On the Mechanism of Pyrrole Formation in the Knorr Pyrrole Synthesis and by Porphobilinogen Synthase

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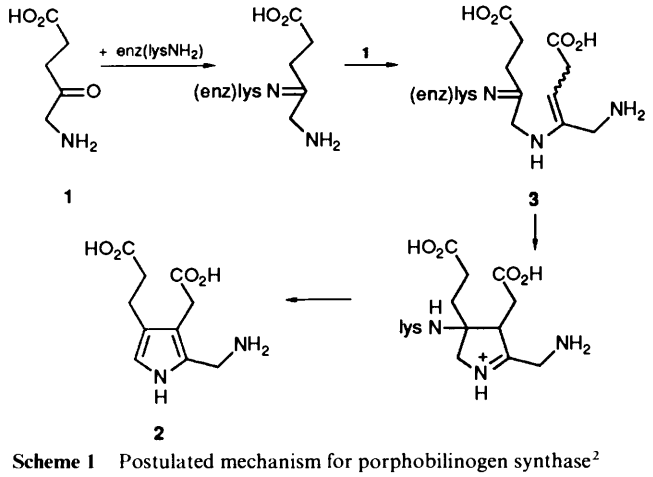
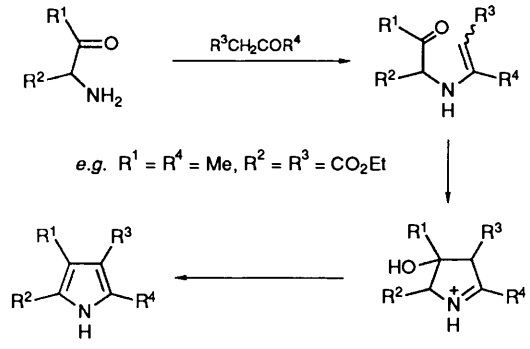
Attempts to synthesise a derivative of *N*-vinylaminoethanal either by oxidation of the corresponding alcohol or by hydrolysis of the corresponding dithioacetal were unsuccessful, but such derivatives were characterised by ^1H and ^{13}C NMR spectroscopy of reactions between ethyl 2-aminoacetoacetate and an excess of ethyl acetoacetate, leading to diethyl 2,4-dimethylpyrrole-3,5-dicarboxylate.

Porphobilinogen synthase (E.C. 4.2.1.24) catalyses the condensation of two molecules of δ-aminolaevulinic acid **1** (δ-ALA) to form porphobilinogen **2**, the precursor of chlorophylls, corrins and porphyrins.[1] The zinc(II)-dependent human and bovine enzyme sequesters one molecule of compound **1** by forming an imine with the ε-amino group of a lysine residue.[2] This enzyme-bound species then reacts with the second molecule of **1** to afford, it is presumed, the intermediate **3** which cyclises and eventually yields the pyrrole **2** with release of the lysine residue (see Scheme 1).[2-4]



Scheme 1 Postulated mechanism for porphobilinogen synthase[2]

The mechanism of Scheme 1 is similar to that proposed for the Knorr pyrrole synthesis (Scheme 2).[5,6] Stereoelectronic



Scheme 2 Postulated mechanism for the Knorr pyrrole synthesis[5]

considerations[7] suggest that the ring closures (5-*endo*-trig[8]) of the enamine intermediates (*e.g.* **3**) in Schemes 1 and 2 should be relatively difficult. Conformational constraints prevent the HOMO of the enamine from approaching the LUMO of the immonium group (Scheme 1) or carbonyl group (Scheme 2) in the preferred manner (assumed to be as in Fig. 1). To explore the validity of this analysis we have attempted to prepare substances like intermediate **3**, so that their chemistry can be studied. Early studies[9] of pyrrole formation by the Knorr route under biomimetic conditions defined a pH optimum of 6.9 for the condensation of an α-amino ketone with a β-keto ester in aqueous solution, but intermediates were not observed. However, Khetan and George[10] have prepared relatively stable enamino ketones by attaching an aryl group to both the nitrogen and keto functions, and have shown that such compounds cyclise to pyrroles on refluxing in acidic methanol. Also, enamino esters (*e.g.* **4**) prepared from 2-amino-2-deoxy-D-glucopyranose and β-keto esters have been shown[11] to produce pyrroles (*e.g.* **4**-**5a**) under mild conditions (*e.g.* in water, 30 h at room temp), presumably *via* the intermediate **6a**.

