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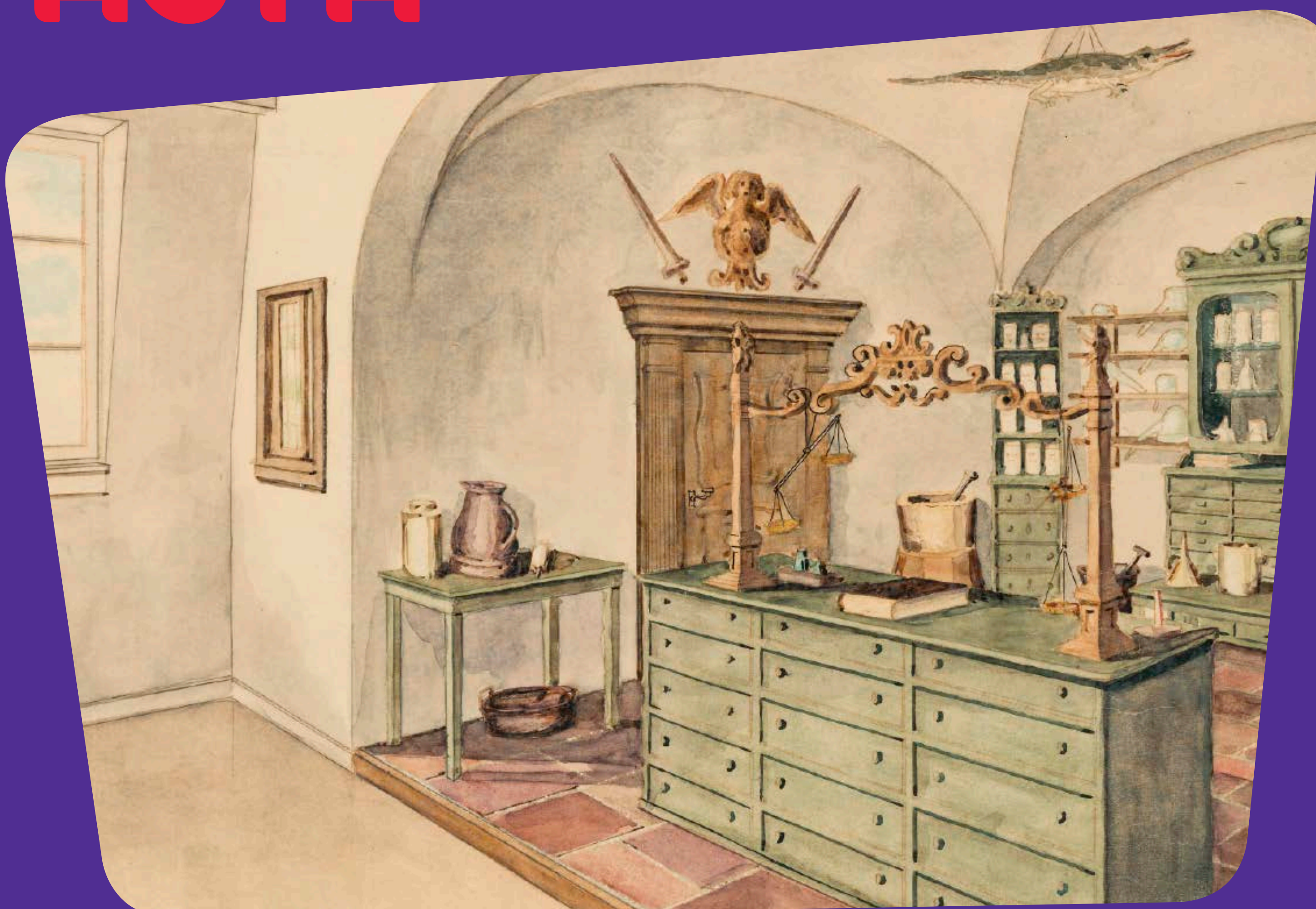

4-Substituted Prolines: Useful Reagents in Enantioselective Synthesis and Conformational Restraints in the Design of Bioactive Peptidomimetics

Recent Advances in Alkene Metathesis for Natural Product Synthesis—Striking Achievements Resulting from Increased Sophistication in Catalyst Design and Synthesis Strategy

ALDRICHIMICA ACTA 50 YEARS 1968 • 2017

From the Editor's Desk

The Aldrichimica Acta's GOLDEN JUBILEE YEAR

Sharbil J. Firsan, Editor
Aldrichimica Acta
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Dr. S. J. Firsan

With this issue of the *Aldrichimica Acta*, we are pleased to introduce our readers to the fresh and vibrant new look of this venerable journal they have come to value and love. This look weds the traditional successful elements of the *Acta* with the design principles of the *Acta*'s new owner, Merck KGaA, Darmstadt, Germany. This makeover couldn't have come at a better time, as we are celebrating the *Aldrichimica Acta*'s golden jubilee year. While this look is new, I'm happy to assure our contributors and readers that the *Acta*'s aim will remain the same: providing the best value and scholarship to the scientific community in fast-changing academic, business, and technology environments. Happily, Merck KGaA, Darmstadt, Germany, has made the same commitment to the chemistry community through the *Acta* that its predecessors Sigma-Aldrich and Aldrich had made: full open access to the entire *Acta* archive, no author or page charges, no subscription fees, topical reviews by some of the best and most active researchers from around the world, top-notch editorial assistance, worldwide print distribution, and a strong presence on the Web, just to name a few. At a time when most scientific publishers have now monetized their digital archives and are charging for access, perhaps no one appreciates what the *Acta* has to offer more than our readers who work at institutions with limited library budgets and, thus, limited access to scientific journals and databases.

As we celebrate this milestone, and as I reflect back on the *Acta*'s journey since its humble beginning in 1968, I am thrilled to let some of those who have helped the *Acta* grow into a leading review journal in the field of organic chemistry express in their own words what the *Acta* means to them.

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—Professor Gerald B. Hammond (University of Louisville)

To all the *Aldrichimica Acta* readers and contributors, I offer a big thank-you from the bottom of my heart and a promise that the *Acta* will continue to aim to be *Best in Science, Best in Business.*

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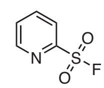
Nielsen, M. K.; Ugaz, C. R.; Li, W.; Doyle, A. G. *J. Am. Chem. Soc.* **2015**, *137*, 9571.



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ABOUT OUR COVER

Alte Apotheke im Museum der Firma Merck (watercolor and ink, 38.5 × 49.5 cm) was painted by an unknown artist in Darmstadt, Germany. It depicts original components of the Merck family's Engel-Apotheke (Engel Pharmacy), such as the pharmacy table within a niche of the company museum. Beneath a cross-ridge vault, the pharmacy is set up as was typical for the early 1800s, stocked with various vessels and grinding implements. A stuffed alligator, an Egyptian symbol against illness, hangs from the ceiling. The scene transports the viewer back in time to an era when apothecarists combined raw animal, plant, and mineral substances to create drugs. After Friedrich Jacob Merck's acquisition of the Engel Pharmacy in 1668, generations of pharmacists strove to refine the pharmaceutical craft into an independent academic discipline. Emmanuel Merck (1794–1855) is generally credited with setting the foundation for his family's pharmacy to grow into a chemical company in the first half of the 19th century. Emmanuel Merck's early research on alkaloids led him to connect with others and seek out pure products rather than raw materials.

Detail from ***Alte Apotheke im Museum der Firma Merck***. Photo courtesy of Merck KGaA, Darmstadt, Germany.

This painting is part of the collection housed at the company museum located at our Darmstadt, Germany, headquarters, where the Engel Pharmacy work tables are still on display. **www.emdgroup.com/history**.

4-Substituted Prolines: Useful Reagents in Enantioselective Synthesis and Conformational Restraints in the Design of Bioactive Peptidomimetics


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Keywords. 4-substituted prolines; peptide conformation; enantioselective synthesis; modified peptides; bioactive compounds.

Abstract. Among the substituted prolines, 4-substituted ones deserve particular attention. The ring conformation and the puckering preference of the prolyl ring are strongly affected by insertion of the substituent, and a great number of peptides containing these noncanonical amino acids have been investigated in the last five years. Moreover, the distance of the substituent from the functions involved in peptide chain formation causes minimal steric hindrance, thus offering the opportunity for conjugation with other chemical groups with little perturbation of the peptide chain.

Outline

1. Introduction

The unusual properties of proline (Pro), in comparison with the other amino acids, are responsible for its protective role toward proteolysis and for the specific conformation constraint it induces in proteins. For the same reasons, proline is commonly inserted into synthetic peptides to modulate the sequence conformation, thus inducing specific affinity towards biological receptors and increasing peptide strength. This unique behavior is due to its cyclic structure; but, attaching a substituent to the pyrrolidine ring may introduce an additional effect: the modulation of ring puckering and cis–trans amide equilibration.[1] Moreover, the introduction of a novel functional group into Pro may offer an opportunity to conjugate proline-containing peptides to other useful entities in the design of drugs or diagnostic agents.

It's well known that Pro usually breaks repeated structural motifs, and changes peptide direction by means of hairpin or reverse-turn folding. The lack of hydrogen-bond-donating capacity prevents Pro from participating in secondary stabilized structures, thus making it poorly compatible with α-helixes and β-sheets. In general, Pro is best located in the (i+1) and (i+2) positions of β- and γ-turns or as the terminal N-residue of an α-helix. Moreover, while for proteinogenic amino acids the trans conformation of the peptide bond is energetically favored, the lower energy gap in the isomerization of the ω-dihedral angle observed for Pro makes the cis conformation of comparable stability (**Figure 1**, Part (a)). Even if the cis and trans isomers are essentially isoenergetic, more than 90% of prolyl amide bonds in proteins adopt the trans conformation.[2] Polyproline or Pro-rich peptide fragments can form right-handed PPI helixes when all-cis peptide bonds are present, or left-handed PPII helixes when all peptide bonds assume the trans conformation. With respect to the pyrrolidine ring, two puckering conformations are favored and are defined as exo (up) and endo (down), depending on the out-of-plane displacement of the C^{γ} atom relative to the carbonyl group, as described by the value of the χ^1 torsion angle (Figure 1, Part (b)).[1] The exo and endo ring puckers influence the trans and cis conformations of prolyl amide bonds, and, consequently, the φ, ψ, and ω main-chain torsion angles that define the backbone of a whole protein.

The introduction of substituents on the pyrrolidine ring provides an exceptional opportunity to exploit the unique properties of proline in the design of complex molecules. The choice of the functionalized position has to be carefully planned, taking into account both the desired effect on the properties of the peptide and the synthetic pathway, since substituent introduction is rarely performed on proline itself. The cis or trans configuration of the novel moiety relative to the carbonyl group would determine the conformation of the five-membered ring.

Insertion of a substituent at position 2 leads to the formation of C_{α}-tetrasubstituted amino acids and induces a dramatic restriction in the allowed torsion angles. 2-Alkylprolines and 2-spiro compounds are of great interest since they have been successfully employed in the synthesis of bioactive synthetic peptides.[3] Attaching a substituent to the pyrrolidine β carbon generates a 3-substituted Pro, and leads to a real "chimera" bearing the side chain of another proteinogenic amino acid and thus possessing characteristics from both molecules.[4] Finally, 5-aryl-substituted prolines are privileged elements in medicinal chemistry,[5] and fused azabicyclo compounds containing the 5-substituted proline scaffold are important structural constraints in several bioactive molecules.[6]

Among the substituted prolines, those bearing a novel moiety at position 4 deserve particular attention. The first advantage is the distance of the substituent from the functions involved in peptide chain formation, resulting in minimal steric hindrance. This offers an opportunity to exploit substitution in order to conjugate peptides to other chemical entities with only a low perturbation of the peptide chain. Moreover, the puckering preference of the prolyl ring is affected by insertion of a substituent at position 4. The presence of an electron-withdrawing group, in particular, changes the relative energies of the ring conformations and leads in some cases to a unique preferred conformation due to the gauche effect (**Figure 2**).[7]

A review of the synthesis of alkyl-substituted prolines has recently been reported,[8] and other interesting surveys have been published, describing specific aspects of these compounds, such as the physiological properties and metabolism of L-Pro analogues or the properties of hydroxyproline derivatives.[9] A more specific review of 4-[^{18}F]-labelled prolines and their applications has also been published relatively recently.[10] In the present review, we highlight the more recent studies (since 2010) of 4-substituted prolines. The synthetic aspects of these unusual amino acids will only be briefly covered, since many of them are commercially available, while major attention will be focused on their insertion into bioactive molecules to induce proper conformations or as point of attachment in conjugation. These topics deserve particular attention since they represent innovative approaches in medicinal chemistry, biomimetic chemistry, and materials chemistry. Moreover, Pro derivatives are very efficient organocatalysts, and the novel applications in this field will also be reported. The great number of papers dealing with the chemistry of 4-substituted prolines has compelled us to choose only a few selected recent examples.

Principal classes of 4-substituted prolines will be presented, each in a separate section. With respect to halogen-substituted prolines, most of the studies reported in the literature over the last five years have been about fluorine, while chlorine, bromine, and iodine have been rarely used, and, for this reason, only the compounds containing fluorine will be described.

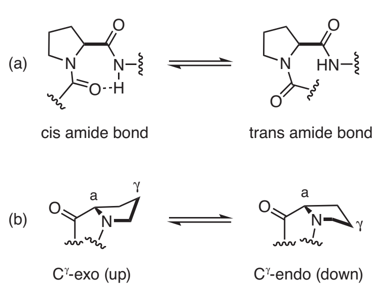


Figure 1. Proline: (a) Cis–Trans Amide Equilibration, and (b) Favored Exo or Endo Puckering of the Pyrrolidine Ring. *(Ref. 1)*

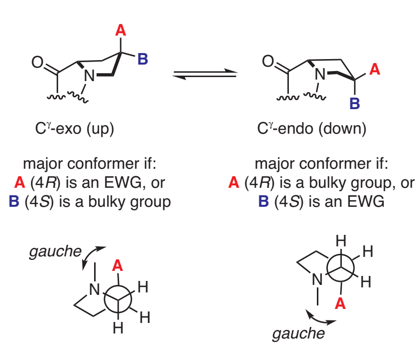


Figure 2. Preferred Conformers and Gauche Effect for 4-Substituted Prolines. *(Ref. 7)*

2. 4-Hydroxyproline (4-Hyp)

2.1. General Properties and Biological Relevance

Among substituted prolines, 4-hydroxyproline (4-Hyp) is present in nature as a fundamental component of collagen proteins, the major extracellular proteins in connective tissues.[11] The biosynthesis of this amino acid takes place as a post-translational event during polypeptide chain elongation by specific prolyl 4-hydroxylases (P4Hs) that are present in the endoplasmic reticulum. The hydroxylation process, in mammalian systems, requires interaction of the enzyme with the unfolded collagen chain in the presence of molecular oxygen, 2-oxyglutarate, ferrous iron, and ascorbate, and leads to the selective formation of *trans*-4-hydroxy-L-proline (T4LHyp). The transformation occurs exclusively in specific positions, resulting in the formation of Gly-Xaa-Yaa consensus tripeptide sequences, where Pro may be found in the Xaa or Yaa position, while 4-Hyp is always found in the Yaa position. The amount of hydroxylated proline in Types I and II collagen is close to 10%, and the regular occurrence of the trimeric sequences is responsible for the triple helical regions of collagen fibrils and for the increased stability of the protein. In collagen's structure, T4LHyp establishes hyperconjugative interactions, inducing a preference for the exo ring-puckering conformation and favoring trans amide bond formation.[6] In bacteria, T4LHyp is metabolized following different enzymatic pathways that involve the formation of *cis*-(4*S*)-D-Hyp (C3DHyp) via deprotonation of the C_α chiral carbon and racemization. Hydroxyprolines have also been detected in nonribosomal peptides, such as pneumocandins, which display antibiotic or antifungal activity. Since the origin of the hydroxylated amino acids in this context is still unclear, Hüttel and co-workers[12] recently investigated the reactivity and regioselectivity of Pro hydrolases (PHs), acting on the isolated amino acid or small congeners. All characterized members of this family have been obtained from bacteria, and biocatalysts able to synthesize three of the four possible Hyp isomers (T4LHyp, C4LHyp, and C3LHyp; this last isomer bearing the hydroxyl group at position 3) are already known. The authors studied the reaction catalyzed by GloF, a hydroxylase obtained by the pneumocandin biosynthesis cluster of the fungus *Glarea lozoyensis* ATCC 74030, with the aim of obtaining preference towards the formation of the elusive T3LHyp. Even if the simultaneous formation of T4LHyp and T3LHyp could be observed for the first time, T4LHyp ended up being the predominant isomer under all conditions tested.

2.2. Novel Syntheses of 4-Hyp

Although all the stereoisomers of 4-Hyp are commercially available, novel enzymatic or synthetic methodologies for their preparation are always of great interest. As mentioned in the preceding section, biocatalysts able to produce T4LHyp, C4LHyp, and C3LHyp are already known, but their use on a preparative scale is limited by their insolubility and low activity. Moreover, they undergo rapid denaturation in vitro, while their use in vivo involves elaborate workup processes. By producing recombinant hydroxylases and optimizing conditions, Klein and Hüttel[13] succeeded in the quantitative and selective conversion of Pro into specific hydroxylated derivatives, easily isolating the products via ion-exchange chromatography. Furthermore, to overcome the milligram-scale limit of the in vitro protocol, the in vivo reaction was also optimized in shake flask culture, leading to a significant increase in productivity.

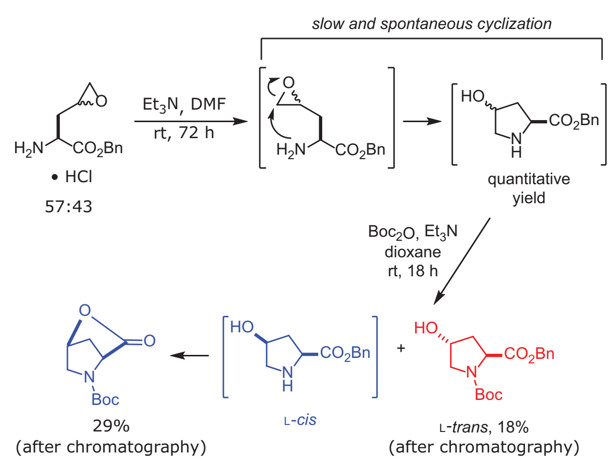
All four stereoisomers of 4-Hyp have been synthesized by Arai and co-workers starting from enzymatically resolved, enantiopure N-protected α-allylglycine benzyl ester that was treated with *m*-CPBA (**Scheme 1**).[14] The reaction afforded a diastereomeric mixture of epoxides, due to the uncontrolled formation of the new C4 stereocenter. Removal of amine protection induced the slow spontaneous intramolecular cyclization to 4-Hyp. The unexpected formation of a bicyclic lactone by reaction of the 4-hydroxyl group with the benzyl ester group was observed for *cis*-Hyp derivatives. Although stereocontrol of the epoxidation step is low, the methodology offers some advantages because all of the stereoisomers of 4-Hyp can be obtained with a simple and cheap procedure.

2.3. Applications of 4-Hyp in Bioactive Compound Synthesis

Recently, an Hyp-based class of histamine H_3 receptor antagonists that play a therapeutic role in CNS disorders has been reported in the literature. Moreover, the (2*R*,4*S*) stereoisomer, T4DHyp, is found at the core of the structures of the members of this class (**Figure 3**).[15] To address the industrial requirements of simplicity and low cost, Pippel and co-workers selected for their synthetic approach the natural and inexpensive T4LHyp as starting material, taking into account that a double-inversion strategy would have to be utilized.[15] The epimerization of C2 was accomplished by optimizing the conditions for the transformation of T4LHyp into La Rosa's lactone, a reactive intermediate, which underwent ring opening by means of the free base homopiperazine. The homopiperazine amide product reductively aminated cyclobutanone, resulting in elongation of the side chain. The last step was a Mitsunobu reaction, which caused inversion of configuration at C4 and yielded the target H_3 receptor antagonist (104 g, 57% (crude); 96 g, 53% (after chromatography)).

