Ring-Closing Metathesis

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Bismacrocyclic Inhibitors of Hepatitis C NS3/4a Protease**

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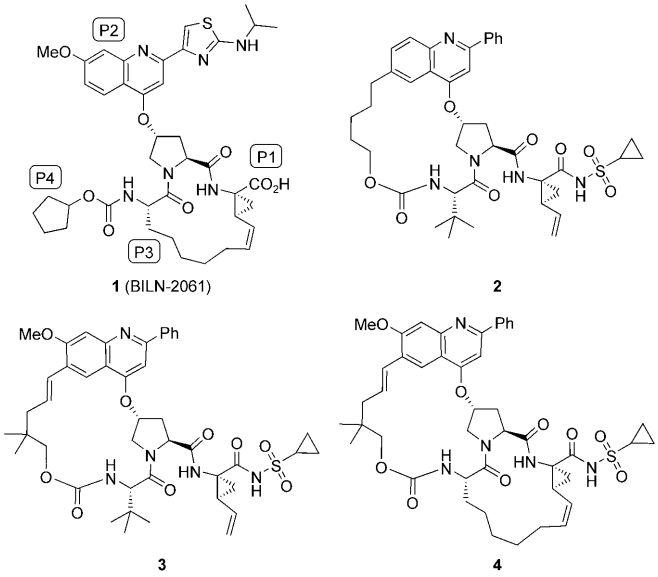
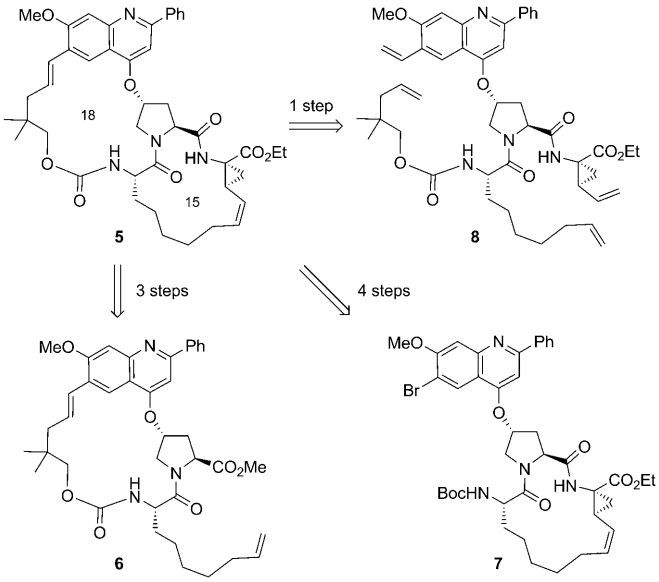
Hepatitis C virus (HCV) is a chronic infection that affects an estimated 170–200 million people worldwide.[1] The positive-RNA-strand virus of the *Flaviviridae* family replicates primarily in the liver, and although disease progression is typically a slow process, a significant fraction of those infected develop serious liver disease, including cirrhosis and hepatocellular carcinoma.[2] HCV is currently a leading cause of death in HIV-coinfected patients[3] and is the most common indication for liver transplantation.[4] Of several promising antiviral targets for HCV that have emerged in recent years,[5] NS3/4a protease inhibitors show perhaps the most dramatic antiviral effects.[6] Clinical proof of concept for this mechanism was first established with BILN-2061 (**1**, Scheme 1).[7]

We recently disclosed our molecular-modeling-derived strategy that led us to design HCV NS3/4a protease inhibitors, such as **2**, which contain a P2–P4 macrocyclic constraint (Scheme 1).[8] These compounds were prepared by using a ring-closing metathesis (RCM) reaction as the key step, and further refinement of this series resulted in the synthesis of compound **3**. With the success of this alternative macrocycle design strategy, we were intrigued by the possibility of accessing a compound that contained both the P2–P4 macrocycle found in compounds **2** and **3** and the P1–P3 macrocycle seen in compounds such as **1**.

