E- and Z-Trisubstituted macrocyclic alkenes for natural product synthesis and skeletal editing

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Abstract

Many therapeutic agents are macrocyclic trisubstituted alkenes, and yet, preparation of these structures is typically inefficient and nonselective. A possible solution would entail catalytic macrocyclic ring-closing metathesis, but these transformations require high catalyst loading, conformationally rigid precursors, and are often low yielding and/or non-stereoselective. Here, we introduce a ring-closing metathesis strategy for synthesis of trisubstituted macrocyclic olefins in either stereoisomeric form, regardless of the level of entropic assistance. The goal was achieved by addressing several unexpected difficulties, including complications arising from pre-ring-closing metathesis alkene isomerization. The power of the method is highlighted by two examples. One being the near-complete reversal of substrate-controlled selectivity in the formation of a macrolactam related to an anti-fungal natural product. The other is a late-stage stereoselective generation of an E-trisubstituted alkene in a 24-membered ring, en route to cytotoxic natural product dolabelide C.

Graphical Abstract
It is a sign of the power of macrocyclic ring-closing metathesis (MRCM)\textsuperscript{1,2} that, despite being often inefficient, it is used regularly to synthesize bioactive trisubstituted cyclic alkenes\textsuperscript{3}. What makes this still more remarkable is that only in rare cases does MRCM deliver one alkene isomer preferentially\textsuperscript{4,5,6,7}, and even then it might not be the desired one\textsuperscript{8,9} (see Supplementary Information for extended bibliography). The stereochemical outcome of a MRCM hinges on subtle energy differences between a substrate’s conformers, making it difficult – if not impossible – to predict whether it will be selective and, if so, to what degree and/or which isomer might be favored. Making matters more difficult, typically, high catalyst loadings (for example, up to 60 mol %), elevated reaction temperatures (up to 100 °C), and/or extended reaction times (up to a week) are necessary\textsuperscript{6,7,10,11,12}.

Progress toward development of kinetically controlled MRCM\textsuperscript{13} has been confined to transformations that afford a disubstituted alkene\textsuperscript{14,15}. While there are less than a handful of methods for preparation of trisubstituted olefins by cross-metathesis\textsuperscript{16,17,18}, to the best of our knowledge, there is no kinetically controlled MRCM method for accessing trisubstituted macrocyclic olefins. One possible approach might involve alkyne metathesis, followed by stereoselective conversion of a macrocyclic alkyne to trisubstituted alkene derivatives\textsuperscript{19}. However, a directing group (such as a propargylic alcohol) is needed for converting the alkyne to a trisubstituted alkene, only one of the two stereoisomers can be accessed, and a macrocyclic alkyne might be too strained to form in high yield.

In an earlier foray, in connection to synthesis of fluvirucin B\textsubscript{1} (Fig. 1a), we showed that MRCM of diene \textbf{1a} can be performed with 20 mol % bis-alkoxide complex \textbf{Mo-1} (see Fig. 1e) to afford 14-membered ring \textbf{2a} in 91% yield and \textgreater{}98:2 \textit{Z}:\textit{E} ratio\textsuperscript{20}; catalytic reduction afforded the desired methyl-substituted carbon stereogenic center with complete stereocontrol. Later investigations revealed that the high \textit{Z}:\textit{E} ratio, resulting from conformational preferences of the starting material (substrate control), was a coincidence and is atypical. For instance, MRCM of regioisomeric \textbf{1b} with 20 mol % \textbf{Ru-1} (see Fig. 1e) afforded \textbf{2b} (C7–C8 vs. C6–C7 alkene in \textbf{2a}) with just a slight preference for the \textit{E} isomer\textsuperscript{21–22}.

