Formal Total Synthesis of (±)-Tetrodotoxin

by

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## Abstract

This dissertation details a formal total synthesis of (±)-tetrodotoxin, a potent sodium channel blocker, based on a transformation developed in these laboratories: the bimolecular oxidative amidation of phenols. The present route leads to the Du Bois intermediate in 27 steps from a commercial starting material. Because the Du Bois intermediate can be elaborated to tetrodotoxin in 4 steps, this work constitutes a formal synthesis of the natural product in 31 steps. This is competitive with the best known alternatives. A structural revision of the Sato tetrodotoxin intermediate is also provided. Finally, an even more concise avenue to a new bis-lactonic precursor of the natural product is described, and potential enantioselective routes to tetrodotoxin are discussed.

## Preface

This thesis was written by S. Xu. All the experiments and data analyses in the main part *i.e.* Chapter 4 and 5 were performed by S. Xu, except for the crystallographic and HPLC analyses of **5.35** and **5.36**, which were conducted by Prof. J. Hein and Dr. C. Rougeot. Dr. M. A. Ciufolini and S. Xu are responsible for planning the synthetic strategy. Dr. M. A. Ciufolini carried out the Molecular Mechanics calculation (HyperChem<sup>®</sup>), provided tactical and technical suggestions, and thoroughly edited this thesis.

A portion of the material in Chapter 4 has been published in two papers. Specifically:

The first part of Chapter 4, elaboration from **3.6** to **4.36**, has been published in: Chau, J.; Xu, S.; Ciufolini, M. A. *J. Org. Chem.* **2013**, *78*, 11901–11910. Dr. M. A. Ciufolini wrote the manuscript. J. Chau performed the experiments included in Chapter 3. S. Xu reproduced the results by J. Chau, performed the experiments included in Chapter 4 and prepared the supporting information.

The rest of Chapter 4, elaboration from **4.36** to **4.88**, **4.88** to **4.123** and **4.88** to **1.109** has been published in: Xu, S.; Ciufolini, M. A. *Org. Lett.* **2015**, *17*, 2424–2427. Dr. M. A. Ciufolini wrote the manuscript. S. Xu performed all experiments and wrote the supporting information.

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## List of Abbreviations

Ac	acetyl
addn.	addition
anh.	anhydrous
aq.	aqueous
Ar	generic aryl group
Bn	benzyl
Boc	<i>t</i> -butyloxycarbonyl
BOM	benzyloxymethyl
BOX	bis(oxazoline)
br	broad
BRSM	based on recovered starting material
Bu	butyl
Bz	benzoyl
calcd.	calculated
CAN	ceric ammonium nitrate
cat.	catalytic
CBS	Corey-Bakshi-Shibata catalyst

CDI	1,1'-carbonyldiimidazole
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
DABCO	1,4-diazabicyclo[2.2.2]octane
d. b.	double bond
DBU	1,8-diazabicycloundec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
dec	decomposition
DI	deionized
DIB	(diacetoxyiodo)benzene
DIBAL	diisobutylaluminum hydride
DIPA	diisopropylamine
DIPEA	N,N-diisopropylethylamine
dis	disrotatory
DMAP	4-dimethylaminopyridine

DMDO	dimethyldioxirane
DMF	N,N-dimethylformamide
DMF-DMA	<i>N</i> , <i>N</i> -dimethylformamide dimethyl acetal
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
dr	diastereomeric ratio
E1cB	elimination, unimolecular, conjugate base
elim.	elimination
epi	epimeric
equiv	equivalent
Et	ethyl
Hex	hexanes
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HMBC	heteronuclear multiple bond correlation
HMDS	hexamethyldisilazide
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
IBX	2-iodoxybenzoic acid
imid.	imidazole

INOC	intramolecular nitrile oxide cycloaddition
IR	infrared spectroscopy
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
mCPBA	meta-chloroperoxybenzoic acid
Me	methyl
Men	menthyl
MMTr	(4-methoxyphenyl)diphenylmethyl
MOM	methoxymethyl
mp	melting point
MS	mass spectrometry
Ms	methanesulfonyl
Na <sub>v</sub>	voltage-gated sodium (channel)
NMM	N-methylmorpholine
NMO	N-methylmorpholine N-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
OA	oxidative amidation

Р	generic protecting group
PCC	pyridinium chlorochromate
Ph	phenyl
PIFA	phenyliodine bis(trifluoroacetate)
Piv	pivaloyl
ppm	part per million
PPTS	pyridinium <i>p</i> -toluenesulfonate
Pr	propyl
Pyr	pyridine
Ру	1-pyridyl
quant.	quantitative
R <sub>f</sub>	retardation factor
RSM	recovered starting material
rt	room temperature
sat.	saturated
SM	starting material
TBAF	tetra-n-butylammonium fluoride
TBDPS	t-butyldiphenylsilyl
TBS	t-butyldimethylsilyl

TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Tr	triphenylmethyl
Ts	<i>p</i> -toluenesulfonyl
TTX	tetrodotoxin
UV	ultraviolet
XRD	X-ray diffraction

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Dedication

To My Grandparents

## **Chapter 1 Introduction**

#### 1.1 Tetrodotoxin: Background

Tetrodotoxin (TTX, **1.1**) is an exceedingly toxic guanidine alkaloid that displays a densely oxygenated 2,4-dioxoadamantane framework.<sup>1</sup> Its name derives from the order *tetraodontiformes*, which includes the pufferfishes. Indeed, the compound was first isolated in 1909 by Tahara<sup>2</sup> from the viscera of a pufferfish species called *Fugu*, which is considered a delicacy in Japan, in spite of the risks associated with its consumption.<sup>3</sup> The livers and ovaries of *Fugu* and of other pufferfish contain enough TTX to kill several humans. Less than extreme care in the preparation of *Fugu* for human consumption may result in contamination of edible parts with TTX, and potentially fatal poisoning of those who consume them. Intoxications and deaths that are due to TTX occur to this day.<sup>3</sup> Tetrodotoxin has since been isolated from other geographically and phylogenetically unrelated organisms, both marine and terrestrial, such as crabs, mollusks (flatworms, octopus, sea slugs), newts and frogs.<sup>4</sup>



**1.1** (–)-tetrodotoxin **Figure 1.1** Structure and numbering of tetrodotoxin

TTX is an extremely polar substance that is virtually insoluble in water or organic solvents.<sup>5</sup> However, it dissolves instantly in weak aqueous acid, where it exists in equilibrium with 4,9-anhydro-4-*epi*-TTX, **1.2**, also known as anhydro-TTX.



Scheme 1.1 Equilibrium between TTX and anhydro-TTX

Tetrodotoxin acts by blocking voltage-gated sodium channels (Na<sub>v</sub>), causing paralysis of voluntary muscles, including the diaphragm. This leads to respiratory arrest and may result in death by asphyxiation.<sup>3</sup> Other marine guanidine alkaloids; *e.g.*, the saxitoxins (**Figure 1.2**), exhibit a similar mechanism of action.<sup>6-7</sup> The guanidine moiety present in all of these alkaloids is required for activity.<sup>8</sup> The role of the other heteroatoms has not been fully elucidated, but it is very likely that they contribute significantly to the high affinity of the compounds for the Na<sub>v</sub> channels, probably through H-bonding and dipolar interactions.<sup>9-10</sup>

The tendency of TTX to modulate the function of voltage-gated sodium channels renders it a valuable tool in neurophysiology.<sup>11</sup> Furthermore, TTX is neither mutagenic nor genotoxic.<sup>12</sup> Consequently, potential application in human medicine may be envisioned.<sup>13</sup> To illustrate, the compound, and/or less toxic congeners thereof, could be useful as neuroprotective agents to



Figure 1.2 Some naturally occurring analogues of tetrodotoxin and saxitoxin

contain damage to brain tissue during ischemic events. They could also serve to prevent of post-traumatic epileptogenesis, and, especially, as non-addictive analgesics for the treatment of opiate-resistant pain in terminally ill cancer patients.<sup>14</sup>

#### **1.2 Biological Origin of Tetrodotoxin**

Various observations suggest that TTX-bearing animals do not produce TTX themselves; rather, they accumulate it from either symbiotic microorganisms that produce it, or feeding on species that support colonies of such microorganisms. Puffer fish in particular may acquire TTX by feeding on colonized mollusks (puffer fish are typical molluscivores). Indeed, puffer fish containing low levels of TTX concentrate significant amounts of the toxin in their liver when fed a TTX-containing diet. Conversely, individual species containing a high concentration of TTX slowly lose their toxicity when placed in filtered sea water and fed a non-TTX containing diet.<sup>15</sup>

This has inspired the establishment of fish farms to raise TTX-free, food-quality fugu in Japan.<sup>15</sup> On the other hand, the origin of TTX in newts remains a matter of dispute, because the animals become progressively more toxic over time even when fed non-TTX containing diets; yet no evidence of symbiosis with TTX-producing microorganisms has been uncovered.<sup>16</sup>

As alluded to in a recent review,<sup>17</sup> the biosynthetic origin of TTX remains to be elucidated and is still under dispute.<sup>18</sup> Shimizu and coworkers proposed that TTX is probably biosynthesized from isopentenyl pyrophosphate **1.17** and arginine **1.18** (Scheme 1.2).<sup>19</sup> However, newts fed <sup>14</sup>C labeled precursors produced no <sup>14</sup>C labeled TTX.



Scheme 1.2 Proposed biosynthesis pathway by Shimizu and coworkers

Given the unusual structure of **1.1**, it seems likely that biosynthetic investigations would reveal novel reactions. Such studies would also enable the cloning and expression of genes that are responsible for the production of TTX, securing a reliable source of the material.

#### **1.3 Elucidation of the Structure of Tetrodotoxin**

The long history and fearsome reputation of TTX stimulated much research toward its structural elucidation. Efforts in that sense were launched immediately following its isolation, but given the primitive nature of structural tools available prior to the 1960's, this objective

presented unsurmountable difficulties. Complicating factors were that elemental analysis showed roughly the same number of heteroatoms as carbons, causing difficulties to analyze chemical degradation, and that infrared spectroscopy (available only after 1950)<sup>20</sup> indicated the absence of carbonyl groups. Furthermore, the  $pK_a$  of protonated TTX is 8.5, which is significantly lower than that of a protonated guanidine ( $pK_a$  11~13),<sup>21</sup> suggesting the existence of a more acidic functional group.



Scheme 1.3 The lactone-orthoacid equilibrium in two TTX derivatives

This state of affairs changed with the advent of more powerful structural elucidation methods; especially X-ray diffractometry. Thus, in the mid-1960s several groups disclosed the structure of TTX independently and virtually simultaneously.<sup>21-23</sup> In particular, Woodward and collaborators determined that treatment of TTX with HCl/acetone or HCl/acetone/MeOH furnished two products that were named Rajappa hydrochloride **1.19** and Gougoutas hydrochloride **1.22**,

respectively. The structure of these products was unambiguously determined by X-ray diffractometry, which revealed an unprecedented orthoacid motif in **1.19**. Moreover, IR spectroscopy showed that **1.19** remained in the orthoacid form under any conditions, while **1.22** existed as a lactone in the solid state, but it partially equilibrated with its orthoacid form when dissolved in DMSO or  $D_2O$ . This diverging chemical behavior is attributed to the fact that the hemiaminal ether linkage in **1.19** brings C10 and C5-OH closer and rigidifies the adamantane-like structure; while the absence of such constraints in **1.22** facilitates equilibration with a lactonic structure.



A noteworthy discovery made during the structural elucidation of TTX is the epimerization at C9. Tsuda found by heating TTX in water followed by treatment with HBr affords substance **1.23**, which was named tetradonic acid hydrobromide.<sup>22</sup> The *anhydro* linkage and the epimeric configuration at C9, relative to TTX, prevent the formation of the orthoacid structure altogether.

We note that the carbon center adjacent to the hemiaminal function, C4a, is also epimerizable. This may provide a synthetic strategy based on a 4a-*epi* TTX intermediate.

#### **1.4 Previous Syntheses of Tetrodotoxin**

A synthesis of TTX became a coveted goal as soon as the structure of the alkaloid was determined. Chemically, the intricate structure of **1.1** constituted a challenge that promised to test the limits of the best available synthetic technologies, and possibly stimulate the development of new ones. At a practical level, it was initially hoped that a chemical synthesis would provide a more stable supply of the alkaloid and diminish the environmental impact connected to its isolation from natural sources. Indeed, the demand of TTX for scientific research places a significant pressure on marine ecosystems. The process of extraction from puffer fish roe, developed in the 1960's, is fairly straightforward, spares the animals, but it is inefficient. Thus, only 8 to 9 grams of TTX can be isolated from 1000 kg of fugu roe.<sup>24</sup> Finally, a chemical route adaptable also to the synthesis of congener would surely be of considerable assistance in determining the structure-activity relationship of the natural product, and possibly in generating an analogue with more desirable properties (diminished toxicity, better therapeutic window, *etc.*).

As a consequence, numerous synthetic approaches were explored over the years, but only few of these led to a total synthesis, or even a formal one. This is unquestionably a reflection of the extreme difficulty of the task at hand. The sections that follow summarize the strategies that translated into successful routes to TTX. Throughout the discussion that follows, the tetrodotoxin numbering is used to identify positions of atoms in the various synthetic intermediates.

### 1.4.1 The Kishi Synthesis

Published in 1972, the Kishi synthesis represents a milestone in the field of total synthesis.<sup>25-28</sup> Kishi's elegant approach relies on two key steps: an oxidative cleavage of a cyclohexene moiety arising through a Diels-Alder reaction, and a Beckmann rearrangement to install N1 at an early stage. Various oxidative transformations were employed for the introduction of oxygen functionalities. Racemic **1.1** was thus reached in 31 steps from **1.24** (33 steps overall from commercial materials): a remarkable accomplishment even by today's standards.



Scheme 1.5 Kishi synthesis: Beckmann rearrangement

The synthesis started with the oximination of 4-acetyltoluhydroquinone **1.24** followed by oxidation to quinone **1.25**. Lewis acid catalyzed, regioselective Diels-Alder reaction with butadiene furnished adduct **1.26**. All carbons in the tetradonic acid backbone are already in place in this compound. Activation of **1.26** with methanesulfonyl chloride triggered a Beckmann



Scheme 1.6 Kishi synthesis: cyclohexane functionalization

rearrangement leading to **1.27**, thereby installing the crucial N1 atom at the C8a position. Chemo- and diastereoselective reduction of the C5 ketone served as a prelude to a number of transformations that provided advanced intermediate **1.30**.

The C11-OH was installed by oxidation with selenium dioxide, and epoxidation of the C6/C7 olefin, followed by appropriate protection-deprotection maneuvers, afforded **1.32**. This ketone was converted to vinyl ether **1.33**, which then underwent facially selective oxidation upon reaction with *m*CPBA. The stereoselectivity of this reaction is attributable to preferential attack onto the convex face of the bowl-shaped **1.33**. This sequence terminated with a Baeyer-Villiger oxidation of **1.34** to give caprolactone **1.35**.

Exposure of **1.35** to AcOH/AcOK followed by heating under vacuum triggered a series of events that delivered compound **1.36**. Racemic tetrodotoxin was rapidly reached from **1.36** by a sequence involving the release of the *N*-acetyl group, introduction of the guanidine moiety, oxidative cleavage of the dihydrofuran and a global deprotection with aqueous ammonia.



Scheme 1.7 Kishi synthesis: final stages

### 1.4.2 The First Isobe Synthesis

Research aimed at the total synthesis of TTX abated considerably following Kishi's landmark accomplishment, but never came to a halt. Indeed, various synthetic approaches (though no new syntheses) were disclosed after 1972, but it was not until 30 years later that the next major advance in the field was recorded, in the form of Isobe's first enantioselective synthesis of (–)-1.1.<sup>29</sup> This effort produced (–)-1.1 in 71 steps from 2-acetoxy-tri-*O*-acetyl-D-glucal **1.39** and after various improvements it opened synthetic avenues to a number of TTX analogues.



Scheme 1.8 The First Isobe synthesis: preparation of a homochiral precursor
Isobe's first generation approach relied on the carbohydrate derivative **1.39** as the source of chirality (**Scheme 1.8**). This material was advanced to the vinyl iodide **1.42** in 6 steps, in preparation for the coupling with segments that would construct the cyclohexane ring of TTX. Thus, a Sonogashira coupling installed an acetylenic fragment, while a Claisen rearrangement served to introduce an acetonyl unit. The resulting ketone **1.44** was then converted to **1.45** by  $Pb(OAc)_4$  oxidation of the corresponding silyl enol ether.



Scheme 1.9 The first Isobe synthesis: cyclohexane construction

The sequence of steps outlined in **Scheme 1.9** served to elaborate **1.45** into cyclohexenone **1.52** via an intramolecular aldol reaction, and ultimately into triol **1.55**. It should be noted that the

latter compound lacks the crucial N1 nitrogen functionality and three hydroxyl groups. Furthermore, the configuration of the OBz-bearing carbon center is incorrect and must be inverted.

The N1 functionality was introduced by an intramolecular aza-Michael reaction. Triol **1.55** was advanced to carbamate **1.58**, which upon treatment with *t*-BuOK cyclized to **1.59**. The practical consideration of this approach then required a reduction of the ester in **1.59** to an alcohol, followed by the protection to give **1.60** (Scheme 1.10).



Scheme 1.10 The first Isobe synthesis: N1 installation

The introduction of the remaining oxygen functionalities on **1.60**, the adjustment of configuration of the benzoate-bearing carbon, and the production of a fully functionalized TTX precursor were accomplished as illustrated in **Scheme 1.11**. Noteworthy in this sequence is the intramolecular *O*-alkylation of the enolate derived from aldehyde **1.64** with the C6-C7 epoxide, leading to dihydropyran **1.65**. This transformation was achieved by treatment of **1.64** with DBU at 130  $\degree$ , and resulted in inversion of the C7 configuration as required for the natural product. Also worth mentioning, for reasons that will become apparent later, is the facially selective



Scheme 1.11 The first Isobe synthesis: a fully functionalized TTX precursor

dihydroxylation of compound **1.65**, which served to introduce the C9 OH group. The shape of **1.65** is such that the incoming oxidant attacked from the convex face of molecule, resulting in the incorrect C9 configuration. This outcome dictated an oxidation of **1.66** to  $\alpha$ -ketolactone **1.67**, followed by selective reduction of the ketone, also from the convex face, to furnish **1.68**.



Scheme 1.12 The first Isobe synthesis: the endgame

Removal of the acetyl group in **1.68** triggered formation of an orthoacid, which was protected as the benzoate ester **1.69**. Release of *tert*-butyl carbamate and acetonide moieties set the stage for guanidinylation of **1.70**, and the resultant **1.71** was advanced to a mixture of **1.1** and **1.2** in seven additional steps (**Scheme 1.12**).

#### 1.4.3 The Second Isobe Synthesis

It seems likely that efforts to shorten and ameliorate the route just outlined were underway even before the first synthesis was completed. In fact, just one year later, in 2004, Isobe described a considerably more efficient total synthesis in 39 steps from (–)-levoglucosenone **1.74**.<sup>30</sup> The new route involved a strategy that was quite different from the previous one. A significant aspect of the new approach was the use of the Overman rearrangement to introduce the N1 atom: a transformation that had failed in the previous attempt.



Scheme 1.13 The second Isobe synthesis: Overman rearrangement

The synthesis started with bromination of **1.74** and BF<sub>3</sub>-catalyzed Diels-Alder reaction of the bromoenone **1.75** with isoprene (**Scheme 1.13**). The emerging **1.76** contains all the skeletal 14

carbons of the final product, except C10. This material was then elaborated to allylic alcohol **1.78**, which underwent the Overman rearrangement to produce trichloroacetyl amide **1.79**. Exposure of **1.79** to pyridinium tribromide caused selective bromination of the endocyclic olefin. Subsequent treatment of the resulting dibromide with DBU produced **1.80**, via selective E2 elimination of the secondary bromide and  $S_N2$ ' displacement of the tertiary halogen.



Scheme 1.14 The second Isobe synthesis: oxygenation of the cyclohexane core

Oxazoline **1.80** was opened under acidic condition, leaving a hydroxyl group at C8. This OH group directed the selective epoxidation of the more electron rich endocyclic olefin to oxirane **1.81**, which in turn suffered regioselective elimination to **1.82** upon reaction with  $Ti(O-i-Pr)_4$ . The configuration of the two hydroxyl groups in **1.82** is opposite to that required for TTX. A redox sequence similar to that employed in the previous synthesis corrected the problem (*cf.* **1.66**  $\rightarrow$  **1.68**; **Scheme 1.11**, p. 13). Compound **1.84** lent itself to the introduction of the remaining oxygen functionalities by selective SeO<sub>2</sub> oxidation of the methyl group and reduction of the corresponding aldehyde, followed by diastereoselective epoxidation of the endocyclic olefin.



Scheme 1.15 The second Isobe synthesis: the dioxaadamantane intermediate

The vinyl group in compound **1.86** was then oxidatively cleaved to give an aldehyde, which reacted with [(trimethylsilyl)ethynyl]magnesium bromide to furnish **1.87** as the major product diastereomer. The propargylic alcohol was acetylated and the terminal alkyne was oxidatively cleaved to carboxylic acid **1.89**, which rapidly opened the epoxide at C5 to afford **1.90**. Release of the silyl groups and global *O*-acetylation afforded dioxaadamantane intermediate **1.91**.



Scheme 1.16 The second Isobe synthesis: the endgame

The final stages of the synthesis are shown in **Scheme 1.16**. Thus, oxidative cleavage of the acetonide in **1.91** with orthoperiodic acid produced an aldehyde that was protected as a dimethyl

acetal. Ammonium hydroxide selectively removed the C9 and C10 acetates, and TBSOTf catalysis promoted cyclization to the *anhydro*-type intermediate **1.93** (*cf.* **Scheme 1.1**, p.2). Deprotection, *N*-guanidinylation and final treatment with aqueous acid successfully converted **1.94** to a mixture of synthetic (–)-**1.1** and **1.2**.

#### 1.4.4 The Du Bois Synthesis

An even shorter total synthesis of (-)-**1.1** was announced by Du Bois and coworkers almost at the same time of Isobe's first one.<sup>31</sup> The Du Bois route required 33 steps from D-isoascorbic acid **1.95**, and rested on two crucial Rh-catalyzed reactions: the C-H insertion of a carbene to construct the cyclohexane core, and that of a nitrene, to install the N1 functionality.



Scheme 1.17 The Du Bois synthesis: carbene C-H insertion

Oxidative degradation of **1.95** afforded lactone **1.96**, which was elaborated to diazoketone **1.99**. The action of dirhodium tetrakis(triphenylacetamide) on **1.99** induced selective carbenoid insertion into the tertiary C-H bond, efficiently providing the cyclohexane core of the natural <sup>17</sup>

product. Diastereoselective reduction of the ketone and the double bond in **1.100** resulted in the isomerization of the lactone, and protection the resultant vicinal diol delivered lactone **1.101** (Scheme 1.17).



Scheme 1.18 The Du Bois synthesis: nitrene C-H insertion

The lactone was opened with dimethylamine and the resulting cyclohexanol was oxidized to a ketone, in preparation for conversion into exomethylene compound **1.103**. The latter transformation was not as straightforward as anticipated. Thus, Wittig and Tebbe reagents were both ineffective but the application of the Lombardo-Takai protocol successfully produced **1.103**. This material was oxidatively converted into the  $\alpha$ , $\beta$ -unsaturated ketone **1.104**, which in turn underwent copper(I)-promoted 1,4-addition of vinylmagnesium bromide. The intermediate allyl ketone was diastereoselectively reduced to alcohol **1.105** with borane-*tert*-butylamine complex, which upon exposure to hot pivalic acid (200 °C) underwent lactonization. The lactone thus obtained was elaborated to **1.106** in a conventional fashion.



Scheme 1.19 The Du Bois synthesis: the endgame

Intermediate **1.106** was advanced to **1.107**, which is the substrate for the key transformation in the synthesis: the insertion of a nitrenoid into the C8a C–H bond. Thus, reaction of **1.107** with (diacetoxyiodo)benzene (DIB) and a catalytic amount of dirhodium tetrakis(trifluoroacetamide) directly afforded **1.108**. A four-step sequence advanced **1.108** to **1.109**, which was *N*-deprotected in preparation for the introduction of the guanidine. The release of the *N*-Boc group was achieved by heating of the substrate in water at 110 °C. In contrast, the guanidine moiety was assembled in a customary fashion and the guanidine **1.110** was elaborated to a mixture of (–)-TTX and anhydro-TTX.

### 1.4.5 The Sato Synthesis of rac-TTX

For the purpose of future discussion, it is appropriate to summarize two syntheses of **1.1** described by Sato and coworkers.<sup>32-33</sup> In 2005, these researchers disclosed a route to racemic TTX starting with *myo*-inositol, **1.111** (Scheme 1.20).<sup>32</sup> A series of protection- and redox steps produced ketone **1.113**, which underwent diastereoselective addition of the anion of  $CH_2Cl_2$  to

furnish **1.114**. Hydrolysis of the germinal dichloride and reduction of the intermediate aldehyde provided **1.115**. This compound was elaborated to alkene **1.118**, hydroboration of which occurred with excellent diastereoselectivity. The resulting diol was advanced to ketone **1.120** in a short sequence culminating with a Dess-Martin oxidation of a cyclohexanol. Compound **1.120** is a key intermediate in the Sato's synthesis as well as in a noteworthy approach described some years later by Alonso, *et al.* (*vide infra*).<sup>34</sup>



Scheme 1.20 The Sato synthesis of rac-TTX: assembly of the crucial ketone

Scheme 1.21 outlines the conversion of ketone 1.120 into *rac*-TTX. The anion of  $CH_2Cl_2$  added to 1.120 diastereoselectively to yield 1.121. Reaction of the latter with NaN<sub>3</sub> resulted in introduction of an azido group at the tertiary carbon. This interesting transformation is believed to involve cyclization of alcohol 1.121 to chloroepoxide 1.122, which subsequently reacts with azide ion selectively at the non-halogenated center. The resulting aldehyde reacted with TMSCN



Scheme 1.21 The Sato synthesis of *rac*-TTX: final stages

with modest diastereoselectivity and the intermediate cyanohydrin was protected to form **1.124**, which was then transformed into lactone **1.125**. Reduction of the azide and desilylation furnished **1.126**, which may well be described as the *Sato lactone*, and this compound underwent a sequence of guanidinylation, PCC oxidation, and acid-catalyzed deblocking to a mixture of *rac*-TTX and *rac*-anhydro-TTX. It will be seen in the course of this dissertation that the originally assigned structures of **1.126** and **1.127** required revision. The racemate of the natural product was thus produced in 33 steps from **1.111**.

#### 1.4.5 The Sato Synthesis of (-)-TTX

Sato and collaborators subsequently transposed the results of the successful effort just described an enantioselective route. <sup>33</sup> This entailed the assembly of enantiopure ketone **1.120** with glucose serving as the source of chirality. The target (–)-TTX was thus prepared in 33 steps  $^{21}$ 

from glucose.



Scheme 1.22 The Sato synthesis of (-)-TTX: preparation of enantiopure ketone 1.120

The synthesis started with the bis-acetonide derivative of glucofuranose, **1.128**, which was rapidly advanced to aldehyde **1.131** (Scheme 1.22). The latter underwent Henry condensation with nitromethane, and the emerging **1.132** reacted in a 1,4-sense with the anion of formaldehyde bis-phenylthioacetal. Acid treatment of the product of the latter step, followed by exposure to aqueous NaHCO<sub>3</sub>, induced formation of the nitrocyclohexane intermediate **1.133**. A series of transformations culminating with an oxidative Nef reaction produced ketone **1.136**, which was converted into enantioenriched **1.120** by transprotection. The remainder of the synthesis retraced the previous approach.

#### 1.4.7 The Alonso Formal Synthesis of rac-TTX

It seems appropriate to conclude this introductory section with a discussion of recent work by the Alonso group, leading to the Sato ketone **1.120** in an unusually concise manner.<sup>34</sup> The Alonso approach relies on the union of dihydroxyacetone acetonide, **1.138**, with nitroaldehyde **1.140** to form cyclohexanone **1.141** (Scheme 1.23). The actual merger of **1.138** and **1.140** was achieved under catalysis by pyrrolidine, and it is likely to involve an initial 1,4-addition of the enamine derived from **1.138** into **1.140**, followed by enamine-aldol cyclization. Alonso reports that this reaction may be efficiently carried out in batches of more than 10 grams.



Scheme 1.23 The Alonso synthesis: convergent construction of the cyclohexane core

Compound **1.141** was converted into the Sato ketone as shown in **Scheme 1.24**. The free alcohol was protected and the ketone was converted to olefin **1.142**. The exposure of **1.142** to a catalytic amount of TsOH in acetone induced the release of the 2-methoxyisopropyl group and isomerization of the acetonide segment to furnish **1.143**. Upjohn dihydroxylation of the latter occurred highly diastereoselectively from the face of the alkene *anti* to the allylic OH group (Kishi

effect).<sup>35</sup> Protection of the newly introduced diol as an acetonide and oxidative degradation of the furan, followed by borane reduction of the intermediate acid, provided compound **1.145**. Appropriate protection steps and oxidative Nef reaction then furnished the target **1.120**. Having successfully reached **1.120**, Alonso and coworkers claimed a formal synthesis of TTX based on the earlier work by Sato and coworkers.



Scheme 1.24 The Alonso synthesis: preparation of the Sato ketone, 1.120

Whereas cyclohexanone **1.120** is highly functionalized, it still lacks skeletal carbons C9 and C10 as well as N1. More recent work, also by Alonso and collaborators, provides an interesting solution to the problem, and introduces a unique way to build the dioxaadamantane structure.<sup>36</sup>

The previously synthesized ketone **1.141** was converted to olefin **1.146** and the acetonide protecting group was exchanged with a methyl orthoester to afford **1.147**. The latter compound was obtained with excellent diatereoselectivity. The benzoyl group was removed and the primary alcohol was oxidized to an aldehyde, in preparation for an intramolecular Henry reaction, which also proceeded with high diastereoselectivity, thereby securing the correct C9 configuration in



Scheme 1.25 The Alonso strategy for dioxaadamantane assembly

**1.149**. The stereochemical course of the Henry step is attributed to hydrogen bonding between furan oxygen, water and aldehyde. The exomethylene unit was dihydroxylated with KMnO<sub>4</sub> and protected to produce **1.150**. It is worthy of note that OsO<sub>4</sub> reacted with **1.149** to give the incorrect diastereomer of the diol as the major product (*ca.* 1:2 ratio). The use of KMnO<sub>4</sub> resulted in the preferential formation of the correct diol isomer, but in moderate yield and diastereoselectivity (*ca.* 4.3:1 ratio). The furan was then oxidatively degraded to an aldehyde, which was protected as its dimethyl acetal **1.151**. The nitro group was reduced and the resulting amine was guanidinylated to afford **1.152**, which was anticipated to advance to TTX upon acid treatment. Unfortunately, the unusual stability of the methyl orthoester prevented this from happening, and acid treatment induced a considerable degree of decomposition. The authors indicated that a similar avenue with a more labile orthoester group would be investigated. As of this writing, however, no additional details have been reported.

The paragraphs above summarize only the reported total or formal syntheses of TTX. However, the literature contains numerous synthetic studies that have yet to lead to a synthesis (total or formal) of the natural product. These efforts explore a range of noteworthy strategies, underscoring the significance of a chemical synthesis of TTX at both chemical and biological levels. Furthermore, published work leaves the reader with the distinct impression that new methodologies and novel strategies may be key to future, more concise syntheses of tetrodotoxin. Beyond the purely academic dimension, a rapid, efficient route that might be adaptable also to the synthesis of congeners would enable, for instance, a structure-activity relationship study based on the properties of suitably designed analogues. This would be of great interest for the development of analgesic agents based on the natural product. Analogues could also be employed to map voltage-gated sodium channels; adding considerably to our understanding of these important cellular features. It is with an eye toward such objectives that our group launched a total synthesis effort on the basis of new synthetic methodology devised in our laboratories: the oxidative amidation of phenols.

## **Chapter 2 The Oxidative Amidation of Phenols**

#### 2.1 The Oxidative Amidation of Phenols: A Method for C-N Bond Formation

The creation of nitrogen-carbon bonds is a key objective in synthetic organic and medicinal chemistry, because the molecular structures of countless substances of academic and practical interest, including pharmaceutical drugs, incorporate nitrogen atoms. Yet, much of the technology for the formation of N–C bonds relies on traditional reactions, such as nucleophilic substitution, reductive amination, and so on, or on more modern refinements of these classical methods. In response to various problems in synthetic organic chemistry, our group has researched new techniques for the construction of N–C bonds. A transformation that has emanated from these efforts is the oxidative amidation (OA) of phenols (Scheme 2.1): an oxidative dearomatization process that converts a phenol, typically 4-substituted such as 2.3, into a nitrogen-substituted dienone, 2.6.<sup>37-39</sup> As detailed below, compounds of type 2.6 have proven to be valuable intermediates for the chemical synthesis of diverse natural products.



Scheme 2.1 General mechanism of oxidative amidation of phenols

The "N" group in 2.3 represents a suitable nitrogen nucleophile, while the dotted semicircle indicates that "N" may be connected to the phenolic ring or be independent. The elaboration of 2.3 into 2.6 involves exposure to a hypervalent iodine(III) oxidant, often (diacetoxyiodo)benzene (DIB) or phenyliodine bis(trifluoroacetate) (PIFA). Interestingly, such iodine(III) species are the sole reagents yet identified that perform adequately in this context. On a side note, other modes of oxidative dearomatization are also recognized as valuable methods for the construction of complex polycyclic ring systems, and are adaptable to a variety of oxidants.<sup>40-42</sup> Mechanistically, the reaction is thought to proceed via an initial ligand exchange at the iodine center, leading to formation of complex 2.4. Fragmentation of the latter yields an electrophilic species, perhaps 2.5, which is then nucleophilically intercepted by "N" to form 2.6. In all known cases, the N atom in 2.6 emerges as part of an amide functionality; hence the terminology "oxidative amidation" of phenols. It should be noted that intact phenols are electron rich species that tend to react with electrophilic agents. In contrast, this technique induces the initial phenol to express electrophilic character and react with a nucleophile, thereby achieving a reversal of polarity ("umpolung")<sup>43</sup> of the aromatic nucleus.



Scheme 2.2 First-generation oxidative amidation: cyclization of phenolic oxazolines

Practical necessities provided an incentive to develop three main modes of oxidative amidation, the first of which is the oxidative cyclization of phenolic oxazolines (**Scheme 2.2**).<sup>44</sup> This method, which in our laboratory is described as the "first generation" OA technology, forms the centerpiece of enantiocontrolled total syntheses of (–)-FR901483<sup>45-46</sup> and of *Erythrina* alkaloids<sup>47</sup> (**Scheme 2.3**).



Scheme 2.3 Synthetic applications of the first generation of oxidative amidation

Difficulties encountered in the application of oxazoline technology to the synthesis of cylindricine alkaloids stimulated the development of a "second generation" solution, which entails the oxidative cyclization of sulfonamides (**Scheme 2.4**, eq. a.).<sup>48-49</sup> Related transformations include the analogous reaction of phosphoramides (eq. b.) and *N*-acylsulfonamides (eq. c.),<sup>50-51</sup> as well as the *ortho*-variants of the process (eq. d.). The chemistry shown in eq. a. enabled a straightforward synthesis of cylindricine, **2.26**,<sup>52</sup> and it is proving to be valuable in an ongoing

approach to himandrine, **2.29**.<sup>53</sup>



Scheme 2.4 Second-generation oxidative amidation: cyclization of phenolic sulfonamides and related substrates

It is important to point out that a common theme in of all the above synthetic endeavors is the stereocontrolled desymmetrization of the "locally symmetrical" dienones produced by OA chemistry as a means to create a tetrasubstituted, N-bearing, stereogenic carbon center of a given configuration. In most cases, this was achieved by harnessing a stereochemical property of the substrate in order to induce a particular reaction selectively at the level of one of the diastereotopic double bonds of the dienone (substrate-controlled asymmetric transformation).<sup>54</sup> For instance, the synthesis of *Erythrina* alkaloids relied on a nucleophilic desymmetrization, whereby the



Scheme 2.5 Synthetic applications of the second generation of oxidative amidation

chirality of the serine-derived segment of **2.14** directed the 1,4-addition of the OH group selectively (> 40:1 dr) to the pro-*R* double bond of the dienone (**Scheme 2.6**).<sup>47</sup> A similar strategy is apparent in the synthesis of **2.26**.<sup>52</sup>



Scheme 2.6 Nucleophilic desymmetrization of a dienone obtained through OA

Likewise, an ongoing synthesis of **2.29** relies on a pericyclic mode of desymmetrization via a diastereoselective Diels-Alder cyclization of **2.28** to **2.31** (Scheme 2.7, > 40:1 dr).



Scheme 2.7 Pericyclic desymmetrization of a dienone obtained through OA

#### 2.2 Bimolecular Oxidative Amidation

First- and second-generation OA methods illustrate intramolecular modes of reactivity, leading to spiropyrrolidine products. A limitation of the methodology is that it affords generally poor results in the formation of spiropiperidines. A bimolecular mode of OA was developed in order to circumvent such difficulties. In that connection, it became necessary to identify a nitrogen nucleophile that would be compatible with strong oxidants such as DIB or PIFA. It ultimately transpired that oxidative activation of a phenolic substrate in acetonitrile triggers a Ritter-like reaction, whereby the presumed phenoxonium ion **2.33** is captured by the solvent,



Scheme 2.8 The bimolecular oxidative amidation and its improvement

leading to acetamido dienones **2.34** (Scheme 2.8).<sup>55-57</sup> Substrates such as 2.35 may thus be advanced to acetamides 2.36, which cyclize efficiently upon *N*-deptotonation with NaH.<sup>55</sup>

### 2.3 Tetrodotoxin: Retrosynthetic Logic

Products of the type 2.34 nicely map onto a diversity of interesting, and challenging, natural products, a case in point being tetrodotoxin (Scheme 2.9). Disconnection of ortholactone and guanidine units reveals a highly substituted cyclohexane precursor, 2.38, which is known as tetrodamine. Among all of the possible ways to reach 2.38, one could imagine a double dihydroxylation of precursor 2.39, wherein symbols "P" represent suitable protecting groups. Dienes such as 2.39 are known to undergo faster osmylation at the exomethylene segment. A well-known example is found in Corey's landmark synthesis of longifolene<sup>58-59</sup> Accordingly, the reaction of 2.39 with  $OsO_4$ , perhaps under Upjohn conditions,<sup>60</sup> would initially install a diol system at carbons 6 and 11. This reaction may be anticipated to take place diastereoselectively in the desired sense on the basis of the so-called Kishi *anti*-effect. Thus, the β-configuration of the C-5 oxygenated functionality should direct the incoming  $OsO_4$  to the  $\alpha$ -face of the exomethylene, so long as protecting group P is silvl or alkyl, but not carbonyl-based.<sup>35</sup> A slower dihydroxylation reaction could now take place at the endocyclic C=C double bond, and the  $\alpha$ -configuration of the C-6 OH group should promote Kishi diastereoselectivity in the  $\beta$ -sense, as required for **2.38**. We note that it was unclear, at this juncture, whether the nitrogenous functionality in 2.39 would reinforce or hinder Kishi selectivity.



Scheme 2.9 Retrosynthetic analysis of tetrodotoxin

The exomethylene motif in **2.39** could be introduced by a Wittig reaction of ketone **2.40**. This material may be seen as derived from **2.41** via the stereo- and regiocontrolled introduction of an oxygenated functionality and of a formyl group equivalent onto one of the dienone double bonds. Notice that such an operation would desymmetrize **2.41** and cause the N-bearing carbon to become stereogenic. Finally, retron **2.41** is recognized as the product of bimolecular oxidative amidation of phenol **2.42**. An evaluation of the number of steps that would be needed to elaborate **2.42** into TTX returned an attractive answer: about 25. This would be shorter than the best available alternatives (see section 1.4). A synthesis of TTX according to such principles would further demonstrate the value of OA technology and illustrate the first synthetic application of the bimolecular reaction.

This dissertation focuses on the translation of the above hypotheses into practice.

# **Chapter 3 Previous Work in Our Group**

The successful route to ( $\pm$ )-tetrodotoxin described herein rests on the pioneering studies carried out by a former group member, Ms. Jaclyn Chau.<sup>61</sup> Building upon even earlier work by one of her predecessor, Mr. (now Dr.) Brian Mendelsohn,<sup>62</sup> Chau optimized the route to nitroketone **3.1** and demonstrated that the latter can be advanced to nitrile **3.4** by treatment with a catalytic amount of Cu(OAc)<sub>2</sub> and *N*-ethylpiperidine, followed by fragmentation of the resultant isooxazoline **3.3** with methanolic Li<sub>2</sub>CO<sub>3</sub> (**Scheme 3.1**). The conversion of **3.1** into **3.3** may be described as a Machetti-De Sarlo reaction, after the researchers who studied this general type of transformation.<sup>63</sup> Nitrile **3.4** was then elaborated to more advanced TTX intermediates.



Scheme 3.1 The Machetti-De Sarlo reaction on the  $\alpha$ -nitroketone

The Chau sequence leading to **3.1** is outlined in **Scheme 3.2**. Bimolecular oxidative amidation of commercial **3.5** furnished **3.6**,<sup>57</sup> which was selectively reduced to a 2.4 : 1 mixture of  $\alpha$ - and  $\beta$ -alcohol diastereomers upon reaction with borane-Me<sub>2</sub>S complex and a catalytic amount of (*S*)-(–)-2-methyl-CBS-oxazaborolidine<sup>64</sup>. The CBS method is the only one that provided selectivity, however weak, for  $\alpha$ -alcohol diastereomer **3.7**, which was later revealed to perform

better than its isomer **3.8** in the synthetic sequence. By contrast, reagents such as DIBAL preferentially afforded the  $\beta$ -alcohol diastereomer.<sup>62</sup> Alcohols **3.7** and **3.8** are sensitive, polar compounds that do not withstand purification by chromatography. Accordingly, the mixture of the two was directly subjected to protection, leading to TBDPS ethers **3.9** and **3.10**. Notice that **3.7/3.8** lack the hydroxyl group corresponding to the ultimate C9 OH. The reason is that analogues of **3.5** bearing a benzylic oxygen functionality were poor substrates for the oxidative amidation step. Work by Canesi and coworkers<sup>65</sup> suggests that this may be due to the unwanted occurrence of pinacol-type reactions, leading to a range of byproducts.



Scheme 3.2 Preparation of a Machetti-De Sarlo substrate 3.1

Without separation, compounds **3.9** and **3.10** were saponified and the emerging carboxylic acids **3.11** and **3.12** were advanced to nitroketones **3.1** and **3.2**. It is now obvious that the ketone in **3.6** had to be reduced prior to nitroketone construction to prevent undesired Michael type cyclizations.

