

Olefin metathesis for chemical biology

Joseph B Binder¹ and Ronald T Raines^{1,2}

Chemical biology relies on effective synthetic chemistry for building molecules to probe and modulate biological function. Olefin metathesis in organic solvents is a valuable addition to this armamentarium, and developments during the previous decade are enabling metathesis in aqueous solvents for the manipulation of biomolecules. Functional group-tolerant ruthenium metathesis catalysts modified with charged moieties or hydrophilic polymers are soluble and active in water, enabling ring-opening metathesis polymerization, cross metathesis, and ring-closing metathesis. Alternatively, conventional hydrophobic ruthenium complexes catalyze a similar array of metathesis reactions in mixtures of water and organic solvents. This strategy has enabled cross metathesis on the surface of a protein. Continuing developments in catalyst design and methodology will popularize the bioorthogonal reactivity of metathesis.

Addresses

¹ Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, WI 53706-1322, USA

² Department of Biochemistry, University of Wisconsin–Madison, 433 Babcock Drive, Madison, WI 53706-1544, USA

Corresponding author: Raines, Ronald T (rraines@wisc.edu)

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Introduction

Olefin metathesis is a versatile, chemoselective means to create carbon–carbon bonds in a range of molecules including natural products, polymers, and biomolecules [1–6]. In the metathesis reaction, carbon–carbon multiple bonds are broken and made through the intermediacy of transition metal carbenes. This process requires no additional reagents beyond the alkene starting materials and a metal catalyst and produces simple alkene byproducts, such as ethylene. Modern ruthenium olefin metathesis initiators, such as complexes **1–4**, tolerate many functional groups common in biomolecules, including amides, alcohols, and carboxylic acids (Figure 1). Under some circumstances, metathesis occurs even in the presence of amines and sulfur-containing moieties. Moreover, olefin metathesis is highly chemoselective. Most func-

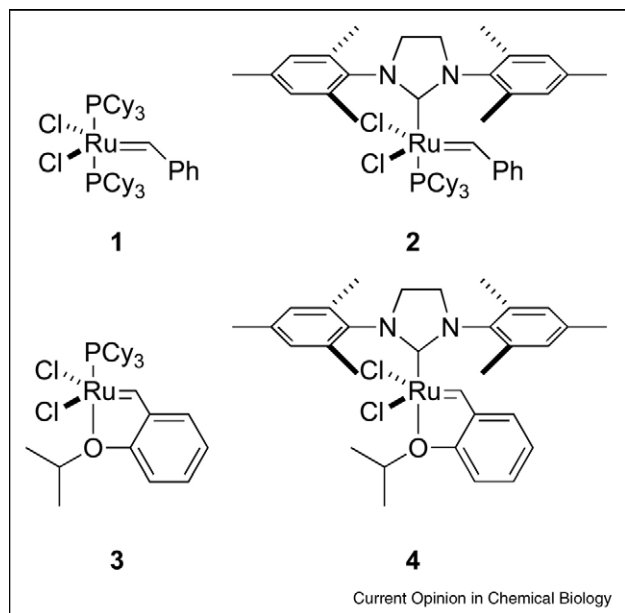
tional groups other than alkenes and alkynes are not modified by ruthenium metathesis catalysts, and the reaction forms only carbon–carbon bonds. These characteristics suggest that olefin metathesis is a candidate for a ‘bioorthogonal’ reaction [7]. Solvent-exposed alkenyl groups are uncommon in living systems, so biomolecules labeled with alkenes could be modified selectively by olefin metathesis.