T4LHyp has also been employed by Hanessian's group as a building block in the synthesis of the model tetracyclic core present in calyciphylline B, a member of the family of alkaloids of the *Daphniphyllum* genus.[16] These alkaloids possess seven contiguous stereogenic carbons in their common backbone. As depicted in **Figure 4**, the Pro ring is conserved in the final structure, while nitrogen, carboxylate, and the hydroxyl moiety are used to build the tetracyclic structure through a stereocontrolled multistep synthesis.

An efficient synthesis of β-arabinofuranosylated-4-Hyp oligomers was reported in 2014 by Xie and Taylor,[17] with the aim of defining the minimal epitope of Art v 1, an allergenic glycoprotein present in the most common pollen and responsible for hay fever. A detailed study on coupling methodologies and peptide capping was performed in order to prepare the dimer, trimer, and tetramer starting from the glycosylated



Scheme 1. Synthesis of L-*cis*- and L-*trans*-4-Hyp from Epoxy Amino Acids Derived from Allylglycines. The Same Approach Was Also Employed for the Synthesis of the D-*cis* and D-*trans* Stereoisomers. *(Ref. 14)*

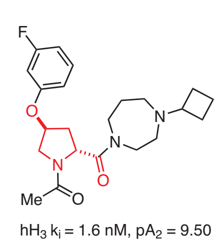
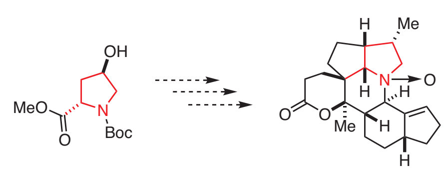


Figure 3. Selective Histamine H_3 Receptor Antagonist. *(Ref. 15)*

monomer. The trimer and tetramer were analyzed with circular dichroism, which showed the presence of a typical PPII-type helical structure that is usually observed only for longer Hyp-rich peptides. This showed that glycosylation of the 4-hydroxyl group stabilizes and promotes the formation of this ordered secondary structure.

2.4. Conformational Studies on Peptides Containing 4-Hyp

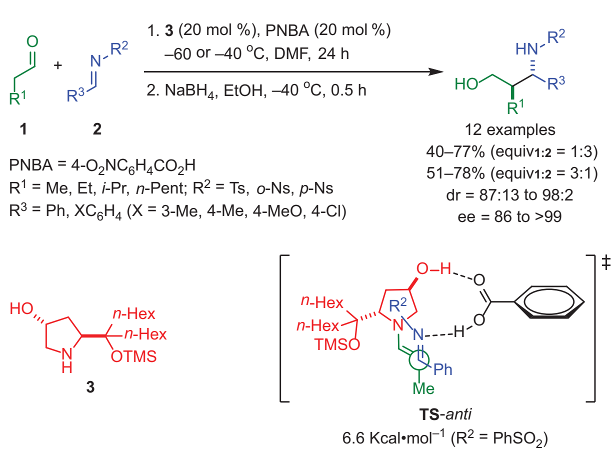
The secondary structure assumed by 4-Hyp-rich peptides has been extensively investigated due to the fundamental role of these sequences in animal and plant structural proteins. The preference of 4-Hyp for a γ-exo pucker conformation, due to the gauche effect and to hyperconjugative interactions, induces the formation of trans peptide bonds, thus stabilizing left-handed PPII helixes. The rigid PPII secondary structure is also typical of Hyp-rich glycoproteins (HRGPs), in which Hyp residues are extensively O-glycosylated. A well-defined model to evaluate the stabilizing effect of Hyp and O-glycosylated-Hyp was developed by Schweizer and co-workers[18] by comparing the homooligomers Ac-(Pro)$_9$-NH$_2$, Ac-(Hyp)$_9$-NH$_2$, and Ac-[Hyp(β-D-Gal)]$_9$-NH$_2$. Although a decrease in the PPII typical signals in the circular dichroism spectrum was observed for the O-glycosylated peptide, the presence of contiguous sugar groups increased the stability of the secondary structure owing to the tendency to form hydrogen bonds with the peptide backbone and with the solvent.



N-Boc-4-(*R*)-hydroxy-L-proline (T4LHyp)

calyciphylline B

Figure 4. Structure of Calyciphilline B, an Alkaloid of the *Daphniphyllum* Genus. *(Ref. 16)*



Scheme 2. *anti*-Selective Mannich Reaction between Aldehydes and *N*-Sulfonylimines Catalyzed by *trans*-4-Hydroxy-L-proline Derivatives with Brønsted Acid as Cocatalyst and Representation of the Transition State, with Corresponding Single-Point Value at B3LYP Level. *(Ref. 20)*

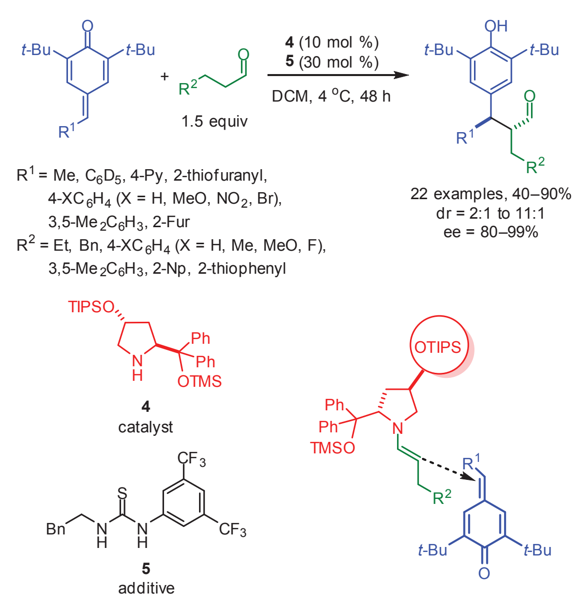
2.5. Application of 4-Hyp and Its Derivatives as Organocatalysts

In the past few years, Pro was extensively studied as an organocatalyst because of its ability to act with high stereoselectivity in aldol condensations, Mannich and Diels–Alder reactions, Michael additions, and other transformations.[19] This privileged role in organocatalysis has led, in the last fifteen years, to the development of a great number of derivatives, that have been successfully applied in asymmetric synthesis. Among them, Hyp and its reduced alcohol form deserve particular attention, since the hydroxyl group may be exploited for enhancing selectivity by introducing novel constraints into the catalytic complex, or for supporting the catalyst on a solid carrier.

The catalytic efficiency of *trans*-4-hydroxyprolinamides had been established by several research groups, but Palomo and co-workers have recently reported an *anti*-selective Mannich reaction performed in the presence of a Brønsted acid.[20] They have also demonstrated that the reaction occurs via a three-component arrangement, where the Pro nitrogen activates the aldehyde donor substrate while the 4-hydroxyl group, together with the added Brønsted acid, activate the imine acceptor component, controlling in this way the stereochemistry of the process. Among the screened catalysts, the best results were obtained with 4-hydroxy-dialkylprolinol derivatives (**Scheme 2**).

Similar, sterically demanding catalysts were successfully synthesized by Jørgensen and co-workers and applied to the enantioselective α-alkylation of aldehydes. In addition, these 4-hydroxyprolinol derivatives, possessing two bulky silyloxy moieties, afforded excellent stereocontrol in the addition of aldehydes to *p*-quinone methides to give α-diarylmethine-substituted aldehydes (**Scheme 3**).[21]

The importance of the 4-hydroxyl moiety was also demonstrated by Kelleher and co-workers in the optimization of the asymmetric Michael addition of aldehydes to nitro-olefins (**Scheme 4**).[22] The reaction of valeraldehyde with *trans*-β-nitrostyrene was performed in



Scheme 3. α-Alkylation of Aldehydes with *p*-Quinone Methides Catalyzed by *trans*-4-Hydroxy-L-proline Derivatives and Model of the Reaction Pathway. *(Ref. 21)*

the presence of a range of 4-hydroxyprolinamide catalysts, differing only in the presence or absence of an α-methyl group in the proline ring or in the steric hindrance of the amide group. The study showed that the *trans*-4-hydroxy group controls the facial stereoselectivity, and the simplest chiral prolinamide affords the best results. Additional substituents on the ring or at the amide nitrogen seem to be detrimental to the efficient control of the stereoselectivity, since small changes in the reagent and catalyst structures lead to a significant decrease of the enantiomeric excess.

Gauchot and Schmitzer sulfonylated the hydroxyl group to obtain a chiral counterion for the imidazolium cation, with the aim of exploring organocatalysis in ionic solvents such as [Bmim]NTf_2. This study afforded two efficient catalysts for the asymmetric aldol reaction (**Figure 5**, Part (a)).[23]

Since proline itself is an excellent organocatalyst for many reactions, the 4-hydroxyl group has also been considered a good handle for attaching the catalyst to a solid support.[24] By following this approach, silica nanoparticles—grafted with polymeric chains and linked to 4-Hyp via ester formation—were prepared to explore modulating the performance of the polymeric organocatalyst by ion-specific effects. This study, performed by Liu and co-workers, provided useful information about the salting-in or out effect induced by different counteranions, as well as about the stabilization of the transition state in proline-catalyzed reactions, induced by the anion polarization of hydrogen bonds (Figure 5, Part (b)).

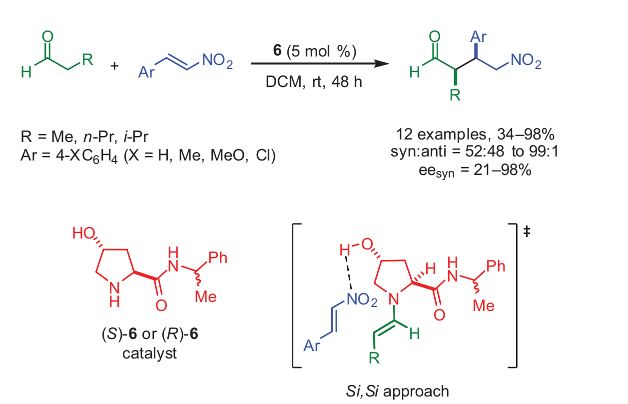
Heterogeneous catalysts with a unique architecture were prepared by Toste, Somorjai, and co-workers by immobilizing on mesoporous SiO_2 self-assembled monolayers (SAM) of chiral molecules such as Pro, T4LHyp, or diproline (Figure 5, Part (c)).[25] It is worth noting that the attachment of T4LHyp to the silica surface occurred via the carboxylic acid function. Au nanoclusters were then synthesized within the dendrimeric matrixes. The reactivity and selectivity of the catalysts were studied in the cyclopropanation reaction. For the T4LHyp containing catalyst, an increase in enantioselectivity was observed in comparison to proline, due to better packing of the SAM around the gold nanoclusters via hydrogen-bond formation. Another heterogeneous synergistic catalyst system was recently reported by Córdova and co-workers.[26] It consisted of a diamino-Pd complex on silica and a 4-hydroxyprolinol anchored to the silica surface via azide–alkyne click chemistry. This system proved to be effective in cascade Michael–carbocyclization reactions as a result of the concerted action of the two catalytic sites (Figure 5, Part (d)).

2.6. 4-Hyp as Synthetic Building Block

The reactivity of Hyp may involve the 4-hydroxyl substituent or the pyrrolidine ring. Zondlo and co-workers[7] have prepared a large number of 4-substituted prolyl amino acids, with 4*R* or 4*S* stereochemistry, starting from T4LHyp via Mitsunobu, oxidation, reduction, acylation, and substitution reactions. These residues may be inserted into peptides as other amino acid mimetics, recognition motifs, handles for spectroscopic studies (NMR, fluorescence, IR) or EWG-containing fragments capable of inducing stereoelectronic effects.

Substitution of the 4-OH group with a sulfur-containing moiety and Mitsunobu inversion were also exploited by Otaka and co-workers[27] for a previously unreported sequential coupling of peptide fragments via a 4-sulfanylproline-mediated native chemical ligation (NCL). The original NCL protocol requires two peptide segments, one possessing a thioester group and the other possessing an N-terminal cysteinyl residue. The limitation of the original protocol to peptides containing cysteine (Cys) was overcome by the authors using a Pro surrogate possessing a sulfanylated tether in position 4 of the proline ring.

Five-membered-ring systems are basic building blocks in heterocyclic chemistry, and pyrrolidines have gained particular prominence due to their presence in a wide variety of fundamental structural motifs. With



Scheme 4. Michael Addition of Valeraldehyde and *trans*-β-Nitrostyrene Catalyzed by 4-Hydroxy-L-prolinamides. *(Ref. 22)*

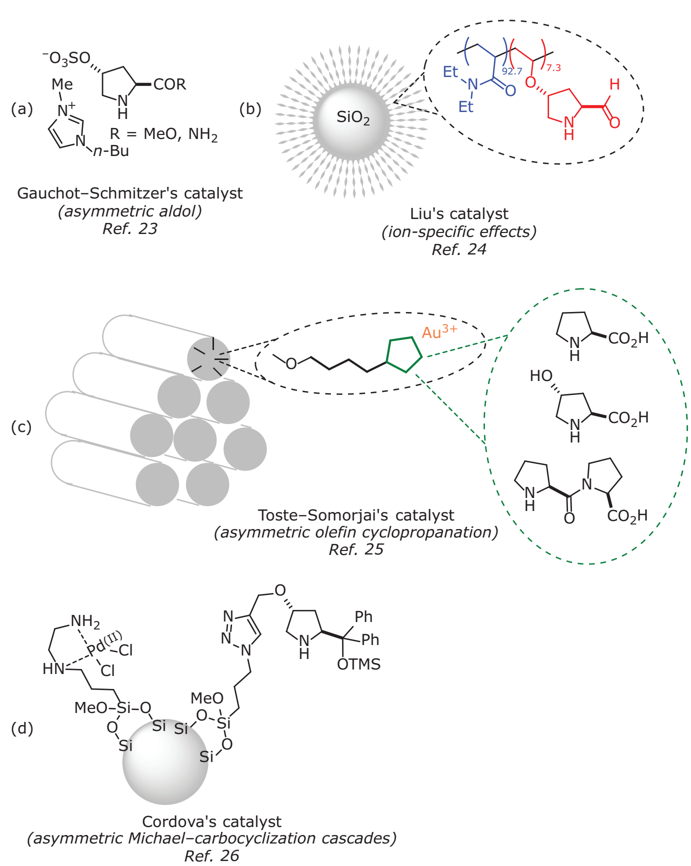
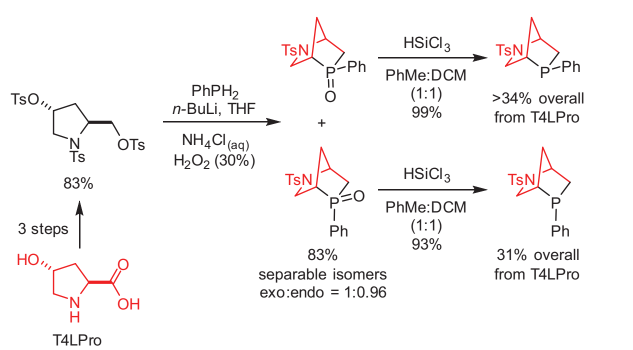
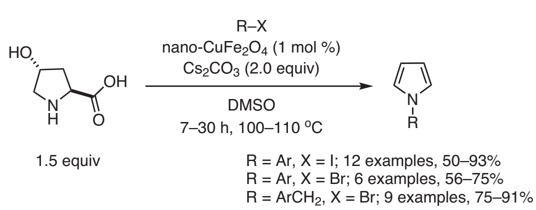


Figure 5. *trans*-4-Hydroxy-L-proline Derivatives in Heterogeneous Catalysis. *(Ref. 23–26)*

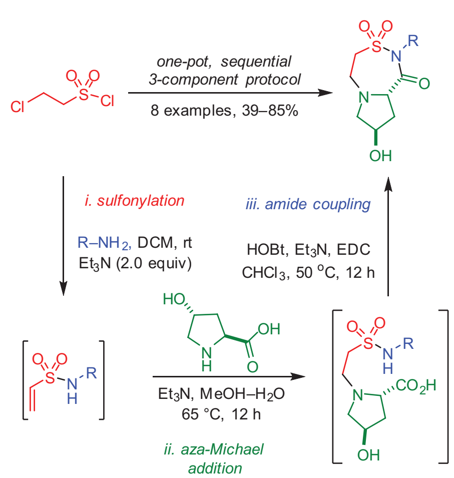
the aim of developing novel chiral phosphine catalysts to facilitate allene–imine [3 + 2] annulations leading to pyrrolines, Kwon's group designed rigid, bridged bicyclic structures using T4LPro as a source of chirality and as the origin of the catalyst pyrrolidine core (**Scheme 5**).[28] The key step of the synthesis is the bisalkylation of dilithium phenylphosphide with tritosylated hydroxyprolinol that affords *exo*- and *endo*-2-aza-5-phosphabicyclo[2.2.1]heptanes. These two diastereoisomeric phosphines are highly effective catalysts for



Scheme 5. Synthesis of Pseudoenantiomeric Bicyclic Phosphines Derived from *trans*-4-Hydroxy-L-proline. *(Ref. 28)*



eq 1 *(Ref. 30)*



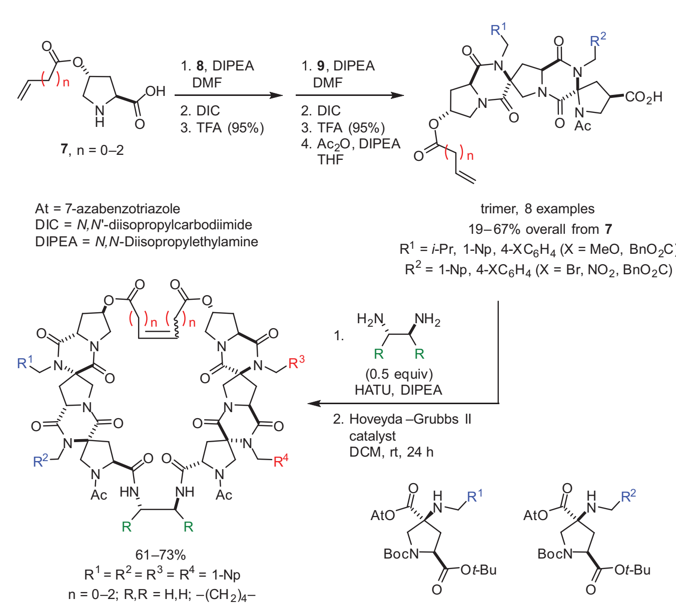
Scheme 6. Synthesis of Bicyclic Sultams via a One-Pot, Sequential, Three-Component Protocol. *(Ref. 31)*

the asymmetric synthesis of 1,2,3,5-tetrasubstituted pyrrolines, and function as pseudoenantiomers, producing the products in opposite enantiomeric forms.

A new synthesis of *N*-arylpyrroles from T4LPro and aryl iodides in the presence of CuI has recently been reported by Rao and co-workers.[29] Moderate-to-good yields were obtained, and the procedure was operationally simple and used inexpensive reagents and a readily available metal catalyst. Later, Nageswar's group optimized a ligand-free C–N cross-coupling reaction by employing recyclable and magnetically separable nanoparticles of $CuFe_2O_4$ as catalyst (**eq 1**).[30] Under these conditions, aryl iodides and bromides, as well as benzyl bromides, were reacted with T4LPro to give the corresponding aromatized 1-aryl- and 1-benzyl-1*H*-pyrroles.