The next phase of the work centered on the cyclization of **3.1** to **3.3**; formally, a nitrile oxide cycloaddition. This type of reaction is often carried out by dehydrating the nitro compound to a nitrile oxide with appropriate reagents.<sup>66</sup> In the case of **3.1**, however, it was found that the best way to achieve conversion into **3.3** was through a Machetti-De Sarlo reaction. It should be stressed that, in its original form, this step afforded variable yields of isooxazoline, necessitating a thorough study of reaction conditions (*vide infra*). Regardless, the preferred diastereomer **3.3** could be isolated in pure form at this stage. A subsequent Kemp fragmentation produced *cis*- $\beta$ -hydroxynitrile **3.4** in excellent yield.



Scheme 3.3 Preparation of 3.19

The elaboration of **3.4** to more advanced intermediates started with the protection of the secondary alcohol with BOMCl. The methyl ester in **3.13** and later intermediates tended to promote aromatization through elimination of the acetamido functionality during base-promoted

reactions. Consequently, **3.13** was reduced with lithium borohydride and the resultant primary alcohol **3.14** was also protected as a BOM ether. The TBDPS group was then released, the liberated alcohol was oxidized to  $\alpha$ , $\beta$ -unsaturated ketone **3.17** with Dess-Martin periodinane, and a Wittig reaction delivered **3.18**. The latter compound would have to undergo double dihydroxylation to install a tetraol system. It will be recalled that the stereochemical outcome of such osmylation reactions was anticipated to be controlled by the Kishi effect. Indeed, exposure of **3.18** to Upjohn-type conditions produced diol **3.19** with the correct configuration. Unfortunately, the endocyclic olefin was unreactive under Upjohn condition, and diol **3.19** was the only product recovered from the reaction.



Scheme 3.4 Dihydroxylation of 3.20 directed by Donohoe effect

The diol was then protected as acetonide **3.20**, in preparation for the dihydroxylation of the endocyclic olefin under more vigorous conditions, with stoichiometric osmium tetroxide in pyridine. Under these conditions, the alkene did react; however, the resulting diol **3.21** emerged with the incorrect configuration. This stereochemical outcome is in accord with observations by

Donohoe and coworkers, who determined that under anhydrous conditions, an allylic acetamido unit tends to direct the incoming  $OsO_4$  to the *syn* face of the  $\pi$  bond, arguably through a hydrogen bonding interaction (*cf.* **3.22**).<sup>67</sup> Evidently, the Donohoe effect overrides the Kishi one in the present case.



Scheme 3.5 Exploratory research from 3.21

The undesired diol stereoisomer served to explore a number of more advanced reactions. In particular, the compound was protected as acetonide **3.21** and then the BOM groups were released. Primary alcohol **3.24** reacted with the Dess-Martin periodinane to afford a presumed aldehyde, which instantly cyclized to lactol **3.25**. This product seemed useful for the introduction of the still missing C9 OH group. Accordingly, it was advanced to the corresponding vinyl ether **3.26**, which arguably could react with appropriate oxidants (OsO<sub>4</sub>, *m*CPBA, *etc.*) from the more exposed  $\beta$ -face, ultimately enabling the installation of the C9 hydroxyl with the correct configuration. Preliminary experiments along these lines yielded promising results. However, the products emanating from such reactions were not thoroughly characterized. It is worthy of note that all

acetamide-containing compounds shown above were quite polar. In many cases, this complicated chromatographic operations.

Whereas the foregoing demonstrates the potential of our strategy, it also highlights a series of issues that needed to be corrected if our efforts were to be successful. Specifically:

- i) The Machetti-De Sarlo reaction had to be optimized;
- ii) The diastereoselectivity of the reduction of dienone **3.6** had to be improved;
- iii) A way to avoid carrying the diastereomeric mixture of 3.7/3.8 on for 4 steps had to be devised, in order to simplify purification and analytical work;
- iv) A viable substitute for the acetamide unit had to be identified, to facilitate handling and purification of the various synthetic intermediates;
- v) The incorrect facial selectivity of the osmylation of the endocyclic alkene needed to be rectified;
- vi) Some redundant steps had to be eliminated;
- vii) The cyano group needed to be reduced to an aldehyde, or equivalent thereof, at an appropriate stage.

Solutions to all these issues, culminating in a successful formal synthesis of tetrodotoxin, will be discussed in Chapter 4.

# Chapter 4 Formal Synthesis of rac-Tetrodotoxin

#### 4.1 Optimization of the Machetti-De Sarlo Step

The best conditions for Machetti-De Sarlo reaction of nitroketone 3.1/3.2 entailed heating at 30 °C for 168 h in a 0.2 M CHCl<sub>3</sub> solution in the presence of 5 mol% of Cu(OAc)<sub>2</sub> and 10 mol% of *N*-ethylpiperidine, resulting in 40-50% of a mixture **3.3** and its diastereomer (Scheme 3.1, p. 35). Attempts to accelerate the reaction by the use of more Cu(OAc)<sub>2</sub>, and especially more base, promoted formation of byproducts, as did higher concentrations. The above protocol was satisfactory on scales of up to 6 g of substrate, but it suffered from a number of shortcomings. First, the yield of isooxazoline was moderate. Second, the reaction required an induction period of 15-20 h before the isooxazoline would begin to form. Third, the reaction failed to reach completion, unreacted nitroketone remaining even after 7 days. Fourth, it tended to stall, and addition of more catalyst to stalled reactions failed to revive them. Fifth, significant quantities of acids **3.11/3.12** accompanied the desired isooxazolines. The acids could only form by hydrolysis of the nitroketone through a process that would release nitromethane, but because reagents and solvents had been carefully dried, it seemed likely that the water molecule required to cleave 3.1 was the one liberated by the reaction itself. Numerous small-scale reactions were thus carried out in CDCl<sub>3</sub> solutions (in regular NMR tubes), with monitoring by <sup>1</sup>H NMR, in order to garner a better understanding of the process. These reactions were carried out with ca. 30 mg of nitroketone and 0.6 mg of Cu(OAc)<sub>2</sub>·H<sub>2</sub>O (weighed as a solid) dissolved in 0.6 mL of a stock

solution of *N*-ethylpiperidine (3.0  $\mu$ L) in CDCl<sub>3</sub> (2 mL). This solution thus contained 0.9  $\mu$ L of *N*-ethylpiperidine. The amount of paramagnetic Cu(II) present in the mixture induced an insignificant extent of line broadening, enabling close monitoring of the progress of the reaction by 300 MHz <sup>1</sup>H NMR. These experiments revealed that release of MeNO<sub>2</sub> (singlet at 4.33 ppm) began to occur after about 15-20 h, at about the same time that the isooxazoline was becoming apparent in the <sup>1</sup>H NMR spectrum. In an effort to contain / suppress MeNO<sub>2</sub> release, *i.e.*, the formation of acids **3.11/3.12**, the effect of adding drying agents to the reaction mixture was examined. Molecular sieves inhibited isooxazoline formation. It seems likely that this was due to protonation of *N*-ethylpiperidine by the acidic molecular sieves, an event that would deny the



Scheme 4.1 Formation of byproducts during the Machetti-De Sarlo reaction

reaction an essential basic agent. Powdered, anhydrous  $Na_2SO_4$  had no effect, but  $CaSO_4$ (powdered white Drierite<sup>®</sup> activated by heating under vacuum) significantly diminished  $MeNO_2$ formation, without fully suppressing it. However, reactions run in the presence of  $CaSO_4$  still tended to stall, and could not be resurrected by the addition of more catalyst.

A byproduct detected during NMR monitoring of the reaction, compound **4.6** (Scheme **4.1**), and the observation that running the reaction at higher temperatures in the presence of CaSO<sub>4</sub> induced only a greater extent of formation of **4.6** without accelerating isooxazoline formation, provided a clue as to the source of the above problems. A sensible mechanism for the formation of **4.6** starts with reversible deprotonation of the AcNH group, either in the free nitroketone (*cf.* **4.1**) or in a Cu(II) chelate thereof (*cf.* **4.2**). Such events lead to the release of nitroacetone, a good ligand for Cu(II) that can sequester the metal and bring the catalytic cycle leading to the isooxazolines to a halt. Moreover, the *O*-terminus of the anion of the acetamide could add to the keto carbonyl (*cf.* **4.1** to **4.3**), triggering release of MeNO<sub>2</sub> and formation of acids **3.11/3.12** upon hydrolysis of azlactones **4.4**. On such a basis, it seemed desirable to replace the *N*-acetyl with a less N–H acidifying and less *O*-nucleophilic Boc group.<sup>68-69</sup>

#### 4.2 Preparation of *N*-Boc Protected Analogues

The exchange of the *N*-acetyl with an *N*-Boc group was anticipated to address issues ii-v above (p. 40) simultaneously. Indeed, *N*-Boc-protected intermediates were expected to be considerably less polar, and therefore more readily handled, than the corresponding acetamides.

The poorer H-bonding ability of an *N*-Boc group could well restore Kishi selectivity during the dihydroxylation of substrates of the type **3.20** (**Scheme 3.4**, p. 38). The bulk of the *N*-Boc group should ensure a greater degree of diastereoselectivity in the desired sense during dienone reduction. Furthermore, the *N*-Ac group would have to be released in any event toward the end of the synthesis. In all likelihood, such an objective would be achieved by activation of the acetamide as an *N*-Boc imide, followed by selective release of the acetyl group.<sup>70</sup> Finally, advanced TTX intermediates wherein the N1 nitrogen is Boc-protected have been successfully employed by both Sato and Du Bois, providing us with an opportunity to connect with other syntheses.<sup>31-33</sup> Our first objective thus became the conversion of **3.6** into its *N*-Boc analogue.



Scheme 4.2 Protection of 3.6 and the side products

Reaction of **3.6** with Boc<sub>2</sub>O and DMAP afforded the desired **4.7** in 61% yield, along with byproducts **4.8**, **4.9** and **4.10** (16%, 10%, 4% yield respectively, **Scheme 4.2**). The use of trimethylamine (1 equiv) in addition to DMAP greatly increased the extent of formation of **4.8** at the expenses of **4.7**, which became a minor component of the product mixture. This reaction

would typically be complete within 48 h at room temperature. Prolonged exposure was to be avoided, as it promoted conversion of **4.7** into **4.9**.

It seems appropriate to comment about the presumed mechanism of the formation of the various byproducts. Substance **3.2** appears to result through the sequence of events outlined in **Scheme 4.3**. Thus, reversible deprotonation of the *N*-Boc group triggers Michael cyclization of anion **4.11** and *O*-acylation of the resultant enolate **4.12** by Boc<sub>2</sub>O present in the reaction medium.



Scheme 4.3 Presumed mechanism of formation of 4.8

The genesis of compound **4.9** may be rationalized based on work by Carreño and collaborators.<sup>71</sup> Thus, reversible deprotonation of the ester, Michael cyclization of the nascent enolate and *O*-acylation of the product enolate may produce intermediate **4.15**, which is likely to undergo disrotatory electrocyclic opening<sup>72-73</sup> to the observed **4.9** (Scheme 4.4).



Scheme 4.4 Presumed mechanism of formation of byproduct 4.9

On a side note, byproduct **4.9** was easily converted into tropone **4.17** by acid-catalyzed release of the Boc group and oxidation of the resultant ketone **4.16** with DDQ in hot toluene (**Scheme 4.5**). Compounds **4.9**, **4.16**, and **4.17** are all potentially valuable for the construction of polycyclic systems.<sup>71</sup>



The mechanism of **Scheme 4.4** implies that the deprotonation of ester **4.7** is facile. Indeed, numerous side reactions observed in the course of this research are attributable to the intervention of enolate **4.13**. In the present context, formation of **4.13** also accounts for the appearance of byproduct **4.10**. Thus, E1cB elimination of the imide group yields presumed dienone **4.18**, which may react with more **4.13** to furnish **4.19**. The *O*-acylation of **4.19** sets the stage for a second E1cB event that produces **4.22**, and ultimately **4.10** (**Scheme 4.6**).

The desired **4.7** was isolated in pure form by column chromatography and subjected to CBS reduction under the same conditions detailed earlier (**Scheme 3.2**, p. 36). The reaction took place with an improved 4.9:1 diastereoselectivity, and the mixture of alcohols thus obtained was immediately advanced to *O*-TBDPS derivatives **4.25** and **4.26**. Owing to the presence of the more lipophilic Boc group, these intermediate were considerably easier to handle and purify


Scheme 4.6 Tentative sequence leading to byproduct 4.10

relative to the corresponding Chau compounds. Furthermore, **4.25** was a highly crystalline material that was obtained in stereochemically pure form upon crystallization from methanol. The mother liquor contained a 1:1 mixture of **4.25** and **4.26**, which were readily separated by column chromatography, followed by further crystallization of the desired **4.25** from MeOH. In this manner, all subsequent operations were carried out with diastereochemically homogeneous material (p. 40). Furthermore, the structure of **4.25** was ascertained by X-ray diffractometry (**Figure 4.1**).



On a final note, the silvlation of **4.23/4.24** took place at an appreciably slower rate compared to the analogous reaction of **3.7/3.8** (Scheme 3.2 p. 36). Whereas the protection of **3.7/3.8** was

conveniently carried out in  $CH_2Cl_2$  at rt, that of **4.23/4.24** required heating in DMF at 70 °C order to achieve an acceptable rate. Also, the reduction of dienone **4.7** with only  $BH_3$ 'SMe<sub>2</sub> complex afforded virtually the same diastereoselectivity as that observed under CBS conditions. However, the yield of **4.23/4.24** was appreciably lower, and variable quantities of very polar byproducts were observed. These secondary products might have resulted from hydroboration of the ring alkenes. The CBS method was thus employed only to maximize chemoselectivity. Even so, it was very important not to exceed a 1 h contact time during the CBS reduction, because longer exposure again resulted in diminished yields of desired alcohols and the formation of polar materials.



Figure 4.1 X-ray crystal structure of 4.25

In preparation for the crucial Machetti-De Sarlo reaction, compound **4.25** had to be advanced to acid **4.27**. To that end, substance **4.25** was treated with aqueous LiOH in order to achieve

simultaneous release of the *N*-acetyl group and saponification of the methyl ester. However, the acidity of the  $\alpha$ -proton of the methyl ester undermined this plan. Thus, contact of **4.25** with aqueous LiOH resulted in formation of a 1:3:2 mixture of **4.27**, **4.28** and **4.29** (Scheme 4.8). The use of a milder base, such as Na<sub>2</sub>CO<sub>3</sub>, resulted only in slow conversion of **4.25** into aromatic products. Evidently,  $\alpha$ -deprotonation of the ester and consequent elimination of the imide system competed effectively with nucleophilic attack on carbonyl systems.



Scheme 4.8 Proposed mechanism of the formation of 4.29 during hydrolysis of 4.25

The problem was circumvented by selective *N*-deacetylation of **4.25** with hydrazine monohydrate, followed by aqueous LiOH treatment of **4.32** and workup under gently acidic conditions. The desired **4.27** was thus obtained in quantitative crude yield (**Scheme 4.9**).



Scheme 4.9 Preparation of acid 4.27

The now familiar nitroketone **4.33** was prepared by the same method detailed earlier (activation of **4.27** by CDI followed by addition of potassium nitromethanide, **Scheme 3.2**, p. 36), except that the condensation of the imidazole derivative of **4.27** with potassium nitromethanide required slightly higher temperatures (40  $\degree$  instead of rt) for best results. Further optimization of the Machetti-De Sarlo reaction was carried out with substrate **4.33**.



Scheme 4.10 Preparation of 4.34 without purification of intermediates

The behavior of *N*-Boc nitroketone **4.33** under the Machetti-De Sarlo conditions of **Scheme 3.1** (p. 35) paralleled that of the *N*-acetyl congener. However, and in sharp contrast to **3.1**, an increase in the amount of base (30 mol% of *N*-ethylpiperidine vs. the original 10 mol%) accelerated the conversion of **4.33** into the desired isooxazoline with no significant increase in formation of the byproducts shown in **Scheme 4.10**. This it is consistent with the diminished N–H acidity and *O*-nucleophilicity of the *N*-Boc compounds relative to *N*-Ac materials. As before, an increase in the amount of Cu(OAc)<sub>2</sub> (10 mol% vs. 5 mol%) resulted only in the formation of more of **4.35** while the addition of CaSO<sub>4</sub> diminished the extent of MeNO<sub>2</sub> release without affecting rates and yields. More beneficial was the use of MeCN as a solvent in lieu of  $CHCl_3$ : a modification that induced the reaction to proceed to completion. Finally, the addition of silica gel to the reaction medium and – more importantly – conducting of the reaction in more dilute solutions (0.04 M) produced the best results. Just as seen earlier in the *N*-acetyl series, **4.25** was reproducibly advanced to isooxazoline **4.34** in 34% overall yield after chromatography over a 4-step sequence encompassing *N*-deacetylation, ester saponification, nitroketone synthesis and Cu(II)-catalyzed cyclization, without purification of intermediate products. With respect to the latter, we stress that that ester **4.32** and nitroketone **4.33** are not particularly stable, and are prone to undergo retro-Mannich aromatization to substances of type **4.35**. It is much better to process these intermediates through the sequence without purification beyond a rapid filtration through a plug of silica gel, to minimize loss of material.

Chromatography of **4.34** also returned quantities of acid **4.27** (typically 25-30% yield based on **4.25**), which was conveniently recycled. Contrary to **4.32** and **4.33**, acid **4.27** is reasonably stable and may be stored at -20 °C for several weeks without any obvious sign of decomposition.

A final refinement of the sequence leading to **4.34** was achieved by avoiding the aqueous workup of the Machetti-De Sarlo reaction. While in the earlier workup the reaction mixture would be washed with aqueous 0.02 M HCl solution to remove *N*-ethylpiperidine and inorganic species, it was found that simply concentrating it to dryness, applying the residue directly to a silica gel column, and eluting with 10% EtOAc/hexanes, reproducibly afforded pure isooxazoline **4.34** in 40-50% yield over four steps from ester **4.25**.

It should be noted that the Machetti-De Sarlo reaction converts an achiral substrate, **4.33**, into a chiral product, **4.34**, thereby breaking the symmetry of the starting material. An asymmetric variant of the process, leading to enantioenriched **4.34** and ultimately to enantioenriched TTX, might be possible through the use of appropriate chiral Cu(II) complexes.<sup>74-75</sup> Some preliminary experiments in that sense were carried out, but the issue was not thoroughly explored. A summary of our results is provided in Chapter 5.



Scheme 4.11 Fragmentation of 4.34 and preparation of 4.38

Nucleophilic fragmentation of isooxazoline **4.34** (Li<sub>2</sub>CO<sub>3</sub>/MeOH) took place in quantitative crude yield to afford **4.36** (Scheme 4.11), which was free from contaminants (NMR) and therefore required no further purification. The practicalities of the synthesis imposed a reduction of the methyl ester at this point. This was achieved in moderate yield (62%) by the use of LiBH<sub>4</sub>. A contact time of approximately 24 h was necessary to achieve complete reduction of the ester. Such a long reaction time promoted the formation of some byproducts, the structures of which were not determined. It is noted, however, that the major byproduct (*ca.* 15% yield) exhibited a

chromatographic mobility very similar to that of the desired **4.37**. Its <sup>1</sup>H NMR and Mass spectra were also similar to those of **4.37**. This suggests that the major byproduct is a diastereomer – possibly a nitrile epimer – of **4.37**.



Scheme 4.12 Attempted formation of aldehyde 4.41

A comment is in order at this juncture. Nitrile epimerization might have been suppressed if a more readily reducible carbonyl group, *e.g.*, an aldehyde, were to replace the ester in **4.36**. In principle, isooxazoline **4.34** could be advanced to aldehyde **4.41** by reduction of the ketone and fragmentation of the resulting **4.40** (Scheme **4.12**). Reaction of **4.34** with NaBH<sub>4</sub> rapidly afforded alcohol **4.40** as a single diastereomer (within the limits of 300 MHz <sup>1</sup>H NMR spectroscopy). The convex shape of the molecule makes it very likely that the OH group in **4.40** was of  $\beta$ -configuration, but this was not ascertained. Unfortunately, various attempts to induce fragmentation of **4.40** to **4.41/4.42** were unsuccessful. Consequently, the matter was not pursued at the present stage.

Diol **4.37** was protected as the bis-BOM derivative **4.38** in 88% yield (Scheme **4.11**). It was essential to employ DIPEA freshly distilled from  $CaH_2$  in this step, in order to suppress formation of byproducts **4.39** (up to 15% of the product mixture). In all likelihood, these formed upon

hydrolysis of BOM-Cl by adventitious water introduced with "moist" DIPEA, release of formaldehyde, reaction of one or both OH groups in **4.37** with HCHO, and BOM protection of the resulting methylol derivatives. Whereas the formation of **4.39** is not prejudicial to the success of the synthesis, their presence complicated analytical work and disallowed the conduct of subsequent operations with pure intermediates.

## 4.3 Facial Selectivity of Dihydroxylation at C6, C7, C8 and C11 Positions

It should be recalled that Chau found that *N*-acetyl endocyclic olefins of the type **3.20** (Scheme 3.4, p. 38) are poorly reactive toward OsO<sub>4</sub>. Identical difficulties were observed in the Boc series. Thus, elaboration of **4.38** to diene **4.45** followed by catalytic osmylation produced only diol **4.46**. Either in free (**4.46**) or protected (**4.47**) form, this material resisted further Upjohn type dihydroxylation.

Fortunately, compound **4.38** itself proved to be a reasonably competent substrate for catalytic dihydroxylation (**Scheme 4.14**, p. 56). The original Upjohn procedure performed poorly with **4.38**, and this for a number of reasons. First, the reaction was extremely slow at room temperature. Operation at 35  $^{\circ}$  improved the rate slightly. However, the relatively high vapor pressure of OsO<sub>4</sub> (7 mmHg, 20  $^{\circ}$ C)<sup>76</sup> resulted in sublimation of the reactant over the long periods of time required for the reaction. As observed also by other researchers,<sup>77</sup> a black stain of probably Os(IV) species appeared on the upper surface of reaction flasks. Portions of this stain redissolved upon contact with the reaction mixture (which contained NMO) and sonication.



Scheme 4.13 Preparation of 4.47, a discontinued route

However, osmium deposits in the neck of the flask and the plastic cap (rubber septa cannot be used because  $OsO_4$  reacts with double bonds present in rubber) could not be recovered this way. Moreover, a dark, highly insoluble residue of osmium species deposited on Teflon-coated stirring bars, the use of which should be avoided. The absence of the stirring bar was not an issue, since the reaction mixture was homogeneous. Even so, only a low 10% conversion was achieved after 7 days at 35 °C in the presence of 5 mol%  $OsO_4$  and 3 equiv of NMO in 10:1 acetone/H<sub>2</sub>O. Furthermore, the reaction slowed down almost to a stall after 96 h, indicating that the osmium might be sequestered by product **4.50** itself, thus bringing the catalytic cycle to a halt. Alternative co-oxidants and/or additives, such as  $K_3[Fe(CN_6)]/K_2CO_3^{78}$ ,  $K_3[Fe(CN_6)]/K_2CO_3/DABCO^{79}$ , NMO/MsNH<sub>2</sub><sup>80</sup>, were tested, but these gave consistently inferior results. The action of RuCl<sub>3</sub>/NaIO<sub>4</sub><sup>81-82</sup> or (*n*-C<sub>16</sub>H<sub>33</sub>NMe<sub>3</sub>)MnO<sub>4</sub><sup>83</sup> on **4.38** resulted only in partial decomposition. A

good solution eventually emerged as follows.



Scheme 4.14 Upjohn dihydroxylation on 4.38

Sharpless and collaborators discovered that citric acid can boost the rate of Upjohn dihydroxylation reactions.<sup>77</sup> The reasons for this are not entirely clear, but the following rational has been offered. An "ordinary" Upjohn osmylation proceeds via the two simultaneous catalytic cycles shown in Scheme 4.15. An initial addition of  $OsO_4$  to the alkene affords 4.52, which is oxidized to 4.53 upon reaction with NMO. Complex 4.53 can either undergo hydrolysis, and consequent liberation of the product diol, or react with a second molecule of 4.51, leading to 4.55. The latter, a green substance, may also undergo hydrolytic release of the diol ligands, and indeed, in either case, the rate-limiting step is the hydrolysis of diolato complexes 4.53 and 4.55. However, the rate of release of the diol from 4.53 and 4.55 may become so slow that the latter will react with water to form preferentially dihydroxo complex 4.56. This material may be deprotonated by N-methylmopholine to yield the red-brown dianion 4.57, which being an 18-electron complex, is extremely stable toward hydrolysis. If the rate of diol release from 4.53 and 4.55 is slow, the metal will advance to virtually inert complex 4.57 and the catalytic cycle will come to a halt.



Scheme 4.15 Simultaneous reaction pathways in the Upjohn osmylation of an alkene

Citric acid acts first of all by keeping the pH of the medium sufficiently low that deprotonation of **4.56** is no longer a problem. At lower pH values, however, Os(VI) complexes such as **4.55** are prone to undergo disproportionation to generate insoluble, inert Os(IV) species.<sup>77, 84</sup> A second, equally important role of citric acid is to combine with  $OsO_4$  to yield complex **4.58**. The reaction of the latter with the substrate leads to **4.59** (Scheme 4.16), which resists disproportionation. Either **4.59** or its hydrated form **4.60** may now undergo hydrolysis.

In accord with the foregoing, compound **4.38** was treated with 4 mol%  $OsO_4$ , 1.1 equiv NMO, 2 equiv citric acid, 1:1:2 H<sub>2</sub>O/*t*-BuOH/acetone (acetone was required to homogenize the mixture) at rt. A slow reaction ensued, which stalled after 10 days. Nonetheless, the desired diol **4.50**, the correct configuration of which was ascertained later, was isolated in 35% yield, together with unreacted **4.38** (60% yield). In the present case, omission of *t*-BuOH proved to be beneficial to retain osmium within the solution; furthermore, the rate of the reaction could be accelerated by operating at 50 °C in a sealed, thick-walled glass vessel. Still, the reaction stalled after about 2 days, and by the start of the third day, the mixture had become chartreuse-colored. This was



Scheme 4.16 Mechanistic proposal for alkene dihydroxylation with OsO4/NMO/citric acid

attributed to the build-up of diol **4.50** (in accordance with **Scheme 4.16**, diol **4.54**) in the reaction medium and the consequent shifting of the equilibrium between **4.60** and **4.61** toward the green-colored diolato complex **4.60**. It is known that the stability of species such as **4.60** increases with the increasing electron-deficient character of the diol ligand.<sup>77</sup> The desired **4.50** is undoubtedly electron-deficient due to the inductive effect of the heteroatomic functionalities flanking the diol system. Regardless, the introduction of one additional equivalent each of citric



Scheme 4.17 Dihydroxylation of 4.38 and stereochemical proof

acid and NMO on the third and fourth days resulted in loss of color and reactivation of the catalytic system. By the fifth day, the catalyst seemed to be no longer recoverable and the reaction was worked up to afford diol **4.50** in 49% yield, as well as unreacted **4.38** in 44% yield (**Scheme 4.17**). On the plus side, it was determined that only 3 mol%  $OsO_4$  were necessary to attain such a level of conversion. We conclude this section by noting that **4.46** resisted dihydroxylation even under the new conditions (**Scheme 4.18**).

Diol **4.50** thus obtained was clearly a single diastereomer (within the limits of <sup>1</sup>H NMR spectrometry), but its relative configuration was unknown. An extensive 2D NOESY NMR study of derivative **4.63** provided a stereochemical proof. Compound **4.63** was prepared by protection of the diol as an acetonide, followed by release of the silyl group. As shown in **Scheme 4.17**, the configuration of **4.63** was unequivocally established thanks to the indicated NOE correlations, which are consistent only with diastereomer **4.63**. Such an assignment was later corroborated by single crystal X-ray diffractometry of more advanced intermediates.



Scheme 4.18 Failure of the dihydroxylation of 4.46 even under Sharpless conditions

The next phase of the research aimed to advance **4.63** to ketone **4.64**, which was destined to undergo Wittig methylenation in preparation for conversion into compound **4.65** (Scheme **4.19**).

The oxidation of **4.63** to **4.64** proceeded efficiently with the Dess-Martin periodinane.<sup>85</sup> Indeed, ketone **4.64** was obtained in 87% overall yield over three steps from **4.50**. However, and in contrast to enone **4.44** (**Scheme 4.13**, p. 55), ketone **4.64** was entirely inert toward methylene triphenylphosphorane.



Scheme 4.19 Proposed elaboration of compound 4.63

Methylenation was therefore attempted though a Peterson reaction.<sup>86</sup> Commercial trimethylsilylmethylmagnesium chloride also failed to add to **4.64**, but the more reactive trimethylsilylmethyllithium, another article of commerce, reacted with **4.64** exclusively (within the limits of 300 MHz <sup>1</sup>H NMR spectroscopy) from the  $\alpha$ -face to form alcohol **4.68**. Alkoxide **4.67** was protonated with AcOH at –78 °C and the configuration of alcohol **4.68** was ascertained by a 2D NOESY NMR study. The observed NOE correlations shown in **Scheme 4.20** are in accord only with diastereomer **4.68**. The stereochemical course of this reaction is in accord with the Felkin-Anh model<sup>87</sup> and in the present case may be also enforced by the convex shape of the molecule.



Scheme 4.20 Transformation and its mechanism from 4.63 to 4.68

Elimination of trimethylsilanol from 4.68 was induced by treatment with PPTS<sup>88</sup> in 1,2-dichloroethane at 60 °C. This reaction was slow, requiring about 24 h to complete. Remarkably, the product was not the expected nitrile 4.65, but rather carboxamide 4.69 (Scheme 4.21). A clue as to the reason for this unanticipated outcome emerged from an experiment, in which a reaction mixture containing alkoxide 4.67 was allowed to warm to above -50 °C, whereupon iminolactone 4.70 resulted. This material was not thoroughly purified or fully characterized, but its mass, <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded, and when treated with PPTS



Scheme 4.21 Transformation of 4.68 into carboxamide 4.69 and mechanistic proposal

in DCE at 60 °C, it rapidly (*ca*. 0.5 h) yielded carboxamide **4.69**. A mechanistic proposal for the formation of **4.69** upon reaction of **4.68** with PPTS in DCE thus invokes a slow acid-promoted cyclization to **4.71**, followed by rapid fragmentation to the final carboxamide.

The Upjohn dihydroxylation of amide **4.69** was briefly examined at this stage. The reaction was slower than that of substrates **4.76/4.79** (*vide infra*). Furthermore, the highly polar nature of **4.72** complicated isolation and purification, while attempted acetonide protection resulted in partial formation of the *N*-methoxyisopropyl derivative of the amide. These technical difficulties forced us to turn instead to a reduction of the amide to a primary alcohol.



Scheme 4.22 Dihydroxylation of 4.69

A number of methods for the reduction of a carboxamide to an alcohol have been described,<sup>89-95</sup> one of which involves the conversion of the substrate to an *N*-acylformamidine, reaction of the latter with methanol, leading to an ester, and its final reduction.<sup>95</sup> The first step of the sequence involves the reaction of the primary amide with dimethylformamide dimethylacetal (DMF-DMA). However, such a treatment of **4.69** produced only **4.73** (**Scheme 4.23**) and we were unable to advance it to **4.75**.



Scheme 4.23 An unsuccessful attempt to convert 4.69 into 4.75

It seemed more logical to activate **4.69** as a Boc derivative prior to reduction.<sup>90</sup> To that end, amide **4.69** was treated with Boc<sub>2</sub>O and Et<sub>3</sub>N, in the presence of DMAP. This step afforded no mono-Boc derivative of **4.69**, but only the bis-Boc analogue **4.76** (Scheme **4.24**). This outcome is consistent with precedent suggesting that greater the N–H acidity of the mono-Boc derivative promotes a faster attachment of the second Boc group.<sup>96</sup> The crude reaction product was of reasonably good quality (it seemed to be contaminated only by a small amount of Boc<sub>2</sub>O) and it was advanced into the synthetic sequence without purification. It should be noted that the mass recovery for this step, hence the crude yield of **4.76**, was not satisfactory, and that the reaction was very inefficient in the absence of triethylamine or when substoichiometric quantities of DMAP were employed. No significant improvement was observed when a substantial excess of reagents were used. Thus, the action of 4 equiv Boc<sub>2</sub>O, 7 equiv Et<sub>3</sub>N, and 1 equiv DMAP on **4.69** still gave a mass recovery of about 70%. At this time, the reasons for this remain unclear.



Scheme 4.24 Activation of 4.69 and reduction of bis-Boc derivative 4.76

Compound **4.76** seemed to be a better substrate for reduction, given the stronger activation provided by the two Boc groups. Contrary to a literature report,<sup>90</sup> bis-Boc derivative **4.76** proved to be entirely inert to NaBH<sub>4</sub>. Exposure to stronger reducing agents, DIBAL or LiBHEt<sub>3</sub>, promoted only migration of one of the Boc groups to the N1 nitrogen to afford compound **4.77** (Scheme **4.24**). By way of mechanism, one may presume that the metal hydride deprotonated the N1 carbamate, which elicited Boc group migration to produce a less basic *N*-anion. Compound **4.77** resisted further reduction, probably because its significant N–H acidity, and consequent deprotonation upon exposure to metal hydrides, leads to an anion that makes the carbonyl insufficiently electrophilic to undergo reduction. The action of LiBH<sub>4</sub> on **4.76** was more successful, resulting in conversion into a mixture of desired alcohol **4.78** and rearranged product **4.77**. The ratio between **4.77** and **4.78** varied and larger scale operation favored the formation of **4.77**.

Although the reduction sequence was clearly unsatisfactory, the search for a better solution was postponed in favor of a study of the osmylation of the alkene. Alcohol **4.78** was

chromatographically separated from **4.77**, which was recycled as detailed in the next section. Protection of **4.78** as a TBDPS ether provided **4.79**, which was subjected to Upjohn dihydroxylation conditions (no citric acid was required in this case). This step was carried out with some trepidation, as Alonso and coworkers had observed that the stereochemical course of the same reaction of a related substrate was opposite the Kishi reactivity model (**Scheme 1.25**, p. 25). Fortunately, **4.79** reacted with exclusive (within the limits of 300 MHz <sup>1</sup>H NMR spectroscopy) Kishi selectivity, as determined by a later single-crystal X-ray diffractometric analysis, and the resulting diol was protected as acetonide **4.81**.



Scheme 4.25 Kishi selectivity in the dihydroxylation of 4.79

In principle, substance **4.77** could be recycled by introducing a second Boc group on the imide segment, followed by reduction. This possibility was explored, but it was determined that the reduction of **4.77** afforded a *ca*. 3:1 ratio of desired **4.82** plus starting **4.83** (Scheme 4.26), which were extremely difficult to separate.



Scheme 4.26 Synthesis of 4.83 from byproduct 4.77

An approach aimed to convert **4.76** into a more easily reduced ester was rapidly abandoned, because **4.76** failed to react with  $K_2CO_3/MeOH$  even at reflux temperature, while the more reactive, but more basic, MeONa/MeOH afforded significant quantities of a product of elimination of the OBOM group, **4.84** (not purified nor characterized beyond a <sup>1</sup>H NMR analysis).



Scheme 4.27 Unsuccessful denitrogenation of 4.76 using methoxide

Parallel work revealed that dihydroxylation of **4.76** before reduction was advantageous, because it facilitated the recovery of byproducts arising from the migration of the Boc group. Thus, Upjohn osmylation of **4.76** and protection of the emerging **4.85** as an acetonide furnished **4.86**, which was reduced with LiBH<sub>4</sub> to provide undesired **4.87** (34% over 4 steps from **4.69**) and desired **4.88** (29% over 4 steps from **4.69**). It is worthy of note that like **4.76**, reactions run on small scale (10 mg, 0.01 mmol) afforded a more favorable ratio of **4.88** to **4.87**. In any event, the two products were easily separated by column chromatography, and a small portion of the desired

**4.88** was *O*-protected as a TBDPS ether (**Scheme 4.28**). Compound **4.81** thus obtained was identical in all respects the one prepared earlier as per **Scheme 4.25**. The reason that only a small portion of **4.88** was converted into **4.81** will be apparent soon (**Scheme 4.32**, p. 70).



Scheme 4.28 The actual route from 4.76 to 4.88

The undesired **4.87** was also converted into **4.88** by the 3-step sequence outlined in **Scheme 4.29**. Thus, imide **4.87** was elaborated to tetra-Boc derivative **4.89**, which reacted slowly (5 days), but cleanly, with LiBH<sub>4</sub> to afford a 1:3 mixture of **4.88** and **4.91**. The formation of **4.91** is attributable to the Boc group transfer from the *N*1-bis-Boc imide to alkoxide **4.90** formed in the course of the reduction. This, however, was inconsequential, in that treatment of the crude product mixture  $K_2CO_3$ /MeOH at reflux released the Boc group from **4.91** and delivered clean **4.88**.

The mixture of **4.83** and **4.77** described in **Scheme 4.26** above could also be advanced to **4.95** in a similar manner. Indeed, separation of the two components became possible after osmylation,



Scheme 4.29 Recovery of 4.87

which again proceeded with Kishi selectivity to deliver **4.92** and **4.93**. Chromatographically purified **4.92** was subjected to acetonide formation by treatment with Me<sub>2</sub>C(OMe)<sub>2</sub>/TsOH. The product of this reaction was the overprotected intermediate **4.94**, which was transformed to the desired **4.95** by careful treatment with PPTS/MeOH. Compound **4.95** proved to be useful for the preparation of Du Bois-type advanced intermediates exhibiting a vinyl group at C4a (*vide infra*, **Scheme 4.44**, p. 86).



Scheme 4.30 Synthesis of 4.95 from byproduct 4.83

An important lesson that emerged from the experiments detailed above is that all key dihydroxylation reactions of substrates derived from oxidative amidation products took place with the anticipated Kishi selectivity, leading to products that were useful for elaboration into tetrodotoxin.

## 4.4 C9 Hydroxylation: Synthesis of the Sato Lactone and Reassignment of Its Structure

The next objective of the research was to advance bis-acetonide type intermediates to lactones such as **4.96**, which incorporate the critical C9 hydroxyl group. The approach we chose initially retraced the Chau sequence (**Scheme 3.5**, p. 39) and envisioned the elaboration of **4.88** to vinyl ether **4.96**, followed by dihydroxylation to **4.97** and selective oxidation to **4.98**. The correct configuration of the C9 OH group was anticipated to arise through a selective approach of OsO<sub>4</sub> from the convex face of the molecule (**Scheme 4.31**). A potential advantage of creating compound **4.98** was that it could be advanced to Sato lactone, **1.126**, thereby connecting with the Sato synthesis (pp. 20-22).<sup>32</sup>



Scheme 4.31 Initial strategy for the preparation of lactone 1.126

Our first goal was therefore the release of the BOM groups, and for that purpose we chose to explore this reaction with compound **4.81**. Contrary to the case of the Chau intermediate **3.23** (**Scheme 3.5**, p. 39), hydrogenolytic deblocking of **4.81** with either Pearlman's catalyst or Pd/C in various solvent (*e.g.* EtOAc, EtOH) was unsuccessful, even under high H<sub>2</sub> pressure (700 psi).

Birch-type deprotection worked well, except that the phenyl substituents on the TBDPS group were also partially reduced,<sup>97</sup> giving a mixture of cyclohexenyl/cyclohexadienyl derivatives **4.99** (**Scheme 4.32**) and greatly complicating analytical / spectroscopic work. This is the reason why only a small amount of TBDPS derivative **4.81** was prepared during this research (p. 67).



Scheme 4.32 Difficulties encountered during Birch release of BOM protecting groups in 4.81

The problem was corrected by blocking the primary alcohol in **4.88** as a TBS ether (**Scheme 4.33**). The resulting **4.100** performed well in the Birch deprotection; except that the desired **4.102** was accompanied by variable quantities of mono- and bis-methylol derivatives as **4.101**. The crude reaction mixture was therefore redissolved in hot aqueous *i*-PrOH, whereupon the various methylol derivatives converged to **4.102**, which was obtained in 82% yield.



Scheme 4.33 Successful Birch deprotection in the TBS series

It should be recalled that a compound similar to 4.102 was selectively oxidized to an aldehyde with Dess-Martin periodinane (cf. 3.24, Scheme 3.5, p. 39), and that the aldehyde cyclized immediately to the corresponding lactol, which was then dehydrated to a vinyl ether. An analogous sequence of reactions proved to be unsatisfactory with substrate 4.102. Carefully controlled DMP oxidation produced an inseparable 3:1 mixture of undesired hemiketal 4.105 (major product) and desired lactol **4.106**. Product **4.105** clearly resulted through the preferential oxidation of the secondary alcohol. This phenomenon had been previously observed,  $^{98}$  and it suggests that the rate limiting step in the DMP oxidation is the fragmentation of the substrate-periodinane complex, which could be sterically accelerated, just as in case of chromate No hemiketal 4.105 was detected upon Swern oxidation of 4.102, which provided esters.<sup>99</sup> instead a 2:3 mixture of desired 4.106 and lactone 4.104, plus some unreacted starting material. 4.102 failed to react under Parikh-Doering conditions,<sup>100</sup> while Ley-Griffith oxidation<sup>101</sup> converted it into lactone 4.104 in nearly quantitative yield. Lactol 4.106 was clearly present during the Ley-Griffith oxidation, monitored by TLC; however, no effort was performed to stop

the reaction at the stage of lactol. In all cases, the lactol was obtained as a single diastereomer, which is believed to be of  $\beta$ -configuration. The Swern method produced the most of **4.106**, albeit in disappointing yield. The quantities of **4.106** thus obtained were still sufficient to explore the crucial C9 hydroxylation via vinyl ether **4.107**, which was smoothly obtained upon dehydration of with MsCl/Et<sub>3</sub>N (**Scheme 4.34**).



Scheme 4.34 Different products derived from the oxidation of 4.102

It was anticipated that an electrophile, *e.g.*, OsO<sub>4</sub>, would attack the  $\pi$  system in **4.107** preferentially / exclusively from the *Si*\*-face, since an approach from the *Re*\*-face is hampered by the concave shape of the molecule and obstructed by the ring acetonide group. This surmise is rooted in observations by Isobe and collaborators concerning the reactivity of regioisomeric vinyl ether **1.65**, which undergoes dihydroxylation exclusively from the convex face, and opposite the axial acetoxy group, to furnish **1.66** (Scheme 1.11, p. 13).<sup>29</sup> In that case, the forces mentioned above operated in the opposite direction, promoting excellent facial selectivity in the incorrect sense.

