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ScienceDirect
Tetrahedron 63 (2007) 5300–5311
Tetrahedron

Mechanistic studies leading to a new procedure for rapid, microwave assisted generation of pyridine-3,5-dicarbonitrile libraries

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Received 14 December 2006; revised 1 March 2007; accepted 22 March 2007
Available online 27 March 2007

Abstract—Mechanistic investigations into the multi-component synthesis of pyridine-3,5-dicarbonitriles have established a defined reaction pathway, particularly clarifying the role of aerobic oxidation in conversion of the intermediate 1,4-dihydropyridines into the final products. Based on such improved understanding of the reaction mechanism, optimised conditions for the preparation of compound libraries based on this core structure have been developed and represent a significant improvement in yield over existing protocols. Particularly, microwave assisted synthesis was found to provide a procedure suitable for high-throughput synthesis of pyridine-3,5-dicarbonitrile libraries.

1. Introduction

Multi-component reactions (MCRs) are powerful tools in modern medicinal chemistry, enabling straightforward access to large libraries of structurally related, drug-like compounds and thereby facilitating lead generation. Hence, combined with the use of combinatorial chemistry and high-throughput parallel synthesis, such reactions have constituted an increasingly valuable approach to drug discovery efforts in recent years.[1,2] Pyridine-3,5-dicarbonitriles of general structure **1** (Fig. 1) represent such a class of medicinally significant compounds, libraries of which demonstrate activity against a wide range of biological targets, for example, displaying efficacy as selective human adenosine receptor modulators (**2** and **3**)[3,4]—relevant for treatment of a range of conditions—and as anti-prion agents[5] (**4**).

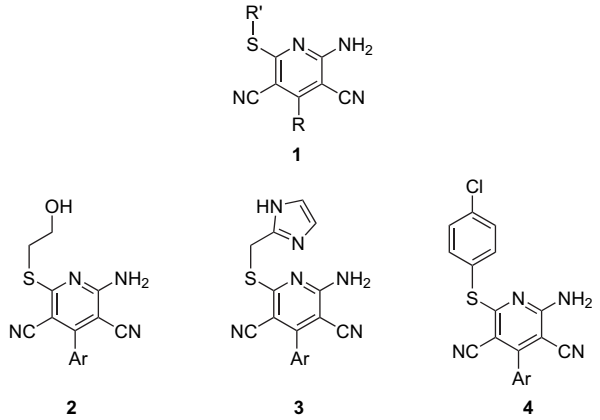
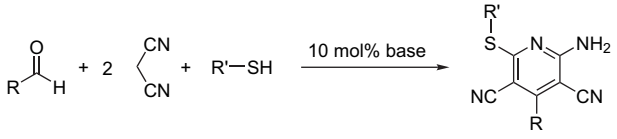


Figure 1. General structure of pyridine-3,5-dicarbonitriles **1** accessible via MCR and related structures of **2**–**4** showing significant biological activity.

As part of an ongoing medicinal chemistry programme aiming to identify novel prion disease therapeutics, we recently reported[6] the synthesis and screening of a small library of such compounds. Both ourselves and others,[3,4] however, have found these efforts hampered by the inefficiency of the MCR used to assemble such libraries (Scheme 1), which typically proceeds in only low to moderate yields. The development of an improved, reliable protocol for this reaction is thus of considerable importance to medicinal chemistry research programmes requiring libraries of pyridine-3,5-dicarbonitriles for lead generation and optimisation.



Scheme 1. Multi-component synthesis of pyridine-3,5-dicarbonitriles.

The MCR in question—as reported by Elghandour et al.[7]—comprises reaction of an aldehyde, a thiol and 2 equiv of malononitrile in the presence of a catalytic amount of base

Keywords: Pyridine-3,5-dicarbonitriles; Drug-like molecules; Aerobic oxidation; Mechanistic study; Library synthesis; Microwave assisted reactions.
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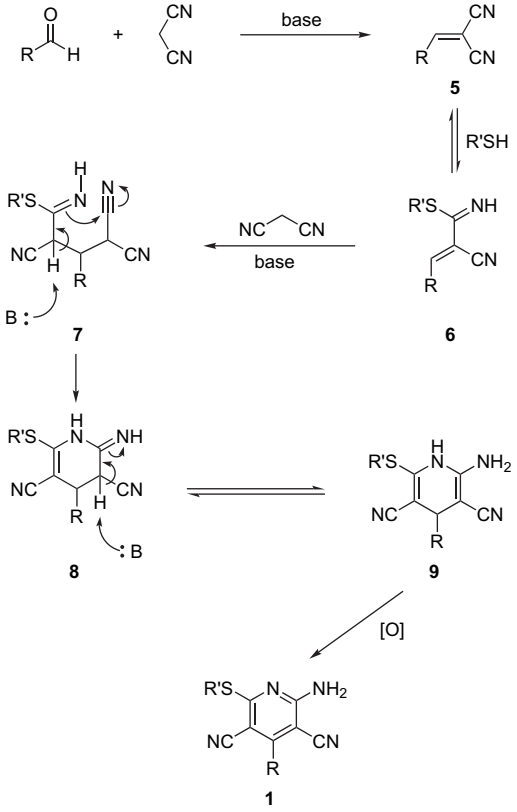
0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.03.139

(usually piperidine or triethylamine) and is ordinarily carried out at reflux in ethanol. However, the mechanism of this process has not been resolved beyond doubt and different interpretations are present in the literature to date. We therefore sought to undertake a thorough mechanistic study of this MCR with the intention of developing a more reliable, higher-yielding synthesis permitting access to as wide a range as possible of pyridine-3,5-dicarbonitriles.

The reported mechanism[6,8] of pyridine dicarbonitrile formation (Scheme 2) involves initial base-catalysed reaction of the aldehyde with 1 equiv of malononitrile resulting in formation of the Knoevenagel adduct **5**. Subsequent addition of the thiol to one of the nitrile groups of this adduct leads to intermediate **6**, to which a second equivalent of malononitrile may add in a 1,4-fashion giving **7**. This species is assumed to cyclise rapidly in the presence of a base to give ring-closed intermediate **8**, tautomeric with penultimate 1,4-dihydropyridine product **9**, which upon aromatisation yields the desired pyridine-3,5-dicarbonitrile **1**. Kambe et al.[8] initially proposed that this final conversion proceeds with loss of molecular hydrogen from the 1,4-dihydropyridine, but in our earlier work[6] we found no evidence to support such a proposition. Rather, we ascertained that chemical oxidation to the final pyridine product was necessary and concluded that this process occurred via aerobic oxidation under the standard reaction conditions. Following reflux, the cooled reaction mixtures were stirred with exposure to air for at least a further 3 h to ensure complete oxidation to the pyridine.

More recently—at around the same time as we first published our results[6]—Evdokimov et al. independently reported



Scheme 2. Reported mechanism of MCR preparation of pyridine-3,5-dicarbonitriles.[6,8]

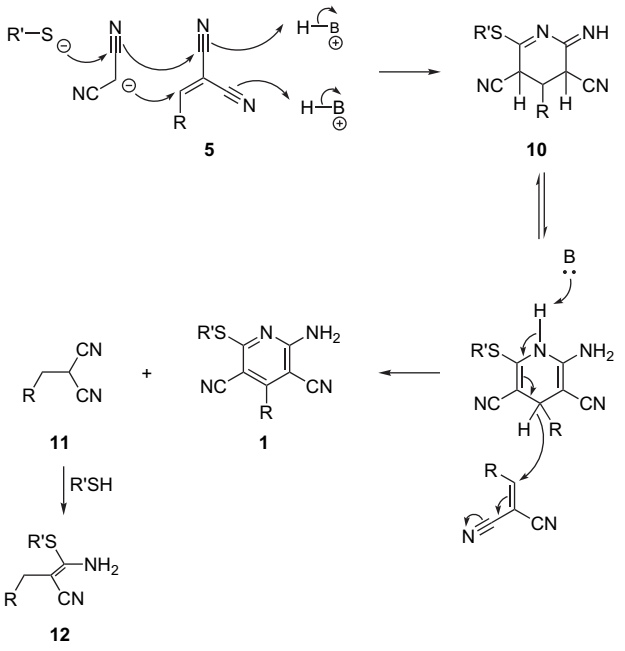


Figure 2. Alternative mechanism proposed by Evdokimov et al.

different findings,[9] suggesting an alternative mechanism (Fig. 2) to that assumed previously. These authors proposed a concerted ring-forming step involving the thiol, malononitrile and Knoevenagel adduct **5**; the product of this process **10**, as with **8**, will then tautomerise to give 1,4-dihydropyridine **9**. Their major findings, however, centred on the discovery that under base catalysis, adduct **5** may act as an oxidising agent for the dihydropyridine (Fig. 2), itself being reduced to **11**. Two key findings were advanced in support of such a process: firstly, isolation of the addition product **12** between the thiol and reduced adduct **11**; and secondly, the observation that though the yield of **1** was always below 50%, it essentially doubled when using a hindered aldehyde (e.g., 2,6-dichlorobenzaldehyde), which causes the reaction to stop at the 1,4-dihydropyridine stage.