A scalable, one-pot protocol for the preparation of stereochemically rich mono- and bicyclic acyl sultam libraries was recently reported by Hanson and co-workers.[31] This general methodology relies on the complementary ambiphilic pairing (CAP) of vinyl sulfonamides and unprotected amino acids. Vinyl sulfonamides may undergo both hetero-Michael additions and N-alkylations, and are ideal substrates for CAP. By using T4LPro and C4DPro, together with the enantiomers of α-methylbenzylamine-derived vinyl sulfonamides, a collection of all possible four diastereoisomers of the sultam were efficiently obtained in good yields and without any sign of racemization (**Scheme 6**).[31] The versatility of the sultam products allows further in situ transformations to be carried out as sequential 3-, 4-, and 5-component reactions.

T4LHyp is a fundamental building block in the convergent synthesis of a novel class of macrocyclic spiroligomers designed to create molecular surfaces and pockets with applications in supramolecular recognition and catalysis. Preactivated, alkylated proline derivatives, possessing a quaternary stereocenter, were synthesized by Zhao and Schafmeister in a few steps starting from T4LHyp, and were then coupled with the 4-pentenoate ester of T4LHyp (**Scheme 7**).[32] The reaction follows a complex mechanism—involving direct acylation



Scheme 7. Synthesis of Spiroligomer-Containing Macrocycles Starting from *trans*-4-(4-Pentenoyloxy)proline. *(Ref. 32)*

of the prolyl amine and an acyl-transfer coupling—that leads to the formation of hexasubstituted spiro-diketopiperazines, whose stereocenters and functional groups are determined during product design. Insertion of a diamine linker and olefin cross-metathesis led to spiroligomer-based macromolecules possessing highly preorganized structures that are suitable for binding proteins and for creating enzyme-like active sites.

Irradiation of Hyp with visible light in the presence of (diacetoxy)-iodobenzene (DIB) and iodine induces a mild, metal-free domino process involving ring opening of pyrrolidine, oxidation of the alcohol to the aldehyde, and addition of acetate ion to the iminium ion (**Scheme 8**). This particular reactivity has been exploited by Romero-Estudillo and Boto for the generation of a customizable unit for the site-selective modification of peptides with the aim of creating peptide libraries starting from a single, parent peptide.[33] The reaction, which occurs with negligible epimerization of the Hyp stereocenter at C2, generates a molecule possessing two reactive chains, which can be transformed independently or through concerted mechanisms. A reductive amination–lactamization took place with amino acids and peptides, leading to α-amino-γ-lactams (Agl, Freidinger–Veber lactams), that are very useful conformational constraints for rigidifying peptide backbones and for inducing conformations mimicking β-turns. The process afforded good results both with the free Hyp and with peptides containing one or more Hyp units in terminal or internal positions. Moreover, elaboration of the *N*-acetoxymethyl moiety permits the isolation of free amines or N-methylated units, which are also useful for the modification of peptide conformation and activity.

3. Conformational Studies of Peptides Containing 4-Aminoproline (Amp)

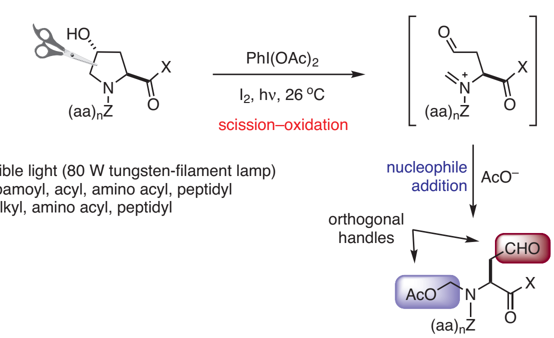
As already reported, a substituent at C4 of the Pro residue has a large influence on the puckering of the pyrrolidine ring and on the trans:cis conformer ratio of its amide bond. This effect is mainly due to steric effects, stereoelectronic gauche effects, repulsive interactions, and transannular hydrogen bonding.

Wennemers and co-workers have determined the behavior of (4*S*)-aminoproline under different pH conditions.[34] They demonstrated that (4*S*)-Amp adopts a C4-*endo* ring pucker in an acidic environment due to the formation of a transannular hydrogen bond and to the electron-withdrawing nature of the ammonium group, which induces an *endo* ring pucker. The uncharged amino group should exert a similar steric effect, and favor a C4-*exo* ring pucker without a transannular hydrogen bond. Thus varying the pH induces a flip between the two ring puckers of the pyrrolidine and the on-off switch of the transannular hydrogen bond. These observations have been confirmed by studying the effect of changes in the protonation state of (4*S*)-Amp-containing collagen on triple-helix formation. The stability of the supramolecular assembly has also been studied by switching between single- and triple-helical states of collagen (**Figure 6**). These results would help with the design of pH-sensitive, collagen-based materials that are capable of specifically releasing drugs in an acidic environment.

A new class of collagen analogues defined as "chimeric cationic collagens" was reported by Ganesh and co-workers.[35] In this case, both the X and Y residues of the collagen triad were simultaneously substituted by a combination of 4(*R*/*S*)-(OH/NH_2/NH_3^+/NHCHO)-prolyl units, and triplex stabilities were measured at different pHs. The observed unique effect of the 4-amino substituent on triplex stability allowed the critical combination of factors that dictate triplex strength to be defined (C4 puckering, intra-residue hydrogen bonding, stereoelectronic (*R*/*S*) and electronic interactions).

Among the prolines substituted with nitrogen at C4, 4-azido-Pro has been inserted by Bernardes's group into the structure of collagen as a chemical reporter for tagging.[36] The metabolic incorporation of this residue by foetal ovine osteoblasts, obtained by supplementation of the growth medium with *cis*-4-azido-L-proline, was followed by labelling with the fluorescent probe dibenzooctyne DIBO via azide–alkyne cycloaddition. Even if the methodology suffers from unspecific reaction of the strained alkyne DIBO with sulfhydryl-rich proteins, the strategy could be particularly useful for monitoring collagen accumulations in fibrotic diseases.

The epimerization of position 2 of N-protected *trans*-4-AMP methyl ester under basic conditions was immediately and irreversibly followed by an intramolecular cascade reaction to form a bridged lactam intermediate, which could be easily converted into various derivatives of 2,5-diazabicyclo[2.2.1]heptane (DBH).[37] This bicyclic scaffold has been utilized in structure–activity relationship studies in medicinal chemistry and as chiral ligand in asymmetric synthesis. Key



Scheme 8. Site-Selective Conversion of the *trans*-4-Hyp Unit Can Lead to Optically Pure Amino Acids, Including Valuable *N*-Alkyl Amino Acids, as Well as Homoserine Lactones and Agl Lactams. *(Ref. 33)*

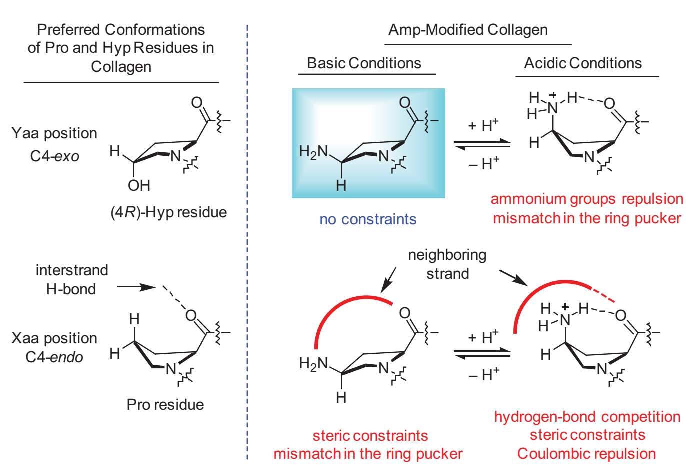


Figure 6. Effects of Introducing Amp in the Yaa or Xaa Position in Triple-Helical Collagen on the Stability of the Supramolecular Assembly under Different pH Conditions. *(Ref. 34)*

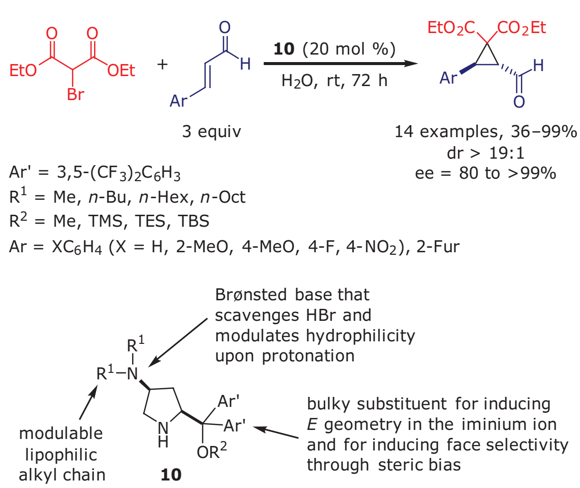
factors controlling the cascade transformation are the N-protective group being an electron-withdrawing group and promotion of the reaction by a strong base.

4-Dialkylamino-α,α-diarylprolinol ethers have been proposed by Vicario and co-workers[38] as efficient organocatalysts in the enantioselective cyclopropanation of α,β-unsaturated aldehydes. The presence of the 4-dialkylamino substituent, possessing linear alkyl chains of different lengths, is crucial for modulating the formation of micellar aggregates in disperse aqueous solutions. Moreover, the C4 amine represents an additional Brønsted basic moiety that may play a role in the activation of the substrate. The results obtained in the Michael–intramolecular α-alkylation cascade confirm that this novel class of catalyst is active in reactions in aqueous media (**eq 2**).[38]

4. 4-Fluoroprolines

4.1. General Properties

Due to the unique physicochemical properties of fluorine, such as highest electronegativity, strong lipophilicity, extremely low polarizability, and the ability to participate in a hydrogen bond, organofluorine compounds have been utilized in a range of applications in medicinal chemistry. In particular, fluorine has been found to have great potential in modulating the properties of peptides and proteins, and to increase stability and bioavailability by affecting the folding.[39] As a consequence of the introduction of substituents at C4 of the pyrrolidine ring, the energies of the conformers can be significantly altered, which results in stabilizing a different arrangement of the protein backbone. When the electron-withdrawing fluorine is incorporated at C4 of the pyrrolidine ring, the hyperconjugative interactions lead to a strong gauche preference for the β-fluoro amide moiety.[40] An exo pucker and a high trans:cis ratio are preferred when the fluorine-substituted C4 has an *R* configuration, while an endo pucker is favored with the *S* configuration of C4. The endo conformation, however, does not lead to an overall preference of cis over trans, even if it exhibits an increased probability of forming a cis peptide bond. These stereoelectronic effects can influence protein folding, thermostability, ligand binding, and catalytic activity. Therefore, investigating the effects of 4-fluoroproline is extremely important both in theoretical studies and in medicinal and diagnostic applications.



eq 2 *(Ref. 38)*

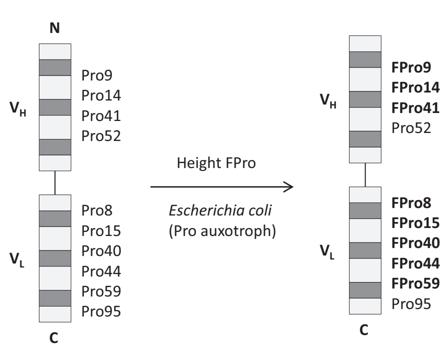


Figure 7. Approach for Improving the Stability of scFv Proteins with FPro. The Available Conserved Pro Residues in the Target scFv´s Can Be Easily Replaced with FPro by a Residue-Specific Substitution Method in the Cytoplasm of *E. coli*. *(Ref. 43)*

4.2. Effects of Fluorination on Protein Folding and Stability

The single-chain Fv protein (scFv) is the smallest functional antigen-binding unit of an antibody; it is composed of two domains, named variable heavy (V_H) and variable light (V_L), joined together by a flexible peptide linker. scFv fragments are powerful tools in radiotherapy and medicinal chemistry applications, owing to their increased rate of blood clearance and low immunogenicity as compared to the parent monoclonal antibodies.[41] However, the scFv fragments suffer from lower binding affinity and insufficient thermostability, which limit their therapeutic potential.[42] The approach proposed by Lee and co-workers to enhance the thermal stability of scFv incorporates fluorinated prolines in the target protein, in order to take advantage of the stereoelectronic changes induced by the new amino acids on the folding and thermal stability of the protein.[43] hu-MscFv, a bacterial cytoplasmic protein containing a total of eight Pro residues in the framework region (FR) affecting the folding and stability, was used as the model system (**Figure 7**).[43]

Only the replacement of Pro with (4*R*)-FPro (not the 4*S* epimer) had a positive influence on folding and activity. The thermal stability of the fluorinated protein was also improved, although only for temperatures below 50 °C. The explanation for this improved thermal stability was provided with the help of modeling studies, which showed ten new dipole interactions between fluorine and other polar groups in the protein.

Wild-type ubiquitin (wt-ub) is a small thermally and chemically stable protein of eukaryotes that regulates the cellular processes of other proteins in the body. Crespo and Rubini studied the effects of introducing 4-FPro in wt-ub, and found that (4*R*)-FPro exerts a positive effect on the stability, while (4*S*)-FPro is not incorporated into the protein.[44] The authors initially evaluated the structure of the modified enzyme by circular dichroism (CD), and discovered that its far UV-CD spectrum was practically identical, at pH 2.0 and 5.0, to that of wt-ub, suggesting that the secondary structure is not modified by incorporation of (4*R*)-FPro. The thermal stability after denaturation at acidic pH, and the equilibrium against denaturation in guanidine-induced unfolding experiments were also investigated, indicating a stabilizing effect of (4*R*)-FPro-ub. The folding–unfolding kinetics of (4*R*)-FPro-ub and wt-ub were also determined by stopped-flow analysis, which proved that (4*R*)-FPro exerts a positive effect, accelerating the folding process of the protein. This effect was attributed to the ability of the fluorine atom to establish stabilizing interactions with the amino groups of the protein backbone, made possible by the Cγ-endo pucker and by the energetically favored trans conformation of the protein.

Lantibiotics are peptides synthesized by Gram-positive bacteria as secondary metabolites typically containing an intramolecular ring structure and the thioether cross-linked amino acids lanthionine or methyllanthionine.[45] In recent years and to date, many variants have been engineered and characterized, with the aim of obtaining compounds with activity against multidrug-resistant pathogens. Budisa, Süssmuth, and co-workers reported the possibility of obtaining lantibiotics containing noncanonical amino acids (ncAAs) by heterologous expression from *Bacillus licheniformis* in *E. coli* as host.[46] In their ribosomal peptide synthesis (RPS) of the two-component lantibiotic lichenicidin, they selected Pro and tryptophan (Trp) as the amino acids to be replaced in JM83 and ATCC 49980 auxotrophic *E. coli* strains, respectively. (4*R*)- and (4*S*)-FPro were successfully embedded at high levels into the peptide, as demonstrated by mass spectrometry, thus opening the way for obtaining new lantibiotics with improved chemical and biological properties.

Collagen mimetic peptides (CMPs) are small synthetic peptides that mimic natural collagen and that were developed as synthetic models to study the structure and folding behavior of collagen, as well as to generate new collagen-like scaffolds for use in many biomedical fields. In this context, Raines and co-workers found that replacing—in the typical Gly-Xaa-Yaa consensus tripeptide—of Hyp in the Yaa position with (4*R*)-FPro and Pro in the Xaa position with (4*S*)-FPro enhanced the collagen triple helix stability by adopting a favorable ring puckering (C^{γ}-exo for Yaa and C^{γ}-endo for Xaa), as a consequence of electronic inductive effects.[47] The same research group implemented these discoveries by investigating the annealing of some fluoroproline-based CMPs to natural collagen.[48] They synthesized Ac-[(4*S*)-FPro-(4*R*)-FPro-Gly]$_7$-(Gly-Ser)$_3$-LysOH and other CMPs as controls, and evaluated them by using fluorescently labeled peptides to establish (i) the annealing of collagen and the time-dependent retention in vitro, (ii) the annealing of collagen in vivo, and (iii) the cytotoxicity toward human dermal fibroblasts. They found that the selected peptides were effectively included into the collagen and that the annealing took place without any preheating. The annealing strategy described in this work could have useful applications in the medical field such as in the treatment of highly traumatized or slowly healing wounds.

The type 1 DNA polymerase from *Thermus aquaticus* (KlenTaq) is a single-chain polypeptide with a molecular mass of 94 kDa containing 32 Pro residues dispersed throughout the whole structure. A (4*R*)-FPro-modified peptide was expressed and purified in *E. coli*, together with wild-type KlenTaq, and extensive studies on crystallization, prolyl ring puckering, cis–trans properties, and structural noncovalent interactions were performed.[49] (4*R*)-FPro-KlenTaq was crystallized in a ternary complex with DNA primer/template duplex and 2',3'-dideoxycytidine-5'-triphosphate (ddCTP) as a chain terminator. It was observed that the crystallization of the fluorinated KlenTaq was faster than that of the wild-type enzyme and occurred under a broader range of conditions. Comparison of the two structures did not show substantial differences, and the authors reported that (4*R*)-FPro-KlenTaq establishes a new network of noncovalent interactions and tends to assume an exo puckering, while the wild-type enzyme has a high percentage of endo puckering, and this conformational change could explain the better crystallization results from the fluorinated enzyme.

Employing a similar approach and aiming to enhance the tendency to adopt the PPII helix conformation, Borgogno and Ruzza carried out the synthesis in the solid phase of analogues of the proline-rich decapeptide PPPLPPKPKF (P2) by replacing some prolines with (4*R*)-FPro and (4*R*)-FHyp.[50] CD spectroscopy revealed a higher content of PPII in the fluorinated compounds, but an unfavorable binding interaction with GST-SH3$_{\text{m-cort}}$ fusion protein, probably due to a decrease in the flexibility of the peptide. The Src homology 3 region (SH3)[51] is a peptide recognition domain of about 60 amino acids that typically interacts with Pro-rich motives, in particular regular periodic PPII helices.

The lipase from the anaerobic bacterium *Thermoanaerobacter thermohydrosulfuricus* (TTL) is widely used as biocatalyst in many industrial processes due to its thermal stability and resistance to solvents and proteases. These properties make TTL a very attractive target to study the effects of incorporation of noncanonical amino acids (ncAAs). Among TTL Pro congeners, the fluorinated ones exhibited highest catalytic activity in *tert*-butyl alcohol and isooctane used as co-solvents for lipase-based biodiesel production.[52]

Enhanced thermal and chemical stabilities were also observed by incorporation of (4*R*)-FPro into the monomeric red fluorescent protein (mRFP1), a versatile biological indicator for monitoring gene expression and protein localization, providing valuable insights on physiological processes.[53] Found in prokaryotes and eukaryotes, thioredoxins (Trx's) are protein disulfide reductases that promote the fast oxidation of two Cys thiol groups to a disulfide with concomitant transfer of two electrons and two protons in a hydrophobic environment. *E. coli* Trx, the prototype of the family, contains a *cis*-Pro76 which is important for stability and function of the enzyme, and which is located in close proximity to Cys32 and Cys35 that form the active site at the N-terminal end of α-helix 1.[54] Trx1P (a variant containing Pro76 and other prolines replaced by Ala) and fluorinated analogues Trx1P-(4*R*)-FPro and Trx1P-(4*S*)-FPro were expressed in *E. coli* and crystallized by Rubini and co-workers.[54] The three enzymes were found practically identical, and, in particular, the Pro ring pucker adopted an endo conformation to avoid unfavorable steric interactions, as demonstrated by a modeling study. The effects on thermodynamic stabilities were determined by guanidium chloride dependent unfolding–refolding equilibria by taking into account both the oxidized and reduced forms of the three enzyme variants. However, all variants showed similar catalytic activities, slightly higher than the wild-type enzyme.

