# De Novo Synthesis of a Potent LIMK1 Inhibitor

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*Received 27 November 2009*

**Abstract:** A potent LIMK1 inhibitor, BMS4, was synthesised in six steps starting from pyrazine-2-carboximidamide, offering a significant improvement over current methods available in the literature.

**Key words:** kinase inhibitor, nitrogen heterocycles, pyrimidine, Suzuki coupling, cyclization

An important component of any drug discovery programme is the availability of tool compounds to validate functional assays and biological targets. Further, these tool compounds are also required as controls in biochemical assays.

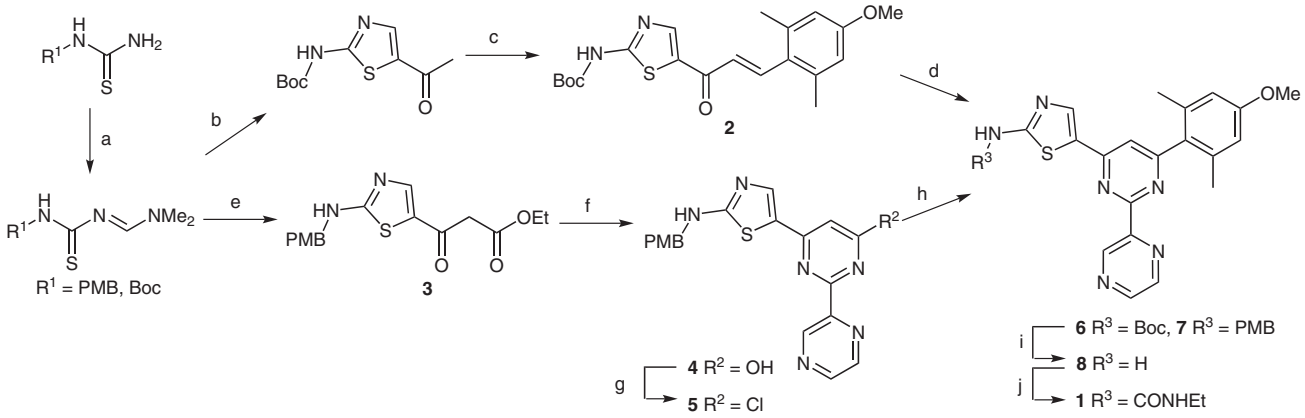
The recent inclusion of LIMK1 in our in-house kinase panel has prompted the requirement for a LIMK1 inhibitor for assay validation purposes. Specifically, LIMK1 is known to phosphorylate cofilin, which deactivates the cell's ability to transform globular actin to filamentous actin. This disrupts the cytoskeleton equilibrium, and thus the cell's motile ability. Overexpression of LIMK1 in multiple invasive and metastatic cell lines has seen LIMK1 emerge as a potential target for cancer therapeutics.[1]

In addition to confirming whether our LIMK1 biochemical assay was performing as desired, the LIMK1 tool compound could also be utilised potentially to validate other in-house assays, such as invasion and migration functional assays.

At the time, Bristol-Myers Squibb pharmaceuticals (BMS) was the only organisation to disclose a LIMK1 inhibitor.[2,3] BMS4 (**1**)[4] (Scheme 1) was chosen as a biochemical tool compound because it demonstrated potent inhibition of LIMK1 ($IC_{50}$ = 22 nM) and was devoid of any off-target cytotoxic effects.[3]

The synthesis of BMS4 (**1**) has been previously described via two pathways (Scheme 1).[2,3] The synthesis of BMS4 (**1**) via **2** has not been successful in our hands. The key step that prevented BMS4 (**1**) from being synthesised by this pathway was the cyclisation of acryloylthiazole **2** with pyrazine-2-carboximidamide. Various conditions were attempted but without success.[5]

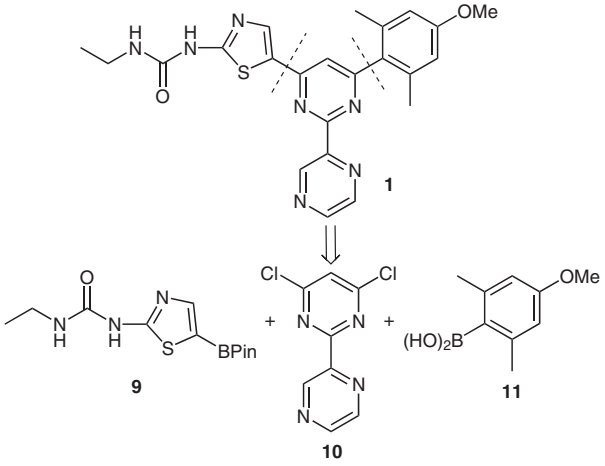
The second pathway described to access BMS4 (**1**) proceeds via the malonylthiazole **3**.[3] It was reported for this synthesis that the key step, cyclisation of **3** to form pyrimidine **4**, proceeded in a high yield (72%). However, it was noted that the efficiency of the pathway to produce BMS4 (**1**) was poor, with a 1% overall yield (Scheme 1). As substantial quantities of BMS4 (**1**) were required, it was decided not to follow this pathway.




