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Predictive Bioinformatic Assignment of Methyl-Bearing Stereocenters, Total Synthesis, and an Additional Molecular Target of Ajudazol B

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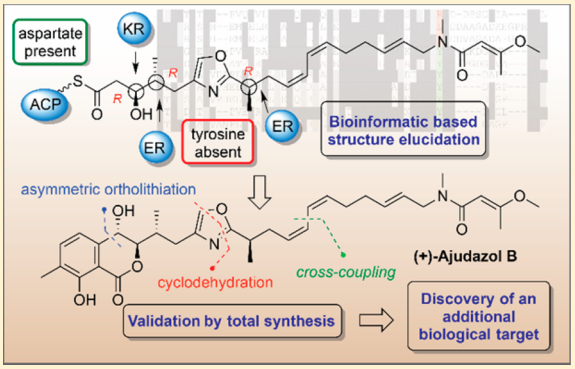
Supporting Information

ABSTRACT: Full details on the evaluation and application of an easily feasible and generally useful method for configurational assignments of isolated methyl-bearing stereocenters are reported. The analytical tool relies on a bioinformatic gene cluster analysis and utilizes a predictive enoylreductase alignment, and its feasibility was demonstrated by the full stereochemical determination of the ajudazols, highly potent inhibitors of the mitochondrial respiratory chain. Furthermore, a full account of our strategies and tactics that culminated in the total synthesis of ajudazol B, the most potent and least abundant of these structurally unique class of myxobacterial natural products, is presented. Key features include an application of an asymmetric ortholithiation strategy for synthesis of the characteristic *anti*-configured hydroxyisochromanone core bearing three contiguous stereocenters, a modular oxazole formation, a flexible cross-metathesis approach for terminal allyl amide synthesis, and a late-stage *Z*,*Z*-selective Suzuki coupling. This total synthesis unambiguously proves the correct stereochemistry, which was further corroborated by comparison with reisolated natural material. Finally, 5-lipoxygenase was discovered as an additional molecular target of ajudazol B. Activities against this clinically validated key enzyme of the biosynthesis of proinflammatory leukotrienes were in the range of the approved drug zileuton, which further underlines the biological importance of this unique natural product.



INTRODUCTION

Myxobacteria present extremely rich sources of structurally diverse natural products with unique molecular architectures.[1] They often show a wide range of potent biological activities[1b] and in many cases address selectively molecular targets with high specificity.[2] The genera *Sorangium cellulosum* and *Chondromyces crocatus* take special places among these Gram-negative bacteria as they have synthesized approximately half of the secondary metabolites isolated from myxobacteria so far.[1] Being genetically closely related, both belong to the suborder *Sorangiineae*[3] that contains the largest bacterial genome sequenced to date.[4] Among these fascinating and eye-catching structures, the ajudazols (Figure 1) constitute a completely novel type of a structurally unusual and stereochemically elaborate class of compounds, isolated from *Chondromyces crocatus*, strain *Cm* c5.[5] From a biological perspective, the ajudazols are potent antifungal agents and show antifungal activities against *Botrytis cinerea*, *Trichoderma koningii*, *Giberella fujikuroi*, and *Ustilago maydis*.[6] In contrast, only weak antibacterial and antiproliferative activities were reported, demonstrating a selective biological interaction profile. On a molecular level, the ajudazols are described as highly effective inhibitors of the mitochondrial respiratory chain by selective binding to complex I (NADH-dehydrogenase). The NADH oxidation level in beef heart submitochondrial particles was inhibited at an IC_{50} value of 13.0 ng/mL (22.0 nM) for ajudazol A (**1**) and 10.9 ng/mL (18.4 nM) for ajudazol B (**2**).

The aerobic production of energy in the mitochondrial respiratory chain presents a key regulatory mechanism in a wide variety of cellular processes.[7] Consequently, malfunctions of this central pathway are correlated with a high number of inherited as well as acquired diseases.

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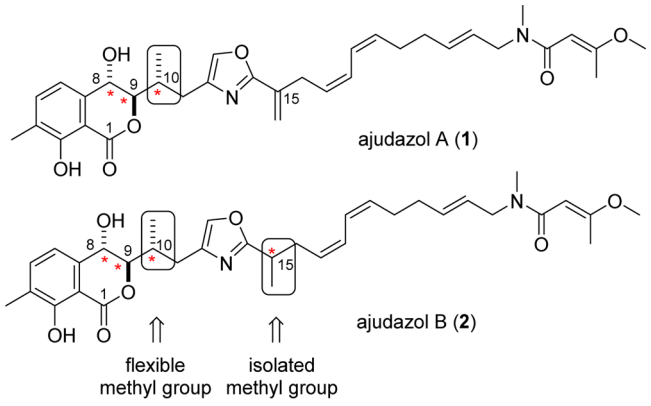

Figure 1. Ajudazols A (**1**) and B (**2**): potent inhibitors of the mitochondrial respiratory chain from the myxobacterium Chondromyces crocatus of initially unknown configuration.

Genetic disorders include Leber's hereditary optic neuropathy (LHON), the Kearns−Sayre syndrome (KSS), different mitochondrial myopathies (CPEO, MERRF, MELAS, MNGIE), Morbus Pearson and diabetes mellitus, and deafness syndrome (DAD).[8]

More recently, respiratory chain defects have been increasingly associated with neurodegenerative disorders, such as Leigh's disease, spastic paraplegia, the Mohr−Tranebjærg syndrome, Friedreich's ataxia, Huntington's chorea, Wilson's, Parkinson's, and Alzheimer's diseases, and reduced activities of the respiratory complexes in these maladies has been unambiguously shown.[9] Animal experiments have shown that the treatment with substances that protect mitochondria like flavonoids, statins, methylene blue, or radical scavengers lead to a significant increase in life expectancy.[9d] The high relevance of respiratory chain defects in a broad range of diseases renders the development of small molecules that interact with these processes of high importance. In addition to the treatments with vitamins or radical scavengers, inhibitors may also become increasingly important as therapeutic agents.[10] In the past, compounds like oligomycin, rotenone, or antimycin A have mainly been used as chemical tools for functional and structural studies of the respiratory chain.[10] But respiratory chain inhibitors have also been specifically developed to induce apoptosis or to generate oxidative stress.[11] Only a few of these inhibitors have already been evaluated in clinical trials,[12] and among these, elesclomol is presently in different phase III clinical trials,[13] indicating the prospective pharmaceutical potential of this inhibitor class.

In addition to myxothiazol,[14] stigmatellin,[15] and crocacin D,[16] the ajudazols have been reported as the fourth compound class of respiratory chain inhibitors from myxobacteria. Stigmatellin has been used specifically for structural studies of complex III,[17] and crocacin D has been used more recently as a lead structure for analogue design.[18] Being very potent inhibitors of complex I, the ajudazols may become similarly successful in the future. This renders more detailed chemical and biological studies of high importance from the perspective of medicinal and biological chemistry.

As shown in Figure 1, the unique three-dimensional architectures of the ajudazols are distinguished by a characteristic isochromanone heterocycle with two vicinal *anti*-configured hydroxyl groups (C_8 and C_9) together with an extended side chain that contains an oxazole, a *Z*,*Z*-diene, and a 3-methoxybutenoic acid amide as typical structural features. So far, two ajudazols have been reported.[5] The main metabolite, ajudazol A (**1**), bears an exomethylene group next to the oxazole, while ajudazol B (**2**) has a methyl group at this position (C_{15}). Ajudazol B (**2**) is less abundant but has been shown to be slightly more active in the biological systems evaluated so far. While oxazole systems and *Z*,*Z*-diene motives have been described as common structural features in natural products, the 3-methoxybutenoic acid has so far only been reported for one other natural product.[19] In contrast, the *anti*,*anti*-configured hydroxylisochromanone system is unique. The ajudazols contain up to four stereocenters of originally unknown absolute configuration.

All initial efforts directed toward a first total synthesis were therefore hampered by this lack of full stereochemical knowledge available in combination with apparent difficulties in establishing an efficient route to the unique isochromanone subunit.[20] Importantly, this subunit was shown to be labile toward translactonizations.[5,21] Herein, we report in full detail a bioinformatics approach that was used for the stereochemical determination of the ajudazols, including the design, evaluation, and application of an easily feasible and generally useful method for configurational assignment of isolated methyl bearing centers. Furthermore, the application of an efficient method for isochromanone synthesis based on an asymmetric ortholithiation strategy[22] leading to the first total synthesis of ajudazol B (**2**) will be reported.[23] Finally, with a synthetic access to ajudazol B (**2**) in hand, we could identify a novel potent molecular target of this unique polyketide.

RESULTS AND DISCUSSION

General Method for Configurational Assignment of Isolated Methyl Bearing Centers: Stereochemical Determination of the Ajudazols by Biosynthetic Gene-Cluster Analysis. The constitutions of the ajudazols were convincingly determined by Jansen et al. by a combination of 1D- and 2D-NMR techniques at the beginning of this century.[5] In addition, a relative stereochemistry of the vicinal stereogenic centers in the isochromanone part was tentatively proposed by a comparison of ^{1}H NMR coupling constants with those of the related natural product benaphthamycin.[24] However, the configuration of this isochromanone had not been unambiguously assigned. In addition, the reported assignment of the methyl-bearing center at C_{10} relative to the stereocenters at C_8 and C_9 appeared disputable, as the observed coupling constants between H_9 and H_{10} (5.6 and 4.1 Hz for ajudazols A and B) suggest a high degree of conformational flexibility. Therefore, reported arguments based on NOE data appeared to be not fully convincing. In addition, efforts to assign the absolute configuration by Mosher ester analysis[25] were not successful due the lability of the isochromanone core, and no information on the absolute configuration of C_{15} of ajudazol B (**2**) was available, as this center is too far away from the remaining chiral centers and too flexible to allow for a correlation by NMR methods.

In general, assignment of isolated or flexible methyl bearing stereocenters poses a particular challenge in structure determination, and currently no generally applicable methods are available. So far, the only solution to resolve this issue involves anomalous X-ray dispersion.[26] However, such an approach is strictly limited to suitable crystals, which very often cannot be obtained. In addition, reported NMR methods are

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limited and restricted to rigid cyclic systems or require a close vicinity to other chiral centers.[27] In many cases, alternative methods that would rely on degradation were not possible due to the lack of available material. Alternatively, a synthesis of all potential isomers may be possible, and such an approach has been pursued by all synthetic groups initially working on the ajudazols.[20] Interestingly, all of them have been targeting the stereoisomer of the ajudazols that was shown in the isolation paper.[5] However, this isomer finally was proved to be an enantiomer of the correct structure as assigned by our study.[23] It may be speculated that this may be partially caused by an implicit understanding that the relatively shown stereochemistry in the isolation paper may also present the absolute stereochemistry. Consequently, all these groups have only prepared stereoisomeric fragments of the ajudazols underlining the risks of such approaches. In combination with existing doubts about the reported relative configuration and the fact that even comparison of optical rotation data of stereoisomeric products may be misleading,[28] we turned our attention to an alternative method. This relies on the information available from the analysis of the genes which are involved in the biosynthesis of the ajudazols to enable a configurational prediction of the hydroxyl-bearing and methyl-bearing stereogenic centers. Polyketide biosynthesis in myxobacteria has been studied in detailed by analysis of the genome and the proteome of the producing organisms.[3,29] According to the collinearity principle or "Celmer's rule", a correlation between the molecular structure of the underlying biosynthetic multienzyme complexes, i.e.. the polyketide synthases and the chemical structure of the metabolite, is possible.[30] This principle enables the elucidation of polyketide syntheses by sequencing and bioinformatics analysis of the underlying gene clusters and also the genetic manipulation of polyketide production.[31]

The genome of the myxobacterium *Chondromyces crocatus Cm* c5, the natural producer of the ajudazols, has been analyzed, and a biosynthesis has been postulated on the basis of the investigated gene clusters.[32] As shown in Figure 2 for a part of this biosynthesis, the stereogenic centers of the ajudazols are set in four distinct enzymatic steps. The methyl-bearing stereocenters at C_{15} and C_{10} are constructed by two enoyl reductases encoded in the AjuC and the AjuE gene cluster, while a ketoreductase in AjuF installs the hydroxyl-bearing stereocenter at C_9. Finally, the benzylic hydroxylgroup at C_8 is introduced as a post-PKS-modification by a cytochrome P450 enzyme.

While no general transferable information on the stereoselective outcome of reactions catalyzed by P450 enzymes are reported,[33] the domain motives of the two other enzymatic systems are highly conserved and may be used for a predictive analysis of the resulting stereochemistries.

On the basis of extensive bioinformatics analysis, the groups of Caffrey[34] and McDaniel[35] have studied in detail the stereoselectivity of ketoreductases of a high number of polyketide systems. On the basis of this evaluation, they discovered characteristic amino acid patterns in highly conserved domain motives with a decisive influence on the stereochemistry of the resulting hydroxyl bearing stereogenic center.[34b] According to a model proposed by Caffrey, a D-configuration is expected when an LDD motive is present upstream of the conserved GVxHxA motive. This assignment becomes more reliable when a proline (P144) or an aspartate (N148) is present at specific places of the sequence. In contrast, the absence of these amino acids and presence of a tryptophan residue (W141) results in the formation of an L-configured

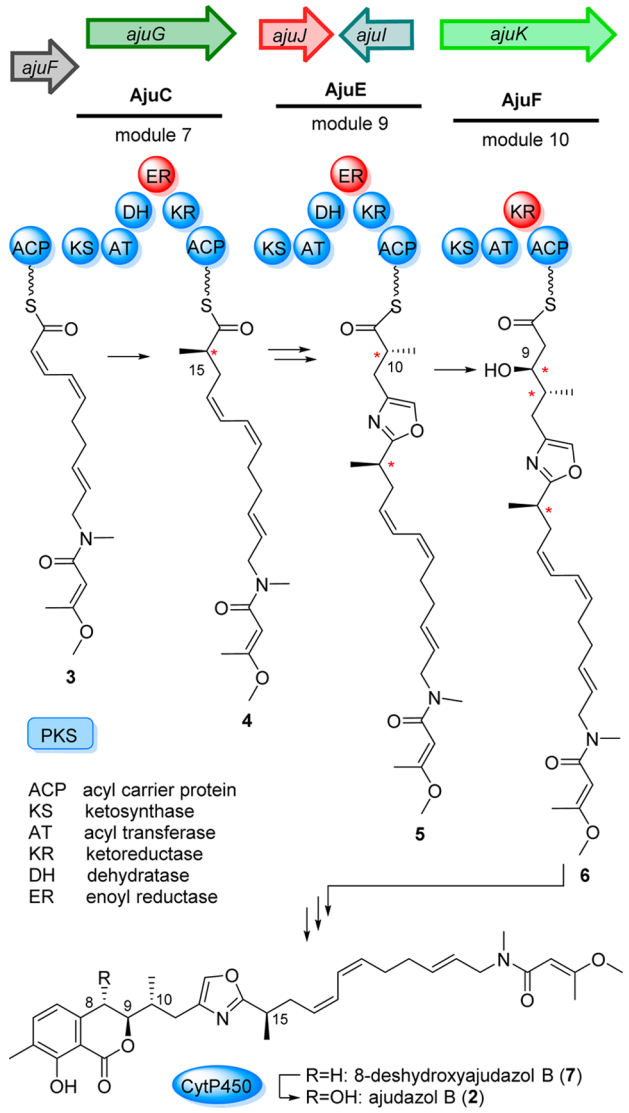

Figure 2. Essential parts of the biosynthesis of ajudazol B (**2**): the stereogenic centers are installed by enoylreductases, a ketoreductase and cytochrome P450 oxidation.

secondary alcohol. In a parallel fashion, the group of McDaniel has proposed a simpler model.[35] Following their analysis, a single characteristic aspartate residue (D95) in the LDD motive is sufficient to deduce a D-configuration of the resulting alcohol, while the absence of this residue results in an L-configuration (Scheme 1).

The crucial influence of these key amino acids was confirmed by mutagenesis and engineering studies and was further rationalized by a mechanistic model.[37] Since the original publications in 2003, the reliability of these models has been confirmed several times in the stereochemical determination of complex polyketides. However, in all these examples, these bioinformatics analyses have been mainly used as complementary confirmations of configurational conclusions drawn from conventional methods, and only in a few examples they have also been used for a prediction of stereocenters, where assignments were difficult by alternative methods.[27d,31c,38]

Alcohols deriving from ketoreduction can have additional methyl branches when 2-methylmalonyl-CoA is used as an extension unit. Different models were discussed in the past to explain the resulting stereochemical outcome of these branches

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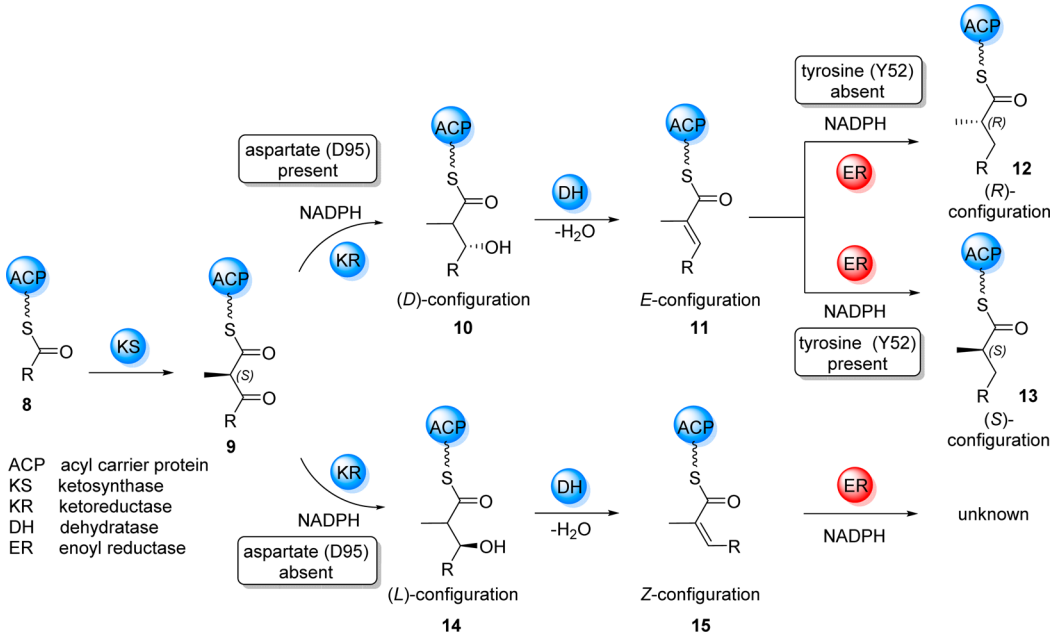
Scheme 1. Configurational Assignment of Hydroxyl- and Methyl-Bearing Stereocenters by Bioinformatic Gene Cluster Analysis According to the Models of Caffrey[34a] and McDaniel[35] for Ketoreductases and the Model of Leadlay[36] for Enoylreductases



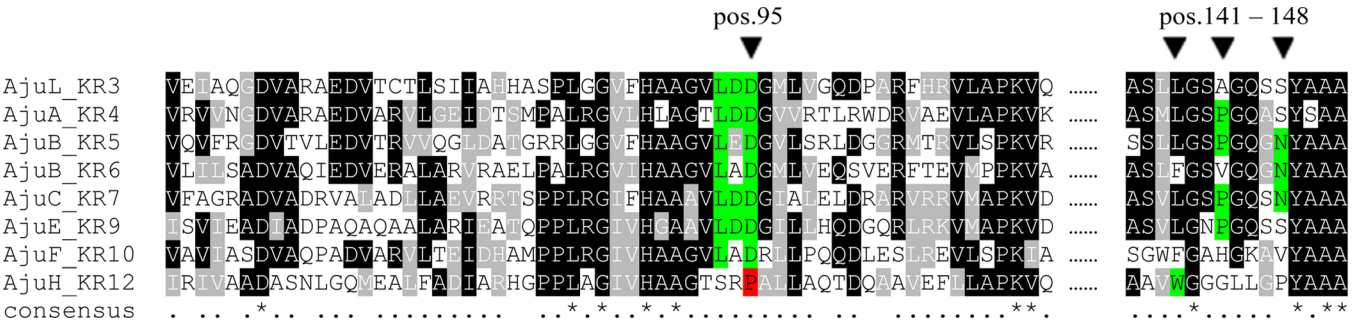


Figure 3. Analysis of the selected ketoreductase core regions of the ajudazol B biosynthesis according to the models of McDaniel[35] and Caffrey[34a] showing the presence (green) or absence (red) of indicative aspartate residue D95 and the presence (green) of other indicative residues (L93, D94, W141, P144, N148).

after ketoreduction.[39] One model explains the stereochemistry of methyl-branched polyketides by epimerization during the ketoreduction step.[39c,d] This model has been experimentally studied in detail for some polyketides,[39c,40] and the group of Keatinge-Clay have combined bioinformatics and structural approaches in order to identify indicative residues for the prediction of the stereochemical outcome of such ketoreductions.[39c,41] They therefore divided ketoreductases into four groups based on the presence of the LDD motive and the two possible configurations of the methyl-branched stereocenter and identified tryptophan (W141), histidine (H146), and proline (P151) as indicative residues. Kitsche and Kalesse have extended and refined this analysis and designed a bioinformatics tool by using profile- hidden Markov models (HMMs) in combination with a derived score difference.[42]

The configuration of the hydroxyl-bearing stereocenters that results from ketoreduction then controls the stereoselectivity of the subsequent biosynthetic step, and the elimination of water by dehydratases. In detail, it could be shown that a D-configured alcohol results in an *E*-configured double bond (**11**) while an L-configured alcohol results in a *Z*-configuration of the alkene (**15**, Scheme 1).[30b,34a,35]

Subsequent reduction of these enones by enoylreductases forms methyl-bearing stereocenters. In contrast to ketoreductases, the stereoselectivity of this enzyme class have been much less evaluated. A first study was reported in 2008 by the group of Leadlay.[36] By alignment of several ER sequences, mainly from actinomycetes, they identified a single characteristic tyrosine residue (Y52) in the vicinity of the NADPH-binding motive, i.e., the HAAAGGVGMA-consensus sequence. According to their model, the presence of this tyrosine results in the formation of an (*S*)-configured methyl-bearing stereocenter (**13**). In contrast, enoylreductases resulted in (*R*)-configured stereocenters (**12**) often showed valine, alanine, or phenylalanine residues at this position. This model was further confirmed by mutagenesis experiments.[43] As shown in Scheme 1, all studied ER systems involved reductions of *E*-configured alkenes (**11**). So far, no data for enoylreductases involving the corresponding *Z*-configured alkenes (**15**) have been reported.

In contrast to the models of Caffrey and McDaniel, predictive applications of this method are largely absent. Only one evaluation has been reported in the context of the structural determination and total synthesis of pellasoren (**17**).[44]

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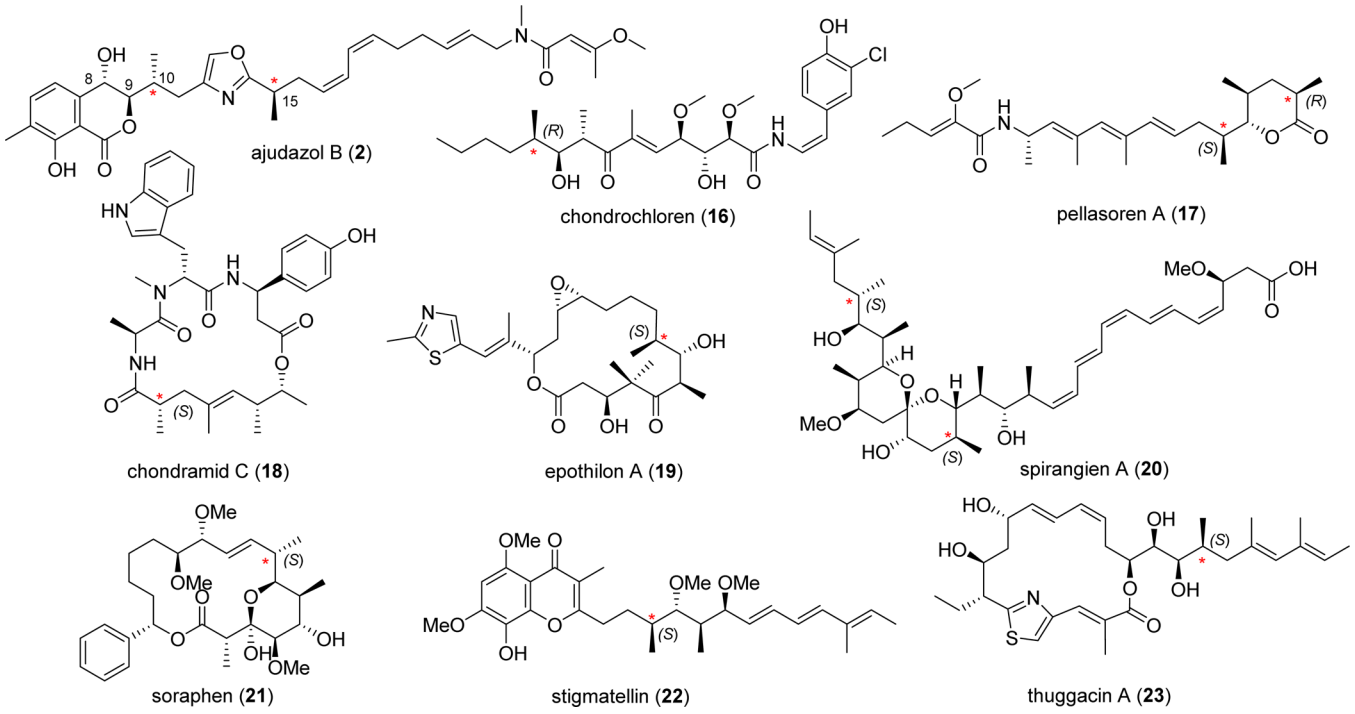

Figure 4. Myxobacterial polyketides with methyl-bearing stereocenters that are derived by enoylreductases. Except for ajudazol B (**2**) the absolute stereochemistry of these compounds were determined by NMR and/or X-ray methods.