Finally, an interesting CD study by Lin and Horng explored the impact of C-terminal FPro on polyproline helices of type I (PPI, all-cis amide bonds) and type II (PPII, all-trans amide bonds).[55] In particular, (4*R*)-FPro significantly stabilized the PPII conformation and reduced the rate of conversion of PPII to PPI. Thus, these stereoelectronic effects favor a C^{γ}-*exo* pucker and trans peptide bonds.

4.3. 4-Fluoroproline in Catalysis

The incorporation of fluorine at C4 of Pro changes its stereoelectronic properties, and permits control of the conformation and modulation of the reactivity, stereoselectivity, and catalysis mechanism.[56] In a study by Yun and co-workers,[57] FPro and DOPA (two ncAAs) were incorporated into the industrially important biocatalyst ω-transaminase (ω-TA) through a combination of residue-specific and site-specific incorporation methods.[58] Two variants were obtained, ω-TAdopa and ω-TAdp[(4*R*)-FP], exhibiting enhanced thermal stability and half-life. ω-TAdopa and ω-TAdp[(4*R*)-FP] were then immobilized onto soluble chitosan or polystyrene (PS) beads, and the PS-immobilized ω-TAdp[(4*R*)-FP] proved to be the most suitable catalyst for the biocatalytic synthesis of chiral amines.[57]

Coxon's group has reported the synthesis and X-ray crystal structure of two fluorinated analogues of the well-known nickel(II) Schiff base complex of (*S*)-2-[*N*-(*N'*-benzylprolyl)amino]benzophenone (BPB) and Ala, namely L-Ala-Ni-(*S*)-F-BPB and L-Ala-Ni-(*R*)-F-BPB.[59] These complexes are artificial analogues of pyridoxal 5'-phosphate (PLP) dependent enzymes, and have been used in the asymmetric synthesis of α-amino acids as glycine (Gly) equivalents. The authors highlighted, on the basis of DFT calculations, a novel fluorine–nickel interaction that may enhance the stability of the complexes.

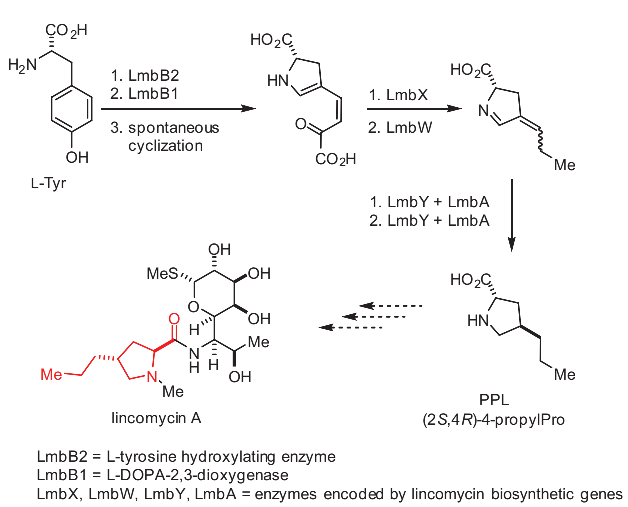
4.4. Applications of 4-Fluoroproline in Medicinal Chemistry

Many wet-adhesion proteins produced by mussels are biomaterials with high biomedical relevance, and are used as "biological glues" for bone surgery and bone regeneration. Budisa's group developed a co-translational method for incorporating FPro into a mussel Pro-rich protein Fp151, with stereoselective preference for *R* isomers. This methodology gives access to a class of biomaterials with new adhesive and mechanical properties.[60]

β-Sheet breaker peptides are fluorinated small peptides that can act against β-amyloid aggregation and fibrillogenesis in Alzheimer's disease.[61] Based on the sequence LVFFD of β-amyloid 1–42 peptide that plays an important role in β-amyloidogenesis, the peptides were obtained by substituting valine (Val) with 4,4,4-trifluoroVal or 4FPro, or substituting phenylalanine (Phe) in the 3 position with 3,4,5-trifluorophenylalanine. These modified fragments induced an α-helix structure on β-amyloid, a conformation which delays the aggregation process.

5. 4-Alkylprolines

4-Alkyl-L-proline derivatives (APD) are present in at least three groups of natural compounds: the anticancer agents pyrrolo[1,4]-benzodiazepines, the bacterial hormone hormaomycin, and the antibiotic lincomycin.[62] These compounds share a specialized biosynthetic pathway encoded by five or six homologous genes. Janata and co-workers revised the biosynthesis of APD on the lincomycin model on the basis of gene inactivation experiments and in vitro assays with recombinant enzymes.[63] The proposed pathway (**Scheme 9**) could be utilized in the preparation of biologically active lincomycin derivatives and other compounds with specific modifications of the APD moiety.



Scheme 9. Structure of Lincomycin A and Proposed Pathway for the Synthesis of Its 4-PropylPro Precursor from Tyrosine. *(Ref. 63)*

Other recent studies have dealt mainly with molecules containing 4-methyl- or 4-cyclohexylproline. 4-Methylproline (4-MePro) is a rare nonproteinogenic amino acid found in secondary metabolites from cyanobacteria and actinobacteria, and is produced from leucine (Leu) through the action of a long-chain dehydrogenase and a pyrroline-5-carboxylic acid (P5C) reductase.[64] The conformational preferences and prolyl cis–trans isomerizations of Ac-4*S*/4*R*-MePro-NHMe in water and in the gas phase were calculated using DFT with a self-consistent reaction field (SCRF) method.[65]

Recently, Sivonen and co-workers[66] developed a method for detecting bioactive peptides in cyanobacteria through biosynthetic 4-MePro, by using a combination of polymerase chain reaction (PCR) and liquid chromatography–mass spectroscopy (LC-MS) methods. They screened 116 cyanobacteria strains from 8 genera, and confirmed the presence of eleven new 4-MePro-containing nonribosomal cyclic depsipeptides, nostoweipeptins and nostopeptolides, from two *Nostoc* strains. On the other hand, spumigins J and A are 4-MePro-containing linear peptides that were extracted from the freshwater cyanobacterium *Anabaena compacta*, characterized, and examined for thrombin and cathepsin B inhibitory activities.[67]

To evaluate the effect of 4-MePro on peptide stability, other cyclic peptides have been investigated. Griselimycin (GM) is a natural cyclic peptide that was isolated from *Streptomyces* a half century ago, and developed by Rhône-Poulenc as an antituberculosis drug. Metabolic studies identified the Pro residue in the 8 position as a main site of metabolic reactions. Kling et al. reinitiated studies on GM, and increased the metabolic stability by alkylation of 8-Pro; the resulting methyl GM (MeGM) and cyclohexyl GM (CGM), obtained by total synthesis, showed improved pharmacokinetic properties.[68]

A54556 cyclic acyldepsipeptides are antibacterial natural products that have been isolated from *Streptomyces hawaiiensis*. Novel analogues of A54556, containing a 4-MePro residue, have been synthesized and evaluated against a variety of Gram-positive and Gram-negative bacteria.[69] Optimization of the macrocyclic core residues and the *N*-acyl side chain of the peptides led to the development of a lead analogue that showed potent activity against all Gram-positive strains tested.

6. Dehydroprolines

Dehydroprolines (DHPs), proline analogues possessing a carbon–carbon double bond in the pyrrolidine ring, may be used as intermediates for the preparation of functionalized prolines or may be introduced into peptides to induce conformational restraints. The specific properties depend, of course, on the position of the unsaturated bond.

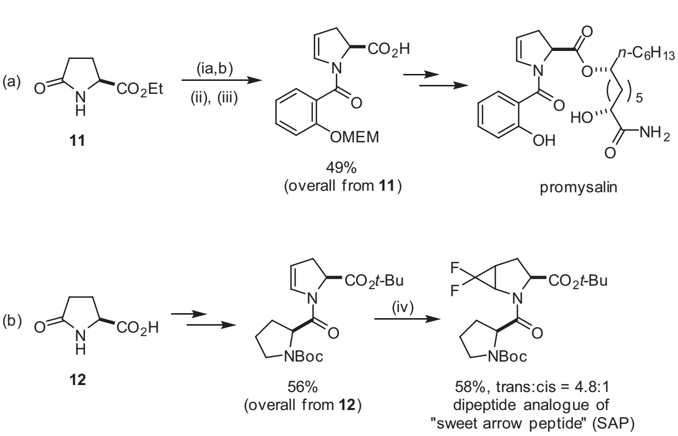
Traces of 3,4-dehydroproline (3,4-DHP) have been found in Sandal leaves (*Santalum album L.*), together with *cis*- and *trans*-4-Hyp.[70] The detected amount changes depending on the season of sample collection, and it has been proven that, in this environment, the amino acid has a short half-life. This suggests a role as intermediate in the biosynthesis of the more abundant 4-Hyp or as a metabolite of the parent compounds. On the other hand, 3,4-DHP has been identified as a lead compound for the control of fire blight, a disease occurring in apples and pears, that leads to significant losses in fruit production. The disease is caused by the Gram-negative bacterium *Erwinia amylovora*. An SAR study was developed by Sarojini and co-workers, by testing the antibacterial activity of a library of heterocyclic compounds selected for their similarity to quorum-sensing signalling molecules in Gram-negative bacteria.[71] Among them, 3,4-DHP showed excellent in vitro and good in vivo inhibition of biofilm formation, as compared to streptomycin. Decreased or loss of activity was observed for pyrrolidine and other derivatives, indicating that the complete structure of 3,4-DHP is essential for bioactivity.

Although the substitution of proline with DHP in proteins or bioactive peptides has been performed in the past with the aim of modifying their chemical, physical, and biological properties, a limited number of studies on the conformational preference of 3,4-DHP have been reported. Kang and Park have performed a computational investigation on 3,4-DHP-containing dipeptide models to verify the conformational preferences and the cis–trans isomerization of this molecule in the gas phase and in chloroform solution.[72] The study suggested that 3,4-DHP is considerably less puckered than the prolyl ring, and the barriers for cis–trans isomerization in different environments are considerably different from the corresponding ones for proline. It also suggested that 3,4-DHP appears to favor a PPII-like helical conformation in a nonpolar environment.

4,5-DHP, another unsaturated proline analogue, has been found in bioactive compounds such as promysalin, an antimicrobial agent that is isolated from *Pseudomonas putida* and which exhibits a remarkable spectrum of growth inhibition activity in other *Pseudomonas*. The total synthesis of this novel antibiotic has been reported by Musso's group, who employed a straightforward approach to obtain the 4,5-DHP-containing fragment (**Scheme 10**, Part (a)).[73] Kubyshkin et al. exploited the same approach to obtain 4,5-DHP for the preparation of 4,5-difluoromethanoprolines, which can exert a substantial influence on the three-dimensional structure of proteins. The crucial step of this synthesis was the difluorocyclopropanation through the in situ generated difluorocarbene (Scheme 10, Part (b)).[74]

7. Conclusion and Outlook

In the past few years, the unique conformational properties of 4-substituted prolines and the possibility of exploiting the substituent at C-4 for further functionalization have offered new research opportunities in synthetic and medicinal chemistry, as well as in catalysis and chemical biology. Examples include interesting transformations of the pyrrolidine ring that lead to polyfunctionalized cores of bioactive products, the successful use of 4-substituted proline derivatives in enantioselective organocatalysis, and the significant influences of these ncAAs on the structures and properties of modified peptides. In particular, the commercially available 4-OH and 4-F amino acids have been successfully introduced into bioactive sequences to modulate their binding affinity to proper receptors and to enhance their physicochemical properties, providing new insights in the field of protein–protein interaction. Moreover, conjugation of peptides to nanoparticles or site-selective chemical ligation via Pro branches opens new avenues in materials science and medicinal chemistry.



(i) (a) 2-MEMC$_6$H$_4$CO$_2$H, Ghosez's reagent, CH$_2$Cl$_2$, 0 °C–rt, 1 h; (b) Et$_3$N, PhMe, 80 °C, 3 h; 82%. (ii) (a) LiBHEt$_3$, PhMe, –78 °C, 1 h; (b) TFAA, DIPEA, DMAP (cat.), –78 °C–rt, 3 h; 62%; (iii) LiOH, EtOH–H$_2$O, 0 °C–rt, 5 h; 97%; (iv) F$_2$ClCCO$_2$Na (27 equiv), diglyme, 177 °C

Scheme 10. Synthesis and Reactivity of 4,5-Dehydroproline (4,5-DHP). *(Ref. 73,74)*

8. Acknowledgment

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9. References

(1) Milner-White, E. J.; Bell, L. H.; Maccallum, P. H. *J. Mol. Biol*. **1992**, *228,* 725.
(2) (a) Dever, T. E.; Gutierrez, E.; Shin, B.-S. *Crit. Rev. Biochem. Mol. Biol*. **2014**, *49*, 413. (b) Renner, C.; Alefelder, S.; Bae, J. H.; Budisa, N.; Huber, R.; Moroder, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 923.
(3) De Poli, M.; Moretto, A.; Crisma, M.; Peggion, C.; Formaggio, F.; Kaptein, B.; Broxterman, Q. B.; Toniolo, C. *Chem.—Eur. J.* **2009**, *15*, 8015.
(4) Mothes, C.; Caumes, C.; Guez, A.; Boullet, H.; Gendrineau, T.; Darses, S.; Delsuc, N.; Moumné, R.; Oswald, B.; Lequin, O.; Karoyan, P. *Molecules* **2013**, *18*, 2307.
(5) Van Esseveldt, B. C. J.; Vervoort, P. W. H.; van Delft, F. L.; Rutjes, F. P. J. T. *J. Org. Chem.* **2005**, *70*, 1791.
(6) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.
(7) Pandey, A. K.; Naduthambi, D.; Thomas, K. M.; Zondlo, N. J. *J. Am. Chem. Soc.* **2013**, *135*, 4333.
(8) Tolomelli, A.; Ammazzalorso, A.; Bruno, I.; Amoroso, R. *Curr. Bioact. Compd*. **2016**, *12*, 146 (DOI: 10.2174/1573407212666160511155230).
(9) Bach, T. M. H.; Takagi, H. *App. Microbiol. Biotechnol*. **2013**, *97*, 6623.
(10) Geisler, S.; Ermert, J.; Stoffels, G.; Willuweit, A.; Galldiks, N.; Filss, C. P.; Shah, N. J.; Coenen H. H.; Langen K. J. *Curr. Radiopharm*. **2014**, *7*, 123.
(11) Krane, S. M. *Amino Acids* **2008**, *35*, 703.
(12) Houwaart, S.; Youssar, L.; Huettel, W. *ChemBioChem* **2014**, *15*, 2365.
(13) Klein, C.; Huettel, W. *Adv. Synth. Catal.* **2011**, *353*, 1375.
(14) Krishnamurthy, S.; Arai, T.; Nakanishi, K.; Nishino, N. *RSC Adv*. **2014**, *4*, 2482.
(15) Pippel, D. J.; Young, L. K.; Letavic, M. A.; Ly, K. S.; Naderi, B.; Soyode-Johnson, A.; Stocking, E. M.; Carruthers, N. I.; Mani, N. S. *J. Org. Chem.* **2010**, *75*, 4463.
(16) Chattopadhyay, A. K.; Menz, H.; Ly, V. L.; Dorich, S.; Hanessian, S. *J. Org. Chem.* **2016**, *81*, 2182, and references therein.
(17) Xie, N.; Taylor, C. M. *J. Org. Chem*. **2014**, *79*, 7459.
(18) Owens, N. W.; Stetefeld, J.; Lattová, E.; Schweizer, F. *J. Am. Chem. Soc.* **2010**, *132*, 5036.
(19) Gaunt, M. J.; Johansson, C. C. C.; McNally, A.; Vo, N. T. *Drug Discovery Today* **2007**, *12*, 8.

