Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 62 (2006) 10528–10540
Tetrahedron

Studies directed toward the synthesis of the scabrosins: validation of a tandem enyne metathesis approach

Mark D. Middleton, Brian P. Peppers and Steven T. Diver*

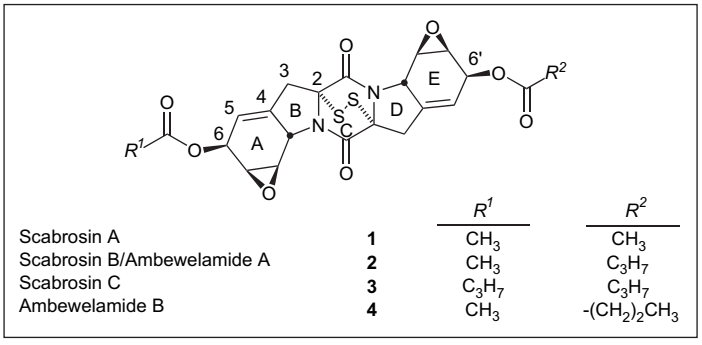
Department of Chemistry, University at Buffalo, State University of New York, Buffalo, NY 14260-3000, USA

Received 20 January 2006; revised 6 May 2006; accepted 10 May 2006
Available online 17 August 2006

Abstract—A synthetic approach to the scabrosin family of antibiotics using a ruthenium carbene-catalyzed tandem metathesis and a Pd(II)-catalyzed cyclization is described. The chiral propargyl amino acid is furnished through enantioselective phase-transfer propargylation. The synthesis of the cyclohexadiene ring system is achieved through ring synthesis using tandem enyne metathesis, previously developed in our lab. The complementary methods of methylene-free and 1,5-hexadiene-alkyne metatheses are compared. The indoline heterocycles are formed using a two-step chloroacetoxylation (Bäckvall reaction) with subsequent nucleophilic attack by an amide nucleophile. The indoline subunits were joined and cyclized to furnish the core diketopiperazine ring. The stereochemical assignment of intermediates is also discussed.

1. Introduction

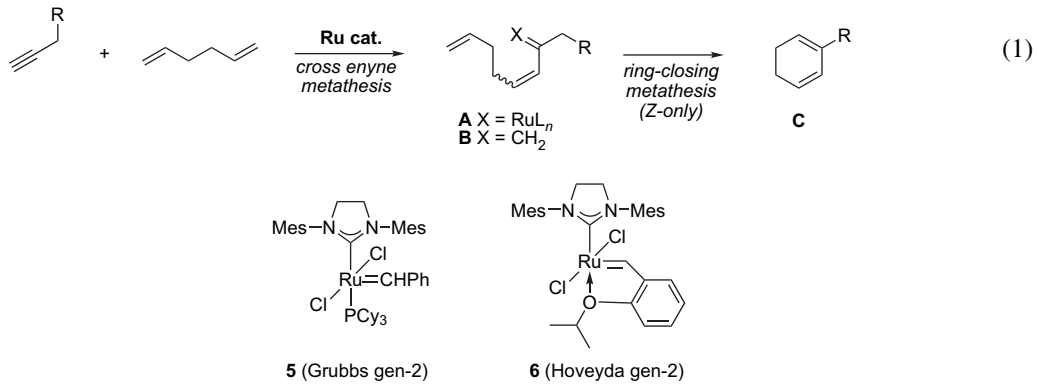
The scabrosins are a group of epidithiadiketopiperazines (edtdkp) symmetrically flanked by two highly functionalized cyclohexene rings (Scheme 1). Scabrosins **1–3** and ambewelamides **2** and **4** share the same core pentacarbocyclic framework with the symmetrical disposition of epoxide functionality. Scabrosin A was isolated from a lichen *Xanthoparmelia scabrosa* isolated on a coastal cliff face in New South Wales, Australia; it features acetyl groups at the flanks and is symmetrical. The same pentacyclic carbocycle was isolated in 1998 by Williams et al. from a lichen *Usnea* sp. found on a rotting tree in Ambewela, Sri Lanka; these molecules, the ambewelamides, bear different acyl



Scheme 1. Scabrosin/ambewelamide group of epidithiadiketopiperazines.

Keywords: Enyne metathesis; Tandem metathesis; 1,3-Cyclohexadienes; Scabrosin; Epidithiadiketopiperazine; Grubbs' catalyst; Hoveyda catalyst; Bäckvall reaction.
* Corresponding author. Tel.: +1 716 645 6800x2201; fax: +1 716 645 6963; e-mail: diver@buffalo.edu

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.05.089

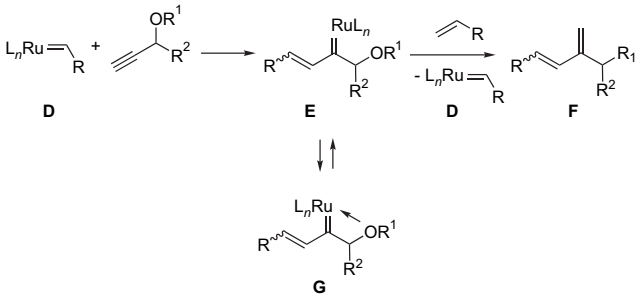


Scheme 2. Cyclohexadiene synthesis by tandem enyne metathesis.

substitution at the C6 and C6′ positions.[1] The best known relative of ambewelamides is gliotoxin. Additional fungal metabolites belonging to the edtdkp family have been isolated including aranotin,[2–4] epicorazine,[5] the rostratins,[6] and others. The central diketopiperazine ring is bridged by a disulfide. This latter functionality spanning the diketopiperazine core (the C-ring) gives this family of natural products their root name: epidithiadiketopiperazines. Of the natural products in Scheme 1, only gliotoxin has been synthesized despite an enormous amount of synthetic activity directed toward the delicate introduction of the disulfide moiety. Kishi and Fukuyama's synthesis of gliotoxin stands as a landmark achievement.[7,8] In this report, we detail our synthetic approach to the scabrosins relying on metal-catalyzed transformation. In particular, ring building of the A and E cyclohexene rings was accomplished through a Grubbs' ruthenium carbene-catalyzed tandem enyne metathesis, developed in our labs (Eq. 1) and the nitrogen heterocycle formed by Pd(II)-catalyzed Bäckvall reaction. The conjunction of the cyclohexadiene ring synthesis with the Pd(II) chemistry for 1,4-difunctionalization validates our approach and offers a powerful sequence of metal catalysis for synthesis (Scheme 2).

The ambewelamide/scabrosins have potential as anticancer agents. In general, the epidithiadiketopiperazine class of fungal natural products display a range of biological activities. For instance, aranotin inhibits viral RNA polymerase[9,10] and gliotoxin is a reverse transcriptase inhibitor.[11–13] However, the scabrosin/ambewelamide group displays unique cytotoxicity and therefore has potential as anticancer agents. This chemical biology is unique due to its dense functionality, the presence of the epidisulfide and because of the flanking epoxides. These factors combined are thought to give scabrosins their unique and potent anticancer activity.[1] Recent studies suggest that the epoxides may not be a major determinant in the unusual cytotoxicity observed for these agents.[14] Ambewelamide A is one of the best studied in the scabrosin family, with an acetyl and butanoyl group at the flanks. Ambewelamide A is toxic to cancer cell lines with an IC_{50} of 8.6 ng/mL (15 nM) for cytotoxicity against the murine P388 leukemia cell line.[1] Waring et al. have also shown potent cytotoxicity against the MCF7 human breast cancer cell line with an IC_{50} of 1 nM.[15] Moreover, tritiated thymidine incorporation was inhibited with an IC_{50} of 0.5 μM in the P815 mastocytomia cell line (gliotoxin gives IC_{50} of 2.9 μM in the same assay). Scabrosin A gives an IC_{50} of 0.56 μM in the same assay, showing identical inhibition profile as found for ambewelamide.[14,15] Because of their identical core structure and similar biological profiles, the ambewelamide/scabrosin family will be hereafter called scabrosins.

Metathesis has become a powerful method for carbon–carbon bond construction in synthesis.[16–18] Enyne metathesis is an adaptation of the parent reaction that provides conjugated dienes as products. In particular, the cross, or intermolecular, enyne metathesis[19] offers a simple coupling of unsaturated reactants to furnish diene products in a single catalytic operation. The power of metathetic processes in synthesis is amplified when metathesis reactions are used in tandem. Our group developed the tandem diene–alkyne metathesis as a cyclohexadiene ring synthesis as illustrated above (Eq. 1). This methodology is attractive because it generates a useful 2-substituted-1,3-cyclohexadiene from simple alkynes and diene starting materials. There are few methods for 1,3-cyclohexadiene synthesis and none that offer a direct, catalytic synthesis achieved on mixing of acyclic reactants. However, this tandem process, triggered by intermolecular enyne metathesis, is not stereoselective. The reaction between ruthenium carbene **D** and alkyne produces *E*- and *Z*-vinyl carbene isomers **E** (Scheme 3). The lack of *Z*-selectivity in intermolecular metathesis is a general problem in alkene metathesis research. Intermolecular enyne metatheses can be more difficult than the intramolecular ring-closing metathesis. The difficulty of the intermolecular or 'cross' enyne metathesis is thought to arise due to the slow catalyst turnover step **E** to **F**. The kinetics are known for only a limited number of cross metatheses and the rate-determining step can change depending on reactants and catalyst employed.[20]



Scheme 3. The vinyl carbene intermediate in enyne metathesis.

