Intramolecular Diels–Alder/Tsuji Allylation Assembly of the Functionalized *trans*-Decalin of Salvinorin A and Sea Lamprey Migratory Pheromone Structure Activity Relationship Studies

A DISSERTATION
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Most importantly, I would like to acknowledge Professor Thomas Hoye for providing an embodiment of three vital attributes of an outstanding scientist, which I can only aspire to approach: an unmatched attention to detail, an exceptional work ethic, and a pronounced sense of professionalism. I will take these seedlings and further cultivate them throughout my career. I am also indebted to Professor Craig Forsyth for encouraging my creativity and independence as a chemist; without it, I would be a fraction of the scientist. In addition, I am grateful to Professor Peter Sorensen for highlighting the joys of an independent research career, as he has demonstrated in his *de novo* development of a research facility, while enjoying an almost surreal level of freedom.

Dedication

This work, and everything I do for that matter, is dedicated to my wife Rachel and my children: Jimmy, Anna, Mary, … Without them nothing I have done, or will do, would be possible. They comprise the *one* aspect of my life that I love more than chemistry.

Abstract

In the first portion of this work, an intramolecular Diels–Alder/Tsuji allylation assembly of functionalized *trans*-decalin systems will be discussed (Chapter I). This is presented in the context of synthetic studies toward the psychoactive (κ-opioid agonist) natural product salvinorin A. Synthetic studies of the sea lamprey (*Petromyzon marinus*) migratory pheromone will be discussed in Chapter II. In the third chapter, a structure activity relationship study of the sea lamprey migratory pheromone will be presented in which results suggest that the sea lamprey olfactory system is not uniquely and specifically recognizing the *allo* (i.e., 5α-H) configuration in bile acid derivatives.

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List of Abbreviations

Ac	Acetyl
AIBN	Azobisisobutyronitrile
3Å MS	3-Angstrom molecular sieves
4Å MS	4-Angstrom molecular sieves
BINAP	2,2'-*Bis*(diphenylphosphino)-1,1'-binaphthyl
BHB	Butylated hydroxy benzene
Bn	Benzyl ($C_6H_5CH_2$-)
BOMCl	Benzyloxymethyl chloride
BT	Benzothiazole
°C	Degrees Celsius
CBS	Corey–Bakshi–Shibata
δ	Chemical shift, in NMR spectroscopy
d	Doublet, in NMR spectroscopy
DCC	*N*,*N*-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHP	3,4-dihydro-2H-pyran
DIAD	Diisopropyl azodicarboxylate
DIBAL	Diisobutylaluminum hydride
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DMP	Dess–Martin Periodinane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide

dppf	1, 1'-*Bis*(diphenylphosphino)
dr	Diastereomeric ratio
equiv	Equivalent
ESI	Electrospray ionization
g	Gram(s)
HEK	Human embryonic kidney
HMPA	Hexamethylphosphoric triamide
HRMS	High resolution mass spectrometry
5-HT_{2A}	5-Hydroxytryptamine receptor 2A
Hz	Hertz (cycles per second)
IBX	Iodoxybenzoic acid
IR	Infrared
i-Pr	Isopropyl
J	Coupling constant (NMR)
K_i	Binding affinity
KHMDS	Potassium *bis*(trimethylsilyl)amide
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LiHMDS	Lithium *bis*(trimethylsilyl)amide
LUMO	Lowest occupied molecular orbital
m-CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl
MHz	Megahertz

mmol	milliMole
MNBA	*meta*-Nitrobenzoic anhydride
mol	Mole(s)
MOM	Methoxymethyl
mp	Melting point
MTPA	α-Methoxytrifluoromethylphenylacetyl
NaHMDS	Sodium *bis*(trimethylsilyl)amide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser Effect/Enhancement
PDC	Pyridinium dichromate
Ph	Phenyl
PMB	*para*-Methoxybenzyl
PMHS	Polymethylhydrosiloxane
ppm	Parts per million
PPTS	Pyridinium *p*-toluenesulfonic acid
PT	1-Phenyl-1H-tetrazole
PTSA or *p*-TsOH	*p*-Toluenesulfonic acid monohydrate
q	Quartet, in NMR spectroscopy
R	Rectus (configurational)
R_f	Ratio to front
rt	Room temperature
S	Sinister (configurational)

s	Singlet, in NMR spectroscopy
t	Triplet, in NMR spectroscopy
TBAF	Tetrabutylammonium fluoride
TBS	*tertiary*-Butyldimethylsilyl
TCDI	Thiocarbonyldiimidazole
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
THF	Tetrahydrofuran
TIPS	triisopropylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts or *p*-Ts	*para*-Toluenesulfonyl

Chapter I. Intramolecular Diels–Alder/Tsuji Allylation Assembly of the Functionalized *trans*-Decalin of Salvinorin A

I.A. Introduction and Background

Salvia divinorum, is a member of the mint (*Lamiaceae*) family that is indigenous to the forest ravines of the Sierra Mazateca between the altitude of 750 m and 1500 m.[1] It has been used for many centuries by the Mazatec people of Oaxaca, Mexico in religious ceremonies and various spiritual practices.[2] *S. divinorum* has been cultivated and used as a legal hallucinogen in California for years: currently, there are no U.S. laws prohibiting the sale and use of *S. divinorum* or any of its active constituents.[3]

Despite its long history of use, *S. divinorum* was not identified until 1962 by Wasson and Hoffman.[4] The presumed active ingredient in *S. divinorum*, salvinorin A was isolated and identified using ^{1}H NMR and single crystal X-ray studies by Alfredo Ortega (1982) and Leander Valdes soon thereafter. These findings were independent of one another and conclusively defined the structure and absolute stereochemistry of salvinorin A (Figure I-1, **1**).[5,6]

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[1] Valdes L. J. III; Diaz, J.; Paul A. G. "Ethnopharmacology of Ska Maria Pastora (*Salvia Divinorum*, Epling and Jativa-m.)" *J. Ethnopharmacology* **1983**, *7*, 287–312.
[2] Valdes L. J. III "*Salvia Divinorum* and the unique diterpene hallucinogen Salvinorin (Divinorin) A. *J. Psychoactive Drugs* **1994**, *26*, 277–283.
[3] Sheffler, D. J.; Roth, B. L. "Salvinorin A: The 'magic mint' hallucinogen finds a molecular target in the kappa opioid receptor" *Trends Pharmacol. Sci.* **2003**, *24*, 107–109.
[4] Wasson, W. G. "A new Mexican psychoactive drug from the mint family" In *Botanical Museum Leaflets, Harvard University Press* **1962**, *20*, 77–84.
[5] Ortega, A.; Blount, J. F.; Manchand, P. S. "Salvinorin, a new *trans*-neoclerodane diterpene from *Salvia divinorum* (Labiatea)" *J. Chem. Soc. Perkins Trans. 1* **1982**, 2505–2508.

Salvinorin A (**1**) is a neoclerodane[5] diterpene, which is unique in that it is the only known non-nitrogenous κ-opioid agonist.[3] It has been reported to be the most potent naturally occurring hallucinogen with an effective dose of 200-1000 μg in humans.[1,2] Salvinorin A (**1**) induces an intense, short-lived hallucinogenic experience qualitatively distinct from that of classical hallucinogens lysergic acid diethylamide (LSD), psilocybin, and mescaline. It may be ingested by: (a) mastication and swallowing the leaves; (b) crushing the leaves to obtain the extract and subsequently ingesting it; or (c) smoking the leaves.[7]

Shortly after the recognition of the psychoactive properties of salvinorin A (**1**) it was submitted for screening to NovaScreen™ to discover the molecular target: it showed no significant inhibition at any receptors screened. Various biogenic amine receptors, cannabinoid receptors and sigma receptors were included in this screening process.[7] Recently, the pharmacological profile **1** was reexamined by screening various human G-protein-coupled receptors (GPCRs), ligand-gated ion channels, and transporters via the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). It was discovered that **1** is a potent and selective kappa opioid receptor (KOR) agonist with no appreciable affinity for μ-opioid receptors (MOR) or δ-opioid receptors (DOR). It was also shown in the same study that no appreciable binding is observed with 5-HT_{2A} serotonin receptors, which are the main molecular

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[6] Valdes, L. J. III; Butler, W. M.; Hatfield, G. M.; Paul, A. G.; Koreeda, M. "Divinorin A, a psychotropic terpenoid, and divinorin B from the hallucinogenic Mexican mint *Salvia divinorum*" *J. Org. Chem.* **1984**, *49*, 4716–4720.
[7] Siebert, D. J. "*Salvia divinorum* and salvinorin A: new pharmacologic findings" *J. Ethnopharmacol.* **1994**, *43*, 53–56.

target responsible for the hallucinogenic properties of LSD, *N*,*N*'-dimethyltryptamine, psilocybin, mescaline, and 4-bromo-2,5-dimethyoxyphenylisoproplyamine.[8]

The κ-opioid receptor (KOR) is a member of the above mentioned G-protein-coupled receptors (GPCRs). GPCRs represent the largest and the most important family of transmembrane receptors characterized by amino acid sequences that contain seven hydrophobic domains. These hydrophobic domains represent the transmembrane-spanning regions of these proteins and gives rise to the second name for this family: 7TM or heptahelical receptors.[9] In mammals, this family contains more than 600 members. These receptors are involved in many biological processes by mediating the signals of a wide variety of stimuli such as peptide hormones (glucagons, angiotensin, bradykinin), neurotransmitters (adrenalin, serotonin, dopamine), neuropeptides (neuropeptide Y), as well as light and odorants.[10] KORs are members of the G-protein subfamily G_i/G_0 and are expected to elicit psychomimetic effects that involve inhibition of adenylyl cyclases, the activation of inward rectifying K^+ channels, and the inhibition of N-, P-, Q- and R-type voltage-activated Ca^{2+} channels.[3] Malfunction of these receptors naturally leads to many disorders. It is now well established that the activation of the KOR induces a large number of behavioral effects that include analgesia,

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[8] Roth, B. L.; Baner, K.; Westkaemper, R.; Siebert, D.; Rice, K. C.; Steinberg, S.; Ernsberger, P.; Rothman, R. B. "Salvinorin A: a potent naturally occurring nonnitrogenous κ opioid selective agonist" *Proc. Nat. Acad. Sci., U.S.A.* **2002**, *99*, 11934–11939.

[9] LefKowitz, R. J. "A superfamily of heptahelical receptors" *Nat. Cell. Biol.* **2000**, *2*, E133–E136.

[10] Dinger, M. C.; Beck-Sickinger, A. G. "Reporter Gene Assay Systems for the Investigation of G-protein-coupled Receptors" in *Molecular Biology in Medicinal Chemistry* (Eds.: Mannhold, R.; Kubinyi, H.; Folkers, G.), Wiley–VHC, New York, **2004**, chap. 3.

sedation, and perceptual distortion.[8] Thus, molecules that target the KOR selectively provide a tool for the better understanding of the GCPRs mechanism of action, which in turn could provide useful for developing KOR specific *antagonists*. Diseases that have been implicated in the involvement of the up-regulation of the KORs include schizophrenia, dementia, and bipolar disorders. Although, with respect to schizophrenia there seem to be conflicting results, two well-controlled studies have demonstrated an up-regulation of KORs in Alzheimer's disease, whereas MOR's and DOR's were down-regulated or remained unchanged.[8,11,12]

A recent structure-activity relationship (SAR) study aimed at gaining understanding of the pharmacophore of salvinorin A (Figure I-1, **1**), produced interesting results.[13] Using a radioligand binding assay, the binding coefficients (K_i) of **1** and its analogs were examined at cloned rat KORs expressed in HEK 293T cells. Compounds isolated from *S. divinorum* (e.g.; **1**, **5**, **7**, and **8**), as well as semisynthetic derivatives of **1** (e.g., **2**–**4**, **6**, and **9**) were interrogated. Notably, it was found that the ketone oxygen at C1 or the lactone oxygen at C17 were not necessary for tight binding (e.g., **2**, **3**, and **4**); compounds lacking these groups had equal, or even enhanced binding affinities. The acetate ester at C2 was found to be important for tight binding, although

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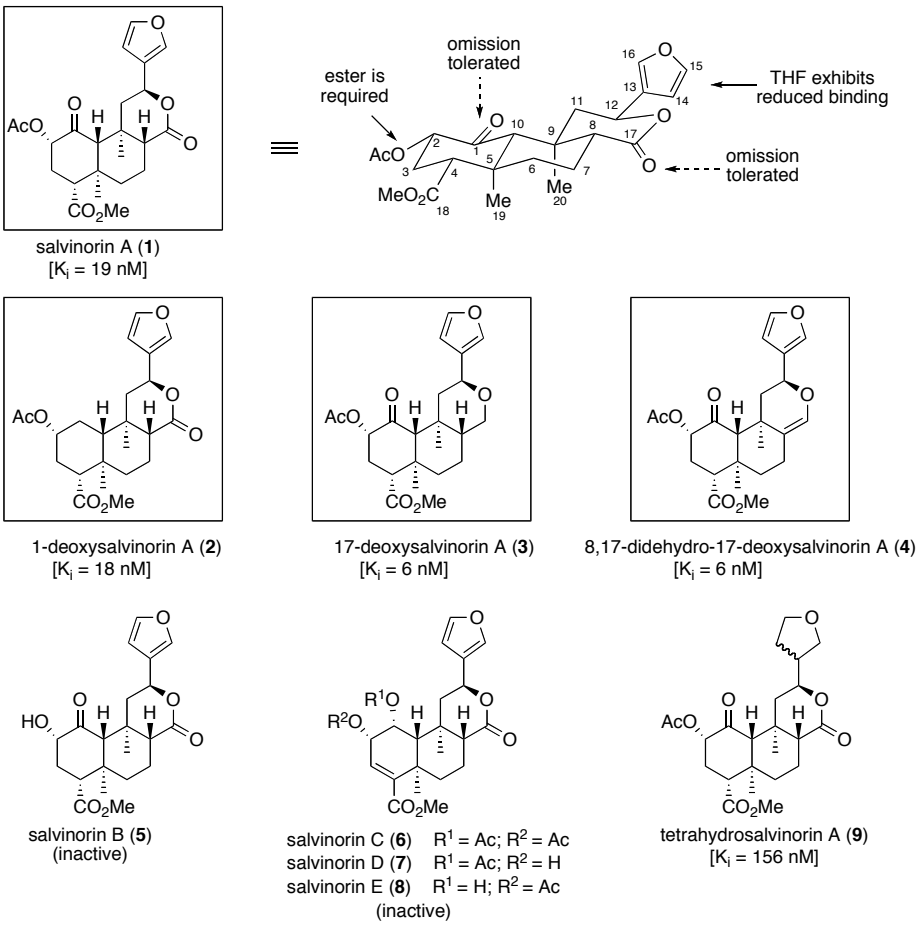
[11] Bard, J.; Belcheva, M.; Rowinski, J.; Ho, A.; Burke, W. J.; Chung, H. D.; Schmidt, C. A.; Coscia, C. J. "Opioid receptor density in Alzheimer amygdala and putamen" *Brain Res.* **1993**, *632*, 209–215.

[12] Mathieu-Kia, A. M.; Fan, L. Q.; Kreek, M. J.; Simon, E. J.; Hiller, J. M. "μ-,δ-, and κ-opioid receptor populations are differentially altered in distinct areas of postmortem brains of Alzheimer's disease patients" *Brain Res.* **2001**, *893*, 121–134.

[13] Munro, T. A.; Rizzacasa, M. A.; Roth, B. L.; Toth, B. A.; Yan, F. "Studies toward the pharmacophore of Salvinorin A: A potent κ Opioid receptor agonist" *J. Med. Chem.* **2005**, *48*, 345–348.

the smaller formate ester retained some activity (i.e., K_i=18 nM). The furan moiety was deemed necessary for binding, as the tetrahydrofuran analog **9** showed reduced activity.

Figure I-1. Structure-activity relationship study of isolated salvinorins and semi-synthetic derivatives



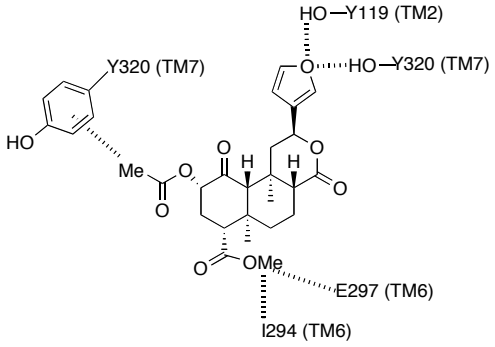
In addition, a study designed to probe the pharmacophore of **1** was conducted in which it was found that replacement of the C2 acetate with a benzoate ester led to loss of KOR activity, but remarkably led to a selective MOR binding.[14] This is an exciting

[14] Harding, W. W.; Tidgewell, K.; Byrd, N.; Howard, C.; Dersch, C. M.; Butelman, E. R.; Rothman, R. B.; Prisinzano, T. E. "Neoclerodane Diterpenes as a Novel Scaffold for μ Opioid receptor ligands" *J. Med. Chem.* **2005**, *48*, 4765–4771.

result which justifies further SAR studies centered around synthetic analogs of **1**; analogs not available through isolation or semi-synthetic methods. Examining such compounds—making small changes on the neoclerodane core—could lead to a greater understanding of KOR agonist–antagonist mechanisms, as well as that of other opioid receptors (i.e., MOR and DOR).

Using a combination of radioligand binding studies, functional assays, and molecular modeling; researchers have recently proposed the binding mode of **1** and they are in good agreement with the results previously mentioned (Figure I-1). Employing a chemically modified salvinorin analog—one in which the C2 acetate was replaced with a thiol group—the activity of cysteine mutants were examined. The proposed binding mode as determined from this study is highlighted below in which KOR side chains [e.g., Y119(TM2)] are indicated (Figure I-2).[15]

Figure I-2. Proposed binding mode of **1** as depicted by Roth et al.



[15] Yan, F.; Mosier, P. D.; Westkaemper, R. B.; Stewart, J.; Zjawiony, J. K.; Vortherms, T. A.; Shettler, D. J.; Roth, B. L. "Identification of the Molecular Mechanisms by Which the Diterpenoid Salvinorin A Binds to κ-Opioid Receptors" *Biochemistry* **2005**, *44*, 8643–8651.

Structural analogs of **1** offer unique molecular targets for synthetic studies: it seems that the salvinorin (neoclerodane) structural core represents a highly specific ligand system in which small changes in structure (e.g., benzoate substitution) can lead to loss of activity at the KOR, but remarkably lead to activity at other opioid receptors.[14] Another case that supports this conclusion is the observed activity of salvinicins A and B (Figure I-3, **10** and **11**). It was observed that relatively small differences in the tetrahydrofuran moiety of the salvinicins led to unexpected results in binding activity. The salvinicins were also isolated from the dried leaves of *S. divinorum* and bear structural resemblance to **1**, differing only in that the furan moiety is further oxygenated. Salvinicin A (**10**) showed partial activity as a KOR agonist, but interestingly salvinicin B (**11**) showed activity as a MOR *antagonist*. Thus, **11** represents the first neoclerodane diterpene with opioid *antagonist* activity.[16]

These findings clearly indicate that salvinorin A, and related compounds offer a unique and informative probe for opioid receptor agonists as well as antagonists. The development of *KOR* antagonists could have an impact on many human diseases that involve the over-expression of KORs: Alzheimer's disease and schizophrenia have been hypothesized to be influenced by an over-expression of KORs in patients.[8,11,12] Thus, an efficient synthetic route that is easily modified to incorporate desired structural modifications on the neoclerodane core could elicit further understanding from a chemical (i.e., synthetic methods), biological (i.e., structure–activity relationship), and medicinal (e.g., schizophrenia) standpoint.

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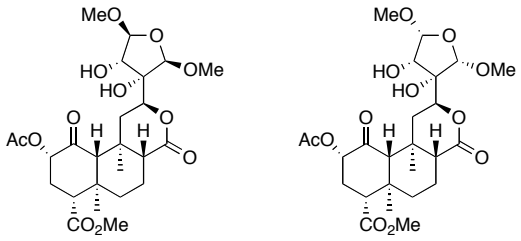
[16] Harding, W. W.; Tidgewell, K.; Schmidt, M.: Shah, K.; Dersch, C. M.; Snyder, J.; Parrish, D.; Deschamps, J. R.; Rothman, R. B.; Prisinzano, T. E. "Salvinicins A and B,

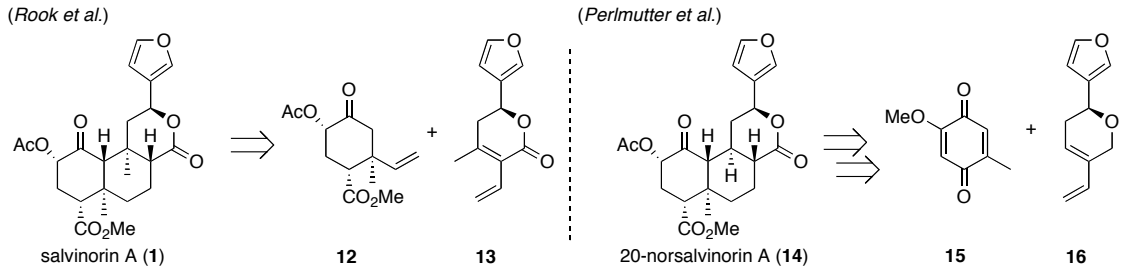
Figure I-3. Salvinicins A (**10**) and B (**11**)



I.B. Previous Synthetic Efforts

At the outset of our synthetic explorations, there were no reports of synthetic efforts toward the total synthesis of **1**. In 2006 a strategy toward **1** was published by Rook et al. (Figure I-4) in which the intermediates **12** and **13** were planned to be used in the construction of **1**: no forward progress of these intermediates was reported in this publication.[17]

Figure I-4. Synthetic efforts toward the construction of salvinorin A (**1**) and analog **14**



Perlmutter et al. reported a synthesis of an intermediate deemed useful in the synthesis of 20-norsalvinorin A (Figure I-4, **14**); an intermolecular Diels–Alder

New Neoclerodane Diterpenes from Salvia divinorum" *Org. Lett.* **2005**, *7*, 3017–3020.
[17] Lingham, A. R.; Hügel, H. M.; Rook, T. J. "Studies Towards the Synthesis of Salvinorin A" *Aust. J. Chem.* **2006**, *59*, 340–348.

cycloaddition was the key step, although there was no mention of employing this strategy toward **1**.[18]

In 2007 Evans et al. published the first total synthesis of salvinorin A (**1**) (Scheme I-1).[19] The synthesis began with the Ni(II)-promoted asymmetric orthoester alkylation of the thiazolidinethione **17**, followed by an acylation–decarboxylation sequence to give the β-ketoester **18**. Treatment of **18** with LiHMDS–$ClPO(OEt)_2$ to give the *Z*-enol phosphonate and subsequent Fe(II)-catalyzed cross-coupling with MeMgCl provided the enoate **19**. After a reduction–oxidation sequence, a stereoselective acetate aldol addition was employed using thiazalidinone **20**, $Sn(OTf)_2$, and *N*-ethylpiperidine to provide **21**. The secondary alcohol was silylated and the auxiliary was exchanged with methanol to provide the methyl ester; the terminal olefin was oxidatively cleaved to provide an intermediate aldehyde that was subjected to Carreira's asymmetric acetylide addition protocol to give **22**. The propargylic alcohol was protected as the BOM ether (i.e., BOMCl; NaHMDS) by conducting the reaction at low temperature (i.e., –78 °C) under Barbier-type conditions. Partial hydrogenation of the alkyne and oxidative cleavage of the resulting olefin, provided the aldehyde **23**. Synthesis of the vinyl iodide **26** was effected in four steps from ynone **24**: CBS reduction, Brown's alkyne isomerization, carbometallation–iodine exchange, and TES protection.

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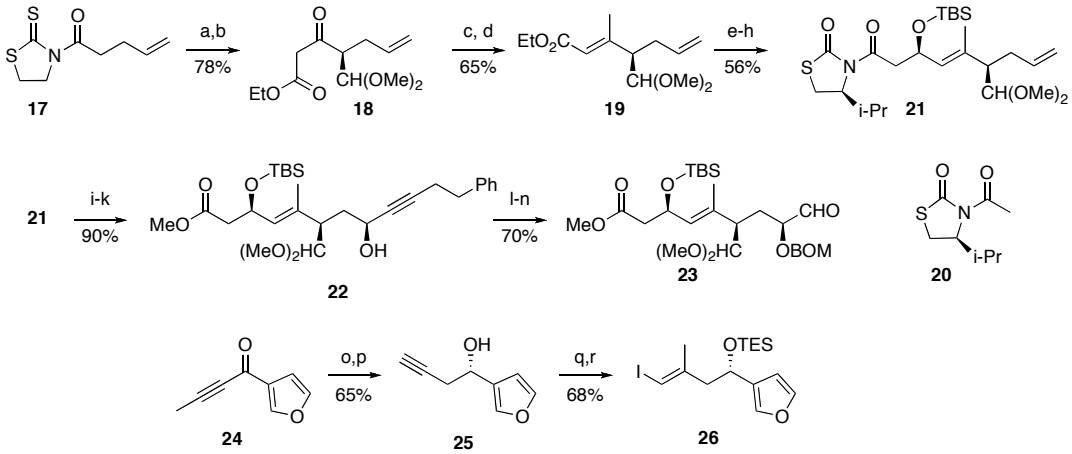
[18] Bergman, Y. E.; Mulder, R.; Perlmutter, P. "Total Synthesis of 20-Norsalvinorin A. 1. Preparation of a Key Intermediate" *J. Org. Chem.* **2008**, *74*, 2589–2591.

Scheme I-1. Synthesis of building blocks **23** and **26**[a]



[a] Conditions: (a) Ni-(R)-BINAP(OTf)$_2$, 2,6-lutidine, BF_3•OEt_2, $HC(OMe)_3$; (b) $HO_2CCH_2CO_2Et$, *i*-PrMgCl, 65 °C; (c) LiHMDS; $ClPO(OEt)_2$; (d) $Fe(acac)_3$, MeMgCl, –20 °C; (e) DIBAL-H, –78 °C; (f) MnO_2; (g) $Sn(OTf)_2$, *N*-ethylpiperdine, **20**, –78 °C; (h) TBSOTf, 2,6-lutidine; (i) K_2CO_3, MeOH; (j) OsO_4, NMO; $NaIO_4$; (k) $Zn(OTf)_2$, (–)-*N*-Me-ephedrine, Et_3N, 4-phenyl-1-butyne; (l) BOMCl, NaHMDS, –78 °C; (m) Lindlar catalyst, H_2; (n) K_2OsO_4, NMO, citric acid, 50 °C; $Pb(OAc)_4$, K_2CO_3 (o) (R)-*B*-Me-CBS catalyst, BH_3•SMe_2, –30 °C; (p) KH, $H_2N(CH_2)_3NH_2$, 0 °C; (q) Me_3Al, Cp_2ZrCl_2; I_2, –45 °C; (r) TESCl, imidazole.

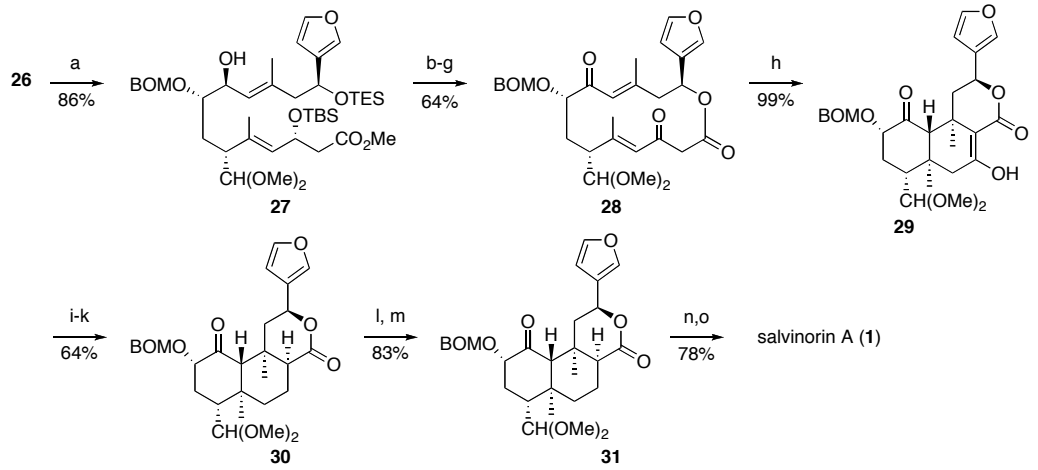
After lithium-halogen exchange of **26** with *t*-BuLi, the vinyl lithium species was transmetallated (i.e., $MgBr_2 \cdot OEt_2$) with magnesium and allowed react with the aldehyde **23** in a Cram-chelation sense to give **27**. The newly created carbinol was silylated (i.e., TBSOTf) and the secondary TES ether was selectively cleaved with PPTS in methanol. Macrolactonization under Shina's protocol (i.e.; MNBA, DMAP) and silyl ether cleavage (i.e., TBAF) gave a carbinol, which was oxidized with DMP to provide the transannular Michael cascade substrate **28**.

The Michael–Michael cascade (Scheme I-2) was effected with TBAF to produce a single diastereomer **29**; which was converted to the enol triflate and reduced, first to the enoate (i.e.; $Pd(OAc)_2$, Et_3SiH), and subsequently to the saturated lactone **30** (i.e.; L-

[19] Scheerer, J. R.; Lawrence, J. F.; Wang, G. C.; Evans, D. A. " Asymmetric Synthesis of Salvinorin A, A Potent κ Opioid Receptor Agonist" *J. Am. Chem. Soc.* **2007**, *129*, 8968–8969.

Selectride, *t*-BuOH). The BOM ether and the dimethyl acetal were cleaved simultaneously with lithium tetrafluoroborate and the resulting aldehyde was oxidized under the Pinnick conditions to give the carboxylic acid, which was converted to the methyl ester with TMS-diazomethane. The resulting product was epimerized (i.e.; K_2CO_3, MeOH)—unfortunately the undesired C8 epimer appears to be thermodynamically favored—and acetylated to give **1**.

Scheme I-2. Total synthesis of **1** by Evans et al.[a]



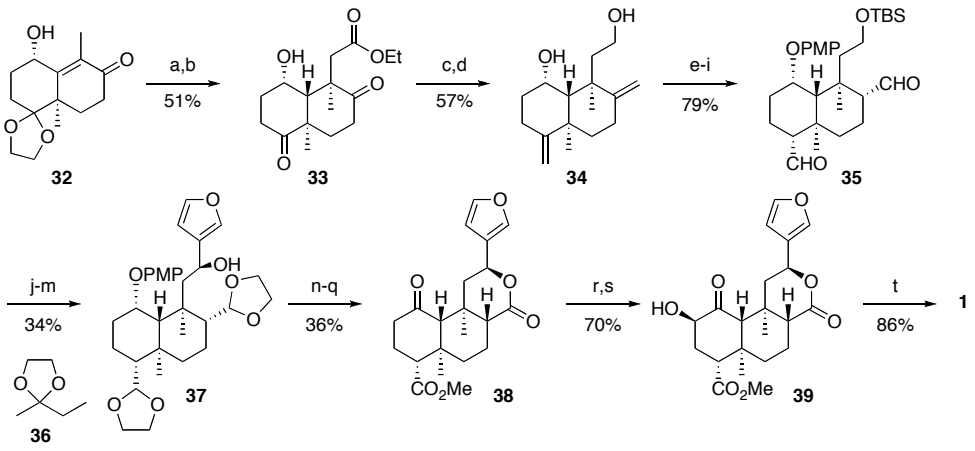
[a] Conditions: (a) *n*-BuLi, $MgBr_2 \cdot OEt_2$, –78 °C, then **23**, $MgBr_2 \cdot OEt_2$, CH_2Cl_2, –78 °C to 0 °C; (b) TBSOTf, 2,6-lutidine; (c) PPTS, MeOH; (d)LiOH, *i*-PrOH, H_2O; (e) MNBA, DMAP, [0.0015 M]; (f) TBAF; (g) Dess–Martin periodinane; (h) TBAF, –78 °C to 5 °C; (i) NaH, Comins reagent; (j) $Pd(OAc)_2$, dppf, Et_3SiH; (k) L-selectride, *t*-BuOH, –78 to –55 °C; (l) $LiBF_4$, $MeCN/H_2O$; (m) $NaClO_2$; $TMSCHN_2$; (n) K_2CO_3, MeOH, quant. mass recovery; (o) Ac_2O, pyridine, DMAP.

Subsequent to our communication concerning this work,[20] Hagiwara et al. published a total synthesis of **1** starting from the Wieland–Miescher ketone derived compound **32** (Scheme I-3), which was reduced using dissolving metal conditions to provide an enolate that was alkylated in situ to provide—after hydrolysis of the ketal—diketone **33**. *Bis*-methylenation and reduction of the ester with LAH provided the

[20] Burns, A. C.; Forsyth, C. J. "Intramolecular Diels–Alder/Tsuji Allylation Assembly of the Functionalized *trans*-Decalin of Salvinorin A" *Org. Lett.* **2008**, *10*, 97–100.

primary alcohol **34**, which was regioselectively protected as the TBS ether; the remaining alcohol was protected as the PMB ether. A *bis*-hydroboration–oxidation–oxidation sequence provided the doubly equatorially disposed dialdehyde **35**.

Scheme I-3. First-generation synthesis of salvinorin A (**1**) by Hagiwara et al.[a]



[a] Conditions: (a) Li, THF/NH_3, –78 °C; ICH_2CO_2Et; (b) 3M HCl, EtOH; (c) NaHMDS, Ph_3PCH_3Br, THF; (d) LAH, Et_2O, 0 °C; (e) TBSCl, DMAP, Et_3N, CH_2Cl_2; (f) NaH, PMBCl, DMF (g) BH_3•THF, THF; 3M NaOH, H_2O_2; (h) PDC, NaOAc, 4Å MS, CH_2Cl_2; (i) NaOMe, MeOH; (j) ethylene glycol, **36**, PTSA, 40 °C; (k) TBAF, THF; (l) PDC, NaOAc, 4Å MS, CH_2Cl_2; (m) 3-bromofuran, *t*-BuLi, THF, –78 °C; (n) PTSA, acetone, H_2O, Δ; (o) DDQ, H_2O, CH_2Cl_2, 0 °C; (p) PDC, 2-methyl-2-butene, DMF; (q) DCC, DMAP, MeOH, CH_2Cl_2; (r) NaHMDS, TESCl, THF, –78 °C; (s) *m*-CPBA, $NaHCO_3$, toluene, H_2O, 0 °C; AcOH; (t) PPh_3, DIAD, AcOH, CH_2Cl_2.

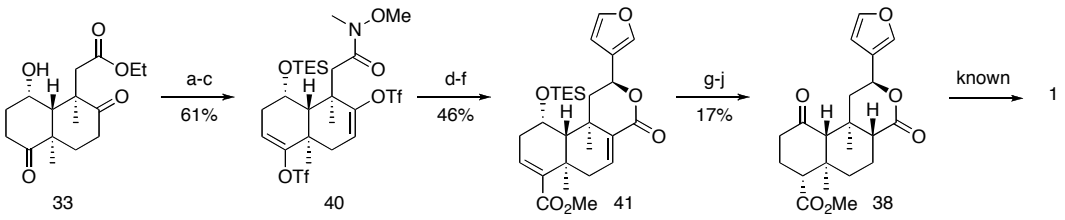
The authors mention that attempts to introduce α,β-unsaturated ester groups by palladium-catalyzed carbonyl insertion of the corresponding trifluoromethanesulfonylenol ether were not fruitful, probably due to the steric hinderance of the C8–C4 centers. This effect was overcome in a second-generation synthesis by this group (*vide infra*, Scheme I-4).

The dialdehyde **25** was protected as an ethylene glycol *bis*-acetal prior to revelation of the primary alcohol, which was oxidized to the C12-aldehyde with PDC. To this aldehyde, 3-lithiofuran was nucleophilically added in a partially selective manner (i.e.; *S*:*R*, 2:3) with preference for the undesired *R*-isomer. The ethylene glycol

acetals were hydrolyzed—PMB ether was oxidatively cleaved (i.e., DDQ)—and further oxidized with PDC and oxidized to a C1-ketone–C4-acid–C17-lactone, which was converted to the C4-methyl ester **38** (i.e.; DCC, DMAP, MeOH). Chemoselective enolate formation (i.e., in the presence of the methyl ester and lactone) followed by the reaction with TESCl effected the formation of a silyl enol ether which was converted to the α-hydroxy ketone **39** via the Rubottom procedure (i.e., *m*-CPBA); it was necessary to invert the carbinol stereocenter by the Mitsunobu conditions (i.e.; PPh_3, DIAD, AcOH) to provide **1**.

Recently, Hagiwara et al. have published a second-generation synthesis of salvinorin A (**1**).[21] The key finding is this report is that the *bis*-enol triflate (Scheme I-4, **40**)—after the addition of 3-furyllithium to the Wienreb amide—could be employed in a palladium-mediated carbonylation reaction (i.e.; $Pd(PPh_3)_4$, CO, MeOH) to provide a *bis*-methyl enoate.

Scheme I-4. Hagiwara's second-generation synthesis of **1**[a]



a Conditions: (a) TESOTf, pyridine, DMAP, DMF, 100 °C; (b) Comins' reagent, NaHMDS, THF; (c) i-PrMgCl, (MeO)MeNH•HCl, THF; (d) 3-bromofuran, t-BuLi, THF; (e) $Pd(PPh_3)_4$, CO, dppf, Et_3N, MeOH, DMF; (f) K-selectride, t-BuOH, THF; (g) SmI_2, Et_3N, AcOH; (h) TBAF, THF; (i) K_2CO_3, MeOH; (j) Dess–Martin periodinane, CH_2Cl_2.

The keto function (i.e., furyl ketone) of the resultant *bis*-methyl enoate could be chemo- and diastereoselectively (i.e., K-Selectride) reduced–lactonized to give **41** as a

single diastereomer. The authors provide a chelation-model to rationalize the selectivity of this provocative transformation. It was discovered that samarium diiodide effected *bis*-enoate reduction to provide a compound that was treated with TBAF, oxidized with DMP, and epimerized at C8 (7:3, in favor of undesired) to provide intermediate **38**; the conversion of **38** to **1** had been previously established.

As a prelude to the next section (Synthetic Design), the time-line of the previous synthetic efforts and total syntheses relative to our synthetic development should be noted. At the outset of our studies, there were no reports of the total synthesis or progress toward salvinorin A (**1**). Our initial synthetic design was subsequently affected by the synthesis of a related natural product, methyl barbascoate (Section I.C, Scheme I-5, **52**), in which the Hagiwara group employed a strategy that hinged upon the use of the Wieland–Miescher ketone [Section I.C, Figure I-5, (–)-**47**] as a the starting material.[22] This led to our reexamination of the synthetic plan with respect to its potential contribution to the chemical field, as judged by the introduction and study of new chemical phenomena.

I.C. Synthetic Design

Our initial synthetic design was centered on the development of an efficient synthesis of salvinorin A (**1**) and analogs thereof. It was reasoned that 1-deoxysalvinorin A (Figure I-5, **2**) would be a more approachable target synthetically due to the reduced complexity (e.g., C1 oxygenation). Furthermore, this target is

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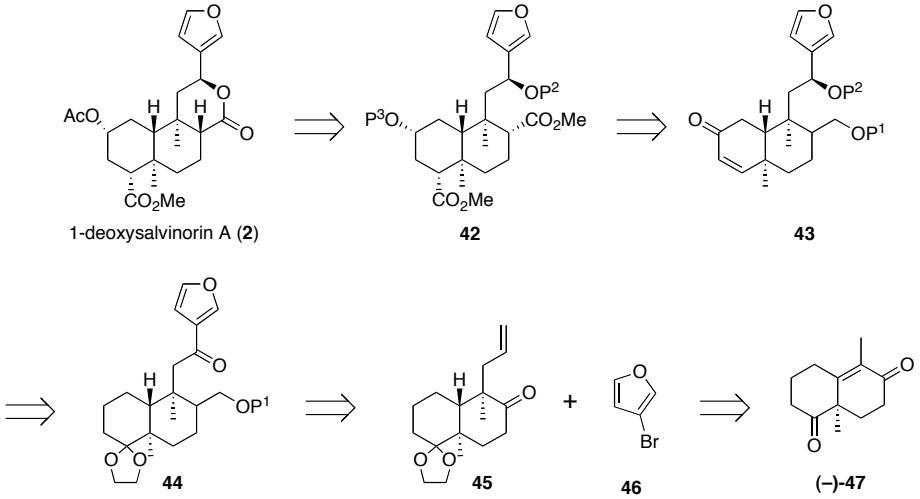
[21] Hagiwara, H.; Suka, Y.; Nojima, T.; Hoshi, T.; Suzuki, T. "Second-generation synthesis of salvinorin A" *Tetrahedron* **2009**, *65*, 4820–4825.
[22] Hagiwara, H.; Hamano, K.; Nozawa, M.; Hoshi, T.; Suzuki, T.; Kido, F. "The First Total Synthesis of (–)-Methyl Barbascoate" *J. Org. Chem.* **2005**, *70*, 2250–2255.

biologically justified due to potency as discussed above (cf.; Section I.A, Figure I-1). The synthesis of **2** could derive from the known Wieland-Miescher type ketone (–)-**47** in either the racemic or enantiomeric form. Either enantiomer of **47** is readily available from the amino acid catalyzed asymmetric Robinson annulation.[23] During initial studies we chose to operate in the more economical racemic series, which could be modified at a later stage for the desired enantiomeric series.

Figure I-5. Initial retrosynthetic analysis of 1-deoxysalvinorin A (**2**)



The target compound **2** could be disconnected to the diester **42** by acetylation of the P^3-deprotected alcohol and lactone formation. Diastereoselective axial conjugate addition of a cyanide nucleophile to the α,β-unsaturated ketone,[24] followed by saponification (with concomitant epimerization to the equatorial carboxylic acid) of the

[23] Hagiwara, H.; Uda, H. "(4a*R*)-(–)-1,4a-Dimethyl-4,4a,7,8-tetrahydronaphthalene-2,5(3H, 6H)-dione and Its Use in the Synthesis of an Inhibitor of Steroid Biosynthesis" *J. Org. Chem.* **1988**, *53*, 2308–2311.

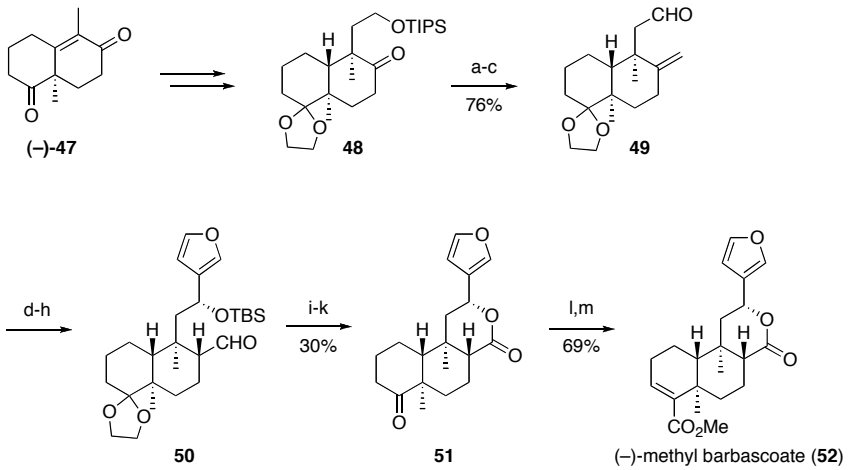
[24] Ziegler, F. E.; Hwang, K.; Kadow, J. F.; Klein, S. I.; Pati, U. K.; Wang, T. "Practical Routes to Two Functionalized Decalones for the Synthesis of Quassinoids" *J. Org. Chem.* **1986**, *51*, 4573–4579.

nitrile, axial reduction [protection of resultant alcohol (P^3)] of the C2 ketone, oxidation of the P^1-deprotected primary alcohol, and *bis*-esterification of the diacid could provide **42** from **43**. One need not worry about the α-stereocenters of the methyl esters: they are equatorially disposed. A Wharton rearrangement of a α,β-unsaturated ketone derived from **44** (e.g., Saegusa oxidation on ketone derived from **44**) could be used to transpose the enone. The furyl ketone would be a likely candidate for a CBS reduction to establish the C12 stereocenter. Organometallic addition of the furan core to an aldehyde derived from a Johnson-Lemieux oxidation or ozonolysis of the terminal alkene, would establish the required furan ring of **44** that would most likely need to be oxidized to facilitate subsequent establishment of the requisite stereocenter via an asymmetric reduction.

