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RCM of Tripeptide Dienes Containing a Chiral Vinylcyclopropane Moiety: Impact of Different Ru-Based Catalysts on the Stereochemical Integrity of the Macrocyclic Products

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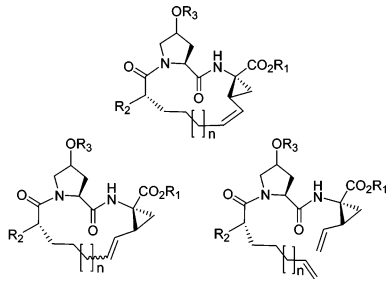

Tripeptide dienes containing an (1*R*,2*S*)-vinyl aminocyclopropylcarboxylate residue were cyclized to β-strand scaffolds under ring-closing metathesis (RCM). Conformational factors, ligand effects, and reaction conditions were evaluated. A protocol was developed for the efficient synthesis of 15-membered ring peptides in high diastereomeric purity. These peptides are key synthetic precursors to antiviral agents that target the hepatitis C virus and represent the first class of clinically validated pharmaceutical agents that are synthesized in large scale using RCM.

Introduction

In recent years, ring-opening/ring-closing metathesis (ROM/RCM) reactions have had an enormous impact in synthetic organic chemistry.[1] Although the metal-catalyzed redistribution of carbon−carbon double bonds has been under investigation for nearly half a century,[2] it is the recent development of very stable organometallic reagents that has revolutionized this field. Some of the most impressive examples of RCM reactions include the formation of polyfunctional, large heterocyclic rings that are often challenging or impossible to prepare efficiently by any other synthetic method. Peptidic scaffolds, such as those typified by the antiviral agent BILN 2061 (**1**),[3,4] a clinically validated inhibitors of the hepatitis C virus (HCV),[3,5] can be prepared efficiently using ruthenium-based catalysts.[6] Key requirements for the antiviral potency of these compounds are the structure and stereochemistry of the rigid macrocyclic peptide that is composed of three unnatural amino acid residues (P1, P2, P3)[7] and designed to mimic the β-strand conformation

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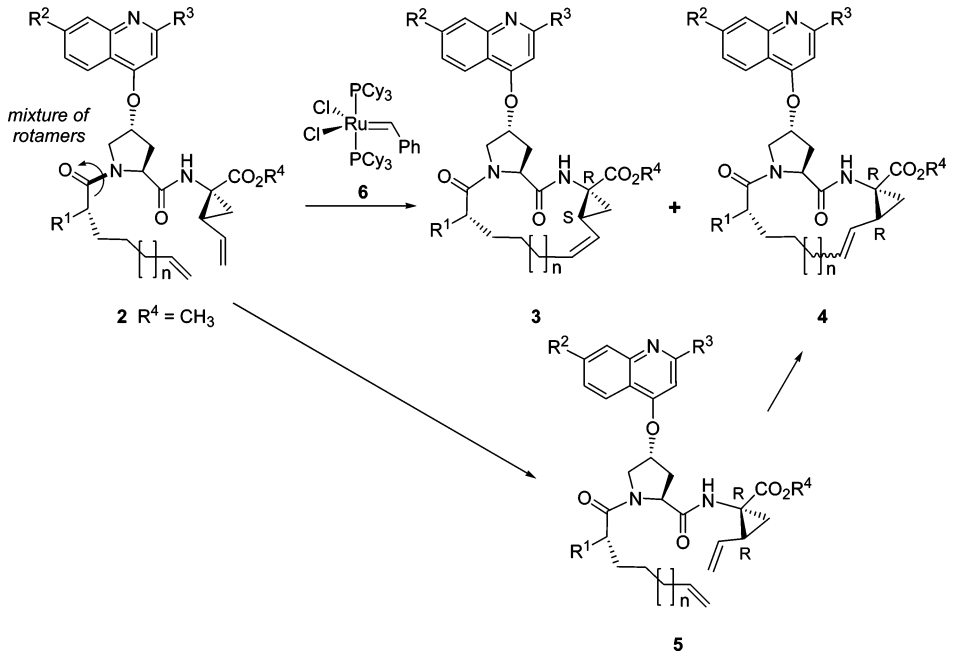
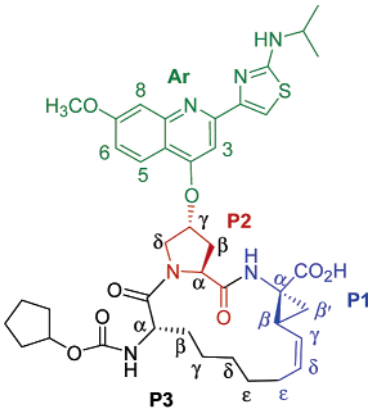
TABLE 1. Ring Side and Conformational Effects of the Acyclic Diene on the RCM Products[a]



entry	2	n	R1	R2	R3	3[b,c]	4[b,c] (E/Z)	5[b,c]	total RCM conversion (%)
1	**a**	3	H	H	H	<10[d]	<15[d]	BDL	<30
2	**b**	3	NHBoc	H	H	40	ND	BDL	~60
3	**c**	3	NHBoc	OCH_3	Ph	80	<5[e]	BDL	~85
4	**d**	2	NHBoc	OCH_3	Ph	BDL	45:0	<5	<50
5	**e**	1	NHBoc	OCH_3	Ph	BDL	41:0	21	<45

[a] All reactions were run at a 10 μM concentration of the diene peptide methyl ester ($R^4 = CH_3$) **2a**−**e**, 30% molar equiv of catalyst **6** in CH_2Cl_2 at reflux for 3 h. [b] Isolated yields after flash column chromatography. [c] Final confirmation of structure and stereochemistry was carried out on the corresponding carboxylic acid of each analogue ($R^4 = H$). [d] Two macrocyclic products were isolated from the RCM reaction of diene **2a**. One product was unambiguously assigned the structure of *E*-**4a**. The NMR data of the second product clearly suggested a *Z* olefinic moiety; however, due to overlapping signals and rotamer effects, the stereochemistry of the vinyl ACCA moiety could not be anambiguously assigned (i.e., the second product may have the structure of **3a** or that of *Z*-**4a**). Below detection limit (BDL) indicated cases where the amount of a product was too small to be isolated and fully characterized. [e] Inseparable mixture characterized by NMR and LC−MS.

of the target enzyme's natural substrates (i.e., the NS3 cleavage sites of the HCV nonstructural polyprotein).[8]



BILN 2061 (**1**)

The (1*R*,2*S*)-vinylaminocyclopropylcarboxylic acid (ACCA) moiety (P1) of antiviral agents such as BILN 2061 (**1**) plays a critical role in the conformation of the macrocyclic scaffold and, consequently, the affinity of these compounds for their intended biological target.[4,5,6a,9] Therefore, maintaining absolute stereochemical fidelity during the RCM conversion of an acyclic diene (e.g., **2** or **9**) to the desired macrocyclic product (e.g., **3** or **10**) was critical to our drug discovery efforts. In this report, our initial evaluation of the factors that modulated the outcome of the RCM reaction is described and includes (a) the backbone conformation of the acyclic diene, (b) catalyst effects, and (c) reaction conditions.

