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Olefin ring-closing metathesis as a powerful tool in drug discovery and development – potent macrocyclic inhibitors of the hepatitis C virus NS3 protease

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Abstract

Peptidomimetic inhibitors of the hepatitis C NS3 protease often exhibit poor biopharmaceutical properties. Structure modification of a substrate-based tripeptide into a β-strand 15-membered ring scaffold provided a new class of peptidomimetics that are significantly superior as drug candidates to their acyclic precursors. Tripeptide dienes composed of three unnatural amino acid residues with numerous chiral centers were efficiently converted to macrocyclic peptides, in high diastereomeric purity, using ring-closing metathesis (RCM). The conformation of the acyclic diene and the protocol for the RCM reaction were investigated and optimized extensively in order to achieve an efficient synthesis of potential therapeutic agents for the treatment of hepatitis C infections. These studies provided the fist small molecule (BILN 2061) that was clinically validated for the treatment of hepatitis C infection in man and opened the door to a plethora of new pre-clinical pharmaceutical agents that can be made in multi kilogram quantities using RCM chemistry.

1. Introduction

Small molecules which can attenuate the function of a biological process by mimicking the secondary structure of proteins, or the critical molecular recognition element (structural *hot spots*) between polypeptides and proteins, are of enormous scientific interest [1]. Such interactions play a key role in numerous cellular functions (e.g. protein glycosylation [2], intracellular recognition [3], proteolysis and others) and can be of particular relevance in drug discovery. However, unlike the structurally unique peptides found at the contact points between two large biomolecules, short peptides are conformationally heterogeneous and exhibit low affinity for their intended binding sites. In addition, these peptides often suffer from poor metabolic stability and low bioavailability, further magnifying the challenge of designing a small peptidomimetic compound as a therapeutic drug. Nonetheless, during the last decade, considerable effort has been devoted to the design and synthesis of substrate-based peptidomimetic inhibitors that target specifically the NS3 serine protease enzyme of the hepatitis C virus (HCV). HCV is a severe medical problem with a global infection afflicting more than 170 million people that can cause cirrhosis of the liver and liver cancer. Therefore, disrupting the catalytic function of its essential enzyme NS3 protease can provide a treatment option of significant socioeconomic benefit.

Initially, the quest for the discovery of a substrate-based, small-molecule inhibitor targeting the HCV NS3

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serine protease was taken on by a number of pharmaceutical companies. However, to the dismay of medicinal chemists, the shallow and solvent-exposed active site of this enzyme proved to be an exceedingly difficult target for drug design and many companies abandoned their efforts. At Boehringer Ingelheim, medicinal chemistry activities were initiated by the discovery that N-terminal peptide products, derived from sequences of the NS3 protease polypeptide substrate, were competitive inhibitors of the HCV NS3 protease [4]. Hexapeptides, such as model compound **1** (Fig. 1), were synthesized and used as NMR probes to investigate the critical interactions between the active site of the enzyme and its polypeptide substrate [5]. Armed with the insight provided by these studies, we designed macrocyclic β-strand tripeptides (e.g. compounds **3b–d**) that adopt in the *free state* the enzyme-bound secondary structure of the hexapeptides and bind tightly to the active site of the enzyme [6]. The key ring-closing step was performed on the acyclic tripeptide dienes **2** using RCM chemistry. While this work was in progress, other investigators also reported similar efforts towards the design of macrocyclic peptides as potential inhibitors of metallo, aspartic, cysteine, and serine proteases [7].

The design of macrocyclic drugs which are peptidic in nature is undoubtedly inspired by nature's non-ribosomally formed secondary metabolites, which are often composed of unusual amino acid fragments and are constrained into cyclic peptides with unique bioactive conformations; well-known pharmaceuticals include cyclosporin, vancomycin and chloropeptin. However, unlike the production of natural products via fermentation, the chemical manufacturing of structurally complex macrocyclic peptides is often very challenging and the synthetic methodologies employed usually hinder the development of such compounds into commercial products. Therefore, following our initial discovery of macrocyclic compounds inhibiting the HCV NS3 protease [6], two of our key objectives were (a) the optimization of the biopharmaceutical properties and (b) the development of a synthetic process that was economically feasible and could support clinical trials of a therapeutic agent. In this report, we examine the effects of substitution along the hydrocarbon linker moiety of the prototype compound **3d**, in light of the structural data previously published for this compound [6]. In addition, we provide an account of the synthetic developments that led to the first pharmaceutical application of ring-closing metathesis in multi-kilogram scale.

2. Results and discussion

2.1. Medicinal chemistry

Over the last 10 years, the developments of stable organometallic reagents that catalyze ring-closing metathesis (RCM) reactions have revolutionized the field of organic synthesis [8]. These reagents provide new means of synthesizing polyfunctional, large heterocyclic rings that are exceptionally challenging to prepare by any other synthetic methods. Peptide scaffolds, such as those typified by the HCV inhibitor **3d**, were originally synthesized using the first-generation Grubbs catalyst, bis-(tricyclohexyl-phosphine)benzylidene-ruthenium dichloride (**4**) [9]. This