The bismacrocycle **4** (Scheme 1) is an interesting target from a biological perspective and, we quickly realized, a formidable synthetic challenge. Compound **4** contains both 18- and 15-membered-ring macrocycles, as well as a *trans* alkene and a *cis* alkene. Our synthetic planning began with the retrosynthetic analysis of ester **5** (Scheme 2), which could be transformed in a straightforward manner into the ultimate target **4**. Initial disconnection of the P1–P3 macrocycle gives **6**, which could theoretically be transformed into **5** in three steps. The synthesis of compound **6**, however, would be nontrivial, as it would require selective RCM of a triene, protection of the terminal alkene contained in the P3 side chain, or exploration of non-RCM synthetic routes. Alter-



Scheme 1. Macrocyclic NS3/4a protease inhibitors.



Scheme 2. Retrosynthetic analysis of the bismacrocyclic core. Boc = *tert*-butoxycarbonyl.

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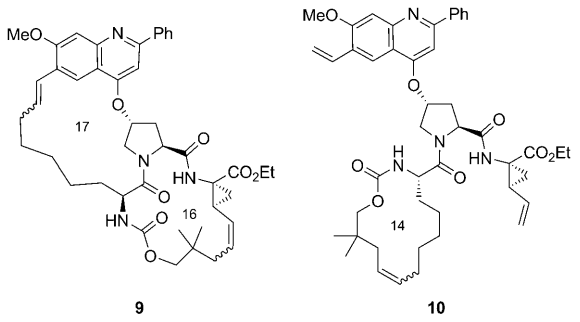
natively, initial disconnection of the P2–P4 macrocycle gives the more readily accessible target **7**. We felt that this route would most likely deliver the target **4**, but only after a mostly linear sequence of more than 10 steps. A third possibility would be to form both rings simultaneously through a double ring-closing metathesis reaction of the tetraene **8**. This route was attractive to us, because **8** could be synthesized in a convergent manner by formation of the central amide bond from readily available fragments. Furthermore, each macrocycle had been formed independently through RCM reactions previously, and the alkene geometry had been controlled in both cases.[7a,8]

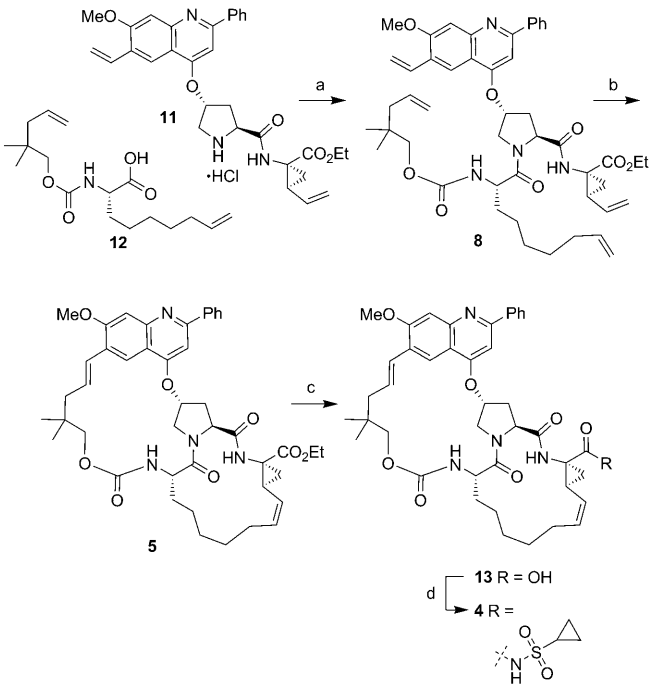
Although this planned synthesis would be a very convergent and rapid approach to a difficult target, the double RCM reaction could have a number of selectivity issues that would make the isolation of the desired product technically difficult, if indeed it was formed. For example, an alternative cyclization of **8** may lead to compound **9** (Scheme 3), which



Scheme 3. Potential alternative cyclization modes for the bismacrocyclization reaction.

contains 16- and 17-membered rings; in this case, the alkene geometry in both rings would be in question. Another possibility we considered was the initial reaction of the two alkene functionalities at the termini of the more flexible carbon chains to give the 14-membered ring present in compound **10**. A recent review of metathesis reactions of tri- and tetraene substrates has shown the utility of these reactions in the synthesis of certain natural products, although many of the examples involve the formation of smaller rings, and the potential selectivity issues that may occur with **8** do not apply.[9]