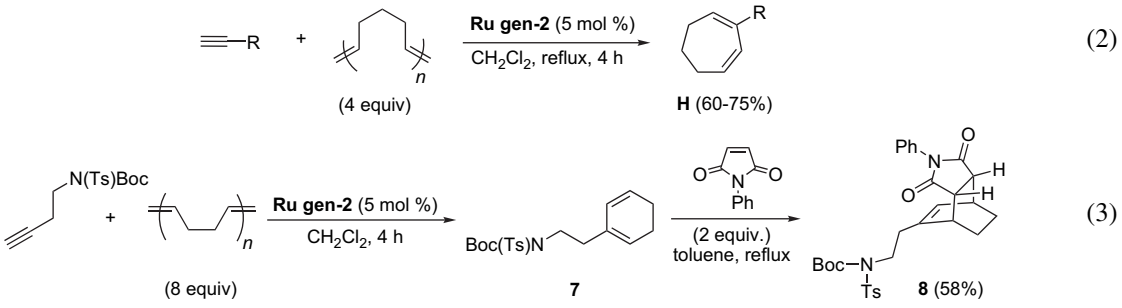
Reaction conditions must be appropriate to kinetically drive the reaction forward to nullify functional group coordination (such as **G**) or chelative decomposition pathways. Functional group chelation to the vinyl carbene intermediate and catalyst decomposition occur by largely unstudied and unknown pathways. Chelates have been suggested in order to account for reduced efficiency in certain enyne metatheses. Though plausible (see carbene **6**), there is no experimental or kinetic evidence of their formation, and further studies are needed to elucidate their relevance to catalytic efficiency. For a typical intermolecular enyne metathesis, excess alkene is used to drive the reaction. High alkene concentration may also help propel catalysis forward relative to unwanted interactions by functional groups (chelative traps are leading to metal carbene decomposition).

Tandem enyne metathesis has evolved as a useful procedure for ring synthesis. Our synthetic goal has been focused on cyclohexadiene synthesis. The one-step cyclohexadiene synthesis was achieved in our original report.[21] However, this study also identified weaknesses. In particular, the lack of stereoselection in the cross metathesis step of the tandem process presented a difficult challenge. We developed a workable solution to this problem using methylene-free conditions (Scheme 4). These conditions proved effective for cycloheptadiene (Eq. 2) and cyclohexadiene synthesis (Eq. 3). The cycloheptadiene ring synthesis engendered a net two-carbon ring expansion of cyclopentene by alkyne insertion, giving **H**. This overall process is actually the result of a threefold metathesis: ring-opening metathesis, cross metathesis, and ring-closing metathesis. The cyclohexadiene synthesis was accomplished from strain-free polybutadiene to form **7**, which was subsequently trapped in a thermal Diels–Alder reaction with *N*-phenyl maleimide to provide **8** in good yield.

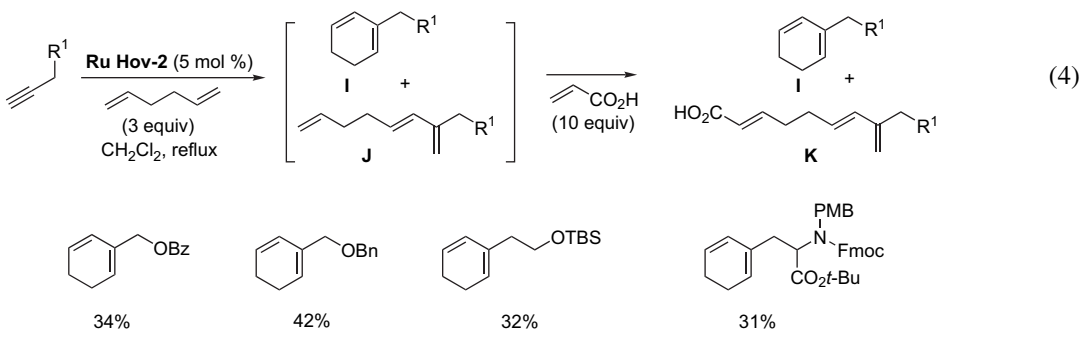
The methylene-free conditions provide higher yields of cyclohexadienes but the reactions are generally slower than the metatheses of 1,5-hexadiene and alkynes.

For alkynes with certain functional groups, the tandem cross metathesis using 1,5-hexadiene provides a complementary ring synthesis to that of the methylene-free metathesis conditions. The cross 1,5-hexadiene-alkyne metatheses are faster in comparison, usually being done in minutes. We have proposed that the 1,5-hexadiene-alkyne metatheses[21] proceed quickly due to a fast vinyl carbene turnover step, due to better binding of a 1-alkene and the high alkene concentration used. These conditions are attractive for ring synthesis where functional group interactions might pose a problem or where low catalyst loadings are desired. In contrast, the conditions of methylene-free metathesis were designed to be slow, and in our original study,[23] we speculated that this might reduce functional group tolerance. Going into the synthesis of the scabrosins, we wanted to have two alternative procedures in hand to tackle potential difficulties in the metathesis step.

The fast cross metathesis with 1,5-hexadiene improves functional group scope but this comes with reduced yield. The yield is compromised by the triene by-product arising from nonstereoselective cross metathesis (Eq. 4). Moreover, the triene by-product **J** is difficult to separate from the desired cyclohexadiene **I**. We developed a simple procedure to separate the undesired triene from cyclohexadiene **I**. This 'one-pot' clean-up procedure transforms the triene **J** into a polar, separable by-product **K** by alkene cross metathesis (Scheme 5). The procedure can be executed with a variety of alkenes in the second step of this sequential one-pot transformation. To increase the polarity of the triene, we typically



Scheme 4. Methylene-free ring synthesis from polyalkenes.[22,23]



Scheme 5. Tandem enyne metathesis and subsequent alkene cross metathesis.[25]

used acrylic acid. The procedure was designed with alkene cross selectivity in mind, using the Grubbs model for alkene reactivity.[24]

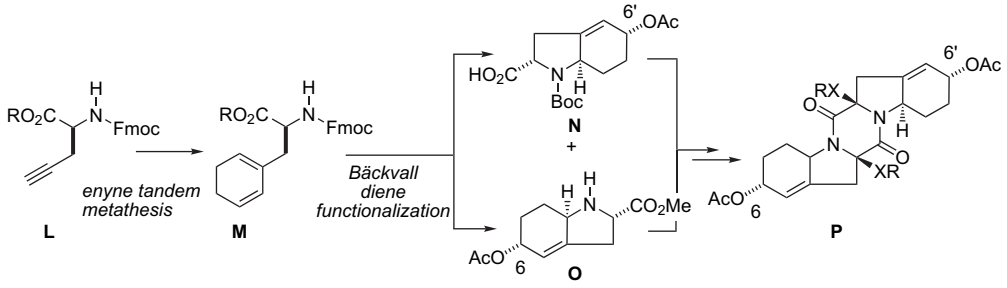
The procedure is remarkably efficient, including a cross enyne metathesis, a ring-closing metathesis, and a cross alkene metathesis, all under the same reaction conditions. In the best cases, a single charge of catalyst was needed. The theoretical yield of cyclohexadienes in these tandem transformations is 50% (based on nonstereoselective cross metathesis), and isolated yields are in the range of 35–45%. Though this is not an ideal solution to the problem of cyclohexadiene ring synthesis by tandem metathesis, the clean-up procedure is attractive due to its simplicity and practicality. The clean-up procedure can be used in cases where functional groups interfere with the efficiency of stereoselective, methylene-free enyne metathesis.

2. Synthesis

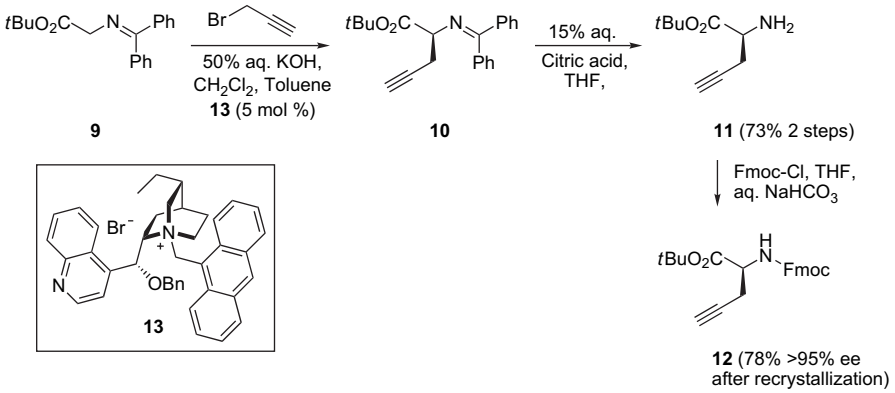
Our approach to the ambewelamides is shown in Scheme 6. Scabrosin is a symmetrical molecule, but we wanted to develop a synthetic scheme that was applicable to the unsymmmetrical ambewelamides (e.g., **2**, **4**). In these cases, two different halves with differing acyl groups on the flanking A and E rings would be synthesized and then joined. The two metal-catalyzed reactions appear early in the synthesis to make the indoline halves **N** and **O**. For this study, we have chosen the desepoxycongener of scabrosin **P** as the synthetic target in this study. First, the ring synthesis by tandem enyne metathesis will be used to generate the cyclohexadiene in **M**. The next metal-catalyzed reaction is the Pd(II)-promoted Bäckvall reaction, which will be used to effect the 1,4-*N*,*O*-difunctionalization of the 1,3-cyclohexadiene ring (Scheme 6). To make unsymmetrical ambewelamides, there are two possibilities. The cognate carboxylic acid could be used in separate Bäckvall reactions to give different acyl groups at C6 and C6′ on the two indolines. Alternatively, the product of Bäckvall cyclization can be manipulated at the C6/C6′ positions to install the desired acyl groups. Our synthesis employs the latter approach. The Bäckvall product **M** will be transformed to **N** and **O** in two separate reactions. Indolines **N** and **O** are differentially protected and ready for peptide coupling and cyclization to produce the pentacycle **P**.

The first goal in the synthesis was the development of a reliable synthetic route that would deliver significant quantities of the metathesis precursor, chiral alkyne **12**. After considering a number of possible methods for the synthesis of propargylated amino acids, we decided to use the phase-transfer-catalyzed alkylation approach (Scheme 7). The phase-transfer-catalyzed alkylation of glycine imine ester **9** has been well studied by the groups of O'Donnell,[26,27] Maruoka,[28,29] Lygo,[30] and others.[31–33] We were attracted to this approach because it is both operationally simple and amenable to scale-up. We elected to use *O*-benzylated cinchona catalyst **13**[30] due to the simplicity of the procedure, the ready availability of phase-transfer catalyst **13** and the low cost of starting materials.

High chemical and optical yields of chiral propargyl amino acid **12** were obtained. Alkylation of **9** using a slight variation on the published conditions[30] gave a good yield of the propargylated glycine imine **10**. Though **10** could be purified and analyzed at this stage (e.g., for enantiomeric excess), it proved sensitive to hydrolysis. The imine was



Scheme 6. Synthetic plan.