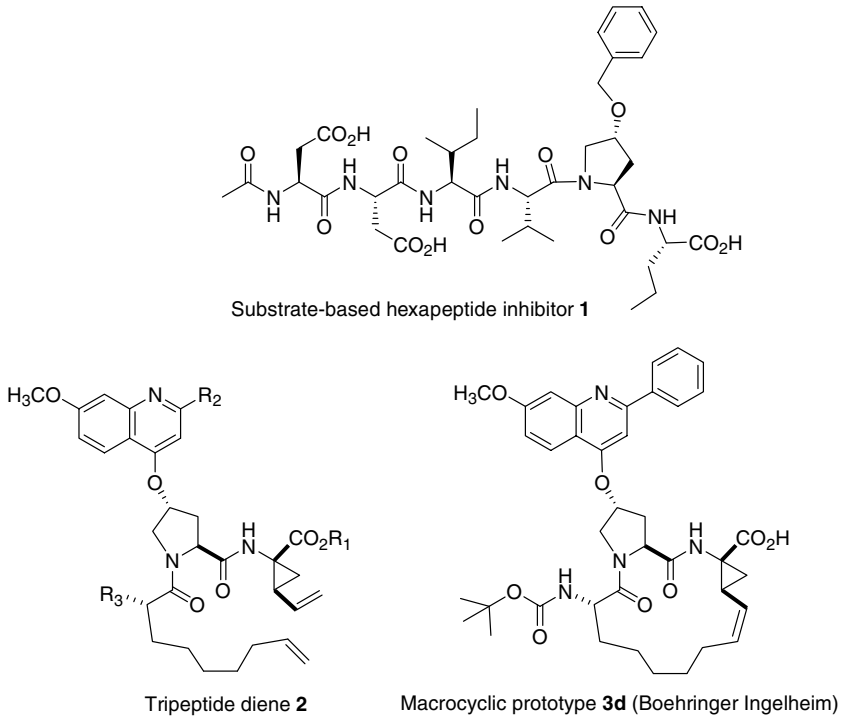
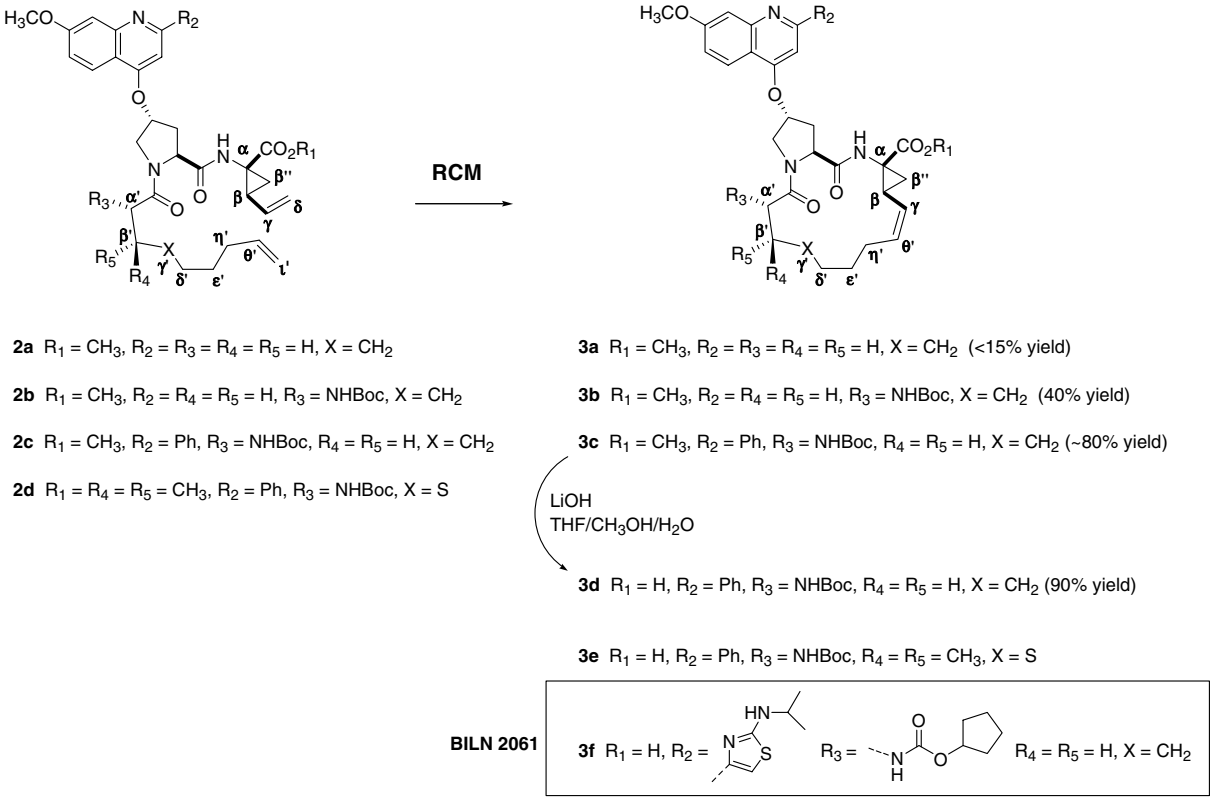


Fig. 1. Substrate-based peptidomimetic inhibitors of HCV NS3 protease.

15-membered macrocyclic scaffold is characterized by the (1*R*,2*S*)-vinyl aminocyclopropyl-carboxylic acid moiety (vinyl ACCA moiety; P1) [10], a proline unit substituted at Cγ with a 4-hydroxy-7-methoxyquinoline (P2) [11] and a linker moiety composed of (2*S*)-*N*-Boc-amino-8-nonenoic acid (P3) [9,12,13]. The backbone conformation of the acyclic diene precursor **2**, and in particular the ratio of the proline *cis:trans* rotamers, was found to be crucial for the yield of the RCM reaction and diastereomeric purity of the macrocyclic product(s) formed [14]. Recently, we reported that when the Cα of the olefinic "linker" moiety (P3) was unsubstituted (Scheme 1: diene **2a**, $R_3 = H$), the *cis:trans* rotamer ratio of the P2–P3 amide bond was approximately 1:1 and the outcome of the RCM reaction was very poor (mixtures of product were obtained with <30% overall RCM conversion) [14]. In contrast, when the bulky –NHBoc group was attached to the Cα of the olefinic linker (Scheme 1: diene **2b**, $R_3 = NHBoc$), the *cis:trans* ratio was approximately 1:9 and the overall conversion of **2b** to macrocyclic ester **3b** was achieved in 40% yield (~60% of total RCM conversion) [14]. Variable temperature ^1H NMR data of the acyclic tripeptide **2a** suggested a tertiary amide rotational barrier of >13 kcal/mol. Finally, the overall conformational preference of diene **2c** clearly pre-organized the peptidic backbone in the same β-strand conformation as that of the desired macrocyclic product (**3c**), thus facilitating the RCM reaction; RCM-induced macrocyclization of diene **2c** was achieved in 80% yield to the desired diastereomer **3c** (Scheme 1). These observations were analogous to those initially reported by Grubbs and coworkers on template-directed RCM reactions [15] and more recently by Lubell's group for the RCM macrocyclization of dipeptides having secondary or tertiary amide bonds [16]. Remote control effects on the outcome of the RCM reaction were also previously reported in the synthesis of macrocyclic natural products, such as salicylihalamide [17] and epothilones [18].

Our initial success with RCM-mediated macrocyclization of tripeptide dienes [9] encouraged medicinal chemistry efforts to focus on exploring the structure–activity relationship (SAR) of these analogs. Our SAR studies included evaluation of modifications along the hydrocarbon linker connecting the P1 to the P3 moiety. Previously, the position of the double bond was found to play a significant role in the potency of the compounds [9]. For example, inhibitor **3d** ($IC_{50} = 10$ nM, $EC_{50} = 77$ nM) was found to be 5–7-fold more potent than **6a** (Scheme 2; $IC_{50} = 50$ nM, $EC_{50} = 410$ nM) in our in vitro and cell-based assays [19]. Therefore, the presence of a *trans* double bond connecting the Cε′ of (2*S*)-*N*-Boc-amino-6-heptenoic acid (P3) to the Cε of a homoallyl ACCA unit at P1 was decreasing the binding affinity of **6a** for its target (Scheme 2) [9]. However, saturation of the double bond to give analog **6b** ($IC_{50} = 16$ nM, $EC_{50} = 120$ nM) resulted in the regain of potency in both the enzymatic and the cell-based assays [9].



