, OCH_3), 6.98 (2H, d, J 7.0, Ar-H), 7.05 (1H, d, J 5.6, $\text{CH}=\text{CH}$), 7.40 (2H, d, J 7.0, Ar-H), 7.75 (1H, d, J 5.6, $\text{CH}=\text{CH}$); EI-MS m/z (203.057 [$\text{C}_{11}\text{H}_9\text{NO}_3(\text{M})^+$ < 0.7 ppm]. Anal. Calcd for $\text{C}_{11}\text{H}_9\text{NO}_3$: C, 64.90 ; H, 4.43; N, 6.90. Found: C, 64.44 ; H, 4.50 ; N, 6.53 .

4-(4-*tert* Butoxycarbonylaminophenyl)maleamic acid (10). *N*-(4-Aminophenyl)maleamic acid (1) (0.43 g, 2.04 mmol) was dissolved in a mixture of dioxane, water and a 1M solution of sodium hydroxide (10 mL:5 mL:5 mL) at 0 °C. Di-*tert*-butyl pyrocarbonate (0.48 g, 2.20 mmol) was added to the cooled solution and stirred for 45 min at rt. The reaction mixture was concentrated under reduced pressure to 10 mL, cooled in an ice bath, covered with a layer of ethyl acetate (10 mL) and acidified with dilute aqueous potassium hydrogen sulphate (1M) to pH 2-3. The aqueous phase was then extracted with ethyl acetate (3 x 15 ml) and the combined organic phase washed with water (2 x 30 mL), dried over anhydrous sodium sulphate and evaporated *in vacuo*. The residue was dissolved in EtOAc, filtered and the filtrate triturated with hexane to give the title compound as a bright yellow powder (0.48 g, 85 %), mp 165 °C. $R_f(\text{C})$ 0.05. ^1H NMR $\delta_{\text{H}}(\text{DMSO}-d_6)$: 1.40 (9H, s, 3 x CH_3), 6.29 (1H, d, J 12.0, CHCHCO_2H), 6.45 (1H, d, J 12.0, CHCHCO_2H), 7.39 (2H, d, J 8.9, Ar-H), 7.50 (2H, d, J 8.9, Ar-H), 9.32 (1H, br s, NHCO), 10.40 (1H, br

s, *NHCO*), 13.36 (1H, br s, *CO₂H*), CI-MS *m/z* (307.12950 [*C₁₈H₁₆N₂O₅* (*M*+*H*)⁺ <2.8 ppm]. Anal. Calcd for *C₁₈H₁₆N₂O₅*: C, 58.80; H, 5.90; N, 9.15. Found: C, 58.65; H, 5.71; N, 8.96.

4-(4-*tert*-Butoxycarbonylaminophenyl)maleimide (11). Compound (**10**) (0.25 g, 0.8 mmol) was added to DCM (50 mL) and the suspension cooled to 0°C. To the cooled stirred solution was added in order 1-hydroxybenzotriazole hydrate (0.13 g, 0.8 mmol) and morpho CDI (1.40 g, 3.3 mmol). After 5 h of stirring, the reaction mixture was diluted with DCM (30 mL) and extracted successively with water (3 x 50 mL), 1M hydrochloric acid (1 x 40 mL) and sodium bicarbonate (1M, 1 x 40 mL). The organic phase was dried (sodium sulfate) and the solvent removed. The title compound was obtained by recrystallisation from methanol as a pale yellow powder (0.19 g, 83%), mp 179 °C. *R_f* (C) 0.67. ¹H NMR δ_H(DMSO-*d*₆): 1.45 (9H, s, 3 x CH₃), 7.14 (2H, s, *CH=CH*), 7.18 (2H, d, *J* 8.7, Ar-H), 7.52 (2H, d, *J* 8.7, Ar-H), 9.48 (1H, br s, *NHCO*). CI-MS *m/z* 289.11883 [*C₁₅H₁₇N₂O₄* (*M*+*H*)⁺ <0.6 ppm]. Anal. Calcd for *C₁₅H₁₆N₂O₄*: C, 62.49; H, 5.59; N, 9.17. Found: C, 61.97; H, 5.42; N, 9.76.

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