**Scheme 1** Synthesis of BMS4 (**1**) according to the literature.[2,3] *Reagents and conditions*: (a) DMFDMA, EtOH, reflux; (b) chloroacetone, MeCN, reflux; (c) 4-methoxy-2,6-dimethylbenzaldehyde, KOH, EtOH; (d) pyrazine-2-carboximidamide, NaOEt; (e) $ClCH_2COCH_2CO_2Et$, MeCN, reflux, 94%; (f) pyrazine-2-carboximidamide hydrochloride, DBU, DMF, reflux, 76%; (g) $POCl_3$, DIPEA, 90 °C, 22%; (h) 4-methoxy-2,6-dimethylboronic acid, $Pd(PPh_3)_4$, $K_2CO_3$, 23%; (i) TFA, 60 °C, 73%; (j) EtNCO, 39%.

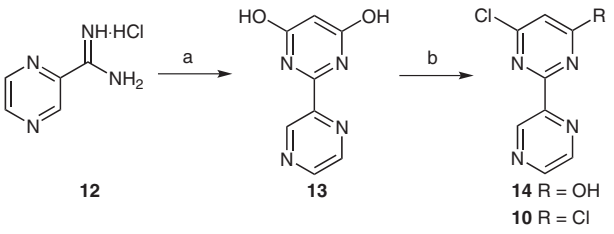
**SYNTHESIS** 2010, No. 7, pp 1091–1096
Advanced online publication: 20.01.2010
DOI: 10.1055/s-0029-1219230; Art ID: P16009SS

It was therefore proposed to develop a novel pathway to access BMS4 (**1**). Retrosynthetic analysis revealed that BMS4 (**1**) could be divided into three accessible components (Scheme 2). The union of the components could be performed by two palladium-mediated Suzuki couplings with the core 4,6-dichloropyrimidine **10**.



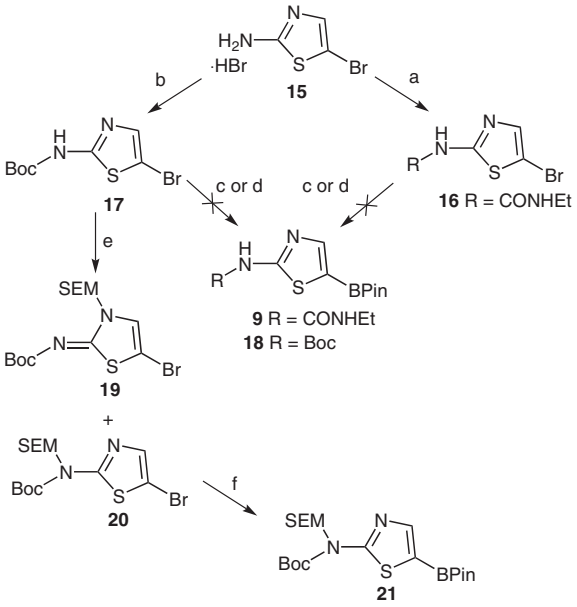

**Scheme 2** Retrosynthetic analysis of BMS4 (**1**)

The core 4,6-dichloro-2-pyrazin-2-ylpyrimidine (**10**) was formed via a Pinner pyrimidine synthesis. This process involved the condensation of pyrazine-2-carboximidamide hydrochloride (**12**) under basic conditions with diethyl malonate to provide dihydroxypyrimidine **13** in high yield (94%) (Scheme 3). Chlorination of pyrimidine **13** was more troublesome than expected. Chlorination in neat phosphoryl chloride resulted in decomposition of the starting pyrimidine **13**. Addition of *N*,*N*-diisopropylethylamine to pyrimidine **13** in toluene, followed by addition of phosphoryl chloride, also resulted in decomposition of the starting material. However, slow addition of *N*,*N*-diisopropylethylamine to pyrimidine **13** in a mixture of toluene and phosphoryl chloride at 0 °C gave dichloropyrimidine **10** in good yield (68%). However, the reaction did not proceed to completion, and a small amount of the singly chlorinated pyrimidine **14** (20%) was also isolated (Scheme 3).




**Scheme 3** Synthesis of the core 4,6-dichloro-2-pyrazin-2-ylpyrimidine (**10**). *Reagents and conditions*: (a) diethyl malonate, NaOEt, 94%; (b) $POCl_3$, toluene, DIPEA, 0–90 °C, 68% (**10**).

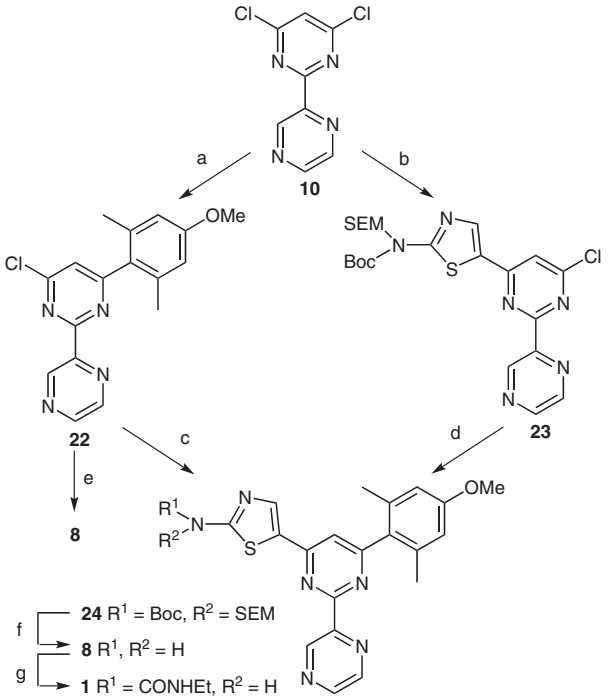
The initial approach to preparing the 2-aminothiazole-5-boronic acid component **9** was to not protect the 2-amino functionality (Scheme 4). It was conceived that maintaining the 2-amino ethylurea moiety through the entirety of the sequence would considerably shorten the synthetic sequence if successful. The ethylurea moiety was installed by reaction of the 2-amino-5-bromothiazole **15** with ethyl isocyanate to give the 2-ureidothiazole **16** in high yield. Unfortunately, subjecting the 2-ureidothiazole **16** to various palladium-mediated conditions did not yield the desired 5-boronate **9** (HBPin = pinacolborane) (Scheme 4). It was decided that protection of the 2-aminothiazole was required to form the boronate. The boronate formation was then attempted with Boc protection. The installation of the Boc group on the 2-aminothiazole **15** proceeded as expected, to yield **17**. However, subsequent boronate formation of the 5-bromo-2-[(*tert*-butoxycarbonyl)amino]thiazole **17** under various palladium-mediated conditions was not successful (Scheme 4).