2a $R_1 = CH_3$, $R_2 = R_3 = R_4 = R_5 = H$, $X = CH_2$

2b $R_1 = CH_3$, $R_2 = R_4 = R_5 = H$, $R_3 = NHBoc$, $X = CH_2$

2c $R_1 = CH_3$, $R_2 = Ph$, $R_3 = NHBoc$, $R_4 = R_5 = H$, $X = CH_2$

2d $R_1 = R_4 = R_5 = CH_3$, $R_2 = Ph$, $R_3 = NHBoc$, $X = S$

3a $R_1 = CH_3$, $R_2 = R_3 = R_4 = R_5 = H$, $X = CH_2$ (<15% yield)

3b $R_1 = CH_3$, $R_2 = R_4 = R_5 = H$, $R_3 = NHBoc$, $X = CH_2$ (40% yield)

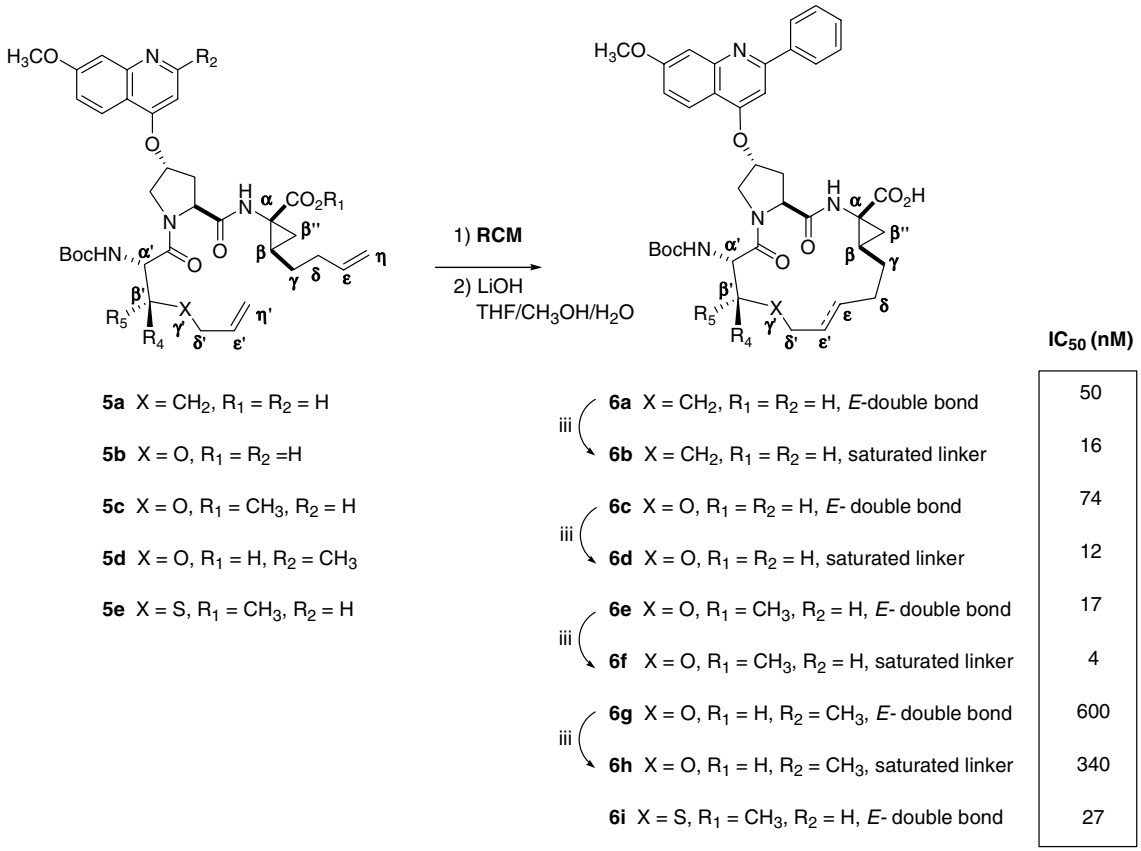
3c $R_1 = CH_3$, $R_2 = Ph$, $R_3 = NHBoc$, $R_4 = R_5 = H$, $X = CH_2$ (~80% yield)

3d $R_1 = H$, $R_2 = Ph$, $R_3 = NHBoc$, $R_4 = R_5 = H$, $X = CH_2$ (90% yield)

3e $R_1 = H$, $R_2 = Ph$, $R_3 = NHBoc$, $R_4 = R_5 = CH_3$, $X = S$

3f $R_1 = H$, $R_2 =$ $R_3 =$ $R_4 = R_5 = H$, $X = CH_2$

Scheme 1. RCM-induced macrocyclization of dienes **2**.



Scheme 2. SAR of covalent linker connecting P1–P3.

A co-crystal structure of inhibitor **3d** with the NS3 protease was also obtained, revealing the binding interactions between the inhibitor and the active site of the enzyme [6]. This structure indicated that the Cγ′ of P3 was located near the sulfur atom of residue cysteine 159 (~3.9 Å from P3-**Cγ** to the **S** of Cys159) and suggested that binding affinity could plausibly be increased through a H-bond, or a dipole–dipole interaction with the Cys159 thiol. Hence, a variety of new tripeptide dienes were synthesized, replacing the Cγ of P3 with oxygen or sulfur (compounds **2d** and **5b–e**). In addition, in order to compare the SAR effects with those previously reported for acyclic tetrapeptides [5a,10b,11] and tripeptides [20], as well the activated carbonyl inhibitors VX-950 (Vertex Pharmaceuticals) [21] and SCH 503034 (Schering-Plough) [22], the effects of branching at the Cβ of the P3 moiety were also evaluated.

The building blocks required for the synthesis of the new dienes were prepared from commercially available N-Boc protected L-serine, L-threonine, (2*S*,3*S*)-allo threonine and L-penicillamine [23]. RCM-induced macrocyclization of dienes **2d** and **5b–e** was catalyzed by the first generation Ru-based Grubbs catalyst (**4**) using the same protocol as previously reported [9]. Interestingly, neither substitution of R_4 by a methyl (Scheme 2), nor replacement of the Cγ by oxygen or sulfur, had any dramatic effects on the in vitro potency (IC_{50}) of the corresponding macrocyclic compounds (Scheme 1 and 2). However, when the Cβ of P3 was substituted with a methyl group at the R_5 position (Scheme 1, compound **3e** and Scheme 2, compounds **6g** and **6h**), a dramatic loss in potency was observed (e.g. >20-fold loss in potency was observed between compound **6b** and **6h**). This observation is consistent with our X-ray data, suggesting that placing a methyl group (i.e. $R_5 = CH_3$) directly into the binding pocket of the enzyme would disrupt many of the favorable interactions observed in the co-crystal of the NS3 protease-**3d** complex [6]. Finally, inhibitor **6f** was found to be only slightly more potent than **3d** in both the enzymatic and cell-based assay, most likely due to further rigidification of the linker moiety by the introduction of the Cβ-methyl at P3. However, based on the overall biopharmaceutical properties of these inhibitors, optimization of the critical macrocyclization step via RCM chemistry was focused on analogs of **3d**. Furthermore, SAR optimization of the P2 quinoline and the *N*-Boc capping group of the P3 moiety [24] led to the discovery of the first small molecule inhibitor of HCV NS3 protease, BILN 2061 (Scheme 1, **3f**), that exhibited cellular potency of ~1 nM, good metabolic stability and oral bioavailability suitable for a drug substance. BILN 2061 (**3f**), was the first clinically relevant NS3 protease inhibitor that achieved dramatic viral load reduction in HCV-infected humans after treatment of only 48 h [25].