Results and Discussion

RCM reactions of tripeptide dienes were initially investigated using the first-generation Grubbs' catalyst, bis(tricyclohexylphosphine)benzylideneruthenium dichloride (**6**),[10] to synthesize a variety of 13- to 15-membered macrocyclic scaffolds (Table 1). The backbone conformation of each acyclic tripeptide, and in particular the ratio of the proline *cis*/*trans* rotamers, was found to be crucial

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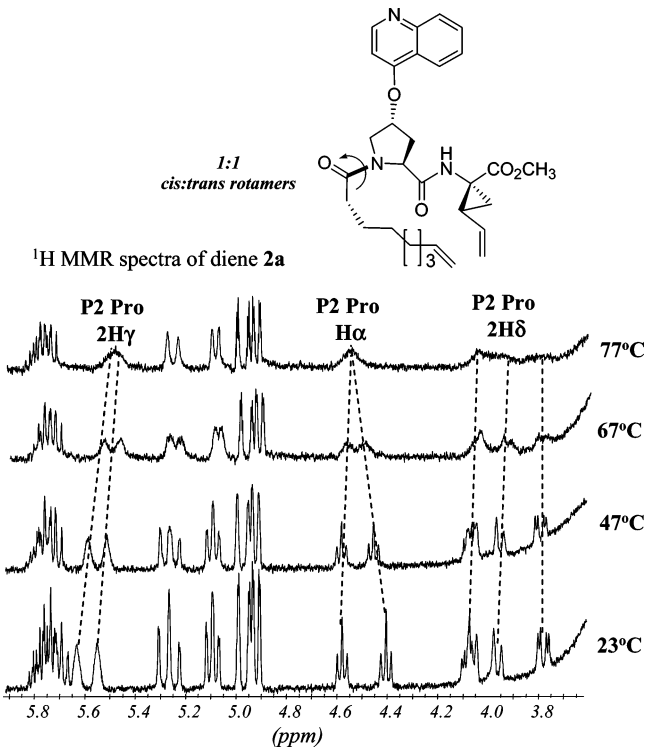
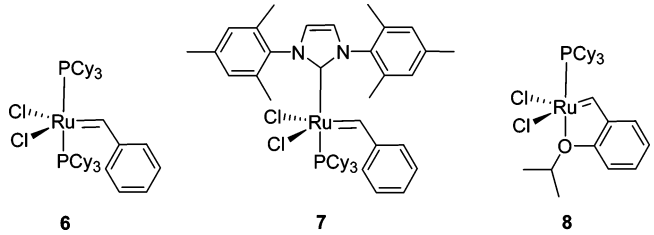


FIGURE 1. Subregion of the ^{1}H NMR spectra of diene **2a** at various temperatures.

to the reaction rate, yield, and diastereomeric purity of the macrocyclic product(s). For example, it was noted that when the Cα of the olefinic carboxylic acid "linker" moiety (P3) that is attached to the proline moiety (P2) was unsubstituted, the proline *cis/trans* rotamer ratio was approximately 1:1 and the outcome of the RCM reaction was very poor (Table 1, entry 1, R^1 = H). In contrast, when a bulky −NHBoc group was attached to the Cα of that olefinic linker (Table 1, entry 2) the *cis/trans* ratio was ∼1:9 and the overall conversion of the diene to macrocyclic products was significantly better (Table 1, entry 2, R^1 = −NHBoc, ∼60% yield). Variable-temperature ^{1}H NMR spectroscopy revealed that coalescence of resonances could not be achieved at the temperatures typically used for RCM reactions; the observed data was consistent with a tertiary amide rotational barrier of >13 kcal/mol (Figure 1). The lack of a conformational preference for the *cis* or *trans* rotamer of the peptide backbone in **2a** was presumed to be responsible for the poor outcome of the attempted macrocyclization reaction; RCM of **2a** produced only small amounts of **3a/4a** as mixture of *E/Z* olefins (Table 1, entry 1). In contrast, the NMR data of **2b** clearly suggested that the bulky −NHBoc capping group at the Cα of the P3 moiety preorganized the peptidic backbone in the same β-strand conformation as that of the desired macrocyclic product **3** (Table 1), thus facilitating the RCM reaction. RCM of diene **2b** gave **3b** as the major compound (40% isolated yield), in addition to some *E/Z*-**4b** macrocyclic olefins (Table 1, entry 2). These observations are analogous to those initially reported by Grubbs and co-workers on template-directed RCM reactions[11] and similar to those reported by Lubell's group for the RCM macrocyclization of dipeptides having secondary or tertiary amide bonds.[12] Remote control effects on the outcome of the RCM reaction were also observed in the synthesis of macrocyclic natural products, such as salicylihalamide[13] and epothilones.[14]



Ring size was another critical factor governing the outcome of macrocyclization (Table 1). Rigid dienes having a C_9 P3 linker attached to the proline were efficiently cyclized to the desired 15-membered ring peptide. A typical example was the conversion of tripeptide **2c** to the macrocyclic product **3c** in approximately 80% isolated yield (Table 1: entry 3). In this reaction, only minor amounts of side products, corresponding to epimerized macrocyclic peptides, were detected by LC−MS (Table 1, entry 3).[15] In contrast, formation of the energetically more strained 14- and 13-membered ring systems from dienes **2d** and **2e** (Table 1, entries 4 and 5, respectively), failed to produce macrocyclic compounds with the desired stereochemistry (Table 1, entries 4 and 5). In both of the latter reactions (Table 1, entries 4 and 5), extensive epimerization at the Cβ of the vinyl ACCA moiety (P1) was observed resulting in *E/Z* mixtures of **4**. Surprisingly, in the case of the shortest P3 moiety, diene **2e** (Table 1, entry 5), a significant amount of epimerized acyclic peptide was also formed (diene **5e**), clearly suggesting that (at least in this case) the competing epimerization reaction preceded the RCM reaction and was more predominant when RCM was energetically disfavored and/or the catalyst had increased accessibility of the P1 vinyl moiety, leading to stereomutation of the vinyl ACCA unit. It should be noted that in each of these reactions, the stereochemistry of the vinyl ACCA moiety was confirmed from the ROESY NMR data of the isolated products (Figure 2).[16] Characteristic chemical shift differences between products of general structures **3** and **4** (or **5**) were easily identified, including a strong correlation between the P1 amide NH and Hβ of the cyclopropyl ring in the ROESY NMR spectrum of **3**, which was completely absent from the ROESY spectrum of products **4** and **5**

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(15) The minor amounts of side products formed in this reaction (Table 1: entry 3) were analyzed by LC/MS. The MS data suggested the formation of dimers and some macrocyclic products having the same mass as **3c** but different retention times on chiral HPLC (i.e., *E/Z* mixtures of **4c**).

