Synthetic Approaches to C-1 Derivatives of Pancratistatin

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ABSTRACT

The contents of this thesis describe a synthetic approach towards C-1 derivatives of pancratistatin, utilizing a previously published pathway to access a late-stage *cis*-diol. The key steps of the approach include enzymatic dihydroxylation to provide the C-ring backbone, Myers' transposition to convert an allylic alcohol into an olefin, and nucleophilic substitution of a tosylate to insert carbon-based nucleophiles at C-1. Experimental and spectral data are provided for the novel compounds.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

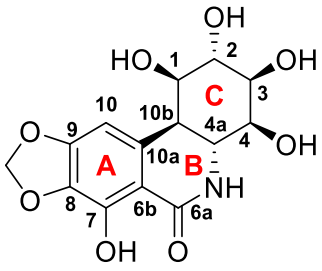
2,2-DMP	2,2-dimethoxypropane
18-C-6	18-crown-6
(±)-CSA	camphorsulfonic acid
AIBN	azobisisobutyronitrile
AcOH	acetic acid
Ac_2O	acetic anhydride
BnBr	benzyl bromide
brsm	based on recovered starting material
Bz_2O	benzoic anhydride
BzCl	benzoyl chloride
COSY	Correlation Spectroscopy
DBAD	di-*tert*-butyl azodicarboxylate
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPHOS	1,2-Bis(diphenylphosphino)ethane
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DME	dimethoxyethane
DMF	dimethylformamide
DPPA	diphenylphosphoryl azide
E. coli	*Escherichia coli*
EtOH	ethanol
EtOAc	ethyl acetate
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HMBC	heteronuclear multiple bond correlation

HMDS	hexamethyldisilazane
HRMS	high resonance mass spectrometry
HSQC	heteronuclear single quantum coherence
IR	infrared spectrum
IPNBSH	2-nitro-*N'*-(propan-2-ylidene)benzenesulfonohydrazide
KCN	potassium cyanide
KOAc	potassium acetate
KOBz	potassium benzoate
LHMDS	lithium *bis*(trimethylsilyl)amide
LRMS	low resonance mass spectrometry
m-CPBA	*meta*-chloroperbenzoic acid
MeCN	acetonitrile
MeOH	methanol
MocNHOH	methoxycarbonyl-protected hydroxylamine
MsCl	mesyl chloride
NaOAc	sodium acetate
NaOBz	sodium benzoate
NBSH	2-nitrobenzenesulfonyl hydrazide
NEt_3	triethylamine
NMO	N-methylmorpholine N-oxide
NMR	nuclear magnetic resonance
PhH	benzene
PMBBr	4-methoxybenzyl bromide
PPh_3	triphenylphosphine
PPTS	pyridinium *para*-toluenesulfonate
p-TsOH	*para*-toluenesulfonic acid
py	pyridine
SDI	1,1′-sulfonyldiimidazole
SMEAH	sodium *bis*(2-methoxyethoxy)aluminium hydride
TBAF	tetra-*n*-butylammonium fluoride
TBSCl	tert-butyldimethylsilyl chloride

t-BuLi	*tert*-butyllithium
t-BuOH	*tert*-butanol
TDO	toluene dioxygenase
TFA	trifluoroacetic anhydride
THF	tetrahydrofuran
TFE	2,2,2-trifluoroethanol
TIPS	triisopropylsilyl ether
TIPSOTf	triisopropylsilyl trifluoromethanesulfonate
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TsCl	tosyl chloride

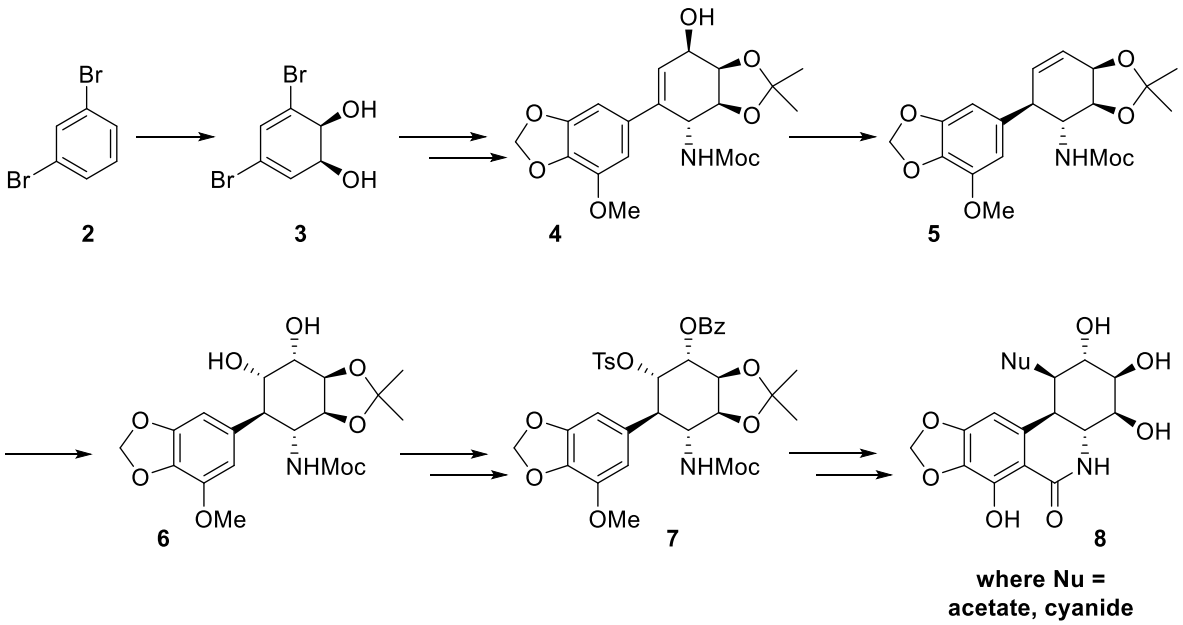
1. INTRODUCTION

The Amaryllidaceae family of flowering plants contains up to 500 alkaloids known for their potential anticancer activity.[1] Of these alkaloids, pancratistatin **(1)**, isolated in 1984 by Pettit from the roots of *Pancratium littorale*,[2] exhibits nanomolar inhibitory activity against a range of cancers, including lymphocytic leukemia.[3] Alterations of the compound have resulted in studies of the pharmacophore, and substitutions at the C-1 position have shown the greatest positive impact on the biological activity. For this reason, several synthetic groups have synthesized C-1 derivatives in the past.



pancratistatin (1)

Herein, a synthetic approach to C-1 derivatives of isocarbostyril **1** will be presented **(Scheme 1)**, utilizing a key intermediate allylic alcohol **4** that was previously employed in the chemoenzymatic total formal synthesis of pancratistatin **(1)**.[4] The chemoenzymatic sequence used to provide late-stage *cis*-diol **6** was repeated for the purpose of this project. The key steps of the current approach include enzymatic dihydroxylation to provide disubstituted dihydrodiol **3**, Myers transposition to generate olefin **5**, and nucleophilic substitution at C-1 of tosylate **7** to reverse stereochemistry to that of natural pancratistatin **(1)**.



Scheme 1. Synthetic approach to C-1 derivatives of pancratistatin **(8)**.

A proof-of-concept for the approach will employ acetate as the nucleophile: this will complete the synthesis of pancratistatin **(1)**. The usage of other carbon nucleophiles, such as cyanide, will provide an approach to C-1 derivatives of pancratistatin **(8)**. Following the installation of a novel substituent at C-1, a Banwell-modified Bischler-Napieralski closure of ring B and global deprotection will provide C-1 derivatives of pancratistatin **(8)**.

The next chapter briefly discusses previous work in this field.

2. HISTORICAL

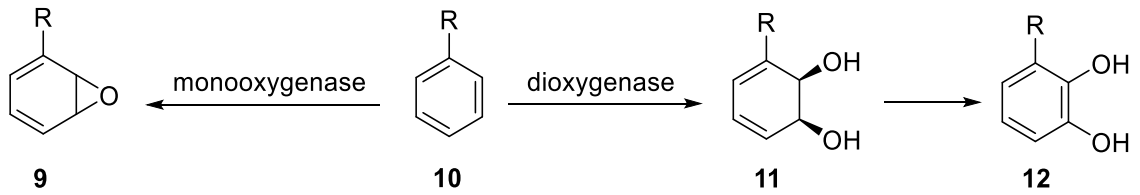
2.1. Introduction

This section will cover the historical background for the chemistry discussed in this thesis. The three topics covered are the enzymatic dihydroxylation of arenes, which pertains to the synthesis of the necessary building block for the C-ring of pancratistatin; the biological activity and selected syntheses of pancratistatin; and selected syntheses of the unnatural derivatives of pancratistatin that most relate to the contents of this thesis.

2.2. Microbial oxidation of arenes

The processing of aromatic compounds by microbes was first described by Stormer in 1908, who showed that *Bacillus hexacarovorum* grew on toluene and xylene.[5] Much later, in 1935, the first dihydrodiol, dihydroxydihydroanthracene, was isolated as a product of the mammalian metabolism of anthracene.[6] The metabolic pathway of the bacterial oxidation of benzene by *Pseudomonas aeruginosa* was proposed by Marr and Stone in 1961.[7]

By that time it was evident that both eukaryotes and prokaryotes were capable of arene oxidation, however, researchers found that different metabolic pathways were involved in the two cases **(Scheme 2)**.[8] In eukaryotes, cytochrome monooxygenases oxidize arenes **10** to arene oxides **9**, which are subsequently hydrolysed to the corresponding *trans*-dihydrodiols, as in the case of anthracene.[8] Conversely, delivery of dioxygen to the arene by dioxygenases produces the *cis*-dihydrodiol **11** in prokaryotes, which is then further oxidized to the catechol **12**.[8]



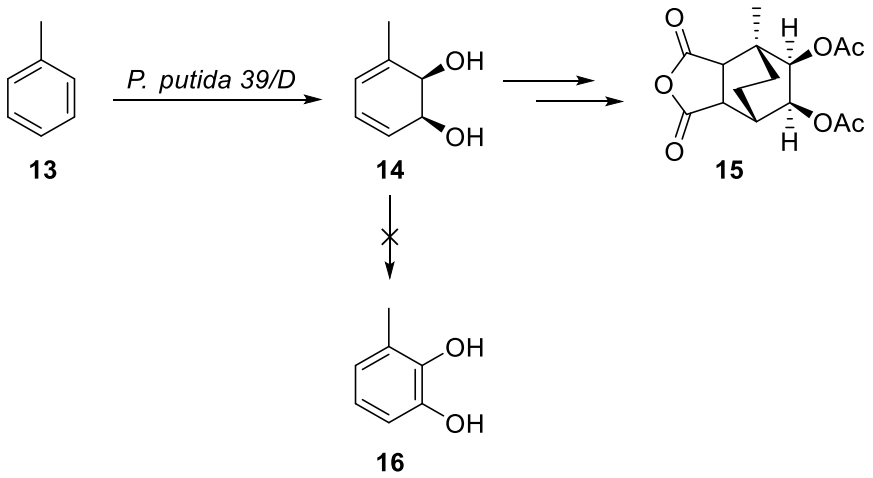
Scheme 2. Comparison of prokaryotic and eukaryotic metabolic pathways of arene oxidation.

2.2.1. Discovery of toluene dioxygenase

In 1968, Gibson showed that *Pseudomonas putida* oxidized benzene, toluene, and ethylbenzene at rapid and equivalent rates.[9] Halogenated aromatic derivatives such as

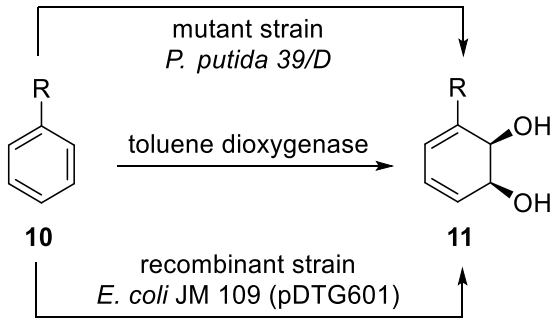
halobenzenes and *p*-chlorotoluene were explored next, as their catechol counterparts showed reduced biodegradability.[10] At this point, the relative stereochemistry of the intermediate *cis*-dihydrodiol **11** had not yet been assigned.

In 1970, Gibson developed a mutant strain of *Pseudomonas putida*, strain 39/D, that allowed for the accumulation and isolation of *cis*-2,3-dihydroxy-1-methylcyclohexa-4,6-diene **(14)** from the microbial oxidation of toluene **(13)**.[11] Further oxidation to the corresponding catechol **16** was not observed.[11] At the same time, Gibson assigned the relative stereochemistry of dihydrodiol **14** as *cis* by preparing the rigid tricyclic derivative **15 (Scheme 3)**.[11]



Scheme 3. Microbial oxidation of toluene by mutant strain *Pseudomonas putida* 39/D and assignment of relative stereochemistry of dihydrodiol **14**.

In 1977, the enzyme catalyzing toluene dihydroxylation, toluene dioxygenase (TDO), was isolated by Gibson.[12] As TDO showed similarities to benzene dioxygenase, Gibson concluded that both enzyme systems were composed of three components: a flavoprotein and two non-heme-iron proteins.[12] Isolation and characterization of the genes encoding for TDO allowed Gibson to prepare recombinant strains of *Escherichia coli* JM109, which were capable of overexpressing TDO.[13] One of these strains, *E. coli* JM109 (pDTG601), is now used to prepare *cis*-dihydrodiols **11** from the corresponding arene **10**[13] **(Scheme 4)**. Soon after, disubstituted arenes also began to show prominence as substrates for enzymatic dihydroxylation.



Scheme 4. The metabolic pathways for arene oxidation by various bacterial strains.

2.2.2. Applications in organic synthesis

The wide range of functionalities present in halogen-substituted *cis*-dihydrodiols **17** make these compounds valuable synthetic intermediates by way of the reactivity patterns displayed in **Figure 1**.[8,14]

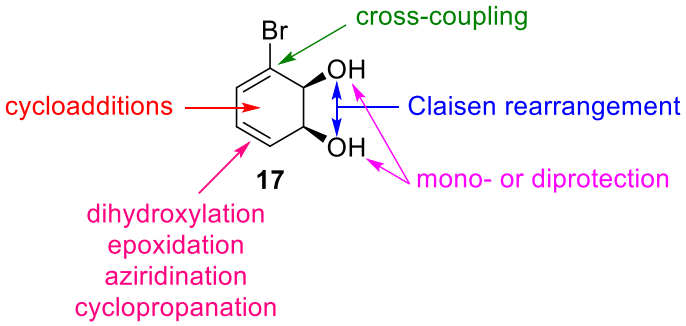
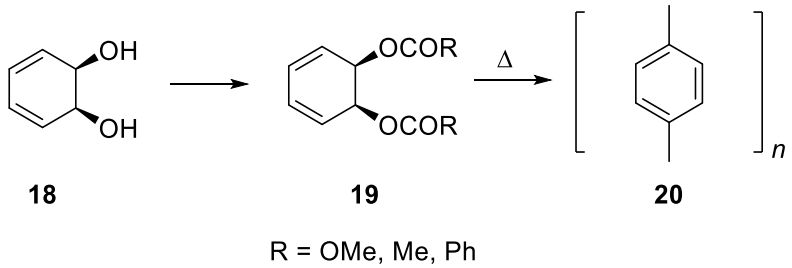


Figure 1. Reactivity patterns of halogen-substituted *cis*-dihydrodiols.[8,14]

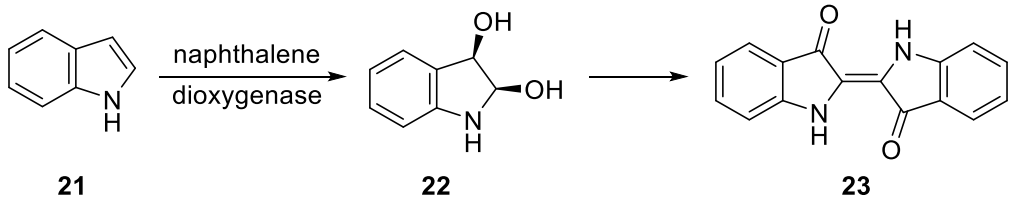
However beneficial, these intermediates come with two major drawbacks: the tendency to undergo dehydrative re-aromatization,[14] and in the case of acetonide-protected diols, dimerization by a Diels-Alder reaction.[14,15] Both drawbacks can be prevented by manipulating the diol toward a stable intermediate prior to storage for long periods of time.

The first synthetic manipulation of *cis*-dihydrodiols was performed by Imperial Chemical Industries (ICI) in 1983 **(Scheme 5)**.[16] From *cis*-dihydrodiol **18**, ICI prepared polyphenylene **(20)** through intermediates of type **19** (a carbonate ester if R = OMe, an ester if R = Me or Ph).[16]



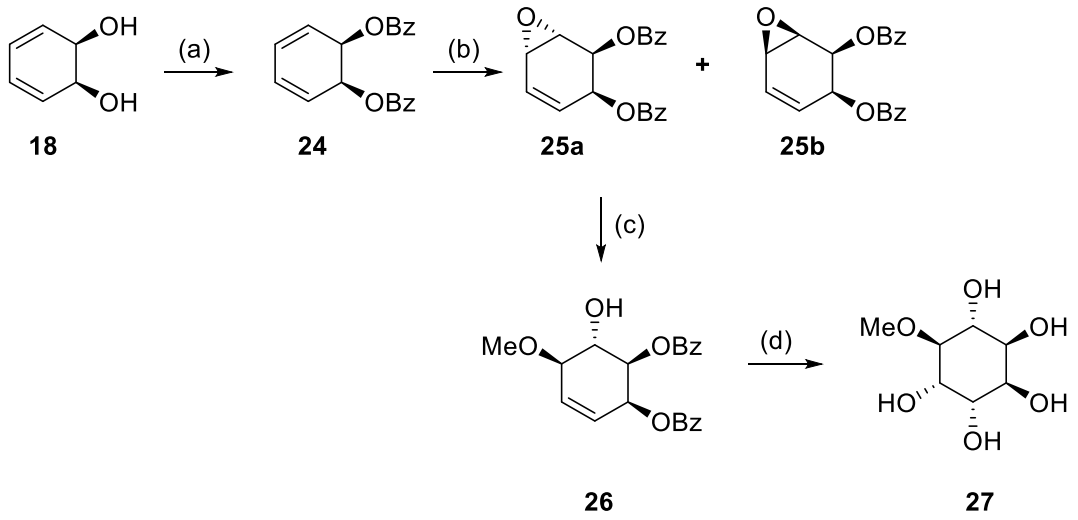
Scheme 5. Imperial Chemical Industries' synthesis of polyphenylene.[16]

In the same year, Gibson produced synthetic indigo **(23)** by way of a recombinant strain of *E. coli* **(Scheme 6)**.[17] Naphthalene dioxygenase oxidation of indole **(21)** produced *cis*-2,3-dihydroxy-2,3-dihydroindole **(22)**, which was dehydrated to indoxyl and further oxidized by air to indigo **(23)**.[17]



Scheme 6. Gibson's synthesis of indigo.[17]

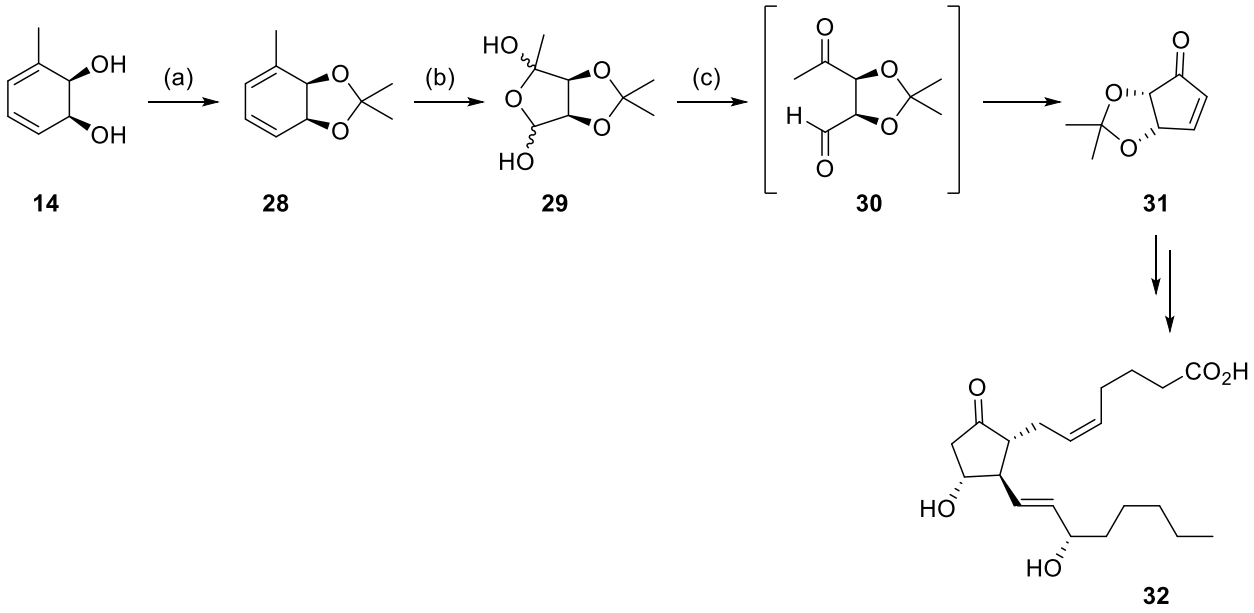
The first use of *cis*-dihydrodiols in natural product synthesis was reported in 1987 with Ley's synthesis of (+)-pinitol **(27) (Scheme 7)**.[18] *cis*-Dihydrodiol **18** was protected as dibenzoate **24** and subjected to epoxidation, which produced a 5:1 mixture of diastereomers **25a** and **25b**.[18] Nucleophilic opening of epoxide **25a** furnished alkene **26**, which was subjected to catalytic osmylation and global deprotection to provide (+)-pinitol **(27)**.[18]



Reagents and conditions: (a) BzCl, py., DMAP, 0 °C; (b) *m*-CPBA, DCE, pH 8 phosphate buffer, rt, 71h; (c) MeOH, (±)-CSA, rt, 13h; (d) (i) 0.1% OsO_4, NMO, t-BuOH/THF/H_2O (10:3:1), rt, 84h; (ii) NEt_3/MeOH/H_2O (1:5:1), rt, 3h.

Scheme 7. Ley's synthesis of (+)-pinitol.[18]

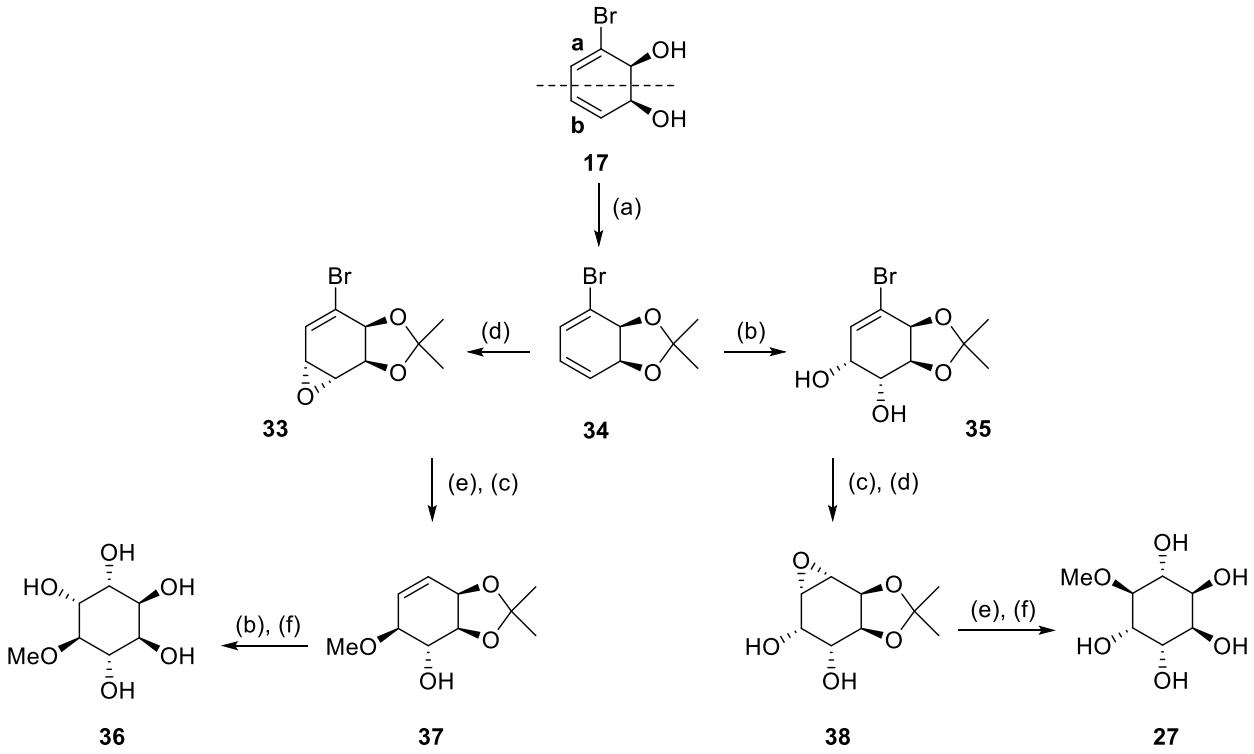
The first use of *cis*-dihydrodiols by the Hudlicky group was reported in 1988 with the formal synthesis of prostaglandin $E_2\alpha$ ($PGE_2\alpha$) **(32) (Scheme 8)**.[19] *cis*-Dihydrodiol **14**, derived from toluene **(13)**, was subjected to acetonide protection followed by ozonolysis to provide hemiacetal **29**.[19] Controlled dehydration on neutral alumina provided ketoaldehyde **30**, which was subjected to further dehydration to provide enone **31**.[19] This intermediate could then be readily converted to $PGE_2\alpha$ **(32)** by a known method.[19]



Reagents and conditions: (a) 2,2-DMP, cat. *p*-TsOH, rt, 10 min; (b) (i) O_3 (excess), EtOAc -78 °C; (ii) Me_2S, 0 °C; (c) Al_2O_3 (neutral), PhH or DME, Δ.

Scheme 8. Hudlicky's formal synthesis of prostaglandin $E_2\alpha$.[19]

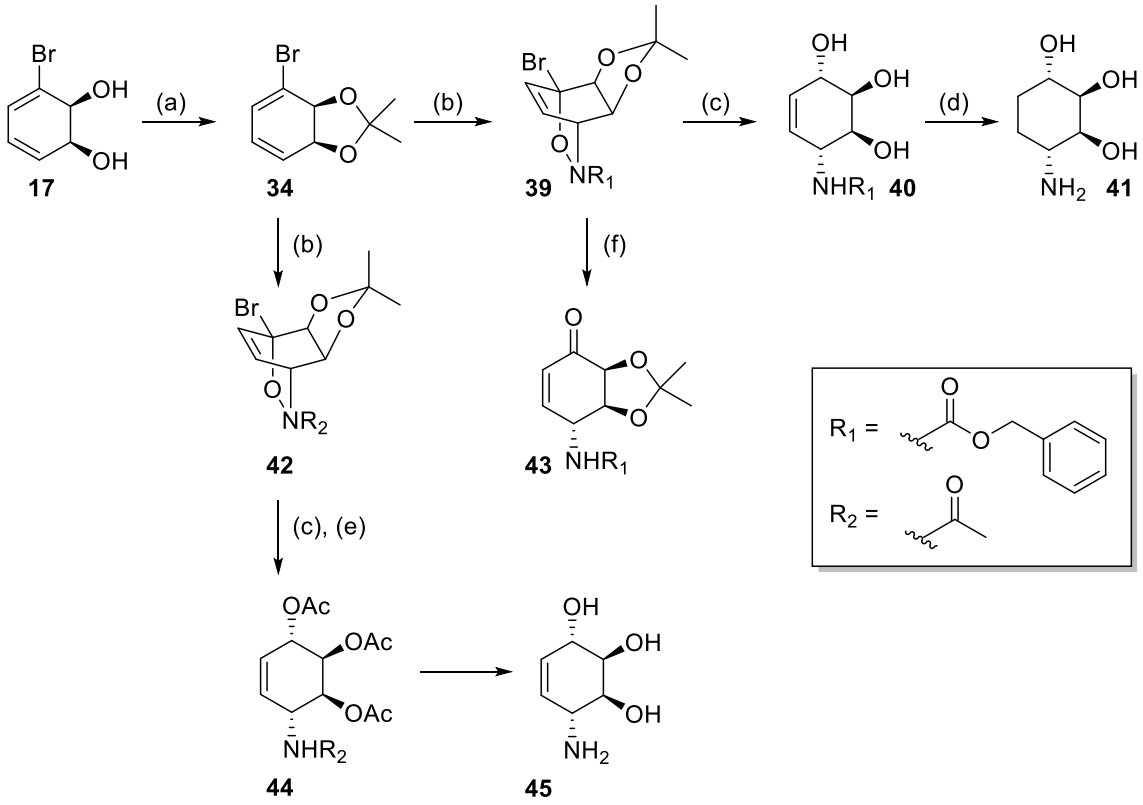
In 1990, Hudlicky's synthesis of (+)-pinitol **(27)** and (-)-pinitol **(36) (Scheme 9)** showed that both enantiomers were available from a single metabolite obtained by biocatalysis.[20] More specifically, the proenantiotopic plane in bromodihydrodiol **17** can be manipulated by altering the order of operations.[20] Osmylation of olefin **b**, followed by halogen reduction and epoxidation of olefin **a** leads to (+)-pinitol **(27)**, whereas epoxidation of olefin **b**, followed by halogen reduction and osmylation of olefin **a** leads to (-)-pinitol **(36)**.[20]



Reagents and conditions: (a) 2,2-DMP, cat. *p*-TsOH, rt, 10 min; (b) OsO_4, NMO, H_2O, acetone; (c) $LiAlH_4$, THF; (d) *m*-CPBA, DCM; (e) MeOH, Al_2O_3; (f) HCl, H_2O, acetone.

