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Nature-inspired approaches to chemical synthesis

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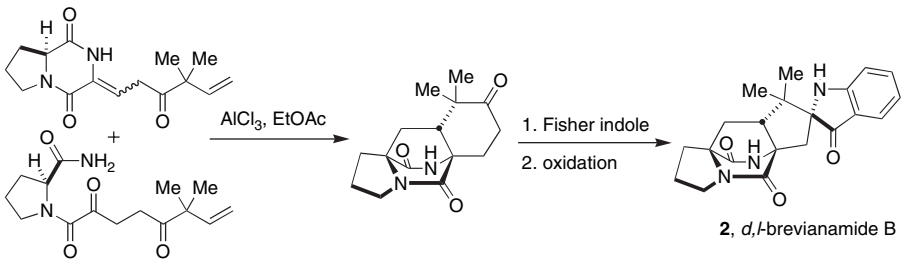
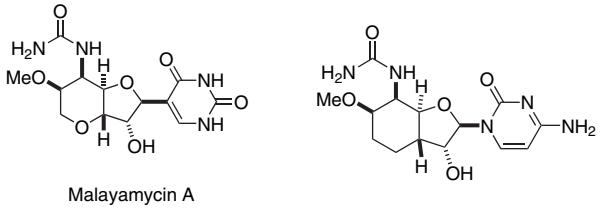
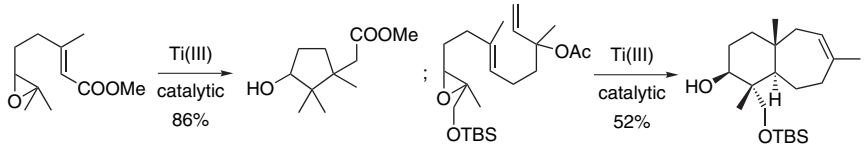
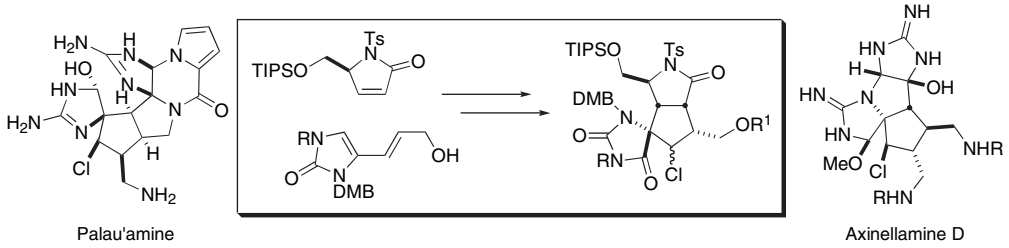
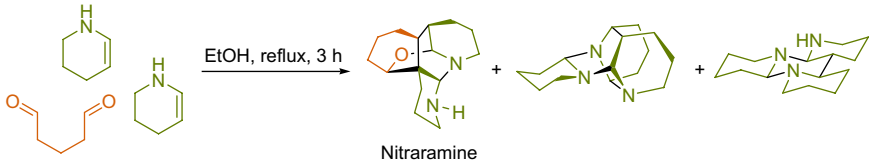
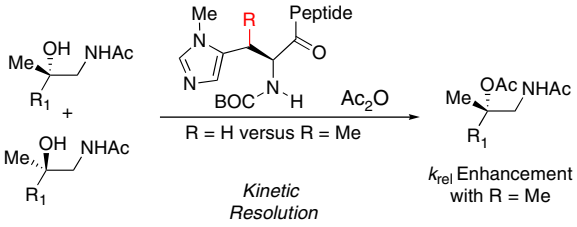




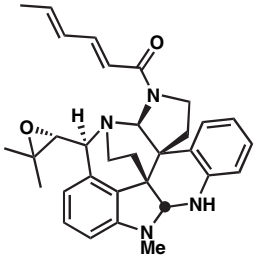


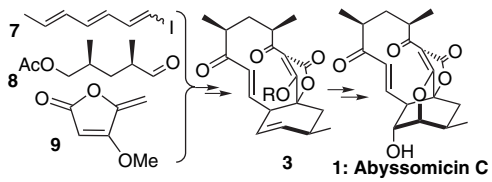




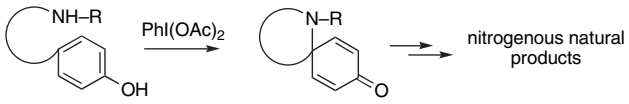


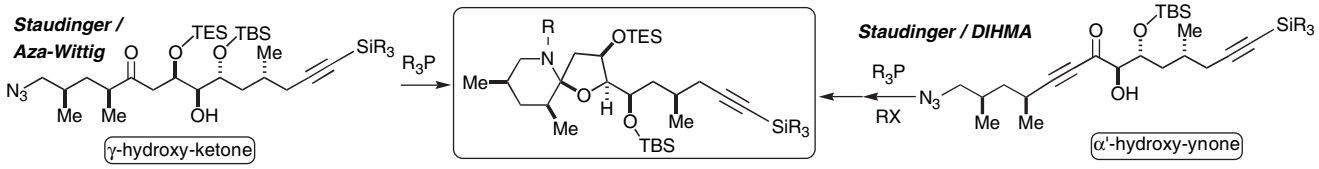
A comparison is made between the calycanthaceous alkaloids, nomofungin, and the communesins using structural and biosynthetic information from studies of the former to shed light on the structural ambiguity of the two latter species.

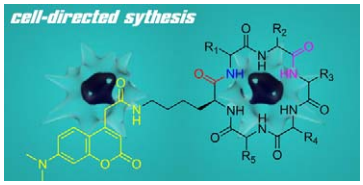




Preparation of key intermediate **3**, by assembling fragments **7**–**9**, is described. In the last step, isomerization of the *Z* to the desired *E* isomer and concomitant intramolecular Diels-Alder cyclization furnished **3** as a single isomer in high yield.







By the appropriate placement within a synthetic scheme, cells can be used not only as an endpoint in probe development but also as practical vehicle to identify and process materials. Examples developed in this study illustrate the synthesis of probes whose intracellular trafficking are regulated by metabolic processing. The development of fluorescent cyclic peptide and depsipeptide probes can now be directed by cells.

*Corresponding author

COVER

The cover picture highlights the stereocontrolled polycyclization of squalene oxide to dammaradienol. This reaction, which represents a central step in the biosynthesis of steroids, has inspired the development of biomimetic polyolefinic cyclizations as viable strategies toward the synthesis of polycyclic natural products. Ideas about the structural origin and biogenesis of natural products are at the heart of biomimetic or nature-inspired syntheses and constitute the theme of this *Tetrahedron* Symposium-in-Print.

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Preface

Nature-inspired approaches to chemical synthesis

The roots of 'nature-inspired' chemical synthesis are found in Sir Robert Robinson's influential synthesis of tropinone. This achievement brimmed with modern ideas about synthesis design, featured a spontaneous union of succindialdehyde, methylamine, and acetone dicarboxylic acid, and clearly showed that efforts to find nature's way of working can lead to powerful syntheses of complex molecular architectures. As an early example of a covalent self-assembly process, Robinson's tropinone synthesis may be viewed as a forerunner of a large and growing number of achievements in organic synthesis that are distinguished by efficient, even beautiful, complexity-generating molecular transformations. While many of these achievements were not meant to be nature-inspired, others were clearly founded on ideas about the structural origins of natural products. Fifty years ago, two landmark publications, 'The Stereochemical Interpretation of the Biogenetic Isoprene Rule for the Triterpenes' by Eschenmoser, Ruzicka, Jeger, and Arigoni and 'The Stereochemistry of Polyene Cyclization' by Stork and Burgstahler, clearly explained the stereospecific, cascade cyclization behavior that squalene and related polyolefins are inclined to undergo. These papers preceded the discovery of the enzymes that nature uses to transmute squalene and oxidosqualene to the polycyclic triterpenes and the incisive chemical reasoning that they contain provided a theoretical and empirical foundation for the magnificent accomplishments in steroid synthesis by the groups of W. S. Johnson, E. van Tamelen, and subsequent generations of organic chemists. These pioneering studies are deeply inspirational to those who have harnessed the reactivity of polyunsaturated molecules in syntheses of constitutionally and stereochemically complex polycycles.

Of course, biomimetic, carbocation-initiated π-cyclizations constitute just one type of reaction that has had a broad impact on the field of chemical synthesis. Corey's early synthesis of friedelin featuring a remarkable cascade of stereospecific Wagner–Meerwein shifts, Barton's fundamental studies of oxidative phenolic radical couplings that culminated in his elegant, two-step synthesis of usnic acid, Woodward's dramatic porphyrin $\rightarrow$ chlorin transformation in his synthesis of the plant pigment chlorophyll, Eschenmoser's experiments concerning the structural origin of vitamin B_{12}, Nicolaou's biomimetic syntheses of the endiandric acids featuring cascades of pericyclic reactions, and Heathcock's brilliant conversion of dihydrosqualene dialdehyde to the complex molecular architecture of the *Daphniphyllum* alkaloids are also salient achievements that have inspired others to derive lessons from nature in their own work in organic synthesis.

Organic chemists continue to be remarkably creative in how they take clues from nature in the development of important, new chemical reactions and innovative ideas for performing syntheses of structurally complex natural products. The theme of this *Symposium-in-Print* appeals to many scientists who are actively engaged in research problems at the frontiers of the field of organic chemistry. As the co-editors of this special issue, we are deeply indebted to all colleagues who kindly agreed to convey aspects of their exciting research that touches on this theme. The research articles described herein show how inspirations from nature continue to strengthen the dynamic field of chemical synthesis. As this fundamental part of organic chemistry continues to evolve into increasingly powerful states, we anticipate that Robinson's legacy of building molecular complexity rapidly with the help of a few 'nature-inspired' ideas will be reflected in many more future achievements. In the course of organizing this *Symposium-in-Print*, we received expert assistance from Ms. Teresa Abendroth-Silva; we are sincerely grateful to her as well.

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Synthesis of the 5-hydroxymethyl-6-aryl-8-oxabicyclo[3.2.1]oct-3-en-2-one natural products descurainin and cartorimine

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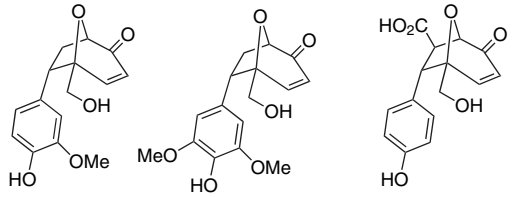
Abstract—Reaction of pyranulose **6** with styrenes **12c** or **13** and Et_3N in CH_2Cl_2 at 25 °C afforded the [5+2] cycloadducts **14c** and **15**, which were hydrolyzed to give the natural products **1** and descurainin (**2**) in 24 and 27% overall yield, respectively. Heating pyranulose **6** with cinnamate ester **21** in the presence of 2,6-di-*t*-butylpyridine in CH_3CN at 175 °C afforded the [5+2] cycloadduct, which was hydrolyzed to give cartorimine (**3**) in 13% yield.

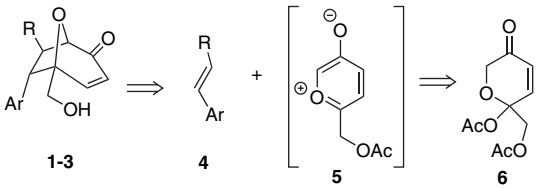
1. Introduction

Wen, He, Xue and Cao isolated the 8-oxabicyclo[3.2.1]oct-3-ene-2-one **1** in 1986 from *Ligusticum chuanxing*.[1] Li and co-workers isolated descurainin (**2**) with an additional methoxy group from the seeds of *Descurainia sophia* (L.) Webb ex Prantl which are used as a Chinese traditional medicine.[2] The structures of both the compounds were assigned by spectroscopic analysis.[3] Yin, He and Ye isolated the oxabicyclic acid cartorimine (**3**) from *Carthamus tinctorius* L., which is used as a traditional Chinese medicine to promote blood circulation. The structure was established from extensive NMR spectral data interpretation and single crystal X-ray analysis.[4,5]



1 **2** (descurainin) **3** (cartorimine)

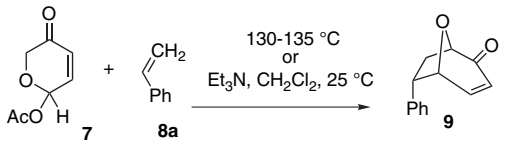
We thought that compounds **1**–**3** could be prepared by the [5+2] cycloaddition of the appropriate styrene derivative **4** with oxypyrylium zwitterion **5**, which could be generated in situ from pyranulose **6** (Scheme 1). This sequence is probably related to the biosynthesis of **1**–**3** because the required styrenes are natural products and **6** is generated by the dehydration and oxidation of fructose.[6]



1-3 **4** **5** **6**

Scheme 1. Retrosynthesis of **1**–**3**.

Hendrickson and Farina discovered that these [5+2] cycloadditions can be carried out by simply heating **7** and a dipolarophile at 130–135 °C to afford adducts analogous to **9** (Scheme 2).[7a] This reaction has been extensively developed by Sammes, who found that electron rich dipolarophiles were more reactive and that the reactions can also be carried out using Et_3N to generate the oxypyrylium zwitterion at room temperature.[8] Further examples of [5+2] cycloadditions have been reported by Heathcock and Ohmori.[9–11] Sammes reported that reaction of **7**, styrene (**8a**, 6 equiv) and Et_3N in CH_2Cl_2 at 25 °C afforded 65% of **9**, which lacks the hydroxymethyl and aryl substituents of **1** and descurainin (**2**).[8b] Oxypyrylium zwitterion **5** had not been previously prepared, but **6** should react similarly to **7** in these reactions. The para oxygen substituents on styrenes **12** and **13** should make them more electron rich and therefore more reactive than styrene (**8a**) itself.



7 **8a** **9**

Scheme 2.

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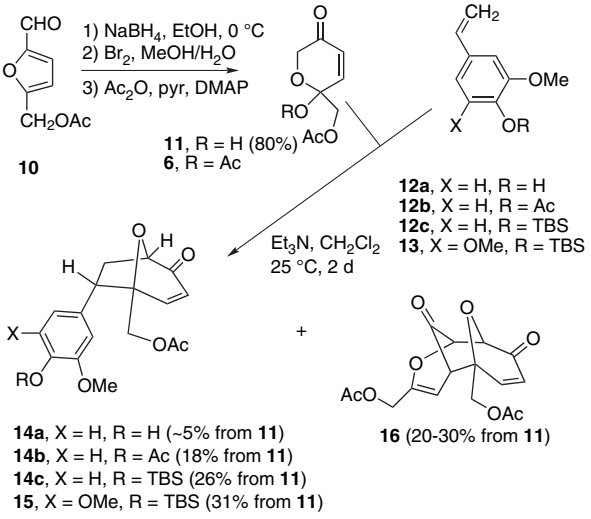
2. Results and discussion

5-(Acetoxymethyl)furfural (**10**) was reduced with $NaBH_4$ in EtOH for 10 min at 0 °C. The solution was quenched dropwise with HOAc and concentrated. The residue was taken up in water and treated with bromine in MeOH to give 80% of **11**.[6,12] Acetylation with Ac_2O, pyridine, and DMAP in CH_2Cl_2 afforded the unstable acetate **6**, which was used without purification (Scheme 3). 4-Hydroxy-3-methoxystyrene (**12a**) was prepared by decarboxylation of the corresponding cinnamic acid with Cu powder and quinoline at 210–240 °C.[13] Acetylation of **12a** with Ac_2O, DMAP, and pyridine gave **12b**[14] in 92% yield, and reaction of **12a** with TBSCl and imidazole afforded **12c**[14] in 91% yield. Styrene derivative **13**[15] was prepared in 99% yield by a Wittig reaction on the corresponding benzaldehyde.

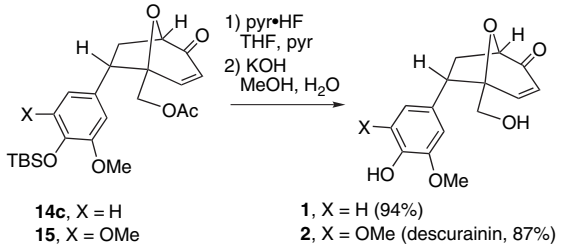


Scheme 3. Preparation of adducts **14** and **15**.

Our initial attempt at cycloaddition by treating **6** and **12a** with 2 equiv of Et_3N in CH_2Cl_2 at 25 °C afforded only ~5% of the desired adduct **14a** (see Scheme 3). We suspected that the free phenol interfered with this reaction, so we examined the reaction of **6** with the acetate **12b**, which gave the desired adduct **14b** in 18% yield. Use of TBS ether **12c** gave adduct **14c** in better (26%) yield as expected from Sammes observations that more electron rich dipolarophiles are more reactive.[8] The yield of cycloadduct **15** from TBS ether **13** improved to 31%. These reactions were carried out using 1 equiv of both **6** and **12** or **13**. Using 6 equiv of styrene **12** or **13** as reported by Sammes for the reaction of **7** and styrene (**8a**) did not improve the yield significantly, complicated purification, and was wasteful of styrene **12** or **13**.

No isomeric adducts were detected, but we did obtain 20–30% of a non-polar dimer tentatively assigned structure **16** based on similar dimers obtained from **7** with Et_3N in CH_2Cl_2 at 25 °C by Hendrickson and Farina[7b,16] and 10–15% of a polar dimer that was not fully characterized. These dimers were most easily isolated by treatment of **6** with Et_3N in CH_2Cl_2 in the absence of dipolarophile, which gave dimer **16** in 25% yield and the uncharacterized dimer in 13% yield. The uncharacterized dimer has two CH_2OAc groups, but no alkene protons. Formation of dimer **16** was suppressed by slow addition of **6** over 1 d to a solution of **13** and Et_3N in CH_2Cl_2. However, the yield of **15** did not improve significantly. We obtained lower yields of **15** at 0 or 60 °C in a sealed tube. Yields also decreased using CH_3CN as the solvent or *i*-Pr(Et_2)N as the base.

Hydrolysis of the TBS ether of **14c** with pyridine·HF in THF and pyridine, followed by hydrolysis of the acetate ester with KOH in aqueous MeOH afforded natural product **1** in 94% yield (see Scheme 4). The ^{13}C NMR spectrum in pyridine-d_5 corresponds well to that reported,[1] except that all peaks absorb downfield by 0.4–0.6 ppm from the literature data. The ^1H NMR spectrum in $CDCl_3$ does not correspond well to that reported, but the spectrum in pyridine-d_5 does correspond well to literature data suggesting that it may have been recorded in pyridine-d_5, rather than $CDCl_3$ as indicated.[1]



Scheme 4. Preparation of **1** and descurainin (**2**).

Similar hydrolysis of **15** provided 87% of descurainin (**2**) with spectral data in DMSO-d_6 identical to those reported. The *endo* stereochemistry of the synthetic material was expected based upon earlier studies with **7**.[7–11] It was confirmed unambiguously by the NOEs between the aromatic hydrogens and H_2 and H_3 (see Fig. 1). The two H_7's can be assigned based on their coupling constants to H_1. H_{7exo} is coupled to H_1 with J=8.9 Hz (25°), while H_{7endo} is coupled to H_1 with J=<1 Hz (94°). The coupling constants between H_6 and H_{7endo} (J=7.3 Hz, 130°) and H_{7exo} (J=10.1 Hz, 8°) are consistent with those expected. An NOE between H_6 and H_{7exo} and a much larger NOE between H_1 and H_{7exo} than between H_1 and H_{7endo} confirm the stereochemical assignment. The assignment of the opposite stereochemistry in natural descurainin appears to result from switched assignments for the two H_7's.[2]

We examined the reaction of **6** with styrene (**8a**) to compare the reactivity of the oxypyrylium zwitterions formed from **6** and **7** because Sammes obtained **9** in 65% yield from **7** and styrene (6 equiv). Reaction of **6** and styrene (1 equiv) with Et_3N in CH_2Cl_2 gave only 14% of a 7:1 mixture of *endo*

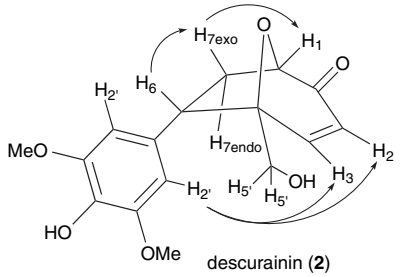
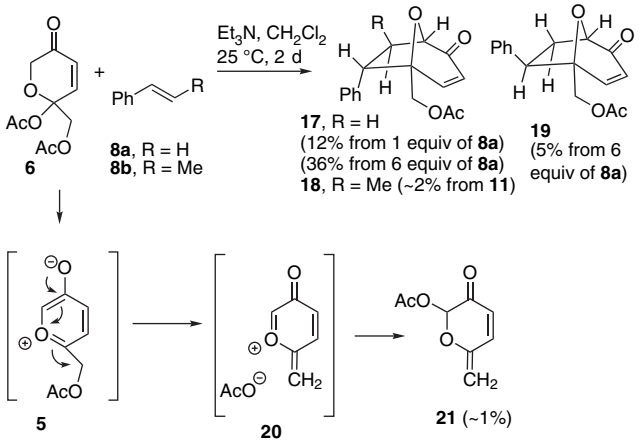


Figure 1. NOE's in synthetic descurainin (**2**).

adduct **17** and the unexpected *exo* adduct **19**. We isolated ~1% of acetoxy dienone **21** as a byproduct in these reactions. The ^{1}H NMR spectral data of **21** are similar to those of analogous compounds.[17–19] Oxypyrylium zwitterion **5** can eliminate acetate to give **20**. Attack of acetate on the cationic center will give acetoxy dienone **21**. Even though **21** is isolated in only 1% yield, this may be a major reaction pathway since **21** should polymerize readily under the basic reaction conditions. The competing formation of **21** may be responsible for the lower yields of [5+2] cycloadducts obtained from **6** than from **7**. Heathcock postulated that 2-methyl-6-methylene-2*H*-pyran-3(6*H*)-one, which differs from **21** only in the 2-substituent, was formed from 2,6-dimethyl-3-oxypyrylium zwitterion by internal proton transfer.[9] This compound was not isolated, but the dimer formed by the [5+2] cycloaddition of the *exo*-methylene group to the oxypyrylium zwitterion was formed in 52% yield.

Reaction of **6** with styrene (**8a**, 6 equiv) under Sammes conditions gave 41% of a 7:1 mixture of **17** and **19** indicating that lower yields of [5+2] cycloadducts are obtained from the oxypyrylium zwitterion **5** obtained from **6** (41% of **17** and **19**) than from the parent oxypyrylium zwitterion obtained from **7** (65% of **9**).[8b] Careful chromatography provided 36% of a ~10:1 mixture rich in the *endo* adduct **17** and 5% of a ~10:1 mixture rich in the *exo* adduct **19**, whose structure was established by COSY and 1D NOESY experiments. The spectra of **1**–**3**, **14**, **15**, and **17** are very similar indicating that they all have the same stereochemistry, whereas the chemical shifts and coupling constants of **19** are quite different (Scheme 5).

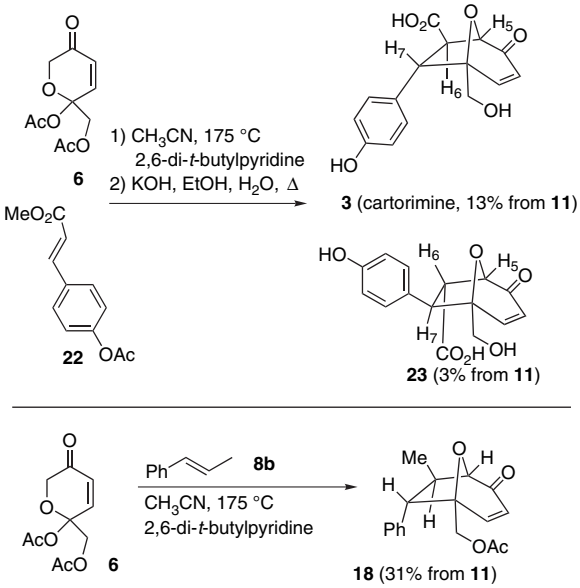


Scheme 5.

A competition experiment was carried out by reaction of **6** and Et_3N with 1 equiv of both **13** and styrene (**8a**). We obtained a 5:1 mixture of **15** and **17**, indicating that the sigma withdrawing, but pi donating, *para* oxygen substituent on the phenyl ring of **13** makes it five times as reactive as the parent styrene.

We now turned our attention to the preparation of cartorimine (**3**), which required the use of a less reactive cinnamate dipolarophile. Introduction of even a methyl substituent on the double bond of the styrene dipolarophile decreases its reactivity. Reaction of **6** with 6 equiv β-methylstyrene (**8b**) and Et_3N at 25 °C gave adduct **18** in only ~2% yield.

Acetoxy ester **22** was prepared in 94% yield from 4-hydroxycinnamic acid by esterification with methanolic HCl at reflux and acetylation with Ac_2O, pyridine, and DMAP in CH_2Cl_2. Reaction of **6** and **22** with Et_3N in CH_2Cl_2 at 25 °C or with $EtN(i\text{-}Pr)_2$ in CH_3CN at 80 °C did not afford the desired cycloadduct. Thermal reaction in CH_3CN at 150–175 °C was more successful, but not completely reproducible. Eventually, we concluded that residual pyridine from the preparation of **22** was important for the success of the reaction. Heating a 0.2 M solution of crude **6** in CH_3CN with 6 equiv of **22** and 1 equiv of 2,6-di-*t*-butylpyridine in a sealed tube in a 175 °C oil bath for 14 h afforded the crude bis acetoxymethyl ester of **3**. Hydrolysis with KOH in 4:1 EtOH/H_2O at reflux for 20 h and preparative TLC afforded 16% (from **11**) of a 4:1 mixture of cartorimine (**3**) and the stereoisomer **23**, which were separated by reverse phase HPLC. A similar reaction using pyridine, instead of 2,6-di-*t*-butylpyridine, afforded only 4% of a 3:1 mixture of **3** and **23**. The analogous cycloaddition of **6** with **8b** (6 equiv) provided 31% (from **11**) of **18** regio- and stereospecifically, confirming that the electron-withdrawing carbomethoxy group of **22** retards the reaction (Scheme 6).



Scheme 6. Preparation of cartorimine (**3**) and **18**.

The spectral data of **3** are identical to those previously reported.[4] Small NOEs from the aromatic hydrogens to the hydroxymethyl group of both **3** and **23** established that the minor product is a stereo- rather than a regioisomer. The vicinal coupling constants support this assignment. $J_{H5,H6}$= 1.5 Hz in **3** and 7.9 Hz in **23**, while $J_{H6,H7}$=7.5 Hz in **3** and 4.3 Hz in **23**. These coupling constants are consistent with those expected from MM2 calculations and analogous to those in the related stereoisomeric adducts formed from oxypyrylium zwitterions and dimethyl fumarate.[10]

Although natural products **1**–**3** are probably biosynthesized by similar [5+2] cycloadditions of achiral compounds, they were isolated with small $[\alpha]_D$ (+1.7° for **2**, −2.6° for **3**) or $\Delta\varepsilon$ (+0.01 for **1**) indicating that they are not completely racemic. However, a [5+2] cycloaddition in a chiral environment could lead to an optically enriched product as was observed.

In conclusion, we have completed the first syntheses of **1**, descurainin (**2**), and cartorimine (**3**) using a possibly biomimetic [5+2] cycloaddition to efficiently construct the fully functionalized 8-oxabicyclo[3.2.1]octenone skeleton. Reaction of **6** with styrenes **8a**, **12**, or **13** and Et_3N in CH_2Cl_2 proceeds at 25 °C, while reaction of **6** with cinnamate ester **21** is best carried out at 175 °C with 2,6-di-*t*-butylpyridine as a proton scavenger.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 400 MHz in $CDCl_3$ unless otherwise indicated, chemical shifts are reported in δ, and coupling constants in Hertz. The silica gel used for chromatography was deactivated with methanol unless otherwise indicated. IR spectra are reported in cm^{-1}.

3.1.1. 6-[(Acetyloxy)methyl]-6-hydroxy-2*H*-pyran-3(6*H*)-one (11). A solution of 5-acetoxymethyl-2-furancarboxyaldehyde (**10**, 1.006 g, 5.98 mmol) in EtOH (17 mL) was added to a suspension of $NaBH_4$ (113 mg, 2.99 mmol) in EtOH (13 mL) at 0 °C and the resulting solution was stirred for 10 min at 0 °C. HOAc was added dropwise to quench the reaction and the EtOH was removed under reduced pressure. The brown residue was dissolved in H_2O (40 mL) and a solution of Br_2 (0.31 mL, 6.0 mmol) in MeOH (3 mL) was added dropwise. After 2 h, the solution was basified to pH 5 with saturated $NaHCO_3$ solution. The resulting aqueous solution was saturated with NaCl and extracted with EtOAc (4×40 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure (<30 °C) to give 894 mg (80%) of **11** with data identical to those previously reported.[6,12]

3.1.2. 6-Acetyloxy-6-[(acetyloxy)methyl]-2*H*-pyran-3(6*H*)-one (6). Ac_2O (20 mL), dry pyridine (10 mL), and DMAP (130 mg, 1.06 mmol) were added in succession to a solution of **11** (1.967 g, 10.6 mmol) in dry CH_2Cl_2 (120 mL) under N_2 and the resulting solution was stirred for 30 min at 0 °C. The solution was washed with 10% $CuSO_4$ solution (100 mL), H_2O (100 mL), saturated $NaHCO_3$ solution (100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give 1.541 g of crude brown **6** (~90% pure) that was used directly for the cycloadditions: ^1H NMR 7.24 (d, 1, *J*=10.4), 6.23 (d, 1, *J*=10.4), 4.61 (d, 1, *J*=17.4), 4.56 (d, 1, *J*=11.6), 4.44 (d, 1, *J*=11.6), 4.31 (d, 1, *J*=17.4), 2.11 (s, 3), 2.10 (s, 3); ^{13}C NMR 193.3, 170.2, 169.4, 143.4, 128.2, 97.4, 68.1, 65.1, 21.3, 20.8.

3.1.3. 2-Methoxy-4-vinylphenol acetate (12b).[14] Ac_2O (2.8 mL), dry pyridine (1.4 mL), and DMAP (17 mg, 0.140 mmol) were added in succession to a solution of 2-methoxy-4-vinylphenol (**12a**)[13] (210 mg, 1.40 mmol) in dry CH_2Cl_2 (14 mL) under N_2 and the resulting solution was stirred for 35 min at 0 °C. The solution was washed with 10% $CuSO_4$ solution (15 mL), saturated $NaHCO_3$ solution (15 mL), and brine (15 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give 248 mg (92%) of **12b** as a yellow oil: ^1H NMR 7.01 (d, 1, *J*=1.2), 6.99 (br s, 2), 6.68 (dd, 1, *J*=17.6, 10.8), 5.70 (d, 1, *J*=17.6), 5.25 (d, 1, *J*=10.8), 3.86 (s, 3), 2.32 (s, 3); ^{13}C NMR 169.1, 151.0, 139.3, 136.6, 136.2, 122.7, 118.9, 114.1, 109.8, 55.8, 20.6; IR (neat) 1764.

3.1.4. (1,1-Dimethylethyl)(4-ethenyl-2-methoxyphenoxy)dimethylsilane (12c).[14] TBSCl (524 mg, 3.48 mmol) and imidazole (494 mg, 7.26 mmol) were added in succession to a solution of 2-methoxy-4-vinylphenol (**12a**)[13] (435 mg, 2.90 mmol) in dry CH_2Cl_2 (15 mL) under N_2 and the resulting solution was stirred for 2 h at 25 °C. The solution was diluted with CH_2Cl_2 (20 mL), washed with brine (2×20 mL) and dried over $MgSO_4$. The solution was concentrated under reduced pressure to give 762 mg of a yellow liquid. Flash chromatography on silica gel (9:1 hexanes/EtOAc) yielded 698 mg (91%) of **12c** as a yellow oil: ^1H NMR 6.93 (d, 1, *J*=1.8), 6.87 (dd, 1, *J*=8.2, 1.8), 6.80 (d, 1, *J*=8.2), 6.64 (dd, 1, *J*=17.1, 10.4), 5.60 (d, 1, *J*=17.1), 5.14 (d, 1, *J*=10.4), 3.83 (s, 3), 0.99 (s, 9), 0.15 (s, 6); ^{13}C NMR 150.9, 145.1, 136.6, 131.5, 120.8, 119.3, 111.7, 109.5, 55.4, 25.7 (3C), 18.4, −4.7 (2C); IR (neat) 1414, 1278.

3.1.5. 4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-3,5-dimethoxybenzaldehyde.[15] TBSCl (1.015 g, 6.73 mmol) and imidazole (952 mg, 14.0 mmol) were added in succession to a solution of 4-hydroxy-3,5-dimethoxybenzaldehyde (1.022 g, 5.61 mmol) in dry CH_2Cl_2 (40 mL) under N_2. The resulting solution was stirred for 30 min at 25 °C. The solution was diluted with CH_2Cl_2 (60 mL), washed with brine (2×100 mL) and dried over $MgSO_4$. The solution was concentrated under reduced pressure to give 1.980 g of a clear solid. Flash chromatography on silica gel (4:1 hexanes/EtOAc) yielded 1.261 g (76%) of the protected aldehyde as a white solid: mp 69–71 °C; ^1H NMR 9.83 (s, 1), 7.10 (s, 2), 3.87 (s, 6), 1.01 (s, 9), 0.16 (s, 6); ^{13}C NMR 191.0, 151.9 (2C), 140.6, 129.3, 106.6 (2C), 55.8 (2C), 25.6 (3C), 18.8, −4.6 (2C); IR (KBr) 1684.

3.1.6. (1,1-Dimethylethyl)(4-ethenyl-2,6-dimethoxyphenoxy)dimethylsilane (13).[15] LiHMDS (1.0 M) in THF (3.5 mL) was added dropwise to a suspension of $MeP(Ph)_3Br$ in dry THF (20 mL) at 0 °C under N_2 and the resulting solution was allowed to stir at 0 °C for 5 min. The above aldehyde (733 mg, 2.47 mmol) in dry THF (8 mL) was added dropwise to the cooled solution and the resulting solution was stirred for 4 h at 25 °C. Saturated NH_4Cl solution (40 mL) was added to the solution and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3×40 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography on silica gel (9:1 hexanes/EtOAc) yielded 681 mg (99%) of **13** as a clear solid: mp 31–33 °C; ^1H NMR 6.62 (dd, 1, *J*=17.4, 11.0), 6.61 (s, 2), 5.61 (d, 1, *J*=17.4), 5.15 (d, 1, *J*=11.0), 3.81 (s, 6), 1.00 (s, 9), 0.13 (s, 6); ^{13}C NMR 151.6 (2C), 137.0, 134.5, 130.3, 111.9, 103.4 (2C), 55.7 (2C), 25.8 (3C), 18.7, −4.6 (2C); IR (KBr) 1409, 1253.

3.1.7. *endo*-5-[(Acetyloxy)methyl]-6-(4-acetyloxy-3-methoxyphenyl)-8-oxabicyclo[3.2.1]oct-3-en-2-one (14b). Et_3N (0.16 mL, 1.1 mmol) was added dropwise to a solution of crude **6** (128 mg, 0.561 mmol) and **12b** (108 mg, 0.561 mmol) in dry CH_2Cl_2 (2 mL) under N_2 at 0 °C. The resulting solution was stirred at 25 °C for 2 d and was

concentrated under reduced pressure. Flash chromatography on silica gel (4:1–1:1 hexanes/EtOAc) yielded 36 mg (18% from **11**) of **14b** as a yellow wax: ^{1}H NMR 6.96 (d, 1 J=8.0), 6.74 (d, 1, J=9.8), 6.73–6.72 (m, 2), 6.29 (d, 1, J=9.8), 4.69 (br d, 1, J=8.9), 4.49 (d, 1, J=12.2), 4.31 (d, 1, J=12.2), 3.80 (s, 3), 3.52 (dd, 1, J=9.8, 7.0), 3.00 (ddd, 1, J=13.7, 9.8, 8.9), 2.31 (s, 3), 2.10 (s, 3), 2.04 (br dd, 1, J=13.7, 7.0); ^{13}C NMR 196.1, 170.6, 168.9, 151.1, 151.0, 139.3, 135.0, 128.6, 122.9, 120.8, 112.8, 83.5, 80.8, 64.8, 55.9, 49.7, 34.1, 20.7, 20.6; IR (neat) 1765, 1745, 1703.

3.1.8. *endo*-5-[(Acetyloxy)methyl]-6-[3-methoxy-4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-phenyl]-8-oxabicyclo[3.2.1]oct-3-en-2-one (14c). Et_3N (0.25 mL, 1.8 mmol) was added dropwise to a solution of crude **6** (204 mg, 0.894 mmol) and **12c** (237 mg, 0.896 mmol) in dry CH_2Cl_2 (4 mL) under N_2. The resulting solution was stirred for 2 d at 25 °C and was concentrated under reduced pressure. Flash chromatography on silica gel (9:1 hexanes/EtOAc) gave 26 mg of an impure 5:2:1 mixture of **14c**, **12c**, and **21**, respectively, followed by 129 mg (26% from **11**) of **14c** as a clear wax. Elution with EtOAc gave 19 mg of impure **16**.

Data for **14c**: ^{1}H NMR 6.75 (d, 1, J=8.6), 6.68 (d, 1, J=10.4), 6.60 (m, 2), 6.27 (d, 1, J=10.4), 4.67 (br d, 1, J=8.9), 4.49 (d, 1, J=12.2), 4.28 (d, 1, J=12.2), 3.77 (s, 3), 3.47 (dd, 1, J=9.8, 6.7), 2.97 (ddd, 1, J=13.4, 9.8, 8.9), 2.10 (s, 3), 2.02 (br dd, 1, J=13.4, 6.7), 0.99 (s, 9), 0.14 (s, 6); ^{13}C NMR 196.3, 170.6, 151.4, 150.8, 144.7, 129.2, 128.4, 121.0, 120.8, 112.5, 83.5, 80.8, 65.0, 55.6, 49.4, 33.9, 25.6 (3C), 20.7, 18.4, −4.6 (2C); IR (KBr) 1743, 1704, 1605, 1043; HRMS (DCI/NH_3) calcd for $C_{23}H_{36}NO_6Si$ (MNH_4^+) 450.2312, found 450.2302.

3.1.9. *endo*-5-[(Acetyloxy)methyl]-6-[3,5-dimethoxy-4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-phenyl]-8-oxabicyclo[3.2.1]oct-3-en-2-one (15). Et_3N (0.36 mL, 2.6 mmol) was added dropwise to a solution of crude **6** (292 mg, 1.28 mmol) and **13** (357 mg, 1.28 mmol) in dry CH_2Cl_2 (6 mL) under N_2. The resulting solution was stirred for 2 d at 25 °C and was concentrated under reduced pressure. Flash chromatography on silica gel (4:1 hexanes/EtOAc) gave 18 mg of an impure 2:1 mixture of **15** and **21**, followed by 237 mg (31% from **11**) of **15** as a clear wax. Elution with EtOAc gave 78 mg of an impure 2:1 mixture of **16** and **15**.

Data for **15**: ^{1}H NMR 6.70 (d, 1, J=10.4), 6.29 (s, 2), 4.67 (br d, 1, J=8.9), 4.49 (d, 1, J=11.9), 4.31 (d, 1, J=11.9), 3.75 (s, 6), 3.44 (dd, 1, J=10.1, 7.3), 2.98 (ddd, 1, J=14.0, 10.1, 8.9), 2.10 (s, 3), 2.01 (ddd, 1, J=14.0, 7.3, 1.7), 1.00 (s, 9), 0.12 (s, 6); ^{13}C NMR 196.3, 170.6, 151.54 (2C), 151.46, 134.0, 128.27, 128.25, 105.8 (2C), 83.6, 80.8, 65.0, 55.8 (2C), 49.9, 33.9, 25.7 (3C), 20.7, 18.7, −4.6 (2C); IR (KBr) 1745, 1703, 1588, 1031; HRMS (DCI/NH_3) calcd for $C_{24}H_{38}NO_7Si$ (MNH_4^+) 480.2418, found 480.2398.

3.1.10. (1α,2α,6α,7α)-4,7-Di(acetyloxy)methyl-3,11-dioxa-tricyclo[5.3.1.1.^{2,6}]dodeca-4,8-diene-10,12-dione (16) Et_3N (0.20 mL, 1.4 mmol) was added dropwise to a solution of crude **6** (163 mg, 0.714 mmol) in dry CH_2Cl_2 (3 mL) under N_2 and the resulting solution was stirred for 2 d at 25 °C and was concentrated under reduced pressure. Flash chromatography on silica gel (22:3 hexanes/EtOAc) yielded 39 mg (25% from **11**) of **16** as a clear oil, followed (EtOAc as eluent) by 20 mg (13% from **11**) of a dimer of unknown structure.

Data for **16**: ^{1}H NMR 6.85 (d, 1, J=10.4), 6.33 (d, 1, J=10.4), 5.01 (d, 1, J=9.2), 4.81 (d, 1, J=7.3), 4.80 (dd, 1, J=9.2, 2.9), 4.45 (d, 1, J=13.4), 4.384 (d, 1, J=13.4), 4.38 (d, 1, J=12.2), 4.29 (d, 1, J=12.2), 3.29 (dd, 1, J=7.3, 2.9); ^{13}C NMR 198.3, 189.0, 170.4, 170.2, 150.8, 147.9, 129.6, 100.9, 81.5, 81.4, 75.9, 66.7, 61.7, 48.5, 20.7, 20.6; IR (neat) br 1745, br 1698, 1043.

Data for the dimer of unknown structure: ^{1}H NMR 4.88 (d, 1, J=6.1), 4.74 (d, 1, J=4.3), 4.61 (d, 1, J=13.4), 4.56 (d, 1, J=13.4), 4.46 (br s, 1), 4.27 (d, 1, J=12.2), 4.23 (d, 1, J=12.2), 4.02 (br s, 1), 2.80 (d, 1, J=16.5), 2.72 (dd, 1, J=16.5, 4.3), 2.68 (br d, 1, J=6.1), 2.12 (s, 3), 2.11 (s, 3).

3.1.11. *endo*-6-(4-Hydroxy-3-methoxyphenyl)-5-(hydroxymethyl)-8-oxabicyclo[3.2.1]oct-3-ene-2-one (1). Pyridine·$(HF)_x$ (1.4 M, 3.5 mL) (5 mL of pyridine·$(HF)_x$ in 20 mL of pyridine and 20 mL of THF) was added to a solution of **14c** in THF (5 mL) and the resulting solution was stirred at 25 °C for 12 h. The solution was neutralized with saturated $NaHCO_3$ solution and the resulting aqueous solution was extracted with EtOAc (3×100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give a brown residue. The residue was dissolved in 4:1 MeOH/H_2O (5 mL), KOH (18 mg, 0.321 mmol) was added, and the resulting solution was stirred at 25 °C for 1 h. The solution was acidified to pH 5 using saturated NaH_2PO_4 solution and the MeOH was removed under reduced pressure. The residue was diluted with H_2O (2 mL), saturated with NaCl, and extracted with EtOAc (3×15 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to yield 69 mg (94% from **14c**) of **1** as a tan powder: mp 168–170 °C; ^{1}H NMR (C_5D_5N) 7.19 (d, 1, J=7.9), 7.06 (d, 1, J=9.5), 7.05 (br s, 1), 6.92 (dd, 1, J=7.9, 1.8), 6.46 (dd, 1, J=9.5, 1.2), 5.02 (br, 1, OH), 4.89 (br d, 1, J=8.9), 4.30 (d, 1, J=12.5), 4.13 (d, 1, J=12.5), 3.98 (dd, 1, J=10.1, 7.3), 3.73 (s, 3), 2.90 (ddd, 1, J=12.8, 10.1, 8.9), 2.19 (br d, 1, J=12.8, 7.3); ^{13}C NMR (C_5D_5N) 197.8, 155.0, 148.9, 147.9, 129.2, 128.5, 122.3, 116.9, 113.9, 87.5, 81.5, 63.6, 56.3, 48.1, 34.5; IR (KBr) 3482, 1679, 1608, 1037; UV (EtOH) λ_{max} (log ε) 210 (3.58), 230 (sh 3.53), 282 nm (sh 2.85); HRMS (DCI/NH_3) calcd for $C_{15}H_{20}NO_5$ (MNH_4^+) 294.1341, found 294.1335. The spectral data match well with those reported for the natural product, which was isolated as white needles: mp 184–185 °C; CD $\Delta\varepsilon$ +0.01 (355 nm, MeOH).[1]

3.1.12. *endo*-6-(4-Hydroxy-3,5-dimethoxyphenyl)-5-(hydroxymethyl)-8-oxabicyclo[3.2.1]oct-3-ene-2-one (Descurainin, 2). Pyridine·$(HF)_x$ (1.4 M, 7.0 mL) (5 mL of pyridine·$(HF)_x$ in 20 mL of pyridine and 20 mL of THF) was added to a solution of **15** in THF (10 mL) and the resulting solution was stirred at 25 °C for 12 h. The solution was neutralized with saturated $NaHCO_3$ solution and the resulting aqueous solution was extracted with EtOAc (3× 200 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to a brown residue. The residue was dissolved in 4:1 MeOH/H_2O (10 mL), KOH (18 mg, 0.321 mmol) was added, and the resulting solution was stirred at 25 °C for 1 h. The solution was

acidified to pH 5 using saturated NaH_2PO_4 solution and the MeOH was removed under reduced pressure. The residue was diluted with H_2O (2 mL), saturated with NaCl, and extracted with EtOAc (3×30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to yield 137 mg (87% from **15**) of **2** as a tan powder: mp 183–185 °C; ^{1}H NMR (DMSO-d_6) 8.29 (s, 1, OH), 6.82 (d, 1, J=10.1), 6.41 (s, 2), 6.19 (br d, 1, J=10.1), 5.13 (t, 1, J=6.1, OH), 4.50 (br d, 1, J=8.9), 3.76–3.67 (m, 1), 3.71 (s, 6), 3.60 (dd, 1, J=12.8, 6.1), 3.46 (dd, 1, J=10.1, 7.3), 2.82 (ddd, 1, J=13.1, 10.1, 8.9), 1.92 (br dd, 1, J=13.1, 7.3); ^{13}C NMR (DMSO-d_6) 196.9, 154.5, 147.6 (2C), 134.7, 127.2, 127.1, 106.3 (2C), 85.8, 79.7, 62.3, 56.0 (2C), 47.2, 32.9; HRMS (DCI/NH_3) calcd for $C_{16}H_{22}NO_6$ (MNH_4^+) 324.1447, found 324.1451. The spectral data match those reported for the natural product which was isolated as colorless needles: mp 193–195 °C; $[\alpha]_D^{20}$ +1.7° (*c* 0.23, MeOH).[2] The 1D NOESY experiments showed: (a) NOEs from H_3 at δ 6.82 to the phenyl protons at δ 6.41, H_2 at δ 6.19, and the CH_2OH group at δ 5.13, 3.76–3.67 and 3.60. (b) NOEs from the phenyl protons at δ 6.41 to H_3 at δ 6.82, H_2 at 6.19, H_6 at δ 3.46, and H_{7endo} at δ 1.92. (c) NOEs from H_2 at δ 6.19 to H_3 at δ 6.82 and the phenyl protons at δ 6.41. (d) NOEs from H_1 at δ 4.50 to H_{7exo} at δ 2.82 (larger) and H_{7endo} at δ 1.92 (smaller). (e) NOEs from H_6 at δ 3.46 to the phenyl protons at δ 6.41, the CH_2OH group at δ 5.13, 3.76–3.67, and H_{7exo} at δ 2.82. (f) NOEs from H_{7exo} at δ 2.82 to H_1 at δ 4.50, H_6 at δ 3.46, and H_{7endo} at δ 1.92. (g) NOEs from H_{7endo} at δ 1.92 to the phenyl protons at δ 6.41, H_1 at δ 4.50, and H_{7exo} at δ 2.82.

3.1.13. *endo*-5-[(Acetyloxy)methyl]-6-phenyl-8-oxabicyclo[3.2.1]oct-3-en-2-one (17) and *exo*-5-[(acetyloxy)-methyl]-6-phenyl-8-oxabicyclo[3.2.1]oct-3-en-2-one (19). Et_3N (0.24 mL, 1.7 mmol) was added dropwise to a solution of crude **6** (196 mg, 0.859 mmol) and styrene (0.10 mL, 0.859 mmol, 1 equiv) in dry CH_2Cl_2 (4 mL) under N_2. The resulting solution was stirred for 2 d at 25 °C and was concentrated under reduced pressure. Flash chromatography on silica gel (22:3 hexanes/EtOAc) yielded 37 mg (12% from **11**) of a 7:1 mixture of **17** and **19** as a clear oil.

Purification of an identical reaction on a larger amount of silica gel (9:1 hexanes/EtOAc) gave **17** in lower yield preceded by 2 mg (1% from **11**) of ~90% pure 2-acetoxy-6-methylene-2*H*-pyran-3(6*H*)-one (**21**): ^{1}H NMR 7.15 (d, 1, J=10.4), 6.28 (s, 1), 6.21 (br d, 1, J=10.4), 5.17 (dd, 1, J=1.5, 1.5), 4.91 (br s, 1), 2.12 (s, 3). A 1D NOESY experiment showed NOE's between the *exo*-methylene hydrogen at δ 4.91 and H_3 at δ 7.15 and the other *exo*-methylene hydrogen at δ 5.17.

Et_3N (0.27 mL, 1.97 mmol) was added dropwise to a solution of crude **6** (298 mg, 1.31 mmol) and **8a** (0.90 mL, 7.9 mmol, 6 equiv) in dry CH_2Cl_2 (0.65 mL) under N_2 at 0 °C. The resulting solution was stirred at 25 °C for 16 h and was concentrated under reduced pressure. Flash chromatography on silica gel (22:3 hexanes/EtOAc) gave 162 mg (36% from **11**) of a 10:1 mixture of **17** and **19** as a clear oil, followed by 23 mg (5% from **11**) of a 10:1 mixture of **19** and **17** as a yellow oil.

Data for **17** were determined from the 10:1 mixture: ^{1}H NMR 7.34–7.25 (m, 3), 7.15 (br d, 2, J=7), 6.65 (d, 1, J=10.1), 6.29 (d, 1, J=10.1), 4.70 (br d, 1, J=9.2), 4.51 (d, 1, J=11.9), 4.25 (dd, 1, J=11.9), 3.55 (dd, 1, J=9.8, 6.7), 3.00 (ddd, 1, J=14.0, 9.8, 9.2), 2.10 (s, 3), 2.10–2.05 (m, 1); ^{13}C NMR 196.1, 170.6, 150.9, 136.1, 128.74, 128.66 (4C), 127.8, 83.6, 80.9, 64.9, 49.7, 33.7, 20.7; IR (neat) 1744, 1704, 1602, 1030.

Data for **19** were determined from the 10:1 mixture: ^{1}H NMR 7.35–7.21 (m, 6), 6.11 (d, 1, J=9.8), 4.83 (br d, 1, J=8.5), 4.07 (d, 1, J=11.9), 3.65 (d, 1, J=11.9), 3.42 (dd, 1, J=8.9, 3.4), 2.59 (ddd, 1, J=14.0, 8.5, 3.4), 2.49 (ddd, 1, J=14.0, 8.9, 1.9), 2.02 (s, 3); ^{13}C NMR 196.2, 170.3, 152.9, 139.5, 128.7 (2C), 128.3 (2C), 127.6, 126.2, 84.2, 81.5, 65.3, 49.2, 36.4, 20.6. The 1D NOESY experiments showed: (a) NOEs from H_1 at δ 4.83 to H_{7exo} at δ 2.59 (larger) and H_{7endo} at δ 2.49 (smaller). (b) NOEs from H_6 at δ 3.42 to the phenyl protons at 7.35–7.21, the CH_2OH group at 4.07, H_{7exo} at δ 2.59 (smaller) and H_{7endo} at δ 2.49 (larger). (c) NOEs from H_{7exo} at δ 2.59 to the phenyl protons at δ 7.35–7.21, H_1 at δ 4.83, H_6 at δ 3.42, and H_{7endo} at δ 2.49. (d) NOEs from H_{7endo} at δ 2.49 to H_1 at δ 4.83, H_6 at δ 3.42, and H_{7exo} at δ 2.59.

3.1.14. Methyl (2*E*)-3-[4-(acetyloxy)phenyl]-2-propenate (22). AcCl (13 mL, 0.18 mol) was added dropwise to MeOH (36 mL) at 0 °C and the resulting solution was stirred at room temperature for 30 min. 4-Hydroxycinnamic acid (6.014 g, 36.6 mmol) was added and the solution was heated to reflux for 2 h. The reaction was cooled and concentrated under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (360 mL) and cooled to 0 °C under N_2. Ac_2O (74 mL), dry pyridine (36 mL) and DMAP (448 mg, 3.37 mmol) were added in succession and the resulting solution was stirred for 35 min at 0 °C. The solution was washed with 10% $CuSO_4$ solution (400 mL), water (400 mL), saturated $NaHCO_3$ solution (400 mL), and brine (400 mL). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give 7.610 g (94%) of **22**: ^{1}H NMR 7.67 (d, 1, J=16.2), 7.54 (d, 2, J=8.5), 7.12 (d, 2, J=8.5), 6.40 (d, 1, J=16.2), 3.81 (s, 3), 2.32 (s, 3); ^{13}C NMR 169.3, 167.4, 152.2, 143.9, 132.3, 129.3 (2C), 122.3 (2C), 118.1, 51.9, 21.3.

3.1.15. Cartorimine (3). 2,6-Di-*t*-butylpyridine (0.45 mL, 2.0 mmol) was added to a solution of crude **6** (459 mg, 2.01 mmol) and **22** (2.664 g, 12.1 mmol, 6 equiv) in dry CH_3CN (10 mL). The resulting solution was degassed using the freeze-thaw method[20] and heated to 175 °C for 14 h in a sealed tube. The reaction was cooled and concentrated under reduced pressure to give 3.119 g of a black solid. Most of the unreacted **22** was removed by filtering the black solid through 50 g of silica gel (3:2 hexanes/EtOAc) to afford 239 mg of crude bis acetoxy ester, which was dissolved in 4:1 EtOH/H_2O (50 mL). KOH (178 mg, 3.18 mmol) was added, and the resulting red solution was heated at reflux for 20 h and cooled. The solution was acidified to pH 3 using a saturated NaH_2PO_4 solution and the EtOH was then removed under reduced pressure. The resulting aqueous solution was extracted with CH_2Cl_2 (3×50 mL) to remove less polar impurities. The resulting aqueous solution was saturated with NaCl and extracted with EtOAc (4×50 mL). The EtOAC solution was dried over $MgSO_4$ and concentrated

under reduced pressure to give 159 mg of crude **3**. Preparative TLC (7:3 $CHCl_3$/acetone) yielded 110 mg (16% from **11**) of a 4:1 mixture of (1*R**,5*R*,6*R*,7*S*)-1-(hydroxymethyl)-7-(4-hydroxyphenyl)-4-oxo-8-oxabicyclo[3.2.1]oct-2-ene-6-carboxylic acid (cartorimine, **3**) and its stereoisomer (1*R**,5*R*,6*S*,7*R*)-1-(hydroxymethyl)-7-(4-hydroxyphenyl)-4-oxo-8-oxabicyclo[3.2.1]oct-2-ene-6-carboxylic acid (**23**). Separation of **3** and **23** was achieved by HPLC on a Zorbex Eclipse XDB-C18 4.6×250 mm column (9:1 H_2O/MeOH) flow rate=1 mL min^{-1} with sample loadings of 0.5 mg: t_R=12.3 (**23**), t_R=18.3 (**3**).

Data for **3**: ^1H NMR (CD_3OD) 7.07 (d, 2, *J*=8.5), 6.80 (d, 1, *J*=10.4), 6.71 (d, 2, *J*=8.5), 6.18 (br d, 1, *J*=10.4), 4.70 (br s, 1), 3.84 (d, 1, *J*=6.7), 3.82 (d, 1, *J*=12.5), 3.73 (d, 1, *J*=12.5), 3.13 (br d, 1, *J*=6.7); ^{13}C NMR (CD_3OD) 197.9, 158.2, 155.6, 131.1 (2C), 128.9, 128.4, 116.4 (2C), 88.4, 86.1, 64.2, 54.2 (2C) (one quaternary carbon was not observed). The spectral data matches those reported for the natural product: mp 206–207 °C; $[\alpha]_D^{25}$ −2.6° (*c* 0.005, MeOH)[4]

Data for **23**: ^1H NMR (CD_3OD) 7.47 (d, 1, *J*=9.5), 7.09 (d, 2, *J*=7.6), 6.73 (d, 2, *J*=7.6), 6.05 (d, 1, *J*=9.5), 3.83–3.64 (m, 2), 3.19 (d, 1, *J*=12.2) (one proton is under the OH peak at δ 4.8 and one is proton under the MeOH peak at δ 3.31); ^1H NMR ($(CD_3)_2CO$) 7.57 (d, 1, *J*=10.1), 7.15 (d, 2, *J*=8.5), 6.81 (d, 2, *J*=8.5), 6.01 (br d, 1, *J*=10.1), 4.89 (br d, 1, *J*=7.9), 3.83–3.79 (m, 1), 3.77 (d, 1, *J*=4.3), 3.37 (d, 1, *J*=11.6), 3.26 (d, 1, *J*=11.6); ^{13}C NMR (CD_3OD) 157.8, 156.8, 132.1, 131.2 (2C), 127.8, 116.3 (2C), 88.7, 85.2, 65.5 (four carbons were not observed).

3.1.16. (1*R,5*R*,6*S*,7*R*)-5-[(Acetyloxy)methyl]-7-methyl-6-phenyl-8-oxabicyclo[3.2.1]oct-3-en-2-one (18).** 2,6-Di-*t*-butylpyridine (0.19 mL, 0.86 mmol) was added to a solution of crude **6** (196 mg, 0.86 mmol) and *trans*-β-methylstyrene (0.69 mL, 5.2 mmol, 6 equiv) in dry CH_3CN (5 mL). The resulting solution was degassed using the freeze-thaw method[20] and heated to 175 °C for 14 h in a sealed tube. The reaction was cooled and concentrated under reduced pressure to give 738 mg of a black liquid. Flash chromatography on silica gel (22:3 hexanes/EtOAc) yielded 76 mg (31%) of **18**: ^1H NMR 7.32–7.26 (m, 3), 7.14 (dd, 2, *J*=7.0, 1.8), 6.70 (d, 1, *J*=9.8), 6.26 (d, 1, *J*=9.8), 4.46 (d, 1, *J*=11.9), 4.27 (br s, 1), 4.21 (d, 1, *J*=11.9), 3.00 (d, 1, *J*=6.7), 2.54 (br dq, 1, *J*=6.7, 6.7), 1.36 (d, 3, *J*=6.7); ^{13}C NMR 195.8, 170.6, 151.1, 135.6, 128.9 (2C), 128.7 (2C), 128.3, 127.9, 88.0, 84.7, 65.3, 59.4, 43.0, 20.7, 19.6.

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20. A resealable tube with a threaded Teflon seal containing a solution of dipolarophile and pyranone in CH_3CN was frozen in a dry ice/acetone bath. The sample was placed under vacuum at 0.2 torr for 5 min. The tube was sealed and warmed to room temperature. This process was repeated twice.

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Evaluation of possible intramolecular [4+2] cycloaddition routes for assembling the central tetracyclic core of the potent marine antiinflammatory agent mangicol A

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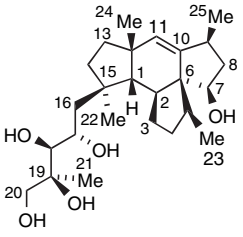
Abstract—A plan for enantioselective construction of the mangicol A framework by means of intramolecular Diels–Alder cycloaddition is outlined. First to be assembled is the enantiopure cyclopentenecarboxylic acid **16**. Of the several approaches targeting the 1,3-diene component **56**, only that involving palladium-catalyzed enyne cyclization proved successful. Following the coupling of **16** to **56**, we were unable to bring about any detectable level of ($4\pi+2\pi$) cycloaddition. Activation of the diene by incorporation of an $OSiEt_3$ substituent on a terminal sp^2-hybridized center likewise proved unsuccessful. Further facilitation was sought in the form of cyclopentenonecarboxylate **66**. However, thermal activation, Lewis acid catalysis, and high-pressure conditions proved ineffective and did not lead to C–C bond formation. These studies serve to underscore the extent to which steric complications can complicate matters and the extent to which they must be skirted to arrive at the title compound.

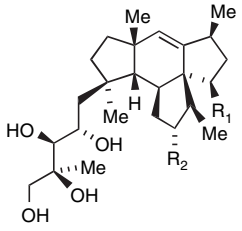
1. Introduction

Two reports emanating from the Fenical group in 1998[1] and 2000[2] described the isolation, structural elucidation, biological properties, and biosynthesis of a new class of spirotetracyclic sesterterpenoids named mangicols **A**–**G** (**1**–**7**). The highly complex carbon framework of these fascinating metabolites is generated by a marine fungus believed to be *Fusarium heterosporum*, which was collected from driftwood discovered in a mangrove habitat at Sweetings Cay in the Bahamas. Also identified were the neomangicols **8**–**10** whose potent cytotoxic and antibiotic activity profiles differentiate them from **1**–**7**. Mangicol A (**1**) has shown very significant antiinflammatory activity in the phorbol myristate acetate (PMA) mouse ear edema assay.[3] On the basis of its medicinal potential, **1** has come to be regarded as an attractive target for total synthesis.[4]

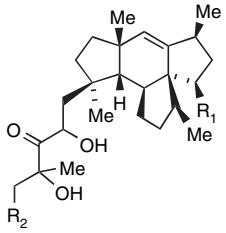
Any plan for the de novo elaboration of **1** must take into account its intertwined rings and 11 stereogenic centers, several of which are quaternary in nature. The biogenetic proposal that has been advanced[2] is of little value in suggesting an avenue by which to attack this challenging problem. To us, the presence of a central six-membered ring suggested the possible utilization of a protocol based upon an intramolecular (4+2) reaction.



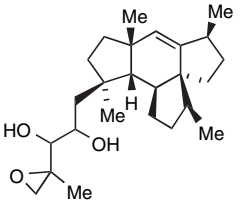
1, Mangicol A



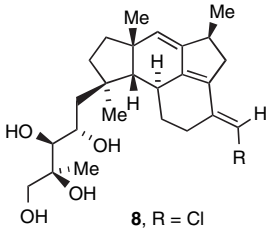
2, Mangicol B: R_1 = H, R_2 = OH
3, Mangicol C: R_1 = H, R_2 = H



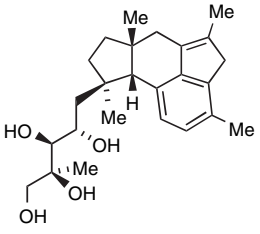
4, Mangicol D: R_1 = OH, R_2 = H
5, Mangicol E: R_1 = H, R_2 = OH
6, Mangicol F: R_1 = H, R_2 = H



7, Mangicol G



8, R = Cl
9, R = Br



10

Keywords: Mangicols; Pd-catalyzed enyne cyclization; Intramolecular aldol cyclizations; Chiral nonracemic substituted cyclopentadienones; Enantioselective diene synthesis.
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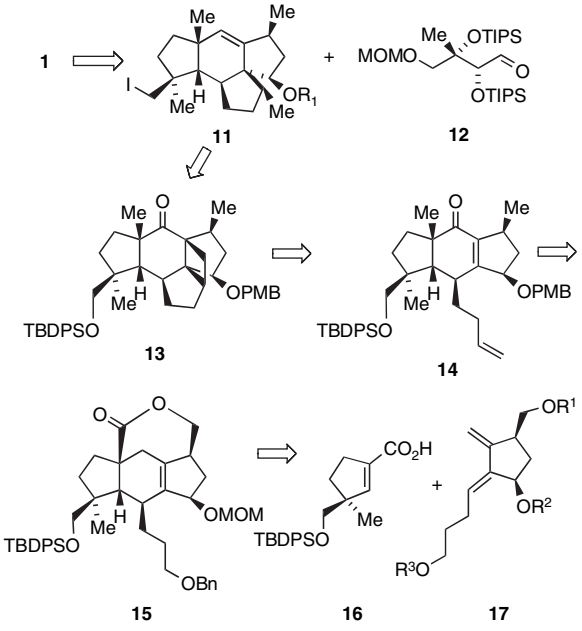
This type of cycloaddition is recognized to be amenable to catalysis by enzymes known collectively as Diels–Alderases,[5] and to play a useful role in select biosynthetic applications.[6] In this light, the source of our inspiration converges nicely with the general theme of this Symposium-in-Print.

2. Synthetic analysis

From the outset, it was envisioned that the final diastereoselective addition of an organometallic intermediate generated from iodide **11** to aldehyde **12**[7] would result in eventual arrival at the target molecule (Scheme 1).

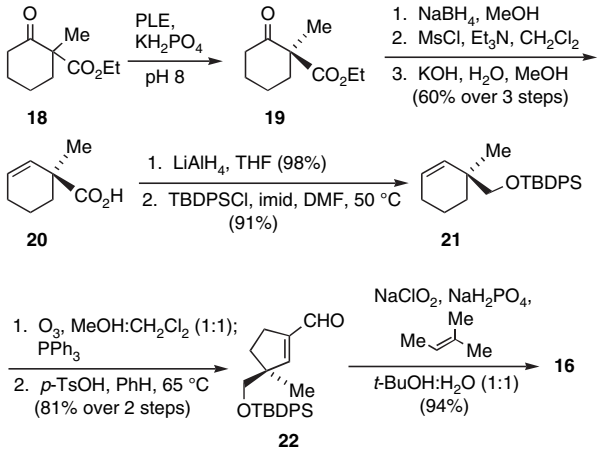


Scheme 1.

The framework of **11** was to be made available by samarium diiodide-promoted cleavage of the cyclobutane ring in **13**[8] followed by a Shapiro reaction.[9] A key feature of this reductive step is its capability for unveiling the C(23) methyl group in its proper configuration. Cyclobutane **13** was to stem from an intramolecular photochemical (2+2) cycloaddition involving the terminal alkene and α,β-unsaturated ketone subunits resident in **14**.[10,11] It was further conjectured that **14** would be accessible by suitable chemical modification of **15**, the construction of which was to be realized by thermally induced or Lewis acid-catalyzed cycloaddition involving the ester to be formed from **16** and **17** or activated forms thereof. As will be seen, provision was made for the anticipated need that modest structural variations might be necessary at this stage. Should the cyclization proceed as required, the lactone bridge was to serve the role of progenitor to the two cis-related angular methyl groups positioned at C(9) and C(12).

2.1. Construction of (+)-cyclopentenecarboxylic acid 16

Pig liver esterase-mediated kinetic resolution of the racemic keto ester **18** made available the desired *S*-enantiomeric **19** at the 91% ee level as determined by chiral HPLC[12] (Scheme 2). The action of sodium borohydride on **19** gave rise to two diastereomeric alcohols, which converged to the identical cyclohexenecarboxylic acid **20** by mesylate-mediated elimination with subsequent saponification.[13] Treatment of **20** with lithium aluminum hydride furnished the primary carbinol that was protected as the *tert*-butyldiphenylsilyl ether **21**. At this point, the stage was set for a ring cleavage-reclosure sequence[14] involving ozonolysis to the dialdehyde and *p*-toluenesulfonic acid-promoted intramolecular aldolization. As a result of the substitution plan, only that reaction channel leading to **22** is operative (81% for the two steps). Oxidation of **22** to **16** was readily and efficiently accomplished with sodium chlorite.[15]



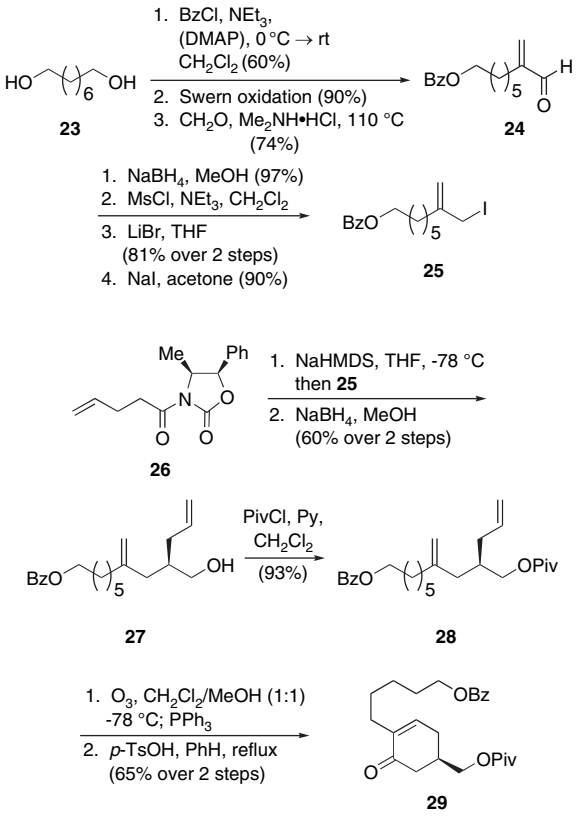
Scheme 2.

2.2. Exploration of a possible enantioselective route to a diene of type 17

This phase of our investigation began with the generation from commercially available 1,8-octanediol (**23**) of its monobenzoylated derivative. The latter was subjected to Swern oxidation and homologated to produce α,β-unsaturated aldehyde **24** via an improved Mannich reaction[16] (Scheme 3). The reduction of **24** with sodium borohydride gave rise to the primary carbinol, which was sequentially transformed via the mesylate and bromide into allylic iodide **25**. The Finkelstein displacement step[17] was performed immediately prior to the utilization of **25** in subsequent chemical maneuvers.

Next to be explored was the enantioselective alkylation of the sodium enolate derived from **26**[18] with **25**. In this instance, C–C bond formation proceeded very smoothly, thus allowing direct reductive cleavage of the chiral oxazolidinone with sodium borohydride in methanol. The configuration depicted for alcohol **27** formed in this two-step sequence (de>95%) is founded on extensive prior precedent.[19] Esterification of the unmasked hydroxyl group in **27** with pivaloyl chloride afforded **28** (93%). With this intermediate in hand, its ozonolytic conversion to a keto aldehyde and ensuing acid-promoted aldol cyclization to generate cyclohexenone **29** were accomplished without event.

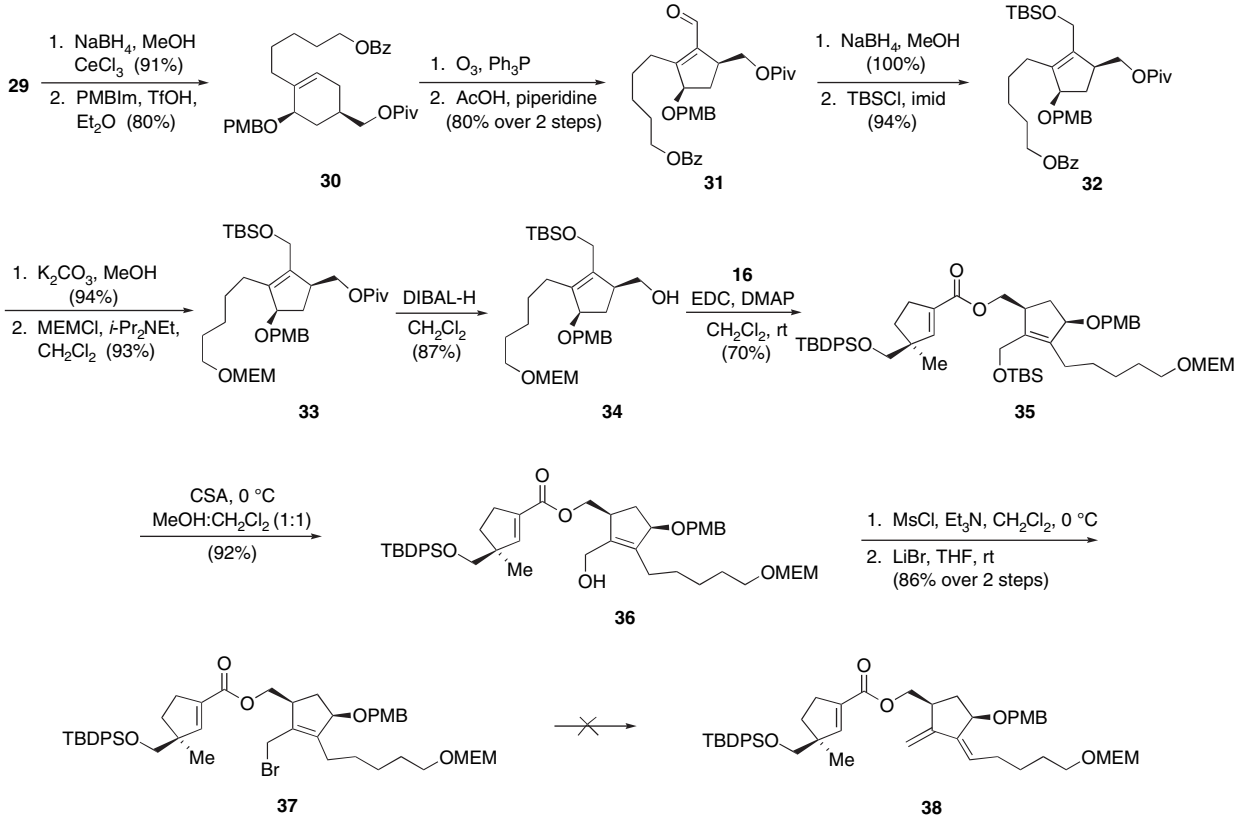
Reduction of enone **29** under Luche conditions[20] gave rise to the equatorial alcohol[21] as the only detectable product (Scheme 4), where protection as the PMB ether was affected



Scheme 3.

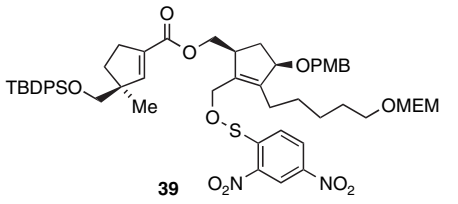
by way of trichloroacetimidate technology.[22] Aldehyde **31** was in turn produced by careful ozonolysis followed directly by aldol ring closure in the presence of piperidine and acetic acid.[14] The primary alcohol obtained from **31** by borohydride reduction reacted smoothly with *tert*-butyldimethylsilyl chloride and imidazole to furnish the quadruply functionalized (−)-cyclopentene **32**. When stirred with potassium carbonate in methanol at rt, this intermediate was efficiently debenzoylated, thus allowing replacement with an MEM protecting group at that site as in **33**. Subsequent removal of the pivaloyl ester with DIBAL-H resulted in formation of the key building block **34** whose role was to be coupled to **16**. The utilization of EDC[23] was quickly found to be well suited to the generation of ester **35**. In line with expectation, the OTBS group in **35** could subsequently be cleaved selectively without incurring unwanted side reactions.[24] This step lent itself to the uncomplicated generation of alcohol **36** (92%), whose hydroxyl group proved quite amenable to activation as the bromide **37** as well as other derivatives.

Quite unexpectedly, all attempts to accomplish the conversion of **37** into diene **38** were to no avail. Recovery of unchanged starting material or complete decomposition[25] was the alternative encountered. In an effort to bypass this complication, **36** was treated with 2,4-dinitrobenzenesulfenyl chloride in the expectation that **39** so formed would undergo [1,3] sigmatropic shift to generate the sulfoxide that would, in turn, experience elimination.[26] However, the

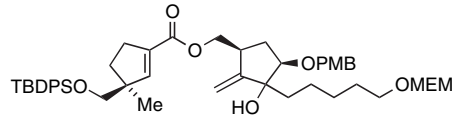


Scheme 4.

intended sequential transformation did not occur and no allylic alcohol was detected.[27]



39

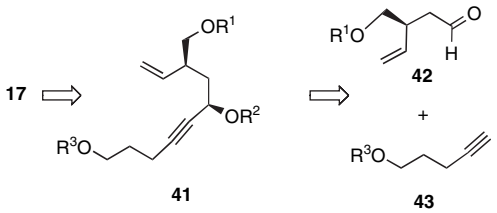


40

We also undertook exploration of the conversion of **36** to the rearranged allylic alcohol **40** via a three-step sequence involving Sharpless epoxidation,[28] treatment of the resulting epoxy alcohol with triphenylphosphine, imidazole, and iodine,[29] and finally warming to 40 °C in water. While this conversion proceeded satisfactorily, **40** resisted xanthate formation at every turn,[27] thereby thwarting its eliminative transformation into **38**. At this point, the decision was made to undertake an alternate approach to subunit **17**.

2.3. Implementation of alternative palladium-mediated diene construction

The observations detailed above led us to entertain a series of retrosynthetic disconnections within **17** that would involve somewhat less complex intermediates (Scheme 5). More specifically, the applicability of an appropriate transition metal-mediated cycloisomerization as a direct route from **41** to **17** could constitute a more straightforward route to its generation. Success here would open up the possibility that precursor compounds such as **42** and **43**[30] might also contribute to greater expediency. To this end, lactone **44**, readily available in two steps from ascorbic acid,[31] was used to define the absolute configuration of **45** via an established one-pot procedure[32] (Scheme 6). The ester was reduced with DIBAL-H and the carbinol so formed was protected as the *tert*-butyldiphenylsilyl ether. Although attempts to hydrolyze the acetonide in **46** chemoselectively with methanolic HCl caused concomitant desilylation, diol **47** could be secured in 90% yield following treatment with 80% acetic acid at rt. Continued success was realized with site-specific introduction of a second OTBDPS group as long as coupling to the silyl chloride was performed in THF at −10 °C.[33] Subsequent acetylation delivered **48** in excellent overall yield.



Scheme 5.

Application of Ireland–Claisen conditions[34] to **48** resulted in smooth conversion to the silyl ether, direct hydrolysis of which with 8 N lithium hydroxide in THF and ensuing exposure to methyl iodide and potassium carbonate gave rise to **49** in 73% yield over the three steps. Advantage was next taken of the ease of conversion of **49** to aldehyde **50**. The subsequent hurdle was the enantioselective 1,2-addition of 5-benzyloxy-1-pentyne[30] to **50**. Unfortunately, the standard conditions reported by Carreira et al.[35] proved inapplicable to our system. Attempts to bring about effective scale-up of the reaction (to a maximum of 1.8 g) furnished only a 1:1 mixture of diastereomers.

To overcome this problem, the mixture of alcohols was oxidized to **51** by means of activated manganese dioxide. At this juncture, recourse was made to the *R*-CBS reagent,[36] whose reactivity potential could be commandeered to deliver the *R* alcohol quantitatively with a de=88%.

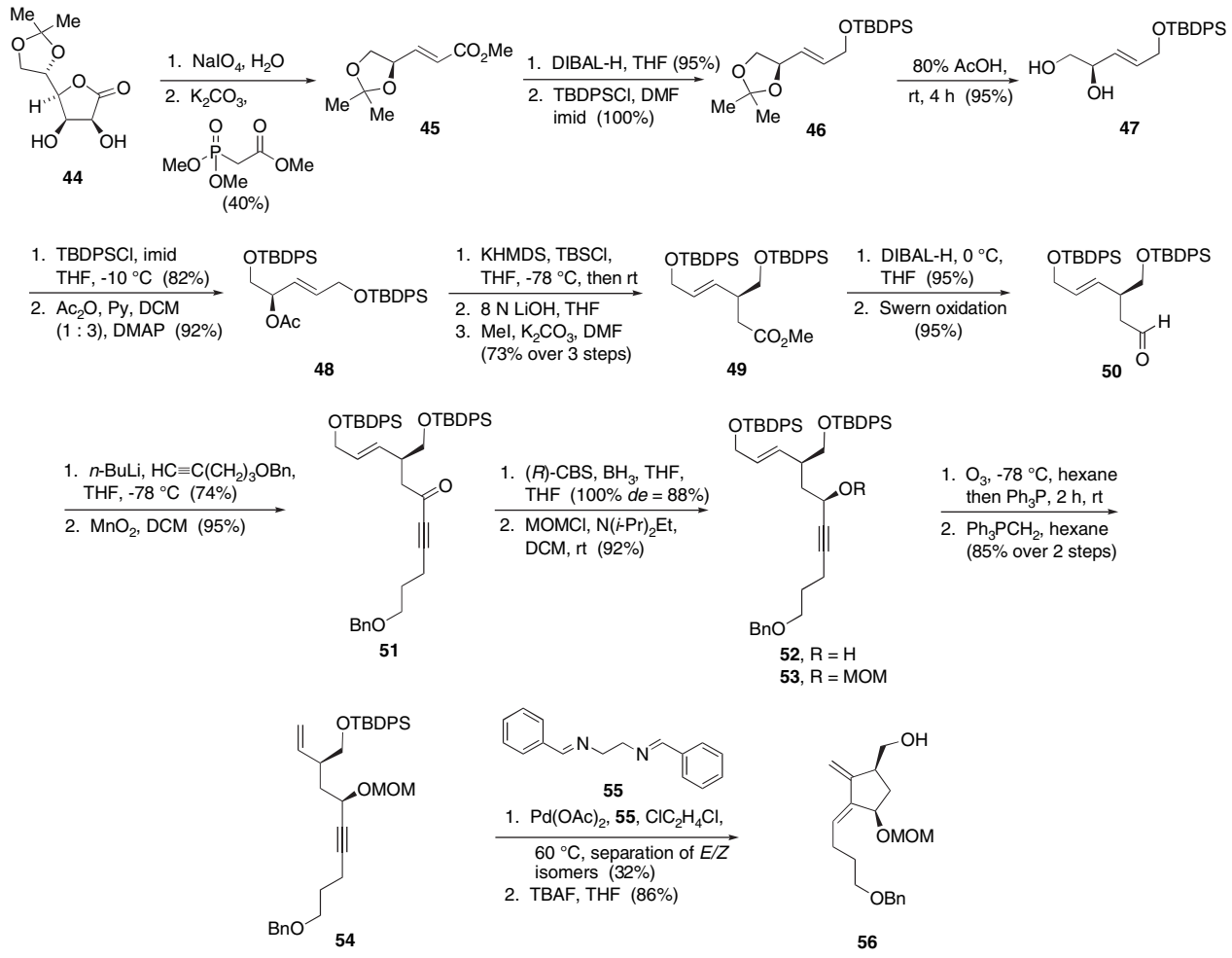
Formation of the MOM ether **53** allowed ozonolytic cleavage of the olefinic bond to be performed. Since the resulting aldehyde is prone to β-elimination, the Wittig olefination with methylenetriphenylphosphorane must be performed under controlled conditions. A particularly attractive option is to conduct the ozonolysis in hexane at −78 °C, to stir with triphenylphosphine at rt for 2 h, and to introduce the $Ph_3P{=}CH_2$ reagent in ether without further delay.

The availability of **54** led us to explore the possibility for palladium-catalyzed cycloisomerization.[37] Under conditions recommended for comparable applications[38,39] (Table 1), three catalysts were examined in a move to achieve optimization. Unsatisfactory conversion was observed in the first three runs. Recourse instead of palladium(II) acetate in combination with *N,N*-bis(benzylidene)ethylenedimine (**55**) as the ligand in 1,2-dichloroethane solution did prove effective (*E*:*Z*=4:1). The minimum temperature to realize complete conversion was 60 °C. Subsequent treatment with TBAF generated **56** without incident.

This result made possible the opportunity to esterify **56** with **16**, thereby generating **57**, the substrate desired for validation of its Type II Diels–Alder reactivity[40] (Scheme 7). We next sought to transform **57** into **58** by purely thermal means (e.g., toluene, 220 °C, 7 h) or under milder Lewis acid-catalyzed conditions such as with Et_2AlCl in CH_2Cl_2 at rt. Under no circumstance was conversion into **58** seen. This body of experiments provided convincing evidence that conditions for intramolecular cycloaddition within **57** as in **57'** were not likely to be found. Our attention was consequently directed instead to the incorporation of activated components.

2.4. Development of routes to a diene and a dienophile with intent to enhance reactivity

We hoped to solve the total synthesis problem via the intermediacy of either a substituted cyclopentenone or a 1-silyloxybutadiene derivative. It is widely recognized that rendering dienophiles more electron-deficient and dienes more electron-rich contributes in utilitarian fashion because of decreases in HOMO–LUMO gaps. With the availability of alcohol **36**, it proved an easy matter to achieve oxidation to the aldehyde level with manganese dioxide as a prelude to



Scheme 6.

O-silylation as in **60** (Scheme 8). At this point, it quickly became apparent that the hurdle of engaging **60** in intermolecular cycloaddition was not to be surmounted. All attempts to achieve cyclization under basic, acidic, purely thermal, or high-pressure conditions failed to come to fruition.[41] Simply stated, **60** proved to be too sensitive for our purposes. When intermolecular variants designed to avoid the incorporation of a lactone bridge were also found to lead to decomposition,[42] dienophile activation was pursued.

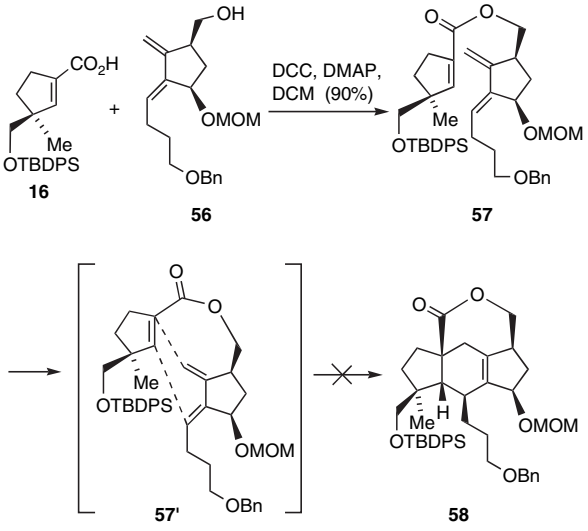
To arrive at the levorotatory keto ester **62**, the racemic cyclopentanonecarboxylate **61** was kinetically resolved by application of Brown's (−)-DIPCl methodology[43] (Scheme 9). Cyclopentene **63** was subsequently generated by mesylate-mediated dehydration.

After adjustment of the substitution plan about the quaternary carbon as in **64**, sequential allylic oxidation[44] and α-iodination[45] followed to provide **65**. Finally, the carbomethoxy group was incorporated in palladium-catalyzed carbonylation.[46] The diactivated dienophile **66** was isolated in 61% yield.

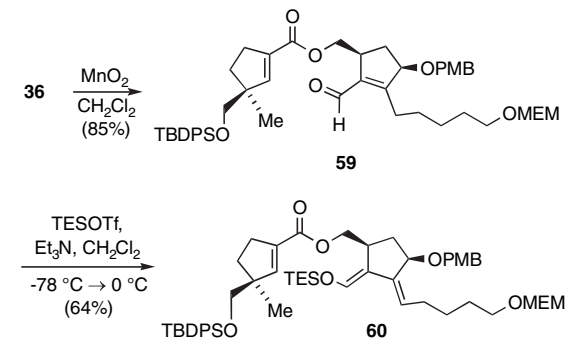
The recalcitrance of **66** to function as a dienophile in intermolecular Diels–Alder reactions involving **67** soon became apparent. Decomposition and/or polymerization were clearly operative under conditions involving either purely thermal conditions (e.g., toluene, sealed tube, 160 °C),[47] catalysis by select Lewis acids (e.g., $AlBr_3$/$AlMe_3$, −10 to 0 °C),[48] or high-pressure conditions (e.g., CH_2Cl_2, rt).[49]

Table 1. Conditions employed for the Pd(II)-catalyzed cycloismerization of **54**

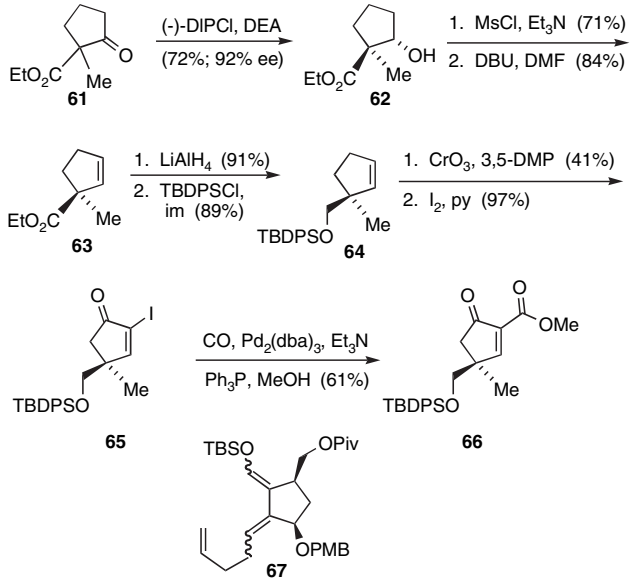
Run	Catalyst	Ligand	Additive	Solvent	Time, h	Temperature, °C	% Conversion	*E/Z* ratio
1	$Pd_2(dba)_3 \cdot CHCl_3$	Ph_3P	HOAc	Benzene	48	60	30	—
2	$Pd_2(dba)_3 \cdot CHCl_3$	Ph_3P	HOAc	Benzene	24	90	40	—
3	$Pd[P(o\text{-tol})_3]_2(OAc)_2$	—	—	$ClCH_2CH_2Cl$	48	60	0	—
4	$Pd(OAc)_2$	**55**	—	Benzene	22	63	100	2:1
5	$Pd(OAc)_2$	**55**	—	$ClCH_2CH_2Cl$	22	63	100	3–4:1



Scheme 7.



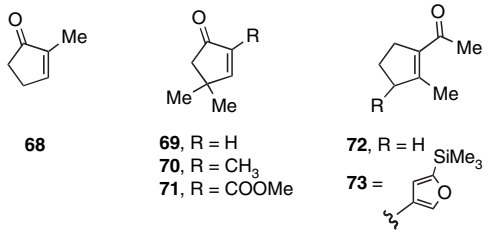
Scheme 8.



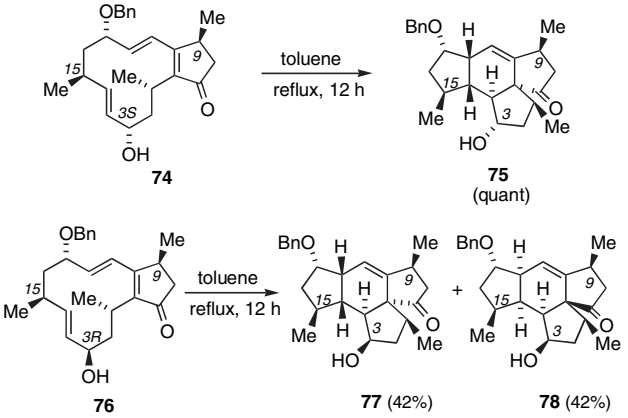
Scheme 9.

3. Overview

The history dealing with the participation of substituted 2-cyclopentenones and other electron-deficient cyclopentenes in intermolecular (4+2) cycloadditions is a checkered one. In general, this compound class performs poorly and as a result has not been made recourse to with a great deal of frequency. The presence of a 2-methyl group as in **68** has no apparent deleterious consequences[50] relative to the parent system.[51] In contrast, exceptionally low reactivity has been noted for **69**[52] and **70**.[53] Their complete failure to react has been attributed to the effective shielding brought on by the quaternary nature of C-4. The significant transition state destabilizing effect operative in these substrates can be offset to some degree by the proper positioning of a carbomethoxy group as in **71**.[47,53] Seemingly more advantageous yet is the acetylcyclopentene motif present in **72** and **73**,[48] and esters of similar type.[54]



Although intramolecular Diels–Alder cycloadditions have long been recognized to offer heightened reactivity advantages stemming from favorable entropic contributions,[55] these effects were not apparent in transition states typified by **57′**. In contrast, Uemura and co-workers found **74** and **76** to cyclize efficiently in refluxing toluene with formation of the mangicol core (Scheme 10).[4] An added bonus was the co-discovery of a remarkable interrelationship between stereoselectivity and the configuration of the secondary hydroxyl group. Both possible *endo* avenues of approach are clearly subject to subtle nonbonded steric interactions. Not withstanding, the kinetic advantages associated with the preformation of a 12-membered cyclic trienone framework are notable and promising.



Scheme 10.

The studies that have been detailed above serve to underscore the sorts of steric complications that may beset intramolecular cycloaddition reactions. These complexities obviously need to be avoided, and we hope to report on one or more alternative synthetic routes to mangicol A in the near future.

4. Experimental

4.1. (*S*)-1-Methyl-2-oxo-cyclohexanecarboxylic acid ethyl ester (19)

To a rapidly stirred solution of racemic **18** (10.4 g, 0.06 mol) in KH_2PO_4 buffer (250 mL, 0.1 M) was added pig liver esterase (0.17 g, 20,000 Units) at pH 8.0 and rt. The mixture was stirred for 24 h while the pH was maintained at 8.0 by pH stat-controlled addition of 1.0 N aqueous NaOH solution, and extracted with CH_2Cl_2 (2×800 mL). The combined organic phases were dried and evaporated to give a residue that was purified by column chromatography on silica gel (elution with 10:1 hexane–ethyl acetate) to give 3.16 g (61%) of **19** as a colorless liquid; IR (neat, cm^{-1}) 1714, 1260, 1158; ^1H NMR (300 MHz, $CDCl_3$) δ 4.24–4.15 (m, 2H), 2.55–2.45 (m, 3H), 2.04–2.02 (m, 1H), 1.75–1.64 (m, 3H), 1.51–1.44 (1H), 1.29 (s, 3H), 1.26 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.7, 172.2, 60.4, 56.3, 39.9, 37.5, 26.9, 22.0, 20.5, 13.4; HRMS ES *m/z* $(M+Na)^+$ calcd 207.0992, obsd 207.1000; $[\alpha]_D^{25}$ +109.0 (*c* 1.0, $CHCl_3$).

4.2. (*S*)-1-Methylcyclohex-2-enecarboxylic acid ethyl ester (20)

To a cold (−78 °C) suspension of $NaBH_4$ (1.23 g, 32.6 mmol) in anhydrous MeOH (30 mL) was added dropwise a solution of **19** (5.0 g, 27.2 mmol) in the same solvent (50 mL). The mixture was stirred at −78 °C for 2 h, quenched with 10% aqueous HCl solution (50 mL), warmed to rt, and stirred for 15 min. This solution was extracted with CH_2Cl_2 (3×100 mL), and the combined organic phases were dried and evaporated to give a mixture of diastereomeric hydroxy esters.

To a cold (0 °C) solution of the above material in dry CH_2Cl_2 (100 mL) was added Et_3N (5.49 g, 54.3 mmol) and MsCl (6.22 g, 54.3 mmol) in sequence. The cloudy solution was stirred at 0 °C for 1.5 h before being quenched with 10% aqueous HCl (50 mL) and diluted with H_2O (100 mL). The separated aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were dried and concentrated to give a mixture of mesylates as yellow oil.

To a solution of the mesylate mixture from above in MeOH (200 mL) and H_2O (25 mL) was added KOH (10.67 g, 190.19 mmol). The mixture was heated to 60 °C for 16 h, cooled to rt, evaporated under vacuum, and acidified to pH 1 by careful addition of 1 N HCl solution. The aqueous layer was extracted with CH_2Cl_2 (4×100 mL). The combined organic phases were dried and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 5:1 hexane–ethyl acetate) to give 2.28 g (60% over three steps) of **20** as a colorless liquid; IR (neat, cm^{-1}) 1697, 1296, 1188; ^1H NMR (250 MHz, $CDCl_3$) δ 5.85–5.67 (m, 2H), 2.20–2.14 (m, 1H), 2.05–1.98 (m, 2H), 1.70–1.66 (m, 2H), 1.53–1.48 (m, 1H), 1.32–1.24 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.7, 130.0, 128.3, 42.8, 32.6, 26.1, 24.6, 19.4; HRMS ES *m/z* $(M+Na)^+$ calcd 140.0832, obsd 140.0824; $[\alpha]_D^{25}$ −100.9 (*c* 1.2, $CHCl_3$).

4.3. *tert*-Butyl-((*S*)-1-methylcyclohex-2-enylmethoxy) diphenylsilane (21)

To a cold (0 °C) suspension of $LiAlH_4$ (1.25 g, 32.86 mmol) in dry THF (40 mL) was added a solution of **20** (2.0 g, 14.29 mmol) in dry THF (40 mL) via an addition funnel. After the addition was complete, the cold bath was removed and the reaction mixture was stirred at rt for 14 h, cooled to 0 °C, carefully quenched with 1 N NaOH solution (1.25 mL) followed by H_2O (3.75 mL), and stirred at 0 °C for 1 h during which time a white precipitate formed. The precipitate was filtered off and washed with Et_2O (100 mL). The filtrate was concentrated under vacuum to leave a light yellow residue that was purified by column chromatography on silica gel (elution with 5:1 hexane–ethyl acetate) to give 1.77 g (98%) of the primary alcohol as a colorless oil; IR (neat, cm^{-1}) 3355; ^1H NMR (300 MHz, $CDCl_3$) δ 5.90–5.78 (m, 1H), 5.41–5.37 (m, 1H), 3.43–3.30 (m, 2H), 2.00–1.95 (m, 2H), 1.71–1.60 (m, 2H), 1.40–1.27 (m, 2H), 0.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.0, 128.1, 70.8, 36.7, 31.5, 25.0, 24.1, 18.8; $[\alpha]_D^{25}$ +8.5 (*c* 1.0, $CHCl_3$).

To a solution of the above alcohol (1.77 g, 14.05 mmol) in dry DMF (7 mL) were added TBDPSCl (4.63 g, 16.86 mmol) and imidazole (2.87 g, 42.15 mmol). The mixture was heated to 50 °C for 2 h before being cooled to rt and diluted with H_2O (100 mL). The aqueous layer was extracted with Et_2O (5×100 mL), and the organic layers were combined, dried, and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 40:1 hexane–ethyl acetate) to obtain 4.65 g (91%) of **21** as a colorless oil; IR (neat, cm^{-1}) 1470, 1361; ^1H NMR (250 MHz, $CDCl_3$) δ 7.70–7.66 (m, 4H), 7.46–7.27 (m, 6H), 5.71–5.64 (m, 1H), 5.46–5.31 (m, 1H), 3.44–3.33 (m, 2H), 1.97–1.70 (m, 2H), 1.70–1.53 (m, 3H), 1.41–1.33 (m, 1H), 1.08–1.05 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7 (4C), 134.0 (2C), 133.8, 129.5 (2C), 127.6 (4C), 127.1, 71.5, 37.2, 31.9, 26.9 (3C), 25.4, 24.8, 19.5, 19.0; HRMS ES *m/z* $(M+Na)^+$ calcd 387.2115, obsd 387.2092; $[\alpha]_D^{25}$ −28.2 (*c* 1.4, $CHCl_3$).

4.4. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methyl-cyclopent-1-enecarbaldehyde (22)

Ozone was passed through a cold (−78 °C) solution of **21** (4.0 g, 10.98 mmol) in a 1:1 mixture of CH_2Cl_2 and MeOH (130 mL each). After the reaction was over, PPh_3 (3.17 g, 12.09 mmol) was added and the mixture was stirred at rt for 4 h. The solvent was evaporated under vacuum; the residue was further evaporated with benzene (250 mL), and redissolved in benzene (250 mL). To this solution was added *p*-TsOH (0.63 g, 3.29 mmol) and the mixture was heated to 65 °C for 20 h. The solvent was removed and the residue was purified by column chromatography on silica gel (elution with 5:1 hexane–ethyl acetate) to give 3.36 g (81% over two steps) of **22** as a colorless oil; IR (neat, cm^{-1}) 1681, 1105; ^1H NMR (250 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.71–7.63 (m, 4H), 7.48–7.36 (m, 6H), 6.90–6.61 (m, 1H), 3.55 (s, 2H), 2.58–2.52 (m, 2H), 2.02–1.94 (m, 1H), 1.73–1.59 (m, 1H), 1.17 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.4, 158.5, 146.3, 135.7 (4C), 133.5

(2C), 129.7 (2C), 127.7 (4C), 70.4, 52.6, 33.4, 27.6, 26.8 (3C), 22.7, 19.3; HRMS ES *m/z* $(M+Na)^+$ calcd 401.1907, obsd 401.1900; $[\alpha]_D^{25}$ −40.4 (*c* 0.6, $CHCl_3$).

4.5. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methyl-cyclopent-1-enecarboxylic acid (16)

To a stirred mixture of **22** (3.0 g, 7.94 mmol) and 2-methyl-2-butene (5.57 g, 79.4 mmol) in *t*-BuOH (20 mL) was added an aqueous solution of $NaClO_2$ (2.87 g, 31.8 mmol) and NaH_2PO_4 (3.29 g, 23.8 mmol) in H_2O (20 mL). The resulting solution was stirred at rt overnight before it was quenched with 10% HCl solution to reach pH 5.0. The aqueous layer was extracted with EtOAc (5×50 mL), and the combined organic phases were dried and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 5:1 hexane–ethyl acetate) to afford 2.94 g (94%) of **16** as a colorless liquid; IR (neat, cm^{-1}) 2858, 1684, 1280; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67–7.63 (m, 4H), 7.46–7.35 (m, 6H), 6.73–6.71 (m, 1H), 3.53–3.46 (m, 2H), 2.64–2.58 (m, 2H), 2.05–1.96 (m, 1H), 1.71–1.61 (m, 1H), 1.15 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 152.1, 135.6 (4C), 135.1, 133.5 (2C), 129.6 (2C), 127.6 (4C), 70.4, 52.5, 33.7, 30.3, 26.8 (3C), 22.7, 19.3; HRMS ES *m/z* $(M+Na)^+$ calcd 417.1856, obsd 417.1876; $[\alpha]_D^{25}$ +18.3 (*c* 1.3, $CHCl_3$).

4.6. Benzoic acid 7-formyl-oct-7-enyl ester (24)

To a cold (−78 °C), stirred solution of oxalyl chloride (48.2 g, 0.38 mol) in dry CH_2Cl_2 (400 mL) was added a solution of DMSO (44.5 g, 0.57 mol) in dry CH_2Cl_2 (300 mL) under N_2. After 30 min of stirring, a solution of the monobenzoate of 1,8-octanediol (48.0 g, 0.19 mol) in dry CH_2Cl_2 (300 mL) was added over 30 min at −78 °C. The resulting cloudy solution was stirred at −78 °C for 1 h before Et_3N (96.0 g, 0.95 mol) was introduced. The cold bath was removed and the mixture was allowed to warm to 0 °C before being quenched with a saturated solution of NH_4Cl (500 mL). The separated aqueous phase was extracted with CH_2Cl_2 (3×500 mL). The organic layers were combined, dried, and evaporated to leave a yellow residue that was purified by column chromatography on silica gel (elution with 5:1 petroleum ether–ethyl acetate) to afford 42.4 g (90%) of the saturated aldehyde as a colorless oil.

A mixture of above aldehyde (13.0 g, 52.4 mmol), $Et_2NH\cdot HCl$ (5.6 g, 68.2 mmol), and CH_2O (5.53 mL of 37% aqueous solution, 68.2 mmol) was heated to 110 °C for 16 h, cooled to rt, diluted with H_2O (200 mL), and extracted with CH_2Cl_2 (4×200 mL). The organic layers were combined, dried, and evaporated to leave a yellow residue that was purified by column chromatography on silica gel (elution with 10:1 petroleum ether–ethyl acetate) to yield 10.6 g (74%) of **24** as colorless oil; IR (neat, cm^{-1}) 1718, 1692, 1117; ^1H NMR (300 MHz, $CDCl_3$) δ 9.53 (s, 1H), 8.05–8.02 (m, 2H), 7.58–7.53 (m, 1H), 7.46–7.41 (m, 2H), 6.25 (s, 1H), 5.99 (s, 1H), 4.33–4.29 (m, 2H), 2.28–2.23 (m, 2H), 1.79–1.72 (m, 2H), 1.51–1.35 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.4, 166.3, 149.9, 133.8, 132.6, 130.2, 129.3 (2C), 128.1 (2C), 64.7, 28.7, 28.4, 27.5, 27.4, 25.6; HRMS ES *m/z* $(M+Na)^+$ calcd 283.1305, obsd 283.1306.

4.7. Benzoic acid 7-iodomethyloct-7-enyl ester (25)

To a cold (−20 °C), stirred solution of **24** (31.7 g, 115.8 mmol) in anhydrous MeOH (250 mL) was added $NaBH_4$ (4.4 g, 115.8 mmol) in portions. The resulting mixture was stirred at −20 °C for 30 min before being quenched with 10% HCl solution (200 mL). The mixture was extracted with CH_2Cl_2 (4×300 mL). The combined organic layers were dried and evaporated to leave a residue that was purified by column chromatography (elution with 4:1 petroleum ether–ethyl acetate) to afford 31.0 g (91%) of the alcohol as a colorless oil; IR (neat, cm^{-1}) 3422, 1720, 1118; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07–8.03 (m, 2H), 7.57–7.54 (m, 1H), 7.48–7.42 (m, 2H), 5.02 (s, 1H), 4.88 (s, 1H), 4.33 (t, *J*=6.6 Hz, 2H), 4.08 (d, *J*=5.8 Hz, 2H), 2.12–2.06 (m, 2H), 1.81–1.76 (m, 2H), 1.54–1.40 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 148.7, 132.5, 130.0, 129.2 (2C), 128.0 (2C), 108.5, 65.1, 64.7, 32.5, 28.7, 28.3, 27.2, 25.5; HRMS ES *m/z* $(M+Na)^+$ calcd 285.1461, obsd 285.1473.

To a cold (−78 °C), stirred solution of the above alcohol (14.7 g, 53.15 mmol) in dry CH_2Cl_2 (150 mL) was added Et_3N (10.8 g, 106.3 mmol) followed by MsCl (12.2 g, 106.3 mmol) under N_2. The resulting solution was slowly warmed to 0 °C and stirred at 0 °C for 1.5 h before being quenched with 10% HCl (100 mL). The separated aqueous layer was extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were dried and evaporated to leave a residue that was dissolved in dry THF (100 mL) and treated with anhydrous LiBr (9.2 g, 106.2 mmol) at 0 °C. The mixture was stirred at rt for 2 h before it was filtered through a pad of silica gel. The solid residue was washed with Et_2O (4×100 mL) and the combined filtrates were evaporated under vacuum to afford a yellow residue, which was purified by column chromatography on silica gel (elution with 40:1 hexane–ethyl acetate) to furnish 14.60 g (81% over two steps) of the allylic bromide as a light yellow oil; IR (neat, cm^{-1}) 1721, 1451, 1275; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07–8.03 (m, 2H), 7.59–7.53 (m, 1H), 7.47–7.41 (m, 2H), 5.16 (s, 1H), 4.96 (s, 1H), 4.32 (t, *J*=6.64 Hz, 2H), 3.97 (s, 2H), 2.25–2.20 (m, 2H), 1.83–1.74 (m, 2H), 1.56–1.35 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 145.3, 132.6 (2C), 130.3, 129.4, 128.2 (2C), 114.8, 64.8, 36.7, 33.0, 28.7, 28.5, 27.0, 25.7.

To a stirred solution of the above product (10.0 g, 30.8 mmol) in acetone (50 mL) was added NaI (6.92 g, 46.15 mmol) at rt. The resulting cloudy solution was stirred in the dark for 24 h before being quenched with H_2O (50 mL). The resulting solution was extracted with Et_2O (3×100 mL). The combined organic layers were evaporated under vacuum to give 11.0 g of crude **25** as an orange oil; IR (neat, cm^{-1}) 1716, 1451, 1271; ^1H NMR (250 MHz, $CDCl_3$) δ 8.08–8.04 (m, 2H), 7.57–7.54 (m, 1H), 7.48–7.42 (m, 2H), 5.24 (s, 1H), 4.92 (s, 1H), 4.34 (t, *J*=6.61 Hz, 2H), 3.94 (s, 2H), 2.27–2.24 (m, 2H), 1.80–1.78 (m, 2H), 1.55–1.43 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 145.9, 132.4 (2C), 130.1, 129.1, 128.0 (2C), 113.1, 64.6, 33.5, 28.5, 28.3, 26.8, 25.5; HRMS ES *m/z* $(M+Na)^+$ calcd 395.0478, obsd 395.0475.

4.8. Benzoic acid (*R*)-9-hydroxymethyl-7-methylenedodec-11-enyl ester (27)

To a cold (−78 °C), stirred solution of **26** (1.8 g, 6.95 mmol) in dry THF (24 mL) was added NaHMDS (7.3 mL of 1.0 M solution in THF, 7.3 mmol) slowly over 5 min under N_2. After 45 min of stirring at this temperature, a solution of **25** (1.27 g, 3.42 mmol) in THF (12 mL) was introduced. The resulting mixture was stirred for 5 h at −78 °C before being quenched with saturated NH_4Cl solution (30 mL). The mixture was allowed to warm to rt, stirred for an additional 10 min, and diluted with H_2O (50 mL). The separated aqueous layer was extracted with CH_2Cl_2 (3×30 mL) and the combined organic layers were dried and evaporated to leave a residue that was dissolved in anhydrous MeOH (90 mL). To a cold (0 °C) flask of this solution was added $NaBH_4$ (1.3 g, 34.4 mmol) in portions and the resulting solution was allowed to stir at 0 °C overnight prior to quenching with saturated NH_4Cl solution (100 mL) followed by H_2O (100 mL). The mixture was extracted with CH_2Cl_2 (4×200 mL) and the combined organic layers were dried and evaporated to yield a residue that was purified by column chromatography on silica gel (elution with 5:1 hexane–ethyl acetate) to afford 0.68 g (60% over two steps) of **27** as a colorless oil; IR (neat, cm^{-1}) 3424, 1721, 1641, 1176; ^1H NMR (250 MHz, $CDCl_3$) δ 8.07–8.03 (m, 2H), 7.56–7.52 (m, 1H), 7.47–7.27 (m, 2H), 5.84–5.77 (m, 1H), 5.10–5.01 (m, 2H), 4.80–4.77 (m, 2H), 4.32 (t, J=6.6 Hz, 2H), 3.55 (d, J=5.3 Hz, 2H), 2.13–2.00 (m, 6H), 1.84–1.75 (m, 3H), 1.50–1.37 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 148.1, 136.8, 132.8, 130.4, 129.5 (2C), 128.3 (2C), 116.4, 110.9, 65.5, 65.0, 38.14, 38.10, 35.7, 35.4, 28.9, 28.6, 27.4, 25.9; HRMS ES *m/z* $(M+Na)^+$ calcd 353.2087, obsd 353.2061; $[\alpha]_D^{20}$ +0.3 (*c* 1.0, $CHCl_3$).

4.9. Benzoic acid (*R*)-9-(2,2-dimethylpropionyloxymethyl)-7-methylenedodec-11-enyl ester (28)

To a cold (0 °C), stirred solution of **27** (1.7 g, 5.15 mmol) in dry CH_2Cl_2 (30 mL) was added pyridine (1.2 g, 15.5 mmol) followed by PivCl (1.24 g, 10.3 mmol). The mixture was stirred at rt overnight prior to quenching with 10% HCl (30 mL). The separated aqueous layer was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were dried and evaporated to obtain a residue that was purified by column chromatography on silica gel (elution with 20:1 hexane–ethyl acetate) to provide 1.98 g (93%) of **28** as a colorless oil; IR (neat, cm^{-1}) 1724, 1641, 1160; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06–8.03 (m, 2H), 7.58–7.53 (m, 1H), 7.46–7.41 (m, 2H), 5.82–5.70 (m, 1H), 5.06–4.99 (m, 2H), 4.77 (d, J=15.5 Hz, 2H), 4.32 (t, J=6.62 Hz, 2H), 3.99–3.91 (m, 2H), 2.13–1.94 (m, 7H), 1.80–1.73 (m, 2H), 1.55–1.33 (m, 6H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 166.4, 146.8, 135.8, 132.6 (2C), 131.3, 129.4 (2C), 128.2, 116.7, 111.3, 65.8, 64.8, 38.9, 37.6, 35.4, 35.3, 35.2, 28.8, 28.6, 27.4, 27.1 (3C), 25.8; HRMS ES *m/z* $(M+Na)^+$ calcd 437.2662, obsd 437.2681; $[\alpha]_D^{20}$ −3.0 (*c* 1.2, $CHCl_3$).

4.10. Benzoic acid 5-[(*R*)-4-(2,2-dimethylpropionyloxy methyl)-6-oxocyclohex-1-enyl]-pentyl ester (29)

Ozone was passed through a cold (−78 °C) solution of **28** (0.82 g, 1.99 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (20 mL each). After the reaction was complete, PPh_3 (3.17 g, 12.09 mmol) was added and the mixture was stirred at rt for 6 h. The solvent was evaporated under vacuum, the residue was further evaporated with benzene (60 mL), and redissolved in benzene (60 mL). To this solution was added *p*-TsOH (0.20 g, 1.04 mmol) and the mixture was heated to 65 °C for 15 h. The solvent was removed and the residue was purified by column chromatography on silica gel (elution with 5:1 hexane–Et_2O) to afford 0.52 g (65% over two steps) of **29** as a colorless oil; IR (neat, cm^{-1}) 1720, 1674, 1480; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06–8.03 (m, 2H), 7.59–7.53 (m, 1H), 7.47–7.42 (m, 2H), 6.70–6.68 (m, 1H), 4.32 (t, J=6.6 Hz, 2H), 4.03–4.00 (m, 2H), 2.60–2.53 (m, 1H), 2.46–2.39 (m, 2H), 2.28–2.18 (m, 4H), 1.81–1.77 (m, 2H), 1.48–1.43 (m, 4H), 1.22 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.0, 178.2, 166.6, 143.2, 139.7, 132.8 (2C), 130.5, 129.5 (2C), 128.3, 66.9, 64.9, 41.2, 38.8, 34.9, 29.2, 28.8, 28.5, 28.2, 27.2 (3C), 25.7; HRMS ES *m/z* $(M+Na)^+$ calcd 423.2142, obsd 423.2112; $[\alpha]_D^{20}$ −3.0 (*c* 1.2, $CHCl_3$).

4.11. Benzoic acid 5-[(4*R*,6*R*)-4-(2,2-dimethylpropionyloxy methyl)-6-(5-methoxybenzyloxy) cyclohex-1-enyl]-pentyl ester (30)

To a mixture of **29** (1.0 g, 2.5 mmol) and $CeCl_3 \cdot 7H_2O$ (1.4 g, 3.8 mmol) in anhydrous MeOH (20 mL) was added $NaBH_4$ (0.12 g, 3.0 mmol) in portions at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was quenched with saturated NH_4Cl solution (40 mL). The aqueous layer was extracted with CH_2Cl_2 (3×40 mL). The combined organic layers were dried and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 5:1 petroleum ether–ethyl acetate) to afford 0.93 g (92%) of the allylic alcohol as a colorless oil; IR (neat, cm^{-1}) 3503, 1721, 1480; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07−8.03 (m, 2H), 7.59–7.53 (m, 1H), 7.48–7.42 (m, 2H), 5.49–5.46 (m, 1H), 4.36–4.28 (m, 3H), 3.97 (d, J=6.0 Hz, 2H), 2.30–2.28 (m, 1H), 2.16–2.00 (m, 5H), 1.82–1.78 (m, 3H), 1.49–1.31 (m, 4H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 165.8, 139.5, 131.9 (2C), 130.2, 128.6 (2C), 127.4, 121.5, 67.5, 67.2, 64.1, 38.0, 35.4, 32.1, 31.5, 27.8, 27.7, 26.5, 26.3 (3C), 24.9; HRMS ES *m/z* $(M+Na)^+$ calcd 425.2298, obsd 425.2285; $[\alpha]_D^{20}$ −24.1 (*c* 1.1, $CHCl_3$).

To a cold (0 °C), stirred mixture of the above alcohol (2.15 g, 5.35 mmol) and *p*-methoxybenzyl trichloroacetimidate (2.27 g, 8.02 mmol) in dry Et_2O (72 mL) was added TfOH (0.16 mL, 0.16 mmol). The resulting mixture was stirred at rt for 5 h prior to quenching with a saturated $NaHCO_3$ solution (20 mL) followed by H_2O (100 mL). The separated aqueous layer was extracted with Et_2O (3×50 mL), the combined organic layers were dried and evaporated, and the residue was purified by column chromatography on silica gel (elution with 10:1 petroleum ether–ethyl acetate) to afford 2.23 g (80%) of **30** as a colorless oil; IR (neat, cm^{-1}) 1721, 1612, 1160; ^1H NMR (250 MHz, $CDCl_3$) δ 8.07–8.03 (m, 2H), 7.57–7.53 (m, 1H), 7.47–7.41 (m, 2H), 7.29–7.25 (m, 2H), 6.89–6.85 (m, 2H), 5.52–5.49 (m, 1H), 4.59 (d, J=11.3 Hz, 1H), 4.40 (d, J=11.3 Hz, 1H), 4.38–4.29 (m, 2H), 4.03–3.98 (m, 3H), 3.79 (s, 3H), 2.30–1.76 (m, 8H), 1.49–1.36 (m, 5H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 166.6, 159.0,

139.2, 132.8, 130.7, 130.4, 129.5 (2C), 129.2 (2C), 128.3 (2C), 123.0, 113.7, (2C), 74.4, 70.1, 68.2, 65.0, 55.2, 38.8, 32.73, 32.70, 31.5, 28.6, 28.5, 27.4, 27.2 (3C), 25.8; HRMS ES *m/z* $(M+Na)^+$ calcd 545.2874, obsd 545.2842; $[\alpha]_D^{20}$ −34.8 (*c* 0.8, $CHCl_3$).

4.12. Benzoic acid 5-[(3*S*,5*R*)-3-(2,2-dimethylpropionyloxy methyl)-2-formyl-5-(4-methoxybenzyloxy)cyclopent-1-enyl]pentyl ester (31)

To a stirred solution of **30** (1.3 g, 2.49 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (30 mL each) was passed O_3 at −78 °C. After reaction was complete, PPh_3 (0.72 g, 2.74 mmol) was added and the resulting mixture was stirred at rt for 4 h. Solvent was evaporated under vacuum, the residue was further evaporated with Et_2O (75 mL), and redissolved in Et_2O (75 mL). To this solution was added piperidine (78 mg, 0.92 mmol) and the resulting mixture was stirred at rt before HOAc (97 mg, 1.62 mmol) was added. The mixture was heated to reflux for 20 h, the solvent was removed under vacuum, and the residue was purified by column chromatography on silica gel (elution with 5:1 petroleum ether–ether) to afford 1.06 g (80%) of **31** as a colorless liquid; IR (neat, cm^{-1}) 1719, 1671, 1611; ^1H NMR (250 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.06–8.02 (m, 2H), 7.57–7.54 (m, 1H), 7.48–7.42 (m, 2H), 7.28–7.24 (m, 2H), 6.90–6.86 (m, 2H), 4.60 (d, *J*=11.5 Hz, 1H), 4.51–4.48 (m, 1H), 4.42–4.29 (m, 4 H), 4.20–4.13 (m, 1H), 3.79 (s, 3H), 3.20–3.12 (m, 1H), 2.79–2.54 (m, 2H), 2.35–2.21 (m, 1H), 1.76–1.45 (m, 7H), 1.18 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 178.3, 166.5, 165.1, 137.7, 132.9, 130.3, 129.9, 129.54 (2C), 129.50 (2C), 128.3 (2C), 113.8 (2C), 82.7, 71.4, 65.5, 64.5, 55.2, 41.4, 38.8, 32.2, 28.4, 28.1, 27.1 (3C), 26.0, 25.8; HRMS ES *m/z* $(M+Na)^+$ calcd 559.2666, obsd 559.2646; $[\alpha]_D^{20}$ +7.1 (*c* 0.7, $CHCl_3$).

4.13. Benzoic acid 5-[(3*S*,5*R*)-2-(*tert*-butyldimethylsilanyloxy methyl)-3-(2,2-dimethylpropionyloxymethyl)-5-(4-methoxybenzyloxy)cyclopent-1-enyl]pentyl ester (32)

To a cold (0 °C), stirred solution of **31** (0.49 g, 0.91 mmol) in anhydrous MeOH (25 mL) was added $NaBH_4$ (70 mg, 1.85 mmol) in portions. The resulting mixture was stirred at 0 °C for 20 min prior to quenching with saturated NH_4Cl solution (30 mL), and extracted with CH_2Cl_2 (4×50 mL). The organic layers were combined, dried, and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 4:1 petroleum ether–ethyl acetate) to afford 0.49 g (100%) of the allylic alcohol as a colorless oil; IR (neat, cm^{-1}) 3475, 1722, 1612; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06–8.03 (m, 2H), 7.59–7.54 (m, 1H), 7.47–7.42 (m, 2H), 7.27–7.23 (m, 2H), 6.89–6.84 (m, 2H), 4.54 (d, *J*=11.4 Hz, 1H), 4.44–4.11 (m, 8H), 3.79 (s, 3H), 3.00–2.95 (m, 1H), 2.33–2.18 (m, 3H), 1.77–1.72 (m, 3 H), 1.47–1.42 (m, 4H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 166.3, 158.8, 141.8, 138.0, 132.5 (2C), 130.5, 130.1, 129.2 (2C), 128.9 (2C), 128.0 (2C), 113.4, 83.3, 70.4, 66.7, 64.5, 57.0, 54.8, 43.2, 38.5, 32.5, 28.2, 27.5, 26.8 (3C), 25.6; HRMS ES *m/z* $(M+Na)^+$ calcd 561.2823, obsd 561.2800; $[\alpha]_D^{20}$ −20.0 (*c* 0.1, $CHCl_3$).

To a stirred solution of above alcohol (0.88 g, 1.63 mmol) in dry CH_2Cl_2 (15 mL) was added imidazole (0.33 g, 4.89 mmol) followed by TBSCl (0.37 g, 2.44 mmol). The mixture was stirred at rt for 1 h before it was quenched with H_2O (20 mL). The separated aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The organic layers were combined, dried, and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 15:1 petroleum ether–ethyl acetate) to afford 1.0 g (94%) of **32** as a colorless oil; IR (neat, cm^{-1}) 1713, 1613, 1169; ^1H NMR (300 MHz, $CDCl_3$) δ 8.09–8.03 (m, 2H), 7.57–7.53 (m, 1H), 7.47–7.41 (m, 2H), 7.27–7.24 (m, 2H), 6.88–6.85 (m, 2H), 4.53 (d, *J*=11.6 Hz, 1H), 4.38–4.27 (m, 6 H), 4.16 (d, *J*=12.5 Hz, 1H), 4.08–3.91 (m, 1H), 3.79 (s, 3 H), 3.02–3.95 (m, 1H), 2.29–2.12 (m, 3 H), 1.78–1.70 (m, 2H), 1.64–1.55 (m, 1H), 1.48–1.39 (m, 4H), 1.19 (s, 9H), 0.88 (s, 9 H), 0.07–0.03 (m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 166.5, 159.0, 140.5, 138.2, 132.7 (2C), 130.9, 130.4, 129.4, 129.2 (2C), 128.2 (2C), 113.6 (2C), 83.8, 70.4, 66.6, 64.6, 58.1, 54.9, 43.5, 38.6, 32.6, 28.4, 27.6, 27.0 (3C), 25.9, 25.7 (3C), 25.6, 18.0, −5.6 (2C); HRMS ES *m/z* $(M+Na)^+$ calcd 675.3688, obsd 675.3660; $[\alpha]_D^{20}$ −17.4 (*c* 0.06, $CHCl_3$).

4.14. 2,2-Dimethylpropionic acid (1*S*,4*R*)-2-(*tert*-butyldimethylsilanyloxymethyl)-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy) pentyl]cyclopent-2-enylmethyl ester (33)

To a stirred solution of **32** (1.0 g, 1.53 mmol) in anhydrous MeOH (60 mL) was added K_2CO_3 (1.06 g, 7.65 mmol). The mixture was stirred at rt overnight before it was quenched with saturated NH_4Cl solution (30 mL). The mixture was extracted with EtOAc (4×40 mL). The combined organic layers were dried and evaporated to leave a residue that was purified by column chromatography on silica gel (elution with 2:1 petroleum ether–ethyl acetate) to afford 0.78 g (94%) of the primary alcohol as a colorless oil; IR (neat, cm^{-1}) 3443, 1731, 1613; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.23 (m, 2H), 6.89–6.84 (m, 2H), 4.53 (d, *J*=11.5 Hz, 1H), 4.43–4.26 (m, 4H), 4.15 (d, *J*=12.4 Hz, 1H), 4.05–3.80 (m, 1H), 3.80 (s, 3 H), 3.62–3.57 (m, 2H), 2.99–2.95 (m, 1H), 2.30–2.20 (m, 1H), 2.15–2.11 (m, 2H), 1.64–1.50 (m, 3H), 1.37–1.18 (m, 13H), 0.88 (s, 9H), 0.06–0.03 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 159.0, 140.6, 138.1, 130.9, 129.2 (2C), 113.6 (2C), 83.8, 70.5, 66.7, 62.6, 58.2, 55.1, 43.5, 38.7, 32.7, 32.5, 27.8, 27.1 (3C), 26.0, 25.76 (3C), 25.70, 18.1, −5.5 (2C); HRMS ES *m/z* $(M+Na)^+$ calcd 571.3425, obsd 571.3434; $[\alpha]_D^{20}$ −21.3 (*c* 0.08, $CHCl_3$).

To a stirred solution of the above alcohol (1.29 g, 2.35 mmol) in dry CH_2Cl_2 (7 mL) was added *i*-Pr_2NEt (0.91 g, 7.05 mmol) followed by MEMCl (0.59 g, 4.70 mmol) at 0 °C under N_2. The resultant solution was stirred at rt for 18 h before it was quenched with 10% HCl (10 mL). The separated aqueous phase was extracted with CH_2Cl_2 (2×10 mL). The combined organic layers were dried and evaporated to provide a residue, which was purified by column chromatography on silica gel (elution with 5:1 petroleum ether–ether) to afford 1.39 g (93%) of **33** as a colorless oil; IR (neat, cm^{-1}) 1726, 1612, 1251; ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.27 (m, 2H), 6.94–6.90 (m, 2H), 4.76 (s, 2H), 4.60–4.56 (m, 1H), 4.49–4.31 (m, 4H), 4.23–4.19 (m, 1H), 4.10–4.04 (m, 1H), 3.86 (s, 3H),

3.79–3.73 (m, 2H), 3.64–3.45 (m, 4H), 3.45 (s, 3H), 3.11–2.94 (m, 1H), 2.35–2.25 (m, 1H), 2.20–2.16 (m, 2H), 1.70–1.60 (m, 3H), 1.49–1.27 (m, 4H), 1.24 (s, 9H), 0.93 (s, 9 H), 0.12–0.08 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 159.0, 140.6, 138.1, 131.0, 129.1 (2C), 113.6 (2C), 95.4, 83.9, 71.7, 70.5, 67.8, 66.8, 66.6, 58.9, 58.2, 55.2, 43.6, 38.7, 32.8, 29.5, 28.1, 27.2 (3C), 26.3, 26.1, 25.8 (3C), 18.2, −5.4, −5.5; HRMS ES *m/z* $(M+Na)^+$ calcd 659.3949, obsd 659.3914; $[\alpha]_D^{20}$ −15.1 (*c* 1.7, $CHCl_3$).

4.15. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methylcyclopent-1-enecarboxylic acid (1*S*,4*R*)-2-(*tert*-dimethylsilanyloxymethyl)-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy)pentyl]cyclopent-2-enylmethyl ester (34)

To a stirred solution of **33** (0.62 g, 0.97 mmol) in dry CH_2Cl_2 (10 mL) was added DIBAL-H (2.43 mL of 1.0 M solution in hexane, 2.43 mmol) at −78 °C under N_2. The resultant solution was stirred at −78 °C for 0.5 h and at 0 °C for 1 h before it was quenched with a saturated solution of potassium sodium tartrate (10 mL). The resultant cloudy solution was stirred at rt overnight, diluted with water (20 mL), and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried, and evaporated to provide a residue that was purified by column chromatography on silica gel (elution with 2:1 petroleum ether–ethyl acetate) to furnish 0.42 g (78%) of **34** as a colorless oil; IR (neat, cm^{-1}) 3457, 1612, 1514, 1249; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.23 (m, 2H), 6.91–6.84 (m, 2H), 4.71 (s, 2H), 4.58–4.53 (m, 1H), 4.39–4.29 (m, 3H), 4.15–4.11 (m, 1H), 3.80 (s, 3H), 3.74–3.66 (m, 2H), 3.57–3.48 (m, 6H), 3.40 (s, 3H), 2.82–2.68 (m, 1H), 2.27–2.09 (m, 3H), 1.61–1.50 (m, 3H), 1.43–1.29 (m, 4H), 0.88 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.8, 141.7, 138.7, 130.5, 129.3 (2C), 113.6 (2C), 95.4, 82.9, 71.7, 70.6, 67.7, 66.6, 64.5, 58.9, 58.5, 55.2, 47.6, 32.5, 29.5, 28.0, 26.3, 26.0, 25.8 (3C), 18.2, −5.4, −5.5; HRMS ES *m/z* $(M+Na)^+$ calcd 575.3375, obsd 575.3362; $[\alpha]_D^{20}$ −31.5 (*c* 0.72, $CHCl_3$).

4.16. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methylcyclopent-1-ene carboxylic acid (1*S*,4*R*)-2-(*tert*-butyldimethylsilanyloxymethyl)-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy)pentyl]cyclopent-2-enyl methyl ester (35)

To a mixture of alcohol **34** (0.25 g, 0.46 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.26 g, 1.38 mmol), and DMAP (0.06 g, 0.46 mmol) in dry CH_2Cl_2 (5 mL) was added a solution of **34** (0.18 g, 0.46 mmol) in dry CH_2Cl_2 (3 mL) via cannula. The resulting mixture was stirred at rt for 24 h, freed of solvent, and subjected to column chromatography on silica gel (elution with 5:1 petroleum ether–ethyl acetate) to give 0.30 g (70%) of **35** as a colorless oil; IR (neat, cm^{-1}) 1714, 1613, 1514, 1251; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.63 (m, 4H), 7.44–7.34 (m, 6H), 7.26–7.22 (m, 2H), 6.89–6.84 (m, 2H), 6.57 (s, 1H), 4.71 (s, 2H), 4.55–4.16 (m, 6H), 4.08–4.02 (m, 1H), 3.80 (s, 3H), 3.71–3.68 (m, 2H), 3.58–3.47 (m, 6H), 3.40 (s, 3H), 3.09–2.98 (m, 1H), 2.61–2.56 (m, 2H), 2.28–2.23 (m, 1H), 2.14–2.11 (m, 2H), 1.99–1.95 (m, 1H), 1.69–1.54 (m, 4H), 1.34–1.26 (m, 4H), 1.12 (s, 3H), 1.05 (s, 9H), 0.88 (s, 9H), 0.05–0.04 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 158.9, 148.9, 140.7, 138.3, 135.7, 135.6 (4C), 133.5, 133.4, 130.8, 129.52, 129.49, 129.1 (2C), 127.5 (4C), 113.6 (2C), 95.3, 83.7, 71.7, 70.4, 67.7, 67.1, 66.5, 58.9, 58.2, 55.1, 52.1, 43.6, 33.7, 32.8, 30.6, 30.2, 29.5, 27.9, 26.7 (3C), 26.3, 26.1, 25.8 (3C), 22.7, 19.2, 18.1, −5.5 (2C); HRMS ES *m/z* $(M+Na)^+$ calcd 951.5233, obsd 951.5210.

4.17. (*S*)-3-(*tert*-Butyldiphenylsilanyloxy)-3-methylcyclopent-1-enecarboxylic acid (1*S*,4*R*)-2-hydroxymethyl-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy)pentyl]cyclopent-2-enylmethyl ester (36)

To a stirred solution of **35** (0.57 g, 0.61 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (10 mL each) at 0 °C was added CSA (0.14 g, 0.61 mmol). The mixture was stirred at 0 °C for 1 h, quenched with saturated $NaHCO_3$ solution (20 mL), and extracted with CH_2Cl_2 (3×20 mL). The organic layers were combined, dried, and evaporated to leave a residue, purification of which by chromatography on silica gel (elution with 1:1 petroleum ether–ether) afforded 0.46 g (92%) of **36** as a colorless oil; IR (neat, cm^{-1}) 3482, 1714, 1613; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65–7.61 (m, 4H), 7.44–7.34 (m, 6H), 7.26–7.23 (m, 2H), 6.91–6.83 (m, 2H), 6.60–6.59 (m, 1H), 4.70 (s, 2H), 4.55–4.51 (m, 1H), 4.41–4.16 (m, 6H), 3.79 (s, 3H), 3.70–3.66 (m, 2H), 3.57–3.48 (m, 6H), 3.39 (s, 3H), 3.08–2.92 (m, 1H), 2.61–2.56 (m, 2H), 2.27–2.26 (m, 1H), 2.20–2.15 (m, 2H), 2.00–1.96 (m, 1H), 1.66–1.55 (m, 4H), 1.44–1.26 (m, 3H), 1.26 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 159.1, 149.5, 142.3, 138.6 (2C), 135.5 (2C), 133.5, 130.8, 129.5 (4C), 129.2 (4C), 127.6 (2C), 113.7 (2C), 95.4, 83.4, 71.8, 70.6, 70.4, 67.7, 67.3, 66.7, 58.9, 57.7, 55.2, 52.2, 43.5, 33.7, 32.8, 30.6, 29.6, 27.7, 26.8 (3C), 26.0, 25.7, 22.7, 19.3 (2C); HRMS ES *m/z* $(M+Na)^+$ calcd 837.4368, obsd 837.4348; $[\alpha]_D^{20}$ −24.5 (*c* 1.08, $CHCl_3$).

4.18. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methylcyclopent-1-ene carboxylic acid (1*S*,4*R*)-2-bromomethyl-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy)pentyl]cyclopent-2-enyl methyl ester (37)

To a solution of **36** (30 mg, 0.036 mmol) in dry CH_2Cl_2 (1 mL) was added freshly distilled Et_3N (0.01 mL, 0.074 mmol) followed by MsCl (6 μL, 0.074 mmol) at 0 °C under N_2. The mixture was stirred at 0 °C for 30 min and quenched with 1 N HCl (2 mL) followed by H_2O (1 mL). The separated aqueous phase was washed with CH_2Cl_2 (2×5 mL). The combined CH_2Cl_2 layers were dried and concentrated under vacuum to provide the allyl mesylate as yellowish oil.

The above material was dissolved in dry THF (2 mL) and anhydrous LiBr (5 mg, 0.06 mmol) was added at rt. The mixture was stirred at rt for 1.5 h and filtered through a pad of silica gel, where the pad was further washed with ether (50 mL). The filtrate was concentrated under vacuum to afford a yellowish oil, which was purified by chromatography on silica gel (elution with 3:1 petroleum ether–ethyl acetate) to furnish 27 mg (86%) of **37** as a colorless liquid; IR (neat, cm^{-1}) 1711, 1658, 1513, 1249; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.61 (m, 4H), 7.45–7.34 (m, 6H), 7.27–7.23 (m, 2H), 6.89–6.84 (m, 2H), 6.63–6.62 (m, 1H), 4.71

(s, 2H), 4.55–4.51 (m, 1H), 4.43–4.34 (m, 2H), 4.27–4.14 (m, 3 H), 4.05–4.02 (m, 1H), 3.80 (s, 3H), 3.71–3.68 (m, 2H), 3.58–3.48 (m, 6H), 3.39 (s, 3H), 3.15–3.08 (m, 1H), 2.63–2.57 (m, 2H), 2.37–2.33 (m, 1H), 2.21–2.18 (m, 2H), 2.04–1.95 (m, 1H), 1.68–1.54 (m, 4H), 1.40–1.32 (m, 4H), 1.12 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 159.2, 149.8, 146.1, 135.7 (2C), 135.6, 133.6 (2C), 130.6, 129.6 (4C), 129.3 (4C), 127.6 (2C), 113.8 (2C), 95.5, 82.9, 71.8, 70.8, 70.5, 67.8, 66.7, 66.6, 59.0, 55.3, 52.3, 42.7, 38.9, 33.7, 32.5, 30.7, 29.7, 27.7, 26.9 (3C), 26.8, 26.4, 26.2, 22.8, 19.4; HRMS ES *m/z* $(M+Na)^+$ calcd 899.3524, obsd 899.3547; $[\alpha]_D^{20}$ −47.0 (*c* 0.83, $CHCl_3$).

4.19. (*E*)-(*R*)-5-(*tert*-Butyldiphenylsilanyloxy)pent-3-ene-1,2-diol (47)

A solution of **46** (3.86 g, 1.8 mmol) in 170 mL of 80% HOAc at 0 °C was allowed to warm to rt, stirred for 12 h, poured into a slurry of 200 g of $NaHCO_3$ suspended in 1 L of water, and extracted with ether (4×150 mL). The combined organic phases were dried and evaporated to leave a residue that was purified by chromatography on silica gel (hexane–ethyl acetate 2:1) to give **47** as a colorless oil (2.78 g, 84%); IR (neat, cm^{-1}) 3416, 1472, 1427; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.13 (m, 4H), 7.50–7.32 (m, 6H), 5.57 (dt, *J*=10.2, 2.2 Hz, 1H), 5.23 (dd, *J*=10.2, 6.3 Hz, 1H), 4.32–4.20 (m, 3H), 3.63 (dd, *J*=9.4, 1.8 Hz, 1H), 3.47 (dd, *J*=8.1, 10.3 Hz, 1H), 2.95–2.15 (bm, 1H), 1.07 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.5, 132.3, 131.8, 129.7, 128.2, 127.7, 72.6, 67.1, 63.7, 26.8, 18.5; HRMS ES *m/z* $(M+Na)^+$ calcd 379.1700, obsd 379.1713; $[\alpha]_D^{20}$ −7.0 (*c* 3.46, $CHCl_3$).

4.20. Acetic acid (*E*)-(*R*)-4-(*tert*-butyldiphenylsilanyloxy)-1-(*tert*-butyldiphenylsilanyloxymethyl)-but-2-enyl ester (48)

Diol **47** (10.7 g, 41.2 mmol) was dissolved in dry THF (80 mL) containing imidazole (3.65 g, 53.6 mmol), cooled to −15 °C, and treated with a solution of TBDPSCl (9.51 mL, 37.1 mmol) in 10 mL of dry THF via syringe pump over 2.5 h. Stirring was continued at −15 °C for another 2 h and a second portion of TBDPSCl (0.76 mL, 2.96 mmol) was added slowly at the same temperature within 30 min. The reaction mixture was stirred for another hour and a third portion of TBDPSCl (0.76 mL, 2.96 mmol) was introduced at −15 °C, quenched an hour later with saturated ammonium chloride solution (50 mL), and extracted with ether (4×80 mL). The combined organic layers were dried and evaporated to dryness to give a colorless oil, which was purified by chromatography on silica gel (gradient elution with hexane–ethyl acetate 25:1 to 7:1) to give 20.02 g (82%) of pure alcohol in the form of a colorless oil; IR (neat, cm^{-1}) 3420, 1472, 1427; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.60 (m, 8H), 7.50–7.28 (m, 12H), 5.83 (dt, *J*=18.1, 2.5 Hz, 1H), 5.70 (dd, *J*=18.1, 8.4 Hz, 1H), 4.30–4.10 (m, 1H), 4.18 (d, *J*=5.8 Hz, 2H), 3.66 (dd, *J*=8.2, 5.6 Hz, 1H), 3.53 (dd, *J*=8.2, 5.9 Hz, 1H), 2.65–2.54 (m, 1H), 1.08 (s, 9 H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.54, 135.48, 134.8, 133.5, 133.1, 133.0, 131.4, 129.8, 129.6, 129.5, 127.79, 127.78, 127.63, 127.60, 72.3 (d), 67.8, 63.7, 26.83, 26.79, 19.2, 18.9 (s); HRMS ES *m/z* $(M+Na)^+$ calcd 617.2878, obsd 617.2861; $[\alpha]_D^{20}$ +0.6 (*c* 1.33, $CHCl_3$).

The alcohol from above (20.02 g, 33.7 mmol) was dissolved in a mixture of pyridine–CH_2Cl_2=1:4 (375 mL) and cooled to 0 °C. DMAP (411 mg, 3.37 mmol) was introduced followed by acetic anhydride (31.6 mL, 0.34 mmol) in dropwise fashion. The mixture was allowed to warm to rt, stirred overnight, and freed of solvent. The residue was purified by column chromatography on silica gel (gradient elution with hexane–ethyl acetate 50:1 to 25:1) to give 19.7 g (92%) of **48** as a colorless oil; IR (neat, cm^{-1}) 1738, 1472, 1369; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78–7.63 (m, 8H), 7.50–7.32 (m, 12H), 5.87–5.81 (m, 2H), 5.52 (dd, *J*=4.2 Hz, 1H), 4.23 (s, 2H), 3.82–3.68 (m, 2H), 2.09 (s, 3H), 1.08 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 135.7, 135.6, 135.5, 135.3, 133.5, 127.74, 127.71, 124.6, 74.6, 65.6, 63.5, 26.94, 26.87, 21.2, 19.3, 19.1; HRMS ES *m/z* $(M+Na)^+$ calcd 659.2983, obsd 659.2948; $[\alpha]_D^{20}$ −8.3 (*c* 1.34, $CHCl_3$).

4.21. (*E*)-(*S*)-6-(*tert*-Butyldiphenylsilanyloxy)-3-(*tert*-diphenylsilanyloxymethyl)hex-4-enoic acid methyl ester (49)

The above acetate (4.00 g, 6.28 mmol) dissolved in 80 mL of dry THF was treated with TBDMSCl (3.92 g, 25.1 mmol) and cooled to −78 °C. KHMDS (28.5 mL, 18.8 mmol) was introduced and the mixture was kept for 3 h at −78 °C, allowed to warm to rt overnight prior to quenching with a mixture of saturated NaCl solution (200 mL) and 1 N HCl (40 mL). The aqueous phase was extracted with ether (3× 60 mL), and the combined organic phases were filtered and freed of solvent to give a mixture of ester and the corresponding carboxylic acid. To obtain pure acid, the crude product was dissolved in 80 mL of THF and 4 N LiOH (20 mL) was added at 0 °C. After 1 h or stirring at 0 °C, the TBS ester was cleaved quantitatively to the carboxylic acid and the reaction was stopped by acidification with 1 N HCl (160 mL) and extraction with ether (3×60 mL) and CH_2Cl_2 (3× 60 mL). The combined organic phases were dried, filtered, and distilled to give almost pure carboxylic acid. The acid was further purified by column chromatography on silica gel (gradient elution with ethyl acetate–hexane 1:10 to 1:7) to give 3.98 g (100%) of colorless oil; IR (neat, cm^{-1}) 2856, 1710, 1471; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.58 (m, 8H), 7.47–7.28 (m, 12H), 5.62–5.58 (m, 2H), 4.12 (s, 2H), 3.65 (dd, *J*=9.7, 4.6 Hz, 1H), 3.51 (dd, *J*=9.7, 6.9 Hz, 1H), 2.86–2.73 (m, 1H), 2.72 (dd, *J*=15.4, 5.9 Hz, 1H), 2.37 (dd, *J*=15.5, 7.6 Hz, 1H), 1.04 (s, 9 H), 1.03 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8, 135.6, 135.5, 133.7, 133.5, 130.8, 129.7, 129.6, 129.4, 127.7, 127.6, 66.5, 64.2, 40.9, 36.2, 26.8, 19.8, 19.3, 19.2; HRMS ES *m/z* $(M+Na)^+$ calcd 659.2984, obsd 659.3016; $[\alpha]_D^{20}$ +5.3 (*c* 0.87, $CHCl_3$).