Subsequent efforts towards identifying a more general strategy led to the discovery of \textbf{Mo-2} (see Fig. 1e). With 7.5 mol % \textbf{Mo-2}, triene \textbf{3} was converted to \textit{Z}-trisubstituted macrocyclic alkene \textbf{4} (Fig. 1b), precursor to epothilone D, in 73% yield and 91:9 \textit{Z}:\textit{E} selectivity\textsuperscript{23}.
Stereoselective epoxidation of 4 afforded epothilone B24. This was an improvement compared to the previous attempts, which demanded 20 mol % Mo-1 (86% yield)24 and led to the formation of isomeric mixtures. Nonetheless, the Mo bisaryl oxides reactions proved not to be broadly applicable catalysts, as manifested by severe inefficiency of MRCM with sparsely functionalized dienes. As an example, none of the desired products were detected in the reaction with 5 to afford macrolactone 6 (Fig. 1c), namely, the macrocyclic core of epothilones sans substituents. It did not matter whether a Mo or Ru complex was used. Only homocoupling byproducts, generated from reaction at the monosubstituted alkene terminus, were observed. Unlike processes that afford disubstituted macrocyclic alkenes25,26,27,28, MRCM reactions that afford a trisubstituted alkene without significant entropic assistance29 are rare and the known examples are either low yielding or hardly stereoselective20,30. This shortcoming is consequential because minimally functionalized trisubstituted macrocyclic olefins are musks31,32. Furthermore, facile and stereoselective preparation of unsaturated large rings, regardless of their substitution pattern and/or stereochemical identity, is important to the art of framework editing33,34 and drug development35,36. Whether a macrocycle contains an E- or a Z-alkene impacts its three-dimensional shape and ability to recognize and bind a particular receptor site.

The reliability of MRCM is especially crucial when it is intended as a late stage operation in a multistep route that leads to a complex molecule, such as the naturally occurring anti-cancer compounds dolabelides A–D (Fig. 1d)37,38. There are two known total syntheses of these 24- and 22-membered unsaturated lactone macrocyclic alkenes, one delivering dolabelide C39 and the other dolabelide D40. In both cases, portionwise addition of 20–25 mol % Ru-2 was needed, there was extensive olefin isomerization, and stereocontrol was nearly non-existent. Additionally, isomeric mixtures, separation of which proved to be difficult, were generated. Stereoisomerically pure macrocyclic alkenes were therefore obtained in just 21% and 31% yield, respectively. In a related initiative, linear fragments that might be used to synthesize dolabelide C were generated in high stereoisomeric purity41,42. Notably, the necessary trisubstituted olefin was prepared by RCM of a silyl-tethered diene, affording a six-membered ring siloxane42, which was then transformed to the desired linear compound. Considering the number of transformations needed to obtain the requisite acyclic precursors such as 7a and 7b (49 and 32 steps, respectively), the existing state-of-the-art is particularly wasteful. Stereodivergency is crucial as well. This is manifested by the fact that, while fluvirucins precursors and epothilones contain a Z-trisubstituted alkene, dolabelides feature an E-olefin.

**Challenges in reaction development**

We first entertained the possibility of a stereoretentive MRCM strategy as a way of synthesizing an E- or a Z-trisubstituted macrocyclic alkene (Fig. 2a). A diene containing an E-trisubstituted alkene (e.g., E-i) could be converted to alkylidene E-ii through reaction with the pendant E- and/or Z-disubstituted olefin. The transformation would begin by the generation of mcb-i (mcb, metallacyclobutane), which is expected to form preferentially on account of the steric pressure in mcb-i, engendered by the proximity of the Cα methyl group and the aryloxide ligand. (For clarity, the less crucial interactions involving Cβ metallacyclobutane substituents will be discussed in later sections43 – see Tables 1 and
2) The following cycloreversion would furnish trisubstituted macrocyclic alkene \( E\text{-}iv \) and ethylidene \( iii \) would be released. Analogously, \( Z\text{-}i \) may be transformed to \( Z\text{-}iv \) (Fig. 2a). A stereoretentive approach would afford products that are of higher stereoisomeric purity because of the larger energy gap between the competing metallacyclobutanes compared to those derived from a 1,1-disubstituted and a monosubstituted olefin (that is, a stereoselective process). Additionally, the latter transformation would proceed via a relatively short-lived Mo methylidene, resulting in low efficiency. Formation of the initial Mo alkylidene (see \( E\text{-}ii \) or \( Z\text{-}ii \)) through reaction at a 1,2-disubstituted olefin, rather than a monosubstituted olefin, would lead to a longer-living alkyl-substituted complex (e.g., methyl-substituted alkylidene \( iii \)). Finally, we expected that there would be much less homocoupling without a terminal alkene.