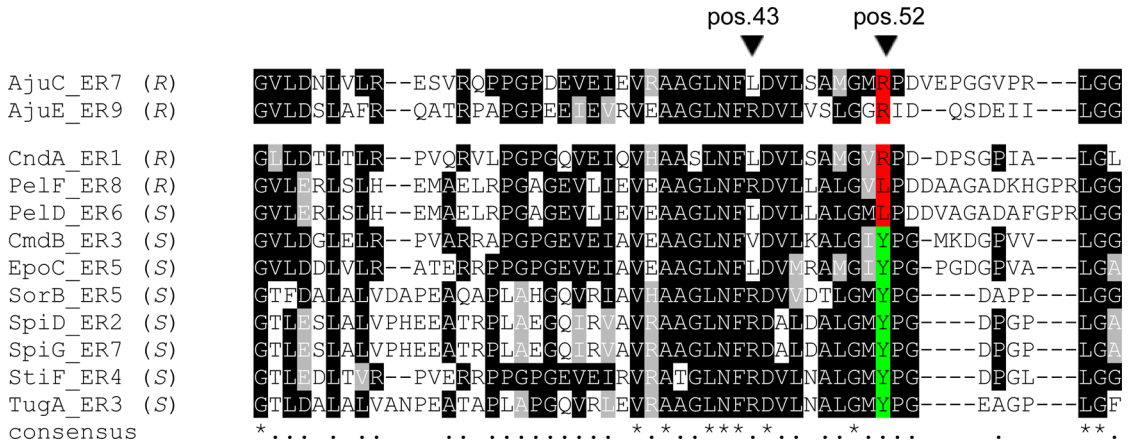

Figure 5. Analysis of the enoylreductase core regions of the polyketides shown in Figure 4 according to the model of Leadlay.[36]

In detail, two enoylreductases were investigated that are involved in the biosynthesis of this polyketide, but only for one of them was the configuration of the resulting methyl-bearing center correctly predicted and in agreement with the independently performed NMR and total synthesis experiments. Therefore, a reevaluation of this method using a broader set of myxobacterial metabolites seemed to be necessary (vide infra).

As shown in Figure 3, we first focused on the more established alignment of the ketoreductase core regions of KR 3−12 of the ajudazol B biosynthesis. Following the model of McDaniel,[35] indicative aspartate residues (D95) were present at the pivotal positions for KR 3−11, while a proline was observed at this position for KR 12. While most of these alcohols are subsequently eliminated by dehydratases, the hydroxyl-bearing stereocenter at C_9 resulting from KR10 is retained. Due to the presence of an aspartate, this hydroxyl should therefore be D-(= *R*)-configured. For further confirmation, the alignment data were then also analyzed according to the more detailed model of Caffrey.[34] In all cases, major parts of the consensus sequences were present. While for some ketoreductases (KR5, KR6, KR10) the LDD motive was not completely present and also the additional indicative amino acids proline or arginine could not be found in all cases (KR3, KR10), there is a precedent that small deviations from the Caffrey consensus residues are tolerable.[27d,31c,38] Therefore, both analyses come to the same conclusions and jointly propose a D-(= *R*)-configuration for C_9. In combination with the proposal of Jansen[5] for the relative stereochemistry, the configuration of the isochromanone part of ajudazol B (**2**) should therefore be (*S*)-C_8, (*R*)-C_9, (*R*)-C_{10}. As discussed above, elimination of the D-configured alcohols would result in *E*-alkenes, while the L-configured hydroxyl would give rise to a *Z*-alkene (KR12). This is in agreement with the postulated biosynthesis of the aromatic ring based on a *Z*-configured alkene (KR12) and the *E*-configured double bond between C_{23}/C_{24} (KR3). It is

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interesting to note that *E*-configured double bonds would have also been expected between C_{17}/C_{18} (KR6) and C_{19}/C_{20} (KR7) and not a (*Z*,*Z*)-diene as observed. This discrepancy could not be explained and may require a more detailed study of the responsible dehydratase enzyme. We then turned our attention to a predictive analysis of the enoyl reductases following the model of Leadlay.[36] Given the limited number of examples discussed in the original publication, which are mainly derived from actinomycetes in combination with a predictive failure of the model for the myxobacterial metabolite pellasoren A (**17**),[44] we first evaluated the general reliability of the method for a range of myxobacterial metabolites. Accordingly, we analyzed the biosynthetic gene cluster of the myxobacterial polyketides chondrochloren (**16**),[31d] chondramid C (**18**),[45] pellasoren A (**17**),[44] epothilon A (**19**),[31a,46] spirangien A (**20**),[47] soraphen (**21**),[48] stigmatellin (**22**),[49] and thuggacin A (**23**)[50] (Figure 4). In all of these metabolites, one or two methyl bearing centers are derived by an enolyreductase. The full stereochemistries of these compounds have been rigorously assigned in all cases by X-ray structure analysis or NMR-based methods and confirmed in most cases by total synthesis.[38b,44,51]

As shown in Figure 5, analysis of the respective enoylreductase core regions by the method of Leadlay[36] revealed tyrosine residues in the critical ER region for the methyl-bearing stereocenters of chondramid C (**18**), epothilon A (**19**), spirangien A (**20**), soraphen (**21**), stigmatellin (**22**), and thuggacin A (**23**). This suggests these methyl-bearing centers to be (*S*)-configured. Correspondingly, the absence of this amino acid residue in the respective enoylreductases of chondrochloren (**16**) and PelF and PelD of pellasoren A (**17**) propose these centers to be (*R*)-configured. In detail, the enoylreductase of chondrochloren (**16**) reveals an arginine residue, and in the case of pellasoren (**17**) two leucine residues can be observed. Comparing these predictions to the configurations independently derived resulted in an almost perfect match. Only for one of the methyl groups of pellasoren A (**17**) (PelD) was the configuration incorrectly proposed. While a reason for this discrepancy cannot be explained at this stage, the assumption[44] that arginine at position 43 might be act as additional indicative residue seems to be not robust in our alignments, the comparison in general validates the method of Leadlay[36] and proves the viability of such a bioinformatics approach for configurational assignment of the ajudazols. The group of Leadlay[36] had only correlated valine, alanine, and phenylalanine to an (*R*)-configuration in their examples. However, they have designed their model in such a way that the absence of a tyrosine is the decisive criterion.

Accordingly, alignment of the critical enoylreductases ER9 and ER7 that are responsible for installation of the methyl-bearing centers at C_{10} and C_{15} reveals the absence of a tyrosine. In agreement with chondrochloren (**16**), again an arginine residue was observed. Therefore, these two centers are proposed to be (*R*)-configured as shown in Figure 6. Notably, the assignment of C_{10} to be (*R*)-configured is in combination with the conclusions drawn above for the assignment of C_9 to be (*R*)-configured in full agreement with the proposed relative stereochemistry.[4] This further corroborates the viability of this approach.

Figure 6 summarizes our proposal of the full relative and absolute configuration for ajudazol A (**1**) as (*S*)-C_8, (*R*)-C_9, (*R*)-C_{10} and for ajudazol B (**2**) as (*S*)-C_8, (*R*)-C_9, (*R*)-C_{10}, (*R*)-C_{15}. Importantly, this assignment was based on a bioinformatics based approach only and did not require material for the

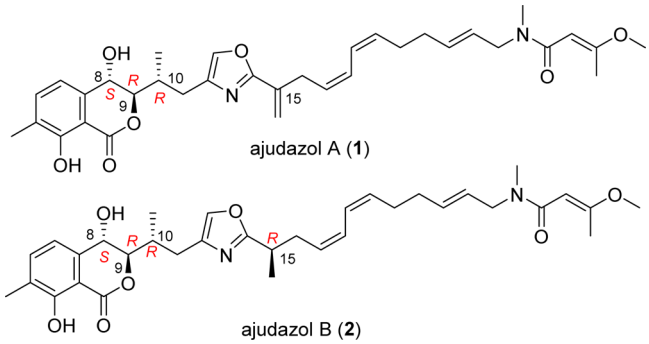


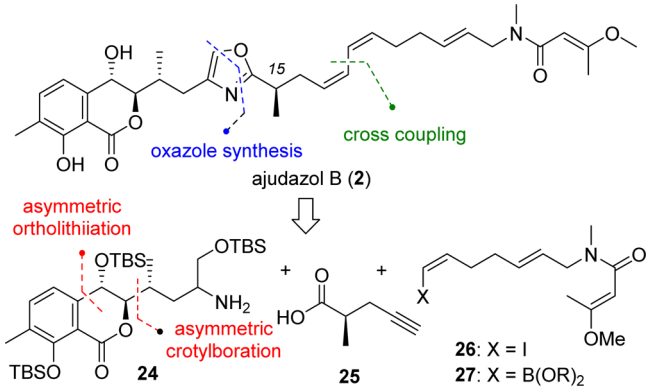
Figure 6. Absolute and relative configuration of ajudazol A (**1**) and B (**2**).

natural product. This presents the first example where the full stereochemistry of a natural product was assigned purely by bioinformatics methods. For a final proof of this assignment and validation of this bioinformatics analysis a total synthesis was required.

Total Synthesis of Ajudazol B: Confirmation of the Stereochemical Assignment. As a synthetic target for an unambiguous confirmation of our bioinformatic stereochemical assignment we chose ajudazol B (**2**), the less abundant and more potent ajudazol. This would also allow the verification of the proposed configuration of the isolated methyl bearing center (C_{15}) adjacent to the oxazole.

As shown in Scheme 2, our retrosynthetic approach relied on a modular introduction of this center at a late stage of our

Scheme 2. Retrosynthetic Analysis for a Total Synthesis of Ajudazol B



synthesis. A reliable cyclodehydration strategy of protected amino alcohol **24** with methyl-bearing alkynylic acid **25** was therefore pursued. For construction of the *Z*,*Z*-diene motive, the group of Taylor has reported an elegant double-carbocupration strategy.[20a] However, such a route is only applicable for ajudazol A (**1**). Alternatively, the group of Rizzacasa has reported a Sonogashira coupling−Lindlar reduction sequence.[20b−d] While this strategy has often been applied for generation of *Z*-alkenes, the reported yields are variable and often overreductions are observed. In agreement with these observations, only low yields[20c,d] were reported by the Rizzacasa group in their synthesis of 8-deshydroxy-9,10-*ent*-ajudazole A and B. Therefore, we chose an alternative approach involving a *Z*-selective sp^2-sp^2 cross-coupling strategy. Accordingly, the terminal alkyne should serve as basis of an *Z*,*Z*-selective sp^2-sp^2 cross-coupling strategy with a suitably

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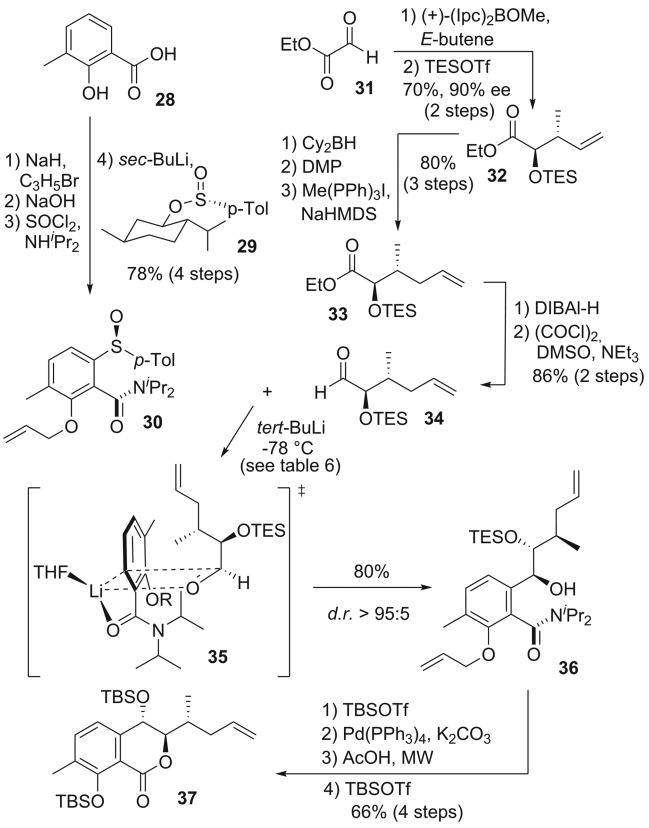
functionalized Eastern fragment, i.e., vinylic iodide **26** or boronate **27**.

Synthesis of the Western Fragment of the Ajudazols by an Asymmetric Ortholithiation Approach. Various strategies for construction of isochromanones have been described in the literature.[20c−g,52] However, these existing approaches appeared not to be directly applicable to the characteristic substitution pattern of the authentic isochromanone core structure of the ajudazols and/or do not enable a flexible and fully stereochemical control. Additionally, C_8-hydroxyl-substituted isochromanones like the ajudazols are known to be labile under basic conditions and can undergo translactonization reactions to form a thermodynamically more stable five-membered ring analogue.[21] For these reasons, we have designed a conceptually novel approach for the construction of isochromanones based on an asymmetric ortholithiation key step.[22] This reaction type allows a modular functionalization of 3-methylsalicylic acid **28**, which already contains nearly the complete aromatic substitution pattern of the ajudazols in parallel to the stereoselective introduction of the C_8-hydroxyl group by an aldehyde electrophile. The two C_9 and C_{10} stereocenters can then be generated independently by asymmetric crotylboration (Scheme 2). Chiral induction during ortholithiation can be achieved by a combination of a tertiary amide as directed metalation group (DMG) and a chiral sulfoxide acting as easy removable temporary stereogenic center.[53] The chiral sulfoxide leads thereby via chirality transfer to a preorientation of the nonplanar amide axis[54] which is retained after cleavage of the sulfoxide by *tert*-BuLi during the ortholithiation reaction at low temperatures (chiral memory). Electrophilic attack of the resulting atropochiral aryllithium species with an aldehyde allows finally the "self-regeneration of the stereocenter" (SRS-principle).[55] For the applicability of this reaction type for construction of hydroxyisochromanones we have previously developed suitable orthogonal protecting group strategies for both the C_8 and phenolic alcohol and efficient protocols for cleavage of sterically highly hindered tertiary amides.[22]

As shown in Scheme 3, we applied these protocols to the Western fragment synthesis of ajudazol B (**2**). First, the phenol group of 3-methylsalicylic acid **28** was protected in a two-step sequence by an allyl group which appeared to be a robust protecting group for the subsequent steps. After conversion to the diisopropyl amide, the amide axis was subsequently fixed by ortholithiation with Andersen reagent **29**[56] to yield *S*-sulfoxide **30**. The required aldehyde reaction partner **34** was then built up from ethyl glyoxalate **31** by asymmetric crotylboration (70%, dr = 98:2, 90% ee).[57] The absolute configuration was confirmed by Mosher ester analysis.[25] For the protection of the newly generated alcohol a triethylsilyl (TES) group was chosen. TES-protected ester **32** had then to be homologated before the ortholithiation reaction by a three-step sequence. For the first step of this sequence a careful choice of the hydroboration reagent, 9-BBN, BH_3·THF, and BH_3·Me_2S, did not form the desired product, and optimization of workup conditions for cyclohexylborane[58] was necessary to prevent saponification of the ester function. After oxidation with Dess−Martin periodinane and Wittig reaction, the homologated ester **33** could be obtained in reasonable yields of 80%. Transformation to aldehyde **34** (86%, two steps) and asymmetric ortholithiation with sulfoxide **30** generated *anti,anti*-product **36**. The highly stereoselective formation of **36** (dr >95:5) can be rationalized in accordance with the literature[54a,59] by transition state **35** in

Scheme 3. Asymmetric Ortholithiation and Isochromanone Synthesis



which the silyl protecting group points away from the aromatic core and the attack on the electrophile occurs from the diisopropylamide containing site due to the formation of a space demanding Li−THF cluster shielding the opposite site. Differentiation of the two hydroxyl groups was then achieved by the orthogonal protection of the C_8-hydroxyl group as *tert*-butyldimethylsilyl (TBS) ether. Transformation to the corresponding *anti,anti*-configured isochromanone was subsequently realized in 90% by removal of the phenolic allyl group under basic conditions by $Pd(PPh_3)_4$ catalysis and application of our optimized microwave-assisted one-pot amide and TES group cleavage protocol.[22] The TBS group of the C_8-alcohol remains thereby intact. Reprotection and unification of the protecting group strategy with TBSOTf leads then to *anti,anti*-isochromanone **37** in 12 steps and 25% overall yield.

For the pivotal asymmetric ortholithiation, the original protocol had to be further optimized to achieve the shown yields of this reaction (Scheme 3) with complex aldehydes like **34** (Table 1). In our initial efforts, we tried to reduce the amount of aldehyde. Simple aldehydes like acrolein are normally added in large access of up to 6 equiv.[53] For complex aldehydes like **34**, the reaction can also be successfully performed with an equimolar ratio of **34** and chiral sulfoxide **30** (entry 1, Table 1). The yield could then be improved by the increase of either the aromatic compound **30** (entries 2 and 3, Table 1) or the aldehyde **34** (entry 4, Table 1). Further improvements were achieved by reduction of the amount of *t*-BuLi (entries 5−7, Table 1). A large excess of *t*-BuLi probably causes decomposition of the aldehyde electrophile prior to attack of the aryllithium species. The use of 1.2 equiv of *t*-BuLi was still suitable to cleave the sulfoxide completely. In addition,

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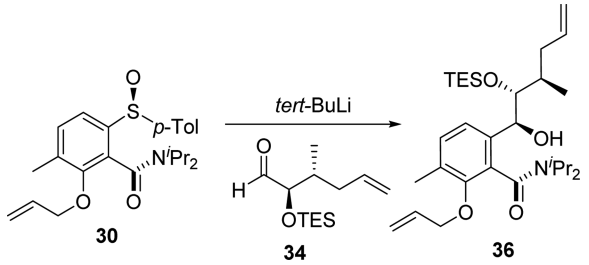
Table 1. Optimization of the Asymmetric Ortholithiation Protocol for Complex Aldehyde Electrophiles



entry	30 (equiv)	34 (equiv)	*t*-BuLi (equiv)	*T* (°C)	time	yield (%)
1	1.0	1.0	3.0	−90	1 h	25
2	1.5	1.0	3.0	−90	1 h	41
3	2.5	1.0	3.0	−90	1 h	51
4	1.0	2.0	3.0	−90	1 h	39
5	1.0	2.0	2.0	−90	1 h	57
6	1.0	2.0	1.5	−90	0.5 h	67
7	1.0	1.5	1.3	−90	10 min	71
				−78	30 min	
8	1.0	1.35	1.2	−90	10 min	69
				−78	30 min	
9	1.0	1.35[a]	1.2	−90	10 min	80
				−78	20 min	

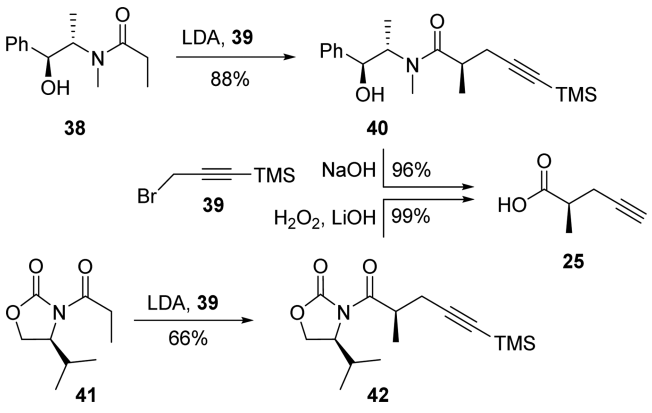
[a]Traces of water were removed by evaporation of anhydrous toluene.

the equivalents of aldehyde **34** could be further reduced along with the amount of *t*-BuLi and the reaction time (entries 7 and 8, Table 1).

Because of the isolation of significant amounts of *ortho*-unsubstituted aromatic compound, we speculated that this may be caused by residual water in the aldehyde starting material. Therefore, we reduced the water content by multiple coevaporation with anhydrous toluene. Under these conditions, the yield of the asymmetric ortholithiation was optimized to 80% (entry 9, Table 1).

Synthesis of the Oxazole Motive by a Cyclodehydration Approach. For synthesis of the required methyl-bearing pentynoic acid **25** an asymmetric α-alkylation was used. As shown in Scheme 4, two main strategies were evaluated that coupled TMS-protected propargyl bromide **39** with either the Myers auxiliary **38**[60] or the Evans auxiliary **41**.[61] Both couplings gave the required alkylated products (**40** and **42**) with excellent

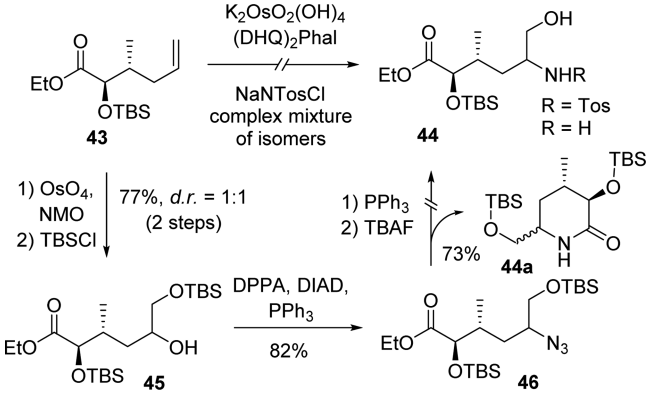
Scheme 4. Synthesis of Middle Fragment 25 by an Asymmetric α-Alkylation



stereoselectivities (dr > 20:1). However, on a large scale the reaction with Myers auxiliary **38** was more robust and reliable in our hands. With 2.0 equiv of the bromide, the desired methyl-substituted amide **40** could be obtained from **38** and **39** in 88% yield. For cleavage of the pseudoephedrine-derived auxiliary, a basic procedure with NaOH proved optimal among those evaluated (H_2SO_4, $MeSO_3H/LiBH_4$), giving the desired *R*-configured alkynecarboxylic acid **25** in 81% yield in three steps in a readily scalable and robust fashion.

