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Concise synthesis and CDK/GSK inhibitory activity of the missing 9-azapaullones

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ABSTRACT

A remarkably concise, chromatography-free route to the parent compound of the paullone family of cyclin-dependent kinase (CDK) inhibitors is reported. A similar strategy allowed the synthesis of the hitherto missing 9-azapaullone and its protonated, N-oxidised and N-alkylated derivatives. Screening studies identified an active and strongly selective inhibitor of CDK9/cyclin T.

As our appreciation of the molecular mechanisms that control the cell cycle matures, it is becoming clear that the molecules which control the checkpoints dividing the cell-cycle phases are interesting targets for the design of anti-cancer chemotherapeutic agents.[1,2] Cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases which, on binding to an appropriate regulatory protein (cyclin), become catalytically competent and trigger key switches during the cell cycle. When operating normally these complex control mechanisms allow smooth passage between cell-cycle phases and controlled proliferation.[3,4] Mutations leading to the misregulation of CDK-cyclin complexes are a characteristic of most human tumour cells[5] and thus the design of (selective) CDK inhibitors is an attractive strategy to combat cancer in cells where division is unregulated due to (for e.g.) either the overexpression of a cyclin component or the malfunctioning of a CDK (or CDK-cyclin complex).[6–8] In addition the inhibition of CDKs has been identified as a potential strategy in a variety of other processes in which cell proliferation plays an important role — such as anti-viral and anti-bacterial chemotherapy, CNS disorders and cardiovascular diseases.[9,6]

Paullones (Fig. 1) are fused tetracyclic compounds identified from analysis of data from an anti-cancer drug screen of the National Cancer Institute's tumour cell lines using the COMPARE algorithm to identify similarities (in terms of mode of action) between screened library compounds and the known CDK inhibitor flavopiridol.[10] The parent compound **1**[11] (paullone) possessed moderate inhibitory activity of CDK1/cyclin B (IC_{50}, 7.0 μM), however, the C-9 brominated analogue **2** (kenpaullone) proved a potent inhibitor with an activity IC_{50} of 0.4 μM. Subsequent SAR-type studies resulted in the identification of the corresponding C-9 nitro compound (alsterpaullone, **3**) as a powerful CDK inhibitor at close to nanomolar concentrations.[12] Further optimisation led to the discovery of the 2-cyanoethyl substituted compound **4** which is active at picomolar concentrations.[13] It is noteworthy that both **3** and **4** are also highly active inhibitors of glycogen synthase kinase-3 (GSK-3α and β) — an enzyme with roles in a number of biological processes such as glucose metabolism, the regulation of microtubule stability and cell-cycle control (*inter alia*).[14–16]

Seminal studies on these compounds by Kunick et al. repeatedly demonstrated that the fused tetracyclic unit incorporating three aromatic rings and an ε-lactam component (including the methylene group), the indole proton and a strongly electron withdrawing group at C-9 (preferably one with at least one lone pair of electrons capable of accepting a hydrogen bond) are all prerequisites for strong CDK1/cyclin B (and GSK 3α/β-inhibitory activity.[10–12]

A diverse array of these compounds have been synthesised (including azapaullone **5a** and 12-azapaullone derivative **5b**[17]—a potent and selective inhibitor of GSK-3),[10–12] however, we were intrigued by the absence of the azapaullone derivative **6** from all screened libraries. 9-Azapaullone (**6**) would be a compound of considerable interest — the pyridine ring nitrogen renders the aromatic ring electron deficient in a similar manner to the nitro group of alsterpaullone (**3**), and as such is an interesting target for its potential potency/specificity alone, however it could also be either alkylated or most importantly (given the SAR identified