Since the disclosure of the first series of macrocyclic inhibitors by Boehringer Ingelheim[26] and particularly the

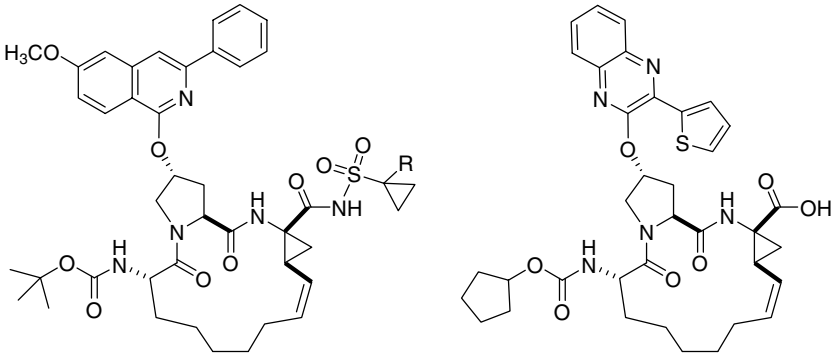
Phase I clinical data of BILN 2061 (**3f**) [25], many compounds characterized by the same 15-member ring peptidomimetic scaffold have appeared in the drug-discovery pipelines of the pharmaceutical industry [27]; these include (Fig. 2) derivatives from Bristol-Myers Squibb (e.g. compound **7**), Enanta-Chiron (e.g. compound **8**), Intermune-Array Biopharma (e.g. compound **9**) and Pfizer (e.g. compound **10**).

2.2. RCM optimization chemistry

Faced with the problem of producing large amounts of BILN 2061 (**3f**) and related analogs, and considering the synthetic difficulties associated with a total re-design of the synthesis, we considered defining a scalable process around the RCM reaction. The (1*R*,2*S*)-vinyl ACCA moiety (P1) of **3**, as well as compounds **7–10**, plays a critical role in the conformation of the macrocyclic scaffold and, consequently, the affinity of these compounds for their intended biological target [6,9,10b]. Therefore, maintaining absolute stereochemical fidelity during the RCM conversion of an acyclic diene to the desired macrocyclic product was critical not only to our drug discovery program, but also and especially to the process development of these compounds. Our initial evaluation of the factors that modulate the outcome of the RCM reaction, using the unique tripeptide precursors from this class of compounds, evidenced a Ru-mediated epimerization reaction involving the vinyl ACCA moiety [14,28]. To the best of our knowledge, the reaction pathway leading to this epimerizion was unprecedented in the literature. In fact, RCM reactions involving vinylcyclopropanes had been previously reported in the synthesis of radicicol-type macroclides [29], coronanes [30], and oligo-*gem*-difluorocyclopropanes [31], without any evidence of stereomutation.

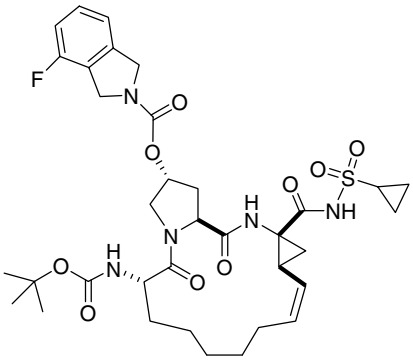
This led us to the examination of other Ru-based catalysts (Fig. 3), which included the Hoveyda catalyst **11** [32], as well as the second-generation, imidazolium-based catalysts **12** and **13**, which normally provide better RCM reactivity and higher turnover number (TON) [33]. With none of these catalysts was the epimerization reaction observed, and this led us to abandon catalyst **4** in our optimization/scale-up studies, focusing instead on **11**, **12**, and **13**. Faced with developing a route to BILN 2061 (**3f**) and related analogs that could be implemented at least up to the 100 kg scale, we had to address a number of complex challenges:

1. *Throughput*: this is the most important factor in large-scale execution of a reaction that requires high dilution. Our ability to prepare multikilo amounts of BILN 2061 is tied to the effective molarity[34] of the particular ring-closure to the 15-membered ring. Because the kinetic and thermodynamic effective molarities of these cyclization may differ, both had to be elucidated.

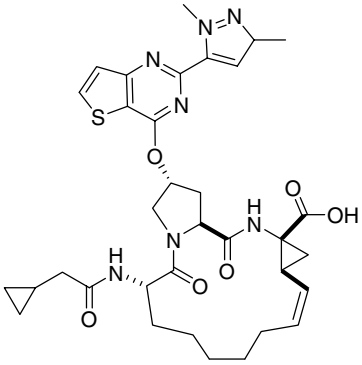


7 (Bristol-Myers Squibb)

8 (Enanta-Chiron)



9 (Intermune-Array Biopharma)



10 (Pfizer)

Fig. 2. Examples of macrocyclic peptides currently under investigation as potential therapeutic agents for the treatment of hepatitis C infections.

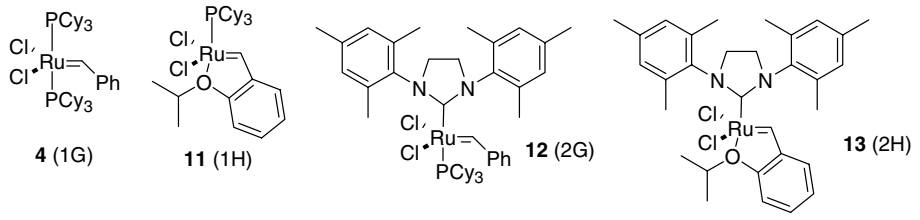


Fig. 3. Ruthenium catalysts described in this study.