The carboxylic acid (4.49 g, 7.06 mL) was dissolved in 44 mL of dry DMF and methyl iodide (5.27 mL, 84.7 mmol) was added. The reaction was started by the addition of dry K_2CO_3 (5.85 g, 42.3 mmol). The mixture was stirred at rt for 1.5 h and stopped by the addition of hexane (100 mL) and water (300 mL). The aqueous phase was extracted with hexane (4×100 mL), dried, and evaporated to afford a crude oil that was purified by chromatography on silica gel (hexane–ethyl acetate 25:1) to afford 3.33 g (73%) of **49** as a colorless oil; IR (neat, cm^{-1}) 1730, 1428, 1112; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.60 (m, 8H), 7.49–7.29 (m, 12H), 5.62 (s, 1H), 5.61 (d, *J*=0.5 Hz, 1H),

4.13 (s, 2H), 3.63 (s, 3H), 3.68–3.53 (m, 1H), 3.53 (dd, J=9.5, 6.6 Hz, 1H), 2.69 (dd, J=9.5, 5.8 Hz, 1H), 2.27 (dd, J=15.2, 8.4 Hz, 1H), 1.08 (s, 9 H), 1.06 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 135.6, 135.5, 133.8, 133.6, 130.7, 129.7, 129.6, 127.7, 127.6, 66.5, 643, 51.5, 41.3, 36.4, 26.9, 26.8, 19.3, 19.2; HRMS ES m/z $(M+Na)^+$ calcd 673.3140, obsd 673.3101; $[\alpha]_D^{20}$ +4.8 (c 3.38, $CHCl_3$).

4.22. (*E*)-(*S*)-6-(*tert*-Butyldiphenylsilanyloxy)-3-(*tert*-butyldiphenylsilanyloxymethyl)hex-4-enal (50)

A solution of **49** (2.09 g, 3.21 mmol) in dry THF was cooled to −78 °C and treated with DIBAL-H (9.64 mL, 9.64 mmol, 1 M in hexane). The reaction mixture was stirred at −78 °C for 10 min, warmed to 0 °C, and stirred for an additional 40 min in ice and 30 min at rt. Then 2 mL of methanol was added to quench and the resulting mixture was stirred for 10 min at rt prior to the introduction of saturated KNa-tartrate solution (60 mL) and ether (60 mL). Stirring was continued until the mixture was clear (2–4 h). The mixture was extracted with ether (4×50 mL), dried, and freed of solvents. The crude product was purified by chromatography on silica gel (hexane–ethyl acetate 7:1) to furnish the alcohol as a colorless oil (1.91 g, 95%); IR (neat, cm^{-1}) 3440, 1644, 1471; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76–7.60 (m, 8H), 7.50–7.29 (m, 12H), 5.62–5.53 (m, 2H), 4.20–4.12 (m, 2H), 3.75–3.52 (m, 4H), 2.47–2.33 (m, 1H), 2.10–1.88 (dt, J=19.8, 6.8 Hz, 1H), 1.61 (dt, J=22.0, 6.0 Hz, 1H), 1.08 (s, 9H), 1.06 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 135.5, 133.8, 133.6, 131.4, 130.4, 129.7, 129.6, 127.7, 127.6, 67.6, 64.4, 61.2, 42.4, 34.8, 26.99, 26.86, 19.3, 19.2; HRMS ES m/z $(M+Na)^+$ calcd 645.3191, obsd 645.3161; $[\alpha]_D^{20}$ +2.9 (c 1.13, $CHCl_3$).

Oxalyl chloride (70.1 μL, 0.83 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and cooled to −78 °C. Dry DMSO (117.5 μL, 1.66 mmol) was slowly added and after 1 h the above alcohol (258 mg, 0.41 mmol) dissolved in dry CH_2Cl_2 (5 mL) was introduced. The reaction mixture was stirred for 15 min at −78 °C, triethylamine (349 μL, 2.49 mmol) was added, and the reaction mixture was warmed to 0 °C, stirred for 30 min, and quenched with saturated NH_4Cl solution (10 mL). The product was extracted into ether (3×15 mL) and the combined organic phases were dried, evaporated to dryness, and quickly filtered through silica (hexane–ethyl acetate 7:1) to give pure **50** (259 mg, 100%); IR (neat, cm^{-1}) 1727, 1427, 1112; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (t, J=2.25 Hz, 1H), 7.69–7.56 (m, 8H), 7.47–7.28 (m, 12H), 5.58 (d, J=3.9 Hz, 2H), 4.13 (s, 2H), 3.64 (dd, J=5.02, 7.5 Hz, 1H), 3.49 (dd, J=7.5, 9.9 Hz, 1H), 2.33–2.96 (m, 1H), 2.66 (ddd, J=16.5, 6.1, 2.2 Hz, 1H), 2.43 (ddd, J=16.5, 7.7, 2.2 Hz, 1H), 1.04 (s, 9H), 1.03 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 135.6, 135.5, 133.7, 131.1, 129.7, 129.6, 129.2, 127.7, 127.6, 66.8, 64.1, 45.7, 39.6, 26.8, 19.22, 19.19; HRMS ES m/z $(M+Na)^+$ calcd 643.3034, obsd 643.3030; $[\alpha]_D^{20}$ +1.6 (c 0.57, $CHCl_3$).

4.23. (*E*)-(*S*)-11-Benzyloxy-1-(*tert*-butyldiphenylsilanyloxy)-4-(*tert*-butyldiphenylsilanyloxymethyl)undec-2-en-7-yn-6-one (51)

Benzyloxy-1-pentyne (7.88 g, 45.2 mmol) was dissolved in 200 mL of dry THF (200 mL) and n-BuLi (28.3 mL, 45.2 mmol, 1.6 N in hexane) was added at −65 °C. The mixture was warmed to −40 °C and stirred at that temperature for 1 h and returned to −78 °C. At this point, **50** (12.48 g, 20.1 mmol) was added via cannula as a solution in dry THF (50 mL), the temperature was raised slowly to −40 °C within 1 h, saturated NH_4Cl solution was added and the product was extracted into ether (3×150 mL). The combined organic layers were dried and evaporated to leave a residue that was purified by flash chromatography on silica gel (gradient elution with hexane–ethyl acetate 25:1 to 10:1) to yield 14.15 g (87%) of the alcohol as a 1:1 mixture of diastereomers.

The above alcohol mixture (94.3 mg, 0.119 mmol) was dissolved in CH_2Cl_2 (2.2 mL) and activated MnO_2 (104 mg, 1.19 mmol) was added. The reaction mixture was stirred for 15 h, treated with fresh MnO_2 (208 mg, 2.38 mmol), stirred for an additional 4 h, filtered through Celite, and purified by chromatography on silica gel (gradient elution with hexane–ethyl acetate 25:1 to 10:1) to afford 89.6 mg (95%) of pure **51**; IR (neat, cm^{-1}) 1673, 1472, 1428; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77–7.60 (m, 10H), 7.52–7.25 (m, 14H), 5.67–5.57 (m, 2H), 4.53 (s, 2H), 4.18 (d, J=1.5 Hz, 2H), 3.67 (dd, J=10.1, 5.3 Hz, 1H), 3.59 (t, J=5.8 Hz, 2H), 3.52–3.50 (m, 1H), 3.10–2.97 (m, 1H), 2.90 (dd, J=15.9, 5.8 Hz, 1H), 2.57 (dd, J=15.9, 7.8 Hz, 1H), 2.53 (t, J=7.0 Hz, 2H), 1.90 (q, J=7.0 Hz, 2H), 1.10 (s, 9H), 1.09 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.3, 135.7, 135.6, 133.74, 133.71, 133.5, 130.9, 129.7, 129.6, 129.4, 128.4, 127.74, 127.71, 127.67, 93.6, 81.4, 73.1, 68.4, 66.5, 64.3, 46.4, 40.7, 28.1, 26.9, 19.32, 19.26, 16.0; HRMS ES m/z $(M+Na)^+$ calcd 815.3922, obsd 815.3908; $[\alpha]_D^{20}$ +5.0 (c 4.21, $CHCl_3$).

4.24. [(*E*)-(4*S*,6*S*)-11-Benzyloxy-4-(*tert*-butyldiphenylsiloxy)-6-methoxymethoxyundec-2-en-7-ynyloxy-*tert*-butyldiphenylsilane (53)

Ketone **51** (112 mg, 0.14 mmol) was dissolved in dry THF (2.7 mL) and the CBS reagent (0.4 mL, 0.4 M solution in THF, 0.16 mmol) was added. The reaction mixture was cooled to −78 °C, treated with borane (0.71 mL, 0.71 mmol, 1 M in THF), warmed to −40 °C within 15 min and kept at that temperature for an hour. When reaction was found to be complete (TLC analysis), MeOH (0.7 mL) was added and stirring was continued for another 10 min. After the addition of saturated NH_4Cl solution, the mixture was extracted with ether (3×5 mL), and the combined organic layers were dried and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane–ethyl acetate 10:1) to give 112 mg (100%) of **52** as a colorless oil; IR (neat, cm^{-1}) 3433, 1472, 1428; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55–7.54 (m, 10H), 7.46–7.21 (m, 14H), 5.67–5.46 (m, 2H), 4.50 (s, 2H), 4.38–4.28 (m, 1H), 4.14 (d, J=4.0 Hz, 2H), 3.48–3.10 (m, 4 H), 2.62–2.47 (m, 1H), 2.35 (dt, J=1.6, 7.0 Hz, 2H), 2.12–1.98 (m, 1H), 1.96–1.80 (m, 1H), 1.81 (q, J=6.8 Hz, 2H), 1.75–1.63 (m, 1H), 1.05 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 135.7, 135.6, 133.7, 133.6, 131.1, 131.0, 129.7, 128.4, 127.72, 127.69, 127.6, 84.5, 82.0, 68.8, 67.6, 64.4, 60.8, 41.8, 40.6, 28.9, 26.93, 26.91, 19.3, 15.7; HRMS ES m/z $(M+Na)^+$ calcd 817.4079, obsd 817.4060; $[\alpha]_D^{20}$ +2.5 (c 6.51, $CHCl_3$).

This enantiomerically enriched alcohol (2.45 g, 3.09 mmol) was dissolved in CH_2Cl_2 (50 mL) and Hünig's base (2.64 mL, 15.4 mmol) was added. The mixture was cooled to 0 °C, treated with MOMCl (1.16 mL, 15.4 mmol), stirred for 8.5 h, and quenched with saturated NH_4Cl solution (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3×30 mL), the combined organic layers were dried, and evaporated to give a crude product that was purified by chromatography on silica gel (gradient elution with hexane–ethyl acetate 25:1 to 10:1) to afford 2.48 g (96%) of **53**; IR (neat, cm^{-1}) 1472, 1428, 1112; ^1H NMR (300 MHz, $CDCl_3$) δ 7.83–7.67 (m, 10H), 7.50–7.26 (m, 14H), 5.73–5.62 (m, 2H), 5.00 (d, J=6.7 Hz, 1H), 4.62 (d, J=6.7 Hz, 1H), 4.54 (s, 2H), 4.50–4.38 (m, 1H), 4.25 (s, 2H), 3.72–3.58 (m, 4 H), 3.38 (s, 3H), 2.54–2.58 (m, 1H), 2.5–2.37 (m, 2H), 2.18–2.04 (m, 1H), 1.87 (q, J=6.7 Hz, 2H), 1.78–1.55 (m, 1H), 1.14 (s, 18 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 135.7, 133.8, 131.1, 129.6, 128.4, 127.68, 127.65, 127.58, 94.0, 85.4, 79.4, 73.0, 68.9, 65.4, 64.5, 63.9, 55.8, 41.4, 35.6, 28.9, 26.9, 19.4, 19.3, 15.8; HRMS ES m/z $(M+Na)^+$ calcd 861.4341, obsd 861.4389; $[\alpha]_D^{20}$ +36.9 (c 4.39, $CHCl_3$).

4.25. ((2*S*,4*R*)-9-Benzyloxy-4-methoxymethoxy-2-vinylnon-5-ynyloxy)-*tert*-butyldiphenylsilane (54)

Enyne **53** (500 mg, 0.596 mmol) was dissolved in hexane (34 mL) and ozone was bubbled through the solution at −78 °C for 15 min. The solution was purged with oxygen for 15 more minutes, treated with a solution of PPh_3 (469 mg, 1.79 mmol) in ether (1 mL), and stirred at 0 °C for 3.5 h at rt. Simultaneously, methylenetriphenylphosphorane was prepared in a separate flask by adding n-BuLi (2.98 mL, 4.17 mol, 1.4 N solution in hexane) to a suspension of $PPh_3CH_3^{\ddagger}Br^-$ (2.34 g, 6.56 mmol) in ether (9.8 mL). The suspension was stirred at 0 °C for 4 h and then added to the phosphorane over a period of 10 min at 0 °C. Stirring was continued overnight and the reaction mixture was allowed to warm to rt, quenched with saturated NH_4Cl solution (30 mL), and extracted with ether (4×20 mL) and CH_2Cl_2 (1×20 mL). The combined organic layers were dried and freed of solvent prior to chromatography on silica gel (gradient elution with pure hexane to hexane–ethyl acetate 3:1) to afford 291 mg (85%) of **54**; IR (neat, cm^{-1}) 1468, 1527, 1112; ^1H NMR (300 MHz, $CDCl_3$) δ 7.80–7.67 (m, 5H), 7.50–7.25 (m, 10H), 5.84–5.78 (m, 1H), 5.64 (s, 1H), 5.00 (d, J=9.7 Hz, 1H), 4.90 (d, J=6.8 Hz, 1H), 4.58 (d, J=6.8 Hz, 1H), 4.52 (s, 2H), 4.44–4.34 (m, 1H), 3.13 (d, J=5.6 Hz, 2H), 3.57 (t, J=6.2 Hz, 2H), 3.38 (s, 3H), 2.64–2.50 (m, 1H), 2.36 (t, J=7.0 Hz, 2H), 2.05 (ddd, J=9.3, 4.6, 4.6 Hz, 1H), 1.82 (q, J=6.5 Hz, 2H), 1.72 (ddd, J=9.3, 4.6, 4.6 Hz, 1H), 1.08 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.3, 138.5, 135.70, 135.67, 135.5, 134.8, 133.8, 129.7, 129.6, 128.4, 127.7, 127.63, 127.58, 116.5, 94.0, 85.4, 79.3, 73.0, 68.8, 67.3, 63.9, 55.8, 42.8, 37.9, 28.9, 26.9, 19.3, 15.6; HRMS ES m/z $(M+Na)^+$ calcd 593.3058, obsd 593.3070; $[\alpha]_D^{20}$ +53.4 (c 0.99, $CHCl_3$).

4.26. {(1*S*,4*R*)-3-[4-Benzyloxybut-(*Z*)-ylidene]-4-methoxymethoxy-2-methylenecyclopentyl}methanol (56)

A solution of **54** (221 mg, 0.39 mmol) in deoxygenated dichloroethane was treated with $Pd(OAc)_2$ (17.4 mg, 0.08 mmol) and *N,N*-bis-(benzylidene)ethylenediamine (**55**) (18.3 mg, 0.08 mmol) and stirred at 60 °C for three days until all starting material was consumed. The solvent was removed to leave a residue (*E:Z*=3.5:1) from which it was possible to separate the major part of the desired *E*-isomer **56** (72.5 mg, 31%) from the *E:Z* mixture (63.7 mg, 32%) (silica gel, gradient elution from 25:5 hexane–ethyl acetate; IR (neat, cm^{-1}) 1468, 1427, 1112; ^1H NMR (300 MHz, $CDCl_3$) δ 7.80–7.60 (m, 5H), 7.51–7.26 (m, 10H), 6.04 (t, J=7.5 Hz, 1H), 5.45 (s, 1H), 4.81 (s, 2H), 4.72 (d, J=6.9 Hz, 1H), 4.56 (d, J=6.9 Hz, 1H), 4.52 (s, 2H), 3.85–3.68 (m, 2H), 3.51 (t, J=6.3 Hz, 2H), 3.36 (s, 3H), 2.90–2.74 (m, 1H), 2.52–2.24 (m, 2H), 2.14–2.00 (m, 1H), 1.99–1.58 (m, 3 H), 1.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 140.9, 135.6, 134.1, 129.5, 128.4, 127.64, 127.61, 127.55, 127.48, 104.0, 94.4, 75.6, 72.9, 69.8, 68.1, 55.6, 45.7, 32.9, 29.7, 26.9, 26.0, 19.3; HRMS ES m/z $(M+Na)^+$ calcd 593.3058, obsd 593.3080; $[\alpha]_D^{20}$ −32.0 (c 3.63, $CHCl_3$).

The above material (66.9 mg, 0.12 mmol) was dissolved in dry THF (3.9 mL) and TBAF (123 μL, 1 M in THF, 0.12 mmol) was added. The reaction mixture was stirred at rt for 4 h and diluted with water (5 mL) and ether (2 mL). The mixture was extracted with ether (3×4 mL) and the combined organic layers were dried and evaporated. The residue was purified by chromatography on silica gel (gradient elution with hexane–ethyl acetate 10:1 to 2:1) to give 34 mg (88%) of pure **56**; IR (neat, cm^{-1}) 3428, 1453, 1147; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.18 (m, 5 H), 6.07 (dt, J=7.2, 1.0 Hz, 1H), 5.41 (d, J=1.8 Hz, 1H), 4.91 (d, J=1.3 Hz, 1H), 4.72 (d, J=5.4 Hz, 1H), 4.63 (dd, J=18.2, 6.9 Hz, 2H), 4.50 (s, 3H), 3.70 (d, J=6.3 Hz, 2H), 3.37 (s, 3H), 2.76–2.88 (m, 1H), 2.53–2.22 (m, 2H), 2.01 (ddd, J=18.9, 14.4, 5.3 Hz, 1H), 1.94–1.63 (m, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.5, 140.3, 138.5, 128.4, 128.2, 127.6, 127.5, 127.4, 104.3, 94.3, 75.1, 72.9, 69.7, 66.3, 55.8, 45.3, 33.9, 29.6, 26.1; HRMS ES m/z $(M+Na)^+$ calcd 355.1880, obsd 355.1861; $[\alpha]_D^{20}$ −20.6 (c 1.81, $CHCl_3$).

4.27. (*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-3-methylcyclopent-1-enecarboxylic acid (1*S*,4*R*)-3-[4-benzyloxybut-(*Z*)-ylidene]-4-methoxymethoxy-2-methylenecyclopentyl methyl ester (57)

Diene **56** (7.3 mg, 0.014 mmol) was dissolved in dry CH_2Cl_2 (1.8 mL) and carboxylic acid **16** (28.3 mg, 0.072 mmol) and DMAP (20.5 mg, 0.168 mmol) were added. Finally DCC (34.7 mg, 0.168 mmol) was added and the reaction mixture was stirred for three days. The reaction was arrested by the addition of water (5 mL) and extraction with CH_2Cl_2 (3×5 mL). The combined organic layers were dried and evaporated to leave a residue that was purified by flash chromatography on silica gel (gradient elution with hexane–ethyl acetate 25:1 to 10:1) to give 9.2 mg (90%) of **57** as a colorless oil; IR (neat, cm^{-1}) 1715, 1646, 1456; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71–7.61 (m, 4H), 7.47–7.25 (m, 11H), 6.62 (t, J=2.0 Hz, 1H), 6.08 (t, J=7.6 Hz, 1H), 5.91 (d, J=1.6 Hz, 1H), 5.40 (d, J=1.6 Hz, 1H), 4.75–4.69 (m, 1H), 4.65 (s, 2H), 4.52 (s, 2H), 4.31 (dd, J=10.4, 7.2 Hz, 1H), 4.36 (dd, J=10.2, 8.4 Hz, 1H), 3.53–3.47 (m, 4H), 3.37 (s, 3H), 3.00–2.90 (m, 1H), 2.68–2.58 (m, 4H), 2.97–2.25 (m, 2H),

2.24–1.60 (m, 6H), 1.16 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 165.4, 154.0, 149.3, 148.0, 140.2, 139.4, 139.0, 135.6, 133.6 (2C), 129.6, 128.4, 127.6, 127.1, 105.0, 100.0, 99.9, 94.5, 75.5, 72.9, 70.5, 70.2, 69.7, 68.0, 58.8, 55.7, 33.7, 33.0, 31.0, 29.7, 26.9, 26.8, 26.6, 24.6, 22.8, 22.7, 14.1; HRMS ES *m/z* $(M+Na)^+$ calcd 731.3738, obsd 731.3740; $[\alpha]_D^{20}$ −8.9 (*c* 0.46, $CHCl_3$).

4.28. (*S*)-3-(*tert*-Butyldiphenylsilanyloxy)-3-methyl-cyclopent-1-enecarboxylic acid (1*S*,4*R*)-2-formyl-4-(4-methoxybenzyloxy)-3-[5-(2-methoxyethoxymethoxy) pentyl]cyclopent-2-enylmethyl ester (59)

To a solution of **36** (179 mg, 0.19 mmol) in dry CH_2Cl_2 (17 mL) was added MnO_2 (334 mg, 3.84 mmol). After 15 h of stirring at rt, the MnO_2 was removed by filtration over a pad of Celite. The filtrate was evaporated and the residue was purified by flash chromatography on silica gel (hexane–ethyl acetate 4:1) to give 122 mg (85%) of pure **59** as a colorless oil; IR (neat, cm^{-1}) 1714, 1672, 1250; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.68–7.59 (m, 4H), 7.47–7.34 (m, 6H), 7.24 (d, *J*=11.6 Hz, 2H), 6.86 (d, *J*=11.6 Hz, 2H), 6.55 (s, 1H), 4.70 (s, 2H), 4.58 (d, *J*=11.5 Hz, 1H), 4.48 (dd, *J*=10.7, 4.3 Hz, 1H), 4.36 (d, *J*=11.5 H, 1H), 4.17 (dd, *J*=10.7, 7.7 Hz, 1H), 3.79 (s, 3H), 3.70–3.64 (m, 2H), 3.58–3.47 (m, 4H), 3.45 (s, 3H), 3.39 (s, 3H), 3.26–3.14 (m, 1H), 2.74–2.41 (m, 4H), 2.35–2.22 (m, 1H), 2.01–1.87 (m, 1H), 1.78–1.16 (m, 8H), 1.12 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.8, 165.3, 159.4, 149.3, 137.9, 135.7, 135.6, 133.6, 133., 130.0, 129.6, 129.5, 127.6, 113.8, 95.5, 82.8, 71.8, 71.4, 70.5, 67.6, 66.7, 65.8, 59.0, 55.2, 41.5, 33.8, 32.3, 31.9, 30.7, 29.4 (2C), 29.2, 28.4, 26.9, 26.3, 26.0, 22.7 (2C), 19.4, 14.1; HRMS ES *m/z* $(M+Na)^+$ calcd 835.4212, obsd 835.4236; $[\alpha]_D^{20}$ −0.77 (*c* 4.81, $CHCl_3$).

4.28.1. (*S*)-3-(*tert*-Butyldiphenylsilanyloxy)-3-methyl-cyclopent-1-enecarboxylic Acid (1*S*,4*R*)-2-[1-(*tert*-butyl-dimethylsilanyloxy)-meth-(*E*)-ylidene]-4-(4-methoxy-benzyloxy)-3-[5-(2-methoxyethoxymethoxy)pent-(*Z*)-ylidene]cyclopentylmethyl ester (60).

A solution of **59** (21.2 mg, 0.026 mmol) and triethylamine (40 μL, 0.29 mmol) in dry CH_2Cl_2 (1 mL) was cooled to −78 °C and triethylsilyl triflate (22 μL, 0.098 mmol) was added. The reaction mixture was warmed to 0 °C and kept at that temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (2 mL) and evaporated, and the residue was purified by flash chromatography (hexane–ethyl acetate 10:1) to give 15.5 mg (64%) of **60** as a colorless oil; IR (neat, cm^{-1}) 1716, 1652, 1457; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.62 (m, 4H), 7.48–7.34 (m, 6H), 7.26 (d, *J*=8.4 Hz, 2H), 6.86 (d, *J*=8.4 Hz, 2H), 6.82 (s, 1H), 6.60 (s, 1H), 5.67 (t, *J*=7.6 Hz, 1H), 4.73 (s, 2H), 4.64 (dd, *J*=10.4, 4.8 Hz, 1H), 4.50 (d, *J*=10.8 Hz, 1H), 4.43–4.51 (m, 1H), 4.11 (t, *J*=10.4 Hz, 1H), 3.80 (s, 3 H), 3.72–3.67 (m, 2H), 3.61–3.47 (m, 4H), 3.42 (s, 3H), 3.32–3.11 (m, 1H), 2.68–2.49 (m, 2H), 2.31 (d, *J*=20.8 Hz, 1H), 2.23–2.07 (m, 2H), 2.03–1.93 (q, *J*=7.6 Hz, 1H), 1.75–1.20 (m, 9H), 1.17 (s, 3H), 1.07 (s, 9H), 1.00 (t, *J*=6.5 Hz, 6H), 0.70 (q, *J*=6.5 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 159.0, 139.5, 136.1, 135.7, 135.6, 134.2, 133.7, 133.6, 130.8, 129.6, 129.5, 129.1, 127.7, 127.6, 126.0, 125.8, 123.8, 121.4, 120.2, 113.7, 95.5, 78.6, 71.8, 70.6, 69.7, 67.8, 66.7, 65.9, 60.4, 59.0, 55.2, 52.2, 39.4, 37.1, 33.8, 29.7, 29.4, 29.3, 26.5, 22.9, 22.7, 22.6, 19.4, 14.2, 14.1, 11.4, 6.5, 4.5; HRMS ES *m/z* $(M+Na)^+$ calcd 949.5077, obsd 949.5119; $[\alpha]_D^{20}$ −15.7 (*c* 0.78, $CHCl_3$).

4.29. (*S*)-1-Methylcyclopent-2-enecarboxylic acid ethyl ester (63)

Dry triethylamine (237 mL, 1.70 mol) and methanesulphonyl chloride (57 mL, 0.74 mol) were added to a stirred solution (2*S*)-hydroxy-(1*S*)-methylcyclopentanecarboxylic acid ethyl ester (97.45 g, 0.567 mol) in dry CH_2Cl_2 (1.15 L) at 0 °C under N_2. The mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2 (800 mL), and washed with brine (1 L). The aqueous phase was extracted with CH_2Cl_2 (500 mL), the combined organic layers were dried and freed of solvent to leave a residue that was purified by chromatography on silica gel (gradient elution with pure hexane to hexane–ethyl acetate 3:1) to afford the mesylate (101.1 g, 71%) as a yellow oil; IR (neat, cm^{-1}) 3548, 1725, 1466; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.32–5.23 (m, 1H), 3.80 (q, *J*=7.1Hz, 2H), 3.03 (s, 3 H), 2.32–1.58 (m, 6H), 1.31 (s, 3H), 1.27 (t, *J*=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 89.3, 60.6, 52.1, 37.5, 34.7, 30.7, 19.8, 17.8, 13.6; HRMS ES *m/z* $(M+Na)^+$ calcd 273.0767, obsd 273.0771; $[\alpha]_D^{20}$ +46.7 (*c* 1.4, $CHCl_3$).

1,8-Diazabicyclo[5.4.0]undec-7-ene (91 mL, 0.61 mol) was added to a solution of the above mesylate (101.1 g, 0.404 mol) in dry DMF (300 mL) at rt under N_2. The reaction mixture was heated at 160 °C for 12 h, diluted with ether (800 mL), and washed with water (2×800 mL). The combined organic layers were dried and evaporated to leave a brown oil, which was purified by chromatography on silica gel (hexane–ethyl acetate 99:1) to afford **63** (35.05 g, 84%) as a pale yellowish oil; IR (neat, cm^{-1}) 1731, 1464, 1372; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (dt, *J*=5.5, 2.1 Hz, 1H), 5.69 (dt, *J*=5.5, 2.0 Hz, 1H), 4.13 (q, *J*=7.1 Hz, 2H), 2.51–2.35 (m, 4H), 1.31 (s, 3H), 1.25 (t, *J*=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 134.8, 131.0, 89.3, 59.9, 55.1, 34.6, 31.3, 24.2, 13.7; HRMS ES *m/z* $(M+Na)^+$ calcd 177.0886, obsd 177.0885; $[\alpha]_D^{20}$ −78.5 (*c* 1.1, $CHCl_3$).

4.30. *tert*-Butylmethyl-((*S*)-1-methylcyclopent-2-enyl-methoxy) phenylsilane (64)

Lithium aluminum hydride (11.2 g, 0.295 mol) was added portion wise over a period of 2 h to a stirred solution of **63** (35.05 g, 0.227 mol) in dry ether (450 mL) at rt. The mixture was stirred for 30 min, cooled to 0 °C, treated with 1.0 N NaOH solution until the mixture turned white, and extracted with CH_2Cl_2 (3×1 L). The combined organic phases were dried and freed of solvent. The residue was chromatographed on silica gel (hexane–ethyl acetate 5:1) to give the primary carbinol (23.25 g, 91%) as a pale yellow oil; IR (neat, cm^{-1}) 3370, 1457, 1379; ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.81 (dt, *J*=5.6, 2.3 Hz, 1H), 5.46 (dt, *J*=5.6, 2.2 Hz, 1H), 3.50–3.31 (m, 2H), 2.45–2.33 (m, 2H), 2.00–1.87 (m, 1H), 1.65–1.49 (m, 1H), 1.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.6, 131.0 69.9, 50.8, 33.3, 31.7, 23.0; HRMS ES molecular ion too fleeting for accurate mass measurement; $[\alpha]_D^{20}$ −32.0 (*c* 1.3, $CHCl_3$).

Imidazole (28.3 g, 0.416 mmol) was added to a solution of the above alcohol (23.2 g, 0.208 mol) in DMF (100 mL) at 0 °C. After 20 min, *tert*-butyldiphenylsilyl chloride (60 mL, 0.23 mol) was added, and the resulting solution was stirred for 15 h at rt, diluted with ether (600 mL), and washed with water (2×500 mL). The organic layer was dried and the solvent was removed to leave a residue that was purified by chromatography on silica gel (hexane) to give **64** as a colorless foam (64.33 g, 89%); IR (neat, cm^{-1}) 1656, 1589, 1486; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73–7.63 (m, 4H), 7.47–7.32 (m, 6H), 5.70 (dt, J=5.6, 2.2 Hz, 1H), 5.57 (dt, J=5.6, 2.1 Hz, 1H), 3.48 (d, J=9.4 Hz, 1H), 3.44 (d, J=9.4 Hz, 1H), 2.40–2.31 (m, 2H), 1.93–1.82 (m, 1H), 1.54–1.48 (m, 1H), 1.12 (s, 3 H), 1.08 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 135.7, 134.0, 130.2, 129.5, 127.6, 71.3, 51.4, 33.9, 31.8, 26.9, 23.8, 19.4; HRMS ES *m/z* $(M+Na)^+$ calcd 373.1958, obsd 373.1967; $[\alpha]_D^{20}$ −31.9 (*c* 1.6, $CHCl_3$).

4.31. (*S*)-4-(*tert*-Butylmethylphenylsilanyloxymethyl)-2-iodo-4-methylcyclopent-2-enone (65)

3,5-Dimethylpyrazole (179 g, 1.87 mol) was added to a suspension of chromium(VI) oxide (189.9 g, 1.899 mol) in dry CH_2Cl_2 (1 L) at −15 °C and stirred at this temperature for 20 min prior to the introduction of a solution of **64** (43.67 g, 0.125 mol) in dry CH_2Cl_2 (200 mL). The mixture was stirred for 1 h at −15 °C, filtered through a pad of SiO_2 (ethyl acetate 1:hexane 3), and freed of solvent. The residue was purified by chromatography on silica gel (hexane–ethyl acetate 5:1) to afford the enone as a pale orange oil (18.74 g, 41%); IR (neat, cm^{-1}) 1717, 1589, 1472; ^1H NMR (250 MHz, $CDCl_3$) δ 7.65–7.58 (m, 4H), 7.49–7.32 (m, 7H), 6.12 (d, J=5.6 Hz, 1H), 3.56 (s, 2H), 2.46 (d, J=18.4 Hz, 1H), 2.08 (d, J=18.4 Hz, 1H), 1.21 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.9, 169.9, 135.4, 133.0, 132.8, 129.6, 127.5, 69.4, 47.2, 45.0, 26.6, 22.3, 19.0; HRMS ES *m/z* $(M+Na)^+$ calcd 387.1751, obsd 387.1752; $[\alpha]_D^{20}$ −55.4 (*c* 0.8, $CHCl_3$).

To a solution of iodine (41.2 g, 0.162 mol) in dry CH_2Cl_2 (100 mL) and dry pyridine (80 mL) at 0 °C was added via cannula to a solution of the above ketone (27.54 g, 0.076 mol) in a l:l mixture of dry CH_2Cl_2 and pyridine (300 mL). The resulting mixture was stirred for 5 min at 0 °C and at rt for 22 h prior to dilution with H_2O (500 mL) and cooling to 0 °C. 2 M HCl was slowly added until pH 4 was reached, at which point the separated aqueous layer was extracted with CH_2Cl_2 (600 mL). The combined organic phases were washed with saturated sodium thiosulphate solution (1 L) and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried and the solvent evaporated to leave a brown oil, which was purified by chromatography on silica gel (hexane–ethyl acetate 1:1) to give **65** as a pale yellow oil (35.9 g, 97%); IR (neat, cm^{-1}) 1721, 1578, 1472; ^1H NMR (250 MHz, $CDCl_3$) δ 7.68–7.58 (m, 4H), 7.50–7.32 (m, 6H), 7.37 (s, 1H), 3.55 (s, 2H), 2.62 (d, J=18.3 Hz, 1H), 2.19 (d, J=18.3 Hz, 1H), 1.19 (s, 3H), 1.03 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.6, 174.6, 135.4, 132.6, 129.7, 127.6, 101.7, 68.9, 49.6, 42.1, 26.6, 21.9, 14.0; HRMS ES *m/z* $(M+Na)^+$ calcd 513.0717, obsd 513.0727; $[\alpha]_D^{20}$ 55.4 (*c* 2.6, $CHCl_3$).

4.32. (*S*)-3-(*tert*-Butylmethylphenylsilanyloxymethyl)-3-methyl-5-oxocyclopent-1-enecarboxylic acid methyl ester (66)

Triethylamine (31 mL, 0.22 mol) and anhydrous MeOH (6.0 mL, 0.15 mol) were added to a stirred solution of **65** (35.92 g, 73.3 mmol), $Pd_2(dba)_3 \cdot CHCl_3$ (2.62 g, 2.86 mmol), and Ph_3P (3.0 g, 0.01 mol) in dry DMF (260 mL) at rt under a carbon monoxide atmosphere. The resulting mixture was heated at 70 °C for 20 h, cooled to rt, diluted with ether (1.2 L), and washed with water (2×1 L). The organic phase was dried and the solvent was removed to give a black residue that was purified by chromatography on silica gel (hexane–ethyl acetate 5:1) to afford **66** as a yellow oil (18.98 g, 61%); IR (neat, cm^{-1}) 1755, 1727, 1624; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.67–7.54 (m, 4H), 7.49–7.34 (m, 6H), 3.86 (s, 3H), 3.59 (s, 2H), 2.66 (d, J=18.5 Hz, 1H), 2.27 (d, J=18.5 Hz, 1H), 1.23 (s, 3 H), 1.03 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 176.7, 162.0, 135.7, 135.4, 132.5, 129.7, 127.6, 69.0, 49.6, 51.7, 46.4, 44.8, 26.5, 21.7, 19.0; HRMS ES *m/z* $(M+Na)^+$ calcd 445.1806, obsd 445.1784; $[\alpha]_D^{20}$ −38.4 (*c* 1.0, $CHCl_3$).

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A concise synthesis of *d*,*l*-brevianamide B via a biomimetically-inspired IMDA construction

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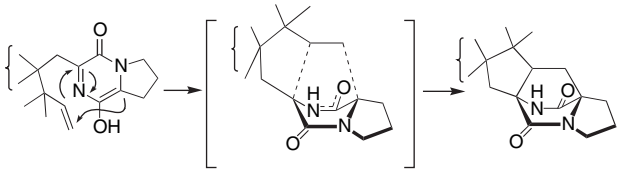
This paper is dedicated to Professor Stephen F. Martin on the occasion of his 60th birthday

Abstract—A concise synthesis of brevianamide B has been accomplished using a biomimetically-inspired intramolecular Diels–Alder reaction to diastereoselectively form the characteristic bicyclo[2.2.2]diazooctane core.

1. Introduction

Birch and co-workers reported the isolation of brevianamides A and B (**1** and **2**, Fig. 1) from *Penicillium brevicompactum* in 1969.[1] This event, and the structural elucidation and biosynthetic studies, which followed, marked the birth of a unique family of prenylated indole alkaloids containing a characteristic, but hitherto unknown bicyclo[2.2.2]diazaoctane core.[2] This family has since grown to include the paraherquamides, marcfortines, sclerotamides, VM55599, asperparalines, asperagamides, avrainvillamide, and the stephacidins (Fig. 1).[3,4] While many of these alkaloids demonstrate interesting biological activities, one of the most intriguing features of this class of compounds is the proposed biosynthesis of the bicyclo[2.2.2]diazaoctane core via a hetero-intramolecular Diels–Alder reaction (Scheme 1).[5] While the [4+2] cycloaddition reaction is perhaps the most powerful and common method to rapidly construct six-membered rings in synthetic chemistry, there are relatively few examples of natural products that have been proven to arise via a biological Diels–Alder reaction, although the number of natural substances postulated to arise via a true Diels–Alder construction has recently exploded.[6] Several groups have claimed the discovery and identification of a 'Diels–Alderase' enzyme, but rigorous proof of catalysis of the pericyclic Diels–Alder transition state has yet to be established.[7] In fact, Jorgensen has recently refuted an earlier claim by Oikawa and associates based on theoretical modeling of the transition states and has proffered an alternative step-wise mechanism in the biosynthesis of macrophomic acid.[8] Significant provocative, yet indirect evidence (mostly stereochemical) has been used to invoke the intermediacy of enzymatic mediation in the biosynthesis of several natural products that appear to fit the Diels–Alder biogenetic structural manifold.[6] We have proposed enzyme mediation to be involved in the biogenesis of the brevianamides and related alkaloids, since there is discriminate diastereoselectivity in the formation of either the brevianamide-type (*anti*-diastereomer at C19) or the paraherquamide-type (*syn*-diastereomer at C20) of stereochemistry, along with intriguing, divergent enantiofacial selectivity (brevianamide A vs brevianamide B).[3,9,10]



Scheme 1. Proposed biosynthetic [4+2] cycloaddition constructing the bicyclo[2.2.2] ring system.[3]

Our laboratory has continued to be interested in the biomimetic total synthesis and biosynthesis of these fascinating compounds.[11] These studies have evolved a new hypothesis on the genesis of the redox biochemistry required to convert the saturated amino acid building blocks into the putative azadiene species that culminate in the formation of the bicyclo[2.2.2]diazaoctane core.[12] Here, we report a concise and diastereoselective synthesis of *d*,*l*-brevianamide B, that was inspired by a new twist on the genesis of the azadiene species.[12]

Keywords: Fisher indole; Intramolecular Diels–Alder; Azadiene; Brevianamide B.
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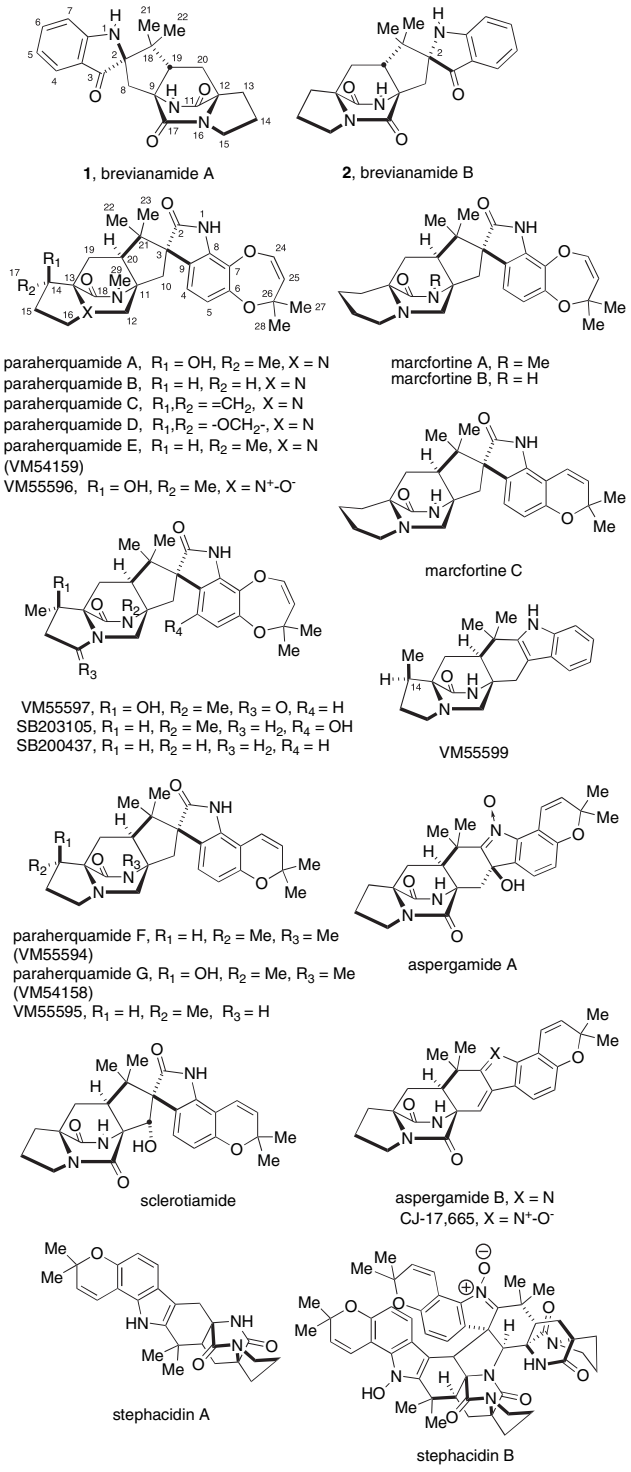
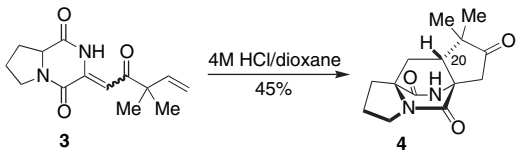


Figure 1. Structures of the prenylated bicyclo[2.2.2] indole alkaloids.

2. Results and discussion

We recently reported an IMDA reaction of azadiene progenitor **3** that formed the spiro-fused five-membered ring species **4** exclusively as the *anti*-diastereomer at C20 (Scheme 2).[12]

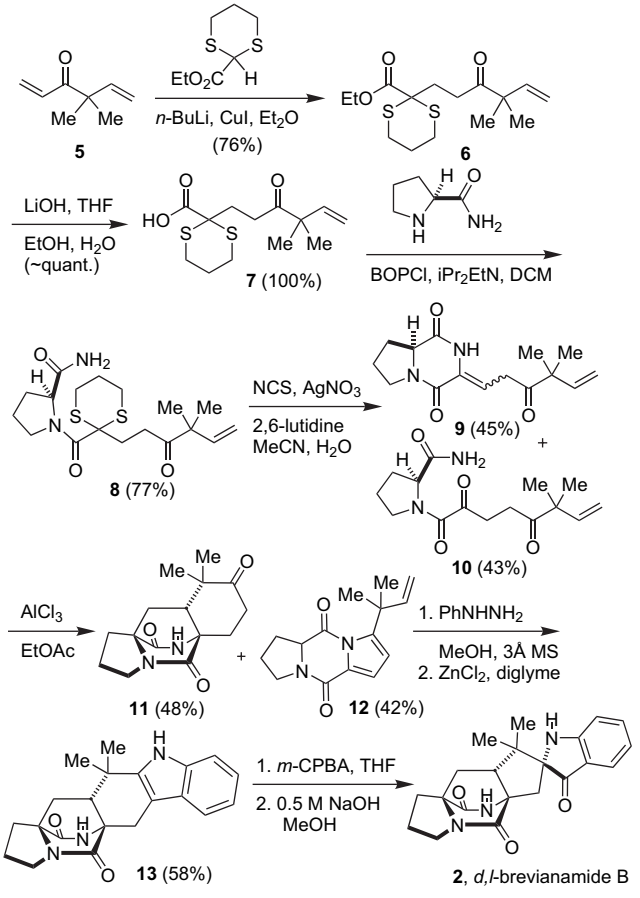
Somewhat conflicting theoretical calculations[10] and experimental results[11,12] in various systems to be discussed below, motivated us to further explore this approach to construct a



Scheme 2. Formation of the *anti*-spiro-5 system of the bicylo[2.2.2] by the Diels–Alder reaction.[12]

spiro-fused six-membered ring system, and to evaluate the intrinsic facial bias of the IMDA reaction of these azadiene species.

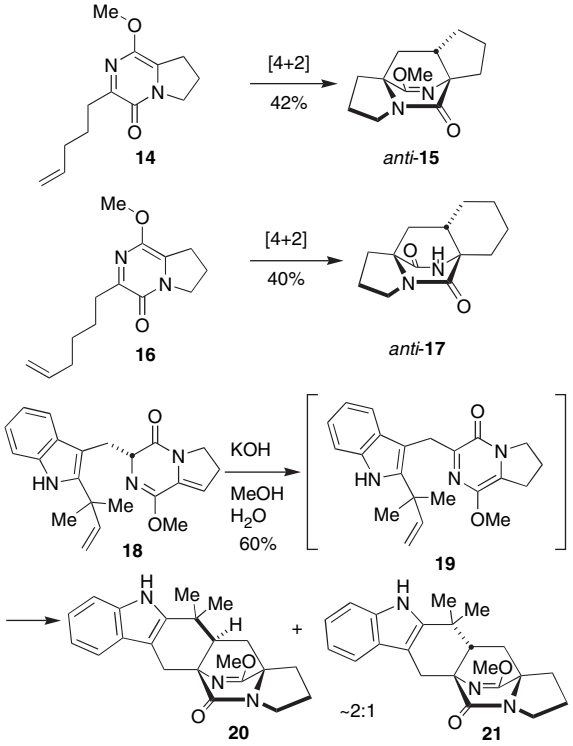
As shown in Scheme 3, the synthesis commenced with the known ketone **5**,[13] which was subjected to conjugate addition of ethyl 1,3-dithiane-2-carboxylate to provide the ester **6** in 76% yield.[14] Basic hydrolysis of the ester to the acid **7** was followed by BOPCl coupling with L-prolinamide, to provide the protected peptide **8** in 77% yield. Oxidative deprotection of the dithiane **8** gave a mixture of diketopiperazine **9** (45%) and the uncylized amide **10** (43%).[15] Subjecting this mixture to 3 equiv of $AlCl_3$ in refluxing EtOAc for 24 h, yielded the desired Diels–Alder product **11** in 48% yield, accompanied by the pyrrole by-product **12** in 42% yield. The relative stereochemistry of cycloadduct **11** turned out to possess the *anti*-configuration as evidenced through conversion into *d,l*-brevianamide B (**2**).[1,17] Thus, the cyclic ketone **11** was converted into the corresponding phenyl hydrazone, which without purification, was rearranged



Scheme 3. IMDA reaction of **9/10** and a concise synthesis of brevianamide B (**2**).

to the indole **13** by the Fischer indole reaction in an overall yield of 58%. This substance proved to be identical to a species previously prepared in our laboratory.[11a,b,16,17] Using conditions previously deployed, the 2,3-disubstituted indole **13** was stereoselectively oxidized to the corresponding 3-hydroxyindolenine, which suffered pinacol-type rearrangement under basic conditions to provide racemic brevianamide B (**2**).[17] Thus, brevianamide B was obtained in nine concise steps from the known ketone **5** (12 steps from commercially available materials), and was identical by ^{1}H NMR, ^{13}C NMR, IR, and TLC to an authentic sample of brevianamide B obtained from *P. brevicompactum*.[17]

The *anti*-stereoselectivity of the IMDA reaction of **3** and **9** is consistent with the simple model systems **14** and **16**, which produced the corresponding spiro-5 (**15**) and spiro-6 (**17**) cycloadducts, respectively, where in both instances a single diastereomer was formed with the *anti*-configuration at the C20 position (brevianamide numbering, Scheme 4).[11d] However, these systems are in stark contrast to the IMDA reaction we reported via azadiene species **18** that led (through the observable and isolable entity **19**) to the cycloadducts **20** and **21** where ~2:1 *syn*-selectivity was observed.[11a,b] Comparison of **3** with **14** and **9** with **16** reveal that the *gem*-dimethyl group does not significantly affect the intrinsic *anti*-bias of these systems. Substrates **3** and **9**, which each contain the single trigonal carbon atom of the ketone function, retain the *anti*-selectivity bias in the IMDA reaction of the derived azadiene tautomers. Thus, the fused 2,3-disubstituted indole moiety in the tethering dienophile chain of **19**, which contains two trigonal atoms as opposed to the more saturated chain of atoms in **3**, **9**, **14**, and **16**, reveals that the modest *syn*-bias of the former substrate is likely governed by the additional conformational rigidity imposed by the indole nucleus relative to the more saturated counterparts.



Scheme 4. Synthetic model systems.[11]

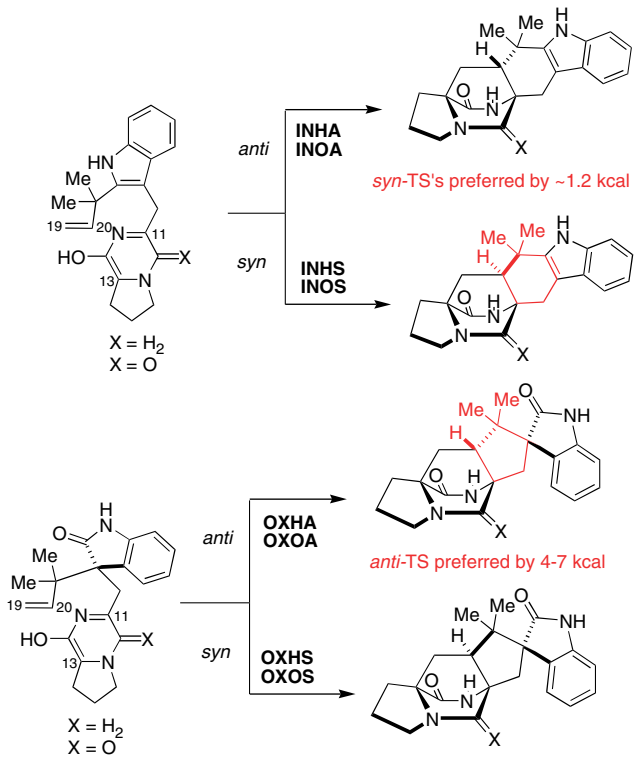


Figure 2. Ab initio calculations on the spiro-5 and spiro-6 modes of IMDA cycloaddition.[10b]

Ab initio calculations previously reported on the 2,3-disubstituted indole and oxindole substrates, predict a modest *syn*-selective bias for the 2,3-disbstituted indole species of ~1.2 kcal/mol furnishing the spiro-6 products, whereas the oxindole substrates that yield the spiro-5 products are substantially favored by 4–7 kcal/mol for the *anti*-stereochemistry (Fig. 2).[10] Additional substrates are currently being prepared to examine other structural and electronic parameters of the azadiene, the dienophile and the tethering chain of atoms, to build a deeper understanding of the subtle ground-state conformational and transition state energies that govern the important diastereoselectivity of these reactions, which may have significant biogenetic implications.

3. Conclusion

We have developed a concise and convergent synthesis of *d*,*l*-brevianamide B via an IMDA construction that has been inspired by our biogenetic proposal for the formation of the bicyclo[2.2.2]diazaoctane core.[12] The tricyclic ketone substrate **11**, should prove to be a versatile intermediate from which a host of brevianamide and C20-*epi*-stephacidin analogs might be readily constructed using Fisher indole and related methodologies. Current efforts in our group are exploring these opportunities and related issues with respect to controlling the facial bias, in both a relative and absolute sense, of these IMDA constructions.

4. Experimental

4.1. General methods

Commercially available reagents were used as received without further purification except where stated. Thin layer

chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with potassium permanganate solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230–400 mesh, Merck). Melting points were obtained using a MEL-TEMP melting point apparatus. IR absorptions on NaCl plates were run on a Perkin–Elmer FTIR 1600. ^{1}H NMR spectral data were obtained using Varian 300 or 400 MHz instruments (500 MHz for synthetic brevianamide B). ^{13}C NMR spectral data were obtained using a Varian 75.5 MHz or 100 MHz spectrometer (125 MHz for synthetic brevianamide B). Chemical shifts are reported in parts per million relative to $CHCl_3$ at δ 7.27 (^{1}H NMR) and δ 77.0 (^{13}C NMR). For all NMR spectra, δ values are given in parts per million and J values in Hertz.

4.1.1. Ethyl 2-(4,4-dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carboxylate (6). To a stirred, cooled (−60 °C) solution of ethyl 1,3-dithiane-2-carboxylate (13.3 mL, 84.2 mmol) in anhydrous Et_2O (100 mL), was added *n*-butyl lithium (52 mL, 84.2 mmol, 1.6 M solution in hexane) quickly. This mixture was stirred at 0 °C under argon for 45 min, cooled to −78 °C and then added via cannula to a cooled (−78 °C) suspension of CuI (8.55 g, 44.9 mmol) in Et_2O (200 mL). After stirring at this temperature for 30 min the white suspension was warmed to −15 °C for 5 min, cooled to −40 °C and stirred for 30 min, prior to the addition (via cannula) of a solution of ketone **5**[13] (3.48 g, 28.1 mmol) in anhydrous Et_2O (150 mL). The reaction was allowed to warm to rt slowly over 2.5 h. TLC (10:90 EtOAc/hexane) showed ethyl 1,3-dithiane-2-carboxylate (R_f 0.30) and dithiane **6** (R_f 0.19) potassium permanganate active. The reaction was quenched by the addition of saturated aqueous NH_4Cl (500 mL), stirred for 15 min and then filtered through Celite. The filter was washed with EtOAc (500 mL), the filtrate layers were separated and the aqueous phase extracted with EtOAc (3×500 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by column chromatography on silica using a gradient of EtOAc/hexane (4:96 EtOAc/hexane to 10:90 EtOAc/hexane) as eluent. The mixed fractions were purified by column chromatography on silica using a gradient of EtOAc/hexane (5:95 EtOAc/hexane to 25:75 EtOAc/hexane) as eluent to give hexenyl dithiane **6** (6.63 g, 75%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24 (6H, s), 1.32 (3H, t, J=7.2 Hz), 1.82–1.94 (1H, m), 2.10–2.20 (1H, m), 2.23–2.31 (2H, m), 2.63–2.77 (4H, m), 3.25–3.37 (2H, m), 4.25 (2H, q, J=7.2 Hz), 5.14 (1H, d, J=17.5 Hz), 5.15 (1H, d, J=10.7 Hz), 5.92 (1H, dd, J=17.5, 10.7 Hz). ^{13}C NMR δ: 14.1, 23.5 (2C), 24.4, 27.7 (2C), 32.6 (2C), 50.8, 51.5, 61.9, 114.4, 142.3, 170.7, 211.5. IR (NaCl): 3525, 2932, 1717, 1421, 1356, 1280, 1225, 1173, 1083, 1017, 917 cm^{-1}. HRMS (FAB+): Calcd for $C_{15}H_{25}O_3S_2$: 317.1245. Found: 317.1230 (MH^+).

4.1.2. 2-(4,4-Dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carboxylic acid (7). To a stirred solution of dithiane ester **6** (3 g, 9.49 mmol) in THF (15 mL), ethanol (15 mL) and water (15 mL) was added lithium hydroxide (4.55 g, 190 mmol). The reaction mixture was heated under argon at 70 °C for 11 h and then the solvent was concentrated under reduced pressure. To the resultant aqueous mixture were added water (125 mL) and Et_2O (125 mL), the layers were separated and the aqueous phase was further extracted with Et_2O (125 mL). EtOAc (250 mL) was added to the aqueous layer, which was then acidified to pH 1 with concd HCl. The layers were separated and the aqueous phase was further extracted with EtOAc (3×250 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, then azeotroped with $CHCl_3$ to give the acid **7** (2.92 g, quantitative) as an off-white solid with a mp of 109–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25 (6H, s), 1.78–1.95 (1H, m), 2.12–2.22 (1H, m), 2.28 (2H, br t, J=7.9 Hz), 2.65–2.74 (2H, m), 2.79 (2H, br t, J=7.9 Hz), 3.28–3.39 (2H, m), 5.16 (1H, d, J=17.2 Hz), 5.17 (1H, d, J=10.5 Hz), 5.93 (1H, dd, J=17.2, 10.5 Hz). ^{13}C NMR: 23.5 (2C), 24.1, 27.6 (2C), 32.4, 32.5, 50.6, 50.8, 114.5, 142.2, 176.8, 211.5. IR (NaCl): 2971, 2929, 1703, 1414, 1364, 1254, 1081, 998, 919, 681 cm^{-1}. HRMS (FAB+): Calcd for $C_{13}H_{21}O_3S_2$: 289.0932. Found: 289.0928 (MH^+). Elemental analysis: Calcd: 54.13% C, 6.99% H, 22.23% S. Found: 54.07% C, 7.30% H, 21.80% S.

4.1.3. (*S*)-1-(2-(4,4-Dimethyl-3-oxohex-5-enyl)-1,3-dithiane-2-carbonyl)pyrrolidine-2-carboxamide (8). To a cold (0 °C) solution of acid **7** (1.6 g, 5.5 mmol) in anhydrous CH_2Cl_2 (20 mL) were added BOPCl (1.7 g, 6.6 mmol) and *i*-Pr_2EtN (1.16 mL, 6.6 mmol). The mixture was stirred for 10 min before (L)-prolinamide (634 mg, 5.5 mmol) was added. The reaction was allowed to warm to rt and stirred for 48 h. After quenching the mixture with saturated aqueous NH_4Cl, the layers were separated and the aqueous solution was extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4, then evaporated under reduced pressure. TLC analysis in EtOAc showed the peptide **8** (R_f 0.20) as potassium permanganate active. This crude mixture was purified by column chromatography on silica using (EtOAc) as eluent to give peptide **8** (1.65 g, 77%) as a white solid with a mp of 111–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (3H, s), 1.22 (3H, s), 1.86–2.08 (6H, m), 2.39 (2H, br t, J=7.8 Hz), 2.64–2.85 (4H, m), 2.91–2.99 (1H, m), 3.13 (1H, br t, J=11.1 Hz), 3.65–3.73 (1H, m), 4.17 (1H, br s), 4.52 (1H, br s), 5.12 (1H, d, J=17.4 Hz), 5.12 (1H, d, J=10.7 Hz), 5.66 (1H, br s), 5.91 (1H, dd, J=17.4, 10.7 Hz), 6.34 (1H, br s). ^{13}C NMR δ: 23.5 (2C), 24.5, 25.9, 27.6, 28.0, 31.5, 32.8, 49.2, 50.8 (2C), 57.8, 62.3, 114.5, 142.2, 168.3, 174.2, 212.2. IR (NaCl): 3327, 2925, 2851, 1627, 1575, 1439, 1311, 1242, 1088, 643 cm^{-1}. HRMS (FAB+): Calcd for $C_{18}H_{29}N_2O_3S_2$: 385.1620. Found: 385.1629 (MH^+).

4.1.4. 3-(4,4-Dimethyl-3-oxohex-5-enylidene)-hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (9) and (*S*)-1-(6,6-dimethyl-2,5-dioxooct-7-enoyl)pyrrolidine-2-carboxamide (10). To a stirred, cooled (0 °C) suspension of silver nitrate (1.08 g, 6.36 mmol) in 4:1 MeCN/water (25 mL) were added sequentially 2,6-lutidine (1.32 mL, 11.3 mmol), *N*-chlorosuccinimide (1.13 g, 8.49 mmol, recrystallized from AcOH), and a solution of dithiane **8** (543 mg, 1.41 mmol) in MeCN (3.5 mL) quickly. The reaction mixture was stirred at rt for 70 min, quenched by the addition of saturated aqueous NH_4Cl (10 mL), stirred for 15 min, and filtered through

Celite. The filter was washed with Et_2O (100 mL), the filtrate layers were separated and the aqueous phase extracted with Et_2O (3×100 mL). The combined organic extracts were washed with 2% aqueous $Na_2S_2O_3$ (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was dissolved in the minimum amount of EtOAc, salts were removed by filtration, and the filtrate was purified by column chromatography on silica gel using (EtOAc) as eluent to give the diketopiperazine **10** (219 mg, 56%, 70:30 diastereomeric mixture of unassigned *E*/*Z*-geometry) as a white solid with a mp of 115–116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08 (3H, s (isomer B)), 1.09 (3H, s (isomer B)), 1.21 (3H, s (isomer A)), 1.22 (3H, s (isomer A)), 1.89–2.38 (4H, m), 2.63 (1H, t, J=6.7 Hz (isomer B)), 2.68 (1H, t, J=6.7 Hz (isomer A)), 2.82 (1H, t, J=6.7 Hz (isomer A)), 2.86 (1H, t, J=6.7 Hz (isomer B)), 3.52–3.60 (2H, m), 4.27 (1H, dd, J=9.7, 6.9 Hz (isomer B)), 4.34 (1H, dd, J=9.5, 7.0 Hz (isomer A)), 5.05–5.15 (3H, m), 5.88 (1H, dd, J=17.4, 10.4 Hz (isomer A)), 5.97 (1H, dd, J=17.9, 10.6 Hz (isomer B)), 7.64 (1H, br s (isomer A)), 7.68 (1H, br s (isomer B)). ^{13}C NMR isomers A and B δ: 21.4, 21.7, 22.4, 22.5, 22.6, 23.5, 28.3, 28.3, 32.5, 32.7, 43.9, 44.0, 45.3, 45.6, 58.9, 59.0, 111.6, 113.9, 114.5, 114.7, 143.3, 143.7, 163.1, 164.9, 172.0, 172.0, 172.5, 178.3, 214.6 (2C). IR (NaCl): 3234, 2977, 1774, 1708, 1429, 1362, 1294, 1182, 1605, 919, 816, 639 cm^{-1}. HRMS (FAB+): Calcd for $C_{15}H_{21}N_2O_3$: 277.1552. Found: 277.1553 (MH^+), and amide **10** (43 mg, 10%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, s), 1.23 (3H, s), 1.92–2.14 (5H, m), 2.34–2.44 (1H, m), 2.66–2.72 (2H, m), 3.48–3.67 (2H, m), 4.28 (1H, dd, J=9.0, 7.2 Hz), 4.83 (1H, br s), 5.15 (1H, d, J=17.3 Hz), 5.16 (1H, d, J=10.7 Hz), 5.91 (1H, dd, J=17.3, 10.7 Hz), 6.76 (1H, br s). ^{13}C NMR δ: 22.7, 23.6 (2C), 28.6, 30.8, 33.3, 45.9, 50.7, 59.9, 114.6, 142.2, 166.9, 167.6, 212.1 (2C). IR (NaCl): 3338, 2974, 1696, 1636, 1445, 1297, 1178, 1074, 923 cm^{-1}. HRMS (FAB+): Calcd for $C_{15}H_{23}N_2O_4$: 295.1658. Found: 295.1662 (MH^+).

4.1.5. Tetracycle (11) from diketopiperazine (9). To a solution of the diketopiperazine **9** (110 mg, 398 μmol) in EtOAc (11 mL) was added aluminum trichloride (159 mg, 1.19 mmol). The solution was heated to reflux for 19 h, then the solution was allowed to cool to rt. TLC analysis (EtOAc) showed the tetracycle **11** (R_f 0.18); UV and potassium permanganate active. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL), then filtered through Celite. The filter was washed with EtOAc (50 mL), the filtrate layers were separated and the aqueous phase extracted with EtOAc (3×50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by column chromatography on silica gel using (EtOAc) as eluent to give the tetracycle **11** (48 mg, 44%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (3H, s), 1.10 (3H, s), 1.76–2.07 (5H, m), 2.17 (1H, dd, J=10.1, 5.9 Hz), 2.23 (1H, ddd, J=14.9, 5.7, 4.4 Hz), 2.40 (1H, dt, J=13.8, 4.4 Hz), 2.57 (1H, ddd, J=14.9, 13.8, 5.7 Hz), 2.73 (1H, dd, J=13.0, 5.9 Hz), 2.93 (1H, td, J=13.8, 5.7 Hz), 3.45 (2H, t, J=6.8 Hz), 8.78 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.5, 23.7, 24.5, 26.1, 28.8, 32.2, 32.8, 44.0, 47.1, 47.2, 59.9, 66.8, 168.9, 174.4, 213.1. IR (NaCl): 3230, 2926, 1692, 1411, 1297, 1073 cm^{-1}. HRMS (FAB+): Calcd for $C_{15}H_{21}N_2O_3$: 277.1552. Found: 277.1550 (MH^+).

4.1.6. Tetracycle (11) from amide (10). To a solution of the amide **10** (20 mg, 67.7 μmol) in EtOAc (2 mL) was added aluminum trichloride (27 mg, 203 μmol). The solution was heated at 85 °C in a sealed reaction vessel for 19 h; TLC analysis (EtOAc) showed the tetracycle **11** (R_f 0.18); UV and potassium permanganate active. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (1 mL), EtOAc (10 mL) was added, the layers were separated and the aqueous phase extracted with EtOAc (3×10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. This crude mixture was purified by PTLC on silica gel using (EtOAc) as eluent to give the tetracycle **11** (5.9 mg, 31%) as a colorless oil. See spectral data above.

4.1.7. Indole (13). To a solution of cyclic ketone **11** (100 mg, 360 μmol) in anhydrous methanol (3 mL), under argon, were added 3 Å molecular sieves followed by phenylhydrazine (71 μL, 720 μmol). The mixture was heated at 90 °C in a sealed reaction vessel for 90 min, then it was allowed to cool to rt and the solvent was evaporated under reduced pressure to give the crude hydrazone that was taken on without further purification. The crude oil was dissolved in anhydrous 2-methoxyethyl ether (2 mL) under argon, and anhydrous zinc chloride (99 mg, 720 μmol) was added. The reaction mixture was heated at 172 °C in a sealed reaction vessel for 18 h; TLC analysis (EtOAc) showed the indole **13** (R_f 0.31); UV and potassium permanganate active. The reaction mixture was filtered through Celite, the filter was washed with toluene and the solvent was removed by short path distillation (5 mmHg, 30–40 °C) to leave a crude residue that was brought up in EtOAc and washed with water. The aqueous phase was extracted with EtOAc (3×5 mL) and the combined organic extracts were washed with brine (10 mL) and dried over anhydrous Na_2SO_4. Concentration under reduced pressure gave the crude indole, which was purified by column chromatography on silica using (EtOAc) as eluent to give the indole **13** (73 mg) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (3H, s), 1.35 (3H, s), 1.89 (1H, ddd, J=13.4, 6.8, 6.8 Hz), 2.02–2.08 (2H, m), 2.10 (1H, dd, J=13.4, 4.2 Hz), 2.18 (1H, dd, J=13.4, 9.9 Hz), 2.38 (1H, dd, J=9.9, 4.2 Hz), 2.83 (1H, ddd, J=13.4, 6.8, 6.8 Hz), 2.93 (1H, d, J=18.0 Hz), 3.57 (2H, t, J=6.8 Hz), 3.95 (1H, d, J=18.0 Hz), 5.78 (1H, br s), 7.12–7.23 (2H, m), 7.34 (1H, d, J=7.8 Hz), 7.54 (1H, d, J=7.8 Hz), 8.84 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.08, 24.68, 25.43, 29.60, 32.86, 34.72, 43.39, 45.96, 59.23, 61.82, 67.28, 103.82, 110.92, 118.55, 119.86, 122.28, 127.40, 136.64, 139.86, 169.29, 172.98. IR (NaCl): 3299, 2926, 2360, 1683, 1457, 1406, 1294, 1140, 1014, 702 cm^{-1}. HRMS (FAB+): Calcd for $C_{21}H_{23}N_3O_2$: 350.17903. Found: 350.18588 (MH^+).

4.1.8. *d,l*-Brevianamide B (2). To a solution of indole **13** (25 mg, 70 μmol) in anhydrous THF (2.5 mL) under argon, was added dry 3-chloroperoxybenzoic acid (16 mg, 90 μmol). The reaction mixture was stirred at rt for 1.5 h and was quenched by the addition of dimethyl sulphide (three drops). The mixture was stirred for 5 min and the solvent evaporated under reduced pressure to give the crude

hydroxyindolenine, which was observed by TLC (EtOAc) with a R_f 0.15; UV and potassium permanganate active. To a stirred solution of the crude hydroxyindolenine in MeOH (2.5 mL) was added 0.5 M sodium hydroxide (5 mL). The bright yellow reaction mixture was stirred at rt for 18 h, and then heated to reflux for 2 h. The MeOH was removed under reduced pressure, the aqueous phase was neutralized to pH 6/7 with 1 M HCl, and finally extracted with CH_2Cl_2 (4×5 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a crude mixture that was purified by PTLC on silica gel using (10:90 MeOH/CH_2Cl_2) as eluent to give *d,l*-**2** (4 mg) as a yellow oil. This material was identical to natural brevianamide B by TLC (10:90 MeOH/CH_2Cl_2; R_f 0.35; fluorescent under UV), ^{1}H NMR, ^{13}C NMR, IR, and HRMS. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, s), 1.13 (3H, s), 1.76–2.07 (5H, m), 2.71–2.78 (1H, m), 3.23–3.33 (1H, m), 3.25 (1H, d, J=16 Hz), 3.49 (2H, t, J=6.7 Hz), 5.10 (1H, br s), 6.51 (1H, br s), 6.79 (1H, t, J=7.5 Hz), 6.84 (1H, d, J=8), 7.42 (1H, t, J=7.5 Hz), 7.55 (1H, d, J=7.5). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 20.20, 22.45, 24.95, 28.60, 29.09, 36.20, 43.97, 46.46, 49.59, 66.38, 67.98, 77.57, 111.30, 118.70, 119.72, 125.04, 137.27, 160.46, 169.14, 173.73. IR (NaCl): 3583, 3259, 2971, 1690, 1618, 1469, 1392, 1326, 1197, 923, 731, 666 cm^{-1}. HRMS (FAB+): Calcd for $C_{21}H_{23}N_3O_3$: 366.17394. Found: 366.181498 (MH^+).

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Total synthesis of malayamycin A and analogues

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Abstract—The total synthesis of the bicyclic *C*-nucleoside malayamycin A is described starting with D-ribonolactone. A new method was developed to obtain preparatively important quantities of β-pseudouridine, which was used as an intermediate. The synthesis of a carba *N*-nucleoside analogue of malayamycin A is also described.

1. Introduction

Nucleosides are traditionally associated with the fascinating chemistry and biology of DNA and RNA.[1] Indeed, the intricate three-dimensional arrays of *N*-nucleotidic sequences in DNA constitute the basis of the alphabet of life that is responsible for all the vital processes.[2] Nature has also produced a group of *N*-nucleosides with potent chemotherapeutic activities, which have been the source of extensive chemical modification.[3] In this regard, several antitumor, antiviral, and antibiotic nucleosides are known. In contrast, *C*-nucleosides[4] constitute a smaller subgroup of carbon-linked anomeric heterocycles that nature has also provided with much less exploited potential for biological activities.[5] Pseudouridine, a constituent of various RNAs,[6,7] was the first naturally occurring 5-uracilyl β-D-*C*-ribofuranoside.[8] Its biosynthesis continues to elicit proposals for fascinating pathways.[9] Curiously, pseudouridine isolated from beer has shown antimutagenic properties against *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.[10]

A unique example of bicyclic *C*-nucleoside having antifungal and antibiotic properties was reported by Sakata and co-workers in 1977.[11] Degradative and spectroscopic work[12] revealed the structures of ezomycin B_2 and ezomycin C_2 as constrained pseudouridine-type *C*-nucleoside disaccharides (Fig. 1). The perhydrofuropyran motif was also revealed in the structures of *N*-nucleosides such as ezomycin A1,[13] miharamycin,[14] and octosyl acid A.[15] Quantamycin[16] is a structure-based bicyclic synthetic hybrid of lincomycin

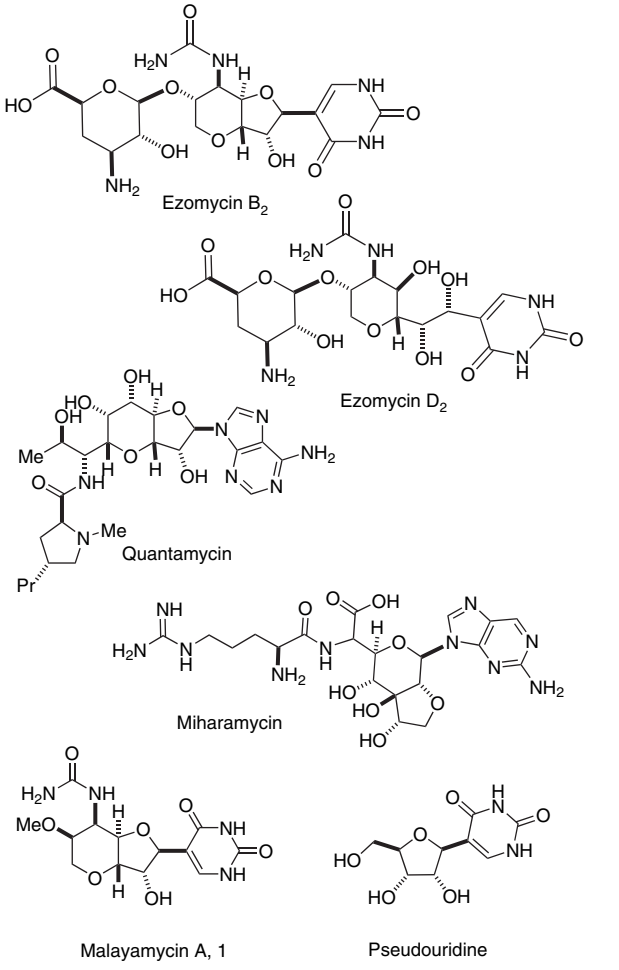

Figure 1. Structures of perhydrofuropyran *C*-nucleosides and β-pseudouridine.

Keywords: *C*-Nucleoside; Thioglycoside; Ring closure metathesis; Perhydrofuropyran.
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and a natural 'starter' nucleotide involved in the process of protein biosynthesis. The field of naturally occurring *C*-nucleosides lay dormant for nearly 25 years before the discovery of a new member by a group at the Syngenta Crop Protection Laboratories in Jeallott's Hill, UK.[17] Malayamycin A (**1**) was isolated from the soil organism *Streptomyces malaysiensis*, and its structure was proposed by detailed NMR studies and by degradation (Fig. 1). As in the case of ezomycin B_2 and pseudouridine, malayamycin A was found to be unstable under strongly acidic and basic conditions. In contrast to the ezomycins that exhibit antifungal and antibiotic activities,[11] malayamycin A is a potent fungicide.[17] The perhydrofuropyran motif in **1**, which is also common to the ezomycins and octosyl acid A, has different functionality and differs by the absence of a carboxylic acid and a disaccharide unit. Recently, the proposed structure and absolute stereochemistry of **1**[17] were confirmed by a stereocontrolled total synthesis.[18] Herein we give details of various aspects of this synthesis, as well as the preparation of semi-synthetic analogues intended to probe the importance of some functional groups. The total synthesis of *N*-malayamycin A and related purine and pyrimidine nucleosides was recently reported.[19]

2. The synthesis plan

As previously remarked,[18] the synthesis plan for **1** has to consider several challenges that include as follows: (a) the stereocontrolled formation of the anomeric *C*-5-uracilyl bond, (b) the construction of a trans-fused bicylic perhydrofuropyran ring, and (c) incorporation of usable functionality to achieve the desired stereochemical arrangement.

The disconnection shown in Figure 2 capitalizes on the formation of an enantiopure unsaturated *C*-pyrimidinyl perhydrofuropyran via a ring closure metathesis of a pseudouridine precursor that would be prepared from D-ribonolactone **2**. Although the first phase of the synthesis had precedents (see below), a main challenge was the stereo- and regio-

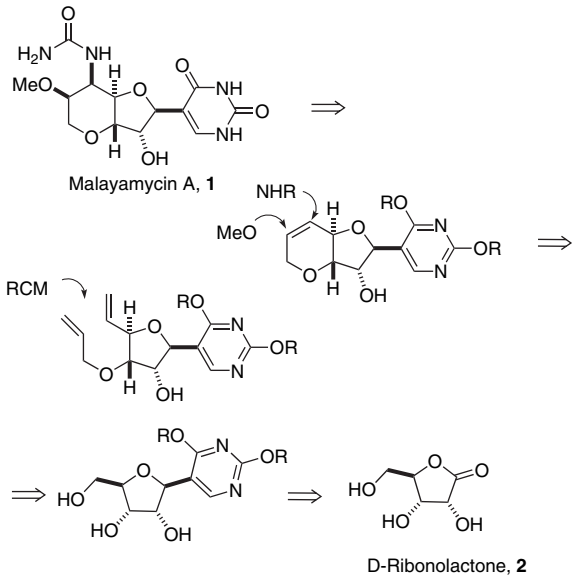


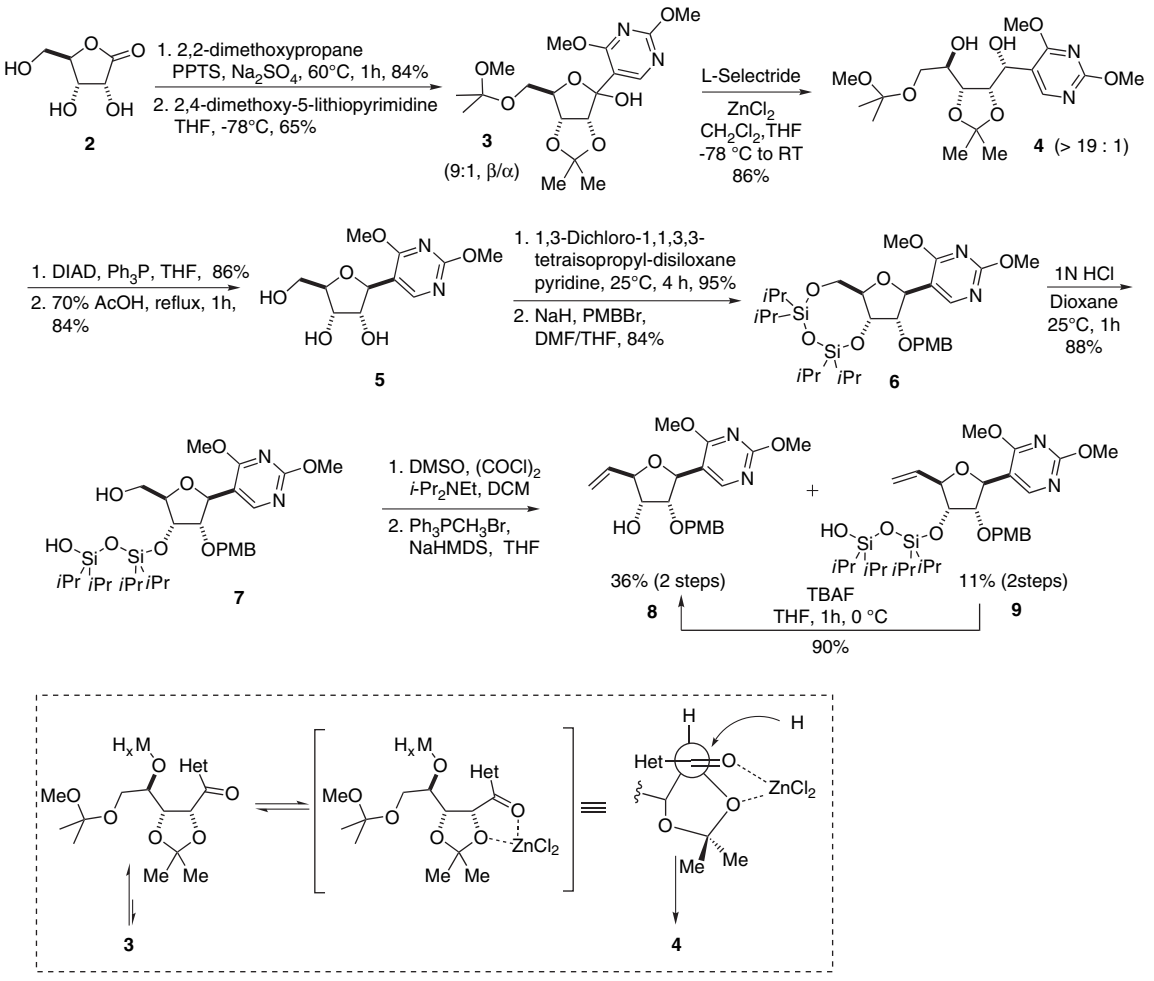
Figure 2. Disconnective analysis of malayamycin A.

controlled introduction of a vicinally disposed *cis*-amino alcohol. In considering pseudouridine as a starting material, we would already resolve the *C*-nucleoside issue. The prohibitive cost of pseudouridine was an initial deterrent that was soon dismissed with the knowledge that a number of total syntheses have been reported over the years.[20] The most recent of these[20a] offered a preparatively viable protocol, although a mixture of anomers was obtained. The need to start with a relatively large amount of **2** instigated the search for a highly stereocontrolled synthesis of **1** and its α-anomer from a common precursor. In our synthetic approach to a new synthesis of pseudouridine,[21] we used a 5-(2,4-dimethoxypyrimidinyl) β-D-ribofuranose as an intermediate.[22]

3. Results and discussion

The readily available D-ribonolactone **2** was treated with 2,2-dimethoxypropane in refluxing CH_2Cl_2 in the presence of PPTS and excess Na_2SO_4 to give the corresponding mixed acetal, which was surprisingly stable to chromatographic purification. In the absence of Na_2SO_4 the bis-acetal lactone was obtained in 40% yield only.[23] Addition of 5-lithio 2,4-dimethoxypyridine[20b] to the protected lactone at −78 °C led to a 75% yield of a mixture of anomers **3** in an α/β ratio of 1:9. Treatment with L-Selectride in the presence of $ZnCl_2$ in a mixture of CH_2Cl_2/THF led to the alcohol **4** as a major isomer in 86% yield on a 1–5 mmol scale. However, on a larger scale (22 mmol), the yield was 54%. Reduction with $NaBH_4$ in MeOH gave a 1:1 mixture of diastereomers. The quasi-exclusive stereocontrolled reduction to the D-*allo* alcohol **4** can be explained by the initial coordination of an intermediate alkoxy ketone followed by *Si*-face-selective hydride attack from the bulky reagent (Scheme 1).[21] Interestingly, the epimeric D-*altro* alcohol, a precursor to α-pseudouridine was the major product in the absence of $ZnCl_2$.[21] Similar observations have been reported in a related case.[24] Treatment of **4** under Mitsunobu conditions led, via a site-selective intramolecular cycloetherification, to the corresponding 1,4-anhydro-D-ribitol *C*-nucleoside.[25] Removal of the acetonide in the presence of 70% acetic acid gave 5-(2,4-dimethoxypyrimidinyl) β-D-ribofuranose **5** in excellent overall yield. The original pseudouridine synthesis involved the corresponding 2,4-dibutoxypyridine as intermediate in order to facilitate the final deprotection step without anomerization.[21] In the case of **1**, however, the more robust dimethoxypyrimidine analogue was used.

With **5** in hand, we devised a protecting group selection protocol that would allow preferential manipulation of the triol system. Thus, the 3′,5′-diol could be protected as the disiloxane acetal, and the 2′-hydroxyl group was treated with PMBBr to give **6** in an 86% overall yield for the two steps. Quite unexpectedly, the disiloxane was selectively cleaved with 1 N HCl in dioxane, exposing the primary alcohol in **7**. Moreover, the primary alcohol could be oxidized to the aldehyde under Swern conditions and the latter converted to the 5′-*C*-vinyl intermediate **8** and its monosilylated derivative **9**, which could be transformed to **8** in the presence of TBAF as expected. Thus, the disiloxane served a dual purpose as a protective group in three operationally different reactions. Allylation of **8** under standard conditions afforded



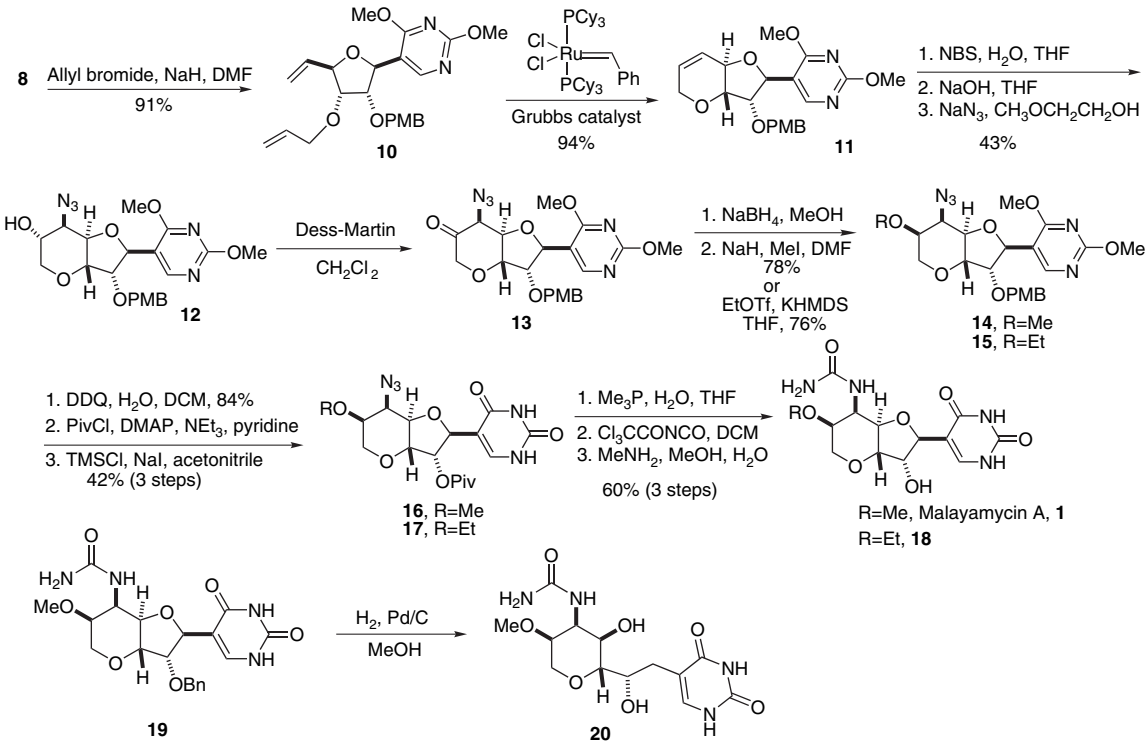
Scheme 1.

10, which was subjected to the Grubbs ring closure metathesis reaction (Scheme 2).[26] In the presence of 5 mol % first generation catalyst[27] and a concentration of 0.05 M, smooth cyclization took place to afford an 89% yield of crystalline product **11**. The reaction could be scaled up to 2 g without appreciable loss in efficiency.[28] It is of interest that in an analogous RCM cyclization of 1,2-*O*-isopropylidene-3-*O*-allyl-5-*C*-vinyl-α-D-ribofuranose to afford a tricyclic intermediate, there was a significant improvement in yield when the concentration was 0.01 M (92%)[19] compared to 0.02 M (63%).[28]

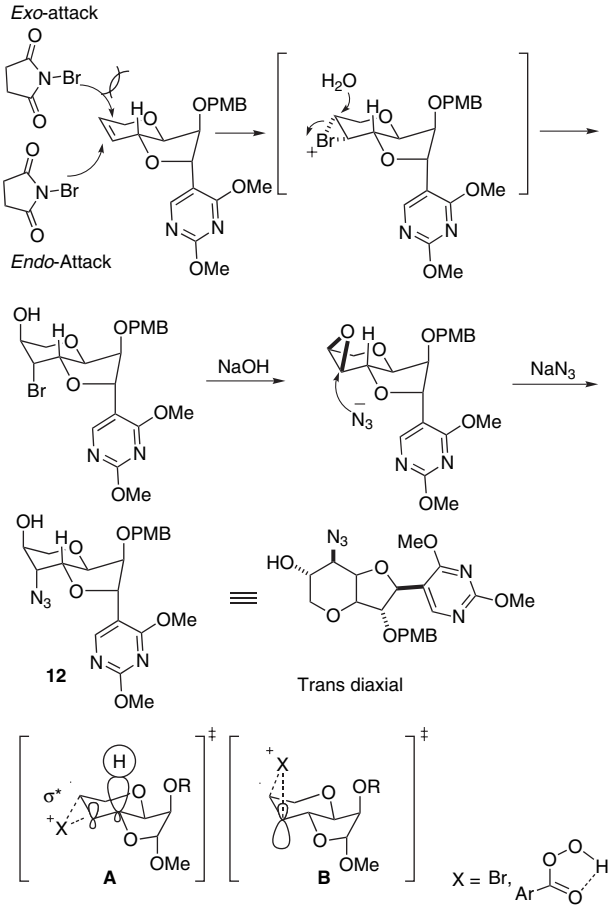
Our initial efforts to introduce an amino function relied on an epoxidation of **11** with mCPBA followed by ring opening with sodium azide. However, this protocol led to a regioisomeric azide.[18] We adopted a different route involving initial treatment with NBS in aqueous THF,[29] which led to the corresponding diaxial bromohydrin via the epibromonium ion (Scheme 3). Base-catalyzed epoxide formation followed by treatment with sodium azide in refluxing methoxyethanol afforded the trans diaxial azido alcohol **12** as a major product (5:1 ratio). The preference for the *endo*-attack of NBS is not clear, although there are precedents for related reactions.[30] It is possible that the *endo*-bromohydrin may benefit from a favorable electron-donation from the C–H σ bond[31] with the developing antibonding orbital in the transition state model A, compared to B. The same rationale can be advanced for the corresponding *endo*-epoxide when mCPBA is used even in the presence of steric bulk.

Oxidation of **12** with the Dess–Martin reagent[32] afforded **13**, without evidence of partial racemization of the pseudoaxial azide group (Scheme 2). Reduction with $NaBH_4$ in MeOH followed by methylation gave **14** in excellent overall yield. In order to probe the spatial importance of the ether group, we also prepared the ethyl ether **15** by treatment of the alcohol with ethyl triflate and KHMDS.

In an earlier version of the synthesis we had reached intermediate **19** having a benzyl ether instead of PMB (Scheme 2). However, when we attempted to remove the benzyl ether in the penultimate step, we were dismayed to find that a second hydrogenolysis had taken place resulting in ring opening of the ribosyl moiety to give **20**. This led us to use the PMB group instead. Model studies showed that the PMB group would not be stable to TMSCl and NaI required to generate the pyrimidinone from the dimethoxy precursor **14**. Thus, a protective group adjustment was needed. Cleavage of the PMB with DDQ proceeded smoothly and the resulting alcohol was esterified as a pivalate, which proved to be stable to the demethoxylation in the presence of TMSCl and NaI (Scheme 2). The methyl and ethyl ethers **16** and **17** were thus obtained in good overall yield. Reduction of the azide under Staudinger conditions,[33] followed by formation of the



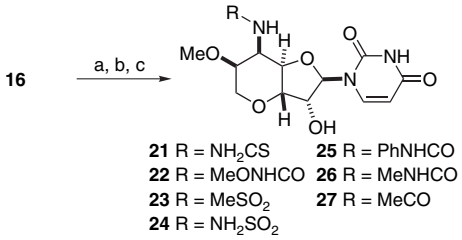
Scheme 2.



Scheme 3.

trichloroacetyl urea,[34] and deprotection with methylamine[35] afforded malayamycin A **1** and its 6-*O*-ethyl analogue **18**. Synthetic **1** was found to be identical to the natural sample in all respects including fungicidal activity. The ethyl ether analogue, however, was considerably less effective.

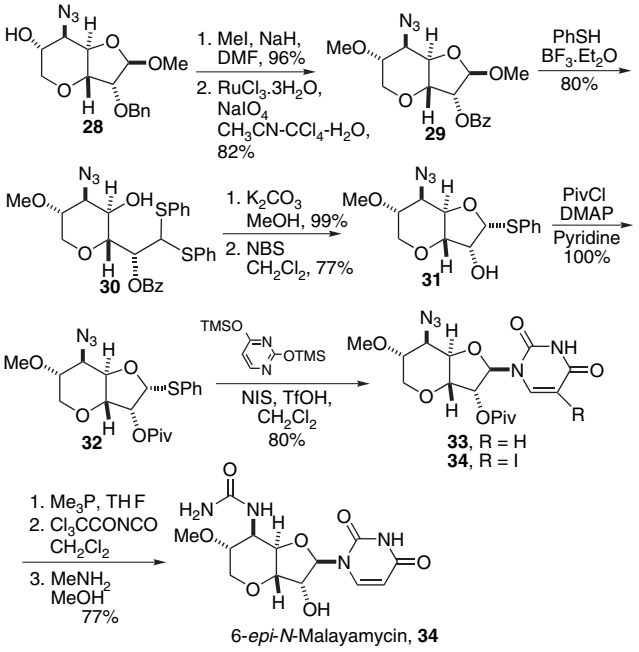
With the azide intermediate **16** in hand, we deemed it appropriate to introduce diversity at the original urea site, hoping to gain some information regarding the nature of the functional groups and activity. Thus, we prepared a series of analogues **21**–**27** ranging from ureas to sulfonamides, to carbamates, and to a simple acetamide (Scheme 4). Unfortunately, these and related modifications resulted in loss of fungicidal activity.



Scheme 4. Reagents and conditions: (a) Me_3P, THF–H_2O; (b) BzNCS, CH_2Cl_2 for **21**; carbonyl diimidazole, CH_2Cl_2, then $MeONH_2 \cdot HCl$, Et_3N, CH_2Cl_2 for **22**; $MeSO_2Cl$, pyridine for **23**; $FmocNHSO_2Cl$, pyridine, then piperidine, DMF for **24**; PhNCO, CH_2Cl_2 for **25**; MeNCO, CH_2Cl_2 for **26**; 40% v/v $MeNH_2$, MeOH for **27**; (c) 40% v/v $MeNH_2$, MeOH for **21**, 56%; **22**, 45%; **23**, 46%; **24**, 22%; **25**, 27%; **26**, 46%; Ac_2O, $NaHCO_3$, MeOH for **27**, 30%.

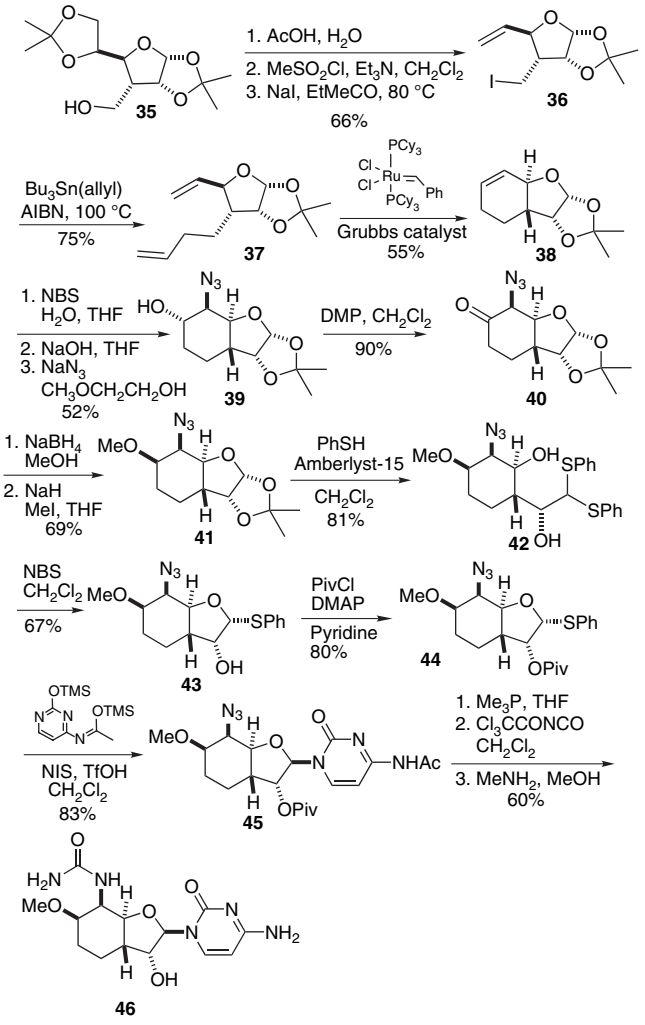
To further establish an SAR, we developed a strategy to do functional and structural modifications on *N*-malayamycin, which could be easily obtained from D-ribose or D-glucose.[19]

The known intermediate **28**[19] was methylated and the product was subjected to an oxidative transformation of the benzyl ether to the benzoate **29** in excellent overall yield[36] (Scheme 5). Treatment with benzenethiol in the presence of BF_3-etherate gave the diphenyl dithioacetal **30**, which was converted to the phenyl thioglycoside **31** as previously described.[19,37] Protection as the pivalate ester **32** and *N*-glycoside formation[19,34,38] via neighboring group participation, gave the 1′,2′-*trans*-bicyclic nucleoside **33** in excellent overall yield accompanied by a small amount of the 5-iodonucleoside **34**. Finally, Staudinger reduction of **33** and manipulation of the amine as described above gave 6′-*epi*-*N*-malayamycin **34**, which had diminished fungicidal activity compared to **1**. Thus, epimerization at C_6 was not tolerated.



Scheme 5.

To probe the tolerance of *N*-malayamycin for deep-seated structural modifications, we embarked on the preparation of the *N*-cytosinyl carba analogue **46** in which the tetrahydro-2*H*-pyran ring was replaced by a cyclohexane (Scheme 6). The synthesis started from the known and easily accessible **35**.[39] Regioselective removal of the 5′,6′-isopropylidene group followed by per-mesylation of the crude triol intermediate and treatment with NaI in ethylmethyl ketone at 80 °C triggered the concomitant formation of a terminal olefin and a primary iodide in 66% yield. Allylation of **36**, under free radical conditions was best performed in neat allyltributyltin in the presence of AIBN, to give **37** in 75% yield. In the presence of 5 mol % of first generation Grubbs catalyst[27] and a concentration of 0.05 M, cyclization took place to afford the volatile tricyclic olefin **38** in 55% yield. Application of the bromohydrin-epoxidation and azide opening protocol, followed by inversion of the C_6–OH stereocenter and O-methylation, led to **41** in 32% yield for six steps. Cleavage of the acetal with concomitant dithioacetal formation in **41** was most efficiently done with benzenethiol in the presence of Amberlyst-15 suspended in CH_2Cl_2 to give the corresponding product **42** in 81% yield. Thioglycoside formation in the presence of NBS followed by pivaloylation proceeded in good overall yield to deliver **44**. N-Glycosidation and subsequent functional group manipulations proceeded uneventfully to give the final *N*-cytosinyl nucleoside **46**. Surprisingly, the seemingly benign replacement of a tetrahydro-2*H*-pyranyl by a cyclohexyl moiety resulted in a complete loss of fungicidal activity.



Scheme 6.

4. Experimental

4.1. General procedure

Solvents were distilled under positive pressure of dry argon before using and dried by standard methods: toluene, THF, and ether from sodium/benzophenone ketyl; CH_2Cl_2 from calcium hydride. All commercially available reagents were used without further purification. All non-aqueous reactions were performed under argon atmosphere with oven-dried glassware. NMR (^1H, ^{13}C, COSY, NOESY) spectra were recorded on AV-400 and ARX-400 spectrometers. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. High-resolution mass spectra were recorded using fast atom bombardment (FAB), (TOF CI+). Melting points are uncorrected. Optical rotations were recorded in a 1 dm cell

at ambient temperature with a sodium lamp (wavelength of 589 nm). Analytical thin-layer chromatography was performed on Merck $60F_{254}$ pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or potassium permanganate. Chromatographic purifications were performed on a column with 230–400 mesh silica gel with the indicated solvent system.

4.1.1. (3a*R*, 4*R*/*S*, 6*R*, 6a*R*)-4-(2′,4′-Dimethoxypyrimidin-5′-yl)-6-(1″-methoxy-1″-methyl-ethoxymethyl)-2,2-dimethyltetrahydro-furo[3,4-*d*][1,3]dioxol-4-ol (3). To a mixture of (+)-D-ribonolactone **2** (6.7 g, 45 mmol) and pyridinium *p*-toluenesulfonate (0.84 g, 3.3 mmol) in 2,2-dimethoxypropane (167 mL) in 500 mL round-bottomed flask was added anhydrous Na_2SO_4 (67 g, 0.47 mol) at room temperature under argon atmosphere. The mixture was stirred at 60 °C with a condenser for 1 h, then cooled to room temperature and concentrated. The residue was purified by flash chromatography on silica gel column to afford the protected lactone (10 g, 38.1 mmol, 84%) as a colorless solid; mp 92–94 °C; $[\alpha]_D$ −45.9 (*c* 0.44, EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.31 (s, 3H), 1.38 (s, 3H), 1.47 (s, 3H), 3.14 (s, 3H), 3.52 (d, 1H, *J*=9.0 Hz), 3.75 (d, 1H, *J*=8.44 Hz), 4.7 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 24.1, 25.0, 26.0, 28.0, 62.2, 78.5, 80.4, 83.9, 103.7, 114.8, 172.2, 173.4; MS (ESI): 261.5 [M+H].

To a solution of 5-bromo-2,4-dimethoxy-pyrimidine (5.7 g, 26 mmol) in 200 mL dry THF was added *t*-butyllithium (1.7 M in hexanes) (31.8 mL, 54 mmol) at −78 °C under argon atmosphere. After stirring for 30 min, a solution of protected lactone (5.2 g, 20 mmol) in 100 mL dry THF was added to this mixture via cannula. The mixture was stirred for 1.5 h at −78 °C, and then quenched by addition of brine (100 mL). The mixture was warmed to room temperature and extracted with EtOAc (200 mL×3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 4:1) afforded **3** as a white solid (5.2 g, 13 mmol, 65%); mp 39–41 °C; $[\alpha]_D$ −56.9 (*c* 0.55, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (m, 6H), 1.43 (s, 4H), 3.30 (s, 3H), 3.62 (d, 2H, *J*=6.8 Hz), 3.91 (s, 3H), 4.1 (s, 3H), 4.29 (m, 2H), 4.35 (t, 1H, *J*=5.83 Hz), 4.9 (m, 2H), 8.44 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 24.5, 25.1, 26.9, 54.8, 55.8, 64.3, 85.0, 87.3, 88.4, 102.0, 107.3, 114.6, 116.9, 158.2, 166.8, 171.2; MS (ESI): 401.1 [M+H].

4.1.2. (1*R*, 4″*R*, 5″*S*)-2-(2′-Methoxypropan-2′-yloxy)-1-(5″-[(*R*)-hydroxy(2‴,4‴-dimethoxypyrimidin-5‴-yl-methyl)-2″,2″-dimethyl-1″,3″-dioxolan-4″-yl] ethanol (4). To a solution of **3** (22 g, 55 mmol) in 2 L CH_2Cl_2 was added slowly a solution of $ZnCl_2$ (1 M in Et_2O) (74.8 mL, 74.8 mmol) at −78 °C under argon atmosphere. After stirring for 30 min, L-Selectride (1 M in THF) (190 mL, 0.19 mol) was added slowly to this solution. The mixture was warmed slowly to room temperature and stirred overnight. The reaction was quenched by adding MeOH (50 mL), and then H_2O (25 mL), 30% H_2O_2 (25 mL), 6 M NaOH (25 mL). The mixture was extracted with CH_2Cl_2 (400 mL×3). The combined organic layer was washed with satd $NaHCO_3$ sodium bicarbonate and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 2:1) afforded **4** as a crystalline solid (12 g, 30 mmol, 54%); mp 35–37 °C; $[\alpha]_D$ −115.6 (*c* 1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 1.31 (s, 3H), 1.40 (s, 6H), 1.60 (s, 3H), 3.29 (s, 3H), 3.48 (m, 1H), 3.52 (m, 1H), 4.11 (s, 6H), 4.35 (m, 3H), 5.40 (s, 1H), 8.41 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 24.8 (2), 25.5, 26.8 (2), 54.7, 55.3, 64.4, 65.5, 70.1, 77.9, 79.4, 101.4, 109.8, 117.1, 158.5, 165.8, 169.1; MS (ESI): 403.1 [M+H].

4.1.3. (2*S*, 3*S*, 4*R*, 5*R*)-2-(2,4-Dimethoxy-pyrimidin-5-yl)-5-hydroxymethyl-tetrahydro-furan-3,4-diol (5). To a solution of **4** (12 g, 30 mmol) in THF (1.2 L) was added Ph_3P (15.6 g, 59.4 mmol) at 0 °C under argon atmosphere. Diisopropyl azadicarbonate (12 mL, 59.4 mmol) was added, and the mixture was stirred overnight and concentrated. The residue was purified by flash chromatography on silica gel column (2:1 hexanes/EtOAc) to afford the bis-acetal as a colorless oil (10 g, 26.4 mmol, 86%). R_f=0.27 (1:1 hexanes/EtOAc); $[\alpha]_D$ −3.4 (*c* 0.9, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 1.34 (s, 9H), 1.60 (s, 3H), 3.19 (s, 3H), 3.54 (dd, 1H, *J*=15.0, *J*=3.9 Hz), 3.63 (dd, 1H, *J*=14.3, *J*=3.5 Hz), 3.94 (s, 3H), 4.07 (s, 3H), 4.18 (m, 1H), 4.72 (s, 2H), 5.01 (s, 1H), 8.32 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 24.3 (2), 26.0, 28.1 (2), 54.1, 55.1, 63.1, 82.2, 83.4, 85.0, 87.1, 102.3, 114.9, 115.0, 158.5, 167.3, 170.1; MS (ESI): 386.1 [M+H].

The bis-acetal was then treated with 70% AcOH (300 mL) at reflux for 2 h. The mixture was then brought to room temperature and concentrated to give a yellow oil. The oil was diluted in water (150 mL) and evaporated (repeated two times). It was then diluted in MeOH (150 mL) and evaporated (repeated two times). The residue was purified by flash chromatography on silica gel column (10:1 EtOAc/MeOH) to afford the product **5** as a white solid (6.0 g, 22 mmol, 84%); mp 128–130 °C; $[\alpha]_D$ +10.0 (*c* 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 3.68 (dd, 1H, *J*=7.42, *J*=4.62 Hz), 3.81 (dd, 1H, *J*=8.88, *J*=3.2 Hz), 3.90 (m, 1H), 3.95 (s, 4H), 4.05 (s, 4H), 4.80 (s, 1H), 8.39 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 54.6, 55.3, 62.9, 75.1, 76.9, 80.0, 85.0, 114.9, 157.8, 166.3, 170.2; MS (ESI): 273.3 [M+H].

4.1.4. (2*S*, 3*S*, 4*R*, 5*R*)-2,4-Dimethoxy-5-[5,5,7,7-tetraisopropyl-3-(4-methoxy-benzyloxy)-tetrahydro-1,4,6,8-tetraoxa-5,7-disila-cyclopentacycloocten-2-yl]-pyrimidine (6). Compound **5** (1.10 g, 4.04 mmol) was dissolved in dry pyridine (42 mL) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.42 mL, 4.44 mmol) was added dropwise at room temperature in a 100 mL round-bottomed flask. The colorless mixture was stirred at room temperature for 4 h under argon and then concentrated (*T*<45 °C). The white solid was diluted with Et_2O (50 mL), then with water (50 mL), the two phases were separated, the aqueous phase was extracted with Et_2O (3×50 mL), and the combined organic phases were washed with brine and dried over Na_2SO_4. After concentration under reduced pressure, the colorless oil was purified by flash chromatography (eluant, 3:1 hexanes/EtOAc) to afford the mono-alcohol as a colorless oil (1.97 g, 3.83 mmol, 95%). R_f=0.21 (3:1 hexanes/EtOAc), $[\alpha]_D$ −1.31 (*c* 1.45, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (m, 28H), 2.91 (s, 1H), 3.95 (s, 3H), 4.0 (s, 3H),

4.12 (m, 4H), 4.35 (m, 1H), 4.95 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 12.6, 12.9, 13.2, 16.8, 16.9, 17.1, 53.6, 53.9, 54.6, 54.7, 60.2, 61.4, 71.1, 75.2, 75.3, 79.9, 79.9, 80.9, 113.2, 156.5, 164.9, 168.0; MS (ESI): 515.7 [M+H].

In a 25 mL flame-dried round-bottomed flask, NaH (135 mg, 3.4 mmol, 60% in mineral oil) was washed with dry hexane (15 mL) under argon atmosphere. After removal of hexane, dry DMF (1.5 mL) was added to NaH and the white heterogeneous mixture was cooled to 0 °C. The mono-alcohol (1.25 g, 2.43 mmol) was dissolved in dry THF (5 mL), added to NaH in DMF at 0 °C, and the mixture was stirred for 10 min at 25 °C after which PMBBr (3.2 mL, 4.13 mmol, 1.3 M in toluene) was added dropwise. The white heterogeneous mixture was stirred for 15 h and then quenched with water (5 mL), and then diluted with Et_2O (15 mL). The two phases were separated, the aqueous phase was extracted with Et_2O (3×10 mL), and the combined organic phase was washed with brine, dried with Na_2SO_4, and concentrated to give an oil. Purification by flash chromatography (eluant, 3:1 hexanes/EtOAc) gave **6** as a colorless oil (1.3 g, 2.05 mmol, 84%). R_f=0.34 (3:1 hexanes/EtOAc). $[\alpha]_D$ −5.9 (*c* 5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (m, 28H), 3.80 (s, 3H), 3.96 (s, 6H), 4.00 (dd, 1H, *J*=17.0, *J*=3.1 Hz), 4.10 (m, 2H), 4.20 (d, 1H, *J*=4.47 Hz), 4.29 (m, 1H), 4.66 (d, 1H, *J*=11.9 Hz), 4.85 (d, 1H, *J*=11.9 Hz), 5.04 (s, 1H), 6.85 (d, 2H, *J*=6.77 Hz), 7.30 (d, 2H, *J*=8.6 Hz), 8.45 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 12.5, 13.3, 13.7, 14.2, 16.9, 17.2, 53.7, 53.8, 54.5, 54.6, 55.1, 55.2, 60.1, 70.1, 71.6, 78.6, 78.7, 80.2, 81.5, 81.6, 113.5, 113.9, 128.9, 130.5, 156.3, 158.9, 164.7, 167.3; MS (ESI): 635.4 [M+H].

4.1.5. (2*S*, 3*S*, 4*R*, 5*R*)-2,4-Dimethoxy-5-[5,5,7,7-tetraisopropyl-3-(4-methoxy-benzyloxy)-tetrahydro-1,4,6,8-tetraoxa-5,7-disila-cyclopen-tacycloocten-2-yl]-pyrimidine (7). Compound **6** (1.12 g, 1.77 mmol) was dissolved in dioxane (38 mL) followed by the addition of 1 N HCl (19 mL) and the mixture was stirred at room temperature for 1 h. The colorless mixture was quenched with Et_3N (4.2 mL, 30 mmol) and diluted with water (38 mL). The organic phase was extracted with CH_2Cl_2 (3×50 mL) and the combined organic phase was dried with Na_2SO_4. After concentration, the colorless oil was purified by flash chromatography (eluant, 1:1 hexanes/EtOAc) to afford **7** as a white solid (1.01 g, 1,55 mmol, 88%). R_f=0.29 (1:1 hexanes/EtOAc); mp 62–65 °C; $[\alpha]_D$ −6.23 (*c* 0.69, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (m, 28H), 3.75 (s, 3H), 3.90 (s, 4H), 4.01 (s, 4H), 4.04 (m, 1H), 4.10 (m, 1H), 4.61 (m, 3H), 5.01 (d, 1H, *J*=4.26 Hz), 6.79 (d, 2H, *J*=8.65 Hz), 7.14 (d, 2H, *J*=8.66 Hz), 8.25 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.59, 13.3, 13.4, 13.5, 14.3, 14.8, 16.5, 17.2, 53.7, 53.8, 54.7, 54.8, 55.0, 55.1, 60.2, 69.9, 71.7, 78.3, 78.4, 81.6, 82.8, 112.9, 113.5, 129.4, 129.6, 157.3, 159.1, 164.8, 167.8; MS (ESI): 653.4 [M+H].

4.1.6. (2*S*, 3*S*, 4*R*, 5*R*)-5-(2,4-Dimethoxy-pyrimidin-5-yl)-4-(4-methoxy-benzyloxy)-2-vinyl-tetrahydro-furan-3-ol (8). In a 25 mL flame-dried round-bottomed flask, dry CH_2Cl_2 (6.5 mL) and dry DMSO (0.63 mL, 8.87 mmol) were added under argon atmosphere. Mixture was cooled to −78 °C and oxalyl chloride (0.37 mL, 4.29 mmol) was added dropwise. The colorless mixture was stirred at −78 °C for 15 min and **7** (935 mg, 1.43 mmol) dissolved in dry CH_2Cl_2 (8.5 mL) was added and stirred at −78 °C for 45 min. ^{i}Pr$_2$NEt (1.99 mL, 11.44 mmol) was added and mixture was stirred at −30 °C for 1 h 30 min. It was then quenched with satd NH_4Cl. (15 mL), diluted with Et_2O (15 mL) and with water (15 mL). The organic phase was separated and concentrated. It was then dissolved in Et_2O (50 mL) and hexane (50 mL), then washed with water (5×20 mL), brine (1×20 mL), and dried over Na_2SO_4. A pale yellow oil was obtained after concentration of the organic phase.

In a 100 mL round-bottomed flask, Ph_3PCH_3Br (1.02 g, 2.86 mmol) was put in suspension in dry THF (29 mL) and cooled to 0 °C. NaHMDS (2.86 mL, 2.86 mmol, 1 M in THF) was added and the yellow mixture was stirred at 0 °C for 1 h. The above obtained aldehyde (930 mg, 1.43 mmol) in dry THF (25 mL) was added to the ylide at −40 °C and stirred for 2 h. After stirring at 0 °C for 16 h, the orange-brown mixture was quenched with satd NH_4Cl and the aqueous phase was extracted with Et_2O (3×40 mL). The combined organic phase was dried with Na_2SO_4 and concentrated to give an orange oil. Purification by flash chromatography (eluant, 1:1 hexanes/EtOAc) afforded compound **9** (204 mg, 0.31 mmol, 11%) as an oil and the desired product **8** as a white solid (200 mg, 0.51 mmol, 36% (two steps)). R_f=0.28 (1:1 hexanes/EtOAc), mp 74 °C; $[\alpha]_D$ +23.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 1H), 3.80 (s, 3H), 3.87 (m, 1H), 3.93 (dd, 1H, *J*=5.5, *J*=2.8 Hz,), 3.98 (s, 3H), 4.00 (s, 3H), 4.24 (dd, 1H, *J*=7.5, *J*=6.5 Hz), 4.53 (d, 1H, *J*=11.3 Hz),, 4.70 (d, 1H, *J*=11.3 Hz), 5.05 (d, 1H, *J*=2.6 Hz), 5.28 (d, 1H, *J*=10.0 Hz), 5.44 (d, 1H, *J*=17.1 Hz), 5.9–6.05 (m, 1H), 6.86 (d, 2H, *J*=8.6 Hz), 7.22 (d, 2H, *J*=8.6 Hz), 8.21 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.2, 55.1, 56.0, 57.8, 72.3, 74.6, 82.5, 84.1, 113.1, 114.8, 117.5, 128.2, 130.1, 136.1, 156.3, 159.1, 165.0, 167.2; MS (ESI): 411.4 [M+Na]. Compound **9** (204 mg) was treated with TBAF in THF (1 h, 0 °C) to give **8** (108 mg, 0.28 mmol, 90%).

4.1.7. (2*S*, 3*S*, 4*R*, 5*R*)-4-Allyloxy-2-(2′,4′-dimethoxypyrimidin-5′-yl)-3-(*p*-methoxy-benzyloxy)-5-vinyl-tetrahydrofuran (10). To a solution of **8** (2.5 g, 6.44 mmol) in 30 mL dry DMF was added sodium hydride (0.387 g, 9.66 mmol) at 0 °C under argon atmosphere. After stirring for 10 min, allyl bromide (0.817 mL, 9.66 mmol) was added to this mixture. The mixture was stirred overnight and quenched by adding $NaHCO_3$. The mixture was extracted with EtOAc (200 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 4:1) afforded **10** as a colorless oil (2.5 g, 5.84 mmol, 91%); $[\alpha]_D$ +37.7 (*c* 0.97, CHCl3), IR (thin film) ν 2956, 1603, 1571, 1514 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (dd, 1H, *J*=7.5, *J*=4.8 Hz), 3.77 (s, 3H, –OMe), 3.92 (m, 3H), 3.97 (s, 6H, 2×–OMe), 4.52 (t, 1H, *J*=7.5 Hz), 4.59 (d, 1H, *J*=11.9 Hz), 4.65 (d, 1H, *J*=11.9 Hz), 5.15–5.3 (m, 5H), 5.45 (d, 1H, *J*=17.1 Hz), 5.75–6.0 (m, 2H), 6.82 (d, 2H, *J*=8.6 Hz), 7.23 (d, 2H, *J*=8.6 Hz), 8.21 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.8, 54.7, 55.1, 71.2 (2C), 78.1, 79.4, 80.8, 81.3, 113.5, 113.5, 117.3, 117.9, 129.3,

129.7, 134.1, 135.9, 156.3, 159.1, 164.8, 167.9; MS (FAB) 429.2 ($M+H^+$); HRMS (FAB) calcd for $C_{23}H_{29}N_2O_6$ 429.2025; found 429.2045.

4.1.8. (2*S*, 3*S*, 3a*R*, 7a*R*)-2-(2′,4′-Dimethoxy-pyrimidin-5′-yl)-3-(*p*-methoxy-benzyloxy)-3,3a,5,7a-tetrahydro-2*H*-furo[3,2-*b*]pyran (11). To a solution of **10** (2.5 g, 5.84 mmol) in 1.2 L dry degassed CH_2Cl_2 was added the first generation Grubbs catalyst (240 mg, 0.29 mmol) at room temperature under argon atmosphere. The mixture was refluxed for 5 h and concentrated. The residue was purified by flash chromatography on silica gel column (hexanes/EtOAc, 4:1) to afford the bicyclic product **11** as a white solid (2.2 g, 5.5 mmol, 94%); mp. 80 °C, $[\alpha]_D$ −33.2 (*c* 1.1, $CHCl_3$), IR (thin film) ν 2956, 1603, 1602, 1575 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.42 (dd, 1H, *J*=8.9, *J*=4.5 Hz), 3.77 (s, 3H, –OMe), 3.93 (m, 1H), 3.95 (s, 3H, –OMe), 3.96 (s, 3H, –OMe), 4.40 (m, 2H), 4.54 (m, 1H), 4.65 (d, 1H, *J*=11.9 Hz), 4.74 (d, 1H, *J*=11.9 Hz), 5.11 (m, 1H), 5.69 (d, 1H, *J*=10.3 Hz), 6.28 (d, 1H, *J*=9.7 Hz), 6.85 (d, 2H, *J*=8.7 Hz), 7.28 (d, 2H, *J*=8.7 Hz), 8.18 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.9, 54.7, 55.1, 68.6, 71.3, 71.6, 78.8, 79.5, 81.7, 113.4, 113.6, 127.1, 127.4, 129.1, 130.0, 156.1, 159.1, 164.8, 167.6; HRMS (MAB) calcd for $C_{21}H_{25}N_2O_6$ ($M+H^+$) 401.1710; found 401.1699.

4.1.9. (2*S*, 3*S*, 3a*R*, 6*R*, 7*R*, 7a*R*)-7-Azido-2-(2′,4′-dimethoxy-pyrimidin-5′-yl)-6-hydroxyl-3-(*p*-methoxy-benzyloxy)-hexahydro-2*H*-furo[3,2-*b*]pyran (12). To a solution of **11** (1.0 g, 2.5 mmol) in 60 mL THF and 60 mL H_2O was added NBS (0.53 g, 2.95 mmol) at room temperature. The mixture was stirred for 1.5 h and quenched by adding 100 mL H_2O containing 2 g $Na_2S_2O_3$. The mixture was extracted with EtOAc (100 mL×3), the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was dissolved in 200 mL THF and 1 N NaOH (33 mL). The solution was refluxed for 1 h and cooled to room temperature, then extracted with EtOAc (200 mL×3). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The intermediate epoxide was dissolved in 150 mL methoxyethanol. The solution was refluxed for 1 h, cooled to room temperature, then poured into 200 mL brine, and extracted with EtOAc (100 mL×3). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 3:1) afforded the *trans*-azido alcohol **12** as a white solid (500 mg, 1.1 mmol, 43%); mp 119 °C; $[\alpha]_D$ +93.1 (*c* 0.98, $CHCl_3$), IR (thin film) ν 3400, 2915, 2105 (N3), 1604, 1572 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.40 (br s, 1H, OH), 3.58 (dd, 1H, *J*=10.0, 4.7 Hz), 3.68 (m, 1H), 3.73 (d, 1H, *J*=12.7 Hz), 3.78 (s, 3H, –OMe), 3.89 (m, 2H), 3.97 (s, 6H, 2×–OMe), 4.33 (m, 2H), 4.67 (s, 2H), 5.10 (m, 1H), 6.86 (d, 2H, *J*=8.7 Hz), 7.27 (d, 2H, *J*=8.7 Hz), 8.33 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.9, 54.8, 55.1, 61.6, 68.3, 69.3, 71.4, 73.3, 74.2, 79.8, 80.3, 113.3, 113.7, 129.2, 129.7, 155.8, 159.2, 164.8, 167.4; HRMS (FAB) calcd for $C_{21}H_{26}N_5O_7$ ($M+H^+$) 460.1832; found 460.1842.

4.1.10. (2*S*, 3*S*, 3a*R*, 7*R*, 7a*R*)7-Azido-2-(2,4-dimethoxy-pyrimidin-5-yl)-3-(4-methoxy-benzyloxy)-tetrahydro-furo[3,2-*b*]pyran-6-one(13). To a solution of **12** (1.0 g, 2.2 mmol) in 50 mL anhydrous CH_2Cl_2 was added Dess–Martin periodinane (2.22 g, 7.0 mmol) at room temperature under argon atmosphere. The mixture was stirred for 4 h and quenched by adding satd $NaHCO_3$ (30 mL) and $Na_2S_2O_3$ (30 mL). The mixture was stirred for 30 min, extracted with EtOAc (200 mL×3), the combined organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. Compound **13** was used immediately in the next step without any purification.

4.1.11. (2*S*, 3*S*, 3a*R*, 6*S*, 7*R*, 7a*R*)-7-Azido-2-(2′, 4′-dimethoxy-pyrimidin-5′-yl)-6-methoxy-3-(*p*-methoxy-benzyloxy)-hexahydro-2*H*-furo[3,2-*b*]pyran (14). Compound **13** was dissolved in 40 mL MeOH and $NaBH_4$ (840 mg, 22.7 mmol) was added to this mixture. After stirring for 1 h, the mixture was concentrated at room temperature. The residue was dissolved in 200 mL EtOAc and washed with H_2O (50 mL×3), brine (30 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was dissolved in 20 mL anhydrous DMF and NaH (131 mg, 3.27 mmol) was added at 0 °C under argon atmosphere. The resulting mixture was stirred for 10 min, iodomethane (0.638 mL, 99 mmol) was added, and the mixture was stirred overnight. After adding satd $NaHCO_3$ (30 mL), the mixture was extracted with EtOAc (50 mL×3), the organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 4:1) afforded **14** as a white solid (800 mg, 1.7 mmol, 78%); mp 89 °C; $[\alpha]_D$ +60.6 (*c* 0.88, $CHCl_3$), IR (thin film) ν 2913, 2105 (N_3), 1602, 1572 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.47 (s, 3H, –OMe), 3.61 (m, 2H), 3.75 (m, 1H), 3.76 (s, 3H, –OMe), 3.85 (d, 1H, *J*=4.63 Hz), 3.96 (s, 3H, –OMe), 3.97 (s, 3H, –OMe), 4.01 (dd, 1H, *J*=10.04, *J*=2.91 Hz), 4.67 (m, 3H), 5.16 (s, 1H), 6.86 (d, 2H, *J*=8.6 Hz), 7.26 (d, 2H, *J*=8.6 Hz), 8.44 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 53.9, 54.8, 55.1, 57.1, 57.4, 59.7, 65.7, 71.2, 74.0, 75.0, 76.4, 78.9, 81.5, 113.3, 113.7, 129.7, 156.0, 159.1, 164.9, 167.2; HRMS (FAB) calcd for $C_{22}H_{28}N_5O_7$ ($M+H^+$) 474.1988; found 474.2002.

The corresponding 6-ethoxy analogue **15** was similarly prepared (76%); $[\alpha]_D$ +68 (0.6, $CHCl_3$); MS (FAB): 488.2 ($M+H^+$).

4.1.12. (2*S*, 3*S*, 3a*R*, 6*S*, 7*R*, 7a*R*)-7-Azido-2-(2′,4′-dioxo-1′,2′,3′,4′-tetrahydro–pyrimidin-5′-yl)-6-methoxy-3-pivaloyloxy)-hexahydro-2*H*-furo[3,2-*b*]pyran (16). To a solution of **14** (800 mg, 1.69 mmol) in 20 mL CH_2Cl_2 and 1 mL H_2O was added DDQ (1.18 g, 5.2 mmol) at room temperature. The mixture was stirred for 6 h and quenched by adding satd $NaHCO_3$ (200 mL). The mixture was extracted with CH_2Cl_2 (50 mL×4). The combined organic phase was washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel column (EtOAc) to afford the alcohol as a colorless oil (580 mg, 1.65 mmol, 97%); $[\alpha]_D$ +93 (*c* 0.3, $CHCl_3$); IR (thin film) ν 3540, 2920, 2105, 1603, 1574 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.49 (s, 3H, OMe), 3.57 (ddd, 1H, *J*=10.4, *J*=4.85, *J*=2.91 Hz), 3.65 (t, 1H, *J*=10.4 Hz), 3.76 (dd, 1H, *J*=9.8, *J*=4.64 Hz), 3.94 (m, 2H), 3.98 (m, 4H, –OMe), 4.02 (s, 3H, –OMe), 4.10 (d, 1H, *J*=4.6 Hz),

4.67 (m, 2H), 5.06 (s, 1H), 8.41 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.1, 54.8, 57.4, 59.4, 65.7, 73.0, 73.7, 74.7, 76.3, 83.5, 112.9, 155.8, 164.4, 167.6; HRMS (FAB) calcd for $C_{14}H_{20}N_5O_6$ ($M+H^+$) 353.1335; found 353.1341.

The alcohol (580 mg, 1.64 mmol) was dissolved in 30 mL anhydrous pyridine, and DMAP (122 mg, 1 mmol) and pivaloyl chloride (0.4 mL, 3.32 mmol) were added at room temperature under argon atmosphere. The mixture was stirred overnight, concentrated, and the residue was purified by flash chromatography on silica gel column (hexanes/EtOAc, 4:1) to afford the pivalate ester as a colorless oil (650 mg, 1.49 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (s, 9H, –OPiv), 3.47 (s, 3H, $-OCH_3$), 3.53 (m, 1H), 3.56 (t, 1H, J=10.2 Hz), 3.79 (dd, 1H, J=10.2, J=2.6 Hz), 3.90 (ddd, 2H, J=10.5, J=5.2, J=2.8 Hz), 3.97 (s, 3H, $-OCH_3$), 3.99 (s, 3H, $-OCH_3$), 4.67 (s, 1H), 5.05 (s, 1H), 5.27 (d, 1H, J=3.7 Hz), 8.41 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 39.3, 54.5, 55.2, 57.9, 59.5, 66.2, 73.4, 73.8, 75.7, 76.1, 76.7, 77.0, 81.8, 112.8, 156.5, 165.7, 168.1, 177.5; MS (FAB): 438.2 ($M+H^+$).

To a solution of the pivalate ester (650 mg, 1.49 mmol) in 27 mL dry MeCN were added NaI (740 mg, 5.22 mmol) and TMSCl (565 mg, 5.22 mmol) at room temperature under argon atmosphere. The mixture was stirred for 24 h and quenched by adding 10% sodium metabisulfite (10 mL) and satd $NaHCO_3$ (10 mL). The mixture was stirred for 10 min and extracted with EtOAc (30 mL×3), the combined organic layer was washed with brine (35 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography on silica gel column (hexanes/EtOAc, 1:1) afforded **16** as a colorless oil (214 mg, 35%). Starting material (150 mg) and non-demethylated intermediate (145 mg, 34%) were recovered. The latter could be recycled as described above. For **16**, $[\alpha]_D$ +100.75 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22, (s, 9H, –OPiv), 3.46 (s, 3H, –OMe), 3.47 (ddd, 1H, J=10.7, J=2.9, J=4.0 Hz), 3.59 (t, 1H, J=10.7 Hz), 3.75 (dd, 1H, J=10.0, J=2.7 Hz), 3.86 (m, 2H), 4.65 (s, 1H), 4.91 (s, 1H), 5.31 (d, 1H, J=4.7 Hz), 7.55 (s, 1H), 10.00 (br, 2H, 2×NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7, 38.7, 57.4, 59.1, 65.6, 72.6, 72.8, 75.2, 76.4, 80.9, 111.6, 138.8, 152.2, 162.5, 171.1; HRMS (FAB) calcd for $C_17H_{24}N_5O_7$ ($M+H^+$) 410.1675; found 410.1693.

4.1.13. Malayamycin A (1). Compound **16** (32 mg, 0.076 mmol) was dissolved in dry THF (6 mL) and argon was bubbled in the solution for 10 min. Water (6 mL) and PMe_3 (88 mL, 1 M solution in toluene, 0.088 mmol) were added. After 5 min at room temperature, the solution was refluxed for 30 min, then concentrated and held under vacuum for 1 h. It was then dissolved in dry CH_2Cl_2 (8 mL) and trichloroacetylisocyanate (10 mL, 0.082 mmol) was added. After 30 min, the solution was concentrated. The oily residue was dissolved in MeOH (2 mL), 40% $MeNH_2$ in water (4 mL) was added and the solution was stirred for 52 h. Concentration gave a solid that was purified by flash chromatography (9:1 CH_2Cl_2/MeOH) to give pure malayamycin A as a white solid (16 mg), 60%; mp 158 °C (dec) $[\alpha]_D$ +120 (*c* 0.19, MeOH); (authentic sample $[\alpha]_D$ +126 (*c* 0.36, MeOH)). ^{1}H NMR (D_2O, 400 MHz) identical to the authentic sample δ 3.30 (s, 3H, OMe), 3.38 (t, 1H, J=10.7, H-7ax′.), 3.51 (dd, 1H, J=10.7, J=5.1 Hz, H-3′), 3.69 (ddd, 1H, J=5.2, J=3.5, J=10.7 Hz, H-6′), 3.85 (dd, 1H, J=3.5, J=11.8 Hz, H-7eq′), 3.93 (dd, 1H, J=10.7, J=5.4 Hz, H-6′), 4.16 (d, 1H, J=2.1 Hz, H-2′), 4.74 (s, 1H, H-1′), 4.82 (s, 1H, H-5′), 7.24 (s, 1H, H-6). ^{13}C NMR (100 MHz, D_2O) δ 48.1, 57.3, 66.4, 73.2, 75.0, 76.6, 85.2, 113.8, 141.2, 154.8, 163.1, 167.5, 179.8. HRMS (FAB) MH+ calcd 342.1176; found 342.1181.

4.1.14. (2*R*, 3*R*, 3a*R*, 6*R*, 7*R*, 7a*R*)-7-Azido-3-benzyloxy-2,6-dimethoxy-hexahydrofuro[3,2-*b*]pyran (29). To a solution of **28** (600 mg, 1.9 mmol) in 30 mL dry DMF were added NaH (60% dispersion in mineral oil) (200 mg, 5.0 mmol) and MeI (0.5 mL, 8.0 mmol) at 0 °C under argon atmosphere. After stirring overnight, the reaction was quenched by adding satd $NaHCO_3$ and extracted with EtOAc (30 mL×4). The combined organic layer was washed with satd $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash silica chromatography (hexanes/EtOAc, 10:1) afforded the product as colorless oil (600 mg, 1.8 mmol, 96%); $[\alpha]_D$ −16.0 (*c* 1.36, $CHCl_3$); IR (neat): 2960, 2098, 1490 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.22 (m, 1H, H-6); 3.38 (s, 3H, –OMe); 3.41 (s, 3H, –OMe); 3.64 (d, 1H, J=12.8 Hz, H-5); 3.86 (dd, 1H, J=10.0, 4.2 Hz, H-3a); 3.95 (d, 1H, J=4.2 Hz, H-2); 4.03 (d, 1H, J=12.8 Hz, H-5); 4.40 (d, 1H, J=3.0 Hz, H-7); 4.45 (dd, 1H, J=10.0, J=3.1 Hz, H-7a); 4.62 (d, 1H, J=12.2 Hz, $PhCH_2$–); 4.84 (d, 1H, J=12.2 Hz, $PhCH_2$–); 4.86 (s, 1H, H-2); 7.26–7.38 (m, 5H, Ph–); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 56.1, 57.7, 59.3, 66.9, 72.7, 74.8, 75.1, 77.8, 79.8, 128.1, 128.2, 128.8, 138.4. FABMS *m*/*z* (relative intensity): 335 (M^+, 15), 275, 184 (100). HRMS (FAB) calcd for $C_{16}H_{21}N_3O_5$ (M^+) 335.1481; found 335.1481.

To a solution of benzyl ether (590 mg, 1.84 mmol) in 25 mL CCl_4–MeCN–H_2O (1:1:1.5) were added $RuCl_3\cdot 3H_2O$ (87.5 mg, 0.35 mmol) and $NaIO_4$ (464 mg, 2.2 mmol) at 16 °C under argon atmosphere, and then 1.2 g of $NaIO_4$ was added in portions over 24 h. After stirring over 24 h, the reaction was quenched by adding 3 mL isopropanol. The mixture was extracted with CH_2Cl_2 (50 mL×3) and the combined organic layer was washed with satd $NaHCO_3$ and brine, filtered, and concentrated. Purification by flash silica chromatography (hexanes/EtOAc, 10:1) afforded benzoate **29** (500 mg, 1.43 mmol, 82%); $[\alpha]_D$ +0.78 (*c* 1.15, $CHCl_3$); IR (neat, cm^{-1}): 2916, 2103, 1727, 1452, 1269, 1199, 1115, ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 3.22 (m, 1H, H-6); 3.36 (s, 3H, –OMe); 3.47 (s, 3H, –OMe); 3.65 (d, 1H, J=12.9 Hz, H-5); 4.0 (d, 1H, J=12.9 Hz, H-5); 4.03 (dd, 1H, J=10.0, J=4.4 Hz, H-8); 4.40 (m, 2H, H-7 and H-9); 5.03 (s, 1H, H-2); 5.40 (d, 1H, J=4.4 Hz, H-3), 7.42–8.05 (m, 5H, Ph–). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 56.4, 57.7, 59.5, 66.7, 72.6, 75.0, 75.5, 77.9, 107.6, 128.8, 129.6, 130.4, 133.7, 170.0. FABMS *m*/*z* (relative intensity): 349 (M^+, 10).

4.1.15. (1′*R*, 2*R*, 3*R*, 4*S*, 5*R*)-4-Azido-2-(1′-benzoyloxy-2′,2′-bisphenylsulfanylethyl)-3-hydroxyl-5-methoxy-tetrahydropyran (30). To a solution of **29** (390 mg, 1.1 mmol) in 10 mL dry CH_2Cl_2 was added benzenethiol (490 mg, 4.5 mmol), then $BF_3\cdot Et_2O$ (0.17 mL, 1.34 mmol) at

−78 °C under argon atmosphere. The mixture was stirred for 3 h, warmed to 0 °C, and quenched by adding satd $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (30 mL×3), and the combined organic layer was washed with satd $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash silica chromatography (hexanes/EtOAc, 5:1) afforded the dithioacetal **30** as a colorless oil (473 mg, 0.88 mmol, 80%); $[\alpha]_D$ +41.0 (*c* 0.56, $CHCl_3$); IR (neat): λ 2918, 2109, 1723, 1439, 1268 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 2.54 (d, 1H, *J*=5.6 Hz, –OH); 3.35 (s, 3H, –OMe); 3.40 (m, 1H, H-5); 3.49 (dd, 1H, *J*=12.5, *J*=3.5 Hz, H-6), 3.56 (dd, 1H, *J*=12.5, *J*=2.4 Hz, H-6); 3.94 (t, 1H, *J*=4.2 Hz, H-4); 4.07 (m, 1H, H-3); 4.18 (t, 1H, *J*=7.6 Hz, H-7), 4.95 (d, 1H, *J*=2.5 Hz, H-2′); 5.73 (dd, 1H, *J*=7.6, *J*=2.5 Hz, H-1′); 7.25–8.08 (m, 15H, Ph–); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 57.1, 61.5, 62.3, 63.6, 67.9, 74.6, 74.7, 76.7, 127.7, 128.3, 128.33, 128.9, 129.0, 129.3, 130.1, 132.3, 133.3, 133.7, 134.5, 166.2; FABMS *m*/*z* (relative intensity): 537 (M+, 3), 428 (M^+−PhS, 16); HRMS (FAB) calcd for $C_{27}H_{28}N_3O_5S_2$ ($M+H^+$) 538.1470; found 538.1453.

4.1.16. (2*R*, 3*R*, 3a*S*, 6*R*, 7*R*, 7a*R*)-7-Azido-6-methoxy-2-phenylsulfanyl-hexahydrofuro[3,2-*b*]pyran-3-ol (31). To a solution of **30** (420 mg, 0.78 mmol) in 10 mL MeOH was added anhydrous K_2CO_3 (5 mg) at room temperature and the mixture was stirred for 30 min. The mixture was evaporated under vacuum and the residue was purified by flash silica chromatography (hexanes/EtOAc, 3:1) to afford diol as a colorless oil (335 mg, 0.77 mmol). To a solution of above diol in 10 mL dry CH_2Cl_2 was added NBS (205 mg, 1.16 mmol) at room temperature under argon atmosphere. After stirring for 30 min, the reaction was quenched by adding satd $Na_2S_2O_3$ (10 mL), and the mixture was extracted with CH_2Cl_2 (20 mL×3). The combined organic layer was washed with satd $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash silica chromatography (hexanes/EtOAc, 10:1) afforded alcohol as a colorless oil (190 mg, 0.59 mmol, 77%); $[\alpha]_D$ +150.5 (*c* 0.54, $CHCl_3$); IR (neat): 3402, 2918, 2103, 1584, 1482, 1069 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 2.59 (d, 1H, *J*=2.0 Hz, –OH); 3.31 (dd, 1H, *J*=2.4, *J*=1.3 Hz, H-6); 3.73 (m, 1H, H-7); 3.78 (dd, 1H, *J*=12.9, *J*=1.6 Hz, H-5); 4.06 (d, 1H, *J*=12.9 Hz, H-5); 4.46 (m, 2H, H-3a and H-7a), 4.58 (m, 1H, H-3); 5.77 (d, 1H, *J*=4.0 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 57.6, 58.7, 66.7, 70.6, 70.61, 73.4, 74.7, 92.8, 127.3, 129.4, 130.8, 132.5; FABMS *m*/*z* (relative intensity): 323 (M^+, 25); HRMS (FAB) calcd for $C_{14}H_{18}N_3O_4S$ ($M+H^+$) 324.1018; found 324.1012.

To a solution of **31** (170 mg, 0.53 mmol) in 5 mL dry pyridine was added DMAP (200 mg), then pivaloyl chloride (0.2 mL) at room temperature under argon atmosphere. The mixture was stirred overnight and pyridine was removed under reduced pressure. The residue was dissolved with 50 mL CH_2Cl_2, washed with satd $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash silica chromatography (hexanes/EtOAc, 5:1) afforded the pivalate ester **31** as a colorless oil (214 mg, 0.53 mmol, quant.); $[\alpha]_D$ +149.6 (*c* 0.51, $CHCl_3$); IR (neat): 2977, 2931, 2104, 1742, 1480, 1146 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 1.30 (s, 9H, PivO–); 3.28 (m, 1H, H-6); 3.37 (s, 3H, –OMe); 3.63 (d, 1H, *J*=12.0 Hz, H-5), 3.73 (d, 1H, *J* =10.0, *J*=4.3 Hz, H-3a); 3.98 (d, 1H, *J*=12.0 Hz, H-5); 4.42 (m, 2H, H-7 and H-7a); 5.69 (t, 1H, *J*=4.3 Hz, H-3); 5.82 (d. 1H, *J*=4.3 Hz, H-2), 7.24–7.51 (m, 5H, Ph–); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 26.9, 39.2, 56.6, 58.5, 65.0, 70.5, 73.5, 73.6, 76.9, 89.9, 126.9, 128.9, 130.5, 134.8, 176.8; FABMS *m*/*z*: 408 (M+H^+, 20), 298 (M^+−PhS, 100); HRMS (FAB) calcd for $C_{19}H_{26}N_3O_5S$ ($M+H^+$) 408.1593; found 408.1601.

4.1.17. (2*R*, 3*R*, 3a*R*, 6*R*, 7*R*, 7a*R*)-7-Azido-2-(2′,4′-dioxo-3′,4′-dihydro-2*H*-pyrimidin-1′-yl)-6-methoxy-3-pivaloyl-oxy-hexahydrofuro[3,2-*b*]pyran and (2*R*, 3*R*, 3a*R*, 6*R*, 7*R*, 7a*R*)-7-azido-2-(5′-iodo-2′,4′-dioxo-3′,4′-dihydro-2*H*-pyrimidin-1′-yl)-6-methoxy-3-pivaloyloxy-hexahydrofuro [3,2-*b*]pyran (32, 33). To a solution of **31** (41 mg, 0.1 mmol), bis-TMS-uracil (40 mg, 0.16 mmol) and NIS (45 mg, 0.2 mmol) in 4 mL dry CH_2Cl_2 was added TfOH (10 μL) over several minutes. After stirring for 5 h, the mixture was quenched by adding satd $Na_2S_2O_3$, and extracted with CH_2Cl_2 (30 mL×3). The combined organic layer was washed with satd $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash silica chromatography (CH_2Cl_2/MeOH, 10:1) afforded the **32** (20 mg) and iodonucleoside **33** (8 mg) as colorless oils, the combined yield was 80% based on recovered starting material (10 mg). For **32**: $[\alpha]_D$ +114.5 (*c* 1.6, $CHCl_3$); IR (neat): 2971, 2106, 1695, 1458, 1147 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 1.25 (s, 9H, PivO–); 3.39 (m, 1H, H-6); 3.40 (s, 3H, –OMe); 3.68 (d, 1H, *J*=13.4 Hz, H-5); 3.80 (dd, 1H, *J*=9.8, *J*=5.1 Hz, H-7a); 4.08 (d, 1H, *J*=13.4 Hz, H-5); 4.21 (dd, 1H, *J*=9.8, *J*=3.0 Hz, H-3a); 4.45 (m, 1H, H-7); 5.30 (d, 1H, *J*=3.0 Hz, H-3); 5.79 (d, 1H, *J*=8.1 Hz, H-5′); 5.85 (s, 1H, H-1); 7.44 (d, 1H, *J*=8.1 Hz, H-6′); 8.29 (s, 1H, –NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 26.9, 38.8, 56.8, 59.2, 65.2, 71.9, 73.0, 74.4, 76.6, 89.6, 102.9, 139.4, 149.4, 162.5, 176.9; FABMS *m*/*z* (relative intensity): 410 (M+H^+, 10), 391 (M^+−H_2O, 16), 298 (M^+−uracilyl, 16); HRMS (FAB) calcd for $C_{17}H_{24}N_5O_7$ ($M+H^+$) 410.1675; found 410.1662. For **33**: $[\alpha]_D$ +123.4 (*c* 0.87, $CHCl_3$); IR (neat): 2978, 2104, 1691, 1611, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 1.25 (s, 9H, PivO–); 3.40 (s, 3H, –OMe); 3.42 (m, 1H, H-6); 3.72 (d, 1H, *J*=13.1 Hz, H-5); 3.79 (dd, 1H, *J*=10.1, 5.0 Hz, H-3a); 4.08 (d, 1H, *J*=13.1, H-5); 4.25 (dd, 1H, *J*=10.1, *J*=2.4 Hz, H-7a); 4.47 (s, 1H, H-7); 5.36 (d, 1H, *J*=5.0 Hz, H-3); 5.80 (s, 1H, H-2); 8.03 (s, 1H, H-6′); 8.59 (s, 1H, –NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 27.5, 39.4, 57.4, 59.9, 65.8, 68.8, 72.2, 73.4, 75.4, 77.3, 90.3, 144.7, 149.5, 159.8, 177.2; FABMS *m*/*z* (relative intensity): 536 (M+H^+, 10), 298 (M^+−iodouracilyl, 26); HRMS (FAB) calcd for $C_{17}H_{23}N_5O_7I$ (M^+) 536.0642; found 536.0664.