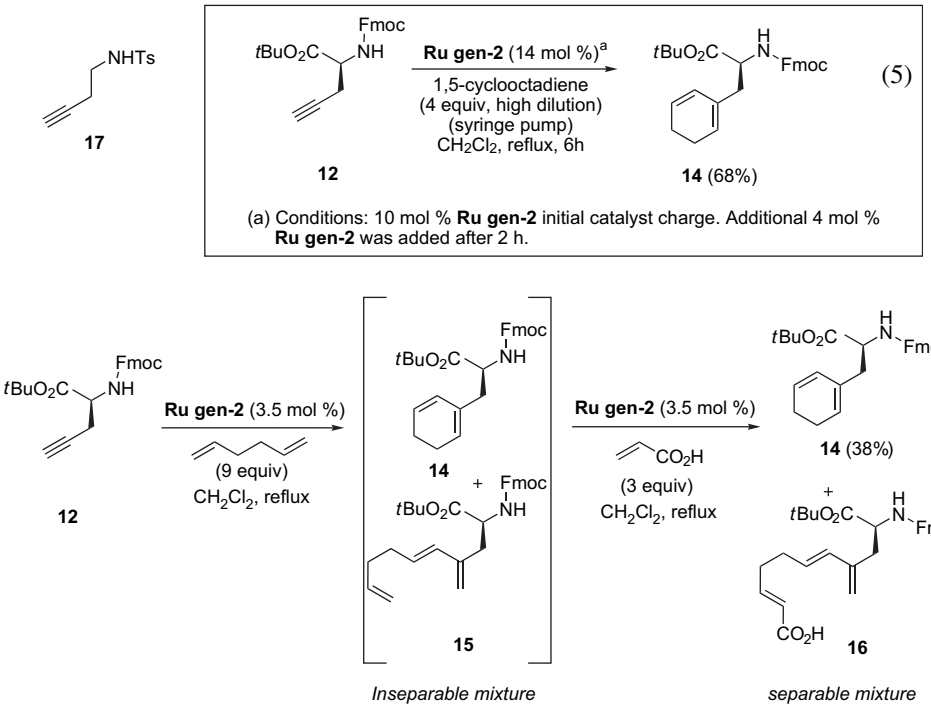
Scheme 7. Synthesis of propargylated amino acid **12**.

isolated after the asymmetric propargylation, and the crude material taken through to hydrolysis with aqueous citric acid in THF. In this way, amine **11** was obtained in 73% isolated yield over the two steps. For stereochemical assignment, imine **10** was analyzed directly. The only absolute configuration data available on chiral propargyl amino acid derivatives comes from alkyne **10**. The optical rotation of **10** was determined on an analytically pure sample (column chromatography), which established the absolute configuration as *S*, based on the literature precedence[29–31,34] for the identical compound. The amine was then protected with (9-fluorenylmethyl)chloroformate under Schotten–Baumann conditions to give **12**.[27] At this point, the enantiopurity of the material was established by HPLC using a chiral stationary phase. The material derived directly from **10** had 89% ee, assigned as the *S*-configuration on the basis of the absolute configuration correlation of imine **10** above. With a single recrystallization, the enantiomeric excess was improved to greater that 95% ee, with a chemical yield of 78% (after a single recrystallization). The sequence outlined in Scheme 7 proved amenable to scale-up and delivered up to 40 g batches of **12**.

The next challenge was the synthesis of cyclohexadiene **14**. The successive alkene metathesis 'clean-up' procedure offered an expedient solution to cyclohexadiene synthesis for the scabrosin synthesis. When **12** was treated with 5 equiv of 1,5-hexadiene and **Ru gen-2**, a 1:1.2 mixture of diene **14** and triene **15** was produced (Scheme 8). The clean-up procedure was then applied. Without isolation or solvent exchange, the mixture of **14** and **15** was directly treated with a second portion of **Ru gen-2** and acrylic acid. Continued reflux in dichloromethane solvent effected the alkene cross metathesis, which occurred with high chemoselectivity on the terminal alkene of **15**. The new mixture of **14** and alkene cross metathesis product **16** could be separated. Separation was readily achieved through extractive work-up using a basic aqueous wash followed by column chromatography, which yielded **14** in 38% overall yield. Initially we ran the two metathesis reactions with 5 mol % each of Grubbs' second-generation carbene catalyst. However, on scale-up this was optimized to a lower loading of 3.5 mol % each, or 7% overall. Lowering the catalyst loading further was unsuccessful: for example, the first cross metathesis stalled at 3 mol % catalyst loading.

Kulkarni and Diver have recently developed the methylene-free metathesis conditions, which deliver good yields of 1,3-cyclohexadienes.[23] Our group is interested in catalytically efficient tandem processes and we typically do not screen reaction conditions employing catalyst loadings above 5 mol % Grubbs carbene complex. In this instance, we were interested in comparatively evaluating the methylene-free method with the clean-up procedure above, which gave modest yields. When alkyne **17** was treated with the second-generation Grubbs carbene complex and polybutadiene, only trace conversion to the cyclohexadiene was detected at high (20 mol %) carbene catalyst loadings.[25] Some conversion was obtained by treating the reaction mixture with successive portions of catalyst, but the loading was too high for practical use in the early stages of total synthesis.

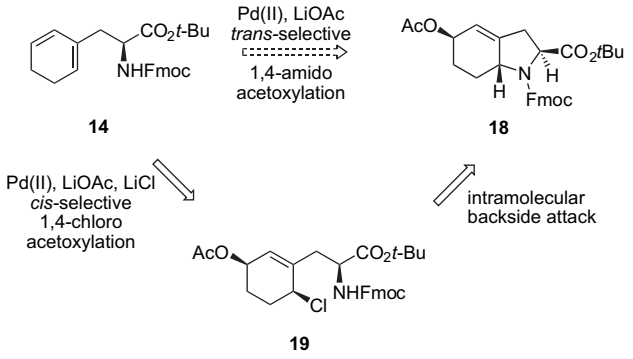
Cyclohexadiene **14** can be accessed by methylene-free enyne metathesis using 1,5-COD as the alkene. In these runs, higher catalyst loadings (10–20 mol %) were required in small-scale reactions.[35] The high catalyst loadings are needed because the free N–H is considered problematic for cross enyne metathesis.[36] Full conversion of alkyne **12** was achieved with 4 equiv COD at 14 mol % total loading of **Ru gen-2** complex (Eq. 5).[37] The cyclodiene was obtained in 68% isolated yield after treatment of the crude reaction with DMSO (the Georg protocol).[38] Typically the reaction requires the addition of a fresh portion of



Scheme 8. Clean-up procedure applied to synthesis of **14**.

catalyst. Currently, the best results are obtained with an initial charge of 10 mol % **Ru gen-2** followed by addition of additional 4 mol % halfway through the 4 h syringe pump addition. We do not fully understand the nature of catalyst decomposition. Further experiments to optimize the reaction conditions are underway.[39]

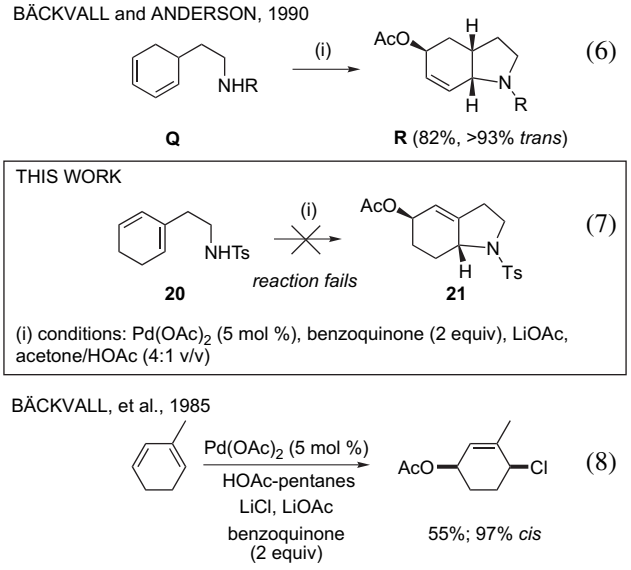
With cyclohexadiene **14** accessible by two different approaches, we proceeded to investigate cyclization to the indoline. Ideally, direct transformation of **14** into indoline **18** would be desired through use of the Bäckvall reaction[40–43] (top path, Scheme 9). Ultimately, we found that an indirect two-step sequence via **19** was necessary to obtain desired indoline **18**.



Scheme 9. Direct and indirect paths to indoline **18**.

Bäckvall's original paper describing the cyclization showed that the amidoacetoxylation[42] proceeds efficiently using diene **Q** (Eq. 6). Our earlier efforts to effect an analogous cyclization on the constitutional isomer **20** failed. The tethered amine must have enough flexibility to attack the η^4-diene–Pd(II) complex; in diene **20** there is too much ring strain for the out-of-plane deformation needed for C–N bond formation to produce **21**. To overcome this difficulty, we imagined that a two-step sequence could be used. Bäckvall has also described Pd(II)-catalysis of a stereoselective *syn*-chloroacetoxylation[44–46] (Eq. 8). Interestingly, the reaction is completely regioselective. Since the direct amidoacetoxylation in Eq. 7 failed, we examined the two-step approach described in Scheme 9 above. The *syn*-selective chloroacetoxylation would be followed by a base-catalyzed intramolecular displacement by an amide nucleophile. This process results in the desired, net 1,4-trans amidoacetoxylation (lower pathway in Scheme 9).

The synthesis of the indoline relied on the Bäckvall chloroacetoxylation–cyclization two-step sequence. Exposing **14** to *syn*-1,4-chloroacetoxyation (LiCl and LiOAc in THF–acetic acid) to Pd(II) under the oxidative conditions (2 equiv benzoquinone) yielded functionalized alkene product **19** and a diastereomer **22**. The diastereomers arise from nondiscriminant complexation to the Pd(II). The α-chiral center is too remote to have any effect on facial selectivity. As a result, both diastereomers were produced in equal yields. During purification of **19** and **22**, phenylalanine **29** was isolated in 17% yield. It is presumed that this oxidation product arises directly from the 1,3-cyclohexadiene **14**. Several observations support this rationalization. Under the extended reaction time, the ratio of the 1,4-acetoxychlorination products **19** and **22** and the oxidation product **29** did not vary significantly. Diene **14** could be undergoing air oxidation or could be undergoing activation by Pd(II) with a β-hydride elimination competing with nucleophilic attack (Scheme 10).