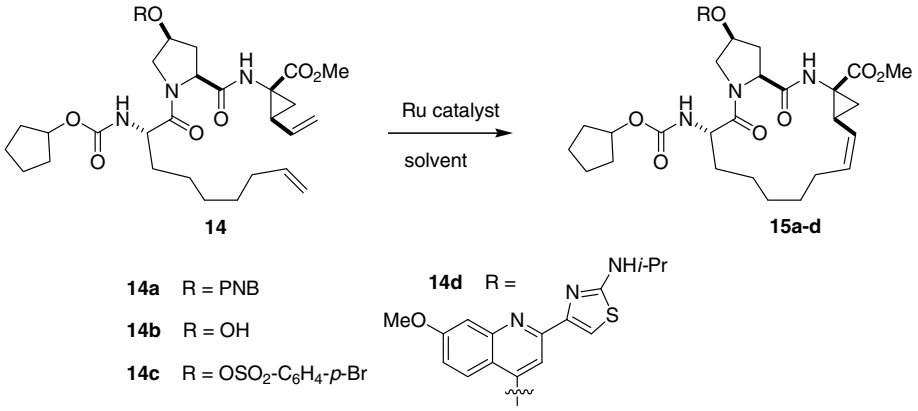
2. *Catalyst load*: in initial experiments, a load of 5–10 mol% catalyst **11** had been necessary. This may prove prohibitive from a cost standpoint. In addition, removal of such large levels of Ru (to <10 ppm levels) from the drug substance is quite challenging.
3. *Reaction time*: initial runs required 20–24 h reaction times. This adds to the cost of the process in terms of plant utilization. A shorter reaction time is needed.
4. *Reproducibility*: this turned out to be a major issue in many runs, especially as the TON improved from 5–10% to <1%. The epimerization reaction described above re-surfaced even with catalysts such as **11**, and this required tight quality control of the starting materials, solvent and all reagents. The need to perform acid washes on the solvent used, to eliminate traces of amines and/or phosphines, has been described already [35].

Several complementary strategies for the assembly of dienes as substrates to the RCM reaction, as well as the installation of the quinoline moiety at P2, have been carefully examined, and the results are discussed elsewhere [36]. For the purpose of the RCM studies, it suffices to note that a number of related diene precursors were available for development studies (Scheme 3).

Substrates **14a–d** represent potential intermediates where the hydroxyl group on the P2 moiety is protected (**14a**), unprotected (**14b**), activated for later introduction of the quinoline moiety by S_N2 reaction (**14c**), and already bearing the complete P2 construct (**14d**). Clearly, the relative efficiency of each substrate assembly must be weighed against their performance in the RCM reaction under a variety of catalytic conditions. These are collected in Table 1. It must be noted that, for fair comparison, all these substrates were used in the crude state, i.e. as emerging from the assembly sequence of the tripeptide diene without further purification. The dienes are typically rather pure (>95% w/w), and further purification, in our experience, only affected the TON obtained and not the course of the reaction, with one major exception: the presence of even small amounts (1–2% mol) of phosphines or amines, as mentioned above, can cause appreciable amounts of epimerization even with catalyst **11**.

None of the reactions shown in Table 1 suffered from this problem. In all experiments the catalyst load represents the amount of catalyst (added in portions) needed to bring the particular reaction to completion (<1–2% starting material).

As shown in the table, RCM of diene **14a** proceeds uneventfully in excellent yield with Hoveyda catalyst **11** (entries 1–2). As long as the concentration does not exceed 10 mM, no cyclic dimers are detected by LC-MS. However, the reaction requires as much as 3.5–5 mol% catalyst as well as prolonged reaction times (20–24 h) to go to completion. When more active catalysts **12** and **13** are used, loads of 0.5–1 mol% suffice for completion in only 1–4 h, but the product is accompanied by a number of cyclic dimers totaling 8–10% in yield; these are problematic to remove and require extensive recrystallization steps (entries 3–4). A control experiment (entry 5) indicates that the reaction reaches an apparent equilibrium in about 5 min, reaching 95% conversion only as long as ethylene is not allowed to evaporate and shift the equilibrium to the right. About 7% dimer content seems to be the thermodynamic value at this concentration with this substrate. It appears therefore that, under the conditions of entries 1 and 2, the reaction is under kinetic



Scheme 3. RCM reaction development using substrates **14a–d**.

Table 1
Development of RCM reaction for the pilot plant (Scheme 3)

Entry	Substrate (10 mM)	Catalyst (equiv.) + additives	Solvent, Temperature (°C)	Time (h)	Yield of **15a–d** (HPLC assay)	% Cyclic dimers (HPLC area %)	Unreacted substrate (%)
1	14a	**11** (5%)	CH_2Cl_2, 40	24	90	<1	<2
2	**14a**	**11** (3.5%)	PhMe, 60	20	90	<1	<2
3	**14a**	**12** (0.5%)	PhMe, 60	4	87	8	<1
4	**14a**	**13** (1%)	PhMe, 55	1	85	10	<1
5	**14a**[a]	**12** (2.5%)	CH_2Cl_2, 40	0.5	88	7	5
6	**14b**	**11** (2.5%)	CH_2Cl_2, 40	20	80	13	<2
7	**14b**	**12** (2%)	CH_2Cl_2, 40	20	78	13	<1
8	**14b**	**12** (2%)	PhMe, 60	1	72	18	<1
9	**14b**	**13** (1%)	PhMe, 55	1	65	10	<1
10	**14b**	**13** (2%)	THF, 60	22	77	9	<2
11	**14b**	**12** (0.7%)	EtOAc, 60	6.5	80	12	<1
12	**14b**	**12** (0.5%)	EtOAc/PhMe 3:2, 60	4	81	12	<1
13	**14b**	**13** (1.2%)	THF/PhMe 4:1, 60	8	88	6	<1
14	**14c**	**11** (4%)	CH_2Cl_2, 40	20	83	5	2
15	**14c**	**11** (4%)	PhMe, 80	20	87	5	<1
16	**14c**	**11** (2%)	PhMe, 60	20	85	5	<1
17	**14c**	**12** (2%)	PhMe, 60	2	72	15	<1
18	**14c**	**13** (2%)	PhMe, 60	2	69	17	<1
19	**14d**	**11** (7%) + 1 equiv. CuI	CH_2Cl_2, 40	40	50	<2	50
20	**14d**	**12** (10%) + 1 equiv. CuI	CH_2Cl_2, 40	24	93	<2	n.d.
21	**14d**	**12** (5%)	PhMe, 60	1	86	5	<2

[a] Experiment carried out in sealed tube.

control, whereas under the conditions in entry 5 the control is thermodynamic; evidently, the kinetic effective molarity of this reaction under catalysis by **11** is slightly more favorable than the thermodynamic one.

Other substrates perform less effectively than **14a**. Thus **14b**, a very desirable starting material because it eliminates the C-4 protection/deprotection of the hydroxyproline subunit, apparently has a less favorable thermodynamic effective molarity and it is kinetically easier to equilibrate than **14a**. The conformational/energetic difference versus **14a** must be slight, but is enough to cause more dimer formation under all conditions tested: thus, 13% dimers are observed even with the milder catalyst **11**, whereas higher proportions of dimers are obtained with second-generation catalysts (entries 6–9). This led to an extensive solvent screen aimed at utilizing this reaction for large-scale production: of all the solvent tested, THF and EtOAc gave the best yields (77–80%, entries 10–11), but led to very slow RCM rates. Better results were obtained with solvent mixtures: use of THF/PhMe gave the best results, achieving a yield of 88%, with only 6% dimers (entry 13). The difference in effective molarity (both kinetic and thermodynamic) between **14a** and **14b** is very hard to explain, given the small structural difference and the remote nature of the substituent at P2.