In practice, compounds **11**[10] and **12**[10] could be coupled under standard conditions in high yield to give the bis-RCM precursor **8** (Scheme 4). When **8** was exposed to the Zhan 1b catalyst[11] and *p*-benzoquinone[12] in 1,2-dichloroethane (5 mM) at 70 °C for 1 h, a single major peak was observed by HPLC, and one compound was isolated in 45 % yield. ^{1}H and ^{13}C NMR spectroscopic analysis, including 2D experiments, confirmed that the major reaction product was the desired bismacrocycle **5**.[10] HPLC–MS analysis of the reaction mixture showed the presence of three other products with masses consistent with bismacrocyclic structures; however, **5** was formed with approximately 10:1 selectivity over each of these compounds. Thus, the 18- and 15-membered rings of **5**, which contain a *trans* and a *cis* alkene, respectively,



Scheme 4. Synthesis of **4** through a selective bis-RCM reaction: a) HATU, DIPEA, DMF, 79 %; b) Zhan 1b catalyst,[11] DCE, 1 mM, 70 °C, 1 h, 66 %; c) LiOH, H_2O, THF, EtOH, 86 %; d) CDI, THF; cyclopropylsulfonamide, DBU, 40 °C, 68 %. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DIPEA = *N*,*N*-diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, DCE = 1,2-dichloroethane, CDI = 1,1′-carbonyldiimidazole, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

were formed preferentially over at least nine other possible products with macrocyclic rings of similar sizes. These potential products include the undesired alkene isomers of **5** and the six compounds represented by structures **9** and **10** (Scheme 3).

We then undertook optimization studies of the bis-RCM step. When the dilution of the reaction mixture was increased to 1 mM, compound **5** was obtained in a remarkable yield of 66 %, and the approximately 10:1 selectivity was maintained. Presumably, this high dilution leads to a decrease in the quantity of oligomeric products that may have been formed under our standard reaction conditions at a concentration of 5 mM.[13] Compound **5** was then hydrolyzed to the carboxylic acid **13**, which was coupled to cyclopropylsulfonamide[14] to complete the target **4**.

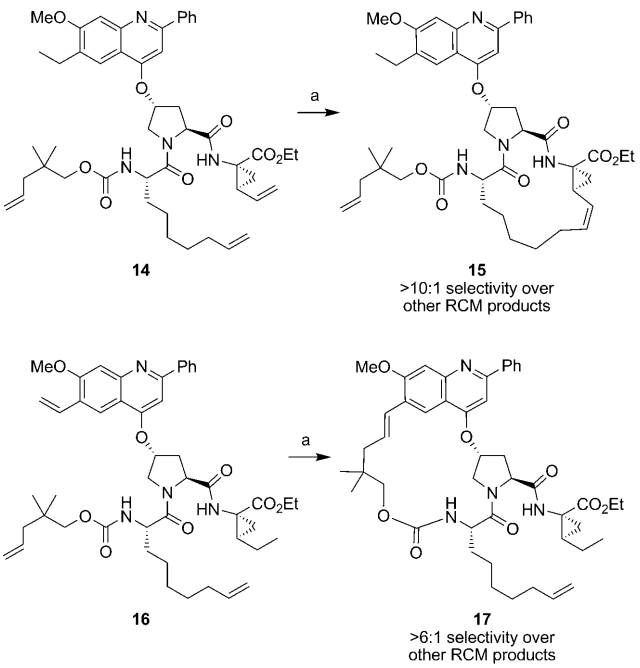
When compared with the P2–P4 monomacrocycles **2** and **3** and the P1–P3 monomacrocycle **1**, bismacrocycle **4** maintains excellent enzyme potency against the genotype 1b NS3/4a protease (Table 1).[15] Compound **4**, however, showed improved potency in the cellular replicon assay under both high- and low-serum conditions.[16] Notably, in comparison to compound **3**, the addition of the P1–P3 macrocycle in compound **4** leads to a fivefold increase in potency against the genotype 3a NS3/4a protease enzyme. Compound **13**, which lacks the potency-enhancing acyl sulfonamide, also maintains good activity, comparable to that of carboxylic acid **1**, against the genotype 1b enzyme.