Additionally, Evdokimov et al. observed that the reaction yield was unaffected under anaerobic conditions, implying that the extent of oxidation to the final product by atmospheric oxygen is negligible using the standard protocol. In contrast—albeit with limited experimentation—we had previously concluded that product formation was not seen in the absence of air.

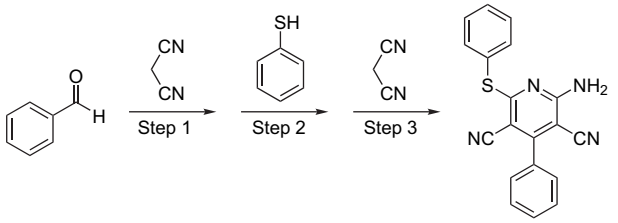
Given such conflicting observations, we considered it necessary to investigate the reaction mechanism of this MCR more fully, seeking first to clarify which intermediates were present in the reaction pathway and secondly to better understand the relative roles of aerobic oxygen and Knoevenagel adduct **5** as oxidising agents in the final step.

2. Results

2.1. Analysis of reaction pathway by mass spectroscopy

In order to establish which species were present during the MCR, the procedure was initially carried out in a stepwise

manner (Scheme 3) with requisite MS (electrospray) analysis of aliquots of the reaction mixtures being performed during each stage. Model reactions were carried out using benzaldehyde, thiophenol and malononitrile in ethanol at 50 °C, containing 10 mol % of piperidine. Reactions were performed either open to the air or under N_2 in rigorously degassed ethanol for comparison.



Scheme 3. Stepwise reactions were carried out to allow analysis of components of the reaction mixture.

Step 1—reaction of benzaldehyde with 1 equiv of malononitrile—predominantly resulted in formation of the Knoevenagel adduct **5** after only 5 min, though small amounts of higher adducts were observed (e.g., **5**+malononitrile). Thiophenol was then added (step 2) and after a further 45 min, the major component of the reaction mixture was seen to be the addition product **6** between **5** and the thiol. Addition of the second equivalent of malononitrile (step 3) then followed, and either with or without the presence of air, the reaction mixtures were found to contain both the pyridine **1** and 1,4-dihydropyridine products **9** after an additional 2 h. The identities of these key intermediates along the reaction pathway (**1**, **5**, **6** and **9**) were confirmed by HRMS analysis.

Aside from the major intermediates known to be present in the reaction mixtures, other components were identified, which helped to shed light on the processes taking place during this MCR. None of the reduced Knoevenagel adducts **11** supposed to be present by Evdokimov et al. could be detected, however, other reduced species were seen (Fig. 3). Both under air and under N_2, compound **12**—the reduced form of **6**, or the addition product of **11** and the thiol—was present together with other reduction products. Structure **13**, again seen under both sets of conditions, is a reduced form of **14**, an addition product between benzaldehyde and **15**, the free amino tautomer of reactive intermediate **7**.

Interestingly, under N_2 only, a further reduced product **16** (Fig. 4) was seen to be present in the reaction mixture during step 3. Finally, under all conditions, malononitrile self-addition product **17** was seen together with its reduced form **18**. Thus, we concluded that a base-catalysed oxidation process of the 1,4-dihydropyridine in the manner proposed by Evdokimov et al. (Fig. 2) was indeed taking place, but that more than one oxidising agent was involved. Our observations established that the predominant oxidising agent was the key reaction intermediate **5**, but that secondary contributions to this process arose from side products also present within the mixture, specifically **6**, **14** and **17**.

One further sequence of addition was investigated (Scheme 4), which served to underscore the observations already noted. Reaction of benzaldehyde with 2 equiv of malononitrile for 5 min resulted predominantly in formation of the

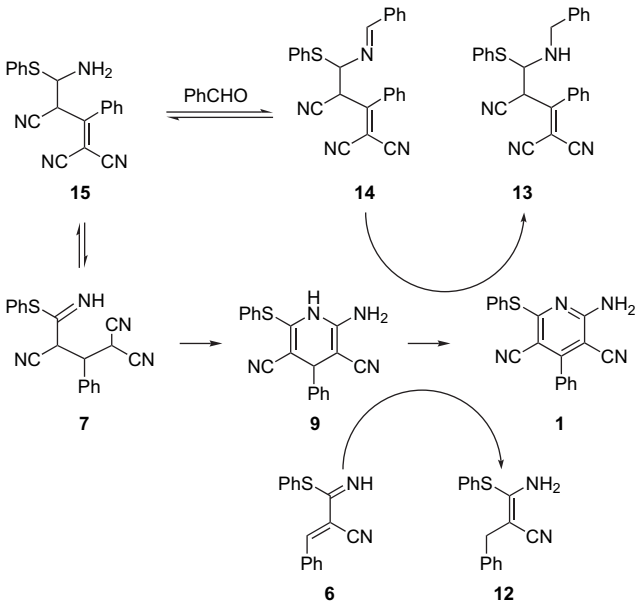


Figure 3. Intermediates seen to be present in step 3 by MS analysis.

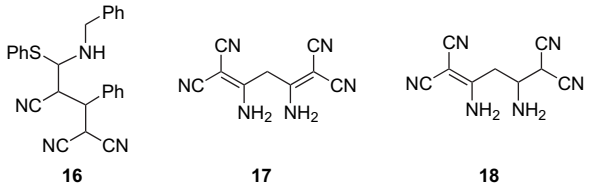
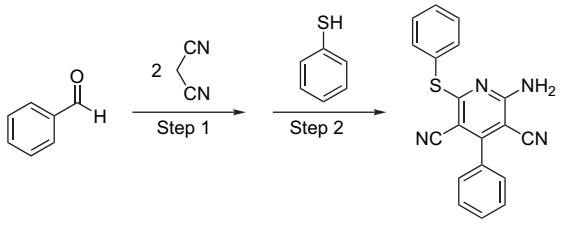


Figure 4. Additional species detected contributing to 1,4-dihydropyridine oxidation.

Knoevenagel adduct **5**, with small amounts of higher addition products again seen to be present. The reaction mixture was treated with thiophenol, and after 2 h more a mixture of pyridine **1** and 1,4-dihydropyridine products **9** was observed as before. Also present were reduced species **12** and **13**, together with a small amount of unreacted adduct **5**.



Scheme 4. Alternative addition sequence investigated to allow analysis of components of the reaction mixture.

2.2. Investigation of the role of aerobic oxidation

In the above examples, we reported that oxidation of the penultimate 1,4-dihydropyridine product **9** to the desired pyridine-3,5-dicarbonitrile **1** did not reach completion under anaerobic conditions. Accordingly, we supposed that in addition to oxidation of these species by the reaction intermediate **5** (and other minor components of the reaction mixture), aerobic oxidation does play a role in formation of the final products under the one-pot conditions usually employed for library synthesis. We thus planned to investigate the relative

Table 1. One-pot reactions carried out at 50 °C, either with or without bubbling of a stream of air through the reaction mixture

Reagent ratio[a]	T (°C)	Entry	t (h)	Gas	Yield[b] (%)	Product ratio[c]	Entry	Gas[d]	Yield[b] (%)	Product ratio[c]
1:2:1	50	1.1	1	None	23	1.2				
		1.2	2	None	25	1.2	1.5	Air	30	1.3
		1.3	3	None	29	1.2	1.6	Air	37	1.7
		1.4	24	None	40	3.8	1.7	Air	42	3.2
2:3:1	50	1.8	1	None	41	P				
		1.9	2	None	65	P	1.12	Air	76	P
		1.10	3	None	81	P	1.13	Air	82	P
		1.11	24	None	83	P	1.14	Air	88	P

[a] Ratio of benzaldehyde/malononitrile/thiophenol.
[b] HPLC yields.
[c] Ratio of pyridine/1,4-dihydropyridine; 'P' denotes pyridine product only.
[d] Air bubbling was initiated after 1 h of the reaction.

contributions to product formation of these two major competing pathways, though given that the oxidation step was still incomplete after 2 h in the presence of air, we surmised that aerobic oxidation was most likely the minor pathway.

Model one-pot reactions between benzaldehyde, malononitrile and thiophenol (Scheme 1, R=R′=Ph) were carried out in parallel under a variety of conditions using a 24-well Büchi Syncore® apparatus. Variables considered were ratio of reagents, reaction time and the presence or absence of air (Table 1). Progress was followed by LC–MS analysis of aliquots of the reaction mixture at the same intervals in each case (1, 2, 3 and 24 h).