As far back as 1975, olefin metathesis was exploited to create molecules to modulate biological function. The laboratories of Rossi and Streck independently recognized the opportunity of metathesis to create complex olefins and used primitive ill-defined metathesis catalysts to prepare insect pheromones from simple alkene precursors [8,9]. Although these initial examples employed sensitive early-transition metal catalysts, major advances in catalyst development since 1975 have produced well-defined metathesis initiators such as air-stable and moisture-stable ruthenium complexes **1–4**. These user-friendly initiators have popularized metathesis among organic chemists and are being used to address biological questions. Nonetheless, olefin metathesis in biological contexts remains limited because of the difficulty of enacting the reaction in aqueous solvents. Conventional metathesis catalysts are insoluble in water, and metathesis with water-soluble complexes in water tends to be far less efficient than in organic solvents. After briefly surveying applications of metathesis in chemical biology, this review will focus on recent progress toward metathesis in homogenous aqueous solutions in order to modify biomolecules in their native environment.

Applications of olefin metathesis in chemical biology

One of the advantages of the metathesis reaction is the wide variety of structures that can be created through its many variations [1,2]. Ring-opening metathesis polymerization (ROMP) converts a cyclic olefin into an unsaturated polymer (Figure 2a). Kiessling and co-workers were the first to use ROMP to synthesize bio-active polymers, including multivalent displays of carbohydrates and other bio-active ligands [10–12]. Such polymers have recently been used to modulate immune responses *in vivo* [13]. Cross metathesis (CM) exchanges two linear alkene fragments, providing a means to connect two molecules (Figure 2b). The chemoselectivity and functional group-tolerance of ruthenium metathesis catalysts enabled Diver and Schreiber to dimerize unprotected FK506 in a single step to form a small-molecule ligand that activated signal transduction and protein expression *in vivo* [14]. More recently, Miller and Raines and co-workers examined

Figure 1



Conventional ruthenium catalysts for olefin metathesis.

protein folding using a *trans* peptide bond mimic made by CM of two alkene-containing fragments (Figure 2b) [15,16]. Ring-closing metathesis (RCM) transforms a diene (or alkene–alkyne) into a cyclic alkene and has proven to be a potent method for creating macrocycles, including bioactive cyclic peptidomimetics (Figure 2c). Demonstrating the robustness of peptide RCM, Liskamp and co-workers have constructed alkene mimics of the five peptide-thioether rings of the lantibiotic nisin [17,18]. Such carba analogs are anticipated to be more stable than native linkages such as disulfide bonds. Vederas and co-workers confirmed this prediction with the disulfide-bridged peptide macrocycle atosiban, finding that a carba-atosiban generated through RCM had a significantly longer half-life in placental tissue and only moderately lower activity as an oxytocin antagonist (Figure 2c) [19,20]. RCM offers an alternative approach to improve peptide stability by creating crosslinks between otherwise flexible portions of a peptide chain. Blackwell and Grubbs were the first to demonstrate that RCM could be used to tether residues of helical peptides [21]. From this initial report, the field of peptide ‘stapling’ has blossomed and is reviewed elsewhere in this issue [22]. In one sense, the maturity of metathesis as a tool for chemical biology is evident in Aileron Therapeutics, which is seeking to commercialize bio-active peptides stabilized by RCM-crosslinks [23]. On the contrary, the use of aqueous metathesis for chemical biology is just beginning.

Water-soluble phosphine complexes

Grubbs and co-workers led the development of ruthenium olefin metathesis catalysts and recognized that the

