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Azine and Diazine Functionalization Using 2,2,6,6-Tetramethylpiperidino-Based Lithium–Metal Combinations: Application to the Synthesis of 5,9-Disubstituted Pyrido[3′,2′:4,5]pyrrolo[1,2-c]pyrimidines

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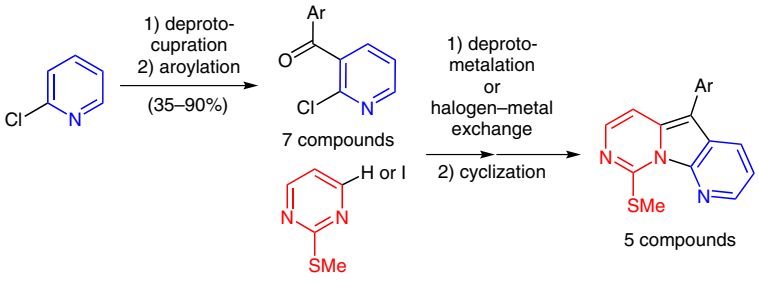

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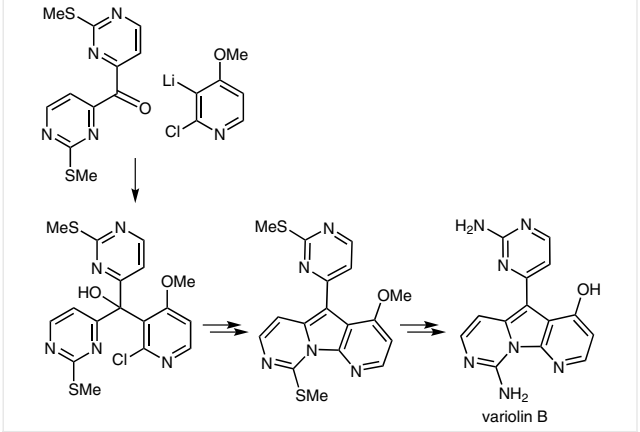
Abstract The synthesis of triaryl methanols was investigated by reacting different 4-metalated 2-substituted pyrimidines with diaryl ketones, the latter being generated by deprotocupration–aroylation of azine and diazine substrates. Cyclization of the triaryl methanols thus obtained afforded pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidines, which were evaluated for kinase inhibition and antiproliferative activities in melanoma cells.

Key words diaryl ketone, pyrimidine, deprotometalation, mixed-metal amide, variolin analogue

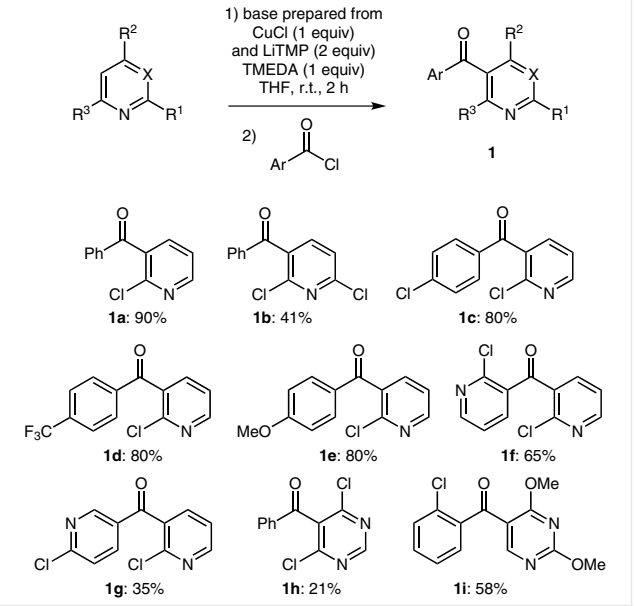
Variolins are a family of marine alkaloids isolated from the Antartic sponge *Kirkpatrickia varialosa*.[1] Among them, variolin B is a tricyclic system bearing a substituent at the 5-position endowed with biological properties such as antitumor and antiviral.[1a,2] Several total syntheses of variolin B and analogues have been reported.[2b,3] In the convergent synthesis of Morris,[3b,3h,4] the key step involves the tandem deoxygenation–cyclization of a triaryl methanol, the latter being for example obtained by reaction of 2-chloro-3-lithio-4-methoxypyridine on a symmetrical ketone (Scheme 1).