It is not unreasonable to expect the organometallic addition to proceed with a moderate level of stereoselectivity, although it may be difficult to predict the stereochemical course of this addition. If the major diastereomer of the addition is the desired one—in reasonable excess—the CBS reduction would be obviated. One could effect convergence of the diastereomers by separation and subsequent Mitsunobu inversion. Homologation at the C8 ketone can be accomplished by many known methods (e.g.; $Ph_3PCHOMe$, Wittig–hydroboration–oxidation, or palladium-mediated carbonylation of a vinyl triflate). Known ketone **45**[24] and can be prepared in two steps from the Wieland-Miescher analog (–)-**47**, which in turn, is prepared from the asymmetric Robinson annulation of the commercially available 2-methyl-1,3-cyclohexanedione and ethyl vinyl ketone.

With the synthesis of **45** underway, a previously mentioned publication by the Hagiwara group surfaced: the first total synthesis of (–)-methyl barbascoate (Scheme I-5, **52**).[23] With this synthesis resembling our synthetic plan—the authors also mentioned using this strategy toward the construction of salvinorin A (**1**)—we chose to redesign our strategy. Before discussing the revised design, it is worth noting an inspiration coming from this work. The authors used a step-saving strategy to effect homologation of the C4 ketone moiety via the enol triflate (i.e., palladium mediated carbonylation); this was incorporated, and furthered in our revised synthetic strategy by planning to use a *bis*-homologative strategy (*vide infra*). Coincidentally, the Hagiwara group was not without this realization of this strategy as they latter used this (i.e., *bis*-homologation) in a second-generation synthesis of **1** (cf.; Section I.B, Scheme I-4).

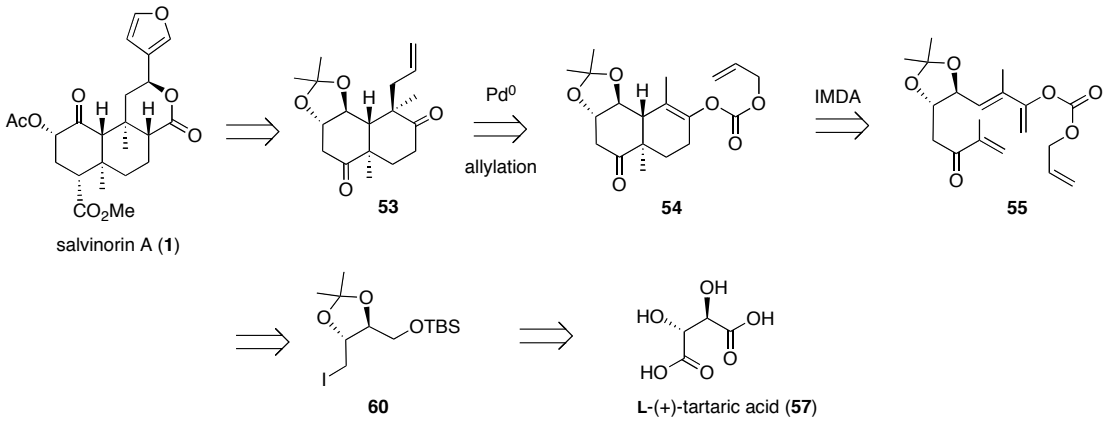
Scheme I-5. Hagiwara's synthesis of (–)-methyl barbascoate (**52**)[a]



[a] Conditions: (a) Ph_3PCH_3Br, NaHMDS, THF, Δ; (b) TBAF, THF; (c) SO_3•Py, DMSO, Et_3N, CH_2Cl_2; (d) 3-bromofuran, *t*-BuLi, THF, –78 °C; (e) TBSCl, Imidazole, DMAP, DMF; (f) BH_3•THF; NaOH, H_2O_2; (g) PDC, NaOAc, 4Å MS, CH_2Cl_2; (h) NaOMe, MeOH; (i) TBAF, THF; (j) PDC, NaOAc, 4Å MS, CH_2Cl_2; (k) PPTS, acetone, H_2O, Δ; (l) NaHMDS, Comins' reagent, THF –78 to –30 °C; (m) $Pd(OAc)_2$, dppf, *n*-Bu_3N, CO, MeOH, DMF 90 °C.

In the revised retrosynthetic analysis it was envisioned that an intramolecular Diels–Alder cycloaddition and a palladium-mediated allylation could serve as the key steps in construction of the functionalized core of **1** (Figure I-6).

Figure I-6. Revised retrosynthetic analysis of salvinorin A (**1**)



Salvinorin (**1**) could be disconnected, via the above mentioned *bis*-carbonylative strategy to the diketone **53**. The terminal olefin present in **53** lends a handle for addition of a furan nucleophile via the aldehyde. The enol carbonate **54** could be transformed into **53** by a palladium mediated allylation (i.e., Tsuji allylation):[25] the selectivity being either substrate controlled, or if necessary the substrate bias could be overridden by reagent control.[26,27] The enol carbonate—a regioselective enolate equivalent—could be constructed by an intramolecular Diels–Alder (IMDA) cycloaddition. This IMDA strategy for the construction of regioselective enolate equivalents was unknown in the

[25] Tsuji, J.; Minami, I.; Shimizu, I. "Palladium-catalyzed allylation of ketones and aldehydes via allyl enol carbonates" *Tetrahedron Lett.* **1983**, *24*, 1793–1796.
[26] Behenna, D. C.; Stoltz, B. M. "The enantioselective Tsuji Allylation" *J. Am. Chem. Soc.* **2004**, *126*, 15044–15045.
[27] Trost, B. M.; Xu, J. "Regio- and Enantioselective Pd-Catalyzed Allylic Alkylation of Ketones through Allyl Enol Carbonates" *J. Am. Chem. Soc.* **2005**, *127*, 2846–2847.

literature prior to our publication.[20] The tetraene **55** could be forged in three steps from known iodide **60**[28] which in turn could be made from L-tartaric acid (**57**) in four steps.

I.D. Results and Discussion

At the outset of this synthetic effort the diastereoselectivity of the IMDA cycloaddition and the substrate controlled Tsuji allylation were predicted based on stereoelectronic models, but remained to be tested as there was no analogous transformation in the literature. Furthermore, there was only one example of a Diels–Alder cylcloaddition with a 2-enol carbonate substituted diene as the substrate;[29] thus, even the reactivity of the tetraene **55** (cf., Section I.C, Figure I-6) was unknown.

The synthesis of the substrate for which the hypothesis involving the IMDA will be tested **55**, commenced with L-tartaric acid (**57**) (Scheme I-6). Ketalization was brought about by the action of 2,2-dimethoxypropane and *p*-toluene sulfonic acid on the diol moiety to provide the dicarboxylic acid **58**. Exhaustive reduction with lithium aluminum hydride and selective *mono*-protection of the C_2-symmetrical diol provided the silyl ether **59**. This compound could be transformed into the primary iodide **60** by either of two methods: one-pot conversion (i.e.; I_2, PPh_3, imidazole) or a two-step protocol (i.e.; TsCl, pyridine; NaI, acetone). The former method suffered from a difficult purification as a result of the R_f similarity of the product with triphenylphosphine, which is used in excess.

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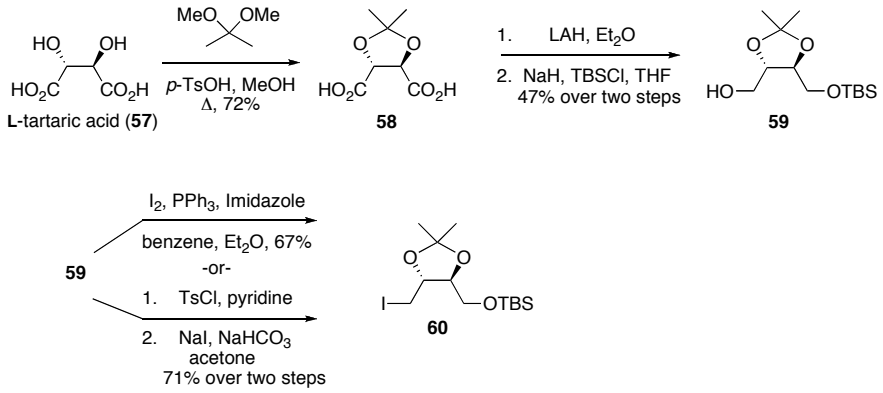
[28] Enders, D.; Lenzen, A.; Backes, C.; Catlin K.; Lannou, M.-I.; Runsink, J.; Raabe, G. "Asymmetric Total Synthesis of the 1-*epi*-Aglycon of the Cripowellins A and B" *J. Org. Chem.* **2005**, *70*, 10538–10551.

[29] Becacalli, E. M.; Marchesini, A. "Diels–Alder Reactions of (*Z*)-Ethyl 3-[(1-ethoxycarbonyloxy-2-methoxy)ethenyl]-2-(ethoxycarbonyloxy)indole-1-carboxylate.

Scheme I-6. Synthesis of iodide **60** for use in dithiane alkylation



It was expected that the primary iodide **60** could be engaged in a nucleophilic displacement with an allylic dithiane anion such as **62** (Scheme I-7). Although no direct precedent for the formation of **62** via methacrolein (**61**) existed, it was attempted using common conditions used in the formation of dithiane acetals. The standard borontrifluoride–diethyl etherate mediated conditions failed with the formation of intractable reaction mixtures most likely due to polymerization (i.e., **61** is a Michael acceptor). Using a modified protocol using acetic acid as a co-solvent at low temperature provided **62** in a low, but temporarily useful yield.[30]

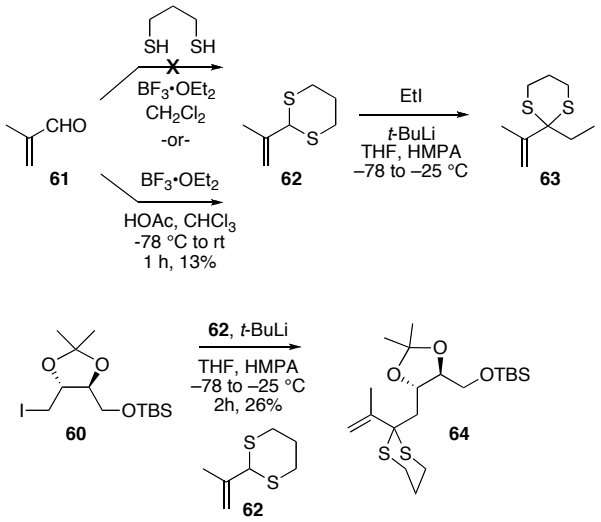
With a workable amount of **62**, alkylation was first attempted with the primary iodide **60**. Base-promoted E_2-elimination of the substrate was anticipated to be a potential problem with substrate **60**. Thus, when TLC analysis of the reaction mixture indicated the formation of a more polar "by-product" (i.e., smaller R_f value) it was

Synthesis of the Carbazole Alkaloid Carbazomycin B" *Tetrahedron* **1996**, *52*, 3029–3036.

[30] Ziegler, F. E.; Fang, J. –M.; Tam, C. C. "Conjugate Addition of Dithianylidene Anions to α,β-Unsaturated Ketones. An Application to the Total Synthesis of (±)-Aromatin and (±)-Confertin" *J. Am. Chem. Soc.* **1982**, *104*, 7174–7181.

initially discounted as an elimination "by-product". In an effort to conduct and optimize this reaction on a model system (e.g., EtI) it was discovered that the product **63** was actually more polar than expected. Substrate **60** was then reexamined under this protocol and it was confirmed that the alkylation product **64** was more polar than the starting iodide **60** or the allylic dithiane **62**. The yield of this alkylation was much too low to be used in a multi-step synthesis, but it did help to provide authentic material **64** and to define "optimum" conditions.

Scheme I-7. Synthesis and alkylation of allylic dithiane **62**

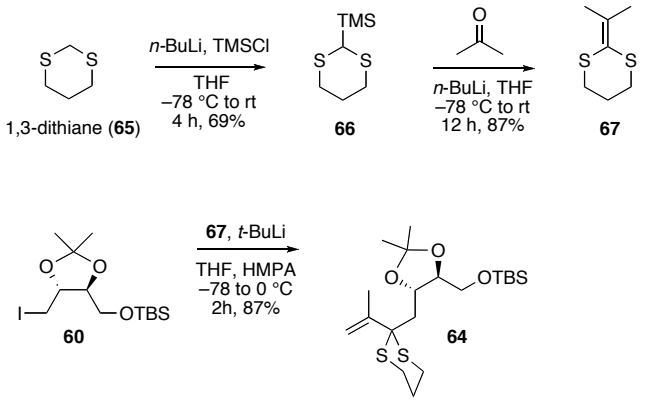


Fortunately, it was realized that due to the allylic nature of the alkylating anion derived from **62** it would be possible to use the isomeric compound **67** as the precursor to the allylic anion (Scheme I-8). This compound was synthesized in short order from 1,3-dithiane (**65**) in two steps as highlighted below.[31]

[31] Page, P. C.; McKenzie, M. J.; Buckle D. R. "Highly Chemoselective Osmium-Mediated Dihydroxylation of 2-Vinyl and 2-Allyl-1,3-dithiane 1-Oxides" *Tetrahedron* **1998**, *54*, 14581–14596.

When **67** was subjected to the conditions developed above using the primary iodide **60** as the substrate, a much improved yield (i.e., 87%) of the product **64** was obtained (Scheme I-8). This result can be directly contrasted with that of substrate **62** under almost identical conditions (Scheme I-7).

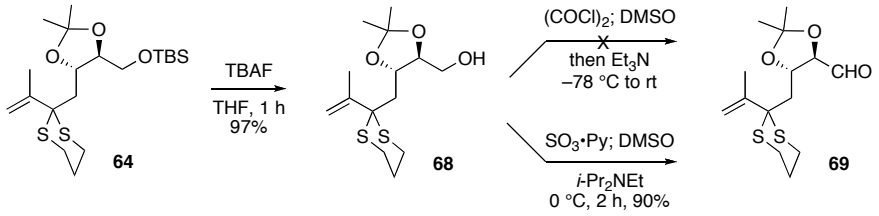
Scheme I-8. Synthesis of dithioketene acetal **67** and subsequent use in an alkylation reaction with the primary iodide **60**



With the silyl ether **64** obtained in good yield, the next obstacle was the somewhat problematic oxidation of the primary alcohol **68** derived from the silyl ether cleavage (i.e., TBAF) of compound **64** (Scheme I-9). Standard Swern conditions failed with the production of overoxidized by-products presumably arising from prior cleavage of the acetonide moiety. It was posited that the non-acidic Parikh–Doering[32] oxidation would be amenable to this substrate; this proved to be true providing the aldehyde **69** in good yield (i.e., 90%).

[32] Parikh, J. P.; Doering, W. E. "Sulfur trioxide in the oxidation of alcohols by dimethyl sulfoxide" *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

Scheme I-9. Activated DMSO-based oxidation of the primary alcohol **68**



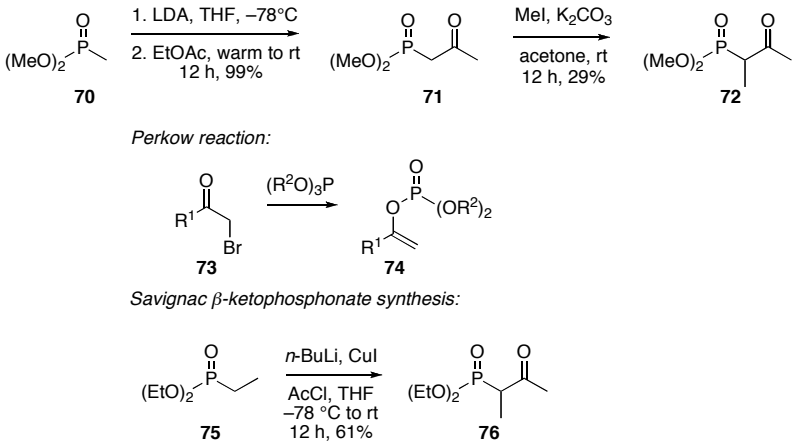
Substrate **69** was expected to engage in a Horner–Wadsworth–Emmons olefination with the appropriate β-ketophosphonate **72** (Scheme I-10). The synthesis of this compound was first conducted by way of *mono*-alkylation of **71** (e.g.; MeI, K_2CO_3, acetone). This reaction suffered from low yields as a result of *bis*-alkylation, however the product could be isolated in pure form by flash chromatography.

Although one may view the Michaelis–Arbuzov (i.e., bromoketone and trialkylphosphite) as an alternative route to **74**, it is well known that bromoketones produce a different product under the Michaelis–Arbuzov conditions: vinyl phosphates (e.g., **74**). This reaction is known as the Perkow reaction.[33] This problem can be avoided by employment of the Savignac protocol in which a copper phosphonate anion is treated with a acid chloride.[34] Presumably, the copper anion possesses attenuated basicity compatible even with an acid chloride (i.e., only one equivalent of *n*-BuLi is required): seemingly precluding the involvement of ketene.

[33] Kürti, L.; Czakó, B. "Arbuzov Reaction" in *Strategic Applications of Named Reations in Organic Synthesis*, Elsevier Academic Press, London, **2005**, pp. 16–17.

[34] Mathey, F.; Savignac, P. "A Direct Synthesis of Diethyl 1-Alkyl-2-oxoalkane-phosphonates" *Synthesis* **1976**, 766–767.

Scheme I-10. Synthesis of mono-substituted β-ketophosphonates (e.g., **72**)

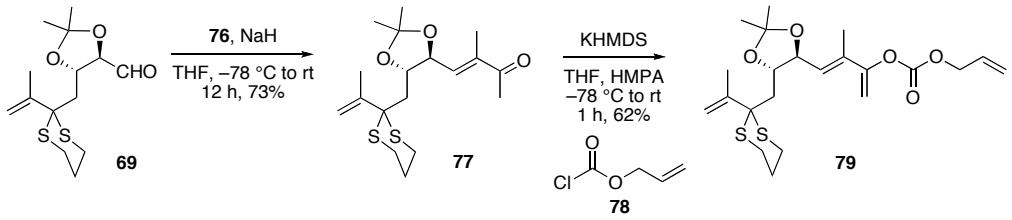


The β-ketophosphonate **76** cleanly reacted under HWE conditions with the aldehyde **69** to provide the *E*-trisubstituted enone **77**: one isomer was observed as determined by ^{1}H NMR chemical shift of the β-hydrogen as well as an nOe experiment (Scheme I-11).

Acylation of the methyl ketone **77** proved to be challenging; the temperature, solvent, and reaction time prior to the addition of allyl chloroformate (**78**) were important. The problem encountered during this reaction was the formation of the C-acylation product, which further complicated the matter by producing an acidic β-ketoester that would quench the starting material enolate. Thus, a mixture of **77**, **79**, and the undesired C-acylation product would form under non-ideal conditions. This problem was predominately solved by the addition of HMPA as a co-solvent, but if was also found that the reaction worked better under more dilute conditions (i.e., 0.01M) and if the enolate was only allowed to stir for short periods (i.e., 15 min) after its formation.

Both of these results indicate that the enolate aggregation state is highly important in this transformation.

Scheme I-11. HWE olefination and subsequent acylation with allyl chloroformate (**58**)

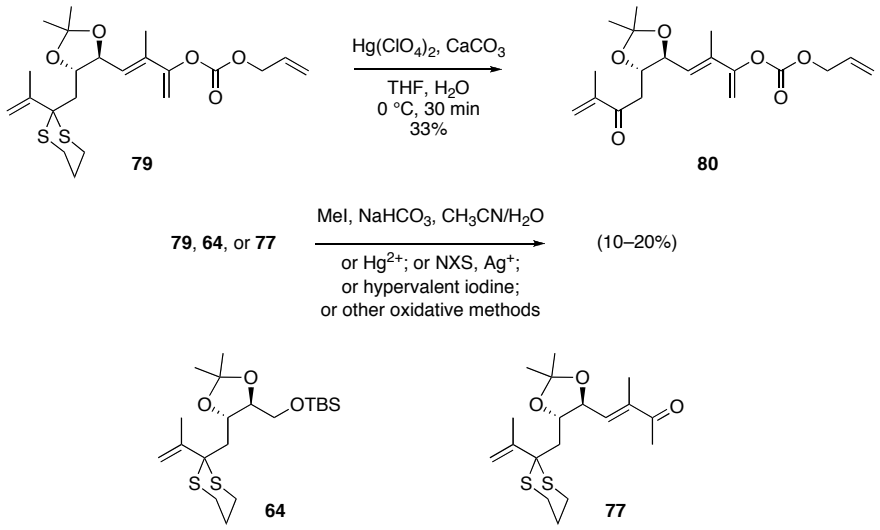


With no success, the IMDA reaction was first attempted on the dithiane **79** (i.e.; xylenes, Δ, sealed tube) with the intention of enhancing the desired *exo*-selectivity in the cycloaddition event. To lower the LUMO of the dienophile—leading to a lower activation barrier, and hence reasonable reaction temperature—the enone **80** was revealed by removal of the dithiane. This event turned out to be one of the most difficult obstacles during this work. Many known methods for dithiane removal were examined and all suffered from low yields, the best of which gave *reproducible* yields in the low thirties (Scheme I-12; $Hg(ClO_4)_2$, $CaCO_3$, THF/H_2O). Although it is not conclusively known what the by-products of this reaction were—poor crude mass recovery could be an indicator of the formation of insoluble salts and/or polymerization—it has been noted in the literature that allylic dithianes tend to be problematic in their deprotection.[35] Even one of the mildest protocols (i.e.; MeI, CH_3CN/H_2O) not involving a metal or oxidant, gave similar results. Experiments were conducted with compounds **77** and **64** in an

[35] Langille, N. F.; Dakin, L. A.; Panek, J. S. "A Mild, Chemoselective Protocol for the Removal of Thioketals and Thioacetals Mediated by Dess–Martin Periodinane" *Org. Lett.* **2003**, *5*, 575–578.

attempt to single out the problematic functional group, which seems to the allylic group of the dithiane, based on the observation of similar yields with these two substrates.

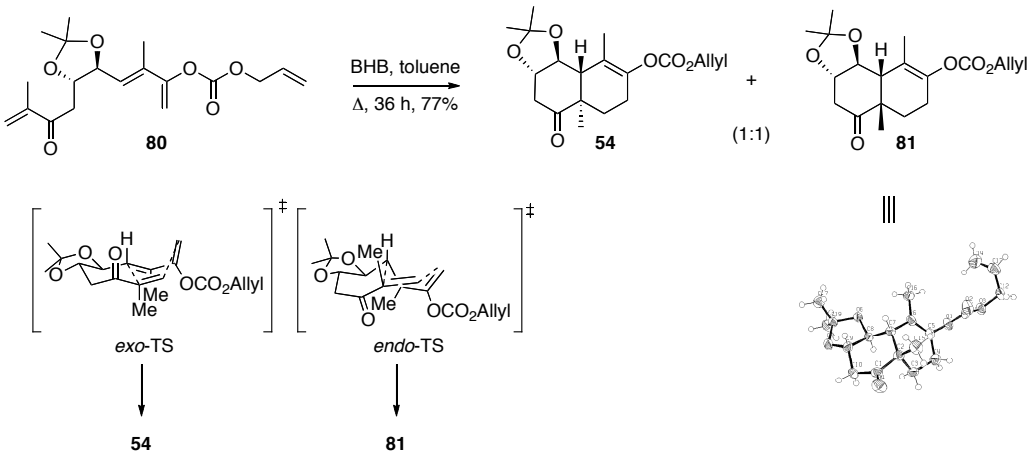
Scheme I-12. Hydrolysis of dithiane **79**



It was unknown whether the tetraene **80** would engage in an IMDA cycloaddition; furthermore, the diastereoselectivity of the event remained to the tested (i.e., four products are possible). Indeed, the cycloaddition proved feasible in refluxing toluene (butylated hydroxybenzene was added as a radical inhibitor and was necessary to avoid decomposition) giving a mixture of diastereomers in a one to one ratio (Scheme I-13, **54** and **81**). Fortunately these compounds could be separated by recrystallization; the crystalline *cis*-isomer **81** could be separated from the desired product in this way. At this time the absolute configuration of the *cis*-isomer **81** was determined by single-crystal X-ray analysis. The configuration of the remaining isomer was proven at a later stage by single-crystal X-ray analysis of a subsequent intermediate (*vide infra*); although it was not conclusive, an nOe study along with the observed

broadening of the angular methyl group in the ^{1}H NMR spectrum pointed to a *trans*-decalin system (two isomers remained possible).

Scheme I-13. Diastereoselective IMDA reaction



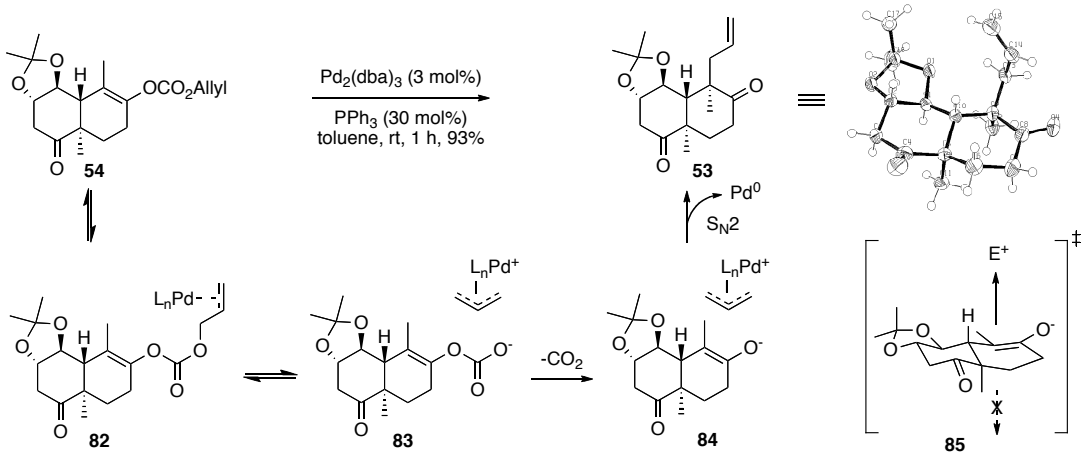
The Tsuji allylation was conducted on the enol carbonate **54** and cleanly produced the diketone **53** in good yield as a single diastereomer. Gratifyingly, this compound could be recrystallized to allow for single-crystal X-ray analysis which indicated that: (a) we had obtained the correct isomer from the IMDA cycloaddition and (b) the allylation occurred with the desired diastereoselectivity via substrate control (i.e.; via transition state **85**, Scheme I-14).

The accepted mechanism—recently enhanced by the Trost group[36]—is indicated in Scheme I-14. Coordination to the olefin by Pd^0 and subsequent allyl insertion of Pd^0 provides carbonate anion–allyl-palladium cation ion-pair **83**. The rate-determining step (i.e., loss of CO_2) occurs to produce and enolate–allyl-palladium cation ion-pair **84** that

[36] Trost, B. M.; Xu, J.; Schmidt, T. "Palladium-catalyzed Decarboxylative Asymmetric Allylic Alkylation of Enol Carbonates" *J. Am. Chem. Soc.* **2009**, *131*, 18343–18357.

reacts via an "outer-sphere" mechanism (i.e.; S_N2, via **85**) to provide the product **53** and reproduce Pd^0. It is worth pointing out that the alkylation reaction is a net equatorial alkylation presumably arising from a boat-like transition state **85** in which the steric thermodynamic penalty of interaction with the axial methyl substituent (i.e., 1,3-interaction) is avoided in the transition state leading to the desired product **53**.

Scheme I-14. Proposed Mechanism of the Tsuji allylation



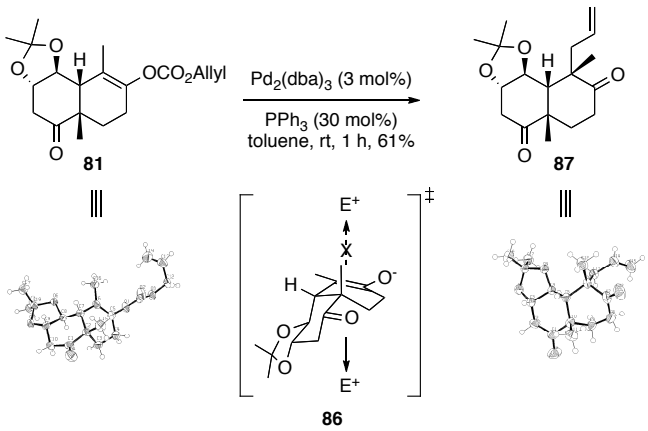
Trost et al. conducted a series of experiments that unified the accepted mechanism with the observed results in the literature. A double-labeled crossover experiment was used to show that there is, in fact crossover in the ion-paired compounds. The authors reasoned that if there was crossover then the presence of a "naked" enolate was implied. If so, acidic additives would be expected to quench the enolate; it was found that an acid with PK_a = 13 (DMSO) (e.g., acetoacetone) would quench the "enolate", while dimethylmalonate [PK_a = 15.9 (DMSO)] would not. Based on this, it was reasoned that the carbonate anion—not the enolate—was being

protonated and that the rate-determining step was the loss of carbon dioxide to produce the ion-pair, which rapidly alkylates in a S_N2 to generate the product and Pd^0.

In addition to the allylation highlighted in Scheme I-14 involving the *trans*-IMDA product **54**, the Tsuji allylation was conducted on the *cis*-IMDA product (Scheme I-15, **81**). One can track the selectivity of this reaction from X-ray structure to X-ray structure: allylation occurs from the face opposite the angular methyl substituent. The selectivity is similar with the above case (i.e., *trans*-IMDA substrate **54**), but with the added effect of the 2-position (i.e., methine annulation point) being in an axial orientation. One could expect that the selectivity may not be as great for this substrate, but in fact it remains to be so: thermodynamically, 1,2-interactions are generally less energetically costly than the 1,3-diaxial interaction.

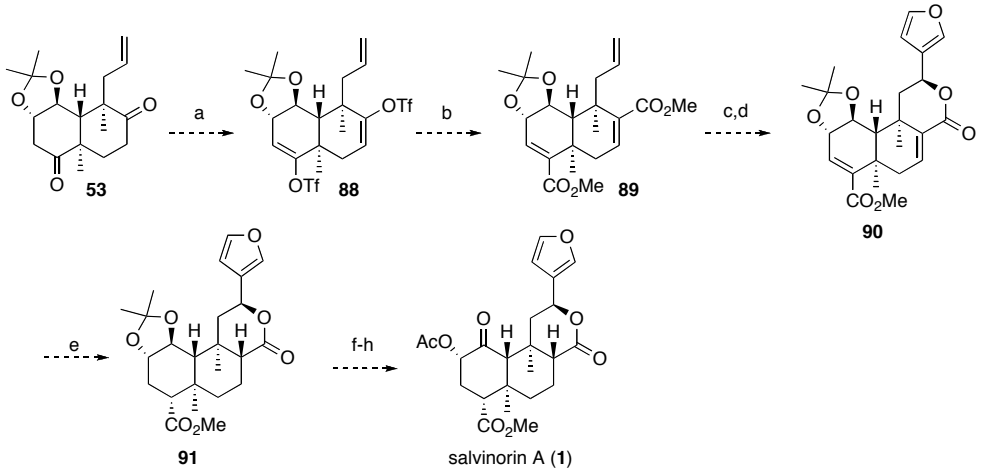
The synthesis of **87** could be used as more than simply a tool to explore our chemical intrigues and a selectivity hypothesis; it could be used as a model study for forward reactions toward salvinorin A (**1**), as it contains identical functional groups.

Scheme I-15. Tsuji allylation with *cis*-IMDA substrate **81**



Future work toward the total synthesis of **1** will be guided by the rough strategy as presented in Scheme I-16. A *bis*-carbonylation of the ditriflate **88** could give the diester **89** which could be transformed into **90** by chemoselective addition of a 3-furanyl nucleophile to an aldehyde derived from oxidative cleavage of the terminal olefin. The carbonylation reaction necessitates further discussion. During the first generation salvinorin A (**1**) campaign, Hagiwara et al. mentioned that, "Attempts to introduce α,β-unsaturated ester groups at C4 and C8 by palladium-catalyzed carbonyl insertion of the corresponding trifluoromethanesulfonylenol ether[s] were not fruitful…" yet the group managed to effect this transformation on substrate **50** (cf., Section I.B, Scheme I-4). The furyl ketone in **50** may have been serving as a ligand (i.e., directing group) helping to facilitate the transformation at the sterically congested (i.e., *neo*-pentyl) C8 center. Therefore, it may be advantageous to add the furan core prior to the palladium-mediated carbonylation.

Scheme I-16. Future work toward the total synthesis of salvinorin A (**1**)[a]

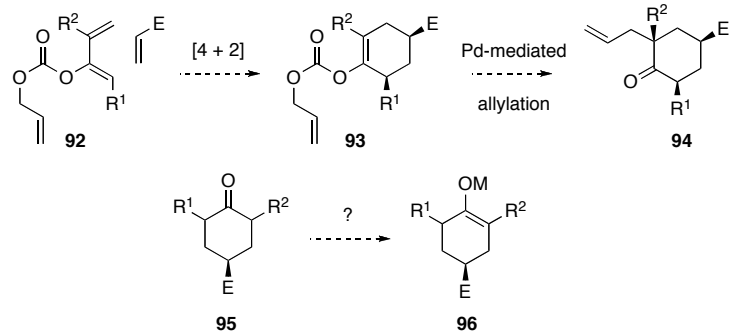


[a] Conditions: (a) Comins' reagent, NaHMDS, THF; (b) $Pd(PPh_3)_4$, CO, dppf, Et_3N, MeOH, DMF; (c) O_3 or OsO_4, $NaIO_4$; (d) 3-bromofuran, *t*-BuLi, THF; (e) SmI_2, Et_3N, AcOH; (f) PTSA, MeOH; (g) acetylation; (h) [O].

Moving forward from **90**, samarium diiodide could the used to simultaneously effect *bis*-enoate reduction—taking Hagiwara's lead (cf., Section I.B, Scheme I-4)—to provide **91**. After revelation of a diol derived from the acetonide **91**, one is still left with the task of differentiating the diols and oxidation to provide **1**.

Perhaps even more interestingly, future work can be directed toward the development of an intermolecular Diels–Alder/Tsuji allylation sequence. This transformation has the potential to address a fundamental issue in enolate chemistry: selective enolate formation at a position that shares a similar steric environment to that of the undesired site. On can see that the potential not only lies on the side of the intramolecular case as above, but opportunities are available in the intermolecular case as in **92** (Scheme I-17). Using this sequence, one can formally differentiate ketones such as **95** to produce enolate equivalents that are currently unobtainable by simple deprotonation: the "enolate" regioisomer is defined by the cycloaddition. In theory both the cycloaddition could be deemed enantioselective via asymmetric catalysis, and the facial selectivity of the substrate could be overridden with asymmetric catalysis; this could offer selective access to many of the possible stereoisomers.

Scheme I-17. Intramolecular Diels–Alder/Tsuji allylation sequence



I.D. Conclusion

A novel strategy toward the construction of regioselective enolates (via IMDA) and their subsequent involvement in diastereoselective palladium-mediated allylations has been discovered and successfully implemented. This strategy was inspired by the molecular architecture of salvinorin A (**1**), which displays a unique biological effects arising from an intriguing mode of action: only known non-nitrogenous κ-opioid agonist.[3]

Salvinorin A (**1**), and related compounds offer a unique and informative probe for opioid receptor agonists as well as antagonists. The development of KOR antagonists could have an impact on many human diseases that involve the over-expression of KORs: Alzheimer's disease and schizophrenia have been hypothesized to be influenced by an over-expression of KORs in patients.[8,11,12] A potential relationship between KOR agonists and antagonists could be identified by further examination of the mechanism in which **1** and its analogs effect pharmacological responses via opioid receptors. An efficient synthetic route that is easily modified to incorporate desired structural modifications on the neoclerodane core could elicit further understanding from biological standpoint.

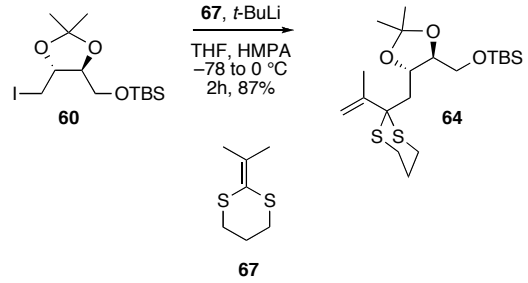
With the intramolecular Diels–Alder/Tsuji allylation established,[20] the scope of this transformation might be broadened to include the intramolecular variants. Such a transformation would—as in the IMDA/Tsuji allylation—allow for the regioselective generation of enolates that are currently not obtainable by simple deprotonation and their subsequent diastereoselective palladium-catalyzed allylation. Asymmetric catalysis could be useful during both transformations: to provide enantiomerically pure

Diels–Alder adducts and during the allylation one could potentially override the substrate selectivity. If realized, this approach could be useful in the construction of many biologically interesting natural products.

I.G. Experimental Section

Unless noted otherwise, all oxygen and moisture-sensitive reactions were executed in oven-dried glassware sealed under a positive pressure of dry argon or nitrogen. Moisture-sensitive solutions and anhydrous solvents were transferred via standard syringe and cannula techniques. All commercial reagents were used as received. Organic solvents were dried under nitrogen: tetrahydrofuran (THF) and diethyl ether were distilled over Na-benzophenone; CH_2Cl_2, HMPA, toluene, and triethylamine were distilled from CaH_2; DMSO was stored over freshly activated 4Å molecular sieves under N_2. Flash chromatography was performed using Baker Flash silica gel 60 (40 μm); analytical TLC was performed using 0.25 mm EM silica gel 60 F_{254} plates that were visualized under UV light (254 nm) or by staining with anisaldehyde reagent (450 mL of 95% ethanol, 25 mL conc. H_2SO_4, 15 mL acetic acid, and 25 mL anisaldehyde) and heating. Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. IR spectra were recorded using a Perkin-Elmer 683 infrared spectrophotometer. NMR spectra were obtained using INOVA 500 and 300 MHz Varian instruments. High-resolution mass spectrometric data were obtained using a Bruker BioTOF II (ESI) mass spectrometer.

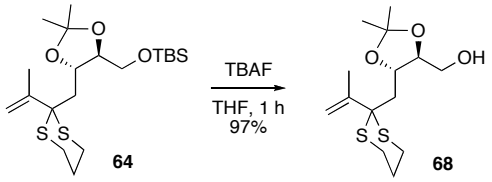
(–)-tert-Butyl(((4S,5S)-2,2-dimethyl-5-((2-(prop-1-en-2-yl)-1,3-dithian-2-yl)methyl)-1,3-dioxolan-4-yl)methoxy)dimethylsilane (**64**):



The allylic dithiane, 2-(Propan-2-ylidene)-1,3-dithiane[32] (2.0 g, 13 mmol) was dissolved in a mixture of THF (25 mL) and HMPA (3.3 mL, 19 mmol) and the resultant solution was cooled to –78 °C. *t*-BuLi (7.7 mL, 1.7M in pentane, 13 mmol) was added over 5 min with stirring and under an atmosphere of argon. The solution was allowed to reach 25 °C over 1 h. The temperature was reduced to –78 °C, and *tert*-butyl(((4*S*,5*R*)-5-(iodomethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-dimethylsilane (**60**,[29] 5.8 g, 15 mmol) was added in one portion (neat). The reaction mixture was allowed to warm to 0 °C over 2 h, saturated aqueous NH_4Cl (25 mL) was added, and the separated aqueous layer was extracted with ether (3 x 25 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (hexanes-ethyl acetate, 50:1, v/v) of the residue provided **64** (4.6 g, 11 mmol, 87%) as a colorless oil: R_f 0.32 (hexanes-ethyl acetate, 10:1, v/v); $[\alpha]^{25}_D$ -4.2 (*c* 0.47, $CHCl_3$); IR (neat) 2986, 2932, 2859, 1462, 1372, 1253, 1088; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.58 (d, *J*=1.5 Hz, 1H), 5.29 (dq, *J*=1.5, 1.5 Hz, 1H), 4.05 (dt, *J*=10.8, 3.6 Hz, 1H), 3.78-3.63 (m, 3H), 2.94-2.80 (m, 2H), 2.67-2.59 (m, 2H), 2.23-2.21 (m, 2H), 2.08-1.86 (m, 5H), 1.40 (s, 3H), 1.35 (s, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.0,

119.2, 109.3, 81.6, 75.1, 63.9, 58.2, 44.4, 27.6 (2C), 27.5, 27.2, 26.1 (3C), 25.3, 20.2, 18.6, -5.2, -5.1; HRMS (ESI) calc. for $[M + Na]^+$ 441.1924, found 441.1935.

(–)-((4S,5S)-2,2-Dimethyl-5-((2-(prop-1-en-2-yl)-1,3-dithian-2-yl)methyl)-1,3-dioxolan-4-yl)methanol (**68**):

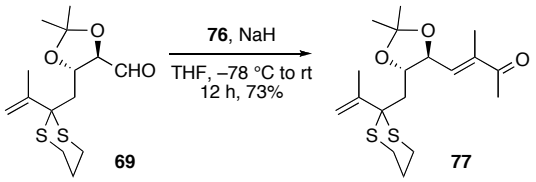


TBAF (12.0 mL, 1.0 M in THF, 12 mmol) was added to a solution of silyl ether **64** (4.6 g, 11 mmol) in THF (40 mL) while stirring at rt. After 1 h, the reaction mixture was diluted with diethyl ether (40 mL) and washed with brine (3 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Flash chromatography (hexanes-ethyl acetate, 2:1, v/v) of the residue provided alcohol **68** (3.21 g, 10.5 mmol, 97%) as a colorless oil: R_f 0.32 (hexanes-ethyl acetate, 2:1, v/v); $[\alpha]^{25}_D$ -30.9 (*c* 1.65, $CHCl_3$); IR (neat) 3468, 2985, 2933, 1374, 1246, 1061; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.56 (d, *J*=1.5 Hz,1H), 5.31 (dq, *J*=1.5, 1.5 Hz, 1H), 4.06 (dt, *J*=7.5, 3.0 Hz, 1H), 3.84-3.76 (m, 2H), 3.66 (ddd, *J*=11.8, 7.5, 4.8 Hz, 1H), 2.90-2.84 (m, 2H), 2.65 (dd, *J*=4.5, 3.0 Hz, 1H), 2.62 (J=5.0, 3.5, 1H), 2.28 (dd, *J*=15.5, 7.0 Hz, 1H), 2.10 (dd, *J*=15.0, 3.0 Hz, 1H), 2.02 (dtt, *J*=8.0, 5.0, 3.0 Hz, 1H), 1.92-1.83 (m, 5H), 1.44 (s, 3H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.9, 119.3, 109.4, 81.9, 73.6, 61.9, 58.0, 44.3, 27.6, 27.5, 27.4, 27.3, 25.1, 20.1; HRMS (ESI) calc. for $[M + Na]^+$ 327.1059, found 327.1050.

(–)-(4R,5S)-2,2-Dimethyl-5-((2-(prop-1-en-2-yl)-1,3-dithian-2-yl)methyl)-1,3-dioxolane-4-carbaldehyde (**69**):

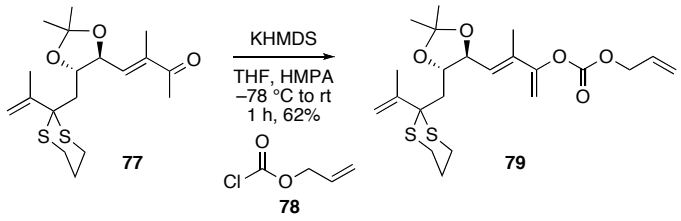
A solution of alcohol **68** (3.8 g, 12.5 mmol) in CH_2Cl_2 (12.5 mL) was added to a suspension of SO_3·Py (7.96 g, 50.0 mmol), triethylamine (8.7 mL, 62.5 mmol), and DMSO (8.9 mL, 125 mmol) in CH_2Cl_2 (100 mL) at 0 °C with stirring. The mixture was allowed to stir at this temperature for 2 h before saturated aqueous NH_4Cl (50 mL) was added. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The crude aldehyde was taken forward without purification. Flash chromatography of an analytical sample (hexanes-ethyl acetate, 2:1, v/v, *p*-anisaldehyde stain) provided aldehyde **69** as a colorless oil: R_f 0.53-0.10 (hexanes-ethyl acetate, 2:1, v/v); $[\alpha]^{25}_D$ -30.7 (*c* 1.35, $CHCl_3$); IR (neat) 2986, 2932, 2857, 1734, 1377, 1249, 1080; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.69 (d, *J*=2.4 Hz, 1H), 5.53 (dq, *J*=1.2, 0.3 Hz, 1H), 5.28 (dq, *J*=1.4, 1.4 Hz, 1H), 4.24 (ddd, *J*=7.5, 5.4, 4.7 Hz, 1H), 4.00 (dd, *J*=7.4, 2.4 Hz, 1H), 2.91-2.76 (m, 2H), 2.72-2.61 (m, 2H), 2.32 (d, *J*=4.8 Hz, 1H), 2.31 (d, *J*=5.4 Hz, 1H), 2.01-1.85 (m, 5H), 1.46 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.2, 142.8, 119.1, 111.5, 84.9, 73.6, 57.4, 43.1, 27.5, 27.4, 27.2, 26.5, 25.0, 19.9; HRMS (ESI) calc. for $[M + Na + MeOH]^+$ 357.1165, found 357.1170.