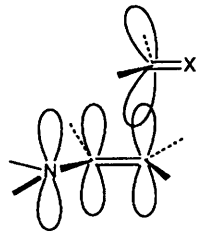
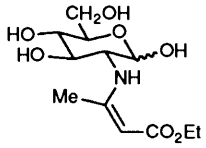


Fig. 1 Stereoelectronically favourable approach of an enamine to a carbonyl group (X = O) or imine (X = N)

Results and Discussion

Attempted Synthesis of Enamines Related to Structure **3**.—(*i*) *From threonine.* The strategy for attempting the preparation of enamines **6b** and **6c** required condensation of threonine methyl ester with diethyl 2-oxobutanedioate and ethyl acetoacetate, respectively, to afford the corresponding intermediates **7a** and **7b**. Oxidation of the hydroxy group of these compounds affords the desired enamines **6b** and **6c**. The condensations of threonine methyl ester with diethyl 2-oxobutanedioate and ethyl acetoacetate were carried out at room temperature in dichloromethane to give intermediates **7a** and **7b** in yields of 13 and 84%, respectively. These compounds were obtained as oils, characterised spectroscopically (see Experimental section).

The selection of a suitable oxidant for compounds **7a** and **7b** must take account of their susceptibility to acidic hydrolysis. Thus, the *N*-benzyl compound **7c** was found to be more than 50% hydrolysed (to benzylamine and ethyl acetoacetate) after 24 h in 1:1 tetrahydrofuran (THF)–0.2 mol dm^{-3} aq acetic acid (pH 3.4) at 20 °C. Attempted oxidation (Swern, pyridinium dichromate, Collin's reagent, Oppenauer or silver carbonate on Celite) of alcohol **7a** or **7b** failed to give the desired ketone **6b** or **6c**, or the pyrrole **5b** or **5c**, respectively.



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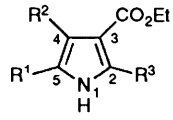
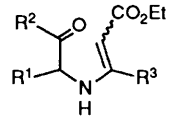
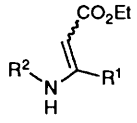


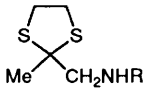
5a ; $R^1 = (CHOH)_3CH_2OH$, $R^2 = H$, $R^3 = Me$
5b ; $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = CO_2Et$
5c ; $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = Me$
5d ; $R^1 = H$, $R^2 = Me$, $R^3 = CO_2Et$
5e ; $R^1 = CO_2Et$, $R^2 = Me$, $R^3 = Me$
5f ; $R^1 = CO_2Et$, $R^2 = Me$, $R^3 = Me^{13}C$ at C –2
5g ; $R^1 = CO_2Et$, $R^2 = Me$, $R^3 = Me^{13}C$ at C –4



6a – 6g; R^1, R^2 and R^3 as for **5a – 5g**
6f ^{13}C at $NC{=}CH$
6g ^{13}C at R^2CO



7a ; $R^1 = CO_2Et$, $R^2 = MeCHOHCHCO_2Me$
7b ; $R^1 = Me$, $R^2 = MeCHOHCHCO_2Me$
7c ; $R^1 = Me$, $R^2 = PhCH_2$
7d ; $R^1 = Me$, $R^2 = PhCH_2$, ^{13}C at $NHCMe$



8a ; R = H
8b ; R = Ac
8c ; R = $MeC{=}CHCO_2Et$
8d ; R = $EtO_2CC{=}CHCO_2Et$

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(*ii*) *From acetamidoacetone.* BF_3-catalysed reaction of acetamidoacetone with ethane-1,2-dithiol gave the ethanedithioacetal **8b**, which was saponified to aminoacetone ethylenedithioacetal **8a**, a new protected form of aminoacetone. This was condensed with a small excess of ethyl acetoacetate in dichloromethane to give ethyl 3-*N*-(2-oxopropylamino)but-2-enoate ethylenedithioacetal **8c**. The enamine **8d** was similarly obtained from amine **8a** and diethyl 2-oxobutanedioate. Although cerium(IV) ammonium nitrate in acetonitrile–water (3:1)[12] (20 °C, 3 min) converted dithioacetal **8b** into acetamidoacetone (84%), with compound **8d** this oxidant failed to give either the ketone **6d** or the pyrrole **5d**. Similarly, reactions of compound **8c** or **8d** with either trimethyloxonium borofluorate[13] or sulphuryl chloride–wet silica gel[14] were unsuccessful for achieving selective hydrolysis of the dithioacetal group.