Where the standard 1:2:1 ratio of thiol, malononitrile and thiophenol was used and reactions carried out at 50 °C,[9] yields were always below 50%. If a stream of air was bubbled through the reaction mixture, commencing after 1 h, small enhancements in yield were obtained in line with our initial supposition that aerobic oxidation makes only a minor contribution to product formation (compare entries 1.5–1.7 and 1.2–1.4, Table 1). Further, we reasoned that changing the ratio of reactants to 2:3:1 would generate an additional equivalent of Knoevenagel adduct **5** in situ thereby allowing the oxidation step to reach completion, were this species the predominant oxidising agent involved. These reactions indeed displayed greatly enhanced yields (entries 1.8–1.11, Table 1) compared to the previous case, and none of the 1,4-dihydropyridine intermediates was present in these reaction mixtures even after 1 h. Introducing a stream of air after 1 h of reaction, as before, again resulted in only slight enhancements in product yield. Thus, we appeared to have established that both the competing pathways of oxidation are present in the final step to give the pyridine product, and that chemical oxidation by reaction intermediate **5** is the major pathway by which this final conversion proceeds.

To confirm these findings, a series of reactions were carried out with strict exclusion of air—in degassed ethanol under nitrogen—with the expectation that the results would closely match those seen under the standard reaction protocol (where the reaction mixture is open to the air). We also looked at raising the reaction temperature to reflux, as this is how our previous library syntheses[6,10] had been carried out.

Raising the reaction temperature to reflux (Table 2) proved advantageous in every case, leading to improved yields and an increased ratio of pyridine products in the reaction mixture (compare entries 2.1–2.7, Table 2, with 1.1–1.7 in Table 1). It was pleasing to note that when using a 1:2:1 ratio of reactants, a yield of over 50% was seen for the first time when a stream of air was bubbled through the refluxing reaction mixture (66% yield, entry 2.7, Table 2); the contribution of aerobic oxidation presumably 'freed up' more of reactive intermediate **5** for product formation compared to the case when no air stream was used (48% yield, entry 2.4). Using a ratio of reactants of 2:3:1, yields were again high with essentially complete conversion to product after 24 h (entries 2.15, 2.18 and 2.22, Table 2) and no 1,4-dihydropyridine intermediate detected.

In the reactions carried out under nitrogen, however (entries 2.8–2.11, 2.19–2.22, Table 2)—where a stream of nitrogen was bubbled through the reaction mixture—the reaction

Table 2. One-pot reactions carried out at reflux, either exposed to air or with bubbling of a stream of air or nitrogen through the reaction mixture

Reagent ratio[a]	T (°C)	Entry	t (h)	Gas	Yield[b] (%)	Product ratio[c]	Entry	Gas[d]	Yield[b] (%)	Product ratio[c]	Entry	Gas	Yield[b] (%)	Product ratio[c]
1:2:1	Reflux	2.1	1	None	31	2.1					2.8	N_2	15	1.6
		2.2	2	None	35	1.9	2.5	Air	33	2.6	2.9	N_2	20	1.1
		2.3	3	None	40	2.0	2.6	Air	38	2.2	2.10	N_2	18	1.4
		2.4	24	None	48	P	2.7	Air	66	P	2.11	N_2	56	P
2:3:1	Reflux	2.12	1	None	62	P					2.19	N_2	72	P
		2.13	2	None	78	P	2.16	Air	77	P	2.20	N_2	71	P
		2.14	3	None	99	P	2.17	Air	86	P	2.21	N_2	68	P
		2.15	24	None	98	P	2.18	Air	99	P	2.22	N_2	99	P

[a] Ratio of benzaldehyde/malononitrile/thiophenol.
[b] HPLC yields.
[c] Ratio of pyridine/1,4-dihydropyridine; 'P' denotes pyridine product only.
[d] Air bubbling was initiated after 1 h of the reaction.

Table 3. One-pot reactions carried out at a 50-fold dilution to prevent product precipitation

Reagent ratio[a]	T (°C)	Entry	t (h)	Gas	Yield[b] (%)	Product ratio[c]	Entry	Gas	Yield[b] (%)	Product ratio[c]	Entry	Gas	Yield[b] (%)	Product ratio[c]
1:2:1	Reflux	3.1	1.5	None	5	0.5	3.4	Air	5	0.5	3.7	N_2	3	0.4
		3.2	2.5	None	8	1.1	3.5	Air	10	1.5	3.8	N_2	6	0.8
		3.3	3.5	None	12	1.8	3.6	Air	17	2.8	3.9	N_2	10	1.4
2:3:1	Reflux	3.10	1.5	None	21	2.6	3.13	Air	30	3.8	3.16	N_2	13	1.6
		3.11	2.5	None	28	5.4	3.14	Air	45	7.2	3.17	N_2	23	3.5
		3.12	3.5	None	37	9.2	3.15	Air	58	24	3.18	N_2	28	5.7

[a] Ratio of benzaldehyde/malononitrile/thiophenol.
[b] HPLC yields.
[c] Ratio of pyridine/1,4-dihydropyridine.

appeared to be slower in the earlier stages compared to the instances when air was present. In addition, when the ratio of reagents of 1:2:1 was used (entries 2.8–2.11), a significantly lower ratio of pyridine product was seen under nitrogen than in the reactions, which were open to air (entries 2.1–2.4). These observations led us to suppose that the role of aerobic oxidation in the reaction pathway may be more significant than we had earlier assumed.

In order to reach a firm conclusion, a similar series of reactions to those already detailed were carried out but at a 50-fold dilution relative to the previous examples (Table 3). This ensured that precipitation of the product was prevented and that all components of the reaction mixture remained in solution for the duration of the reaction. The data so obtained reveal a significant role for aerobic oxidation, although reactions did not reach completion within a reasonable time at this dilution, as might be expected.

Using the classical 1:2:1 ratio of benzaldehyde, malononitrile and thiophenol, it can be clearly seen that pyridine yields and product ratios were highest when air was bubbled through the reaction mixture (entries 3.4–3.6, Table 3), lowest with exclusion of air (entries 3.7–3.9) and intermediate when open to air but without a bubbled stream (entries 3.1–3.3). This trend was repeated when using the 2:3:1 ratio of reactants (entries 3.10–3.18), and the fact that yields were more than doubled under these conditions is enough to implicate a significant role for aerobic oxidation alone. The extent of the contribution from oxidation by air is much more pronounced in these dilute reactions than it is under the standard conditions—so, under the usual, more concentrated conditions used for library synthesis it may be assumed that the small contribution by aerobic oxidation in these cases is due to the low, limiting solubility of oxygen in the solvent (ethanol).

2.3. Variation of substitution at 2- and 6-position

Having gained a good understanding of the reaction between benzaldehyde, malononitrile and thiophenol, investigation of the use of other aldehydes and thiols was necessary to establish the generality of our observations. Two aldehydes (3-hydroxybenzaldehyde and 4-chlorobenzaldehyde) and two thiols (3-[carboxymethyl]thiophenol and 4-hydroxythiophenol) were chosen, and reacted in the four possible combinations for their use in this MCR (Table 4). The general trend matched that seen in earlier examples: best yields were obtained when a stream of air was bubbled through the reaction mixture (entries 4.5–4.8, Table 4), lowest yields were seen with exclusion of air (entries 4.9–4.12), and the case where the reaction mixture was left open to the air, but without a bubbled stream, gave intermediate results (entries 4.1–4.4). For reactions involving 4-hydroxythiophenol, though, bubbling of air through the reaction mixture offered no improvement in yield over cases where this mixture was simply left open to the air (compare entries 4.6 and 4.8 with entries 4.2 and 4.4, respectively).

Table 4. One-pot reactions using a variety of aldehydes (RCHO) and thiols (R′SH)

Reagent ratio[a]	T (°C)	Entry	R	R′	t (h)	Gas	Yield[b] (%)
1:2:1	Reflux	4.1	3-OH-C_6H_4	3-CO_2Me-C_6H_4	3	None	29
		4.2	3-OH-C_6H_4	4-OH-C_6H_4	3	None	35
		4.3	4-Cl-C_6H_4	3-CO_2Me-C_6H_4	3	None	27
		4.4	4-Cl-C_6H_4	4-OH-C_6H_4	3	None	50
1:2:1	Reflux	4.5	3-OH-C_6H_4	3-CO_2Me-C_6H_4	3	Air	42
		4.6	3-OH-C_6H_4	4-OH-C_6H_4	3	Air	35
		4.7	4-Cl-C_6H_4	3-CO_2Me-C_6H_4	3	Air	34
		4.8	4-Cl-C_6H_4	4-OH-C_6H_4	3	Air	43
1:2:1	Reflux	4.9	3-OH-C_6H_4	3-CO_2Me-C_6H_4	3	N_2	27
		4.10	3-OH-C_6H_4	4-OH-C_6H_4	3	N_2	24
		4.11	4-Cl-C_6H_4	3-CO_2Me-C_6H_4	3	N_2	27
		4.12	4-Cl-C_6H_4	4-OH-C_6H_4	3	N_2	21
2:3:1	Reflux	4.13	3-OH-C_6H_4	3-CO_2Me-C_6H_4	3	None	66
		4.14	3-OH-C_6H_4	4-OH-C_6H_4	3	None	61
		4.15	4-Cl-C_6H_4	3-CO_2Me-C_6H_4	3	None	59
		4.16	4-Cl-C_6H_4	4-OH-C_6H_4	3	None	57

[a] Ratio of benzaldehyde/malononitrile/thiophenol.
[b] HPLC yields.