Scheme 10. Cyclization to indoline based on Bäckvall reactions (Eqs. 6 and 8 from Bäckvall[42,44]).

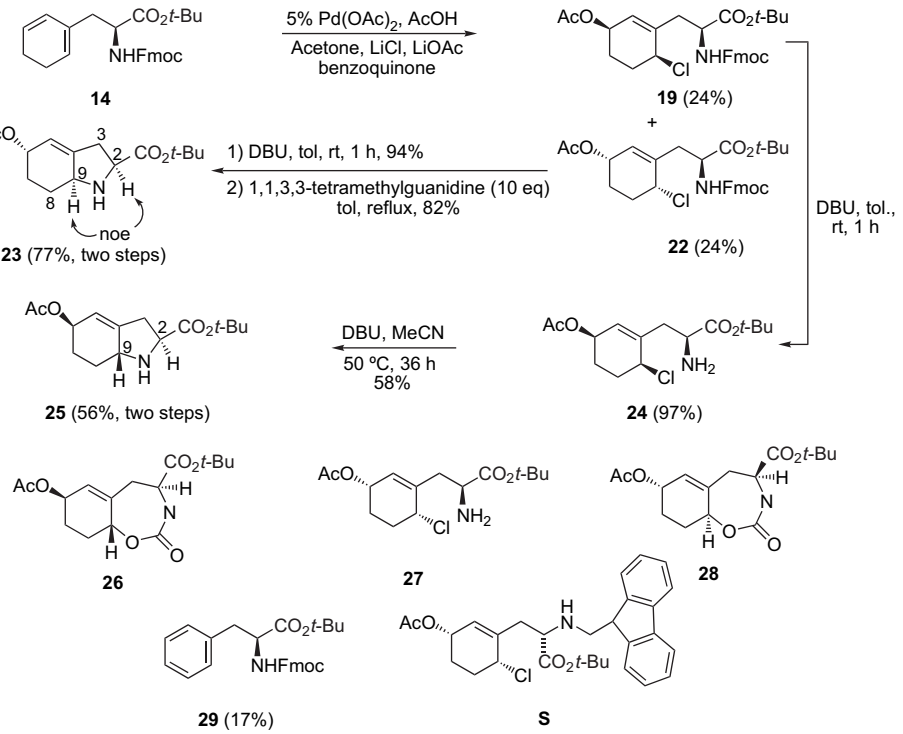
Deprotection of the fluorenylmethyloxycarbonyl (Fmoc) group was accomplished over two discrete treatments with base. Initial experiments using 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in acetonitrile on the mixture of diastereomers **19/22** gave Fmoc deprotection with the formation of up to 30% of **26** and **28**. The formation of cyclic carbamates was surprising. This is unusual, seldom observed probably because most carbamic acids formed in situ during Fmoc deprotection do not have a cyclization path. During the deprotection of **19/22** with DBU in acetonitrile, the intermediate carbamic acids gave cyclization by substitution reaction on the allylic chloride providing the cyclic carbamates **26** and **28**. Other solvents such as tetrahydrofuran, dichloromethane, dichloroethane, and benzene all result in the formation of **26** and **28** in decreasing amounts, respectively. Each diastereomer **19/22** gave cleaner deprotection in toluene without the formation of the cyclic carbamates. Treatment of **19** or **22** with DBU in toluene at room temperature afforded the corresponding primary amino acids in 97% and 94% yields, respectively. The addition of DBU too quickly similarly produced trace amounts of **26** and **28** in toluene. Last, when the primary amine was cyclized in the same pot as the Fmoc deprotection, there were several products formed including a nonpolar adduct **S** between the primary amine and fulvene. The fulvene is formed as a by-product of Fmoc protecting group removal. For instance it is known that the primary amines can add into the fulvene to form the adduct.[8] To optimize the yield of cyclization, it proved necessary to remove the nonpolar fulvene before the allylic chloride **27** was subjected to the more forcing conditions required for the cyclization.

The cyclization of **19** and **22** had to be conducted under different conditions. Treatment of **22** with 10 equiv of 1,1,3,3-tetramethylguanidine in refluxing toluene for 15 h

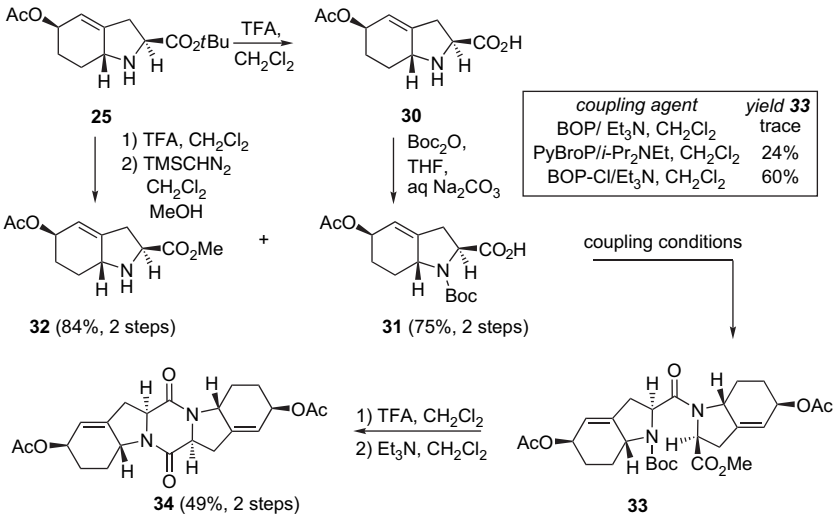
yielded 82% of **23**. In contrast, the 2,5-*trans*-pyrrolidine substructure found in indoline **25** (2,9-*anti*-configuration based on scabrosin numbering) makes it more strained than **23**. As a result, indoline **25** was found to be much harder to synthesize. Treatment of **24** with 1,1,3,3-tetramethylguanidine under identical conditions resulted in trace conversion to indoline **25**. After screening several bases, DBU in acetonitrile at 50 °C for 36 h yielded the desired indoline **25** in 58% isolated yield. Access to both diastereomers of the indolines proved advantageous for the assignment of relative stereochemistry. The stereochemistry of **23** was established directly by NOE experiments. Irradiation of the resonance at δ 3.41 (C9 proton) gave an NOE of the resonances at δ 3.73 (C2 proton, scabrosin numbering) and δ 1.70. Irradiation of the proton at δ 3.73 ppm showed enhancement in the resonances at δ 3.41 (C9 proton) and δ 2.85 (C3 proton). This was expected for a 2,5-*cis*-disubstituted pyrrolidine,[47] and led to the assignment of the indoline relative stereochemistry shown for **23** (Scheme 11). For diastereomer **25**, the analogous NOE between δ 3.77 (C2 proton) and δ 3.64 (C9 proton) of **25** was not observed, suggestive of trans-orientation. The two observations taken together lead to an assignment of the 2,5-*trans*-pyrrolidine substructure in **25**. Ultimately this conclusion was corroborated by crystal structure of the pentacycle (vide supra).

Completion of the synthesis of the pentacyclic core of scabrosin is summarized in Scheme 12. Indoline **25** was divided into two portions and manipulated separately for eventual union to form the diketopiperazine ring. The *tert*-butyl ester was deprotected with trifluoroacetic acid, then esterified with trimethylsilyldiazomethane in 1:1 v/v CH_2Cl_2–methanol to produce methyl ester **32** in 84% yield. This amino ester has a free amine in the proline ring ready for peptide bond coupling. A second portion of amino acid **25** was deprotected and converted (di-*tert*-butyl dicarbonate in aqueous sodium carbonate) to Boc-protected carboxylic acid **31** in 75% yield. Indoline half **31** features the carboxylic acid to be activated in the peptide coupling step. Next, the two pieces were joined through conventional peptide coupling. Conventional peptide coupling agents used for difficult couplings gave poor results. Both BOP reagent and PyBroP were attempted but gave low conversion to dipeptide **33**. The hindered nature of the secondary amine in **32** hampered the efficiency of coupling. Activation of the carboxylic acid **31** with bis(oxazolidinyl)-phosphoryl chloride (BOP-Cl)[48,49] gave an improved yield, providing amide **33**, isolated in 60% yield. Amide **33** appeared as a mixture of amide and carbamate rotamers in the NMR. The final cyclization required intramolecular aminolysis of the methyl ester and was accomplished by deprotection of the *N*-Boc group with TFA in CH_2Cl_2. The excess TFA was then removed and the secondary ammonium salt neutralized with triethylamine, refluxed in CH_2Cl_2 overnight to furnish the pentacycle **34** in 49% yield.

The structure of **34** was confirmed by single crystal X-ray structure (Fig. 1). The pentacyclic diketopiperazine **34** was crystallized from ethyl acetate and hexanes to provide white colorless crystals, mp 259–261 °C. Importantly, the structure determination corroborated the diastereomeric assignment of the indolines based on observed NOE's (Scheme 11 describing the Bäckvall chloroacetoxylation–cyclization sequence). Since the molecule **34** does not contain any heavy atoms and since it crystallized in a centrosymmetric space group, the structure determination was not used to corroborate absolute configuration. The structure determination of **34** illustrates relative configuration. Furthermore, the crystal structure showed disorder in the ester moieties, with the



Scheme 11. Synthesis of diastereomeric indolines **23** and **25**.



Scheme 12. Completion of scabrosin pentacycle synthesis.

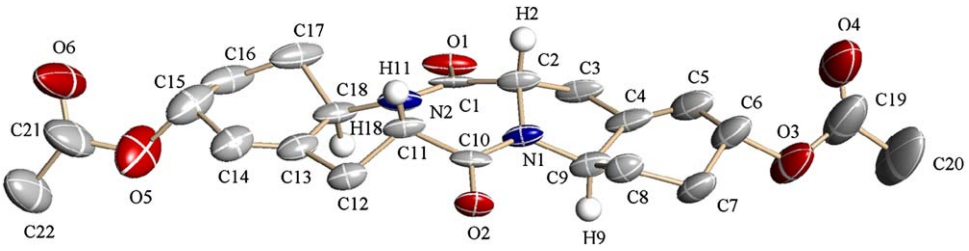


Figure 1. ORTEP drawing of pentacycle **34**. The major conformer (63%) found in the unit cell is shown. Thermal ellipsoids are drawn at the 50% probability level. The numbering on the right half is the same as scabrosin numbering. The crystallographic numbering C10–C18 corresponds to C1′–C9′ (scabrosin numbering).

major (63% population) isomer shown. Both structures have the same relative stereochemistry. A further discussion is provided in Section 3.