(–)-(E)-4-((4S,5S)-2,2-Dimethyl-5-((2-(prop-1-en-2-yl)-1,3-dithian-2-yl)methyl)-1,3-dioxolan-4-yl)-3-methylbut-3-en-2-one (**77**):



Dimethyl 3-oxobutan-2-ylphosphonate[35] (**76**, 2.6 g, 12.5 mmol) in THF (12 mL) was added to a cooled (0 °C) suspension of NaH (550 mg, 60% in mineral oil, 13.8 mmol) in THF (25 mL). The mixture was allowed to stir at this temperature for 1 h before crude aldehyde **69** (~12.5 mmol) in THF (12 mL) was added in one portion. The solution was allowed to warm to rt over 3 h, at which time saturated aqueous NH_4Cl (50 mL) was added. The layers were separated and the aqueous layer was extracted with diethyl ether (3 x 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (hexanes-ethyl acetate, 5:1, v/v) provided **77** (2.45 g, 6.87 mmol, 55% over two steps) as a colorless oil: R_f 0.42 (hexanes-ethyl acetate, 5:1, v/v); $[\alpha]^{25}_D$ -18.0 (*c* 0.59, $CHCl_3$); IR (neat) 2986, 2932, 2917, 1677, 1424, 1371, 1241; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.40 (dq, *J*=8.7, 1.4 Hz, 1H), 5.51 (dq, *J*=1.6, 0.6 Hz, 1H), 5.28 (dq, *J*=1.3, 1.3 Hz, 1H), 4.42 (t, *J*=8.4 Hz, 1H), 3.94 (ddd, *J*=8.4, 7.0, 2.2 Hz, 1H), 2.88-2.77 (m, 2H), 2.62-2.55 (m, 2H), 2.32 (s, 3H), 2.27 (dd, *J*=15.2, 7.0 Hz, 1H), 2.00-1.94 (m, 2H), 1.86-1.77 (m, 7H), 1.44 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 199.6, 142.9, 141.4, 137.3, 119.1, 110.0, 77.5, 77.3, 57.8, 42.2, 27.5 (2C), 27.3, 27.1, 25.9, 25.0, 20.0, 12.3; HRMS (ESI) calc. for $[M + Na]^+$ 379.1372, found 379.1387.

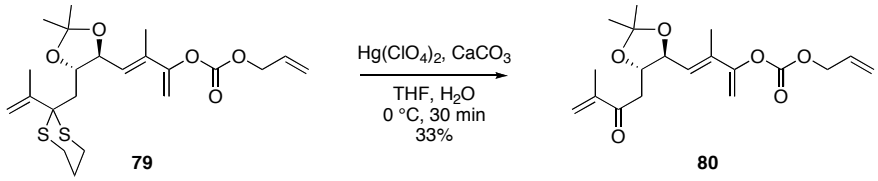
Allyl (–)-(E)-4-((4S,5S)-2,2-dimethyl-5-((2-(prop-1-en-2-yl)-1,3-dithian-2-yl)methyl)-1,3-dioxolan-4-yl)-3-methylbuta-1,3-dien-2-yl carbonate (**79**):



Ketone **77** (670 mg, 1.88 mmol) was dissolved in a mixture of THF (190 mL) and HMPA (990 μL, 5.64 mmol) and the resultant solution was cooled to –78 °C. With stirring, KHMDS (5.64 mL, 0.5M-toluene, 13 mmol) was added over 1 min and allowed to stir an additional 15 min at –78 °C. To this solution was added allylchloroformate (300 μL, 2.82 mmol) in one portion. The resultant solution was allowed to reach rt over 1, at which time saturated aqueous NH_4Cl (50 mL) was added and the aqueous layer was extracted with ether (3 x 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (200 mL), brine (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (hexanes-ethyl acetate, 10:1, v/v) provided **79** (417 mg, 0.946 mmol, 62%) as a colorless oil: R_f 0.42 (hexanes-ethyl acetate, 5:1, v/v); $[\alpha]^{25}_D$ -21.3 (*c* 0.015, $CHCl_3$); IR ($CHCl_3$) 2984, 2932, 2901, 1763, 1220; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.97 (ddt, *J*=17.2, 10.5, 5.8 Hz, 1H), 5.72 (dd, *J*=9.0, 1.2 Hz, 1H), 5.52 (dd, *J*=1.5, 0.4 Hz, 1H), 5.40 (dq, *J*=17.2, 1.5 Hz, 1H), 5.30 (dq, *J*=10.5, 1.2 Hz, 1H), 5.26 (dq, *J*=1.3, 1.3 Hz, 1H), 5.15 (d, *J*=2.4 Hz, 1H), 5.02 (d, *J*=2.5 Hz, 1H), 4.68 (t, *J*=1.4, 1H), 4.67 (t, *J*=1.4, 1H), 4.37 (t, *J*=8.3 Hz, 1H), 3.83 (ddd, *J*=10.2, 8.0, 2.1, 1H), 2.89-2.78 (m, 2H), 2.66-2.58 (m, 2H), 2.21 (dd, *J*=15.0, 7.4 Hz, 1H), 2.03 (dd, *J*=15.0, 2.1 Hz, 1H), 2.00-1.95 (m, 1H), 1.94 (d, *J*=1.3 Hz, 3H), 1.91-1.85 (m, 1H), 1.83 (d, *J*=1.0 Hz, 3H), 1.41 (s, 3H), 1.37 (s, 3H); ^{13}C

NMR ($CDCl_3$, 125 MHz) δ 154.0, 153.1, 142.9, 133.8, 131.4, 124.5, 119.4, 119.1, 109.5, 103.8, 77.6, 77.5, 69.1, 57.8, 42.0, 27.6, 27.5, 27.4, 27.3, 25.2, 19.9, 14.2; HRMS (ESI) calc. for $[M + Na]^+$ 463.1583, found 463.1582.

Allyl (–)-(E)-4-((4S,5S)-2,2-dimethyl-5-(3-methyl-2-oxobut-3-enyl)-1,3-dioxolan-4-yl)-3-methylbuta-1,3-dien-2-yl carbonate (**80**):

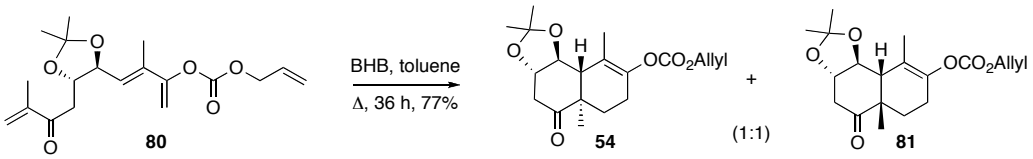


79 **80**

To a cooled (0 °C) suspension of dithiane **79** (6.4 g, 14.5 mmol) and $CaCO_3$ (3.2 g, 31.9 mmol), in THF (40 mL) and H_2O (6 mL), was added a solution of $Hg(ClO_4)_2 \cdot 3H_2O$ (7.25 g, 16.0 mmol) in H_2O (4 mL) in one portion. The resultant mixture was allowed to stir at 0 °C for 30 min, at which time saturated aqueous $NaHCO_3$ (50 mL) was added then the separated aqueous layer was extracted with ether (3 x 50 mL). The combined organic layers were washed with saturated aqueous, brine (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography (hexanes-ethyl acetate, 5:1, v/v) of the residue provided **80** (1.68 g, 4.79 mmol, 33%) as a colorless oil: R_f 0.29 (hexanes-ethyl acetate, 3:1, v/v); $[\alpha]^{25}_D$ -135.4 (*c* 0.0007, $CHCl_3$); IR (neat) 2986, 2900, 1765, 1225; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.05-5.91 (m, 2H), 5.83 (s, 1H), 5.76 (d, *J*=8.8 Hz, 1H), 5.41 (dq, *J*=17.2, 1.3 Hz, 1H), 5.31 (dq, *J*=10.4, 1.2 Hz, 1H), 5.16 (d, *J*=2.5 Hz, 1H), 5.05 (d, *J*=2.4 Hz, 1H), 4.71 (q, *J*=1.3 Hz, 1H), 4.69 (q, *J*=1.3 Hz, 1H), 4.51 (t, *J*=8.3 Hz, 1H), 4.21 (dt, *J*=7.8, 3.9 Hz, 1H), 3.04 (dd, *J*=15.9, 7.8 Hz, 1H), 2.78 (dd, *J*=15.8, 4.0 Hz, 1H), 1.94 (d, *J*=1.2 Hz, 3H), 1.93 (t, *J*=1.3 Hz, 3H), 1.44 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 125

MHz) δ 198.6, 153.9, 153.1, 145.0, 134.0, 131.4, 124.7, 124.0, 119.6, 109.5, 103.9, 77.3, 77.1, 69.2, 39.6, 27.4 (2C), 17.7, 14.1; HRMS (ESI) calc. for $[M + Na]^+$ 373.1622, found 373.1632.

Allyl (+)-(3aS,5aR,9aR,9bS)-2,2,5a,9-tetramethyl-5-oxo-3a,4,5,5a,6,7,9a,9b-octahydronaphtho[1,2-d][1,3]dioxol-8-yl carbonate (**54**):



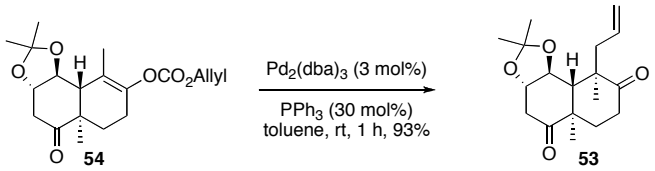
80 **54** **81**

A solution of tetraene **80** (3.3 g, 9.4 mmol) and butylated hydroxybenzene (194 mg, 0.94 mmol) in toluene (940 mL) was heated at reflux for 36 h, at which time the reaction mixture was cooled to rt and subsequently concentrated in vacuo. Flash chromatography (hexanes-ethyl acetate, 10:1, v/v) of the residue provided **54** and **81** (1:1, 2.54 g, 7.25 mmol, 77%) as an amorphous solid. Subsequent removal of the *cis* isomer **81** (containing 8% of **54**; one additional recrystallization provided pure **81** as well as the remaining 8% of **54**) by recrystallization (95% EtOH), provided pure **54** (1.00 g, 2.85 mmol, 30%) as a colorless oil: R_f 0.52 (hexanes-ethyl acetate, 3:1, v/v); $[\alpha]^{25}_D$ +43.1 (*c* 0.0065, $CHCl_3$); IR (neat) 2985, 2932, 1757, 1714, 1238; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.96 (ddt, *J*=17.1, 10.5, 5.7 Hz, 1H), 5.39 (dq, *J*=17.2, 1.5 Hz, 1H), 5.31 (dq, *J*=10.4, 1.2 Hz, 1H), 4.67 (q, *J*=1.3 Hz, 1H), 4.66 (q, *J*=1.3 Hz, 1H), 3.93 (dd, *J*=11.6, 8.5 Hz, 1H), 3.51 (ddd, *J*=13.7, 8.4, 5.0 Hz, 1H), 3.04 (t, *J*=13.4 Hz, 1H), 2.82 (dd, *J*=13.4, 5.0 Hz, 1H), 2.55 (ddq, *J*=11.6, 4.0, 1.3, 1H), 2.36-2.23 (m, 2H), 2.01 (ddd, *J*=13.8, 6.6, 2.2 Hz, 1H), 1.77 (q, *J*=2.2 Hz, 3H), 1.65 (ddd, *J*=13.8, 11.0, 8.0 Hz, 1H), 1.48 (s, 3H), 1.44 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.4,

153.2, 142.5, 131.5, 120.8, 119.3, 111.1, 79.1, 77.4, 69.0, 47.9, 44.4, 41.6, 29.7, 27.2, 27.1, 24.0, 17.8, 14.1; HRMS (ESI) calc. for $[M + Na]^+$ 373.1622, found 373.1624.

Data for: Allyl (+)-(3a*S*,5a*S*,9a*R*,9b*S*)-2,2,5a,9-tetramethyl-5-oxo-3a,4,5,5a,6,7,9a,9b-octahydronaphtho[1,2-*d*][1,3]dioxol-8-yl carbonate (**81**): R_f 0.51 (hexanes-ethyl acetate, 3:1, v/v); m.p. 95-98 ^{0}C; $[\alpha]^{25}_D$ +32.2 (*c* 0.0057, $CHCl_3$); IR (neat) 2985, 2936, 1752, 1713, 1239; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.96 (ddt, *J*=17.2, 10.5, 5.8 Hz, 1H), 5.39 (dq, *J*=17.2, 1.5 Hz, 1H), 5.31 (dq, *J*=10.5, 1.2 Hz, 1H), 4.68 (q, *J*=1.3 Hz, 1H), 4.66 (q, *J*=1.3 Hz, 1H), 3.73 (dd, *J*=10.8, 9.0 Hz, 1H), 3.61 (ddd, *J*=13.6, 9.0, 4.7 Hz, 1H), 3.04 (t, *J*=13.7 Hz, 1H), 2.90 (dd, *J*=14.6, 4.7 Hz, 1H), 2.73 (dd, *J*=14.5, 13.7, 1H), 2.41-2.32 (m, 1H), 2.20 (dddq, *J*=17.4, 6.8, 3.3, 3.3 Hz, 1H), 2.10 (d, *J*=10.8 Hz, 1H), 2.01 (ddd, *J*=13.1, 11.4, 6.8 Hz, 1H), 1.76 (t, *J*=2.0 Hz, 3H), 1.47 (s, 3H), 1.45 (s, 3H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 211.1, 153.0, 141.9, 131.5, 121.9, 119.3, 111.4, 82.9, 76.9, 69.0, 48.1, 47.0, 43.0, 29.6, 27.3 (2C), 23.2, 20.0, 16.1; HRMS (ESI) calc. for $[M + Na]^+$ 373.1622, found 373.1621.

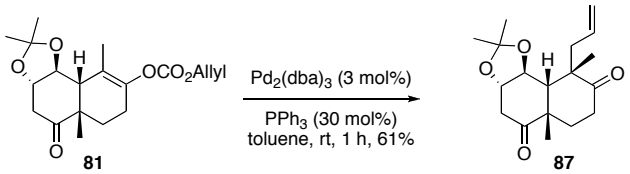
(–)-(3aS,5aR,9R,9aS,9bS)-9-Allyl-2,2,5a,9-tetramethyl-hexahydronaphtho[1,2-d][1,3]dioxole-5,8(5aH,9bH)-dione (**53**):



A solution of enol-carbonate **54** (100 mg, 0.285 mmol) in toluene (1 mL) was added in one portion to a solution of triphenylphosphine (37 mg, 0.14 mmol) and $Pd_2(dba)_3$ (13 mg, 0.014 mmol) in toluene (10 mL). The resultant solution was allowed to stir at rt for 1 h, at which time the reaction mixture was concentrated in vacuo. Flash

chromatography (hexanes-ethyl acetate, 5:1, v/v) of the residue provided **53** (75 mg, 0.245 mmol, 86%) as a colorless solid. Recrystallization from 95% EtOH provided a colorless crystalline solid: mp 69-70 °C; R_f 0.41 (hexanes-ethyl acetate, 3:1, v/v); $[\alpha]^{25}_D$ -8.3 (*c* 0.0035, $CHCl_3$); IR ($CHCl_3$) 2983, 2932, 1713, 1701, 1232; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.56 (dddd, *J*=16.7, 10.1, 8.8, 6.4 Hz, 1H), 5.08 (dd, *J*=10.2, 2.2 Hz, 1H), 5.01 (dq, *J*=17.0, 1.3 Hz, 1H), 3.95 (dd, *J*=11.5, 8.6 Hz, 1H), 3.55 (ddd, *J*=11.8, 8.6, 6.6 Hz, 1H), 2.91-2.83 (m, 3H), 2.53-2.42 (m, 5H), 2.29 (d, *J*=11.5 Hz, 1H), 1.99-1.87 (m, 3H), 1.49 (s, 3H), 1.45 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 215.1, 209.2, 134.1, 119.7, 111.3, 77.8, 77.5, 50.1, 47.6, 45.8, 44.5, 42.4, 35.0, 30.5, 27.0, 26.9, 21.6, 19.6; HRMS (ESI) calc. for $[M + Na]^+$ 329.1723, found 329.1718.

Data for (+)-(3aS,5aS,9S,9aS,9bS)-9-Allyl-2,2,5a,9-tetramethyl-hexahydro-naphtho[1,2-d][1,3]dioxole-5,8(5aH,9bH)-dione (**87**):



81 **87**

R_f 0.41 (hexanes-ethyl acetate, 3:1, v/v); $[\alpha]^{25}_D$ +5.37 (*c* 0.0054, $CHCl_3$); IR ($CHCl_3$) 2983, 2932, 1706, 1701, 1234; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.59 (dddd, *J*=16.9, 10.0, 7.7, 6.9 Hz, 1H), 5.06 (dq, *J*=16.6, 1.4 Hz, 1H), 5.04 (dq, *J*=10.0, 1.0 Hz, 1H), 3.98 (ddd, *J*=11.3, 9.2, 6.5 Hz, 1H), 3.77 (dd, *J*=11.4, 9.2 Hz, 1H), 3.11 (dd, *J*=17.7, 6.5 Hz, 1H), 2.56 (dd, *J*=17.7, 11.3 Hz, 1H), 2.54-2.29 (m, 5H), 1.95 (d, *J*=11.3 Hz, 1H), 1.86-1.81 (m, 1H), 1.48 (s, 3H), 1.46 (s, 3H), 1.24 (s, 3H), 1.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.9, 211.6, 133.6, 118.4, 111.3, 78.3, 74.7, 53.3, 50.0, 48.0, 43.0, 38.7, 34.2, 28.5, 27.4, 27.3, 26.6, 23.4; HRMS (ESI) calc. for $[M + Na]^+$ 329.1723, found 329.1716.

Chapter II. Sea Lamprey Migratory Pheromone: Synthetic Explorations

II.A. Introduction

About a century ago the sea lamprey invaded the Great Lakes of North America, where it triggered the collapse of many fisheries.[38] This effect was primarily confronted by implementation of a toxicant-based program using 3-trifluoromethyl-4-nitrophenol (TFM) as a larvicide, literally pumping this compound into freshwater lakes, streams, and rivers.[37] Other methods that are currently used in an attempt to control undesired sea lamprey include maintaining barriers to migration, trapping of adults, and the controlled release of sterilized males.[38] Although TFM does exhibit selective toxicity to larval sea lamprey, it stands to reason that large-scale introduction of fluorinated hydrocarbons into the aquatic system is less than ideal.

The anadromous (i.e., fish that migrate up-stream from the sea to breed in fresh water) sea lamprey spawns in fresh water where its larvae spend 3–20 years filter-feeding prior to their metamorphosis, beginning the parasitic phase of their lifecycle. During the parasitic phase of the sea lamprey lifecycle it feeds off of host fish by direct attachment. After approximately one year, the parasitic lamprey transition into the migratory phase of their lifecycle in which they detach from the host and migrate to suitable streams to spawn and subsequently die.[39]

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[37] Appelgate, V. C.; Smith, M. A.; Willeford, B. R. "Molecular characteristics Versus Biological Activity" *Chemistry* **1967**, *40*, 28–30.
[38] Li, W.; Twohey, M.; Jones, M.; Wagner, M. "Research to Guide Use of Pheromones to Control Sea Lamprey" *J. Great Lakes Res.* **2007**, *33*, 70–86.
[39] Stacey, N.; Sorensen, P. "Reproductive Pheromones" *Fish Physiology* **2006**, *24*, 359–412.

Sea Lamprey pheromones have been extensively studied from a fundamental standpoint (i.e., their olfactory behavior), as well as the practical stance that the results may prove useful in the development of chemical strategies to control these invasive (e.g., Great Lakes) fish that threaten the natural fish populations.

Many species of aquatic vertebrates perform extensive migrations between specific locales relying on chemical cues. Pheromones—in this context—are defined as a subset of chemical cues that pass between members of the same species (i.e., conspecific) and elicit a unique and adaptive response that does not require learning.[39,40] It should be reiterated that all chemical cues are not pheromonal; sea lamprey are known to respond to the amino acid **L**-arginine and are thought to use this as a method of finding nearby prey during the parasitic phase of their lifecycle.[41] A better understanding of these behavioral responses—on a molecular level—has been sought after by several research groups. Sorensen et al. pioneered the implementation of the electro-olfactogram (EOG) method[42] with live sea lamprey demonstrating that lamprey can distinguish bile acids at extremely low concentrations (e.g., 10^{-12} M). In addition, it was shown by cross-adaptation studies that the substrates eliciting strong EOG responses [i.e.; allocholic acid (**100**), petromyzonol sulfate (**101**), and taurolithocholic acid 3-sulfate (**102**), were likely doing so via different olfactory mechanisms (Figure II-

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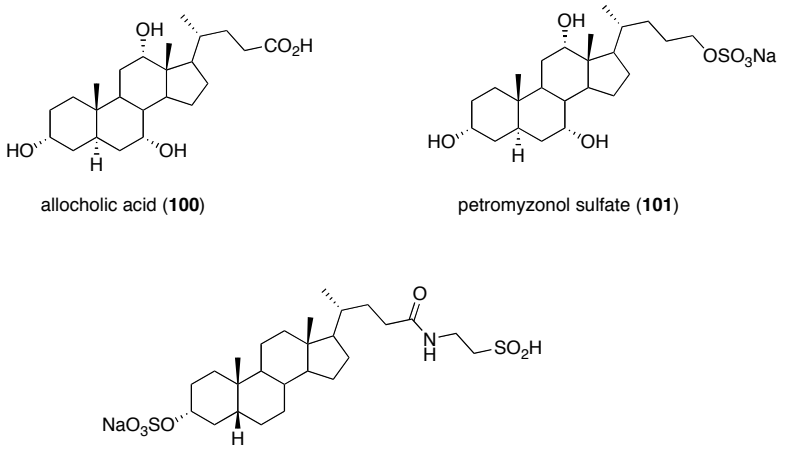
[40] Sorensen, P. W.; Fine, J. M.; Dvornikovs, V.; Jeffrey, C. S.; Shao, F.; Wang, J.; Vrieze, L. A.; Anderson, K. R.; Hoye, T. R. "Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey" *Nat. Chem. Biol.***2005**, *1*, 324–328.
[41] Kleerekoper, H.; Mogensen, J. "Role of Olfaction in the Orientation of *Petromyzon marinus*. I. Response to a Single Amine in Prey's Body Odor" *Physiological Zoology* **1963**, *36*, 347–360.
[42] Scott, J. W.; Scott-Johnson, P. E. "The electroolfactogram: A Review of Its History and Uses" *Microsc. Res. Tech.* **2002**, *58*, 152–160.

1).[43] The Sorensen group later provided evidence for the presence of at least four receptor classes as determined by the cross-adaptation studies of ten bile acids/derivatives.[44]

Figure II-1. Highly independent olfactory responses elicited by naturally occurring bile acids identified in Sorensen's landmark EOG study



Behavioral studies have established that the adult sea lamprey is more attracted to water from streams with larvae than those from streams without larvae.[45] It is interesting to note that a typical larva (i.e., ~1 g) can activate approximately 400 L of water in an hour: consistent with the attractive nature of stream waters. This paints a picture depicting migratory sea lamprey using the pheromone produced by larvae

[43] Li, W.; Sorensen, P. W.; Gallaher, D. D. "The Olfactory System of Migratory Adult Sea Lamprey (*Petromyzon marinus*) is Specifically and Acutely Sensitive to Unique Bile Acids Released by Conspecific Larvae" *J. Gen. Physiol.* **1995**, *105*, 569–587.
[44] Li, W.; Sorensen, P. W. "Highly independent olfactory receptor sites for naturally occurring bile acids in the sea lamprey, *Petromyzon marinus*" *J. Comp. Physiol. A* **1996**, *180*, 429–438.

residing in the freshwater streams as a guide to a suitable spawning environment. It is known that the biosynthesis of both allocholic acid (**100**) and petromyzonol sulfate (**101**) occurs in the liver—with subsequent storage in the gall bladder—of larvae. It has also been established that adult lamprey do not synthesize these compounds.[40]

The males arrive earlier than the females and build nests in areas where the flow rates are 0.5 to 1.5 $m \cdot s^{-1}$; shortly thereafter the males are joined by ovulating females at the site of the newly constructed nest.[46] This has been indicated to be the result of a sex pheromone (i.e., 3-keto petromyzonol sulfate) that is released by sperminating males and that attracts ovulated female lamprey upstream to the newly built nest for subsequent mating and hence proliferation.[47]

During the previously mentioned behavioral studies conducted by Sorensen et al., in which the effects of larval containing water were explored; it was noted that, although mixtures of **100** and **101** showed behavioral activity, they were consistently less attractive than larval holding waters. This led to an early hypothesis that the pheromone comprises additional components. In an effort to define the additional components of the sea lamprey pheromone, Sorensen et al. initiated a massive isolation campaign involving the large scale purification of 8000 L of larval water to yield three compounds in 0.2 to 1 mg quantities, the most potent of which petromyzonamine disulfate (Figure II-2, **102**) showed behavioral and physiological activity at

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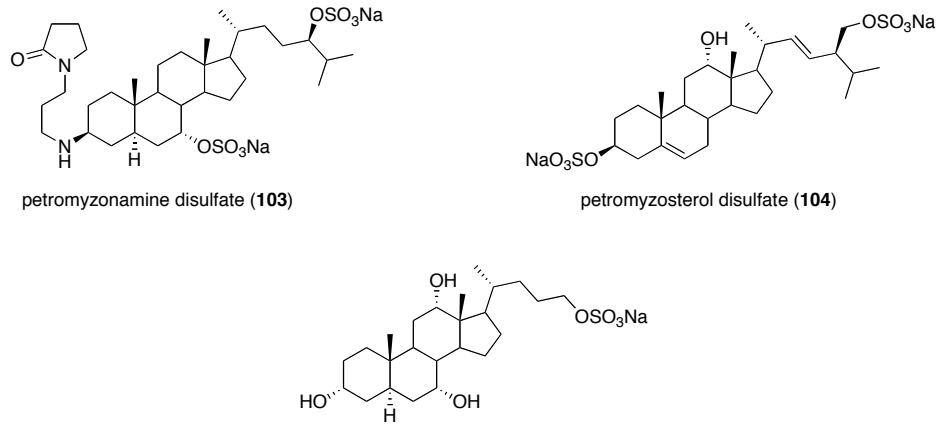
[45] Vrieze, L. A.; Sorensen, P. W. "Laboratory assessment of the role of a larval pheromone and natural stream odour in spawning stream localisation by migratory sea lamprey (*Petromyzon marinus*) *Can. J. Fish. Aquatic Sci.* **2001**, *58*, 2374–2385.
[46] Li, W.; Scott, A. P.; Siefkes, M. J.; Yan, H.; Liu, H.; Yun, S.; Gage, D. A. "Bile Acid Secreted by Male Sea Lamprey That Acts as a Sex Pheromone" *Science* **2002**, *296*, 138–141.

concentrations as low as 10^{-13} M (a possible record for fish). In a joint effort with the Hoye group, the structures of these compounds were deduced using a combination of high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) experiments (Figure II-2).[41,47]

Figure II-2. Pheromone components isolated by Sorensen et al.



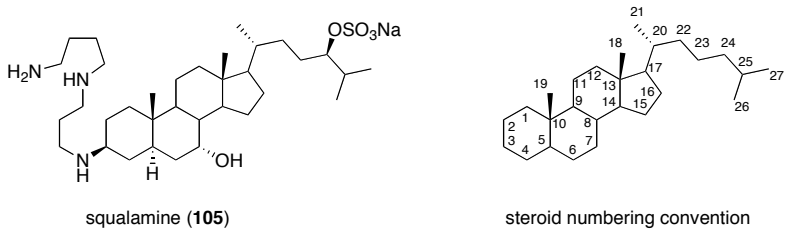
The structure of petromyzonamine disulfate (**103**) was established as follows. First MS, MS-MS, and high-resolution MS studies indicated that there were two sulfur atoms present in the molecule in the form of ionizable sulfate esters. Furthermore, there was only one exchangeable proton in which deuteration studies pointed to the existence of an NH-group in the molecule containing six units of unsaturation.

The ^{1}H NMR spectrum exhibited the characteristic—two methyl singlets and three methyl doublets—pattern of cholesterol-like steroids. Literature searching for

[47] Hoye, T. R.; Dvornikovs, V.; Fine, J. M.; Anderson, K. R.; Jeffrey, C. S.; Muddiman, D. C.; Shao, F.; Sorensen, P. W.; Wang, J. "Details of the Structure Determination of

nitrogenous steroids revealed squalamine (Figure II-3, **105**): a strikingly similar natural product isolated from the dogfish shark (*Squalus acanthias*), another ancient fish. Interestingly, **105** has been shown to be a potent antimicrobial agent and an antiangiogenic agent finding much interest in the medical community.[48]

Figure II-3. Structure of an antimicrobial and antiangiogenic aminosterol natural product: squalamine (**105**)



squalamine (**105**) steroid numbering convention

After the similarity to **105** was established by NMR analysis, remaining differences in the ^{1}H NMR spectra between **105** and **104** could be used to "adjust" the structure of **105** to provide **103** [e.g., the chemical shift of the C7 carbinol of squalamine (δ 3.79 ppm) was shifted downfield in **103**, hence the C7-sulfate). The side chain was posited to be an *N*-(3-aminopropyl)pyrrolidin-2-one based on 1D and 2D ^{1}H NMR analysis. It was subsequently further established through synthesis of model substrates and ultimately **103**. It is interesting to note that Hoye et al. found an interesting acidity effect on the chemical shifts of the protons adjacent to the secondary amine unit. When **103** was isolated as the HCl salt, the chemical shifts were downfield shifted by about 0.5 ppm in each case relative to the free base. At intermediate pH

the Sulfated Steroids PSDS and PADS: New Components of the Sea Lamprey (*Petromyzon marinus*) Migratory Pheromone" *J. Org. Chem.* **2007**, *72*, 7544–7550.

[48] Brunel, J. M.; Salmi, C.; Loncle, C.; Vidal, N.; Letourneux, Y. "Squalamine: A Polyvalent Drug of the Future?" *Current Cancer Drug Targets* **2005**, *5*, 267–272.

values the chemical shifts were observed as the weighted average of the protonated and unprotonated forms via rapid proton exchange at room temperature.

During the chemical synthesis of **103**—discussed in more detail later—Hoye et al. made both isomers of the C24-carbinol, which were independently subjected to Mosher ester analysis, thus corroborating the assignment of the C24-stereochemistry. In addition, synthetic **103** was shown to: (a) elicit electrophysiological activity from the sea lamprey olfactory system as judged by the electro-olfactogram (EOG) method; and (b) attract adult sea lamprey in behavioral test mazes with activity comparable to the isolated **103**.

The structure of petromyzosterol disulfate (**104**) was deduced in a similar way, but with comparison to another known sulfated steroidal natural product asteriidoside L. However, the authors mention that an unambiguous proof of the absolute configuration at C24 must await resolution by chemical synthesis, which is being currently pursued.

Petromyzonol sulfate (**101**) was a known compound, thus its structural proof halted at the stage of this realization. However, Hoye et al. did provide a more thorough ^{1}H and ^{13}C NMR spectroscopic analysis of the non-sulfated petromyzonol and that of **101** than had been previously reported in the literature.

At this point two goals—not mutually exclusive—were formulated: (a) to develop a synthesis toward analogs of **103**, and hence **103** itself; and (b) to interrogate the activity of these analogs by the electro-olfactogram (EOG) method. The results of the latter would feed into the former in the form of a feedback loop; the results of which would further the understanding of the olfactory receptor activation on a molecular level: the olfactophore.

II.B. Previous Syntheses of Squalamine (105)

In order to follow the logic behind the initial synthetic design and subsequent modifications, it is necessary to review what was known concerning the synthesis of squalamine (**105**). The pros and cons will be evaluated for each synthetic route from a practical standpoint (i.e., efficiency of the synthesis). Furthermore, it almost goes without mention to state that the so-called "*de novo*" steroid synthetic routes in which the steroid nucleus is prepared by successive annulation reactions, will not be considered. For example the de novo synthesis of (+)-chenodeoxycholic acid (a common *starting material* used in the synthesis of **105**) was prepared in around 40 steps from commercially available materials.[49] For an additional recent example of the de novo synthesis of *ent*-cholesterol in 16 steps from readily available starting materials.[50]

The first synthesis of squalamine (**105**) was reported by Moriarty et al. and began with the expensive starting material acetylcholenic acid [Scheme II-1, **106** (available in one step from cholenic acid which is currently about $500/g–Aldrich)].[51] After converting the carboxylic acid **106** to the acid chloride, addition of isopropylcadmium bromide (i.e., *i*-PrMgBr + $CdBr_2$) effected the conversion to a ketone; reduction of the ketone with calcium borohydride (i.e., $CaCl_2$ + $NaBH_4$) provided a carbinol compound. The authors do not mention the presence of selectivity in the reduction event, thus one can conclude—as would be expected—there was none.

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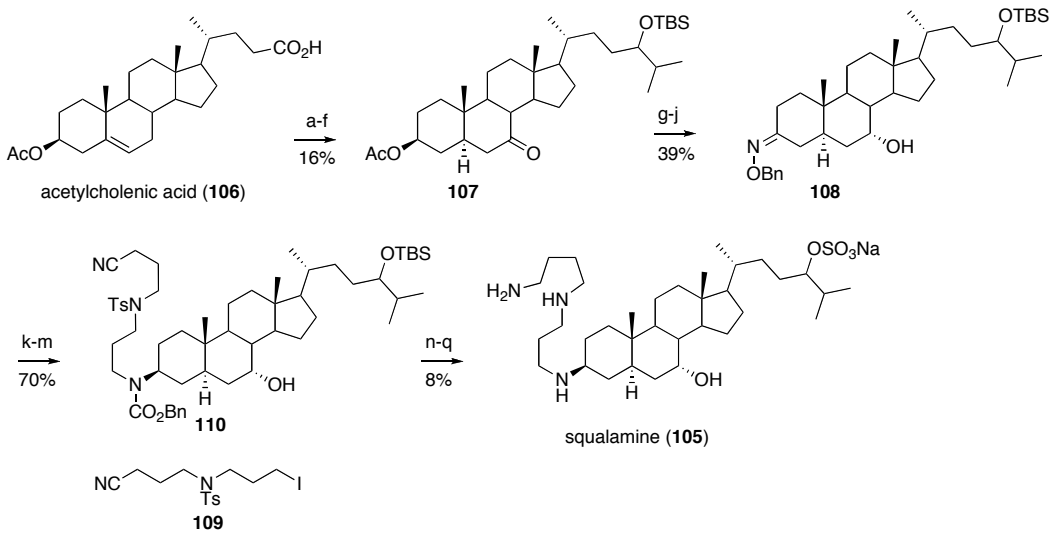
[49] Kametani, T.; Suzuki, K.; Nemoto, H. "First Total Synthesis of (+)-Chenodeoxycholic acid" *J. Am. Chem. Soc.* **1981**, *103*, 2890–2891.
[50] Belani, J. D.; Rychnovsky, S. D. "A Concise Synthesis of *ent*-Cholesterol" *J. Org. Chem.* **2008**, *73*, 2768–2773.
[51] Moriarty, R. M.; Tuladhar, S. M.; Guo, L.; Wehrli, S. "Synthesis of Squalamine. A steroidal antibiotic from the Shark" *Tetrahedron Lett.* **1994**, *35*, 8103–8106.

Silylation (i.e.; TBSCl, imidazole, CH_2Cl_2) of the resultant carbinol, allylic oxidation (i.e.; $Cr(CO)_6$, *t*-BuOOH), and dissolving metal reduction of the enone provided the *trans*-A,B-ring steroid **107**.

Scheme II-1. Moriarty's synthesis of squalamine (**105**)[a]



[a] Conditions: (a) $(COCl)_2$, CH_2Cl_2, Δ, 2 h; (b) *i*-PrCdBr, benzene, rt, 1 h; (c) $Ca(BH_4)_2$, THF, rt; (d) TBSCl, imidazole, CH_2Cl_2, rt, 16 h; (e) $Cr(CO)_6$, *t*-BuOOH, CH_3CN, Δ, 12 h; (f) Li, NH_3, Et_2O, –78 °C, 10 min; (g) K-selectride, THF, –50 °C, 5 h; (h) NaCN, MeOH, Δ, 8 h; (i) $(t\text{-BuO})_3Al$, toluene, Δ, 20 h; (j) $BnONH_2$•HCl, pyridine, EtOH, Δ, 16 h; (k) LAH, Et_2O, Δ, 16 h; (l) **109**, K_2CO_3, CH_3CN, Δ, 16 h; (m) BnOCOCl, NaOH, THF, 0 °C to rt, 4 h; (n) Na, NH_3, THF, –78 °C to rt; (o) LAH, Et_2O, Δ, 6 h; (p) HCl, EtOH, rt, 3 h; (q) SO_3•Py, pyridine, 75 °C, 2 h.

Equatorial hydride delivery (i.e., K-selectride) yielded an axial alcohol which was first deacetylated (i.e.; NaCN, MeOH) and regioselectively oxidized at the C3 carbinol position—an equatorial alcohol—to provide a ketoalcohol that was condensed with *O*-benzyl hydroxylamine hydrochloride salt to provide the oxime ether **109**. The oxime ether was subsequently reduced with lithium aluminum hydride to provide a primary amine, which was employed as a substrate for a *mono*-selective nucleophilic displacement reaction with the primary iodide **109** (i.e.; K_2CO_3, CH_3CN). Functionalization of the secondary amine as the carbamate was done as a means of

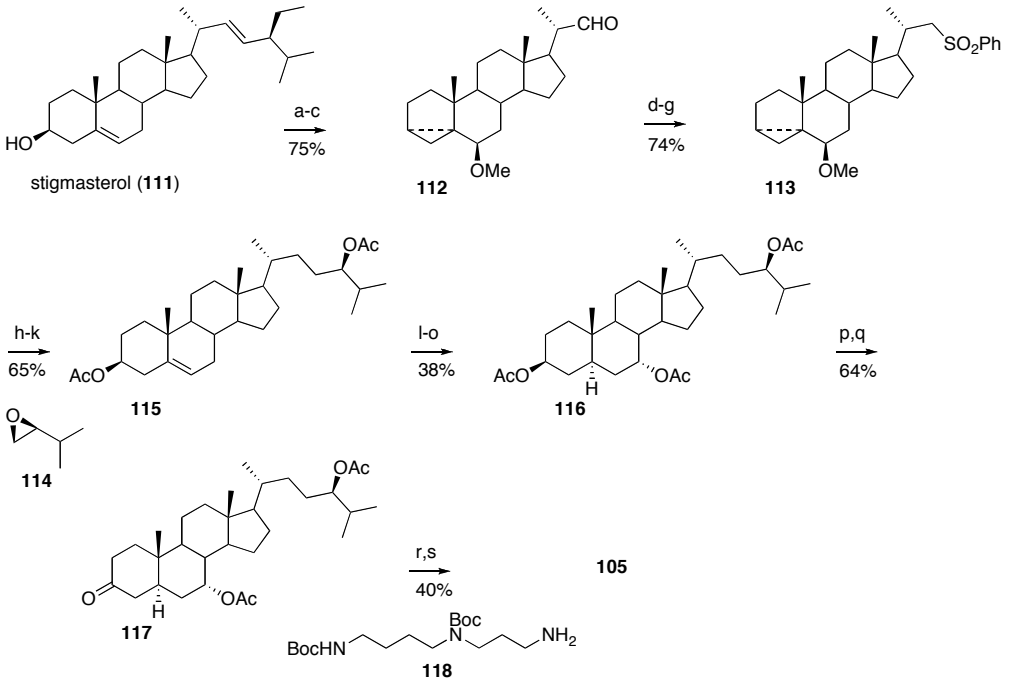
purification and was subsequently removed—along with the sulfonamide—under dissolving metal conditions (i.e.; Na, NH_3). Reduction of the cyanide followed by the acid assisted silyl ether cleavage generated polyamine product "protected" as the hydrochloride salt, which was employed in the final chemo- and regioselective sulfation (i.e.; SO_3·Py, pyridine) reaction providing squalamine (**105**).

The use of a prohibitively expensive starting material (i.e., cholenic acid) makes the direct use of this sequence impractical, but much can be learned from this work. First, allylic oxidation at C7 of $\Delta^{5,6}$-steroids (i.e., cholesterol like) is an important strategy to consider. Second, the net reductive amination sequence employed (i.e., oxime to primary amine) alkylation strategy must be considered—especially for a divergent analog strategy—as a means of employing the side chain. Third, one should be able to effect C24-selective (i.e., regioselective) sulfation in the presence of the protonated amine functionality (i.e., chemoselective). In **103** the C7-alcohol is in the form of a sulfate ester as well (i.e., *bis*-sulfate), however it is useful to know that analogs in which the C7-alcohol is not sulfated should be accessible. It should also be noted that **105** was prepared as a mixture of C24-epimers.

Moriarty subsequently reported a second-generation synthesis of **105**, starting from the cheap and readily available stigmasterol (Scheme II-2, **111**).[52] Protection of the $\Delta^{5,6}$-olefin as the *isomeric* cyclopropylmethyl ether (i.e., *i*-stigmasterol methyl ether) and subsequent ozonolysis of the disubstituted olefin provided the aldehyde **112**. The aldehyde was converted to the sulfone **113**: reduction (i.e., $NaBH_4$), conversion to the

primary iodide (i.e.; NaI, acetone), and displacement with sodium benzenesulfinate. An anion derived from the sulfone **113** (i.e.,**113** + *n*-BuLi) was employed as a nucleophile in the reaction with the electrophilic epoxide **114** incorporating the squalamine (**105**) C24 side chain with the appropriate stereochemistry.

Scheme II-2. Moriarty's second-generation synthesis of **105**[a]



[a] Conditions: (a) TsCl, pyridine, 14 h; (b) MeOH, KOAc, Δ, 4h; (c) O_3, MeOH, –78 °C; (d) $NaBH_4$, MeOH, 0 °C to rt; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 2 h; (f) NaI, acetone, Δ, 17 h; (g) $PhSO_2Na$, DMF, rt, 32 h; (h) *n*-BuLi, **114**, THF, –78 °C, 2 h; (i) Li, NH_3, –78 °C, 30 min; (j) TsOH, dioxane, H_2O, 80 °C, 1 h; (k) Ac_2O, pyridine, 14 h; (l) CrO_3, 1,3-dimethylpyrazole, CH_2Cl_2, –20 °C, 24 h; (m) Li, NH_3, –78 °C, 10 min; (n) K-selectride, THF, –50 °C, 6 h; (o) Ac_2O, DMAP, CH_2Cl_2, 14 h; (p) NaCN, MeOH, rt, 48 h; (q) Jones oxidation; (r) **118**, $NaBH_3CN$, 14 h; (s) HCl, MeOH, 14 h.

The *i*-steroid was converted to the homoallylic diacetate **115** by treatment with *p*-toluene sulfonic acid in the presence of water and subsequent acetylation. Another variant of the C7-allylic oxidation (i.e.; CrO_3, 3,5-dimethylpyrazole) was used, followed

[52] Moriarty, R. M.; Enache, L. A.; Kinney, W. A.; Allen, C. S.; Canary, J. W.; Tuladhar, S. M.; Guo, L. "Stereoselective Synthesis of Squalamine Dessulfate" *Tetrahedron Lett.* **1995**, *36*, 5139–5142.

by a dissolving metal enone reduction—with concomitant cleavage of the sulfur–carbon bond—to establish the *trans*-A,B-ring. Equatorial hydride delivery (i.e., K-selectride) and re-acetylation gave the triacetate **116**. Interestingly, the C3-acetate could be selectively removed by the action of sodium cyanide in methanol at room temperature for 48 hours; Jones oxidation (i.e.; CrO_3, H_2SO_4, acetone) provided the C3-ketone **117**. This time around a reductive amination was used to provide the secondary amine by way of the ketone, primary amine, and sodium cyanoborohydride; acid mediated removal of the carbamate groups provided **105**.

In this synthesis, the use of stigmasterol (**111**) as a starting material highlights an approach that is economical and can be considered for scale-up and/or the production of analogs. The sulfone anion–epoxide coupling and the reductive amination make the divergent incorporation of either side-chains manageable (i.e., C24-carbinol or amine side chains).

From a chemical standpoint, it is interesting to note that the steroid $\Delta^{5,6}$-olefin—when homoallylic alcohol is present—can be protected as the isomeric steroid methyl ether (i.e., *i*-steroid methyl ether). This can allow for oxidative manipulations to be conducted on the C21–C24 steroid side chain. It is also interesting to note the use of the Salmond oxidation (i.e., chromium trioxide and 3,5-dimethylpyrazole) to form the C7-enone which remains to be the best method for C7-allylic oxidation of steroids, if one

does not consider the toxicity of chromium (VI).[53] Finally, the fact that the triacetate can be selectively saponified at C3, should be noted.