Table 5. Reactions performed under microwave irradiation

Reagent ratio[a]	T (°C)	Entry	t (min)	Yield[b] (%)	Product ratio[c]
1:2:1	90	5.1	10	36	4.0
		5.2	20	41	5.7
		5.3	30	42	6.5
		5.4	90	56	17
2:3:1	90	5.5	5	70	P
		5.6	10	75	P
		5.7	15	76	P
		5.8	30	82	P

[a] Ratio of benzaldehyde/malononitrile/thiophenol.
[b] HPLC yields.
[c] Ratio of pyridine/1,4-dihydropyridine; 'P' denotes pyridine product only.

As was expected, best results were obtained when using a 2:3:1 ratio of aldehyde, malononitrile and thiol, though optimum yields were lower than in the model reactions carried out earlier using benzaldehyde and thiophenol. Use of more highly functionalised starting materials naturally increases the likelihood of side reactions, thereby compromising the efficacy of the MCR, but nonetheless, the yields obtained represented an improvement over the classical method.

2.4. Effect of microwave heating

We have previously applied the use of microwave irradiation (MWI) to the MCR preparation of pyridine-3,5-dicarbonitrile libraries[10] and found that this technique offered some advantage compared to classical solution-phase reactions in most cases. Consequently, it seemed pertinent to investigate the use of MWI in these reactions further as part of the present study. Microwave assisted reactions between benzaldehyde, malononitrile and thiophenol were carried out in a sealed tube at 90 °C and the results are summarised in Table 5.

Using a 1:2:1 ratio of reactants, the microwave reaction was found to follow a similar course to the classical method (entries 2.1–2.4, Table 2) but with an approximately 20-fold acceleration rate and a higher ratio of pyridine to 1,4-dihydropyridine at every stage (entries 5.1–5.4, Table 5). When the same reagents were combined in a 2:3:1 ratio under the microwave conditions, a high degree of conversion to product was seen after just 10 min and only the desired pyridine product could be detected in the reaction mixtures. Use of MWI was thus found to offer significant potential for a greatly accelerated version of this MCR, amenable to high-throughput library generation.

2.5. Isolated yields of pyridine-3,5-dicarbonitriles

Though combining the reagents in the MCR in a ratio of 2:3:1 may be considered wasteful in terms of the aldehyde and malononitrile, our LC–MS analysis of model reactions (entries 2.12–2.22, Table 2) showed clearly that this procedure results in a clean, high degree of conversion to the desired pyridine-3,5-dicarbonitriles. We assumed that such conditions would facilitate isolation of pure products in good yields—the most important criteria in library synthesis—and set out to test this assertion by applying our optimised conditions to the preparation of a small range of pyridine-3,5-dicarbonitriles **1** (Table 6). At reflux in ethanol, improved yields of precipitated, pure products were obtained; where significant discrepancies between calculated (HPLC) and isolated yields are seen, this may be due to partial solubility of such products resulting in incomplete precipitation. The aliphatic aldehyde, propionaldehyde (entry 6.1, Table 6), appeared to give a more complex mixture due to its relatively higher reactivity and column chromatography was required in this case to allow isolation of the pure product. The isolated yields reported here represent a significant improvement over existing protocols, for example, our previous best isolated yields for the 4-(thiophen-2-yl) compounds (entries 6.6 and 6.7) were 18[6] and 23%[10], respectively.

Of most interest, however, are the results obtained using microwave heating (entries 6.8–6.14, Table 6). Combining the starting materials in a 2:3:1 ratio led to high yields after only 10 min of reaction and the products could be collected simply by filtration. In most cases, the microwave assisted reaction noticeably outperformed the classical method, but its attractiveness lies primarily in the rapid and expedient route to pyridine-3,5-dicarbonitriles offered by this technique.

3. Discussion

The observations detailed above provide new insight into the mechanism of pyridine-3,5-dicarbonitrile formation via the MCR shown in Scheme 1. Direct observation of the reaction intermediates along the stepwise pathway (Scheme 3) by mass spectrometry, coupled with that of reduced species **12** and **13** (derived from **6** and **7**, respectively), suggests that ring formation occurs through a sequential rather than concerted pathway. At the outset of our studies we considered the single-step mechanism (Fig. 2) to be less feasible, requiring as it does in a five-molecule concerted process (as proposed). In order for such a process to occur, the thiol

Table 6. Comparison of HPLC and isolated yields in reactions using a variety of aldehydes (RCHO) and thiols (R′SH)

R	R′	Entry	t[a] (h)	Yield (HPLC)	Yield (Isol.)	Entry	t[b] (min)	MWI yield (HPLC)	MWI yield (Isol.)
Et	Ph	6.1	3	42	23[c]	6.8	10	10	7[c]
4-F-C_6H_4	Ph	6.2	3	76	72	6.9	10	69	62
4-Cl-C_6H_4	Ph	6.3	3	71	63	6.10	10	89	78
4-Cl-C_6H_4	4-OH-C_6H_4	6.4	3	71	53	6.11	10	82	73
Ph	Ph	6.5	3	99	82	6.12	10	87	81
Thiophen-2-yl	Ph	6.6	3	54	41	6.13	10	85	60
Thiophen-2-yl	4-Cl-C_6H_4	6.7	3	79	75	6.14	10	80	70

In each case, reactions were performed using a ratio of 2:3:1 of aldehyde/malononitrile/thiol.
[a] Conventional heating at reflux.
[b] Microwave heating at 90 °C.
[c] Yield after column chromatography.

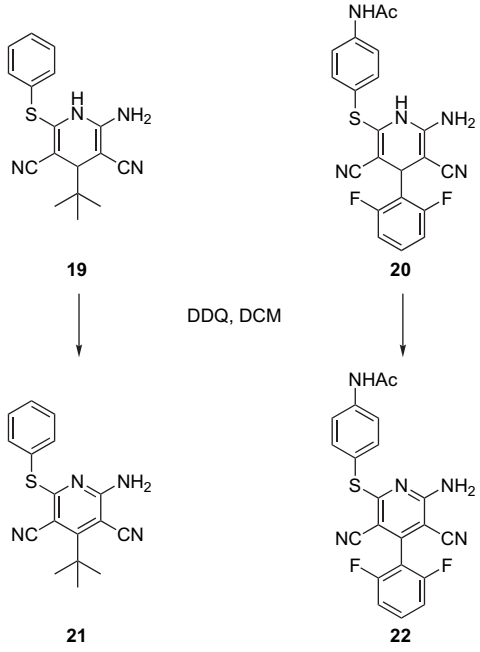
and malononitrile species must be present in their deprotonated states, yet in the presence of only a catalytic amount of base. Hence, we considered the likelihood of all species illustrated coinciding as necessary to be negligible.

Previous work[4] within our group revealed that increasing the amount of base used led almost exclusively to a decrease, rather than an increase, in yield as might be expected were the pathway illustrated in Figure 3 significant. The putative single-step ring formation presents a further objection: attack of a thiolate anion upon a negatively charged species (the malononitrile anion) is unlikely given the coulombic repulsion, which would exist between these two entities. It was therefore assumed that the stepwise assembly of the ring (Scheme 2)—which we have published previously[6]—was more plausible. The data reported herein, particularly the observation of intermediates **6** and **13**, confirm such a mechanism as a viable route to 1,4-dihydropyridines **9**, though they do not entirely rule out the possibility of a contribution to product formation arising from other reaction pathways, such as that illustrated in Figure 3.

The present results further clarify the nature of oxidation of the penultimate 1,4-dihydropyridine products **9**, formed initially under the reaction conditions, to the final pyridine-3,5-dicarbonitriles **1**. The greater extent of this conversion arises through a base-catalysed oxidative process, first described by Evdokimov et al.,[9] in which there is a net transfer of H_2 from **9** to Knoevenagel adduct **5** with concomitant aromatisation to form the pyridine ring. It is apparent that aerobic oxidation also contributes to this process, and that when the conventional 1:2:1 ratio of aldehyde/malononitrile/thiol is used, approximately one third of the product formation may be accounted for through this avenue. This is obvious in the case of the dilute, homogeneous reactions (compare entries 3.7–3.9 with 3.4–3.6 in Table 3) and also apparent in the early stages (first 3 h) of reactions run at higher concentration (compare entries 2.9 and 2.10 with 2.5 and 2.6 in Table 2). In addition, the fact that a yield of over 50% was obtained on bubbling air through the reaction mixture implies that a degree of aerobic oxidation was taking place (entry 2.7, Table 2).