(20) Gómez-Bengoa, E.; Maestro, M.; Mielgo, A.; Otazo, I.; Palomo, C.; Velilla, I. *Chem.—Eur. J.* **2010**, *16*, 5333.
(21) Caruana, L.; Kniep, F.; Johansen, T. K.; Poulsen, P. H.; Jørgensen, K. A. *J. Am. Chem. Soc*. **2014**, *136*, 15929.
(22) Watts, J.; Luu, L.; McKee, V.; Carey, E.; Kelleher, F. *Adv. Synth. Catal.* **2012**, *354*, 1035.
(23) Gauchot, V.; Schmitzer, A. R. *J. Org. Chem*. **2012**, *77*, 4917.
(24) Xu, Y.; Hua, Z.; Zhang, J.; Yang, J.; Cao, Z.; Zhang, D.; He, L.; Craig, V. S. J.; Zhang, G.; Liu, G. *Chem. Commun*. **2016**, *52*, 3392.
(25) Gross, E.; Liu, J. H.; Alayoglu, S.; Marcus, M. A.; Fakra, S. C.; Toste, F. D.; Somorjai, G. A. *J. Am. Chem. Soc.* **2013**, *135*, 3881.
(26) Deiana, L.; Ghisu, L.; Afewerki, S.; Verho, O.; Johnston, E. V.; Hedin, N.; Bacsik, Z.; Córdova, A. *Adv. Synth. Catal.* **2014**, *356*, 2485.
(27) Ding, H.; Shigenaga, A.; Sato, K.; Morishita, K.; Otaka, A. *Org. Lett.* **2011**, *13*, 5588.
(28) Henry, C. E.; Xu, Q.; Fan, Y. C.; Martin, T. J.; Belding, L.; Dudding, T.; Kwon, O. *J. Am. Chem. Soc*. **2014**, *136*, 11890.
(29) Reddy, V. P.; Kumar, A. V.; Rao, K. R. *Tetrahedron Lett*. **2011**, *52*, 777.
(30) Satish, G.; Reddy, K. H. V.; Ramesh, K.; Kumar, B. S. P. A.; Nageswar, Y. V. D. *Tetrahedron Lett.* **2014**, *55*, 2596.
(31) Asad, N.; Samarakoon, T. B.; Zang, Q.; Loh, J. K.; Javed, S.; Hanson, P. R. *Org. Lett*. **2014**, *16*, 82.
(32) Zhao, Q.; Schafmeister, C. E. *J. Org. Chem*. **2015**, *80*, 8968.
(33) Romero-Estudillo, I.; Boto, A. *J. Org, Chem.* **2015**, *80*, 9379.
(34) Siebler, C.; Erdmann, R. S.; Wennemers, H. *Angew. Chem., Int. Ed*. **2014**, *53*, 10340.
(35) Umashankara, M.; Sonar, M. V.; Bansode, N. D.; Ganesh, K. N. *J. Org. Chem*. **2015**, *80*, 8552.
(36) Amgarten, B.; Rajan, R.; Martinez-Sáez, N.; Oliveira, B. L.; Albuquerque, I. S.; Brooks, R. A.; Reid, D. G.; Duer, M. J.; Bernardes, G. J. L. *Chem. Commun*. **2015**, *51*, 5250.
(37) Cui, B.; Yu, J.; Yu, F.-C.; Li, Y.-M.; Chang, K.-J.; Shen, Y. *RSC Adv*. **2015**, *5*, 10386.
(38) Martínez, J. I.; Reyes, E.; Uria, U.; Carrillo, L.; Vicario, J. L. *ChemCatChem* **2013**, *5*, 2240.
(39) Salwiczek, M.; Nyakatura, E. K.; Gerling, U. I. M.; Ye, S.; Koksch, B. *Chem. Soc. Rev.* **2012**, *41,* 2135.
(40) Hobart, D. B., Jr.; Merola, J. S. *Acta Cryst.* **2012**, E68, o2490.
(41) Ahmad, Z. A.; Yeap, S. K.; Ali, A. M.; Ho, W. Y.; Alitheen, N. B. M.; Hamid, M. *Clin. Dev. Immunol.* **2012**, Article ID 980250, 15 pages, DOI: 10.1155/2012/980250.
(42) Garber, E.; Demarest, S. J. *Biochem. Biophys. Res. Commun*. **2007**, *355*, 751.
(43) Edwardraja, S.; Sriram, S.; Govindan, R.; Budisa, N.; Lee, S.-G. *Mol. BioSyst*. **2011**, *7*, 258.
(44) Crespo, M. D.; Rubini, M. *PLoS One* **2011**, *6*, e19425.
(45) Bierbaum, G.; Sahl, H. G. *Curr. Pharm. Biotechnol*. **2009**, *10*, 2.
(46) Oldach, F.; Al Toma, R.; Kuthning, A.; Caetano, T.; Mendo, S.; Budisa, N.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2012**, *51*, 415.
(47) Hodges, J. A.; Raines, R. T. *J. Am. Chem. Soc*. **2005**, *127*, 15923.
(48) Chattopadhyay, S.; Murphy, C. J.; McAnulty, J. F.; Raines, R. T. *Org. Biomol. Chem*. **2012**, *10*, 5892.
(49) Holzberger, B.; Obeid, S.; Welte, W.; Diederichs, K.; Marx, A. *Chem. Sci*. **2012**, *3*, 2924.
(50) Borgogno, A.; Ruzza, P. *Amino Acids* **2013**, *44*, 607.
(51) Marchiani, A.; Antolini, N.; Calderan, A.; Ruzza, P. *Curr. Top. Pept. Protein Res*. **2009**, *10*, 45.
(52) Acevedo-Rocha, C. G.; Hoesl, M. G.; Nehring, S.; Royter, M.; Wolschner, C.; Wiltschi, B.; Antranikian, G.; Budisa, N. *Catal. Sci. Technol*. **2013**, *3*, 1198.
(53) Deepankumar, K.; Nadarajan, S. P.; Ayyadurai, N.; Yun, H. *Biochem. Biophys. Res. Commun.* **2013**, *440*, 509.
(54) Rubini, M.; Schärer, M. A.; Capitani, G.; Glockshuber, R. *ChemBioChem* **2013**, *14*, 1053.
(55) Lin, Y.-J.; Horng, J.-C. *Amino Acids* **2014**, *46*, 2317.
(56) Cahard, D.; Bizet, V. *Chem. Soc. Rev*. **2014**, *43*, 135.
(57) Deepankumar, K.; Nadarajan, S. P.; Mathew, S.; Lee, S.-G.; Yoo, T. H.; Hong, E. Y.; Kim, B.-G.; Yun, H. *ChemCatChem* **2015**, *7*, 417.
(58) Ayyadurai, N.; Deepankumar, K.; Prabhu, N. S.; Lee, S.-G.; Yun, H. *Chem. Commun*. **2011**, *47*, 3430.
(59) Tatum, N. J.; Yufit, D. S.; Cobb, S. L.; Coxon, C. R. *J. Fluorine Chem.* **2015**, *173*, 77.
(60) Larregola, M.; Moore, S.; Budisa, N. *Biochem. Biophys. Res. Commun.* **2012**, *421*, 646.
(61) Loureiro, J. A.; Crespo, R.; Börner, H.; Martins, P. M.; Rocha, F. A.; Coelho, M.; Carmo Pereira, M.; Rocha, S. *J. Mater. Chem. B* **2014**, *2*, 2259.
(62) Giampietro, L.; Amoroso, R.; Chiarini, M. *Curr. Bioact. Compd*. **2016**, *12*, 191.
(63) Jiraskova, P.; Gazak, R.; Kamenik, Z.; Steiningerova, L.; Najmanova, L.; Kadlcik, S.; Novotna, J.; Kuzma, M.; Janata, J. *Front. Microbiol.* **2016**, *7*, Article 276; DOI: 10.3389/fmicb.2016.00276.
(64) Fujii, K.; Sivonen, K.; Adachi, K.; Noguchi, K.; Sano, H.; Hirayama, K.; Suzuki, M.; Harada, K. *Tetrahedron Lett*. **1997**, *38,* 5525.
(65) Kang, Y. K.; Byun, B. J.; Park, H. S. *Biopolymers* **2010**, *95*, 51 (DOI: 10.1002/bip.21534).
(66) Liu, L.; Jokela, J.; Herfindal, L.; Wahlsten, M.; Sinkkonen, J.; Permi, P.; Fewer, D. P.; Døskeland, S. O.; Sivonen, K. *ACS Chem. Biol*. **2014**, *9*, 2646.
(67) Anas, A. R. J.; Kisugi, T.; Umezawa, T.; Matsuda, F.; Campitelli, M. R.; Quinn, R. J.; Okino, T. *J. Nat. Prod.* **2012**, *75*, 1546.
(68) Kling, A.; Lukat, P.; Almeida, D. V.; Bauer, A.; Fontaine, E.; Sordello, S.; Zaburannyi, N.; Herrmann, J.; Wenzel, S. C.; König, C.; Ammerman, N. C.; Barrio, M. B.; Borchers, K.; Bordon-Pallier, F.; Brönstrup, M.; Courtemanche, G.; Gerlitz, M.; Geslin, M.; Hammann, P.; Heinz, D. W.; Hoffmann, H.; Klieber, S.; Kohlmann, M.; Kurz, M.; Lair, C.; Matter, H.; Nuermberger, E.; Tyagi, S.; Fraisse, L.; Grosset, J. H.; Lagrange, S., Müller, R. *Science* **2015**, *348*, 1106.
(69) Goodreid, J. D.; Janetzko, J.; Santa Maria, J. P., Jr.; Wong, K. S.; Leung, E.; Eger, B. T.; Bryson, S.; Pai, E. F.; Gray-Owen, S. D.; Walker, S.; Houry, W. A.; Batey, R. A. *J. Med. Chem.* **2016**, *59*, 624.
(70) Kuttan, R.; Panikkar, B.; Binitha, P. P. *SpringerPlus* **2015**, *4*, 546.
(71) De Zoysa, G. H.; Washington, V.; Lewis, G.; Sarojini, V. *Plant Pathol.* **2013**, *62*, 767.
(72) Kang, Y. K.; Park, H. S. *Pept. Sci.* **2009**, *92,* 387.
(73) Kaduskar, R. D.; Dhavan, A. A.; Dallavalle, S.; Scaglioni, L.; Musso, L. *Tetrahedron* **2016**, *72*, 2034.
(74) Kubyshkin, V. S.; Mykhailiuk, P. K.; Afonin, S.; Ulrich, A. S.; Komarov, I. V. *Org. Lett.* **2012**, *14*, 5254.

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Alessandra Tolomelli was born in Bologna, Italy. She completed her master's degree in chemistry in 1994 and her Ph.D. degree in chemical sciences in 1999 at the Alma Mater Studiorum - University of Bologna, working in the group of Professor G. Cardillo. In 1997, she joined Professor J. Konopelski's laboratories at the University of California, Santa Cruz, as a visiting student working on the synthesis of pharmacologically active polypeptides. Alessandra has been Assistant Professor in the Department of Chemistry "Giacomo Ciamician" at the Alma Mater Studiorum - University of Bologna since 1999, focusing her research work on bioactive peptides and peptidomimetics containing five-membered-ring heterocycles and on β-amino acid synthesis and functionalization.

Lucia Ferrazzano was born in Foggia, Italy. She began her undergraduate study in chemistry in Bologna, where she received her B.Sc. degree in 2011 (under the supervision of Prof. M. Lombardo) and her M.Sc. degree in organic chemistry in 2013 (under the supervision of Dr. A. Tolomelli). Her Ph.D. studies (with Dr. A. Tolomelli at Alma Mater Studiorum - University of Bologna) focused on the synthesis of N-heterocycles as scaffolds for more complex bioactive compounds, from antibiotics to targeting ligands. Lucia is currently a research fellow in the Department of Chemistry "Giacomo Ciamician" at the Alma Mater Studiorum - University of Bologna. She is presently working on the synthesis of fluorescent, self-assembled amphiphilic nanoparticles as imaging agents for conjugation with targeting ligands in collaboration with the group of Prof. Luc Brunsveld at the Eindhoven University of Technology.

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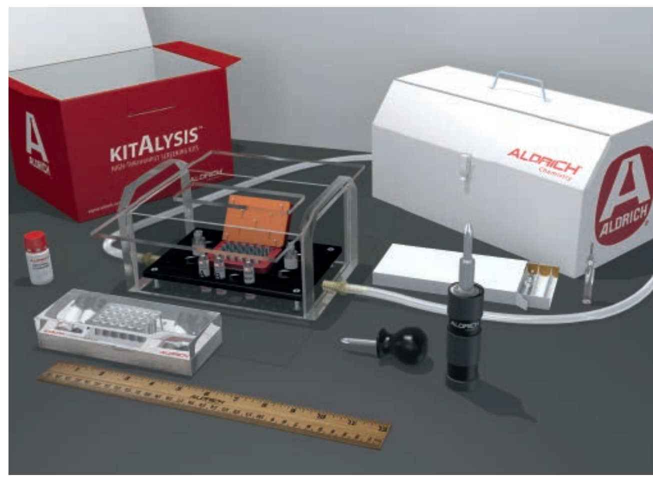

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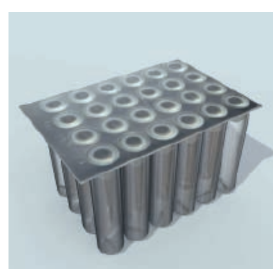

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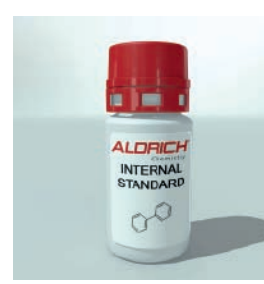

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Recent Advances in Alkene Metathesis for Natural Product Synthesis—Striking Achievements Resulting from Increased Sophistication in Catalyst Design and Synthesis Strategy

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Keywords. metathesis; natural product synthesis; strategy; catalysts; stereocontrol.

Abstract. Every year, advances in the design of metathesis catalysts and insightful strategic applications of alkene metathesis work in concert to drive the field into new and exciting directions. From ring-closing to enyne and cross-metathesis, and from late-stage steps that directly furnish natural products to early transformations that supply starting materials, metathesis can play a role at every stage of a synthesis. This review will highlight some of the particularly innovative or surprising ways in which alkene metathesis has been implemented in natural product synthesis.

Outline

1. Introduction

The importance of alkene metathesis to synthetic chemistry cannot be overstated. The pioneering work by Chauvin, Grubbs, and Schrock on improving our mechanistic understanding of metathesis, on developing novel metathesis catalysts, and on developing metathesis applications garnered them the 2005 Nobel Prize in Chemistry.[1] For over two decades, organic chemists have harnessed the power of alkene metathesis in academic and industrial settings, and new applications continue to be reported on a regular basis. In the decade following Nicolaou's extensive review of the use of alkene metathesis in natural product synthesis,[2] the number of examples of such uses has grown at a dizzying rate. Books[3–7] and reviews[8–11] have been written to chronicle the breadth of applications of alkene metathesis. Herein, we aim to provide a structured look at a select group of alkene metathesis reactions that are employed in natural product synthesis. We discuss accomplishments from the past ten years that exemplify groundbreaking strategic applications of alkene metathesis and/or a particularly impressive reactivity or selectivity in metathesis processes. We have organized the discussion by transformation type: (i) ring-closing metathesis for normal- and medium-size rings, (ii) metathesis in the synthesis of macrocycles, (iii) tandem metatheses, (iv) ring-opening metathesis, and (v) cross-metathesis.

We note here that our purpose is only to demonstrate the power of metathesis for complex-molecule synthesis using select examples.

Furthermore, although closely related, alkyne metathesis[12] is not a prime focus of this article, and select examples are provided only to provide context. In this short review, only a small number of the myriad and incredibly versatile metathesis catalysts developed to date are showcased (**Figure 1**).

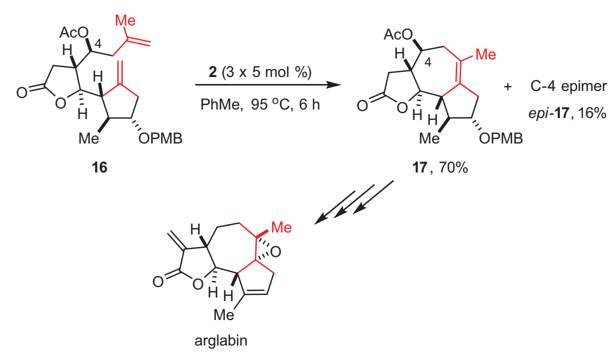
2. Ring-Closing Metathesis

By the mid-2000s, ring-closing metathesis (RCM) to form unstrained, normal-size rings was a well-established, reliable tool for synthesis. The examples below were selected because they have pushed forward the frontiers of what was thought possible in terms of reactivity and/or selectivity.

Reiser's group reported the first enantioselective synthesis of the complex sesquiterpenoid arglabin (**Scheme 1**).[13] A challenging RCM of two 1,1-disubstituted alkenes to forge a tetrasubstituted alkene within a 7-membered ring serves as a key step. A particularly direct, stereoselective synthesis of the RCM precursor, **16**, set the stage for this challenging ring closure. This metathesis required three separate charges (5 mol %) of the Grubbs second-generation catalyst[14] (**2**) and inert-gas sparging at 95 °C to successfully provide the tetrasubstituted alkene in **17**. Epoxidation of the tetrasubstituted alkene and installation of the requisite functional groups completed the synthesis of arglabin. At the time, and to this day, this RCM is striking for its efficient generation of a ring size that can often be slow to form, while simultaneously forging a tetrasubstituted alkene.

Stoltz's group was among the first to employ the RCM of alkenyl chlorides in natural product synthesis in the course of their innovative synthesis of elatol.[15] The authors built on Weinreb's earlier work which had demonstrated the general feasibility and utility of this type of RCM.[16] To access the salient chlorinated cyclohexene of elatol, the researchers utilized an RCM between two 1,1-disubstituted alkene groups in **18** (**Scheme 2**), one with two carbon substituents and one bearing an alkyl substituent and a chlorine atom. Substrate **18** underwent RCM in the presence of **3** to provide intermediate **19** containing a tetrasubstituted alkene. Following its introduction, catalyst **3** has become an important addition to the arsenal of available metathesis catalysts.[3,17]

In one of a few reported examples of stereochemical equilibration–RCM, Tang, Chen, Yang and co-workers reported the synthesis



Scheme 1. Efficient Formation by RCM of a Cycloheptene with a Tetrasubstituted Double Bond in Reiser's Enantioselective Synthesis of Arglabin. *(Ref. 13)*

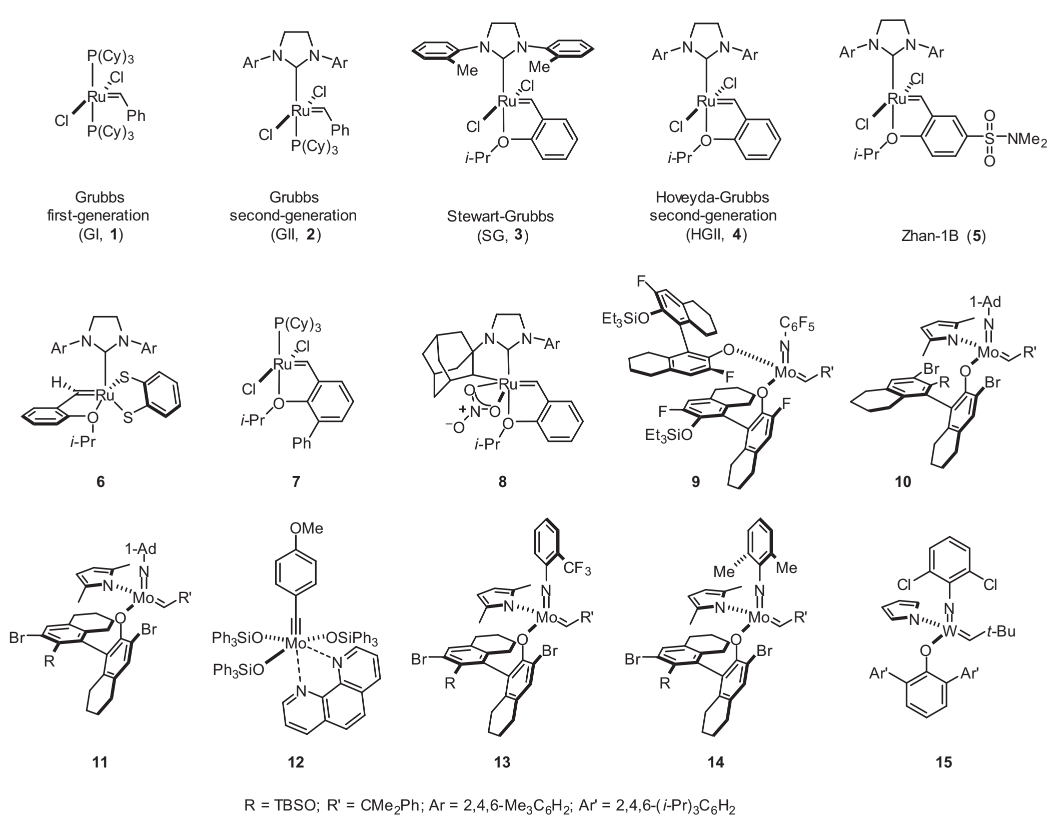
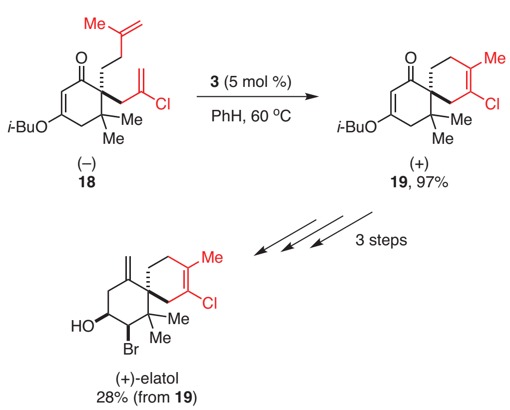


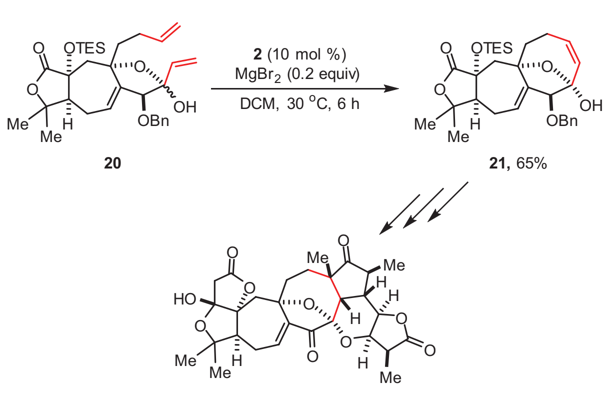
Figure 1. Structures of Metathesis Catalysts Featured in This Review.

of schindilactone A, in which that process provided the central oxabicyclo[4.2.1]nonane ring system of the target (**Scheme 3**).[18] In the presence of GII (**2**), the desired RCM gave **21**, with in situ epimerization producing a single diastereomer at the hemiketal carbon.[19] The resulting cyclooctene later participated in a Pauson–Khand reaction to annulate another one of the rings of the natural product. Other examples of stereochemical equilibration during RCM mostly involve epimerization of stereogenic centers adjacent to ketones.[20]

In their synthesis of sculponeatin N, a bioactive diterpene, Thomson and co-workers accomplished the equivalent of a butadiene–cyclopentenone Diels–Alder cycloaddition by a sequence featuring an unusual equilibrating diastereoselective RCM reaction (**Scheme 4**).[21] When **24** could not be made by the more straightforward Diels–Alder approach, sequential alkene installation converted the spiro-cyclopentenone precursor to the triene-containing RCM substrate **22**. Subjecting triene **22** to metathesis conditions with GII (**2**) afforded the desired cis-fused cyclohexene-containing **24**. The authors commented that spirocyclopentene **23** could be isolated by stopping the reaction early; however, the trans-fused cyclohexene was never observed. This particularly creative workaround to the unsuccessful cycloaddition also sets a key quaternary stereogenic center of the target.