NMR Spectroscopic Studies of Knorr Pyrrole Condensations.—(*i*) ^1H *NMR spectroscopic studies.* Mixing the hydrochloride of ethyl 2-aminoacetoacetate **9**[15] with ethyl acetoacetate (10 mol equiv.) in ethanol containing triethylamine (1 mol equiv.) (initial molarity of **9** = 0.18) gave within 3 h at 20 °C the pyrrole **5e** (89%), identical with the substance obtained by the standard Knorr synthesis[16] [*in situ* generation of amino keto ester **9** from ethyl acetoacetate (by nitrosation, followed by zinc reduction of the intermediate oxime)[16]]. Therefore, zinc ions are not essential for pyrrole generation in the Knorr synthesis (see ref. 2 concerning the question as to whether zinc plays a catalytic or structural role in porphobilinogen synthase). A large excess of ethyl acetoacetate was used in the reactions described herein to suppress self-condensation of amino keto ester **9**, leading eventually to diethyl 3,6-dimethylpyrazine-2,5-dicarboxylate **10**. Trial experiments showed that a ratio of 10:1 [ethyl acetoacetate:**9**) gave a ratio of 6.3:1 (pyrrole **5e**:**10**).

The reaction between amino keto ester **9** and ethyl acetoacetate was also conducted in [$^2H_4$]methanol, with monitoring by 300 MHz ^1H NMR spectroscopy. The reactants (initial molarity of **9** = 0.2; 1 mol equiv. triethylamine, 4 mol equiv. ethyl acetoacetate) were mixed at 233 K. The temperature was then raised to 263 K where it was held for 2.5 h before being heated to 283 K. The appearance and disappearance of singlet resonances in the δ 2–3 region are interpreted as the formation (at 263 K) of the (*E*)- and (*Z*)-isomers of enamino ester **6e**, and their transformation (at 283 K over several hours) into pyrrole **5e**. It is known that enamino esters of type $R^1NHCR^2{=}CHCO_2R^3$ exist predominantly as the hydrogen-bonded (*Z*)-isomer in chloroform, but both the (*E*)- and (*Z*)-isomers are observed in methanol.[17] We observed an *E*/*Z* ratio of 1:5 for freshly dissolved ethyl 3-*N*-benzylaminobut-2-enoate **7c** in methanol. The half-life for formation of this compound from 1 mol dm^{-3} benzylamine and 1 mol dm^{-3} ethyl acetoacetate in methanol was *ca.* 30 min at 294 K. The formation of the pyrrole **5e** (methyl resonances at δ 2.50 and 2.55) from **6e** paralleled the disappearance of resonances attributed to the isomers of enaminoester **6e**.

(*ii*) ^{13}C *NMR spectroscopic studies.* To confirm the identity of resonances observed in ^1H NMR spectroscopic studies of the reaction between the amino keto ester **9** and ethyl acetoacetate (see above), ^{13}C NMR spectroscopic studies were performed with ^{13}C-enriched materials.

The reaction between amino keto ester **9** and ethyl [3-^{13}C]acetoacetate* (90 atom % ^{13}C) in [$^2H_4$]methanol was monitored by 75 MHz $^{13}C\{^1H\}$ NMR spectroscopy (con-

* We thank Dr. I. M. Lockhart, BOC Limited, for a gift of this compound.

centration of reactants and temperatures as given above for ^1H NMR experiments). Signals that appeared and then disappeared at δ 161 and 164 (3:2 ratio) are assigned to the α-enamino carbon of the (*E*)- and (*Z*)-isomers of enamino ester **6f**, by comparison with data (δ 162 and 164) for ethyl [3-^{13}C]-3-*N*-benzylaminobut-2-enoate **7d**, prepared from ethyl [3-^{13}C]-acetoacetate and benzylamine. The disappearance of the resonances at δ 161 and 164 corresponded with the appearance of a resonance at δ 141 for pyrrole **5f** (see Table 1 in Experimental section). In another reaction, ethyl [3-^{13}C]-2-aminoacetoacetate (prepared from ethyl [3-^{13}C]acetoacetate by the procedure described[15] for the unlabelled compound) was condensed with ethyl acetoacetate in [$^2H_4$]methanol. The intermediate enamino ester **6g** was characterised by a resonance at δ 201 which gradually gave way to a peak at δ 132 for pyrrole **5g**. In the experiments described no other major ^{13}C resonances were observed and therefore none of the other intermediates between reactants and pyrrole accumulated to detectable concentrations.

