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RCM Macrocyclization Made Practical: An Efficient Synthesis of HCV Protease Inhibitor BILN 2061

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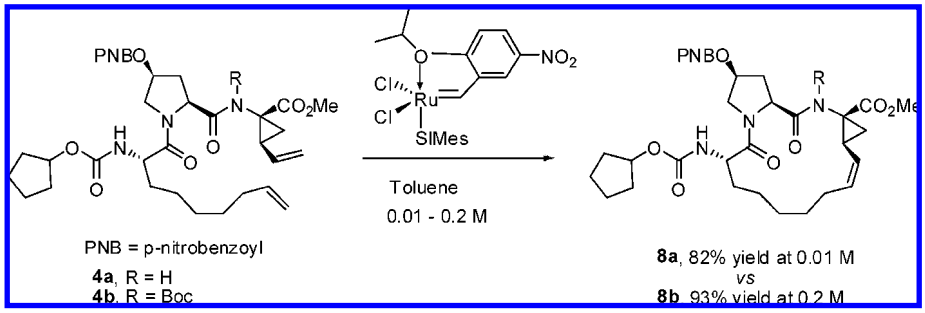
ABSTRACT



We report here that dramatic improvement of the key RCM reaction in the synthesis of HCV protease inhibitor BILN2061 can be achieved by N-substitution of the diene substrate with an electron-withdrawing group. Mechanistic studies using ^{1}H NMR spectroscopy showed an unprecedented switch of the initiation sites and the correlation between such switch and the results of RCM, from the unmodified to the modified substrates. We also provided theoretical evidence that such modification may also increase the thermodynamic preference of the macrocyclic product over the diene substrate.

Macrocycles are prevalent structural motifs in natural products, and they are increasingly popular scaffolds in drug design.[1] For example, new potent hepatitis C protease inhibitors containing a 15-membered macrocyclic lactam have been recently shown to be clinically active.[2] Thus, the development of macrocyclization strategies,[3] without the use of impractical high-dilution conditions, is of great interest to both industrial and academic scientists.[4] A useful concept that has been employed to describe the difficulty of forming large rings is that of "effective molarity" (EM), which depends on the size of the ring to be formed and also on any conformational constraints that may affect the system.[5]

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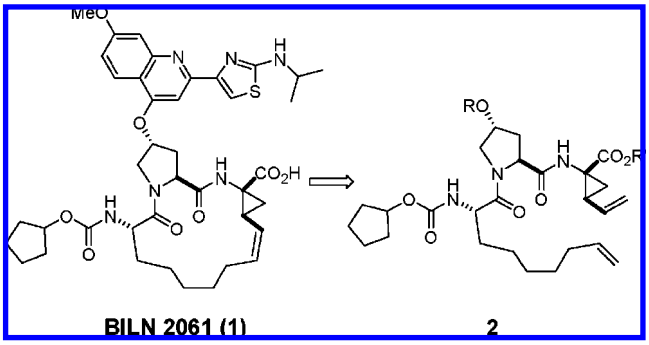
Scheme 1. Approach to **1** by RCM Macrocyclization



In recent years, the ring-closing metathesis (RCM) reaction has emerged as a very attractive approach to the synthesis of macrocycles, due to its neutral reaction conditions, broad functional group tolerance, and simple reaction procedure.[6]

Nonetheless, implementation of RCM macrocyclizations in an industrial setting is extremely problematic and very uncommon. A recent review highlights the fact that, in the large number of macrocyclizations that have been effected by RCM, the substrate concentration range was 0.2−8.5 mM, and the catalyst loading 2−10 mol %.[6c] Our recently disclosed process for BILN 2061, (**1**, Scheme 1) which was scaled to >100 kg, suffered from high dilution (10 mM) and large catalyst loading (3−5 mol %), thus rendering the approach unsuitable for production-scale manufacturing.[7]

We are pleased to report here our solution to this problem, that is, the development of an RCM macrocyclization that can operate at "acceptable" plant concentrations (>0.2 M) and at low catalyst loadings (≤0.1 mol %), thus making this *the first example of a truly practical RCM macrocyclization*.