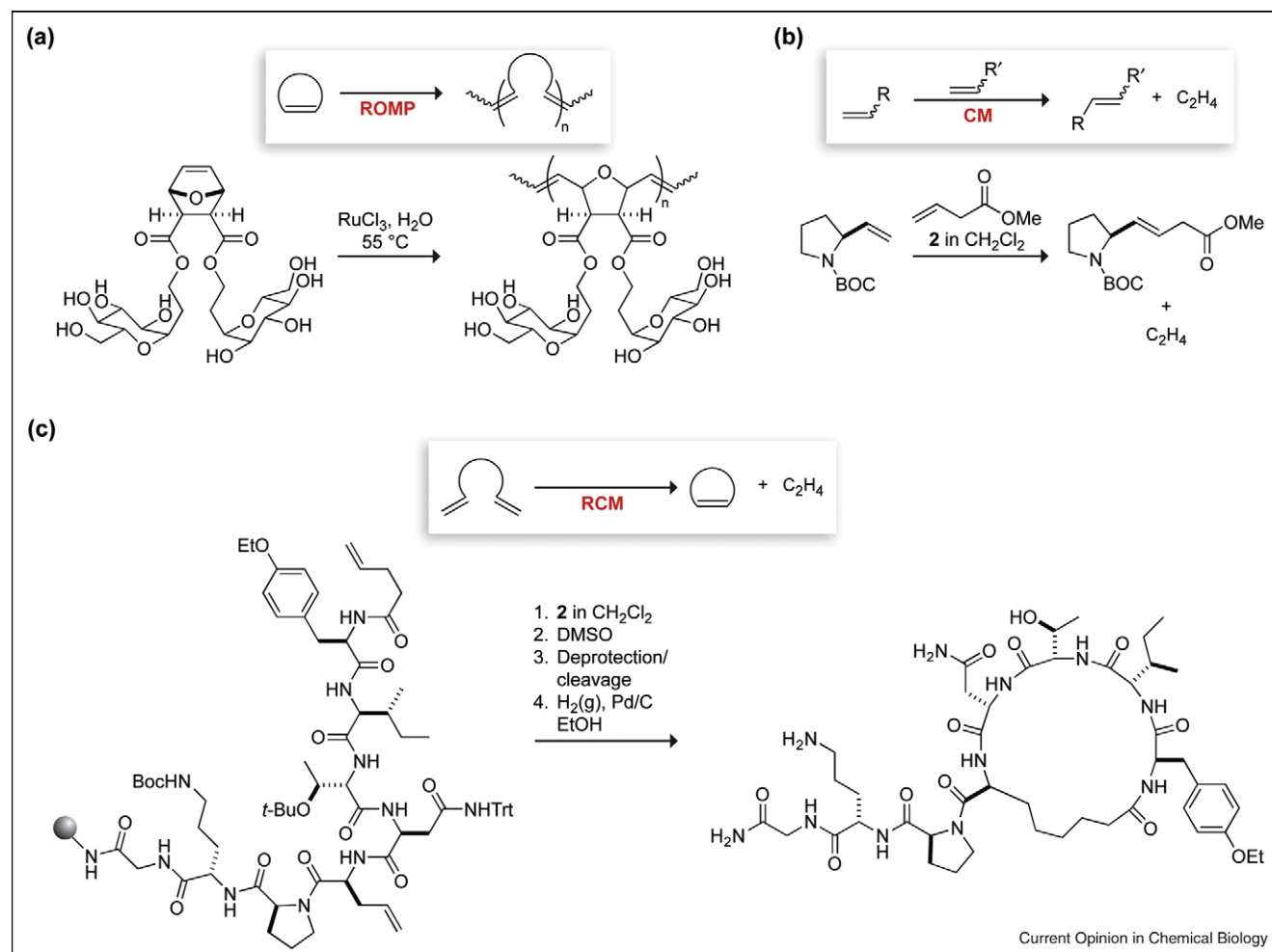
catalytic ruthenium species were actually water-tolerant, in contrast to other metathesis-active metals. In fact, they noted that ill-defined ruthenium ROMP catalysts initiated faster in water than in organic solvents [24]. These observations led to the subsequent development of well-defined ruthenium initiators such as **1**. Grubbs and co-workers returned to water by substituting the hydrophobic phosphines in **1** with electron-rich, cationic phosphines to create complexes such as **5**, which are capable of catalyzing ROMP and RCM in water (Figure 3). Notably, complex **5** accomplishes living ROMP in water when activated with one equivalent of hydrochloric acid. These phosphine-based catalysts, were, however, highly air-sensitive and required that one of the alkene partners in RCM be substituted with a terminal alkyl group [25–27]. These problems limited the practicality of water-soluble phosphine-based initiators. More recently, Schanz and co-workers reported benzylidene-functionalized catalysts, such as **6**, that catalyze ROMP in mixtures of 2,2,2-trifluoroethanol and water, but are also air-sensitive (Figure 3) [28].

