Hidden signatures: new reagents for developing latent fingerprints†

M. John Plater,*[a] Paul Barnes,[a] Lauren K. McDonald,[a] Sandy Wallace,[a] Nia Archer,[b] Thomas Gelbrich,‡[c] Peter N. Horton[c] and Michael B. Hursthouse[c]

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Aldehydes substituted with a quaternised pyridinium or quinolinium ring have been investigated for the development of latent fingerprints. Two routes were developed to a novel *in situ* formed azacyanine dye. This dye might form in the fingerprint where reagents are concentrated but does not form appreciably in solution experiments as evidenced by the lack of an absorption band at 600 nm. *N*-Alkyl and *N*-aryl substituted benzimidazole-2-carboxaldehydes give stable fluorescent fingerprints.

Introduction

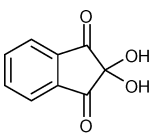
Every time we touch an object or surface we leave behind an invisible signature made from the sweat that our skin constantly secretes and other materials that contaminate the skin surface (*e.g.* cosmetics, foodstuffs *etc.*). The signature is a fingerprint and can be used for identification purposes to help catch criminals in cases of burglary, the handling of stolen money, forged documents and other serious crimes. Sweat contains small quantities of amino acids which can be reacted with a colour developer such as ninhydrin **1**,[1–2] 1,8-diazafluorenone (DFO) **2**,[3–5] indan-1,2-dione **3**[6] or alloxan **4**[7] to create a print that can be photographed and stored. DFO, indanedione and *para*-dimethylaminocinnamaldehyde[8] form products which exhibit fluorescence thereby improving sensitivity.

Ninhydrin **1** and alloxan **4** are the oldest colour developers for amino acids. Alloxan **4** was the earliest discovered by Strecker in 1862.[7] However, the product it forms from reaction with amino acids, called murexide, is less strongly colored than Ruhemann's purple **8**, which forms on reaction of an amino acid with ninhydrin **1**. All four reagents and related compounds have a similar elegant mechanism of action which involves stripping out a single nitrogen atom from the amino acid and incorporating it into a dye (Scheme 1). The dyes are all structurally similar. Development conditions for the reagents can involve heating at temperatures up to 100 °C. New, more sensitive reagents are desirable, particularly those that react under milder development conditions enabling use at crime scenes early in an investigation.

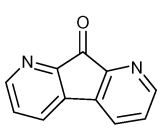
The carbonyl groups in reagents **1**–**4** are good electron sinks owing to their planarity, which allows effective stabilisation of an enol or enolate formed by decarboxylation of an amino acid. The old addage taught to undergraduates that 'β-keto acids readily decarboxylate' could well be applied here to the imines formed by condensation of these reagents with α-amino acids. The mechanism of reaction of ninhydrin **1**,[2] DFO **2**[3] and indan-1,2-dione **3**[9] with α-amino acids has been studied in some detail and involves azomethine ylids. In search of new systems that would react with α-amino acids and allow their ready decarboxylation, we considered the structure of DFO **2** in which the C=O functionality has been replaced by C=N. The carbonyl group of DFO **2** is not hydrated as it is in ninhydrin **1** and the reaction of an isomer with an amine can be facilitated with a Lewis acid.[10]

Discussion

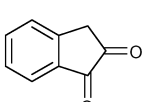
Our attention turned to *N*-methylpyridine-4-carboxaldehyde iodide **9**.[1] The aldehyde is electrophilic evidenced by the fact that it readily hydrates and the quaternised pyridinium ring should facilitate ready decarboxylation of imine **10** into enamine **11** formed by reaction with an amino acid (Scheme 2). This could re-protonate and hydrolyse to give 4-aminomethyl-*N*-methylpyridinium iodide **13**. Alternatively, imine **10** could tautomerise to imine **15** *via* dihydropyridine **14** and hydrolyse to give the same product **13**. This route would involve a transamination reaction. Scheme 3 shows a proposed route to an azacyanine dye which might form by a condensation of product **13** with starting material **9**. This is a bimolecular step similar to the bimolecular step required for the formation of dyes from ninhydrin **1** or DFO **2** reacting with amino acids. Transamination reactions have an interesting



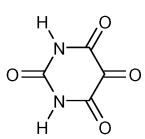
Ninhydrin **1**



1,8-Diazafluorenone (DFO) **2**



Indan-1-2-dione **3**

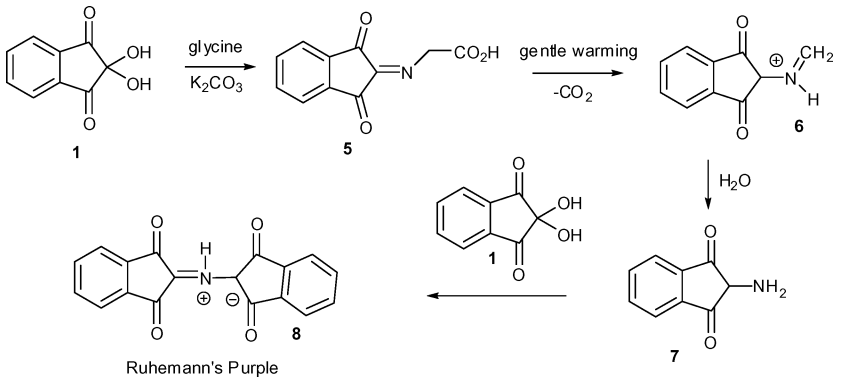


Alloxan **4**

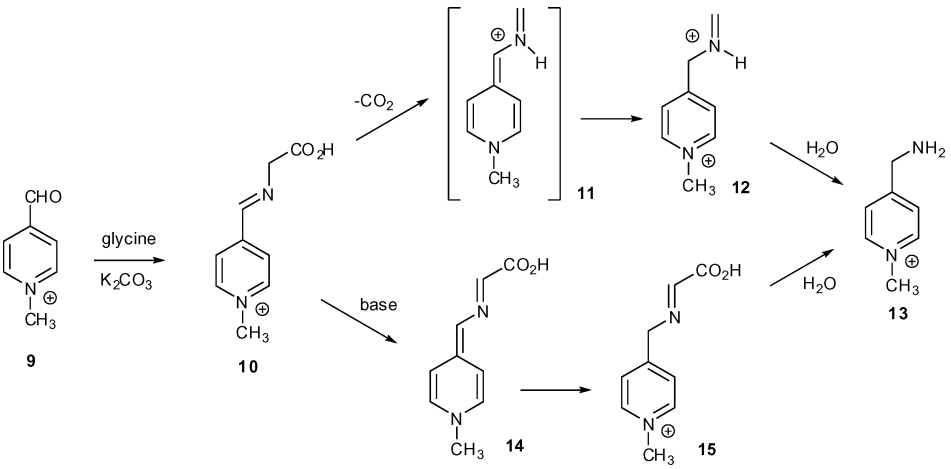
[a]*Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen, UK AB24 3UE. E-mail: m.j.plater@abdn.ac.uk; Fax: +44 (0)1224 272921; Tel: +44 (0)1224 272927*
[b]*Trident Court, 2920 Solihull Parkway, Birmingham, Business Park, Birmingham, UK B37 7YN*
[c]*Department of Chemistry, University of Southampton, Highfield, Southampton, UK SO17 1BJ*