(16) Refer to the Supporting Information for experimental details and representative NMR spectra.

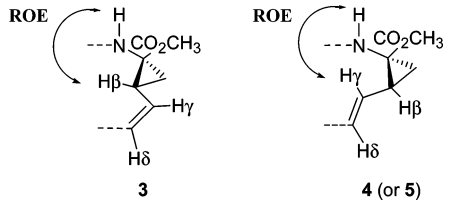

FIGURE 2. Characteristic ROE differences observed in the ROESY NMR spectra of compounds containing the (1*R*,2*S*)- or the (1*R*,2*R*)-vinyl ACCA moiety (i.e., general structure **3**, **4**, or **5**).

(Figure 2).[16] In contrast, the ROESY spectrum of **4** and **5** contained strong ROESY correlations between the P1 amide NH and the olefinic Hγ/Hδ, which were absent in the ROESY spectrum of **3** (Figure 2).[16] In the more strained 13- and 14-membered ring systems, only the epimerized *E* olefin macrocyclic product **4** was observed, suggesting that the *Z* olefin was energetically disfavored.

The reaction pathway leading to the epimerized products **4** and **5** is unprecedented in the literature. It should be noted that RCM reactions involving vinylcyclopropanes have been previously reported in the synthesis of radicicol-type macrolides,[17] coronanes,[18] and oligo-*gem*-difluorocyclopropanes[19] without any evidence of stereomutaion. A Michael-type attack on the vinyl ACCA by the metal-dissociated PCy_3 ligand was ruled out, since epimerization was only observed at Cβ of the vinyl ACCA moiety (P1), whereas the stereochemistry of the Cα was unaffected under the RCM conditions. Furthermore, exposure of diene **2** to PCy_3, under strict anaerobic and anhydrous conditions in refluxing CH_2Cl_2 over a period of 24 h in the absence of any Ru-based catalyst, failed to give any epimerized products.

The enantiomers of vinyl ACCA, (1*R*,2*S*)-ACCA and (1*S*,2*R*)-ACCA, were isolated in high enantiomeric purity[20] and were used independently in the synthesis of model tripeptides missing the P3 olefinic moiety. These compounds were independently used to study the epimerization phenomenon under RCM conditions and the products formed (i.e., all four diasteromers of the vinyl ACCA moiety in mixtures of two for each reaction) were clearly distinguishable by their retention time on chiral HPLC, as well as their ^{1}H and ROESY NMR spectra. In each case, the presence of only two compounds was observed, one corresponding to the starting material and the second to its epimer at Cβ of the cyclopropyl ring. The results obtained from these model reactions were consistent with the observations made with dienes **2a**−**e** and confirmed that the mechanism of epimerization (a) does not affect the Cα of the vinyl ACCA moiety and involves a yet unclear interaction between the Ru-based catalyst and the P1 olefinic moiety.

A number of plausible mechanisms were considered that could involve a π-allyl-type shift of ruthenium-hydride (formed as decomposition byproduct of the catalyst),[21] an intermediate metal carbene or ruthenacyclopentene (formed via a metal-mediated expansion of the strained cyclopropyl ring),[22] or even an electron-transfer oxidation mechanism.[23] However, the studies required in order to gain support for, or against, any one of these mechanisms were beyond the scope of the current investigation.[24] Our main goal was to optimize the RCM reaction in order to provide high yields of the 15-membered ring scaffold with the desired (2R,3S) stereochemistry of the vinyl ACCA moiety. To this end, we embarked on a study of the effects of the Ru-based catalyst and the reaction conditions.

Mindful of numerous advances in catalyst design, as well as mechanistic investigations that shed light on the role of ligands in modulating the stability and reactivity of the ruthenium catalysts,[21,25] two distinctly different catalysts, **7** and **8**, in addition to **6**, were compared in the RCM reaction of diene **9** (Table 2). The "naked" tripeptide backbone **9** was selected as a model compound since its macrocyclic product **10** is a key synthetic precursor for numerous HCV NS3 protease inhibitors typified by the antiviral agent BILN 2061 (**1**)[4,26] and for closely related macrocyclic aza-peptides.[27] Diene **9** was also free of conformational effects imposed by a quinoline/heteroaryl substituent commonly found in the bioactive compounds and attached to the pyrrolidine ring of the P2 moiety. However, it should be noted that even the free hydroxyl group of diene **9** imposed complex steric, electronic and conformational effects on the backbone of the tripeptide that are difficult to evaluate in the context of the RCM reaction.[28]

RCM of diene **9** under the same reaction conditions as those used for dienes **2** (Table 1) gave excellent overall conversion to macrocyclic products. The desired 15-membered scaffold **10** was isolated as the major product in ~80% yield (Figure 3a). However, the amount of

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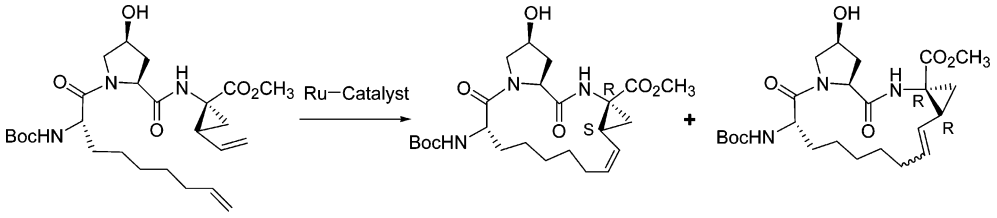
TABLE 2. Catalyst Effect on the RCM Reaction of Diene 9[a]



9 **10** **11**

entry	catalyst (mol %)	solvent	T (°C)	t (h)	**10**[b] (%)	**11** (% *E*:*Z*)[b]	conversion[c] (%)
1	**6** (5)	CH_2Cl_2	40	2.5	83	12.5	>99
2	**6** (10)	THF	50	4.0	36	13:8	~60
3	**6** (10)	THF	23	24	38	7:4	~50
4	**6** (10)	PhMe	50	4.0	56	25:11	~90
5	**6** (10)	PhMe	23	24	55	12:5	~70
6	**7** (2)[d]	CH_2Cl_2	40	20	52	BDL	<60
7	**8** (5)	CH_2Cl_2	40	20	85	BDL	85

[a] Reactions were run at a 10 μM concentration of the diene peptide under standard RCM conditions. [b] Ratios were determined by HPLC at $\lambda = 205$ nm using a chiral column. [c] Yields were determined by HPLC at $\lambda = 220$ nm using a C_{18} reversed-phase column. [d] RCM of similar dienes to **9** in PhMe at 60 °C, when catalyzed by **7**, usually resulted in the formation of side products before completion of the reaction. Below detection limit (BDL).