Given the oxidative role of Knoevenagel adduct **5** in product formation, we attempted combining the reactants in a 2:3:1 ratio to generate an additional equivalent of this intermediate in situ, such that its consumption in the oxidation step would no longer compromise product yields. As was anticipated, a marked increase in yield resulted in every case. Interestingly though, when using the 2:3:1 ratio in the homogeneous (dilute) reactions, observed yields were doubled when air was bubbled through the solutions compared to the cases where air was excluded (compare entries 3.16–3.18 with 3.13–3.15, Table 3). These results—together with those reported above—suggest that under the classical (higher concentration) conditions employed for this MCR, the noted role of aerobic oxidation in the final aromatisation step is limited by the low solubility of oxygen in ethanol. Our experimental evidence indicates that solubility aside, the reactivities of **5** and oxygen in this step are comparable, but as Knoevenagel adduct **5** remains in solution it is present at higher concentration and thus makes the dominant contribution to the oxidation process.

When a sterically hindered aldehyde is used in the MCR, the reaction stops at the 1,4-dihydropyridine stage, a phenomenon that has already been noted by both ourselves[6] and others.[9] This occurred during the attempted synthesis of compound **21**[6] (Scheme 5), derived from trimethylacetaldehyde, and is also seen where hindered aromatic aldehydes—such as 2,6-dichlorobenzaldehyde[9]— are used. An intriguing intermediate result was seen in the course of trying to prepare **22** from 2,6-difluorobenzaldehyde, which we previously reported[6] as providing a 3% yield of the pyridine. Further examination of this case, however, revealed that the 1,4-dihydropyridine **20** is the major species formed, the desired pyridine **22** being a minor product in this example.



Scheme 5. DDQ-mediated oxidation of hindered 1,4-dihydropyridines.

The isolation of **19** and **20** plainly provided direct evidence for the position of 1,4-dihydropyridines **9** as the penultimate product in the reaction pathway and it is self-evident that neither Knoevenagel adduct **5** nor atmospheric oxygen is strong enough oxidant to cause aromatisation when the 4-position is sufficiently sterically crowded. Conversion into the pyridines is quite possible by other means, however, and we have found 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to be an efficient oxidant for this purpose.[6] Thus, using this reagent, preparation of the desired pyridine-3,5-dicarbonitriles **21** and **22** proved straightforward (Scheme 5).

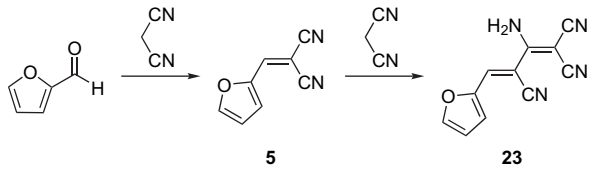
Since its role as an efficient oxidant for 1,4-dihydropyridines had been established, the low-concentration reactions in Table 2 were repeated with addition of DDQ after 90 min. In these cases only the pyridine product was detected, though yields remained more or less unchanged. Analysis of LC–MS traces from aliquots of the reaction mixtures revealed that though DDQ was responsible for efficient 1,4-dihydropyridine oxidation, several extra species arising from side reactions were detected in the presence of this reagent.

The case of microwave irradiation is intriguing given that a yield of over 50% was observed using a 1:2:1 ratio of reagents

(56% yield, entry 5.4, Table 5). Since these reactions were carried out in a sealed system aerobic oxidation was not possible, but as already noted, other minor components of the reaction mixture are now known to contribute to the 1,4-dihydropyridine oxidation, which may serve to explain this apparent discrepancy. Under microwave conditions, yields were almost exactly doubled when using a 2:3:1 ratio, underlining the role of **5** as an active oxidising agent in the MCR (compare entries 5.6 with 5.1 and 5.8 with 5.3, Table 5).

Regrettably, absolute confirmation of the proposed mechanism through direct isolation of **11** (the reduced form of **5**) remains elusive. It has already been noted that this species' thiol addition product, **12**, has been observed by both ourselves and others,[9] warranting the consideration that **6** may be the active oxidising agent, undergoing direct reduction to **12**. Were this the case, the thiol would be consumed unproductively, precluding the high extent of conversion to products which is seen in most cases. So, although reduction of **9** by **6** might well occur to some small extent where product yields do not approach 100%, it must be concluded that Knoevenagel adduct **5** is the prevailing—though not the only—oxidant involved in the final step of the reaction mechanism. Moreover, the observations reported herein imply that ring formation (i.e., of the 1,4-dihydropyridine **9**) must be relatively fast compared to the subsequent oxidation step, otherwise **12** would be formed to a greater extent than is usually observed. Finally, Evdokimov et al. have noted that reaction of **12** with malononitrile (and a catalytic amount of base) in the presence of DDQ results in pyridine-3,5-dicarbonitrile formation.[11] We attribute this finding to the oxidation of **12** back to reactive intermediate **6** under such conditions, re-opening the established reaction pathway back to **9** followed by DDQ-mediated oxidation to give **1**.

Despite the now defined role of **5** in the reaction pathway, the loss of yield observed when using a range of more functionalised aldehydes and thiols requires explanation. Even using the 2:3:1 ratio, which proved highly efficient in model reactions, the extent of conversion to product is typically in the range 55–80% and would be expected to be higher (though such results do represent a significant improvement over existing protocols, nonetheless). Direct reduction of **9** by reaction intermediate **6** would consume thiol unproductively, as already discussed, and likely results in a small loss of yield in some cases. One further possibility merits discussion, however. In earlier work[10] we found 2-furaldehyde to be ineffective as a component of the MCR and isolated **23**, the product of 1,2-addition of a second equivalent of malononitrile to reaction intermediate **5**, as a major component of the complex mixture formed when using this aldehyde (Scheme 6). Such an unwanted side reaction diverted the reaction away from a fruitful pathway with the result that desired product formation failed. In this instance the problem was circumvented by addition of the Knoevenagel adduct to a premixed solution of malononitrile and twofold excess of thiol containing catalytic base, suppressing the formation of **23** and successfully directing the reaction towards formation of the desired products. We assume that a similar reaction of adducts of general structure **5** with malononitrile, rather than the thiols, would occur in some cases where a low yield is observed, most likely when other heteroaromatic aldehydes are used.



5 **23**

Scheme 6. An unproductive side reaction of **5** observed during the use of 2-furaldehyde in MCR.

Similarly, another obvious possibility needs to be considered. In order for product formation to occur, a 1,2-addition of thiol to adduct **5** is necessary, though one would expect 1,4-addition to be the prevailing process in the case of such a soft nucleophile. This 1,4-addition is reversible though, and unavailing in terms of product formation, so we consequently assume the subsequent (ultimately irreversible) steps following 1,2-addition, leading to pyridine-3,5-dicarbonitrile products, drive the reaction down that pathway in the course of this MCR. Finally, it must be considered that Knoevenagel adducts **5** derived from differing aldehydes may vary in their efficiency as oxidising agents in the final step of formation of the pyridine products.

4. Conclusions

Mechanistic studies have established that in the multi-component reaction of an aldehyde, a thiol and malononitrile, the route to product formation is most likely as proposed in Scheme 2 (vide supra), with the final oxidation step being mediated primarily by the intermediate Knoevenagel adducts present in the reaction pathway (Fig. 2). This is not the only mode of oxidation of the penultimate 1,4-dihydropyridine products, however, since a notable contribution from aerobic oxidation is observed where reaction mixtures are exposed to air. Optimal yields are obtained when the aldehyde, malononitrile and thiol components are combined in a 2:3:1 ratio, thereby generating sufficient Knoevenagel adduct in situ to permit both 1,4-dihydropyridine ring formation and subsequent oxidation to the final aromatised products.

Significant acceleration of the MCR was observed under microwave irradiation, allowing isolation of precipitated products after short reaction times and so providing much more straightforward access to large pyridine-3,5-dicarbonitrile libraries than has previously been possible. The results reported herein thus represent an important step forward for medicinal chemistry programmes requiring increased library sizes of these potential therapeutic compounds for lead generation and optimisation.