4.1.18. (2*R*, 3*R*, 3a*S*, 6*S*, 7*R*, 7a*R*)-[2-(2′,4′-Dioxo-3′,4′-dihyro-2*H*-pyrimidin-1′-yl)-3-hydroxyl-6-methoxy-hexahydrofuro[3,2-*b*]pyran-7-yl]-urea (6-*epi*-*N*-malayamycin) (34). To a solution of **32** (37 mg, 0.09 mmol) in 4 mL anhydrous THF was added Me_3P (1 M in toluene) (250 μL, 0.25 mmol) at room temperature under argon atmosphere. After stirring for 30 min, 10 μL of H_2O was added, and the resulting mixture was refluxed for 40 min, then evaporated. The residue was dried under reduced

pressure (1 mmHg) for 1.5 h and dissolved in 2 mL dry CH_2Cl_2. To this solution was added trichloroacetylisocyanate (20 μL) at room temperature under argon atmosphere. After stirring for 60 min, CH_2Cl_2 was removed and the residue was dissolved with MeOH (3 mL) and 40% $MeNH_2$ in H_2O (3 mL), and stirred over 3 days. The mixture was evaporated and purified by flash silica chromatography (CH_2Cl_2/MeOH, 9:1) to afford 6-*epi*-*N*-malayamycin **34** as a white solid (25 mg, 0.073 mmol, 77%); $[\alpha]_D$ +30.0 (*c* 0.45, MeOH); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 3.39 (m, 1H, H-6′); 3.44 (s, 1H, –OMe); 3.59 (dd, 1H, *J*=10.7, *J*=5.2 Hz, H-3a); 3.79 (d, 1H, *J*=13.2 Hz, H-5); 4.12 (d, 1H, *J*=13.2 Hz, H-5); 4.22 (dd, 1H, *J*=10.7, *J*=4.2 Hz, H-7a); 4.31 (d, 1H, *J*=5.2 Hz, H-3); 4.65 (m, 1H, H-7); 5.62 (s, 1H, H-2); 5.70 (d, 1H, *J*=8.1 Hz, H-5′); 7.62 (d, 1H, *J*=8.1 Hz, H-6′); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ 48.7, 56.4, 66.6, 72.6, 73.5, 74.1, 78.7, 93.8, 101.7, 141.7, 150.9, 160.6, 165.2; FABMS *m*/*z* 343 ($M+H^+$); HRMS (FAB) calcd for $C_{13}H_{19}N_4O_7$ ($M+H^+$) 343.1253; found 343.1284.

4.1.19. (3a*R*, 5*R*, 6*R*, 6a*R*)-Dihydro-6-(iodomethyl)-2,2-dimethyl-5-vinyl-5*H*-furo[2,3-*d*][1,3]dioxole (36). A solution of **35**[39] (10 g, 36.5 mmol) in AcOH/H_2O 75:25 v/v (70 mL) was kept at room temperature for 15 h, and then concentrated in vacuo at 30 °C to afford the triol as a colorless oil, which did not require further purification. R_f=0.27 (100:3 AcOEt/MeOH).

To a solution of the above triol, triethylamine (21 mL, 151 mmol) and DMAP (400 mg, 3.27 mmol) in CH_2Cl_2 (45 mL) at −20 °C under an argon atmosphere was added dropwise methanesulfonic chloride (10 mL, 129 mmol) (strongly exothermic). The reaction mixture was stirred at room temperature for 1 h. Aq satd $NaHCO_3$ (50 mL) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic extracts were combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash silica gel chromatography (hexanes/EtOAc 7:3) afforded the intermediate trimesylate as a colorless oil (13.8 g, 81% for two steps). R_f=0.42 (8:3 AcOEt/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.85 (d, 1H, *J*=3.6 Hz), 4.92 (td, 1H, *J*=6.4, *J*=2.7 Hz), 4.79 (dd, 1H, *J*=4.1, *J*=3.6 Hz), 4.57 (dd, 1H, *J*=11.9, *J*=2.7 Hz), 4.53–4.45 (m, 2H), 4.41 (dd, 1H, *J*=11.9, *J*=6.4 Hz), 4.18–4.10 (m, 1H), 3.18 (s, 3H), 3.09 (s, 3H), 3.08 (s, 3H), 2.65–2.52 (m, 1H), 1.52 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 113.2, 105.2, 80.4, 78.6, 77.2, 67.6, 65.8, 46.6, 39.0, 37.7, 37.2, 26.9, 26.4.

A solution of the above trimesylate (9.5 g, 20.3 mmol) in ethylmethyl ketone (80 mL) was treated with NaI (15.2 g, 101 mmol). The resulting suspension was refluxed for 15 h, whereas a dark-brown coloration corresponding to the formation of iodine developed gradually. The reaction mixture cooled down to room temperature was treated with satd $Na_2S_2O_3$ (200 mL). After stirring for 15 min, *t*-butyl methyl ether (200 mL) was added and the phases were separated. The aqueous layer was extracted with *t*-butyl methyl ether (100 mL×2). The organic extracts were combined, washed with brine (100 mL×2), dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash silica gel chromatography (hexanes/EtOAc 9:1) afforded the diene **36** as a yellowish oil (5.2 g, 82%). R_f=0.68 (7:3 AcOEt/hexanes); $[\alpha]_D$ +14.1 (*c* 1.11 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (d, 1H, *J*=3.7 Hz), 5.76 (ddd, 1H, *J*=17.8, *J*=10.5, *J*=7.8 Hz), 5.35 (d, 1H, *J*=17.8 Hz), 5.29 (d, 1H, *J*=10.5 Hz), 4.72 (app. t, 1H, *J*=4.1 Hz), 4.10 (dd, 1H, *J*=10.0, *J*=7.8 Hz), 3.25 (dd, 1H, *J*=11.9, *J*=9.6 Hz), 3.06 (dd, 1H, *J*=9.6, *J*=3.7 Hz), 2.14 (ddt, 1H, *J*=11.9, *J*=10.0, *J*=4.1 Hz), 1.54 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.8, 119.2, 111.7, 103.9, 81.2, 81.1. 53.4, 26.4, 26.1, −2.9; HRMS calcd for $C_{10}H_{16}IO_3$ $[M+H]^+$ 311.0144; found 311.0146.

4.1.20. (3a*R*, 5*R*, 6*R*, 6a*R*)-6-(But-3-enyl)-dihydro-2,2-dimethyl-5-vinyl-5*H*-furo[2,3-*d*][1,3]dioxole (37). A mixture of **36** (3.0 g, 9.7 mmol), allyltributyltin (54 mL, 174 mmol) and AIBN (159 mg, 0.97 mmol) under an argon atmosphere was heated at 100 °C for 1 h. An additional portion of AIBN (159 mg, 0.97 mmol) was then added and the reaction mixture was heated for 1 h. The product was isolated by two successive flash silica gel chromatography (CH_2Cl_2), which afforded 1.86 g of **37** (75%) as a colorless oil. R_f=0.52 (3:1.5:0.5 toluene/hexanes/AcOEt); $[\alpha]_D$ +26.4 (*c* 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89–5.76 (m, 1H), 5.80 (d, 1H, *J*=3.7 Hz), 5.73 (ddd, 1H, *J*=17.4, *J*=10.5, *J*=7.8 Hz), 5.31 (ddd, 1H, *J*=10.5, *J*=1.8, *J*=0.9 Hz), 5.03 (dm, 1H, *J*=17.4 Hz), 4.97 (dm, 1H, *J*=10.5 Hz), 4.13 (app. t, 1H, *J*=3.7 Hz), 2.35–2.19 (m, 1H) 2.18–2.02 (m, 1H), 1.76–1.63 (m, 2H), 1.52 (s, 3H), 1.47–1.35 (m, 1H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 136.0, 118.5, 114.9, 111.4, 105.0, 83.1, 80.7, 49.1, 31.8, 26.7, 26.6, 23.3.

4.1.21. (3a*R*, 4a*R*, 8a*R*, 8b*R*)-7,8,8a,8b-Tetrahydro-2,2-dimethyl-4a*H*-benzofuro[2,3-*d*][1,3]dioxole (38). A solution of **37** (7.0 g, 31.2 mmol) and Grubbs first generation catalyst (1.28 g, 1.56 mmol) in CH_2Cl_2 (620 mL) saturated with argon was refluxed under a slight flow of argon for 2 h. The reaction mixture was concentrated at 400 mbar without heating. Purification of the residue by flash silica gel chromatography (CH_2Cl_2) afforded 3.37 g of **38** (55%) as a brown oil, which is volatile. R_f=0.38 (3:1.5:0.5 toluene/hexanes/AcOEt); $[\alpha]_D$ −14.1 (*c* 1.01 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.04 (dm, 1H, *J*=10.5 Hz), 5.90 (d, 1H, *J*=3.7 Hz), 5.64 (ddd, 1H, *J*=10.5, *J*=6.4, *J*=3.2 Hz), 4.62 (app. t, 1H, *J*=3.7 Hz), 4.34–4.25 (m, 1H), 2.30–2.19 (m, 2H), 2.03–1.92 (m, 1H), 1.85–1.68 (m, 1H), 1.65–1.51 (m, 1H), 1.53 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.7, 127.3, 111.5, 106.6, 80.0, 75.9, 47.0, 30.9, 26.1, 25.9, 19.6; HRMS calcd for $C_{11}H_{17}O_3$ $[M+H]^+$ 197.1178; found 197.1176.

4.1.22. (3a*R*, 4a*S*, 5*R*, 6*S*, 8a*R*, 8b*R*)-5-Azido-hexahydro-2,2-dimethyl-4a*H*-benzofuro[2,3-*d*][1,3]dioxol-6-ol (39). To a solution of **38** (2.80 g, 14.3 mmol) in THF–H_2O (1:1 v/v, 340 mL) at room temperature was added NBS (4.82 g, 27.1 mmol). The mixture was stirred at room temperature for 2 h and then diluted with aq satd $Na_2S_2O_3$ (100 mL) and extracted with EtOAc (100 mL×3). The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated in vacuo. The resulting brown oil was engaged in the next reaction. R_f=0.68 (AcOEt).

A solution of the above residue in THF (240 mL) was treated with aq 1 N NaOH (120 mL) and then refluxed for 1 h. The

mixture was poured into H_2O (120 mL) and extracted with EtOAc (150 mL×2). The combined organic layer was washed with brine (150 mL), dried over $MgSO_4$, and concentrated in vacuo to give a brown oil, which was engaged in the next reaction. R_f=0.60 (AcOEt).

The above material was dissolved in 2-methoxyethanol (300 mL) and sodium azide (13.9 g, 214 mmol) was added. The mixture was heated at 130 °C for 2 h, diluted with brine (300 mL), and extracted with EtOAc (100 mL×3). The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash silica gel chromatography (hexanes/EtOAc 2:1) to afford 1.9 g of azide **39** (52%) as an orange oil. R_f=0.24 (2:1 hexanes/AcOEt); $[\alpha]_D$ −7.5 (*c* 0.80 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.85 (d, 1H, *J*=3.7 Hz), 4.60 (app. t, 1H, *J*=3.7 Hz), 4.22–4.15 (m, 2H), 4.09–4.05 (br m, 1H), 2.10 (d, 1H, *J*=3.7 Hz), 1.89–1.79 (m, 1H), 1.79–1.63 (m, 4H), 1.52 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 112.0, 105.3, 80.2, 76.8, 69.3, 62.3, 42.1, 28.0, 26.3, 26.0, 18.3; HRMS calcd for $C_{11}H_{18}N_3O_4$ $[M+H]^+$ 256.1297; found 256.1299.

4.1.23. (3a*R*, 4a*S*, 5*S*, 8a*R*, 8b*R*)-5-Azido-tetrahydro-2,2-dimethyl-7*H*-benzofuro[2,3-*d*][1,3]dioxol-6(8b*H*)-one (40). To a solution of **39** (1.80 g, 7.05 mmol) in CH_2Cl_2 (32 mL) at room temperature was added Dess–Martin periodinane (3.59 g, 8.46 mmol). The mixture was stirred for 2.5 h. Aq satd $NaHCO_3$ (50 mL) and $Na_2S_2O_3$ (50 mL) were added sequentially. The mixture was stirred for 20 min and the phases were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by flash silica gel chromatography (hexanes/EtOAc, 4:1) afforded the product **40** as a colorless oil (1.6 g, 90%). R_f=0.25 (4:1 hexanes/AcOEt); $[\alpha]_D$ +12.4 (*c* 0.95 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (d, 1H, *J*=3.7 Hz), 4.65 (app. t, 1H, *J*=3.7 Hz), 4.35 (d, 1H, *J*=3.7 Hz), 3.88 (dd, 1H, *J*=11.0, 3.7 Hz), 2.60 (ddd, 1H, *J*=15.0, *J*=13.2, *J*=7.3 Hz), 2.38 (ddm, 1H, *J*=15.1, *J*=5.0 Hz), 2.37–2.24 (m, 1H), 2.06–1.96 (m, 1H), 1.65 (ddd, 1H, *J*=26.0, *J*=13.2, *J*=5.0 Hz), 1.48 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.4, 112.5, 106.8, 79.4, 79.2, 68.4, 41.0, 36.2, 26.3, 26.0, 18.8; HRMS calcd for $C_{11}H_{16}N_3O_4$ $[M+H]^+$ 254.1141; found 254.1144.

4.1.24. (3a*R*, 4a*S*, 5*R*, 6*R*, 8a*R*, 8b*R*)-5-Azido-hexahydro-6-methoxy-2,2-dimethyl-4a*H*-benzofuro[2,3-*d*][1,3]dioxole (41). To a solution of **40** (3.4 g, 13.4 mmol) in MeOH (68 mL) at 0 °C was added $NaBH_4$ (761 mg, 20.1 mmol). The resulting mixture was stirred at room temperature for 3 h, and then concentrated in vacuo without heating. The residue was taken up with EtOAc (30 mL) and washed with water (30 mL). The aqueous layer was extracted with EtOAc (30 mL×2). The organic extracts were combined, dried over $MgSO_4$, and concentrated in vacuo to afford a crude oil that did not require further purification. R_f=0.13 (4:1 hexanes/AcOEt).

To a solution of the above alcohol in THF (20 mL) at 0 °C under an argon atmosphere was added NaH (60% suspension, 1.07 g, 26.7 mmol). After stirring for 1 h at 0 °C, MeI (1.71 mL, 27.5 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and was quenched by adding satd NH_4Cl (50 mL). The mixture was extracted with EtOAc (30 mL×2). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash silica gel chromatography (hexanes/EtOAc, 4:1) to afford **41** (2.5 g, 69%) as a colorless oil, which crystallized upon conservation at 4 °C. R_f=0.32 (4:1 hexanes/AcOEt); $[\alpha]_D$ +16.1 (*c* 0.86 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (d, 1H, *J*=3.2 Hz), 4.54 (app. t, 1H, *J*=3.7 Hz), 4.45 (br app. t, 1H, *J*=2.7 Hz), 3.63 (dd, 1H, *J*=11.0, *J*=2.7 Hz), 3.41 (s, 3H), 3.32 (ddd, 1H, *J*=11.4, *J*=4.6, *J*=3.2 Hz), 1.97–1.75 (two m, 3H), 1.58 (ddd, 1H, *J*=24.7, *J*=12.8, *J*=4.1 Hz), 1.48 (s, 3H), 1.41–1.23 (1H, obscured by CH_3), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.9, 106.4, 79.9, 79.5, 78.5, 60.0, 56.6, 41.7, 26.3, 26.0, 25.9, 14.1; HRMS calcd for $C_{12}H_{20}N_3O_4$ $[M+H]^+$ 270.1454; found 270.1460.

4.1.25. (1*S*, 2*S*, 3*R*, 6*R*)-2-Azido-6-((*R*)-1-hydroxy-2,2-bis(phenylthio)ethyl-3-methoxycyclohexanol (42). To a solution of **41** (1.00 g, 3.71 mmol) in CH_2Cl_2 (22 mL) at 5 °C, PhSH (1.73 mL, 16.9 mmol) was added followed by Amberlyst-15 (1.0 g). The mixture was stirred at 5 °C for 3.5 h and at room temperature for 2 h before being filtrated and concentrated. Purification of the crude product by flash silica gel chromatography (hexanes/EtOAc, 4:1) afforded the product **42** as a colorless oil (1.30 g, 81%). R_f=0.10 (4:1 hexanes/AcOEt); $[\alpha]_D$ −18.5 (*c* 1.15 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.50 (m, 2H), 7.43–7.29 (m, 8H), 4.61 (d, 1H, *J*=3.2 Hz), 4.23 (d, 1H, *J*=3.7 Hz), 4.15 (br app. t, 1H, *J*=3.7 Hz), 3.88 (d, 1H, *J*=2.3 Hz), 3.70 (app. dt, 1H, *J*=8.2, *J*=2.7 Hz), 3.64 (app. dt, 1H, *J*=10.0, *J*=3.2 Hz), 3.38 (s, 3H), 3.16 (ddd, 1H, *J*=11.4, *J*=4.6, *J*=2.7 Hz), 2.40–2.25 (m, 1H), 1.78–1.65 (m, 1H), 0.84–0.68 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.5, 133.3, 133.0, 132.6, 129.4, 129.2, 128.5, 128.1, 79.0, 77.2, 74.5, 64.8, 64.3, 56.3, 39.6, 24.4, 23.4; HRMS calcd for $C_{21}H_{25}N_3O_3S_2Na$ $[M+Na]^+$ 454.1235; found 454.1239.

4.1.26. (2*R*, 3*R*, 3a*S*, 6*R*, 7*R*, 7a*S*)-7-Azido-octahydro-6-methoxy-2-(phenylthio)benzofuran-3-ol (43). To a solution of **42** (1.00 g, 2.32 mmol) in CH_2Cl_2 (58 mL) at 0 °C, NBS (615 mg, 3.46 mmol) was added and the mixture was stirred at 0 °C for 1 h. A saturated solution of $Na_2S_2O_3$ (50 mL) was added and the solution was stirred at room temperature until it became colorless. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (25 mL×2). The combined organic phase was washed with brine (50 mL), dried with $MgSO_4$, filtrated, and concentrated. Flash silica gel chromatography (hexanes/EtOAc, 6:4) afforded the product **43** as pale yellowish oil (499 mg, 67%). R_f=0.46 (1:1 hexanes/AcOEt); $[\alpha]_D$ −12.1 (*c* 1.09 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.48 (m, 2H), 7.38–7.22 (m, 8H), 5.74 (d, 1H, *J*=3.7 Hz), 4.52 (br app. t, 1H, *J*=2.7 Hz), 4.44 (app. t, 1H, *J*=4.1 Hz), 3.95 (dd, 1H, *J*=11.4, *J*=2.7 Hz), 3.44 (s, 3H), 3.33 (ddd, 1H, *J*=11.4, *J*=4.6, *J*=3.2 Hz), 2.33 (d, 1H, *J*=3.2 Hz), 2.10 (app. tt, 1H, *J*=11.4, *J*=4.1 Hz), 1.92 (dm, 1H, *J*=12.3 Hz), 1.88–1.77 (m, 1H), 1.63 (ddd, 1H, *J*=12.8, *J*=11.4, *J*=4.1 Hz), 1.53–1.40 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.3, 131.1, 129.1, 127.2, 95.4, 79.8, 79.6, 72.8, 60.3, 56.7, 42.2, 25.8, 19.1; HRMS calcd for $C_{15}H_{20}N_3O_3S$ $[M+H]^+$ 322.1225; found 322.1229.

4.1.27. (2*R*, 3*R*, 3a*R*, 6*R*, 7*R*, 7a*S*)-7-Azido-octahydro-6-methoxy-2-(phenylthio)benzofuran-3-yl pivalate (44). To the alcohol **43** (360 mg, 1.12 mmol) in pyridine (6.5 mL), DMAP (684 mg, 5.60 mmol) and pivaloyl chloride (1.38 mL, 11.2 mmol) was added and the mixture was stirred at 50 °C for 4 h. Aq satd $NaHCO_3$ (10 mL) and EtOAc (10 mL) were added and the two phases were separated. The organic phase was washed with NH_4Cl (10 mL) and brine (10 mL×2), dried over $MgSO_4$, and concentrated in vacuo. Purification of the crude residue by flash silica gel chromatography (EtOAc) afforded the product **44** as pale yellowish oil (363 mg, 80%). R_f=0.77 (1:1 hexanes/AcOEt); $[\alpha]_D$ −12.1 (*c* 1.09 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53–7.47 (m, 1H), 7.35–7.20 (m, 4H), 5.83 (d, 1H, *J*=4.1 Hz), 5.65 (app. t, 1H, *J*=4.6 Hz), 4.44 (br app. t, 1H, *J*=2.7 Hz), 3.87 (dd, 1H, *J*=11.4, *J*=2.7 Hz), 3.43 (s, 3H), 3.32 (ddd, 1H, *J*=11.4, *J*=4.6, *J*=3.2 Hz), 2.22 (app. tt, 1H, *J*=11.9, *J*=4.1 Hz), 1.96–1.85 (m, 1H), 1.85–1.74 (m, 1H), 1.69–1.53 (m, 1H), 1.30 (s, 9H), 1.16–0.99 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 135.3, 131.1, 129.1, 127.2, 95.4, 83.1, 79.6, 72.8, 60.3, 56.7, 42.2, 19.5, 27.4, 25.8, 19.5; HRMS calcd for $C_{20}H_{28}N_3O_4S$ $[M+H]^+$ 406.1801; found 406.1803.

4.1.28. (2*R*, 3*R*, 3a*R*, 6*R*, 7*R*, 7a*S*)-2-(4′-Acetylamino-2′-oxo-2*H*-pyrimidin-1′-yl)-7-azido-octahydro-6-methoxy-benzofuran-3-yl pivalate (45). To a solution of **44** (180 mg, 0.43 mmol), *N*-4-acetyl bis-*O*-TMS cytosine (316 mg, 1.06 mmol) and NIS (238 mg, 1.06 mmol) in CH_2Cl_2 (3.0 mL) at room temperature, TfOH (92 μL, 1.06 mmol) was added portionwise over a period of 20 min. The mixture was stirred at room temperature for 6 h, which was followed by the addition of a saturated solution of $Na_2S_2O_3$ (3 mL) and the two phases were separated. The aqueous phase was extracted with CH_2Cl_2 (6 mL×2), the organic layer was washed with aq satd $NaHCO_3$ (6 mL), and dried with $MgSO_4$. After concentration, the crude product was purified by flash silica gel chromatography (EtOAc/MeOH, 99:1) to afford the product **45** as pale yellowish oil (160 mg, 83%). R_f=0.35 (100:2 AcOEt/MeOH); $[\alpha]_D$ +6.5 (*c* 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.18 (br s, 1H), 8.31 (d, 1H, *J*=7.8 Hz), 7.46 (d, 1H, *J*=7.8 Hz), 5.94 (s, 1H), 5.23 (d, 1H, *J*=4.6 Hz), 4.60 (br app. t, 1H, *J*=2.7 Hz), 3.74 (dd, 1H, *J*=11.9, *J*=2.7 Hz), 3.44 (s, 3H), 3.39 (ddd, 1H, *J*=11.4, *J*=4.6, *J*=2.7 Hz), 2.26 (s, 3H), 2.12–1.99 (m, 1H), 1.99–1.88 (m, 1H), 1.87–1.55 (m, 1H), 1.73–1.55 (m, 1H), 1.23 (s, 9H), 1.04–0.87 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 171.1, 163.0, 154.7, 144.2, 96.6, 91.9, 81.4, 76.6, 67.9, 60.3, 56.8, 38.9, 38.5, 30.3, 27.2, 25.6, 24.8, 19.1; MS (ESI) 449 $[M+H]^+$.

4.1.29. (2*R*, 3*R*, 3a*S*, 6*R*, 7*R*, 7a*S*)-[2-(4′-Amino-2′-oxo-2*H*-pyrimidin-1′-yl)-octahydro-3-hydroxy-6-methoxy-benzofuran-7-yl]urea (46). The azide **45** (100 mg, 0.22 mmol) was dissolved in dry THF (6.6 mL) and argon was bubbled in the solution for 10 min. Water (17 μL) and Me_3P (0.66 mL, 1 M solution in toluene, 0.66 mmol) were added. After 5 min at room temperature, the solution was refluxed for 30 min, then concentrated, and kept in vacuo for 1 h. The residue was then dissolved in dry CH_2Cl_2 (6.6 mL) and trichloroacetylisocyanate (30 μL, 0.25 mmol) was added. After 1 h at room temperature, the solution was concentrated. The oily residue was dissolved in MeOH (6.6 mL), 40% $MeNH_2$ in water (6.6 mL) was added and the solution was stirred for 30 h. Concentration gave a solid, which was purified successively by flash silica gel chromatography (CH_2Cl_2/MeOH/25% aq NH_3, 10:3:0.3) and reverse phase HPLC (LiChrospher 100 RP 18, CH_3CN 0–100% gradient) to give **46** as a colorless crystalline solid (45 mg, 0.0233 mmol, 60%). R_f=0.18 (10:3:0.3 CH_2Cl_2/MeOH/NH_3 aq 25%); mp 278–288 °C (decomposition); $[\alpha]_D$ +8.1 (*c* 0.56 in $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, 1H, *J*=7.8 Hz), 5.83 (d, 1H, *J*=7.8 Hz), 5.62 (s, 1H), 5.94–4.86 (br m, 1H), 4.09 (d, 1H, *J*=4.6 Hz), 3.92 (dd, 1H, *J*=11.0, *J*=3.0 Hz), 3.47–3.40 (m, 1H), 3.37 (s, 3H), 1.95–1.89 (br m, 1H), 1.78–1.71 (m, 1H), 1.63–1.53 (br m, 1H), 1.52–1.38 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.9, 162.5, 158.4, 142.0, 96.0, 95.2, 83.7, 79.1, 76.8, 56.5, 49.5, 40.5, 27.9, 19.5; HRMS calcd for $C_{14}H_{22}N_5O_5$ $[M+H]^+$ 340.1621; found 340.1624.

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Synthesis of five- to seven-membered polyfunctional terpenic carbocycles via Ti(III)-catalyzed radical cyclizations of epoxypolyprenes

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Abstract—Ring size on Ti(III)-mediated radical cyclization of monoepoxypolyprenoids can be controlled by varying the substitution pattern and/or the electronic distribution of the double bond involved in the ring closure. The feasibility of applying this idea to tandem cyclizations has also been proven. Besides, when a silyloxy function is located in α position to the oxirane ring of the acyclic polyprene, the cyclization leads to carbocyclic terpenoids doubly functionalized in the A ring with acceptable yields. These results widen significantly the scope of this methodology.

1. Introduction

The bis(cyclopentadienyl)titanium(III) chloride-mediated radical cyclization of suitable unsaturated epoxides, first reported by RajanBabu and Nugent using Ti(III) in equimolecular quantities,[1] and then by Gansauer in its catalytic version,[2] has recently proved to be an efficient tool in the synthesis of natural products. In this context, Barrero et al. developed a new biomimetic strategy for the synthesis of cyclic terpenoids containing six- and seven-membered rings, based on the radical opening of acyclic epoxypolyprenes.[3] This and other cyclization strategies have also been applied to numerous cyclic terpenic structures, either by using stoichiometric or catalytic protocols.[4] Most of the above-mentioned synthetic efforts have led to the formation of terpenic structures with six-membered rings, although Fernández-Mateos et al. have achieved cyclizations leading to rings containing three to seven members.[5] The substrates used by these latter authors were mostly monocyclic terpenic compounds with carbonyls, nitriles, and α,β-unsaturated carbonyls as radical acceptors, although structurally different from the natural acyclic polyprenes.

Considering the synthesis in nature of polycyclic isoprenoids, it is thought that the specific cyclizations occur in a four-step-sequence, the first step being the generation of a carbocation.[6] However, two radical-mediated synthetic strategies, namely, the aforementioned Ti(III)-based opening of epoxypolyprenes, and the photoinduced cascade cyclizations of polyalkenes via radical cationic intermediates,[7] proved to induce a number of impressive biomimetic polycyclizations. Furthermore, these radical protocols permitted not only to improve the results obtained in certain cationic processes, but also to induce some transformations, which could not be achieved via cationic intermediates.[8] Both methods could be considered as illustrations of the 'minimal enzymatic assistance' theory.[9]

2. Results and discussion

We describe here our results using mono- and tandem cyclization processes carried out with acyclic epoxypolyprenes possessing α,β-unsaturated esters as radical traps (Fig. 1a). Previous reports using α,β-unsaturated esters as radical acceptors in intermolecular processes let anticipate good yields in this kind of cyclizations.[10] A point of special interest in this type of cyclizations lies in the regioselectivity of the process: 5-*exo*-trig versus 6-*endo*-trig ring closures.

Furthermore, the likelihood of obtaining polyfunctionalized structures with five- to seven-membered rings prompted us also to test the influence of oxygenated functions in α position to the oxirane ring not only regarding the efficiency of the cyclization process, but concerning the stereochemical

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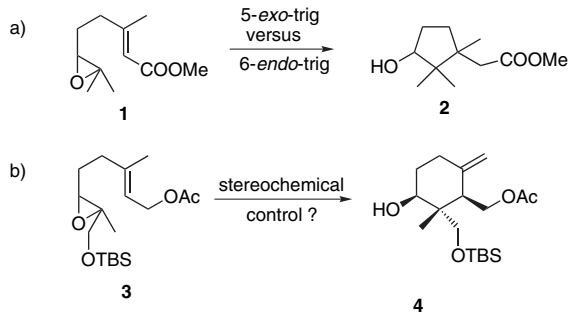
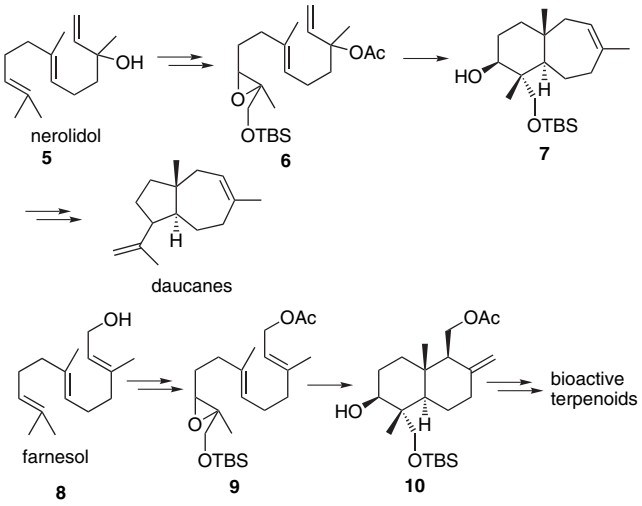


Figure 1.

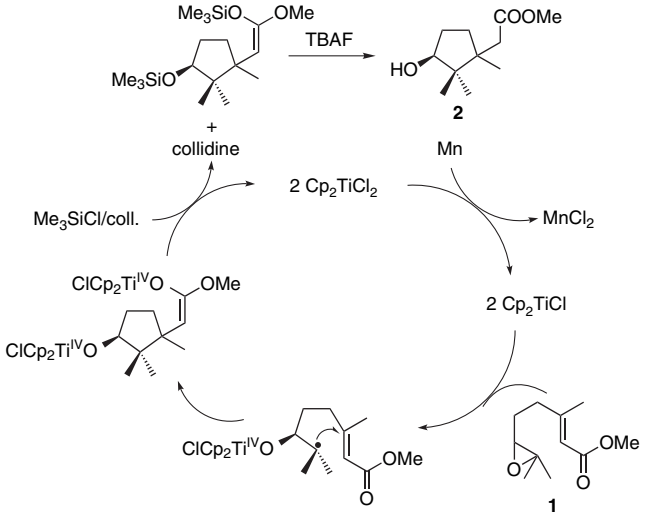
outcome of the process, since three or more stereogenic centers are created (Fig. 1b). These functionalizations can be incorporated using Sharpless asymmetric epoxidation protocol, which increases the interest of this study. Thus, efficient regio- and stereoselectively controlled cyclizations of suitable substrates such as **6** and **9**, easily obtained from commercially available farnesol and natural nerolidol,[11] would facilitate the enantioselective access to polyfunctionalized structures such as **7** and **10** (Scheme 1).



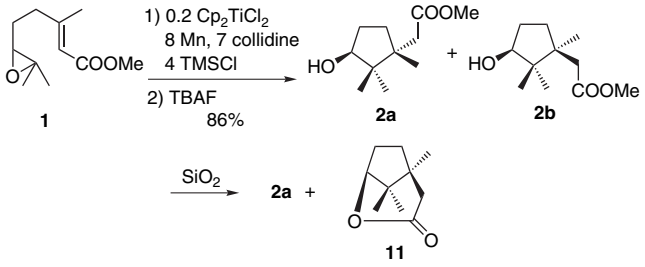
Scheme 1.

These bicyclic compounds might be advanced intermediates in the synthesis of compounds of interest. Thus, **7** could be used as a precursor of daucanes with an isopropenyl group,[12] whilst **10** could lead to functionalized drimanes (whose main skeleton is already contained in **10**) and advanced synthons for the preparation of A and B rings of bioactive terpenoids, including triterpenic structures.[13]

We began by reacting methyl 6,7-epoxygeraniate (prepared from commercial geraniol) with catalytic Cp_2TiCl_2[14] to afford, after 10 min and subsequent trimethylsilylether deprotection, an 86% yield of 5-*exo*-trig cyclization products **2a** and **2b**. The complete catalytic cycle for this transformation is shown in Scheme 2. These compounds could be easily separated since **2b** is quantitatively transformed into **11** by spontaneous lactonization on silica gel or by treatment with *p*TsOH in DCM (Scheme 3). Two features of these reactions are particularly noteworthy: firstly, the regioselectivity of the cyclization towards 5-*exo* products, caused by the substitution pattern and the electronic distribution of the α,β-unsaturated ester, and secondly, the remarkable increase in yield (approx. 25%) as compared to that obtained when a six-membered ring is created.[4i]

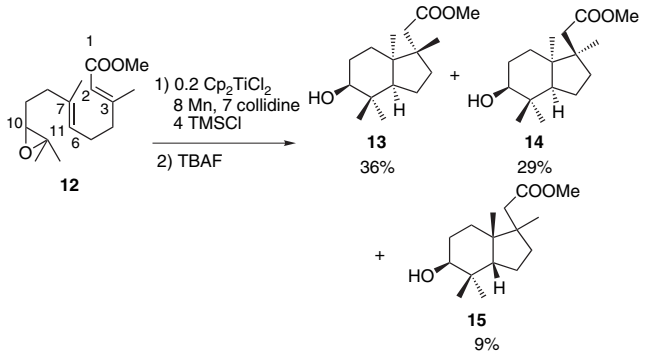


Scheme 2.



Scheme 3.

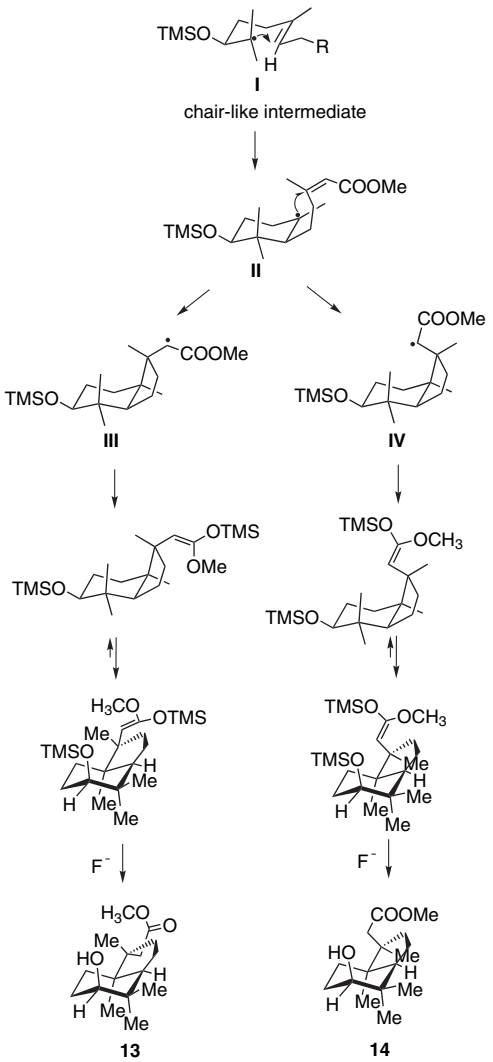
This gratifying result encouraged us to try the corresponding tandem process starting from methyl 10,11-epoxyfarnesoate, **12**, obtained from commercial farnesol. The exposure of **12** to the same experimental conditions used for **1** led to a 75% yield of compounds **13**–**15**, as a result of a tandem 6-*endo*-trig and 5-*exo*-trig cyclization (Scheme 4). As happened with **1**, the presence of an α,β-unsaturated ester caused a change in the regioselectivity on the closing of the second cycle. The yield also increased considerably



Scheme 4.

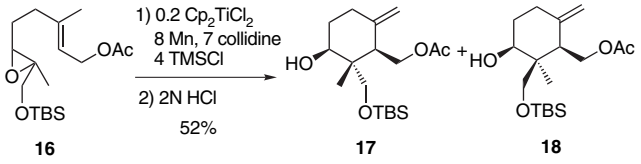
(approx. 20%) compared to that obtained with 10,11-epoxyfarnesyl acetate, which gave a double 6-*endo* tandem cyclization.[4i] Apart from this we also observed a pronounced stereoselectivity towards cis interannular junction anti to the hydroxyl group (7:1).

We postulate the mechanism shown in Scheme 2 to account for the regio- and stereochemical outcome of this reaction. An epoxide reductive opening originates radical **I**, the stable chair-like conformation of this intermediate determining the stereoselectivity of the first 6-*endo* cyclization.[3] The monocyclic radical thus generated is trapped by the conjugated double bond via a 5-*exo*-trig process, the regioselectivity of which is now determined by the electronic distribution of this olefin.[15] With respect to the stereochemistry of the interannular junction, proven to be cis,[16] this must be determined by the size of the new ring created. No stereoselectivity was observed at C-8, on the other hand, since there appears to be no preference for the ensuing attack of the carbon-centered radical against the double bond Δ^2. Finally, a conformational change leads to the most stable conformation, as depicted in Scheme 4. MM2 theoretical calculations confirm the most stable conformation proposed for compounds **13** and **14** (Scheme 5).[17]



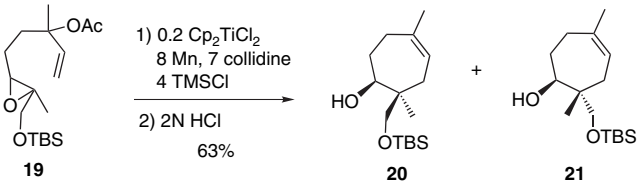
Scheme 5.

To test the influence of an oxygenated function in the α position upon the oxirane ring, which initiates the radical cyclization, epoxide **16** was prepared from geraniol. Thus the Ti(III)-induced homolytic opening of the oxirane ring and subsequent radical cyclization afforded the 6-*endo*-trig products **17** and **18** in relative proportions of 7:1, favoring the isomer possessing the equatorially oriented CH_2OTBS group, together with minor quantities of *endo*-isomers (Scheme 6). This result agrees with those reported previously by Takahashi et al.,[18] who proposed the existence of an association between the CH_2OTBS group and the titanoxy group at C-3 to account for the stereochemical control of the process.



Scheme 6.

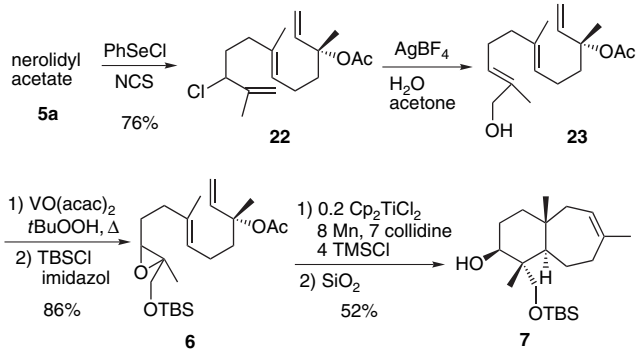
When we carried out this reaction with the corresponding epoxy derivative of linalyl acetate, 7-*endo* cyclization products **20** and **21** were formed in a 63% yield (Scheme 7).[19]



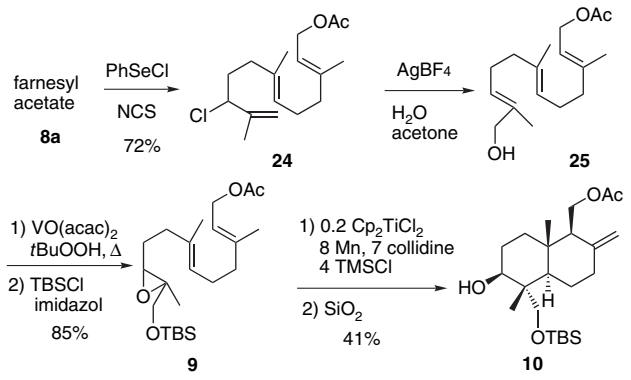
Scheme 7.

Nevertheless, no stereoselectivity was observed in this case, which may be put down to the higher conformational mobility of the seven-membered ring.

Bearing these results in mind, it would be interesting to test the stereochemical outcome of the tandem cyclizations of the α-oxygenated derivatives of farnesol and nerolidol (Schemes 8 and 9).



Scheme 8.



Scheme 9.

Allylic oxidation at the C-12 position of nerolidyl acetate was achieved via allylic chlorination with catalytic PhSeCl,[20] and a noteworthy yield of 75% of the desired chloro derivative **22** was obtained, which was then transformed into the primary alcohol **23** (40%) by treatment with $AgBF_4$.[21] Hydroxyl-directed epoxidation using Sharpless conditions[22] and protection with TBSCl let us to obtain the key intermediate **6** in 86% yield. Exposure of **6** to catalytic Ti(III) led, after silica gel separation, to the isolation of a noticeable 65% yield of bicyclic compound **7**. Regarding the stereochemical outcome of the reaction, it is worth mentioning that only one stereoisomer could be detected in this radical process where up to four contiguous stereogenic centers are created.

Following a synthetic route parallel to that used with nerolidyl acetate farnesyl acetate was converted to the desired 6+6 bicyclic sesquiterpenoid **10**. Again the reaction took place with high diastereoselectivity, and only isomer **10** could be isolated in a 41% yield, although in this case the formation of minor quantities of other stereoisomers was detected.

In conclusion, with the present work we have shown that, in radical cyclizations of epoxypolyprenes catalyzed by Cp_2TiCl, the employment of α,β-unsaturated methyl esters as radical acceptors provokes 5-*exo*-trig ring closures. A significant increase of the reaction yield (20–25%) was also observed in these cyclizations, as compared to the yield obtained when a six-membered ring is created. Furthermore, in the cases where a silyloxy function is located in the α position to the oxirane ring, the cyclizations lead to carbocyclic terpenoids doubly functionalized in the A ring with acceptable yields. Besides, a good stereoselectivity is observed in most cases, the major stereoisomers presenting the CH_2OTBS group disposed equatorially. Thus, this methodology permits an easy access to advanced intermediates in the synthesis of both polyfunctionalized terpenoids and terpenoids possessing unusual skeletons.

3. Experimental

3.1. General methods

All air- and water-sensitive reactions were performed in flasks flame-dried under a positive flow of argon and conducted under an atmosphere of argon. Tetrahydrofuran (THF) was freshly distilled immediately prior to use from sodium/benzophenone and strictly deoxygenated for 30 min under argon for each of the Cp_2TiCl_2/Mn reactions. Reagents were purchased at the higher commercial quality and used without further purification, unless otherwise stated. Silica gel SDS 60 (35–70 μm) was used for flash column chromatography. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as the visualizing agent and a solution of phosphomolybdic acid in ethanol and heat as developing agent. IR spectra were recorded with a Matson model Satellite FTIR instrument as NaCl plates (films). NMR studies were performed with a Bruker ARX 400 (^{1}H 400 MHz/^{13}C 100 MHz) spectrometer. The accurate mass determination was carried out with an AutoSpec-Q mass spectrometer arranged in an EBE geometry (Micromass Instrument, Manchester, UK) and equipped with a FAB (LSIMS) source.

3.1.1. General procedure for catalytic Ti(III)-mediated cyclization of epoxides. A mixture of Cp_2TiCl_2 (62 mg, 0.24 mmol) and Mn dust (532 mg, 9.68 mmol) in strictly deoxygenated THF (7 mL) was stirred at room temperature until the red solution turned green. Then, a solution of the corresponding oxirane (1.20 mmol), 2,4,6-collidine (1.1 mL, 8.33 mmol), and TMSCl (0.6 mL, 4.76 mmol) in strictly deoxygenated THF (3 mL) was added to the solution of Cp_2TiCl. The reaction mixture was stirred until dispartition of the starting material (20 min–3 h), quenched with 2 N HCl, extracted with *t*-BuOMe, washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure.

3.2. Cylization of epoxypolyprenes possessing α,β-unsaturated methyl esters as radical acceptors

3.2.1. Cyclization of 1. After subjecting commercially available **1** (150 mg, 0.76 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography (hexane/*t*-BuOMe, 4:1) on silica gel to afford 130 mg (86%) of an equimolecular mixture of **2a** and **2b**. A solution of this mixture (100 mg, 0.59 mmol) in Et_2O was treated with an excess of *p*TsOH for 26 h at room temperature. The reaction mixture was then washed with saturated $NaHCO_3$ and brine. Removal of the solvent afforded a crude residue, which was purified by flash chromatography (hexane/*t*-BuOMe, 3:1) to give 16 mg of **11**, 25 mg of **2a**, and 52 mg of a mixture of these two compounds. Compound **2a**, colorless oil. IR (film) ν: 3464, 2957, 2878, 1735, 1462, 1438, 1326, 1205, 1014 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.87 (s, 3H), 1.05 (s, 3H), 1.45–2.25 (m, 4H), 2.19 (s, 3H), 3.64 (s, 3H), 3.88 (dd, J=8.2, 5.2 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.4, 21.7, 22.7, 31.0, 34.3, 42.3, 44.8, 47.3, 51.3, 81.4, 173.6 ppm. HRFABMS calcd for $C_{11}HO_3Na$ [M+Na]$^+$ 223.1310, found 223.1317. Compound **11**, colorless oil. IR (film) ν: 2960, 2878, 1736, 1468, 1370, 1211, 1040 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H), 0.86 (s, 3H), 0.95 (s, 3H), 1.63–1.88 (m, 2H), 1.89–2.11 (m, 2H), 2.30 (d, J=18.8 Hz, 1H), 2.48 (dd, J=18.8, 3.2 Hz, 1H), 4.16 (d, J=4.5 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.8, 19.2, 20.5, 29.7, 36.1, 41.9,

43.6, 44.9, 89.1, 171.5 ppm. HRFABMS calcd for $C_{10}H_{16}O_2Na$ $[M+Na]^+$ 191.1048, found 191.1060.

3.2.2. Cyclization of 12. After subjecting **12**[23] (300 mg, 1.13 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography (hexane/*t*-BuOMe, 4:1) on silica gel to afford 109 mg (36%) of **13**, 88 mg (29%) of **14**, and 27 mg (9%) of **15**. Compound **13**, colorless oil. IR (film) ν: 3515, 2950, 2879, 1731, 1470, 1439, 1212, 1124, 1013 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 3H), 0.92 (s, 3H), 0.93 (s, 3H), 1.00 (s, 3H), 1.50–1.98 (m, 10H), 2.11 (d, J=12.8 Hz, 1H), 2.22 (d, J=12.9 Hz, 1H), 3.46 (t, J=2.8 Hz, 1H), 3.61 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) ™18.8, 20.8, 23.4, 24.9, 26.3, 26.5, 29.6, 31.8, 36.3, 40.7, 45.2, 49.1, 51.3, 51.8, 75.1, 174.1 ppm. HRFABMS calcd for $C_{16}H_{28}O_3Na$ $[M+Na]^+$ 291.1936, found 291.1943. Compound **14**, colorless oil. IR (film) ν: 3514, 2949, 2879, 1731, 1471, 1439, 1212, 1001 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 1.01 (s, 3H), 1.35–2.18 (m, 10H), 2.16 (d, J=12.8 Hz, 1H), 2.22 (d, J=12.8 Hz, 1H), 3.47 (t, J=2.8 Hz, 1H), 3.62 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 21.6, 23.2, 25.0, 26.4, 26.6, 29.7, 34.5, 36.2, 40.9, 45.0, 47.9, 51.3, 51.4, 75.1, 174.2 ppm. HRFABMS calcd for $C_{16}H_{28}O_3Na$ $[M+Na]^+$ 291.1936, found 291.1935. Compound **15**, colorless oil. IR (film) ν: 3515, 2949, 2878, 1731, 1471, 1438, 1321, 1212, 1124, 1001 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 3H), 0.93 (s, 3H), 0.95 (s, 3H), 0.98 (s, 3H), 1.15–1.90 (m, 10H), 2.13 (d, J=13.0 Hz, 1H), 2.16 (d, J=13.0 Hz, 1H), 3.58 (dd, J=5.7, 10.5 Hz, 1H), 3.61 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 22.2, 22.3, 24.0, 27.3, 28.0, 29.9, 34.2, 37.1, 40.8, 45.2, 47.8, 51.3, 54.6, 74.1, 174.0 ppm. HRFABMS calcd for $C_{16}H_{28}O_3Na$ $[M+Na]^+$ 291.1936, found 291.1937.

3.3. Monocyclizations of epoxypolyprenes possessing a CH_2OTBS group in the α position to the oxirane ring

3.3.1. Synthesis of 16. To a solution of 229 mg (1.03 mmol) of the primary alcohol in DCM (7 mL) resulting from subjecting geranyl acetate to the SeO_2/*t*-BuOOH system at 0 °C was added *m*CPBA (557 mg, 2.26 mmol) in 7 mL of DCM. The reaction mixture was stirred under argon at this temperature for 1 h 15 min. Then, the mixture was diluted with DCM, washed with 1 N NaOH, and brine. The solvent was removed to afford a crude product, which was used in the next reaction. To a solution of the above crude in 33 mL of DCM at 0 °C and under argon was added imidazole (334 mg, 4.91 mmol) and TBSCl (570 mg, 3.78 mmol). The reaction was stirred at room temperature for 1 h and then diluted with *t*-BuOMe and washed with H_2O, 2 N HCl, saturated $NaHCO_3$, and brine and worked up as usual. The product obtained was purified by chromatography on silica gel (hexane/*t*-BuOMe, 4:1) to give 239 mg (68% overall yield) of **16** as a colorless oil. IR (film) ν: 2955, 2930, 2857, 1741, 1472, 1366, 1233, 1097, 838, 778 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.27 (s, 3H), 1.69 (q, J=7.6 Hz, 2H), 1.73 (s, 3H), 2.06 (s, 3H), 2.19 (m, 2H), 2.86 (t, J=6.2 Hz, 1H), 3.58 (s, 2H), 4.59 (d, J=7.1 Hz, 2H), 5.40 (t, J=7.1 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.4, 14.2, 16.5, 18.3, 21.0, 25.9, 26.6, 36.2, 60.4, 61.0, 61.2, 67.7, 118.9, 141.2, 171.1 ppm. HRFABMS calcd for $C_{18}H_{34}O_4SiNa$ $[M+Na]^+$ 365.2124, found 365.2105.

3.3.2. Cyclization of 16. After subjecting **16** (260 mg, 0.76 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography on silica gel. Eluting with hexane/*t*-BuOMe (4:1) afforded **17** and **18**, 135 mg (52%) in a 7:1 ratio. This mixture was re-subjected to column chromatography on $AgNO_3$ (20%)–silica gel to isolate **17** in a pure state as colorless oil. IR (film) ν: 3491, 2954, 2929, 2857, 1741, 1463, 1252, 1091, 838 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.90 (s, 9H), 1.19 (s, 3H), 1.70 (q, J=7.6 Hz, 2H), 2.05 (s, 3H), 2.20 (br s, 1H), 3.53 (d, J=9.9 Hz, 1H), 3.71 (d, J=9.9 Hz, 1H), 3.83 (dd, J=8.5, 5.6 Hz, 1H), 4.15 (dd, J=11.9, 4.3 Hz, 1H), 4.29 (dd, J=11.9, 4.3 Hz, 1H), 5.41 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.5, 12.1, 18.3, 21.2, 22.0, 25.8, 30.8, 41.4, 43.1, 62.9, 69.4, 71.6, 121.3, 132.3, 170.9 ppm. HRFABMS calcd for $C_{18}H_{34}O_4SiNa$ $[M+Na]^+$ 365.2124, found 365.2101. Compound **18** was characterized from a mixture of this compound with **17**. ^1H NMR (400 MHz, $CDCl_3$) δ (only distinctive signals) 1.02 (s, 3H), 4.41 (s, 1H), 4.85 (s, 1H) ppm.

3.3.3. Synthesis of 19. This compound was prepared by the same procedure used for **16** with an overall yield of 66%. Compound **19**, colorless oil. IR (film) ν: 2955, 2930, 2857, 1740, 1472, 1368, 1251, 1098, 838, 778 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.90 (s, 9H), 1.25 (s, 3H), 1.54 (s, 3H), 1.40–1.95 (m, 4H), 1.97 (s, 3H), 2.83 (t, J=6.2 Hz, 1H), 3.56 (s, 2H), 5.08 (d, J=11.0 Hz, 1H), 5.14 (d, J=17.3 Hz, 1H), 5.92 (dd, J=17.3, 11.0 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, 14.1, 16.5, 18.4, 23.0, 23.7, 25.9, 26.4, 36.5, 60.6, 67.8, 82.4, 113.6, 141.6, 170.0 ppm. HRFABMS calcd for $C_{18}H_{34}O_4SiNa$ $[M+Na]^+$ 365.2124, found 365.2111.

3.3.4. Cyclization of 19. After subjecting **19** (213 mg, 0.62 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography on silica gel. Eluting with hexane/*t*-BuOMe (6:1) afforded 111 mg (63%) of **20** and **21** in an equimolecular ratio. This mixture was re-subjected to column chromatography on $AgNO_3$ (20%)–silica gel to isolate **21** in a pure state as a colorless oil. IR (film) ν: 3487, 3437, 2956, 2930, 2857, 1471, 1255, 1086, 1068, 836, 777 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.86 (s, 3H), 0.88 (s, 9H), 1.35 (q, J=13.0 Hz, 1H), 1.45 (dd, J=8.5, 14.1 Hz, 1H), 1.66 (m, 1H), 1.71 (s, 3H), 1.76 (m, 1H), 1.89 (dd, J=6.9, 14.7 Hz, 1H), 2.12 (t, J=13.1 Hz, 1H), 3.35 (d, J=9.6 Hz, 1H), 3.55 (d, J=9.6 Hz, 1H), 3.69 (dd, J=3.9, 10.9 Hz, 1H), 4.35 (s, 1H), 5.26 (t, J=5.1 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.6, −5.5, 14.0, 18.1, 25.3, 25.9, 29.0, 29.4, 32.4, 40.1, 75.9, 81.8, 120.1, 141.3 ppm. HRFABMS calcd for $C_{16}H_{32}O_2SiNa$ $[M+Na]^+$ 307.2069, found 307.2059. Compound **20** was characterized from a mixture of this compound with **21**. ^1H NMR (400 MHz, $CDCl_3$) δ (only distinctive signals) 0.89 (s, 3H), 0.91 (s, 3H), 1.58 (m, 1H), 1.63 (m, 1H), 1.73 (s, 3H), 1.76 (m, 1H), 1.86 (m, 1H), 2.36 (m, 1H), 3.42 (d, J=9.6 Hz, 1H), 3.57 (m, 1H), 3.67 (d, J=9.6 Hz, 1H), 4.16 (s, 1H) ppm.

3.4. Biscyclizations of epoxypolyprenes possessing a CH_2OTBS group in the α position to the oxirane ring

3.4.1. Synthesis of the 6+7 bicyclic structure 7.

3.4.1.1. Chlorination of nerolidyl acetate. To a stirred solution of PhSeCl (222 mg, 1.14 mmol) in DCM (75 mL) was added under argon and at room temperature 2500 mg of nerolidyl acetate (9.47 mmol). The mixture was further stirred for 5 min and then, NCS (1420 mg, 10.42 mmol) was added. The resulting mixture was stirred for 50 min and then, most of the DCM was evaporated resulting in the formation of a white solid. Et_2O was added to the above mixture and the liquid was separated from the white solid by decantation. This operation was repeated three times. The combined organic extracts were washed with brine. Evaporation of the solvent followed by column chromatography (hexane/*t*-BuOMe, 20:1) afforded 2144 mg of **22** (76%) as a colorless oil. IR (film) *ν*: 3487, 3437, 2956, 2930, 2857, 1471, 1255, 1086, 1068, 836, 777 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.47 (s, 3H), 1.52 (s, 3H), 1.60–2.05 (m, 8H), 1.74 (s, 3H), 1.94 (s, 3H), 4.26 (t, *J*=6.8 Hz, 1H), 4.82 (m, 1H), 4.93 (br s, 1H), 5.05 (d, *J*=10.9 Hz, 1H), 5.08 (d, *J*=17.5 Hz, 1H), 5.08 (m, 1H), 5.90 (dd, *J*=17.5, 10.9 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 15.9, 17.1, 22.2, 22.3, 23.7, 34.9, 36.6, 39.7, 66.3, 82.9, 113.2, 114.2, 124.9, 133.9, 141.8, 144.4, 169.9 ppm.

3.4.1.2. Reaction of 22 with $AgBF_4$ in acetone/H_2O. To a solution of **22** (1337 mg, 4.49 mmol) collidine (2.4 mL, 17.95 mmol) in acetone/H_2O (120 mL, 1:1) under argon was added $AgBF_4$ (2623 mg, 13.47 mmol). The mixture was stirred under reflux for 1 h. Then acetone was removed under reduced pressure, and the resulting mixture was extracted with EtOAc. The combined organic extracts were washed with 1 N HCl and brine and worked up as usual. The residue was purified by column chromatography. Eluting with hexane/*t*-BuOMe, 2:1, afforded 502 mg of **23** (40%). Eluting with hexane/*t*-BuOMe, 4:1, gave 565 mg (45%) of the isomer possessing a secondary alcohol. Compound **23**, colorless oil. IR (film) *ν*: 3425, 2973, 2920, 2859, 1737, 1646, 1449, 1370, 1250, 1018, 923, 842 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.47 (s, 3H), 1.52 (s, 3H), 1.59 (s, 3H), 1.65–2.10 (m, 8H), 1.94 (s, 3H), 3.92 (br s, 2H), 4.93 (br s, 1H), 5.04 (m, 1H), 5.05 (d, *J*=10.9 Hz, 1H), 5.08 (d, *J*=17.5 Hz, 1H), 5.31 (br t, *J*=8.3 Hz, 1H), 5.90 (dd, *J*=17.5, 10.9 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 13.7, 15.9, 22.2, 22.3, 23.7, 26.2, 39.3, 39.7, 69.0, 83.0, 113.2, 124.0, 126.0, 134.9, 135.1, 141.9, 170.0 ppm. HRFABMS calcd for $C_{17}H_{28}O_3Na$ $[M+Na]^+$ 303.1936, found 303.1901.

3.4.1.3. Synthesis of 6. A mixture of allylic alcohol **23** (350 mg, 1.25 mmol) and $VO(acac)_2$ (10 mg) in benzene (40 mL) was refluxed for 10 min under argon. Addition of *t*-BuOOH in decane (0.3 mL, 2 mmol) followed, and stirring continued at this temperature for 20 min. After cooling, the mixture was diluted with EtOAc, washed with saturated $NaHCO_3$, and brine, worked up as usual, and chromatographed on silica gel (hexane/*t*-BuOMe, 1:1) to give 335 mg of the desired epoxide. To a solution of the above epoxide in 11 mL of DCM at 0 °C and under argon was added imidazole (177 mg, 2.6 mmol) and TBSCl (285 mg, 1.89 mmol). The reaction was stirred at room temperature for 1 h and then diluted with *t*-BuOMe and washed with H_2O, 2 N HCl, saturated $NaHCO_3$, and brine, and worked up as usual. The product obtained was purified by chromatography on silica gel (hexane/*t*-BuOMe, 4:1) to give 440 mg (86% overall yield) of **6** as a colorless oil. IR (film) *ν*: 2956, 2930, 2857, 1739, 1462, 1368, 1250, 1097 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.05 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 1.27 (s, 3H), 1.55 (s, 3H), 1.61 (s, 3H), 1.65–2.20 (m, 8H), 2.01 (s, 3H), 2.84 (t, *J*=6.3 Hz, 1H), 3.57 (s, 2H), 5.13 (d, *J*=10.9 Hz, 1H), 5.15 (t, 1H), 5.15 (d, *J*=17.5 Hz, 1H), 5.97 (dd, *J*=17.5, 10.9 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* −5.3, 14.2, 15.9, 18.3, 22.3, 23.6, 25.9, 26.9, 28.6, 36.3, 39.7, 60.6, 61.0, 67.8, 82.8, 113.1, 124.3, 134.5, 141.8, 169.9 ppm. HRFABMS calcd for $C_{23}H_{42}O_4SiNa$ $[M+Na]^+$ 433.2750, found 433.2751.

3.4.1.4. Cyclization of 6. After subjecting **6** (244 mg, 0.59 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography (hexane/*t*-BuOMe, 8:1) on silica gel to afford 109 mg (52%) of **7** as a colorless oil. IR (film) *ν*: 3450, 2953, 2928, 2857, 1739, 1640, 1462, 1384, 1252, 1093, 837, 776 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.08 (s, 6H), 0.81 (s, 3H), 0.86 (s, 3H), 0.91 (s, 9H), 1.21–2.10 (m, 11H), 1.73 (s, 3H), 3.36 (d, *J*=9.5 Hz, 1H), 3.47 (br s, 1H), 3.62 (dd, *J*=10.3, 5.8 Hz, 1H), 3.71 (d, *J*=9.5 Hz, 1H), 5.35 (m, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* −5.6, 11.3, 19.6, 21.9, 25.4, 25.9, 26.9, 34.6, 35.3, 40.5, 43.0, 45.9, 53.5, 72.3, 76.3, 113.2, 122.6, 141.0 ppm. HRFABMS calcd for $C_{21}H_{40}O_2SiNa$ $[M+Na]^+$ 375.2695, found 375.2690.

3.4.2. Synthesis of the 6+6 bicyclic structure 10.

3.4.2.1. Chlorination of farnesyl acetate. To a stirred solution of PhSeCl (222 mg, 1.14 mmol) in DCM (75 mL) was added under argon and at room temperature 2500 mg of nerolidyl acetate (9.47 mmol). The mixture was further stirred for 5 min and then, NCS (1420 mg, 10.42 mmol) was added. The resulting mixture was stirred for 50 min and then, most of the DCM was evaporated resulting in the formation of a white solid. Et_2O was added to the above mixture and the liquid was separated from the white solid by decantation. This operation was repeated three times. The combined organic extracts were washed with brine. Evaporation of the solvent followed by column chromatography (hexane/*t*-BuOMe, 20:1) afforded 2032 mg of **24** (72%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.60 (s, 3H), 1.71 (s, 3H), 1.81 (s, 3H), 1.75–2.20 (m, 8H), 2.06 (s, 3H), 4.34 (t, *J*=6.9 Hz, 1H), 4.59 (d, *J*=7.1 Hz, 2H), 4.90 (s, 1H), 5.0 (s, 1H), 5.14 (t, *J*=6.5 Hz, 1H), 5.35 (t, *J*=7.2 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 16.0, 16.5, 17.1, 21.1, 26.2, 34.8, 36.7, 39.5, 61.4, 66.4, 114.3, 118.5, 124.9, 133.9, 142.1, 144.4, 171.2 ppm.

3.4.2.2. Reaction of 24 with $AgBF_4$ in acetone/H_2O. To a solution of **24** (1070 mg, 3.59 mmol) collidine (1.9 mL, 14.36 mmol) in acetone/H_2O (96 mL, 1:1) under argon was added $AgBF_4$ (2098 mg, 10.78 mmol). The mixture was stirred under reflux for 1 h. The acetone was removed under reduced pressure, and the resulting mixture was extracted with EtOAc. The combined organic extracts were washed with 1 N HCl and brine and worked up as usual. The residue was purified by column chromatography. Eluting with hexane/*t*-BuOMe, 2:1, afforded 402 mg of **25**

(40%). Eluting with hexane/*t*-BuOMe, 4:1, gave 452 mg (45%) of the isomer possessing a secondary alcohol. Compound **25**, IR (film) *ν*: 3425, 2973, 2920, 2859, 1737, 1646, 1449, 1370, 1250, 1018, 923, 842 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 3H), 1.66 (s, 3H), 1.70 (s, 3H), 1.95–2.25 (m, 9H), 2.05 (s, 3H), 3.99 (br s, 2H), 4.60 (d, J=7.1 Hz, 2H), 5.10 (t, J=6.7 Hz, 1H), 5.34 (t, J=7.1 Hz, 1H), 5.38 (t, J=7.0 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 15.8, 16.2, 20.8, 26.0, 26.1, 39.1, 39.3, 61.2, 68.4, 118.2, 123.7, 125.4, 134.7, 134.9, 141.9, 171.0 ppm. HRFABMS calcd for $C_{17}H_{28}O_3Na$ $[M+Na]^+$ 303.1936, found 303.1903.

3.4.2.3. Synthesis of 9. A mixture of allylic alcohol **25** (245 mg, 0.87 mmol), $VO(acac)_2$ (8 mg) in benzene (33 mL) was refluxed for 10 min under argon. Addition of *t*-BuOOH in decane (0.2 mL, 1.40 mmol) followed and stirring continued at this temperature for 20 min. After cooling, the mixture was diluted with EtOAc, washed with saturated $NaHCO_3$, and brine, worked up as usual, and chromatographed on silica gel (hexane/*t*-BuOMe, 1:1) to give 234 mg of the desired epoxide. To a solution of the above epoxide in 8 mL of DCM at 0 °C and under argon was added imidazole (124 mg, 1.82 mmol) and TBSCl (199 mg, 1.32 mmol). The reaction mixture was stirred at room temperature for 1 h and then diluted with *t*-BuOMe and washed with H_2O, 2 N HCl, saturated $NaHCO_3$, and brine, and worked up as usual. The product obtained was purified by column chromatography on silica gel (hexane/*t*-BuOMe, 4:1) to give 303 mg (85% overall yield) of **9** as a colorless oil. IR (film) *ν*: 2955, 2930, 2857, 1741, 1472, 1382, 1232, 1097, 838, 778 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ −0.01 (s, 3H), 0.00 (s, 3H), 0.84 (s, 9H), 1.21 (s, 3H), 1.56 (s, 3H), 1.50–1.60 (m, 2H), 1.64 (s, 3H), 1.95–2.15 (m, 6H), 1.99 (s, 3H), 2.78 (t, J=6.3 Hz, 1H), 3.51 (s, 2H), 4.53 (d, J=7.1 Hz, 2H), 5.09 (t, J=6.7 Hz, 1H), 5.27 (t, J=7.1 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, 14.2, 14.2, 16.1, 16.5, 18.4, 21.1, 25.9, 26.2, 27.1, 36.4, 39.5, 60.8, 61.5, 68.0, 118.4, 124.3, 134.5, 142.1, 170.9 ppm. HRFABMS calcd for $C_{23}H_{42}O_4SiNa$ $[M+Na]^+$ 433.2750, found 433.2740.

3.4.2.4. Cyclization of 9. After subjecting **9** (233 mg, 0.57 mmol) to the catalytic procedure conditions, the resulting crude was purified by column chromatography (hexane/*t*-BuOMe, 2:1) on silica gel to afford 96 mg (41%) of **10** as a colorless oil. IR (film) *ν*: 3493, 2931, 2857, 1740, 1471, 1386, 1366, 1252, 1092, 1036, 853, 776 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.80 (s, 3H), 0.85 (s, 3H), 0.91 (s, 9H), 1.21–1.80 (m, 10H), 2.02 (s, 3H), 2.39 (dt, J=13.1, 3.3 Hz, 1H), 3.35 (d, J=9.6 Hz, 1H), 3.64 (d, J=9.2, 5.8 Hz, 1H), 3.66 (m, 1H), 4.19 (dd, J=11.2, 8.6 Hz, 1H), 4.31 (dd, J=11.2, 3.8 Hz, 1H), 4.54 (br s, 1H), 4.86 (br s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.6, −5.5, 11.8, 15.5, 18.2, 21.2, 23.7, 25.9, 26.7, 36.7, 37.3, 38.7, 42.2, 48.9, 54.6, 61.4, 72.6, 76.0, 107.8, 146.0, 170.2 ppm. HRFABMS calcd for $C_{23}H_{42}O_4Si$ $[M+H]^+$ 411.2931, found 411.2925.

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A unified synthetic strategy toward oroidin-derived alkaloids premised on a biosynthetic proposal☆

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Abstract—Details of the evolution of a synthetic strategy toward the spirocyclic chlorocyclopentane core of oroidin-derived alkaloids, including the axinellamines and potentially adaptable to palau'amine, are described. A proposed refinement of the Kinnel–Scheuer biosynthetic proposal for palau'amine is posited. Studies undertaken to improve the regioselectivity and efficiency of a key Diels–Alder reaction utilizing a novel protecting group strategy, microwave chemistry, and other strategies are described. Further insights regarding the suitability of different protecting groups during the epoxidation/chlorination/ring contraction sequence are disclosed. Several interesting by-products from this reaction sequence are reported. These studies have led to a unified synthetic strategy to the axinellamines and palau'amine.

1. Introduction

Organisms produce a wide diversity of natural products, and while a common view is that they are produced as a defense mechanism against predators, the complexity of these structures, their ability to potently interact with mammalian proteins, and the metabolic mechanisms and processes that are known to produce them, raise a number of questions that may only be answered by a more holistic approach to natural products research. One such intriguing hypothesis named the 'screening hypothesis' seeks to overcome the inherent shortcomings of traditional evolutionary thought regarding the incredible diversity of metabolites produced by a given organism.[1]

While classical synthetic routes toward these complex and unusual architectures are possible, the convergent and efficient construction of these intriguing metabolites, building on knowledge garnered from consideration of possible biosynthetic origins, opens the possibility of discovering new chemistry and testing theories regarding their biosynthesis. In this regard, the oroidin family of marine alkaloids contains a large number of biogenetically related molecules that have inspired many to propose intriguing biosynthetic hypotheses.[2] The simple heterocyclic system, oroidin (**5a**), is thought to be a common precursor to this alkaloid family (Fig. 1). Among this family of alkaloids, axinellamine and palau'amine contain a common structural feature, namely a spirocyclic chlorocyclopentane ring. Several groups have described synthetic approaches to these hexacyclic oroidin-derived metabolites including those of Overman,[3] Carreira,[4] Lovely,[5] Austin,[6] and Harran.[7] Our synthetic approach to this class of bisguanidine alkaloids is premised on the biosynthetic hypothesis proposed by Kinnel and Scheuer.[8] Alternative biosynthetic proposals have been posited by Al Mourabit and Potier[9] and most recently by Baran.[10]

Kinnel and Scheuer proposed that palau'amine originates from a Diels–Alder reaction of dehydrophakellin (**7a**), which has not been isolated, and a truncated oroidin (AAPE, **5b**), which has been isolated. This is followed by a presumed chloroperoxidase-mediated chlorination initiating a 1,2-shift/ring contraction (Fig. 2), a process with some precedent.[11] In our synthetic studies of phakellstatin,[12] we found that aminals **6a** bearing a potential leaving group at C10 were unstable likely due to acyliminium formation while aminals **6b** bearing a leaving group at C6 were quite stable. The latter findings were in accord with concurrent work by Evans demonstrating the utility of pyrrolocarbinolamines as aldehyde surrogates.[13] These findings led to our successful enantioselective synthesis of phakellstatin and a proposed refinement of the biosynthetic proposal of Kinnel and Scheuer. The initial idea that dehydrophakellin (**7a**) could serve as a dienophile was unsettling as its reactivity would be expected to be low in this regard. However, if one considers the ring-opened form of dehydrophakellin (**7b**) consisting of an allylic acyliminium species, which may indeed be promoted in an enzyme active site, the expected high reactivity of the resulting acyliminium as

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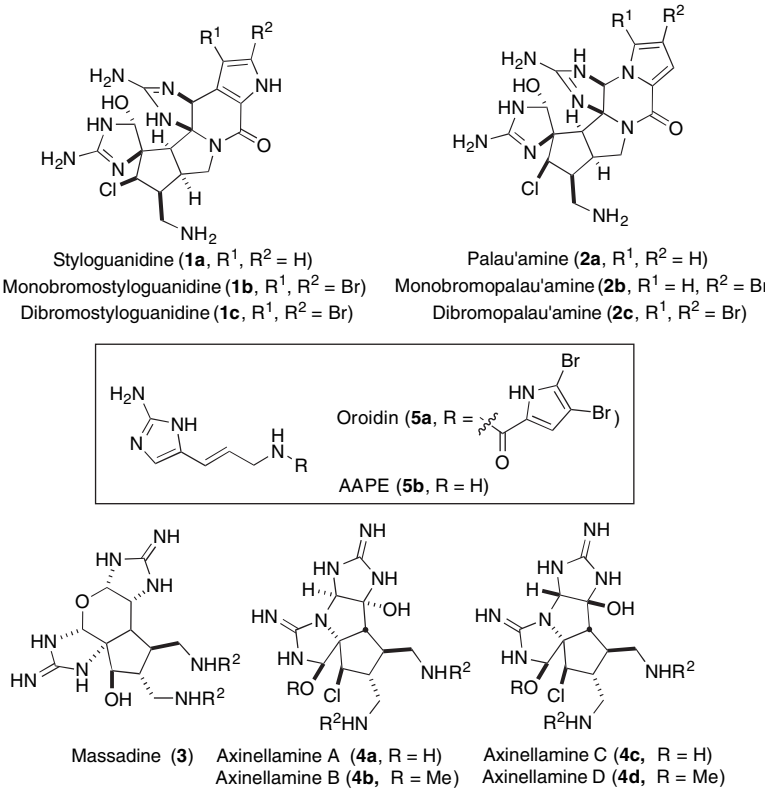


Figure 1. Oroidin-derived natural products.

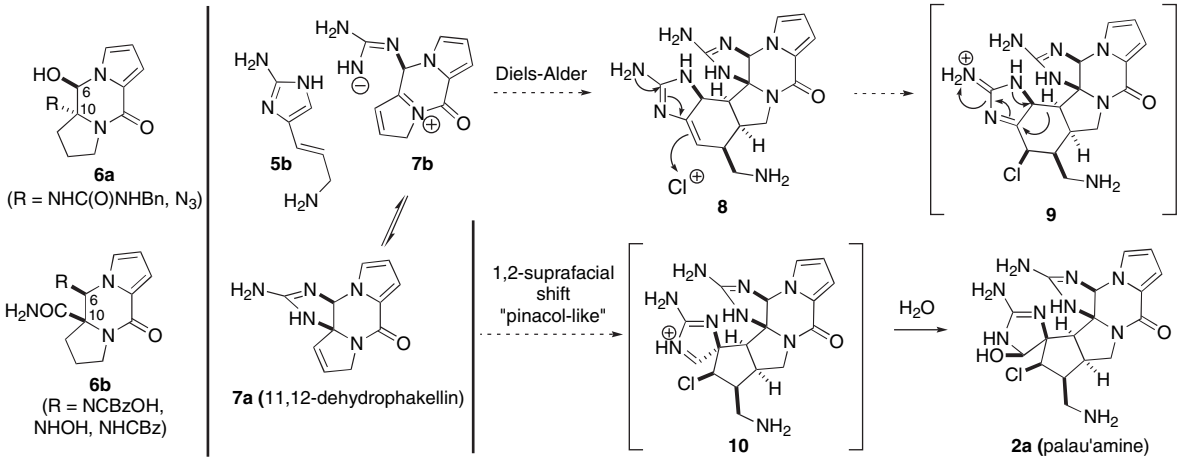


Figure 2. Stable/unstable aminals and carbinolamines **6** and Kinnel and Scheuer's proposed biosynthesis of palau'amine.

a dienophile is reminiscent of the allylic carbocations studied by Gassman and Singleton.[14] Thus, we propose a refinement to the Kinnel–Scheuer biosynthetic proposal that proceeds through the ring-opened acyliminium species **7b** leading to Diels–Alder adduct **8**. Chlorination initiates the 1,2-pinacol-like shift/ring contraction leading to cyclopentane bearing the iminium species **10**, which is trapped with water to deliver palau'amine.

1.1. Overall synthetic strategy premised on a proposed biosynthesis

Our initial interest in the oroidin alkaloids stemmed from the unique architecture presented by palau'amine in conjunction with its potent immunosuppressive activity. This parlayed into our group's interest in natural products displaying potent, cell-specific, physiological properties and the structurally distinct marine sponge isolate, pateamine A, is an example.[15] In the course of our studies toward palau'amine, a unified strategy toward both axinellamine and palau'amine evolved proceeding through a Diels–Alder reaction and then diverging to either axinellamine or palau'amine by virtue of either an inter- or intramolecular chlorination, respectively (Fig. 3).

This divergence addresses the proposed difference in relative stereochemistry between axinellamine and palau'amine at the chlorine bearing carbon C1 and that of C3. The

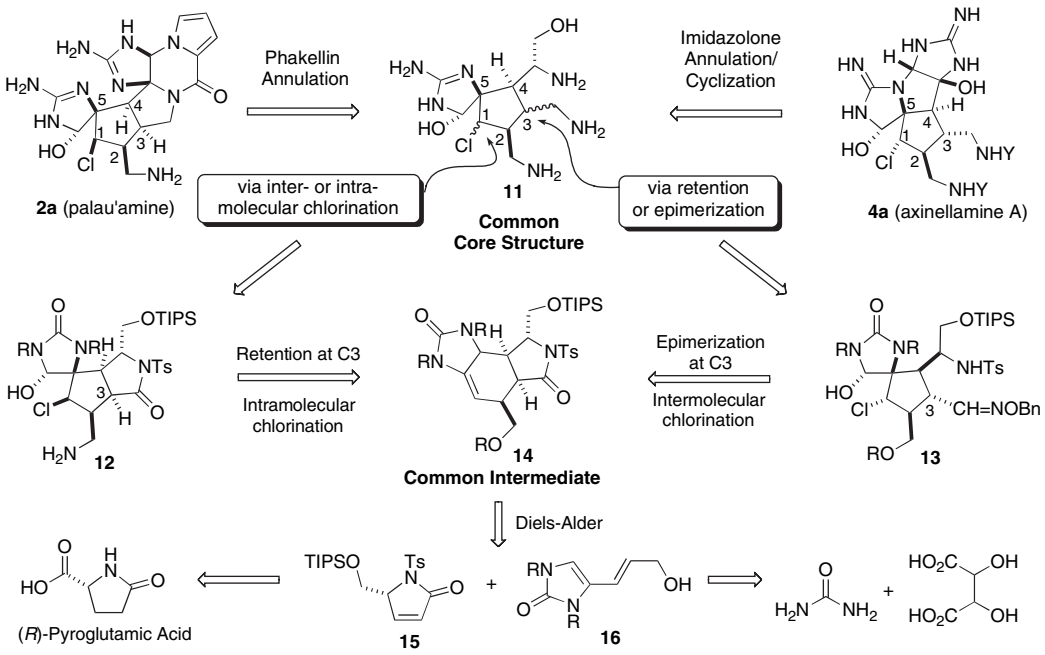


Figure 3. A unified synthetic strategy to the oroidin-derived alkaloids, axinellamine, and palau'amine.

anti-relationship between C1 and C2 found in axinellamine could be accessed by intermolecular chlorination as a result of the topography of the tricyclic common intermediate **14**. This chlorination of enamine **8** would be expected to initiate a pinacol-like, 1,2-shift of iminium **9** resulting in a ring contraction, as proposed by Kinnel and Scheuer.[16]

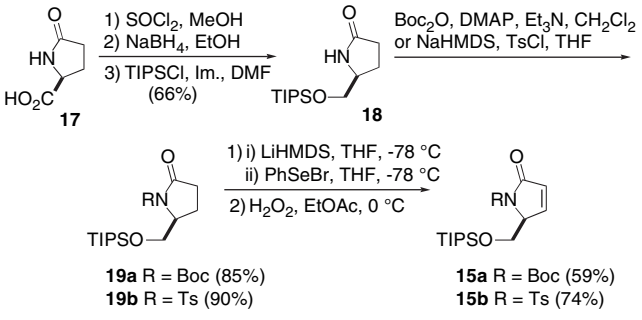
Common intermediate **14** would be obtained by a Diels–Alder reaction between the vinyl imidazolone **16** and a dienophile **15** derived from pyroglutamic acid. The absolute stereochemistry of axinellamine and palau'amine has not been confirmed, however, based on similarities in the CD spectra of palau'amine and dibromophakellin, the absolute configuration has been proposed to be as shown.[8] Based on biosynthetic considerations, one might predict that axinellamine will possess a related absolute configuration. Thus, to obtain the natural configuration of these targets would require the use of (*R*)-pyroglutamic acid and ultimately all stereocenters in these natural products would be derived from this single stereocenter. Due to cost considerations, the (*S*) enantiomer has been utilized in initial studies. The required diene is ultimately derived from urea and tartaric acid. A subsequent epimerization at C3 via an imine would be required to obtain the all *anti*-configuration about the cyclopentane as found in axinellamine (i.e., **13**). Subsequent imidazolone annulation, guanidinylation, and an oxidative cyclization are proposed to provide the final ring and deliver axinellamine.

Toward palau'amine, an intramolecular chlorination would be required to set the required stereochemistry between C1 and C2 (cf. **12**). In this case, the Diels–Alder process sets the required *syn*-stereochemistry at C3 and C4, thus epimerization is not required. Guanidinylation and annulation of the phakellin substructure onto core structure **11** would deliver palau'amine. Details of our synthetic studies in this area culminating in a unified strategy toward the oroidin alkaloids, axinellamine, and palau'amine are described herein.

2. Results and discussion

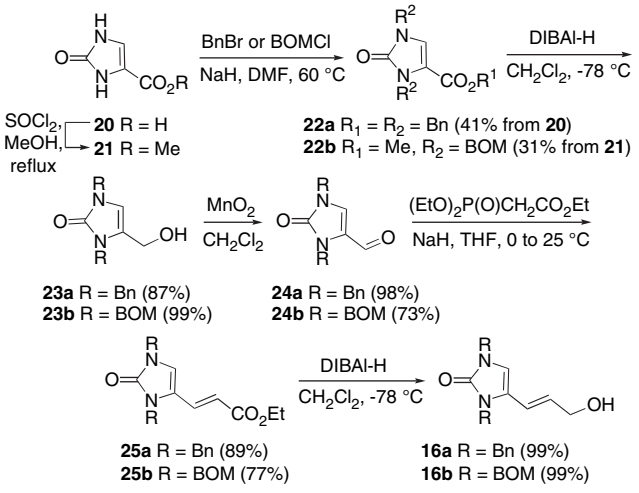
2.1. Studies of the Diels–Alder reaction toward common intermediate 14

2.1.1. Synthesis of the dienophiles and dienes. A series of dienophiles were prepared from L-(*S*)-pyroglutamic acid (**17**), following modified literature procedures,[17] to test their reactivity in the Diels–Alder reaction. Esterification was followed by reduction and protection of the resulting alcohol. Initially a *tert*-butylcarbamate (Boc, **19a**) was used to protect the lactam nitrogen, however, low reactivity as a dienophile subsequently led to the use of the *p*-toluenesulfonyl (Ts) protected lactam **19b**. A two-step sequence to introduce the unsaturation involving enolization with LDA, selenation, and finally oxidation with H_2O_2 provided enamides **15a**,**b** efficiently on ~1 g scale with no loss of optical purity as determined by Mosher ester analysis of the alcohol following TIPS deprotection. However, this reaction was only reproducible and scaleable when LiHMDS was used for enolization and ethyl acetate was used for the oxidation–elimination. In this way, the enamide **15b** could be obtained in high and reproducible yield on a large scale (~20 g) (Scheme 1).



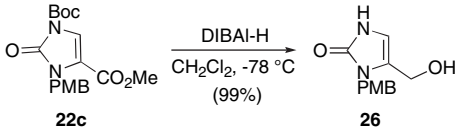
Scheme 1.

Formation of the diene commenced with imidazolone acid **20** prepared in one step from urea and tartaric acid.[18] Again, a number of different permutations of the diene were synthesized. Perbenzylation of the acid, followed by diisobutylaluminum hydride (DIBAl-H) reduction of the benzyl ester gave alcohol **23a** in good overall yield (Scheme 2 and Table 1). Oxidation with manganese dioxide followed by *E* selective olefination and reduction of the ester **25a** furnished the desired bis-benzylated diene **16a**. A benzyloxymethylene (BOM) protected diene **16b** was synthesized in a similar manner starting from the methyl ester **21**.



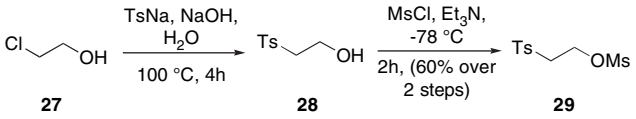
Scheme 2.

All other dienes synthesized (i.e., **16c–f**) incorporated orthogonal protecting groups requiring two-step protection but would allow for timed deprotection at later stages in the synthesis (Table 1). The synthesis of these dienes commenced from known methyl ester **21**.[18] Regioselective nitrogen protection is readily achieved due to steric (ester substituent) and electronic (increased acidity) properties of ester **21**.[19] Subsequent homologation was achieved in a manner similar to that described above for dienes **16a**,**b** (Table 1). While the Boc/*p*-methoxy (PMB) protected ester **22c** could be prepared, the acyl carbamate was not stable to DIBAl-H reduction conditions leading to alcohol **26** precluding its homologation (entry 1, Table 1). Dienes successfully synthesized in this manner include the Tse (*p*-tolylsulfonylethyl)/DMB-protected diene **16d**, Ts/PMB-protected diene **16e**, and the Ts/DMB (3,4-dimethoxybenzyl)-protected diene **16f** (Scheme 3).



Scheme 3.

The Tse group was installed using tosylethyl mesylate **29** (TseOMs) prepared in two steps from chloroethanol **27** on 100 g scale (Scheme 4). Importantly, the Tse/DMB-protected diene **16d** could be readily synthesized on 50–60 g scale with no need for chromatographic purification throughout the entire sequence.

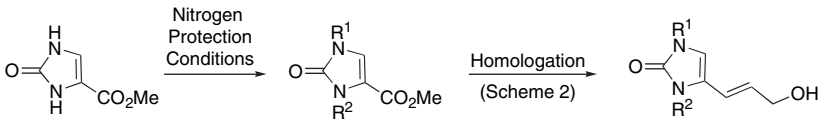


Scheme 4.

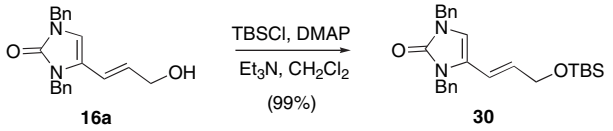
Dienes **16a–f** displayed varied acid sensitivity due to the presence of the allylic/benzylic alcohol and thus were typically prepared by DIBAl-H reduction and used directly in the Diels–Alder reaction without purification. Those dienes possessing an electron-withdrawing group (R^1) were notably more stable as expected. Protection of the hydroxyl moiety of diene **16a** as the *tert*-butyldimethylsilyl ether prior to the cycloaddition was briefly investigated in efforts to increase convergency (Scheme 5). While the silylation and subsequent purification proceeded smoothly, the instability of the silylated diene **30** appeared even more pronounced than that of the parent alcohol.

To further streamline the synthetic approach, incorporation of the pendant amine functionality, eventually required for

Table 1. Synthesis of dienes **16c–f** bearing orthogonal, nitrogen protecting groups

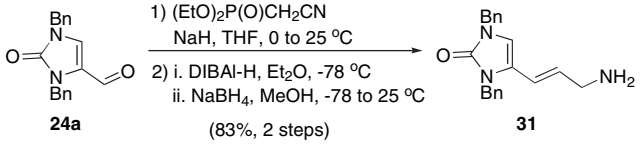


Entry	Nitrogen protection conditions (% yield, **22c–f**)	R^1	R^2	Esters **22**	Dienes **16c–f** (% yield)
1	i) Boc_2O, K_2CO_3, MeCN, 25 °C (54) ii) PMBBr, K_2CO_3, MeCN, 70 °C (53)	Boc	PMB	**22c**	**16c** (0)
2	i) TseOMs, $NaHCO_3$, DMSO, 70 °C (90) ii) DMBCl, K_2CO_3, DMF, 65 °C (100)	Tse	DMB	**22d**	**16d** (80)
3	i) TsCl, NaH, DMF, 25 °C (58) ii) PMBBr, K_2CO_3, MeCN, 70 °C (83)	Ts	PMB	**22e**	**16e** (64)
4	i) TsCl, NaH, DMF, 25 °C (58) ii) DMBCl, K_2CO_3, MeCN, 50 °C (64)	Ts	DMB	**22f**	**16f** (61)



Scheme 5.

the synthesis of palau'amine and axinellamine, into the diene was studied (Scheme 6). This approach involved a slight modification of the Horner–Wadsworth–Emmons olefination previously employed allowing installation of a vinyl cyanide, which was subsequently reduced to yield the amine diene **31** in excellent overall yield.



Scheme 6.

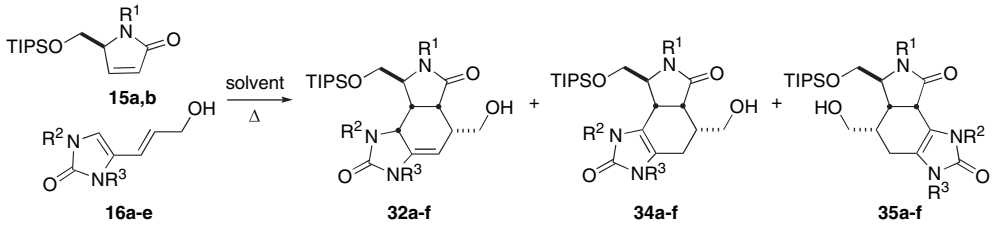
2.1.2. Facility and regioselectivity of the Diels–Alder reaction: effects of dienophile electronics. Building on precedence in the literature involving Diels–Alder reactions of related pyroglutamic acid derived dienophiles, the proposed cycloaddition was expected to proceed with high facial selectivity and *endo*-selectivity.[20] However, the regioselectivity was less certain given the fact that the diene is substituted with two nitrogens and each was expected to direct to opposite regioisomers (cf. diene **16**). A slight preference for the desired regiochemistry would be expected due to the presence of the alkyl substituent at the terminus. We recognized several possible strategies to overcome potential regiochemical issues including the use of Lewis acids, modifying the electronics of nitrogen protecting groups, and templated reactions.[21]

Our initial studies began with the Boc-protected lactam **15a** and bis-benzyl diene **16a** and while the cycloaddition did proceed, it required elevated temperatures providing cycloadduct **34a** in modest yields (28%, Table 2, entry 1). However, the expected initial Diels–Alder adduct **32a**, was not isolated, as the pseudoaromatic imidazolone was regenerated via double bond migration. Since the reactivity of the Boc-protected lactam **15a** was low, requiring high temperatures (~140 °C) for cycloaddition, the synthesis and use of a more reactive dienophile was warranted. The high reaction temperatures also contributed to the rather rapid degradation of diene **16a**. When the tosylated dienophile **15b** was used, the reaction proceeded at lower temperatures and provided higher yields (47%) of cycloadducts **34b**. Subsequent reactions performed in benzene and in the presence of 2,6-lutidine showed a marked improvement in yield (79%, Table 2, entry 3) suggestive of the acid instability of the dienes previously observed. Two regioisomers were produced in a ~4.3:1 ratio and the major isomer was determined to be the desired regioisomer based on extensive NMR studies including NOE data for the major regioisomer (Fig. 4a). The regioisomer **35b** proved to be crystalline and suitable for X-ray analysis providing further, albeit indirect, support for the structural assignment of the desired regioisomer (Fig. 4b).

Addition of Lewis acids to improve regioselectivity was investigated briefly as this led to rapid decomposition of diene **16a** even at low temperature. An exception was $MgBr_2 \cdot OEt_2$, which gave a slightly improved yield and regioisomeric ratio but importantly using only 1 equiv of diene (Table 2, entry 5 vs 6).

Other dienes were studied in efforts to facilitate protecting group removal late in the synthesis and also to improve convergency. The bis-BOM-protected diene **16b** provided a similar ratio of regioisomers in an unoptimized 36% yield (Table 2, entry 4). Cycloaddition of amino diene **31** and lactam **15b** was studied only briefly and provided low yields of the amino-substituted Diels–Alder adduct (not shown) due to extensive diene decomposition. Acylation or sulfonylation of this diene will reduce basicity and increase stability possibly rendering it more useful in Diels–Alder reactions. The

Table 2. Diels–Alder reactions providing the tricyclic common intermediates **34a–f**



Entry	Dienophile	R^1	Diene[a]	R^2	R^3	Reaction conditions	Products	% Yield[b]
1	**15a**	Boc	**16a** (1.1)	Bn	Bn	*o*-Xylene, 140 °C, 14 h	**34a**	28
2	**15b**	Ts	**16a** (2.5)	Bn	Bn	*o*-Xylene, 2,6-lut. (1.2 equiv), 90 °C, 28 h	**34b**	47
3	**15b**	Ts	**16a** (1.41)	Bn	Bn	PhH, 2,6-lut. (0.66 equiv), 95 °C, 4 d	**34b**, **35b**	64 (15)[c]
4	**15b**	Ts	**16b** (0.9)	BOM	BOM	PhH, 2,6-lut. (0.57 equiv), 95 °C, 24 h	**34c**, **35c**	30 (6)[c]
5	**15b**	Ts	**16d** (2.5)	Tse	DMB	PhH, 2,6-lut. (0.75 equiv), 95 °C, 3 d	**34d**, **35d**	54 (20)[c]
6	**15b**	Ts	**16d** (1.0)	Tse	DMB	PhH, $MgBr_2 \cdot OEt_2$ (0.5 equiv), 100 °C, 3 d	**34d**, **35d**	67 (12)[c]
7	**15b**	Ts	**16e** (1.0)	Ts	PMB	PhH, 2,6-lut. (0.55 equiv), 114 °C, 4 d	**34e**	59
8	**15b**	Ts	**16f** (1.45)	Ts	DMB	PhH, 2,6-lut. (0.46 equiv), 110 °C, 4 d	**34f**	74

[a] Equivalents of diene employed in parentheses.
[b] Refers to isolated yields.
[c] Yield for isolated regioisomers **35a–f** given in parentheses.

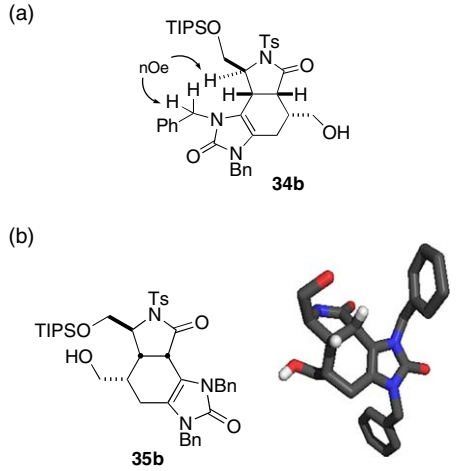
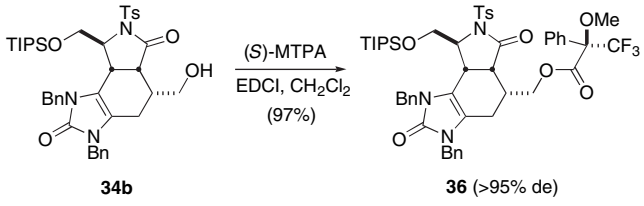


Figure 4. Regiochemical assignment of Diels–Alder adducts **34b** and **35b**. (a) Key NOE correlations observed confirming regiochemistry. (b) POV-chem rendering of the X-ray crystal structure of regioisomeric cycloadduct **35b** (protecting groups removed for clarity).

use of Tse/DMB diene **16d** was also studied for correlation to a product obtained from a tosylvinyl (Tsv) protected diene (**16g**, vide infra) and this gave diminished regioselectivity (~2.7:1, Table 2, entry 5). The Tse group was also advantageous from the standpoint of orthogonality with respect to the lactam Ts group.

Erosion of optical purity of the dienophile **15b** during the Diels–Alder reaction was possible due to the potential for pyrrole tautomer formation. Thus, the Mosher ester **36** of alcohol **34b** was prepared and integration of the diastereomeric methoxy protons by ^{1}H NMR spectroscopy indicated a diastereomeric excess of >95% for the cycloadduct (in comparison to that prepared from racemic dienophile **15b**). This confirmed ~2.5% epimerization of lactam **15b** under the Diels–Alder reaction conditions, which was >99% at the outset of the reaction as determined by chiral HPLC analysis (Scheme 7).



Scheme 7.

Notably, in all Diels–Alder reactions, the majority of unreacted dienophile **15b** could be cleanly recovered (~80–97% mass recovery) with minimal loss of optical purity (chiral HPLC analysis) after the cycloadditions, while the bis-alkylated dienes had decomposed.

2.1.3. Regioselectivity of the Diels–Alder reaction: effects of diene electronics. While Diels–Alder reactions employing bis-alkylated dienes **16a** and **16b** proceeded with high facial and *endo*-selectivity as anticipated, we sought alternative means to improve the modest regioselectivity (~4:1). One strategy involved varying the electronics of protecting groups on the nitrogen atoms of the diene imidazolone. We reasoned that use of an electron-withdrawing group on N^1 while maintaining an electron-donating group on N^2 would perturb the orbital coefficients of the diene leading to improved regioselectivity (Fig. 5).

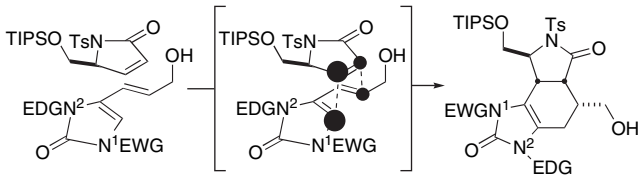


Figure 5. Electronics of vinyl imidazolones as dienes.

Indeed, initial studies employing the Ts/PMB diene **16e** provided for the first time, the initial Diels–Alder adduct **32e** that had not undergone olefin isomerization. Continued heating for a further two days resulted in conversion to the isomerized cycloadduct (**34e**) and importantly as a single regioisomer (59%, Table 2, entry 7). Further optimization was performed with Ts/DMB diene **16f** and ultimately led to a highly stereo- and regioselective Diels–Alder reaction providing adduct **34f** in good yield (74%, Table 2, entry 8).

2.1.4. Development of an electronically adjustable tosylvinyl (Tsv) nitrogen protecting group. In subsequent studies, we determined that while the Ts/DMB diene **16f** produced only one regioisomer in the Diels–Alder reaction, the resulting adduct and deprotected derivatives did not participate in the chlorination/rearrangement process (vide infra). Through our studies, we determined that an electron-withdrawing group (EWG) on N^1 was required for high regioselectivity in the Diels–Alder reaction while successful chlorination/rearrangement required an electron-donating group (EDG) on N^1 (Fig. 6). A typical way to address this issue would entail a protecting group switch, however, we were attracted to the possibility of utilizing a protecting group with readily adjusted electronics. This led us to consider the use of a *p*-tolylsulfonylvinyl (Tsv) group, which in a vinylogous manner provides the electron-withdrawing ability of the Ts group. However, reduction of the alkene provides the Tse group leading to an EDG suitable for the chlorination/rearrangement sequence.

A range of electrophiles were studied to install the Tsv group on imidazolone **21**. The use of commercially available *p*-toluenesulfonyl acetylene in the presence of a range of bases resulted in disubstituted product **37**. Similar methods were used by Arjona and Vilarrasa to protect thiols with the Tsv group (Scheme 8).[22]

Ultimately, only (*Z*)-1,2-di-*p*-toluenesulfonylethylene was found to be useful to install the Tsv group via an addition–elimination sequence using NaH as base.[23] Subsequent protection of the remaining nitrogen as the DMB amine provided ester **38**. Attempted reduction of ester **38** with DIBAl-H resulted in concomitant reduction of the Tsv group. Chemoselective reduction was finally achieved by activation of the acid functionality as either an acid chloride or a mixed anhydride and reduction with lithium borohydride. Following oxidation and olefination, this process was necessary

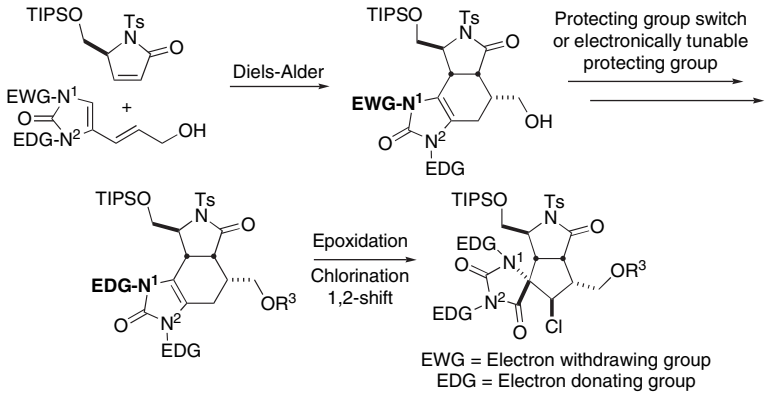
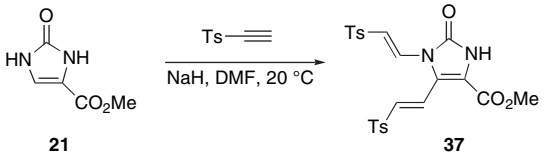
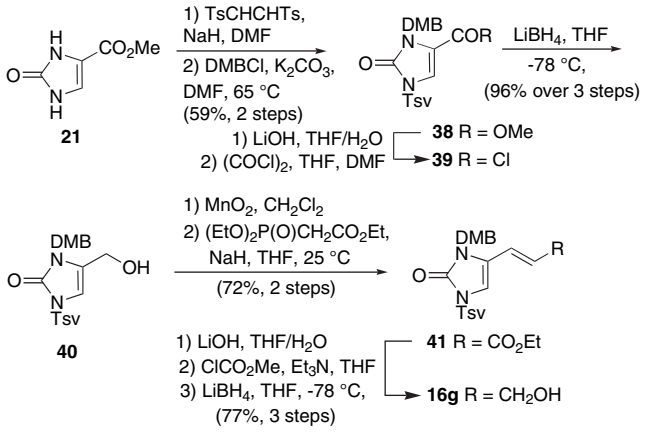


Figure 6. Electronic requirements for the Diels–Alder reaction and chlorination/rearrangement sequence and possible solutions.



Scheme 8.

once again to prevent Tsv reduction. However, formation of diene **16g** by this route was possible without recourse to silica gel chromatography throughout the entire sequence on large scale (Scheme 9).

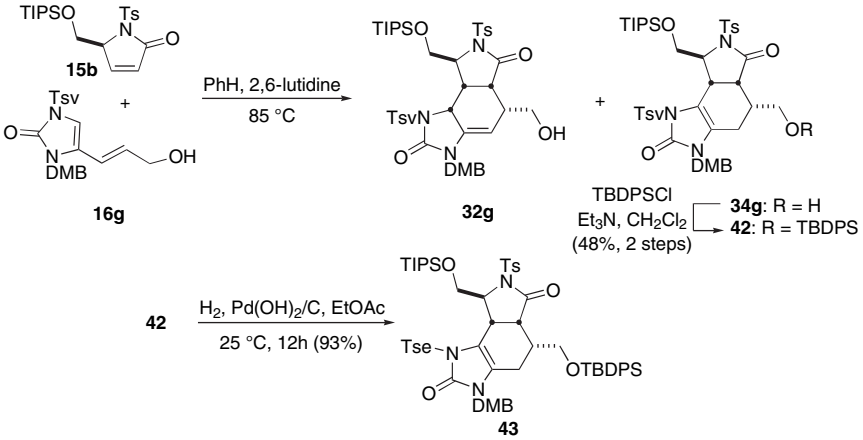


Scheme 9.

Use of the Tsv/DMB diene **16g** in the Diels–Alder reaction at 85 °C under the previously described conditions gave initial Diels–Alder adduct **32g** and regioisomer **34g** (Scheme 10). Upon increasing the reaction temperature to 95 °C, adduct **34g** was obtained as a single isomer as determined by ^{1}H NMR analysis of the crude reaction mixture (~20–30% conversion). A by-product isolated from the reaction mixture was derived from competing dimerization of the diene at the Tsv group (not shown). Attempts to improve the yield by the addition of multiple equivalents of diene at various reaction time points were unsuccessful. Following alcohol protection of adduct **34g**, the Tsv group of silyl ether **42** was hydrogenated to give the reduced product in 93% yield. This compound was correlated to the same cycloadduct (i.e., **34d**, Table 2) obtained by direct Diels–Alder reaction with the Tse/DMB diene **16d** and found to be identical thus confirming its structure.

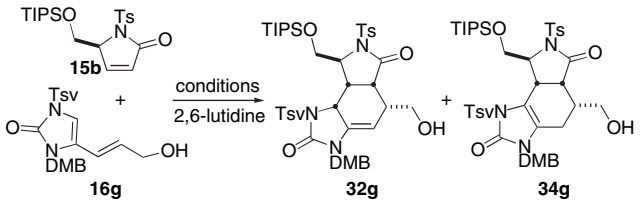
2.1.5. Microwave-assisted Diels–Alder reactions. The long reaction times for several of the Diels–Alder reactions described above, led us to consider the use of microwave irradiation to accelerate these cycloadditions. Microwave chemistry has developed into a very useful technique, and has proven particularly useful for accelerating sluggish cycloaddition reactions.[24]

Initial studies into the Diels–Alder reaction of Tsv-diene **16g** with dienophile **15b** under microwave conditions, employed 1 equiv of diene relative to dienophile and the crude reaction



Scheme 10.

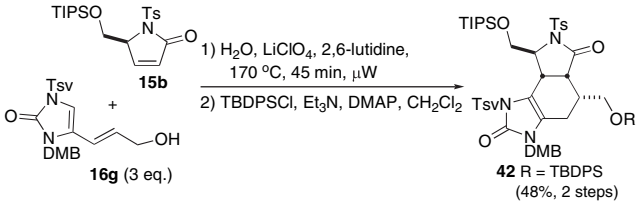
Table 3. Microwave-assisted Diels–Alder reactions



Entry	Reagents and conditions (with 0.6 equiv of 2,6-lutidine)	Ratio of **15b/32g/34g**
1	PhH/H_2O (1:4), 150 °C, 1 h	1:0.07:0.18
2	PhH/H_2O (1:1), $LiClO_4$, 170 °C, 5 h	1:0.07:0.44
3	H_2O, $LiClO_4$, 170 °C, 45 min	1:0:1.38
4	H_2O, $LiClO_4$, 170 °C, 45 min, 2 equiv of **16g**	1:0:2
5	H_2O, $LiClO_4$, 170 °C, 45 min, 3 equiv of **16g**	1:0:6

mixture was analyzed (^{1}H NMR) for the ratio of initial cycloadduct **32g**, isomerized cycloadduct **34g**, and dienophile **15b** (Table 3). In analogy to conventional thermal conditions, the regioisomer of **34g** was not observed under microwave conditions. When aqueous benzene was used, some degree of non-isomerized Diels–Alder adduct **32g** was observed (entry 1). Addition of $LiClO_4$ led to further conversion but only with extended reaction times (entry 2). Ultimately, using only water as the heating medium resulted in much shorter reaction times possibly due to hydrophobic effects,[25] and the initial cycloadduct **32g** was not observed (entry 3). As expected, increasing to 2.0 or 3.0 equiv of diene **16g** further improved the resulting ratio of product to dienophile **15b** (entries 4 and 5).

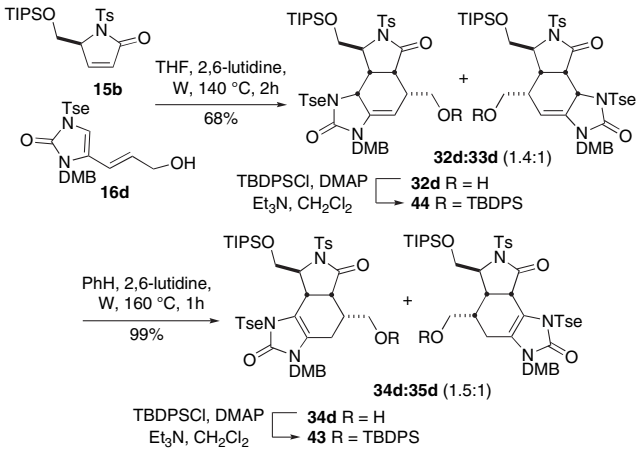
Under optimal microwave conditions, the Diels–Alder reaction of Tsv-diene **16g** gave a maximum overall yield of 48% yield after TBDPS protection (Scheme 11). Again the major side product was determined to be the competing dimerization of the Tsv-diene **16g**. The competing dimerization requiring excess diene to reach reaction completion has limited the use of this substrate in our synthetic efforts toward the axinellamines and palau'amine.



Scheme 11.

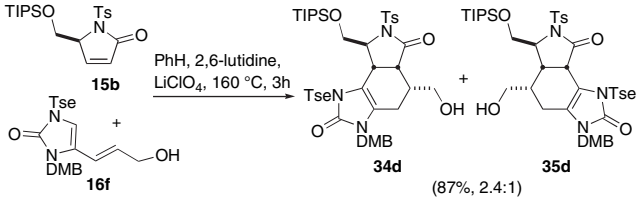
The cycloaddition of the Tse/DMB diene **16d** was also investigated under microwave conditions in THF at 140 °C for 2 h and this gave exclusively the initial Diels–Alder adduct **32d** and regioisomer **33d** (Scheme 12). Adduct **32d** is a useful substrate for direct chlorination and rearrangement as originally proposed in our overall synthetic strategy. These adducts could be converted to isomerized adducts **34d** and **35d** under microwave conditions in benzene in the presence of 2,6-lutidine.

Interestingly, conducting the Diels–Alder reaction of Tse/DMB diene **16d** in benzene under microwave conditions



Scheme 12.

with the weak Lewis acid, $LiClO_4$, resulted in the formation of isomerized adduct **34d** as the major product along with regioisomer **35d** (2.4:1 ratio) in 87% overall yield using only 1.1 equiv of diene (Scheme 13). This contrasts to conventional heating, which required 2–2.5 equiv of diene to achieve similar conversions. The addition of water to the reaction medium had no effect on conversion or regioselectivity.

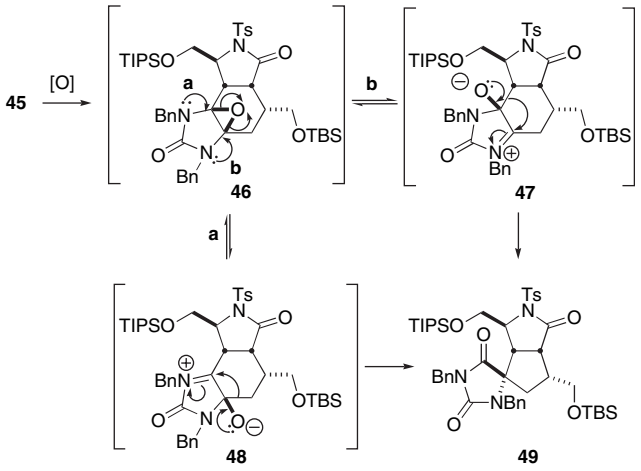


Scheme 13.

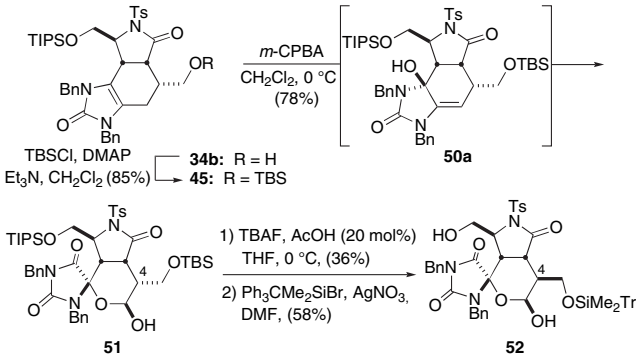
2.2. Attempted epoxidation/rearrangement of the Diels–Alder adduct leading to serviceable allylic alcohols for chlorination/rearrangement

Given that double bond isomerization of the initial Diels–Alder adduct was observed, we proceeded to study the viability of the proposed 1,2-shift/ring contraction sequence to furnish a deschlorospirocycle (i.e., **49**, Scheme 14). We reasoned that highly facial selective epoxidation on the convex face of the tricyclic imidazolone alkene would initiate the desired rearrangement. Following epoxidation of the electron-rich alkene of imidazolone **45** (cf. Scheme 15) or direct formation of a hydroxy iminium species **47** via enamine chemistry, carbinolurea **47** or **48** was expected to undergo subsequent 1,2-shift/ring contraction to yield deschlorospirocycle **49**. Importantly, it was inconsequential which C–C bond participated in the 1,2-shift since both pathways (**a** and **b**) would ultimately deliver spirohydantoin **49** provided high facial selectivity was achieved in the initial oxidation.

Toward deschlorospirocycle **49**, silyl protection of alcohol **34b** provided silyl ether **45**, which was then treated with excess *m*-chloroperbenzoic acid (*m*-CPBA) furnishing what



Scheme 14.



Scheme 15.

we initially believed to be allylic alcohol **50a** and not the rearranged spirohydantoin (Scheme 15). This structure was quickly excluded, since mass spectrometric analysis of the reaction product revealed incorporation of three oxygen atoms rather than one. Notably, the formation of the major reaction product was instantaneous at 0 °C, and lowering the reaction temperature appeared to have an effect on the reaction rate but not the reaction outcome suggestive of an

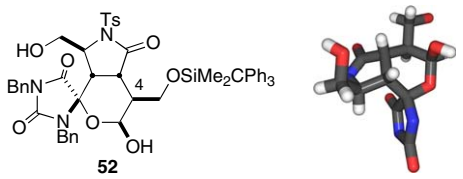
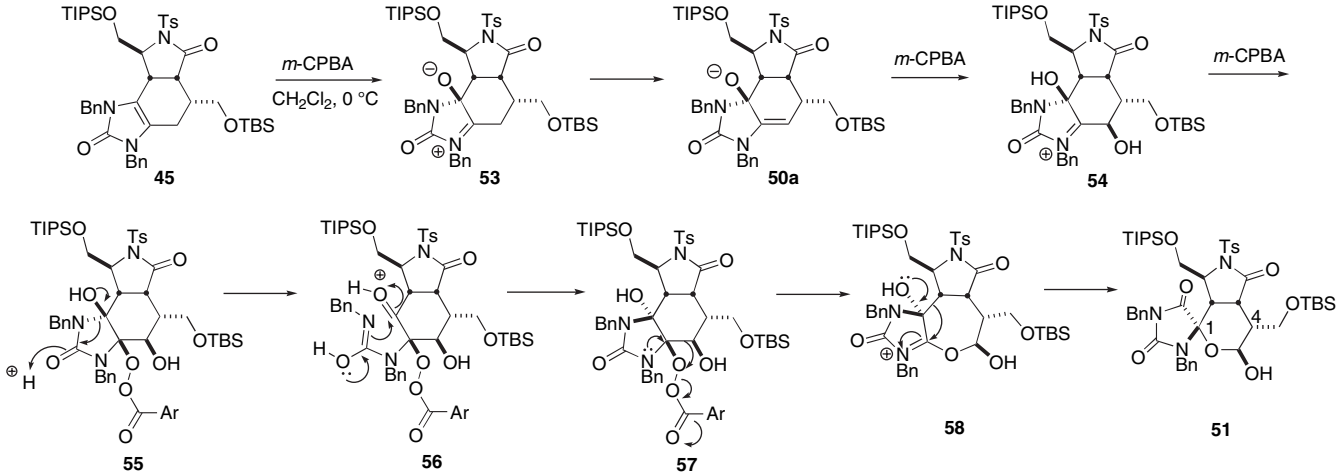


Figure 7. Structure of the derivatized over-oxidation product **52** and POV-chem rendering of the X-ray crystal structure (protecting groups are removed for clarity).

extremely facile transformation with *m*-CPBA. Indeed, use of 1.0 equiv of *m*-CPBA led to 33% yield of **51** and 66% recovered starting material.

The structure of this highly rearranged product could not be confirmed despite extensive spectral analysis although we recognized the presence of two acetal/aminal carbons and a hydantoin. Consequently, we attempted preparation of a crystalline derivative to confirm the proposed structure derived from plausible mechanistic scenarios. Toward this end, desilylation and mono-silylation with trityldimethylsilylbromide, a protecting group known to frequently produce crystalline derivatives,[26] provided colorless needles suitable for X-ray analysis. Thus, the structure of spirocycle **52** was confirmed as the lactol hydantoin (Fig. 7) and retrospectively the structure of the over-oxidation product was confirmed to be lactol **51** following careful analysis of changes occurring during the sequence leading to lactol **52** as described below (Fig. 7).

The hydroxymethylene bearing center (C4) had epimerized during the sequence and this was found to occur during the desilylation step presumably via the aldehyde. The stereochemistry at the spirocenter was unexpected for a suprafacial 1,2-shift, which was expected for this rearrangement. However, reasonable explanations for this inversion of stereochemistry could be conceived when considering possible mechanistic scenarios involved in the generation of this over-oxidation product (Scheme 16). One possible mechanism involves initial epoxidation of alkene **45** with *m*-CPBA or direct iminium ion formation by oxidation (cf. Scheme 14) followed by deprotonation to give alkene **50a**. A second epoxidation/ring opening sequence followed by



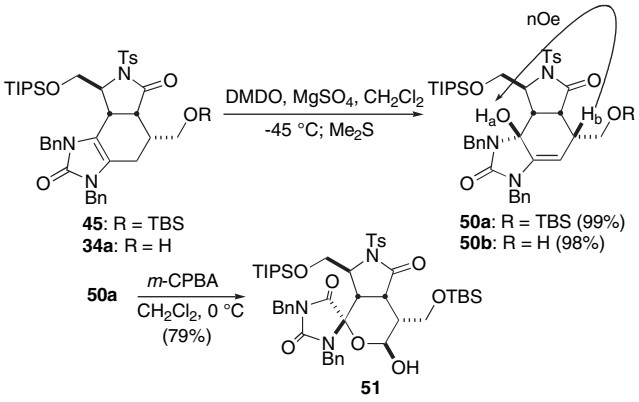
Scheme 16.

a Baeyer–Villiger type oxidation of the resulting iminium intermediate **54** yields oxepane **58**. Notably, while transformations of this type have not been reported for iminium species, analogous Baeyer–Villiger type oxidations of oxocarbenium intermediates have been described previously.[27] Considering this mechanistic proposal, the inverted stereochemistry at the quaternary center may be rationalized by invoking an acid-catalyzed epimerization of carbinolamine **55**. The epimerization may proceed via ketone **56** by reversible ring cleavage and readdition of the urea from the convex face. Subsequent 1,2-shift/ring contraction provides spirocyclic hydantoin **51** containing a tetrahydropyran. Another possible rationale for inversion at the quaternary center involves epimerization at the stage of the final hydantoin **51** via ring-opened intermediates (not shown).

While not useful toward the synthesis of the target core structure of oroidin-derived alkaloids, the over-oxidation product proved quite valuable. The formation of the spirohydantoin **51** lent credence to our proposed 1,2-shift/ring contraction. Furthermore, given the potential intermediacy of allylic alcohol **50a**, we recognized the utility of this substrate for the projected chlorination/rearrangement sequence. The placement of the alkene moiety in carbinol **50a** would facilitate the incorporation of a chlorine atom at C5 by electrophilic addition. Furthermore, C=O bond formation leading to a spirocyclic hydantoin as observed would provide an added driving force for the 1,2-shift/ring contraction. Therefore, we examined other oxidants with the expectation that milder conditions might allow isolation of alcohol **50a**. While initial oxidations with DMDO[28] gave complex reaction mixtures when performed at 22 °C, we ultimately generated allylic alcohol **50a** in a highly diastereoselective manner and in excellent yield by careful treatment of imidazolone **45** with DMDO at −45 °C, followed by quenching of excess DMDO with dimethylsulfide at low temperature. While alcohol **50a** could be purified by silica gel chromatography, substantial decomposition was observed. However, it could be stored neatly in the freezer for several days and it was of sufficient purity to be used directly in subsequent reactions. The structural and stereochemical proof of carbinolurea **50a** was provided by detailed spectral analysis including COSY and HMQC. Key data included the presence of a carbinolurea carbon in the ^{13}C NMR spectrum (δ 86.5, acetone-d_6). This chemical shift correlated well with that of known carbinolurea substructures found in related natural products.[29] In addition, a key NOE was observed between the hydroxyl proton H_a and the methine proton H_b (Scheme 17). Interestingly, the hydroxyl proton H_a appeared as a sharp singlet at δ 5.57 but was exchangeable with D_2O.

In support of the aforementioned mechanism for the generation of over-oxidation product **51**, treatment of alcohol **50a** with *m*-CPBA also led to the same oxepane **51** observed using *m*-CPBA alone (Scheme 17). Notably, DMDO oxidation of the unprotected Diels–Alder adduct **34a** with a pendant free hydroxyl furnished the analogous carbinolurea. Alternative oxidants that were briefly investigated included peracetic acid and hydrogen peroxide, which led to desilylation (−TBS) and recovery of starting material, respectively.

While the synthesis of allylic alcohol **50a** enabled further investigations toward the chlorination/ring contraction

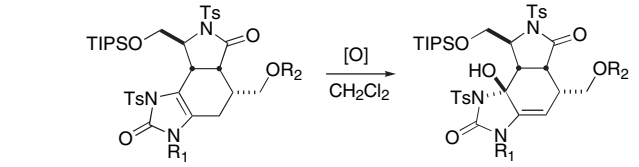


Scheme 17.

sequence (vide infra), synthesis of the corresponding carbinolureas derived from bis-tosyl Diels–Alder adducts **59** and **60** were also investigated since these had been obtained as single regioisomers in the cycloaddition. Not surprisingly, oxidation of the imidazolone alkene, now rendered less electron rich due to the tosyl substituent, did not proceed but only returned starting material (Table 4, entry 1). Increasing reaction temperature only furnished starting material contaminated to a varying degree with unidentified oxidation products (Table 4, entry 2). After unsuccessful oxidation attempts with H_2O_2 (Table 4, entry 3) and *m*-CPBA (Table 4, entry 4), the more reactive DMDO analog, methyl(trifluoromethyl)dioxirane[30] was utilized and gratifyingly yielded the desired allylic alcohol **50c** in nearly quantitative yield (Table 4, entry 5). The same reaction conditions could be employed to furnish the corresponding carbinolurea **50d** derived from the TBS-protected Diels–Alder adduct **60** (Table 4, entry 6).

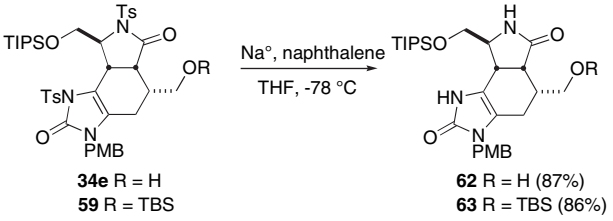
While these results were pleasing, the use of expensive and volatile methyl(trifluoromethyl)dioxirane on large scale would ultimately prevent the viability of this route. Therefore, we explored the possibility of removing the electron-withdrawing tosyl group prior to oxidation. Selective

Table 4. Oxidation of bis-sulfonylated Diels–Alder adducts



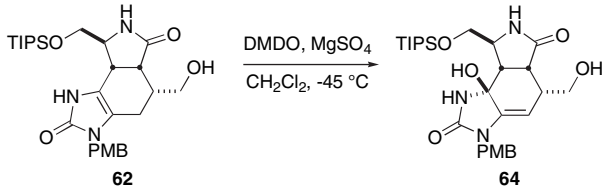
Entry	Imidazolone	Reaction conditions	Reaction outcome
1	**34e**	DMDO, $MgSO_4$, −45 °C	No reaction
2	**34e**	DMDO, $MgSO_4$, 22→41 °C	Mixture of products
3	**34e**	H_2O_2, 0 °C	No reaction
4	**59**	*m*-CPBA, 0 °C	Mixture of products
5	**59**	Methyl(trifluoromethyl)dioxirane, $MgSO_4$, −45 °C	**50c** (99%)
6	**60**	Methyl(trifluoromethyl)dioxirane, $MgSO_4$, −45 °C	**50d** (99%)

detosylation of Diels–Alder adduct **34e** and its silylated analog **59** proceeded smoothly with sodium naphthalenide at low temperature (Scheme 18).



Scheme 18.

Initial oxidation of these deprotected systems was conducted with imidazolone **62** devoid of the silyl protecting group. Treatment with DMDO did indeed produce a compound tentatively assigned as the desired allylic alcohol **64**, as confirmed by mass spectrometry, albeit not reproducibly (Scheme 19).



Scheme 19.

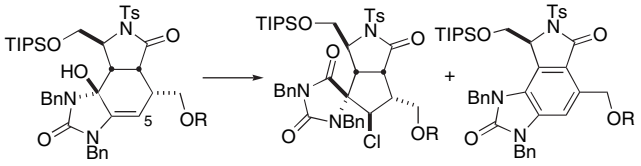
Ultimately, these attempted oxidation studies of *N*-unprotected Diels–Alder adducts (cf. **62**, **63**) in conjunction with findings made in subsequent chlorination studies (vide infra) led us to conclude that efficient formation of spirocyclic systems mandated nitrogen protection.

2.3. Development of a chlorination/1,2-shift/ring contraction sequence: synthesis of the spirocyclic core useful for axinellamine synthesis

Due to the ideal disposition of the double bond in allylic alcohol **50a** and encouraged by the spirohydantoin formation during the *m*-CPBA over-oxidation, we proceeded with investigation of the proposed halogenation/ring contraction sequence. It was expected that treatment of the distinctly cup-shaped carbinolurea **50a** with a source of electrophilic halogen would result in halide incorporation from the convex face of the tricyclic system. Initial attempts to effect the ring contraction by treatment of allylic alcohol **50a** with *N*-chlorosuccinimide (NCS) at −12 °C pleasingly gave a compound assigned as the desired spirohydantoin **65a** (49%) but contaminated with a painfully obtained aromatic system **66a** (loss of four stereocenters! Table 5, entry 1).[31] The formation of spirohydantoin likely involves initial chlorination, generating an iminium species, which subsequently undergoes a suprafacial 1,2-alkyl migration driven by C=O bond formation to yield the desired ring contracted spirocycle **65a**,**b** (cf. Fig. 2).

We postulated that acid-mediated eliminations of the carbinolurea **50a** may lead to cyclohexadiene intermediates, which could then be oxidized further by NCS to give

Table 5. Chlorination/1,2-shift/ring contractions of allylic alcohols **50a**,**b**



Entry	Substrate	Reaction conditions	**65a**,**b** (% yield)	**66a**,**b** (% yield)
1	**50a** (R=TBS)	NCS, −12 °C	49	16
2	**50a**	NCS, C_6H_{10} −78→−45 °C	75	Trace
3	**50b** (R=H)	NCS, C_6H_{10} −78→−45 °C	78	Trace

aromatic product **66a**. To minimize the generation of these side products, we employed cyclohexene as an acid buffer, a technique utilized by Hoye to absorb HOCl.[32] Pleasingly, the addition of cyclohexene, in conjunction with lowering the reaction temperature, furnished the desired spirocycle in good yield with only trace amounts of the aromatized by-product **66a**. Subsequent rearrangements of allylic alcohol **50a** were performed at −45 °C, affording consistent yields (75%) of spirocycle **65** (Table 5, entry 2). The sequence could also be performed on the unprotected allylic alcohol **50b** in similar yield (Table 5, entry 3).

Extensive NMR experiments were conducted to support the proposed relative stereochemistry of the halogenated spirohydantoins **65**. Most diagnostic were NOE experiments, which could be observed for chlorospirocycle **65** and its desilylated analog **67** (Fig. 8). Desilylation of the spirocycle **65** proved necessary since overlapping peaks precluded several key NOE's from being observed.

The *N*-benzyl protons on the series of compounds including the Diels–Alder adduct **34b**, allylic alcohol **50a**, and chlorocyclopentane **65** proved to be diagnostic markers for identifying the nature of the tricyclic system (Fig. 9). As often observed with benzyl protons in asymmetric environments, differences in chemical shift between the diastereotopic protons, *h*/*h*′ and *i*/*i*′, are reflective of the degree of steric congestion (i.e., conformational mobility) about these methylene groups. Thus, the benzylic protons are useful as probes for the nature of the tricyclic system in these and other systems in this series as the benzyl groups are readily distinguished (confirmed by NOE experiments) based on the

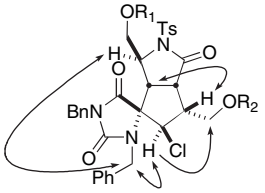


Figure 8. Key NOE enhancements observed for chlorinated spirocycles **65** and **67**.

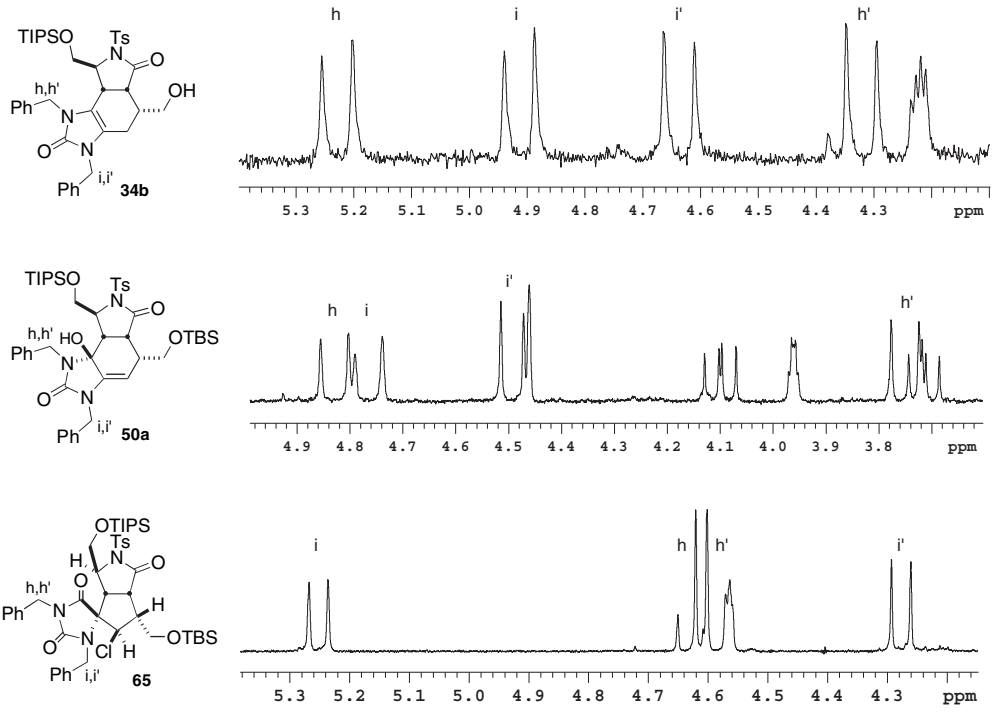
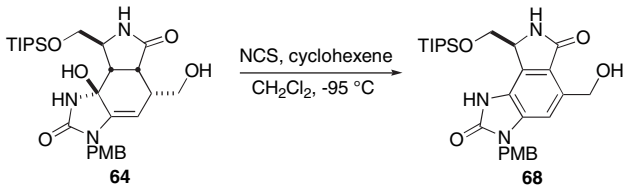


Figure 9. Benzyl protons of intermediates **34b**, **50a**, and **65** as diagnostic markers of steric congestion and structure.

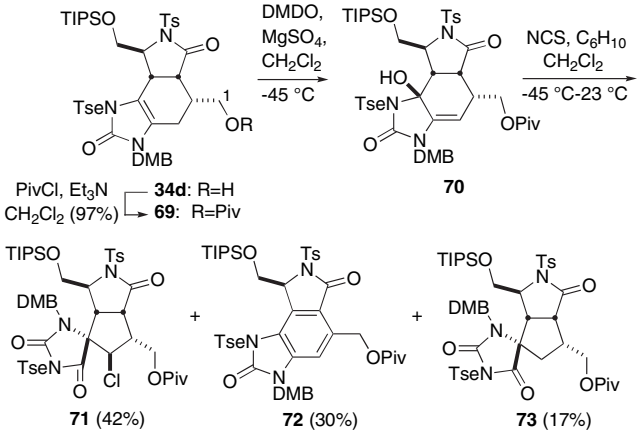
$\Delta\delta$ for these protons. For example, on conversion of the allylic alcohol **50a** to the spirocycle **65**, one observes a complete reversal in the $\Delta\delta$ for protons *h* and *i*. Namely, protons *h* of alcohol **50a**, which are present in the bay region of the tricycle have a large $\Delta\delta$ relative to protons *i* since these have greater conformational mobility. A complete reversal in this effect is observed following rearrangement/ring contraction since protons *i* are situated in the concave face of the spirocyclic system.

In analogy to the oxidation sequence, we were again interested in applying the rearrangement to the bis-sulfonylated Diels–Alder adducts (i.e., **61**, **50c**,**d**) as they were obtained as single regioisomers. NCS, 2,3,4,5,6,6-hexachlorocyclohexa-2,4-dien-1-one, sodium hypochlorite, chloramine-T, and chlorine gas were all studied in attempts to effect the rearrangement of allylic alcohol **50d**. All these reaction conditions furnished either starting allylic alcohol **50d** or produced only traces of the desired rearranged compound, frequently only identifiable after mass spectrometric analysis of the crude product. Attempted rearrangement of the detosylated analog **64** led to the aromatized product even at low (−95 °C) temperature (Scheme 20).



Scheme 20.

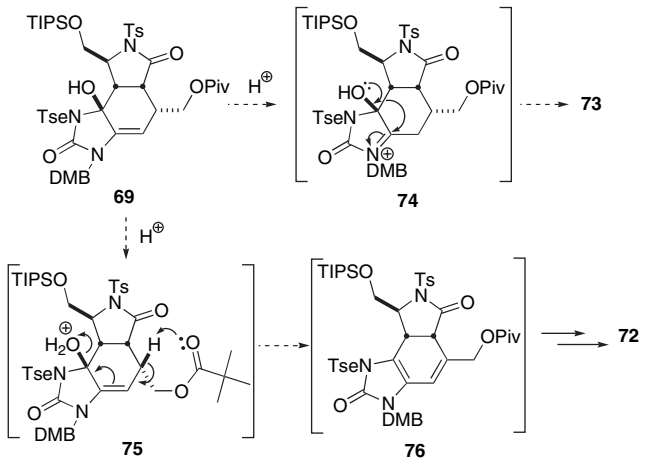
These results reinforced the need for electron-donating groups on N^1/N^2 of the Diels–Alder adduct for the oxidation/chlorination/rearrangement sequence (cf. Fig. 6). This added further impetus to development of the Tsv/Tse protecting group strategy described above. In further studies, it was also found that the choice of protecting group for the alcohol at C1 was also critical to this reaction (Scheme 21). Previous studies toward the chlorocyclopentane ring had been conducted using a TBS-protected alcohol, however, further endeavors toward axinellamine required an acidic stable protecting group. This led to the use of the pivaloate group, however, conducting the rearrangement under conditions previously utilized for the Tse/DMB-protected imidazolone resulted in non-reproducible yields of the spirocycle **71**.



Scheme 21.

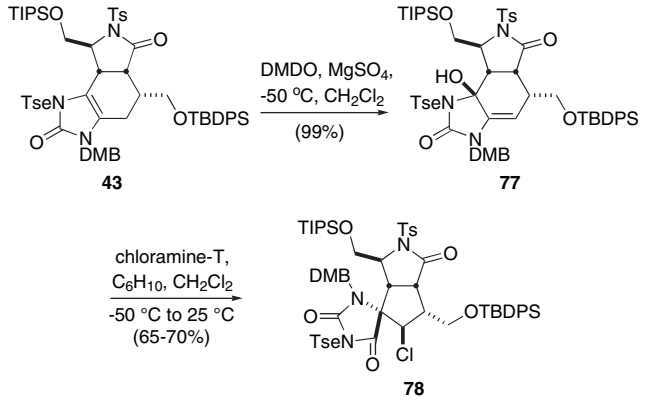
The desired chlorocyclopentane **71** (42%) remained as the major component and two by-products were identified as the aromatized compound **72** (30%) and the deschlorospirocycle **73** (17%). The presence of adventitious acid may lead

to protonation of enamine **69** rather than chlorination leading to deschlorocyclopentane **73** via iminium **74**. Alternatively, the acid presumably leads to the loss of the alcohol and formation of an iminium intermediate, for example, **75**. The increased amount of aromatized product **72** produced in this case compared to other protecting groups studied leads us to speculate that the pivaloate group may assist in diene formation by intramolecular deprotonation via intermediate **75** (Scheme 22). Subsequent α-chlorination and elimination or air oxidation of diene **76** may lead to the aromatized adduct **72**.



Scheme 22.

These findings led us to explore the more acid stable *tert*-butydiphenylsilyl (TBDPS) protecting group at C1 and as previously observed with the TBS ether, the chlorination/rearrangement sequence proceeded without incident (Scheme 23). However, while NCS gave isolable quantities of the aromatized product, chloramine-T nearly eliminated the formation of this by-product and gave highly reproducible yields of chlorocyclopentane **81** on gram scale (65–70%, Scheme 23).



Scheme 23.

3. Conclusion

We have developed a unified synthetic strategy to the oroidin alkaloids that will allow access to several members of this family of marine natural products. While Diels–Alder reactions of vinyl imidazolones provide adducts that have undergone facile olefin isomerization, subsequent oxidation provides a surprisingly stable allylic alcohol that serves as an excellent substrate for the key halogenation/ring contraction sequence. This involves a sequential chlorination/1,2-shift/ring contraction sequence allowing rapid access to a tricyclic spirocyclopentane (i.e., **78**), which serves as a point of departure for current synthetic studies toward the axinellamines. The requirement of imidazolone nitrogen protecting groups with differing electronics led to the development of several strategies for controlling regioselectivity in the Diels–Alder process and promoting the chlorination/rearrangement sequence. One strategy involved the development of a Tsv/Tse protecting group strategy, which allows for facile electronic tuning of this protecting group without recourse to a protecting group switch. The described synthetic studies have paved the way for our ongoing synthetic approaches to several members of the oroidin family of bioactive marine natural products that will ultimately enable further study of their precise biological modes of action.

4. Experimental

4.1. General

All non-aqueous reactions were carried out under a nitrogen atmosphere in oven-dried (120 °C) glassware unless noted otherwise. Tetrahydrofuran (THF, EM Science) and diethyl ether (Et_2O, EM Science) were distilled immediately prior to use from sodium metal/benzophenone ketyl. Methylene chloride (CH_2Cl_2, EM Science) and benzene (PhH, EM Science) were distilled from calcium hydride prior to use. Methanol (MeOH, EM Science) was distilled from magnesium methoxide. *N*-Chlorosuccinimide (NCS) was recrystallized from glacial acetic acid prior to use. Solutions of dimethyldioxirane[28] (DMDO) in acetone and methyl (trifluoromethyl)dioxirane[30] in 1,1,1-trifluoroacetone were prepared according to literature procedures. All other commercially available reagents were used as received unless specified otherwise.

Infrared spectra were recorded with a Nicolet Impact 410 FTIR spectrometer. ^1H NMR and ^{13}C NMR spectra were obtained on a Varian Unity-500, Inova-500, Unity-300 or VXR-300 spectrometer. Mass spectra were obtained on a VG analytical 70S high resolution, double focusing, sectored (EB) mass spectrometer (for FAB), an MDS Sciex (Concord, Ontario, Canada) API Qstar Pulsar (for ESI) or a ThermoFinnigan (San Jose, CA) LCQ Deca Mass Spectrometer (for APCI) at the Mass Spectrometry Application and Collaboration Facility (Texas A&M University). Flash column chromatography was performed using 60 Å Silica Gel (EM Science, 230–400 mesh) as a stationary phase. Enantiomeric excess (ee) was determined by HPLC analysis (RAININ SD-200 with DYANMAX UV-C DETECTOR) using a Chiralpak® AD column. Microwave reactions were carried out in a CEM® Explorer™/Discover™ microwave system.

4.1.1. General procedure for N-alkylation of imidazol-2-one as described for 1,3-bis-(benzyl)-4-(benzyloxy-carbonyl)imidazol-2-one (22a). To a slurry of NaH (3.79 g,

158 mmol) in anhydrous DMF (90 mL) was added imidazolone carboxylic acid **20** (4.01 g, 31.2 mmol) at 0 °C. The ice bath was removed and the reaction was allowed to warm to 22 °C. After stirring for 30 min, benzyl bromide (19.0 mL, 160 mmol) was added and the reaction was stirred for an additional 10 h at 22 °C. Water was added and the mixture was extracted with Et_2O. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→1:9) gave benzyl ester **22a** (5.1 g, 41%) as a light yellow solid: R_f=0.30 (hexanes/EtOAc, 7:3); IR (thin film) 1724, 1695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.25 (m, 15H), 7.01 (s, 1H), 5.25 (s, 2H), 5.15 (s, 2H), 4.86 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.0, 153.5, 137.8, 135.7, 135.4, 129.0, 128.4, 128.36, 128.3, 128.2, 128.0, 127.9, 127.8, 127.3, 120.5, 113.6, 66.1, 47.6, 45.6; HRMS (FAB) Calcd for $C_{25}H_{23}N_2O_3$ [M+H]: 399.1781. Found: 399.1726.

4.1.2. 1,3-Bis(benzyloxymethyl)-4-(methoxycarbonyl)-imidazol-2-one (22b). R_f=0.40 (hexanes/EtOAc, 7:3); IR (thin film) 1731, 1593 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.49 (s, 1H), 7.38–7.21 (m, 10H), 5.49 (s, 2H), 5.19 (s, 2H), 4.60 (s, 2H), 4.59 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 160.1, 154.5, 139.2, 138.5, 129.0, 128.9, 128.5, 128.4, 128.2, 128.17, 122.3, 114.4, 73.5, 71.8, 71.4, 71.3, 51.8; HRMS (FAB) Calcd for $C_{21}H_{23}N_2O_5$ [M+H]: 383.1607. Found: 383.1597.