Polymeric water-soluble *N*-heterocyclic carbene complexes

Application of *N*-heterocyclic carbene (NHC) ligands to ruthenium olefin metathesis catalysts has led to remarkable increases in stability and activity in both organic solvents and water. The laboratories of Herrmann and co-workers [29], Nolan and co-workers [30], and Grubbs and co-workers [31] nearly concurrently reported ruthenium complexes bearing one tightly bound NHC ligand and one labile phosphine, such as **2**. Although these complexes initiate more slowly than does **1**, they transform much faster within the metathesis catalytic cycle. In addition, the NHC ligand increases the stability of the ruthenium complex. Hoveyda and co-workers reported another important advance in catalyst design with complexes **3** and **4** [32,33]. In these initiators, a chelating ether replaces the phosphine ligand, further decreasing the air-sensitivity of the catalyst and improving catalyst stability [34,35].

Many researchers have capitalized on these improvements in organic-soluble complexes to develop more effective metathesis catalysts for aqueous solution, chiefly by substituting complexes with poly(ethylene glycol) (PEG) or quaternary ammonium moieties. In an early development, Cannon and Blechert immobilized a complex similar to **4** on hydrophilic PEGA-NH₂ resin (amino-functionalized dimethylacrylamide and mono-2-acrylamidoprop-1-yl polyethyleneglycol) [36]. This heterogeneous catalyst was capable of CM of a variety of alcohols in water but was less effective with charged substrates. By contrast, Grubbs and co-workers have developed soluble polymer-supported complexes. Complex **7**, in which PEG is attached to an *N*-benzyl NHC, is more active than **5** for ROMP of hindered cyclic olefins in

Figure 2



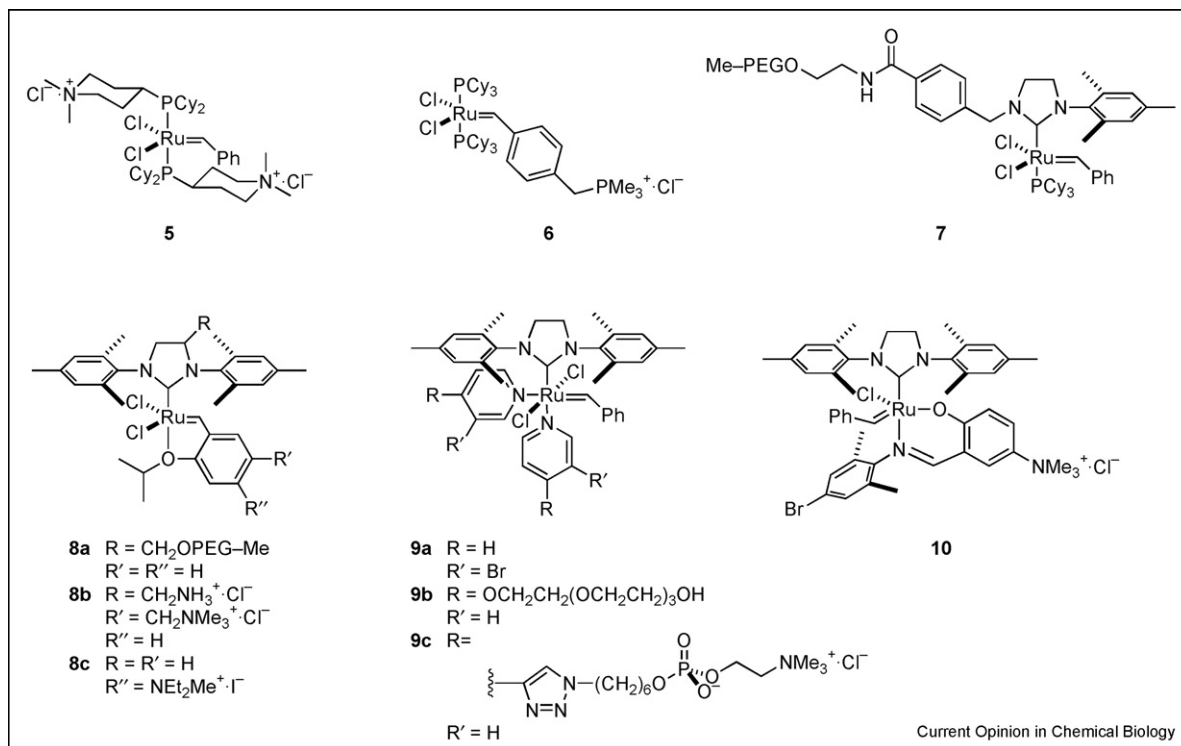
Olefin metathesis reactions and their application to chemical biology. **(a)** Ring-opening metathesis polymerization can produce highly functionalized and well-defined macromolecules, such as this carbohydrate-displaying polymer [10]. **(b)** Cross metathesis links two alkene fragments to create increasingly complex molecules, including this proline-glycine dipeptide mimic [15,16]. **(c)** Ring-closing metathesis is an effective reaction for producing cyclic molecules with constrained conformations, such as this oxytocin hormone mimic [20].

