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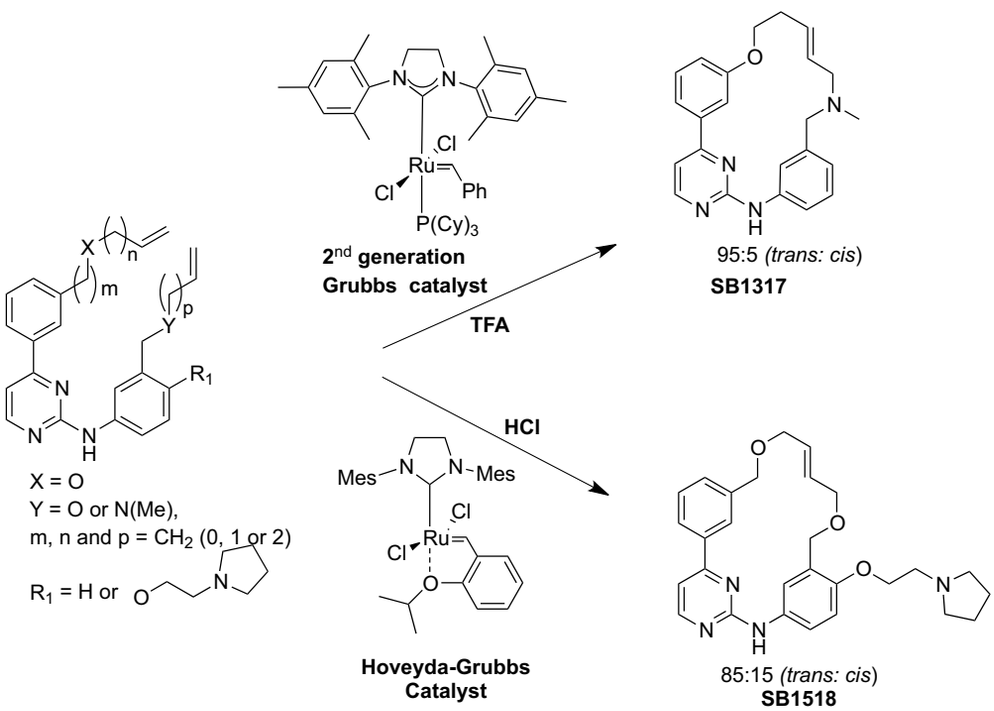
Acid Mediated Ring Closing Metathesis: A Powerful Synthetic Tool Enabling the Synthesis of Clinical Stage Kinase Inhibitors

Anthony D. William*[a] and Angeline C.-H. Lee

Abstract: The powerful olefin metathesis reaction was employed for the construction of late-phase clinical agents SB1317 and SB1518. In both cases RCM seems to proceed only in the presence of an acid and to predominantly furnish *trans* isomers. In case of SB1518 it proceeded in the presence of acid HCl, while for SB1317, it mainly proceeds in the presence of TFA (trifluroacetic acid).



Keywords: Cyclin-dependent kinases (CDKs) · Grubbs catalyst · Janus kinase 2 (JAK2) · Ring closing metathesis (RCM) · Zhan-1B catalyst

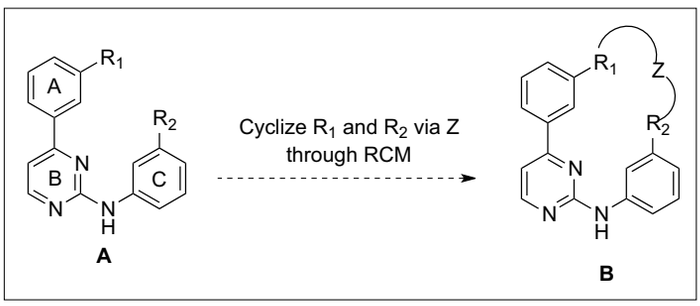
Introduction

The term macrocycle is usually associated with high molecular weight molecules that are primarily natural products.[1] Due to their useful pharmacological properties many macrocycles have been developed into drugs.[2] Despite their proven therapeutic potential macrocycles remain an under-explored chemical class in small molecule drug discovery probably due to their perceived structural complexity and poor drug-like properties.[3] However, we envisioned that macrocycles could be an excellent source of innovative small molecules in the competitive arena of kinase inhibitor drug discovery.