In conclusion, we have achieved a stereocontrolled synthesis of the scabrosin pentacycle using tandem diene–alkyne metathesis conjoined with the Pd(II)-promoted chloroacetoxylation–base-catalyzed cyclization. The combination of the two metal-catalyzed reactions demonstrates the value and versatility of the metathesis chemistry when linked with 1,4-difunctionalization. The cyclohexadiene ring synthesis can be achieved under either 1,5-hexadiene-alkyne cross metathesis or under methylene-free conditions using cyclooctadiene as the alkene. The Bäckvall 1,4-*N*,*O*-difunctionalization chemistry of the 1,3-cyclohexadiene results in the formation of the indoline ring system. The synthetic approach is amenable to the synthesis of unsymmetrical epidithiadiketopiperazines of this family of natural products. Further studies directed toward the stereospecific sulfurization of the diketopiperazine ring are in progress.

3. Experimental

3.1. General information

Reactions were conducted under argon atmosphere unless otherwise noted. Solvents were dried and degassed under argon by a solvent purification system and drawn immediately prior to use. Dichloromethane, tetrahydrofuran, and ether were dried by passage through alumina and toluene was dried and deoxygenated using columns of alumina and Q5. Ruthenium [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidine]dichloro(phenylmethylene)(tricyclohexylphosphine) (Grubbs' second-generation catalyst) was obtained from Materia, Inc. (Pasadena, CA) or purchased from Aldrich Chemical Co. 1,5-Hexadiene was purified by distillation from sodium metal. All other chemicals were purchased from Aldrich Chemical Co. and used as received. Column chromatography was carried out on Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded at 300, 400, or 500 MHz and ^{13}C NMR spectra at either 75 or 125 MHz in the indicated solvent. ^{1}H NMR spectra were referenced on the TMS signal for $CDCl_3$. The ^{13}C NMR spectra were referenced at 77 ppm for $CDCl_3$. Enantiomeric excesses were determined by HPLC using a Chiralcel OD-H column (4.6 mm×250 mm, 5 μm particle size) using UV detection. Proton and carbon NMR data can be found in Supplementary data file.

3.1.1. 2-(Benzhydrylidene-amino)-pent-4-ynoic acid *tert*-butyl ester (10). In a 5 L Erlenmeyer flask equipped with magnetic stirbar, *tert*-butyl *N*-(diphenylmethylene)glycinate **9** (53 g, 0.18 mol) was dissolved in toluene (1.3 L) and CH_2Cl_2 (0.54 L). The solution was treated sequentially with catalyst **13** (5.9 g, 9.0 mmol), propargyl bromide (80% in toluene, 24 mL, 0.22 mol), and 50% aqueous potassium hydroxide (0.36 L, 3.2 mol). The mixture was stirred vigorously for 14 h at room temperature, the phases were separated and the aqueous layer was extracted with Et_2O

(2×300 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo (rotary evaporator). The residue was then passed though a short plug of silica gel, eluted with 20% ethyl acetate–hexanes, and concentrated to give crude imine **10** (57 g, 95%), which was used directly in the subsequent hydrolysis step.

An analytical sample of **10** was obtained in a separate small-scale reaction, conducted analogous to the procedure above on 10 mmol scale. After the usual work-up, the residue was further purified by flash column chromatography on silica gel (gradient elution with 1:30 ethyl acetate–hexane to 1:9 ethyl acetate–hexane) to give **10** (2.38 g, 71%) as a green oil: R_f=0.39 (1:9 ethyl acetate–hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.64 (m, 2H), 7.45–7.25 (m, 8H), 4.19–4.15 (m, 1H), 2.83–2.71 (m, 2H), 1.94 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 169.6, 139.7, 136.3, 130.4, 129.0, 128.7, 128.4, 128.3, 128.1, 81.6, 81.3, 70.1, 64.8, 28.1, 23.4; FTIR (thin film, cm^{-1}) 3297, 2978, 1732, 1624, 1446, 1368, 1285, 1152, 696; high-resolution MS (ESI$^+$) calcd for $C_{22}H_{24}O_2N_1$ (M^++H): 334.1802, found: 334.1809; $[\alpha]_D^{25}$ −96.8 (*c* 1.0, $CHCl_3$). The negative sign of optical rotation established the *S*-configuration in agreement with the literature assignment.[31,34]

3.1.2. 2-Amino-pent-4-ynoic acid *tert*-butyl ester (11). A 2 L rb flask equipped with magnetic stirbar and rubber septum was charged with imine **10** (57 g, 0.17 mol) and 650 mL THF. To the stirred solution was added 15% aqueous citric acid (350 mL) and stirring was continued for 12 h. The mixture was diluted with 1 M HCl (200 mL), extracted with Et_2O (3×350 mL), and the combined organics were subsequently washed with water (2×300 mL). The combined aqueous layers were basified to pH 11 by the addition of K_2CO_3. The aqueous layer was then extracted with ethyl acetate (3×400 mL) and all of the organic layers were then combined, dried (Na_2SO_4), filtered, and concentrated in vacuo (rotary evaporator). The residue was purified by flash column chromatography on silica gel (elution with 3:1 ethyl acetate–hexanes) to give **11** (22.3 g, 77%) as a clear oil: R_f=0.32 (3:1 ethyl acetate–hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (dd, *J*=9.0, 9.0 Hz, 1H), 2.61–2.58 (m, 2H), 2.06–2.05 (m, 1H), 1.67 (br s, 1H), 1.48 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 81.6, 79.8, 71.0, 53.6, 28.0, 25.0; FTIR (thin film, cm^{-1}) 2979, 2362, 1730, 1394, 1368, 1251, 1221, 1153; high-resolution MS (ESI$^+$) calcd for $C_9H_{15}O_2N_1Na$ (M^++Na): 192.0995, found: 192.0994; $[\alpha]_D^{25}$ −23.8 (*c* 1.0, $CHCl_3$).

3.1.3. (2*S*)-*tert*-Butyl 2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-pent-4-ynoate (12). A 2 L rb flask containing magnetic stirbar and rubber septum was charged with amine **11** (22.3 g, 0.132 mol) in THF (500 mL) to which 9-(fluorenylmethoxycarbonyl)chloride (38.0 g, 0.145 mol) was added. A solution of 10% aqueous Na_2CO_3 (500 mL) was added and the mixture was stirred for 16 h. After this time, the mixture was diluted with ethyl acetate (400 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (2×400 mL) and the organic layers were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo (rotary evaporator). The residue was subsequently purified by flash column chromatography on silica gel (gradient elution with 1:20 ethyl acetate–hexanes to 1:5 ethyl acetate–hexanes) to afford a white solid, which was recrystallized (ethyl acetate–hexanes) to give **12** as white needles (39.2 g, 76%): mp 68–70 °C; R_f=0.53 (1:3 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J*=8.0 Hz, 2H), 7.64 (d, *J*=7.5 Hz, 2H), 7.4 (dd, *J*=7.5, 7.5 Hz, 2H), 7.34 (dd, *J*=8.0, 7.5 Hz, 2H), 5.5 (d, *J*=8.0 Hz, 1H), 4.48–4.38 (m, 3H), 4.27 (dd, *J*=7.0 Hz, 1H), 2.81–2.78 (m, 2H), 2.09 (br s, 1H), 1.53 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 155.6, 143.9, 143.8, 141.3, 127.7, 127.1, 125.2, 120, 82.8, 78.6, 71.6, 67.2, 52.6, 47.1, 28.0, 23.0; FTIR (thin film, cm^{-1}) 3298, 2977, 1721, 1507, 1450, 1349, 1223, 1156; high-resolution MS (EI$^+$) calcd for $C_{24}H_{25}O_4N$ (M^+): 391.1778, found: 391.1776; enantiomeric excess determination by HPLC (0.6 mL/min, gradient elution 15% 2-propanol–hexane to 40% 2-propanol–hexane over 40 min, t_R=15.1, 1.6% (*R*), 26.0, 98.4% (*S*) min) indicated 97% ee of the *S*-enantiomer; $[\alpha]_D^{25}$ +31.6 (*c* 1.0, $CHCl_3$).

3.1.4. (2*S*)-*tert*-Butyl 3-cyclohexa-1,5-dienyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionate (14).

3.1.4.1. Method A: 1,5-cyclooctadiene. A 50 mL air-free Schlenk tube was charged with dichloromethane (5 mL) and 1,5-cycloctadiene (188 μL, 1.5 mmol). Argon was bubbled through this solution for 10 min. **Ru gen-2** (21.2 mg, 0.025 mmol, 10 mol %) was then added to the room temperature solution and subsequently placed into a 55 °C oil bath. To this solution was added **12** (100 mg, 0.256 mmol) in 2 mL dichloromethane over 4 h via gas-tight syringe (addition rate 0.5 mL/h).[50] After 2 h of addition, an additional 4 mol % of **Ru gen-2** (8.7 mg, 0.010 mmol, 4 mol %) was added to the reaction. After 2 h, the addition was complete and the flask was heated for another 6 h. The reaction was allowed to cool to room temperature, concentrated in vacuo (rotary evaporator) diluted with 5 mL of dichloromethane and 182 μL of dimethyl sulfoxide (2.56 mmol, 1000 mol %), and stirred for 12 h. The solution was then concentrated in vacuo (rotary evaporator) and the residue purified by column chromatography on silica gel, eluting with 1:7 ethyl acetate–hexanes to obtain 75 mg of **14** (68%) as a pale yellow oil: R_f=0.27 (1:5 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J*=7.5 Hz, 2H), 7.60 (m, 2H), 7.40 (t, *J*=7.0 Hz, 2H), 7.31 (t, *J*=7.5 Hz, 2H), 5.83 (m, 2H), 5.55 (s, 1H), 5.27 (d, *J*=8.0 Hz, 1H), 4.41 (m, 1H), 4.34 (m, 2H), 4.23 (m, 1H), 2.48 (m, 2H), 2.10 (m, 4H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 155.5, 143.9, 141.3, 130.9, 127.7, 127.3, 127.0, 126.6, 125.2, 124.4, 120.0, 82.1, 66.1, 53.6, 47.2, 38.5, 28.0, 22.3, 22.1; FTIR (thin film, cm^{-1}) 3326, 2933, 1718, 1507, 1450, 1367, 1224, 1155, 1050; high-resolution ESI molecular ion calcd for $C_{28}H_{31}O_4N$+Na 468.2145, found: 468.2152; $[\alpha]_D^{25}$ +10.0 (*c* 1.6, $CHCl_3$).