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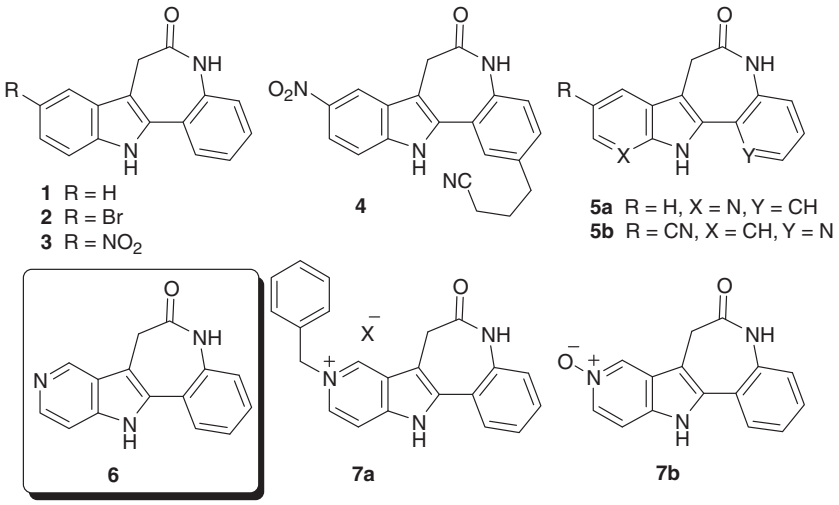


Figure 1. Selected paullone derivatives and aza-paullone **6**.

by Kunick) oxidised to afford compounds such as **7a**–**b**, respectively. These, together with **6**, would be potentially useful in further exploring chemical space around **3** and in particular offer new opportunities for the facile modification of the C-9 substituent while remaining firmly within the orbit of the pharmacophore. For example, Lemcke et al.[12b] constructed a 3D-QSAR picture of **3** bound to the ATP-binding site of CDK1 and identified regions of space where steric bulk (blue), negative charge (black) and positive charge (red) lead to increased inhibitory activity (Fig. 2). We were thus intrigued as to the potential activity of compounds such as **7a**,**b** — which are structurally quite distinct from **1**–**4** and possess (due to the innate modifiability of the pyridine ring at the nitrogen atom) functionality in these key areas *predicted* to be consistent with efficient binding.

Speculating that the absence of **6** from the literature was related to a synthetic challenge, we therefore set out to develop a new, concise and readily modifiable route to the paullone core and then to utilise this to prepare and evaluate the potency of 9-azapaullone. The route to the basic paullone structure originally developed by Kunick et al. (Scheme 1) involves a three step conversion of anthranilic acid ethyl ester (**8**) into lactam **9** after an acylation, Dieckmann condensation and hydrolysis/decarboxylation sequence. A Fischer indole synthesis with phenylhydrazine (**10**) then affords the product. This route has proven satisfactory for the synthesis of a wide range of paullone derivatives, however in the case of the archetypal paullone **1** it requires the use of an anthranilic acid derivative (a DEA list 1 restricted chemical) and it also involves a Fischer indole synthesis[18] in the final reaction step — a reaction of wide general

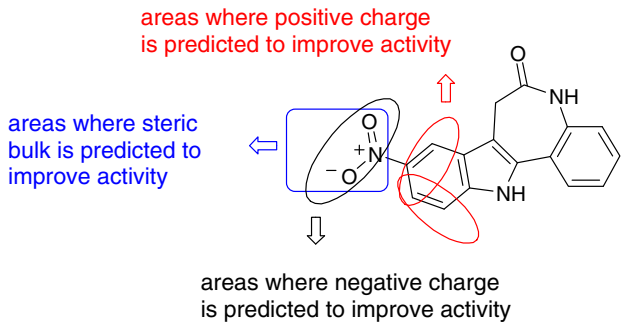
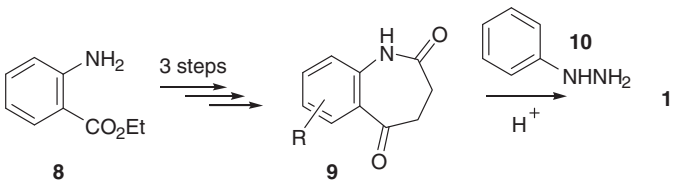


Figure 2. Pictorial representation of the CoMSIA contour map for steric and electrostatic fields reported by Lemcke et al. for the binding of **3** to CDK1 (indole section only).