Jaffe and Markham[4] (see also ref. 18) have used [4-^{13}C] and [5-^{13}C] δ-ALA to confirm that δ-ALA and a lysine residue of porphobilinogen synthase form an imine intermediate (as shown in Scheme 1 with lys CH_2N *anti* to the δ-ALA CH_2NH_2, but with the protonation site of the imine uncertain — either lys or δ-ALA nitrogen). They have also characterised enzyme-bound porphobilinogen, but no intermediates other than the imine described were observed.

Conclusions

The above experiments show that compounds of type **6** undergo cyclisation to pyrroles **5** with a half-life of several hours at 10 °C in methanol, despite the stereoelectronic caveat in the introduction of this paper. Thus, by proper design it should be possible to prepare compounds of type **6**. Future experiments need to compare the rates of cyclisation of enamines **6** with analogues leading to six-membered ring products, and with intermolecular analogues. The synthesis of closer models for porphobilinogen synthase is also required.

Experimental

Materials and Instruments.—See ref. 19. *J* Values in Hz.

Diethyl 2-N-(2-Hydroxy-1-methoxycarbonylpropylamino)-but-2-enedioate **7a**.—To a solution of (*S*)-threonine methyl ester (200 mg, 1.5 mmol) in dichloromethane (2 cm^3) was added a solution of diethyl 2-oxobutanedioate (380 mg, 2 mmol) in dichloromethane (1 cm^3). Sodium sulphate (280 mg) was added and the mixture was stirred at room temperature for 65 h. Dichloromethane (40 cm^3) was added and after filtration the solvent was removed. The residue was chromatographed on silica gel with ethyl acetate–dichloromethane (1:5) as eluent to give the *title compound* as an oil (58 mg, 13%): ν_{max}(film)/cm^{-1} 3490, 3309, 1780, 1743, 1662 and 1607; δ_H 1.15–1.43 (9 H, m, $CHCH_3$ and 2 × CH_2CH_3), 3.02 (1 H, s, OH), 3.76 (3 H, s, OCH_3), 3.92–4.49 (6 H, m, OCH, NCH and 2 × CH_3CH_2), 5.37 (1 H, s, =CH) and 8.60 (1 H, br NH) (Found: M$^+$, 303.1345. $C_{13}H_{21}NO_7$ requires *M*, 303.1318).

Ethyl 3-N-(2-Hydroxy-1-methoxycarbonylpropylamino)but-2-enoate **7b**.—To a solution of ethyl acetoacetate (5.3 g, 40.7 mmol) in dichloromethane (5 cm^3) was added a solution of (*S*)-threonine methyl ester (1.35 g, 10.2 mmol) in dichloromethane (5 cm^3). The mixture was stirred at room temperature for 3 h and then evaporated. The excess of ethyl acetoacetate was removed in high vacuum with warming. The residue was chromatographed on silica gel with ethyl acetate–dichloromethane (1:5) as eluent to give the *title compound* as an oil (2.1 g, 84%); ν_{max}(film)/cm^{-1} 3425, 3290, 1737, 1654 and 1600; δ_H 1.23 (3 H, t, CH_2CH_3), 1.27 (3 H, d, $CHCH_3$), 1.90 (3 H, s, $=CCH_3$), 3.32 (1 H, br s, OH), 3.78 (3 H, s, OCH_3), 3.88–4.33 (4 H, m, CH_3CH_2, OCH, and NCH), 4.58 (1 H, s, =CH) and 8.95 (1 H, d, *J* 10, NH) (Found: M$^+$, 245.1246. $C_{11}H_{19}NO_5$ requires *M*, 245.1263).

N-Acetyl-2-oxopropylamine Ethylenedithioacetal **8b**.—*Method 1.* To a solution of *N*-acetyl-*N*-acetamidoacetone[20] (5.75 g, 36 mmol) in dichloromethane (50 cm^3) was added ethane-1,2-dithiol (6 cm^3, 72 mmol) followed by boron trifluoride–ether (4.4 cm^3, 36 mmol). After 16 h at room temperature the mixture was poured into water (20 cm^3) and extracted with ethyl acetate (300 cm^3). The organic extract was washed with a saturated aqueous sodium hydrogen carbonate (until pH > 7), water (3 × 50 cm^3) and brine (50 cm^3), dried (Na_2SO_4), filtered and evaporated to give the title compound as white plates, m.p. 140–142 °C, after recrystallisation from ethyl acetate (3.7 g, 53%). The product was identical with that obtained in Method 2.