3.1.4.2. Method B: preparative scale using 1,5-hexadiene. Into a 250 mL rb flask equipped with a condenser, magnetic stirbar, and rubber septum was added alkyne **12** (15.6 g, 40 mmol) and 1,5-hexadiene (24 mL, 200 mmol), dissolved in CH_2Cl_2 (80 mL). The Grubbs' catalyst **Ru gen-2** (1.2 g, 1.4 mmol, 3.5 mol %) was added and the solution was then brought immediately to reflux by immersion in a 50 °C oil bath. Heating was maintained for 5 h, the mixture was subsequently cooled and concentrated to a volume of 10 mL (rotary evaporator). The residue was passed though

a short plug of silica gel (eluting with 20% ethyl acetate–hexanes) and concentrated. The crude mixture was then dissolved in CH_2Cl_2 (80 mL) and acrylic acid (11.0 mL, 160 mmol) was then added. The Grubbs' catalyst **Ru gen-2** (1.2 g, 1.4 mmol, 3.5 mol %) was then added and the solution was then brought immediately to reflux by immersion in a 50 °C oil bath. Heating was maintained for 12 h, the mixture was subsequently cooled to room temperature, diluted with CH_2Cl_2 (200 mL), and washed with saturated aqueous $NaHCO_3$ (2×250 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and the solvent removed in vacuo (rotary evaporator). Purification was accomplished by flash column chromatography on silica gel (elution with 1:5 ethyl acetate–hexanes) afforded **14** (6.8 g, 38%), as a brown oil. Spectral data matched that reported for **14** above (using COD), though 17–20% butadiene was present by proton NMR. This by-product proved difficult to remove, so the diene was carried through to the next step.

3.1.5. Preparation of 19, 22, and 29. To a stirred solution of $Pd(OAc)_2$ (149 mg, 0.664 mmol), LiCl (111 mg, 2.6 mmol), LiOAc–$2H_2O$ (667 mg, 6.64 mmol), and benzoquinone (2.87 g, 26.6 mmol) in glacial acetic acid (3.9 mL) and acetone (37 mL) was added two solutions via syringe pump over 12 h. Solution 1 was cyclohexadiene **14** (5.9 g, 13.2 mmol) in acetone (5.7 mL). Solution 2 was LiCl (1.0 g, 23.9 mmol) in glacial acetic acid (5.7 mL). After 15 h, the reaction was concentrated in vacuo (rotary evaporator), diluted with ethyl acetate (300 mL), transferred to a separatory funnel, and washed with 1 M $NaOH_{(aq)}$ (3×200 mL). The aqueous layers were then combined and back-extracted with ethyl acetate (3×100 mL), the organic layers were then combined, dried ($MgSO_4$), concentrated in vacuo (rotary evaporator) to give a viscous black sludge. The black residue was dissolved in a minimal amount of chloroform and passed through a short column of silica gel (3.7 cm×12 cm) eluted with 1:10 ethyl acetate–hexanes ramping up to 1:5 ethyl acetate–hexanes to remove palladium and other polar by-products (this operation removes most of **29** from **19** and **22**). The desired fractions (containing **29** R_f=0.27 in 1:5 ethyl acetate–hexanes) were collected and concentrated into a viscous yellow oil. This oil was further purified by column chromatography on silica gel (3.7 cm×30 cm), eluting with 1:10 ethyl acetate–hexanes ramping polarity to 1:6 ethyl acetate–hexanes giving 1.7 g of **19** (24%), 1.7 g of **22** (24%), and 1.0 g of **29** (17%), each obtained as viscous pale yellow oils. If **19** and **22** are dissolved in a minimal amount of ether (at rt) and diluted with five volumes of pentane and concentrated, a pale yellow solid is obtained in quantitative yield. The pale yellow product is clean enough to be taken on to the next reaction. If another column is performed it is possible to obtain **19** and **22** as a clear oil or white powder as described above. The **19**, **22**, and **29** are stable at room temperature in a vial in regular lab light for months.

Compound **19**: mp=51–54 °C. R_f=0.11 (1:5 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J=7.5 Hz, 2H), 7.60 (d, J=7.0 Hz, 2H), 7.40 (t, J=7.5 Hz, 2H), 7.32 (t, J=7.5 Hz, 2H), 5.63 (s, 1H), 5.34 (d, J=8.0 Hz, 1H), 5.30 (m, 1H), 4.52 (s, 1H), 4.38 (m, 3H), 4.23 (t, J=7.0 Hz, 1H), 2.76 (dd, J=14.5, 5.5 Hz, 1H), 2.49 (dd, J=14.5, 8.0 Hz, 1H), 2.19 (m, 1H), 2.01 (m, 6H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 155.5 (143.8, 143.7, rotamers), 141.8, 137.2, 129.2, 127.7, 127.0, 125.0, 120.0, 82.7, 69.1, 67.0, 55.8, 53.0, 47.1, 37.7, 30.2, 28.0, 23.2, 21.1; FTIR (thin film, cm^{-1}) 3339, 2977, 1733, 1520, 1450, 1369, 1237, 1154, 1033; high-resolution ESI molecular ion calcd for $C_{30}H_{34}O_4ClN$+Na 562.1967, found: 562.1982; $[\alpha]_D^{25}$ −4.4 (*c* 2.00, $CHCl_3$).

Compound **22**: mp=52–54 °C. R_f=0.15 (1:5 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J=12.0 Hz, 1H), 7.58 (d, J=11.5 Hz, 1H), 7.40 (t, J=12.0 Hz, 1H), 7.32 (t, J=12.0 Hz, 1H), 5.61 (s, 1H), 5.29 (d, J=13.0 Hz, 1H), 5.20 (m, 1H), 4.66 (s, 1H), 4.31 (m, 4H), 2.94 (m, 1H), 2.10 (m, 6H), 1.48 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.4, 155.9, 143.8, 141.3, 137.4, 129.1, 127.7, 127.0, 125.0, 120.0, 82.7, 69.1, 67.1, 55.7, 52.3, 47.1, 38.4, 30.1, 28.0, 23.3, 21.1; FTIR (thin film, cm^{-1}) 3338, 2978, 1734, 1525, 1450, 1369, 1240, 1156; high-resolution ESI molecular ion calcd for $C_{30}H_{34}O_4ClN$+Na 562.1967, found: 562.1978; $[\alpha]_D^{25}$ +18.5 (*c* 2.00, $CHCl_3$).

Compound **29**: R_f=0.27 (1:5 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J=7.5 Hz, 2H), 7.57 (t, J=7.0 Hz, 2H), 7.40 (t, J=7.5 Hz, 2H), 7.28 (m, 5H), 7.15 (d, J=7.0 Hz, 2H), 5.28 (d, J=7.5 Hz, 1H), 4.55 (m, 1H), 4.44 (dd, J=11.0, 7.5 Hz, 1H), 4.32 (dd, J=11.0, 7.5 Hz, 1H), 4.21 (t, J=7.0 Hz, 1H), 3.10 (d, J=5.5 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 155.4, 143.7, 141.1, 136.0, 129.3, 128.2, 127.5, (126.8, 127.7 rotomers), 124.9, 119.8, 82.0, 66.7, 55.0, 47.0, 38.2, 27.8; FTIR (thin film, cm^{-1}) 3334, 2979, 1723, 1511, 1368, 1253; high-resolution ESI molecular ion calcd for $C_{28}H_{29}O_4N$+Na 466.1989, found: 466.1988; $[\alpha]_D^{25}$ +19.6 (*c* 2.00, $CHCl_3$).

3.1.6. Preparation of 24. A 100 mL Schlenk tube was charged with **19** (780 mg, 1.44 mmol) and 74 mL of toluene. To this stirring solution was added dropwise 1,8-diazabicyclo[5.4.0]undec-7-ene (208 μL, 1.44 mmol) over 2 min, then stirred for 1 h. The color changed from yellow to a pink/red on addition of DBU. The reaction was concentrated in vacuo (rotary evaporator) to a volume of ∼1 mL and purified by column chromatography on silica gel, eluting with 1:1 ethyl acetate–hexane ramping polarity to 100% ethyl acetate (once the fulvene has been eluted, the solvent polarity is increased from 1:1 to 4:1 ethyl acetate–hexane and then to 100% ethyl acetate after **24** starts to elute), to yield 436 mg of **24** (96%) as a pale yellow oil: R_f=0.17 (4:1 ethyl acetate–hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (s, 1H), 5.82 (t, J=7.0 Hz, 1H), 4.57 (t, J=3.5 Hz, 1H), 3.5 (t, J=7.0 Hz, 1H), 2.45 (m, 2H), 2.04 (m, 7H), 1.81 (s, 2H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 170.7, 138.4, 128.4, 81.5, 69.2, 56.5, 54.0, 40.2, 30.3, 28.0, 23.2, 21.2; FTIR (thin film, cm^{-1}) 2919, 2850, 1734, 1369, 1241, 1154, 1026; high-resolution ESI molecular ion calcd for $C_{15}H_{24}O_4NCl$+H 318.1467, found: 318.1456; $[\alpha]_D^{25}$ −16.4 (*c* 3.70, $CHCl_3$).

