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Synthesis and preliminary antibacterial evaluation of simplified thiomarinol analogs

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Dedicated to Professor Jon Ellman on receipt of the 2006 Tetrahedron Young Investigator Award in Bioorganic and Medicinal Chemistry

Abstract—A series of simplified thiomarinol derivatives was prepared by way of catalytic enantioselective inverse electron demand hetero [4 + 2] cycloaddition/allylboration tandem reaction. As a preliminary evaluation, these analogs were tested for antimicrobial activity using a standard disk diffusion assay. Whereas amide analogs were less active, the simple ester analogs **3** and **4** were demonstrated to be more active than thiomarinol H.

1. Introduction

Mupirocin (pseudomonic acid A, Fig. 1) is one of the worlds' leading topical antibiotics commercialized under the name Bactroban® by GlaxoSmithKline.[1] It is produced by *Pseudomonas fluorescens*, a soil isolate reported to possess antibacterial activity as early as 1887.[2] While the mixture of pseudomonic acids was found to be the active component in the 1960s,[3] the major constituent was characterized later and named pseudomonic acid A (mupirocin).[4] Mupirocin is active against Gram-positive aerobic bacteria and a few Gram-negative strains. It is prescribed for treating skin infections such as impetigo, candidiasis, burn wounds, and cuts.

Mupirocin inhibits the bacterial isoleucyl tRNA synthetase enzyme responsible for loading the aminoacid isoleucine (Ile) onto its cognate tRNA required for ribosomal protein synthesis (Fig. 2).[5] Aminoacyl tRNA synthetases belong to a superfamily of nucleotidyl transferase enzymes related to other ATP-binding proteins such as dehydrogenases and photolyase. There is at least one discrete synthetase per specific aminoacid. These enzymes are divided into two related classes, I or II, depending on whether they acylate the 2′ or 3′ end of the tRNA (Fig. 2).[6] Some synthetases, such as $tRNA^{Ile}$ synthetase (a class I synthetase), even possess an editing site for cor-

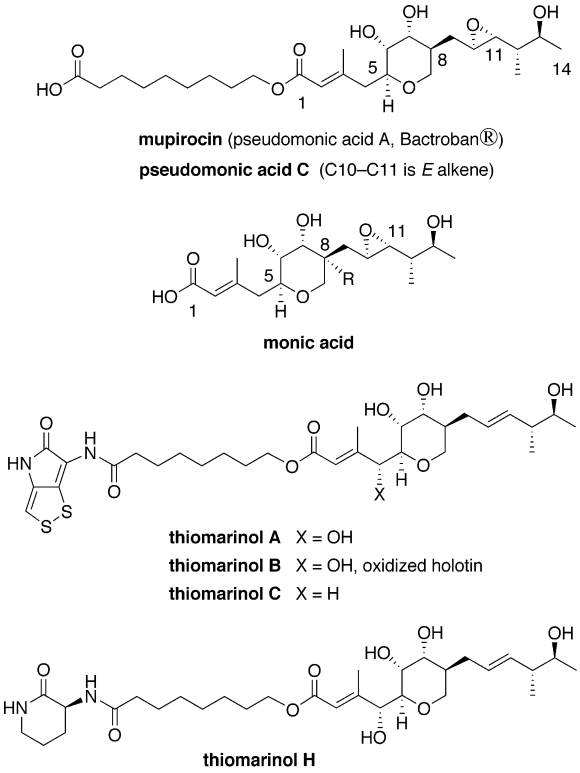

Figure 1. Structures of mupirocin (pseudomonic acid A) and selected members of the thiomarinol family.

Keywords: Thiomarinol analogs; Antibacterial; Mupirocin; Cycloaddition/allylboration tandem reaction.
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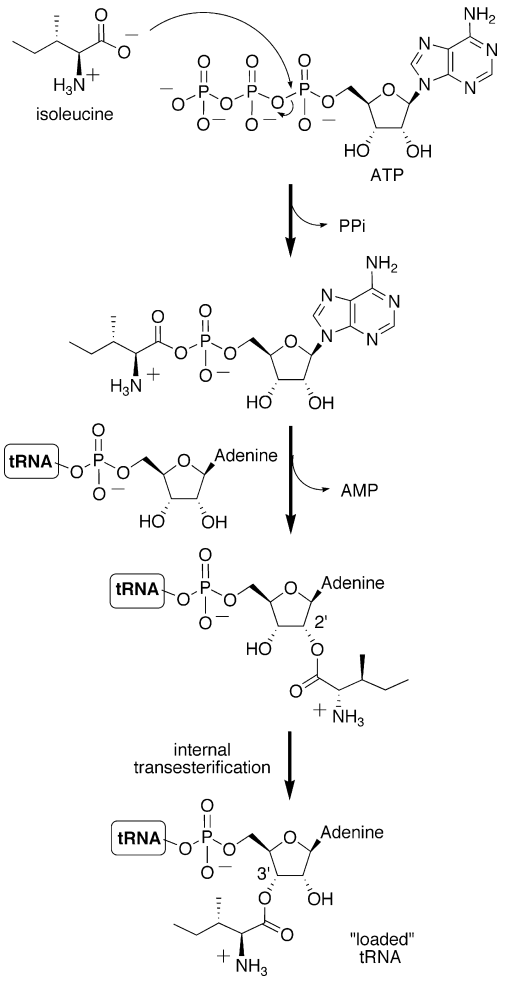


Figure 2. Biomechanism of action for $tRNA^{Ile}$ synthetase, a class I aminoacyl tRNA synthetase.

recting misloaded aminoacids. The synthetase site of these enzymes holds substrates in place without involving functional groups of the enzyme in a chemical sense (i.e., without the involvement of any covalent bonds or proton exchange). Instead, it stabilizes with very conserved residues (the so-called HIGH box) the transition state for cleavage of the α-phosphate upon nucleophilic attack by the aminoacid carboxylate.[7]

These enzymes are characterized by the presence of a Rossman-like dinucleotide-binding fold consisting in α/β tertiary structure, which is very common amongst adenylyl and cytidylyl triphosphate binding enzymes. Mupirocin is approximately 10,000 times more potent on the bacterial enzyme than the human homolog, which ensures its selectivity as a pharmaceutical drug.[8] The binding of mupirocin to the enzyme–tRNA complex is competitive with isoleucine and ATP, and also with Ile-AMP derivatives. Surprisingly, tryptophan fluorescence studies revealed that the epoxide-containing side chain does not occupy the site of the other enzyme substrate, isoleucine, but rather an adjacent site slightly off from the residues normally involved in phosphate anion stabilization.[9] This information was elegantly employed in a recent report describing the preparation of subnanomolar chimeric analogs embodying the monate core and a sulfonamido-isoleucyl side chain.[10] The X-ray crystal structure of mupirocin bound to the complex between $tRNA^{Ile}$ synthetase and its cognate tRNA was solved in 1999.[11] The structure confirmed that the right-hand side chain of mupirocin does not occupy the site that would normally stabilize the phosphatidyl isoleucine. The structure also confirmed that mupirocin acts mainly as an ATP mimic and occupies the site normally occupied by the ribose ring. A map of the main molecular interactions in the mupirocin–$tRNA^{Ile}$ complex is shown in Figure 3. Critical to the inhibitor complex is a π–π^* interaction between Phe587 and the acrylate moiety, which is also held in place with a backbone hydrogen bond between Val588 and the carbonyl oxygen.

Due to their structure and their biological activity, the pseudomonic acids have attracted considerable attention from synthetic and medicinal chemists.[12] Unfortunately, mupirocin displays poor oral absorptivity and low metabolic stability. In the bloodstream, its ester linkage is rapidly hydrolyzed to an inactive product, monic acid (Fig. 1).[13] Consequently, there has been significant interest in the development of improved analogs that could also be suitable as oral antibiotics. Thiomarinol A[14] and thiomarinol H[15] (Fig. 1) are rare marine natural products recently isolated from the bacterium *Alteromonas rava* sp. nov. SANK 73390. These closely related families of naturally occurring antibiotics display minor structural differences. The structures of thiomarinols A and H differ from mupirocin by the presence of a C4-hydroxyl, a shorter C1-alkoxy chain, and the replacement of the C10–C11 epoxide with an *E* alkene unit. Based on the X-ray crystal structure of the mupirocin–$tRNA^{Ile}$ synthetase complex, we speculated that the additional 4-hydroxyl group in thiomarinols might be able to form hydrogen bonds with His64 and Asp557 in the bacterial enzyme (Fig. 4). The role of the additional 4-hydroxyl group is intriguing and deserves further investigation because thiomarinol analogs might demonstrate improved antimicrobial activity. Thiomarinols A and H are distinguishable by their respective holotin and anhydroornithine C1 amide end-groups. These natural substances were found to be equally po-

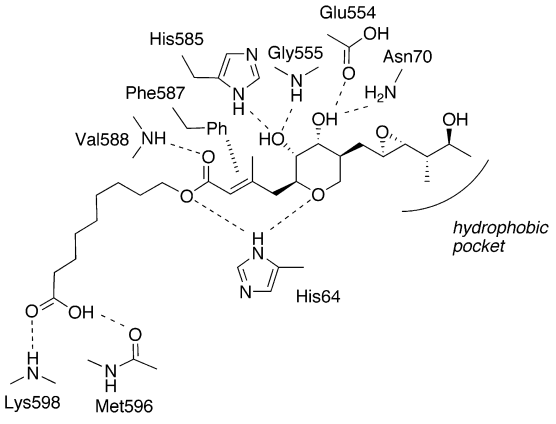


Figure 3. Map of main molecular interactions in the mupirocin–$tRNA^{Ile}$ complex.[11]