Scheme 9. Hudlicky's enantiodivergent synthesis of (+)-pinitol and (-)-pinitol.[20]

Hudlicky's 1991 synthesis of aminocyclitols **(Scheme 10)** reported the first usage of regio- and stereospecific Diels-Alder cycloadditions between bromodihydrodiol **17** and nitrosyl derivatives such as hydroxamic acids.[21] Reduction of oxazines **39** and **42** furnished allylic alcohol **40** and reprotected allylic acetate **44**, respectively.[21] Hydrogenation of the olefin in allylic alcohol **40** produced dihydroconduramine A-1 **(41)**, whereas deprotection of allylic acetate **44** produced conduramine A-1 **(45)**.[21]

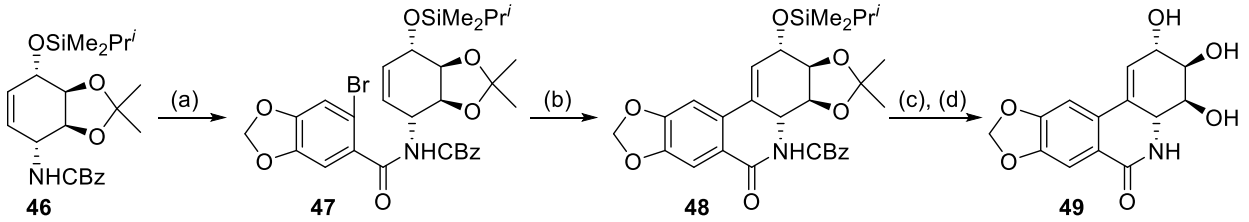


Reagents and conditions: (a) DMP, *p*-TsOH, acetone; (b) Bu_4NIO_4 and benzyl-N-hydroxycarbamate or acetohydroxamic acid; (c) (i) Al/Hg, THF/H_2O; (ii) AcOH/THF/H_2O; (d) H_2/Pd, MeOH; (e) Ac_2O, py.; (f) Bu_3SnH, AIBN, toluene.

Scheme 10. Hudlicky's synthesis of aminocyclitols.[21]

The aminocyclitols conduramine A-1 **(45)** and dihydroconduramine A-1 **(41)** contain the structural moiety of the C-ring of pancratistatin **(1)** and its isocarbostyril congeners; this route has thus been employed by Hudlicky in many syntheses, including the 1992 synthesis of (+)-lycoricidine[22] **(49) (Scheme 11)** and the 1999 synthesis of (+)-narciclasine[23] **(59) (Scheme 13)**.

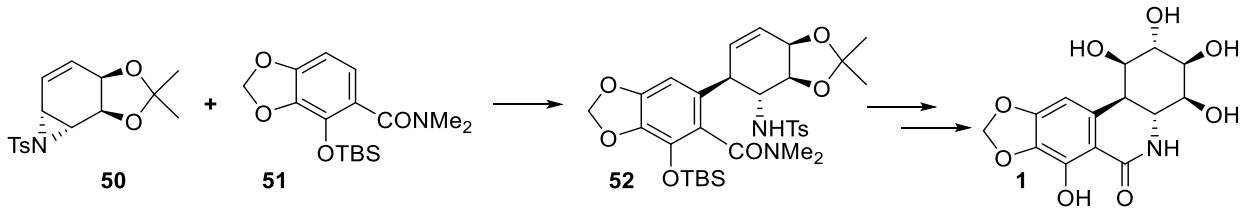
The key steps of the synthesis of (+)-lycoricidine[22] **(49)** by Hudlicky are as follows. CBz-protected aminoconduritol **46**, obtained by the chemoenzymatic route discussed previously, was subjected to acylation followed by a modified Heck cyclization to afford silyl ether **48**.[22] Hydrogenation followed by global deprotection afforded (+)-lycoricidine **(49)**.[22]



Reagents and conditions: (a) BuLi, THF, -78 °C; then Br-piperonyloyl chloride; (b) $Pd(OAc)_2$, Tl(OAc), DIPHOS, anisole; (c) H_2, Pd/C, cyclohexene, EtOH; (d) TFA, 0 °C.

Scheme 11. Hudlicky's synthesis of (+)-lycoricidine.[22]

The first enantioselective chemoenzymatic approach to (+)-pancratistatin **(1)** was published by Hudlicky in 1995.[24] Amide **52** was produced through a sequence of *ortho*-lithiation and cupration of amide **51**, followed by an S_N2-type opening of aziridine **50**.[24] This synthesis will be discussed further in the following chapter, in relation to the evolution of syntheses of pancratistatin **(1)**.

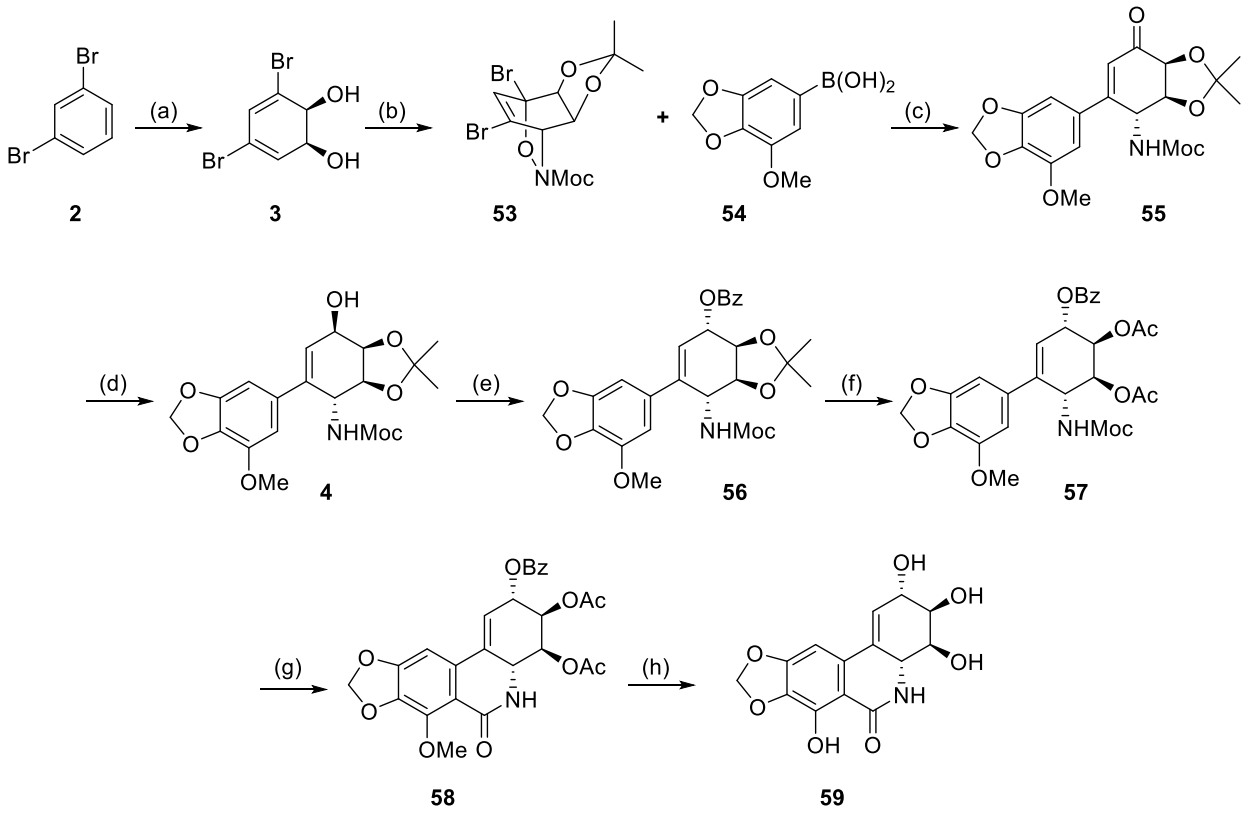


Scheme 12. Hudlicky's enantioselective synthesis of (+)-pancratistatin.[24]

Apart from the multiple syntheses of pancratistatin **(1)** by various groups, Hudlicky's enantioselective synthesis of (+)-narciclasine **(59)** in 1999[23] is most relevant to this thesis, for the construction of the A, B, and C rings (pancratistatin numbering) are equivalent.

Bicylic oxazine **53** was prepared in one-pot conditions from *m*-dibromobenzene **(2)**.[23] Suzuki coupling with boronic acid **54** resulted in enone **55**, which was subjected to Luche reduction to provide allylic alcohol **4**.[23] This sequence was repeated in the current work to access allylic alcohol **4**. The Mitsunobu inversion of C-2 position (pancratistatin numbering) provided benzoate **56** with the natural stereochemistry.[23] Acetonide deprotection and reprotection with acetates allowed for

the smooth cyclization of ring B under Banwell-modified Bischler-Napieralski conditions.[23] Finally, global deprotection provided (+)-narciclasine **(59)** in twelve steps.[23]

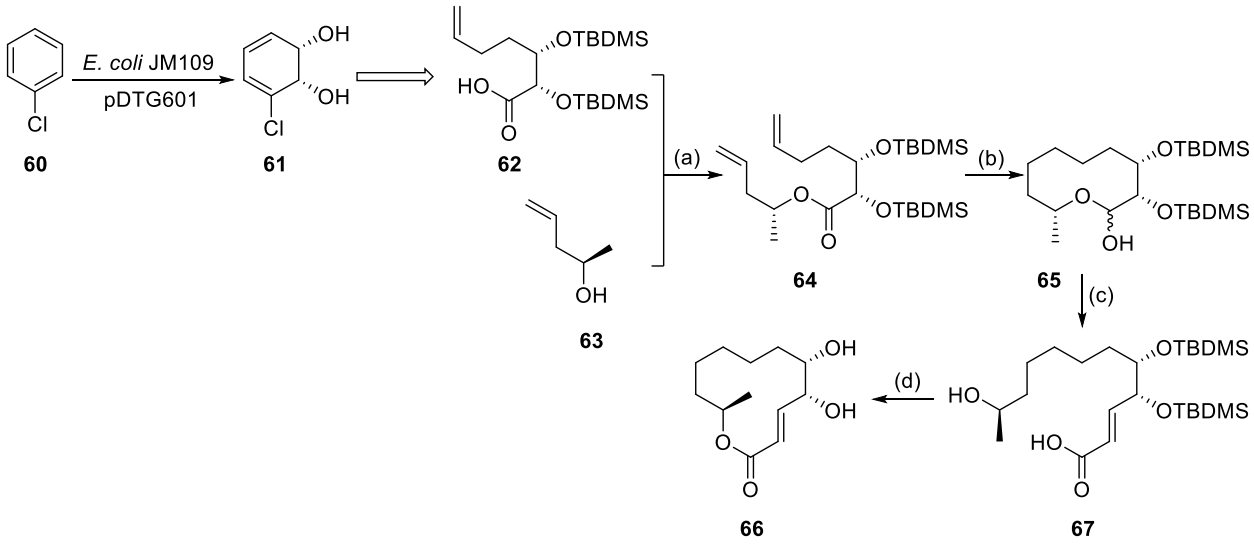


Reagents and conditions: (a) *E. coli* JM109 (pDTG601); (b) 2,2-DMP, acetone, *p*-TsOH, rt; then MocNHOH, $NaIO_4$, rt; (c) (i) $Pd(PPh_3)_4$, aq. Na_2CO_3, PhH, reflux; (ii) $Mo(CO)_6$, MeCN, H_2O, reflux; (d) $NaBH_4$, $CeCl_3$, MeOH, 0 °C; (e) BzOH, Bu_3P, DEAD, THF, rt; (f) (i) Dowex-$50X_{8\text{-}100}$, MeOH, rt; (ii) Ac_2O, py., DMAP, rt; (g) Tf_2O, DMAP, DCM, 0 °C; (h) (i) Amberlyst A21, MeOH, rt; (ii) LiCl, DMF, 120 °C.

Scheme 13. Hudlicky's enantioselective synthesis of (+)-narciclasine.[23]

Banwell's synthesis of the macrolide (-)-cladospolide A **(66)** in 2002 **(Scheme 14)** employed the chemoenzymatic dihydroxylation of chlorobenzene **(60)** to acquire *cis*-dihydrocatechol **61**.[25] Yonemitsu esterification of acid **62** and alcohol **63** provided diene **64**.[25] This compound was subjected to ring closing metathesis followed by DIBAL reduction to provide lactol **65**.[25] Finally,

the acid **67** was subjected to Yamaguchi lactonization and deprotection to afford (-)-cladospide **(66)**.[25]

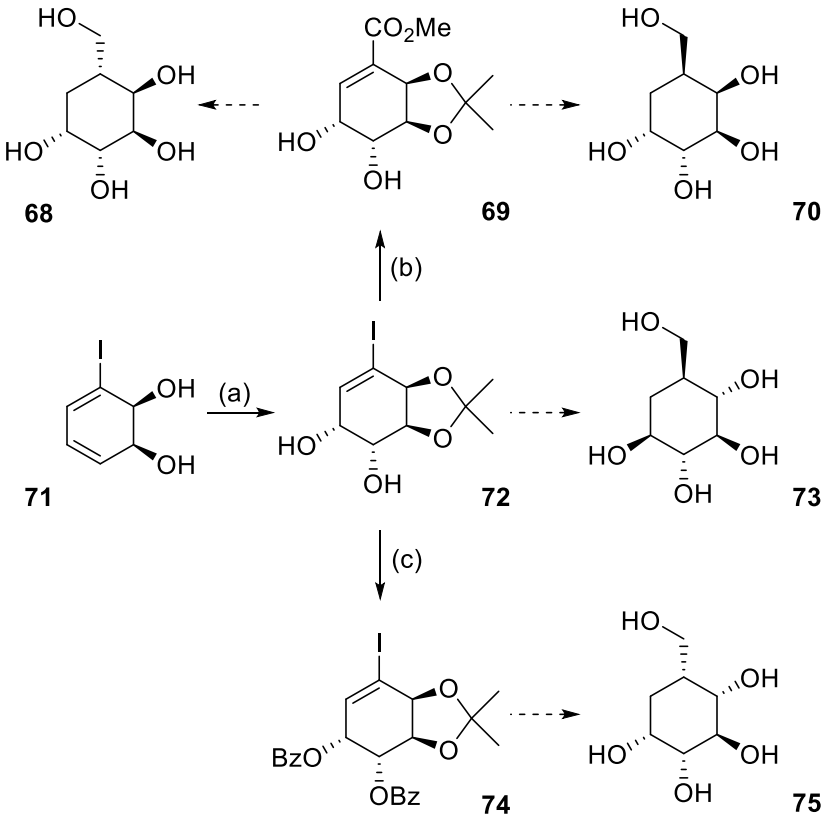


Reagents and Conditions: (a) NaOH, 9:1 v/v EtOH-H_2O, 60 °C, 2h, then 1M HCl, then 2,4,6-trichlorobenzoyl chloride, **63**, NEt_3, DMAP, toluene, 1h; (b) (i) tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene](benzylidene)ruthenium(IV) dichloride, DCM, 18 °C, 24 h; (ii) H_2 (1 atm), 10% Pd/C, EtOH, 6h; (iii) DIBAL-H, toluene, -78 °C, 10 min (c) (i) $(MeO)_2P(O)CH_2CO_2Me$, NaH, THF, 0 °C to rt, 3h, then **65**, 0 °C to rt, 1 h; (ii) NaOH, 4:1 v/v EtOH-H_2O, 18 °C, 18 h, then 1 M HCl; (d) (i) 2,4,6-trichlorobenzoyl chloride, NEt_3, THF, 18 °C, 2h, then DMAP, toluene, 111 °C, 1h; (ii) $Zn(BF_4)_2$, MeCN, 18 °C, 24 h.

Scheme 14. Banwell's synthesis of (-)-cladospolide A.[25]

In 2005, Boyd subjected iodobenzene to chemoenzymatic dihydroxylation to synthesize iododihydrodiol **71**.[26] This metabolite was subjected to acetonide protection followed by osmylation to furnish diol **72**.[26] From diol **72**, carba-*β*-L-glucopyranose **(73)** can be accessed in 6 steps.[26] Free diol **72** is protected as dibenzoate **74**, allowing access to carba-*β*-D-idopyranose **(75)** in 9 steps.[26] Ester **69** can be separately manipulated to provide carba-*β*-D-altropyranose **(68)** in 4

steps and carba-α-L-galactopyranose **(70)** in 4 steps.[26] Thus, iodobenzene was uniquely employed to provide four pyranose carbasugars **(Scheme 15)**.[26]



Reagents and Conditions: (a) (i) 2,2-DMP, (ii) OsO_4, acetone, H_2O; (b) $Pd(OAc)_2$, CO, NaOAc, MeOH; (c) BzCl, C_6H_5N.

Scheme 15. Boyd's chemoenzymatic synthesis of pyranose carbasugars.[26]

The synthetic utility of metabolites derived from enzymatic dihydroxylation of arenes has been illustrated by the few syntheses described above. Several other chemoenzymatic approaches that use *cis*-dihydrodiols have resulted in the syntheses of sugars, terpenes, and multiple natural products; these applications of microbial oxidation have been extensively reviewed.[8,14] Microbial oxidation thus provides a biocatalytic approach toward starting materials that can be altered in a highly stereo- and regioselective manner.

2.3. Pancratistatin: An Amaryllidaceae Constituent

2.3.1. Introduction

Plants of the Amaryllidaceae family have long been used as remedies for various ailments.[27] Since the isolation of lycorine in 1877, more than 300 Amaryllidaceae constituents have been identified and some classified as potential anticancer compounds.[27] The isolation of pancratistatin **(1)** by Pettit in 1984[2] was thus regarded as a great success.

Preliminary investigations showed that pancratistatin **(1)** selectively targets and induces apoptosis in tumor cells, while remaining non-cytotoxic to healthy cells.[28] However, more advanced investigations are hindered by the low natural abundance and low aqueous solubility of the title compound. To bypass these issues, several research groups have targeted the chemical synthesis of isocarbostyril **1**. The following chapters will discuss these as well as syntheses of the unnatural derivatives of pancratistatin **(1)**.

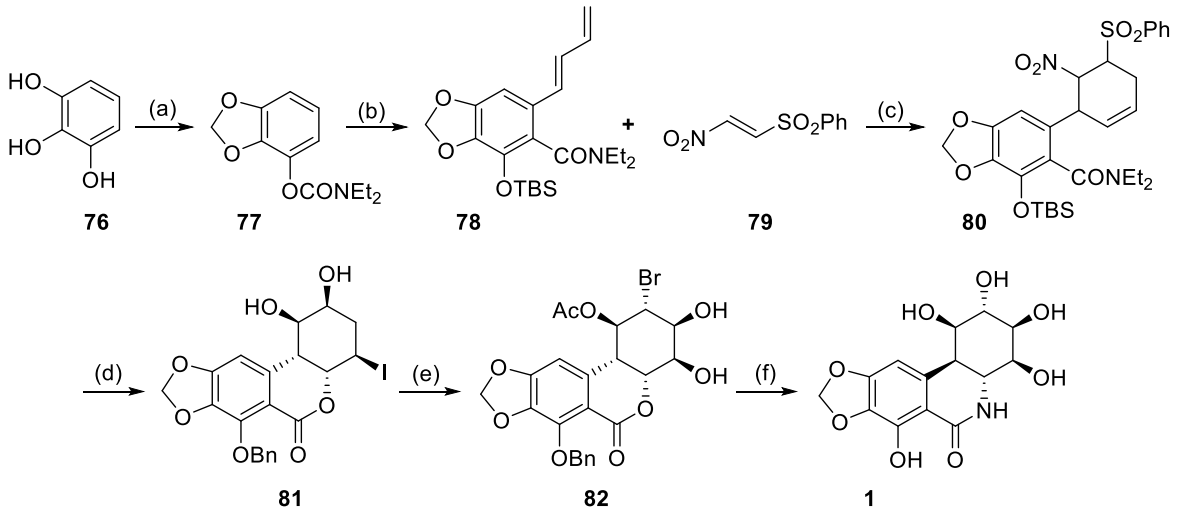
2.3.2. Selected Syntheses of Pancratistatin

The first total synthesis of racemic pancratistatin **(1)** was achieved by Danishefsky in 1989.[29] Since then, seventeen total syntheses of pancratistatin **(1)** have been published and extensively reviewed **(Table 1)**.[27,30,31] A few of these syntheses will be discussed in detail: Danishefsky's,[29] Hudlicky's,[4,24] Magnus',[32] Rigby's,[33] Pettit's,[34] Kim's,[35] Alonso's,[36] Sato's,[37] and Sarlah's.[38]

No.	Author	Year	Step Count
1.	Danishefsky[29]	1989	27
2.	Hudlicky[24]	1995	14
3.	Trost[39]	1995	19
4.	Haseltine[40]	1997	15
5.	Magnus[32]	1998	22
6.	Rigby[33]	2000	23
7.	Pettit[34]	2001	10
8.	Kim[35]	2002	16
9.	Li[41]	2006	13
10.	Madsen[42]	2009	18
11.	Cho[43]	2011	16
12.	Alonso[36]	2012	14
13.	Sato[37]	2013	18
14.	Cho[44]	2013	13
15.	Ellmann[45]	2017	10
16.	Hudlicky[4]	2017	14
17.	Sarlah[38]	2017	7
18.	Sarlah[46]	2019	9

Table 1. Total syntheses of pancratistatin **(1)** accomplished since 1989.

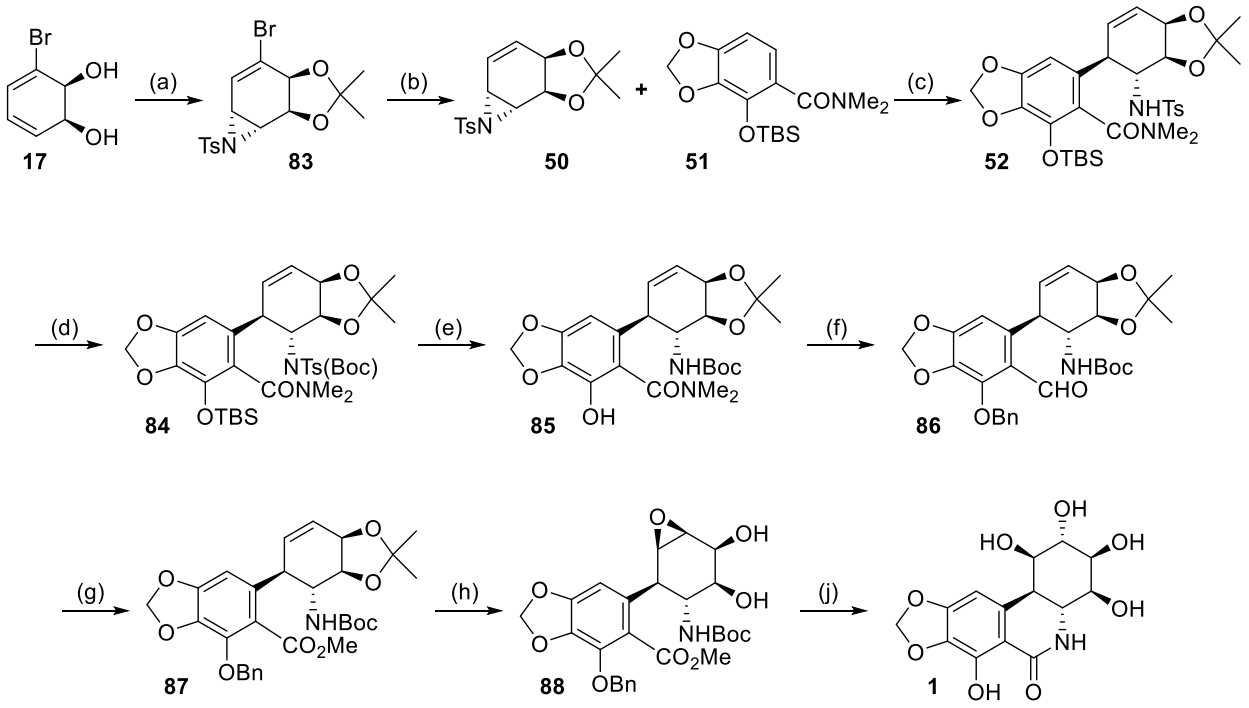
Danishefsky's total synthesis of racemic pancratistatin **(Scheme 16)** utilized pyrogallol **(76)** as the starting material to construct the A-ring.[29] The carbamate at C-7 (pancratistatin numbering) was rearranged to the neighbouring position by way of the Snieckus rearrangement.[29] The Diels-Alder reaction of diene **78** and dienophile **79** installed the necessary C-10a/C-10b linkage and ring B was closed via lactonization.[29] Further manipulations produced the necessary stereocenters on ring C, successfully completing the synthesis of racemic pancratistatin (0.13% yield and 27 steps).[29]



Reagents and Conditions: (a) (i) $CH(EtO)_3$; (ii) $ClCONEt_2$, NaH, DMAP; (iii) *p*-TsOH, MeOH; (iv) CH_2Br_2, K_2CO_3, CuO; (b) (i) *s*-BuLi; (ii) TBSCl, imidazole; (iii) *s*-BuLi, TMEDA, DMF; (iv) allyl magnesium bromide; (v) MsCl; (vi) DBU; (c) Bu_3SnH, AIBN; (d) (i) Bu_4NF; (ii) $(Bu_3Sn)_2O$; (iii) I_2; (iv) BnBr, Ag_2O; (v) OsO_4, NMO; (e) (i) DBU; (ii) 1-bromo-2-methyl-1-oxopropan-2-yl acetate; (iii) OsO_4, NMO; (f) (i) $(Bu_3Sn)_2O$; (ii) PMBBr; (iii) BnBr, Ag_2O; (iv) DDQ; (v) Zn dust; (vi) cat. NaH, CCl_3CN; (vii) 100 °C, high vacuum; (viii) OsO_4, NMO; (ix) K_2CO_3, MeOH; (x) Amerlite IR-120; then DCC; (xi) H_2, $Pd(OH)_2/C$.

Scheme 16. Danishefsky's total synthesis of racemic pancratistatin.[29]

The first asymmetric synthesis of pancratistatin **(1)** was achieved by Hudlicky in 1995 **(Scheme 17)**.[24] Three of the stereocenters in ring C of pancratistatin **(1)** were readily established using microbial oxidation,[24] thus greatly reducing the number of synthetic conversions required to complete the synthesis. S_N2-type opening of aziridine **50** furnished the correct stereochemistry at C-10b (pancratistatin numbering).[24] Treatment of epoxide **88** with sodium benzoate for 6 days surprisingly accomplished epoxide opening, thermal cleavage of the Boc protecting group, cyclization of ring B, and debenzylation at C-7 (pancratistatin numbering).[24] Quenching of the reaction after 48 hours resulted in C-10 benzylated pancratistatin, which was subjected to hydrogenation to furnish pancratistatin **(1)**.[24] Overall, the first enantioselective synthesis of pancratistatin **(1)** was achieved in 14 steps (2% yield).[24]



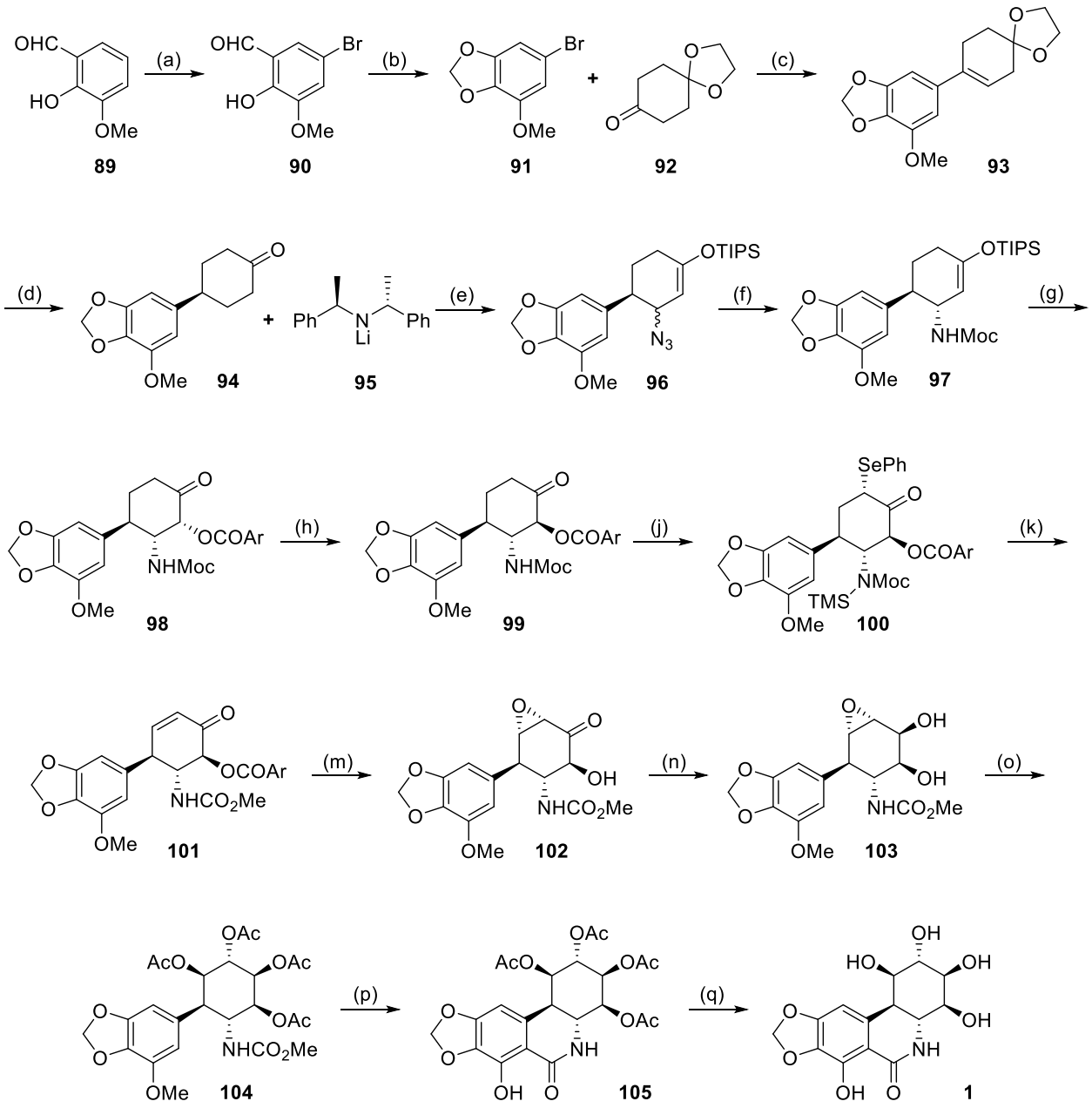
Reagents and conditions: (a) (i) DMP, *p*-TsOH, DCM; (ii) PhI=NTs, $Cu(acac)_2$, MeCN; (b) Bu_3SnH, AIBN, THF; (c) (i) *s*-BuLi, TMEDA, THF, -90 °C, (ii) CuCN, -90 °C to -20 °C; (iii), **51**, -78 °C to rt; (d) (i) *s*-BuLi, THF; (ii) Boc_2O; (e) (i) Na/anthracene, DME, -78 °C; (ii) TBAF, THF; (f) (i) SMEAH/morpholine; (ii) BnBr, K_2CO_3; (g) (i) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O; (ii) CH_2N_2; (h) (i) AcOH, THF, H_2O; (ii) *t*-BuOOH, $VO(acac)_2$, C_6H_6; (j) NaOBz (cat.), H_2O, 100 °C.