In our quest for kinase inhibitors with an amino-pyrimidine ring system as the core-binding motif, we screened our in-house library with structures of type 'A' that demonstrated a broad kinase inhibitory activity in numerous cancer cell lines. However, the pyrimidine-based cores are heavily patented limiting the development of proprietary compounds. We envisioned that by fusing the open ends R_1 and R_2 to form macrocycles would access new intellectual property (Scheme 1).

Thus, using RCM as a synthetic tool we embarked on the synthesis of novel small molecule macrocycles.

*Correspondence: Dr. A. D. William[a]
S*BIO Pte Ltd,
250 North Bridge Road
#28-00 Raffles City Tower
Singapore 179101
Tel.: + 65 67 998 526
E-mail: wanthony11@gmail.com
[a]Current Address: Institute of Chemical and Engineering Sciences (ICES)
Agency for Science, Technology, and Research (A*STAR)
11 Biopolis Way, Helios Block, #03-08
Singapore 138667, Singapore



Scheme 1. Concept for small molecule macrocycles.

Results and Discussions

Small Molecule Macrocycle SB1317

Kinase inhibitors which inhibit more than one kinase have yielded better clinical results than selective compounds as they block more than one pathway critical for tumour growth.[4] SB1317 not only inhibits its key Cyclin-dependent kinases (CDKs) but other kinases such as Janus kinase 2 (JAK2) and Fms-like tyrosine kinase 3 (FLT-3) that are implicated in the pathogenesis of hematological malignancies. While most of the CDK, JAK2 and Flt3 inhibitors in clinical trials have open chain structures,[5] SB1317 is unusual in being a macrocyclic compound exhibiting the above spectrum of activities combined in a single molecule.[6]

