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Divergent Synthesis and Chemical Reactivity of Bicyclic Lactone Fragments of Complex Rearranged Spongian Diterpenes

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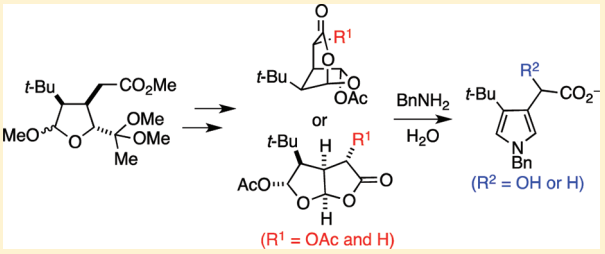
Supporting Information

ABSTRACT: The synthesis and direct comparison of the chemical reactivity of the two highly oxidized bicyclic lactone fragments found in rearranged spongian diterpenes (8-substituted 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one and 6-substituted 7-acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-one) are reported. Details of the first synthesis of the 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one ring system, including an examination of several possibilities for the key bridging cyclization reaction, are described. In addition, the first synthesis of 7-acetoxy-2,8-dioxabicyclo[3.3.0]octanones containing quaternary carbon substituents at C6 is disclosed. Aspects of the chemical reactivity and Golgi-modifying properties of these bicyclic lactone analogs of rearranged spongian diterpenes are also reported. Under both acidic and basic conditions, 8-substituted 2,7-dioxabicyclo[3.2.1]octanones are converted to 6-substituted-2,8-dioxabicyclo[3.3.0]octanones. Moreover, these dioxabicyclic lactones react with primary amines and lysine side chains of lysozyme to form substituted pyrroles, a conjugation that could be responsible for the unique biological properties of these compounds. These studies demonstrate that acetoxylation adjacent to the lactone carbonyl group, in either the bridged or fused series, is required to produce fragmented Golgi membranes in the pericentriolar region that is characteristic of macfarlandin E.



INTRODUCTION

Skeletal rearrangement and oxidation of spongian diterpene precursors of general structure **1** (Figure 1A) provides a structurally diverse group of marine-derived natural products referred to as rearranged spongian diterpenes.[1] These natural products are isolated from sponges and dorid nudibranchs, the latter of which are believed to acquire these diterpenes from sponge sources as a chemical defense mechanism.[1] Among the most structurally complex of the rearranged spongian diterpenes is a structurally unique group that contains a polycyclic hydrocarbon fragment joined to an oxidized lactone unit (Figure 1). The biological activity of this group of spongian-derived diterpenes has been characterized to only a limited extent. Antimicrobial,[2−4] cytotoxic,[4] and nematocidal[4] activities have been reported, and norrisolide (**9**) has been shown to induce irreversible fragmentation and delocalization of Golgi membranes throughout the cytosol in human cell lines.[5] In addition, we reported in 2010 that macfarlandin E (**4**, MacE) and a simplified analog **13** (*t*-Bu-MacE, see eq 1) induce a novel Golgi organization phenotype that is characterized by small, pericentriolar Golgi fragments and blockage of protein transport from the Golgi to the plasma membrane.[6]

A central feature of the group of rearranged spongian natural products depicted in Figure 1 is the presence of highly oxygenated and hydrophobic subunits. The oxygenated fragment is structurally diverse and includes monocyclic variants, such as that found in shahamin K (**2**),[7] and more complex dioxabicyclic lactone fragments. The 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one subunit is particularly rare; at the onset of our studies, it was known only in the diterpene macfarlandin E (**4**)[2,8] and 13 additional rearranged spongian diterpenes such as aplyviolene (**3**),[8] chromodorolide A (**5**),[4a] shahamin I (**6**),[9] and norrlandin (**7**)[10] (Figure 1B). The isomeric 7-acetoxy(or hydroxy)-2,8-dioxabicyclo[3.3.0]octan-3-one fragment is somewhat more abundant in rearranged spongian diterpenes,[11] as exemplified by dendrillolide A (**8**),[12] norrisolide (**9**),[13] cheloviolenes A (**10**) and B (**11**),[14] and omriolide A (**12**)[15] (Figure 1C). The substituted dioxabicyclo[3.3.0]octanone ring system of these diterpenes has been the subject of limited synthetic efforts,[16] highlighted by two total syntheses of norrisolide.[17,18]

Last year we disclosed the first synthesis of the 4,6-diacetoxy-2,7-dioxabicylo[3.2.1]octan-3-one moiety of MacE and evidence obtained by evaluation of related diterpenes and various analogs that the entire oxygenated subunit—including both acetoxy substituents—is required to elicit the MacE Golgi phenotype.[6] Additionally, we showed that *t*-Bu-MacE (**13**) and its deacetoxy congener **14** are converted to substituted pyrroles such as **15** and **16** in the presence of primary amines under mild conditions

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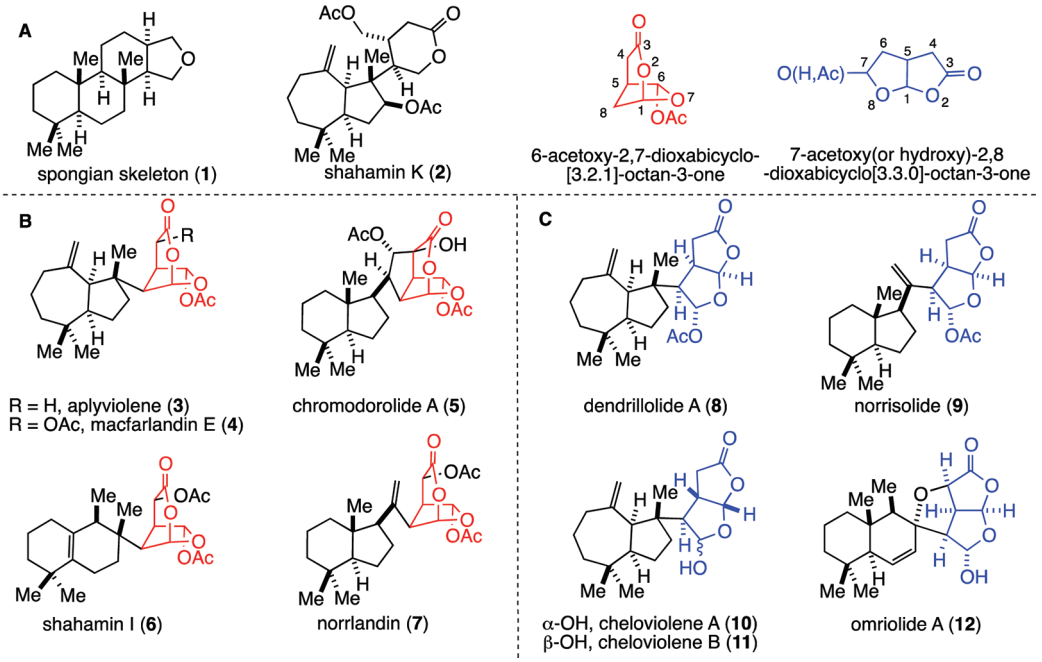
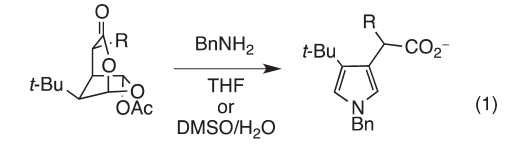


Figure 1. Representative rearranged spongian diterpene natural products containing lactone fragments.