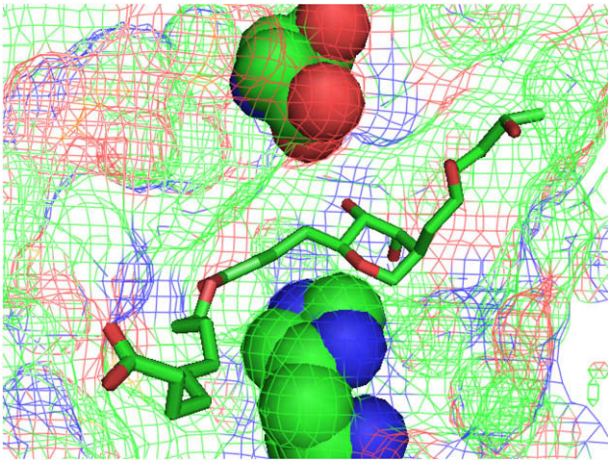


Figure 4. His64 (bottom spheres) and Asp557 (top spheres) near the C4 center of mupirocin (sticks) complexed to the bacterial tRNA isoleucyl synthetase.[11]

tent to pseudomonic acid A, and thiomarinol A was found to possess a wider spectrum of activity (against both Gram-positive and -negative bacteria).[14] For example, the activity of thiomarinol H against *Staphylococcus aureus* was shown to be comparable to that of tetracycline and streptomycin.[15] These impressive properties justify efforts to develop synthetic routes to the thiomarinols. Our group has recently synthesized the first member of the thiomarinol family, thiomarinol H, using a synthetic route that could be amenable to the design of simplified analogs.[16] At the onset of this project on the evaluation of thiomarinol analogs, several questions arose: (1) What is the contribution of the left-hand side chain and the heterocyclic end-group in the thiomarinols? (2) What is the contribution of the C4-hydroxy group of thiomarinols. (3) Is the right-hand side chain necessary in its integral form? These questions can be addressed with simple analogs that are easily accessible as a result of our unique synthetic approach to the thiomarinol skeleton. We hoped that the information gained could lead to further improvements through the design of second-generation analogs.

2. Results

2.1. Design of simplified analogs

It is believed that mupirocin and the thiomarinols share the same target, bacterial isoleucine tRNA synthetase. Extensive medicinal chemistry studies have been performed on pseudomonic acid analogs and the outcome of these studies can be of help with the thiomarinol family. A precise pharmacophore model of mupirocin was developed even before the availability of X-ray crystallographic information (Fig. 5). The dihydroxypyran (monate) unit is essential for the antibacterial activity. It was shown that the left-hand side chain and the acrylate linker are quite variable provided that a suitable isoelectronic replacement such as a *N*,*O*-heterocyclic unit is used as the linker.[17] While most of these analogs possessed potent IC_{50} values when assayed enzymatically,

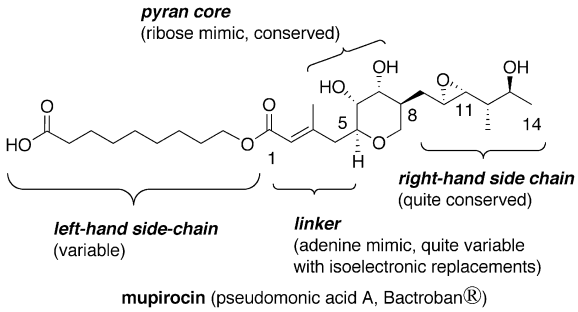


Figure 5. Qualitative pharmacophore model for mupirocin.

they did not display good in vitro activity (i.e., high MIC values) probably because of their lipophilic character. Monic ester analogs with additional substituents in the 2-position, like the 2-fluoro and 2-methyl derivatives, retain most of the antimicrobial activity provided that the olefin geometry is not inverted.[18] To improve the oral bioavailability of the compounds, the left-hand alkoxy group on the hydrolizable ester was replaced with various carboxyl group surrogates such as heterocycles like diazoles and triazoles.[19] Unfortunately, most of these derivatives are inactive. Allylic ethers and ketones were also evaluated.[20] Whereas the ethers demonstrated good IC_{50} values but low in vitro activity, some of the ketones were active in vitro. Modifications of the right-hand side chain (C9–C14) were also examined.[21] Surprisingly, even subtle changes to the terminal C12–C14 unit are not well tolerated. Thus, both the C13-deoxy analog and the analog with inverted stereochemistry showed weak activity.

To address the effect of the structural differences between mupirocin and the thiomarinols on their respective antimicrobial activity, we planned the design of simple analogs **1**–**6** with a simplified ester or an amide as left-hand side chain (Fig. 6). It was hoped that amide analogs, such as **5** and **6**, could preserve a high antibacterial activity without the hydrolytic susceptibility of

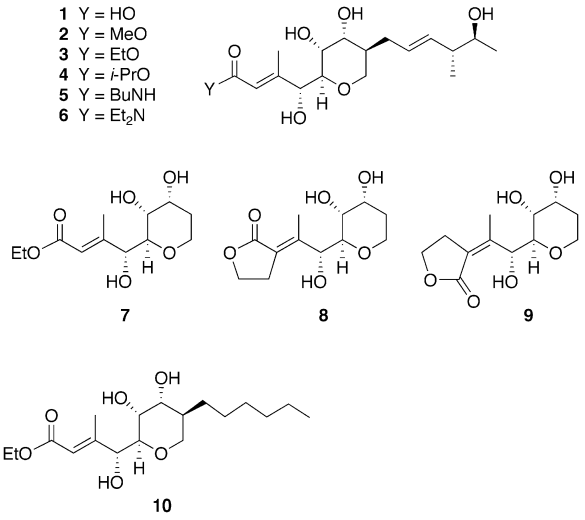


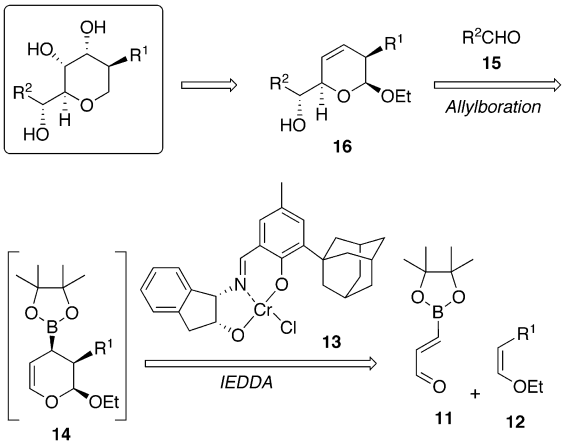
Figure 6. Structures of simplified thiomarinol analogs evaluated in this study.

mupirocin. Furthermore, we designed analogs **7**–**9** lacking a right-hand (C9–C14) side chain and analog **10** with a simplified side chain devoid of the C13 hydroxyl and other substituents. The preliminary evaluation of these analogs will reveal the extent to which the thiomarinol core can be simplified toward designing more potent analogs.

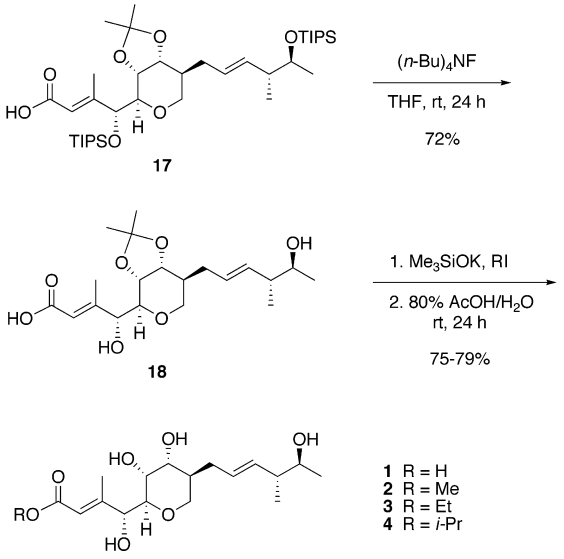
All of these analogs were prepared by way of our catalytic enantioselective inverse electron demand hetero [4 + 2] cycloaddition/allylboration tandem reaction between boronate-containing heterodiene **11**, enol ethers (**12**), and aldehydes (**15**) (Scheme 1).[22] The cycloaddition step is catalyzed by Jacobsen's Cr(III) complex **13**.[23] This multicomponent reaction functions well with a wide range of enol ethers and aldehydes, and was demonstrated in the context of complex target synthesis, as exemplified by thiomarinol H.[16] Acetal reduction and a stereoselective dihydroxylation of dihydropyrans **16** would afford the desired analogs.