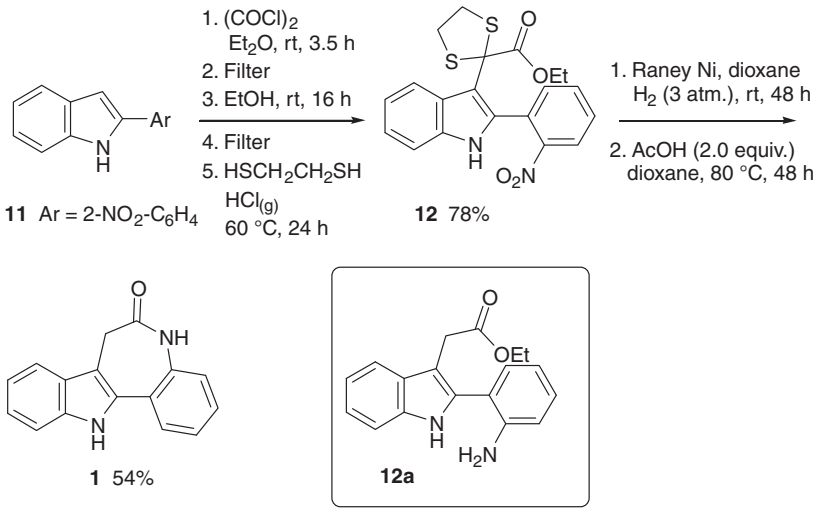
Scheme 1. Original Kunick paullone synthesis.

scope but which is notorious for variable product yields and harsh conditions.

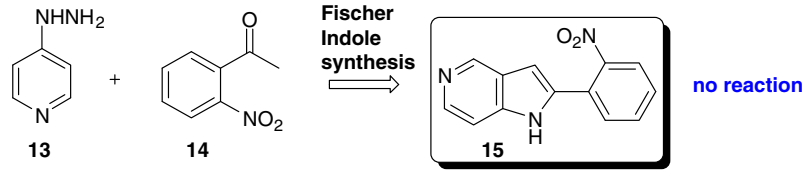
In developing a new route to **1**, our choice of disconnections were guided by a desire to (a) start the synthesis with a Fischer indole synthesis product rather than end with it, (b) take advantage of the inherent reactivity of indoles towards electrophiles at the C-3 position and (c) close the lactam ring as late as possible in the synthesis, in order to exploit the rigidity afforded by the two aromatic rings to facilitate ring closure.

Our route to **1** is outlined in Scheme 2. Starting from the known indole **11** (readily available on multigram scale[19] from the Fischer indole synthesis involving *o*-nitroacetophenone and phenylhydrazine), reaction with oxalyl chloride[20] in ether produced the Friedel-Crafts acid halide product, which then underwent alcoholysis in ethanol. The product precipitated from solution in pure form during both operations. Protection of the ketone moiety as the corresponding dithiolane by acid catalysed reaction with 1,2-ethanedithiol gave **12** in 78% overall yield.[21]

Treatment of **12** with Raney nickel followed by heating in dioxane in the presence of acetic acid allowed (without purification) the reduction of the nitro functionality, hydrogenolysis of the dithiolane to a methylene group[22] and cyclisation via attack of the in situ formed aniline on the ester to afford paullone **1** in 54% yield.[23] Analysis of this reaction as it progressed revealed that the cyclisation is the final step (i.e., ring closure of **12a**[24]). The route is concise and efficient: it utilises a readily available starting material, it takes advantage of multiple reaction steps in one pot and requires no chromatography.[25] In addition, since the Fischer indole synthesis is relocated to the beginning of the route, this strategy offers (in our view) a greater degree of certainty regarding versatility. One can synthesise the indole with the required substitution pattern on both aromatic rings at the beginning of the sequence and then form the ε-lactam (which is essential for activity) via a novel, short, two-pot sequence, instead of necessitating that potential modifications to the paullone core be compatible with



Scheme 2. A new concise route to **1** involving a one-pot reduction, hydrogenolysis and lactamisation sequence.