Subsequently, we tried to provide the required Western fragment **24** with a terminal amino alcohol functionality. As a first approach, an aminohydroxylation of a terminal alkene following the protocol developed by Sharpless et al.[62] was evaluated (Scheme 5). This would present the simplest and

Scheme 5. Evaluation of a Regioselective Aminohydroxylation of Alkene 43



most direct method for construction of the required functionality. To allow a high degree of overall convergence in the route, this would ideally be installed early on in the sequence, i.e., by derivatization of TBS-protected ester **43**. However, in all attempts only low regioselectivities were obtained leading to a complex product mixture which is in agreement with related observations.[63] This outcome could not be altered by varying the protocol, omission of the $(DHQ)_2$Phal-Ligands, or addition of various additives like chlorohydantoin[64] or replacement of chloraminT (NaTosNCl) with *N*-bromoacetamide[65] as nitrogen source. Therefore, a more conventional stepwise sequence was used, which first involves introduction of the amine via azide **46**. Accordingly, TBS-ester **43** was first dihydroxylated, and the resulting diol was obtained in 77% yield with a diastereomeric ratio of 1:1. After selective protection of the primary hydroxyl group as a TBS-ether, the resulting secondary alcohol **45** was converted to azide **46** by treatment with diphenylphosphorylazid (DPPA) under Mitsunobu conditions,[66] which proved to result in higher yields as compared to a likewise tested alternative by using DBU.[67] However, subsequent Staudinger reduction of the azide **46** did not give rise to the required amine **44**. In contrast, a direct formation of lactam **44a** was observed by cleavage of the ethyl ester, which could not be suppressed by modification of the reaction conditions. Therefore, an early introduction of the oxazole was no longer studied, but rather a formation of the heterocycle after isochromanone synthesis was pursued.

Accordingly, the same sequence as before was applied to isochromanone **37**, i.e., dihydroxylation, to give **47** again in a diastereomeric ratio of 1:1, selective TBS-protection of the terminal hydroxyl, introduction of the azide, and reduction of

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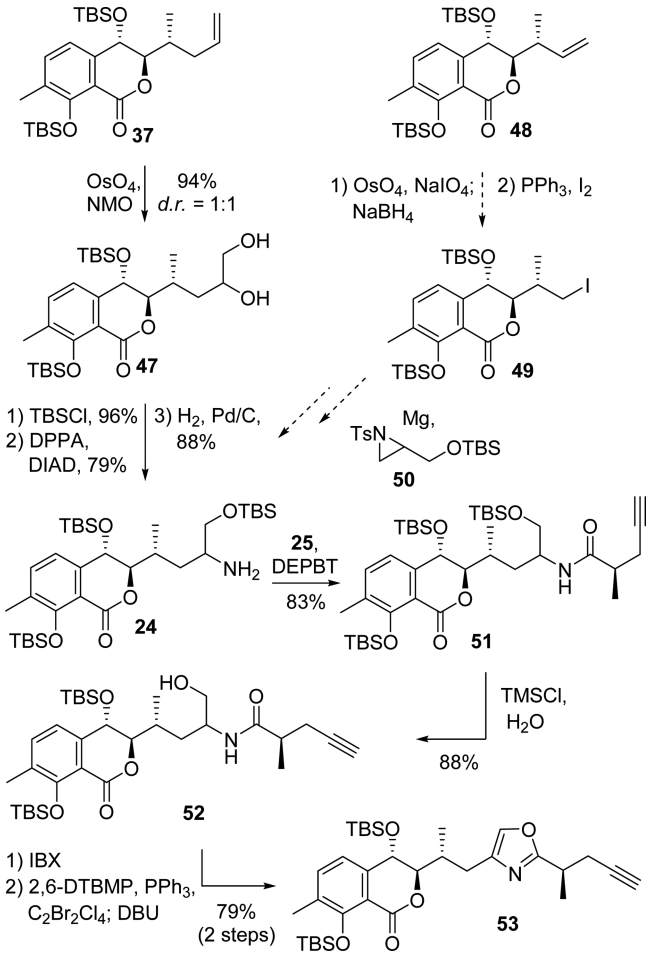
the amine (Scheme 6). In contrast to the protocols used above, here a hydrogenation with Pd on charcoal was used for

Scheme 6. Synthesis of Oxazole 53 by Cyclodehydration



reduction in order to facilitate isolation of the polar amine **24**. On occasion, partial deprotection of the phenolic TBS group during azide substitution was observed, requiring a reprotection in these cases. While this may be avoided by using $Zn(N_3)_2 \cdot Pyr_2$,[68] the yields (74%) that were obtained reliably after optimization (4.0 equiv of $Zn(N_3)_2 \cdot Pyr_2$ and 6.0−10.0 equiv of PPh_3 and DIAD) were not as high compared to the ones obtained with DPPA (79%, 89% brsm).[69] Additionally, we also tested a mono-TES protection strategy for the primary hydroxyl. This would have reduced the overall sequence by one step, since a TES groups may be directly removed under the chosen slightly acidic hydrogenation conditions with Pd/C.[70] However, a selective introduction of a TES ether without affecting the secondary hydroxyl could not be achieved.

As an alternative to this route, also a more elegant and more convergent route was evaluated that relied on a regioselective opening of aziridines. While such aziridine cleavages have been described,[71] the utilization of this sequence for oxazole synthesis has to our best knowledge not been reported and would therefore present a novel access to this heterocycle. The drafted approach starts from isochromanone **48**, which is more readily available as compared to homologated derivative **37**.[22] The terminal alkene of **48** would first be converted to iodide **49** by periodate cleavage, reduction to the resulting alcohol using $NaBH_4$, and an Appel reaction involving iodide and PPh_3.[72]

Introduction of the amino alcohol motive **24** should then proceed after transformation of the iodide into the corresponding Grignard reagent by regioselective opening of a suitable aziridine like **50**.[73] For this opening, a tosyl-protected aziridine was first selected, as this would enable a deprotection under radical conditions (SmI_2).[74] While aziridine **50** could be readily opened with commercial EtMgB solution (not shown), the implementation of this sequence for authentic **49** as well as related model substrates could not be realized, presumably by apparent difficulties of accessing the required Grignard reagent, using diverse activating agents like dibromoethane and iodine, and by the use of ^{i}PrMgBr or turbogrignard.[75] Therefore, we finally decided to use the more conventional four -step route starting from **37** and prepared sufficient quantities of Western fragment **24** following this sequence.

For coupling with the central methyl-bearing subunit by a Robinson−Gabriel cyclodehydration,[76] we first evaluated an *O*,*N*-shift strategy which was reported several times[77] and also used by Rizzacasa for the synthesis of 8-deshydroxy-9,10-*ent*-ajudazole A and B.[20c] This involves selective esterification of the primary hydroxyl of diol **47** with **25** and substitution of the secondary hydroxyl with an azide, followed by reduction to the amine with concomitant to give **52**. While selective esterification and azide formation worked reliably after optimization of the equivalents, the final reduction/rearrangement sequence did not proceed smoothly in our hands, giving a range of products that could not be efficiently separated. We therefore turned our attention to a more conventional cyclodehydration sequence.[78]

Accordingly, amine **24** was first coupled with acid **25**, which was readily effected with either IBCF (80%) or DEPBT (83%).[79] Subsequent selective deprotection of primary TBS ether of amide **51** was a considerable challenge. Application of standard reagents like CSA, HF·Pyr, or TBAF also resulted in partial removal of the aromatic TBS group or no conversion. While selective TBS deprotections have been well documented,[80] we could not find a literature precedent of a selective deprotection of a primary TBS group in the presence of a secondary aliphatic and an aromatic TBS group. It has been described that ZnBr in water,[81] $CeCl_3 \cdot (H_2O)_7$,[82] I_2 in MeOH,[83] or catalytic amounts of TMSCl in water[84] may selectively cleave primary TBS ethers in the presence of aromatic TBS ethers. However, no details on a potential deprotection of secondary aliphatic TBS ethers under the reported conditions have been reported. Since the secondary TBS group of the isochromanone system has proven to be stable against acetic acid, we evaluated various acidic deprotection protocols. Using 0.2 equiv of TMSCl and 1.0 equiv of water in acetonitrile according to a method of Grieco[84] gave the best results, yielding the corresponding free alcohol **52** in 88% yield together with small amounts of reisolated starting material (96% brsm).

For completion of the synthesis of oxazole **53**, mild two-step protocols were applied based on the oxidation of the terminal alcohol, cyclization and formal elimination of water (cyclodehydration). These were originally developed by Wipf in the 1990s[78] and have since then been further improved and expanded by using alternative oxidizing agents like DMP or IBX as an alternative to Burgess reagent or by using other reagents to effect the cyclization step like DAST or Deoxo-Fluor[85] in addition to iodine, $BrCCl_3$, $C_2Br_2Cl_4$, and C_2Cl_6.[86] This wide range of mild reagents contributed to numerous applications of this sequence in complex natural product syntheses.

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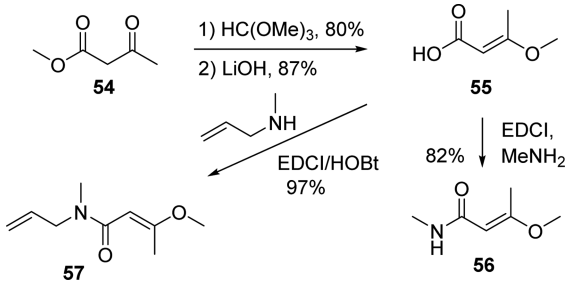
For the initial oxidation of the terminal alcohol **52**, a procedure using IBX in refluxing ethyl acetate was applied, allowing for a facile isolation of the resulting aldehyde by simple filtration. The subsequent cyclodehydration was best performed using $C_2Br_2Cl_4$, 2,6-DTBMP, PPh_3, and DBU, while protocols with I_2, PPh_3, and NEt_3 or C_2Cl_6, NEt_3, and PPh_3 led to significant amounts of halogenated intermediates.[87] It should be noted that reproducibly high yields could only be obtained on scales larger than 15 mg.[88]

Synthesis of the Eastern Fragment I: Evaluation of a Cross-Metathesis Approach for Allyl Amide Synthesis. With oxazole **53** in hand, we next performed the synthesis of Eastern fragments **26** and **27** (Scheme 3). As shown in Scheme 7, the terminal 3-methoxybutenoic acid (**55**) of **26** and **27** was prepared from methyl acetoacetate **54** in 70% overall yield by treatment with trimethyl orthoformate followed by hydrolysis of the intermediate ester with lithium hydroxide according to a literature procedure.[89] Subsequent coupling with *N*-allylmethylamine and EDCI gave amide **57** in an excellent yield. Alternatively, amide **56** was obtained from acid **55** by EDCI-mediated coupling with methylamine (Scheme 7).

Scheme 7. Synthesis of Terminal 3-Methoxybutenoic Acid Fragments 56 and 57 for Subsequent Cross-Metathesis Strategies

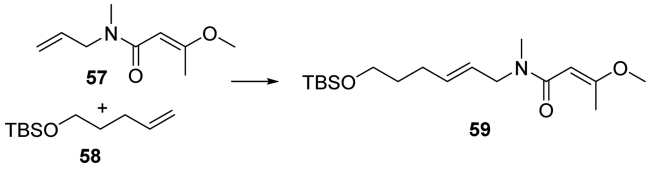


Two main strategies were then evaluated for construction of the central C_{22}–C_{23} alkene of the required side-chain coupling partners **26** and **27**. The first one involved cross-metathesis approaches (Tables 2 and 3) and would also enable a modular and simple modification of the side chain. As an alternative also, a more conventional Wittig coupling was studied (see Scheme 8).

We first tested the cross-metathesis reaction of allylic amide **57** with TBS-protected alkene **58** (Table 2). As shown in Table 2, initial studies indicated that Grubbs catalyst of the first generation had the highest activity of the tested catalysts for this kind of metathesis (entries 1–3). The low yields were thought to arise from a chelation of the amide functionality with the metal carbene intermediate.[90] Therefore, different Lewis acids were added in order to inhibit such a coordination of the oxygen atom to the ruthenium.[91] However, all Lewis acids that were evaluated led to a decline of yields or gave no product at all (entries 4–8, Table 2). Next, we tried an increase of the catalytic loading (entries 9–11, Table 2). The required yields are nearly stoichiometric in relation to the used ruthenium catalyst, which indicates an inhibition of the catalytic center after each catalytic cycle. This result could not be improved by a stepwise addition of the catalyst, but notably both **57** and **58** could be reisolated in considerable amounts.

Therefore, in total, a yield of 71% based on recovered starting material could be achieved (entry 12, Table 2). For small-scale reactions, nearly exclusively the desired *E*-product was formed, but on a larger scale (1.5 g of **57**) variable amounts (2–16%) of the *Z*-isomer of **59** were also detected.

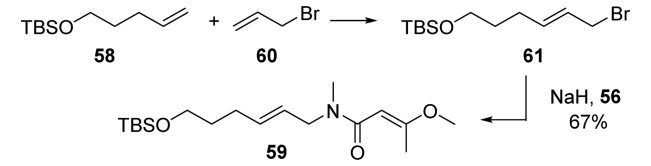
Table 2. Synthesis of the Eastern Fragment by Cross-Metathesis with Allylamide 57[a]



entry	catalyst	additive (mol %)	yield (%)
1	Grubbs I (5)	DDQ (10)	10
2	Grubbs II (5)	DDQ (10)	4
3	Hoveda–Grubbs II (5)	DDQ (10)	4
4	Grubbs I (5)	BCl (10)	6
5	Grubbs I (5)	Cy_2BCl (10)	8
6	Grubbs I (5)	$Ti(O\text{-}i\text{-}Pr)_4$ (10)	
7	Hoveda–Grubbs II (5)	ArO_2BCl (10)	
8	Hoveda–Grubbs II (5)	Cy_2BCl (10)	
9	Grubbs I (15)	DDQ (10)	14
10	Grubbs I (20)	DDQ (10)	21
11	Grubbs I (30)	DDQ (10)	34
12	Grubbs I[b] (5 × 5)	DDQ (10)	30(71 brsm)

[a]All reactions were carried out in refluxing DCM overnight. Equimolar amounts of both starting materials were used. [b]5 mol % of Grubbs I was added every hour. After the final addition, stirring was continued overnight.

Table 3. Synthesis of the Eastern Fragment by Cross-Metathesis with Allyl Bromide 60[a]



entry	olefin **58**	allyl bromide (equiv)	catalyst (mol %)	yield (%)
1	4 equiv (4.0 mmol)	1	Grubbs II (2)	96[b]
2	1 equiv (1.0 mmol)	1	Grubbs II (2)	50
3	1 equiv (2.5 mmol)	1	Grubbs I (2)	8
4	1 equiv (2.5 mmol)	1	Hoveda–Grubbs II (2)	20
5	1 equiv (2.5 mmol)	2	Hoveda–Grubbs II (2)	23
6	1 equiv (2.5 mmol)	2	Hoveda–Grubbs II (5)	10
7	1 equiv (5.0 mmol)	1.5	Hoveda–Grubbs II (2)	22

[a]All reactions were carried out in refluxing DCM overnight. [b]Yield calculated in reference to allyl bromide.

Because of the aforementioned difficulties to perform this metathesis in viable yields, we decided to evaluate the coupling of **58** with allyl bromide (**60**) instead of allylic amide **57** and introduction of the amide after this coupling.[92] As outlined in Table 3, the best results were achieved by using Grubbs II catalyst in small-scale reactions (entry 2, Table 3). The use of excess allyl bromide did not significantly improve the yields (compare entries 4/5, Table 3) and a higher catalyst loading

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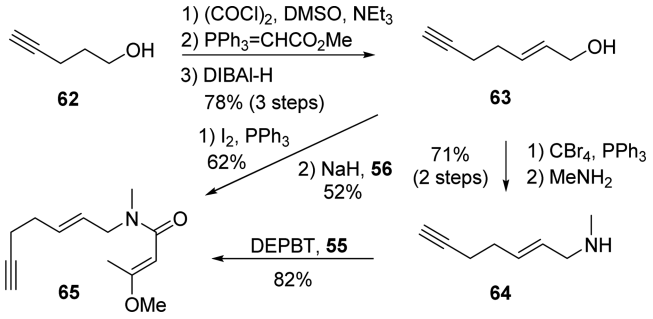
even lowered the yield (entry 6, Table 3). In all reactions, a 5:1 *E*/*Z* ratio for the new formed double bond was observed. Allylic bromide **61** was then coupled with amide **56** to give desired compound **59** in a straightforward sequence.

Synthesis of the Eastern Fragment II: A Convergent Combination of Conventional Wittig Reaction and Rhodium-Catalyzed *Z*-Selective Hydroboration. In parallel, also a more conventional approach to this building block was explored that relied on an olefination reaction. Following the studies of Krebs and Taylor,[20a] we planned to install the C_{23}/C_{24}-double bond by an *E*-selective Wittig reaction. As shown in Scheme 8, we aimed for an early introduction of a

Scheme 8. Synthesis of the Side-Chain Fragment 65 by Wittig Reaction

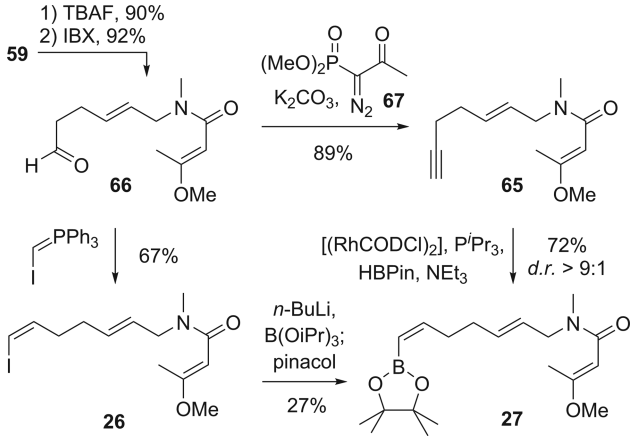


terminal alkyne, which may then serve as a suitable handle for hydroboration. Accordingly, pentynol **62** was oxidized using the Swern procedure[93] to the corresponding volatile aldehyde, which was immediately used in the Wittig reaction with ethyl(triphenylphosphoranylidene)acetate. The resulting (*E*)-configured unsaturated ester was directly reduced with DIBAl-H to give the desired *E*-configured allylic alcohol **63** in good yields. In accordance with our experiences above (see Table 3), the allylic alcohol was first transformed into the corresponding iodide and then coupled with deprotonated methoxybuteneamide **56**. While the convergence of this sequence was very high (5 steps), the yields of these two last steps were not satisfactory. Therefore, an alternative route was pursued. This involved an Appel reaction with CBr_4 and PPh_3 and treatment with methylamine to give the secondary amine **64** in 71% yield over two steps. Initial difficulties during the isolation of this polar amine could be resolved by using a chromatographic purification with basic aluminum oxide. Finally, amine **64** was coupled with 3-methoxybutenoic acid **55** by DEPBT[79] to give required amide **65** in a reliable manner. Notably, the overall yield of this sequence is almost twice as high as compared to the previous one (45% vs 25%) despite the additional step.

In agreement with our general retrosynthetic approach, two side-chain fragments were targeted. These contained either a terminal vinyl iodide or a boronate to enable a certain degree of flexibility in the final fragment connection. Therefore, side chains that are terminating with a vinyl iodide (i.e., **26**) and a boronate (i.e., **27**) were targeted.

As shown in Scheme 9, both the terminal TBS ether **59** and the terminal alkyne **65** could be converted to the desired Eastern fragments **26** and **27**. For conversion of **59**, the TBS ether was first deprotected with TBAF and the resulting alcohol was oxidized to **66** by IBX in DMSO. The obtained aldehyde could then be transformed to vinyl iodide **26**[94] by performing a Stork–Zhao–Wittig reaction[95] or homologated to **66** by using the Ohira–Bestmann reagent **67**.[96] This reagent was best

Scheme 9. Completion of the Synthesis of the Eastern Fragments 26 and 27



prepared by diazo-transfer to dimethyl 2-oxo-propyl-phosphonate, while an alternative more recently described one-pot procedure[97] did not prove to be reliable in our hands. Two different strategies for introduction of the (*Z*)-configured boronate were then pursued. The first involved conversion of iodide **26**. However, despite considerable efforts the desired boronate **27**, could only be obtained in 27% yield by treatment of **26** with *n*-BuLi/B(O-*i*-Pr)$_3$ and pinacol.[98] Alternative bases (*t*-BuLi) or boron sources (^{i}PrOBPin, B(OMe)$_3$) resulted in even lower yields. In addition, isolation of **27** appeared to be difficult, since various unidentified side products could not be removed by conventional chromatographic techniques. Application of a Miyaura borylation strategy failed completely.[99] Despite variations of the base, solvent, or boron equivalents, no conversion to the desired product could be observed. Therefore, we turned our attention to a transformation of the terminal alkyne to the desired *Z*-boronate **27**. A very elegant way for such a transformation was reported by Miyaura in 2000 by application of a rhodium catalyst[100] leading to *E*-configured compounds.[101]

Following this approach, the desired Eastern fragment **27** could indeed be obtained in 72% yield, and a diastereoselectivity (*Z*/*E*) > 9:1 after few optimizations of the originally reported protocol regarding the equivalents of the catalyst, the type of ligand, and final purification. The required configuration was clearly assigned by the vicinal coupling constants (J = 13.5 and 19.3 Hz). Minor amounts of the undesired *E*-isomer could be removed by careful chromatography on silica gel. Overall, the boronated Eastern fragment **27** was obtained in 32% yield following the sequences described in Schemes 8 and 9. This presents the shortest synthesis of this fragment reported so far. The central rhodium catalyzed *Z*-selective hydroboration enables a short and elegant access to the desired *Z*-configuration.

Completion of the Total Synthesis of Ajudazol B and Analytical Comparison to Natural Ajudazol B. With all main fragments of the ajudazol skeleton in hand, we focused on a completion of the total synthesis. According to our main strategy involving a *Z*-selective sp^2–sp^2 cross coupling, also the terminal alkyne of isochromanone building block **53** had to be transformed either into boronate **68** or the terminal vinyl iodide **70** (Scheme 10). In contrast to studies with the Eastern fragment **27**, however, introduction of the boronate could not be effected for **53** by Miyaura's rhodium-catalyzed hydro-

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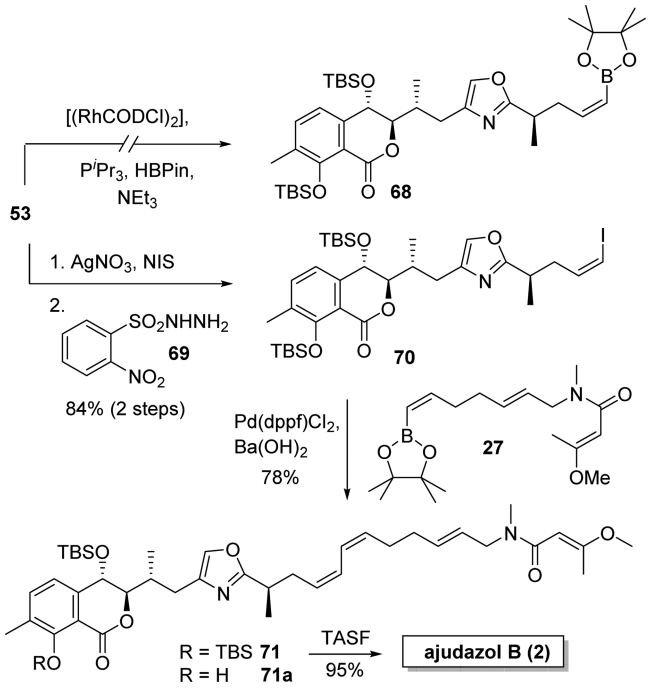
Scheme 10. Completion of the Total Synthesis of Ajudazol B (2)



boration protocol.[100] Despite variations of the ligand system ($PiPr_3$, PCy_3), the reaction time, or replacement of pinacolborane with catecholborane, no conversion was observed and the starting material could be reisolated. In addition, a stepwise procedure failed, as the starting material proved to be labile under the conditions required for terminal borylation of the alkyne and additional procedures appeared to be not applicable.[98b,102] Therefore, we focused on the transformation of the oxazole fragment **53** to a vinyl iodide **70**. The desired conversion of the terminal alkyne could be effected in good yields (84%) by treatment with $AgNO_3$ and iodine and reduction of the resulting alkynylic iodide with *o*-nitrobenzylsulfonyl hydrazide (NBSH, **69**).[103]

After optimization on structurally simplified Western fragments, the fragment coupling of the resulting terminal vinyl iodide with the side chain boronate could be effected using $Pd(dppf)Cl_2$ as catalyst in combination with $Ba(OH)_2$ as base.[104] Alternative catalysts [$Pd(PPh_3)_4$], bases (Ag_2O, K_2CO_3, Cs_2CO_3), or ligands ($AsPh_3$) were also tested but resulted in lower yields. During this fusion, partial deprotection of the phenolic TBS group was observed, giving TBS-protected **71** together with the deprotected analogue **71a** in a combined yield of 78% with a ratio of **71** to the deprotected analog **71a** of 1:14. Finally, for complete deprotection, TASF[105] as a particularly mild desilylation reagent was used, giving ajudazol B (**2**) without traces of translactonizations or epimerization in 95% yield after purification by HPLC on reverse phase.[106]

Comparison of the MS data of the synthetic material with those reported for the natural product resulted in a perfect agreement. In addition, the NMR data were almost identical to the ones given for the natural product (see table in the SI). However, a chemical shift difference of 0.2 ppm for the methyl group at C_{10} was observed which could not be explained by a possible misassignment or a different calibration. Importantly, the absolute configuration at this center had been proposed on the basis of the enoylreductase alignment. Such discrepancy therefore questioned our stereochemical proposal and the reliability of the bioinformatics approach for stereochemical determination of isolated methyl groups in general. Gratifyingly, this deviation could finally be resolved by a type-setting error in the isolation manuscript. For comparison of the ^{13}C NMR spectroscopic data, a detailed analysis of the $^{1}H/^{13}C$-HMBC spectrum was applied, as only very weak ^{13}C signals were observed in the amide region of the side chain. The signals of C_{26} and C_{28} could not be observed at all without their $^{1}H/^{13}C$-HMBC correlations. This appeared to have not been realized during the structural determination of ajudazol B (**2**), as these two signals had been mistakenly suggested to be overlaid by the signal of C_1. Within this study these signals could be correctly assigned. In addition, the occurrence of an additional ^{13}C signal for the methyl group at the amide nitrogen was corrected in agreement with the two amide resonances possible. Finally, the full spectral identity between natural and synthetic ajudazol B was confirmed by an overlay of the NMR spectra of synthetic ajudazol B with an authentic spectrum (see the SI). Depending on the experimental conditions, the OH signals in the ^{1}H NMR spectra may be exchanged (also see the SI). For a first confirmation of the absolute configuration, the optical rotation of the synthetic material ($[\alpha]_D^{21}$ = +7.9 (*c* 0.9, MeOH)) was in agreement with the data reported for natural ajudazol B[5] ($[\alpha]_D^{21}$ = +6.1 (*c* 1.34, MeOH). For an unambiguous proof also of the remote stereocenter at C_{15} a CD spectrum was recorded, as comparison of optical rotations alone may be misleading.[28] This was compared to a CD spectrum of natural ajudazol B, which was reisolated. As shown in Figure 7, we obtained a perfect match of the overlaid curves

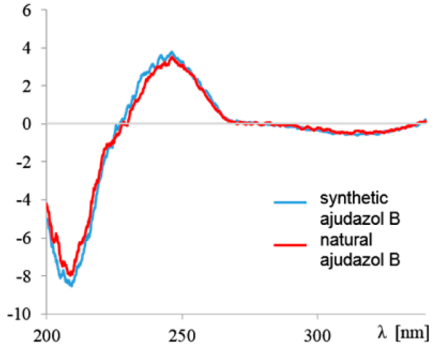


Figure 7. Overlay of the CD spectra of synthetic (blue) and natural (red) ajudazol B (**2**).

unambiguously confirming our assignment of the relative and absolute configuration of ajudazol B (**2**). In summary, this validates the reliability of our bioinformatics approach for stereochemical determination of ketoreductase derived hydroxyl-bearing stereocenters and enoylreductase-derived configurations of methyl-bearing centers.