(eq 1). This latter finding suggests a functional role for the oxygenated ring system of MacE, formation of a protein-bound pyrrole species, which could be responsible for the unique biological properties of these compounds.

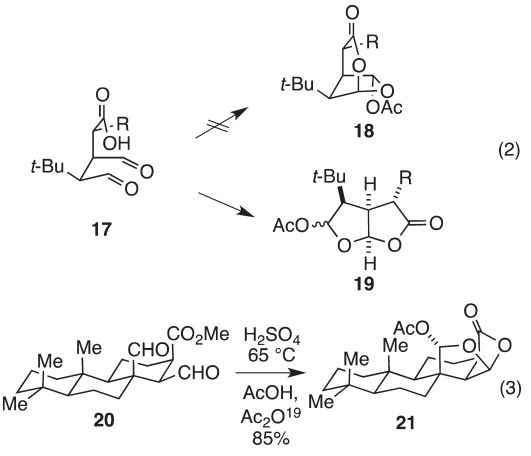


Although both MacE and norrisolide have pronounced effects on the structure and function of the Golgi, their phenotypes are different: MacE causes the conversion of the Golgi ribbon into membrane fragments that remain in the pericentriolar region, whereas norrisolide induces fragmentation and dispersal of Golgi membranes throughout the cytoplasm.[5,6] In addition, the structure−activity relationships reported to date for these two rearranged spongian natural products are quite distinct: the hydrophobic fragment is suggested to be essential for norrisolide's activity,[5] whereas the full 4,6-diacetoxy-2,7-dioxabicylo[3.2.1]-octan-3-one subunit, and not the hydroazulene fragment, is believed to be essential for eliciting the MacE-Golgi phenotype.[6]

This article reports the synthesis of simplified analogs of the oxygenated subunits of MacE (**4**) and dendrillolide A (**8**) and a survey of their chemical reactivity and Golgi-modifying properties. We find that both 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-ones and 7-acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-ones react under mild conditions with benzylamine or lysine side chains of lysozyme to form substituted pyrrole products. Moreover, we show that the presence of acetoxy substitution adjacent to the lactone carbonyl in either the bridged or fused dioxabicyclooctanone ring system induces a nearly identical Golgi organization phenotype and greater reactivity with lysine side chains.

RESULTS AND DISCUSSION

Enantioselective Synthesis of *t*-Bu-MacE. As an appropriate initial target for developing a chemical synthesis of the 4,6-diacetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one ring system, we chose *t*-Bu-MacE (**13**) which possesses a *tert*-butyl group in place of the hydroazulene subunit of MacE. Our synthetic approach to *t*-Bu-MacE was based on the prospect that kinetically controlled cyclization of dialdehyde acid **17** would not form the desired dioxabicyclo[3.2.1]octanone but rather the 2,8-dioxabicyclo[3.3.0]octan-3-one isomer **19** (eq 2). Moreover, we anticipated, and later established experimentally (see below), that the fused isomer also would be favored thermodynamically. Bolstering our expectation that direct cyclization of **17** was unlikely to generate the 2,7-dioxabicyclo[3.2.1]octan-3-one ring system is the conversion outlined in eq 3. In this example, even the enforced 1,3-diaxial proximity of the carboxyl nucleophile and the distal aldehyde of precursor **20** did not result in forming the bridged dioxabicyclo product, but rather fused isomer **21**.[19]



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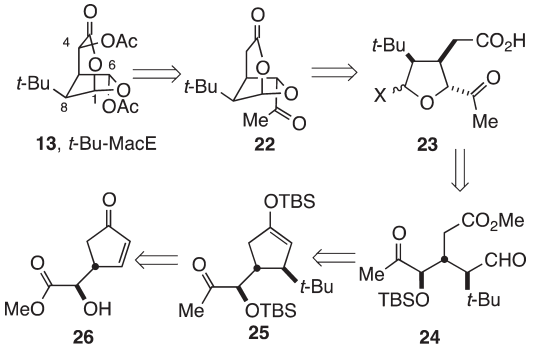
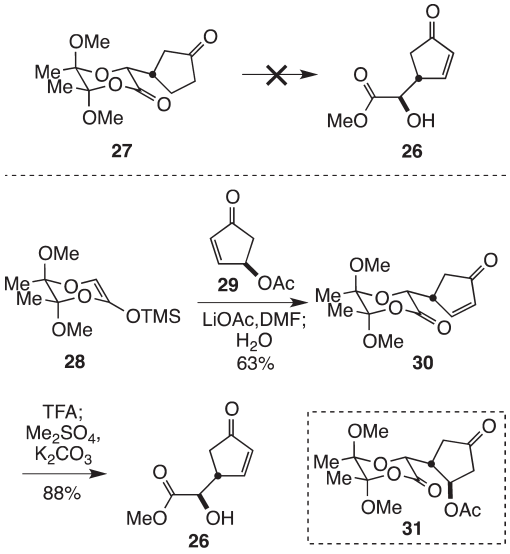
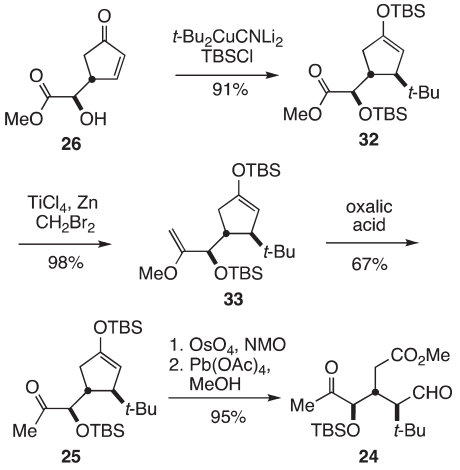
Scheme 1. Retrosynthetic Analysis of *t*-Bu-MacE