4.1.3. 1-*tert*-Butoxycarbonyl-4-(methoxycarbonyl)imidazol-2-one (Boc-21). To a slurry of imidazolone methyl ester **21** (943 mg, 6.64 mmol) in 50 mL CH_3CN was added K_2CO_3 (873 mg, 6.32 mmol) and Boc_2O (1.42 g, 6.50 mmol). Upon completion of the reaction as monitored by TLC the reaction mixture was concentrated in vacuo and purified by flash chromatography on SiO_2 eluting with CH_2Cl_2/MeOH (10:0→9.5:0.5) to yield the Boc-protected imidazolone methyl ester **Boc-21** (872 mg, 54%) as an off-white solid. In a separate reaction the crude product was found to be sufficiently pure for alkylation: R_f=0.43 (MeOH/CH_2Cl_2, 1:9); IR (thin film) 1796, 1753, 1738 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 9.90 (br s, 1H), 7.35 (s, 1H), 3.81 (s, 3H), 1.57 (s, 9H); ^{13}C NMR (75 MHz, acetone-d_6) δ 160.0, 150.2, 147.9, 116.4, 115.9, 84.9, 52.3, 27.9; HRMS (ESI) Calcd for $C_{10}H_{15}N_2O_5$ [M+H]: 243.0981. Found: 243.0889.

4.1.4. Methanesulfonic acid 2-(toluene-4-sulfonyl)-ethyl ester (29). To a stirred solution of the sodium salt of toluene sulfinic acid (100 g, 561 mmol) and sodium hydroxide (45 g, 1.12 mol) in water (1000 mL) was added chloroethanol (75.0 mL, 1.12 mol) at 100 °C and stirring continued for 5 h. The reaction was cooled to room temperature and extracted with ethyl acetate (3×500 mL). The combined organic layers were dried over anhydrous $MgSO_4$. Solvents were removed in vacuo and the crude alcohol isolated as an orange oil. Triethylamine (69.6 mL, 499 mmol) was added to a stirred solution of the crude alcohol in dichloromethane (1000 mL) at 0 °C, followed by the addition of MsCl (38.6 mL, 499 mmol) and stirring continued for 30 min. The organic layer was washed with water and dried over anhydrous $MgSO_4$ and concentrated in vacuo to give the crude mesylate. Recrystallization from ethyl acetate/hexane gave mesylate **29** (81.0 g, 52%) as a colorless solid: mp 89–91 °C; R_f=0.38 (hexanes/EtOAc, 6:4); IR (thin film) 1593 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J=8.1 Hz, 2H), 7.40 (d, J=8.1 Hz, 2H), 4.55 (t, J=6.0 Hz, 2H), 3.53 (t, J=6.0 Hz, 2H), 2.98 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.8, 136.3, 130.4, 128.4, 62.2, 55.5, 38.0, 22.0; LRMS (APCI) Calcd for $C_{10}H_{15}S_2O_5$ [M+H]: 279. Found: 279.

4.1.5. Synthesis of 2-oxo-1-[2-(toluene-4-sulfonyl)-ethyl]-2,3-dihydro-1*H*-imidazole-4-carboxylic acid methyl ester (Tse-21). A solution of imidazolone (45.0 g, 317 mmol) in 900 mL DMSO at 25 °C was treated with sodium hydrogen carbonate (53.2 g, 634 mmol). After 10 min at 70 °C, TseOMs (88.1 g, 317 mmol) was added to the resulting solution. The reaction was further stirred for 12 h, and poured onto ice (300 g). The resulting solution was filtered to give a brown solid. The crude solid was purified by dissolution of impurities in ethyl acetate and the solid residue was filtered to afford **Tse-21** (>95% purity, 78.0 g, 76%) as a tan solid. R_f=0.34 (EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 8.24 (br s, 1H), 7.76 (AB, J=8.5 Hz, 2H), 7.35 (AB, J=8.5 Hz, 2H), 7.03 (d, J=2.0 Hz, 1H), 4.16 (t, J=6.5 Hz, 2H), 3.84 (s, 3H), 3.55 (t, J=6.5 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.4, 152.2, 145.3, 136.0, 130.1, 127.8, 120.1, 113.1, 54.1, 51.9, 38.5, 21.6; MS (ESI) 331 [M+Li], 325 [M+H]. HRMS (ESI) Calcd for $C_{14}H_{16}N_2O_5SLi$ [M+Li]: 331.0940. Found: 331.0942.

4.1.6. Synthesis of 2-oxo-1,5-bis-[2-(toluene-4-sulfonyl)-vinyl]-2,3-dihydro-1*H*-imidazole-4-carboxylic acid methyl ester (37). A solution of imidazolone **21** (25.0 mg, 0.18 mmol) in 3 mL DMF at 0 °C was treated with potassium carbonate (1.20 mg, 0.0087 mmol). A solution of 1-ethynesulfonyl-4-methylbenzene (32 mg, 0.18 mmol) in 2 mL DMF was added to the stirred solution dropwise over the period of an hour. The mixture was allowed to warm to room temperature over 12 h. Solvents were removed in vacuo and the crude solid was purified by flash chromatography (SiO_2, EtOAc/hexane, 4:6) to afford **37** (29.0 mg, 66%) as a colorless solid: mp 160–165 °C (dec); R_f=0.60 (EtOAc/hexane, 2:3); IR (thin film) 3587, 1721, 1703, 1633 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.82–7.78 (m, 5H), 7.35 (d, J=8.0 Hz, 2H), 7.32 (d, J=8.0 Hz, 2H), 7.23 (s, 1H), 6.86 (d, J=8.5 Hz, 1H), 6.83 (d, J=13.5.5 Hz, 1H), 6.40 (d, J=8.5 Hz, 1H), 3.85 (s, 3H), 2.44 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 149.1, 145.3, 144.9, 137.8, 137.0, 132.3, 130.2, 130.0, 128.6, 128.3, 123.2, 127.8, 18.4, 117.3, 116.6, 52.7, 21.82, 21.77; MS (ESI) 509 [M+Li]. HRMS (ESI) Calcd for $C_{23}H_{22}N_2O_7S_2Li$ [M+Li]: 509.1028. Found: 509.1016.

4.1.7. 1-Toluenesulfonyl-4-(methoxycarbonyl)imidazol-2-one (Ts-21). To a stirred solution of NaH (219 mg, 9.14 mmol) in 50 mL anhydrous DMF at 0 °C was added imidazolone methyl ester **21** (1.29 g, 9.06 mmol) followed by TsCl (1.74 g, 9.12 mmol). The reaction mixture was heated at 65 °C for 19 h, diluted with pH 7 buffer and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4. Solvents were removed in vacuo and the crude solid

was purified by flash chromatography on SiO_2, eluting with CH_2Cl_2/MeOH (10:0→9:1) to furnish tosylated methyl ester **Ts-21** (1.55 g, 58%) as an off-white solid: mp 191–192 °C (EtOAc/hexanes); R_f=0.40 (hexanes/EtOAc, 6:4); IR (thin film) 1724 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 10.06 (br s, 1H), 8.01 (d, J=8.5 Hz, 2H), 7.50 (d, J=8.5 Hz, 2H), 7.46 (s, 1H), 3.86 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.6, 149.7, 147.2, 134.9, 130.8, 117.0, 116.1, 52.4, 21.6; HRMS (ESI) Calcd for $C_{12}H_{13}N_2O_5S$ [M+H]: 297.0539. Found: 297.0560.

4.1.8. General procedure for the N-alkylation of the acylated or sulfonylated imidazol-2-one as described for 1-*tert*-butoxycarbonyl-3-(4-methoxybenzyl)-4-(methoxycarbonyl)imidazol-2-one (22c). To crude Boc imidazolone methyl ester **Boc-21** (3.41 g, 14.1 mmol) in 100 mL acetonitrile was added K_2CO_3 (6.15 g, 44.5 mmol) and PMBBr (4.00 mL, 28.7 mmol). The reaction mixture was heated to reflux for 16 h, diluted with pH 7 buffer, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (10:0→7:3) gave PMB-protected imidazolone **22c** (2.69 g, 53%) as a light yellow solid: R_f=0.52 (hexanes/EtOAc, 6:4); IR (thin film) 1796, 1753, 1724 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.36 (s, 1H), 7.31 (d, J=8.5 Hz, 2H), 6.77 (d, J=8.5 Hz, 2H), 5.08 (s, 2H), 3.77 (s, 3H), 3.72 (s, 3H), 1.56 (s, 9H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.3, 158.9, 150.9, 146.7, 129.5, 129.3, 116.5, 115.1, 113.6, 85.3, 55.0, 51.8, 44.6, 27.3; HRMS (ESI) Calcd for $C_{18}H_{23}N_2O_6$ [M+H]: 363.1556. Found: 363.1454.

4.1.9. 3-(3,4-Dimethoxybenzyl)-2-oxo-1-[2-(toluene-4-sulfonyl)-ethyl]-2,3-dihydro-1*H*-imidazole-4-carboxylic acid methyl ester (22d). R_f=0.50 (EtOAc); IR (neat) 1721, 1692 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.59 (d, J=8.0 Hz, 2H), 7.23 (m, 2H), 6.66 (d, J=8.0 Hz, 2H), 6.56 (m, 2H), 5.19 (s, 2H), 3.63 (t, J=7.0, 5.5 Hz, 2H), 3.52 (s, 3H), 3.334 (s, 3H), 3.332 (s, 3H), 3.11 (t, J=7.0, 5.5 Hz, 2H), 1.85 (s, 3H); ^{13}C NMR (125 MHz, C_6D_6) δ 159.8, 153.0, 150.1, 149.7, 144.4, 137.3, 131.3, 129.8, 128.0, 121.2, 121.1, 113.3, 113.1, 112.3, 55.6, 55.5, 53.7, 50.8, 45.3, 38.7, 21.1; HRMS (ESI) Calcd for $C_{23}H_{27}N_2O_7S$ [M+H]: 475.1539. Found: 475.1529.

4.1.10. 1-Toluenesulfonyl-3-(4-methoxybenzyl)-4-(methoxycarbonyl)imidazol-2-one (22e). R_f=0.32 (hexanes/EtOAc, 7:3); IR (thin film) 1726 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.01 (d, J=8.5 Hz, 2H), 7.55 (s, 1H), 7.52 (d, J=8.5 Hz, 2H), 7.12 (d, J=9.0 Hz, 2H), 6.79 (d, J=9.0 Hz, 2H), 4.98 (s, 2H), 3.80 (s, 3H), 3.74 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 160.1, 159.9, 150.4, 147.5, 134.6, 130.9, 130.1, 129.9, 129.4, 117.1, 114.6, 55.4, 52.4, 45.5, 21.6; HRMS (ESI) Calcd for $C_{20}H_{21}N_2O_6S$ [M+H]: 417.1120. Found: 417.1078.

4.1.11. 1-Toluenesulfonyl-3-(3,4-dimethoxybenzyl)-4-(methoxycarbonyl)imidazol-2-one (22f). R_f=0.36 (hexanes/EtOAc, 6:4); IR (thin film) 1731, 1724 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.03 (d, J=8.5 Hz, 2H), 7.56 (s, 1H), 7.51 (d, J=8.5 Hz, 2H), 6.78 (m, 1H), 6.77 (s, 1H), 6.71 (m, 1H), 4.98 (s, 2H), 3.80 (s, 3H), 3.74 (s, 3H), 3.61 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.8, 150.3, 150.1, 149.8, 147.5, 134.6, 130.8, 130.7, 130.6, 129.4, 120.9, 117.0, 112.4, 112.3, 55.9, 55.8, 52.4, 45.7, 21.6; HRMS (ESI) Calcd for $C_{21}H_{23}N_2O_7S$ [M+H]: 447.1226. Found: 447.1205.

4.1.12. General procedure for DIBAl-H reduction of imidazolone ester as described for 1,3-bis(benzyl)-4-(hydroxymethylene)imidazol-2-one (23a). To a cooled (−78 °C) solution of benzyl ester **22a** (8.61 g, 21.6 mmol) in 165 mL CH_2Cl_2 was added a 0.99 M solution of DIBAl-H in CH_2Cl_2 (54.6 mL, 53.8 mmol). The reaction was stirred for 3 h at −78 °C and additional DIBAl-H was added (11.0 mL, 1.0 M, 11.2 mmol). After 4 h MeOH (40 mL) was added at −78 °C followed by a solution of Rochelle's salt and the heterogeneous mixture was allowed to warm to 22 °C overnight. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→1:9) afforded alcohol **23a** (5.55 g, 87%) as an off-white solid: R_f=0.28 (hexanes/EtOAc, 3:7); IR (thin film) 3360, 1687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35–7.20 (m, 10H), 6.01 (s, 1H), 4.95 (s, 2H), 4.73 (s, 2H), 4.15 (s, 2H), 2.92 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.8, 137.6, 136.7, 128.8, 128.7, 128.0, 127.9, 127.5, 127.2, 122.3, 109.0, 55.3, 47.1, 44.9; HRMS (ESI) Calcd for $C_{18}H_{19}N_2O_2$ [M+H]: 295.1497. Found: 295.1584.

4.1.13. 1,3-Bis(benzyloxymethyl)-4-(hydroxymethylene)imidazol-2-one (23b). R_f=0.09 (hexanes/EtOAc, 6:4); IR (thin film) 3491, 1702 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.37–7.25 (m, 9H), 7.13–7.07 (m, 1H), 6.54 (s, 1H), 5.30 (s, 2H), 5.09 (s, 2H), 4.59 (s, 2H), 4.56 (s, 2H), 4.51 (s, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 154.8, 138.8, 138.7, 130.1, 128.94, 128.93, 128.43, 128.41, 128.25, 128.23, 126.5, 124.1, 109.8, 72.9, 71.2, 70.9, 70.8, 55.0; HRMS (FAB) Calcd for $C_{20}H_{23}N_2O_4$ [M+H]: 355.1650. Found: 355.1658.

4.1.14. 3-(3,4-Dimethoxybenzyl)-4-hydroxymethyl-1-[2-(toluene-4-sulfonyl)-ethyl]-1,3-dihydro-imidazol-2-one (23d). R_f=0.15 (EtOAc); IR (film) 3367, 1670 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.75 (d, J=8.0 Hz, 2H), 7.32 (d, J=8.0 Hz, 2H), 6.85 (s, 1H), 6.78 (m, 2H), 6.22 (s, 1H), 4.80 (s, 2H), 4.22 (s, 2H), 4.01 (t, J=6.5 Hz, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.50 (t, J=7.0, 6.5 Hz, 2H), 2.42 (s, 3H), 2.32 (br s, 1H); ^{13}C NMR (125 MHz, C_6D_6) δ 153.2, 149.1, 148.5, 145.1, 136.0, 130.0, 129.8, 127.8, 122.5, 119.6, 111.1, 110.8, 109.6, 55.9, 55.8, 55.1, 54.3, 44.6, 38.0, 21.6; HRMS (ESI) Calcd for $C_{22}H_{27}N_2O_6S$ [M+H]: 453.1672. Found: 453.1647.

4.1.15. 3-(4-Methoxybenzyl)-1-toluenesulfonyl-4-(hydroxymethylene)imidazol-2-one (23e). R_f=0.07 (hexanes/EtOAc, 7:3); IR (thin film) 3411, 1709 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.95 (d, J=8.5 Hz, 2H), 7.46 (d, J=8.5 Hz, 2H), 7.10 (d, J=8.5 Hz, 2H), 6.81 (d, J=8.5 Hz, 2H), 6.77 (s, 1H), 4.74 (s, 2H), 4.29 (s, 2H), 3.75 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 160.1, 151.3, 146.6, 135.6, 130.6, 129.9, 129.4, 128.8, 114.7, 105.9, 55.4, 55.2, 44.7, 21.5; HRMS (ESI) Calcd for $C_{19}H_{20}N_2O_5SLi$ [M+Li]: 395.1253. Found: 395.1234.

4.1.16. 3-(3,4-Dimethoxybenzyl)-1-toluenesulfonyl-4-(hydroxymethylene)imidazol-2-one (23f). Mp 135–136 °C (EtOAc/hexanes); R_f=0.09 (hexanes/EtOAc, 6:4); IR (thin film) 3418, 1716 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.96 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H), 6.81 (d, J=7.5 Hz, 1H), 6.77 (app t, J=1.5 Hz, 1H), 6.68 (dd, J=7.5, 1.5 Hz, 1H), 4.74 (s, 2H), 4.29 (d, J=5.5 Hz, 2H), 3.75 (s, 3H), 3.62 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 150.7, 149.8, 149.2, 146.0, 135.1, 130.9, 129.9, 128.3, 126.9, 119.7, 112.0, 111.3, 105.2, 55.4, 55.2, 54.6, 44.4, 20.9; HRMS (ESI) Calcd for $C_{20}H_{23}N_2O_6S$ [M+H]: 419.1277. Found: 419.1189.

4.1.17. General procedure for oxidation of 4-(hydroxymethylene)imidazol-2-one as described for 1,3-bis(benzyl)4-(carboxaldehyde)imidazol-2-one (24a). To activated MnO_2 (13.1 g, 151 mmol) was added alcohol **23a** (5.55 g, 18.9 mmol) in 40 mL CH_2Cl_2, rinsing twice with 20 mL CH_2Cl_2 to complete the transfer. After stirring for 10 h at ambient temperature the reaction mixture was filtered through Celite and concentrated in vacuo to yield aldehyde **24a** (5.38g, 98%) as a yellow viscous oil. No further purification was required: R_f=0.67 (hexanes/EtOAc, 3:7); IR (thin film) 1702, 1658 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.43–7.23 (m, 10H), 6.89 (s, 1H), 5.23 (s, 2H), 4.87 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 153.3, 137.4, 135.1, 129.1, 128.5, 128.4, 128.2, 127.6, 127.2, 47.8, 45.9; HRMS (FAB) Calcd for $C_{18}H_{17}N_2O_2$ [M+H]: 293.1290. Found: 293.1279.

4.1.18. 1,3-Bis(benzyloxymethyl)-4-(carboxaldehyde)-imidazol-2-one (24b). R_f=0.25 (hexanes/EtOAc, 6:4); IR (thin film) 1731, 1673 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 9.36 (s, 1H), 7.72 (s, 1H), 7.36–7.28 (m, 10H), 5.49 (s, 2H), 5.23 (s, 2H), 4.62 (br s, 4H); ^{13}C NMR (125 MHz, acetone-d_6) δ 178.3, 156.3, 139.1, 138.3, 129.7, 129.0, 128.9, 128.5, 128.4, 128.16, 128.13, 124.0, 73.6, 72.1, 71.5, 71.3; HRMS (FAB) Calcd for $C_{20}H_{21}N_2O_4$ [M+H]: 353.1501. Found: 353.1507.

4.1.19. 3-(3,4-Dimethoxybenzyl)-2-oxo-1-(2-tosylethyl)-2,3-dihydro-1*H*-imidazole-4-carbaldehyde (24d). Mp 150–152 °C; R_f=0.39 (EtOAc); IR (KBr) 2837, 1708, 1660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 9.21 (s, 1H), 7.72 (d, J=8.5 Hz, 2H), 7.29 (d, J=8.5 Hz, 2H), 7.14 (s, 1H), 7.02 (d, J=2.0 Hz, 1H), 6.97 (dd, J=8.0, 2.0 Hz, 1H), 6.77 (d, J=8.0 Hz, 1H), 5.04 (s, 2H), 4.19 (t, J=6.5, 5.5 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.56 (t, J=6.5, 5.5 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.8, 152.6, 148.8, 148.6, 145.4, 136.0, 130.1, 129.8, 128.3, 127.6, 122.8, 121.0, 111.9, 110.9, 55.8, 53.7, 45.6, 38.9, 21.6; HRMS (ESI) Calcd for $C_{22}H_{24}N_2O_6SLi$ [M+Li]: 451.1515. Found: 451.1496.

4.1.20. 3-(4-Methoxybenzyl)-1-toluenesulfonyl-4-(carboxaldehyde)imidazol-2-one (24e). R_f=0.31 (hexanes/EtOAc, 7:3); IR (thin film) 2836, 1731, 1673 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 9.45 (s, 1H), 8.02 (d, J=8.5 Hz, 2H), 7.99 (s, 1H), 7.52 (d, J=8.5 Hz, 2H), 7.17 (d, J=8.5 Hz, 2H), 6.80 (d, J=8.5 Hz, 2H), 4.97 (s, 2H), 3.74 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 179.9, 159.6, 149.6, 147.1, 133.8, 130.3, 129.5, 128.9, 124.1, 113.9, 54.8, 45.2, 21.0; HRMS (ESI) Calcd for $C_{19}H_{18}N_2O_5SLi$ [M+Li]: 393.1096. Found: 393.1081.

4.1.21. 3-(3,4-Dimethoxybenzyl)-1-toluenesulfonyl-4-(carboxaldehyde)imidazol-2-one (24f). R_f=0.23 (hexanes/EtOAc, 6:4); IR (thin film) 2836, 1731, 1673 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 9.42 (s, 1H), 8.01 (d, J=8.4 Hz, 2H), 7.98 (s, 1H), 7.51 (d, J=8.4 Hz, 2H), 6.82–6.74 (m, 3H), 4.96 (s, 2H), 3.73 (s, 3H), 3.62 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.0, 150.21, 150.17, 150.0, 147.7, 134.4, 130.9, 130.5, 129.5, 125.5, 124.7, 121.1, 112.6, 112.5, 56.0, 55.8, 46.0, 21.6; HRMS (ESI) Calcd for $C_{20}H_{21}N_2O_6S$ [M+H]: 417.1120. Found: 417.1089.

4.1.22. General procedure for Horner–Wadsworth–Emmons reaction as described for 1,3-bis(benzyl)-4-(ethylpropenoate)imidazol-2-one (25a). To a cooled (0 °C) suspension of NaH (11.8 mg, 0.49 mmol) in 2.5 mL THF was slowly added triethylphosphonoacetate (98.0 μL, 0.49 mmol). After stirring the reaction at 0 °C for 5 min the ice bath was removed and the mixture was stirred at 22 °C for 1 h. Aldehyde **24a** (131 mg, 0.45 mmol) was added at ambient temperature in 2 mL THF. After 2 h pH 4 buffer was added and the layers were separated. The aqueous layer was extracted with Et_2O and the combined organics were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give a mixture of product and starting material. The crude reaction mixture was resubjected to the reaction conditions and furnished upon purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→4:6) ester **25a** (44.7 mg, 89%) as a light yellow solid: R_f=0.34 (hexanes/EtOAc, 6:4); IR (thin film) 1687, 1629 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.20 (m, 10H), 7.14 (d, J=15.9 Hz, 1H), 6.57 (s, 1H), 5.90 (d, J=15.9 Hz, 1H), 5.02 (s, 2H), 4.86 (s, 2H), 4.12 (q, J=7.2 Hz, 2H), 1.22 (t, J=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 153.5, 136.5, 135.9, 129.8, 128.8, 128.7, 128.0, 127.9, 127.5, 126.6, 120.1, 114.4, 114.2, 60.2, 47.3, 45.1, 14.1; HRMS (FAB) Calcd for $C_{22}H_{23}N_2O_2$ [M+H]: 363.1709. Found: 363.1691. Generally, the HWE olefination proceeded smoothly and all the aldehyde was consumed, such that the crude reaction mixture did not have to be resubjected.

4.1.23. 1,3-Bis(benzyloxymethyl)-4-(ethylpropenoate)-imidazol-2-one (25b). R_f=0.40 (hexanes/EtOAc, 6:4); IR (thin film) 1702, 1636 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.39–7.25 (m, 12H), 6.39 (d, J=16.0 Hz, 1H), 5.31 (s, 2H), 5.17 (s, 2H), 4.62 (s, 2H), 4.59 (s, 2H), 4.18 (q, J=7.0 Hz, 2H), 1.26 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 167.2, 154.8, 138.6, 138.56, 131.0, 129.0, 128.6, 128.5, 128.3, 121.4, 118.0, 115.8, 73.3, 71.7, 71.3, 70.9, 66.6, 14.6; HRMS (FAB) Calcd for $C_{24}H_{27}N_2O_5$ [M+H]: 423.1920. Found: 423.1922.

4.1.24. 3-{3-(3,4-Dimethoxybenzyl)-2-oxo-1-[2-(toluene-4-sulfonyl)-ethyl]-2,3-dihydro-1*H*-imidazol-4-yl}-acrylic acid ethyl ester (25d). A solution of triethylphosphonoacetate (10.3 mL, 52.0 mmol) in 100 mL THF was cooled to 0 °C and treated with 80% NaH (1.43 g, 49.5 mmol), allowed to warm to 25 °C and further stirred for 40 min. To the resulting solution was added dropwise a solution of

aldehyde **49** (22.0 g, 49.5 mmol) in 300 mL THF and then stirring was continued for 2 h. The reaction was quenched with 100 mL of pH 4 buffer solution. THF was removed in vacuo and the aqueous phase was extracted with EtOAc (3×300 mL). The organic layers were combined and dried ($MgSO_4$). Solvents were removed in vacuo and the crude ester **50** was isolated as a yellow oil. The ester was generally used without purification.

4.1.25. 3-(4-Methoxybenzyl)-1-toluenesulfonyl-4-(ethylpropenoate)imidazol-2-one (25e). R_f=0.25 (hexanes/EtOAc, 7:3); IR (thin film) 1724, 1716 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.99 (d, J=8.5 Hz, 2H), 7.56 (s, 1H), 7.51 (d, J=8.5 Hz, 2H), 7.22 (d, J=16.5 Hz, 1H), 7.04 (d, J=8.5 Hz, 2H), 6.84 (d, J=8.5 Hz, 2H), 6.36 (d, J=16.5 Hz, 1H), 4.87 (s, 2H), 4.14 (q, J=7.0 Hz, 2H), 3.75 (s, 3H), 2.48 (s, 3H), 1.23 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 166.3, 160.2, 150.9, 147.1, 135.1, 130.8, 129.9, 129.3, 129.1, 128.9, 123.7, 119.9, 115.0, 110.7, 61.0, 55.5, 44.8, 21.6, 14.5; HRMS (ESI) Calcd for $C_{23}H_{24}N_2O_6S$ [M+H]: 457.1433. Found: 457.1415.

4.1.26. 3-(3,4-Dimethoxybenzyl)-1-toluenesulfonyl-4-(ethylpropenoate)imidazol-2-one (25f). R_f=0.25 (hexanes/EtOAc, 6:4); IR (thin film) 1724, 1716 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.01 (d, J=8.5 Hz, 2H), 7.57 (app t, J=0.5 Hz, 1H), 7.51 (d, J=8.5 Hz, 2H), 7.25 (dd, J=16.0, 0.5 Hz, 1H), 6.83 (d, J=8.0 Hz, 1H), 6.73 (d, J=2.0 Hz, 1H), 6.62 (dd, J=8.0, 2.0 Hz, 1H), 6.37 (dd, J=16.0, 0.5 Hz, 1H), 4.87 (s, 2H), 4.14 (q, J=7.0 Hz, 2H), 3.75 (s, 3H), 3.63 (s, 3H), 2.48 (s, 3H), 1.22 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 166.3, 150.8, 150.5, 149.9, 147.1, 135.1, 130.8, 129.9, 129.8, 129.2, 123.7, 119.9, 119.8, 112.8, 111.5, 110.6, 61.0, 56.0, 55.8, 45.1, 21.6, 14.8; HRMS (ESI) Calcd for $C_{24}H_{27}N_2O_7S$ [M+H]: 487.1539. Found: 487.1568.

4.1.27. General procedure for preparation of diene as described for 1,3-bis(benzyl)-4-(3-hydroxypropenyl)-imidazol-2-one (16a). To a cooled (−78 °C) solution of α,β-unsaturated ester **25a** (1.10 g, 3.05 mmol) was added a 1.0 M solution of DIBAl-H in CH_2Cl_2 (9.15 mL, 9.15 mmol). After 2 h, MeOH (15 mL) was added followed by Rochelle's salt solution. Upon stirring for 8 h the layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organics were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give diene **16a** (977 mg, 99%) as a yellow foam. No further purification was required: R_f=0.17 (hexanes/EtOAc, 3:7); IR (thin film) 3404, 1673 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.39–7.25 (m, 10H), 6.64 (s, 1H), 6.24 (d, J=15.5 Hz, 1H), 6.04 (dt, J=5.5, 15.5 Hz, 1H), 4.96 (s, 2H), 4.85 (s, 2H), 4.07 (app t, J=5.5 Hz, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 154.3, 139.3, 139.0, 130.3, 129.4, 129.3, 128.5, 128.3, 128.0, 127.6, 122.7, 116.5, 108.3, 62.8, 47.3, 45.1; HRMS (ESI) Calcd for $C_{20}H_{21}N_2O_2$ [M+H]: 321.1603. Found: 321.1595.

4.1.28. 1,3-Bis(benzyloxymethyl)-4-(3-hydroxypropenyl) imidazol-2-one (16b). R_f=0.31 (hexanes/EtOAc, 3:7); IR (thin film) 3404, 1687 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) 7.20–7.09 (m, 10H), 6.51 (s, 1H), 6.23 (d, J=16.0 Hz, 1H), 6.14 (dt, J=5.0, 16.0 Hz, 1H), 5.03 (s, 2H), 4.93 (s, 2H), 4.42 (s, 2H), 4.39 (s, 2H), 4.05 (br s, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 154.7, 138.8, 138.7, 136.9, 131.3, 130.2, 128.94, 128.93, 128.4, 128.3, 126.5, 123.2, 116.2, 109.1, 73.2, 71.3, 70.9, 70.8, 62.9; HRMS (FAB) Calcd for $C_{22}H_{25}N_2O_4$ [M+H]: 381.1814. Found: 381.1800.

4.1.29. 3-(3,4-Dimethoxybenzyl)-4-(3-hydroxypropenyl)-1-[2-(toluene-4-sulfonyl)-ethyl]-1,3-dihydro-imidazol-2-one (16d). Mp 81–83 °C (EtOAc/hexanes); R_f=0.16 (EtOAc); IR (film) 3432, 1713 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.70 (d, J=8.0 Hz, 2H), 6.90 (d, J=2.0 Hz, 1H), 6.82 (dd, J=8.0, 2.0 Hz, 1H), 6.73 (d, J=8.0 Hz, 2H), 6.54 (d, J=8.0 Hz, 1H), 6.09 (d, J=16.0 Hz, 1H), 5.90 (s, 1H), 5.78 (dt, J=16.0, 5.5 Hz, 1H), 4.73 (s, 2H), 3.86 (br s, 2H), 3.86 (t, J=6.5, 6.0 Hz, 2H), 3.50 (s, 3H), 3.33 (s, 3H), 3.21 (t, J=6.5, 6.0 Hz, 2H), 1.86 (s, 3H); ^{13}C NMR (125 MHz, C_6D_6) δ 153.4, 150.4, 149.6, 137.5, 130.6, 129.8, 129.4, 128.3, 127.9, 122.1, 119.7, 116.6, 112.4, 111.9, 108.1, 62.9, 55.7, 55.5, 54.4, 44.8, 38.2, 21.1; HRMS (ESI) Calcd for $C_{24}H_{28}N_2O_6SNa$ [M+Na]: 473.1746. Found: 473.1704.

4.1.30. 3-(4-Methoxybenzyl)-1-toluenesulfonyl-4-(3-hydroxypropenyl)imidazol-2-one (16e). R_f=0.36 (hexanes/EtOAc, 3:7); IR (thin film) 3491, 1709 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.96 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H), 7.02 (d, J=8.5 Hz, 2H), 6.95 (s, 1H), 6.80 (d, J=8.5 Hz, 2H), 6.33 (dt, J=4.0, 16.5 Hz, 1H), 6.24 (m, 1H), 4.71 (s, 2H), 4.14 (m, 2H), 3.75 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 160.0, 151.0, 146.6, 135.5, 135.3, 130.6, 129.7, 129.0, 128.9, 126.3, 114.7, 114.6, 104.0, 62.3, 55.4, 44.5; HRMS (ESI) Calcd for $C_{21}H_{23}N_2O_5S$ [M+H]: 415.1328. Found: 415.1297.

4.1.31. 3-(3,4-Dimethoxybenzyl)-1-toluenesulfonyl-4-(3-hydroxypropenyl)imidazol-2-one (16f). R_f=0.24 (hexanes/EtOAc, 3:7); IR (thin film) 3433, 1716 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.77 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H), 6.95 (s, 1H) 6.79 (d, J=8.0 Hz, 1H), 6.72 (d, J=2.0 Hz, 1H), 6.62 (dd, J=8.0, 2.0 Hz, 1H), 6.33 (dt, J=16.0, 4.0 Hz, 1H), 6.27 (m, 1H), 4.71 (s, 2H), 4.18 (br s, 2H), 3.75 (s, 3H), 3.62 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 151.0, 150.4, 149.8, 146.6, 135.6, 135.3, 130.6, 130.3, 128.9, 126.3, 120.0, 114.7, 112.7, 111.7, 103.9, 62.4, 56.0, 55.8, 44.8, 21.5; HRMS (ESI) Calcd for $C_{22}H_{25}N_2O_6S$ [M+H]: 445.1433. Found: 445.1452.

4.1.32. Diels–Alder adduct 34a. A heterogeneous mixture of diene **16a** (33.9 mg, 0.11 mmol) and dienophile **15a** (36.3 mg, 0.10 mmol) in 500 μL *o*-xylene was heated to 140 °C for 24 h. The reaction mixture was concentrated in vacuo and purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (7:3→3:7) furnished Diels–Alder adduct **34a** (19.1 mg, 28%) as a light yellow solid: R_f=0.54 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ −55.3° (*c* 1.64, CH_2Cl_2); IR (thin film) 3418, 1724, 1680, 1651 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34–7.22 (m, 8H), 7.12 (m, 2H), 5.16 (d, J=16.2 Hz, 1H), 4.93 (d, J=15.9 Hz, 1H), 4.69 (d, J=15.9 Hz, 1H), 4.65 (d, J=16.2 Hz, 1H), 3.96–3.85 (m, 4H), 3.69 (dd, J=6.3, 14.4 Hz, 1H), 3.43 (dd, J=3.6, 7.5 Hz, 1H), 3.14 (d, J=7.5 Hz, 1H), 2.50 (ddd,

J=2.7, 12.0, 15.9 Hz, 1H), 2.22 (m, 1H), 2.03–1.95 (m, 1H), 1.40 (s, 9H), 0.98–0.95 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 154.7, 149.4, 137.3, 137.1, 128.9, 128.8, 127.7, 127.6, 127.3, 126.6, 120.5, 114.7, 88.7, 64.5, 63.7, 62.0, 46.1, 45.4, 44.9, 36.8, 33.9, 27.9, 19.2, 17.9, 11.8; HRMS (ESI) Calcd for $C_{39}H_{56}N_3O_6Si$ [M+H]: 690.3938. Found: 690.3939.

4.1.33. Diels–Alder adduct 34b. To a solution of dienophile **15b** (2.77 g, 6.55 mmol) in 50 mL PhH was added diene **16a** (2.81 g, 6.52 mmol) and 2,6-lutidine (450 μL, 3.95 mmol). The reaction mixture was heated to 95 °C in a sealed tube for three days. Upon cooling additional diene **16a** (891 mg, 2.79 mmol) was added. After stirring at 95 °C for an additional 24 h, the reaction was concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (8:2→1:9) gave 3.09 g (64%) Diels–Alder adduct **34b** as a yellow foam along with 714.1 mg (15%) of regioisomer **35b**. **34b**: R_f=0.69 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ −53.0° (*c* 2.53, CH_2Cl_2); IR (thin film) 3475, 1738, 1680 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J=8.1 Hz, 2H), 7.34–7.10 (m, 12H), 5.23 (d, J=16.2 Hz, 1H), 4.91 (d, J=16.2 Hz, 1H), 4.62 (d, J=16.2 Hz, 1H), 4.32 (d, J=16.2 Hz, 1H), 4.21 (dd, J=2.7, 5.1 Hz, 1H), 4.04 (dd, J=5.1, 10.5 Hz, 1H), 3.92 (dd, J=2.7, 10.5 Hz, 1H), 3.74 (app t, J=3.9 Hz, 2H), 3.41 (dd, J=3.0, 6.9 Hz, 1H), 3.18 (app br d, J=6.9 Hz, 1H), 2.40 (s, 3H), 2.00–1.90 (m, 2H), 1.88–1.80 (m, 1H), 1.05–0.95 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6, 154.6, 145.1, 137.03, 137.0, 135.0, 129.4, 128.9, 128.8, 128.5, 128.4, 127.9, 127.6, 127.1, 127.0, 120.8, 114.0, 65.2, 64.3, 64.1, 45.6, 45.0, 44.7, 36.4, 35.5, 21.7, 18.9, 17.9, 11.8; HRMS (FAB) Calcd for $C_{41}H_{53}N_3O_6SSiNa$ [M+Na]: 766.3322. Found: 766.3314. **35b**: R_f=0.42 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ −24.1° (*c* 1.85, CH_2Cl_2); mp 76.0–78.0 °C; IR (thin film) 3385, 1737, 1690 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.91 (d, J=8.0 Hz, 2H), 7.36–7.26 (m, 10H), 7.10 (d, J=8.0 Hz, 2H), 5.09 (d, J=16.5 Hz, 1H), 5.01 (d, J=16.5 Hz, 1H), 4.91 (d, J=16.0 Hz, 1H), 4.71 (d, J=16.0 Hz, 1H), 4.36 (br s, 1H), 4.25 (dd, J=3.5, 11.0 Hz, 1H), 4.02 (m, 1H), 3.85 (m, 1H), 3.62 (d, J=7.5 Hz, 1H), 3.52–3.49 (m, 1H), 3.22 (m, 1H), 3.00–2.97 (m, 1H), 2.69 (dd, J=4.0, 16.5, 1H), 2.42 (s, 3H), 2.34 (ddd, J=2.5, 4.0, 16.5 Hz, 1H), 2.27–2.22 (m, 1H), 0.94–0.93 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 172.4, 154.7, 146.1, 139.4, 139.1, 136.5, 130.2, 129.3, 129.2, 128.9, 128.0, 127.9, 127.7, 127.1, 118.8, 111.2, 66.1, 63.1, 60.6, 45.2, 44.9, 40.2, 39.2, 37.5, 21.5, 20.8, 18.2, 12.5; HRMS (FAB) Calcd for $C_{41}H_{54}N_3O_6SSi$ [M+H]: 744.3503. Found: 744.3508.

4.1.34. Diels–Alder adduct 34c. To dienophile **15b** (275 mg, 0.65 mmol) and diene **16b** (230 mg, 0.61 mmol) was added 5 mL PhH and 2,6-lutidine (40.0 μL, 0.34 mmol). The reaction mixture was heated to 95 °C in a sealed vial for 17 h, concentrated in vacuo, and purified by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→1:1) to furnish 148 mg (30%) Diels–Alder adduct **34c** along with the 31.1 mg presumed regioisomer: R_f=0.62 (hexanes/EtOAc, 3:7); IR (thin film) 3476, 1738 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.64 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H), 7.37–7.24 (m, 10 H), 5.15 (d, J=11.0 Hz, 1H), 5.11 (d, J=11.0 Hz, 1H), 5.09 (d, J=11.0 Hz, 1H), 5.05 (d, J=11.0 Hz, 1H), 4.80 (d, J=12.5 Hz, 1H), 4.74 (app t, J=4.0 Hz, 1H), 4.71 (d, J=12.5 Hz, 1H), 4.50 (d, J=12.0 Hz, 1H), 4.43 (d, J=12.0 Hz, 1H), 4.17 (d, J=4.0 Hz, 2H), 3.90–3.80 (m, 2H), 3.65 (dd, J=3.0, 7.0 Hz, 1H), 3.60 (m, 1H), 2.41 (ddd, J=1.0, 4.5, 15.5 Hz, 1H), 1.99–1.91 (m, 1H), 1.71 (ddd, J=3.0, 11.5, 15.5 Hz, 1H), 1.14–1.12 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 174.6, 155.2, 145.6, 138.8, 138.7, 136.4, 130.0, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 121.1, 115.9, 71.7, 71.4, 70.9, 70.8, 65.8, 64.2, 63.3, 42.4, 38.1, 35.6, 21.5, 19.6, 18.4, 12.6; HRMS (MALDI) Calcd for $C_{43}H_{58}N_3O_8SSi$ [M+H]: 804.3708. Found: 804.3698.

4.1.35. Diels–Alder adduct 34e. To dienophile **15b** (663 mg, 1.57 mmol) and diene **16e** (650 mg, 1.57 mmol) was added 7 mL PhH and 2,6-lutidine (100 μL, 0.86 mmol). The reaction mixture was heated to 114 °C in a sealed vial for 19 h, concentrated in vacuo and purified by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→1:1) to furnish 494 mg (59%) Diels–Alder adduct **34e** as a single regioisomer: R_f=0.25 (hexanes/EtOAc, 6:4); IR (thin film) 3476, 1731 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.94 (d, J=8.0 Hz, 2H), 7.62 (d, J=8.5 Hz, 2H), 7.51 (d, J=8.0 Hz, 2H), 7.31 (d, J=8.5 Hz, 2H), 6.67 (s, 4H), 4.66 (d, J=16.0 Hz, 1H), 4.41 (t, J=2.0 Hz, 1H), 4.36 (dd, J=2.5, 6.0 Hz, 2H), 4.05 (d, J=16.0 Hz, 1H), 3.87 (m, 1H), 3.81–3.75 (m, 2H), 3.73 (s, 3H), 3.71 (m, 1H), 2.51 (s, 3H), 2.46 (s, 3H), 2.01 (br s, 1H), 2.00–1.96 (m, 1H), 1.72 (m, 1H), 1.14–1.11 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 175.0, 159.9, 152.6, 146.7, 146.0, 138.6, 135.1, 130.6, 130.5, 129.1, 129.0, 128.6, 127.9, 127.8, 114.7, 114.6, 67.2, 66.0, 63.2, 55.4, 44.1, 43.8, 39.0, 37.0, 21.7, 21.6, 20.5, 18.4, 12.6; HRMS (ESI) Calcd for $C_{42}H_{56}N_3O_9S_2Si$ [M+H]: 838.3227. Found: 838.3303.

4.1.36. Diels–Alder adduct 34f. R_f=0.44 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ −71.9° (*c* 1.66, CH_2Cl_2); IR (thin film) 3440, 1724 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.96 (d, J=8.5 Hz, 2H), 7.63 (d, J=8.5 Hz, 2H), 7.49 (d, J=7.5 Hz, 2H), 7.32 (d, J=7.5 Hz, 2H), 6.66 (d, J=8.0 Hz, 1H), 6.57 (d, J=2.0 Hz, 1H), 6.23 (dd, J=8.0, 2.0 Hz, 1H), 4.68 (d, J=16.0 Hz, 1H), 4.42 (s, 1H), 4.37 (m, 2H), 4.03 (d, J=16.0 Hz, 1H), 3.87 (m, 1H), 3.82–3.75 (m, 2H), 3.76–3.71 (m, 2H), 3.74 (s, 3H), 3.58 (s, 3H), 2.49 (s, 3H), 2.47 (s, 3H), 2.10 (m, 1H), 2.01–1.95 (m, 1H), 1.74 (m, 1H), 1.14–1.23 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 175.0, 152.3, 150.4, 149.8, 146.8, 146.0, 136.6, 135.8, 130.6, 130.5, 129.7, 129.2, 127.9, 127.5, 119.6, 114.8, 112.4, 111.5, 67.3, 66.1, 63.2, 56.0, 55.9, 44.2, 44.1, 39.0, 37.1, 21.7, 21.6, 20.5, 18.41, 18.40, 12.7; HRMS (ESI) Calcd for $C_{43}H_{57}N_3O_{10}S_2SiNa$ [M+Na]: 890.3152. Found: 890.3201.

4.1.37. 3-(3,4-Dimethoxybenzyl)-2-oxo-1-[2-(toluene-4-sulfonyl)-vinyl]-2,3-dihydro-1*H*-imidazole-4-carboxylic acid methyl ester (38). To a stirred solution of **21** (3.80 g, 26.7 mmol) in 200 mL of DMF at 25 °C was added 80% sodium hydride (722 mg, 24.1 mmol), the resulting mixture was stirred for 15 min. To the slurry was added dropwise a solution of *cis*-1,2-di-*p*-toluenesulfonylethylene (6.30 g, 18.7 mmol) in 100 mL of DMF. Stirring was continued for 13 h, the reaction was cooled to 0 °C and then ethyl acetate (200 mL) and water (200 mL) were added to quench the

reaction. The organic layer was washed with water (6×100 mL) and dried ($MgSO_4$). Solvents were removed in vacuo and the crude solid was purified by dissolution of impurities in ethyl acetate and filtering the residue to afford the Tsv-protected imidazolone ester **Tsv-21** (>95% purity, 3.41 g, 56%) as a light yellow solid (mp 239–241 °C (EtOAc/hexanes)). To a solution of **Tsv-21** (13.4 g, 41.4 mmol) in 300 mL of DMF at 25 °C was added potassium carbonate (6.30 g, 45.6 mmol) followed by DMBCl (8.10 g, 43.5 mmol). The reaction was heated to 60 °C and stirring continued for 16 h. On cooling to 25 °C the reaction was diluted with 200 mL of ethyl acetate and washed with water (6×100 mL). The organic layer was dried ($MgSO_4$). Solvents were removed in vacuo to afford the ester **38** (19.6 g, 99%), which was of sufficient purity to be taken onto the next step without purification. A small sample of crude **38** was taken and purified by flash chromatography for analysis on SiO_2, eluting with EtOAc/hexanes (2:5) affording ester **38** as a colorless foam: R_f=0.31 (EtOAc/hexanes, 2:5); IR (neat) 1716 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J=14.0 Hz, 1H), 7.77 (d, J=8.0 Hz, 2H), 7.32 (d, J=8.0 Hz, 2H), 7.12 (s, 1H), 6.97 (d, J=2.0 Hz, 1H), 6.91 (dd, J=8.5, 2.0 Hz, 1H), 6.85 (d, J=14.0 Hz, 1H), 6.76 (d, J=8.5 Hz, 1H), 5.13 (s, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 151.3, 148.8, 148.6, 144.5, 137.8, 132.9, 130.0, 129.3, 127.5, 120.8, 117.0, 116.6, 115.8, 111.7, 110.9, 55.9, 55.8, 52.1, 45.4, 21.6; HRMS (ESI) Calcd for $C_{23}H_{25}N_2O_7S$ [M+H]: 473.1382. Found: 473.1410.

4.1.38. 3-(3,4-Dimethoxybenzyl)-4-hydroxymethyl-1-[2-(toluene-4-sulfonyl)-vinyl]-1,3-dihydro-imidazol-2-one (40). To a stirred solution of crude ester **38** (19.6 g, 41.4 mmol) in 400 mL of THF/H_2O (v/v; 3:1) at 25 °C was added LiOH (1.14 g, 47.5 mmol) and stirred continuously for 2 h and then the THF was removed in vacuo. EtOAc (400 mL) was added and the mixture was extracted (the organic phase was discarded). HCl (1 M, 500 mL) was added to the aqueous phase and extracted with EtOAc (3×500 mL). Organic layers were combined and dried ($MgSO_4$). Solvents were removed in vacuo to yield the crude acid as a yellow foam (18.2 g, 96%). The crude acid (18.1 g, 39.5 mmol) was dissolved in 400 mL of THF and 0.5 mL of DMF at 25 °C, oxalyl chloride (3.70 mL, 43.4 mmol) was added dropwise to the solution, and the mixture was stirred for 1 h. The reaction mixture was cooled to −78 °C and $LiBH_4$ in THF (2 M, 59.2 mL, 118 mmol) was added dropwise. The reaction mixture was stirred for 2 h, 100 mL of H_2O was added to quench the reaction, and then THF was removed in vacuo. HCl (1 M, 500 mL) was added to the aqueous phase and extracted with EtOAc (3×500 mL). Organic layers were combined and dried ($MgSO_4$). Solvents were removed in vacuo and the crude alcohol **40** (17.1 g, 97%) isolated as a yellow solid, which was of sufficient purity to be taken onto the next step without purification. A small amount was taken and purified by flash chromatography for analysis on SiO_2 eluting with EtOAc/hexanes (2:5) to afford alcohol **40** as a yellow solid: mp 138–140 °C (EtOAc/hexanes); R_f=0.14 (EtOAc/hexanes, 4:1); IR (film) 3466, 1711 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J=14.0 Hz, 1H), 7.75 (d, J=8.0 Hz, 2H), 7.30 (d, J=8.0 Hz, 2H), 6.85 (d, J=2.0, 1H), 6.81 (dd, J=8.0, 2.0 Hz, 1H), 6.77 (d, J=8.0 Hz, 1H), 6.46 (d, J=14.0 Hz, 1H), 6.31 (s, 1H), 4.87 (s, 2H), 4.29 (s, 2H), 3.827 (s, 3H), 3.825 (s, 3H), 2.50 (br s, 1H), 2.42 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.2, 149.5, 149.0, 144.6, 138.5, 133.9, 130.2, 129.0, 127.5, 127.4, 120.1, 113.2, 111.4, 111.1, 105.4, 56.2, 56.1, 55.3, 45.2, 21.8; HRMS (ESI) Calcd for $C_{22}H_{25}N_2O_6S$ [M+H]: 445.1407. Found: 445.1407.

4.1.39. 3-{3-(3,4-Dimethoxybenzyl)-2-oxo-1-[2-(toluene-4-sulfonyl)-vinyl]-2,3-dihydro-1*H*-imidazol-4-yl}-acrylic acid ethyl ester (41). To a slurry of activated manganese dioxide (heated with a heat gun under vacuum) (32.4 g, 373 mmol) in 60 mL of dichloromethane at 25 °C was added a solution of **40** (13.8 g, 31.0 mmol) in 400 mL of dichloromethane. The resulting solution was stirred for 12 h and filtered through a pad of Celite®. Solvents were removed in vacuo and the crude aldehyde (9.90 g, 72%) was isolated as a yellow solid (mp 150–152 °C). A solution of triethylphosphonoacetate (4.78 mL, 24.1 mmol) in 100 mL of THF was cooled to 0 °C and treated with 80% NaH (658 mg, 21.9 mmol), after warming to 25 °C and stirring for an additional 40 min. The resulting solution was added dropwise to a solution of the crude aldehyde (9.70 g, 21.9 mmol) in 300 mL of THF and stirring was continued for 30 min. The reaction was quenched with 50 mL of pH 4 buffer solution. THF was removed in vacuo and the aqueous phase was extracted with EtOAc (3×300 mL). The organic layers were combined and dried ($MgSO_4$). Solvents were removed in vacuo and the crude ester **41** isolated as a yellow oil (13.2 g). A small amount of the crude ester was taken and purified by flash chromatography for analysis on SiO_2, eluting with EtOAc/hexanes (2:3) to afford ester **41** as a colorless foam: R_f=0.41 (EtOAc/hexanes, 2:3); IR (film) 1712 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J=14.0 Hz, 1H), 7.79 (d, J=8.5 Hz, 2H), 7.33 (d, J=8.5 Hz, 2H), 7.22 (d, J=16.0 Hz, 1H), 6.80–6.76 (m, 4H), 6.67 (d, J=14.0 Hz, 1H), 6.14 (d, J=16.0 Hz, 1H), 4.89 (s, 2H), 4.20 (q, J=7.0 Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 2.43 (s, 3H), 1.28 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 151.8, 149.6, 149.1, 144.7, 138.7, 133.2, 130.2, 128.8, 128.3, 127.7, 124.3, 119.74, 119.69, 115.3, 111.5, 110.6, 108.5, 61.2, 56.2, 56.1, 45.5, 21.9, 14.4; HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_7S$ [M+H]: 513.1695. Found: 513.1692.

4.1.40. 3-(3,4-Dimethoxybenzyl)-4-(3-hydroxypropenyl)-1-[2-(toluene-4-sulfonyl)-vinyl]-1,3-dihydro-imidazol-2-one (16g). To a stirred solution of crude **41** (13.2 g, 25.8 mmol) in 400 mL of THF/H_2O (v/v; 3:1) at 25 °C was added LiOH (788 mg, 32.9 mmol) and stirring was continued for 12 h and then THF was removed in vacuo. EtOAc (150 mL) was added and the mixture extracted, the organic phase was discarded. HCl (1 M, 150 mL) was added to the aqueous phase and extracted with EtOAc (3×300 mL). Organic layers were combined and dried ($MgSO_4$). Solvents were removed in vacuo and the crude acid was isolated (8.10 g, 76%) as a yellow solid (mp 99–102 °C (EtOAc/hexanes)). Due to the instability of the diene, a portion of the acid (1.00 g, 2.06 mmol) was dissolved in 100 mL of THF at 0 °C. To the solution was added triethylamine (288 μL, 2.06 mmol), followed by methyl chloroformate (174 μL, 2.06 mmol), and the mixture was stirred for 15 min or until

TLC indicated complete formation of the mixed anhydride. The reaction mixture was cooled to −78 °C and $LiBH_4$ in THF (2 M, 2.06 mL, 4.13 mmol) was added dropwise. The reaction mixture was stirred for 15 min, after which 50 mL of H_2O was added to quench the reaction and the solvents were removed in vacuo. HCl (1 M, 100 mL) was added to the aqueous phase and extracted with CH_2Cl_2 (3×200 mL). The organic layers were combined and dried ($MgSO_4$) and solvents were removed in vacuo and the crude alcohol **16g** (972 mg, 99%) was isolated as a yellow foam: R_f=0.16 (EtOAc); IR (film) 3391, 1721 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 8.11 (d, J=14.0 Hz, 1H), 7.82 (d, J=8.0 Hz, 2H), 6.80 (d, J=2.0 Hz, 1H), 6.77–6.73 (m, 4H), 6.48 (d, J=8.0 Hz, 1H), 6.02 (m, 1H), 5.74 (dt, J=16.0, 2.0 Hz, 1H), 5.45 (s, 1H), 4.55 (s, 2H), 3.77 (dd, J=4.5, 2.0 Hz, 2H), 3.40 (s, 3H), 3.30 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (125 MHz, C_6D_6) δ 151.7, 150.3, 149.8, 143.3, 140.1, 133.6, 132.7, 129.8, 129.75, 129.71, 125.9, 119.8, 114.9, 113.9, 112.1, 111.8, 102.9, 62.3, 55.5, 55.4, 44.7, 21.0; HRMS (ESI) Calcd for $C_{24}H_{27}N_2O_6S$ [M+H]: 471.1590. Found: 471.1609.

4.1.41. TBDPS-protected Diels–Alder adduct 42. To a solution of crude diene **16g** (10.0 mg, 0.02 mmol) in 1 mL of benzene at 25 °C in a sealed tube was added dienophile **15b** (9.0 mg, 0.02 mmol), followed by 2,6-lutidine (1.85 μL, 0.02 mmol). The resulting solution was stirred for 24 h at 95 °C. Another portion (10.0 mg, 0.02 mmol) of diene **16g** was added every 24 h until 4.0 equiv had been added. Solvents were removed in vacuo and the crude oil was purified by passing through a plug of SiO_2, eluting with EtOAc to afford recovered dienophile **15b** (5.0 mg), and a single Diels–Alder regioisomer as a colorless oil (~80% purity). A solution of the Diels–Alder adduct in 3 mL of dichloromethane at 25 °C was treated with TBDPSCl (3.20 μL, 0.01 mmol), followed by triethylamine (1.7 μL, 0.01 mmol) and DMAP (catalytic). After 24 h at 25 °C, solvents were removed in vacuo and the crude oil was purified by flash chromatography on SiO_2, eluting with EtOAc/hexanes (7:3) to afford silyl ether **42** (11.5 mg, 48%) as a clear oil: R_f=0.73 (EtOAc/hexanes, 2:3); IR (film) 1723 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.93 (d, J=8.5 Hz, 2H), 7.79 (s, 2H), 7.76–7.73 (m, 4H), 7.65 (d, J=8.5 Hz, 2H), 7.27–7.24 (m, 6H), 6.88 (d, J=2.0 Hz, 1H), 6.85 (d, J=8.5 Hz, 2H), 6.71 (dd, J=8.0, 2.0 Hz, 1H), 6.61 (d, J=8.5 Hz, 2H), 6.55 (d, J=8.0 Hz, 1H), 4.68 (d, J=15.0 Hz, 1H), 4.27 (dd, J=10.5, 3.5 Hz, 1H), 4.23 (dd, J=7.0, 3.5 Hz, 1H), 4.19 (app d, J=8.0 Hz, 2H), 3.97 (dd, J=10.5, 7.0 Hz, 1H), 3.68 (d, J=15.0 Hz, 1H), 3.43 (s, 3H), 3.36 (s, 3H), 3.29 (dd, J=6.0, 3.5 Hz, 1H), 3.20 (m, 1H), 2.13 (m, 1H), 1.95 (s, 3H), 1.88 (m, 1H), 1.84 (s, 3H), 1.71 (m, 1H), 1.17–1.13 (m, 30H); ^{13}C NMR (125 MHz, C_6D_6) δ 172.4, 151.9, 150.5, 150.1, 145.0, 143.6, 140.0, 135.8, 135.4, 133.7, 133.6, 132.4, 130.1, 130.0, 129.5, 129.2, 128.1, 127.5, 127.4, 123.9, 120.3, 116.6, 112.14, 112.09, 112.07, 64.8, 64.5, 62.4, 55.6, 55.5, 44.5, 41.4, 36.7, 34.3, 27.0, 21.3, 21.0, 19.8, 19.3, 18.3, 18.2, 12.0; HRMS (ESI) Calcd for $C_{61}H_{78}N_3O_{10}S_2Si_2$ [M+H]: 1132.4667. Found: 1132.4666.

4.1.42. TBDPS-protected Diels–Alder adduct (43). To a stirred solution of Tsv-protected adduct **42** (56.0 mg, 0.05 mmol) in 3 mL of EtOAc at 25 °C was added $Pd(OH)_2/C$ (spatula tip). The reaction was placed under an atmosphere of hydrogen and the reaction stirred for 24 h. The reaction mixture was filtered through a pad of Celite® and solvents were removed in vacuo to afford a yellow oil. Purification by flash chromatography on SiO_2, eluting with EtOAc/hexanes (3:2) afforded Tse-protected Diels–Alder adduct **43** (52.0 mg, 93%) as a colorless oil. R_f=0.40 (EtOAc/hexanes, 2:5); IR (film) 1738 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.79 (d, J=8.0 Hz, 2H), 7.73–7.70 (m, 4H), 7.63 (d, J=8.0 Hz, 2H), 7.25–7.20 (m, 6H), 6.97 (d, J=1.5 Hz, 1H), 6.78 (dd, J=8.0, 1.5 Hz, 1H), 6.72 (d, J=8.0 Hz, 2H), 6.66 (d, J=8.0 Hz, 2H), 6.61 (d, J=8.0 Hz, 1H), 4.79 (d, J=15.5 Hz, 1H), 4.39 (m, 2H), 4.24 (app t, J=7.5 Hz, 1H), 4.20–4.16 (m, 2H), 4.14–4.07 (m, 2H), 3.99 (d, J=15.5 Hz, 1H), 3.97–3.91 (m, 2H), 3.82 (br d, J=6.0 Hz, 1H), 3.56 (s, 3H), 3.39 (s, 3H), 3.25 (dd, J=6.0, 2.5 Hz, 1H), 2.95 (m, 1H), 2.23 (m, 1H), 1.98 (m, 1H), 1.86 (m, 1H), 1.84 (s, 3H), 1.22–1.07 (m, 30H); ^{13}C NMR (125 MHz, C_6D_6) δ 173.1, 153.9, 150.7, 149.7, 144.6, 144.1, 137.9, 136.2, 135.9, 133.94, 133.92, 130.4, 130.1, 130.0, 129.7, 129.5, 127.9, 120.4, 119.6, 114.7, 112.3, 111.7, 65.2, 65.1, 64.6, 55.8, 55.6, 53.3, 53.0, 44.4, 41.9, 37.8, 36.5, 35.0, 27.1, 21.3, 21.1, 20.1, 19.4, 18.30, 18.28, 12.2; HRMS (ESI) Calcd for $C_{61}H_{80}N_3O_{10}S_2Si_2$ [M+H]: 1134.4824. Found: 1134.4818.

4.1.43. Typical procedure for microwave-assisted Diels–Alder reactions. To a specially designed reaction tube equipped with a magnetic stirrer was added dienophile **15b** (122 mg, 0.29 mmol), Tse-diene **16d** (204 mg, 0.43 mmol), and lithium perchlorate (3.0 mg, 0.03 mmol) sequentially, followed by benzene (2.0 mL) and 2,6-lutidine (20 μL, 0.17 mmol). The reaction vessel was sealed and heated under microwave conditions. The temperature was set to 160 °C and the reaction time was 3 h. After 3 h, the reaction mixture was cooled down and concentrated in vacuo. Flash chromatography (75% EtOAc/hexanes to 90% EtOAc/hexanes) afforded isomerized Diels–Alder adduct **34d** along with its regioisomer (224 mg, 87%) as light yellow foam. The regioisomeric ratio was 2.4:1 in favor of **34d** based on ^1H NMR integration. Compound **34d** can be separated from its regioisomer by automated MPLC purification.

4.1.44. Initial Diels–Alder adduct 32d and TBDPS-protected adduct 44. To a microwave reaction tube equipped with a magnetic stirrer was added dienophile **15b** (12 mg, 0.028 mmol) and Tse-diene **16d** (16 mg, 0.034 mmol), followed by anhydrous THF (0.3 mL) and 2,6-lutidine (2 μL, 0.017 mmol). The reaction mixture was heated to 140 °C under microwave for 2 h. Then the reaction mixture was cooled down and concentrated. Silica gel flash chromatography (50% EtOAc/hexanes to 75% EtOAc/hexanes) gave initial Diels–Alder adduct **32d** and its regioisomer **33d** (17 mg, 68%) as off-white foam. The regioisomeric ratio was 1.4:1 in favor of **32d** based on NMR integration. Compound **32d** cannot be separated from its regioisomer at this stage. They have same R_f values (0.34, 60% EtOAc/hexanes). The mixture was subjected to TBDPS protection. To a solution of **32d** and its regioisomer (33.0 mg, 0.037 mmol) in CH_2Cl_2 (0.50 mL) was added Et_3N (20 μL, 0.142 mmol) at room temperature, followed by TBDPSCl (11 μL, 0.041 mmol) and catalytic amount of DMAP. The reaction mixture was stirred at ambient temperature for 24 h. The

solvent was removed in vacuo and purified by flash chromatography on SiO_2 (20% → 40% EtOAc/hexanes) afforded the silyl-protected adduct **44** as a colorless film (25.0 mg, 60%). R_f=0.74 (60% EtOAc/hexanes); ^{1}H NMR (500 MHz, C_6D_6) δ 8.02 (d, J=8.5 Hz, 2H), 7.82 (m, 2H), 7.75 (m, 4H), 7.16–7.29 (m, 6H), 6.98 (d, J=2.0 Hz, 1H), 6.81 (dd, J=8.0, 2.0 Hz, 1H), 6.78 (d, J=8.5 Hz, 2H), 6.75 (d, J=8.5 Hz, 2H), 6.59 (d, J=8.0 Hz, 1H), 4.93 (d, J=15.0 Hz, 1H), 4.49 (dd, J=8.5, 10.0 Hz, 1H), 4.39 (t, J=3.5 Hz, 1H), 4.36 (m, 1H), 4.05–4.16 (m, 5H), 3.58 (s, 3H), 3.52 (m, 1H), 3.48 (d, J=15.0 Hz, 1H), 3.38 (m, 1H), 3.32 (s, 3H), 3.22 (dd, J=3.5, 9.0 Hz, 1H), 2.97–3.04 (m, 2H), 2.31 (m, 1H), 1.86 (s, 3H), 1.85 (s, 3H), 1.17 (s, 9H), 1.12–1.16 (m, 21H); ^{13}C NMR (125 MHz, C_6D_6) δ 172.9, 158.4, 150.6, 149.7, 144.5, 144.2, 137.17, 137.11, 137.0, 136.0, 135.87, 135.83, 134.1, 134.0, 129.9, 129.88, 129.87, 129.6, 129.14, 128.61, 128.23, 119.7, 112.4, 111.5, 93.22, 66.42, 63.78, 59.23, 55.7, 55.5, 54.0, 52.39, 44.70, 42.36, 39.52, 36.57, 36.32, 27.06, 21.04, 19.4, 18.23, 18.21, 18.2, 12.2; IR (thin film) 2940, 2858, 1726, 1680, 1255, 1117 cm^{-1}; HRMS (ESI) calculated for $C_{61}H_{79}N_3O_{10}S_2Si_2Li$ [M+Li]: 1140.4905. Found: 1140.4898.

4.1.45. Mosher ester 36. To a solution of (*S*)-MTPA (58.2 mg, 0.25 mmol) and EDCI (68.3 mg, 0.36 mmol) in 500 μL CH_2Cl_2 was added Diels–Alder adduct **34b** (8.80 mg, 0.01 mmol) in 500 μL CH_2Cl_2. The reaction mixture was stirred for 18 h, washed with H_2O, brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1 → 7:3) gave 11.0 mg (97%) ester **36** as an off-white solid: R_f=0.37 (hexanes/EtOAc, 6:4); $[\alpha]_D^{25}$ −27.7° (*c* 1.26, CH_2Cl_2); IR (thin film) 1745, 1702, 1658 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.58 (d, J=8.7 Hz, 2H), 7.45–7.24 (m, 15H), 7.11 (m, 2H), 5.09 (d, J=15.9 Hz, 1H), 4.87 (d, J=15.9 Hz, 1H), 4.81 (d, J=15.9 Hz, 1H), 4.73 (d, J=10.5 Hz, 1H), 4.54 (dd, J=5.4, 10.5 Hz, 1H), 4.34 (d, J=15.9 Hz, 1H), 4.25 (dd, J=3.3, 6.0 Hz, 1H), 4.13–4.09 (m, 1H), 3.49 (m, 1H), 3.45 (m, 1H), 3.35 (s, 3H), 2.50 (s, 3H), 2.41 (m, 1H), 2.26–2.14 (m, 1H), 1.78 (m, 1H), 1.07–1.05 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 173.9, 166.7, 155.2, 146.2, 139.0, 138.8, 136.2, 133.0, 130.6, 130.4, 129.5, 129.4, 129.3, 128.4, 128.2, 128.1, 128.0, 127.7, 120.2, 114.9, 68.0, 65.8, 64.7, 55.8, 45.6, 44.7, 43.0, 35.7, 34.4, 21.7, 20.6, 18.32, 18.31, 12.6; HRMS (ESI) Calcd for $C_{51}H_{61}F_3N_3O_8SSi$ [M+H]: 960.3901. Found: 960.3988.

4.1.46. General procedure for the silylation of Diels–Alder adducts as described for *tert*-butyldimethylsilyl ether 45. To a solution of Diels–Alder adduct **34b** (24.8 mg, 0.03 mmol) in 400 μL CH_2Cl_2 was added DMAP (catalytic), Et_3N (35.0 μL, 0.25 mmol), and TBSCl (26.3 mg, 0.18 mmol). The reaction was stirred at ambient temperature for 8 h, diluted with CH_2Cl_2, and washed with H_2O and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1 → 7:3) gave 27.5 mg (96%) silylated Diels–Alder adduct **45** as an off-white foam: R_f=0.73 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ +8.7° (*c* 1.0, CH_2Cl_2); IR (thin film) 1695 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, 8.4 Hz, 2H), 7.34–7.20 (m, 10H), 7.10 (m, 2H), 5.15 (d, J=15.9 Hz, 1H), 4.93 (d, J=15.6 Hz, 1H), 4.71 (d, J=15.9 Hz, 1H), 4.25 (d, J=15.6 Hz, 1H), 4.21 (dd, J=3.6, 6.0 Hz, 1H), 4.02–3.95 (m, 2H), 3.87 (dd, J=6.9, 9.6 Hz, 1H), 3.75 (dd, J=7.2, 9.6 Hz, 1H), 3.31–3.24 (m, 2H), 2.44 (s, 3H), 2.06 (dd, J=3.9, 15.0 Hz, 1H), 1.89–1.78 (m, 1H), 1.67 (m, 1H), 1.04–1.02 (m, 21H), 0.80 (s, 9H), 0.00 (s, 3H), −0.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 154.5, 144.8, 137.2, 137.0, 135.3, 129.3, 128.8, 128.7, 127.9, 127.6, 127.5, 127.3, 127.0, 120.6, 114.5, 64.7, 63.6, 63.4, 45.4, 44.6, 41.3, 37.6, 34.8, 25.8, 21.7, 19.6, 17.9, 11.8, −5.5; HRMS (FAB) Calcd for $C_{47}H_{68}N_3O_6SSi_2$ [M+H]: 858.4367. Found: 858.4355.

4.1.47. *tert*-Butyldimethylsilyl ether 59. R_f=0.68 (hexanes/EtOAc, 6:4); $[\alpha]_D^{25}$ +25.3° (*c* 0.95, CH_2Cl_2); IR (thin film) 1726 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.96 (d, J=8.5 Hz, 2H), 7.62 (d, J=8.5 Hz, 2H), 7.51 (d, J=8.0 Hz, 2H), 7.31 (d, J=8.0 Hz, 2H), 6.66 (s, 4H), 4.69 (d, J=15.5 Hz, 1H), 4.38 (m, 3H), 4.03 (d, J=15.5 Hz, 1H), 3.89 (d, J=7.5 Hz, 1H), 3.73 (s, 3H), 3.60 (dd, J=3.0, 7.5 Hz, 1H), 2.51 (s, 3H), 2.46 (s, 3H), 2.03–2.02 (m, 1H), 1.99–1.95 (m, 1H), 1.67 (ddd, J=3.0, 11.0, 16.0 Hz, 1H), 1.44–1.12 (m, 21H), 0.82 (s, 9H), 0.00 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 174.1, 159.3, 151.9, 146.1, 145.4, 136.0, 134.5, 130.0, 129.9, 128.54, 128.51, 128.0, 127.2, 126.8, 114.2, 114.1, 66.6, 65.4, 63.7, 54.8, 43.3, 42.9, 38.2, 36.6, 25.6, 21.1, 17.8, 12.1, −5.8, −5.9; HRMS (ESI) Calcd for $C_{48}H_{70}N_3O_9S_2Si_2$ [M+H]: 952.4092. Found: 952.4079.

4.1.48. Lactol 51. To a cooled (0 °C) solution of silylated Diels–Alder adduct **45** (16.7 mg, 0.02 mmol) in 250 μL CH_2Cl_2 was added *m*-CPBA (4.50 mg, 0.03 mmol). Additional *m*-CPBA (5.0 mg, 4.8 mg) was added after 1.5 and 3.5 h, respectively. Following the addition of *m*-CPBA the reaction was stirred for 1.5 h and diluted with saturated $NaHCO_3$ (2.0 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1 → 6:4) furnished 13.7 mg (78%) lactol **51** as a light yellow solid: R_f=0.30 (hexanes/EtOAc, 8:2); $[\alpha]_D^{25}$ −52.0° (*c* 0.34, CH_2Cl_2); IR (thin film) 3476, 1789, 1724 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J=8.0 Hz, 2H), 7.40–7.25 (m, 12H), 5.69 (dd, J=2.5, 8.5 Hz, 1H), 4.92 (d, J=17.0 Hz, 1H), 4.69 (d, J=15.0 Hz, 1H), 4.54 (d, J=15.0 Hz, 1H), 4.43 (d, J=2.5 Hz, 1H), 4.35 (dd, J=9.5, 11.0 Hz, 1H), 4.28 (d, J=17.0 Hz, 1H), 3.98 (app d, J=11.0 Hz, 1H), 3.97 (dd, J=6.0, 11.0 Hz, 1H), 3.62 (br s, 1H), 3.55 (dd, J=7.0, 8.5 Hz, 1H), 2.68 (dd, J=1.5, 11.0 Hz, 1H), 2.51 (d, J=8.5 Hz, 1H), 2.41 (s, 3H), 1.98–1.91 (m, 1H), 0.87–0.84 (m, 30H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 167.8, 156.9, 144.8, 137.8, 135.8, 135.1, 129.3, 129.0, 128.8, 128.7, 128.6, 128.1, 127.9, 127.2, 94.2, 87.7, 65.3, 61.3, 59.6, 42.55, 42.47, 42.1, 41.1, 38.7, 25.8, 21.6, 17.8, 11.6, −5.5, −5.6; HRMS (FAB) Calcd for $C_{47}H_{68}N_3O_9SSi_2$ [M+H]: 906.4215. Found: 906.4207.

4.1.49. Trityldimethylsilyl ether 52. To a cooled (0 °C) solution of lactol **51** (303 mg, 0.34 mmol) in 3.0 mL THF was

added a solution of TBAF containing 20 mol % AcOH in THF (3.5 mL). After 1 h pH 7 buffer was added and the layers were separated. The aqueous layer was extracted with Et_2O and the combined organic layers were dried over Na_2SO_4 and concentrated. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (7:3→0:1) gave 75.4 mg (36%) desilylated triol as a white solid: R_f=0.28 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ −23.1° (*c* 3.67, CH_2Cl_2); IR (thin film) 3437, 1726 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J*=8.1 Hz, 2H), 7.45–7.26 (m, 12H), 5.39 (d, *J*=1.8 Hz, 1H), 4.91 (d, *J*=15.6 Hz, 1H), 4.65 (d, *J*=14.7 Hz, 1H), 5.54 (d, *J*=14.7 Hz, 1H), 4.20 (d, *J*=15.6 Hz, 1H), 3.80 (d, *J*=5.1 Hz, 2H), 3.64 (dd, *J*=2.1, 12.0 Hz, 1H), 3.50 (br s, 1H), 3.22–3.12 (m, 2H), 2.70–2.62 (m, 3H), 2.41 (s, 3H), 1.82 (dd, *J*=1.2, 12.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4, 170.0, 156.7, 145.4, 138.4, 135.3, 134.6, 129.4, 129.1, 128.9, 128.8, 128.7, 128.2, 128.1, 94.1, 87.5, 63.3, 62.2, 60.9, 42.5, 42.3, 39.8, 37.1, 36.0, 21.7; HRMS (ESI) Calcd for $C_{32}H_{34}N_3O_9S$ [M+H]: 636.2016. Found: 636.2078.

To a solution of the desilylated triol obtained above (10.5 mg, 0.02 mmol) in 220 μL anhydrous DMF was added Ph_3CSiMe_2Br (34.0 mg, 0.09 mmol) and $AgNO_3$ (17.5 mg, 0.10 mmol). The reaction was stirred in the dark for 20 h and filtered through cotton. The filtrate was diluted with Et_2O and washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (8:2→6:4) gave monosilylated lactol **52** (9.0 mg, 58%) as an off-white film, which was recrystallized from hexanes/Et_2O/EtOAc: R_f=0.65 (hexanes/EtOAc, 6:4); $[\alpha]_D^{25}$ −40.0° (*c* 1.43, CH_2Cl_2); mp 98.0–100.0 °C; IR (thin film) 3426, 1726, 1680 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J*=8.5 Hz, 2H), 7.41–7.16 (m, 25H), 6.99 (m, 2H), 4.94 (d, *J*=2.0 Hz, 1H), 4.86 (d, *J*=15.5 Hz, 1H), 4.63 (d, *J*=14.5 Hz, 1H), 4.54 (d, *J*=14.5 Hz, 1H), 4.12 (d, *J*=15.5 Hz, 1H), 3.86 (dd, *J*=3.5, 10.0 Hz, 1H), 3.75 (dd, *J*=6.5, 10.0 Hz, 1H), 3.63 (dd, *J*=2.0, 12.0 Hz, 1H), 3.45 (br s, 1H), 2.95 (d, *J*=10.0 Hz, 1H), 2.86 (t, *J*=10.0 Hz, 1H), 2.60–2.54 (m, 1H), 2.41 (s, 3H), 1.82 (d, *J*=12.0 Hz, 1H), 0.22 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 169.8, 156.8, 145.4, 145.2, 138.6, 135.4, 134.9, 130.1, 129.3, 129.0, 128.89, 128.86, 128.8, 128.21, 128.19, 128.15, 128.11, 128.0, 125.7, 94.5, 87.6, 63.8, 62.3, 60.3, 54.8, 42.5, 42.2, 39.4, 36.0, 35.6, 29.7, 21.7, 0.3, −0.7; LRMS (ESI) Calcd for $C_{53}H_{54}N_3O_9SSi$ [M+H]: 935. Found [M+H−Tr]: 692.

4.1.50. Allylic alcohol 50a. To a cooled (−45 °C) solution of silylated Diels–Alder adduct **45** (87.3 mg, 0.10 mmol) in 1.1 mL CH_2Cl_2 was added a 0.09 M solution of DMDO (1.15 mL, 0.103 mmol). After 4 h the reaction was quenched with Me_2S, filtered through cotton, and concentrated in vacuo to give 88.9 mg (99%) allylic alcohol **50a** as an off-white foam: R_f=0.75 (hexanes/EtOAc, 6:4); $[\alpha]_D^{25}$ −115.4° (*c* 1.41, CH_2Cl_2); IR (thin film) 3273, 1731, 1680 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.80 (d, *J*=8.5 Hz, 2H), 7.54 (d, *J*=7.5 Hz, 2H), 7.48 (d, *J*=8.0 Hz, 2H), 7.42 (app t, *J*=7.5 Hz, 2H), 7.36–7.22 (m, 6H), 5.57 (s, 1H), 4.83 (d, *J*=15.5 Hz, 1H), 4.76 (d, *J*=15.5 Hz, 1H), 4.50 (d, *J*=16.0 Hz, 1H), 4.47 (d, *J*=3.3 Hz, 1H), 4.10 (dd, *J*=8.0, 9.5 Hz, 1H), 3.97 (app s, 1H), 3.76 (d, *J*=16.0 Hz, 1H), 3.72 (dd, *J*=8.0, 9.5 Hz, 1H), 3.53 (dd, *J*=2.5, 11.0 Hz, 1H), 3.20 (dd, *J*=4.5, 9.0 Hz, 1H), 3.09–3.03 (m, 1H), 2.89 (d, *J*=9.0 Hz, 1H), 2.56 (d, *J*=11.0 Hz, 1H), 2.50 (s, 3H), 0.99–0.97 (m, 21H), 0.90 (s, 9H), −0.05 (s, 6H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.9, 158.8, 145.8, 141.1, 139.7, 137.7, 130.6, 129.8, 129.4, 128.8, 128.2, 127.6, 98.2, 86.4, 66.0, 63.8, 62.5, 62.1, 46.5, 45.2, 44.6, 43.8, 38.4, 26.3, 21.7, 18.5, 13.6, −5.0; HRMS (FAB) Calcd for $C_{47}H_{67}N_3O_7SSi_2Na$ [M+Na]: 896.4136. Found: 896.4139.

4.1.51. Allylic alcohol 50b. R_f=0.55 (hexanes/EtOAc, 6:4); $[\alpha]_D^{25}$ −38.7° (*c* 2.08, CH_2Cl_2); IR (thin film) 3396, 1721, 1680 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 7.78 (d, *J*=8.5 Hz, 2H), 7.53 (d, *J*=7.5 Hz, 2H), 7.47 (d, *J*=8.5 Hz, 2H), 7.41 (app t, *J*=7.5 Hz, 2H), 7.35–7.24 (m, 6H), 5.54 (s, 1H), 4.79 (d, *J*=16.0 Hz, 1H), 4.74 (d, *J*=15.5 Hz, 1H), 4.59 (d, *J*=3.5 Hz, 1H), 4.51 (d, *J*=15.5 Hz, 1H), 4.00–3.98 (dd, *J*=1.5, 2.5 Hz, 1H), 3.85 (d, *J*=16.0 Hz, 1H), 3.82–3.78 (m, 1H), 3.76–3.71 (m, 1H), 3.58 (app t, *J*=6.0 Hz, 1H), 3.55 (dd, *J*=2.5, 10.5 Hz, 1H), 3.21 (dd, *J*=4.5, 9.0 Hz, 1H), 3.06–3.02 (ddd, *J*=3.5, 7.5, 11.0 Hz, 1H), 2.93 (d, *J*=9.0 Hz, 1H), 2.63 (dd, *J*=1.5, 10.5 Hz, 1H), 2.49 (s, 3H), 1.01–0.97 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 174.4, 158.8, 145.8, 141.1, 139.7, 137.8, 137.5, 130.5, 129.7, 129.5, 129.3, 128.8, 128.3, 128.0, 127.9, 98.6, 86.3, 65.9, 63.3, 62.3, 46.2, 45.8, 45.1, 43.9, 38.1, 21.6, 18.4, 18.3, 12.5; HRMS (ESI) Calcd for $C_{41}H_{53}N_3O_7SSiLi$ [M+Li]: 766.3534. Found: 766.3632.

4.1.52. Allylic alcohol 50c. To a cooled (−40 °C) slurry of silylated Diels–Alder adduct **59** (83.3 mg, 0.09 mmol) and $MgSO_4$ was added 0.7 M methyl(trifluoromethyl)dioxirane (450 μL, 0.32 mmol) via Teflon cannula. Me_2S was added after 5 h and the mixture was filtered through cotton and concentrated in vacuo to give 84.6 mg (99%) allylic alcohol **50c** as a light orange residue: R_f=0.25 (hexanes/EtOAc, 8:2); $[\alpha]_D^{25}$ −12.4° (*c* 1.80, CH_2Cl_2); IR (thin film) 3432, 1731, 1650 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.06 (d, *J*=8.5 Hz, 2H), 7.83 (d, *J*=8.5 Hz, 2H), 7.45 (d, *J*=8.5 Hz, 2H), 7.42 (d, *J*=8.5 Hz, 2H), 7.03 (d, *J*=8.5 Hz, 2H), 6.77 (d, *J*=8.5 Hz, 2H), 4.68 (d, *J*=8.5 Hz, 1H), 4.56 (br s, 1H), 4.53 (d, *J*=16.0 Hz, 1H), 4.33 (br s, 2H), 4.12 (dd, *J*=8.0, 9.5 Hz, 1H), 3.77 (d, *J*=16.0 Hz, 1H), 3.72 (s, 3H), 3.65 (dd, *J*=1.5, 9.0 Hz, 1H), 3.36 (dd, *J*=4.5, 9.0 Hz, 1H), 3.00–2.95 (m, 1H), 2.48 (s, 3H), 2.45 (s, 3H), 1.10–1.08 (m, 21H), 0.84 (s, 9H), 0.01 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 173.6, 160.0, 152.1, 145.8, 145.7, 138.2, 137.4, 137.2, 130.6, 130.02, 129.96, 129.4, 128.6, 128.0, 114.7, 101.3, 89.1, 66.6, 63.5, 63.4, 55.4, 48.0, 44.6, 44.4, 37.9, 26.2, 21.57, 21.51, 18.4, 18.3, 12.7, −5.2, −5.3; LRMS (ESI) Calcd for $C_{48}H_{70}N_3O_{10}S_2Si_2$ [M+H]: 968. Found: 968.

4.1.53. Allylic alcohol 50d. R_f=0.26 (hexanes/EtOAc, 7:3); $[\alpha]_D^{25}$ +3.8° (*c* 2.12, CH_2Cl_2); IR (thin film) 3426, 1724, 1690 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.10 (d, *J*=8.0 Hz, 2H), 7.80 (d, *J*=8.0 Hz, 2H), 7.66 (dd, *J*=8.0, 1.5 Hz, 2H), 7.65 (dd, *J*=8.0, 1.5 Hz, 2H), 7.46–7.38 (m, 10H), 6.71 (d, *J*=8.5 Hz, 1H), 6.65 (dd, *J*=8.5, 2.0 Hz, 1H), 6.52 (d, *J*=2.0 Hz, 1H), 5.61 (s, 1H), 4.71 (d, *J*=4.0 Hz, 1H), 4.58 (app d, *J*=1.5, 1H), 4.54 (d, *J*=16.0 Hz, 1H), 4.35 (d, *J*=1.5 Hz, 2H), 4.23 (dd, *J*=9.5, 8.0 Hz, 1H), 3.80 (dd, *J*=9.5, 8.0 Hz, 1H), 3.70 (s, 3H),

3.67 (dd, J=9.0, 1.5 Hz, 1H), 3.61 (d, J=16.0 Hz, 1H), 3.47 (s, 3H), 3.45 (dd, J=9.0, 4.0 Hz, 1H), 3.12–3.08 (m, 1H), 2.46 (s, 3H), 2.45 (s, 3H), 1.16–1.02 (m, 21H), 0.91 (s, 9H); ^{13}C NMR (125 MHz, acetone-d_6) δ 173.6, 152.2, 145.9, 145.7, 138.1, 137.5, 137.3, 136.2, 134.3, 130.6, 130.5, 130.1, 130.0, 128.6, 128.5, 120.7, 112.3, 111.7, 101.2, 89.1, 66.9, 64.5, 63.5, 56.0, 55.5, 48.3, 44.8, 44.3, 37.9, 27.2, 21.6, 21.5, 19.7, 18.42, 18.39, 17.8, 12.7; HRMS (ESI) Calcd for $C_{59}H_{75}N_3O_{11}S_2SiNa$ [M+Na]: 1144.4279. Found: 1144. 4290.

4.1.54. Lactam 63. To a cooled (−78 °C) solution of silylated Diels–Alder adduct **59** (50.3 mg, 0.53 mmol) in 1 mL THF was added a 0.27 M solution of sodium naphthalenide in THF (1.0 mL, 0.27 mmol). After stirring the resulting yellow solution for 3.5 h pH 7 buffer was added and the mixture was diluted with CH_2Cl_2. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography on SiO_2 eluting with hexanes/EtOAc (8:2→0:10) gave 29.1 mg (86%) detosylated cycloadduct **63** as an off-white residue: R_f=0.14 (hexanes/EtOAc, 3:7); $[\alpha]_D^{25}$ +14.4° (c 1.55, CH_2Cl_2); IR (thin film) 1685 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 10.00 (br s, 1H), 7.23 (d, J=8.5 Hz, 2H), 6.86 (d, J=8.5 Hz, 2H), 6.76 (br s, 1H), 4.74 (d, J=15.5 Hz, 1H), 4.66 (d, J=15.5 Hz, 1H), 4.08 (d, J=6.5 Hz, 2H), 3.88 (d, J=5.0 Hz, 2H), 3.76 (s, 3H), 3.63 (m, 1H), 3.34 (d, J=7.0 Hz, 1H), 3.16 (dd, J=2.5, 7.0 Hz, 1H), 2.46 (d, J=11.5 Hz, 1H), 2.08–2.01 (m, 2H), 1.08–1.07 (m, 21H), 0.89 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (acetone-d_6) δ 176.9, 159.9, 155.6, 131.4, 129.5, 119.8, 115.7, 114.7, 66.7, 65.2, 59.2, 55.4, 43.8, 40.7, 38.8, 37.0, 26.3, 21.1, 18.3, 12.6, −5.2; LRMS (ESI) Calcd for $C_{34}H_{58}N_3O_5Si_2$ [M+H]: 644. Found: 644.

4.1.55. Chlorospirohydantoin 65a. To a cooled (−45 °C) solution of allylic alcohol **50a** (19.0 mg, 0.02 mmol) in 200 μL CH_2Cl_2 was added cyclohexene (11.0 μL, 0.11 mmol). A cooled (−45 °C) solution of *N*-chlorosuccinimide (9.60 mg, 0.07 mmol) in 100 μL CH_2Cl_2 was added and the resulting reaction mixture was stirred at ambient temperature overnight. Water was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash chromatography on SiO_2 eluting with hexanes/EtOAc (9:1→8:2) and gave 14.7 mg (75%) spirohydantoin **65a** as a white foam: R_f=0.47 (hexanes/EtOAc, 8:2); $[\alpha]_D^{25}$ −8.4° (c 0.75, CH_2Cl_2), IR (thin film) 1782, 1745, 1716 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.08 (d, J=8.5 Hz, 2H), 7.49 (d, J=8.5 Hz, 2H), 7.43 (d, J=8.0 Hz, 2H), 7.38 (d, J=8.5 Hz, 2H), 7.34–7.30 (m, 6H), 5.32 (d, J=16.0 Hz, 1H), 4.70 (d, J=15.0 Hz, 1H), 4.65 (d, J=15.0 Hz, 1H), 4.63 (m, 1H), 4.37 (d, J=16.0 Hz, 1H), 4.10 (d, J=12.5 Hz, 1H), 4.09 (dd, J=3.5, 10.5 Hz, 1H), 4.05 (dd, J=8.5, 10.0 Hz, 1H), 3.92 (dd, J=1.5, 10.5 Hz, 1H), 3.79 (dd, J=4.0, 10.0 Hz, 1H), 3.48 (t, J=8.5 Hz, 1H), 3.29 (d, J=8.5 Hz, 1H), 3.15–3.07 (m, 1H), 2.44 (s, 3H), 0.98–0.83 (m, 30H), 0.07 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 173.8, 171.9, 157.8, 146.8, 138.4, 137.3, 136.9, 130.8, 129.5, 129.44, 129.39, 129.2, 128.8, 128.7, 128.6, 76.8, 65.7, 61.3, 60.6, 59.7, 48.7, 47.7, 46.9, 46.3, 43.4, 41.0, 26.4, 18.4, 18.3, 12.7, −5.2; HRMS (FAB) Calcd for $C_{47}H_{66}ClN_3O_7SSi_2Na$ [M+Na]: 930.3746. Found: 930.3785. An aromatic side product **66a** was also isolated in this reaction: R_f=0.35 (hexanes/EtOAc, 8:2); $[\alpha]_D^{25}$ −24.5° (c 0.44, CH_2Cl_2); IR (thin film) 1711; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.82 (d, J=7.8 Hz, 2H), 7.43–7.31 (m, 12H), 5.63 (d, J=16.2 Hz, 1H), 5.36 (d, J=15.9 Hz, 1H), 5.30 (dd, J=1.5, 3.0 Hz, 1H), 5.25 (d, J=15.9 Hz, 1H), 5.16 (d, J=16.2, 1H), 5.07 (br s, 2H), 4.30 (dd, J=3.0, 10.8 Hz, 1H), 4.03 (dd, J=1.5, 10.8 Hz, 1H), 2.39 (s, 3H), 0.90 (s, 9H), 0.68–0.64 (m, 21H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 166.8, 156.2, 145.6, 138.1, 138.0, 137.7, 137.2, 135.0, 130.3, 129.9, 129.6, 128.7, 128.6, 128.5, 128.0, 127.4, 126.3, 122.9, 121.0, 107.0, 65.4, 62.4, 61.4, 46.8, 45.6, 26.3, 18.8, 17.9, 17.8, 12.3, −5.3; HRMS (ESI) Calcd for $C_{47}H_{64}N_3O_6SSi_2$ [M+H]: 854.4054. Found: 854.3971.

4.1.56. Chlorospirohydantoin 65b. R_f=0.39 (hexanes/EtOAc, 7:3); $[\alpha]_D^{25}$ −16.8° (c 2.13, CH_2Cl_2), IR (thin film) 3454, 1716 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.05 (d, J=8.5 Hz, 2H), 7.49 (d, J=8.5 Hz, 2H), 7.43 (d, J=7.0 Hz, 2H), 7.38 (d, J=7.5 Hz, 2H), 7.34–7.26 (m, 6H), 5.23 (d, J=16.0 Hz, 1H), 4.71 (d, J=15.0 Hz, 1H), 4.66 (d, J=15.0 Hz, 1H), 4.63 (br s, 1H), 4.39 (d, J=16.0 Hz, 1H), 4.25 (d, J=12.5 Hz, 1H), 4.02 (dd, J=3.5, 11.0 Hz, 1H), 3.84 (dd, J=2.0, 11.0 Hz, 1H), 3.78–3.72 (m, 2H), 3.52 (app t, J=9.0 Hz, 1H), 3.36 (d, J=9.0 Hz, 1H), 3.30 (m, 1H), 3.08 (m, 1H), 2.46 (s, 3H), 0.98–0.90 (m, 21H); ^{13}C NMR (125 MHz, acetone-d_6) δ 174.0, 173.6, 157.8, 146.8, 138.2, 137.1, 136.4, 130.7, 129.4, 129.3, 129.2, 129.0, 128.7, 128.6, 128.4, 76.6, 65.2, 61.6, 60.0, 58.6, 48.2, 47.5, 46.8, 46.2, 43.2, 21.5, 18.2, 18.1, 12.5; HRMS (ESI) Calcd for $C_{41}H_{53}ClN_3O_7SSi$ [M+H]: 794.3062. Found: 794.2923.

4.1.57. Chlorocyclopentane 71 and deschlorocyclopentane 73. A solution of **69** (320 mg, 0.33 mmol) and magnesium sulfate (30 mg) in 10 mL dichloromethane was cooled to −60 °C and treated with dimethyldioxirane (0.09 M in acetone, 3.99 mL, 0.36 mmol) over 2 h. Stirring continued for a further 2 h, after which the reaction was quenched with 100 μL of methyl sulfide. Solvents were removed in vacuo to afford crude allylic alcohol **40** (320 mg, 99%) as a colorless foam. A solution of crude allylic alcohol (150 mg, 0.15 μmol) in 10 mL dichloromethane was cooled to −50 °C and treated with cyclohexene (76.0 μL, 0.75 mmol) and sodium hydrogen carbonate (25.3 mg, 0.30 mmol) followed by NCS (50.0 mg, 0.38 mmol). The reaction mixture was allowed to warm to 25 °C, and further stirred for a total of 26 h. The reaction was quenched with 2 mL of pH 7 buffer. The aqueous layer was extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were dried ($MgSO_4$). Solvents were removed in vacuo and the crude oil was purified by flash chromatography (SiO_2, EtOAc/hexane, 4:6) to afford a mixture of the chlorospirocyclic pentane **71** and the aromatic compound **72** (52.0 mg, 50%). These were further purified to obtain analytically pure samples. Chlorocyclopentane **71**: R_f=0.47 (EtOAc/hexane, 2:3); ^{1}H NMR (500 MHz, C_6D_6) δ 8.19 (d, J=8.5 Hz, 2H), 7.87 (d, J=8.5 Hz, 2H), 7.47 (d, J=2.0, 1H), 7.39 (dd, J=8.5, 2.0 Hz, 1H), 6.86 (d, J=8.5 Hz, 2H),

6.74 (d, J=8.5, 2H), 6.53 (d, J=8.5 Hz, 1H), 5.83 (d, J=15.5, 1H), 4.88 (s, 1H), 4.79 (dd, J=11.0, 9.5 Hz, 1H), 4.71 (d, J=15.5 Hz, 1H), 4.58 (d, J=12.0 Hz, 1H), 4.55 (dd, J=11.0, 4.0 Hz, 1H), 4.20 (dd, J=11.0, 2.5 Hz, 1H), 4.08 (dd, J=11.0, 1.3 Hz, 1H), 3.75 (m, 3H), 3.66 (s, 3H), 3.55 (ddd, J=15.0, 9.0, 3.0 Hz, 1H), 3.38 (ddd, J=16.0, 7.0, 3.0 Hz, 1H), 3.33 (s, 3H), 3.22 (ddd, J=15.0, 9.0, 3.0 Hz, 1H), 2.91 (ddd, J=16.0, 7.0, 2.5 Hz, 1H), 1.87 (s, 3H), 1.82 (s, 3H), 1.19 (s, 9H), 0.96–0.88 (m, 21H); ^{13}C NMR (125 MHz, C_6D_6) δ 177.1, 173.2, 171.4, 156.8, 150.5, 150.1, 145.1, 144.8, 136.3, 135.8, 129.9, 129.7, 129.1, 127.6, 127.4, 127.2, 121.5, 113.2, 112.3, 76.6, 65.5, 61.3, 60.3, 55.8, 55.4, 50.5, 48.3, 47.5, 46.1, 45.4, 38.8, 33.4, 27.3, 21.2, 21.1, 18.2, 12.2; HRMS (ESI) Calcd for $C_{50}H_{68}N_3O_{12}S_2SiClLi$ [M+Li]: 1036.3862. Found: 1036.3912. Further elution gave the deschloro spirocyclic pentane **73**: R_f=0.38 (EtOAc/hexane, 2:3); IR (thin film) 1713 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.22 (d, J=8.5 Hz, 2H), 7.88 (d, J=8.5 Hz, 2H), 7.27 (d, J=2.0 Hz, 1H), 7.15 (dd, J=8.5, 2.0 Hz, 1H), 6.86 (d, J=8.5 Hz, 2H), 6.76 (d, J=8.5, 2H), 6.53 (d, J=8.5 Hz, 1H), 5.83 (d, J=16.0, 1H), 4.87 (s, 1H), 4.69 (d, J=16.0 Hz, 1H), 4.66 (dd, J=11.0, 8.5 Hz, 1H), 4.28 (dd, J=11.0, 6.5 Hz, 1H), 4.18 (dd, J=11.0, 2.5 Hz, 1H), 4.09 (dd, J=11.0, 2.5 Hz, 1H), 3.62 (m, 3H), 3.60–3.50 (m, 3H), 3.43–3.37 (m, 5H), 3.36 (s, 3H), 3.27 (ddd, J=15.0, 8.5, 3.0 Hz, 1H), 3.04 (ddd, J=15.0, 7.0, 3.0 Hz, 1H), 2.12 (app t, J=13.5 Hz, 1H), 1.87 (m, 2H), 1.86 (s, 3H), 1.82 (s, 3H), 1.13 (s, 9H), 0.97–0.90 (m, 21H); ^{13}C NMR (125 MHz, C_6D_6) δ 177.2, 177.0, 172.5, 156.4, 150.5, 149.8, 144.9, 144.8, 136.4, 135.9, 130.7, 129.9, 129.6, 129.2, 128.3, 128.1, 127.9, 120.4, 112.5, 112.4, 71.9, 65.5, 63.3, 61.6, 55.8, 55.5, 50.9, 50.3, 49.6, 45.8, 40.4, 38.7, 35.7, 33.1, 27.2, 21.14, 21.08, 18.2, 18.1 12.2; MS (ESI) 1002 [M+Li]. HRMS (ESI) Calcd for $C_{50}H_{69}N_3O_{12}S_2SiLi$ [M+Li]: 1002.4252. Found: 1002.4262.

4.1.58. Spirocycle 78. A slurry of alkene **43** (500 mg, 0.44 mmol) and magnesium sulfate (100 mg) in 40 mL of dichloromethane was cooled to −50 °C and treated with dimethyldioxirane (~0.06 M in acetone, 8.1 mL, 0.48 mmol). After 3 h, the reaction was quenched with 100 μL of methyl sulfide at −50 °C, filtered through a pad of Celite® and concentrated in vacuo to afford crude allylic alcohol **77** (500 mg, 99%) as a colorless foam. The crude allylic alcohol **77** was redissolved in 40 mL of dichloromethane and cooled to −50 °C. Addition of cyclohexene (223 μL, 2.20 mmol) and magnesium sulfate (100 mg) was followed by treatment with chloramine-T (200 mg, 0.88 mmol). The reaction mixture was allowed to warm to 25 °C, and then stirred for a total of 12 h. The reaction was quenched with 20 mL of water and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo, and the crude oil was purified by flash chromatography on SiO_2, eluting with EtOAc/hexanes (1:3) to afford cyclopentane **78** (339 mg, 65%) as a colorless foam: R_f=0.35 (EtOAc/hexanes, 1:2); IR (film) 1773, 1718 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.26 (d, J=8.5 Hz, 2H), 7.96–7.94 (m, 2H), 7.88 (d, J=8.5 Hz, 2H), 7.81–7.79 (m, 2H), 7.46 (d, J=2.0 Hz, 1H), 7.39 (dd, J=8.0, 2.0 Hz, 1H), 7.28–7.20 (m, 6H), 6.84 (d, J=8.5 Hz, 2H), 6.77 (d, J=8.5 Hz, 2H), 6.39 (d, J=8.0 Hz, 1H), 5.87 (d, J=16.0 Hz, 1H), 4.90 (s, 1H), 4.74 (d, J=16.0 Hz, 1H), 4.55 (t, J=10.0 Hz, 1H), 4.50 (d, J=12.5 Hz, 1H), 4.23 (dd, J=10.0, 2.0 Hz, 1H), 4.11 (m, 2H), 3.96 (t, J=8.0 Hz, 1H), 3.80 (d, J=8.0 Hz, 1H), 3.79–3.73 (m, 1H), 3.62 (s, 3H), 3.58–3.53 (ddd, J=15.0, 9.0, 3.0 Hz, 1H), 3.39–3.34 (ddd, J=15.5, 7.0, 3.0 Hz, 1H), 3.27 (s, 3H) 3.26–3.21 (ddd, J=15.0, 9.0, 3.0 Hz, 1H), 2.95–2.90 (ddd, J=15.5, 7.0, 3.0 Hz, 1H), 1.87 (s, 3H), 1.84 (s, 3H), 1.20 (s, 9H), 0.98–0.90 (m, 21H); ^{13}C NMR (125 MHz, C_6D_6) δ 173.3, 171.8, 156.8, 150.3, 149.9, 145.0, 144.8, 136.5, 136.3, 136.0, 135.9, 133.9, 133.7, 129.91, 129.88, 129.8, 129.6, 129.14, 129.12, 128.0, 121.5, 113.1, 112.2, 76.8, 65.5, 61.2, 60.6, 60.0, 55.8, 55.3, 50.6, 49.1, 48.6, 47.2, 46.0, 33.4, 27.1, 21.2, 21.1, 19.5, 18.18, 18.15, 18.11, 12.2; HRMS (ESI) Calcd for $C_{61}H_{78}N_3O_{11}S_2Si_2ClLi$ [M+Li]: 1190.4465. Found: 1190.4366.

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Biomimetic investigations from reactive lysine-derived C_5 units: one step synthesis of complex polycyclic alkaloids from the *Nitraria* genus

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Abstract—A single biomimetic cascade sequence featuring intermolecular followed by intramolecular cyclizations allowed the biomimetic synthesis of nitraramine and the formation of an interesting original cage-like structure from simple C_5 lysine-derived metabolites. In the course of the study, the structure and spectral data of 1-epinitraramine were also revisited.

1. Introduction

1.1. The *Nitraria* genus

Plants from the *Nitraria* genus (Zygophyllaceae) are bushes that can grow up to 2 m high. They have fleshy leaves, little white flowers (with five petals and 15 stamens), and their fruits have three pyramidal chambers. Well adapted to arid climate, species of *Nitraria* are found in desert regions of south-east Europe (*Nitraria komarovii*, *Nitraria sibirica*) and middle-east (*Nitraria schoberi*) but also in Australia (*Nitraria billardieri*) and Africa, in northern and occidental parts of the Sahara desert, and in Mauritania (*Nitraria tridendata* or *Nitraria retusa*).

1.2. *Nitraria* alkaloids

Isolated from aerial parts of the plants, alkaloids of the *Nitraria* genus are classically classified into three major groups (Fig. 1):[1] tripiperidine alkaloids (e.g., schoberine), indole alkaloids (e.g., nitrarine), and the group of spiro alkaloids. The latter can be divided in two sub-groups: simple spiro alkaloids (e.g., sibirine) and complex spiro alkaloids (e.g., nitraramine **1**, 1-epinitraramine **2**).

These piperidine alkaloids from *Nitraria* species constitute a singular class of natural compounds both in terms of chemical diversity (e.g., many 3-spiropiperidines) and biosynthetic origin. In fact, a particularly primitive lysine-based metabolism can account for the biogenesis of these alkaloids. Moreover, the fact that numerous *Nitraria* alkaloids are present as chiral but as racemic forms in nature strongly suggests a minimum enzymatic intervention, at least for the key diversity-generating cyclizations.[2]

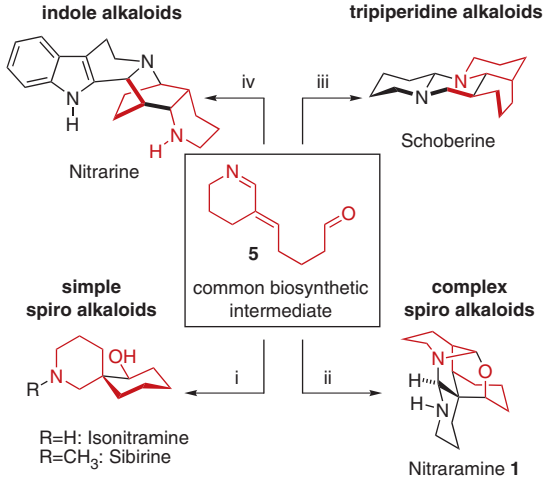

Figure 1. Central biosynthetic role of intermediate **5**.

1.3. Biosynthesis

The biosynthesis of many of these compounds can be explained by the assembly of simple C_5 units derived from L-lysine, such as 2-piperideine **3** and glutaraldehyde **6** (Scheme 1). The formation of an endocyclic enamine **3** from lysine is a known step in the biosynthesis of many alkaloids such as

Keywords: Nitraria; Nitraramine; Biosynthesis; Biomimetic synthesis; Polycyclic alkaloids; Epinitraramine; Structure reassignment.
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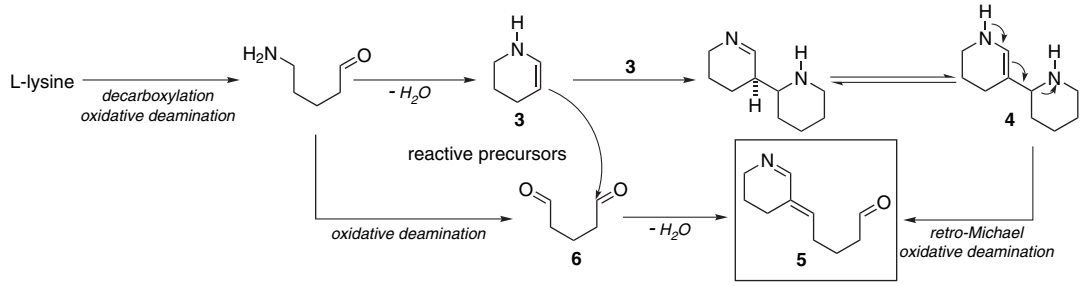
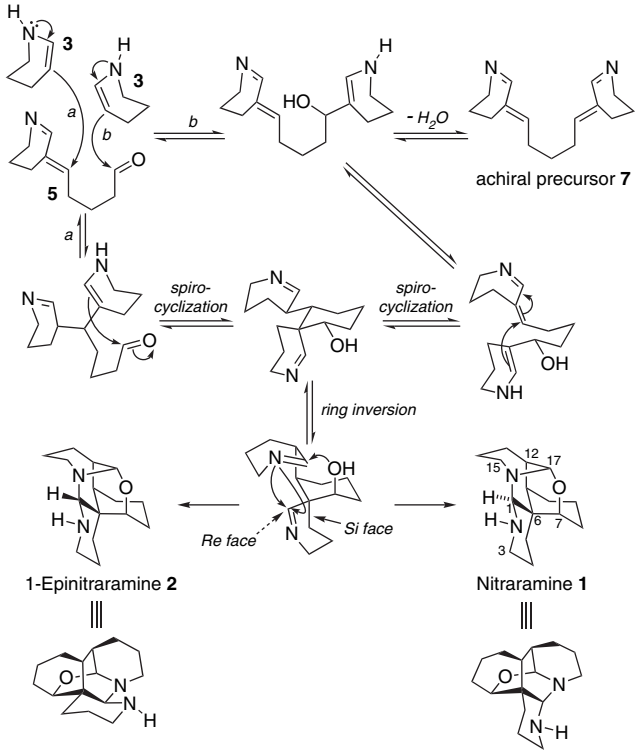


Scheme 1. Different possibilities for the biosynthesis of intermediate **5**.

those of the *Lupinus* genus. Dimerization of 2-piperideine **3** into tetrahydroanabasine **4** followed by opening and oxidation could lead to an intermediate **5**,[1,3] but the attack of a 2-piperideine unit on a molecule of glutaraldehyde followed by a dehydration step could also be a potential path for the formation of this pivotal key precursor (Scheme 1) that spiro, indole, and tripiperidine alkaloids share. Compound **5** can then be reduced and undergo a spirocyclization process, leading to the formation of simple spiro alkaloids (Fig. 1, i). It can also react with an additional molecule of 2-piperideine, either through C-alkylation, in which case it will afford the required intermediates for the formation of complex spiro alkaloids (Fig. 1, ii), or through N-alkylation and in that case eventually leads to the formation of tripiperidine alkaloids (Fig. 1, iii). Finally, intermediate **5** can react with a molecule of tryptamine via a Pictet–Spengler reaction to afford indole alkaloids such as nitrarine (Fig. 1, iv).

One of the products derived from **5** is nitraramine **1**,[4] a small but complex intricate structure, isolated from *Nitraria schoberi*.[5] From **5**, the formation of **1** can be explained through a series of virtually equilibrated reactions essentially consisting of imine/enamine tautomerism, Michael/retro-Michael, Mannich/retro-Mannich reactions, and hydrations/dehydrations. In the last step, following a ring inversion, a nucleophilic attack of the hydroxyl group, and a last aza-Mannich reaction onto the remaining iminium system afforded **1**. In this context, the formation of 1-epinitraramine, which was assigned the structure **2**,[6] in conjunction with **1**, has been explained by a likely attack on the *Si*-face or the *Re*-face of the iminium, during the aminal formation to produce **1** and **2**, respectively (Scheme 2).



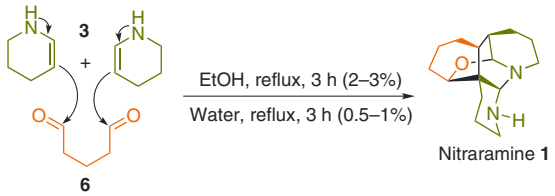
Scheme 2. Proposed biogenetic pathway to nitraramine **1**.

In this paper we discuss a full account of the assembly of **1** from simple precursors[7] as well as the absence of formation of **2**, which states the high stereoselectivity of the reaction cascade. Other molecules formed during the 'dynamic combinatorial process' have also been characterized and are presented herein.

2. Chemistry

2.1. Biomimetic total synthesis of nitraramine 1

In connection with their biosynthetic hypothesis, Koomen and Wanner targeted the plausible achiral intermediate **7** by a stepwise approach in their pioneering beautiful synthesis of **1** (7 steps, ~0.5% yield).[5] On the basis of the proposed biosynthetic pathway and considering the different possibilities for the formation of intermediate **5** discussed above, we reasoned that it should be possible to access directly the nitraramine **1** from much simpler precursors than the ones chosen by Koomen and Wanner (Scheme 3). A reaction between 2 equiv of 2-piperideine **3** and 1 equiv of glutaraldehyde **6** should logically lead to the assembly of **1**, as no oxidation or reduction step is required for starting the reaction from these precursors.



Scheme 3. Biomimetic synthesis of **1**.

From this observation, we decided to develop a one-step synthesis of this particular original molecule, by reacting commercial glutaraldehyde with 2 equiv of 2-piperideine formed

in situ from its crystalline trimeric form, which was simply obtained from piperidine[8] treated with *N*-chloro-succinimide and sodium methanoate.

To carry out the reaction in a polar solvent that could be easily removed under reduced pressure, we figured that ethanol was the most convenient solvent. The mixture was stirred under reflux to allow a better dissociation of the trimer into 2-piperideine **3** but also to provide the energy required for the ring inversion step of the biomimetic cascade sequence. After 3 h of reaction, the crude reaction mixture showed a complex TLC profile.

By mixing the reactive precursors without any control on the reaction outcome to select specific associations, we knew that we would obtain many different products amongst which we would have to identify and isolate the desired alkaloid. To guide our search for nitraramine within the mixture, we had limited but relatively precise information at our disposal consisting mainly in ^{13}C and ^{1}H NMR data. Despite certain differences, all ^{1}H NMR spectra displayed a singlet at 3.5 ppm, attributed to the aminal H-1, which permitted the identification of the product and enabled the exact location of **1** to be determined on TLC (CH_2Cl_2/MeOH 9:1, R_f=0.35).

Relying on what had been previously published in literature,[5] we at first opted for purification on silica gel column eluted with methylene chloride/methanol 95:5. Even though silica gel made it possible to refine the crude mixture by providing, after several columns, fractions enriched with nitraramine **1**, it appeared virtually impossible to obtain the latter totally free of impurities. We therefore decided to change the solid phase to basic alumina and to use an elution gradient. After different tries, optimal conditions were defined and it turned out that pure nitraramine **1** could be obtained by two consecutive basic alumina columns eluted with a gradient of methylene chloride/methanol from 99:1 to 96:4. This method provided pure **1** as colorless crystals in 2–3% yield.

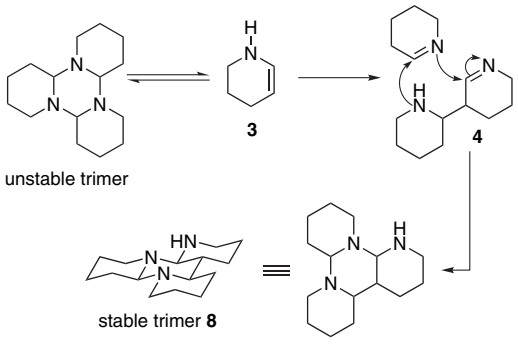
At first sight this yield may seem rather deceiving but one needs to consider that it is the global yield of a total synthesis in the course of which five new bonds (including three carbon–carbon bonds) and six chiral centers (including one spiro-carbon) were formed in a single vessel. Starting material is inexpensive and experimental procedure is very simple, permitting the whole synthesis to be carried out in less than a day.

In ideal biomimetic conditions the reaction should be conductible in water; the same condensation protocol was therefore tried in boiling water (citrate buffer 4%) and the resulting TLC profile of the crude reaction mixture was similar to the one obtained in ethanol. After purification, pure nitraramine **1** was obtained but in lower yield (0.5–1%), probably due to an important loss of material in water during workup.

2.2. Other molecules formed during the process

Given the yield of just a few percents, one can rightfully wonder how all the residual material has reacted. Even though it was not our priority to do so, we decided to investigate the reaction products other than the expected alkaloid **1**. Thus, we were able to characterize two molecules formed in significant proportions during the reaction process: stable trimer **8** (15–20%) and the much more complex and original molecule **9** (8–10%).

Trimer **8** is a known product that we expected in the reaction mixture and it was easily identified by comparison of the obtained spectral data with that of prior literature data (^{1}H NMR and ^{13}C NMR spectra).[8] The formation of this compound can be easily explained by the reaction of one 2-piperideine **3** unit on a molecule of tetrahydroanabasine **4** (which is itself obtained from two 2-piperideine units), as shown in Scheme 4.



Scheme 4. Proposed formation path for trimer **8**.

Moreover, it was observed that when a solution of trimeric **3** in ether was left at room temperature, the rearrangement took place, resulting in the formation of **8**, and that within 48 h all the material was converted.

For the structural determination of compound **9** the process was more complicated, especially because of numerous ^{1}H NMR signals, which were overlapping one another in the CH_2 region (1–2 ppm), making exploitation of the information impossible.

The product was slightly less polar than compound **8** and its IR spectrum showed the absence of N–H bonds. Mass spectroscopy (ESI-MS) gave a quasi-molecular ion peak $[M+H]^+$ at *m*/*z* 248 and a unique fragment ion peak at *m*/*z* 165.

The ^{13}C NMR spectrum showed the presence of five CH and 10 CH_2 that constituted a mass of 205 to which three nitrogen atoms had to be added to get the right molecular weight and give the molecular formula $C_{15}H_{25}N_3$, requiring five degrees of unsaturation. Given the absence of ethylenic signals, it was obvious that these came from the presence of cycles.

The analysis of the COSY spectrum turned out not to be as informative as it should have been. Most signals were overlapping one another between 1 and 2 ppm and it was impossible to determine any sequence between CH_2. Nevertheless, it was possible to identify the most substituents of tertiary carbons, thanks to the correlation signals on the HSQC spectrum, and three segments were determined. Considering the number of nitrogen atoms and the different couplings observed on the ^{1}H NMR spectra, we were able to assemble these fragments in a bicyclic system, as shown in Figure 2.

Relative configuration of carbons 2′ and 3″ was given by the coupling constant that existed between the ^{1}H NMR signals

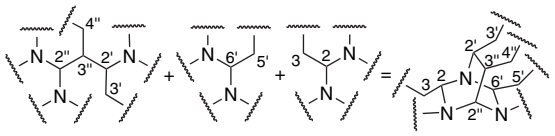


Figure 2. Construction of the central bicyclic system of molecule **9**.

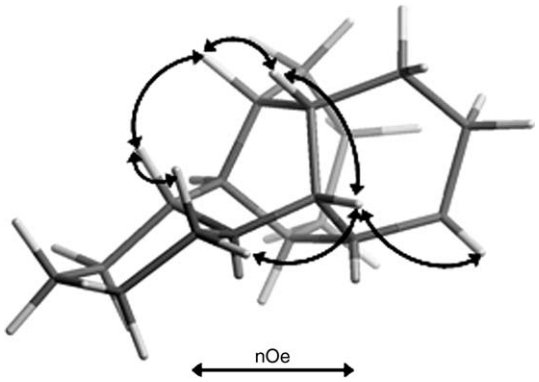


Figure 3. Selected NOESY correlations for molecule **9**.

(J=11.5 Hz) and the difference between carbons 2 and 6′ was made by an analysis of the NOESY spectrum (see Fig. 3). The central bicyclic structure was confirmed by the HMBC correlations (the most significant ones are shown on Fig. 4).

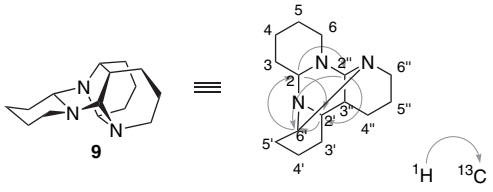
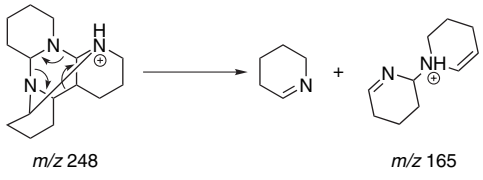


Figure 4. Selected HMBC correlations for molecule **9**.

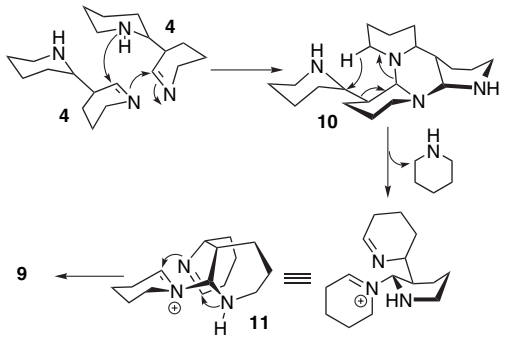
At that point, there were still six residual CH_2 to assign in order to complete the last three cycles of the structure. Keeping in mind the reagents and preferring six-membered rings, we realized that only one structure could be considered. Configurations of the peripheral cycles were determined on the basis of the observation of the ^1H NMR signals (i.e., multiplicities and coupling constants).

Moreover, the obtained structure could explain the observed fragment ion peak at m/z 165 on the ESI-MS mass spectrum, through a mechanism shown in Scheme 5.



Scheme 5. Proposed fragmentation mechanism.

A plausible hypothesis for the formation of compound **9** from piperideine-derived units is shown in Scheme 6. Two tetrahydroanabasine units react to give rise to compound **10** which is rearranged by a hydride transfer to yield molecule **11**.[9] The attack of the imine by the free secondary amine followed by the reduction of the iminium completes the synthesis of compound **9**.



Scheme 6. Possible formation mechanism proposed for molecule **9**.

Our structural hypothesis is strengthened by the fact that the molecule could result from the association of two units of tetrahydroanabasine **4**, which also contributes to the formation of compound **8** found in large quantity in the mixture. This observation led us to think that the low yield for the formation of **1** was probably widely due to the formation of a large amount of **4**.

2.3. Revision of the previously reported 1-epinitraramine 2

It appeared clearly, when comparing compiled NMR data,[10] that relatively important variations existed in chemical shifts observed for the same product. We simply could not find two totally superimposable spectra. Various hypotheses were considered to explain the phenomena until the NMR analysis of a diluted solution of nitraramine **1** gave us a spectrum equivalent to the one attributed by Quirion and colleagues to 1-epinitraramine **2**, in their 1995 article.[6]

After a closer study of the spectra obtained during our experiments, we realized that a relation existed between the concentration of **1** and the corresponding NMR signals (regarding chemical shifts as well as signal shape/multiplicity). We therefore decided to carry out an NMR study on samples of different nitraramine concentrations. The results clearly demonstrated that the compound previously described as 1-epinitraramine **2** was in fact diluted nitraramine **1** in $CDCl_3$ (Fig. 5 shows the most significantly variable area of the spectrum, under different concentration conditions). As a matter of fact, the observed spectrum for a solution of about 6 mg mL^{-1} is equivalent to the one attributed to 1-epinitraramine,[6] and the spectrum observed for about 24 mg mL^{-1} equivalent to the one attributed to nitraramine by Quirion and colleagues.[6]

The phenomena still needed an explanation and we came to think that it could come from the influence of acid traces, found in $CDCl_3$, on the protonation state of the molecule (the most variable signals were the ones attributed to protons that are close to nitrogen atoms). To confirm the hypothesis we decided to collect the contents of all NMR tubes that gave ‘epinitraramine-like’ spectra and made a single batch that

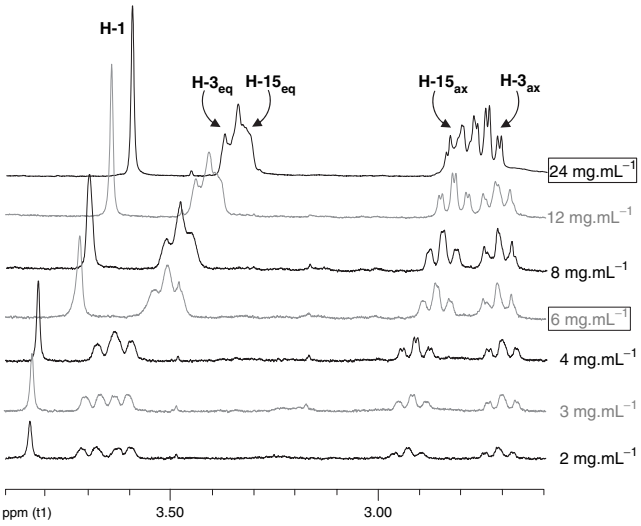


Figure 5. Concentration influence on chemical shifts of **1** (400 MHz, 25 °C).

we treated with K_2CO_3. The dried product was then dissolved in $CDCl_3$ (that had previously been filtered on basic alumina to remove traces of acid), and an NMR analysis was performed. The obtained spectrum was the one corresponding to what had been reported by Koomen and Wanner as **1**. The product was then sequentially diluted with $CDCl_3$ (previously filtered on basic alumina) until no product could be detected by NMR analysis and all observable spectra were identical to the one obtained with concentrated nitraramine.

Each spectrum on Figure 5 probably reflects a different degree of protonation, showing different average of the two chemical forms. In fact, according to the ^{1}H NMR chemical shift time scale, protonated/deprotonated states are considered in fast exchange giving rise to a weighted averaged spectrum of extreme values (i.e., δ (protonated) and δ (free base)).

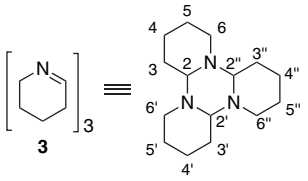
For these reasons, 1-epinitraramine **2**, which was only characterized upon NMR analysis by Quirion and colleagues, is likely to be an experimental artefact. The existence of 1-epinitraramine cannot be totally excluded but if it does exist, it's rather surprising that it has never been encountered in plants containing nitraramine. In terms of chemical assembly, the cascade involved in the formation of **1** appears therefore to be highly stereoselective.

3. Conclusion

The low yield of **1** reflects the multiple biomimetic coupling possibilities of lysine-derived precursors **3** and **6** but states for the incontestable spontaneous formation of **1** from fundamental C_5 units presumably derived from lysine. Bearing in mind that the poor yield of our synthesis is a significant drawback, one can add that the easiness and reliability of the reaction conditions from costless reagents permit the preparation of pure **1** in a few hour time scale. Therefore, the described total synthesis of **1** competes largely with scarce extraction from natural sources (which are present only in restricted areas) and with the previous total synthesis. Enough material has been prepared for needed biological screening of this intriguing complex small molecule. Furthermore, total synthesis unexpectedly led to a structural reassignment of 1-epinitraramine **2**.[11] Finally, the chemistry of simple C_5 lysine-type molecules[12] presented in this paper offers further opportunities toward biomimetic synthesis[13] and molecular self-assembly of complex natural product-like skeletons,[14] as illustrated by the formation of compound **9**.

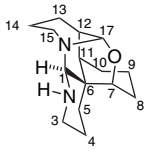
4. Experimental

4.1. Synthesis of 3 from piperidine



In a round bottom flask (1 L), *N*-chloro-succinimide (65 g, 0.5 mol) was stirred in ether (450 mL) and the suspension was cooled to 4 °C in an ice bath. Piperidine (40 mL, 0.47 mol) was then slowly added. After 2 h under agitation at room temperature, the mixture was filtered and the filtrate was washed with water (2×250 mL), and then dried. After filtration, three-third of the solvent was removed under reduced pressure and sodium methanoate (267 mL of a 2 N solution in methanol) was added carefully. The mixture was stirred under reflux for 45 min after which water was added until total dissolution of the formed salt (NaCl). An extraction with ether was performed and the organic phase was dried. After solvent evaporation, a thick yellow oil (crystallizing at 4 °C) was obtained (27 g, 70%). Compound **3**: yellow crystals; ^{13}C NMR (100 MHz, $CDCl_3$): δ 82.1 (C-2), 46.5 (C-6), 29.5 (C-3), 26.3 (C-5), 22.8 ppm (C-4); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.86 (3H, m, H-2), 2.55 (3H, m), 1.65–1.95 (6H, m), 1.25–1.62 (12H, m), 1.18 ppm (3H, m); Mass (ESI-MS) $[M+H]^+$ 250.

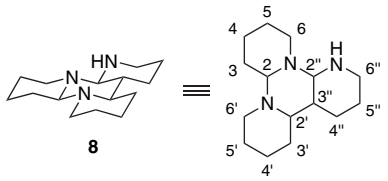
4.2. Synthesis of 1 from 3



2-Piperideine **3** (in its trimeric form, 5 g, 0.06 mol) was dissolved in ethanol (500 mL). Immediately after dissolution, glutaraldehyde (0.5 equiv, 0.03 mol, 12 mL of 25% aqueous solution) was added. The mixture was stirred under reflux for 3 h and then concentrated under reduced pressure. Purification of the crude mixture was performed on two consecutive alumina columns (CH_2Cl_2/MeOH 99:1, 98:2, 97:3, and finally 96:4) and monitored by TLC on silica gel (CH_2Cl_2/MeOH 9:1) to furnish pure nitraramine **1** (165–225 mg, 2–3%). Compound **1**: colorless crystals; R_f=0.35 (silica gel, CH_2Cl_2/MeOH 9:1); IR (film, $CHCl_3$): ν_{max}=2928, 1635, 1066 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$ filtered through

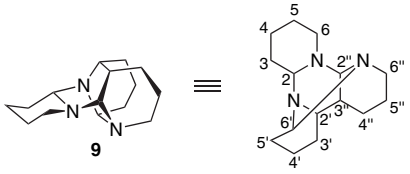
activated alumina) δ 82.2 (C-17), 75.9 (C-1), 66.5 (C-7), 50.5 (C-15), 45.1 (C-3), 38.8 (C-11), 38.0 (C-12), 32.4 (C-6), 30.4 (C-5), 28.4 (C-8), 25.2 (C-13), 24.1 (C-10), 21.6 (C-4), 15.3 (C-9), 14.6 ppm (C-14); ^{1}H NMR (400 MHz, $CDCl_3$ filtered through activated alumina) δ 4.43 (1H, m, H-7), 4.06 (1H, d, J=2 Hz, H-17), 3.36 (1H, s, H-1), 3.10 (2H, m, H-3_{eq}, H-15_{eq}), 2.68 (2H, m, H-15_{ax}, H-3_{ax}), 2.17–2.13 (1H, m, J_{gem}=13.7 Hz, H-5_{eq}), 2.00 (1H, m, H-12), 1.87–1.38 (10H, m), 1.26–1.22 (1H, m, H-10_{ax}), 1.16 (1H, m, H-11), 1.09–1.00 ppm (2H, m, H-14_{ax}, H-5_{ax}); HRMS (ES) calculated for $C_{15}H_{24}N_2OH^+$ $[M+H]^+$: 249.1967, found: 249.1969.

4.3. Trimer 8



Compound ***8***: $C_{15}H_{27}N_3$; waxy yellow solid; R_f=0.70 (silica gel, CH_2Cl_2/MeOH 90:10); IR (film, $CHCl_3$) ν_{max}=3300, 2935, 1651, 1442 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$): δ 83.7 (C-2), 81.7 (C-2″), 63.9 (C-2′), 48.2 (C-6), 47.7 (C-6′), 45.4 (C-6″), 43.3 (C-3″), 29.4 (C-3′), 28.3 (C-3), 27.3 (C-5), 26.1 (C-5′), 25.8 (C-5″), 25.0 (C-4′), 23.1 (C-4″), 22.9 ppm (C-4); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.56 (1H, m, H-6_{eq}), 2.95 (2H, m, H-$6'_{eq}$, H-$6''_{eq}$), 2.42 (3H, m, H-2, H-2′, H-$6''_{ax}$), 1.05–1.72 (19H, m), 0.78 ppm (1H, qd, J_{ax-ax}=12.5 Hz, J_{ax-eq}=5 Hz, H-$5''_{ax}$); Mass (ESI-MS) $[M+H]^+$ 250.

4.4. Compound 9



Compound ***9***: $C_{15}H_{25}N_3$; yellow wax; R_f=0.75 (silica gel, CH_2Cl_2/MeOH 90:10); IR (film, $CHCl_3$) ν_{max}=3020, 1215 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$): δ 83.8 (C-2″), 83.5 (C-6′), 61.5 (C-2), 57.4 (C-2′), 56.2 (C-6), 50.2 (C-3), 46.7 (C-6″), 35.4 (C-3″), 33.0, 31.0, 27.4, 26.4, 25.3, 24.7, 20.8 ppm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 (1H, t/dd, J=10 Hz, H-2), 3.69 (1H, sd, J=2 Hz, H-2″), 3.54 (1H, t, J=8 Hz, H-6_{eq}), 3.53 (1H, s large, H-6′), 3.34 (1H, dd, J=14, 6 Hz, H-$6''_{eq}$), 3.10 (1H, td, J=14, 6 Hz, H-$6''_{ax}$), 2.71 (1H, m, H-6_{ax}), 2.63 (1H, ddd, J=11, 5 Hz, H-2′), 2.51 (1H, ddd, J=11 Hz, H-3_{eq}), 2.44 (1H, m), 2.05 (1H, td, J=11, 2 Hz, H-3_{ax}), 1.68–1.98 (5H, m), 1.60 (1+1H, ddd, J=11, 5 Hz, H-3″), 1.09–1.55 ppm (7H, m); HRMS (ES) calculated for $C_{15}H_{25}N_3H^+$ $[M+H]^+$ 248.2082, found $[M+H]^+$ 248.2085.

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Dihedral angle restriction within a peptide-based tertiary alcohol kinetic resolution catalyst

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Abstract—Kinetic resolution of several tertiary alcohols has been evaluated with a peptide-based catalyst that was designed to probe the role of dihedral angle restrictions for certain bonds within the catalyst. In particular, the nucleophilic residue (π-Me)–His has been replaced with the (β-Me)–(π-Me)–His. A synthesis of the key residue is presented, along with characterization data that suggests the substituent exerts a substantial ground state conformational effect. In addition, kinetic resolution data indicate that the H- to Me-substitution confers enhanced stereoselectivity in several tertiary alcohol resolutions.

1. Introduction

Biomimetic chemistry can encompass a staggering array of objectives, reflecting the enormity of the field of biochemistry.[1] Certainly, the goal of achieving 'artificial enzymes' is one avenue that has drawn inspiration from nature's catalysts.[2] Among the many features of macromolecular catalysts is the ability to control conformation through a wide range of factors that mirror the complexity of the protein folding (and nucleic acid folding) problem.

Our laboratory has been studying short sequences of peptides as catalysts for an array of synthetically relevant reactions.[3] Since even a short peptide sequence has a wide array of conformational possibilities available within its low energy conformational ensembles,[4] we have sought strategies to reduce the complexity of the conformational equilibria.[5] While in some cases this appears relevant to stereoselective catalysts, and in others it does not, we do view the simplification of the conformational equilibria as one strategy that may facilitate the rational design of catalytically active and selective peptide-based catalysts.

Among the strategies available for limiting the conformational dynamics, engineered intramolecular hydrogen bonds, and the incorporation of secondary structure-promoting sequence modules have had a major impact.[6] A less often applied approach in the context of peptide-based catalysts is the strategy of dihedral angle restriction.[7] Perhaps inspired by the proteinogenic amino acids threonine and isoleucine (in comparison to serine and leucine, respectively), the synthesis of β-substituted amino acids, and their incorporation into bioactive peptides, has resulted in enhanced biological activity in a variety of contexts. For example, Hruby improved a synthetic agonist for pain treatment (**1a**) by synthesizing isomers of β-methyl-2′,6′-dimethyltyrosine (TMT) and then substituting each analogue for the original exocyclic tyrosine moiety (Fig. 1).[8] The (2*S*,3*R*)-TMT analogue **1b** produced a seven-fold increase in binding affinity and a two-fold improvement in selectivity whereas the (2*S*,3*S*)-TMT analogue **1c** did not alter the binding affinity and impaired selectivity.

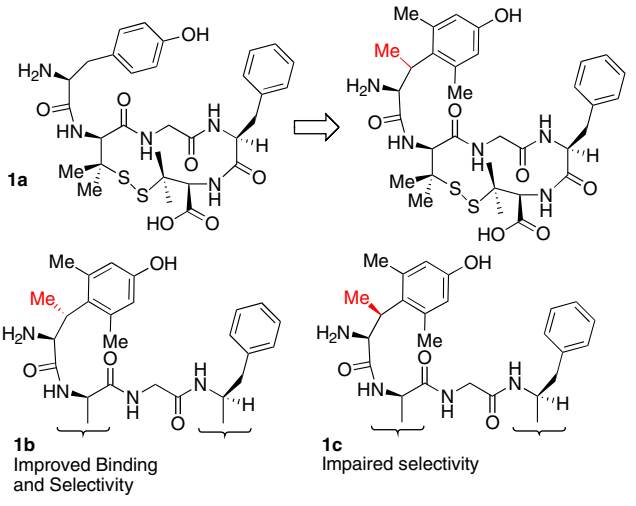

Figure 1. Synthetic agonist for pain treatment and Hruby's optimization through β-substitution within an unnatural tyrosine derivative.

Hruby has shown that the basis of the constraining effect of β-methylation may be two-fold.[9] First, β-methylation of

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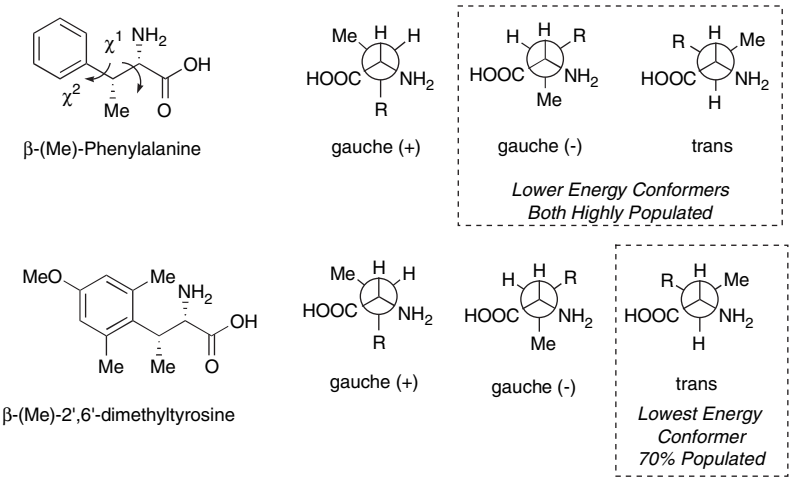
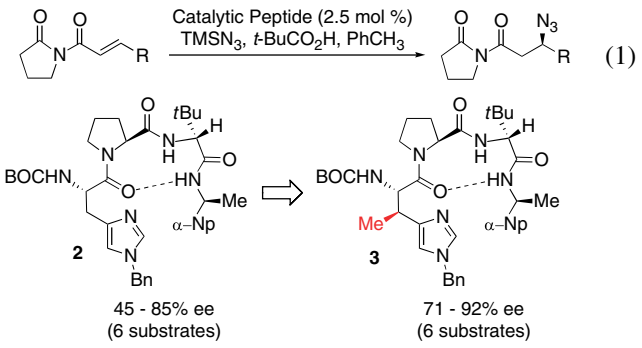


Figure 2. Hruby's analysis of β-substituted amino acid conformational equilibria.

phenylalanine and tyrosine analogues altered the population of rotamers around the Cα–Cβ bond (χ1 angle) such that one or two of the normally available conformations, *gauche* (−), *gauche* (+), or trans, were significantly disfavored (Fig. 2). In the case of the (β-Me)-phenylalanine analogue shown in Figure 2, β-methylation was the only change with respect to the naturally occurring (*S*)-amino acid. The two lowest energy conformations, *gauche* (−) and trans, were highly populated when compared with the wild type. In the case of the (β-Me)-tyrosine analogue shown in Figure 2 the 2′ and 6′ positions of the aromatic moiety were also methylated. Only the lower energy trans conformation was highly populated in this case. The second possible constraining effect of β-methylation is inhibited by aryl ring rotation around the Cβ–Cγ bond (χ2 angle). This category of restricted rotation will be discussed later in the context of our results.

In our earlier studies, we applied the β-substitution strategy to the development of peptide-based catalysts for asymmetric conjugate addition of an azide (Eq. 1).[10] In these experiments we found a subtle, but significant improvement in the enantioselectivity (and overall activities) of the catalysts that contained an appropriately configured substituent in the histidine residue. As shown in Eq. 1, comparison of catalysts **2** and **3** showed that the β-methyl catalyst **3** outperformed catalyst **2** in the enantioselective conjugate addition reactions in each of the six substrate cases we explored. While the precise basis for the ee and rate enhancements was not fully determined; NMR studies of the two catalysts revealed significant differences that supported a more conformationally defined secondary structure for catalyst **3** in comparison to that adopted by catalyst **2**.



In order to evaluate the potential generality of this approach, we sought to explore strategic methyl group insertions into catalysts for a particularly difficult reaction, the kinetic resolution of tertiary alcohols.[11,12] Although the enzymatic kinetic resolution of secondary alcohols is a well-studied area, there are far fewer examples of tertiary alcohol resolution.[13,14] One likely reason for this lack of success is due to the steric demand of the substrate.[15] Among the literature reports of biocatalytic resolution of tertiary alcohols, the degree of enantiodiscrimination ranges from small to very high, although cases of the latter are quite limited.[16,17]

Our own studies of tertiary alcohol kinetic resolution with nonenzymatic peptide-based catalysts reflected these challenges as well. We recently reported catalyst **4**,[18] which provides the only example for a small-molecular catalyst for kinetic resolution that directly resolves tertiary alcohols through nonenzymatic asymmetric acylation.[19] Catalyst **4** was identified by fluorescence-based screening and was successfully utilized to resolve a highly specific class of acetamido-substituted tertiary alcohol substrates (Eq. 2). Six substrates (**5**–**10**, Table 1) were resolved with k_{rel} values ranging from 9 to 22 at 4 °C and from 19 to >50 at −23 °C.[20] However, reactions were generally quite sluggish in comparison with the related secondary alcohol substrates, and k_{rel} values were lower when reactions were conducted at room temperature. Substrates outside this class of tertiary alcohol were not found to undergo significant kinetic resolution with catalyst **4**. We therefore set out to examine the possible advantages of dihedral angle restriction in catalysts for these challenging processes.

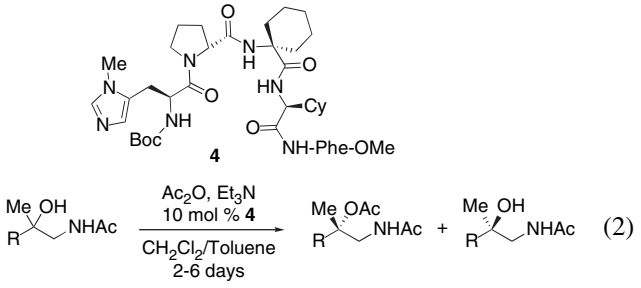
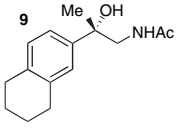
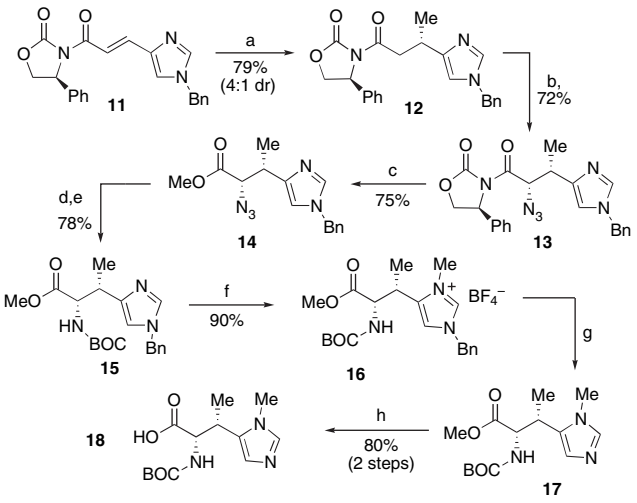


Table 1. Kinetic resolutions of tertiary alcohols with catalyst **4**[a]

Entry	(±)-Substrate	Temp (°C)	k_{rel} (% Conv.)[b]
1	**5**, R=Ph	4	20 (47)
		−23	40 (37)
2	**6**, R=*p*-Tol	4	22 (52)
		−23	>50 (48)
3	**7**, R=*p*-NO_2–Phe	4	15 (54)
		−23	32 (40)
4	**8**, R=β-Nap	4	14 (47)
		−23	40 (35)
5	**9** 	4	20 (51)
		−23	39 (38)
6	**10**, R=Cy	4	9 (51)
		−23	19 (35)

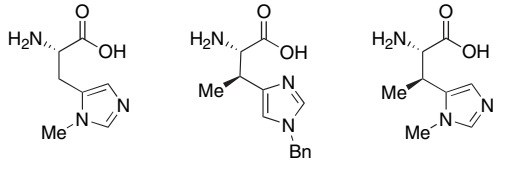
[a] Conditions found in Ref. 18.
[b] Calculated according to the method of Kagan.



Scheme 1. (a) CH_3MgBr, CuBr·DMS, THF, −40 °C; (b) KHMDS, THF, −50 °C; then trisyl azide, −78 °C; (c) NaOMe, MeOH/CH_2Cl_2, −10 °C; (d) H_2, Pd/C, AcOH/MeOH; (e) Boc_2O, CH_2Cl_2; (f) Me_3OBF_4, CH_2Cl_2, 25 °C; (g) H_2, 10% Pd/C, MeOH/AcOH; (h) LiOH, MeOH/H_2O, 25 °C; H-ion exchange, NH_4OH.

2. Results and discussion

Evaluation of β-methyl substitution in the context of tertiary alcohol kinetic resolutions through asymmetric acylation required a synthesis of (β̃-Me)–(π-Me)–His to be accomplished. Our earlier studies in azide conjugate addition relied on the incorporation of (β̃-Me)–(τ-Bn)–His into the peptides. Our synthetic approach to this residue followed Hruby's initial synthetic procedure. In the context of the π-methylated version as required, we opted to simply convert (π-Me)–His to (β-Me)–(π-Me)–His.