Ajudazol B Is a Potent and Direct Inhibitor of 5-Lipoxygenase. Isochromanones are key structural features in a variety of natural products and bioactive agents, and a wide variety of potent biological activities have been reported for these compounds. These include cytostatic potencies (mellein and hydroxymellein),[107] ACE inhibitory effects (7,8-dihydroxy-3-methylisochromanone),[108] plant growth regulation (sclerotinin C),[109] as well as antiallergic and antidiabetic properties (hydrangeol and phyllodulcin),[110] carcinogenic and nephrotoxic activities (ochratoxin),[111] mutagenic effects and inhibition

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of topoisomerase (both alternariol),[112] influence on the endothelin-converting-enzyme production (benaphthamycin **24**),[24] antiplasmodic activity (bacillosarcin B),[113] and effects on the central nervous system (AI-77-B).[114] Among all isochromanonic compounds, bergenin (**27**) has been analyzed in more detail and possesses hepatoprotective, immunomodulatory, antinoceptive, and antinarcotic properties,[115] shows regeneratory effects on β-cells, and has been considered to be valuable in ulcustherapy.[116,117] Finally, two isochromanone-based structures (AC-7954 and FL 68) are presently in clinical phase II studies for cardiovascular diseases and diabetes.[118]

Given this wide range of biological activities and with synthetic material in hand, we were interested in a further biological evaluation of ajudazol B (**2**). The groups of Höfle and Reichenbach had already evaluated the ajudazols in conventional antifungal, antibacterial, and antiproliferative test systems and had discovered complex I of NADH-dehydrogenase as a potent molecular target of the ajudazols.[6] Given the promiscuous biological potency that is frequently observed for natural products, we were searching for alternative targets. In particular, we were interested in whether ajudazol B may possess also anti-inflammatory potential. We therefore evaluated the effect of ajudazol B on pro-inflammatory cytokine release and eicosanoid biosynthesis. As shown in Table 4,

Table 4. Effect of Ajudazol B (2) on Cytokine Release and Eicosanoid Biosynthesis[a]

entry	biological target	conc of ajudazol B (**2**)
1	HL-60 viability (MTT)	67.2 ± 0.6
2	PBMC viability (MTT)	n.i.[b]
3	IL-1β release	145.9 ± 11.4*
4	TNF-α release	89.1 ± 7.6
5	IL-6 release	72.1 ± 6.1*
6	IL-8 release	74.5 ± 5.1*
7	COX-1 (enzyme)	n.i.[b]
8	COX-2 (enzyme)	n.i.[b]
9	COX-1 (platelets)	86.3 ± 5.8
10	mPGES-1	80.3 ± 3.6*
11	5-LO (enzyme)	***6.9 ± 1.5***[c]
12	5-LO (neutrophils)	***1.6 ± 0.2***[c]
13	12-LO (neutrophils)	123.1 ± 16.2
14	15-LO (neutrophils)	162.9 ± 12.3*

[a]Residual activity/release as percentage of control (at 10 μM ajudazol B) and IC_{50} values (μM) are given. [b]Not determined. [c]IC_{50} values; n = 3−4 except viability (triplicate), (*) $P < 0.05$, (**) $P < 0.01$; student t-test. HL-60 = human promyelocytic leukemia cells; IL = interleukin; TNF = tumor necrosis factor; COX = cyclooxygenase; LO = lipoxygenase.

ajudazol B inhibits 5-lipoxygenase (5-LO), the key enzyme in leukotriene biosynthesis, in a cell-free assay as well as in intact human neutrophils with IC_{50} = 6.9 and 1.6 μM, respectively. Of interest, the related 12- and 15-lipoxygenases were not inhibited by ajudazol B (in neutrophils). On the other hand, moderate effects on the viability of human promyelocytic leukemia HL-60 cells as well as repression of interleukin (IL)-6 and -8 release and microsomal prostaglandin E synthase (mPGES)-1 were observed at 10 μM ajudazol B, while no activity against peripheral blood mononuclear cells (PBMC) and cyclooxygenase enzymes was detected. 5-LO is a clinically validated molecular target for treatment of asthma and allergic rhinitis.[119] In addition, malfunctions of 5-LO or leukotrienes have been correlated with atherosclerosis and cancer, rendering the identification of novel inhibitors an important discovery. Note that ajudazol B blocked 5-LO in neutrophils in the low micromolar range with potency comparable to that of the drug zileuton (IC_{50} = 1.3 μM, data not shown) that reached the market for treatment of asthma.[120] This finding implies anti-inflammatory potential of ajudazol B and renders further evaluations of ajudazols and structural variants thereof as 5-LO inhibitors to a promising research area.

CONCLUSION

In summary, we have reported the evaluation and application of a generally useful method for stereochemical assignment of methyl-bearing stereogenic centers that relies on gene cluster alignment of enoylreductases. The procedure can be easily performed by using freely available genomic data and correlates the presence or absence of a single indicative amino acid to the configuration of the methyl bearing center. High degrees of fidelity of this method were shown by evaluation of a broad range of myxobacterial compounds. The procedure may also be used for isolated, labile, or flexible stereocenters, which are very difficult to assign by other means or may not be assigned at all. A double application of this method enabled a determination of one remote and of one conformationally flexible methyl group of the ajudazols, highly potent inhibitors of mitochondrial respiratory chain. The full stereochemistry of this unique class of myxobacterial polyketides was assigned by a bioinformatics approach that also included analysis of ketoreductases. Importantly, stereochemical determination was purely based on a bioinformatics analysis and did not require an access to the authentic natural products.

Furthermore, a convergent total synthesis of ajudazol B, the most potent and least abundant ajudazol, has been reported, which presents the only total synthesis of a member of this natural product family so far. The scalable route proceeds in an overall yield of 8.2% and 22 steps in the longest linear sequence and unequivocally confirms the full stereochemistry of this unique class of natural products validating our bioinformatics based proposal. The total synthesis of a natural product where the stereochemistry has been assigned by bioinformatics analysis only also underlines the high fidelity this novel bioinformatic approach. Key synthetic strategies of our efficient route include an flexible route to the stereochemical elaborate isochromanone core based on an asymmetric ortholithiation strategy, a highly effective cross coupling approach to *Z*,*Z*-dienes, a useful protocol for cross-metathesis of allyl bromides, and a versatile oxazole formation strategy for complex substrates. These tactics may be readily applied also to various other synthetic strategies.

Finally, the synthetic access to ajudazol B was used for a deeper biological characterization, and we identified 5-LO as additional biological target of this natural product class. 5-LO, a clinically validated molecular target for treatment of asthma and allergic rhinitis, was efficiently inhibited by ajudazol B with potencies in the range of the pharmaceutically used drug zileuton. This finding indicates that a more general evaluation of the ajudazols, structural analogues, as well as respiratory chain inhibitors within an immunological context may be rewarding.

EXPERIMENTAL SECTION

Materials and Methods. Starting materials and reagents were obtained from commercial sources and used as received unless

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otherwise specified. The following reagents and building blocks were prepared according to literature procedures: Andersen reagent **29**,[121] IBX,[122] Dess–Martin periodinane,[123] Myers' auxiliary **38**,[60b] Evans auxiliary **41**,[124] alkine **42**,[61,125] aziridine **50**,[126] Ohira–Bestmann reagent **67**,[127] and *o*-nitrobenzenesulfonyl hydrazide (NBSH, **69**).[103a] Unless stated otherwise, all nonaqueous reactions were performed in flame-dried glassware under an atmosphere of argon. Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Polygram Sil G/UV254 on plastic). Flash column chromatography was performed by using silica gel S (pore size 60 Å, 0.040–0.063 mm, Sigma-Aldrich). Preparative high performance liquid chromatography (PHPLC) was carried out on a Knauer Eurospher II 100 RP C-18, 5 μm, 250 × 16.0 mm column with precolumn (30 × 16.0 mmg). Optical rotations were measured in a 1 dm cuvette using a sodium lamp. ^{1}H and ^{13}C NMR spectra were recorded at room temperature with ^{1}H operating frequencies of 300, 400, 500, and 600 MHz or with ^{13}C operating frequencies of 75, 100, 125, and 150 MHz, respectively. The chemical shifts are reported in parts per million (ppm) and are given in δ units relative to deuterated solvents as internal standard ($CDCl_3$ 7.27 ppm, 77.0 ppm). Coupling constants are given in hertz (Hz). Chemical shifts associated with the major rotamer are marked with an asterisk (*); the minor rotamer are marked with a hash (#); the major diastereomer are marked with an a ([a]); the minor diastereomer are marked with a b ([b]); both diastereomers are marked with an c ([c]). IUPAC names and atom numbering were generated using the program ChemBioDraw Ultra 13.0.

Allyl 2-(Allyloxy)-3-methylbenzoate (28a). 3-Methylsalicylic acid **28** (12.0 g, 78.8 mmol) was dissolved in DMF (160 mL) and cooled to 0 °C, and NaH (60% in mineral oil, 7.57 g, 189 mmol, 2.4 equiv) was added in three portions over a period of 20 min. The reaction mixture was stirred at this temperature for 1.5 h before allyl bromide (18.8 mL, 236 mmol, 3.0 equiv) was added dropwise. After complete conversion of the starting material (1.5 h), water (200 mL) and Et_2O (100 mL) were added. The organic layer was separated, and the aqueous layer was extracted with 3 × 150 mL of Et_2O. The combined organic phases were washed with brine (2 × 100 mL), dried over $MgSO_4$, filtered, and evaporated to give a yellow liquid of crude **155** in quantitative yield (18.3 g, 78.9 mmol). TLC: R_f = 0.62 (petroleum ether/ethyl acetate = 15:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.32 (3 H, s), 4.45 (2 H, dt, J = 5.6 Hz, 1.4 Hz), 4.81 (2 H, dt, J = 5.8 Hz, 1.4 Hz), 5.22–5.32 (2 H, m), 5.35–5.46 (2 H, m), 5.91–6.24 (2 H, m), 7.06 (1 H, dd, J = 7.6 Hz), 7.35 (1 H, d, J = 7.0 Hz), 7.67 (1 H, d, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 16.3, 65.7, 75.0, 117.5, 118.5, 123.6, 125.0, 129.1, 132.2, 133.0, 133.9, 135.1, 157.1, 166.1.HR-MS (EI-TOF): calcd for $[M]^+$ = $C_{14}H_{16}O_3$ 232.1099, found 232.1122 (Δ = +2.3 mmu). The data are in accordance with the literature.[128]

2-(Allyloxy)-3-methylbenzoic Acid (28b). To ester **28a** (18.0 g, 77.5 mmol, 1.0 equiv) was added to methanol (300 mL) followed by aqueous sodium hydroxide (6 M, 78 mL, 465 mmol, 6.0 equiv), and the mixture was heated under reflux for 4 h. The solvent was then removed under reduced pressure to leave a dense white residue. This was dissolved in water (150 mL), and the solution was acidified to pH = 3 with aqueous sulfuric acid (2 N) and extracted with 3 × 100 mL Et_2O. The combined organic extracts were washed with water (100 mL), dried with $MgSO_4$, and concentrated under reduced pressure to provide the product **28b** as a white solid in quantitative yield (14.9 g, 77.5 mmol). TLC: R_f = 0.42 (petroleum ether/ethyl acetate = 9:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.39 (3 H, s), 4.53 (2 H, dt, J = 5.9 Hz, 1.2 Hz), 5.39 (1 H, dd, J = 10.6 Hz, 1.1 Hz), 5.48 (1 H, dq, J = 17.2, 1.3 Hz), 6.15 (1 H, ddt, J = 16.9 Hz, 10.6 Hz, 6.0 Hz), 7.19 (1 H, dd, J = 7.7 Hz), 7.46 (1 H, m), 7.96 (1 H, d, J = 7.8 Hz), 10.71 (1 H, br s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.1, 75.8, 120.0, 122.7, 124.8, 130.5, 131.9, 131.9, 136.8, 156.4, 167.1. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_{11}H_{12}O_3$ 192.0786, found 192.0795 (Δ = +0.8 mmu). Mp: 52–54 °C. The data are in accordance with the literature.[129]

2-(Allyloxy)-*N*,*N*-diisopropyl-3-methylbenzamide (28c). A solution of acid **28b** (14.5 g, 75.5 mmol, 1.0 equiv) in dry CH_2Cl_2 (150 mL) was treated with freshly distilled $SOCl_2$ (16.4 mL, 226 mmol, 3.0 equiv), and the mixture was refluxed for 6 h. After evaporation of unreacted $SOCl_2$, the residual solution was resolved in dry CH_2Cl_2 (150 mL) and cooled to 0 °C. A solution of diisopropylamine (31.8 mL, 226 mmol, 3.0 equiv) in dry CH_2Cl_2 (100 mL) was added dropwise, and the mixture was stirred for 12 h at rt overnight. Then water (100 mL) was added, the organic layer was separated, and the aqueous layer was extracted with 3 × 100 mL of CH_2Cl_2. The combined organic layers were washed with brine (100 mL) and water (100 mL) and were dried over $MgSO_4$. After removal of the solvent, the resultant yellow oil was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate 9:1 → 5:1) to give the title compound **28c** (18.0 g, 65.4 mmol) in 87% yield as a white solid. TLC: R_f = 0.30 (petroleum ether/ethyl acetate =9:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.03 (3 H, d, J = 6.7 Hz), 1.18 (3 H, d, J = 6.6 Hz), 1.55 (3 H, d, J = 6.7 Hz), 1.56 (3 H, d, J = 6.6 Hz), 2.29 (3 H, s), 3.49 (1 H, spt, J = 6.8 Hz), 3.68 (1 H, spt, J = 6.7 Hz), 4.34 (1 H, ddt, J = 12.2 Hz, 5.5 Hz, 1.4 Hz), 4.57 (1 H, ddt, J = 12.2 Hz, 5.4 Hz, 1.4 Hz), 5.20 (1 H, dq, J = 10.5 Hz, 1.4 Hz), 5.38 (1 H, dq, J = 17.2 Hz, 1.7 Hz), 6.05 (1 H, ddt, J = 17.2 Hz, 10.6 Hz, 5.4 Hz), 6.91–7.08 (2 H, m), 7.10–7.22 (1 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.2, 20.2, 20.5, 20.7, 20.8, 45.6, 51.0, 74.6, 116.9, 124.2, 124.6, 131.0, 131.7, 133.4, 134.1, 152.9, 168.9. HR-MS (ESI-TOF): calcd for $[M + H]^+$ = $C_{17}H_{26}O_2N$ 276.1958, found 276.1957 (Δ = −0.1 mmu). Mp: 95–96 °C.

(*S*)-2-(Allyloxy)-*N*,*N*-diisopropyl-3-methyl-6-(*p*-tolylsulfinyl)-benzamide (30). To a stirred solution of amide **28c** (7.00 g, 21.8 mmol, 1.0 equiv) and TMEDA (3.65 mL, 24.0 mmol, 1.1 equiv) in dry THF (110 mL) at −78 °C (acetone/dry ice) was injected dropwise *s*-BuLi (1.4 M in hexane, 17.1 mL, 24.0 mmol, 1.1 equiv) within 15 min. The lithiated solution was stirred at −78 °C for 20 min, and then it was cannulated to a solution of (1*R*,2*S*,5*R*,*SS*)-(−)-menthyl-*p*-toluenesulfinate **29** (12.8 g, 43.6 mmol, 2.0 equiv) in dry THF (110 mL). After 1.5 h, the mixture was quenched with saturated aqueous NH_4Cl solution (200 mL) at −78 °C, brought up to rt, and extracted with 3 × 200 mL of Et_2O, and the combined organic layers were dried over $MgSO_4$, filtrated, and evaporated under reduced pressure to give a residue which was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate 5:1–1:1) to afford the white crystalline sulfoxide **30** (7.90 g, 19.1 mmol) in 88% yield. TLC: R_f = 0.15 (petroleum ether/ethyl acetate = 3:1). $[\alpha]_D^{23}$ = −94.6 (c 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.23 (3 H, d, J = 7.3 Hz), 1.25 (3 H, d, J = 7.4 Hz), 1.61 (3 H, d, J = 6.9 Hz), 1.64 (3 H, d, J = 6.9 Hz), 2.29 (3 H, s), 2.35 (3 H, s), 3.60 (1 H, spt, J = 6.9 Hz), 3.76 (1 H, spt, J = 6.7 Hz), 4.32 (1 H, ddt, J = 12.1 Hz, 5.5 Hz, 1.4 Hz), 4.57 (1 H, ddt, J = 12.1 Hz, 5.5 Hz, 1.4 Hz), 5.22 (1 H, dq, J = 10.4 Hz, 1.4 Hz), 5.38 (1 H, dq, J = 17.3 Hz, 1.6 Hz), 6.03 (1 H, ddt, J = 17.1 Hz, 10.6 Hz, 5.5 Hz), 7.16–7.29 (3 H, m), 7.45 (1 H, d, J = 8.0 Hz), 7.73 (2 H, d, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 76 MHz): δ = 16.4, 20.2, 20.4, 20.6, 21.0, 21.3, 46.2, 51.7, 74.9, 117.3, 120.7, 124.5 (2 C), 129.7 (2 C), 132.3, 132.4, 133.5, 135.4, 140.7, 142.1, 142.2, 152.2, 165.3. HR-MS (ESI-TOF): calcd for $[M + H]^+$ = $C_{24}H_{32}O_3NS$ 414.2097, found 414.2099 (Δ = +0.2 mmu). Mp: 99–102 °C.

Ethyl (2*R*,3*R*)-2-Hydroxy-3-methylpent-4-enoate (31a). To a stirred mixture of KO-*t*-Bu (7.76 g, 16.0 mmol, 1.03 equiv, dried at 1.0 mbar/80 °C/12 h) in dry THF (35 mL) was added liquid *trans*-2-butene (10.5 g, 188 mmol, 2.8 equiv) via transfer cannula at −78 °C (acetone/dry ice). Then a solution of *n*-BuLi (2.5 M in hexane, 26.8 mL, 67.0 mmol, 1.0 equiv) was added dropwise within 30 min via syringe driver. Thirty minutes after complete addition of *n*-BuLi, the mixture was stirred at −45 °C (acetone/dry ice) for 10 min. The resulting orange solution was recooled to −78 °C, and it was added dropwise a solution of (+)-$(Ipc)_2$BOMe (25.1 g, 79.2 mmol, 1.18 equiv) in dry Et_2O (80 mL). After the reaction mixture was stirred at −78 °C for 30 min, $BF_3{\cdot}OEt_2$ (12.1 mL, 96.0 mmol, 1.4 equiv) was added dropwise within 20 min via syringe driver followed by a technical solution of ethyl glyoxalate in toluene (ca. 4.9 M, 34.2 mL, 168 mmol, 2.5 equiv) within 30 min. The mixture was then stirred at −78 °C for 4 h and after the removal of the cooling bath treated with an aqueous NaOH solution (1 N, 150 mL, 2.25 equiv) and carefully with H_2O_2 (30%, 21.0 mL). The contents were stirred for 2 h at rt. The organic layer was separated, the aqueous layer was extracted with

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3 × 100 mL Et_2O, and the combined organic layers were washed with water (30 mL) and brine (30 mL) and dried over $MgSO_4$. After removal of the solvents, the residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to yield 70% of **31a** (7.43 g, 47.0 mmol, dr = 98:2, ee = 90% determined by Mosher ester analysis) as a colorless liquid with a fruity odor. TLC: R_f = 0.33 (petroleum ether/ethyl acetate = 10:1). $[\alpha]_D^{23}$ = −4.9 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.16 (3 H, d, *J* = 7.0 Hz)[a], 1.30 (3 H, t, *J* = 7.1 Hz)[a], 1.37 (1 H, d, *J* = 7.0 Hz)[b], 1.51 (1 H, t, *J* = 7.1 Hz)[b], 2.17−2.28 (1 H, m)[b], 2.49 (1 H, br s)[b], 2.59−2.72 (1 H, m)[a], 2.74 (1 H, br. s)[a], 4.11 (1 H, d, *J* = 3.3 Hz)[a], 4.16 (1 H, d, *J* = 2.6 Hz)[b], 4.14−4.33 (2 H, m)[a], 4.34−4.50 (1 H, m)[b], 5.01−5.07 (1 H, m)[a], 5.07−5.11 (1 H, m)[a], 5.11−5.13 (1 H, m)[b], 5.14−5.18 (1 H, m)[b], 5.68−5.82 (1 H, m)[a], 5.79−5.92 (1 H, m)[b]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 13.5[b], 14.2[a], 15.1[b], 16.3[a], 41.6[b], 41.9[a], 61.6[a], 64.1[b], 73.8[b], 74.3[a], 115.5[b], 116.4[a], 137.6[a], 139.4[b], 174.2[a]. HR-MS (EI-TOF) calculated for $[M]^+$ = $C_8H_{14}O_3$ 158.0943, found 158.0951 (Δ = +0.8 mmu). The data are in accordance with the literature.[130]

(2*R*,3*R*)-Ethyl 3-Methyl-2-((triethylsilyl)oxy)pent-4-enoate (32). To an ice-cooled solution of the ester **31a** (4.6 g, 29.1 mmol, 1.0 equiv) in dry CH_2Cl_2 (100 mL) were added 2,6-lutidine (8.45 mL, 72.9 mmol, 2.5 equiv) and TESOTf (8.22 mL, 36.4 mmol, 1.25 equiv). The resulting mixture was stirred at rt for 3 h until TLC control indicated complete consumption of the starting material. The reaction was quenched with water (80 mL) and extracted with 3 × 50 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 50:1−30:1) afforded 99% of silyl ether **32** (7.85 g, 28.8 mmol) as a colorless liquid. TLC: R_f = 0.60 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +10.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.63 (6 H, m), 0.96 (9 H, t, *J* = 7.8 Hz), 1.06 (3 H, d, *J* = 7.0 Hz), 1.27 (3 H, t, *J* = 7.2 Hz), 2.52−2.68 (1 H, m), 4.08 (1 H, d, *J* = 4.9 Hz), 4.17 (2 H, qd, *J* = 7.0 Hz, 2.4 Hz), 4.95−5.08 (2 H, m), 5.73−5.93 (1 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 4.6 (3 C), 6.7 (3 C), 14.3, 16.4, 42.7, 60.5, 76.1, 115.3, 139.0, 172.8. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{14}H_{29}O_3Si$ 273.1881, found 273.1880 (Δ = −0.1 mmu).