Scheme 1 Morris synthesis of variolin B

Because of our interest in the synthesis of diaryl ketones by deprotocupration–aroylation,[5] we decided to evaluate the reactivity of these ketones toward different 4-metalated 2-substituted pyrimidines in order to reach different triaryl methanols. Thus, the required diaryl ketones were prepared from azines or diazines as reported previously.[5] The latter were deprotocuprated at room temperature in tetrahydrofuran (THF) containing *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) by using $(TMP)_2CuLi{\cdot}LiCl$ (TMP = 2,2,6,6-tet-

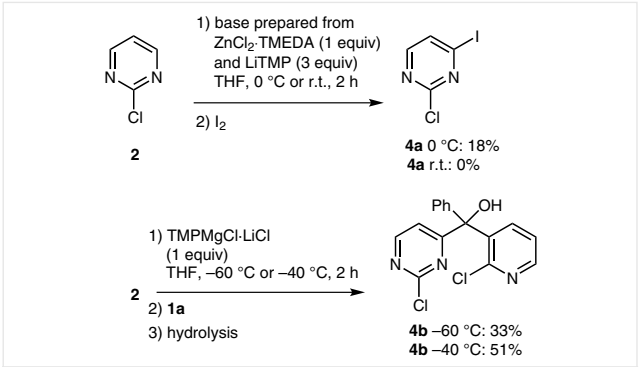
ramethylpiperidino), prepared in situ from CuCl and LiTMP (2 equiv). Trapping with aroyl chlorides after two hours furnished the compounds **1a–i** (Scheme 2).



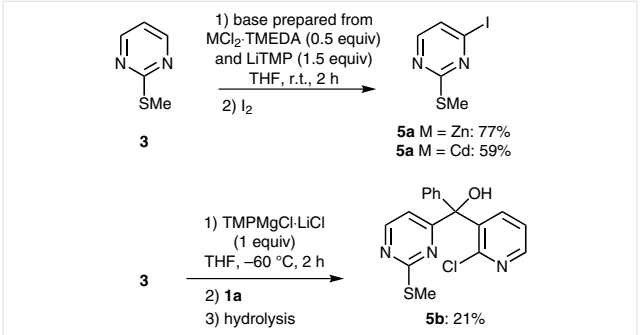
Scheme 2 Synthesis of the diaryl ketones **1a–i**

We next considered the formation of 4-metalated 2-chloropyrimidine from **2** and its trapping. The pyrimidine **2** being prone to nucleophilic attacks,[6] we first tried to use the base prepared in situ by mixing $ZnCl_2$·TMEDA and LiTMP in a 1:3 ratio,[7] and supposed to be a 1:1 LiTMP·2LiCl(±TMEDA)–$Zn(TMP)_2$ mixture.[8] It proved not appropriate, with the iodide **4a** isolated in a low 18% yield when the deprotonation step was performed at 0 °C and only degradation noticed at higher temperatures. TMPMgCl·LiCl being a suitable base for **2** in THF at –60 °C, as evidenced by subsequent iodolysis after two hours,[9] we thus employed it in order to attempt the interception of the deprotomagnesated species with the ketone **1a**. The expected triaryl methanol **4b** was obtained in a moderate 33% yield by carrying out the reaction at –60 °C, but its formation could be improved with a deprotonation step at –40 °C (Scheme 3).

4-Metalated derivatives of 2-(methylthio)pyrimidine (**3**) could be formed at room temperature in THF by using either the previous lithium–zinc base or the corresponding lithium–cadmium base, prepared in situ by mixing $CdCl_2$·TMEDA (0.5 equiv) and LiTMP (1.5 equiv).[10] This was evidenced by iodolysis to afford **5a** in correct yields. When TMPMgCl·LiCl was employed at –60 °C, the alcohol **5b** resulting from a quenching with the ketone **1a** was obtained in a moderate 21% yield (Scheme 4).



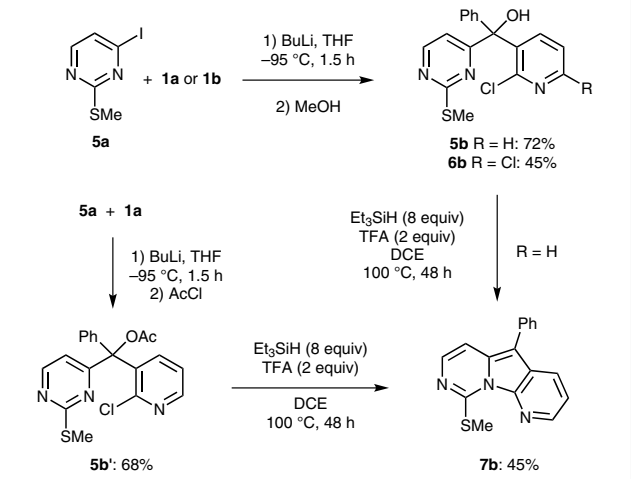
Scheme 3 Functionalization of 2-chloropyrimidine (**2**)