† Electronic supplementary information (ESI) available: Photographs of fingerprints developed using *N*-methylpyridine-4-carboxaldehyde iodide **9**. CCDC reference numbers 702015 and 702097. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b820257e

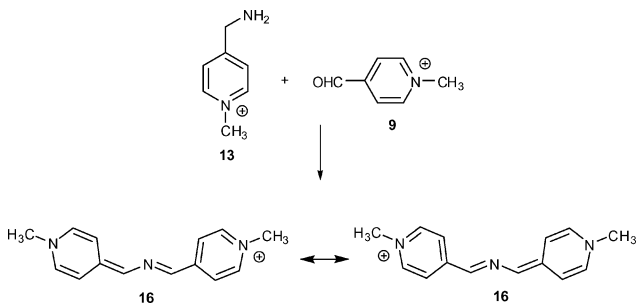
‡ Current address: Institut für Pharmazie, Universität Innsbruck, Innrain 52, 6020 Innsbruck, Austria.



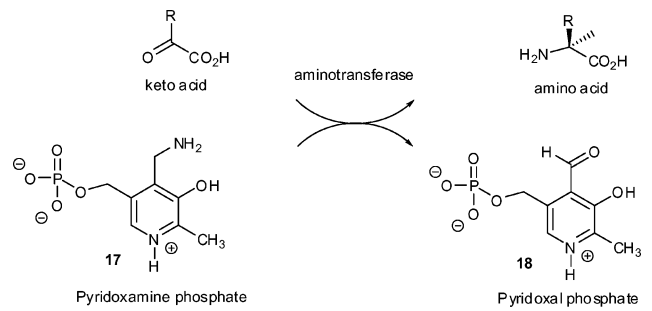
Scheme 1



Scheme 2



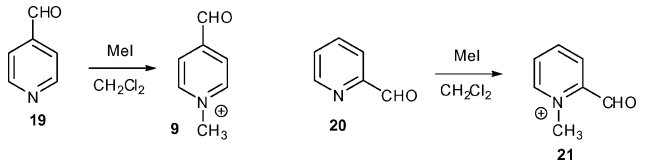
Scheme 3



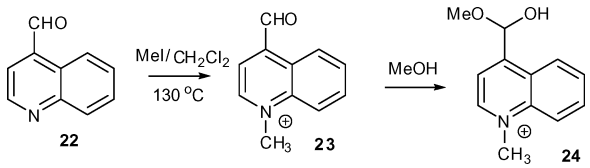
Scheme 4

biological precedent which is shown in Scheme 4. Pyridoxamine phosphate **17** and an α-keto acid are converted by an enzyme into pyridoxal phosphate **18** and an α-amino acid or *vica versa.* The conversion of pyridoxal phosphate into pyridoxamine phosphate resembles the stepwise conversion of reagent **9** into intermediate **13**. Both co-factors **17** and **18** and reagent **9** have a common pyridine ring although it is not alkylated in the co-factor. Biological models for transamination have been investigated in a number of systems.[11–14] The transamination reaction between *N*-methylpyridine-4-carboxaldehyde **9** and glycine or alanine gives a visible colouration that is attributed to a transient dihydropyridine tautomer, such as **14** formed in the case of glycine, on route to glyoxalic and pyruvic acid respectively. However, for alanine a species absorbing at 600 nm was also observed, which might be owing to a larger chromophore such as species **16** proposed here. These experiments and theory provided precedent that a colour forming reaction might occur between pyridinium carboxaldehyde salts and amino acids for finger printing.

Pyridine-4-carboxaldehyde **19** was alkylated with methyl iodide in DCM with stirring for two days at room temperature (Scheme 5). Stirring gives a more crystalline product and is an improvement on the literature procedure[15] especially when the reaction is scaled up. Heating in a pressure bomb at 60 °C was not used for this substrate but heat was necessary to alkylate the 2-isomer **20** and quinoline-4-carboxaldehyde **22** (Scheme 6). These aldehydes are readily hydrated. With alcohols hemiacetals form.[15] Salt **9** slowly begins to turn from orange to pale yellow as it hydrates under air filtration on a sinter. The salts are



Scheme 5



Scheme 6

soluble in methanol and easily characterised by NMR as the deuterated methanol hemiacetals. These would form when dissolved in deuterated methanol. Interestingly *N*-methylpyridine-2-carboxaldehyde iodide **21** was characterised by NMR as a mixture of a hydrate and deuterated methanol hemiacetal suggesting that the hydrate has some stability in methanol. In the ^{1}H NMR, two peaks for the N-CH_3 group occur (4.44 and 4.47) and two CH(OR) peaks occur (6.00 and 6.30). The hydrated or hemiacetal carbon is close to the *N*-methyl group so two resonances are observed in this case for the *N*-methyl group. Crystallisation of salt **23** from methanol gave hemiacetal **24** which was characterised by a single crystal structure determination (Scheme 6 and Fig. 1).

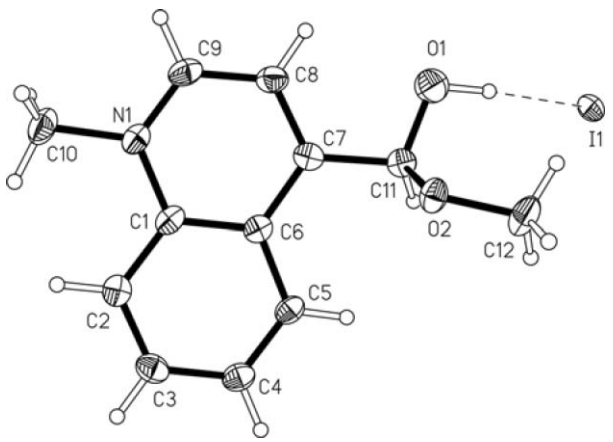


Fig. 1 Molecular structure of **24** with displacement ellipsoids drawn at the 50% probability level.