Scheme 2. Synthesis of Enantiopure Cyclopentenone 26



Scheme 3. Synthesis of Tricarbonyl Intermediate 24

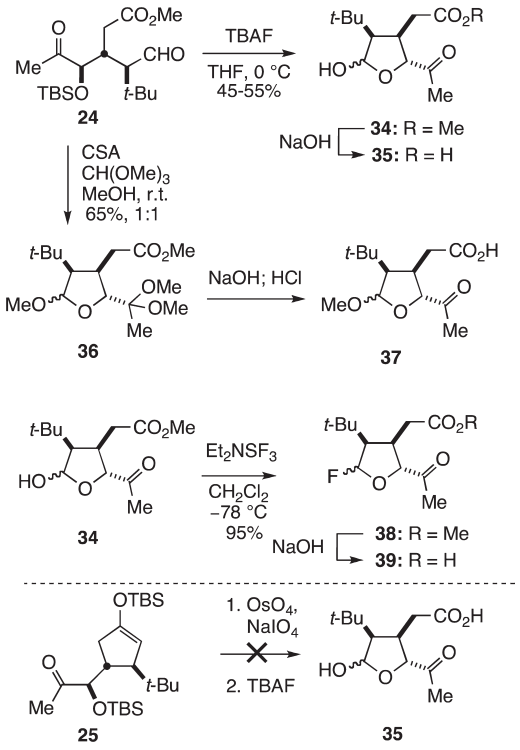


The analysis that guided our initial synthesis of *t*-Bu-MacE (**13**) is summarized in Scheme 1. Late stage Baeyer–Villiger oxidation was anticipated to form the C6-acetoxy substituent from an acetyl precursor.[17,20] It was anticipated initially that the C4 acetate could be installed at a late stage as well. Bridged-bicyclic intermediate **22** was seen arising from the cyclic acetal **23**, wherein X is a leaving group. Acyclic tricarbonyl intermediate **24** was seen resulting from oxidative cleavage of cyclopentyloxysilane **25**, which in turn would arise from facial-selective conjugate addition of a *tert*-butylcuprate to cyclopentenone **26** in the presence of a silyl electrophile.

Guided by these considerations, we initiated the synthesis of *t*-Bu-MacE by targeting cyclopentenone **26**. Attempts to access enone **26** from known cyclopentanone **27**[21] were unsuccessful, as regioselective installation of the double bond proved problematic under a variety of conditions (Scheme 2).[22] Consequently, enantiopure cyclopentenone **29**, which is readily available in four steps from cyclopentadiene, was used as the Michael acceptor.[23] The union of silyl ketene acetal **28**[24] and enone **29** to afford cyclopentenone **30** was realized in good yield in dry DMF in the presence of lithium acetate.[25] Earlier attempts to promote this reaction with Lewis acids ($SnCl_4$ or $TiCl_4$ in CH_2Cl_2) or tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) were less successful. After consumption of the starting materials at 0 °C, addition of a slight molar excess of water to the reaction mixture and allowing the reaction to warm to room temperature prior to aqueous workup delivered the enone product in reproducibly good yield. If this step was omitted, conjugate addition product **31** was obtained in 30–60% yield.[26] We speculate that the trimethylsilyl group is only partially transferred in the initial 1,4-addition; addition of water and warming to room temperature is believed to transform any enoxysilane intermediate to the corresponding ketone, allowing enolate equilibration and β-elimination to take place. Cyclopentenone **26** was then obtained in high yield by cleavage of the butane diacetal group with aqueous trifluoroacetic acid and methylation of the resulting carboxylic acid.[27]

In five steps, enone **26** was elaborated in good overall yield to tricarbonyl intermediate **24** (Scheme 3). The *tert*-butyl substituent was first incorporated by stereoselective addition of the cuprate reagent generated from *tert*-butyllithium and CuCN (2:1 molar ratio) in the presence of *tert*-butyldimethylsilyl chloride (TBSCl) to provide exclusively trans-substituted cyclopentenyl silyl ether **32**.[28] At this stage, we needed to transform the methyl ester to an acetyl group without cleaving the enoxysilane. A two-step sequence, proceeding via methyl enol ether intermediate **33**, was eventually developed. Although methylenation of **32** with the Tebbe reagent[29] proceeded sluggishly, resulting in incomplete conversion, the Takai methylenation conditions developed by Rainer and co-workers produced methyl enol ether **33** in nearly quantitative yield.[30] After examining several conditions for hydrolyzing the methyl enol ether, the use of 1.5 equiv of oxalic acid in aqueous *i*-PrOH at 0 °C was identified as particularly effective in achieving the conversion to methyl ketone **25** with minimal cleavage of the enoxysilane. We ascribe this rare, if not unprecedented, selective acidic cleavage of a methyl enol ether in the presence of a silyl enol ether to steric shielding of protonation of the cyclopentenyl double bond by the two bulky trans-oriented substituents.[31] In our initial efforts, we cleaved the double bond of **25** by reaction with OsO_4 and $NaIO_4$, followed by addition of trimethylsilyldiazomethane[32] to the crude product mixture to provide tricarbonyl product **24** in useful, albeit variable, yields (55–84%).[6] To improve the reproducibility of this conversion and avoid the undesirable use of $TMSCHN_2$, an

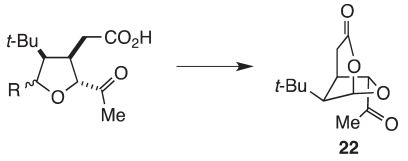
Scheme 4. Formation of Tetrahydrofuryl Cyclization Precursors



alternate sequence was developed. In this improved procedure, enoxysilane **25** was oxidized with OsO_4 (0.05 equiv) and *N*-methylmorpholine-*N*-oxide (NMO, 2.0 equiv), followed by cleavage of the resulting α-hydroxyketone with 1.3 equiv of methanolic $Pb(OAc)_4$, a sequence that reproducibly delivered tricarbonyl intermediate **24** in 95% yield.

As a prelude to examining the pivotal bridging reaction, acyclic intermediate **24** was transformed to several potential tetrahydrofuryl cyclization precursors (Scheme 4). Cleavage of the silyl ether by reaction of **24** with 1.5 equiv of TBAF provided tetrahydrofuryl lactol **34** in moderate yield, which upon careful saponification with 1.5 equiv of NaOH at 0 °C gave the crude carboxylic acid **35** in sufficient purity for subsequent cyclization studies. Attempts to access intermediate **35** more expeditiously from cyclopentenyl precursor **25** by sequential reaction with $OsO_4/NaIO_4$ and TBAF were unsuccessful, providing only complex mixtures of products. Reaction of tricarbonyl intermediate **24** with 1.6 equiv of camphorsulfonic acid (CSA) and trimethylorthoformate in methanol at room temperature generated diacetal **36** as a 1:1 mixture of separable methoxy anomers in 65% yield. The α epimer of **36** could be converted to the β epimer by equilibration in methanol in the presence of camphorsulfonic acid, allowing the β-**36** to be obtained in 66% overall yield from acyclic precursor **24** after two recycles. Subsequent saponification of the ester and selective cleavage of the dimethyl acetal with aqueous HCl at 0 °C yielded crude tetrahydrofuryl ketoacid **37**. As glycosyl fluorides are used extensively as glycosyl donors because of their stability and the mild, orthogonal methods available for their activation,[33] hemiacetal **34** was transformed to anomeric fluoride **38** by reaction with 1.6 equiv of diethylaminosulfur trifluoride (DAST) at