Scheme 4 Functionalization of 2-(methylthio)pyrimidine (**3**)

To reach the triaryl methanols, it also proved possible to involve the iodide **5a** in a butyllithium-mediated halogen–metal exchange reaction in the presence of the ketone **1a** or **1b**. Performing this reaction in THF at –95 °C, as previously documented by Morris,[3b,h,4] led to the alcohol **5b** or **6b** in 72% or 45% yield, respectively. Replacing methanol quenching with acetyl chloride afforded the corresponding acetate **5b′**. Finally, treating both **5b** and **5b′** with triethylsilane and trifluoroacetic acid at 100 °C in 1,2-dichloroethane furnished the phenyl-substituted tricycle **7b** in 45% yield (Scheme 5). Because no improvement was here noted by using the acetate **5b′** as intermediate,[4] we kept the sequence involving triaryl methanols for the rest of the study.

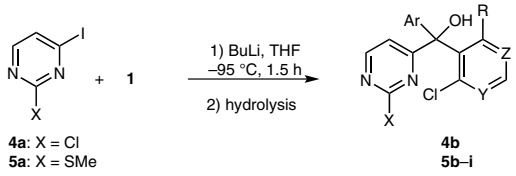
In order to prepare analogues of 9-(methylthio)-5-phenylpyrido[3′,2′;4,5]pyrrolo[1,2-*c*]pyrimidine (**7b**), we aimed at synthesizing various triaryl methanols (Table 1). When 2-chloro-4-iodopyrimidine (**4a**) was involved instead of **5a** in the reaction with **1a**, a lower 24% yield was noticed (Table 1, entries 1 and 2). With the other aryl(2-chloro-3-pyridyl)methanones **1c–e**, the expected alcohols were isolated in yields ranging from 52–70% (Table 1, entries 3–5). The position of chlorine on the (2-chloro-3-pyridyl)(chloro-3-pyridyl)methanone is an important parameter for the success of the reaction. Indeed, whereas a good 75% yield was registered by using **1f** (compound **5f**, Table 1, entry 6), a complex mixture without the expected derivative togeth-



Scheme 5 Synthesis of 9-(methylthio)-5-phenylpyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (**7b**)

er with starting material was obtained from **1g** (Table 1, entry 7). With the ketones **1h** and **1i** bearing a pyrimidyl group, things become difficult, probably in relation with ring sensitivity to nucleophilic attacks; as a consequence, the alcohol **5i** was the only to be formed, in a very low 8% yield (Table 1, entries 8 and 9).

Table 1 Synthesis of the Triaryl Methanols **4b** and **5b–i**



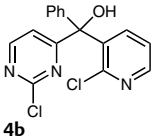
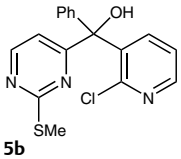
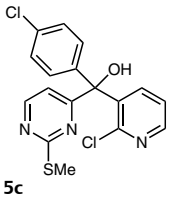
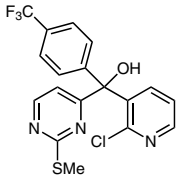
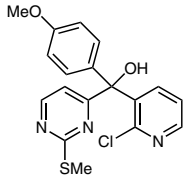
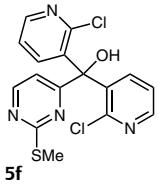
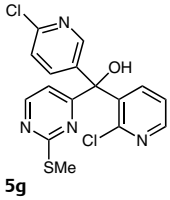
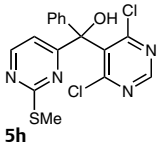
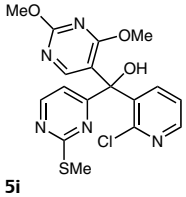
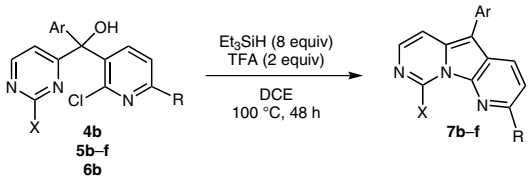
Entry	Substrate X	1	Product	Yield (%)
1	**4a** Cl	**1a**	 **4b**	24
2	**5a** SMe	**1a**	 **5b**	72
3	**5a** SMe	**1c**	 **5c**	70