The geometric parameters of compound **24**, which are shown in Fig. 1, are unexceptional. The planes of the hydroxymethyl and methoxymethyl fragments form angles of 12.1(2)° and 70.4(2)°, respectively, with the mean plane of the methylquinolinium moiety, and they form an angle of 58.8(3)° with one another. Each iodide anion is O–H···I bonded to one hydroxyl group with O···I = 3.424(2) Å and O–H···I = 174(4)°. The cation molecules, related by centres of inversion, are stacked, with their methylquinolinium fragments face-to-face, in columns parallel to the *a* axis. Adjacent columns of this kind are then packed in a parallel fashion along the *c* axis. Furthermore, consecutive 2D structure fragments formed thereby are related by 2_1 screw axes and organised in a herringbone fashion along the *b* axis.

A preliminary evaluation of the three pyridinium salts **9**, **21** and **24** for the development of latent fingerprints was carried out. Typically a damp finger was pressed onto a silica plate for a few seconds to create a print. The dampness facilitates transfer of amino acids from the skin onto the plate and increases the chances of observing a fingerprint. Each reagent was dissolved slowly in ethanol with vigorous stirring (50–100 mg made up to 100 mL). The reagents are salts so require a polar solvent for dissolution. The aldehyde **9** will slowly dissolve in ethanol but the hydrate is less soluble. Methanol is a better solvent for these salts. The silica plate was dipped briefly in the solvent then dried in an oven for a minute or so or heated with a heat gun to develop. Both salts **9** and **24** developed sandy orange prints but salt **21** was poorer. The prints were not stable and showed a tendency to fade over a day or so. The failure of reagent **21** to work suggests that the *N*-methyl group interferes with conjugation to the pyridine ring by twisting the aldehyde group. Reagent **9** was examined in more detail using a dry fingerprint on paper and the developed print was photographed using an argon ion laser as light source with an orange filter (see ESI section for photograph†).

The reaction of *N*-methylpyridine-4-carboxaldehyde **9** with three amino acids glycine, leucine and alanine was attempted in hot water in a test tube with some K_2CO_3. When the reagents were warmed the solution turned orange then red. The UV/VIS spectra of the three dyes all showed a broad absorption in the range 400–500 nm but whereas the spectra of the dye from leucine and alanine were identical, the spectrum of the dye formed from glycine was different (Fig. 2, 3 and 4). It was much broader and showed a stronger absorption that stretched further into the visible region. If the three dyes had been the same, it would be evidence that the substituents of the amino acids had been lost leaving a single nitrogen atom to be incorporated into a dye. Substituted amino acids appear to give dyes with similar UV/VIS spectra that are different from the dye formed from an unsubstituted amino acid,

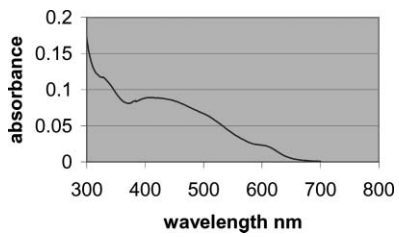


Fig. 2 UV/VIS spectrum of a red dye formed from heating *N*-methylpyridine-4-carboxaldehyde iodide **9**, glycine and K_2CO_3 in water.

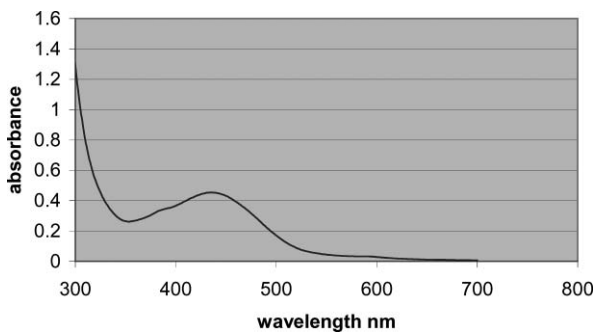


Fig. 3 UV/VIS spectrum of a red dye formed from heating *N*-methylpyridine-4-carboxaldehyde iodide **9**, leucine and K_2CO_3 in water.

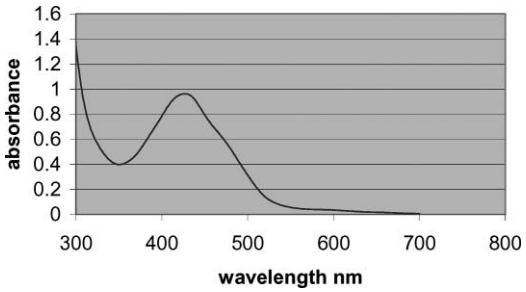


Fig. 4 UV/VIS spectrum of a red dye formed from heating *N*-methylpyridine-4-carboxaldehyde iodide **9**, alanine and K_2CO_3 in water.

glycine. This suggests that the alkyl side chain may be retained in the dye. The dyes fade quickly and it was not possible to isolate and characterise them. These observations are in line with previous studies suggesting that in solution an unstable dihydropyridine tautomer such as compound **14** accounts for the colouration.[14] For substituted amino acids this may be more hydrolytically stable but less stable when formed from glycine and more prone to hydrolyse giving other products displaying a broader absorption profile.

To cast further light on possible structures for the dyes that might form in solution or in a fingerprint, two independent synthetic routes to the azacyanine dye **16** were attempted. The first route, shown in Scheme 7, involved a synthesis of the key intermediate 4-aminomethyl-*N*-methylpyridinium iodide **13** as the hydrochloride salt. The second route, described shortly, is described in Scheme 8. 4-Aminomethylpyridine **25** was acylated with acetic anhydride in water to give the acylated product **26**. Performing the reaction in water was an improvement on the literature procedure in pyridine.[16] Alkylation was achieved with methyl iodide and deprotection by boiling in dilute HCl. Compound **27** was characterised by a single crystal structure determination (Fig. 5).

The geometric parameters of the molecule are unexceptional. The amide fragment forms an angle of 73.9(2)° with the mean plane of the methylpyridinium fragment. The amide hydrogen forms a hydrogen bond to the iodide ion (N···I = 3.5949(17) Å and N–H···I = 173.9°. The simple 'L-shaped' nature of the cation molecule results in corrugated sheets in the YZ-plane. Apart from the hydrogen bond, the other close contacts are between the amide methyl group and the face of the pyridinium ring, and the pyridinium methyl group with the amide oxygen atom.