The above plan appeared promising until further analysis pointed to the following possible complications (Fig. 2b): (1) For maximum efficiency, a cross-metathesis reaction is generally performed at 2.0 M concentration (solvent is used only to add the Mo complex to the reaction mixture).\(^{16-18}\) These conditions are not suitable for MRCM, for which, typically, a 0.5–2.0 mM solution is needed to favor ring formation (see Fig. 1). (2) In cross-metathesis one reactant is usually present in excess, an advantage not extendable to MRCM. (3) Most known cross-metathesis reactions that afford a trisubstituted olefin proceed via a Mo alkylidene bearing a small and activating substituent (such as a halogen or a nitrile).\(^{16,18}\)

The less common transformations involving (non-cyano) carbon-substituted alkylidenes, that is, MRCM intermediates, are far less efficient. In fact, a cross-metathesis intended to deliver an all-carbon trisubstituted alkene did not furnish any desired product under conditions that are still five to ten times more concentrated (10 mM) than a typical macrocyclization where homocoupling is a major issue; this was despite the fact that, unlike an MRCM, one substrate was present in excess (i.e., 5.0 equiv.; Fig. 2b).

**Unexpected problem and mechanism-guided solution**

Lacking a better option, we pinned our hopes on the possibility that enhanced effective concentration because of the intramolecularity of ring-closing would result in appreciable turnover rates. As the model process, we selected the reaction of diene \( E\text{-}8 \) to generate 20-membered macrocyclic lactone \( E\text{-}9 \) (Fig. 3a) in the presence of bisaryloxide Mo-2 or monoaryloxide pyrrolide (MAP) alkylidenes, such as Mo-3a-b. Due to the concerns regarding efficiency, transformations were run at 10 mM concentration, and the initial data were indeed encouraging. Contrary to the aforementioned cross-metathesis reactions (Fig. 2b), there was 18–58% conversion to \( 9 \) without any detectable homocoupling; the desired macrocyclic alkene was isolated in up to 46% yield (with complex Mo-3b). Nonetheless, the near complete lack of stereochemical control (52:48–66:34 \( E\text{,}Z \); Fig. 3a) was surprising. This kind of precipitous erosion in stereocontrol was unprecedented and considered unlikely, especially for reactions involving a trisubstituted alkene, moieties that are relatively inert to post-metathesis isomerization.
In all likelihood, the MRCM reaction proceeds via alkylidene $E$-10 and mcb-iii, the collapse of which affords $E$-9 and syn-iii (Fig. 3b). (The absolute stereochemistry of Mo alkylidenes alternates with each metallacyclobutane formation, inconsequential to the final outcome.) The resulting ethylidene complex (syn-iii) can combine with $E$-8 to re-generate $E$-10 and Z-butene, the latter of which may react with syn-iii via mcb-iv to afford $E$-butene and anti-iii, which is probably higher in energy and more reactive than syn-iii. The reason the aforementioned sequence occurs is that the $\alpha$-methyl group in mcb-iv is not sufficiently large to cause the steric repulsion with the aryloxide ligand that is needed to inhibit $E$-butene and anti-iii formation completely. Unlike the previous studies, where short-lived methylidene complexes had to be avoided, here, the major problem is the generation of an anti-alkylidene. The presence of anti-iii may lead to loss of stereoisomeric purity because it can react more readily with substrate $E$-8 via mcb-v to give Z-8, which would be converted to Z-9 and Z-10. Moreover, the concomitantly formed $E$-butene can react with syn-iii to give ent-anti-iii, causing further diminution in stereoselectivity. There is therefore a direct correlation between ligand structure and the level of stereoretentivity (Fig. 3a). Steric hindrance in mcb-v is less severe owing to a smaller aryloxide. Accordingly, there is further loss in stereochemical purity with a smaller ligand (e.g., Mo-3a compared to Mo-3b) because there is less steric pressure in the corresponding metallacyclobutane (mcb-iv–vi; see details in mcb-v’ and mcb-v’’; Fig. 3b). Isomerization of this type is not an issue in cross-metathesis. One reason for this is that the less substituted substrate is present in excess and can react with any anti-alkylidene formed to release the desirable syn isomer (see anti-iii $\rightarrow$ mcb-v $\rightarrow$ Z-8 $+$ syn-iii, Fig. 3b). A smaller amount of isomerized cross partner has less impact on cross-metathesis where often one olefin is available in greater concentration. Another factor is that most reported cross-metathesis cases afford a trisubstituted alkenyl halide or nitrile, and the olefin byproduct has the same electron-withdrawing unit and is as a result less reactive and less likely to cause problem.