Upjohn dihydroxylation of the surprisingly stable vinyl ether **4.107** proceeded very slowly, requiring 10 days to reach 50% conversion, but with complete diastereoselectivity (within the limits of 300 MHz <sup>1</sup>H NMR spectroscopy) to afford diol **4.108**, the configuration of which was proven by an extensive 1D NOE NMR study (**Scheme 4.35**)



Scheme 4.35 Stereochemical course of the dihydroxylation of vinyl ether 4.107

All that remained to do in order to advance **4.108** to a Sato-like TTX intermediate was to achieve a selective oxidation of the lactol to a lactone. Various oxidants were examined for this purpose, with uniformly disappointing results. Freshly prepared F  $\pm$  or reagent (Ag<sub>2</sub>CO<sub>3</sub> on Celite<sup>®</sup>)<sup>102</sup> failed to react with the substrate even in refluxing dry toluene or heptane, reflecting the inaccessibility of the lactol hydrogen. The same was true of aq. Br<sub>2</sub>/NaHCO<sub>3</sub><sup>103</sup> and BaMnO<sub>4</sub>.<sup>104</sup> Activated MnO<sub>2</sub> slowly cleaved the vicinal diol to afford **4.109**. TPAP/NMO swiftly converted the substrate into  $\alpha$ -ketoester **4.110** without any intermediate products being detected. An attempt to protect the less hindered secondary alcohol as alkoxymethyl ether prior to oxidation of

the lactol also failed, giving **4.111** as the only product, probably because of the higher acidity of the anomeric -OH.<sup>105</sup> It rapidly became apparent that the strategy depicted in **Scheme 3.5** was doomed on account of all such difficulties.



Scheme 4.36 Attempts to selectively oxidize the lactol in 4.108

The only product that was available efficiently upon oxidation of **4.102** was lactone **4.104**. In principle, this material could be advanced to the desired **4.103** by reaction of the corresponding enolate with a suitable carrier of electrophilic OH (**Scheme 4.37**), *e.g.*, a Davis oxaziridine,<sup>106-107</sup> the MoOPH reagent,<sup>108</sup> and so on. A worrisome aspect of this hypothesis was that Isobe and coworkers found that regioisomeric lactone **4.113** was entirely resistant to enolate formation.<sup>29</sup> On the other hand, an inspection of molecular models indicated that in our lactone **4.104** the more accessible *exo*-hydrogen atom at the  $\alpha$ -position of the carbonyl group (the one pointing toward the CH<sub>2</sub>OTBS moiety) was nearly perpendicular to the plane containing the C=O system; therefore it should be readily abstracted by a base.<sup>109-110</sup>



Scheme 4.37 Second-generation strategy based on lactone 4.104

Initial experiments aiming to probe the foregoing prediction gave very promising results. Thus, deprotonation of 4.104 with NaHMDS followed by D<sub>2</sub>O quenching returned partially deuterated (*ca.* 25% by <sup>1</sup>H NMR) lactone. Deuteration proceeded to completion (*ca.* 95% by <sup>1</sup>H NMR) upon deprotonation with LDA, also followed by D<sub>2</sub>O quenching. The following observations suggest that deuterium incorporation took place exclusively from the convex face of the molecule, resulting in formation of diastereomer 4.115. First, the hydrogens at the  $\alpha$ -position of the carbonyl in 4.104 appear as an apparent AB system, the doublets of which are centered at 2.25 and 2.91 ppm ( ${}^{2}J_{AB} = 17.8$  Hz). A 2D COSY spectrum revealed the presence of a W-coupling between the signal at 2.91 ppm and that of the C4a hydrogen at 3.25 ppm, signifying that the *endo*-proton resonates at 2.91 ppm and the *exo*-proton at 2.25 ppm. Deuterated product 4.115 still exhibited the 2.91 ppm signal, now appearing as a singlet, but that at 2.25 ppm resonance had disappeared. Thus, only the signal of the *endo*-H was visible, indicating that the deuterium had entered with the exo orientation. Furthermore, when 4.115 was treated with LDA and the enolate quenched with H<sub>2</sub>O the original **4.104** was recovered, demonstrating that only the

*exo*- $\alpha$ -C=O-proton is abstractable by base.



Scheme 4.38 Deprotonation of lactone 4.104 and deuteration of the resulting enolate

These observations encouraged us to examine some hydroxylation methods. Bubbling of  $O_2$  into a solution of enolate, followed by introduction of  $P(OEt)_3$ ,<sup>111-112</sup> returned only starting lactone. Interception of the enolate with TMSCl, followed by *in situ* treatment of the presumed silyl ketene acetal with DMDO also returned starting **4.104**.<sup>113-114</sup> However, the reaction of the enolate with Davis oxaziridine **4.116** successfully produced the desired hydroxylactone (**Scheme 4.39**).<sup>106-107</sup> Best results were obtained when the oxaziridine was thoroughly purified by column chromatography prior to use. This process removed all contamination by sulfonylimine **4.117**, which appeared to react rapidly with the enolate of **4.104** (**4.112**), leading to the formation of the undesired byproduct **4.118**.<sup>106</sup> Greater than 60% conversion of **4.104** was consistently achieved under optimized conditions, with no formation of **4.118**.

The polarities of **4.104** and **4.103** were very similar, complicating the chromatographic isolation of the desired **4.103**. Separation of the two products, however, became possible after protection of the hydroxyl group in **4.103**. In that connection, a strategic decision was made at



Scheme 4.39 α-Hydroxylation of lactone 4.104

this juncture: compound **4.103** would be advanced to the Sato lactone, **1.126** (Scheme **4.40**). Because the latter can be converted into TTX in 4 straightforward steps, a synthesis of **1.126** is tantamount to a formal synthesis of tetrodotoxin (Scheme 1.21, p. 21).<sup>32</sup> This course of action was attractive, first of all because it avoided several safety and regulatory issues that we would have had to face before actually making the exceedingly toxic natural product. Second, it embodied a time-saving measure, it that it bypassed, among other things, the cumbersome purification of TTX.



Scheme 4.40 Planned advancement of 4.103 to the Sato lactone 1.126

The elaboration of 4.103 to the Sato lactone required protection of the hindered, unreactive C9

hydroxyl as a MOM ether. No reaction occurred when **4.103** was treated with MOM-Cl and Et<sub>3</sub>N, but successful protection was achieved upon *O*-deprotonation with NaHMDS and reaction of the corresponding alkoxide with MOM-Cl (**Scheme 4.41**). It is worthy of note that no C9 epimerization was observed during these operations, even upon prolonged contact of **4.119** with excess base at rt. This is believed to be yet another consequence of the inaccessibility of the *endo*-H at the  $\alpha$ -position of the lactone carbonyl. In any event, the resultant **4.119** was now readily separated from **4.104**, and was obtained in 49% over two steps from **4.104** after chromatography. The *exo* configuration of the MOMO group is supported by the presence of dipolar coupling (2D NOESY) between the *endo*-H and one of the methyl groups of the abutting acetonide, as well as a W-coupling (2D COSY) with the C4a hydrogen (**Scheme 4.41**).



Scheme 4.41 MOM protection of the C9 hydroxyl group

Silyl and Boc groups now needed to be released from **4.119** in order to reach the Sato lactone. Treatment of **4.119** with TBAF/HOAc provided a 3:1 mixture (<sup>1</sup>H NMR) of two inseparable products, which turned out to be **4.120** and **4.121**. All batches of this deblocking step gave the same ratio of **4.120** and **4.121**, indicating that this corresponded to the tautomeric mixture in thermodynamic equilibrium. The structure of the two lactones was assigned based on the characteristic doublet at 2.74 ppm of **4.121** (C5-O*H*, *cf*. **4.123** in **Figure 4.3**, p. 83 and **4.135** in **Figure 4.9**, p. 94).



Scheme 4.42 Desilylation of 4.119 leading to two tautomeric lactones

The next reaction, the release of the Boc group, was carried out with the 3:1 mixture of **4.120** and **4.121**. Treatment of the mixture with acids, *e.g.*, BF<sub>3</sub>  $OEt_2$ , resulted in complex mixtures of unidentifiable products. Du Bois and coworkers achieved the deprotection of a Boc carbamate similar to **4.120** by dissolution in boiling water.<sup>31</sup> However, heating of **4.120** up to 110  $^{\circ}C$  in deaerated water resulted in hydrolytic loss of one of the acetonides along with release of the Boc group (detected by mass spectrometry). It seemed likely that no such problem would be encountered if Boc release were to be carried out under water-free conditions; *e.g.*, in a purely thermal fashion.<sup>115</sup> Accordingly, a small amount of solid mixture of **4.120/4.121** was placed in a round bottom flask and heated in a sand bath under high vacuum. One or both of **4.120** and **4.121** started to sublime at a bath temperature of approximately 170  $^{\circ}C$ , escaping from the high-temperature zone of the flask and condensing on the cooler upper surfaces. Yet, it was exhilarating to discover that the <sup>1</sup>H NMR spectrum of the mixture recovered from this experiment

exhibited all the signals corresponding to the Sato lactone, although the latter was clearly present only in trace amounts. Evidently, sublimation of the substrate was preventing complete deblocking.



Scheme 4.43 Thermal decomposition of Boc amine

When a round bottom flask containing solid **4.120/4.121** under Ar atmosphere, instead of high vacuum, was immersed in an oil bath pre-heated to 190 °C, material still sublimed and deposited above the oil level. In this case, a slightly more polar product was isolated from the reaction mixture, along with starting **4.120/4.121** and some Sato lactone. The new substance was identified as **4.122**, and its structure ascertained by X-ray diffractometry, confirming that it, and all prior intermediates, were of correct relative configuration.

The technical problems described above were resolved by completely immersing a sealed melting point capillary containing solid **4.120/4.121** into an oil bath pre-heated to at least 200 °C. A suitable procedure was devised on the basis of the following safety tests. First of all, the bath containing polydimethylsiloxane silicone oil was loosely covered with two pieces of glass to minimize temperature fluctuations, evaporation of the oil, and – especially – contact of the hot oil with the atmosphere. This precaution was implemented despite the high boiling- and flash points



Figure 4.2 X-ray crystal structure of byproduct 4.122

(275 °C and 250 °C respectively) of silicone oil. The bath was heated on an electric hot plate until the internal temperature stabilized at the desired level. The ability of melting points capillaries to withstand pressure was determined by placing several sealed, empty tubes in the oil bath pre-heated to 230 °C. Throughout this and all later experiments, the entire apparatus was kept behind a blast safety shield and the hood sash was fully lowered. A rough calculation suggested that the pressure inside the empty tubes would increase to about 1.8 atm under these conditions, while the gases liberated upon decomposition of 2 mg of **4.120** would bring the inner pressure to just below 3 atm. After 15 min at 230 °C the tubes were retrieved using lab tweezers and found to be all intact.

Solid **4.120/4.121** (10 mg) was thus dissolved in a small amount (*ca.* 400  $\mu$ L) of CH<sub>2</sub>Cl<sub>2</sub> and the solution was distributed into 5 glass melting point capillaries (*ca.* 70  $\mu$ L in each tube,

corresponding to 2 mg of substrate) using a microsyringe. It was essential to use capillaries supplied by the Kimble-Chase Co., article no. 34505-99: capillaries provided by other manufacturers, *e.g.*, Pvrex<sup>®</sup> 9530-3, promoted undesirable side reactions, possibly because of the more basic nature of the glass composition. The open capillaries were kept upright in a small Erlenmeyer flask placed inside the fume hood and the solvent was allowed to evaporate. This required 2-3 days. The tubes were then placed in a round-bottom flask, which was evacuated (high vacuum line) and then flushed with argon. The capillaries were quickly taken out and the open ends were flame-sealed by heating using a natural gas Bunsen burner. The tubes now containing solid **4.120/4.121** under Ar at 1 atm were then placed in a polydimethylsiloxane oil bath pre-heated to 215  $^{\circ}$ C (the optimal temperature) and configured as detailed above (glass covers, safety shield, etc.). The tubes were nearly completely submerged in the hot oil. A lower temperature (200  $^{\circ}$ C) resulted in incomplete reaction, while a higher temperature (230  $^{\circ}$ C) caused significant decomposition. A slow disappearance of the solid was observed and the contents of the capillaries became slightly darker. After 10 min (the optimal contact time), the tubes were retrieved, cooled to rt, rinsed thoroughly with hexanes and DCM, wrapped in a Kimwipe<sup>TM</sup>, placed over a conical glass funnel, and ground with pliers. The Kimwipe<sup>TM</sup> containing the glass fragments was placed in a glass funnel and thoroughly rinsed with DCM, and the combined washes were concentrated to afford the crude product. Chromatographic purification afforded Sato lactone.


**Figure 4.3** Expansion of <sup>1</sup>H NMR of Sato lactone as obtained by Sato and coworkers (top) and in this work (bottom, contaminated with *ca.* 5% **4.122**)

The spectrum of the Sato lactone thus produced was identical to the one published by Sato and collaborators.<sup>32</sup> In either case, the spectra were recorded from  $CDCl_3$  solutions, and in that respect it is important to note that the  $CDCl_3$  employed as the NMR solvent had to be stored over anhydrous  $K_2CO_3$  for at least 3 hours before use. This removed traces of D-Cl, which otherwise



**Figure 4.4** Expansion of <sup>1</sup>H NMR of Sato lactone (contaminated with 15% **4.120/4.121** and 25% **4.122**) before (top) and after (bottom) being shaken with D<sub>2</sub>O

would partially protonate the amino group in the Sato lactone, resulting in broadening of NMR signals.



Figure 4.5 Presumed (1.126) and actual (4.123) structure of the Sato lactone

A feature of the 1H NMR spectra of the Sato lactone (either the original or the one obtained in this study) that captured our attention was the presence of a sharp doublet at 2.91 ppm (1H, J = 12 Hz). It was not at all clear why the NMR spectrum of **1.126** should exhibit such a doublet. A 2D-COSY spectrum of the same solution indicated that the doublet at 2.91 ppm correlated only

with a doublet of doublets appearing at 3.80 ppm (1H,  $J_1 = 12$  Hz,  $J_2 = 5$  Hz). A 2D HSQC spectrum indicated that the proton producing the doublet at 2.91 ppm was not connected to a carbon atom. Furthermore, shaking the NMR solution with D<sub>2</sub>O caused disappearance of the doublet at 2.91 ppm and collapse of the doublet of doublets at 3.80 ppm into a doublet, J = 5 Hz (**Figure 4.4**). Evidently, the doublet at 2.91 ppm is produced by the proton of the OH group coupled to a single vicinal proton. This is entirely inconsistent with structure **1.126**, but in accord with **4.123**. This suspicion was confirmed by single crystal X-ray diffractometry, which conclusively proved that the Sato lactone was actually **4.123**. Thus, the structure of Sato lactone had been misassigned.



Figure 4.6 X-ray crystal structure of actual Sato lactone, 4.123

#### 4.5 Formal Synthesis of *rac*-TTX Through the Du Bois Intermediate

The structural discrepancy just unveiled raised questions about the validity of a claim that a synthesis of **4.123** corresponds to a formal synthesis of TTX (more about this later). Rather than shedding more light on the issue at this point, our priority was to set our claim of a total synthesis on firmer grounds. This was done by elaborating **4.88** to the racemate of the Du Bois intermediate, **1.109** (**Scheme 1.19**, p. 19), a compound of secure structure that may be advanced to TTX in 4 steps.<sup>31</sup>



Scheme 4.44 Formal synthesis of TTX through the elaboration of 4.88 to the Du Bois intermediate

The effort to reach **1.109** from **4.88** commenced with the installation of the C4a vinyl group. This transformation was initially explored using bis-Boc imide **4.95**, recognized as a byproduct obtained in a previous sequence (**Scheme 4.30**, p. 68). Both Dess-Martin oxidation to aldehyde **4.124** and Wittig methylenation proceeded efficiently to furnish the desired **4.125**. However, scale-up of the reaction was carried out with mono-Boc protected compound **4.88**, whereupon an annoying difficulty was uncovered. Whereas the oxidation of **4.88** to **4.126** occurred uneventfully, Wittig methylenation of the resultant **4.126** cleanly produced substance **4.129** as the sole product. This may be rationalized by invoking deprotonation of the carbamate, intramolecular deprotonation of the aldehyde (*cf.* **4.127**), and consequent E1cB elimination of the OBOM moiety, followed by Wittig reaction of the emerging **4.128** (Scheme 4.45).



Scheme 4.45 E1cB elimination during the olefination of aldehyde 4.126

Fortunately, the more reactive Peterson organometallic species added to 4.126 with no evidence of elimination. Thus, the addition of TMSCH<sub>2</sub>Li to 4.126 provided 4.130 in good crude yield. Without purification or thorough characterization, the latter was treated with excess KHMDS to yield 4.131 as the only product.



Scheme 4.46 Successful Peterson olefination of 4.126

An unanticipated difficulty was encountered during the next step of the sequence: the release of BOM groups by Birch-type reduction. This reaction was initially tested with compound 4.125. The same procedure employed earlier for the deprotection of **4.100** led to the formation of a 1:2 mixture of desired 4.132 (minor product) and undesired 4.133, wherein the vinyl group had undergone reduction to an ethyl group (Scheme 4.47). Analogous vinyl group reductions are documented, but they tend to occur upon prolonged reaction times.<sup>116</sup> It was somewhat surprising to observe that the reduction of alkene took place so rapidly (10 min or so). A shorter reaction time (1 min) resulted in incomplete deprotection, but it suppressed the formation of 4.133. Interestingly, no mono-deprotected product seemed to be present in the crude reaction mixture; furthermore, BOM-deprotected products had also lost one of the N1 Boc groups. It would seem that the release of Boc group took place only after the departure of the BOM groups, in that no **4.131** (the mono-Boc version of **4.125**) was observed in the crude product mixture. Complete BOM deprotection was achieved by increasing the reaction time to 12 min (10 min after the reaction media turned blue); however, some reduction of the vinyl group occurred under these conditions. Substance 4.133 constituted a dead end of this synthesis, but it was nicely crystalline; more so than 4.132. Its structure was therefore ascertained by X-ray diffractometry to confirm that it possessed the correct relative configuration.

Larger-scale work was carried out with substrate **4.131**, which was subjected to Birch conditions over a short reaction time. As soon as the solution turned blue (3-5 min), solid  $NH_4Cl$  was added to quench the reaction and prevent reduction of the vinyl group. As seen earlier for



Scheme 4.47 Birch deprotection of 4.125 and 4.131

**4.100** (Scheme 4.33, p. 71), the resultant crude was actually obtained as a mixture of methylol derivatives, necessitating a hydrolytic workup (*i*-PrOH/H<sub>2</sub>O) to release H-CHO from the product. The desired 4.132 was obtained in 80% yield from 4.88 over 4 steps.



Figure 4.7 X-ray crystal structure of 4.133

The oxidation of **4.132** to **4.134** worked perfectly well and the  $\alpha$ -hydroxylation of **4.134** worked even better that that of **4.104**, in the sense of the resulting Du Bois intermediate **1.109** has an extremely limited solubility in hexanes, while **4.134** is moderately soluble (a large quantity of hexanes is necessary for complete dissolution). This greatly facilitated the recovery of **1.109** and **4.134** in pure form and the ultimate recycling of the latter. The spectral data recorded for **1.109** coincided with those reported by Du Bois and Hinman.<sup>31</sup> Moreover, the already secure structure of **1.109** was confirmed by single-crystal X-ray diffractometry.



Scheme 4.48 Formal synthesis of TTX

Because the Du Bois intermediate may be advanced to TTX in 4 steps, the preparation of **1.109** is tantamount to a formal synthesis of the natural product, and because **1.109** was thus reached in 27 steps from **3.5**, this work constitutes a formal synthesis of tetrodotoxin in 31 steps from **3.5**: a sequence competitive with known alternatives. The effort also demonstrates the first major synthetic application of the bimolecular oxidative amidation of phenols, and it shows that

the methodology can sustain the chemical synthesis of complex alkaloidal architectures, even when employed at an early stage.



Figure 4.8 X-ray crystal structure of the Du Bois intermediate 1.109

## 4.6 Further Observations on the Chemistry of the Sato Lactone

Having set our claim of a formal synthesis of **1.1** on firmer grounds, the decision was made to shed light on the issues raised by the discovery that the structure of the Sato lactone had been misassigned. Scrutiny of all data available in the Sato publications produced incontrovertible evidence that these workers had indeed obtained tetrodotoxin from **4.123**. This meant that at some point of the Sato synthesis lactone **4.123** had isomerized back to **1.126** (Scheme 4.49).



Scheme 4.49 Possible equilibration of actual (4.123) with presumed (1.126) Sato lactone

A Molecular Mechanics calculation<sup>117</sup> showed that structure **4.123** is more stable than **1.126** by *ca.* 4.6 kcal/mol, suggesting that at equilibrium it should preponderate by a factor of about 2400:1 at rt. Indeed, extensive NMR studies indicated that if **1.126** were present at all in a solution of Sato lactone, it was below detection. This led to the hypothesis that greater steric bulk around the N1 atom might decrease the energy difference between **4.123** and **1.126** and possibly favor equilibration.



Scheme 4.50 Guanidinylation of the Sato lactone and equilibration of the products

To explore such a possibility, compound **4.123** was *N*-guanidinylated by reaction with  $N,N^{\circ}$ -bis-Boc-*S*-methyl-isothiourea in the presence of HgCl<sub>2</sub> and Et<sub>3</sub>N. This resulted in formation of a *ca*. 2:1 mixture (<sup>1</sup>H NMR) of two products. The <sup>1</sup>H NMR spectrum of this mixture was identical to the one reported by Sato, except for the ratio of products (2:1 in our case, 3:1 in Sato's). Sato described the two products as "conformational isomers" of compound **1.127**, but

this appears to be inconsistent with the Sato's own spectroscopic data. In particular, the OH proton of the major component is observed as a doublet at 2.71 ppm (J = 11.7 Hz). Sato reported that this doublet is coupled to a neighboring proton that resonates at 3.83 ppm (ddd, J = 11.7, 6.2, 1.0 Hz), which in turn was attributed to the C5-H. Therefore, C5 must bear an OH group. But only structure 4.135 is consistent with the observed NMR pattern. Furthermore, in 4.135 the hydrogen at C9 appears as a singlet at 5.18 ppm, as anticipated. But in the minor component, the C9-*H* resonates as a doublet, J = 6.4 Hz, at 5.60 ppm, and it is reportedly coupled to the C4a-*H*, which appears at 3.82 ppm (dddd, J = 7.4, 6.4, 3.4, 2.4 Hz). Evidently, C9 in 1.127 experiences a W-type coupling with C4a: this is possible only in structure **1.127**. Additionally, the C5-*H* in the major component appears at 3.83 (ddd, J = 11.7, 6.2, 1.0 Hz). The coupling pattern is consistent with the presence of a W-coupling with the C7-H (J = 1.0 Hz), and the chemical shift is typical a cyclohexanol. But in the minor component, C5-H resonates at 4.36 ppm (d, J = 3.2 Hz). This chemical shift is more consistent with an ester / lactone. Indeed, a very similar chemical shift and a small coupling to the neighboring C4a of C5-*H* are also seen in the Du Bois intermediate, **1.109**. Clearly, the signals of the minor component are consistent only with isomeric structure **1.127**. In conclusion, the two products obtained from this reaction are in all probability 4.135 (major) and 1.127 (minor) [Figure 4.9, wherein "S" stands for Sato structure, and "T" for tetrodotoxin structure, respectively], indicating that lactone equilibration occurs once the guanidinyl moiety is in place.



**Figure 4.9** Expansion (2.6-5.7 ppm) and assignment of <sup>1</sup>H NMR spectrum of a 2:1 mixture of **4.135** ("*S*ato" type guanidine) and **1.127** ("*T*TX" type guanidine)

An important comment is in order at this point. Sato and collaborators experienced major difficulties with the oxidation of purported **1.127** (actually the minor component of a 3:1 mixture of **4.135** and **1.127**). Among many oxidants tried, only the use of the relatively acidic PCC led to the conversion of the substrate into an aldehyde, which instantly cyclized to **4.136**.<sup>32</sup> In retrospect, this seems logical, to the extent that lactone isomerization is probably catalyzed either by acid or by base. One may speculate that the well documented acidity of PCC<sup>118</sup> was essential in promoting isomerization of **4.135** into **1.127** as the latter was being oxidized to **4.136**.



Scheme 4.51 Possible explaination of the viability of PCC in the oxidation of 4.135 to 4.136

Chapter 5 The Search for an Avenue to Enantioenriched TTX and an Improved Route to Advanced Synthetic Intermediates: a New Strategy Based on Early Oxygenation at C7, C8 and C9

The successful conclusion of the studies detailed in Chapter 4 encouraged us to investigate possible routes to enantioenriched **1.1**. In addition, improved reaction sequences that would circumvent some of the difficulties described earlier, such as the formation of amide **4.69** and the consequent requirement for Boc activation, were also desirable. The present chapter summarizes our efforts in that sense.

### 5.1 General Aspects of a Possible Route to (-)-Tetrodotoxin

Early synthetic intermediates in the formal synthesis of TTX just described are achiral, up to and including **4.33**. This substance is the substrate for the crucial Machetti-De Sarlo reaction, which in its current form leads to the racemate of chiral isooxazoline **4.34**. Compound **4.34** is the progenitor of all later, chiral TTX intermediates. Scalemic **4.34** could become available through an enantioselective variant of the Machetti-De Sarlo reaction, which might result if a chiral, enantiopure Cu(II) complex were employed in lieu of achiral Cu(OAc)<sub>2</sub> (**Scheme 5.1**). Hence, an asymmetric Machetti-De Sarlo reaction is key to an enantioselective synthesis of **1.1**.



Scheme 5.1 Hypothetical asymmetric Machetti-De Sarlo reaction

An alternative might be envisioned in the form of a directed nitrile oxide cycloaddition. For instance, oxidative cyclization of an enantioenriched substrate of general structure **5.1** would produce **5.2**, which could be elaborated to **5.3**. The configuration of **5.3** is such that an ensuing intramolecular nitrile oxide cycloaddition (INOC) could generate only product **5.4**, wherein all the stereogenic carbons possess the configuration required for (–)-TTX (**Scheme 5.2**). Thus the chirality initially present at the future C9 of **1.1** would be relayed to all the other stereocenters.



Scheme 5.2 Possible avenue to 5.4 via scalemic 5.1

Yet another option involves a Sharpless asymmetric dihydroxylation of, *e.g.*, **4.32** (Scheme **5.3**). It should be noted, however, that no information was available at this point regarding the facial selectivity of the osmylation of **4.32** and congeners. Also, poor enantiomeric excess often observed in the asymmetric dihydroxylation of cyclohexenes<sup>119-121</sup> overshadowed this approach.



Scheme 5.3 The diastereomeric recognition of the cyclohexadiene

The results thus obtained are presented below.

## 5.2 Enantioselective Machetti-De Sarlo Reaction

Certain substrates undergo intramolecular Machetti-De Sarlo reaction spontaneously, even in the absence of copper catalysts.<sup>122</sup> This would negate the possibility of an asymmetric variant of the process. Fortunately, it was determined that Machetti-De Sarlo cyclization of, *e.g.*, **4.33** (**Scheme 5.4**), does not proceed without a copper(II) catalyst. Additionally, an acetonitrile solution of Cu(OAc)<sub>2</sub>, or Cu(OAc)<sub>2</sub> and **4.33**, or Cu(OAc)<sub>2</sub> and *N*-ethylpiperidine, was blue. However, an acetonitrile solution containing all three components was yellow, suggesting that a



Scheme 5.4 The participation of a chiral ligand in the Machetti-De Sarlo reaction

new complex, probably **5.8**, had been formed. Thus, isooxazoline **4.34** could emerge in enantioenriched form, if a chiral ligand were present on the metal.

The complex of anhydrous Cu(II) triflate with readily available *i*-Pr-BOX (**5.11**) was employed to test the idea. The reaction proceeded as smoothly as in the racemic version, but the product isooxazoline **4.34** appeared to be racemic, showing no significant optical rotation at 589 nm or 436 nm on a polarimeter.



Scheme 5.5 A Machetti-De Sarlo reaction with *i*-Pr-BOX

It is known that different BOX ligands show different selectivities in a particular reaction.<sup>123</sup> Furthermore, the solvent plays an important role, influencing the relative energies of the possible transition states. Given the multitude of parameters that would have to be addressed to achieve a successful enantioselective reaction, the Machetti-De Sarlo approach was set aside, to be revisited at a more opportune time.

### 5.3 Approach Based on Chirality Relay from C9

The oxygen functionality that would ultimately become the C9-OH in TTX had to be introduced prior to oxidative amidation. Indeed, an experiment had revealed that neither **4.25** nor

**4.32** lent themselves to enolate formation, precluding, for example, a possible Davis asymmetric hydroxylation.<sup>107</sup> To wit, **4.25** undergoes rapid aromatization to **4.31** upon contact with base, probably via E1cB expulsion of the imido group and tautomerism of the transient **4.30**. Likewise, the action of base on **4.32** leads to **4.35**, probably via a retro Mannich-type reaction (**Scheme 5.6**).



Scheme 5.6 The implausibility of C9 functionalization at different stages

Fortunately, compound **5.13** was available to us, prepared by another group member in connection with an unrelated project.<sup>124</sup> A highly enantioselective reduction of **5.13** to alcohol **5.14** may be readily achieved using biocatalytic methods.<sup>124-125</sup> However, the aim of the present investigation was to ascertain the feasibility of an oxidative cyclization of **5.16**, for which purpose racemic material was perfectly sufficient. Consequently, **5.13** was reduced to *rac*-**5.14**, and the latter was acylated with tosyl isocyanate to give **5.15**. Desilylation furnished **5.16**. Unfortunately, none of desired **5.17** seemed to be present in the complex reaction mixture that resulted upon treatment of **5.16** with DIB.



Scheme 5.7 Effort with N-tosyl carbamate as nitrogenous nucleophile in oxidative amidation

A similar approach evolving from methyl *p*-coumarate, **5.18**, also gave disappointing results. Compound **5.19**<sup>126</sup> would efficiently undergo Sharpless asymmetric dihydroxylation to give scalemic **5.20**;<sup>127</sup> however, racemic **5.20** was again employed in the course of this study. DIBAL reduction of the acetonide derivative of **5.20** released the acetyl group and converted the ester to



Scheme 5.8 Attempt of a tandem oxidative amidation-INOC reaction

aldehyde **5.22**, which existed in equilibrium with the geminal diol **5.23**, and that reacted with hydroxylamine to yield oxime **5.24** as a single geometric isomer, probably of *trans*-configuration. Our intent was to induce a tandem oxidative amidation-INOC reaction of **5.24**<sup>128</sup> but once again, exposure of **5.24** to the action of DIB in acetonitrile generated a complex mixture of products containing none of the desired **5.27**.

In a similar vein, an attempt to carry out oxidative amidation and INOC reactions as independent steps met with failure. This possibility was explored with substrate **5.28**, which also yielded no **5.29** upon exposure to DIB in MeCN.



Scheme 5.9 An unsuccessful oxidative amidation on 5.28

Recent work by Canesi<sup>65</sup> suggests that the failure of these reactions may be due to the occurrence of undesired pinacol-like rearrangements orchestrated by the benzylic oxygen functionality. In any event, the approach based on C9 stereorelay was abandoned in light of all such difficulties.

#### 5.4 The Early Dihydroxylation Approach

Turning now to **Scheme 5.3** (p. 98), our first objective was to determine the facial selectivity of the dihydroxylation reaction of an early 1,4-cyclohexadiene intermediate. The substrate

chosen for this study was **4.25**. The absence of an N–H unit in **4.25** would hopefully suppress Donohoe-type effects<sup>67</sup> and cause the substrate to undergo reaction in a Kishi sense.<sup>35</sup>



Scheme 5.10 Products obtained from the dihydroxylation of 4.25 and 4.32

Upon treatment with catalytic  $OsO_4$  and NMO in the presence of citric acid, **4.25** was slowly converted into an approximately 80:10:10 mixture of diol **5.30**, lactone **5.31**, and acetate **5.32** (**Scheme 5.10**). The product ratio varied with contact time and temperature, and the indicated yields reflect a typical outcome. In all cases, the substrate has undergone dihydroxylation in accord with the Kishi model. The reaction, however, stalled at about 65% conversion after 6 days. The three products were isolated in pure form by column chromatography that also returned 33% of unreacted **4.25**. The formation of **5.31** and **5.32** is attributed to transesterification reactions catalyzed by the citric acid present in the medium. Thus, attack of the C8 hydroxyl group on the (reversibly protonated) methyl ester or acetyl group in **5.30** leads to **5.31** or **5.32**, respectively. Substrate **4.32** also underwent dihydroxylation with complete Kishi selectivity. As in the case of **4.32**, the reaction was slow and it also stalled at 65% conversion. In the present case, no diol was detected in the reaction, but only lactone **5.33**, suggesting that lactonization is faster in the -NHBoc series of compounds. Chromatography provided **5.33** in 44% yield and unreacted **4.32** in 33% yield. *p*-Aminophenol derivative **4.35** was a significant byproduct and was isolated in *ca*. 20% yield.



Scheme 5.11 Unreactivity of 4.25 in the Sharpless asymmetric dihydroxylation reaction

Disappointingly, **4.25** failed to undergo dihydroxylation upon exposure to AD-mix- $\beta$ . No effort was made to promote the reaction by the use of citric acid, because it is known that the latter completely surpresses enantioinduction.<sup>77</sup> The desymmetrization of a more reactive analogue of **4.25** was postponed, to be revisited in the future.

### 5.5 Attempted Separation of Diastereomeric Menthyl Esters

The inertness of **4.25** toward Sharpless AD reagents induced us to explore an alternative relying on a possible *diastereoselective* dihydroxylation of a chiral ester. Readily available chiral terpene alcohols, such as menthol, have been widely employed as chiral auxiliaries for asymmetric reactions,<sup>129</sup> or for the resolution of racemates via diastereomeric derivatives.<sup>130</sup> One of our

tetrodotoxin intermediates, acid **4.27**, contains a carboxylic acid that could be esterified with menthol. The resulting **5.34** could undergo dihydroxylation to selectively produce diastereomer **5.35** or **5.36** of the product diol. Alternatively, the menthol unit could enable separation of **5.35** and **5.36**, either as such or as appropriate derivatives.



Scheme 5.12 Menthyl as a chiral side chain for the diastereomeric separation

Acid **4.27** was thus esterified with (–)-menthol using DCC as a coupling agent. Menthyl ester **5.34** underwent slow, facially selective (*ca.* 3:1 in the correct sense), but non-diastereoselective, dihydroxylation. Separation by a combination of recrystallization and column chromatography returned a 1:1 mixture (<sup>1</sup>H NMR) of **5.35** and **5.36** (42% yield), a 1:1 mixture (<sup>1</sup>H NMR) of possible **5.37** and **5.38** (14%), and unreacted **5.34** admixed with some **4.35**. The diols **5.35** and **5.36** exhibited no apparent tendency to lactonize to **5.33**, even when heated in refluxing methanol. Unfortunately, the polarity of **5.35** and **5.36** was virtually identical,

preventing chromatographic separation even by HPLC. Furthermore, recrystallization of this mixture from various solvents, such as hexanes, DCM/hexanes, toluene/hexanes, methanol, or methanol/water, always produced crystals consisting of **5.35** and **5.36** in an exact 1:1 ratio, as revealed by an X-ray diffractometric study. Likewise, no diastereomeric enrichment was detected in the mother liquor.



Figure 5.1 X-ray structure of a co-crystal of 5.35 and 5.36

The crystal lattice of **5.35/5.36** displayed only hydrogen bonded pairs of **5.35** and **5.36** (**Figure 5.1**). An attempt to break up the H-bonding network by converting the diols to bis-4-bromobenzoate ester was of no avail: esters **5.39** and **5.40** again proved to be inseparable either by chromatography or by crystallization. In the present case, no X-ray diffractometric study of the resulting crystals was carried out.



Scheme 5.13 Preparation of the bis-4-bromobenzoyl derivative of 5.35/5.36

### 5.6 Toward an Improved Route to Advanced TTX Intermediates

The separation of diastereomeric derivatives of the menthol esters, at least in the manner outlined above, seemed to be implausible. Yet, the resistance of **5.35/5.36** to lactonization, proved useful in a different context. Considerations rooted in the findings summarized in the previous chapter suggested that a route to *rac*-TTX involving an early dihydroxylation of substrates such as **5.41**, and proceeding, *e.g.*, as indicated in **Scheme 5.14**, would suppress the need for some protection/deprotection steps, as well as avoid the formation of amide **4.69** 



Scheme 5.14 Advantageous route to TTX via early dihydroxylation

(Scheme 4.21, p. 61) and all attendant difficulties.

Consistent with the above expectations, the mixture of diastereomeric diols **5.35** and **5.36** was protected as acetonides **5.47**. No lactonization to compounds of the type **5.35**/**5.36** occurred under the acidic conditions of this step. In contrast, the analogous reaction of compound **5.30** took place with a substantial extent of lactonization (**Scheme 5.15**). The configuration of **5.35** and **5.36** was proved at this stage. Saponification of the menthyl ester took place slowly (3 days at 60 °C) and occurred simultaneously with silyl group release to yield acid **5.48** as a single (racemic) isomer. This substance was identical in all respects to the acid prepared from compound **5.49** (*cf.* **Scheme 5.18**, p. 111), the configuration of which was secure.



Scheme 5.15 Protection of 5.35 and 5.36 and structural proof by chemical correlation with 5.49

The 1:1 mixture (<sup>1</sup>H NMR) of acetonides **5.47** was further advanced to a single isomer of acid **5.44** (**Scheme 5.16**). Removal of the TBDPS group with TBAF generated a 1:1 mixture of diastereomers of alcohol **5.50**, which was oxidized using Dess-Martin periodinane in preparation

for Wittig methylenation to a 1:1 mixture of diastereomers of diene **5.52**. Subsequent Upjohn osmylation took place exclusively at the exocyclic olefin, resulting in a 1:1 mixture of diastereomers of crystalline diol **5.53**. Once again, all attempts to separate the two diastereomers by recrystallization were unfruitful: the two components were present in a strictly 1:1 ratio both in the crystals and in the mother liquor (<sup>1</sup>H NMR). The diol was thus protected as an acetonide and the ester was saponified. The latter reaction was quite sluggish, but removal of the menthyl group caused the convergence of the 1:1 mixture of diastereomers of **5.54** to a single diastereomer of racemic carboxylic acid **5.44** (<sup>1</sup>H NMR).



Scheme 5.16 Elaboration of 5.47 into a new TTX intermediate 5.44

Acid **5.44** is, of course, a plausible substrate for the now familiar Machetti-De Sarlo reaction. However, the elaboration of **5.44** to more advanced intermediates did not seem to shorten the overall synthetic sequence. Moreover, no enantiomeric enrichment was achieved at this point. Rather than exploring the Machetti-De Sarlo chemistry of **5.44**, a more interesting question was addressed: does lactone **5.33** constitute a dead end of this approach, or could it be the starting point of an even better route to TTX? Compound **5.33** is more readily accessible than **5.35/5.36**, and it could be elaborated to advanced TTX intermediates as indicated in **Scheme 5.17**.



Scheme 5.17 Possible route to advanced TTX intermediates from lactone 5.33

The translation of such hypotheses into practice required knowledge of the chemistry of the lactone. Investigations in that sense led to a concise route to an advanced bis-lactonic TTX intermediate that promises to simplify the overall avenue to the natural product.

### 5.7 The Chemistry of Lactone 5.33

Initial forays into the chemistry of **5.33** started with reduction to triol **5.59** and selective protection of the vicinal dihydroxyl system as acetonide **5.60** (Scheme **5.18**). Alcohol **5.60** was subsequently reoxidized to **5.61**.<sup>131</sup> This circuitous approach was mandated by the proclivity of diol **5.30** to cyclize to **5.33** during acetonide formation (Scheme **5.15**, p. 108), making the yield of **5.62** rather poor. Acid **5.62** underwent the Machetti-De Sarlo sequence smoothly to yield <sup>110</sup>

isooxazoline **5.7**, the relative configuration of which was ascertained by a 2D NOESY experiment. Unfortunately, the subsequent fragmentation of **5.7** with  $Li_2CO_3$ /MeOH produced a fair amount of side product that had the same molecular mass as the desired  $\beta$ -hydroxynitrile **5.63**. We suspect that this byproduct is an epimer of **5.63**. However, it remains unclear whether **5.64** is epimeric at C4a (the result of reversible deprotonation of the nitrile) or at C5 (reversible retro-aldol ring opening).



Scheme 5.18 Proof of the viability of the early dihydroxylation strategy

Parallel investigations revealed that the mixture of compound **5.30-5.32** arising upon dihydroxylation of **4.25** (Scheme 5.10, p. 103) could be advanced to a deacetylated lactone by

heating in MeOH solution in the presence of a 1:1 mixture of NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>. Interestingly, this treatment also promoted partial migration of the TBDPS group to the C7 alcohol to furnish a 5:6 (<sup>1</sup>H NMR) mixture of lactones **5.33** and **5.67** after acidic workup (the crude reaction mixture before acidification actually contained carboxylates **5.65** and **5.66**). Furthermore, the two were readily separable, in that **5.67** crystallized from MeOH-H<sub>2</sub>O in pure form. The mother liquor of the crystallization contained a mixture of **5.33** and some **5.67**. This mixture was recycled through the basic MeOH treatment, resulting in accumulation of **5.67**, which was obtained in 64% overall yield after 3 cycles of this operation. It would appear that the observed 6:5 ratio of **5.67** and **5.33** corresponds to the thermodynamic equilibrium mixture, in that **5.67** and **5.33** were obtained always in the same proportion after a sufficiently long contact time.



Scheme 5.19 Conversion of the mixture of 5.30-5.32 into lactone 5.67

Initially, the serendipitous formation of **5.67** was regarded as a breakthrough, in that it simplified the construction of the exomethylene segment. Unfortunately, the Witting reaction of

ketone **5.68** was capricious, leading to diene **5.69** in variable yield, typically 20%, and often failing to provide any **5.69** at all. The reasons for this failure are not fully clear; however scrutiny of the <sup>1</sup>H NMR spectra of crude reaction mixtures revealed the presence of signals that might have arisen from product **5.70** of E1cB elimination and its cyclized isomer **5.71**. However, no sound structural proof for these byproducts is available at this time. Peterson or Lombardo-Takai<sup>132</sup> olefination of **5.68** fared even worse, returning no **5.69** ever.



Scheme 5.20 Problematic C6 olefination

# 5.8 A Rapid Synthesis of an Advanced Bis-lactonic Tetrodotoxin Intermediate

More promising results were obtained during a study of the enolization of the bis-TBS analogue of **5.33/5.67**: compound **5.75**. Whereas this substance was readily prepared from **5.33/5.67**, a more direct route that avoids unnecessary protection/deprotection steps is delineated in **Scheme 5.21**. The mixture of diastereomers of alcohol **4.23/4.24** (3 steps from commercial **3.5**; **Scheme 4.7**, p. 47) underwent dihydroxylation much faster than the TBDPS protected analogue **4.25** to afford the very polar triol as a mixture of C6 epimers. Without purification, this mixture

was subjected to basic MeOH to induce *N*-deacetylation and lactonization, followed immediately by acetonide formation. Only the minor diastereomer in **5.72** reacted to yield **5.73**, enabling facile separation from **5.74** by chromatography. The latter was recrystallized from  $CHCl_3$  in pure form and it was doubly protected to afford **5.75**.



Scheme 5.21 A compressed preparation of 5.75

It should be noted that Kishi dihydroxylation of the minor,  $\beta$ -isomer of alcohol **4.24** might have produced some triol **5.76**, which upon ketalization would have advanced to **5.77** (or related compounds) At this time, we are unable to determine whether either byproduct was present in the crude mixture of **5.72** and **5.74**. Certainly, minor components of unknown structure were present.