3.1.7. Preparation of 27. A 100 mL Schlenk tube was charged with **22** (511 mg, 0.927 mmol) and 46 mL of toluene. To this stirring solution was added dropwise 1,8-diazabicyclo (5.4.0) undec-7-ene (130 μL, 0.927 mmol) over 2 min and then stirred for 1 h. The color changed from yellow to a pink/red on addition of DBU. The reaction was

concentrated in vacuo (rotary evaporator) to a volume of ~1 mL and purified by column chromatography on silica gel, eluting with 1:1 ethyl acetate–hexanes ramping polarity to 100% to ethyl acetate (once the fulvene been eluted, the solvent polarity is changed from 1:1 to 4:1 ethyl acetate–hexanes and then to straight ethyl acetate after **27** starts to elute), to yield 286 mg of **27** (94%) as a pale yellow oil: R_f=0.10 (4:1 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.63 (s, 1H), 5.34 (t, J=7.5 Hz, 1H), 4.56 (t, J=3.5 Hz, 1H), 3.53 (dd, J=9.5, 4.5 Hz, 1H), 2.76 (dd, J=14.5, 4.5 Hz, 1H), 2.20 (m, 2H), 2.03 (m, 6H), 1.54 (s, 2H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 170.6, 138.2, 128.3, 81.4, 69.2, 56.0, 53.0, 39.4, 30.3, 28.0, 23.3, 21.2; FTIR (thin film, cm^{-1}) 3380, 2977, 1733, 1369, 1243, 1157, 1027; high-resolution ESI molecular ion calcd for $C_{15}H_{24}O_4NCl$+H 318.1467, found: 318.1466; $[\alpha]_D^{25}$ +18.0 (c 2.00, $CHCl_3$).

3.1.8. Preparation of 26 and 28. Substituting toluene for acetonitrile in the procedure above will result in the formation of 20–30% **26** and **28** as a clear oil (R_f=0.30, R_f=0.32, respectively, 4:1 ethyl acetate–hexanes). The use of tetrahydrofuran, dichloromethane, dichloroethane or benzene all resulted in the formation of **26** and **28** in decreasing amounts, respectively. The addition of DBU too quickly will also produce trace amounts of **26** and **28** in toluene. Work-up is the same as for **24** and **27**.

Compound **28**: R_f=0.32 (4:1 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (m, 2H), 5.26 (d, J=4.0 Hz, 1H), 4.72 (t, J=4.0 Hz, 1H), 4.08 (ddd, J=12.5, 8.5, 4.5 Hz, 1H), 2.78 (m, 2H), 2.1 (m, 5H), 1.73 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 168.9, 157.7, 136.5, 127.3, 83.4, 73.6, 66.7, 53.6, 37.8, 27.9, 25.8, 24.1, 21.1; FTIR (thin film, cm^{-1}) 3314, 2967, 1728, 1376, 1244, 1155, 1014; high-resolution ESI molecular ion calcd for $C_{16}H_{23}O_6N$+Na 348.1418, found: 348.1414; $[\alpha]_D^{25}$ −118.0 (c 0.50, $CHCl_3$).

3.1.9. Preparation of 25. To a 100 mL rb flask was added **24** (252 mg, 0.795 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (112 μL, 0.795 mmol) and 40 mL of acetonitrile. This solution was heated to 50 °C for 36 h. After 36 h, the reaction was concentrated in vacuo (rotary evaporator) and purified by column chromatography on silica gel, eluting with 1:1 ramping polarity to 3:1 ethyl acetate–hexane, to yield 130 mg of **25** (58%) as a pale yellow oil: R_f=0.28 (4:1 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.48 (s, 1H), 5.43 (m, 1H), 3.77 (m, 1H), 3.64 (m, 1H), 2.84 (m, 1H), 2.63 (m, 1H), 2.16 (m, 2H), 2.03 (s, 1H), 1.77 (s, 1H), 1.47 (m, 11H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 170.9, 146.4, 118.0, 81.5, 71.1, 58.1, 56.3, 30.1, 28.0, 27.1, 21.3; FTIR (thin film, cm^{-1}) 3343, 2935, 1731, 1370, 1242, 1156; high-resolution ESI molecular ion calcd for $C_{15}H_{23}O_4N$+H 282.1700, found: 282.1704; $[\alpha]_D^{25}$ +79.3 (c 1.20, $CHCl_3$).

3.1.10. Preparation of 23. To a 50 mL rb flask was added **27** (149 mg, 0.47 mmol), 1,1,3,3-tetramethylguanidine (112 μL, 0.795 mmol) and 24 mL of toluene. This solution was gently refluxed for 15 h. After this time, the reaction was filtered, concentrated in vacuo (rotary evaporator), and purified by column chromatography on silica gel, eluting with 1:1 ethyl acetate–hexane ramping polarity to 3:1 ethyl acetate–hexane, to yield 110 mg of **23** (82%) as a pale yellow oil: R_f=0.22 (4:1 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.43 (m, 2H), 3.73 (dd, J=6.5, 2.5 Hz, 1H), 3.41 (d, J=8.0 Hz, 1H), 2.85 (ddd, J=17.0, 9.5, 2.0 Hz, 1H), 2.43 (ddd, J=17.0, 6.0, 2.0 Hz, 1H), 2.24 (m, 2H), 2.10 (s, 3H), 1.7 (s, 1H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 170.8, 146.5, 117.4, 81.4, 71.1, 58.6, 58.3, 35.4, 28.9, 27.9, 27.4, 21.2; FTIR (thin film, cm^{-1}) 2977, 2939, 2867, 1733, 1369, 1244, 1155, 1020; high-resolution ESI molecular ion calcd for $C_{15}H_{23}O_4N$+H 282.1700, found: 282.1706; $[\alpha]_D^{25}$ −118.2 (c 1.43, $CHCl_3$).

3.1.11. 5-Acetoxy-2,3,5,6,7,7a-hexahydro-1*H*-indole-2-carboxylic acid methyl ester (32). Into a 10 mL rb flask containing magnetic stirbar and rubber septum was placed indoline **25** (30 mg, 0.11 mmol) dissolved in CH_2Cl_2 (1.25 mL) at room temperature. To the stirred solution was added trifluoroacetic acid (1.25 mL), stirred at ambient temperature for 12 h then concentrated in vacuo and placed under high vacuum for 2 h. The residue was dissolved in 1:1 v/v CH_2Cl_2–MeOH (1 mL of each) and (trimethylsily)-diazomethane (2.0 M in Et_2O, 205 μL) was added dropwise by microliter syringe until gas evolution ceased and the solution remained yellow. The mixture was stirred for 8 h, concentrated in vacuo (rotary evaporator) and the residue was subsequently purified by flash column chromatography on silica gel (gradient elution with 3:1 ethyl acetate–hexane to 100% ethyl acetate) to provide **32** (14 mg, 84%), as a brown oil: R_f=0.15 (3:1 ethyl acetate–hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.49 (s, 1H), 5.43–5.42 (m, 1H), 3.91 (dd, J=9.0, 5.0 Hz, 1H), 3.74 (s, 3H), 3.64–3.62 (m, 1H), 2.84 (ddd, J=16.5, 9.0, 1.5 Hz, 1H), 2.70 (ddd, J=16.5, 4.5, 2.0 Hz, 1H), 2.49 (br s, 1H), 2.23–2.13 (m, 2H), 2.05 (s, 3H), 1.56–1.48 (m, 1H), 1.39–1.31 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.6, 171.0, 146.0, 118.1, 71.0, 57.4, 56.4, 52.3, 33.9, 29.9, 27.1, 21.4; FTIR (thin film, cm^{-1}) 2950, 1734, 1441, 1373, 1243, 1138; high-resolution MS (ESI$^+$) calcd for $C_{12}H_{18}O_4N_1$ (M^++H): 240.1230, found: 240.1235; $[\alpha]_D^{25}$ +43.6 (c 0.5, $CHCl_3$).

3.1.12. 5-Acetoxy-2,3,5,6,7,7a-hexahydro-indole-1,2-dicarboxylic acid 1-*tert*-butyl ester (31). Into a 10 mL rb flask containing magnetic stirbar and rubber septum was placed indoline **25** (45 mg, 0.16 mmol) dissolved in CH_2Cl_2 (2 mL) at room temperature. To the stirred solution was added trifluoroacetic acid (2 mL), stirred at ambient temperature for 8 h, then concentrated in vacuo (rotary evaporator) and placed under high vacuum for 2 h. The residue was then dissolved in a mixture of THF (4 mL) and 10% aqueous Na_2CO_3 (4 mL). To the stirred solution was added di-*tert*-butyl dicarbonate (Boc_2O, 42 mg, 0.19 mmol), the mixture was stirred at ambient temperature for 11 h, acidified to pH 3 with 1 M aq HCl and then extracted with ethyl acetate (3×15 mL). The combined organic layers were then dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was subsequently purified by flash column chromatography on silica gel (gradient elution with 5% MeOH–CH_2Cl_2 to 10% MeOH–CH_2Cl_2) to afford **31** (50 mg, 96%) as a yellow gum: R_f=0.18 (5% MeOH–CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.56 (s, 1H), 5.41–5.34 (m, 1H), 4.28–4.18 (m, 2H), 2.87–2.81 (m, 1H), 2.65–2.49 (m, 1H), 2.09–2.22

(m, 1H), 2.04 (s, 3H), 1.48–1.22 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.8, 171.1, 154.9, 153.8, 142.8, 142.3, 120.3, 120.2, 81.1, 81.0, 70.4, 70.2, 67.7, 67.5, 58.6, 58.4, 57.1, 56.7, 34.3, 33.8, 29.7, 29.5, 29.0, 28.4, 28.3, 28.2, 26.9, 26.7, 23.8, 21.3; FTIR (thin film, cm^{-1}) 2978, 1733, 1698, 1478, 1418, 1368, 1243, 1145; high-resolution MS (ESI$^+$) calcd for $C_{16}H_{23}O_6NNa$ (M^++Na): 348.1418, found: 348.1418; $[\alpha]_D^{25}$ −23.2 (*c* 1.0, $CHCl_3$).

3.1.13. Amide 33. Into a 10 mL rb flask containing magnetic stirbar and rubber septum was placed acid **31** (18 mg, 0.55 mmol) and amino ester **32** (13 mg, 0.055 mmol), dissolved in CH_2Cl_2 (1 mL). To this solution was added successively BOP-Cl (21 mg 0.083 mmol) then Et_3N (11 mg, 0.11 mmol). The mixture was then stirred at room temperature for 18 h, water (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were then dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was subsequently purified by flash column chromatography on silica gel (gradient elution with 1:2 ethyl acetate–hexanes to 2:1 ethyl acetate–hexanes) to provide **33** (18 mg, 60%) as a yellow gum: R_f=0.47 (3:1 ethyl acetate–hexanes); The NMR spectra showed a mixture of amide and carbamate rotamers that did not coalesce on heating in the NMR probe. The compound was therefore partially characterized and taken on to the cyclization step. High-resolution MS (ESI$^+$) calcd for $C_{28}H_{38}O_9N_2Na$ (M^++Na): 569.2470, found: 569.2462.