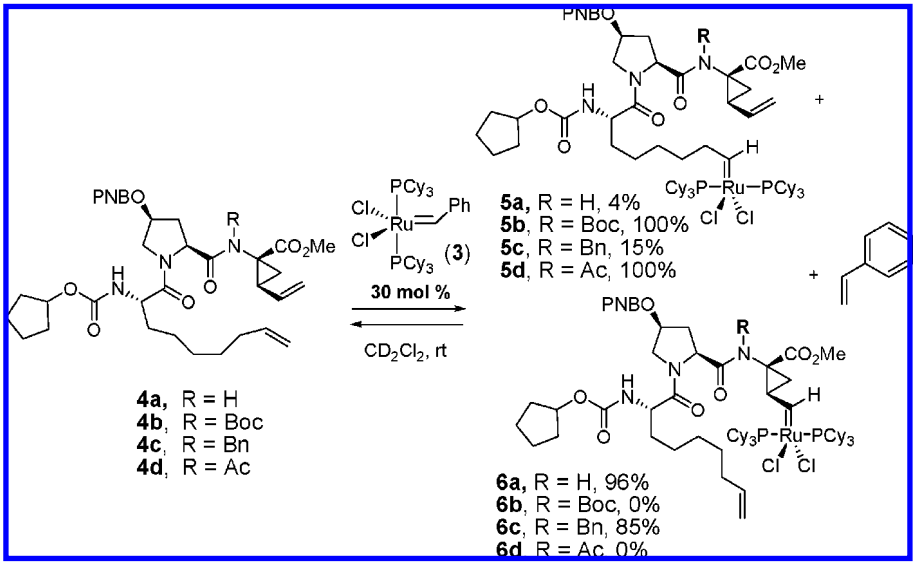
Our approach stems from various initial observations on our early RCM process: First, we noticed that the remote substituent at the C-4 position of the hydroxyproline moiety had a small but detectable effect on the RCM rate and therefore on the EM. This small effect was tentatively ascribed to subtle conformational factors. Second, we noticed that, when the initiation of the reaction was monitored using a stoichiometric amount of Grubbs catalyst **3**, carbene transfer occurred to a large extent (96%) at the vinylcyclopropane moiety, where the Ru may be stabilized by chelation. Such stabilization, in turn, may reduce the concentration of the active Ru catalyst in the reaction and negatively affect the rate of the RCM step.[7c]

To exploit these clues, we decided to prepare a number of derivatives in which the amide bond had been protected with various removable groups. We expected that such substitution may interrupt the coordinative stabilization by the ester group through steric interaction ($A^{1,3}$ strain) and therefore lead to initiation at the nonenoic acid moiety, which may be beneficial to the RCM.[8] Gratifyingly, our first insight found experimental confirmation (Scheme 2).

The resting state of the Grubbs catalyst (**3**) was readily determined by ^{1}H NMR spectroscopy as already reported.[7c] Indeed, whereas *N*-benzyl substitution (**4c**) had a relatively minor effect on the site of initiation, acylation of the N atom led to inhibition of carbene transfer to that position. Instead, carbene transfer took place completely (>98%) at the nonenoic acid moiety (**5b** and **5d** were produced in >90% conversion).

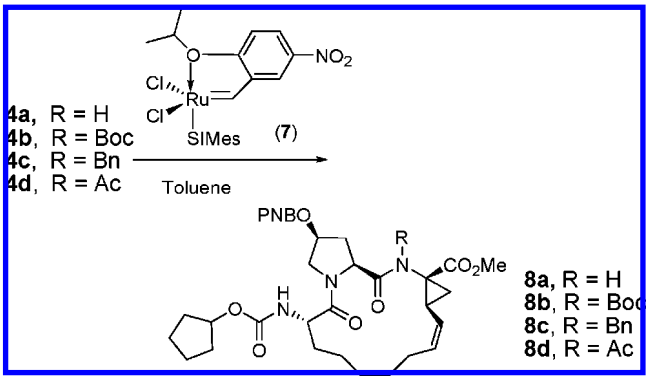
With these initial observations on hand, we proceeded to attempt the RCM of substrate **4b** under standard conditions (10 mM, toluene, 60 °C), but employing a second-generation Ru catalyst **7**.[9] The desired RCM took place with an initial rate that was 3−4 times faster than that for substrate **4a** under identical conditions, leading to the desired product in quantitative yield (>98%), without formation of dimers. This is a significant improvement over the RCM reaction of **4a** (*vide infra*). Reactions with our key substrates were then

Scheme 2. RCM Initiation with Modified Substrates



tested, under what we presume to be thermodynamic conditions,[10] on the basis of our previous work (Scheme 3 and Table 1).