The synthesis began in analogy to the literature precedent for Boc–(2*S*,3*S*)–(β-Me)–(τ-Bn)–His–OH (Scheme 1).[21,22] Starting from urocanic acid-derived imide **11**, diastereoselective conjugate addition using CH_3MgBr and CuBr·DMS afforded **12** in a 4:1 mixture of the diastereomers with the (*S*)-methyl configuration as the major product. The major diastereomer was carried on and subjected to diastereoselective azidation as described by Evans[23] to afford azido imide **13** with complete diastereocontrol. The chiral auxiliary was removed by methanolysis to yield the corresponding methyl ester **14**. Reduction of the azide **14** (H_2, 10% Pd/C, AcOH/MeOH) followed by Boc-protection afforded Boc–(2*S*,3*S*)–(β-Me)–(τ-Bn)–His–OMe **15**.

In order to prepare the desired π-methyl derivative, a procedure was developed in which the π-nitrogen of **15** was alkylated using trimethyloxonium tetrafluoroborate to yield imidazolium salt **16** (90%). Subsequent hydrogenolysis of the benzyl group using 10% Pd/C under 1 atm of H_2 for 24 h afforded **17**; hydrolysis of the methyl ester (LiOH, MeOH/H_2O) and isolation by elution with ammonium hydroxide on ion exchange resin afforded the desired Boc–(2*S*,3*S*)–(β-Me)–(π-Me)–His–OH **18** (80%, two steps: hydrogenolysis/hydrolysis). Final purification of **18** was easily carried out by preparative reverse phase HPLC to yield the key amino acid derivative as a fine white powder.

The ^1H NMR spectrum of amino acid derivative **18** (300 MHz, D_2O) exhibited some evidence for restricted rotation about the Cβ–Cγ bond. As shown in Figure 3, two peaks of unequal height for the imidazole C2-proton (H_A) were observed at 25 °C; the peaks could be observed to coalesce reversibly upon heating the sample to 55 °C. The existence of signal doubling for the proton corresponding to H_A on the imidazole ring may be consistent with restricted rotation around the Cβ–Cγ bond (χ2) of **18**. The barrier to interconversion was estimated to be 16 kcal/mol according to the standard analysis of coalescence temperatures.[24] Of note, the temperature dependent ^1H NMR spectra were also observed for methyl ester derivative **17** when the ^1H NMR spectrum was analyzed in D_2O. However, when compound **17** was examined in CD_2Cl_2, the effect was not observed. While these observations are not fully understood at this time, it appears that strong solute–solvent interactions in protic versus aprotic media influence the dynamics of compounds like **17** and **18**. Of note, these observations of temperature dependent NMR spectra and their relationship to dihedral angle restriction are quite reminiscent of the observations of Hruby in the β-substituted tyrosine series.[25]

In the case of (2*S*,3*S*)–(β-Me)–(π-Me)–His–OH (**19**), however, we also were able to show that the dynamic NMR behavior was coupled to the Boc-protection state of the backbone nitrogen. Deprotection under standard conditions produced free amino acid **19** (Fig. 4). The ^1H NMR spectrum of the protecting group-free residue did not exhibit evidence of restricted rotation about any torsional angles. Based on these data, we speculate that either a remote steric effect, or possibly a hydrogen bonding interaction is associated with the Boc-group of **18**, which is related to the dihedral

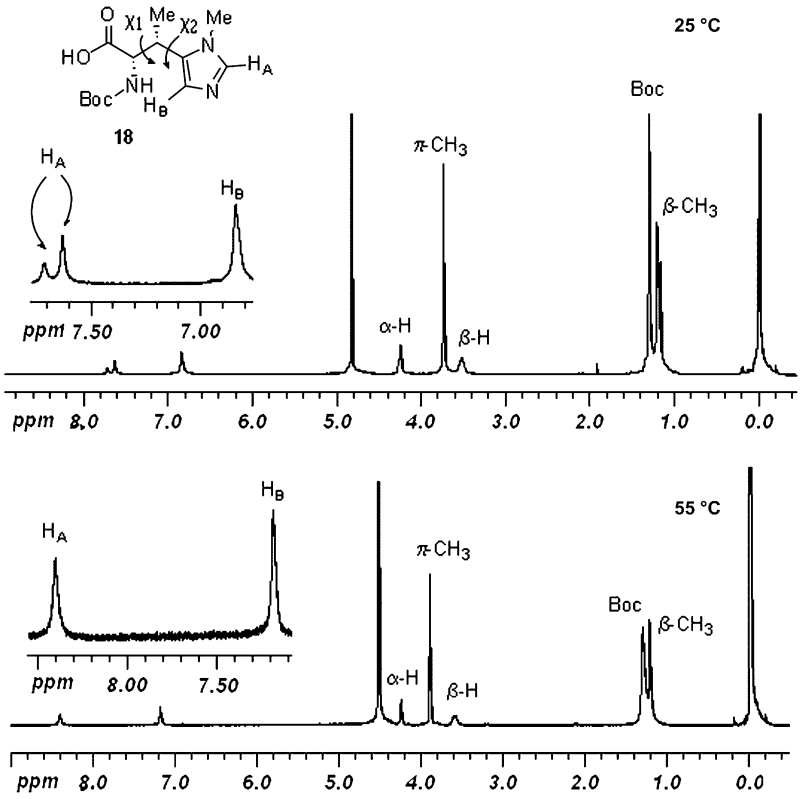


Figure 3. ^{1}H NMR spectra (300 MHz, D_2O) of amino acid derivative **18** recorded at 25 °C (top) and 55 °C (bottom).

angle restriction. This possibility is notable in the context of peptide-based catalysts wherein residues like **19** are incorporated into peptide sequences where nonbonding interactions within the catalyst structure may contribute strongly to the overall structure and function.

These structural effects do indeed translate to improved catalysts for the kinetic resolution of tertiary alcohols. Initially, we sought to examine the direct comparison of catalyst **4**, lacking the β-methyl group in the His-residue, to catalyst **20**, an analog that contained this perturbation. We began with a comparison of kinetic resolutions of tertiary alcohol **21** (Eq. 3), and the results are presented in Table 2.

A significant improvement in catalyst performance was observed with catalyst **20** on comparison with **4**. Not only enantioselectivity (k_{rel}) was improved, but also performance was excellent at the higher temperature, reaction times were greatly diminished, and improved selectivity was sustained at lower catalyst loadings. For example, catalyst **4** delivers

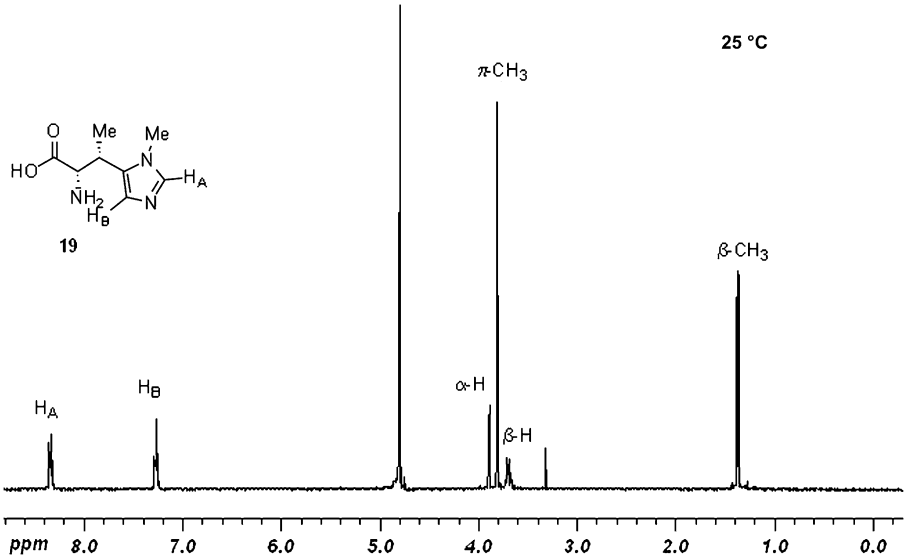


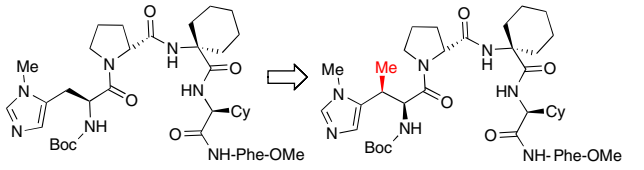
Figure 4. ^{1}H NMR spectrum (400 MHz, D_2O) of acid **19**.

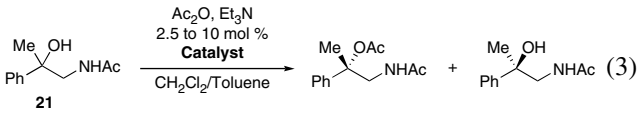
Table 2. Kinetic resolutions of substrate **21** with catalysts **4** versus **20**[a]

Entry	Catalyst	Temp (°C)	Time (h)	k_{rel} (% Conv.)[b]
1	**4** (10 mol %)	25	24	14 (29)
2	**20** (10 mol %)	25	24	>50 (51)
3	**4** (10 mol %)	4	24	21 (26)
4	**20** (10 mol %)	4	24	>50 (50)
5	**20** (10 mol %)	25	12	>50 (46)
6	**20** (2.5 mol %)	25	12	49 (27)

[a] Conditions found in Section 4.
[b] Calculated according to the method of Kagan.

a k_{rel} of 14 at 29% conversion when the reaction is conducted at 25 °C (Table 2, entry 1), whereas catalyst **20** results in a k_{rel} of >50 at 51% conversion under identical conditions (entry 2). Furthermore, while peptide **4** may be induced to deliver a slight boost in selectivity at lower temperature (k_{rel}=21, 4 °C, entry 3), catalyst **20** performs in a nearly identical fashion within this moderate temperature range (k_{rel}>50, 4 °C, entry 4). Because of the apparent increase in activity and selectivity with catalyst **20**, we examined its performance within shorter reaction times and at lower catalyst loading. Notably, within 12 h, the kinetic resolution of **21** may be performed such that a k_{rel} of >50 at 46% conversion may be achieved (entry 5). In addition, when the catalyst loading is reduced to 2.5 mol %, the reaction may be performed such that k_{rel} of 49 is observed, but at 27% conversion within 12 h (entry 6).[26]





Given the marked improvement of catalyst **20** in comparison with **4**, we sought to examine if these effects would translate to other substrates (Eq. 4). As shown in Table 3, the performance enhancements indeed translated to a range of cases, although they were not universally achieved. Compound **22** (entry 1a–b), bearing a replacement of the phenyl ring of **21** with a cyclohexyl group, presents a particularly interesting case. Whereas a sluggish reaction occurs with catalyst **4** to give modest stereoselectivity (k_{rel}=9, 33% conv.), catalyst **20** induces a substantial rate enhancement, with a dramatic improvement in stereoselectivity (k_{rel}>50). Compound **23** (entry 2a–b) also undergoes improved kinetic resolution with catalyst **20**, although the selectivity enhancement is less pronounced (k_{rel}=10, catalyst **4**; k_{rel}=24, catalyst **20**).

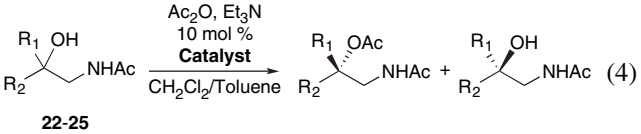
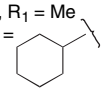
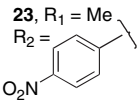
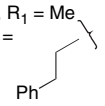


Table 3. Kinetic resolutions of tertiary alcohols with catalysts **4** versus **20**[a]

Entry	(±)-Substrate	Catalyst	Time (h)	Temp (°C)	k_{rel} (% Conv.)[b]
1a	**22**, R_1 = Me, R_2 = 	**4**	52	4	9 (33)
1b		**20**	52	4	>50 (53)
2a	**23**, R_1 = Me, R_2 = 	**4**	20	25	10 (39)
2b		**20**	20	25	24 (60)
3a	**24**, R_1 = Me, R_2 = 	**4**	20	25	3 (32)
3b		**20**	20	25	18 (65)
4a	**25**, R_1=CO_2Me	**4**	18	25	1.6 (21)
4b	R_2=Ph	**20**	18	25	2.8 (43)

[a] Conditions found in Section 4.
[b] Calculated according to the method of Kagan.

Substrate **24** (entry 3a–b) provides another case of aryl group replacement with an aliphatic chain (Ph, **21** to C_2H_4Ph, **24**). Once again, catalyst **20** provides a substantial improvement (k_{rel}=3, catalyst **4**; k_{rel}=18, catalyst **20**), and once again overall activity is improved as conversion increases from 32 to 65% within a 20 h window. Finally, to illustrate that catalyst **20** is by no means a universal solution to the challenging substrate class of tertiary alcohol resolution, we note that substrate **25** (entry 4a–b) exhibits low selectivity, and only a small improvement in k_{rel} was observed when catalyst **4** is exchanged for **20** (k_{rel}=1.6, catalyst **4**; k_{rel}=2.8, catalyst **20**).

3. Conclusions

In combination with our earlier results in the area of peptide-catalyzed asymmetric conjugate addition of azide, the results presented above constitute a second example wherein the rational incorporation of a β-branch into a catalytically significant histidine residue results in an improvement in the overall stereoselectivity and activity exhibited by a peptide-based catalyst. The exact mechanistic basis of the rate and selectivity enhancements for catalysts remains a topic of investigation. In combination with precedents in the literature, and our own observations of solution conformational behavior, it may be noted that the dihedral angle restriction is indeed at the heart of the improvements in catalysis we have observed. If these effects are in fact manifested in conformational features, it may be noted that we have leveraged in some way nature's evolution of β-branched amino acids as complements of those that are simply '–CH_2–' in the β-position.

4. Experimental

4.1. General procedures

Proton NMR spectra were recorded on Varian 400 or 300 spectrometers. Proton chemical shifts are reported in parts per million (δ) relative to an internal tetramethylsilane (TMS, 0.00 δ) or with the solvent reference relative to

TMS employed as the internal standard ($CDCl_3$, δ 7.26 ppm; D_2O, δ 4.79 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), broad doublet (br d), broad singlet (br s), and multiplet (m)], coupling constants [Hz], integration). Carbon NMR spectra were recorded on Varian 400 (100 MHz), or 300 (75 MHz) spectrometers with complete proton decoupling. Carbon chemical shifts are reported in parts per million (δ) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ 77.0; D_2O/methanol, δ 49.15). NMR data were collected at 25 °C, unless otherwise indicated. Infrared spectra were obtained on a Nicolet 210 spectrometer. Diagnostic bands are reported (cm^{-1}). Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 pre-coated plates (0.25 mm thickness). TLC R_f values are reported. Visualization was accomplished by irradiation with a UV lamp and/or staining with cerium ammonium molybdenate (CAM) or $KMnO_4$ solutions. Silica gel chromatography was performed using Silica Gel 60A (32–63 μm) from Scientific Adsorbants, Inc. Optical rotations were performed on a Rudolf Research Analytical IV Automatic polarimeter at the sodium D line (path length 50 mm) at 20 °C. High resolution mass spectra were obtained at the Mass Spectrometry Facilities at Boston College (Chestnut Hill, MA, USA). The method of ionization is given in parentheses. Analytical GC was performed on a Hewlett–Packard 6890 employing a flame ionization detector and the column was specified in the individual experiment. Analytical and preparative reverse phase HPLC were performed on a Rainin SD-200 chromatograph equipped with a single wavelength UV detector (214 nm). Analytical normal-phase, chiral HPLC was performed on a Hewlett–Packard 1100 or Agilent 1100 Series chromatograph equipped with a diode array detector. (The wavelengths of 214 and 254 nm were used for compound detection.)

All solvents, triethylamine (Et_3N), and diisopropylethylamine (DIPEA) were distilled from appropriate drying agents prior to use. Acetic anhydride was also distilled over an appropriate drying agent prior to use and stored in a Schlenk tube over molecular sieves. Amino acid derivatives (unless otherwise specified) and coupling reagents (EDC, HOBt) were used as received from NovaBiochem or Advanced ChemTech. Trimethyloxonium tetrafluoroborate (Meerwein salt, $Me_3O^+\ BF_4^-$) was used as received from Aldrich Chemical Company. Palladium on carbon (10% Pd/C) was purchased from Strem Chemical Company and used as received.

Racemic substrates **21**–**23** and **25** were prepared from the corresponding epoxides.[27] Substrate **24** was prepared according to the method of Simona.[28] The following compounds have been also previously prepared in the literature: **4**, **11**–**15**,[10a] **21**–**23**, and **25**.[18]

4.1.1. Preparation of 16. Boc–(2*S*,3*S*)–(β-Me)–(τ-Bn)–His–OMe (**15**, 690 mg, 1.90 mmol) was dissolved in methylene chloride and then trimethyloxonium tetrafluoroborate (410 mg, 2.80 mmol) was added. The reaction mixture was stirred for 1 h at 25 °C and then quenched by washing with 2×15 mL of distilled water. The organic layer was dried, filtered, and evaporated to give **16** as a white solid (710 mg, 1.5 mmol, 80% yield). ^1H NMR ($CDCl_3$, 300 MHz) δ 8.86 (s, 1H), 7.38 (m, 5H), 7.27 (s, 1H), 6.98 (br s, 1H), 5.27 (m, 3H), 4.53 (m, 1H), 3.92 (s, 3H), 3.76 (s, 3H), 3.47 (dd, J=3.9, 6.8 Hz, 1H), 1.29 (s, 9H), 1.21 (d, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 155.5, 137.0, 136.8, 132.8, 129.7, 129.2, 119.4, 80.9, 55.9, 53.8, 53.4, 34.3, 32.8, 28.5, 14.2; IR (film, cm^{-1}) 3376, 3144, 3093, 2974, 1740, 1715, 1161, 1061, 746; TLC R_f 0.24 (ethyl acetate); $[\alpha]_D$ +30.7 (*c* 2.0, CH_2Cl_2); exact mass calcd for $[C_{21}H_{30}N_3O_4]^+$ requires *m*/*z* 388.2236. Found 388.2239. (ESI+).

4.1.2. Preparation of 17. Compound **16** (115 mg, 0.24 mmol) was dissolved in 5 mL of methanol and 200 μL of acetic acid. Then the flask was flushed with N_2 and after 5 min 10% Pd/C was carefully added. The flask was evacuated and purged with H_2 two times before being left to stir under 1 atm of H_2. After 24 h the reaction mixture was filtered through Celite, washed with methanol, and the solvent was evaporated. Crude product was purified using a reverse phase HPLC [RP-18 X Terra (Waters) column], eluting with 20% methanol/H_2O at a flow rate of 4 mL/min, to give a yellow oil. The purified oil was then taken up in CH_2Cl_2 and washed twice with saturated $NaHCO_3$. The organic layer was dried, filtered, and evaporated to give **17**. (83 mg, 0.22 mmol, 90% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (s, 1H), 6.80 (s, 1H), 5.24 (d, J=8.8 Hz, 1H), 4.56 (dd, J=9.0, 4.6 Hz, 1H), 3.76 (s, 3H), 3.66 (s, 3H), 3.38 (m, 1H), 1.36 (s, 9H), 1.27 (d, J=3.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2, 155.0, 138.0, 131.6, 127.1, 80.1, 56.7, 52.6, 32.6, 31.6, 28.4, 15.1; IR (film, cm^{-1}) 3371, 3207, 2974, 1753, 1709, 1507, 1369, 1161; TLC R_f 0.33 (1% methanol/CH_2Cl_2); $[\alpha]_D$ +30.7 (*c* 2.0, CH_2Cl_2); exact mass calcd for $[C_{14}H_{23}N_3O_4+H]^+$ requires *m*/*z* 298.1767. Found 298.1769. (ESI+).

4.1.3. Preparation of 18. Compound **17** (85 mg, 0.22 mmol) was dissolved in 3 mL of methanol and 1 mL of distilled H_2O. Lithium hydroxide monohydrate ($LiOH\cdot H_2O$) was added (36 mg, 0.86 mmol) and the reaction was stirred for 3 h. The solvent was evaporated, and the crude product was then taken up in 10% NH_4OH and eluted with the same solvent through a column of Dowex-500 hydrogen ion exchange resin. The solvent was then evaporated to give a crude off-white powder. The crude material was purified using a reverse phase RP-18 X Terra (Waters) column, eluting with 5% methanol/H_2O at a flow rate of 4 mL/min to give **18** as a white powder (55 mg, 0.19 mmol, 88%). ^1H NMR (D_2O, 55 °C, 300 MHz) δ 8.88 (br s, 1H), 7.59 (br s, 1H), 4.58 (d, J=3.60 Hz, 1H), 4.26 (s, 1H), 3.94 (m, 1H), 1.63 (br s, 9H), 1.55 (d, J=7.20 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 175.6, 156.8, 136.7, 134.7, 117.1, 81.0, 56.8, 33.4, 32.3, 27.7, 12.6; IR (film, cm^{-1}) 3320, 2974, 1690, 1614, 1400, 1161, 1029; TLC R_f 0.13 (15% methanol/CH_2Cl_2); $[\alpha]_D$ +8.1 (*c* 1.0, methanol); exact mass calcd for $[C_{13}H_{21}N_3O_4+H]+$ requires *m*/*z* 284.1610. Found 284.1605. (ESI+).

4.1.4. Preparation of 19. Compound **18** was dissolved in 1 mL of trifluoroacetic acid (TFA) and 1 mL of CH_2Cl_2. The reaction was stirred for 40 min. The solvent was evaporated, and the crude oil was redissolved in toluene, and subsequently concentrated three times, under conditions of azeotrope to remove residual water. ^1H NMR (D_2O,

400 MHz) δ 8.33 (s, 1H), 7.26 (s, 1H), 3.89 (d, J=4.8 Hz, 1H), 3.80 (s, 3H), 3.69 (m, 1H), 1.36 (d, J=7.2 Hz, 3H).

4.1.5. Data for purified peptide 20. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32–7.20 (overlapping of s and m, 5H), 7.16 (br d, J=6.8 Hz, 2H), 6.89 (s, 1H), 6.65 (br d, J=8.4 Hz, 1H), 6.44 (m, 1H), 6.29 (br s, 1H), 4.92 (m, 1H), 4.65 (t, J=10.2 Hz, 1H), 4.30 (m, 1H), 4.16 (m, 1H), 4.03 (m, 1H), 3.75 (m, 1H), 3.66 (s, 3H), 3.62 (s, 3H), 3.31 (m, 1H), 3.17 (dd, J=14.0, 5.6 Hz, 1H), 3.08 (dd, J=14.0, 6.2 Hz, 1H), 2.18–1.64 (m, 11), 1.45 (s, 9H), 1.33 (d, J=7.2 Hz, 3H), 1.29–1.09 (m, 10H), 1.00–0.81 (m, 4H); TLC R_f 0.35 (4% methanol/CH_2Cl_2); exact mass calcd for $[C_{43}H_{63}N_7O_8+H]^+$ requires m/z 806.4816. Found 806.4821 (ESI+).

4.2. Standard conditions for kinetic resolution

A stock solution of racemic alcohol (0.015 M) was made by dissolving the alcohol in 2:3 CH_2Cl_2/toluene. The stock solution was distributed in 1 mL aliquots to reaction vessels, and then 20 μL of a stock solution of peptide catalyst (0.0015 mmol in CH_2Cl_2) was introduced. Acetic anhydride (70.0 μL, 0.74 mmol) and triethylamine (42 μL, 0.30 mmol) were then introduced. The reaction mixture was allowed to stir at 25 or 4 °C. Then the reaction mixture was quenched with methanol, and the solvent and amine were removed in vacuo. The peptide catalyst was removed by running the residue through a 1 cm silica plug in a Pasteur pipette, eluting with ethyl acetate. Solvent was again removed. The residue was dissolved in 10% 2-propanol in hexane and assayed by chiral HPLC analysis or dissolved in ethyl acetate and assayed by chiral GC analysis as previously described[18] (substrates **21**–**23**, and **25**) or as described below.

4.2.1. Data for substrate 24. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31–7.25 (m, 2H), 7.19–7.16 (m, 3H), 6.26 (br s, 1H), 3.31 (dd, J=15.0, 5.9 Hz, 1H), 3.28 (dd, J=13.2, 5.9 Hz, 1H), 3.15 (s, 1H), 2.70 (m, 2H), 2.00 (s, 3H), 1.84–1.70 (m, 2H), 1.23 (s, 3H); IR (film, cm^{-1}) 3452, 3050, 2987, 1671, 1426, 1269, 730; TLC R_f 0.15 (60% ethyl acetate/hexane); exact mass calcd for $[C_{13}H_{19}NO_2+Na]^+$ requires m/z 244.1313. Found 244.1319 (ESI+).

4.2.2. Data for acylation product 24–Ac. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26–7.16 (m, 5H), 6.33 (br s, 1H), 3.68 (dd, J=14.65, 6.59 Hz, 1H), 3.58 (dd, J=14.65, 5.62 Hz, 1H), 2.74–2.57 (m, 2H), 2.23–2.13 (m, 1H), 2.00–1.93 (m, 1H), 2.02 (s, 3H), 1.46 (s, 3H); IR (film, cm^{-1}) 3295, 2924, 1734, 1652, 1558; TLC R_f 0.44 (60% ethyl acetate/hexane); exact mass calcd for $[C_{13}H_{21}NO_3+Na]^+$ requires m/z 286.1419. Found 286.1418 (ESI+).

4.2.3. Assay of enantiomeric purity for 24 and 24–Ac. Enantiomers of the starting material **24** and the enantiomers of the corresponding acylated product (**24**–**Ac**) were separated by chiral HPLC using a chiral OD column (Alltech) by eluting with 2% ethanol/hexane at a flow rate of 0.75 mL/min for 90 min. Retention times: **24**: 67 and 86 min, **24**–**Ac**: 43 and 51 min.

The absolute configurations of **21**, **21**–**Ac**, **22**, and **22**–**Ac** were determined by correlation of the observed optical rotations with literature reports.[18] The absolute configurations of the major and minor enantiomers of the other substrates were not determined, but are rather drawn in analogy to the observed fast and slow reacting enantiomers of substrate **21** and **22** with catalysts **4** and **20**.

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The structural and synthetic implications of the biosynthesis of the calycanthaceous alkaloids, the communesins, and nomofungin

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Abstract—A comparison is made between the calycanthaceous alkaloids, nomofungin, and the communesins using structural and biosynthetic information from studies of the former to shed light on the structural ambiguity of the two latter species. Also, a novel biosynthetic approach for the communesins is presented that involves coupling of tryptamine with the ergot alkaloid aurantioclavine that is suitable for synthetic emulation. Preliminary synthetic studies and intermediates are reported.

1. Introduction

Natural product isolation, biosynthetic studies, and natural product synthesis are often used synergistically to illuminate molecular structure and to broaden understanding of small molecule/biological interactions. Chemical transformations, purifications, conformational analysis, and structural characterization are all shared aspects of these approaches to describe natural products. Often, knowledge of the biosynthesis and the natural function of isolated compounds can suggest approaches to their synthesis and application to novel targets. Synthetic efforts can resolve ambiguities in structural assignment as well as provide material for in depth analysis. Because of this interconnectedness, comprehensive background knowledge of structural classes of compounds is essential for the practicing chemist.

Thus, organic chemists have become well acquainted with the calycanthaceous alkaloids, which were first isolated from the plant genus *Calycanthus*.[1] Early isolations of individual members showed a closely related skeleton that differed only in the aminal connectivity for the various natural products. In fact, five structural isomers are possible through aminal construction from the hypothetical intermediate **1** (see Fig. 1), though only four of these arrangements (**3**, **4**, **5**, and **6**) are currently represented by natural products. The subtle differences in possible structures mandated substantial effort to establish the relative and absolute stereochemistry of these compounds; chemical degradation studies, nuclear magnetic resonance (NMR) techniques, X-ray diffraction analysis, and total synthesis techniques were required to elucidate unambiguously the structures of these alkaloids.

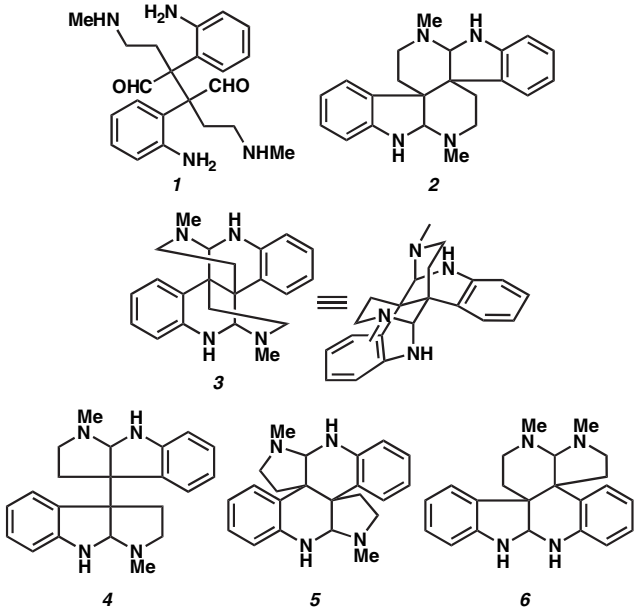

Figure 1. Possible topologies for the calycanthaceous alkaloids.

The first calycanthoid to be characterized was (+)-calycanthine (**3**, Fig. 2). The structure was established chemically by Robinson[2] and Woodward[3] and crystallographically by

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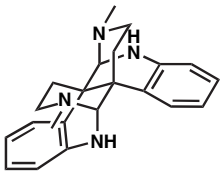


Figure 2. Calycanthine.

Hamor and Robertson using the dihydrobromide dihydrate salt.[4] Two appended bridged bicycles comprise this C_2-symmetric structure, the most intricate of the five regioisomers. The absolute stereochemistry of calycanthine was determined using circular dichroism analysis by Mason soon after the publication of the crystal structure.[5]

The structure of (−)-chimonanthine (**7**, Fig. 3), isolated from *Chimonanthus fragrans* (Hodson et al.[6]), was subsequently elucidated by Hamor and Robertson through X-ray analysis of the dihydrobromide salt.[7] Chimonanthine contains two indoline units, each with an annulated pyrrolidine, that are 3,3′-connected. Interestingly, both the C_2-symmetric and *meso* isomers have been identified. The absolute stereochemistry of the vicinal quaternary carbon centers of (−)-chimonanthine is identical to that of calycanthine, and the two equilibrate under acidic conditions. Two other ambiguous structures, those of calycanthidine[8] (**8**) and folicanthine[9] (**9**), were also confirmed by the chimonanthine crystal structure, as these compounds had been shown to be the successive methylation products of chimonanthine.[10]

Later, in 1992, (−)-calycanthine, *meso*-chimonanthine, and the novel *iso*-calycanthine (**11**, Fig. 4) were isolated from *Psychotria forsteriana*.[11] The first two compounds were identified by comparison to previously isolated compounds. The structure of *iso*-calycanthine was assigned based on available spectroscopic data and contrast to known isomers. Compound **11**, a *meso*-compound, bears the same diastereomeric relationship about its quaternary carbon centers as *meso*-chimonanthine. An oxidized version of skeleton **2** was later observed in extracts of *Psychotria colorata* in addition to the compounds isolated from *P. forsteriana*.[12] (8-8a),(8′-8′a)-Tetradehydroisocalycanthine (**12**) was characterized spectroscopically and via a crystal structure using the dihydrobromide salt. If performed, oxidation of **11** to **12** or reduction of **12** to **11** would further confirm the structure of **11**.

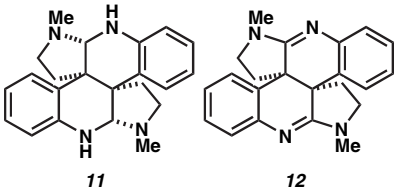


Figure 4. Representatives of framework **5**.

Many closely related compounds have been isolated from sources other than plants. In fact, the antipodes for the *Calycanthus*-derived structures, (−)-calycanthine and (+)-chimonanthine, were isolated from the Colombian poison-dart frog, *Phyllobates terribilis*.[13]

Similarly, skeleton **6** (Fig. 1) appears in the communesins[14] and nomofungin.[15] Communesins A and B (**14** and **15**, Fig. 5) were isolated from a strain of *Penicillum sp*. marine fungus in 1993. Communesin B had cytotoxic effects on P-388 lymphocytic leukemia cells with an effective dose of 0.45 μg mL^{-1}, while communesin A had an effective dose of 3.5 μg mL^{-1}. Later, isolations of communesins C–H showed varied substitution on the nitrogens and pendant isobutene.[16] Communesins C–F also showed antiproliferative and insecticidal activity, but G and H did not.[16c] Nomofungin (**13**) was found to disrupt microtubule formation in cultured mammalian cells. It is somewhat cytotoxic and demonstrates minimum inhibitory concentrations of 2 and 4.5 μg mL^{-1} with LoVo and KB cells, respectively.

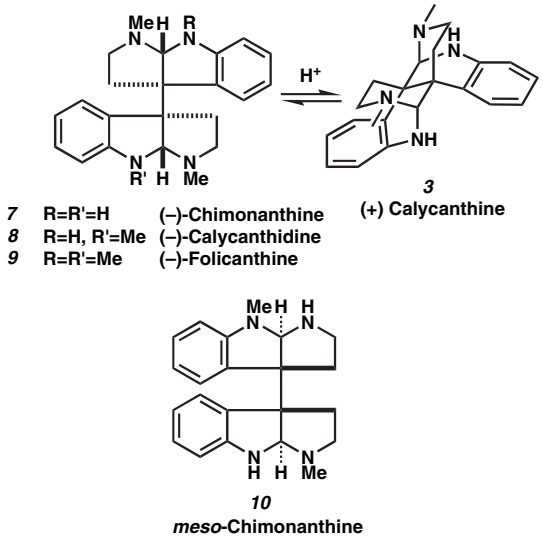


Figure 3. Compounds representative of scaffold **4**.

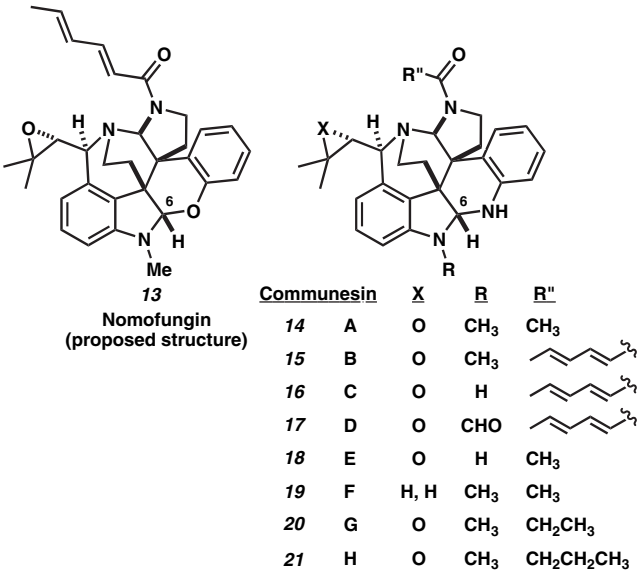
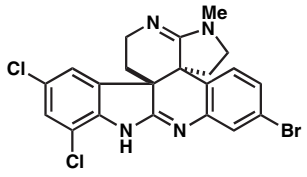


Figure 5. The communesins and nomofungin.

An additional recent isolate from fungal cultures (*Perophora namei* ascidia), reminiscent of the calycanthaceous alkaloids and similar in connectivity to the communesins, is perophoramidine (**22**, Fig. 6).[17] This compound demonstrated cytotoxicity with the HCT116 colon carcinoma cell line with an IC_{50} of 60 μM and induced apoptosis via PARP cleavage within 24 h. Note that the arrangement of the vicinal quaternary carbon centers in perophoramidine is diastereomeric with respect to the orientation found in the communesins. Perophoramidine mimics the relative stereochemistry found in *meso*-chimonanthine, while communesin mirrors that of (±)-chimonanthine at those centers.

The apparently convergent development of the calycanthaceous alkaloids by such disparate species indicates they are a readily accessed family of compounds for many organisms and thus are likely to be important as research targets. The similarities between nomofungin and communesin B are especially striking, prompting an examination of the reported spectral data for the two compounds. Interestingly, the chemical shifts and coupling constants are essentially identical in all respects.[14,15] In particular, the chemical shift of the C(6) proton is reported to be 4.70 ppm for communesin B and 4.69 ppm for nomofungin. Analogously, the ^{13}C NMR chemical shift for C(6) is 82.4 ppm for both compounds. From these data, and the similarity of the full NMR data set, we concluded that communesin B and nomofungin must be the same molecule.[18]



22
Perophoramidine

Figure 6. Perophoramidine.

Fortunately, structural similarity to the calycanthaceous alkaloids can potentially shed light on a potential biosynthesis of this compound and can thus help to determine which proposed structure, if any, correctly describes communesin B and nomofungin. Such a comparison of structures obviously favors the communesin structural assignment. Even so, comparison to known chemical shift values for aminal and hemiaminal functionalities confirms the tendency for the ^{1}H NMR chemical shifts of the former to reside upfield relative to the latter. For example, the aminal protons in isochimonanthine (**23**) and calycanthine (**3**) are 4.32 and 4.42 ppm, respectively, whereas the shifts for hemi-aminals **24**, **25**, and **27** are 5.0, 5.4, and 6.3 ppm (Fig. 7).[19] The nomofungin and communesin B value of 4.7 ppm resides closer to that for an aminal. Similarly, ^{13}C NMR chemical shifts for aminal carbons are typically in the range of 71.0 to 84.0 ppm, while those for hemi-aminal carbons are shifted downfield to between 97.0 and 106.0 ppm.[20] The case of the proposed and actual structures for diazonamide A, **26** and **27**, respectively, shows a general trend that additional oxygenation shifts the signals further downfield. The nomofungin and communesin shift, 82 ppm, is clearly in the range for aminal carbons. Given the ^{1}H and ^{13}C NMR chemical shift data, we have assumed that the assignment for communesin B best represents the true structure.

Biosynthetic comparison to the calycanthaceous alkaloids can also provide insight to the atomic makeup of

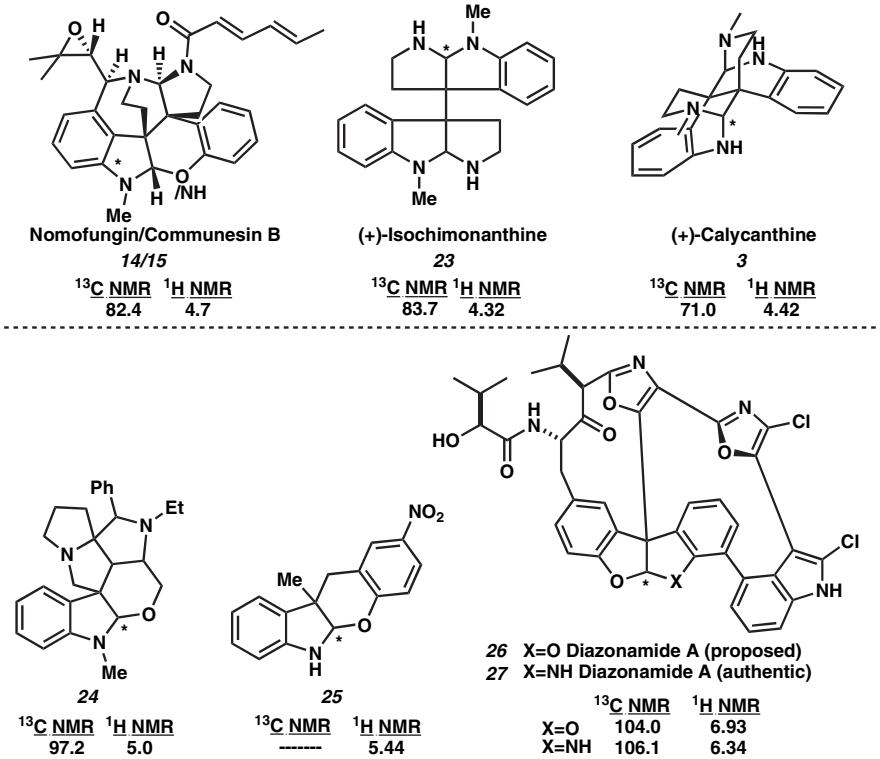
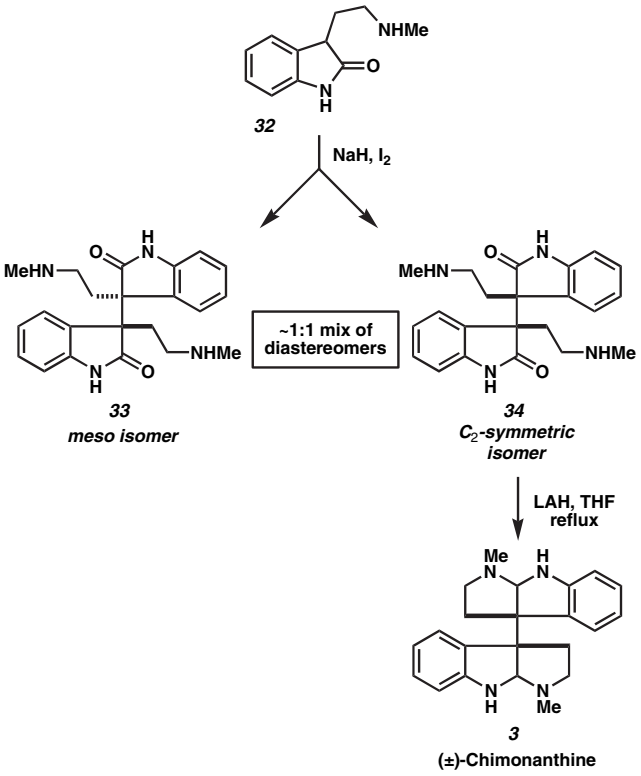


Figure 7. Peak frequencies of known compounds.[19,20]

communesin and nomofungin. Both biosynthetic labeling and biomimetic syntheses of the calycanthoids have established structural assignments and an accepted biosynthesis, which is predicated on an oxidative dimerization of *N*-methyl tryptamine (**28**, Scheme 1). The newly formed bis-indolenine can undergo hydrolysis to access hypothetical intermediate **1**, or it can easily form the chimonanthine scaffold **2** or scaffold **4** by attack of the free amines on the indolenine imines. As scaffold **2** remains unrepresented by isolated natural products, the chimonanthine scaffold **4** is likely the favored product of aminal closure. This scaffold can then be modified to provide the various chimonanthine derivatives. The other scaffolds (i.e., **3** and **5**) are accessible from intermediate **1**.

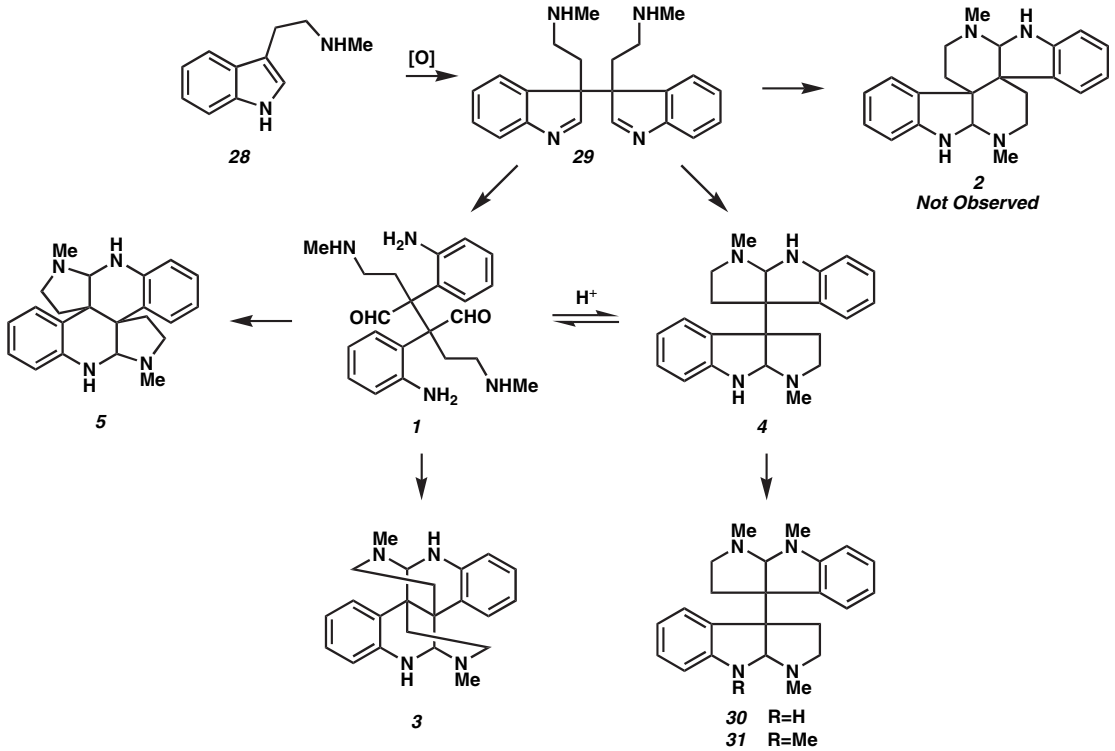
The oxidative coupling of tryptamine equivalents has been replicated in the laboratory using several oxidants. In an early study, Hendrickson et al. performed the coupling using sodium hydride and iodine to afford the bis-oxindoles **33** and **34** as a 1:1 mixture of diastereomers (C_2-symmetric and *meso*, Scheme 2). The C_2-symmetric isomer can be reductively cyclized with LAH in refluxing THF to form (±)-chimonanthine.[1]

In a later study, Scott et al. prepared the magnesium salt of methyl tryptamine and oxidatively dimerized it using iron (III) chloride to produce the indolenine dimer **29** (Scheme 3).[21] Subsequent aminal formation by the tryptamine side chains under the reaction conditions afforded *meso*- and (±)-chimonanthine in one step. Moreover, (±)-calycanthine is accessible by treating (±)-chimonanthine with aqueous acid, demonstrating that scaffold **3** is thermodynamically preferred to scaffold **4**.[1,21]

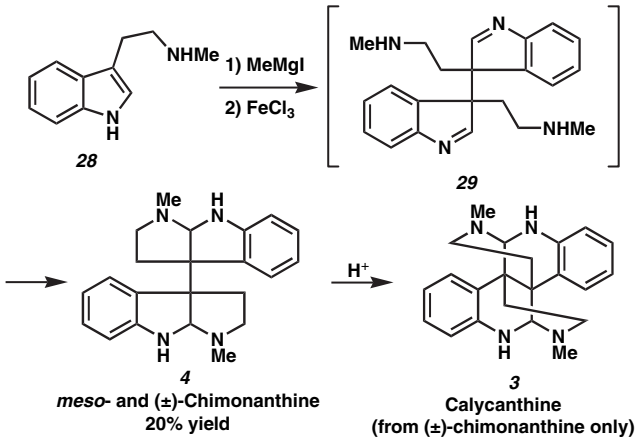


Scheme 2. Biomimetic synthesis studies by Hendrickson.

Both of these sequences, though characterized by low yields of the desired product,[22] demonstrate the feasibility of the proposed biosynthesis.[23,24] The model by Scott et al., however, better approximates the actual biosynthetic pathway,



Scheme 1. Biosynthetic pathway to the calycanthaceous alkaloids.



Scheme 3. Scott's direct biomimetic synthesis.

as Kirby has shown that 2-tritio-tryptophan and 2-tritio-tryptamine (**35**) retain their label when processed by *Calycanthus floridus* into chimonanthine (Fig. 8).[25] The retention of the 2-tritio label excludes the possibility of oxindole intermediates.

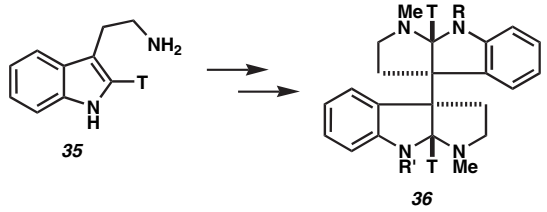
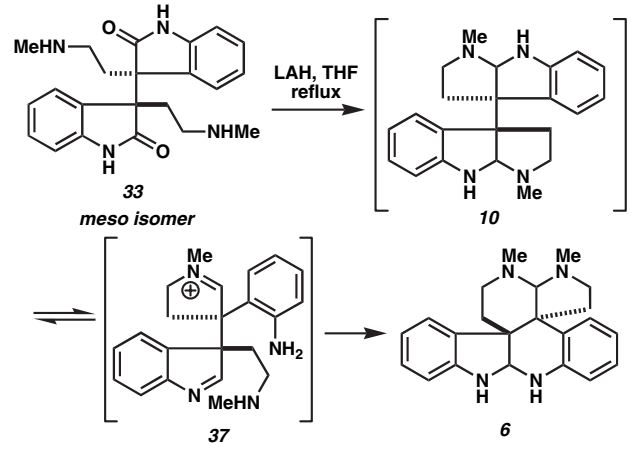


Figure 8. Label studies with chimonanthine.

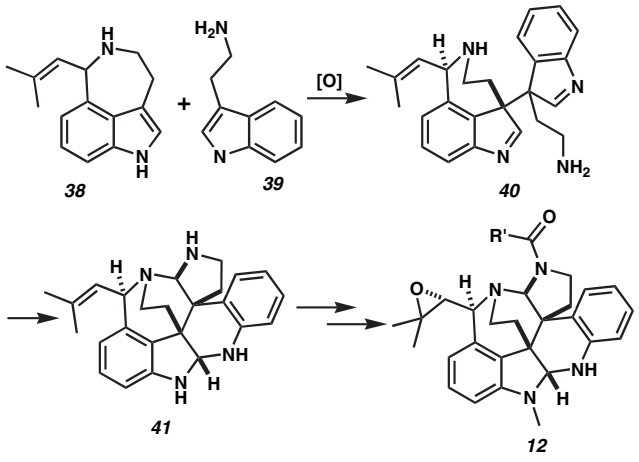
An interesting side-note emerges from the oxidative indole coupling reported by Hendrickson, in which the C_2-symmetric oxindole dimer **34** produces (±)-chimonanthine when reduced. Unusually, the *meso*-dimer **33** does not produce *meso*-chimonanthine upon treatment with LAH, but affords the isomeric hexacycle **6** (Scheme 4) under LAH treatment. Presumably, reduction occurs similarly to the C_2-symmetric adduct, but the Lewis acidic nature of LAH promotes an aminal rearrangement to a preferred geometry. This study suggests that a biosynthetic pathway optimized to produce a *meso*-tryptamine or tryptophan adduct could produce indole alkaloids with framework **6**.



Scheme 4. Formation of the framework **6**.

The defining feature of construct **6** is the unique presence of non-equivalent *N*-methyl groups, a trait easily recognized by proton NMR. This skeletal arrangement was unknown for naturally occurring alkaloids until the isolation of the communesins, nomofungin, and perophoramidine. These structures represent appended or oxidized versions of **6**. Hendrickson's findings suggest these compounds could arise from dimerization of tryptamine derivatives in a similar fashion to the calycanthus alkaloids.[1,26,27]

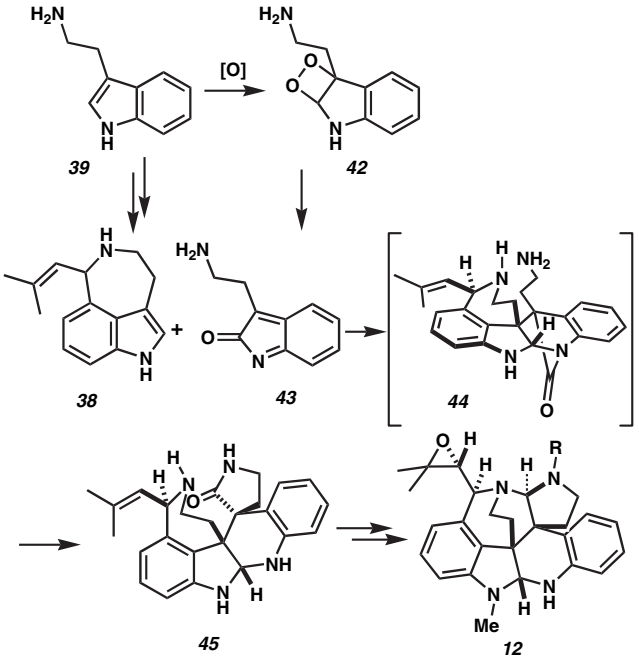
Specifically, the product of combination of the aminal formation from a dimer of the *Penicillum* fungal alkaloid aurantioclavine[28] and tryptamine would then be the communesin core **41**, and functionalization of the appropriate nitrogens and oxidation of the butenyl group to generate the epoxide yields communesin B (Scheme 5). However, a concern with the formation of **41** exists. Unlike perophoramidine, the vicinal quaternary carbon centers of the communesins possess a diastereomeric relationship akin to that of (±)-chimonanthine, which preferentially forms the connectivity found in calycanthine, not **6**. However, the additional carbocycle of intermediate **40** might bias the system toward formation of the less symmetrical framework **41**. Importantly, nomofungin cannot be formed through this biosynthetic pathway from known biogenic compounds. The precedent of *Calycanthus* biosynthesis and synthetic efforts with model systems[18] indicate that **15** likely represents the correct structure for both communesin B and nomofungin.



Scheme 5. Communesin biosynthesis.

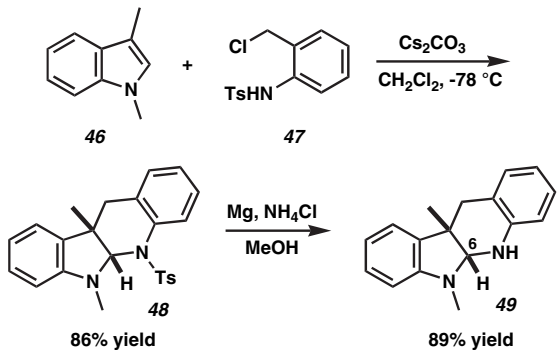
Unfortunately, directly adapting the theoretical biosynthesis outlined above to a synthetic approach to the communesins is unlikely because of the need for a selective dimerization event. However, an alternate biosynthetic pathway was proposed for the communesin family in 2003, where tryptophan or tryptamine is transformed into the quinone methide imine **43** (Scheme 6).[18] This electron deficient diene can then react with aurantioclavine (**38**) through an *exo* inverse-demand Diels–Alder reaction to generate the bridged lactam **44**. This intermediate, possessing a highly twisted lactam (analogous to the strained quinuclidone ring system) should readily undergo transamidation with the residual primary amine to produce the spiro lactam **45**. Biosynthetic reduction of the lactam, aminal closure, epoxidation, and acylation affords the communesins.[29] Again, a reasonable, equivalent biosynthetic pathway that would lead to the proposed structure for

nomofungin cannot be formulated. Furthermore, this hypothetical biosynthesis allowed for a plausible synthetic approach to communesin B.[30,31]



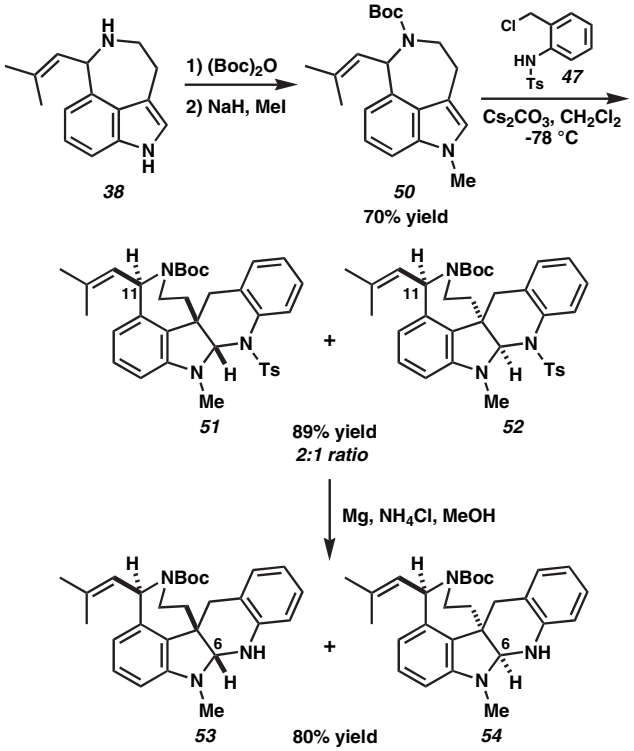
Scheme 6. An alternate communesin biosynthesis.

As a model system for the proposed Diels–Alder type cycloaddition of **38** and **43**, we investigated the cyclization of 1,3-dimethylindole (**46**) and chloroaniline **47** (Scheme 7) using conditions previously developed by Corey for the cycloaddition of **47** with electron-rich olefins.[32] To our delight, upon slow addition of chloride **47** to a mixture of indole **46** and Cs_2CO_3 in CH_2Cl_2 at −78 °C, a reaction immediately occurred to provide a single diastereomer of adduct **48** in 86% yield. Cleavage of the sulfonamide group by exposure of tetracycle **48** to Mg, MeOH, and NH_4Cl produced aminal **49**. Interestingly, the ^1H NMR chemical shift of the proton at C(6) is at 4.14 ppm, and the ^{13}C resonance is at 83.9 ppm. These shifts are in good accord with the values of 4.69–4.70 ppm (^1H) and 82.4 ppm (^{13}C) reported for both communesin B and nomofungin, strongly suggesting that the communesin structure is the appropriate representation for the natural product.[33,34]



Scheme 7. The inverse-demand Diels–Alder reaction with indole.

Furthermore, we have prepared (±)-aurantioclavine (**38**) by known methods[35] and utilized the *N*-Boc-1-methyl derivative **50** in the cycloaddition reaction with **47** (Scheme 8). Cycloaddition again proceeded smoothly upon treatment with Cs_2CO_3 to produce the adducts **51** and **52**. Unfortunately, the putative cycloadduct was produced as a 2:1 mixture of diastereomers with respect to the methylpropenyl side chain at C(11). Following cleavage of the sulfonyl group with Mg and NH_4Cl in MeOH, separation of the diastereomers was possible by preparative thin-layer silica gel chromatography. Importantly, the ^{13}C NMR residues for C(6) of diastereomers **53** and **54** were at 84.8 and 83.9 ppm, again in full accord with the data for the communesins and nomofungin.



Scheme 8. The Diels–Alder reaction with aurantioclavine.

Fortunately, the minor diastereomer, **54**, could be recrystallized by slow cooling from refluxing heptane and subsequent slow evaporation of the solvent to provide crystals suitable for single-crystal X-ray diffraction studies. The structure thus obtained is shown in Figure 9.[36] Here, the isobutenyl group is on the same face as electrophile addition. Thus, the major isomer was generated by addition of the electrophile to the aurantioclavine face opposite the pendant olefin, indicating a small capacity for the olefin to direct the reaction. One possible reason for the modest selectivity is also seen in the crystal structure; the Boc group resides on the face opposite the pendant olefin and thus competes for directional control. A smaller protecting group is likely to improve the selectivity of the reaction.

In conclusion, we have proposed that the natural products nomofungin and communesin B are, in fact, identical molecules and that the structure of communesin B more correctly represents the actual structure. We initially came to this

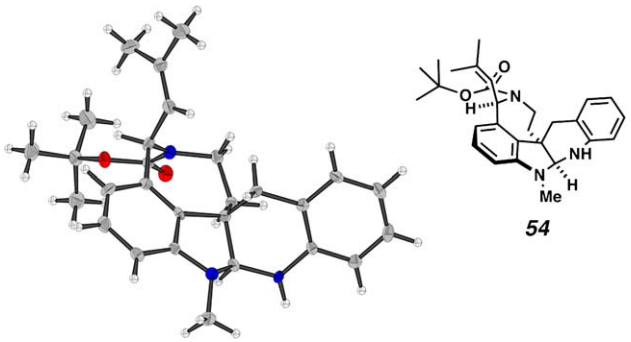


Figure 9. Structure confirmation by single-crystal X-ray diffraction.

conclusion based on comparison to the calycanthaceous alkaloids and biosynthetic hypotheses. Moreover, comparison of the reported data for the compounds to similar species and to the ^{1}H NMR chemical shift data of synthetic analogs have bolstered this argument. Finally, a potential intermediate (**51**) in our synthesis of communesin B has been prepared by a [4+2] cycloaddition route (Scheme 8) that is similar to the biosynthetic proposal outlined in Scheme 6. Efforts to complete the total synthesis of communesin B (also known as nomofungin) by such biomimetic routes are ongoing.

2. Experimental

2.1. General

Reactions were performed in flame-dried glassware under a nitrogen atmosphere using freshly purified solvents. Solvents were purified by passing through an activated alumina column. All other reagents were used as received from commercial sources. Reaction temperatures were controlled by an IKAmag temperature modulator. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV, *p*-anisaldehyde staining, or ceric ammonium molybdate staining (CAM). ICN silica gel (particle size 0.032–0.063 mm) was used for flash chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer (at 300 MHz and 75 MHz, respectively) in $CDCl_3$ and are internally referenced to the residual chloroform peak (7.27 ppm and 77.23 ppm, respectively) relative to Me_4Si. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in frequency of absorption (cm^{-1}). High resolution mass spectra were obtained from the California Institute of Technology Mass Spectrometry Facility.

2.1.1. Indoline 48. To a cooled solution of 1,3-dimethylindole (**46**, 23 mg, 0.16 mmol) and Cs_2CO_3 (168 mg, 0.360 mmol) in 0.2 mL anhydrous CH_2Cl_2 at −78 °C was added chloroaniline **47** (53 mg, 0.179 mmol) in anhydrous CH_2Cl_2 (0.8 mL) via syringe pump over 4 h. The solution was then warmed to 23 °C for 30 min, immediately filtered over a Celite plug, rinsing with CH_2Cl_2 (3×10 mL), concentrated under reduced pressure, and subjected to flash column chromatography (6:1 hexane/ethyl acetate eluent) to provide the Diels–Alder adduct **48** (54.7 mg, 86% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) 7.55 (dd, J=7.9, 1.2 Hz, 1H), 7.48 (d, J=8.5 Hz, 2H), 7.22 (d, J=8.2 Hz, 2H), 7.11 (t, J=7.6 Hz, 1H), 6.98 (td, J=7.6, 4.4 Hz, 1H), 6.88 (dd, J=7.6, 7.6 Hz, 1H), 6.76 (dd, J=7.6, 7.6 Hz, 2H), 6.45 (dd, J=7.3, 7.3 Hz, 1H), 6.14 (d, J=7.9 Hz, 1H), 5.66 (s, 1H), 2.99 (s, 3H), 2.52 (d, J=14.1 Hz, 1H), 2.42 (s, 3H), 1.62 (s, J=14.1 Hz, 1H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 150.0, 143.8, 137.9, 135.3, 135.1, 132.8, 129.8, 128.4, 128.2, 128.0, 127.3, 127.2, 126.8, 121.6, 117.0, 104.5, 86.5, 51.0, 38.1, 29.9, 29.2, 21.8; IR (neat) 3052, 3028, 2951, 2920, 1608, 1494 cm^{-1}; MS *m*/*z* calcd for $[C_{24}H_{24}N_2O_2S+H]^+$: 405.1637, found 405.1634.

2.1.2. Indoline 49. A vial (20 mL) equipped with a Teflon stirbar was charged with tosylamine **48** (28 mg, 0.069 mmol), which was subsequently dissolved in MeOH (2.7 mL). To this solution was added solid NH_4Cl (131 mg, 2.45 mmol) and Mg (131 mg, 5.39 mmol). Equal masses of NH_4Cl and Mg were added every few hours (usually to hundreds of equivalents) until all the starting material was converted to product as visualized by TLC. The solution was then filtered over a Celite plug, rinsing with CH_2Cl_2 (3×10 mL), and concentrated under reduced pressure. Purification was performed via flash column chromatography (9:1 hexanes/ethyl acetate eluent) to afford **49** (21.2 mg, 0.0847 mmol, 89% yield) as a white solid. R_f 0.44 (3:1 hexane/ethyl acetate eluent); ^{1}H NMR (300 MHz, $CDCl_3$) 7.17–7.12 (comp. m, 2H), 7.06 (dd, J=7.3, 7.9 Hz, 1H), 7.00 (d, J=7.3 Hz, 1H), 6.79 (dd, J=7.0, 7.6 Hz, 1H), 6.69 (dd, J=7.3, 7.3 Hz, 1H), 6.64 (d, J=7.9 Hz, 1H), 6.54 (d, J=7.6 Hz, 1H), 4.65 (br s, 1H), 4.14 (s, 1H), 2.81 (d, J=15.2 Hz, 1H), 2.78 (s, 3H), 2.51 (d, J=15.2 Hz, 1H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 149.2, 141.1, 137.2, 129.2, 127.9, 127.1, 121.5, 121.4, 118.8, 118.0, 113.5, 108.1, 83.9, 39.1, 37.7, 32.5, 21.7; IR (neat) 3404, 2956, 2851, 1609 cm^{-1}; MS *m*/*z* calcd for $[C_{17}H_{18}N_2+H]^+$: 250.1470, found 250.1461.

2.1.3. Aurantioclavine 38. A flame-dried flask (50 mL) equipped with a Teflon stirbar was charged with 2-terbutyldimethylsilyl-4-(3-methyl-3-hydroxy)but-1-enylgramine[35] (20.4 g, 49.19 mmol), which was subsequently dissolved in benzene (100 mL). To the resulting solution was added methyl iodide (12.4 mL, 199 mmol). The mixture was stirred for 12 h. The reaction was then concentrated under reduced pressure. The resulting salt was redissolved in THF (100 mL) and dry nitromethane (30 mL). A solution of TBAF (1 M in THF, 74 mL, 74 mmol) was added slowly after the reaction had been cooled to 0 °C. After allowing the reaction to come to room temperature and stir for 15 min, the reaction was quenched with water and extracted three times with ether. The organic layers were combined, the solution was dried over magnesium sulfate, and the solvent was then removed under reduced pressure. Purification was performed via flash column chromatography (5:1 to 0:1 gradient of hexanes/ethyl acetate) and washing the resulting solid with dichloromethane to afford (*E*)-2-methyl-4-(3-(2-nitroethyl)-1*H*-indol-4-yl)but-3-en-2-ol[35] (3.25 g, 11.85 mmol, 24% yield) as a yellow solid.

A flame-dried flask (250 mL) equipped with a Teflon stirbar was charged with (*E*)-2-methyl-4-(3-(2-nitroethyl)-1*H*-indol-4-yl)but-3-en-2-ol[35] (400.1 mg, 1.458 mmol), which

was subsequently dissolved in MeOH (104 mL) and 2 N HCl (36 mL). To this solution was added amalgamated zinc, which had been formed from zinc dust (5.33 g, 62.42 mmol) and mercuric chloride (793 mg, 2.92 mmol) in 2 N HCl (36 mL) and subsequently rinsed with MeOH. The mixture was stirred at reflux for 3 h. The reaction was then decanted from the remaining amalgam and basified to pH>10. The solid was removed by filtration, and the resulting solution was extracted five times with dichloromethane. The organic layers were combined, the solution was dried over magnesium sulfate, and the solvent was then removed under reduced pressure. Purification was performed via flash column chromatography (18:1 dichloromethane/methanol with 0.5% NH_4OH) to afford aurantioclavine **38** (204.7 mg, 0.904 mmol, 62% yield) as a yellow foam that had spectral characteristics identical to those previously reported.[35]

2.1.4. *N*-Boc-1-methylaurantioclavine (50). A flame-dried flask (10 mL) equipped with a Teflon stirbar was charged with aurantioclavine (**38**, 100 mg, 0.442 mmol). Dioxane (4.5 mL) was then added, and the solution was cooled to 10 °C. $(Boc)_2O$ (164.2 mg, 0.752 mmol) was then added, and the solution was warmed to room temperature. After 30 min, water (100 μL) was added to quench any excess reagent, then brine was added. The mixture was extracted three times with ethyl acetate, the combined organic layers were dried over Na_2SO_4, and the solution was filtered and concentrated under vacuum. Purification by flash column chromatography (3:1 hexanes/ethyl acetate eluent) yielded *N*-Boc-aurantioclavine (127.4 mg, 0.390 mmol, 88% yield) as a light yellow oil; R_f 0.23 (3:1 hexanes/ethyl acetate eluent). A flame-dried flask (25 mL), equipped with a Teflon stirbar, was charged with NaH (35.7 mg, 0.893 mmol) and THF (1 mL), and the mixture was cooled to 0 °C. Boc-aurantioclavine (125.2 mg, 0.384 mmol) was then added in a solution of THF (2 mL) dropwise. MeI (119 μL, 1.90 mmol) was added and the solution was warmed to room temperature. After 30 min, water (100 μL) was added to quench any excess reagent, then brine was added. The mixture was extracted three times with ethyl acetate, the combined organic layers were dried over Na_2SO_4, and the solution was filtered and concentrated under vacuum. Purification by flash column chromatography (9:1 hexanes/ethyl acetate eluent) yielded **50** (102.9 mg, 0.302 mmol, 79% yield) as a yellow oil; R_f 0.40 (3:1 hexanes/ethyl acetate eluent).

2.1.5. Diels–Alder adducts 51 and 52. A flame-dried flask (5 mL) equipped with a Teflon stirbar was charged with Cs_2CO_3 (62.1 mg, 0.191 mmol) and *N*-Boc-*N*-methylaurantioclavine (**50**, 19.3 mg, 0.0567 mmol). Dichloromethane was then added, and the mixture was cooled to −78 °C. 2-Chloromethyltosylaniline (**47**, 24.5 mg, 0.0828 mmol) was added dropwise in a solution of dichloromethane (400 μL). The combined solution was stirred for 1 h and then allowed to warm to room temperature slowly (30 min) before quenching with water (500 μL). The mixture was extracted three times with ethyl acetate, the combined organic layers were dried over Na_2SO_4, and the solution was filtered and concentrated under vacuum. Purification by flash column chromatography (15:1 to 9:1 hexanes/ethyl acetate eluent) gave an inseparable mixture of **51** and **52** (30.1 mg, 0.0502 mmol, 89% yield) as a white solid.

2.1.6. Indoline alkaloids 53 and 54. A vial (20 mL) equipped with a Teflon stirbar was charged with the mixture of tosylamines **51** and **52** (30 mg, 0.05 mmol), which were subsequently dissolved in MeOH (5 mL). To this solution was added solid NH_4Cl (137 mg, 2.56 mmol) and Mg (137 mg, 5.64 mmol). Equal masses of NH_4Cl and Mg were added every few hours (usually to hundreds of equivalents) until all the starting material was converted to product as visualized by TLC with stain. Purification was performed via preparative thin-layer chromatography (4:1 hexanes/ethyl acetate eluent run four times) to afford **51** (13.4 mg, 0.0301 mmol, 60% yield) and **52** (4.8 mg, 0.0108 mmol, 22% yield) as white solids.

2.1.7. Indoline 51. R_f 0.33 (3:1 hexane/ethyl acetate eluent); ^{1}H NMR (300 MHz, $CDCl_3$, 50 °C) 7.10–7.05 (comp. m, 3H), 6.79 (dd, *J*=7.1, 7.7 Hz, 1H), 6.63 (d, *J*=8.2 Hz, 1H), 6.54 (br s, 1H), 6.42 (d, *J*=7.7 Hz, 1H), 6.15 (br d, *J*=6.5 Hz, 1H), 5.34 (br s, 1H), 4.59 (br s, 1H), 4.01 (br s, 2H), 3.20–3.12 (m, 1H), 2.77 (app. s, 2H), 2.73 (s, 3H), 1.82 (s, 3H), 1.77 (s, 3H), 1.69–1.62 (comp. m, 3H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, 50 °C) 155.1, 141.2, 139.6, 132.6, 129.8, 129.4, 128.0, 127.2, 124.4, 121.0, 118.0, 113.2, 106.7, 84.8, 80.0, 57.6, 56.4, 40.0, 33.2, 32.1, 29.0, 25.9, 18.7; IR (neat) 3371, 2975, 2245, 1672, 1600 cm^{-1}; MS *m*/*z* calcd for $[C_{28}H_{35}N_3O_2-H]^+$: 444.2651, found 444.2640.

2.1.8. Indoline 52. Crystals suitable for X-ray diffraction studies could be grown from heptane.[36] R_f 0.32 (3:1 hexane/ethyl acetate eluent); ^{1}H NMR (300 MHz, $CDCl_3$, 50 °C), 7.25 (d, *J*=4.0 Hz, 1H), 7.09–7.01 (comp. m, 2H), 6.69 (dd, *J*=6.0, 7.7 Hz, 1H), 6.63 (d, *J*=7.1 Hz, 1H), 6.52 (br s, 1H), 6.45 (d, *J*=7.7 Hz, 1H), 6.0 (d, *J*=57.7 Hz, 1H), 5.43 (br s, 1H), 4.58 (br s, 1H), 3.96 (s, 1H), 3.91–3.70 (m, 1H), 3.50–3.35 (m, 1H), 3.09–2.80 (comp. m, 2H), 2.74 (s, 3H), 1.84 (s, 3H), 1.77 (s, 3H), 1.57–1.29 (comp. m, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, 23 °C) 154.9, 150.3, 141.4, 141.2, 140.6, 138.5, 138.3, 132.6, 132.2, 129.8, 128.1, 127.8, 127.5, 124.3, 120.1, 119.9, 118.8, 117.9, 113.6, 113.5, 107.5, 83.9, 79.9, 79.5, 59.0, 57.7, 41.7, 41.5, 38.7, 38.1, 33.2, 33.1, 32.5, 32.4, 31.2, 28.7, 26.1, 18.6, 18.5; IR (neat) 3363, 2973, 2242, 1672, 1594 cm^{-1}; MS *m*/*z* calcd for $[C_{28}H_{35}N_2O_2]^+$: 445.2729, found 445.2731.

Acknowledgments

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23. (a) Non-biomimetic synthetic efforts have also been pursued. Overman has synthesized many calycanthine alkaloids through the formation of the vicinal quaternary carbon centers with a bisoxindole bisenolate and an enantiopure C_2-symmetric chiral electrophile. Both the *meso*- and C_2-symmetric products can be selectively formed. See: Overman, L. E.; Paone, D. V.; Stearns, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 7702–7703; Overman, L. E.; Larrow, J. F.; Stearns, B. A.; Vance, J. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 213; (b) The MacMillan lab has developed an enantioselective, organocatalytic Michael addition of indoles into α,β-unsaturated aldehydes. This addition forms one of the key quaternary carbon centers, and subsequent manipulation builds the desired molecule. See: Austin, J. F.; Kim, S.-G.; Sinz, C. J.; Xiao, W.-J.; MacMillan, D. W. C. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5482–5487.
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29. Subsequent to our initial report, the Funk lab proposed a similar biosynthesis in a report on synthetic efforts toward perophoramidine (Ref. 31), differing only in the use of 4-prenyltryptamine in place of aurantioclavine and addition of a late-stage oxidative amino cyclization with the prenyl group is proposed.
30. (a) The Weinreb lab has a synthetic strategy toward perophoramidine using an oxindole-forming carbonylative Heck reaction, building one of the quaternary carbon centers in the process (Ref. 26); (b) A successful synthesis of perophoramidine was reported by the Funk lab that utilized chemistry related to the biosynthesis proposed in Scheme 6 (Ref. 31).
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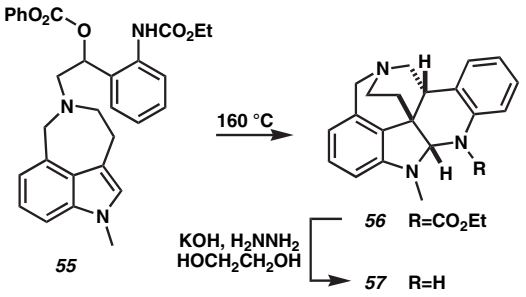
33. As additional confirmation, the Funk lab generated indole **55**, which upon heating in dichlorobenzene and subjection to hydrolysis generated a polycyclic framework **57** similar to that of the communesins. NMR data from this study further confirmed the structural assignment of communesin B (Ref. 34).



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36. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 220259.

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Formal synthesis of Abyssomicin C

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Abstract—An alternative strategy towards Abyssomicin C (**1**) is described. The key ene-diene intermediate is synthesized via a Kishi type coupling of an *E*/*Z* mixture of triene-iodide **7** and a suitably functionalized derivative of 2,4-dimethylglutaric acid. A final in situ isomerization/intramolecular Diels–Alder cyclization resulted in the formation of the known intermediate **3** as a single isomer in high yield. Further heating of **3** using excess of iodine, afforded iodo-derivative **23**, having the entire carbon skeleton of Abyssomicin D.

1. Introduction and retrosynthetic analysis

Antibiotics with high potency against pathogenic antibiotic-resistant bacterial strains will be of value in the clinic. Abyssomicin C (**1**, Fig. 1) recently discovered by Süssmuth et al.,[1] is a serious candidate since it possess an unprecedent complicated structure and an impressive biological profile. Abyssomicin C, inhibits the biosynthesis of *p*-amino-benzoic acid[2] (a pathway existing in bacteria but not in humans) and it is highly potent against resistant *Staphylococcus aureus* strains (methicillin-resistant MIC=4 μg mL^{-1}; vancomycin-resistant MIC=13 μg mL^{-1}).[2] Efficient chemical routes for the preparation of Abyssomicin C, as well as of related analogues, will have to be invented in order to facilitate possible pharmaceutical application of the new architecture. Not surprisingly many synthetic chemistry groups have already been alerted[3–5] and one year after its discovery an elegant total synthesis has been released by Sorensen et al.[6]

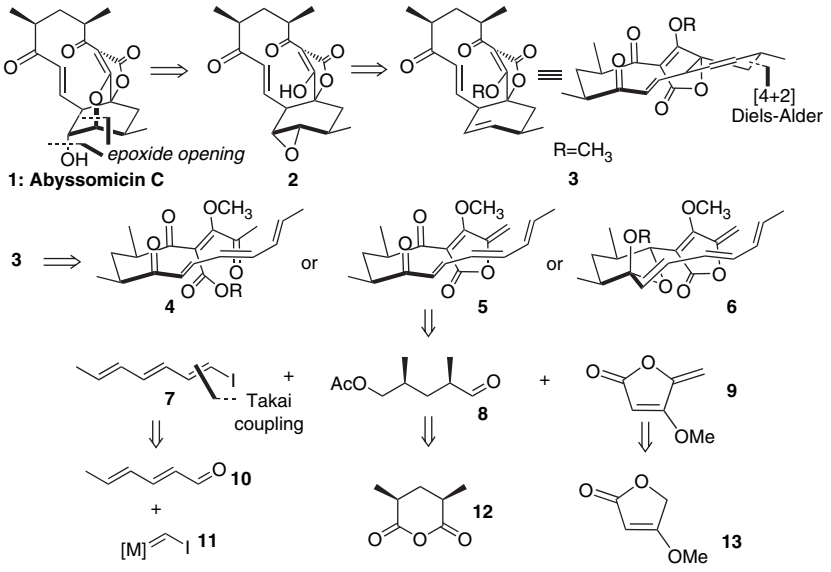

Figure 1. Retrosynthetic analysis towards a biomimetic route to Abyssomicin C.

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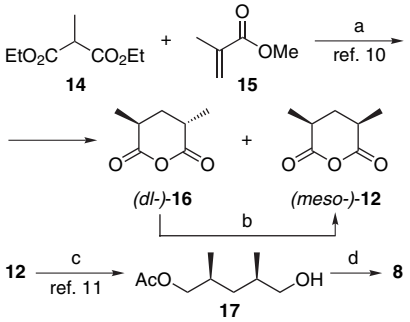
Aspiring to meet the challenge of constructing this impressive architecture, we focused our attention on synthetic strategies resembling a plausible biomimetic route as much as possible (Fig. 1). Thus, assuming that an intramolecular epoxide opening is possible, **1** can be disconnected to spirotetronate precursor **2**, which may be formed from **3** upon selective epoxidation and deprotection. The cyclohexenyl moiety of **3** called for an ene-diene precursor, is suitably designed to yield an intramolecular Diels–Alder cyclization. Although an in situ enolization of the terminal methyl ketone and concomitant stereoselective Diels–Alder seemed precarious, linear polyketidic triene **4**, was considered as a possible biomimetic retron of **3**. On the other hand, the alternative bicyclic precursor **6**, bearing an electron deficient exocyclic double bond and a hemiketalic connection between the adjacent α-methyl-carbonyls, is ideally preorganized for an efficient [4+2] cyclization. However, **6** features two new stereocenters and as a consequence, its synthesis can be anticipated to be lengthy. Thus, **5** was targeted as a more realistic compromise. Indeed, while our work was in progress, Sorensen et al.[6] and Snider and Zou[5] did prove independently that **5** may be transformed smoothly and in high yield to cyclized intermediate **3**. Moreover, the former succeeded in converting **3** to Abyssomicin C, following the depicted sequence. We report herein an alternative synthesis of key intermediate **3**.

Since the central core of **5** resembles reported *meso*-2,4-dimethylglutaric acid, the readily available aldehyde **8** was chosen to be the building block, as well as the chirality source. The tetronate moiety could be attached by a direct alkylation of **8** with anion of **9**.[7] Finally, the triene part was planned to be mounted after transformation of the terminal acetate to aldehyde, followed by a Kishi type coupling[8] with vinyl-iodide **7**, derived via a Takai olefination[9] of commercial hexadienal **10**.

2. Synthesis of key intermediate 3

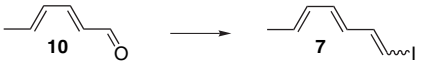
The key starting material, *meso*-2,4-dimethylglutaric anhydride **12**, was prepared on a multi-gram scale from diethylmethyl malonate and methyl methacrylate within three steps adopting two related syntheses[10] (Scheme 1). *meso*-Isomer **12**, selectively crystallizes out of the derived mixture of *dl*- and *meso*-forms. We were pleased to observe that the isomeric ratio of the crystallization mother liquor (which was enriched in the *dl*-form) could be reversed upon equilibration by heating with a mild base, thus raising the reported yield from 30 to 65%. The optically pure alcohol **17** was then prepared within two steps[11] and Swern oxidation[12] of the latter furnished aldehyde **8**, which was taken forward to the next step without further purification.



Scheme 1. Reagents and conditions: (a) i. Na, EtOH, rt, ii. HCl, AcOH, 105 °C, iii. Ac_2O, reflux, and iv. recrystallization (30% of **12**, three steps); (b) i. Et_3N, THF, reflux and ii. recrystallization, 52%; (c) i. LAH, THF, 0 °C and ii. AcOCH=CH_2, Amano lipase AK, THF, 0 °C (58%, two steps); (d) $(COCl)_2$, DMSO, DCM, Et_3N, 95%.

The remaining coupling partner, vinyl-iodide **7** was prepared from commercially available sorbaldehyde **10**,[†] in good yield (Table 1). However, under all conditions tried (Takai[9] or Evans[13,14] conditions for *E* selectivity or Stork's[15] conditions for *Z* selectivity), the obtained stereoselectivity was very poor. Moreover, all efforts to isomerize[16,17] resulted in 1:1 *E*/*Z* mixtures, indicating the presence of an equilibrium.[18] Nonetheless, since possible double bond in situ isomerization during the IMDA reaction would result in a very short scheme, we opted to continue our efforts employing vinyl-iodide **7** as a mixture of isomers (approximately 2:1 *E*/*Z* ratio).

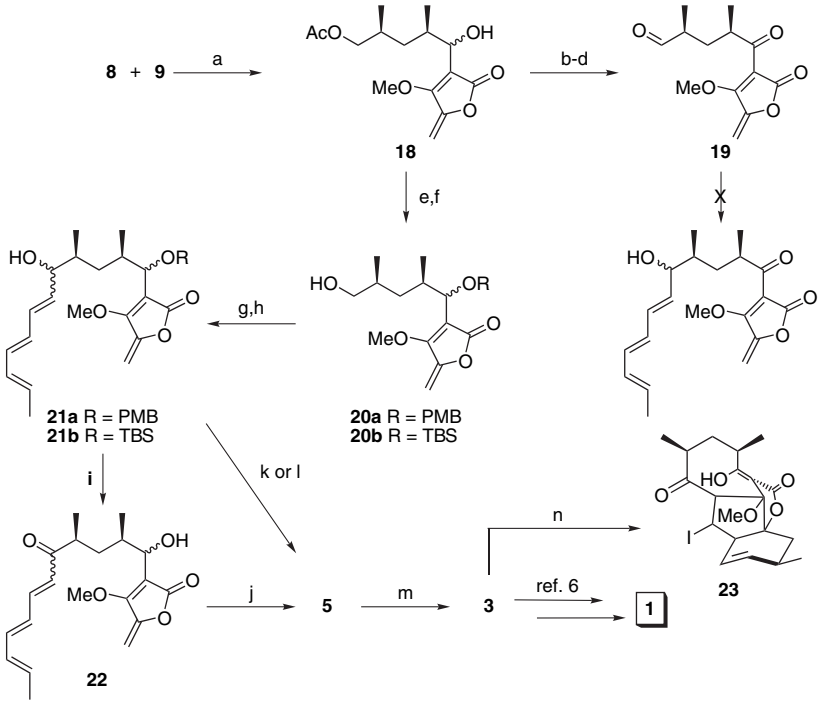
Table 1. Synthesis of vinyl-iodide **7**



Entry	Reagents/conditions	Solvents	*E*/*Z*	Yield (%)
1	$CrCl_2$, CHI_3, 0 °C	THF	2/1	85
2	$CrCl_2$, CHI_3, 0 °C	THF/dioxane 6/1	2/1	75
3	$Ph_3P^+(I^-)CH_2I$, NaHMDS, −78 °C	THF	1.2/1	87

The assembly of intermediate **5** commenced with coupling between aldehyde **8** and tetronate **9** (Scheme 2). After some experimentation, alcohol **18** was synthesized in relatively high yield along with partial recover of the starting materials. Alcohol **18** was formed as a diastereomeric mixture in 1:1 ratio, however oxidation of this stereocenter at a subsequent step would alleviate this problem. Thus, oxidation of the allylic alcohol with IBX,[19] enzymatic cleavage[20] of the acetate and subsequent oxidation of the resulting primary alcohol furnished keto-aldehyde **19**. Deacylation of all tetronate derivatives under alkaline conditions resulted usually in very low yields. We found that Novozyme 435 was quite efficient in this case. Though the chemical yields of these sequence were good, the stability of all intermediates upon chromatographic manipulations and storage was low. In addition, any further attempt to introduce the triene moiety (using $CrCl_2$ or the lithium anion of the respective triene) led only to immediate polymerization of aldehyde **19**. Thus, alcohol **18** was protected as the corresponding *p*-methoxy benzyl ether, anticipating that upon treatment with DDQ the allylic alcohol would be concomitantly transformed to the required ketone. Since Novozyme 435 reacted very slowly on this substrate, alcohol **20a** was prepared using Amano Lipase AK. It should be noted that all PMB-protected intermediates were by far more stable than the respective keto derivatives. In addition, Swern oxidation of **20a** and subsequent coupling

[†] Commercial sorbaldehyde contains up to 10% of the Z isomer. As it has been already reported by Snider[5] the wrong isomer remains unreacted in the final cyclization step. For clarity reasons this isomer is not depicted in the schemes or mentioned in the text.



Scheme 2. Reagents and conditions: (a) **9** (3 equiv), LDA, THF, −100 °C, then **8**, 45–58%, (20% recover of **8**, 50% recover of **9**); (b) IBX, DMSO, 78%; (c) Novozyme 435, toluene/phosphate buffer, 52% (16% recover); (d) IBX, DMSO, 70%; (e) PMBO(C=NH)CCl_3, cyclohexane/DCM, cat. CSA, 98% or TBS-Cl, imid., DMF, 85%; (f) Lipase AK, acetonitrile/phosphate buffer, 81% for R=PMB, 15% for R=TBS (recover 70%) or guanidine hydrochloride, EtOH/4 N NaOH, 78% for R=TBS; (g) IBX, DMSO, 88%; (h) **7**, $CrCl_2$, $NiCl_2$, THF/DMSO, 40% (recover 47%) for R=PMB, 50% (recover 30%) for R=TBS; (i) DDQ, DCM/H_2O, 20% for R=PMB; (j) Dess–Martin, DCM, 95%; (k) for R=PMB: i. MnO_2, Et_2O, 90%. ii. DDQ, DCM/$NaHCO_{3aq}$, 35%. iii. Dess–Martin, DCM, 95%; (l) for R=TBS: i. TBAF, THF, 83%. ii. IBX, DMSO, 85%; (m) toluene, cat. I_2, 100 °C, 3 h, 75%, (*n*) toluene, excess I_2, 100 °C, 3 h, 87%.

of the resulted aldehyde with vinyl-iodide **7**, under Kishi conditions, successfully lead to the targeted linear analogue of Abyssomicins **21a**. At this point we attempted an one pot, direct transformation of alcohol **21a** to di-keto-ene-diene **5** by a PMB deprotection and subsequent double oxidation of both allylic alcohols using DDQ.[21] Unfortunately, this reaction was messy and only keto-alcohol **22** could be isolated in low yield (5–30%). The latter was oxidized to diketone **5**.[5] Alternatively, **5** could be derived from **21a** following a three steps protocol (MnO_2 oxidation, DDQ deprotection and Dess–Martin periodinate oxidation),[22] yet in low overall yield (20%).

In order to improve the total yield towards **6**, the previous sequence was repeated using *t*-$BuMe_2Si$ instead of PMB for the protection of the hydroxyl group. This time, saponification of the acetate moiety was performed by employing guanidine under buffered conditions, since both of the previously used enzymes reacted very slowly. Alcohol **21b**, upon desilylation with TBAF and subsequent double oxidation of the resulting diol using IBX, afforded the targeted precursor **5** in very good yield. It should be pointed out that the aforementioned diol is a mixture of two diasteromeric centers and possesses the *E*/*Z* double bond stereochemistry originating from the used vinyl-iodide (total eight isomers). However, the derived diketone **5** was only a mixture of the *E*/*Z* isomers and, to our delight, this mixture upon heating at 100 °C in toluene with a catalytic amount of I_2, was smoothly converted in high yield to the cyclized advanced intermediate of Abyssomicin C, **3**, as a single isomer. Interestingly, by adding excess of I_2 and heating for additional 3 h, iodo-derivative **23** was isolated as the only product, giving rise to the Abyssomicin D carbon skeleton in a similar manner that Snider observed for a sulfide analogue.[5]

3. Conclusion

In conclusion we have presented herein an efficient and straightforward synthesis of a key advanced intermediate towards **1**. We are currently working towards a scaled up synthesis of Abyssomicin C as well as of related derivatives in order to explore their antibacterial activities.

4. Experimental

4.1. General techniques

All reactions were carried out under anhydrous conditions and an argon atmosphere using dry, freshly distilled solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, dichloromethane (DCM) from CaH_2, and toluene from sodium. Yields refer to chromatographically and spetroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. All reagents were purchased at highest commercial quality and used without further purification, unless otherwise stated. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck silica gel plates (60 F254) using UV light as visualizing agent and ethanolic phosphomolybdic acid or *p*-anisaldehyde solution and heat as developing agents. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. NMR spectra

were recorded on Bruker Avance DRX-500 or AC-250 instruments. The following abbreviations were used to explain NMR signal multiplicities: br s = broad singlet, br d = broad doublet, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublets of doublets, dt = doublet of triplets. IR spectra were recorded on a Perkin–Elmer 1600 series FTIR or Nicolet Magna system 550 FTIR instruments. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions and matrix-assisted (MALDI-FTMS) mass spectra were recorded on a PerSeptive Biosystems Voyager IonSpect mass spectrometer.

4.1.1. *meso*-2,4-Dimethylglutaric anhydride 12. To a solution of *dl*-2,4-dimethylglutaric anhydride (52.0 g, 0.366 mol) in THF (270 mL) was added triethylamine (255 mL, 1.83 mol) under argon. The reaction mixture was heated under reflux for 60 h and the solvents were removed under reduced pressure. The residue was distilled under high vacuum using air-cooled condenser to afford a colorless solid. This material was dissolved in AcOEt (50 mL) and the solution was allowed to stand at room temperature for 12 h, in the freezer for 6 h and at −20 °C for 2 h. The colorless solid was recrystallized from AcOEt (25 mL), to give *meso*-2,4-dimethylglutaric anhydride (27 g, 52%) as a colorless solid. Mp 90.5–91.9 °C (lit.[23] 91.4–92.8 °C).

4.1.2. Aldehyde 8. To a stirred solution of oxalyl chloride (1.53 mL, 0.018 mol) in dry DCM (20 mL) at −78 °C, DMSO (2.90 mL, 0.038 mol) was added drop-wise under an argon atmosphere. After 30 min, a solution of alcohol **17** (2.0 g, 0.014 mol) in dry DCM (20 mL) was added to the reaction mixture. After 30 min of stirring at −78 °C, Et_3N (13.8 mL, 0.10 mol) was added and the reaction was stirred for 1 h at ambient temperature. The reaction mixture was quenched with saturated NH_4Cl_{aq} solution (30 mL) and extracted with DCM (30 mL). The combined organic extracts were washed with water (30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude aldehyde, **8**, was used in the next step without further purification.

4.1.3. Alcohol 18. To a stirred solution of 4-methoxy-5-methylene-2(5*H*)-furanone, **9**, (2.07 g, 16.4 mmol) in THF (235 mL), at (−100 °C) under an argon atmosphere was added, via cannula, a cooled (−100 °C) LDA solution [prepared from *i*-Pr_2NH (2.7 mL, 19.05 mmol) and *n*-BuLi (1.6 M in Hexane; 10.3 mL, 16.53 mmol) in THF (83 mL) after stirring for 1 h at 0 °C] over a period of 2 min. After stirring for 6 min, a solution of the crude aldehyde **8** (943 mg, 5.48 mmol) in THF (46 mL) was added, over a period of 2 min via cannula. After being stirred at −100 °C −90 °C for 15 min, the reaction was quenched with saturated NH_4Cl_{aq} (50 mL) and then allowed to warm at room temperature. The mixture was extracted with ethyl acetate (2×40 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture was subjected to flash chromatography (SiO_2, Hexane/AcOEt 8:2 to 7:3) to afford alcohol **18** (mixture of two diastereoisomers, ratio 1:1; 948 mg, 3.18 mmol; 58%) as a yellow oil, and recovered starting materials: aldehyde **8** (188 mg, 1.09 mmol; 20%) and 4-methoxy-5-methylene-2(5*H*)-furanone, **9** (1.0 g, 7.93 mmol). R_f=0.22 (Hexane/AcOEt 8:2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.07 (s, 4H, =CH_2), 4.46 (d, ^3J(H,H)=7.5 Hz, 1H, C*H*OH), 4.40 (d, ^3J(H,H)=8.6 Hz, 1H, C*H*OH), 4.12 (s, 6H, OCH_3), 3.96 (dd, ^3J(H,H)=4.6, 10.3 Hz, 1H, AcOCH_2), 3.92–3.79 (m, 3H, AcOCH_2), 3.41–3.09 (br s, 2H, *OH*), 2.03 (s, 3H, *Ac*O), 1.99 (s, 3H, *Ac*O), 1.99–1.90 (m, 2H, CH_3C*H*CHOH), 1.89–1.79 (m, 2H, AcOCH_2C*H*CH_3), 1.42–1.13 (m, 4H, CHCH_2CH), 1.05 (d, ^3J(H,H)=6.3 Hz, 3H, *CH_3*), 0.97 (d, ^3J(H,H)= 6.9 Hz, 3H, *CH_3*), 0.93 (d, ^3J(H,H)=6.3 Hz, 3H, *CH_3*), 0.85 (d, ^3J(H,H)=6.9 Hz, 3H, *CH_3*) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 171.7, 171.6, 169.9, 162.1, 149.7, 107.4, 107.3, 93.9, 71.6, 70.9, 69.6, 69.1, 68.5, 68.3, 60.9, 37.9, 37.7, 37.6, 37.5, 33.4, 31.8, 30.6, 30.5, 30.4, 30.0, 21.3, 21.1, 19.1, 18.8, 17.6, 16.3 ppm; FTIR (neat): $\overline{\nu}_{max}$ 3492, 2964, 2933, 2877, 1767, 1736, 1669, 1624, 1460, 1392, 1281, 1245, 1156, 1036, 979, 878, 784 cm^{-1}; HRMS (ESI) calculated for $C_{15}H_{22}O_6$ ([M+Na]$^+$): *m*/*z* 321.1308, found 321.1309.

4.1.4. Aldehyde 19. To a solution of alcohol **18** (247 mg, 0.828 mmol) in DMSO (1.6 mL) was added IBX (463 mg, 1.654 mmol) at room temperature under an argon atmosphere. After stirring for 2 h the reaction was quenched with water (5 mL) and AcOEt (5 mL). The iodosocarboxylic acid, which precipitated as a white amorphous solid, was removed by filtration through Celite. The filtrate was extracted with AcOEt (2×10 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was subjected to flash chromatography (silica gel, Hexane/AcOEt 8:2) to afford the respective ketone (191 mg, 0.646 mmol; 78%) as yellow oil. R_f=0.44 (Hexane/AcOEt 8:2); $[\alpha]_D^{2.5}$ −5° (*c* 7.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.27 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 5.22 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 4.11 (s, 3H, OCH_3), 3.95 (dd, ^3J(H,H)=5.7, 10.9 Hz, 1H, AcOCH_2), 3.89 (dd, ^3J(H,H)=6.3, 10.9 Hz, 1H, AcOCH_2), 3.72 (dd, ^3J(H,H)=6.9, 13.8 Hz, 1H, C*H*CO), 2.06 (s, 3H, *Ac*O), 1.95–1.77 (m, 1H, CH_2C*H*CH_2), 1.32–1.22 (m, 2H, CHCH_2CH), 1.15 (d, ^3J(H,H)=7.5 Hz, 3H, CH_3), 0.97 (d, ^3J(H,H)=6.9 Hz, 3H, CH_3) ppm; ^{13}C NMR (62.8 MHz, $CDCl_3$, 25 °C): δ 200.5, 171.2, 168.8, 166.2, 148.9, 104.7, 95.8, 69.0, 62.8, 41.8, 36.5, 30.5, 29.7, 17.5, 17.4 ppm; FTIR (neat): $\overline{\nu}_{max}$ 2964, 2934, 2876, 2855, 1772, 1738, 1684, 1666, 1592, 1456, 1390, 1366, 1287, 1241, 1155, 1040, 994, 882, 793, 738 cm^{-1}; HRMS (ESI) calculated for $C_{15}H_{20}O_6$ ([M+Na]$^+$): *m*/*z* 319.1152, found 319.1152.

Novozyme 435 (Candida Antarctica lipase immobilized on a macroporous acrylic resin, Novo Nordisk; 441 mg) was added in a well-stirred solution of the previous ketone (245 mg, 0.827 mmol), in toluene (6 mL) and sodium phosphate buffer (pH 6.2; 6 mL, 0.1 M), at room temperature. After 48 h (TLC indicated no further conversion) the reaction mixture was filtered and the retained enzyme was washed with AcOEt (20 mL) and water (20 mL). The organic layer was separated and the aqueous layer of the filtrate was extracted with AcOEt (2×20 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and

concentrated under reduced pressure. The residue was subjected to column chromatography (SiO_2, Hexane/AcOEt 8:2–7:3) to afford the respective alcohol (110 mg, 0.433 mmol; 52%) as yellow oil, along with recovered starting material (40 mg, 0.157 mmol; 16%). R_f=0.28 (Hexane/AcOEt 7:3); ^{1}H NMR (500 MHz, Acetone-d_6, 25 °C): δ 5.30 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 5.27 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 4.14 (s, 3H, OCH_3), 3.70–3.60 (m, 1H, C*H*CO), 3.46–3.40 (m, 1H, CH_2OH), 3.35–3.29 (m, 1H, CH_2OH), 1.97–1.88 (m, 1H, HOCH$_2$C*H*), 1.71–1.61 (m, 1H, CHCH_2CH), 1.19–1.04 (m, 1H, CHCH_2CH), 1.14 (d, ^3J(H,H)=6.9 Hz, 3H, CH_3), 0.94 (d, ^3J(H,H)=6.9 Hz, 3H, CH_3) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 202.2, 170.1, 167.9, 150.9, 130.1, 96.7, 68.4, 64.3, 43.6, 38.4, 35.5, 18.8, 18.5 ppm.

To a solution of the previous alcohol (37 mg, 0.146 mmol) in DMSO (1 mL) was added IBX (160 mg, 0.571 mmol) at room temperature under an argon atmosphere. After stirring for 2 h the reaction was quenched with water (3 mL) and AcOEt (3 mL). The iodosocarboxylic acid, which precipitated as a white amorphous solid, was removed by filtration through Celite. The filtrate was extracted with AcOEt (2×5 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was subjected to flash chromatography (SiO_2, Hexane/AcOEt 8:2) to afford the aldehyde **19** (26 mg, 0.102 mmol; 70%) as colorless oil. R_f=0.37 (Hexane/AcOEt 7:3); $[\alpha]_D^{25}$ +31° (*c* 0.15 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 9.62 (s, 1H, C*H*O), 5.30 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 5.24 (d, ^2J(H,H)=2.9 Hz, 1H, =CH_2), 4.13 (s, 3H, OCH_3), 3.77–3.65 (m, 1H, CH$_2$C*H*CO), 2.48–2.37 (m, 1H, C*H*CHO), 2.34–2.24 (m, 1H, CHCH_2CH), 1.35–1.25 (m, 1H, CHCH_2CH), 1.18 (d, ^3J(H,H)=7.5 Hz, 3H, CH_3), 1.15 (d, ^3J(H,H)=6.9 Hz, 3H, CH_3) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 204.4, 199.9, 148.8, 141.8, 133.3, 127.9, 96.1, 62.9, 44.4, 41.9, 32.9, 17.9, 13.9 ppm; FTIR (neat): $\overline{\nu}_{max}$ 2962, 2924, 2875, 2852, 1769, 1726, 1686, 1667, 1585, 1455, 1390, 1286, 996, 738 cm^{-1}.

4.1.5. Alcohol 20a. A solution of *p*-methoxy benzyl trichloroacetimidate (2.06 g, 7.29 mmol) in cyclohexane (3.9 mL) was added to a solution of alcohol **18** (726 mg, 2.43 mmol) in DCM (1.9 mL) under an argon atmosphere. The resulting mixture was cooled to 0 °C and treated with (±)-camphorsulfonic acid (120 mg, 0.504 mmol). The reaction mixture was warmed to room temperature and stirred for 7 h, slowly developing a white precipitate. The suspension was filtered and washed with CCl_4 (2×15 mL). The filtrate was washed with saturated $NaHCO_{3aq}$ (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, Hexane/AcOEt 9:1) to afford the PMB-ether (mixture of two diastereoisomers, ratio 1:1; 1.0 g, 2.39 mmol; 98%) as colorless oil. R_f=0.40 (Hexanes/AcOEt 8:2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.25–7.17 (m, 4H, Ar*H*), 6.86 (d, ^3J(H,H)=8.5 Hz, 4H, Ar*H*), 5.10–5.03 (m, 4H, =CH_2), 4.48 (d, ^2J(H,H)=11.3 Hz, 1H, OC*H*HAr), 4.45 (d, ^2J(H,H)=11.3 Hz, 1H, OCH*H*Ar), 4.28 (d, ^2J(H,H)=11.3 Hz, 2H, OCH_2Ar), 4.14 (s, 6H, OCH_3), 4.11–4.05 (m, 2H, C*H*OPMB), 3.96 (dd, ^3J(H,H)=4.9, 10.8 Hz, 1H, C*H*HOAc), 3.90–3.81 (m, 2H, CH_2OAc), 3.79 (s, 6H, Ar-OCH$_3$), 3.76–3.71 (m, 1H, CH*H*OAc), 2.03–1.95 (m, 8H, C*H*CHOPMB, OA*c*), 1.90–1.75 (m, 2H, C*H*CH$_2$OAc), 1.33–1.22 (m, 2H, CHCH_2CH), 1.08 (d, ^3J(H,H)=6.6 Hz, 3H, CH_3), 1.04–0.99 (m, 2H, CHCH_2CH), 0.97 (d, J=6.6 Hz, 3H, CH_3), 0.92 (d, J=6.8 Hz, 3H, CH_3), 0.83 (d, J=6.8 Hz, 3H, CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 171.1, 169.6, 169.5, 163.6, 159.3, 149.8, 129.7, 129.5, 129.4, 128.2, 113.8, 110.3, 104.8, 92.6, 92.5, 77.5, 76.8, 71.2, 71.15, 68.7, 68.0, 61.7, 55.4, 55.2, 38.4, 37.3, 36.9, 36.6, 34.9, 30.4, 29.9, 20.8, 20.7, 18.6, 18.3, 17.2, 16.6; HRMS (ESI) calculated for $C_{23}H_{30}O_7$ ([M+Na]$^+$): *m*/*z* 441.1882, found 441.1883.

Amano Lipase AK (*Pseudomonas fluorescens*, Aldrich; 1.05 g) was added in a well-stirred solution of the previous PMB–ether (950 mg, 2.27 mmol) in acetonitrile (12.6 mL) and sodium phosphate buffer (pH 6.2; 50.5 mL; 0.1 M), at room temperature. The reaction was completed after 12 h (TLC), was filtered through Celite and the retained enzyme was washed with AcOEt (50 mL) and water (30 mL). The aqueous layer of the filtrates was extracted with AcOEt (2×30 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was filtered through a silica pad (SiO_2, Hexane/AcOEt 7:3) to afford alcohol **20a** (mixture of two diastereoisomers, ratio 1:1; 692 mg, 1.838 mmol; 81%) as yellow oil. R_f=0.25 (Hexane/AcOEt 7:3); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.29–7.18 (m, 4H, Ar*H*), 6.86 (d, ^3J(H,H)=8.0 Hz, 4H, Ar*H*), 5.10–5.04 (m, 4H, =CH_2), 4.54–4.44 (m, 2H, OCH_2Ar), 4.33–4.26 (m, 2H, OCH_2Ar), 4.14 (s, 6H, OCH_3), 4.14–4.10 (m, 1H, C*H*OPMB), 3.80–3.86 (m, 1H, C*H*OPMB), 3.80 (s, 6H, Ar-OCH$_3$), 3.50–3.43 (m, 2H, CH_2OH), 3.42–3.36 (m, 2H, CH_2OH), 2.16–1.97 (m, 2H, C*H*CHOPMB), 1.87–1.72 (m, 2H, C*H*CH$_2$OH), 1.71–1.49 (m, 2H, CHCH_2CH), 1.36–1.19 (m, 2H, CHCH_2CH), 1.09 (d, ^3J(H,H)=6.6 Hz, 3H, CH_3), 0.96 (d, ^3J(H,H)=6.5 Hz, 3H, CH_3), 0.93 (d, ^3J(H,H)=6.7 Hz, 3H, CH_3), 0.85 (d, ^3J(H,H)=6.9 Hz, 3H, CH_3) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 169.6, 163.9, 159.3, 149.8, 146.2, 139.1, 129.7, 129.6, 113.9, 113.8, 92.7, 92.6, 71.3, 71.1, 67.6, 66.9, 61.7, 59.6, 55.3, 38.4, 37.5, 36.6, 36.4, 32.8, 31.2, 29.7, 18.4, 18.1, 17.3, 17.2 ppm; HRMS (ESI) calculated for $C_{21}H_{28}O_6$ ([M+Na]$^+$): *m*/*z* 399.1778, found 399.1779.

4.1.6. Alcohol 20b. To a solution of alcohol **18** (200 mg, 0.670 mmol) in DMF (0.1 mL) was added imidazole (91 mg, 1.34 mmol), TBDMS-Cl (152 mg, 1.00 mmol) and DMAP (8.18 mmol, 0.067 mmol) at room temperature under argon an atmosphere. After being stirred for 12 h the reaction was quenched with MeOH (0.5 mL) and saturated NH_4Cl_{aq} (10 mL). The mixture was extracted with AcOEt (15 mL) and organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, Hexane/AcOEt 95:5) to afford the corresponding silyl ether (mixture of two diastereoisomers, ratio 1:1; 235 mg, 0.569 mmol; 85%) as colorless oil. R_f=0.65 (Hexanes/AcOEt 9:1); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.08–5.02 (m, 4H, =CH_2), 4.31–4.26 (m, 2H, C*H*OTBS), 4.26 (s, 3H, OCH_3), 4.25 (s, 3H, OCH_3), 3.98 (dd,

^2J(H,H)=3.9 Hz, ^3J(H,H)=10.8 Hz, 1H, CH*H*OAc), 3.91 (m, 3H, CH_2OAc), 2.03 (s, 3H, *Ac*), 1.99 (s, 3H, *Ac*), 1.96–1.78 (m, 4H, C*H*CH$_3$), 1.28–1.17 (m, 2H, CHCH_2CH), 1.06 (d, ^3J(H,H)=6.5 Hz, 3H, CH_3), 0.99 (d, ^3J(H,H)=6.4 Hz, 3H, CH_3), 0.98–0.90 (m, 2H, CHCH_2CH), 0.93 (d, ^3J(H,H)=6.8 Hz, 3H, CH_3), 0.88 (s, 18H, *TBS*), 0.81 (d, ^3J(H,H)=6.8 Hz, 3H, CH_3), 0.09 (s, 6H, *TBS*), −0.04 (s, 6H, *TBS*) ppm; ^{13}C NMR (62.9 MHz, $CDCl_3$, 25 °C): δ 202.9, 171.3, 169.7, 162.9, 149.9, 107.4, 92.6, 92.6, 71.2, 70.9, 68.3, 67.6, 61.8, 39.4, 39.3, 38.0, 36.1, 30.1, 29.7, 25.8, 20.9, 20.8, 18.9, 18.6, 17.9, 17.4, 15.9, 3.6, 3.1 ppm.

To a solution of the previous acetyl ester (155 mg, 0.375 mmol) in EtOH (1.5 mL) was added guanidine (1 M in EtOH; 0.48 mmol) [prepared from guanidine hydrochoride (525 mg, 5.50 mmol) and NaOH (4 N; 1.3 mL) in EtOH (3.93 mL)] at 0 °C and stirred for 1.5 h. The reaction mixture was quenched with saturated NH_4Cl_{aq} (10 mL) and extracted with AcOEt (2×10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, Hexane/AcOEt 8:2) to afford alcohol **20b** (mixture of two diastereoisomers, ratio 1:1; 109 mg, 0.293 mmol; 78%) as yellow oil. R_f=0.45 (Hexane/AcOEt 7:3); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.08–5.02 (m, 4H, =CH_2), 4.39–4.23 (m, 2H, C*H*OTBS), 4.27 (s, 3H, OCH_3), 4.26 (s, 3H, OCH_3), 3.59–3.51 (m, 1H, CH_2OH), 3.51–3.44 (m, 1H, CH_2OH), 3.44–3.28 (m, 2H, CH_2OH), 2.06–1.86 (m, 2H, C*H*CH$_3$), 1.88–1.66 (m, 2H, C*H*CH$_3$), 1.25–1.16 (m, 2H, CHCH_2CH), 1.06 (d, ^3J(H,H)=6.5 Hz, 3H, *CH*$_3$), 1.05–0.95 (m, 2H, CHCH_2CH), 0.98 (d, ^3J(H,H)=6.4 Hz, 3H, *CH*$_3$), 0.92 (d, ^3J(H,H)=6.7 Hz, 3H, CH_3), 0.83 (d, ^3J(H,H)=6.8 Hz, 3H, *CH*$_3$), 0.1 (s, 3H, *TBS*), 0.09 (s, 3H, *TBS*), −0.04 (s, 6H, *TBS*) ppm.

4.1.7. Triene-iodide 7. Anhydrous $CrCl_2$ (1.9 g, 0.015 mol) was suspended in THF (25 mL) under an argon atmosphere. A solution of 2,4-hexadienal, **10** (0.3 mL, 2.72 mmol) and iodoform (1.5 g, 3.81 mmol) in THF (13.6 mL) was added drop-wise to the suspension at 0 °C via cannula. After stirring at 0 °C for 1 h the reaction was completed (TLC), the mixture was filtered through Celite and washed with Et_2O (50 mL). The combined filtrates were washed with water (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash chromatography (Al_2O_3, Hexane) to afford the iodide, **7** (510 mg, 2.31 mmol; 85%, mixture of isomers *E*/*Z* 2:1) as a yellow solid. R_f=0.82 (Hexane); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.51–7.41 (m, 1H), 7.18–7.06 (m, 1H), 7.02 (dd, ^3J(H,H)=14.3, 14.3 Hz, 1H), 6.83–6.74 (m, 1H), 6.71 (dd, ^3J(H,H)=10.0, 10.0 Hz, 1H), 6.57–6.48 (m, 1H), 6.44 (dd, ^3J(H,H)=14.9, 14.9 Hz, 1H), 6.38–6.08 (m, 7H), 6.08–5.95 (m, 4H), 5.93–5.76 (m, 3H), 5.75–5.60 (m, 3H), 1.79 (d, ^3J(H,H)=6.8 Hz, 3H, *CH*$_3$), 1.76 (d, ^3J(H,H)=7.0 Hz, 3H, *CH*$_3$), 1.83–1.75 (m, 6H, *CH*$_3$) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 145.4, 138.4, 137.3, 133.8, 132.8, 132.3, 132.0, 131.7, 131.5, 130.9, 129.8, 129.6, 129.5, 129.2, 128.7, 128.6, 128.3, 127.5, 81.9, 81.2, 78.4, 77.5, 29.7, 18.5 ppm.

4.1.8. Alcohol 21a. To a solution of alcohol **20a** (380 mg, 1.011 mmol) in DMSO (7.2 mL) was added IBX (1.38 g, 3.225 mmol) at room temperature under argon an atmosphere. After stirring for 2 h the reaction was quenched with water (5 mL) and AcOEt (5 mL). The iodosocarboxylic acid, which precipitated as a white amorphous solid, was removed by filtration through Celite. The filtrate was extracted with AcOEt (2×10 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture was subjected to flash chromatography (SiO_2, Hexane/AcOEt 8:2) to afford the corresponding aldehyde (mixture of two diastereoisomers, ratio 1:1; 334 mg, 0.892 mmol; 88%) as yellow oil. R_f=0.40 (Hexane/AcOEt 7:3); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 9.53 (d, ^3J(H,H)=2.3 Hz, 1H, C*H*O), 9.52 (d, ^3J(H,H)=2.3 Hz, 1H, C*H*O), 7.22 (d, ^3J(H,H)=8.6 Hz, 2H, Ar*H*), 7.21 (d, ^3J(H,H)=8.6 Hz, 2H, Ar*H*), 6.87 (d, ^3J(H,H)=8.6 Hz, 2H, Ar*H*), 6.86 (d, ^3J(H,H)=8.6 Hz, 2H, Ar*H*), 5.12 (d, ^2J(H,H)=2.4 Hz, 1H, =CH_2), 5.09 (d, ^2J(H,H)=2.4 Hz, 1H, =CH_2), 5.08–5.05 (m, 2H, =CH_2), 4.48 (d, ^3J(H,H)=11.5 Hz, 1H, OCH_2Ar), 4.47 (d, ^3J(H,H)=11.5 Hz, 1H, OCH_2Ar), 4.28 (d, ^3J(H,H)=11.5 Hz, 2H, OCH_2Ar), 4.19 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 4.12 (d, ^3J(H,H)=6.9 Hz, 1H, C*H*OPMB), 4.10 (d, ^3J(H,H)=6.9 Hz, 1H, C*H*OPMB), 3.81 (s, 3H, Ar-OCH_3), 3.80 (s, 3H, Ar-OCH_3), 2.56–2.57 (m, 1H, C*H*CHO), 2.47–2.38 (m, 1H, C*H*CHO), 2.31–2.24 (m, 1H, CHCH_2CH), 2.04–1.93 (m, 2H, C*H*CHOPMB), 1.70–1.61 (m, 1H, CHCH_2CH), 1.20–1.09 (m, 2H, CHCH_2CH), 1.11 (d, ^3J(H,H)=6.9 Hz, 3H, *CH*$_3$), 1.07 (d, ^3J(H,H)=7.5 Hz, 3H, *CH*$_3$), 1.05 (d, ^3J(H,H)=6.9 Hz, 3H, *CH*$_3$), 0.82 (d, ^3J(H,H)=6.9 Hz, 3H, *CH*$_3$) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 205.6, 205.0, 170.4, 170.1, 164.2, 159.8, 150.3, 150.2, 130.1, 130.0, 128.7, 114.3, 114.2, 93.3, 93.2, 77.1, 71.7, 71.5, 62.3, 62.2, 55.7, 44.7, 44.1, 38.0, 37.8, 35.9, 34.2, 30.1, 17.2, 16.9, 15.3, 15.1 ppm.

A solution of the previous aldehyde (230 mg, 0.614 mmol) and freshly prepared vinyl-iodide **7** (338 mg, 1.53 mmol) in THF/DMSO (5.4 mL/2.4 mL) was treated under an argon atmosphere with $CrCl_2$ (798 mg, 6.148 mmol) and $NiCl_2$ (11.5 mg, 0.082 mmol) (thoroughly premixed and flame-dried in vacuo). After stirring for 12 h at room temperature, the reaction mixture was diluted with water (20 mL) and extracted with Et_2O (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (SiO_2, Hexanes/AcOEt/Et_3N 8:2:0.1) to afford alcohol **21a** (mixture of four diastereoisomers; 115 mg, 0.246 mmol; 40%) as colorless oil, along with recovered aldehyde (108 mg, 0.288 mmol; 47%). R_f=0.55 (Hexanes/AcOEt 7:3); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.25–7.18 (m, 32H, Ar*H*), 6.90–6.83 (m, 32H, Ar*H*), 6.59–6.41 (m, 3H), 6.41–5.89 (m, 60H), 5.89–5.44 (m, 30H), 5.44–5.26 (m, 3H), 5.16–4.97 (m, 32H, C=CH_2), 4.57–4.40 (m, 18H), 4.39–4.24 (m, 18H), 4.24–3.92 (m, 75H), 3.92–3.70 (m, 49H), 2.18–1.97 (m, 16H), 1.97–1.81 (m, 10H), 1.81–1.74 (m, 36H), 1.74–1.53 (m, 10H), 1.52–1.17 (m, 32H), 1.17–1.04 (m, 24H), 1.04–0.73 (m, 80H); FTIR (neat): $\bar{\nu}_{max}$ 3514, 3058, 3018, 2964, 2928, 2873, 2853, 1769, 1669, 1622, 1513, 1462, 1383, 1282, 1251, 1175, 1078, 1035, 1002, 977, 824, 740, 707 cm^{-1}.

4.1.9. Alcohol 21b. To a solution of alcohol **20b** (86 mg, 0.231 mmol) in DMSO (1.8 mL) was added IBX (130 mg,

0.462 mmol) at room temperature under an argon atmosphere. After stirring for 1.5 h the reaction was quenched with water (5 mL) and AcOEt (5 mL). The iodosocarboxylic acid, which precipitated as a white amorphous solid, was removed by filtration through Celite. The filtrate was extracted with AcOEt (2×10 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture was filtered through a small pad of SiO_2 (Hexane/AcOEt 8:2) to afford the corresponding aldehyde (73 mg) as yellow oil, which was subjected to the next step without further purification.

A solution of the aldehyde (73 mg, 0.197 mmol) and freshly prepared vinyl-iodide **7** (107 mg, 0.486 mmol) in THF/DMSO (1.73 mL/0.72 mL) was treated under an argon atmosphere with $CrCl_2$ (264 mg, 2.034 mmol) and $NiCl_2$ (11 mg, 0.0071 mmol) (thoroughly premixed and flame-dried in vacuo). After stirring for 12 h at room temperature, the reaction mixture was diluted with water (15 mL) and extracted with Et_2O (3×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (SiO_2, Hexanes/AcOEt/Et_3N 85:15:0.1) to afford **21b** (mixture of four diastereoisomers; 45 mg, 0.098 mmol; 50%) as colorless oil, along with recovered aldehyde (22 mg, 0.059 mmol; 30%). R_f=0.55 (Hexanes/AcOEt 7:3).

4.1.10. Alcohol 22. To a solution of alcohol **21a** (95 mg, 0.203 mmol) in Et_2O (8.6 mL) was added activated MnO_2 (1.51 g) and the mixture was stirred at room temperature for 3 h (TLC indicated the end of the reaction). The solution was filtrated through Celite, to remove MnO_2 and concentrated under reduced pressure to provide the corresponding trienone (82 mg, 0.180 mmol; 90%) as yellow oil, which was subjected to the next step without further purification. R_f=0.40 (Hexane/AcOEt 8:2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.78–7.68 (m, 8H), 7.57–7.49 (m, 8H), 7.44–7.31 (m, 6H), 7.27–7.13 (m, 22H), 6.86 (dd, ^3J(H,H)=8.9, 9.9 Hz, 16H), 6.60–6.49 (m, 6H), 6.25–6.07 (m, 8H), 6.01–5.89 (m, 4H), 5.81–5.73 (m, 2H), 5.09 (d, ^2J(H,H)=2.5 Hz, 4H), 5.07 (d, ^2J(H,H)=2.2 Hz, 4H), 5.05–5.02 (m, 8H), 4.46 (d, ^3J(H,H)=10.9 Hz, 4H), 4.44 (d, ^3J(H,H)=11.2 Hz, 4H), 4.33–4.29 (m, 1H), 4.28 (d, ^3J(H,H)=10.5 Hz, 4H), 4.26 (d, ^3J(H,H)=11.4 Hz, 4H), 4.21–4.16 (m, 1H), 4.16 (s, 6H), 4.16 (s, 6H), 4.12 (s, 6H), 4.12 (s, 6H), 4.10–4.03 (m, 6H), 3.80 (s, 12H), 3.79 (s, 12H), 2.94–2.79 (m, 6H), 2.35–2.25 (m, 2H), 1.93–1.77 (m, 4H), 1.84 (d, ^3J(H,H)=6.9 Hz, 24H), 1.77–1.57 (m, 10H), 1.50–1.32 (m, 6H), 1.21–1.10 (m, 2H), 1.11 (d, ^3J(H,H)=6.9 Hz, 9H), 1.05 (d, ^3J(H,H)=6.9 Hz, 9H), 1.02 (d, ^3J(H,H)=6.4 Hz, 3H), 0.99–0.82 (m, 14H), 0.79 (d, ^3J(H,H)=6.9 Hz, 9H) ppm; ^{13}C NMR (125.8 MHz, $CDCl_3$, 25 °C): δ 143.5, 143.2, 142.7, 142.5, 136.0, 135.9, 131.8, 131.7, 131.3, 130.0, 129.9, 129.4, 129.3, 128.8, 128.6, 128.5, 127.5, 127.2, 114.2, 93.1, 92.9, 71.7, 71.5, 67.9, 66.0, 62.3, 62.2, 55.7, 42.9, 42.1, 40.3, 38.5, 38.3, 38.1, 36.4, 31.0, 30.9, 30.1, 19.6, 19.1, 19.0, 18.8, 17.6, 16.9, 14.1 ppm; HRMS (ESI) calculated for $C_{28}H_{34}O_6$ ([M+Na]$^+$): *m*/*z* 489.2245, found 489.2244.

A solution of the previous *p*-methoxy benzyl ether (82 mg, 0.180 mmol) in DCM/0.5% $NaHCO_{3aq}$ (9:1; 5.8 mL) was treated with DDQ (120 mg, 0.528 mmol) at 0 °C for 2.5 h. The reaction mixture was filtered through a short pad of silica gel and washed with Hexanes/AcOEt/Et_3N (7:3:0.1; 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (SiO_2, Hexane/AcOEt/Et_3N 8:2:0.1) to afford **22** (mixture of two diastereoisomers; 23 mg, 35%) as colorless oil. R_f=0.35 (Hexanes/AcOEt 7:3); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.40–7.27 (m, 3H), 7.00–6.89 (m, 5H), 6.89–6.80 (m, 2H), 6.73–6.64 (m, 2H), 6.64–6.52 (m, 4H), 6.39–6.25 (m, 8H), 6.25–6.09 (m, 16H), 6.05–5.90 (m, 4H), 5.86–5.72 (m, 4H), 5.08 (br s, 16H), 4.32–4.03 (m, 4H), 4.14 (br s, 24H), 3.82–3.76 (m, 1H), 3.68–3.60 (m, 1H), 3.47–3.37 (m, 2H), 2.97–2.80 (m, 8H), 1.92–1.80 (m, 24H), 1.80–1.71 (m, 8H), 1.45–1.38 (m, 8H), 1.29–1.23 (m, 8H), 1.17–1.08 (m, 24H), 1.04–0.95 (m, 24H); HRMS (ESI) calculated for $C_{20}H_{26}O_5$ ([M+Na]$^+$): *m*/*z* 369.1672, found 369.1673.

4.1.11. Alcohol 22. (One pot oxidation-deprotection of **21a**.) A solution of the crude trienone (90 mg, 0.193 mmol) in DCM/0.5% $NaHCO_{3aq}$ (9:1; 6.4 mL) was treated with DDQ (131.4 mg, 0.579 mmol) at 0 °C for 2.5 h. The reaction mixture was filtered through a short pad of silica gel and washed with Hexanes/AcOEt/Et_3N (7:3:0.1; 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (SiO_2, Hexane/AcOEt/Et_3N 8:2:0.1) to afford alcohol **22** (16 mg, 25%) as colorless oil.

4.1.12. Diketone 5 from 22. A solution of alcohol **22** (20 mg, 0.058 mmol) in DCM (1.3 mL) was treated with Dess–Martin periodinate (30 mg, 0.071 mmol) at ambient temperature for 1 h. The reaction was quenched with saturated $Na_2S_2O_3$ and stirred for 30 min. The mixture was separated and the aqueous layer was extracted with DCM (2×10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, Hexanes/AcOEt/Et_3N 8:2:0.1) to afford **5** as colorless oil (mixture of *E*/*Z* isomers; 19 mg, 95%). R_f=0.32 (Hexanes/AcOEt 7:3); $[\alpha]_D^{2.5}$ −20.1° (*c* 0.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 7.26 (dd, ^3J(H,H)=10.7, 15.0 Hz, 1H), 6.59 (dd, ^3J(H,H)=10.8, 14.8 Hz, 1H), 6.28–6.09 (m, 3H), 6.01–5.89 (m, 1H), 5.27 (br d, ^2J(H,H)=2.7 Hz, 1H), 5.21 (br d, ^2J(H,H)=2.7 Hz, 1H), 4.12 (s, 3H), 3.71–3.59 (m, 1H), 2.81 (ddq, ^3J(H,H)=6.9, 6.9, 6.9 Hz, 1H), 2.27–2.16 (m, 1H), 1.84 (br d, *J*=6.0 Hz, 3H), 1.37–1.25 (m, 1H), 1.15 (d, ^3J(H,H)=7.1 Hz, 3H), 1.13 (d, ^3J(H,H)=7.0 Hz, 3H); (ZEE, partial) 7.32 (dd, ^3J(H,H)=11.4, 15.0 Hz, 1H), 6.94 (dd, ^3J(H,H)=12.3, 15.1 Hz, 1H), 6.38–6.28 (m, 1H), 5.81–5.73 (m, 1H), 1.87 (br d, ^3J(H,H)=7.2 Hz, 3H); HRMS (ESI) calculated for $C_{20}H_{24}O_5$ ([M+H]$^+$): *m*/*z* 337.1515, found 337.1518.

4.1.13. Diketone 5 from 21b. To a solution of silyl ether **21b** (32 mg, 0.069 mmol) in THF (0.23 mL) was added TBAF (0.21 mL of 1 M solution in THF, 0.207 mmol) at 0 °C. After 5 min the ice bath was removed, the reaction mixture was stirred for additional 1 h at ambient temperature (TLC indicated the end of the reaction). The reaction mixture was extracted with AcOEt (2×5 mL). The combined organic layers were washed with saturated NH_4Cl_{aq} (5 mL) and brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture was subjected to flash

chromatography (SiO_2, Hexane/AcOEt 1:1) to afford the corresponding diol (mixture of diastereoisomers; 20 mg, 0.057 mmol; 83%) as colorless oil. R_f=0.45 (Hexanes/AcOEt 1:1); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 6.60–6.46 (m, 1H), 6.38–5.95 (m, 31H), 5.83–5.50 (m, 15H), 5.49–5.28 (m, 1H), 5.18–4.98 (m, 16H), 4.62–4.28 (m, 10H), 4.20–3.92 (m, 30H), 3.65–3.48 (m, 2H), 3.45–3.26 (m, 4H), 2.22–1.84 (m, 12H), 1.84–1.64 (m, 26H), 1.58–1.47 (m, 2H), 1.45–1.35 (m, 2H), 1.33–1.14 (m, 8H), 1.12–0.76 (m, 48H).

To a solution of the previous diol (18 mg, 0.052 mmol) in DMSO (0.4 mL) was added IBX (44 mg, 0.156 mmol) at room temperature under an argon atmosphere. After stirring for 1.5 h the reaction was quenched with water (1 mL) and AcOEt (1 mL). The iodosocarboxylic acid, which precipitated as a white amorphous solid, was removed by filtration through Celite. The filtrate was extracted with AcOEt (2×5 mL) and the combined organic layers were washed with saturated $NaHCO_{3aq}$ (4 mL) and brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude mixture was subjected to flash chromatography (SiO_2, Hexanes/AcOEt/Et_3N 8:2:0.1) to afford **5** as colorless oil (mixture of *E*/*Z* isomers; 15 mg, 0.044 mmol 85%).

4.1.14. Diels–Alder product 3. A solution of trienone **5** (5 mg, 0.015 mmol) in degassed toluene (2.5 mL; 0.006 M) was stirred at 100 °C in the presence of catalytic iodine under an argon atmosphere. After 3 h (TLC indicated no further conversion) the solvent was removed under reduced pressure and the residue was subjected to flash chromatography (SiO_2, Hexanes/AcOEt 8:2) to afford **3** (4 mg, 0.011 mmol, 75%). as colorless oil. R_f=0.45 (Hexanes/AcOEt 8:2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 6.48 (dd, ^3J(H,H)=6.8, 16.8 Hz, 1H, C*H*═), 6.25 (dd, ^4J(H,H)=1.2 Hz, ^3J(H,H)=16.8 Hz, 1H, C*H*═), 5.86 (dt, ^3J(H,H)=3.1, 9.9 Hz, 1H, C*H*═), 5.67 (dt, ^3J(H,H)=2.4, 9.9 Hz, 1H, C*H*═), 3.91 (s, 3H, OCH_3), 3.48–3.42 (m, 1H, ═CHC*H*CH═), 3.16–3.08 (m, 1H, C*H*CH_3), 3.00–2.90 (m, 1H, COC*H*CH_3), 2.69–2.59 (m, 1H, C*H*CH_3), 2.40 (dd, ^2J(H,H)=14.4 Hz, ^3J(H,H)=7.9 Hz, 1H, CH*H*CHCH_3), 1.88 (ddd, ^3J(H,H)=4.2, 5.9 Hz, ^2J(H,H)=15.2 Hz, 1H, CHC*H*HCH), 1.82 (dd, ^3J(H,H)=4.6 Hz, ^2J(H,H)=14.4 Hz, 1H, C*H*HCHCH_3), 1.24–1.12 (m, 1H, CHCH*H*CH), 1.21 (d, ^3J(H,H)=6.8 Hz, 3H, CH_3), 1.19 (d, ^3J(H,H)=6.8 Hz, 3H, CH_3), 1.15 (d, ^3J(H,H)=7.3 Hz, 3H, CH_3).

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The design, synthesis, and characterization of a PAMAM-based triple helical collagen mimetic dendrimer

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Abstract—The synthesis and characterization of a collagen mimetic dendrimer composed of the Gly-Pro-Nleu sequence is described. The dendrimer is built on a 'first generation' poly(amidoamine) core and is synthesized in 38% yield. This dendrimer exhibits a melting temperature of 25 °C, which is in between previously studied analogous molecules of identical sequence and length.

1. Introduction

Collagen is the most abundant protein in vertebrates comprising roughly one-third of all proteins in animals[1] and is an integral component of the extracellular matrix upon which tissues are built. The extracellular matrix promotes the differentiation and proliferation of many cell types. For example, fibroblasts within the dermis form a complex three-dimensional network while in tissue culture fibroblasts cease to proliferate once a two-dimensional monolayer is formed.[2] When collagen biosynthesis was stimulated in vitro by a vitamin C derivative the dermal fibroblasts formed a three-dimensional tissue similar to that of natural skin.[3] The biosynthesis of collagen and other extracellular matrix proteins is vital for the differentiation of cells in a developing fetus. The formation of cartilage is one of the earliest morphogenetic events in the development of an embryo. The ultimate transformation of this cartilage into skeletal tissue is brought about by the biosynthesis of various collagens. A signaling pathway is initiated that causes the vascularization of the cartilage. The resulting blood flow infuses osteoblasts into this site and the process of replacing the cartilage with mineralized bone begins.[4]

Collagen functions as the major load-bearing constituent of the connective tissues that comprise teeth, bone, skin, cartilage, and tendons among others. Much of collagen's high tensile strength is derived from its triple helical structure. Three polypeptide chains, each in a left-handed polyproline II type helix, coil around one another about a common axis to form a right-handed triple helix. The sterically hindered environment created by this triple helical conformation results in a repeating Gly-Xaa-Yaa sequence where Xaa and Yaa are frequently populated by imino acids.[5]

Collagen represents a nearly ideal target for the preparation of biomaterials due to its ubiquity, low cytotoxicity, low immunogenicity, and high durability. Natural collagen has been utilized for cartilage replacement, surgical sutures, hemostatic agents, and tissue replacements for blood vessels and valves to name a few applications.[6–9] However, there are problems associated with the use of natural collagen as a biomaterial. Many of the methods used to purify and sterilize natural collagen results in the disruption of its structural integrity. Also, the chemical methods utilized to crosslink the collagen may result in cytotoxicity upon implantation. Frequently, collagens are prepared from bovine sources, which can cause allergic and inflammatory responses.[10] If the collagen is obtained from a human cadaver, there are concerns of disease transmission. The issues stated above underscore the need for the preparation of artificial and synthetic collagen-based biomaterials.

Inspired by the enormous significance of collagen in nature and its remarkable biological and physiological properties, we sought to develop new artificial collagens. We hypothesized that such compounds could be tuned to have the desired biological properties and be devoid of any side effects related to the use of natural collagen. With this idea in mind, we developed a collagen–dendrimer conjugate research program. The use of dendrimers in biomaterial and therapeutic applications is becoming increasingly significant.[11–13] Dendrimers represent a relatively new class of polymer with a more defined and monodisperse molecular structure than their traditional linear polymeric analogues. Because dendrimers are considered to be spherical, they have a distinct periphery where a multitude of functional groups can be displayed. Researchers have derivatized these groups with various structures that drive the assembly of

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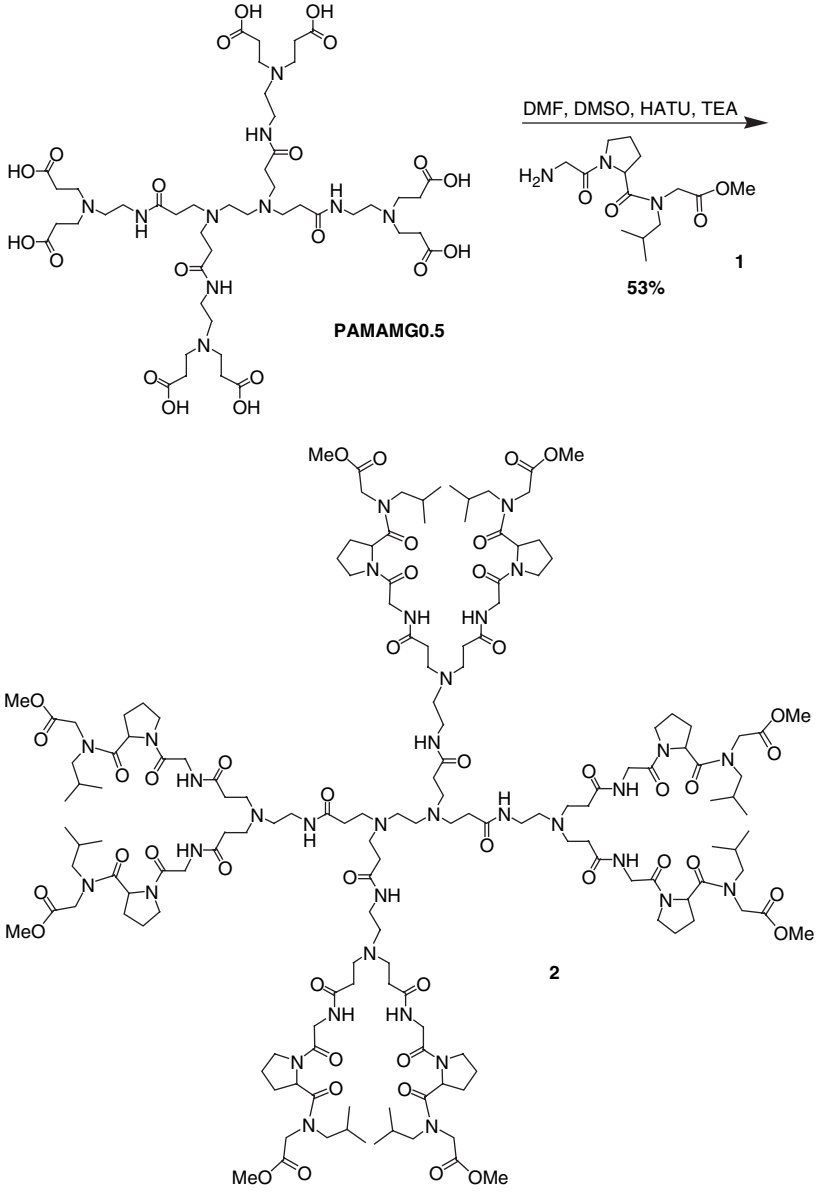
dendrimers to fashion nanostructures[14] and nanodevices[15] having applications as biomaterials.[16] For example, Higashi and Niwa utilized a third generation poly(amidoamine) (PAMAM) dendrimer to assemble synthetic α-helical peptides into a closely packed array. The densely packed protein-like environment induced by the dendritic structure resulted in a nearly 50% enhancement in α-helical character of the peptides. It is known that collagen triple helices associate with one another to form collagen fibrils. Therefore, we chose to use Higashi's approach in our collagen mimetics program. In this prototype the PAMAM dendrimer will act not only as a scaffold to promote intramolecular triple helical formation but also may act as a nucleation source for the interaction of intramolecular triple helical arrays.

PAMAM dendrimers represent the first dendrimer family to be made commercially available. Consequently, PAMAM dendrimers are widely utilized in the biomedical field. Applications range from immunodiagnostics,[17,18] gene transfection,[19–21] drug delivery,[18,22–24] and magnetic resonance imaging contrast agents.[25–28]

Previously we reported the synthesis of collagen mimetic dendrimers based on a trimesic acid (TMA) core structure and a tris-based scaffold.[29] In this publication we describe the preparation and characterization of a collagen mimetic dendrimer based on a PAMAM dendrimer core. The collagen–PAMAM conjugates were synthesized to prepare collagen mimetic dendrimers with increased functionality with respect to the TMA-based structures and to study the effect of different dendritic architecture on collagen mimetic melting transitions.

2. Results and discussion

The synthesis of PAMAM–collagen conjugate structures was undertaken by utilizing the first generation PAMAM

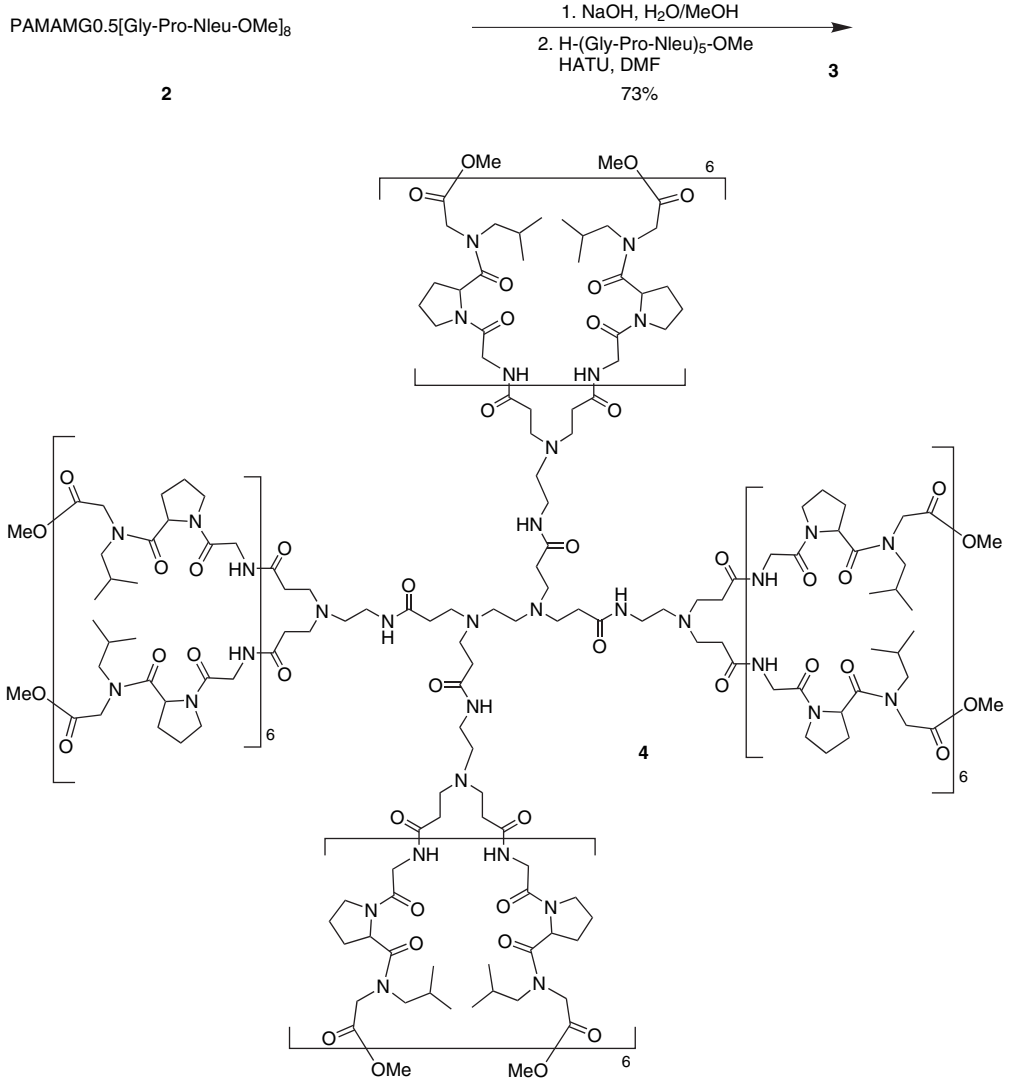


Scheme 1.

dendrimer containing eight sodium carboxylate groups (PAMAMG0.5) as shown in Scheme 1. The PAMAMG0.5 dendrimer was converted to the free acid by the addition of TFA in methanol. The salts were removed by GPC and the solvent was removed under reduced pressure. The reaction was then carried out according to Scheme 1 where the PAMAMG0.5 dendrimer was allowed to react with an excess of H-Gly-Pro-Nleu-OMe (**1**) in the presence of HATU. The product, PAMAMG0.5[Gly-Pro-Nleu-OMe]$_8$ (**2**), was obtained after purification by anion exchange chromatography in the carbonate form and gel permeation chromatography (GPC) in a yield of 53%. The product was characterized by analytical HPLC and mass spectrometry.