2.2. Preparation of analogs

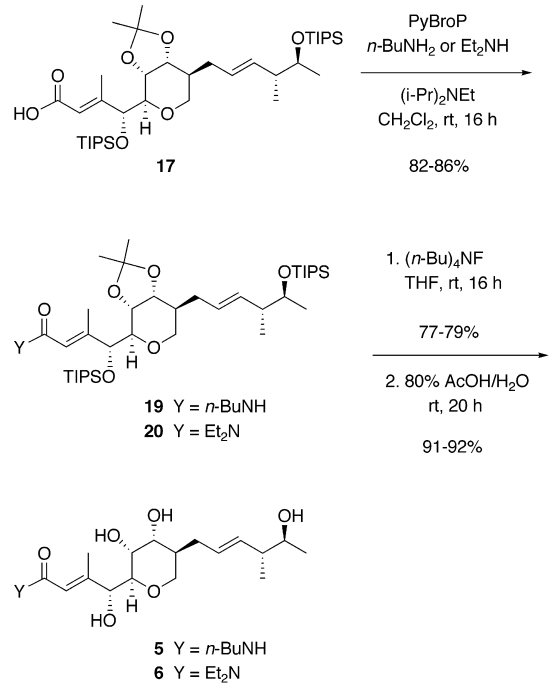
Analogs **2**–**4** with a simplified ester as well as the amide analogs **5** and **6** were prepared from an advanced synthetic intermediate, carboxylic acid **17** (Scheme 2), made in our total synthesis of thiomarinol H.[16] Intermediate **17** can be reached in a dozen steps following the initial key multicomponent reaction depicted in Scheme 1. Then, the siloxy groups of **17** were removed using tetrabutylammonium fluoride to give intermediate **18**. The latter was subjected to an alkylative esterification with different alkyl iodides to provide the ester analogs **2**–**4** after hydrolysis of the acetonide with aqueous acetic acid. The free carboxylic acid analog **1** was made from **18** by direct treatment with aqueous acetic acid. The respective secondary and tertiary amide analogs **5** and **6** were synthesized with a similar sequence that commenced with a PyBroP-promoted amidation to provide protected intermediates **19** and **20** (Scheme 3). These two intermediates were treated as above to remove the siloxy groups and unblock the acetonide-protected diol, providing analogs **5** and **6**.



Scheme 1. Three-component hetero [4 + 2] cycloaddition/allylboration reaction approach to thiomarinol analogs.

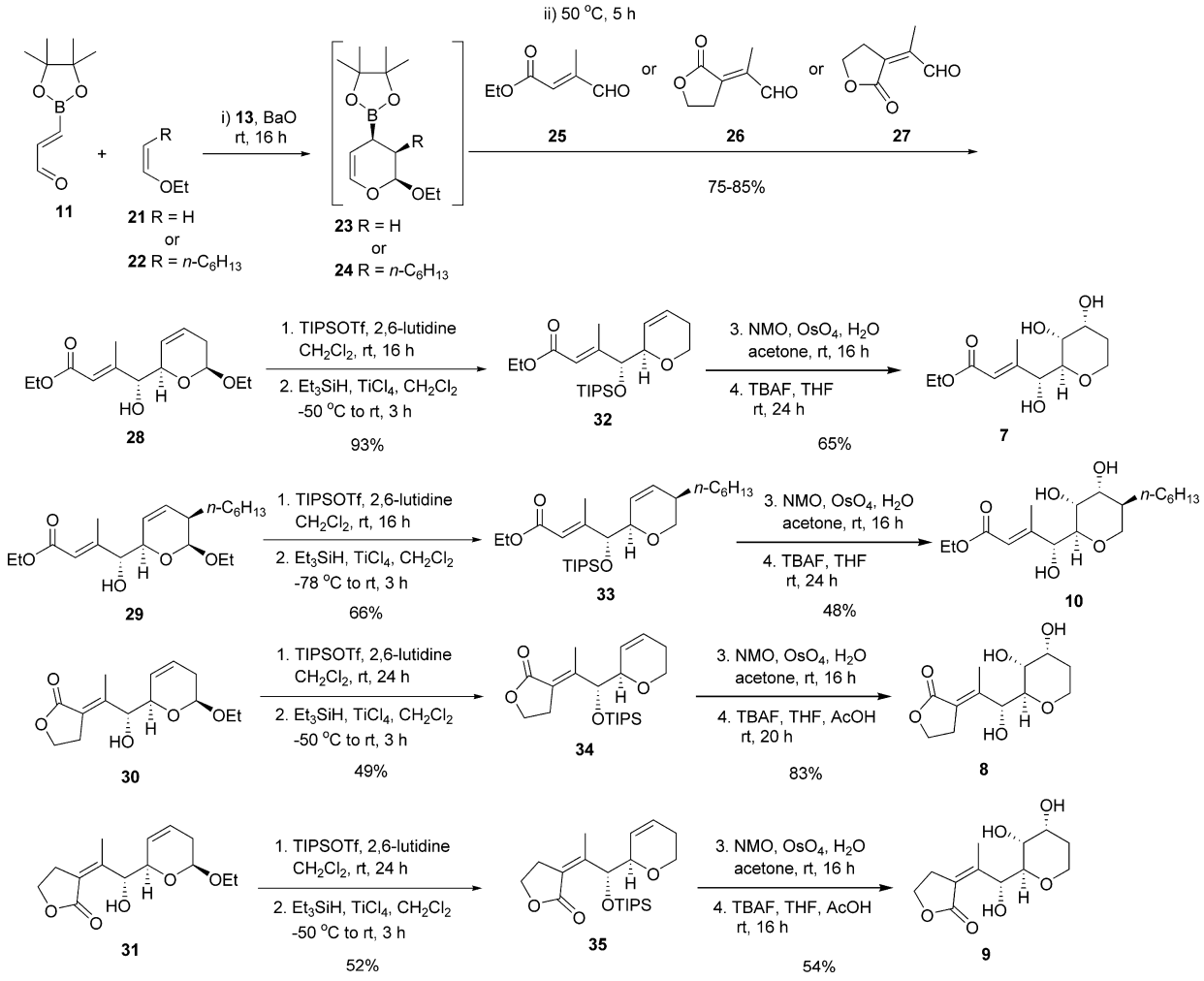


Scheme 2. Preparation of acid **1** and simplified ester analogs **2**–**4**.



Scheme 3. Preparation of amide analogs **5** and **6**.

Analogs **7**–**9** with a simplified ester and no right-hand side chain as well as analog **10** with a simplified right-hand chain were prepared from the [4 + 2] cycloaddition between heterodiene **11** and enol ethers **21** and **22** to produce adducts **23** and **24**, which were reacted with aldehydes **25**–**27** to generate allylboration products **28**–**31** in good yields and high ee (Scheme 4). The hydroxyl groups of intermediates **28**–**31** were protected as siloxy ether prior to the silane-promoted reduction of the acetal to generate intermediates **32**–**35**. Stereo-selective dihydroxylation of the pyran's alkene was achieved by reaction with osmium tetraoxide. The resulting four intermediates were then treated with TBAF (in the presence of acetic acid in the case of the furanone analogs) as above to provide the desired analogs **7**–**10**.



Scheme 4. Preparation of simplified analogs **7**–**10**. NMO = *N*-methyl morpholine oxide, TABF = tetra-*n*-butylammonium fluoride, TIPS = triisopropylsilyl.

2.3. Evaluation of antibacterial activity

As a preliminary evaluation, analogs **1**–**10** were tested for antimicrobial activity against *S. aureus* using a standard disk diffusion assay.[24] The diameter of zones with complete growth inhibition induced by commonly used antibiotics and our simplified thiomarinol analogs were compared. The larger the diameter of the zone, the more active the analogs. Control antibiotics were streptomycin, penicillin G, and pseudomonic acid A.

Using this simple but reliable assay, it was found that synthetic thiomarinol H was more active than tetracycline–HCl, but less active than streptomycin, penicillin G, and pseudomonic acid A (Table 1). Among the simplified analogs, not surprisingly, the acid derivative **1** did not show any antimicrobial activity, an outcome similar to monic acid.[13] While the amide analogs **5** and **6** are inferior to thiomarinol H in terms of activity, the simplified ester analogs, especially the ethyl and isopropyl esters **3** and **4**, demonstrated stronger inhibition. Although the simplified ester analogs of thiomarinols exhibit slightly lower antimicrobial in vitro activity than pseudomonic acid A, it does not necessarily mean that thiomarinols are inferior ligands for the bacterial isoleucyl *t*RNA synthetase because there are other factors such as bioavailability that might be limiting (e.g., solubility and the ability to cross the bacterial membrane). Thus, to clarify the role of the 4-hydroxyl group in thiomarinols, more studies are required.

Analogs lacking the right-hand side chain **7**–**9** as well as the ester analog with the simplified right-hand side chain **10** did not show any antimicrobial activity in the disk diffusion assay (i.e., no zone of inhibition). It was hoped that analog **8** (with the *trans* double bound) would show some in vitro activity on the basis of the inherent proteolytic stability of the lactone ring. On the other hand, it was expected that analog **9** would be less active based on previous studies on the olefin geometry of mupirocin analogs.[18] The fact that analog **10** did not inhibit bacterial growth strongly suggests that the right-hand side chain of the natural molecule must remain mostly intact to keep the activity. This conclusion is in agreement with results obtained with the C12–C14 mupirocin analogs.[21]

The information gained in this study will help us design more potent thiomarinol analogs. Key targets for a second-generation of derivatives would be esters similar to analogs **3** and **4**. Analogs bearing a lactone ring on the

Table 1. Disk diffusion assay against *Staphylococcus aureus*

Compound	Amount loaded on disk ($\times 10^{-2}$ μmol)	Strains Inhibition zone[a] (mm)	
		ATCC#6538	ATCC#25923
Streptomycin sulfate	0.686	16	14
Tetracycline–HCl	6.02	28	29
Penicillin G Na salt	1.9	40	32
Pseudomonic acid A Ca salt	1.72	32	26
Thiomarinol H	1.72	16	10
1	1.72	No zone	No zone
2	1.72	16	13
3	1.72	22	21
4	1.72	22	21
5	3.44	14	Slight
6	3.44	15	Slight
7	1.72	No zone	No zone
8	1.72	No zone	No zone
9	1.72	No zone	No zone
10	1.72	No zone	No zone

[a] Diameter of zones with complete growth inhibition. One representative set of data is shown.

left-hand side chain and the unmodified right-hand side chain would be of particular interest considering the probable in vivo stability of the heterocycle. Changes to the right-hand side chain could be inspired by the studies of Pope and co-workers.[10] Finally, the preparation of C4–deoxy thiomarinol analogs would greatly help in clarifying the role of this particular hydroxyl group in the biological activity of this class of antibiotics.