Method 2. To a solution of *N*-acetamidoacetone[20] (2.3 g, 20 mmol) in dichloromethane (25 cm^3) was added ethane-1,2-dithiol (3.4 cm^3, 40 mmol) followed by boron trifluoride–ether (2.4 cm^3, 20 mmol). The reaction conditions and work-up were as in Method 1 above to give the *title compound* (1.87 g, 67%); ν_{max}(KBr)/cm^{-1} 3251, 3071, 1644 and 1573; δ_H 1.73 (3 H, s, CH_3), 2.04 (3 H, s, $COCH_3$), 3.34 (4 H, s, SCH_2CH_2S), 3.59 (2 H, d, *J* 6 NCH_2) and 6.13 (1 H, br, NH) (Found: *M*$^+$, 191.0449. $C_7H_{13}NOS_2$ requires *M*, 191.0438) (Found: C, 43.9; H, 6.9; N, 7.25. $C_7H_{13}NOS_2$ requires C, 44.0; H, 6.85; N, 7.3%).

2-Oxopropylamine Ethylenedithioacetal **8a**.—A mixture of dithiolane **8b** (0.96 g, 5 mmol), lithium hydroxide (1.68 g, 40 mmol), ethanol (5 cm^3) and water (15 cm^3) was boiled under reflux under nitrogen for 20 h. The solvent was removed and the residue was dissolved in methanol (10 cm^3). The mixture was agitated with diethyl ether (40 cm^3) and filtered. The solvent from the filtrate was removed to give the *title compound* as a colourless oil (0.39 g, 52%), which was used directly for the preparations of compounds **8c** and **8d**: δ_H 1.73 (3 H, s, CH_3), 2.23 (2 H, s, NH_2), 2.86 (2 H, s, NCH_2) and 3.31 (4 H, s, SCH_2CH_2S) (Found: M$^+$, 149.0324. $C_5H_{11}NS_2$ requires *M*, 149.0333).

Ethyl 3-N-(2-Oxopropylamino)but-2-enoate Ethylenedithioacetal **8c**.—To a solution of the amine **8a** (222 mg, 1.49 mmol) in dichloromethane (2 cm^3) was added a solution of ethyl acetoacetate (213 mg, 1.64 mmol) in dichloromethane (2 cm^3) and the mixture was stirred at room temperature for 17 h. The solvent was evaporated and the residue was pumped in high vacuum to remove the excess of ethyl acetoacetate. Chromatography of the residue on silica gel with ethyl acetate–dichloromethane (1:9) as eluent gave the *title compound* as an oil (330 mg, 85%): λ_{max}(EtOH)/nm 287 (ε 30 400 dm^3 mol^{-1} cm^{-1}); ν_{max}(film)/cm^{-1} 3285, 1656 and 1603; δ_H 1.24 (3 H, t, CH_2CH_3), 1.76 (3 H, s, CH_3), 1.96 (3 H, s, $=CCH_3$), 3.35 (4 H, s, SCH_2CH_2S), 3.50 (2 H, d, *J* 7, NCH_2), 4.13 (2 H, q, CH_3CH_2), 4.47 (1 H, s, =CH) and 9.11 (1 H, br NH) (Found: M$^+$, 261.0834. $C_{11}H_{19}NO_2S_2$ requires 261.0857).

Diethyl 2-N-(2-Oxopropylamino)but-2-enedioate Ethylenedithioacetal **8d**.—To a solution of the amine **8a** (386 mg, 2.59 mmol) in dichloromethane (5 cm^3) was added a solution of diethyl 2-oxobutanedioate (536 mg, 2.85 mmol) in dichloromethane (5 cm^3) and the mixtue was boiled under reflux for 18 h. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel with dichloro-

methane as eluent to give the *title compound* as an oil (727 mg, 88%): λ_{max}(EtOH)/nm 315 (ε 9500 dm^3 mol^{-1} cm^{-1}); ν_{max}(film)/cm^{-1} 3280, 1729, 1662 and 1605; δ_H 1.27 and 1.33 (6 H, dt, 2 × CH_2CH_3), 1.73 (3 H, s, CH_3), 3.26 (4 H, s, SCH_2CH_2S), 3.68 (2 H, d, *J* 7, NCH_2), 4.13 and 4.26 (4 H, dq, 2 × CH_3CH_2), 5.15 (1 H, s, =CH) and 8.57 (1 H, br, NH) (Found: M^+, 319.0912. $C_{13}H_{21}NO_4S_2$ requires *M*, 319.0912).