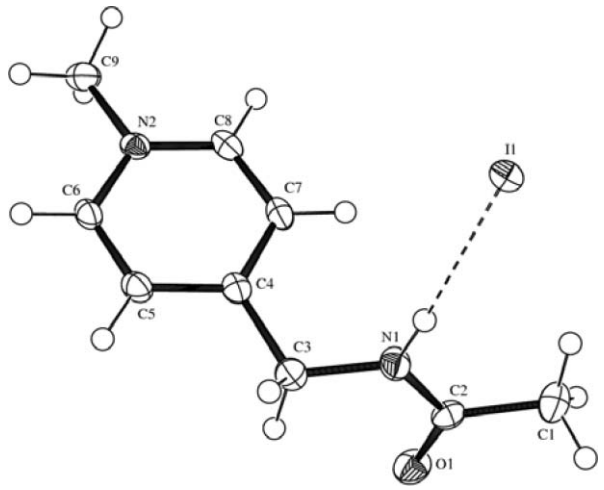
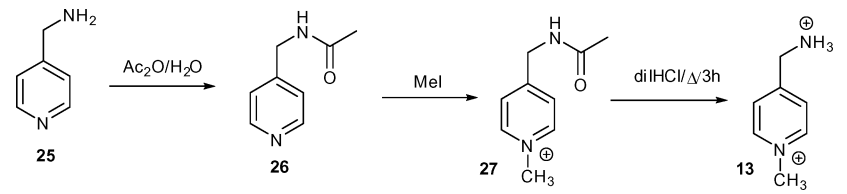


Fig. 5 Molecular structure of **27** with displacement ellipsoids drawn at the 50% probability level.

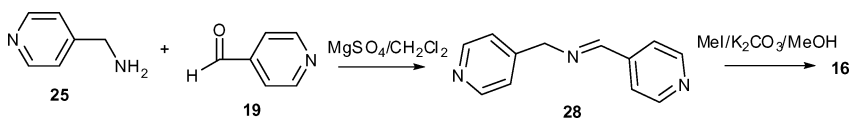
The product **13** was isolated as a HCl salt which gave satisfactory NMR data in CD_3OD. Mixing salt **13** with aldehyde **9** in water with K_2CO_3 gave a green dye which is proposed to be the azacyanine dye **16** (Scheme 3). The UV/VIS spectrum shows a new absorption at 600 nm. All attempts to isolate the dye and obtain good NMR data were unsuccessful so a second independent synthesis was carried out to provide evidence for it's structure (Scheme 8). 4-Aminomethylpyridine **25** was condensed with pyridine-4-carboxaldehyde **19** to give imine **28** using $MgSO_4$ to absorb water. Treatment of imine **28** with methyl iodide in methanol with K_2CO_3 gave a green precipitate that was washed with methanol to give an emerald green product that began to fade in colour after a few days. Again satisfactory NMR spectra were not obtained. The UV/VIS spectrum (Fig. 7) was the same as before (Fig. 6) exhibiting an absorption at 600 nm suggesting that this dye is also azacyanine dye **16**.

The dye is formed reversibly. The colour is quenched with dilute acid (Fig. 8) but returns on neutralisation with alkali (Fig. 9). Treatment with acid presumably hydrolyses the dye back into its component parts **9** and **13**, which can recondense hence this experiment is further evidence for the structure of the dye.

These experiments provide further characterisation of the dyes that may form in solution upon reaction of amino acids with *N*-methylpyridine-4-carboxaldehyde **9**. Our work suggests that the



Scheme 7



Scheme 8

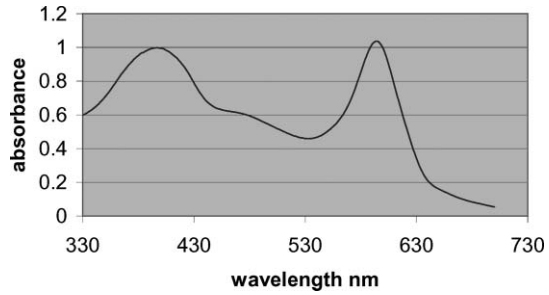


Fig. 6 UV/VIS of a green dye **16** formed by mixing *N*-methylpyridine-4-carboxaldehyde iodide **9** with [4-aminomethyl-*N*-methylpyridinium iodide] hydrochloride **13** with K_2CO_3 in water (Scheme 3) (route A).

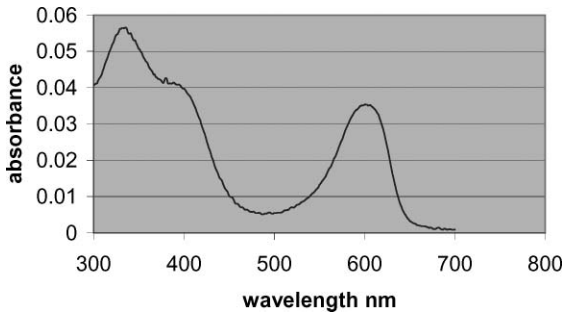


Fig. 7 UV/VIS spectrum of a green dye **16** formed by mixing the imine **28** (formed from 4-aminomethylpyridine **25** with pyridine-4-carboxaldehyde **19**) followed by alkylation with MeI and K_2CO_3 in MeOH (route B).

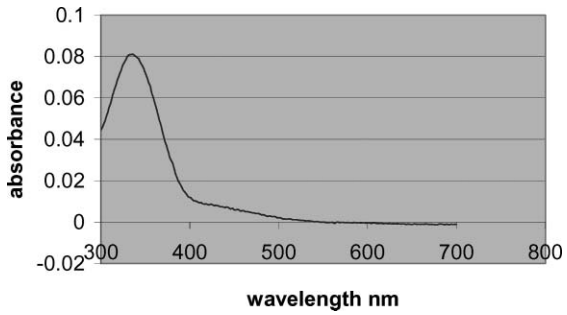


Fig. 8 UV/VIS spectrum of a green dye **16** (route B) after decolourisation with dilute HCl.

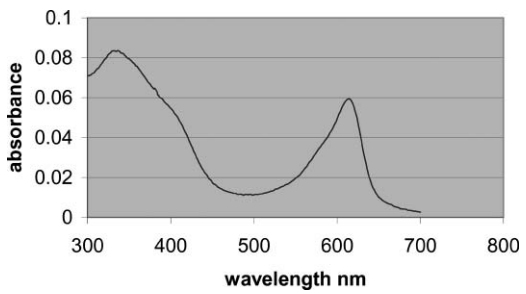
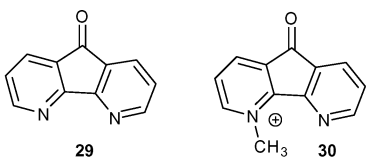


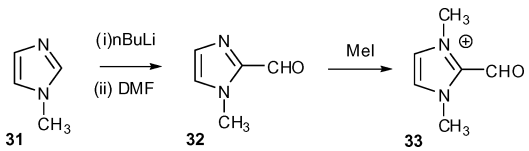
Fig. 9 UV/VIS spectrum of a green dye **16** (route B) after decolourisation with dilute HCl then neutralisation with alkali restoring the green colour.