Our analysis suggested that butene removal would counter subsequent formation of $E$-butene and anti-iii complexes, allowing for better retention of an alkene’s stereochemical identity. We substantiated this scenario experimentally (Fig. 3c). Analysis of unreacted 8 after 58% conversion to the 20-membered ring macrocycle (5.0 mol % Mo-3b) indicated pre-RCM isomerization, that is, loss of stereochemical purity of the trisubstituted alkene (from >98:2 to 68:32 E:Z). There was also complete isomerization at the more reactive disubstituted olefin (from >98:2 to <2:98 Z:E), but, as we would later ascertain, this can only influence the rate of Mo alkylidene formation (see $E$-10) and not the stereoisomeric purity of the product. Not surprisingly then, under mild vacuum (100 Torr; efficient butene removal) $E$-9 was generated in higher stereoisomeric purity (95:5 E:Z). The low efficiency of $E$-9 formation under reduced pressure might be attributed to competitive reaction of Mo alkylidene $E$-10 with substrate $E$-8 in a homocoupling or nonproductive process (despite the fact that the homocoupled byproduct can, in principle, react with the catalytically active ethylidene and re-enter the catalytic cycle). Also damaging could be that at high concentration (10 mM) Mo alkylidene dimerization and catalyst decomposition is probably more facile. Typically, the solution to these complications would be to improve yield by lowering substrate concentration. What made this situation unusual was whether, under higher dilution, less $E$-butene and anti-iii
would be formed (i.e., to give $Z$-$9$; see Fig. 3b) or the intermolecular reaction of an anti alkylidene with $E$-$8$ would be less competitive with MRCM, allowing the ring formation to be more stereoretentive.

When the macrocyclization was performed under increasingly dilute conditions, without resorting to reduced pressure, not only the efficiency but also – more uniquely – the stereochemical purity of the macrocycle improved steadily (Fig. 3c). With 5.0 mol % Mo-$3b$, which is commercially available and may be used directly at ambient pressure, a 1.0 mM solution of $E$-$8$ was converted to $E$-$9$ in 76% yield and with complete retention of stereochemistry (>98:2 $E$:$Z$). At 3.0 mM or lower concentration not only was there less homocoupling, as expected, but the yield of $E$-$8$ increased also, probably on account of reduced catalyst dimerization/decomposition. Cyclization of an isomeric substrate, which contained an $E$-1,2-disubstituted alkene was likewise efficient and selective (at 1.0 mM, 80% conv., 72% yield, 95:5 $E$:$Z$), confirming that the stereochemical identity of the disubstituted alkene is inconsequential. Equally notable, compared to a traditional MRCM involving a monosubstituted and a 1,1-disubstituted olefin (e.g., Fig. 1b), ring formation can be performed at a relatively high concentration (up to 10 mM) without much homocoupling.

**Range of $E$-trisubstituted macrocyclic alkenes**

The anticipation that MRCM leading to $E$-trisubstituted alkenes (Table 1) would be more stereoretentive is based on the additional steric repulsion in mcb-$i'$ (compared to mcb-$i$). Whereas in mcb-$i$ the Cβ methyl unit is proximal to the macrocyclic moiety, mcb-$i'$ contains more severe steric interactions with a Cα moiety pointing towards an aryloxide ligand and macrocyclic fragments being in a trans relationship. Various macrocyclic $E$-trisubstituted lactones (11–17, Table 1), ranging from a 22-membered ring to a 12-membered macrolactone, were obtained in 48–93% yield and 90:10 to >98:2 $E$:$Z$ ratios. Synthesis of the more strained 12- and 13-membered rings 16 and 17 required higher catalyst loading and longer reaction times (10 mol % and 12 h vs. 5.0 mol % and 4 h). The reduced $E$:$Z$ ratio for 13-membered ring lactone 16 may be due to the slower rate of cyclization of this relatively strained ring (thus 48% conv. and 48% yield), allowing for some isomerization to occur at the trisubstituted site of the substrate diene prior to ring formation (see Fig. 3b–c). Although most naturally occurring bioactive macrocycles contain a methyl-substituted olefin, those bearing other alkyl substituents might be of interest in drug discovery as well. Apropos, ethyl-substituted 18-membered macrolactone 18 was obtained in 49% yield and 80:20 $E$:$Z$ ratio, and only when 10 mol % Mo-$3c$, which bears a sterically less congested and electronically activated tetraphenyl-p-bromo aryloxide ligand, was used. With Mo-$3b$, a more hindered aryloxide, conversion was low (<10%), and with Mo-$3d$, an electronically less activated aryloxide, there was only 10% conversion to the desired product. Therefore, while in most cases, reactions with Mo-$3b$ are efficient and highly stereoretentive, with more demanding substrates, sterically and/or electronically altered catalysts will probably be needed.