water (Figure 3). Nonetheless, complex **7** is rather ineffective for RCM of simple unsubstituted dienes in methanol and was not reported to catalyze RCM in water [37]. Despite these disappointing results with the first PEG-derivatized initiator, minor modifications led to much more efficient water-soluble catalysts. In particular, shifting the point of attachment of the PEG to the NHC backbone and replacing the phosphine with an ether chelate dramatically improved the activity of **8a** over that of **7** (Figure 3) [38^{*}]. Complex **8a** is stable in water at room temperature for more than one week under inert atmosphere and is faster at ROMP than is complex **7**. Moreover, the modified complex enables both RCM of water-soluble α,ω -dienes in neat water in good yields and CM of allyl alcohol. Gratifyingly, complex **8a** accomplishes the challenging formation of trisubstituted olefins through RCM. Unfortunately, CM using this catalyst fails

with some alkenes substituted with carboxylic acids or quaternary ammonium groups. The basis for the improved activity of this PEG-based initiator is probably its maintaining the crucial 1,3-diaryl NHC structure while incorporating an ether chelate. Furthermore, the amphiphilic nature of the PEG-complex conjugate leads to aggregation of the complexes into micelle-like structures. The resulting sequestration in a PEG shell protects the catalyst from water, but also has the deleterious consequence of preventing metathesis of bulky, water-soluble substrates such as proteins.

An additional problem with these early water-soluble complexes is that the **NHC slows initiation of the complex**, preventing the synthesis of well-defined polymers via ROMP. Grubbs and co-workers overcame this problem in organic solvents by creating the fast-initiating

Figure 3



Ruthenium catalysts for aqueous olefin metathesis.

bis(3-bromopyridine) complex **9a**, and Emrick and co-workers have sought to do the same in water with water-soluble bis(pyridine) complexes such as **9b** and **9c** (Figure 3) [39,40^{*}]. *O*-Linked PEG initiator **9b** is capable of ROMP at in water low pH, which is presumably required for dissociation of the basic ligands. On the contrary, the phosphorylcholine-based **9c** is active under both neutral and acidic conditions, partly because of the decreased basicity of the pyridine nitrogen. Unfortunately, these catalysts produce polymers with polydispersity indices (PDI) between 1.3 and 2.4, whereas well-controlled ROMP results in PDI < 1.1. Hence, the production of well-defined ROMP polymers in water remains challenging.