**Scheme 4** Synthesis of the 2-amino-5-borylthiazole component. *Reagents and conditions*: (a) EtNCO, py, 83%; (b) $Boc_2O$, py, 92%; (c) $[PdCl_2(dppf)_2]$, $(BPin)_2$, KOAc, dioxane, 90 °C; (d) $Pd_2(dba)_3$, X-Phos, $(BPin)_2$, KOAc, dioxane, 90 °C; (e) SEMCl, DIPEA, THF, 20% (**19**), 73% (**20**); (f) BuLi, *i*-PrOBPin, THF, –78 to 0 °C, 72%.

It was hypothesised that the 2-NH moiety on thiazole was deprotonated under the basic conditions of the palladium-mediated coupling, thus deactivating the system towards palladium insertion. Therefore, the acidic NH functionality was removed by protection with [2-(trimethylsilyl)ethoxy]methyl (SEM) (Scheme 4). Protection with [2-(trimethylsilyl)ethoxy]methyl chloride and *N*,*N*-diisopropylethylamine provided the bis-protected 2-aminothiazole **20** in high yield (73%), and, as expected, a small amount (20%) of the regioisomer **19**. To simplify subsequent reactions, it was decided to proceed with the one regioisomer **20**. With full protection of the amino functionality in place, base metalation was then possible. The boronate ester **21** was thereby obtained in high yield (72%) (Scheme 4).

The 4-methoxy-2,6-dimethylphenylboronic acid (**11**) component was commercially available. All three coupling components **10**, **11**, and **21** (protected **9**) (Scheme 2), were now at hand. Coupling of the two boronic acids **11** and **21** with dichloropyrimidine **10** could be undertaken via two pathways, depending on which boronic acid was coupled first (Scheme 5).




**Scheme 5** Synthesis of BMS4 (**1**). *Reagents and conditions*: (a) **11**, $Pd(PPh_3)_4$, $Na_2CO_3$, toluene, $H_2O$, 90 °C, 49%; (b) **21**, $Pd(PPh_3)_4$, $Na_2CO_3$, toluene, EtOH, $H_2O$, 90 °C, 74%; (c) **21**, $Pd(PPh_3)_4$, $Na_2CO_3$, toluene, EtOH, $H_2O$, 90 °C, 68%; (d) **11**, $Pd(PPh_3)_4$, $Na_2CO_3$, toluene, EtOH, $H_2O$, 90 °C, 90%; (e) **21**, $Pd(PPh_3)_4$, $Na_2CO_3$ (4.2 equiv), DME, EtOH, $H_2O$, 90 °C, 46%; (f) TFA, $CH_2Cl_2$, 60%; (g) EtNCO, py, 72%.

Initially, anisole **11** was coupled to dichloropyrimidine **10** in the presence of tetrakis(triphenylphosphine)palladium[6] in a heterogeneous medium to afford the coupling product **22** in a mediocre yield (49%) (Scheme 5). Very little disubstituted 4,6-pyrimidine was observed (LC–MS). One assumption for the low yield was the hydrolysis of the activated dichloropyrimidine under the aqueous basic conditions. Non-aqueous coupling conditions[7] were then attempted, but led to no improvement. The coupling product **22** was then reacted under Suzuki conditions with the thiazole **21** to give the disubstituted product **24** in a good yield (68%) (Scheme 5).

The opposite order of coupling partners gave better results (Scheme 5). The reaction of thiazole **21** with dichloropyrimidine **10** over two hours gave product **23** in high yield (74%). The subsequent Suzuki reaction with anisole **11** gave **24** in excellent yield (90%).

It was noticed that extended reaction times gave poorer yields for both coupling pathways. This was attributed to slow hydrolysis of the 2-aminothiazole protective groups Boc and SEM under the heterogeneous aqueous conditions. To assess this theory, homogeneous Suzuki conditions were examined for the coupling of chloropyrimidine **22** with the thiazole-5-boronate **21** with a twofold excess of base. It was observed that the Boc and the SEM groups were, indeed, slowly hydrolysed, and **8** could be isolated in a 46% yield (Scheme 5).