Synthesis of carbocycles 19 and 20 shows that the method can be extended to all-carbon ring structures. As far as we know, there are no other previously reported cases, leading to the recent claim$^{47}$ that synthesis of carbocyclic alkenes through MRCM is inherently
problematic (see Table 2 for more examples). These data confirm that this is not the case. Reactions of amides are more complicated, largely because of the ability of this strongly Lewis basic moiety to chelate with a Mo complex, diminishing efficiency. Thus, under typical conditions, there was no conversion to the 19-membered ring secondary amide 21. In contrast, when the transformation was performed with tris(pentafluorophenyl)borane \( \text{B(C}_6\text{F}_5)_3 \), a Lewis acid that can associate with the amide carbonyl\(^{18}\), we were able to isolate the desired product in 80% yield and >98:2 \( E:Z \) ratio. Still, further study revealed that the presence of a Lewis acid does not always lead to high efficiency. For instance, there was just 16% conversion to secondary amide 22a despite the presence of \( \text{B(C}_6\text{F}_5)_3 \) (see Fig. 4a for more examples). With the derived Boc-amide, 22b was isolated in 82% yield and 91:9 \( E:Z \) ratio (no Lewis acid added). The optimal results for MRCM leading to 22b were obtained with the less sterically demanding tetraphenylaryloxide Mo-3d; the reaction with Mo-3b, while more stereoretentive, was less efficient (38% conv., >98:2 \( E:Z \)).

**Scope of Z-trisubstituted macrocyclic alkenes**

There is likely a smaller energy difference between competing mcb intermediates for MRCM reactions involving a \( Z \)-trisubstituted alkene (mcb-ii vs. mcb-ii', Table 2). There is slightly less steric pressure in mcb-ii' compared to mcb-i' (a \( C_\beta \) methyl group being \( cis \) to the ring section compared to two \( cis \) ring fragments, respectively), and the steric repulsion in mcb-ii is more severe than in mcb-i (two \( cis \) ring segments compared to a \( C_\beta \) methyl group being \( cis \) to the ring structure). Consequently, the stereoisomeric purity of the corresponding macrocyclic lactones, while generally high, was somewhat lower than for the transformations generating the corresponding \( E \)-trisubstituted olefins (see Table 1). The two sets of MRCM processes were similarly efficient. Synthesis of \( Z \)-trisubstituted alkenes 30 and 31 further confirms suitability for all-carbon macrocycle synthesis. The use of electronically activated bromoaryloxide Mo-3e\(^{48}\) was necessary for achieving efficient formation of 31, albeit with some loss of stereochemical purity (65% conv., 90:10 \( Z:E \) vs. 10% conv., >98:2 \( Z:E \) with Mo-3b). As noted above vis-à-vis the impact of the Lewis acidic additive, the more active Mo complexes can cause partial isomerization at the trisubstituted alkene site of the substrate diene prior to ring formation.

Unlike the aforementioned 19-membered ring \( E \)-macrolactam 21 (Table 1; >98:2 \( E:Z \)), for the corresponding \( Z \) isomer (32a; Table 2), an equivalent of \( \text{B(C}_6\text{F}_5)_3 \) improved efficiency but eroded stereochemical purity almost completely (55:45 \( Z:E \)). Analogous to \( E \)-lactam 22b, Boc-protected amide 32b was isolated in 70% yield and 94:6 \( Z:E \) ratio without needing a Lewis acidic additive. Along the same lines, tertiary amide 33 was obtained in 40% yield and as a single alkene isomer (>98:2 \( Z:E \)).