The PAMAM–collagen conjugate composed of eight discrete tripeptide units of Gly-Pro-Nleu (PAMAMG0.5[Gly-Pro-Nleu-OMe]$_8$) (**2**) was allowed to react with aqueous sodium hydroxide in methanol (Scheme 2). The reaction was followed by analytical C4 RP-HPLC. The reaction mixture was acidified to pH 0 and the sodium salts were removed by GPC. The conjugate, which contained eight carboxylic acids, obtained in 95% yield, was allowed to react with the free amine pentadecapeptide ester H-(Gly-Pro-Nleu)$_5$-OMe in the presence of the coupling reagent HATU as shown in Scheme 2. The product (**4**) was purified by anion exchange and GPC as describe above and isolated in 77% yield. The core PAMAM dendrimer structure possesses six tertiary amines. This resulted in facile ionization and characterization of the desired product by mass spectrometry. The desired product was identified by analytical HPLC and MALDI-TOF mass spectrometry.

The characterization of the triple helical structure of the PAMAM–collagen mimetic conjugate consisted of circular dichroism (CD) spectroscopy and thermal denaturation monitored by optical rotation. Triple helical collagen-like structures exhibit a unique CD spectrum consisting of a minimum, crossover, and maximum around 197, 213, and 220 nm, respectively.[30,31] When the triple helical structure of collagen mimetics composed of the Gly-Pro-Nleu sequence is denatured, a characteristic blue shift is observed in the above spectral positions. In addition, when observed by temperature dependent optical rotation, the disruption of triple helicity is coincident with a cooperative melting



Scheme 2.

transition. The results of the biophysical characterization for the triple helical PAMAM–collagen chimera are shown in Figures 2 and 3. For comparison, Figures 2 and 3 also display the characterization of the TMA dendrimer and its constituents, which was reported previously (Fig. 1).[29] The molecules studied were incubated in H_2O (0.1–0.2 mg/mL) at 4 °C for 1–7 days prior to characterization. The scaffold-assembled and dendritic collagen structures were incubated for 24 h prior to analysis because the intramolecular association is rapid, while the single chain collagen mimetic (**5**) was incubated for 7 days at high concentration (10 mg/mL) to facilitate intermolecular strand association.[32] This solution was diluted to 0.2 mg/mL 1 day prior to analysis. Figure 2A and B show the CD spectra of compounds **4**–**7** at 8 and 22 °C, respectively. At 8 °C the single chain collagen structure **5** is not triple helical as shown by its blue shifted positive peak at 215 nm. The other three mimetics are triple helical and exhibit nearly superimposable CD spectra with maximum, crossover, and minimum near 220, 213, and 199 nm, respectively. When the spectra are reacquired at 22 °C (Fig. 2B) the single chain **5** remains non-triple helical as expected but the tris-assembled collagen mimetic **6** is nearly completely denatured. The PAMAM (**4**) and TMA (**7**) collagen mimetic dendrimers remain triple helical in structure. While the tris-assembled structure clearly possesses a melting temperature (T_m) below room temperature, an aspect of the dendritic architecture of compounds **4** and **7** impart an added thermal stability which allows for triple helical structure above room temperature.

Optical rotation measurements of compounds **4** through **7** are shown in Figure 3. The single chain compound (**5**) shows no melting transition over the temperature range tested and is therefore not triple helical in H_2O. The tris-assembled mimetic (**6**) has a T_m of 19 °C, thus explaining the denatured CD spectrum of this molecule at 22 °C. Melting transitions for the PAMAM (**4**) and TMA (**7**) collagen mimetic dendrimers are shown in Figure 3 with T_m of 25 and 28 °C,

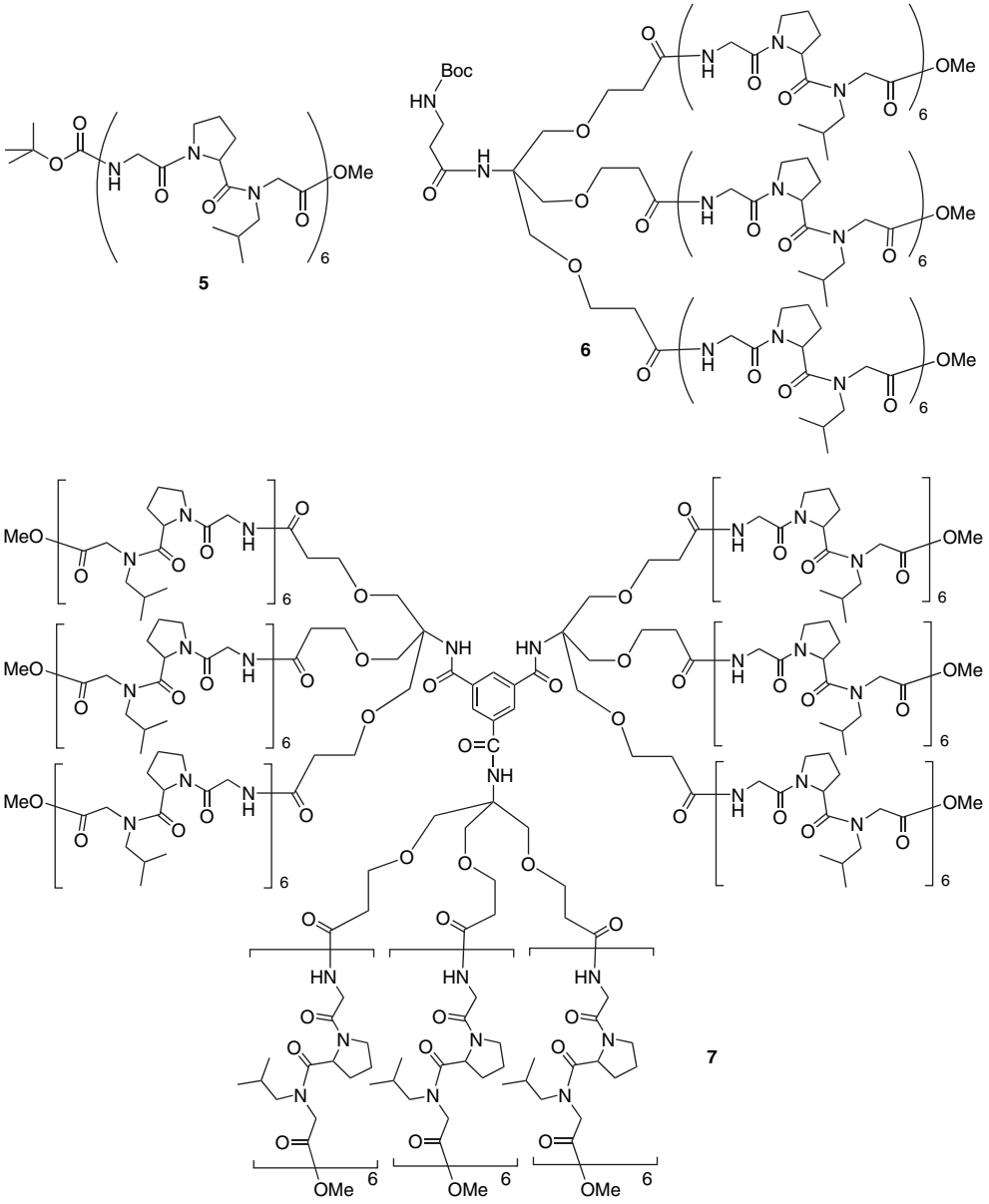


Figure 1. Boc-(Gly-Pro-Nleu)$_6$-OMe (**5**), Boc-βAla-tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (**6**), and TMA[tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$ (**7**).

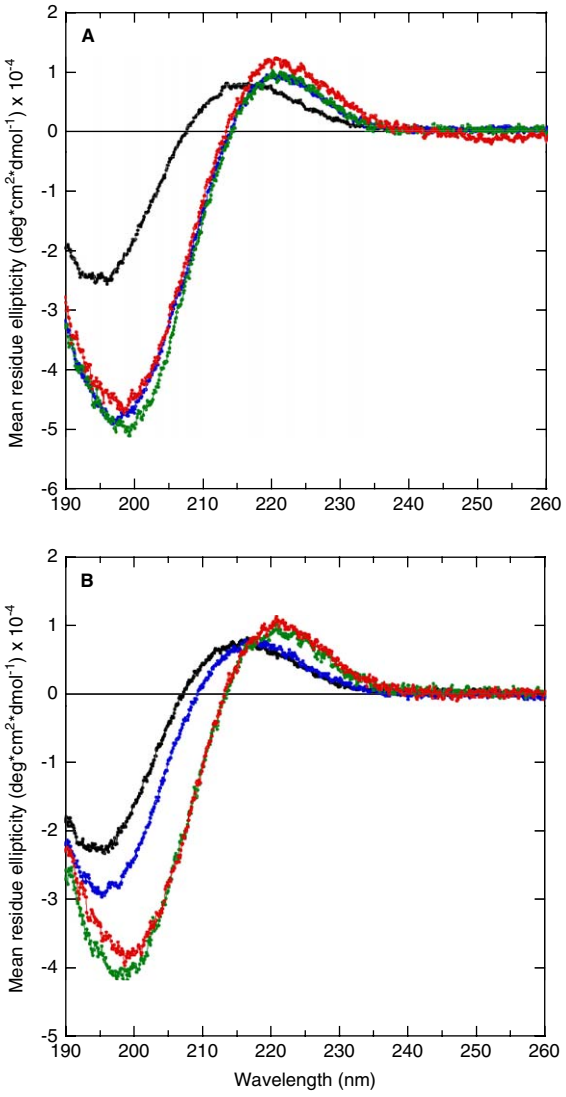


Figure 2. CD spectra at 8 °C (A) and 22 °C (B) of Boc-(Gly-Pro-Nleu)$_6$-OMe (*black*, 0.2 mg/mL), Boc-βAla-tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (*blue*, 0.2 mg/mL), TMA[tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$ (*green*, 0.1 mg/mL), and PAMAMG0.5[(Gly-Pro-Nleu)$_6$-OMe]$_8$ (*red*, 0.1 mg/mL). Measurements were carried out in H_2O.

respectively. Results obtained from the CD studies and the optical rotation measurements are consistent with each other.

3. Conclusion

It is interesting to note that the T_m of the PAMAM–collagen conjugate PAMAMG0.5[(Gly-Pro-Nleu)$_6$-OMe]$_8$, at 25 °C, is between that of the tris-assembled structure Boc-β-Ala-tris[(Gly-Pro-Nleu)$_6$-OMe], at 19 °C, and the TMA dendrimer TMA[tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$, at 28 °C. In a previous publication we asserted that the TMA dendrimer (**7**) possesses a higher melting temperature due to an intramolecular clustering of triple helices.[29] This results in a packed structure that excludes solvent from the interior of the dendrimer and imbues a higher triple helical thermal stability. The clustered ensemble responsible for the enhanced thermal stability of the triple helices of the TMA

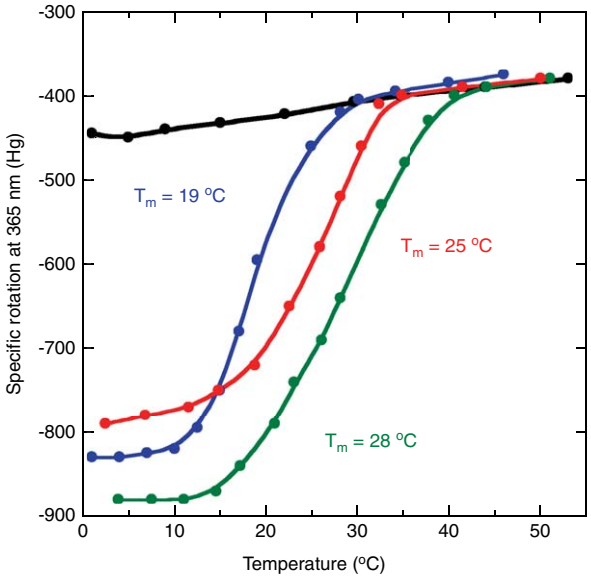


Figure 3. Thermal denaturation monitored by optical rotation of Boc-(Gly-Pro-Nleu)$_6$-OMe (*black*, 0.2 mg/mL), Boc-βAla-tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (*blue*, 0.2 mg/mL), TMA[tris[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$ (*green*, 0.1 mg/mL), and PAMAMG0.5[(Gly-Pro-Nleu)$_6$-OMe]$_8$ (*red*, 0.1 mg/mL). Measurements were carried out in H_2O.

dendrimer also may be present in the PAMAM–collagen conjugate. However, the conjugate structure has eight peptidomimetic chains and can form only two complete triple helical arrays. This results in a different intramolecular structure that less effectively shields the triple helices from solvent invasion, hence a lower melting temperature relative to the TMA-assembled dendrimer. It appears that the thermal stability of collagen mimetic dendrimers can be attenuated by inhibiting the interaction between the triple helical arrays or by decreasing the number of triple helical arrays that associate with each other.

PAMAM dendrimers have been shown to complex a variety of transition metals, particularly Cu^{2+} and Ni^{2+}, by the donation of amine and amide electrons.[33–35] Copper(II) is an important enzyme cofactor in lysyl oxidase, an enzyme that carries out the oxidation of lysine and hydroxylysine to the corresponding aldehyde for the crosslinking of fibril collagens. This crosslinking is vital for the structural integrity of connective tissues.[36] In addition, many biological events involving collagen such as wound healing and bone mineralization are facilitated by metal cofactors.[37,38] Therefore, the synthesis of collagen–PAMAM conjugates and their subsequent complexation with biologically relevant metals may lead to compounds with novel biological properties.

4. Experimental

4.1. General

All amino acids used were of L-configuration unless otherwise specified. Protected amino acids were purchased from Novabiochem. The chemicals HOAt and HATU were purchased from Perseptive Biosystems. Reagent grade and HPLC-grade solvents were purchased from Fisher Scientific with DCM, THF, and TEA being distilled before use when

appropriate. All other reagents were purchased either from Aldrich or Acros.

Reactions carried out in solution were monitored by HPLC. Both preparatory and analytic HPLC were carried out on two instruments. One was a Waters (two Waters 510 pumps and 2487 Dual λ Absorbance detector) system. The other was a Waters Millennium 2010 system (715 Ultra WISP sample processor, 996 photodiode array detector, and two Waters 510 pumps) with a NexStar PC compatible computer interface. The solvents used in HPLC were (A) water with 0.1% TFA and (B) acetonitrile with 0.1% TFA. The flow rate for preparatory purification was 10 mL/min (Vydac, C-18 and C-4, 25×2.2 cm) and 1 mL/min (Vydac, C-18 and C-4, 25×0.46 cm).

Circular dichroism (CD) measurements were carried out on a Cary 61 spectropolarimeter, which was modified by replacing the original Pockel cell with a 50 kHz photoelastic modulator (Hinds International FS-5/PEM-80). The original Cary linear polarizer was replaced with a MgF_2 linear polarizer supplied by AVIV Inc. An EGG Princeton Applied Research model 128A lock in amplifier was used to integrate the phase-detected output of the original end-on photomultiplier tube and preamp. System automation, multiple scan signal averaging, and base line subtraction were accomplished with an AT286 PC interfaced directly to both the Cary 61 and the 128A amplifier. The system software and custom hardware interfaces were designed by Allen Micro-Computer Services Inc. and the UC San Diego, Department of Chemistry and Biochemistry Computer Facility. The CD spectra were obtained using a 0.02–0.5 cm path length cell by signal averaging 10 scans from 190 to 300 nm at a scan speed of 0.8 nm/s.

Optical rotations were measured with a Perkin–Elmer 241 Polarimeter equipped with a Model 900 isotemp refrigerator circulator (Fisher Scientific). Data were collected at 365 nm (Hg). The solutions were stored at 4 °C for at least 24 h for equilibration of triple helix formation. Before recording an optical rotation, the sample was equilibrated 30 min at the initial temperature. At each subsequent temperature point, the samples were allowed to equilibrate for 30 min.

Mass spectra were obtained at UC San Diego or the Scripps Research Institute. Fast atom bombardment (FAB), electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) methods were used to determine product mass.

4.2. Synthesis

4.2.1. PAMAMG0.5[Gly-Pro-Nleu-OMe]$_8$ (2). The PAMAMG0.5 sodium salt starting material was converted to the free acid using TFA. The inorganic salts were removed by GPC. The PAMAMG0.5 free acid was concentrated and used in the coupling reaction. To a solution of PAMAMG0.5 (43.5 mg, 0.0343 mmol), H-Gly-Pro-Nleu-OMe (**1**) (138 mg, 0.411 mmol), and HOAt (23.3 mg, 0.171 mmol), stirred under Ar gas in a solution of DMSO/DMF/DCM (3:3:2, 800 μL) was added HATU (130.3 mg, 0.3428 mmol). After 2 min TEA (142 mg, 1.405 mmol) was added and the reaction was allowed to continue for 12 h. The solvents were removed under reduced pressure and the resulting residue was dissolved in MeOH (3 mL) and passed through an anion exchange column. This solution was then passed through a gel permeation column (Sephadex LH-20) twice to effect purification. The fractions containing the desired product were combined and the solvent was removed under reduced pressure. The product was obtained as a white solid (61 mg, 53%). MS-ESI (*m*/*z*) calculated for $C_{158}H_{264}N_{34}O_{44}$ 3342.0, found 1115.9 $[M+3H]^{3+}$, reconstruction 3345.9 [M+3H]. Analytical C4 RP-HPLC t_R=20.85 min (20–80% solvent B over 30 min).

4.2.2. PAMAMG0.5[Gly-Pro-Nleu-OH]$_8$. To a solution of **2** in MeOH (0.2 mL) was added NaOH (2.75 M, 0.2 mL) solution in water. The reaction was allowed to stir for 1 h. The reaction mixture was acidified to pH 0 by using 2 N HCl. The solution was passed down a GPC column (LH-20). The fractions containing the product were concentrated to obtain a white solid (60 mg, 95%). This compound was used without further purification or characterization.

4.2.3. PAMAMG0.5[(Gly-Pro-Nleu)$_6$-OMe]$_8$ (4). To a solution of PAMAMG0.5[Gly-Pro-Nleu-OH]$_8$ (60 mg, 17.4 μmol), **3** (293 mg, 209 μmol), and HOAt (19 mg, 139 μmol), stirred under Ar gas in DMF (800 μL) at 50 °C was added HATU (66 mg, 174 μmol). After 2 min TEA (77 mg, 765 μmol) was added and the reaction was allowed to continue for 12 h. The solvents were removed under reduced pressure and the resulting residue was dissolved in MeOH (3 mL) and passed through an anion exchange column. The remaining material was passed down a gel permeation column (Sephadex LH-60) twice to effect purification. The fractions containing the desired product were combined and the solvent was removed under reduced pressure. The product was obtained as a white solid (187 mg, 77%). MALDI-TOF (*m*/*z*) calculated for $C_{678}H_{1104}N_{154}O_{164}K$ 14,076, found 14,077 $[M+K]^+$. Analytical C4 RP-HPLC t_R=26.0 min (nontriple helical peak) and 27.4 min (triple helical peak) (20–90% solvent B over 35 min).

The syntheses of compounds **1**, **3**, **5**, **6**, and **7** have been previously reported.[29]

Acknowledgments

We would like to dedicate this manuscript to the memory of our mentor Professor Murray Goodman. This research was funded by the NSF Biomaterial Division, DMR-0111617.

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Dimerization of (+)-myrmicarin 215B. A potential biomimetic approach to complex myrmicarin alkaloids

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Abstract—The acid promoted diastereoselective dimerization of myrmicarin 215B is described. The reactivity of these sensitive alkaloids, structural assignment, and a possible mechanism for the observed dimerization is discussed. These finding raise the intriguing possibility of the synthesis of the highly sensitive myrmicarin alkaloids based on a strategy involving the direct dimerization of functional tricyclic myrmicarin derivatives.

1. Introduction

A series of structurally fascinating alkaloids have been isolated from the poison gland secretion of the African *Myrmicaria opaciventris* ant species (Fig. 1).[1] Through a series of elegant spectroscopic studies, the relative stereochemistry of myrmicarins 430A (**1**) and 663 (**2**) has been assigned. These complex alkaloids were found to be highly sensitive to air-oxidation, complicating their isolation. The high sensitivity of myrmicarin 430A (**1**) required its structural assignment as a crude mixture using phase-sensitive spectroscopic techniques.[1c] The relative stereochemistry of myrmicarin 645 (**5**) in addition to the absolute stereochemistry of complex myrmicarin alkaloids (i.e., **1**, **2**, and **5**) is unknown. Another isomeric myrmicarin 430 was detected in the poison gland secretion but no structural information was reported. Interestingly, the paralytic activity of the poison gland secretion has been attributed to these intriguing alkaloids.[1b] A combination of their alluring molecular structure and the plethora of challenges associated with their sensitivity has prompted us to initiate a program directed toward the synthesis of the complex myrmicarin alkaloids. Herein we report our findings that raise the possibility of an efficient strategy for the synthesis of complex myrmicarins based on a vinyl pyrrole dimerization strategy.

Schröder and Francke have reported the spontaneous dehydration of a C1′–C3 unsaturated derivative of myrmicarin 237 (Fig. 1) to give myrmicarin 217 (**6**),[2] which they cite as strong evidence for the formation of tricyclic myrmicarins from simpler indolizine derivatives. Furthermore, they propose the possible dimerization of a doubly unsaturated indolizine derivative **9** (Scheme 1) to afford myrmicarin 430A (**1**) and other complex myrmicarin alkaloids.[1b]

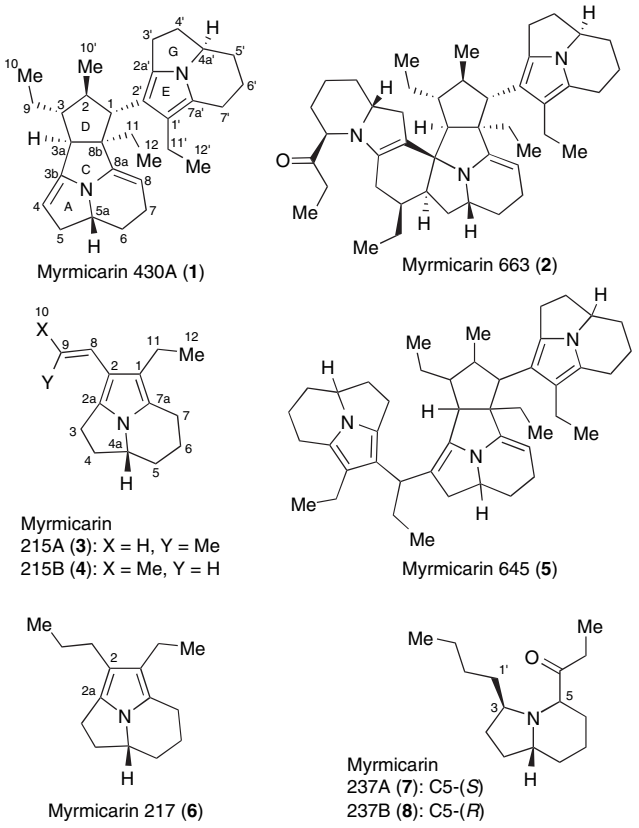

Figure 1. Representative members of the myrmicarin family of alkaloids. The relative stereochemistry of **5** is unknown; see Ref. 1d.

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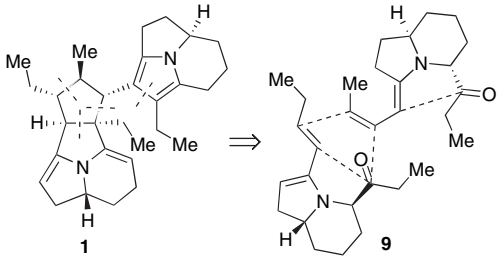
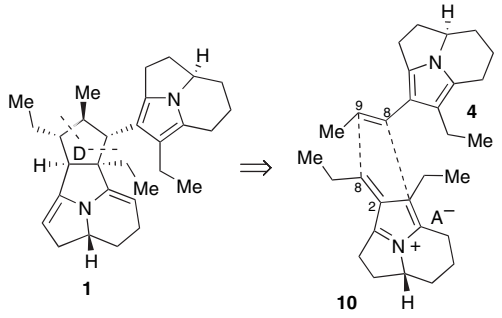


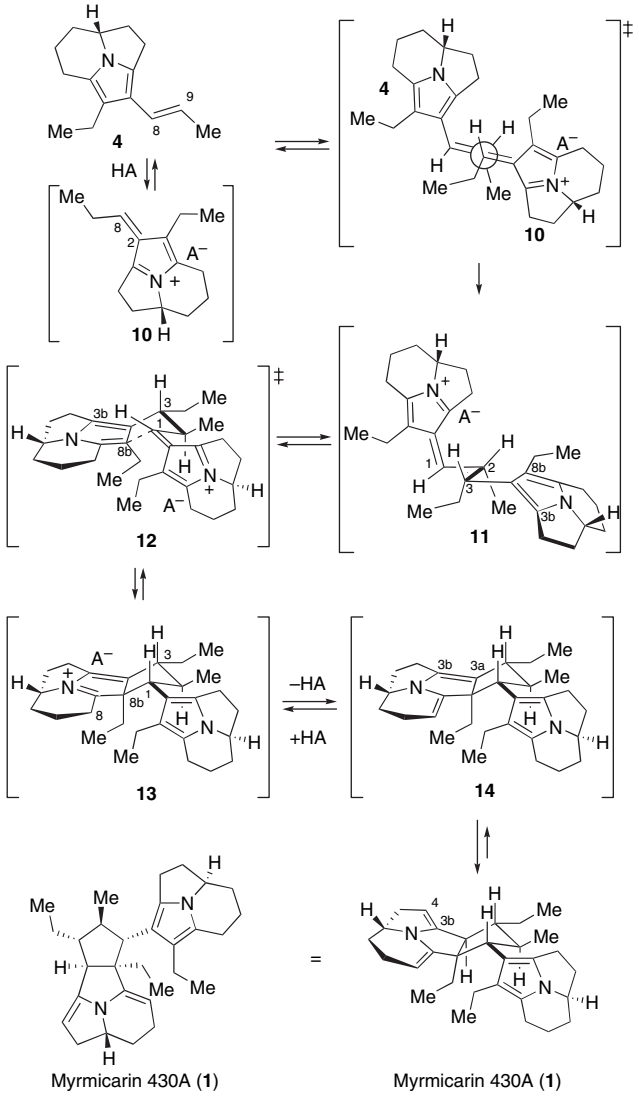
Scheme 1. Francke's proposed dimerization of bicyclic diene **9** to give myrmicarin 430A (**1**).

While the dimerization of **9** to give myrmicarin 430A (**1**) is plausible, we considered the possibility of tricyclic myrmicarin derivatives serving as direct precursors to **1**. We propose that an acid promoted dimerization of a suitable vinyl pyrroloindolizine derivative may lead directly to the fully substituted cyclopentane D-ring of myrmicarin 430A (**1**) as shown in Scheme 2.



Scheme 2. Our proposed potential biomimetic dimerization of pyrroloindolizines to give myrmicarin 430A (**1**).

Specifically, our approach to the complex myrmicarin alkaloids is based on the hypothesis that C9-protonation of myrmicarin 215B (**4**) by a Brönsted acid (HA) would lead to reversible formation of the highly reactive azafulvenium ion **10** (Schemes 2 and 3).[3] We envisioned that C9-nucleophilic addition of myrmicarin 215B (**4**) to the C8-electrophilic center of the proposed azafulvenium ion **10** would result in the hexacyclic azafulvenium ion **11** (Scheme 3), exchanging a π-bond for a σ-bond. Intramolecular trapping of the intermediate azafulvenium ion **11** by C8b-nucleophilic[4] addition to C1-electrophilic center via transition state structure **12** was envisioned to give the iminium ion **13** as a heptacyclic precursor to myrmicarin 430A (**1**). The C8-deprotonation of iminium ion **13** would afford bis-enamine **14**. Acid catalyzed tautomerization of the C3b-enamine would provide the expected *cis*-azabicyclooctane core (the CD-ring system of **1**) of myrmicarin 430A (**1**). Central to this strategy for the synthesis of complex myrmicarins was our proposed acid promoted cyclopentane annulation of vinyl pyrroloindolizine **4** (myrmicarin 215B), an unknown mode of reactivity for vinyl pyrroles. We envisioned isolation of the highly unstable myrmicarin 430A (**1**) either as an iminium ion salt (i.e., **13**, Scheme 3) or as an appropriate derivative masking the sensitive enamine functional groups.



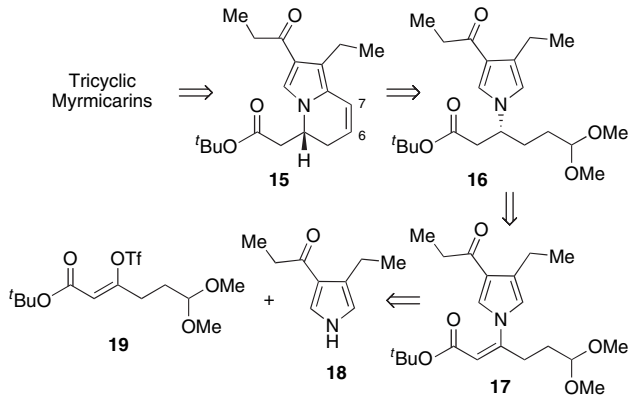
Scheme 3. Our proposed dimerization of myrmicarin 215B (**4**) to give **1**.

2. Results and discussion

2.1. Enantioselective synthesis of the tricyclic myrmicarins

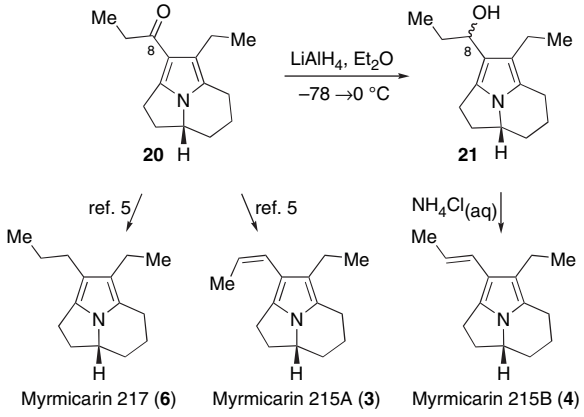
The implementation of this strategy for the preparation of complex myrmicarin alkaloids required the development of a versatile synthesis of tricyclic myrmicarin derivatives. We have recently reported a concise, enantioselective synthesis of all tricyclic myrmicarins (Scheme 4).[5,6] The key steps involve palladium-catalyzed coupling between triflate **19** and pyrrole **18**,[7,8] copper-catalyzed enantioselective conjugate reduction of the resulting *N*-vinyl pyrrole **17**,[9,10] and a regioselective acid catalyzed Friedel–Crafts cyclization to provide the dihydroindolizine **15** (Scheme 4).[5]

Hydrogenation of the C6–C7 alkene in **15** (Scheme 4) and selective conversion of the *tert*-butyl ester to the corresponding primary iodide followed by a silver tetrafluoroborate promoted cyclization provided the tricyclic ketone **20** (Scheme 5).[5] The final stages of the synthesis of myrmicarin 215B (**4**) involved reduction of the ketone **20** with lithium



Scheme 4. Synthesis of key bicyclic intermediate **15**.

aluminum hydride at 0 °C to afford the acid sensitive tricyclic alcohol **21** as an equal mixture of C8-epimers (Scheme 5). Acid catalyzed dehydration of alcohol **21** gave exclusively myrmicarin 215B (**4**). Alternatively, the reduction of ketone **20** to the C8-alcohol followed by an acidic work-up (pH 2) directly gave **4**.[5] The synthesis of the acid sensitive C8–C9 *Z*-alkene of myrmicarin 215A (**3**) was possible by an initial dehydration of ketone **20**, using 2-chloro-3-ethylbenzoxazolium tetrafluoroborate,[11] followed by partial hydrogenation with Lindlar catalyst.[5] These stereoselective routes to the vinyl pyrroloindolizine structure provided access to the first pure samples of myrmicarins 215A (**3**) and 215B (**4**). Complete reduction of the C3-carbonyl of **20** using lithium aluminum hydride at high temperature provided myrmicarin 217 (**6**). The spectroscopic data for the synthetic alkaloids **3**, **4**, and **6** was found to be identical with those reported for natural isolates.[1b]

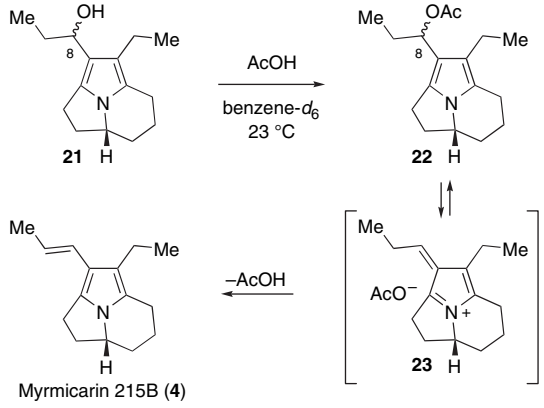


Scheme 5. Enantioselective synthesis of the tricyclic myrmicarin alkaloids.

During the late operations in the synthesis of the tricyclic myrmicarins, we found the intermediates lacking the C8-ketone to be highly sensitive to both air-oxidation and acid catalyzed decomposition. As noted in the isolation reports of myrmicarins 215 and 217, these compounds show significant air-oxidation to the corresponding C6–C7 didehydro derivatives 213A/B and 215C.[1b] In fact, we observed direct conversion of benzene-d_6 solutions of myrmicarins 215A, 215B, and 217 to myrmicarins 213A, 213B, and 215C, respectively, upon exposure to oxygen. Prolonged exposure to oxygen led to complete decomposition of both the tricyclic myrmicarins and their dehydrogenated derivatives. As a result, all manipulations of the tricyclic myrmicarin derivatives were conducted strictly under an argon atmosphere.[12] Indeed, as a testament to their acid sensitivity, thin layer chromatography showed that brief exposure of myrmicarin 215A (**3**) to silica gel effected partial conversion to myrmicarin 215B (**4**).

2.2. Acid promoted reactivity of myrmicarin 215

As an alternative to performing an acid catalyzed C8–C9 dehydration using an acidic aqueous work-up, completely stereoselective conversion of the alcohol **21** (~1:1, C8-epimers) to myrmicarin 215B (**4**) was achieved by treatment of a benzene-d_6 solution (0.05 M) of alcohol **21** with acetic acid (1.5 equiv) at ambient temperature (Scheme 6). ^{1}H NMR monitoring of the reaction mixture led to the detection of the intermediate C8-acetate **22**, likewise as an approximately equal mixture of C8-epimers. After 1 h, trace amounts of myrmicarin 215B (**4**) were detected. Consumption of approximately 90% of the alcohol **21** occurred in 9 h, at which point an equal mixture of acetate **22** and myrmicarin 215B (**4**) was observed. After an additional 61 h, myrmicarin 215B (**4**) was the only significant component remaining in the sample. Failure to detect the putative azafulvenium ion **23** by ^{1}H NMR is consistent with its expected high reactivity and short lifetime.[3]



Scheme 6. Acetic acid catalyzed dehydration of alcohol **21** to myrmicarin 215B (**4**).

In contrast, treatment of a benzene-d_6 solution (0.05 M) of alcohol **21** with trifluoroacetic acid (TFA, 1.10 equiv) effected full and clean conversion (≥90% by ^{1}H and ^{13}C NMR) to a single new product within 45 min. ^{1}H NMR (500 MHz) monitoring of the reaction mixture revealed that myrmicarin 215B (**4**) was formed immediately upon introduction of TFA and persisted in rapidly diminishing quantities until complete conversion to the new compound had occurred. Attempts to isolate this product after proper work-up or by direct crystallization were not successful due to rapid decomposition. However, under strictly moisture- and oxygen-free atmosphere the reaction mixture could be stored at subambient temperatures for 24 h without significant decomposition. ^{13}C NMR (125 MHz) showed this compound to possess 30 chemically distinct carbons, the number expected for the desired dimerization product. Importantly, the same dimeric product could be obtained

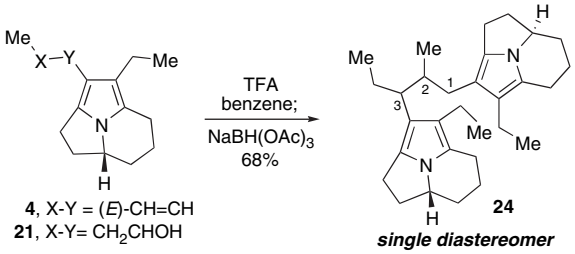
cleanly (≥90% by ^{1}H NMR) by direct treatment of a benzene-d_6 solution (0.05 M) of myrmicarin 215B (**4**) with TFA (1.10 equiv). ^{1}H NMR (500 MHz) monitoring of a benzene-d_6 solution (0.05 M) of myrmicarin 215B (**4**) with substoichiometric quantities of TFA revealed that the extent of the conversion to the dimeric product was approximately equal to the amount of Brönsted acid additive, suggesting an acid promoted dimerization. By comparison, monitoring a dilute benzene-d_6 solution (0.003 M) of myrmicarin 215B (**4**) exposed to a large excess of TFA (>100 equiv) gave less than 5% of the dimeric product. Instead we observed a mixture of pyrrole-ring protonated species over a period of several days.[13,14] A basic work-up returned the starting myrmicarin 215B (**4**). Under these conditions, the neutral myrmicarin 215B (**4**) required to serve as the nucleophile in the dimerization process was not present in sufficient concentration for the reaction to occur. Notably, the dimerization of myrmicarin 215B (**4**) proceeds at low concentrations (i.e., 0.005 M) in the presence of stoichiometric quantities of acid.

Significantly, subjection of myrmicarin 215A (**3**) to these dimerization conditions (benzene-d_6, 0.01 M, 23 °C, 5.5 h) provided a compound that was identical by ^{1}H NMR to the dimeric product obtained from myrmicarin 215B (**4**). While a trace amount of myrmicarin 215B (**4**) was observed immediately after treatment with TFA, the monomer persisting throughout the reaction was exclusively myrmicarin 215A (**3**), suggesting that the C8–C9 alkene isomerization was slower than the subsequent acid promoted dimerization of myrmicarin 215B (**4**). Interestingly, the major myrmicarin 215 isomer isolated from the poison gland secretion is myrmicarin 215A (**3**) and not myrmicarin 215B (**4**).[1b]

2.3. Synthesis and isolation of dimeric myrmicarins

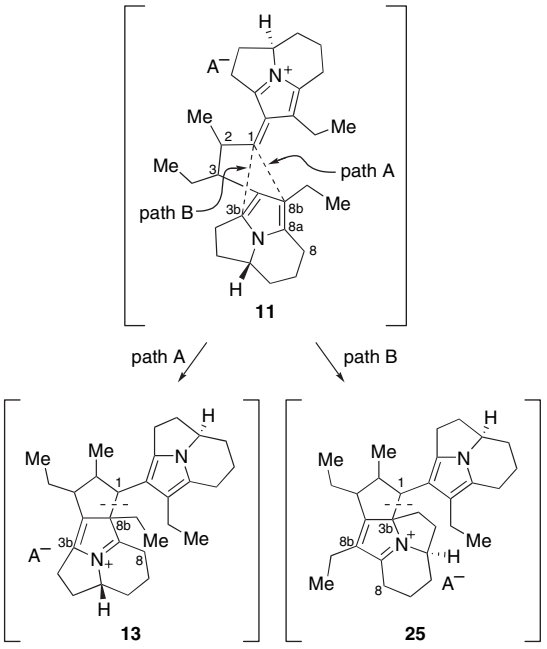
Due to the aforementioned instability of the acid promoted dimerization product of myrmicarin 215B (**4**) toward isolation, we attempted to assign its structure using a combination of gradient correlation (gCOSY) and heteronuclear single quantum correlation (HSQC) NMR experiments. Hence, we found the C_{30}-dimeric compound to possess four methyl, 13 methylene, and five methine units, as well as eight quaternary carbons. Compellingly, all of the resonances in the ^{1}H NMR spectrum occurred in the upfield region ($<\delta$ 3.57 ppm), attesting to the absence of the C8–C9 alkene present in the monomeric myrmicarins 215A (**3**) and 215B (**4**). Furthermore, the ^{1}H and ^{13}C NMR spectra contained one subset of closely matched tricyclic portion of myrmicarin 217 (**6**). Considering the intermediates in our proposed acid promoted dimerization of myrmicarin 215B (**4**), we speculated that the immediate dimeric product might be related to the iminium ion **13** (Scheme 3), a protonated tautomer of myrmicarin 430A (**1**). As the instability of the immediate dimerization product precluded an aqueous work-up, chromatographic purification, or crystallization, we examined reaction conditions that would provide a more stable, isolable derivative for thorough characterization.

Addition of hydrogen cyanide to a solution of the dimeric product to trap the putative iminium ion did not provide a stable derivative.[15] We reasoned that introduction of mild reducing agents would result in reduction of a reactive iminium ion or enamine functional group(s) that may be present in the dimerization product (i.e., C8a-iminium ion **13** or bis-enamine **14**, Scheme 3). Interestingly, sequential treatment of a benzene solution (0.02 M) of either alcohol **21** or myrmicarin 215B (**4**) with TFA (1.10 equiv) for 4 h at 23 °C followed by addition of sodium triacetoxyborohydride (6.50 equiv) in acetonitrile and mixing of the mixture (3.5:1, benzene–acetonitrile) for 3.5 h at 23 °C, provided a compound with sufficient stability to undergo aqueous work-up (saturated aqueous ammonium hydrogen chloride solution), isolation, and chromatographic purification on silica gel. Significantly, this oxygen-sensitive compound obtained in 66% isolated yield as a single diastereomer was consistent in all respects (HR-CIMS, ^{1}H, ^{13}C, gCOSY, HSQC) with the hexacyclic dimer **24** (Scheme 7). The isolation of the dimer **24** as the sole product suggests a highly diastereoselective dimerization of myrmicarin 215B (**4**) in the initial bond forming event. The C2- and C3-stereochemistry was later shown to be (2*S*,3*R*), as described below. The isolation of compound **24** is consistent with hydride reduction at C1 in either azafulvenium ion **11** (Scheme 3) or iminium ion **13** (Scheme 3).[16]



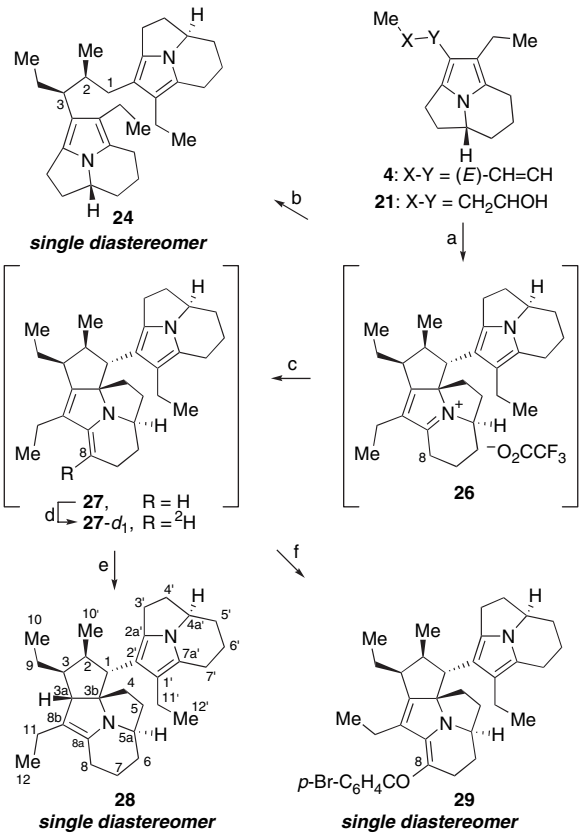
Scheme 7. Acid promoted dimerization of myrmicarin 215B (**4**) followed by immediate reduction to provide the first isolable dimeric product **24**. The C2- and C3-stereochemistry was later assigned; see below.

Although evidence for the first of the two carbon–carbon bond forming events in our proposed dimerization was compelling, we required a means for isolating a suitable derivative of the putative heptacyclic iminium ion to validate the formation of the second carbon–carbon bond, and to secure the presence of the fully substituted cyclopentane. The difficulties in the full structural characterization of the immediate TFA-promoted dimerization product (due to its limited longevity) not withstanding, in situ NMR correlation experiments of this compound provided valuable information. Importantly, while the assignment of all 30 signals in the ^{13}C NMR spectrum agreed reasonably well with the predicted values based on those of myrmicarin 430A (**1**), the resonance assigned to position C8b in the putative iminium ion **13** (Scheme 4) occurred at an anomalously high chemical shift (δ 96.1 ppm). As this value was higher than expected for a quaternary carbon, we systematically considered alternative structures that would be more consistent with the data for the putative iminium ion (Scheme 8). While the anticipated C1–C8b bond formation would result in the heptacyclic iminium ion **13** (Scheme 8, path A), an alternate bond formation between C1 and C3b would provide the isomeric heptacyclic iminium ion **25** (Scheme 8, path B).[17] In this scenario, the signal at δ 96.1 ppm would be due to the C3b in the iminium ion **25** (Scheme 8).



Scheme 8. Two possible modes of intramolecular azafulvenium ion trapping leading to isomeric heptacyclic iminium ions **13** and **25**.

The spectroscopic data that we had obtained for the heptacyclic iminium ion was more consistent with a C1–C3b second bond formation to yield **25** (Scheme 8). To firmly establish the connectivity of the heptacyclic dimerization product, we investigated possible derivatives for further spectroscopic analysis. We reasoned that C8-deprotonation of the putative intermediate **25** (Scheme 8) with a strong base would provide the corresponding enamine that may exhibit enhanced stability relative to the iminium ion **25**. Gratifyingly, we found that treatment of a benzene-d_6 solution of the dimeric iminium ion, prepared as described above, with excess resin-bound BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine, 10 equiv) under strictly oxygen-free conditions cleanly yielded a new C_{30}-product as a solution in benzene-d_6. Importantly, the ^{1}H NMR (500 MHz) of this species possessed a single resonance at δ 4.72 ppm (1H, dd), which was consistent with an expected C8-methine of enamine **27** (Scheme 9, for stereochemical assignment see Fig. 2). Addition of methanol-d_4 (15 equiv) to the solution of enamine **27** resulted in complete disappearance of the resonance at δ 4.72 ppm over a period of 1.5 h, consistent with the ^{2}H/H-exchange of an enamine. Unfortunately, the enamine **27** was unstable toward an aqueous work-up or attempted purification. While this presented challenges with regard to isolation or derivatization of enamine **27**, its sensitivity was not surprising based on the reported difficulties associated with the closely related myrmicarin 430A (**1**). However, an isolable derivative could be obtained by hydrogenation (1 atm of dihydrogen over 5% palladium on carbon) of the diene **27** in benzene, which cleanly afforded the enamine **28**.[18] Thus without the need for isolation of sensitive intermediates, alcohol **21** was converted to enamine **28** in a single operation (87%).[19] Enamine **28** was sufficiently stable toward isolation in neat form but required storage strictly under an inert atmosphere to avoid oxidative decomposition.



Scheme 9. TFA promoted dimerization of myrmicarin 215B (**4**) and related derivatives. For stereochemical assignment please see below. (a) TFA, benzene-d_6, 23 °C, 3 h, 90% (by ^{1}H NMR). (b) NaBH(OAc)$_3$, MeCN-d_3, benzene-d_6, 23 °C, 3.5 h, 68% from **21**. (c) resin bound-BEMP, benzene-d_6, 23 °C, 30 min. (d) methanol-d_4 (~15 equiv), benzene-d_6, 23 °C, 1.5 h, complete C8 deuterium incorporation. (e) Pd–C, H_2, 87% from **21**. (f) *p*-BrPhCOCl, 30 min; ^{i}Pr$_2$NH, 67% from **21**.

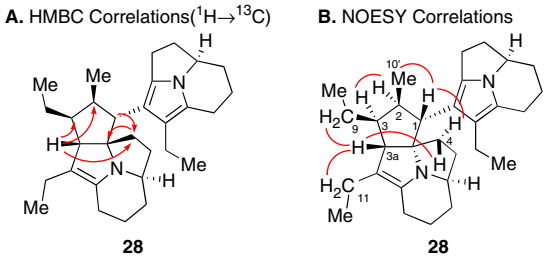


Figure 2. Key HMBC (^{1}H→^{13}C) and NOESY correlations for the enamine **28**.

Similar to the previously isolated hexacycle **24** (Scheme 7), the ^{1}H and ^{13}C NMR spectra of both diene **27** and enamine **28** contained one set of resonances that closely resembled those of the tricyclic core of myrmicarin 217 (**6**). Furthermore, the ^{13}C and ^{1}H–^{13}C HSQC NMR spectra of each compound showed the correct number of methine, methylene, methyl, and quaternary carbon units for structures **27** and **28** (Scheme 9). The presence of distinct C4–C8 and C1–C3 spin systems was recognized in the gCOSY spectra of both diene **27** and enamine **28**. However, this data alone was not sufficient to distinguish between the two modes of cyclization (path A vs path B, Scheme 8) in the formation

of these heptacyclic products. Additional data obtained using heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) NMR (600 MHz) experiments allowed structural verification of products **27**–**29** (Scheme 9). The HMBC correlations (Fig. 2A) in the spectrum of the enamine **28** were entirely consistent with the heptacyclic structure depicted in Scheme 9 (path B, Scheme 8). Correlations between C1–H/C4–H_c, C3a–H/C4–H_t, and C3a–H/C11–H in the NOESY spectrum of enamine **28** validated this structural assignment. Additionally, NOESY correlations between C1–H/C10′–H, C2–H/C3–H, and C3a/C9 provided the relative stereochemistry about the newly formed fully substituted cyclopentane ring (Fig. 2B).[20]

We also sought derivatives that would be amenable to X-ray crystallographic analysis. Unfortunately, numerous attempts to crystallize the enamine **28** were unsuccessful. Even under strictly inert conditions extensive decomposition was observed within two days. Similar complications were present during manipulations of the corresponding protonated salts of enamine **28** formed upon treatment of **28** with a variety of Brönsted acids. In attempts to obtain derivatives with greater stability, we found that treatment of a benzene solution of the enamine **27** (Scheme 9) with benzoyl chlorides in the presence of excess diisopropylethylamine cleanly provided the corresponding C8-benzoylated products, many of which could be purified by silica gel chromatography. For example, in a single operation, the C8-*p*-bromobenzoylated product **29** was obtained in 67% yield starting with the alcohol **21** without isolation of iminium ion **26** or diene **27**. Although the C8-*p*-bromobenzoylated and C8-*p*-iodobenzoylated products were found to be storable in the absence of oxygen, crystallization and co-crystallization attempts did not provide samples suitable for single crystal X-ray analysis. However, 2D-NMR analysis of these samples provided additional data that paralleled our earlier results with the enamine **28** (Fig. 2). Specifically, the data obtained using the isolable *p*-bromobenzoylated product **29** (gCOSY, HSQC, HMBC, and NOESY) provided further support for the structural assignment of compounds **26**–**29**. The signals at 96.1, 86.4, 83.6, and 86.9 ppm in the ^{13}C NMR spectra of **26**–**29**, respectively, were consistent with the C3b-tertiary amine. Since the hexacyclic dimer **24** is obtained by treatment of the iminium ion intermediate **26** with sodium triacetoxyborohydride, the C2- and C3-stereochemistry of **24** is assignment based on the relative stereochemistry found in the heptacyclic compounds **26**–**29** (Table 1).

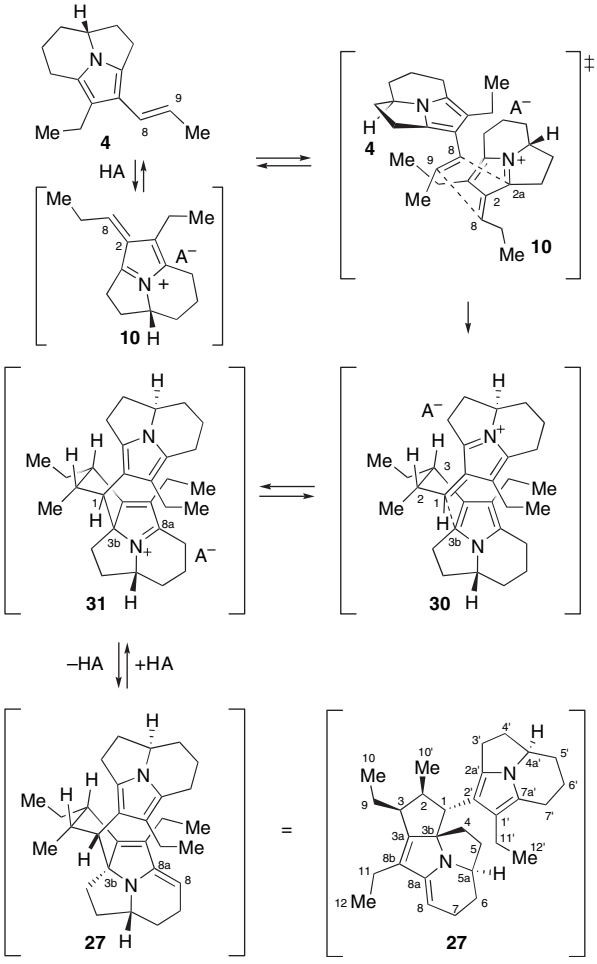
A possible mechanism for the dimerization of myrmicarin 215B (**4**) to enamine **27** is presented in Scheme 10. The overall process may involve a stepwise C9–C8 bond formation to give **30** followed by C3b–C1 bond formation to provide the iminium ion **31**.[21] The relative stereochemistry of the newly

Table 1. ^1H and ^{13}C NMR data in parts per million for myrmicarin 430A[a] (**1**), iminium salt **26**, heptacyclic diene **27**, heptacyclic enamine **28**, and bromophenylketone **29** in benzene-d_6[b]

Position	Myrmicarin 430A (**1**)		Iminium salt **26**[c]		Heptacyclic diene **27**[c]		Heptacyclic enamine **28**[c,d]		Bromophenylketone **29**[c,e]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	55.3	3.21	45.9	2.54	49.4	2.60	51.9	2.85	48.5	2.58
2	43.4	1.97	51.7	2.29[d]	50.3	2.26	40.1	2.68	49.4	2.20
3	54.7	1.66	45.2	2.35	44.8	2.48	50.1	1.72	45.1	2.48
3a	62.1	—	181.0	—	152.3	—	62.0	—	162.6	—
3b	145.8	—	96.1	—	86.4	—	83.6	2.92	86.9	—
4	88.3	4.38	38.0	1.47, 1.54	41.3	1.75, 1.85	38.2	1.90[t], 2.14[c]	41.0	1.61[t], 1.65[c]
5	41.4	2.65[t], 3.10[c]	36.7	1.68, 2.31	36.3	1.68, 2.16	28.0	1.06[c], 1.56[t]	36.7	1.52[c], 2.15[t]
5a	55.1	3.94	55.7	2.73	54.8	3.00	58.8	2.65	54.6	2.80
6	28.1	1.30[t], 1.42[c]	27.0	0.88, 1.35	30.6	1.09, 1.71	27.7	1.51[t], 1.81[c]	30.4	0.82[t], 1.43[c]
7	22.3	1.93[t], 2.16[c]	18.6	1.22, 1.81	22.7	2.15, 2.21	20.6	1.24[t], 1.42[c]	28.8	2.24[c], 2.38[t]
8	90.1	4.39	26.4	2.77, 2.82	88.0	4.72	24.9	1.90[c], 2.38[t]	104.2	—
8a	48.7	2.54	187.1	—	132.9	—	140.5	—	162.6	—
8b	154.1	—	137.2	—	132.7	—	114.8	—	136.6	—
9	27.5	1.35, 1.85	21.0	1.08, 1.29	20.8	1.31, 1.50	22.9	1.25, 1.67	20.8	1.23, 1.44
10	12.8	1.16	13.3	0.81	13.9	1.00	13.6	1.07	13.6	0.93
10′	17.1	0.98	16.7	0.74	16.3	1.04	14.7	1.03	16.3	0.95
11	26.7	1.47, 1.73	18.3	2.24 (2H)	19.4	2.26, 2.34	19.9	2.05, 2.26	20.9	2.25, 3.16
12	17.1	0.99	14.0	1.06	14.8	1.24	14.9	1.14	14.8	1.19
1′	123.6	—	121.7	—	121.9	—	123.2	—	122.0	—
2′	111.7	—	111.3	—	114.2	—	111.2	—	113.6	—
2a′	127.6	—	127.1	—	128	—	128	—	128	—
3′	27.7	2.56[t], 2.69[c]	27.0	2.05, 2.29	27.9	2.70, 3.00	28.6	2.87[t], 3.10	27.5	2.65[t], 2.78[c]
4′	37.1	1.67[t], 2.07[c]	37.7	1.60, 2.34	37.3	1.65, 2.08	37.5	1.70[t], 2.17[c]	37.3	1.63[t], 2.13[c]
4a′	54.8	3.38	55.6	3.57	55.5	3.46	55.5	3.54	55.6	3.48
5′	30.0	0.90[t], 1.57[c]	29.7	0.77, 1.64	30.2	0.88, 1.53	30.5	0.96[t], 1.63[c]	30.1	0.89[t], 1.56[c]
6′	22.9	1.41[c], 1.70[t]	22.7	1.45, 1.68	23.3	1.39, 1.69[t]	23.3	1.41[c], 1.71[t]	23.2	1.38[c], 1.69[t]
7′	21.0	2.46[t], 2.69[c]	20.9	2.29, 2.54	21.3	2.50, 2.68	21.3	2.47[t], 2.64[c]	21.2	2.44[t], 2.62[c]
7a′	118.1	—	119.4	—	116.7	—	117.4	—	117.5	—
11′	18.8	2.68, 2.93	18.6	2.39, 2.45	19.2	2.67	19.3	2.66, 2.68	19.0	2.59
12′	16.6	1.32	17.1	1.19	17.6	1.38	17.3	1.34	17.4	1.32

[a] From Ref. 1c.
[b] The superscripts ‘c’ and ‘t’ refer to the protons cis and trans, respectively, to the C4a′ or C5a methine in each spin system.
[c] Signal assignments were made through analyses of ^1H NMR, ^{13}C NMR, gCOSY, and HSQC spectra.
[d] Signal assignments were made through analyses of HMBC and NOESY spectra.
[e] Signal assignments were made through analysis of HMBC and ROESY spectra.

formed cyclopentane ring that is shared in compounds **26**–**29** suggests a convex face to convex face approach (i.e., **30**, Scheme 10). Under the reaction conditions described above, π-stacking interactions between the putative electron deficient azafulvenium ion **10** and the approaching electron rich vinyl pyrroloindolizine (**4**), or the positioning of the counter ion (A^-, Scheme 10) with respect to the dimerization precursors may be responsible for the observed stereoselectivity. Hence, ongoing efforts are directed at modifying the reaction conditions and identifying an appropriate counter ion (i.e., formate) that may influence the mode of dimerization (Scheme 8). While the involvement of any biosynthetic machinery in the dimerization of C15 myrmicarin monomers to the more complex myrmicarins is unknown at this time, the observed high level of diastereoselection and efficiency in our acid promoted dimerization of myrmicarin 215B (**4**) highlights the possible direct dimerization of a pyrroloindolizine (i.e., **4**) as the first step toward C30 and C45 derivatives.



Scheme 10. The proposed mechanism for the diastereoselective dimerization of (+)-myrmicarin 215B (**4**) to an isomer of myrmicarin 430A (**1**), enamine **27** (isomyrmicarin 430A).

3. Conclusions

TFA-promoted dimerization of (+)-myrmicarin 215B (**4**) leads to a sequence of highly efficient and stereoselective carbon–carbon bond forming events, providing the heptacyclic dimeric enamine **27** (Scheme 9). A possible mechanism for the diastereoselective dimerization of myrmicarin 215B (**4**) to this isomer of myrmicarin 430A (**1**), isomyrmicarin 430A (**27**), is presented (Scheme 10). The isolation of a single diastereomer of the heptacyclic products via the TFA-promoted dimerization of myrmicarin 215 is noteworthy. These observations provide experimental data relevant to our proposed vinyl pyrroloindolizine dimerization strategy for the synthesis of the heptacyclic portion of complex myrmicarin alkaloids. Current efforts are directed at controlling the mode of dimerization (Scheme 8) for implementation of this strategy toward these highly sensitive compounds: a strategy with potential implications regarding the biogenesis of these structurally fascinating alkaloids.

4. Experimental

4.1. General procedures

Reactions were performed in oven-dried or flame-dried round bottomed flasks or modified Schlenk (Kjeldahl shape) flasks. The flasks were fitted with rubber septa and reactions were conducted under a positive pressure of argon. NMR experiments were performed in vacuum-dried Wilmad Glass Co., Inc. 528-PP NMR tubes. Stainless steel syringes or cannulae were used to transfer air- and moisture-sensitive liquids. Flash column chromatography was performed as described by Still[22] using silica gel (60-Å pore size, 32–63 μm, standard grade, Sorbent Technologies) or non-activated alumina gel (80–325 mesh, chromatographic grade, EM Science). Analytical thin layer chromatography was performed using glass plates pre-coated with 0.25 mm 230–400 mesh silica gel or neutral alumina gel impregnated with a fluorescent indicator (254 nm). Thin layer chromatography plates were visualized by exposure to ultraviolet light and/or by exposure to an ethanolic phosphomolybdic acid (PMA), an acidic solution of *p*-anisaldehyde (anis), an aqueous solution of ceric ammonium molybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$) or an ethanolic solution of ninhydrin followed by heating (<1 min) on a hot plate (~250 °C). Organic solutions were concentrated on Büchi R-200 rotary evaporators at ~20 Torr (house vacuum) at 25–35 °C, then at ~1 Torr (vacuum pump) unless otherwise indicated. Commercial reagents and solvents were used as received with the following exceptions: dichloromethane, diethyl ether, tetrahydrofuran, acetonitrile, and toluene were purchased from J.T. Baker (Cycletainer™) and were purified by the method of Grubbs et al. under positive argon pressure.[23] Proton nuclear magnetic resonance (^1H NMR) spectra were recorded with a Varian inverse probe 500 INOVA spectrometer or a Bruker inverse probe 600 Avance spectrometer. Chemical shifts are recorded in parts per million from internal tetramethylsilane on the δ scale and are referenced from the residual protium in the NMR solvent (C_6H_6: δ 7.16). Data are reported as follow: chemical shift [multiplicity (s = singlet, d = doublet, q = quartet, m = multiplet), coupling constant(s) in Hertz, integration, assignment]. Carbon-13 nuclear magnetic resonance spectra were recorded with a Varian 500 INOVA spectrometer and are recorded in parts per million from internal tetramethylsilane on the δ scale and are referenced from the carbon resonances of the solvent

(benzene-d_6: δ 128.0). We thank Dr. Li Li for obtaining HRMS data at the Department of Chemistry Instrumentation Facility (MIT-DCIF). Infrared data were obtained with a Perkin–Elmer 2000 FTIR and are reported as follow: [frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad), assignment].

4.1.1. Tricyclic alcohol 21. Solid lithium aluminum hydride (11.6 mg, 306 μmol, 6.00 equiv) was added in a single portion to a solution of tricyclic ketone **20** (11.8 mg, 51.0 μmol, 1 equiv) in Et_2O (950 μL) at −78 °C followed by immediate placement of the reaction flask on an ice-water bath. After 40 min, the vigorously stirred gray suspension was cooled to −78 °C and excess hydride was quenched by the slow addition of water (1.20 mL) via syringe. The cold bath was immediately removed and the mixture allowed to warm to 23 °C. The pale gray suspension was diluted sequentially with a 6-mL portion of Et_2O and a saturated aqueous solution of Rochelle salt (6 mL), and the two-phase mixture was vigorously stirred. After 3 h, the resulting slightly opaque aqueous layer was separated from the clear and colorless organic layer and was extracted with EtOAc (3×5 mL). The combined organic layers were washed with a 5-mL portion of brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give exclusively the alcohol **21** as a colorless oil (10.9 mg, 100%). ^{1}H NMR analysis revealed the product to be a mixture of C8-epimers (1:1). The alcohol **21** was found to be exceedingly sensitive toward dehydration and decomposition upon treatment with silica or alumina gel and required storage under an argon atmosphere. ^{1}H NMR (500 MHz, C_6D_6, 23 °C, 3:2 mixture of epimers, major epimer denoted by *), δ: 4.71 (dd, 1H, J=7.6, 5.8 Hz, C8–H*), 4.70 (dd, 1H, J=7.2, 6.3 Hz, C8–H), 3.19–3.27 (m, 2H, C4a–H*, C4a–H), 2.74 (dd, 1H, J=14.6, 7.9 Hz, C7–H*), 2.53–2.70 (m, 9H, C3–H*, C3–H, C3–H*, C3–H, C11–H*, C11–H, C11–H′*, C11–H′, C7–H), 2.31–2.41 (m, 2H, C7–H*, C7–H), 2.00–2.09 (m, 2H, C4–H*, C4–H), 1.90–1.99 (m, 4H, C9–H*, C9–H, C9–H′*, C9–H′), 1.65–1.72 (m, 2H, C6–H, C6-H), 1.50–1.60 (m, 4H, C4–H*, C4–H, C5–H*, C5–H), 1.31 (t, 3H, J=7.5 Hz, C12–H*), 1.28 (t, 3H, J=7.6 Hz, C12–H), 1.12 (t, 3H, J=7.3 Hz, C10–H), 1.11 (t, 3H, J=7.3 Hz, C10–H*), 0.79–0.90 (m, 2H, C5–H*, C5–H). ^{13}C NMR (500 MHz, C_6D_6, 23 °C, 3:2 mixture of epimers, major epimer denoted by *), δ: 127.7*, 127.6, 121.6*, 121.6, 118.9*, 118.5, 118.3*, 118.3, 70.0*, 69.7, 55.3*, 55.2, 37.2*, 37.4, 31.5*, 32.4, 30.2*, 30.2, 26.3*, 26.3, 23.2*, 23.1, 20.9*, 20.9, 19.2*, 19.1, 17.2*, 17.3, 11.8, 11.7*. FTIR (neat), cm^{-1}: 3417 (br s, O–H), 2597 (s, C–H), 2854 (s, C–H), 1454, 1320, 1044. HRMS (ESI): m/z calcd for $C_{15}H_{23}NONa$ $[M+Na]^+$: 256.1672; Found: 256.1677. TLC (alumina gel, 30% EtOAc–hexanes), R_f: 0.26, 0.30 (UV, $KMnO_4$).

4.1.2. Hexacyclic dimer 24. A sample of alcohol **21** (3.4 mg, 14.6 μmol, 1 equiv) was dried by concentration from anhydrous benzene-d_6 (3×350 μL). The residue was dissolved in benzene-d_6 (650 μL) and was purged by a stream of argon for 3 min. A solution of TFA (1.2 μL, 16.0 μmol, 1.10 equiv) in benzene-d_6 (51.2 μL) was added drop-wise via syringe. The resulting solution became intense yellow immediately upon addition of TFA and faded to a tan color within 2 min. The mixture was mixed and maintained under an argon atmosphere for 4.5 h. A suspension of sodium triacetoxyborohydride (20.1 mg, 94.9 μmol, 6.50 equiv) in acetonitrile-d_3 (200 μL) was then added via syringe and the resulting pale burgundy suspension was stirred under an inert atmosphere. After 3.5 h, the suspension was diluted with EtOAc (7.5 mL) and the resulting mixture was washed with saturated aqueous ammonium chloride solution (4 mL). The clear, colorless aqueous layer was separated and extracted with ethyl acetate (3×3 mL) and the combined pale yellow organic solution was washed with saturated aqueous sodium bicarbonate (3 mL), and brine (3 mL). The organic layer was dried over anhydrous sodium sulfate, and then concentrated under reduced pressure to give a deep brown residue. Purification of the residue by silica gel column chromatography (5% EtOAc–hexanes; diameter 0.5 cm, height 4.5 cm) gave the hexacyclic dimer **24** as a yellow oil (2.1 mg, 68%).[24] ^{1}H NMR (500 MHz, C_6D_6, 23 °C), δ: 3.36 (m, 2H, C4a′–H, C5a′–H), 3.15 (dd, 1H, J=13.4, 2.7 Hz, C1–H), 2.80 (m, 1H, C3–H), 2.79 (m, 1H, C3′–H/C4–H), 2.63–2.73 (m, 9H, C3′–H/C4–H, C11–H, C11′–H, C3′–H/C4–H, C7′–H, C8–H, C3′–H/C4–H), 2.46 (m, 2H, C7′–H, C8–H), 2.35 (dd, 1H, J=13.4, 10.8 Hz, C1–H′), 2.20 (m, 1H, C2–H), 2.06 (m, 1H, C4′–H/C5–H), 2.01 (m, 1H, C4′–H/C5–H), 2.01 (m, 1H, C9–H), 1.93 (m, 1H, C9–H′), 1.71 (m, 2H, C6′–H/C7–H), 1.67 (m, 1H, C4′–H/C5–H), 1.59 (m, 1H, C4′–H/C5–H), 1.57 (m, 2H, C5′–H, C6–H), 1.46 (t, 3H, J=7.5 Hz, C12–H/C12′–H), 1.43 (t, 3H, J=7.5 Hz, C12–H/C12′–H), 1.42 (m, 1H, C6′–H/C7–H), 1.35 (m, 1H, C6′–H/C7–H), 1.26 (d, 3H, J=6.7 Hz, C10′–H), 1.11 (t, 3H, J=7.3 Hz, C10–H), 0.91 (m, 1H, C5′–H/C6–H), 0.88 (m, 1H, C5′–H/C6–H). ^{13}C NMR (125.8 MHz, C_6D_6, 23 °C), δ: 128 (C2a′/C3b), 126.8 (C2a′/C3b), 122.7 (C1′/C8b), 121.8 (C1′/C8b), 118.6 (C7a′/C8a), 118.3 (C7a′/C8a), 116.5 (C2′/C3a), 114.1 (C2′/C3a), 55.4 (C4a′/C5a), 55.2 (C4a′/C5a), 44.9 (C3), 42.1 (C2), 37.6 (C4′/C5), 31.3 (C1), 30.6 (C5′/C6), 30.3 (C5′/C6), 27.4 (C3′/C4), 25.6 (C3′/C4), 25.2 (C9), 23.3 (C6′/C7), 23.2 (C6′/C7), 21.3 (C7′/C8), 21.2 (C7′/C8), 19.3 (C11/C11′), 19.3 (C11/C11′), 18.2 (C10′), 17.0 (C12, $C12_2'$), 13.8 (C10). FTIR (neat), cm^{-1}: 2928 (s, C–H), 2852 (s, C–H), 1737, 1688, 1458, 1321, 1261, 1197. HRMS (ESI): m/z calcd for $C_{30}H_{44}N_2$ $[M+H]^+$: 433.3577; Found: 433.3441. TLC (silica gel, 10% EtOAc–hexanes), R_f: 0.53 (UV, anis).

4.1.3. Iminium salt 26. A sample of myrmicarin 215B (**4**, 5.5 mg, 25.6 μmol, 1 equiv) was dried by concentration from anhydrous benzene (3×350 μL). The residue was dissolved in benzene-d_6 (600 μL) in an NMR tube fitted with a rubber septum and was purged by a gentle stream of argon for 3 min. A solution of TFA (2.2 μL, 29.4 μmol, 1.15 equiv) in benzene-d_6 (52.7 μL) was added drop-wise via syringe. The resulting reaction mixture became intense yellow immediately upon addition of the TFA solution and gradually turned brown over 30 min. The sample was mixed and maintained under an argon atmosphere for 4.5 h at ambient temperature. ^{1}H NMR analysis revealed complete conversion of myrmicarin 215B (**4**) to the highly air-sensitive iminium salt **26**. This compound was found to be unstable towards isolation. The same dimerization product **26** was obtained starting with the alcohol **21** in place of myrmicarin 215B (**4**) following a similar protocol. ^{1}H NMR (500 MHz, C_6D_6, 23 °C), δ: 3.57 (tdd, 1H, J=10.5, 4.9, 3.7 Hz, C4a′–H), 2.82 (m, 1H, C8–H), 2.77 (m, 1H, C8–H′), 2.73 (m, 1H,

C5a–H), 2.54 (m, 1H, C7′–H), 2.54 (d, 1H, J=9.8 Hz, C1–H), 2.45 (m, 1H, C11′–H), 2.39 (m, 1H, C11′–H′), 2.35 (m, 1H, C3–H), 2.34 (m, 1H, C4′–H), 2.31 (m, 1H, C5–H), 2.29 (m, 1H, C7′–H), 2.29 (m, 1H, C2–H), 2.29 (m, 1H, C3′–H), 2.24 (q, 2H, J=7.6 Hz, C11–H), 2.05 (tdd, 1H, J=14.3, 10.7, 6.3 Hz, C3′–H), 1.81 (m, 1H, C7–H), 1.68 (m, 1H, C6′–H), 1.68 (m, 1H, C5–H), 1.64 (m, 1H, C5′–H), 1.60 (m, 1H, C4′–H), 1.54 (m, 1H, C4–H), 1.47 (m, 1H, C4–H′), 1.45 (m, 1H, C6′–H), 1.35 (m, 1H, C6–H), 1.29 (m, 1H, C9–H), 1.22 (m, 1H, C7–H′), 1.19 (t, 3H, J=7.5 Hz, C12′–H), 1.08 (m, 1H, C9–H′), 1.06 (t, 3H, J=7.6 Hz, C12–H), 0.88 (m, 1H, C6–H′), 0.81 (t, 3H, J=7.5 Hz, C10–H), 0.77 (m, 1H, C5′–H), 0.74 (d, 3H, J=6.7 Hz, C10′–H). ^{13}C NMR (125.8 MHz, C_6D_6, 23 °C), δ: 187.1 (C8a), 181.0 (C3a), 137.2 (C8b), 127.1 (C2a′), 121.7 (C1′), 119.4 (C7a′), 111.3 (C2′), 96.1 (C3b), 55.7 (C5a), 55.6 (C4a′), 51.7 (C2), 45.9 (C1), 45.2 (C3), 38.0 (C4), 37.7 (C4′), 36.7 (C5), 29.7 (C5′), 27.0 (C6), 27.0 (C3′), 26.4 (C8), 22.7 (C6′), 21.0 (C9), 20.9 (C7′), 18.6 (C7), 18.6 (C11′), 18.3 (C11), 17.1 (C12′), 16.7 (C10′), 14.0 (C12), 13.3 (C10).

4.1.4. Heptacyclic diene 27. A sample of alcohol **21** (5.1 mg, 21.9 μmol, 1 equiv) was dried by concentration from anhydrous benzene (3×350 μL). The residue was dissolved in benzene-d_6 (600 μL) in an NMR tube fitted with a rubber septum and was purged by a gentle stream of argon for 3 min. A solution of TFA (1.8 μL, 24.1 μmol, 1.10 equiv) in benzene-d_6 (52.1 μL) was added drop-wise via syringe. The resulting reaction mixture became intense yellow immediately upon addition of the TFA and faded to a tan color within 2 min. The sample was mixed and maintained under an argon atmosphere for 4.5 h at ambient temperature. Monitoring of the reaction mixture by ^{1}H NMR revealed complete consumption of alcohol **21** and myrmicarin 215B (**4**, generated in situ). The contents of the sample were transferred to a recovery flask under an inert atmosphere (glove-box, nitrogen atmosphere) and the transfer completed with three 50-μL benzene-d_6 rinses. A single portion of resin-bound BEMP (99.0 mg, 2.2 μmol/mg on 200–400 mesh polystyrene resin, 10.0 equiv) was added to the solution of the iminium salt **26** and the resulting pale yellow suspension was stirred for 30 min under strictly inert conditions. The suspension was filtered through a cotton plug, the filter-cake was rinsed with benzene-d_6 (3×150 μL), and the pale yellow solution was sealed in a recovery flask under a nitrogen atmosphere, placed on a vacuum manifold and concentrated to ~350 μL. The transfer of this solution via cannula into a sealed, argon-purged NMR tube and using two additional portions of benzene-d_6 (150 μL) to complete the transfer, provided a clear, faintly yellow solution of the exceedingly air-sensitive diene **27** suitable for ^{1}H NMR and ^{13}C NMR analysis. ^{1}H NMR (500 MHz, C_6D_6, 23 °C), δ: 4.72 (dd, 1H, J=7.2, 3.2 Hz, C8–H), 3.46 (tdd, 1H, J=10.9, 5.4, 3.8 Hz, C4a′–H), 3.00 (m, 1H, C3′–H), 3.00 (m, 1H, C5a–H), 2.70 (dd, 1H, J=15.4, 7.5 Hz, C3′–H), 2.68 (m, 1H, C7′–H), 2.67 (q, 2H, J=7.6 Hz, C11′–H), 2.60 (d, 1H, J=10.7 Hz, C1–H), 2.50 (ddd, 1H, J=16.0, 11.8, 6.3 Hz, C7′–H), 2.48 (m, 1H, C3–H), 2.34 (dq, 1H, J=13.8, 7.5 Hz, C11–H), 2.26 (dq, 1H, J=13.8, 7.5 Hz, C11–H′), 2.26 (m, 1H, C2–H), 2.21 (m, 1H, C7–H), 2.16 (m, 1H, C5–H), 2.15 (m, 1H, C7–H′), 2.08 (dt, 1H, J=11.5, 5.8 Hz, C4′–H), 1.85 (td, 1H, J=11.5, 7.8 Hz, C4–H), 1.75 (m, 1H, C4–H′), 1.71 (m, 1H, C6–H), 1.69 (m, 1H, C6′–H), 1.68 (m, 1H, C5–H′), 1.65 (m, 1H, C4′–H), 1.53 (m, 1H, C5′–H), 1.50 (m, 1H, C9–H), 1.39 (m, 1H, C6′–H), 1.38 (t, 3H, J=7.6 Hz, C12′–H), 1.31 (m, 1H, C9–H′), 1.24 (t, 3H, J=7.5 Hz, C12–H), 1.09 (tdd, 1H, J=11.9, 4.9, 1.4 Hz, C6–H′), 1.04 (d, 3H, J=6.7 Hz, C10′–H), 1.00 (t, 3H, J=7.3 Hz, C10–H), 0.88 (tdd, 1H, J=12.8, 10.9, 2.0 Hz, C5′–H). ^{13}C NMR (125.8 MHz, C_6D_6, 23 °C), δ: 152.9 (C8a), 152.3 (C3a), 132.7 (C8b), 128 (C2a′), 121.9 (C1′), 116.7 (C7a′), 114.2 (C2′), 88.0 (C8), 86.4 (C3b), 55.5 (C4a′), 54.8 (C5a), 50.3 (C2), 49.4 (C1), 44.8 (C3), 41.3 (C4), 37.3 (C4′), 36.2 (C5), 30.6 (C6), 30.2 (C5′), 27.9 (C3′), 23.3 (C6′), 22.7 (C7), 21.3 (C7′), 20.8 (C9), 19.4 (C11), 19.2 (C11′), 17.6 (C12′), 16.3 (C10′), 14.8 (C12), 13.9 (C10).

4.1.5. Heptacyclic enamine 28. A sample of alcohol **21** (10.0 mg, 42.9 μmol, 1 equiv) was dried by concentration from anhydrous benzene (3×500 μL). The residue was dissolved in benzene (1.06 mL) and was purged by a stream of argon for 3 min. A solution of TFA (3.5 μL, 47.2 μmol, 1.10 equiv) in benzene (23.5 μL) was added drop-wise via syringe. The resulting solution became intense yellow immediately upon addition of TFA and faded to a clear tan color within 2 min. The mixture was mixed and maintained under an argon atmosphere for 4.5 h. The contents of the sample were transferred to a recovery flask under an inert atmosphere (glove-box, nitrogen atmosphere) and the transfer completed with three 50-μL benzene-d_6 rinses. A single portion of resin-bound BEMP (195 mg, 2.2 μmol/mg on 200–400 mesh polystyrene resin, 10.0 equiv) was added to the solution of the iminium ion salt **26** and the resulting pale yellow suspension was stirred for 30 min under strictly inert conditions. The suspension was filtered through a cotton plug and the filter-cake was rinsed with benzene (3×450 μL) to give a solution of the diene **27**. Palladium on activated carbon (20.0 mg, 5%-Pd–C) was added and the flask was sealed under nitrogen and removed from the glove-box. The reaction vessel was flushed with dihydrogen (~1 atm) for 5 min and then maintained under a balloon-pressure of dihydrogen for an additional 30 min at ambient temperature. Dilution of the black suspension with EtOAc (2 mL), and filtration of the mixture through a plug of Celite (diam 0.6 cm, ht. 4.0 cm), followed by an EtOAc rinse (15 mL), yielded a clear yellow solution of the desired enamine **28**. Removal of the volatiles under reduced pressure gave the pure enamine **28** (8.1 mg, 87%) as a clear yellow oil with marginal stability. This enamine was sufficiently stable toward isolation in neat form but required storage strictly under an inert atmosphere to avoid oxidative decomposition; spectroscopic characterization was conducted shortly after isolation. ^{1}H NMR (500 MHz, C_6D_6, 23 °C), δ: 3.54 (tdd, 1H, J=10.7, 5.5, 3.6 Hz, C4a′–H), 3.10 (ddd, 1H, J=15.3, 10.9, 5.9 Hz, C3′–H_c), 2.92 (s, 1H, C3a–H), 2.87 (dd, 1H, J=15.3, 7.6 Hz, C3′–H_t), 2.85 (d, 1H, J=12.8 Hz, C1–H), 2.68 (m, 1H, C2–H), 2.68 (m, 1H, C11′–H), 2.66 (m, 1H, C11′–H), 2.65 (m, 1H, C5a–H), 2.64 (m, 1H, C7′–H_c), 2.47 (ddd, 1H, J=15.9, 11.8, 6.4 Hz, C7′–H_t), 2.38 (dt, 1H, J=14.4, 4.2 Hz, C8–H_t), 2.26 (dq, 1H, J=14.3, 7.3 Hz, C11–H), 2.17 (dt, 1H, J=10.7, 5.7 Hz, C4′–H_c), 2.14 (td, 1H, J=11.9, 7.6 Hz, C4–H_c), 2.05 (dq, 1H, J=14.3, 7.3 Hz, C11–H′), 1.90 (m, 1H, C4–H_t), 1.90 (m, 1H, C8–H_c), 1.81 (tdd, 1H, J=13.6, 5.5, 4.7 Hz, C6–H_c),

1.72 (m, 1H, C3–H), 1.71 (m, 1H, C6′–H_t), 1.70 (m, 1H, C4′–H_t), 1.67 (m, 1H, C9–H), 1.63 (m, 1H, C5′–H_c), 1.56 (td, J=11.7, 3.6 Hz, 1H, C5–H_t), 1.51 (dddd, J=13.5, 5.2, 3.2, 1.9, 1H, C6–H_t), 1.42 (m, 1H, C7–H_c), 1.41 (m, 1H, C6′–H_c), 1.34 (t, 3H, J=7.5 Hz, C12′–H), 1.25 (m, 1H, C9–H′), 1.24 (m, 1H, C7–H_t), 1.14 (t, 3H, J=7.3 Hz, C12–H), 1.07 (t, 3H, J=7.3 Hz, C10–H), 1.06 (m, 1H, C5–H_c), 1.03 (d, 1H, J=7.0 Hz, C10′–H), 0.96 (tdd, 1H, J=12.8, 10.7, 2.4 Hz, C5′–H_t). ^{13}C NMR (125.8 MHz, C_6D_6, 23 °C), δ: 140.5 (C8a), 128 (C2a′), 123.2 (C1′), 117.4 (C7a′), 114.8 (C8b), 111.2 (C2′), 83.6 (C3b), 62.0 (C3a), 58.8 (C5a), 55.5 (C4a′), 51.9 (C1), 50.1 (C3), 40.1 (C2), 38.2 (C4), 37.5 (C4′), 30.5 (C5′), 28.6 (C3′), 28.0 (C5), 27.7 (C6), 24.9 (C8), 23.3 (C6′), 22.9 (C9), 21.3 (C7′), 20.6 (C7), 19.9 (C11), 19.3 (C11′), 17.3 (C12′), 14.9 (C12), 14.7 (C10′), 13.6 (C10). FTIR (neat), cm^{-1}: 2955 (s, C–H), 1680, 1455, 1376, 1321, 1166. HRMS (ESI): m/z calcd for $C_{30}H_{44}N_2$ [M+H]$^+$: 433.3577; Found: 433.3566. TLC (silica gel pre-treated with Et_3N, 1.5% Et_3N, 2.5% EtOAc–hexanes), R_f: 0.27 (UV, anis).

4.1.6. Bromophenylketone 29. A sample of alcohol **21** (9.6 mg, 41.2 μmol, 1 equiv) was dried by concentration from anhydrous benzene (3×500 μL). The residue was dissolved in benzene (1.0 mL) and was purged by a stream of argon for 3 min. A solution of TFA (3.4 μL, 45.3 μmol, 1.10 equiv) in benzene (25.0 μL) was added drop-wise via syringe. The pale yellow mixture became intense yellow immediately upon addition of TFA and faded to a clear tan color solution within 2 min. The reaction mixture was mixed and maintained at ambient temperature for 4.5 h strictly under an inert atmosphere to allow complete conversion of the myrmicarin 215B (**4**) to the iminium ion **26**. A single portion of resin-bound BEMP (187 mg, 2.2 μmol/mg on 200–400 mesh polystyrene resin, 10.0 equiv) was added under an inert atmosphere (glove-box, nitrogen atmosphere) to the solution of the iminium salt **26** and the resulting pale yellow suspension was stirred under strictly inert conditions. After 30 min, the yellow suspension was filtered through a cotton plug into a recovery flask and the transfer was completed using additional benzene (3×450 μL) for rinsing. The flask was sealed under nitrogen and removed from the glove-box. The pale yellow solution was concentrated partially under reduced pressure (to ~750 μL). Diisopropylethylamine (89.7 μL, 515 μmol, 12.5 equiv) was added via syringe at ambient temperature, followed by 4-bromobenzoyl chloride (11.3 mg, 51.5 μmol, 1.25 equiv) as a solid in a single portion and the flask was immediately resealed and flushed with argon. The resulting intense yellow, slightly opaque mixture was vigorously stirred at ambient temperature. After 30 min, diisopropylamine (150 μL, 1.07 mmol, 26.1 equiv) was introduced via syringe to quench the excess acid chloride. After 5 min, the suspension was concentrated under reduced pressure to give the crude product as a bright yellow semi-solid. Purification of the residue by column chromatography on silica gel (5% Et_3N, 5% EtOAc–hexanes, diameter 1.5 cm, height 15 cm) afforded the benzoylated derivative **29** as a bright yellow oil (8.4 mg, 67%). ^{1}H NMR (500 MHz, C_6D_6, 23 °C), δ: 7.67 (d, 2H, J=8.2 Hz, C15–H, C15′–H), 7.29 (d, 2H, J=8.2 Hz, C16–H, C16′–H), 3.48 (tdd, 1H, J=10.7, 5.0, 3.7 Hz, C4a′–H), 3.16 (dq, 1H, J=14.0, 7.3 Hz, C11–H), 2.80 (m, 1H, C5a–H), 2.78 (m, 1H, C3′–H_c), 2.65 (m, 1H, C3′–H_t), 2.62 (m, 1H, C7′–H_c), 2.59 (m, 2H, C11′–H), 2.48 (m, 1H, C3–H), 2.44 (m, 1H, C7′–H_c), 2.38 (dt, 1H, J=13.8, 3.3 Hz, C7–H_t), 2.25 (m, 1H, C11–H′), 2.24 (td, 1H, J=13.8, 2.9 Hz, C7–H_c), 2.20 (m, 1H, C2–H), 2.15 (m, 1H, C5-H_t), 2.13 (m, 1H, C4′–H_c), 1.69 (m, 1H, C6′–H_t), 1.65 (m, 1H, C4–H_c), 1.63 (m, 1H, C4′–H_t), 1.61 (m, 1H, C4–H_t), 1.56 (m, 1H, C5′–H_c), 1.52 (m, 1H, C5–H_c), 1.44 (m, 1H, C9–H), 1.43 (m, 1H, C6–H_c), 1.38 (m, 1H, C6′–H_c), 1.32 (t, 3H, J=7.6 Hz, C12′–H), 1.23 (m, 1H, C9–H′), 1.19 (t, 3H, J=7.3 Hz, C12–H), 0.95 (d, 3H, J=6.7 Hz, C10′–H), 0.93 (t, 3H, J=7.6 Hz, C10–H), 0.89 (tdd, 1H, J=12.6, 10.4, 2.2 Hz, C5′–H_t), 0.82 (dtd, 1H, J=13.8, 11.9, 2.9 Hz, C6–H_t). ^{13}C NMR (125.8 MHz, C_6D_6, 23 °C), δ: 190.4 (C13), 162.6 (C3a), 162.6 (C8a), 141.8 (C14), 136.6 (C8b), 131.8 (C15), 131.4 (C16), 128 (C2a′), 125.2 (C17), 122.0 (C1′), 117.5 (C7a′), 113.6 (C2′), 104.2 (C8), 86.9 (C3b), 55.6 (C4a′), 54.6 (C5a), 49.4 (C2), 48.5 (C1), 45.1 (C3), 41.0 (C4), 37.3 (C4′), 36.7 (C5), 30.4 (C6), 30.1 (C5′), 28.8 (C7), 27.5 (3′), 23.2 (C6′), 21.2 (C7′), 20.9 (C11), 20.8 (C9), 19.0 (C11′), 17.4 (C12′), 16.3 (C10′), 14.8 (C12), 13.6 (C10). FTIR (neat), cm^{-1}: 2958 (s, C–H), 1733, 1635, 1523, (s, C═O), 1437, 1338, 1201. HRMS (ESI): m/z calcd for $C_{37}H_{45}BrN_2O$ [M+H]$^+$: 613.2788; Found: 613.2771. TLC (silica gel, 20% EtOAc–hexanes), R_f: 0.41 (UV, ninhydrin).

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19. Hydrogenation of this enamine under forcing conditions (160 psi H_2, 23 °C, 60 h) did provide further reduction of the enamine to the corresponding amine as a single diastereomer with similar chemical lability to **28**.
20. The designations C4–H_c and C4–H_t refer to the protons cis and trans to the C4a methine, respectively.
21. (a) While equilibrium of **30** and **31** is neither supported by spectroscopic data nor by enthalpic considerations, the presence of this equilibrium cannot be ruled out at this time. (b) Alternative cycloaddition type processes for the direct union of **4** and **10** to provide iminium ion **31** have been considered, and as with other possibilities necessarily await further experimental data.
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(±)-Diinsininone: made nature's way

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Abstract—We report the synthesis of diinsininone (**33**), the aglycone of (±)-diinsinin (**2**). Thereby, we complete the first construction of a proanthocyanidin (PA) type-A compound incorporating a [3.3.1]-bicyclic ketal as its characteristic core. Our strategy utilizes a coupling between a benzopyrilium salt and a flavanone that proves applicable to other PA type-A compounds. During this undertaking, treatment of naringenin (**9**) with 2-iodoxybenzoic acid (IBX) followed by reductive work-up affords eriodictyol (**10**). This reactivity mirrors that of catechol hydroxylase (F3H) found in the flavonoid pathway. Other interesting transformations include the formation of flavonoids through an *ortho*-quinone methide (*o*-QM) cycloaddition–oxidation sequence and regioselective β-glycosidations of several unprotected flavanones suggesting a likely synthesis of **2** from the aglycone **33**.

1. Introduction

Chemists should ponder probable biosynthetic pathways leading to their target, because few things are more humbling to one's best-laid plans than nature's simpler solution. Nature has been conducting and optimizing chemical experiments for millions of years; therefore, matching or exceeding nature's accumulated wisdom is a daunting challenge. However, the precise sequence used by nature to assemble its products is rarely known. In these instances, synthetic chemists are empowered with the ability to illuminate nature's hidden pathways by determining the reactivity inherent to its intermediates.

Consider the [3.3.1]-bicyclic compounds diinsininol (**1**) and diinsinin (**2**) shown in Figure 1. In nature's quest to build these compounds in an efficient manner, it might fashion a single flavan component using genomics, and then cause this entity to dimerize, forming bonds *i* and *ii*, whereupon oxidases and lipases would complete its creation. By mimicking this purported pathway, we hoped to reveal some of nature's more closely guarded secrets regarding chemoselectivity, diastereoselectivity and enantioselectivity during the assembly process leading to **1** and **2**.

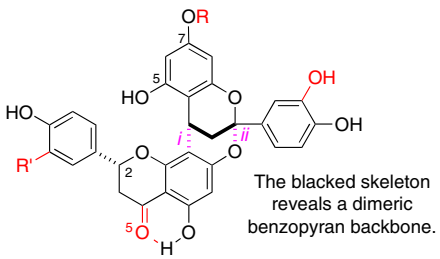

Figure 1. PA type-A structures, diinsininol (**1**) and diinsinin (**2**).

In 1996, the compounds **1** and **2** were discovered in the rhizome of *Sarcophyte piriei* Hutch, a parasitic plant that grows on the roots of the *Acacia* species of tropical shrubs and trees.[1] A decoction of its tuber is used in Somalian folk medicine; an activity that led to their eventual isolation and evaluation.[2] Diinsininol (**1**) and diinsinin (**2**) inhibit prostaglandin synthesis (IC_{50} values of 9.20 μM and 13.14 μM, respectively) and platelet activating factor-induced exocytosis (IC_{50} values of 49 μM and 39 μM, respectively). These biological activities are common among drugs that inhibit cyclooxygenase-2 (COX-2) and protect cells from tumor growth; tumors with high COX-2 levels are usually larger and metastasize faster than those without.[3]

2. Biological relevance

The flavonoid carbon skeleton encompasses a range of structural and biological diversity and is widely distributed throughout the plant kingdom.[4] Because of its abundance, it often proves useful as a starting material for synthesis. Its structural diversity on the other hand enables evaluation of an assortment of pharmacological targets. More than 5000 flavonoids have been isolated varying in the hydroxylation, methoxylation, glycosylation, and acylation patterns

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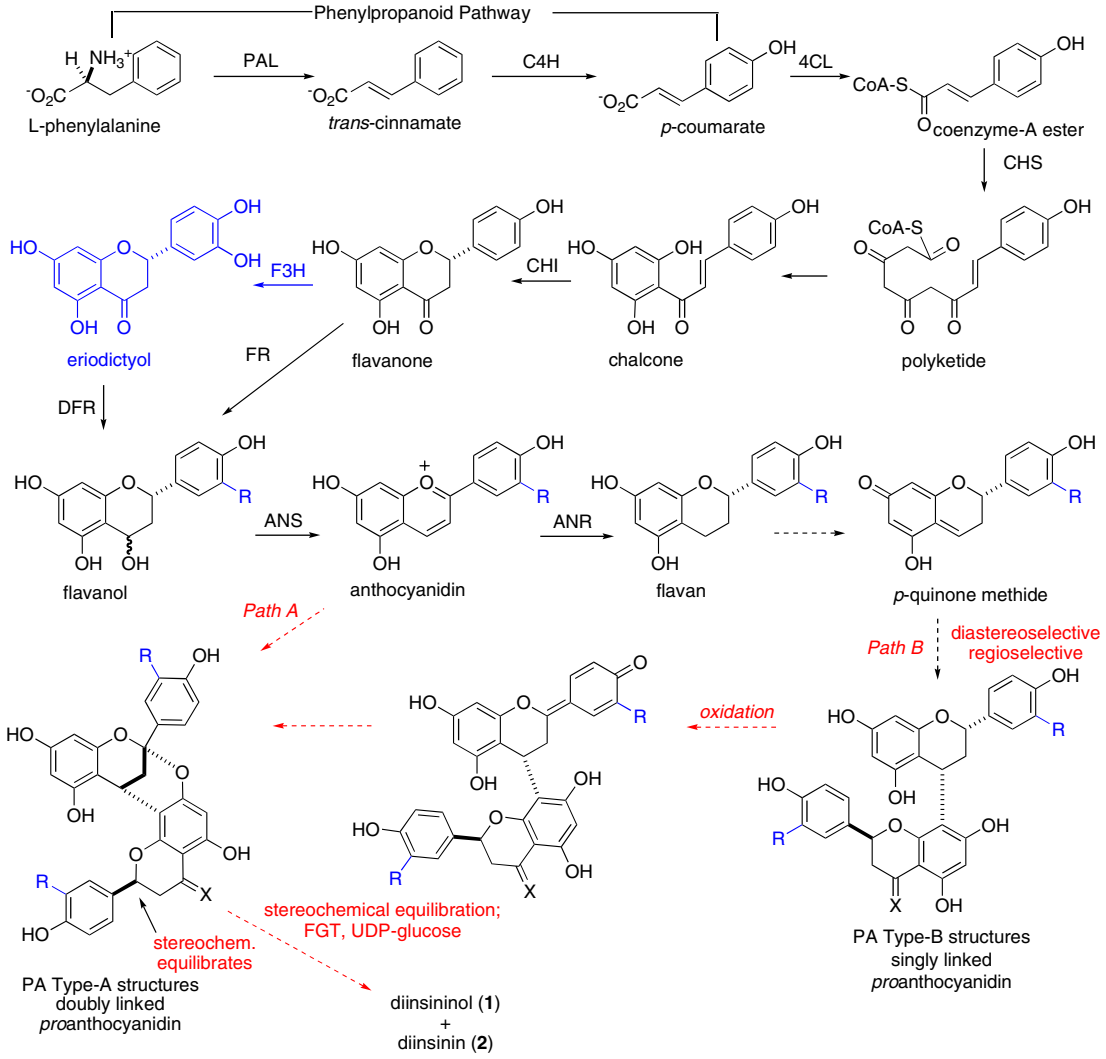
of the carbon backbone.[5] This arsenal of structures evolved as a defense to oxidative stress and hungry herbivores.[6]

Flavonoids protect organisms from oxidative stress by destroying reactive oxygen species that would have otherwise caused cell damage. It has long been known that foods high in antioxidants lower the risk of many diseases, including cancer, cataract, heart disease, and rheumatoid arthritis.[7] The so-called 'French Paradox' stems from the observation that French people consuming as much as 1200 mg of flavonoids per liter of red wine with structures similar to **1** and **2** display lower incidences of coronary disease despite a higher intake of saturated fat.[8–12]

In addition to these suppositious medical benefits, compounds **1** and **2** resemble antihyperglycemic components found within cinnamon, which have been studied for their ability to potentiate insulin, maintain normal blood sugar levels, and assist in fat metabolism.[13] Currently insulin resistance, which is referred to as metabolic syndrome X, affects 50 million people. Syndrome X is linked to an unhealthy lifestyle, genetic factors, and the progression to type-II diabetes.[14] In 2004, several powerful antihyperglycemic compounds were identified in cinnamon and determined to be trimer and tetramers of PA type-A compounds displaying the characteristic [3.3.1]-bicyclic ketal core.[15]

3. Relevance of the speculative biosynthetic pathways

The biosyntheses of **1** and **2** initially follows the flavonoid pathway, which proceeds from phenylalanine through *trans*-cinnamate, chalcones, flavanones, anthocyanidins, to flavans. It is among the best studied of biosynthetic pathways in nature.[16–18] The optically active compounds **1** and **2** suggest a double union between two products emerging from the flavonoid pathway. It is reasonable to speculate that an organism would utilize an identical path for these structurally related natural products. Paths A and B have been proposed (Scheme 1). In our minds, these proposed routes are profoundly different and suggest different consequences for reactivity and stereochemistry leading to the [3.3.1]-bicyclic ketal. Path B, which has been shown to afford the singly linked type-B PA structures, was proposed by Nonaka to



Scheme 1. Known (solid arrows) and speculative (dashed arrows) biosynthetic pathways leading to **1** and **2**. PAL: phenylalanine ammonia lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumarate: CoA ligase; CHS: chalcone synthase; CHI: chalcone flavanone isomerase; F3H: flavanone 3-hydroxylase; FR: flavanone reductase; DFR: dihydroxyflavanone reductase; ANS: anthocyanidin synthase; ANR: anthocyanidin reductase.

yield the [3.3.1]-bicyclic ketal structure found in type-A PA compounds by an oxidative cyclization. A catechin type-B dimer has been transformed into the corresponding type-A structure with picrylhydrazyl radical and hydrogen peroxide.[19] Path B appears to entail the chemoselective addition of a flavanone or flavan nucleophile to an electrophile arising from the *para*-quinone methide or its corresponding cation. This initial coupling should be inherently diastereoselective. However, if a Path B-oxidation sequence does result in the biosyntheses of **1** and **2**, it is remarkable because the original stereocenters ultimately become compromised when carried to the optically active natural products. Path A, on the other hand, suggests that a benzopyrylium salt can undergo a net [3+3]-cycloaddition in an enantioselective fashion chaperoned by an unknown enzyme,[20] or perhaps proceeds in a diastereoselective fashion with the directing stereochemistry again compromised when forming the final natural products.

The oxidation state of the components at the time of coupling remains unclear as does the timing of the oxidation that distinguishes between the two natural products. Flavanone 3-hydroxylase (F3H) may catalyze hydroxylation of (2*S*)-flavanone to eriodictyol. A combination of di-hydroxylated and mono-hydroxylated materials leads to diinsininone (**33**), while a combination of di-hydroxylated systems provides diinsininolone (**34**). Alternatively, F3H or some derivative of this enzyme may establish divergence in oxidation states (catechol vs phenol) in the final or penultimate step of the biosynthesis. Similarly, glycosylation may occur as the final or penultimate step of the biosynthesis. Divergence among the pathways for similar components seems energetically wasteful for an organism.

4. Synthetic relevance

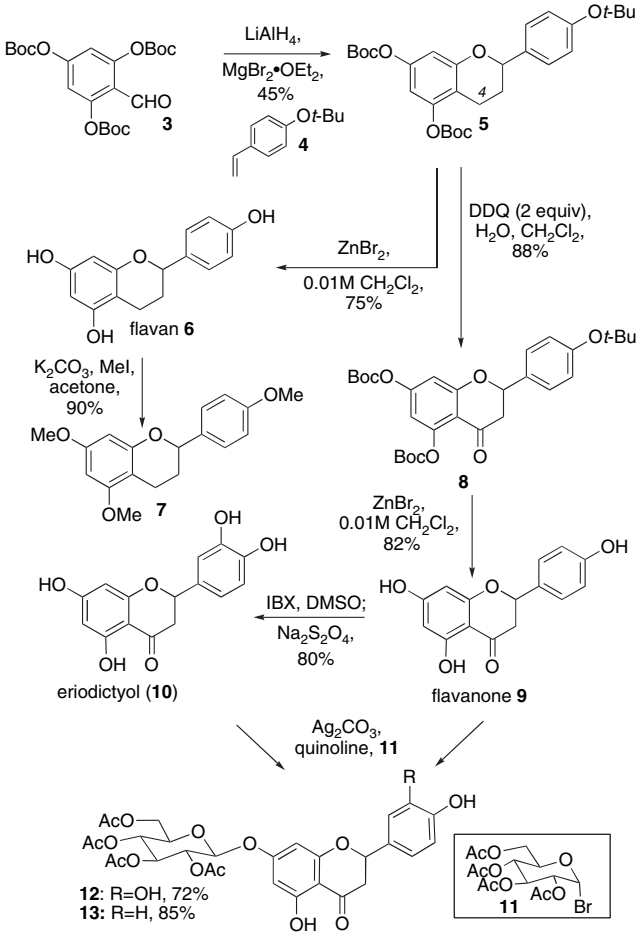
To the best of our knowledge, there are no previous synthetic endeavors addressing diinsininol (**1**), diinsinin (**2**), or similar type-A PA compounds in the literature. While there are numerous methods for the construction of chromans, the enantioselective construction of flavonoids is a surprisingly difficult task and it is difficult to improve upon their biosynthetic pathway.[21] Recently, our group devised a strategy for the enantioselective assembly of non-natural flavonoids by a [4+2]-cycloaddition between an *o*-QM and a chiral enol ether. We utilized this process in the first total synthesis of the flavonoid (+)-mimosifoliol.[22] We initially envisioned extending this strategy to the chroman functionality embedded within diinsininol (**1**) and diinsinin (**2**). The additional hydroxyl residue that distinguishes **1** from **2** could be introduced using our IBX oxidation–reduction sequence for the construction of *o*-quinols from phenols.[28]

5. Related path A and B synthetic endeavors

Previous syntheses of the singly linked PA type-B structures have followed the putative biosynthetic route, Path B.[23–26] However, unless other stereochemical substituents adjoin the site of reactivity and direct the process Path B couplings appear to result in a complex diastereomeric mixture of products. Moreover, regioselectivity and chemoselectivity prove problematic for most systems. Far less information is available regarding the likelihood of a successful Path A coupling. However, Jurd reports a simple benzopyrylium salt undergoes coupling with phloroglucinol to result in a similar [3.3.1]-bicyclic ketal in 25% yield.[27]

6. Our synthetic studies

We began surveying these problems by constructing the flavans, flavanones, and dihydroxyflavanones, which might arise in the putative biosynthetic pathway, and then proceeded to investigate the inherent ability of these structures to form type-A and B PA adducts (Scheme 2). Recently, our group had shown that *o*-QMs generated from *o*-Boc salicyl-alcohols would undergo cycloadditions with electron-rich olefins forming chroman adducts that resemble compound **5**.[22] However, our original two-pot process, involving reduction and coupling proved unfruitful with aldehyde **3**. This substrate mandates the use of an in situ reduction–cycloaddition protocol. The tris-*o*-Boc salicylaldehyde **3** (0.10 M diethyl ether, −78 °C), styrene **4** (40 equiv), and magnesium bromide dietherate (1.0 equiv) are subjected to lithium aluminum hydride (1.0 equiv) and thereby produce the flavan **5** in a 45% isolated yield.



Scheme 2. Synthesis of flavan, flavanone, and catechol.

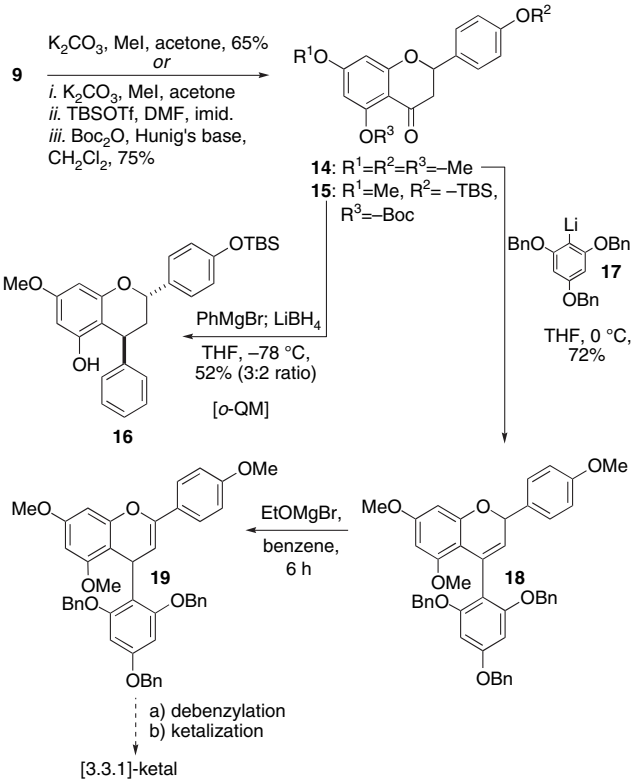
C(4)-oxidation of flavan **5** (0.10 M methylene chloride) occurs with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2 equiv) in the presence of water (1.0 equiv) to afford the flavanone **8** in 88% yield. Cleavage of the *t*-butyl residues

found in compounds **5** and **8** (0.01 M methylene chloride) is accomplished with zinc bromide (10 equiv) to afford the flavan **6** and the flavanone **9**, respectively. Per-methylation of **6** (0.10 M acetone) using methyl iodide (5.0 equiv) and potassium carbonate (4.0 equiv) affords tris-methylated flavan ether **7** and facilitates its structural identification.

Next, we used our one-pot two-step chemical process that mimics the enzyme F3H found in the flavan biosynthesis.[28] The unprotected flavanone **9** (0.03 M dimethyl sulfoxide) is subjected to 2-iodoxybenzoic acid (2.0 equiv) and forms a highly reactive *o*-quinone intermediate, which is reduced during work-up with sodium sulfite (3.0 equiv) to provide eriodictyol (**10**) in an isolated 80% yield. As previously observed, phenols bearing electron-withdrawing groups such as –C(O)R, –CHO, and $-NO_2$ fail to undergo oxidation with 2-iodoxybenzoic acid.[28] This reaction can be viewed as electrophilic aromatic substitution.[29] The –C(O)R substituent of flavanone **9** deactivates the A-ring and effectively prevents its oxidation. With the phenol **9** and catechol **10** in hand, methods for their regioselective glycosidation were explored. The insolubility of substrates **9** and **10** severely limited the conditions that could be employed. After some experimentation, we found that phenol **9** and catechol **10** (0.10 M quinoline) undergo regioselective β-glycosidation with acetobromo-α-D-glucose **11** (1.50 equiv) and silver carbonate (1.50 equiv) to produce compounds **13** and **12** in good yield.[30] The following order of reactivity was observed for flavanone **9** and appears to follow the relative acidity of each phenol: C(5)–OH<c(4′)–OH<c(7)–OH. Given this result, we expect the glycosidation of the aglycones of **1** and **2** to be fairly straightforward.

Next, we turned our attention to the formation of the C(4)–C(8) bond following a putative Path B biosynthesis. Given our experiences with diastereoselective additions to *o*-QMs, we decided to explore this reaction within the context of a flavanone (Scheme 3). The glycosidation reaction revealed that the hydroxyl residues of flavanones would also undergo regioselective alkylation. We decided to exploit this inherent reactivity for selective protection. Sequential treatment of flavanone **9** with methyl iodide, *tert*-butyldimethylsilyl triflate, and di-*tert*-butyl dicarbonate and the appropriate bases affords compound **15** in good yield (75%).

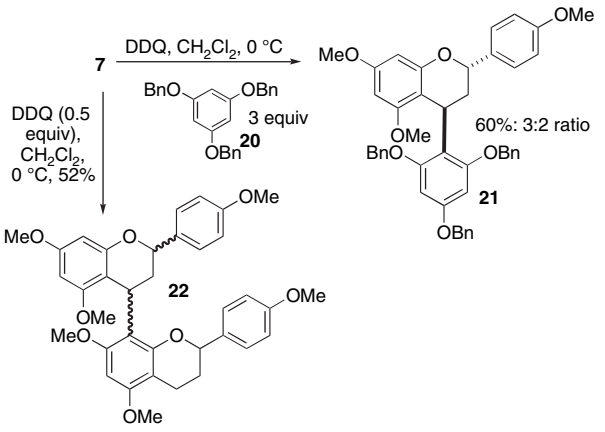
Reaction of the *o*-OBoc flavanone **15** (0.05 M tetrahydrofuran) with phenylmagnesium bromide (1.0 equiv) followed by lithium borohydride (1.0 equiv) results in flavan **16** formation in a 52% yield and a 3:2 ratio of diastereomers. We presume that the reaction proceeds through an *o*-QM intermediate. The lack of stereoselectivity was somewhat troubling and did not bode well for applying any of these Path B strategies to the syntheses of **1** and **2**. Therefore, we sought another method for carbon–carbon bond formation. The flavanone **9** was per-methylated to afford compound **14**. Treatment of flavanone **14** (0.2 M tetrahydrofuran, −78 °C) with the phloroglucinol lithium **17** (3.0 equiv) affords the chromene **18** by spontaneous elimination of the tertiary alcohol intermediate. Isomerization of the double bond within compound **18** (0.07 M benzene, 65 °C) occurs upon treatment with ethoxymagnesium bromide (2.0 equiv) to form the enol ether **19**.[31] We envisioned that debenzylation of compound **19** and subsequent ketalization would afford the



Scheme 3. *o*-QM reaction with flavanone backbone.

desired [3.3.1]-bicyclic system. However, treatment of the debenzylated adduct with strong acid results in loss of the phloroglucinol fragment from adduct **19**.

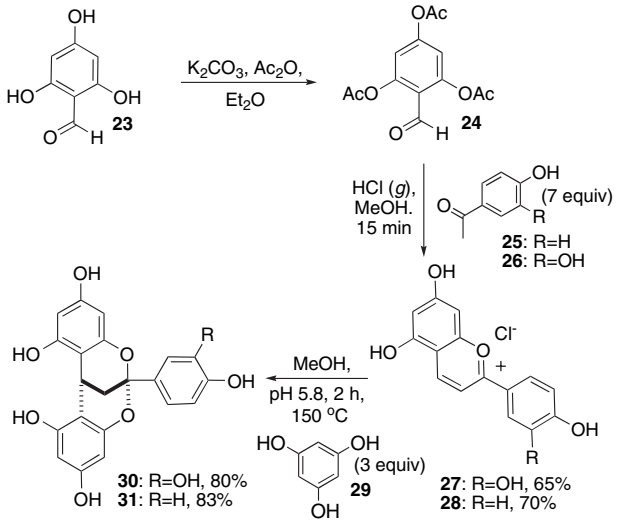
With compound (±)–**7** in hand, we considered other strategies mimicking Path B (Scheme 4).[32] Treatment of the flavan (±)–**7** (0.10 M methylene chloride) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.5 equiv) affords a mixture of more than four stereoisomers of the dimer **22** in a combined 52% yield. Given this result, we expected optically pure **7** to give more than two diastereomers; there must be atropisomerism among the trans and/or cis adducts. To partially circumvent this stereochemical problem, we examined the oxidation of **7** (0.1 M methylene chloride) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.0 equiv) and in



Scheme 4. DDQ dimerization and coupling.

the presence of tribenzylated phloroglucinol (**20**) (3 equiv). The reaction proceeds in a 60% yield and affords the type-B PA structure **21** in a 3:2 ratio favoring the trans diastereomer. In addition to the stereochemistry issues raised by this strategy, there are numerous protecting group issues that would have to be resolved as well. Therefore, we began to investigate a Path A inspired synthesis.

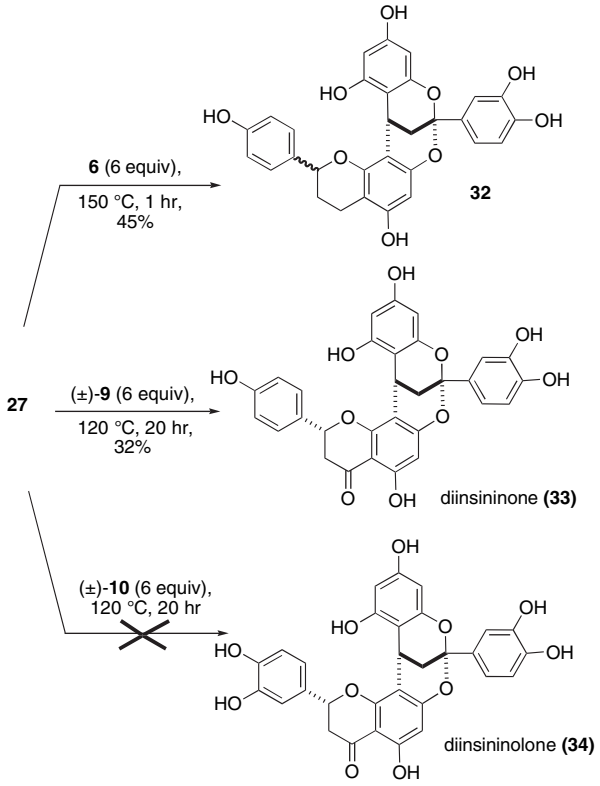
A Path A strategy requires rapid and easy access to the corresponding benzopyrylium salts. We initially considered methods to construct these systems from **7**, **9**, and **10**. However, careful scrutiny of the literature reveals oxidative methods are plagued by low yields, crude mixtures.[33] Instead, we opted to explore a new protocol recently reported by Mas (Scheme 5).[34] 2,4,6-Triacetoxybenzaldehyde (**24**) undergoes condensation with hydroxyacetophenones **25** and **26** in a saturated hydrochloric acid solution in methanol to result in the benzopyrylium salts **27** and **28** in good yields.



Scheme 5. Mas's procedure provides benzopyrylium salts that readily undergo addition with phloroglucinol.

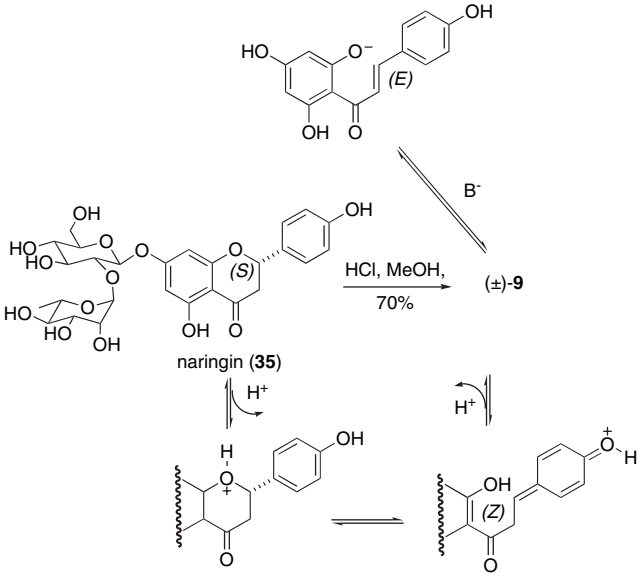
We find that addition of an aqueous buffered solution of phloroglucinol **29** (3 equiv) to the respective benzopyryliums **27** and **28** (0.20 M methanol) affords the ketals **30** and **31**, respectively, in outstanding yields. Optimal conditions employ a Emrys microwave reactor with heating to 150 °C for 120 min. Isolation of non-ketalized products from reactions conducted at lower temperatures reveals that the carbon–carbon bond formation precedes the oxygen–carbon bond formation. While we entertained the possibility of desymmetrizing the *meso* compounds **30** and **31**, we first examined the addition of flavans and flavanones to the benzopyrilium salts **27** and **28**.

The benzopyrilium salt **27** (0.10 M methanol) combines with flavan **6** (6 equiv) using the previously described microwave equipment and results in the regioselective formation of the type-A PA compound **32** in 42% yield (Scheme 6). However, a 1:1 mixture of diastereomers is observed. Upon lowering the temperature to 120 °C and lengthening the reaction time to 20 h for the subsequent combination of the benzopyrilium **27** with the flavanone **9**, diinsininone (**33**) forms. To our delight, compound **33** emerges from this extended reaction as a single diastereomer. However, the similar conditions proved entirely unproductive for the combination of the benzopyrilium **27** with the hydroxylated flavanone **10**.



Scheme 6. Reactions of the benzopyrilium salt **27** with flavan and flavanone.

The formation of a single diastereomer of diinsininone (**33**) is explained by the equilibria shown in Scheme 7. It should be noted that we had originally imagined degrading naringin (**35**) to afford optically active (*S*)-C(2) flavanone **9**. However, in our hands, hydrolysis of the sugar results in

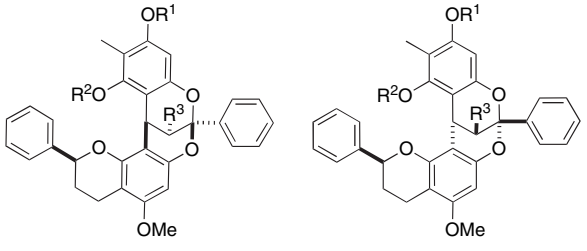


Scheme 7. Acid and base-catalyzed racemization of a C(2)-stereocenter.

formation of racemic **9** as determined by a Chiralcel OD-H column.[35]

7. Conclusions

One day, a better understanding of the PA biosynthetic pathway may allow the genetic engineering of plants for the production of compounds coveted for their antioxidant and antihyperglycemic activities. Until that day arrives, chemical synthesis remains the best method for the construction of these compounds and enables the exploration of their biological activities. The preceding synthetic studies have illuminated inherent reactivity of several intermediates belonging to the flavonoid pathway. These studies suggest that compounds **1** and **2** most likely arise from an identical biosynthetic pathway proceeding through a Path A benzopyrilium coupling. Whether the oxidative divergence among these structures arise as the ultimate or penultimate step remains to be determined. Related natural products belonging to the Dragon's Blood family of flavanones, a name that derives from the red resin plant exudates, can also be accessed by appropriately adapting the above strategy (Fig. 2).[32b]



36: R^1=H;R^2=Me;R^3=OH
37: R^1=Me;R^2=Me;R^3=OH
38: R^1=Me;R^2=Me;R^3=OAc
39: R^1=H;R^2=Me;R^3=H

40: R^1=Me;R^2=Me;R^3=H
41: R^1=Ac;R^2=Me;R^3=H
42: R^1=R^2=R^3=H
43: R^1=R^2=R^3=Me

Figure 2. Natural products of the Dragon's Blood family.

8. Experimental

8.1. Aldehyde 3

A flame-dried flask equipped with a stir-bar and a nitrogen line containing 2,4,6-trihydroxybenzaldehyde (2 g, dehydrated with P_2O_5, 0.05 M CH_2Cl_2), Hunig's Base (1.36 mL, 0.6 equiv), DMAP (four crystals), and Boc_2O (7.74 g, 3.2 equiv) was stirred overnight. The reaction was quenched with 1 M NH_4Cl and extracted twice with CH_2Cl_2. The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The crude mixture was then chromatographed on silica gel, eluting with petroleum ether/EtOAc (95:5) to afford the desired product in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.24 (s, 1H), 6.88 (s, 2H), 1.46 (s, 18H), 1.42 (s, 9H).

8.2. Tris-protected flavan 5

A flame-dried flask equipped with stir-bar and nitrogen line, was charged with aldehyde **3** (50 mg, 0.1 M Et_2O), 1-*tert*-butoxy-4-vinylbenzene (0.19 mL, 40 equiv), and $MgBr_2\cdot OEt_2$ (28.4 mg, 1.00 equiv). The solution was cooled to −78 °C and $LiAlH_4$ (4 mg, 1.00 equiv) was added. The reaction was warmed to rt over 3 h then quenched with 1 M $NaHCO_3$ and extracted with Et_2O. The ether layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (95:5) to afford the desired product in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (d, 2H, J=9 Hz), 6.70 (d, 2H, J=9 Hz), 6.39 (s, 1H), 6.34 (s, 1H), 5.20–4.90 (m, 1H), 2.60–2.20 (m, 4H), 1.45 (s, 9H), 1.40 (s, 18H).

8.3. Flavan 6

A flame-dried flask equipped with stir-bar and nitrogen line, was charged with flavan **5** (20.0 mg, 0.01 M CH_2Cl_2) and $ZnBr_2$ (87.5 mg, 10 equiv). The solution was stirred at rt for 6 h. The reaction was quenched with 1 M HCl and extracted twice with EtOAc. The ether layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (50:50) to afford the desired product in 75% yield. ^{1}H NMR (400 MHz, CD_6CO): δ 7.27 (d, 2H, J=9 Hz), 6.85 (d, 2H, J=9 Hz), 6.00 (s, 1H), 5.87 (s, 1H), 4.89–4.86 (m, 1H), 2.72–2.66 (m, 2H), 2.11–1.88 (m, 2H).

8.4. Tris-methylated chroman 7

A round-bottom flask equipped with a stir-bar was charged with flavan **6** (1.67 g, 0.2 M acetone), K_2CO_3 (340 mg, 4 equiv), and MeI (0.19 mL, 5 equiv). The reaction was stirred overnight and filtered. The reaction was diluted with H_2O and extracted twice with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (80:20) to afford the desired product in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (d, 2H, J=9 Hz), 6.93 (d, 2H, J=9 Hz), 6.13 (s, 1H), 6.09 (s, 1H), 4.93–4.92 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.77 (s, 3H), 2.80–2.60 (m, 4H), 2.20–1.97 (m, 4H).

8.5. Flavanone 8

Flavan **5** (30 mg, 1 equiv, 0.01 M CH_2Cl_2), H_2O (1 μL, 1 equiv), and DDQ (recrystallized from hot $CHCl_3$, 26.5 mg, 2 equiv) were added to a round-bottom flask equipped with a stir-bar, a septum and stirred under N_2 (g) at rt overnight. The reaction was diluted with H_2O and extracted twice with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (70:30) to afford the desired product in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (d, 2H, J=9 Hz), 6.70 (d, 2H, J=9 Hz), 6.54 (s, 1H), 6.48 (s, 1H), 5.55–5.50 (m, 1H), 3.38–3.13 (m, 2H), 1.56 (s, 18H), 1.40 (s, 9H).

8.6. 4′,5,7-Trihydroxyflavanone (9)

A flame-dried flask equipped with stir-bar and nitrogen line, was charged with flavanone **8** (20.0 mg, 0.01 M CH_2Cl_2) and $ZnBr_2$ (85.2 mg, 10 equiv). The solution was stirred at rt for

6 h. The reaction was quenched with 1 M HCl and extracted twice with EtOAc. The ether layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (50:50) to afford the desired product in 82% yield. ^{1}H NMR (400 MHz, CD_6CO): δ 12.19 (s, 1H), 9.59 (s, 1H), 8.55 (s, 1H), 7.39 (d, 2H, J=9 Hz), 6.90 (d, 2H, J=9 Hz), 5.95 (s, 1H), 5.94 (s, 1H), 5.48–5.44 (m, 1H), 3.22–3.15 (m, 2H), 2.75–2.70 (m, 2H); ^{13}C NMR (100 MHz, CD_6CO): δ 197.4, 167.3, 165.4, 164.5, 158.8, 130.9, 129.1, 116.3, 103.3, 96.8, 95.9, 80.0, 43.6, 30.5; HRMS (ESI-TOF) (M^++H^+) m/z calcd for $C_{15}H_{12}O_5$ 273.0685, found 273.0765.

8.7. Eriodictyol (10)[28]

To a solution of flavanone **9** (1.011 g, 0.03 M DMSO) was added IBX[36] (2.135 g, 2 equiv) and stirred at rt for 5 h. As the reaction progressed, the color changed from a translucent yellow solution to an opaque brown solution. $Na_2S_2O_4$ (1.94 g, 3 equiv) was added to the reaction and stirred for an additional 4 h. The reaction was quenched with water (300 mL) and extracted with EtOAc (3×100 mL). The combined organic extracts stirred with pH 8.5 buffer solution to remove *o*-iodobenzoic acid. The organic layer was washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (50:50) to afford the desired product in 80% yield. ^{1}H NMR (400 MHz, CD_6CO): δ 7.03 (s, 1H), 6.87 (s, 2H), 5.96–5.94 (m, 2H), 5.41–5.38 (m, 1H), 3.18 (m, 1H), 2.75 (m, 1H); ^{13}C NMR (100 MHz, CD_6CO): δ 167.7, 137.8, 135.7, 134.8, 116.8, 116.5, 102.0, 89.7, 86.5, 85.2, 73.6, 67.2, 66.3, 50.4, 14.0; HRMS (ESI-TOF) (M^++Na^+) m/z calcd for $C_{15}H_{12}O_6$ 311.0633, found 311.0525.

8.8. Acetobromo-α-D-glucose (11)

D-Glucose (2.05 g, 0.40 M pyridine) and Ac_2O (12 mL, 0.44 M) were stirred at rt overnight. The reaction was quenched with H_2O, extracted with $CDCl_3$, washed with $NaHCO_3$, dried with Na_2SO_4, and afforded pentaacetylglucose. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72–5.71 (m, 1H), 5.28–5.24 (m, 1H), 5.16–5.11 (m, 2H), 4.32–4.28 (m, 1H), 4.13–4.10 (m, 1H), 3.86 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.3, 169.6, 169.5, 169.2, 91.9, 73.0, 72.9, 70.4, 67.9, 61.6, 21.0, 20.9, 20.8, 20.7; HRMS (ESI-TOF) (M^++Na^+) m/z calcd for $C_{16}H_{22}O_{11}$ 413.1162, found 413.1057. When the pentaacetoglucose (2 g, 0.57 M acetic acid) was dissolved, HBr (0.64 mL, 48% in acetic acid) was added drop-wise. The reaction was stirred overnight, poured over an ice/H_2O slurry, extracted with $CHCl_3$, neutralized with $NaHCO_3$, washed with H_2O, dried with Na_2SO_4, and afforded acetobromo-α-D-glucose (**11**). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62–6.61 (m, 1H), 5.59–5.55 (m, 1H), 5.19–5.15 (m, 1H), 4.86–4.83 (m, 1H), 4.36–4.30 (m, 2H), 4.15–4.12 (m, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.1, 170.0, 169.7, 86.8, 72.3, 70.8, 70.4, 67.4, 61.1, 20.9, 20.9, 20.8, 20.7; HRMS (ESI-TOF) (M^++Na^+) m/z calcd for $C_{14}H_{19}O_9Br$ 433.0212, found 433.0126.

8.9. Naringenin glucoside 13[30]

A solution of flavanone **9** (9.4 mg, 0.14 M quinoline), bromide **11** (21 mg, 1.50 equiv), and Ag_2CO_3 (13.8 mg, 1.5 equiv) was stirred for 6 h at rt. The reaction was poured into methanol, filtered through a short pad of Celite, and evaporated in vacuo. The residue was dissolved into EtOAc, washed with 1 N HCl, washed with brine, and dried over $MgSO_4$. After evaporation, the resulting crude product was purified by flash chromatography eluting with hexane/EtOAc (1:1) to afford the desired product in 85% yield. The product was an inseparable mixture of diastereomers. ^{1}H NMR (400 MHz, DMSO): δ 12.03 (s, 1H), 9.65 (s, 1H), 7.31 (d, 2H, J=9 Hz), 6.79 (d, 2H, J=9 Hz), 6.16–6.09 (m, 2H), 5.67–5.63 (m, 1H), 5.53–5.50 (m, 1H), 5.37–5.34 (m, 1H), 5.06 (m, 2H), 4.30–4.26 (m, 1H), 4.15–4.05 (m, 2H), 3.40–3.33 (m, 1H), 2.76–2.70 (m, 1H), 2.00–1.95 (m, 12H); (ESI-TOF) (M^++Na) m/z calcd for $C_{29}H_{30}O_{14}$ 625.1636, found 625.1528.

8.10. Trimethylated naringenin 14

Naringenin (**9**, 1 g, 0.2 M acetone), K_2CO_3 (3.05 g, 6 equiv), and MeI (1.83 mL, 8 equiv) were stirred overnight. The reaction was filtered and concentrated in vacuo. The resulting crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (70:30) to afford the desired product in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, 2H, J=9 Hz), 6.98 (d, 2H, 9 Hz), 6.18 (s, 1H), 6.16 (s, 1H), 5.45–5.40 (m, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.03–2.85 (m, 2H), 2.64–2.58 (m, 2H).

8.11. Tris-protected naringenin 15

Naringenin (**9**, 162 mg, 0.1 M DMF), Na_2CO_3 (69 mg, 1.10 equiv), and MeI (0.4 mL, 1.10 equiv) were stirred at rt overnight. The reaction was quenched with H_2O and extracted with EtOAc. The organic layer was washed repeatedly with H_2O, washed with brine, and dried over $MgSO_4$. The resulting crude product was purified by flash chromatography (petroleum ether/EtOAc 50:50) to afford mono-protected naringenin. ^{1}H NMR (400 MHz, CD_6CO): δ 12.15 (s, 1H), 8.57 (s, 1H), 7.40 (d, 2H, J=8 Hz), 6.89 (d, 2H, J=8 Hz), 6.04 (s, 1H), 6.03 (s, 1H), 5.39–5.35 (m, 1H), 3.82 (s, 3H), 3.10–3.03 (m, 2H), 2.78–2.70 (m, 2H). mono-Protected narigenin (66 mg, 0.1 M THF), 2,6-lutidine (0.028 mL, 1.05 equiv), and TBSOTf (0.056 mL, 1.05 equiv) were stirred at rt for 4 h. The reaction was quenched with H_2O, extracted with EtOAc, and dried with Na_2SO_4. The resulting crude product was purified by flash chromatography (petroleum ether/EtOAc 80:20) to afford bis-protected naringenin. ^{1}H NMR (400 MHz, CD_6CO): δ 12.15 (s, 1H), 7.47 (d, 2H, J=8 Hz), 6.96 (d, 2H, J=8 Hz), 6.07 (s, 1H), 6.05 (s, 1H), 5.55–5.51 (m, 1H), 3.86 (s, 3H), 3.27–3.19 (m, 2H), 2.81–2.77 (m, 2H), 1.00 (s, 9H), 0.33 (s, 6H). The bis-protected compound (0.1 M CH_2Cl_2), DMAP (one crystal), Hunig's Base (0.037 mL, 1.0 equiv), and Boc_2O (100 mg, 1.0 equiv) were stirred at rt for 1 h. The reaction was quenched with H_2O, extracted with EtOAc, and dried with Na_2SO_4. The resulting crude product was purified by flash chromatography (petroleum ether/EtOAc 90:10) to afford tris-protected naringenin **15** in 75% yield. ^{1}H NMR (400 MHz, CD_6CO): δ 7.50–7.48

(d, 2H, J=8 Hz), 6.98–6.95 (d, 2H, J=8 Hz), 6.50 (s, 1H), 6.37 (s, 1H), 5.52–5.49 (s, 1H), 3.90 (s, 3H), 3.08–3.05 (m, 2H), 2.85 (m, 2H), 1.54 (s, 9H), 1.01 (s, 9H), 0.25 (s, 6H).

8.12. Compound 16

A flame-dried flask equipped with stir-bar and nitrogen line, was charged with flavanone **15** (8.0 mg, 1 equiv, 0.05 M THF) and cooled to −78 °C. Phenylmagnesium bromide (8.0 μL, 1.50 equiv, 3 M THF) was added drop-wise and stirred for 1 h. $LiBH_4$ (1.0 mg, 1.00 equiv) was added to the solution, warmed to rt, quenched with H_2O, and extracted with EtOAc. The ether layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (90:10) to afford the desired product in 52% yield.

8.13. Chromene 18

Bromotribenzylated phloroglucinol (65 mg, 0.3 M THF, 3 equiv) was cooled to −78 °C, *n*-BuLi (0.052 mL, 2.5 M THF, 3.00 equiv) was added drop-wise, and the reaction stirred until complete by TLC (eluting with petroleum ether/EtOAc 90:10). This solution was added drop-wise to a stirring solution of flavan **15** (13.7 mg, 0.2 M THF) at −78 °C. After 1 h, the reaction was quenched with H_2O, extracted EtOAc, washed with brine, and dried with $MgSO_4$. The reaction was purified by flash chromatographed with EtOAc/hexane (70:30) to afford the desired product in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.22 (m, 17H), 6.61–6.59 (m, 2H), 6.35–6.30 (m, 2H), 6.14–6.12 (m, 1H), 5.92–5.91 (m, 1H), 4.91 (d, 1H, J=5 Hz), 4.76 (d, 1H, J=5 Hz), 5.05–4.96 (m, 6H), 3.73 (s, 3H), 3.72 (s, 3H), 3.30 (s, 3H); HRMS (ESI-TOF) $(M-H)^+$ *m/z* calcd for $C_{45}H_{40}O_7$ 691.7900, found 691.2711.

8.14. Enol ether 19[31]

A flame-dried flask equipped with a stir-bar and a nitrogen line containing EtOH (2 equiv, 0.5 M THF) was cooled to 0 °C. Phenylmagnesium bromide (2 equiv) was added to the solution warmed to rt, then evaporated to dryness. The chromene **18** (1 equiv, 0.7 M benzene) was added to the EtOMgBr, equipped with a reflux condenser, stir-bar, nitrogen line, and refluxed for 6 h. The reaction was quenched with H_2O, extracted EtOAc, washed with brine, and dried with $MgSO_4$. The reaction was purified by flash chromatography eluting with EtOAc/hexane (70:30) to afford the desired product in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.05 (m, 15H), 7.03 (d, 2H, J=8 Hz), 6.66 (d, 2H, J=8 Hz), 6.47 (s, 1H), 6.36 (d, 1H, J=10 Hz), 6.28–6.27 (m, 1H), 6.00 (s, 1H), 4.94 (s, 1H), 4.98 (s, 2H), 4.88–4.83 (m, 4H), 4.66 (d, 1H, J=10 Hz), 3.74 (s, 3H), 3.72 (s, 3H), 3.33 (s, 3H).

8.15. Chroman 21

To a flame-dried flask equipped with a stir-bar and a nitrogen line containing flavan **7** (20 mg, 0.10 M CH_2Cl_2) and tribenzylphloroglucinol (33.6 mg, 3 equiv) was added DDQ (recrystallized from hot $CHCl_3$, 15.8 mg, 1 equiv) at rt. The reaction was stirred for 3 h, quenched with H_2O, extracted with EtOAc, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (70:30) to afford the desired product in 60% yield.

8.16. Dimer 22

To a flame-dried flask equipped with a stir-bar and a nitrogen line containing flavan **7** (30 mg, 0.10 M CH_2Cl_2) stirring at rt was added DDQ (recrystallized from hot $CHCl_3$, 11.2 mg, 0.5 equiv). The reaction was stirred for 3 h, quenched with H_2O, extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture was then chromatographed through silica gel, eluting with petroleum ether/EtOAc (40:60) to afford the desired product in 52% yield.

8.17. 2,4,6-Triacetoxybenzaldehyde (24)

A flame-dried flask equipped with a stir-bar and a nitrogen line containing phenol **23**, dehydrated with P_2O_5, (2.58 g, 1 equiv, 0.15 M Et_2O), K_2CO_3 (8.41 g, 3.6 equiv), and Ac_2O (16.8 mL, 11 equiv) was stirred overnight. The reaction was filtered and concentrated to afford the desired product in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.13 (s, 1H), 6.97 (s, 2H), 2.38 (s, 6H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.6, 168.7, 167.9, 155.2, 1530.0, 118.3, 114.9, 21.4, 21.0; δ IR (CH_2Cl_2, υ_{max} cm^{-1}) 1778, 1697, 1616, 1580, 1433, 1370, 1298, 1180, 1122, 1051, 1022; HRMS (ESI-TOF) $(M^+$+Na) *m/z* calcd for $C_{13}H_{12}O_7$ 303.2302, found 303.0484.

8.18. General method for benzopyrilium salt formation[34]

A stream of anhydrous gaseous HCl was first passed through a solution of hydroxyacetophenone (1.6 g, 7 equiv, 0.12 M MeOH) at 0 °C, until the concentration of HCl rose to 15% by mass. A solution of aldehyde **24** (0.43 g, 1 equiv, 0.06 M MeOH) was then added drop-wise at 0 °C. At the end of the addition, the reaction mixture was kept for a few minutes at rt and evaporated to dryness. The resulting residue was vigorously stirred in Et_2O, which afforded the crude product after filtration and washing with the same solvent. The pure product was obtained by recrystallization in MeOH–HCl (95:5) in 65 and 70% yields for compounds **27** and **28**, respectively. *Luteolinidin Chloride* (**27**). ^{1}H NMR (400 MHz, CD_3OD–TFA 99:1): δ 8.95 (d, 1H, J=9 Hz), 7.94 (d, 1H, J=9 Hz), 7.78 (dd, 1H, J=9, 2 Hz), 7.65 (d, 1H, J=2 Hz), 6.98 (d, 1H, J=9 Hz), 6.87 (d, 1H, J=2 Hz), 6.63 (d, 1H, J=2 Hz); ^{13}C NMR (100 MHz, CD_3OD–TFA 99:1): δ 172.4, 171.9, 160.2, 159.6, 156.1, 149.0, 148.0, 125.3, 121.6, 117.9, 115.9, 113.5, 110.6, 103.3, 96.1. *Apigeninidin Chloride* (**28**). ^{1}H NMR (400 MHz, CD_3OD–TFA 99:1): δ 9.06 (dd, 1H, J=9, 1 Hz), 8.27 (d, 2H, J=9 Hz), 8.01 (d, 1H, J=9 Hz), 7.06 (d, 2H, J=9 Hz), 6.92 (dd, 1H, J=2, 1 Hz), 6.64 (d, 1H, J=2 Hz); ^{13}C NMR (100 MHz, CD_3OD–TFA 99:1): δ 172.7, 172.4, 167.3, 160.6, 160.0, 149.5, 133.1, 121.3, 118.4, 113.8, 110.3, 103.2, 96.1.

8.19. General method for the condensation of benzopyrilium salt with phloroglucinol[27]

Benzopyrilium salt **27** (20 mg, 0.6 M MeOH/pH 5.8 buffer (1:1) and phloroglucinol (26.2 mg, 3 equiv) were heated at 150 °C for 2 h in an Emrys microwave reactor. The reaction was concentrated to dryness on silica gel and purified by flash chromatographed with EtOAc/hexane (80:20) to afford the desired product in 80% yield. *Compound* **30**. ^{1}H NMR (400 MHz, CD_6CO): δ 7.19 (d, 1H, 2 Hz), 7.05 (dd, 1H, J=2, 8 Hz), 6.88 (d, 1H, J=2 Hz), 6.06 (s, 4H), 4.33 (t, 1H, J=3 Hz), 2.22 (d, 3H, J=3 Hz); ^{13}C NMR (100 MHz, CD_6CO): δ 158.1, 154.7, 154.3, 146.4, 145.6, 134.9, 118.4, 115.8, 114.2, 107.5, 99.4, 97.3, 96.7, 34.5, 21.4; HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{21}H_{16}O_8$ 419.0843, found 419.0758. An alternative method for easier purification is as follows. The reaction is concentrated to dryness and submitted to pyridine (0.3 mL) and Ac_2O (0.5 mL) overnight. The reaction is quenched with 1 M HCl, repeatedly extracted with EtOAc, and dried with $MgSO_4$. The reaction was purified with flash chromatography eluting with EtOAc/hexane (60:40) and the ketal and non-ketal compounds were isolated. *Acetylated compound* **30**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56–7.27 (m, 3H), 6.74 (d, 2H, J=2 Hz), 6.54 (d, 2H, J=2 Hz), 4.28 (t, 1H, J=3 Hz), 2.42 (s, 6H), 2.33 (s, 3H), 2.32 (s, 3H), 2.29 (s, 3H, J=3 Hz), 2.26 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 169.0, 168.3, 153.7, 150.2, 147.8, 142.9, 142.1, 139.0, 124.3, 123.7, 121.6, 115.7, 109.8, 108.5, 98.4, 33.0, 22.9, 21.7, 21.3, 20.9, 20.8; IR (CH_2Cl_2, υ_{max} cm^{-1}) 1772, 1618, 1369, 1198, 1222, 1024; HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{33}H_{28}O_{14}$ 671.1479, found 671.1378. *Enol ether* **30**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.22 (m, 3H), 6.90–6.82 (m, 4H), 5.32 (d, 1H, J=4 Hz), 4.90 (d, 1H, J=4 Hz), 2.48–2.25 (m, 21H); HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{35}H_{30}O_{15}$ 713.1585, found 713.1511.

8.20. Acetylated compound of 31

Benzopyrilium salt **28** (30 mg, 0.6 M MeOH/pH 5.8 buffer (1:1) and phloroglucinol (39.0 mg, 3 equiv) were heated at 150 °C for 2 h in an Emrys microwave reactor. The reaction was concentrated to dryness and submitted to pyridine (0.3 mL) and Ac_2O (0.5 mL) overnight. The reaction is quenched with 1 M HCl, repeatedly extracted with EtOAc, and dried with $MgSO_4$. The crude product was purified by flash chromatography with EtOAc/hexane (60:40) and the ketal and non-ketal product were isolated in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, 2H, J=9 Hz), 7.18 (d, 2H, J=9 Hz), 6.75 (d, 2H, J=2 Hz), 6.55 (d, 2H, J=2 Hz), 4.27 (t, 1H, J=3 Hz), 2.43 (s, 6H), 2.29 (d, 3H, J=3 Hz), 2.33 (s, 3H), 2.27 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 169.2, 169.0, 153.8, 151.4, 150.2, 147.8, 137.8, 127.3, 121.8, 115.8, 109.7, 108.5, 98.9, 33.1, 23.0, 21.7, 21.4, 21.3; δ IR (CH_2Cl_2, υ_{max} cm^{-1}) 1768, 1620, 1434, 1369, 1201, 1184, 1130, 1116, 1072, 1022, 904; HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{31}H_{26}O_{12}$ 613.1424, found 613.1337. *Enol ether* **31**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, 2H, J=9 Hz), 7.10 (d, 2H, J=9 Hz), 6.90–6.60 (m, 4H), 5.30 (d, 1H, J=4 Hz), 4.91 (d, 1H, J=4 Hz), 2.38–2.13 (m, 18H); HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{33}H_{28}O_{13}$ 655.1530, found 655.1423.