(2*R*,3*R*)-Ethyl 3-Methyl-5-oxo-2-((triethylsilyl)oxy)-pentanoate (32a). To a solution of cyclohexene (4.30 mL, 42.4 mmol, 2.1 equiv) in dry Et_2O (200 mL) at 0 °C was added dropwise $BH_3{\cdot}SMe_2$ (2 M in THF, 10.6 mL, 21.2 mmol, 1.05 equiv). The solution was stirred at 0 °C for 1.5 h and at rt for another 1.5 h until a white precipitate occurred. Then the solution was recooled to 0 °C and cannulated to solution of **32** (5.50 g, 20.2 mmol, 1.0 equiv) in Et_2O (100 mL) at 0 °C. The reaction mixture was stirred for 2 h at this temperature before water (20 mL) was added, followed by the simultaneous addition of aqueous NaOH (1 M, 110 mL, 110 mmol, 5.0 equiv) and H_2O_2 (30%, 12.5 mL, 110 mmol, 5.0 equiv). After being stirred for 1 h at 0 °C and 1 h at rt, the reaction mixture was quenched with 50 mL of saturated aqueous $Na_2S_2O_3$ solution. The aqueous layer was separated and extracted with 3 × 100 mL Et_2O. The combined organic extracts were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure.

The obtained crude product was resolved in dry CH_2Cl_2 (100 mL) and cooled to 0 °C, and Dess−Martin periodinane (25.6 g, 60.5 mmol, 3.0 equiv) was added in three portions over a period of 10 min. The white slurry was stirred at this temperature for 1 h and warmed to rt, and stirring was continued for an additional 3 h. Silica gel (60 g) was added, and the solvent was evaporated. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 15:1−9:1) afforded 86% of aldehyde **32a** (5.03 g, 17.3 mmol) as a colorless liquid. TLC: R_f = 0.57 (petroleum ether/ethyl acetate = 9:1). $[\alpha]_D^{23}$ = +9.5 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): 0.51−0.73 (6 H, m), 0.96 (9 H, t, *J* = 7.9 Hz), 1.03 (3 H, d, *J* = 7.0 Hz), 1.30 (3 H, t, *J* = 7.2 Hz), 2.32 (1 H, m), 2.43−2.67 (2 H, m), 4.06 (1 H, d, *J* = 4.8 Hz), 4.19 (2 H, q, *J* = 7.2 Hz), 9.75 (1 H, t, *J* = 1.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.5 (3 C), 6.6 (3 C), 14.2, 16.9, 33.1, 46.1, 60.9, 75.9, 172.8, 201.8. HR-MS (ESI-TOF, arginine): calcd for $[M + Na]^+$ = $C_{14}H_{28}O_4SiNa$ 311.1649, found 311.1649 (Δ = 0 mmu).

(2*R*,3*R*)-Ethyl 3-Methyl-2-((triethylsilyl)oxy)hex-5-enoate (33). A suspension of methyltriphenylphosphonium iodide (4.71 g, 11.7 mmol, 1.35 equiv) in THF (60 mL) at 0 °C was treated with NaHMDS (1 M in THF, 10.4 mL, 10.4 mmol, 1.20 equiv) within 30 min and then cooled to −78 °C. To the yellow solution was added **32a** (2.49 g, 8.63 mmol, 1.0 equiv) in THF (12 mL). After being stirred for 3 h at −78 °C, the reaction mixture was allowed to warm to rt over 1 h, subsequently poured into brine (40 mL), and extracted with 3 × 40 mL of Et_2O. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) afforded 93% of olefin **33** (2.29 g, 7.99 mmol) as a pale yellow liquid. TLC: R_f = 0.42 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +6.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.58−0.68 (6 H, m), 0.91 (3 H, d, *J* = 6.6 Hz), 0.97 (9 H, t, *J* = 7.9 Hz), 1.29 (3 H, t, *J* = 7.1 Hz), 1.79−2.03 (2 H, m), 2.18−2.39 (1 H, m), 4.02 (1 H, d, *J* = 5.2 Hz), 4.19 (2 H, q, *J* = 7.1 Hz), 4.84−5.17 (2 H, m), 5.67−5.85 (1 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.6, 6.7, 14.2, 15.5, 36.0, 37.8, 60.5, 76.1, 116.2, 136.9, 173.3. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{15}H_{31}O_3Si$ 287.2037, found 287.2041 (Δ = +0.4 mmu).

(2*R*,3*R*)-3-Methyl-2-((triethylsilyl)oxy)hex-5-en-1-ol (33a). A stirred solution of the TES-protected ester **33** (1.95 g, 7.85 mmol, 1.0 equiv) in dry CH_2Cl_2 (60 mL) was cooled to −78 °C (acetone/dry ice). Then a solution of DIBAl-H (1.0 M in CH_2Cl_2, 14.3 mL, 14.3 mmol, 2.1 equiv) was injected via syringe driver over a period of 30 min. The reaction mixture was allowed to warm to rt overnight (12 h) and poured into a saturated solution of potassium sodium tartrate (150 mL). Et_2O (80 mL) was added, and the mixture was stirred vigorously until two phases appeared. Extraction with 3 × 50 mL CH_2Cl_2, drying over $MgSO_4$, and evaporation of the solvent yielded a nearly quantitative amount of the colorless TES-protected alcohol **33a**, which was used in the following reaction without further purification. TLC: R_f = 0.16 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = −6.7 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.62 (6 H, m), 0.90 (3 H, d, *J* = 6.8 Hz), 0.94−1.03 (9 H, m), 1.70 (1 H, m), 1.94−2.09 (1 H, m), 2.05 (1 H, br s.), 2.34 (1 H, dq, *J* = 6.8 Hz, 6.1 Hz), 3.40−3.61 (2 H, m), 3.73 (1 H, d, *J* = 8.6 Hz), 4.98−5.11 (2 H, m), 5.72−5.90 (1 H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): 4.3, 5.1, 5.8, 6.6, 6.7, 6.9, 15.3, 35.8, 37.3, 64.7, 75.8, 116.4, 136.9. HR-MS (ESI-TOF, arginine) calculated for $[M + H]^+$ = $C_{13}H_{29}O_2Si$ 245.1931, found 245.1929 (Δ = −0.2 mmu).

(2*R*,3*R*)-3-Methyl-2-((triethylsilyl)oxy)hex-5-enal (34). Oxalyl chloride (0.76 mL, 8.95 mmol, 1.35 equiv) was dissolved in dry CH_2Cl_2 (30 mL). The mixture was cooled to −78 °C, and a solution of DMSO (1.22 mL, 17.2 mmol, 2.6 equiv) in dry CH_2Cl_2 (10 mL) was added within 5 min. The mixture was stirred at this temperature for 25 min before a solution of TES-protected alcohol **33a** (1.62 g, 6.63 mmol, 1.0 equiv) in dry CH_2Cl_2 (10 mL) was added dropwise. After being stirred for 1 h, the mixture was treated with dry NEt_3 (3.67 mL, 26.5 mmol, 4.0 equiv), and stirring was continued at −78 °C for 30 min before the mixture was slowly warmed to rt over 1.5 h. Water was added, the phases were separated, and the aqueous phase was extracted with 3 × 30 mL of CH_2Cl_2. The combined organic layers were washed with brine (2 × 30 mL), and the solvent was evaporated under reduced pressure. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 70:1) yielded 86% of the TES-protected aldehyde **34** (1.39 g, 5.73 mmol) as a pale yellow liquid. TLC: R_f = 0.47 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +21.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.62 (6 H, q, *J* = 7.9 Hz), 0.97 (9 H, t, *J* = 7.9 Hz), 0.97 (3 H, d, *J* = 6.9 Hz), 1.89−2.08 (2 H, m), 2.21−2.32 (1 H, m), 3.80 (1 H, dd, *J* = 4.6 Hz, 2.1 Hz), 4.98−5.12 (2 H, m), 5.72 (1 H, ddt, *J* = 17.0 Hz, 10.1 Hz, 6.9 Hz), 9.62 (1 H, d, *J* = 2.1 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 4.8, 6.7, 15.7, 35.9, 37.3, 81.0, 116.8, 136.8, 205.0. HR-MS (ESI-TOF, HPmix) calcd for $[M + Na]^+$ = $C_{13}H_{26}O_2SiNa$ 265.1594, found 265.1596 (Δ = +0.2 mmu).

2-(Allyloxy)-6-((1*S*,2*R*,3*R*)-1-hydroxy-3-methyl-2-(triethylsilyloxy)hex-5-enyl)-*N*,*N*-diisopropyl-3-methylbenzamide (36). A solution of *t*-BuLi (1.7 M in pentane, 2.80 mL, 4.77 mmol, 1.2 equiv) was added dropwise to a stirred solution of sulfoxide

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30 (1.64 g, 3.97 mmol, 1.0 equiv) in dry THF (40 mL) at −90 °C (acetone/liquid nitrogen). After 5 min, the TES-protected aldehyde **34** (1.30 g, 5.36 mmol, 1.35 equiv, dried by evaporation of dry toluene) in THF (5 mL) was added dropwise within 4 min. The mixture was allowed to warm to −78 °C and stirred for 20 min at this temperature. Then an aqueous solution of NH_4Cl (40 mL) was added at −78 °C, and the mixture was allowed to warm to rt. Extraction with 3 × 50 mL of Et_2O, drying over $MgSO_4$, and evaporation of the solvent gave a residue which was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate 12:1−5:1) to afford the ortholithiation product **36** (1.65 g, 3.19 mmol) in 80% yield as a colorless oil. TLC: R_f = 0.23 (petroleum ether/ethyl acetate = 9:1). $[\alpha]_D^{23}$ = +53.4 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.44 (6 H, q, *J* = 8.0 Hz), 0.77 (9 H, t, *J* = 7.9 Hz), 1.02 (3 H, d, *J* = 6.3 Hz), 1.08 (3 H, d, *J* = 6.7 Hz), 1.25 (3 H, d, *J* = 6.3 Hz), 1.56 (3 H, d, J = 6.8 Hz), 1.59 (3 H, d, *J* = 6.8 Hz), 1.98−2.10 (2 H, m), 2.04 (1 H, br s.), 2.28 (3 H, s), 2.38−2.50 (1 H, m), 3.55 (1 H, spt, *J* = 6.8 Hz), 3.80 (1 H, spt, *J* = 6.5 Hz), 4.18 (1 H, d, *J* = 8.8 Hz), 4.27 (1 H, dd, *J* = 12.3 Hz, 5.3 Hz), 4.46 (1 H, dd, *J* = 11.8 Hz, 5.3 Hz), 4.49 (1 H, d, *J* = 9.0 Hz), 4.93−5.12 (2 H, m), 5.21 (1 H, d, *J* = 10.5 Hz), 5.38 (1 H, d, *J* = 17.2 Hz), 5.78−5.94 (1 H, m), 6.04 (1 H, ddt, *J* = 16.7 Hz, 10.9 Hz, 5.4 Hz), 7.17−7.21 (1 H, d, *J* = 7.5 Hz), 7.22−7.26 (1 H, d, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 5.4 (3 C), 6.7 (3 C), 15.5, 15.9, 20.1, 20.4, 20.4, 20.8, 36.3, 37.3, 46.0, 51.7, 71.1, 74.4, 76.1, 115.4, 117.0, 122.8, 130.4, 131.2, 133.9, 138.5, 139.0, 152.2, 156.4, 169.1. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{30}H_{52}O_4NSi$ 518.3660, found 518.3662 (Δ = +0.2 mmu).

2-(Allyloxy)-6-((5*S*,6*R*)-8,8-diethyl-2,2,3,3-tetramethyl-6-((*R*)-pent-4-en-2-yl)-4,7-dioxa-3,8-disiladecan-5-yl)-*N*,*N*-diisopropyl-3-methylbenzamide (36a). To an ice-cooled solution of the alcohol **36** (1.50 g, 2.90 mmol, 1.0 equiv) in dry CH_2Cl_2 (50 mL) were added 2,6-lutidine (1.66 mL, 7.24 mmol, 5.0 equiv) and TBSOTf (1.68 mL, 14.5 mmol, 2.5 equiv). The resulting mixture was allowed to warm to rt and stirred overnight (14 h). The mixture was quenched with water (15 mL) and extracted with 3 × 20 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) afforded 77% of silyl ether **36a** (1.41 g, 2.23 mmol) as a colorless oil. TLC: R_f = 0.43 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +13.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): −0.30 (3 H, s), 0.11 (3 H, s), 0.22−0.49 (6 H, m), 0.74 (9 H, t, *J* = 7.9 Hz), 0.82 (9 H, s), 1.03 (3 H, d, *J* = 7.0 Hz), 1.17 (3 H, d, *J* = 6.6 Hz), 1.13 (3 H, d, *J* = 6.6 Hz), 1.58 (6 H, d, *J* = 6.7 Hz), 1.78−1.95 (1 H, m), 1.96−2.14 (1 H, m), 2.26 (3 H, s), 2.38 (1 H, dd, *J* = 12.8 Hz, 5.2 Hz), 3.52 (1 H, dt, *J* = 13.6 Hz, 6.8 Hz), 3.72−3.92 (2 H, m),4.19 (1 H, dd, *J* = 12.4 Hz, 5.4 Hz), 4.45−4.60 (1 H, m), 4.55 (1 H, d, *J* = 8.9 Hz), 4.94−5.10 (2 H, m), 5.17 (1 H, d, *J* = 10.5 Hz), 5.31- 5.41 (1 H, m), 5.82 (1 H, ddt, *J* = 17.0 Hz, 10.0 Hz, 7.0 Hz), 6.04 (1 H, ddt, *J* = 16.8 Hz, 11.0 Hz, 5.4 Hz), 7.06−7.19 (2 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.7, −5.1, 5.8 (3 C), 6.6 (3 C), 15.9, 17.1, 17.9, 20.3, 20.6, 20.9, 21.4, 25.9 (3 C), 33.6, 35.7, 45.7, 50.7, 70.7, 74.7, 81.4, 115.3, 116.6, 123.5, 130.1, 130.7, 134.0, 134.2, 138.5, 139.3, 152.6, 167.6. HR-MS (ESI-TOF, arginine) calcd for $[M + H]^+$ = $C_{36}H_{66}O_4NSi_2$ 632.4530, found 632.4574 (Δ = +4.4 mmu).

(3*R*,4*S*)-4-(*tert*-Butyldimethylsilyloxy)-8-hydroxy-7-methyl-3-((*R*)-pent-4-en-2-yl)-isochroman-1-one (36c). Allylether **36a** (1.31 g, 2.79 mmol, 1.0 equiv) was dissolved in dry MeOH (30 mL), followed by the addition of $[Pd(Ph_3)_4]$ (23.9 mg, 20.7 μmol, 1 mol %). After being stirred for 10 min at rt, K_2CO_3 (859 mg, 6.22 mmol, 3.0 equiv) was added to the resulting yellow solution, and stirring was continued for 4 h until TLC control indicated complete consumption of the starting material. Then the solvent was evaporated under reduced pressure and the resulted slurry was resolved in water (50 mL), acidified with a solution of HCl (1 N) to pH = 6, and extracted with 3 × 50 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to afford phenol **36b** (1.23 g, 2.99 mmol) in quantitative yield as a pale yellow solid.

Compound **36b** (1.10 g, 1.86 mmol, 1.0 equiv) was dissolved in dry toluene (15 mL) and placed in a septum-sealed microwave vessel, and acetic acid (99.9%, 2.44 mL, 30 equiv) was added. The resulting mixture was heated to 150 °C in a microwave reactor (ca. 60 W continuous power) for 3.5 h. Then the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to yield 90% the TBS-protected hydroxyisochromanone **36c** (633 mg, 1.68 mmol) as colorless oil. TLC: R_f = 0.36 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +61.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = −0.01 (3 H, s), 0.14 (3 H, s), 0.87 (9 H, s), 0.93 (3 H, d, *J* = 6.9 Hz), 1.59−1.82 (1 H, m), 2.08 (1 H, m), 2.28 (3 H, s), 2.40 (1 H, m), 4.33 (1 H, dd, *J* = 8.3 Hz, 3.4 Hz), 4.76 (1 H, d, *J* = 3.6 Hz), 5.02−5.14 (2 H, m), 5.61−5.81 (1 H, m), 6.72 (1 H, d, *J* = 7.4 Hz), 7.34 (1 H, d, *J* = 7.4 Hz), 11.25 (1 H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −4.4, −4.3, 15.6, 15.8, 18.0, 25.7 (3 C), 34.0, 36.2, 66.5, 88.6, 106.8, 117.1, 117.5, 127.1, 135.2, 136.7, 137.4, 160.1, 168.6. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{21}H_{33}O_4Si$ 377.2143, found 377.2164 (Δ = +2.1 mmu).

(3*R*,4*S*)-4,8-Bis(*tert*-Butyldimethylsilyloxy)-7-methyl-3-((*R*)-pent-4-en-2-yl)isochroman-1-one (37). To an ice-cooled solution of isochromanone **36c** (600 mg, 3.79 mmol, 1.0 equiv) in dry CH_2Cl_2 (15 mL) were added NEt_3 (516 μL, 6.37 mmol, 4.0 equiv) and TBSOTf (732 μL, 3.19 mmol, 2.0 equiv). The resulting mixture was stirred at rt overnight (15 h), and then water (10 mL) was added followed by extraction with 3 × 10 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 60:1 to 30:1) afforded 96% of bis-silyl ether **37** (754 mg, 1.54 mmol) as a colorless liquid. TLC: R_f = 0.40 (petroleum ether/ethyl acetate = 30:1).$[\alpha]_D^{23}$ = +55.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.01 (3 H, s), 0.14 (3 H, s), 0.15 (3 H, s), 0.18 (3 H, s), 0.87 (9 H, s), 0.91 (3 H, d, *J* = 7.1 Hz), 1.05 (9 H, s), 1.57−1.78 (1 H, m), 2.06 (1 H, m), 2.27 (3 H, s), 2.41−2.60 (1 H, m), 4.15 (1 H, dd, *J* = 8.3 Hz, 3.9 Hz), 4.73 (1 H, d, *J* = 3.9 Hz), 4.95−5.15 (2 H, m), 5.59−5.87 (1 H, m), 6.85 (1 H, d, *J* = 7.6 Hz), 7.33 (1 H, d, *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = −4.4, −4.2, −3.6, −3.5, 15.5, 17.5, 18.0, 18.6, 25.7 (3 C), 26.0 (3 C), 33.8, 36.1, 67.5, 86.6, 116.3, 117.3, 119.3, 131.8, 135.5, 135.6, 139.0, 154.7, 161.9. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{27}H_{47}O_4Si_2$ 491.3007, found 491.3029 (Δ = +2.2 mmu).

(*R*)-*N*-((1*S*,2*S*)-1-Hydroxy-1-phenylpropan-2-yl)-*N*,2-dimethyl-5-(trimethylsilyl)pent-4-ynamide (40). A mixture of lithium chloride (6.67 g, 157 mmol, 6.0 equiv, dried at 1.0 mbar/150 °C/24 h) and diisopropylamine (8.3 mL, 59.0 mmol, 2.25 equiv) in dry THF (40 mL) was cooled to −78 °C. After the mixture was stirred for 10 min, a solution of *n*-BuLi in hexane (2.5 M, 21.8 mL, 54.5 mmol, 2.1 equiv) was added. The suspension was warmed briefly (ca. 3 min.) to 0 °C and was then cooled to −78 °C. An ice-cooled solution of propionyl pseudophedrine **38** (5.8 g, 26.2 mmol, 1.0 equiv) in dry THF (120 mL) was added to the reaction flask. The reaction mixture was stirred at −78 °C for 1 h, in an iced bath at 0 °C in for 15 min, at rt for 5 min, and finally cooled to −78 °C, where upon 3-bromoprop-1-ynyltrimethylsilane (**39**) (8.5 mL, 52.4 mmol, 2.0 equiv) was added within 15 min via syringe driver. The mixture was stirred at −78 °C for 3 h, for 1 h at 0 °C in an iced bath, and then quenched by the addition of saturated aqueous NH_4Cl solution (10 mL). The mixture was partitioned between saturated aqueous ammonium chloride solution (100 mL) and ethyl acetate (70 mL), and the aqueous layer was separated and extracted with 2 × 25 mL ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and concentrated to afford a yellow solid. Column chromatography on silica gel (ethyl acetate/petroleum ether 1:1) furnished the title compound **40** (7.67 g, 26.2 mmol) in 88% yield as a colorless crystalline solid. TLC: R_f = 0.50 (petroleum ether/ethyl acetate = 1:1). $[\alpha]_D^{23}$ = +43.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.14 (9 H, s)*, 0.17 (9 H, s)$^{\#}$, 1.04 (3 H, d, *J* = 6.7 Hz)$^{\#}$, 1.10 (3 H, d, *J* = 6.7 Hz,)*, 1.18 (3 H, d, *J* = 6.7 Hz), 2.26−2.55 (2 H, m)*, 2.56−2.66 (2 H, m)$^{\#}$, 2.82−2.92 (1 H,m)*, 2.92 (3H, s)$^{\#}$, 2.93 (3 H, s)*, 3.10−3.18 (1 H, m)$^{\#}$, 3.90 (1 H, br s.), 4.00−4.19 (1 H, m)$^{\#}$, 4.48−4.58 (1 H, m)*, 4.61 (1 H, t, *J* = 7.7 Hz), 7.28−7.41 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 0.0 (3 C)*, 0.1 (3 C)$^{\#}$, 14.4*, 15.5$^{\#}$, 16.9*, 17.4$^{\#}$, 24.5, 27.2$^{\#}$, 32.5*, 35.9$^{\#}$, 36.4*, 58.2, 75.5$^{\#}$, 76.4*, 85.7, 105.1, 126.4, 126.9, 127.7, 128.4, 128.7,

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142.2, 177.2. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_{19}H_{29}O_2NSi$ 331.1958, found 331.1951 (Δ = −0.7 mmu). Mp: 106 °C. The data are in accordance with the literature.[131]

(*R*)-2-Methylpent-4-ynoic Acid (25). *Procedure A.* A mixture of amide **40** (3.50g, 10.6 mmol, 1.0 equiv) in ^{t}BuOH (50 mL), methanol (50 mL), and aqueous NaOH solution (6 N, 17.6 mL, 106 mmol, 10.0 equiv) was heated at reflux for 12 h. After cooling to rt, the mixture was concentrated to remove the organic solvents, and the resulting aqueous solution was partitioned between water (50 mL) and CH_2Cl_2 (50 mL). The aqueous layer was separated and extracted with 3 × 50 mL CH_2Cl_2, the organic extracts were separated, and then the aqueous layer was acidified to pH ≤ 2 by the slow addition of aqueous H_2SO_4 solution (6 N). The acidified aqueous solution was extracted with 3 × 30 mL CH_2Cl_2, and the recent combined organic extracts were dried over $MgSO_4$. The solvent was removed under reduced pressure to afford acid **25** as a clear liquid (1.14 g, 10.2 mmol, 96%).