To complete the synthesis of BMS4 (**1**), the SEM and Boc protecting groups were removed from **24**, by use of a mixture of trifluoroacetic acid and dichloromethane. A good yield (60%) of the 2-aminothiazole **8** was obtained. The ethylureido moiety was then installed by use of ethyl isocyanate; this afforded BMS4 (**1**) in good yield (72%) (Scheme 5).

A sample of the BMS4 (**1**) that was obtained was tested in an in-house LIMK1 Transcreener fluorescence polarisation biochemical assay.[8] The synthesised BMS4 (**1**) exhibited an $IC_{50}$ of 54 nM, which was in good agreement with the $IC_{50}$ of 22 nM reported in the literature,[3] if the slight variation in $K_m$ between the two assay formats is taken into account. This data confirmed that our in-house LIMK1 biochemical assay was performing accordingly. BMS4 (**1**) will continue to be used as an in-house control to verify LIMK1 activity and assay performance over time. Further, BMS4 (**1**) will be utilised as a tool compound to validate functional assays that are currently under development.

In summary, starting from pyrazine-2-carboximidamide hydrochloride (**12**), a six-step process, via the intermediate **23**, gave BMS4 (**1**) in an overall linear yield of 18.4%. The method outlined here offers a near twenty-fold improvement to the synthesis with a 1% overall yield previously described.[2,3] Furthermore, if BMS4 (**1**) does progress further towards the clinic, the synthetic pathway herein allows for a more efficient avenue to manipulate BMS4 (**1**) for optimisation.

All non-aqueous reactions were performed in oven-dried glassware under an atmosphere of anhyd $N_2$, unless otherwise specified. All anhyd solvents were purified by using a Braun purification system. All other solvents were reagent grade. PE describes a mixture of hexanes with bp 40–60 °C. Analytical TLC was performed on aluminum-backed Merck silica gel $60F_{254}$ plates and were visualised by fluorescence quenching under UV light. Flash chromatography was performed using a Teledyne Iso CombiFlash Rf purification system. All melting points were measured with a Standford Research Systems OptiMelt. All IR spectra were recorded on a Bruker Tensor 27 using ATR. All NMR spectra were recorded on a Bruker Avance DRX 300 with the solvents indicated ($^1H$ NMR at 300 MHz and $^{13}C$ at 75 MHz). Chemical shifts are referenced to the appropriate solvent peak. LC–MS was performed on a Finnigan LCQ Advantage MAX, using a Phenomenex Gemini C18-column (3 μm, 20 × 4 mm). HRMS was carried out at the Australian National University Mass Spectrometry Facility using a Waters LCT Premier XE (ESI–TOF mass spectrometer).

**Pyrazine-2-carboximidamide Hydrochloride (12)**

Pyrazine **12** was purchased commercially (Apollo) and also obtained by the following preparation: A 0.24 M soln of NaOMe in MeOH (20 mL, 4.7 mmol) was added slowly to a soln of 2-cyanopy-

razine (Oakwood) (5.0 g, 47.5 mmol) in MeOH (20 mL) at 0 °C under a $N_2$ atmosphere. The soln was allowed to stir at r.t. for 6 h. $NH_4Cl$ (2.69 g, 52.3 mmol) was added and the soln was stirred for 20 h at 40 °C. $Et_2O$ (100 mL) was added to the reaction mixture. The precipitate that formed was collected by filtration, washed with $Et_2O$, and dried in a vacuum oven; this gave amidine **12** as a white solid; yield: 7.2 g (95%). It was of sufficient purity (85% by $^1H$ NMR) to be used in the next transformation.

IR (ATR): 2971, 1700, 1615, 1381, 1021, 761 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 9.77 (br s, 3 H), 9.50 (d, *J* = 1.5 Hz, 1 H), 9.01 (d, *J* = 2.4 Hz, 1 H), 8.90 (dd, *J* = 1.5, 2.4 Hz, 1 H).

$^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ = 161.6, 149.7, 145.0, 144.8, 140.8.

ESI-MS: *m/z* (%) = 123 (100) $[M + H]^+$, 106 (15).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_5H_6N_4$: 123.0671; found: 123.0663.

**2-(Pyrazin-2-yl)pyrimidine-4,6-diol (13)**

Na (1.26 g, 54.7 mmol) was added in one portion to EtOH (20 mL) at 0 °C under a $N_2$ atmosphere. The soln was stirred at r.t. until the Na had dissolved. The NaOEt soln was then added to a soln of the amidine **12** (2.45 g, 16 mmol) and diethyl malonate (Aldrich; 2.6 g, 16.4 mmol) in EtOH (20 mL). The resulting mixture was then allowed to reflux for 20 h. The reaction mixture was concentrated to dryness in vacuo. The solid was then dissolved in $H_2O$ (50 mL). The aqueous layer was then acidified with concd aq HCl to pH 5. The solid that had formed was collected by filtration and washed with $H_2O$; this gave pyrimidine **13**.

Pale brown solid; yield: 2.8 g (94%); $R_f$ = 0.37 ($CH_2Cl_2$–MeOH, 10:1); mp >300 °C.

IR (ATR): 3111, 1632, 1548, 1420, 1270, 1207, 1019, 986, 826 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 9.34 (d, *J* = 1.5 Hz, 1 H), 8.85 (d, *J* = 2.5 Hz, 1 H), 8.79 (dd, *J* = 1.5, 2.5 Hz, 1 H), 5.45 (s, 1 H).