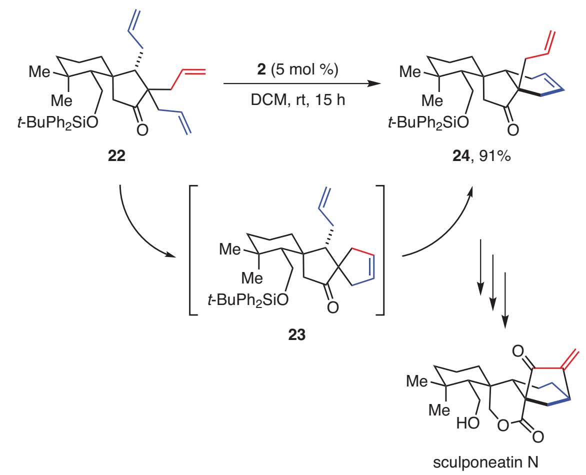
Scheme 2. First Application of the RCM of Alkenyl Chlorides in Natural Product Synthesis as Demonstrated in Stoltz's Asymmetric Total Synthesis of (+)-Elatol. *(Ref. 15)*



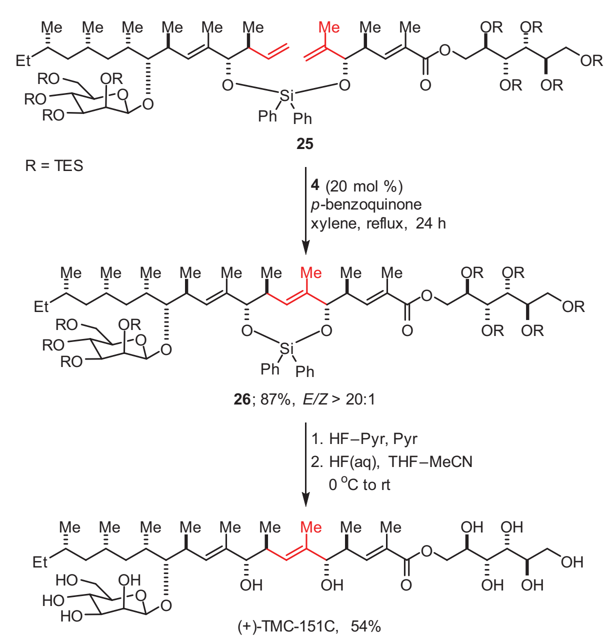
Scheme 3. Yang's Diastereoselective Synthesis of the Fully Functionalized CDE Ring System in (±)-Schindilactone A by RCM. *(Ref. 18)*

3. Tethered Ring-Closing Metathesis

Temporary tethers are enormously useful for increasing the rates of slow bimolecular reactions, and are advantageous with respect to both chemo- and stereoselectivity. In Kobayashi's total synthesis of (+)-TMC-151C, a silicon-tethered RCM reaction convergently assembled the polyketide natural product from two fragments of similar complexity in a reaction that would have been virtually impossible to achieve in a bimolecular setting (**Scheme 5**).[22] Further, the tether served to reinforce the selectivity of the alkene geometry in forming the trisubstituted alkene. The RCM reaction of **25** was effected with the Hoveyda–Grubbs second-generation catalyst (**4**),[23] and global desilylation of **26** afforded (+)-TMC-151C directly.



Scheme 4. Diastereoselective RCM as a Butadiene–Cyclopentenone Diels–Alder Equivalent in Thomson's Total Synthesis of Sculponeatin N. *(Ref. 21)*



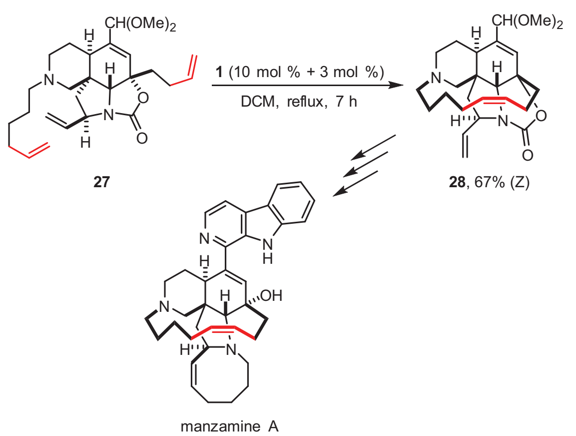
Scheme 5. Silicon-Tethered RCM in Kobayashi's Convergent Total Synthesis of (+)-TMC-151C. *(Ref. 22)*

4. Macrocycles

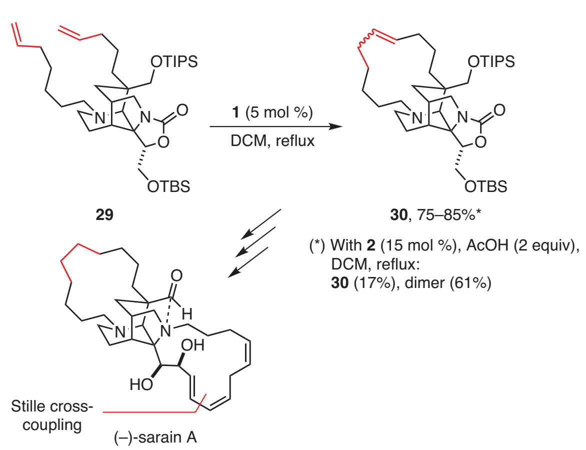
4.1. Macrocyclic Ring Closure by Metathesis

4.1.1. The Alkylpiperidine Alkaloids

A number of structurally related alkaloids, including the manzamines,[24a] sarains,[24b] haliclonacyclamines,[24c] and haliclonins,[24d] belong to the family of alkylpiperidine natural products. In addition to an alkylpiperidine subunit, these secondary metabolites usually contain one or more macrocycles. The synthesis of the macrocycle(s) of the various alkylpiperidines has been accomplished by ring-closing alkene or alkyne metathesis numerous times, by employing various strategies aimed at accomplishing the transformation selectively for the challenging *Z*-configured alkene present in many of the natural products. In 1999, Martin and co-workers were the first to utilize a macrocyclizing RCM reaction in the synthesis of an alkylpiperidine natural product (**Scheme 6**).[25] Since then, few have achieved the same level of substrate-controlled *Z*-selectivity Martin's group observed for the conversion of **27** to **28**, without the use of modern *Z*-selective metathesis catalysts. In the past decade, a number of syntheses of alkylpiperidine natural products relying on RCM reactions for macrocyclic ring-closure have been reported.



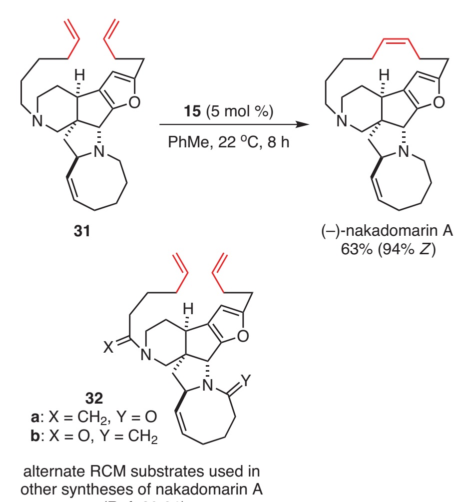
Scheme 6. Macrocycle Ring Closure by Metathesis, as Illustrated in Martin's Total Synthesis of Manzamine A, an Alkylpiperidine Alkaloid. *(Ref. 25)*



Scheme 7. Macrocycle Ring Closure by Metathesis in Overman's Total Synthesis of (–)-Sarain A. *(Ref. 26)*

Overman's group reported the first total synthesis of the incredibly complex alkaloid sarain A, which exhibits antibacterial, insecticidal, and antitumor activities. In this work, the saturated 13-membered-ring of the natural product was formed via RCM and subsequent hydrogenation (**Scheme 7**),[26] while the second, 14-membered ring of sarain A containing the skipped-triene was ultimately constructed by a Stille cross-coupling, and not by metathesis. In the RCM step to generate the saturated macrocycle, the use of catalyst **2** with **29** led to significant quantities of dimeric byproducts, in addition to some of the desired macrocyclization product, **30**. Switching to the less active, first-generation Grubbs catalyst (**1**)[27] diminished the amount of dimer formed and, after optimization, a 75–85% yield of the macrocyclization product, **30**, was obtained. It was postulated that the use of the more active catalyst **2** gave a thermodynamic ratio of products, as supported by subjection of either the isolated macrocycle or dimer to the same reaction conditions, which yielded in each case a similar mixture of macrocycle and dimer. The less active **1**, however, may have been unable to initiate metathesis on the resultant internal alkene, making the RCM step effectively irreversible, and providing only the desired RCM product.

Nishida's first-generation synthesis of nakadomarin A[28] was published in 2003, and, since then, many other groups have contributed impressive syntheses of their own. The *Z*-alkene-containing, 15-membered ring of nakadomarin A has been constructed by ring-closing alkene metathesis,[28–32] ring-closing alkyne (RCAM) metathesis and semi-reduction,[33–34] and by macrolactamization.[35] In their pioneering work, Nishida and co-workers achieved a *Z*:*E* ratio of 1:1.8 by using catalyst **1** to close the 15-membered ring from **32a** (**Scheme 8**). Subsequent syntheses by the groups of Kerr,[29] Dixon,[30] and Zhai,[31] using either substrate **32a**, **32b**, or **31** resulted in only slightly improved *Z*:*E* ratios (up to 2:1). Nilson and Funk[33] were the first to employ a two-step RCAM–semi-reduction to afford the *Z* alkene as the sole product. Dixon's group embraced this approach as well,[34] but later reported a collaborative effort with the Schrock and Hoveyda labs, making use of the recently developed *Z*-selective, tungsten-based metathesis catalyst



Scheme 8. Final Step in the Synthesis of (–)-Nakadomarin A by Catalyst-Controlled *Z*-Selective RCM. *(Ref. 36)*

15.[36] Use of this catalyst with **31** produced (–)-nakadomarin A in 63% yield and 94% *Z*-selectivity, the highest *Z*-selectivity observed in a synthesis of nakadomarin A by RCM.

Huang and co-workers reported the first asymmetric total synthesis of the alkaloid (–)-haliclonin A using catalyst **1** for RCM, followed by hydrogenation, to form the saturated macrocycle of the molecule.[37] At a later point in the synthesis, an RCAM with molybdenum benzylidyne catalyst **12**[38] closed the second, unsaturated, 15-membered ring of compound **33**, affording intermediate **34**. Partial hydrogenation installed the desired *Z*-alkene geometry (**Scheme 9**).[37] It is worth noting that the alkyne metathesis and partial hydrogenation steps were both tolerant of the unconjugated alkene, and no isomerization was noted.

4.1.2. The Ansatrienins

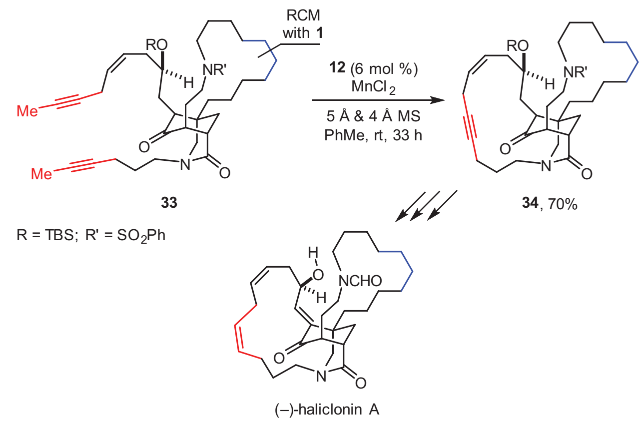
The ansatrienins,[39a–c] a subclass of the ansamycins,[39] bear an all-trans conjugated triene subunit in their macrocyclic core. Cross-coupling[40] and alkenylation[41] approaches to this triene fragment have been successfully employed in syntheses of some of the members of this class of natural products. The application of an RCM between two diene units to furnish an all-trans triene—an approach that certainly would appear to harbor risk—was central to two successful syntheses.[42–43] Hayashi and co-workers reported an asymmetric total synthesis of the anticancer drug (+)-cytotrienin A, where RCM successfully provided the macrocyclic ring containing the all-trans triene (**eq 1**).[42] Krische's group rapidly assembled a related tetraene-containing RCM substrate using their C–H functionalization methodology, and completed the synthesis of trienomycins A and F (not shown).[43]

4.1.3. Other Natural Products

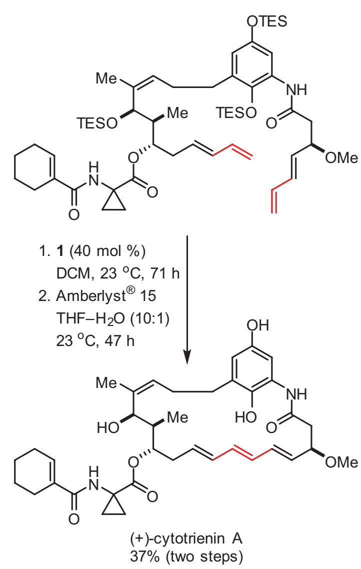
Krische's group utilized a ring-closing enyne metathesis (RCEYM) to form the macrocyclic ring in their total synthesis of 6-deoxyerythronolide B (**Scheme 10**).[44] Some of the challenges that were overcome in this synthesis include: (i) Terminal alkene isomerization from **35** was the only observed product in the absence of ethylene. (ii) Enyne metathesis proceeded at 80 °C, converting the alkyne into a terminal diene but not into the macrocycle. To overcome this second challenge, the ethylene atmosphere was replaced with nitrogen after the enyne metathesis was complete, and the reaction mixture was then heated to 110 °C, accomplishing the desired ring closure. The macrocyclic diene **36** was then converted in a few steps into 6-deoxyerythronolide B. Together with Krische's efficient methods for assembling the precursor, this RCEYM process was strategically advantageous.

In their synthesis of (+)-neopeltolide, Fuwa and co-workers utilized a chemoselective cross-metathesis (CM) to form an α,β-unsaturated ester from a terminal alkene and methyl acrylate, ultimately facilitating pyran formation by an oxa–Michael cyclization (**Scheme 11**).[45] Since the pendant styrenyl group in substrate **37** was also poised to competitively undergo an undesired RCM, the ability of the proximal hydroxyl group to form an intramolecular H….Cl interaction between the OH and the Cl in the substrate-bound catalyst was key to accomplishing the selective CM leading to **38**. When the proximal OH was protected with a BOM group, a significant amount (46–71%) of the ring-closed product was formed. Finally, RCM of intermediate **39** with catalyst **2** afforded the macrocyclic, trisubstituted alkene **40**, which was then hydrogenated to the saturated macrolactone, constituting a formal synthesis of (+)-neopeltolide. Hoveyda, Schrock, and Yu also reported the synthesis of (+)-neopeltolide, making extensive use of alkene metathesis to construct the natural product (not shown).[46]

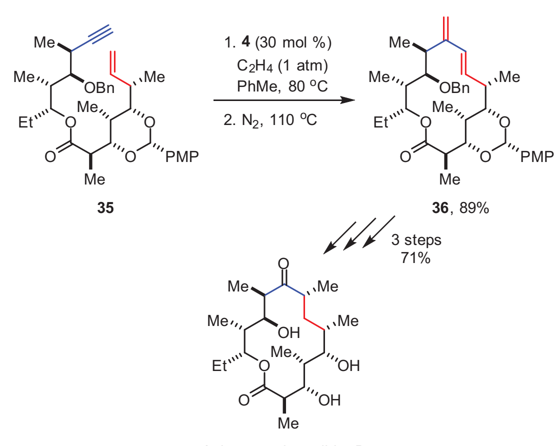
Kita and Kigoshi reported an asymmetric total synthesis of the marine macrolides mycalolides A and B, and evaluated both an RCM



Scheme 9. Ring-Closing Alkyne Metathesis (RCAM) Step in Huang's Asymmetric Total Synthesis of (–)-Haliclonin A. *(Ref. 37)*



eq 1 *(Ref. 42)*



Scheme 10. Ring-Closing Enyne Metathesis (RCEYM) in Krische's Total Synthesis of the Polyketide 6-Deoxyerythronolide B. *(Ref. 44)*

and a CM approach (**Scheme 12**).[47] In their work, the RCM strategy suffered from low selectivity for the desired alkene geometry; even after extensive optimization, it proceeded in only a 63% yield and an *E:Z* ratio of 2.7:1. The CM approach was more successful; the reaction of **41** with **42** proceeded to give **43** in 77% yield and an *E:Z* ratio of 5:1. Although adhering to the rules[48] for best-case CM substrates (Type 1 and Type 2 alkenes), this CM is remarkable for the extreme complexity of both reaction partners used in close to equimolar amounts.

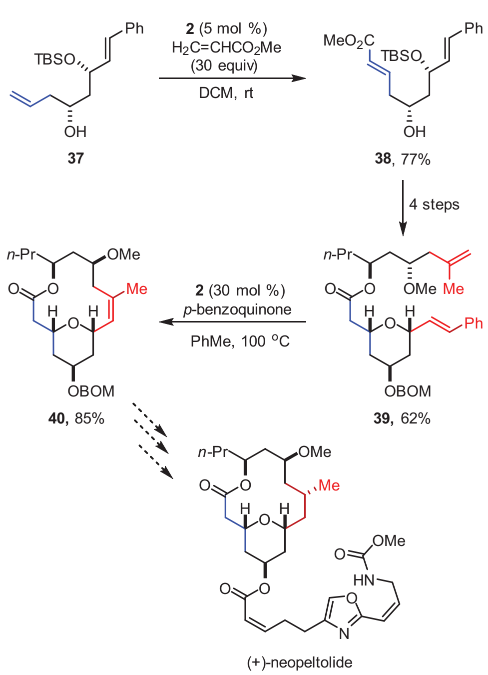
Krische and co-workers synthesized swinholide A using their asymmetric, hydrogen-mediated C–C bond-forming methodology and alkene metathesis each at multiple stages (**Scheme 13**).[49] An enantioselective, iridium-catalyzed allylation provided product **46**, which underwent CM with acrolein catalyzed by **2** to afford dihydropyran hemiacetal **47**; this product was elaborated into a key fragment for convergent coupling. Construction of a second fragment began with a diastereo- and site-selective iridium-catalyzed allylation to supply **48**, which was subjected to CM with *cis*-1,4-diacetoxy-2-butene. The resulting allylic acetate **49** underwent palladium-catalyzed Tsuji–Trost cyclization to give a *cis*-2,4-disubstituted vinyl tetrahydropyran **50**. The two fragments were elaborated and coupled, yielding the final metathesis substrate, **51**. In the presence of **4**, intermediate **51** was converted, via sequential CM–RCM, into the dimeric macrodiolide swinholide A in 25% yield, as well as via an RCM of the monomer, into the macrolide hemiswinholide in 43% yield. This final step that directly affords two natural products is striking for its efficiency in the presence of two other alkenes, a host of unprotected hydroxyl groups, and numerous other Lewis basic sites.