Table 1: Inhibitory activity against HCV NS3/4a protease.[a]

Compound	K_i(genotype 1b) [nM]	K_i(genotype 3a) [nM]	EC_{50}(1b replicon) [nM] 10% FBS/50% NHS
1	0.30	–	3/19
2	< 0.016	35	13/25
3	0.034	6.8	3/14
4	0.029	1.2	2/10
13	0.13	140	10/340

[a] Data are geometric averages of the values for at least three measurements. FBS = fetal bovine serum, NHS = normal human serum.

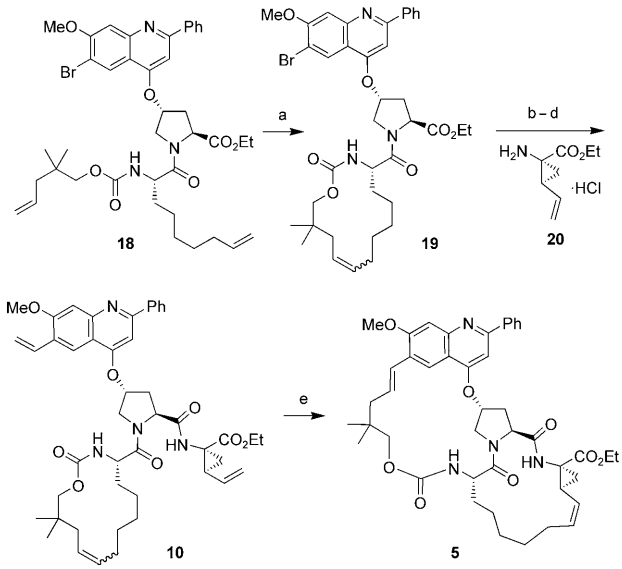
In addition to the excellent biological profile of **4**, we were fascinated by the remarkable selectivity of the bis-RCM reaction. We designed two competition experiments to explore the selectivity of the individual ring-forming reactions (Scheme 5). We first studied the reactivity of triene **14**, which



Scheme 5. Competition experiments: a) Zhan 1b catalyst,[11] *p*-benzoquinone, DCE, 5 mM, 70 °C, 1 h; **15**: 38%; **17**: 42%.

lacks the styryl alkene to eliminate the possibility of forming the P2–P4 macrocycle. Under standard RCM conditions, compound **15** was formed as the major product with greater than 10:1 selectivity over four other RCM products, as determined by HPLC–MS analysis. Thus, the desired P1–P3 macrocyclization was favored.[10] A complementary experiment with **16**, which lacks the alkene in the P1 side chain, also showed selectivity for the desired macrocycle, **17**, under the RCM conditions; however, this reaction is slightly less selective (> 6:1 selectivity over five other products).[10] These experiments demonstrate that the remarkable selectivity observed in the formation of **5** is not a result of a driving of overall efficiency by a single chemoselective reaction, but is instead dictated by the high degree of chemoselectivity in the formation of both the P1–P3 and P2–P4 macrocycles. Furthermore, the conversion of **8** into **5** seems to generate fewer RCM side products, which indicates perhaps that selectivities are reinforced through a form of double chemoselection.

Another possibility for the highly selective formation of **5** from **8** is that **5** could be the thermodynamic product, and intermediate compounds equilibrate to product **5** under the reaction conditions. We initially thought that compound **10** would be a likely product of the RCM reaction of **8** because of the flexibility of the chains containing the relevant alkenes, although, in practice, we did not detect intermediate products in the cyclization of **8**.[17] To probe this possibility, we prepared **10** as shown in Scheme 6. Compound **18** was synthesized in a



Scheme 6. Equilibration experiment: a) Zhan 1b catalyst,[11] DCE, 5 mM, 70 °C, 1 h, 95%; b) $(CH_2{=}CH)SnBu_3$, $[Pd(PPh_3)_4]$, toluene, 100 °C, 74%; c) LiOH, H_2O, THF, EtOH; d) HATU, DIPEA, **20**, DMF, 60% (2 steps); e) Zhan 1b catalyst, DCE, 5 mM, 70 °C, 92 h, 26%.