8.21. Compound 32

Benzopyrilium salt **27** (8.5 mg, 0.6 M MeOH/pH 5.8 buffer (1:1) and flavan **6** (43 mg, 6 equiv) were heated at 150 °C for 2 h in an Emrys microwave reactor. The reaction was concentrated to dryness and submitted to pyridine and Ac_2O overnight. The reaction was quenched with 1 M HCl, repeatedly extracted with EtOAc, and dried with $MgSO_4$. The desired product was purified by gradient chromatography with EtOAc/hexane (60:40–80:20) and the desired product was isolated in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58–7.50 (m, 4H), 7.46–7.38 (m, 4H), 7.16–7.08 (m, 4H), 6.75–6.68 (m, 2H), 6.53–6.51 (m, 2H), 6.29–6.27 (m, 2H), 5.17–4.92 (m, 2H), 4.14 (br s, 2H), 2.97 (m, 8H), 2.44–2.31 (m, 40H); HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{42}H_{36}O_{15}$ 803.2054, found 803.1935.

8.22. Diinsininone (33)

Benzopyrilium salt **27** (8.9 mg, 0.6 M MeOH/pH 5.8 buffer (1:1) and flavanone **9** (47 mg, 6 equiv) were heated at 120 °C for 20 h in an Emrys microwave reactor. The reaction was concentrated to dryness and submitted to pyridine and Ac_2O overnight. The reaction was quenched with 1 M HCl, repeatedly extracted with EtOAc, and dried with $MgSO_4$. The desired product was purified by gradient chromatography with EtOAc/hexane (60:40–80:20) and the desired product was isolated in 32% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57–7.48 (m, 8H), 7.24–7.19 (m, 6H), 6.74–6.72 (m, 2H), 6.51–6.50 (m, 4H), 5.60–5.51 (m, 2H), 4.47 (br s, 1H), 4.44 (br s, 1H), 3.09–3.00 (m, 2H), 2.78–2.27 (m, 2H), 2.39–2.27 (m, 40H); HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{42}H_{34}O_{16}$ 817.1847, found 817.1593.

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The power of singlet oxygen chemistry in biomimetic syntheses

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Dedicated to the memory of Christopher S. Foote, mentor and friend

Abstract—Herein, we describe selected highlights from the successful syntheses of the litseaverticillol family of natural products and from the synthesis of the core of the prunolide molecules, using powerful $^1O_2$-orchestrated biomimetic strategies. In these syntheses, cascade reaction sequences initiated by the reaction of $^1O_2$ with a furan and the ene-reaction of $^1O_2$ with double bonds together facilitated the swift assembly of the targeted compounds from simple precursors. We also introduce our most recent $^1O_2$-facilitated synthetic strategies used in our approach to the synthesis of premnalane A. In this investigation, we explore a number of different reactivities of $^1O_2$, thus completing a brief survey of how $^1O_2$ chemistry may be fruitfully employed in the synthesis of complex secondary metabolites.

1. Introduction

There is perhaps no reagent that could be said to be more synonymous with biomimetic synthetic strategies than singlet oxygen. This situation arises because in plants and living organisms four crucial prerequisites are met, which favor the production and reaction of singlet oxygen. These criteria are: (1) the presence of natural sunlight providing visible spectrum irradiation; (2) the proliferation of photosensitizers (e.g., tannins, porphyrins, and chlorophyll) in the environment; (3) pervasive molecular dioxygen ($\approx$20% of atmospheric air); and, finally, (4) an abundance of oxidizable substrates, such as terpenes, in the immediate vicinity. Biomimetic synthetic strategies, as this special edition of Tetrahedron so aptly illustrates, are admired for their efficiency in the swift construction of molecular complexity. Of particular note, are biomimetic strategies that harness cascade reaction sequences to forge core structures rapidly from simpler precursors. Here, once again, we can see how singlet oxygen is uniquely suited to the paradigm since it willingly participates in complex domino reaction sequences. In the article that follows, we hope to convince you of the veracity of all our introductory statements by giving a brief overview of our work, both past and current, employing singlet oxygen in the field of biomimetically inspired natural product syntheses.

2. Designing biomimetic syntheses using singlet oxygen

Herein, we shall see three different reactions of $^1O_2$, namely, [4+2][1] and [2+2][2] cycloadditions, and the ene-reaction.[3] Before one can consider designing a biomimetic strategy for the synthesis of any given natural product using $^1O_2$, the chief characteristics of each of the various modes of reaction of $^1O_2$ must be fully appreciated; for knowledge about the respective rates and preferences of each reaction mode is an essential prerequisite to the design of cascades that will work smoothly. As we shall soon see, we frequently encounter substrates where each of the different $^1O_2$ modes of reaction could be envisaged as being possible, and, because, $^1O_2$ is a highly reactive electrophilic species, unless we can control the order and timing of such reactions indiscriminate oxidation and degradation are the likely result. Fortunately, the reactions of $^1O_2$ have been studied extensively in simple substrates[4] providing us with key information that may now be used to extend the use of $^1O_2$ chemistry in the synthesis of the more complex molecules. Our first example of the application of a $^1O_2$-orchestrated biomimetic strategy, to the synthesis of a family of naturally occurring sesquiterpenes, the litseaverticillols, perfectly illustrates this point as selective reaction through one reaction mode at a time and regiochemical discretion are both of pivotal importance in this instance.

3. Synthesis of the litseaverticillols

The litseaverticillols are a family of related sesquiterpenes, isolated from a Vietnamese shrub, which possess interesting

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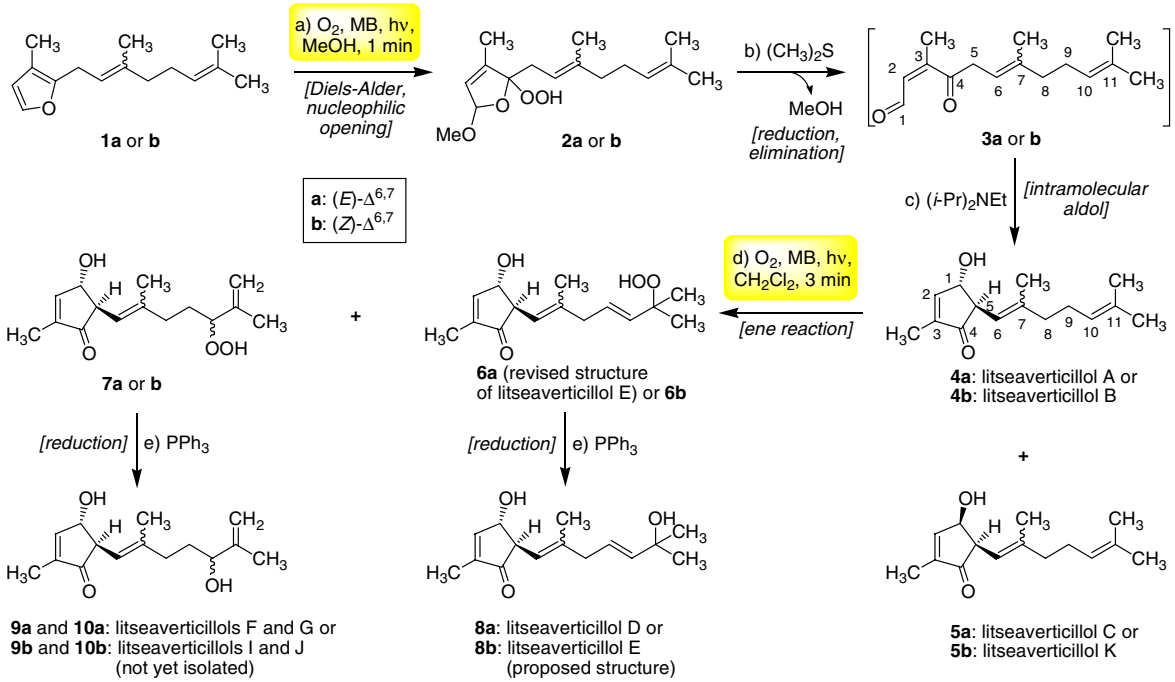
anti-HIV activity.[5] Upon close inspection of their structures, a possible biomimetic synthetic strategy presented itself to us. Our subsequent syntheses of litseaverticillols A–G, I and J[6] and the surprising structural reassignment for litseaverticillol E[7] would seem to provide ample empirical justification for the original hypothesis of their biogenesis (Scheme 1).

The structural features that informed our analysis regarding the natural origin of the litseaverticillol family are as follow: (1) the litseaverticillols could be subdivided into two generations with the second generation compounds (litseaverticillols D, E, F, G, I, and J) conceivably arising from the first generation congeners (litseaverticillols A, B, C, and K) through regioselective $^1O_2$-mediated ene-reactions taking place at the trisubstituted $\Delta^{10,11}$ bond, most distal from the 4-hydroxycyclopentenone core, of the pendent side chains. (2) likewise, the 4-hydroxycyclopentenone core could be envisaged to have been derived via a cascade reaction sequence beginning with the [4+2]-cycloaddition between a furan precursor and $^1O_2$.[8] Notably, at least one of the proposed furan precursors, sesquirosefuran (**1a**), is a known natural product.[9] Furthermore, observations made later on during our syntheses of the litseaverticillols would suggest that this single known furan **1a** might well be the natural progenitor to all the litseaverticillols (vide infra). (3) the litseaverticillols are racemates, a relatively rare occurrence in natural products (which are usually synthesized in a homochiral fashion by enzymes), prompting us to hypothesize that the entire cascade reaction sequence, which we proposed for the synthesis of the litseaverticillol core, does not take place under the orchestration of an enzyme. In summary, it was our belief that all the litseaverticillols are derived in nature from furan precursors via sequential and selective singlet oxygen mediated non-enzymatic reactions. The best way to test and refine this postulate was by synthesizing the compounds in the laboratory and so this is what we did.[6,7]

The furan precursors (**1a** and **b**) were assembled in short order.[6,7] A one-pot, five synthetic operation, biomimetic cascade was then developed (Scheme 1) that directly and efficiently furnished the first generation litseaverticillols A (**4a**), B (**4b**), C (**5a**), and K (**5b**).[10] The biomimetic cascade begins with the [4+2]-cycloaddition between the electron rich diene of the furan **1a** (or **b**) and singlet oxygen (generated using the sensitizer methylene blue and visible light irradiation for 1 min). The resultant endoperoxide adduct is then subjected to nucleophilic attack by the solvent, in this case methanol, to afford hydroperoxide **2a** (or **b**) as a single regio- and stereoisomer (as established by NOE studies). In nature the methanol must be replaced by water, thus affording the hydroxyl-analogue of **2**. Next, reduction of the hydroperoxide **2a** (or **b**) yields the anomeric hemiketals from which methanol is eliminated to furnish the achiral keto aldehyde **3a** (or **b**). Timely addition of Hünigs base to **3a** (or **b**) then promoted an intramolecular aldol reaction to furnish the first generation litseaverticillols A (**4a**) and C (**5a**) in 55% overall yield, or B (**4b**) and K (**5b**) in 51% overall yield depending on the initial substrate. It should be noted that litseaverticillols A (**4a**) and C (**5a**) exist in equilibrium with one another (A/C 19:1), as do litseaverticillols B (**4b**) and K (**5b**, B/K 20:1), thereby attesting to the reversibility of the aldol reaction (**3a** or **b**→**4a** or **b**). Furthermore, both litseaverticillols A (**4a**) and B (**4b**) could be obtained from the reaction of furan (**1b**), especially if litseaverticillol B (**4b**) was not isolated immediately but left in the basic solution for prolonged periods (>12 h), indicating that isomerization of the $\Delta^{6,7}$ bond is facile under the mildly basic reaction conditions. This isomerization most probably occurs via the retroaldol reaction of **4b** (or **5b**) to give the C-5 anion. This process yields a stabilized and extensively conjugated anion in which rotation about the



Scheme 1. Synthesis of the litseaverticillol family using a biomimetic $^1O_2$-orchestrated cascade reaction sequence.

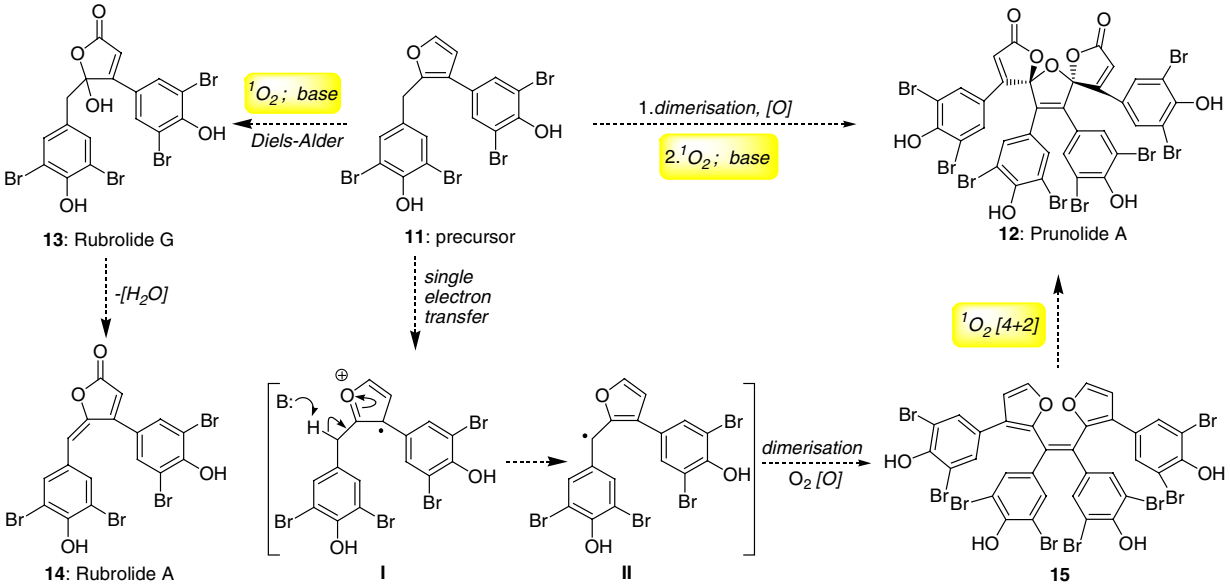
C-6/C-7 bond becomes feasible thus allowing for stereochemical scrambling. This observation is the origin of our proposed amendment of the biomimetic hypothesis to include the possibility that one furan (e.g., sesquirosefuran **1a**) could be the progenitor to all the litseaverticillols. This refinement to our proposal is in accord with the natural distribution seen for the various litseaverticillols.

The second generation litseaverticillols were then synthesized from their first generation parents using a second mode of $^1O_2$ reaction. Thus, a regioselective ene-reaction was employed to produce both of the two possible hydroperoxide-regioisomers from each substrate. A classic ene-reaction mechanism governs the formation of these two products; wherein the perepoxide intermediate forms such that the pendant oxygen atom sits preferentially over the more substituted side of the double bond, in a phenomenon known as the cis-effect,[3] it follows that there are then two positions from which a hydrogen atom can be abstracted. The ene-reaction only took place at the desired $\Delta^{10,11}$ bond, the other two, more electron deficient and/or hindered, olefins in the substrate proved to be unreactive. The hydroperoxide products were reduced to the corresponding alcohols using triphenylphosphine. Each of these so-formed alcohols represented a second generation litseaverticillol. Thus, through this two step procedure, litseaverticillol A (**4a**) fathered the tertiary alcohol litseaverticillol D (**8a**) and the diastereoisomeric secondary alcohols, litseaverticillols F and G (**9a** and **10a**, respectively). Likewise, when litseaverticillol B (**4b**) was subjected to the same two sets of reaction conditions, three new litseaverticillols were synthesized, litseaverticillols I (**9b**) and J (**10b**) [not yet isolated from natural sources, perhaps because of the low abundance of their parent, litseaverticillol B (**4b**)] and a compound possessing the structure proposed for litseaverticillol E (**8b**). In an unexpected turn of events, the spectral data we obtained for tertiary alcohol **8b** did not match those reported for litseaverticillol E.[5] After some detective work involving the reexamination of the reported spectral data for litseaverticillol E and comparison of it with spectral data for our intermediate compounds, it became obvious that the true structure of litseaverticillol E was that of the tertiary hydroperoxide **6a**.

The fact that we were able to make both the entire litseaverticillol family, systematically, and in relative ratios that reflected the natural abundance of the compounds, and reassign the structure of litseaverticillol E as being an intermediate en-route to the final products, strongly supports our biogenetic hypothesis for this sesquiterpene family. Furthermore, the two modes of reaction of $^1O_2$ that we used proved to be highly chemo- and regioselective with the [4+2]-cycloaddition occurring at a much faster rate than the subsequent ene-reaction. It is notable that other non-natural reagents (Br_2/MeOH/H_2SO_4[11] or magnesium monoperoxyphthalate[12]), known in the literature for the oxidation of furans to the corresponding (*Z*)-1,4-enediones, proved to be unselective in their reaction with our substrates, reacting both at the side chain double bonds and the furan core indiscriminately. Once again, this feature would seem to lend credence to the $^1O_2$ biogenesis hypothesis. From a practical standpoint the litseaverticillol synthesis reinforces the comment made at the beginning of this article that a good knowledge and understanding of the relative rates and selectivities for the reactions of $^1O_2$ are vital if it is to be employed successfully in complex biomimetic synthetic strategies.

4. Synthesis of the spirocyclic core of the prunolides

The prunolides are a family of architecturally beautiful cytotoxic natural products isolated in 1999 from a species of Australian colonial ascidian.[13] Our interest in these compounds was piqued not only by their compact and intricate C_2-symmetric bis-spiroketal core, but by the repeating occurrence of the butenolide moiety and by their isolation partners, the rubrolides (Scheme 2). The antibiotic rubrolide A (**14**), which was found within the same colonial ascidian extract as the prunolides, had also been isolated, along with other rubrolides, previously in 1991.[14] These latter features of interest immediately suggested a hypothesis for the



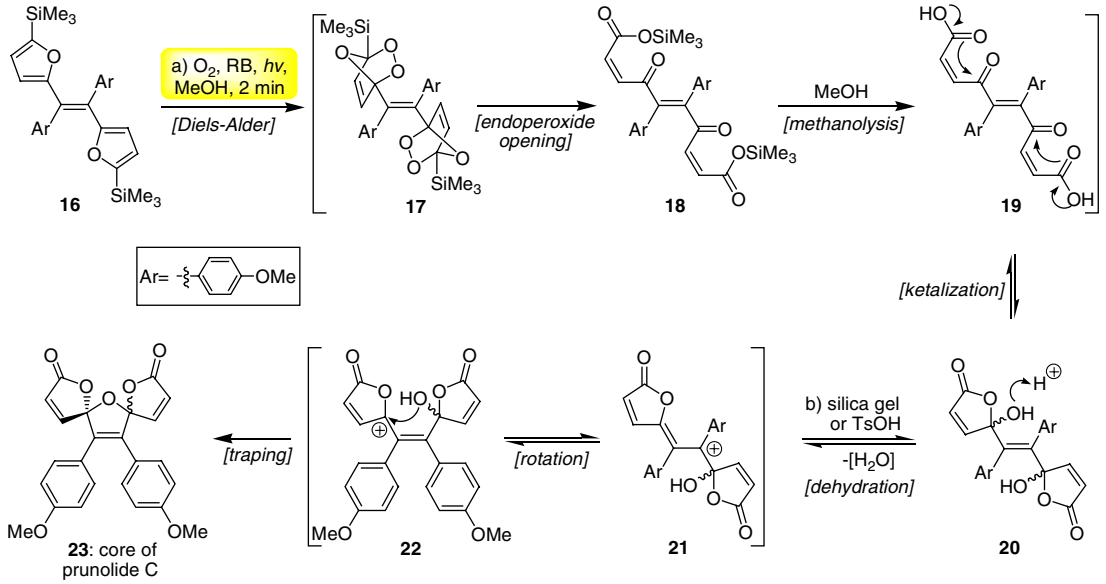
Scheme 2. Proposed biogenesis of the prunolide and rubrolide families of natural products.

prunolide/rubrolide biogenesis to us, which we intended to test through the vehicle of a laboratory synthesis.

At the heart of the biogenetic proposal lay the oxidation of furan precursors by $^1O_2$ and a key dimerization reaction that delineated the relationship, which we were proposing existed between the rubrolides and the prunolides. Thus, if we take the case of rubrolides A (**14**) and G (**13**) and prunolide A (**12**) as the example, we envisaged the existence of a common furan precursor **11** to these compounds (Scheme 2). Two different fates can reasonably be imagined for this precursor **11**. In the first, the furan moiety might be oxidized by $^1O_2$ directly to give the hydroxybutenolide rubrolide G (**13**). The production of hydroxylbutenolides from 2-substituted furans upon oxidation with $^1O_2$ is a well-known and studied reaction.[15,8b] Facile elimination of water from rubrolide G (**13**) would then furnish rubrolide A (**14**). The second possible destiny for the precursor **11** involves a single electron transfer-dimerization sequence. Thus, the furan moiety could possibly donate an electron to a single electron transfer oxidant (of which nature has an abundance) to form the radical cation intermediate I. The radical II may then form upon loss of a proton from the radical cation I. Radical II might conceivably dimerize to form a difuryl compound, which, it is reasonable to expect, might be readily oxidized by molecular dioxygen to afford the cascade precursor **15**. The envisaged cascade sequence is initiated by a double [4+2]-cycloaddition, occurring between the two furan moieties and $^1O_2$, to afford a diendoperoxide (e.g., **17**, Scheme 3) that we proposed might swiftly collapse to furnish a linear unsaturated diacid (e.g., **19**). Following double ketalisation and the elimination of a molecule of water, this diacid might yield prunolide A intact.

Excited by this biogenetic proposal, we immediately set forth on a synthetic program aimed at testing its essential postulates. We begun by working with a compound unencumbered by the peripheral functionalities in order to explore the validity of the concept.[16] A McMurray coupling was chosen to mimic the oxidative coupling step of the biogenetic proposal. Thus, from the corresponding ketone monomer (synthesized rapidly from furan itself[16]), dimer **16** and its *Z*-isomer were synthesized in good yield (72%, $Z/E \approx 1{:}3$) using the standard McMurray coupling conditions. Both the isomers, which were easily separated, were then investigated in the biomimetic reaction cascade sequence, however, for ease of discussion we have chosen to represent only the more interesting (vide infra) *E*-isomer in the scheme delineating the cascade outcome (Scheme 2).

Nature certainly does not include silicon groups in her substrates for the photooxygenation reactions, so why did we? It is known that the unsubstituted furans (where H replaces $SiMe_3$) do undergo the desired [4+2]-cycloaddition reaction[8] with $^1O_2$, however, the transformation of the resultant endoperoxide into the hydroxybutenolide using base[17] is known to be problematic.[18] This problem was confirmed in our case when we first tested the unsubstituted analogue of **16** in the photooxygenation cascade sequence. As a result, we were prompted to include the trimethylsilyl groups from the start. When 1,2-difuryl alkene **16** was subjected to standard photooxygenation conditions (10^{-4} M Rose Bengal as senitizer, O_2, MeOH, and visible spectrum irradiation) for 2 min the beautiful biomimetic cascade took place just as predicted (Scheme 3). Endoperoxide **17** was rapidly transformed through **18**[19] to the linear diacid **19**. The intermediary and labile bis-hydroxybutenolides **20** were observed by ^1H NMR spectroscopy. Upon treatment of butenolides **20** with traces of acid (TsOH), or on contact with silica gel, two readily separable bis-spiroketal products **23** were obtained in high yield (80% overall from **16**). The bis-spiroketals were a mixture of the cis and trans isomers (cis/trans $\approx 1{:}2$), the trans isomer representing the fully intact prunolide core. It should be noted that the *Z*- and *E*-isomer (**16**) of the starting 1,2-difuryl alkene compound produced identical results from the cascade sequence, indicating that the central double bond of **16** is the subject of isomerization during the course of this sequence (**21**→**22**). Hence we were able to access the prunolide core with remarkable ease from a simple dimer via an oxidative



Scheme 3. Synthesis of the core of the prunolide molecules using a biomimetic $^1O_2$-mediated cascade sequence.

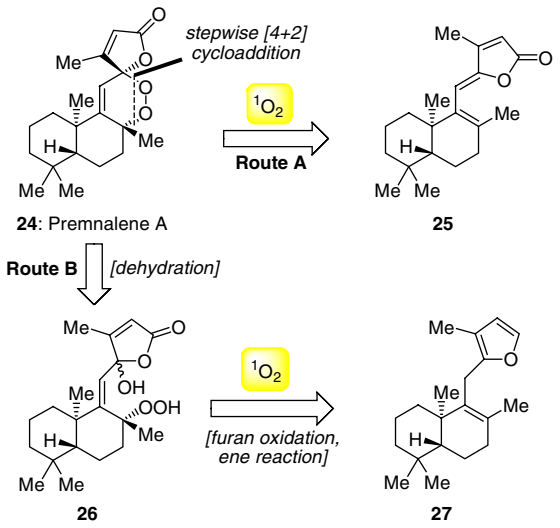
cascade sequence orchestrated by $^1O_2$ during which a linear molecule was zipped up to form this complicated bis-spiroketal core. Unfortunately, the venerable McMurray coupling has not proven to be robust enough to tolerate the more highly functionalized ketone monomers required to apply the elaborated cascade to the total syntheses of all the various prunolides. At present a modified approach to these molecules is, therefore, under investigation in our laboratories.

5. Towards the synthesis of premnalane A

Simple success stories are frequently less instructive than the analogous tales relating surprising and unpredicted results. For the latter can, and often do, inspire new approaches and strategies that otherwise would have remained unexplored. We shall now turn our attention to some recent results obtained in our laboratory, which, although not proceeding quite as planned, have thrown up some didactic observations and very useful ideas that we hope to convert into a new series of $^1O_2$ biomimetic syntheses in the near future.

Premnalane A (**24**, Scheme 4)[20] was isolated in 1991 from a shrub growing at high altitude in the Sidamo Province of Ethiopia. Its gross structure, as revealed by X-ray crystallography, was shown to be based upon an enantiomer of the known labdane skeleton. We were immediately attracted to this synthetic target because of its obvious $^1O_2$ roots. The six-membered peroxide ring bearing a spirocyclic unsaturated lactone was highly suggestive of a $^1O_2$-mediated cascade sequence. Our first retrosynthetic analysis for the key biomimetic $^1O_2$-orchestrated cascade is shown in Scheme 4 (Route A, **24**→**25**). In this analysis, we envisaged that the last step of the synthetic sequence would be a stepwise [4+2]-addition. Although rarer than their concerted cousins, stepwise [4+2]-additions are known, especially in cases where the diene partner cannot easily adopt a planar *s*-cis conformation (true of the hindered diene we were proposing).[21] The intermediate in the stepwise reaction may be either a biradical, or a bipolar species.[22] We proposed a stepwise [4+2]-addition to construct premnalane A's endoperoxide ring not only due to the hindrance of the starting diene, but also because of the trans-stereochemistry desired in the resultant endoperoxide. In order to test our hypothesis, we set forth on a program directed towards the synthesis of $^1O_2$-precursor **25**. It should be noted that it was clear to us from the beginning that a different series of $^1O_2$ reactions might be responsible for the assembly of premnalane A (Scheme 4, Route B). However, only a laboratory study of the various possibilities could shed light on which series of reactions likely to have been used by Nature herself. With this in mind we sought to introduce flexibility, at as many stages as possible, into our synthesis of the photooxygenation precursors.

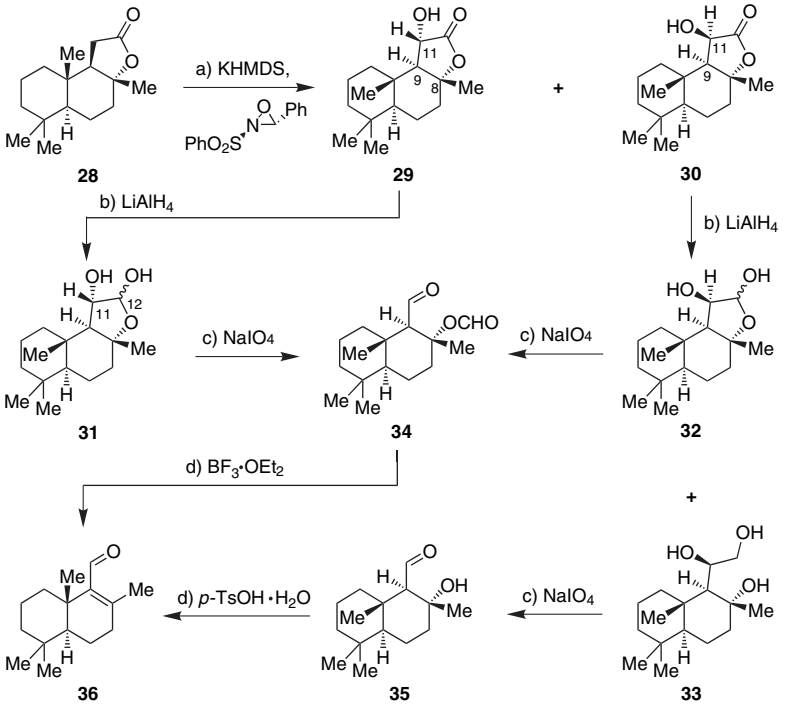
The decalin system of (+)-sclareolide (**28**), a commercially available compound, provided us with a suitable starting point for an initial investigation into our proposed biomimetic strategy for the synthesis of premnalane A. (+)-Sclareolide (**28**) possesses the enantiomeric stereochemistry at the decalin ring junction from premnalane A, but for the purposes of our initial investigations this was not important. We began with the installation of a hydroxyl group α to the



Scheme 4. Retrosynthetic analyses delineating the possible biogenetic origins for premnalane A.

lactone moiety of **28** by reaction of the enolate with Davis oxaziridine[23] (Scheme 5). This reaction proceeded in good yield (93%), with KHMDS as base, to afford a separable mixture of diastereomeric products, **29** and **30** (**29**:**30**≈1:1). The stereochemistry of **29** and **30** was assigned based on the coupling constant of the interaction between the adjacent C-9 and C-11 hydrogens, with the trans-relationship present in the more polar compound **29** having a larger value. These details proved important when we later became aware of the work of Quideau et al. in a similar system.[24] During their work towards the marine sponge metabolite, (+)-puupehenone, they took (+)-sclareolide (**28**) and first epimerized the C-8 stereocenter under acidic conditions. Following this epimerization, their attempts to introduce a hydroxyl group at C-11 (using Vedejs' $MoO_5\cdot$pyridine$\cdot$HMPA reagent system) were fraught with difficulties. LDA failed to deprotonate the precursor and they had to resort to use of magnesium bis(diisopropylamide) as base. Notably, only one C-11 hydroxyl diastereoisomer was seen in their study.

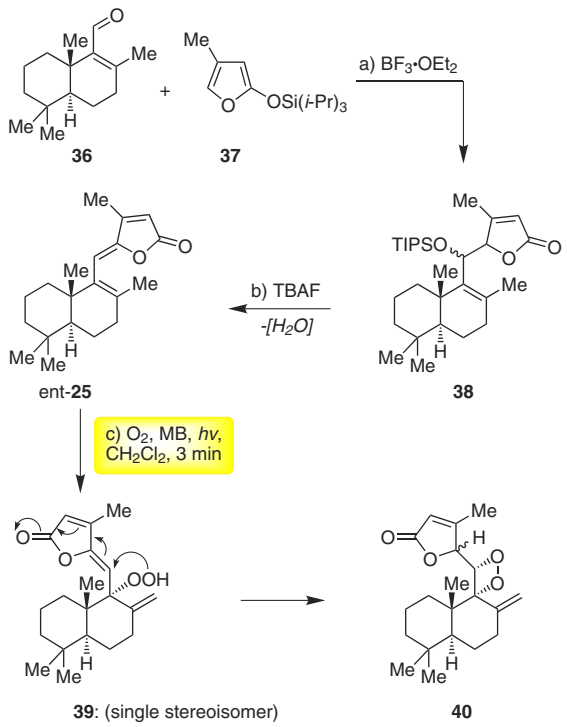
We next wished to reduce the newly acquired hydroxylactones, **29** and **30**, to triol **33** and its diastereoisomer. Surprisingly, however, upon treatment of **29** with $LiAlH_4$, diastereomeric lactols **31** were the sole products formed (H-11, H-12 trans/cis≈1.5:1). Use of excess $LiAlH_4$ had no effect on the outcome. Conversely, when hydroxylactone **30** was treated with $LiAlH_4$ a mixture of diastereomeric lactols **32** and triol **33** resulted (**32a**:**32b**:**33**≈1:1:2). The product ratio in this case was also unaffected by the amount of $LiAlH_4$ employed. Quideau et al.[24] had attempted to reduce their hydroxylactone (differing from **29** in the stereochemistry at C-8) to the corresponding triol using $LiAlH_4$, but had found that the reaction stopped at the intermediate lactol and could not be forced further. When they switched reducing agent and employed Dibal-H for the reduction a mixture of the lactol and the desired triol (lactol/triol≈1.2:1) was obtained, albeit with a low yield. We next examined the oxidative cleavages, using silica-supported $NaIO_4$,[25] of substrates **31**, **32**, and **33**. Cleavage of the lactols **31** and **32** furnished the formate **34**, whilst triol **33** afforded the aldehyde **35**. The divergence of the synthesis that we



Scheme 5. Preparation of α,β-unsaturated aldehyde **36**.

now had was of no concern to us because we were able, following the dehydration of **34** and **35** (combined with formate hydrolysis in the former case), to converge upon a single compound, the α,β-unsaturated aldehyde **36**. Furthermore, we optimized the sequence such that the mixtures obtained from the preceding reactions (installation of hydroxyl functionality and reduction) could be carried through the subsequent transformations without separation up to the dehydration step. Thus, reduction with $LiAlH_4$ of the mixture of diastereoisomers **29** and **30** afforded a mixture of **31**, **32**, and **33** (overall yield 93%) that was subjected, without separation, to oxidative cleavage using silica-supported $NaIO_4$ to furnish a mixture of **34** and **35** in 92% combined yield. Hydroxy aldehyde **35** could then be dehydrated by warming it up in toluene in the presence of catalytic *p*-TsOH to furnish α,β-unsaturated aldehyde **36** (yield 54%). In a similar fashion, formate **34** could be hydrolyzed and dehydrated using $BF_3 \cdot OEt_2$ at ambient temperature to afford **36** in excellent yield (95%).

With α,β-unsaturated aldehyde **36** in hand, we next sought to install the requisite unsaturated lactone moiety. This task was readily accomplished by using a $BF_3 \cdot OEt_2$-mediated Mukaiyama aldol to couple the aldehyde **36** with an excess of 2-triisopropylsilyloxyfuran **37** to give diastereoisomeric unsaturated lactones **38** (3:1 mixture of isomers) in a yield of 63% (Scheme 6). Deprotection with concomitant dehydration of **38**, under the influence of TBAF, afforded the $^1O_2$ reaction precursor diene *ent*-**25** as a single geometric isomer in high yield (89%). The stage was now set to test the hypothesis regarding the stepwise [4+2]-addition of singlet oxygen to the diene. When diene *ent*-**25** was treated with $^1O_2$, generated using methylene blue as a sensitizer and visible spectrum irradiation, in dichloromethane for 3 min, diastereomeric dioxetanes **40** were the only products (isolated yield 96%, major/minor isomer ≈ 1.5:1). Once again, just as we saw in the synthesis of the litseaverticillols (vide supra), this result underscores the importance of garnering an intricate knowledge about the relative rates and preferences of the possible modes of $^1O_2$ reaction in a given substrate. For, without this information the correct biomimetic cascade sequence toward the synthetic target cannot readily be identified. In this instance, the desired product (i.e., *ent*-

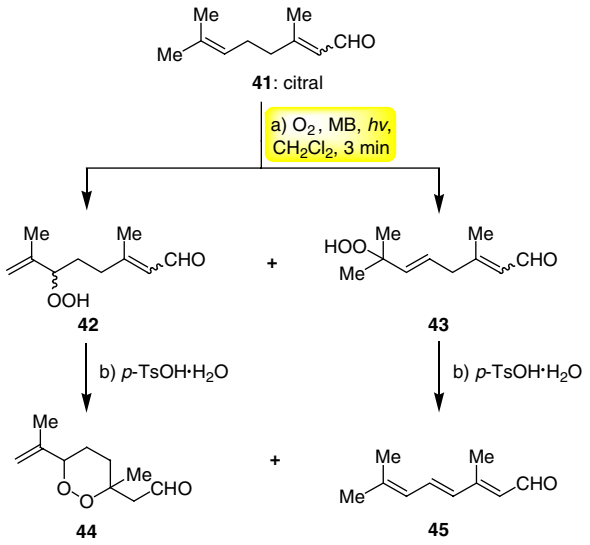


Scheme 6. Abortive attempts to synthesize *ent*-premnalane A: synthesis of dioxetane **40**.

premnalane A *ent*-**24**) was not obtained because a stereoselective ene-reaction between $^1O_2$ and the endocyclic double bond of *ent*-**25** was faster than the corresponding stepwise [4+2]-addition reaction. The product of the ene-reaction, hydroperoxide **39**, then underwent an intramolecular conjugate addition reaction to afford **40**. If the reaction was carried out in benzene at 6 °C, intermediate hydroperoxide **39** could be separated by column and analyzed by ^{1}H NMR spectroscopy, because a mixture of **39** and **40** was obtained (**39**:**40** ≈ 1:1.6).

We have now redesigned and refined our hypothesis regarding the details of the biomimetic cascade sequence, which might afford premnalane A (**24**), taking into account the new information that was revealed by our initial foray. Thus, we now believe that premnalane A (**24**) might arise in nature when a furan precursor, such as **27** (Scheme 4), is subjected to a $^1O_2$-orchestrated cascade reaction sequence. We expect based upon our litseaverticillol work that first the furan moiety of **27** will undergo a [4+2]-cycloaddition with $^1O_2$. In the presence of a base the labile endoperoxide so-formed should collapse to afford the hydroxybutenolide.[17] We then anticipate an ene-reaction might occur with the endocyclic double bond to regio- and stereoselectively form hydroperoxide **26**. It is our postulate that the negative steric interactions between the methyl group, situated at the ring junction, and the butenolide moiety of the ene-reaction substrate will force the latter group to sit above the face of the decalin system opposite to this large axial group. This confirmation will then govern the stereoselectivity of the initial addition of $^1O_2$ to the double bond through steric and electronic interactions.[3,26] Modeling and mechanistic precedent regarding the cis-effect[3] and the large group effect[27] would indicate that hydrogen abstraction from this intermediate would then occur to yield regioisomer **26** exclusively.

This new and exciting analysis of the biogenetic origins of premnalane A (**24**) has now become the subject of an investigation in our laboratory and we hope to be in a position to communicate the initial results soon. Meanwhile, the fact that we obtained isomer **40** during our first foray towards premnalane A (**24**) has prompted us to explore this type of conjugate addition further. A host of interesting and biologically active natural products contain five[28]- or six[28,29]-membered endoperoxide rings. We propose that this motif arises in nature following conjugate addition of the hydroperoxide obtained from the ene-reaction between $^1O_2$ and a specified fatty acid, or terpenoid, unsaturated precursor. We have now completed a proof of concept study for this hypothesis (Scheme 7), and, intend, in the near future, to apply it towards the synthesis of a new set of natural products.



Scheme 7. Proof of principle: synthesis of six-membered peroxide moieties using a biomimetic $^1O_2$-faciltated approach.

6. Conclusion

Herein, we have described highlights from the successful syntheses of the litseaverticillol family of natural products and from the synthesis of the core of the prunolide molecules, using powerful $^1O_2$-orchestrated biomimetic strategies. In these syntheses, cascade reaction sequences initiated by the reaction of $^1O_2$ with a furan and the ene-reaction of $^1O_2$ with double bonds proved to be crucial tools that allowed the respective molecules to be rapidly assembled from simple precursors. We also introduced our most recent $^1O_2$-facilitated synthetic strategies used in our approaches to the synthesis of premnalane A. Here we use a number of different reactivities of $^1O_2$, thus completing a brief survey of how $^1O_2$ chemistry may be fruitfully employed in the synthesis of complex secondary metabolites.

$^1O_2$ is a benign and environmentally sound biomimetic reagent that is extremely versatile in a synthetic capacity. Furthermore, the use of $^1O_2$ avoids unnecessary waste because protecting groups are rarely, if ever, required in $^1O_2$ reaction cascades. Despite the obvious utility of $^1O_2$, its application in natural product synthesis is rarer than might be expected. One suspects the reason being that the relative rates and chief characteristics of its various modes of reaction are not widely appreciated. We hope that our examples described herein will go some way to rectify this situation so that the beautiful and powerful chemistry of $^1O_2$ will in the future find many more applications in biomimetic natural product syntheses.

7. Experimental

7.1. Diastereomeric 11-hydroxysclareolides 29 and 30

To KHMDS (1.53 g, 7.68 mmol), in anhydrous THF (40 mL), under argon, and at −20 °C, was added dropwise a solution of sclareolide (1.20 g, 4.8 mmol) in anhydrous THF (40 mL). The reaction mixture was allowed to warm from −20 to −10 °C over 50 min. Afterwards the reaction mixture was recooled to −30 °C and a solution of Davis oxaziridine (2.13 g, 8.16 mmol) in anhydrous THF (50 mL) was added dropwise. The mixture was then allowed to warm from −30 to −10 °C over 40 min. The reaction was quenched upon addition of H_2O (4 mL), warmed to 0 °C, and Et_3N (4 mL) added. After stirring for 5 min, 5% aq HCl (150 mL) was added and stirring was continued for further 20 min. The reaction mixture was diluted with Et_2O, washed with saturated aq Na_2CO_3 and then brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane/

EtOAc 6:1→2:1) to afford 0.59 g of the less polar epimer **30** and 0.60 g of the more polar epimer **29** (93% combined yield).

7.2. Lactols 31 and 32, and triol 33

To a solution of $LiAlH_4$ (228 mg, 6.0 mmol) at 0 °C in anhydrous THF (5 mL) was added dropwise a solution of the two diastereomeric 11-hydroxysclareolides **29** and **30** (1.04 g, 3.91 mmol) in anhydrous THF (10 mL). The reaction mixture was allowed to warm to ambient temperature with stirring over 30 min, before a few drops of EtOAc were added as a quench. The reaction mixture was diluted with EtOAc and washed two times with a saturated solution of Rochelle's salt. The combined aqueous layers were extracted with EtOAc. The combined organic layers were then dried (Na_2SO_4) and concentrated in vacuo. The crude material was employed in the next step without further purification (0.98 g, 93%).

7.3. Formate 34 and hydroxyl aldehyde 35

A suspension of silica gel-supported $NaIO_4$ reagent (7.34 g) was stirred vigorously in dry CH_2Cl_2 (18 mL). To this suspension was added a dropwise solution of the crude mixed of **31**, **32**, and **33** obtained from the previous reaction (see above) in dry CH_2Cl_2 (18 mL). The reaction mixture was stirred for 5 min. The mixture was then filtered through a sintered glass funnel to remove the silica gel, which was washed with copious quantities of EtOAc. The solvent was removed from the combined filtrates and the residue purified by flash column chromatography (silica gel, hexane/EtOAc 9:1→4:1) to afford two products—formate **34** (0.0.69 g, 71%) and hydroxyl aldehyde **35** (0.18 g, 21%).

34: ^{1}H NMR (500 MHz, $CDCl_3$): δ=9.98 (d, J=3.9 Hz, 1H), 7.91 (s, 1H), 2.55 (td, J_1=12.8 Hz, J_2=3.5 Hz, 1H), 2.49 (d, J=3.9 Hz, 1H), 1.85 (s, 3H), 1.84 (m, 1H), 1.76 (m, 1H), 1.64 (m, 2H), 1.42 (m, 3H), 1.20 (m, 2H), 1.17 (s, 3H), 0.99 (dd, J_1=12.4 Hz, J_2=2.1 Hz, 1H), 0.88 (s, 3H), 0.82 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=204.0, 159.8, 85.6, 68.6, 54.8, 41.4, 39.7, 39.6, 38.8, 33.2, 33.0, 22.0, 21.3, 19.8, 17.9, 17.0 ppm.

35: ^{1}H NMR (500 MHz, $CDCl_3$): δ=9.98 (d, J=1.4 Hz, 1H), 3.20 (br s, OH), 2.04 (br s, 1H), 1.90 (br d, J=12.6 Hz, 1H), 1.78 (td, J_1=12.6 Hz, J_2=3.2 Hz, 1H), 1.66 (m, 2H), 1.44 (m, 3H), 1.35 (s, 3H), 1.29 (dq, J_1=12.3 Hz, J_2=3.2 Hz, 1H), 1.17 (tt, J_1=13.3 Hz, J_2=3.8 Hz, 2H), 1.08 (s, 3H), 0.93 (dd, J_1=12.2 Hz, J_2=2.0 Hz, 1H), 0.86 (s, 3H), 0.80 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=208.0, 72.7, 71.2, 55.0, 42.7, 41.5, 39.7, 37.3, 33.2, 33.1, 25.2, 21.3, 19.8, 18.1, 17.4 ppm.

7.4. α,β-Unsaturated aldehyde 36 (from formate 34)

To a solution of formate **34** (0.43 g, 1.62 mmol) in dry CH_2Cl_2 (60 mL) was added dropwise $BF_3\cdot Et_2O$ (0.1 mL, 0.81 mmol). The reaction mixture was allowed to stir for 24 h at ambient temperature. The mixture was washed with saturated aq $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo to afford the α,β-unsaturated aldehyde **36** (0.34 g, 95%).

7.5. α,β-Unsaturated aldehyde 36 (from hydroxy aldehyde 35)

To a solution of hydroxy aldehyde **35** (30 mg, 0.126 mmol) in toluene (2 mL) in a sealed tube was added p-TsOH·H_2O (3.0 mg, 12 mol %). The reaction mixture was allowed to stir at 50 °C for 1 h. It was then diluted with Et_2O and washed two times with saturated aq $NaHCO_3$ and with brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc 8:1→5:1) to afford α,β-unsaturated aldehyde **36** (15 mg, 54%).

36: ^{1}H NMR (500 MHz, $CDCl_3$): δ=10.04 (s, 1H), 2.55 (br d, J=12.0 Hz, 1H), 2.26 (m, 2H), 2.02 (s, 3H), 1.71 (m, 1H), 1.62 (m, 1H), 1.45 (m, 3H), 1.18 (s, 3H), 1.17 (m, 1H), 1.08 (dd, J_1=12.6 Hz, J_2=1.9 Hz, 1H), 0.97 (dt, J_1=13.2 Hz, J_2=3.7 Hz, 1H), 0.89 (s, 3H), 0.86 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=192.6, 153.5, 143.6, 51.5, 41.5, 37.5, 36.5, 36.2, 33.4, 33.2, 21.6, 20.1, 19.1, 18.8, 18.2 ppm.

7.6. Mukaiyama aldol product 38

To a solution of α,β-unsaturated aldehyde **36** (110 mg, 0.5 mmol) in dry CH_2Cl_2 (5 mL) was added a solution of 2-triisopropylsilyloxyfuran **37** (378 mg, 1.5 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was cooled to −78 °C and $BF_3\cdot Et_2O$ (63 μL, 0.5 mmol) was added dropwise. The resulting mixture was allowed to warm to −40 °C and it was then quenched with saturated aq $NaHCO_3$. Following this quench, the reaction mixture was allowed to warm to ambient temperature. The solution was diluted with CH_2Cl_2 and washed two times with saturated aq $NaHCO_3$ and then brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc 8:1→6:1) to afford the coupled product **38** (150 mg, 63%).

7.7. Diene *ent*-25

To a solution of **38** (70 mg, 0.15 mmol) at 0 °C in anhydrous THF (2 mL), under an argon atmosphere, was added TBAF (0.3 mL, 1.0 M solution in anhydrous THF). The resulting solution was allowed to warm to ambient temperature and was then stirred for a further 12 h. The solution was diluted with Et_2O and washed with brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc 20:1→18:1) to afford the diene *ent*-**25** (40 mg, 89%).

ent-**25**: ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.89 (s, 1H), 5.75 (br s, 1H), 2.18 (s, 3H), 2.13 (br m, 2H), 1.70 (br m, 1H), 1.60 (br m, 3H), 1.53 (s, 3H), 1.43 (m, 3H), 1.20 (m, 2H), 1.02 (s, 3H), 0.90 (s, 3H), 0.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.0, 154.4, 150.1, 134.5, 132.4, 115.7, 110.2, 50.9, 41.6, 38.5, 38.2, 33.3 (2C), 33.1, 21.7, 21.4, 20.4, 18.9, 18.6, 12.2 ppm.

7.8. Diastereomeric dioxetanes 40

A solution of diene *ent*-**25** (20 mg, 0.066 mmol) in CH_2Cl_2 (3 mL), containing methylene blue (10^{-4} M), was placed

in a test tube with O_2 gently bubbling through it. Irradiation with a Xenon Variac Eimac Cermax 300 W lamp for 2.5 min at –40 °C leads to complete tranformation of the starting material (based on TLC). The solvent was removed in vacuo to yield the diastereomeric dioxetanes **40** (21 mg, 96%).

Alternatively, the reaction could be carried out in benzene using TTP (tetraphenylporphyrin) as sensitizer. In this case, irradiation for 3 min at 6 °C afforded a mixture of **39**:**40** that could be separated and purified by column chromatography (silica gel, hexane/EtOAc 10:1→6:1)

39: ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.71 (s, –OOH), 5.97 (s, 1H), 5.50 (s, 1H), 5.14 (s, 1H), 4.83 (s, 1H), 2.62 (dt, J_1=13.4 Hz, J_2=8.1 Hz, 1H), 2.35 (qd, J_1=13.4 Hz, J_2=2.3 Hz, 1H), 2.21 (d, J=1.3 Hz, 3H), 1.99 (dd, J_1=12.8 Hz, J_2=3.0 Hz, 1H), 1.75 (m, 2H), 1.50–1.13 (m, 6 H), 1.01 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=169.0, 155.9, 149.5, 147.9, 115.8, 113.5, 110.3, 92.3, 46.0, 45.2, 41.6, 33.9, 33.8, 33.7, 32.5, 23.1, 22.5, 18.9, 18.3, 12.5 ppm.

40: ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.83 (br t, J=1.4 Hz, 1H, minor), 5.79 (br t, J=1.4 Hz, 1H, major), 5.78 (d, J=9.5 Hz, 1H, minor), 5.60 (d, J=9.5 Hz, 1H, major), 5.06 (br d, J=1.5 Hz, 1H major plus 1H minor), 4.91 (br t, J=1.8 Hz, 1H, major), 4.80 (d, J=9.5 Hz, 1H, major), 4.76 (d, J=9.5 Hz, 1H, minor), 4.54 (br t, J=1.9 Hz, 1H, minor), 2.55 (qd, J_1=13.0 Hz, J_2=2.2 Hz, 1H, major), 2.50 (qd, J_1=13.0 Hz, J_2=2.2 Hz, 1H, minor), 2.15 (m, 1H, minor), 2.00 (s, 3H, minor), 1.96 (s, 3H, major), 1.79 (m, 1H major plus 1H minor), 1.68–1.06 (m, 9H major plus 8H minor), 0.99 (s, 3H major plus 3H minor), 0.89 (s, 3H, major), 0.88 (s, 3H, minor), 0.87 (s, 3H, major), 0.86 (s, 3H, minor) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, major isomer): δ=173.9, 169.0, 162.5, 144.6, 116.3, 113.4, 111.9, 83.4, 54.1, 42.4, 41.7, 37.7, 37.5, 34.2, 33.6, 23.5, 22.2, 22.1, 19.9, 14.3 ppm; ^{13}C NMR (125 MHz, $CDCl_3$, minor isomer): δ=173.5, 169.0, 161.5, 146.3, 117.1, 112.7, 112.5, 82.7, 53.2, 42.2, 41.8, 37.5, 37.2, 34.2, 33.5, 23.6, 22.8, 22.5, 21.2, 14.3 ppm.

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Synthetic ventures inspired by biosynthetic hypotheses: the evolution of a method for the oxidative amidation of phenols

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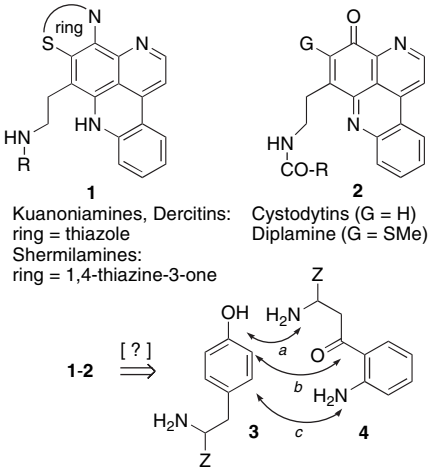
Abstract—We describe the development of a technique for the oxidative conversion of 4-alkyl phenols to derivatives of the corresponding 4-alkyl-4-amino-2,5-cyclohexanediones. This transformation, which was inspired by biogenetic considerations, constitutes a key step in the total syntheses of FR-901483, TAN-1251C, and cylindricine C.

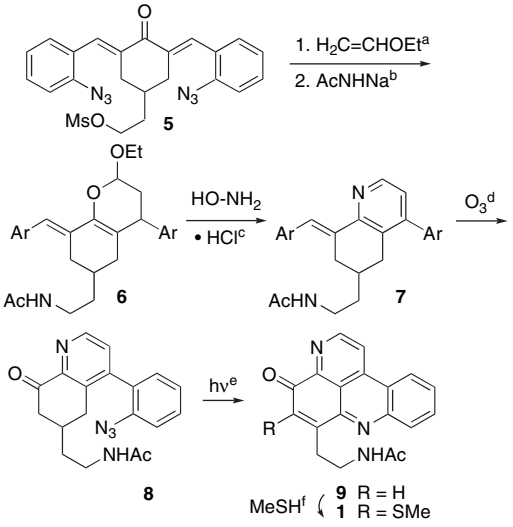
1. Introduction

The senior author of this article discovered the fascinating world of biosynthesis through an elective course in bioorganic chemistry that he attended as a graduate student.[1] Since then, almost as a reflex, he has routinely engaged in the exercise of imagining how an organism's biosynthetic machinery could possibly assemble the structure of an architecturally appealing natural product, even though he is not involved in the study of biosynthesis per se.

Such an exercise does not constitute mere intellectual overkill. Indeed, biogenetic considerations often suggest interesting strategies for the synthesis of structurally novel natural products, and may even lead to the development of valuable new methodology. For instance, our work on pyridoacridine alkaloids[2] was inspired by the supposition that the ring system of these substances, which are exemplified by structural types **1**–**2** in Scheme 1, may result through oxidative condensation of a molecule of tyramine (**3**, Z=H, a product of degradation of tyrosine, Z=COOH) with one of kynurenin or kynuramine (**4**, Z=COOH or Z=H), as outlined in Scheme 1. This surmise, details of which may have been misconstrued as embodying claims,[3] led to the development of techniques that accomplish the equivalent of bond-forming processes *a*–*c* through a new pyridine-forming reaction (*a*–*b*)[4] and a Meth-Cohn nitrene insertion into a C–H bond (*c*). Scheme 2



Scheme 1.



Scheme 2. (a) cat. $Yb(fod)_3$, $(CH_2Cl)_2$, reflux, 99%; (b) NaH, DMF, 97%; (c) MeCN, reflux, 62%; (d) O_3, CH_2Cl_2/MeOH, −78 °C, then Me_2S, 67%; (e) 250 W sunlamp, PhCl, 110 °C, then titration with DDQ, 30%; (f) 4:1 CH_2Cl_2/AcOH, then removal of volatiles and titration with DDQ in CH_2Cl_2, 94%.

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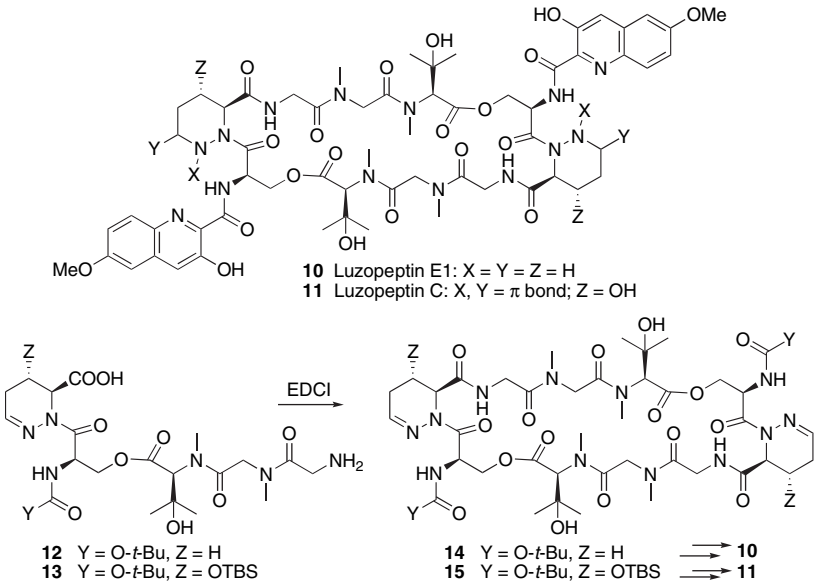


Scheme 3.

highlights these methods (**5**→**7** and **8**→**9**, respectively) in the context of the total synthesis of diplamine.[5]

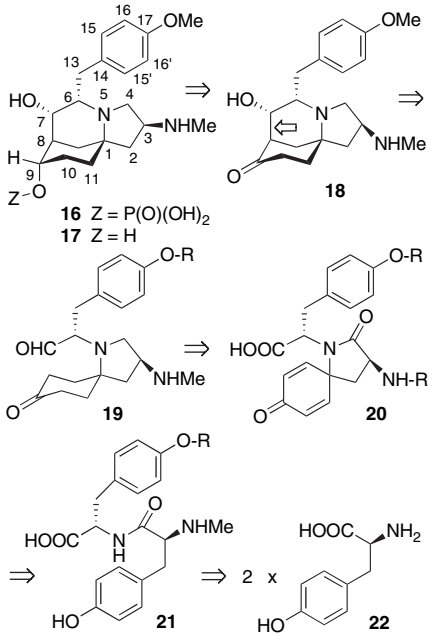
Other times, the pursuit of a 'biomimetic' approach to a target molecule may reveal unexpected chemical properties of an advanced intermediate that greatly facilitate endgame planning. This is what happened during our synthesis of luzopeptins (Scheme 3). Our strategy was influenced by the hypothesis that the macrocyclic portion of **10**–**11** is likely to emerge upon enzymatic cyclodimerization of a pentapeptide of the type **12**–**13**. It was ultimately discovered that C-terminus activation of **12**–**13** themselves triggers spontaneous assembly of macrocycles **14**–**15**. Yields are moderate (25% and 26%, respectively), but comparable to those observed in a stepwise sequence relying on preparation and cyclization of a discrete linear decapeptide.[6] Substances **14**–**15** were elaborated to luzopeptins E1 and C, respectively.[7]

2. FR-901483 and TAN-1251: development of a novel oxidative amidation of phenols

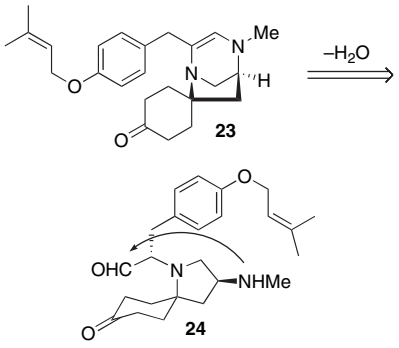
Unique opportunities for the development of methodology materialized in the mid-1990s with the discovery of a fungal metabolite termed FR-901483, **16** (Scheme 4).[8,9] This remarkable compound displays potent immunosuppressive activity, which is intimately associated with the presence of the labile C-9 phosphate ester. Unfortunately, phosphatases rapidly convert **16** to the corresponding diol **17**, which is inactive. Prospects for further development of **16** as a drug, therefore, seem modest.

Regardless, the structural novelty of **16** provided an irresistible opportunity for biogenetic speculation, and the fact that many nitrogenous natural products derive from aminoacids led to the conclusion that FR-901483 must be a dimer of tyrosine. Indeed, if the oxygen atom anchoring the phosphate ester were at some point part of a keto group, then the C-7–C-8 bond in the hypothetical intermediate **18** could be created through a regio- and diastereoselective intramolecular aldol cyclization of ketoaldehyde **19**, R=Me. The latter aldehyde might well emanate from biosynthetic precursor **20**, which is the product of oxidative spirolactamization of tyrosinyltyrosine **21**. If one could duplicate the conversion of **21** to **20** in the laboratory, then the synthesis of **16** would become relatively straightforward, and it could be carried out using enantiopure, inexpensive tyrosine, **22**, as the starting material.

An analogous reaction might also facilitate the synthesis of a group of fungal metabolites discovered at the Takeda Pharmaceutical Co. in Japan, and christened the TAN-1251 family of compounds.[10] These substances appear to share a common biogenetic precursor with **16** in the form of a variant of aldehyde **19** wherein R=prenyl. This material, described in Scheme 5 as compound **24**, may advance to TAN-1251C, **23**, via intramolecular enamine formation.

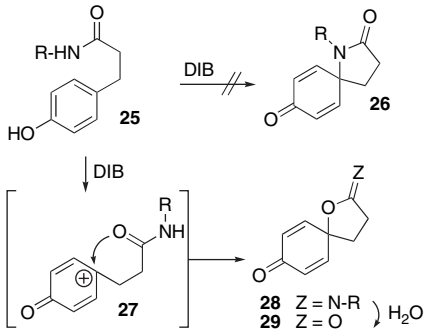


Scheme 4.



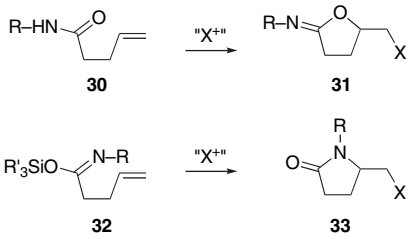
Scheme 5.

A search of the literature revealed that the oxidative cyclization of phenolic amides such as **21** to spirolactams of the type **20** was unknown,[11] even though the interest (and the synthetic potential!) of this transformation had obviously been recognized years earlier. For instance, in 1987 Kita described the reaction of **25** with $PhI(OAc)_2$ ('DIB'), or $PhI(OCOCF_3)_2$ ('PIFA').[12] Perhaps the intent was to reach spirolactams of the type **26**. However, oxidative attack of **25** furnished only lactone **29**, arguably through capture of an electrophilic intermediate arising from the phenol, and naively represented in Scheme 6 as **27**, by the carbonyl oxygen of the amide group. Hydrolysis of the intervening iminolactone **28** during workup then produces **29**.



Scheme 6.

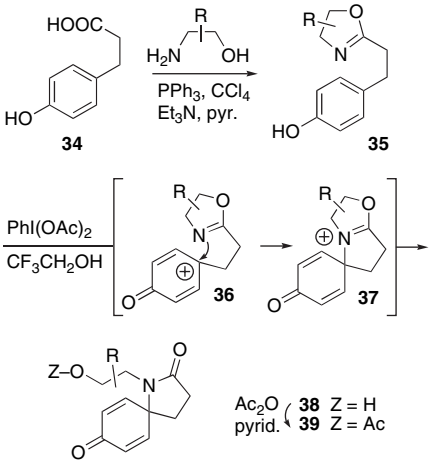
Knapp encountered analogous difficulties during work on the iodo-amidation of olefins.[13] Thus, reaction of **30** with I_2 resulted in the formation of **31** instead of the desired **33** (Scheme 7). These observations, and Kita's, are consistent with the notion that resonance interactions between the N atom and the carbonyl system in an amide promote accumulation of electronic density on the oxygen atom, which therefore becomes nucleophilic at the expenses of the N atom. Knapp circumvented such difficulties by the use of iminoethers **32** as substrates for iodolactamization. Resonance



Scheme 7.

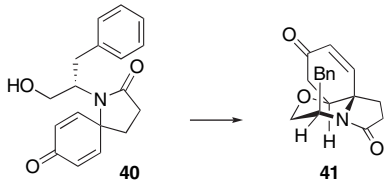
interactions in **32** now promote accumulation of electronic density on the nitrogen atom, which therefore becomes capable of expressing the desired nucleophilic reactivity.

It seemed to us that the same logic could be put to profit in our case, provided that an iminoether-type functionality resistant to the action of oxidants (e.g., DIB) could be identified. Oxazolines ultimately emerged as suitable amide equivalents in these transformations.[14] Such heterocycles are prepared by condensation of a phenolic carboxylic acid with a suitable 1,2-aminoalcohol. The Vorbrüggen oxazoline synthesis[15] proved to be especially effective for this purpose because, contrary to other methods for oxazoline formation,[16] it required no protection of the phenol. This removed the need for a supplementary protection/deprotection sequence. An initial version of the (formal) oxidative spirolactamization of phenolic amides thus emerged as indicated in Scheme 8 for the conversion of **35** to **38**[17] under Kita-type[18] conditions.



Scheme 8.

Compounds of general structure **38** are prone to undergo spontaneous Michael cyclization to morpholine derivatives. The proclivity to cyclize appears to depend on structural details, but the resulting morpholines always form in a highly diastereoselective manner. For instance, compound **40** cyclized to give **41** exclusively (Scheme 9; structure ascertained by X-ray crystallography). The stereoselective formation of **41** is attributable to the strong preference for the axial orientation on the part of alkyl substituents flanking the nitrogen atom in *N*-acyl piperidines and related six-membered heterocycles.[19]

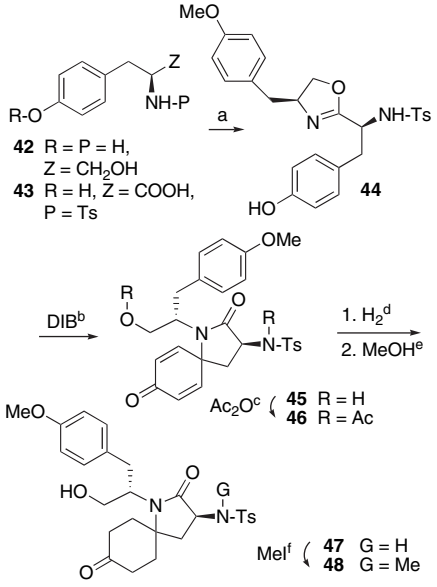


Scheme 9.

Morpholine formation is helpful in certain cases, because it leads to fully stereocontrolled desymmetrization of the 'locally symmetrical' dienone segment of the primary products, in a manner that secures a specific configuration of the now stereogenic spirocenter. This principle constitutes a

pivotal point in the synthesis of cylindricines (vide infra). In other instances, cyclization is problematic and must be suppressed. Acetylation of the OH group in **38** prior to purification readily accomplishes this objective. Overall yields of acetates **39** are typically between 45–50%. Such moderate yields must be weighed against the fact that the reaction rapidly converts inexpensive aminoacid-derived substances to valuable enantiopure intermediates.

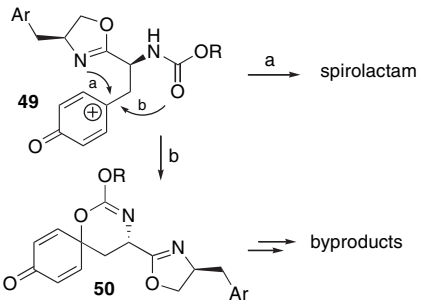
The opening moves of our syntheses of FR-901483 and TAN-1251C appear in Scheme 10. The key oxazoline **44** was prepared through the union of aminoalcohol **42** with acid **43**, both of which are readily available from L-tyrosine. Oxidative spirocyclization and acetylation of the primary product **45**, which was prone to Michael cyclization, provided **46**. The latter intermediate was uneventfully elaborated to **48**, at which stage the routes to TAN-1251C and FR-901483 diverged.



Scheme 10. (a) PPh_3, CCl_4, MeCN, pyridine, 25 °C, Et_3N, 73%; (b) CF_3CH_2OH, 25 °C; (c) pyridine, 4-DMAP, 25 °C, 41% b–c; (d) PtO_2, EtOAc, 25 °C, 96%; (e) K_2CO_3, 25 °C, 79%; (f) K_2CO_3, Me_2CO/DMF, 25 °C, 97%.

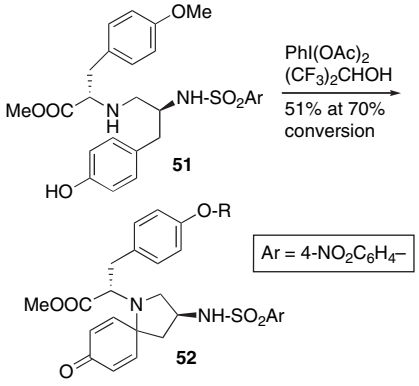
A comment is in order regarding our choice of an *N*-tosyl protecting group for **44**. Initial experiments carried out with variants of **44** displaying carbonyl-type *N*-protection furnished complex mixtures containing only some of the desired spirolactams. Byproducts were detected, the genesis of which is consistent with the following mechanistic picture. Oxidative activation of the phenol leads to an electrophilic intermediate represented in Scheme 11 as cation **49**. Capture by the oxazoline (pathway a) leads to the desired spirolactam. However, interception by the carbonyl group (pathway b) competes effectively, causing formation of iminocarbamate **50**, which then evolves to a variety of secondary products. It was necessary to subdue the nucleophilic aptitude of the *N*-blocking group in order to maximize formation of **45**. The use of an *N*-sulfonamido group emerged as an eminently effective solution.

A brief digression is appropriate at this juncture. The landmark Sorensen synthesis of **16** relied on an unprecedented



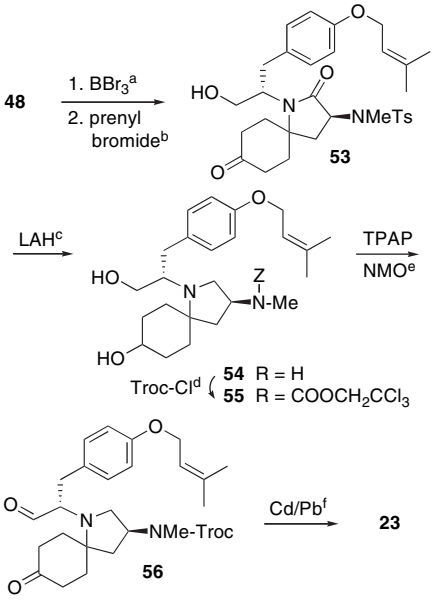
Scheme 11.

oxidative cyclization of phenolic amine **51** to spiropyrrolidine **52** (Scheme 12).[9b,20] Significantly, a sulfonamido protecting group was present on the spectator amino functionality in **51**. We presume that this choice was dictated by the difficulties adumbrated in Scheme 12.



Scheme 12.

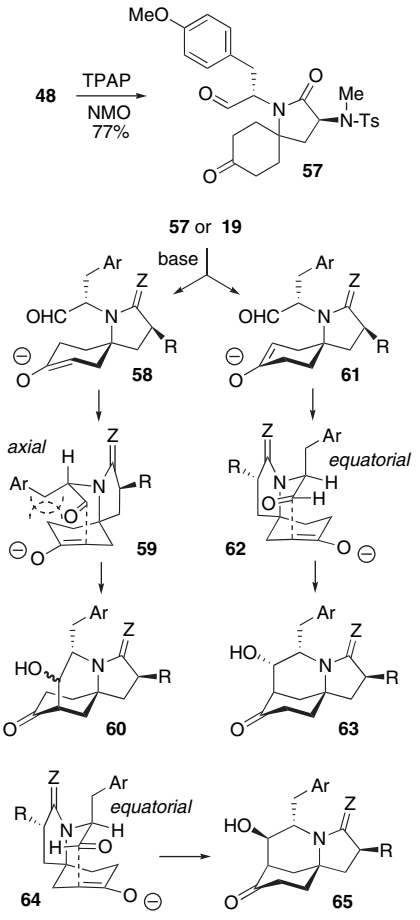
The operations leading from **48** to fully synthetic TAN-1251C[21] are depicted in Scheme 13. Phenolic ether exchange and vigorous LAH reduction provided **54**, which



Scheme 13. (a) CH_2Cl_2, −60 °C, 87%; (b) Cs_2CO_3, Me_2CO, 25 °C, 98%; (c) THF, 0 °C to reflux, 48 h, 88%; (d) aq THF, $NaHCO_3$, 25 °C, 62%; (e) CH_2Cl_2, 25 °C, 1 h, 63%; (f) THF, NH_4OAc buffer, 25 °C, 79%.

for ease of processing was *N*-blocked as the Troc derivative. Subsequent Ley oxidation[22] furnished ketoaldehyde **55**. Release of the free amino function with Cd/Pb couple[23] precipitated instant enamine formation leading to **23**. The yield of **23** from tyrosine was 4% over 16 steps.[24]

The synthesis of FR-901483 presented the complex issue of the aldol cyclization of ketoaldehyde **57**, prepared from **48** as detailed in Scheme 14, or of one of the type **19**. The success of this step is subordinate to the occurrence of the following sequence of events: the substrate must undergo chemoselective enolization of the cyclohexanone segment, leading to regioselective formation of enolate **61** (Z=H, H or O; P=prot. group), which must add diastereoselectively to the *Si*-face of the aldehyde to yield **65**. Superficially, any hope to entice **57**/**19** to behave as required may seem overly optimistic. A careful analysis leads to a more favorable prognosis.



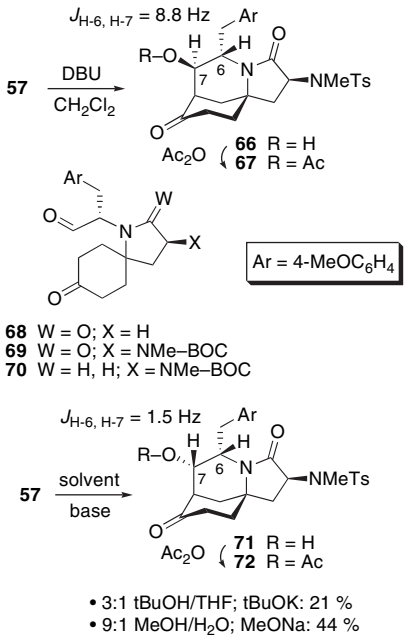
Scheme 14.

Important work by Myers indicates that the kinetics of enolization of protected α-amino aldehydes are not as rapid as one might expect.[25] This principle has been incorporated in a number of brilliant syntheses.[26] If this were true for **57**/**19**, then selective enolization of the cyclohexanone may be possible under gently basic conditions. Exposure to mild bases would promote reversible, non-regioselective formation of the ketone enolate and prime the molecule for aldol cyclization via isomeric chair-like transition states.[27] Scheme 14 illustrates that for a fixed (*S*)-configuration of the stereogenic center at the α-position of the aldehyde, aldol cyclization from the incorrect regioisomer of the cyclohexanone enolate, **58**, forces the 4-methoxybenzyl substituent into an axial orientation in transition states such as **59**. This generates severe compression against a methylene group of the enolate ring (dashed semicircles). No such problems subsist in regioisomeric transition state **62**, wherein the substituent in question is pseudoequatorial in the developing ring. This should favor selective formation of the correct regioisomer of the aldol product, both on kinetic and on thermodynamic grounds. However, a potential complication loomed if substituent Z were a carbonyl group. The ring system emerging from the aldol reaction is now an *N*-acyl piperidine. As mentioned earlier, substituents at the α-position of the nitrogen atom in such structures prefer the *axial* orientation.[18] The incorrect aldol regioisomer would then be favored under thermodynamic conditions, and possibly even under kinetic conditions, if the transition state for the aldol cyclization were product-like. Avoiding entanglement with these complications entailed execution of the aldol step with substrates in which substituent Z is a pair of H atoms; i.e., with a variant of **19**. This is the pathway that Sorensen chose.[9b] On the other hand, a sequence proceeding through direct aldol closure of **57** would be shorter than the one requiring prior elaboration to a protected form of **19**.

A computational simulation (MM+) carried out with simplified variants of transition state structures **59** and **62**, in which R=H, Z=O, and the distance between enolate and formyl carbons (dashed bonds) was arbitrarily fixed at 2.3 Å, suggested that **62** would still be less energetic than **59** by about 0.8 kcal/mol. This value is below the confidence level of the calculation; still, it engendered measured optimism concerning the feasibility of the aldol step in the desired *regiochemical* mode. The question of *diastereoselectivity* constituted a more delicate issue. Indeed, MM+ revealed that transition state models **62** and **64**, which reflect the desired (cf. **63**) and undesired (cf. **65**) epimers of the aldol product, are essentially isoenergetic, as are **63** and **65** themselves. This intimated that no substrate-directed diasterocontrol could be exerted during the aldol step in the absence of external influences.

In an attempt to reach a tricyclic intermediate, compound **57** was treated with DBU in CH_2Cl_2 (Scheme 15). This led to the stereoselective formation of a compound (accompanied by diastereomeric products) that was ultimately shown to be **66** by comparison with analogs of secure constitution. The sole structural parameter initially available to us was the magnitude of the vicinal coupling constant, $^3J_{H\text{-}6,H\text{-}7}$= 8.8 Hz (FR-901483 numbering), for acetate ester **67**. This suggested a trans-diaxial arrangement of the H's in question, i.e., formation of the incorrect aldol diastereomer. Still, it was apparent that the use of a weak base indeed promotes kinetically faster enolization of the cyclohexanone.

It did not escape our attention that the formation of **66** was consistent with the so-called Seebach rule.[28] This reactivity model holds for nonprotic solvents and it may break down in protic ones, leading to an erosion, or even a reversal, of diastereoselectivity, that may be ascribed to solvation of reactive species through H-bonding. One might hope that conduct of the reaction in a protic medium could afford reasonable



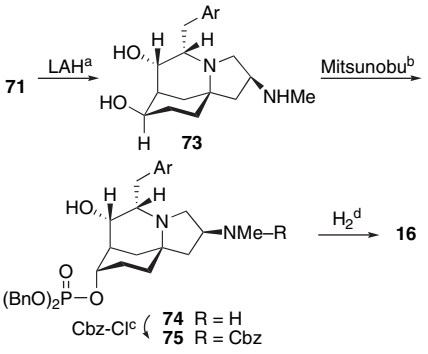
Scheme 15.

quantities of the desired aldol diastereomer. Indeed, pioneering work by Snider appearing in the literature at this juncture proved that model system **68**[9g] as well as 'real' substrate **69**,[9a] which differs from **57** only at the level of the *N*-protecting group (BOC in lieu of a tosylamide), cyclize diastereoselectively to the correct aldol isomer upon reaction with *t*-BuOK in *t*-BuOH. The same outcome obtained upon treatment of **70** with MeONa/MeOH, as reported shortly thereafter by Sorensen.[9b] Interestingly, Snider had determined that the aldol cyclization of **69** proceed with slightly lower diastereoselectivity in MeOH/NaOMe,[9a] suggesting that optimal conditions for this step are intimately dependent on structural details.

Ketoaldehyde **57** did not perform well under Snider-type conditions. First, the compound was poorly soluble in plain *t*-BuOH, necessitating the use of a 3:1 mixture of *t*-BuOH/THF to effect aldol cyclization (*t*-BuOK). This reaction furnished **71** as a significant component of a mixture of diastereomeric aldol products in 21% chromatographed yield: unacceptable in terms of both yield and diastereoselectivity. The structural assignment of **71** initially rested on the coupling constant, $^3J_{H\text{-}6\text{–}H\text{-}7}$=1.5 Hz, measured for acetate **72**, implying a cis relative configuration, and it was ultimately confirmed by an X-ray study of a derivative. Efforts to improve this step led us to examine alternative alcohol/alkoxide combinations. The reaction became increasingly more efficient as we switched to EtOH/EtONa and then to MeOH/MeONa. This again contrasted with the behavior of the Snider substrate, but it was consonant with Sorensen's choice of conditions.

The fact that **57** produced more of the desired **71** in media of higher polarity[29] and/or of higher hydrogen bonding ability[30] crystallized an interesting question: how could one render methanol even more polar and more apt to establish strong hydrogen bonds? A plausible answer was to add some water to the methanolic medium. We rapidly determined that a methanolic solution of **57** remained homogeneous upon dilution with up to 10 vol % of water. Addition of solid NaOMe triggered rapid aldol cyclization and diastereoselective formation of **71** in 44% chromatographed yield. It is tempting to speculate that if the biosynthetic pathway leading to **16** indeed involves aldol cyclization of a species akin to **19**, then the occurrence of this event within an enzyme with a hydrophilic active site hosting numerous water molecules could facilitate formation of the 'good' diastereomer.

With a reliable avenue to **71** in hand, the synthesis was completed rapidly and without incident (Scheme 16). Vigorous LAH reduction of **71** produced **73** (82%) plus a small amount of what appeared to be the epimeric alcohol. Compound **73** may also be obtained with complete diastereoselectivity (within the limits of 500 MHz ^{1}H NMR) by reduction of **71** with L-Selectride® and converted to fully synthetic **16**[31] by the Snider method.[9a] A more rapid endgame relied on regioselective Mitsunobu reaction of **73** with dibenzylphosphate à la Sorensen.[9b] The emerging **74** was extremely polar and difficult to purify. To palliate such difficulties, we installed an *N*-CBZ group prior to purification. Hydrogenolysis of pure **75** yielded totally synthetic FR-901483, the bis-hydrochloride salt of which was found to be identical to that of natural **16**.[24,32] The longest linear sequence in this synthesis is 17 steps from tyrosine, and the overall yield approaches 1.5%.

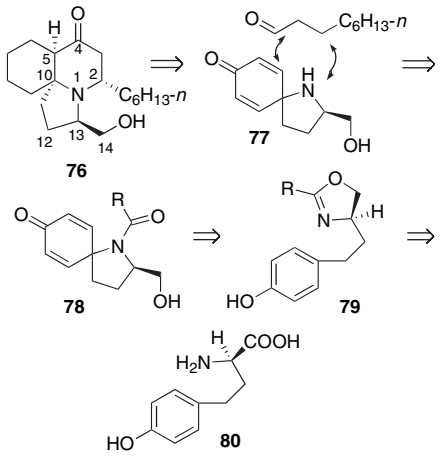


Scheme 16. (a) THF, −78 °C to reflux, 12 h, 91%; (b) DIAD, $(BnO)_2P(O)OH$, $(4\text{-}ClC_6H_4)_3P$; (c) Cbz/Cl, aq THF, $NaHCO_3$, 25 °C, 26% a–c; (d) Pd(C), MeOH, HCl, 25 °C, 3 h, 94%.

3. Second-generation methodology: oxidative cyclization of phenolic sulfonamides

We rapidly realized that a generic oxidative amidation of phenols holds considerable potential in the chemical synthesis of nitrogenous spirocycles. The biosynthetically inspired hypotheses that led to the chemistry of Scheme 8 thus stimulated the evolution of the methodology in a direction that no longer paralleled biomimetic pathways. A case in point is that of the cylindricines,[33] exemplified in Scheme 17 by (−)-cylindricine C, **76**.[34] These moderately cytotoxic, but structurally unique, secondary metabolites of the ascidian, *Clavelina cylindrica*, have engendered considerable interest in the synthetic community.[35] An approach to **76** based on oxazoline technology envisions annulation of the piperidinone ring onto spirodienone **77** (Scheme 17) with concomitant differentiation of the pair of diastereotopic olefins present in the substrate. Compound **77** is available through

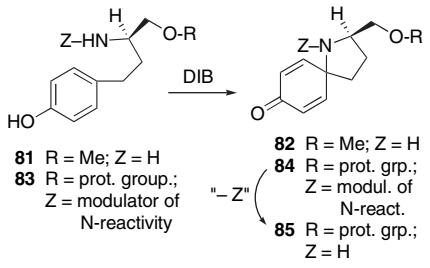
oxidative cyclization of oxazoline **79**, which in turn derives from homotyrosine, **80**.[36]



Scheme 17.

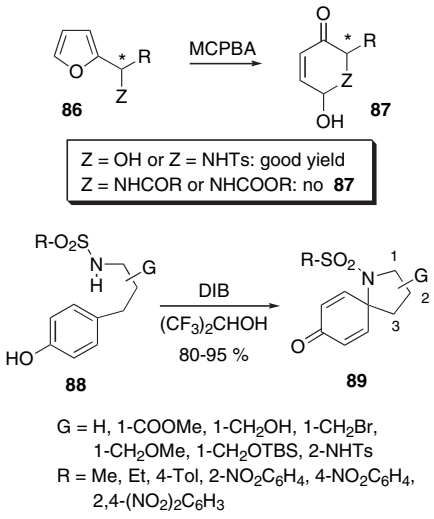
A number of technical difficulties conspired to render the above strategy entirely impractical. First of all, and contrary to previous cases, oxazoline **79** was difficult to purify due to its propensity to retain debris of reagents used in its preparation. Second, oxidative cyclization to **78** was low yielding (25–30%). Third, deacylation of the pyrrolidine nitrogen at the stage of **78**, in preparation for piperidone ring assemblage, was problematic and low yielding. Finally, free aminodienone **77** was prone to polymerization, presumably through cascade Michael-type reactions.

Some of the foregoing obstacles could have been circumvented through direct oxidative cyclization of an *N*-unprotected homoalaninol derivative. Such reactions proceed reasonably well with phenolic *secondary* amines[9b,19] but not so with *primary* ones. Thus, in our hands **81** never afforded the desired **82** in more than 6% yield (Scheme 18). A corrective measure for this unpleasant behavior could involve derivatization of the primary amine with a suitable group Z that modulates the reactivity of the nitrogen center (cf. **83**). Unit Z could then be removed at an opportune post-oxidation stage (cf. **84**→**85**). Kita had established that phenolic 3-arylpropanols cyclize efficiently to spirotetrahydrofurans upon oxidative attack with DIB.[11,17] If group Z in **83** imparted alcohol-like reactivity to the primary amino function, i.e., moderate nucleophilicity and low basicity, then conversion to **84** might become efficient.



Scheme 18.

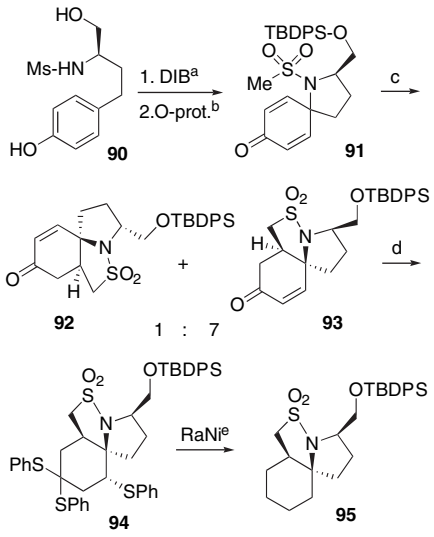
Recent literature precedents indicate that the reactivity of sulfonamides is comparable to that of alcohols. Especially significant in this regard is the work of Zhou,[37] who observed that furylsulfonamides (**86**, Z=NHTs; Scheme 19) undergo aza-Achmatowicz reaction[38] as efficiently as furylcarbinols (the original substrates for Achmatowicz reactions; **86**, Z=OH). This stands in sharp contrast to furylamides and furylcarbamates, which are poor substrates for this transformation. Sulfonamides **88** might well be competent substrates for the desired transformation.



Scheme 19.

It was most pleasing to discover that contrary to the case of oxazolines, which rarely afford yields of spirocycles greater than 50%, the oxidative cyclization of sulfonamides is nearly quantitative. The reaction tolerates a wide range of functional groups and it proceeds very cleanly: in most cases, there is no need for subsequent purification of products **89**.[39] Application of Fukuyama nitrobenzenesulfonamide technology[40] in this reaction yields materials that may be *N*-deblocked under gentle conditions. The resulting *N*-unprotected spirodienones are formal products of cyclization of primary amines. We have already alluded to the poor stability of such educts, which we prefer to avoid. Fortunately, an appropriate sulfonamide can function not only as a modulator of the reactivity of the nitrogen atom during oxidative spirocyclization, but also as a potential nucleophilic handle for elaboration of products **89** to advanced intermediates. This adds an interesting new dimension to the newly devised methodology.

In the context of the synthesis of **76** (Scheme 20),[41] a methanesulfonamide, was effectively employed as a carbonyl anion equivalent. To illustrate, oxidative cyclization of homotyrosine-derived **90** proceeded with no interference from the free alcohol. The highly polar spirodienone thus obtained was *O*-protected with a sterically demanding silyl ether, which directed a subsequent Michael-type cyclization regioselectively to the pro-*S* double bond of **91** (dr=7),[42] thereby exerting stereocontrol at the emerging spiro-stereogenic center through desymmetrization of the dienone. This technique for stereoselective spirocenter formation was inspired by the results of Scheme 9. Unusual in the realm of synthetic endeavoring, compound **93** contained surplus functionality relative to the required cylindricine intermediate **95**. Conversion to **95**, therefore, continued with

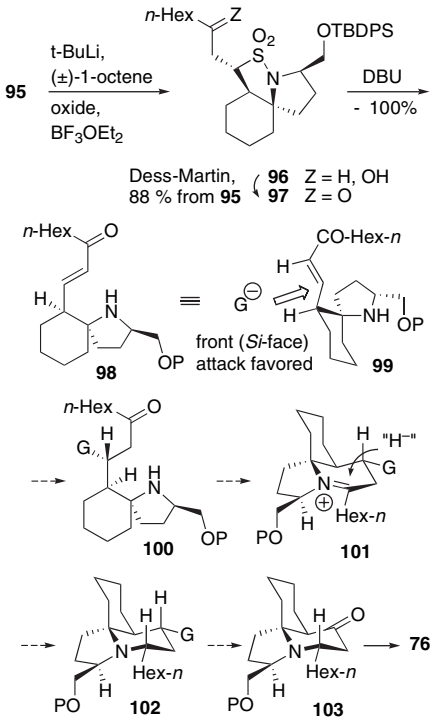


Scheme 20. (a) $(CF_3)_2CHOH$, 25 °C, 30 min; (b) DMF, imidazole, *t*-$BuPh_2$-SiCl, 3 h, 87% a–b; (c) KHMDS/toluene, THF, −100 °C, 3 h, 89%; (d) PhSH, BF_3OEt_2, CH_2Cl_2, 25 °C, 12 h, 77%; (e) 2:1 EtOAc/EtOH, 25 °C, 3 h, 77%.

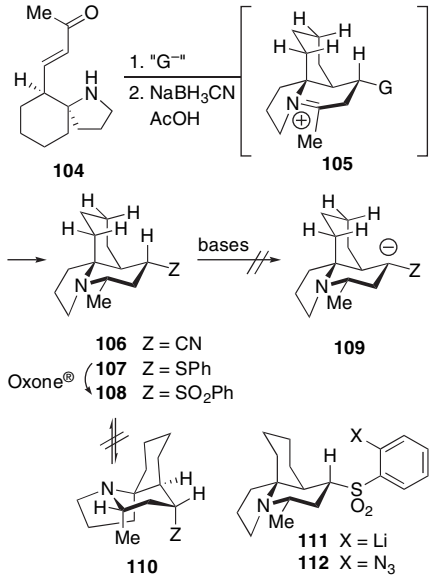
elaboration to trisulfide **94** and RaNi desulfurization.[43] It is worthy of note that the desilylated analog of **94** was crystalline and it was characterized by X-ray diffractometry. This confirmed the regiochemical outcome of the sulfonamide Michael cyclization, as well as the *R*-configuration of the sulfenylated stereogenic carbon. This configuration was expected based on an *anti*-selective conjugate addition of thiophenol directed by the spirosulfonamide nitrogen through a Felkin–Anh-type effect.[44]

Extensive model work aiming to define the best approach for the installation of the piperidinone ring on compound **95** revealed an optimal endgame strategy in the form of an intramolecular reductive amination of ketone **100** (Scheme 21). Group G in **100** stands for an appropriate precursor to the keto function of cylindricine C (cf. **102**→**76**). The steric demand of G must be sufficiently large to enforce a significant preference for conformer **101** of the iminium ion involved in the reductive amination step. Nucleophilic addition to such species tends to occur under stereoelectronic control in the axial mode;[45] therefore, reduction of **101** was expected to produce the correct (*S*)-configuration at C-2 in **102** (cylindricine numbering). Substituent G could be introduced by diastereoselective conjugate addition to enone **98**, which may be anticipated to react in the correct stereochemical sense on the basis of well documented conformational properties of a $C(sp^2)$–$C(sp^3)$ junction. Minimization of $A^{1,3}$-interactions would strongly favor conformer **99**. Consequently, a nucleophilic form of G should selectively add to the more exposed *Si*-face of the enone π bond.

The conversion of **95** to **98** again relied on transformations orchestrated by the sulfonamide unit, which therefore played a triple role during the synthesis: modulator of N-atom reactivity, agent for the desymmetrization of the 'locally symmetrical' dienone, and now implement for the annulation of the piperidinone segment. Deprotonation of **95** with *t*-BuLi[46] and reaction with (±)-1-octene oxide activated by BF_3OEt_2[47] resulted in fully diastereoselective (300 MHz ^{1}H NMR) α-face alkylation to yield **96**. Of course, this product was a 1:1 mixture of alcohol epimers, due to the racemic nature of the epoxide. However, Dess–Martin oxidation[48] of this mixture afforded stereochemically homogeneous ketone **97** (88% from **95**), which upon exposure to DBU experienced retro-Michael extrusion of SO_2 to furnish **98**. Unpleasant difficulties could be anticipated with the 1,4-addition of an actual oxygen nucleophile to **98**. Besides, one may foresee mediocre levels of conformational control if group G in **100** were an alcohol or a derivative thereof. Exploratory work aiming to reach ketones of the type **103**, therefore, relied on intermediates derived from racemic model enone **104** (Scheme 22) through conjugate addition



Scheme 21.



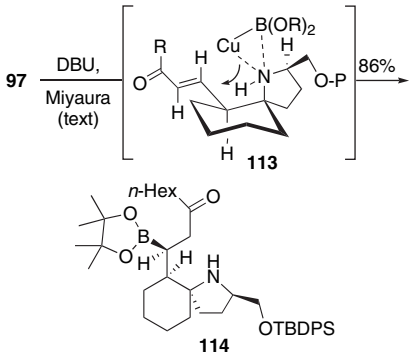
Scheme 22.

of a cyano or a phenylsulfenyl group, either one of which can function as a precursor to a carbonyl.

Nagata cyanation[49] of **104** took place in the anticipated sense, but with a modest 4:1 facial selectivity. The subsequent reductive amination likewise proceeded with moderate diastereocontrol. Conjugate addition of thiophenoxide ion fared much better, occurring with essentially complete stereoselectivity. The bulkier phenylsulfenyl substituent also performed exceptionally well as an element of stereocontrol during the subsequent reductive amination. However, to our utter dismay, the emerging tricyclic intermediates **106**–**108** resisted conversion to the corresponding ketone. Thus, failure was the result of attempted α-chlorination (PCl_5) of nitrile **106**, or deprotonation (cf. anion **109**, Z=CN)/oxidation, e.g., with the Davis oxaziridine. The same fate awaited attempts to induce Pummerer-type reactions of **107**, via the corresponding sulfoxide or through an *S*-halosulfonium ion, or to deprotonate sulfone **108**[50] as a prelude to oxygenation of anion **109** (Z=SO_2Ph). We imputed this string of failures to the conformational rigidity of structures **106**–**108**, which exist exclusively as the depicted conformers (NMR). The proton that must be abstracted in the course of the foregoing, unsuccessful reactions is inaccessible to external bases, situated as it is at an axial position *and* between the pair of axial protons on the second cyclohexane ring of the *cis*-decaline-type system. Interconversion of **106**–**108** with conformer **110**, in which the proton in question, being equatorial, would be more readily accessible, might facilitate deprotonation. However, a triad of problematic 1,3-diaxial interactions, especially severe if Z=SO_2Ph, all but precludes access to **110**. Even *intramolecular* reactions requiring abstraction of the recalcitrant proton were unfruitful. For instance, treatment of **108** with *t*-BuLi promoted *ortho*-lithiation to **111**. A solution of **111** was warmed to just above rt, in the hope to induce intramolecular proton transfer from the aliphatic C–H bond, and formation of a more stable anion. This only resulted in decomposition. Interception of **111** with trisyl azide furnished **112** (90%). Photochemical activation of the azido group was expected to form a triplet nitrenoid that might undergo insertion into the aliphatic C–H bond à la Meth-Cohn:[51] hydrolytic cleavage of the resultant would produce the target ketone. None of the desired product was obtained upon any form of azide activation.

At this juncture, we turned our attention to a bulky boronic ester as an alternative for group G in **100**, and we were not to regret this choice. The recently developed Miyaura conjugate borylation of enones[52] allowed us to subject **97** to an unprecedented transformation involving treatment with DBU, CuCl, KOAc, and bis-pinacolyldiboronate, leading directly to **114** in 86% yield (Scheme 23). Isolable enone **98** forms transiently through the action of DBU on **97**, the other reagents convert it to **114** in situ.

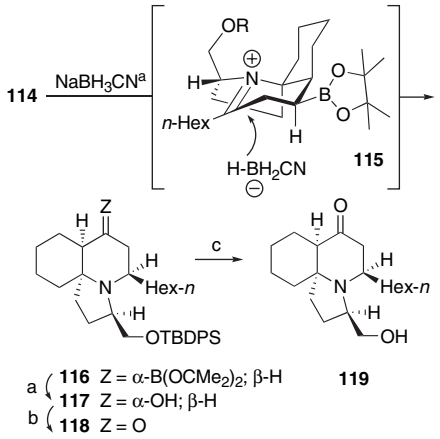
This second step, the Miyaura borylation proper, took place at an unusually fast rate (10 min instead of the normal 15 h or longer) and it occurred with complete reversal of facial selectivity at the level of **98**. Experiment determined that kinetic and stereochemical aspects of the reaction may be ascribed to the directing effect of the nitrogen atom: coordination of the Miyaura borylcopper complex (at the Cu atom,



Scheme 23.

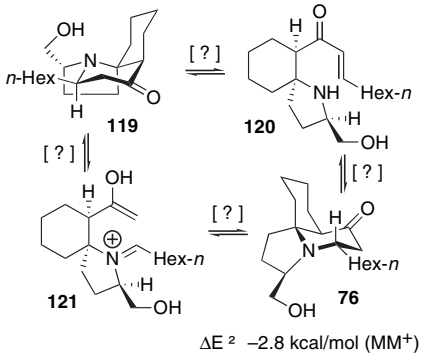
at the B atom, or at both) promotes directed delivery to the *Re* face of the alkene (cf. **113**).[53]

The stereochemical outcome of the Miyaura borylation created an interesting problem at the stage of the subsequent reductive amination step. As shown in Scheme 24, the boronic ester segment favors conformer **115** of the intermediate iminium ion. Axial delivery of hydride[44] upon $NaBH_3CN$/AcOH reduction produced the unnatural 2-(*R*)-configuration of tricyclic intermediate **116**. The latter was not isolated, but it was converted in situ to alcohol **117**, which was uneventfully elaborated to (−)-2-*epi*-cylindricine, **119**. Our synthetic material $[\alpha]_D^{25}$ −39 (*c* 0.5, CH_2Cl_2), was thus obtained in 15 steps and 18% overall yield from homotyrosine, and it was spectrally identical to the racemic **119** described by Weinreb.[35h]



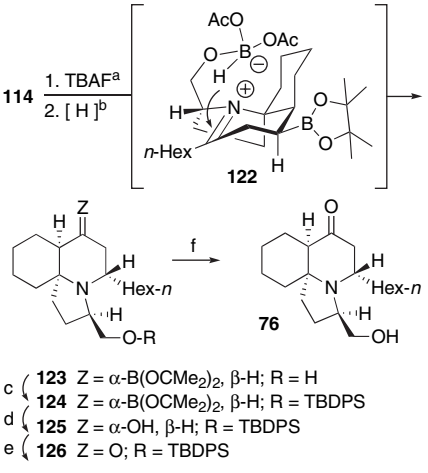
Scheme 24. (a) MeOH, cat. AcOH, 0 °C, 3 h, then aq NaOH, 30% H_2O_2, 0 °C, 30 min, 80%; (b) Dess–Martin periodinane, CH_2Cl_2, 1 h, 94%; (c) TBAF, THF, 25 °C, 3 h, 96%.

A computational simulation revealed that natural cylindricine contains at least 2.8 kcal/mol less steric energy than 2-*epi*-cylindricine. Furthermore, aspects of recorded syntheses of cylindricines may lead one to infer that it should be possible to induce isomerization of **119** to **76** through reversible opening of the piperidinone ring, either in a *retro*-Michael mode (cf. **120**, Scheme 25) or in a *retro*-Mannich sense (cf. **121**)—with a caveat. Observations recorded by Weinreb[35h] cast doubt on this surmise. We must go with Weinreb on this issue: extensive experimentation aiming to promote the desired isomerization, in the interest of reaching the natural product, was entirely unfruitful.



Scheme 25.

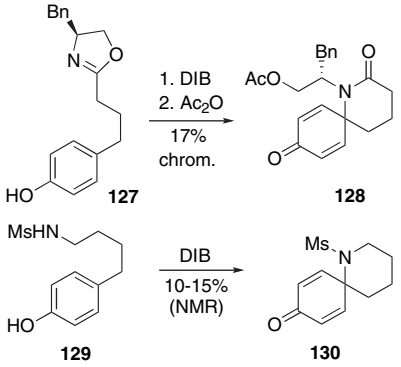
A felicitous development allowed us to exert complete reversal of facial selectivity in the reductive amination step, permitting access to intermediates displaying the natural C-2-(*S*)-configuration. Thus, ketone **114** was desilylated and treated with $NaBH(OAc)_3$ and AcOH. Analogy with the presumed course of the Evans directed carbonyl reduction[54] crystallized the idea that the reductant should rapidly anchor itself to the now free hydroxymethyl group of the substrate. Intramolecular hydride transfer from the resultant complex **122** (Scheme 26) would secure the correct C-2 configuration. The fact that such a hydride transfer must occur through a seven-centered cyclic transition state was a potential obstacle, but inspection of a molecular model of **122** allayed our concerns. Indeed, the borohydride agent is perfectly positioned to deliver H^- to the iminium ion with the correct orientation. Experiment vindicated this hypothesis: compound **123** emerged from the reaction as essentially the sole product. We are not aware of literature precedent for this type of directed *iminium ion* reduction. The synthesis of (−)-cylindricine C was thus completed as shown in Scheme 26.[55]



Scheme 26. (a) THF, 25 °C, 3 h, 95%; (b) $NaBH(OAc)_3$, cat. AcOH, CH_2Cl_2, −78° to 25 °C, 12 h, 73%; (c) *t*-$BuPh_2SiCl$, imidazole, DMF, 25 °C, 95%; (d) aq NaOH, 30% H_2O_2, 0 °C, 30 min, 97%; (e) Dess–Martin periodinane, CH_2Cl_2, 1 h, 94%; (f) TBAF, THF, 25 °C, 3 h, 96%.

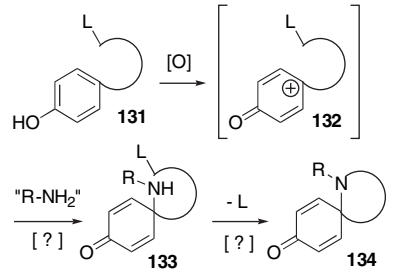
4. Third generation oxidative amidation of phenols: the bimolecular reaction

Oxazoline- and sulfonamide-based techniques of oxidative amidation of phenols suffer from a major limitation: formation of six-membered rings is problematic.[56] To illustrate, oxidative cyclization of **127** or **129** (Scheme 27) produced spiropiperidines **128** and **130** in a disheartening 10–15% yield. Spiranes **128–130** are synthetically valuable building blocks for many alkaloids: a more efficient avenue to these materials seemed highly desirable.



Scheme 27.

Efforts to correct the problem led to a 'third generation' method of oxidative amidation of phenols in the *bimolecular* regime. The genesis of the new process is rooted in the hypothesis sketched in Scheme 28. Oxidative activation of a 4-substituted phenol **131** (L=leaving group) and capture of the electrophilic intermediate, perhaps **132**, with the equivalent of a primary amine would surrender **133**, which upon nucleophilic displacement of L should produce the desired **134**.

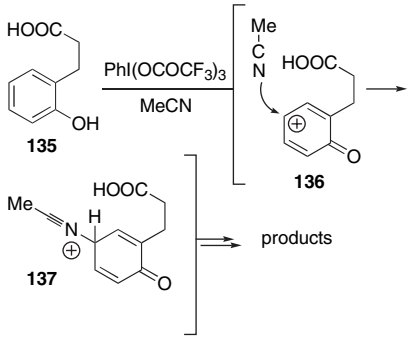


Scheme 28.

It must be stressed that a bimolecular variant of the oxidative amidation of phenols is problematic for several reasons. First of all, it is difficult to identify a competent nitrogen nucleophile that is also resistant to the action of oxidants such as DIB. Second, the kinetics of capture of the electrophilic resultant of phenol activation (cf. hypothetical cation **132**) by an external nitrogen nucleophile are generally unfavorable. Thus, all attempts to intercept the electrophilic agent produced through DIB activation of the phenol with amines (primary or secondary), pyridine, imidazole, and primary sulfonamides,[57] were entirely unsuccessful. Indeed, phenols **131** are customarily converted to products **133** through reaction with electrophilic nitrogen species,[58] but until recently, consensus had it that it was not possible to effect the same transformation with a nucleophilic nitrogenous agent.

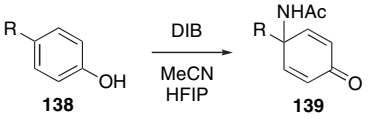
An interesting result disclosed by Wood (Scheme 29)[59] suggested a possible way to attain our objective. Thus, phenol **135** reacted with PIFA in MeCN to furnish products presumed to originate from nitrilium ion **137**, which forms

upon Ritter-type addition of MeCN to **136**. This induced us to study the oxidative amidation of 4-substituted phenols in the presence of nitriles, with the expectation that these might play the role of agent 'R–NH_2' of Scheme 28.



Scheme 29.

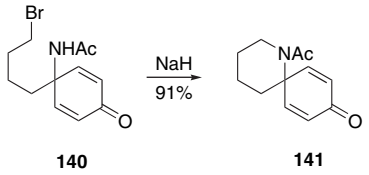
Oxidative attack of several 4-substituted phenols with PIFA in MeCN resulted in the formation of complex mixtures of products, whereas treatment of the same substrates with DIB in MeCN induced no reaction. However, acetamides **139** were obtained in fair to good yield upon DIB oxidation of phenols **138** in a mixture of MeCN and HFIP (Scheme 30).[60] Nitrile solvents other than MeCN may be used in the reaction, which also tolerate a range of useful functionality on the side chain of the substrates. Halogenated substrates performed quite well and afforded the expected products without incident. This is especially relevant to the present discussion: as seen in Scheme 31, exposure of, e.g., **140**, to NaH triggered cyclization to **141** in excellent yield, thereby circumventing the limitation alluded to earlier.



R	yield[a] %	R	yield[a] %
Me	56	$(CH_2)_nCN$	67 (n=2)
Pr-*n*	54		71 (n=3)
Pr-*i*	62		71 (n=4)
CH_2COOMe	58	$(CH_2)_nN_3$	42 (n=3)
$(CH_2)_2NHTs$	53		49 (n=4)
$(CH_2)_3OPiv$	67	$(CH_2)_nBr$	65 (n=3)
$(CH_2)_3CO_2TFE$	57		72 (n=4)

[a]chromatographed

Scheme 30.



Scheme 31.

The bimolecular oxidative amidation of phenols is the centerpiece of a number of ongoing synthetic efforts, disclosure of which is premature at this point. We find it interesting that a hypothesis formulated in connection with our effort toward **16** and **23**, and that falls squarely under the rubric of 'biomimetic chemistry,' ultimately spawned research that retains little of the original 'biomimetic' flavor. Venturing into the *terra incognita* of the reactions of Schemes 19 and 30, however, produced a great deal of new chemistry. Biosynthetic considerations are undeniably a major source of inspiration: the marriage thereof with the élan toward ever shorter avenues to molecular architectures that only synthesis can engender is a powerful motor to drive the progress of organic chemical technology.

5. Experimental

5.1. Experimental protocols

Unless otherwise noted, NMR spectra were recorded in $CDCl_3$ at 300 MHz for ^1H and 75 MHz for ^{13}C. Chemical shift (δ) are in parts per million, and coupling constants (J) are in Hertz. Multiplicities are reported as: 's' (singlet), 'd' (doublet), 'dd' (doublet of doublets), 't' (triplet), 'q' (quartet), 'm' (multiplet), 'c' (complex), 'br' broad. IR spectra (cm^{-1}) were measured on a Perkin Elmer 1720-X FTIR from $CHCl_3$ solutions. Low- and high-resolution mass spectra (*m/e*) were obtained in the CI (isobutane), EI (70 eV), LSIMS (Cs^+), or ESI mode, as specified. Optical rotations were measured in $CHCl_3$, with concentrations (*c*) expressed in g/100 mL. All reactions were run under argon, and monitored by TLC. Reagents and solvents were commercial products and were used as received, including trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), except THF (freshly distd Na/benzophenone); CH_2Cl_2, Et_3N (distd CaH_2). Ground 4 Å molecular sieves used for TPAP–NMO oxidations were activated at 120 °C under vacuum.

5.2. General procedure for the oxidative cyclization of phenolic oxazoline

A solution of DIB (0.4 g, 1.2 mmol) in TFE (5 mL) was added dropwise at rt over 5 min to a solution of a phenolic oxazoline (1.0 mmol) in TFE (5 mL), and the mixture was stirred for 30 min (argon). Solid $NaHCO_3$ (0.3 g) was added and after brief stirring the resulting suspension was filtered over glass wool and concentrated. The crude product was immediately taken up in anhydrous pyridine (0.8 g, 10.0 mmol) and treated with Ac_2O (1.0 g, 10.0 mmol) and DMAP (6.1 mg, 50 μmol) at rt for 12 h with good stirring. Finally, the mixture was evaporated and the residue was purified by chromatography and/or recrystallization.

5.2.1. Oxazoline 44. A solution of PPh_3 (8 g, 30 mmol) in 1:1 acetonitrile/pyridine (20 mL, warming necessary to completely dissolve PPh_3) was added dropwise at rt over 3 h to a solution of **42** (1.81 g, 10 mmol), **43** (3.35 g, 10 mmol), NEt_3 (3.05 g, 30 mmol), and CCl_4 (6.22 g, 40 mmol) in 1:1 MeCN/pyridine (20 mL) at 30 °C. Stirring was continued for 24 h at 30 °C (thermostat). Aqueous 0.5 M NaOH (200 mL) was added with good stirring and the organic layer was discarded. The aqueous layer was successively washed with Et_2O (2×50 mL) and CH_2Cl_2 (2×50 mL). These extracts were also discarded, then EtOAc (50 mL) was added to the aqueous layer and the mixture was carefully acidified to pH 6 with solid NH_4Cl. The layers were separated and the aqueous phase was extracted with

more EtOAc (4×50 mL). The combined extracts were dried ($MgSO_4$) and evaporated to yield 3.5 g (73%) of **44**, orange foam, $[\alpha]_D^{25}$ +13.3 (*c* 1.00, EtOH). ^{1}H: 7.73 (2H, d, *J*=8.5), 7.26 (2H, d, *J*=8.5), 6.92 (2H, d, *J*=8.5), 6.83 (2H, d, *J*=8.5), 6.76 (2H, d, *J*=8.5), 6.44 (2H, d, *J*=8.5), 5.51 (1H, d, *J*=9.6, NH), 4.40–4.25 (1H, m), 4.15–4.00 (2H, m), 3.87–3.80 (1H, m), 3.73 (3H, s), 2.93 (2H, d, *J*=5.5), 2.60 (1H, dd, *J*=13.8, 5.5), 2.39 (3H, s), 2.10 (1H, dd, *J*=13.8, 7.7). ^{13}C: 166.2, 158.4, 155.5, 143.4, 137.3, 130.5, 129.9, 129.5, 129.1, 127.4, 125.7, 115.4, 114.0, 72.5, 66.7, 55.2, 52.2, 40.2, 38.9, 21.5. IR: 3260, 1515, 1445, 1340, 1305. MS (CI): 481 (MH^+). HRMS (CI): calcd for $C_{26}H_{28}N_2O_5S$ (MH^+) 481.1797; found: 481.1770.

5.2.2. Spirolactam 46. A solution of DIB (0.74 g, 2.3 mmol) in TFE (10 mL) was added dropwise over 5 min to a solution of oxazoline **44** (0.96 g, 2 mmol) in TFE (10 mL). The mixture was stirred for 30 min at rt; then solid $NaHCO_3$ (0.50 g, 6 mmol) was added. The suspension was filtered through glass wool and evaporated under reduced pressure. Crude **45** thus obtained was redissolved in pyridine (1.58 g, 20 mmol) and treated with Ac_2O (2.03 g, 20 mmol) and DMAP (24.4 mg, 0.2 mmol) at rt. After stirring overnight, the solution was diluted with EtOAc and washed with satd aq NH_4Cl (3×15 mL). The organic layer was dried ($MgSO_4$) and concentrated. Chromatography of the residue (10%→60% EtOAc/hexane) afforded 0.48 g (41%) of **46**, yellow foam, $[\alpha]_D^{25}$ −22.7 (*c* 1.21). ^{1}H: 8.04 (2H, d, *J*=8.1), 7.42 (2H, d, *J*=8.1), 7.10 (2H, d, *J*=8.5), 6.83 (3H, m), 6.24 (1H, dd, *J*=9.9, 1.8), 6.17 (1H, dd, *J*=10.3, 2.8), 6.07 (1H, dd, *J*=10.3, 1.8), 5.24 (1H, t, *J*=8.8), 4.57 (1H, dd, *J*=11.3, 8.3), 4.28 (1H, dd, *J*=11.3, 4.4), 3.78 (3H, s), 3.18 (1H, dd, *J*=12.1, 7.0), 3.23–3.05 (1H, m), 3.00 (1H, dd, *J*=12.1, 5.5), 2.54 (1H, dd, *J*=13.2, 5.5), 2.47 (3H, s), 2.42 (1H, dd, *J*=13.2, 5.2), 2.28 (3H, s), 2.03 (3H, s). ^{13}C: 184.1, 170.3, 169.8, 169.6, 158.6, 149.0, 148.6, 145.5, 136.2, 130.8, 130.2, 130.1, 129.6, 128.9, 127.6, 113.9, 62.5, 60.0, 57.5, 55.2, 34.7, 34.4, 25.1, 21. 7, 20.9. IR: 1745, 1705, 1675. MS (EI): 580 (M^+), 375, 323, 206 (100), 163, 147, 121, 91, 43. HRMS (EI): calcd for $C_{26}H_{28}N_2O_5S$ (M^+) 580.1879; found: 580.1884.

5.2.3. Cyclohexanone 47. A solution of **46** (0.548 g, 0.95 mmol) and PtO_2 (22 mg) in EtOAc (5 mL) was stirred at rt under 1 atm of H_2 overnight. Filtration (Celite) and concentration afforded 0.530 g (96%) of the corresponding saturated ketone, white foam, $[\alpha]_D^{25}$ −53.2 (*c* 1.25). ^{1}H: 8.07 (2H, d, *J*=8.1), 7.42 (2H, d, *J*=8.1), 7.15 (2H, d, *J*=8.5), 6.82 (2H, d, *J*=8.5), 5.20–5.06 (1H, m), 4.66 (1H, dd, *J*=11.0, 8.1), 4.31 (1H, dd, *J*=11.0, 5.5), 3.77 (3H, s), 3.41–3.28 (1H, m), 3.14 (2H, dd, *J*=7.0, 3.3), 2.79 (1H, dd, *J*=12.3, 10.1), 2.56–2.20 (5H, m), 2.46 (3H, s), 2.27 (3H, s), 2.18–2.07 (1H, m), 1.99 (3H, s), 1.89–1.54 (3H, m). ^{13}C: 208.3, 169.7, 169.6, 158.4, 145.3, 130.6, 130.4, 130.2, 127.6, 127.3, 114.0, 62.9, 60.2, 57.4, 55.4, 55.2, 38.0, 37.2, 35.2, 35.0, 33.9, 33.7, 25.2, 21.7, 20.9. IR: 3377, 1715, 1695. MS (CI): 585 (MH^+). HRMS (CI): calcd for $C_{30}H_{36}N_2O_8S$ (MH^+) 585.2271; found: 585.2285. A mixture of this material (0.530 g, 0.91 mmol) and K_2CO_3 (0.025 g, 0.18 mmol) in MeOH (18 mL) was stirred overnight, and then it was concentrated. The residue was taken up with EtOAc (30 mL) and acidified with 1 M HCl (20 mL). The layers were separated and the aqueous layer was extracted with more EtOAc (2×30 mL). The combined EtOAc extracts were washed with brine (30 mL) and dried ($MgSO_4$). Concentration afforded 0.360 g (79%) of **47**, yellow foam, $[\alpha]_D^{25}$ +2.7 (*c* 1.1). ^{1}H: 7.80 (2H, d, *J*=8.5), 7.32 (2H, d, *J*=8.5), 7.02 (2H, d, *J*=8.5), 6.79 (2H, d, *J*=8.5), 5.84 (1H, s, NH), 3.98 (1H, dd, *J*=11.1, 7.0), 3.87–3.72 (1H, m), 3.75 (3H, s), 3.69 (1H, dd, *J*=11.1, 3.3), 3.28–3.16 (1H, m), 3.11 (1H, dd, *J*=14.0, 8.1), 2.98 (1H, dd, *J*=14.0, 6.6), 2.88 (1H, dd, *J*=13.2, 8.1), 2.53–2.15 (4H, m), 2.43 (3H, s), 2.13–1.91 (1H, m), 1.90–1.56 (3H, m), 0.81–0.65 (1H, m). ^{13}C: 208.1, 171.8, 158.5, 144.0, 135.9, 130.4, 130.0, 129.9, 127.2, 114.0, 62.8, 61.5, 58.3, 55.2, 52.4, 38.0, 37.6, 37.1, 35.1, 33.8, 32.9, 21.5. IR: 3261, 1715, 1682. MS (CI): 501 (MH^+). HRMS (CI): calcd for $C_{26}H_{32}N_2O_6S$ (MH^+) 501.2059; found: 501.2058.

5.2.4. Compound 48. Iodomethane (caution: suspect carcinogen, corrosive, poison; 0.68 g, 4.8 mmol;) was added to a solution of **47** (0.785 g, 1.60 mmol) in acetone/DMF (14 mL/2 mL) containing K_2CO_3 (0.261 g, 1.92 mmol). After stirring for 12 h, the solution was concentrated and the residue was diluted with EtOAc (40 mL) and washed with 1 M HCl (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated to yield 0.780 g (97%) of **48**, yellow foam, $[\alpha]_D^{25}$ −76.0 (*c* 1.0). ^{1}H: 7.84 (2H, d, *J*=8.5), 7.31 (2H, d, *J*=8.5), 7.02 (2H, d, *J*=8.5), 6.75 (2H, d, *J*=8.5), 5.03 (1H, dd, *J*=10.7, 9.2), 4.12 (1H, dd, *J*=11.2, 7.2), 3.74 (3H, s), 3.72 (1H, dd, *J*=11.2, 3.3), 3.31–3.19 (1H, m), 3.13 (1H, dd, *J*=13.6, 8.8), 2.98 (1H, dd, *J*=13.6, 5.9), 2.76 (3H, s), 2.57 (1H, dd, *J*=12.7, 9.2); 2.45–2.35 (2H, m), 2.42 (3H, s), 2.21–2.09 (2H, m), 2.02–1.91 (2H, m), 1.74–1.63 (1H, m), 1.56 (1H, dd, *J*=12.7, 10.7), 0.68–0.57 (1H, m). ^{13}C: 208.4, 171.4, 158.5, 143.8, 136.1, 130.6, 130.4, 129.8, 127.6, 113.8, 63.1, 60.2, 58.5, 57.2, 55.3, 38.2, 37.2, 35.7, 34.1, 32.9, 31.8, 29.8, 21.6. IR: 3415, 1703. MS (CI): 515 (MH^+). HRMS (CI): calcd for $C_{27}H_{34}N_2O_6S$ (MH^+) 515.2216; found: 515.2215.

5.2.5. Prenyl ether 53. Commercial 1 M BBr_3 in CH_2Cl_2 (2.45 mL, 2.45 mmol) was added dropwise to a cold (−60 °C) solution of **48** (0.360 g, 0.7 mmol) in CH_2Cl_2 (2.3 mL). The mixture was stirred for 30 min, then it was warmed to 0 °C and stirring was continued for another 30 min. Aqueous 10% HCl solution (5 mL) was added and the layers were separated. The aqueous layer was extracted with EtOAc (2×5 mL) and the combined extracts were dried ($MgSO_4$) and concentrated. Purification by column chromatography (silica gel, EtOAc/hexane: 70:30→90:10) afforded 0.306 g (87%) of the free phenol, yellow foam, $[\alpha]_D^{25}$ −76.5 (*c* 0.65). ^{1}H: 8.26 (1H, s), 7.86 (2H, d, *J*=8.3), 7.40 (2H, d, *J*=8.3), 7.02 (2H, d, *J*=8.7), 6.73 (2H, d, *J*=8.7), 4.99 (1H, dd, *J*=10.7, 9.0), 3.99 (1H, dd, *J*=11.3, 6.9), 3.72 (1H, dd, *J*=11.3, 5.1), 3.48–3.35 (1H, m), 3.11 (1H, dd, *J*=13.4, 9.0), 2.97 (1H, dd, *J*=13.4, 6.0), 2.78 (3H, s), 2.69 (1H, dd, *J*=13.0, 9.0), 2.68–2.53 (1H, m), 2.41 (3H, s), 2.32 (1H, dd, *J*=14.7, 9.0), 2.31–2.16 (1H, m), 2.11–1.92 (3H, m), 1.81 (1H, ddd, *J*=17.8, 14, 4.4), 1.70 (1H, t, *J*=11.7), 0.79–0.69 (1H, m). ^{13}C: 208.9, 171.8, 157.3, 144.5, 138.4, 131.9, 131.1, 130.8, 128.8, 116.3, 63.0, 61.1, 59.7, 58.5, 39.0, 38.1, 36.6, 35.2, 33.9, 32.5, 30.6, 21.9. IR: 3372, 1681. MS (CI): 501 (MH^+). HRMS (CI): calcd for $C_{26}H_{32}N_2O_6S$ (MH^+) 501.2059; found: 501.2049. A solution of this compound (0.354 g,

708 μmol), Cs_2CO_3 (0.346 g, 106 μmol), and 1-bromo-3-methyl-but-2-ene (122 μL, 106 μmol) in acetone (10 mL) was stirred at 50 °C for 2 h. After cooling to 25 °C, the mixture was filtered through Celite with additional CH_2Cl_2 (2×10 mL). The filtrates were concentrated and the residue was purified by column chromatography (silica gel, EtOAc/hexane: 60:40) to yield 0.394 g (98%) of **53**, colorless foam, $[\alpha]_D^{25}$ −75.0 (*c* 1.00). ^{1}H: 7.81 (2H, d, *J*=8.3), 7.32 (2H, d, *J*=8.3), 7.02 (2H, d, *J*=8.5), 6.77 (2H, d, *J*=8.5), 5.44 (1H, br t, *J*=6.7), 5.03 (1H, dd, *J*=10.6, 9.1), 4.44 (2H, d, *J*=7.0), 4.12 (1H, dd, *J*=11.8, 7.0), 3.73 (1H, dd, *J*=11.8, 3.2), 3.30–3.19 (1H, m), 3.13 (1H, dd, *J*=13.5, 9.7), 2.98 (1H, dd, *J*=13.5, 6.0), 2.77 (3H, s), 2.57 (1H, dd, *J*=12.7, 8.9), 2.45–2.35 (2H, m), 2.42 (3H, s), 2.25–2.09 (2H, m), 2.03–1.92 (2H, m), 1.77 (3H, s), 1.71 (3H, s), 1.70–1.59 (1H, m), 1.56 (1H, t, *J*=12.0), 0.60 (1H, d, *J*=12.8). ^{13}C: 209.3, 171.6, 158.0, 144.1, 138.6, 136.4, 130.9, 130.8, 130.2, 127.9, 119.9, 114.9, 65.2, 62.9, 60.5, 58.9, 57.5, 38.6, 37.6, 36.0, 34.3, 33.2, 31.4, 30.2, 26.3, 21.9, 18.6. IR: 3434, 1716, 1686. MS (CI): 569 (MH^+). HRMS (CI): calcd for $C_{31}H_{40}N_2O_6S$ (MH^+) 569.2685; found: 569.2682.

5.2.6. Compound 54. Commercial 1 M $LiAlH_4$ in THF (1.8 mL, 1.76 mmol) was added dropwise to a cold (−78 °C) solution of **53** (0.20 g, 352 μmol) in THF (3.5 mL). The mixture was warmed to 0 °C, stirred for an additional 15 min and then heated at reflux for 2 days. After cooling to 0 °C EtOAc was added (caution: vigorous reaction) followed by H_2O (30 μL), 15% NaOH (30 μL), and H_2O (90 μL). The precipitate was filtered through Celite and rinsed with EtOAc. The filtrates were concentrated to yield 0.124 g of crude **54**, colorless foam. ^{1}H: 7.00 (2H, d, *J*=8.6), 6.81 (2H, d, *J*=8.6), 5.47 (1H, t, *J*=6.7), 4.46 (2H, *J*=6.8), 3.57–3.46 (1H, m), 3.29 (1H, dd, *J*=9.8, 4.9), 3.19–3.05 (3H, m), 3.04–2.80 (2H, m), 2.62 (1H, br t, *J*=7.9), 2.43 (3H, s), 2.47–2.38 (1H, m), 2.2 (1H, ddd, *J*=14.3, 10.9, 2.9), 1.98–1.85 (3H, m), 1.77 (3H, s), 1.72 (3H, s), 1.67–1.19 (5H, m). ^{13}C: 157.8, 138.5, 131.2, 130.0, 120.1, 115.1, 70.2, 65.2, 62.9, 60.9, 57.8, 56.7, 49.4, 43.0, 36.6 (two resonances), 35.3, 34.1, 32.9 (two resonances), 26.2, 18.6. IR: 3379. MS (LSI): 403 (MH^+). HRMS (LSI): calcd for $C_{24}H_{38}N_2O_3$ (MH^+) 403.2961; found: 403.2959.

5.2.7. Carbamate 55. Trichloroethyl chloroformate (0.142 g, 0.67 mmol) was added to a solution of **54** (0.245 g, 0.61 mmol) and $NaHCO_3$ (0.154 g, 1.83 mmol) in THF/H_2O (3 mL/3 mL). After stirring at rt overnight the mixture was extracted with EtOAc (2×10 mL). The combined extracts were dried ($MgSO_4$) and concentrated. Filtration of the residue through silica gel (EtOAc/hexane: 70:30) afforded 0.22 g (62%) of **55**, white foam, $[\alpha]_D^{25}$ −8.8 (*c* 1.05). ^{1}H: 7.01 (2H, d, *J*=8.4), 6.82 (2H, d, *J*=8.4), 5.47 (1H, t, *J*=6.7), 4.75 (2H, s), 4.66–4.54 (1H, m), 4.47 (2H, *J*=6.7), 3.64–3.52 (1H, m), 3.33 (1H, dd, *J*=10.1, 4.4); 3.16 (1H, t, *J*=10.1), 3.05–2.92 (3H, m), 2.95 (3H, s), 2.88 (1H, dd, *J*=13.5, 2.9), 2.44 (1H, t, *J*=13.5), 1.99–1.91 (2H, m), 1.78 (3H, s), 1.73 (3H, s), 1.76–1.54 (6H, m), 1.44–1.27 (2H, m). ^{13}C: 157.8, 155.0, 138.6, 130.9, 130.0, 120.1, 115.2, 96.0, 75.6, 70.1, 65.2, 62.7, 61.1, 57.1, 54.3, 45.0, 36.6, 36.1, 34.0, 33.3, 33.1, 32.7, 30.6, 26.2, 18.6. IR: 3410, 1712. MS (CI): 583 (w), 581, 579, 577 (MH^+, Cl cluster). HRMS (CI): calcd for $C_{27}H_{39}N_2O_5^{35}Cl_3$ (MH^+) 577.2003; found: 577.2006.

5.2.8. Aldehyde 56. A solution of **55** (65 mg, 112 μmol), 4-methylmorpholine-*N*-oxide (52 mg, 448 μmol), and Pr_4NRuO_4 (4 mg, 11.2 μmol) in CH_2Cl_2 (2.2 mL) containing suspended powdered 4 Å molecular sieves (56 mg, 0.5 g/mmol) was stirred at rt for 1 h. Filtration over silica gel (EtOAc) and concentration afforded 40 mg (63%) of **56**, colorless foam, $[\alpha]_D^{25}$ −38.6 (*c* 0.95). ^{1}H: 9.66 (1H, s), 7.06 (2H, d, *J*=8.6), 6.81 (2H, d, *J*=8.6), 5.52–4.41 (1H, m), 4.92–4.70 (3H, m), 4.47 (2H, *J*=6.7), 3.84 (1H, t, *J*=8.8), 3.44–3.39 (2H, m), 3.00 (3H, s), 2.67–2.57 (3H, m), 2.43–2.29 (2H, m), 2.26–2.18 (2H, m), 1.93–1.67 (3H, m), 1.78 (3H, s), 1.73 (3H, s), 1.51–1.40 (2H, m). ^{13}C: 210.6, 201.4, 157.9, 154.9, 138.5, 131.3, 130.8, 120.1, 114.9, 96.0, 75.7, 71.2, 65.2, 62.4, 59.6, 59.1, 41.3, 36.6, 39.1, 38.4, 30.4, 26.2, 18.6. IR: 3411, 1716. MS (CI): 579 (weak), 577, 575, 573 (MH^+, Cl cluster). HRMS (CI): calcd for $C_{27}H_{34}N_2O_5^{35}Cl_3$ (MH^+) 573.1690; found: 573.1690.

5.2.9. Synthetic TAN-1251C, 23. A mixture of **56** (50 mg, 87 μmol), THF (0.4 mL), aq 1 M NH_4OAc solution (0.4 mL), and 10% Cd/Pb couple (435 μmol of Cd) was vigorously stirred at rt for 1 h. The solid was filtered off and rinsed with EtOAc. The filtrate was basified (aq NaOH) and the product was extracted with EtOAc. Concentration and purification of the residue by preparative TLC (100% EtOAc) afforded 26 mg (79%) of TAN-1251C, pale yellow oil, $[\alpha]_D^{25}$ +23.4 (*c* 0.48, MeOH) (lit. $[\alpha]_D^{25}$ +24 (*c* 0.44, MeOH), Refs. 10 and 21b). ^{1}H: 7.08 (2H, d, *J*=8.7), 6.82 (2H, d, *J*=8.7), 5.53–5.46 (1H, m), 5.22 (1H, d, *J*=1.1), 4.47 (2H, d, *J*=6.7), 3.42–3.37 (1H, m), 3.22 (2H, s), 3.21 (1H, dd, *J*=11.5, 2.8), 2.80 (1H, dd, *J*=11.5, 1.9), 2.61–2.56 (1H, m), 2.51 (3H, s), 2.50–2.44 (1H, m), 2.41–2.17 (4H, m), 2.00 (1H, ddd, *J*=13.6, 10.7, 5.4), 1.89 (1H, *J*=13.6, 5.4), 1.86–1.81 (2H, m), 1.79 (3H, s, CH3); 1.73 (3H, s, CH3). ^{13}C: 212.0, 157.5, 138.4, 132.2, 130.2, 128.5, 128.2, 120.2, 114.8, 71.9, 65.1, 59.4, 52.5, 43.2, 41.8, 40.7, 39.9, 38.2, 37.6, 35.0, 26.2, 18.6. IR: 3393, 1716. MS (CI): 381 (MH^+). HRMS (CI): calcd for $C_{24}H_{33}N_2O_2$ (MH^+) 381.2542; found: 381.2543.

5.2.10. Aldehyde 57. A solution of **48** (0.340g, 0.66 mmol), 4-methylmorpholine-*N*-oxide (0.154 g, 1.32 mmol), and Pr_4NRuO_4 (23 mg, 66 μmol) in CH_2Cl_2 (13.2 mL) containing suspended powdered 4 Å molecular sieves (330 mg, 0.5 g/mmol), was stirred at rt for 15 min, then it was filtered over silica gel (EtOAc) and concentrated to afford 0.263 g (77%) of **57**, colorless foam, $[\alpha]_D^{25}$ −56.6 (*c* 1.25). ^{1}H: 9.57 (1H, s), 7.84 (2H, d, *J*=8.1), 7.32 (2H, d, *J*=8.1); 7.01 (2H, d, *J*=8.6), 6.75 (2H, d, *J*=8.6), 5.01 (1H, dd, *J*=11.0, 9.2), 3.76 (3H, s), 3.56–3.45 (1H, m), 3.36 (1H, d, *J*=7.7), 2.77 (3H, s), 2.75–3.70 (1H, m), 2.47–2.37 (2H, m), 2.42 (3H, s), 2.31–1.65 (5H, m), 1.47–1.33 (1H, m), 0.60–0.46 (1H, m). ^{13}C: 207.6, 197.6, 170.4, 158.8, 143.8, 136.0, 132.3, 130.6, 129.8, 127.7, 114.1, 63.2, 59.2, 57.0, 55.4, 37.9, 37.1, 35.6, 32.9, 32.9, 32.7, 29.9, 21.7. IR: 1736, 1717, 1698. MS (CI): 513 (MH^+). HRMS (CI): calcd for $C_{27}H_{32}N_2O_6S$ (MH^+) 513.2059; found: 513.2054.

5.2.11. Tricyclic intermediate 71. Commercial sodium methoxide (42 mg, 780 μmol) was added to a solution of **57** (200 mg, 390 μmol) in MeOH/H_2O (7.2 mL/0.8 mL), and the mixture was stirred at rt for 30 min. The solution was concentrated and the residue was diluted with EtOAc

(40 mL). This organic phase was sequentially washed with 1 M HCl (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated, and the residue was purified by preparative TLC (EtOAc/hexane: 60:40) to afford 90 mg (44%) of **71**, colorless foam, $[\alpha]_D^{25}$ −10.6 (*c* 1.41, EtOH). ^{1}H: 7.86 (2H, d, *J*=8.1), 7.31 (2H, d, *J*=8.1), 7.10 (2H, d, *J*=8.8), 6.76 (2H, d, *J*=8.8), 5.00 (1H, dd, *J*=11.0, 8.8), 3.81–3.69 (2H, m), 3.75 (3H, s), 3.38 (1H, dd, *J*=14.0, 8.8), 3.32–3.25 (1H, m), 2.82–2.76 (1H, m), 2.65–2.37 (3H, m), 2.61 (3H, s), 2.41 (3H, s), 2.29 (1H, dd, *J*=12.9, 8.8), 2.25–1.90 (2H, m), 1.80 (1H, dd, *J*=13.6, 3.3), 1.72 (1H, dd, *J*=12.9, 11.0). ^{13}C: 211.8, 171.5, 158.1, 143.5, 136.3, 130.7, 129.9, 129.8, 127.6, 113.9, 68.5, 59.8, 58.4, 57.2, 55.2, 50.7, 36.9, 35.61, 33.1, 32.3, 29.6, 28.3, 21.6. IR: 3428, 1702. MS (CI): 513 (MH^+). HRMS (CI): calcd for $C_{27}H_{32}N_2O_6S$ (MH^+) 513.2059; found: 513.2061.

5.2.12. Acetate 72. A solution of **71** (0.140 g, 0.27 mmol), pyridine (0.064 g, 0.81 mmol), acetic anhydride (0.033 g, 0.33 mmol), and DMAP (catalytic amount) in CH_2Cl_2 (2.7 mL) was stirred at rt for 3 h. The mixture was concentrated and the residue was purified by preparative TLC (EtOAc/hexane: 60:40) to afford 0.140 g (93%) of **72**, colorless foam, $[\alpha]_D^{25}$ −27.8 (*c* 1.41). ^{1}H: 7.82 (2H, d, *J*=8.1), 7.30 (2H, d, *J*=8.1), 7.02 (2H, d, *J*=8.8), 6.74 (2H, d, *J*=8.8), 4.94–4.85 (2H, m), 3.80 (1H, dd, *J*=14.0, 7.4), 3.73 (3H, s), 3.41–3.32 (1H, m), 3.05 (1H, dd, *J*=14.0, 7.4), 2.92–2.86 (1H, m), 2.65–2.97 (2H, m), 2.57 (3H, s), 2.41 (3H, s), 2.30 (1H, dd, *J*=12.8, 8.8), 2.19 (3H, s), 2.04–1.84 (2H, m), 1.77 (1H, dd, *J*=12.8, 11.0). ^{13}C: 209.5, 170.4, 169.7, 158.3, 143.5, 136.3, 130.1, 129.7, 127.6, 113.9, 68.9, 58.3, 58.1, 56.6, 55.2, 47.5, 36.9, 35.2, 33.2, 32.1, 29.5, 28.9, 21.6, 21.0. IR: 3405, 1742, 1708. MS (CI): 555 (MH^+). HRMS (CI): calcd for $C_{29}H_{34}N_2O_7S$ (MH^+) 555.2165; found: 555.2163.

5.2.13. Diol 73. Commercial 1 M LAH in THF (0.96 mL, 0.96 mmol) was added dropwise to a cold (−78 °C) solution of compound **71** (98 mg, 191 μmol) in THF (2 mL). The mixture was warmed to 0 °C and stirred for 15 min, then it was refluxed overnight. The reaction was cooled to 0 °C and quenched with EtOAc (caution: vigorous reaction), followed by H_2O (30 μL), 15% NaOH (30 μL), and H_2O (50 μL). The precipitate was filtered through Celite and rinsed with EtOAc. The filtrate was concentrated to yield 60 mg of **73** (contaminated with some of its diastereomer) as a yellow foam. The two isomers were not separated at this point. ^{1}H: 7.17 (2H, d, *J*=8.6), 6.77 (2H, d, *J*=8.6), 3.93–3.81 (1H, m), 3.73 (3H, s), 3.65–3.39 (3H, m), 3.16–3.05 (1H, m), 2.77 (2H, d, *J*=5.0), 2.50 (1H, dd, *J*=9.4, 5.0), 2.29 (3H, s), 2.23–2.07 (2H, m), 2.01–1.87 (2H, m), 1.82 (1H, dd, *J*=12.7, 9.0), 1.73–1.64 (1H, m), 1.34 (1H, dd, *J*=12.7, 5.6), 1.29–1.19 (2H, m). ^{13}C: 156.7, 130.2, 129.3, 112.6, 68.8, 63.2, 60.2, 57.8, 55.3, 54.2, 53.6, 45.0, 43.1, 34.8, 33.7, 30.4, 29.8, 29.0. IR: 3384. MS (CI): 347 (MH^+). HRMS (CI): calcd for $C_{20}H_{30}N_2O_3$ (MH^+) 347.2335; found: 347.2338.

5.2.14. Synthetic FR-901483 bis-hydrochloride: 16·2HCl. Dibenzylphosphate (145 mg, 520 μmol), tris(4-chlorophenyl)phosphine (98 mg, 270 μmol), DIAD (54 μL, 270 μmol), and Et_3N (0.121 mL, 0.87 mmol) were added to a solution of **73** (30 mg, 86.7 μmol) in THF (1.7 mL). The mixture was stirred at rt for 1 h then it was concentrated. The residue was taken up with EtOAc, and the solution was washed (H_2O, then brine), dried ($MgSO_4$), and concentrated. The highly polar, crude Mitsunobu product was converted, taken up in CH_2Cl_2 (1 mL) and treated with NEt_3 (30 μL, 208 μmol), and benzyl chloroformate (20 μL, 135 μmol). After stirring at rt until TLC showed complete conversion, the solution was concentrated and the residue was purified by preparative TLC (10% MeOH in CH_2Cl_2) to afford **75** in an overall 26% yield from compound **73**. Aqueous 3 M HCl (6 μL) was added to a solution of **75** (13 mg, 17.5 μmol) in MeOH (1 mL). The mixture was concentrated and the residue was redissolved in MeOH (0.2 mL), treated with 10% Pd/C (5 mg) and stirred at rt under 1 atm of H_2 (balloon) for 3 h. Filtration through Celite and concentration yielded 7.6 mg (94%) of FR-901483 bis-hydrochloride, $[\alpha]_D^{25}$ +4.0 (*c* 0.35, MeOH, lit. [Ref. 9a] +5, rotation very sensitive to the amount of water and of residual HCl). ^{1}H (500 MHz, CD_3OD): 7.35 (2H, d, *J*=8.6), 6.92 (2H, d, *J*=8.6), 4.52 (1H, dd, *J*=13.6, 9.9), 4.36 (1H, br d, *J*=7.7), 4.33–4.26 (1H, m), 3.97 (1H, dd, *J*=13.6, 2.9), 3.93–3.88 (1H, m), 3.80 (3H, s), 3.67 (1H, br s), 3.34 (1H, m), 3.10 (1H, dd, *J*=12.4, 3.6), 2.81 (3H, s), 2.65 (1H, dd, *J*=14.0, 8.9), 2.48 (1H, br s), 2.35–2.07 (6H, m), 1.93 (1H, br d, *J*=14.2). ^{13}C (125 MHz, CD_3OD): 159.5, 130.7, 127.4, 114.3, 71.4, 67.9, 63.9, 61.0, 54.7, 54.1, 50.9, 41.8, 40.7, 33.0, 31.4, 27.2, 26.6, 21.7. MS (LSI): 427 (MH^+). HRMS (LSI): calcd for $C_{31}H_{38}N_2O_8S$ (MH^+) 427.1998; found: 427.2000.

5.3. General procedure for oxidative cyclization of phenolic sulfonamides 88

A solution of DIB (2.2 mmol) in HFIP (3.5 mL) was added to a solution of a phenolic sulfonamide (2 mmol) in HFIP (5 mL) during 5 min. After 30–60 min, a color change from yellow to green occurred and TLC showed complete conversion. The mixture was concentrated in vacuo and the residue was chromatographed (silica gel, typically 60:40 EtOAc/cyclohexane) to furnish the product, which is often obtained as a foam.