4.1.4. Tiacumicin B Aglycon

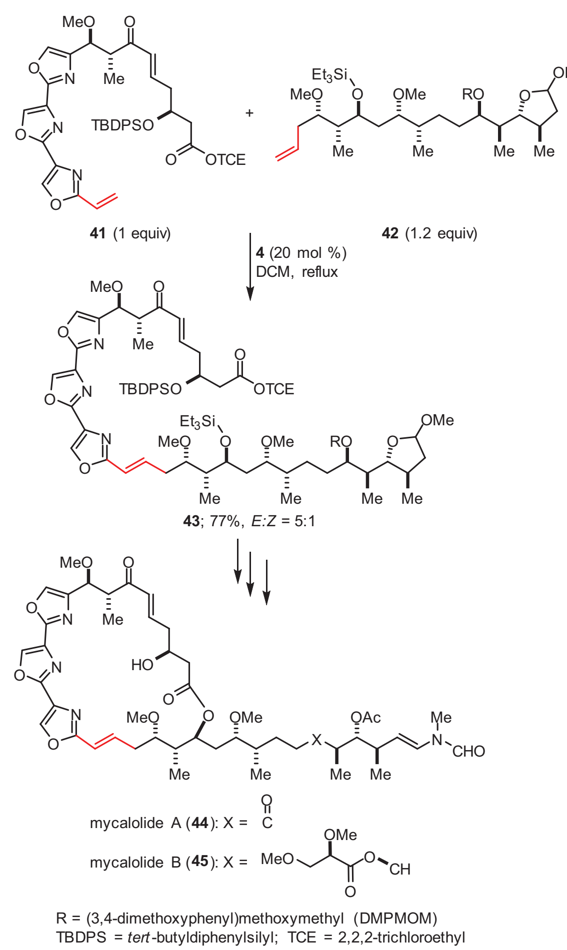
In 2015, three research groups concurrently reported syntheses of tiacumicin B, with each group utilizing alkene metathesis in their synthesis (**Scheme 14**).[50–52] Zhu and co-workers targeted a protected form of the tiacumicin B aglycon,[50] whereby ester-linked ring-closing metathesis precursor **52a** underwent the desired RCM macrocyclization in the presence of **2**, with only deprotection required to complete the synthesis. Gademann's group targeted the protected tiacumicin B aglycon and utilized a macrocyclic RCM similar to that used in Zhu's synthesis, closing the diene fragment of **52b**.[51] Gademann's substrate underwent a more efficient ring closure, reminding us of the significant effect that different protecting group strategies can have on macrocyclization by RCM (and indeed by any method). The authors also reported a procedure to isomerize the undesired *Z* alkene to the *E* alkene allowing material to be recycled. Altmann's group reported a synthesis of the tiacumicin B aglycon, in which they employed a CM to assemble the linear precursor to the natural product.[52] Complex **4** catalyzed the synthesis of tetraene fragment **53**, attaining the highest *E/Z* ratio of the three syntheses (6.7:1). Yamaguchi esterification with a vinyl boronate containing fragment and Suzuki macrocyclization completed the synthesis.

4.2. Macrocyclic Ring Closure by Other Means

Hoye and co-workers synthesized (+)-peloruside A—a cytotoxic marine macrolide that is being evaluated for use against paclitaxel-resistant cancers—by utilizing a relay RCM between the tethered alkenes to



Scheme 11. CM and RCM Key Steps in Fuwa's Concise Total Synthesis of (+)-Neopeltolide. *(Ref. 45)*

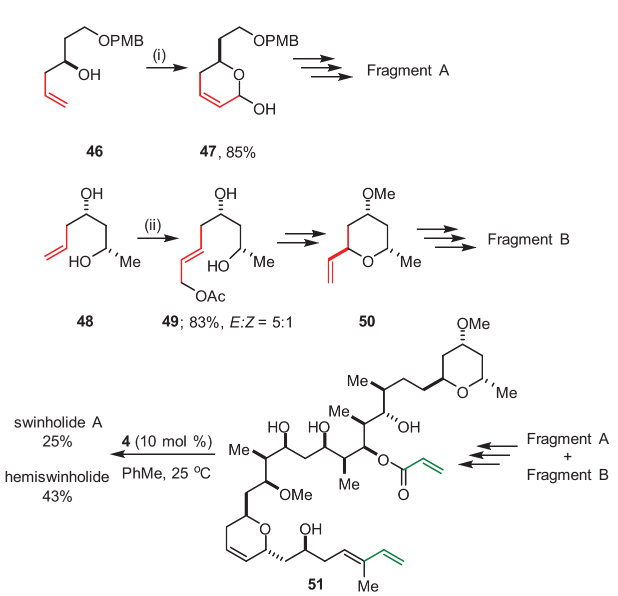


Scheme 12. CM between Two Advanced Intermediates in Kita and Kigoshi's Total Synthesis of Mycalolides A and B. *(Ref. 47)*

construct the trisubstituted alkene group present in the natural product (**Scheme 15**).[53] Two tethering approaches, one via a silaketal (**54**) and the other via an ester linkage (**55**), were investigated. Each tether contained an (*R*)-citronellene derived tail, initially incorporated to enable efficient enzymatic resolution of the diastereomeric mixtures of alcohols. This tail was cleaved during the metathesis step and the two routes converged to a substrate with differentially protected alcohol groups. This fragment was coupled to a polyol fragment via an aldol addition, after which a macrolactonization–deprotection sequence completed the synthesis of peloruside A.

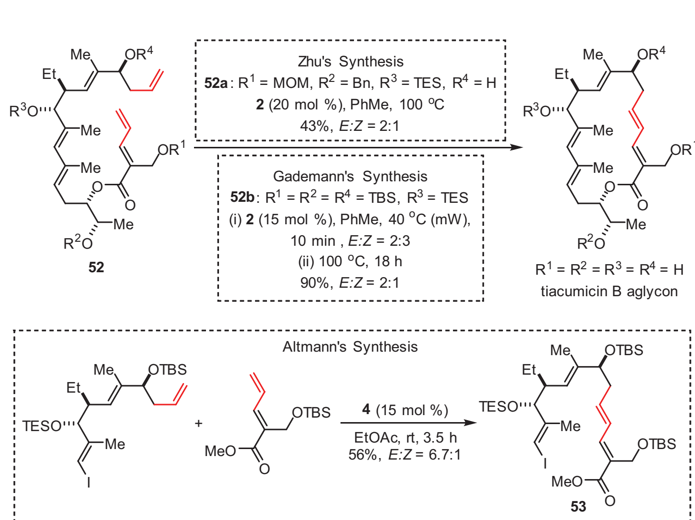
Volchkov and Lee completed the asymmetric total synthesis of (–)-amphidinolide V by employing metatheses at multiple stages in the synthesis (**Scheme 16**).[54] RCEYM of silyl-tethered enyne **56** formed the desired silacycle **57**, which was ring-opened and coupled to provide polyene **58**. RCM of the latter compound led to an 8-membered silacycle (**59**). Subsequent elaboration, including allylic transposition, gave a fragment corresponding to roughly half of the target. Acetylenic intermediate **60** was subjected to enyne metathesis with ethylene, catalyzed by **2** to afford the salient 1,3-diene, **61**. Ultimately, fragments **59** and **61** were combined to complete the synthesis of (–)-amphidinolide V. Another member of the amphidinolide family, (–)-amphidinolide K, was synthesized by Lee and co-workers, whose work featured strategic use of enyne metathesis (not shown).[55]

In their total synthesis of disorazole C_1, an antifungal and anticancer macrocyclic natural product, Hoveyda and co-workers employed a number of metathesis steps to tackle the construction of the C_2-symmetric dimeric macrocycle (**Scheme 17**).[56] The disorazole C_1 ring contains two conjugated triene units, within which are found four *Z*-configured carbon–carbon double bonds. The convergent, stereoselective route employed required the RCM of a *Z*-vinyl iodide tethered to a *Z*-vinylborane in the precursor **68**, which was assembled with the help of a number of *Z*-selective cross-metathesis steps. A

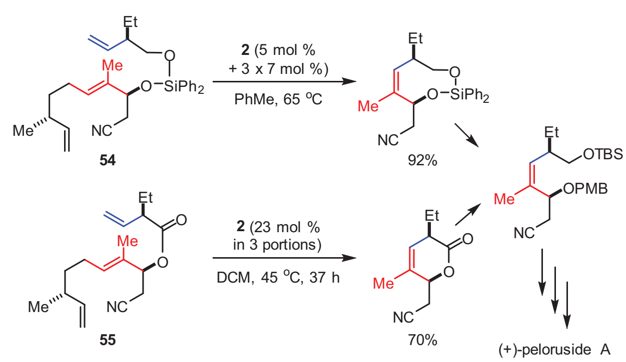


(i) acrolein (3 equiv), **2** (2 mol %), DCM, 25 °C.
(ii) *cis*-($AcOCH_2CH)_2$ (4 equiv), **2** (3 mol %), DCM, 50 °C.

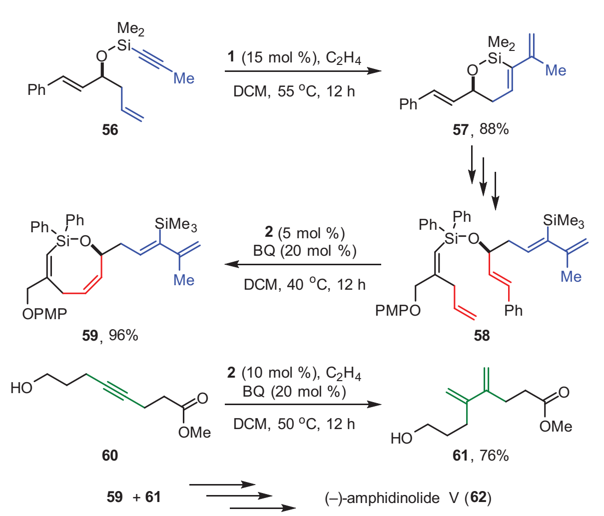
Scheme 13. CMs and CM–RCM Sequences Employed in Krische's Total Synthesis of the Actin-Binding Marine Polyketide Swinholide A. *(Ref. 49)*



Scheme 14. RCM and CM in the Synthesis of Tiacumicin B Aglycon. *(Ref. 50–52)*



Scheme 15. Relayed, Tethered RCMs in Hoye's Total Synthesis of Peloruside A. *(Ref. 53)*



Scheme 16. Enyne Metatheses and RCM in Lee's Asymmetric Total Synthesis of (–)-Amphidinolide V. *(Ref. 54)*

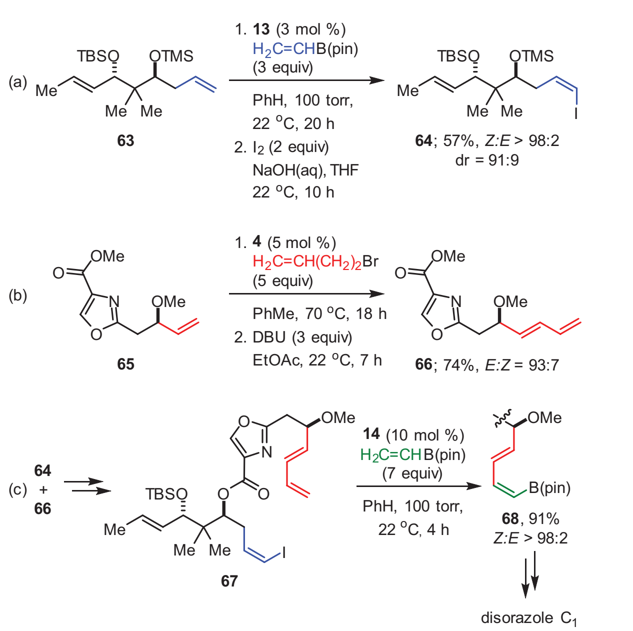
double Suzuki cross-coupling strategy served to dimerize compound **68**, affording less than 2% of the unimolecular cross-coupling product. A careful deprotection led to completion of the synthesis of disorazole C_1.

5. Tandem Metatheses

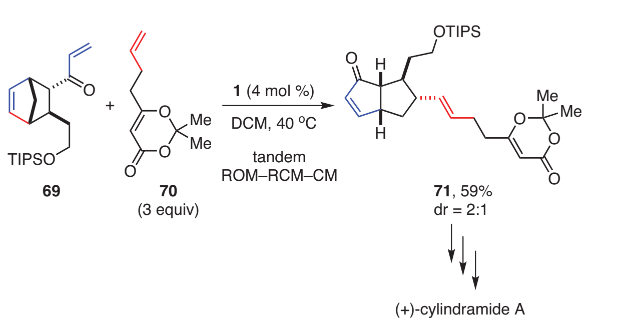
In their synthesis of (+)-cylindramide A, Hart and Phillips developed a tandem ROM–RCM–CM sequence to rearrange a bicyclo[2.2.1]-heptene into the bicyclo[3.3.0]octene core of (+)-cylindramide A (**eq 2**).[57] In the presence of GI (**1**) and the butenyl-substituted dioxinone **70**, the metathesis cascade substrate **69** was transformed into the desired bicyclo[3.3.0]octene **71** with incorporation of the butenyl-substituted dioxinone. This work showcases how the reliably predictable stereochemical relationships generated in the course of Diels–Alder reactions can be transferred to very different ring topologies via tandem metathesis chemistry.

5.1. Enyne Metatheses

Ramonanins A–D are spirocyclic phenylpropanoid tetramers that show cytotoxic activity against lines of human breast cancer cells. In the



Scheme 17. *Z*-Selective CM and RCM in Hoveyda's Convergent, Diastereoselective, and Enantioselective Total Synthesis of Disorazole C_1. *(Ref. 56)*



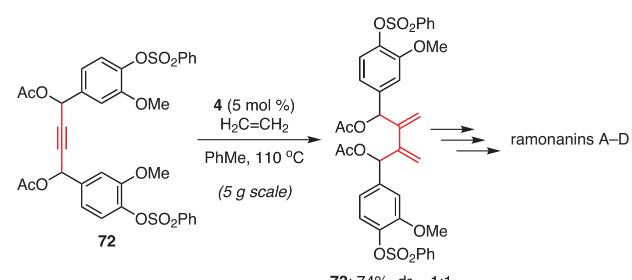
eq 2 *(Ref. 57)*

first total synthesis of these lignan natural products, Sherburn's group targeted a dimethylene tetrahydrofuran intermediate that could be dimerized to a mixture of the different ramonanins (**eq 3**).[58] Starting from vanillin, the authors arrived at **72**, the alkyne-bridged diacetate substrate for enyne metathesis, after four steps. Enyne metathesis with ethylene, catalyzed by **4**, furnished the diene diacetate **73**. Hydrolysis of the diacetate, tetrahydrofuran ring formation from the resultant diol, and cleavage of the benzenesulfonate protecting groups afforded the desired dimerization precursor, which was taken to generate the natural product targets by intermolecular Diels–Alder cycloadditions.

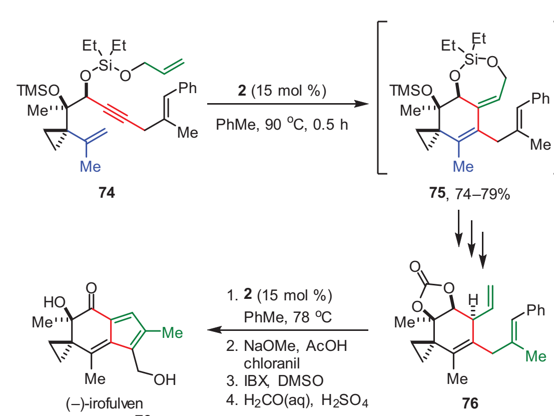
5.2. Ene-yne-ene Metatheses

Tandem, ring-closing ene-yne-ene metathesis (RCEYEM) sequences are powerful for the construction of bicyclic ring systems and have been applied in a number of different natural product syntheses in the past decade.

Movassaghi's group reported particularly non-obvious metathesis-based approaches to the semisynthetic illudin derivatives (–)-acylfulvene and (–)-irofulven, the latter being an especially active antitumor agent against a variety of solid tumors (**Scheme 18**).[59] Silyl-tethered RCEYEM substrate **74** underwent the desired metathesis cascade catalyzed by GII (**2**) providing **75**, which was then converted into **76** via a reductive allylic transposition. The resulting intermediate **76** underwent RCM in the presence of **2** to form the cyclopentane ring of the illudins. Oxidation with DDQ or chloranil and IBX (*ortho*-iodoxybenzoic acid) furnished (–)-acylfulvene (not shown), and reaction of (–)-acylfulvene with aqueous formaldehyde provided (–)-irofulven.



eq 3 *(Ref. 58)*



Scheme 18. RCEYEM and RCM in Movassaghi's Enantioselective Total Synthesis of (–)-Irofulven. *(Ref. 59)*

Spectacular use of RCEYEM was demonstrated in the enantioselective total synthesis of three tetracyclic kempene diterpenes (**Scheme 19**).[60] Tandem RCEYEM substrate **77**, when heated in the presence of GII (**2**), gave rise to intermediate **78**, possessing the tetracyclic core of the kempenes. The key to the success of this complexity-building transformation was the substitution pattern of each of the unsaturated reaction partners, which was carefully considered so that the order of reaction was the proper one to yield the desired product outcome. Following the synthesis of **78**, protecting group exchange, ketone reduction, and acylation afforded the kempene natural products.

Yang, Li, and co-workers reported the stereoselective total syntheses of the alkaloids (–)-flueggine A and (+)-virosaine B, derived in a biomimetic fashion from (–)-norsecurinine and (+)-allonorsecurinine, which were each constructed via relay RCEYEM.[61] An *N*-Boc-protected, commercially available, D-proline-derived Weinreb amide served as the starting material to construct the RCEYEM substrate possessing a heptadienoate chain. This tethering strategy successfully controlled the direction of ring closure in the cascade process. Both diastereomers of the RCEYEM substrate could be successfully carried through the reaction sequence to furnish both (–)-norsecurinine and (+)-allonorsecurinine, which were ultimately converted into their more complex relatives.

In 2016, Smith's group reported a total synthesis of (±)-morphine that makes use of an RCEYEM sequence (**Scheme 20**).[62] In the presence of catalyst HGII (**4**), the desired RCEYEM proceeded to give the intermediate tetracycle **81**, which, after amine deprotection, underwent an intramolecular 1,6-addition forming **82**, a final reduction away from morphine.

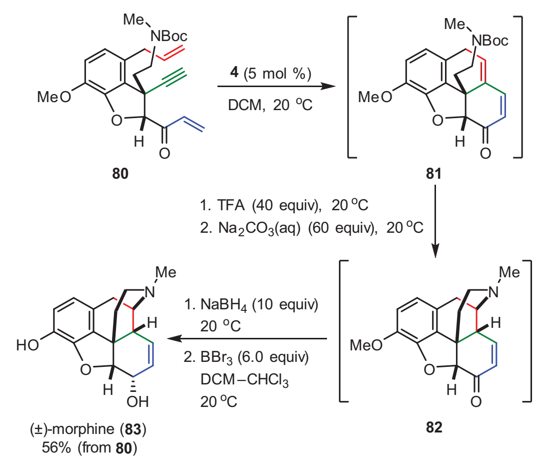
6. Ring-Opening Cross-Metathesis

A collaborative synthesis of the potent antifungal agent (±)-hippolachnin A has been disclosed by the groups of Wood and Brown. This complex natural product has an unusual structure that features six contiguous stereocenters, a quaternary center, and a congested compact core. The two groups had arrived at similar and "complementary" approaches and sought to design a collaborative synthesis playing to the strengths of each of their separate syntheses. In their combined strategy, the [2 + 2] photocycloaddition of quadricyclane and an α,β-unsaturated acid chloride ultimately forged the tricyclic ROCM precursor **84**, which underwent ethylenolysis catalyzed by **1** to give **85** (**Scheme 21**).[63] Strategically, the use of ring-opening metathesis to afford the bis-alkenyl bicycle served to introduce two two-carbon groups that would give rise to two of the four ethyl groups present in the natural product. This method of ethyl group introduction is distinct from other approaches to hippolachnin A.