The enolate of **5.75** was readily generated by the action of LDA at -78 °C. An important aspect of this reaction was that at least 4 equivalent of LDA were required to achieve complete deprotonation. As seen before with **4.104**, the enolate failed to undergo oxygenation upon



Scheme 5.22 A possible facial isomer 5.76 and its corresponding acetonide

exposure to  $O_2$  followed by P(OEt)<sub>3</sub>,<sup>112</sup> but it reacted with Davis oxaziridine **4.116** from the convex  $\alpha$ -face, resulting in the formation of hydroxylactone **5.79** as a single diastereomer. The configuration of the newly installed OH group was incorrect, necessitating an inversion of configuration. Moreover, substance **5.79** was accompanied by **5.80** (the product of addition of the enolate to the sulfonylimine derived from **4.116**, see also **Scheme 4.39**, p. 77) and by an unidentified byproduct **5.81**, in a 6:8:3 mole ratio (<sup>1</sup>H NMR).



Scheme 5.23 Hydroxylation of the enolate of 5.73 with Davis oxaziridine 4.116

The Davis step was not optimized further, nor was **5.79** extensively purified, on account of the very similar polarity of all products, unreacted **5.75** and **4.116**, and the sulfonylimine

corresponding to **4.116**. Careful column chromatography did provide some **5.79** contaminated with only 10-15% of **5.81**. However, it was more expedient to process the crude mixture through a redox sequence that served to invert the C9 configuration. Alcohol **5.79** was oxidized to ketone **5.82** with Dess-Martin periodinane (**Scheme 5.24**), and the ketone was immediately reduced with NaBH<sub>4</sub> at -50 °C to deliver diastereomeric alcohol **5.83** of exclusive (within the limits of 300 MHz <sup>1</sup>H NMR spectroscopy)  $\beta$ -configuration. Unlike previous synthetic intermediates, **5.83** was significantly more polar than other byproducts, permitting facile purification by column chromatography. The relative configuration of **5.83** was confirmed by a 2D NOESY experiment (**Scheme 5.24**).



Scheme 5.24 Inversion of the C9 configuration by a redox scheme

In accord with **Scheme 5.17**, our next objective was to generate a formal nitrile oxide connected to the OH group of **5.83**. A few options were explored for this purpose. The nitroacetate ester of **5.84** was prepared<sup>133-134</sup> as a possible substrate for a Machetti-De Sarlo reaction. However, under our standard condition, **5.84** (difficult to be isolated from **5.83**) yielded none of the desired isooxazoline, slowly reverting to **5.83** instead. Carreira-type *O*-silyl

hydroxycarbamate **5.86** was assembled as a prelude to dehydration with triflic anhydride.<sup>135</sup> The latter treatment resulted in formation of a complex mixture of unidentified products.



Scheme 5.25 Failed methods to generate nitrile oxides

Oximes may be oxidized to nitrile oxides by a number of methods.<sup>66</sup> Accordingly, alcohol **5.83** was converted into hydroxyiminoacetate ester **5.90** (Scheme 5.27) in preparation for oxidation of the hydroxyiminoacetyl unit to a carbalkoxylformonitrile oxide<sup>136-139</sup> and subsequent INOC reaction. This was done starting with esterification of the alcohol with acid **5.88**. This compound appears to be unknown in the literature, and it was prepared by the condensation between glyoxylic acid monohydrate **5.87** and *O*-TBDPS hydroxylamine **5.85** (Scheme 5.26)



Scheme 5.26 Preparation of acid 5.88

The condensation of **5.83** with **5.88** was achieved in the presence of DCC and the oxime in **5.89** was selectively desilylated by reaction of with a controlled amount of TBAF. A slow but noticeable decay of **5.90** back to **5.83** was observed even at room temperature, signaling that it was prudent to subject **5.90** to the next reaction after only a simple filtration through silica gel to remove silyl debris.



Scheme 5.27 Synthesis of the  $\alpha$ -hydroxyimino acetate 5.90

The oxidation of **5.90** with aqueous sodium hypochlorite gave almost only furoxan **5.92** as the product. Because the starting material was racemic, the furoxan (nitrile oxide dimer) emerged as a mixture of diastereomers. It subsequently transpired that a small amount of isooxazoline **5.93** had also formed. This was discovered upon a later comparison of the NMR spectra of crude reaction mixtures with those of an authentic sample of **5.93**.



Scheme 5.28 Dimerization of nitrile oxide 5.91
Padwa and coworkers had observed that INOC reactions analogous to the one shown above are problematic on account of conformational effects.<sup>140</sup> Specifically, the ester linkage in **5.91** and related species favor conformation **5.91A** very strongly (**Scheme 5.29**). This conformer does not allow the occurrence of an INOC reaction, dimerizing rapidly to furoxan **5.92** instead. The unfavorable conformer **5.91B** is conducive to the formation of the INOC product, but its instant concentration in solution is so low that only trace amounts of isooxazoline are produced. A Molecular Mechanics estimate<sup>117</sup> of the conformational energy difference between **5.91A** and **5.91B** returned a value of 8-9 kcal/mol, suggesting that, in a vacuum and at rt, the favored conformer **5.91A** dominates to the tune of *ca*.  $10^6$ :1. In solution, of course, the ratio can change greatly. On the upside, it was observed that upon contact with silica gel or mild base, furoxan **5.92** was converted back into starting **5.83**. Therefore, the formation of **5.92** did not constitute a total loss.



Scheme 5.29 Conformational rationale for the crucial INOC reaction

This state of affairs was not as desperate as it might seem, because (i) trace amounts of **5.93** were indeed formed, and (ii) the cyclization of **5.91B** to **5.93** is kinetically a first-order reaction, while the dimerization of **5.91A** is second-order. If the nitrile oxide were to be generated under conditions of high dilution, the rate of dimerization may well decrease to an acceptably low level compared to the rate of formation of **5.93**.



Scheme 5.30 Another side product from the fragmentation of nitrile oxide

A method developed in our laboratory appeared to be particularly well adaptable to the creation of **5.91** in dilute solutions. It had previously been found that oximes of various kind, including ethyl hydroxyliminoacetate, are easily oxidized to nitrile oxides upon exposure to DIB in methanol.<sup>141</sup> Surprisingly, however, slow addition of **5.90** into a dilute methanol solution of DIB yielded a roughly 1:3 mixture of **5.83** and **5.94**. This suggested that the  $\alpha$ -oxo nitrile oxide was not only prone to dimerization, but also to solvolysis with the attendant departure of fulminate. The latter, being a pseudohalide, is probably a good leaving group, and its departure may be accelerated through hydrogen bonding with (moderately nucleophilic) methanol.



Scheme 5.31 Successful INOC reaction under dilute conditions

The use of a polar, aprotic, and poorly nucleophilic solvent that dissolves DIB well could circumvent the above problems. In that connection we explored the use of acetonitrile. Slow addition of a MeCN solution of **5.90** (syringe pump, 6.5 h) to a dilute solution of DIB in dry MeCN (0.003 M final substrate concentration) resulted in formation of about 30% of the desired isooxazoline **5.93**, the balance of recovered material being furoxan **5.92** (<sup>1</sup>H NMR of crude reaction mixture). When the addition of the substrate was carried out over 25 h (syringe pump), the ratio of **5.93** to **5.92** improved to about 1:1. This exciting development proved that an INOC reaction was possible in a homogeneous dilute solution in an aprotic, poorly nucleophilic solvent.



Scheme 5.32 The decomposition pathways of 4.53 and 4.54

The isolation of **5.93** was not straightforward, being accompanied by a major loss of material upon flash chromatography. Such an operation promoted slow conversion of **5.92** to more polar

**5.83**, which would then contaminate all of later chromatographic fractions. More interestingly, the tension present in the tetracyclic system of **5.93** rendered the molecule prone to cleavage of the 6-membered ring lactone, resulting in formation of extremely polar acid **5.95** (not fully characterized). Upon heating at 80 °C in DMF, the latter sustained decarboxylative fragmentation to **5.96**.<sup>139</sup> The two operations could be combined simply by adding triethylamine and water to the reaction mixture in which the INOC step had been carried out, and heating to 50 °C, resulting slow decomposition of **5.92** to **5.83** and of **5.93** to **5.96** over 2 days. Compound **5.96** was also difficult to isolate due to its propensity to streak on silica gel. This was ultimately attributed to facile cyclization to polar, streaky iminolactone **5.97**.



Scheme 5.33 One-pot route to 4.58 from 4.51

The facile formation of the iminolactone was significant, in that it provided a straightforward solution for the creation of the C4a  $CH_2OH$  subunit through hydrolysis to a lactone and selective reduction. A similar sequence had been successfully employed in a total synthesis of (+)-camptothecin.<sup>142</sup> It proved possible to elaborate crude **5.93** directly to bis-lactone **5.97** in a one-pot operation. The reaction mixture from the INOC step was treated

with water and trimethylamine, heated to 50  $^{\circ}$ C for 2 days, and evaporated. The residue was taken up in THF and acidified with aqueous HCl, resulting in formation of a *ca*. 1:3 mixture of now readily separable, well behaved **5.83** and **5.98** (Scheme 5.33).



Scheme 5.34 Selective deprotection of 5.98

A short contact time of **5.98** with TBAF (1 equiv) cleanly produced a mono-TBS ether. Circumstantial evidence based on an inspection of the 2D COSY spectrum of the product suggests that the compound may be the C6 alcohol **5.99**. In fact, the resonance of the OH proton at 3.00 ppm exhibits a weak, but detectable, correlation with the C6 proton at 4.18 ppm.



Figure 5.2 COSY correlation in 5.99

#### **5.9 Future Work**

A priority objective in our group is the elaboration of a more concise route to tetrodotoxin through bis-lactonic intermediate **5.98**. This material is now available in 15 steps from

commercial methyl (*p*-hydroxyphenyl)acetate **3.5**, and in principle, it may be advanced to  $(\pm)$ -tetrodotoxin in 9 steps, as per **Scheme 5.35**.



Scheme 5.35 A proposed sequence to (±)-tetrodotoxin from 5.98

One appealing aspect to this route is that – as stated on p. 114 – alcohol 4.23/4.24 is significantly more reactive than 4.25 toward OsO<sub>4</sub>. It is possible that, unlike 4.25, 4.23/4.24 will undergo asymmetric dihydroxylation to furnish enantioenriched 5.105, thereby achieving desymmetrization of the 1,4-cyclohexadiene system.<sup>121</sup> This would ultimately enable an asymmetric synthesis of 1.1. Provided that the hydroxyl groups in 5.100 can be appropriately differentiated, a route to TTX from 3.5 would encompass as few as 24 steps: by far the shortest avenue to the natural product ever devised.



Scheme 5.36 A possible desymmetrization of 4.23/4.24 using asymmetric dihydroxylation

A few tetrodotoxin analogues are readily accessible from our synthetic intermediates. For example, 11-nortetrodotoxin-6-(S)-ol **5.107**, an unnatural tetrodotoxin derivative, is known to be only about 8% as potent as natural **1.1** as a sodium channel blocker.<sup>9</sup> However, its biological activity was not extensively studied, because of the inefficiency of its semisynthesis from **1.1**.<sup>9</sup> Our intermediate **5.98** could be easily advanced to **5.100** (Scheme 5.35), which is only 5 steps away from **5.107** (Scheme 5.37). A relatively straightforwad avenue to the latter would thus result.



Scheme 5.37 A proposed sequence to 5.107 from 5.100

In a similar vein, 9-deoxytetrodotoxin **5.109**, a novel analogue of the natural product, is now available in 4 steps from Du Bois-type intermeditate **4.134** (**Scheme 5.38**).<sup>31</sup> The biological activity of this compound is currently being evaluated to assess the importance of the 9-hydroxyl functional group.



Scheme 5.38 A proposed sequence to 5.109 from 4.134

#### **5.10** Conclusions

The work presented in this dissertation demonstrated the first synthetic application of the bimolecular oxidative amidation of phenols, and it proves that the methodology can sustain efforts toward complex molecules even when incorporated at an early stage of the synthetic sequence. The structural correction of the Sato lactone is likely to be of interest to ther researchers currently pursuing a total synthesis of the natural product. Furthermore, the new route to intermediates of the type **5.98** illustrates a new strategy and presages the advent of a more concise, stereocontrolled route to TTX.

A significant dimension of the work described herein is that the synthetic intermediates prepared in the course of this effort could provide access to analogues of tetrodotoxin that enable mapping of voltage-gated sodium channels, and that might exhibit more desirable biological properties relative to **1.1**.

# References

- 1. Chau, J.; Ciufolini, M. A. Mar. Drugs 2011, 9, 2046.
- 2. Lago, J.; Rodr guez, L. P.; Blanco, L.; Vieites, J. M.; Cabado, G. Mar. Drugs 2015, 13, 6384.
- 3. Hwang, D.-F.; Noguchi, T. Adv. Food Nutr. Res. 2007, 52, 141.
- 4. Noguchi, T.; Arakawa, O. Mar. Drugs 2008, 6, 220.
- 5. TETRODOTOXIN : Biotoxin; CDC The Emergency Response Safety and Health Database,

The National Institute for Occupational Safety and Health (NIOSH). Available from http://www.cdc.gov/niosh/ershdb/emergencyresponsecard\_29750019.html.

- 6. Bane, V.; Lehane, M.; Dikshit, M.; O'Riordan, A.; Furey, A. Toxins 2014, 6, 693.
- 7. Cusick, K. D.; Sayler, G. S. Mar. Drugs 2013, 11, 997.
- 8. Satin, J.; Limberis, J. T.; Kyle, J. W.; Rogart, R. B.; Fozzard, H. A. Biophys. J. 1994, 67, 1007.
- 9. Yotsu-Yamashita, M.; Sugimoto, A.; Takai, A.; Yasumoto, T. J. Pharm. Exp. Therap. 1999, 289, 1688.
- 10. Lipkind, G. M.; Fozzard, H. A. Biophys. J. 1994, 66, 1.
- 11. Hucho, F. Angew. Chem. Int. Ed. Engl. 1995, 34, 39.
- 12. Guzmán, A.; Fernández de Henestrosa, A. R.; Mar ń, A.-P.; Ho, A.; González Borroto, J. I.; Carasa, I.; Pritchard, L. *Mutat. Res. Gen. Tox. En.* **2007**, *634*, 14.

13. Berde, C. B.; Athiraman, U.; Yahalom, B.; Zurakowski, D.; Corfas, G.; Bognet, C. *Mar. Drugs* **2011**, *9*, 2717.

- 14. Nieto, F. R.; Cobos, E. J.; Tejada, M. A.; S ánchez Fern ández, C.; Gonz ález-Cano, R.; Cend án, C. M. *Mar. Drugs* **2012**, *10*, 281.
- 15. Noguchi, T.; Arakawa, O.; Takatani, T. Comp. Biochem. Physiol. Part D Genom. Proteom. 2006, 1, 153.
- 16. Hanifin, C. T.; Brodie, E. D., III; Brodie, E. D., Jr. Toxicon 2002, 40, 1149.
- 17. Chau, R.; Kalaitzis, J. A.; Neilan, B. A. Aquat. Toxicol. 2011, 104, 61.
- 18. Matsumura, K.; Kim, D.-S.; Kim, C.-H. Appl. Environ. Microbiol. 2001, 67, 2393.
- 19. Shimizu, Y.; Kobayashi, M. Chem. Pharm. Bull. 1983, 31, 3625.
- 20. Fellgett, P. B. J. Opt. Soc. Am. 1952, 42, 872.
- 21. Woodward, R. B. Pure Appl. Chem. 1964, 9, 49.
- 22. Tsuda, K.; Ikuma, S.; Kawamura, M.; Tachikawa, R.; Sakai, K.; Tamura, C.; Amakasu, O. *Chem. Pharm. Bull.* **1964**, *12*, 1357.
- 23. Tomiie, Y.; Furusaki, A.; Kasami, K.; Yasuoka, N.; Miyake, K.; Haisa, M.; Nitta, I. *Tetrahedron Lett.* **1963**, *4*, 2101.
- 24. Tsuda, K. Naturwissenschaften 1966, 53, 171.
- 25. Kishi, Y.; Nakatsubo, F.; Aratani, M.; Goto, T.; Inoue, S.; Kakoi, H.; Sugiura, S. *Tetrahedron Lett.* **1970**, *11*, 5127.
- 26. Kishi, Y.; Nakatsubo, F.; Aratani, M.; Goto, T.; Inoue, S.; Kakoi, H. Tetrahedron Lett. 1970, 11,

5129.

- 27. Kishi, Y.; Aratani, M.; Fukuyama, T.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura,
- S.; Kakoi, H. J. Am. Chem. Soc 1972, 94, 9217.
- 28. Kishi, Y.; Fukuyama, T.; Aratani, M.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura,
- S.; Kakoi, H. J. Am. Chem. Soc 1972, 94, 9219.
- 29. Ohyabu, N.; Nishikawa, T.; Isobe, M. J. Am. Chem. Soc 2003, 125, 8798.
- 30. Nishikawa, T.; Urabe, D.; Isobe, M. Angew. Chem. Int. Ed. 2004, 43, 4782.
- 31. Hinman, A.; Du Bois, J. J. Am. Chem. Soc 2003, 125, 11510.
- 32. Sato, K.; Akai, S.; Sugita, N.; Ohsawa, T.; Kogure, T.; Shoji, H.; Yoshimura, J. J. Org. Chem. **2005**, *70*, 7496.
- 33. Sato, K.; Akai, S.; Shoji, H.; Sugita, N.; Yoshida, S.; Nagai, Y.; Suzuki, K.; Nakamura, Y.;
- Kajihara, Y.; Funabashi, M.; Yoshimura, J. J. Org. Chem. 2008, 73, 1234.
- 34. Cagide-Fag n, F.; Alonso, R. Eur. J. Org. Chem. 2010, 6741.
- 35. Cha, J. K.; Christ, W. J.; Kishi, Y. Tetrahedron 1984, 40, 2247.
- 36. Lago-Santomé, H.; Meana-Pañeda, R.; Alonso, R. J. Org. Chem. 2014, 79, 4300.
- 37. Ciufolini, M. A.; Canesi, S.; Ousmer, M.; Braun, N. A. Tetrahedron 2006, 62, 5318.
- 38. Ciufolini, M. A.; Braun, N. A.; Canesi, S.; Ousmer, M.; Chang, J.; Chai, D. *Synthesis* **2007**, 3759.
- 39. Liang, H.; Ciufolini, M. A. Tetrahedron 2010, 66, 5884.
- 40. Wessely, F.; Lauterbach-Keil, G.; Sinwel, F. Monatsh. Chem. Verw. Tl. 1950, 81, 811.
- 41. Roche, S. P.; Porco, J. A., Jr. Angew. Chem. Int. Ed. 2011, 50, 4068.
- 42. Varghese, V.; Hudlicky, T. Angew. Chem. Int. Ed. 2014, 53, 4355.
- 43. Seebach, D. Angew. Chem. Int. Ed. Engl. 1979, 18, 239.
- 44. Braun, N. A.; Ousmer, M.; Bray, J. D.; Bouchu, D.; Peters, K.; Peters, E.-M.; Ciufolini, M. A. *J. Org. Chem.* **2000**, *65*, 4397.
- 45. Ousmer, M.; Braun, N. A.; Ciufolini, M. A. Org. Lett. 2001, 3, 765.
- 46. Ousmer, M.; Braun, N. A.; Bavoux, C.; Perrin, M.; Ciufolini, M. A. J. Am. Chem. Soc 2001, 123, 7534.
- 47. Paladino, M.; Zaifman, J.; Ciufolini, M. A. Org. Lett. 2015, 17, 3422.
- 48. Canesi, S.; Belmont, P.; Bouchu, D.; Rousset, L.; Ciufolini, M. A. *Tetrahedron Lett.* **2002**, *43*, 5193.
- 49. Liang, H.; Ciufolini, M. A. Chem. Eur. J. 2010, 16, 13262.
- 50. Jain, N.; Ciufolini, M. A. Synlett 2015, 26, 631.
- 51. Tang, D. unpublished results from our laboratory.
- 52. Canesi, S.; Bouchu, D.; Ciufolini, M. A. Angew. Chem. Int. Ed. 2004, 43, 4336.
- 53. Kasahara, T.; Ciufolini, M. A. Can. J. Chem. 2013, 91, 82.
- 54. Masamune, S.; Choy, W.; Petersen, J. S.; Sita, L. R. Angew. Chem. Int. Ed. Engl. 1985, 24, 1.
- 55. Canesi, S.; Bouchu, D.; Ciufolini, M. A. Org. Lett. 2004, 7, 175.
- 56. Liang, H.; Ciufolini, M. A. J. Org. Chem. 2008, 73, 4299.
- 57. Chau, J.; Ciufolini, M. A. Org. Synth. 2013, 90, 190.

- 58. Corey, E. J.; Ohno, M.; Vatakencherry, P. A.; Mitra, R. B. J. Am. Chem. Soc 1961, 83, 1251.
- 59. Corey, E. J.; Ohno, M.; Mitra, R. B.; Vatakencherry, P. A. J. Am. Chem. Soc 1964, 86, 478.
- 60. VanRheenen, V.; Kelly, R. C.; Cha, D. Y. Tetrahedron Lett. 1976, 17, 1973.
- 61. Chau, J. Thesis, The University of British Columbia, 2012.
- 62. Mendelsohn, B. A.; Ciufolini, M. A. Org. Lett. 2009, 11, 4736.
- 63. Cecchi, L.; De Sarlo, F.; Machetti, F. Chem. Eur. J. 2008, 14, 7903.
- 64. Corey, E. J.; Bakshi, R. K.; Shibata, S. J. Am. Chem. Soc 1987, 109, 5551.
- 65. Gu érard, K. C.; Chapelle, C.; Giroux, M.-A.; Sabot, C.; Beaulieu, M.-A.; Achache, N.; Canesi,
- S. Org. Lett. 2009, 11, 4756.

66. Belen'kii, L. I. In Nitrile Oxides, Nitrones, and Nitronates in Organic Synthesis: Novel

*Strategies in Synthesis*, 2 ed.; Feuer, H., Ed. John Wiley & Sons, Inc.: Hoboken, New Jersey, 2008; pp 4.

67. Donohoe, T. J.; Blades, K.; Moore, P. R.; Waring, M. J.; Winter, J. J. G.; Helliwell, M.;

- Newcombe, N. J.; Stemp, G. J. Org. Chem. 2002, 67, 7946.
- 68. Du Vigneaud, V.; Meyer, C. E. J. Biol. Chem. 1932, 99, 143.
- 69. Halpern, B.; Chew, L. F.; Westley, J. W. Anal. Chem. 1967, 39, 399.
- 70. Flynn, D. L.; Zelle, R. E.; Grieco, P. A. J. Org. Chem. 1983, 48, 2424.
- 71. Carre ño, M. C.; Ortega-Guerra, M.; Ribagorda, M.; Sanz-Cueta, M. J. *Chem. Eur. J.* **2007**, *14*, 621.
- 72. Woodward, R. B.; Hoffmann, R. J. Am. Chem. Soc 1965, 87, 395.
- 73. Hoffmann, R.; Woodward, R. B. Acc. Chem. Res. 1968, 1, 17.
- 74. Fritschi, H.; Leutenegger, U.; Pfaltz, A. Angew. Chem. Int. Ed. Engl. 1986, 25, 1005.
- 75. Evans, D. A.; Miller, S. J.; Lectka, T.; von Matt, P. J. Am. Chem. Soc 1999, 121, 7559.
- 76. Švorc, Ľ.; Tomčík, P.; Durdiak, J.; Rievaj, M.; Bustin, D. Pol. J. Environ. Stud. 2012, 21, 7.
- 77. Dupau, P.; Epple, R.; Thomas, A. A.; Fokin, V. V.; Sharpless, K. B. Adv. Synth. Catal. 2002, 344, 421.
- 78. Minato, M.; Yamamoto, K.; Tsuji, J. J. Org. Chem. 1990, 55, 766.
- 79. Döbler, C.; Mehltretter, G. M.; Sundermeier, U.; Beller, M. J. Organomet. Chem. 2011, 621, 70.
- 80. Junttila, M. H.; Hormi, O. O. J. Org. Chem. 2009, 74, 3038.
- 81. Shing, T. K. M.; Tai, V. W.-F.; Tam, E. K. W. Angew. Chem. Int. Ed. Engl. 1994, 33, 2312.
- 82. Plietker, B.; Niggemann, M. J. Org. Chem. 2005, 70, 2402.
- 83. Bhushan, V.; Rathore, R.; Chandrasekaran, S. Synthesis 1984, 431.
- 84. Greedan, J. E.; Willson, D. B.; Haas, T. E. Inorg. Chem. 1968, 7, 2461.
- 85. Dess, D. B.; Martin, J. C. J. Org. Chem. 1983, 48, 4155.
- 86. Peterson, D. J. J. Org. Chem. 1968, 33, 780.
- 87. Mengel, A.; Reiser, O. Chem. Rev. 1999, 99, 1191.
- 88. Miyashita, M.; Yoshikoshi, A.; Grieco, P. A. J. Org. Chem. 1977, 42, 3772.
- 89. Hutchins, R. O.; Learn, K.; El-Telbany, F.; Stercho, Y. P. J. Org. Chem. 1984, 49, 2438.
- 90. Ragnarsson, U.; Grehn, L.; Monteiro, L. S.; Maia, H. L. S. Synlett 2003, 2386.

- 91. Spletstoser, J. T.; White, J. M.; Rao Tunoori, A.; Georg, G. I. J. Am. Chem. Soc 2007, 129, 3408.
- 92. Pelletier, G.; Bechara, W. S.; Charette, A. B. J. Am. Chem. Soc 2010, 132, 12817.
- 93. Balaraman, E.; Gnanprakasam, B.; Shimon, L. J. W.; Milstein, D. J. Am. Chem. Soc **2010**, *132*, 16756.
- 94. Szostak, M.; Spain, M.; Eberhart, A. J.; Procter, D. J. J. Am. Chem. Soc 2014, 136, 2268.
- 95. Anelli, P. L.; Brocchetta, M.; Palano, D.; Visigalli, M. Tetrahedron Lett. 1997, 38, 2367.
- 96. Hansen, M. M.; Harkness, A. R.; Coffey, D. S.; Bordwell, F. G.; Zhao, Y. *Tetrahedron Lett.* **1995**, *36*, 8949.
- 97. Kögl, M.; Brecker, L.; Warrass, R.; Mulzer, J. Eur. J. Org. Chem. 2008, 2714.
- 98. Dess, D. B.; Martin, J. C. J. Am. Chem. Soc 1991, 113, 7277.
- 99. Westheimer, F. H.; Nicolaides, N. J. Am. Chem. Soc 1949, 71, 25.
- 100. Parikh, J. R.; v. E. Doering, W. J. Am. Chem. Soc 1967, 89, 5505.
- 101. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. J. Chem. Soc., Chem. Commun. 1987, 1625.
- 102. F dizon, M.; Golfier, M.; Mourgues, P. Tetrahedron Lett. 1972, 13, 4445.
- 103. Williams, D. R.; Klingler, F. D.; Allen, E. E.; W., L. F. Tetrahedron Lett. 1988, 29, 5087.
- 104. Firouzabadi, H.; Ghaderi, E. Tetrahedron Lett. 1978, 19, 839.
- 105. Feng, S.; Bagia, C.; Mpourmpakis, G. J. Phys. Chem. A 2013, 117, 5211.
- 106. Davis, F. A.; Vishwakarma, L. C.; Billmers, J. G.; Finn, J. J. Org. Chem. 1984, 49, 3241.
- 107. Davis, F. A.; Chen, B.-C. Chem. Rev. 1992, 92, 919.
- 108. Vedejs, E.; Engler, D. A.; Telschow, J. E. J. Org. Chem. 1978, 43, 188.
- 109. Corey, E. J.; Sneen, R. A. J. Am. Chem. Soc 1956, 78, 6269.
- 110. Behnam, S. M.; Behnam, S. E.; Ando, K.; Green, N. S.; Houk, K. N. J. Org. Chem. 2000, 65, 8970.
- 111. Corey, E. J.; Ensley, H. E. J. Am. Chem. Soc 1975, 97, 6908.
- 112. Wasserman, H. H.; Lipshutz, B. H. Tetrahedron Lett. 1975, 16, 1731.
- 113. Rubottom, G. M.; Vazquez, M. A.; Pelegrina, D. R. Tetrahedron Lett. 1974, 15, 4319.
- 114. Hassner, A.; Reuss, R. H.; Pinnick, H. W. J. Org. Chem. 1975, 40, 3427.
- 115. Rawal, V. H.; Cava, M. P. Tetrahedron Lett. 1985, 26, 6141.

116. Huffman, J. W. In *Comprehensive Organic Synthesis - Selectivity, Strategy & Efficiency in Modern Organic Chemistry*, 1 ed. Vol. 8; Flaming, I., Ed. Pergamon Press: Oxford, United Kingdoms, 1991; p 114.

- 117. This work was carried out using the MM+ program provided with the HyperChem<sup>®</sup> suite.
- 118. Corey, E. J.; Suggs, J. W. Tetrahedron Lett. 1975, 16, 2647.
- 119. Wang, L.; Sharpless, K. B. J. Am. Chem. Soc 1992, 114, 7568.
- 120. Andersson, P. G.; Sharpless, K. B. J. Am. Chem. Soc 1993, 115, 7047.
- 121. Wang, Z.-M.; Kakiuchi, K.; Sharpless, K. B. J. Org. Chem. 1994, 59, 6895.
- 122. Guideri, L.; De Sarlo, F.; Machetti, F. Chem. Eur. J. 2013, 19, 665.
- 123. Rechavi, D.; Lemaire, M. Chem. Rev. 2002, 102, 3467.

- 124. Paladino, M. unpublished results from our laboratory.
- 125. Yadav, J. S.; Nanda, S.; Thirupathi Reddy, P.; Bhaskar Rao, A. J. Org. Chem. 2002, 67, 3900.
- 126. Snider, B. B.; Grabowski, J. F. Tetrahedron 2006, 62, 5171.
- 127. Liu, P.; He, W.; Zhao, Y.; Wang, P.-A.; Sun, X.-L.; Li, X.-Y.; Zhang, S.-Y. *Chirality* **2008**, *20*, 75.
- 128. Mendelsohn, B. A.; Lee, S.; Kim, S.; Teyssier, F.; Aulakh, V. S.; Ciufolini, M. A. Org. Lett. **2009**, *11*, 1539.
- 129. Blaser, H.-U. Chem. Rev. 1992, 92, 935.
- 130. Nambara, T.; Ikegawa, S.; Hasegawa, M.; Goto, J. Anal. Chim. Acta 1978, 101, 111.
- 131. Lindgren, B. O.; Nilsson, T. Acta Chem. Scand. 1973, 27, 888.
- 132. Takai, K.; Kakiuchi, T.; Kataoka, Y.; Utimoto, K. J. Org. Chem. 1994, 59, 2668.
- 133. Sylvain, C.; Wagner, A.; Mioskowski, C. Tetrahedron Lett. 1999, 40, 875.
- 134. Vanier, S. F.; Larouch, G.; Wurz, R. P.; Charette, A. B. Org. Lett. 2010, 12, 672.
- 135. Muri, D.; Bode, J. W.; Carreira, E. M. Org. Lett. 2000, 2, 539.
- 136. Skinner, G. S. J. Am. Chem. Soc 1924, 46, 731.
- 137. Panizzi, L. Gazz. Chim. Ital. 1939, 69, 332.
- 138. Vaughan, W. R.; Spenser, J. L. J. Org. Chem. 1960, 25, 1160.
- 139. Kozikowski, A. P.; Adamczyk, M. J. Org. Chem. 1983, 48, 366.
- 140. Hassner, A.; Murthy, K. S. K.; Padwa, A.; Chiacchio, U.; Dean, D. C.; Schoffstall, A. M. J. Org. Chem. **1989**, *54*, 5277.
- 141. Jen, T.; Mendelsohn, B. A.; Ciufolini, M. A. J. Org. Chem. 2011, 76, 728.
- 142. Ciufolini, M. A.; Roschangar, F. Angew. Chem. Int. Ed. Engl. 2003, 35, 1692.
- 143. Davis, F. A.; Chattopadhyay, S.; Towson, J. C.; Lal, S.; Reddy, T. J. Org. Chem. **1988**, *53*, 2087.
- 144. Burchat, A. F.; Chong, J. M.; Nielsen, N. J. Organomet. Chem. 1997, 542, 281.
- 145. Hinman, A. W. Dissertation, Stanford University, 2004.

# Appendices

## **Appendix A: Complete Sequence to Tetrodotoxin**

## A.1 Sequence to 4.88, a Crucial TTX Intermediate from 3.6





A.2 Sequence to Sato Lactone, 4.123 from 4.88



#### A.3 Sequence to Du Bois Intermediate, 1.109 from 4.88



## A.4 Sequence to 5.98, a New TTX Intermediate from 4.23





# Appendix B: Experimental

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#### **B.2 Experimental Protocols**

Unless otherwise indicated, 1D <sup>1</sup>H (300 MHz), 1D <sup>13</sup>C (75 MHz), and 2D (300 MHz for <sup>1</sup>H) NMR spectra were obtained from CDCl<sub>3</sub> solutions at rt. Chemical shifts are reported in parts per million (ppm) on the  $\delta$  scale; coupling constants, J, in hertz (Hz); multiplicities as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "dd" (doublet of doublets), "m" (multiplet), "br" (broad), "ABq" (AB quartet), "app" (apparent). Infrared (IR) spectra  $(cm^{-1})$  were recorded from films (Perkin Elmer<sup>®</sup> Universal ATR Sampling Accessories). Low- and high-resolution mass spectra (m/z) were obtained in the electrospray (ESI) mode in methanol or acetonitrile solution. Melting points (uncorrected) were measured on a Mel-Temp apparatus. Commercial reagents and solvents were used without further purification except THF (freshly distilled from Na/benzophenone under N<sub>2</sub>), CH<sub>2</sub>Cl<sub>2</sub> (freshly distilled from CaH<sub>2</sub> under N<sub>2</sub>), MeOH (freshly distilled from Mg/I<sub>2</sub> under Ar), 1,2-DCE (distilled from CaH<sub>2</sub> under argon then stored over 4Å molecular sieves), MeCN (distilled from CaH<sub>2</sub> under argon then stored over 3Å molecular sieves), triethylamine, diisopropylamine (both distilled from CaH<sub>2</sub> under Ar then stored over KOH) and diisopropylethylamine (freshly distilled from CaH<sub>2</sub> under Ar). The Davis oxaziridine **4.116** was prepared by a literature method.<sup>143</sup> Commercial *n*-BuLi was titrated against *N*-benzylbenzamide in THF at -40 °C until persistence of a light blue color.<sup>144</sup> Flash chromatography was performed on Silicycle<sup>®</sup> 230 – 400 mesh silica gel unless otherwise noted. Analytic TLC was carried out with Merck<sup>®</sup> silica gel 60 plates with fluorescent indicator. Spots were visualized with UV light or permanganate stain. Preparative TLC was carried out with premade Analtech TLC 151

Uniplates<sup>TM</sup> (silica gel, fluorescent indicator, 500 or 1000 microns). Unless otherwise indicated, all reactions were performed under Ar atmosphere in oven-dried flasks fitted with rubber septa for the introduction of substrates/reagents/solvents via syringe, and equipped with Teflon<sup>TM</sup> stirring bars.

#### **B.3 Experiments and Data**



**Preparation of 4.7** 

Solid DMAP (82 mg, 0.67 mmol, 0.04 equiv) and solid Boc<sub>2</sub>O (6.6 g, 30.3 mmol, 1.8 equiv) were added to a solution of dienone **3.6**<sup>57</sup> (3.75 g, 16.8 mmol, 1.0 equiv) in THF (100 mL). The mixture was stirred under Ar at rt for 48 h, then it was concentrated under vacuum. The residue was dissolved in EtOAc (50 mL) and the resulting solution was washed with 0.02 M HCl solution (2x30 mL), DI water (10 mL) and brine (10 mL), then dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The residue was purified by chromatography (gradient EtOAc:Hex, 30:70~50:50~70:30) to afford, in order of elution: **4.9** (0.72 g, 1.7 mmol, 10%;  $R_f$ =0.50 in 30:70 EtOAc/Hexanes) as pale yellow oil; **4.10** (0.17 g, 0.3 mmol, 4%;  $R_f$ =0.34 in 30:70 EtOAc/Hexanes) as white plates; desired **4.7** (3.20 g, 9.9 mmol, 61%;  $R_f$ =0.24 in 30:70 EtOAc/Hexanes) as a pale yellow oil, and **4.8** (0.88 g, 2.7 mmol, 16%;  $R_f$ =0.52 in EtOAc) as a reddish oil.

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 7.26 (d, 2H, *J*=10.2); 6.24 (d, 2H, *J*=10.2); 3.66 (s, 3H); 3.22 (s, 2H); 2.28 (s, 3H); 1.46 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 184.6, 172.2, 169.0, 152.7, 148.4, 128.0, 85.2, 58.7, 52.0, 42.5, 27.5, 26.3.
IR (film): 1740, 1690, 1670, 1631.

**MS**: 346 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{16}H_{21}NO_6Na [M + Na]^+$  346.1267; found 346.1265.

<sup>1</sup>H NMR spectrum of 4.7 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.7 (75 MHz, CDCl<sub>3</sub>)




Data for 4.8

<sup>1</sup>H (**300 MHz, CDCl<sub>3</sub>**): 5.85 (d, 1H, *J*=9.8); 5.77 (dd, 1H, *J*=10.2, 1.9); 5.67 (app. d, 1H, *J*=5.1);

5.77 (d, 1H, *J*=5.1); 3.64 (s, 3H); 2.79, 2.64 (ABq, 2H, *J*<sub>AB</sub>=14.9); 1.96(s, 3H); 1.49 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.0, 166.1, 150.8, 148.1, 132.4, 120.8, 106.3, 84.0, 80.4, 69.0, 52.0, 45.5,

27.8, 14.1.

**IR (film**): 1754, 1739, 1662.

**MS**: 346 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{16}H_{22}NO_6 [M + H]^+$  324.1447; found 324.1453.

# <sup>1</sup>H NMR spectrum of 4.8 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.8 (75 MHz, CDCl<sub>3</sub>)





Data for 4.9

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 6.36 (dd, 1H, *J*=7.0, 1.2); 6.21 (app. d, 1H, *J*=9.7); 6.05 (d, 1H, *J*=7.0);
5.63 (dd, 1H, *J*=9.7, 6.7); 3.69 (s, 3H); 3.28 (d, 1H, *J*=6.7); 2.47 (s, 3H); 1.51(s, 9H); 1.43 (s, 9H).
<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 173.3, 170.9, 152.3, 151.5, 151.3, 124.2, 123.9, 122.8, 122.3, 117.2, 83.8,
83.7, 52.3, 47.8, 27.9, 27.7, 26.1.

IR (film): 1739, 1708 (shoulder).

**MS**: 446 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{21}H_{29}NO_8Na [M + Na]^+ 446.1791$ ; found 446.1793.

<sup>1</sup>H NMR spectrum of 4.9 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.9 (75 MHz, CDCl<sub>3</sub>)





Data for 4.10

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 7.10, 7.03 (app. ABq, 8H, *J*<sub>AB</sub>=8.8); 3.82 (s, 6H); 1.54(s, 18H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 168.2, 151.5, 151.2, 138.2, 131.5, 131.0, 121.2, 83.9, 52.9, 27.8.

**IR (film)**: 1752, 1718.

**MS**: 551 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{28}H_{32}O_{10}Na [M + Na]^+ 551.1893$ ; found 551.1888.

**mp (Hexanes)**: 171 ℃ (dec)

## <sup>1</sup>H NMR spectrum of 4.10 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.10 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.25** 

Commercial BH<sub>3</sub>'SMe<sub>2</sub> complex (1.0 mL, 10.0 mmol, 1.0 equiv) was carefully syringed over 5 min into a cold (0 °C) solution of 4.7 (3.20 g, 9.9 mmol, 1.0 equiv) and (S)-CBS catalyst (28 mg, 0.1 mmol, 0.01 equiv) in THF (74 mL), with good stirring under Ar. The ice bath cooling the mixture was removed and stirring was continued for an additional 50 min. The reaction mixture was quenched by careful dropwise addition of MeOH (1.5 mL, 37 mmol, 3.7 equiv; CAUTION: H<sub>2</sub> evolution). When gas evolution stopped, more MeOH (10 mL) was added and stirring was continued for another 15 min. The solution was concentrated, the residue was redissolved in MeOH (15 mL) and the solution was again concentrated to dryness. The latter operation was repeated once to ensure complete decomposition of organoboron species. The residue was then filtered through a short silica gel plug with 50% EtOAc/Hexanes until no product (R<sub>f</sub>=0.13 in 30:70 EtOAc/Hexanes) was observed in the eluate by TLC. The filtrate was concentrated to dryness under vacuum and the residue was subjected to azeotropic evaporation with toluene (15 mL) and then dried under high vacuum to constant mass (3.0 g). The crude reduction product 4.23/4.24 was dissolved in dry DMF (20 mL) and treated with imidazole (1.30 g, 19.1 mmol, 1.9 equiv) and TBDPSCl (2.9 mL, 11.2 mmol, 1.1 equiv). The reaction flask was then immersed into an oil bath maintained at 70 °C and the solution stirred for 24 h. The mixture was then cooled to rt and most DMF was removed under vacuum. The residue was taken up in EtOAc (60 mL) and the

solution was successively washed with 0.02 M HCl solution (3x30 mL), DI water (15 mL) and brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The residue was redissolved in MeOH (15 mL) and the solution was again concentrated to dryness. The latter operation was repeated once to ensure complete removal of volatiles. The residue thus obtained was dissolved in refluxing MeOH (15 mL) and the hot solution was allowed to stand overnight, whereupon **4.25** crystallized as transparent prisms. This solid was filtered and rinsed with cold MeOH (2x5 mL), then recrystalized again from MeOH (10 mL) to afford pure **4.25** (2.82 g, 5.0 mmol, 51%) as white prisms. The combined mother liquors from such recrystallizations were concentrated and the residue was subjected to chromatography (gradient EtOAc:Hex, 10:90~20:80) to obtain more **4.25** (0.45 g, 0.8 mmol, 8%; R<sub>f</sub>=0.38 in 20:80 EtOAc/Hexanes), **4.26** (0.44 g, 0.8 mmol, 8%; R<sub>f</sub>=0.31 in 20:80 EtOAc/Hexanes) as pale yellow oil (crystallized at  $-20^{\circ}$ C), and a mixture of the two (0.56 g, 1.0 mmol, 10%; containing 48% of **4.25**). In summary, a 76% yield of both diastereomer was obtained in 4.9:1 diastereomeric ratio.

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.71-7.67 (m, 4H); 7.46-7.36 (m, 6H); 6.16 (dd, 2H, *J*=10.3, 1.8); 5.81 (dd, 2H, *J*=10.3, 2.9); 4.46-4.42 (m, 1H); 3.53 (s, 3H); 3.08 (s, 2H); 2.16 (s, 3H); 1.53 (s, 9H); 1.07 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.5, 170.1, 153.9, 134.0, 133.7, 130.0, 129.5, 128.0, 127.9, 84.5, 63.1,
57.3, 51.6, 44.0, 27.6, 27.0, 25.2, 19.3.