$^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ = 167.3, 154.72, 147.6, 144.98, 144.32, 144.29, 91.1.

ESI-MS: *m/z* (%) = 191 (100) $[M + H]^+$.

ESI-HRMS: *m/z* $[M - H]^-$ calcd for $C_8H_6N_4O_2$: 189.0413; found: 189.0408.

**4,6-Dichloro-2-(pyrazin-2-yl)pyrimidine (10)**

$POCl_3$ (Aldrich; 16.9 mL, 185.0 mmol) was added slowly to a mixture of pyrimidine **13** (2.2 g, 11.6 mmol) and anhyd toluene (20 mL) at 0 °C. DIPEA (Aldrich; 9.9 mL, 57.8 mmol) was added dropwise over 1 h at 0 °C. The resulting mixture was then allowed to stir at 90 °C under an inert atmosphere for 16 h. The reaction mixture was concentrated to dryness in vacuo. Ice water (50 mL) and $CH_2Cl_2$ (50 mL) were added to the reaction mixture. The precipitate that formed was collected by filtration, and washed with $H_2O$ followed by $CH_2Cl_2$; this gave singly substituted chloropyrimidine **14**.

**Compound 14**

Pale brown solid; yield: 20%; $R_f$ = 0.24 (MeOH–$CH_2Cl_2$, 0.5:10); mp 268 °C.

IR (ATR): 2900, 1703, 1566, 1296, 1138, 985, 864 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 9.36 (d, *J* = 1.5 Hz, 1 H), 8.88 (d, *J* = 2.5 Hz, 1 H), 8.82 (d, *J* = 1.5, 2.5 Hz, 1 H), 6.68 (s, 1 H).

$^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ = 162.7, 162.4, 158.0, 148.0, 145.2, 144.7, 144.5, 122.2.

LC–MS: $t_R$ = 4.47 min; *m/z* (%) = 209 (100) $[M + H]^+$, 211 (65).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_8H_5{}^{35}ClN_4O$: 207.0074; found: 207.0071.

**Compound 10**

The filtrate layers were then separated. The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give a solid. The solid was subjected to column chromatography (silica gel, EtOAc–PE, 5:95 to 50:50); this gave dichloropyrimidine **10**.

White solid (68%); $R_f$ = 0.38 (EtOAc–PE, 1:1); mp 138 °C.

IR (ATR): 1771, 1524, 1363, 1160, 1017, 815 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 9.70 (d, *J* = 1.5 Hz, 1 H), 8.82 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.75 (d, *J* = 1.5 Hz, 1 H), 7.48 (s, 1 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 162.9, 162.8, 147.6, 146.7, 145.8, 144.7, 121.3.

LC–MS: $t_R$ = 5.43 min; *m/z* (%) = 229, 227 (100 each) $[M + H]^+$, 231 (40).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_8H_4{}^{35}Cl_2N_4$: 226.9891; found: 226.9895.

**1-(5-Bromothiazol-2-yl)-3-ethylurea (16)**

A mixture of 5-bromothiazole-2-amine hydrobromide (**15**; Aldrich; 5 g, 19.4 mmol) and EtNCO (Aldrich; 3.1 mL, 38.8 mmol) in py (15 mL) was allowed to stir for 2 h at 25 °C. A 10% citric acid soln was added to the reaction mixture. The precipitate that formed was collected by filtration, washed with $H_2O$, and dried in a vacuum oven; this gave urea **16**.

Off-white solid; yield: 4.0 g (88%); $R_f$ = 0.42 (MeOH–$CH_2Cl_2$, 0.5:10); mp 189 °C.

IR (ATR): 2971, 1669, 1551, 1344, 1149, 997, 800 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 10.59 (s, 1 H), 7.34 (s, 1 H), 6.46 (t, *J* = 5.6 Hz, 1 H), 3.17–3.08 (dq, *J* = 7.20, 5.7 Hz, 2 H), 1.04 (t, *J* = 7.20 Hz, 3 H).

$^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ = 160.9, 154.2, 138.76, 99.9, 34.7, 15.6.

LC–MS: $t_R$ = 5.70 min; *m/z* (%) = 252, 250 (100 each) $[M + H]^+$, 179 (100), 181 (100).

ESI-HRMS: *m/z* $[M - H]^+$ calcd for $C_6H_7{}^{79}BrN_3OS$: 247.9493; found: 247.9490.

***tert*-Butyl 5-Bromothiazol-2-ylcarbamate (17)**

A mixture of 5-bromothiazole-2-amine hydrobromide (**15**; Aldrich; 5 g, 15.5 mmol), and $Boc_2O$ (Aldrich; 4.06 g, 18.6 mmol) in py (10 mL) was allowed to stir for 2 h at 25 °C. A 10% citric acid soln was added to the reaction mixture. The precipitate that formed was collected by filtration, washed with $H_2O$, and dried in a vacuum oven; this gave thiazole **17**.

Off-white solid; yield: 4.0 g (92%); $R_f$ = 0.61 (EtOAc–PE, 1:5); mp 149 °C.

IR (ATR): 2977, 1710, 1562, 1274, 1152, 768 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 7.41 (s, 1 H), 1.46 (s, 9 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 161.8, 152.7, 137.5, 101.7, 82.6, 28.2.