Scheme 17. Hudlicky's chemoenzymatic synthesis of (+)-pancratistatin.[24]

In 1998, Magnus applied the *β*-azidonation reaction to establish the C-ring of (+)-pancratistatin **(Scheme 18)**.[32] The A-ring was synthesized from *o*-vanillin **(89)** via bromination, Baeyer-Villiger oxidation, and formation of the methylenedioxy bridge.[32] This protocol to establish the A-ring has been used in several syntheses of Amaryllidaceae alkaloids, including in the work presented in this thesis.

The prochiral arylcyclohexanone **94** was treated with lithium (+)-bis(α-methylbenzyl)amide **(95)** and the resulting enolate was trapped with a triisopropylsilyl ether (TIPS) protecting group.[32] This allowed directed *β*-azidonation, which was followed by reduction and acylation to furnish

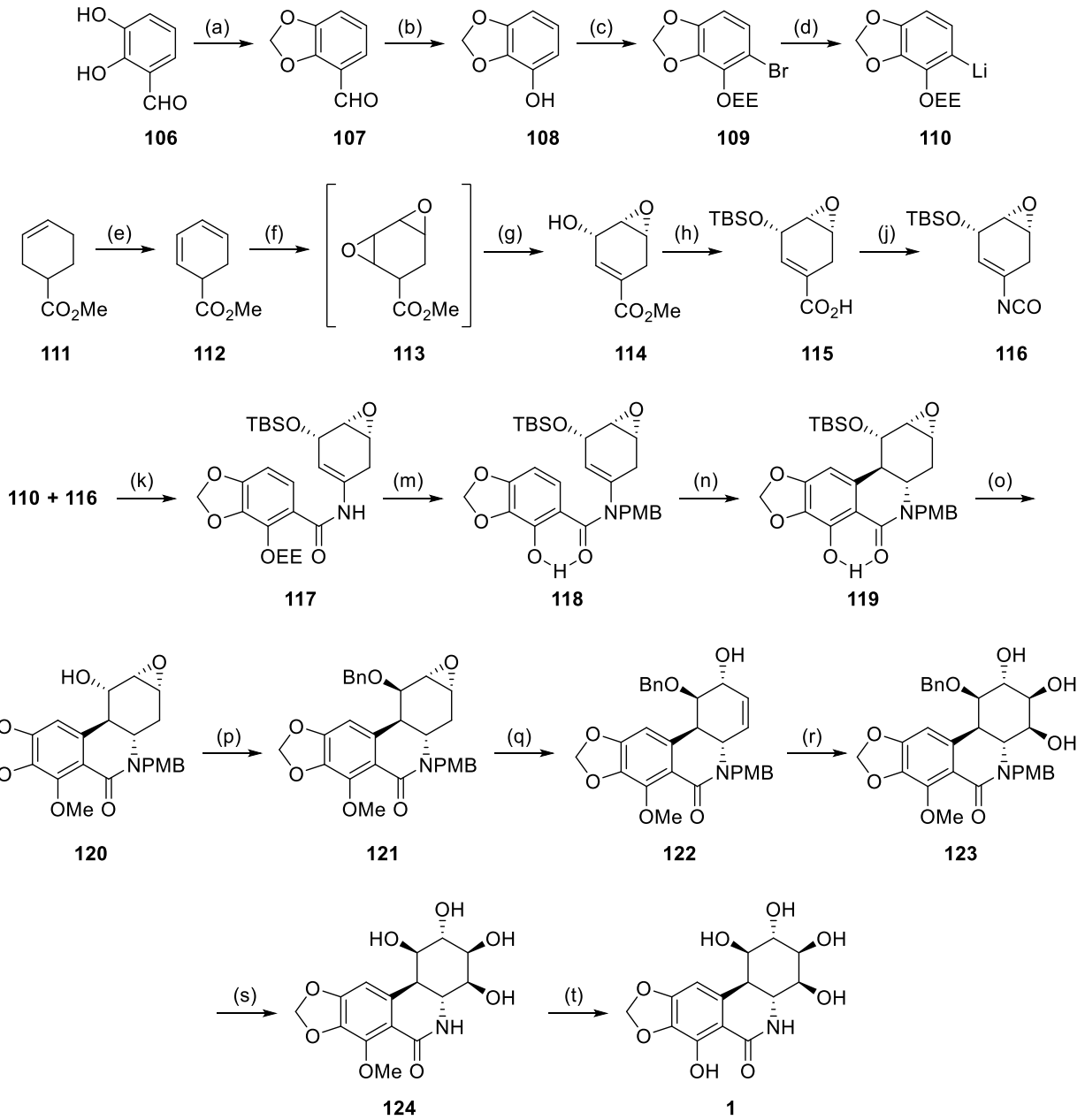
silyl enol ether **97**.[32] The enone **101** was subjected to a similar protocol as that in Hudlicky's synthesis: epoxidation followed by nucleophilic attack with sodium benzoate.[32] Finally, a Bischler-Napieralski cyclization and global deprotection provided pancratistatin **(1)** in 22 steps (1.2% yield).[32]



Reagents and conditions: (a) Br_2, AcOH, NaOAc; (b) (i) H_2O_2, NaOH; (ii) $BrCH_2Cl$, K_2CO_3; (c) (i) *n*-BuLi/THF, then **92**; (ii) $POCl_3$, DBU, py.; (d) (i) H_2/Pd-C; (ii) dioxane/H_3O^+; (e) (i) **95**, LiCl, TIPSOTf; (ii) PhIO, $TMSN_3$; (f) (i) $LiAlH_4$, Et_2O; (ii) MeOCOCl, py.; (g) (i) *m*-CPBA; (ii) H_3O^+; (h) KO^tBu/HMPA; (j) (i) TMSOTf, NEt_3; (ii) $PhSeOCOCF_3$; (k) H_2O_2, py.; (m) $NaHCO_3$, H_2O_2, MeOH; (n) L-selectride, THF; (o) (i) $PhCO_2Na$, H_2O; (ii) Ac_2O, py.; (p) (i) Tf_2O, DMAP; (ii) BBr_3; (q) NaOMe/MeOH.

Scheme 18. Magnus' enantioselective synthesis of (+)-pancratistatin.[32]

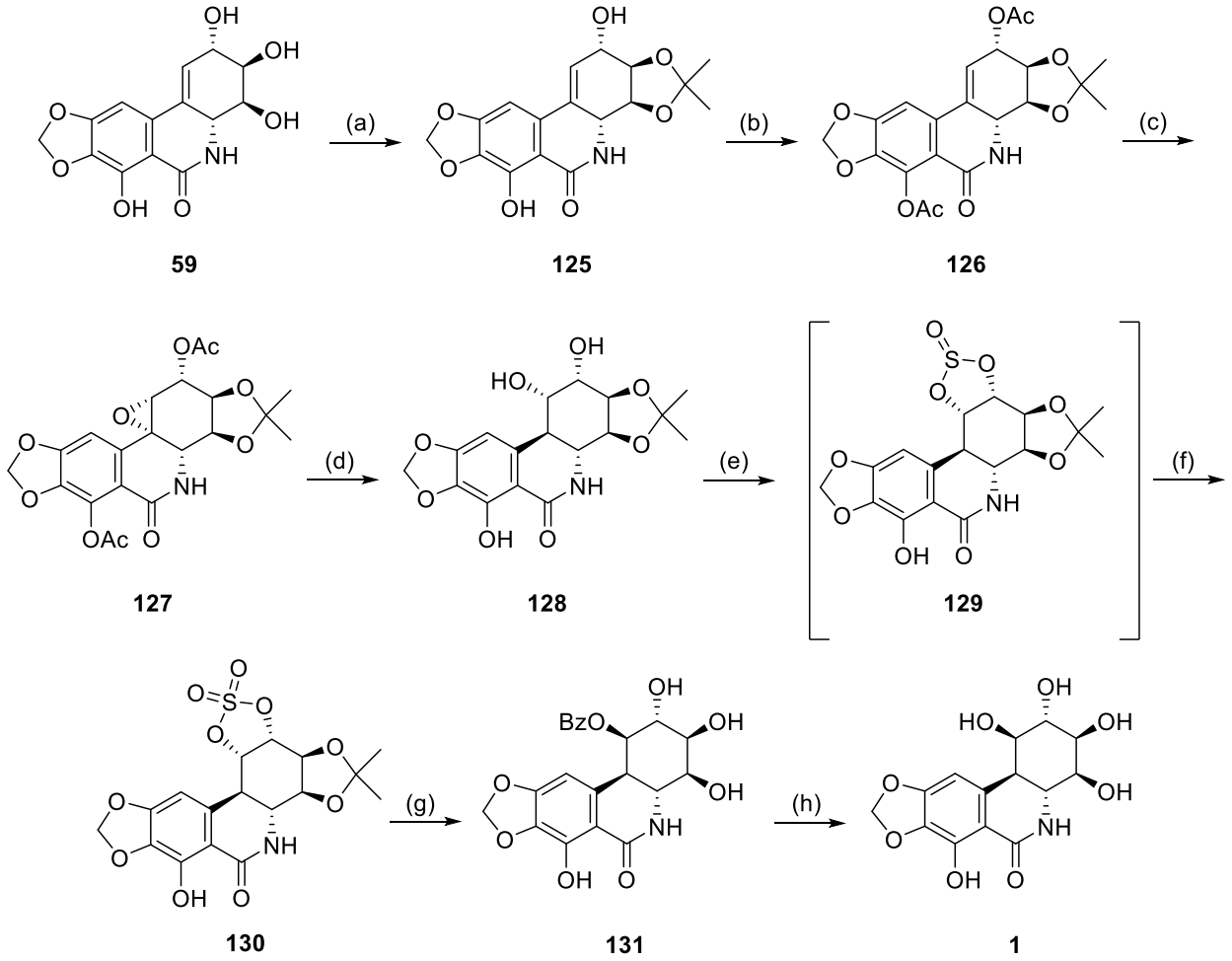
In 2000, Rigby's enantioselective approach to both (+)-pancratistatin **(Scheme 19)** and (+)-narciclasine utilized an advanced intermediate **(119)** derived from an aryl enamide photocyclization.[33] Coupling of isocyanate **116** and bromide **109** established the necessary elements of the phenanthridone backbone.[33] Photocyclization furnished the correct stereochemistry at C-10b (pancratistatin numbering).[33] Selenoxide elimination provided allylic alcohol **122**, which was then subjected to osmylation, furnishing all the correct stereocenters of ring C.[33] Finally, global deprotection provided (+)-pancratistatin **(1)** in 23 steps (0.35% yield).[33]



Reagents and conditions: (a) CH_2Br_2, K_2CO_3; (b) *m*-CPBA, then KOH; (c) (i) CF_3CO_2Ag, Br_2; (ii) ethyl vinyl ether, PPTS; (d) *n*-BuLi, -78 °C; (e) (i) NBS, AIBN; (ii) Bu_3SnH; (f) (i) O_2, Rose Bengal, hν; (ii) $RuCl_2(PPh_3)_2$; (g) (i) NaOMe, MeOH; (ii) *n*-PrCOCl, NEt_3; (iii) cholesterol esterase; (h) TBSCl, then LiOH; (j) DPPA, then toluene, 110 °C; (k) **116** was added dropwise to freshly prepared **110** at -100 °C; (m) (i) NaH, PMBBr; (ii) PPTS; (n) hν, PhH; (o) (i) NaH, MeI; (ii) TBAF; (p) (i) Dess-Martin periodinane; (ii) $NaBH_4$, -20 °C; (iii) NaH, BnBr; (q) $(PhSe)_2$, $NaBH_4$; then H_2O_2, reflux; (r) OsO_4, *t*-BuOH; (s) $Pd(OH)_2/H_2$; (t) LiCl, DMF.

Scheme 19. Rigby's enantioselective synthesis of (+)-pancratistatin.[33]

In 2001, Pettit reported a remarkable synthesis of (+)-pancratistatin **(1)** from closely related and more naturally abundant isocarbostyril (+)-narciclasine **(59) (Scheme 20)**.[34] The necessary C-10b stereocenter (pancratistatin numbering) was installed by way of epoxidation of the C-1/C-10b olefin.[34] Nucleophilic opening of cyclic sulfate **130** with cesium benzoate followed by acid hydrolysis provided benzoate **131**.[34] Finally, hydrolysis of benzoate **131** provided (+)-pancratistatin **(1)** in 10 steps (3.6% yield) from (+)-narciclasine **(59)**.[34] The remarkability of the synthesis arises from Pettit's preparation of benzoate **131**, which was found to have increased activity compared to the parent compound.[34] This pioneering event led to the future syntheses of pancratistatin derivatives with substitutions at C-1. This will be further discussed in the next section.

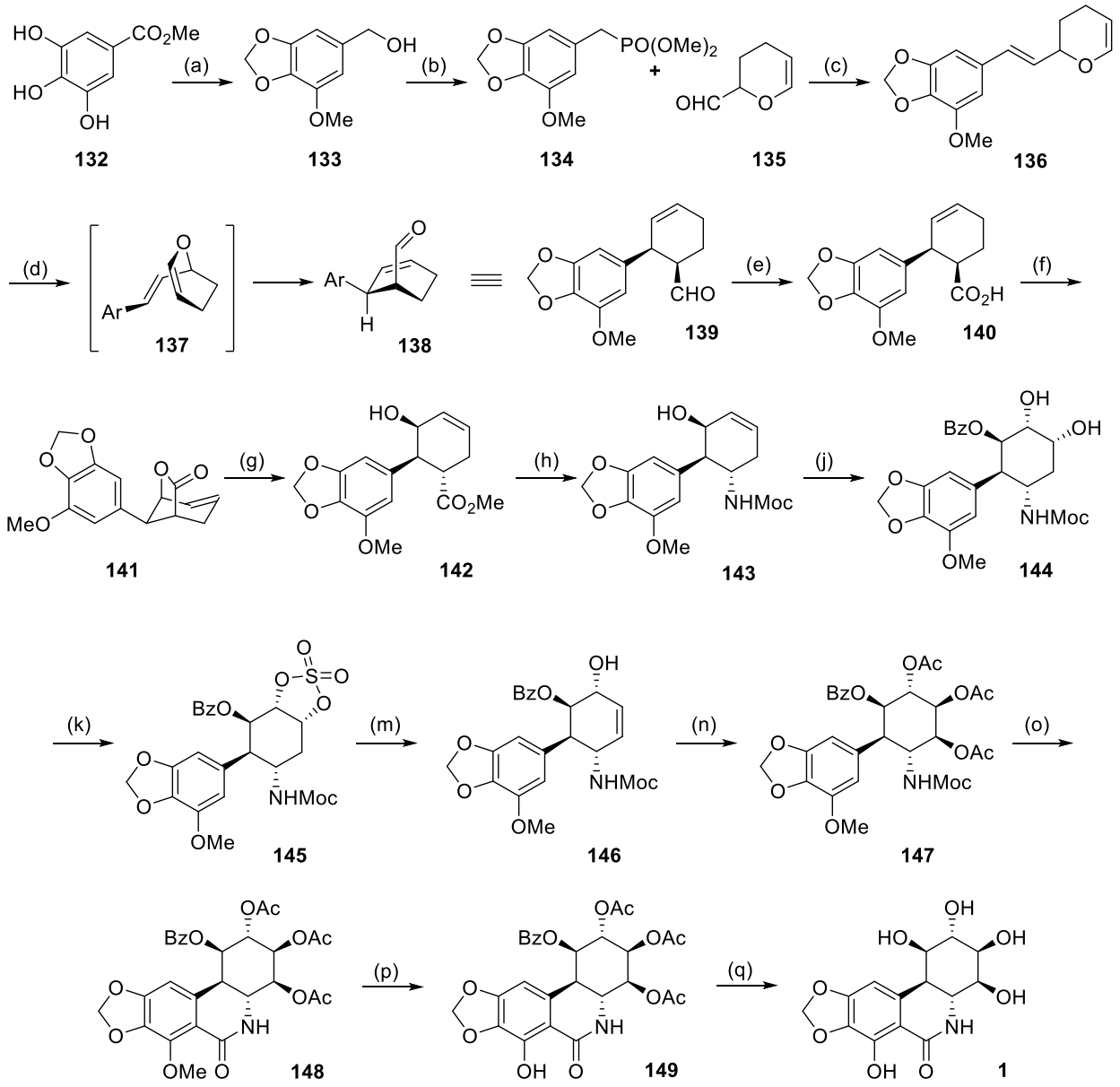


Reagents and Conditions: (a) DMP, *p*-TsOH, DMF; (b) Ac_2O, py.; (c) *m*-CPBA, phosphate buffer; (d) (i) H_2, 10% Pd/C; (ii) K_2CO_3, aq. CH_3OH; (e) $SOCl_2$, NEt_3; (f) $RuCl_3$, $3H_2O/NaIO_4$, CH_3CN, CCl_4, H_2O; (g) (i) BzOH, Cs_2CO_3; (ii) THF/H_2O, cat. H_2SO_4; (h) K_2CO_3, CH_3OH.

Scheme 20. Pettit's conversion of (+)-narciclasine to (+)-pancratistatin.[34]

A year later, in 2002, Kim reported an enantioselective synthesis of (+)-pancratistatin **(Scheme 21)** in which a Claisen rearrangement was employed to install the necessary C-10b stereocenter of pancratistatin **(1)**.[35] As epoxidation of olefin **143** was unsuccessful, a sequence of osmylation, formation of a cyclic sulfate, and dehydration were used to install the C-2 hydroxyl

group.[35] A Bischler-Napieralski cyclization and global deprotection were employed under Magnus' conditions[32] to complete the synthesis of pancratistatin **(1)** in 16 steps (3.6% yield).[35]

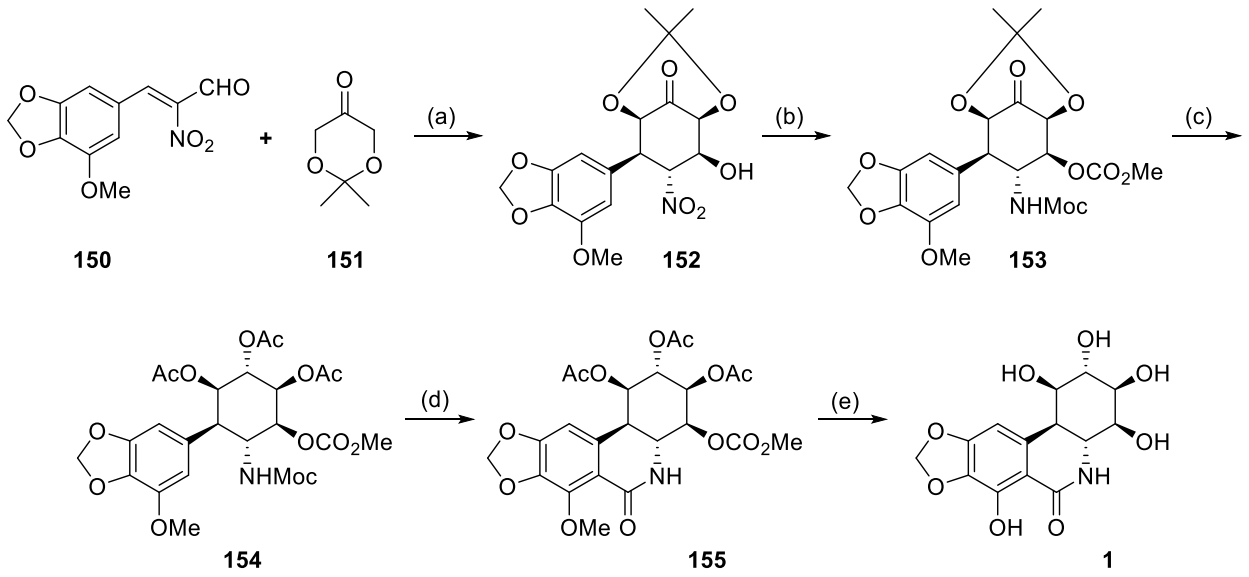


Reagents and Conditions: (a) (i) Borax, Me_2SO_4; (ii) CsF, CH_2Br_2; (iii) LAH; (b) PBr_3, $P(OMe)_3$, toluene, sealed tube, 180 °C, 2 h; (c) **135**, LHMDS, THF, 0 °C to rt, 22 h; (d) toluene, sealed tube, 250 °C, 20 h; (e) $NaClO_2$, $NaH_2PO_4 \cdot 2H_2O$, 2-methyl-2-butene, THF, *t*-BuOH, H_2O; (f) (i) KI_3, aq. $NaHCO_3$, DCM; (ii) DBU, PhH, reflux; (g) NaOMe, MeOH, reflux; (h) (i) 1N LiOH, THF; (ii) DPPA, NEt_3, toluene, reflux; (iii) NaOMe, MeOH, reflux; (j) (i) BzCl, NEt_3, DMAP, DCM; (ii) OsO4, NMO, THF/H2O; (k) (i) $SOCl_2$, NEt_3, DCM, 0 °C; (ii) oxone, $RuCl_3 \cdot 3H_2O$,

EtOAc/CH_3CN, H_2O; (m) DBU, toluene, reflux; then H_2SO_4, H_2O/THF; (n) (i) OsO_4, NMO, THF/H_2O; (ii) Ac_2O, DMAP, py., DCM; (o) Tf_2O, DMAP, DCM; (p) BBr_3, DCM, -78 °C; (q) NaOMe, MeOH, THF.

Scheme 21. Kim's enantioselective synthesis of pancratistatin.[35]

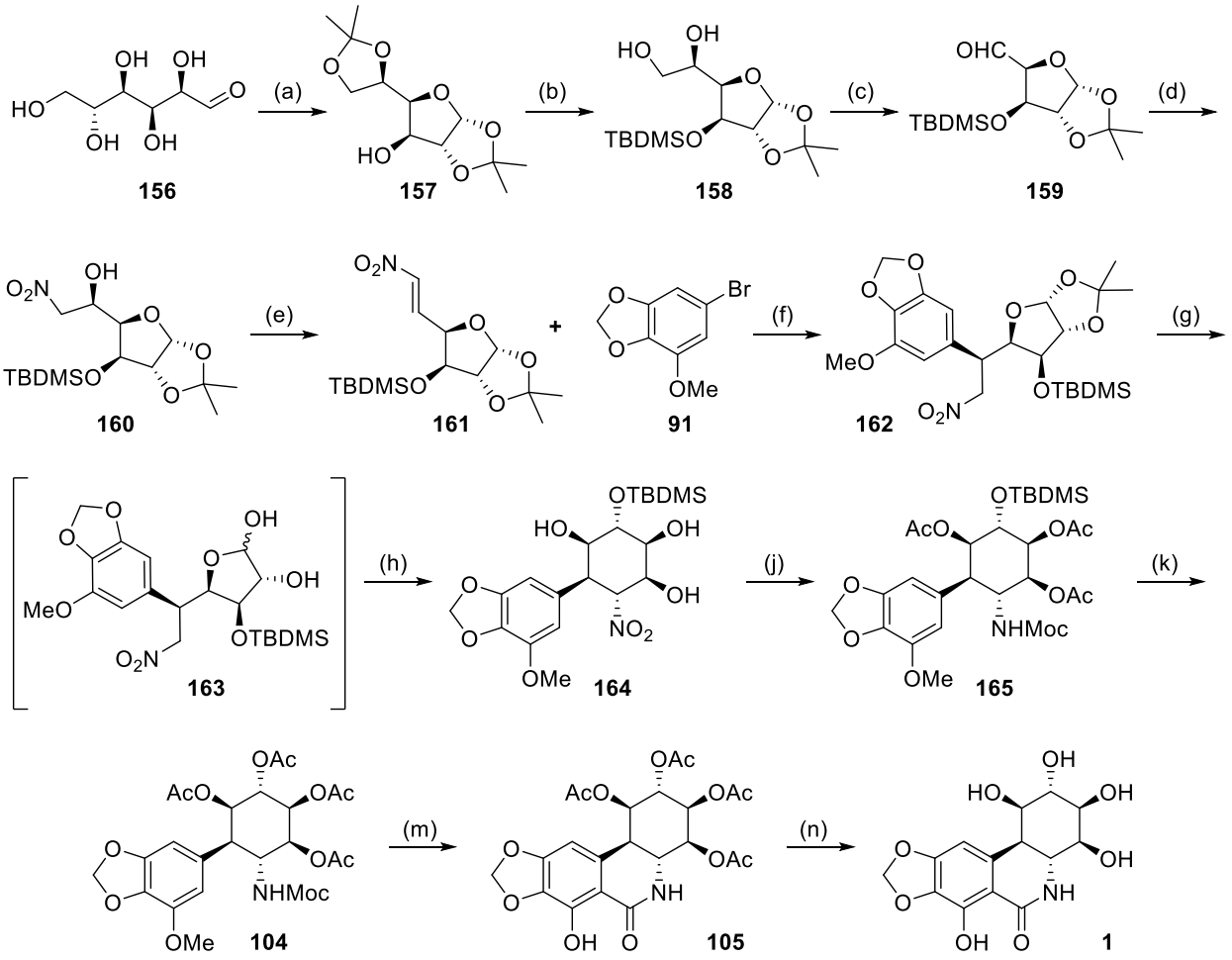
In 2012, Alonso presented a notable synthesis of (+)-pancratistatin **(1)**, employing a Robinson annulation that furnished the five contiguous stereocenters of the C-ring of the title compound **(Scheme 22)**.[36] The aromatic component **150** was synthesized from vanillin by a known procedure.[47] Stereoselective reduction of ketone **153** provided the C-2 stereocenter (pancratistatin numbering).[36] Finally, a Bischler-Napieralski cyclization and global deprotection provided (+)-pancratistatin **(1)** in 14 steps.[36]



Reagents and conditions: (a) **151** + (*R*)-2-(methoxymethyl)-pyrrolidine, anh. Na_2SO_4; then **150**, PPTS; (b) (i) NH_4CO_2H, 10% Pd/C, MeOH; (ii) $ClCO_2Me$, DMAP, DCM; (c) (i) Dowex 50WX, MeOH; (ii) $Na(AcO)_3BH$, DCE/THF; (iii) Ac_2O, DMAP, NEt_3, DCM; (d) (i) Tf_2O, DMAP, DCM, 0 °C; (ii) HCl, 1,4-dioxane, rt; (e) (i) BBr_3, DCM, -78 °C to rt; (ii) NaOMe, MeOH/THF.