Procedure B. A 0.05 M solution of compound **42** (2.67 g, 9.04 mmol, 1.0 equiv) in a 3:1 THF/H_2O mixture (135 mL THF, 45 mL H_2O) was treated at 0 °C with 30% H_2O_2 (8.20 g, 72.2 mmol, 8.0 equiv) followed by (0.75 g, 18.1 mmol, 2.0 equiv) LiOH. The resulting mixture was stirred at 0 °C for 2 h, and the excess peroxide was quenched at 0 °C with Na_2SO_3 (1.5 N, 13 mL, 10.0 mmol, 1.1 equiv). After buffering to pH 10 with aqueous $NaHCO_3$ and evaporation of the THF, the oxazolidinone chiral auxiliary was recovered by CH_2Cl_2 extraction. The carboxylic acid was isolated by EtOAc extraction of the acidified aqueous phase. The crude product was purified by silica gel column chromatography with hexane/ethyl acetate (1:1) to give **25** (1.00 g, 8.95 mmol, 99%) as a colorless liquid. TLC: R_f = 0.25 (petroleum ether/ethyl acetate = 3:1). $[\alpha]_D^{23}$ = +4.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.33 (3 H, d, *J* = 7.0 Hz), 2.03 (1 H, t, *J* = 2.7 Hz), 2.40 (1 H, ddd, *J* = 16.7 Hz, 7.7 Hz, 2.7 Hz), 2.57 (1 H, ddd, *J* = 16.8 Hz, 5.9 Hz, *J* = 2.7 Hz), 2.64−2.78 (1 H, m), 10.24 (1 H, br. s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.1, 22.3, 38.5, 70.1, 81.1, 181.0. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_6H_8O_2$ 112.0524, found 112.0536 (Δ = +1.2 mmu). The data are in accordance with the literature.[131,132]

Ethyl (2*R*,3*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-methylpent-4-enoate (31b). To an ice-cooled solution of the ester **33a** (2.43 g, 15.4 mmol, 1.0 equiv) in dry CH_2Cl_2 (120 mL) were added 2,6-lutidine (7.13 mL, 61.4 mmol, 4.0 equiv) and TBSOTf (10.6 mL, 46.1 mmol, 3.0 equiv). The resulting mixture was stirred at rt for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (40 mL) and extracted with 3 × 40 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 60:1) afforded 93% of silyl ether **31b** (3.9 g, 14.4 mmol) as a pale yellow liquid. TLC: R_f = 0.17 (petroleum ether/ethyl acetate = 60:1). $[\alpha]_D^{23}$ = +20.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.04 (3H, s), 0.08 (3H, s), 0.92 (s, 9 H), 1.07 (3 H, d, *J* = 7.0 Hz), 1.27 (3 H, t, *J* = 7.1 Hz), 2.55−2.70 (1 H, m), 4.07 (1 H, d, *J* = 4.8 Hz), 4.17 (2 H, m_c), 4.99 (1 H, s), 5.01−5.06 (1 H, m), 5.82 (1 H, ddd, *J* = 17.2, 10.4, 8.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ = −5.3, −4.9, 14.3, 16.8, 18.3, 25.7 (3 C), 42.8, 60.5, 76.3, 115.3, 139.0, 172.9. HRMS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{14}H_{29}O_3Si$ 273.1880, found 273.1881 (Δ = −0.1 mmu).

(2*R*,3*R*)-Ethyl 2-((*tert*-Butyldimethylsilyl)oxy)-3-methylhex-5-enoate (43). The hydroboration and oxidation procedure described above for **32** was carried out with alkene **31b** (3.18 g, 11.6 mmol, 1.0 equiv) to yield aldehyde **31c** (2.57 g, 8.91 mmol, 76% (two steps)) after flash column chromatography on silica gel (petroleum ether/ethyl acetate 30:1). Wittig reaction of **31b** (2.24 g, 7.77 mmol, 1.0 equiv) according to the procedure given for **33** led to alkene **43** (2.01 g, 7.02 mmol, 90%) after flash column chromatography on silica gel (petroleum ether/ethyl acetate 60:1). TLC: R_f = 0.37 (petroleum ether/ethyl acetate = 30:1). $[\alpha]_D^{23}$ = +29.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.05 (3 H, s), 0.06 (3 H, s), 0.89−0.95 (3 H, d, *J* = 6.7 Hz), 0.94 (9 H, s), 1.29 (3 H, t, *J* = 7.1 Hz), 1.84−2.09 (2 H, m), 2.20−2.30 (1 H, m), 4.01 (1 H, d, *J* = 4.8 Hz), 4.10−4.25 (2 H, m), 4.93−5.09 (2 H, m), 5.62−5.87 (1 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.3, −4.9, 14.2, 15.9, 18.3, 25.7 (3C), 35.9, 37.7, 60.5, 76.2, 116.2, 136.9, 173.3. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{15}H_{31}O_3Si$ 287.2038, found 287.2037 (Δ = −0.1 mmu).

Ethyl (2*R*,3*R*)-2,6-Bis((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-3-methylhexanoate (45). The dihydroxylation procedure described below for **47** was carried out with alkene **43** (692 mg, 2.42 mmol, 1.0 equiv) to yield diole **43a** (675 mg, 2.42 mmol, 87%, d.r. = 1:1) after flash column chromatography on silica gel (petroleum ether/ethyl acetate 1:1 to pure ethyl acetate).

Subsequent selective TBS protection of diole **43a** (627 mg, 1.96 mmol, 1.0 equiv) was performed as described for compound **47a** to yield **45** (751 mg, 1.73 mmol) in 88% after flash column chromatography on silica gel (petroleum ether/ethyl acetate 4:1−1:1). TLC: R_f = 0.31[a]/0.39[b] (petroleum ether/ethyl acetate = 3:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = −0.02 (3 H, s), 0.00 (9 H, s), 0.83 (9 H, s), 0.85 (9 H, s), 0.94 (3 H, d, *J* = 6.9 Hz), 1.13−1.20 (1 H, m), 1.21 (3 H, t, *J* = 7.2 Hz), 1.33−1.44 (1 H, m), 2.14−2.23 (1 H, m), 2.25 (1 H, d, *J* = 3.8 Hz), 3.28−3.38 (1 H, m), 3.47−3.57 (1 H, m), 3.57−3.68 (1 H, m), 3.99−4.06 (1 H, m), 4.12 (2 H, q, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.4[b], −5.4[b], −5.4 (2 C)[a], −5.4 (2 C)[b], −4.9 (2 C)[a], 14.2[b], 14.3[a], 16.1[b], 17.3[a], 18.3[c], 18.3[c], 25.6 (3 C)[b], 25.7 (3 C)[b], 25.7 (3 C)[a], 25.9 (3 C)[a], 34.0[a], 34.1[a], 34.8[b], 35.1[b], 60.5[a], 60.6[b], 67.2[b], 67.9[a], 69.3[a], 69.8[b], 76.3[b], 76.8[a], 173.2[b], 173.2[a]. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{21}H_{47}O_5Si_2$ 435.2957, found 435.2969 (Δ = +1.2 mmu).

Ethyl (2*R*,3*R*)-5-Azido-2,6-bis((*tert*-butyldimethylsilyl)oxy)-3-methylhexanoate (46). The procedure described below for azide substitution of **47b** was carried out with alcohol **45** (40 mg, 92 μmol, 1.0 equiv), PPh_3 (41 mg, 156 μmol, 1.7 equiv), DIAD (30 μL, 156 μmol, 1.7 equiv), and DPPA (34 mg, 138 μmol, 1.5 equiv) leading to azide **46** (34.7 mg, 75 μmol, 82%) after flash column chromatography on silica gel (petroleum ether/ethyl acetate 9:1−5:1). TLC: R_f = 0.21 (petroleum ether/ethyl acetate = 9:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.05 (3 H, s), 0.07 (3 H, s), 0.08 (6 H, s), 0.91 (9 H, s), 0.92 (9 H, s), 1.01 (3 H, d, *J* = 7.0 Hz), 1.23−1.27 (1 H, m), 1.29 (3 H, t, *J* = 7.1 Hz), 1.55−1.65 (1 H, m), 2.04−2.23 (1 H, m), 3.34−3.44 (1 H, m), 3.49−3.58 (1 H, m), 3.66−3.77 (1 H, m), 4.05 (1 H, d, *J* = 5.2 Hz), 4.19 (2 H, q, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz) δ = −5.6 (2 C)[c], −5.4[b], −5.4[a], −5.0[a], −4.9[b], 14.2[a], 16.1[b], 17.6[c], 18.2[b], 18.2[a], 18.2[a], 18.3[b], 25.7 (3 C)[a], 25.7 (3 C)[b], 25.8 (3 C)[b], 25.8 (3 C)[a], 31.2[b], 32.1[a], 34.4[b], 35.2[a], 60.7[a], 61.2[b], 62.1[c], 66.7[a], 67.2[b], 75.8[a], 76.5[b], 173.0[b], 173.1[a]. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{21}H_{45}N_3O_4Si_2$ 460.3017, found 460.3013 (Δ = −0.4 mmu).

(3*R*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methylpiperidin-2-one (44a). Azide **46** (37.9 mg, 82 μmol, 1.0 equiv) was dissolved in THF (2.5 mL) followed by the addition of water (14 μL, 825 μmol, 10 equiv) and PPh_3 (75 mg, 288 μmol, 3.5 equiv). The resulting mixture was then heated to 50 °C and stirred at this temperature for 6 h. After the mixture was cooled to rt, water (5 mL) was added, and the resulting mixture was extracted with 3 × 15 mL of ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 9:1−3:1) furnished lactam **44a** (23.6 mg, 61 μmol) in 73% yield. TLC: R_f = 0.33 (petroleum ether/ethyl acetate = 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.05 (3 H, s), 0.07 (3 H, s), 0.13 (3 H, s), 0.18 (3 H, s), 0.90 (9 H, s), 0.91 (9 H, s), 1.04 (3 H, d, *J* = 6.9 Hz), 1.37−1.49 (1 H, m), 1.82 (1 H, dt, *J* = 13.5, 6.6 Hz), 2.04−2.22 (1 H, m), 3.26−3.46 (1 H, m), 3.50−3.61 (2 H, m), 4.02 (1 H, d, *J* = 4.4 Hz), 5.81 (1 H, br s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.6, −5.4, −4.5 (2 C), 14.0, 18.4 (2 C), 25.8 (3 C), 25.8 (3 C), 27.9, 32.1, 51.1, 67.2, 72.5, 171.9. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{19}H_{42}NO_3Si_2$ 388.2699, found 388.2693 (Δ = −0.4 mmu).

(3*R*,4*S*)-4,8-Bis((*tert*-Butyldimethylsilyl)oxy)-3-((2*R*)-4,5-dihydroxypentan-2-yl)-7-methylisochroman-1-one (47). To a solution of bis-TBS-protected hydroxyisochromanone **37** (740 mg, 1.51 mmol, 1.0 equiv) in acetone/H_2O (4:1, 15 mL) at 0 °C was added a solution of OsO_4 (2.5 mol % in *tert*-butyl alcohol, 15 mg, 60 μmol, 0.04 equiv) followed by the addition of NMO (353 mg, 3.02 mmol, 2.0 equiv) in three portions. The mixture was stirred at 0 °C for 2 h and at rt overnight (12 h). Then water (15 mL) was added, and the

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mixture was extracted with 3 × 15 mL of ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 1:1) provided diol **47** (745 mg, 1.42 mmol) in 94% yield as mixture of diastereomers (d.r. = 1:1) as a colorless oil. TLC: R_f = 0.43[a]/0.55[b] (petroleum ether/ethyl acetate = 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = −0.03 (3 H, s)[b], 0.01 (3 H, s)[a], 0.13 (3 H, s)[b], 0.15 (3 H, s)[a], 0.17 (3 H, s)[c], 0.18 (3 H, s)[c], 0.85 (9 H, s)[b], 0.88 (9 H, s)[a], 1.01 (3 H, d, J = 6.9 Hz)[b], 1.03 (3 H, d, J = 6.9 Hz)[a], 1.04 (9 H, s)[c], 1.22−1.37 (1 H, m)[c], 1.46−1.60 (1 H, m)[b], 1.73 (1 H, m)[b], 1.76−1.91 (1 H, m)[a], 1.94−2.10 (3 H, m)[a,c], 2.26 (3 H, s)[c], 3.36−3.48 (1 H, m)[c], 3.58 (1 H, dd, J = 10.9 Hz, 3.2 Hz)[b], 3.65 (1 H, dd, J = 10.9, 3.0 Hz)[a], 3.75−3.86 (1 H, m)[c], 4.16 (1 H, dd, J = 7.9, 4.2 Hz)[a], 4.27 (1 H, dd, J = 8.7, 3.2 Hz)[b], 4.73 (1 H, d, J = 3.2 Hz)[b], 4.75 (1 H, d, J = 4.2 Hz)[a], 6.84 (1 H, d, J = 7.3 Hz)[b], 6.86 (1 H, d, J = 7.3 Hz)[a], 7.34 (1 H, d, J = 7.6 Hz)[a], 7.35 (1 H, d, J = 7.6 Hz)[b]. ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −4.5[b], −4.4[b], −4.3[a], −4.1[a], −3.5[a], −3.4[a], −3.4[b], −3.3[b], 15.8[a], 16.1[b], 17.5[b], 17.6[a], 18.0[b], 18.1[a], 18.6[a], 18.6[b], 25.6 (3 C)[a], 25.6 (3 C)[b], 26.0 (3 C)[c], 30.4[a], 31.0[b], 34.7[a], 35.1[b], 67.4[b], 67.6[a], 69.0[a], 69.2[b], 70.5[b], 70.8[a], 87.4[b], 87.5[a], 106.6[b], 106.7[a], 119.3[a], 119.7[b], 131.8[a], 132.1[b], 135.7[a], 135.8[b], 138.6[b], 139.3[a], 154.8[a], 154.9[b], 161.8[b], 162.0[a]. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{27}H_{49}O_6Si_2$ 525.3062, found 525.3068 (Δ = +0.6 mmu).

(3*R*,4*S*)-4,8-Bis(*tert*-butyldimethylsilyloxy)-3-((2*R*)-5-((*tert*-butyldimethylsilyl)oxy)-4-hydroxypentan-2-yl)-7-methylisochroman-1-one (47a). Diol **47** (296 mg, 0.563 mmol, 1.0 equiv) was dissolved in dry CH_2Cl_2 (10 mL), and DMAP (3.5 mg, 28 μmol, 0.05 equiv) was added, followed by the addition of imidazole (80 mg, 1.18 mmol, 2.1 equiv). The mixture was cooled to 0 °C, and TBSCl (106 mg, 0.70 mmol, 1.25 equiv) was added. The resulting mixture was stirred at this temperature for 3 h until TLC indicated complete consumption of the starting material. The reaction was quenched with water (15 mL) and extracted with 3 × 20 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 15:1) afforded 96% of tris-TBS-protected diol **47a** (347 mg, 0.542 mmol) as a colorless oil. TLC: R_f = 0.23[a]/0.38[b] (petroleum ether/ethyl acetate = 30:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (diastereomer a) = −0.21 (3 H, s), −0.12 (3 H, s), −0.12 (3 H, s), −0.05 (3 H, s), −0.03 (3 H, s), 0.00 (3 H, s), 0.67 (9 H, s), 0.71 (9 H, s), 0.86 (9 H, s), 0.87 (3 H, d, J = 6.7 Hz), 1.23 (1 H, dt, J = 14.2 Hz, 8.6 Hz), 1.49 (1 H, br s), 1.50−1.62 (1 H, m), 1.62 (1 H, dt, J = 14.2 Hz, 3.5 Hz), 2.09 (3 H, s), 3.16 (1 H, dd, J = 9.8 Hz, 8.0 Hz), 3.36 (1 H, dd, J = 9.8 Hz, 3.4 Hz), 3.43−3.52 (1 H, m), 4.05 (1 H, dd, J = 9.2 Hz, 3.0 Hz), 4.56 (1 H, d, J = 3.0 Hz), 6.65 (1 H, d, J = 7.4 Hz), 7.15 (1 H, d, J = 7.6 Hz); δ (diastereomer b) = 0.02 (3 H, s), 0.08 (6 H, s), 0.14 (3 H, s), 0.17 (6 H, s), 0.88 (9 H, s), 0.91 (9 H, s), 1.01 (3 H, d, J = 6.7 Hz), 1.05 (9 H, s), 1.16−1.33 (1 H, m), 1.62 (1 H, br s), 1.84 (1 H, ddd, J = 13.9 Hz, 10.9 Hz, 3.1 Hz), 1.95−2.11 (1 H, m), 2.26 (3 H, s), 3.37 (1 H, dd, J = 9.8 Hz, 6.9 Hz), 3.63 (1 H, dd, J = 9.9 Hz, 3.3 Hz), 3.68−3.80 (1 H, m), 4.15 (1 H, dd, J = 7.7 Hz, 4.4 Hz), 4.74 (1 H, d, J = 4.4 Hz), 6.86 (1 H, d, J = 7.6 Hz), 7.33 (1 H, d, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (diastereomer a) = −4.4, −4.2, −3.6 (2 C), −3.3, −3.2, 15.9, 16.9, 17.7, 18.1, 18.7, 25.7 (3 C), 25.7 (3 C), 26.0 (3 C), 33.0, 36.0, 67.2, 67.6, 70.9, 87.4, 116.0, 119.7, 132.1, 135.8, 138.7, 155.0, 161.8;δ (diastereomer b) = −5.4, −5.3, −4.2, −4.1, −3.6, −3.5, 15.7, 17.5, 18.1, 18.3, 18.6, 25.8 (3 C), 25.9 (3 C), 26.0 (3 C), 30.2, 34.0, 67.4, 67.9, 68.4, 87.6, 116.2, 119.2, 131.7, 135.6, 139.5, 154.7, 162.1. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{33}H_{63}O_6Si_3$ 639.3927, found 639.3949 (Δ = +2.2 mmu).

(3*R*,4*S*)-3-((2*R*)-4-Azido-5-((*tert*-butyldimethylsilyl)oxy)-pentan-2-yl)-4,8-bis((*tert*-butyldimethylsilyl)oxy)-7-methylisochroman-1-one (47b). To a solution of the tris-TBS-protected diol **47a** (420 mg, 420 μmol, 1.0 equiv) in dry THF (10 mL) at 0 °C were added PPh_3 (275 mg, 1.05 mmol, 2.5 equiv) and DIAD (210 μL, 1.07 mmol, 2.55 equiv), followed by the addition of DPPA (153 mg, 630 μmol, 1.5 equiv). The mixture was allowed to warm to rt and stirred for 5 h before another 1 equiv of PPh_3 and DIAD at 0 °C was added. After the mixture was stirred overnight (14 h) at rt, the solvent was evaporated in vacuo. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 30:1−15:1) gave 79% of azide **47b** (214 mg, 332 μmol) and also 26 mg of the starting material (89% brsm) as a colorless liquid. TLC: R_f = 0.36[a]/0.48[b] (petroleum ether/ethyl acetate = 30:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = −0.03 (3 H, s)[a], 0.02 (3 H, s)[b], 0.06 (3 H, s)[b], 0.08 (3 H, s)[a], 0.09 (3 H, s)[c], 0.13 (3 H, s)[a], 0.15 (3 H, s)[b], 0.16 (3 H, s)[a], 0.17 (3 H, s)[b], 0.19 (3 H, s)[c], 0.86 (9 H, s)[b], 0.89 (9 H, s)[a], 0.90 (9 H, s)[a], 0.92 (9 H, s)[b], 1.01 (3 H, d, J = 6.9 Hz)[b], 1.03 (3 H, d, J = 6.9 Hz)[a], 1.05 (9 H, s)[a], 1.05 (9 H, s)[b], 1.26−1.35 (1 H, m)[b], 1.35−1.46 (1 H, m)[a], 1.61−1.75 (1 H, m)[c], 1.84 (1 H, dt, J = 14.4 Hz, 4.2 Hz)[a], 1.89−1.99 (1 H, m)[b], 2.27 (3 H, s)[c], 3.38 (1 H, tt, J = 8.0 Hz, 4.2 Hz)[a], 3.52−3.63 (2 H, m)[cb], 3.65−3.77 (1 H, m)[c], 4.11 (1 H, dd, J = 7.4 Hz, 4.8 Hz)[b], 4.16 (1 H, dd, J = 8.7, 3.3 Hz)[a], 4.70 (1 H, d, J = 4.8 Hz)[b], 4.72 (1 H, d, J = 3.3 Hz)[a], 6.83 (1 H, d, J = 7.6 Hz)[a], 6.87 (1 H, d, J = 7.6 Hz)[b], 7.35 (1 H, d, J = 7.4 Hz)[c]. ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −5.6[c], −5.6[c], −4.4[a], −4.3[c], −4.0[b], −3.6[a], −3.4[b], −3.3[a], −3.3[b], 15.7[a], 16.7[b], 17.5[b], 17.6[a], 18.0[a], 18.1[b], 18.2[a], 18.2[b], 18.6[b], 18.7[a], 25.7 (3 C)[a], 25.7 (3 C)[b], 25.8 (3 C)[a], 25.8 (3 C)[b], 26.0 (3 C)[b], 26.0 (3 C)[a], 30.6[a], 31.8[b], 33.2[b], 33.6[a], 61.2[b], 62.8[a], 67.1[b], 67.5[a], 67.6[c], 87.1[b], 87.3[a], 116.0[b], 116.2[a], 119.0[b], 119.5[a], 131.7[b], 132.0[a], 135.6[b], 135.7[a], 138.6[a], 139.4[b], 154.8[b], 154.9[a], 161.5[a], 161.8[b]. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{33}H_{62}O_5N_3Si_3$ 664.3992, found 664.4011 (Δ = +1.9 mmu).

(3*R*,4*S*)-3-((2R)-4-Amino-5-((*tert*-butyldimethylsilyl)oxy)-pentan-2-yl)-4,8-bis((*tert*-butyldimethylsilyl)oxy)-7-methylisochroman-1-one (24). Azide **47b** (120 mg, 180 μmol, 1.0 equiv) in dry MeOH (10 mL) was treated with Pd on activated charcoal (10%, 38 mg, 36 μmol, 0.2 equiv) under a hydrogen atmosphere (1.013 bar) at rt. The mixture was stirred for 6 h and monitored by TLC, and the solvent was evaporated under reduced pressure. Evaporation of the solvent in vacuo and filtration over silica gel (CH_2Cl_2/MeOH 97:3 as eluent) afforded amine **24** (101 mg, 158 μmol) in 88% yield as a pale yellow oil. TLC: R_f = 0.18[b]/0.21[a] (CH_2Cl_2/MeOH = 97:3). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = −0.02 (3 H,s)[a], −0.02 (3 H, s)[b], 0.07 (3 H, s)[c], 0.16 (3 H, s)[c], 0.17 (6 H, br. s)[c], 0.18 (3 H, s)[c], 0.84 (9 H, s)[b], 0.86 (9 H, s.)[c], 0.89 (9 H, s)[a], 1.01 (3 H, d, J = 7.2 Hz)[a], 1.03 (9 H, s)[c], 1.04 (3 H, d, J = 7.2 Hz)[b], 1.40−1.52 (1 H, m)[c], 1.63 (2 H, br s.)[a], 1.66−1.85 (1 H, m)[c], 1.69 (2 H, br s.)[b], 1.88−2.00 (1 H, m)[a], 2.06 (1 H, m)[b], 2.26 (3 H, s)[c], 3.14−3.31 (1 H, m)[a], 3.46−3.52 (1 H, m)[c], 3.52−3.60 (1 H, m)[b], 3.67−3.80 (1 H, m)[c], 4.11−4.17 (1 H, m)[b], 4.21 (1 H, dd, J = 8.5 Hz, 3.1 Hz)[a], 4.70−4.74 (1 H, m)[b], 4.76 (1 H, d, J = 3.6 Hz)[a], 6.86 (1 H, d, J = 7.2 Hz)[c], 7.34 (1 H, d, J = 7.4 Hz)[c]. ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −5.4[c], −5.4[c], −4.3[c], −4.2[c], −3.1[c], −3.1[c], 15.9[b], 16.7[a], 17.6[c], 18.0[c], 18.2[c], 18.7[c], 25.7 (3 C)[c], 25.9 (3 C)[c], 26.1 (3 C)[c], 31.1[b], 31.3[b], 32.1[a], 34.0[a], 51.5[a], 51.7[b], 61.6[b], 65.9[a], 67.6[b], 67.9[a], 87.4[c], 115.7[c], 119.5[c], 131.9[c], 135.9[c], 139.2[c], 155.2[c], 161.7[c]. HR-MS (ESI-TOF, HPmix): calcd for $[M + Na]^+$ = $C_{33}H_{63}O_5NSi_3Na$ 660.3912, found 660.3923 (Δ = +1.1 mmu).