LC–MS: $t_R$ = 7.47; *m/z* (%) = 279, 281 (10 each) $[M + H]^+$, 223 (100), 225 (100).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_8H_{11}{}^{79}BrN_2O_2S$: 278.9803; found: 278.9801.

***tert*-Butyl (5-Bromothiazol-2-yl){[2-(trimethylsilyl)ethoxy]methyl}carbamate (20) and *tert*-Butyl 5-Bromo-3-{[2-(trimethylsilyl)ethoxy]methyl}thiazol-2(3*H*)-ylidenecarbamate (19)**

A mixture of thiazole **17** (3.15 g, 11.3 mmol), SEMCl (Aldrich; 2.45 g, 14.7 mmol), and DIPEA (Aldrich; 3.0 mL, 15.8 mmol) in THF (30 mL) was allowed to stir for 20 h at 25 °C. The mixture was

partitioned between EtOAc (30 mL) and brine (50 mL). The layers were then separated. The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave the 3-thiazole isomer **19**. Further elution gave the 2-thiazole isomer **20**.

**Isomer 19**

Pale yellow oil; yield: 0.9 g (19%) (**19** was found to be unstable at r.t. over a period of 1 month); $R_f$ = 0.75 (EtOAc–PE, 1:5).

IR (ATR): 2953, 1708, 1248, 1157, 1060, 832 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 7.34 (s, 1 H), 5.49 (s, 2 H), 3.69–3.63 (m, 2 H), 1.58 (s, 9 H), 0.97–0.91 (m, 2 H), –0.02 (s, 9 H).

$^{13}C$ NMR: not obtained due to lack of stability of **19**.

ESI-MS: *m/z* (%) = 410, 409 (6 each) $[M + H]^+$, 279 (4), 192 (50).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{14}H_{25}{}^{79}BrN_2O_3SSi$: 409.0617; found: 409.0609.

**Isomer 20**

Colourless oil; yield: 3.35 g (73%); $R_f$ = 0.5 (EtOAc–PE, 1:5).

IR (ATR): 2952, 1706, 1508, 1249, 1090, 833 $cm^{-1}$.

$^1H$ NMR (300 MHz,$CDCl_3$): δ = 6.98 (s, 1 H), 5.43 (s, 2 H), 3.64–3.59 (m, 2 H), 1.54 (s, 9 H), 0.98–0.93 (m, 2 H), 0.00 (s, 9 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 161.0, 138.5, 103.7, 84.2, 75.2, 67.0, 28.1, 18.1, –1.4.

ESI-MS: *m/z* (%) = 410, 409 (7 each) $[M + H]^+$, 279 (4), 192 (38).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{14}H_{25}{}^{79}BrN_2O_3SSi$: 409.0617; found: 409.0616.

***tert*-Butyl [5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)thiazol-2-yl]{[2-(trimethylsilyl)ethoxy]methyl}carbamate (21)**

A 2.06 M soln of BuLi in hexanes (Fluka; 3.92 mL, 8.1 mmol) was added slowly to a mixture of thiazole **20** (3.0 g, 7.3 mmol) in THF (30 mL) at –78 °C under a $N_2$ atmosphere. Isopropoxypinacolborane (*i*-PrOBPin; Boron Molecular; 1.91 g, 10.3 mmol) was added and the resulting mixture was then allowed to warm to 0 °C under a $N_2$ atmosphere for 2 h. A 2 M $NH_4Cl$ soln (30 mL) was added to the reaction mixture. The mixture was partitioned between EtOAc (30 mL) and brine (30 mL). The layers were then separated. The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave borate **21**.

Pale yellow oil, which solidified on standing; yield: 2.4 g (72%); $R_f$ = 0.6 (EtOAc–PE, 1:5); mp 59 °C.

IR (ATR): 2900, 1712, 1370, 1242, 1132, 1087, 837 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 7.87 (s, 1 H), 5.58 (s, 2 H), 3.70–3.64 (m, 2 H), 1.59 (s, 9 H), 1.32 (s, 12 H), 0.97–0.91 (m, 2 H), –0.02 (s, 9 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 166.4, 152.7, 147.9, 137.8, 114.3, 84.0, 76.1, 66.8, 28.2, 24.7, 18.1, –1.4.

ESI-MS: *m/z* (%) = 457 (100) $[M + H]^+$, 375 (50), 330 (58).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{20}H_{37}BN_2O_5SSi$: 457.2364; found: 457.2374.

**4-Chloro-6-(4-methoxy-2,6-dimethylphenyl)-2-(pyrazin-2-yl)pyrimidine (22)**

A mixture of dichloropyrimidine **10** (790 mg, 0.035 mmol), 4-methoxy-2,6-dimethylphenylboronic acid (**11**; Chembridge; 688 mg, 0.38 mmol), $Pd(PPh_3)_4$ (Strem; 5 mol%), and $Na_2CO_3$ (737 mg, 0.44 mmol) in a mixture of toluene (16 mL) and $H_2O$ (4 mL) was allowed to stir for 5 h at 90 °C. A 10% citric acid soln (20 mL) was added to the reaction mixture. The aqueous soln was extracted with EtOAc (2 × 10 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave borate **22**.

White solid (49%); $R_f$ = 0.41 (EtOAc–PE, 1:1).

IR (ATR): 1554, 1521, 1323, 1156, 849, 802 $cm^{-1}$.