3. Conclusions

Thiomarinols are rare marine natural products closely related to mupirocin. The preparation of simplified thiomarinol analogs will help in the quest to generate an orally available (with high metabolic stability) derivative of mupirocin. In this study, the efficient enantioselective inverse electron demand hetero [4 + 2] cycloaddition/allylboration tandem reaction developed in our laboratory was used to prepare rationally designed thiomarinol analogs. As a preliminary evaluation of their antimicrobial activity, these simplified analogs were tested against two strains of *S. aureus* using a simple disk diffusion assay. The evaluation of these analogs has provided insight into the extent to which the thiomarinols can be simplified while maintaining a potent activity. Interestingly, the simplified ester analogs **3** and **4** were demonstrated to be more active than thiomarinol H, showing that the left-hand side chain and the heterocyclic end-group are not essential for the antimicrobial activity. On the other hand, analogs **7**–**10** did not show any antimicrobial activity suggesting that the right-hand chain is necessary in its integral form. Also, amide analogs **5** and **6** were inferior to thiomarinol H in the disk diffusion assay. Although more studies will be required to clarify the role of the C4-hydroxyl group of the thiomarinols, the information gained in this study will be very useful in the design of improved second-generation analogs.

4. Experimental

4.1. General

Unless otherwise stated, all reactions was performed under a nitrogen atmosphere using flame-dried glassware. Toluene and CH_2Cl_2 were distilled from CaH_2. All aldehydes were purified by bulb-to-bulb distillation prior to use. Analytical thin layer chromatography were performed on Merck Silica Gel 60 F254 plates and were visualized with UV light and 1% $KMnO_4$ (aq). Deactivated silica-gel refers to the silica-gel washed with triethylamine prior to use. NMR spectra were recorded on Varian INOVA-300, INOVA-400 or INOVA-500 MHz instruments. The residual solvent protons (^1H) or the solvent carbon (^{13}C) were used as internal standards. ^1H NMR data are presented as follows: chemical shift in ppm (δ) downfield from tetramethylsilane (multiplicity, coupling constant, integration). The following abbreviations are used in reporting NMR data: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dq, doublet of quartets; dd, doublet of doublets; m, multiplet. High-resolution mass spectra were recorded by the University of Alberta mass spectrometry service laboratory using either electron impact (EI) or electrospray ionization (ESI) techniques. Infrared spectra were obtained on a Nicolet Magna-IR 750 with frequencies expressed in cm^{-1}. X-ray crystallography was performed using a Bruker P4/RA/SMART 1000 CCD diffractometer. Catalyst **13** was prepared according to the procedure of Jacobsen and co-workers.[23] Boronate **11** was prepared according to our previously published procedure and purified by bulb-to-bulb distillation (<1 mm Hg, 94%).[25] The preparation of intermediate **17** was reported before.[16] BaO (Acros) was used as supplied (90% tech-powder). Optical rotations were measured using a 1-mL cell with a 1-dm length on a Perkin-Elmer 241 polarimeter.

4.2. Preparation of analogs

4.2.1. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid (18). To the solution of TIPS protected alcohol **17** (0.210 g, 0.301 mmol) in THF (3 mL) was added TBAF (2.41 mL. 1.00 M solution in THF, 2.410 mmol). The reaction mixture was stirred at ambient temperature for 24 h and quenched with an saturated aqueous solution of NH_4Cl (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2× 20 mL). The combined organic layers were washed with water (20 mL) brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (EtOAc/MeOH, 9:1) to afford the title compound **18** (0.083 g, 81%) as a yellow oil. $[\alpha]_D^{23}$ −7.8 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3420, 1690, 1646; ^1H NMR (500 MHz, $CDCl_3$) δ 6.02 (s, 1 H), 5.41–5.57 (m, 2H), 4.12–4.22 (m, 3H), 3.75 (dd, J = 3.1, 11.5 Hz, 1H), 3.66 (dd, J = 1.6, 11.5 Hz, 1H), 3.55–3.63 (m, 1H), 3.41 (dd, J = 2.3, 8.4 Hz, 1H), 2.00–2.32 (m, 7H), 1.48 (s, 3H), 1.38 (s, 3H), 1.16 (d, J = 6.3 Hz, 3H), 1.01 (d, J = 6.9 Hz, 3H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 170.9, 159.7, 134.9, 128.9, 115.9, 108.8, 77.7, 75.6, 75.3, 71.2, 70.2, 66.5, 44.4, 36.5, 34.0, 28.3, 26.3, 20.3, 16.6, 16.0; HRMS (ESI, *m*/*z*) calcd for $C_{20}H_{33}O_7$ [M + H] 385.22208, found 385.22213.

4.2.2. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid (1). A solution of **18** (32.0 mg, 0.056 mmol) in 80% aqueous AcOH (0.65 mL) was allowed to stir at ambient temperature for 20 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (EtOAc/MeOH, 9:1) to afford the title compound **1** (27.0 mg, 90%) as a colorless oil. $[\alpha]_D^{23}$ 1.33 (*c* 1.4, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3385, 1693, 1655; ^1H NMR (500 MHz, CD_3OD) δ 6.02 (s, 1H), 5.39–5.51 (m, 2H), 4.36 (s, 1H), 3.92 (dd, *J* = 3.0, 3.0 Hz, 1H), 3.82 (dd, *J* = 3.0, 9.5 Hz, 1H), 3.76 (dd, *J* = 2.6, 11.3 Hz, 1H), 3.68 (dd, *J* = 1.6, 9.5 Hz, 1H), 3.60 (dq, *J* = 5.7, 5.7 Hz, 1H), 3.53 (d, *J* = 11.3 Hz, 1H), 3.41 (dd, *J* = 2.3, 8.4 Hz, 1H), 2.23–2.29 (m, 1H), 2.08–2.18 (m, 5H), 1.70–1.76 (m, 1H), 1.08 (d, *J* = 6.3 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.5, 160.9, 135.7, 129.9, 116.6, 77.6, 74.4, 72.2, 71.9, 66.0, 65.8, 45.4, 43.9, 33.5, 20.3, 26.7, 16.2; HRMS (ESI, *m*/*z*) calcd for $C_{17}H_{28}O_7Na$ 367.17273, found 367.17258.

4.2.3. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoyl-oxy-methane (2). To a solution of compound **18** (0.130 g, 0.340 mmol) in DMF (1.5 mL) at ambient temperature was added $KOSiMe_3$ (0.047 g, 0.370 mmol). After 10 min at ambient temperature, MeI (0.145 g, 1.00 mmol) was added. The mixture was then stirred overnight at ambient temperature. After that time, the mixture was diluted with EtOAc (20 mL) and washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to afford crude ester which was used without purification.

The ketal protecting group in the crude ester was removed as described in the synthesis of **1**. $[\alpha]_D^{23}$ 9.02 (*c* 0.57, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3420, 1705, 1655; ^1H NMR (500 MHz, $CDCl_3$) δ 6.04 (s, 1H), 5.41–5.53 (m, 2H), 4.31 (s, 1H), 3.99 (br s, 1H), 3.85–3.91 (m, 2H), 3.72 (s, 3H), 3.66 (dd, *J* = 2.0, 9.6 Hz, 1H), 3.53–3.59 (m, 2H), 3.41 (dd, *J* = 2.3, 8.4 Hz, 1H), 2.06–2.26 (m, 6H), 1.82–1.88 (m, 1H), 1.16 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3, 158.7, 134.4, 129.0, 115.3, 75.8, 73.7, 71.4, 70.4, 65.3, 64.6, 51.1, 44.5, 41.5, 32.4, 20.6, 16.8, 16.0; HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{31}O_7$ [M+H] 359.20643, found 359.20673.

4.2.4. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoyl-oxy-ethane (3). Prepared as described for compound **2**. $[\alpha]_D^{23}$ 13.86 (*c* 3.1, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3419, 1700, 1653; ^1H NMR (500 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.36–5.52 (m, 2H), 4.30 (s, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.96 (br s, 1H), 3.82–3.89 (m, 2H), 3.66 (dd, *J* = 1.5, 9.5 Hz, 1H), 3.52–3.58 (m, 2H), 2.03–2.23 (m, 6H), 1.82–1.88 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.16 (d, *J* = 6.1 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.9, 158.2, 134.5, 129.1, 115.8, 75.8, 73.8, 71.4, 70.4, 65.3, 64.6, 59.8, 44.6, 41.5, 32.4, 20.6, 16.8, 16.0, 14.3; HRMS (ESI, *m*/*z*) calcd for $C_{19}H_{33}O_7$ 373.22208, found 373.22238.