5. Experimental

5.1. General procedures

All reagents were purchased from commercial sources and used as supplied. Flash column chromatography was carried out using Fluorochem silica gel 60 Å. Accurate mass and nominal mass measurements were determined using a Waters Micromass LCT electrospray mass spectrometer (ES mode), or a VG Autospec electron impact mass spectrometer (EI mode). LC–MS analysis was carried out under the following conditions: HPLC—Gemini 5 μ C18 column,

250×2.0 mm, 50–72% MeCN (0.1% TFA) in water (0.1% TFA) over 20 min, then 72–95% MeCN (0.1% TFA) in water (0.1% TFA) over 5 min, then 95–50% MeCN (0.1% TFA) in water (0.1% TFA) over 5 min, flow rate 0.2 mL min^{-1}, detection at 254 nm, total run time 30 min; MS—measured using a Waters Micromass LCT electrospray mass spectrometer in ES^+ mode for 30 min. Where parallel reactions are described, these were carried out in 50 mL glass test tubes using a Büchi Syncore® 24-well parallel synthesis apparatus. This equipment permits reactions to be carried out under an inert atmosphere of nitrogen across all wells where necessary. Microwave reactions were carried out using a Smith-Creator™ Optimiser EXP reactor (Personal Chemistry, Inc.). The machine consists of a continuous focussed microwave power delivery system. Reaction times and temperatures are operator selectable. Sample temperature is constantly monitored by IR, pressure by a transducer on the top of the vial's septum, and the microwave power automatically adjusted to maintain programmed temperature profiles. For the experiments reported here, 'Fix Hold Time' was set to 'On' and 'Absorption Level' set to 'Normal'. Reactions were carried out in Smith Process Vials™ (2.0–5.0 mL).

5.2. Analysis of reaction pathway (Section 2.1)

5.2.1. Three-step reaction (Scheme 3).

(i) *With no exclusion of air*: piperidine (15 μL, 13 mg, 0.15 mmol) was added to a stirred solution of benzaldehyde (152 μL, 159 mg, 1.5 mmol) and malononitrile (94 μL, 99 mg, 1.5 mmol) in absolute ethanol (5 mL). The reaction mixture was warmed to 50 °C and after 5 min an aliquot was removed for MS analysis (step 1). Thiophenol (154 μL, 165 mg, 1.5 mmol) was added and heating continued at 50 °C for a further 45 min, at which point a second aliquot was taken for MS analysis (step 2). A second equivalent of malononitrile (94 μL, 99 mg, 1.5 mmol) was added and after 2 h more at the same temperature, a final aliquot was sampled for MS analysis (step 3). Observed in step 1: 2-benzylidenemalononitrile **5**, HRMS (ES^-) *m/z* calcd for $C_{10}H_5N_2$ $[M]^-$ 154.0530, obsd 154.0532. Step 2: 2-cyano-3-phenylthioacrylimidic acid phenyl ester **6** (R=R′=Ph), HRMS (ES^+) *m/z* calcd for $C_{16}H_{13}N_2S$ $[M+H]^+$ 265.0799, obsd 265.0806. Step 3: 2-amino-4-phenyl-6-sulfanylpyridine-3,5-dicarbonitrile **1** (R=R′=Ph), HRMS (ES^+) *m/z* calcd for $C_{19}H_{13}N_4S$ $[M+H]^+$ 329.0861, obsd 329.0860; 2-amino-4-phenyl-6-phenylsulfanyl-1,4-dihydropyridine-3,5-dicarbonitrile **9** (R=R′=Ph), HRMS (ES^+) *m/z* calcd for $C_{19}H_{15}N_4S$ $[M+H]^+$ 331.1017, obsd 331.1027; **6**; 3-amino-2-benzyl-3-phenylsulfanylacrylonitrile **12** (R=R′=Ph), HRMS (ES^+) *m/z* calcd for $C_{16}H_{15}N_2S$ $[M+H]^+$ 267.0956, obsd 267.0952; 4-(benzylaminophenylsulfanylmethyl)-2-cyano-3-phenylpent-2-enedinitrile **13**, HRMS (ES^+) *m/z* calcd for $C_{26}H_{21}N_4S$ $[M+H]^+$ 421.1487, obsd 421.1483; 2,6-dicyano-3,5-diiminoheptanedinitrile **17**; MS (ES^+) *m/z* 199 $[M+H]^+$; 3-amino-2,6-dicyano-5-iminoheptanedinitrile **18**, MS (ES^+) *m/z* 201 $[M+H]^+$. Signals for **17** and **18** were too weak for HRMS measurement to be carried out, presumably as only a trace of these structures were present in the reaction mixture.

(ii) *With exclusion of air*: a two-neck round-bottomed flask fitted with a suba-seal and reflux condenser was flushed with nitrogen and charged with degassed ethanol (5 mL). Thorough exclusion of air from the solvent was ensured by bubbling a stream of nitrogen (via a needle inserted through the suba-seal) through the ethanol for 15 min. Benzaldehyde (152 μL, 159 mg, 1.5 mmol), malononitrile (94 μL, 99 mg, 1.5 mmol) and piperidine (15 μL, 13 mg, 0.15 mmol) were then added and the solution warmed to 50 °C. After 5 min an aliquot of the reaction mixture was removed for MS analysis (step 1). Thiophenol (154 μL, 165 mg, 1.5 mmol) was added and heating continued at 50 °C for a further 45 min, at which point a second aliquot was taken for MS analysis (step 2). Additional malononitrile (94 μL, 99 mg, 1.5 mmol) was then added and after an extra 2 h at the same temperature, a final aliquot was withdrawn for MS analysis (step 3). Observed in step 1: **5**. Step 2: **6**. Step 3: **1**, **9**, **6**, **12** (all with R=R′=Ph), **17** and **18**, HRMS analyses of which concurred with those above; 2-(benzylaminophenylsulfanylmethyl)-4-cyano-3-phenylpentanedinitrile **16**, MS (ES^+) 422 $[M]^+$. The signals for **16** were too weak for HRMS measurement to be carried out, presumably as only a trace of this structure was present in the reaction mixture. Aliquots were subjected to MS analysis as soon as possible after removal from the reaction mixture. Repeated MS analysis of the final sample (from step 3) revealed an apparently increasing amount of pyridine product relative to the 1,4-dihydropyridine over time due to air exposure.

5.2.2. Two-step reaction (Scheme 4). Piperidine (15 μL, 13 mg, 0.15 mmol) was added to a stirred solution of benzaldehyde (152 μL, 159 mg, 1.5 mmol) and malononitrile (188 μL, 198 mg, 3.0 mmol) in absolute ethanol (5 mL). The reaction mixture was warmed to 50 °C and after 5 min an aliquot was removed for MS analysis (step 1). Thiophenol (154 μL, 165 mg, 1.5 mmol) was added and heating continued at 50 °C for a further 3 h, after which time a second aliquot was taken for MS analysis (step 2). Observed in step 1: **5**, HRMS analysis of which was in agreement with that above; 2,4-dicyano-3-phenylglutaronitrile, MS (EI^+) *m/z* 220, $[M]^+$. Again, this signals was too weak for HRMS measurement to be carried out. Step 2: **1**, **9**, **6** (all with R=R′=Ph) and **13**, HRMS analyses of which all concurred with those reported above.

5.3. Study of the role of aerobic oxidation (Section 2.2)

5.3.1. Model one-pot reactions at 50 °C (Table 1). Parallel reactions were used to investigate the two different ratios of starting materials. In the first run, entries 1.1–1.7 (Table 1) were carried out in parallel at 50 °C using benzaldehyde (30.5 μL, 31.8 mg, 0.30 mmol), malononitrile (37.8 μL, 39.6 mg, 0.60 mmol), thiophenol (30.7 μL, 33.1 mg, 0.30 mmol) and piperidine (3 μL, 2.6 mg, 0.03 mmol) in absolute ethanol (1 mL) in each case, with air bubbling used where indicated. In a second run, entries 8–14 were carried out similarly using benzaldehyde (71 μL, 62.6 mg, 0.60 mmol), malononitrile (56.7 μL, 59.4 mg, 0.90 mmol), thiophenol (30.7 μL, 33.1 mg, 0.30 mmol) and piperidine (6 μL, 5.2 mg, 0.06 mmol) in absolute ethanol (1 mL) in each case, again with air bubbling used wherever

indicated. In every case above, acetonitrile (1 mL) was added at the point of analysis to ensure complete dissolution of all materials present. Of this final reaction mixture, an aliquot of 40 (entries 1.1–1.7) or 20 μL (entries 1.8–1.14) was made up to 1 mL in acetonitrile to provide a stock solution for LC–MS analysis.

5.3.2. Model one-pot reactions at reflux (Table 2). Reactions corresponding to entries 2.1–2.7 and 2.12–2.18 (Table 2) were carried out in the same way as those detailed above for entries 1.1–1.7 and 1.8–1.14 in Table 1, respectively, except the reaction temperature was set to 90 °C rather than 50 °C. Entries 2.8–2.11 were carried out as for entries 2.1–2.4 except using degassed ethanol and performed under a nitrogen atmosphere; likewise, entries 2.19–2.22 were carried out as for entries 2.12–2.15 but under nitrogen and using degassed ethanol.