Small-molecule water-soluble *N*-heterocyclic carbene complexes

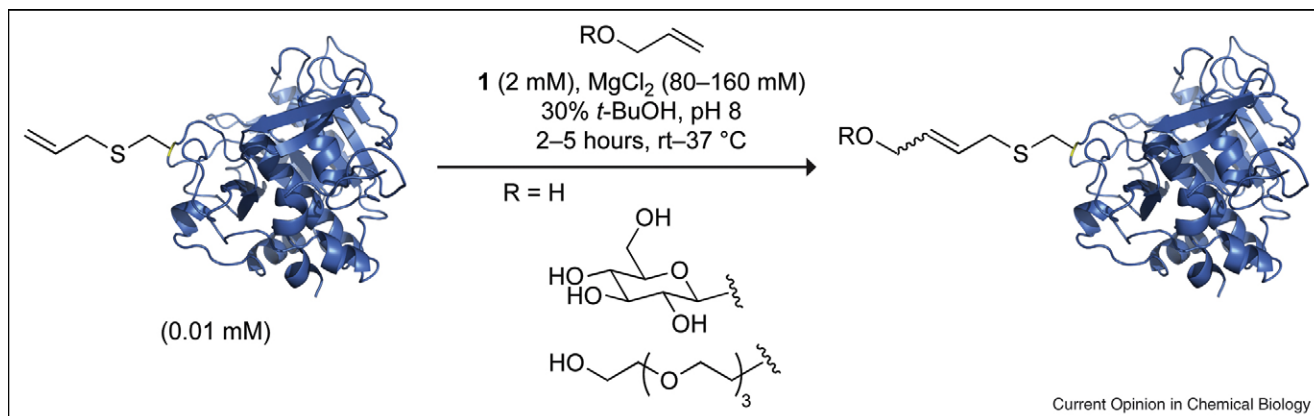
Small-molecule aqueous-soluble catalysts avoid the heterogeneity problems of PEG-based catalysts and, in fact, are typically as active as PEG-based catalysts. To date, most of these complexes have been made hydrophilic by the attachment of a quaternary ammonium groups. Taking advantage of the well-documented stability of ruthenium salicylaldimine complexes, we reported complex **10**, which is soluble and stable in 2:1 methanol/water (Figure 3) [41]. This robust initiator

requires elevated temperatures for activation but enables RCM and ring-closing enyne metathesis of hydrophobic and hydrophilic substrates in methanol/water under air at 55 °C.

The laboratories of both Grubbs [42^{••}] and Grela [43–45,46^{••}] have developed chelating ether complexes functionalized with a range of quaternary ammonium substituents. Jordan and Grubbs reported complex **8b**, which bears two cationic centers and is readily soluble in water (Figure 3). This initiator is as active as **8a** for aqueous ROMP, RCM, and CM of water-soluble substrates, but does display a greater propensity for promoting isomerization side reactions of challenging substrates. As with **8a**, the reported CM substrate scope of **8b** is limited to allylic alcohols.

Grela and co-workers examined the activity of complex **8c** [43,46^{••}] as well as other benzylidene-tagged initiators [44,45] in water and water/alcohol mixtures (Figure 3). This diethylmethylammonium-substituted catalyst is moderately soluble in water and displays a substrate scope similar to that of **8a** and **8b** for RCM and CM, though this catalyst has not been reported to mediate RCM of water-soluble substituted dienes. Complex **8c** also accomplishes RCM and CM of a variety of substrates in homogeneous

Figure 4



Olefin cross metathesis for site-specific protein modification at allyl sulfide substituents [49**]. The allyl group is appended to Cys156 of the S156C variant of subtilisin *Bacillus lentus*. The protein structure is based on the atomic coordinates in PDB entry 1st3.

alcohol/water mixtures. Notably, metathesis by **8c** often can be achieved with lower catalyst loadings (1–2.5 mol% versus 5 mol%), a finding Grela and co-workers attribute to the **electronic activation provided by the electron-withdrawing quaternary ammonium group**. Nonetheless, all the multiple water-soluble metathesis catalysts now available, have similar activities and substrate scopes which, though impressive, have not yet been shown to be sufficient for the synthesis of complex molecules in water.