In 1998, Selinsky et al. reported a synthesis of **105** using stigmasterol (**111**) as an economic starting material (Scheme II-3). The authors—interestingly one of which (William A. Kinney) was a co-author in the second-generation Moriarty synthesis—criticize the previous syntheses with respect to step economy and price of starting materials (i.e., cholenic acid). Although this work is divided in two publications, the overall step count remains high.[54,55] Despite this fact, the reactions are conducted on a gram-scale by Kinney's process effort at Magainin Pharmaceuticals [Michael Zasloff (Georgetown University) was the founder and president at the time].

This synthesis begins with a unique allylic oxidation—without requiring protection of the C3-carbinol—protocol employing *N*-hydroxyphthalamide, benzoyl peroxide, with air as the stoichiometric oxidant conducted in refluxing ethyl acetate–acetone while bubbling air through the system for 48 hours. In a common laboratory setting (i.e., without the use of complicated reactors) this reaction is difficult to use: one must add ethyl acetate–acetone to match the rapid rate of solvent loss as a result of the vigorous bubbling of air through a refluxing solution for 48 hours. The resultant allylic

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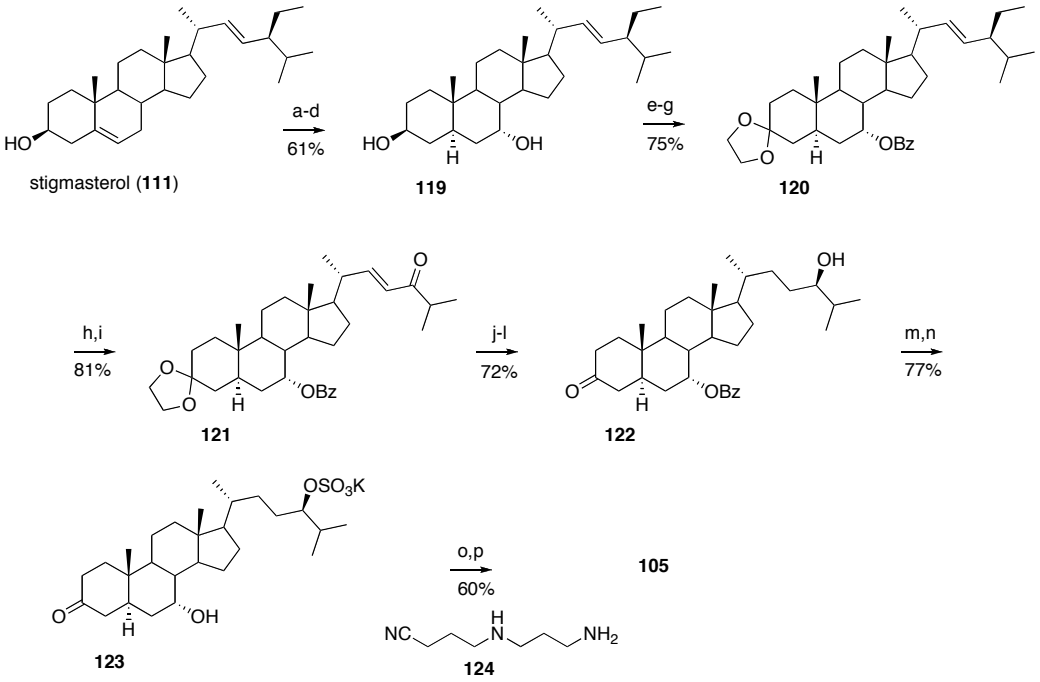
[53] Salmond, W. C.; Barta, M. A.; Havens, J. L. "Allylic Oxidation with 3,5-Dimethylpyrazole. Chromium Trioxide Complex. Steroidal Δ^5-7-ketones" *J. Org. Chem.* **1978**, *43*, 2057–2059.

[54] Jones, S. R.; Selinsky, B. S.; Rao, M. N.; Zhang, X.; Kinney, W. A.; Tham, F. S. "Efficient Route to 7α-(Benzoyloxy)-3-dioxolane Cholestan-24(R)-ol, a Key Intermediate in the Synthesis of Squalamine" *J. Org. Chem.* **1998**, *63*, 3786–3789.

[55] Zhang, X.; Rao, M. N.; Jones, S. R.; Shao, B.; Feibush, P.; McGuigan, M.; Tzodikov, N.; Feibush, B.; Sharkansky, I.; Snyder, B.; Mallis, L. M.; Sarkahian, A.; Wilder, S.; Turse, J. E.; Kinney, W. A. "Synthesis of Squalamine Utilizing a Readily Accessible Spermidine Equivaline" **1998**, *63*, 8599–8603.

peroxide is subsequently treated with cupric chloride in pyridine to provide the enone. This enone was reduced—to establish the *trans*-A,B-ring—under dissolving metal conditions to give a saturated ketone that was reduced with K-selectride to give the axial C7-carbinol **119**.

Scheme II-3. Selinsky's synthesis of **105**[a]



[a] Conditions: (a) *N*-hydroxyphthalamide, $(BzO)_2$, EtOAc, acetone, air, Δ, 48 h; (b) $CuCl_2$, pyridine, 0 °C; (c) Li, NH_3, THF, –78 °C; (d) K-selectride, THF, –20 °C; (e) Ag_2CO_3, celite, toluene, Δ; (f) BzCl, DMAP, pyridine, 0 °C; (g) ethylene glycol, PTSA, toluene, Δ; (h) O_3, CH_2Cl_2, EtOH, –78 °C, then $(MeO)_3P$; (i) $(EtO)_2POCH_2COCH(CH_3)_2$, NaH, THF, Δ; (j) *(R)*-CBS, BH_3•THF, toluene, –20 °C; (k) $Pd(OH)_2$ (20 mol%), THF, $NaNO_2$, H_2; (l) Amberlyst 15, acetone; (m) SO_3•Py, pyridine, 80 °C; (n) KOH, MeOH; (o) **124**, $(MeO)_3CH$, $NaBH_4$, MeOH, –78 °C; (p) PtO_2, TFA, EtOH, H_2.

The diol was selectively oxidized at the C3-position via the Fetizon oxidation (i.e., Ag_2CO_3 on celite); the ketone was functionalized as the ketal (i.e.; PTSA, ethylene glycol) and acylated with benzoyl chloride to give **120**. Ozonolysis, interestingly using a phosphite as the reductive quench, and a Horner–Wadsworth–Emmons olefination provided the enone **121**. The enone was subjected to the Itsuno–CBS asymmetric

reduction protocol to set the C24 stereocenter; the olefin was subsequently hydrogenated (i.e.; $Pd(OH)_2$, $NaNO_2$, H_2) to give **122**. Revelation of the ketone (i.e.; Amberlyst 15, acetone), sulfation (i.e.; SO_3·Py, pyridine), and saponification of the benzoate (i.e.; KOH, MeOH) gave the two-step reductive amination substrate. Imine formation (i.e.; **124**, trimethylorthoformate) followed by hydrogenation (i.e.; PtO_2, H_2) effected the formation of **105**.

The allylic oxidation method is interesting in that it is not necessary to protect the C3-carbinol, although the protection (e.g., acetylation) of the C3-position and deprotection (e.g., saponification) are easily conducted and are high yielding; this was previously demonstrated in Moriarty's second-generation synthesis (cf., Scheme I-2). With the procedural difficulties previously mentioned, this protocol was determined not to warrant further consideration.

As demonstrated in this synthesis it is interesting to note the method for stereoselective introduction of the C24-carbinol center via the Itsuno–CBS reduction. However, it goes without mention in the publication that the (*R*)-CBS reagent is derived from **D**-proline, which is the unnatural stereoisomer and hence expensive. Corey et al. have reported a more economic route to the (*R*)-CBS reagent from the inexpensive pyroglutamic acid,[56] but the above mentioned reaction requires a stoichiometric amount of the catalyst to acquire acceptable *de*'s, which make this strategy difficult, although not impossible to use.

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[56] Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C.; Signh, V. K. "A Stable and Easily Prepared Catalyst for the Enantioselective Reduction of Ketones. Applications to multistep Syntheses" *J. Am. Chem. Soc.* **1987**, *109*, 7925–7926.

The two-step reductive amination protocol that the authors opted to use in place of the already known one-step reaction is not discussed in the publication. The reasons for this choice—in the current discussion—have to be left to conjecture.

An altogether different approach was reported by Zhou et al in 2003: starting from the readily available bile acid derivative methyl chenodeoxycholate (**125**).[57,58] This synthesis provides the best step economy and employs a catalytic asymmetric C–C bond forming reaction to establish the C24-stereocenter and isopropyl unit (Scheme II-4).

Selective C3 Fetizon oxidation (i.e., Ag_2CO_3 on celite) and protection of the C7-alcohol as the MOM-ether (i.e.; MOMCl, NaI, *i*-Pr_2NEt) provided the ketone **126**, which was subjected to IBX in the presence of trifluoroacetic acid in dimethylsulfoxide effecting the selective formation of the $\Delta^{2,3}$-enone **127**. Dissolving metal conditions (i.e.; Li, NH_3, THF) astonishingly afforded the saturated ketoaldehyde **128** in good yield. An aminoalcohol catalyzed asymmetric alkylzinc addition provided the secondary alcohol as a single disastereomer in good yield. The MOM-ether was cleaved under acidic conditions (i.e., PPTS, *t*-BuOH) and reductive amination of the primary amine **118** and the ketodiol **130** (i.e.; 3Å MS, $NaBH_4$, MeOH, –78 °C) provided the diol **131**; regioselective sulfation provided **105**.

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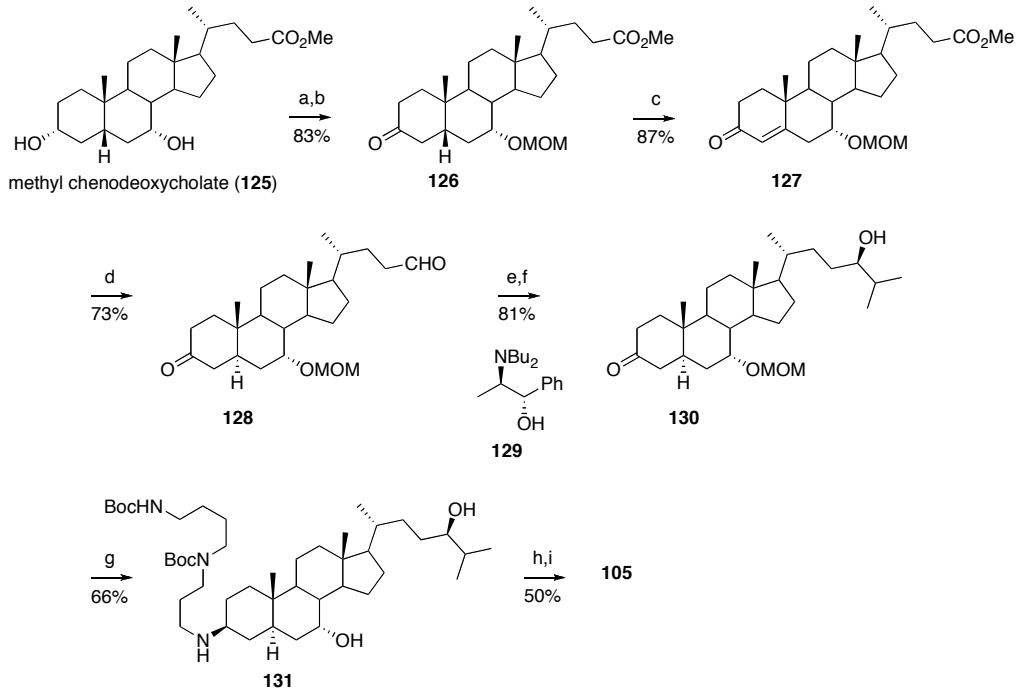
[57] Zhang, D.; Cai, F.; Zhou, X.; Zhou, W. "A Concise and Stereoselective Synthesis of Squalamine" *Org. Lett.* **2003**, *5*, 3257–3259.
[58] Zhou, X.; Cai, F.; Zhou W. "A stereoselective synthesis of squalamine" *Tetrahedron* **2002**, *58*, 10293–10299.

Scheme II-4. Zhou's synthesis of **105**[a]



[a] Conditions: (a) Ag_2CO_3 on Celite, toluene, Δ; (b) MOMCl, *i*-Pr_2NEt, cat. NaI, CH_2Cl_2, Δ; (c) IBX, TFA, DMSO, 24 h; (d) Li, NH_3, THF, –78 °C, 1 h; (e) **9** (20 mol%), *i*-Pr_2Zn (2.2 equiv), toluene, 0 °C, 4 h; (f) PPTS, *t*-BuOH, Δ; (g) **118**, 3Å MS 18 h, then $NaBH_4$, MeOH, –78 °C, 4 h; (h) HCl, MeOH, 29 h; (i) SO_3•Py, pyridine, 40 °C, 2 h.

Besides requiring far fewer synthetic steps, this route comprises several interesting transformations. The regioselective ketone oxidation with IBX was shown to be accelerated by the addition of trifluoroacetic acid (TFA). In fact, it was reported not to occur in the absence of TFA; the enone was generated in excellent yield under this protocol.

Perhaps the most interesting transformation was the dissolving metal reduction of the enone ester **127** to provide the saturated ketoaldehyde **128** as the product in good yield. Furthermore, this aldehyde was used in a catalytic asymmetric alkylzinc addition and provided the desired stereoisomer in excellent yield. The dissolving metal reduction of an ester to an aldehyde does not have strong precedent in the literature.

The authors chose to reduce an in situ generated imine with sodium borohydride at low temperature; this protocol is different than the above mentioned reductive amination strategies and the reduction exhibits better selectivity at reduced temperature.

II.C. Synthetic Design

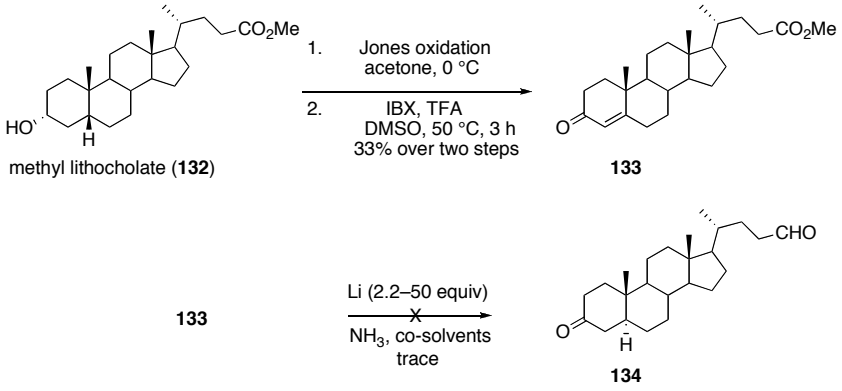
During the synthesis of petromyzonamine disulfate (**103**) by our group using a route similar to that described by Zhou et al., difficulty was met with the dissolving metal conditions: transformation of **127** to the ketoaldehyde **128** (Scheme II-4). Although the product **128** was formed and could be isolated, the maximum yield was around 20% (sometimes as low as 5%). This could be scaled up in a brute force campaign that was sufficient for the previous isolation studies in which **103** was only desired as a structural proof; for present purposes, this would not be acceptable.

A model enone **133** (Scheme II-5) was prepared in two steps starting from lithocholic acid. The iodoxybenzoic acid (IBX) oxidation was low yielding with considerable formation of the regioisomeric enone (i.e., ~20%) as well as other undetermined by-products.

Using enone **133**, various dissolving metal conditions were examined and all were met with almost complete failure, with trace amounts of the ketoaldehyde detected in the crude ^{1}H NMR spectrum. Various co-solvents were screened (e.g., toluene, ether, and THF); a large excess of lithium (i.e., 50 equiv) was also used followed by first quenching the excess lithium with a diene (e.g., isoprene) and then a proton source (e.g., NH_4Cl). At this time it was believed that this strategy would not be workable, so an alternate approach was envisioned. However, conditions were discovered to improve

the efficiency of a related enone reduction and this will be discussed later in more detail.

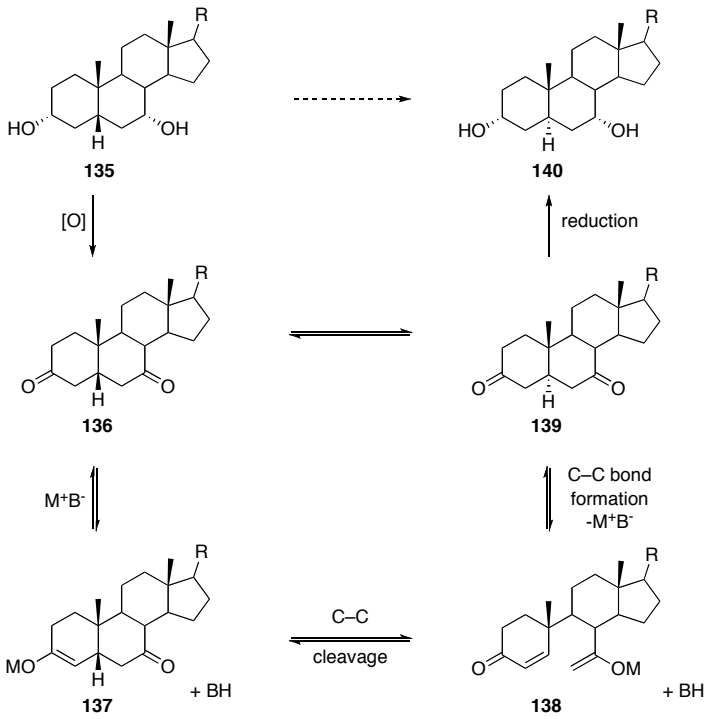
Scheme II-5. Enone **133** reduction using dissolving metal conditions



With the difficulty met during both the reaction of ketone **132** with IBX (i.e., enone formation) and the dissolving metal reduction—an overall formal epimerization of the 5-position—a more direct route was desired.

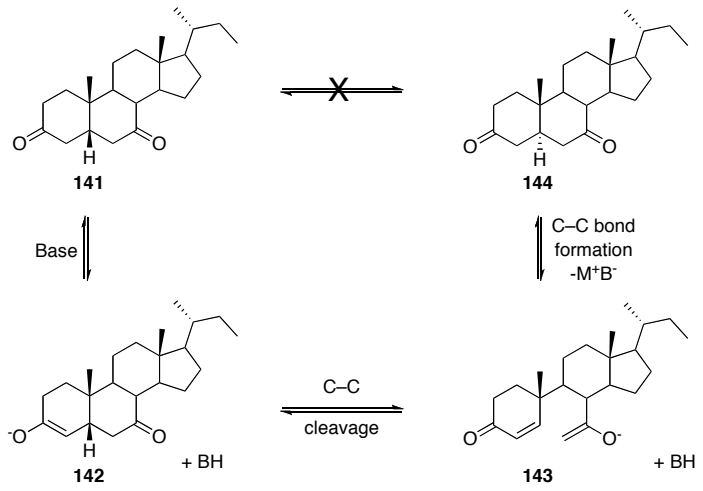
It was envisioned that a 1,5-diketone (Scheme II-6, **136**) could be deprotonated to generate an enolate **137**, which could possibly fragment to provide a *seco*-enolate **138**; reformation of the C–C bond could lead to the potentially more stable *trans*-diketone **139**. This overall process would constitute a retro-Michael–Michael epimerization, of which there is obvious precedent for in the literature.

Scheme II-6. Potential strategy for epimerization the C5-stereocenter in bile acids



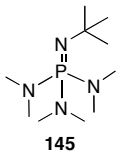
The retro-Michael–Michael isomerization was first attempted under various basic conditions (Scheme II-7). Under conditions expected to produce a low-level concentration of an enolate (e.g., **142**) capable of fragmentation at elevated temperatures (i.e.; NaOH, triglyme, 220 °C), only starting material was recovered after extended reaction periods (e.g., 48 h). Stronger bases (e.g.; KH or NaHMDS) that would be expected to generate a molar equivalent of the enolate were examined, both in excess and with slightly less than one equivalent [e.g., KH (0.95 equiv), triglyme, 220 °C]: starting material was recovered.

Scheme II-7. Retro-Michael–Michael 1,5-diketone isomerization: basic attempts



conditions:

NaOH or KH, triglyme, 220 °C; NaHMDS, THF, 70 °C; LiHMDS/LiCl, THF, 70 °C; Schwesinger's P_1-*t*-Bu (**145**), THF, 70 °C



145

The effect of added lithium chloride was studied to test the hypothesis that perhaps added lithium salts could speed up the potential retro-Michael fragmentation by dual Lewis acid–Lewis base activation. The analogous and well known retro-aldol reaction is know to occur through a six-membered transition state with the cation simultaneously acting as a counter cation for the enolate and a Lewis acid activating the β-carbonyl group: retro-Aldol fragmentation leads directly to a enolate–cation pair. A retro-Michael reaction cannot realistically adopt a cyclic transition state, thus added lithium cations could enhance the fragmentation. Unfortunately, this approach also led to recovered starting material.

In addition, it was speculated that a loosely coordinated anion could be more susceptible to C–C bond cleavage. Thus, the non-metallic and non-nucleophilic

Schwesinger's P_1-*t*-Bu base (**145**)[59] was used; however, starting material was again unaffected.

A Norrish type-II photochemical C–C bond cleavage, followed by an intramolecular Michael addition would comprise another strategy for isomerization (Scheme II-7). Despite the few examples of preparative Norrish type-II synthetic protocols in the literature and the appreciation for the requirements of orbital overlap in the C–C bond cleavage event, photochemical conditions were examined. Conditions were examined with or without sensitizers (e.g., acetone or benzophenone) and at various wavelengths of radiation (e.g., 254 nm or 310 nm) in different solvents (e.g., THF, CH_3CN, cyclohexane). The diketone **141** was fairly resilient to these conditions, with the observation only of by-products arising from oxidation of the solvent (e.g., Cy_2O was observed by GC-MS when the reaction was conducted in cyclohexane). In benzene (310 nm), functionalization of the starting material occurred in one week to provide a product that was 18 mass units higher (i.e., most likely due to the incorporation of water). The structure of this complicated steroid was not fully elucidated, but it was most likely a result of the known α-cleavage-hydration pathway of ketones that yields carboxylic acids as the products.[60] This preliminary analysis is based on the ^1H NMR of the crude reaction mixture in which the 6β-proton adjacent to the C7-ketone was gone and a new methyl singlet appeared; the polarity was also indicative of transformation to a carboxylic acid (i.e., more polar).

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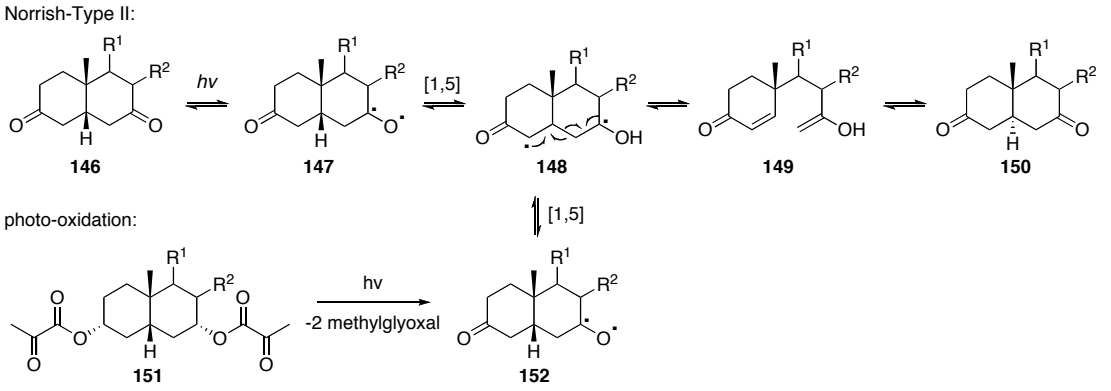
[59] Schwesinger, R.; Schlemper, H. "Peralkylated Polyaminophosphazenes–Extremely Strong, Neutral Nitrogen Bases" *Angew. Chem., Int. Ed.* **1987**, *26*, 1167–1169.
[60] Schaffner, K.; Jeger, O. "A Chapter on Ketone Photochemistry" *Tetrahedron* **1974**, *30*, 1891–1902.

The triplet ketone state was also envisioned to be accessible through a photochemical oxidation method (Scheme II-7).[61] Besides generating the triplet ketone from a better chromophore (i.e., longer wavelength and higher extinction coefficient), the oxidation could be done in situ (i.e., saving one step). Unfortunately, the all acylation attempts led to the C3 monopyruvyl ester and the C7 axial alcohol could not be acylated even under forcing conditions [e.g., pyruvyl chloride (20 equiv), DMAP (1 equiv)].

Scheme II-7. Attempted isomerization via photochemical Norrish type-II cleavage



In studies directed toward an efficient synthesis of petromyzonamine disulfate (**103**) and analogs, a strategy was desired that avoided the use of a dissolving metal reduction to establish the *trans*-A,B-steroid system. This was largely in part due to the previous difficulty in obtaining good results as discussed above (cf., Scheme II-5). It is well known that C7-enones (i.e., the enone is in the B-ring) of steroids can be

[61] Carless, H. A. J.; Fekarurhobo, G. K. "A Photochemical Synthesis of β,γ-Epoxycyclohexanones" *Tetrahedron Lett.* **1983**, *24*, 107–110.

hydrogenated (i.e.; Pd/C, H_2) to give the *trans*-A,B-steroid; C3-enone hydrogenation give the *cis*-A,B-steroid.

Three objectives were addressed in the revised synthetic design of **103** and analogs (Figure II-4): (a) to develop a potentially efficient strategy toward **103**; (b) to develop a strategy amenable to analog synthesis via a divergent late-stage intermediate; and (c) to develop a strategy that does not require the use of dissolving metal conditions to establish the *trans*-A,B ring system.

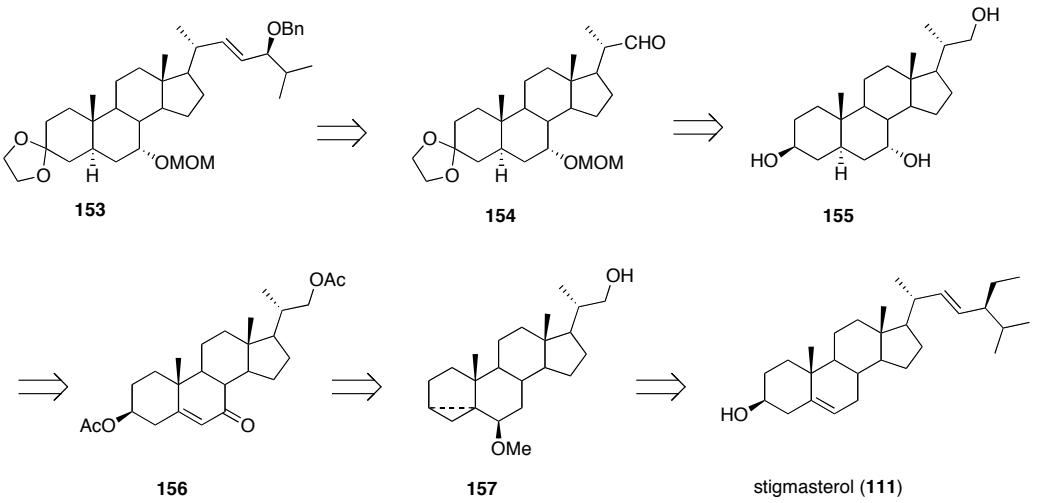
Compound **153** could serve as good candidate for the divergent synthesis of analogs differing in the amino functionality at C3, established by reductive amination protocols on an amino alcohol (i.e., after one-pot deprotection of the ketal and MOM ether under acidic conditions). Furthermore, the aldehyde precursor **154** could be functionalized in a divergent manner via olefination protocols.

The aldehyde **154** could be accessed from the selective Oppennauer oxidation of the triol **155**, selective protection of the primary alcohol, and MOM ether formation at C7. As previously discussed, the Oppennauer oxidation is known to be selective for oxidation of C3 equatorial carbinols in the presence of C7 axial alcohols (Section II.B, Scheme II-1). Furthermore, the Oppennauer conditions (i.e., $(t\text{-BuO})_3Al$, cyclohexanone, benzene) will not oxidize primary alcohols obviating the need for protection at this stage.

The triol **155**, could be obtained from the hydrogenation of the B-ring enone in **156** (i.e.; Pd/C, H_2) and selective axial hydride reduction of the saturated ketone with concomitant removal of the acetate protection groups. Acetate protection of the homoallylic alcohol derived from acidic hydrolysis of the *i*-steroid **157**, is necessary to

guard against undesired oxidation during the C7 allylic oxidation transformation (i.e.; CrO_3, 3,5-dimethylpyrazole). The *i*-steroid **157** is known and can be prepared via stigmasterol (**111**) in three steps (i.e.; sulfonylation, cyclopropyl methyl ether formation, and ozonolysis with a sodium borohydride work-up).

Figure II-4. Revised synthetic route to petromyzonamine disulfate (**103**) and analogs

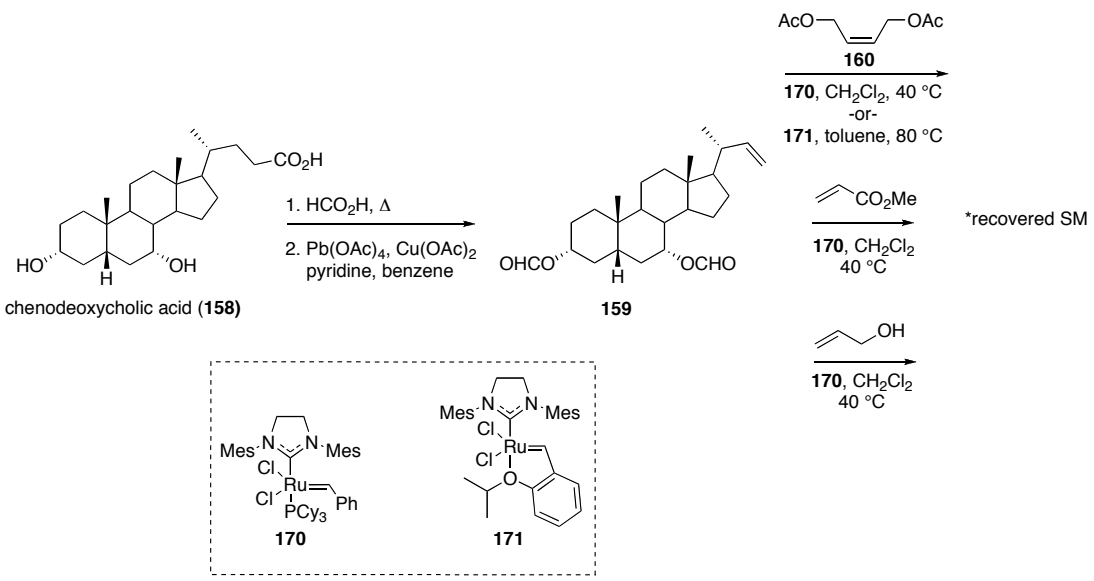


The proposal highlighted in Figure II-4 was dependent on the establishment of conditions to effect the required olefination. Two strategies were considered to this end: (a) alkene cross-metathesis (CM) and (b) the modified Julia olefination.

II.D. Results and Discussion

To test the feasibility of using olefin cross-metathesis to install the side-chain, the model olefin **159** was prepared in two steps from chenodeoxycholic acid (Scheme II-8, **158**).

Scheme II-8. Olefin cross-metathesis approach for the installation of the C23–C27 side chain of **103**

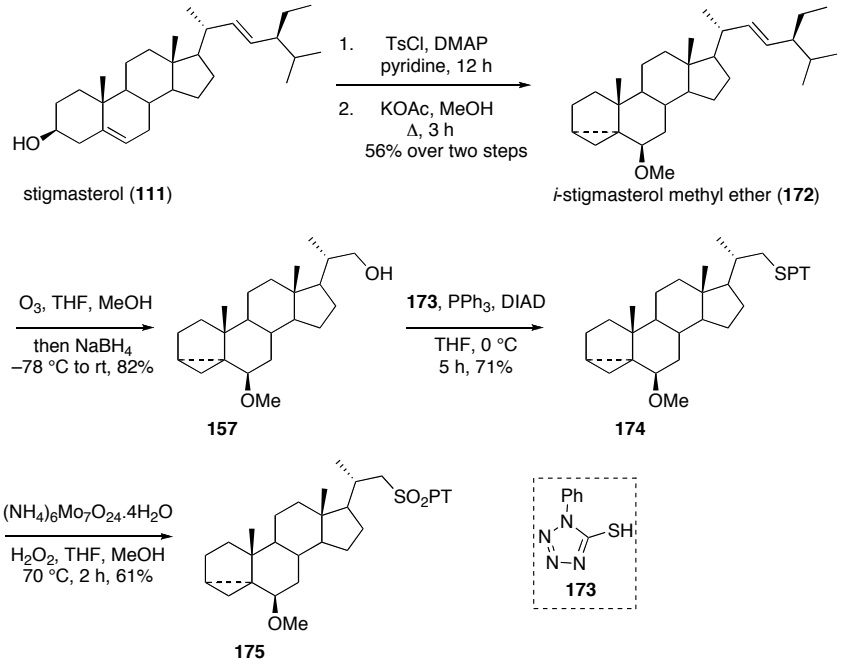


The alkene **159** appeared to be too hindered for successful participation in cross-metathesis reactions using either the Grubbs second-generation metathesis initiator (**170**) or the Hoveyda–Grubbs second-generation metathesis initiator (**171**): even substrate **160**—one of the best substrates for CM—failed to yield cross-metathesis products.

To test the feasibility of an olefination strategy, the phenyltetrazole (PT) sulfone **175** was prepared in five steps starting with stigmasterol (Scheme II-9, **111**). The *i*-steroid **172** was first prepared to protect the trisubstituted olefin during ozonolysis (i.e.; TsCl, DMAP, pyridine; KOAc, MeOH). Ozonolysis–reduction (i.e.; O_3, $NaBH_4$) of the disubstituted olefin provided the primary alcohol **157**. The PT-thioether **174** was

prepared using the Mitsunobu protocol (i.e.; PTSH, DIAD, PPh_3) and subsequently oxidized to the sulfone (i.e.; ammonium paramolybdate, H_2O_2).

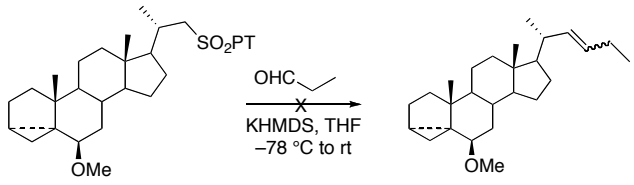
Scheme II-9. Synthesis of phenyltetrazole (PT) sulfone **175**



The first attempts at C–C bond formation—conducted with the potassium anion derived from the PT-sulfone **175**—was met with complete failure (Scheme II-10). Formation of the anion at low-temperature (i.e.; KHMDS, THF, –78 °C) prior to the addition of propional did not lead to observable olefination as determined by crude ^{1}H NMR spectral analysis. Instead, the PT-sulfone anion was rapidly (i.e., in minutes) decomposing most likely by route of *ipso* dimerization of sulfone anions: a known by-product that can be observed during modified Julia olefinations. However, it is noted

that PT-sulfones are less prone to this dimerization relative to the benzothiazole (BT) sulfones.[62]

Scheme II-10. Julia–Kocienski olefination: PT-sulfone **175**

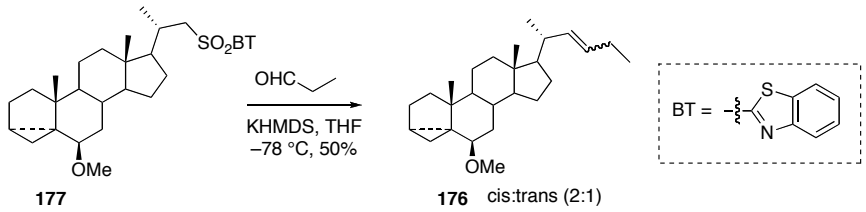


175 **176**

The benzothiazole (BT) sulfone **177** was synthesized in a manner analogous to that of the PT sulfone (Scheme II-9, **175**). First attempts were conducted under the standard conditions with the sulfone anion being preformed at low temperature prior to the addition of the aldehyde; this protocol did not produce the desired olefin, but again highlighted the instability of the sulfone anion. In response to this, a Barbier-type approach was implemented (i.e., base is added to the mixture of sulfone and aldehyde); this protocol led to an observable olefination product **176** in low yield, but interestingly the selectivity was in favor of the *cis* olefin (Scheme II-11). It is also worth mentioning that the Barbier-type protocol did not work with the PT-sulfone (Scheme II-10, **175**).

[62] Blakemore, P. R. "The modified Julia olefination: alkene synthesis *via* the condensation of metallated heteroarylalkylsulfones with carbonyl compounds" *J. Chem. Soc., Perkin Trans. 1* **2002**, 2563–2585.

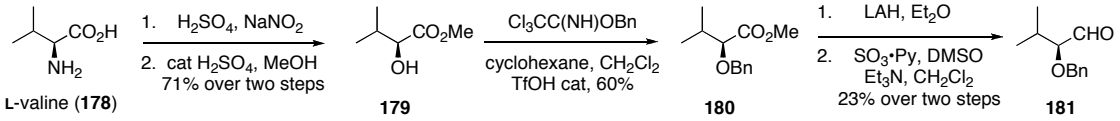
Scheme II-11. Modified Julia olefination: benzothiazole (BT) sulfone **177**



177 **176** cis:trans (2:1)

Concurrently, the synthesis of the aldehyde—corresponding to the petromyzonamine disulfate (**103**) side chain—was underway as depicted in Scheme II-12.[63] The aldehyde was prepared by diazotization–double inversion (i.e., net retention) with water acting as the nucleophile on a cyclopropyl lactone derived from L-valine (**178**). Fisher esterification of the α-hydroxycarboxylic acid provided the α-hydroxyester **179**. Conversion to the benzyl ether **180** (i.e.; benzyl trichloroacetimidate, TfOH cat, CH_2Cl_2) followed by a exhaustive reduction–oxidation protocol (i.e.; LAH, Parihk–Doering oxidation) provided the aldehyde **181**. The one-step conversion of **180** to **181** was briefly examined (i.e., DIBAL, toluene, –78 °C), but a mixture of **181** and the carbinol was obtained.

Scheme II-12. Synthesis of petromyzonamine disulfate (**103**) based aldehyde **181**

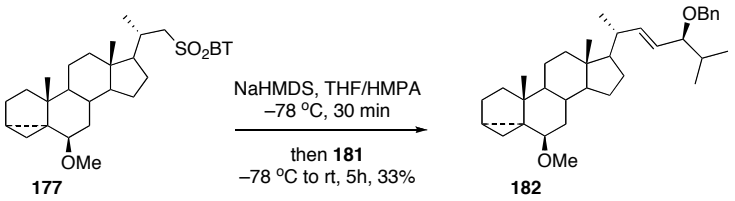


L-valine (**178**) **179** **180** **181**

[63] Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullié, M. M. "Total Synthesis and Structural Investigations of Didemnins A, B, and C" *J. Am. Chem. Soc.* **1990**, *112*, 7659–7672.

The modified Julia olefination with the BT-sulfone **177** was optimized with the aldehyde substrate **181** (Scheme II-13). The optimum yield obtained remained low (i.e., 33%) for this transformation; it was reasoned that this was due to the sterically encumbered nature of the sulfone.

Scheme II-13. Modified Julia olefination with aldehyde **181**



Although it may seem like an uphill battle (i.e., β-elimination), the inverse Julia-Kocienski olefination was briefly considered (Scheme II-14). This was motivated by Markó's publication in which β-silyloxy PT-sulfones were employed in the modified Julia reaction and silyl allyl ethers were obtained in high yields.[64]

The aldehyde **183** was produced by a low-yielding ozonolysis reaction: several conditions were examined including Dussault's recent *N*-oxide method.[65] It is also worth noting that the olefin is seemingly to hindered to allow for facile osmium tetroxide mediated dihydroxylation; the reaction took 6 d to reach completion even with added pyridine which is known to accelerate osmate ester formation.[66] In retrospect, problems associated with the ozonolysis of *i*-steroids have been discussed in the

[64] Pospísil, J.; Markó, I. E. "Efficient and Stereoselective Synthesis of Allylic Ethers and Alcohols" *Org. Lett.* **2006**, *8*, 5983–5986.
[65] Schwartz, C.; Raible, J.; Mott, K.; Dussault, P. H. "Fragmentation of Carbonyl Oxides by *N*-Oxides: An Improved Approach to Alkene Ozonolysis" *Org. Lett.* **2006**, *8*, 3199–3201.

literature and are thought to involve the oxidation of the methine C–H bond at the C6-ether.[67] Interestingly, the reductive ozonolysis conditions (i.e.; O_3, THF, MeOH, $NaBH_4$) described in Scheme II-9 worked well (i.e., 82%). Recent results indicate that using THF as the solvent during ozonolysis reactions may provide a "buffer" to over oxidation with THF serving as a sacrificial reductant (i.e., γ-butyrolactone is formed). Under these conditions even when the reaction is run for long periods of time (i.e., after the starting material has been completely consumed) no additional oxidation of the steroid nucleus was observed; this may prove to be a general solution to the problem of overoxidation by-products during ozonolysis.

The PT-sulfone **185** was prepared in two steps from previously discussed primary alcohol **184** (i.e., Mitsunobu and oxidation). Unfortunately, **185** did not serve as an acceptable substrate for the Julia–Kocienski olefination: β-elimination is predominate.

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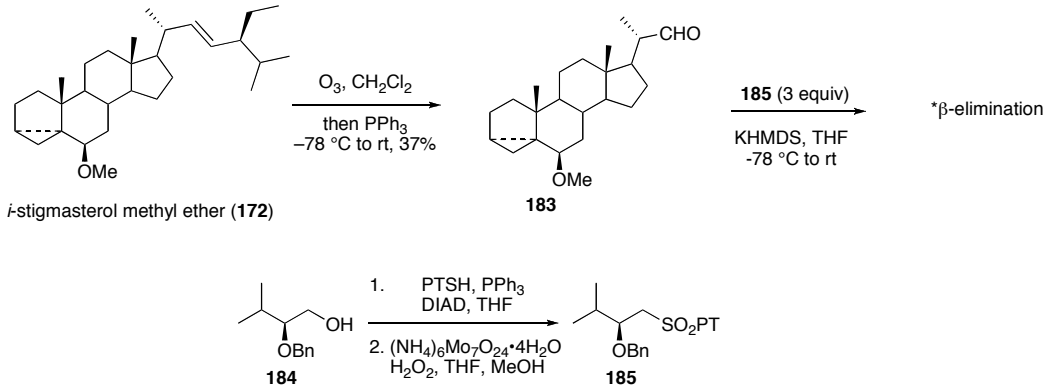
[66] Schröder, M. "Osmium tetroxide Cis hydroxylation of Unsaturated Substrates" *Chem. Rev.* **1980**, *80*, 187–213.

Scheme II-14. Sulfone–aldehyde inverse Julia–Kocienski strategy



The classical Julia-Lythgoe protocol was also explored (Scheme II-15). It was hypothesized that a less stable sulfone anion (i.e., $–SO_2Ph$ vs. $–SO_2Het$) would be more thermodynamically encouraged to add to an aldehyde in an irreversible manner. Furthermore, *in situ* capping of the resultant alkoxide anion could obviate the retro-addition of the sulfone anion.

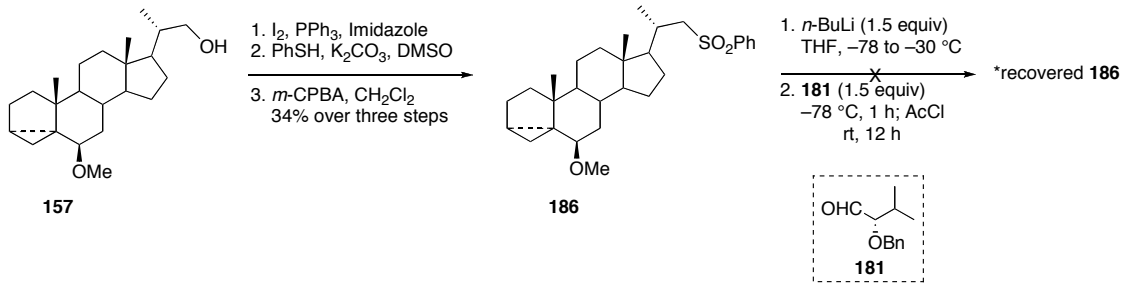
The phenyl sulfone **186** was synthesized in three steps from the primary alcohol **157** (i.e.; I_2, PPh_3, imidazole; PhSH, K_2CO_3; *m*-CPBA); the iodide could have been directly displaced with sodium benzene sulfinate to reduce the need for an oxidation step, but the chosen sequence enjoyed closely related precedent.[68]

Attempts of addition of the anion derived from the sulfone **186** (e.g., KHMDS or *n*-BuLi) to the aldehyde **181** were met with failure. To ensure deprotonation of the

[67] Spencer, T. A.; Li, D.; Russel, J. S. "Further Studies on the Synthesis of 24(*S*),25-Epoxycholesterol. A New, Efficient Preparation of Desmosterol" *J. Org. Chem.* **2000**, *65*, 1919–1923.
[68] Gao, L,-J.; Zhao, X.-Y.; Vandewalle, M.; Clercq, P. D. "Convergent Synthesis of 1α-Hydroxyvitamin D_5" *Eur. J. Org. Chem.* **2000**, 2755–2759.

sulfone, the base–sulfone mixture was warmed to –30 °C prior to re-cooling to –78 °C for subsequent addition to the aldehyde substrate. Therefore, the lack of addition of the sulfone anion to the aldehyde is most likely not the result of inefficient deprotonation of the sulfone. Furthermore, acetyl chloride was added directly to the reaction mixture prior to warming the mixture to room temperature; these conditions should render retro-addition less likely. Based on the above reasoning, the difficulty met during this transformation was believed to be a result of the steric hinderance of the steroid D-ring.