4.2.5. 2-[(4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoyl-oxy]-propane (4). Prepared as described for compound **2**. $[\alpha]_D^{23}$ 10.94 (*c* 3.1, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3412, 197, 1655; ^1H NMR (500 MHz, $CDCl_3$) δ 5.99 (s, 1H), 5.38–5.53 (m, 2H), 5.04 (septet, *J* = 6.3 Hz, 1H), 4.28 (d, *J* = 8.8 Hz, 1H), 3.96 (br s, 1H), 3.82–3.88 (m, 2H), 3.67 (dd, *J* = 1.6, 9.4 Hz, 1H), 3.52–3.58 (m, 2H), 3.39 (d, *J* = 5.5 Hz, 1H), 3.12 (d, *J* = 9.4 Hz, 1H), 3.01 (s, 1H), 2.20–2.35 (m, 6H), 1.82–1.88 (m, 1H), 1.26 (d, *J* = 6.3 Hz, 6H), 1.16 (d, *J* = 6.2 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4, 157.8, 134.6, 129.1, 116.3, 75.9, 73.9, 71.4, 70.4, 67.1, 65.3, 64.7, 44.6, 41.5, 32.5, 22.0, 20.7, 16.8, 15.9; HRMS (ESI, *m*/*z*) calcd for $C_{20}H_{34}O_7Na$ 409.21968, found 409.21960.

4.2.6. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-triisopropylsilyloxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-triisopropylsilyl-oxy-but-2*E*-enoic acid butylamide (19). To a solution of **17** (0.220 g, 0.320 mmol) in CH_2Cl_2 (1.5 mL) at ambient temperature were added sequentially PyBroP (0.180 g, 0.380 mmol) and butylamine (0.028 g, 0.380 mmol) followed by Hunig's base (0.130 g, 1.00 mmol). After being stirred at ambient temperature overnight, the mixture was diluted with EtOAc (30 mL) and washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (hexanes/EtOAc, 5:1) to afford **19** (0.195 g, 82%) as yellow oil. $[\alpha]_D^{23}$ 3.13 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3294, 1662, 1653; ^1H NMR (500 MHz, $CDCl_3$) δ 5.78 (s, 1 H), 5.59 (t, *J* = 5.5 Hz, 1H), 5.32–5.48 (m, 2H), 4.09–4.15 (m, 2H), 3.85–3.93 (m, 2H), 3.59–3.62 (m, 2H), 3.22–3.38 (m, 3H), 2.26–2.32 (m, 1H), 2.16–2.22 (m, 2H), 2.05 (s, 3H), 1.90–1.96 (m, 1H), 1.43–1.55 (m, 5H), 1.31–1.42 (m, 5H), 1.03–1.10 (m, 45H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.1, 149.9, 135.7, 127.2, 120.3, 108.3, 80.2, 79.1, 75.6, 71.7, 71.0, 66.0, 44.1, 38.9, 36.8, 33.8, 31.8, 28.3, 26.4, 20.2, 19.2, 18.2, 18.2, 18.1, 18.0, 15.4, 14.1, 13.7, 12.6, 12.6; HRMS (ESI, *m*/*z*) calcd for $C_{42}H_{82}NO_6Si_2$752.56752, found 752.56731.

4.2.7. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-triisopropylsilyloxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-triisopropylsilyl-oxy-but-2*E*-enoic acid diethylamide (20). Prepared as described for compound **19**. $[\alpha]_D^{23}$ −9.65 (*c* 0.97, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2942, 1656, 1631; ^1H NMR (500 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.38–5.46 (m, 2H), 4.20 (d, *J* = 4.5 Hz, 1H), 4.08 (dd, *J* = 2.3, 5.0 Hz, 1H), 3.86–3.98 (m, 2H), 3.58–3.62 (m, 2H), 3.34–3.46 (m,

4H), 3.28 (dd, J = 4.3, 8.9 Hz, 1H), 2.26–2.32 (m, 1H), 2.16–2.22 (m, 2H), 1.88–2.00 (m, 4H), 1.46 (s, 3H), 1.32 (s, 3H), 1.12–1.16 (m, 51H), 1.00 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6, 146.7, 135.6, 127.2, 119.9, 108.3, 80.3, 78.7, 75.7, 71.7, 70.7, 65.8, 44.1, 36.9, 33.7, 28.3, 26.4, 19.2, 18.2, 18.1, 18.1, 18.1, 15.4, 14.1, 12.7, 12.6; HRMS (ESI, m/z) calcd for $C_{42}H_{82}NO_6Si_2$ 752.56752, found 752.56780.

4.2.8. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid butylamide (19-*O*-TIPS deprotected). To the solution of **19** (0.220 g, 0.235 mmol) in THF (3 mL) was added TBAF (1.50 mL. 1.00 M solution in THF, 1.50 mmol). The reaction mixture was stirred at ambient temperature for 11 h and quenched with a saturated aqueous solution of NH_4Cl (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2× 20 mL). The combined organic layers were washed with water (20 mL) brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (EtOAc/MeOH, 9:1) to afford the title compound **19**-*O*-TIPS deprotected (0.119 g, 81%) as a white foam. $[\alpha]_D^{23}$ −3.86 (*c* 0.84, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3320, 1669, 1635; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.90 (s, 1H), 5.42–5.60 (m, 3H), 4.13–4.20 (m, 2H), 4.06 (s, 1H), 3.73 (dd, J = 1.7, 11.5 Hz, 1H), 3.66 (d, J = 11.5 Hz, 1H), 3.58 (t, J = 6.1 Hz, 2H), 3.38–3.43 (m, 1H), 3.26–3.32 (m, 2H), 2.02–2.26 (m, 7H), 1.46–1.53 (m, 5H), 1.32–1.40 (m, 5H), 1.16 (d, J = 6.2 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 150.6, 134.9, 128.9, 119.0, 108.7, 77.5, 75.7, 74.9, 71.1, 70.4, 66.4, 44.5, 39.0, 36.5, 34.1, 31.7, 28.3, 26.3, 20.5, 20.1, 16.7, 15.3, 13.7; HRMS (ESI, m/z) calcd for $C_{24}H_{42}NO_6$ 440.30066, found 440.30006.

4.2.9. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-*O*-Isopropylidene-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid diethylamide (20-*O*-TIPS deprotected). Prepared as described for compound **19**-*O*-TIPS deprotected. $[\alpha]_D^{23}$ −12.89 (*c* 0.63, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3393, 1653, 1607; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.16 (s, 1H), 5.45–5.57 (m, 2H), 4.18–4.22 (m, 2H), 4.10 (s, 1H), 3.74 (dd, J = 3.2, 11.7 Hz, 1H), 3.66 (d, J = 1.4, 11.7 Hz, 1H), 3.58 (dq, J = 6.3, 6.3 Hz, 1H), 3.35–3.43 (m, 5H), 2.18–2.26 (m, 2H), 2.08–2.12 (m, 1H), 2.02–2.08 (m, 1H), 1.96 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H), 1.12–1.28 (m, 9H), 1.02 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 146.3, 134.6, 129.0, 119.2, 108.5, 77.6, 75.7, 74.6, 71.0, 70.5, 66.5, 44.3, 42.4, 39.4, 36.6, 34.3, 28.3, 26.3, 20.4, 16.5, 15.4, 14.3, 13.1; HRMS (ESI, m/z) calcd for $C_{24}H_{42}NO_6$ 440.30066, found 440.30033.

4.2.10. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid butylamide (5). Prepared as described for compound **1**. $[\alpha]_D^{23}$ 14.37 (*c* 0.16, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3346, 1667, 1634; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.98 (s, 1H), 5.95 (br s, 1H), 5.40–5.52 (m, 2H), 4.26 (s, 1H), 3.96 (s, 1H), 3.82–3.88 (m, 2H), 3.67 (d, J = 8.9 Hz, 1H), 3.52–3.58 (m, 2H), 3.25–3.32 (m, 2H), 2.02–2.12 (m, 6H), 1.82–1.88 (m, 1H), 1.46–1.53 (m, 2H), 1.32–1.40 (m, 2H), 1.16 (d, J = 6.1 Hz, 3H), 1.00 (d, J = 6.7 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 151.2, 134.6, 128.8, 118.7, 76.2, 73.3, 71.3, 70.7, 65.0, 64.8, 44.4, 41.5, 39.2, 32.3, 31.6, 20.7, 20.2, 16.9, 15.5, 13.8; HRMS (ESI, m/z) calcd for $C_{21}H_{38}NO_6$ 400.26936, found 400.26975.

4.2.11. (4*R*)-{(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[(4*R*,5*S*)-4-methyl-5-hydroxy-hex-2-enyl]-tetrahydro-pyran-2-yl}-3-methyl-4-hydroxy-but-2*E*-enoic acid diethylamide (6). Prepared as described for compound **1**. $[\alpha]_D^{23}$ 25.39 (*c* 0.48, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3391, 1653, 1600; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H), 5.39–5.53 (m, 2H), 4.25 (s, 1H), 3.95 (s, 1H), 3.86 (dd, J = 2.5, 11.4 Hz, 1H), 3.83 (dd, J = 3.1, 9.6 Hz, 1H), 3.65 (dd, J = 2.8, 9.3 Hz, 1H), 3.52–3.58 (m, 2H), 3.36–3.50 (m, 5H), 2.06–2.26 (m, 3H), 1.86–1.91 (m, 4H), 1.12–1.19 (m, 9H), 0.99 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 146.1, 134.3, 129.3, 119.0, 76.2, 73.3, 71.1, 70.6, 65.3, 64.9, 44.5, 42.8, 41.4, 39.5, 32.5, 20.4, 16.6, 15.7, 14.2, 13.0; HRMS (ESI, m/z) calcd for $C_{21}H_{38}NO_6$ 400.26936, found 400.26975.