Deprotection of N-*Acetyl*-2-*oxopropylamine Ethylenedithioacetal*.—*Method 1*. Ammonium cerium(IV) nitrate (2.19 g, 4 mmol) was added to a solution of compound **8b** (191 mg, 1 mmol) in 75% acetonitrile–water (12 cm^3). After being stirred for 3 min at room temperature, the mixture was diluted with water (12 cm^3) and extracted with diethyl ether (2 × 100 cm^3). The combined ether extracts were dried (Na_2SO_4), filtered, and evaporated to give *N*-acetamidoacetone (97 mg, 84%), the ^1H NMR spectrum of which was identical with authentic material.

Method 2. To a mixture of compound **8b** (191 mg, 1 mmol), silica gel (0.5 g), water (0.5 cm^3), and dichloromethane (6 cm^3) was added dropwise a solution of sulphuryl chloride (0.1 cm^3, 1.24 mmol) in dichloromethane (3 cm^3). The mixture was stirred at room temperature for 1 h after which finely powdered potassium carbonate (138 mg, 1 mmol) was added to it. The mixture was then stirred for a further 1 h after which it was filtered and evaporated. The crude product was dissolved in dichloromethane (30 cm^3) and the solution washed with water (5 cm^3). The organic layer was dried (Na_2SO_4), filtered and evaporated to give *N*-acetamidoacetone (102 mg, 89%).

Ethyl [3-^{13}C]-2-*Oximinoacetoacetate*.—A mixture of ethyl [3-^{13}C]acetoacetate (42 mg, 0.32 mmol) and acetic acid (0.1 cm^3, 1.74 mmol) was cooled (ice–salt bath) to −6 °C and a solution of sodium nitrite (25 mg, 0.36 mmol) in water (0.1 cm^3) was added to it. After 4 h at 0 °C the mixture was taken up in diethyl ether (50 cm^3), washed with water (10 × 2 cm^3), and dried (Na_2SO_4). Solvent evaporation gave the title compound as an oil (39 mg, 77%) which was used directly for the reduction described below: δ_H 1.35 (3 H, t, CH_2CH_3), 2.42 (3 H, d, *J* 6, $COCH_3$), 4.35 (2 H, q, CH_3CH_2) and 10.10 (1 H, br s, OH).

Ethyl [3-^{13}C]*Aminoacetate Hydrochloride*.—A mixture of ethyl [3-^{13}C]-2-oximinoacetoacetate (39 mg, 0.24 mmol) and 10% palladium–charcoal (8 mg) in a 0.5 mol dm^{-3} solution of hydrochloric acid in ethanol (10 cm^3) was hydrogenated until no more hydrogen was taken up. The mixture was filtered (Celite) and evaporated. The residue was recrystallised from ethanol–diethyl ether to afford the title compound (31 mg, 70%): m.p. 126–128 °C; δ_C (75.4 MHz; CD_3OD) 14.3 (CH_2CH_3), 28.3 ($COCH_3$), 64.9 (CH_3CH_2), 96.7 (NCH), 166.8 (CO_2) and 197.1 (CH_3CO) [*c.f.* ^{13}C NMR spectrum of ethyl [3-^{13}C]acetoacetate: 14.6 (CH_2CH_3), 30.3 ($COCH_3$), 62.6 (CH_3CH_2), 98.1 (CH_2), 176.2 (CO_2) and 203.5 (CH_3CO)].

Diethyl 2,4-*dimethylpyrrole*-3,5-*dicarboxylate* **5e**.—A solution of triethylamine (371 mg, 3.67 mmol) in ethanol (5 cm^3) was slowly added (30 min) to a mixture of ethyl acetoacetate (4.78 g, 36.7 mmol) and the amine salt **9** (665 mg, 3.67 mmol) in ethanol (20 cm^3). The mixture was stirred at room temperature for **3** h and then evaporated. Ethyl acetate (50 cm^3) was added to the residue and the mixture was filtered to remove triethylamine hydrochloride. The filtrate was triturated with light petroleum (b.p. 40–60°C). The precipitate was collected, washed with light petroleum, and recrystallised from ethanol to give compound **5e** (780 mg, 89%): m.p. 137–138 °C (lit.,[16] m.p. 136–137 °C); ν_{max}(KBr)/cm^{-1} 3264, 1690, 1670, 1270 and 1200; δ_H 1.37 (6 H, dt, 2 × CH_2CH_3), 2.52 and 2.57 (6 H, ds, 2 × =CCH_3), 4.34 (4 H, dq, 2 × CH_3CH_2), and 9.89 (1 H, br s, NH); δ_C (75.4 MHz; CD_3OD, broadband) 13.2 (=CCH_3), 14.8 (CH_2CH_3), 15.8 ($NCCH_3$), 60.6 and 61.2 (CH_3CH_2), 114.1 (COC=C), 119.3 (COCN), 131.4 (CH_3C=C), 141.0 (CH_3CN), 162.5 and 166.5 (CO_2) (Found: M^+, 239.1176. $C_{12}H_{17}NO_4$ requires *M*, 239.1157).