Scheme II-15. Julia-Lythgoe olefination approach



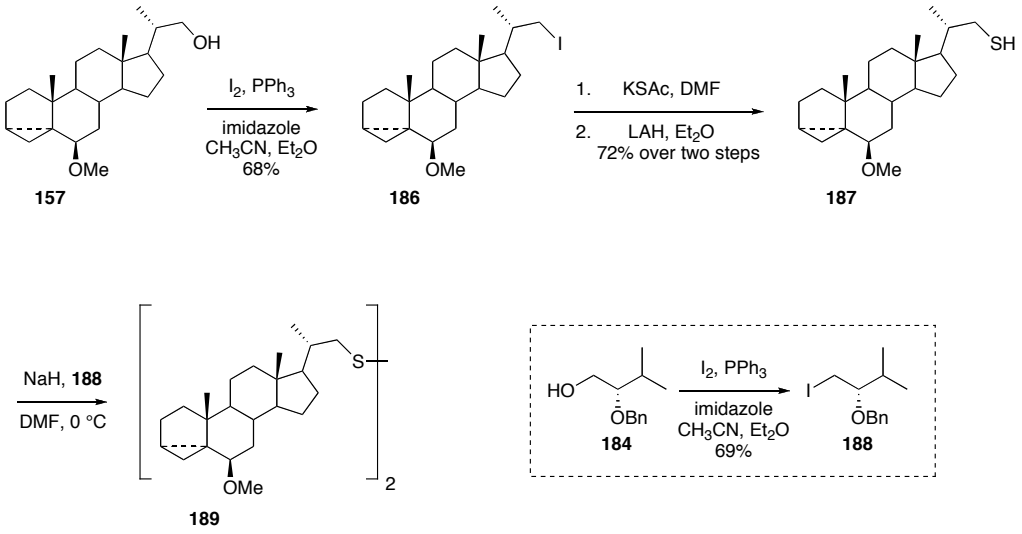
Guided by the Julia olefination studies in which the difficulty is most likely due to the steric bulk of the highly branched sulfone anion, or aldehyde in Scheme II-15; a linchpin strategy was envisioned, effectively homologating the site of alkylation by one heteroatom (i.e., Ramberg–Bäcklund olefination). This approach would facilitate the alkylation in two ways: (a) the alkylation site is further removed from the steric bulk of the molecule and (b) the alkylation is at a heteroatom which is kinetically more feasible than a C–C bond forming event.

The Ramberg–Bäcklund thiol **188** was prepared from the primary alcohol **157** by way of the primary iodide **187** (i.e.; I_2, PPh_3, imidazole). Nucleophilic displacement

of the iodide with potassium thioacetate and reduction of the thioester (i.e., LAH) provided the thiol **187** (Scheme II-16). Care must be taken to quench the LAH reduction with 1M HCl: disulfide formation predominates if a basic quench is employed.

The primary iodide **189** was prepared from the previously mentioned primary alcohol **184** (i.e.; I_2, PPh_3, imidazole) and added to a solution of the sodium salt of the thiol **188** (i.e.; NaH, DMF) to cleanly generate the *disulfide* **190** via oxidative dimerization of the thiolate. At this point—also with the added synthetic manipulations required—this approach was lowered in priority. However, in preparation of this document it is realized that milder (i.e., less basic) alkylation conditions should have been attempted (e.g.; K_2CO_3, MeOH). This would have increased the rate of alkylation to that of oxidative dimerization; thiols are nucleophilic enough to alkylate in the protonated form, with the proton being subsequently consumed in a Schotten–Baumann sense.

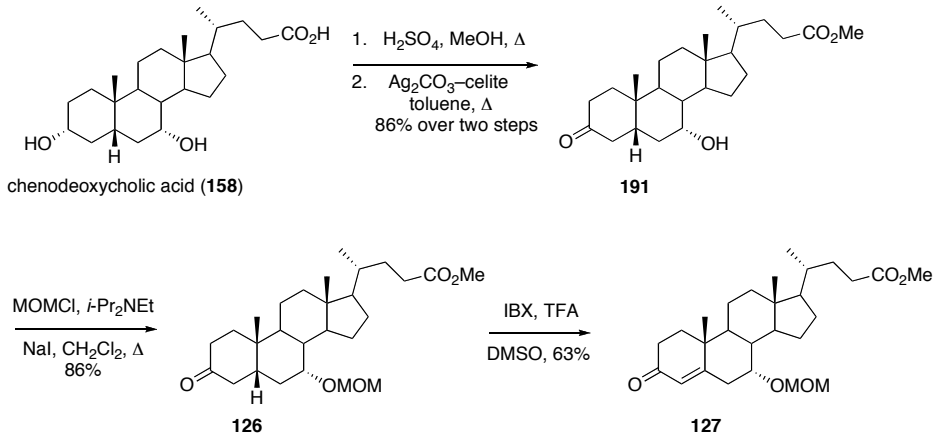
Scheme II-16. Ramberg–Bäcklund approach



At this point the chenodeoxycholic acid (Scheme II-17, **158**) route (cf., Section II.B, Scheme II-4) involving the establishment of the *trans*-A,B ring by way of A-ring enone reduction was further examined. It was envisioned that perhaps other methods (i.e.; not Li, NH_3) could be used to effect the enone reduction.

The enone **127** was synthesized in four steps starting from chenodeoxycholic acid (**158**): Fisher esterification (i.e.; MeOH, H_2SO_4), Fetizon oxidation (i.e., Ag_2CO_3–celite), MOM-ether formation (i.e., MOMCl, NaI, *i*-Pr_2NEt), and modified Nicolaou–Baran oxidation[69] (i.e.; IBX, TFA, DMSO).

Scheme II-17. Synthesis of enone **127**

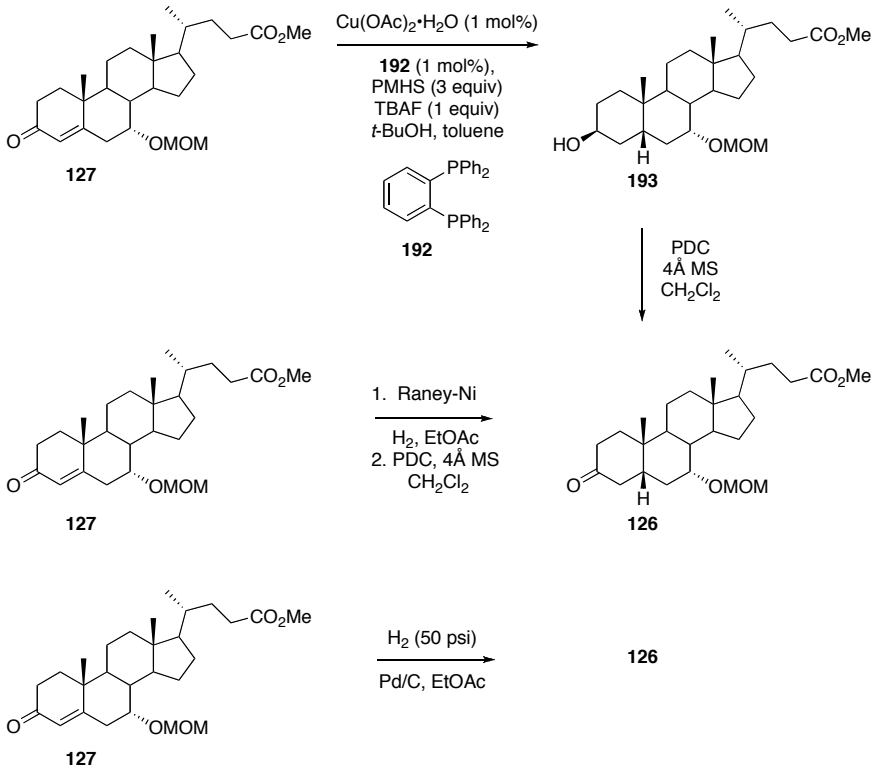


With enone **127**, several non-dissolving metal protocols were examined. The recently developed "copper hydride" protocol developed by Lipshutz et al. was first

[69] Nicolaou, K. C.; Montagnon, T.; Baran, P. S.; Zhong, Y.-L. "Iodine(V) Reagent in Organic Synthesis. Part 4. *o*-Iodoxybenzoic Acid as a Chemospecific Tool for Single Electron Transfer-Based Oxidation Processes" *J. Am. Chem. Soc.* **2002**, *124*, 2245–2258.

attempted (Scheme II-18).[70] Following the standard conditions (i.e., no TBAF), no reaction occurred; it was serendipitously found that the addition of TBAF effected the transformation.

Scheme II-18. Non-dissolving metal based attempts to establish the *trans*-A,B ring system



This was first done based on the hypothesis that perhaps an unusually stable (e.g., perhaps polymeric) silyl enol ether was being formed and running with the excess polymethylhydrosiloxane (PMHS) on the TLC plate leading to a misconception of no conversion. This did not turn out to be the case, but TBAF did accelerate the reaction. A

[70] Baker, B. A.; Boskovic, Z. V.; Lipshutz, B. H. "(BDP)CuH: A "Hot" Stryker's Reagent for Use in Achiral Conjugate Reductions" *Org. Lett.* **2008**, *10*, 289–292.

control experiment was conducted without the cupric acetate under otherwise identical conditions: only 1,2-reduction of the ketone was observed.

Although this modification of the Lipshutz protocol may be interesting in its own right, the *cis*-A,B ring system was obtained as proven by oxidation the ketone and comparison to the known ketone **126**. Thus, this approach was not further explored.

Hydrogenation with Raney–nickel W-2—after oxidation of the saturated carbinol—also delivered **126**; hydrogenation catalyzed by palladium on carbon provided the *cis*-decalin exclusively. It is worth noting that magnesium in methanol[71] and the recently described titanocene-catalyzed conjugate reduction[72] resulted in no reaction (i.e., recovered starting material).

In was posited that a hydroxyl-directed hydrogenation could be used to obtain the *trans*-A,B ring (Scheme II-19). To this end, the homoallylic alcohol **194** was prepared from the unprotected ketoalcohol **191** using the procedure described by Nicolaou et al. (i.e., HIO_3, DMSO).[73] Unfortunately, the olefin of **194** proved to be too hindered for effective hydrogenation.

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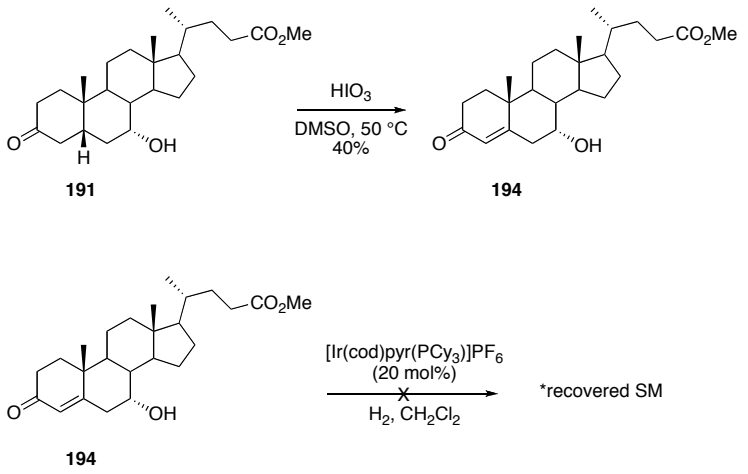
[71] Lee, G. H.; Youn, I. K.; Choi, E. B.; Lee, H. K.; Yon, G. H.; Yang, H. C.; Pak, C. S. "Magnesium in Methanol (Mg/MeOH) in Organic Synthesis" *Current Organic Chemistry* **2004**, *8*, 1263–1287.

[72] Kosal, A. D.; Ashfeld, B. L. "Titanocene-Catalyzed Conjugate Reduction of α,β-Unsaturated Carbonyl Derivatives" *Org. Lett.* **2010**, *12*, 44–47.

[73] Nicolaou, K. C.; Montagnon, T.; Baran, P. S. "HIO_3 and I_2O_5: Mild and Selective Alternative Reagents to IBX for the Dehydrogenation of Aldehydes and Ketones" *Agew. Chem. Int. Ed.* **2002**, *41*, 1386–1389.

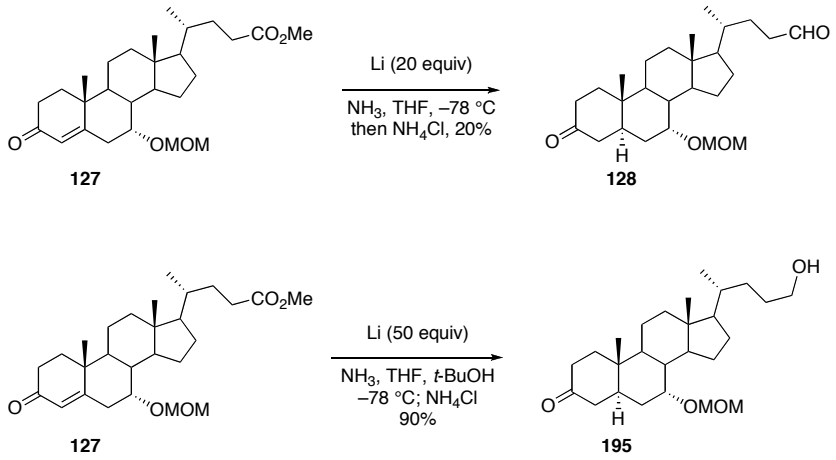
Scheme II-19. Attempted hydroxyl-directed hydrogenation employing the Crabtree catalyst



While reading a compendium on partial reductions of enones and related systems, it became evident that two concepts are important to consider when conducting an dissolving metal enone reduction: (a) one should distill the ammonia from Na–$Fe(NO_3)_3$ to remove residual water and (b) a proton source (e.g., *t*-BuOH) can be beneficial.[74] One can observe the effects of these concepts in Scheme II-20. With prior distillation of the ammonia from sodium and ferric nitrate but without *t*-butanol, the saturated ketoaldehyde **128** was obtained in a reproducible 20% yield. In previous attempts (cf., Section IC, Scheme II-5) only trace amounts of the ketoaldehyde could be obtained. By combining the purification of ammonia with the addition of *t*-butanol (10 equiv) a 90% yield of the keto *alcohol* **195** was obtained.

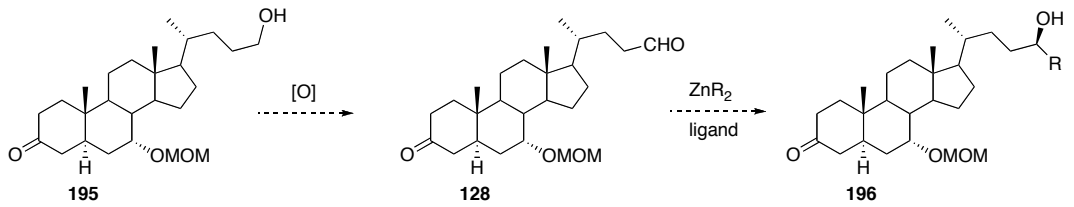
[74] Keinan, E.; Greenspoon, N. "Partial Reduction of Enones, Styrenes and Related Systems" In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon: New York, **1991**; Vol VIII, 523–578.

Scheme II-20. Dissolving metal reduction revisited: effect of distillation of ammonia and an adventitious proton source



With a reliable and scalable route to access the alcohol **195** established, attention was now turned to the development of an asymmetric method for installation of various alkyl groups (Scheme II-21). Using this approach petromyzonamine disulfate (**103**) could be accessed as well as various structural analogs; this approach could eliminate the need for the separation of C24 diastereomers.

Scheme II-21. Strategy for asymmetric installation of C24 alkyl groups



There were two examples in the literature of catalytic asymmetric diisopropyl zinc addition to an aliphatic aldehyde: the previously mentioned synthesis of

squalamine (**105**) by Zhou et al.[58] and a related report by Tanaka et al.[75] Coincidentally they were both involved in the installation of the C24 steroid side chains. Zhou et al. employed the norephedrine based Soai catalyst [(–)-(*N*,*N*)-di-*n*-butylamino-1-phenylpropane-1-ol or (–)-DBNE][76] in the *chemoselective* asymmetric diisopropyl zinc addition to a ketoaldehyde; Tanaka et al. used both Soai's catalyst and Noyori's catalyst [(–)-3-*exo*-(dimethylamino)isoborneol or (–)-DAIB][77] to effect the formation of the isopropyl appended carbinol. The selectivity was slightly better with (–)-DAIB (Scheme II-22, **200**); more importantly, the amount of reduction product (i.e., carbinol) was reduced. The reduction of carbonyl compounds by dialkyzinc–ligand complexes to provide the zinc-alkoxide and an olefin, is a common by-product associated with these conditions. Therefore, Noyori's ligand **200** was first studied; recently Nugent has reported a related ligand that can be preferred over **200** in specific cases.[78]

The synthesis of **200** is highlighted below in Scheme II-22. Starting from (+)-camphor (**197**), condensation with isoamylnitrite provided an oxime, which was subsequently reduced to the amino alcohol **198** (i.e., LAH). The amino alcohol **198** was converted to the cyclic carbamate (i.e.; triphosgene, KOH) and *N*-methylated (i.e.; KH,

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[75] Okamoto, M.; Tabe, M.; Fujii, T.; Tanaka, T. "Asymmetric Isopropylation of Steroidal 24-Aldehydes for the Synthesis of 24(R)-Hydroxycholesterol" *Tetrahedron: Asymmetry* **1995**, *6*, 767–778.

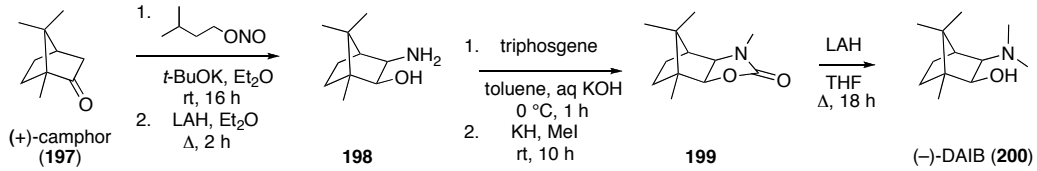
[76] Soai, K.; Yokoyama, S.; Hayasaka, T. "Chiral *N*,*N*-Dialkylnorephedrines as Catalysts of the Highly Enantioselective Addition of Dialkylzincs to Aliphatic and Aromatic Aldehydes. The Asymmetric Synthesis of Secondary Aliphatic and Aromatic Alcohols of High Optical Purity" *J. Org. Chem.* **1991**, *56*, 4264–4268.

[77] White, J. D.; Wardrop, D. J.; Sundermann, K. F. "(2*S*)-(–)-3-*exo*-(dimethylamino)isoborneol [(2*S*)-(–)-DAIB]" *Org. Synth.* **2002**, *79*, 130.

[78] Nugent, W. A. "MIB: an advantageous alternative to DAIB for the addition of organozinc reagents to aldehydes" *Chem. Commun.* **1999**, 1369–1370.

MeI) to provide **199**; lithium aluminum hydride reduction provided the tertiary amino alcohol **200**, isolated by Kugelrohr distilation.

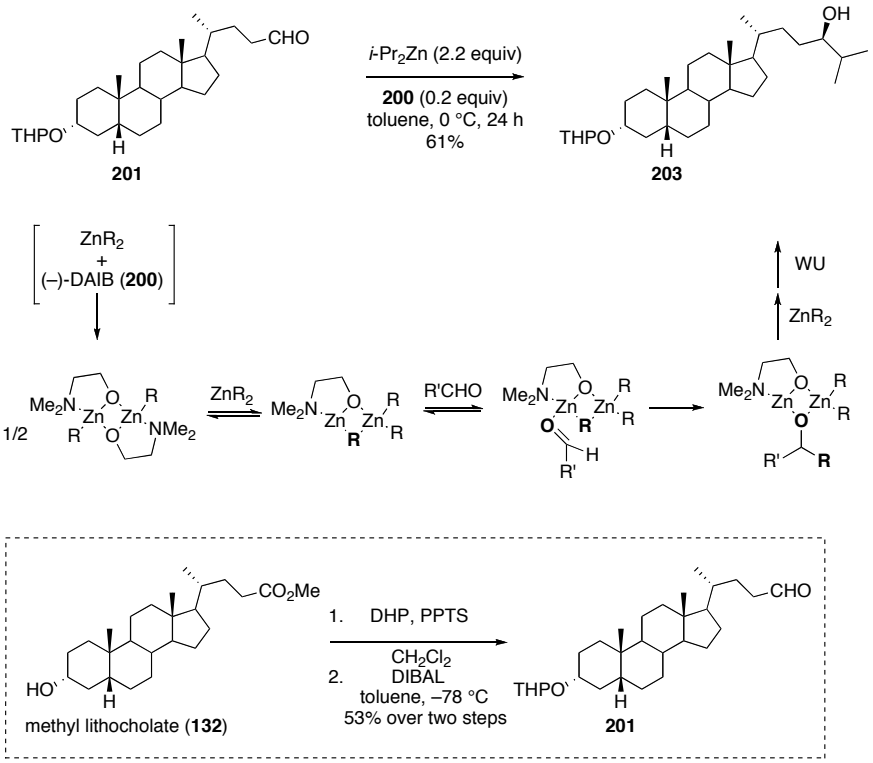
Scheme II-22. Synthesis of Noyori's (–)-3-*exo*-(dimethylamino)isoborneol (DAIB) ligand



The asymmetric isopropylation reaction was first studied on the model aldehyde **201** as depicted in Scheme II-23. This reaction went to complete conversion with the mass balance being provided by the primary alcohol by-product (i.e., reduction). The absolute stereochemistry assignment was made by analogy to the literature precedent[77] and corroborated by simple Mosher ester analysis.[79]

[79] Dale, J. A.; Mosher, H. S. "Nuclear Magnetic Resonance Enantiomer Reagents. Configurational Correlations *via* Nuclear Magnetic Resonance Chemical Shifts of

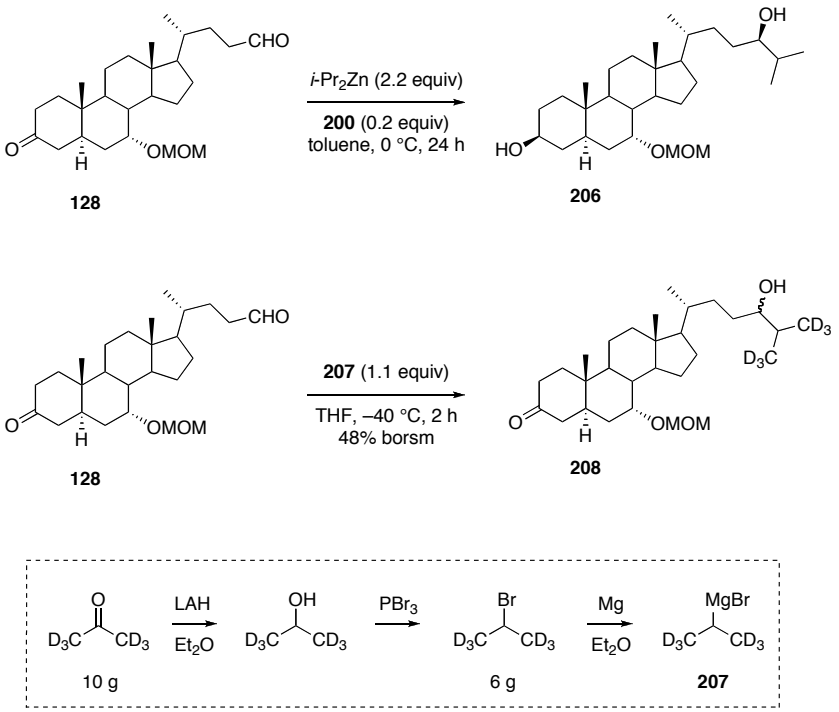
Scheme II-23. Asymmetric dialkylzinc addition: model substrate **201**



Attention was next turned to the catalytic asymmetric dialkylzinc addition to ketoaldehyde **128** (Scheme II-24). Unfortunately, following the previously developed protocol led to the diol **206** as the major product arising from concomitant reduction of the ketone. This diol would be difficult, if not impossible to differentiate. The by-product(s) (e.g., a considerable amount of the primary alcohol diol was also formed) formation was competitive (i.e., rate) with the asymmetric addition, thus reducing the equivalents of diisopropyzinc and/or temperature would not likely have a dramatic effect.

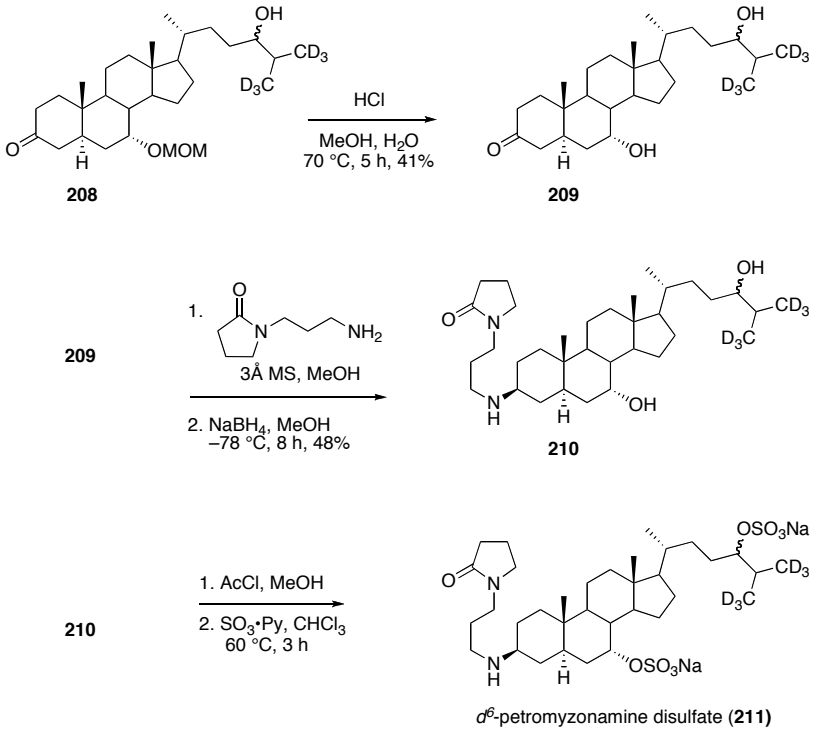
Diastereomeric Mandelate, *O*-Methylmandelate, and α-Methoxy-α-trifluoromethylphenylacetate (MTPA) Esters" *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

Scheme II-24. Nucleophilic alkyl addition: ketoaldehyde **128**



In the pursuit of a synthesis of d_6-petromyzonamine disulfate (**211**), which may find use as an ESI-MS internal standard for petromyzonamine disulfate concentration determination (*vide infra*), the nucleophilic addition of d^6-isopropyl magnesium bromide to the ketoaldehyde **128** was next examined. The reaction was explored using (e.g., 1.0–3.0 equivalents of the grignard) at various temperatures (e.g., –78–25 °C). The best result that was obtained (i.e., –40 °C, THF) gave the desired ketoalcohol **208** in 48% borsm. An almost 1:1:1 mixture (**128**: **208**: reduction products) was observed, but the desired product was isolated (1:1 mixture of C24 diastereomers) and carried on to the next step.

Scheme II-25. Synthesis of d^6-petromyzonamine disulfate (**211**)



The ketoalcohol **208** provided d^6-petromyzonamine disulfate (**211**) in two steps: reductive amination followed by sulfation (Scheme II-25). The reductive amination at low temperature (i.e., –78 °C) with sodium borohydride effected the diastereoselective formation of the equatorial amine; Borsch conditions or sodium triacetoxyborohydride lead to a mixture of diastereomers. The newly formed secondary amine **210** was first protected as the hydrochloride salt (i.e.; AcCl, MeOH) and subsequently converted to **211** with sulfur trioxide–pyridine complex in refluxing, ethanol-free chloroform.

II.E. Conclusion

In synthetic studies directed toward the synthesis of petromyzonamine disulfate (**103**) several interesting findings have been made one of which being a modified Julia

olefination strategy to install steroid side chains; if this transformation could be further optimized and generalized it could find broad use in the steroid field.

The synthesis of d^6-petromyzonamine disulfate (**211**) has also been achieved. This deuterated natural product could find practical use as an internal standard for the direct determination of **103** in natural waters by mass spectrophotometry. In theory, one could spike the test water with a known amount of **211**, which should ionize in a similar manner as that of **103** (i.e., it is structurally almost identical), and use this information to calculate the concentration of **103** in the test water. This information would be informative in that the presence of **103** in stream water would indicate that there are sea lamprey larvae present and that adult migration is likely to occur at this site. This information could provide useful in control strategies.[80]

II.F. Experimental Section

Unless noted otherwise, all oxygen and moisture-sensitive reactions were executed in oven-dried glassware sealed under a positive pressure of dry argon or nitrogen. All commercial reagents were used as received. Anhydrous THF and methylene chloride were tapped immediately prior to use after being passed through a column of activated alumina. Flash chromatography was performed using Baker Flash silica gel 60 (40 μm); analytical TLC was performed using 0.25 mm EM silica gel 60 F_{254} plates that were visualized under UV light (254 nm) or by staining with anisaldehyde reagent (450 mL of 95% ethanol, 25 mL concd H_2SO_4, 15 mL acetic acid, and 25 mL anisaldehyde) and

[80] Sorensen, P. W.; Hoye, T. R. "A critical review of the discovery and application of a migratory pheromone in an invasive fish, the sea lamprey *Petromyzon marinus* L." *J. Fish Biol.* **2007**, *71*, 100–114.

heating. Optical rotations were obtained using a Perkin-Elmer-241 polarimeter. IR spectra were recorded using a Perkin-Elmer 683 infrared spectrophotometer. NMR spectra were obtained using an INOVA 500 Varian (^{1}H data recorded at 500 MHz, ^{13}C data recorded at 125 MHz), INOVA 300 Varian, or Unity-plus 400 Varian instruments. ^{1}H chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to tetramethylsilane (0.00 ppm) when $CDCl_3$ was employed or to CD_2HOD (3.31 ppm) when *d*^{4}-methanol was used. ^{13}C chemical shifts are reported in delta (δ) units, in parts per million (ppm) relative to $CDCl_3$ (77.23 ppm) or to CD_3OD (49.15 ppm). High-resolution mass spectrometric data was obtained using a Bruker BioTOF II (ESI) mass spectrometer.

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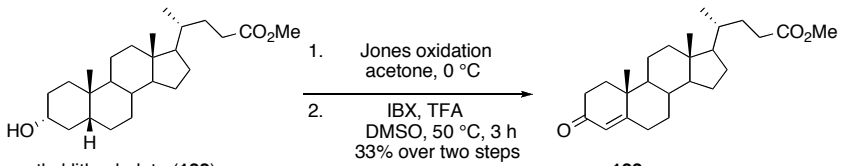
methyl 3-oxo-chol-4-ene-24-oate (**133**):



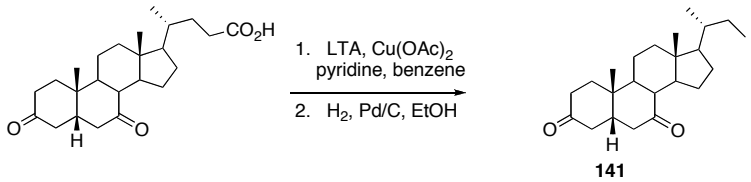
methyl lithocholate (**132**) **133**

To a cooled (0 °C) solution of methyl lithocholate (**132**) (1.0 g, 2.56 mmol) in 8.5 mL of acetone was added 2.5 M Jones reagent (3.0 mL, 7.68 mmol). The solution was allowed to stir at 0 °C for one hour prior to quenching the excess oxidant with neat isopropanol until the characteristic orange–green color change occurred at which point the solution was warmed to room temperature and eluted through a plug of silica with ethyl acetate to provide the ketone (650 mg, 65%): R_f 0.71 (hexanes–ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.67 (s, 3H), 2.70 (t, *J* = 14.5 Hz, 1H), 2.39–2.30 (m, 2H), 2.26–2.14 (m, 2H), 2.06–2.01 (m, 3H), 1.92–1.77 (m, 4 H), 1.61 (ddd, *J* = 9.5,

5.0, 3.0 Hz, 1H), 1.53–1.57 (m, 15 H), 1.02 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.69 (s, 3H).

A mixture of 2-iodoxybenzoic acid (1.08 g, 3.84 mmol) in 20 mL of dimethylsulfoxide was heated to 50 °C for 30 min at which time the homogeneous solution was cooled to room temperature and was added the ketone (745 mg, 1.92 mmol) and trifluoroacetic acid (43 μL, 0.576 mmol). The resultant solution was heated to 70 °C for 3 h and subsequently cooled to room temperature. The reaction was quenched upon the addition of an aqueous solution of sodium bicarbonate–sodium sulfite. The aqueous layer was extracted with ethyl acetate and subsequently washed with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to provide the crude material that was purified by flash chromatography to provide **133** (180 mg, 50% borsm). White amorphous solid; R_f 0.31 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.74 (s, 1H), 3.67 (s, 3H), 2.47–2.33 (m, 4H), 2.29–2.20 (m, 3H), 2.04–2.00 (m, 2H), 1.92–1.77 (m, 3H), 1.69 (ddd, *J* = 14.5, 14.5, 5.0 Hz, 1H), 1.62 (dddd, *J* = 12.0, 10.0, 7.0, 3.0 Hz, 1H), 1.56–1.49 (m, 2H), 1.47–1.38 (m, 2H), 1.37–1.26 (m, 2H), 1.26–0.99 (m, 4H), 1.18 (s, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.71 (s, 3H).

24-nor-3,7-dioxo-5β-cholane (**141**):



141

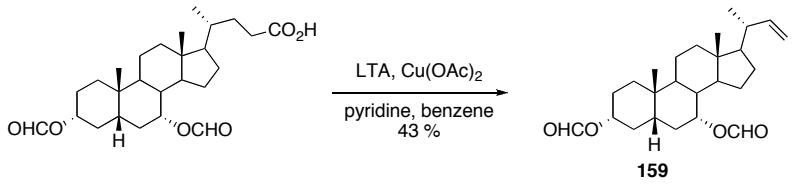
To a solution of lead tetraacetate (890 mg, 2.01 mmol), cupric acetate monohydrate (10 mg, 52 μmol), and pyridine (63 μL, 0.773 mmol) in 13 mL of benzene was added 3,7-diketo-5β-cholan-24-oic acid (200 mg, 0.515 mmol) in one portion. The resultant mixture was allowed to stir at room temperature for 24 h and subsequently eluted with ethyl acetate through a silica plug to provide the crude material which was purified by flash chromatography to provide the terminal olefin (55 mg, 31%). White amorphous solid; R_f 0.70 (2:1, hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.66 (dd, *J* = 17.0, 10.0 Hz, 1H), 4.90 (ddd, *J* = 17.0, 2.0, 1.5 Hz, 1H), 4.82 (dd, *J* = 10.0, 3.5 Hz, 1H), 2.87 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.49 (dd, *J* = 11.0, 11.0 Hz, 1H), 2.34–1.44 (m, 16H), 1.37–1.11 (m, 3H), 1.30 (s, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 1.00–0.87 (m, 1H), 0.71 (s, 3H).

To a solution of the terminal olefin (55 mg, 161 μmol) in 5 mL absolute ethanol was added 6 mg of palladium on carbon. The reaction flask was equipped with a hydrogen balloon and allowed to stir at room temperature for 12 h and subsequently filtered through a plug of celite. The volatiles were removed in vacuo to provide the crude material which did not require further purification. It was found that the C3 ketone was spontaneously ketalized to provide the diethyl ketal product. White amorphous solid; R_f 0.45 (5:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ

3.46 (dddd, *J* = 5.0, 2.0, 2.0, 2.0 Hz, 2H), 3.38 (dddd, *J* = 9.5, 1.5, 1.5, 1.5 Hz, 2H), 2.83 (dd, *J* = 12.5, 6.0 Hz, 1H), 2.38 (dd, *J* = 11.0, 11.0 Hz, 1H), 2.14–2.02 (m, 1H), 2.04 (dddd, *J* = 13.5, 6.0, 4.0, 2.5 Hz, 1H), 1.99 (ddd, *J* = 13.0, 3.0, 3.0 Hz, 1H), 1.92–1.84 (m, 2H), 1.81–1.73 (m, 3H), 1.64 (dddd, *J* = 11.5, 11.5, 4.0, 4.0 Hz, 1H), 1.54–1.01 (m, 12H), 1.21 (s, 3H), 1.17 (dd, *J* = 7.0, 7.0 Hz, 3H), 1.13 (dd, *J* = 7.0, 7.0 Hz, 3H), 0.93 (dd, *J* = 12.5, 7.0 Hz, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.81 (dd, *J* = 7.0, 7.0 Hz, 3H), 0.64 (s, 3H).

The diethyl ketal (37mg, 89 μmol) was dissolved in 900 μL of acetone and to this solution was added Amberlite IR120 acidic resin (44 mg, 195 μmol). The resultant solution was allowed to stir for 1 h at room temperature at which time TLC analysis indicated complete conversion. The solution was filtered and concentrated to provide the crude material that was purified by flash chromatography to provide **141** (30 mg, 98%). White amorphous solid; R_f 0.24; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.89 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.50 (dd, *J* = 11.0, 11.0 Hz, 1H), 2.33–2.03 (m, 8H), 1.98–1.85 (m, 3H), 1.71–0.76 (m, 11H), 1.31 (s, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.82 (dd, *J* = 7.0, 7.0 Hz, 3H), 0.69 (s, 3H).

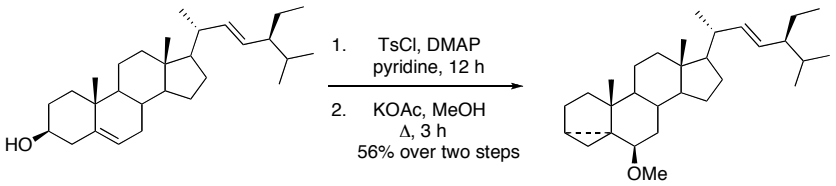
3α,7α-diformyloxy-24-nor-5β-chol-22-en (**159**):



159

To a solution of lead tetraacetate (33 g, 74.9 mmol), cupric acetate monohydrate (373 mg, 1.87 mmol), and pyridine (2.3 mL, 28.1 mmol) in 375 mL of pyridine was added 3α,7α-diformyloxy-5β-cholan-24-oic acid (8.4 g, 18.7 mmol) in one portion. The solution was allowed to stir at room temperature for 24 h at which time the reaction mixture was eluted through a plug of celite/silica to provide the crude material on concentration. The crude material was purified by flash chromatography to provide **159** (3.2 g, 43%). White amorphous solid; R_f 0.50 (5:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (s, 1H), 8.02 (s, 1H), 5.64 (ddd, *J* = 17.5, 10.5, 8.5 Hz, 1H), 5.02 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H), 4.89 (dd, *J* = 17.0, 2.0 Hz, 1H), 4.81 (dd, *J* = 10.5, 2.5 Hz, 1H), 4.72 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H), 2.13 (ddd, *J* = 13.0, 13.0, 13.0 Hz, 1H), 2.09–1.96 (m, 3H), 1.90 (ddd, *J* = 14.5, 3.5, 3.5 Hz, 1H), 1.85 (ddd, *J* = 12.0, 12.0, 4.5 Hz, 1H), 1.75 (ddddd, *J* = 12.0, 12.0, 12.0, 3.5, 3.5 Hz, 1H), 1.70–1.59 (m, 4H), 1.53–1.48 (m, 3H), 1.39–1.15 (m, 6H), 1.11–1.02 (m, 5H), 0.95 (s, 3H), 0.68 (s, 3H).

i-stigmasterol methyl ether (**172**):



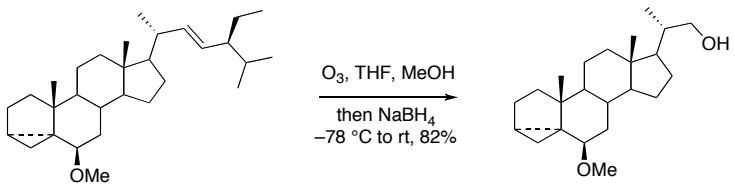
stigmasterol (**111**) *i*-stigmasterol methyl ether (**172**)

To a solution of *p*-toluenesulfonyl chloride (969 mg, 2.42 mmol) and 4-dimethylamino pyridine (30 mg, 242 μmol) in 15 mL of pyridine was added stigmasterol (1 g, 2.42 mmol). The resultant mixture was allowed to stir for 12 h at room temperature at which time the solution was poured into saturated aqueous sodium bicarbonate (100 mL) and filtered to provide the crude solid. Recrystallization from acetone provided the tosylate (970 mg, 71%). White amorphous solid; R_f 0.67 (5:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 5.30 (ddd, *J* = 5.5, 2.0, 2.0 Hz, 1H), 5.14 (dd, *J* = 15.5, 9.0 Hz, 1H), 5.01 (dd, *J* = 15.5, 9.0 Hz, 1H), 4.32 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H), 2.47–2.41 (m, 4H), 2.27 (dd, *J* = 13.0, 3.5 Hz, 1H), 2.07–1.90 (m, 3H), 1.84–1.79 (m, 2H), 1.74–1.66 (m, 2H), 1.57–1.38 (m, 10H), 1.28–1.11 (m, 4H), 1.06–1.02 (m, 1H), 1.01 (d, *J* = 6.5 Hz, 3H), 0.97 (s, 3H), 0.89 (dddd, *J* = 6.0, 6.0, 6.0, 6.0 Hz, 1H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.80 (dd, *J* = 8.5, 8.5 Hz, 3H), 0.79 (d, *J* = 8.0 Hz, 3H), 0.68 (s, 3H).

To a solution of the tosylate (7.1 g, 13.0 mmol) in 355 mL of methanol was added potassium acetate (7.1 g). The resultant solution was heated to reflux and maintained at this temperature for 3 h. The solution was cooled to room temperature, concentrated in vacuo, and diluted with 100 mL of water. The aqueous layer was extracted with 100 mL of ethyl acetate (3X) and the combined organic layer was

washed with brine and dried over anhydrous sodium sulfate prior to concentration in vacuo to provide the crude material. Purification by flash chromatography provided **172** (5 g, 90%). White amorphous solid; R_f 0.70 (5:1, hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.15 (dd, *J* = 15.0, 8.5 Hz, 1H), 5.02 (dd, *J* = 15.0, 8.5 Hz, 1H), 3.33 (s, 3H), 2.77 (dd, *J* = 2.5, 2.5 Hz, 1H), 0.74 (s, 3H), 0.65 (dd, *J* = 4.5, 4.5 Hz, 1H), 0.44 (dd, *J* = 8.0, 5.0 Hz, 1H).