4.2.12. (*R*,2*E*)-ethyl 4-((2*R*,6*S*)-6-ethoxy-5,6-dihydro-2*H*-pyran-2-yl)-4-hydroxy-3-methylbut-2-enoate (28). In a flame-dried round-bottomed flask was placed a mixture of 3-boronoacrolein pinacolate **11** (1.07 g, 5.88 mmol) (freshly distilled by Kugelrohr) and ethoxyethene **21** (2 mL, 24.7 mmol). To this solution were added the Jacobsen catalyst (1*S*,2*R*) **13** (100 mg, 0.21 mmol) and barium oxide powder (2.4 g). The reaction mixture was allowed to stir at 20 °C overnight and then was directly purified to removed the catalyst through a short chromatography column (Et_3N deactivated silica-gel, hexanes/ether 9:1). The desired compound **23** (1.28 g) was obtained and was directly used in the next reaction.

A mixture of hetero Diels–Alder cycloadduct (1.28 g, 5.00 mmol) and the aldehyde **25** (2.0 g, 14.1 mmol) was stirred at 50 °C for 5 h in a sealed tube under nitrogen. Upon completion, the mixture was cooled to ambient temperature and a saturated aqueous $NaHCO_3$ solution (10 mL) was added to the reaction mixture, which was stirred for 30 min. The resulting mixture was extracted with ether. The ethereal layers were combined, washed with brine and then dried over anhydrous $MgSO_4$. After filtration and concentration in vacuo, the residue was purified by flash column chromatography (Et_3N deactivated silica-gel, hexane/EtOAc, 1:1) to yield 1.1 g (81%) of the title compound **28** as a colorless oil. $[\alpha]_D^{25}$ +65.95 (*c* 2.42, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3444, 1714, 1654, 1377, 1215, 1149, 1061; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.62 (dq, J = 2.0, 10.2 Hz, 1H), 4.76 (dd, J = 3.6, 6.0 Hz, 1H), 4.44 (m, 1H), 4.16 (q, J = 7.2 Hz, 2H), 4.01 (t, J = 5.4 Hz, 1H), 3.90 (dq, J = 7.2, 9.6 Hz, 1H), 3.52 (dq, J = 7.2, 9.4 Hz, 1H), 3.15 (d, J = 6.4 Hz, 1H), 2.22 (m, 5H), 1.28 (t, J = 7.0 Hz, 3H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7,

156.6, 125.9, 125.1, 117.4, 97.9, 78.1, 74.9, 64.7, 59.8, 30.6, 15.3, 15.1, 14.3; HRMS (EI, *m*/*z*) calcd for $C_{14}H_{21}O_5$ 269.13889, found 269.13890.

4.2.13. (*R*,2*E*)-ethyl 4-((2*R*,5*R*,6*S*)–6-ethoxy-5-hexyl-5,6-dihydro-2*H*-pyran-2-yl)-4-hydroxy-3-methylbut-2-enoate (29). Prepared as described for compound **28**. $[\alpha]_D^{25}$ +4.25 (*c* 2.62, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3418, 2927, 1716, 1214, 1150, 1044; ^1H NMR (500 MHz, $CDCl_3$) δ 6.05 (t, *J* = 2.0 Hz, 1H), 5.83 (dt, *J* = 3.0, 10.5 Hz, 1H), 5.62 (dt, *J* = 2.0, 10.5 Hz, 1H), 4.72 (d, *J* = 3.5 Hz, 1H), 4.50 (m, 1H), 4.16 (dq, *J* = 1.5, 7.0 Hz, 2H), 3.97 (t, *J* = 5.3 Hz, 1H), 3.90 (dq, *J* = 7.0, 9.5 Hz, 1H), 3.48 (m, 2H), 2.24 (m, 1H), 2.18 (d, *J* = 1.5 Hz, 2H), 1.57 (m, 1H), 1.28 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 157.1, 129.7, 124.9, 116.9, 98.9, 77.9, 74.3, 65.3, 59.7, 38.1, 31.8, 29.9, 29.5, 26.9, 22.7, 15.4, 14.9, 14.4, 14.1; HRMS (ESI, *m*/*z*) calcd for $C_{20}H_{34}O_5Na$ 377.22985, found 377.22963.

4.2.14. 3-((*R*)-1-((2*R*,6*S*)-6-ethoxy-5,6-dihydro-2*H*-pyran-2-yl)-1-hydroxypropan-2-ylidene)-dihydrofuran-2(3*H*)-one (30) and (31). Prepared as described for compound **28** from a mixture of **26** and **27** and obtained as an inseparable mixture. IR ($CHCl_3$, cast, cm^{-1}) 3460, 1740, 1377, 1180, 1060, 1025; ^1H NMR (400 MHz, $CDCl_3$) δ 5.70 (m, 2H), 4.78 (m, 1H), 4.36 (m, 3H), 3.96 (m, 1H), 3.59 (m, 1H), 3.04 (m, 2H), 2.32 (t, *J* = 2.4, Hz, *trans* Me), 2.26 (m, *2*H), 2.03 (t, *J* = 1.8, Hz, *cis* Me), 1.27 (m, 5H).

4.2.15. (*R*,2*E*)-ethyl 4-((2*R*,6*S*)-6-ethoxy-5,6-dihydro-2*H*-pyran-2-yl)-4-triisopropylsilyloxy-3-methylbut-2-enoate (28-*O*-TIPS). To a solution of alcohol **28** (1.00 g, 3.70 mmol) and 2,6-lutidine (1.19 g, 11.10 mmol) in dichloromethane (20 mL) at 0 °C was added triisopropylsilyl trifluoromethanesulfonate (2.27 g, 7.40 mmol). The reaction mixture was allowed to slowly warm to room temperature and was left to react overnight. An aqueous saturated $NaHCO_3$ solution (10 mL) was added and the reaction mixture was stirred for another 30 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2× 100 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (Et_3N deactivated silica-gel, pentane/ether, 9:1) to yield 1.5 g of the title compound **28**-*O*-TIPS (yield 95%) as colorless oil. $[\alpha]_D^{25}$ +97.78 (*c* 2.45, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2944, 2868, 1719, 1216, 1144, 1108, 1063; ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 1H), 5.72 (m, 1H), 5.64 (dq, *J* = 1.6, 10.0 Hz, 1H), 4.62 (q, *J* = 3.4 Hz, 1H), 4.38 (m, 2H), 4.16 (q, *J* = 7.0 Hz, 2H), 3.95 (dq, *J* = 7.2, 9.6 Hz, 1H), 3.54 (dq, *J* = 7.2, 9.6 Hz, 1H), 2.15 (d, *J* = 1.2 Hz, 3H), 2.13 (m, 2H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.07 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 158.2, 125.9, 124.6, 117.2, 99.0, 78.6, 63.9, 59.6, 31.3, 18.0, 17.8, 16.5, 15.3, 14.4, 12.4; HRMS (EI, *m*/*z*) calcd for $C_{23}H_{42}O_5Si$ 426.28015, found 426.28069.

4.2.16. (*R*,2*E*)-ethyl 4-((2*R*,5*R*,6*S*)-6-ethoxy-5-hexyl-5,6-dihydro-2*H*-pyran-2-yl)-4-triisopropylsilyloxy-3-methylbut-2-enoate (29-*O*-TIPS). Prepared as described for compound **28**-*O*-TIPS. $[\alpha]_D^{25}$ +38.78 (*c* 1.47, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2944, 2868, 1720, 1216, 1149, 1112, 1047; ^1H NMR (500 MHz, $CDCl_3$) δ 5.87 (s, 1H), 5.84 (ddd, *J* = 2.0, 6.0, 10.0 Hz, 1H), 5.64 (dt, *J* = 1.2, 10.5 Hz, 1H), 4.58 (d, *J* = 2.5 Hz, 1H), 4.34 (m, 2H), 4.15 (q, *J* = 7.0 Hz, 2H), 3.88 (dq, *J* = 7.2, 9.8 Hz, 1H), 3.49 (m, 1H), 2.15 (d, *J* = 1.5 Hz, 2H), 2.07 (m, 1H), 1.66 (m, 1H), 1.23 (m, 16H), 1.07 (m, 21H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 158.1, 130.4, 125.2, 117.5, 100.8, 78.9, 78.7, 64.1, 59.6, 39.3, 31.9, 29.8, 28.9, 27.1, 22.7, 18.0, 16.7, 15.3, 14.4, 14.1, 12.3; HRMS (EI, *m*/*z*) calcd for $C_{29}H_{54}O_5Si$ 510.37405, found 510.37434.

4.2.17. 3-((*R*)-1-((2*R*,6*S*)-6-ethoxy-5,6-dihydro-2*H*-pyran-2-yl)-1-triisopropylsilyloxypropan-2-ylidene)-dihydrofuran-2(3*H*)-one (30-*O*-TIPS) and (31-*O*-TIPS). Prepared as described for compound **28**-*O*-TIPS from a mixture of **30** and **31** and obtained as a hard to separate mixture. IR ($CHCl_3$, cast, cm^{-1}) 2943, 2867, 1749, 1376, 1181, 1105, 1063, 1042, 1028; ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 (m, 3H), 4.65 (m, 1H), 4.30 (m, 2H), 3.92 (m, 1H), 3.53 (m, 1H), 2.88 (m, 1H), 2.23 (t, *J* = 2.4, Hz, *trans* Me), 2.15 (m, *2*H), 1.96 (t, *J* = 1.8, Hz, *cis* Me), 1.27 (m, 5H), 1.06 (m, 21H); HRMS (EI, *m*/*z*) calcd for $C_{23}H_{40}O_5Si$ 424.26450, found 424.26254.