**IR (film)**: 1741, 1682.

**MS**: 586 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{32}H_{41}NO_6NaSi [M + Na]^+ 586.2601$ ; found 586.2600.

**mp (methanol)**: 94-95 °C

## <sup>1</sup>H NMR spectrum of 4.25 (300 MHz, CDCl<sub>3</sub>)



## <sup>13</sup>C NMR spectrum of 4.25 (75 MHz, CDCl<sub>3</sub>)





Data for 4.26

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.71-7.66 (m, 4H); 7.44-7.35 (m, 6H); 6.20 (dd, 2H, *J*=10.3, 2.1); 5.89 (dd, 2H, *J*=10.3, 2.7), 4.58-4.53 (m, 1H); 3.67 (s, 3H); 3.19 (s, 2H); 2.04 (s, 3H); 1.29 (s, 9H); 1.07 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.4, 169.8, 153.6, 136.0, 133.9, 132.3, 129.9, 128.4, 127.9, 84.1, 63.8,

57.1, 51.8, 43.1, 27.4, 27.1, 24.9, 19.3.

IR (film): 1741, 1682.

**MS**: 586 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{32}H_{41}NO_6SiNa [M + Na]^+ 586.2601$ ; found 586.2598.

**mp (methanol)**: 82-84 ℃.

### <sup>1</sup>H NMR spectrum of 4.26 (300 MHz, CDCl<sub>3</sub>)



## <sup>1</sup>H NMR spectrum of 4.32 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.32 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.34** 

Hydrazine monohydrate (225 µL, 4.4 mmol, 1.7 equiv) was added (by a syringe) to a suspension of 4.25 (1.47 g, 2.61 mmol, 1.0 equiv) in THF (26 mL) maintained under Ar in a heavy-walled glass tube fitted with a screw cap container. The mixture was heated to 60  $\,^{\circ}$ C and stirred for 24 h, then it was cooled to rt and concentrated in vacuo, and the residue was filtered through a short silica gel plug (10 mL) with 1:1 EtOAc/Hexanes until no deacetylated 4.32 (R<sub>f</sub>=0.72 in 30:70 EtOAc/Hexanes) eluted. The filtrate was concentrated to dryness and the residual light yellow oil was dissolved in THF (14 mL). DI water (14 mL) was added with good stirring, followed by solid LiOH<sub>H2</sub>O (332 mg, 7.90 mmol, 3.0 equiv, added in one portion). The resulting suspension became a clear monophasic solution within 4 h. Stirring was continued for another hour until all starting ester had been consumed, then the mixture was cooled in an ice bath and acidified with 0.4 M HCl solution (20 mL, 8.0 mmol, 3.1 equiv), added slowly and with vigorous stirring. The acidic solution was extracted with EtOAc (2x35 mL), the combined extracts were washed with DI water (20 mL) and brine (20 mL), then dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The residue of crude **4.27** was dissolved in THF (17 mL) and treated with carbonyldiimidazole (467 mg, 2.88 mmol, 1.1 equiv). After 3 h, to the mixture was added MeNO<sub>2</sub> (840 µL, 15.6 mmol, 6.0 equiv) and t-BuOK (1.17g, 10.4 mmol, 4.0 equiv), then the reaction flask was immersed in an oil bath maintained at 40  $\,^{\circ}$ C for

30 min. The mixture was cooled to rt and quenched by adding 1:9 HOAc/H<sub>2</sub>O (20 mL) solution, then it was extracted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL, 2x10 mL). The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. Complete removal of AcOH was achieved by azeotropic distillation with toluene (3x10 mL) under vacuum. The residue was filtered through a short silica gel plug (10 mL) with 50:50 EtOAc/Hexanes (removal of imidazonium salts) until no more 4.33 ( $R_f= 0.46$  in 30:70 EtOAc/Hexanes, streak) eluted. Concentration of the filtrate afforded crude 4.33, which was dissolved in freshly distilled MeCN (64 mL) containing Cu(OAc)<sub>2</sub>H<sub>2</sub>O (25 mg, 0.13 mmol, 0.05 equiv), silica gel (14 mg), and N-ethylpiperidine (105 µL, 0.76 mmol, 0.3 equiv). The resulting suspension was stirred (Ar) at 35 °C (oil bath) for 168 h, whereupon TLC indicated complete consumption of 4.33. The mixture was concentrated under vacuum, the residue was dissolved in EtOAc (70 mL) and the solution washed with 0.02 M HCl (3x20 mL) and brine (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. Purification of the residue by flash chromatography (gradient EtOAc:Hex, 10:90~20:80) furnished 4.34 as an off-white foam (470 mg, 0.88 mmol, 34%; R<sub>f</sub>=0.56 in 30:70 EtOAc/Hexanes). Further elution with 50:50 EtOAc/Hexanes returned acid 4.27 (340 mg, 0.67 mmol, 26%;  $R_{f}$ = 0.37 in 70:30 EtOAc/Hexanes, streak), which was conveniently recycled. For the last reaction, instead of applying the aqueous workup, the crude reaction mixture could be concentrated under reduced pressure and the residue was directly applied to a silica gel column, which was eluted with 10% EtOAc in hexanes. In this case, no 4.27 was recovered from the chromatography. Several runs of this reaction gave yields ranging from 40 to 50%, making the average yield 45% over 4 steps.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.70-7.61 (m, 4H); 7.52-7.38 (m, 6H); 6.15 (dd, 1H, J=9.9, 6.0); 5.81 (dd, 1H, J=9.9, 1.0); 5.09-5.02 (m, 2H); 4.83 (d, 1H, J=11.1); 4.17 (dd, 1H, J=6.0, 2.0); 3.91, 3.40 (ABq, 2H, J<sub>AB</sub>=18.5); 1.45 (s, 9H); 1.09 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 191.5, 161.7, 155.01, 135.8, 135.7, 134.7, 134.5, 132.6, 132.5, 130.6, 130.4, 128.2, 128.1, 85.1, 80.7, 62.9, 55.4, 54.7, 52.0, 28.4, 27.1, 19.1.

**IR (film**): 1747, 1711.

**MS**: 555 [M+Na]<sup>+</sup>, 587 [M+MeOH+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{30}H_{36}N_2O_5NaSi [M + Na]^+ 555.2291$ ; found 555.2300.



# <sup>1</sup>H NMR spectrum of 4.34 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.34 (75 MHz, CDCl<sub>3</sub>)





Data for 4.27

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 7.71-7.66 (m, 4H); 7.48-7.36 (m, 6H); 5.93 (dd, 2H, *J*=10.3, 1.6); 5.81 (dd, 2H, *J*=10.3, 2.5); 4.52 (m, 1H); 2.64 (br, 2H); 1.45 (s, 9H); 1.07 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 173.1, 136.0, 133.9, 130.0, 129.5 (br, 2 signals), 127.8, 81.2 (br), 63.5, 51.1, 44.2 (br), 28.5, 27.0, 19.32. The carbonyl carbon of the Boc group at *ca*. 155 ppm was barely visible, presumably due to spin saturation / slow relaxation. No attempt was made to obtain a better spectrum by altering the relaxation delay between pulses.

IR (film): 3325, 1709, 1656.

**MS**: 530  $[M+Na]^+$ ; negative ion mode: 506  $[M-H]^-$ .

**HRMS**: calcd. for  $C_{29}H_{37}NO_5NaSi [M + Na]^+ 530.2339$ ; found 530.2346.

**mp (DCM):** 157-158 ℃

## <sup>1</sup>H NMR spectrum of 4.27 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.27 (75 MHz, CDCl<sub>3</sub>)





Data for 4.33

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.71-7.67 (m, 4H); 7.46-7.37 (m, 6H); 6.73 (s, 0.15H, presumed enol tautomer); 5.99 (app. dd, 2H, *J*=10.3 Hz, 1.8); 5.81 (app. dd, 2H, *J*=10.3, 2.6); 5.26 (s, 1.7H); 4.61 (br, 1H); 4.56 (m, 1H); 3.02 (s, 1.7H); 2.77 (s, 0.3H, presumed enol tautomer); 1.45 (s, 9H); 1.08 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 193.3, 171.3 (presumed enol tautomer), 155.1, 154.6 (presumed enol tautomer), 136.0, 133.8, 130.3, 130.0, 128.3, 127.8, 118.1 (presumed enol tautomer), 83.9, 80.5, 63.5 (presumed enol tautomer), 63.4, 52.2 (presumed enol tautomer), 51.1, 48.1, 42.4 (presumed enol tautomer), 28.4, 27.0, 19.3.

IR (film): 3409, 1703, 1560, 1493.

**MS**: 573 [M+Na]<sup>+</sup>.

**HRMS**: calcd. for  $C_{30}H_{36}N_2O_5NaSi [M + Na]^+ 573.2397$ ; found 573.2390.

### <sup>1</sup>H NMR spectrum of 4.33 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.33 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.36** 

Solid Li<sub>2</sub>CO<sub>3</sub> (5 mg, 0.07 mmol, 0.5 equiv) was added to a solution of **4.34** (72 mg, 0.14 mmol, 1.0 equiv, dried to constant weight under high vacuum and then stored in a desiccator overnight) in dry MeOH (2.5 mL). The suspension was stirred under Ar for 1 h, then it was diluted with CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL), filtered through Celite<sup>®</sup> with more CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and concentrated *in vacuo*. Compound **4.36** (76 mg, 0.13 mmol, 99%;  $R_f$ =0.46 in 30:70 EtOAc/Hexanes) was obtained as an off-white foam. The NMR spectra of this material revealed no impurities; consequently, no further purification was carried out.

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.73-7.69 (m, 4H); 7.48-7.38 (m, 6H); 5.85 (d, 1H, *J*=10.3); 5.68 (dd, 1H, *J*=10.2, 2.4); 4.77 (br, 1H); 4.46 (dd, 1H, *J*=4.8, 2.0); 4.13-4.09 (m, 2H); 3.67 (s, 3H); 3.39, 2.82 (ABq, 2H, *J*<sub>AB</sub>=15.4); 2.14 (d, 1H, *J* = 3.5) ; 1.45 (s, 9H); 1.10 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.4, 153.9, 136.4, 135.8, 133.3, 133.3, 132.1, 130.4, 130.3, 128.2, 128.1, 127.9, 117.53, 80.8, 72.2, 70.2, 54.8, 51.9, 40.00, 39.8, 28.4, 27.1, 19.4.

IR (film): 3418, 2246, 1721 (broad).

**MS**: 571 [M+Li]<sup>+</sup>.

**HRMS**: calcd. for  $C_{31}H_{40}N_2O_6NaSi [M + Na]^+ 587.2553$ ; found 587.2551.

### <sup>1</sup>H NMR spectrum of 4.36 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.36 (75 MHz, CDCl<sub>3</sub>)





## <sup>1</sup>H NMR spectrum of 4.35 (300 MHz, CDCl<sub>3</sub>)



#### **Preparation of 4.37**

Solid LiBH<sub>4</sub> (73 mg, 3.3 mmol, 2.1 equiv) was added to a solution of crude 4.37 (880 mg, 1.6 mmol, 1 equiv) in dry THF (20 mL) and the resulting clear solution was stirred at rt under N<sub>2</sub> for 15 h. The reaction mixture was quenched by careful addition of aq. sat. NH<sub>4</sub>Cl solution (15 mL) (CAUTION: evolution of flammable H<sub>2</sub> gas) followed by stirring until no more H<sub>2</sub> evolved (ca. 15 min). The mixture was extracted with EtOAc (50 mL), and the extract was washed successively with deionized water (10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. The white solid residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>/hexanes (1:5, 12 mL) at reflux then cooled to rt overnight. The precipitate was recovered by filtration through a fritted Büchner funnel (10-16 microns), the crystals were rinsed with cold CH<sub>2</sub>Cl<sub>2</sub> (2x3 mL) then recovered by dissolution in EtOAc (20 mL), followed by evaporation under vacuum. Diol 4.37 (340 mg) was thus obtained as white powder. The combined filtrates from the recrystallization were concentrated under vacuum and the residue was purified by flash chromatography (EtOAc:Hex, 30:70) to obtain additional **4.37** (180 mg). A total of 520 mg of **4.37** (0.97 mmol, 62%, R<sub>f</sub>=0.45 in 50:50 EtOAc:Hex) was thus obtained.

<sup>1</sup>**H** (**300 MHz**, **CDCl**<sub>3</sub>): 7.69-7.73 (m, 4H), 7.51-7.37 (m, 6H), 5.77 (dd, 1H, *J* = 10.2, 1.3), 5.64 (dd, 1H, *J* = 10.2, 2.4), 4.75 (s, 1H), 4.48-4.44 (m, 1H), 4.09-4.04 (m, 2H), 3.79 (app br s, 2H), 2.47

(app dt, 1H, *J* = 14.9, 5.5, 5.5), 2.11-2.01 (m, 2H), 1.45 (s, 9H), 1.10 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 154.0, 136.1, 135.8, 133.4 (2C), 131.1, 130.4, 130.3, 129.5, 128.2, 128.0, 118.0, 80.7, 72.3, 70.6, 58.8, 56.0, 40.1, 38.7, 28.5, 27.1, 19.4.

**IR (film)**: 3448(br), 3254(br), 2257, 1682.

**MS**: 559 [M + Na<sup>+</sup>].

**HRMS**: calcd. for  $C_{30}H_{40}N_2O_5NaSi^+$ : 559.2604, found 559.2599.

**mp (Hexanes)**: 154-156 ℃





<sup>13</sup>C NMR spectrum of 4.37 (75 MHz, CDCl<sub>3</sub>)





#### **Preparation of 4.38**

Substance **4.37** (520 mg, 0.97 mmol, 1 equiv) was dried under vacuum for 6 h in a heavy-walled pressure tube sealable with a Teflon screwcap and equipped with a Teflon stirring bar. The tube was then flushed with Ar and  $(CH_2Cl)_2$  (3.8 mL) and *i*-Pr<sub>2</sub>NEt (1.2 mL, 6.9 mmol, 7.1 equiv) were introduced. Benzyl chloromethyl ether (75%, contains ca. 25% of (BnO)<sub>2</sub>CH<sub>2</sub>, 0.68 mL, 3.7 mmol, 3.8 equiv) was added over 5 min at rt into the resulting solution under vigorous stirring. Stirring at rt was continued for 5 min, then the tube was sealed with a Teflon screw cap and immersed into an oil bath maintained at 80 °C. After 69 h, the mixture was cooled to rt and partitioned between aq. sat. NH<sub>4</sub>Cl solution (10 mL) and EtOAc (30 mL). The organic layer was retained and the aqueous phase was extracted with more EtOAc (15 mL). The combined extract were successively washed with deionized water (10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum to afford an orange syrup. This material was subjected to column chromatography (gradient EtOAc:Hex, 10:90~15:85~20:80) to afford **4.38** (660 mg, 0.85 mmol, 88%, R<sub>f</sub>=0.48 in 20:80 EtOAc:Hex) as a colorless oil.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.77-7.70 (m, 4H), 7.45-7.29 (m, 16H), 5.72 (d, 1H, J = 10.3), 5.64 (dd, 1H, J = 10.3, 3.2), 5.13 (br, 1H), 4.75-4.59 (m, 5H), 4.63-4.47 (m, 5H), 4.15 (dd, 1H, J = 5.7, 3.5), 3.78-3.62 (m, 2H), 2.45 (ddd, 1H, J = 14.7, 7.0, 4.6), 2.20 (ddd, 1H, J = 14.8, 6.8, 4.4), 1.43 (s, 9H),

1.09 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 153.8, 137.8, 137.6, 136.1, 136.0, 134.0, 133.3, 130.3 (2C), 130.0, 129.9, 128.6, 128.5, 128.1, 128.0, 127.9(2C), 127.8, 127.7, 118.8, 94.9, 94.7, 80.2, 77.2 (by HSQC), 70.0, 69.8, 68.8, 63.8, 55.4, 38.0, 36.4, 28.4, 27.0, 19.5.

**IR (film)**: 3384 (br), 2243, 1716.

**MS**: 799  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>46</sub>H<sub>56</sub>N<sub>2</sub>O<sub>7</sub>NaSi<sup>+</sup>: 799.3755, found 799.3768.



<sup>1</sup>H NMR spectrum of 4.38 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.38 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum





#### **Preparation of 4.50**

Aqueous solution of OsO<sub>4</sub> (4% w/v, 265 µL, 42 µmol, 0.030 equiv) and N-methylmorpholine N-oxide (50% w/w, 570 µL, 2.8 mmol, 2.0 equiv) was added to a suspension of 4.38 (1.1 g, 1.4 mmol, 1 equiv) and anhydrous citric acid (534 mg, 2.8 mmol, 2.0 equiv) in 4:1 acetone:water (18 mL) in a heavy-walled pressure tube sealable with a Teflon screw cap. No stirring bar was necessary. More acetone (4.0 mL) was added, resulting in a homogeneous chartreuse-colored solution, which turned colorless upon heating in an oil bath maintained at 50  $^{\circ}$ C. After 47 h at 50 °C, more citric acid (267 mg, 1.39 mmol, 1.0 equiv) and 50% aqueous 4-methylmorpholine N-oxide solution (285 µL, 1.39 mmol, 1.0 equiv) was added. Addition of the same amounts of citric acid and 50% aqueous 4-methylmorpholine N-oxide solution was repeated after 68 h at 50  $^{\circ}$ C. After 110h, the olive-green mixture was cooled to rt and treated with solid Na<sub>2</sub>SO<sub>3</sub> (800 mg, 6.3 mmol, 4.6 equiv), water (10 mL) and EtOAc (10 mL). The solution was stirred for 1 h, then more EtOAc (30 mL) was added. The organic layer was sequentially washed with aq. sat. NH<sub>4</sub>Cl solution (15 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. The residual thick oil was purified by column chromatography (gradient EtOAc:Hex, 20:80~30:70~50:50) to provide unreacted **4.38** (470 mg, 0.60 mmol, 44%) and desired **4.50** (550 mg, 0.68 mmol, 49%, R<sub>f</sub>=0.20 in 30:70 EtOAc:Hex) as colorless oil.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.80-7.72 (m, 4H), 7.45-7.29 (m, 16H), 4.79-4.44 (m, 10H), 4.30 (app t, 1H, J = 9.0), 3.76 (dd, 1H, J = 9.4, 4.9), 3.68-3.62 (m, 4H), 3.20 (d, 1H, J = 3.6), 2.47-2.43 (m, 2H), 2.14 (d, 1H, J = 7.4), 1.41 (s, 9H), 1.11 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 153.7, 137.7, 137.5, 135.9, 135.8, 134.1, 133.8, 129.7, 129.6, 128.6, 128.3, 128.1, 127.8, 127.7, 127.6 (3 C total), 118.0, 95.9, 95.2, 80.9, 76.5 (by HSQC), 73.5, 72.6, 72.1, 70.3, 70.2, 63.3, 58.5, 37.7, 31.6, 28.2, 27.1, 19.7.

IR (film): 3600-3250 (br), 2247, 1714, 1698.

**MS**: 833 [M + Na<sup>+</sup>].

**HRMS**: calcd. for C<sub>46</sub>H<sub>58</sub>N<sub>2</sub>O<sub>9</sub>NaSi<sup>+</sup>: 833.3809, found 833.3803.





<sup>13</sup>C NMR spectrum of 4.50 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum





#### **Preparation of 4.64**

A solution of **4.50** (550 mg, 0.68 mmol, 1 equiv) in 2,2-dimethoxypropane (7 mL), containing TsOHH<sub>2</sub>O (*ca.* 3 mg, 0.016 mmol, 0.02 equiv) was stirred at rt for 45 min, then it was treated with aq. sat. NaHCO<sub>3</sub> (15 mL) and extracted with EtOAc (50 mL). The extract was washed with brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. Crude 4.62 thus obtained (600 mg, colorless oil, R<sub>f</sub>=0.59 in 30:70 EtOAc:Hex) was dissolved in dry THF (9 mL) and treated with 1.0 M acetic acid solution in THF (1.2 mL, 1.2 mmol, 1.8 equiv) followed by dropwise addition over 5 min (rt, vigorous stirring) of commercial 1.0 M TBAF solution in THF (1.2 mL, 1.2 mmol, 1.8 equiv). After stirring at rt for 15 h, the solvents were removed *in vacuo*. The residue was dissolved in EtOAc (30 mL) and successively washed with aq. sat. NaHCO<sub>3</sub> solution (10 mL), deionized water (10 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. The residue of crude 4.63 (0.61 g, colorless oil, R<sub>f</sub>=0.47 in 50:50 EtOAc:Hex, contained TBDPSF) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (11 mL), and treated with solid Dess-Martin periodinane (390 mg, 0.92 mmol, 1.35 equiv) added in one portion. After stirring at rt for 2 h 45 min, the reaction was quenched by addition of a 1:1 (vol/vol) mixture of aq. sat.  $Na_2S_2O_3$  and aq. sat.  $NaHCO_3$  solutions (10 mL), whereupon the mixture became clear. The solution was diluted with EtOAc (30 mL), the aqueous phase was drained and retained, and the EtOAc layer was washed sequentially with aq. sat.

NaHCO<sub>3</sub> solution (10 mL) and brine (5 mL). The combined aqueous layers were extracted with more EtOAc (10 mL), and the extract was washed with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and brine (5 mL). The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. Flash chromatography of the residue (EtOAc:Hex, 30:70) yielded ketone **4.64** (360 mg, 0.59 mmol, 87% over 3 steps,  $R_f$ =0.20 in 30:70 EtOAc:Hex) as white waxy solid.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.40-7.30 (m, 10H), 4.92, 4.89 (AB q, 2H, J<sub>AB</sub> = 7.3), 4.81-4.73 (m, 4H),
4.71-4.66 (m, 3H), 4.64 (s, 2H), 4.44 (d, 1H, J = 5.3), 4.20 (d, 1H, J = 4.4), 3.80-3.73 (m, 2H),
2.47-2.40 (m, 2H), 1.52 (s, 3H), 1.44 (s, 9H), 1.37 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 202.1, 154.8, 138.2, 137.4, 128.8, 128.6, 128.3, 128.1 (2C), 127.9, 115.7, 111.6, 95.5, 94.5, 81.5, 80.4, 78.3, 72.9, 70.7, 70.6, 63.5, 56.7, 40.6, 32.3, 28.4, 26.8, 26.1.
IR (film): 3351 (br), 2248, 1752, 1714.

**MS**: 633  $[M + Na^+]$ .

**HRMS**: calcd. for  $C_{33}H_{42}N_2O_9Na^+$ : 633.2788, found 633.2786.

### <sup>1</sup>H NMR spectrum of 4.64 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.64 (75 MHz, CDCl<sub>3</sub>)





Data for 4.63

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.40-7.29 (m, 10H), 4.88, 4.85 (AB q, 2H, J<sub>AB</sub> = 7.3), 4.77-4.69 (m, 4H),
4.60 (app s, 2H), 4.56 (d, 2H, J = 4.2), 4.09-4.01 (m, 2H), 3.91 (d, 1H, J = 3.9), 3.81 (dd, 1H, J = 9.8, 4.5), 3.75-3.68 (m, 2H), 3.11 (s, 1H), 2.52-2.44 (m, 2H), 1.62 (s, 3H), 1.41 (s, 9H), 1.35 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 154.1, 138.2, 137.1, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 117.5, 110.6, 95.3, 95.1, 81.1, 78.9, 77.3 (by HSQC), 75.4, 73.6, 70.7, 70.2, 63.2, 57.4, 37.4, 32.2, 28.3, 27.9, 26.4.

**IR (film)**: 3469 (br), 3334 (br), 2245, 1712.

**MS**: 635 [M + Na<sup>+</sup>].

**HRMS**: calcd. for  $C_{33}H_{44}N_2O_9Na^+$ : 635.2945, found 635.2950.
# <sup>1</sup>H NMR spectrum of 4.63 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.63 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum



Expansion of COSY spectrum of 4.63 (CDCl<sub>3</sub>)



Expansion of NOESY spectrum of 4.63 (CDCl<sub>3</sub>)





**Preparation of 4.69** 

Commercial (trimethylsilyl)methyllithium solution (1.0 M in pentane, 1.53 mL, 1.53 mmol, 3.3 equiv) was added dropwise over 5 min to a cold (-78 °C), vigorously stirred solution of **4.64** (0.28 g, 0.46 mmol, 1 equiv) in dry THF (7 mL), under Ar. After 15 min, a 1M solution of acetic acid in THF (2.5 mL) was added via a syringe. After 5 min, the reaction flask was removed from the Dry Ice/acetone bath and the mixture was allowed to warm to room temperature. The solution was poured into a 2:1 mixture of saturated NaHCO<sub>3</sub>/ water (15 mL), and extracted with EtOAc (30 mL). The extract was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated in vacuo. The residue was loaded on silica gel (3 g) and eluted with EtOAc (25 mL). Rotary evaporation gave alcohol 4.68 (310 mg, nearly colorless oil,  $R_f=0.66$  in 30:70 EtOAc:Hex, contaminated with ca. 15% of **4.64**), which was used in the next step without further purification. The bulk of crude alcohol 4.68 was dissolved in 1,2-dichloroethane (11 mL; Ar-flushed round bottom flask equipped with a condenser). Solid pyridinium p-toluenesulfonate (115 mg, 0.46 mmol, 1.0 equiv) was added and the solution was warmed to 60  $\,^{\circ}$ C (oil bath). After 14.5 h, the mixture was cooled to rt, treated with aq. sat. NaHCO<sub>3</sub> (10 mL), stirred for 10 min, and finally extracted with EtOAc (30 mL). The organic phase was successively washed with saturated NaHCO<sub>3</sub> (5 mL) and brine (5 mL). The combined aqueous phases were extracted with more EtOAc (10 mL) and the extract was

successively washed with aq. sat. NaHCO<sub>3</sub> (3 mL) and brine (3 mL). The combined organic phases were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo*. Chromatography of the residue (gradient EtOAc:Hex, 30:70~50:50) returned unreacted **4.64** (46 mg, 0.075 mmol, 16%) and desired **4.69** (131 mg, 0.21 mmol, 46%,  $R_f$ =0.16 in 50:50 EtOAc:Hex) as a colorless oil.

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 7.37-7.27 (m, 10H), 6.95 (br s, 1H), 5.56, 5.53 (AB q, 2H, *J*<sub>AB</sub> = 1.8), 5.42 (br s, 1H), 4.92, 4.82 (AB q, 2H, *J*<sub>AB</sub> = 7.1), 4.75-4.53 (m, 10H), 3.81-3.67 (m, 2H), 3.56 (d, 1H, *J* = 6.2), 2.56-2.34 (m, 2H), 1.53 (s, 3H), 1.42 (s, 9H), 1.40 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.1, 154.2, 142.6, 137.9, 137.8, 128.5, 128.4, 128.0, 127.8 (2C), 127.7, 112.3, 109.5, 94.6, 93.2, 80.3, 78.0, 75.0, 70.7, 70.1, 69.5, 63.5, 56.7, 56.3, 31.9, 28.3, 27.5, 26.0.
IR (film): 3460 (br), 3346 (br), 1717, 1694, 1669.

**MS**: 649  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>34</sub>H<sub>46</sub>N<sub>2</sub>O<sub>9</sub>Na<sup>+</sup>: 649.3101, found 649.3109.





<sup>13</sup>C NMR spectrum of 4.69 (75 MHz, CDCl<sub>3</sub>)





Data for 4.68

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.38-7.28 (m, 10H), 4.93, 4.88 (AB q, 2H, J<sub>AB</sub> = 7.2), 4.80-4.69 (m, 4H),
4.62, 4.60 (AB q, 2H, J<sub>AB</sub> = 12.0), 4.42 (d, 1H, J = 4.0), 4.17 (d, 1H, J = 5.8), 4.05 (d, 1H, J = 5.9),
3.91-3.82 (m, 2H), 3.74 (ddd, 1H, J = 10.7, 6.3, 4.3), 2.71 (s, 1H) 2.58-2.43 (m, 2H), 1.62 (s, 3H),
1.42, 0.76 (AB q, 2H, J<sub>AB</sub> = 14.5), 1.42 (s, 9H), 1.37 (s, 3H), 0.12 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 154.5, 138.0, 137.6, 128.7, 128.5, 128.0 (2C), 127.9 (2C), 118.2, 109.9, 95.5, 94.9, 80.7, 79.0, 78.7, 76.7 (by HSQC), 73.3, 70.6, 70.0, 64.0, 57.2, 35.0, 33.3, 29.4, 28.4, 25.9, 25.4, 0.3.

IR (film): 3584 (br), 3328 (br), 2244, 1711, 1695.

**MS**: 721  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>37</sub>H<sub>54</sub>N<sub>2</sub>O<sub>9</sub>NaSi<sup>+</sup>: 721.3496, found 721.3483.

**mp (Hexanes):** 115-117 °C

# <sup>1</sup>H NMR spectrum of 4.68 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.68 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum





**Preparation of 4.88** 

Solid di-t-butyl dicarbonate (233 mg, 1.07 mmol, 4.4 equiv) was added to a solution of 4.69 (152 mg, 0.24 mmol, 1 equiv), 4-dimethylaminopyridine (30 mg, 0.25 mmol, 1.0 equiv), and triethylamine (235 µL, 1.69 mmol, 6.9 equiv) in dry THF (8.5 mL), under Ar, and the mixture was stirred at rt for 14 h. The volatiles were carefully removed *in vacuo*, and the residue was dissolved in EtOAc (25 mL), sequentially washed with aq. sat. NH<sub>4</sub>Cl (10 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure to afford crude 4.76 (200 mg, thick yellow syrup, R<sub>f</sub>=0.32 in 20:80 EtOAc:Hex), which was employed in the next step without further purification. Crude 4.76 obtained as described above was dissolved in 4:1 acetone/water (7 mL) and treated with 4% aqueous OsO<sub>4</sub> solution (5 drops, ca. 50 µL, 8 µmol, 0.03 equiv) and 50% aqueous 4-methylmorpholine N-oxide solution (75 µL, 0.28 mmol, 1.2 equiv). The reaction was stirred for 23 h, then water (10 mL), EtOAc (10 mL), and solid Na<sub>2</sub>SO<sub>3</sub> (100 mg, 0.79 mmol, 3.3 equiv) were added, and the mixture was stirred for another 90 min, then it was extracted with EtOAc (20 mL). The extract was washed with aq. sat. NH<sub>4</sub>Cl (10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give crude **4.85** (190 mg, faintly yellow oil, R<sub>f</sub>=0.43 in 50:50 EtOAc:Hex), which was employed in the next step without further purification. Crude 4.85 obtained as described above was dissolved in 2,2-dimethoxypropane (7 mL) and treated with TsOHH<sub>2</sub>O (ca. 5 mg, 0.026 mmol, 0.1 equiv) and the solution was stirred for 90 min. The mixture was quenched with aq. sat. NaHCO<sub>3</sub> solution (20 mL) then stirred for another 15 min. The aqueous layer was discarded and the organic phase was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to yield crude **4.86** (201 mg, off white oil,  $R_f=0.62$  in 30:70 EtOAc:Hex). The compound was dissolved in dry THF (4.5 mL), then LiBH<sub>4</sub> (20 mg, 0.92 mmol, 3.8 equiv) was added. The mixture was stirred at room temperature for 16 h and quenched by addition of aq. sat. NH<sub>4</sub>Cl (6 mL; CAUTION: evolution of flammable H<sub>2</sub> gas). When gas evolution subsided, EtOAc (20 mL) and water (4 mL) were added and the organic layer was separated and washed with brine (5 mL). The combined aqueous phases were extracted with more EtOAc (5 mL). The new organic phase was then washed with brine (2 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. Gradient chromatography (EtOAc:Hex, 20:80~30:70) afforded desired **4.88** (48 mg, 0.070 mmol, 29%, R<sub>f</sub>=0.21 in 30:70 EtOAc:Hex) as a waxy white solid with **4.87** (75 mg, 0.083 mmol, 34%, R<sub>f</sub>=0.37 in 30:70 EtOAc:Hex).

**4.87** was recovered as follow. **4.87** (75 mg, 0.083 mmol, 1 equiv) and DMAP (10 mg, 0.082 mmol, 1.0 equiv) was dissolved in dry acetonitrile (3 mL) followed by the addition of triethylamine (46  $\mu$ L, 0.33 mmol, 4.0 equiv). Solid Boc<sub>2</sub>O (55 mg, 0.25 mmol, 3.0 equiv) was added into the mixture. The mixture was stirred for 3 h and the solvents were removed under vacuum. The residue was loaded on a short silica gel plug (2 mL) then eluted with EtOAc/Hexs (20:80) until the disappearance of product in the eluate (R<sub>f</sub>=0.68 in 30:70 EtOAc:Hex). The

eluate was concentrated *in vacuo* to afford crude **4.89** (82 mg) as yellow oil. This crude product was dissolved in THF (5 mL) then solid LiBH<sub>4</sub> (9 mg, 0.41 mmol, 5 equiv) was added. The reaction mixture was warmed to 40 %, stirred for 120 h, and then cooled to room temperature. To this solution was added aq. sat. NH<sub>4</sub>Cl (CAUTION: evolution of flammable H<sub>2</sub> gas) followed by stirring until no more H<sub>2</sub> evolved (ca. 15 min). The solution was extracted with EtOAc (20 mL), then the organic layer was successively washed with aq. sat. NH<sub>4</sub>Cl (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated to yield a crude as colorless foam. The crude material consisted mainly of a 3:1 mixture of 4.91 (R<sub>f</sub>=0.43 in 30:70 EtOAc:Hex) and 4.88 (alcohol) with some HNBoc<sub>2</sub> ( $R_f=0.60$  in 30:70 EtOAc:Hex). Finally, this crude mixture and solid Na<sub>2</sub>CO<sub>3</sub> (69 mg, 0.50 mmol, 6.0 equiv) were dissolved in methanol (7 mL) in a round bottom flask equipped with a reflux condenser. The solution was heated to 60 °C, stirred for 46 h, cooled to room temperature and poured into aq. sat. NH<sub>4</sub>Cl (10 mL). The aqueous solution was successively extracted with DCM (3x10 mL). The DCM layer was combined, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated and the residue was purified by flash chromatography (EtOAc:Hex, 30:70) to yield **4.88** as a white waxy solid (38 mg, 0.055 mmol, 67% over 3 steps). In total, 86 mg (0.125 mmol) of 4.88 was produced through this process to account for a 52% yield from 4.69.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.35-7.27 (m, 10H), 4.98 (s, 1H), 4.89 (app s, 2H), 4.76-4.57 (m, 7H), 4.31 (d, 1H, J = 6.6), 4.24 (d, 1H, J = 4.0), 4.21 (app s, 2H), 3.83-3.74 (m, 3H), 3.71-3.65 (m, 1H), 3.19-2.69 (br m, 2H), 2.36-2.24 (m, 2H), 1.54 (s, 3H), 1.45 (s, 3H), 1.40 (app s, 12H), 1.35 (s, 3H).
<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 154.3, 137.9, 137.6, 128.6 (2C), 128.0 (2C), 127.9 (2C), 109.1, 108.7, 96.8,

94.6, 84.3, 81.1, 79.8, 77.6 (by HSQC), 77.5, 70.2, 69.7, 66.6, 64.7, 59.3, 58.0, 45.3, 31.7, 28.5, 26.9, 26.7, 26.2, 25.1.

**IR (film**): 3484 (br), 3374 (br), 1716, 1693.

**MS**: 710 [M + Na<sup>+</sup>].

**HRMS**: calcd. for C<sub>37</sub>H<sub>53</sub>NO<sub>11</sub>Na<sup>+</sup>: 710.3516, found 710.3516.

# <sup>1</sup>H NMR spectrum of 4.88 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.88 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum





Data for 4.76

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.40-7.26 (m, 10H), 6.34 (br s, 1H), 5.47-5.42 (m, 2H), 5.08-5.04 (m, 2H), 4.91-4.56 (m, 10H), 4.12 (td, 1H, *J* = 10.0, 10.0, 1.5), 3.74 (ddd, 1H, *J* = 10.4, 5.8, 2.9), 2.91 (ddd, 1H, *J* = 15.5, 9.4, 2.9), 2.21 (ddd, 1H, *J* = 15.6, 5.6, 1.6), 1.50 (app s, 21H), 1.41-1.36 (m, 12H).
<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 169.8, 154.9, 149.7, 143.7, 137.9 (2C), 128.4 (2C), 128.1,127.9, 127.7, 127.6, 111.8, 109.7, 95.1, 94.2, 84.4, 79.2, 78.7, 74.7, 73.7, 70.3, 69.4, 65.7, 59.6, 48.2, 30.5, 28.3, 27.6, 27.1, 25.6.

**IR**: 3376 (br), 1783, 1747, 1717.

**MS**: 850  $[M + Na^+]$ .

**HRMS**: calcd. for  $C_{44}H_{62}N_2O_{13}Na^+$ : 849.4150, found 849.4149.





<sup>13</sup>C NMR spectrum of 4.76 (75 MHz, CDCl<sub>3</sub>)





Data for 4.85

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.39-7.27 (m, 10H), 6.62 (br s, 1H), 5.24 (d, 1H, J = 6.7), 4.95 (s, 1H),
4.88-4.53 (m, 8H), 4.31-4.24 (m, 2H), 4.19 (s, 1H), 4.15-4.09 (m, 1H), 3.70-3.64 (m, 1H), 3.59 (dd,
1H, J = 11.5, 7.7), 3.26 (s, 1H), 3.19-3.14 (m, 1H), 3.03 (dd, 1H, J = 16.0, 9.7) 2.32 (dd, 1H, J = 16.3, 5.2), 1.52 (s, 18H), 1.44 (s, 3H), 1.39 (s, 9H), 1.33 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 174.0, 155.1, 150.5, 137.9, 137.2, 128.7, 128.5, 128.3, 128.2, 128.0, 127.9, 108.4, 97.0, 94.3, 84.7, 79.5, 79.1, 79.0, 77.2, 74.0, 70.8, 69.7, 67.1, 64.1, 60.6, 43.6, 32.5, 28.6, 27.7, 26.0, 25.0.

IR (film): 3477 (br), 3372 (br), 1780 (shoulder), 1737, 1714.

**MS**: 884  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>44</sub>H<sub>64</sub>N<sub>2</sub>O<sub>15</sub>Na<sup>+</sup>: 883.4204, found 883.4203.

# <sup>1</sup>H NMR spectrum of 4.85 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.85 (75 MHz, CDCl<sub>3</sub>)



# <sup>1</sup>H NMR spectrum of 4.89 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.102** 

Solid imidazole (30 mg, 0.44 mmol, 5.3 equiv) and t-butyldimethylsilyl chloride (29 mg, 0.19 mmol, 2.3 equiv) were added to a solution of **4.88** (57 mg, 0.083 mmol, 1 equiv) in dry DMF (0.80 mL), under Ar. The mixture was stirred at rt for 15 min, then it was warmed to 70  $\,^{\circ}$ C (oil bath temperature) for 20 h. The cooled mixture was poured into deionized water (3 mL) and extracted with EtOAc (20 mL). The extract was washed successively with aq. sat. NH<sub>4</sub>Cl (3 mL), deionized water (4x3 mL) and brine (3 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. Crude TBS ether 4.100 thus obtained (71 mg, colorless oil, R<sub>f</sub>=0.59 in 30:70 EtOAc:Hex) was dissolved in dry THF (3.0 mL), the flask containing the solution was cooled to -78 °C (Ar atmosphere) and liquid NH<sub>3</sub> (ca. 7 mL) was condensed into the same flask. Metallic sodium (58 mg, 2.5 mmol, 30 equiv) was added in to the reaction flask with vigorous stirring. After 3 min, the solution turned dark blue and was maintained stirring at -78 °C for another 30 min followed by the addition of solid NH<sub>4</sub>Cl (203 mg, 3.8 mmol, 46 equiv). The resulting slurry was stirred for 5 min until the blue color disappeared, then the flask was removed from the Dry Ice bath and uncapped to allow the NH<sub>3</sub> to evaporate. The residue was taken up with EtOAc and filtered through Celite<sup>®</sup> (removal of solids) with more EtOAc. Occasionally, the <sup>1</sup>H NMR and mass spectra of this material revealed the presence of variable quantities of mono- and bis-hydroxymethyl derivatives of **4.102**, probably compounds **4.101**. In such cases, crude **4.102** thus obtained was redissolved in 1:1 2-propanol:water (15 mL), the solution was stirred at 50  $^{\circ}$ C for 1 h, then the solvents were removed *in vacuo* to afford a product free from **4.101**. Flash chromatography (EtOAc:Hex, 30:70) of the residue, concentration of the combined fractions containing the product, addition of hexanes to the waxy residue and again rotary evaporation returned pure **4.102** (38 mg, 0.068 mmol, 82% over 2 steps, R<sub>f</sub>=0.20 in 30:70 EtOAc:Hex), as a white powder.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.68 (s, 1H), 4.80 (d, 1H, *J* = 6.7), 4.33 (d, 1H, *J* = 6.7), 4.28, 4.26 (AB q, 2H, *J*<sub>AB</sub> = 9.2), 4.05-4.02 (m, 1H), 4.00-3.89 (m, 3H), 3.85-3.77 (m, 1H), 3.37 (d, 1H, *J* = 5.6), 2.74-2.67 (m, 1H), 2.41-2.24 (m, 2H), 2.06 (br s, 1H), 1.66 (br s, 1H), 1.48 (s, 3H), 1.43 (app s, 12H), 1.41 (s, 3H), 1.35 (s, 3H), 0.91 (s, 9H), 0.11 (app s, 6H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 155.0, 110.1, 108.6, 81.6, 79.5 (shoulder), 79.4, 77.8, 72.6, 68.2, 62.3, 59.9, 57.9, 44.3, 35.3, 28.6, 27.0, 26.8, 26.0, 25.9, 24.7, 18.2, -5.4, -5.5.

**IR**: 3472 (br), 3354 (br), 1714, 1694.

**MS**: 584  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>27</sub>H<sub>51</sub>NO<sub>9</sub>NaSi<sup>+</sup>: 584.3231, found 584.3233.

**mp (Hexanes)**: 96-97 °C



<sup>1</sup>H NMR spectrum of 4.102 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.102 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.104** 

Tetrapropylammonium perruthenate (TPAP, *ca.* 1 mg, 3  $\mu$ mol, 0.04 equiv) was rinsed (dry CH<sub>2</sub>Cl<sub>2</sub>, 2.5 mL) into a flask containing a solution of diol **4.102** (38 mg, 0.068 mmol, 1 equiv) and 4-methylmorpholine *N*-oxide (30 mg, 260  $\mu$ mol, 4 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL), and the mixture was stirred at rt. After 3 h, the starting material **4.102** and the corresponding lactol **4.106** (R<sub>f</sub>=0.33 in 30:70 EtOAc:Hex) were no longer detectable by TLC. The solution was loaded on a silica gel (1.5 g) column and eluted with 50:50 EtOAc:Hex (20 mL). Pure **4.104** (35 mg, 0.063 mmol, 93%, R<sub>f</sub>=0.30 in 20:80 EtOAc:Hex) was obtained as a white powder.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 4.88 (br d, 1H, J = 5.3), 4.62 (s, 1H), 4.43, 4.19 (AB q, 2H, J<sub>AB</sub> = 9.8),
4.34-4.29 (m, 2H), 3.83 (dd, 1H, J = 10.2, 3.9), 3.72 (dd, 1H, J = 10.1, 8.4), 3.26-3.23 (m, 1H),
2.91, 2.25 (AB q, 2H, J<sub>AB</sub> = 17.8), 1.53 (s, 3H), 1.45 (s, 9H), 1.43 (s, 3H), 1.41 (s, 3H), 1.37 (s, 3H),
0.88 (s, 9H), 0.06 (app s, 6H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 167.3, 153.6, 111.4, 109.4, 80.2, 79.4, 79.3, 78.7, 76.1, 68.9, 59.3, 54.6,
35.2, 34.9, 28.3, 27.2, 26.0, 25.8, 25.0, 24.7, 18.1, -5.5, -5.7.