5.3.3. Lower concentration reactions (Table 3).

(i) *1:2:1 ratio*: for entries 3.1–3.3—carried out in parallel—benzaldehyde (1.2 M in ethanol, 25 μL, 30 μmol), malononitrile (1.8 M in ethanol, 33 μL, 60 μmol), thiophenol (0.6 M in ethanol, 50 μL, 30 μmol) and piperidine (0.12 M in ethanol, 25 μL, 3 μmol) were combined in ethanol (5 mL) and heated to reflux. After 1.5 (entry 3.1), 2.5 (entry 3.2) and 3.5 h (entry 3.3), a 0.5 mL aliquot of the reaction mixture was withdrawn and diluted with 0.5 mL acetonitrile to prepare a stock solution for LC–MS analysis. Entries 3.4–3.6 were performed in the same manner, except stream of air was bubbled through the reaction mixtures. Likewise, entries 3.7–3.9 were carried out the same way but with a stream of nitrogen used, rather than air.

(ii) *2:3:1 ratio*: entries 3.10–3.18 were carried out as for the 1:2:1 reactions except that the following quantities of reagents were used: benzaldehyde (1.2 M in ethanol, 50 μL, 60 μmol), malononitrile (1.8 M in ethanol, 50 μL, 90 μmol), thiophenol (0.6 M in ethanol, 50 μL, 30 μmol) and piperidine (0.12 M in ethanol, 50 μL, 6 μmol) in ethanol (5 mL).

5.4. Model microwave assisted reactions (Section 2.4)

5.4.1. Model reactions (Table 5).

(i) *1:2:1 ratio (entries 5.1–5.4, Table 5)*: benzaldehyde (30.5 μL, 32 mg, 0.30 mmol), malononitrile (38 μL, 40 mg, 0.60 mmol), thiophenol (31 μL, 33 mg, 0.30 mmol) and piperidine (3.0 μL, 2.6 mg, 30 μmol) were combined in ethanol (1 mL) and the microwave vial sealed. The vessel was irradiated at 90 °C for 10 (entry 5.1), 20 (entry 5.2), 30 (entry 5.3) or 90 min (entry 5.4). After the reaction was completed, acetonitrile (4 mL) was added and a 100 μL portion of the resultant solution diluted further with 900 μL of acetonitrile to provide a stock solution for LC–MS analysis.

(ii) *2:3:1 ratio (entries 5.5–5.8, Table 5)*: benzaldehyde (71 μL, 64 mg, 0.60 mmol), malononitrile (57 μL, 60 mg, 0.90 mmol), thiophenol (31 μL, 33 mg, 0.30 mmol) and piperidine (6 μL, 5.2 mg, 60 μmol) were combined in ethanol and the microwave vial sealed. The reaction mixture was irradiated at 90 °C for 5 (entry 5.5), 10 (entry 5.6), 15 (entry 5.7) or 30 min (entry 5.8). Solutions for LC–MS analysis were then prepared as detailed above (for entries 5.1–5.4).

5.5. Variation of substitution at 2- and 6-position (Sections 2.3 and 2.5)

5.5.1. Parallel reactions (Table 4). Firstly, stock solutions of reagents were made up in ethanol/aldehydes at 2.4 M (3-hydroxybenzaldehyde and 4-chlorobenzaldehyde), thiols at 1.2 M (methyl-3-mercaptobenzoate and 4-mercaptophenol), malononitrile at 3.6 M and piperidine at 0.24 M. For each reaction of entries 4.1–4.4 (1:2:1 reactions), carried out in parallel, the relevant aldehyde (125 μL of stock solution, 0.3 mmol), malononitrile (167 μL of stock solution, 0.6 mmol), the relevant thiol (250 μL of stock solution, 0.3 mmol) and piperidine (125 μL of stock solution, 0.03 mmol) were combined in ethanol (1 mL) and the reaction heated at reflux for 3 h (temperature set to 90 °C). After this time, 4 μL of each reaction mixture was taken and made up to 1 mL in acetonitrile to provide a stock solution for LC–MS analysis. Reactions 4.5–4.8 were carried out in the same way but with a stream of air bubbled through the reaction mixture; similarly, reactions 4.9–4.12 were approached in the same manner with bubbling of a stream of nitrogen through the mixture. Entries 4.10–4.13 were carried out as for entries 4.1–4.4 but using the following quantities (2:3:1 ratio): aldehyde (250 μL of stock solution, 0.6 mmol), malononitrile (250 μL of stock solution, 0.9 mmol), thiol (250 μL of stock solution, 0.3 mmol) and piperidine (250 μL of stock solution, 0.06 mmol) in ethanol (1 mL).

5.5.2. Isolated products (Table 6).

5.5.2.1. Classical solution-phase method. Reactions were carried out in parallel. Aldehyde (3.0 mmol), malononitrile (283 μL, 297 mg, 4.5 mmol), thiol (1.5 mmol) and piperidine (30 μL, 26 mg, 0.3 mmol) were combined in ethanol (5 mL) and the solution heated at reflux for 3 h. After allowing the mixtures to cool to room temperature, the precipitated products were collected by filtration, washed with ethanol and dried thoroughly. We have reported the products for entries 6.5 and 6.6 (Table 6) previously,[6] and characterisation data were in agreement with those already published.

5.5.2.1.1. 2-Amino-4-ethyl-6-phenylsulfanylpyridine-3,5-dicarbonitrile (entry 6.1). It was prepared using propionaldehyde (221 μL, 174 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol), isolated as a white solid (56 mg, 23%) after purification of 300 mg of the 521 mg crude product by flash column chromatography on silica gel (eluted with 3:1 hexane/ethyl acetate): mp 148–150 °C; ν_{max} (solid)/cm^{-1} 3156, 2361, 2342, 2220, 1636, 1559, 1533, 1472, 1440, 1266, 1237, 1172, 1098, 1057, 1021; δ_H/ppm (250 MHz, DMSO-d_6) 7.74 (br s, 2H), 7.59–7.44 (m, 5H), 2.73 (q, 2H, J=7.6 Hz), 1.22 (t, 3H, J=7.6 Hz); δ_C/ppm (62.8 MHz, DMSO-d_6) 162.6, 159.7, 134.8, 129.6, 129.4, 127.2, 114.5, 93.0, 86.5, 26.9, 13.5; HRMS (EI$^+$), m/z calcd for $C_{15}H_{12}N_4S$ [M]$^+$ 280.0783, obsd 280.0784.

5.5.2.1.2. 2-Amino-4-(4-fluorophenyl)-6-phenylsulfanylpyridine-3,5-dicarbonitrile (entry 6.2). It was obtained as

a yellow solid (372 mg, 72%) using 4-fluorobenzaldehyde (328 μL, 372 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol): mp 248–250 °C; ν_{max} (solid)/cm^{-1} 3491, 3343, 3225, 2362, 2340, 2215, 1630, 1603, 1553, 1505, 1474, 1421, 1318, 1259, 1230, 1156, 1097; δ_H/ppm (250 MHz, DMSO-d_6) 7.86 (br s, 2H), 7.68–7.59 (m, 4H), 7.53–7.40 (m, 5H); δ_C/ppm (62.8 MHz, DMSO-d_6) 165.7, 159.6, 157.5, 136.7, 135.4, 134.8, 132.7, 130.4, 129.5, 128.9, 125.9, 115.1, 114.8, 93.2, 87.2; HRMS (EI$^+$), *m/z* calcd for $C_{19}H_{11}FN_4S$ [M]$^+$ 346.0688, obsd 346.0682.

5.5.2.1.3. 2-Amino-4-(4-chlorophenyl)-6-phenylsulfanylpyridine-3,5-dicarbonitrile (entry 6.3). It was obtained as a yellow solid (343 mg, 63%) using 4-chlorobenzaldehyde (435 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol): mp 230–232 °C; ν_{max} (solid)/cm^{-1} 3485, 3340, 3219, 2211, 1631, 1595, 1574, 1541, 1520, 1493, 1420, 1316, 1257, 1235, 1178, 1114, 1093; δ_H/ppm (250 MHz, DMSO-d_6) 7.88 (br s, 2H), 7.69–7.47 (m, 9H); δ_C/ppm (62.8 MHz, DMSO-d_6) 166.4, 159.6, 157.9, 157.5, 135.3, 132.8, 130.4, 130.3, 128.9, 127.6, 125.3, 117.0, 115.2, 114.9, 93.4, 87.1; HRMS (EI$^+$), *m/z* calcd for $C_{19}H_{11}ClN_4S$ [M]$^+$ 362.0393, obsd 362.0376.