The data on MRCM of secondary amides represented several instances where the addition of \( \text{B(C}_6\text{F}_5)_3 \) diminishes efficiency and, more often, caused considerable loss of stereochemical purity. This suggested that there might be an unfavorable interaction between the catalyst and the Lewis acid additive. Experimental data support the latter notion. When the transformations leading to macrolactones 25, 26, or 28 were carried out with one equivalent of the Lewis acid, cyclic alkenes were generated in 66:34, 58:42, and 67:33 \( Z:E \) ratio respectively (compared to 88:12–92:8 \( Z:E \) without the Lewis acid). While the precise
nature of the interaction between a pentafluorophenyl imido Mo alkylidene and B(C₆F₅)₃ must await more detailed investigations, the resulting complex likely promotes rapid isomerization of the trisubstituted alkene moiety of the diene substrate, as described above (see Fig. 3b–c), causing diminution of stereoselectivity. The low conversion for the smaller 14-membered ring 22a may be attributed to reduced catalyst lifetime, and perhaps the 19-membered ring in 21 is less strained and its more facile formation can outpace catalyst decomposition.

Reversing substrate-controlled selectivity

As discussed earlier, a key question was the extent to which it might be possible to counter substrate-controlled selectivity through stereoretentive MRCM. Unlike the cases reported thus far²⁵–²⁸, where turning a nonselective or minimally selective process into a highly selective one was the sole objective, the aim here would be to cause stereoselectivity reversal. To explore this, we chose the transformation affording Z-trisubstituted alkene 2a, the 14-membered macrolactam precursor to anti-fungal agent fluvirucin B₁ (Fig. 1a)²⁹.

Subjection of diene 34a (Fig. 4a) to different MRCM conditions with different Mo MAP complexes did not lead to any detectable transformation. However, when B(C₆F₅)₃ was introduced, as described previously (see Tables 1–2), E-2c was formed in low selectivity (23:77 Z:E; 55% yield). We reasoned that a more flexible tertiary amide moiety, as opposed to a more rigid secondary variant, would mean that a larger number of conformations are available, some of which may more readily undergo RCM to afford the desired E isomer. This indeed proved to be the case: reaction of benzyl amide 34b delivered E-2c with 8:92 Z:E selectivity (66% yield). Considering the influence of olefin stereochemistry on the overall shape of a macrocycle, this approach offers an attractive option for framework editing of not only epothilones⁴⁹ but also of any macrocyclic drug candidate³⁵.

Stereoselective synthesis of dolabelide C

The remaining question was whether the catalytic approach is dependable enough for it to be performed towards the end of a multi-step complex molecule synthesis. We set out to determine whether we might be able to carry out the first fully stereoselective total synthesis of dolabelide C (Fig. 4b). A complex molecule that contains 11 stereogenic centers, ten of which can be found in a 24-membered ring macrolactone, and two trisubstituted E olefins, one of which is within the large ring, would allow us to probe the applicability and reliability of the MRCM approach.

The precursor to the macrocyclic alkene (triene 45) was prepared convergently (see Supplementary Information, Section 5). It was key that the requisite trisubstituted alkenes, one for the side chain of the target molecule and the other needed for the stereoretentive MRCM, would be synthesized efficiently, stereoselectively, and through the use of scalable and cost-effective protocols. Trisubstituted olefins 37 and 41 were synthesized in multigram quantities and >98:2 E:Z ratio in two steps from inexpensive starting materials, catalysts, and reagents. The aldol addition involving aldehyde 36 and ketone 38⁵⁰ afforded β-hydroxy ketone 39 in 73% yield and 91:9 diastereomeric ratio (d.r.), owing to partial epimerization
at C2 en route to 36, probably at the aldehyde stage. The stereoisomeric impurity was chromatographically removed at the final stages of the total synthesis, allowing us to secure the target molecule as the pure E-alkene.