6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholan-22-ol (**157**):

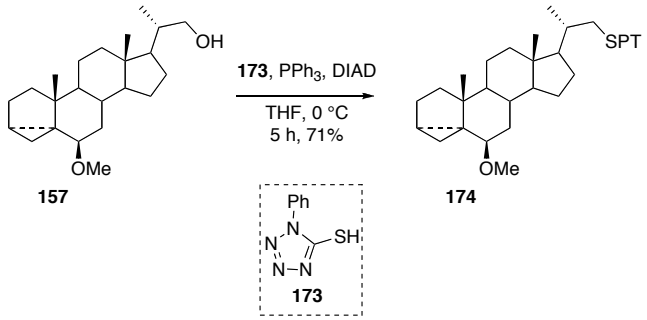


i-stigmasterol methyl ether (**172**) **157**

To a solution of **172** (1.45 g, 3.40 mmol) and sodium bicarbonate (1.45 g) in 70 mL THF and 7 mL methanol at –78 °C was passed ozone until TLC analysis indicated consumption of the starting material. Oxygen was passed through the solution for an addition 5 min at which time sodium borohydride (721 mg, 20.4 mmol) was added in one portion and the solution was allowed to reach room temperature over 1 h. The reaction mixture was quenched by the slow addition of saturated ammonium chloride and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate and subsequently concentrated in vacuo. The crude material was purified by flash chromatography to provide **157** (966 mg, 83%). White amorphous solid; R_f 0.20 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.64 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.37 (dd, *J* = 10.5, 6.5 Hz, 1H), 3.33 (s, 3H), 2.78 (dd, *J* = 3.0, 3.0 Hz, 1H), 1.98 (ddd, *J* = 12.0, 3.0, 3.0 Hz, 1H), 1.89 (ddd, *J* = 13.5,

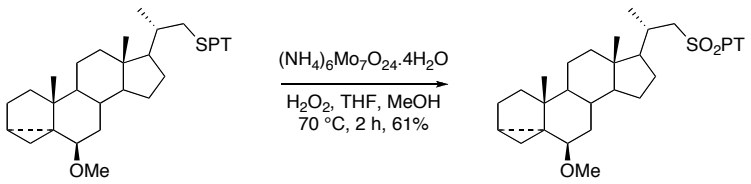
3.0, 3.0 Hz, 1H), 1.85–0.97 (m, 15H), 1.05 (d, *J* = 6.5 Hz, 3H), 0.95–0.77 (m, 4H), 0.74 (s, 3H), 0.65 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.43 (dd, *J* = 8.0, 5.0 Hz, 1H).

24-[22-(6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholanyl)-sulfanyl]-1-phenyl-1H-tetrazole (**174**):



To a solution of alcohol **157** (190 mg, 548 μmol), phenyl-1H-tetrazole-5-thiol (127 mg, 712 μmol), and triphenylphosphine (187 mg, 712 μmol) in 5.5 mL of THF was added diisopropyl azodicarboxylate (162 μL, 822 μmol) dropwise at 0 °C and allowed to stir at this temperature for an additional hour. The volatiles were removed in vacuo to provide the crude material that was purified by flash chromatography to provide **174** (196 mg, 71%). White amorphous solid; R_f 0.63 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.61–7.53 (m, 5H), 3.73 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.33 (s, 3H), 3.13 (dd, *J* = 12.5, 3.0 Hz, 1H), 2.77 (dd, *J* = 2.5, 2.5 Hz, 1H), 1.98–1.88 (m, 4H), 1.78–1.71 (m, 2H), 1.68–1.62 (m, 1H), 1.55–0.96 (m, 10H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.91–0.79 (m, 3H), 0.65 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.44 (dd, *J* = 8.0, 5.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.1, 134.0, 130.2, 130.0 (2C), 124.1 (2C), 82.5, 56.8, 56.5, 55.6, 48.1, 43.6, 43.4, 41.2, 40.3, 36.4, 35.5, 35.2, 33.6, 30.7, 28.2, 25.2, 24.4, 22.9, 21.7, 19.5, 19.0, 13.3, 12.6.

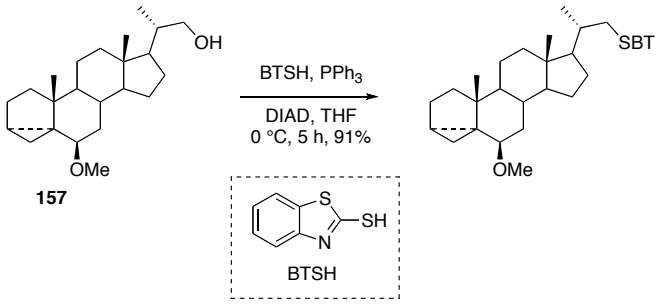
24-[22-(6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholanyl)-sulfonyl]-1-phenyl-1H-tetrazole (**175**):



157 **175**

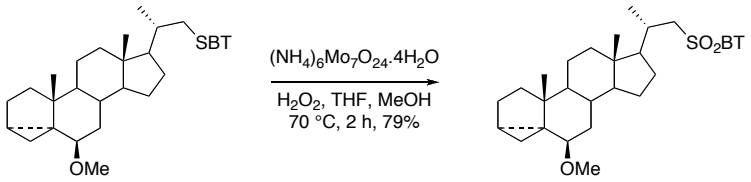
A solution of **157** (50 mg, 99 μmol), ammonium paramolybdate tetrahydrate (49 mg, 39 μmol), and 30% hydrogen peroxide (338 μL, 2.97 mmol) in 670 μL ethanol and 330 μL of tetrahydrofuran was heated at 70 °C for 2 h. The reaction mixture was cooled to room temperature and diluted with 5 mL of water and extracted with ethyl acetate. The combined organic layer was washed with saturated aqueous sodium thiosulfate, brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography to provide **175** (33 mg, 61%). White amorphous solid; R_f 0.62 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70–7.59 (m, 5H), 3.95 (dd, *J* = 14.5, 2.0 Hz, 1H), 3.50 (dd, *J* = 14.5, 10.5 Hz, 1H), 3.33 (s, 3H), 2.78 (dd, *J* = 2.5, 2.5 Hz, 1H), 2.39–2.31 (m, 1H), 1.97 (ddd, *J* = 12.5, 3.0, 3.0 Hz, 1H), 1.93–1.85 (m, 2H), 1.79–1.72 (m, 2H), 1.67–1.63 (m, 1H), 1.53 (dd, *J* = 12.5, 5.0 Hz, 1H), 1.49 (dd, *J* = 12.5, 8.0 Hz, 1H), 1.46–1.31 (m, 4H), 1.26 (d, *J* = 6.5 Hz, 3H), 1.24–1.15 (m, 2H), 1.13–1.05 (m, 2H), 1.02 (s, 3H), 0.92–0.79 (m, 3H), 0.77 (s, 3H), 0.66 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.45 (dd, *J* = 8.0, 5.0 Hz, 1H).

24-[22-(6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholanyl)-sulfanyl]-benzothiazole:



To a solution of **157** (1 g, 2.89 mmol), triphenylphosphine (985 mg, 3.76 mmol), and 2-mercaptobenzothiazole (629 mg, 3.76 mmol) in 30 mL of tetrahydrofuran at 0 °C was added dropwise diisopropyl azodicarboxylate (852 μL, 4.33 mmol). The resultant solution was allowed to stir at 0 °C for an additional 5 h at which time the reaction mixture was concentrated in vacuo and purified by recrystallization from acetone to give the BT-thioether (1.3 g, 91%). White amorphous solid; R_f 0.62 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (ddd, *J* = 8.0, 0.5, 0.5 Hz, 1H), 7.75 (ddd, *J* = 8.0, 1.0, 1.0 Hz, 1H), 7.41 (ddd, *J* = 8.5, 8.5, 1.5 Hz, 1H), 7.29 (ddd, *J* = 8.0, 8.0, 1.0 Hz, 1H), 3.65 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.33 (s, 3H), 3.07 (dd, *J* = 12.5, 8.5 Hz, 1H), 2.78 (dd, *J* = 3.0, 3.0 Hz, 1H), 2.02–1.89 (m, 4H), 1.80–1.72 (m, 2H), 1.67 (dddd, *J* = 12.0, 9.0, 9.0, 3.5 Hz, 1H), 1.56–1.48 (m, 3H), 1.46–1.36 (m, 2H), 1.31 (ddd, *J* = 9.5, 9.5, 9.5 Hz, 1H), 1.25–1.06 (m, 7H), 1.03 (s, 3H), 0.92–0.81 (m, 3H), 0.75 (s, 3H), 0.66 (dd, *J* = 4.5, 4.5 Hz, 1H), 0.44 (dd, *J* = 8.0, 5.5 Hz, 1H).

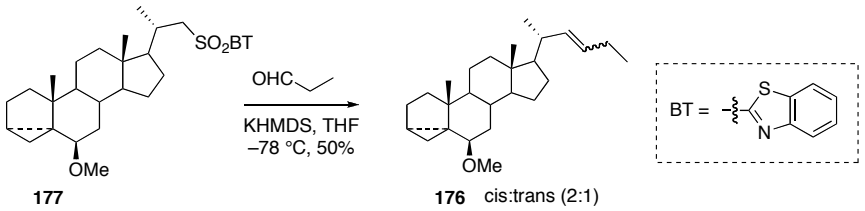
24-[22-(6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholanyl)-sulfonyl]-benzothiazole (**177**):



177

A solution of the BT-thioether (230 mg, 454 μmol), ammonium paramolybdate tetrahydrate (112 mg, 91 μmol), and 30% hydrogen peroxide (774 μL, 6.81 mmol) in 6.8 mL of tetrahydrofuran and 2.3 mL methanol was heated to 70 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with 10 mL of water with subsequent extraction with ethyl acetate. The combined organic layer was washed with saturated aqueous sodium thiosulfate, brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography to give **177** (180 mg, 79%). White amorphous solid; R_f 0.39 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (d, *J* = 7.5 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.63 (ddd, *J* = 8.5, 8.5, 1.5 Hz, 1H), 7.58 (ddd, *J* = 7.5, 7.5, 1.5 Hz, 1H), 3.63 (dd, *J* = 14.5, 1.5 Hz, 1H), 3.26 (dd, *J* = 15.0, 10.0 Hz, 1H), 3.75 (dd, *J* = 2.0, 2.0 Hz, 1H), 2.36–2.27 (m, 1H), 1.95 (ddd, *J* = 12.5, 3.0, 3.0 Hz, 1H), 1.88–1.68 (m, 5H), 1.62–1.56 (m, 1H), 1.53–1.21 (m, 6H), 1.24 (d, *J* = 6.5 Hz, 3H), 1.17–1.00 (m, 5H), 0.94 (s, 3H), 0.88–0.76 (m, 4H), 0.72 (s, 3H), 0.63 (dd, *J* = 4.5, 4.5 Hz, 1H), 0.42 (dd, *J* = 8.0, 5.0 Hz, 1H).

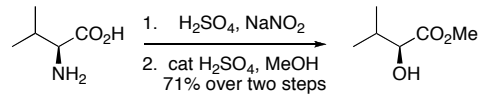
cis,*trans*-23-(6β-methoxy-3α,5-cyclo-5α-24-norchol-22-enyl)-ethane (**176**):



177 **176** cis:trans (2:1)

To a cooled solution (–78 °C) solution of **177** (10 mg, 19 μmol) and propionaldehyde (1.2 μL, 17 μmol) in 380 μL of tetrahydrofuran was added dropwise 0.5 M potassium *bis*-(trimethylsilyl)amide (60 μL, 30 μmol) until the –78 °C for 1 h and subsequently allowed to reach room temperature. After 12 h the reaction was quenched upon addition of saturated ammonium chloride and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography to provide an inseparable mixture of *cis* and *trans* isomers (2:1) **176** (3.5 mg, 50%). Colorless oil; R_f 0.89 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (dd, *J* = 15.0, 6.0 Hz, 1H), 5.22 (dd, *J* = 15.5, 9.0 Hz, 1H), 5.18 (d, *J* = 10.5 Hz, 1H), 5.10 (dd, *J* = 10.5, 10.5 Hz, 1H), 1.03 (s, 3H), 1.02 (s, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.76 (s, 3H), 0.73 (s, 3H).

methyl (*S*)-2-hydroxy-3-methylbutyrate (**179**):

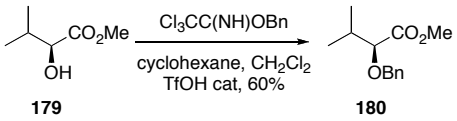


L-valine (**178**) **179**

To a stirred suspension of L-valine (20 g, 171 mmol) in 120 mL of water was added 2 N sulfuric acid (94 mL) at 0 °C at which time the solution became homogeneous. A 2 M aqueous solution of sodium nitrite (94 mL) was added over 3 h at 0 °C via an addition funnel and the solution was subsequently allowed stir at room temperature for an additional 12 h. The reaction mixture was extracted with ethyl acetate (5 X 200 mL) and the combined organic layer was dried over anhydrous sodium sulfate. The volatiles were removed to provide the crude solid, which was recrystallized from ether–petroleum ether to provide the α-hydroxy acid (14.3 g, 71%). White crystalline solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.15 (d, J = 3.5 Hz, 1H), 2.14 (septd, J = 7.0, 3.5 Hz, 1H), 1.04 (d, J = 6.5 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H).

A solution of the α-hydroxy acid (14 g, 119 mmol) and concentrated sulfuric acid (500 μL) in 140 mL of methanol was heated to reflux for 5 h. The solution was cooled to room temperature and concentrated in vacuo to approximately 5 mL and then diluted with 100 mL of ether. The organic layer was washed with saturated sodium bicarbonate and brine and dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo provided the crude material **179** (15.7 g, quant) that was used in the next step without purification.

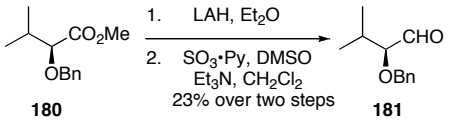
methyl (*S*)-2-benzyloxy-3-methylbutyrate (**180**):



179 **180**

To a stirred solution of **179** (14 g, 106 mmol) and freshly prepared benzyl 2,2,2-trichloroacetimidate (19.7 mL, 106 mmol) in 140 mL of cyclohexane and 70 mL of methylene chloride was added triflic acid (938 μL, 11 mmol). The resultant mixture was allowed to stir at room temperature for 2 h and subsequently filtered and concentrated. The crude material was purified by flash chromatography to provide **180** (14.1 g, 60%). Colorless oil; R_f 0.59 (5:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.30 (m, 5H), 4.72 (d, J = 12.0 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 3.76 (s, 3H), 3.72 (d, J = 5.5 Hz, 1H), 2.16–2.05 (m, 1H), 0.99 (d, J = 7.0 Hz, 3H), 0.97 (d, J = 7.0 Hz, 3H).

(*S*)-2-benzyloxy-3-methylbutyraldehyde (**181**):



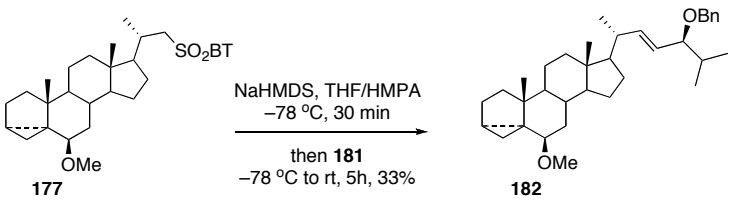
180 **181**

To a solution of lithium aluminum hydride (7.9 g, 208 mmol) in 210 mL of ethyl ether was added a 1 M solution of **180** (63 mL) dropwise at 0 °C via an addition funnel. The solution was warmed to room temperature and allowed to stir for 3 h. The solution was cooled to 0 °C and carefully quenched according to the Fieser and Fieser protocol (i.e.; 7.9 mL water, 7.9 mL 3N NaOH, and 23.7 mL water) and was subsequently filtered and dried over anhydrous sodium sulfate. The volatiles were removed in vacuo

and the crude material was purified by flash chromatography to provide the primary alcohol (5.7 g, 47%). Colorless oil; R_f 0.30 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.34 (m, 5H), 4.63 (d, *J* = 11.0 Hz, 1H), 4.57 (d, *J* = 11.5 Hz, 1H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.61 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.26 (ddd, *J* = 6.5, 6.5, 3.5 Hz, 1H), 2.02–1.94 (m, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 7.0 Hz, 3H).

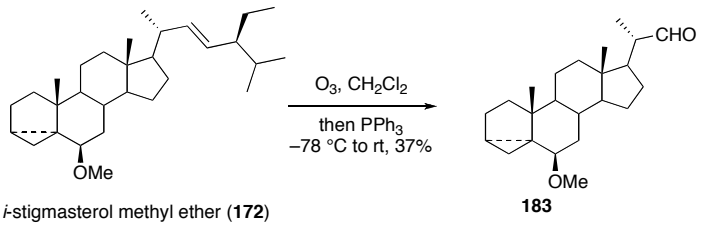
To a solution of sulfur trioxide–pyridine (1.63 g, 10.3 mmol) and triethylamine (2.9 mL, 20.6 mmol) in dimethylsulfoxide (1 mL)–methylene chloride (9 mL) at 0 °C was added dropwise the primary alcohol (500 mg, 2.57 mmol in 3 mL methylene chloride). The resultant solution was allowed to stir for 3 h at 0 °C and subsequently quenched upon the addition of saturated ammonium chloride and diluted with ethyl acetate. The aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Purification of the crude material by flash chromatography provided **181** (240 mg, 49%). Colorless oil; R_f 0.74 (3:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.66 (d, *J* = 3.0 Hz, 1H), 7.38–7.31 (m, 5H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 3.47 (dd, *J* = 6.0, 3.0 Hz, 1H), 2.18–2.02 (m, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 7.0 Hz, 3H).

(22*E*,24*S*)-6β-methoxy-3α,5-cyclo-5α-24-benzyloxychol-22-ene (**182**):



To a stirred solution of **177** (33 mg, 62 μmol) in tetrahydrofuran (96 μL)–hexamethylphosphoramide (125 μL) at –78 °C was added 2 M (THF) sodium *bis*(trimethylsilyl)amide (39 μL, 78 μmol). The resultant solution was allowed to stir at –78 °C for 30 min followed by the addition of **181** (10 mg, 52 μmol, in 96 μL THF). The mixture was allowed to reach room temperature and allowed to stir for an additional 5 h at which time the reaction was quenched upon the addition of saturated ammonium chloride. The aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with saturated sodium bicarbonate, brine, and dried over anhydrous sodium sulfate. The crude material was purified by flash chromatograpy to provide **182** (10.5 mg, 33%). Colorless oil; R_f 0.83 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35–7.32 (m, 5H), 5.42 (dd, *J* = 15.0, 8.5 Hz, 1H), 5.24 (dd, *J* = 15.5, 8.5 Hz, 1H), 4.58 (d, *J* = 12.0 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H), 3.34 (s, 3H), 3.30 (dd, *J* = 8.5, 7.0 Hz, 1H), 2.78 (dd, *J* = 2.5, 2.5 Hz, 1H), 2.20–2.10 (m, 1H), 1.99 (ddd, *J* = 12.5, 3.5, 3.5 Hz, 1H), 1.89 (ddd, *J* = 13.5, 3.0, 3.0 Hz), 1.81–1.64 (m, 3H), 1.62–1.50 (m, 3H), 1.46–1.37 (m, 2H), 1.34–0.78 (m, 10H), 1.10 (d, *J* = 7.0 Hz, 3H), 1.04 (s, 3H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.76 (s, 3H), 0.66 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.45 (dd, *J* = 7.5, 5.0 Hz, 1H).

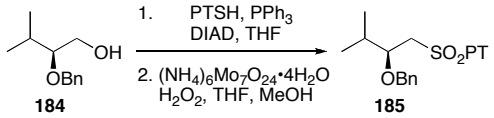
6β-methoxy-3α,5-cyclo-5α-23,24-bisnorcholan-22-al (**183**):



i-stigmasterol methyl ether (**172**) **183**

To a solution of **172** (200 mg, 469 μmol) in 9 mL methylene chloride at –78 °C was passed ozone until the blue color persisted. Oxygen was passed through the solution for an addition 5 min until the blue color had diminished at which triphenylphosphine (369 mg, 1.41 mmol) was added in one portion and the solution was allowed to reach room temperature over 1 h. The reaction mixture was concentrated in vacuo and the crude material was purified by flash chromatography to provide **183** (60 mg, 37%). White amorphous solid; R_f 0.41 (10:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.58 (d, *J* = 3.5 Hz, 1H), 3.33 (s, 3H), 2.79 (dd, *J* = 2.5, 2.5 Hz, 1H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.03 (s, 3H), 0.77 (s, 3H), 0.66 (dd, *J* = 4.0, 4.0 Hz, 1H), 0.45 (dd, *J* = 8.0, 5.5 Hz, 1H).

1-[(*S*)-2-benzyloxy-3-methylbutylsulfonyl]-benzothiazole(**185**):

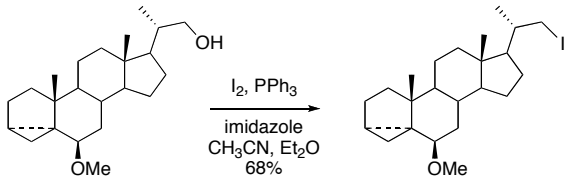


184 **185**

To a solution of **184** (500 mg, 2.57 mmol), triphenylphosphine (876 mg, 3.34 mmol), and phenyl-1H-tetrazole-5-thiol (595 mg, 3.34 mmol) in 26 mL of tetrahydrofuran at 0 °C was added dropwise diisopropyl azodicarboxylate (759 μL, 3.86 mmol). The resultant solution was allowed to stir at 0 °C for one hour at which time the reaction mixture was concentrated in vacuo and purified by flash chromatograpy to give the BT-thioether (685 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63–7.52 (m, 5H), 7.39–7.26 (m, 5H), 4.66–4.57 (m, 2H), 3.79 (dd, *J* = 12.5, 5.5Hz, 1H), 3.64–3.51 (m, 3H), 2.15–1.99 (m, 1H), 1.05 (d, *J* = 7.0Hz, 3H), 1.01 (d, *J* = 7.0Hz, 3H).

To a stirred solution of the BT-thioether (625 mg, 1.76 mmol) in 15 mL of tetrahydrofuran and 5 mL of methanol was added 30% hydrogen peroxide (3 mL, 26.4 mmol) followed by ammonium paramolybdate tetrahydrate (436 mg, 353 μmol). The solution was allowed to stir at room temperature for 5 h and subsequently diluted with 100 mL of ethyl acetate and washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and removed in vacuo to provide the crude material which was purified by flash chromatography to give **185**. Colorless oil; R_f = 0.58 (3:1 hexanes/ethyl acetate).

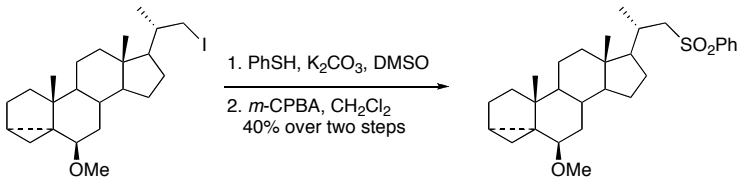
6β-methoxy-3α,5-cyclo-5α-23,24-bisnor-22-iodocholane (**186**):



157 **186**

To a solution **157** (330 mg, 952 μmol), imidazole (130 mg, 1.90 mmol), and triphenylphosphine (275 mg, 1.05 mmol) in 7.1 mL ethyl ether and 2.4 mL acetonitrile was added iodine (181 mg, 1.43 mmol) in one portion. After 5 h the reaction was quenched with aqueous sodium thiosulfate and extracted with ethyl ether. The combined organic layer was washed with water and brine and dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo provided the crude material which was purified by flash chromatography to give **186** (295 mg, 68%). White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.34 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.33 (s, 3H), 3.18 (dd, *J* = 9.0, 5.0 Hz, 1H), 2.77 (dd, *J* = 3.0, 3.0 Hz, 1H), 1.94 (ddd, *J* = 12.5, 12.5, 3.5 Hz, 1H), 1.89 (ddd, *J* = 13.0, 13.0, 3.5 Hz, 1H), 1.88–1.82 (m, 1H), 1.78–1.62 (m, 3H), 1.51 (ddd, *J* = 13.5, 13.5, 8.0 Hz, 1H), 1.45–1.35 (m, 2H), 1.29–1.04 (m, 8H), 1.02 (d, *J* = 5.5 Hz, 3H), 1.02 (s, 3H), 0.91–0.80 (m, 3H), 0.76 (s, 3H), 0.65 (dd, *J* = 4.5, 4.5 Hz, 1H), 0.44 (dd, *J* = 8.0, 5.0 Hz, 1H).

6β-methoxy-3α,5-cyclo-5α-23,24-bisnor-22-(phenylsulfonyl)cholane (**186**):



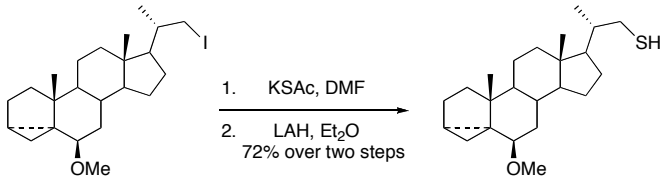
186 **186**

A solution of **186** (650 mg, 1.42 mmol), phenylthiol (292 μL, 2.85 mmol), and potassium carbonate (589 mg, 4.26 mmol) in 15 mL of dimethylsulfoxide was allowed to stir at room temperature for 12 h. The solution was diluted with 150 mL of ethyl acetate and washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and subsequently concentrated in vacuo to provide the crude material which was purified by flash chromatography to give the phenyl thioether (382 mg, 61%). White amorphous solid; R_f 0.59 (10:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.30 (m, 2H), 7.28–7.24 (m, 2H), 7.14 (dddd, *J* = 7.5, 1.5 Hz, 1H), 3.32 (s, 3H), 3.14 (dd, *J* = 12.0, 3.0 Hz, 1H), 2.77 (dd, *J* = 2.5, 2.5 Hz, 1H), 2.64 (dd, *J* = 12.0, 3.0 Hz, 1H), 1.98 (ddd, *J* = 12.5, 3.0 Hz, 1H), 1.93–1.83 (m, 2H), 1.79–1.69 (m, 3H), 1.65–1.57 (m, 2H), 1.55–1.00 (m, 8H), 1.12 (d, *J* = 7.0 Hz, 3H), 1.02 (s, 3H), 0.91–0.79 (m, 3H), 0.71 (s, 3H), 0.65 (dd, *J* = 5.0, 5.0Hz, 1H), 0.43 (dd, *J* = 8.0, 5.5Hz, 1H).

To a stirred solution of 70% *meta*-chloroperoxybenzoic acid (457 mg, 1.85 mmol) in 28 mL of methylene chloride was added a solution of the phenyl thioether (370 mg, 843 μmol, in 2.8 mL CH_2Cl_2). The solution was allowed to stir for 3 h at which time the solution was diluted with 100 mL of saturated sodium bicarbonate and extracted with ethyl acetate. The combined organic layer was washed with brine, dried

over anhydrous sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography to provide the sulfone **186** (261 mg, 66%). Colorless oil; R_f 0.43 (3:1 hexanes/ethyl acetate)

6β-methoxy-3α,5-cyclo-5α-23,24-bisnor-22-mercaptocholane (**186**):



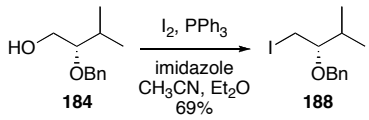
186 **187**

To a solution of **186** (260 mg, 570 μmol) in 11.4 mL of *N*,*N*-dimethylformamide at room temperature was added potassium thioacetate (325 mg, 2.85 mmol) in one portion and the resultant solution was allowed to stir for 1 h. The reaction mixture was diluted with 100 mL of ethyl acetate and washed with water (3 X 100 mL), brine (3 X 100 mL), and the organic layer was dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo provided the crude material which was purified by flash chromatography to give the thioacetate (187 mg, 81%). White amorphous solid; R_f 0.37 (10:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.33 (s, 3H), 3.16 (dd, *J* = 13.0, 3.0 Hz, 1H), 2.77 (dd, *J* = 3.0, 3.0 Hz, 1H), 2.59 (dd, *J* = 13.0, 8.5 Hz, 1H), 2.33 (s, 3H), 1.99–0.77 (m, 20H), 1.02 (s, 3H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.72 (s, 3H), 0.65 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.43 (dd, *J* = 8.0, 5.5 Hz, 1H).

To a solution of the thioacetate (100 mg, 247 μmol) in 2.5 mL of ethyl ether at –78 °C was added dropwise 1 M (toluene) lithium aluminum hydride (494 μL, 494 μmol). The solution was warmed to room temperature over 30 min and subsequently quenched at 0 °C with 1 M hydrochloric acid (120 μL). The solution was warmed to

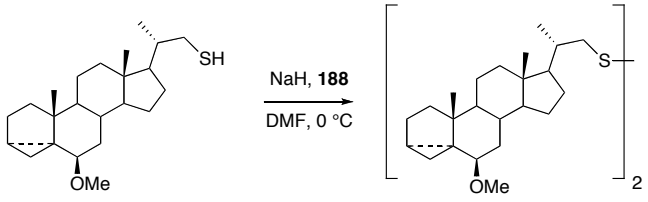
room temperature and filtered through celite and dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo provided the crude material which was purified by flash chromatography to give **187** (80 mg, 89%). Colorless oil; R_f 0.42 (10:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.33 (s, 3H), 2.77 (dd, *J* = 3.0, 3.0 Hz, 1H), 2.66 (ddd, *J* = 13.0, 8.5, 3.0 Hz, 1H), 2.36 (ddd, *J* = 13.0, 7.5, 7.5 Hz, 1H), 1.96 (ddd, *J* = 12.0, 3.0, 3.0 Hz, 1H), 1.89 (ddd, *J* = 13.5, 3.0, 3.0 Hz, 1H), 1.84–1.36 (m, 9H), 1.33–1.06 (m, 7H), 1.04 (d, *J* = 6.5 Hz, 3H), 1.02 (s, 3H), 0.92–0.77 (m, 3H), 0.73 (s, 3H), 0.65 (dd, *J* = 5.0, 5.0 Hz, 1H), 0.43 (dd, *J* = 8.0, 5.5 Hz, 1H).

1-iodo-(2*S*)-benzyloxy-3-methylbutane (**188**):



To a solution **184** (300 mg, 1.54 mmol), imidazole (315 mg, 4.62 mmol), and triphenylphosphine (444 mg, 1.69 mmol) in 12 mL diethyl ether and 4 mL acetonitrile was added iodine (586 mg, 4.62 mmol) in one portion. After 5 h the reaction was quenched with aqueous sodium thiosulfate and extracted with diethyl ether. The combined organic layer was washed with water and brine and dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo provided the crude material which was purified by flash chromatography to give **188** (323 mg, 69%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.29 (m, 5H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 3.37 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.30 (dd, *J* = 11.0, 5.5 Hz, 3H), 3.05 (dd, *J* = 5.5, 5.5 Hz, 1H), 2.05–1.92 (m, 1H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H).

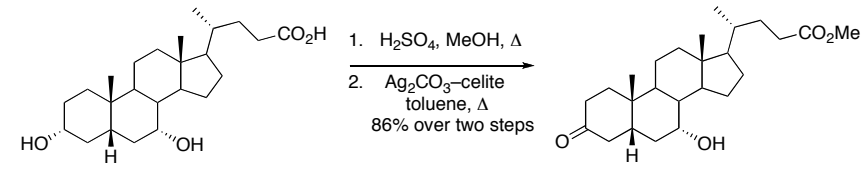
6β-methoxy-3α,5-cyclo-5α-23,24-bisnor-22,22'-dithiocholane (**189**):



187 **189**

To a stirred solution of **187** (25 mg, 69 μmol) in 1.4 mL of *N*,*N*-dimethylformamide at 0 °C was added in one portion 60% sodium hydride dispersion in mineral oil (4.1 mg, 104 μL). The solution was allowed to stir for 30 min at 0 °C at which time the sodium thiolate precipitated out of solution. The iodide **188** (23 mg, 76 μmol) was added (100 μL of DMF) to the suspension in one portion at room temperature and subsequently the mixture was heated to 60 °C for 5 h at which time TLC analysis indicated consumption of **187**. The mixture was cooled to room temperature, quenched with saturated ammonium chloride, and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. Removal of the volatiles in vacuo and purification of the crude material by flash chromatography provide the iodide **188** unaffected and **189** (5 mg, 20%). The conversion was nearly spot to spot, but was isolated in low yield due to solubility of the product disulfide. Colorless oil; R_f 0.31 (10:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.34 (s, 6H), 2.93 (dd, *J* = 13.0, 2.0 Hz, 2H), 2.79 (dd, *J* = 3.0, 3.0 Hz, 2H), 2.40 (dd, *J* = 13.0, 2.0 Hz, 2H), 1.09 (d, *J* = 7.0 Hz, 6H), 1.04 (s, 6H), 0.76 (s, 6H), 0.67 (dd, *J* = 5.0, 5.0 Hz, 2H), 0.45 (dd, *J* = 8.0, 5.5 Hz, 2H).

methyl 3-oxo-7α-hydroxy-5β-cholan-24-oate (**191**):



chenodeoxycholic acid (**158**) **191**

A solution of chenodeoxycholic acid (15 g, 38.7 mmol) and 1.3 mL of concentrated sulfuric acid in 130 mL of methanol was heated to reflux for 5 h. The solution was cooled to room temperature and carefully quenched with solid sodium bicarbonate (1.5 g) and subsequently concentrated in vacuo. The residue was triturated with methylene chloride and the organic phase was dried over anhydrous sodium sulfate followed by concentrated in vacuo. The crude material was sufficiently pure for use in the subsequent step. White amorphous solid; R_f 0.47 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.85 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H), 3.67 (s, 3H), 3.42 (dddd, *J* = 7.0, 7.0, 4.0, 4.0 Hz, 1H), 2.36 (ddd, *J* = 15.5, 10.0, 5.0 Hz, 1H), 0.98 (ddd, *J* = 14.5, 14.5, 3.5 Hz, 1H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.90 (s, 3H), 0.66 (s, 3H).

A stirred suspension of freshly prepared Fetizon's reagent (50% w/w Ag_2CO_3–celite) (59.3 g, 107.7 mmol) and the diol (14.6 g, 35.9 mmol) in 720 mL of toluene was heated to reflux (Dean–Stark trap) for 3 h. The suspension was cooled to approximately 50 °C and filtered; the filter cake was washed several times with ethyl acetate and the combined organic layer was concentrated in vacuo. Flash chromatography of the crude material afforded **191** (12.5 g, 86%). White amorphous solid; R_f 0.39 (3:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H), 3.67 (s,

3H), 3.40 (dd, *J* = 14.5, 14.5 Hz, 1H), 1.01 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.70 (s, 3H).

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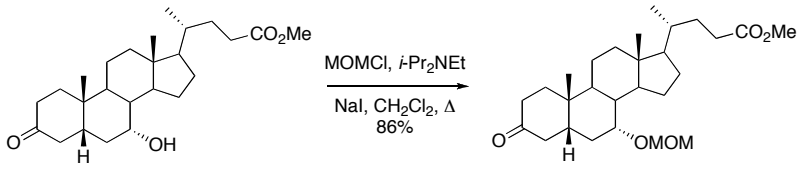
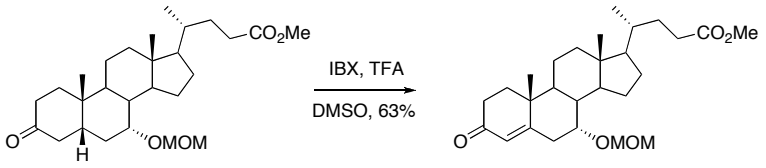
methyl 3-oxo-7α-(methoxymethoxy)-5β-cholan-24-oate (**126**):



191 **126**

A stirred suspension of **191** (9.0 g, 22.2 mmol), methoxymethylchloride (8.1 g, 50.2 mmol), *N*,*N*-diisopropylethylamine (35 mL, 201 mmol), and sodium iodide (603 mg, 4.02 mmol) in 70 mL of methylene chloride was heated to reflux at which time the solution became homogeneous. After 12 h, the reaction mixture was cooled to room temperature and diluted with 200 mL of saturated aqueous ammonium chloride and extracted with ethyl acetate (3 X 200 mL). The combined organic layer was washed with water, brine, and dried over anhydrous sodium sulfate. The organic layer was removed in vacuo to provide the crude material which was purified by flash chromatography to afford **126** (8.6 g, 86%). White amorphous solid; R_f 0.67 (3:1 hexanes/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.67 (d, *J* = 7.0 Hz, 1H), 4.54 (d, *J* = 7.0 Hz, 1H), 3.67–3.66 (m, 4H), 3.37 (s, 3H), 3.32 (dd, *J* = 14.0, 14.0 Hz, 1H), 2.39 (ddd, *J* = 15.0, 15.0, 5.0 Hz, 1H), 2.34 (dd, *J* = 10.0, 5.0 Hz, 1H), 2.21 (ddd, *J* = 16.0, 10.0, 6.5 Hz, 1H), 1.01 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.68 (s, 3H).

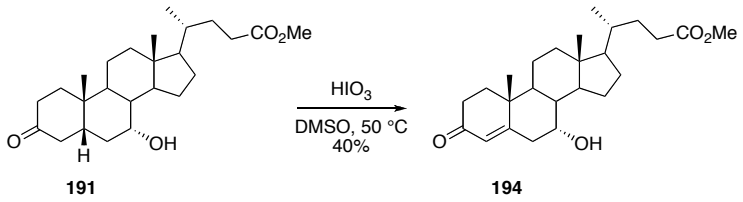
methyl 3-oxo-7α-(methoxymethoxy)-5β-chol-5-en-24-oate (**126**):



126 **127**

A suspension of 2-iodoxybenzoic acid (9.24 g, 33.0 mmol) in 165 mL of dimethylsulfoxide was heated at 50 °C for 1 h at which time the solution became homogeneous. The solution was cooled to room temperature followed by the addition of **127** (7.4 g, 16.5 mmol) and 2,2,2-trifluoroacetic acid (12.3 mL, 165 mmol). The resultant mixture was allowed to stir at room temperature for 36 h at which time the solution was carefully quenched by the addition of solid sodium bicarbonate (1.7 g, 33 mmol). The suspension was diluted with 500 mL of ethyl acetate and washed with water (3 X 250 mL), saturated aqueous sodium bicarbonate (250 mL), and brine (250 mL). The combined organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to provide the crude material. Purification by flash chromatography afforded **127** (4.6 g, 63%). White amorphous solid; R_f 0.53 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75 (s, 1H), 4.67 (d, *J* = 7.0 Hz, 1H), 4.61 (d, *J* = 7.0 Hz, 1H), 3.76 (ddd, *J* = 2.5, 2.5, 2.5 Hz, 1H), 3.67 (s, 3H), 3.35 (s, 3H), 2.63 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.47–2.33 (m, 4H), 2.23 (ddd, *J* = 16.5, 10.0, 6.5 Hz, 1H), 2.04 (ddd, *J* = 13.5, 5.0, 3.0 Hz, 1H), 1.99 (ddd, *J* = 13.0, 3.5, 3.5 Hz, 1H), 1.90 (dddd, *J* = 13.5, 9.5, 9.5, 6.5 Hz, 1H), 1.84–1.75 (m, 2H), 1.68–1.52 (m, 4H), 1.48–1.29 (m, 4H), 1.21–1.07 (m, 4H), 1.19 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.71 (s, 3H).

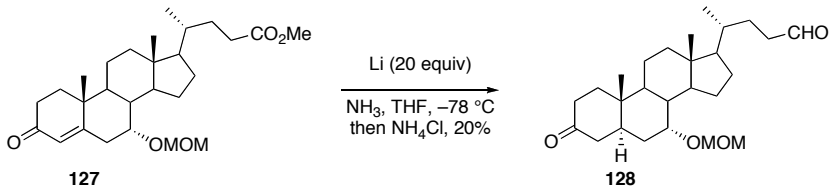
methyl 3-oxo-7α-hydroxy-5β-chol-5-en-24-oate (**194**):



191 **194**

A suspension of iodic acid (339 mg, 1.93 mmol) and cyclohexene (323 μL) in 1.9 mL dimethylsulfoxide was heated to 80 °C for 1 h. The solution was cooled to 50 °C at which time **191** (520 mg, 1.29 mmol) in 1.3 mL of dimethylsulfoxide was added to the solution. The reaction mixture was maintained at 50 °C for 12 h and subsequently cooled to room temperature followed by the addition of 5 mL saturated aqueous thiosulfate. The mixture was extracted with ethyl acetate and the combined organic layer was washed with saturated sodium bicarbonate, brine, and dried over anhydrous sodium sulfate. Concentration in vacuo provided the crude material which was purified by flash chromatography to afford **194** (207 mg, 40%). White amorphous solid; R_f 0.40 (1:2 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.80 (s, 1H), 3.97 (ddd, J = 2.0, 2.0, 2.0 Hz, 1H), 3.67 (s, 3H), 2.62 (ddd, J = 15.0, 3.5, 3.5 Hz, 1H), 1.17 (s, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H).

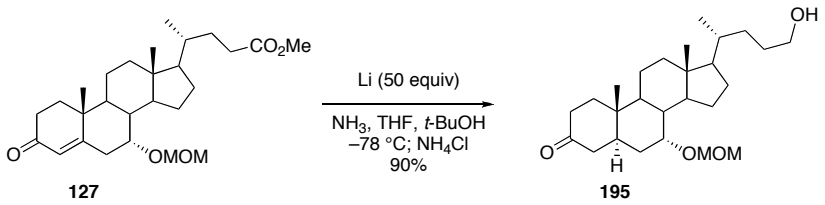
3-oxo-7α-(methoxymethoxy)-5α-cholan-24-al (**128**):



127 **128**

Lithium wire (31 mg, 4.48 mmol) was added at –78 °C to 3 mL of liquid ammonia [distilled from $Fe(NO_3)$/Na] with stirring. After the solution was homogeneous, **127** (100 mg, 224 μmol) in 1 mL of tetrahydrofuran was added dropwise. The reaction mixture was allowed to stir for 1 h at –78 °C prior to quenching with solid ammonium chloride (1 g). The suspension was warmed to room temperature with concomitant evaporation of the ammonia to provide a residue which was taken up in diethyl ether and washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to provide the crude material. Purification by flash chromatography afforded the aldehyde **128** (21 mg, 20%). Colorless oil; R_f 0.55 (1:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.77 (s, 1H), 4.67 (d, *J* = 6.5 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 3.62 (s, 1H), 3.35 (s, 3H), 1.01 (s, 3H), 0.68 (s, 3H).

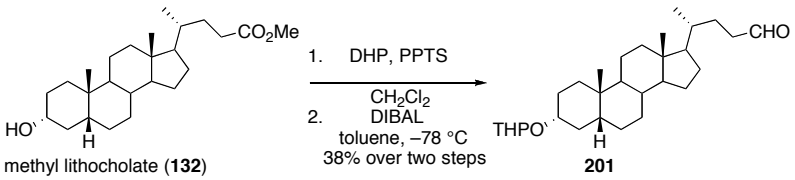
3-oxo-7α-(methoxymethoxy)-5α-cholan-24-ol (**195**):



127 **195**

Lithium wire (78 mg, 11.2 mmol) was added at –78 °C to 16 mL of liquid ammonia [distilled from $Fe(NO_3)$/Na] with stirring. After the solution was homogeneous, **127** (100 mg, 224 μmol) in 8 mL of tetrahydrofuran and *t*-butanol (210 μL, 2.24 mmol) was added dropwise. The reaction mixture was allowed to stir for 1 h at –78 °C prior to quenching with solid ammonium chloride (1.2 g). The suspension was warmed to room temperature with concomitant evaporation of the ammonia to provide a residue which was taken up in diethyl ether and washed with water and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to provide the crude material. Purification by flash chromatography afforded **195** (85 mg, 90%). Colorless oil; R_f 0.30 (1:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.67 (d, J = 7.0 Hz, 1H), 4.60 (d, J = 7.0 Hz, 1H), 3.63–3.60 (m, 3H), 1.01 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.68 (s, 3H).

3α-(tetrahydropyranyloxy)-5β-cholan-24-al (**201**):

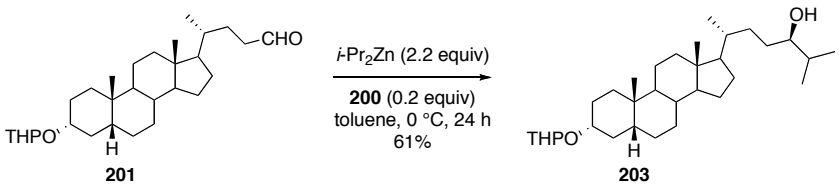


To a solution of methyl lithocholate (1.0 g, 2.56 mmol) and 3,4-dihydro-2H-pyran (351 μL, 3.84 mmol) in 8.5 mL of methylene chloride was added pyridinium *para*-toluenesulfonate (32 mg, 128 μmol). The resultant solution was allowed to stir at room temperature for 8 h at which time the volatiles were removed in vacuo to provide the crude material. Purification by flash chromatography provided the THP-ether (1.1 g, 91%). White amorphous solid; R_f 0.63 (5:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHZ, $CDCl_3$): δ 4.73–4.71 (m, 1H), 3.94–3.90 (m, 1H), 3.67 (m, 4H), 3.51–3.46 (m, 1H), 2.35 (ddd, J = 15.0, 10.0, 5.0 Hz, 1H), 2.21 (ddd, J = 16.0, 10.0, 6.5 Hz, 1H), 1.93 (ddd, J = 15.0, 3.0, 3.0 Hz, 1H), 0.90 (s, 3H), 0.89 (d, J = 7.0 Hz, 3H), 0.63 (s, 3H).