Olefin metathesis in homogeneous aqueous solvent mixtures

The intricate synthesis of water-soluble ligands presents a further limitation of specially designed aqueous metathesis catalysts. The use of conventional hydrophobic ruthenium complexes in mixtures of organic solvents and water avoids this difficulty, yet effective metathesis reactions of water-soluble substrates typically require homogeneous conditions. Blechert and co-workers initially demonstrated the possibility of using chelating ether complexes under these conditions, finding that a complex similar to **4** enabled RCM of a hydrophobic substrate in moderate yields in 3:1 *N,N*-dimethylformamide/water [47]. We extended these results through a more comprehensive survey of reaction conditions [48*]. By testing a variety of water-miscible aprotic solvents, we found that acetone, 1,2-dimethoxyethane (DME), and PEG are superior cosolvents for aqueous RCM. In addition, **4** is superior to complexes **1–3** in DME/water, probably for the same reasons that NHC ligands and chelating ethers enhance water-soluble catalyst performance. In DME/water and acetone/water, complex **4** enables RCM of a range of hydrophobic and hydrophilic substrates, as well as CM of allyl alcohol, and its performance in these mixed solvents is similar to that of the best water-soluble complexes in water. Thus, the main

advantage of the **specialized ligands of water-soluble complexes is their hydrophilicity rather than their effects on stability or activity**. The efficiency of both catalyst systems is still far lower than that of conventional complexes in organic solvents. Further advances in aqueous metathesis will probably require ligands that provide even greater water-tolerance.

Olefin metathesis on proteins

Using a similar strategy, Davis and co-workers have further developed aqueous CM and achieved metathesis on a protein [49**]. Screening a variety of alkene partners for CM with allyl alcohol in 1:1 water/*t*-butyl alcohol, they found that alkenes with allylic heteroatoms are privileged participants in CM and that sulfur substitution is the most effective. The effectiveness of CM diminishes with butenyl and pentenyl sulfides, leading Davis and co-workers to hypothesize that the **allylic heteroatom plays a role as a directing group, while the more distant sulfides form unproductive chelates**. Incorporation of *S*-allylcysteine on the surface of a model protein, subtilisin *Bacillus lentus*, provides a potential protein CM partner [50]. Although CM fails with allyl alcohol and 200 equivalents of **4** in 7:3 phosphate buffer/*t*-butyl alcohol, the addition of MgCl_2 (presumably to mask protein carboxylate groups) enables CM with allyl alcohol and allylic ethers in 50–90% conversion (Figure 4). This methodology allowed Davis and co-workers to functionalize the model protein with carbohydrate and PEG moieties while maintaining the enzymatic activity of subtilisin. It is noteworthy that *S*-allylcysteine can be incorporated genetically as a methionine surrogate [49**].

Conclusions

The olefin metathesis reaction is a highly versatile tool for synthetic chemical biologists. Modern ruthenium-based catalysts are readily available and can be easily used in

organic solution to construct molecules for probing and manipulating biological function. On the contrary, metathesis on biomolecules in water remains challenging. Advances in catalyst design during the past decade have enabled simple ROMP, RCM, and CM in water, but controlled ROMP to create low polydispersity polymers is still difficult and the CM of many substrates remains impossible. Many of the reactions made possible by water-soluble catalysts are also catalyzed by conventional complexes in aqueous-organic solutions amenable to biological substrates. This hybrid approach has enabled the first CM on the surface of a protein using complex **4**, a success that heralds olefin metathesis in water as a method for chemical biology. Nonetheless, more complex applications of metathesis, such as demanding ROMP or CM in water, will probably require the endowment of ruthenium catalysts with additional stability and activity in aqueous environments.

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