Scheme 3. RCM Reaction of Modified Substrates.



As entries 1−4 show, in the case of **4a**, the yield of the RCM product **8** decreases as the substrate concentration increases. **4b**, on the other hand, operating at a 10-fold higher concentration (0.10 M, entry 8), provided essentially the same yield as the best result obtained with **4a**. Furthermore, by running the reaction at higher temperatures,[11] both the yield and the EM increased, and we were able to lower the catalyst loading to 0.1%. *Entry 11 demonstrates that we have achieved our goal of operating with ≤0.1 mol % catalyst, as well as under standard concentrations (≥0.2 M).* At even higher concentration (0.4 M, entry 12), up to 20% yield of dimer was detected, therefore limiting the yield of the RCM.[12] The *N*-benzyl derivative **4c** behaved similarly to **4a**, whereas the acetyl derivative **4d** mirrored **4b**. These data

Table 1. Effect of Concentration on RCM of **4a**−**d**[a]

entry	substrate	concn (M)	**7**, mol %	temp (°C)	yield (convn)
1	**4a**	0.01	1	60	82 (95)
2	**4a**	0.02	1	60	70 (91)
3	**4a**	0.05	1	60	52 (80)
4	**4a**	0.10	1	60	35 (72)
5	**4b**	0.01	1	60	98 (98)
6	**4b**	0.02	1	60	97 (98)
7	**4b**	0.05	1	60	87 (92)
8	**4b**	0.10	1	60	80 (87)
9	**4b**	0.05	0.1	110	97 (100)
10	**4b**	0.10	0.1	110	95 (100)
11	**4b**	0.20	0.1	110	93 (100)
12	**4b**	0.40	0.1	110	80 (99)
13	**4c**	0.01	1	60	85 (95)
14	**4c**	0.10	1	110	42 (77)
15	**4d**	0.01	1	60	99 (100)
16	**4d**	0.10	0.1	110	89 (98)

[a] Reactions were run on 1 mmol scale. Yields were determined by quantitative HPLC assay. Conversion refers to starting material consumed (HPLC assay). See Supporting Information for details.

indicate that the increase of EM by N-substitution with an electron-withdrawing group was closely related to the shift of initiation from **6** to **5**.[13]

The reversible nature of metathesis and high reactivity of **7**, however, suggest that at least part of the effect of the electron-withdrawing substituent is to increase the thermodynamic EM, possibly by reducing the ring strain. Indeed, both amide and the cyclopropane ring represent trans elements, which strain the ring system in **8a**. It is possible that removal of the enforced planarity in **8a** by *N*-Boc substitution may reduce ring strain and improve the thermodynamic EM. Thus, a theoretical analysis was carried out to (1) calculate the strain energy of the macrocycle and (2) gain an understanding of the conformational characteristics of the open chain and ring molecules.

The strain energy content of the macrocycle was calculated by first determining the conformational energy change between the *open chain* molecules with and without Boc substitution, **4a** and **4b**, and then comparing with the energy change between *ring* molecules **8a** and **8b** with the same chemical modifications. The difference between these two energy changes, that is, $\Delta\Delta E$, is the contribution of Boc substitution to the strain energy of the molecule. Because we were only interested in the strain energy of the core structure, we omitted the PNB group from our calculations for simplicity. The conformational energy of the open chain and ring molecules was calculated by molecular mechanics (MM) and quantum chemical (QM) DFT methods. MM methods are computationally efficient. Using the powerful torsion-scan/low-mode search[14] MC algorithm from the Macromodel program,[15] we identified the unique energy

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(8) Change of initiation site is known to affect RCM. See: (a) Wallace, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 1912. (b) Hoye, T. R.; Jeffrey, C. S.; Tennakoon, M. A.; Wang, J.; Zhao, H. *J. Am. Chem. Soc.* **2004**, *126*, 10210.