$^1H$ NMR (300 MHz,$CDCl_3$): δ = 9.72 (d, *J* = 1.5 Hz, 1 H), 8.81 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.71 (d, *J* = 2.4 Hz, 1 H), 7.35 (s, 1 H), 6.70 (s, 2 H), 3.84 (s, 3 H), 2.16 (s, 6 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 170.3, 162.7, 162.4, 160.0, 149.1, 146.1, 145.9, 144.5, 137.3, 129.6, 122.7, 113.6, 55.2, 20.5.

LC–MS: $t_R$ = 7.25 min; *m/z* (%) = 327, 329 (100 each) $[M + H]^+$.

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}{}^{35}ClN_4O$: 327.1013; found: 327.1011.

***tert*-Butyl {5-[6-Chloro-2-(pyrazin-2-yl)pyrimidin-4-yl)thiazol-2-yl}{[2-(trimethylsilyl)ethoxy]methyl}carbamate (23)**

A mixture of dichloropyrimidine **10** (350 mg, 1.54 mmol), thiazole **21** (704 mg, 1.54 mmol), $Pd(PPh_3)_4$ (Strem; 5 mol%), and $Na_2CO_3$ (327 mg, 3.1 mmol) in a mixture of toluene (16 mL), EtOH (2 mL), and $H_2O$ (4 mL) was allowed to stir for 2 h at 90 °C. A 10% citric acid soln (20 mL) was added to the reaction mixture. The aqueous soln was extracted with EtOAc (2 × 10 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave pyrimidine **23**.

White solid (74%); $R_f$ = 0.5 (EtOAc–PE, 1:1); mp 155 °C.

IR (ATR): 2900, 1707, 1516, 1415, 1215, 1085, 832 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 9.76 (d, *J* = 1.5 Hz, 1 H), 8.80 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.72 (d, *J* = 2.4 Hz, 1 H), 8.22 (s, 1 H), 7.56 (s, 1 H), 5.61 (s, 2 H), 3.74–3.69 (m, 2 H), 1.64 (s, 9 H), 1.00–0.95 (m, 2 H), 0.00 (s, 9 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 164.9, 162.6, 162.6, 160.3, 152.7, 148.9, 146.1, 145.8, 144.47, 140.5, 130.7, 114.7, 84.8, 75.7, 67.3, 28.2, 18.3, –1.3.

ESI-MS: *m/z* (%) = 521 (100) $[M + H]^+$, 282 (95).

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{22}H_{29}{}^{35}ClN_6O_3SSi$: 521.1558; found: 521.1561.

***tert*-Butyl {5-[6-(4-Methoxy-2,6-dimethylphenyl)-2-(pyrazin-2-yl)pyrimidin-4-yl)thiazol-2-yl}{[2-(trimethylsilyl)ethoxy]methyl}carbamate (24)**

Compound **24** was obtained from **22** by one route, and from **23** by another route, according to the two procedures outlined below.

*From 22*: A mixture of chloropyrimidine **22** (20 mg, 0.06 mmol), thiazole **21** (28 mg, 0.06 mmol), $Pd(PPh_3)_4$ (Strem; 5 mol%), and $Na_2CO_3$ (13 mg, 0.12 mmol) in a mixture of toluene (3 mL), EtOH (0.3 mL), and $H_2O$ (0.3 mL) was allowed to stir for 2 h at 90 °C. A 10% citric acid soln (10 mL) was added to the reaction mixture. The aqueous soln was extracted with EtOAc (2 × 10 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave pyrimidine **24**.

Oil, which solidified on standing; yield: 68%.

*From 23*: A mixture of chloropyrimidine **23** (70 mg, 0.13 mmol), 4-methoxy-2,6-dimethylphenylboronic acid (**11**; 36 mg, 0.20 mmol), $Pd(PPh_3)_4$ (Strem; 5 mol%), and $Na_2CO_3$ (30 mg, 0.28 mmol) in a mixture of toluene (4 mL), EtOH (0.5 mL), and $H_2O$ (0.5 mL) was allowed to stir for 2 h at 90 °C. A 10% citric acid soln (15 mL) was

added to the reaction mixture. The aqueous soln was extracted with EtOAc (2 × 10 mL). The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was then subjected to column chromatography (silica gel, EtOAc–PE, 0:100 to 40:60); this gave pyrimidine **24**.

Off-white solid; yield: 90%; $R_f$ = 0.56 (EtOAc–PE, 1:1); mp 91 °C.

IR (ATR): 2900, 1708, 1581, 1412, 1156, 1064, 835 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 9.80 (d, *J* = 1.5 Hz, 1 H), 8.76 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.67 (d, *J* = 2.4 Hz, 1 H), 8.19 (s, 1 H), 7.46 (s, 1 H), 6.67 (s, 2 H), 5.61 (s, 2 H), 3.81 (s, 3 H), 3.74–3.68 (m, 2 H), 2.15 (s, 6 H), 1.63 (s, 9 H), 0.99–0.93 (m, 2 H), 0.01 (s, 9 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 169.0, 164.2, 162.5, 159.7, 158.9, 152.7, 150.3, 145.8, 145.4, 144.4, 139.4, 137.2, 132.0, 131.2, 116.1, 113.2, 84.5, 75.7, 67.1, 55.2, 28.2, 20.6, 18.2, –1.4.

LC–MS: $t_R$ = 7.0 min; *m/z* (%) = 621 (100) $[M + H]^+$.