Table 1. Cyclization Reactions To Form 22



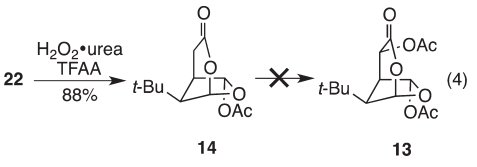
Entry	R	Conditions	Yield **22**[a]
1	OH (**35**)	DEAD, PPh_3, CH_2Cl_2	0%
2	OH (**35**)	0.2 equiv of CSA, $CHCl_3$, rt, 12 h	54%
3	OMe (**37**)	1.2 equiv of BF_3•OEt_2, CH_2Cl_2, 0 °C, 1 h	66%
4	F (**39**)	2 equiv of $SnCl_2$, DMF, rt, 18 h	71%

[a] Yield reported as conversion from methyl ester precursor (**34**, **36**, and **38**, respectively).

−78 °C in CH_2Cl_2. Saponification of this product with 1.5 equiv of 1 N NaOH in MeOH at room temperature provided carboxylic acid **39**.

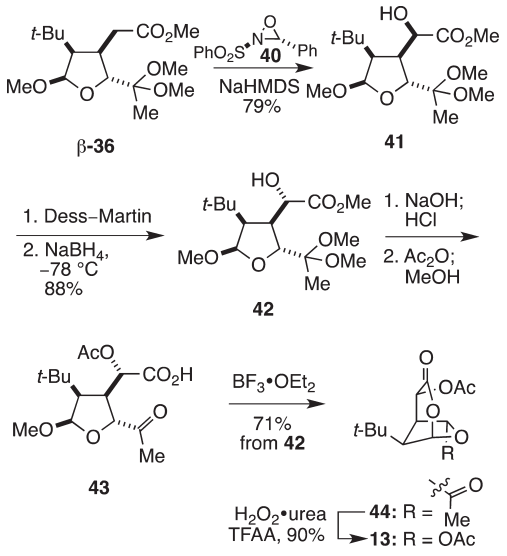
With cyclization substrates **35**, **37**, and **39** in hand, their transformation to 2,7-dioxabicyclo[3.2.1]octan-3-one **22** was studied (Table 1). Mitsunobu conditions failed to promote cyclization of hemiacetal **35** (entry 1); however, in the presence of 1 equiv of camphorsulfonic acid (CSA) in chloroform, lactol **35** was converted at room temperature to dioxabicyclooctanone **22** in 54% overall yield from ester precursor **34** (entry 2). In a similar fashion, methoxy acetal **37** was transformed in the presence of 1 equiv of BF_3•Et_2O to dioxabicyclooctanone **22** in 66% yield (entry 3). Anomeric fluoride **39** cyclized with slightly enhanced efficiency when exposed to 2 equiv of $SnCl_2$ in DMF at room temperature, generating bicyclic lactone **22** in 71% yield (entry 4).[34]

With several complementary methods for accomplishing the critical bridging lactonization reaction identified, all that remained was installing the additional oxygen functionality of *t*-Bu-MacE. The C6 acetoxy substituent was readily introduced by reaction of dioxabicyclooctanone **22** with 4 equiv of trifluoroperacetic acid at room temperature,[35] providing the *tert*-butyl analog of aplyviolene, 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one (**14**), in 88% yield. We had hoped that the enhanced acidity of lactones relative to esters would allow the remaining acetoxy substituent to be incorporated at this stage.[36] However, all attempts to directly introduce a hydroxyl substituent adjacent to the lactone carbonyl group were unsuccessful. For example, enolization of **14** with 1−2 equiv of LDA or KHMDS, followed by oxidation (Davis oxaziridine[37] or O_2), silylation, or acylation resulted in either recovered starting material or extensive decomposition.[38]

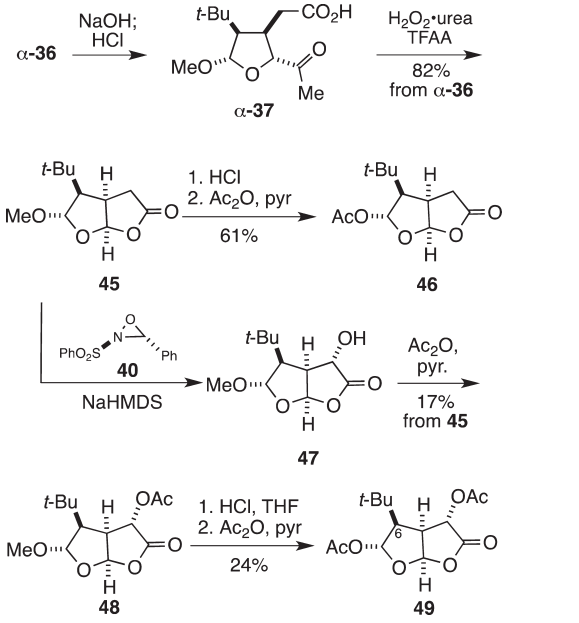


As a result of our inability to selectively oxidize bicyclic lactone **14**, we turned to examine introduction of the α-acetoxy

Scheme 5. Completion of the Synthesis of *t*-Bu-MacE (13)