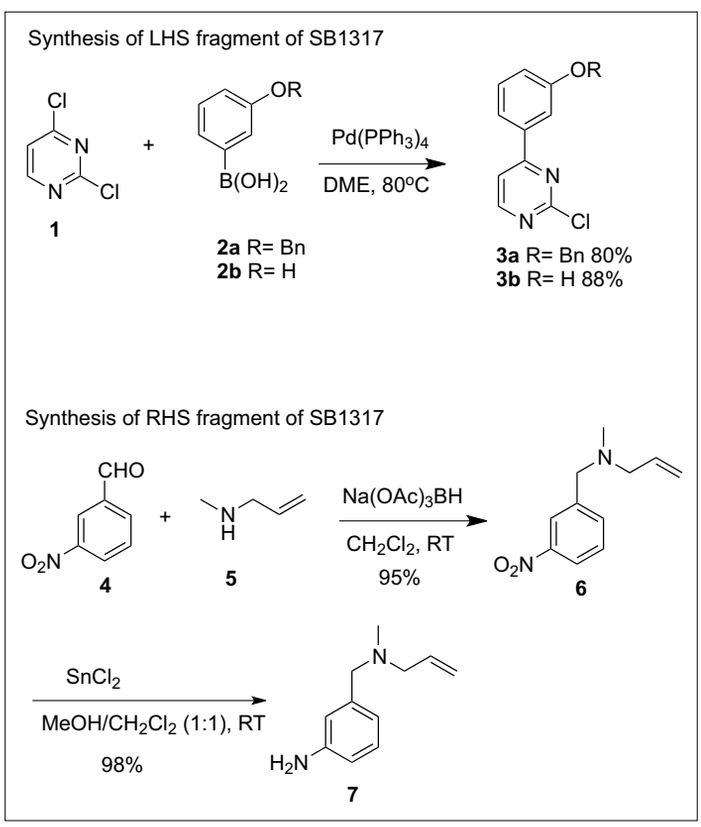
Synthesis

The synthesis (Scheme 2) was initiated from 2,4-dichloropyrimidine (**1**). Suzuki coupling with boronic acid **2a** afforded the left-hand fragment, biaryl **3a** in 80% yield.[7] The right-hand half of SB1317 was constructed from 3-nitrobenzadehyde (**4**) and *N*-methyl allylamine (**5**) *via* reductive amination which proceeded smoothly affording allyl-benzylamine **6** in good yield.[8] Aniline **7** was obtained in quantative yield under $SnCl_2$ reduction conditions.[9]

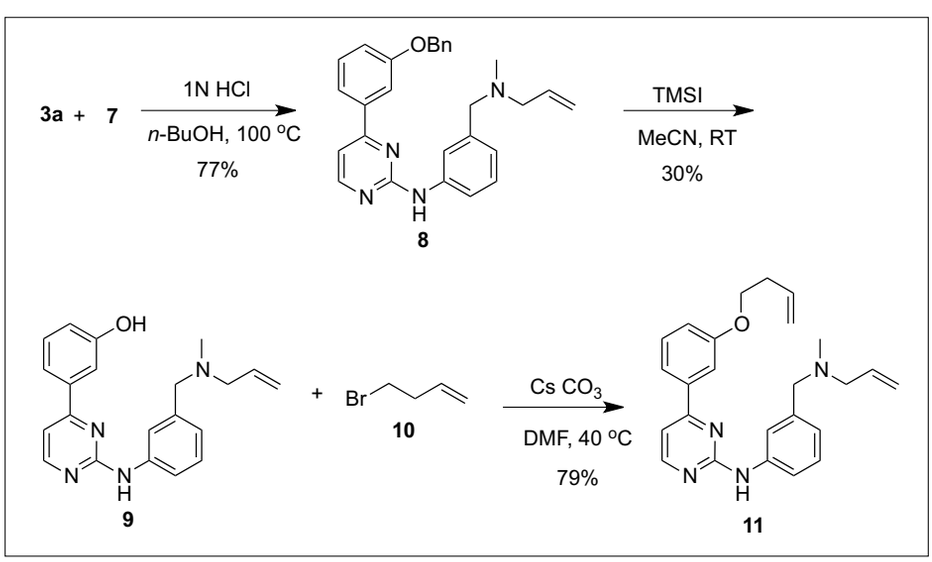
Coupling of **3a** with **7** was accomplished in the presence of HCl in *n*-butanol (Scheme 3).[10] De-benzylation of **8** was attempted under various standard conditions but without much success.[11] Finally, it was achieved in 50% yield using TMSI under normal reported conditions.[12] Alkylation of the resulting phenol with homoallyl bromide **10** proceeded efficiently in the presence of cesium carbonate to furnish RCM precursor diene **11**.[13]

To circumvent low yields associated with the benzyl protecting group and to aid scale-up activities, an atom-efficient synthesis of **11**, without the use of a protecting group, was attempted. Suzuki coupling of **1** with phenol **2b** proceeded pleasingly well (Scheme 2) to furnish **3b** in 88% yield. Alkylation of **3b** with bromide **10** afforded **13** in good yield (Scheme 4). The coupling of the two halves **13** and **7** proceeded efficiently in the presence of HCl to afford **11** in a much improved overall yield.