**IR (film)**: 3354 (br), 1752, 1713.

**MS**: 580  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>27</sub>H<sub>47</sub>NO<sub>9</sub>NaSi<sup>+</sup>: 580.2918, found 580.2918.

**mp (Hexanes)**: 236-238 ℃

# <sup>1</sup>H NMR spectrum of 4.104 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.104 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.119** 

Commercial *n*-BuLi solution (1.43 M in hexanes, 53 µL, 75 µmol, 7 equiv) was added to a cold (-78 °C) solution of diisopropylamine (15 µL, 110 µmol, 10 equiv) in THF (50 µL). The mixture was stirred at −78 °C for 15 min, then it was removed from the Dry Ice/acetone bath, stirred for another 10 min, and finally transferred (syringe) into a cold (-78 °C) solution of lactone 4.104 (6 mg, 11  $\mu$ mol, 1 equiv) in THF (100  $\mu$ L). The resulting solution was stirred at -78 °C for 1h, then a THF (0.10 mL) solution of 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (9 mg, 340 µmol, 3.2 equiv) was added dropwise. The mixture was warmed to -25 °C during 30 min and stirred at  $-25\pm5$  °C for 2 h, then it was cooled to -78 °C prior to slow addition of a 1 M THF solution of acetic acid (200 µL). The reaction mixture was warmed to rt, diluted with hexanes (1 mL), and eluted through a silica gel column (1 mL) using 50:50 EtOAc/hexanes (15 mL). The eluate was concentrated in vacuo. The residue was purified with flash chromatography (EtOAc:Hex, 10:90) to give an inseparable mixture of **4.104** and **4.103** (3:7 mol/mol, 6 mg, R<sub>f</sub>(**4.103**)=0.38 in 20:80 EtOAc:Hex). A 1.0 M THF solution of NaHMDS (20 µL, 20 µmol, 1.9 equiv) was added to a cold (-78 %) solution of the foregoing mixture in dry THF (150  $\mu$ L), and the resulting solution was stirred at -78 °C for 1h. Finally, a stock solution (20 µL) of chloromethyl methyl ether (12 μL; CAUTION: cancer suspect agent) in THF (170 μL), was slowly added (addition of 1.3 μL of MOMCl, 15  $\mu$ mol, 1.4 equiv) into the alkoxide solution. The mixture was stirred at -78 °C for 2 h, gently warmed to rt during 1h, and stirred at rt for 16 h. The reaction mixture was poured into aq. sat. NH<sub>4</sub>Cl solution (5 mL) and extracted with EtOAc (15 mL). The extract was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The residue was purified by gradient chromatograph (EtOAc:Hex, 10:90~20:80) to afford unreacted **4.104** (2mg, 4  $\mu$ mol, 33 %) and desired **4.119** (3.3 mg, 5  $\mu$ mol, 49%, R<sub>f</sub>=0.38 in 20:80 EtOAc:Hex). Compound **4.119** was recrystallized from MeOH to give white plates.

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 5.45 (br s, 1H), 5.25 (br d, 1H, J = 5.4), 4.99, 4.73 (AB q, 2H,  $J_{AB} = 6.5$ ), 4.70 (app s, 1H), 4.43, 4.19 (AB q, 2H,  $J_{AB} = 9.8$ ), 4.32 (dd, 1H, J = 6.3, 1.2), 4.27 (s, 1H), 3.85 (app t, 1H, J = 10.8), 3.66 (dd, 1H, J = 10.6, 3.7), 3.48 (s, 3H), 3.26 (dd, 1H, J = 11.0, 3.7), 1.52 (s, 3H), 1.47 (s, 9H), 1.43 (s, 3H), 1.40 (s, 3H), 1.35 (s, 3H), 0.88 (s, 9H), 0.05 (app s, 6H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 167.1, 154.3, 111.6, 109.5, 98.5, 79.9, 79.4, 78.6, 78.4, 75.0, 73.6, 69.1, 58.1, 57.0, 56.6, 35.8, 28.5, 27.3, 26.2, 26.0, 24.9, 24.7, 18.3, -5.2, -5.3.

**IR (film)**: 3382 (br), 1754, 1717.

**MS**: 640  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>29</sub>H<sub>51</sub>NO<sub>11</sub>NaSi<sup>+</sup>: 640.3129, found 640.3124.

**mp** (**MeOH**): 148-149 ℃

### <sup>1</sup>H NMR spectrum of 4.119 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.119 (75 MHz, CDCl<sub>3</sub>)





Expansion of COSY spectrum of 4.119 (CDCl<sub>3</sub>)

### Expansion of NOESY spectrum of 4.119 (CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 4.103 (contains *ca.* 10% 4.104) (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.123, the Sato lactone** 

A 1.0 M solution of acetic acid in THF (32 µL, 32 µmol, 6 equiv) was added at rt to a dry THF (0.5 mL) solution of **4.119** (3.3 mg, 5.3 µmol, 1 equiv), followed by commercial 1.0 M TBAF solution in THF (32 µL, 32 µmol, 6 equiv). After stirring at rt for 25 h, the solvents were removed in vacuo and the residue was loaded on a silica gel plug (1 mL) and eluted with 50:50 EtOAc/hexanes (20 mL). Evaporation of the eluate under vacuum gave a 3:1 mixture of 4.120 and 4.121 (2.9 mg,  $R_f=0.11$  in 30:70 EtOAc:Hex). This material was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 µL) and *ca*. 70 µL of thus solution was transferred into a borosilicate melting point capillary (1.1-1.4 mm inner diameter, 90 mm length, 0.2 mm wall thickness). The solvent was allowed to evaporate (ca. 48 h), then the tube was placed inside a 1-necked round-bottom a flask. The flask was evacuated and kept under high vacuum for 3 h, then it was flushed with argon. The open end of the tube was carefully sealed (Bunsen burner). The sealed tube was immersed for 10 min into an oil bath preheated to 215  $^{\circ}$ C behind a blast shield, then it was retrieved, cooled down, and repeatedly rinsed with hexanes and then with  $CH_2Cl_2$ . The tube was then wrapped in a Kimwipe <sup>TM</sup> and ground over a cotton-plugged glass funnel using a pair pliers. Glass fragments were allowed to fall inside the funnel, then the Kimwipe was rinsed with CH<sub>2</sub>Cl<sub>2</sub> (5x2 mL), and the rinsings were allowed to percolate through the glass fragments. The filtrate was concentrated to provide a mixture of

**4.120/4.121**, **4.122** and **4.123** (1:3:16, mol/mol by <sup>1</sup>H NMR). The combined residues obtained from two runs of the above reaction were purified with flash chromatography (EtOAc:Hex, 50:50; fractions monitored by <sup>1</sup>H NMR) to provide **4.123** (*ca.* 1 mg, *ca.* 2  $\mu$ mol, *ca.* 60%, R<sub>f</sub>=0.49 in 70:30 EtOAc:Hex), contaminated with *ca.* 5% of cyclic carbamate **4.122**. Recrystallization of **4.123** from hexanes (1 mL, slow evaporation) afforded colorless prisms.

<sup>1</sup>**H** (**600 MHz**, **CDCl**<sub>3</sub>): 5.01, 4.83 (AB q, 2H,  $J_{AB} = 6.7$ ), 4.76 (s, 1H), 4.59 (br s, 1H), 4.45-4.39 (m, 2H), 4.36 (dd, 1H, J = 11.9, 5.1), 4.33, 4.23 (AB q, 2H,  $J_{AB} = 9.7$ ), 3.80 (dd, 1H, J = 11.8, 5.0), 3.48 (s, 3H), 2.91 (d, 1H, J = 12.0), 2.64 (br s, 1H), 1.64 (br s, 4H, exchangeable with D<sub>2</sub>O, NH<sub>2</sub> + H<sub>2</sub>O), 1.48 (s, 3H), 1.41 (app s, 6H), 1.39 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 170.7, 111.7, 109.2, 96.3, 79.6, 78.1 (by HSQC), 77.2 (by HSQC), 72.9, 70.2, 69.1, 66.7, 56.9, 51.8, 43.3, 27.6, 26.2, 25.7, 23.7.

IR (film): 3486 (br), 3352 (br), 1759.

**MS**: 404  $[M + H^+]$ .

HRMS: calcd. for C<sub>18</sub>H<sub>30</sub>NO<sub>9</sub>: 404.1921, found 404.1927.

**mp (Hexanes)**: 146-147 °C



<sup>1</sup>H NMR spectrum of 4.123 (600 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.123 (75 MHz, CDCl<sub>3</sub>)









<sup>1</sup>H NMR spectrum of a *ca.* 3:1 mixture of 4.120/4.121 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 4.122** 

A solution of the crude 3:1 mixture of 4.120 and 4.121 in CH<sub>2</sub>Cl<sub>2</sub> and hexanes was evaporated to dryness inside a 10 mL, 1-necked round bottom flask to leave 5 mg (10 µmol, 1 equiv) of a white solid residue. The flask was sealed with a rubber septum, then it was thoroughly flushed with argon and immersed into an oil bath preheated to 200  $^{\circ}$ C. After 15 min, the bath temperature was raised to 230  $^{\circ}$  over *ca*. 5 min, and the flask was maintained at that temperature for another 5 min. Sublimation of a white solid, which condensed at the cooler zones of the flask, was observed during this time. The flask was removed from the bath and cooled to rt. The contents were retrieved by dissolution in CH<sub>2</sub>Cl<sub>2</sub> and evaporation of the resulting solution in vacuo. The residue thus obtained consisted of a 12:5:3 mixture (<sup>1</sup>H NMR) of compound **4.120/4.121**, **4.122** and **4.123**. This material was loaded onto a plug of silica gel (2 mL). Elution with 50:50 EtOAc/hexanes (10 mL) returned a 3:1 mixture of 4.120/4.121 (3 mg, 6 µmol, 60%). Further elution with EtOAc (10 mL) provided a 5:3 (<sup>1</sup>H NMR) mixture of 4.122 and 4.123 (white solid). Recrystallization from hexanes (3 mL), and rinsing of the crystalline material thus obtained with more hexanes (2x3 mL), gave pure **4.122** (*ca.* 1 mg, *ca.* 2 µmol, *ca.* 20%, R<sub>f</sub>=0.44 in 70:30 EtOAc:Hex) as a white powder. X-ray quality crystals of 4.122 (colorless plates) were obtained by diffusion crystallization of hexanes vapors into a toluene (0.1 mL) solution of the above powder.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.23 (s, 1H), 5.06, 4.64 (AB q, 2H, J<sub>AB</sub>= 6.9), 4.79 (dd, 1H, J = 12.9, 10.7),
4.49 (d, 1H, J = 1.2), 4.44, 4.25 (AB q, 2H, J<sub>AB</sub>= 10.2), 4.37 (d, 1H, J = 6.2), 4.36 (dd, 1H, J = 10.6,
6.2), 4.29 (dd, 1H, J = 6.2, 0.9), 4.23 (s, 1H), 3.45 (s, 3H), 2.60 (dd, 1H, J = 12.9, 6.2), 1.56 (s, 3H),
1.46 (s, 3H), 1.44 (s, 3H), 1.38 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 165.6, 152.5, 112.1, 111.2, 96.8, 79.5, 79.1, 78.3, 75.6, 70.1, 68.8, 65.7, 57.3, 55.9, 28.2, 27.3, 26.0, 25.0, 24.6.

**IR**: 3222 (br), 3119, 1744, 1701.

**MS**: 452  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>19</sub>H<sub>27</sub>NO<sub>10</sub>Na<sup>+</sup>: 452.1533, found 452.1527.

**mp** (Hexanes/Toluene): 251-254 °C



<sup>1</sup>H NMR spectrum of 4.122 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.122 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.126** 

Solid Dess-Martin periodinane (24 mg, 57  $\mu$ mol, 1.4 equiv) was added at rt to a stirred solution of **4.88** (27 mg, 39  $\mu$ mol, 1 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL), under Ar. After 75 min, a 3:7 mixture of aq. sat. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and aq. sat. NaHCO<sub>3</sub> solutions (5 mL) and EtOAc (3 mL) were added and stirring was continued for another 10 min, during which time the mixture became clear. The solution was extracted with EtOAc (15 mL) and the extract was successively washed with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under vacuum to afford crude aldehyde **4.126** (27 mg, 39  $\mu$ mol, quant. colorless oil, R<sub>f</sub>=0.56 in 30:70 EtOAc:Hex). This material was of excellent quality and required no further purification.

<sup>1</sup>**H** (**300 MHz, CDCl**<sub>3</sub>): 9.92 (d, 1H, *J* = 1.9), 7.37-7.26 (m, 10H), 5.83 (s, 1H), 4.87-4.81 (m, 2H), 4.75(d, 1H, *J* = 6.7), 4.73, 4.70 (AB q, 2H, *J*<sub>AB</sub>= 6.8), 4.66-4.59 (m, 3H), 4.54 (d, 1H, *J* = 11.8), 4.44, 4.22 (AB q, 2H, *J*<sub>AB</sub>= 9.4), 4.36 (d, 1H, *J* = 6.8), 4.31 (d, 1H, *J* = 4.3), 3.93-3.86 (m, 2H), 3.67-3.59 (app dt, 1H, *J* = 10.5, 5.8, 5.8), 2.31-2.23 (m, 2H), 1.51 (s, 3H), 1.45 (s, 3H), 1.41 (app s, 12H), 1.38 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 202.1, 154.4, 137.8, 137.6, 128.6 (2C), 128.1, 128.0, 127.9 (2C), 110.9, 108.9, 96.5, 94.6, 80.8, 80.0, 79.8, 77.7 (by HSQC), 76.8 (by HSQC), 70.3, 69.7, 68.8, 64.8, 58.3,
53.5, 34.0, 28.5, 26.9, 26.8, 25.7, 24.6.

**IR (film)**: 3373 (br), 1715.

**MS**: 709  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>37</sub>H<sub>51</sub>NO<sub>11</sub>Na<sup>+</sup>: 708.3360, found 708.3353.





<sup>13</sup>C NMR spectrum of 4.126 (75 MHz, CDCl<sub>3</sub>) with expansion of HSQC spectrum





**Preparation of 4.132** 

Commercial (trimethylsilyl)methyllithium solution (1.0 M in pentane, 360 µL, 360 µmol, 9 equiv relative to 4.88), was diluted with dry THF (0.20 mL) and cooled to -78 °C (argon atmosphere). A solution of crude aldehyde 4.126 (27 mg) in dry THF (250 µL) was added (syringe) over 30 sec to the cold, vigorously stirred organolithium solution. The flask containing starting 4.126 was rinsed with more dry THF (250 µL), which was transferred into the reaction flask as well. After 15 min, a 1 M THF solution of acetic acid (500 µL) was added slowly, then the reaction mixture was warmed to room temperature, diluted with hexanes (2 mL), eluted through silica gel (1 mL) using 50:50 EtOAc/hexanes (15 mL). The eluate was concentrated in vacuo to afford crude 4.130 (32 mg, white solid,  $R_f=0.38$  in 20:80 EtOAc:Hex). A cold (0 °C) solution of this material in dry THF (2.5 mL) was treated with commercial potassium bis(trimethylsilyl)amide (0.5 M toluene solution, 330 µL, 165 µmol, 4.2 equiv relative to **4.88**), added carefully, dropwise and with good stirring. After 15 min, the reaction flask from removed from the ice bath and the yellow solution was stirred for another 15 min. The ice bath was applied again, aq. sat. NH<sub>4</sub>Cl solution (10 drops) was added, and the mixture was stirred for another 10 min. The mixture was warmed to rt, diluted with hexanes (2.5 mL), and eluted through silica gel (1 mL) using 50:50 EtOAc/ hexanes (20 mL). The eluate was concentrated under reduced pressure to afford crude 4.131 (25 mg, colorless oil,

 $R_f=0.30$  in 20:80 EtOAc:Hex). Finally, liquid ammonia (*ca.* 3 mL) was condensed into a cold (-78 °C) THF solution (2.5 mL) of this crude compound, and metallic sodium (24 mg, in two pieces, 1 mmol, 27 equiv relative to **4.88**) was added with vigorous stirring. After 6 min, a homogeneously dark blue solution resulted. At this exact moment, solid NH<sub>4</sub>Cl (150 mg, 2.8 mmol, 72 equiv relative to **4.88**) was added, causing the blue color to fade completely. After stirring for another 5 min, the Dry Ice/acetone bath was removed and the ammonia was allowed to evaporate. The residual solution was filtered through Celite<sup>®</sup> with EtOAc and the filtrate was evaporated *in vacuo* to afford crude **4.132**. Occasionally, the <sup>1</sup>H NMR and mass spectra of this material revealed the presence of variable quantities of mono- and bis-hydroxymethyl derivatives. In such cases, crude **4.132** thus obtained was redissolved in 4:1 2-propanol:water (5 mL), warmed to 55 °C and stirred for 1 h at that temperature. The volatiles were removed *in vacuo* to afford a product free from contaminants. Flash chromatography (EtOAc:Hex, 50:50) afforded **4.132** (14 mg, 32 µmol, 80% over 4 steps,  $R_f=0.23$  in 50:50 EtOAc:Hex) as a white powder.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.80 (app dt, 1H, *J* = 16.8, 10.1, 10.1), 5.27-5.18 (m, 2H), 5.10 (br s, 1H),
4.61, 4.35 (AB q, 2H, *J*<sub>AB</sub>= 6.4), 4.19, 4.10 (AB q, 2H, *J*<sub>AB</sub>= 8.8), 4.00 (app t, *J* = 5.7), 3.79-3.74 (m,
2H), 3.19 (dd, 1H, *J* = 10.0, 4.8), 2.40 (br s, 1H), 2.26-2.20 (m, 2H), 1.69 (br s, 1H), 1.55 (s, 3H),
1.45 (s, 3H), 1.43 (s, 9H), 1.41 (s, 3H), 1.37 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 154.6, 134.1, 120.4, 109.3, 108.8, 83.9, 79.9, 79.6, 78.3, 71.0, 67.1, 59.0,
57.2, 50.7, 35.8, 28.5, 26.8 (2C), 26.2, 24.6.

**IR (film)**: 3457 (br), 3363 (br), 1715 (shoulder), 1692, 1639.

**MS**: 466  $[M + Na^+]$ .

**HRMS**: calcd. for  $C_{22}H_{37}NO_8Na^+$ : 466.2417, found 466.2425.

**mp (Hexanes)**: 53-54 °C



<sup>1</sup>H NMR spectrum of 4.132 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.132 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.134** 

Solid TPAP (*ca.* 0.5 mg, 1  $\mu$ mol, 0.05 equiv) was rinsed (dry CH<sub>2</sub>Cl<sub>2</sub>; 0.5 mL) into a solution of diol **4.132** (13 mg, 0.029 mmol, 1 equiv) and solid 4-methylmorpholine *N*-oxide (*ca.* 15 mg, 130  $\mu$ mol, 4 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) and the mixture was stirred at for 3 h. Additional TPAP (*ca.* 0.5 mg, 0.05 equiv) was rinsed into the flask (dry CH<sub>2</sub>Cl<sub>2</sub>, 0.5 mL). After stirring for another 2 h, starting **4.132** and the corresponding lactol (R<sub>f</sub>=0.21 in 30:70 EtOAc:Hex) were no longer detectable by TLC. The solution was loaded on a column of silica gel (1.0 mL, *ca.* 0.5 g) and the desired product was eluted with 50:50 EtOAc:Hexanes (20 mL). Compound **39** thus obtained was recrystallized from hexanes (1.5 mL), and the resulting crystals were rinsed with ice cold hexanes (2x1 mL) to yield pure **4.134** (9 mg) as colorless prisms. The combined mother liquor and washes were concentrated *in vacuo* and the residue was purified by column chromatography (gradient EtOAc:Hexanes, 15:85~20:70) to give additional **4.134** (2 mg; 11 mg in total, 0.025 mmol, 85%, R<sub>f</sub>=0.45 in 30:70 EtOAc:Hex) as white powder.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.88 (ddd, 1H, J = 17.4, 10.6, 7.1), 5.44-5.32 (m, 2H), 5.01 (br d, 1H, J = 6.2), 4.44-4.39 (m, 2H), 4.35-4.31 (m, 2H), 4.21 (d, 1H, J = 9.9), 3.91 (br d, 1H, J = 7.0), 2.89 (dd, 1H, J = 17.6, 1.8), 2.16 (dd, 1H, J = 17.6, 1.2), 1.53 (s, 3H), 1.45 (s, 9H), 1.43 (s, 3H), 1.42 (s, 3H), 1.37 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 167.2, 153.9, 132.0, 119.9, 111.7, 109.6, 80.6, 80.4, 79.4, 79.2, 75.8, 69.1,

55.7, 36.6, 34.8, 28.4, 27.4, 26.1, 25.1, 24.9.

**IR**: 3347 (br), 1755, 1713, 1641.

**MS**: 462  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>22</sub>H<sub>33</sub>NO<sub>8</sub>Na<sup>+</sup>: 462.2104, found 462.2111.

**mp (Hexanes)**: 194-195 °C



<sup>1</sup>H NMR spectrum of 4.134 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.134 (75 MHz, CDCl<sub>3</sub>)





#### Preparation of 1.109, the Du Bois intermediate

Commercial *n*-BuLi solution (1.43 M in hexanes, 72 µL, 0.10 mmol, 5 equiv) was added to a cold (-78 °C) solution of diisopropylamine (20 µL, 0.14 mmol, 7 equiv) in THF (70 µL). The mixture was stirred at -78 °C for 10 min, then it was removed from the Dry Ice/acetone bath, stirred for another 10 min, and finally transferred (syringe) into a cold (-78 °C) solution of lactone 4.134 (9 mg, 0.021 mmol, 1 equiv) in THF (100 µL). The resulting solution was stirred at -78 °C for 1 h, then a THF (0.10 mL) solution of 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (16 mg, 60 µmol, 3 equiv) was added dropwise. The mixture was warmed to -25 °C during 45 min and stirred at -25±5 °C for 2 h, then it was cooled to -78 °C prior to slow addition of a 1 M THF solution of acetic acid (250  $\mu$ L). The reaction mixture was warmed to rt, diluted with hexanes (1 mL), and eluted through silica gel (0.5 mL) using 50:50 EtOAc/hexanes (15 mL). The eluate was concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc:Hex, 10:90) to give a white solid consisting of an inseparable mixture of starting 4.134 and product 1.109 in a 1:3 ratio (8 mg,  $R_{f(1,109)}=0.18$  in 20:80 EtOAc:Hex). This white solid was dissolved in a 1:9 mixture (vol/vol) of CH<sub>2</sub>Cl<sub>2</sub> and hexanes (2 mL) in a loosely capped scintillation vial, and the solution was allowed to evaporate slowly at rt to a volume of about 0.5 mL, whereupon colorless crystals of 1.109 formed. The mother liquor was decanted and the

crystals were rinsed with hexanes (2x2 mL). This process was repeated for 4 times until virtually no **4.134** was present in the concentrated mother liquor (<sup>1</sup>H NMR). Pure **1.109** (6 mg, 0.013 mmol, 64%) was thus obtained as colorless prisms. The mother liquor and the foregoing washes were combined and concentrated to return starting **4.134** (2 mg, 5  $\mu$ mol, 22%, containing *ca*. 5% **1.109** by <sup>1</sup>H NMR).

<sup>1</sup>H (600 MHz, CDCl<sub>3</sub>): 6.07 (ddd, 1H, J = 17.2, 10.3, 8.5), 5.53 (s, 1H), 5.36 (d, 1H, J = 6.4), 5.33 (app dt, 1H, J = 17.2, 1.1, 1.1), 5.28 (app d, 1H, J = 10.4), 4.49 (dd, 1H, J = 5.0, 0.9), 4.44 (app t, 1H, J = 1.1), 4.39, 4.19 (AB q, 2H, J<sub>AB</sub> = 10.0), 4.35 (dd, 1H, J = 6.4, 1.4), 3.85 (app d, 1H, J = 8.5),
2.93 (d, 1H, J = 5.0), 1.49 (s, 3H), 1.44 (s, 9H), 1.43 (s, 3H), 1.41 (s, 3H), 1.36 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 169.5, 154.7, 132.8, 119.4, 111.7, 109.4, 81.3, 79.9, 79.0, 78.5, 74.3, 68.7, 66.9, 57.5, 36.7, 28.3, 27.2, 25.9, 24.8(2C).

**IR**: 3419 (br), 1754, 1717, 1641.

**MS**: 478  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>22</sub>H<sub>33</sub>NO<sub>9</sub>Na<sup>+</sup>: 478.2053, found 478.2051.

**mp (Hexanes)**: 211-213 °C



## <sup>1</sup>H NMR spectrum of 1.109 (600 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 1.109 (75 MHz, CDCl<sub>3</sub>)



### Expansion of COSY spectrum of 1.109 (CDCl<sub>3</sub>)







## HSQC spectrum of 1.109 (CDCl<sub>3</sub>)









**Preparation of 5.7** 

Diene 4.32 (crude, 195 mg, 0.36 mmol, 1 equiv) and citric acid (54 mg, 0.28 mmol, 0.77 equiv) was dissolved in 4:1 acetone/water (4 mL) in a heavy-wall sealable tube and 50% aqueous NMO (92 µL, 0.49 mmol, 1.2 equiv) and 4% aqueous OsO<sub>4</sub> (7 drops, ca. 70 µL, 0.011 mmol, 0.030 mmol) was added. The vessel was warmed to 50  $\,^{\circ}$ C then stirred for 69 hours. The oxidants were quenched with solid sodium sulfite (0.3 g), and water (3 mL) stirred for another 1 hour. The reaction mixture was diluted with water (20 mL) then extracted with EtOAc (20 mL). The organic layer was washed with saturated NH<sub>4</sub>Cl solution (10 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) then concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient EtOAc:Hex, 20:80~30:70) to yield 5.33 (86 mg, contains ca. 15%) impurity) and returned 4.32 (64 mg, 0.12 mmol, 34%). Such impure 5.33 was dissolved in dry THF (5 mL) followed by the addition of solid LiBH<sub>4</sub> (18 mg, 0.83 mmol, 2.3 equiv) under argon atmosphere. After 21 hours, the septum was removed and saturated aqueous NH<sub>4</sub>Cl (5 mL) was added dropwise (CAUTION: evolution of flammable H<sub>2</sub> gas) to quench the excess LiBH<sub>4</sub>. After no H<sub>2</sub> gas was evolving, the mixture was extracted with ethyl acetate (20 mL), washed successively with water (5 mL) and brine (5 mL). Combined aqueous layer was again extracted with ethyl acetate (5 mL) then washed with water (2 mL) and brine (2 mL). Organic layers were

combined, dried with Na<sub>2</sub>SO<sub>4</sub> then concentrated *in vacuo*. Flash chromatography (EtOAc:Hex, 50:50) furnished pure triol **5.59** (65 mg, 0.12 mmol, 34% over 2 steps,  $R_f$ =0.20 in 50:50 EtOAc:Hex) which was not fully characterized.

Triol 5.59 (65 mg, 0.12 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (3 mL) then solid TsOH<sup>+</sup>H<sub>2</sub>O was added at rt. After 1 hour, saturated aqueous NaHCO<sub>3</sub> was added to basify the solution and stirred for 30 minutes then extracted with EtOAc (15 mL). The organic layer was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under vacuum. The crude product was dissolved in methanol (10 mL) in a round bottom flask then gently rotated on a rotary evaporator for 3 h and methanol was removed in vacuo to produce crude 5.60 (67 mg, R<sub>f</sub>=0.38 in 30:70 EtOAc:Hex) as a white wax. This crude alcohol was dissolved in dry dichloromethane (4 mL) then solid Dess-Martin periodinane (65 mg, 0.15 mmol, 1.2 equiv) was added at room temperature. The solution turned cloudy and was stirred for 3.5 h. The reaction was quenched by addition of 3:7 saturated Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/NaHCO<sub>3</sub> solution (5 mL) followed by 30-minute agitation until solid disappeared. The mixture was extracted with ethyl acetate (15 mL) and the extract was successively washed with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under vacuum to yield crude aldehyde 5.61 (69 mg, R<sub>f</sub>=0.67 in 30:70 EtOAc:Hex) of good quality. Without further purification, the aldehyde was dissolved in *t*-BuOH (2 mL) then 2-methyl-2-butene (125 µL, 1.2 mmol, 10 equiv) was added. Ice bath was applied and the solution was frozen. A solution of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (29 mg, 0.24 mmol, 2.0 equiv) and NaClO<sub>2</sub> (40 mg, 0.44 mmol, 3.6 equiv) in water (1.2 mL) was added. The frozen solution was

slowly warmed to room temperature and the reaction was stirred for 5.5 h. The reaction mixture was poured into 0.02M HCl (15 mL) then successively extracted with DCM (10+5+5 mL). The extract was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum to yield crude acid **5.62** (70 mg,  $R_f=0.21$  in 30:70 EtOAc:Hex) as colorless film. This material was dissolved in dry THF (1.8 mL) under argon. Solid CDI (23 mg, 0.14 mmol, 1.2 equiv) was added then the flask was immersed in a 40 °C oil bath for 1.5 h. The reaction was cooled to rt, dry nitromethane  $(38 \,\mu\text{L}, 0.55 \,\text{mmol}, 4.4 \,\text{equiv})$  and solid *t*-BuOK (53 mg, 0.47 mmol, 3.8 equiv) was added. The suspension was again warmed up to 40 °C then stirred for 6 hours. The reaction was cooled to rt, then a HOAc (0.3 mL) in water (5 mL) was added dropwise. The biphasic solution was extracted successively with DCM (3x5 mL) and the organic layer was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a mixture of starting acid **5.62** and nitroketone **5.6**. Flash chromatography (EtOAc:Hex, 10:90) produced pure nitroketone 5.6 (40 mg, 0.064 mmol, 52%, R<sub>f</sub>=0.59 in 30:70 EtOAc:Hex) and flush the silica gel with EtOAc recovered carboxylic acid 5.62 (30 mg, 0.051 mmol, 42%). Nitroketone 5.6 was not fully characterized.

Cu(OAc)<sub>2</sub>:H<sub>2</sub>O (2.0 mg, 0.010 mmol, 0.16 equiv) and *N*-ethylpiperidine (8.0  $\mu$ L, 0.058 mmol, 0.91 equiv) was dissolved in dry acetonitrile (12.0 mL). This blue solution (4.0 mL) that contained Cu(OAc)<sub>2</sub> (0.05 equiv) and *N*-ethylpiperidine (0.30 equiv) was added into the flask containing **5.6** (40 mg, 0.064 mmol, 1 equiv) and the color turned yellow immediately. This solution was agitated at 40 °C for 5 days, and then solvents were removed under reduced pressure. Purification by chromatography (EtOAc:Hex, 10:90) of the residue provided isooxazoline **5.7** (20

mg, 0.033 mmol, 51%, 9% over 7 steps, Rf=0.62 in 30:70 EtOAc:Hex) as colorless oil.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 7.70-7.61 (m, 4H), 7.53-7.39 (m, 6H), 5.46 (s, 1H), 4.89 (app dt, 1H, *J* = 12.8, 2.0, 2.0), 4.58 (dd, 1H, *J* = 7.8, 1.3), 4.43 (app dt, 1H, *J* = 7.7, 1.9, 1.9), 4.29 (dd, 1H, *J* = 12.8, 0.7), 4.15 (app t, 1H, *J* = 2.2), 3.78, 3.08 (AB q, 2H, *J*<sub>AB</sub>= 17.8), 1.44 (s, 9H), 1.26 (s, 3H), 1.17 (s, 3H), 1.13 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 190.4, 159.7, 154.4, 135.7, 135.6, 131.9, 131.4, 130.7, 130.5, 128.3, 128.1, 108.5, 80.6 (2C by HSQC and HMBC), 74.7, 74.2, 66.6, 56.4, 54.2, 52.2, 28.3, 27.1, 23.3, 22.5, 18.9.

IR (film): 3305 (br), 1754, 1717.

**MS**: 629  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>33</sub>H<sub>42</sub>N<sub>2</sub>O<sub>7</sub>NaSi<sup>+</sup>: 629.2659, found 629.2667.

<sup>1</sup>H NMR spectrum of 5.7 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 5.7 (75 MHz, CDCl<sub>3</sub>)



### Expansion of COSY spectrum of 5.7 (CDCl<sub>3</sub>)



Expansion of NOESY spectrum of 5.7 (CDCl<sub>3</sub>)



HSQC spectrum of 5.7 (CDCl<sub>3</sub>)



## <sup>1</sup>H NMR spectrum of 5.33 (300 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 5.59 (300 MHz, CDCl<sub>3</sub>)



## <sup>1</sup>H NMR spectrum of 5.60 (300 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 5.61 (300 MHz, CDCl<sub>3</sub>)





<sup>1</sup>H NMR spectrum of 5.62 (300 MHz, CDCl<sub>3</sub>)

<sup>1</sup>H NMR spectrum of 5.6 (300 MHz, CDCl<sub>3</sub>)





#### **Preparation of 5.67**

To an Erlenmeyer flask equipped with a plastic stopper was added **4.25** (1.55g, 2.75 mmol, 1 equiv) and citric acid (529 mg, 2.76 mmol, 1.0 equiv). The solid was suspended in 60 mL 5:1 acetone/water with mild agitation. 4% aqueous osmium tetroxide (525 µL, 0.083 mmol, 0.030 equiv) and 50% aqueous NMO (735 µL, 3.58 mmol, 1.3 equiv) was then slowly added to the mixture. All crystals of starting materials dissolved within 24 hours and after a total of 165 hours, solid Na<sub>2</sub>SO<sub>3</sub> (350 mg, 2.78 mmol, 1.0 equiv) was used to quench the reaction mixture followed by carefully evaporation of acetone. Ethyl acetate (50 mL) was used to extract the remaining aqueous layer then the extract was washed successively with saturated NH<sub>4</sub>Cl solution (15 mL) and brine (10 mL). The combined aqueous layer was again extract with ethyl acetate (10 mL) then washed successively with water (3 mL), saturated NH<sub>4</sub>Cl solution (3 mL) and brine (3 mL). The combined ethyl acetate layer was dried with Na<sub>2</sub>SO<sub>4</sub> and solvents were removed on a rotary evaporator. Besides the starting material, the crude (1.61 g) contained mostly 5.30 (R<sub>f</sub>=0.10 in 30:70 EtOAc:Hex) with approximately 10% each of 5.31 (R<sub>f</sub>=0.24 in 30:70 EtOAc:Hex) and 5.32 (R<sub>f</sub>=0.36 in 30:70 EtOAc:Hex). And this material should be kept at -20 °C to prevent acyl migration. A simple flash chromatography (10:90 EtOAc:Hexanes) was performed to remove the nonpolar starting material (0.77 g, 1.37 mmol, 49%) then ethyl acetate was applied to the column

to rapidly flush all dihydroxylated products out. Solvents were evaporated to afford the mixture of three compounds as white solid [0.85 g, 1.42 mmol (calculated as 5.30), 52%]. The dihydroxylated products was dissolved in 4:1 MeOH/water (75 mL), then solid NaHCO<sub>3</sub> (239 mg, 2.85 mmol, 2.0 equiv) and Na<sub>2</sub>CO<sub>3</sub> (302 mg, 2.85 mmol, 2.0 equiv) was added to the solution then warmed up in a 50  $^{\circ}$  coil bath. After 48 hours of agitation, the reaction mixture was cooled to 0 °C and 1.0 M HCl (10.0 mL, 10.0 mmol, 7.0 equiv) was slowly added to the solution to acidify the reaction mixture. The cloudiness disappeared and the solution was warmed to room temperature for 6 hours of further stirring. Two spots on TLC plate appeared for 5.33 (R<sub>f</sub>=0.45 in 30:70 EtOAc:Hex) and 5.67 (R<sub>f</sub>=0.53 in 30:70 EtOAc:Hex). When the reaction finished, normally prisms would be seen in the solution. Methanol was then removed in vacuo. The residual aqueous suspension was extracted with ethyl acetate (25 mL) then washed with brine (10 mL). Combined aqueous layers from extraction were extracted again with ethyl acetate (10 mL) and washed with brine (5 mL). Combined ethyl acetate layers were dried with Na<sub>2</sub>SO<sub>4</sub> and ethyl acetate was removed under reduced pressure to yield light yellow solid as crude (0.76 g). This material was dissolved in 4:1 methanol/water (125 mL) in a 250 mL round bottom flask then the solution line was immersed under a 40  $\,^{\circ}$ C oil bath with the neck open to the atmosphere. Crystals formed at the bottom of flask upon the evaporation of methanol. Should the solution turn slightly cloudy, it could be made clear again by careful addition of methanol (less than 10 mL). Typically the final volume of the solution was about 60 mL. Mother liquor was decanted and 2:1 methanol/water (21 mL) was added, the suspension was brought to refluxing, then air-cooled to

room temperature. Mother liquor was again decanted then the crystals were washed with 1:1 MeOH/water (3x5 mL). The solid was dried under high vacuum to afford pure **5.67** (215 mg, 0.41 mmol, 15%) as colorless needles. The combined mother liquor and washes were evaporated to recover the mixture of **5.33** and **5.67** (500 mg) and then were subjected to two same reaction cycles with proportional amount of reagents and solvents to produce additional **5.67** (160 + 100 mg, 0.31 + 0.19 mmol, 11% + 7%) and recovered the mixture of **5.33** and **5.67** (230 mg, *ca*. 0.44 mmol, 16%) from the last batch of mother liquor. In summary, **5.67** was furnished in two steps (3 reaction cycles) with 33% isolated yield, with 16% of potentially recyclable material and 49% of starting material.

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.74-7.62 (m, 4H), 7.49-7.37 (m, 6H), 5.78 (dd, 1H, *J* = 9.7, 4.9), 5.73 (d, 1H, *J* = 9.7), 5.15 (app s, 1H), 4.76 (s, 1H), 4.29 (app s, 1H), 3.87-3.72 (m, 1H), 3.71-3.55 (m, 1H), 2.97, 2.62 (AB q, 2H, *J*<sub>AB</sub>= 17.3), 1.39 (s, 9H), 1.04 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 174.3, 154.5, 136.2, 135.8, 133.7, 131.9, 130.3, 130.2, 129.5, 128.5, 128.1, 128.0, 81.6, 80.5, 72.3, 66.3, 57.1, 41.8, 28.3, 27.0, 19.3.

**IR**: 3347 (br), 1788, 1689.

**MS**: 546  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>29</sub>H<sub>37</sub>NO<sub>6</sub>NaSi<sup>+</sup>: 546.2288, found 546.2285.

**mp** (**MeOH/water**): 168-169 ℃



### <sup>1</sup>H NMR spectrum of 5.67 (300 MHz, CDCl<sub>3</sub>)

# <sup>13</sup>C NMR spectrum of 5.67 (75 MHz, CDCl<sub>3</sub>)



## <sup>1</sup>H NMR spectrum of 5.30 (300 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 5.31 (contains *ca.* 10% 5.32) (300 MHz, CDCl<sub>3</sub>)





# <sup>1</sup>H NMR spectrum of 5.32 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 5.68** 

Solid **5.67** (30 mg, 0.057 mmol, 1 equiv) was dissolved in dry dichloromethane (1.5 mL) then solid Dess-Martin periodinane (34 mg, 0.080 mmol, 1.4 equiv) was added at room temperature. The solution turned cloudy and was stirred for 80 minutes. The reaction mixture was quenched by addition of 3:7 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/NaHCO<sub>3</sub> solution (5 mL) followed by 15-minute agitation until solid disappeared. The mixture was extracted with ethyl acetate (15 mL) and the extract was successively washed with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under vacuum to afford crude enone **5.68**. Flash chromatography (EtOAc:Hex, 30:70) afforded pure **5.68** (30 mg, 0.058 mmol, quant.,  $R_f$ =0.31 in 30:70 EtOAc:Hex) as colorless film that provided white waxy solid with hexanes trituration.

<sup>1</sup>**H** (**300 MHz, CDCl<sub>3</sub>**): 7.75-7.67 (m, 4H), 7.48-7.35 (m, 6H), 6.54 (dd, 1H, *J* = 10.2, 1.3), 6.03 (d, 1H, *J* = 10.2), 4.69-4.64 (m, 1H), 4.64-4.59 (m, 1H), 4.54 (s, 1H), 3.13, 2.88 (AB q, 2H, *J*<sub>AB</sub>= 17.3), 1.38 (s, 9H), 1.13 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 192.4, 171.8, 153.6, 141.8, 135.9, 132.7, 132.3, 130.2, 130.1, 128.5, 127.9, 127.7, 83.2, 81.5, 72.8, 57.7, 41.7, 28.2, 26.8, 19.4.

**IR**: 3340 (br), 1793, 1710.

**MS**: 560  $[M + K^+]$ .

**HRMS**: calcd. for C<sub>29</sub>H<sub>35</sub>NO<sub>6</sub>NaSi<sup>+</sup>: 544.2131, found 544.2148.

**mp (Hexanes)**: 70-72 °C

200









#### **Preparation of 5.74**

4.7 (0.47 g, 1.45 mmol, 1 equiv) was transformed into a mixture of diastereomeric dienols 4.23 and 4.24 (0.37 g, 1.14 mmol, 4.6:1 dr, 78% total) with the CBS/Borane reduction aforementioned with an additional flash chromatography (gradient EtOAc:Hex, 30:70~50:50). The mixture of dienol epimers and citric acid (220 mg, 1.15 mmol, 0.79 equiv) was dissolved with 4:1 acetone/water (25 mL) in an Erlenmeyer flask with a plastic stopper. 4% aqueous osmium tetroxide (215 µL, 8.6 mg, 0.034 mmol, 2.3%) and 50% aqueous NMO (300 µL, 1.46 mmol, 1.0 equiv) was added to the solution. At this point the solution turned from colorless to dark yellow quickly. After 48 hours, roughly 90% of the material was converted presumably to the corresponding triols (R<sub>f</sub>=0.19 in EtOAc) and some tentative translactonized compounds (0.33<Rf<0.57 in EtOAc) were also observed through TLC. A total of 72 hours of agitation was applied and the reaction was quenched by the addition of solid Na<sub>2</sub>SO<sub>3</sub> (0.144 g, 1.14 mmol, 0.78 equiv) followed by a 30-minute agitation. Acetone was removed in vacuo and brine (1 mL) was added before the successive extraction with ethyl acetate (10+5 mL). To the aqueous layer was added brine (2 mL) and successively extracted with *n*-butanol (3x5 mL) until no products were monitored from the aqueous layer. The combined organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum to yield a crude mixture of triol derivatives as beige solid (0.44 g).