5.3.1. Spirodienone 91. A 1 M solution of $PhI(OAc)_2$ ('DIB') in hexafluoroisopropanol ('HFIP', 4.05 mL, 1.05 equiv of DIB) was added dropwise to a well stirred solution of (D)-(−)-*N*-methanesulfonyl-homotyrosinol (1.0 g, 3.86 mmol) in HFIP (15 mL) at rt. A color change from yellow to green occurred and no starting material was apparent (TLC) after 30 min. The volatiles were removed and the highly polar, crude product, tan foam, was used directly in the next step. $[\alpha]_D^{25}$ +10 (*c* 0.5, acetone) ^{1}H (acetone-d_6): 7.26 (1H, dd, *J*=9.8, 3.0), 7.03 (1H, dd, *J*=10.2, 3.0), 6.16 (1H, dd, *J*=10.2, 2.3), 6.10 (1H, dd, *J*=10.2, 2.3), 4.07 (1H, m), 3.76 (2H, m), 3.01 (3H, s), 2.52 (1H, m), 2.48 (1H, m), 2.18 (1H, m), 1.92 (1H, m). ^{13}C (acetone-d_6): 185.4, 153.7, 149.6, 128.6, 128.2, 65.2, 64.8, 63.9, 40.2, 38.6, 27.3. IR: 3414, 1666. HRMS (CI): calcd for $C_{11}H_{15}NO_4S$ (MH^+): 258.0800; found 258.0801. A solution of this material (0.9 g), imidazole (1 g, 4 equiv), and TBDPSCl (1.10 g, 1.1 equiv) in DMF (15 mL) was stirred at rt for 3 h, then it was diluted with EtOAc (20 mL) and washed with brine (15 mL). The organic phase was separated and the aqueous layer was extracted with more EtOAc (10 mL). The combined extracts were washed

with brine (4×15 mL), and then they were concentrated. Silica gel chromatography of the residue (1:1:0.1, EtOAc/hexanes/NEt3) afforded pure **91** (pale yellow foam, 1.56 g, 3.17 mmol, 82% from homotyrosinol mesylamide). $[\alpha]_D^{25}$ +8 (*c* 0.5, acetone). ^{1}H (acetone-d_6): 7.74 (m, 4H), 7.47 (m, 6H), 7.27 (1H, dd, *J*=10.2, 3.0), 6.94 (1H, dd, *J*=9.8, 3.0), 6.20 (1H, dd, *J*=10.2, 1.9), 6.09 (1H, dd, *J*=10.2, 2.3), 4.17 (1H, m), 4.03 (1H, dd, *J*=10.2, 3.8), 3.93 (1H, dd, *J*=10.2, 7.5), 2.95 (3H, s), 2.48 (2H, m), 2.30 (1H, m), 1.93 (1H, m), 1.10 (9H, s). ^{13}C (acetone-d_6): 185.2, 153.7, 148.7, 136.4, 134.0, 130.8, 129.1, 128.7, 128.2, 66.7, 65.3, 63.3, 39.6, 38.3, 27.3, 27.1, 19.8. IR: 1668. HRMS (CI): calcd for $C_{27}H_{33}NO_4SSi$ (MH^+): 496.1978; found: 496.1972.

5.3.2. Compounds 92 and 93. A 0.5 M solution of KHMDS in toluene (8 mL, 4 mmol, 1.3 equiv) was added to a cold (−100 °C), stirred solution of **91** (1.5 g, 3.00 mmol) in dry THF (100 mL), under argon. The reaction was allowed to warm up to 0 °C during 3 h, then it was quenched with aq satd NH_4Cl (50 mL) and diluted with EtOAc (50 mL). The aqueous phase was discarded and the organic layer was washed with brine (50 mL) and concentrated. A proton NMR spectrum of the crude product revealed that **93** was the major product of a 7:1 mixture of regioisomers (de=75%). Silica gel chromatography (3:7:0.1, EtOAc/hexanes/NEt3) afforded an inseparable mixture of **93** and its regioisomer (1.33 g, 2.70 mmol, 89%). Separation was effected at the stage of compound **95**. $[\alpha]_D^{25}$ +30 (*c* 0.5, CH_2Cl_2). ^{1}H: 7.67 (4H, m), 7.40 (6H, m), 6.62 (1H, dd, *J*=10.2, 1.9), 6.03 (1H, d, 10.2), 4.27 (1H, m), 3.67 (1H, dd, *J*=10.5, 4.9), 3.62 (1H, dd, *J*=7.6, 2.3), 3.43 (1H, dd, *J*=13.2, 7.9), 3.25 (1H, t, 12.4), 3.00 (1H, m), 2.70 (1H, dd, *J*=17.3, 5.3), 2.63 (1H, dd, *J*=16.2, 2.3), 2.14 (4H, m), 1.07 (9H, s). ^{13}C: 194.5, 150.2, 135.5, 134.7, 132.8, 129.8, 127.7, 71.1, 66.3, 61.1, 53.3, 40.6, 36.7, 34.8, 27.2, 26.8, 19.1. IR: 1682. HRMS (CI): calcd for $C_{27}H_{33}NO_4SSi$ (MH^+): 496.1978; found: 496.1975.

5.3.3. Thiophenol adduct 94 and regioisomer. A cold (0 °C) solution of the 7:1 mixture of regioisomers **92** and **93** (1.30 g, 2.62 mmol,), BF_3Et_2O (20 mol %, 70 mg), and thiophenol (1.8 g, 6 equiv) in CH_2Cl_2 (40 mL) was stirred overnight, during which time it was allowed to warm to rt. The reaction was quenched with aq satd K_2CO_3 (40 mL) and diluted with EtOAc (80 mL). The aqueous layer was separated and extracted with more EtOAc (30 mL). The combined extracts were washed with brine (50 mL) and concentrated. Silica gel chromatography (15:85, EtOAc/hexanes) of the residue provided **94** (1.63 g, 2.02 mmol, 77%, 7:1 mixture of regioisomers) as a foam. $[\alpha]_D^{25}$ +12 (*c* 0.5 CH_2Cl_2) ^{1}H: 7.80–7.00 (15H, m), 4.41 (1H, t, *J*=12.8), 4.25 (1H, m), 3.90 (2H, m), 3.65 (1H, t, *J*=8.3), 3.31 (1H, dd, *J*=13.2, 8.3), 2.59 (1H, m), 2.32 (1H, d, *J*=14.7), 2.24–1.23 (7H, m), 1.06 (9H, s). ^{13}C: 137.1, 136.3, 135.6, 134.6, 133.5, 133.4, 133.1, 130.8, 129.7, 129.5, 129.4, 129.0, 128.9, 128.7, 127.5, 127.4, 76.2, 65.5, 62.0, 61.0, 53.5, 51.9, 42.9, 42.2, 35.0, 30.9, 27.2, 26.7, 19.2. IR: 1474, 1436, 1307. MS (LSI): 808 (MH^+) for $C_{45}H_{45}NO_4S_4Si$.

5.3.4. Sulfonamide 95. A solution of **94** (1.35 g, 1.67 mmol, 7:1 mixture of regioisomers) in a 1:3 mixture of EtOAc/EtOH (100 mL) containing suspended activated Raney nickel (50% slurry in water, Acros, decanted and added as a wet metallic powder, 15 g) was stirred at rt for 4 h, then it was carefully filtered over Celite (caution: RaNi is pyrophoric when dry). The metallic residue was washed with more EtOAc (3×30 mL), and the combined organic phases were concentrated. Silica gel chromatography (1:9 EtOAc/hexanes) of the residue afforded pure **95** (pale yellow foam, 623 mg, 1.29 mmol, 77%), uncontaminated by the regioisomer formed during cyclization of **91**. $[\alpha]_D^{25}$ −13 (*c* 0.5, CH_2Cl_2) ^{1}H: 7.69 (4H, m), 7.39 (6H, m), 4.13 (1H, m), 3.65 (1H, dd, *J*=10.2, 4.9), 3.53 (1H, dd, *J*=10.2, 6.8), 3.48 (3H, t, *J*=13.2), 3.25 (1H, dd, *J*=13.2, 7.5), 2.50 (1H, m), 2.10–1.00 (12H, m), 1.08 (9H, s). ^{13}C: 135.6, 133.2, 129.6, 127.6, 73.8, 66.3, 59.5, 53.1, 40.8, 35.5, 35.1, 27.9, 26.8, 24.6, 23.4, 19.6, 19.2. IR: 1307. HRMS (CI): calcd for $C_{27}H_{37}NO_3SSi$ (MH^+): 484.2342; found: 484.2349.

5.3.5. Compounds 96. A 1.5 M pentane solution of *t*-BuLi (0.5 mL, 0.75 mmol, 1.1 equiv) was added to a cold (−78 °C), stirred solution of **95** (345 mg, 0.71 mmol) in THF (5 mL) under argon. After 15 min, $BF_3\cdot Et_2O$ (100 mg, 1.1 equiv) was added, followed 5 min later by (±)-octene oxide (120 mg, 1.3 equiv). The mixture was stirred for 3 h, during which time it was allowed to warm to 0 °C, then it was quenched with aq satd NH_4Cl (10 mL) and diluted with EtOAc (10 mL). The organic layer was separated, washed with brine (10 mL), and concentrated. Crude **97**, foam, mixture of diastereomers, was advanced to the next step without further purification. IR: 3479, 1428, 1301. HRMS (CI): calcd for $C_{35}H_{53}NO_4SSi$ (MH^+): 612.3543; found: 612.3547.

5.3.6. Ketone 97. A 0.5 M solution of Dess–Martin periodinane in CH_2Cl_2 (Acros, 2.1 mL, 1.5 equiv) was added at rt to a solution of crude **96** (430 mg) in CH_2Cl_2 (4 mL) and the mixture was stirred for 2 h. The solution was diluted with EtOAc (15 mL) and washed with a 1:1 mixture of aq satd K_2CO_3 and aq satd $Na_2S_2O_3$ (3×10 mL), and then it was concentrated. Crude **97** (yellow foam, 381 mg, 0.62 mmol, 88% from **95**) required no further purification. $[\alpha]_D^{25}$ +3 (*c* 0.5, CH_2Cl_2). ^{1}H: 7.69 (4H, m), 7.39 (6H, m), 4.13 (2H, m), 3.69 (1H, dd, *J*=10.2, 4.9), 3.51 (1H, dd, *J*=10.2, 3.0), 3.12 (1H, dd, *J*=18.5, 5.7), 2.60 (1H, dd, *J*=18.5, 6.4), 2.50 (2H, m), 2.38 (3H, t, 7.5), 2.07–1.49 (11H, m), 1.28 (8H, m), 1.09 (9H, s), 0.90 (3H, t, *J*=5.4). ^{13}C: 206.2, 135.6, 134.7, 129.6, 127.6, 71.8, 66.2, 59.6, 56.9, 54.8, 47.5, 45.0, 42.9, 40.8, 35.9, 34.9, 31.4, 30.2, 28.7, 27.7, 26.6, 23.6, 22.4, 19.1, 13.9. IR: 1720, 1462, 1428, 1305. HRMS (CI): calcd for $C_{35}H_{51}NO_4SSi$ (MH^+): 610.3386; found: 610.3386.

5.3.7. Borylketone 114. A stirred solution of **97** (370 mg, 0.61 mmol) in dry DMF (5 mL) under argon was treated with DBU (100 mg, 1.05 equiv), followed, after 10 min, by bis(pinacolyl)diboronate (175 mg, 1.1 equiv), CuCl (70 mg, 1.1 equiv), and KOAc (70 mg, 1.1 equiv). Stirring was continued for 20 min, then the mixture was diluted with EtOAc (10 mL), sequentially washed with aq concd NH_4OH (2×5 mL) and brine (3×10 mL), and concentrated. Silica gel chromatography (1:9:0.1, EtOAc/hexanes/NEt_3) of the residue afforded pure **144** (pale yellow oil, 352 mg, 0.52 mmol, 86%). $[\alpha]_D^{25}$ +10 (*c* 0.5, CH_2Cl_2). ^{1}H: 7.59 (4H, m), 7.39 (6H, m), 4.01 (1H, dd, *J*=10.5, 2.6), 3.68 (1H, d, *J*=10.5), 3.51 (2H, br m), 2.62 (1H, dd, *J*=15.8, 3.0), 2.40 (2H, m), 2.30 (1H, dd, *J*=13.9, 3.4), 2.12–1.12 (15H, m),

1.09 (21H, s), 0.90 (3H, t, J=6.4). ^{13}C: 214.9, 135.6, 130.0, 127.7, 127.6, 72.1, 63.9, 58.0, 47.5, 45.1, 42.1, 34.5, 32.7, 31.5, 28.9, 26.9, 26.7, 24.5, 24.0, 23.7, 22.4, 19.5, 19.0, 13.9. HRMS (ESI): calcd for $C_{41}H_{64}NO_4BSi$ (MH^+): 674.4784; found: 674.4792.

5.3.8. Tricyclic intermediate 117. A cold (0 °C) solution of **114** (35 mg, 0.052 mmol), AcOH (1 drop), and $NaBH_3CN$ (10 mg, 3 equiv) in dry MeOH (3 mL) was stirred under argon for 4 h, then it was treated with aq 2 N NaOH (0.3 mL) and aq 35% H_2O_2 (0.2 mL) and stirred at 0 °C for another 30 min. The mixture was diluted with EtOAc (10 mL). The aqueous layer was separated and washed with more EtOAc (5 mL), and the combined organic phases were washed with brine (10 mL) and concentrated. Silica gel chromatography (1:9:0.1, EtOAc/hexanes/NEt_3) of the residue yielded pure **117** (pale yellow oil, 23 mg, 0.042 mmol, 80%). $[\alpha]_D^{25}$ +6 (c 0.5, CH_2Cl_2). ^{1}H: 7.69 (4H, m), 7.39 (6H, m), 4.14 (1H, m), 3.65 (1H, br), 3.41 (1H, br), 3.0 (1H, br), 2.48 (1H, br), 2.15–1.10 (25H, m), 1.08 (9H, s), 0.86 (3H, t, J=6.8). ^{13}C: 135.6, 129.5, 127.6, 127.5, 71.3, 66.9, 68.6, 63.8, 57.0, 39.7, 38.6, 37.4, 36.2, 33.6, 31.9, 31.8, 29.4, 26.9, 26.8, 26.5, 25.6, 23.7, 22.6, 19.2, 14.1. IR: 3417. HRMS (CI): calcd for $C_{35}H_{53}NO_2Si$ (MH^+): 548.3929; found: 548.3928.

5.3.9. Piperidinone 118. A 0.5 M solution of Dess–Martin periodinane in CH_2Cl_2 (Acros, 0.15 mL, 2.0 equiv) was added to a solution of **117** (20 mg, 0.036 mmol) in CH_2Cl_2 (1 mL) at rt. The mixture was stirred for 2 h, then it was diluted with EtOAc (5 mL), washed with a 1:1 mixture of aq satd K_2CO_3 and aq satd $Na_2S_2O_3$ (3×5 mL), and concentrated. Silica gel chromatography of the residue (1:9:0.1, EtOAc/hexane/NEt3) delivered pure **118** (pale yellow oil, 19 mg, 0.034 mmol, 94%). $[\alpha]_D^{25}$ +3 (c 0.5, CH_2Cl_2). ^{1}H: 7.69 (4H, m), 7.39 (6H, m), 3.60 (1H, dd, J=10.2, 4.5), 3.40 (1H, t, J=8.7), 3.13 (1H, m), 3.00 (1H, m), 2.51 (1H, dd, J=15.4, 5.3), 2.44 (1H, br), 2.24 (1H, d, J=10.5), 2.10 (1H, dd, J=15.8, 7.2), 2.05–1.00 (16H, m), 1.08 (9H, s), 0.85 (3H, t, J=7.2). ^{13}C: 212.6, 135.6, 134.8, 129.6, 127.7, 127.6, 68.1, 68.0, 66.2, 58.5, 50.9, 43.0, 40.5, 36.9, 36.2, 31.8, 29.2, 26.9, 26.5, 26.0, 24.3, 23.1, 22.6, 21.6, 19.2, 14.1. IR: 1707. HRMS (CI): calcd for $C_{35}H_{51}NO_2Si$ (MH^+): 546.3767; found: 546.3768.

5.3.10. Synthetic (−)-2-epicylindricine C, 119. A solution of **118** (18 mg, 33 μmol) and TBAF (32 mg, 3 equiv) in THF (1 mL) was stirred at rt for 4 h. Concentration and chromatography of the residue (1:9:0.1, EtOAc/hexanes/NEt_3) afforded pure (−)-**119** (pale yellow oil, 10 mg, 0.032 mmol, 96%), whose ^{1}H NMR spectra were superimposable to those published by Weinreb (Ref. 35h). $[\alpha]_D^{25}$ −39 (c 0.5, CH_2Cl_2). ^{1}H: 3.53 (1H, dd, J=10.5, 4.1), 3.40–3.20 (3H, m), 2.63 (1H, dd, J=15.3, 5.7), 2.52 (1H, br), 2.27 (1H, m), 2.19 (1H, dd, J=15.4, 6.0), 2.03 (2H, m), 1.83 (2H, m), 1.70–1.20 (19H, m), 0.87 (3H, t, J=6.5). ^{13}C: 211.5, 68.9, 64.6, 63.7, 57.9, 50.9, 42.7, 39.4, 36.9, 36.7, 31.7, 29.7, 29.2, 26.6, 26.2, 24.3, 22.6, 22.6, 14.1. IR: 3445, 1707. HRMS (CI): calcd for $C_{19}H_{33}NO_2$ (MH^+): 308.2589; found: 308.2587.

5.3.11. Boronic ester 123. A solution of **114** (122 mg, 0.18 mmol) and TBAF (175 mg, 3 equiv) in THF (4 mL) was stirred at rt for 5 h, and then it was concentrated. The residue was quickly chromatographed (silica gel) by sequential elution with 1:9:0.1 EtOAc/hexanes/NEt_3 (silicon-containing byproducts) and 95:5 EtOAc/NEt_3 (elution of desilylated **123**). The spectra of the product (pale yellow oil, 75 mg, 0.17 mmol, 95%) suggest that it exists largely as the open-chain, instead of the hemiaminal, tautomer. $[\alpha]_D^{25}$ +11 (c 0.5, CH_2Cl_2). ^{1}H: 3.90 (1H, d, J=9.4), 3.61 (2H, m), 3.04 (1H, d, J=8.7), 2.71 (1H, dd, J=16.2, 3.8), 2.40 (2H, m), 2.31 (1H, dd, J=13.2, 3.8), 2.20–1.20 (21H, m), 1.15 (12H, d, J=12.4), 0.87 (3H, t, J=6.5). ^{13}C: 214.9, 79.3, 72.9, 63.3, 59.5, 47.2, 46.3, 42.3, 34.0, 32.7, 31.5, 28.9, 27.4, 26.8, 26.0, 24.9, 24.4, 24.0, 23.6, 22.4, 19.5, 14.0. IR: 3253, 1704. MS (ESI): 436 (MH^+) for $C_{25}H_{46}NO_4B$. A cold (−78 °C) solution of this material (70 mg, 0.16 mmol), AcOH (two drops), and $NaBH(OAc)_3$ (50 mg, 1.5 equiv) in CH_2Cl_2 (4 mL) was stirred under argon overnight, during which time it was allowed to warm up to rt. The mixture was diluted with EtOAc (10 mL) and washed with aq satd K_2CO_3 (10 mL). The organic phase was further washed with brine (10 mL) and concentrated. Silica gel chromatography of the residue (2:8:0.1 EtOAc/hexanes/NEt3) provided pure **123** (49 mg, 0.12 mmol, 73%) as a pale yellow oil, $[\alpha]_D^{25}$ −22 (c 0.5, CH_2Cl_2). ^{1}H: 3.44–3.22 (4H, m), 2.28 (1H, dt, J=12.8; 4.1), 2.22–1.90 (3H, m), 1.80–1.20 (22H, m), 1.25 (12H, s), 0.87 (3H, t, J=6.5). ^{13}C: 82.7, 66.3, 66.2, 56.1, 51.0, 37.0, 36.7, 35.4, 34.0, 31.9, 30.0, 29.7, 29.4, 28.9, 27.1, 24.9, 24.0, 22.7, 21.7, 19.0, 14.1. IR: 3409. MS (ESI): 420 (MH^+) for $C_{25}H_{46}NO_3B$.

5.3.12. Compound 124. A solution of **123** (42 mg, 0.10 mmol), imidazole (25 mg, 4 equiv), and TBDPSCl (28 mg, 1.1 equiv) in DMF (2 mL) was stirred at rt for 3 h, then it was diluted with EtOAc (5 mL) and brine (5 mL). The aqueous layer was removed and washed with more EtOAc (5 mL). The combined organic phases were washed with brine (4×15 mL) and concentrated. Silica gel chromatography of the residue (1:9:0.1 EtOAc/hexanes/NEt3) yielded pure **22** (pale yellow oil, 0.63 mg, 0.095 mmol, 95%). $[\alpha]_D^{25}$ +9 (c 0.5, CH_2Cl_2). ^{1}H: 7.70 (4H, m), 7.39 (6H, m), 3.52 (1H, br), 3.15 (3H, br), 2.20–1.00 (25H, m), 1.25 (12H, s), 1.06 (9H, s), 0.87 (3H, t, J=6.5). ^{13}C: 135.6, 129.4, 127.5, 82.6, 69.3, 65.3, 57.8, 50.7, 37.3, 35.6, 35.3, 34.2, 31.9, 29.7, 29.3, 27.1, 26.9, 26.4, 24.9, 24.8, 24.2, 22.6, 21.6, 19.2, 14.1. MS (ESI): 658 (MH^+) for $C_{41}H_{64}NO_3SiB$.

5.3.13. Alcohol 125. A cold (0 °C) solution of **124** (56 mg, 0.085 mmol), 2 N aq NaOH (0.5 mL), and aq 35% H_2O_2 (0.3 mL) in THF (2 mL) was stirred for 30 min, and then it was diluted with EtOAc (5 mL). The aqueous layer was discarded and the organic phase was washed with brine (3×3 mL) and concentrated. Crude **125** (45 mg, 0.083 mmol, 97%), pale yellow oil, was used without further purification. $[\alpha]_D^{25}$ +6 (c 0.5, CH_2Cl_2). ^{1}H: 7.66 (4H, m), 7.37 (6H, m), 4.14, (1H, br), 3.57 (1H, br), 3.16 (3H, br), 2.15–1.00 (25H, m), 1.08 (9H, s), 0.84 (3H, t, J=6.5). ^{13}C: 135.6, 134.3, 134.0, 129.4, 127.5, 73.6, 68.9, 65.1, 57.5, 46.9, 37.8, 36.2, 35.1, 35.0, 33.8, 31.8, 29.3, 27.2, 26.9, 26.2, 24.4, 23.1, 22.6, 19.2, 14.0. IR: 3417. HRMS (CI): calcd for $C_{35}H_{53}NO_2Si$ (MH^+): 548.3929; found: 548.3929.

5.3.14. Protected cylindricine C, 126. A 0.5 M CH_2Cl_2 solution of Dess–Martin periodinane (Acros, 0.5 mL,

1.5 equiv) was added to a solution of **125** (41 mg, 0.075 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at rt for 2 h, then it was diluted with EtOAc (5 mL), washed with a 1:1 mixture of aq satd K_2CO_3 and aq satd $Na_2S_2O_3$ (3×5 mL), and concentrated. Silica gel chromatography of the residue (1:9:0.1 EtOAc/hexanes/NEt3) afforded pure **126** (38 mg, 0.070 mmol, 94%) as a (pale yellow oil, $[\alpha]_D^{25}$ −6 (*c* 0.5, CH_2Cl_2). ^{1}H: 7.68 (4H, m), 7.39 (6H, m), 3.63, (1H, dd, *J*=6.4, 1.9), 3.32 (1H, m), 3.29 (1H, t, *J*=9.0), 3.09 (1H, m), 2.30–2.00 (7H, m), 1.70–1.00 (18H, m), 1.08 (9H, s), 0.84 (3H, t, *J*=7.2). ^{13}C: 211.4, 135.6, 134.0, 133.8, 129.6, 127.6, 70.2, 68.4, 57.7, 55.3, 50.1, 42.9, 35.8, 34.9, 34.8, 31.7, 29.7, 29.0, 26.9, 25.9, 24.4, 22.9, 22.5, 21.9, 19.2, 14.0. IR: 1707. HRMS (CI): calcd for $C_{35}H_{51}NO_2Si$ (MH^+): 546.3767; found: 546.3768.

5.3.15. Synthetic (−)-cylindricine C, 76. A solution of **126** (29 mg, 0.053 mmol) and TBAF (50 mg, 3 equiv) in THF (1 mL) was stirred at rt for 4 h, and then it was concentrated. Silica gel chromatography of the residue (1:9:0.1 EtOAc/hexanes/NEt3) provided pure (−)-**76** (pale yellow oil, 16 mg, 0.051 mmol, 96%), whose ^{1}H NMR spectra were superimposable to those published by Molander (Ref. 35a). The optical rotation measured for **3**, $[\alpha]_D^{25}$ −66 (*c* 0.5, CH_2Cl_2), matched that reported by Molander for (−)-cylindricine C, $[\alpha]_D^{25}$ −64 (*c* 0.2, CH_2Cl_2), and, in absolute value, that reported by Trost (Ref. 35b) for (+)-cylindricine C, $[\alpha]_D^{25}$ +61 (*c* 0.4, CH_2Cl_2). ^{1}H: 3.52, (2H, m), 3.44 (1H, m), 3.29 (1H, m), 2.86 (1H, br m), 2.30–2.07 (5H, m), 1.83 (1H, m), 1.77–1.20 (19H, m), 0.88 (3H, t, *J*=7.2). ^{13}C: 210.4, 70.6, 66.3, 56.5, 55.3, 50.2, 42.5, 36.4, 35.9, 35.2, 31.7, 29.2, 28.7, 27.1, 24.2, 22.7, 22.5, 21.8, 14.0. IR: 3445, 1707. HRMS (CI): calcd for $C_{19}H_{33}NO_2$ (MH^+): 308.2589; found: 308.2589.

5.4. General procedure for bimolecular oxidative amidation of phenols

A solution of $PhI(OAc)_2$ ('DIB', 232.0 mg, 0.7 mmol, 1.2 equiv) in $(CF_3)_2CHOH$ ('HFIP', 0.5 mL) was added dropwise over 30 s to a vigorously stirred solution of a phenol (0.6 mmol, 1 equiv) in MeCN (2.0 mL) and HFIP (1.5 mL) kept at 15 °C (bath temperature). The mixture was stirred for 20 min, and then it was concentrated. Silica gel chromatography of the residue, first with 1:1 AcOEt/Hexanes (removal of gross contaminants), and then with 5–10% MeOH in CH_2Cl_2, provided the pure product.

5.4.1. Amidodienones 139. *R=CH₃*. Yield=56%. ^{1}H: 6.82 (2H, d, *J*=10.2), 6.31 (1H, br s), 6.19 (2H, d, *J*=10.2), 1.91 (3H, s), 1.43 (3H, s). ^{13}C: 185.5, 170.3, 152.5, 127.6, 52.5, 26.1, 23.1. HRMS (CI): calcd for $C_9H_{12}O_2N_1$ (MH^+): 166.0868; found:166.0871.

R=(CH₂)₂CH₃. Yield=54%. ^{1}H: 6.77 (2H, d, *J*=10.2), 6.67 (1H, br s), 6.24 (2H, d, *J*=10.2), 1.96 (3H, s), 1.69 (2H, m), 1.22 (2H, m), 0.84 (3H, t, *J*=7.2). ^{13}C: 185.8, 170.0, 151.3, 128.6, 55.9, 40.4, 23.2, 16.4, 13.9. HRMS (CI): calcd for $C_{11}H_{16}O_2N_1$ (MH^+): 194.1181; found: 194.1182.

R=CH(CH₃)₂. Yield=62%. ^{1}H: 6.81 (2H, d, *J*=10.2), 6.31 (1H, br s), 6.26 (2H, d, *J*=10.2), 2.21 (1H, h, *J*=7.0), 1.91 (3H, s), 0.89 (6H, d, *J*=7.0). ^{13}C: 185.8, 170.1, 149.8, 129.3, 58.9, 34.7, 23.3, 16.9. HRMS (CI): calcd for $C_{11}H_{16}O_2N_1$ (MH^+): 194.1181; found: 194.1181.

R=CH₂COOMe. Yield=58%. ^{1}H: 6.95 (2H, d, *J*=10.2), 6.80 (1H, br s), 6.29 (2H, d, *J*=10.2), 3.72 (3H, s), 2.76 (2H, s), 1.97 (3H, s). ^{13}C: 184.7, 170.2, 170.1, 148.4, 128.8, 53.2, 52.3, 42.2, 23.5.

R=(CH₂)₂NHTs. Yield=53%. ^{1}H: 7.62 (2H, d, *J*=7.9), 7.24 (2H, d, *J*=7.9), 6.98 (1H, br s), 6.84 (2H, d, *J*=9.8), 6.21 (2H, d, *J*=9.8), 5.83 (1H, br s), 2.83 (2H, br m), 2.38 (3H, s), 2.03 (2H, br m), 1.91 (3H, s). ^{13}C: 185.6, 170.8, 150.4, 143.8, 136.1, 129.8, 128.9, 126.9, 54.8, 38.2, 37.7, 23.3, 21.5.

R=(CH₂)₃OPiv. Yield=67%. ^{1}H: 6.80 (2H, d, *J*=10.2), 6.30 (2H, d, *J*=10.2), 6.21 (1H, br s), 3.99 (2H, t, *J*=6.0), 1.95 (3H, s), 1.88 (2H, m), 1.53 (2H, m), 1.16 (9H, s). ^{13}C: 185.4, 178.4, 169.9, 150.1, 129.1, 63.3, 55.5, 38.6, 34.2, 27.0, 23.3, 22.6. HRMS (CI): calcd for $C_{16}H_{24}O_4N_1$ (MH^+): 294.1705; found 294.1705.

R=(CH₂)₃CO₂TFE. Yield=57%. ^{1}H: 6.84 (2H, d, *J*=10.2), 6.43 (1H, br s), 6.28 (2H, d, *J*=10.2), 4.47 (2H, q, *J*=8.3), 2.41 (2H, t, *J*=6.8), 1.94 (3H, s), 1.83 (2H, m), 1.62 (2H, m). ^{13}C: 185.4, 171.4, 170.0, 150.0, 129.2, 60.5 (q), 55.6, 37.2, 32.7, 23.4, 18.4.

R=(CH₂)₂CN. Yield=67%. ^{1}H: 6.89 (2H, d, *J*=10.2), 6.70 (1H, br s), 6.31 (2H, d, *J*=10.2), 2.26 (4H, br), 1.91 (3H, s). ^{13}C: 184.7, 170.5, 148.0, 130.0, 118.7, 55.0, 32.4, 23.4, 11.9.

R=(CH₂)₃CN. Yield=71%. ^{1}H: 6.91 (1H, br s), 6.82 (2H, d, *J*=10.2), 6.26 (2H, d, *J*=10.2), 2.31 (2H, t, *J*=6.8), 1.97 (2H, m), 1.89 (3H, s), 1.52 (2H, m). ^{13}C: 185.3, 170.2, 149.9, 129.1, 118.8, 55.3, 36.1, 23.2, 19.2, 16.8.

R=(CH₂)₄CN. Yield=71%. ^{1}H: 6.86 (2H, d, *J*=10.2), 6.60 (1H, br s), 6.26 (2H, d, *J*=10.2), 2.31 (2H, t, *J*=7.2), 1.92 (3H, s), 1.87 (2H, m), 1.60 (2H, q, *J*=7.2), 1.35 (2H, m). ^{13}C: 185.4, 170.1, 150.2, 129.0, 119.2, 55.7, 36.9, 25.0, 23.4, 22.4, 16.8.

R=(CH₂)₃N₃. Yield=42%. ^{1}H: 6.82 (2H, d, *J*=10.2), 6.29 (2H, d, *J*=10.2), 5.89. (1H, br s), 3.31 (2H, t, *J*=6.4), 1.98 (2H, m, 3H, overlapping a s), 1.47 (2H, m). ^{13}C: 185.4, 170.1, 150.0, 129.2, 55.6, 50.9, 34.9, 23.4, 22.9.

R=(CH₂)₄N₃. Yield=49%. ^{1}H: 6.79 (2H, d, *J*=10.2), 6.25 (1H, br s), 6.24 (2H, d, *J*=10.2), 3.20 (2H, t, *J*=7.0), 1.91 (3H, s), 1.78 (2H, m), 1.47 (2H, quintuplet, *J*=7.0), 1.25 (2H, m). ^{13}C: 185.6, 170.2, 150.7, 128.8, 55.8, 50.8, 37.4, 28.4, 23.2, 20.4.

R=(CH₂)₃Br. Yield=65%. ^{1}H: 6.81 (2H, d, *J*=10.2), 6.37 (1H, br s), 6.30 (2H, d, *J*=10.2), 3.35 (2H, t, *J*=6.0), 2.01 (2H, m), 1.96 (3H, s), 1.73 (2H, m). ^{13}C 185.3, 170.0, 149.9, 129.4, 55.5, 36.4, 32.8, 26.3, 23.5.

R=(CH₂)₄Br. Yield=72%. ^{1}H: 6.84 (2H, d, *J*=10.2), 6.68 (1H, br s), 6.26 (2H, d, *J*=10.2), 3.32 (2H, t, *J*=6.8), 1.91 (3H, s), 1.80 (4H, m), 1.37 (2H, m). ^{13}C: 185.6, 170.1, 150.6, 128.9, 55.8, 37.0, 32.9, 32.1, 23.3, 21.7.

5.4.2. Spirane 141. A solution of **140** (0.2 mmol) in dry THF (2.0 mL) was treated with solid NaH (1.1 equiv) and stirred at rt (Ar). After 1 h, TLC showed complete conversion. Satd aq NH_4Cl solution (two drops) was added and the mixture was concentrated. The residue was taken up with $CHCl_3$ (3×2 mL). Filtration and concentration provided essentially pure **141** (91%) as a slightly yellow oil. ^1H: 7.01 (2H, d, J=10.2), 6.19 (2H, d, J=10.2), 3.58 (2H, app t, J=5.7), 2.08 (3H, s), 1.80 (2H, m), 1.63 (4H, m). ^{13}C: 185.3, 171.8, 152.0, 125.9, 56.8, 38.2, 23.9, 23.2 (two overlapping signals), 19.5.

Note added in proof

A noteworthy synthesis of (−)-cylindricine has recently been disclosed by Swidorski, J. J.; Wang, J.; Sung, R. P. *Org. Lett.* **2006**, *8*, 777.

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References and notes

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Facile biomimetic syntheses of the azaspiracid spiroaminal

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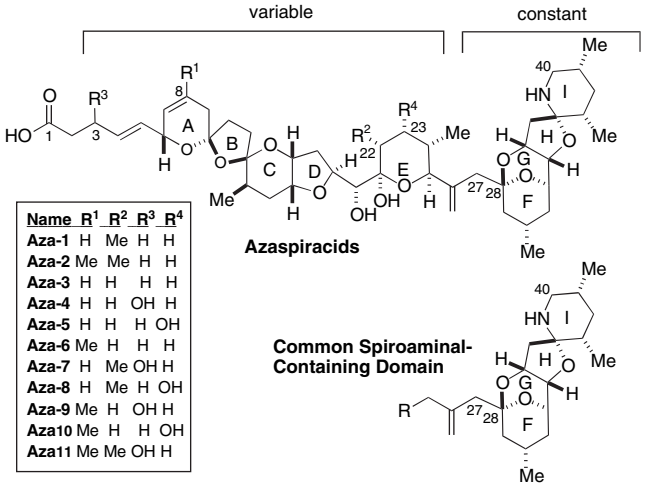
Abstract—The azaspiracid natural products display a common spiroaminal-containing terminal domain that has inspired the development of two new methods for spiroaminal syntheses—a Staudinger reduction–aza-Wittig process and a double intramolecular hetero-Michael addition. These effective laboratory approaches proceed through imine and enamine intermediates that may reflect transient biogenetic species.

1. Introduction

The azaspiracids are the causative agents of a recently defined class of human poisoning resulting from consumption of tainted shellfish. As such, intense surveillance programs have been established to monitor the occurrence and extent of azaspiracid contamination in edible shellfish. The archetypal member of this novel class of marine toxins, azaspiracid-1 (AZA1, Fig. 1), was reported by Yasumoto and co-workers as an isolate from the cultivated Irish mussel *Mytilus edulis*.[1] Since then, AZA1 has also been found in the dinoflagellate *Protoperidinium crassipes*, which may represent the primary biogenic source of the azaspiracid toxins.[2] Recently, the complete structure of AZA1 was established by a comprehensive total synthesis—correlation effort by the Nicolaou group.[3] To date, five azaspiracids have been isolated and structurally elucidated by extensive NMR analysis and FABMS–MS experiments (AZA1–5),[4] and six others (AZA6–11) have been detected using a combination of liquid chromatography and multi-tandem mass spectrometry (LC–MS^n).[5] Among the 11 known azaspiracids, none show any structural variation within the spiroaminal-terminated C27–C40 domain. Hence, we have focused on developing efficient synthetic entries to this common portion of the natural products to support the generation of an ELISA for environmental monitoring,[6] total syntheses of the natural products, and the laboratory exploration of putative biomimetic pathways.[7] Here, we summarize the development and application of two novel methods for the formation of the common azaspiracid spiroaminal.



Name	R^1	R^2	R^3	R^4
Aza-1	H	Me	H	H
Aza-2	Me	Me	H	H
Aza-3	H	H	H	H
Aza-4	H	H	OH	H
Aza-5	H	H	H	OH
Aza-6	Me	H	H	H
Aza-7	H	Me	OH	H
Aza-8	H	Me	H	OH
Aza-9	Me	H	OH	H
Aza10	Me	H	H	OH
Aza11	Me	Me	OH	H

Figure 1. Structures of the azaspiracids and the common spiroaminal-containing domain.

2. Results and discussion

2.1. Synthetic design

Our established route to the dioxabicyclononane system, comprising the C28–C34 F-G rings of the azaspiracids, involves a double intramolecular hetero-Michael addition (DIHMA) upon an ynone.[8] Retrosynthetically, the G ring may be disconnected to enone **1** by a retro-Michael disconnection of the C28 ketal releasing a C34 hydroxyl group (Scheme 1).

A second retro-Michael disconnection of the β-oxyenone reveals dihydroxy ynone **2**, which, in turn may arise via the coupling of a C27 acetylide anion equivalent (**3**) upon a generalized C26 carbonyl derivative. Advanced intermediate **3**

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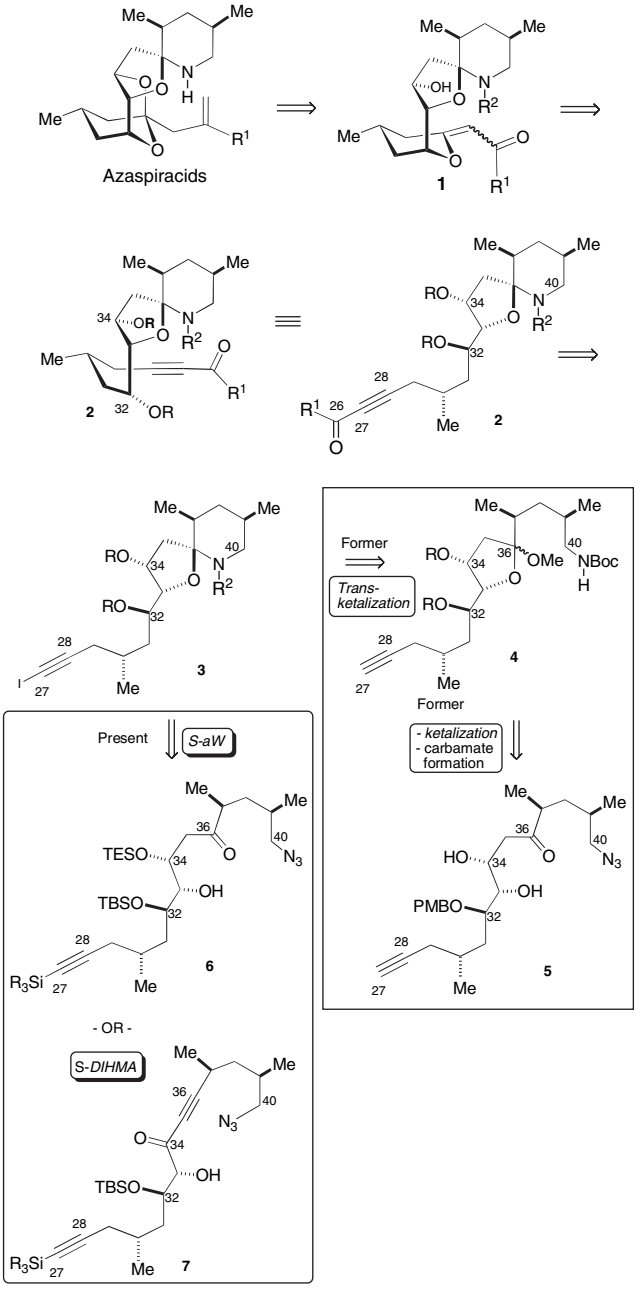
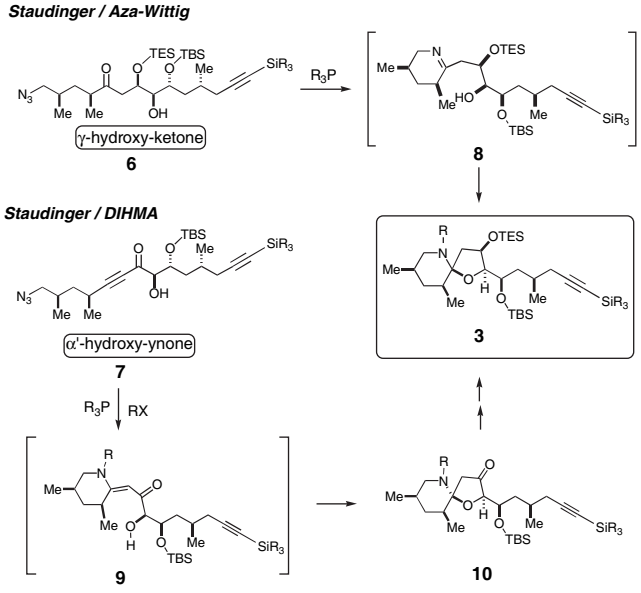


Scheme 1. Retrosynthetic analysis for the C27–C40 fragment.

contains the piperidine–tetrahydrofuran fused spiroaminal (H-I rings) and latent nucleophilic oxygens at C32 and C34 masked as silyl ethers. The previously published assemblies of the azaspiracid spiroaminal moiety used a stepwise sequence of ring-closings.[8–10] These involved the initial formation of the C33–C36 H ring as a cyclic mixed methyl ketal. A C40 terminal azide was then reduced and protected as a carbamate (cf. **4**, Scheme 1). Finally, the spiroaminal was formed by closure of the C36–C40 piperidine I ring upon treatment with a Lewis acid, such as $Yb(OTf)_3$[8,10] or $BF_3\cdot OEt_2$.[9] We have since designed more facile assemblies of spiroaminal **3** from acyclic intermediates γ-hydroxy-δ′-azidoketone **6** or α-hydroxy-α′,β′-ynone **7**.

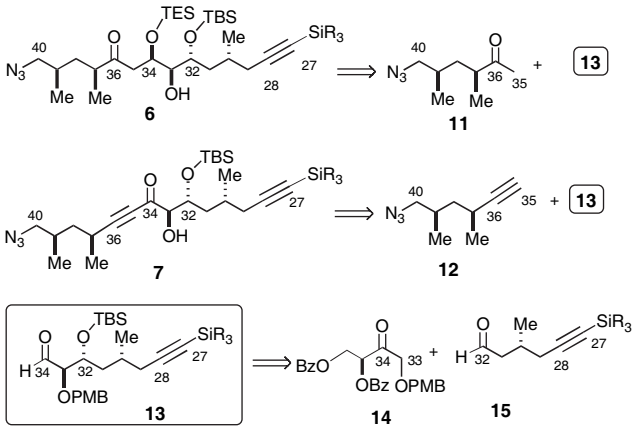
The appropriately functionalized acyclic intermediate **6** would be subjected to Staudinger reduction under anhydrous conditions to induce an intramolecular aza-Wittig reaction (S–aW) to form the I ring as an imine (**8**, Scheme 2), followed by engagement of the tethered hydroxyl group to close the H ring and generate **3**. Both kinetic stereoelectronic and thermodynamic effects should favor the selective formation of the natural products' C36 spiroaminal configuration. Alternatively, liberation of a primary amine by Staudinger reduction of **7** in the presence of a carbamate-forming reagent (RX, Scheme 2) might allow formal successive intramolecular hetero-Michael additions upon the α,β-unsaturated ketone (DIHMA) to ensue. In the latter case, an initial 6-*exo* addition of a carbamate nitrogen upon the ynone would generate a hydroxy-enamine (**9**) that could isomerize to spiroaminal **10**. The residual ketone at C34 of **10** would provide a functional handle for the stereoselective installation of the corresponding silyl ether in **3**. Although Staudinger reactions are unlikely to be involved in the actual biogenesis of spiroaminals, the postulated types of hydroxy-imine or hydroxy-enamine intermediates (**8** and **9**) derived from Staudinger reactions in our laboratory syntheses may well be. Furthermore, these designed Staudinger reaction-initiated cascade sequences should be accessible under essentially neutral reaction conditions that are tolerant of the extensive functionality found in the azaspiracid natural products.



Scheme 2. Two proposed methods for the formation of the azaspiracid spiroaminal.

Retrosynthetically, we maintained the same type of triply convergent approach employed in our previously disclosed synthesis of the acyclic precursor to the azaspiracid C27–C40 domain, but with a few significant changes.[8] Ynones **6** and **7** could be derived from methyl ketone **11** or alkyne **12**, respectively, and the common C34 aldehyde **13** (Scheme 3). A chelation-controlled Mukaiyama aldol reaction was envisioned to join a silyl enol ether derivative of **11** with **13** while simultaneously setting the C33,C34 *syn*-stereochemistry in **6**. Addition of an acetylide nucleophile derived from **12** to aldehyde **13** followed by oxidation and PMB ether cleavage would provide **7**. The common aldehyde **13** arises from a Paterson aldol reaction of a boron enolate obtained from ketone **14** and C32 aldehyde **15**, with subsequent oxidative excision of the benzoyl chiral directing moiety

resident in **14**. Importantly, a chelating α-*p*-methoxybenzyl (PMB) ether was incorporated in aldehyde **13** at the stage of ketone **14** to effect the desired chelation-controlled Mukaiyama reaction. This specific protecting group array would also simplify the functional group manipulations required to enable the late-stage cyclization cascades via either the S–aW or the S–DIHMA processes to form the H-I ring.

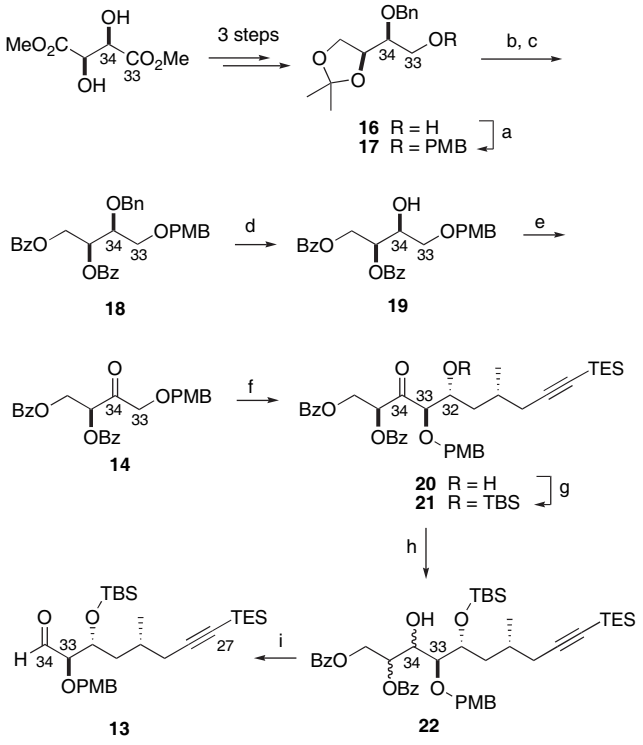


Scheme 3. Key disconnections of ynones **6** and **7**.

2.2. Preparative chemistry

The synthesis of the C27–C34 aldehyde **13** common to both spiroaminal formation strategies began with the preparation of ketone **14**, which ultimately provides only carbons 33 and 34 (Scheme 4). However, **14** also bears the essential α-benzyloxy stereogenic center that defines the absolute stereochemistry of the ensuant C32 and C33 centers via the Paterson boron aldol reaction.[11] Dimethyl D-tartrate was converted into primary alcohol **16** according to the procedure of Ohno.[12] The C32 PMB ether was formed at this stage to generate **17**. Cleavage of the acetonide moiety followed by bis-benzoylation of the resultant diol provided **18**. Selective hydrogenolysis of the benzyl ether of **18** in the presence of the vicinal PMB was accomplished using Pd–$BaSO_4$ in ethyl acetate to yield secondary alcohol **19**. Oxidation of the alcohol then delivered ketone **14** in five steps and 40% overall yield from **16**.

A boron-mediated aldol reaction between ketone **14** and aldehyde **15**[8] following Paterson's procedure[11] proceeded smoothly to afford the desired *syn/anti* product **20** (Scheme 4) in high yield and diastereoselectivity (ds >9:1, by ^{1}H NMR spectroscopy). The anticipated C32,C33 *anti*-relationship was confirmed by the relatively large coupling constant (5 MHz) between C32–H and C33–H. It should be noted that attempts to use the analogous 1,4-bis-(*p*-methoxybenzyl)oxy-3-benzoyl-butanone under the same reaction conditions only resulted in a complicated mixture of inseparable diastereomeric aldol products. The resultant β-hydroxyl group in **20** was protected as a TBS ether to give **21**. Exhaustive reduction of the ketone and the bis-benzoyloxy groups in **21** using $LiAlH_4$ resulted in a low yield of the expected triol. This may be attributed to a net retro-aldol reaction leading to the fragmentation of the oxy-ketone. A stepwise approach was thus pursued. Hence ketone **21** was reduced non-stereoselectively to alcohol **22** using $NaBH_4$. Removal

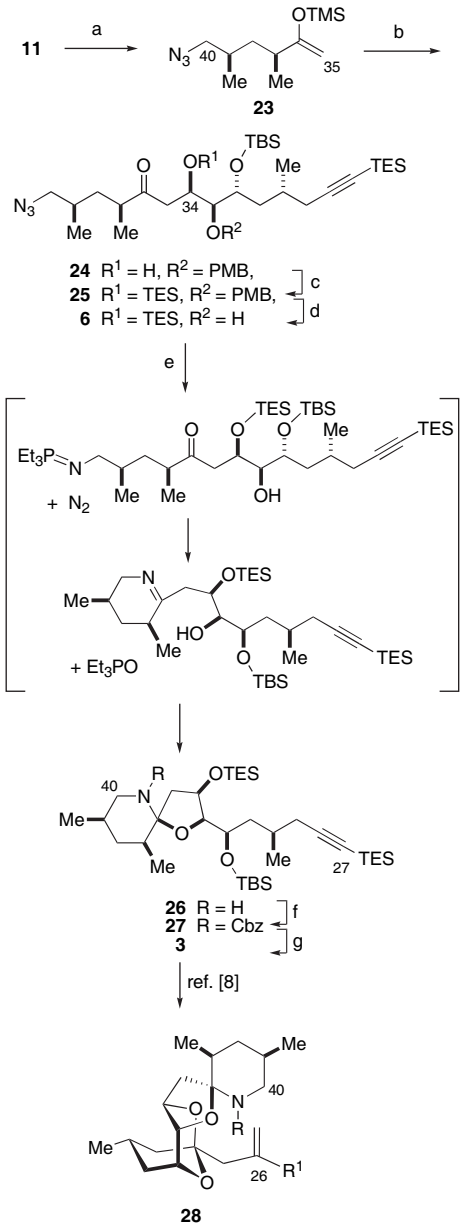


Scheme 4. Synthesis of the C27–C34 aldehyde. a) NaH, PMBCl, TBAI, DMF, THF, 0 °C to rt, 6 h (96%); b) CSA (cat.), MeOH, 30 min; c) BzCl, triethylamine, DMAP, CH_2Cl_2, 14 h (93% from **17**); d) H_2, Pd–$BaSO_4$, EtOAc, 14 h (58%); e) TPAP (cat.), NMO, CH_2Cl_2, 2 h (78%); f) $Chex_2BCl$, triethylamine, 0 °C, 2 h, then **15**, −78 °C, 5 h, (69%); g) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 30 min (83%); h) $NaBH_4$, MeOH, 0 °C, 1 h, (92%); i) K_2CO_3, MeOH, 5 h, then AcOH, $NaIO_4$, 30 min (70%).

of both benzoyl groups was then accomplished by treatment of **22** with K_2CO_3 in methanol. Thereafter, the crude triol solution was neutralized with acetic acid and subsequently treated with $NaIO_4$. This one-pot procedure reliably provided the desired C27–C34 aldehyde **13**.

To implement the S–aW spiroaminal formation strategy, methyl ketone **11**[5] (Scheme 3), representing C35–C40 of the azaspiracids, was joined with aldehyde **13** via a chelation-controlled Mukaiyama aldol reaction. (Scheme 5). For this, ketone **11** was converted to the TMS vinyl ether **23** by treatment with NaHMDS followed by TMSCl. Various Lewis acids were screened for the aldol reaction, including $SnCl_4$, $TiCl_4$, $ZnCl_2$, $MgCl_2$, and $MgBr_2\cdot OEt_2$. Among these, $MgBr_2\cdot OEt_2$ best delivered the desired *anti*-Felkin–Anh product **24**. The absolute configuration of (34*R*) in **24** was confirmed by application of the advanced Mosher ester analysis.[13] Silylation of the C34 hydroxy group and removal of the PMB protective group left only the C33 hydroxyl and C36 ketone of **6** free to participate in spiroaminal formation, while the latent nucleophilic terminal nitrogen remained masked as an azide.

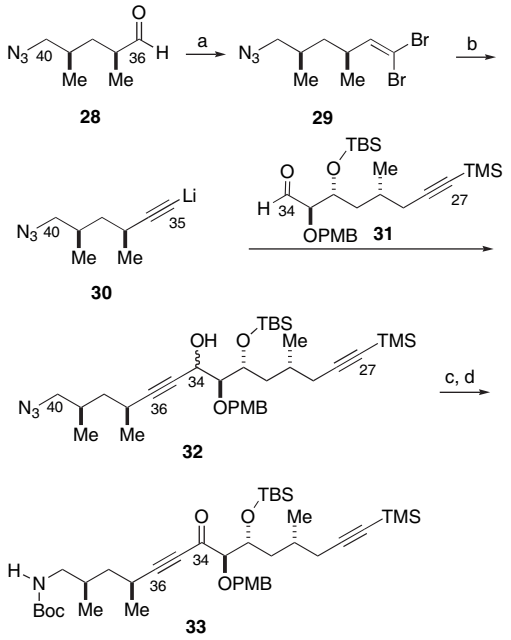
The anticipated spiroaminal moiety in **26** was initially formed in moderate yield upon treatment of azide **6** with *n*-Bu_3P in benzene (Scheme 5). After surveying various conditions it was found that Et_3P in benzene provided better results, reproducibly giving **26** in ca. 75% yield as a ca. 4:1 ratio of C36 epimers. The sequence likely proceeds via reaction of the trialkyphosphine with the azide to generate an



Scheme 5. Spiroaminal assembly via a Staudinger–aza-Wittig process. a) NaHMDS, THF, −78 °C, 15 min, then TMSCl, 1 h (~90%, crude); b) **13**, $MgBr_2 \cdot OEt_2$, CH_2Cl_2, −78 °C to −25 °C, 14 h (66%, 74% borsm); c) TBSOTf, 2,6-lutidine, CH_2Cl_2, −10 °C (92%); d) DDQ, *t*-BuOH, CH_2Cl_2, H_2O, 2 h, (82%); e) Et_3P, PhH, 6 h (75%); f) CbzCl, K_2CO_3, 4 Å MS, CH_2Cl_2, 14 h (73%); g) AgTFA, NIS, DMF (87%).

iminophosphorane that undergoes an intramolecular aza-Wittig reaction with the C36 ketone to form the six-membered cyclic imine. Addition of the C33 hydroxyl group to the imine completes the cascade. The spiroaminal nitrogen of **26** was converted into the corresponding benzylcarbamate **27** for characterization. ^{1}H NMR experiments, including NOE, firmly established the relative configuration of the C36 spiroaminal center in the major diastereomer as indicated. This result is consistent with either a kinetically controlled pseudoaxial addition of the C33 hydroxyl upon the piperidine imine, or post-addition thermodynamic equilibration, or both.

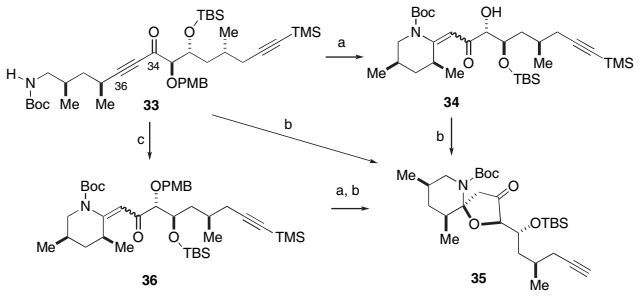
The alternative DIHMA approach to the azaspiracid spiroaminal required the synthesis of a conjugated ynone of type **7** (Scheme 2). For this, δ-azido-aldehyde **28** (Scheme 6) was converted to geminal dibromo-olefin **29** under Corey–Fuchs conditions. Transformation of **29** to the C35–C40 lithium acetylide **30** with *n*-butyllithium allowed addition to C27–C34 aldehyde **31** to generate propargylic alcohols **32**. It was determined that Staudinger reduction and carbamate formation best preceded propargylic alcohol oxidation to yield C27–C40 ynone **33**.



Scheme 6. Assembly of the DIHMA precursor. a) CBr_4, Ph_3P, CH_2Cl_2 (91%); b) *n*-BuLi, THF, −78 °C, then **31** (70%); c) (i) Et_3P, toluene, (ii) BocON, −10 °C to rt (61%); d) MnO_2, pentane (100%).

To parlay carbamate-ynone **33** into the azaspiracid spiroaminal the carbamate nitrogen must conjugatively add to the ynone and the α-keto PMB ether needs to be cleaved so that the liberated C33 secondary hydroxyl group may add to the C34–C36 enone system. An initial attempt to engage in this sequence involved PMB ether cleavage from **33** with DDQ (Scheme 7). Concomitant with removal of the PMB group under the DDQ reaction conditions, however, was the conjugate addition of the carbamate nitrogen upon the ynone to generate hydroxyl enamine **34**. Application of conditions intended to convert the terminal alkynyl silyl group of **34** into the corresponding iodo-alkyne also led to an unanticipated secondary transformation—spiroaminal formation via addition of the C33 hydroxyl upon C36 of the enamine–enone to result in the target **35**. Intramolecular conjugate addition of the carbamate nitrogen of **33** upon the ynone system could also be initiated under the influence of the Lewis acid $MgBr_2 \cdot OEt_2$ to form enone **36**. Thereafter, cleavage of the PMB ether of **36** revealed the C33 hydroxyl, which completed the second conjugate addition to form the spiroaminal. Finally, the direct conversion of C33 PMB ether–ynone **33** into spiroaminal **36** could be accomplished in one operation and moderate yield using AgTFA followed by addition of ethanolic KI. Four functional group transformations are accomplished in this remarkable one-pot process: hetero-Michael addition of the terminal carbamate nitrogen upon the C34–C36 ynone, scission of both C27

terminal alkyne and C33 secondary alcohol protecting groups, and the stereoselective addition of the C33 oxygen upon the C36 center to generate spiroaminal **36**. Presumably, the silver cation activates the internal alkyne towards an initial intramolecular addition of the nitrogen to initiate this cascade.



Scheme 7. Spiroaminal formation via DIHMA. a) DDQ, CH_2Cl_2, *t*-BuOH, pH 7 buffer; b) (i) AgTFA, CH_2Cl_2, (ii) EtOH, H_2O, KI (**33** to **35**=56%). c) $MgBr_2 \cdot OEt_2$, CH_2Cl_2.

3. Conclusion

We have developed two complementary second-generation syntheses of the C27–C40 fragment of the azaspiracids that are both efficient and stereoselective. Both routes utilize a common C27–C34 aldehyde that is assembled with a Paterson aldol reaction to establish the C32 and C33 configurations.

For the Staudinger–aza-Wittig-based assembly of the spiroaminal, the C27–C34 aldehyde was subjected to a chelation-controlled Mukaiyama aldol reaction to set the C34 configuration and provide γ-hydroxy-δ′-azidoketone **6**. Subsequent one-pot formation of the spiroaminal moiety involved treatment with triethylphosphine to initiate the cyclization cascade leading to **26**. Compound **26** represents the fully functionalized C27–C40 azaspiracid intermediate that is amenable to elaboration into the complete F-G-H-I ring domain via our established DIHMA process. The synthetic sequence leading to **27** via the Staudinger–aza-Wittig process started with three simple subunits and was completed in nine linear steps with 11% overall yield.

The complementary DIHMA closure of the azaspiracid spiroaminal required an α-hydroxy-α′,β′-ynone (cf. **7**) that was derived from the C27–C34 aldehyde (**31**) and a C35–C40 acetylide (**30**). The emergent conjugated ynone **33** could also be converted into the corresponding spiroaminal in a one-pot process. In this case, an Ag^+ species initiated the cyclization sequence. Each of these spiroaminal forming processes demonstrated here in the context of the azaspiracids may find broader applications in organic synthesis.

4. Experimental

4.1. General

Unless noted otherwise, all oxygen and moisture-sensitive reactions were executed in oven-dried glassware sealed under a positive pressure of dry argon or nitrogen and moisture-sensitive solutions and anhydrous solvents were transferred via standard syringe and cannula techniques. Unless stated otherwise, all commercial reagents were used as received. Organic solvents were dried under nitrogen atmosphere: tetrahydrofuran (THF) and diethyl ether were distilled over Na-benzophenone; CH_2Cl_2, *N*,*N*-di-*iso*-propyl-*N*-ethylamine, and pyridine were distilled from CaH_2. Flash chromatography was performed using Baker Flash silica gel 60 (40 μM); analytical TLC was performed using 0.25 mm EM silica gel 60 F_{254} plates that were visualized by irradiation (254 nm) or by staining (450 mL of 95% ethanol, 25 mL concd. H_2SO_4, 15 mL acetic acid, and 25 mL anisaldehyde). Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. IR spectra were recorded using a Perkin–Elmer 683 infrared spectrophotometer. NMR spectra were obtained using INOVA 500 and 300 MHz Varian instruments. High-resolution mass spectrometric data were obtained using a VG Analytical Sector-Field mass spectrometer.

4.1.1. (2*S*,3*S*)-3-(*O*-Benzyl)-4-(*O*-[*p*-methoxy]benzyl)-1,2,3,4-buaneteteraol-1,2-di-*O*-acetonide (17). To a stirred solution of **16** (2.35 g, 9.32 mmol) in THF (95 mL) at 0 °C was added in portions NaH (1.12 g, 60% in mineral oil, 28 mmol). The suspension was warmed to rt. After 30 min, PMBCl (2.35 mL, 18.6 mmol) and TBAI (0.68 g, 1.9 mmol) were added sequentially. The reaction mixture was stirred for 24 h, cooled to 0 °C, and H_2O (30 mL) was added slowly. The resulting mixture was transferred to a separatory funnel and extracted with diethyl ether (3×70 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (2×30 mL) and dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude residue by silica gel column chromatography (hexanes–ethyl acetate, 10:1 to 5:1, v/v) provided **17** (3.32 g, 8.93 mmol, 96%) as a colorless oil: R_f 0.61 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ −5.8 (*c* 0.63, CH_2Cl_2); IR (thin film): 3063–2840, 1612, 1586, 1513, 1455, 1370, 1301, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.39–7.28 (m, 5H), 7.24 (d, *J*=8.7 Hz, 2H), 6.87 (d, *J*=8.7 Hz, 2H), 4.77 (d, *J*=11.4 Hz, 1H), 4.74 (d, *J*=11.4 Hz, 1H), 4.47 (d, *J*=12 Hz, 1H), 4.42 (d, *J*=12 Hz, 1H), 4.26 (q, *J*=9.0 Hz, 1H), 3.97 (dd, *J*=8.4, 6.6 Hz, 1H), 3.81 (s, 3H), 3.72 (dd, *J*=8.4, 7.4 Hz, 1H), 3.64–3.55 (m, 3H), 1.40 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.2, 138.6, 130.2, 129.3, 128.3, 127.9, 127.5, 113.8, 78.4, 76.9, 73.2, 72.9, 70.0, 65.9, 55.3, 26.6, 25.6; HRMS (ESI) calcd for $[C_{22}H_{28}O_5+Na]^+$ 395.1829, found 395.1833.

4.1.2. (2*R*,3*S*)-2-(*O*-Benzyl)-1-(*O*-[*p*-methoxy]benzyl)-1,2,3,4-buaneteteraol (17b). To a stirred solution of **17** (1.00 g, 2.68 mmol) in methanol (26 mL) was added Amberlite (IR 120H^+ C.P., Mallinckrodt; 1.0 g). After the mixture was stirred for 14 h, the resin was removed by filtration. The filtrate was evaporated in vacuo to give **17b** (0.84 g, 2.5 mmol, 94%) as a colorless oil: R_f 0.32 (hexanes–ethyl acetate, 1:1, v/v); $[\alpha]_D^{23}$ +17.6 (*c* 2.13, CH_2Cl_2); IR (thin film): 3417 (br), 3064, 3032, 2933, 2870, 1613, 1587, 1514, 1456, 1365, 1302, 1249 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.38–7.29 (m, 5H), 7.26 (d, *J*=8.5 Hz, 2H), 6.89 (d, *J*=8.5 Hz, 2H), 4.75 (d, *J*=11.0 Hz, 1H), 4.55 (d, *J*=11.0 Hz, 1H), 4.51 (d, *J*=13.0 Hz, 1H), 4.47 (d, *J*=13.0 Hz, 1H),

3.82 (s, 3H), 3.79 (m, 2H), 3.66 (m, 4H), 2.74 (br d, J=4.5 Hz, 1H), 2.31 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.3, 137.9, 129.7, 129.5, 128.6, 128.0, 113.9, 78.2, 73.3, 72.7, 71.9, 68.9, 63.5, 55.3; MS (ESI) calcd for $[C_{19}H_{24}O_5+Na]^+$ 355.15, found 355.21.

4.1.3. (2*R*,3*S*)-1,2-Dibenzoyl-3-(*O*-benzyl)-4-(*O*-[*p*-methoxy]benzyl)-1,2,3,4-buaneteteraol (18). To a stirred solution of **17b** (0.80 g, 2.4 mmol) in CH_2Cl_2 (24 mL) were added Et_3N (1.33 mL, 9.60 mmol), benzoyl chloride (0.84 mL, 7.2 mmol), and DMAP (29 mg, 0.24 mmol) sequentially. The reaction mixture was stirred for 1.5 h before saturated aqueous NH_4Cl (10 mL) was added. The mixture was extracted with CH_2Cl_2 (2×10 mL). The combined organic extracts were washed with saturated aqueous NaCl (10 mL) and dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude residue by silica gel column chromatography (hexanes–ethyl acetate, 4:1 to 2:1, v/v) provided **18** (1.18 g, 2.25 mmol, 93%) as a colorless oil: R_f 0.52 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ +3.3 (c 0.92, $CDCl_3$); IR (thin film): 3065, 3033, 2933, 2864, 1782, 1724, 1611, 1586, 1514, 1453, 1368, 1316, 1253 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (d, J=7.1 Hz, 2H), 7.96 (dd, J=7.1 Hz, 2H), 7.56 (m, 2H), 7.41 (m, 4H) 7.36–7.27 (m, 5H), 7.25 (d, J=8.7 Hz, 2H), 6.84 (d, J=8.7 Hz, 2H), 5.55 (m, 1H), 4.81 (d, J=11.8 Hz, 1H), 4.69 (d, J=11.8 Hz, 1H), 4.66 (d, J=4.2 Hz, 1H), 4.62 (d, J=4.2 Hz, 1H), 4.48 (s, 2H), 4.02 (dd, J=9.9, 5.4 Hz, 1H), 3.79 (s, 3H), 3.72 (d, J=5.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.0, 165.6, 159.0, 137.7, 133.0, 132.9, 129.7, 129.5, 129.3, 128.2, 128.1, 127.8, 127.6, 113.6, 76.3, 73.1, 72.9, 71.3, 68.7, 63.1, 55.1; HRMS (ESI) calcd for $[C_{33}H_{32}O_7+Na]^+$ 563.2041, found 563.2035.

4.1.4. (2*R*,3*S*)-1,2-Dibenzoyl-4-(*O*-[*p*-methoxy]benzyl)-1,2,3,4-buaneteteraol (19). To a solution of **18** (1.00 g, 1.85 mmol) in ethyl acetate (100 mL) was added Pd–$BaSO_4$ (0.2 g, 10% w/w). The mixture was stirred under a H_2 atmosphere (~1 atm) for 6 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 5:1 to 2:1, v/v) to give recovered **18** (0.25 g, 25%) and the product **19** (0.49 g, 1.1 mmol, 58%) as a colorless oil: R_f 0.33 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ −8.7 (c 1.1, $CDCl_3$); IR (thin film): 3489 (br), 3065, 2935, 2865, 1727, 1611, 1586, 1514, 1452, 1361, 1316, 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, J=8.7 Hz, 2H), 7.98 (d, J=9.0 Hz, 2H), 7.56 (m, 2H), 7.42 (m, 4H), 7.20 (d, J=8.7 Hz, 2H), 6.82 (d, J=8.7 Hz, 2H), 5.62 (ddd, J=7.0, 4.5, 4.5 Hz, 1H), 4.71 (dd, J=11.7, 4.5 Hz, 1H), 4.59 (dd, J=11.7, 6.9 Hz, 1H), 4.46 (s, 2H), 4.17 (m, 1H), 3.78 (s, 3H), 3.65 (dd, J=9.9, 4.5 Hz, 1H), 3.59 (dd, J=9.9, 6.3 Hz, 1H), 2.61 (d, J=6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.3, 165.9, 159.3, 133.3, 133.2, 129.8, 129.7, 129.5, 128.4, 113.8, 73.3, 72.1, 70.4, 69.6, 63.2, 55.2; MS (ESI) calcd for $[C_{26}H_{26}O_7+Na]^+$ 473.16, found 473.25.

4.1.5. (3*S*)-3,4-Dibenzoyl-4-(*O*-[*p*-methoxy]benzyl)-1,3,4-trihydroxybuanone (14). To a solution of **19** (1.67 g, 3.71 mmol) in CH_2Cl_2 (35 mL) was added 4 Å molecular sieves (0.4 g) and TPAP (65 mg, 0.18 mmol), followed by NMO (0.65 g, 5.6 mmol). After 30 min, the reaction mixture was transferred to a silica gel column and eluted with hexanes–ethyl acetate (9:1 to 4:1 to 2:1, v/v) to provide **14** (1.45 g, 3.24 mmol, 87%) as a colorless oil: R_f 0.37 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ +54.2 (c 0.74, benzene); IR (thin film): 3065, 2956, 2847, 1726, 1611, 1586, 1514, 1452, 1374, 1253 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, J=7.5 Hz, 2H), 7.98 (d, J=7.5 Hz, 2H), 7.58 (q, J=7.5 Hz, 2H), 7.44 (q, J=7.5 Hz, 4H), 7.27 (d, J=9.0 Hz, 2H), 6.86 (d, J=9.0 Hz, 2H), 5.89 (t, J=4.0 Hz, 1H), 4.87 (d, J=4.0 Hz, 2H), 4.61 (d, J=12.0 Hz, 1H), 4.55 (d, J=12.0 Hz, 1H), 4.38 (d, J=15.0 Hz, 1H), 4.30 (d, J=15.0 Hz, 1H), 3.79 (s, 3H; ^{13}C NMR ($CDCl_3$, 75 MHz): δ 202.0, 166.0, 165.3, 159.3, 133.7, 133.4, 130.0, 129.7, 128.6, 128.5, 114.0, 102.8, 75.2, 73.4, 73.3, 62.8, 55.3; HRMS (ESI) calcd for $[C_{26}H_{24}O_7+Na]^+$ 471.1420, found 471.1431.

4.1.6. Aldol adduct (20). To a stirred, 0 °C solution of dicyclohexylchloroborane (0.79 mL, 3.6 mmol) and triethylamine (0.63 mL, 4.5 mmol) in diethyl ether (40 mL) was added a solution of **14** (1.34 g, 3.0 mmol) in diethyl ether (2 mL). The initially colorless solution became a yellow suspension. After 2 h, the reaction mixture was cooled to −78 °C, and a solution of **15** (0.49 g, 2.2 mmol) in diethyl ether (3 mL) was added. After 10 min, the reaction mixture was allowed to warm slowly to −25 °C, and stirring was continued at this temperature for 4 h. A solution of pH 7 aqueous phosphate buffer (10 mL), methanol (10 mL), and 30% aqueous H_2O_2 solution (5 mL) were added sequentially. The mixture was allowed to warm to rt and stirred for 1 h before diethyl ether (20 mL) was added. The aqueous phase was separated and extracted with diethyl ether (2×10 mL), and the combined organic extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated in vacuo. The oily residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1 to 4:1 to 2:1, v/v) to provide **20** (1.02 g, 1.51 mmol, 69%) as a colorless oil: R_f 0.50 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ +37.5 (c 2.5, CH_2Cl_2); IR (thin film): 3491.8, 2955.9, 2871.4, 2170, 1727, 1613, 1586, 1514, 1453, 1262 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.05 (d, J=7.5 Hz, 2H), 8.01 (d, J=7.5 Hz, 2H), 7.58 (q, J=7.5 Hz, 2H), 7.44 (q, J=7.5 Hz, 4H), 7.27 (d, J=9.0 Hz, 2H), 6.85 (d, J=9.0 Hz, 2H), 5.93 (dd, J=4.5, 3.0 Hz, 1H), 4.89 (m, 2H), 4.63 (d, J=11.5 Hz, 1H), 4.61 (d, 11.5 1H), 4.13 (m, 1H), 4.04 (d, J=5.5 Hz, 1H), 3.77 (s, 3H), 2.90 (d, J=7.5 Hz, 1H) 2.29 (dd, J=12.0, 5.0 Hz, 1H), 2.22 (dd, J=12.0, 6.5 Hz, 1H), 2.00 (m, 1H), 1.64 (ddd, J=14.0, 8.5, 3.5 Hz, 1H), 1.50 (ddd, J=14.0, 1.0, 5.0 Hz), 1.04 (d, J=7.0 Hz, 3H), 0.97 (t, J=7.5 Hz, 9H), 0.56 (q, J=7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.1, 166.4, 166.1, 159.7, 133.9, 133.4, 130.2, 130.0, 129.8, 128.6, 114.0, 106.8, 86.7, 72.8, 69.9, 63.0, 55.9, 38.7, 28.9, 26.1, 20.4, 7.6, 4.6; HRMS (ESI) calcd for $[C_{39}H_{48}O_8Si+Na]^+$ 695.3013, found 695.2990.

4.1.7. TBS ether (21). To a solution of **20** (0.69 g, 1.0 mmol) in CH_2Cl_2 (30 mL) at 0 °C were added 2,6-lutidine (0.36 mL, 3.1 mmol) and *t*-butyldimethylsilyl triflate (0.47 mL, 2.0 mmol) sequentially. After 1 h, saturated aqueous NH_4Cl (10 mL) was added slowly. The aqueous phase was separated and extracted with diethyl ether (2×10 mL) and the combined organic extracts were washed with

saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 10:1 to 7:1, v/v) to provide **21** (0.66 g, 0.85 mmol, 83%) as a colorless oil: R_f 0.69 (hexanes–ethyl acetate, 2:1, v/v); $[\alpha]_D^{23}$ −15 (*c* 0.97, $CDCl_3$); IR (thin film): 2957, 2923, 2875, 2175, 1725, 1613, 1595, 1512, 1446, 1256 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.05 (d, *J*=8.4 Hz, 2H), 7.99 (d, *J*=8.4 Hz, 2H), 7.57 (m, 2H), 7.43 (m, 4H), 7.29 (d, *J*=8.7 Hz, 2H), 6.84 (d, *J*=8.7 Hz, 2H) 6.06 (dd, *J*=5.1, 2.7 Hz, 1H), 4.93 (dd, *J*=12.3, 5.1 Hz, 1H), 4.82 (d, *J*=10.8 Hz, 1H), 4.80 (dd, *J*=12.3, 2.7 Hz, 1H) 4.58 (d, *J*=10.8 Hz, 1H), 4.26 (m, 2H), 3.78 (s, 3H), 2.30 (dd, *J*=16.8, 3.6 Hz, 1H), 2.01 (dd, *J*=16.8, 8.1 Hz, 1H), 1.80 (m, 1H), 1.70–1.60 (m, 2H), 1.03 (d, *J*=6.3 Hz, 3H), 0.98 (t, *J*=7.8 Hz, 9H), 0.91 (s, 9H), 0.56 (q, *J*=7.8 Hz, 6H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.2, 138.6, 130.2, 129.3, 128.3, 127.9, 127.5, 113.8, 78.4, 76.9, 73.2, 72.9, 70.0, 65.9, 55.3, 26.6, 25.6; HRMS (ESI) calcd for $[C_{45}H_{62}O_8Si_2+Na]^+$ 809.3881, found 809.3904.

4.1.8. Alcohol (22). To a stirred, 0 °C solution of **22** (0.80 g, 1.0 mmol) in methanol (11 mL) was slowly added $NaBH_4$ (21 mg, 0.55 mmol). After 1 h, saturated aqueous NH_4Cl (10 mL) was added dropwise and the reaction mixture was allowed to warm to rt. The aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were washed with saturated aqueous NaCl (5 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 10:1 to 7:1, v/v) to provide the corresponding alcohol **22** (0.73 g, 0.93 mmol, 92%, mixture of diastereomers) as a colorless oil: R_f 0.48 (hexanes–ethyl acetate, 5:1, v/v); IR (thin film): 3493, 2954, 2170, 1723, 1611, 1586, 1514, 1454, 1255 cm^{-1}; MS (ESI) calcd for $[C_{45}H_{64}O_8Si_2+Na]^+$ 811.39, found 811.45.

4.1.9. Aldehyde (13). To a solution of **22** (0.73 g, 0.93 mmol) in methanol (5 mL) was added K_2CO_3 (0.74 g, 5.4 mmol). After 5 h, TLC analysis showed complete conversion to triol: HRMS (ESI) calcd for $[C_{31}H_{56}O_6Si_2+K]^+$ 619.3253, found 619.3249. To the solution of triol was slowly added acetic acid (ca. 0.5 mL) to achieve pH 9. $NaIO_4$ (1.14 g, 5.35 mmol) was then added portion-wise. After 4 h, saturated aqueous $NaHCO_3$ (5 mL) and CH_2Cl_2 (15 mL) were added, and stirring was continued for 15 min. The separated aqueous phase was extracted with CH_2Cl_2 (4×10 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to give **13** (3.33 g, 0.65 mmol, 70%) as a colorless oil: R_f 0.58 (hexanes–ethyl acetate, 4:1, v/v); $[\alpha]_D^{23}$ +30.9 (*c* 0.55, $CDCl_3$); IR (thin film): 2956, 2933, 2876, 2172, 1735, 1614, 1587, 1514, 1463, 1379, 1302, 1252 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.64 (d, *J*=2.5 Hz, 1H), 7.27 (d, *J*=8.7 Hz, 2H), 6.88 (q, *J*=8.7 Hz, 2H), 4.58 (d, *J*=11.0 Hz, 1H), 4.55 (d, *J*=11.0 Hz, 1H), 4.07 (ddd, *J*=8.0, 6.0, 2.5 Hz, 1H), 3.81 (s, 3H), 3.64 (dd, *J*=2.5, 2.5 Hz, 1H) 2.25 (dd, *J*=16.5, 5.0 Hz, 1H), 2.12 (dd, *J*=16.5, 7.0 Hz, 1H), 1.78 (m, 1H), 1.56 (m, 2H), 1.00 (d, *J*=5.0 Hz, 3H), 0.98 (t, *J*=8.0 Hz, 9H), 0.57 (q, *J*=7.5 Hz, 6H), 0.09 (s, 3H), 0.07 (s, 3H) ^{13}C NMR ($CDCl_3$, 75 MHz): δ 203.2, 159.3, 129.5, 129.2, 113.7, 106.0, 86.6, 86.3, 72.3, 72.1, 55.1, 39.8, 28.5, 26.5, 25.7, 19.8, 7.7, 4.4, 0.9, −4.4, −4.9; HRMS (ESI) calcd for $[C_{29}H_{50}O_4Si_2+Na]^+$ 541.3140, found 541.3138.

4.1.10. TMS enol ether (23). To a solution of **11** (0.30 g, 1.8 mmol) in THF (6 mL) at −78 °C was added NaHMDS (2.66 mL, 1 M in THF). After 30 min, trimethylsilyl chloride (0.44 mL, 3.6 mmol) was added. After 1 h, a solution of pH 7 phosphate buffer (6 mL) was added and the reaction mixture was allowed to warm to rt. The separated aqueous phase was extracted with ether, and the ether extract was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated by rotary evaporation at 20 °C. The residue was passed quickly through a short pad of silica gel with pentane–ether (3:1 v/v). The solvent was removed by rotary evaporation to yield **23** as a pale yellow oil (0.49 g). The product was used for the next step without further purification: R_f 9.0 (hexanes–ethyl acetate, 5:1, v/v); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.01 (d, *J*=0.9 Hz, 1H), 3.96 (s, 1H), 3.17 (dd, *J*=12.0, 6.0 Hz, 1H), 3.08 (dd, *J*=12.0, 6.9 Hz, 1H), 2.19 (m, 1H), 1.75 (m, 1H), 1.58 (ddd, *J*=13.5, 9.6, 4.5 Hz, 1H), 1.02 (d, *J*=6.9 Hz, 3H), 0.95 (d, *J*=6.6 Hz, 3H), 0.19 (d, *J*=9.9 Hz, 9H); IR (thin film): 2963, 2097, 1655, 1624, 1461, 1319, 1253, 1092, 1020 cm^{-1}.

4.1.11. Mukaiyama aldol product (24). To a stirred solution of **23** (0.49 g) and **13** (144 mg, 355 μmol) in CH_2Cl_2 (6 mL) at −78 °C was added a fine powder of $MgBr_2 \cdot OEt_2$ (275 mg, 1.07 mmol). Stirring was continued for 4 h before the reaction flask was placed in a freezer for 14 h. A solution of pH 7 phosphate buffer (10 mL) was added and the temperature was allowed to rise to rt. The aqueous phase was extracted with diethyl ether and the combined organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to provide **24** (161 mg, 234 μmol, 66%) as a colorless oil: R_f 0.19 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{23}$ +25.4 (*c* 0.51, CH_2Cl_2); IR (thin film): 3491, 2962, 2933, 2877, 2170, 2100, 1713, 1614, 1514, 1461, 1380, 1356, 1286, 1251, 1177, 1105, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.28 (d, *J*=8.7 Hz, 2H), 6.89 (d, *J*=8.7 Hz, 2H), 4.70 (d, *J*=11.4 Hz, 1H), 4.38 (m, 2H), 4.14 (dd, *J*=6.9, 1.8 Hz, 1H), 3.84 (d, *J*=1.5 Hz, 1H), 3.82 (s, 3H), 3.30 (m, 1H), 3.20 (dd, *J*=12.0, 5.1 Hz, 1H), 3.07 (dd, *J*=12.0, 6.6 Hz, 1H), 2.79 (dd, *J*=17.4, 6.3 Hz, 1H), 2.55 (m, 1H), 2.52 (dd, *J*=17.1, 5.7 Hz, 1H), 2.29 (dd, *J*=16.8, 4.5 Hz, 1H), 2.17 (dd, *J*=16.8, 6.0 Hz, 1H), 1.90–1.55 (m, 6H), 1.07 (d, *J*=7.5 Hz, 3H), 1.04 (d, *J*=6.0 Hz, 3H), 1.00 (t, *J*=7.8 Hz, 9H), 0.93 (d, *J*=7.2 Hz, 3H), 0.91 (s, 9H), 0.59 (q, *J*=7.8 Hz, 6H), 0.15 (s, 3H), 0.12 (s, 3H); MS (ESI) calcd for $[C_{37}H_{65}N_3O_5Si_2+Na]^+$ 710.43, found 710.43.

4.1.12. TES ether (25). To a stirred solution of **10** (32 mg, 47 μmol) in CH_2Cl_2 (1 mL) at −10 °C were added 2,6-lutidine (22 μL, 0.19 mmol) and triethylsilyl triflate (21 μL, 93 μmol). After 30 min, saturated aqueous $NaHCO_3$ (1 mL) was added. The mixture was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated. The

residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to provide **25** (35 mg, 43 μmol, 92%) as colorless viscous oil: R_f 0.55 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{23}$ +15.4 (*c* 1.4, CH_2Cl_2); IR (thin film): 2957, 2877, 2171, 2098, 1715, 1614, 1514, 1461, 1415, 1380, 1250, 1109, 1071, 1018 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25 (d, *J*=4.2 Hz, 2H), 6.87 (d, *J*=8.0 Hz, 2H), 4.66 (d, *J*=11.0 Hz, 1H), 4.47 (d, *J*=11.0 Hz, 1H), 4.36 (m, 1H), 3.98 (ddd, *J*=8.5, 4.0, 1.5 Hz, 1H), 3.81 (s, 3H), 3.44 (dd, *J*=5.5, 1.5 Hz, 1H), 3.22 (dd, *J*=11.5, 5.0 Hz, 1H), 3.07 (dd, *J*=11.5, 7.0 Hz, 1H), 2.81 (dd, *J*=17.0, 3.5 Hz, 1H), 2.72 (dd, *J*=17.0, 7.5 Hz, 1H) 2.58 (septet, *J*=7.0 Hz, 1H), 2.30 (dd, *J*=16.5, 3.5 Hz, 1H), 2.04 (dd, *J*=8.0, 16.5 Hz, 1H), 1.88 (m, 1H), 1.79 (ddd, *J*=13.5, 8.5, 6.0 Hz, 1H), 1.66 (m, 1H), 1.48 (ddd, *J*=13.5, 9.0, 4.5 Hz, 1H), 1.07 (d, *J*=7.0 Hz, 3H), 1.04 (d, *J*=6.5 Hz, 3H), 0.99 (t, *J*=8.0 Hz, 9H), 0.93 (d, *J*=7.0 Hz, 3H), 0.90 (s, 9H), 0.89 (t, *J*=8.0 Hz, 9H), 0.58 (q, *J*=8.0 Hz, 6H), 0.54 (q, *J*=4.0 Hz, 6H), 0.09 (s, 3H), 0.08 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 211.85, 130.85, 128.99, 113.41, 106.70, 92.68, 83.92, 72.74, 71.56, 68.72, 57.58, 55.11, 45.57, 44.30, 39.57, 36.79, 31.23, 28.69, 25.88, 20.26, 17.81, 16.88, 7.39, 6.86, 4.78, 4.48, −3.72, −5.22; HRMS (ESI) calcd for $[C_{43}H_{79}N_3O_5Si_3+Na]^+$ 824.5220, found 824.5234.

4.1.13. Alcohol (6). To a stirred solution of **25** (42 mg, 61 μmol) in CH_2Cl_2 (3 mL) were added aqueous phosphate buffer (0.3 mL, pH 7), *t*-butanol (0.1 mL) and DDQ (42 mg, 0.18 mmol). The reaction mixture turned dark green. After 30 min, saturated aqueous $NaHCO_3$ (3 mL) was added, the mixture was diluted with CH_2Cl_2, and transferred to a separatory funnel. The separated aqueous phase was extracted with CH_2Cl_2 and the combined organic extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to give **25** (29 mg, 50 μmol, 82%) as a colorless oil: R_f 0.50 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{23}$ +6.5 (*c* 0.39, $CDCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.30 (dd, *J*=10.2, 5.7 Hz, 1H), 3.77 (dd, *J*=12.0, 6.6 Hz, 1H), 3.35 (dd, *J*=10.2, 5.4 Hz, 1H), 3.24 (dd, *J*=12.0, 5.4 Hz, 1H), 3.12 (dd, *J*=12.0, 6.6 Hz, 1H), 2.80 (dd, *J*=16.5, 5.7 Hz, 1H), 2.61 (dd, *J*=16.5, 5.4 Hz, 1H), 2.47 (d, *J*=6.3 Hz, 1H), 2.32 (dd, *J*=16.8, 3.9 Hz, 1H), 2.09 (dd, *J*=16.8, 7.2 Hz, 1H), 1.93 (m, 1H), 1.80 (m, 1H), 1.69 (m, 1H), 1.53 (t, *J*=6.6 Hz, 1H), 1.10 (d, *J*=6.9 Hz, 3H), 1.06 (d, *J*=6.6 Hz, 3H), 0.99 (t, *J*=7.1 Hz, 9H), 0.96 (t, *J*=7.8 Hz, 9H), 0.90 (s, 9H), 0.64 (q, *J*=7.8 Hz, 6H), 0.57 (d, *J*=7.8 Hz, 6H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C ($CDCl_3$, 75 MHz): δ 211.4, 106.7, 82.7, 71.3, 68.1, 57.5, 45.5, 44.5, 38.5, 36.7, 31.2, 29.5, 28.4, 26.3, 25.7, 20.0, 17.8, 16.9, 7.3, 6.7, 4.9, 4.4, 0.8, −4.2, −4.3; IR (thin film): 3551, 2957, 2878, 2172, 2099, 1714, 1613, 1514, 1461, 1414, 1380, 1256, 1102, 1019 cm^{-1}; MS (ESI) calcd for $[C_{35}H_{71}N_3O_4Si_3+Na]^+$ 704.46, found 704.46.

4.1.14. Spiroaminal (26). To a solution of **6** (28 mg, 41 μmol) in toluene was added triethylphosphine (18 μL, 0.12 mmol). After 14 h, solvent was removed under a stream of N_2 (in a hood—stench). The crude product could be used for the next step without purification. For characterization, the residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to yield **26** (19 mg, 30 μmol, 75%, 4:1 mixture of anomers) as a colorless oil: R_f 0.23 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{23}$ −10 (*c* 0.40, $CDCl_3$); IR (thin film): 2956, 2878, 2173, 1461, 1415, 1378, 1256, 1017 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz): δ 4.29 (dd, *J*=12.0, 6.5 Hz, 1H), 4.22 (t, *J*=3.5 Hz, 1H), 3.80 (dd, *J*=4.5, 2.5 Hz, 1H), 2.85 (t, *J*=11 Hz, 1H), 2.71 (m, 1H), 2.60 (dd, *J*=16.5, 3.5 Hz, 1H), 2.39 (m, 1H), 2.19 (m, 2H), 2.04 (dd, *J*=13.5, 4.5 Hz, 1H), 1.95 (m, 2H) 1.81 (m, 2H), 1.73 (d, *J*=13.5 Hz, 1H), 1.48 (m, 1H), 1.33 (d, *J*=6.5 Hz, 3H), 1.14 (t, *J*=8.0 Hz, 9H), 1.04 (s, 9H), 0.98 (t, *J*=7 Hz, 9H), 0.89 (d, *J*=6.5 Hz, 3H), 0.85 (d, *J*=7.0 Hz, 3H), 0.29 (s, 6H); HRMS (ESI) calcd for $[C_{35}H_{71}NO_3Si_3+H]^+$ 638.4815, found 638.4834.

4.1.15. Carbamate (27). To a stirred solution of **12** (8 mg, 0.1 mmol) in CH_2Cl_2 (0.5 mL) were sequentially added powdered 4 Å molecular sieves (30 mg), K_2CO_3 (34 mg, 0.25 mmol, fine powder), and carbobenzyloxy chloride (7.2 μL, 0.05 mmol). After 14 h, the mixture was applied directly onto a silica gel column and eluted with hexanes–ethyl acetate (10:1, v/v). The product collected after evaporation of the solvents by rotary evaporation was placed under high vacuum for 2 h to afford **27** as a colorless oil (7 mg, 9 μmol, 73%, 4:1 mixture of anomers): R_f 0.69 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{23}$ +28 (*c* 1.0, CH_2Cl_2); IR (thin film); 2956, 2876, 2171, 1704, 1460, 1396, 1356, 1259, 1174, 1072, 1019 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.33 (m, 5H), 5.10 (d, *J*=2 Hz, 2H), 4.49 (m, 1H), 4.09 (dd, *J*=8.0, 2.5 Hz, 1H), 3.91 (m, 1H), 3.83 (dt, *J*=9.5, 2.5 Hz, 1H), 3.76 (dd, *J*=13.5, 2.5 Hz, 1H), 3.25 (t, *J*=12.5 Hz, 1H), 2.66 (m, 1H), 2.53 (m, 1H), 2.40 (dd, *J*=16.5, 3.5 Hz, 1H), 1.86 (m, 2H), 1.77 (m, 1H), 1.60 (m, 1H), 1.415 (m, 2H), 1.32 (m, 1H), 1.04 (d, *J*=6 Hz, 3H), 0.99 (t, *J*=5.5 Hz, 9H), 0.94 (t, *J*=8.0 Hz, 9H), 0.91 (s, 9H), 0.80 (d, *J*=6.5 Hz, 3H), 0.77 (d, *J*=7 Hz, 3H), 0.57 (m, 12H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.61, 137.00, 128.48, 127.77, 127.48, 108.42, 95.62, 85.56, 81.82, 72.33, 70.46, 66.50, 59.60, 49.07, 39.54, 38.01, 31.23, 29.87, 26.08, 21.01, 18.73, 16.81, 7.62, 7.13, 4.79, 1.16, −4.14; HRMS (ESI) calcd for $[C_{43}H_{77}NO_5Si_3+Na]^+$ 794.5007, found 794.4999.

4.1.16. Alkynyl iodide (3). To a solution of **27** (5 mg, 6.3 μmol) in DMF (0.3 mL) were added *N*-iodosuccimide (4 mg, 0.09 mmol) and silver trifluoroacetate (1.5 mg, 6.5 μmol). After 10 min, the reaction mixture was diluted with diethyl ether and saturated aqueous $NaHCO_3$ was added. The separated aqueous phase was extracted with diethyl ether. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexanes–ethyl acetate, 6:1, v/v) to provide **3** (4.3 mg, 87%) as a colorless oil: R_f 0.64 (hexanes–ethyl acetate, 5:1, v/v); $[\alpha]_D^{25}$ +29 (*c* 0.32, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.29–7.38 (m, 5H), 5.08 (s, 2H), 4.43–4.50 (m, 1H), 4.09 (dd, *J*=9.0, 4.5 Hz, 1H), 3.83 (dt, *J*=4.5, 2.5 Hz, 1H), 3.75 (dd, *J*=13.5, 3.5 Hz, 1H), 3.22 (dd, *J*=12, 12 Hz, 1H), 2.64 (m, 1H), 2.52 (dd, *J*=16, 12 Hz, 1H), 2.46 (dd, *J*=16.5, 4.5 Hz, 1H), 2.05 (dd, *J*=17.0, 6.0 Hz, 1H), 1.80–1.96 (m, 2H), 1.51–1.59 (m, 2H), 1.2–1.35 (m, 3H), 0.98 (d, *J*=7 Hz, 3H), 0.88–0.91

(m, 9H), 0.80 (d, J=7 Hz, 3H), 0.77 (d, J=7.5 Hz, 3H), 0.55–0.59 (m, 9H), 0.055–0.068 (m, 10H); IR (thin film) 2959 (m), 2929 (m), 2878 (w), 2857 (w), 2361 (m), 1701 (s), 1458 (m), 1397 (m), 1260 (m) cm^{-1}; HRMS (ESI) *m/z* calcd for $(C_{37}H_{62}INO_5Si_2+Na)^+$ 806.3109, found 806.3118.

4.1.17. Ynone (33). To a solution of CBr_4 (471 mg, 1.42 mmol) in CH_2Cl_2 (6 mL) at 0 °C under argon was added triphenylphosphine (745 mg, 2.8 mmol). The resulting orange solution was stirred for 30 min before a solution of aldehyde **28** (110 mg, 0.71 mmol) in CH_2Cl_2 (6 mL) was added. After stirring for 10 min, saturated aqueous $NaHCO_3$ (6 mL) was added and the resulting mixture was extracted with diethyl ether. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexanes–diethyl ether, 5:1, v/v) to provide dibromide **29** (0.20 g, 0.64 mmol, 91%) as a yellow oil: R_f 0.75 (hexanes–ethyl acetate, 5:1, v/v); ^1H NMR (500 MHz, $CDCl_3$) δ 6.12 (d, J=9.5 Hz, 1H), 3.16 (d, J=6.0 Hz, 2H), 2.56–2.61 (m, 1H), 1.66–1.70 (m, 1H), 1.42–1.48 (m, 1H), 1.14–1.20 (m, 1H), 1.02 (d, J=7.0 Hz, 3H), 0.98 (d, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.6, 104.8, 58.0, 40.7, 36.0, 31.6, 19.9, 17.6; LRMS (EI) *m/z* calcd for $(C_8H_{13}Br_2N_3–N_2–H)^+$ 280.9, found 280.9.

To a solution of **29** (24 mg, 77 μmol) in THF (0.8 mL) was added *n*-butyllithium (65 μmol, 2.5 M in hexane) at −78 °C. After stirring for 20 min, a solution of aldehyde **31** (15.1 mg, 31 μmol) in THF (0.2 mL) was added. The resulting mixture was warmed slowly to 0 °C over 1 h and stirred for another 30 min at 0 °C before saturated aqueous NH_4Cl (1 mL) was added. The separated aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexanes–diethyl ether, 10:1, v/v) to afford the alcohol **32** (14.6 mg, 23 μmol, 73%): R_f 0.63 (hexanes–ethyl acetate, 3:1, v/v).

To a solution of **32** (7.2 mg, 11 μmol) in toluene (0.6 mL) under argon was added triethylphosphine (6 mg, 0.05 mmol). The resulting solution was stirred at rt for 50 min and cooled to −20 °C before BocON (7.3 mg, 30 μmol) was added. The reaction mixture was warmed slowly to rt and stirred for 12 h. Ethyl acetate (2 mL) was added and the resulting solution was washed with H_2O (3×1 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexanes–ethyl acetate, 10:1, v/v) to provide carbamate alcohol **32b** (4.8 mg, 61%): R_f 0.44 (hexanes–ethyl acetate, 3:1, v/v).

Alcohol **32b** (3.8 mg, 5.4 μmol) was dissolved in pentane (0.8 mL) and MnO_2 (21 mg, 0.24 mmol) was added. After stirring for 30 min at rt, the reaction mixture was applied directly to a silica gel column and purified by flash column chromatography (hexanes–diethyl ether, 10:1, v/v) to provide ynone **33** (3.8 mg, 100%): R_f 0.53 (hexanes–ethyl acetate, 3:1); ^1H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, J=9.0 Hz, 2H), 6.86 (d, J=9.0 Hz, 2H), 4.63 (d, J=11.5 Hz, 1H), 4.46–4.50 (b, 1H), 4.43 (d, J=11.5 Hz, 1H), 4.13–4.15 (m, 1H), 3.88 (d, J=4.5 Hz, 1H), 3.80 (s, 3H), 2.98–3.06 (m, 1H), 2.90–2.98 (m, 1H), 2.70–2.78 (m, 1H), 2.23 (dd, J=16.5, 4.5 Hz, 1H), 2.05 (dd, J=17.0, 7.0 Hz, 1H), 1.75–1.85 (m, 2H), 1.53–1.62 (m, 3H), 1.43 (s, 9H), 1.22 (d, J=7.0 Hz, 3H), 1.18–1.21 (m, 1H), 0.99 (d, J=7.0 Hz, 3H), 0.89 (d, J=7.0 Hz, 3H), 0.86 (s, 9H), 0.13 (s, 6H), 0.068 (d, J=9.0 Hz, 9H); LRMS *m/z* calcd for $(C_{39}H_{65}INO_6Si_2+Na)^+$ 722.4, found 722.5.

4.1.18. Spiroaminal (35). To a stirred rt solution of ynone **33** (0.5 mg, 0.7 μmol) in CH_2Cl_2 (0.3 mL) was added silver trifluoroacetate (1 mg, 5 μmol). After disappearance of starting material **35** by TLC, ethanol (1 mL) and H_2O (0.5 mL) were added, followed by the addition of KI (2 mg). After stirring for another 5 min, the reaction mixture was applied to a small silica gel column and eluted to afford **38** (0.2 mg, 0.4 μmol, 56%): R_f 0.39 (hexanes–ethyl acetate, 3:1); ^1H NMR (500 MHz, $CDCl_3$) δ 4.48 (d, J=2.0 Hz, 1H), 4.18–4.22 (m, 1H), 3.05–3.08 (m, 1H), 2.85–2.97 (m, 1H), 2.76–2.81 (m, 1H), 2.21 (ddd, J=16.0, 4.5, 2.5 Hz, 1H), 1.98–2.06 (m, 2H), 1.95 (t, J=2.5 Hz, 1H), 1.75–1.85 (m, 1H), 1.63–1.85 (m, 3H), 1.45–1.50 (m, 1H), 1.43 (s, 9H), 1.25–1.27 (m, 1H), 1.02 (d, J=6.5 Hz, 3H), 0.93 (d, J=6.0 Hz, 3H), 0.87–0.90 (m, 12H), 0.11 (d, J=1.5 Hz, 6H). HRMS (ESI) *m/z* calcd for $(C_{18}H_{49}NO_5Si+Na)^+$ 530.3278, found 530.3191.

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Cellular routines in the synthesis of cyclic peptide probes

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Abstract—For even the most primitive microbes, the activation and regulation of biosynthesis is guided by material transport about the cell. It is within these transactions that secondary metabolite biosynthesis orchestrates a key set of chemical transformations. Cellular factors are often as important to the regulation of biosynthesis as the structures of their metabolites and the mechanism of their biosynthesis. In accord with this issue on biologically-inspired synthesis, this manuscript evaluates the adaptation of cells as tools to direct the synthesis of fluorescent nonribosomal peptide-based probes.

1. Introduction

One of the principle differences between biosynthesis and chemical synthesis can be attributed to the reaction vessel.[1] In the lab, synthetic operations are conducted in a flask,[2] on a resin,[3] or on a surface[4]. Once operations are completed, the products of these reactions are extracted from their vessel, purified, and screened against a battery of molecular, cellular, and physiological assays.[5] Data from these assays are then pooled and processed through chemoinformatic systems in order to develop a bioactivity profile.[6] While this method operates at the center of drug discovery, its process is radically different from that used in nature.

In nature, secondary metabolism is translated through a complex network of interplay between synthetic operations and biochemical activity. For the producer, these events must be simultaneously optimized for performance at the level of molecule, cell, organism, and ecosystem.[7] Entry into biosynthesis is carefully timed to provide an optimal relay between synthesis and transmission of bioactivity. The logic contained within these regulatory events has yet to be adapted to laboratory synthesis.

2. Results and discussion

2.1. Model system

Conventionally, cells are used as an endpoint to screen the optimal molecular probe. The development of these probes is often accelerated by targeting a molecule or class of molecules, such as natural products. For this study, peptides and their cyclic variants were chosen as targets, as: (1) their synthesis can be addressed in a modular fashion;[8] (2) they are readily displayed in a combinatorial manner;[9] (3) they can be synthesized by variety of methods;[4] (4) their biosynthesis is understood;[10] and (5) they are metabolized in both eukaryotic and prokaryotic cells.[11]

For an organism, events such as subcellular trafficking and metabolism are scored according to the structural features of individual components within a given library of compounds. Metabolism often participates in activating the signals transposed by these materials as illustrated by the oxidative cascades on actinomycin[12] or the appendage of glycosides such as in vancomycin.[13] The mimicry of metabolic processes has been extensively reviewed with regards to the development of prodrugs[14] and metabolic engineering.[15] While peptide and cyclic peptide prodrugs have been shown to increase the activity and facilitate delivery,[16] cells still appear as an endpoint within these investigations.

By moving the position of the cell in a synthetic scheme one can advance the biological-sophistication of a molecular probe. Through these new positions, metabolic processes are effectively encoded within a probe through a few chemical transitions. This manuscript illustrates how probe development and metabolism can be simultaneously engineered into peptide-based probes to prepare materials whose intracellular localization can be trafficked between the endoplasmic reticulum (ER) and nucleus (N) without an extracellular stimulus. To model such regulation, our studies focused on examining the macrocyclization of peptides, the macrolactonization of depsipeptides, and their respective hydrolytic reversion.

2.2. Synthesis of a linear peptide pool

A pool of linear pentapeptides **5** was prepared on Wang resin using a protocol comparable to that described by Ellman[17]

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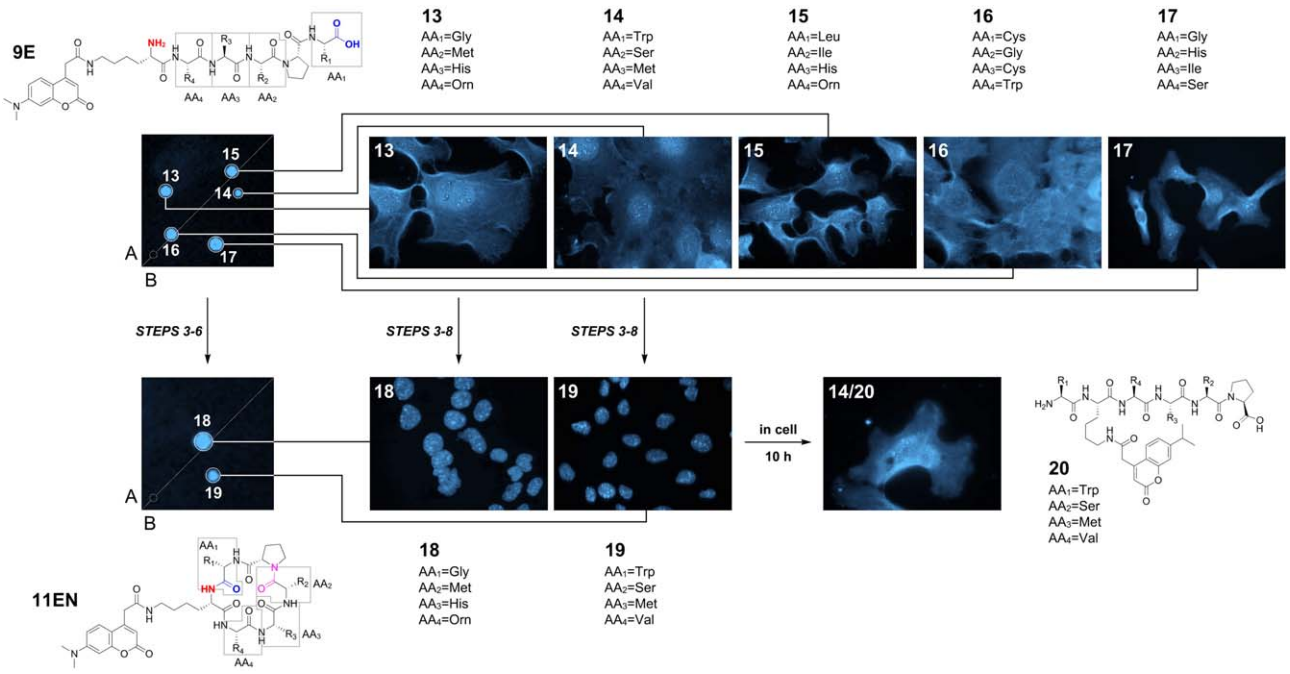


Figure 1. Cell-directed synthesis of cyclic peptide probes. Images depicting the cellular localization of five linear peptides **13**–**17** from sets **9E** denotes the affinity to the ER. Each of these probes was purified by pTLC purification and the images shown were developed from individual TLC spots containing single materials (left). Peptides **13**–**17** were cyclized according to steps 3–6 in Scheme 2 to provide two new cyclic peptides **18**–**19**. The uptake and localization of these peptides was developed using the methods in steps 7–8 in Scheme 2. After incubation at 37 °C for 10 h, cyclic peptide **19** is metabolized in HeLa cells to return peptide **14** and deliver a new linear peptide **20**. For the pTLC analysis (A) denotes acid elution and (B) basic elution.

(Fig. 1). The synthesis was conducted in a parallel-fashion by targeting two pools of peptides, one with a hydroxyl terminus **8** and another with an amine terminus **9**. A pool was developed with 20 sets of peptides from resins **3** containing 0.4–0.6 mmol/g of single amino acid (see Section 4.2). In turn, resins **3** were coupled to Fmoc-proline in a parallel-manner to provide **4**. Resin **5** was prepared from 20 individual dipeptide resins **4** by three sequential couplings with an isokinetic mixture of 20 Fmoc-protected amino acids (see Section 4.2). Resins **5** represented a single set, theoretically containing 8000 peptides. In a parallel-fashion, peptide sets **5** were terminated by capping either with fluorescent-*O*-Fmoc-α-hydroxyacid **1** or fluorescent-Fmoc-lysine **2** providing the respective resins **6** and **7**. Cleavage and deprotection were accomplished by treatment with 20% piperidine in DMF followed by 95% TFA, 2.5% triisopropylsilane, and 2.5% water to afford the corresponding linear peptides **8** and **9**.

2.3. Macrocyclization

Cyclization conditions were identified by screening a series of peptides **8** (AA_1=Ile, AA_2=Ala, AA_3=Ala, AA_4=Ala), **8** (AA_1=Ala, AA_2=Ile, AA_3=Ala, AA_4=Ala), **8** (AA_1=Ala, AA_2=Ala, AA_3=Ile, AA_4=Ala), and **8** (AA_1=Ala, AA_2=Ala, AA_3=Ala, AA_4=Ile). Each peptide was displayed on 256 welled PTFE Teflon plate and screened for macrocyclization with reaction conditions containing combinations of the following reagents: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)uronium tetrafluoroborate (TDBTU), *N*,*N*,*N*′,*N*″-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU), EtN^iPr_2, *sym*-collidine, dimethylaminopyridine (DMAP), and DL-dithiothreitol (DTT). After extensive analysis, a mixture of 5 equiv of HATU with 0.1 equiv of DTT and 10 equiv of *sym*-collidine in DMF routinely delivered the desired depsipeptide in 40–68% yield. The same conditions also provided an effective conversion of the corresponding series of isoleucine and alanine containing peptides **9** (AA_1=Ile, AA_2=Ala, AA_3=Ala, AA_4=Ala), **9** (AA_1=Ala, AA_2=Ile, AA_3=Ala, AA_4=Ala), **9** (AA_1=Ala, AA_2=Ala, AA_3=Ile, AA_4=Ala), and **9** (AA_1=Ala, AA_2=Ala, AA_3=Ala, AA_4=Ile) providing the desired cyclic peptides in 35–78% yield after purification.

The interference from side chain residues was examined by completing an aspartic acid and lysine scan, using the respective sets of peptides; *set A* [**8** (AA_1=Asp, AA_2=Gly, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Asp, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Gly, AA_3=Asp, AA_4=Gly), and **8** (AA_1=Gly, AA_2=Gly, AA_3=Gly, AA_4=Asp)], *set B* [**8** (AA_1=Asp-(OAllyl), AA_2=Gly, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Asp-OAllyl, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Gly, AA_3=Asp-OAllyl, AA_4=Gly), and **8** (AA_1=Gly, AA_2=Gly, AA_3=Gly, AA_4=Asp-OAllyl)], *set C* [**8** (AA_1=Lys, AA_2=Gly, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Lys, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Gly, AA_3=Lys, AA_4=Gly), and **8** (AA_1=Gly, AA_2=Gly, AA_3=Gly, AA_4=Lys)], and *set D* [**8** (AA_1=Lys-N-Alloc, AA_2=Gly, AA_3=Gly, AA_4=Gly), **8** (AA_1=Gly, AA_2=Lys-N-Alloc, AA_3=Gly, AA_4=Gly), **8**

(AA_1=Gly, AA_2=Gly, AA_3=Lys-N-Alloc, AA_4=Gly), and **8** (AA_1=Gly, AA_2=Gly, AA_3=Gly, AA_4=Lys-N-Alloc)]. Even under optimal macrocyclization conditions, HPLC analysis indicated that without protection the aspartic acid residue in *set A* and the lysine residue in *set C* participated in amide bond formation, thereby reducing the yield of the desired cyclic peptide from 50 to 70% in *sets B/D* from 0.1to 8.5% in *sets A/C*. Comparable complications are also expected to interfere with the cyclization of Arg and Gly peptides, while His, Tyr, and Phe containing peptides may exhibit reactivity within their side chains to a lesser extent. Instead of altering our synthetic scheme, we included these materials in the peptide pools.

2.4. Probe processing

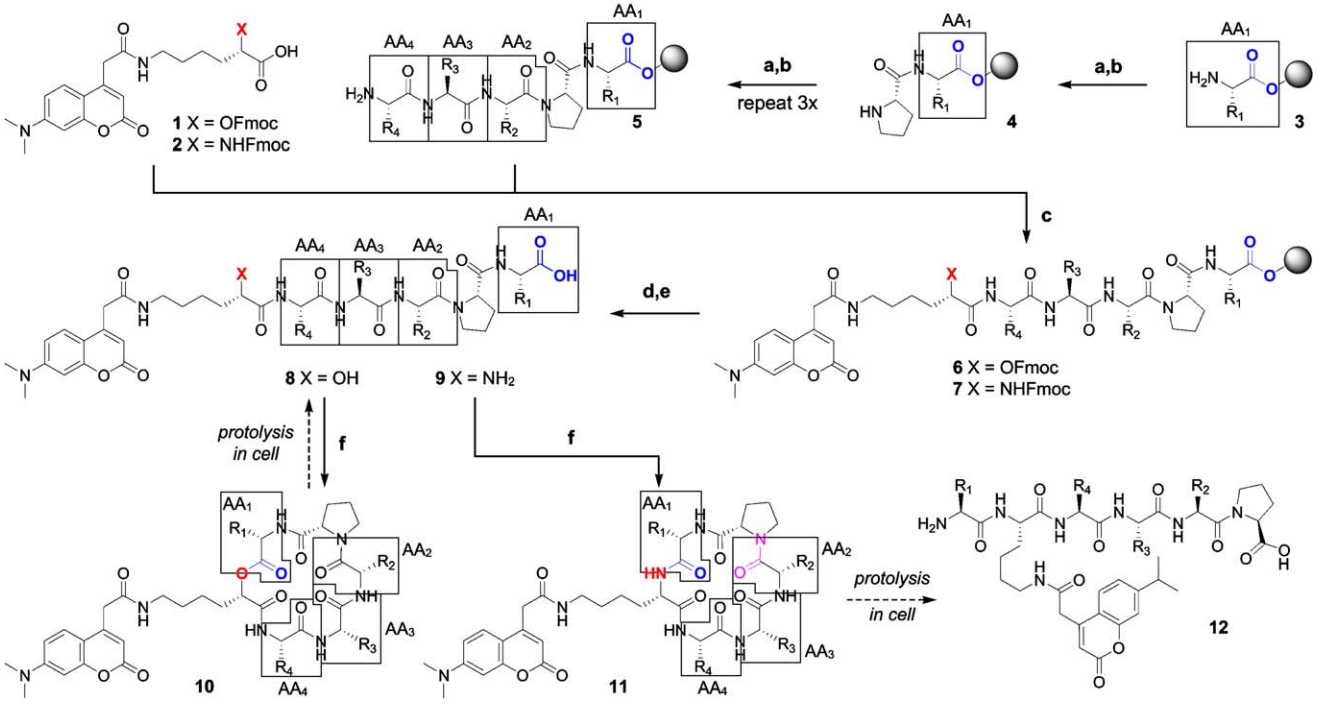
In the cell, depsipeptides **10** and cyclic peptides **11** are subject to proteolysis.[20] For depsipeptides, cleavage arises rapidly at the lactone bond resulting in reversion of **10** to **8**. Cyclic peptides[11] on the other hand can undergo proteolysis at any of the six amide bonds providing reversion to **9** or the formation of a new linear peptide as illustrated by **12** (Scheme 1). The question remained as to the function that can be encoded by the conversion between linear peptides **8**, **9** and their cyclic variants **10**, **11**, respectively. For this study, we were interested in identifying analogs of **10** and **11** that could be trafficked between the ER and nucleus. The procedure outlined in Scheme 2 was selected after examining several approaches.

The process began by evaluating two peptide pools **8** and **9** where **8** was designed to screen depsipeptides **10**, and **9** evaluated the corresponding cyclic peptides **11**. Pools were constructed with 20 sets of peptides as described in Scheme 1. Samples of the sets of peptide **8** or **9** in DMSO were presented to HeLa cells and their fluorescent uptake was imaged (step 1).

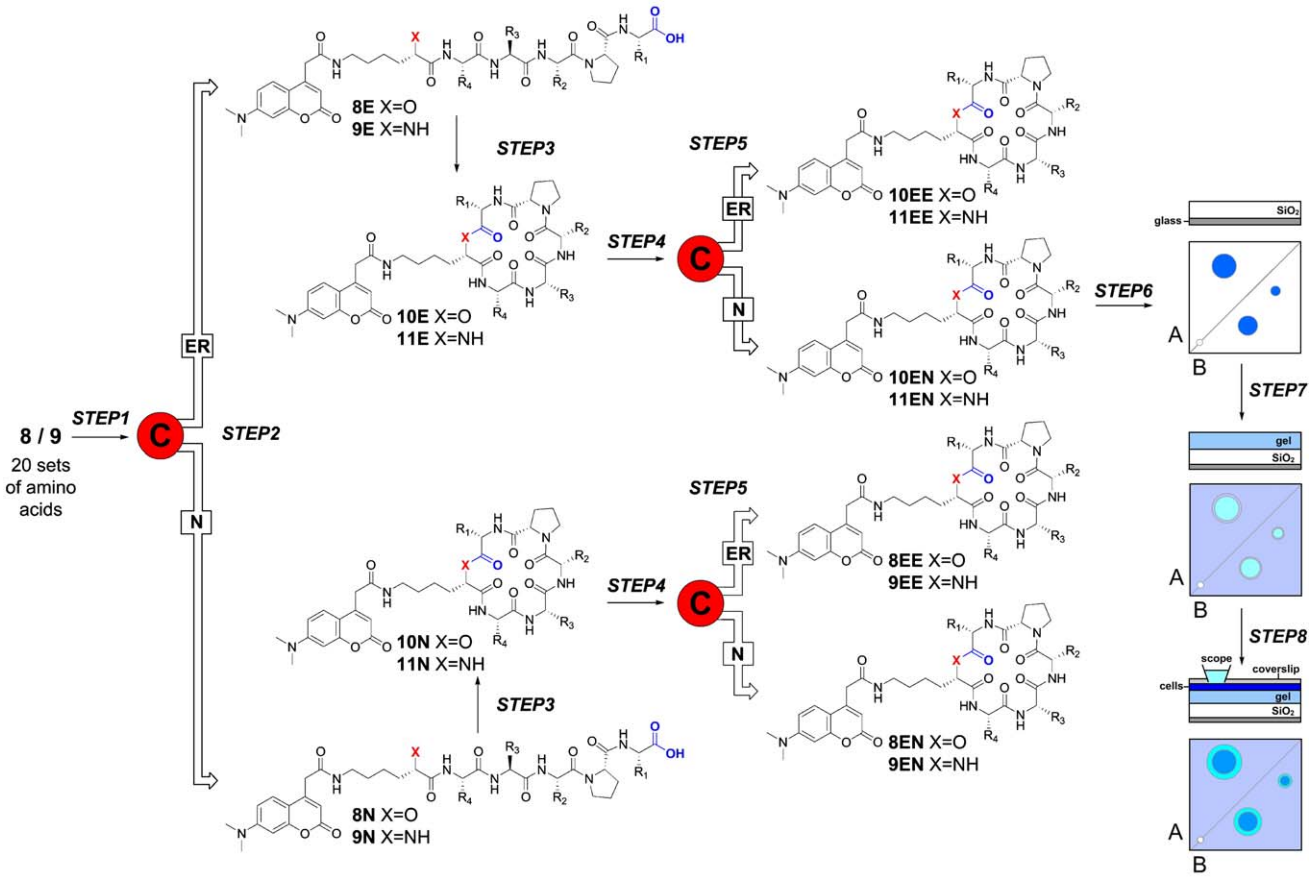
Cells depicting fluorescence in the ER or nucleus were then fractionated into nuclear and endoplasmic reticulum components (step 2). The fluorescent peptides were then extracted from these components to provide the corresponding sets of nuclear-localizing linear peptides **8N** and **9N** and ER-localizing peptides **8E** and **9E**. Samples from each reaction mixture were purified by pTLC K18-reverse phase silica gel or C(18)-reverse phase HPLC to provide pure peptides. The uptake and localization of each purified peptide was determined microscopically.

At this point, the crude sets of peptides were cyclized (step 3). Sets **8E**, **8N**, **9E**, and **9N** at 250±10 μM/peptide (0.05 μmol) in DMF were treated with HATU (0.95 mg, 2.5 μmol), DTT (3.8 μg, 0.025 μmol), and *sym*-collidine (0.2 μL, 2.5 μmol) in DMF. The reaction mixture was stored at rt under argon in the absence of light for 24 h, and the resulting material was filtered through a plug of K18-reverse phase silica gel (300 mg).

Samples of the cyclized reaction mixtures **10E**, **10N**, **11E**, and **11N** in DMSO at 10.0±0.5 μM were presented to HeLa cells and imaged (step 4). Cells were again fractionated and the peptides were extracted from the corresponding cell lysates (step 5). The conditions used for this process were identical to that used to screen for linear peptides in step 2. Samples of the resulting products were purified by 2D-pTLC on Partisil KC18 plates to provide peptide sets **10EE** and **10EN** from **10E** and **11EE** and **11EN** from **11E** (step 6). The corresponding analysis could also be conducted on **10N** and **11N**.



Scheme 1. Reagents and conditions: (a) 20% piperidine, DMF; (b) Fmoc-AA-OH, HATU, *sym*-collidine, DMF; (c) HATU, *sym*-collidine, DMF; (d) 20% piperidine, DMF; (e) 95% TFA, 2.5% triisopropylsilane (TIS), 2.5% water; and (f) HATU, *sym*-collidine, DTT, DMF.



Scheme 2. Cell-directed synthesis. An eight-step process is used. (step 1) Cell uptake and localization; (step 2) nuclear and ER subcellular fractionation; (step 3) macrocyclization as given by reagents (f) in Scheme 1; (step 4) cell uptake and localization; (step 5) nuclear and ER subcellular fractionation; (step 6) 2D-pTLC isolation; (step 7) transfer fluorescent peptides from TLC plate to HeLa cells is conducted through an agarose film by incubation at 37 °C; (step 8) the localization of the cyclic peptides was imaged on an LED-fluorescence microscope. For the pTLC analysis (A) denotes acid elution and (B) basic elution.

The uptake of the probes was imaged directly using a TLC plate. The silica gel was scraped from the plate in regions lacking blue fluorescence. The plate was then coated with a 0.10±0.02 mm layer of low-melt agarose and covered with cover slip containing live HeLa cells (steps 7 and 8). Cells were positioned such that at least ~10^4 cells were placed facing down over a single fluorescent band on the TLC plate. The agarose film served to transfer the peptides from silica gel on plate to the upper layer of the cells. Alternately, the peptide components could be extracted from the pTLC plate and added in a DMSO solution and transferred into the HeLa cell cultures. The uptake and localization of the peptides obtained at this stage were imaged on an LED microscope (step 8). The structures of the peptides identified in sets **10EN** and **11EN** were determined by a combination of TLC–MS[18] and FABMS peptide-sequencing.[19] These assignments were confirmed by synthesizing the peptides individually.

2.5. Discussion

The aim of this study was to develop peptide materials that could be trafficked between the ER and the nucleus.

As indicated in Scheme 2, this transition is depicted by the preparation of probes in sets **10EN** and **11EN**. To simplify matters for this study, sets **10EE**, **10NE**, and **10NN** as well as **11EE**, **11NE**, and **11NN** were collected but not analyzed. The analysis of sets **11EN** and **10EN** is provided in Figures 1 and 2, respectively.

When examined over the entire pool, **9E** contained a total of 121 linear peptides. Images depicting five of these materials **13**–**17** are presented in Figure 1. Upon macrocyclization, only cyclic peptides **18** and **19** localized in the nucleus as given by the requirements of set **11EN**. The remaining 119 peptides either failed to cyclize, as illustrated by **15** or **16**, or failed to provide a cyclic peptide that localized in the nucleus, such as **17**. Interestingly, the nuclear localization of cyclic peptide **19** degraded with time resulting in an indistinguishable signal after 10 h in HeLa cells. A new linear peptide **20** was isolated and its structure was determined by MS peptide-sequencing.

A comparable set of depsipeptides **10EN** was also obtained (Fig. 2). A total of 93 peptides in pool **8** localized in the ER, designated as set **8E**. Only three materials were identified as displaying the conversion of **8E** to **10EN** (Scheme 1). Upon cyclization, peptides **21**–**23**, which were observed in the ER were converted to nuclear-localizing depsipeptides **24**–**26**, respectively. This localization was reverted after 2–8 h in HeLa cells. Cyclic peptide **26** was the fastest, retreating with the ER in 2 h witnessed the formation of **23** by TLC analysis of the corresponding cell lysates. The other two cyclic peptides **24** and **25** were more stable as given by the loss

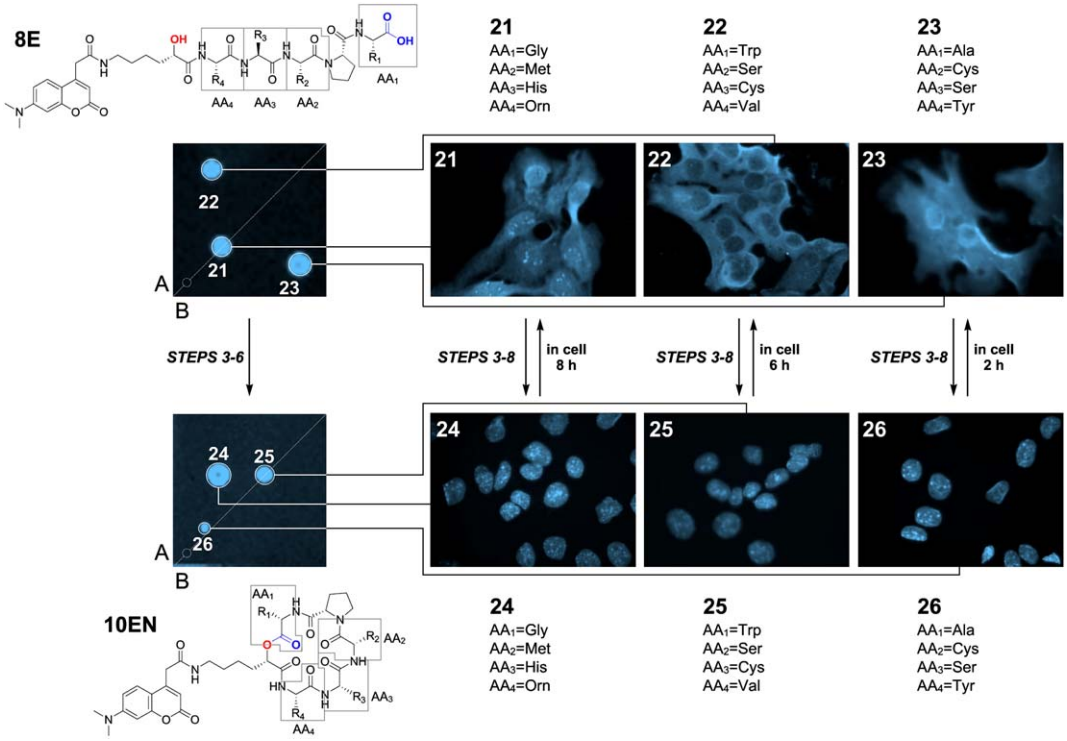


Figure 2. Cell-directed synthesis of depsipeptide probes. Images depicting the cellular localization of three linear peptides **21**–**23** from sets **9E** denotes the uptake in the ER. Each of these probes was purified by pTLC purification (left) and the images shown were developed from individual TLC spots containing single materials. These peptides were cyclized according to steps 3–6 in Scheme 2 to provide three new cyclic peptides **24**–**26**. The uptake and localization of these peptides was developed using the methods in steps 7–8 in Scheme 2. After incubation at 37 °C, depsipeptides **24**–**26** were hydrolyzed to **21**–**23**, respectively, in HeLa cells to afford **14** and **20**. Note that the AA sequence of **21/24** are identical to **13/18** and **22/25** differ from **14/19** by methyl group. For the pTLC analysis (A) denotes acid elution and (B) basic elution.

of nuclear localization after 6 h. Interestingly, the interplay between **21** and **24**, **22** and **25**, and **23** and **26** now provides a set of probes whose localization can be modified over a given time interval.

3. Conclusion

A method was developed that employed cells as a means of selection through the combination of subcellular fractionation and extraction. Through this routine, cyclic peptides **18**, **19** and depsipeptides **24**, **26** were synthesized to provide an effective correlation between nuclear and ER localization.

Advantageously, the proteolysis of probes **18**, **19**, and **24**–**26** provided a signal whose trafficking within the cell could be addressed in a logical fashion. It is within this conduit that methods such as that described herein can be used to expedite the design and synthesis of probes whose activity within a cell is not only regulated in thermodynamic terms but also can act as multidimensional kinetically-regulated signal.

The intracellular trafficking of fluorescent peptide probes was engineered so that the subcellular localization can be guided by in vivo proteolysis. This protocol offers a favorable means to prepare probes whose intermediates and metabolic products can be engineered to target specific organelles. While the molecular target of the peptides described herein was not examined, this manuscript develops syntheses whose function mimics biological selection at the cellular level. It is within this mimetic that new avenues in the development of biologically-inspired synthesis are unveiled.

4. Experimental

4.1. General methods

7-Dimethylaminocoumarin-4-acetic acid was obtained from GFS Chemicals Inc. Fluorescent lysine analogs **1** and **2** were prepared from 7-dimethylaminocoumarin-4-acetic acid using established procedures.[21] Wang resin was obtained from Calbiochem Inc. Fmoc-amino acids were prepared in-house and coupled with Wang resin to provide **3** with a substitution level of 0.40–0.55 mmol/g. Resins **3** could also be purchased from Anaspec Inc. or Polymer Labs Inc. The amine substitution level of each resin was determined by a spectrophotometric Fmoc-quantization.[22] Anhydrous solvents, *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), and methanol (MeOH), from EMD Chemicals were used. EtN^iPr_2, *sym*-collidine, 1-acetylimidazole, dimethylaminopyridine (DMAP), triisopropylsilane (TIS), and DL-Dithiothreitol (DTT) were obtained from TCI Inc. HATU was obtained from Anaspec Inc. Analytical samples of the reagents used to screen the macrocyclization process were obtained from number of laboratories including generous donations from Profs M. M. Joullie and M. Goodman.

HeLa cells (ATCC CCL-2) were cultured in phenol red-free Dulbecco's modification of Eagle's medium (DMEM) with 4.5 g/L glucose, 4.5 g/L L-glutamine, and 5% heat

inactivated fetal calf serum (FCS). Cells were cultured in 250 mL or 1 L culture flasks and as needed were grown on 24×60 mm glass Superslips (VWR Scientific).

4.2. Peptide synthesis

The reaction conditions were corrected for the level of substitution.[22] Fmoc-Ala-resin **3** (1 g, 0.45 mmol, 0.45 mmol/g of resin) was added to a reaction vessel of a self-built peptide synthesizer and solvated with CH_2Cl_2 (10 mL) for 10 min. The resin was filtered and 20% piperidine in DMF (10 mL) was added. After agitation for 30 min, the resin was filtered, and washed three times with DMF (10 mL). The resin was treated twice for 20 h with a solution of Fmoc-proline (379.6 mg, 1.125 mmol), HATU (427.7 mg, 1.125 mmol), and *sym*-collidine (364 μL, 4.5 mmol) in DMF (5 mL). After the second treatment, the resin was washed three times with DMF (5 mL) and capped by treatment with 1-acetylimidazole (743.2 mg, 6.75 mmol) and DMAP (27.5 mg, 0.225) in DMF (5 mL). After 20 h, the resin was washed three times with DMF (5 mL), three times with MeOH (5 mL), and three times with THF (5 mL). The amine substitution level of the resulting resin **4** within three repetitions was determined to be 0.41±0.02 mmol/g by Fmoc-quantization.[22]

An aliquot of resin **4** AA_1=Ala (200 mg, 0.08 mmol, 0.40 mmol/g of resin) was loaded in a self-built peptide synthesizer and solvated with CH_2Cl_2 (3 mL). The resin was filtered and treated with 20% piperidine in DMF (3 mL) for 30 min, filtered, and washed three times with DMF (3 mL). Amino acids AA_2, AA_3, and AA_4 were introduced in series using an isokinetic mixture[23] composed of: Fmoc-Ala, 3.4%; Fmoc-Arg(Pmc), 6.1%; Fmoc-Asn(Trt), 5.6%; Fmoc-Asp(O^{t}Bu), 3.5%; Fmoc-Cys(Trt), 3.6%; Fmoc-D-Orn(Boc)-OH, 3.9%; Fmoc-Gln(Trt), 5.4%; Fmoc-Glu(O^{t}Bu), 2.9%; Fmoc-Gly, 3.3%; Fmoc-His(Trt), 3.5%; Fmoc-Ile, 15.5%; Fmoc-Leu, 4.9%; Fmoc-Lys(Boc), 5.5%; Fmoc-Met, 2.6%; Fmoc-Phe, 2.6%; Fmoc-Pro, 4.3%; Fmoc-Ser(^{t}Bu), 4.2%; Fmoc-Thr(^{t}Bu), 4.1%; Fmoc-Tyr(^{t}Bu), 3.9%; and Fmoc-Val, 11.2%. A sample of this mixture (182 mg, 0.40 mmol) in DMF (4 mL) was activated for 15 min by incubation with HATU (304.2 mg, 0.80 mmol) and *sym*-collidine (162 μL, 2.0 mmol) in DMF (1 mL) and presented to the resin. After agitation for 5 h, the resin was filtered, washed twice with DMF (5 mL), and treated with a second aliquot of Fmoc-AA mixture (182 mg, 0.40 mmol), HATU (304.2 mg, 0.80 mmol), and collidine (162 μL, 2.0 mmol) in DMF (5 mL) and presented to the resin. After agitation for 10 h, the resin was filtered, washed three times with DMF (5 mL), treated with 20% piperidine in DMF (3 mL) for 30 min, filtered, and washed three times with DMF (3 mL). This procedure was repeated twice to incorporate AA_3 and AA_4. Resin **5** was always stored prior to removal of the N-terminal Fmoc protecting group. The amine substitution level of resin **5** AA_1=Ala within three repetitions was determined to be within 0.32±0.04 mmol/g by Fmoc-quantization.[22]

Resin **6** was prepared by solvating Fmoc-protected resin **5** AA_1=Ala (100 mg, 0.03 mmol, 0.3 mmol/g) in 20% piperidine in DMF (3 mL) for 30 min followed by filtering and washing three times with DMF (2 mL). Fluorescent hydroxyacid **1** or amino acid **2** (56.4 mg, 0.15 mmol), HATU (114 mg, 0.30 mmol), and *sym*-collidine (60.5 μL, 0.75 mmol) in DMF (2 mL) was incubated for 10 min and then added to the resin. After 3 h, the resin was filtered, washed three times with DMF (2 mL), and treated with a second aliquot of the fluorescent hydroxyacid **1** or amino acid **2** (56.4 mg, 0.15 mmol), HATU (114 mg, 0.30 mmol), and *sym*-collidine (60.5 μL, 0.75 mmol) in DMF (2 mL). After 6 h of agitation, the resin was filtered, and washed three times with DMF (2 mL), twice with MeOH (2 mL), three times with THF (2 mL), and air dried to provide resin **6** or **7**. The amine substitution level within three repetitions was found to be 0.21±0.03 and 0.26±0.02 mmol/g for resin **6** and **7**, respectively.[22]

Peptides **8** and **9** were cleaved from the resin using the same protocol. Resin **6** AA_1=Ala or **7** AA_1=Ala (100 mg, 0.025 mmol, 0.25 mmol/g) was treated with 20% piperidine in DMF (1 mL) for 30 min, filtered, washed three times with DMF (1 mL), and washed twice with CH_2Cl_2 (2 mL). After drying in vacuo, the resin was incubated with a cleavage solution (2 mL) containing 95:2.5:2.5 TFA/TIS/H_2O for 1 h. The solution was collected and the resin was washed twice with the cleavage solution (0.5 mL). The solution and washes were combined and concentrated in vacuo. The resulting materials were dissolved in 0.2 mL DMSO and filtered through a plug of K18-reverse phase silica gel (300 mg) using a gradient of 10–80% aq CH_3CN. The fluorescent fractions were collected and lyophilized. The amount of peptide obtained from three repetitions of this procedure was determined to contain 0.22±0.03 mmol of **8** or 0.26±0.03 mmol of **9** (see Section 4.3). This procedure provides ~20 mg of net peptide or ~2.5 μg of each peptides at the theoretical production of 8000 peptides per set.

4.3. Fluorescence imaging and quantification

The fluorescence from the 7-dimethylaminocoumarin-4-acetamide provided an effective handle to quantify the concentration of peptide on resins **6** and **7** and the concentration of solutions of **8–11**. Fluorescence was quantified in solution using a spectrophotometer or plate reader with λ_{ex}^{max}=375±6 nm, λ_{em}^{max}=462±5 nm, and ε=23,500±2500 M^{-1} cm^{-1}. The concentration of dye on resin or in cell was determined using quantitative fluorescence microscopy on an LED-fluorescence microscope (Xenobe Research Institute) using fluorescent microspheres as standards (Molecular Probes Inc.).

LED-fluorescence images were collected using the excitation from a 100 mW, 370 nM GaN UV-LED (Roithner Lasertechnik), filtration through a dichroic filter set FF400 (Semrock Inc.) with an excitation at 377±50 nm, a dichroic with >98% reflection at 344–404 nm and >98% transmission at 415–570 nm, an emission filter at 447±60 nm. Images were collected using a 60X Oil Immersion Epiplan Neofluar objective (Zeiss), and image collection on an Electron Bombardment CCD Camera EBCCD (Hamamatsu Corp.).

4.4. Cell uptake

Samples of the peptide sets **8** or **9** were dissolved in DMSO. The concentrations were adjusted to 10 μM/peptide-based on the expected fluorescence from 8000 peptides per set. A 200 μL aliquot of this solution was added to 20 mL of

DMEM in 250 mL culture flask containing ~106 cells/cm^2. After incubation at 37 °C for 30 min, the cells were washed three times with 50 mL of DMEM containing 10% ethanol and the resulting cells were imaged on an LED-fluorescence microscope (see Section 4.3). The cells were then harvested by brief digestion with trypsin, isolated by centrifugation at 1000 rpm, and washed five times with DMEM (10 mL).

Samples of sets of depsipeptides **10** and cyclic peptides **11** were imaged as crude mixtures or directly after TLC analysis. 2D-pTLC was conducted on 2×2 cm Partisil KC18 plates using a 1–2 mm diameter spot. The plates were eluted with 10:80:5:2.5:2.5 CH_2Cl_2/MeOH/H_2O/pyridine/Et_3N in one direction, turned 90°, and run in the other direction with 10:80:5:5 CH_2Cl_2/MeOH/H_2O/acetic acid. The nonfluorescent regions were scraped from the plate and the plate was gently washed with 2:1 mixture of hexane and ethyl acetate to remove silica particles. The plate was wet with 50% aq CH_3CN using an aerosol spray and covered with 0.10±0.02 mm layer of low-melt agarose.[24] A cover slip containing ~10^6 cells/cm^2 was placed on top of the agarose layer, cells facing down into the agarose layer. The agarose layer served to transport the fluorescent peptide to the cells. This sandwich was incubated at 37 °C for 1 h and then imaged. Though effective, this technique required a resolution of at least 2 mm between each spot.

Alternatively, samples of the depsipeptides **10** and cyclic peptides **11** were extracted from a 10×10 cm Partisil KC18 plates using a 1:1 mixture of MeOH and DMF. The resulting aliquots were dried and dissolved at 10 μM/peptide in DMSO. This solution was added to 2 μL/mL of DMEM and placed on a microscope slide containing ~10^6 cells/cm^2 and incubated at 37 °C for 30 min. The cells were washed with 3×50 mL of DMEM containing 10% ethanol and imaged on an LED-fluorescence microscope (see Section 4.3). The cells were harvested by release, by brief digestion with trypsin, isolated by centrifugation at 1000 rpm, and were washed five times with DMEM (10 mL).

4.5. Cell fractionation

Isolation of nuclei was conducted using a procedure developed by Roeder.[25] HeLa cell pellets were suspended in five volumes of 4 °C phosphate buffered saline pH 7.4 and collected by centrifugation for 10 min at 2000 rpm at 4 °C. The subsequent steps were performed at 4 °C. The cells were diluted five-fold with 10 mM HEPES (pH 7.9 at 4 °C) containing 1.5 mM $MgCl_2$, 10 mM KC1, and 0.5 mM DTT; and allowed to stand for 10 min. The cells were collected by centrifugation as before and resuspended by two-fold dilution in 10 mM HEPES (pH 7.9 at 4 °C) containing 1.5 mM $MgCl_2$, 10 mM KC1, and 0.5 mM DTT and lysed by 10 strokes of Dounce all-glass homogenizer with a type B pestle. The homogenate was checked microscopically for cell lysis and centrifuged for 10 min at 2000 rpm (470 g) and 20 min at 25,000 g to provide the crude nuclei. Materials were extracted from these nuclei using the method in Section 4.6.

Subcellular fractionation of the ER was conducted using Endoplasmic Reticulum isolation kit (ER0100) from Sigma Aldrich. Materials were extracted from these ER using the method in Section 4.6.

4.6. Peptide extraction

Nuclear and ER fractions were lysed in four volumes of PBS 7.2 by sonication at 4 °C, extracted twice with cyclohexane (10 mL) and ethyl acetate (10 mL) to remove lipids, and lyophilized. The resulting dried material was extracted twice with THF (1 mL), MeOH (1 mL), CH_3CN (1 mL), DMF (1 mL), and the combined extracts were dried in vacuo. The residue was dissolved in DMF and standardized to concentration of 250±10 μM per peptide using the fluorescence from 7-dimethylaminocoumarin-4-acetamide (see Section 4.3). The number of peptides present was determined by 2D-pTLC on 2×2 cm Partisil KC18 plates using a 1–2 mm diameter spot. The plates were eluted with 10:80:5:2.5:2.5 CH_2Cl_2/MeOH/H_2O/pyridine/Et_3N in one direction, turned 90°, and run in the other direction with 10:80:5:5 CH_2Cl_2/MeOH/H_2O/acetic acid. Preparatory purification was possible by repeating the same purification on a 10×10 cm Partisil KC18 plates using an 8–9 mm diameter spot.

4.7. Macrocyclization

A 200 μL aliquot of solution containing peptides **8** or **9** standardized to 250±10 μM/peptide (0.05 μmol) in DMF was diluted to 1:1 by addition of HATU (0.95 mg, 2.5 μmol), DTT (3.8 μg, 0.025 μmol), and *sym*-collidine (0.2 μL, 2.5 μmol) in DMF. The reaction mixture was stored at rt under argon in the absence of light. After 24 h, the reaction mixture was passed through a plug of K18-reverse phase silica gel (300 mg) using 1 mL of 50% MeOH in DMF and concentrated. The number of peptides present was determined by 2D-pTLC on 10×10 cm Partisil KC18 plates using an 8–9 mm diameter spot. The plates were eluted with 10:80:5:2.5:2.5 CH_2Cl_2/MeOH/H_2O/pyridine/Et_3N in one direction, turned 90°, and run in the other direction with 10:80:5:5 CH_2Cl_2/MeOH/H_2O/acetic acid.

The mass of peptides **13–26** was determined by pTLC-MALDI-MS.[18] Their sequence was determined using established methods for MS based peptide-sequencing[19] after isolating individual peptides from a pTLC plate. The identity of each peptide was established by repeating its synthesis in a single substrate manner at a 20 mg scale. Cell uptake and localization data for each peptide were confirmed by repeating the cellular imaging experiments with this material.

Acknowledgments

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