Cis compound **31**-*O*-*TIPS*: $[\alpha]_D^{25}$ +31.11 (*c* 3.22, CH_2Cl_2); IR (microscope, cm^{-1}) 2943, 2866, 1746, 1185, 1106, 1061, 1042, 1028; ^1H NMR (300 MHz, $CHCl_3$) δ 6.25 (d, *J* = 5.7 Hz, 1H), 5.75 (m, 2H), 4.62 (dd, *J* = 4.0, 8.0 Hz, 1H), 4.35 (m, 3H), 3.91 (dq, *J* = 7.2, 9.8 Hz, 1H), 3.49 (dq, *J* = 7.2, 9.5 Hz, 1H), 2.86 (t, *J* = 7.0 Hz, 2H), 2.12 (m, 3H), 1.90 (t, *J* = 1.8 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 2H), 1.06 (m, 21H); ^{13}C NMR (100 MHz, $CHCl_3$) δ 153.7, 130.6, 127.1, 124.3, 120.5, 98.7, 77.8, 69.1, 64.6, 63.9, 31.4, 28.1, 18.2, 17.9, 15.4, 12.5; HRMS (ESI, *m*/*z*) calcd for $C_{23}H_{40}O_5SiNa$ 447.25372, found 447.25328.

4.2.18. (*R*,2*E*)-ethyl 4-((*R*)-5,6-dihydro-2*H*-pyran-2-yl)-4-triiso-propylsilyloxy-3-methylbut-2-enoate (32). To a solution of pyran **28**-*O*-TIPS (0.80 g, 1.88 mmol) and triethylsilane (0.87 g, 7.50 mmol) in CH_2Cl_2 (10 mL) was added $TiCl_4$ (2.44 mL, 1.0 M solution in CH_2Cl_2, 2.44 mmol) dropwise at −50 °C. After being stirred for 2.5 h at −50 °C, the reaction mixture was allowed to warm up to ambient temperature and quenched with aqueous saturated $NaHCO_3$ solution (3 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2× 15 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (hexanes/EtOAc 7:3) to afford the title compound **32** (0.71 g, 98% yield) as a yellow oil. $[\alpha]_D^{25}$ +61.62 (*c* 2.11, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2944, 2867, 1719, 1217, 1152, 1107, 883; ^1H NMR (400 MHz, $CDCl_3$) δ 5.9 (m, 2H), 5.70 (dq, *J* = 1.6, 10.4 Hz, 1H), 4.31 (d, *J* = 5.6 Hz, 1H), 4.25 (m, 1H), 4.15 (q, *J* = 7.2 Hz, 2H), 3.92 (m, 1H), 3.63 (ddd, *J* = 4.0, 9.2, 11.0 Hz, 1H), 2.2 (m, 1H), 2.14 (d, *J* = 1.6 Hz, 3H), 1.9 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H),

1.06 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 158.5, 126.3, 126.1, 117.1, 78.8, 63.4, 59.6, 25.3, 18.0, 17.8, 16.5, 14.4, 12.4; HRMS (ESI, *m/z*) calcd for $C_{21}H_{38}O_4SiNa$ 405.24316, found 405.24318.

4.2.19. (*R*,2*E*)-ethyl 4-((2*R*,5*R*)-5-hexyl-5,6-dihydro-2*H*-pyran-2-yl)-4-triisopropylsilyloxy-3-methylbut-2-enoate (33). Prepared as described for compound **32**. $[\alpha]_D^{25}$ +27.93 (*c* 1.42, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2928, 2867, 1720, 1215, 1182, 1008; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (m, 2H), 5.69 (dt, *J* = 1.7, 10.0 Hz, 1H), 4.31 (d, *J* = 5.5 Hz, 1H), 4.22 (m, 1H), 4.16 (q, *J* = 7.0 Hz, 2H), 3.66 (m, 2H), 2.15 (d, *J* = 1.5 Hz, 2H), 1.94 (m, 1H), 1.29 (m, 14H), 1.07 (m, 21H), 0.89 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.7, 158.3, 131.0, 125.3, 117.4, 78.9, 77.5, 67.4, 59.6, 34.7, 33.3, 31.8, 29.5, 27.2, 22.7, 18.0, 16.6, 14.4, 14.1, 12.4; HRMS (EI, *m/z*) calcd for $C_{27}H_{50}O_4Si$ 466.34784, found 466.34809.

4.2.20. (3*E*)-dihydro-3-((*R*)-1-((*R*)-5,6-dihydro-2H-pyran-2-yl)-1-triisopropylsilyloxypropan-2-ylidene)furan-2(3*H*)-one (34). Prepared as described for compound **32**. $[\alpha]_D^{25}$ −1.29 (*c* 0.97, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 2944, 2867, 1753, 1375, 1183, 1101, 1068, 1038, 1014, 883; ^{1}H NMR (400 MHz, $CHCl_3$) δ 5.90 (m, 1H), 5.79 (dd, *J* = 3.8, 8.2 Hz, 1H), 4.56 (d, *J* = 6.4 Hz, 1H), 4.27 (m, 3H), 3.85 (m, 1H), 3.62 (m, 1H), 3.05 (m, 1H), 2.88 (m, 1H), 2.22 (t, *J* = 2.2 Hz, 3H), 2.17 (m, 1H), 1.94 (m, 1H), 1.07 (m, 21H); ^{13}C NMR (100 MHz, $CHCl_3$) δ 170.8, 152.7, 126.5, 126.0, 120.4, 77.5, 75.9, 64.7, 63.0, 28.0, 25.3, 18.0, 12.4, 5.9; HRMS (EI, *m/z*) calcd for $C_{21}H_{36}O_4Si$ 380.23828, found 380.23831.

4.2.21. (3*Z*)-dihydro-3-((*R*)-1-((*R*)-5,6-dihydro-2*H*-pyran-2-yl)-1-triisopropylsilyloxypropan-2-ylidene)furan-2(3*H*)-one (35). Prepared as described for compound **32**. $[\alpha]_D^{25}$ +23.08 (*c* 1.92, CH_2Cl_2); IR (microscope, cm^{-1}) 2943, 2866, 1745, 1183, 1102, 1086, 1063, 1042; ^{1}H NMR (500 MHz, $CHCl_3$) δ 6.19 (d, *J* = 5.5 Hz, 1H), 5.92 (m, 1H), 5.69 (dq, *J* = 2.0, 10.5 Hz, 1H), 4.35 (t, *J* = 7.3 Hz, 2H), 4.22 (m, 1H), 4.04 (quint, *J* = 5.5 Hz, 1H), 3.70 (quint, *J* = 5.5 Hz, 1H), 2.89 (t, *J* = 7.5 Hz, 2H), 2.09 (m, 2H), 1.96 (t, *J* = 1.5 Hz, 3H), 1.10 (m, 21H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 169.5, 154.7, 126.9, 126.4, 119.8, 77.3, 70.2, 64.7, 62.3, 27.9, 25.2, 18.0, 17.6, 12.5; HRMS (ESI, *m/z*) calcd for $C_{21}H_{36}O_4SiNa$ 403.22751, found 403.22818.

4.2.22. (*R*,*E*)-ethyl 4-((2*S*,3*R*,4*R*)-tetrahydro-3,4-dihydroxy-2*H*-pyran-2-yl)-4-triisopropylsilyloxy-3-methylbut-2-enoate (dihydroxy-32). The pyran **32** (0.50 g, 1.31 mmol) was dissolved in acetone/water (60 mL, 9:1). Then, the monohydrate of *N*-methylmorpholine *N*-oxide (0.23 g, 1.96 mmol) and osmium tetroxide (2.5 wt% solution in 2-methyl-2-propanol, 0.7 mL) were added and the mixture was stirred at ambient temperature overnight. It was then diluted with aqueous sodium sulfite (50 mL) and extracted with EtOAc (2× 200 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (EtOAc 100%) to afford the title compound dihydroxy-**32** (0.50 g, 91%) as a colorless oil. $[\alpha]_D^{25}$ +14.44 (*c* 1.08, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3486, 2945, 2869, 1718, 1216, 1155, 1096, 883; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (s, 1H), 4.55 (d, *J* = 4.4 Hz, 1H), 4.18 (dq, *J* = 1.2, 7.2 Hz, 2H), 4.03 (q, *J* = 3.2 Hz, 1H), 3.99 (s, 1H), 3.83 (dd, *J* = 4.0, 9.6 Hz, 1H), 3.75 (dt, *J* = 2.8, 11.6 Hz, 1H), 3.66 (ddd, *J* = 1.4, 5.4, 11.2 Hz, 1H), 3.57, (dd, *J* = 3.0, 9.9 Hz, 1H), 2.68 (d, *J* = 1.6 Hz, 1H), 2.21 (d, *J* = 1.2 Hz, 3H), 1.80 (m, 1H), 1.74 (m, 1H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.15 (m, 3H), 1.06 (t, *J* = 6.6 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 157.1, 117.6, 78.8, 76.0, 69.7, 66.3, 62.5, 60.0, 30.7, 18.2, 18.0, 14.5, 12.3; HRMS (EI, *m/z*) calcd for $C_{21}H_{40}O_6Si$ 426.25942, found 426.25026.