The crude solid was dissolved in 2:1 MeOH/water (30 mL), followed by the addition of NaHCO<sub>3</sub> (191 mg, 2.27 mmol, 1.56 equiv) and Na<sub>2</sub>CO<sub>3</sub> (241 mg, 2.27 mmol, 1.56 equiv). The suspension was immersed in a 45  $\,^{\circ}$ C oil bath then stirred for 14 hours. The reaction mixture was then cooled to 0 °C followed by gentle addition of 1.0 M HCl (8.0 mL, 8 mmol, 5.5 equiv). The acidified solution was stirred for 3 hours then methanol was evaporated *in vacuo*. The aqueous layer was successively extracted with ethyl acetate (30+10 mL). Then to the aqueous layer was added brine (5 mL) and successively extracted with *n*-butanol (3x5 mL) until no products were monitored from the aqueous layer. Combined organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and solvent was removed under reduced pressure to yield orange solid as crude. The crude product was dissolved in ethyl acetate and filter through a short silica gel plug (10 mL) with ethyl acetate (30 mL) until no 5.72 (R<sub>f</sub>=0.30 in 70:30 EtOAc:Hex) was observed from TLC. The filtrate was concentrated to yield 5.72 as two epimers (245 mg). To separate the minor epimer, the crude was dissolved in dry acetone (10 mL) under argon then treated with PPTS (54 mg, 0.22 mmol, 0.15 equiv) and 2,2-dimethoxypropane (0.54 mL, 4.4 mmol, 3.0 equiv). After 24 hours, the reaction was quenched with Et<sub>3</sub>N (60  $\mu$ L, 0.43 mmol, 30%) and the solvent was evaporated under reduced pressure. Purification by chromatography (gradient EtOAc:Hex, 50:50~70:30~100:0) provided a fraction mostly contained 5.73 (60 mg, R<sub>f</sub>=0.56 in 50:50 EtOAc:Hex) as colorless oil and pure 5.74 (165 mg, 0.58 mmol, 40% over 3 steps,  $R_f=0.30$  in 70:30 EtOAc:Hex) as white solid. The latter could also be purified by recrystallization from chloroform.

<sup>1</sup>**H** (**300 MHz, acetone**- $d_6$ ): 6.70 (br s, 0.84H, N1-H), 5.90 (dd, 1H, J = 9.9, 3.5), 5.77 (d, 1H, J = 1.000 (d, 1H, J = 1.000) (d, 1H, J = 1.0000) (d, 1H, J = 1.0000) (d, 1H, J = 1.0000) (d, 2H, J = 1.0

9.9), 5.01 (d, 1H, J = 2.0), 4.56 (d, 0.45H, J = 5.0, C7O-H), 4.19 (d, 0.42H, J = 7.5, C6O-H),
4.15-4.08 (m, 1H), 4.04-3.97 (m, 1H), 3.13, 2.68 (AB q, 2H, J<sub>AB</sub>= 17.2), 1.41 (s, 9H).

<sup>13</sup>C (300 MHz, acetone-*d<sub>6</sub>*): 174.8, 156.0, 130.4, 129.3, 82.0, 80.3, 71.4 (2C, *C7*), 68.1 (2C, *C6*),
58.4 (2C, *C8a*), 41.1, 28.6. (Note: C6, C7 and C8a were split into two signals due to its partial deuterium exchange on their adjacent heteroatoms.)

**IR (film)**: 3335 (br), 1775, 1692.

**MS**: 308 [M + Na<sup>+</sup>].

**HRMS**: calcd. for C<sub>13</sub>H<sub>19</sub>NO<sub>6</sub>Na<sup>+</sup>: 308.1110, found 308.1107.

**mp (Hexanes)**: 166-167 ℃



### <sup>1</sup>H NMR spectrum of 5.74 (300 MHz, acetone-*d*<sub>6</sub>)

<sup>13</sup>C NMR spectrum of 5.74 (75 MHz, acetone-*d*<sub>6</sub>)




## Expansion of COSY spectrum of 5.74 (acetone-*d6*)







Diol **5.74** (165 mg, 0.58 mmol, 1 equiv) and imidazole (276 mg, 4.06 mmol, 7.0 equiv) were dissolved in dry DMF (1.5 mL) in a heavy-walled pressure tube sealable with a Teflon screwcap and equipped with a Teflon stirring bar with argon protection. Solid TBSCI (305 mg, 2.02 mmol, 3.5 equiv) was then added in one portion. The flask was sealed then heated to 55  $\,^{\circ}$ C (oil bath temperature) for 45 hours. DMF was removed by high vacuum assisted rotavap then 1:1 ethyl acetate/hexanes (10 mL) was added. The suspension was filtered through celite<sup>®</sup> then washed with more 1:1 EtOAc/hexanes (40 mL). The filtrate was concentrated and a flash chromatograph (gradient EtOAc:Hex, 10:90~15:85) yielded **5.75** (297 mg, 0.58 mmol, quant.,  $R_f$ =0.46 in 20:80 EtOAc:Hex) as white solid.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.80 (d, 1H, J = 10.0), 5.75 (dd, 1H, J = 9.9, 3.1), 4.78 (app s, 1H), 4.69 (s, 1H), 4.17 (dd, 1H, J = 5.1, 3.1), 3.92 (dd, 1H, J = 5.4, 2.5), 3.46, 2.54 (AB q, 2H, J<sub>AB</sub>= 17.0), 1.42 (s, 9H), 0.88 (s, 9H), 0.86 (s, 9H), 0.08 (app s, 12H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 174.9, 154.1, 130.3, 127.5, 81.5, 80.7, 71.6, 68.2, 58.0, 39.7, 28.4, 25.9, 25.8, 18.1, 18.0, -4.4, -4.5, -4.6, -4.9.

**IR (film)**: 3338 (br), 1789, 1711.

**MS**: 536  $[M + Na^+]$ .

**HRMS**: calcd. for  $C_{25}H_{47}NO_6NaSi_2^+$ : 536.2840, found 536.2844.

**mp (Hexanes)**: 166-167 ℃





<sup>13</sup>C NMR spectrum of 5.75 (75 MHz, CDCl<sub>3</sub>)





Commercial *n*-BuLi solution (1.51 M in hexanes, 485  $\mu$ L, 0.73 mmol, 4.1 equiv) was added to a cold (-78 °C) solution of diisopropylamine (140 µL, 0.99 mmol, 5.5 equiv) in dry THF (0.5 mL) under argon. The mixture was stirred at -78 °C for 10 min, then it was switched to an ice bath and stirred for another 10 min. The freshly prepared LDA solution was transferred (syringe) into a cold (-78 °C) THF (0.5 mL) solution of lactone 5.75 (94 mg, 0.18 mmol, 1 equiv). The resulting solution was stirred at -78 °C for 80 minutes, then a THF (0.5 mL) solution of 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (130 mg, 0.50 mmol, 2.8 equiv) was added dropwise. The reaction mixture was agitated at -78 °C for 270 minutes, then slowly warmed to -30 °C in 60 minutes and immediately cooled to -78 °C again. 1 M HOAc in THF (1.0 mL, 1.0 mmol, 5.6 equiv) was added to quench the reaction. The mixture was warmed to rt, diluted with hexanes (2 mL), and eluted through silica gel (3 mL) using 50:50 EtOAc/hexanes (40 mL) then the eluate was concentrated in vacuo. The residue was first left open to the atmosphere at rt for overnight to allow 4.117 decomposed into benzaldehyde and benzene sulfonamide, purified by a careful chromatography (gradient EtOAc:Hex, 10:90~15:85) to give a white solid (45 mg) containing mostly 5.79 ( $R_f=0.44$  in 20:80 EtOAc:Hex), with *ca.* 10~15% of unidentifiable 5.81. Contaminated 5.79 was dissolved in DCM (4 mL) followed by the addition of Dess-Martin periodinane (47 mg, 0.11 mmol, 0.62 equiv). The reaction was stirred for 18 h at rt and quenched by addition of a 7:3 sat. NaHCO<sub>3</sub>/NaS<sub>2</sub>O<sub>3</sub> solution (10 mL). The solution became clear in 10 min then was extracted with ethyl acetate (15 mL). The organic layer was successively washed with saturated NaHCO<sub>3</sub> (5 mL) solution and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under vacuum to afford crude **5.82** (R<sub>f</sub>=0.34 in 20:80 EtOAc:Hex) as yellow solid. Finally, the crude ketolactone was dissolved in methanol (2 mL) then cooled to -50 °C followed by dropwise addition (2 min) of ice cold methanol solution (2 mL) of sodium borohydride (10 mg, 0.26 mmol, 1.5 equiv). The solution was stirred at -50 °C for 20 min before quench acetone (0.5 mL) was slowly added at -78 °C. Sat. aqueous NH<sub>4</sub>Cl solution was added then the mixture was brought to rt. All methanol and acetone were evaporated under vacuum then the remaining aqueous layer was extracted with EtOAc (15 mL). The organic layer was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. Flash chromatography (gradient EtOAc:Hex, 20:80~30:70) furnished pure 5.83 (29 mg, 0.055 mmol, 30% over 3 steps, Rf=0.20 in 20:80 EtOAc:Hex) as a white solid.

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.86 (dd, 1H, J = 10.2, 1.9), 5.69 (d, 1H, J = 10.2), 5.33 (br s, 1H), 4.95 (s, 1H), 4.67 (s, 1H), 4.28 (app d, 1H, J = 7.4), 3.85 (dd, 1H, J = 7.4, 1.6), 3.31 (br s, 1H), 1.45 (s, 9H), 0.92 (s, 9H), 0.91 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), 0.11 (app s, 6H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 174.0, 154.4, 135.9, 120.6, 81.5, 78.7, 71.5, 71.1, 69.0, 61.9, 28.4, 26.0, 25.9, 18.2, 18.1, -4.0, -4.1, -4.6, -4.7.

IR (film): 3359 (br), 1786, 1712.

**MS**: 552  $[M + Na^+]$ .

**HRMS**: calcd. for C<sub>25</sub>H<sub>47</sub>NO<sub>7</sub>NaSi<sub>2</sub><sup>+</sup>: 552.2789, found 552.2781.

**mp (Hexanes)**: 186-187 °C

<sup>1</sup>H NMR spectrum of 5.83 (300 MHz, CDCl<sub>3</sub>)





### Expansion of COSY spectrum of 5.83 (CDCl<sub>3</sub>)



### Expansion of NOESY spectrum of 5.83 (CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 5.79 (contains *ca.* 15 mol% of 5.81) (300 MHz, CDCl<sub>3</sub>)



Solid *O*-(*t*-butyldiphenylsilyl)hydroxylamine (1.09 g, 4.0 mmol, 1 equiv) and glyoxylic acid monohydrate (0.41 g, 4.5 mmol, 1.1 equiv) were placed in a round bottom flask at 0 °C. Acetonitrile (22 mL) was slowly added into the flask with vigorous stirring. The mixture was stirred for 1.5 hours after the ice bath was removed then acetonitrile was removed under reduced pressure to produce colorless oil. Flash chromatography (gradient EtOAc:Hex, 20:80~30:70~50:50) produced **5.88** (1.18 g, 3.6 mmol, 90%,  $R_f$ =0.25 in 69.5:30:0.5 EtOAc:Hex:HOAc) that contains about 10 mol% of TBDPSOH as colorless oil. It solidified by addition of hexanes and standing for 2 hours to produce white flakes.

<sup>1</sup>H (**300 MHz, CDCl**<sub>3</sub>): 8.59 (br s, 1H), 7.82 (s, 1H), 7.70-7.63 (m, 4H), 7.48-7.36 (m, 6H), 1.15 (s, 9H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>):164.1 146.0, 135.6, 131.9, 130.4, 128.0, 27.0, 19.5.

**IR (film**): 1711.

**MS**: 366  $[M + K^+]$ .

**HRMS**: calcd. for C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub>NaSi<sup>+</sup>: 350.1188, found 350.1184.

**mp (Hexanes)**: 77-80 °C





<sup>13</sup>C NMR spectrum of 5.88 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 5.98** 

Alcohol **5.83** (16 mg, 0.030 mmol, 1 equiv), carboxylic acid **5.98** (25 mg, 0.076 mmol, 2.5 equiv) and DMAP (1.0 mg, 0.008 mmol, 0.3 equiv) were dissolved in DCM (0.4 mL) under argon atmosphere at 0 °C. Solid DCC (16 mg, 0.078 mmol, 2.6 equiv) was added in one portion. The reaction mixture was stirred for 30 min at 0  $\,^{\circ}$ C then ice bath was removed. After 20 hours, 3:7 EtOAc/Hexanes (2 mL) was added and the suspension was filtered through a short silica gel plug (2 mL) with more 3:7 EtOAc/Hexanes (10 mL) until no 5.89 (R<sub>f</sub>=0.64 in 20:80 EtOAc:Hex) was observed in the eluent. The solvents were removed in vacuo to yield crude 5.89 (36 mg) as a colorless film. This material was dissolved in THF (4 mL) then added 1 M THF solution of HOAc (50 µL, 0.050 mmol, 1.7 equiv) followed by a slow addition of 1.0 M THF solution of TBAF (50 µL, 0.050 mmol, 1.7 equiv). After 5 min, the solution was quickly evaporated to 1 mL volume by blowing air into the flask within 2 min, diluted with hexanes (3 mL) then filtered through a short silica gel plug (2 mL) with 50:50 EtOAc/hexanes (20 mL). Purification by chromatography (gradient EtOAc:Hex, 10:90~20:80) provided oxime 5.90 (17 mg, containing about 10% of **5.83**, R<sub>f</sub>=0.18 in 20:80 EtOAc:Hex). Since the oxime was less stable, it was carried onto the next reaction immediately. This material was dissolved in dry acetonitrile (5 mL) then monotonously transferred into an acetonitrile (5 mL) solution of DIB (15 mg, 0.047 mmol, 1.6

equiv) over 23 hours via a syringe pump. After the addition, triethylamine (0.3 mL) and water (0.3 mL) was dissolved in acetonitrile (5 mL) then this solution was used to rinse the syringe (3 portions) and the rinse was added into the reaction mixture. The reaction mixture was agitated for another 26 hours at 50  $\,^{\circ}$ C then all solvents were removed *in vacuo*. In the same flask, the crude sample was dissolved in 4:1 THF/water (5 mL), then 1 M aqueous HCl (170 µL, 0.17 mmol, 5.7 equiv) was added. The reaction mixture was stirred for 48 hours then all solvents were removed *in vacuo*. The crude product was dissolved in a small amount of EtOAc, filtered through silica gel (2 mL) with EtOAc (25 mL) to remove ammonium and triethylammonium salts then the eulate was concentrated under vacuum. NMR analysis showed an incomplete conversion of nitrile 5.96. The crude was then again dissolved in 4:1 THF/water (2.5 mL) followed by the addition of 1 M HCl (60 µL, 0.06 mmol, 2.0 equiv). After 20 hours, all solvents were evaporated and the crude was subjected to two consecutive flash chromatographies (gradient EtOAc:Hex, 10:90~20:80) to yield 5.98 (3 mg, 5.2  $\mu$ mol, 17 % over 5 steps, three pots, R<sub>f</sub>=0.53 in 30:70 EtOAc:Hex) as colorless film with recovered alcohol **5.83** (8 mg, 0.015 mmol, 50%).

<sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): 5.10-5.02 (m, 2H), 4.90 (d, 1H, J = 8.5), 4.67 (d, 1H, J = 3.5), 4.22 (app t, 1H, J = 4.1), 4.16-4.06 (m, 2H), 3.47 (d, 1H, J = 9.6), 1.44 (s, 9H), 0.90 (s, 9H), 0.89 (s, 9H), 0.17 (s, 3H), 0.14 (s, 3H), 0.12 (s, 3H), 0.11 (s, 3H).

<sup>13</sup>C (75 MHz, CDCl<sub>3</sub>): 173.3, 168.7, 154.9, 82.8, 82.4 (by HMBC), 78.5, 75.0, 71.2, 70.6, 60.8, 42.5, 28.3, 25.8, 25.7, 18.6, 17.9, -4.8, -5.1, -5.3, -5.4.

**IR (film)**: 3486 (br), 3372 (br), 1794 (shoulder), 1779, 1706.

**MS**: 596 [M + Na<sup>+</sup>].

**HRMS**: calcd. for  $C_{26}H_{47}NO_9NaSi_2^+$ : 596.2687, found 596.2695.



## <sup>1</sup>H NMR spectrum of 5.98 (300 MHz, CDCl<sub>3</sub>)

Expansion of <sup>1</sup>H NMR spectrum of 5.98 (3.3-5.3 ppm, 300 MHz, CDCl<sub>3</sub>)





<sup>13</sup>C NMR spectrum of 5.98 (75 MHz, CDCl<sub>3</sub>) with expansion of HMBC spectrum

<sup>1</sup>H NMR spectrum of 5.89 (300 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 5.90 (300 MHz, CDCl<sub>3</sub>)



Expansion of <sup>1</sup>H NMR spectra of 5.92 (top), a 7:6 mixture of 5.92/5.93 (middle) and a 1:2

mixture of 5.92/5.93 (bottom) (300 MHz, CDCl<sub>3</sub>)





Lactone **5.98** (4 mg, 7.0  $\mu$ mol, 1 equiv) was dissolved in dry THF (1.5 mL) under argon. 1 M THF solution of HOAc (8.0  $\mu$ L, 8.0  $\mu$ mol, 1.1 equiv) and 1.0 M THF solution of TBAF (8.0  $\mu$ L, 8.0  $\mu$ mol, 1.1 equiv) were added. The reaction was run for 30 min until all starting material was consumed monitored by TLC. Silica gel (20 mg) was added to quench the reaction mixture then the solution was diluted with hexanes (1 mL), filtered through a short silica gel plug (1 mL) with EtOAc (10 mL). Rotatory evaporation furnished crude solid **5.99**, which was carefully rinsed with hexanes (2x1 mL) to provide pure **5.99** (3 mg, 6.5  $\mu$ mol, 90%, R<sub>f</sub>=0.28 in 50:50 EtOAc:Hex) as white solid.

<sup>1</sup>**H**: 5.23 (s, 1H), 4.95-4.88 (m, 2H), 4.81 (d, 1H, *J* = 2.9), 4.39-4.27 (m, 2H), 4.18 (app s, 1H), 3.53 (d, 1H, *J* = 8.9), 3.00 (br s, 1H), 1.46 (s, 9H), 0.89 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H).

<sup>13</sup>C: 173.8, 168.6, 154.9, 82.7 (by HMBC), 81.4, 78.1, 75.2, 71.5, 71.0, 60.9, 44.4, 28.2, 25.7, 18.5, -5.4, -5.6.

**IR**: 3348 (br), 1780, 1703.

**MS**: 458  $[M - H^{-}]$ .

**HRMS**: calcd. for C<sub>20</sub>H<sub>32</sub>NO<sub>9</sub>Si<sup>-</sup>: 458.1846, found 458.1859.

**mp (Hexanes)**: 110-112 °C

## <sup>1</sup>H NMR spectrum of 5.99 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 5.99 (75 MHz, CDCl<sub>3</sub>) with expansion of HMBC spectrum



#### **B.4 Experimental Procedures for Compounds that were not Fully Characterized**

Like all total synthesis projects, this research produced numerous intermediates and byproducts that were not fully characterized or thoroughly purified, especially if they constituted dead-ends in the approaches. However, <sup>1</sup>H NMR spectra were obtained for all such compounds and in most cases their mass, <sup>13</sup>C, and 2D NMR spectra were also recorded. This section provides experimental procedures and spectral data for all substances that were not fully characterized or thoroughly purified.



#### **Preparation of 4.16**

Solid **4.9** (20 mg, 0.047 mmol, 1 equiv) was dissolved in dry DCM (0.8 mL) under Ar and TFA (0.2 mL) was added dropwise. The mixture was stirred for 50 min then carefully quenched with saturated NaHCO<sub>3</sub> (1 mL). The mixture was poured into EtOAc (10 mL) and washed successively with water (5 mL), sat. NaHCO<sub>3</sub> (2x5 mL) and brine (5 mL). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo* to afford a yellow oil, which was purified by flash chromatography (EtOAc:Hex, 30:70) to yield pure **4.16** (5 mg, 0.22 mmol, 48%, R<sub>f</sub>=0.21 in 30:70 EtOAc:Hex) as yellow crystals, mp 115-116 °C. <sup>1</sup>H and <sup>13</sup>C NMR spectra are attached.

<sup>1</sup>H NMR spectrum of 4.16 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.16 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.17** 

**4.16** (4 mg, 0.018 mmol, 1 equiv) was dissolved in dry toluene (0.7 mL) under Ar followed by the addition of DDQ (5 mg, 0.22 mmol, 1.2 equiv). The red solution was agitated at 60  $\,^{\circ}$ C for 77 h before the addition of saturated NaHCO<sub>3</sub> (2 mL) and EtOAc (2 mL). After 3 h, more EtOAc (5 mL) was added and the mixture was successively washed with 1:1 water/saturated NaHCO<sub>3</sub> (2x4 mL) and brine (4 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under vacuum. The crude was purified by flash chromatography (EtOAc:Hex, 70:30) to produce pure **4.17** (1 mg, 0.0045 mmol, 25%, R<sub>f</sub>=0.27 in 70:30 EtOAc:Hex) as yellow solid. This material was recrystallized in 1:1 DCM/Hexanes to form yellow needles, mp 141  $\,^{\circ}$ C. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 4.17 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.40** 

Isooxazoline **4.34** (8 mg, 0.015 mmol, 1 equiv) was dissolved in commercial *i*-PrOH (0.5 mL) then the flask was immersed in ice bath. NaBH<sub>4</sub> (*ca.* 1 mg, excess) was added to the solution and ice bath was removed. After 15 min, the reaction was quenched by careful dropwise addition of sat. NH<sub>4</sub>Cl (1 mL; CAUTION: H<sub>2</sub> evolution). Upon the depletion of bubbles, EtOAc (10 mL) and DI water (5 mL) was added. The organic layer was separated, washed successively with DI water (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvents were removed *in vacuo*. Crude **4.40** (8 mg, 0.015 mmol, quant. crude yield,  $R_f$ =0.20 in 10:90 EtOAc:Hex) was colorless film and no further purification was attempted. <sup>1</sup>H and <sup>13</sup>C NMR spectra of the crude product are attached.



<sup>1</sup>H NMR spectrum of 4.40 (300 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C NMR spectrum of 4.40 (75 MHz, CDCl<sub>3</sub>)





Compound **4.38** (33 mg, 0.042 mmol, 1 equiv) was dissolved in THF (0.8 mL) and treated with 1.0 M acetic acid solution in THF (105  $\mu$ L, 0.105 mmol, 2.5 equiv) followed by dropwise addition of commercial 1.0 M TBAF solution in THF (105  $\mu$ L, 0.105 mmol, 2.5 equiv). After stirring at rt for 5.5 h, to the reaction mixture was added EtOAc (15 mL). The solution successively washed with aq. sat. NaHCO<sub>3</sub> solution (5 mL), DI water (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. Flash chromatography (EtOAc:Hex, 30:70) produced **4.43** (21 mg, 0.039 mmol, 93%, R<sub>f</sub>=0.13 in 30:70 EtOAc:Hex) as colorless film. <sup>1</sup>H NMR spectrum is attached.



<sup>1</sup>H NMR spectrum of 4.43 (300 MHz, CDCl<sub>3</sub>)



Enol **4.43** (144 mg, 0.28 mmol, 1 equiv) was dissolved in dry  $CH_2Cl_2$  (4.5 mL), and treated with solid Dess-Martin periodinane (160 mg, 0.38 mmol, 1.35 equiv) added in one portion. After stirring at rt for 15 min, the reaction was quenched by addition of a 2:3 (vol/vol) mixture of aq. sat. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and aq. sat. NaHCO<sub>3</sub> solutions (10 mL), and DI water (5 mL) whereupon the mixture became clear. The solution was diluted with EtOAc (30 mL), and the aqueous phase was drained. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure to yield crude **4.44** (155 mg, R<sub>f</sub>=0.41 in 30:70 EtOAc:Hex) in good quality, no further purification attempts were made. <sup>1</sup>H NMR spectrum of the crude product is attached.



<sup>1</sup>H NMR spectrum of 4.44 (300 MHz, CDCl<sub>3</sub>)



Solid (Ph<sub>3</sub>PMe)Br (73 mg, 0.21 mmol, 5.0 equiv) was suspended in dry THF (0.5 mL) at rt followed by the addition of *n*-BuLi (1.50 M in hexane, 90  $\mu$ L, 0.135 mmol, 3.3 equiv). The orange solution was stirred for 50 min before being cooled to – 78 °C. Crude ketone **4.44** (22 mg, 0.041 mmol, 1 equiv) was dissolved in dry THF (0.4 mL) and added into the phosphonium ylide dropwise via a syringe. This mixture was kept in Dry Ice bath for another 5 min before warmed to rt. After 1 h, this reaction was quenched by the addition of sat. NH<sub>4</sub>Cl solution (5 mL) and EtOAc (15 mL). The organic layer was separated and successively washed with DI water (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. Flash chromatography (EtOAc:Hex, 20:80) was performed to provide pure diene **4.45** (10 mg, 0.019 mmol, 46%, R<sub>f</sub>=0.50 in 30:70 EtOAc:Hex). <sup>1</sup>H NMR spectrum is attached.



# <sup>1</sup>H NMR spectrum of 4.45 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 4.46** 

Diene **4.45** (10 mg, 0.019 mmol, 1 equiv) was dissolved in 4:1 acetone/water (0.5 mL) and treated with 4% aqueous  $OsO_4$  solution (1 drop, *ca.* 10 µL, 1.6 µmol, 0.08 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (8 µL, 0.039 mmol, 2.0 equiv). The reaction was stirred for 24 h, then water (10 mL), EtOAc (10 mL), and solid  $Na_2SO_3$  (*ca.* 10 mg) were added, and the mixture was stirred for another 180 min, then it was extracted with EtOAc (10 mL). The extract was washed with DI water (5 mL) and brine (5 mL), dried ( $Na_2SO_4$ ) and evaporated *in vacuo* to give crude **4.46** (10 mg, quant. crude yield,  $R_f$ =0.28 in 50:50 EtOAc:Hex) as a colorless film and as a single diastereomer, which was not further purified. <sup>1</sup>H NMR spectrum of the crude product is attached.

# <sup>1</sup>H NMR spectrum of 4.46 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.47** 

Crude **4.46** (21 mg, 0.037 mmol, 1 equiv) obtained as described above was dissolved in 2,2-dimethoxypropane (0.4 mL) and treated with TsOH'H<sub>2</sub>O (*ca.* 1 mg, 0.005 mmol, 0.1 equiv) and the solution was stirred for 40 min. The mixture was quenched with aq. sat. NaHCO<sub>3</sub> solution (5 mL) then stirred for another 15 min. The aqueous layer was successively extracted with CHCl<sub>3</sub> (3x5 mL). The combined organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated to yield crude **4.47** (21 mg, quant. crude yield,  $R_f$ =0.70 in 50:50 EtOAc:Hex) as colorless film. <sup>1</sup>H NMR spectrum of the crude product is attached.

<sup>1</sup>H NMR spectrum of 4.47 (300 MHz, CDCl<sub>3</sub>)





Commercial (trimethylsilyl)methyllithium solution (1.0 M in pentane, 3.70 mL, 3.70 mmol, 5.0 equiv) was added dropwise over 5 min to a cold (-78 °C), vigorously stirred solution of **4.64** (448 mg, 0.73 mmol, 1 equiv) in dry THF (9.5 mL), under Ar. After 30 min, sat. NH<sub>4</sub>Cl (6 mL) was added via a syringe. The frozen content was allowed to warm to room temperature in a water bath. The solution was poured into a 2:1 mixture of saturated NaHCO<sub>3</sub> / water (15 mL), and extracted with EtOAc (30 mL). The extract was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo*. The residue was purified by flash chromatography (EtOAc:Hex, 30:70) to provide **4.70** (173 mg, 0.31 mmol, 42%, R<sub>f</sub>=0.24 in 50:50 EtOAc:Hex), as well as recovered **4.64** (49 mg, 0.080 mmol, 11%), and a mixture of **4.64** and **4.70** (163 mg, *ca.* 3:2 mol/mol). <sup>1</sup>H and <sup>13</sup>C NMR spectra are attached.

Another avenue leading to **4.70** is described below. Commercial (trimethylsilyl)methyllithium solution (1.0 M in pentane, 0.16 mL, 0.16 mmol, 5.0 equiv) was added dropwise over 5 min to a cold (-78 °C), vigorously stirred solution of **4.64** (19 mg, 0.031 mmol, 1 equiv) in dry THF (0.7 mL), under Ar. The solution was warmed to -50 °C within 10 min, upon which time **4.70** appeared on TLC plate. The solution was warmed to -40 °C in 10 min then again cooled to -78 °C, quenched by addition of a THF solution of HOAc (1 M, 0.31 mL). The mixture was

warmed to rt and poured into DI water (5 mL), extracted by EtOAc (15 mL). The organic layer was then washed with brine (5 mL), dried with  $Na_2SO_4$  and concentrated under vacuum to provide a residue (21 mg). The crude product contained a mixture of **4.64**, **4.68** and **4.70** (*ca.* 1:1:1).





<sup>13</sup>C NMR spectrum of 4.70 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.72** 

**4.69** (14 mg, 0.022 mmol, 1 equiv) was dissolved in 4:1 acetone/water (0.8 mL) and treated with 4% aqueous  $OsO_4$  solution (1 drop, *ca.* 10 µL, 1.6 µmol, 0.07 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (2 drops, excess). The reaction was stirred for 43 h then quenched by adding solid Na<sub>2</sub>SO<sub>3</sub> (10 mg), DI water and EtOAc (3 mL). After 30 min, the content was extracted with EtOAc (12 mL), and the organic layer was sequentially washed with sat. NH<sub>4</sub>Cl (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated on rotatory evaporator. The crude **4.72** (12 mg, R<sub>f</sub>=0.38 in 50:50 EtOAc:Hex) showed a *ca.* 90% conversion and was not further purified. <sup>1</sup>H NMR spectrum of the crude product is attached.



<sup>1</sup>H NMR spectrum of 4.72 (contains *ca.* 10% 4.69) (300 MHz, CDCl<sub>3</sub>)



Preparation of 4.73

Amide **4.69** (35 mg, 0.056 mmol, 1 equiv) was dissolved in dry MeOH (0.7 mL) in a heavy-walled pressure tube sealable with a Teflon screwcap. The mixture was heated to 68  $\,^{\circ}$ C and stirred for 18 h, then concentrated *in vacuo*. Flash chromatography (EtOAc:Hex, 30:70) followed by recrystallization in DCM/Hex (1:2) provide pure **4.73** (9 mg, 0.016 mmol, 29%, R<sub>f</sub>=0.40 in 30:70 EtOAc:Hex) as a white wax. <sup>1</sup>H and <sup>13</sup>C NMR spectra are attached.

## <sup>1</sup>H NMR spectrum of 4.73 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.73 (75 MHz, CDCl<sub>3</sub>)





Preparation of 4.77 and 4.78

**4.76** (8 mg, 0.011 mmol, 1 equiv) was dissolved in dry THF (0.8 mL), then LiBH<sub>4</sub> (*ca.* 1-2 mg, excess) was added. The mixture was stirred at room temperature for 40 h and quenched by addition of aq. sat. NH<sub>4</sub>Cl (4 mL; **CAUTION**: evolution of flammable H<sub>2</sub> gas). When gas evolution subsided, the solution was successively extracted with CHCl<sub>3</sub> (3x5 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. Gradient chromatography (EtOAc:Hex, 20:80~30:70) afforded desired **4.78** (3 mg, 0.0070 mmol, 64%, R<sub>f</sub>=0.23 in 30:70 EtOAc:Hex) as waxy white solid with **4.77** (1 mg, 0.0013 mmol, 12%, R<sub>f</sub>=0.34 in 30:70 EtOAc:Hex) as colorless film. <sup>1</sup>H and <sup>13</sup>C NMR spectra are attached for **4.77** and <sup>1</sup>H NMR spectrum is attached for **4.78**.





<sup>13</sup>C NMR spectrum of 4.77 (75 MHz, CDCl<sub>3</sub>)


## <sup>1</sup>H NMR spectrum of 4.78 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.79** 

Alcohol **4.78** (33 mg, 0.054 mmol, 1 equiv) and imidazole (25 mg, 0.37 mmol, 6.9 equiv) was dissolved in dry DMF (0.3 mL) under Ar, follow by the addition of TBDPSCl (32  $\mu$ L, 0.11 mmol, 2.0 equiv). The reaction flask was immersed in a 70 °C oil bath and stirred for 24 h. The content was then dilute with EtOAc (15 mL) at rt, then successively washed with DI water (3x3 mL) and brine (3 mL). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under vacuum. Gradient chromatography (EtOAc:Hex, 10:90~15:85) afforded desired **4.79** (32 mg, 0.038 mmol, 70%, R<sub>f</sub>=0.43 in 20:80 EtOAc:Hex) as colorless oil. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 4.79 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.80** 

Alkene **4.79** (32 mg, 0.038 mmol, 1 equiv) was dissolved in 4:1 acetone/water (1 mL) and treated with 4% aqueous OsO<sub>4</sub> solution (1 drop, *ca.* 10  $\mu$ L, 3  $\mu$ mol, 0.04 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (2 drops, *ca.* 20  $\mu$ L, 0.10 mol, 3 equiv). The reaction was stirred for 23 h, then DI water (1 mL), EtOAc (2 mL), and solid Na<sub>2</sub>SO<sub>3</sub> (*ca.* 30 mg) were added, and the mixture was stirred for another 30 min, then it was extracted with EtOAc (13 mL). The extract was washed with 0.1 M HCl (5 mL) and brine (3 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give crude **4.80** (34 mg, quantitative conversion, R<sub>f</sub>=0.26 in 30:70 EtOAc:Hex) as a colorless film, which was employed in the next step without further purification. <sup>1</sup>H NMR spectrum of the crude product is attached.

## <sup>1</sup>H NMR spectrum of 4.80 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.81** 

Crude **4.80** (34 mg) obtained as described above was dissolved in 2,2-dimethoxypropane (0.7 mL) and treated with TsOHH<sub>2</sub>O (*ca.* 3 mg, 0.015 mmol, 0.5 equiv) and the solution was stirred for 10 min. The mixture was quenched with aq. sat. NaHCO<sub>3</sub> solution (3 mL) then stirred for another 15 min. The solution was extracted with EtOAc (15 mL) and the organic layer was washed with brine (3 mL) dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated to yield crude **4.81**. Gradient chromatography (EtOAc:Hex, 10:90~15:85) afforded desired **4.81** (30 mg, 0.032 mmol, 85% over 2 steps,  $R_f$ =0.34 in 20:80 EtOAc:Hex) as colorless oil. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 4.81 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.82** 

**4.77** (35 mg, 0.047 mmol, 1 equiv) and DMAP (5 mg, 0.041 mmol, 0.9 equiv) was dissolved in THF (0.7 mL), follow by the addition of solid Boc<sub>2</sub>O (25 mg, 0.11 mmol, 2.4 equiv). After 90 min, the solution was poured into sat. NH<sub>4</sub>Cl (5 mL), extracted with ethyl acetate (15 mL). The organic layer was successively washed with DI water (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated *in vacuo*. Flash chromatography (EtOAc:Hex, 10:90) thus provided pure **4.82** (32 mg, 0.038 mmol, 81%,  $R_f$ =0.50 in 20:80 EtOAc:Hex) as colorless oil. <sup>1</sup>H NMR spectrum is attached.







**Preparation of 4.92** 

4.82 (32 mg, 0.038 mmol, 1 equiv) was dissolved in dry THF (1.2 mL), then LiBH<sub>4</sub> (ca. 2-3 mg, excess) was added. The mixture was stirred at 36 % for 48 h and guenched by addition of aq. sat. NH<sub>4</sub>Cl (6 mL; CAUTION: evolution of flammable H<sub>2</sub> gas). When gas evolution subsided, the solution was extracted with EtOAc (15 mL). The organic layer was successively washed with DI water (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. Flash chromatography (EtOAc:Hex, 20:80) afforded an inseparable mixture of alcohol 4.83 and 4.77(19 mg, 3:1,  $R_f=0.40$ in 30:70 EtOAc:Hex). This mixture was dissolved in 4:1 acetone/water (0.8 mL) and treated with 4% aqueous OsO<sub>4</sub> solution (1 drop, ca. 10 µL, 3 µmol, 0.07 equiv) and 50% aqueous 4-methylmorpholine N-oxide solution (2 drops, ca. 20 µL, 0.10 mol, 3 equiv). The reaction was proceeded for 22 h, then DI water (1 mL), EtOAc (2 mL), and solid Na<sub>2</sub>SO<sub>3</sub> (ca. 30 mg) were added, and the biphasic mixture was stirred for another 30 min, then it was extracted with EtOAc (10 mL). The extract was washed with sat. NH<sub>4</sub>Cl (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated in vacuo. Flash chromatography (EtOAc:Hex, 50:50) furnished triol 4.92 (13 mg, 0.017 mmol, 46% over 2 steps,  $R_f=0.21$  in 50:50 EtOAc:Hex) as a white solid. <sup>1</sup>H NMR spectrum is attached.

# <sup>1</sup>H NMR spectrum of 4.92 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.95** 

Triol **4.92** (13 mg, 0.017 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (0.7 mL) and treated with TsOHH<sub>2</sub>O (*ca.* 1 mg, 0.005 mmol, 0.3 equiv) and the solution was stirred for 1 h. The mixture was quenched with aq. sat. NaHCO<sub>3</sub> solution (3 mL) then stirred for another 15 min. The solution was extracted with EtOAc (15 mL) and the ester layer was successively washed with sat. NH<sub>4</sub>Cl (3 mL), 0.1 M HCl (3 mL) and brine (3 mL), dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated to yield a mixture of **4.94** and **4.95** (*ca.* 1:1 mol/mol). This crude product was then dissolved in methanol (0.8 mL) treated with PPTS (*ca.* 1mg) and stirred for 3 min. The reaction mixture was poured into sat. NaHCO<sub>3</sub> (5 mL) and extracted with EtOAc (15 mL). The organic layer was washed with brine (5 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The residue was adsorbed on a silica gel column to perform a flash chromatography (EtOAc:Hex, 30:70) and gave **4.95** (12 mg, 0.015 mmol, 88%, R<sub>f</sub>=0.20 in 30:70 EtOAc:Hex) as colorless oil. <sup>1</sup>H and <sup>13</sup>C NMR spectra are attached.

### <sup>1</sup>H NMR spectrum of 4.95 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.95 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.107** 

Diol 4.102 (5 mg, 0.0089 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1.1 mL) under argon, cool to 0 °C and treated with solid Dess-Martin periodinane (5 mg, 0.012mmol, 1.3 equiv) added in one portion. The solution was warmed to rt and stirring for 50 min and quenched by addition of a 1:3 (vol/vol) mixture of aq. sat. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and aq. sat. NaHCO<sub>3</sub> solutions (3 mL). The solution was diluted with EtOAc (10 mL), and the aqueous phase was drained. The organic layer was washed with aq. sat. NaHCO<sub>3</sub> solution (3 mL) and brine (3 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure to yield a crude containing about  $30 \sim 40\%$  of **4.106** (5 mg, R<sub>f</sub>=0.56 in 30:70 EtOAc:Hex), which was used for the next step without further purification. This crude product obtained was dissolved in distilled 1,2-DCE (0.2 mL) and treated with Et<sub>3</sub>N (40 µL, 0.28 mmol, 32 equiv), then MsCl (7 µL, 0.09 mmol, 10 equiv) was added. A large amount of precipitate appeared immediately. This mixture was stirred at rt for 60 min and 40 °C for 30 min then poured into a mixture of DI water (3 mL) and EtOAc (15 mL). Aqueous layer was drained and organic layer was successively washed with DI water (3 mL) and brine (3 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. Gradient chromatography (EtOAc:Hex, 10:90~20:80) afforded desired 4.107 (ca. 2 mg, 0.0037 mmol, ca. 40% over 2 steps,  $R_f=0.50$  in 20:80 EtOAc:Hex) as colorless film. <sup>1</sup>H NMR spectrum is attached. <sup>1</sup>H NMR spectrum of a purified **4.106** sample is also available.

### <sup>1</sup>H NMR spectrum of 4.106 (300 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of 4.107 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.108** 

Dihydropyran **4.107** (2 mg, 0.0037 mmol, 1 equiv) was dissolved in 4:1 acetone/water (0.5 mL) and treated with 4% aqueous  $OsO_4$  solution (1 drop, *ca.* 10 µL, 1.5 µmol, 0.4 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (2 drops, excess). The reaction was stirred for 10 d, then DI water (2 mL), EtOAc (2 mL), and solid  $Na_2SO_3$  (*ca.* 30 mg) were added, and the mixture was stirred for another 30 min. The mixture was extracted with EtOAc (10 mL). The extract was washed with DI water (3 mL) and brine (3 mL), dried ( $Na_2SO_4$ ) and evaporated *in vacuo* to yield a non-fully converted material. Gradient chromatography (EtOAc:Hex, 20:80~30:70) furnished lactol **4.108** (1 mg, 0.0017 mmol, 50%,  $R_f$ =0.27 in 30:70 EtOAc:Hex) as white solid. <sup>1</sup>H, COSY, HSQC and 1D NOE NMR spectra are attached.



# <sup>1</sup>H NMR spectrum of 4.108 (400 MHz, CDCl<sub>3</sub>)

#### Expansion of COSY spectrum of 4.108 (CDCl<sub>3</sub>)



Expansion of HSQC spectrum of 4.108 (CDCl<sub>3</sub>)





NOE spectrum of 4.108, irradiation on C10-H (400 MHz, CDCl<sub>3</sub>)

NOE spectrum of 4.108, irradiation on C9-H (400 MHz, CDCl<sub>3</sub>)





Preparation of 4.110

Lactol **4.108** (1 mg, 0.0017 mmol, 1 equiv) was dissolved in DCM (0.25 mL) at 0  $^{\circ}$  C, followed by the addition of 50% aqueous 4-methylmorpholine *N*-oxide solution (0.5 drops, excess). Solid TPAP (1 grain) was loaded on a metal spatula tip and rinsed into the reaction flask with DCM (0.2 mL). EtOH (20 µL) was added to quench the reaction after 90 min. The solution was directly loaded on a short silica gel plug (*ca.* 1 mL) and eluted with 20:80 EtOAc/Hex until no product was detected. Flash chromatography (EtOAc:Hex, 10:90) furnished **4.110** (<1 mg,  $R_f$ =0.45 in 20:80 EtOAc:Hex). <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 4.110 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 4.111** 

Lactol **4.108** (2 mg, 0.0035 mmol, 1 equiv) was dissolved in 1,2-DCE (0.15 mL) in a 1 mL sealable vessel under argon and treated with Et<sub>3</sub>N (6  $\mu$ L, 0.043 mol, 12 equiv), followed by addition of EtOCH<sub>2</sub>Cl (2  $\mu$ L, 0.022 mmol, 6 equiv). The reaction vessel was capped and stirred for 41 h at 60 ° C. After the solution was cooled to rt, it was directly adsorbed onto a silica gel column and eluted with 10:90 EtOAc/Hex, to yield **4.111** (1 mg, 0.0016 mmol, 46%, R<sub>f</sub>=0.46 in 30:70 EtOAc:Hex) as colorless film and return **4.108** (1 mg, 50%). <sup>1</sup>H, COSY and HSQC NMR spectra are attached.