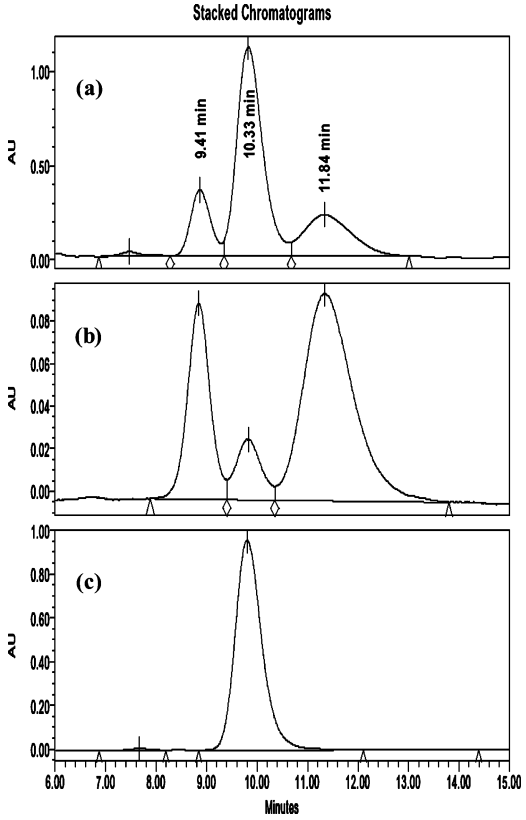


FIGURE 3. Chiral HPLC chromatograms of macrocyclic reaction products formed from the RCM of diene **9** (Table 2): (a) mixture of macrocyclic products formed from the RCM conversion of diene **9** catalyzed by Grubbs' first-generation catalyst **6** (Table 2, entry 1); (b) enriched mixture of epimerized macrocyclic products *E*/*Z*-**11** (Table 2, entries 1 to 5); (c) macrocyclic product **10** formed from the RCM conversion of diene **9** catalyzed by Hoveyda's first-genration catalyst **8** (Table 2, entry 7).

epimerized products formed (i.e., *E*:*Z*-**11** in ratio of ~2:1) was unacceptable for a multikilo production scale of the desired compound **10** in high purity (Table 1, entry 1). Changes in the solvent and temperature of this reaction proved to be futile in eliminating the epimerization pathway (Table 2, entries 2−5). For example, RCM of **9** in THF or toluene at 50 °C resulted in lower yields of **10** and larger amounts of epimerized products **11** as compared to those observed in CH_2Cl_2 at 40 °C (Table 1, entries 2−5). Progress of the reaction in either THF or toluene at rt was slower and resulted in only modest improvement in the ratio of **10**/**11**.

RCM of diene **9** was subsequently investigated using catalysts **7** and **8**, having distinctly different ligands from catalyst **6**. The replacement of one phosphine ligand with the more bulky and strongly electron-donating *N*-heterocyclic carbene 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylide (IMes)[29] is known to result in a significantly more active catalyst than catalyst **6**.[30] Grubbs and co-workers demonstrated that although the initiation step (i.e., dissociation of the PCy_3 ligand to form the initial 14-electron intermediate) was slower for catalyst **7**, as compared to **6**, the RCM propagation step was consider-

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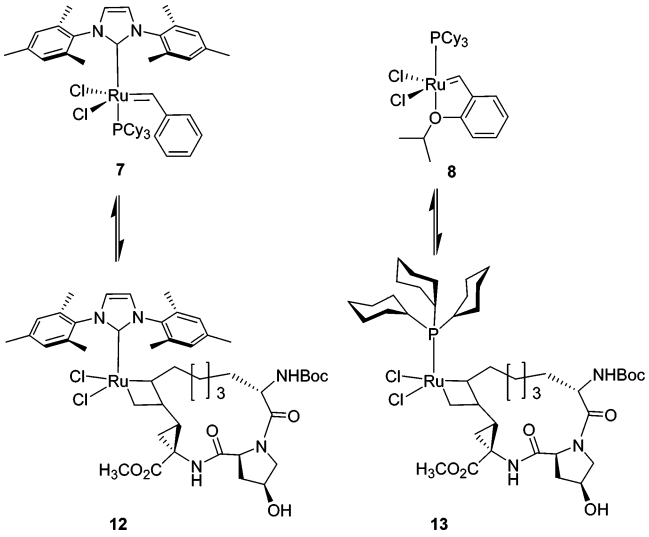
SCHEME 1. RCM-Induced Macrocyclization of Diene 9 to 10: Possible Transition States with Different Catalysts



ably faster.[25e,g] The RCM conversion of diene **9** to the macrocyclic product **10**, in CH_2Cl_2, proceeded cleanly with catalyst **7**, without any noticeable amount of epimerized products. However, the yield of the reaction was much lower with catalyst **7** than with catalyst **6**; even after a 20 h period, compound **10** was isolated in only 50% yield and ~15% of the unreacted diene **9** could be recovered (Table 2, entry 6). Progress of the RCM reaction of **9** (under the same reaction conditions) catalyzed by Hoveyda's first-generation catalyst **8**[25l,m] was slower than the equivalent reaction catalyzed by **6**. However, the desired 15-membered ring scaffold **10** was obtained in 85% isolated yield (Table 2, entry 7) and in excellent purity after a simple filtration through a pad of silica gel. More importantly, analysis of the isolated compound **10** by chiral HPLC confirmed a >99% homogeneity and the absence of any epimerized side products (Figure 3c).

As proposed initially by Hérisson and Chauvin[2a] and later by Chen's group,[31] a key step in the RCM reaction involves formation of the 14-electron metallacyclobutane transition state or intermediate (Scheme 1; **12** and **13**). Recently, Romero and Piers provided convincing evidence for the formation of a 14-electron metallacyclobutane intermediate from Ru(IV) species that are stabilized by a strongly σ-donating *N*-heterocyclic carbene ligand.[32] Some of the key electronic and steric properties of *N*-heterocyclic carbenes, such as the IMes ligand, were initially explored and compared to those of PCy_3 by Nolan's group.[30a] Although the Tolman cone angle of PCy_3 (170°)[33] could not be directly compared to the "fence-like" steric bulk of the IMes group, Nolan and co-workers provided crystallographic evidence that congestion around the metal center was greater with the IMes ligand than with PCy_3.[30a] The steric demands of the IMes ligand and its significantly shorter Ru−C bond distance (bringing the IMes ligand closer to the metal center), suggested that the metallacyclobutane intermediate **12** may be less favorable than the transition state **13**. The steric differences between the IMes and the PCy_3 ligand, in combination with those of the rigid vinyl ACCA moiety, may partly account for the differences observed in the yields of the corresponding RCM reactions (i.e., Table 2, entry 6 vs entry 7). The significant differences in electronic effects between the IMes and the PCy_3 ligand may also contribute in some yet unclear way. Furthermore, it is plausible that the amide and hydroxyl moieties decorating diene **9** could potentially have a coordinating effect toward the metal that would further complicate the mechanism of these reactions.[13a] The differences observed between catalysts **6** and **8** (when presumably they both proceed through the same metallacyclobutane transition state **13**) are more difficult to explain; further studies would be required in order to shed light into the mechanistic differences. Nonetheless, this study provides another example of the profound influence that a complex substrate can have on the mechanism of an RCM-induced macrocyclization reaction.[24a,34]