Scheme 6. Synthesis of 2,8-Dioxabicyclo[3.3.0]octanones 46 and 49



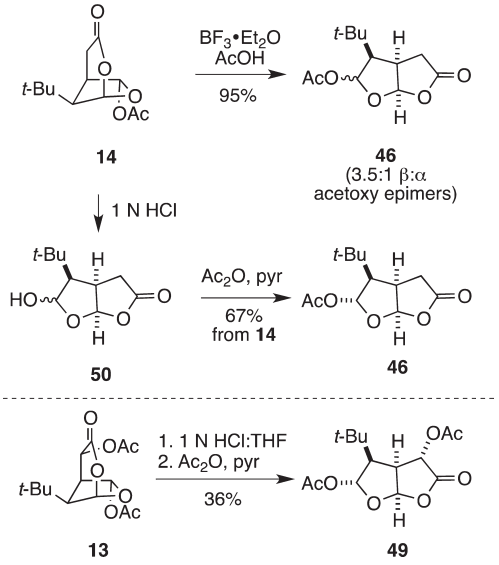
substituent prior to forming the dioxabicyclo[3.2.1]octanone ring system. Hydroxylation of the sodium enolate of the β-methoxy epimer of tetrahydrofuryl acetal **36** with oxaziridine **40**[39] proceeded smoothly to generate α-hydroxy ester **41** as a single stereoisomer (Scheme 5). The stereoselectivity of this oxidation is rationalized by orientation of the enolate away from the tetrahydrofuran ring and delivery of the electrophile to the face opposite the *tert*-butyl group. As the hydroxyl substituent of **41** has the opposite relative configuration to the corresponding acetate of MacE, it was inverted by oxidation with Dess–Martin reagent[40] and subsequent stereoselective reduction of the ketone product with sodium borohydride at −78 °C. This sequence provided alcohol **42** in 88% overall yield. Saponification of the ester and acidic hydrolysis of the ketal, followed by exhaustive acylation and anhydride methanolysis, yielded α-acetoxycarboxylic acid **43**. We were delighted to find that tetrahydrofuryl acetal **43**, when exposed to 1.1 equiv of BF_3•OEt_2 at 0 °C, gave rise to 2,7-dioxabicyclo[3.2.1]octan-3-one **44** in 71% overall yield from tetrahydrofuran **42**. Baeyer–Villiger oxidation of this product with trifluoroperacetic acid then provided *t*-Bu-MacE (**13**) in 90% yield.[41] The synthetic sequence outlined in Schemes 2–5 allowed 0.5 g of *t*-Bu-MacE to be synthesized, enabling the chemical reactivity and biological profile of this compound to be studied in detail.

Enantioselective Synthesis of 7-Acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-ones. Several intermediates prepared during the synthesis of *t*-Bu-MacE provide potential access to related structures in the 2,8-dioxabicyclo[3.3.0]octan-3-one series. We demonstrated this chemistry with the α-methoxy epimer of intermediate **36**, thus allowing both epimers of acetal **36** to be utilized (Scheme 6). Sequential ester saponification and ketal hydrolysis cleanly provided tetrahydrofuran α-**37** from acetal precursor α-**36**. Baeyer–Villiger oxidation of this intermediate took place with concomitant cyclization to provide 7-methoxy-2,8-dioxabicyclo[3.3.0]octanone **45** in 82% overall yield for the three steps. Hydrolysis of **45** with dilute HCl yielded a mixture of bicyclic lactols, which upon acetylation delivered crystalline 7-acetoxy-2,8-dioxabicyclo[3.3.0]octanone **46** as a single stereoisomer. The structure and relative configuration of this product were initially secured by ^{1}H NMR NOE analysis and subsequently confirmed by single crystal X-ray diffraction.[42]

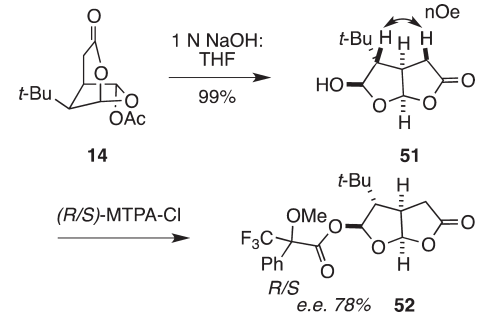
Analog **49**, which possesses an acetoxy group adjacent to the lactone carbonyl, was also prepared. The enolate of bicyclic lactone **45** was generated with NaHMDS in THF at −78 °C and hydroxylated to afford **47** (Scheme 6). Subsequent acetylation of the secondary alcohol gave acetate **48** in low (unoptimized) yield over two steps.[43] The methoxy acetal functional group of **48** was hydrolyzed with dilute HCl, followed by acetylation of the hemiacetal product with acetic anhydride and pyridine, to provide an inseparable mixture of 4,7-diacetoxy-2,8-dioxabicyclo[3.3.0]octan-3-one **49** and an uncharacterized aldehyde byproduct. Pure dioxabicyclo[3.3.0]octanone **49** was obtained in modest yield from this crude product mixture by sodium chlorite oxidation,[44] which allowed for easy removal of carboxylic acid impurities by chromatography. Of note, the syntheses summarized in Scheme 6 are the first of 7-acetoxy-2,8-dioxabicyclo-[3.3.0]octan-3-ones possessing a quaternary-carbon substituent at C6, a structural feature found in many rearranged spongian diterpene natural products (see Figure 1).

Chemical Reactivity of the 6-Acetoxy-2,7-dioxabicylo-[3.2.1]octan-3-one and 7-Acetoxy-2,8-dioxabicylo[3.3.0]-octan-3-one Ring Systems. Our expectation that the 2,7-dioxabicyclo[3.2.1]octan-3-one ring system would be less stable than the isomeric 2,8-dioxabicyclo[3.3.0]octan-3-one ring system was readily confirmed by exposure of dioxabicyclo-[3.2.1]octanone **14** to BF_3•OEt_2 in acetic acid at 0 °C to generate dioxabicyclo[3.3.0]octanone isomer **46** as a 3.5:1 mixture of separable acetal epimers, favoring the β-acetoxy isomer (Scheme 7).[45] Alternatively, hydrolysis of **14** at room temperature with 1 N HCl in THF gave a mixture of dioxabicyclo[3.3.0]octanone lactol epimers **50**,[46] which upon acetylation provided the α epimer of dioxabicyclo[3.3.0]octanone **46** exclusively. This sample was identical to the material prepared by the approach outlined in Scheme 6. In a similar fashion, acidic

Scheme 7. Conversion of Bridged Dioxabicyclic Lactones 13 and 14 to Fused Isomers 46 and 49



Scheme 8. Synthesis of 51 and Its Mosher Ester



hydrolysis of *t*-Bu-MacE (**13**) provided a lactol intermediate, which was identical to the product formed by acidic hydrolysis of **48**. Ensuing acetylation of this lactol intermediate gave α-acetoxylactone **49** in 36% overall yield from *t*-Bu-MacE.

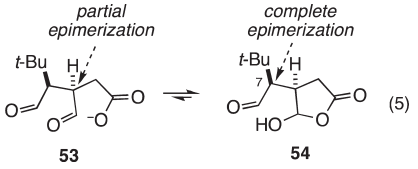
The transformation of the 2,7-dioxabicyclo[3.2.1]octan-3-one ring system to fused bicyclic lactone products could also be accomplished under basic conditions. In the presence of sodium hydroxide at room temperature, dioxabicylo[3.2.1]octanone **14** yielded a single 2,8-dioxabicylo[3.3.0]octan-3-one lactol product. However, in this case, ^{1}H NMR NOE analysis showed that the *tert*-butyl group of product **51** resides on the convex face of the 2,8-dioxabicyclo[3.3.0]octanone ring system (Scheme 8). Confirmation that this product resulted from epimerization of the carbon bearing the *tert*-butyl substituent, and not the methine hydrogens of the ring junction, was obtained by conversion of **51** to the *R* and *S* Mosher esters **52**. Enhanced Mosher analysis established that **52** possesses the 1*R*,5*R*,6*S*,7*R* absolute configuration with a slightly eroded enantiomeric purity of 78% ee.[47]