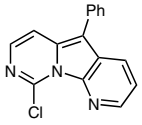
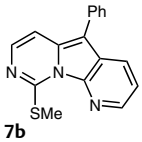
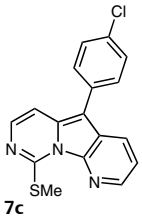
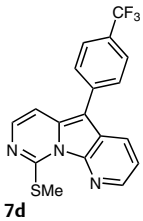
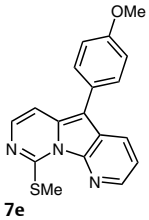
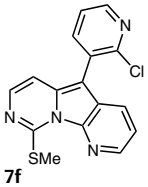
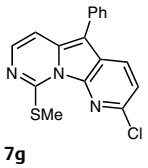
Table 1 (continued)

Entry	Substrate X	1	Product	Yield (%)
4	**5a** SMe	**1d**	 **5d**	59
5	**5a** SMe	**1e**	 **5e**	52
6	**5a** SMe	**1f**	 **5f**	75
7	**5a** SMe	**1g**	 **5g**	0
8	**5a** SMe	**1h**	 **5h**	0
9	**5a** SMe	**1i**	 **5i**	8

In order to progress toward the corresponding 5-aryl tricycles, the triaryl methanols were submitted to the action of triethylsilane and trifluoroacetic acid as before (Table 2). Under these conditions, the targets **7b–f** were generated in moderate yields (Table 2, entries 2–6). No cyclization was noticed from the dichloride **4b** (Table 2, entry 1). Similarly, cyclization of the dichloride **6b** did not take place under the conditions used (Table 2, entry 7). In both cases, starting material was recovered.

Table 2 Synthesis of the Tricycles **7b–f**



Entry	Substrate X, R	Product	Yield (%)
1	**4b** Cl, H	 **7a**	0[a]
2	**5b** SMe, H	 **7b**	45
3	**5c** SMe, H	 **7c**	23
4	**5d** SMe, H	 **7d**	36
5	**5e** SMe, H	 **7e**	18
6	**5f** SMe, H	 **7f**	21
7	**6b** SMe, Cl	 **7g**	0[a]

[a] Only starting material was recovered.

Variolin B was characterized as a potent inhibitor of cyclin-dependent kinases (CDK), key actors involved notably in the regulation of the cell-division cycle, programmed cell-death by apoptosis, transcription as well as differentiation.[2a] This chemical scaffold was consequently used to design new inhibitors of CDK.[11] In this study we thus tested the new derivatives on a panel of nine protein kinases including CDK5: *Hs*Aurora B, *Hs*CDK5/p25, *Hs*RIPK3 (receptor interacting protein kinase), *Hs*Haspin; porcine (*Sus scrofa*) *Ss*GSK-3 (glycogen synthase kinase-3) and *Ss*CK1 (casein kinase 1); kinases from the protozoan parasites, *Leishmania major Lm*CK1, *Plasmodium falciparum Pf*GSK-3, and from *Leishmania donovani LdTLK (tousled-like kinase*). These kinases were not significantly affected by the tested chemical compounds (**5b–e** and **7c–f**) with none of the molecules causing more than 50% inhibition of enzymatic activity at 10^{-5} M.

The antiproliferative activity of the 5,9-disubstituted pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidines **5b–e** and **7c–f** was studied in the A2058 (ATCC® CRL-11147) melanoma cell line (Figure 1). A2058 are highly invasive human epithelial adherent melanoma cells, derived from lymph nodes metastatic cells obtained from a 43 years male patient. They are tumorigenic at 100% frequency in nude mice and considered as very resistant to anticancer drugs.

The compounds **5** exerted low antiproliferative activity in A2058 melanoma cells, with 0–13% growth inhibition in cells treated for 72 h at 10^{-5} M. In contrast, the compounds **7** exhibited 23–44% growth inhibition and were considered as moderately antiproliferative. This activity was not correlated to CDK inhibition, as all molecules were inactive at 10^{-5} M in the CDK inhibition assay. Because of the presence of the sp^3 carbon, the compounds **5** are not planar in contrast to the compounds **7**. This observation indicates that the planar structure improves the antiproliferative activity and suggests that the cytotoxicity of these new compounds may be related to a DNA intercalating activity, as previously reported with variolin analogues.[1a,b] The nature of the ring