Scheme 22. Alonso's synthesis of (+)-pancratistatin.[36]

In his total formal synthesis of (+)-pancratistatin **(Scheme 23)**, Sato employed a chiral pool strategy with *D*-glucose **(156)** as the starting material.[37] The coupling of the A-ring (derived from *o*-vanillin from three steps according to Magnus' protocol)[32] and the C-ring adduct was achieved by Michael addition.[37] An intramolecular Henry reaction provided adduct **163**, which was elaborated to Magnus' tetraacetate intermediate **104** through a sequence of steps.[37] Finally, ring B cyclization and global deprotection under Magnus' conditions[32] provided (+)-pancratistatin in 18 steps (8.5% yield).[37]

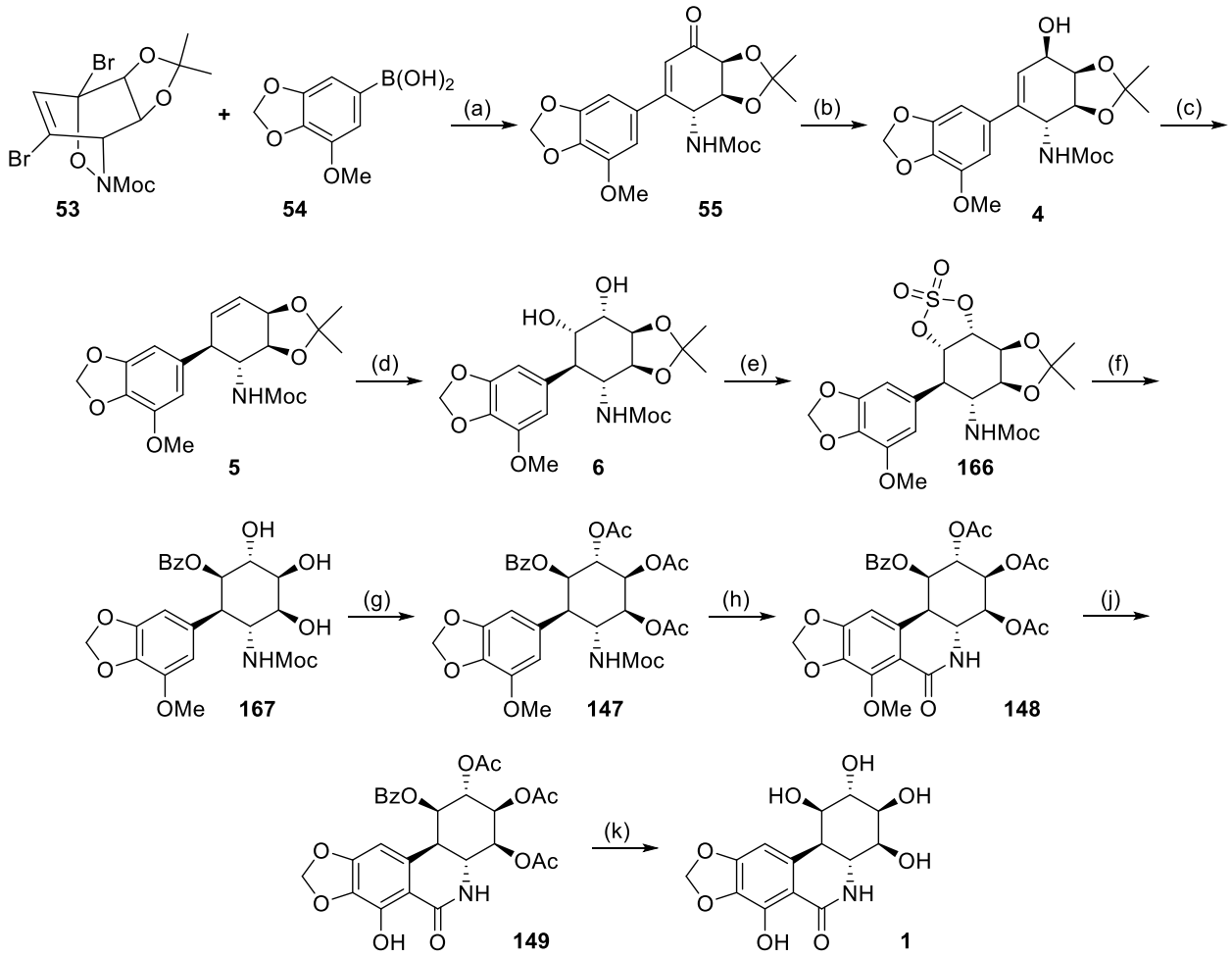


Reagents and conditions: (a) anh. acetone, $Me_2S^+BrBr^-$; (b) (i) TBDMSCl, imidazole, DMF; (ii) 70% aq. AcOH; (c) $NaIO_4$, MeOH/H_2O; (d) $MeNO_2$, NaOMe, MeOH; (e) MsCl, NEt_3, DCM; (f) **66**, Mg, CuI, TMSCl, THF; (g) 70% aq. AcOH; (h) $NaHCO_3$, MeOH/H_2O; (j) (i) 6M aq. HCl, Zn, EtOH; (ii) $ClCO_2Me$, 3% aq. NaOH, THF; (iii) Ac_2O, DMAP, py.; (k) TBAF, THF; then Ac_2O, DMAP, py.; (m) (i) Tf_2O, DMAP, DCM; (ii) BBr_3; (n) NaOMe/MeOH.

Scheme 23. Sato's total formal synthesis of (+)-pancratistatin.[37]

In 2017, Hudlicky published a total formal synthesis of (+)-pancratistatin,[4] which is most related to the contents of this thesis. Allylic alcohol **4** was accessed by the route employed in Hudlicky's 1999 synthesis of (+)-narciclasine **(Scheme 24)**.[23] The key step was the Myers transposition: a conversion of allylic alcohols into their corresponding olefins via a stereospecific 1,3-transposition.[4,48] The original paper by Myers utilized triphenyl phosphine, diethyl azodicarboxylate, and 2-nitrobenzenesulfonyl hydrazide (NBSH) to effect the transformation.[48] Low temperatures and careful handling of the reagents were necessary to provide a clean reaction with fewer byproducts (70-75% isolated yield).[48] To bypass these problems, Movassaghi utilized *N*-isopropylidene-*N'*-2-nitrobenzenesulfonyl hydrazine (IPNBSH), which provided flexibility in solvent choice, reaction temperature, concentration of substrate and reagents, and order of addition.[49] When Myers' original conditions were applied to allylic alcohol **4**, only traces of the product were found.[4] Altering the conditions and using IPNBSH effectively allowed transformation of allylic alcohol **4** into olefin **5**, with simultaneous installation of the necessary C-10b stereocenter.[4]

The cyclic sulfate route employed by Pettit **(Scheme 20)** was utilized,[34] with the substitution of 1,1'-sulfonyldiimidazole (SDI) for thionyl chloride.[4] This allowed access to cyclic sulfate **166** in one step from *cis*-diol **6**, as compared to the two steps required in Pettit's synthesis. Finally, the Bischler-Napieralski cyclization and global deprotection strategies employed by Magnus[32] and Kim[35] were employed, affording (+)-pancratistatin in 14 steps.[4]

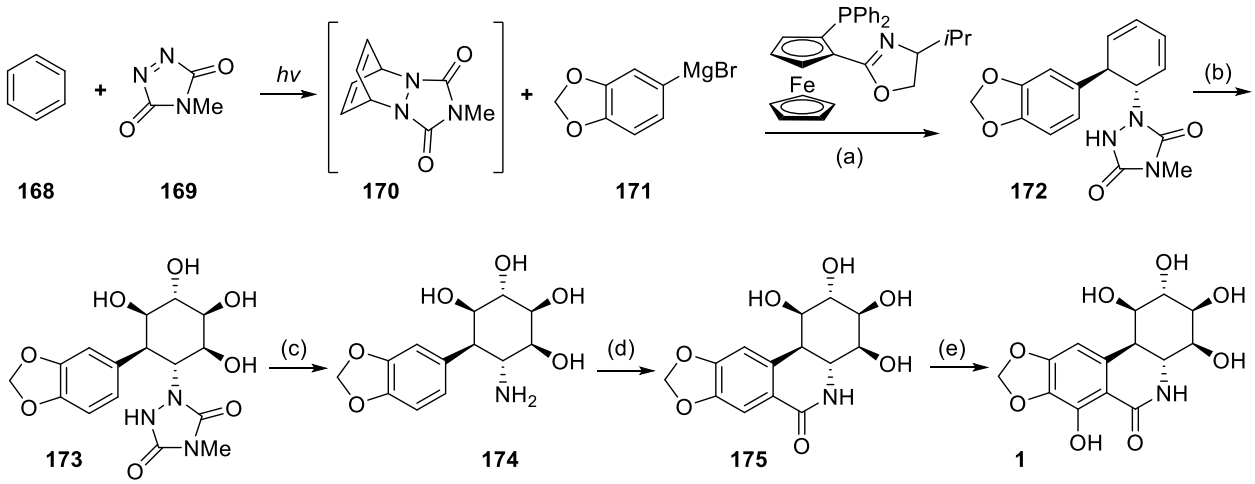


Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, PhH, H_2O, EtOH, then $Mo(CO)_6$, CH_3CN, H_2O; (b) $CeCl_3 \cdot 7H2O$, $NaBH_4$, MeOH; (c) DBAD, IPNBSH, PPh_3, THF; then CF_3CH_2OH, H_2O; (d) OsO_4, NMO, acetone, H_2O; (e) NaH, SDI, THF; (f) $PhCO_2NH_4$, DMF; then H_2SO_4, H_2O, THF; (g) Ac_2O, DMAP, NEt_3, DCM; (h) Tf_2O, DMAP, DCM; (j) BBr_3, DCM, -78 °C; (k) NaOMe, MeOH, THF.

Scheme 24. Hudlicky's total formal synthesis of (+)-pancratistatin.[4]

The shortest synthesis of pancratistatin **(1)** by far is that achieved by Sarlah in 2017 **(Scheme 25)**.[38] The photocatalyzed cycloaddition of benzene **(168)** and the arenophile MTAD **(169)** produced cycloadduct **170**, which was an active substrate in a catalytic cycle.[38] Oxidative addition of the transition metal catalyst to the cycloadduct **170**, followed by transmetalation with aryl

magnesium bromide **171**, reductive elimination, and finally ligand decomplexation to regenerate the catalyst and provide diene **172**.[38] This dearomative *trans*-carboamination procedure allowed Sarlah to prepare upwards of 300 g of diene **172** with the necessary C-10b and C-4a stereocenters correctly installed.[38] Functionalization of the olefins proved to be simplistic, thereby furnishing all of the necessary stereocenters of the C-ring.[38] B-ring closure was achieved via bromination followed by catalytic carbonylation under a carbon monoxide atmosphere, furnishing 7-deoxypancratistatin **(175)**.[38] Finally, Sarlah achieved the first late-stage conversion of 7-deoxypancratistatin **(175)** to pancratistatin **(1)** using a cupration/oxidation sequence.[38] The syntheses of 7-deoxypancratistatin **(175)** and pancratistatin **(1)** were achieved in six and seven steps with overall yields of 19% and 12%, respectively.[38]



Reagents and Conditions: (a) $[Ni(cod)_2]$, Me_2SO_4; (b) (i) *m*-CPBA, TsOH, H_2O; (ii) OsO_4, NMO; (c) (i) $LiAlH_4$; (ii) H_2, Raney-Co; (d) (i) Br_2, AcOH; (ii) $NaCo(CO)_4$, CO, UV light (365 nm); (e) (i) HMDS; (ii) $(TMP)_2Cu(CN)Li_2$, *t*-BuOOH.

Scheme 25. Sarlah's dearomative *trans*-carboamination synthesis of 7-deoxypancratistatin and pancratistatin.[38]

Selected syntheses of natural pancratistatin **(1)** have thus far been discussed. The next chapter will discuss syntheses of unnatural derivatives of pancratistatin **(1)**.

2.3.3. Unnatural Derivatives of Pancratistatin

Pettit's conversion of natural narciclasine **(59)** to pancratistatin **(1)** in 2001 **(Scheme 20)** provided several isocarbostyril intermediates that were tested against human cancer and murine lymphocytic leukemia cell lines.[34] All intermediates showed a decrease in cancer cell growth inhibition compared to pancratistatin, except the C-1 benzoate **131**, which showed enhanced activity.[34] This result pioneered research into developing C-1 derivatives of pancratistatin. In the same year, Pettit synthesized the phosphate prodrug **177**, which exhibited higher aqueous solubility (>230 μg/mL)[34] compared to natural pancratistatin **(1)** (53 μg/mL).[34] This phosphate prodrug **177** also exhibited comparable activity to its parent compound.

In 1994, Pettit accomplished a diastereoselective synthesis of the C-10b-hydroxy epimer of pancratistatin **(176)** from natural narciclasine.[50] Biological testing of this product against cancer cell lines showed its potency was reduced 1000 times compared to the title compound.[50] Moreover, the naturally occurring 7-deoxypancratistatin **(175)**, isolated in 1989 by Ghosal,[51] showed a 10-fold reduction in potency compared to pancratistatin **(1)**.[27]

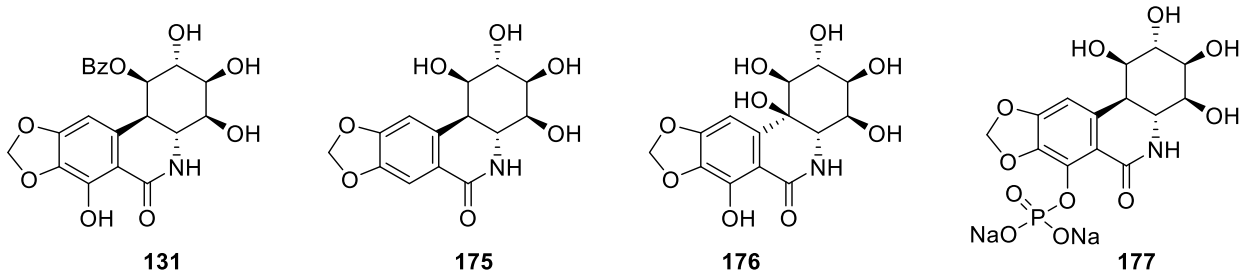


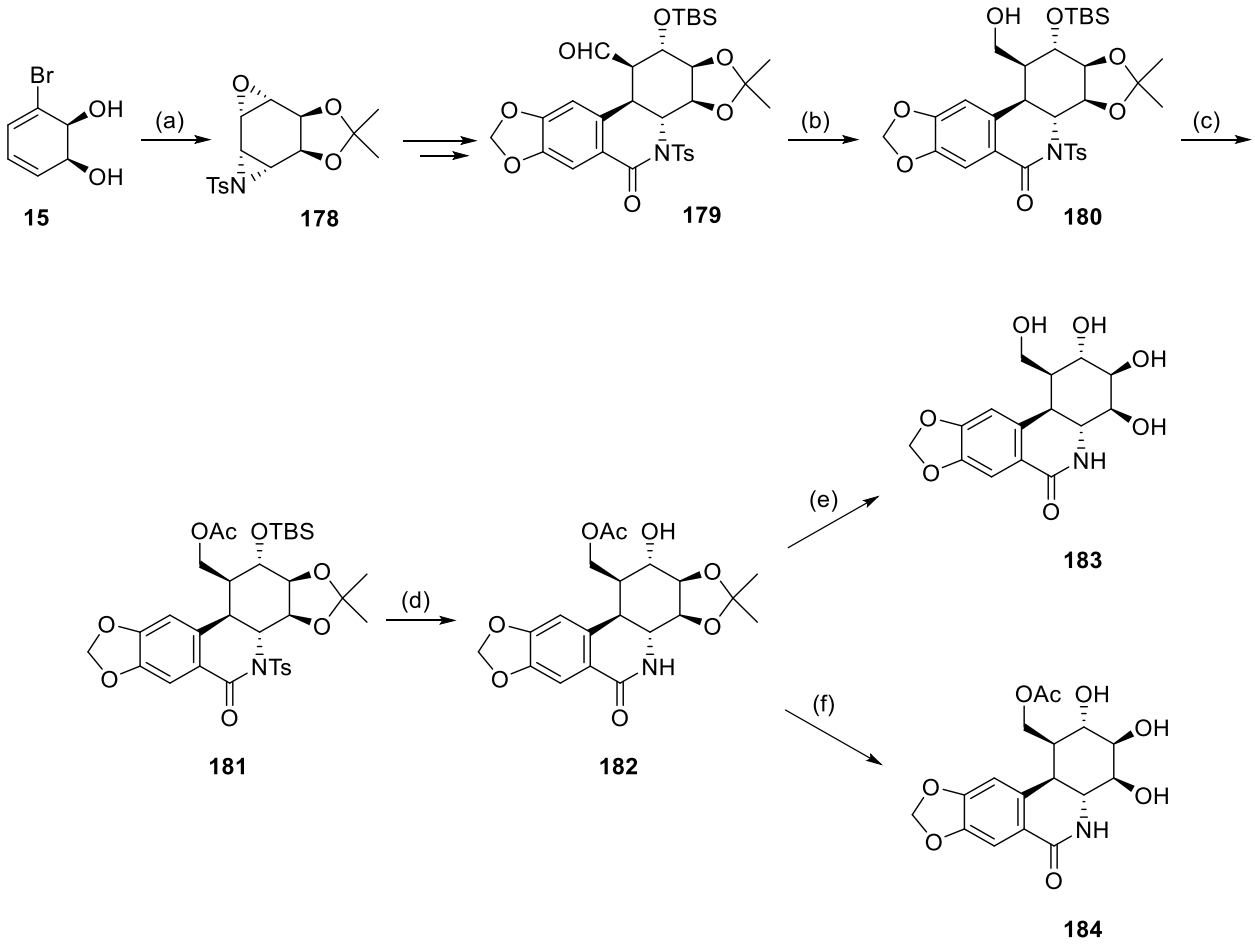
Figure 2. Natural and unnatural derivatives of pancratistatin.

A set of suggestions for future modifications of isocarbostyril **1** emerged with the preparation of such unnatural derivatives.[27] The essential components of the pharmacophore are listed as follows:

1) A phenanthridone skeleton with a *trans* relationship at the B/C ring junction.[27]
2) All C-ring hydroxyls, unprotected, and with their natural stereocenters.[27]
3) The C-7 hydroxyl and unsubstituted N-5 of the B ring are essential.[27]
4) The methylenedioxy functionality may be essential; further studies are required to determine this.[27]

Substitutions at C-7, C-10, and C-1 have resulted in a number of derivatives, the syntheses and biological activities of which have been extensively reviewed.[27,52] Most importantly, it was recognized that derivatives with C-1 substitutions may have equivalent, or higher, bioactivity compared to the parent compound.[27] Moreover, lipophilic substitutions at C-1 were shown not to impede biological activity, and in some cases, have shown to increase activity.[27] Selected syntheses of such C-1 derivatives and homologues will now be discussed.

In 2008, Hudlicky developed a chemoenzymatic approach to the C-1 carboxaldehyde of 7-deoxypancratistatin **(179)**.[53] This intermediate was then employed in 2010 by Hudlicky for the preparation of the C-1 homologues **183** and **184** of 7-deoxypancratistatin **(Scheme 26)**.[54]



Reagents and conditions: (a) (i) DMP, *p*-TsOH, acetone, rt; (ii) PhINTs, $Cu(acac)_2$, CH_3CN, 0 °C to rt; (iii) *n*-Bu_3SnH, THF, AIBN, reflux; (iv) *m*-CPBA, DCE, reflux; (b) $NaBH_4$,

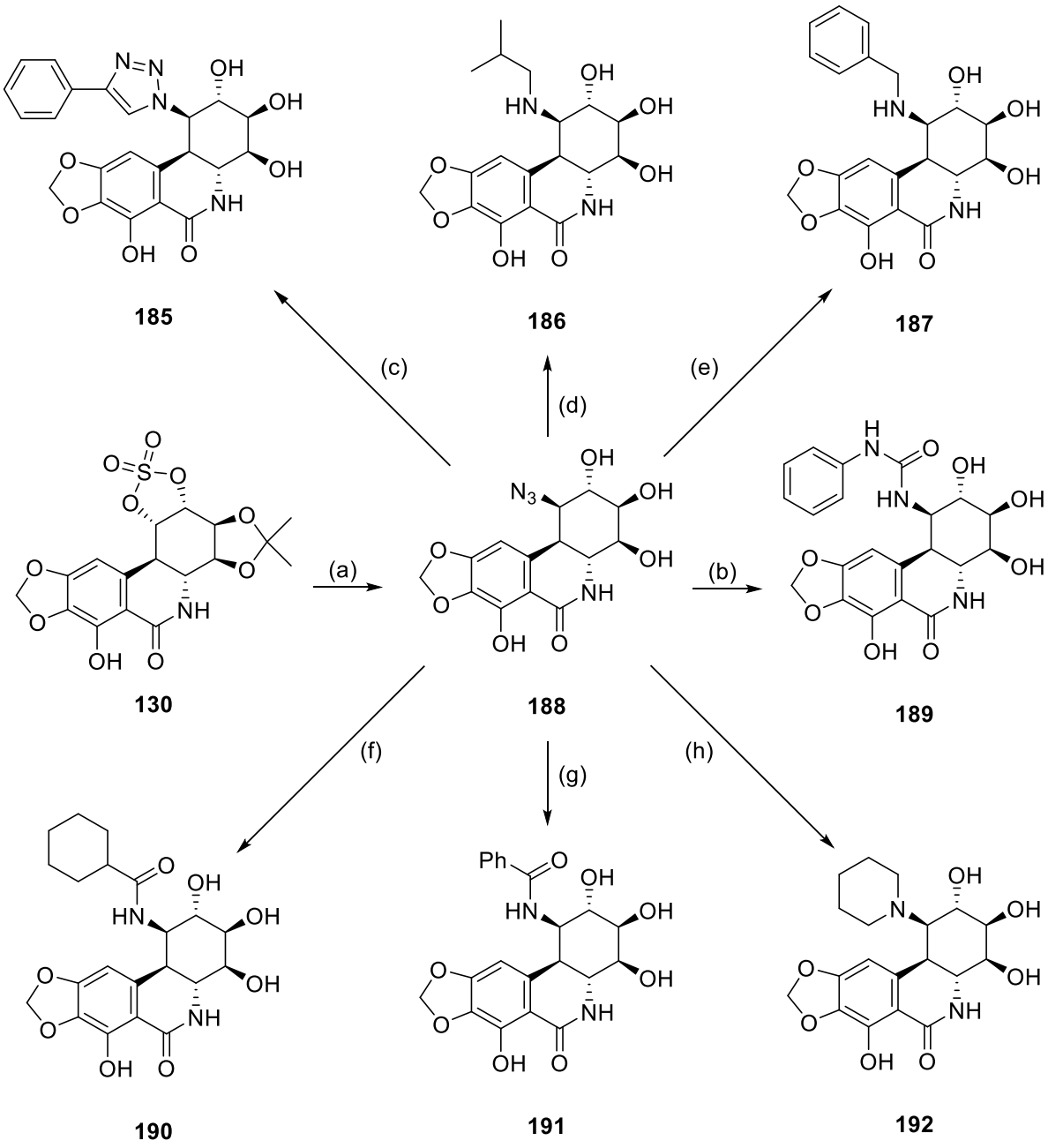
dioxane/EtOH, 0 °C; (c) Ac_2O, py, DMAP, DCM; (d) (i) Na/naphthalene, DME; (ii) TBAF, THF, 0 °C; (e) (i) K_2CO_3, MeOH, H_2O; (ii) HCl, H_2O; (f) 3% HCl, MeOH.

Scheme 26. Chemoenzymatic approach to C-1 derivatives of 7-deoxypancratistatin.[53,54]

The chemoenzymatic approach utilized the microbial oxidation of bromobenzene to produce bromodihydrodiol **17**.[53] A series of functionalizations including intramolecular aziridine opening produced C-1-carboxaldehyde **179**.[53] Reduction, acetylation and deprotection provided C-1-acetoxymethyl-7-deoxypancratistatin **(184)**.[54] Removal of the acetate group followed by full deprotection instead provided C-1-hydroxymethyl-7-deoxypancratistatin **(183)**.[54] Although C-1 homologues **183** and **184** exhibited similar but slightly higher activity against lung cancer cell lines compared to 7-deoxypancratistatin **(175)**, they were still approximately 10-fold less potent than pancratistatin **(1)**.[54]

After Pettit's preparation of C-1 benzoate **131**, success with C-1 substitutions was next recognized in 2009 with Marion's preparation of C-1 aza-derivatives.[55] Both an increase in solubility and biological activity were achieved with the preparation of these derivatives.[27]

From azide **188**, Marion used a divergent synthesis to prepare 35 derivatives, some of which are represented in **Scheme 27**.[27] Most of the derivatives were prepared through reduction of the azide, followed by acylation or reductive amination with an aldehyde.[27] Triazole **185**, however, was prepared from the cycloaddition of azide **188** with phenylacetylene.[27] Benzamide **191** showed the most potent activity against a range of cancer cell lines, and was five times as potent as narciclasine **(59)**.[27]

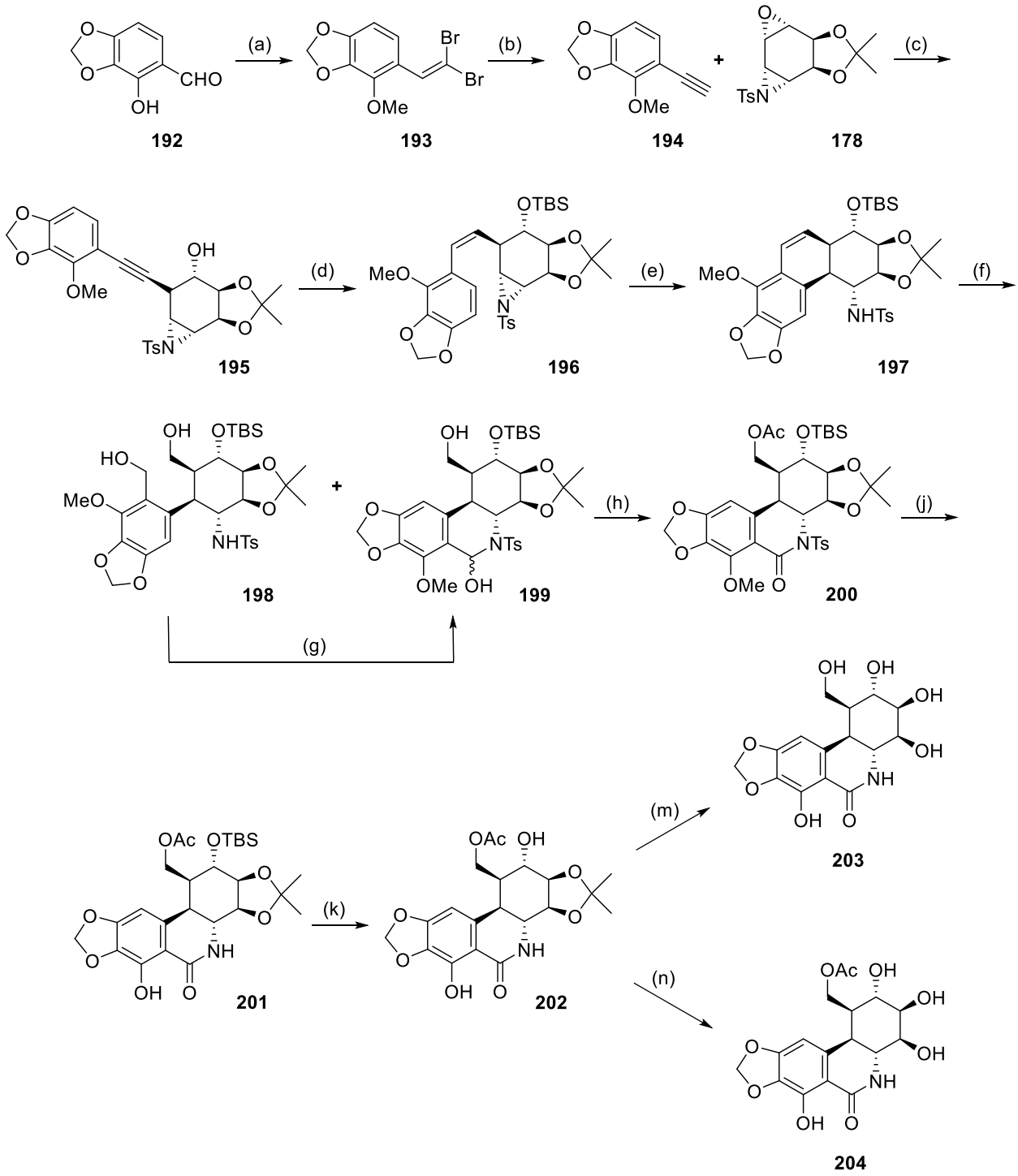


Reagents and conditions: (a) NaN_3, DMF, 80 °C; (b) 10% Pd/C, H_2; then phenyl isocyanate, NEt_3; (c) phenylacetylene, 100 °C; (d) 10% Pd/C, H_2; then isobutyraldehyde, $NaBH_3CN$; (e) 10% Pd/C, H_2; then benzaldehyde, $NaBH_3CN$; (f) 10% Pd/C, H_2; then cyclohezanoic acid chloride, NEt_3; (g) 10% Pd/C, H_2; then benzoyl chloride, NEt_3; (h) 10% Pd/C, H_2; then glutaraldehyde, $NaBH_3CN$.