To a solution of the THP-ether (250 mg, 527 μmol) in 2.6 mL of toluene at –78 °C was added 1M (toluene) diisobutylaluminum hydride (632 μL, 632 μmol) over 15 min. The reaction mixture was allowed to stir at –78 °C for an additional hour followed by the addition of methanol (280 μL–then 15 min). A saturated aqueous solution Rochelle's salt (3 mL) was added and the solution was warmed to room temperature with continued stirring until the biphasic solution was no longer turbid (~3 h). The mixture was extracted with ethyl acetate and the combined organic layer was washed with saturated aqueous ammonium chloride, saturated aqueous sodium bicarbonate, brine, and dried over anhydrous sodium sulfate. Concentration in vacuo provided the

crude material which was purified by flash chromatography to afford **201** (135 mg, 58%). Colorless oil; R_f 0.65 (5:1 hexanes/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.77 (dd, *J* = 2.0, 2.0 Hz, 1H), 4.75–4.69 (m, 1H), 3.96–3.89 (m, 1H), 3.69–3.57 (m, 1H), 3.52–3.45 (m, 1H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.64 (s, 3H).

(24*R*)-3α-(tetrahydropyranyloxy)-24-hydroxy-5β-cholestane (**203**):

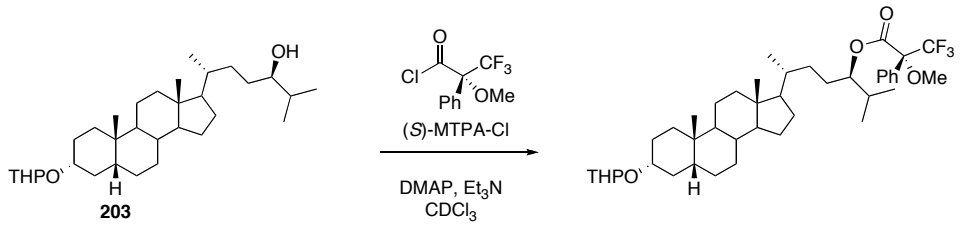


Diisopropyl zinc was prepared by addition of 2M (Et_2O) isopropyl magnesium chloride (15.3 mL, 31.6 mmol) to a suspension of dry zinc bromide (3.6 g, 15.8 mmol) in 35 mL of diethyl ether. The solution was allowed to stir at room temperature for 12 h and subsequently diluted with 50 mL of pentane to provide a suspension that was carefully filtered using a Schlenk frit under reduced pressure. The system was backfilled with nitrogen and a short path distillation apparatus was equipped. Most of the ether–pentane was removed at ambient pressure under nitrogen at 40 °C. The pressure was further reduced to 300 mmHg (digital vacuum regulator) and the temperature was raised to 60 °C and the distillation was continued. The temperature was maintained at 60 °C while the pressure was reduced by 50 mmHg/h until fuming was observed in the receiving flask (~10 mmHg) at which time the receiving flask was exchanged and the remaining material was distilled to near dryness. The *neat* diisopropyl zinc (*Danger*: spontaneously bursts into flames if comes into contact with

air) was diluted with 30 mL of toluene to provide a 0.5 M solution (titrated with iodine to obtain exact molarity).

To a solution of (2*S*)-(–)-3-*exo*-(Dimethylamino)isoborneol (7.5 mg, 38 μmol) in 1 mL of toluene at 0 °C was successively added 0.5 M diisopropyl zinc (841 μL, 421 μmol) and **201** (85 mg, 191 μmol in 100 μL toluene). The reaction mixture was allowed to stir at 0 °C for 24 h and subsequently quenched by the addition of saturated aqueous ammonium chloride with warming to room temperature. The biphasic mixture was extracted with ethyl acetate and the combined organic layer was washed with saturated aqueous sodium bicarbonate, brine, and dried over anhydrous sodium sulfate. Concentration of the organic layer provided the crude material which was purified by flash chromatography to afford **203** (57 mg, 61%). Colorless oil; R_f 0.62 (10:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.74–4.71 (m, 1H), 3.95–3.90 (m, 1H), 3.67–3.59 (m, 1H), 3.51–3.47 (m, 1H), 3.34–3.29 (m, 1H), 1.96 (ddd, J = 12.5, 3.0, 3.0 Hz, 1H), 0.92 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.0 Hz, 3H), 0.90 (s, 3H), 0.64 (s, 3H).

(*R*)-MTPA ester of (24*R*)-3α-(tetrahydropyranyloxy)-24-hydroxy-5β-cholestane:

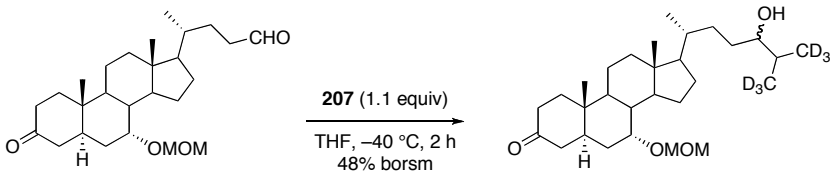


203

To a stirred solution of (*R*)-MTPA-OH (5 mg, 18 μmol) and oxalyl chloride (6 μL, 60 μmol) in 600 μL of hexane was added *N*,*N*-dimethylformamide (2 μL, 18 μmol) at room temperature. After 1 h, the solution was filtered and concentrated and the residue was taken up in 100 μL of deuterochloroform. To this solution was added **203** (3 mg, 6 μmol, in 100 μL $CDCl_3$), triethylamine (3 μL, 21.5 μmol), and *N*,*N*-dimethyl-4-amino pyridine (~1 mg). After 5 h the solution was concentrated and purified with a pipette column to afford the (*R*)-MTPA ester. R_f 0.60 (5:1 hexane/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.92 (ddd, *J* = 9.0, 5.5, 4.0 Hz, 1H), 0.92 [d, *J* = 7.0 Hz, C(26)H_3], 0.91 [d, *J* = 7.5 Hz, C(26')H_3], 0.90 [s, C(19)H_3], 0.80 [d, *J* = 6.5 Hz, C(21)H_3], 0.60 [s, C(18)H_3].

Data for (S)-MTPA ester: ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.94 (ddd, *J* = 9.0, 5.5, 4.0 Hz, 1H), 0.91 [s, C(19)H_3], 0.87 [d, *J* = 6.5 Hz, C(26)H_3], 0.86 [d, *J* = 6.5 Hz, C(26')H_3], 0.84 [d, *J* = 7.5 Hz, C(21)H_3], 0.63 [s, C(18)H_3].

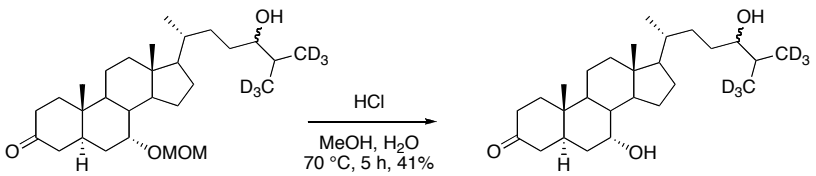
(26,27-2H6)-3-oxo-7α-(methoxymethoxy)-24-hydroxy-5α-cholestane (**208**):



128 **208**

To a cooled (–78 °C) stirred solution of **128** (44 mg, 105 μmol) in 2.1 mL of tetrahydrofuran was added a 2 M (Et_2O) solution of **207** (58 μL, 116 mmol). The reaction mixture was allowed to stir at –78 °C for 5 h and subsequently quenched by the addition of acetic acid (100 μL in 100 μL of THF) and subsequently allowing the solution to reach room temperature by removal of the cooling bath. The mixture was diluted with ethyl acetate and washed with water, brine, and dried over anhydrous sodium sulfate. Concentrating provided the crude material which was purified by flash chromatography to afford **208** (12 mg, 25%) and recovered **128** (10 mg). R_f 0.26 (3:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.67 (d, *J* = 7.0 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 3.62 (ddd, *J* = 2.0, 2.0, 2.0 Hz, 1H), 3.35 (s, 3H), 3.35–3.29 (m, 2H), 2.40 (dd, *J* = 15.5, 7.0 Hz, 1H), 2.26 (dd, *J* = 14.5, 14.5 Hz, 1H), 1.01 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.68 (s, 3H), 0.67 (s, 3H).

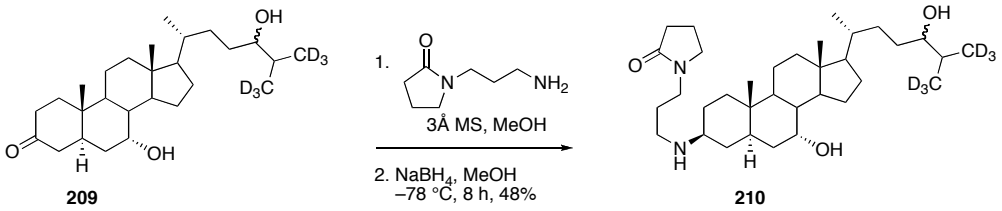
(26,27-2H6)-3-oxo-7α,24-dihydroxy-5α-cholestane (**209**):



208 **209**

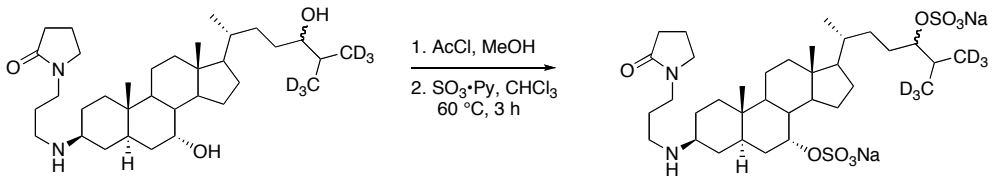
A solution of **208** (27 mg, 58 μmol) and 1 drop of concentrated hydrochloric acid in a mixture of methanol (930 μL) and water (200 μL) was heated to 70 °C in a culture tube for 2 h. The solution was cooled to room temperature and quenched by the addition of saturated sodium bicarbonate followed by extraction with ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. Concentration in vacuo provided the crude material which was purified by flash chromatography to afford **209** (10 mg, 41%). R_f 0.29 (1:1 hexanes/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.87 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H), 3.33–3.29 (m, 1H), 2.39 (dd, *J* = 16.0, 7.0 Hz, 1H), 1.01 (s, 3H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.70 (s, 3H), 0.69 (s, 3H).

(26,27-2H6)-petromyzonamine (**210**):



A solution of **209** (10 mg, 24 μmol) in 100 μL methanol with 100 mg 3Å molecular sieves was allowed to stir at room temperature for 24 h. The solution was diluted with 200 μL of tetrahydrofuran and cooled to –78 °C and sodium borohydride (2.7 mg, 72 μmol) was added. After 8 h the reaction was quenched by the addition of 50 μL of acetic acid and allowed to reach room temperature by removal of the cooling bath. The solution was extracted with ethyl acetate and the combined organic layer was washed with saturated sodium bicarbonate, brine, and dried over anhydrous sodium sulfate. Concentration in vacuo provided the crude material which was purified by flash chromatography to afford **210** (6.3 mg, 48%). R_f 0.59 (80:18:2 chloroform/methanol/conc aq ammonia).

(26,27-2H6)-petromyzonamine disulfate (**211**):



210 d^6-petromyzonamine disulfate (**211**)

To a solution of **210** (6 mg, 11 μmol) in 2 mL of methanol was carefully added acetyl chloride (50 μL) and subsequently the mixture was concentrated in vacuo to provide the hydrochloride salt. This residue was taken up in 5 mL of ethanol-free chloroform and sulfur trioxide–pyridine complex (26 mg, 163 μmol) was added and the temperature of the resultant solution was raised to 60 °C and maintained at this temperature for 3 h. Saturated aqueous sodium hydroxide (100 μL) was added and heating was continued for 30 min at which time the mixture was cooled to room temperature and concentrated in vacuo to provide the crude residue. Purification by flash chromatography afforded **211** (3 mg, 36%). R_f 0.19 (12:4:1 chloroform/methanol/water); ^{1}H NMR (500 MHz, CD_3OD): δ 4.45 (s, 1H), 4.14–4.10 (m, 1H), 3.51 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.40 (ddd, *J* = 6.5, 6.5, 2.5 Hz, 2H), 3.10 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H), 3.04–2.95 (m, 2H), 2.42 (dd, *J* = 8.0, 8.0 Hz, 2H), 2.15 (ddd, *J* = 14.0, 3.0, 3.0 Hz, 1H), 2.09 (dddd, *J* = 7.5, 7.5, 7.5, 7.5 Hz, 2H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.89 (s, 3H), 0.70 (s, 3H).

Chapter III. Sea Lamprey Migratory Pheromone Structure Activity Relationship Studies: Synthesis and Evaluation

III.A. Introduction and Background

It was envisioned that structurally simplified analogs of the individual components of the sea lamprey pheromone [c.f. Section II.A, Figure II-2; petromyzonamine (**103**), petromyzonol sulfate (**101**), and petromyzosterol disulfate (**104**)] would be interesting to examine by the EOG method for at least two reasons. First, a deeper understanding of the olfactophore—defined as the structural features in a molecule that are responsible for its activity in stimulating olfactory receptors of the nasal epithelium—could be better understood; this would lead to a deeper understanding of the pheromone component binding pocket. Second, if synthetically more accessible compounds could be found that elicit a stimulatory response in sea lamprey nasal epithelium, it may be possible to identify a synthetic mimic of the sea lamprey pheromone which would be more economically feasible. With this in mind a structure–activity relationship study was initiated.

Using the electro-olfactogram (EOG) method, one can measure the depolarization of the ciliary surface of olfactory receptor neurons (ORNs) relative to a reference electrode.[81] According to the current understanding, odorants bind receptor proteins on the surface of the ciliary membranes projecting from the ORNs. These receptors act as second messengers to open ion channels which leads to the observed voltage change upon odorant binding; thus, one is actually measuring the depolarization

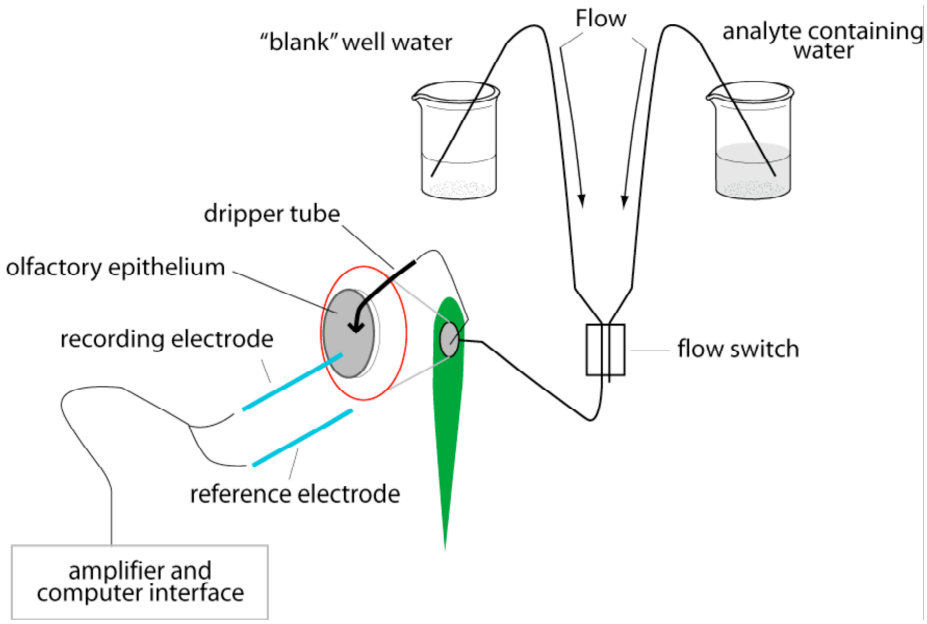
[81] Scott, J. W.; Scott-Johnson, P. E. "The Electroolfactogram: A Review of Its History and Uses" *Microsc. Res. Tech.* **2002**, *58*, 152–160.

process in the cilia during this experiment and not the action potential in the ORN directly.

It should be noted that the EOG is an extracellular measure of olfactory responsiveness that measures across many thousands of neurons comprised of very large, unknown numbers of receptor sites. Although one cannot determine how many or what types of receptors are involved using the EOG method, one *can* demonstrate: (a) that a given compound stimulates the olfactory epithelium and (b) whether a given set of components is stimulating the olfactory epithelium independent of one another (i.e., mixture studies).

The EOG experiment as it relates to the sea lamprey is depicted in Figure III-1. A solution of the analyte to be tested is placed in a beaker equipped with a tube that leads to a flow switch; another reservoir containing well water is equipped with a tube that leads to the same flow switch. During the majority of the experiment, the flow switch in the position of the well water "blank" which is being perfused at the olfactory epithelium via a secondary tube that connects the flow switch to the lamprey olfactory tissue. When a trigger is initiated by the user, the flow switch exchanges with the tube containing the analyte and routes this solution to the olfactory epithelium. At this time the response, or lack of response, is measured by the electrode that is carefully placed on the tissue relative to the standard electrode that is positioned on the dorsal skin. The voltage is amplified and digitized to provide the observed response, which is displayed graphically on a computer screen.

Figure III-1. Schematic depiction of the sea lamprey EOG experiment[82]



It is now well established that certain naturally occurring bile acids are potent olfactory stimulants for the sea lamprey (*Petromyzon marinus*).[83,84] Furthermore, behavioral evidence suggests that specific bile acid derivatives (e.g., petromyzonol sulfate) function as pheromones for migratory adult sea lamprey, which recognize and select the odor of larval conspecifics when choosing spawning streams.[85]

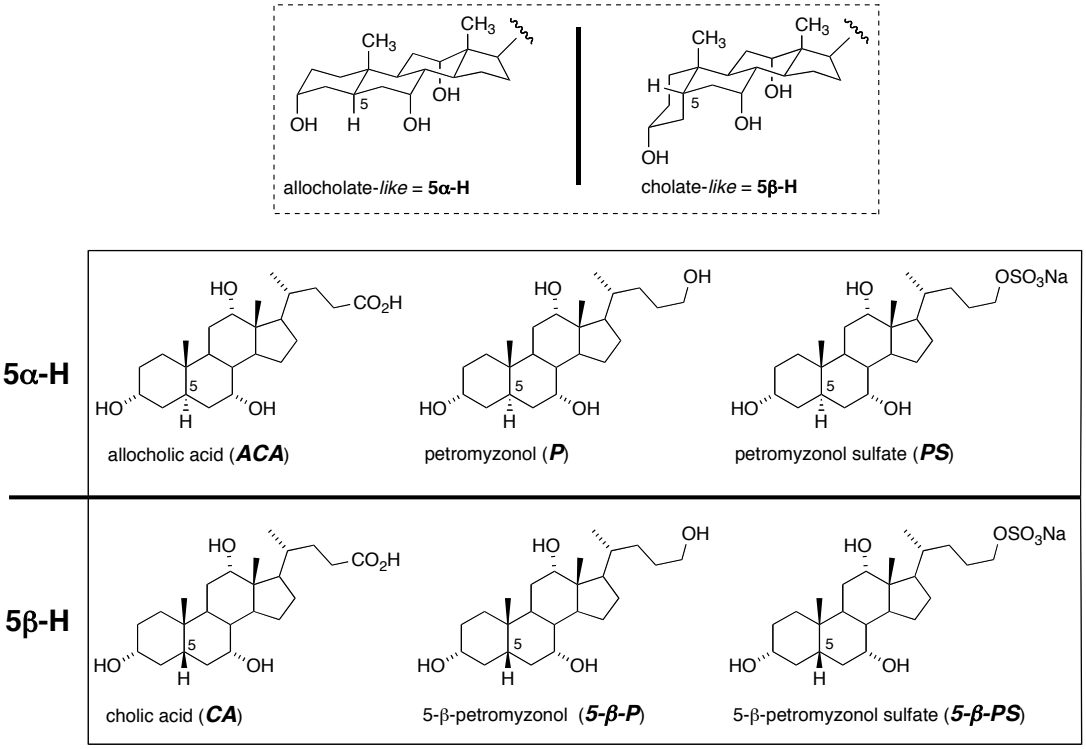
[82] Figure from: C. S. Jeffrey, Ph.D. Thesis, University of Minnesota, **2004**
[83] Li, W.; Sorensen, P. W. "The Olfactory System of Migratory Adult Sea Lamprey (*Petromyzon marinus*) Is Specifically and Acutely Sensitive to Unique Bile Acids Released by Conspecific Larvae" *J. Gen. Physiol.* **1995**, *105*, 569–587.
[84] Li, W.; Sorensen, P. W. "Highly independent olfactory receptor sites for naturally occurring bile acids in the sea lamprey, *Petromyzon marinus*" *J. Comp. Physiol. A* **1997**, *180*, 429–438.
[85] Sorensen, P. W.; Fine, J. M., Dvornikovs, V.; Jeffrey, C. S.; Shao, F.; Wang, J.; Vrieze, L. A.; Anderson, K. R.; Hoye, T. R. "Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey" *Nat. Chem. Biol.* **2005**, *1*, 324–328.

Previous sea lamprey olfaction studies with commercially available bile acids have demonstrated that cholic acid (**CA**, Figure III-2, 5-β configuration) and allocholic acid (**ACA**, Figure III-2, 5-α-cholic acid) each elicit an olfactory response in sea lamprey as measured by the electro-olfactogram (EOG) method.[85] Cross-adaptation and mixture studies[85]—methods that demonstrate whether the olfactory receptor sites are specifically sensitive to the compounds in question—have shown that **CA** and **ACA** are not independently discriminated. This means that the *allo* (i.e., 5-α) configuration for the ACA-reception is not an essential structural requirement. In addition, it has been observed that petromyzonol (**P**, Figure III-2, 5-α configuration) and 5-β-petromyzonol (**5-β-P**, 5-β configuration) elicit an similar EOG response magnitude with respect to one another; although it is unknown whether these compounds are independently discriminated as the cross-adaptation and mixture studies were not explored for this set of components.[84] However, it has been established that petromyzonol sulfate (**PS**) and **P** are independently discriminated (i.e., sulfate is necessary for PS-type reception). Thus, previous studies were unable to directly test the requirement of the *allo* configuration for binding to the PS olfactory receptor class. Only inference from surrogate substrates (e.g., **CA** and **ACA**) could be made: both of which were shown to elicit EOG responses independently of **PS**; therefore, weakening any inference made.

Furthermore, it should be reiterated that **PS** is a known component of the sea lamprey migratory pheromone,[86] thus this study could provide insight into the development of a synthetically more accessible (i.e., fewer chemical steps) pheromone to be used in sea lamprey control strategies. There is a certain irony involved in this approach in that setting the 5-α-configuration is a major obstacle in the synthesis of

PADS and much effort was involved in attempting to solve this problem; it would be fascinating if the inversion of the 5-β-center could be deemed unnecessary.

Figure III-2. Structures of bile acid derivatives



III.B. Synthesis of sulfated bile acid derivatives

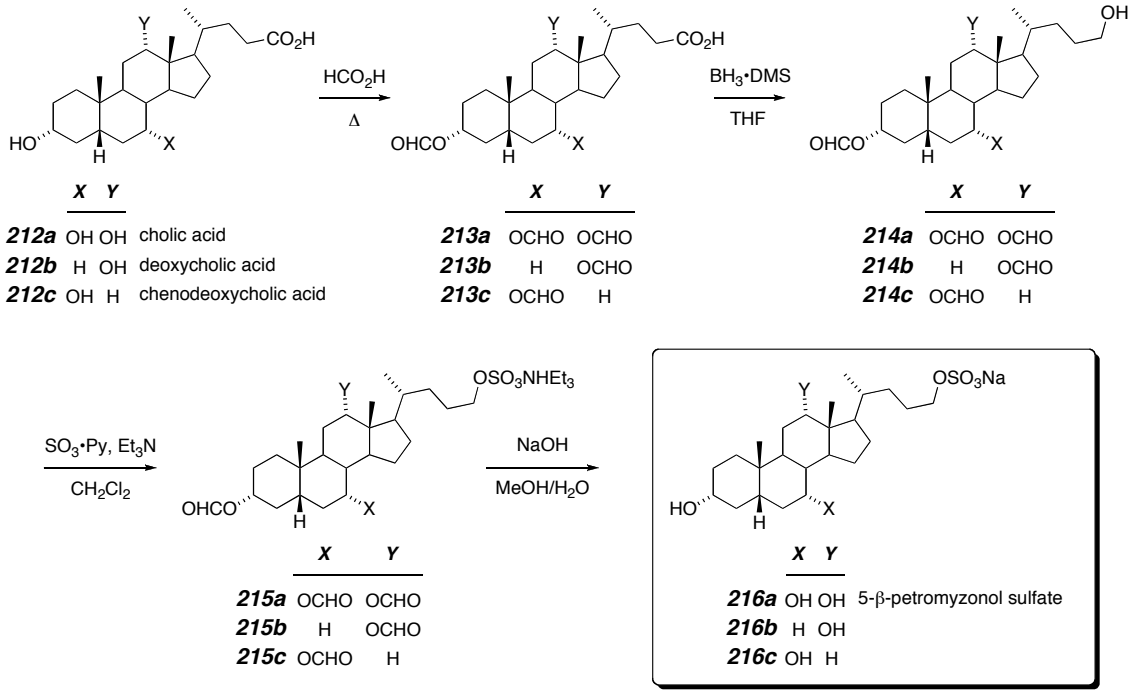
The synthesis of 5-β-petromyzonol sulfate (**216a**)[86] was achieved by protection of cholic acid (**212a)** as the triformate ester **213a** (i.e.; formic acid, 55 °C)[87] and

[86] Kuramoto, T.; Kihira, K.; Hoshita, T. "Determination of the Sulfated Position in 5β-Bufol Sulfate by a Carbon-13 Nuclear Magnetic Resonance Study" *Chem. Pharm. Bull.* **1981**, *29*, 1136–1139.

[87] Cortese, F.; Bauman, L. "A synthesis of Conjugated Bile Acids. I. Glycolic Acid." *J. Am. Chem. Soc.* **1935**, *57*, 1393–1395.

subsequent chemoselective reduction[88] of the carboxylic acid with borane–dimethylsulfide complex to provide the primary alcohol **214a** (Scheme III-1).

Scheme III-1. Synthesis of sulfated bile acid derivatives **216a**–**216c**



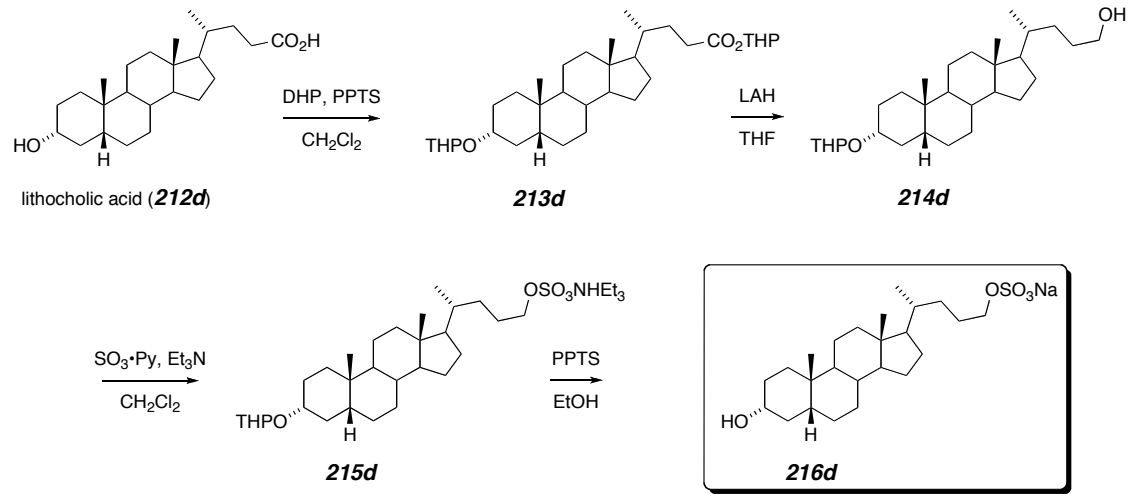
Sulfation of **214a** was effected by a modified procedure in which a suspension sulfur trioxide–pyridine complex in methylene chloride was solubilized by the addition of triethylamine (4 equiv); this permits sulfation to occur at ambient temperature rather than the higher temperatures typically required for sulfation using sulfur–trioxide in pyridine. The triformate **215a** was saponified (i.e.; aq NaOH, MeOH) with concomitant triethylammonium–sodium exchange to provide **216a**. The analogous compounds **216b**

[88] Babu, P.; Maitra, U. "Synthesis and in vitro cholesterol dissolution by 23- and 24-phosphonobile acids" *Steroids* **2005**, *70*, 681–689.

and **216c**, derived from deoxycholic acid (**212b**) and chenodeoxycholic acid (**212c**) respectively, were synthesized using a similar protocol.

Lithocholic acid (**212d**) was converted to the primary alcohol **214d** following a one-flask two-pot reaction sequence (Figure I-2); presumably via the THP-ester **213d** although this compound was not isolated.[89] The primary alcohol **214d** was sulfated (i.e.; SO_3·Py, Et_3N) to give the THP-ether **215d**, which was converted to the secondary alcohol **216d** (i.e.; PPTS, EtOH).

Scheme III-2. Synthesis of lithocholic acid derivative **216d**



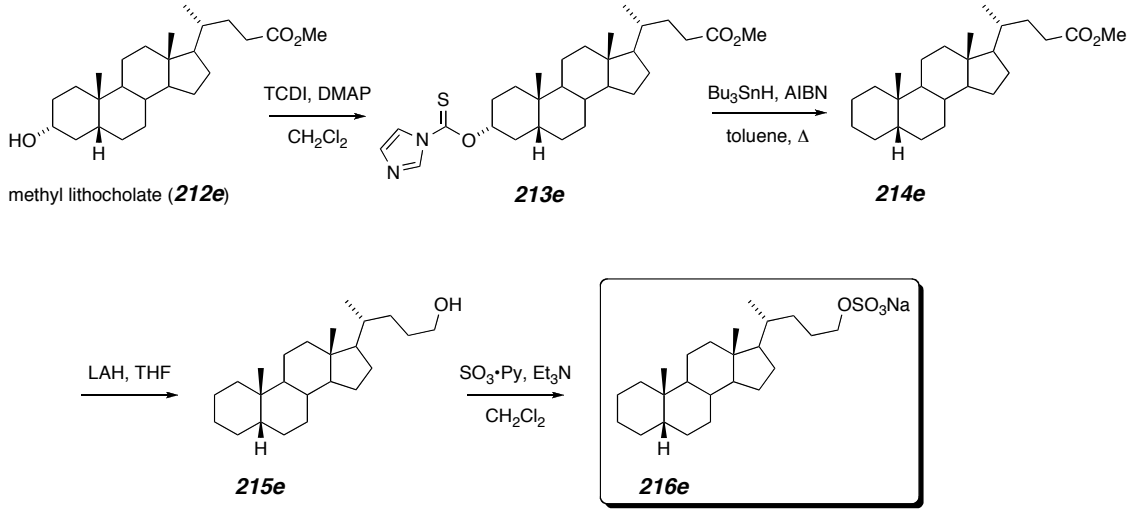
Methyl lithocholate (**212e**) was converted to the thiocarmabate **213e** (Scheme III-3; TCDI, DMAP) followed by a modified Barton-McCombie deoxygenation to provide the methyl ester **214e**.[90,91] The deoxygenation was sensitive to the reaction

[89] Banerjee, S.; Trivedi, G. K. "Synthesis of Potential Spin Probes for Biomembranes–Tempo and Proxyl Nitroxides of Lithocholic Acid" *Tetrahedron Lett.* **1992**, *48*, 9939–9950.

[90] Barton, D. H. R.; McCombie, S. W. "A New Method for the Deoxygenation of Secondary Alcohols" *J. Chem. Soc., Perkin Trans 1* **1975**, 1574–1582.

conditions: formation of a by-product arising from the reduction of the thiocarbamate moiety was observed under the standard Barton-McCombie protocol.[92] This could be overcome by the slow addition (i.e., over 1 h) of the substrate and azobisisobutyronitrile (AIBN) to a dilute solution of an excess of tri-*n*-butyltin hydride (0.06 M, 4.0 equiv) at toluene reflux. The crude methyl ester **214e** was reduced to provide the primary alcohol **215e**.[93] Sulfation of **215e** (i.e.; SO_3·Py, Et_3N) provided the target compound **216e**.

Scheme III-3. Synthesis of cholan-24-sulfate (**216e**)



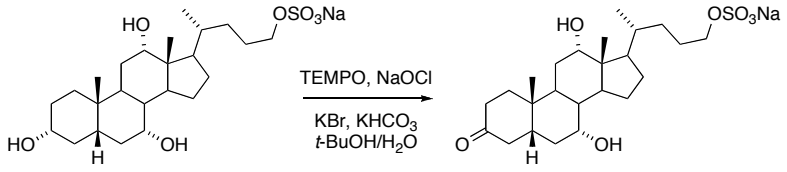
Selective oxidation of 5-β-petromyzonol sulfate (**216a**) with TEMPO under aqueous conditions gave 3-keto-5-β-petromyzonol sulfate (**217**) (Scheme III-4). Under these conditions the regioselective oxidation of the C3 carbinol in the presence of the

[91] Chochrek, P.; Wicha, J. "An Expedited Approach to the Vitamin D *trans*-Hydrindane Building Block from the Hajos Dione" *Org. Lett.* **2006**, *8*, 2551–2553.

[92] The by-product observed is proposed to be the thiohemiacetal based on the ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.80 (d, J = 9.0 Hz, HSC<u>H</u>$_2$O–R) and 2.02 (t, J = 9.0 Hz, <u>H</u>SCH$_2$O–).

unprotected C7 and C12 alcohols is noteworthy; the use of a stoichiometric amount of *inexpensive* TEMPO and the slow addition of the stoichiometric oxidant (i.e., 5% NaOCl) was necessary to prevent an unselective background oxidation effected by sodium hypochlorite.

Scheme III-4. Selective oxidation of 5-β-petromyzonol sulfate (**216a**)



5-β-petromyzonol sulfate (***216a***) 3-keto-5-β-petromyzonol sulfate (***217***)

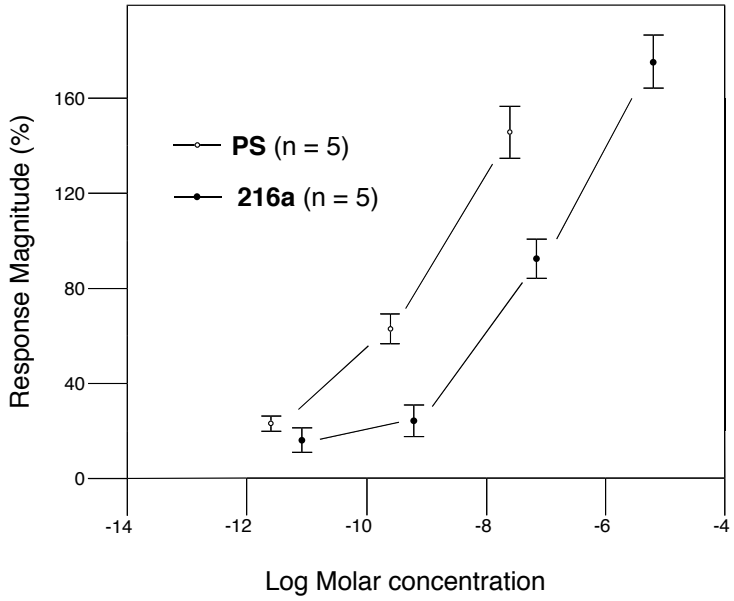
III.C. Results and Discussion

EOG is an extracellular measure of olfactory responsiveness that measures across many thousands of neurons comprised of very large, unknown numbers of receptor sites. Although one cannot determine how many or what types of receptors are involved using the EOG method, one can demonstrate: (a) that a given compound stimulates the olfactory epithelium and (b) whether a given set of components (e.g., **216a** and **PS**) is stimulating the olfactory epithelium independent of one another (mixture studies). In the following EOG studies all recordings were conducted at least twice and the average was calculated relative to the standard (10^{-5} M **L**-arginine).

[93] Cushman, M.; Golebiewski, W. M.; Pommier, Y.; Mazumder, A.; Reymen, D.; Clercq, E. D.; Graham, L.; Rice, W. G. "Cosalane Analogues with Enhanced Potencies as Inhibitors of HIV-1 Protease and Integrase" *J. Med. Chem.* **1995**, *38*, 443–452.

The dose response relationship presented in Figure III-3 indicates that 5-β-petromyzonol sulfate (**216a**) does, in fact, elicit a considerable olfactory response in live sea lamprey at concentrations below 10^{-10}M: the response magnitudes are comparable to that of **PS**.

Figure III-3. Dose-response relationship of **PS** and **216a**. Semi-logarithmic plots of the dose-response relationships. Average response magnitudes are presented as a percentage of 10^{-5} M **L**-arginine. The water blank was not detected. *Vertical Bars* represent one standard error (n = 5 animals)



Furthermore, this result is analogous to the previous findings with respect to **CA/ACA** and **P/5-β-P** (cf., Figure III-2) as mentioned in the introduction.[84,85] This represents another example of tolerance of the 5-β-steroid system in sea lamprey olfaction; moreover, despite the considerable structural differences between cholate-*like* (i.e., 5-β) and allocholate-*like* (i.e., 5-α) systems (cf., Figure III-2), sea lamprey olfactory PS-reception *not* uniquely and specifically recognizing the allo configuration.

With the olfactory response established for **216a**, the next goal was to probe the EOG response as a function of oxygenation of the steroid scaffold. These experiments were conducted to perhaps gain further insight into the olfactory binding pocket of the sea lamprey PS-receptor(s).

The EOG responses were next examined as a function of oxygenation at the 3, 7, and 12 positions of the steroid skeleton. The results presented in Figure III-4 indicate that *tris*-oxygenation is not required to elicit an olfactory response: *bis*-oxygenation is tolerated (e.g., **216c** and **216b**). Even the *mono*-oxygenated compound **216d** generated a meaningful EOG response, albeit with a lower response magnitude relative to the parent compound **216a**. The 3-keto-petromyzonol sulfate[94,95] *mimic* **217** produced an olfactory response. Strikingly however, the *des*-oxygenated compound **216e** did not elicit an olfactory response, which indicates that some oxygenation is required for olfactory stimulation.

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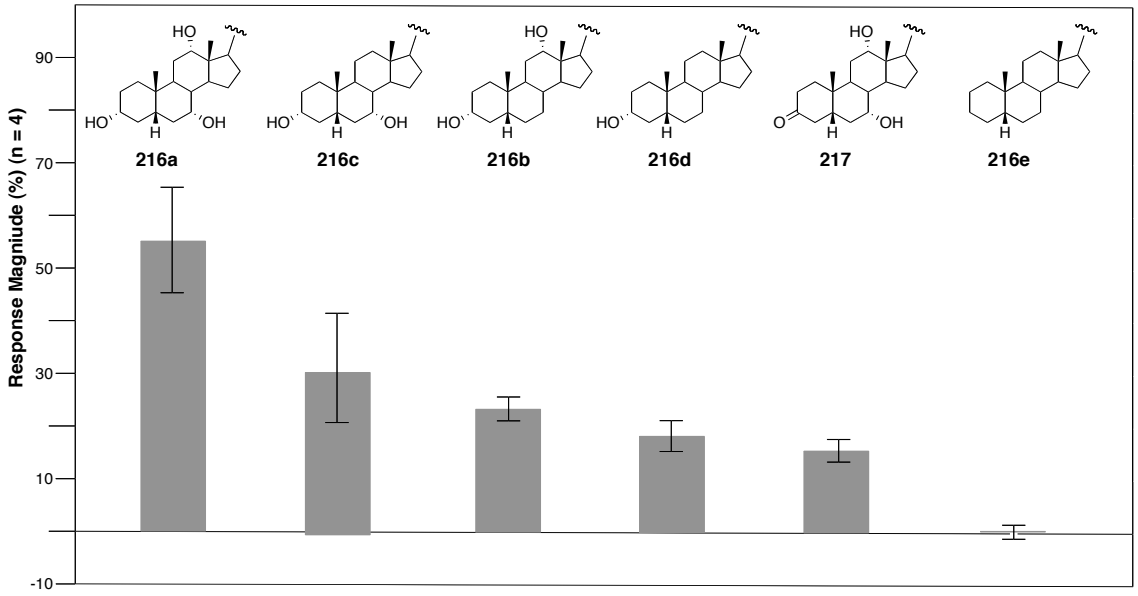
[94] Li, W.; Scott, A. P.; Siefkes, M. J.; Yan, H.; Liu, Q.; Yun, S.; Gage, D. A. "Bile Acid Secreted by Male Sea Lamprey That acts as a Sex Pheromone" *Science* **2002**, *296*, 138–141.

[95] Johnson, N. S.; Yun, S.; Thompson, H. T.; Brant, C. O.; Li, W. "A synthesized pheromone induces upstream movement in female sea lamprey and summons them into traps" *Proc. Natl. Acad. Sci.* **2009**, *106*, 1021–1026.

Figure III-4. EOG response magnitudes of 5-β-petromyzonol sulfate (**216a**) and analogs tested at 10^{-9} M concentrations. Average response magnitudes are presented as a percentage of 10^{-5} M L-arginine. The water blank was not detected. *Vertical Bars* represent one standard error (n = 4 animals)



Binary mixture studies were conducted to gain insight on whether **PS** and **216a** are uniquely and specifically recognized by the sea lamprey olfactory receptors. The independent component index (ICI) and mixture discrimination index (MDI) were calculated as previously described (Figure III-5, Equation 1 and 2).[85] ICI values deviating from 1.0 represent a lack of independence. Conversely, the MDI characterizes whether responsiveness to the components of a mixture are (or are not) discriminated; it equals 1.0 if the mixture of components (i.e., **a** and **b**) is not discriminated from a single component "mixture" (i.e., 2 x **a** or 2 x **b**). Because R_{2a} and R_{2b} will approximately equal one another, as implied by the requirement of equally stimulatory concentrations defining R_a and R_b in Eq (1), one can simply take the average of these two values [i.e.,

½ (R_{2a} + R_{2b})]. MDI values less than 1.0 indicate mixture suppression and values greater than one indicate mixture enhancement.

Figure III-5. Equations that define the independent component index (ICI) and the mixture discrimination index (MDI)

$$\textbf{ICI} = \frac{R_{ab}}{R_a + R_b} \quad (1) \qquad \textbf{MDI} = \frac{R_{ab}}{0.5 \times (R_{2a} + R_{2b})} \quad (2)$$

R_a = response arising from component **a** at a concentration in the near linear portion of the dose response curve

R_b = response arising from component **b** at a equally stimulatory concentration as defined by R_a

R_{ab} = response arising from a mixture of components **a** and **b**, with each component at the same concentration as in R_a or R_b respectively

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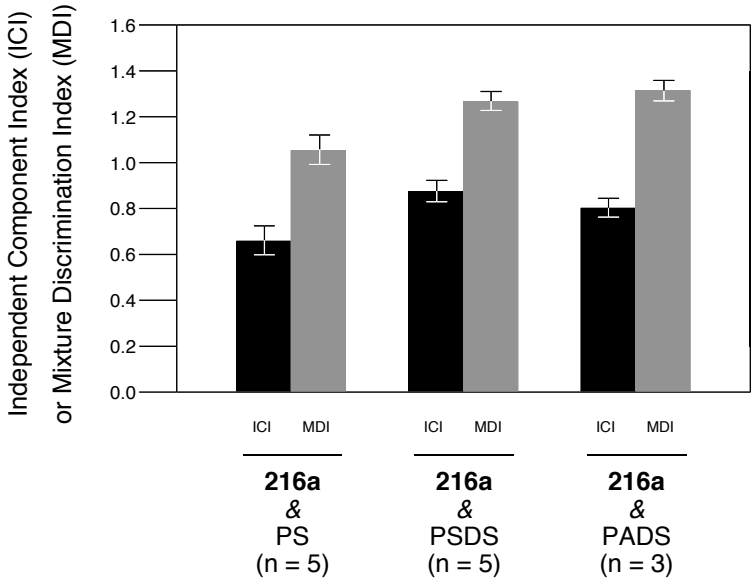
With an ICI that is less than one (i.e., ICI = 0.67) and a MDI = 1.0, it can posited that **PS** and **216a** do not act independently of one another and they are not discriminated when administered as mixtures (Figure III-6). Furthermore, neither mixture suppression nor enhancement was observed. Mixtures of **216a** with the remaining two constituents of the migratory sea lamprey pheromone[86] were also examined. It is clear from the MDI data [i.e.; **216a**/PSDS (MDI = 1.25) or **216a**/PADS (MDI = 1.32)] that mixtures of **216a** and PSDS or PADS *are discriminated* with values greater than one, which is indicative of mixture enhancement.