3.1.14. Acetic acid 9-acetoxy-6,13-dioxo-2,4,4a,6a,7,9,10,11,11a,13,13a,14-dodecahydro-3*H*,6*H*-pyrazino [1,2-*a*;4,5-*a*′] diindol-2-yl ester (34). Into a 10 mL rb flask containing magnetic stirbar and rubber septum was placed dipeptide **33** (9.0 mg, 0.017 mmol), dissolved in CH_2Cl_2 (300 μL), and stirred at room temperature. TFA (60 μL, 0.81 mmol) was then added dropwise over 2 min, the mixture stirred at ambient temperature for 1.5 h, the solution was concentrated in vacuo (rotary evaporator) and placed under high vacuum for 1 h. The residue was dissolved in CH_2Cl_2 (1 mL) to which Et_3N (11.5 μL, 0.083 mmol) was added. The mixture was stirred at ambient temperature for 24 h, concentrated in vacuo (rotary evaporator) and the residue was then purified by flash chromatography (gradient elution with 1:1 ethyl acetate–hexane to 3:1 ethyl acetate–hexane) to afford diketopiperazine **34** (3.3 mg, 49%) as a white solid: mp 259–261 °C; R_f=0.22 (3:1 ethyl acetate–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (s, 1H), 5.43 (m, 1H), 4.40–4.24 (m, 2H), 2.86–2.80 (m, 3H), 2.30–2.25 (m, 1H), 2.07 (s, 3H), 1.67–1.60 (m, 1H), 1.56 (s, 3H), 1.41–1.31 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 166.1, 141.5, 121.6, 70.0, 59.5, 57.4, 33.9, 28.4, 27.0, 21.3; FTIR (thin film, cm^{-1}) 2925, 1738, 1649, 1439, 1249, 915; high-resolution MS (ESI$^+$) calcd for $C_{22}H_{26}O_6N_2Na$ (M^++Na): 437.1683, found: 437.1672; $[\alpha]_D^{25}$ −73.6 (*c* 0.5, $CHCl_3$).

Acknowledgements

We thank the NIH (R01CA090603) for supporting the early stages of this work and the NSF (Career CHE-092434) and SUNY Buffalo for continued support. The authors thank Amol Kulkarni for earlier synthetic studies on the ambewelamides and for advice regarding the methylene-free metathesis. We also thank Dr. Cara L. Nygren for refinement of the crystal structure data, Professor Philip Coppens (UB) for use of his X-ray diffraction equipment, and Materia, Inc. for samples of the Grubbs second-generation complex.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2006.05.089.

References and notes

1. Williams, D. E.; Bombuwala, K.; Lobkovsky, E.; De Silva, E. D.; Veranja, K.; Allen, T. M.; Clardy, J.; Andersen, R. J. *Tetrahedron Lett.* **1998**, *39*, 9579–9582.
2. Nagarajan, R.; Huckstep, L. L.; Lively, D. H.; DeLong, D. C.; Marsh, M. M.; Neuss, N. *J. Am. Chem. Soc.* **1968**, *90*, 2980–2982.
3. Neuss, N.; Nagarajan, R.; Molloy, B. B.; Huckstep, L. L. *Tetrahedron Lett.* **1968**, 4467–4471.
4. Nagarajan, R.; Neuss, N.; Marsh, M. M. *J. Am. Chem. Soc.* **1968**, *90*, 6518–6519.
5. Kleinwachter, P.; Dahse, H.-M.; Luhmann, U.; Schlegel, B.; Dornberger, K. *J. Antibiot.* **2001**, *54*, 521–525.
6. Tan, R. X.; Jensen, P. R.; Williams, P. G.; Fenical, W. *J. Nat. Prod.* **2004**, *67*, 1374–1382.
7. Fukuyama, T.; Kishi, Y. *J. Am. Chem. Soc.* **1976**, *98*, 6723–6724.
8. Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045–2078.
9. Murdock, K. C. *J. Med. Chem.* **1974**, *17*, 827–835.
10. Ho, P. P. K.; Walters, C. P. *Ann. N.Y. Acad. Sci.* **1970**, *173*, 438–443.
11. Ottenheijm, H. C. J.; Herscheid, J. D. M.; Tijhuis, M. W.; Nivard, R. J. F.; De Clercq, E.; Prick, P. A. J. *J. Med. Chem.* **1978**, *21*, 799–804.
12. Ottenheijm, H. C. J.; Herscheid, J. D. M.; Tijhuis, M. W.; Oosterbaan, M.; De Clercq, E. *J. Med. Chem.* **1978**, *21*, 796–799.
13. Ottenheijm, H. C. J. *Chem. Ber.* **1978**, *111*, 2064–2065.
14. Chai, C. L. L.; Elix, J. A.; Huleatt, P. B.; Waring, P. *Bioorg. Med. Chem.* **2004**, *12*, 5991–5995.
15. Ernst-Russel, M. A.; Chai, C. L. L.; Hurne, A. M.; Waring, P.; Hockless, D. C. R.; Elix, J. A. *Aust. J. Chem.* **1999**, *52*, 279–283.
16. Mori, M. Ene–Yne Metathesis. In *Handbook of Metathesis*; Grubbs, R. H., Ed.; Wiley-VCH: Weinheim, 2003; Vol. 2, pp 176–204.
17. Poulsen, C. S.; Madsen, R. *Synthesis* **2003**, 1–18.
18. Diver, S. T.; Giessert, A. J. *Chem. Rev.* **2004**, *104*, 1317–1382.
19. Stragies, R.; Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed.* **1997**, *36*, 2518–2520.
20. Galan, B. R.; Giessert, A. J.; Keister, J. B.; Diver, S. T. *J. Am. Chem. Soc.* **2005**, *127*, 5762–5763.
21. Smulik, J. A.; Diver, S. T. *Tetrahedron Lett.* **2001**, *42*, 171–174.
22. Kulkarni, A. A.; Diver, S. T. *Org. Lett.* **2003**, *5*, 3463–3466.
23. Kulkarni, A. A.; Diver, S. T. *J. Am. Chem. Soc.* **2004**, *126*, 8110–8111.
24. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360–11370.

25. Middleton, M. D.; Diver, S. T. *Tetrahedron Lett.* **2005**, *46*, 4039–4043.
26. O'Donnell, M. J. *Acc. Chem. Res.* **2004**, *37*, 506–517.
27. O'Donnell, M. J.; Delgado, F. *Tetrahedron* **2001**, *57*, 6641–6650.
28. Ooi, T.; Maruoka, K. *Acc. Chem. Res.* **2004**, *37*, 526–533.
29. Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **1999**, *121*, 6519–6520.
30. Lygo, B.; Humphreys, L. D. *Tetrahedron Lett.* **2002**, *43*, 6677–6679.
31. Park, H.-g.; Jeong, B.-S.; Yoo, M.-S.; Lee, J.-H.; Park, M.-k.; Lee, Y.-J.; Kim, M.-J.; Jew, S.-s. *Angew. Chem., Int. Ed.* **2002**, *41*, 3036–3038.
32. Jew, S.-s.; Jeong, B.-S.; Yoo, M.-S.; Huh, H.; Park, H.-g. *Chem. Commun. (Cambridge)* **2001**, 1244–1245.
33. Corey, E. J.; Xu, F.; Noe, M. C. *J. Am. Chem. Soc.* **1997**, *119*, 12414–12415.
34. Okino, T.; Takemoto, Y. *Org. Lett.* **2001**, *3*, 1515–1517.
35. Previously, we have shown that we can decrease catalyst loadings on scale-up.
36. Rodriguez-Conesa, S.; Candal, P.; Jimenez, C.; Rodriguez, J. *Tetrahedron Lett.* **2001**, *42*, 6699–6702.
37. We have shown that the methylene-free metathesis is a scaleable procedure. See: Kulkarni, A. A.; Diver, S. T. *Org. Synth.*, in press.
38. Ahn, Y. M.; Yang, K.; Georg, G. I. *Org. Lett.* **2001**, *3*, 1411–1413.
39. While this manuscript was under review, we found that lower catalyst loadings can be used at higher concentrations of 1,5-cyclooctadiene. For the same substrate in Eq. 5, we utilized 7.5 mol % ruthenium carbene catalyst and 9 equiv COD to obtain an 80% isolated yield of cyclohexadiene product **14**. See: Peppers, B. P.; Kulkarni, A. A.; Diver, S. T. *Org. Lett.* **2006**, *8*, 2539–2542.
40. Bäckvall, J.-E. *Metal-Catalyzed Cross-Coupling Reactions*; Stang, P. J., Diedrich, F., Eds.; Wiley-VCH: Weinheim, 1998; pp 339–385.
41. Andersson, P. G.; Bäckvall, J.-E. *Handbook of Organopalladium Chemistry for Organic Synthesis*; Negishi, E.-i., Ed.; Wiley: Hoboken, NJ, 2002; Vol. 2, pp 1859–1874.
42. Bäckvall, J. E.; Andersson, P. G. *J. Am. Chem. Soc.* **1990**, *112*, 3683–3685.
43. Bäckvall, J. E.; Andersson, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 6374–6381.
44. Bäckvall, J. E.; Nystroem, J. E.; Nordberg, R. E. *J. Am. Chem. Soc.* **1985**, *107*, 3676–3686.
45. Bäckvall, J. E.; Nordberg, R. E.; Nystroem, J. E. *Tetrahedron Lett.* **1982**, *23*, 1617–1620.
46. Bäckvall, J. E.; Vaagberg, J. O. *Org. Synth.* **1990**, *69*, 38–43.
47. Banker, R.; Carmeli, S. *Tetrahedron* **1999**, *55*, 10835–10844.
48. Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350–3354.
49. Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.
50. The Schlenk flask is fitted with a rubber septum on the sidearm. This septum accommodates the argon line and the needle from the syringe pump.