Scheme 27. Marion's preparation of C-1 aza-derivatives of pancratistatin.[55]

Recognizing the success with C-1 homologues **183** and **184** of 7-deoxypancratistatin, and the higher potency of pancratistatin **(1)** compared to its 7-deoxy counterpart **(175)**, Hudlicky then ventured towards the preparation of C-1 homologues of pancratistatin **(1)**. In 2011, Hudlicky prepared the C-1 hydroxymethyl **(171)** and C-1 acetoxymethyl **(172)** analogues of pancratistatin **(Scheme 28)**.[56]

The chemoenzymatic approach utilized was like that of the preparation of C-1 homologues of 7-deoxypancratistatin.[53] Key steps of the synthesis include the Corey-Fuchs homologation of aldehyde **192** to acetylene **194**, ozonolysis of olefin **197**, and reductive detosylation to remove the N-5 protecting group.[56] The C-1 homologues **203** and **204** exhibited potency 10-fold higher than that of their 7-deoxy analogues against prostate cancer cells.[56] A similar relationship is evident between pancratistatin **(1)** and its 7-deoxy counterpart **(175)**.[52]

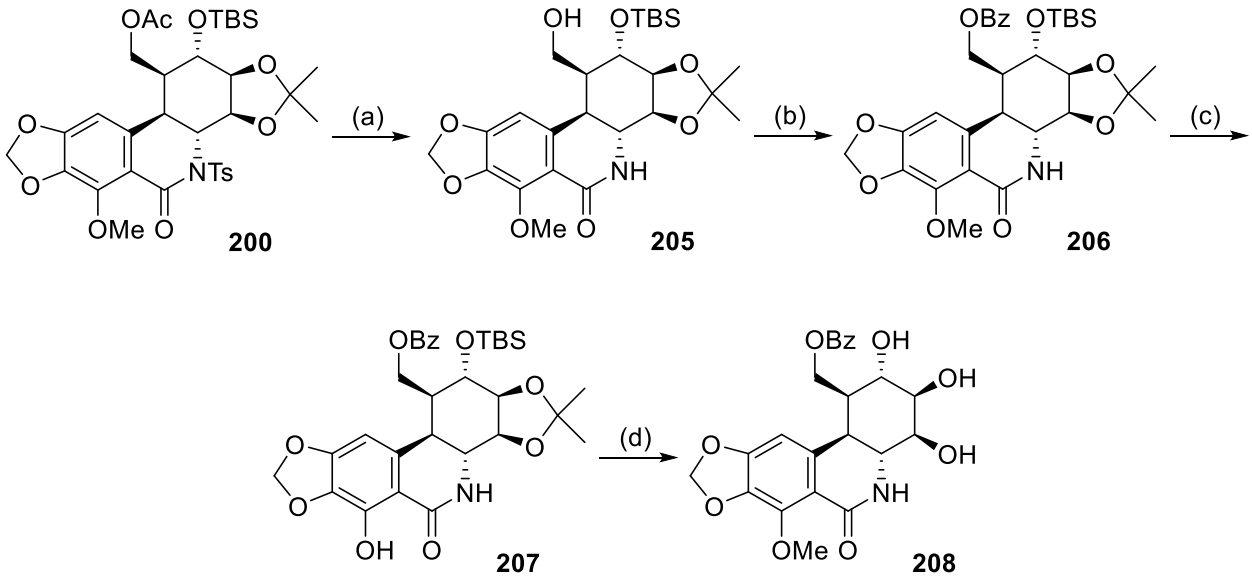


Reagents and conditions: (a) Me_2SO_4, K_2CO_3, acetone, reflux; (b) (i) CBr_4, PPh_3, DCM; (ii) *n*-BuLi, THF, -78 °C; (c) (i) *n*-BuLi, toluene, -50 °C; (ii) Me_2AlCl, -50 °C; (iii) **178**, -25 °C; (d) (i) H_2/Lindlar cat., MeOH; (ii) TBSOTf, NEt_3, DCM, 0 °C; (e) SiO_2, quinolone, 120 °C; (f) (i) O_3/MeOH, Sudan Red 7b, -80 °C; (ii) $NaBH_4$, -80 °C to rt; (g) MnO_2, DCM; (h) (i) Ac_2O, py,

DCM; (ii) TPAP, NMO, DCM; (j) (i) Na, naphthalene, DME, -50 °C; (ii) LiCl, DMF, 120 °C; (k) TBAF, THF, 0 °C; (m) 2% HCl, DCM, MeOH; (n) TFA, DCM, MeOH.

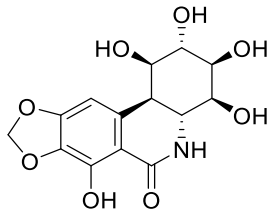
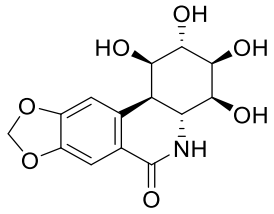
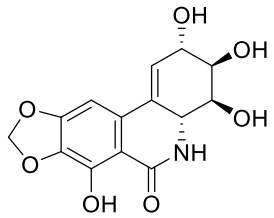
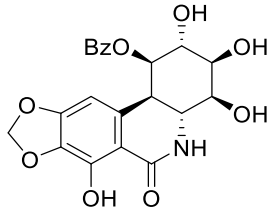
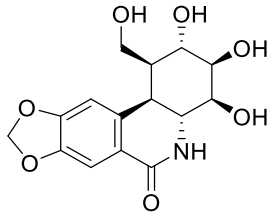
Scheme 28. Hudlicky's chemoenzymatic synthesis of C-1 homologues of pancratistatin.[56]

A year later, Hudlicky prepared the C-1 benzoxymethyl analogue of pancratistatin **(208)** using the advanced C-1 acetoxymethyl intermediate **200** prepared in the prior synthesis **(Scheme 29)**.[57] Deacetylation followed by benzoylation and full deprotection provided the C-1 benzoxymethyl analogue of pancratistatin **(208)**.[56]



Reagents and conditions: (a) (i) Na, naphthalene, DME, -50 °C; (ii) aq. NaOH, MeOH; (b) BzCl, NEt_3, DMAP, DCM; (c) LiCl, DMF, 120 °C; (d) TBAF, THF, 0 °C.

Scheme 29. Hudlicky's synthesis of the C-1 benzyloxymethyl analogue of pancratistatin.[56,57]

Natural Product or Derivative	Inhibitory Activity
 **1**	IC_{50} = 0.12 μM (Colon HCT-116)[34]
 **175**	GI_{50} = 0.29 μg/mL (Lung NCI-H460)[58]
 **59**	IC_{50} = 0.05 μM (Lung NCI-H460)[57]
 **131**	GI_{50} = <0.001 μg/mL (Lung NCI-H460)[34]
 **183**	IC_{50} = 0.40 μM (Lung NCI-H460)[57]

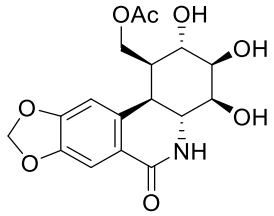
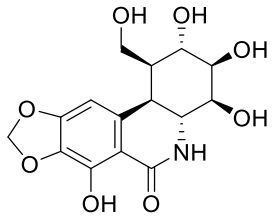
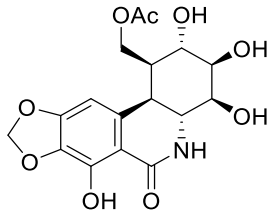
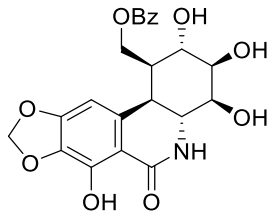
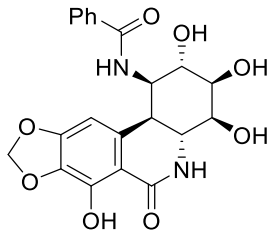
Natural Product or Derivative	Inhibitory Activity
 **184**	IC_{50} = 0.53 μM (Lung NCI-H460)[57]
 **203**	IC_{50} = 0.081 μM (Colon HCT-116)[57]
 **204**	IC_{50} = 0.095 μM (Colon HCT-116)[57]
 **208**	IC_{50} = 0.033 μM (Colon HCT-116)[57]
 **191**	IC_{50} = 0.0047 μM (Colon HCT-116)[57]

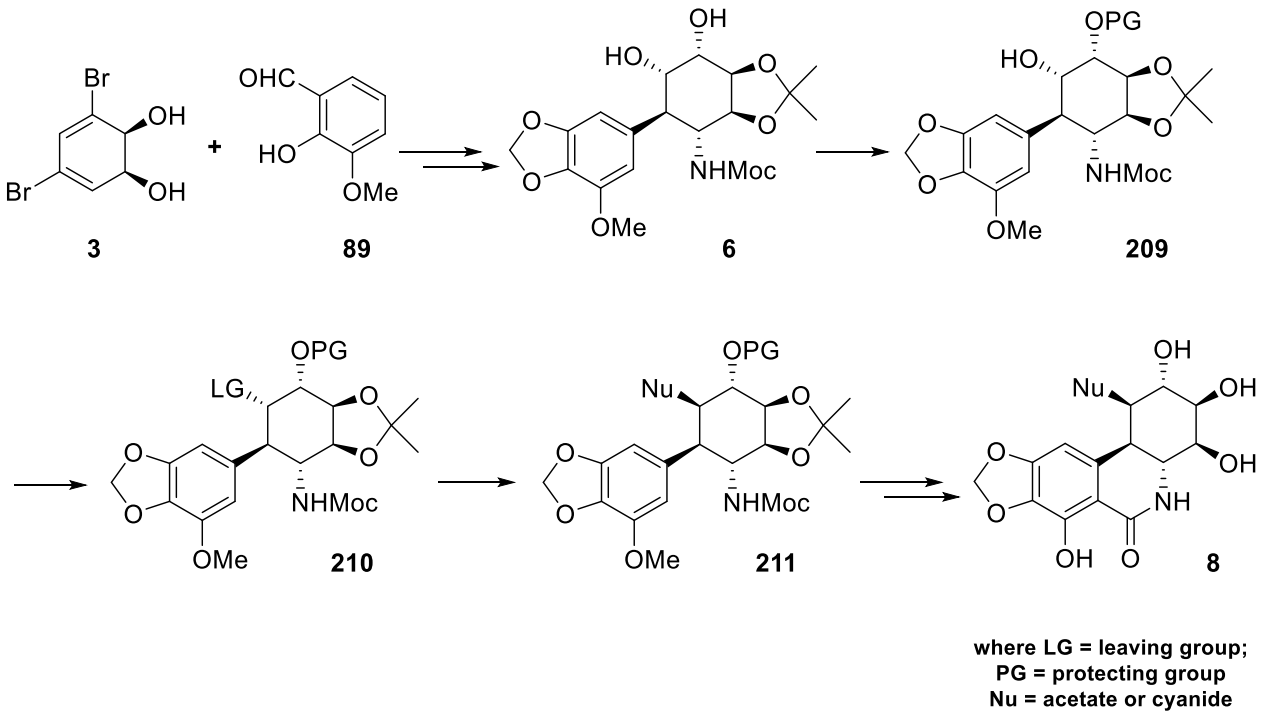
Table 2. Inhibitory activities of natural products and their corresponding derivatives against cancer cell lines.[34,55,57,58]

Table 2 summarizes the inhibitory activities of the discussed compounds and the corresponding natural products against cancer cell lines.[34,55,57,58] From this summary, it is evident that functionalization at C-1 of pancratistatin, especially with large lipophilic groups, has a significant positive effect on the natural product's biological activity. It is presumed that a hydrophobic pocket on the target protein interacts with the lipophilic substitution on the C-1 position, leading to a positive impact on the biological activity. This leads to the discussion of the current research, presented in the following section.

3. DISCUSSION

3.1. Introduction

As discussed in the Historical, the bioactivity of the parent compound, pancratistatin **(1)**, may be improved when the C-1 position is substituted with large lipophilic groups. To target C-1 derivatives of pancratistatin, the late-stage *cis*-diol **6** was prepared in seven steps by repeating the procedures employed in Hudlicky's total formal synthesis of pancratistatin.[4] From late-stage *cis*-diol **6**, the synthetic approach **(Scheme 30)** involved the selective protection of the C-2 position, activation of the C-1 position using a leaving group (mesylate or tosylate), nucleophilic displacement of the leaving group, and finally, cyclization of ring B and removal of all protecting groups to provide C-1 derivatives of pancratistatin **(1)**.

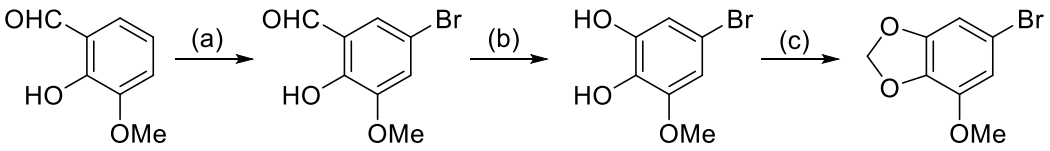


Scheme 30. A synthetic approach towards C-1 derivatives of pancratistatin.

3.2. Synthesis of the late-stage *cis*-diol

3.2.1. Synthesis of the A-ring

The A-ring of pancratistatin was synthesized in 3 steps from commercially available *o*-vanillin **(89)** using a previously established procedure **(Scheme 31)**.[4] First, *o*-vanillin **(89)** was subjected to bromination under acidic conditions. The resulting aryl bromide **90** was then subjected to the Baeyer-Villiger reaction, producing an intermediate formyl ester that was subsequently hydrolyzed. Finally, the methylene dioxy bridge was installed onto catechol **212** to complete the synthesis of the A-ring.



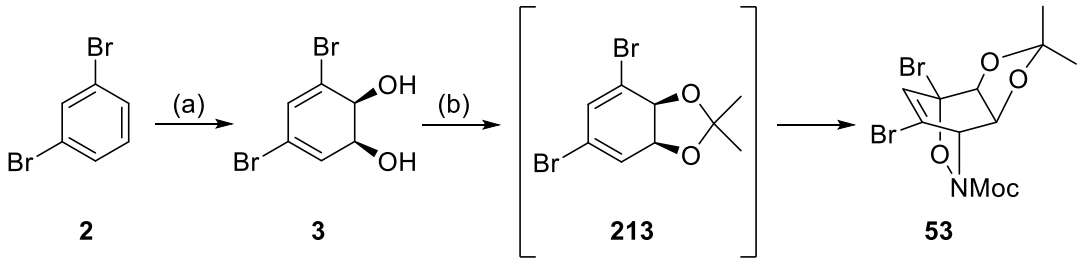
89 **90** **212** **91**

Reagents and Conditions: (a) Br_2, AcOH, NaOAc **(62%)**; (b) (i) 2% NaOH, H_2O_2 in H_2O; (ii) 3M HCl **(76%)**; (c) CH_2I_2, K_2CO_3, DMF, 100 °C, 2h **(66%)**.

Scheme 31. Synthesis of the A-ring of pancratistatin.

3.2.2. Chemoenzymatic preparation of oxazine

The C-ring of pancratistatin was established through the chemoenzymatic oxidation of 1,3-dibromobenzene **(2)** using previously established procedures **(Scheme 32)**.[4] Dihydrodiol **3** was provided through microbial fermentation operated by Mary Ann Endoma-Arias and Helen De La Paz initially; followed by repetition by Juana Goulart Stollmaier and Korey Bedard in later stages. Oxazine **53** was prepared in one-pot conditions from 1,3-dibromodihydrodiol **(3)** to counteract the instability of the intermediate compounds. Dihydrodiol **3** is unstable in air because of its propensity to undergo dehydrative aromatization, and acetonide **213** undergoes dimerization via the Diels-Alder reaction if left open to air. However, these disadvantages can be prevented by a one-pot conversion of dihydrodiol **3** to oxazine **53** in two steps.

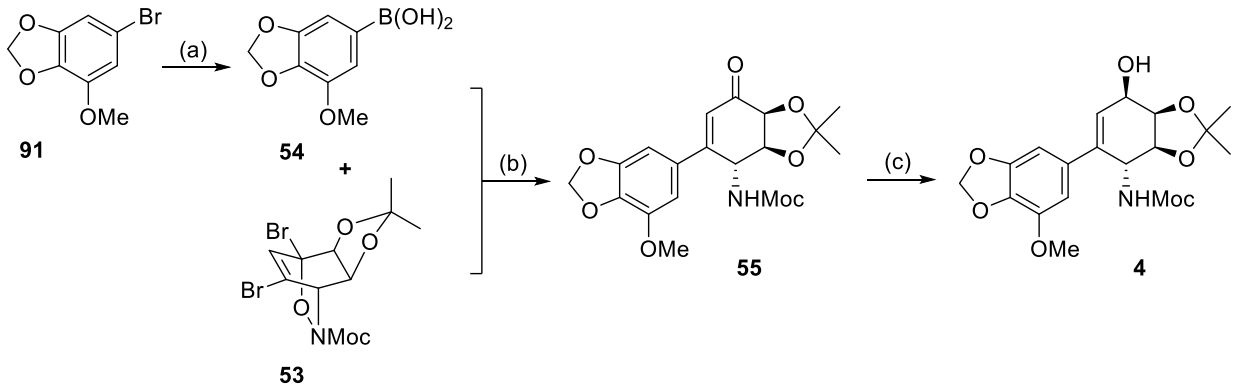


Reagents and Conditions: (a) *E. coli* JM109 (pDTG601); (b) 2,2-DMP, acetone, *p*-TsOH, rt; then $NaIO_4$, MocNHOH, rt **(50% over two steps)**

Scheme 32. Chemoenzymatic preparation of oxazine **53**.

3.2.3. Synthesis of key intermediate allylic alcohol

The C-10b/C-10a tether was installed using a previously established Suzuki coupling reaction.[4] A-ring bromide **91** was subjected to lithium-halogen exchange, followed by quenching with trimethyl borate and hydrolysis to result in boronic acid **54**. Using one-pot conditions, the Suzuki coupling followed by N-O bond reduction with molybdenum hexacarbonyl furnished enone **55** in 2 steps. Initially, crude boronic acid **54** was used immediately in the Suzuki coupling with oxazine **53** without isolation or purification. Further on into the project, a sample of boronic acid **54** prepared previously by Dr. Mukund Ghavre in 2017 was used instead, but this procedure often decreased the yield of enone **55** by 50%. This proved that crude boronic acid had to be used in the Suzuki coupling to achieve good yields of enone **55**. Finally, enone **55** was subjected to the Luche reduction to provide allylic alcohol **4**, the substrate for the key Myers transposition reaction **(Scheme 33)**.

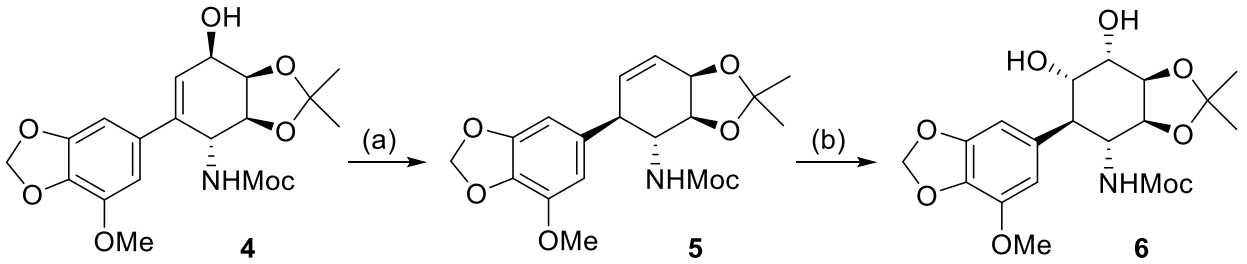


Reagents and Conditions: (a) (i) *t*-BuLi, THF, -78 °C; (ii) $B(OMe)_3$, -78 °C to 0 °C; (iii) H_2O, 0 °C to rt; (b) (i) $Pd(PPh_3)_4$, 2M Na_2CO_3, PhH, EtOH; (ii) CH_3CN, H_2O; then $Mo(CO)_6$, reflux **(74%)**; (c) $CeCl_3$, $NaBH_4$, 0 °C to rt, 3h **(66%)**.

Scheme 33. Synthesis of allylic alcohol **4**.

3.2.4. Myers transposition and synthesis of *cis*-diol

The Myers transposition was envisioned as the key step of this approach because of its versatility. If successful, the usage of the Myers transposition could lead to the syntheses of both pancratistatin (previously published) and C-1 derivatives of pancratistatin (current approach). Olefin **5** was synthesized from allylic alcohol **4**, and the C-10b stereocenter identical to that present in natural pancratistatin **(1)** was correctly established with this reaction. Purification of this compound proved to be difficult as two chromatographic separations were necessary to purify the product. Upjohn dihydroxylation of olefin **5** by way of catalytic osmium tetroxide produced *cis*-diol **6**. The reaction often did not go to completion, but this could be avoided by adding a second portion of NMO to regenerate the osmium catalyst. In this way, the 7-step synthesis towards late-stage *cis*-diol, the substrate for the research content of this thesis, was completed through the repetition of previously applied procedures.[4]



Reagents and Conditions: (a) (i) IPNBSH, PPh_3, THF, 1h; (ii) DBAD, THF, 0 °C to rt, 3h; (iii) 2,2,2-TFE/H_2O, 0 °C to rt, 16h **(80%)**; (b) OsO_4, NMO, H_2O, acetone **(66%)**

Scheme 34. Synthesis of *cis*-diol **6**.

3.3. Selective protection of C-1/C-2 alcohols

Having synthesized *cis*-diol **6**, it was then necessary to elaborate the C-1/C-2 positions through protection and activation. Prior to activation of the C-1 position with a leaving group, protection of the C-2 position was envisioned to prevent possible selectivity issues arising from the two equally available hydroxyl groups. For this purpose, various protecting groups were explored. The first of these attempts was acetylation using acetic anhydride, which produced diacetate **214** exclusively **(Scheme 35)**. Attempts to revise the conditions were not pursued at the time as acetates are known to migrate from less stable positions to more stable positions. To counter this, a protecting group that would be labile, yet larger and less likely to migrate was chosen, namely a benzoate ester.

Benzoate ester formation was attempted using benzoyl chloride and pyridine in dichloromethane. Similar to acetylation, this resulted in primary formation of dibenzoate **215**. However, this could be prevented by careful monitoring for the formation of dibenzoate **215** by TLC, followed by immediate quenching. As the C-4a carbamate group is prone to rotation, characterization of the monobenzoates **216** and **217** proved to be difficult by NMR. The propensity of the molecule to assume different conformers provided broad proton resonances, especially for the resonances associated with the C-ring protons. Acquiring the spectra on the 300 MHz machine showed better resolution of the resonances compared to the 400 or 600 MHz machines. Using COSY, HSQC, and HMBC experiments, the regioisomers were classified as the C-1 monobenzoate **216** and the C-2 monobenzoate **217** with a ratio of 7:1, respectively.

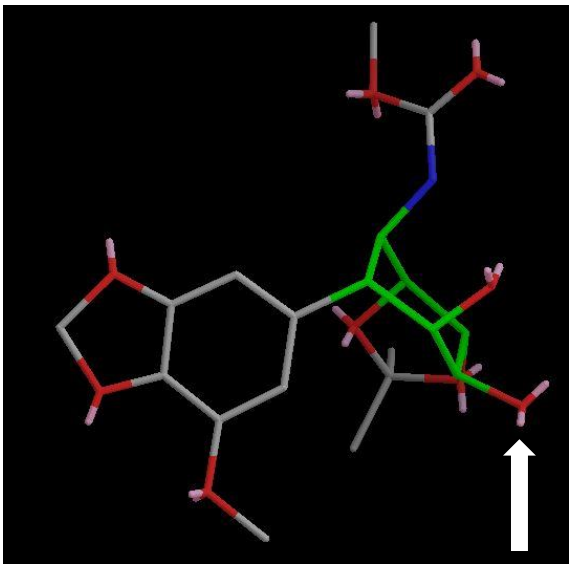
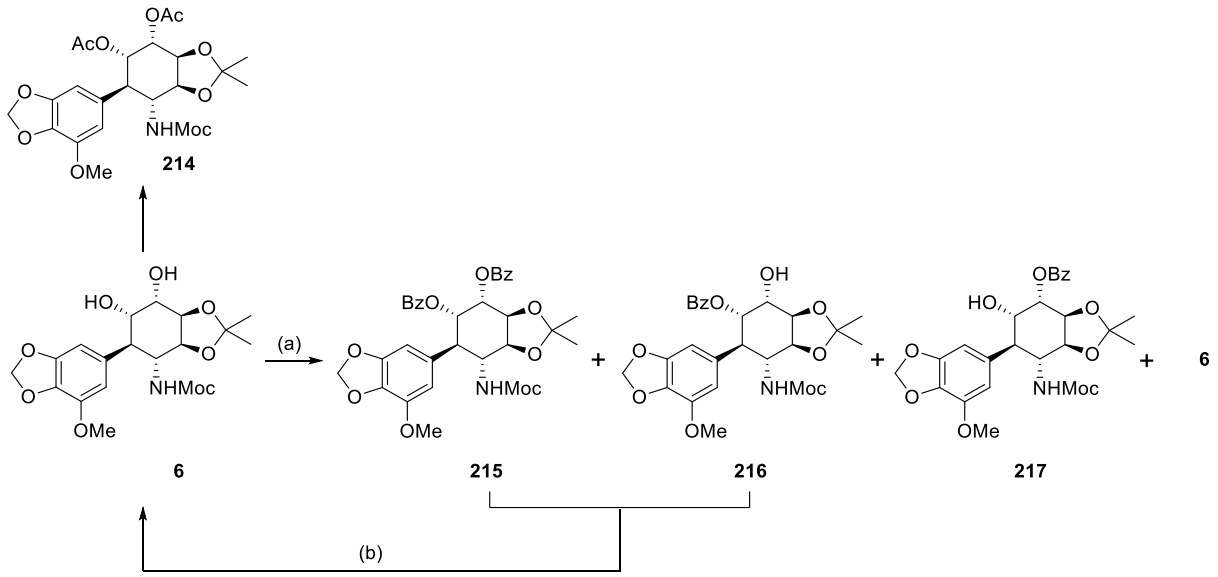


Figure 3. 3D model representing steric hindrance around the C-2 position.

The relative positions of the C-1 and C-2 hydroxyl groups is modelled by **Figure 3** (arrow points to the C-2 hydroxyl). C-1 is likely more accessible than C-2 as the latter is close in proximity to the bulky acetonide protecting group. Yields of the reaction were compromised since the reaction had to be quenched prior to starting material consumption to prevent formation of the undesired dibenzoate **215**. Initially, benzoyl chloride was used, producing C-2 monobenzoate **216** with yields ranging from 5-7% and C-1 monobenzoate **215** with yields ranging from 40-50%; these yields were improved to 10% and 55% respectively by using benzoic anhydride instead **(Scheme 35)**. Moreover, the lability of the benzoate group allowed for the recovery of *cis*-diol **6** through basic hydrolysis of undesired benzoates **215** and **216**.

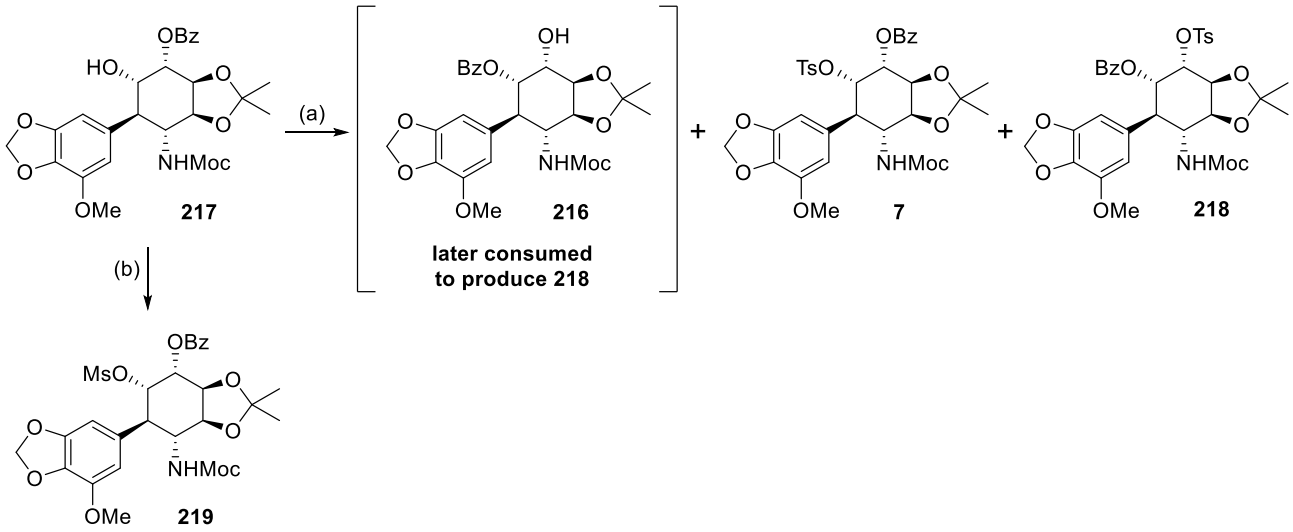


Reagents and Conditions: (a) Bz_2O, NEt_3, DMAP, DCM, 10 min [**215:** yield can be controlled by quenching the reaction early; **216**: 54% isolated yield, 65% brsm; **217**: 10% isolated yield, 12% brsm; **6**: 20% isolated yield]; (b) K_2CO_3, DCM, MeOH, rt, 1h **(70%)**.

Scheme 35. Attempts at selective protection of *cis*-diol **6**.

With enough C-2 monobenzoate **217** synthesized, the activation of the C-1 hydroxyl as a leaving group was explored next. First, the free alcohol of C-2 monobenzoate **217** was subjected to mesylation **(Scheme 36)** at room temperature. The reaction showed complete conversion of starting material and provided mesylate **219** in 10 minutes. To investigate whether mesylates and tosylates would act similarly, the tosylation of the C-1 position was pursued next **(Scheme 36)**. However, the results were not as expected. Tosylation would not proceed at room temperature and required heating to reflux to progress.