The formation of product **51** from dioxabicyclo[3.2.1]-octanone precursor **14** requires further comment. Certainly isomer **51** having the *tert*-butyl group on the convex face of the *cis*-dioxabicyclo[3.3.0]octanone ring system should be more stable than stereoisomer **50**. Under the basic reaction conditions (pH ~14), the predominant aldehyde intermediates generated from hydroxide opening of the lactone (or cleavage of the acetate substituent) of precursor **14** would be expected to be **53** and **54** (eq 5). Base-promoted epimerization of the likely predominant species, uncharged **54**, would lead to the formation of the observed product **51**. The partial erosion of enantiomeric purity observed in the conversion of **14** to **51** indicates that there is some epimerization under these conditions of the central methine carbon of intermediate **53**.[48]

Table 2. Half-life of Hydrolysis at pD 8.3

Compound	$t_{1/2}$ (h)[a]
13	10.2
14	91.9
46	9.7
49	1.5

[a] Incubated at 37 °C in 50 mM phosphate buffer; disappearance of substrate (5 mM) was monitored by ^{1}H NMR.

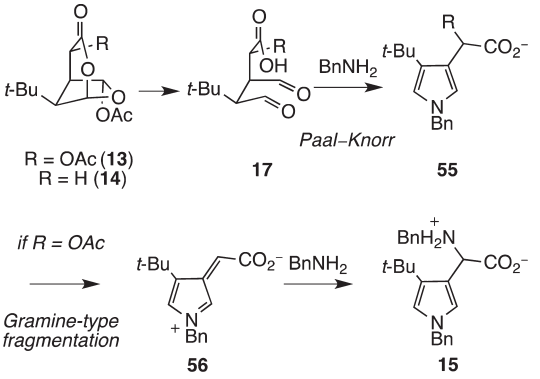


To gain further insight into the reactivity of these dioxabicyclic lactone ring systems, we examined the rate of hydrolysis of compounds **13**, **14**, **46**, and **49**. The hydrolytic rate was measured by NMR observation of the disappearance of the dioxabicyclic ring system in a pD 8.3 phosphate buffer containing 10% DMSO at 37 °C (Table 2). The presence of an acetoxy group adjacent to the lactone carbonyl results in an enhanced hydrolysis rate of both the substituted dioxabicyclo[3.2.1]octanone and dioxabicyclo[3.3.0]octanone ring systems, with the latter ring system hydrolyzing more rapidly than the former.

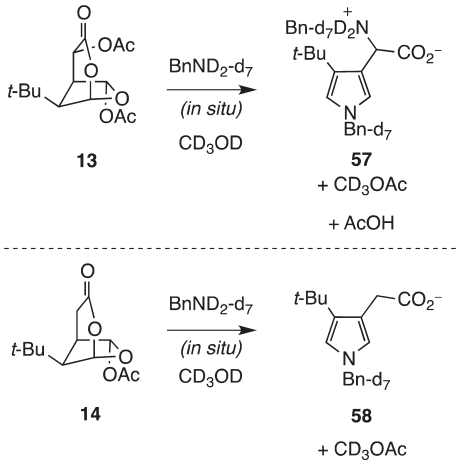
In our initial report, we demonstrated that the reaction of substituted dioxabicyclo[3.2.1]octanones **13** and **14** with benzylamine leads to substituted pyrroles.[6] Specifically, we showed that *t*-Bu-MacE (**13**) was converted to pyrrole **15** in a mixture of perdeutero-benzylamine (2.5 equiv) and THF-d_8 and that dioxabicyclooctanone **14** is transformed to pyrrole **16** in high yield in the presence of 5 equiv of benzylamine in DMSO and water (eq 1). Under these conditions, fragmentation of these precursors undoubtedly generates transient 1,4-dialdehyde intermediates, **17**, which undergo Paal–Knorr pyrrole formation (Scheme 9).[6] In the case of the pyrrole derived from *t*-Bu-MacE (**13**), the oxygen substituent of the acetic acid side chain is exchanged for a benzylamino substituent, likely after formation of the pyrrole by a gramine-type fragmentation/addition pathway.

The conversion of *t*-Bu-MacE (**13**) and analog **14** to pyrrole products was examined in CD_3OD to gain insight into the initial step of the pyrrole-forming process under protic conditions (Scheme 10).[49] NMR analysis of the reaction of **13**, perdeutero-benzylamine (5 equiv), and CD_3OD at room temperature indicated that pyrrole **57** was formed along with equimolar quantities of acetic acid and methyl acetate.[50] Similarly, when **14** was converted to **58** under identical conditions, methyl acetate was observed.[49] The formation of methyl acetate, as well

Scheme 9. Mechanistic Outline for Pyrrole Formation from 13 and 14



Scheme 10. *In Situ* Observation of the Formation of 57 and 58 and Reaction Byproducts

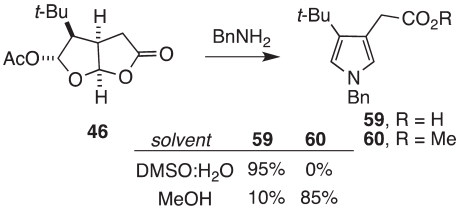


as the exclusive formation of the carboxylates **57** and **58**, demonstrates that fragmentation of the 6-acetoxy-2,7-dioxabicyclo[3.2.1]octanones **13** and **14** is initiated by initial reaction of the protic solvent at the anomeric acetoxy substituent.

Pyrroles are also formed from the reaction of 7-acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-ones with primary amines. For example, 7-acetoxydioxabicyclo[3.3.0]octanone **46** was converted to pyrrole carboxylic acid **59** when exposed at room temperature to 2 equiv of benzylamine in DMSO/H_2O (Scheme 11). When this reaction was conducted in methanol, pyrrole methyl ester **60** was observed as the major product. The formation of methyl ester **60** suggests, in contrast to bridged dioxabicyclo[3.2.1]-octanone compounds **13** and **14**, that fragmentation in this series is initiated by reaction of the protic solvent at the lactone carbonyl group.