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{31}H_{40}N_6O_4SSi$: 621.2679; found: 621.2689.

**5-[6-(4-Methoxy-2,6-dimethylphenyl)-2-(pyrazin-2-yl)pyrimidin-4-yl]thiazol-2-amine (8)**

Compound **8** could be obtained from starting material **24** or **22**, by following one of the two procedures outlined below.

*From **24***: A mixture of the bis-protected 2-aminothiazole **24** (40 mg, 0.06 mol) in TFA (Aldrich; 1 mL) and $CH_2Cl_2$ (1 mL) was allowed to stir for 24 h. The soln was evaporated to dryness in vacuo. EtOAc (10 mL) was added and the soln was washed with sat. aq $NaHCO_3$ (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The oil thus obtained was subjected to column chromatography (silica gel, MeOH–$CH_2Cl_2$, 0:100 to 5:95); this gave 2-aminothiazole **8**.

Pale yellow solid; yield: 15 mg (60%).

*From **22***: A mixture of the substituted pyrimidine **22** (90 mg, 0.28 mmol), the thiazole **21** (128 mg, 0.28 mmol), $[PdCl_2(PPh_3)_2]$ (Strem; 5 mol%), and $Na_2CO_3$ (95 mg, 0.69 mmol) in a mixture of EtOH (2 mL), $H_2O$ (2 mL), and DME (2 mL) was allowed to stir for 20 h at 90 °C. The mixture was partitioned between EtOAc (10 mL) and $H_2O$ (10 mL). The layers were then separated. The organic layer was dried ($MgSO_4$) and the organic layer was concentrated in vacuo to give an oil. The oil was subjected to column chromatography (silica gel, MeOH–$CH_2Cl_2$, 0:100 to 5:95); this gave deprotected thiazole **8**.

Pale yellow solid; yield: 50 mg (46%); $R_f$ = 0.56 (MeOH–$CH_2Cl_2$, 1:10); mp 159 °C.

IR (ATR): 3230, 1581, 1509, 1359, 1154 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ = 9.74 (d, *J* = 1.5 Hz, 1 H), 8.78 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.67 (d, *J* = 2.4 Hz, 1 H), 7.88 (s, 1 H), 7.37 (1H,s), 6.68 (s, 2 H), 5.55 (br s, 2 H), 3.82 (s, 3 H), 2.16 (s, 6 H).

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ = 168.4, 162.5, 159.9, 158.7, 150.4, 145.7, 145.4, 144.5, 141.3, 137.3, 131.2, 127.5, 115.2, 113.3, 55.2, 20.6.

LC–MS: $t_R$ = 6.70 min; *m/z* (%) = 391 (100) $[M + H]^+$.

ESI-HRMS: *m/z* $[M + H]^+$ calcd for $C_{20}H_{18}N_6OS$: 391.1341; found: 391.1342.

**1-Ethyl-3-{5-[6-(4-methoxy-2,6-dimethylphenyl)-2-(pyrazin-2-yl)pyrimidin-4-yl]thiazol-2-yl}urea (1)**

A mixture of the 2-aminothiazole **8** (40 mg, 0.1 mol) and EtNCO (Aldrich; 44 mg, 0.6 mol) in py (1.5 mL) was allowed to stir for 24 h at 80 °C in a sealed vessel. The solvent was removed in vacuo, and 10% citric acid soln (8 mL) was added. The precipitate was collected by filtration and washed with $H_2O$ to give a brown solid. The solid was then subjected to column chromatography (silica gel, MeOH–$CH_2Cl_2$, 0:100 to 5:95); this gave BMS4 (**1**).

Cream-coloured solid; yield: 35 mg (72%); $R_f$ = 0.50 (MeOH–$CH_2Cl_2$, 1:10); mp 241 °C (onset point), 274.9 °C (clear point) (Lit.[3] 274–276 °C).

IR (ATR): 2900, 1687, 1504, 1428, 1224, 1151, 799 $cm^{-1}$.

$^1H$ NMR (300 MHz, DMSO-$d_6$): δ = 10.79 (br s, 1 H), 9.52 (d, *J* = 1.5 Hz, 1 H), 8.85 (dd, *J* = 1.5, 2.4 Hz, 1 H), 8.80 (d, *J* = 2.4 Hz, 1 H), 8.46 (s, 1 H), 8.00 (s, 1 H), 6.79 (s, 2 H), 6.65 (t, *J* = 5.4 Hz, 1 H), 3.80 (s, 3 H), 3.22–3.17 (m, 2 H), 2.13 (s, 6 H), 1.10 (t, *J* = 7.2 Hz, 3 H).

$^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ = 166.6, 163.2, 161.3, 158.7, 158.6, 153.1, 149.5, 145.3, 144.2, 144.1, 141.1, 136.5, 130.4, 128.1, 115.2, 112.6, 54.7, 33.9, 19.8, 14.7.

LC–MS: $t_R$ = 5.17 min; *m/z* (%) = 462 (100) $[M + H]^+$.

ESI-HRMS: *m/z* $[M – H]^+$ calcd for $C_{23}H_{23}N_7O_2S$: 460.1556; found: 460.1568.

## Acknowledgment

The authors acknowledge the financial support of the Cancer Therapeutics CRC, established and supported under the Australian Government's Cooperative Research Centres Program, Hendrik Falk for assistance with biochemical assays, and Shalong Yang for assistance with synthesis.

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