The reaction was monitored by TLC, showing an interesting occurrence. Although pure C-2 monobenzoate **217** was used as the substrate, the addition of heat to the reaction resulted in the migration of the benzoate group, producing C-1 monobenzoate **216** *in situ*. Because of this unforeseen transesterification, both tosylate regioisomers **7** and **218** were isolated in a 1:1.05 ratio, respectively. Tosylate **218** was produced within an hour of heating whereas tosylate **7** was only produced after heating overnight. The migration of the benzoate group from C-2 to C-1 was not observed with mesylation as the reaction did not have to be heated to proceed.

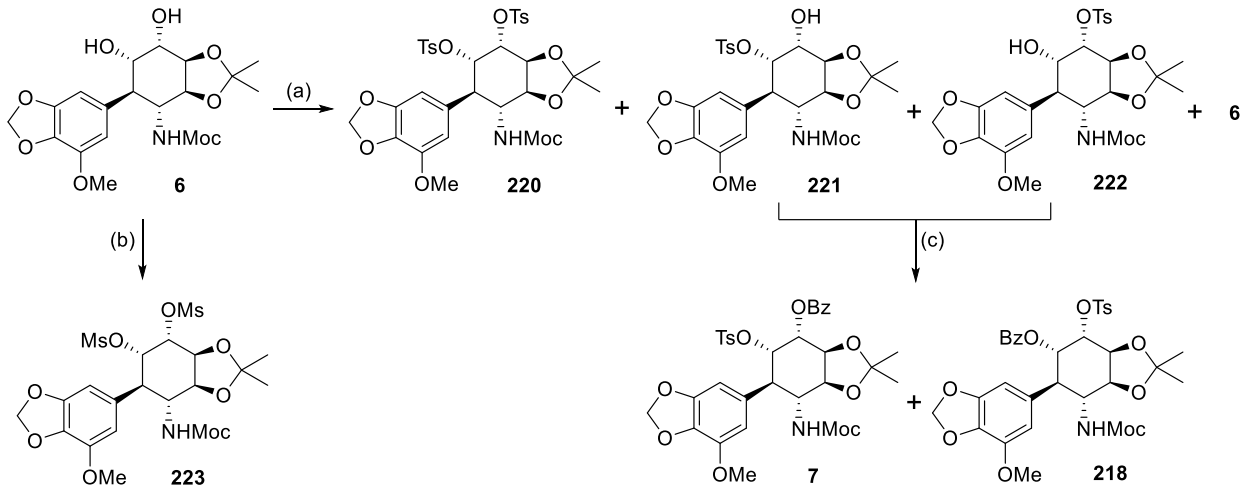


Reagents and Conditions: (a) TsCl, NEt_3, DMAP, DCM, reflux [**7**: 13%, **218**: 28%]; (b) MsCl, NEt_3, DMAP, DCM, 0° C to rt **(47%)**

Scheme 36. Activation of the C-1 position of *cis*-diol **6.**

Since benzoate ester protection followed by tosylation resulted in migration of the benzoate, it was estimated that the opposite sequence, tosylation of *cis*-diol **6** followed by benzoate ester protection, may give better results. *Cis*-diol **6** was subjected to tosylation, proceeding adequately at room temperature without heating **(Scheme 37)**. The reaction was monitored by TLC and quenched upon formation of the ditosylate **220**. Initially, only one spot other than ditosylate **220** was visible on TLC, but isolation of this compound revealed by NMR analysis that it was a mixture of regioisomers (5:6 mixture of C-1 tosylate **221** to: C-2 tosylate **222**).

This was later confirmed by subjecting the mixture of tosylate regioisomers **221** and **222** to benzoate ester protection of the corresponding free hydroxyl group, resulting in tosylates **7** and **218** in a 5:6 ratio. Once again, it was noticed that tosylate **218** formed faster than tosylate **7**, possibly because C-2 is sterically hindered by the neighbouring acetonide group. Although the ratio for the desired tosylate **7** is lower for this route, it was presumed that migration of the benzoate in the previously described route would be more difficult to control.



Reagents and Conditions: (a) TsCl, NEt_3, DMAP, DCM, rt [**220**: yield can be controlled by quenching reaction early; **221 + 222**: 22% combined isolated yield; 45% combined yield brsm; **6:** 50% isolated yield] (b) MsCl, NEt_3, DMAP, DCM, 0º C to rt **(40%)**; (c) Bz_2O, NEt_3, DMAP, DCM, rt, 14 h [**7:** 21%, **218:** 23%]

.

Scheme 37. Activation of the C-1 position prior to protection of the C-2 position of *cis*-diol **6**.

Purification of tosylates **7** and **218** proved to be extremely difficult. Several solvent systems were tested to resolve the compounds on TLC. The only solvent system that appropriately resolved the compounds was when the TLC was developed thrice in [EtOAc/hex (1:2)]. This became an issue when separating the regioisomers via column chromatography. Pure tosylate **218** eluted with no issue, but coelution of **7** with **218** was always apparent. This was the case even when using low polarity solvent [EtOAc/hex (1:4)] and avoiding forced pressure through the column.

All products synthesized including and after enone **55** had to be purified using 10% deactivated silica because the acetonide was acid sensitive. In the same way, tosylates **7** and **218** were subjected to column chromatography using 10% deactivated silica. However, because of their propensity to coelute, and because the necessary tosylate **7** always contained traces of **218**, another mode of purification was necessary: preparatory thin-layer chromatography.

The regioisomers **7** and **218** were dissolved in a small amount of DCM (~1 mL) and applied as a solution to the preparatory TLC plate. The plate was developed thrice in [EtOAc/hex (1:2)], allowing the separation of the regioisomers. The product bands were identified using ultraviolet

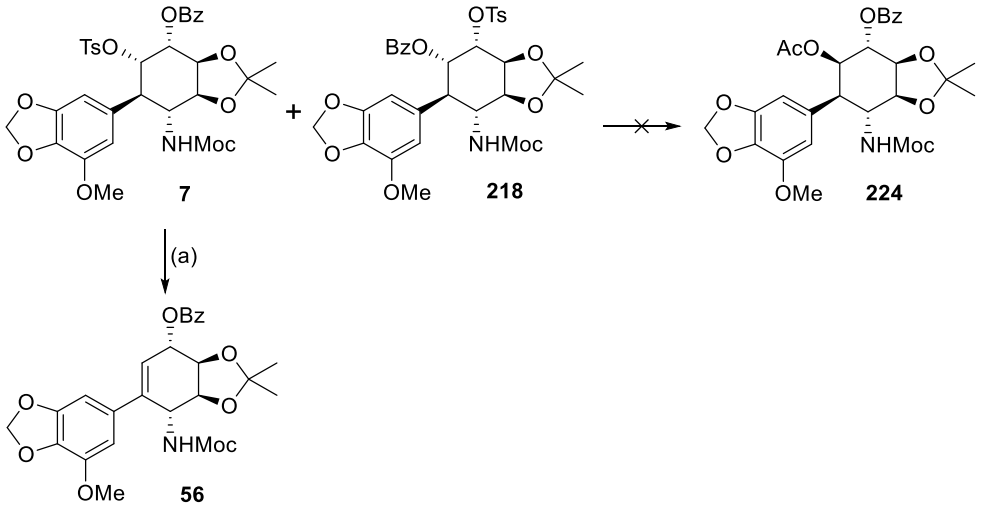
visualization and the silica containing products was removed from the plate using a spatula. From here, two separate outcomes resulted. If the silica was washed with EtOAc and filtered, tosylates **7** and **218** were furnished without coelution or acetonide cleavage. However, if the silica was washed with excess MeOH, acidic cleavage of the acetonide resulted in the free C-3/C-4 hydroxyls of **7** and **218**, but this could be countered by resubjecting the free diols to a quick acetonide protection. In this way, the issue of separation was solved by subjecting the coeluting tosylates **7** and **218** to preparatory TLC, though this posed limits on the scalability of the purification.

As the synthesis of C-2 monobenzoate **217** was low-yielding, *cis*-diol **6** was also subjected to mesylation **(Scheme 37)** to check if the C-1 mesylate could be formed preferentially, but dimesylate **223** was acquired exclusively.

3.5. Nucleophilic displacement at C-1

To reverse the stereochemistry at C-1 to that identical to natural pancratistatin **(1)**, an S_N2 reaction at C-1 with displacement of either mesylate or tosylate was envisioned. Once acetate **224** is formed, cleavage of the acetonide and C-2 benzoate ester, and reprotection with acetates would provide Magnus' late-stage tetraacetate **104**. This would work as a proof-of-concept approach, and if successful, this would complete a formal total synthesis to pancratistatin.

To this end, the mixture of tosylates **7** and **218** was subjected to nucleophilic attack by way of potassium acetate (KOAc) in a suspension of 18-crown-6-ether (18-C-6) and 1,2-dimethoxyethane (DME), but only starting material was isolated. The reaction was repeated, this time with acetonitrile as the solvent **(Scheme 38)**. 18-C-6 was first dissolved in distilled acetonitrile, followed by addition of KOAc to form a suspension. This mixture was stirred for 30 minutes, after which a solution of the tosylates **7** and **218** in acetonitrile was added dropwise. Heating the reaction mixture to reflux for 2 days resulted in a new spot on TLC that was estimated as the C-1 acetate **224**. Only tosylate **7** was consumed while **218** was left unreacted.



Reagents and Conditions: (a) 18-C-6, MeCN, then KOAc, 30 min; **7** + **218**, dropwise, 0 °C to reflux, 2 days.

Scheme 38. Attempts at nucleophilic substitution at the C-1 position with acetate.

However, characterization of the compound confirmed that the product was not the C-1 acetate **224**, but rather the elimination product benzoate **56**. Although the results were disappointing, they confirmed that tosylates **7** and **218** were identified correctly. It is likely that steric hindrance arising from the A-ring disfavours nucleophilic attack at the C-1 position **(Figure 4)**. Tosylate **7** has the propensity to form an elimination product as an 8-electron conjugated system is formed, whereas tosylate **218** does not. Since the C-10b proton and the C-1 tosylate are positioned in a *syn* fashion, it is unlikely that this was the predominant mechanism.

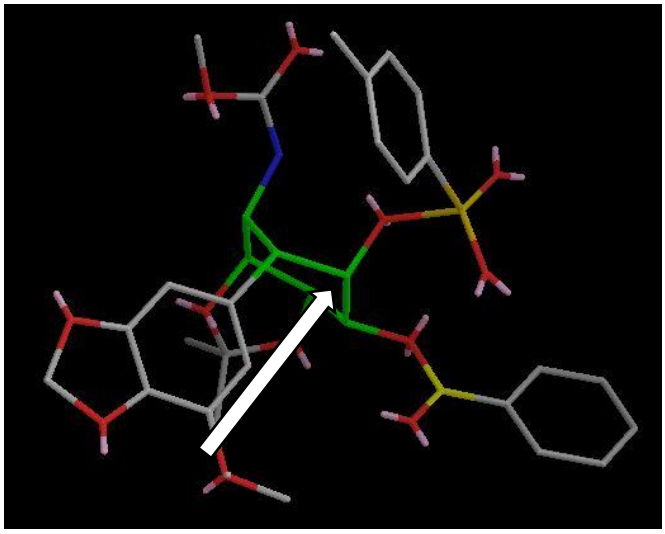
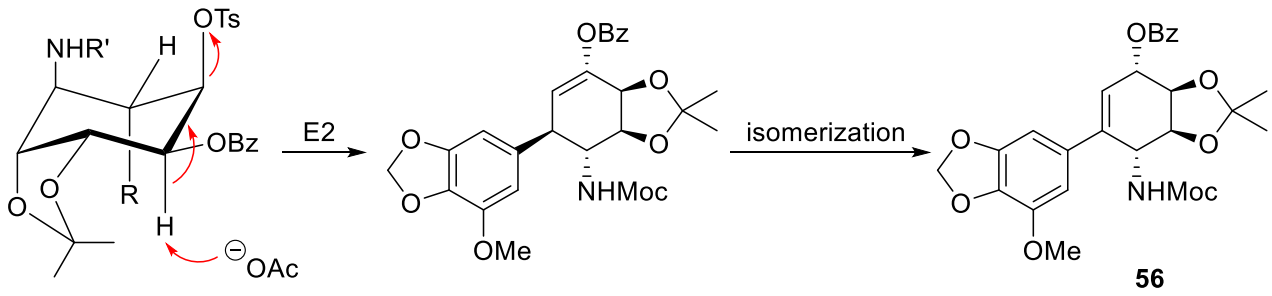


Figure 4. 3D model representing nucleophilic attack at the C-1 position of tosylate **7**.

Two plausible mechanisms of action can be proposed to explain this result. The first is the abstraction of the C-2 proton to form a C-1/C-2 olefin, which isomerizes to the more

stable C-1/C-10b olefin that participates in the 8-electron conjugated system with the A-ring **(Scheme 39)**.



Scheme 39. Proposed mechanism for the formation of olefin **56**.

The second possibility is that the neighbouring aromatic A-ring assists in the formation of a carbocation at C-1, resulting in a phenonium ion. This would be followed by acetate attack to form the C-1 acetate *anti* to the C-10b proton. Subsequent E2 elimination in an antiperiplanar fashion results in olefin **56**. Although acetate should not be able to abstract a proton, it can be assumed that the tosylate's leaving group ability and the propensity for forming an 8-electron conjugated system allowed this reaction to proceed as such. These mechanisms can be explored further through repetition and analysis of the reaction.

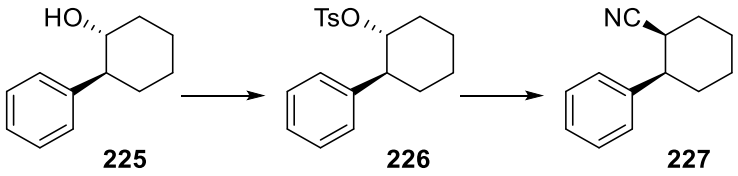
Because the undesired product **56** formed, the conditions were revised to check for other results. The reaction was repeated twice: once at room temperature for three days, and once by substituting potassium benzoate as the nucleophile. Neither reaction produced results. It was predicted that the limited solubility of potassium benzoate in acetonitrile might have prevented the reaction from proceeding.

To form the necessary C-C bond linkage at C-1 to provide derivatives of pancratistatin, sodium malonate was employed as the nucleophile. The sodium anion of dimethyl malonate was generated *in situ* using sodium hydride. Then, the mixture of tosylates **7** and **218** was cannulated into the reaction flask and heated to reflux for 2 days. The reaction was attempted both in THF and toluene, but neither reaction produced results.

To investigate which conditions would result in nucleophilic substitution at C-1 without further sacrifice of late-stage material, a model study involving *trans*-2-phenylcyclohexanol **(225)** was

envisioned. Although the model did not prove to be an accurate model of tosylate **7**, it allowed for facile testing of various reaction conditions without sacrifice of late-stage material.

Alcohol **225** was subjected to tosylation in classic conditions using TsCl, NEt_3, DMAP, and DCM, but these conditions did not result in a product. Instead, dissolving alcohol **225** in dry pyridine, followed by addition of TsCl and stirring for 24 h allowed full conversion of alcohol **225** to its respective tosylate **226 (Scheme 40)**. Tosylate **226** was recrystallized using ethanol and then subjected to nucleophilic substitution using sodium cyanide in dry DMF, forming the required nitrile **227 (Scheme 40)**.

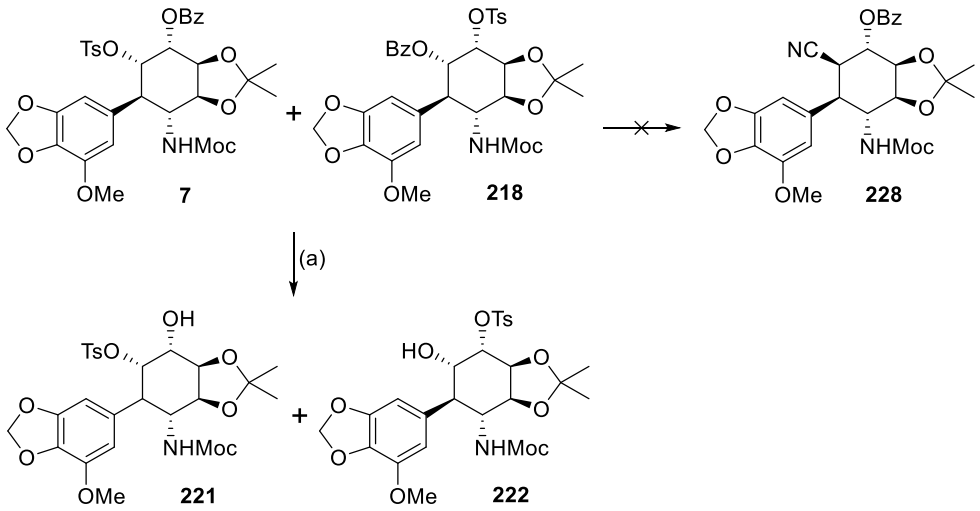


Reagents and Conditions: (a) TsCl, py, rt, 24 h **(60%)**; (b) NaCN, DMF, 100 °C, 14 h **(65%)**.

Scheme 40. Model study to find conditions for nucleophilic substitution of C-1 tosylate **7**.

The conditions used in the model study to produce nitrile **227** were repeated on the mixture of tosylate regioisomers **7** and **218 (Scheme 41)**. A new spot on TLC in 1:1 EtOAc/hex was identified after 14 h of stirring at 100 °C. C-1 tosylate **7** was fully consumed, whereas C-2 tosylate **218** was not. Isolation and purification of the new compound indicated that it was not the expected C-1 nitrile **228**, but rather the free alcohol of tosylate **7**. This result revealed that cyanide performed acyl substitution at the electrophilic benzoate ester of **7**, effectively cleaving it to tosylate **221**.

NMR analysis revealed that the spot isolated was a mixture of the two tosylate regioisomers, **221** and **222**. This indicated that C-2 tosylate **218** was also being deprotected to tosylate **222**, except at a slower rate compared to C-1 tosylate **7**.



Reagents and Conditions: (a) NaCN, DMF, 100 °C, 14 h (**42%** combined yield).

Scheme 41. Nucleophilic substitution at C-1 using the conditions from the model study.

Although the C-1 and C-2 positions are more electrophilic than the carbonyl of the benzoate ester, it can be presumed that the latter position participated in acyl substitution because it is not as sterically hindered as the former positions. This is evidenced by the 3D model present in **Figure 5a** (left arrow points access to the C-1 position; right arrow points to the carbonyl of the benzoate ester at C-2). Removal of the benzoate group provides tosylate **221 (Figure 5b)** and promotes the C-1 position as the only electrophilic position for nucleophilic attack. Thus, this reaction must be repeated to check if cyanide will perform C-1 substitution following cleavage of the benzoate ester.

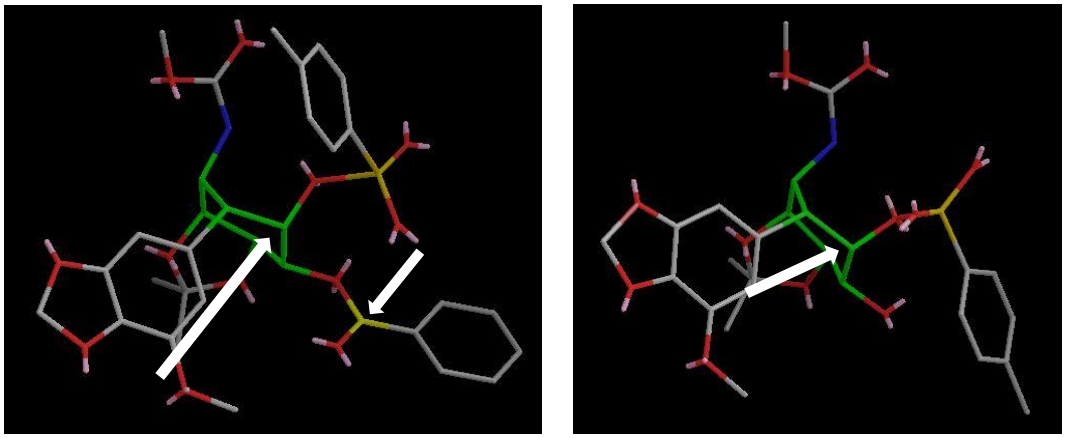
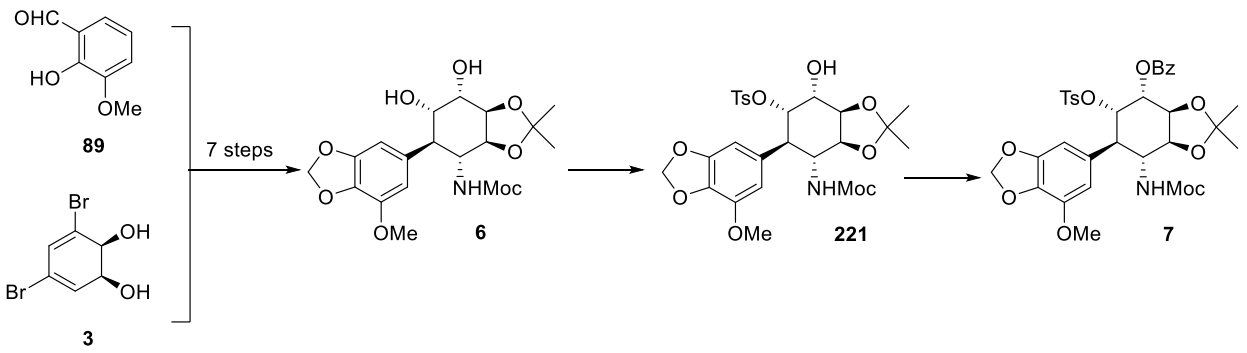


Figure 5. (a) 3D model of tosylate **7**; **(b)** 3D model of tosylate **221**.

4. CONCLUSIONS AND FUTURE WORK

C-1 derivatives of pancratistatin have been regarded as high-value targets for synthetic chemists since the discovery that large lipophilic substitutions at the C-1 position may result in increased inhibitory activity compared to the parent compound. To this effect, a synthetic approach towards C-1 derivatives of pancratistatin was presented in this thesis. This work involved the repetition of a previously published chemoenzymatic route towards late-stage *cis*-diol **6**, performed in 7 steps from commercial isovanillin **(89)** and 1,3-dibromodihydrodiol **(3)**.[4] From *cis*-diol **6**, original research was conducted to provide tosylate **7**. Selectivity issues were the primary hurdle to the project.

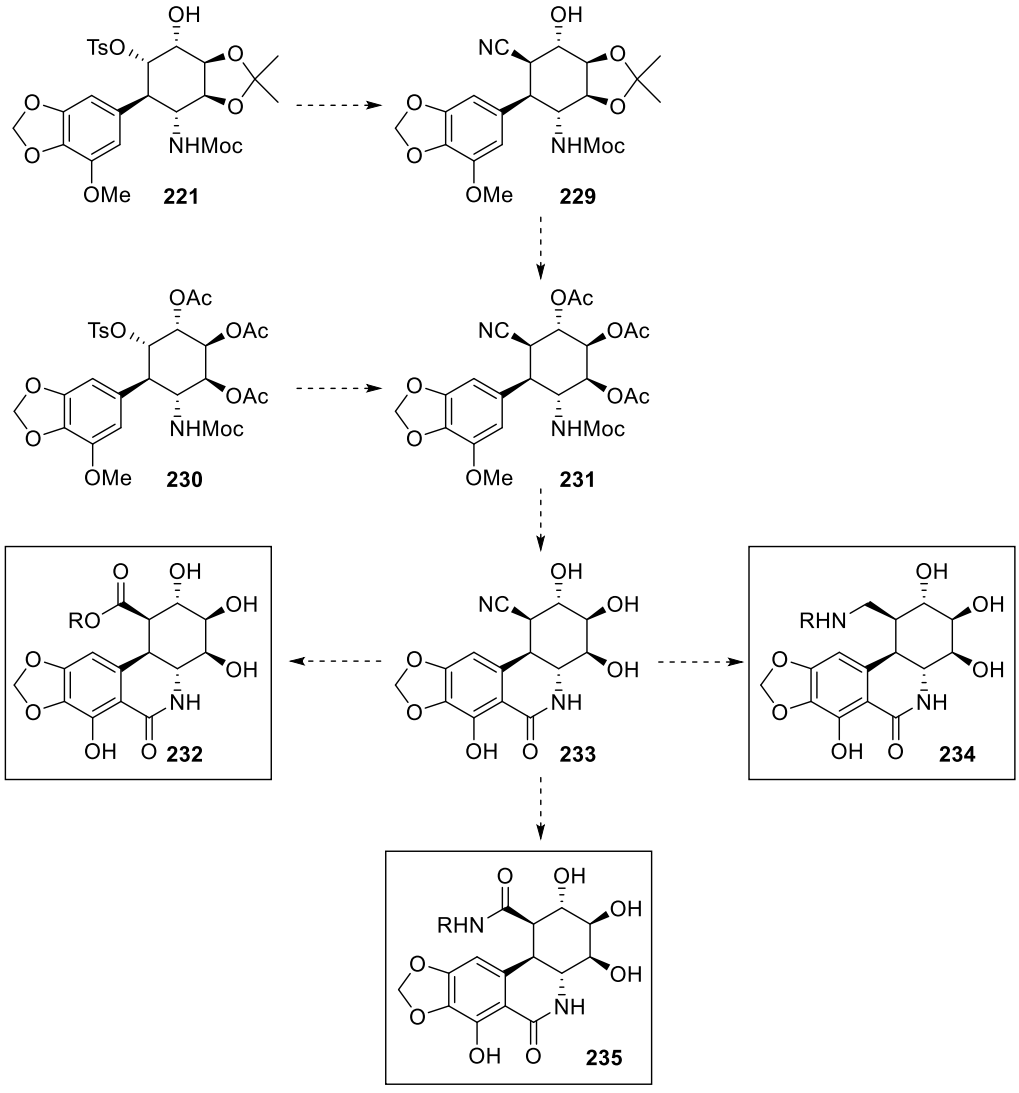


Scheme 42. Synthesis of tosylate **7** from isovanillin and 1,3-dibromodihydrodiol.

Future prospects for this project involve successful nucleophilic substitution at the C-1 position with carbon nucleophiles. Because cyanide participates in acyl substitution of the benzoate ester at C-2 primarily, this reaction can be repeated on tosylate **221** after the benzoate ester is cleaved. Alternatives include replacement of the benzoate group with non-electrophilic protecting groups such as a benzyl ether or a *tert*-butyldimethylsilyl ether. If these alternatives are unsuccessful, the acetonide can be replaced with acetates to check if steric hindrance is the primary reason for nucleophilic substitution failing.

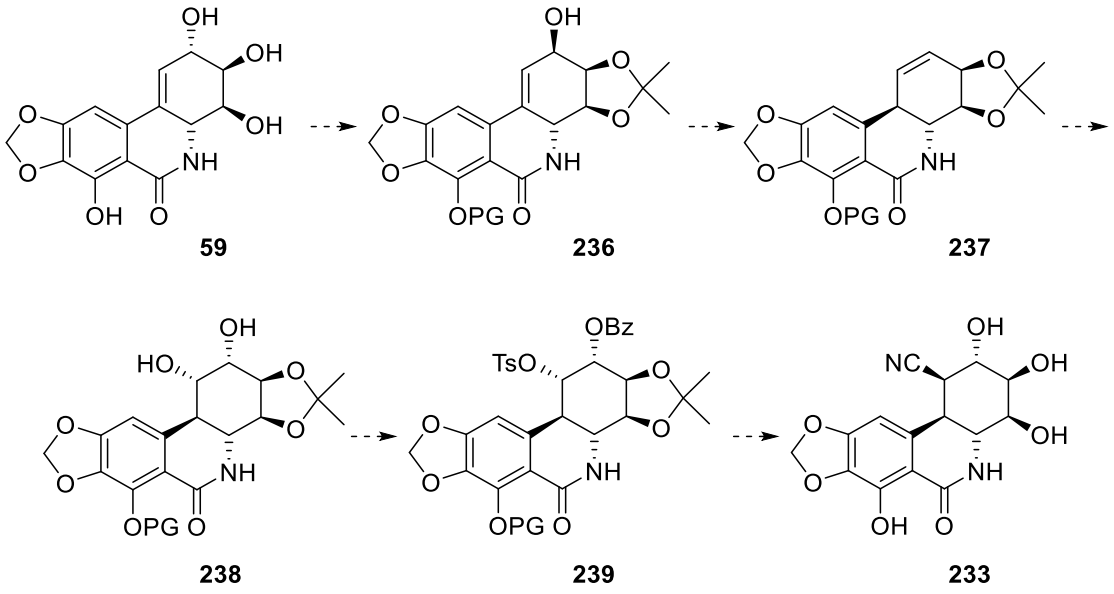
Another option is to attempt selective protection of *cis*-diol with catalytic amounts of stannous chloride ($SnCl_2$), an acylating agent, and *N*,*N*-diisopropylethylamine in acetonitrile.[59] This approach has been evaluated against various carbohydrates and should be attempted on the current substrate.[59] This can be an opportunity to prevent selectivity issues and improve product yields.

If nucleophilic substitution with cyanide is successful, the B-ring can be cyclized using the Banwell-modified Bischler-Napieralski cyclization. The acetonide of nitrile **229** must first be cleaved and the corresponding C-3/C-4 diol must be reprotected as acetates for the cyclization to proceed. Following cyclization, full deprotection would provide nitrile **233.** Hydrolysis of the nitrile forms a carboxylic acid, which can be further manipulated to form ester derivatives of type **232** or amide derivatives of type **235**. Alternatively, reduction of the nitrile can provide amine derivatives of type **234.** In this way, the current approach could be used to synthesize C-1 derivatives of pancratistatin **(Scheme 43)**.