5.5.2.1.4. 2-Amino-4-(4-chlorophenyl)-6-(4-hydroxyphenylsulfanyl)pyridine-3,5-dicarbonitrile (entry 6.4). It was obtained as a yellow solid (32.6 mg, 53%) after further washing of a 50 mg portion of the 464 mg crude product with hexane/ethanol (9:1), from a reaction using 4-chlorobenzaldehyde (435 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol): mp 252–253 °C; ν_{max} (solid)/cm^{-1} 3482, 3396, 3341, 3222, 2213, 1633, 1599, 1574, 1542, 1521, 1492, 1456, 1421, 1365, 1317, 1280, 1257, 1235, 1173, 1092, 1035, 1010, 886, 832, 821, 804; δ_H/ppm (250 MHz, DMSO-d_6) 9.99 (s, 1H), 7.81 (br s, 2H), 7.66 (d, 2H, *J*=8.6 Hz), 7.59 (d, 2H, *J*=8.6 Hz), 7.37 (d, 2H, *J*=8.6 Hz), 6.87 (d, 2H, *J*=8.6 Hz); δ_C/ppm (62.8 MHz, DMSO-d_6) 167.6, 159.6, 159.2, 157.4, 137.1, 135.3, 132.9, 130.5, 128.9, 116.6, 115.2, 115.0, 114.8, 92.7, 86.7; HRMS (EI$^+$), *m/z* calcd for $C_{19}H_{11}ClN_4OS$ [M]$^+$ 378.0342, obsd 378.0355.

5.5.2.1.5. 2-Amino-4-phenyl-6-phenylsulfanylpyridine-3,5-dicarbonitrile (entry 6.5). It was obtained as a yellow solid (402 mg, 82%) using benzaldehyde (305 μL, 318 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol).

5.5.2.1.6. 2-Amino-4-(thiophen-2-yl)-6-phenylsulfanylpyridine-3,5-dicarbonitrile (entry 6.6). It was obtained as a yellow solid (204 mg, 41%) using thiophene-2-carboxaldehyde (280 μL, 336 mg, 3.0 mmol) and thiophenol (156 μL, 165 mg, 1.5 mmol).

5.5.2.1.7. 2-Amino-4-(thiophen-2-yl)-6-[(4-chlorophenyl)sulfanyl]pyridine-3,5-dicarbonitrile (entry 6.7). It was obtained as a yellow powder (417 mg, 75%) using thiophene-2-carboxaldehyde (280 μL, 336 mg, 3.0 mmol) and 4-chlorothiophenol (217 mg, 1.5 mmol): mp 226–227 °C (decomp.); ν_{max} (solid)/cm^{-1} 3475, 3330, 3220, 2209, 1636, 1545, 1526, 1509, 1472, 1432, 1404, 1386, 1356, 1255, 1236, 1176, 1088, 1008, 906, 836, 820; δ_H/ppm (250 MHz, DMSO-d_6) 7.97 (d, 1H, *J*=4.9 Hz), 7.90 (br s, 2H), 7.70–7.50 (m, 5H), 7.30 (t, 1H, *J*=4.4 Hz); δ_C/ppm (62.8 MHz, DMSO-d_6) 166.3, 159.8, 150.9, 136.7, 134.8, 132.7, 131.3, 130.9, 129.5, 127.9, 126.0, 115.4, 115.1, 93.0, 86.9; HRMS (EI$^+$), *m/z* calcd for $C_{17}H_9ClN_4S_2$ [M]$^+$ 367.9957, obsd 367.9949.

5.5.2.2. Microwave method. For entries 1 and 2, aldehyde (3.0 mmol), malononitrile (283 μL, 297 mg, 4.5 mmol), thiol (1.5 mmol) and piperidine (30 μL, 26 mg, 0.3 mmol) were combined in ethanol (5 mL) in the microwave vial, which was then sealed. For entries 3–7, aldehyde (1.8 mmol), malononitrile (170 μL, 178 mg, 2.7 mmol), thiol (0.9 mmol) and piperidine (18 μL, 16 mg, 0.18 mmol) were combined in ethanol (3 mL) in the microwave vial, which was then sealed. In all cases the vessel was then irradiated at 90 °C for 10 min and after allowing cooling back to room temperature, the precipitated products were collected by filtration, washed with ethanol and dried thoroughly. Yields are summarised in Table 6 (entries 6.8–6.14) and characterisation data were in agreement with those reported above. Entry 6.8 required further purification by column chromatography, as described for entry 6.1.

Verification of compound purity: all products reported in Table 6 were subjected to HPLC analysis and found to be >95% pure (except entry 6.10, 94% pure). Conditions: Altima HPLC 3 μ C18 column, 150×4.6 mm, 40–70% MeCN in water over 20 min, then 70–90% MeCN in water over 5 min, then hold for 5 min; flow rate 1.0 mL min^{-1}, 20 μL injection. Detection at 256 nm, total run time 30 min.

5.6. Products derived from 2,6-difluorobenzaldehyde (Scheme 5)

5.6.1. *N*-{4-[6-Amino-3,5-dicyano-4-(2,6-difluorophenyl)-1,4-dihydropyridin-2-ylsulfanyl]phenyl}acetamide (21). Malononitrile (0.66 g, 10.0 mmol) in ethanol (4 mL) and 4-acetamidothiophenol (0.84 g, 5.0 mmol) were added successively to a solution of 2,6-difluorobenzaldehyde (0.54 mL, 0.71 g, 5.0 mmol) in the same solvent (15 mL) containing piperidine (60 μL, 52 mg, 0.61 mmol). After 6 h at reflux the reaction mixture was allowed to cool to room temperature and stirred overnight whilst open to the air. No precipitation of product was observed, so the mixture was poured onto ice (50 mL) and neutralised by dropwise addition of 32% hydrochloric acid. The resultant aqueous solution was extracted into chloroform and the organic layer separated and evaporated to dryness. Trituration with ethyl acetate/hexane (1:1) gave the title compound as a pale brown powder (260 mg, 12%): mp 284–285 °C; ν_{max} (solid)/cm^{-1} 3336, 2966, 2202, 2176, 1735, 1677, 1642, 1621, 1586, 1492, 1469, 1398, 1371, 1312, 1250, 1229, 1182, 1044, 993, 831, 780, 717; δ_H/ppm (250 MHz, DMSO-d_6) 10.16 (s, 1H), 8.99 (s, 1H), 7.67 (d, 2H, *J*=8.8 Hz), 7.48–7.36 (m, 3H), 7.15 (t, 2H, *J*=8.5 Hz), 6.02 (br s, 2H), 4.91 (s, 1H), 2.05 (s, 3H); δ_C/ppm (100 MHz, DMSO-d_6) 169.2, 167.8, 160.1, 159.8, 157.7, 147.7, 141.4, 136.5, 132.5, 120.4, 120.0, 119.5, 114.8, 114.5, 113.2, 93.8, 88.2, 53.2, 24.6; HRMS (ES$^+$), *m/z* calcd for $C_{21}H_{15}F_2N_5NaOS$ [M+Na]$^+$ 446.0863, obsd 444.0879. After the neutralisation with acid, some insoluble material was seen, which was not taken forward into the

organic extraction. Analysis of this material by TLC indicated that it contained a further portion of the title compound together with a small amount of the pyridine (oxidised product).

5.6.2. *N*-{4-[6-Amino-3,5-dicyano-4-(2,6-difluorophenyl)pyridin-2-ylsulfanyl]phenyl}acetamide (22). *N*-{4-[6-Amino-3,5-dicyano-4-(2,6-difluorophenyl)-1,4-dihydropyridin-2-ylsulfanyl]phenyl}acetamide **21** (70 mg, 0.165 mmol) was added to a solution of DDQ (56 mg, 0.24 mmol) in DCM (12 mL). After stirring at room temperature for 24 h, the reaction mixture was evaporated to dryness and the residue purified by flash column chromatography on silica gel (eluted with EtOAc) to provide the title compound as a pale yellow powder (69 mg, 99%): mp 261–263 °C; ν_{max} (solid)/cm^{-1} 3450, 3311, 3172, 3044, 2977, 2857, 2561, 2453, 2336, 2179, 1900, 1688, 1647, 1592, 1531, 1494, 1469, 1428, 1284, 1180; δ_H/ppm (250 MHz, DMSO-d_6) 8.07 (br s, 2H), 7.75–7.70 (m, 3H), 7.54 (d, 2H, J=8.5 Hz), 7.46–7.39 (m, 2H), 2.08 (s, 3H); δ_C/ppm (62.8 MHz, DMSO-d_6) 168.7, 167.3, 164.3, 160.4, 159.3, 147.2, 141.0, 136.0, 124.5, 119.5, 119.1, 114.3, 114.0, 112.7, 112.4, 93.9, 87.8, 24.1; HRMS (ES$^+$), m/z calcd for $C_{21}H_{13}F_2N_5NaOS$ [M+Na]$^+$ 444.0707, obsd 444.0720.

Acknowledgements

The authors acknowledge the Department of Health for their generous funding (Contract No. DH007/0102). We also thank Mr. Simon Thorpe for assistance with mass spectroscopy.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.139.

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