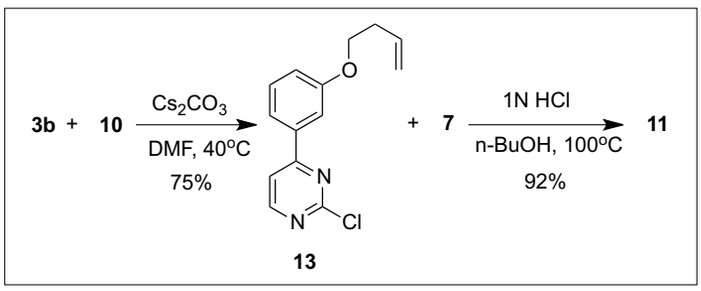
RCM was attempted on **11** as described in Table 1. Grubbs 1st and 2nd generation catalysts failed to afford any product under normal conditions (Table 1, entries 1 and 2). RCM is ineffective with molecules having basic centres as the catalyst probably co-ordinates with the basic nitrogens rendering it inactive.[14] We reasoned that neutralisation of the basic centre could free the catalyst, hence an attempt to close the macrocycle was made with HCl as an additive. We were delighted to find that the desired product had formed with good *trans:cis* ratios albeit in low yield with the 1st generation catalyst (entry 3). An improvement was quickly realised with the 2nd generation catalyst affording a moderate yield (entry 4). However, with TFA as an additive the RCM proceeded most smoothly, full conversion occurred in just



Scheme 2. Synthesis of key intermediates of SB1317.



Scheme 3. Synthesis of key intermediate for RCM.



Scheme 4. Atom efficient synthesis of SB1317.

4 h to furnish SB1317 in 86% yield with 94:6 *trans:cis* ratio on column chromatographic purification. The *trans* isomer purity was increased to >98% by simply washing the solid with cold EtOAc with 90% recovery.

In conclusion, we have shown that RCM is a powerful synthetic tool for preparation of the novel small molecule macrocycle SB1317 which is otherwise very difficult to obtain by other means.

Small Molecule Macrocycle SB1518

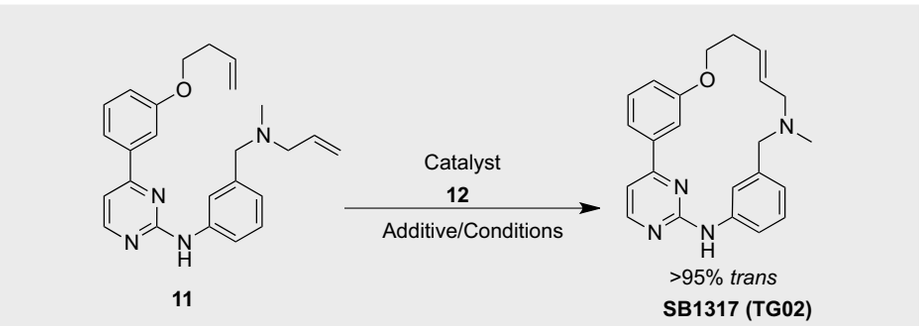
Having completed phase 2 clinical trials and entered phase 3, SB1518 is a promising new therapeutic agent for myeloproliferative neoplasms (MPNs), particularly myelofibrosis (MF), and lymphoma.[15] The synthesis of the precursor of SB1518 diene **14** is reported elsewhere.[15,16] Herein in Table 2 the attempts of RCM with various catalysts are depicted.

Catalysis with Grubbs 1st and 2nd generation catalysts without additives under normal conditions did not proceed to completion, even after 36 h. An attempt to close the macrocycle was made with HCl as an additive. It was encouraging to find that the desired product was formed albeit in low yield with the 1st generation catalyst (entry 3). An improvement was quickly realized employing the 2nd generation Grubbs catalyst. The reaction showed complete consumption of diene **14** in just 3 h to afford 60% of the product with *trans:cis* ratio 85:15 (entry 4). However, with Hoveyda-Grubbs 2nd generation catalyst (entry 5) as well as Zhan-1B catalyst (entry 6) the RCM proceeded most smoothly. A high conversion with comparable *trans:cis* ratios was observed. SB1518 individual *trans/cis* isomers had similar biological activities towards JAK2 kinases and FLT3, hence all preclinical activities, including animal models, were performed with mixtures of *tran/cis* isomers.[15]