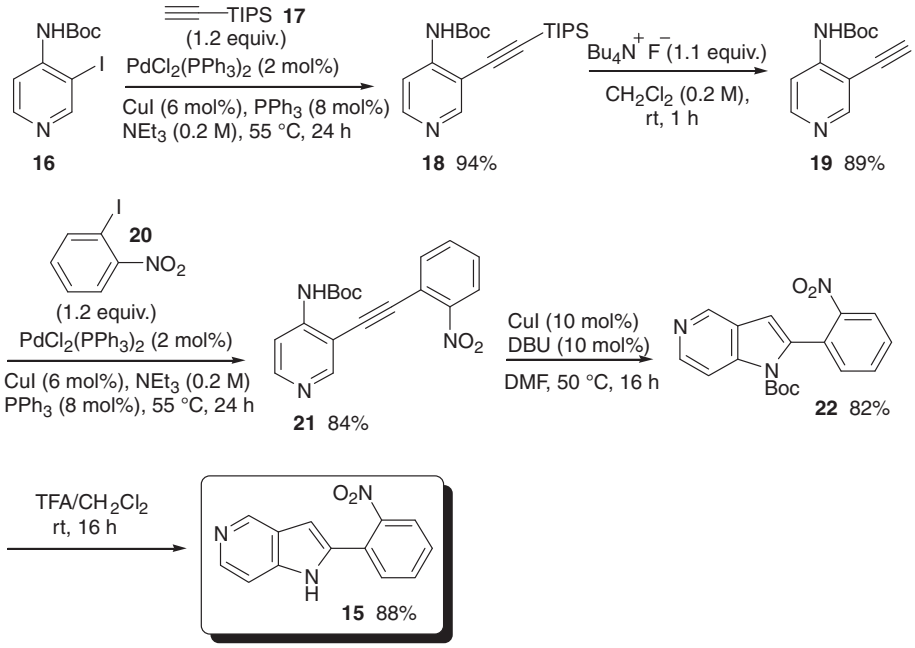


Scheme 3. Failure of the Fischer indole synthesis strategy.

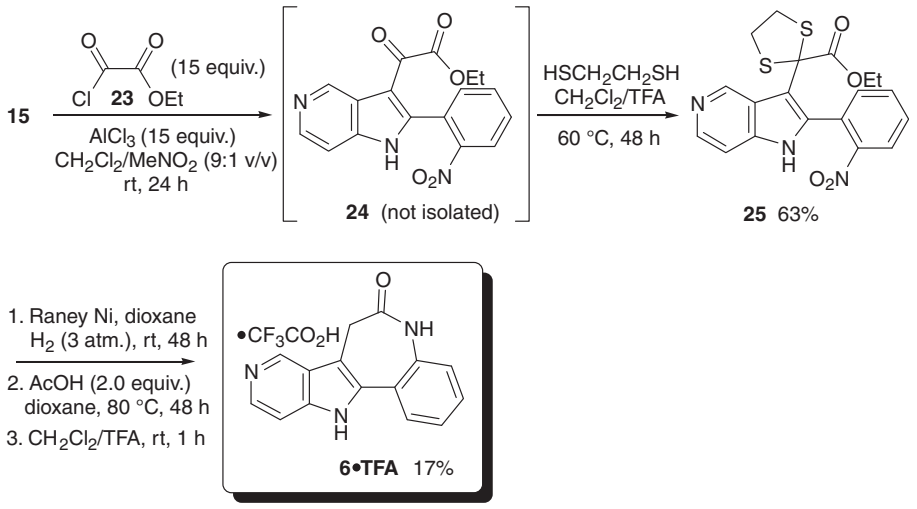
the often harsh conditions associated with Fischer indole synthesis in the final step.

We were next keen to apply this route to the synthesis of the missing azapaullone **6**. Here the prudence of the above sequence became clear: the preparation of **6** requires a Fischer indole synthesis using pyridylhydrazine **13** and acetophenone **14** and in our hands this reaction would not proceed under a variety of different conditions (either thermal or microwave assisted) in the presence of either Brønsted or Lewis acids (Scheme 3). Whilst Fischer indole syntheses utilising **13** have been reported, in our hands this reaction failed repeatedly, despite considerable experimentation.

Thus, it was clear that in order to synthesise **6** using the same general strategy outlined in Scheme 2 an alternative route to the azaindole **15** was required. We therefore developed a straightforward route (Scheme 4) to the azapaullone precursor based on the known protected iodopyridine **16**,[26] which could be coupled with the commercially available terminal alkyne **17** under Sonogashira conditions to give internal alkyne **18**. Fluoride ion-mediated



Scheme 4. Synthesis of azaindole precursor **15**.