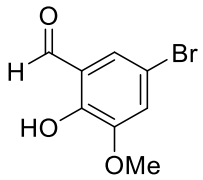
Scheme 43. Future prospects of this project to synthesize C-1 derivatives of pancratistatin.

The completion of the above route could lead to attempting this approach on natural narciclasine **(Scheme 44)**. This would prospectively decrease the number of synthetic manipulations required. With the C-7 hydroxyl and the C-3/C-4 diol protected, allylic alcohol **59** can be subjected to an oxidation-reduction sequence to reverse stereochemistry at C-2 to allow for the Myers transposition to proceed. Following that, a sequence of Upjohn dihydroxylation, selective protection of C-2, activation of C-1 with a leaving group, and nucleophilic substitution at C-1 will furnish nitrile **233**. Following **Scheme 43**, nitrile **233** can be manipulated to furnish C-1 derivatives of pancratistatin, which can then be subjected to biological testing.



Scheme 44. Proposed synthetic approach from natural narciclasine to C-1 derivatives of pancratistatin.

5. EXPERIMENTAL SECTION

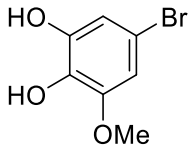


5-bromo-2-hydroxy-3-methoxybenzaldehyde (90)

2-hydroxy-3-methoxybenzaldehyde (33.0 mmol, 5.05 g, 1.00 eq) was charged to a two-neck round-bottomed flask and dissolved in glacial acetic acid (100 mL). Sodium acetate (51.0 mmol, 4.18 g, 1.55 eq) was transferred to the flask and allowed to dissolve. An addition funnel and thermometer were connected to the flask. A solution of Br_2 (1.74 mL, 1.03 eq) in glacial acetic acid (12.3 mL) was added dropwise over a period of 1h 15 min, with the temperature monitored between 25 – 30 °C. After stirring for 3h, a TLC in [EtOAc/hex (1:4)] revealed full consumption of starting material. The reaction was quenched with saturated sodium thiosulfate (10 mL) and left stirring overnight. Excess acetic acid was removed by concentrating *in vacuo* with a 10% NaOH bubbler.

The resulting solid was redissolved in DCM, partitioned with H_2O and extracted 7 times with 30 mL of DCM. Addition of 40 mL of 6M HCl and extraction with DCM resulted in full transfer of the product to the organic layer, which was then dried over sodium sulfate. The organic layer was filtered and concentrated *in vacuo*, followed by drying under high vacuum overnight. Purification was achieved via gradient column chromatography [EtOAc/hex (1:7 to 1:3)]. Concentration *in vacuo* and drying under high vacuum overnight resulted in 4.70 g (62%) of pure bromoaldehyde **90** as a yellow flaky solid.

90: The spectral and physical properties of the product match those reported in the literature.[4,32] **R_f** = 0.5 [EtOAc/hex (1:4)]; **mp** = 119-121 °C (EtOH), lit.[32] 122-124 °C (Ac_2O/H_2O); **IR ($CDCl_3$) *v*** 1651, 1464, 1388, 1256, 903, 719; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 11.00 (s, 1H), 9.86 (s, 1H), 7.31 (d, *J* = 2.2 Hz, 1H), 7.18 (d, *J* = 2.2 Hz, 1H), 3.92 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 195.5, 176.8, 151.1, 126.3, 121.5, 121.0, 111.2, 56.7.

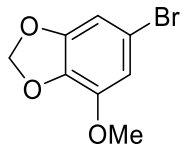


5-bromo-3-methoxybenzene-1,2-diol (212)

5-bromo-2-hydroxy-3-methoxybenzaldehyde (20.34 mmol, 4.69 g, 1.00 eq) was charged to a three-neck round-bottomed flask, then dissolved in a solution of 2% NaOH (86 mL). The suspension was cooled to ~8 °C, then connected to an addition funnel and a thermometer. A solution of hydrogen peroxide (15.2 mL, 6.60 eq) in water (110 mL) was added dropwise over 90 min, with the temperature monitored between 10 – 12 °C. The reaction was warmed slowly to room temperature and stirred for 2.5 h. A small-scale workup was performed using 3M HCl and EtOAc. A TLC in [EtOAc/hex (1:2)] revealed full consumption of starting material. The workup for the compound was achieved with 3M HCl (50 mL) followed by stirring for 10 min. The reaction mixture was quenched with saturated sodium sulfite (5 mL) and checked with iodine potassium starch paper for unreacted peroxides.

The reaction mixture was partitioned with DCM, then extracted 7 times with DCM (75 mL). The organic layer was washed with magnesium sulfate, filtered, and concentrated *in vacuo*. Purification was achieved via gradient column chromatography [EtOAc/hex (1:7 to 1:3)]. Concentration *in vacuo* and drying under high vacuum overnight resulted in 3.38 g (76%) of pure catechol **212** as a light brown solid.

212: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.3 [EtOAc/hex (1:2)]; **mp** = 73-74 °C (EtOAc), lit.[4] 75 °C (EtOAc); **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.77 (d, *J* = 2.0 Hz, 1H), 6.61 (d, *J* = 2.1 Hz, 1H), 5.44 (d, *J* = 14.3 Hz, 2H), 3.86 (s, 3H).

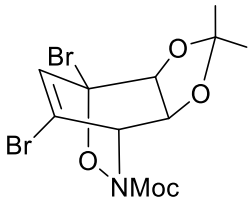


6-bromo-4-methoxybenzo[*d*][1,3]dioxole (91)

5-bromo-3-methoxybenzene-1,2-diol (15.4 mmol, 3.36 g, 1.00 eq) was charged to a round-bottomed flask and connected to a reflux condenser. The system was flushed three times with a vacuum-argon cycle, then placed under argon. Dry DMF (30 mL) was added to the flask, then stirred for 8 min. Oven dried potassium carbonate (30.7 mmol, 3.26 g, 2.00 eq) and diiodomethane (1.85 mL, 1.50 eq) were added to the flask, while maintaining the argon flow. The system was heated to 100 °C for 2 h, then allowed to cool to room temperature and stirred overnight. A TLC the following day in [EtOAc/hex (1:4)] revealed almost full consumption of starting material.

The mixture was diluted with brine, partitioned with DCM, and extracted 8 times with DCM (20 mL). The organic layer was dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude black oily product was triturated 3 times with toluene to remove excess water and DMF. The crude product was purified via column chromatography using [EtOAc/hex (1:20)]. Concentration *in vacuo* and drying under high vacuum overnight afforded 2.33 g (66%) of pure bromide **91** as a white solid.

91: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.7 [EtOAc/hex (1:4)]; **mp** = 79-80 °C (EtOAc/hex), lit.[32] 80-81 °C (Et_2O/hex); **IR ($CDCl_3$) *ν*** 2902, 1629, 1487, 1446, 1420, 1180, 1103, 1036; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.68 (q, *J* = 1.7 Hz, 1H), 5.97 (s, 1H), 3.88 (s, 2H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 149.6, 144.3, 113.4, 111.2, 106.4, 102.0, 56.9.



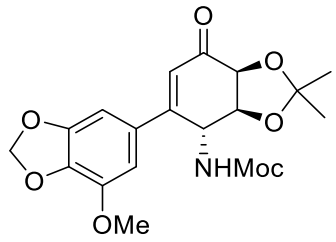
Methyl(7a*S*)-4,6-dibromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino) benzo[*d*][1,3]dioxole-8-carboxylate (53)

Chemoenzymatic oxidation of 1,3-dibromobenzene by *Escherichia coli* JM109 (pDTG601A) yielded (1*S*,2*S*)-3,5-dibromocyclohexa-3,5-diene-1,2-diol, which was extracted with EtOAc and concentrated *in vacuo* to near dryness to obtain ~7.5 g of crude product (28.0 mmol, 1.00 eq). The flask was charged with 2,2-dimethoxypropane (30 mL, 4.00 eq), and DCM was added to help redissolve the diol. Then, *p*-toluenesulfonic acid (cat., spatula tip) was added and a loose septum was placed on the neck of the flask and left stirring overnight. A TLC in [EtOAc/hex (1:4)] the following morning indicated incomplete conversion of the starting material, thus a second spatula tip of *p*-TsOH was added. Stirring for 30 min resulted in full consumption of starting material.

Water (15 mL) and $NaIO_4$ (28.0 mmol, 5.97 g, 1.00 eq) were added, followed by cooling of the system to 0 °C. Methoxycarbonyl-protected hydroxylamine (MocNHOH) (30.8 mmol, 2.80 g, 1.10 eq) was separately dissolved in MeOH (20 mL) and transferred to an addition funnel. The carbamate solution was added dropwise over a period of 1 h, resulting in a viscous solution that was difficult to stir. After the addition was complete, a TLC in [EtOAC/hex (1:4)] revealed full consumption of the starting material. Quenching was achieved with a saturated solution of sodium sulfite (15 mL). The crude material was concentrated *in vacuo* to remove volatile components, then partitioned with DCM and extracted 8 times with 75 mL DCM. The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*.

Purification was achieved via gradient column chromatography with [EtOAC/hex (1:20 to 1:6)]. Concentration *in vacuo* and drying over high vacuum overnight afforded 5.58 g of pure oxazine **53** as a white flaky solid (50% over two steps).

53: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.5 [EtOAc/hex (1:4)]; **mp** = 149-151 °C (EtOAc), lit.[23] 150-152 °C (EtOAc); **IR ($CDCl_3$) *v*** 1728, 1600; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.70 (dd, J = 2.1, 0.8 Hz, 1H), 5.17 (dd, *J* = 4.3, 2.3 Hz, 1H), 4.70 (dd, *J* = 6.9, 4.3 Hz, 1H), 4.59 (d, *J* = 6.9 Hz, 1H), 3.84 (s, 3H), 1.42 (s, 3H), 1.35 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 158.0, 132.7, 120.9, 112.3, 87.2, 81.3, 74.7, 61.4, 54.5, 25.8, 25.6.



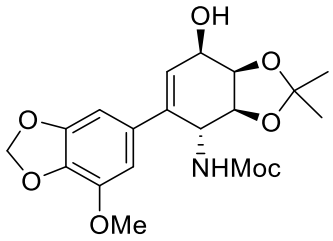
Methyl ((3a*S*,4*R*,7a*S*)-7'-methoxy-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydro-[5,5'-bibenzo[*d*][1,3]dioxol]-4-yl)carbamate (55)

Methyl(7a*S*)-4,6-dibromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino) benzo [*d*] [1,3]dioxole-8-carboxylate (0.63 mmol, 0.25 g, 1.00 eq) was transferred to a round-bottomed flask and flushed with three alternating vacuum-argon cycles, then placed under argon. Distilled benzene (6 mL) was added and the system was degassed by continuous bubbling of argon through the solution for 15 min. $Pd(PPh_3)_4$ (0.03 mmol, 0.036 g, 0.05 eq) was added to the flask and stirred. A degassed solution of 2M Na_2CO_3 (1.25 mmol, 0.13 g, 2.00 eq) in water (0.63 mL) was added, followed by addition of a solution of crude boronic acid (prepared from 0.18 g of 6-bromo-4-methoxybenzo[*d*][1,3]dioxole) in EtOH (2.2 mL). The system was connected to a condenser and heated to reflux for 2.5 h. A TLC in [EtOAc/hex (1:4)] revealed full consumption of starting material.

The mixture was diluted with acetonitrile (1.25 mL) and water (1.25 mL) and $Mo(CO)_6$ (0.69 mmol, 0.18 g, 1.10 eq) was added, followed by refluxing for 2 h. A TLC in [EtOAC/hex (1:2)] revealed full consumption of the starting material, thus the mixture was cooled to room temperature. The product was filtered through a plug of Celite® and rinsed with EtOAc. The resulting filtrate was concentrated *in vacuo* and purified with gradient column chromatography (10% deactivated silica) using [EtOAc/hex (1:4 to 1:1)]. The product was concentrated *in vacuo*

and dried overnight under high vacuum to obtain 0.18 g (74% over 3 steps) of pure enone **55** as a light-yellow oil.

55: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.5 [EtOAc/hex (2:1)]; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.82 (d, *J* = 17.5 Hz, 2H), 6.46 (s, 1H), 6.04 (s, 2H), 5.27 (d, *J* = 17.9 Hz, 1H), 5.00 (d, *J* = 8.7 Hz, 1H), 4.69 (s, 1H), 4.45 (d, *J* = 5.1 Hz, 1H), 3.92 (s, 3H), 3.69 (s, 3H), 1.42 (s, 3H), 1.33 (s, 3H). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 195.6, 153.2, 153.2, 149.8, 138.2, 130.1, 124.0, 110.6, 107.6, 102.4, 101.1, 73.6, 65.8, 56.9, 31.4, 27.6, 26.2, 25.7, 25.3.

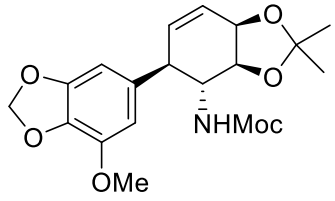


Methyl ((3a*S*,4*R*,7*R*,7a*R*)-7-hydroxy-7'-methoxy-2,2-dimethyl-3a,4,7,7a-tetrahydro-[5,5'-bibenzo[*d*][1,3]dioxol]-4-yl)carbamate (4)

Methyl((3a*S*,4*R*,7a*S*)-7'-methoxy-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydro-[5,5'-bibenzo [*d*][1,3]dioxol]-4-yl)carbamate (0.36 mmol, 0.14 g, 1.00 eq) was dissolved in MeOH (6 mL), and transferred to a round-bottomed flask. The solution was degassed under argon for 5 min prior to addition of $CeCl_3 \bullet 7H_2O$ (0.54 mmol, 0.20 g, 1.50 eq). The flask was cooled to 0 °C after 20 min of stirring under argon flow. $NaBH_4$ (0.39 mmol, 0.015 g, 1.10 eq) was added in four portions and evolution of hydrogen gas was observed. After 3 h, a TLC in [EtOAC/hex (1:1)] indicated full consumption of starting material. The reaction mixture was then diluted with EtOAc and filtered through a Celite® plug. The crude product was purified using gradient column chromatography (10% deactivated silica) with [EtOAc/hex (1:1.5 to 2:1)], and the resulting product was concentrated *in vacuo* and dried under high vacuum, resulting in 0.17 g (66%) of pure allylic alcohol **4** as a foamy white oil.

4: The spectral and physical properties of the product match those reported in the literature.[4]

R_f = 0.1 [EtOAc/hex (1:1)]; **IR ($CDCl_3$) *v*** 3323, 2988, 2937, 1695, 1508, 1211, 1042, 730; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.58 (d, *J* = 4.0 Hz, 2H), 6.08 (s, 1H), 5.96 (s, 2H), 4.68 (dd, *J* = 10.7, 6.4 Hz, 1H), 4.54 (s, 1H), 4.41 (s, 1H), 3.90 (s, 3H), 3.69 (s, 3H), 2.74 (s, 1H), 1.35 (s, 3H), 1.31 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 156.7, 149.3, 143.7, 137.4, 135.5, 133.8, 130.8, 109.4, 105.8, 101.8, 100.1, 75.4, 66.7, 60.5, 56.9, 26.3, 24.8, 21.2, 14.3.

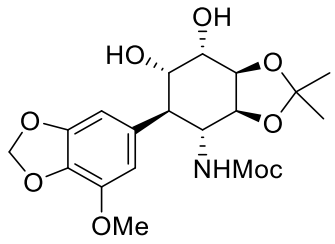


Methyl ((3a*S*,4*R*,5*R*,7a*R*)-7'-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydro-[5,5'-bibenzo[*d*][1,3]dioxol]-4-yl)carbamate (5)

Methyl ((3a*S*,4*R*,7*R*,7a*R*)-7-hydroxy-7'-methoxy-2,2-dimethyl -3a,4,7,7a-tetrahydro-[5,5'-bibenzo[*d*][1,3]dioxol]-4-yl)carbamate (0.64 mmol, 0.20 g, 1.00 eq) was dissolved in DCM (10 mL) and transferred to a Schlenk flask, then concentrated *in vacuo* to obtain ~0.20 g of allylic alcohol. PPh_3 (1.28 mmol, 0.34 g, 2.00 eq) and IP-NBSH (1.28 mmol, 0.33 g, 2.00 eq) were charged to the flask and subjected to alternating cycles of vacuum and argon. The flask was briefly flame-dried and placed under high vacuum for 1 h. The system was placed under argon and distilled THF (2.0 mL) was added to the flask. After cooling the system to 0 °C, a solution of DBAD (1.28 mmol, 0.30 g, 2.00 eq) in dry THF (1.0 mL) prepared separately in anhydrous conditions, was added dropwise to the Schlenk flask. The system was warmed slowly to room temperature and stirred under argon flow for 3 h, after which a mixture of 2,2,2-trifluoroethanol (1.0 mL) in water (1.0 mL) was added dropwise at 0 °C. Stirring overnight under argon flow resulted in full consumption of starting material; checked by TLC in [EtOAc/hex (1:1)].

The reaction mixture was concentrated *in vacuo* using DCM as the solvent. The crude product was concentrated onto 10% deactivated silica and purified using two consecutive gradient columns: the first with [EtOAc/hex (1:4 to 1:1)], then [DCM/MeOH (400:1 to 50:1)]. The fractions containing product were combined and concentrated *in vacuo* to obtain 0.19 g (80%) of pure olefin **5** as a foamy yellow oil.

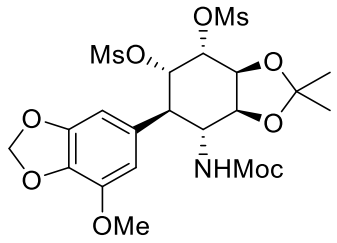
5: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.3 [EtOAc/hex (1:1)]; **IR ($CDCl_3$) *v*** 3339, 2985, 2938, 1704, 1633, 1511, 1451, 1048, 1730; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.45 (m, 1H), 5.96 (s, 2H), 5.95 (m, 2H), 4.74 (m, *J* = 5.4 Hz, 1H), 4.65 (m, 1H), 4.56 (m, 1H), 3.96 (m, 1H), 3.86 (s, 3H), 3.55 (s, 3H), 3.41 (br, 1H), 1.54 (s, 3H), 1.41 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 156.5, 154.3, 148.9, 143.5, 136.1, 135.5, 134.2, 123.6, 109.7, 107.5, 102.2, 101.4, 72.5, 57.2, 56.6, 53.4, 51.9, 28.2, 25.9.



Methyl ((3a*S*,4*R*,5*R*,6*S*,7*S*,7a*R*)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (6)

Methyl((3a*S*,4*R*,5*R*,7a*R*)-7'-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydro-[5,5'-bibenzo[*d*][1,3]dioxol]-4-yl)carbamate (1.57 mmol, 0.59 g, 3.64 eq) was concentrated to dryness and dissolved in acetone (8.5 mL) and distilled water (2.1 mL). NMO (1.57 mmol, 0.18 mg, 3.64 eq) was transferred to the flask and allowed to dissolve. Then, OsO_4 (0.430 mmol, 0.11 mg, 1.00 eq) was added to the flask and a loose septum was placed on top. The reaction was stirred overnight, after which a TLC in EtOAc/hex (2:1) indicated almost full consumption of starting material. The reaction was quenched with $NaHSO_3$ (10 mL), then passed through a Celite plug to remove volatiles. The product was purified by column chromatography (10% deactivated silica) with EtOAC/hex (2:1) to remove impurities, then flushed with EtOAc to acquire 0.43 g (66%) of pure *cis*-diol **6** as a colourless oil.

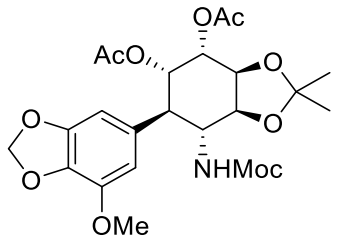
6: The spectral and physical properties of the product match those reported in the literature.[4] **R_f** = 0.1 [EtOAc/hex (2:1)]; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.95 (s, 2H), 5.96 (s, 2H), 4.64 (d, *J* = 9.6 Hz, 1H), 4.34 (m, 2H), 4.20 (m, 2H), 4.02 (m, 2H), 3.90 (s, 3H), 3.54 (s, 3H), 2.93 (t, 1H), 2.71 (s, 1H), 1.86 (s, 1H), 1.39 (s, 3H), 1.25 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 156.7, 149.4, 144.0, 141.0, 132.1, 128.0, 109.3, 101.7, 99.0, 88.3, 72.7, 69.5, 60.6, 56.7, 52.3, 28.0, 26.0, 14.3.



(3aS,4R,5R,6S,7R,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxole]-6,7-diyl dimethanesulfonate (223)

Methyl-((3aS,4R,5R,6S,7S,7aR)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (0.10 mmol, 0.04 g, 1.05 eq) was concentrated to dryness, then backfilled thrice with alternating cycles of vacuum and argon. Distilled DCM (2.5 mL) was added, followed by addition of triethylamine (1.50 mmol, 20 µL, 1.50 eq). The system was cooled to 0 °C and an anhydrous solution of mesyl chloride (0.10 mmol, 7.6 µL, 1.00 eq) in DCM (0.15 mL) was added dropwise. The reaction was stirred for 30 min, then quenched with H_2O (1 mL) and extracted five times with DCM (10 mL). The organic layers were washed with sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was concentrated onto 10% deactivated silica and subjected to gradient column chromatography [EtOAc/hex (1:4 to 1:2)]. The fractions were concentrated *in vacuo* and dried under high vacuum overnight to obtain 20 mg of dimesylate **223** as a white solid (40%).

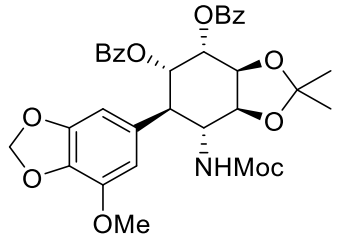
223: $\mathbf{R_f}$ = 0.8 [EtOAc/hex (2:1)]; **mp** = 217-218 °C dec. (DCM); $\mathbf{[\alpha]^{21}_D}$ -16.2 (*c* 0.2, DCM); **IR (DCM)** *v* 3340, 2941, 1708, 1515, 1355, 1177, 1023, 852, 736; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 6.52 (s, 2H), 5.95 (s, 2H), 5.14 – 5.01 (m, 2H), 4.94 (d, *J* = 9.3 Hz, 1H), 4.55 (d, *J* = 5.3 Hz, 1H), 4.32 (t, *J* = 6.1 Hz, 1H), 4.21 – 4.03 (m, 1H), 3.87 (s, 3H), 3.57 (s, 3H), 3.19 (s, 3H), 3.14 (d, *J* = 8.9 Hz, 1H), 2.45 (s, 3H), 1.63 (s, 3H), 1.39 (s, 3H); **^{13}C NMR (75 MHz, CDCl3)** δ 143.9, 128.2, 115.5, 110.7, 108.0, 105.2, 101.7, 98.9, 96.5, 94.5, 91.8, 89.2, 84.8, 71.0, 68.0, 56.7, 52.4, 40.5, 37.8, 27.5, 25.5; **HRMS (EI)** calcd for $C_{21}H_{29}NO_{13}S_2$: 567.1080; found: 567.1071; **LRMS (EI)** *m/z* (relative intensity) 567 (M^+, 3) 535 (25), 472 (55), 317 (36), 296 (57), 277 (68), 211 (25), 197 (35), 169 (49), 153 (100), 141 (63), 127 (74), 113 (78); **anal.** calcd for $C_{21}H_{29}NO_{13}S_2$: C, 44.44; H, 5.15; found C, 48.17; H, 5.86; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



(3aS,4R,5R,6S,7S,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxole]-6,7-diyl diacetate (214)

Methyl-((3aS,4R,5R,6S,7S,7aR)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (0.097 mmol, 0.04 g, 1.00 eq) was concentrated to dryness, charged to a 25-mL round-bottomed flask, and backfilled thrice with alternating cycles of vacuum and argon. Distilled DCM (4.0 mL) was added, followed by cooling of the system to 0° C. Triethylamine (0.25 mL, excess) and acetic anhydride (0.25 mL, excess) were added dropwise to the system at 0° C, followed by a catalytic amount of DMAP (spatula tip). The reaction was stirred with slow warm up to room temperature for 40 min, and a TLC in EtOAc/hex (2:1) showed full consumption of starting material. Saturated sodium bisulfite (2 mL) was added to quench unreacted acetic anhydride, followed by concentration *in vacuo* to obtain the crude product. The crude product was then concentrated onto 10% deactivated silica and applied to a column [EtOAc/hex (2:1)]. Concentration *in vacuo* and drying under high vacuum overnight resulted in 12 mg (26%) of diacetate **214** as a white solid.

214: $\mathbf{R_f}$ = 0.3 [EtOAc/hex (2:1)]; **mp** = 220-222 °C dec. (DCM); $\mathbf{[\alpha]^{22}_D}$ -21.6 (*c* 4.3, DCM); **IR (DCM)** *v* 1800, 1264, 731, 703; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.40 (d, *J* = 5.5 Hz, 1H), 5.94 (s, 2H), 5.53 (t, 1H), 5.42 (dd, *J* = 9.4 Hz, 1H), 4.70 (d, *J* = 4.9 Hz, 1H), 4.40 (s, 1H), 4.28 (s, 1H), 3.87 (s, 3H), 3.74 (d, *J* = 9.4 Hz, 1H), 3.56 (s, 3H), 3.20 (s, 1H), 2.15 (s, 3H), 1.85 (s, 3H), 1.60 (s, 3H), 1.37 (s, 3H); **^{13}C NMR (75 MHz, CDCl3) δ** 169.7, 156.4, 148.9, 143.6, 134.5, 132.5, 110.2, 107.6, 102.4, 101.6, 75.2, 71.7, 69.5, 56.7, 56.6, 52.3, 28.0, 25.8, 21.1, 20.8; **HRMS (EI)** calcd for $C_{23}H_{29}NO_{11}$: 495.1735, found: 495.1733; **LRMS (EI)** *m/z* (relative intensity) 495 (M^+, 3), 463 (27), 435 (57), 303 (36), 302 (81), 288 (35), 275 (96), 260 (76), 243 (29), 233 (100), 232 (55); **anal.** calcd for $C_{23}H_{29}NO_{11}$: C, 55.75; H, 5.90; found C, 63.99; H, 8.18; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



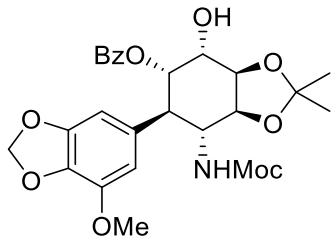
(3aS,4R,5R,6S,7S,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxole]-6,7-diyl dibenzoate (215)

Methyl-((3aS,4R,5R,6S,7S,7aR)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (0.73 mmol, 0.30 g, 1.00 eq) was concentrated to dryness, then backfilled thrice with alternating cycles of vacuum and argon. Distilled DCM (45 mL) was added, followed by addition of triethylamine (0.36 mmol, 40 µL, 0.50 eq) using a micropipette. A catalytic amount of DMAP was added (spatula tip) and the system was cooled to 0° C. An anhydrous solution of benzoic anhydride (0.36 mmol, 0.083 g, 0.50 eq) in 0.3 mL distilled DCM was prepared separately, then added dropwise to the system. A TLC in 2:1 EA/hex was checked immediately, monitoring for the formation of the dibenzoate, rather than the consumption of starting material. After 20 minutes, the reaction was quenched with a saturated solution of $NaHCO_3$ (2 mL), then extracted five times with DCM (20 mL). The organic layers were combined and dried under sodium sulfate, then concentrated *in vacuo*.

The crude product was concentrated on 10% deactivated silica and applied to a gradient column [EtOAc/hex (1:3 to 1:1)] until all benzoylated products eluted, then flushed with EtOAc to recover starting material. The fractions containing product were concentrated *in vacuo* and dried overnight to obtain dibenzoate **215** (12 mg, 3%) as a clear oil.

215: $\mathbf{R_f}$ = 0.6 [EtOAc/hex (2:1)]; $\mathbf{[\alpha]^{21}_D}$ -55.9 (*c* 1.2, EtOAc); **IR (DCM)** *v* 3346, 3056, 2985, 2940, 1733, 1636, 1514, 1451, 1436, 1373, 1045, 733, 703; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.12 – 7.98 (m, 2H), 7.77 – 7.72 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.53 – 7.43 (m, 3H), 7.30 (t, *J* = 7.7 Hz, 2H), 6.49 (s, 2H), 5.95 (t, *J* = 3.1 Hz, 1H), 5.88 (dd, *J* = 6.8, 1.4 Hz, 2H), 5.75 (dd, *J* = 9.4, 2.9 Hz, 1H), 4.80 (d, *J* = 8.7 Hz, 1H), 4.63 – 4.49 (m, 2H), 3.98 – 3.85 (m, 1H), 3.84 (s, 3H), 3.60 (s, 3H), 3.52 (s, 1H), 1.67 (s, 1H), 1.43 (s, 1H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 165.3, 165.2, 152.1,

149.0, 143.7, 134.6, 133.8, 133.6, 133.2, 132.3, 130.3, 130.0, 129.70, 129.66, 129.6, 128.8, 128.6, 128.4, 110.4, 107.8, 102.4, 101.6, 100.7, 75.4, 72.7, 70.2, 56.7, 52.3, 38.8, 28.1, 26.0; **HRMS (EI)** calcd for $C_{33}H_{33}NO_{11}$ $[M\text{-}CH_3]^+$: 604.1704, found: 604.1813; **LRMS (EI)** *m/z* (relative intensity) 604 ($[M\text{-}CH_3]^+$, 3), 497 (36), 364 (80), 352 (20), 337 (68), 296 (76), 211 (18), 197 (25), 169 (34), 153 (60), 141 (43), 113 (48), 105 (100).