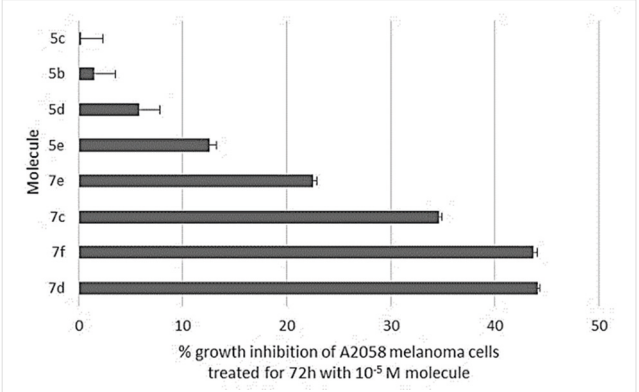


Figure 1 Antiproliferative activity of the compounds **5b–e** and **7c–f** in A2058 human melanoma cells grown for 72 h in a cell-culture medium containing 10^{-5} M molecule

connected to the tricycle also plays an important role, as the absence of cycle leads to a complete loss of activity, as observed with variolin D.[1c] Moreover, the presence of ring nitrogens or/and amino function is also critical for CDK inhibition.[2a]

As a conclusion, 2-substituted pyrimidines could be functionalized at their 4-position by using a lithium–metal TMP-based deprotonating agent.[12–14] The triaryl methanols obtained either after subsequent quenching, or through iodine–lithium exchange with in situ ketone trap, were cyclized to afford new pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidines.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560496.

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(12) **2-Chloro-α-(2-chloro-3-pyridyl)-α-phenyl-4-pyrimidinemethanol (4b)**
i-PrMgCl·LiCl (about 1.3 M THF solution, 1.2 mmol) was stirred with 2,2,6,6-tetramethylpiperidine (0.21 mL, 1.2 mmol) at r.t. for 48 h. The resulting solution was cooled at –60 °C before introduction of a cooled solution of 2-chloropyrimidine (**2**, 0.11 g, 1.0 mmol) in THF (2 mL). After 2 h at –40 °C, a solution of the ketone **1a** (0.26 g, 1.2 mmol) in THF (4 mL) was added at –60 °C. The mixture was stirred overnight at r.t. before addition of H_2O (0.5 mL) and dilution with EtOAc (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was performed by chromatography on silica gel (eluent: heptane–EtOAc, 7:3) to afford **4b** in 51% yield as a yellow powder; mp 90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.97 (s, 1 H), 7.18 (dd, 1 H, *J* = 7.8, 4.7 Hz), 7.33 (dd, 1 H, *J* = 7.8, 1.9 Hz), 7.36 (d, 1 H, *J* = 5.2 Hz), 8.37 (dd, 1 H, *J* = 4.7, 1.9 Hz), 7.35–7.45 (m, 5 H), 8.60 (d, 1 H, *J* = 5.2 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 80.5 (C), 117.6 (CH), 122.3 (CH), 127.5 (2 CH), 128.7 (CH), 128.8 (2 CH), 138.2 (C), 139.9 (CH), 140.7 (C), 149.4 (CH), 150.8 (C), 160.1 (CH), 161.0 (C), 175.0 (C) ppm. ESI-HRMS: *m/z* calcd for $C_{16}H_{11}{}^{35}Cl_2N_3NaO$ $[M + Na]^+$: 354.0177; found: 354.0178.

(13) **4-Iodo-2-(methylthio)pyrimidine (5a)**
To a stirred, cooled (0 °C) solution of 2,2,6,6-tetramethylpiperidine (0.50 mL, 3.0 mmol) in THF (6 mL) were successively added BuLi (about 1.6 M hexanes solution, 3.0 mmol) and, 5 min later, $ZnCl_2$·TMEDA[7] (0.26 g, 1.0 mmol). The mixture was stirred for 15 min at 0 °C before introduction of 2-(methylthio)pyrimidine (**3**, 0.25 g, 2.0 mmol). After 2 h at this temperature, a solution of I_2 (0.76 g, 3.0 mmol) in THF (10 mL) was added. The mixture was stirred overnight before addition of an aqueous saturated

solution of $Na_2S_2O_3$ (10 mL) and extraction with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification was performed by chromatography on silica gel (eluent: heptane–EtOAc, 95:5) to afford **5a** in 77% yield as a beige powder; mp 52 °C (ref. 14: 52–53 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.54 (s, 3 H), 7.40 (d, 1 H, *J* = 5.1 Hz), 8.00 (d, 1 H, *J* = 5.1 Hz) ppm.

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