Substrate **14c** behaved slightly more favorably than **14b**, and here dimer levels could be kept at 5% (entries 14–18), as long as the first-generation Hoveyda catalyst was used (entries 14–16), whereas dimer levels climbed up to 17% otherwise (entry 18).

Finally, use of substrate **14d**, containing the quinoline moiety at P2, gave markedly reduced RCM rates (entry 19). The addition of one full equivalent of CuI (presumably binding the quinoline nitrogen and freeing more catalyst) was necessary in order to reach good conversion (entry 20). Cuprous iodide was the most active co-catalyst of the many Cu-based species screened [33b]. However, CuI is destructive to the catalyst, and even with highly active **12**, as much as 5–10% mol catalyst is needed, as long as the solvent is dichloromethane. Surprisingly, we found that nonpolar solvents like PhMe and higher temperatures obviate the need for CuI, and high yields can be obtained without it. In this case also, however, rather large catalyst loads are needed (entry 21). Dimer levels can be limited to 5%, and this protocol becomes therefore also relatively attractive. We do not fully understand the origin of the dichotomy between experiments in entries 20 and 21: the effect appears to be both solvent- and temperature-related. Perhaps both factors affect binding of the catalyst to the P2 quinoline moiety, which may be the culprit for the slower reaction rate and the higher catalyst load needed.

Clearly, a variety of subtle factors are at play here, and the most surprising finding in this study was the difference in effective molarity between closely related substrates such as **14a–d**, for which we cannot advance a reasonable explanation. In general, catalysts **12** and **13** can be used in much smaller proportions (TONs up to 200 for **12** versus 20–30 for **11**) and yield faster rates. Unfortunately, they always lead to sizable amounts of dimeric product even at 10 mM substrate concentration, probably a result of partial or total thermodynamic control. The observation that minor structural differences can have a major influence on the effective molarity of the reaction suggests a clear strategy for future optimization of this reaction.

3. Conclusion

In summary, the β-strand macrocyclic core of the antiviral agent BILN 2061 (**3f**) was synthesized from a tripeptide diene in excellent yield using ring-closing metathesis. A side reaction that led to epimerized by-products, and was shown to be catalyst dependent, was eliminated by careful selection of the ligands attached to the Ru center. To the best of our knowledge, this is the first application of a Ru-induced RCM reaction to the large-scale synthesis of a class of molecules that have significant potential value as therapeutic agents [27].

It is well known that the frequency at which novel synthetic methodologies make it out of the academic laboratories and into an industrial manufacturing environment is relatively low, particularly when the chemistry involves potentially hazardous metals, complex intellectual property scenarios, costly reagents/catalysts and reactions requiring very high dilutions. In this report, we have provided a brief account of the studies that led to the first practical application of ring-closing metathesis in the pharmaceutical industry and that allowed the production of BILN 2061 (**3f**) in >100 kg quantities.

With further improvements in catalyst performance and reaction throughput, it is our firm belief that the olefin RCM reaction can be used for the manufacturing of BILN 2061, or closely-related analogs, on full production scale i.e. multiton quantities. Such developments will be described in due course.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2006.09.027.

References

[1] (a) A.G. Cochran, Curr. Opin. Chem. Biol. 5 (2001) 654;
(b) M.W. Peczuh, A.D. Hamilton, Chem. Rev. 100 (2000) 2479;
(c) A.G. Cochran, Chem. Biol. 7 (2000) R85.
[2] E. Weerapana, B. Imperiali, Org. Biomol. Chem. 1 (2003) 93.
[3] A. Loregian, H.S. Marsden, G. Palù, Rev. Med. Virol. 12 (2002) 239.
[4] (a) M. Llinàs-Brunet, M. Bailey, G. Fazal, S. Goulet, T. Halmos, S. LaPlante, R. Maurice, M. Poirier, M.-A. Poupart, D. Thibeault, D. Wernic, D. Lamarre, Bioorg. Med. Chem. Lett. 8 (1998) 1713;
(b) M. Llinàs-Brunet, M. Bailey, R. Déziel, G. Fazal, V. Gorys, S. Goulet, T. Halmos, R. Maurice, M. Poirier, M.-A. Poupart, J. Rancourt, D. Thibeault, D. Wernic, D. Lamarre, Bioorg. Med. Chem. Lett. 8 (1998) 2719;
(c) C. Steinkühler, G. Biasiol, M. Brunetti, A. Urbani, U. Koch, R. Cortese, A. Pessi, R. De Francesco, Biochemistry 37 (1998) 8899;
(d) P. Ingallinella, S. Altamura, E. Bianchi, M. Taliani, R. Ingenito, R. Cortese, R. De Francesco, C. Steinkühler, A. Pessi, Biochemistry 37 (1998) 8906.
[5] (a) M. Llinàs-Brunet, M. Bailey, G. Fazal, E. Ghiro, V. Gorys, S. Goulet, T. Halmos, R. Maurice, M. Poirier, M.-A. Poupart, J. Rancourt, D. Thibeault, D. Wernic, D. Lamarre, Bioorg. Med. Chem. Lett. 10 (2000) 2267;
(b) S.R. LaPlante, D.R. Cameron, N. Aubry, S. Lefebvre, G. Kukolj, R. Maurice, D. Thibeault, D. Lamarre, M. Llinàs-Brunet, J. Biol. Chem. 274 (1999) 18618.
[6] Y.S. Tsantrizos, G. Bolger, P. Bonneau, D.R. Cameron, N. Goudreau, G. Kukolj, S.R. LaPlante, M. Llinàs-Brunet, H. Nar, D. Lamarre, Angew. Chem., Int. Ed. Engl. 42 (2003) 1356.
[7] (a) R.C. Reid, L.K. Pattenden, J.D.A. Tyndall, J.L. Martin, T. Walsh, D.P.J. Fairlie, Med. Chem. 47 (2004) 1641;
(b) P.A. Bartlett, M. Rezac, S. Olson, S. Phillips, PTC International Application [The Regents of the University of California, USA] WO 02/099045, December 2002;
(c) E. Dumez, J.S. Snaith, R.F.W. Jackson, A.B. McElroy, J. Overington, M.J. Wythes, J.M. Withka, T.J. McLellan, J. Org. Chem. 67 (2002) 4882;
(d) R.C. Reid, M.J. Kelso, M.J. Scanion, D.P. Fairlie, J. Am. Chem. Soc. 124 (2002) 5673;
(e) M.P. Glenn, L.K. Pattenden, R.C. Reid, D.P. Tyssen, J.D.A. Tyndall, C.J. Birch, D.P. Fairlie, J. Med. Chem. 45 (2002) 371;
(f) J.S. Nowick, E.M. Smith, J.W. Ziller, A.J. Shaka, Tetrahedron 58 (2002) 727;
(g) A.S. Ripka, K.A. Satyshur, R.S. Bohacek, D.H. Rich, Org. Lett. 3 (2001) 2309;
(h) C.C. Mak, V.-D. Le, Y.-C. Lin, J.H. Elder, C.-H. Wong, Bioorg. Med. Chem. Lett. 11 (2001) 219;
(i) J.D.A. Tyndall, D.P. Fairlie, Curr. Med. Chem. 8 (2001) 893;
(j) D.P. Fairlie, M.L. West, A.K. Wong, Curr. Med. Chem. 5 (1998) 29;
(k) Z.-Q. Tian, B.B. Brown, D.P. Mack, C.A. Hutton, P.A. Bartlett, J. Org. Chem. 62 (1997) 514;
(l) J.S. Nowick, M. Pairish, I.Q. Lee, D.L. Holmes, J.W. Ziller, J. Am. Chem. Soc. 119 (1997) 5413;
(m) A. Boumendjel, J.C. Roberts, E. Hu, P.V. Pallai, J. Org. Chem. 61 (1996) 4434;
(n) A.B. Smith, R. Hirschmann, A. Pasternak, M.C. Guzman, A. Yokoyama, P.A. Sprengeler, P.L. Darke, E.A. Emini, W.A. Schleif, J. Am. Chem. Soc. 117 (1995) 11113;
(o) D.P. Fairlie, G. Abbenante, D.R. March, Curr. Med. Chem. 2 (1995) 654;
(p) S.F. Martin, C.J. Oalmann, S. Linas, Tetrahedron 49 (1993) 3532.
[8] (a) Recent reviews on ROM and RCM: R.H. Grubbs, Tetrahedron 60 (2004) 7117;
(b) R.H. Grubbs (Ed.), Handbook of Metathesis, vols. 1–3, Wiley-VCH, 2003;
(c) R.R. Schrock, A.H. Hoveyda, Angew. Chem. Int. Ed. 42 (2003) 4592;
(d) T.M. Trnka, R.H. Grubbs, Acc. Chem. Res. 34 (2001) 18;
(e) A. Fürstner, Angew. Chem., Int. Ed. Engl. 39 (2000) 3012.
[9] N. Goudreau, C. Brochu, D.R. Cameron, J.-S. Duceppe, A.-M. Faucher, J.-M. Ferland, C. Grand-Ma ıtre, M. Poirier, B. Simoneau, Y.S. Tsantrizos, J. Org. Chem. 69 (2004) 6185.
[10] (a) P.L. Beaulieu, J. Gillard, M.D. Bailey, C. Boucher, J.-S. Duceppe, B. Simoneau, X.-J. Wang, L. Zhang, K. Grozinger, I. Houpis, V.