(2*R*)-*N*-((4*R*)-4-((3*R*,4*S*)-4,8-Bis((*tert*-butyldimethylsilyl)oxy)-7-methyl-1-oxoisochroman-3-yl)-1-hydroxypentan-2-yl)-2-methylpent-4-ynamide (52). Carboxylic acid **25** (19 mg, 0.169 mol, 1.30 equiv) and amine **24** (83 mg, 130 μmol, 1.0 equiv) were dissolved in dry THF (4 mL). At rt, dry triethylamine (90 μL, 650 μmol, 5.0 equiv) was added followed by DEPBT (70 mg, 234 μmol, 1.8 equiv). The yellow solution was then stirred overnight, followed by the addition of saturated NH_4Cl solution (5 mL). The mixture was extracted with ethyl acetate (3 × 5 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. After purification by column chromatography on silica gel (petroleum ether/ethyl acetate, 1:1), **51** was obtained as a colorless oil (79 mg, 107 μmol, 83%).

To a solution of amide **51** (70 mg, 95 μmol, 1.1 equiv) in MeCN (1 mL) was added a solution of water in MeCN (1.8 M, 53 μL, 95 μmol, 1.0 equiv) followed by a solution of TMSCl in MeCN (0.2 M, 95 μL, 19 μmol, 0.2 equiv). After 6 h at rt, the reaction was quenched by the addition of pH-7 buffer (3 mL). After extraction with 3 × 5 mL of ethyl acetate, the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 1:1 to pure ethyl acetate) and yielded **52** as colorless oil (52 mg, 84 μmol, 88%) and also 7 mg of unconsumed

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starting material (96% brsm). TLC: R_f = 0.56/0.62 (petroleum ether/ethyl acetate = 1:1). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = −0.07 (3 H, s)[a], −0.06 (3 H, s)[b], 0.12 (3 H, s)[a], 0.13 (3 H, s)[b], 0.15−0.23 (6 H, m)[c], 0.83 (9 H, s)[a], 0.84 (9 H, s)[b], 0.98 (3 H, d, J = 7.2 Hz)[a], 1.00 (3 H, d, J = 7.2 Hz)[b], 1.03 (9 H, s)[a], 1.04 (9H, s)[b], 1.24 (3 H, d, J = 6.7 Hz)[a], 1.25−1.27 (3 H, m)[b], 1.37−1.44 (1 H, m)[a], 1.63−1.70 (1 H, m)[b], 1.84 (1 H, dd, J = 10.1 Hz, 6.0 Hz)[a], 1.87−1.90 (1 H, m)[a], 1.98−2.06 (2 H, m)[b], 2.25 (3 H, s)[a], 2.27 (3 H, s)[b], 2.30−2.41 (2 H, m)[c], 2.42−2.53 (1 H, m)[c], 3.47−3.56 (1 H, m)[c], 3.57−3.67 (1 H, m)[c], 4.00−4.06 (1 H, m)[b], 4.12 (1H, dd, J = 9.7, 2.6 Hz)[a], 4.17 (1 H, dd, J = 8.8 Hz, 2.8 Hz)[b], 4.11−4.18 (1 H, m)[b], 4.70 (1 H, d, J = 3.0 Hz)[a], 4.71 (1 H, d, J = 2.8 Hz)[b], 5.66 (1 H, d, J = 9.1 Hz)[a], 6.20 (1 H, d, J = 7.6 Hz)[b], 6.81 (1 H, d, J = 7.3 Hz)[a], 6.83 (1 H, d, J = 7.6 Hz), 7.34 (1 H, d, J = 7.8 Hz)[b], 7.35 (1 H, d, J = 7.3 Hz)[a]. ^{13}C NMR ($CDCl_3$, 150 MHz):δ = −4.5[a], −4.4[b], −4.3[b], −4.3[a], −3.3[a], −3.2[b], −3.0[a], −3.0[b], 15.9[a], 16.0[b], 16.9[b], 17.0[a], 17.6[a], 17.6[b], 18.0[b], 18.0[a], 18.7[a], 18.8[b], 23.1[a], 23.3[b], 25.6 (3 C)[c], 26.0 (3 C)[a], 26.0 (3 C)[b], 31.9[b], 32.5[a], 33.2[b], 34.0[a], 40.3[a], 40.5[b], 48.6[b], 49.5[a], 65.9[a], 66.5[b], 67.6[a], 67.7[b], 70.1[b], 70.3[a], 81.9[b], 82.1[a], 86.9[a], 87.2[b], 115.6[b], 115.7[a], 119.8[b], 119.9[a], 132.2[b], 132.2[a], 135.9[c], 138.5[a], 138.7[b], 155.1[a], 155.3[b], 161.6[a], 161.6[b], 175.2[b], 175.4[a]. HR-MS (ESI-TOF, arginine): calcd for $[M + H]^+$ = $C_{33}H_{56}O_6NSi_2$ 618.3641, found 618.3643 (Δ = +0.2 mmu).

(3*R*,4*S*)-4,8-Bis((*tert*-butyldimethylsilyl)oxy)-7-methyl-3-((*R*)-1-(2-((*R*)-pent-4-yn-2-yl)oxazol-4-yl)propan-2-yl)isochroman-1-one (53). To a solution of hydroxyamide **52** (68 mg, 110 μmol, 1.0 equiv) in ethyl acetate (5 mL) was added IBX (92 mg, 330 μmol, 3.0 equiv). The reaction mixture was then refluxed for 2 h. After removal of the solvent under reduced pressure, the resulting white slurry was filtered off a short plug of silica gel (petroleum ether/ethyl acetate 2:1) and concentrated in vacuo to furnish the required aldehyde **52a** (62 mg crude product).

The resulting clear oil was immediately dissolved in dry CH_2Cl_2 (4 mL), cooled to 0 °C, and treated with triphenylphosphine (132 mg, 503 μmol, 5.0 equiv) and 2,6-DTBMP (226 mg, 1.10 mmol, 10.0 equiv), followed after 5 min by 1,2-dibromo-1,1,2,2-tetrachloroethane (179 mg, 550 μmol, 5.0 equiv). Afterward, the reaction mixture was stirred at 0 °C for 14 h. Then DBU (247 μL, 1.65 mmol, 15.0 equiv) in MeCN (1 mL) was added dropwise. Stirring was continued at 0 °C for an additional 6 h. The resulting yellowish mixture was finally washed with 3 × 3 mL of an aqueous saturated NH_4Cl solution and with 3 mL of brine. After the mixture was dried over $MgSO_4$ and filtered, the solvent was removed in vacuo. Purification by column chromatography on silica gel (petroleum ether/ethyl acetate = 15:1 to 9:1) gave 52 mg (87 μmol, 79%) of oxazole **53** as a colorless liquid. TLC: R_f = 0.42 (petroleum ether/ethyl acetate = 9:1). $[\alpha]_D^{23}$ = +56.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.00 (3 H, s), 0.15 (3 H, s), 0.16 (3 H, s), 0.19 (3 H, s), 0.85−0.89 (9 H, m), 0.93 (3 H, d, J = 6.8 Hz), 1.05 (9 H, s), 1.43 (3 H, d, J = 7.0 Hz), 1.97 (1 H, t, J = 2.6 Hz), 1.97−2.02 (1 H, m), 2.27 (3 H, s), 2.48−2.58 (2 H, m), 2.68 (1 H, ddd, J = 17.0 Hz, 6.0 Hz, 2.6 Hz), 2.88 (1 H, dd, J = 14.7 Hz, 3.1 Hz), 3.17 (1 H, sxt, J = 7.0 Hz), 4.20 (1 H, dd, J = 8.4 Hz, 3.8 Hz), 4.75 (1 H, d, J = 3.8 Hz), 6.85 (1 H, d, J = 7.6 Hz), 7.34 (1 H, d, J = 7.6 Hz), 7.36 (1 H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −4.5, −4.2, −3.5, −3.4, 15.9, 17.3, 17.5, 18.0, 18.6, 24.2, 25.7 (3 C), 26.0 (3 C), 28.0, 33.1, 33.7, 67.4, 70.1, 81.3, 86.6, 116.2, 119.4, 131.9, 135.3, 135.6, 137.5, 139.0, 154.8, 161.9, 166.1. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{33}H_{52}O_5NSi_2$ 598.3379, found 598.3385 (Δ = +0.6 mmu).

(*E*)-Methyl 3-Methoxybut-2-enoate (54a). In a 50 mL round-bottom flask were added methyl acetoacetonate **54** (20.0 g, 172 mmol, 1.00 equiv), trimethyl orthoformate (18.6 g, 175 mmol, 1.0 equiv), and concentrated H_2SO_4 (6 drops), and the mixture was stirred at 20 °C for 24 h. After this time, a slight excess of quinoline (12 drops) was added to neutralize the acid. Distillation under reduced pressure afforded **54a** (19.5 g, 149 mmol, 87%) as colorless liquid. TLC: R_f = 0.40 (ethyl acetate/hexane =1:15). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.28 (s, 3H), 3.61 (s, 3H), 3.66 (s, 3H), 5.01 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 18.8, 50.6, 55.3, 90.4, 168.2, 173.2. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_6H_{10}O_3$ 130.0630, found 130.0615 (Δ = −1.5 mmu). Bp: 90.0 °C (70.0 mbar). The data are in accordance with the literature.[20a]

(*E*)-3-Methoxybut-2-enoic Acid (55). Ester **54a** (4.00 g, 30.8 mmol, 1.00 equiv) was dissolved in a 250 mL round-bottom flask in THF (150 mL). To this solution were added water (50 mL) and $LiOH·H_2O$ (9.00 g, 214 mmol, 6.9 equiv), and the suspension was vigorously stirred at 67 °C for 24 h. After this time, the reaction was cooled to 0 °C, and concentrated hydrochloric acid was added until pH 3. The mixture was extracted with diethyl ether (3 × 200 mL) and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was recrystallized from diethyl ether/hexane (1:1) to afford the desired acid **55** (2.86 g, 24.6 mmol, 80%) as colorless powder. TLC: R_f = 0.33 (ethyl acetate/hexane = 1:2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.29 (s, 3H), 3.66 (s, 3H), 5.03 (s, 1H), 12.21 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 19.2, 55.6, 90.5, 173.7, 175.3. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_5H_8O_3$ 116.0473, found 116.0500 (Δ = +2.7 mmu). Mp: 130 °C. The data are in accordance with the literature.[20a]

(*E*)-*N*-Allyl-3-methoxy-*N*-methylbut-2-enamide (57). In a flame-dried, 500 mL, round-bottom flask under an argon atmosphere *N*-allylmethylamine (600 mg, 8.44 mmol, 1.0 equiv) was dissolved in dichloromethane (350 mL). Acid **55** (980 mg, 8.44 mmol, 1.0 equiv), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (4.00 g, 13.5 mmol, 1.6 equiv), and 1-hydroxybenzotriazole hydrate (228 mg, 1.69 mmol, 0.2 equiv) were added. The reaction mixture was stirred at room temperature for 4 h, filtrated, and then concentrated by rotary evaporation. The resulting crude product in the remaining slurry was purified by column chromatography on silica gel with ethyl acetate/hexane = 1:2 as eluent, which yielded the desired amide **57** (1.39 g, 8.17 mmol, 97%) as a yellow oil. TLC: R_f = 0.27 (ethyl acetate/hexan = 1:2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.21 (s, 3H), 2.96 (s, 3H,), 3.57 (s, 3H)*, 3.61 (s, 3H)#, 3.94 (bs, 2H)*, 4.02 (bs, 2H)#, 5.13 (m, 2H), 5.19 (s, 1H), 5.80 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.3, 33.4*, 35.1#, 49.7#, 52.7*, 54.7, 90.9, 116.2*, 116.6#, 132.8*, 133.4#, 167.8#, 168.4*, 168.6. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_9H_{15}NO_2$ 169.1103, found 169.1105 (Δ = +0.2 mmu).

(*E*)-3-Methoxy-*N*-methylbut-2-enamide (56). Acid **55** (5.30 g, 45.6 mmol, 1.0 equiv) was dissolved in dry THF (50 mL). The solution was cooled to 0 °C, and a solution of methylamine was added (2.0 M in THF, 34.0 mL, 68.5 mmol, 1.5 equiv), followed by the addition of EDCI·HCl (10.80 g, 57.1 mmol, 1.25 equiv). The mixture was stirred for 0.5 h at 0 °C and was then allowed to warm to rt. Stirring was continued for 6 h at rt before water was added (50 mL), and the reaction mixture was extracted with 3 × 50 mL of EtOAc. After drying over $MgSO_4$, filtration, and removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel (pure ethyl acetate) to afford amide **56** (4.81 g, 37.2 mmol) in 82% yield as a colorless solid. TLC: R_f = 0.33 (ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.31 (3 H, s), 2.82 (3 H, d, J = 4.8 Hz), 3.57 (3 H, s), 4.88 (1 H, s), 5.42 (1 H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.3, 26.1, 54.8, 92.8, 168.0, 169.2. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_6H_{11}NO_2$ 129.0790, found 129.0816 (Δ = +2.6 mmu). Mp: 40 °C.

***tert*-Butyldimethyl-(pent-4-enyloxy)silane (58).** To an ice-cooled solution of pent-4-en-1-ol (500 mg, 5.80 mmol, 1.0 equiv) in dry DMF (20 mL) were added *tert*-butyldimethylsilyl chloride (1.40 g, 9.30 mmol, 1.6 equiv) and imidazole (643 mg, 9.40 mmol, 1.6 equiv). The mixture was stirred at room temperature for 5 h, diluted in water (50 mL), and then extracted with diethyl ether (3 × 100 mL). The combined organic extracts were washed with H_2O (3 × 200 mL), dried over $MgSO_4$, and evaporated to afford protected alcohol **58** as light yellow liquid (1.15 g, 5.74 mmol, 99%). TLC: R_f = 0.87 (ethyl acetate/hexane = 1:2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.06 (s, 6H), 0.91 (s, 9H), 1.62 (quin, J = 7.0 Hz, 2H), 2.11 (q, J = 7.2 Hz, 2H), 3.63 (t, J = 6.5 Hz, 2H), 4.96 (d, J = 11.2 Hz, 1H), 5.02 (d, J = 17.8 Hz, 1H), 5.83 (ddt, J = 6.6 Hz, 10.3 Hz, 17.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.3, 18.3, 26.0, 30.0, 32.0, 62.5, 114.5, 138.5. HR-MS (EI-TOF) calculated for $[M - (t\text{-Bu})]^+$ = $C_7H_{15}OSi$: 143.0892, found 143.0903 (Δ = +1.1 mmu). The data are in accordance with the literature.[133]

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(*E*)-*N*-((*E*)-6-((*tert*-Butyldimethylsilyl)oxy)hex-2-en-1-yl)-3-methoxy-*N*-methylbut-2-enamide (59). *Procedure A.* The reaction was performed in a flame-dried, 25 mL, round-bottom flask equipped with a condenser under an argon atmosphere. Amide **57** (169 mg, 1.00 mmol, 1.0 equiv), protected alcohol **58** (200 mg, 1.00 mmol, 1.0 equiv), and 2,6-dichloro-1,4-benzochinone (17.7 mg, 0.10 mmol, 10 mol %) were dissolved in dichloromethane (20 mL). First-generation Grubbs catalyst (206 mg, 0.25 mmol, 30 mol %) was added in portions. The mixture was heated at 50 °C and stirred overnight and then concentrated by rotary evaporation. The resulting crude product in the remaining slurry was purified by column chromatography on silica gel with ethyl acetate/hexane = 1:2 as eluent, which yielded the desired compound (102 mg, 0.30 mmol, 30%, 71% brsm) together with 96 mg of amide **59** as dark green oil.

Procedure B. Sodium hydride (60% dispersion in mineral oil, 715 mg, 17.9 mmol, 3.5 equiv) was suspended in dry DMF (6 mL) and cooled to 0 °C, and a solution of amide **56** (792 mg, 6.13 mmol, 1.2 equiv) in dry DMF (12 mL) was added. The mixture was allowed to warm to room temperature and stirred for 1.5 h. It was then cooled to 0 °C again, and allyl bromide **61** (1.50 g, 5.11 mmol, 1.0 equiv) in dry DMF (12 mL) was added. After additional stirring at room temperature for 2 h, the reaction mixture was ice-cooled, diluted with diethyl ether (30 mL), and quenched by addition of water (90 mL). The aqueous phase was extracted with diethyl ether (3 × 70 mL), and the combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography (ethyl acetate/petroleum ether 1:5−1:2) yielded the title compound **59** (1.16 g, 3.40 mmol, 67%) as slight yellow liquid. TLC: R_f = 0.27 (ethyl acetate/petroleum ether 1:2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.05 (s, 6H), 0.90 (s, 9H), 1.59 (tt, J = 7.3, 6.5 Hz, 2H), 2.11 (td, J = 7.3, 6.8 Hz, 2H), 2.22 (s, 3H), 2.94 (s, 3H), 3.61 (m, 5H), 3.88 (bs, 2H)*, 3.96 (bs, 2H)#, 5.18 (s, 1H), 5.41 (dt, J = 15.4 Hz, 5.6 Hz, 1H), 5.59 (dt, J = 15.4 Hz, 6.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.4 (2C), 18.3, 18.7, 25.9 (3C), 28.4, 32.3, 33.2*, 34.9#, 49.0#, 52.2*, 54.8, 62.3, 91.2, 124.7*, 125.4#, 132.7*, 133.3#, 167.8, 168.4. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_{18}H_{35}NO_3Si$ 341.2386, found 341.2415 (Δ = +2.9 mmu).

(*E*)-(6-Bromohex-4-enyloxy)-*tert*-butyldimethylsilane (61). Alkene **58** (802 mg, 4.00 mmol, 4.0 equiv) was dissolved in dry DCM (15 mL). Allyl bromide **60** (121 mg, 1.00 mmol, 1.0 equiv) and Grubbs catalyst second generation (17.0 mg, 0.02 mmol, 0.02 equiv) were added and the solution was stirred 15 h at 50 °C. The solvent was then removed *in vacuo* and the residue was purified by flash column chromatography (pure petroleum ether to ethyl acetate/petroleum ether 1:40) to give the title compound **59** (283 mg, 0.96 mmol, 96%) as colorless liquid. TLC: R_f = 0.45 (ethyl acetate/petroleum ether 1:20). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.06 (s, 6H), 0.90 (s, 9H), 1.62 (m, 2H), 2.14 (td, J = 7.3, 6.8 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H), 3.96 (d, J = 6.9 Hz, 2H), 5.76 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = −5.3 (2C), 18.3, 25.9 (3C), 28.4, 31.9, 33.5, 62.3, 126.6, 136.2. HR-MS (EI-TOF) calculated for $[M\text{-}(t\text{-Bu})]^+$ = $C_8H_{16}{}^{79}BrOSi$: 235.0154, found 235.0174 (Δ = +2.0 mmu).

Methyl (*E*)-hept-2-en-6-ynoate (62b). Oxalyl chloride (3.52 mL, 41.0 mmol, 1.15 equiv) was dissolved in dry CH_2Cl_2 (120 mL) and cooled to −78 °C, and a solution of DMSO (6.33 mL, 89.2 mmol, 2.5 equiv) in dry CH_2Cl_2 (12 mL) was added within 5 min. The mixture was stirred at this temperature for 30 min before a solution of 5-pentyn-1-ol **62** (3.0 g, 35.7 mmol, 1.0 equiv) in dry CH_2Cl_2 (10 mL) was added dropwise. After being stirred for 1 h, the mixture was treated with dry NEt_3 (19.8 mL, 142 mmol, 4.0 equiv), and stirring was continued at −78 °C for 30 min before the mixture was warmed slowly to rt over 0.5 h. Then water (50 mL) was added, the phases were separated, and the aqueous phase was extracted with 3 × 30 mL CH_2Cl_2. The combined organic layers were washed with brine (2 × 30 mL) and dried over $MgSO_4$, and the solvent was evaporated carefully under reduced pressure to yielded 86% of the crude aldehyde **62a** (2.52 g, 30.7 mmol) as a pale yellow liquid which was used in the next reaction without further purification. TLC: R_f = 0.35 (petroleum ether/ethyl acetate = 9:1).

Crude aldehyde **62a** (2.52 g, 30.7 mmol, 1.0 equiv) was immediately dissolved in dry CH_2Cl_2 (70 mL) and treated with methyl (triphenylphosphoranylidene)acetate (12.0 g, 36.0 mmol, 1.2 equiv) at rt. After the mixture was stirred overnight (13 h), a saturated solution of NH_4Cl (30 mL) was added, the organic layer was separated, and the mixture was extracted with 3 × 25 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Flash column chromatography on silica gel (petroleum ether/ethyl acetate 50:1−15:1) gave 91% of ester **62b** as a colorless liquid (3.76 g, 27.2 mmol). TLC: R_f = 0.32 (petroleum ether/ethyl acetate = 15:1). ^{1}H NMR ($CDCl_3$, 300 MHz): 2.00 (1 H, t, J = 2.5 Hz), 2.28−2.38 (2 H, m), 2.38−2.49 (2 H, m), 3.74 (3 H, s), 5.90 (1 H, dt, J = 15.7 Hz, 1.4 Hz), 6.98 (1 H, dt, J = 15.7 Hz, 6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 17.4, 31.0, 51.5, 69.4, 82.6, 122.1, 146.6, 166.7. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_8H_{10}O_2$ 138.0681, found 138.0660 (Δ = −2.1 mmu). The data are in accordance with the literature.[134]

(*E*)-Hept-2-en-6-yn-1-ol (63). A stirred solution of ester **142b** (1.70 g, 12.3 mmol, 1.0 equiv) in dry CH_2Cl_2 (60 mL) was treated dropwise with a solution of DIBAl-H (1.0 M in CH_2Cl_2, 30.7 mL, 30.72 mmol, 2.5 equiv) at −78 °C. After the solution was stirred for 1 h at −78 °C, TLC control indicated completion of the reaction, the cooling bath was removed, and the reaction mixture was carefully poured into a saturated solution of potassium sodium tartrate (150 mL). Et_2O (80 mL) was added, and the mixture was stirred vigorously until two phases appeared (2 h). Extraction with 3 × 100 mL of Et_2O, drying over $MgSO_4$, and evaporation of the solvent afforded crude alcohol **143** (1.36 g, 12.3 mmol) in quantitative yield, which was used in the following reaction without further purification. TLC: R_f = 0.24 (petroleum ether/ethyl acetate = 5:1). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.53 (1 H, br s), 1.98 (1 H, t, J = 2.6 Hz), 2.26−2.30 (4 H, m), 4.09−4.17 (2 H, m), 5.71−5.77 (2 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.4, 31.1, 63.5, 68.7, 83.7, 130.5, 130.6. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_7H_{10}O$ 110.0732, found 110.0710 (Δ = −2.2 mmu).[134]

(*E*)-*N*-Methylhept-2-en-6-yn-1-amine (64). To a solution of alcohol **63** (600 mg, 5.45 mmol, 1.0 equiv) and CBr_4 (2.17 g, 6.54 mmol, 1.2 equiv) in dry CH_2Cl_2 (40 mL) at 0 °C was added triphenylphosphine (1.71 g, 6.54 mmol, 1.2 equiv) in three portions over a period of 15 min. After 1 h at 0 °C, the resultant mixture was allowed to warm to rt and stirred for an additional 1 h. Then the reaction mixture was adsorbed on silica gel (30 g), the solvents were carefully removed under reduced pressure, and the crude product was purified by flash column chromatography on silica gel (pure pentane) to afford bromide **63a** (810 mg, 4.68 mmol) in 86% yield as a colorless oil. TLC: R_f = 0.23 (pentane).

Compound **63a** (500 mg, 2.89 mmol, 1.0 equiv) was dissolved in dry THF (5 mL) and cooled to 0 °C, and a solution of methylamine in THF (2.0 M, 14.5 mL, 28.9 mmol, 10.0 equiv) was added dropwise over a period of 10 min. After 1 h at 0 °C, the resultant mixture was allowed to warm to rt and stirred for additional 3 h. The solvents and excess methylamine were removed under reduced pressure, and the crude product was purified by flash column chromatography on neutral Al_2O_3 (CH_2Cl_2/Et_2O 97:3) to afford amine **64** (292 mg, 2.37 mmol) in 82% yield as a yellow oil. TLC: R_f = 0.27 (Al_2O_3 CH_2Cl_2/Et_2O 97:3). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.85 (1 H, s), 1.96 (1 H, t, J = 2.6 Hz), 2.24−2.30 (4 H, m), 2.43 (3 H, s), 3.19 (2 H, d, J = 5.0 Hz), 5.53−5.73 (2 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.6, 31.3, 35.6, 53.4, 68.6, 83.9, 129.3, 130.7. HR-MS (EI-TOF): calcd for $[M]^+$ = $C_8H_{13}N$ 123.1048, found 123.1058 (Δ = +1.0 mmu).