Conclusion

In summary, the β-strand macrocyclic core of the antiviral agent BILN 2061 (**1**) was synthesized from a tripeptide diene in excellent yield using ring-closing metathesis. A side reaction that led to epimerized byproducts, and appeared 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.[35]

Experimental Section

General Protocol for Synthesis and Purification. The synthesis of acyclic tripeptide dienes (such as compounds **2a**−**e** and **9**) and their conversion to macrocyclic compounds was previously reported for structurally very similar compounds.[6a,16] To increase throughput and avoid multiple purification steps for each key analogue, compounds from this study which were also intended for use in our structure−activity relationship (SAR) studies (i.e., evaluation in the HCV NS3 protease drug design program) were first saponified to the corresponding free carboxylic acids and purified by semipreparative C_{18} reversed-phase HPLC (usually to >95% homogeneity) before they were fully characterized by NMR and MS (i.e., unambiguous structural confirmation from 1D and 2D high-field NMR and HRMS data). Examples from the SAR studies that were processed in this way include a few of the acyclic dienes **2** and their corresponding macrocyclic products **3** and **4**.

NMR Assignments. ^{1}H NMR spectra of all synthetic intermediates and products were obtained at 27 °C and chemical shifts are referenced to the internal deuterated

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solvent. Two-dimensional (2D) double-quantum-filtered COSY (DQF-COSY) and ROESY spectra were acquired using standard pulse sequences (more details are available in the Supporting Information).

Carboxylic Acid Analogue of Diene 2a. HPLC purification of the corresponding carboxylic acid of ester **2a** gave an amorphous white solid in >99% homogeneity (based on analytical HPLC method A; t_R = 19.0 min): ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers in 1:1 ratio) δ ppm 1.12−2.75 (m, 15H), 3.73−4.13 (4m, 2H), 4.34−4.65 (2dd, J = ∼8.0 Hz, 1H), 4.88−5.00 (m, 2H), 5.06−5.12 (m, 1H), 5.22−5.33 (m, 1H), 5.52−5.65 (2m, 1H), 5.66−5.83 (m, 2H), 7.42−7.49 (m, 1H), 7.77 (dd, J = 8.1 Hz, 1H), 8.00 (dd, J = 7.2 Hz, 1H), 8.09 (d, J = 8.6 Hz, 1H), 8.24 (dd, J = 8.7 Hz, 1H), 8.55 (s, 1H), 8.93 (s, 1H), 9.04−9.06 (m, 1H); FAB HRMS *m/z* found 506.265610 (M + H)$^+$, calcd for $C_{29}H_{36}N_3O_5$ 506.265497.

Carboxylic Acid Analogues of Macrocyclic Carboxylic Esters 3a and 4a. Two macrocyclic products were isolated as a mixture from the RCM reaction of diene **2a**. Saponification of the product mixture under basic conditions, followed by semipreparative HPLC purification, allowed reasonable separation of the two corresponding carboxylic acids as amorphous white solids and in good purity 87% and 83% homogeneity (based on analytica HPLC; t_R = 16.2 and 16.9 min, compounds **3a** and **4a**, respectively).

Compound at t_R = 16.2 min. Based on the observed coupling constant for the olefinic protons, this compound was assigned a *cis* double bond (J = 10.5 Hz). However, due to extensive overlap of rotamer signals, the stereochemistry of the vinyl ACCA Cβ residue could not be unambiguously assigned. Although the structure of **3a** was assigned to this compound, the NMR and HRMS data could also support a *Z*-**4a** structure: ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers) δ ppm (major rotamer) 0.84−2.26 (m, 14 H), 2.25−2.94 (m, 3H), 3.72−4.09 (2m, 2H), 4.49−4.82 (m, 1H), 4.91 (bs, 1 H), 5.35−5.49 (m, 1H), 5.52 (bs, 1H), 7.32−7.46 (m, 1H), 7.72 (dd, J = 7.8 Hz, 1H), 7.75−7.85 (bs, 1H), 7.94 (dd, J = 7.6 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 8.99 (d, J = 6.0 Hz, 1H); HRMS *m/z* found 478.234030 (M + H)$^+$, calcd for $C_{27}H_{32}N_3O_5$ 478.234197. ^{1}H NMR decoupling experiments, via selective irradiation of the methylene protons adjacent to the olefin (P1Hϵ), as well as the Hβ of vinyl ACCA (i.e., selective irradiation at ∼2.1 ppm), resulted in $J_{H\gamma-H\delta}$ = 10.5 Hz between the two olefinic protons, confirming a cis geometry of the double bond.

Compound *E*-4a (t_R = 16.9 min): ^{1}H NMR (400 MHz, DMSO-d_6) mixture of rotamers in ∼2:1 ratio) δ ppm (major rotamer) 0.95−2.3 (m, 14H), 2.40−2.86 (m, 3H), 3.72−3.77 (dd, J = 4.1 & 13.7 Hz, 1H), 4.07 (d, J = 13.4 Hz, 1H), 4.59−4.63 (dd, J = 7.8 Hz, 1H), 5.03−5.09 (dd, J = 8.1, 15.6 Hz, 1H), 5.53−5.69 (m, 2H), 7.52 (d, J = 3.5 Hz, 1H), 7.81−7.85 (dd, J = 7.0 Hz, 1H), 8.03−8.07 (dd, J = 7.5 Hz, 1H), 8.11 (d, J = 8.6 Hz 1H), 8.27−8.32 (dd, J = 8.6, 12.7 Hz, 1H), 8.63 (s, 1H), 9.11 (d, J = 5.2 Hz, 1H); HRMS *m/z* found 478.234030 (M + H)$^+$, calcd for $C_{27}H_{32}N_3O_5$ 478.234197. ^{1}H NMR homo-decoupling experiments, via selective irradiation of the methylene protons adjacent to the olefin (i.e. P1Hϵ at ∼2.1 ppm), resulted in $J_{H\gamma-H\delta}$ = 15.6 Hz between the two olefinic protons, confirming the trans geometry of the double bond. Furthermore, the ROESY correlation between the vinyl ACCA amide NH proton and the olefinic Hγ clearly suggested epimerization at Cβ of the vinyl ACCA moiety placing the olefinic linker syn to the amide bond.