Conclusion

In conclusion, we have shown that RCM is a powerful synthetic tool for preparation of the novel small molecule macrocycles SB1518 and SB1317. Both have been prepared with atom efficient syntheses, devoid of protecting groups and with an acceptable overall yield. SB1518, a selective JAK2/FLT3 inhibitor, is in phase 3 clinical trials for cancers such as lymphoma and myelofibrosis.[17] SB1317 is undergoing phase 1 clinical trials for treatment of patients with advanced/refractory hematologic malignancies such as advanced leukemias and multiple myeloma.[18] We believe the clinical success of SB1317 and SB1518 will highlight the significance of

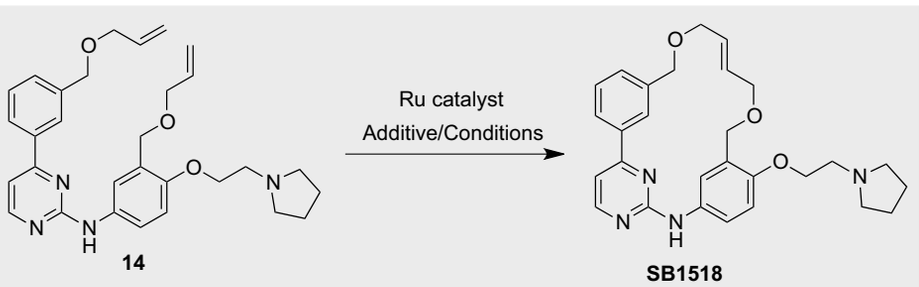
Table 1. Optimal Conditions for an Efficient RCM



Entry	Catalyst[a] **(12)**	Additive/Time[b]	*trans/cis* [%][c]	yield [%][d]
1	Grubbs 1st generation	16 h	-	*nd*
2	Grubbs 2nd generation	16 h	-	*nd*
3	Grubbs 1st generation	HCl, 16 h	86:14	10[f]
4	Grubbs 2nd generation	HCl, 4 h	94:6[e]	70
5	Grubbs 1st generation	TFA, 16 h	85:15	8[f]
6	Grubbs 2nd generation	TFA, 4 h	94:6[e]	86

[a]All reactions employed 10 mol% of Grubbs catalyst. [b]Reactions were carried out in in CH_2Cl_2 in the presence of 5 equiv. of 4 M methanolic HCl or TFA at reflux conditions. [c]Determined by HPLC on crude sample. [d]Isolated combined yields of *cis* and *trans*. [e]The *trans* isomer purity was increased to >98% by simply washing the solid after column purification with cold EtOAc with 90% recovery. [f]% of unreacted diene **(11)** was recovered. *nd,* not determined.

Table 2. Optimal Conditions for an Efficient RCM of **14**



Entry	Catalyst	mol %	Additive/Time[a]	*trans:cis*[b]	yield, %[c]
1	Grubbs 1st generation	25	36 h	-	*nd*
2	Grubbs 2nd generation	25	36 h	-	*nd*
3	Grubbs 1st generation	25	HCl, 36 h	*nd*	35
4	Grubbs 2nd generation	10	HCl, 3 h	85:15	60
5	Hoveyda-Grubbs 2nd generation	10	TFA, 3 h	85:15	68
6	Zhan-1B	10	TFA, 3 h	85:15	67

[a]Reactions were carried out in CH_2Cl_2 in the presence of 5 equiv. of 4 M methanolic HCl or TFA at reflux conditions. [b]Determined by HPLC on crude sample. [c]Isolated combined yields of *cis and trans. nd,* not determined.

RCM in small molecule synthetic drugs and this will spur growth of development of small molecule macrocycles.

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[17] a) For an update on clinical trials with SB1518 see *http://clinicaltrials.gov*; b) SB1518 codenamed Pacritinib has been sold to CTI BioPharma Corp.

[18] a) For an update on clinical trials with SB1317 see *http://clinicaltrials.gov*; b) SB1317 is licensed to Tragara Pharmaceuticals Inc. and entered the clinic as TG02 in mid-2010.