Figure III-6. The independent component index (ICI) and mixture discrimination index (MDI) of binary mixtures of **216a** with **PS** (n = 5 animals), petromyzosterol disulfate (PSDS) (n = 5 animals), and petromyzonamine disulfate (PADS) (n = 3 animals). *Vertical Bars* represent one standard error



III.D. Conclusion

The study has demonstrated that 5-β-petromyzonol sulfate (**216a**) does, in fact, elicit a considerable olfactory response in live sea lamprey at concentrations below 10^{-10} M: the response magnitudes are comparable to that of **PS**. Furthermore, this result is analogous to the previous findings with respect to **CA**/**ACA** and **P**/**5-β-P** (cf., Figure III-2) as mentioned in the introduction.[84,85] The sea lamprey olfactory PS-receptors are *not* uniquely and specifically recognizing the *allo* configuration. This represents another example of tolerance of the 5-β-steroid system in sea lamprey olfaction, and perhaps highlights a general trend present in sea lamprey reception across different receptor classes.

The EOG responses were examined as a function of oxygenation at the 3, 7, and 12 positions of the steroid skeleton. The results indicate that *tris*-oxygenation is not required to elicit an olfactory response as even the *mono* oxygenated compound **216d** produced a meaningful response. This information could provide useful in future developments of *individual* pheromone analogs. Furthermore, a modified synthetic protocol for the room temperature sulfation (i.e.; $SO_3 \cdot Py$, Et_3N, CH_2Cl_2) of alcohols was developed along with a method for the regioselective oxidation (i.e., TEMPO) of a water-soluble triol substrate **216a**.

Mixture studies (i.e., ICI and MDI) provided evidence that **216a** and **PS** are not independent or discriminated, which could imply a shared binding mode at the PS-type receptors. Furthermore, **216a** was shown to be discriminated with *partial* independence in mixtures with either PSDS or PADS.

Taken together with the previous results[84,85] mentioned above, this data suggests that the tolerance of the 5β-configuration may be common; this realization could lead to the development of synthetically more accessible (i.e., less expensive) pheromone mimics comprising 5β-isomers of the individual components.

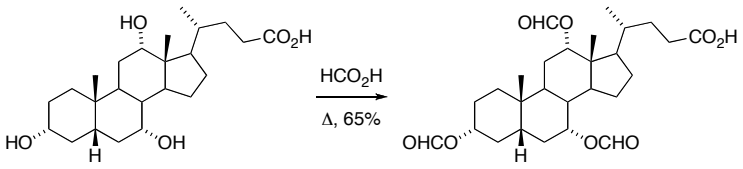
III.E. Experimental Section

For general chemistry expedite details refer to Chapter II.F.

EOG recording was conducted as described previously by Li et al.[2] For testing, animals were anaesthetized with an intramuscular injection of metomidate hydrochloride (Syndel, Vancouver, Canada, 3 mg kg^{-1} body weight), immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide; Sigma, 150 mg kg^{-1} body weight), and secured to a stand where their gills were perfused with well water at 11 °C. Their olfactory epithelium was exposed by surgically removing the dorsal portion of their cartilaginous nasal capsule and immediately perfusing it with well water. Differential EOG responses were recorded using two Ag/AgCl electrodes (type EH-1S; WPI, Sarasota, Fla., USA) filled with 3 $mol \cdot L^{-1}$ KCl and bridged to saline (8%) gelatin-filled glass capillaries with tip diameters of about 400 μm. The recording electrode was placed between two ventral lamellae and adjusted to maximize responses to the standard while minimizing responses to blank water control. The reference electrode was placed on the dorsal skin near the naris. Electrical signals were amplified by a DC-preamplifier (Model P16, Grass, Quincy, Mass., USA), digitized by a MacLab/4 (Analog Digital Instruments, Castle Hill, Australia), and displayed on an Apple Macintosh computer.

After the appropriate blank control response had been subtracted (generally there was no blank response), each set of duplicate responses was averaged and calculated as a percentage of the average response to the most recently tested standard ('response magnitude'); L-Arginine (10^{-5} M) was used as a standard.

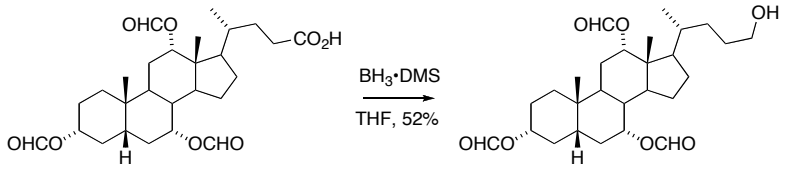
3,7,12-triformyloxy-5-cholan-24-oic acid (**213a**):



212a **213a**

A 10 mL flask with a magnetic stirring bar was charged with cholic acid (1.0 g, 2.45 mmol) and 4 mL of 88% formic acid. The resultant solution was heated for 24 h at 55 °C at which time TLC analysis showed consumption of the cholic acid. The solution was cooled to room temperature and the formic acid was removed in vacuo. The crude material was recrystallized from ethyl acetate/hexane to provide **213a** (785 mg, 65%). White crystalline solid; mp 208–209 °C; R_f = 0.48 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +90.3° (c = 0.050, $CHCl_3$); IR (neat): 2943, 1717 (sharp), 1183 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.17 (s, 1H), 8.11 (s, 1H), 8.03 (s, 1H), 5.27 (dd, *J* = 2.0, 2.0 Hz, 1H, H12), 5.07 (ddd, *J* = 2.5, 2.5, 2.5 Hz, 1H, H7), 4.72 (dddd, *J* = 11.5, 11.5, 4.0, 4.0 Hz, 1H, H3), 2.38 (ddd, *J* = 15.5, 10.0, 5.5 Hz, 1H, H23), 2.25 (ddd, *J* = 16.0, 9.5, 6.5 Hz, 1H, H23), 2.17–2.10 (m, 2H), 2.02 (ddd, *J* = 16.0, 5.0, 5.0 Hz, 1H), 1.97–1.85 (m, 2H), 1.82–1.64 (m, 9H), 1.60–1.52 (m, 2H), 1.49–1.39 (m, 2H), 1.37–1.28 (m, 3H), 1.16–1.06 (m, 2H), 0.95 (s, 3H, $CH_3$19), 0.86 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.76 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.0, 160.8 (2C), 160.7, 75.5, 73.9, 70.9, 47.4, 45.2, 43.2, 41.0, 37.9, 34.9, 34.7, 34.6, 34.5, 31.5, 31.0, 30.6, 28.8, 27.3, 26.8, 25.8, 23.0, 22.6, 17.7, 12.4; HRMS (ESI+) calcd for $C_{27}H_{40}O_8Na^+$ ($M + Na^+$) 515.2615, found 515.2613.

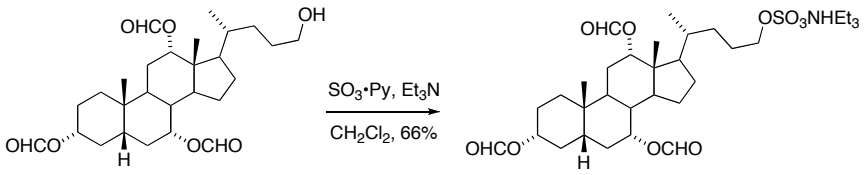
3,7,12-triformyloxy-5-cholan-24-ol (**214a**):



213a **214a**

A 25 mL flask equipped with a magnetic stir bar was charged with **213a** (500 mg, 1.02 mmol, 1.0 equiv) and 10 mL of tetrahydrofuran and cooled to –78 °C. To this solution was added 10 M borane–dimethyl sulfide complex (153 μL, 1.53 mmol, 1.5 equiv) and the resultant solution was warmed to room temperature over 1 h and subsequently allowed to stir for an additional hour. The excess borane was quenched with 200 μL of acetic acid at 0 °C and allowed to stir for 12 h at room temperature. The volatiles were removed in vacuo to give the crude material that was purified by flash chromatography to provide **214a** (253 mg, 52%). White amorphous solid; mp 86–92 °C; R_f = 0.44 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +60.7° (c = 0.018, $CHCl_3$); IR (neat): 2941, 1717 (sharp), 1183 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 8.11 (s, 1H), 8.03 (s, 1H), 5.28 (dd, *J* = 3.0, 3.0 Hz, 1H, H12), 5.07 (ddd, *J* = 2.5, 2.5, 2.5 Hz, 1H, H7), 4.72 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H, H3), 3.64–3.56 (m, 2H), 2.15–2.09 (m, 2H), 2.02 (ddd, *J* = 15.0, 5.0, 3.0 Hz, 1H), 1.97–1.84 (m, 2H), 1.82–1.52 (m, 10H), 1.48–1.25 (m, 6H), 1.15–1.02 (m, 3H), 0.95 (s, 3H, $CH_3$19), 0.85 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.76 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.8 (2C), 160.7, 75.5, 73.9, 70.9, 63.5, 47.5, 45.1, 43.1, 41.0, 37.9, 35.1, 34.7, 34.6, 34.4, 31.7, 31.5, 29.4, 28.7, 27.4, 26.7, 25.7, 23.0, 22.5, 18.0, 12.3; HRMS (ESI+) calcd for $C_{27}H_{42}O_7Na^+$ (M + Na^+) 501.2823, found 501.2820.

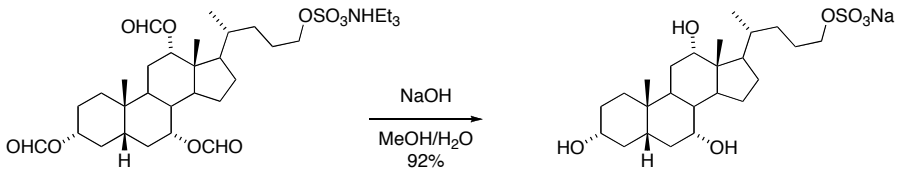
3,7,12-triformyloxy-5-cholan-24-sulfate (**215a**):



214a **215a**

A 10 mL flask equipped with a magnetic stir bar was charged with **214a** (200 mg, 0.418 mmol, 1.0 equiv) and 4.2 mL of methylene chloride. To this solution was added triethylamine (233 μL, 1.67 mmol, 4.0 equiv) and sulfur trioxide–pyridine complex (200 mg, 1.25 mmol, 3.0 equiv). The reaction mixture was allowed to stir at room temperature for 24 h. The volatiles were removed in vacuo to give the crude material that was purified by flash chromatography to provide **215a** (183 mg, 66%). White amorphous solid; mp 95.5–99.0 °C; R_f = 0.41 (80:26:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +51.8° (c = 0.026, MeOH); IR (neat): 2944, 1717 (sharp), 1180 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 8.22 (s, 1H), 8.15 (s, 1H), 8.05 (d, *J* = 1.0 Hz, 1H), 5.28 (dd, *J* = 5.0, 5.0 Hz, 1H, H12), 5.05 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H, H7), 4.68 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H, H3), 3.98–3.92 (m, 2H), 3.22 (q, *J* = 7.0 Hz, 6H, $NC\underline{H}_2CH_3$), 2.20–2.10 (m, 3H), 2.02 (ddd, *J* = 12.5, 12.5, 7.5, 7.5 Hz, 1H), 1.93–1.61 (m, 10H), 1.57–1.41 (m, 5H), 1.40–1.28 (m, 10H), 1.22–1.08 (m, 3H), 1.00 (s, 3H, $CH_3$19), 0.88 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.82 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 162.8 (2C), 162.7, 77.0, 75.2, 72.4, 69.6, 48.1, 46.4 (3C), 44.5, 42.4, 39.1, 36.3, 36.0, 35.7, 35.5, 33.0, 32.6, 29.9, 28.4, 27.9, 27.1, 26.8, 23.9, 22.8, 18.4, 16.4, 12.6, 9.4 (3C); HRMS (ESI–) calcd for $C_{27}H_{41}SO_{10}^-$ (M – $HNEt_3$) 557.2426, found 557.2428.

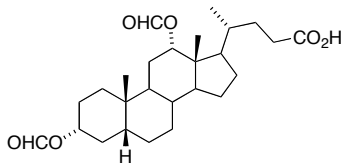
5β-Petromyzonol-24-sulfate (**216a**):



215a **216a**

A 10 mL flask equipped with a magnetic stir bar was charged with **215a** (150 mg, 0.227 mmol, 1.0 equiv) and 8.4 mL of a mixture of methanol/water (4:1). To this solution was added sodium hydroxide (90 mg, 2.25 mmol, 10.0 equiv) and the mixture was heated to 50 °C for 12 h. The solution was cooled to room temperature and the volatiles were removed in vacuo to give the crude material that was purified by flash chromatography to provide **216a** (104 mg, 92%). White amorphous solid; mp 180.0–182.0 °C; R_f = 0.24 (80:26:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +21.1° (c = 0.031, MeOH); IR (neat): 3400 (broad), 2939, 1209 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 3.99–3.94 (m, 3H), 3.79 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H, H7), 3.37 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 2.32–2.22 (m, 2H), 2.01–1.71 (m, 8H), 1.67–1.51 (m, 8H), 1.47–1.36 (m, 3H), 1.33–1.25 (m, 1H), 1.19–1.08 (m, 2H), 1.02 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.98 (ddd, *J* = 14.0, 14.0, 3.5 Hz, 1H), 0.92 (s, 3H, $CH_3$19), 0.72 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 74.2, 73.0, 69.8, 69.2, 48.4, 47.6, 43.3, 43.1, 41.2, 40.6, 37.0, 36.6, 36.0, 35.9, 33.3, 31.3, 29.7, 28.9, 28.0, 27.4, 24.4, 23.3, 18.1, 13.1; HRMS (ESI–) calcd for $C_{24}H_{41}SO_7^-$ ($M - Na^+$) 473.2578, found 473.2585.

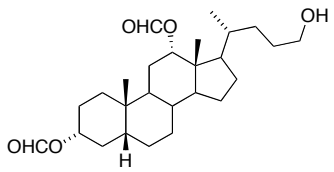
Data for 3α,12α-diformyloxy-5β-cholan-24-oic acid (**213b**):



213b

White crystalline solid; mp 198.0–198.5 °C; R_f = 0.40 (3:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +61.6° (c = 0.500, $CHCl_3$); IR (neat): 3400 (broad), 2939, 1698 (sharp), 1179 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 8.03 (d, *J* = 0.5 Hz, 1H), 5.25 (dd, *J* = 3.0, 3.0 Hz, 1H, H12), 4.84 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 2.39 (ddd, *J* = 15.5, 10.0, 5.5 Hz, 1H, H23), 2.25 (ddd, *J* = 16.0, 9.5, 7.0 Hz, 1H, H23), 2.17–2.10 (m, 2H), 2.02 (ddd, *J* = 15.5, 5.5, 3.5 Hz, 1H), 1.93–1.77 (m, 4H), 1.74–1.57 (m, 8H), 1.51–1.40 (m, 4H), 1.17–1.01 (m, 3H), 0.93 (s, 3H, $CH_3$19), 0.84 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.75 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.2, 160.9, 160.8, 76.2, 74.3, 49.5, 47.6, 45.2, 41.9, 35.8, 35.0, 34.9, 34.4, 34.5, 34.2, 32.3, 31.1, 30.7, 27.6, 27.0, 26.7, 26.1, 26.0, 23.6, 23.1, 17.6, 12.6; HRMS (ESI+) calcd for $C_{26}H_{40}O_6Na^+$ ($M + Na^+$) 471.2717, found 471.2721.

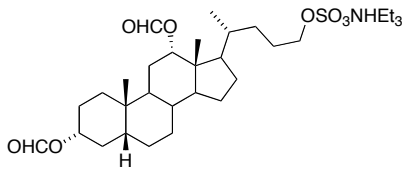
Data for 3α,12α-diformyloxy-5β-cholan-24-ol (**214b**):



214b

White amorphous solid; mp 57.0–62.5 °C; R_f = 0.78 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +85.5° (c = 0.010, $CHCl_3$); IR (neat): 2938, 1721 (sharp), 1178 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 8.03 (d, *J* = 0.5 Hz, 1H), 5.26 (dd, *J* = 3.0, 3.0 Hz, 1H, H12), 4.84 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H, H3), 3.65–3.57 (m, 2H), 1.92–1.01 (m, 27H), 0.93 (s, 3H, $CH_3$19), 0.84 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.75 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.9, 160.8, 76.3, 74.3, 63.6, 49.4, 47.7, 45.1, 41.9, 35.8, 35.2, 34.8, 34.4, 34.2, 32.3, 31.8, 29.5, 27.6, 27.0, 26.6, 26.1, 25.9, 23.6, 23.1, 18.0, 12.5; HRMS (ESI+) calcd for $C_{26}H_{42}O_5Na^+$ (M + Na^+) 457.2924, found 457.2932.

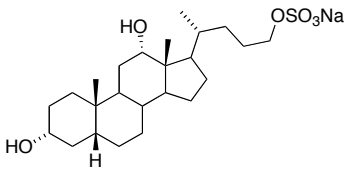
Data for 3α,12α-diformyloxy-5β-cholan-24-sulfate (**215b**):



215b

White amorphous solid; mp 153.2–154.0 °C; R_f = 0.25 (80:18:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +65.5° (c = 0.025, MeOH); IR (neat): 2943, 1718 (sharp), 1176 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 8.20 (s, 1H), 8.05 (d, *J* = 0.5 Hz, 1H), 5.24 (dd, *J* = 3.0, 3.0 Hz, 1H, H12), 4.80 (dddd, *J* = 11.0, 11.0, 5.0, 5.0 Hz, 1H, H3), 3.99–3.92 (m, 2H), 3.21 (q, *J* = 10.0 Hz, 6H, $NC\underline{H_2}CH_3$), 1.97–1.85 (m, 2H), 1.80–1.30 (m, 27H), 1.20–1.05 (m, 4H), 0.98 (s, 3H, $CH_3$19), 0.87 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.80 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 162.8, 162.7, 77.5, 75.4, 69.6, 50.8, 48.1, 46.4 (3C), 43.3, 37.1, 36.4, 35.9, 35.6, 35.3, 33.5, 33.1, 28.6, 28.1, 27.7, 27.3, 27.2, 26.9, 24.7, 23.5, 18.5, 17.9, 12.9, 9.4 (3C); HRMS (ESI–) calcd for $C_{26}H_{41}SO_8^-$ (M – $HNEt_3$) 513.2528, found 513.2533.

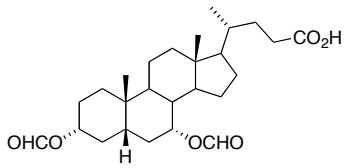
Data for 3α,12α-dihydroxy-5β-cholan-24-sulfate (**216b**):



216b

White amorphous solid; mp 172.7–176.2 °C; R_f = 0.40 (80:26:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +35.0° (c = 0.020, MeOH); IR (neat): 3400 (broad), 2934, 1219 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 3.98–3.93 (m, 3H), 3.52 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 1.92–1.72 (m, 7H), 1.64–1.38 (m, 13H), 1.32–1.23 (m, 2H), 1.21–1.05 (m, 3H), 1.01 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.98 (ddd, *J* = 14.0, 14.0, 3.0 Hz, 1H), 0.93 (s, 3H, $CH_3$19), 0.71 (s, 3H, $CH_3$21); ^{13}C NMR (125 MHz, d^4-methanol) δ 74.2, 72.7, 69.8, 49.4, 48.5, 47.7, 43.8, 37.6, 37.4, 37.0, 36.6, 35.5, 35.0, 33.2, 31.2, 30.0, 28.9, 28.6, 27.6, 27.3, 25.0, 23.9, 18.0, 13.4; HRMS (ESI–) calcd for $C_{24}H_{41}SO_6^-$ ($M - Na^+$) 457.2629, found 457.2628.

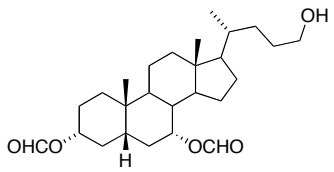
Data for 3α,7α-diformyloxy-5β-cholan-24-oic acid (**213c**):



213c

White crystalline solid; mp 100.0–102.5 °C; R_f = 0.53 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +32.8° (c = 0.017, $CHCl_3$); IR (neat): 2948, 1722 (sharp), 1186 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.08 (s, 1H), 8.03 (d, *J* = 0.5 Hz, 1H), 5.04 (ddd, *J* = 3.5, 3.5, 3.5 Hz, 1H, H7), 4.73 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 2.40 (ddd, *J* = 15.5, 10.0, 5.0 Hz, 1H, H23), 2.26 (ddd, *J* = 16.0, 10.0, 6.5 Hz, 1H, H23), 2.13 (ddd, *J* = 13.0, 13.0, 13.0 Hz, 1H), 2.00 (dddd, *J* = 12.0, 12.0, 4.0, 4.0 Hz), 1.92–1.74 (m, 5H), 1.67–1.62 (m, 3H), 1.54–1.24 (m, 9H), 1.22–1.04 (m, 4H), 0.95 (s, 3H, $CH_3$19), 0.94 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.66 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.3, 161.0 (2C), 74.2, 71.6, 49.5, 55.8, 50.3, 42.9, 41.1, 39.6, 38.0, 35.4, 34.9, 34.7, 34.1, 31.6, 31.1, 30.8, 28.1, 26.9, 23.7, 22.8, 20.8, 18.4, 14.4, 11.9; HRMS (ESI+) calcd for $C_{26}H_{40}O_6Na^+$ ($M + Na^+$) 471.2717, found 471.2721.

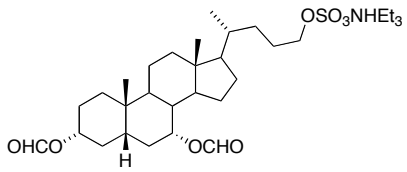
Data for 3α,7α-diformyloxy-5β-cholan-24-ol (**214c**):



214c

White amorphous solid; mp 49.5–52.0; R_f = 0.45 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +11.1° (c = 0.114, $CHCl_3$); IR (neat): 3400 (broad), 2937, 1710 (sharp), 1181 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.08 (s, 1H), 8.03 (s, 1H), 5.03 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H, H7), 4.73 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H, H3), 3.65–3.57 (m, 2H), 2.14 (ddd, 14.0, 14.0, 14.0 Hz, 1H), 2.04–1.98 (m, 2H), 1.91 (ddd, *J* = 14.5, 3.0, 3.0 Hz, 1H), 1.88–1.79 (m, 2H), 1.78–1.73 (m, 1H), 1.66–1.60 (m, 4H), 1.54–1.03 (m, 16H), 0.95 (s, 3H, $CH_3$19), 0.94 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.66 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.9 (2C), 74.2, 71.6, 63.7, 56.1, 50.3, 42.8, 41.1, 39.6, 38.0, 35.7, 35.0, 34.7, 34.2, 32.0, 31.7, 29.5, 28.3, 26.9, 26.1, 23.7, 22.8, 20.8, 18.8, 11.9; HRMS (ESI+) calcd for $C_{26}H_{42}O_5Na^+$ ($M + Na^+$) 457.2924, found 457.2926.

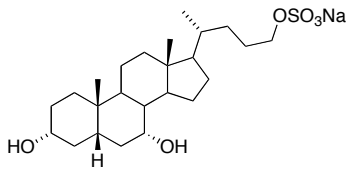
Data for 3α,7α-diformyloxy-5β-cholan-24-sulfate (**215c**):



215c

White amorphous solid; mp 174.9–176.1 °C; R_f = 0.25 (80:18:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +14.0° (c = 0.026, MeOH); IR (neat): 2941, 1718 (sharp), 1187 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 8.13 (s, 1H), 8.05 (d, *J* = 1.0 Hz, 1H), 5.02 (ddd, *J* = 2.0, 2.0, 2.0 Hz, 1H, H7), 4.69 (dddd, *J* = 11.5, 11.5, 4.5, 4.5 Hz, 1H, H3), 3.99–3.92 (m, 2H), 3.22 (q, *J* = 7.0 Hz, 6H, NC$\underline{H_2}CH_3$), 2.18 (ddd, *J* = 13.5, 13.5, 13.5 Hz, 1H), 2.11 (ddd, *J* = 15.5, 5.0, 3.5 Hz, 1H), 2.05 (ddd, *J* = 12.5, 3.5, 3.5 Hz, 1H), 1.96–1.84 (m, 3H), 1.77–1.70 (m, 3H), 1.65–1.61 (m, 2H), 1.57–1.32 (m, 10H), 1.32 (t, *J* = 7.5 Hz, 9H), 1.26–1.09 (m, 5H), 1.00 (s, 3H, $CH_3$19), 0.97 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.72 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 162.9, 162.8, 75.5, 72.9, 69.7, 57.5, 51.6, 48.1 (3C), 43.9, 42.5, 41.0, 39.3, 36.9, 36.1, 36.0, 35.9, 35.5, 33.2, 32.7, 29.2, 28.0, 27.2, 24.6, 23.2, 21.9, 19.2, 12.3, 9.4 (3C); HRMS (ESI–) calcd for $C_{26}H_{41}SO_8^-$ (M – $HNEt_3$) 513.2528, found 513.2530.

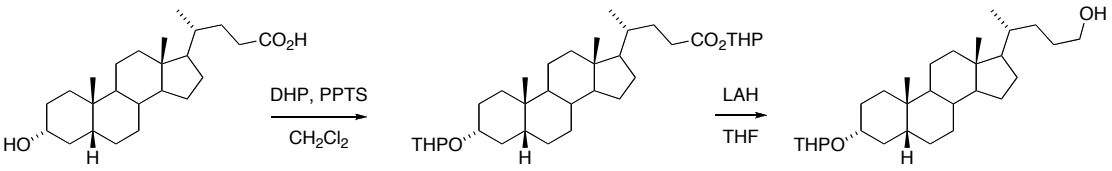
Data for 3α,7α-dihydroxy-5β-cholan-24-sulfate (**216c**):



216c

White amorphous solid; mp 172.7–176.2 °C; R_f = 0.38 (80:26:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +35.0° (c = 0.020, MeOH); IR (neat): 3400 (broad), 2934, 1219 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 3.98–3.93 (m, 3H), 3.52 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 1.92–1.72 (m, 7H), 1.64–1.38 (m, 13H), 1.29 (dddd, *J* = 4.5, 4.5, 2.0, 2.0 Hz, 1H), 1.26 (ddd, *J* = 4.0, 2.0, 2.0 Hz, 1H), 1.21–1.05 (m, 3H), 1.01 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.98 (ddd, *J* = 14.0, 14.0, 3.0 Hz, 1H), 0.93 (s, 3H, $CH_3$19), 0.71 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 73.0, 69.8, 69.2, 57.6, 51.6, 43.8, 43.3, 41.2, 40.9, 40.6, 39.3, 37.0, 36.7, 36.4, 36.0, 34.2, 33.3, 31.5, 29.4, 27.3, 24.8, 23.5, 21.9, 19.3, 12.3; HRMS (ESI–) calcd for $C_{24}H_{41}SO_6^-$ ($M - Na^+$) 457.2629, found 457.2627.

3α-tetrahydropyranyloxy-5β-cholan-24-ol (**214d**):

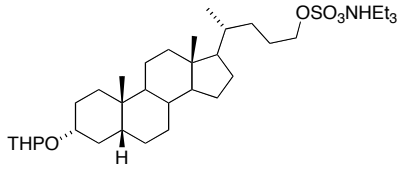


lithocholic acid (***212d***) ***213d*** ***214d***

A 25 mL flask equipped with a magnetic stir bar was charged with lithocholic acid (2.0 g, 5.31 mmol, 1.0 equiv) and 18 mL of methylene chloride. To this solution was added dihydropyran (4.7 mL, 53.1 mmol, 10 equiv) and pyridinium *p*-toluenesulfonate (133 mg, 0.531 mmol, 0.1 equiv). The resultant solution was allowed to stir at room temperature for 24 h at which time the volatiles were removed in vacuo to provide **213d**. To a 100 mL flask, equipped with a magnetic stir bar and the crude residue, was added 53 mL of ethyl ether. The solution was cooled with an ice–water bath and lithium aluminum hydride (1.0 g, 26.4 mmol, 5.0 equiv) was added. The resultant solution was allowed to stir at room temperature for 3 h and quenched at 0 °C with 1 mL of water, 1 mL of 3N sodium hydroxide, and 3 mL of water. The suspension was filtered and the resultant solution was concentrated in vacuo to give the crude alcohol that was purified by flash chromatography to provide **214d** (1.43 g, 60%). Colorless oil; R_f = 0.35 (1:1 hexane/ethyl acetate); IR (neat): 3397 (broad), 2915, 2852, 1449, 1025 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.73–4.71 (m, 1H), 3.94–3.90 (m, 1H), 3.66–3.58 (m, 3H), 3.51–3.46 (m, 1H), 1.95 (ddd, *J* = 12.5, 2.5, 2.5 Hz, 1H), 1.92–1.76 (m, 4H), 1.75–1.60 (m, 4H), 1.57–1.51 (m, 6H), 1.47–1.17 (m, 13H), 1.15–0.87 (m, 7H), 0.92 (d, *J* = 7.0 Hz, 3H, $CH_3$21), 0.91 (s, 3H, $CH_3$19), 0.64 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 97.0, 96.7, 76.2, 76.0, 63.7, 63.0, 62.9, 56.6, 56.4, 42.9,

42.5, 42.2, 40.4, 40.3, 36.6, 36.0, 35.8, 35.7, 35.5, 34.9, 34.7, 34.5, 32.9, 32.4, 32.0, 31.5, 31.0, 30.7, 29.6, 28.7, 28.5, 27.5, 27.4, 26.9, 25.7, 24.4, 23.6, 23.5, 21.0, 20.9, 20.2, 20.1, 18.8, 12.2; HRMS (ESI+) calcd for $C_{29}H_{50}O_3Na^+$ (M + Na^+) 469.3652, found 469.3660.

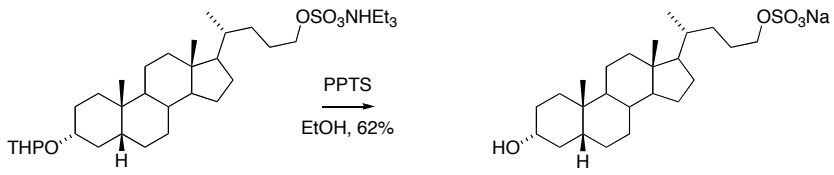
Data for 3α-tetrahydropyranyloxy-5β-cholan-24-sulfate (**215d**):



215d

White amorphous solid; mp 121.5–128.0 °C; R_f = 0.17 (90:9:1 chloroform/methanol/satd aq NH_3); IR (neat): 2927, 2895, 1441, 1253, 1196, 1023 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.77 (bs, 1H), 4.73–4.71 (m, 1H), 4.06–3.98 (m, 2H), 3.94–3.90 (m, 1H), 3.63 (ddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 3.51–3.46 (m, 1H), 3.18 (q, *J* = 7.0 Hz, 6H, $NC\underline{H}_2CH_3$), 1.95 (ddd, *J* = 12.0, 2.5, 2.5 Hz, 1H), 1.89–1.69 (m, 8H), 1.60–1.31 (m, 15H), 1.39 (t, *J* = 7.5 Hz, 9H, $NCH_2C\underline{H}_3$), 1.29–1.17 (m, 3H), 1.12–0.94 (m, 7H), 0.91 (s, 3H, $CH_3$19), 0.90 (d, *J* = 6.0 Hz, 3H, $CH_3$21), 0.63 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 96.9, 76.2, 68.8, 62.9, 56.6, 56.4, 46.6 (3C), 42.8, 42.2, 40.4, 40.3, 36.0, 35.7, 35.6, 34.9, 32.9, 32.0, 31.5, 28.7, 28.4, 27.5, 26.5, 26.2, 25.6, 24.4, 23.5, 20.9, 20.2, 18.7, 12.2, 8.9 (3C); HRMS (ESI–) calcd for $C_{29}H_{49}SO_6^-$ (M – Na^+) 525.3255, found 525.3258.

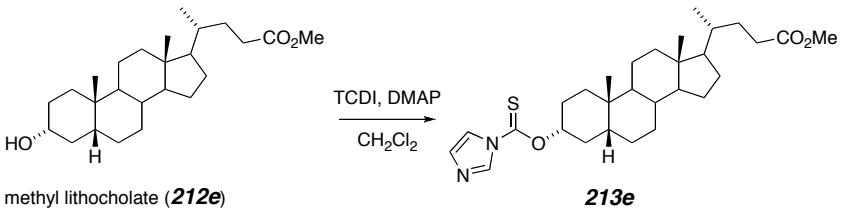
3α-hydroxy-5β-cholan-24-sulfate (**216d**):



215d ***216d***

A 5 mL flask equipped with a magnetic stir bar was charged with **215d** (100 mg, 159 μmol, 1.0 equiv), 1.6 mL of 95% ethanol, and pyridinium *p*-toluenesulfonate (4 mg, 16 μmol, 0.1 equiv). The reaction mixture was heated at 50 °C for 3 h at which time 400 μL of aq sodium hydroxide (15% by weight) solution was added and the mixture was allowed to stir for 15 min at this temperature. The mixture was cooled to room temperature and the volatiles were removed in vacuo to give the crude material that was purified by flash chromatography to provide **216d** (46 mg, 62%). White amorphous solid; mp 210.0–212.0 °C; R_f = 0.45 (80:18:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +24.7° (c = 0.016, MeOH); IR (neat): 2926, 2855, 1441, 1212 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 3.99–3.92 (m, 2H), 3.53 (dddd, *J* = 11.0, 11.0, 5.0, 5.0 Hz, 1H, H3), 2.02 (ddd, *J* = 12.5, 2.5, 2.5 Hz, 1H), 1.94–1.70 (m, 5H), 1.64–1.40 (m, 11H), 1.35 (ddd, *J* = 14.5, 3.0, 3.0 Hz, 1H), 1.34–1.24 (m, 3H), 1.19 (dd, *J* = 12.5, 3.5 Hz, 1H), 1.22–1.04 (m, 5H), 1.02–0.96 (m, 4H), 0.95 (s, 3H, $CH_3$19), 0.69 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 72.6, 69.8, 58.1, 57.8, 44.0, 43.7, 42.0, 41.7, 37.4, 37.3, 36.9, 36.6, 35.8, 33.2, 31.3, 28.7, 29.4, 28.5, 27.8, 27.3, 25.4, 24.1, 22.1, 19.2, 12.6; HRMS (ESI–) calcd for $C_{24}H_{41}SO_5^-$ ($M - Na^+$) 441.2680, found 441.2680.

O-(methyl lithocholate) 1H-imidazole-1-carbothioate (**213e**):



methyl lithocholate (***212e***) ***213e***

A 100 mL flask equipped with a magnetic stir bar was charged with methyl lithocholate (2 g, 5.12 mmol, 1.0 equiv), thiocarbonyldiimidazole (2.7 g, 15.4 mmol, 3.0 equiv), 4-dimethylaminopyridine (63 mg, 512 μmol, 0.1 equiv), and 50 mL of methylene chloride. After 5 h the mixture was quenched with 25 mL of saturated aqueous ammonium chloride. The aqueous layer was extracted twice with 50 mL of ethyl acetate. The combined organic layers were combined and washed with 50 mL of saturated aqueous sodium bicarbonate, 50 mL of saturated brine, and dried over anhydrous sodium sulfate. The volatiles were removed in vacuo to give the crude material that was purified by flash chromatography to provide **213e** (1.62 g, 63%). White amorphous solid; mp 146.0–147.5 °C; R_f = 0.61 (1:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +47.6° (c = 0.029, $CHCl_3$); IR (neat): 2949, 2870, 1740 (sharp), 1465, 1387, 1339, 1285, 1233 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.65 (s, 1H), 7.02 (s, 1H), 5.45 (dddd, *J* = 11.0, 11.0, 4.5, 4.5 Hz, 1H, H3), 3.67 (s, 3H), 2.35 (ddd, *J* = 15.5, 10.0, 5.0 Hz, 1H, H23), 2.20 (ddd, *J* = 16.0, 10.0, 6.5 Hz, 1H, H23), 2.06–1.77 (m, 8H), 1.66–1.53 (m, 3H), 1.45–1.02 (m, 15H), 0.98 (s, 3H, $CH_3$19), 0.92 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.66 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.7, 174.9, 137.0, 130.8, 118.1, 84.6, 56.6, 56.2, 51.7, 42.9, 42.1, 40.7, 40.2, 36.0, 35.5, 35.0, 34.8, 31.6,

31.2, 31.1, 28.4, 27.2, 26.5, 26.1, 24.4, 23.4, 21.0, 18.5, 12.2; HRMS (ESI+) calcd for $C_{29}H_{44}N_2SO_3Na^+$ (M + Na^+) 523.2965, found 523.2963.

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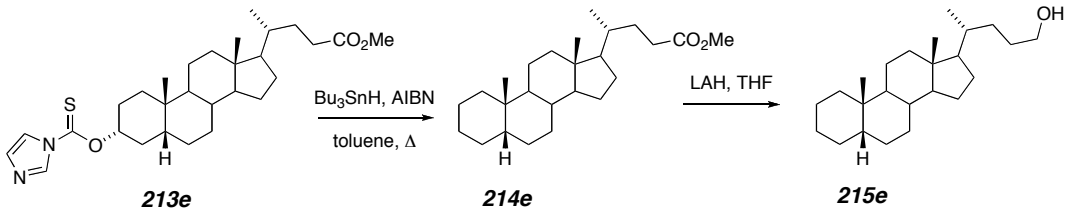
cholanol (**215e**):



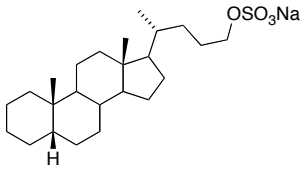
213e ***214e*** ***215e***

A 10 mL flask equipped with a magnetic stir bar was charged with tri-*n*-butyltin hydride (54 μL, 200 μmol, 4.0 equiv) and 3.3 mL of toluene. The flask was equipped with a condenser and heated at reflux while a solution of **213e** (25 mg, 50 μmol, 1.0 equiv) and azobisisobutyronitrile (1.6 mg, 5 μmol, 0.2 equiv) in 1.7 mL of toluene was added over one hour. The solution was maintained at reflux for an additional 3 h and subsequently cooled to room temperature, at which time the volatiles were removed in vacuo to provide the crude material that was used without purification in the subsequent step. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.67 (s, 3H), 2.35 (ddd, *J* = 15.5, 10.5, 5.0, 1H, H23), 2.21 (ddd, *J* = 16.0, 9.5, 6.5, 1H, H23), 1.95 (ddd, *J* = 6.5, 2.5, 2.5 Hz, 1H), 1.89–1.69 (m, 6H), 1.59–1.51 (m, 2H), 1.43–0.93 (m, 19H), 0.92 (s, 3H, $CH_3$19), 0.90 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 0.64 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 64.5, 56.6, 56.1, 51.7, 42.9, 42.2, 40.5, 40.3, 36.0, 35.6, 35.4, 35.0, 33.0, 31.3, 31.2, 28.4, 27.4, 27.1, 26.5, 24.4, 23.6, 21.0, 18.5, 12.2.

A 5 mL flask equipped with a magnetic stir bar was charged with the crude material from the Barton–McCombie deoxygenation and 1 mL of ethyl ether. To this

solution lithium aluminum hydride (9.5 mg, 250 μmol, 5.0 equiv) was added and the solution was allowed to stir for an additional 3 h. The reaction was subsequently quenched 10 μL of water, 10 μL of 3N sodium hydroxide, and 20 μL of water. The suspension was filtered and the resultant solution was concentrated in vacuo to give the crude alcohol that was purified by flash chromatography to provide colonel (**215e**) (11.1 mg, 64%). White amorphous solid; mp 129.5–130.5 °C; R_f = 0.43 (3:1 hexane/ethyl acetate); $[\alpha]_D$ (23 °C): +22.2° (c = 0.019, $CHCl_3$); IR (neat): 2937, 2864, 1472, 1375, 1054 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.65–3.57 (m, 2H), 1.96 (ddd, *J* = 12.0, 3.5, 3.5 Hz, 1H), 1.89–1.53 (m, 7H), 1.45–1.02 (m, 22H), 0.93 (d, *J* = 7.0 Hz, 3H, $CH_3$21), 0.91 (s, 3H, $CH_3$19), 0.65 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 63.8, 56.8, 56.4, 43.9, 42.9, 40.7, 40.5, 37.8, 36.1, 35.8, 35.6, 32.0, 29.6, 28.5, 27.7, 27.4, 27.2, 26.8, 24.5, 24.4, 21.5, 21.0, 18.9, 12.3; LRMS (EI) calcd for $C_{24}H_{42}O$ (M) 346.3, found 346.

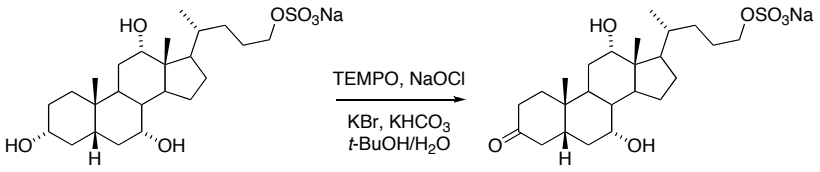
Data for cholan-24-sulfate (**216e**):



216e

White amorphous solid; mp 195.5–198.0 °C; R_f = 0.44 (90:9:1 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +18.3° (c = 0.026, MeOH); IR (neat): 2930, 2860, 1449, 1402, 1213 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 3.99–3.92 (m, 2H, H24), 2.01 (ddd, *J* = 12.5, 3.5, 3.5 Hz, 1H), 1.95–1.71 (m, 6H), 1.61–1.09 (m, 23H), 0.96 (d, *J* = 7.5 Hz, $CH_3$21), 0.95 (s, 3H, $CH_3$19), 0.69 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 69.8, 58.1, 57.8, 45.4, 44.0, 42.0, 41.8, 38.9, 37.4, 36.9, 36.6, 33.3, 29.5, 28.8, 28.5, 28.4, 27.9, 27.3, 25.5, 25.0, 22.5, 22.1, 19.3, 12.7; HRMS (ESI–) calcd for $C_{24}H_{41}SO_4^-$ ($M - Na^+$) 425.2731, found 425.2736.

3-keto-5β-petromyzonol sulfate (**217**):



5-β-petromyzonol sulfate (***216a***) 3-keto-5-β-petromyzonol sulfate (***217***)

A 25 mL flask equipped with a magnetic stir bar was charged with **216a** (300 mg, 604 μmol, 1.0 equiv), 400 μL of water, and 1.6 mL of *t*-butanol. To this solution was added potassium bromide (144 mg, 1.21 mmol, 2.0 equiv), potassium bicarbonate (605 mg, 6.04 mmol, 10.0 equiv), and 2,2,6,6-tetramethylpiperidine-1-oxyl (104 mg, 664 μmol, 1.1 equiv). The solution was cooled to 0 °C and 5% sodium hypochlorite (7.2 mL, 4.83 mmol, 8.0 equiv) was added in 1.8 mL portions at one-hour intervals. After the final addition of sodium hypochlorite, the mixture was allowed to stir for one additional hour (5 h total). The resultant reaction mixture was quenched at 0 °C with 1.2 g sodium thiosulfate pentahydrate in 10 mL of water and allowed to come to room temperature. The aqueous layer was extracted ten times with 10% methanol in chloroform (10 x 40 mL). The volatiles were removed in vacuo to give the crude material that was purified by flash chromatography (80:18:2 to 80:26:2 chloroform/methanol/satd aq ammonia) to provide **217** (188 mg, 63%). White amorphous solid; mp >230 °C; R_f = 0.43 (80:26:2 chloroform/methanol/satd aq NH_3); $[\alpha]_D$ (23 °C): +23.6° (c = 0.035, MeOH); IR (neat): 2946, 2870, 1700 (sharp), 1221 cm^{-1}; ^1H NMR (500 MHz, d^4-methanol): δ 4.02 (dd, *J* = 2.5, 2.5 Hz, 1H, H12), 3.99–3.95 (m, 2H, H24), 3.86 (ddd, *J* = 3.5, 3.5, 3.5 Hz, 1H, H7), 3.54 (dd, *J* = 15.5, 14.0 Hz, 1H,

H4α), 2.60 (ddd, *J* = 14.5, 14.5, 5.5 Hz, 1H, H2α), 2.44 (ddd, *J* = 12.5, 12.5, 4.5 Hz, 1H, H2β), 2.15–1.09 (m, 21H), 1.04 (d, *J* = 6.5 Hz, 3H, $CH_3$21), 1.03 (s, 3H, $CH_3$19), 0.76 (s, 3H, $CH_3$18); ^{13}C NMR (125 MHz, d^4-methanol) δ 216.8, 74.0, 69.8, 69.0, 47.7, 45.1, 45.0, 43.1, 41.2, 38.0, 37.8, 37.1, 36.3, 35.2, 35.1, 33.3, 30.0, 28.9, 28.5, 27.4, 24.3, 22.3, 18.1, 13.2; HRMS (ESI–) calcd for $C_{24}H_{39}SO_7^-$ ($M - Na^+$) 471.2422, found 471.2412.

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