Carboxylic acid derivative of diene 2b: homogeneity >99.5% based on C_{18} reversed-phase analytical HPLC (t_R = 20.25 min); ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers in ∼9:1 ratio) δ ppm (major rotamer) 1.09−1.55 (m, 10H), 1.16 (s, 9H), 1.56−1.65 (m, 1H), 1.98−2.05 (m, 2H), 2.28−2.34 (m, 1H), 2.58−2.68 (m, 1H), 4.02−4.14 (m, 2H), 4.40−4.49 (m, 2H), 4.89−5.27 (m, 4H), 5.66−5.82 (m, 3H), 7.11 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 6.4 Hz, 1H), 7.72−7.76 (dd, J = 7.6 Hz, 1H), 8.01−8.05 (dd, J = 7.6 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 8.35 (d, J = 8.3 Hz, 1H), 8.53 (s, 1H), 9.11 (d, J = 6.2 Hz, 1H); HRMS *m/z* found 621.328600 (M + H)$^+$, calcd for $C_{34}H_{45}N_4O_7$ 621.328825.

Carboxylic acid derivative of macrocyclic peptide 3b: homogeneity of 94% based on C_{18} reversed-phase analytical HPLC (t_R = 16.14 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.05 (s, 9H), 1.13−1.53 (m, 9H), 1.65−1.75 (m, 2H), 2.10−2.17 (m, 1H), 2.40−2.46 (m, 1H), 2.57−2.63 (m, 2H), 3.89 (d, J = 8.6 Hz, 1H), 3.99−3.96 (m, 1H), 4.49−4.54 (dd, J = 8.0, 9.5 Hz, 1H), 4.71 (d, J = 11.8 Hz, 1H), 5.24−5.29 (dd, J = 9.9 Hz, 1H), 5.49−5.54 (m, 1H), 5.76 (bs, 1H), 7.11 (d, J = 6.0 Hz, 1H), 7.61 (d, J = 6.4 Hz, 1H), 7.74−7.79 (dd, J = 7.3, 8.0 Hz, 1H), 8.04−8.08 (dd, J = 7.3, 8.0 Hz, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.66 (s, 1H), 9.19 (d, J = 6.4 Hz, 1H); ^{13}C NMR (400 MHz, DMSO) δ ppm 21.5, 23.9, 25.8, 26.8, 27.2, 27.6, 29.8, 31.7, 33.7, ∼39.5 (Cα of vinyl ACCA under DMSO), 52.1, 52.3, 58.1, 77.8, 80.3, 103.4, 120.7, 122.9, 127.3, 128.3, 132.4, 134.3, 139.5, 146.9, 155.2, 166.0, 170.3, 171.8, 172.0, 172.6; HRMS *m/z* found 593.297540 (M + H)$^+$, calcd for $C_{32}H_{41}N_4O_7$ 593.297525. ^{1}H NMR homo-decoupling experiments, via selective irradiation of the vinyl ACCA Hβ proton (i.e., P1Hβ adjacent to the olefin at δ 2.10−2.20 ppm), resulted in $J_{H\gamma-H\delta}$ = 10.4 Hz between the two olefinic protons, confirming the cis geometry of the double bond. Furthermore, a strong ROESY correlation between the same Hβ proton of the amide NH of the vinyl ACCA moiety was consistent with the desired stereochemistry of the vinyl ACCA moiety having the olefinic linker syn to the carboxylic acid.

Carboxylic Acid Derivatives of Diene 2c and Macrocyclic Product 3c. The synthesis and full characterization of these two compounds was recently reported.[6a] Since **3c** is a key compound in this series of HCV NS3 protease inhibitors, its ^{1}H NMR and ROESY spectra are assigned and available in the Supporting Information, along with important and characteristic NMR ROESY data for this class of compounds. The ^{13}C NMR data was not previously reported: ^{13}C NMR (400 MHz, DMSO) δ ppm 21.4, 24.0, 25.9, 26.9, 27.3 (due to extensive overlap of resonances in the 0.9−1.3 ppm region of the ^{1}H NMR spectrum, some ^{13}C resonances could not be unabmiguously assigned), 27.8 [Boc, −C(***C***H$_3$)$_3$], 29.8 (P1, Cβ), 31.8 (P3, Cβ), 33.9 (P2, Cβ), ∼40 (under DMSO, P1, Cα), 52.0 (P2, Cδ), 52.6 (Ar, −OCH$_3$), 56.0 (P3, Cα), 58.2 (P2, Cα), 77.8 (Boc, −***C***(CH$_3$)$_3$, 79.2 (P2, Cγ), 100.4 (Ar, C-3 and C-8), 114.6 (Ar, C-4′), 119.2 (Ar, C-6), 124.2 (Ar, C-5), 127.3 (P1, Cγ), 128.6 and 129.1 (Ar, C-10, C-11, C-12), 131.5 (Ar, C-9), 132.4 (P1, Cδ), 155.2 (Ar, C-8′), 156.6 (Boc, −CO-), 157.8 (Ar, C-2), 158.1 (Ar, C-4), 162.9 (Ar, C-7), 171.9 (P3, −CO-), 172.0 (P1, −CO$_2$H), 172.8 (P2, −CO−).

Diene tripeptide 2d: homogeneity >95% based on silica gel TLC (3:2 EtOAc/Hexane) R_f = 0.47 and ^{1}H NMR; ^{1}H NMR (400 MHz, CDCl$_3$, mixture of rotamers in ∼9:1 ratio) δ ppm (major rotamer) 1.41 (s, 9H), 1.33−2.09 (m, 9H), 2.11−2.19 (m, 1H), 2.36−2.48 (m, 1H) 2.95−3.03 (m, 1H), 3.67 (s, 3H), 3.92−3.99 (m, 1H), 3.96 (s, 3H), 4.27−4.32 (bd, J = 10.8 Hz, 1H), 4.42−4.50 (m, 1H), 4.82−5.05 (m, 3H), 5.09−5.16 (m, 2H), 5.26−5.34 (m, 1H), 5.44 (bs, 1H), 5.65−5.81 (m, 2H), 7.06 (s, 1H), 7.08−7.11 (dd, J = 2.6 & 8.9 Hz, 1H), 7.42−7.56 (m, 4H), 7.62 (bs, 1H), 8.00−8.09 (m, 4H); ES$^+$ MS *m/z* 727.6 (M + H)$^+$.