Farina, H. Heimroth, T. Krueger, J. Schnaubelt, J. Org. Chem. 70 (2005) 5869;
(b) J. Rancourt, D.R. Cameron, V. Gorys, D. Lamarre, M. Poirier, D. Thibeault, M. Llinàs-Brunet, J. Med. Chem. 47 (2004) 2511.

[11] (a) N. Goudreau, D.R. Cameron, P. Bonneau, V. Gorys, C. Plouffe, M. Poirier, D. Lamarre, M. Llinàs-Brunet, J. Med. Chem. 47 (2004) 123;
(b) M.-A. Poupart, D.R. Cameron, C. Chabot, E. Ghiro, N. Goudreau, S. Goulet, M. Poirier, Y.S. Tsantrizos, J. Org. Chem. 66 (2001) 4743.

[12] Labeling of the unnatural amino acid residues within the peptidic scaffold is based on the protease subsite nonmenclature: I. Schechter, A. Berger, Biochem. Biophys. Res. Commun. 27 (1967) 157.

[13] A.-M. Faucher, M.D. Bailey, P.L. Beaulieu, C. Brochu, J.-S. Duceppe, J.-M. Ferland, E. Ghiro, V. Gorys, T. Halmos, S.H. Kawai, M. Poirier, B. Simoneau, Y.S. Tsantrizos, M. Llinàs-Brunet, Org. Lett. 6 (2004) 2901.

[14] M. Poirier, N. Aubry, C. Boucher, J.-M. Ferland, S. LaPlante, Y.S.J. Tsantrizos, J. Org. Chem. 70 (2005) 10765.

[15] (a) M. Weck, B. Mohr, J.-P. Sauvage, R.H. Grubbs, J. Org. Chem. 64 (1999) 5463;
(b) B. Mohr, M. Weck, J.-P. Sauvage, Grubbs, Angew. Chem., Int. Ed. Engl. 36 (1997) 1308;
(c) M.J. Marsella, H.D. Maynard, R.H. Grubbs, Angew. Chem., Int. Ed. Engl. 36 (1997) 1101.

[16] R. Kaul, S. Surprenant, W.D. Lubell, J. Org. Chem. 70 (2005) 3838.

[17] (a) A. Fürstner, T. Dierkes, O.R. Thiel, G. Blanda, Chem. Eur. J. 7 (2001) 5286;
(b) A. Fürstner, O.R. Thiel, G. Blanda, Org. Lett. 2 (2000) 3731.

[18] D. Meng, D.-S. Su, A. Balog, P. Bertinato, E.J. Sorensen, S.J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S.B. Horwitz, J. Am. Chem. Soc. 119 (1997) 2733.

[19] A. Pause, G. Kukolj, M. Bailey, M. Brault, F. Dô, T. Halmos, L. Lagacé, R. Maurice, M. Marquis, G. McKercher, C. Pellerin, L. Pilote, D. Thibeault, D.D. Lamarre, J. Biol. Chem. 278 (2003) 20374.

[20] M. Llinàs-Brunet, M.D. Bailey, E. Ghiro, V. Gorys, T. Halmos, M. Poirier, J. Rancourt, N. Goudeau, J. Med. Chem. 47 (2004) 6585.

[21] (a) R.B. Perni, S.J. Almquist, R.A. Byrn, G. Chandorkar, P.R. Chaturvedi, L.F. Courtneay, C.J. Decker, K. Dinehart, C.A. Gates, S.L. Harbeson, A. Heiser, G. Kalkeri, E. Kolaczkowski, K. Lin, Y.-P. Luong, B.G. Rao, W.P. Taylor, J.A. Thomson, R.D. Tung, Y. Wei, A.D. Kwong, C. Lin, Antimicrob. Agents Chemother. 50 (2006) 899;
(b) K. Lin, R.B. Perni, A.D. Kwong, C. Lin, Antimicrob. Agents Chemother. 50 (2006) 1813.

[22] B.A. Malcolm, R. Liu, F. Lahser, S. Agrawal, B. Belanger, N. Butkiewicz, R. Chase, F. Gheyas, A. Hart, D. Hesk, P. Ingravallo, C. Jiang, R. Kong, J. Lu, J. Pichardo, A. Prongay, A. Skelton, X. Tong, S. Venkatraman, E. Xia, V. Girijavallabhan, F.G. Njoroge, Antimicrob. Agents Chemother. 50 (2006) 1013.