standard fashion, and the 14-membered ring was formed by RCM to give **19** as a mixture of alkene isomers in good yield. A three-step sequence of vinylation, hydrolysis, and amide coupling with **20**[18] then yielded compound **10**. The extended exposure of **10** to RCM conditions, including the addition of up to 20 mol% of the Zhan 1b catalyst, led to bismacrocycle **5** as the major product. This result demonstrates that **5** is likely to be the thermodynamic product.[19] Since the equilibration of **10** to **5** requires extended heating, as opposed to the direct conversion of tetraene **8** into **5** (92 h versus 1 h), we do not believe that compound **10** is an intermediate in the conversion of **8** into **5**.[10] This result is particularly noteworthy, as the 14-membered ring in **19** is formed quickly and efficiently (1 h, 95% yield) from a substrate, **18**, with only two alkene functionalities.

In summary, we have synthesized the bismacrocyclic HCV NS3/4a protease inhibitor **4** through a highly convergent synthetic sequence of amide-bond formation followed by a chemo- and stereoselective double RCM reaction to form the 18- and 15-membered rings simultaneously. Compound **4**

shows excellent activity against both the genotype 1b and the genotype 3a NS3/4a enzymes as well as very good cellular activity. Further studies on this unique class of NS3/4a protease inhibitors and into the origins of the bismacrocyclization selectivity are ongoing.

Experimental Section

5: A solution of **8** (50 mg, 0.06 mmol) in 1,2-dichloroethane (61 mL; 1 mM) was purged with nitrogen gas for 10 min. The Zhan 1b catalyst (4.5 mg, 0.006 mmol) was then added, and the reaction mixture was heated to 70 °C and stirred for 1 h. The reaction mixture was then cooled, concentrated, and purified by silica-gel chromatography (gradient elution, 10–100 % EtOAc in hexane) to provide **5** (31 mg, 66 %). ^{1}H NMR (600 MHz, CD_3CN): $\delta = 8.22$ (s, 1H), 8.20–8.18 (m, 2H), 7.55–7.52 (m, 2H), 7.50–7.46 (m, 1H), 7.33 (s, 1H), 7.26 (br s, 1H), 7.24 (s, 1H), 6.76 (d, $J = 16.1$ Hz, 1H), 6.36 (ddd, $J = 16.1$, 10.5, 5.6 Hz, 1H), 6.26 (d, $J = 8.3$ Hz, 1H), 5.49 (apparent t, $J = 3.0$ Hz, 1H), 5.44 (apparent q, $J = 8.4$ Hz, 1H), 5.18 (apparent t, $J = 9.8$ Hz, 1H), 4.75 (dd, $J = 11.5$, 1.6 Hz, 1H), 4.53 (ddd, $J = 11.1$, 8.5, 3.2 Hz, 1H), 4.45 (d, $J = 11.1$ Hz, 1H), 4.37 (dd, $J = 9.3$, 7.7 Hz, 1H), 4.02–3.96 (m, 2H), 3.99 (s, 3H), 3.89 (dd, $J = 11.5$, 2.8 Hz, 1H), 3.31 (d, $J = 11.1$ Hz, 1H), 2.63 (ddd, $J = 14.3$, 7.5, 1.6 Hz, 1H), 2.47–2.39 (m, 2H), 2.33 (dd, $J = 13.5$, 5.0 Hz, 1H), 2.19–2.14 (obscured m, 1H), 2.03 (dd, $J = 13.5$, 10.5 Hz, 1H), 1.84–1.79 (m, 2H), 1.61–1.54 (m, 1H), 1.51 (dd, $J = 9.5$, 5.0 Hz, 1H), 1.47 (dd, $J = 8.5$, 5.0 Hz, 1H), 1.45–1.36 (m, 3H), 1.28–1.14 (m, 3H), 1.11 (s, 3H), 1.10 (s, 3H), 0.86 (s, 3H) ppm; HRMS (ESI): m/z calcd for $C_{44}H_{53}N_4O_8$: 765.3858 $[M+H]^+$; found: 765.3880.

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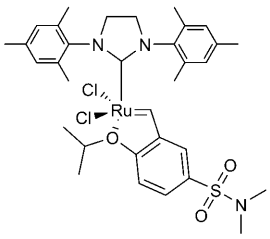

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