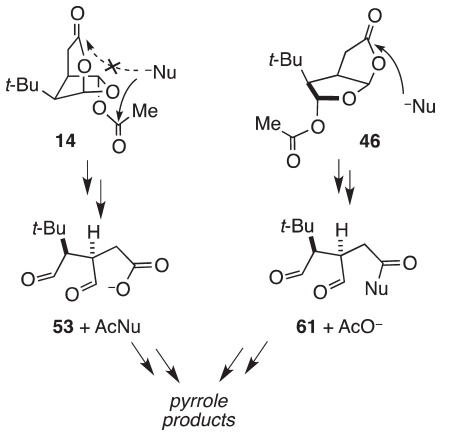
The divergence in fragmentation pathways of the two isomeric bicyclic lactone ring systems (summarized in Scheme 12) is consistent with the reactivity of related simple lactones. Although six-membered lactones are typically more reactive than their five-membered ring counterparts,[51] oxabicyclo[3.2.1]octanone **62** is saponified at a dramatically reduced rate (Figure 2).[52] The reduced rate of saponification of lactone **62** is readily ascribed to developing destabilizing syn-pentane interactions during axial

Scheme 11. Conversion of Dioxabicyclo[3.3.0]octanones 46 to Pyrroles 59 and 60



solvent	59	60
DMSO:H_2O	95%	0%
MeOH	10%	85%

Scheme 12. Regioselectivity of Initial Nucleophilic Attack on Bicyclic Lactones 14 and 46 Leading to 1,4-Dialdehyde Intermediates



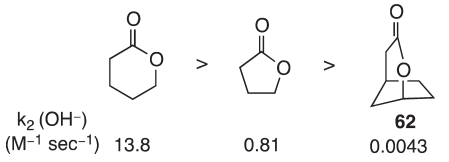


Figure 2. Saponification rates of select lactones.[53]

approach of hydroxide to the lactone carbonyl group. Similar destabilizing interactions would be involved in the tetrahedral intermediate generated from the addition of nucleophiles to the lactone carbonyl of 6-acetoxy-2,7-dioxabicyclo[3.2.1]octanones **14**.

To ascertain whether the oxygenated bicyclic lactones would react with lysine residues of proteins under biologically relevant conditions, we examined the reactivity of these molecules with hen egg white lysozyme (HEWL).[53] In initial experiments, we found that dioxabicyclo[3.2.1]octanones **13** and **14** converted lysine residues of HEWL to pyrrole adducts at room temperature in pH 7 phosphate buffer (Figure 3 and Table 3, entries 1 and 3). Analog **14** provided the pyrrole-3-acetic acid modification (+164 mu), whereas *t*-Bu-MacE (**13**) led to the pyrrole-3-hydroxyacetic acid adduct (+180 mu) resulting from solvolytic incorporation of a hydroxyl substituent at the heterobenzylic site (Figure 3). The reaction of dioxabicyclo[3.3.0]octanones **46** and **49** with HEWL at pH 7 and 8 was also examined. As expected, 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-ones and isomeric 7-acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-ones provided identical pyrrole

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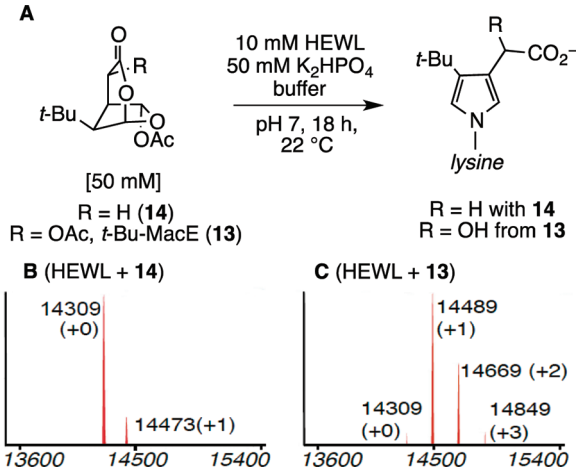

Figure 3. Modification of lysozyme under the conditions shown with **13** and **14** to form lysine adducts (a) and the distribution of alkylated products obtained from **14** (b) and **13** (c). The ESI-MS spectra were reconstructed from charge ladders.

Table 3. Modification of HEWL by Dioxabicyclolactones 13, 14, 46, and 49[a]

Entry	Substrate	pH	Unmod. (%)	+1 (%)	+2 (%)	+3 (%)	+4 (%)
1	**13**	7	9	54	33	4	0
2	**13**	8	0	14	49	32	5
3	**14**	7	88	12	0	0	0
4	**14**	8	43	47	2	0	0
5	**46**	7	61	35	4	0	0
6	**46**	8	7	48	38	7	0
7	**49**	7	13	47	28	12	0
8[b]	**49**	8	0	4	19	43	7

[a] Conditions: 50 μM substrate, 10 μM lysozyme, 50 mM K_2HPO_4 buffer incubated at 22 °C for 20 h. Product distributions were determined from ESI-MS analyses. With **13** and **49**, the addition of +180 mu was observed per modification, and with **14** and **46**, +164 mu was observed. [b] In addition +5 (7%).

adducts: **14** and **46** (+164 mu), **13** and **49** (+180 mu).[54] *t*-Bu-MacE (**13**) and **49**, which possess an acetoxy substituent adjacent to the lactone carbonyl group, modified lysozyme to a greater extent than the corresponding desacetoxy congeners **14** and **46**. Subjecting the modified lysozymes to trypsin digestion, followed by standard MALDI-MS-MS peptide sequencing, revealed that the two most surface-accessible lysines (K-33 and K-97) were the predominant sites of covalent modification.[55]

Effects of 6-Acetoxy-2,7-dioxabicylo[3.2.1]octan-3-ones and 7-Acetoxy-2,8-dioxabicylo[3.3.0]octan-3-ones on Golgi Organization. Synthetic access to 2,8-dioxabicyclo[3.3.0]octan-3-ones **46** and **49** allowed us to compare the Golgi-modifying properties of these structures with those of isomers in the 2,7-dioxabicyclo[3.2.1]octan-3-one series. Normal Rat Kidney (NRK) cells grown on coverslips were incubated with analogs **46** and **49** for 60 min at 37 °C, followed by examination of the Golgi for the MacE-induced reorganization phenotype by immunofluorescence analysis with antibodies to the known Golgi resident protein, mannosidase-II.[56] The 4,7-diacetoxy-2,8-dioxabicyclo[3.3.0]octanone **49** produced a Golgi phenotype

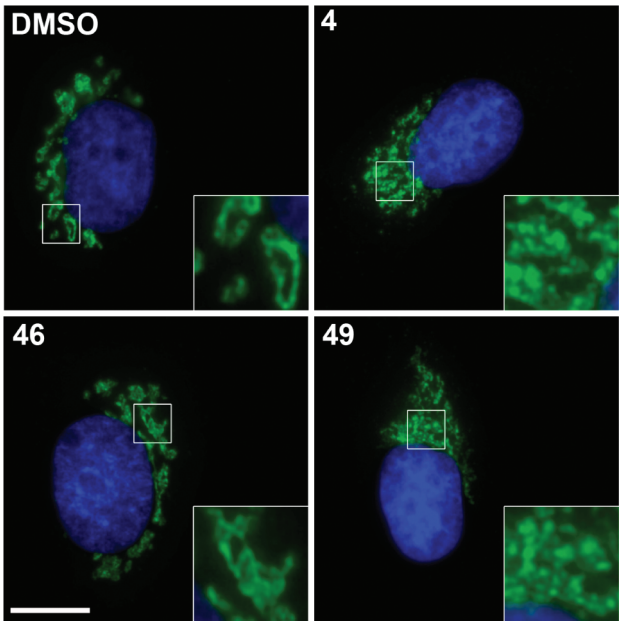

Figure 4. NRK cells were treated with control (DMSO), MacE (**4**) at 20 μg/mL, **49** at 40 μg/mL, or **46** at 80 μg/mL for 1 h at 37 °C. MacE and **49** are shown at the minimal active concentration, and **46** is shown at the maximal concentration tested. Cells were fixed and stained with an antibody to the Golgi resident protein mannosidase II (green) and the DNA dye Hoechst 33342 (blue). Area demarcated by the white square is enlarged in the insets to show detail of Golgi organization following each treatment. Scale bar is 10 μm.