(9) Michrowska, A.; Bujok, R.; Haruytyuyan, S.; Sashuk, V.; Dolgonos, G.; Grela, K. *J. Am. Chem. Soc.* **2004**, *126*, 9318. We are fully aware of the fact that initiation with the Grela catalyst may be different from that with catalyst **3**. On the other hand, only catalysts containing two strong donor ligands give rise to observable intermediates, and those with IMes and SIMes ligands cyclize too quickly to make the carbene transfer products observable.

(10) Our previous studies on diene **4a** showed the reaction to be readily reversible under these conditions. See ref 7a.

(11) Yamamoto, K.; Biswas, K.; Gaul, C.; Danishefsky, S. J. *Tetrahedron Lett.* **2003**, *44*, 3297.

(12) The main cyclic dimeric product was isolated, and separately converted to **8b** under standard RCM conditions. See supporting information for details on the assignment of the structure as well as the conversion of **8b** to **1**.

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(14) Kolossvary, I.; Guida, W. C. *J. Comput. Chem.* **1999**, *20*, 1671.

(15) *Macromodel*, v 9.1; Schrödinger, Inc.: New York, 2006.

minima and the lowest-energy conformations of the relevant molecules. However, since the parametrizations of current force fields are not fully validated for the complex, polar-lipophilic macrocycles like the present system, the calculated energies obtained with the MM method may not reflect the true strain energy well. Therefore, we further used QM DFT methods to calculate the energies of the most representative conformation for the ring and open chain molecules. Table 2 summarizes the calculated strain energy reduction in the macrocycle due to Boc substitution. Our calculations using a number of theoretical methods invariably indicate that Boc substitution at the *P*1/*P*2 N atom reduces the strain energy of the ring molecule by ~2 kcal/mol, which is consistent with the experimental results. By analyzing the QM DFT optimized structures, we determined that the zero point energy of the molecules does not make a net contribution to the ΔG change in the Boc-substituted macrocycle, thus minimizing entropic contributions.

Table 2. Calculated Reduction of Strain Energy by Boc Substitution on Molecule **8b** in Comparison to **8a**

method	OPLS01[a]	MM3[a]	MMFFs[a]	DFT/B3LYP[b]
energy change (kcal/mol)	−3.33	−1.99	−1.10	−2.18

[a] Calculated from Boltzmann average of the energy minima of the chain and ring molecules sampled by Macromodel program in water. Solvation energy was calculated by the generalized Born model provided in the Macromodel program. [b] Calculated from full geometry optimization of single structure for each molecule in gas phase with the Jaguar program.[16] The basis set used in the calculation was 6-31G*. The calculated gas-phase strain energy change is −4.26 kcal/mol. Calculation with Jaguar program using continuous solvation model gave a difference of solvation energy of 2.08 kcal/mol. The sum of gas-phase strain energy and solvation energy is shown in the table.

In summary, we have developed a practical synthesis of BILN 2061 by introducing the first example of practical RCM macrocyclization. The origins of the "*N*-Boc-effect"[17] seem to be grounded in favorable kinetic and thermodynamic effects. We have shown that strategic introduction of removable groups on the RCM linker can direct the initiation site and have a remarkable effect on the RCM, thus complementing the known relay strategy,[8] and can also affect the strain content in the product, thus dramatically increasing the thermodynamic EM. Generalization of these important effects could lead to widespread utilization of the RCM and other macrocyclizations in the industrial plant.

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Supporting Information Available: Experimental Procedures for all compounds described in the paper, the conversion from **8b** into **1**, and detailed information on calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) *Jaguar*, v 6.5; Schrödinger, Inc.: New York, 2006.

(17) We prefer the Boc group over other acyl groups like the acetyl because Boc groups are easily removed from the product, thus making the *N*-Boc a "protecting-activating moiety" in this case.