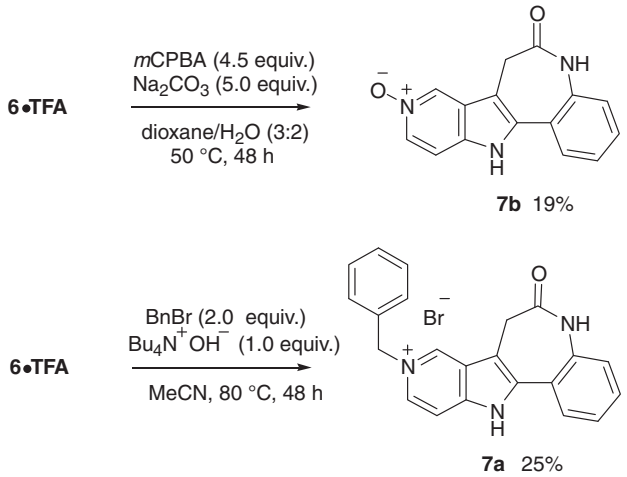


Scheme 5. Synthesis of 9-azapaullone (**6**·**TFA**).

deprotection of **18** afforded **19**, which participated in a second Sonogashira reaction with iodonitrobenzene **20** to afford the cyclisation precursor **21**. After extensive investigation it was found that **21** would undergo a base-catalysed smooth 5-endo-dig cyclisation reaction under relatively mild conditions in the presence of DBU if CuI was added as a co-catalyst.[27] Removal of the Boc-protecting group then furnished **15** in good overall yield.

With azaindole **15** in hand attention now turned to the 9-azapaullone synthesis (Scheme 5). Due to the attenuating properties of the azaindole nitrogen atom on the heterocycle's affinity for electrophiles, more forcing conditions for the Friedel-Crafts reaction than those used to prepare **12** were required. With the assistance of $AlCl_3$, **15** could be converted to **24** (in addition to the corresponding diethylketal if ethanol was used to quench the reaction) in the presence of excess acid chloride **23**. Protection of the ketone moiety as its dithiolane derivative **25** (in good overall yield from **15**) then allowed the same reduction, hydrogenolysis and cyclisation sequence as that used to prepare **1** to give the desired azapaullone **6**, which was isolated as its TFA salt due to the poor solubility of the free base in organic solvents.

Derivatisation of **6**·**TFA** was carried out as shown in Scheme 6. After considerable experimentation, conditions were identified under which the azapaullone salt could be N-oxidised in aqueous media. Treatment of the azapaullone with *m*CPBA in aqueous dioxane under basic conditions led to the isolation of N-oxide **7b**, while alkylation of **6**·**TFA** with benzyl bromide in the presence of base afforded the *N*-alkyl salt **7a**.



Scheme 6. Synthesis of oxidised and alkylated 9-azapaullone derivatives.

The biological activity of paullones **1**, **2**, **6** and **7a, b** was then evaluated on a small panel of standard relevant kinases (Table 1).

The results of this screening study confirm the potency of paullones as CDK and GSK-3 inhibitors. As expected, paullone (**1**) possessed modest activity, however, the performance of 9-azapaullone (**6**) is of interest: it exhibits broadly similar activity to **1**, with the exception that **6** is a considerably more efficient inhibitor of CDK9/cyclin T (entry 4), although neither are as powerful an inhibitor of this kinase/cyclin complex as kenpaullone (**2**).

The evaluation of azapaullone derivatives **7a** and **7b** furnished the most intriguing (and gratifying) results. The *N*-benzyl salt **7a** was inactive in the presence of almost all the kinases. The neutral N-oxide **7b**, however, possesses the most promising activity profile of the compounds evaluated in this study — it exhibits 0.24 μM activity as an inhibitor of CDK9/cyclin T. While it should be pointed out that **7b** is *ca.* four times less active than **2** towards this complex, unlike **2** (which inhibits CDK9/cyclin T and GSK-3 with almost equal potency — entries 4 and 9), **7b** is remarkably selective and is more than an order of magnitude less effective an inhibitor of GSK than CDK9/cyclin T. In addition, it is either essentially inactive or very weakly active against all other kinases utilised in the screen. Thus, while the inhibitory activity of **7b** towards CDK9/cyclin T is respectable but not comparable to that associated with benchmark compounds in the literature, this N-oxide does appear to possess the ability to effectively distinguish CDK9/cyclin T from a range