indistinguishable from that of MacE or *t*-Bu-MacE (**13**), with the conversion of the Golgi ribbon into small fragments that remained localized adjacent to the centrosome (Figure 4). By contrast, 7-acetoxy-2,8-dioxabicyclo[3.3.0]octanone **46**, which lacks an acetoxy substituent adjacent to the lactone carbonyl group, did not affect the Golgi structure at concentrations of up to 80 μg/mL. As 6-acetoxy-2,7-dioxabicyclo[3.2.1]octanone **14** also did not impact the Golgi structure,[6] these findings indicate that the formation of pericentrosomal Golgi fragments characteristic of MacE depends on the presence of oxygenation adjacent to the lactone carbonyl group, in either the substituted dioxabicyclo-[3.2.1]octanone or dioxabicyclo[3.3.0]octanone ring systems.

■ CONCLUSIONS

The synthesis and initial comparison of the chemical reactivity of two highly oxidized bicyclic lactone fragments found in rearranged spongian diterpenes — 8-substituted 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one and 6-substituted 7-acetoxy-2,8-dioxabicyclo[3.3.0]octan-3-one — are reported. The syntheses of *t*-Bu-MacE (**13**) and congener **14** are the first syntheses of the 8-substituted 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-one ring system found in rearranged spongian diterpenes such as macfarlandin E (**4**) and aplyviolene (**3**). A late-stage intermediate, **36**, was diverted to provide the first synthetic entry to isomeric structures (**46** and **49**) in the 7-acetoxy-2,8-dioxabicyclo-[3.3.0]octan-3-one series.

Access to these highly oxidized bicyclic lactones allowed the chemical reactivity and Golgi-modifying activity of these ring systems to be studied. Under both acidic and basic conditions, 8-substituted 6-acetoxy-2,7-dioxabicyclo[3.2.1]octan-3-ones are converted to 6-substituted 7-acetoxy-2,8-dioxabicyclo[3.3.0]-

octan-3-one products. Both dioxabicyclooctan-3-one ring systems are found to react readily with primary amines to form pyrrole products. Of particular significance, lysine side chains of hen egg white lysozyme are converted under physiologically relevant conditions to substituted pyrroles upon exposure to dioxabicyclic lactones **13**, **14**, **46**, and **49**. The presence of an acetoxy substituent adjacent to the lactone carbonyl group, in either the bridged or fused dioxabicyclooctanone series, increases the extent of the lysine to pyrrole conversion and is essential for induction of the macfarlandin E Golgi phenotype. These investigations provide a basis for future studies aimed at identifying the biological target(s) of these Golgi-modifying natural products, as well as initial insight into the reactivity of the family of structurally distinctive rearranged spongian diterpenes depicted in Figure 1.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and copies of ^{1}H and ^{13}C NMR spectra of new compounds; CIF files for compounds **46** and **51**. This material is available free of charge via the Internet at http://pubs.acs.org.

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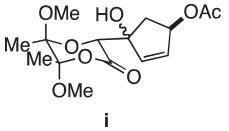

i

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(42) Crystallographic data for this compound were deposited at the Cambridge Crystallographic Data Centre: CCDC 839175.

(43) The low yield observed in this sequence is likely a result of the sensitivity of the α-hydroxylated intermediate **47** to silica gel.

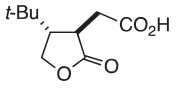
(44) (a) Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091. (b) Kraus, G. K.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175. (c) Lindgren, B. O.; Nilsson, T.; Husebye, S.; Mikalsen, Ø.; Leander, K.; Swahn, C. *Acta Chem. Scand.* **1973**, *27*, 888.

(45) That the acetal mixture reflected a thermodynamic ratio was confirmed by individually exposing the separable acetal diastereomers of **46** to the reaction conditions (BF_3•OEt_2, AcOH, CH_2Cl_2, 0 °C) to provide an identical 3.5:1 α:β mixture of diastereomers.

(46) The ratio of epimeric lactols was somewhat variable (between 1:1 and 3:1).

(47) Further confirmation was obtained by chromatographic separation of the major diastereomer of **52** (possessing the *S*-Mosher-ester) and single crystal X-ray diffraction. Crystallographic data for this compound were deposited at the Cambridge Crystallographic Data Centre: CCDC 839176.

(48) Longer reaction times do not afford fully equilibrated, racemic **51**, as significant quantities of Cannizzaro product **ii** are formed. For example, after 72 h in 1 N NaOH at room temperature 54% of **ii** is isolated from the reaction mixture.



ii

(49) The initial site of nucleophilic attack in protic conditions was not established in ref 6 in which either aprotic ($BnNH_2$, THF) or aqueous ($BnNH_2$, DMSO, H_2O) conditions were used.

(50) Compound **57** was identified by NMR and mass spectroscopic observation. Efforts to isolate **57** were complicated by the formation of additional products during purification. Compound **58** was also identified by NMR and mass spectroscopic observation but could be isolated in pure form. See Supporting Information for details.

(51) For a discussion of the differences in reactivity between five- and six-membered lactones, see: Brown, H. C.; Brewster, J. H.; Shechter, H. *J. Am. Chem. Soc.* **1954**, *76*, 467.

(52) Hall, H. K. *J. Org. Chem.* **1963**, *28*, 2027.

(53) The Modification of Amino Groups. In *Chemical Reagents for Protein Modification*, 3rd ed.; Lundblad, R. L., Ed.; CRC Press: Washington, DC, 2005; pp 31−67.

(54) No products resulting from an acyl transfer reaction to HEWL appeared to be formed by ESI-MS analysis, suggesting that initial hydrolysis, and not protein side chain reactivity, provides the reactive 1,4-dialdehyde intermediate.

(55) A similar lysine modification pattern is observed in the acylation of HEWL by acetic anhydride; see: Suckau, D.; Mak, M.; Przybylski, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5630.

(56) The Golgi staining was also carried out with an antibody to the known Golgi protein giantin, which led to nearly indistinguishable staining patterns (see Supporting Information).