original observation[14] of an absorption at 600 nm from reacting compound **9** with alanine may be due to dye **16** which has an absorption maximum at this value (although no absorption at this value was observed in our reactions of salt **9** with either alanine or leucine suggesting that only a transient intermediate was observed in the previous experiments[14]). In our experiments, reacting compound **9** with glycine, a broad absorption at 400–500 nm was observed as well as a weak shoulder at 600 nm which might be due to dye **16**. However, the picture is complicated further by the fact that treatment of salt **13** with dilute alkali gives an unstable red colouration that will not extract into DCM. In summary, the development of latent fingerprints using compound **9** could give any of these species as well as the azacyanine dye **16** and might represent a mixture, of which all the components have poor stability. Although the azacyanine dye **16** does not form to an appreciable extent in solution reactions of salt **9** with amino acids, it does form from the preformed components, and so this dye may still form in developing fingerprints.

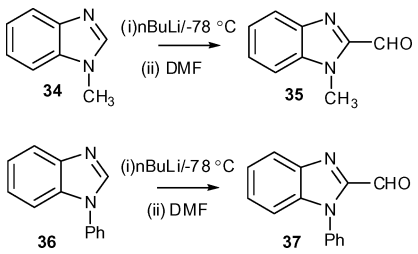
Since 1,8-diazafluorenone **2** (DFO) is a successful reagent for the development of latent fingerprints[4] we examined the isomer 4,5-diazafluorenone **29** which can be readily prepared in one step from phenanthroline.[17] The alkylated derivative **30** was prepared as a red solid.[18] Compound **30** resembles reagents **9** and **23** by having an electrophilic carbonyl group, a ketone instead of an aldehyde, and could follow a similar chemical reaction with an amino acid. A fingerprint developed from a damp finger on a silica plate using compound **29** (50 mg in 100 ml of DCM) gave a weak fluorescence at 366 nm with no discernable detail by eye. Using the quaternised salt **30** (80 mg in EtOH–H_2O 99 ml : 1 ml), only a faint outline was discernable in the UV. It did not give the rapid visible colouration characteristic of salt **9**.

Owing to the facile success of *N*-methylpyridine-4-carboxaldehyde iodide **9** for the development of latent fingerprints, the imidazolium salt **33** was prepared and tested.[19] *N*-Methylimidazole **31** was lithiated and quenched with DMF forming aldehyde **32** (Scheme 9) following a published methodology.[20–21] The imidazole **32** was readily alkylated with methyl iodide. Salt **33** was unfortunately completely inert to amino acids upon heating in the dry state or in solution. The aldehyde group is sterically hindered on both sides by two methyl groups which prevent attack from nucleophiles. However, as a consequence of making this salt, a preliminary fingerprint test was carried out on the precursor imidazole carboxaldehyde **32**. This compound was able to develop fingerprints from a damp finger on a silica sheet to give a pale yellow image. From this encouraging result two further reagents, **35** and **37**, were made by similar methods based on benzimidazoles (Scheme 10). *N*-Phenylbenzimidazole **36** was prepared by refluxing





Scheme 9



Scheme 10

N-phenylorthophenylene diamine in formic acid. Unfortunately, all attempts to react *N*-phenylorthophenylene diamine in dilute boiling HCl with acetic anhydride or glycolic acid gave only recovered starting material. This is in contrast to the reports published by Phillips in which the reaction with acetic anhydride was successful as were reactions of 2-aminodiphenylamine-4-arsinic acid with both acetic anhydride and lactic acid.[22] The use of a benzimidazole in place of imidazole was expected to enhance the absorption and visibility of a fingerprint.

Fingerprints developed from a damp finger on a silica plate using reagents **35** or **37** (50 mg in 100 ml of DCM) are strongly fluorescent under a UV lamp at 254 and 366 nm and show good stability. Development was achieved in under 2 min at 60–100 °C. Fine detail was discerned uniformly over the image at a wavelength of 254 nm. The print fluorescence covered the whole of the original fingerprint which contrasted with similar fingerprints developed with ninhydrin (50 mg dissolved in 100 ml of EtOH). They were often only partially developed. Under these simulated conditions the reagents outperform ninhydrin. Attempts at independent synthesis of the chromophore with amino acids were, however, generally unsuccessful so it has not been possible to deduce the structure of the fluorescent chromophore.

The lithiation route shown in Scheme 10 is suitable for small scale synthesis but is not ideal for scaling up. Scheme 11 describes a literature route that can be used for making larger quantities of reagent **35**. This involves a Phillips condensation[23] of *N*-methylorthophenylene diamine **38** with tartaric acid to give dimer **39** followed by oxidative diol cleavage with $Pb(OAc)_4$.[24] The method is the same as the literature procedure but with a shorter period of reflux and is reported here in English. Purification is straightforward because the protonated product is soluble in water allowing filtration from lead salts.

In summary, reagent **35** should be cheap to supply commercially as it is readily prepared *via* a short high yielding route and shows promise as a reagent for the development of latent fingerprints.

Experimental

X-Ray crystal structure determination

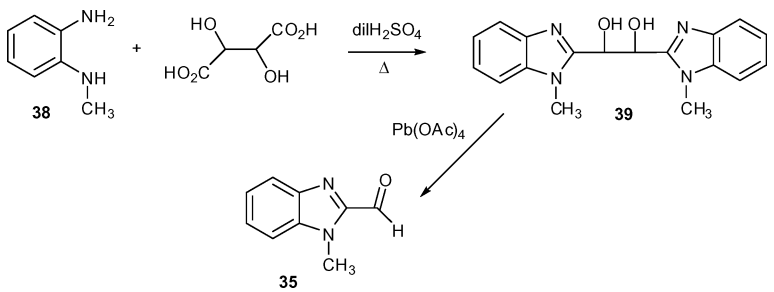
Crystal data: **24** $C_{12}H_{14}INO$, $M = 331.14$, yellow block, monoclinic, $a = 7.9338(2)$, $b = 14.4447(5)$, $c = 11.0343(3)$ Å, $\alpha = 90$, $\beta = 93.66(3)$, $\gamma = 90°$, $U = 1229.10(6)$ Å^3, $T = 120(2)$ K, space group $P2_1/n$, $Z = 4$, $\mu = 2.591$ mm^{-1} reflections collected = 8256, independent reflections = 2342 ($R_{int} = 0.0229$), $R1 = 0.0247$, w$R2 = 0.0783$ [$F^2 > 2\sigma(F^2)$], $R1 = 0.0252$, w$R2 = 0.0788$ (all data) CCDC 702097.