4.2.23. (*R*,*E*)-ethyl 4-((2*S*,3*R*,4*R*,5*S*)-5-hexyl-tetrahydro-3,4-di-hydroxy-2*H*-pyran-2-yl)-4-triisopropylsilyloxy-3-me-thylbut-2-enoate (dihydroxy-33). Prepared as described for compound dihydroxy-**32**. $[\alpha]_D^{25}$ +37.19 (*c* 0.63, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3485, 2929, 2868, 1719, 1215, 1154, 1094, 1047; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (t, *J* = 1.2 Hz, 1H), 4.55 (d, *J* = 4.0 Hz, 1H), 4.15 (m, 3H), 3.84 (s, 1H), 3.81 (m, 1H), 3.73 (s, 1H), 3.66 (dd, *J* = 3.2, 10.0 Hz, 1H), 3.54 (d, *J* = 11.6 Hz, 1H), 2.72 (s, 1H), 2.21 (d, *J* = 1.2 Hz, 2H), 1.72 (m, 1H), 1.27 (m, 14H), 1.09 (m, 21H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 157.1, 117.2, 78.5, 70.2, 66.7, 65.6, 59.8, 40.3, 31.7, 29.3, 28.7, 27.6, 22.6, 18.04, 19.95, 17.5, 14.3, 14.1, 12.1; HRMS (ESI, *m/z*) calcd for $C_{27}H_{52}O_6SiNa$ 523.34254, found 523.34233.

4.2.24. (3*E*)-dihydro-3-((*R*)-1-((2*S*,3*R*,4*R*)-tetrahydro-3,4-dihydroxy-2*H*-pyran-2-yl)-1-triisopropylsilyloxy-propan-2-ylidene)furan-2(3*H*)-one (dihydroxy-34). Prepared as described for compound dihydroxy-**32**. $[\alpha]_D^{25}$ −6.90 (*c* 1.15, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3461, 2945, 2868, 1732, 1184, 1099, 1058, 1038, 1015; ^{1}H NMR (500 MHz, $CHCl_3$) δ 4.84 (d, *J* = 4.5 Hz, 1H), 4.28 (m, 2H), 4.18 (s, 1H), 4.07 (q, *J* = 3.0 Hz, 1H), 3.89 (dd, *J* = 4.5, 10.0 Hz, 1H), 3.76 (dt, *J* = 3.0, 11.7 Hz, 1H), 3.63 (m, 2H), 3.14 (m, 1H), 2.82 (m, 1H), 2.64 (d, *J* = 1.0 Hz, 1H), 2.39 (t, *J* = 2.2 Hz, 3H), 1.79 (m, 2H), 1.13 (m, 21H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 170.6, 149.9, 122.7, 76.8, 76.1, 70.3, 66.2, 64.8, 62.3, 32.7, 30.5, 28.1, 17.9, 12.1; HRMS (ESI, *m/z*) calcd for $C_{21}H_{38}O_6SiNa$ 437.23299, found 437.23286.

4.2.25. (*R*,*E*)-ethyl 4-((2*S*,3*R*,4*R*)-tetrahydro-3,4-dihydroxy-2*H*-pyran-2-yl)-4-hydroxy-3-methylbut-2-enoate (7). Prepared as described for compound **1**. $[\alpha]_D^{25}$ −14.07 (*c* 0.63, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3421, 2929, 2878, 1703, 1218, 1157, 1137, 1112, 1078, 1046, 757; ^{1}H NMR (400 MHz, acetone-d_6) δ 6.02 (s, 1H), 4.32 (d, *J* = 8.0 Hz, 1H), 4.09 (q, *J* = 7.2 Hz, 2H), 4.07 (m, 1H), 3.86 (m, 1H), 3.76 (d, *J* = 8.4 Hz, 1H), 3.69 (m, 3H), 3.55 (ddd, *J* = 2.0, 4.9, 10.9 Hz, 1H), 2.79 (s, 1H), 2.11 (s, 3H), 1.73 (m, 2H), 1.22 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ 167.1, 161.0, 115.7, 76.8, 74.2, 68.2, 67.8, 62.3, 59.7, 33.2, 16.1, 14.6; HRMS (ESI, *m/z*) calcd for $C_{12}H_{20}O_6Na$ 283.11521, found 283.11520.

4.2.26. (3*E*)-dihydro-3-((*R*)-1-((2*S*,3*R*,4*R*)-tetrahydro-3,4-dihydroxy-2*H*-pyran-2-yl)-1-hydroxypropan-2-ylidene)-furan-2(3*H*)-one (8). To the solution of the TIPS protected alcohol dihydroxy-**34** (0.04 g, 0,096 mmol) in THF (8 mL) were added acetic acid (0.01 g, 0.16 mmol) and TBAF (1 M solution in THF, 0.096 mL, 0.096 mmol). The reaction mixture was stirred at rt for 20 h. Upon completion, the solvent was evaporated in vacuo and the residue was used directly purified by flash chromatography (EtOAc/MeOH, 9:1) to yield the title compound **8** as a white solid (25 mg, 100% yield). $[\alpha]_D^{25}$ −60.70 (*c* 0.42, MeOH); IR ($CHCl_3$, cast, cm^{-1}) 3310, 1723, 1181, 1071, 1020; ^1H NMR (500 MHz, CD_3OD) δ 4.60 (s, 1H), 4.30 (m, 2H), 4.08 (m, 1H), 3.68 (m, 3H), 3.55 (dd, J = 2.0, 9.5 Hz, 1H), 2.97 (m, 2H), 2.26 (t, J = 2.3 Hz, 3H), 1.85 (tdd, J = 2.5, 5.5, 12.0 Hz, 1H), 1.72 (dq, J = 1.7, 14.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.5, 157.7, 119.3, 79.1, 72.1, 68.43, 68.39, 66.4, 62.8, 33.6, 28.0, 14.5; HRMS (ESI, *m*/*z*) calcd for $C_{12}H_{18}O_6Na$ 281.09956, found 281.09970.

4.2.27. (3*Z*)-dihydro-3-((*R*)-1-((2*S*,3*R*,4*R*)-tetrahydro-3,4-dihydroxy-2*H*-pyran-2-yl)-1-hydroxypropan-2-ylidene)-furan-2(3*H*)-one (9). The dihydroxy pyran was prepared as described in the synthesis of dihydroxy-**32** and was directly in the next reaction. The TIPS deprotection was achieved as described for compound **8**. $[\alpha]_D^{25}$ −6.90 (*c* 1.15, $CHCl_3$); IR ($CHCl_3$, cast, cm^{-1}) 3315, 2974, 2918, 2880, 1723, 1650, 1182, 1071, 1022, 921; ^1H NMR (400 MHz, acetone-d_6) δ 5.78 (s, 1H), 4.32 (dt, J = 1.6, 4.5 Hz, 2H), 4.10 (m, 1H), 3.89 (d, J = 8.4 Hz, 1H), 3.72 (m, 2H), 3.55 (dd, J = 2.0, 9.5 Hz, 1H), 3.45 (m, 2H), 1.93 (t, J = 2.3 Hz, 3H), 1.85 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100 MHz, acetone-d_6) δ 170.6, 157.2, 120.0, 80.4, 68.4, 67.9, 67.6, 65.7, 62.2, 33.0, 29.4, 18.4; HRMS (EI, *m*/*z*) calcd for $C_{12}H_{14}O_4$ 222.08920, found 222.08933.

4.2.28. (*R*,*E*)-ethyl 4-((2*S*,3*R*,4*R*,5*S*)-5-hexyl-tetrahydro-3,4-di-hydroxy-2*H*-pyran-2-yl)-4-hydroxy-3-methylbut-2-enoate (10). Prepared as described for compound **1**. $[\alpha]_D^{25}$ −4.82 (*c* 0.99, MeOH); IR ($CHCl_3$, cast, cm^{-1}) 3496, 3365, 2926, 2896, 1717, 1214, 1155, 1045; ^1H NMR (400 MHz, $CDCl_3$) δ 6.01 (t, J = 1.4 Hz, 1H), 4.28 (d, J = 9.2 Hz, 1H), 4.16 (q, J = 7.2 Hz, 2H), 3.97 (m, 1H), 3.85 (m, 2H), 3.64 (d, J = 9.6 Hz, 1H), 3.57 (d, J = 11.2 Hz, 1H), 2.52 (d, J = 9.2 Hz, 1H), 2.24 (m, 2H), 1.73 (m, 1H), 1.28 (m, 16H); 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 158.3, 115.9, 75.8, 74.1, 71.3, 65.1, 65.0, 59.8, 41.8, 31.8, 29.3, 28.7, 27.7, 22.7, 15.9, 14.4, 14.1; HRMS (ESI, *m*/*z*) calcd for $C_{18}H_{32}O_6Na$ 367.20911, found 367.20962.

4.3. Bioassay[24]

Staphylococcus aureus strains were ATCC#25923 and ATCC#6538. Mueller–Hinton broth (Difco#275730) was used at 2.1% (w/v) and agar (Difco#214010) was used at 1.7%. Blank 6 mm sterile paper disks were purchased from BDH. Disk diffusion tests were performed following the Clinical and Laboratory Standards Institute guidelines (formerly NCCLS; publication M2-A8). *S. aureus* was grown on trypticase soy agar. Five fresh colonies were inoculated into 4 ml trypticase soy broth and grown about five hours, standing, at 35 °C. Cultures were adjusted to A_{625} of 0.1 (0.5 McFarland's Standard) and streaked on Mueller–Hinton agar plates using a sterile cotton swab. All compounds were dissolved in 25% methanol in water. Disks were loaded with compounds in 15 µl of the appropriate solvents as indicated and placed on the agar plates. Plates were incubated for about 17 h at 35 °C and zones of inhibition were recorded. All plates were done in duplicate for each *S. aureus* strain. One representative set of data is shown.

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Supplementary data

Procedure for the preparation of **22**, **26**, and **27**: structures of additional synthetic intermediates; ^1H and ^{13}C NMR spectra for compounds **1**–**10** and crystallographic data for compound **8** (CCDC #668593) are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.01.001.

References and notes

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