<sup>1</sup>H NMR spectrum of 4.111 (300 MHz, CDCl<sub>3</sub>)



Expansion of COSY spectrum of 4.111 (CDCl<sub>3</sub>)

Expansion of HSQC spectrum of 4.111 (CDCl<sub>3</sub>)





**Preparation of 4.115** 

Lactone **4.104** (4 mg, 0.0072 mmol, 1 equiv) was dissolved in dry THF (0.3 mL) at -78 ° C then a THF solution of LDA (0.2 M, 200 µL, 0.040 mmol, 5.5 equiv) was added dropwise. The enolate was stirred for another 1 h then D<sub>2</sub>O (30 µL, excess) was added dropwise. The solution was warmed to rt, diluted with hexanes (1.5 mL) and filtered through a short silica gel plug (*ca.* 1 mL) with 30:70 EtOAc/Hex until no product was detected. The solution was concentrated under vacuum to yield **4.115** (3 mg, 0.0054 mmol, 75%) An about 95% deuterium exchange was observed through <sup>1</sup>H NMR. The same operation to **4.115**, however, quenched with H<sub>2</sub>O returned **4.104**. Comparison of <sup>1</sup>H NMR in between **4.104** and **4.115** is attached. Expansion of <sup>1</sup>H NMR spectrum of 4.115 (bottom) with comparison to 4.104 (top) (300 MHz, CDCl<sub>3</sub>)



Expansion of COSY spectrum of 4.104 (CDCl<sub>3</sub>)



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**Preparation of 4.124** 

Compound **4.95** (12 mg, 0.015 mmol, 1 equiv) was dissolved in dry  $CH_2Cl_2$  (1 mL), and treated with solid Dess-Martin periodinane (10 mg, 0.023 mmol, 1.5 equiv) added in one portion. After stirring at rt for 2 h, the reaction was quenched by addition of a 3:7 (vol/vol) mixture of aq. sat.  $Na_2S_2O_3$  and aq. sat.  $NaHCO_3$  solutions (4 mL). The solution was diluted with EtOAc (15 mL), the aqueous phase was drained, and the EtOAc layer was successively washed with sat.  $NaHCO_3$  (4 mL) and brine (4 mL). The organic layer was dried ( $Na_2SO_4$ ) and evaporated under reduced pressure to yield crude **4.124** (12 mg, quantitative crude yield,  $R_f$ =0.63 in 30:70 EtOAc:Hex) in good quality, no further purification attempts were made. <sup>1</sup>H NMR spectrum of the crude product is attached.



<sup>1</sup>H NMR spectrum of 4.124 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 4.125** 

Solid (Ph<sub>3</sub>PMe)Br (38 mg, 0.11 mmol, 7.0 equiv) was suspended in dry THF (0.3 mL) at rt followed by the addition of *n*-BuLi (1.05 M in hexane, 60  $\mu$ L, 0.063 mmol, 4.2 equiv). The orange solution was stirred for 30 min before being cooled to -78 °C. Crude ketone **4.124** (12 mg, 0.015 mmol, 1 equiv) was dissolved in dry THF (0.3 mL) and added into the phosphonium ylide dropwise via a syringe. More THF (0.2 mL) was used to rinse the flask containing **4.124** and transferred to the reaction flask using the same syringe. This mixture was kept in Dry Ice/acetone bath for another 5 min before warmed to rt. After 0.5 h, this reaction was quenched by the addition of HOAc in THF (1 M, 1 mL). This mixture was poured into a separatory funnel containing EtOAc (15 mL), sat. NH<sub>4</sub>Cl (5 mL) and DI water (3 mL). The aqueous layer was drained and the organic layer was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. The residue contained NMR quality olefin **4.125** (8 mg, 0.010 mmol, 67%, R<sub>f</sub>=0.38 in 20:80 EtOAc:Hex) as off white oil, which was used without further purification. <sup>1</sup>H NMR spectrum is attached.



<sup>1</sup>H NMR spectrum of 4.125 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 4.129** 

Solid (Ph<sub>3</sub>PMe)Br (33 mg, 0.0.092 mmol, 7.1 equiv) was suspended in dry THF (0.3 mL) at rt followed by the addition of NaHMDS (1.0 M in hexane, 55  $\mu$ L, 0.055 mmol, 4.2 equiv). The orange solution was stirred for 30 min before being cooled to –78 °C. **4.126** (9 mg, 0.013 mmol, 1 equiv) was dissolved in dry THF (0.15 mL) and added into the phosphonium ylide dropwise via a syringe. The same syringe was used to transfer another THF (0.15 mL) rinse of the flask containing **4.126**. This mixture was kept in Dry Ice bath for another 15 min before warmed to rt. After 30 min, this reaction was quenched by the addition of 1 M HOAc in THF (0.10 mL). The solvents were removed under reduced pressure and the residue was purified by flash chromatography (EtOAc:Hex, 10:90) to furnish pure diene **4.45** (7 mg, 0.013 mmol, quant., R<sub>f</sub>=0.37 in 20:80 EtOAc:Hex). <sup>1</sup>H and <sup>13</sup>CNMR spectra are attached.

<sup>1</sup>H NMR spectrum of 4.129 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 4.129 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 4.133** 

Alkene **4.125** thus obtained as above (8 mg, 0.010 mmol, 1 equiv) was dissolved in dry THF (0.2 mL), the flask containing the solution was cooled to -78 °C (Ar atmosphere) and liquid NH<sub>3</sub> (*ca.* 1 mL) was condensed into the same flask. Metallic sodium (13 mg, 0.57 mmol, 57 equiv) was added in to the reaction flask with vigorous stirring. After 15 sec, the solution turned dark blue and was maintained stirring at -78 °C for another 12 min followed by the addition of solid NH<sub>4</sub>Cl (*ca.* 30 mg). The resulting slurry was stirred for 5 min until the blue color disappeared, then the flask was removed from the Dry Ice bath and uncapped to allow NH<sub>3</sub> to evaporate. The residue was taken up with EtOAc and filtered through Celite<sup>®</sup> with more EtOAc. The concentrated material was subjected to flash chromatography (EtOAc:Hex, 40:60) with excessive amount of silica gel (10 mL) to produce pure **4.133** (2 mg, 0.0045 mmol, 45%, R<sub>f</sub>=0.32 in 50:50 EtOAc:Hex), with **4.132** (1 mg, 0.002 mmol, 22%). **4.133** was recrystallized in DCM/Hexs to produce a white solid. <sup>1</sup>H NMR spectrum is attached.



### <sup>1</sup>H NMR spectrum of 4.133 (300 MHz, CDCl<sub>3</sub>)



**Preparation of 4.135/1.127** 

Solid HgCl<sub>2</sub> (17 mg), 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (14 mg) were placed in a roun-bottom flask and dissolved in dry MeCN (0.48 mL). After 10 min, Et<sub>3</sub>N (20 µL) was added to the flask. White precipitate appeared immediately. The stirring was halted after 2 min and an aliquot (70 µL) of the supernatant was taken by a microsyringe, which contained tantamount of HgCl<sub>2</sub> (2.4)0.0087 mmol, 1.8 equiv), a mg, 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (2.0 mg, 0.0068 mmol, 1.4 equiv) and Et<sub>3</sub>N (2.8 µL, 0.020 mmol, 4 equiv). This solution was added into a DCM/MeCN (1:4, 0.5 mL) solution of amine 4.123 (2.0 mg, 0.0050 mmol, 1 equiv) and stirred for 30 min, followed by a workup with methanol (0.5 mL). This suspension was filtered though Celite<sup>®</sup> and the filtrate was concentrated under vacuum. Preparative TLC (EtOAc:Hex, 30:70) afforded a 2:1 mixture of 4.135 and 1.127 (30% conversion by <sup>1</sup>H NMR of the crude sample,  $R_f=0.18$  in 30:70 EtOAc:Hex) with **4.123** (*ca.* 1 mg) recovered. <sup>1</sup>H NMR spectrum is attached, and it is identical to that in Sato's report.<sup>33</sup>



<sup>1</sup>H NMR spectrum of 4.135 and 1.127 (*ca.* 2:1 mol/mol) (300 MHz, CDCl<sub>3</sub>)

Expansion of <sup>1</sup>H NMR spectrum of 4.135/1.127 (ca. 2:1 mol/mol) (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.14** 

Ketone **5.13**<sup>124</sup>(155 mg, 0.50 mmol, 1 equiv) was dissolved in commercial MeOH (5 mL) then NaBH<sub>4</sub> (58 mg, 1.5 mmol, 3.0 equiv) was added to the solution. After 10 min, the reaction was quenched by careful dropwise addition of sat. NH<sub>4</sub>Cl (2 mL; **CAUTION**: H<sub>2</sub> evolution). Upon the depletion of bubbles, EtOAc (15 mL) and DI water (3 mL) was added. The organic layer was separated, washed successively with sat. NH<sub>4</sub>Cl (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvents were removed *in vacuo*. Purification by chromatography (gradient EtOAc:Hex, 20:80~50:50) provided **5.14** (68 mg, 0.22 mmol, 44%, R<sub>f</sub>=0.38 in 20:80 EtOAc:Hex) as a white wax. <sup>1</sup>H and <sup>13</sup>C NMR spectra of the crude product are attached.

### <sup>1</sup>H NMR spectrum of 5.14 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 5.14 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 5.15** 

Alcohol **5.14** (68 mg, 0.22 mmol, 1 equiv) was dissolved in dry DCM (2 mL), followed by the treatment of TsNCO (40  $\mu$ L, 0.26 mmol, 1.2 equiv). The content of was concentrated after 2 h and purified by flash chromatography (EtOAc:Hex, 10:90) to furnish **5.15** (100 mg, 0.20 mmol, 90%, R<sub>f</sub>=0.11 in 20:80 EtOAc:Hex, streak) as colorless oil. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 5.15 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.16** 

Ester **5.15** (100 mg, 0.20 mmol, 1 equiv) was dissolved in THF (5 mL) and treated with 1.0 M acetic acid solution in THF (250  $\mu$ L, 0.25 mmol, 1.3 equiv) followed by dropwise addition of commercial 1.0 M TBAF solution in THF (250  $\mu$ L, 0.25 mmol, 1.3 equiv). After stirring at rt for 5 h, the solvents were evaporated and the crude was loaded on a silica gel plug (3 mL). The silica gel was eluted first with EtOAc (50 mL), then 1% HOAc in EtOAc (25 mL). The latter eluate was concentrated to produce phenol **5.16** (64 mg, 0.16 mmol, 80%, R<sub>f</sub>=0.07 in 50:50 EtOAc:Hex, streak) as colorless oil. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 5.16 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.20** 

Coumaric acid derivative **5.19** (325 mg, 1.47 mmol, 1 equiv) and citric acid (284 mg, 1.48 mg, 1.0 equiv) were dissolved in 2:1 acetone/water (10 mL) and treated with 4% aqueous OsO<sub>4</sub> solution (2 drops, *ca.* 20  $\mu$ L, 3  $\mu$ mol, 0.002 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (0.365 mL, 1.78 mmol, 1.2 equiv). The reaction was stirred for 41 h, then and solid Na<sub>2</sub>SO<sub>3</sub> (93 mg, 0.74 mmol, 0.5 equiv) was added, and the mixture was stirred for another 30 min and concentrated *in vacuo*. The crude product was dissolved in EtOAc (30 mL) and washed with sat. NH<sub>4</sub>Cl (10 mL). The organic layer was collected and the aqueous layer was successively extracted with EtOAc (5x10 mL). The combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to yield crude **5.20** (380 mg, R<sub>f</sub>=0.20 in 50:50 EtOAc:Hex) as a white solid. <sup>1</sup>H NMR spectrum of the crude product is attached.
## <sup>1</sup>H NMR spectrum of 5.20 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.21** 

Crude **5.20** (380 mg) obtained as described above was dissolved in 2,2-dimethoxypropane (10 mL) and DCM (5 mL) and treated with TsOH'H<sub>2</sub>O (*ca.* 5 mg, 0.026 mmol, 0.02 equiv) and the solution was stirred for 4 h, during which time the solid slowly disappeared. The mixture was quenched with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and DI water (5 mL) then stirred for another 30 min. The solution was extracted with EtOAc (15 mL) and the ester layer was washed with brine (5 mL), dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated. The crude compound was loaded on a silica gel plug (7 mL) then flushed with 30:70 EtOAc/Hexs (60 mL). The eluate was concentrated to furnish NMR quality crude **5.21** (440 mg, 1.50 mmol, quant. crude yield,  $R_f$ =0.45 in 30:70 EtOAc:Hex) as white was. <sup>1</sup>H and <sup>13</sup>C NMR spectra of the crude product are attached.

<sup>1</sup>H NMR spectrum of 5.21 (300 MHz, CDCl<sub>3</sub>)



<sup>13</sup>C NMR spectrum of 5.21 (75 MHz, CDCl<sub>3</sub>)





**Preparation of 5.24** 

Ester 5.21 (58 mg, 0.20 mmol, 1 equiv) was dissolved in dry DCM (1.5 mL) under argon atmosphere and the solution was immersed in a Dry Ice/acetone bath (-78 °C). DIBAL (1.0 M in hexanes, 0.80 mL, 0.80 mmol, 4.0 equiv) was slowly added to the solution within 7 mins, followed by 53 min more contact time at the same temperature. Glacial AcOH (3 drops) was added to aq. sat. potassium sodium tartrate (1 mL) and the mixture was slowly added to the reaction mixture using a glass dropper, followed by careful addition of DI water (1 mL), MeOH (1 mL) and EtOAc (2 mL). This solution was warmed to rt and aq. sat. potassium sodium tartrate (1 mL) was applied. The mixture turned transparent after 1 h of agitation, then was extracted with EtOAc (20 mL). The aqueous layer was discarded and the organic layer was successively washed with sat. NH<sub>4</sub>Cl solution (10 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo*. The residue was loaded on a short silica gel plug (ca. 2 mL) then eluted with 50:50 EtOAc:Hex (25 mL). Concentration of the eluate furnished the crude aldehyde 5.22 and hydrate 5.23 (48 mg, R<sub>f</sub>=0.32 in 50:50 EtOAc:Hex, streak). This crude product was dissolved in MeOH (2.5 mL), followed by the addition of solid K<sub>2</sub>CO<sub>3</sub> (54 mg, 0.39 mmol, 2 equiv) and hydroxylamine hydrochloride (11 mg, 0.16 mmol, 0.8 equiv) at rt. After 18 h, the solvents were evaporated and the residue was redissolved in EtOAc (15 mL), washed with 0.1 M HCl (10 mL), DI water (5 mL) and brine (5 mL). The organic layer was dried with  $Na_2SO_4$ , filtered and concentrated to yield crude oxime **5.24**, which was recrystallized in DCM/Hex to provide **5.24** (32 mg, 0.14 mmol, 68%,  $R_f$ =0.68 in 70:30 EtOAc:Hex) as white solid. <sup>1</sup>H NMR spectrum is attached.



<sup>1</sup>H NMR spectrum of 5.24 (300 MHz, acetone-*d6*)



**Preparation of 5.28** 

Ester **5.21** (50 mg, 0.17 mmol, 1 equiv) was dissolved in MeOH (10 mL) and DI water (0.25 mL) and treated with solid NaHCO<sub>3</sub> (100 mg, 1.19 mmol, 7 equiv) and the suspension was stirred for 16 h. The reaction mixture was diluted with DI water (9 mL) and brine (1 mL) then extracted with EtOAc (10 mL). The aqueous layer was discarded and organic layer was washed with brine (3 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, then concentrated on rotatory evaporator to yield crude **5.28** (43 mg, 0.17 mmol, quantitative conversion,  $R_f$ =0.36 in 30:70 EtOAc:Hex) of reasonable purity by NMR analysis as a colorless oil. <sup>1</sup>H NMR spectrum of the crude product is attached.

<sup>1</sup>H NMR spectrum of 5.28 (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.34** 

Crude acid **4.27** (425 mg, 0.84 mmol, 1 equiv), (–)-menthol (218 mg, 1.40 mmol, 1.7 equiv) and DMAP (10 mg, 0.082 mmol, 0.10 equiv) was dissolved in dry DCM (4 mL) under argon atmosphere and immersed in an ice bath (0 °C). With good agitation, solid DCC (224 mg, 1.09 mmol, 1.3 equiv) was added in three portions (98+90+36 mg, every 1 min). The cloudy solution was stirred at 0 °C for 20 min, and then the flask was allowed to be warmed to rt, followed by 19 h more reaction period. The solution was diluted with hexanes (10 mL) then filtered through a short plug of Celite<sup>®</sup> and the filtrate was concentrated *in vacuo*. Flash chromatography (EtOAc:Hex, 5:95) yielded ester **5.34** (495 mg, 0.77 mmol, 91%,  $R_f$ =0.45 in 10:90 EtOAc:Hex) as colorless oil. Notably, this material should be advanced to the next reaction immediately to avoid possible decomposition. <sup>1</sup>H NMR spectrum of the crude is attached.



## <sup>1</sup>H NMR spectrum of 5.34 (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.35/5.36

Aqueous solutions of  $OsO_4$  (4% w/v, 170 µL, 27 µmol, 0.030 equiv) and 4-methylmorpholine N-oxide (50% w/w, 220 µL, 1.07 mmol, 1.2 equiv) were added to a suspension of 5.34 (570 mg, 0.88 mmol, 1 equiv) and anhydrous citric acid (170 mg, 0.89 mmol, 1.0 equiv) in 4:1 acetone:water (12 mL) in an Erlenmever flask without ground glass joint equipped with plastic cap. No stirring bar was necessary. More acetone (2.0 mL) was added to generate a homogeneous solution. The reaction was stirred at rt for 72 h and quenched by the addition of solid Na<sub>2</sub>SO<sub>3</sub> (220 mg) and DI water (10 mL). The solution was stirred for 1 h, extracted with EtOAc (30 mL) and the organic layer was sequentially washed with aq. sat. NH<sub>4</sub>Cl solution (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum. The crude product was dissolved in hexanes (4 mL) then store at -20 °C overnight. White solid formed and the mother liquor was decanted. The remaining solid was washed with hexanes (3x2 mL) to yield a mixture of 5.35 and 5.36 (125 mg, 1:1). The mother liquor and rinsed were combined, evaporated and subjected to a gradient chromatography (EtOAc:Hex, 5:95~8:92~10:90~15:85) to provide more of the mixture of 5.35 and 5.36 (125 mg, 1:1) and recovery of impure starting material 5.34 (167 mg). In total, a mixture of **5.35** and **5.36** (250 mg, 0.37 mmol, 42%, R<sub>f</sub>=0.33 in 20:80 EtOAc:Hex) was obtained through this reaction as white prisms. <sup>1</sup>H NMR spectrum is attached.



<sup>1</sup>H NMR spectrum of 5.35 and 5.36 (1:1) (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.39/5.40

To a pyridine (0.3 mL) solution of **5.35** and **5.36** (1:1, 15 mg, 0.022 mmol, 1 equiv) and DMAP (1 mg, 0.008 mmol, 0.4 equiv) was added solid 4-bromobenzoyl chloride (32 mg, 0.15 mmol, 6.6 equiv). The resulting suspension was immersed into an oil bath maintained at 35 °C for 42 h, then quenched by the addition of DI water (2 mL). The content was poured into a mixture of EtOAc (15 mL) and sat. NaHCO<sub>3</sub> (5 mL), partitioned and the aqueous layer was discarded. The organic layer was then washed with brine (5 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under vacuum. The crude material was then subjected to a gradient chromatography (EtOAc:Hex, 5:95~10:90) to yield a 1:1 mixture of **5.39** and **5.40** (13 mg, 0.013 mmol, 60%,  $R_f$ =0.57 in 10:90 EtOAc:Hex) that was recrystalized in MeOH/H<sub>2</sub>O as colorless prisms. <sup>1</sup>H spectrum is attached.



<sup>1</sup>H NMR spectrum of 5.39 and 5.40 (1:1, contains *ca*. 30% EtOAc) (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.47 and its diastereomer

A mixture of **5.35** and **5.36** (1:1, 75 mg, 0.11 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (2.5 mL) and treated with TsOHH<sub>2</sub>O (*ca.* 2 mg, 0.01 mmol, 0.1 equiv) and the solution was stirred for 50 min then quenched with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and DI water (5 mL). After 10 min, the solution was extracted with EtOAc (10 mL) and the ester layer was washed with brine (5 mL), dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated to furnish NMR quality crude **5.47** and its diastereomer (78 mg, 0.11 mmol, quant. crude yield,  $R_f$ =0.38 in 10:90 EtOAc:Hex) as colorless oil. <sup>1</sup>H NMR spectrum of the crude product is attached.

<sup>1</sup>H NMR spectrum of 5.47 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)





**Preparation of 5.49** 

Diol **5.30** (25 mg, 0.042 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (1.5 mL) and treated with TsOH'H<sub>2</sub>O (*ca.* 1 mg, 0.005 mmol, 0.1 equiv) and the solution was stirred for 2.5 h then quenched with aq. sat. NaHCO<sub>3</sub> solution (5 mL) and DI water (5 mL). After 10 min, the solution was extracted with EtOAc (10 mL) and the ester layer was washed with brine (5 mL), dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated. <sup>1</sup>H NMR of the crude product showed a 3:3:1 ratio of **5.49**, **5.31** and **5.32**. Flash chromatography (EtOAc:Hex, 10:90) yielded **5.49** (10 mg, 0.016 mmol, 37%,  $R_f$ =0.52 in 30:70 EtOAc:Hex) as colorless oil. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 5.49 (300 MHz, CDCl<sub>3</sub>)





Preparation of 5.50 and its diastereomer

A 1:1 mixture of **5.47** and its diastereomer (32 mg, 0.044 mmol, 1 equiv) was dissolved in THF (2 mL) and treated with 1.0 M acetic acid solution in THF (100  $\mu$ L, 0.100 mmol, 2.3 equiv) followed by dropwise addition of commercial 1.0 M TBAF solution in THF (100  $\mu$ L, 0.100 mmol, 2.3 equiv). After stirring at rt for 7 h, the solvents were evaporated and the residue was loaded on silica gel column (EtOAc:Hex, 20:80), producing 1:1 mixture **5.50** and its diastereomer (16 mg, 0.033 mmol, 74%, R<sub>f</sub>=0.13 in 20:80 EtOAc:Hex) as white foam. <sup>1</sup>H NMR spectrum is attached.

<sup>1</sup>H NMR spectrum of 5.50 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)





Preparation of 5.51 and its diastereomer

Enol **5.50** and its diastereomer (16 mg, 0.033 mmol, 1 equiv) was dissolved in dry  $CH_2Cl_2$  (1 mL), and treated with solid Dess-Martin periodinane (18 mg, 0.042 mmol, 1.3 equiv). After stirring at rt for 4 h, the reaction was quenched by addition of a 3:7 (vol/vol) mixture of aq. sat. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and aq. sat. NaHCO<sub>3</sub> solutions (3 mL), whereupon the mixture became clear. The solution was diluted with EtOAc (10 mL), and the aqueous phase was drained. The organic layer was sequentially washed with sat. NaHCO<sub>3</sub> (3 mL), brine (3 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure to yield crude 1:1 mixture of **5.51** and its diastereomer (16 mg, quantitative crude yield,  $R_f$ =0.41 in 20:80 EtOAc:Hex) in good quality, no further purification attempts were made. <sup>1</sup>H NMR spectrum of the crude product is attached.



<sup>1</sup>H NMR spectrum of 5.51 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.52 and its diastereomer

Solid (Ph<sub>3</sub>PMe)Br (83 mg, 0.23 mmol, 7.0 equiv) was suspended in dry THF (1 mL) at rt followed by the addition of *n*-BuLi (1.60 M in hexane, 83  $\mu$ L, 0.13 mmol, 4.0 equiv). The orange solution was stirred for 30 min before being cooled to –78 °C. Crude ketone **5.51** and its diastereomer (16 mg, 0.033 mmol, 1 equiv) was dissolved in dry THF (0.2 mL) and added into the phosphonium ylide dropwise via a syringe. More THF (0.2+0.1 mL) was used to quantitatively transfer the ketones. This mixture was kept in Dry Ice/acetone bath for another 30 min before warmed to 0 °C. After 15 min, this reaction was quenched by the addition of sat. NH<sub>4</sub>Cl solution (1.5 mL) and DI water (1.5 mL). EtOAc (10 mL) was used to extract this solution and the organic layer was successively washed with water (3 mL) and brine (3 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. The crude was loaded on a short silica gel plug (*ca.* 2 mL) and elute with 20:80 EtOAc/Hex (10 mL) to produce 1:1 mixture of **5.52** and its diastereomer (10 mg, 0.021 mmol, 64%, R<sub>f</sub>=0.61 in 20:80 EtOAc:Hex) of good quality (by NMR analysis) as a colorless oil. <sup>1</sup>H NMR spectrum of the crude product is attached.



# <sup>1</sup>H NMR spectrum of 5.52 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.53 and its diastereomer

A 1:1 mixture of crude diene **5.52** and its diastereomer (10 mg, 0.021 mmol, 1 equiv) was dissolved in 4:1 acetone/water (1.2 mL) and treated with 4% aqueous  $OsO_4$  solution (1 drop, *ca.* 10  $\mu$ L, 1.6  $\mu$ mol, 0.08 equiv) and 50% aqueous 4-methylmorpholine *N*-oxide solution (1 drop, *ca.* 10  $\mu$ L, 0.049 mmol, 2 equiv). The reaction was stirred for 52 h, then water (3 mL) and solid Na<sub>2</sub>SO<sub>3</sub> (*ca.* 10 mg) were added, and the mixture was stirred for another 45 min, then it was extracted with EtOAc (10 mL). The organic extract was washed with sat. NH<sub>4</sub>Cl (5 mL) and brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo*. Gradient chromatography (EtOAc:Hex, 20:80~30:70) provided a mixture of **5.53** and its diastereomer (8 mg, 0.016 mmol, 76%, R<sub>f</sub>=0.25 in 30:70 EtOAc:Hex) as white solid. <sup>1</sup>H NMR spectrum of the crude product is attached.



# <sup>1</sup>H NMR spectrum of 5.53 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)



Preparation of 5.54 and its diastereomer

A 1:1 mixture of **5.53** and its diastereomer (8 mg, 0.016 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (1 mL) and treated with TsOHH<sub>2</sub>O (*ca.* 1 mg, 0.005 mmol, 0.3 equiv) and the solution was stirred for 60 min then quenched with aq. sat. NaHCO<sub>3</sub> solution (5 mL). After 15 min, the solution was extracted with EtOAc (10 mL) and the ester layer was washed with brine (5 mL), dried with Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated to furnish crude 1:1 mixture of **5.54** and its diastereomer (8 mg, quant. crude yield,  $R_f$ =0.20 in 5:95 EtOAc:Hex) as a white wax. <sup>1</sup>H spectrum of the crude product is attached.



<sup>1</sup>H NMR spectrum of 5.54 and its diastereomer (1:1) (300 MHz, CDCl<sub>3</sub>)



**Preparation of 5.44** 

A 1:1 mixture of **5.54** and its diastereomer (8 mg, 0.015 mmol, 1 equiv) and LiOHH<sub>2</sub>O (12 mg, 0.29 mmol, 19 equiv) was dissolved in a mixture of THF (1 mL), DI water (1 mL) and MeOH (0.2 mL). The solution was warmed to 50 °C with a reflux condenser and allowed to be stirred for 5 d before being poured into 0.1 M HCl (8 mL), then extracted with EtOAc (15 mL). The aqueous layer was drained and the organic layer was sequentially washed with water (3 mL) and brine (3 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated on a rotatory evaporator. Crude **5.44** (5 mg, R<sub>f</sub>=0.12 in 20:80 EtOAc:Hex) was obtained contaminated with (–)-menthol (5:3 mol:mol). No further attempts were performed to purify the material. <sup>1</sup>H spectrum of the crude is attached.



<sup>1</sup>H NMR spectrum of 5.44 and L-menthol (*ca.* 1:0.6 mol/mol) (300 MHz, CDCl<sub>3</sub>)



#### **Preparation of 5.86**

A dry MeCN solution of **5.83** (9 mg, 0.017 mmol, 1 equiv) was treated with CDI (4.2 mg, 0.025 mmol, 1.5 equiv) under Ar. After 150 min, solid **5.85** (18 mg, 0.070 mmol, 4.0 equiv) was added and the reaction mixture was warmed to 40 °C. After 22 h, all solvents were removed *in vacuo* and loaded on a silica gel column. Gradient chromatography (EtOAc:Hex, 5:95~10:90~30:70) provided **5.86** with *ca*. 30 mol% unidentified contaminant (3 mg,  $R_f$ =0.85 in 20:80 EtOAc:Hex) as colorless film with recovered **5.85** (16 mg) and **5.83** (4 mg, 0.008 mmol, 44%). <sup>1</sup>H NMR spectrum is attached.



<sup>1</sup>H NMR spectrum of 5.86 with *ca.* 30 mol% unidentified contaminant (300 MHz, CDCl<sub>3</sub>)



**Preparation of 5.92** 

A stock aq. solution of NaClO (1.2 % Cl<sub>2</sub> equivalent) was prepared by diluting a concentrated aq. NaClO (13% Cl<sub>2</sub> equivalent, 0.90 mL) in a graduated cylinder (final vol. 10 mL). To a cold (0  $^{\circ}$ C) DCM (4 mL) solution of **5.90** (12 mg, 0.020 mmol, 1 equiv), an aliquot of dilute aq. NaClO (1.0 mL, 0.17 mmol Cl<sub>2</sub> equivalent, 8.5 equiv) was added via a syringe within 2 min. The reaction was allowed to be conducted at 0  $^{\circ}$ C for another 3 min and at rt for 20 min. All contents were transferred into a separatory funnel containing DI water (4 mL) followed by 3 successive extraction with DCM (3x5 mL). Combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under vacuum. <sup>1</sup>H NMR of the crude material revealed a 9:1 mixture of **5.92** and **5.83**, COSY revealed trace amount of **5.93**. These spectra are attached.



<sup>1</sup>H NMR spectrum of 5.92 (contains *ca.* 10 mol% 5.83 and trace 5.93) (300 MHz, CDCl<sub>3</sub>)

Expansion of COSY spectrum of 5.92 (contains ca. 10 mol% 5.83 and trace 5.93) (300 MHz,

CDCl<sub>3</sub>)





**Preparation of 5.94** 

To a DIB (3 mg, 0.009 mmol, 1.8 equiv) solution in dry methanol (2.5 mL), a methanol solution of **5.90** (3 mg, 0.005 mmol, 1 equiv) was added over 7 h with a syringe pump at rt, followed by another 1 h of contact time. The solution was then concentrated under vacuum, and the residue was subjected to a gradient chromatography (EtOAc:Hex, 10:90~20:80) to yield **5.94** (*ca.* 1 mg, 0.02 mmol, 30%,  $R_f$ =0.46 in 20:80 EtOAc:Hex) as colorless film. <sup>1</sup>H NMR spectrum is attached.





### Appendix C: Comparative NMR Data



Comparative NMR data for the revised structure of the Sato lactone, **4.123** 

Position	4.123 synthesized in this work		<b>4.123</b> synthesized by Sato and coworkers <sup>32</sup>	
	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm) <i>, J</i> (Hz)	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm) <i>, J</i> (Hz)
1	N/A	1.64 (br s, exchangeable)	N/A	1.75 (br s)
4	66.7	4.42 (multiplicity unidentifiable)	N/A	4.43 (t, <i>J</i> = 12.4)
		4.36 (dd, <i>J</i> = 11.9, 5.1)		4.35 (dd, <i>J</i> = 12.4, 5.5)
4a	43.3	2.64 (br s)	N/A	2.60 (dt, <i>J</i> =12.4, 5.5, 5.5)
5	70.2	3.80 (dd, <i>J</i> = 11.8, 5.0)	N/A	3.78 (dd, <i>J</i> = 12.4, 5.5)
5'	N/A	2.91 (d, <i>J</i> = 12.0)	N/A	2.96 (d, <i>J</i> = 12.4)
6	79.6	N/A	N/A	N/A
6'	111.7	N/A	N/A	N/A
6''a/6''b	26.2	1.41 (s)	N/A	1.40 (s)
	27.6	1.41 (s)		1.41 (s)
7	77.2	4.44 (multiplicity unidentifiable)	N/A	4.43 (dd, <i>J</i> = 6.9, 1.4)
7'	109.2	N/A	N/A	N/A
7"a/7"b	23.7	1.39 (s)	N/A	1.39 (s)
	25.7	1.48 (s)		1.48 (s)
8	78.1	4.59 (br s)	N/A	4.55 (d, <i>J</i> = 6.9)
8a	51.8	N/A	N/A	N/A
9	72.9	4.76 (s)	N/A	4.74 (s)
9'	96.3	5.01, 4.83 (AB q, J <sub>AB</sub> = 6.7)	N/A	5.00, 4.82 (AB q, J <sub>AB</sub> = 6.9)
9''	56.9	3.48 (s)	N/A	3.48 (s)
10	170.7	N/A	N/A	N/A
11	69.1	4.33, 4.23 (AB q, J <sub>AB</sub> = 9.7)	N/A	4.33, 4.24 (AB q, J <sub>AB</sub> = 9.6)



Comparative NMR data for the Du Bois intermediate, **1.109**.

Position	<b>1.109</b> synthesized in this work		<b>1.109</b> synthesized by Du Bois and Hinman <sup>31, 145</sup>	
	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm) <i>, J</i> (Hz)	<sup>13</sup> C (ppm)	<sup>1</sup> H (ppm) <i>, J</i> (Hz)
1	N/A	5.53 (s)	N/A	5.55 (s)
2	154.7	N/A	154.7	N/A
2′	79.9	N/A	79.9	N/A
2"	28.3	1.43 (s)	28.2	1.44 (s)
4	132.8	6.07 (ddd, <i>J</i> = 17.2, 10.3, 8.5)	132.7	6.08 (ddd, <i>J</i> = 17.2, 10.4, 8.7)
4'	119.4	5.33 (dt, <i>J</i> = 17.2, 1.1, 1.1)	119.3	5.36 (dt, <i>J</i> = 17.1, 1.3, 1.3)
		5.26 (app d, <i>J</i> = 10.4)		5.26 (dt, <i>J</i> = 10.4, 1.3, 1.3)
4a	36.7	3.85 (app d, <i>J</i> = 8.5)	36.8	3.83 (d, <i>J</i> = 8.5)
5	81.3	4.44 (app t, <i>J</i> = 1.1)	81.4	4.42 (t, <i>J</i> = 1.4)
6	78.5	N/A	78.5	N/A
6′	111.7	N/A	111.6	N/A
6"a/6"b	27.2	1.43 (s)	27.2	1.42 (s)
	25.9	1.41 (s)	25.9	1.40 (s)
7	79.0	4.35 (dd, <i>J</i> = 6.4, 1.4)	79.0	4.34 (dd, <i>J</i> = 6.5, 1.6)
7′	109.4	N/A	109.4	N/A
7"a	24.8	1.36 (s)	24.8	1.35 (s)
7‴b	24.8	1.49 (s)	24.7	1.48 (s)
8	74.3	5.36 (d, <i>J</i> = 6.4)	74.2	5.36 (d, <i>J</i> = 6.3)
8a	57.5	N/A	57.4	N/A
9	66.9	4.49 (dd, <i>J</i> = 5.0, 0.9)	66.8	4.48 (dd, <i>J</i> = 4.1, 1.0)
9′	N/A	2.93 (d, <i>J</i> = 5.0)	N/A	3.35 (br t, <i>J</i> = 4.7)
10	169.5	N/A	169.7	N/A
11	68.7	4.39, 4.19 (AB q, J <sub>AB</sub> = 10.0)	68.6	4.38, 4.18 (AB q, J <sub>AB</sub> = 9.9)

#### Appendix D: X-ray Crystal Data

X-ray crystal data of 4.25



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation

Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on  $F^2$ , all data): R1; wR2 Residuals (refined on  $F^2$ ): R1; wR2 Goodness of fit indicator C<sub>32</sub>H<sub>41</sub>NO<sub>6</sub>Si 563.75 colorless, prism 0.15 x 0.24 x 0.35 mm triclinic primitive a = 8.9300(5) Å b = 12.8309(7) Å c = 14.2483(8) Å $\alpha = 97.358(3)^{\circ}$  $\beta = 92.479(3)^{\circ}$  $\gamma = 107.783(3)^{\circ}$  $V = 1535.9(2) Å^3$ *P*-1 (#2) 2  $1.219 \text{ g/cm}^3$ 604.00  $1.20 \text{ cm}^{-1}$ Bruker X8 APEX II MoKα ( $\lambda = 0.71073$  Å) graphite monochromated 3091 exposures @ 10.0 seconds 39.70 mm 60.2° Total: 17699 Unique: 8981 ( $R_{int} = 0.034$ ) 0.050; 0.098 0.034; 0.079 1.03

#### X-ray crystal data of 4.122



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on  $F^2$ , all data): R1; wR2 Residuals (refined on  $F^2$ ): R1; wR2 Goodness of fit indicator C<sub>19</sub>H<sub>27</sub>NO<sub>10</sub> 429.41 colorless, plate 0.02 x 0.10 x 0.10 mm triclinic primitive a = 10.522(2) Åb = 10.667(2) Åc = 10.716(2) Å $\alpha = 74.105(4)^{\circ}$  $\beta = 73.746(4)^{\circ}$  $\gamma = 61.845(4)^{\circ}$  $V = 1003.6(3) Å^3$ *P*-1 (#2) 2  $1.421 \text{ g/cm}^3$ 456.00  $1.16 \text{ cm}^{-1}$ Bruker APEX DUO MoKα ( $\lambda = 0.71073$  Å) 2115 exposures @ 15.0 seconds 38.15 mm 60.2° Total: 22447 Unique: 5879 ( $R_{int} = 0.039$ ) 0.064; 0.115 0.043; 0.104 1.01

#### X-ray crystal data of 4.123



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation

Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on F<sup>2</sup>, all data): R1; wR2 Residuals (refined on F<sup>2</sup>): R1; wR2 Goodness of fit indicator  $C_{18}H_{29}NO_9$ 403.42 colorless, irregular 0.10 x 0.12 x 0.20 mm triclinic primitive a = 8.6171(5) Å b = 10.2536(6) Å c = 11.1944(6) Å $\alpha = 76.353(2)^{\circ}$  $\beta = 79.048(3)^{\circ}$  $\gamma = 78.430(3)^{\circ}$  $V = 931.01(9) Å^3$ *P*-1 (#2) 2  $1.439 \text{ g/cm}^3$ 432.00  $1.15 \text{ cm}^{-1}$ Bruker X8 APEX II MoKα ( $\lambda = 0.71073$  Å) graphite monochromated 2810 exposures @ 10.0 seconds 39.76 mm  $60.2^{\circ}$ Total: 29972 Unique: 5475 ( $R_{int} = 0.023$ ) 0.042; 0.095 0.035; 0.091 1.05

#### X-ray crystal data of 4.133



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on F<sup>2</sup>, all data): R1; wR2 Residuals (refined on F<sup>2</sup>): R1; wR2 Goodness of fit indicator  $C_{22}H_{39}NO_8$ 445.54 colorless, irregular 0.13 x 0.37 x 0.64 mm monoclinic primitive a = 10.7342(7) Å b = 23.158(2) Åc = 19.6917(1) Å $\alpha = 90^{\circ}$  $\beta = 101.797(4)^{\circ}$  $\gamma = 90^{\circ}$  $V = 4791.6(6) \text{ Å}^3$ *P* 21/*n* (#14) 8  $1.235 \text{ g/cm}^3$ 1936.00  $7.69 \text{ cm}^{-1}$ Bruker APEX DUO MoKα ( $\lambda = 0.71073$  Å) 2984 exposures @ 60.0 seconds 49.80 mm 112.5° Total: 61147 Unique: 6243 (R<sub>int</sub> = 0.070) 0.076; 0.176 0.067; 0.170 1.12

#### X-ray crystal data of 1.109



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation

Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on F<sup>2</sup>, all data): R1; wR2 Residuals (refined on F<sup>2</sup>): R1; wR2 Goodness of fit indicator C<sub>22</sub>H<sub>33</sub>NO<sub>9</sub> 455.49 colorless, prism 0.11 x 0.29 x 0.42 mm triclinic primitive a = 9.9044(10) Åb = 11.8847(11) Åc = 11.9608(11) Å $\alpha = 60.570(5)^{\circ}$  $\beta = 81.210(6)^{\circ}$  $\gamma = 75.803(5)^{\circ}$  $V = 1187.9(2) Å^3$ *P*-1 (#2) 2  $1.273 \text{ g/cm}^3$ 488.00  $0.99 \text{ cm}^{-1}$ Bruker X8 APEX II MoKα ( $\lambda = 0.71073$  Å) graphite monochromated 2315 exposures @ 5.0 seconds 39.75 mm  $60.5^{\circ}$ Total: 30831 Unique:  $6971 (R_{int} = 0.035)$ 0.050; 0.103 0.038; 0.096 1.03

#### X-ray crystal data of co-crystal of 5.35 and 5.36



Empirical formula Formula weight Crystal color, habit Crystal dimensions Crystal system Lattice type Lattice parameters

Space group Z value  $D_{calc}$  $F_{000}$  $\mu(MoK\alpha)$ Diffractometer Radiation Data images Detector position  $2\theta_{max}$ No. of reflections measured

Residuals (refined on  $F^2$ , all data): R1; wR2 Residuals (refined on  $F^2$ ): R1; wR2 Goodness of fit indicator C<sub>39</sub>H<sub>57</sub>NO<sub>7</sub>Si (for one diastereomeric unit) 518.61 colorless, prism 0.10 x 0.15 x 0.19 mm triclinic primitive a = 12.8721(15) Å b = 17.014(2) Å c = 18.675(2) Å $\alpha = 98.858(2)^{\circ}$  $\beta = 90.024(2)^{\circ}$  $\gamma = 100.845(2)^{\circ}$  $V = 3967.2(8) Å^3$ *P* 1 (#1) 4  $1.138 \text{ g/cm}^3$ 1472.00  $1.05 \text{ cm}^{-1}$ Bruker APEX DUO MoKα ( $\lambda = 0.71073$  Å) 1963 exposures @ 30.0 seconds 40.09 mm 50.8° Total: 144451 Unique: 28424 (R<sub>int</sub> = 0.044) 0.086; 0.106 0.053; 0.095 1.04