Crystal data: **27** $C_9H_{13}IN_2O$, $M = 292.11$, light yellow block, monoclinic, $a = 8.5840(2)$, $b = 12.9688(3)$, $c = 10.3119(2)$ Å, $\alpha = 90$, $\beta = 100.852(1)$, $\gamma = 90°$, $U = 1127.43(4)$ Å^3, $T = 120(2)$ K, space group $P2_1/c$, $Z = 4$, $\mu = 2.808$ mm^{-1} reflections collected = 12694, independent reflections = 2569 ($R_{int} = 0.0228$), $R1 = 0.0189$, w$R2 = 0.0422$ [$F^2 > 2\sigma(F^2)$], $R1 = 0.0206$, w$R2 = 0.0430$ (all data) CCDC 702015.

For **24** the hydroxyl group was refined with a restrained O–H distance of 0.82(2) Å. For both structures all carbon bonded hydrogen atoms were fixed. Diffractometer: Nonius Kappa CCD area detector (φ scans and ω scans to fill asymmetric unit sphere). Cell determination: *DirAx*.[25] Data collection: *Collect*.[26] Data reduction and cell refinement: *Denzo*[27]absorption correction.[28] The structure was solved by direct methods and refined anisotropically using *SHELX97*.[29]

Infrared spectra were recorded on an ATI Mattson FTIR spectrometer using potassium bromide or sodium chloride discs. Ultraviolet spectra were recorded using a Perkin-Elmer Lambda 25 UV-VIS spectrometer with $CHCl_3$, MeOH or THF as the solvent. ^{1}H and ^{13}C NMR spectra were recorded at 250 MHz and 62.9 MHz respectively using a Brucker AC 250 spectrometer. In some cases ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100.5 MHz respectively using a Varian 400 spectrometer. Solvents used for NMR spectral analyses include $CDCl_3$, CD_3OD, $(CD_3)_2SO$ and D_2O. Chemical shifts, δ, are given in ppm relative to the residual solvent and coupling constants, J are given in Hz. Elemental analysis was carried out at Butterworth Laboratories using a PE.2400 CHN analyser. A number of low resolution mass spectra were obtained at the University of Wales, Swansea, using electron impact ionisation, chemical ionisation and electrospray ionisation methods. Accurate mass spectra were determined at the University of Wales, Swansea, using fast atom bombardment methods. Melting points were determined on a Kofler hot-stage microscope.

Aldrich and Lancaster Synthesis supplied all starting materials. Solvents were used as supplied unless otherwise stated. DCM was



Scheme 11

dried under argon over CaH_2. THF was dried under argon over sodium wire with benzophenone as an indicator.

N-Methylpyridine-4-carboxaldehyde iodide 9[15]

Pyridine-4-carboxaldehyde **19** (5 mL, 56.9 mmol) in dichloromethane (20 mL) was treated with methyl iodide (7 mL, 112.4 mmol) and stirred at ambient temperature for 48 h. This gave the *title compound* (12.2 g, 81%) filtered off as yellow/orange cubic crystals with identical spectroscopic properties to the literature material, mp 104–105 °C (lit.[15] 105–106 °C); ν_{max} (KBr)/cm^{-1} 3250, 3020, 1701, 1642, 1465, 1223, 1042 and 833; (Found: C, 33.5; H, 3.4; N, 5.5. C_7H_8INO requires C, 33.7; H, 3.2; N, 5.6%); δ_H(250 MHz; CD_3OD) (deuterated methanol solvate) 4.43 (3H, s, C*H*$_3$), 5.75 (1H, s, C*H*(OH)(OCD$_3$), 8.13 (2H, d, *J* 6.2, Ar*H*), 8.92 (2H, d, *J* 6.2, Ar*H*); δ_C(62.9 MHz; CD_3OD) 53.0–55.0 (O*C*D$_3$), 95.8, 126.9, 146.9, 161.1 (one resonance is missing); *m*/*z* $C_8H_{12}NO_2$ (methanol solvate from methanol) 154 (M$^+$, 70%) and 122 (M$^+$ – CH_3OH).

N-Methylpyridine-2-carboxaldehyde iodide 21[15]

Pyridine-2-carboxaldehyde **20** (1 mL, 10.5 mmol) in dichloromethane (10 mL) was treated with methyl iodide (2.5 mL, 40.2 mmol) and heated at 60 °C for 48 h. This gave the *title compound* (12.2 g, 81%) filtered off as yellow/orange cubic crystals with identical spectroscopic properties to the literature material, mp 179–182 °C (lit.[15] 180–183 °C); ν_{max} (KBr)/cm^{-1} 3043 (CH), 1698 (ArC=O) and 1614 ArC=C); δ_H(250 MHz; CD_3OD) (mixture of deuterated methanol solvate and hydrate) 4.44 (1.8H, s, C*H*$_3$), 4.47 (1.2H, s, C*H*$_3$), 6.00 (0.6H, s, C*H*(OH)(OCD$_3$)), 6.30 (0.4H, s, C*H*(OH)$_2$), 8.05 (1H, t, *J* 6.8, Ar*H*), 8.35 (1H, d, *J* 7.9, Ar*H*), 8.63 (1H, t, *J* 7.6, Ar*H*), 8.92 (1H, d, *J* 6.1, Ar*H*); δ_C(62.9 MHz; CD_3OD) 45.6, 45.7, 85.8, 92.2, 125.4, 125.9, 127.1, 127.4, 146.2, 146.3, 147.1, 147.4, 155.0 and 157.2; *m*/*z* $C_8H_{12}NO_2$ (methanol solvate from methanol) 154 (M$^+$, 70%) and 122 (M$^+$ – CH_3OH).

4-(1,1-Hydroxymethoxymethyl)-1-methylquinolinium iodide 23

Quinoline-4-carboxaldehyde **22** (1.565 g, 9.96 mmol) in dichloromethane (15 mL) was treated with methyl iodide (2 mL, 32.1 mmol) and heated to 130 °C for 16 h in a PTFE lined pressure bomb. This gave the *title compound* (2.4 g, 74%) as brown cubic crystals, mp >200 °C (from methanol) (Found C, 43.4; H, 4.2; N, 4.3. $C_{12}H_{14}INO_2$ requires C, 43.5; H, 4.2; N, 4.2%); ν_{max}(KBr)/cm^{-1} 3255 (OH), 2822 (CH), 1601 (ArC=C) and 1527 (ArC=C); δ_H(250 MHz; D_6DMSO) 4.59 (3H, s, C*H*$_3$), 6.30 (1H, s), 8.04 (1H, t, *J* 7.8, Ar*H*), 8.14 (1H, d, *J* 6.1, Ar*H*), 8.24 (1H, t, *J* 7.8, Ar*H*), 8.47 (1H, d, *J* 8.9, Ar*H*), 8.59 (1H, d, *J* 8.2, Ar*H*), 9.40 (1H, d, *J* 6.1, Ar*H*); δ_C(62.9 MHz; CD_3OD) 45.5, 93.4, 118.7, 119.0, 127.3, 127.4, 129.9, 135.3, 139.25, 149.8 and 157.6; *m*/*z* 204 (M$^+$ – I, 100%), 172 (M$^+$ – CH_3OHI, 45) Acc. mass $C_{12}H_{14}NO_2$: calculated 204.1019, found 204.1020.