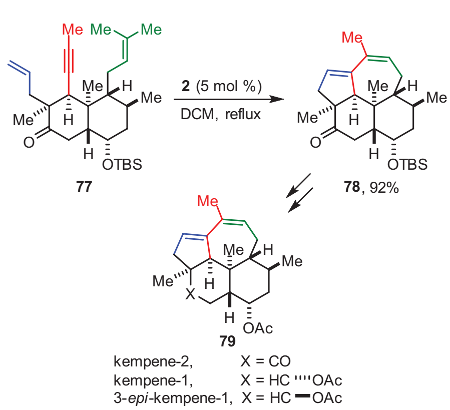
7. Cross-Metathesis

Kim and co-workers developed a procedure for the installation of a *Z*-enyne fragment, which they applied to the synthesis of (+)-3-(*Z*)-laureatin and *ent*-elatenyne (**Scheme 22**).[64] In the key CM step in the synthesis of *ent*-elatenyne, a protected enyne bearing a tethered allyl ether reacted with the terminal alkene of **87** to provide the enyne CM product **88** with high *Z*-selectivity.

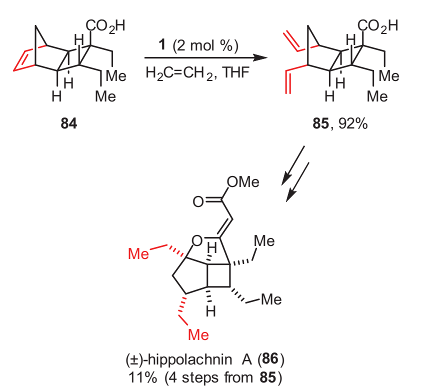
The first applications of Grubbs *Z*-selective ruthenium metathesis catalysts[65] to natural product synthesis were reported by the Grubbs group, when they prepared, in stereochemically pure form, nine lepidopteran female sex pheromones that had been approved by the EPA as insecticide alternatives (**Scheme 23**).[66] Starting from two different seed-oil derivatives, CM with a variety of terminal alkenes provided, either directly or after one step, seven different



Scheme 20. Smith's Use of RCEYEM for a Key Strategic Cyclization That Forms the Tetracyclic Morphine Core. *(Ref. 62)*



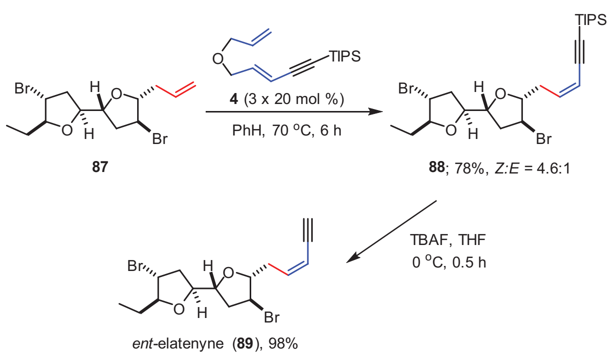
Scheme 19. Spectacular Use of RCEYEM by Schubert and Metz in the Enantioselective Total Synthesis of the Kempene Diterpenes. *(Ref. 60)*



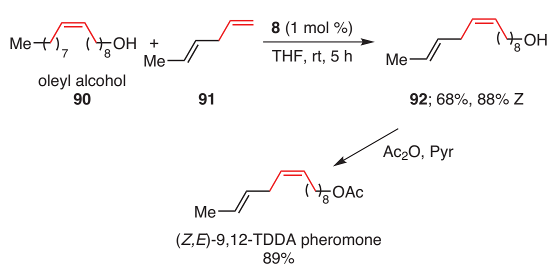
Scheme 21. ROCM in a Collaborative Total Synthesis by Wood and Brown of the Potent Antifungal Agent (±)-Hippolachnin A. *(Ref. 63)*

pheromones. The final two pheromones synthesized each required a total of four steps to complete. Though not structurally complex, these pheromones are otherwise challenging to synthesize because of the remoteness of the functional groups and necessity for control of alkene geometrical isomers. The metatheses employed each required catalyst loadings of 2 mol % or less, with Z-selectivities all greater than 75%. One CM partner, *trans*-1,4-hexadiene (**91**), underwent selective CM at the terminal position to afford **92**, not engaging the (*E*)-alkene moiety, owing to the catalyst's selectivity for *Z* alkenes.

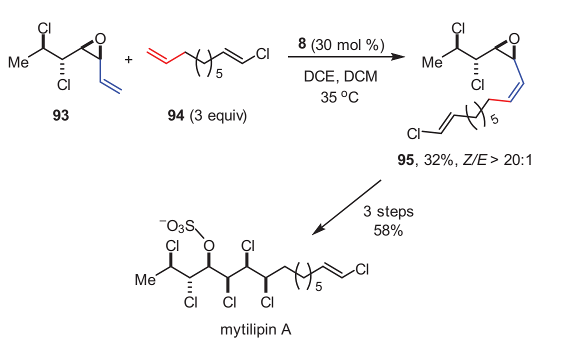
Our group's chlorosulfolipid syntheses took advantage of catalyst **8** for a highly *Z*-selective convergent cross-metathesis of two chlorinated partners (**Scheme 24**).[67] The stereoselective CM between the chlorine-containing vinyl epoxide **93** and the dienyl chloride partner **94** proceeded with very high *Z*-selectivity to give **95**, setting the stage for alkene chlorinolysis, a second dichlorination, and finally sulfation to complete a short synthesis of the chlorosulfolipid mytilipin A. The key aspect of selectivity in reaction partner **94** can be explained in part by the reduced reactivity of alkenyl chlorides and the low rates of cyclooctene formation; however, almost certainly, the key determinant of selectivity involves the recalcitrance of catalyst **8** to engage any kind of *E* alkene, thus sparing the chlorinated alkene moiety of **94** and product **95**.



Scheme 22. Relay RCM-CM in Burton and Kim's Total Synthesis of *ent*-Elatenyne. *(Ref. 64)*



Scheme 23. *Z*-Selective CM in Grubbs's Total Synthesis of Stereochemically Pure Insect Sex Pheromones. *(Ref. 66)*



Scheme 24. *Z*-Selective CM in Vanderwal's Direct Synthesis of Mytilipin A. *(Ref. 67)*

8. Conclusion

Over the past two decades, alkene metathesis has become an essential component of the synthetic chemist's toolbox. The syntheses presented in this review, and the many more which could not be discussed, are evidence of both the objective utility of alkene metathesis as well as the widespread adoption of metathesis as a go-to, reliable reaction in synthetic planning. The featured syntheses also serve to highlight some of the advances made in the field; catalyst and reaction design have overcome supposed limitations of reactivity or selectivity, and implementation in complex settings has illuminated new, non-obvious, and increasingly clever strategies to make use of alkene metathesis. We anticipate continued developments along both of these lines in the coming years.

9. References

(1) The Nobel Prize in Chemistry 2005: Yves Chauvin, Robert H. Grubbs, and Richard R. Schrock; Nobelprize.org; Nobel Media AB **2014**; http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2005/index.html (accessed Jan 16, 2017).
(2) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490.
(3) *Metathesis in Natural Product Synthesis: Strategies, Substrates and Catalysts*; Cossy, J., Arseniyadis, S., Meyer, C., Eds.; Wiley-VCH: Weinheim, Germany, 2010.
(4) *Green Metathesis Chemistry*; Dragutan, V., Demonceau, A., Dragutan, I., Finkelshtein, E. S., Eds.; NATO Science for Peace and Security Series A: Chemistry and Biology; Springer: Netherlands, 2010.
(5) *Olefin Metathesis: Theory and Practice*; Grela, K., Ed.; Wiley: Hoboken, NJ, 2014.
(6) *Handbook of Metathesis*, 2nd ed.; Grubbs, R. H., O'Leary, D. J., Eds.; Wiley-VCH: Weinheim, Germany, 2015; 3 vols.
(7) Yet, L. *Org. React.* **2016**, *89*, 1.
(8) Hoveyda, A. H.; Zhugralin, A. R. *Nature* **2007**, *450*, 243.
(9) Herndon, J. W. *Coord. Chem. Rev.* **2009**, *253*, 86.
(10) Fürstner, A. *Chem. Commun.* **2011**, *47*, 6505.
(11) Werrel, S.; Walker, J. C. L.; Donohoe, T. J. *Tetrahedron Lett.* **2015**, *56*, 5261.
(12) Fürstner, A. *Angew. Chem., Int. Ed.* **2013**, *52*, 2794.
(13) Kalidindi, S.; Jeong, W. B.; Schall, A.; Bandichhor, R.; Nosse, B.; Reiser, O. *Angew. Chem., Int. Ed.* **2007**, *46*, 6361.
(14) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.
(15) White, D. E.; Stewart, I. C.; Grubbs, R. H.; Stoltz, B. M. *J. Am. Chem. Soc.* **2008**, *130*, 810.
(16) (a) Chao, W.; Weinreb, S. M. *Org. Lett.* **2003**, *5*, 2505. (b) Chao, W.; Meketa, M. L.; Weinreb, S. M. *Synthesis* **2004**, 2058.
(17) Stewart, I. C.; Ung, T.; Pletnev, A. A.; Berlin, J. M.; Grubbs, R. H.; Schrodi, Y. *Org. Lett.* **2007**, *9*, 1589.
(18) Xiao, Q.; Ren, W.-W.; Chen, Z.-X.; Sun, T.-W.; Li, Y.; Ye, Q.-D.; Gong, J.-X.; Meng, F.-K.; You, L.; Liu, Y.-F.; Zhao, M.-Z.; Xu, L.-M.; Shan, Z.-H.; Shi, Y.; Tang, Y.-F.; Chen, J.-H.; Yang, Z. *Angew. Chem., Int. Ed.* **2011**, *50*, 7373.

(19) Scholl, M.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 1425.
(20) Wang, J.; Lee, V.; Sintim, H. O. *Chem.—Eur. J.* **2009**, *15*, 2747.
(21) Moritz, B. J.; Mack, D. J.; Tong, L.; Thomson, R. J. *Angew. Chem., Int. Ed.* **2014**, *53*, 2988.
(22) Matsui, R.; Seto, K.; Sato, Y.; Suzuki, T.; Nakazaki, A.; Kobayashi, S. *Angew. Chem., Int. Ed.* **2011**, *50*, 680.
(23) (a) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168. (b) Gessler, S.; Randl, S.; Blechert, S. *Tetrahedron Lett.* **2000**, *41*, 9973.
(24) (a) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404. (b) Cimino, G.; De Stefano, S.; Scognamiglio, G.; Sodano, G.; Trivellone, E. *Bull. Soc. Chim. Belg.* **1986**, *95*, 783. (c) Charan, R. D.; Garson, M. J.; Brereton, I. M.; Willis, A. C.; Hooper, J. N. A. *Tetrahedron* **1996**, *52*, 9111. (d) Jang, K. H.; Kang, G. W.; Jeon, J.; Lim, C.; Lee, H.-S.; Sim, C. J.; Oh, K.-B.; Shin, J. *Org. Lett.* **2009**, *11*, 1713.
(25) (a) Martin, S. F.; Humphrey, J. M.; Ali, A.; Hillier, M. C. *J. Am. Chem. Soc.* **1999**, *121*, 866. (b) Humphrey, J. M.; Liao, Y.; Ali, A.; Rein, T.; Wong, Y.-L.; Chen, H.-J.; Courtney, A. K.; Martin, S. F. *J. Am. Chem. Soc.* **2002**, *124*, 8584.
(26) (a) Garg, N. K.; Hiebert, S.; Overman, L. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2912. (b) Becker, M. H.; Chua, P.; Downham, R.; Douglas, C. J.; Garg, N. K.; Hiebert, S.; Jaroch, S.; Matsuoka, R. T.; Middleton, J. A.; Ng, F. W.; Overman, L. E. *J. Am. Chem. Soc.* **2007**, *129*, 11987.
(27) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **1995**, *34*, 2039.
(28) (a) Nagata, T.; Nakagawa, M.; Nishida, A. *J. Am. Chem. Soc.* **2003**, *125*, 7484. (b) Ono, K.; Nakagawa, M.; Nishida, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2020.
(29) Young, I. S.; Kerr, M. A. *J. Am. Chem. Soc.* **2007**, *129*, 1465.
(30) Jakubec, P.; Cockfield, D. M.; Dixon, D. J. *J. Am. Chem. Soc.* **2009**, *131*, 16632.
(31) Cheng, B.; Wu, F.; Yang, X.; Zhou, Y.; Wan, X.; Zhai, H. *Chem.—Eur. J.* **2011**, *17*, 12569.
(32) Clark, J. S.; Xu, C. *Angew. Chem., Int. Ed.* **2016**, *55*, 4332.
(33) Nilson, M. G.; Funk, R. L. *Org. Lett.* **2010**, *12*, 4912.
(34) Kyle, A. F.; Jakubec, P.; Cockfield, D. M.; Cleator, E.; Skidmore, J.; Dixon, D. J. *Chem. Commun.* **2011**, *47*, 10037.
(35) Bonazzi, S.; Cheng, B.; Wzorek, J. S.; Evans, D. A. *J. Am. Chem. Soc.* **2013**, *135*, 9338.
(36) Yu, M.; Wang, C.; Kyle, A. F.; Jakubec, P.; Dixon, D. J.; Schrock, R. R.; Hoveyda, A. H. *Nature* **2011**, *479*, 88.
(37) Guo, L.-D.; Huang, X.-Z.; Luo, S.-P.; Cao, W.-S.; Ruan, Y.-P.; Ye, J.-L.; Huang, P.-Q. *Angew. Chem., Int. Ed.* **2016**, *55*, 4064.
(38) Heppekausen, J.; Stade, R.; Goddard, R.; Fürstner, A. *J. Am. Chem. Soc.* **2010**, *132*, 11045.
(39) (a) Weber, W.; Zähner, H.; Damberg, M.; Russ, P.; Zeeck, A. *Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Orig. C* **1981**, *2*, 122. (b) Zhang, H.; Kakeya, H.; Osada, H. *Tetrahedron Lett.* **1997**, *38*, 1789. (c) Umezawa, I.; Funayama, S.; Okada, K.; Iwasaki, K.; Satoh, J.; Masuda, K.; Komiyama, K. *J. Antibiot.* **1985**, *38*, 699. (d) Sensi, P.; Margalith, P.; Timbal, M. T. *Farmaco, Ed. Sci.* **1959**, *14*, 146.
(40) Panek, J. S.; Masse, C. E. *J. Org. Chem.* **1997**, *62*, 8290.
(41) Smith, A. B., III; Barbosa, J.; Wong, W.; Wood, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 10777.
(42) Hayashi, Y.; Shoji, M.; Ishikawa, H.; Yamaguchi, J.; Tamura, T.; Imai, H.; Nishigaya, Y.; Takabe, K.; Kakeya, H.; Osada, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 6657.
(43) Del Valle, D. J.; Krische, M. J. *J. Am. Chem. Soc.* **2013**, *135*, 10986.
(44) Gao, X.; Woo, S. K.; Krische, M. J. *J. Am. Chem. Soc.* **2013**, *135*, 4223.
(45) Fuwa, H.; Saito, A.; Sasaki, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 3041.
(46) Yu, M.; Schrock, R. R.; Hoveyda, A. H. *Angew. Chem., Int. Ed.* **2015**, *54*, 215.
(47) Kita, M.; Oka, H.; Usui, A.; Ishitsuka, T.; Mogi, Y.; Watanabe, H.; Tsunoda, M.; Kigoshi, H. *Angew. Chem., Int. Ed.* **2015**, *54*, 14174.
(48) Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360.
(49) Shin, I.; Hong, S.; Krische, M. J. *J. Am. Chem. Soc.* **2016**, *138*, 14246.
(50) Erb, W.; Grassot, J.-M.; Linder, D.; Neuville, L.; Zhu, J. *Angew. Chem., Int. Ed.* **2015**, *54*, 1929.
(51) Miyatake-Ondozabal, H.; Kaufmann, E.; Gademann, K. *Angew. Chem., Int. Ed.* **2015**, *54*, 1933.
(52) Glaus, F.; Altmann, K.-H. *Angew. Chem., Int. Ed.* **2015**, *54*, 1937.
(53) Hoye, T. R.; Jeon, J.; Kopel, L. C.; Ryba, T. D.; Tennakoon, M. A.; Wang, Y. *Angew. Chem., Int. Ed.* **2010**, *49*, 6151.
(54) Volchkov, I.; Lee, D. *J. Am. Chem. Soc.* **2013**, *135*, 5324.
(55) Ko, H. M.; Lee, C. W.; Kwon, H. K.; Chung, H. S.; Choi, S. Y.; Chung, Y. K.; Lee, E. *Angew. Chem., Int. Ed.* **2009**, *48*, 2364.
(56) Speed, A. W. H.; Mann, T. J.; O'Brien, R. V.; Schrock, R. R.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2014**, *136*, 16136.
(57) Hart, A. C.; Phillips, A. J. *J. Am. Chem. Soc.* **2006**, *128*, 1094.
(58) Harvey, R. S.; Mackay, E. G.; Roger, L.; Paddon-Row, M. N.; Sherburn, M. S.; Lawrence, A. L. *Angew. Chem., Int. Ed.* **2015**, *54*, 1795.
(59) Movassaghi, M.; Piizzi, G.; Siegel, D. S.; Piersanti, G. *Angew. Chem., Int. Ed.* **2006**, *45*, 5859.
(60) Schubert, M.; Metz, P. *Angew. Chem., Int. Ed.* **2011**, *50*, 2954.
(61) Wei, H.; Qiao, C.; Liu, G.; Yang, Z.; Li, C. *Angew. Chem., Int. Ed.* **2013**, *52*, 620.
(62) Chu, S.; Münster, N.; Balan, T.; Smith, M. D. *Angew. Chem., Int. Ed.* **2016**, *55*, 14306.
(63) McCallum, M. E.; Rasik, C. M.; Wood, J. L.; Brown, M. K. *J. Am. Chem. Soc.* **2016**, *138*, 2437.
(64) (a) Kim, H.; Lee, H.; Lee, D.; Kim, S.; Kim, D. *J. Am. Chem. Soc.* **2007**, *129*, 2269. (b) Dyson, B. S.; Burton, J. W.; Sohn, T.; Kim, B.; Bae, H.; Kim, D. *J. Am. Chem. Soc.* **2012**, *134*, 11781.
(65) Endo, K.; Grubbs, R. H. *J. Am. Chem. Soc.* **2011**, *133*, 8525.
(66) Herbert, M. B.; Marx, V. M.; Pederson, R. L.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **2013**, *52*, 310.
(67) (a) Chung, W.; Carlson, J. S.; Bedke, D. K.; Vanderwal, C. D. *Angew. Chem., Int. Ed.* **2013**, *52*, 10052. (b) Chung, W.; Carlson, J. S.; Vanderwal, C. D. *J. Org. Chem.* **2014**, *79*, 2226.

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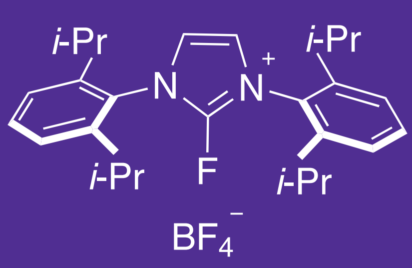

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