When 45 was treated with 10 mol % Mo-3b in benzene at 40 °C for 12 hours, 46 was formed in 66% yield and with >98:2 E:Z selectivity, along with 20% of the recovered starting material. There was no competitive reaction at the more hindered trisubstituted alkene of the side chain. After two more steps, diastereomerically pure dolabelide C was obtained after routine silica gel chromatography, concluding the only fully stereoselective total synthesis of a member of this important class of natural products. The route consists of 39 steps with a longest linear sequence of 19 transformations, affording dolabelide C in 2.0% overall yield (compared to 0.3% overall yield, 50 total steps, and a longest linear sequence of 27 operations formerly). Catalytic MRCM was the thirty-seventh step of the total synthesis, providing strong testament regarding the robustness and reliability of the approach.

Conclusions

We introduce a solution to a key problem regarding one of the most important sets of transformations in chemistry. Specifically, we have developed a general strategy for preparation of a large assortment of macrocyclic trisubstituted alkenes in either stereoisomeric form. In the area of kinetically controlled olefin metathesis for accessing trisubstituted alkenes, a demanding and mostly unchartered territory, this advance is singular because it deals with the formation of large rings (as opposed to linear structures). It will now be possible to synthesize many macrocyclic trisubstituted alkenes efficiently and in high E,Z or Z,E selectivity with a commercially available organometallic complex, without the need for at least one small substituent (such as a halide or a nitrile). Prior to this work, a macrocycle could not be generated efficiently from an unfunctionalized trisubstituted olefin substrate (that is, not an allylic alcohol, enone, or enoate). The feasibility of reliably synthesizing a wide array of macrocyclic trisubstituted alkenes, in either stereoisomeric form, regardless of whether the cyclization is under strong substrate control, means that larger and richer libraries of skeletally diverse bioactive compounds and therapeutic candidates can be accessed.

Additionally, these studies reveal two important mechanistic principles. 1) Stereochemical control, and not only efficiency, may be impacted by substrate and catalyst concentration in kinetically controlled olefin metathesis. 2) Formation of an anti-alkylidene, and not only a methyldiene, can have an adverse effect on stereochemical control. High dilution is needed not just for minimizing substrate homocoupling, but also to disfavor the interaction of a small amount of a highly reactive anti-alkylidene with a trisubstituted olefin. Regarding practicality, the approach offers several advantages compared to other methods for macracycle formation, olefin metathesis based or otherwise. Homocoupling is less preponderant than when substrates containing a mono- and a 1,1-disubstituted olefin are used; this precludes the requirement for conformation-restricting elements to ensure high efficiency. The present strategy will likely be preferred to cyclization through formation of the ester bond of a lactone or the amide bond of a lactam. With target...
compounds like dolabelide, such linkages can be at a sterically congested site, rendering their transformations through a cyclization event low yielding. The MRCM approach obviates the need for a stereochemically defined acyclic trisubstituted alkene[^42], the synthesis of which would typically be more involved compared to those that are at the terminus of a diene substrate. Cross-metathesis would be less attractive also because excess amounts of a highly valuable advanced intermediate would be required. The greater understanding of the above issues, as offered here, paves the way to the development of additional challenging olefin metathesis processes.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1 | Unresolved problems and challenges in macrocyclic ring-closing metathesis (MRCM).

a, Substrate-controlled macrocyclic ring-closing metathesis (MRCM) is possible but stereoselectivity is difficult to predict, as indicated by reactions of 1a, affording 2a en route to antifungal agent fluvirucin B1, and 1b, which may be used for the same purpose. In most cases, low stereoselectivity is observed (1a → 2a is uncommon).

b, Mo bis-aryloxides have been used to promote MRCM that afford trisubstituted alkenes with stereocontrol, but only for a single case.

c, Reactions of sparsely functionalized dienes are particularly inefficient, probably on account of minimal entropic support.

d, Late-stage MRCM in total syntheses of naturally occurring anti-cancer agents dolabelides C and D is nonselective, requiring up to 25 mol % of complexes that contain a precious metal (Ru).

e, Mo and Ru complexes used in the previous studies. TBS, t-butyldimethylsilyl; TES, triethylsilane; Ac, acetyl; Mes, 2,4,6-trimethylphenyl.
Fig. 2. Challenges associated with designing a stereoretentive MRCM that affords a trisubstituted alkene.