N-Pyridin-4-yl-methylacetamide 26[16]

4-Aminomethylpyridine **25** (5.0 g, 0.05 mol) and acetic anhydride (6.12 g, 0.06 mol) in water (50 g), were stirred for two days or until the reaction had gone to completion as monitored by TLC. Water was removed under vacuum leaving a pale yellow oil. The oil was dissolved in DCM (50 mL) and dried with $MgSO_4$. After concentration the oil began to crystallise. The solid sample was then centrifuged to remove the acetic acid that still remained. This formed a white solid, mp 68–70 °C; ν_{max}(KBr)/cm^{-1} 3275, 1648, 1599, 1367; δ_H(400 MHz; $CDCl_3$) 1.98 (3H, s), 4.35 (2H, D, *J* 6.2), 6.59 (1H, s), 7.10 (2H, d, *J* 5.8), 8.44 (2H, d, *J* 5.8); δ_C(100 MHz; $CDCl_3$) 23.0, 42.3, 122.2, 147.6, 149.8, 170.3.

4-(Acetylaminomethyl)-*N*-methylpyridinium iodide 27

N-Pyridin-4-ylmethylacetamide **26** (5.0 g, 0.03 mol) and MeI (5.3 g, 0.04 mol) in DCM (50 mL) were left for two days or until the reaction had gone to completion as monitored by TLC. Crystals were filtered off as green needles, mp 142–144 °C (Found: C, 36.8; H, 4.4; N, 9.4. $C_9H_{13}IN_2O$ requires C, 36.9; H 4.4; N, 9.5%); ν_{max}(KBr)/cm^{-1} 3240, 1664, 1633, 1366, 1180; δ_H(400 MHz; CD_3OD) 2.05 (3H, s), 4.34 (3H, s), 4.60 (2H, d, *J* 5.1), 7.92 (2H, d, *J* 6.5), 8.78 (2H, d, *J* 6.5); δ_C(100 MHz; CD_3OD) 21.3, 42.1, 47.3, 125.7, 145.2, 159.8, 172.8; *m*/*z* 165 (M$^+$, 100%) Acc. mass $C_9H_{13}IN_2O$: calc. 165.1022, found 165.1023.

[4-Aminomethyl-*N*-methylpyridinium chloride] hydrochloride 13

4-(Acetylaminomethyl)-*N*-methylpyridinium iodide **27** (4.0 g, 0.014 mol) was refluxed in 150 mL of dilute HCl for 3 h. Aqueous HCl was then removed *in vacuo* to give a brown precipitate, mp 206–208 °C; ν_{max}(KBr)/cm^{-1} 2863, 1639, 1547, 1391, 1175; δ_H(400 MHz; CD_3OD) 4.42 (3H, s), 4.52 (2H, s), 8.13 (2H, d, *J* 6.5) and 8.98 (2H, d, *J* 6.5); δ_C(100 MHz; CD_3OD) 32.9, 41.5, 127.1, 146.0, 152.5; *m*/*z* 123 (M$^+$, 100%) Acc. mass $C_7H_{11}IN_2$ (M$^+$ – HCl and I): calc. 123.0917, found 123.0916.

1-Methyl-4-[(1-methyl-1*H*-pyridin-4-ylidenemethylimino)-methyl]pyridinium iodide 16

Mixing [4-aminomethyl-*N*-methylpyridinium chloride] hydrochloride **13** and *N*-methylpyridine-4-carboxaldehyde iodide **9** in water with K_2CO_3 gave a green solution, mp >250 °C (UV Fig. 6).

1-Methyl-4-[(1-methyl-1*H*-pyridin-4-ylidenemethylimino)-methyl]pyridinium iodide 16

Pyridine-4-carboxaldyhyde **19** (1.0 g, 9.3 mmol) and 4-aminomethylpyridine **25** (1.0 g, 9.3 mmol) were dissolved in DCM (50 mL). The solution was then dried using magnesium sulfate and the DCM was evaporated *in vacuo* leaving an orange oil. This was then dissolved in MeOH with an excess of MeI and K_2CO_3. After leaving overnight, a precipitate formed which was filtered off. The precipitate was a mixture of green and brown solid which was washed with MeOH. The brown solid disappeared and a bright emerald green product was left; after a few days, the colour of this product began to fade to an olive green colour, mp >250 °C (UV Fig. 7).

N-Methyl-1*H*-imidazole-2-carboxaldehyde 32[20]

n-Butyllithium (32 mmol) (20 mL of a 1.6 mol/dm^{-3} solution in hexanes) was added dropwise to a stirred solution of *N*-methylimidazole **31**[5] (2 mL, 25.8 mmol) in dry THF (30 mL) under argon at –78 °C. After 30 min at –78 °C dimethylformamide (4.6 mL, 59.4 mmol) was added dropwise. The mixture was

then allowed to warm to ambient temperature and stirred for a further 2 h. The reaction was quenched with saturated ammonium chloride (20 mL) and the organic layer separated and washed with brine (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed to yield the *title compound* with identical spectroscopic properties to the literature material (1.59 g, 56%) as colourless oil; ν_{max}(film)/cm^{-1} 3190 (CH), 2844 (CH) and 1682 (C=O); δ_H(250 MHz; $CDCl_3$) 3.89 (3H, s, CH_3), 7.02 (1H, s, Ar*H*), 7.13 (1H, s, Ar*H*), 9.66 (1H, s, C*H*O); δ_C(62.9 MHz; $CDCl_3$) 34.9 (*C*H$_3$), 127.4 (Ar*C*H), 131.4 (Ar*C*H), 143.6 (Ar*C*H), 182.0 (*C*HO).