Diethyl 2,5-*dimethylpyrazine*-3,6-*dicarboxylate* **10**.—A solution of triethylamine (390 mg, 3.85 mmol) in ethanol (5 cm^3) was added to a solution of the amine salt **9** (700 mg, 3.85 mmol) in ethanol (5 cm^3) and the mixture was vigorously stirred at room temperature in an open flask for 6 h. The mixture was evaporated and the residue diluted with ethyl acetate (40 cm^3) and then filtered to remove triethylamine hydrochloride. Evaporation of the filtrate, followed by recrystallisation of the residue yielded the title compound (437 mg, 90%): m.p. 88 °C (lit.,[15] m.p. 88 °C); δ_H (200 MHz; CD_3OD) 1.43 (6 H, t, 2 × CH_2CH_3), 2.73 (6 H, s, 2 × =CCH_3) and 4.46 (4 H, q, 2 × CH_3CH_2); δ_C (50.3 MHz; CD_3OD, broadband) 14.7 (CH_2CH_3), 22.3 (=CCH_3), 63.6 (CH_3CH_2), 145.6 (COC=C), 152.1 (CH_3CN) and 166.5 (CO_2) (Found: M^+, 252.1087. $C_{12}H_{16}N_2O_4$ requires *M*, 252.1109).

Ethyl 3-N-*Benzylaminobut*-2-*enoate* **7c**.—Benzylamine (5.35 g, 50 mmol) was added to a solution of ethyl acetoacetate (6.5 g, 50 mmol) in dichloromethane (20 cm^3) and the mixture was stirred at room temperature for 16 h. It was then evaporated and the residue was chromatographed on silica gel with dichloromethane as eluant to give compound **7c** as an oil (9.69 g, 88%): δ_H(CD_3OD) *inter alia* 1.86 (s, =CCH_3 for *Z*-isomer), 2.28 (s, =CCH_3 for *E*-isomer); *E*:*Z* 1:5 (6 min after solution was prepared), 1:3 (after 24 h) (Found: M^+, 219.1217. $C_{13}H_{17}NO_2$ requires *M*, 219.1260).

Rate of Formation of Ethyl 3-N-*Benzylaminobut*-2-*enoate in Methanol*.—*Experiment 1*. Benzylamine (54 mg, 0.5 mmol) was added to a solution of ethyl acetoacetate (65 mg, 0.5 mmol) in deuteriated methanol (0.5 cm^3) and the extent of the reaction was monitored by ^1H NMR spectroscopy. The reaction was complete with 2.5 h ($t_{\frac{1}{2}}$ *ca.* 30 min at 21 °C).

Experiment 2. Triethylamine (69.5 mm^3, 0.5 mmol) was added to a solution of ethyl acetoacetate (65 mg, 0.5 mmol) and benzylamine hydrochloride (72 mg, 0.5 mmol) in deuteriated methanol (0.5 cm^3) and the reaction was monitored as in Experiment 1. The reaction was also complete within 2.5 h at 21 °C.

Ethyl [3-^{13}C]-3-N-*Benzylaminobut*-2-*enoate* **7d**.—Benzylamine (16 mg, 0.15 mmol) was added to a solution of ethyl [3-^{13}C]acetoacetate (20 mg, 0.15 mmol) in deuteriated methanol (0.5 cm^3) and the mixture was kept at room temperature overnight before its NMR spectra were recorded: δ_H(CD_3OD) *inter alia* 1.88 (CCH_3) for *Z*-isomer), 2.27 (=CCH_3 for *E*-isomer); *E*:*Z* 1:3.4 δ_C (75.4 MHz, CD_3OD, broadband) 15.2 (CH_2CH_3), 18.7 (=CCH_3), 46.7 and 47.8 ($PhCH_2$), 58.6 and 59.7 ($COCH_2$), 128.2–131.1 (8 peaks, Ph), 140.7 (COC=C), 162.2 (NC=C for *E*-isomer); 164.0 (NC=C for *Z*-isomer) and 180.6 (CO_2).