[23] Details on the synthesis of dienes **5b–e** and a typical procedure for kilo-scale RCM reaction can be found in the Supplementary Materials.

[24] M. Llinàs-Brunet, M.D. Bailey, G. Bolger, C. Brochu, A.-M. Faucher, J.-M. Ferland, M. Garneau, E. Ghiro, V. Gorys, C. Grand-Ma ıtre, T. Halmos, N. Lapeyre-Paquette, F. Liard, M. Poirier, M. Rhéaume, Y.S. Tsantrizos, D. Lamarre, J. Med. Chem. 47 (2004) 1605.

[25] (a) H. Hinrichsen, Y. Benhamou, H. Wedemeyer, M. Reiser, R.E. Sentjens, J.L. Calleja, X. Forns, A. Erhardt, J. Crönlein, R.L. Chaves, C.-L. Yong, G. Nehmiz, G.G. Steinmann, Gastroenterology 127 (2004) 1347;
(b) D. Lamarre, P.C. Anderson, M. Bailey, P. Beaulieu, G. Bolger, P. Bonneau, M. Bös, D. Cameron, M. Cartier, M.G. Cordingley, A.-M. Faucher, N. Goudreau, S.H. Kawai, G. Kukolj, L. Lagacé, S.R. LaPlante, H. Narjes, M.-A. Poupart, J. Rancourt, R.E. Sentjens, R. St George, B. Simoneau, G. Steinmann, D. Thibeault, Y.S. Tsantrizos, S.M. Weldon, C.-L. Yong, M. Llinàs-Brunet, Nature 426 (2003) 186;
(c) C.M. Rice, Nature 426 (2003) 129.

[26] Y.S. Tsantrizos, D.R. Cameron, A.-M. Faucher, E. Ghiro, N. Goudreau, T. Halmos, M. Llinàs-Brunet, WO 2000/59929.

[27] (a) S. Venkatraman, V.M. Girijavallabhan, Schering Corporation WO 2005/051980 A1;
(b) L.M. Blatt, S.M. Wenglowsky, S.W. Andrews, Y. Jiang, A.L. Kennedy, K.R. Condroski, J.A. Josey, P.J. Stengel, M.R. Madduru, G.A. Doherty, B.T. Woodard, Intermune, Inc./ Array Biopharma Inc. WO 2005/037214 A2;
(c) M. Llinàs-Brunet, M. Bailey, P. Bhardwaj, P. Forgione, E. Ghiro, N. Goudreau, T. Halmos, J. Rancourt, Boehringer Ingelheim (Canada) Ltd. WO 2005/028501 A1;
(d) S. Nakajima, Y. Sun, D. Tang, G. Xu, B. Porter, Y.S. Or, Z. Wang, Z. Miao, Enanta Pharmaceuticals Inc. WO 2004/093798 A2;
(e) F. McPhee, J.A. Campbell, W. Li, S. D'Andrea, Z.B. Zheng, A.C. Good, D.J. Carini, B.L. Johnson, P.M. Scola, Bristol-Myers Squibb Company WO 2004/094452 A2;
(f) S. Chen, X. Mei, Z. Wang, Boehringer Ingelheim International GMBH. WO 2004/093915 A1;
(g) K. Donsbach, D. Ecker, R.P. Frutos, F. Gallou, D. Gutheil, N. Haddad, R. Hagenkoetter, D. Kemmer, J. Kroeber, T. Nicola, J. Schnaubelt, M. Schul, R.D. Simpson, X. Wei, E. Winter, Y. Xu, N.K. Yee, J. Brandenburg, Boehringer Ingelheim International GMBH. WO 2004/092203 A2;
(h) W. Dersch, W. Samstag, Boehringer Ingelheim International GMBH. WO 2004/089974 A1;
(i) Z. Miao, Y. Sun, F. Wu, S. Nakajima, G. Xu, Z. Wang, Enanta Pharmaceuticals Inc. WO 2004/072243 A2;
(j) M. Llinàs-Brunet, M.D. Bailey, Boehringer Ingelheim (Canada) Ltd. WO 2004/037855 A1;
(k) M. Llinàs-Brunet, V.J. Gorys, Boehringer Ingelheim (Canada) Ltd. WO 2003/064455 A2

[28] X. Zeng, X. Wei, V. Farina, E. Napolitano, Y. Xu, L. Zhang, N. Haddad, N.K. Yee, N. Grinberg, S. Shen, C.H. Senanayake J. Org. Chem., in press.

[29] (a) Z.-Q. Yang, X. Geng, D. Solit, C.A. Pratilas, N. Rosen, S.J. Danishefsky, J. Am. Chem. Soc. 126 (2004) 7881;
(b) K. Yamamoto, K. Biswas, C. Gaul, S. Danishefsky, J. Tetrahedron Lett. 44 (2003) 3297.

[30] A.G.M. Barrett, D. Hamprecht, R.A. James, M. Ohkubo, P.A. Procopiou, M.A. Toledo, A.J.P. White, D.J. Williams, J. Org. Chem. 66 (2001) 2187.

[31] T. Itoh, K. Mitsukura, N. Ishida, K. Uneyama, Org. Lett. 2 (2000) 1431.

[32] J.S. Kingsbury, J.P.A. Harrity, P.J. Bonitatebus, A.H. Hoveyda, J. Am. Chem. Soc. 121 (1999) 791.

[33] (a) M. Scholl, T.M. Trnka, J.P. Morgan, R.H. Grubbs, Tetrahedron Lett. 40 (1999) 2247;
(b) T. Weskamp, F.J. Kohl, W. Hieringer, D. Gleich, W.A. Herrmann, Angew. Chem., Int. Ed. Engl. 38 (1999) 2416;
(c) J. Huang, E.D. Stevens, S.P. Nolan, J.L. Petersen, J. Am. Chem. Soc. 121 (1999) 2674;
(d) S.B. Garber, J.S. Kingsbury, B.L. Gray, A.H. Hoveyda, J. Am. Chem. Soc. 122 (2000) 8168.

[34] G. Illuminati, L. Mandolini, Acc. Chem. Res. 14 (1981) 95.

[35] T. Nicola, M. Brenner, K. Donsbach, P. Kreye, Org. Process Res. Dev. 9 (2005) 513.

[36] (a) N.K. Yee, V. Farina, I. Houpis, N. Haddad, R.P. Frutos, F. Gallou, X.-j. Wang, X. Wei, R.D. Simpson, X. Feng, V. Fuchs, Y. Xu, J. Tan, L. Zhang, J. Xu, L.S. Smith-Keenan, J. Vitous, M.D. Ridges, E.M. Spinelli, M. Johnson, K. Donsbach, T. Nicola, M. Brenner, E. Winter, P. Kreye, W.J. Samstag, J. Org. Chem. 71 (2006) 7133;
(b) N. Haddad, X.-j. Wang, X. Wei, L. Zhang, J. Xu, V. Farina (in preparation).