2-Formyl-1,3-dimethyl-3*H*-imidazolium iodide 33[19]

1-Methyl-1*H*-imidazole-2-carboxaldehyde **32** (200 mg, 1.82 mmol) in dichloromethane (10 mL) was treated with iodomethane (1 mL, 16.1 mmol). The mixture was stirred at ambient temperature for three days after which a colourless precipitate was filtered off and washed with dichloromethane (2 × 50 mL) yielding the *title compound* (0.24 g, 53%) with identical spectroscopic properties to the literature material, mp >200 °C (lit.[17] 264 °C); ν_{max}(KBr)/cm^{-1} 3073 (CH), 1695 (C=O); δ_H(250 MHz; D_6DMSO) 4.09 (6H, s, 2 × CH_3), 7.96 (2H, s, Ar*H*), 9.99 (1H, s, C*H*O); *m*/*z* 157 (M$^+$ + MeOH, 100%), 125 (M$^+$, 10).

N-Methyl-1*H*-benzimidazole-2-carboxaldehyde 35[22,24]

n-Butyllithium (16 mmol) (10 mL of a 1.6 mol/dm^{-3} solution in hexanes) was added dropwise to a stirred solution of *N*-methylbenzimidazole **34** (1.739 g, 13.2 mmol) in dry THF (15 mL) under argon at –78 °C. After 30 min at –78 °C, dimethylformamide (2.3 mL, 29.7 mmol) was added dropwise. The mixture was then allowed to warm to ambient temperature and stirred for a further 2 h. The reaction was quenched with saturated ammonium chloride (15 mL) and the organic layer separated and washed with brine (15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed to yield the *title compound* (1.01 g, 48%) as a yellow solid, mp 110–112 °C (lit.[22] 110 or 124 °C) with identical spectroscopic properties to the literature material; ν_{max}(KBr)/cm^{-1} 2956 (ArC=C), 2861 (ArC=C) and 1693 (C=O); δ_H(250 MHz; $CDCl_3$) 3.99 (3H, s, CH_3), 7.25–7.40 (3H, m, Ar*H*), 7.80 (1H, d, Ar*H*), 9.99 (1H, s, C*H*O); δ_C(62.9 MHz; $CDCl_3$) 31.2, 110.6, 122.2, 124.0, 126.7, 136.8, 142.6, 146.1, 184.9; *m*/*z* 161 (M$^+$ + H, 100%) Acc. mass $C_9H_9N_2O$ calc. 161.0709, found 161.0709.

N-Phenylbenzimidazole 36[31–32]

N-Phenyl-*o*-phenylenediamine (200 mg, 1.1 mmol) in formic acid (10 mL) was heated to reflux for 1.5 h. After cooling it was poured onto water and neutralised with KOH then extracted with diethyl ether (3 × 20 mL). The combined organic extracts were washed with brine (20 mL) and dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed to yield the *title compound* (135 mg, 63%) as a viscous red oil; δ_H(250 MHz; $CDCl_3$) 7.23–7.56 (9H, m, Ar*H*), 8.16 (1H, s, Ar*H*); δ_C(250 MHz; $CDCl_3$) 110.5, 120.6, 122.8, 123.7, 124.0, 128.0, 130.1, 133.7, 136.4, 142.3, 144.1.

N-Phenyl-1*H*-benzimidazole-2-carboxaldehyde 37[32]

n-Butyllithium (2.24 mmol) (1.4 mL of a 1.6 mol/dm^{-3} solution in hexanes) was added dropwise to a stirred solution of *N*-phenylbenzimidazole **36** (400 mg, 2.06 mmol) in dry ether (20 mL) under argon at –78 °C. After 30 min at –78 °C, DMF (0.2 mL, 2.58 mmol) was added dropwise. The mixture was then allowed to warm to ambient temperature and stirred for a further 2 h. The reaction was quenched with saturated ammonium chloride (20 mL) and the organic layer separated and washed with brine (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed to yield the *title compound* (0.3 g, 59%) as an amorphous yellow solid with identical spectroscopic properties to the literature material; ν_{max}(film)/cm^{-1} 3065 (CH), 2855 (CH) and 1701 (C=O); δ_H(250 MHz; $CDCl_3$) 7.23 (1H, m, Ar*H*), 7.35–7.43 (4H, m, Ar*H*), 7.54 (3H, m, Ar*H*), 7.98 (1H,m, Ar*H*), 10.05 (1H, s, C*H*O); δ_C(62.9 MHz; $CDCl_3$) 111.8, 122.3, 124.6, 127.1, 127.4, 129.3, 129.6, 135.2, 137.5, 142.6, 146.0, 182.9; *m*/*z* 222 (M$^+$, 100%) Acc. mass: calculated 223.0866, found 223.0863.

1,2-Bis(1-methyl-1*H*-benzimidazole-2yl)ethane-1,2-diol 39[24]

N-Methyl-1,2-phenylenediamine **38** (2.3 g, 18.9 mmol) and tartaric acid (1.3 g, 8.7 mmol) were added to dilute sulfuric acid (25 mL, 40%). This mixture was refluxed for 24 h then cooled. The solution was dark blue/green in colour. The solution was diluted with water (200 mL) and neutralised to pH 7 with NaOH (2 M). On neutralisation a light green/yellow precipitate formed. The precipitate was filtered off, washed with water and dried in a dessicator to give the *title compound* (2.5 g, 90%) as a lime green solid, mp 242–244 °C (lit.[24] 262 °C) which had identical spectroscopic properties to those reported previously; δ_H(250 MHz; D_6DMSO) 3.9 (6H, s), 5.5 (2H, s), 7.05 (2H, m), 7.13 (2H, m), 7.39 (2H, d, 7.9) and 7.45 (2H, d, 7.9); δ_C(62.9 MHz; $CDCl_3$) 30.6, 67.9, 110.7, 119.4, 122.2, 122.8, 136.0, 141.8 and 154.8.

N-Methyl-1*H*-benzimidazole-2-carboxaldehyde 35[24]

1,2-Bis(1-methyl-1*H*-benzimidazole-2yl)ethane-1,2-diol **39** (1.0 g, 3.1 mmol) was suspended in acetic acid (4 mL) and toluene (8 mL). The mixture was cooled in an ice bath and treated with $Pb(OAc)_4$ over 15 min. The mixture was allowed to warm to rt and stirred for 3 h. Dilute HCl (100 mL) was added which formed a black precipitate. The mixture was filtered and the toluene layer was separated. The aqueous layer was cooled to 15 °C and neutralised with dilute NaOH to pH 6. The majority of the product remained in solution. The aqueous layer was extracted with DCM (3 × 50 mL). The extracts were combined, dried over Na_2SO_4, and concentrated *in vacuo* to give the *title compound* (0.54 g, 54%), mp 110–112 °C, as a light brown solid with identical spectroscopic properties to those reported previously.[24]

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