a, The rationale for why catalytic stereoretentive MRCM should result in the formation of trisubstituted macrocyclic alkenes with high stereoisomeric purity is predicated by the preference for the metallacyclobutane stereoisomer that contains less steric pressure. b, The results of previous studies regarding stereoretentive cross-metathesis indicate that high substrate concentration is required. These data cast doubt on feasibility of the corresponding MRCM processes, which would require considerably more dilute conditions. L (L_{n}), ligand(s); R, aryl or alkyl group; Ar, aryl.
Fig. 3 | Unexpected complications and mechanism-guided solutions.  

a, Preliminary studies show that while stereoretentive MRCM leading to a trisubstituted olefin is reasonably efficient, it is not stereoselective.  
b, Mechanistic analysis suggests that the low stereocontrol might be caused by isomerization of the starting trisubstituted alkene, facilitated by a more reactive anti-iii, adventitiously formed in the reaction mixture. This higher energy anti-alkylidene complex can react with the stereoisomerically pure substrate (E-8) to cause pre-metathesis isomerization via mcb-v, leading to eventual generation of Z-8.  
c, Analysis of the recovered starting material supports that suggested scenario...
and MRCM became more efficient and more selective under more dilute conditions. L, L-n, ligands; Ar, aryl; R, aryl or alkyl substituent. Conversions (formation of the desired macrocycles) and stereoselectivities were measured by $^1$H NMR (±2%); yields are for purified products (±5%). All experiments were performed in triplicate (at least). See Supplementary Information section 3 for details.
Fig. 4 | Reversing substrate-controlled selectivity and late-stage stereoretentive MRCM in total synthesis of dolabelide C.

a, Catalyst-controlled stereoretentive MRCM may be used to override substrate-controlled stereochemical preference. b, The reliability of the approach is highlighted by the late-stage cyclization to generate the 24-membered ring macrocycle of dolabelide C. The total synthesis delivered diastereo- and enantiomerically pure dolabelide C (2.0% vs. 0.3% overall yield, previously; see Ref. 39). d.r., diastereomeric ratio; e.r., enantiomeric ratio; Lpc, isopinocampheyl; PMB, p-methoxybenzyl; DMB, 3,4-dimethoxybenzyl; TBS,
$t$-butyldimethylsilyl; DMAP, $N,N$-dimethyl-4-aminopyridine, Ac, acetyl; brsm, based on recovered starting material. Conversions, referring to formation of the desired macrocycles, and stereoselectivities were measured by $^1$H NMR (±2%); yields are for purified products (±5%). All experiments were performed in triplicate (at least). See Supplementary Information sections 4 and 5 for details.
Table 1 | Stereocontrolled synthesis of $E$-trisubstituted macrocyclic alkenes through catalytic stereoretentive ring-closing metathesis.

Reactions were carried out under N$_2$. Conversion to the desired product as measured by analysis of 400 MHz $^1$H NMR spectra of unpurified mixtures with DMF serving as the internal standard; the variance of values is ±2%. Yield of isolated and purified product, average over at least three runs; the variance of values is estimated to be <5%. $^a$With 10 mol % Mo-3b, 12 h. $^b$With 10 mol % Mo-3c, 12 h. $^c$With 5.0 mol % Mo-3d, 12 h. See the Supplementary Information section 3 for details. TBS, $t$-butyldimethylsilyl; PMB, $p$-methoxybenzyl; Boc, $t$-butyloxycarbonyl; ND, not determined.

$^a$With 10 mol % Mo-3b (commm. avail.), Benene (1.0 mM), 4 h, 40 °C.

$^b$With 10 mol % Mo-3c, 12 h.

$^c$With 5.0 mol % Mo-3d, 12 h. See the Supplementary Information section 3 for details. TBS, $t$-butyldimethylsilyl; PMB, $p$-methoxybenzyl; Boc, $t$-butyloxycarbonyl; ND, not determined.
Reactions were carried out under N$_2$. Conversion to the desired product as measured by analysis of 400 MHz $^1$H NMR spectra of unpurified mixtures with DMF serving as the internal standard; the variance of values is ±2%. Yield of isolated and purified product, average over at least three runs; the variance of values is estimated to be <5%. a With 10 mol % Mo-3e, 12 h. b With 10 mol % Mo-3a, 12 h. See the Supplementary Information section 3 for details. TBS, t-butyldimethylsilyl; PMB, p-methoxybenzyl; Boc, t-butyloxycarbonyl; ND, not determined.