Carboxylic acid derivative of macrocyclic peptide *E*-4d: homogeneity of 99.8% based on C_{18} reversed phase analytical HPLC (t_R = 18.55 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.10 (s, 9H), 1.02−1.38 (m, 6H), 1.55−1.58 (m, 1H), 1.73−1.83 (m, 1H), 1.85−2.00 (m, 2H, P1-*2H*ϵ), 2.24−2.31 (m, 1H, P1-*H*β), 2.43−2.50 (m, 1H), 2.55−2.67 (m, 1H), 3.97 (s, 3H), 4.00−4.09 (m, 2H), 4.51−4.55 (dd, J = 8.1 Hz, 1H), 4.76 (d, J = 10.8 Hz, 1H), 4.90−4.97 (dd, J = 9.0 & 15.3 Hz, 1H, P1-*H*γ), 5.42−5.50 (m, 1H, P1-*H*δ), 5.78 (bs, 1H), 7.12 (d, J = 6.0 Hz, 1H), 7.25 (d, J = 9.2 Hz, 1H), 7.54 (s, 1H), 7.69 (bs, 4H), 8.17 (bs, 2H), 8.25 (d, J = 9.2 Hz, 1H), 8.43 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 20.1, 21.6, 26.4, 27.8, 28.5, 29.6, 31.0, 34.7, 37.8, 51.7, 52.8, 56.0, 58.4, 77.8, 79.2, 101.4, 114.6, 119.0, 119.4, 124.4, 128.6, 128.9, 129.1, 129.9, 130.9,

131.5, 155.1, 156.6, 157.6, 160.0, 172.0, 172.3, 173.2. HRMS *m/z* found 685.323890 $(M + H)^+$ calcd for $C_{38}H_{45}N_4O_8$ 685.323740. ^{1}H NMR homo-decoupling experiments, via selective irradiation of the methylene P1−Hϵ adjacent to the olefin (δ 1.82−2.00 ppm) resulted in $J_{H\gamma-H\delta} = 15.26$ Hz for the two olefinic protons, confirming the trans geometry of the double bond. Furthermore, the lack of ROESY correlation between the P1−Hβ and the P1 amide NH (i.e., ACCA moiety) was consistent with epimerization at the Cβ the vinyl ACCA moiety (P1) placing the olefinic linker syn to the amide bond.

Diene tripeptide ester 2e: homogeneity of 96% based on C_{18} reversed-phase analytical HPLC ($t_R = 22.60$ min); ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers in ~9:1 ratio) δ ppm (major rotamer) 1.41 (s, 9H), 1.35−2.18 (m, 8H), 2.37−2.48 (m, 1H), 2.95−3.05 (m, 1H), 3.66 (s, 3H), 3.96 (s, 3H), 3.90−3.98 (m, 1H), 4.29 (bd, $J = 11.1$ Hz, 1H), 4.43−4.552 (m, 1H), 4.83−4.87 (m, 1H), 4.93−5.02 (m, 2H), 5.10−5.16 (m, 2H), 5.27−5.33 (m, 1H), 5.4 (bs, 1H), 5.68−5.79 (m, 2H), 7.06 (s, 1H), 7.08−7.11 (dd, $J = 2.5$ & 9.1 Hz, 1H), 7.43−7.54 (m, 5H), 7.60 (bs, 1H), 8.01−8.07 (m, 3H); ES^+ MS *m/z* 713.5 $(M + H)^+$; ES^- MS *m/z* 711.5 $(M - H)^-$.

Carboxylic acid derivative of macrocyclic peptide E-4e: homogeneity of 96% based on C_{18} reversed-phase analytical HPLC ($t_R = 18.20$ min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09−1.12 (m, 1H), 1.21 (s, 9H), 1.33−1.48 (m, 1H), 1.63−1.85 (m, 5H), 1.98−2.08 (m, 1H, P1-*H*ϵ), 2.15−2.21 (dd, $J = 8.1$, 15.1 Hz, 1H, P1-*H*β), 2.38−2.47 (m, 1H), 2.65−2.75 (m, 1H), 3.97 (s, 3H), 4.14 (bs, 1H), 4.23 (bd, $J = 9.5$ Hz, 1H), 4.32−4.38 (m, 1H), 4.51 (bd, $J = 11.1$ Hz, 1H), 5.20−5.26 (dd, $J = 7.0$, 16.0 Hz, 1H, P1-*H*γ), 5.45 (bd, $J = 15.0$ Hz, 1H, P1-*H*δ), 5.79 (bs, 1H), 6.83 (d, $J = 4.8$ Hz, 1H), 7.23 (d, $J = 8.5$ Hz, 1H), 7.55 (s, 1H), 7.70 (bs, 4H), 7.90 (s, 1H), 8.16 (bs, 2H), 8.31 (d, $J = 8.5$, 1H); HRMS *m/z* found 671.308350 $(M + H)^+$, calcd for $C_{37}H_{43}N_4O_8$ 671.308090. ^{1}H NMR homo-decoupling experiments, via selective irradiation of one methylene P1−Hϵ adjacent to the olefin (δ 1.98−2.08 ppm), or irradiation of the P1−Hβ (δ 2.15−2.21) resulted in $J_{H\gamma-H\delta} = 15.90$ Hz for the two olefinic protons, confirming the trans geometry of the double bond. Furthermore, strong ROESY correlation between both Hγ/Hδ and the amide NH of the ACCA moiety (P1 moiety) was consistent with epimerization at the Cβ the vinyl ACCA moiety placing the olefinic linker syn to the amide bond.

Epimerized carboxylic acid tripeptide *E*-5e: homogeneity of 94% based on C_{18} reversed-phase analytical HPLC ($t_R = 20.23$ min); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.03−1.11 (m, 2H), 1.06 (s, 9H), 1.36−1.55 (m, 3H), 1.58−1.62 (m, 2H), 1.97−2.02 (m, 2H), 2.28−3.37 (m, 2H, P2-*H*β + P1-*H*β), 2.58−2.67 (m, 1H), 3.94−4.0 (m, 1H), 3.97 (s, 3H), 4.12−4.18 (m, 1H), 4.48−4.52 (m, 2H), 4.92−4.95 (dd, $J = 2.2$ & 10.2 Hz, 2H), 4.98−5.03 (dd, $J = 1.9$ & 17.0 Hz, 1H, P1-*H*δ), 5.20 (d, $J = 17.0$ Hz, 1H, P1-*H*δ), 5.40−5.50 (m, 1H), 5.72−5.78 (m, 2H), 7.12 (bd, $J = 9.5$ Hz, 1H), 7.55 (bs, 1H), 7.68 (bs, 4H), 8.18 (bs, 2H), 8.26 (d, $J = 9.8$ Hz, 1H), 8.42 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 21.5, 24.3, 27.9, 28.2, 30.4, 33.0, 34.6, 37.9, 52.0, 52.7, 56.0, 58.2, 77.8, 78.9, 100.4, 114.6, 114.9, 115.9, 119.3, 124.5, 128.6, 129.1, 131.6, 135.7, 138.5, 155.6, 156.5, 157.9, 158.2, 162.6, 162.8, 171.7, 171.8, 173.0; HRMS *m/z* found 699.339700 $(M + H)^+$, calcd for $C_{39}H_{47}N_4O_8$ 699.339390. Strong ROESY correlation between both P1Hγ and P1Hδ and the P1 amide NH (i.e., vinyl ACCA moiety) was consistent with epimerization at the Cβ the vinyl ACCA moiety placing the vinyl group syn to the amide bond and anti to the carboxylic acid. In contrast, there was no ROESY correlation observed between P1−Hβ and the P1 amide NH, which is characteristic of all tripeptides containing the 1*R*,2*S*-vinyl ACCA fragment such as the dienes **2a**−**e**.