The Effect of Changing the Ratio of Ethyl Acetoacetate to the Amine Salt **9** *on the Yields of the Pyrrole* **5e** *and Diethyl* 3,6-*Dimethylpyrazine*-2,5-*dicarboxylate* **10**.—To a solution of the amine salt **9** (145 mg, 0.8 mmol) and ethyl acetoacetate (104 mg, 0.8 mmol) in ethanol (4 cm^3) was added triethylamine (81 mg, 0.8 mmol) in ethanol (1 cm^3). The mixture was stirred at room temperature for 24 h and then evaporated. Ethyl acetate was added to the residue, and the triethylamine hydrochloride was filtered off. The filtrate was evaporated and a ^1H NMR spectrum of the residue was obtained. The experiment was

Table 1 Relative amounts of pyrrole derivative (δ 141) and intermediate enamino ester (δ 161 and 164) during the reaction of ethyl [3-^{13}C]acetoacetate with ethyl 2-aminoacetoacetate at 283 K

	Intensity of resonance at δ	
t/min	141	161 + 164
0	1.15	7.27
30	3.71	6.41
60	5.55	4.65
90	7.08	3.83
120	8.04	2.52
150	8.91	2.07
180	9.50	1.72
210	9.84	1.27
21h	15.95	0.0

repeated, varying the number of mol equivalents of ethyl acetoacetate in each case. Equimolar reactants gave a ratio of pyrrole–pyrazine of 1.8:1. This rose to 6.3:1 with a ratio of ethyl acetoacetate: **9** of 10:1.

Reaction of Ethyl 2-Aminoacetoacetate Hydrochloride with Ethyl Acetoacetate Monitored by ^{1}H *NMR Spectroscopy.*—A mixture of ethyl 2-aminoacetoacetate hydrochloride (18 mg, 0.1 mmol) and ethyl acetoacetate (52 mg, 0.4 mmol) in deuteriated methanol (0.5 cm^3) was cooled to freezing (liquid nitrogen) in an NMR tube and then triethylamine (13 mm^3, 0.1 mmol) was added. The mixture was quickly warmed to melt, thoroughly mixed, and ^{1}H NMR spectra of the mixture were obtained at 300 MHz at intervals, initially at 263 K and then at 283 K.

Reaction of Ethyl 2-Aminoacetoacetate Hydrochloride with Ethyl [3-^{13}C]*Acetoacetate Monitored by* ^{13}C *NMR Spectroscopy.*—A mixture of ethyl 2-aminoacetoacetate hydrochloride (18 mg, 0.1 mmol) and ethyl [3-^{13}C]acetoacetate (52 mg, 0.4 mmol) in deuteriated methanol (0.5 cm^3) was cooled to freezing (liquid nitrogen) in an NMR tube and then triethylamine (13 mm^3, 0.1 mmol) was added. The mixture was quickly warmed to melt, thoroughly mixed, and ^{13}C NMR spectra of the mixture were obtained at 75.4 MHz at intervals, initially at 233 K and eventually at 300 K. The relative intensities of peaks at δ 141 (CH_3CN of pyrrole), 161 and 164 (NC=C, *E*- and *Z*-isomers of enamino ester intermediate) recorded at 283 K, and after the mixture was kept at room temperature for 21 h, are given in Table 1.

Reaction of Ethyl [3-^{13}C]-2-*Aminoacetoacetate Hydrochloride with Ethyl Acetoacetate Monitored by* ^{13}C *NMR Spectroscopy.*—A mixture of ethyl [3-^{13}C]-2-aminoacetoacetate hydrochloride (14 mg, 0.077 mmol) and ethyl acetoacetate (100 mg, 0.77 mmol) in deuteriated methanol (0.5 cm^3) was cooled to freezing (liquid nitrogen) in an NMR tube and then triethylamine (10.7 mm^3, 0.077 mmol) was added. The mixture was quickly warmed to melt, thoroughly mixed, and ^{13}C NMR spectra of the mixture were obtained at 75.4 MHz at intervals, initially at 233 K and eventually at 283 K. Peaks were observed at δ 49.3 (CD_3OD), 132 (CH_3C=C of pyrrole), 152 (CH_3CN of pyrazine), 197 (CH_3CO of starting amine) and at 200.7 (CH_3CO of intermediate compound).

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