Table 1
Protein kinase inhibitory activity of compounds **1**, **2**, **6**, **7a**, **7b**

Entry	Target	IC_{50} (μM)[a]				
		1	**2**	**6**	**7a**	**7b**
1	CDK1/cyclin B	3.0	0.5	2.1	>10	5.7
2	CDK2/cyclin A	2.7	0.78	2.1	>10	6.2
3	CDK5/p25	4.2	1.0	3.0	>10	7.3
4	CDK9/cyclin T	0.81	0.064	0.22	7.4	0.24
5	CK1	>10	>10	>10	>10	>10
6	CLK1	>10	>10	>10	>10	>10
7	DYRK1A	>10	>10	>10	>10	>10
8	GSK-3	1.1	0.078	0.81	>10	3.4
9	PfGSK-3	>10	>10	>10	>10	>10

[a] Compounds were tested at various concentrations on nine purified kinases as described in Ref. 28. IC_{50} values, calculated from the dose–response curves, are reported in μM.

of related proteins/protein complexes — in particular the challenging case of GSK-3.

In summary, we have developed an efficient, easily modified and concise route to paullone **1** from simple starting materials. A variant of this synthetic strategy was then used to prepare the hitherto unreported 9-azapaullone (**6**), in addition to its *N*-benzyl salt **7a** and N-oxide derivative **7b**. A study to evaluate the inhibitory activity of these compounds against a range of kinases indentified **7b** as a *selective* inhibitor of CDK9/cyclin T. The potential significance of the function of CDK9/cyclin T in a range of medicinally relevant domains such as cell-cycle/transcription regulation,[4,8,29] HIV-1 replication[30] and cardiovascular disease[31] is only beginning to be understood, thus the development of highly selective inhibitors of this complex is a goal of considerable interest. Studies aimed at increasing the potency of **7b** and improving its ability to distinguish between CDK9/cyclin T and other kinases are underway.

Acknowledgments

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21. *Dithiolane* **12***:* In a three-necked round bottomed flask, oxalyl chloride (1.28 mL, 15.11 mmol) was added to anhydrous diethyl ether (16 mL) under an atmosphere of argon. The solution was cooled to 0 °C and indole **11** (1.64 g, 5.87 mmol) was added. The resulting suspension was stirred at ambient temperature for 3.5 h. The resulting pale yellow precipitate was removed by filtration, suspended in ethanol and stirred at ambient temperature for 24 h. The resulting solid was removed by filtration, washed with a mixture of ethanol and diethyl ether (ca. 1:1) yielding 1.89 g of crude ethyl ester which was used without further purification. The ethyl ester was then suspended in ethanol (85 mL) and the suspension saturated with HCl gas. Ethane dithiol (0.59 mL, 7.04 mmol) was added. The solution was heated overnight at 60 °C under an argon atmosphere. The resulting (spectroscopically pure) orange precipitate (1.84 g, 78%) was removed by filtration and used without further purification. mp 228–230 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 0.98 (t, *J* 7.1 Hz, 3H), 3.30 (m, 4H) 3.74 (q, *J* 7.1 Hz, 2H), 7.06 (app. t, 1H), 7.16 (app. t, 1H), 7.33 (d, *J* 8.0 Hz, 1H), 7.57 (d, *J* 7.3 Hz, 1H), 7.75 (m, 2H), 7.82 (app. t, 1H), 8.21 (d, *J* 8.0 Hz, 1H), 11.51 (br s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) 13.4, 39.7, 61.6, 67.7, 108.7, 111.3, 118.9, 120.2, 121.3, 124.4, 126.5, 128.2, 130.4, 131.4, 132.9, 133.7, 135.7, 148.5, 170.0. IR (solid) ν 3265, 1688, 1498, 980 cm^{-1}. HRMS (ESI) calcd for $[C_{20}H_{18}N_2O_4 + Na]^+$ requires 437.0613. Found 437.0606.
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24. Our cyclisation conditions are based on those originally developed by Opatz, who reported a similar cyclisation strategy via the ring closure of in situ generated **12a**. In the Opatz synthesis the generation of **12a** via a 5-exo-trig cyclisation reaction involving a deprotonated α-aminonitrile requires more steps and furnishes **1** in lower yield: Opatz, T.; Ferenc, D. *Synthesis* **2008**, 3941.
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