(*E*)-*N*-((*E*)-Hept-2-en-6-ynyl)-3-methoxy-*N*-methylbut-2-enamide (65). *Synthesis of Compound **65** via Ohira−Bestmann Homologation of Aldehyde **66**.* To a solution of K_2CO_3 (202 mg, 1.46 mmol, 2.2 equiv) and aldehyde **66** (150 mg, 0.67 mmol, 1.0 equiv) in dry methanol (10 mL) at rt was added a solution of dimethyl 1-diazol-2-oxopropylphosphonate (166 mg, 0.87 mmol, 1.3 equiv) in dry methanol (1.5 mL). After being stirred for 5 h, the reaction mixture was diluted with Et_2O (10 mL), washed with a solution of $NaHCO_3$ (5%, 10 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum

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ether/ethyl acetate 1:1) to yield the desired alkene **65** (131 mg, 0.59 μmol, 89%) as colorless oil.

*Synthesis of Compound **65** via Amide Coupling of Amine **64**.* Acid **55** (94 mg, 0.81 mmol, 1.0 equiv) and amine **64** (100 mg, 0.81 mmol, 1.0 equiv) were dissolved in dry THF (7 mL). Then dry NEt_3 (0.56 mL, 4.06 mmol, 5.0 equiv) was added followed by DEPBT (361 mg, 1.22 mmol, 1.5 equiv) at rt. The yellow solution was stirred at rt overnight (14 h) before a saturated solution of NH_4Cl (15 mL) was added. The mixture was extracted with 3 × 20 mL of EtOAc, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. After purification by flash column chromatography on silica gel (petroleum ether/ethyl acetate 1:1), **65** (148 mg, 0.67 mmol) was obtained as a colorless oil in 82% yield. TLC: R_f = 0.43 (petroleum ether/ethyl acetate = 1:1). ^{1}H NMR (acetone-d_6, 400 MHz): δ = 2.14 (3 H, s), 2.26 (4 H, m), 2.34 (1 H, s), 2.87 (3 H, br s)*, 2.97 (3 H, br. s)#, 3.62 (3 H, br. s), 3.95 (2 H, d, J = 5.1 Hz), 5.34 (1 H, br s), 5.42−5.60 (1 H, m), 5.66 (1 H, dt, J = 15.3 Hz, 6.1 Hz). ^{13}C NMR (acetone-d_6, 100 MHz): δ = 17.8, 18.1, 31.1, 32.4*, 34.1#, 48.2#, 51.5*, 54.4, 69.4, 83.4, 91.3, 126.9*, 127.1#, 130.5*, 131.2#, 166.8#, 167.1*, 167.7*, 168.0#. HR-MS (ESI-TOF, HPmix): calcd for $[M + Na]^+$ = $C_{13}H_{19}NO_2Na$ 244.1308, found 244.1308 (Δ = 0 mmu).

(*E*)-*N*-((*E*)-6-Hydroxyhex-2-en-1-yl)-3-methoxy-*N*-methylbut-2-enamide (59a). To an ice-cooled solution of the TBS-protected alcohol **59** (4.00 g, 11.7 mmol, 1.0 equiv) in THF (50 mL) was added TBAF (1 M in THF, 93.7 mL, 93.7 mmol, 8.0 equiv). The mixture was allowed to warm to rt over a period of 16 h. Then water (100 mL) was added, and the aqueous phase was extracted with 3 × 75 mL of EtOAc, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (pure ethyl acetate) afforded alcohol **59a** (2.39 g, 10.5 mmol) in 90% yield as yellow oil. TLC: R_f = 0.16 (ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.65 (2 H, quin, J = 6.9 Hz), 2.15 (2 H, q, J = 7.0 Hz), 2.21 (3H, s), 2.94 (3 H, s), 3.59 (3 H, s), 3.65 (2 H, t, J = 6.5 Hz), 3.88 (2 H, br s)*, 3.95 (2 H, br s)#, 5.17 (1 H, s), 5.44 (1 H, dt, J = 15.3 Hz, 5.6 Hz), 5.60 (1 H, dt, J = 15.4 Hz, 6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.7, 28.5, 32.0, 33.3*, 35.0#, 49.0#, 52.1*, 54.8, 62.0, 91.1, 124.9*, 125.5#, 132.5*, 133.0#, 167.9, 168.5. HR-MS (ESI-TOF): calcd for $[M + H]^+$ = $C_{12}H_{22}NO_3^+$ 228.1600, found 228.1594 (Δ = −0.6 mmu).

(*E*)-3-Methoxy-*N*-methyl-*N*-((*E*)-6-oxohex-2-en-1-yl)but-2-enamide (66). Alcohol **59a** (521 mg, 2.29 mmol, 1.0 equiv) was dissolved in dry DMSO (15 mL), and IBX (1.92 g, 6.87 mmol, 3.0 equiv) was added. The mixture was stirred for 4 h at room temperature, and DCM (150 mL) was added. After additional stirring for 30 min, saturated aqueous sodium hydrogen carbonate solution (100 mL) was added. The organic phase was separated and washed a second time with saturated aqueous sodium hydrogen carbonate solution (100 mL). The combined aqueos phases were extracted with DCM (60 mL), the combined organic phases were dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was then purified by flash column chromatography (pure ethyl acetate) to give the aldehyde **66** (475 mg, 2.11 mmol, 92%) as a yellow liquid. TLC: R_f = 0.35 (ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.21 (3 H, s), 2.39 (2 H, q, J = 6.4 Hz), 2.54 (2 H, tt, J = 7.0 Hz, 1.5 Hz), 2.93 (3 H, s), 3.60 (3 H, s), 3.89 (2 H, s)*, 3.95 (2 H, s)#, 5.17 (1 H, br s), 5.46 (1 H, dt, J = 5.4 Hz, 15.5 Hz), 5.58 (1 H, dt, J = 15.4, 6.2 Hz), 9.77 (1 H, t, J = 1.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.6, 24.6, 33.3*, 35.0#, 43.0, 48.8#, 51.9*, 54.7, 91.0, 126.0*, 126.5#, 130.5*, 130.9#, 167.8, 168.6, 201.3*, 201.7#. HR-MS (ESI-TOF): calcd for $[M + H]^+$ = $C_{12}H_{20}NO_3^+$ 226.1443, found 226.1436 (Δ = −0.7 mmu).

(*E*)-3-Methoxy-*N*-methyl-*N*-((2*E*,6*Z*)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hepta-2,6-dienyl)but-2-enamide (27). To a mixture of $[Rh(COD)Cl]_2$ (6.3 mg, 13 μmol, 0.03 equiv) and P-*i*-Pr_3 (12 μL, 9.7 mg, 61 μmol, 0.14 equiv) in cyclohexane (1.5 mL) were added NEt_3 (300 μL, 2.16 mmol, 5.0 equiv) and pinacolborane (63 μL, 55 mg, 432 μmol, 1.0 equiv). The resultant yellow mixture was stirred at rt for 30 min. To this mixture was added a solution of alkyne **65** (110 mg, 497 μmol, 1.15 equiv) in cyclohexane (1.0 mL). After being stirred at rt for 5 h, the mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/ethyl acetate 5:1 to 3:1 to 1:1) gave (*Z*)-vinyl boronate **27** (109 mg, 312 μmol, 72%, *Z*/*E* > 9:1) as a colorless oil. TLC: R_f = 0.59 (petroleum ether/ethyl acetate = 1:1). ^{1}H NMR (acetone-d_6, 600 MHz): δ = 1.15 (12 H, s), 2.05 (5 H, br. m), 2.40 (2 H, q, J = 7.2 Hz), 2.76 (3 H, br s)*, 2.86 (3 H, br s)#, 3.52 (3 H, br s), 3.83 (2 H, d, J = 5.8 Hz), 5.20 (1 H, d, J = 13.5 Hz), 5.25 (1 H, br s), 5.45−5.56 (2 H, m), 6.31 (1 H, dt, J = 13.4, 7.0 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ = 18.8[a], 19.1[b], 25.2[b], 25.3[a], 32.6[c], 33.0[c], 33.4[c#], 35.0[c]*, 49.3[c]*, 52.6[c#], 55.3[a], 55.4[b], 83.6[a], 84.2[b], 92.3[c], 119.6 (m)[c], 131.4[b], 132.1[b], 132.7[a#], 132.9[a]*, 133.4[a#], 133.5[a]*, 154.5[c], 167.7[c#], 168.1[c]*, 168.6[c]*, 168.9[c#]. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{19}H_{33}BNO_4$ 350.2501, found 350.2496 (Δ = −0.5 mmu).

(*E*)-*N*-((2*E*,6*Z*)-7-Iodohepta-2,6-dienyl)-3-methoxy-*N*-methylbut-2-enamide (26). The whole reaction was carried out in the absence of light. To a suspension of iodomethyltriphenylphosphonium iodide (684 mg, 1.29 mmol, 1.5 equiv) in dry THF (3.3 mL) was slowly added sodium hexamethyldisilazane (1 M in THF, 1.29 mL, 1.29 mmol, 1.5 equiv) at room temperature. After being stirred for 1 min, the mixture was cooled to −60 °C, and DMPU (0.78 mL, 6.46 mmol, 7.5 equiv) was slowly added. The reaction mixture was then cooled to −80 °C, and aldehyde **66** (194 mg, 861 μmol, 1.0 equiv) dissolved in THF (1.5 mL) was then added slowly by allowing it to run down the wall of the cold flask. After the mxiture was stirred at this temperature for 1 h, saturated aqueous ammonium chloride solution (30 mL) was added. The precipitated solid was filtered off, and the filtrate was extracted with diethyl ether (3 × 30 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by flash column chromatography (ethyl acetate/petroleum ether 1:1) to yield vinyl iodide **26** (201 mg, 576 μmol, 67%) as a yellow liquid. TLC: R_f = 0.37 (ethyl acetat/petroleum ether 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.20 (m, 7H), 2.93 (s, 3H), 3.58 (s, 3H), 3.88 (s, 2H)*, 3.95 (s, 2H)#, 5.15 (s, 1H), 5.43 (dt, J = 15.4, 5.4 Hz, 1H), 5.57 (m, 1H), 6.14 (dt, J = 7.3, 5.9 Hz, 1H), 6.21 (d, J = 7.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75.56 MHz): δ = 18.7, 30.4, 33.4*, 34.2, 35.1#, 48.9#, 52.0*, 54.8, 82.8, 83.0, 91.1, 125.6*, 126.2#, 131.5*, 131.9#, 140.1, 140.4, 167.8, 168.5. HR-MS (ESI-TOF): calcd for $[M + H]^+$ = $C_{13}H_{21}INO_2$ 350.0617, found 350.0610 (Δ = −0.7 mmu).

(3*R*,4*S*)-4,8-Bis((*tert*-butyldimethylsilyl)oxy)-3-((*R*)-1-(2-((*R*)-5-iodopent-4-yn-2-yl)oxazol-4-yl)propan-2-yl)-7-methylisochroman-1-one (53a). Alkyne **53** (35 mg, 58 μmol, 1.0 equiv) was dissolved in acetone (0.5 mL) followed by the addition of EtOH (18 μL, 292 μmol, 5.0 equiv), $AgNO_3$ (11 mg, 64 μmol, 1.1 equiv), and NIS (17 mg, 76 μmol, 1.3 equiv). The resulting suspension was stirred vigorously in the dark at rt for 4 h. The solvent was removed, and the residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 9:1) to afford alkynyl iodide **53a** as a light yellow oil (37 mg, 51 μmol, 88% yield). TLC: R_f = 0.45 (petroleum ether/ethyl acetate = 9:1). $[\alpha]_D^{23}$ = 38.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.00 (3 H, s), 0.16 (3 H, s), 0.17 (3 H, s), 0.21 (3 H, s), 0.87 (9 H, s), 0.94 (3 H, d, J = 6.8 Hz), 1.06 (9 H, s), 1.42 (3 H, d, J = 7.0 Hz), 1.96−2.05 (1 H, m), 2.27 (3 H, s), 2.56 (1 H, dd, J = 14.6 Hz, 9.0 Hz), 2.69 (1 H, dd, J = 16.7 Hz, 7.9 Hz), 2.82−2.93 (2 H, m), 3.18 (1 H, sxt, J = 6.7 Hz), 4.20 (1 H, dd, J = 8.3 Hz, 3.8 Hz), 4.75 (1 H, d, J = 3.6 Hz), 6.86 (1 H, d, J = 7.5 Hz), 7.34 (1 H, d, J = 7.6 Hz), 7.37 (1 H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −4.4, −4.2, −3.5, −3.4, 15.9, 17.4, 17.5, 18.0, 18.6, 25.7 (3 C), 26.0 (3 C), 26.5, 27.8, 29.7, 33.2, 33.7, 67.5, 86.6, 91.3, 116.2, 119.4, 131.9, 135.5, 135.6, 137.4, 139.0, 154.8, 161.9, 166.0. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{33}H_{51}O_5NSi_2I$ 724.2345, found 724.2351 (Δ = +0.6 mmu).

(3*R*,4*S*)-4,8-Bis(*tert*-butyldimethylsilyloxy)-3-((*R*)-1-(2-((*R*,*Z*)-5-iodopent-4-en-2-yl)oxazol-4-yl)propan-2-yl)-7-methylisochroman-1-one (70). *o*-Nitrobenzenesulfonylhydrazide (NBSH, **69**) (19 mg, 87 μmol, 2.1 equiv) was added to a solution of alkyne iodide **53a** (30 mg, 41 μmol, 1.0 equiv) in THF/*i*-PrOH (1:1, 1.0 mL) at 0 °C in the dark. Then NEt_3 (19 μL, 145 μmol, 3.5 equiv) was added, and the mixture was allowed to warm to rt overnight (14 h). After evaporation of the solvents, the resulting crude yellow oil was purified by flash column chromatography (petroleum ether/ethyl acetate = 9:1) to afford the title compound **70** (29 mg, 40 μmol) as a yellow

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colorless oil in 96% yield. TLC: R_f = 0.52 (petroleum ether/ethyl acetate = 9:1). $[\alpha]_D^{23}$ = 35.0 (*c* 1.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.00 (3 H, s), 0.16 (3 H, s), 0.16 (3 H, s), 0.20 (3 H, s), 0.87 (9 H, s), 0.92 (3 H, d, *J* = 6.7 Hz), 1.05 (9 H, s), 1.34 (3 H, d, *J* = 7.1 Hz), 1.96−2.04 (1 H, m), 2.27 (3 H, s), 2.43−2.54 (2 H, m), 2.57 (1 H, dtd, *J* = 14.6 Hz, 6.8 Hz, 1.5 Hz), 2.88 (1 H, dd, *J* = 14.6 Hz, 2.8 Hz), 3.09 (1 H, sxt, *J* = 7.0 Hz), 4.21 (1 H, dd, *J* = 8.4 Hz, 3.7 Hz), 4.75 (1 H, d, *J* = 3.8 Hz), 6.15 (1 H, q, *J* = 7.0 Hz), 6.27 (1 H, dt, *J* = 7.4 Hz, 1.3 Hz), 6.85 (1 H, d, *J* = 7.6 Hz), 7.32 (1 H, s), 7.34 (1 H, d, *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = −4.5, −4.2, −3.5, −3.4, 15.9, 17.5, 17.9, 18.1, 18.6, 25.7 (3 C), 26.0 (3 C), 28.2, 32.7, 33.7, 39.9, 67.5, 84.4, 86.6, 116.3, 119.4, 131.9, 135.0, 135.6, 137.8, 138.2, 139.0, 154.7, 161.9, 166.6. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{33}H_{53}O_5NSi_2I$ 726.2502, found 726.2511 (Δ = +0.9 mmu).

8-(*tert*-Butylsilyl)ajudazol B (71a). To a mixture of $Ba(OH)_2 \cdot (H_2O)_8$ (32 mg, 103 μmol, 5.0 equiv) and $Pd(dppf)Cl_2$ (2.2 mg, 3.0 μmol, 0.15 equiv) in degassed DMF (0.5 mL) was added (*Z*)-vinylboronate **27** (10.8 mg, 31.0 μmol, 1.5 equiv) followed after stirring for 10 min by vinyl iodide **70** (15 mg, 21 μmol, 1.0 equiv). The resultant mixture was stirred at rt for 20 h, treated with Et_2O (5 mL), and washed with 3 × 5 mL of water. The aqueous phase was extracted with 3 × 5 mL of Et_2O, and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate = 1:1) afforded a mixture (1:10) of bis-TBS-protected and mono-TBS-protected ajudazol B (**71a**) (11.4 mg, 16.0 μmol) in 78% yield as colorless oils. TLC: R_f = 0.45 (petroleum ether/ethyl acetate = 1:1). $[\alpha]_D^{26}$ = +32.2 (*c* 1.0, MeOH). ^{1}H NMR (acetone-d_6, 600 MHz):δ = 0.04 (3 H, s), 0.23 (3 H, s), 0.87 (9 H, s), 0.91 (3 H, d, *J* = 6.9 Hz), 1.26 (3 H, d, *J* = 7.0 Hz), 2.02−2.06 (1 H, m), 2.10−2.17 (2 H, m), 2.14 (3 H, s), 2.20−2.28 (2 H, m), 2.24 (3 H, s), 2.41−2.53 (2 H, m), 2.53−2.65 (1 H, m), 2.82 (3 H, br. s)*, 2.82−2.89 (1 H, m), 2.92 (3 H, br s)#, 2.92−2.99 (1 H, m), 3.61 (3 H, br s), 3.92 (2 H, d, *J* = 5.0 Hz), 4.61 (1 H, dd, *J* = 8.7 Hz, 2.8 Hz), 5.04 (1 H, d, *J* = 2.9 Hz), 5.33−5.43 (1 H, m), 5.35 (1 H, br s), 5.37−5.47 (2 H, m), 5.54−5.68 (1 H, m), 6.24 (1 H, dd, *J* = 11.7 Hz, 10.3 Hz), 6.31 (1 H, dd, *J* = 11.3 Hz, 9.9 Hz), 6.97 (1 H, d, *J* = 7.7 Hz), 7.48 (1 H, d, *J* = 7.3 Hz), 7.55 (1 H, s), 11.33 (1 H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ = −4.2, −4.0, 15.7, 16.3, 18.4, 18.8, 19.1, 26.2 (3 C), 28.0, 29.3, 32.9, 33.4#, 33.6, 34.7, 34.7*, 34.9, 49.3#, 52.5*, 55.4, 67.4, 89.8, 92.3, 108.0, 118.9, 124.8, 126.3, 127.0*, 127.2#, 127.7, 129.3, 132.4, 132.6, 136.1, 137.9, 138.6, 138.9, 160.8, 168.1, 168.7, 168.9, 169.4. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{40}H_{59}O_7N_2Si$ 707.4086, found 707.4096 (Δ = +1.0 mmu).

(+)-Ajudazol B (2). To a solution of TBS-protected ajudazol B **71** (10.0 mg, 14.0 μmol, 1.0 equiv) in DMF (0.5 mL) and H_2O (5 μL, 282 μmol, 20 equiv) was added tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF, 1.5 M in DMF, 94 μL, 141 μmol, 10.0 equiv). The reaction was monitored by TLC until starting material was consumed (3 h). The reaction mixture was then diluted with EtOAc (3 mL) and washed with pH 7 buffer (3 × 3 mL). The aqueous layer was extracted with 3 × 5 mL of EtOAc, and the combined organic layers were washed again with pH 7 buffer (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford 95% (8.0 mg, 13.5 μmol) of ajudazol B (**2**), which was further purified for an analytical pure sample by preparative HPLC (MeOH/H_2O 85:15, 100 RP C-18, flow: 15.0 mL/min, pressure: 161 bar, retention time: 11.13 min). TLC: R_f = 0.11 (petroleum ether/ethyl acetate 1:1). $[\alpha]_D^{21}$ = +7.9 (*c* 0.9, MeOH). ^{1}H NMR (acetone-d_6, 600 MHz): δ = 1.06 (3 H, d, *J* = 6.6 Hz), 1.29 (3 H, d, *J* = 6.9 Hz), 2.10−2.16 (2 H, m), 2.14 (3 H, s), 2.22 (3 H, s), 2.22−2.27 (2 H, m), 2.42−2.47 (1 H, m), 2.47−2.49 (1 H, m), 2.49−2.53 (1 H, m), 2.61 (1 H, dtd, *J* = 14.4 Hz, 7.3 Hz, 1.2 Hz), 2.84 (3 H, br s)*, 2.89 (1 H, ddd, *J* = 14.5 Hz, 4.4 Hz, 1.2 Hz), 2.94 (3 H, br s)#, 3.03 (1 H, tq, *J* = 7.0 Hz), 3.60 (3 H, br s), 3.92 (2 H, d, *J* = 5.5 Hz), 4.43 (1 H, dd, *J* = 8.3, 4.1 Hz), 4.96 (1 H, d, *J* = 8.5 Hz), 5.33 (1 H, br s), 5.38−5.40 (1 H, m), 5.41−5.44 (1 H, m), 5.44−5.49 (1 H, m), 5.60 (1 H, dtd, *J* = 15.2 Hz, 6.4, Hz, 1.5 Hz), 6.24 (1 H, ddd, *J* = 11.6 Hz, 10.4 Hz, 1.5 Hz), 6.29 (1 H, ddd, *J* = 11.9 Hz, 10.7 Hz, 1.3 Hz), 7.07 (1 H, dd, *J* = 7.4 Hz, 1.0 Hz), 7.47 (1 H, dd, *J* = 7.6 Hz, 1.3 Hz), 7.61 (1 H, d, *J* = 1.3 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ = 15.5, 17.0, 18.4, 18.8, 27.9, 28.0, 32.9, 33.2#, 33.6, 34.0, 34.3*, 34.8, 49.1#, 52.3*, 55.4, 65.4, 88.2, 92.3, 107.3, 116.6, 124.7, 126.3, 126.4, 127.0, 129.1, 132.2, 132.5, 135.9, 138.1, 139.5, 142.2, 160.7, 167.9, 168.3, 168.7, 170.4. HR-MS (ESI-TOF, HPmix): calcd for $[M + H]^+$ = $C_{34}H_{45}O_7N_2$ 593.3221, found 593.3222 (Δ = +0.1 mmu).

Experimental Biological Procedures. The analysis of cell viability of HL-60 and PBMC using MTT assay was performed as described.[135] The release of interleukin-1β, -6, and -8 as well as tumor necrosis factor (TNF) α from lipopolysaccharide-activated human monocytes was conducted according to ref 136. The activities of cyclooxygenases and mPGES-1 in cell-free or cell-based assays were analyzed as described in ref 137. Expression and purification of human recombinant 5-LO was performed as reported in ref 137. The isolated 5-LO was preincubated with ajudazol B for 10 min at 4 °C and prewarmed for 30 s at 37 °C. 5-LO product formation was initiated by addition of 2 mM $CaCl_2$ and 20 μM arachidonic acid. After 10 min at 37 °C, the reaction was terminated by addition of 1 mL of ice cold methanol. Formed 5-LO metabolites (all-trans isomers of LTB_4 and 5-H(P)ETE) were analyzed by RP-HPLC according to ref 138. Lipoxygenase activities in human neutrophils were determined as previously reported. In brief, neutrophils (1 × 10^7) isolated from peripheral blood of adult healthy donors[137] were preincubated with ajudazol B for 15 min at 37 °C. Then, 2.5 μM Ca^{2+}-ionophore A23187 plus 20 μM arachidonic acid were added. The reaction was stopped after 10 min at 37 °C with 1 mL of methanol. Major 5-LO (LTB_4 and its all-trans isomers, and 5-H(P)ETE), 12-lipoxygenase (12-HETE), and 15-lipoxygenase metabolites (15-HETE) were extracted and analyzed by HPLC as described in ref 137. Statistics, biological: Data are expressed as mean ± SEM of single determinations performed in three or four independent experiments on different days. IC_{50} values obtained from at least four different compound concentrations were calculated by nonlinear regression using SigmaPlot 9.0 (Systat Software Inc., San Jose, CA) one-site binding competition. Statistical evaluation of the data was performed by one-way ANOVA followed by a Bonferroni post-hoc test. A *p* value < 0.05 (*) was considered significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02844.

Full bioinformatics alignments, copies of ^{1}H NMR, ^{13}C NMR, and CD spectra (PDF)

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Notes

The authors declare no competing financial interest.

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