Tripeptide Diene 9. The pure acyclic diene **9** was isolated as a white foam after purification by flash column chromatography using a solvent gradient from 20% hexane in EtOAc to 100% EtOAc: homogeneity of 94% based on C_{18} reversed-phase analytical HPLC ($t_R = 21.56$ min); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.24−1.47 (m, 7H), 1.44 (s, 9H), 1.53−1.60 (m, 1H), 1.67−1.75 (m, 1H), 1.85−1.88 (dd, $J = 5.5$, 8.1 Hz, 1H), 2.00−2.18 (m, 4H), 2.39 (d, $J = 14.3$ Hz, 1H), 3.62 (d, $J = 10.8$ Hz, 1H), 3.65 (s, 3H), 3.83−3.87 (dd, $J = 3.8$, 10.8 Hz, 1H), 4.29−4.34 (m, 1H), 4.47−4.52 (m, 1H), 4.72 (d, $J = 8.9$ Hz, 1H), 4.92−5.08 (m, 4H), 5.11−5.15 (dd, $J = 1.3$, 10.5 Hz, 1H), 5.28 (d, $J = 15.3$ Hz, 1H), 5.67−5.82 (m, 2H), 7.86 (s, 1H); ES^+ MS *m/z* 508.3 $(M + H)^+$, 530.3 $(M + Na)^+$; ES^- MS *m/z* 506.2 $(M - H)^-$.

Macrocyclic Products 10 and 11. Following RCM of the acyclic tripeptide diene **9** (in each case, using the reaction conditions and catalyst given in Table 2), the crude reaction mixture was concentrated to a reddish-brown syrup and chromatographed on silica gel using a solvent gradient from 10% EtOAc in CH_2Cl_2 to 100% EtOAc. The macrocyclic product(s) were isolated as a white solid. It should be noted that epimerized products **11** could not be cleanly separated from the desired product **10** by either flash column chromatography of preparative C_{18} reversed-phase HPLC; however, they were easily detected by chiral HPLC (Figure 3). Analysis of the RCM reaction mixtures from entries 1 to 5 (Table 2) by chiral HPLC revealed the presence of three products at t_R = 9.41, 10.33, and 11.84 min (Figure 3a). Analysis by chiral LC MS indicated that all three products had the same mass corresponding to the macrocyclic products **10** and **11**. After repeated C_{18} reversed-phase HPLC purifications of the RCM products from entries 1 to 5 (Table 2), a sample enriched in the epimerized products was isolated (Figure 3b) which was subsequently analyzed by NMR and assigned the structure of products **11**. RCM of diene **9** using catalyst **8** (Table 2, entry 7) gave cleanly the desired product **10**, which was isolated (after simple flash column chromatography) in ≥98% homogeneity based on NMR and C_{18} reversed phase analytical HPLC ($t_R = 18.17$ min). Furthermore, chiral HPLC confirmed that the product was formed as a single stereoisomer; refer to Figure 3c. As expected, coupling of **10** with 2-phenyl-4-hydroxy-7-methoxyquinoline under Mitsunobu conditions gave a product that was identical to compound **3c**.

Compound 10: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.22−1.58 (m, 7H), 1.43 (s, 9H), 1.61−1.69 (m, 1H), 1.76−1.88 (m, 2H), 2.08−2.25 (m, 4H, P1-*2H*ϵ + P1−Hβ + P2-*1H*β), 2.45 (d, $J = 14.3$ Hz, 1H), 3.67 (s, 3H), 3.72 (d, $J = 11.1$ Hz, 1H), 3.88−3.92 (dd, $J = 4.3$ & 11.2 Hz, 1H), 4.44−4.52 (m, 2H), 4.76 (d, $J = 8.6$ Hz, 1H), 4.86 (d, $J = 9.6$ Hz, 1H), 5.20−5.28 (m, 2H), 5.53−5.60 (m, 1H), 7.32 (s, 1H); ^{13}C NMR (400 MHz, DMSO) δ ppm 21.4, 24.1, 25.8, 27.1, 28.2 (28.1 shoulder), 30.2, 31.7, 35.8, 39.6, 51.6, 51.9, 54.7, 58.3, 69.4, 77.9, 126.5, 133.0, 155.1, 170.6, 171.7, 174.1; HRMS *m/z* found 480.270880 $(M + H)^+$, calcd for $C_{24}H_{38}N_3O_7$ 480.270976. ^{1}H NMR homo-decoupling experiments, via selective irradiation of the methylene P1−Hϵ protons, as well as the Hβ of the vinyl ACCA moiety (P1) adjacent to the olefin (δ 1.98−2.08 ppm), resulted in $J_{H\gamma-H\delta} = 9.8$ Hz for the two olefinic protons, confirming the cis geometry of the double bond. Furthermore, strong ROE correlation between Hβ and the amide NH of the ACCA moiety was consistent with the desired stereochemistry at the Cβ of the vinyl ACCA moiety placing the olefinic linker *syn* to ester moiety and *anti* to the amide bond.

Compound *E*/*Z*-11. Superposition of the ^{1}H NMR (400 MHz, $CDCl_3$) of the pure compound **10** and the mixture of compounds **11** (mixture indicated in Figure 3b) indicated a very similar patern of resonances. However, ^{1}H NMR homo-decoupling experiments, via selective irradiation at the chemical shift where the Hβ proton of vinyl ACCA is typically found (δ ~2.4−2.5 ppm), resulted in the decoupling of an olefinic Hγ proton (typically at δ 5.04 ppm) to a doublet with $J = 15.26$ Hz, confirming a *trans* geometry for the double bond of one compound in this mixture, which was assigned the structure of *E*-**11**. Subsequently, this mixture of "naked" macrocyclic scaffolds was reacted with 2-thiazole-4-hydroxy-7-methoxyquinoline, via Mitsunobu reaction at the Cγ-OH of the proline unit,[6a] to product a mixture of derivatives which were slightly more amenable to separation. A product corresponding to a

Z-**11** macrocyclic precursor (i.e., the epimer at the Cβ of vinyl ACCA moiety with a *cis* double bond) was isolated and fully chracterized by NMR (data not shown).

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Supporting Information Available: Some experimental details as well as ^{1}H NMR spectra of key compounds (as their acid analogues) **3b**, **3c**, *E*-**4d**, *E*-**4e**, **5e**, diene **9**, and macrocyclic compound **10**; the chemical shift assignments (as indicated) were confirmed by multiple 2D NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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