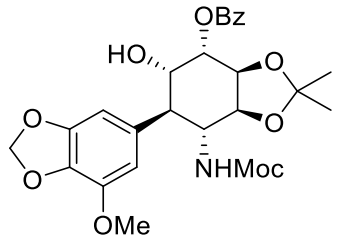


(3aS,4R,5R,6S,7S,7aR)-7-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl benzoate (216)

Methyl-((3aS,4R,5R,6S,7S,7aR)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (0.73 mmol, 0.30 g, 1.00 eq) was concentrated to dryness, then backfilled thrice with alternating cycles of vacuum and argon. Distilled DCM (45 mL) was added, followed by addition of triethylamine (0.36 mmol, 40 µL, 0.50 eq) using a micropipette. A catalytic amount of DMAP was added (spatula tip) and the system was cooled to 0° C. An anhydrous solution of benzoic anhydride (0.36 mmol, 0.083 g, 0.50 eq) in 0.3 mL distilled DCM was prepared separately, then added dropwise to the system. A TLC in 2:1 EA/hex was checked immediately, monitoring for the formation of the dibenzoate, rather than the consumption of starting material. After 20 minutes, the reaction was quenched with a saturated solution of $NaHCO_3$ (2 mL), then extracted five times with DCM (20 mL). The organic layers were combined and dried under sodium sulfate, then concentrated *in vacuo*.

The crude product was concentrated on 10% deactivated silica and applied to a gradient column [EtOAc/hex (1:3 to 1:1)] until all benzoylated products eluted, then flushed with EtOAc to recover starting material. The fractions containing product were concentrated *in vacuo* and dried overnight to obtain C-1-monobenzoate **216** (207 mg, 55%) as a clear oil.

216: $\mathbf{R_f}$ = 0.3 [EtOAc/hex (2:1)]; $\mathbf{[\alpha]^{22}_D}$ -94.3 (*c* 7.1, DCM); **IR (DCM)** *v* 3054, 1724, 1514, 1264, 896, 731, 703; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.85 (d, *J* = 7.1 Hz, 2H), 7.50 (t, 1H), 7.36 (t, 2H), 6.54 (d, *J* = 1.4 Hz, 1H), 6.52 (d, *J* = 1.4 Hz, 1H), 5.86 (d, *J* = 1.5 Hz, 1H), 5.84 (d, *J* = 1.5 Hz, 1H), 5.56 (d, *J* = 12.9 Hz, 1H), 5.24 (d, *J* = 9.7 Hz, 1H), 4.40 (s, 1H), 4.36 (s, 1H), 4.14 – 4.11 (m, 1H), 3.78 (s, 3H), 3.55 (s, 3H), 3.35 (s, 1H), 1.65 (s, 3H), 1.39 (s, 3H); **^{13}C NMR (151 MHz, CDCl3) δ** 165.4, 156.5, 148.9, 143.5, 134.3, 133.3, 129.8, 129.7, 128.5, 109.7, 108.0, 102.7, 101.4, 76.5, 75.1, 69.3, 60.5, 56.5, 55.4, 52.3, 29.8, 27.6, 25.5, 21.1, 14.3; **HRMS (EI)** $C_{26}H_{29}NO_{10}$: 515.1791, found: 515.1786; **LRMS (EI)** *m/z* (relative intensity) 515 (M^+, 4), 483 (54), 393 (86), 369 (58), 364 (31), 318 (43), 296 (76), 293 (63), 260 (50), 231 (36), 211 (25), 197 (35), 183 (34), 169 (48), 153 (100), 127 (74), 105 (70); **anal.** calcd for $C_{26}H_{29}NO_{10}$: C, 60.58; H, 5.67; found C, 62.72; H, 6.25; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



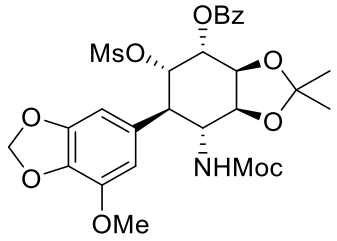
(3aS,4R,5R,6S,7S,7aS)-6-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl benzoate (217)

Methyl-((3aS,4R,5R,6S,7S,7aR)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate (0.73 mmol, 0.30 g, 1.00 eq) was concentrated to dryness, then backfilled thrice with alternating cycles of vacuum and argon. Distilled DCM (45 mL) was added, followed by addition of triethylamine (0.36 mmol, 40 µL, 0.50 eq) using a micropipette. A catalytic amount of DMAP was added (spatula tip) and the system was cooled to 0° C. An anhydrous solution of benzoic anhydride (0.36 mmol, 0.083 g, 0.50 eq) in 0.3 mL distilled DCM was prepared separately, then added dropwise to the system. A TLC in 2:1 EA/hex was checked immediately, monitoring for the formation of the dibenzoate, rather than the consumption of starting material. After 20 minutes, the reaction was quenched with a saturated

solution of $NaHCO_3$ (2 mL), then extracted five times with DCM (20 mL). The organic layers were combined and dried under sodium sulfate, then concentrated *in vacuo*.

The crude product was concentrated on 10% deactivated silica and applied to a gradient column [EtOAc/hex (1:3 to 1:1)] until all benzoylated products eluted, then flushed with EtOAc to recover starting material. The fractions containing product were concentrated *in vacuo* and dried overnight to obtain C-2-monobenzoate **217** (38 mg, 10%) as a clear oil.

217: $\mathbf{R_f}$ = 0.4 [EtOAc/hex (2:1)]; $\mathbf{[\alpha]^{21}_D}$ -157.2 (*c* 1.7, MeOH); **IR (DCM)** *v* 3369, 3055, 1727, 1637, 1514, 1453, 1435, 1373, 1265, 1239, 1046, 932, 851, 732, 702; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.10 (d, *J* = 7.4 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 6.47 (d, *J* = 4.8 Hz, 2H), 5.96 (d, *J* = 4.7 Hz, 2H), 5.83 (s, 1H), 4.71 (d, *J* = 8.1 Hz, 1H), 4.45 – 4.36 (m, 2H), 4.27 (dd, *J* = 9.6, 2.6 Hz, 1H), 3.90 (s, 3H), 3.87 – 3.81 (m, 1H), 3.58 (s, 3H), 3.15 (d, *J* = 64.8 Hz, 1H), 1.81 (s, 1H), 1.63 (s, 3H), 1.40 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)** δ 165.9, 156.6, 149.4, 143.9, 134.8, 133.7, 132.3, 130.0, 129.7, 128.8, 110.1, 102.3, 101.7, 75.2, 71.7, 71.4, 56.8, 56.3, 52.3, 32.1, 29.8, 29.8, 28.2, 26.1, 26.1, 14.3; **HRMS (EI)** $C_{26}H_{29}NO_{10}$: 515.1791, found: 515.1786; **LRMS (EI)** *m/z* (relative intensity) 500 ($[M\text{-}CH_3]^+$, 5) 482 (30), 393 (46), 363 (29), 317 (24), 296 (94), 293 (30), 260 (24), 211 (25), 197 (32), 183 (33), 169 (50), 155 (63), 153 (100), 141 (61), 127 (73), 111 (73); **anal.** calcd for $C_{26}H_{29}NO_{10}$: C, 60.58; H, 5.67; found C, 63.94; H, 6.65; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



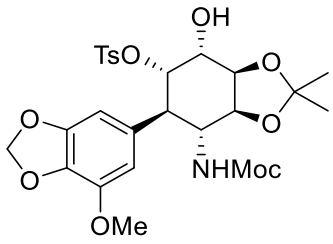
(3aS,4R,5R,6S,7R,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-6-((methylsulfonyl)oxy)-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl benzoate (219)

(3aS,4R,5R,6S,7S,7aS)-6-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl benzoate was charged to a 25-mL round bottomed flask and placed under high vacuum for 1 h to yield (0.025 mmol, 0.013 g, 1.00 eq). The flask was backfilled thrice with alternating cycles of vacuum and argon, then distilled DCM (2.0 mL) was added. Triethylamine was added (0.025 mmol, 4.0 µL, 1.0 eq), and the system was cooled to 0 °C. A solution of mesyl chloride (20 µL) in distilled DCM (1.0 mL) was prepared separately in anhydrous conditions, then 0.1 mL of the solution (0.025 mmol, 2 µL, 1.0 eq) was added dropwise to the system, followed by warming to room temperature. The reaction turned light yellow upon addition of mesyl chloride. A TLC in [EtOAc/hex (1:1)] showed complete conversion of starting material after 10 min. The reaction was extracted five times with DCM, and the organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain 18 mg of crude product as an off-yellow oil.

The crude product was dissolved in 2 mL of DCM and applied to a prep TLC plate. The plate was eluted twice with 1:2 EA/hex, followed by once with 1:1 EA/hex. The major spot visible under UV light was isolated and stirred with DCM for 10 min. Next, vacuum filtration with a fritted funnel, using DCM as the solvent, resulted in a fraction that was concentrated *in vacuo* and placed on high vacuum overnight to result in 7 mg (47%) of mesylate **219** as a clear oil.

219: $\mathbf{R_f}$ = 0.2 [EtOAc/hex (1:1)]; $\mathbf{[\alpha]^{21}_D}$ -134.7 (*c* 3.0, DCM); **IR (DCM)** *v* 3392, 3055, 2986, 2927, 1725, 1515, 1452, 1436, 1360, 1264, 1095, 959, 732, 703; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.11 (d, *J* = 7.2 Hz, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 2H), 6.53 (d, *J* = 1.2 Hz, 2H), 5.96 (d, *J* = 1.6 Hz, 2H), 5.87 (t, *J* = 3.0 Hz, 1H), 5.19 (dd, *J* = 8.7, 2.8 Hz, 1H), 4.84 (d, *J* = 8.8

Hz, 1H), 4.49 (d, *J* = 3.2 Hz, 2H), 3.91 (m, 4H), 3.60 (s, 3H), 3.46 (s, 1H), 2.62 (s, 3H), 1.63 (s, 3H), 1.39 (s, 3H); **^{13}C NMR (75 MHz, CDCl3) δ** 165.2, 156.4, 149.3, 143.9, 135.0, 133.9, 132.0, 130.1, 129.2, 128.8, 110.6, 108.1, 102.7, 101.8, 80.6, 74.9, 70.7, 56.9, 56.1, 52.4, 38.5, 27.9, 25.8; **HRMS (EI)** calcd for $C_{27}H_{31}NO_{12}S$: 593.1561, found: 593.1568; **LRMS (EI)** *m/z* (relative intensity) 593 (M^+, 2), 561 (29), 497 (35), 364 (94), 317 (48), 285 (37), 275 (30), 105 (100).

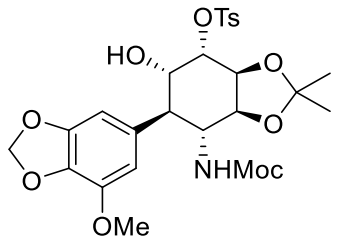


(3aS,4R,5R,6S,7R,7aR)-7-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl 4-methylbenzenesulfonate (221)

Methyl ((3a*S*,4*R*,5*R*,6*S*,7*S*,7a*R*)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate**)** was charged to a 100-mL round bottomed flask and placed under high vacuum for 1 h to yield (0.17 mmol, 0.068 g, 1.00 eq). The flask was backfilled thrice with alternating cycles of vacuum and argon, then distilled DCM (32 mL) was added. Once fully dissolved, triethylamine (0.17 mmol, 23 µL, 1.00 eq) was added, followed by a catalytic amount of DMAP (spatula tip). An anhydrous solution of tosyl chloride (0.17 mmol, 0.032 g, 1.00 eq) in 0.2 mL distilled DCM was prepared separately, then added dropwise to the system at room temperature. The reaction mixture was stirred for 2 h and monitored for the formation of ditosylate.

The reaction was quenched with saturated NH_4Cl and extracted five times with DCM (20 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain the crude product. The crude material was concentrated onto 10% deactivated silica and subjected to a gradient column [EtOAc/hex (1:2 to 2:1)]. The fractions were concentrated *in vacuo* and placed under high vacuum to obtain 21 mg (0.85:1 ratio) of inseparable regioisomers **221** and **222** (22%) as a colourless oil and recovered starting material *cis*-diol **6** as a colourless oil (50%).

221: $\mathbf{R_f}$ = 0.6 [EtOAc/hex (2:1)]; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.31 (d, *J* = 5.5 Hz, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.22 (d, *J* = 12.9 Hz, 2H), 5.90 (s, 2H), 5.38 (d, *J* = 9.8 Hz, 1H), 4.69 (d, *J* = 10.7 Hz, 1H), 4.50 (s, 1H), 4.41 (d, *J* = 6.0 Hz, 1H), 4.21 (d, *J* = 7.0 Hz, 1H), 3.71 (s, 3H), 3.56 (s, 3H), 3.15 (d, *J* = 3.5 Hz, 1H), 3.02 (dd, *J* = 10.4, 5.7 Hz, 1H), 2.41 (s, 3H), 1.70 (s, 1H), 1.58 (s, 3H), 1.36 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 156.4, 156.1, 149.1, 148.3, 145.3, 144.8, 143.7, 143.3, 134.6, 134.1, 133.6, 132.8, 132.1, 131.9, 129.9, 129.2, 128.3, 127.6, 110.0, 109.3, 105.3, 101.5, 101.3, 88.5, 83.1, 79.2, 74.8, 70.7, 70.1, 56.6, 55.9, 52.2, 49.1, 47.6, 27.7, 27.0, 25.8, 24.8, 23.0, 21.7, 21.6; inseparable mixture of regioisomers.



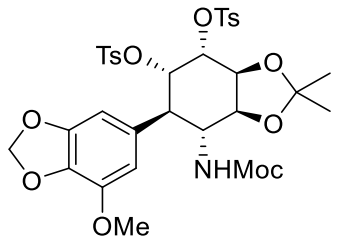
(3aS,4R,5R,6S,7S,7aS)-6-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl 4-methylbenzenesulfonate (222)

Methyl ((3a*S*,4*R*,5*R*,6*S*,7*S*,7a*R*)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate**)** was charged to a 100-mL round bottomed flask and placed under high vacuum for 1 h to yield (0.17 mmol, 0.068 g, 1.00 eq). The flask was backfilled thrice with alternating cycles of vacuum and argon, then distilled DCM (32 mL) was added. Once fully dissolved, triethylamine (0.17 mmol, 23 µL, 1.00 eq) was added, followed by a catalytic amount of DMAP (spatula tip). An anhydrous solution of tosyl chloride (0.17 mmol, 0.032 g, 1.00 eq) in 0.2 mL distilled DCM was prepared separately, then added dropwise to the system at room temperature. The reaction mixture was stirred for 2 h and monitored for the formation of ditosylate.

The reaction was quenched with saturated NH_4Cl and extracted five times with DCM (20 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain the crude product. The crude material was concentrated onto 10% deactivated silica and subjected to a gradient column [EtOAc/hex (1:2 to 2:1)]. The fractions were concentrated *in vacuo*

and placed under high vacuum to obtain 21 mg (0.85:1 ratio) of inseparable regioisomers **221** and **222** (22%) as a colourless oil and recovered starting material *cis*-diol **6** as a colourless oil (50%).

222: $\mathbf{R_f}$ = 0.6 [EtOAc/hex (2:1)]; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.84 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 5.4 Hz, 2H), 6.39 (s, 2H), 5.93 (s, 2H), 4.94 (s, 1H), 4.75 (d, *J* = 9.6 Hz, 1H), 4.37 (s, 1H), 4.25 (d, *J* = 5.2 Hz, 1H), 4.08 (s, 1H), 3.97 – 3.89 (m, 1H), 3.86 (s, 3H), 3.53 (s, 3H), 2.87 (s, 1H), 2.43 (s, 3H), 1.80 (d, *J* = 5.0 Hz, 1H), 1.51 (s, 3H), 1.32 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 156.4, 156.1, 149.1, 148.3, 145.3, 144.8, 143.7, 143.3, 134.6, 134.1, 133.6, 132.8, 132.1, 131.9, 129.9, 129.2, 128.3, 127.6, 110.0, 109.3, 105.3, 101.5, 101.3, 88.5, 83.1, 79.2, 74.8, 70.7, 70.1, 56.6, 55.9, 52.2, 49.1, 47.6, 27.7, 27.0, 25.8, 24.8, 23.0, 21.7, 21.6; inseparable mixture of regioisomers.



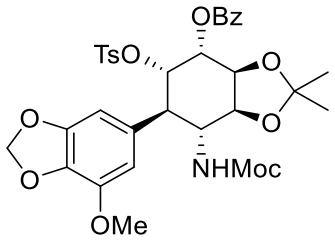
(3aS,4R,6S,7R,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxole]-6,7-diyl bis(4-methylbenzenesulfonate) (220)

Methyl ((3a*S*,4*R*,5*R*,6*S*,7*S*,7a*R*)-6,7-dihydroxy-7'-methoxy-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-4-yl)carbamate**)** was charged to a 100-mL round bottomed flask and placed under high vacuum for 1 h to yield (0.17 mmol, 0.068 g, 1.00 eq). The flask was backfilled thrice with alternating cycles of vacuum and argon, then distilled DCM (32 mL) was added. Once fully dissolved, triethylamine (0.17 mmol, 23 µL, 1.00 eq) was added, followed by a catalytic amount of DMAP (spatula tip). An anhydrous solution of tosyl chloride (0.17 mmol, 0.032 g, 1.00 eq) in 0.2 mL distilled DCM was prepared separately, then added dropwise to the system at room temperature. The reaction mixture was stirred for 2 h and monitored for the formation of ditosylate.

The reaction was quenched with saturated NH_4Cl and extracted five times with DCM (20 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to

obtain the crude product. The crude material was concentrated onto 10% deactivated silica and subjected to a gradient column [EtOAc/hex (1:2 to 2:1)]. The fractions were concentrated *in vacuo* and placed under high vacuum to obtain 10 mg of ditosylate **220** (8%) as a colourless oil.

220: $\mathbf{R_f}$ = 0.93 [EtOAc/hex (2:1)]; $\mathbf{[\alpha]^{21}_D}$ -14.8 (*c* 0.3, DCM); **IR (DCM)** *v* 3386, 2926, 1712, 1515, 1370, 1177, 1019, 849, 671; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.90 (d, *J* = 8.2 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.1 Hz, 2H), 6.14 (d, *J* = 4.7 Hz, 2H), 5.90 (d, *J* = 6.1 Hz, 2H), 5.01 (s, 2H), 4.66 (dd, *J* = 5.8, 2.4 Hz, 1H), 4.54 (d, *J* = 8.7 Hz, 1H), 4.29 (t, *J* = 5.3 Hz, 1H), 4.24 – 4.10 (m, 1H), 3.69 (s, 3H), 3.59 (s, 3H), 2.86 (dd, *J* = 10.1, 5.5 Hz, 1H), 2.47 (s, 3H), 2.38 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 181.3, 172.8, 159.9, 150.8, 149.1, 148.4, 145.8, 144.9, 143.4, 134.3, 132.8, 130.2, 129.2, 128.5, 127.7, 109.8, 101.3, 81.9, 78.3, 77.9, 75.5, 75.0, 68.6, 55.9, 53.4, 52.3, 50.9, 48.1, 29.7, 26.8, 24.6, 21.7, 21.5; **HRMS (EI)** calcd for $C_{33}H_{37}NO_{13}S_2$: 719.1706, found: 719.1707; **LRMS (EI)** *m/z* (relative intensity) 719 (M^+, 4), 687 (37), 663 (35), 547 (100), 435 (41), 317 (60), 153 (53), 141 (36), 127 (43), 113 (40).



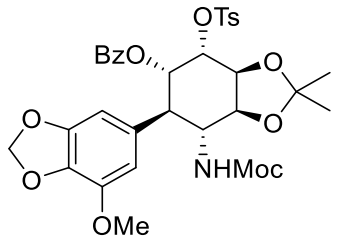
(3aS,4R,5R,6S,7R,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-6-(tosyloxy)-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl benzoate (7)

The mixture of regioisomers (3aS,4R,5R,6S,7R,7aR)-7-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl 4-methylbenzenesulfonate and 3aS,4R,5R,6S,7S,7aS)-6-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl 4-methylbenzenesulfonate was charged to 25-mL round-bottomed flask and placed under high vacuum for 1 h to yield (0.06 mmol, 0.033 g, 1.00 eq). Distilled DCM (5 mL) was added to dissolve

the substrate, followed by NEt_3 (0.06 mmol, 8 µL, 1.00 eq) and a catalytic amount of DMAP (spatula tip). An anhydrous solution of benzoic anhydride (0.06 mmol, 0.013 g, 1.00 eq) was prepared and added dropwise to the reaction mixture at room temperature.

The reaction was stirred for 17 h, quenched with a saturated solution of $NaHCO_3$ (10 mL), then extracted five times with DCM (10 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain a crude mixture. The mixture was applied to prep TLC and eluted four times with [EtOAc/hex (1:2)] to separate the regioisomers. The silica was filtered off using a fritted funnel and the fraction of filtrate was concentrated *in vacuo* to provide 15 mg of tosylate **7** (38%) as a clear oil.

7: $\mathbf{R_f}$ = 0.3 [EtOAc/hex (1:1)]; $\mathbf{[\alpha]^{22}_D}$ -46.6 (*c* 0.7, DCM); **IR (DCM)** *v* 2929, 1734, 1373, 1244, 1047, 847, 714; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.99 (d, *J* = 7.5 Hz, 2H), 7.61 (t, 1H), 7.52 – 7.41 (m, 4H), 7.02 (d, *J* = 7.9 Hz, 2H), 6.32 (d, *J* = 5.5 Hz, 2H), 5.95 (s, 2H), 5.65 (s, 1H), 4.94 (d, *J* = 4.0 Hz, 1H), 4.72 (d, *J* = 8.3 Hz, 1H), 4.58 (t, *J* = 5.5 Hz, 1H), 4.42 (t, *J* = 7.3 Hz, 1H), 3.99 – 3.88 (m, 1H), 3.84 (s, 3H), 3.56 (s, 3H), 3.24 (s, 1H), 2.27 (s, 3H), 1.58 (s, 3H), 1.39 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 165.2, 156.4, 148.9, 145.0, 143.7, 134.7, 133.7, 132.8, 132.2, 130.1, 129.7, 129.3, 128.6, 127.8, 110.5, 107.4, 102.5, 101.6, 81.0, 76.4, 74.6, 71.5, 56.5, 55.2, 53.6, 52.4, 29.8, 27.7, 25.5, 21.7; **HRMS (EI)** calcd for $C_{33}H_{35}NO_{12}S$: 669.1880, found: 669.1868; **LRMS (EI)** *m/z* (relative intensity) 669 (M+, 1.3), 637 (100), 515 (11), 497 (49), 364 (47), 337 (34), 317 (21), 285 (16), 105 (33); **anal.** calcd for $C_{33}H_{35}NO_{12}S$: C, 59.19; H, 5.27; found C, 51.42; H, 5.54; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



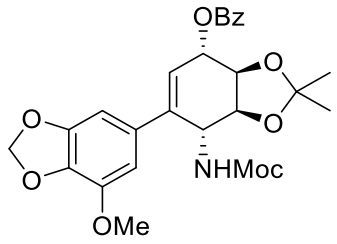
(3aS,4R,5R,6S,7S,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-7-(tosyloxy)-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl benzoate (218)

The mixture of regioisomers (3aS,4R,5R,6S,7R,7aR)-7-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl 4-methylbenzenesulfonate and 3aS,4R,5R,6S,7S,7aS)-6-hydroxy-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl 4-methylbenzenesulfonate was charged to 25-mL round-bottomed flask and placed under high vacuum for 1 h to yield (0.06 mmol, 0.033 g, 1.00 eq). Distilled DCM (5 mL) was added to dissolve the substrate, followed by NEt_3 (0.06 mmol, 8 µL, 1.00 eq) and a catalytic amount of DMAP (spatula tip). An anhydrous solution of benzoic anhydride (0.06 mmol, 0.013 g, 1.00 eq) in distilled DCM (0.1 mL) was prepared and added dropwise to the reaction mixture at room temperature.

The reaction was stirred for 17 h, quenched with a saturated solution of $NaHCO_3$ (10 mL), then extracted five times with DCM (10 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain a crude mixture. The mixture was applied to prep TLC and eluted four times with [EtOAc/hex (1:2)] to separate the regioisomers. The silica was filtered off using a fritted funnel and the filtrate was concentrated *in vacuo* to provide 5 mg of tosylate **218** as a clear oil (13%).

218: $\mathbf{R_f}$ = 0.3 [EtOAc/hex (1:1)]; $\mathbf{[\alpha]^{22}_D}$ -43.2 (*c* 0.95, DCM); **IR (DCM)** *v* 2924, 1725, 1374, 1179, 1048, 929, 840; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.68 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.51 (t, *J* = 7.3 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 6.93 (d, *J* = 8.1 Hz, 2H), 6.45 (s, 2H), 5.85 (d, *J* = 2.2 Hz, 2H), 5.41 (dd, *J* = 11.0, 2.1 Hz, 1H), 5.02 (s, 1H), 4.79 (s, 1H), 4.57 (d, *J* = 5.1 Hz, 1H), 4.37 (s, 1H), 4.15 (s, 1H), 3.80 (s, 3H), 3.59 (s, 3H), 3.23 (s, 1H), 2.11 (s, 3H), 1.65 (s, 3H), 1.39 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 165.0, 156.4, 149.0, 145.3, 143.6, 134.5, 133.3, 132.6, 132.2, 130.0, 129.8, 129.2, 128.3, 128.1, 110.5, 107.9, 102.5, 101.5, 75.5, 71.6, 56.6,

53.6, 52.4, 46.7, 32.1, 29.9, 29.5, 27.7, 25.8, 22.9, 22.8, 21.7, 14.3; **HRMS (EI)** calcd for $C_{33}H_{35}NO_{12}S$: 669.1880, found: 669.1873; **LRMS (EI)** *m/z* (relative intensity) 669 (M^+, 1.7), 637 (15), 547 (75), 515 (100), 317 (14), 243 (11); **anal.** calcd for $C_{33}H_{35}NO_{12}S$: C, 59.19; H, 5.27; found C, 50.01; H, 5.00; decomposition of the material at room temperature is likely the reason for failed combustion analysis.



(3aS,4R,7S,7aR)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-3a,4,7,7a-tetrahydro-[5,5'-bibenzo[d][1,3]dioxol]-7-yl benzoate (56)

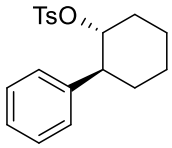
A 15-mL round-bottom flask was subjected to Schlenk technique with flame drying, then placed under argon for 5 min. 18-C-6 was added (0.05 mmol, 0.012 g, 0.43 eq), then dissolved with distilled CH_3CN (0.3 mL, 0.15 M concentration of 18-C-6). Once the crown ether was fully dissolved, KOAc was added (0.21 mmol, 0.021 g, 2.00 eq) and stirred for 30 min until the solution became cloudy.

In a separate flask, the regioisomer mixture of (3aS,4R,5R,6S,7R,7aS)-7'-methoxy-4-((methoxycarbonyl)amino)-2,2-dimethyl-6-(tosyloxy)-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo [d][1,3]dioxol]-7-yl benzoate and (3aS,4R,5R,6S,7S,7aS)-7'-methoxy-4-((methoxycarbonyl) amino)-2,2-dimethyl-7-(tosyloxy)-3a,4,5,6,7,7a-hexahydro-[5,5'-bibenzo[d][1,3]dioxol]-6-yl benzoate was subjected to Schlenk technique without flame drying, then dissolved with CH_3CN (0.3 mL). This solution was added dropwise to the crown ether/KOAc suspension at 0 °C. The reaction mixture was heated to reflux for 2 days, showing consumption of starting material.

The reaction was quenched with H_2O (5 mL) and extracted five times with DCM (10 mL). The organic layer was washed with sodium sulfate, filtered, and concentrated *in vacuo* to obtain the crude product. This crude product was concentrated onto 10% deactivated silica and subjected to column chromatography with [EtOAc/hex (1:3)] to elute the product. The fractions were

concentrated *in vacuo* and placed under high vacuum overnight to obtain ~1 mg of benzoate **56** as a light-yellow oil.

56: The spectral and physical properties of the product match those reported in the literature.[23] **R_f** = 0.3 [EtOAc/hex (1:2)]; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.01 (d, *J* = 7.5 Hz, 2H), 7.60 (t, 1H), 7.46 (t, 2H), 6.71 (d, *J* = 11.7 Hz, 2H), 6.45 (d, *J* = 6.5 Hz, 1H), 5.97 (s, 2H), 5.69 (d, *J* = 6.3 Hz, 1H), 5.29 (d, *J* = 9.7 Hz, 1H), 4.99 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 8.9 Hz, 1H), 4.67 (d, *J* = 6.4 Hz, 1H), 3.90 (s, 3H), 3.70 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H); **^{13}C NMR (151 MHz, $CDCl_3$)** δ 156.3, 146.4, 143.9, 133.7, 129.4, 128.6, 121.4, 108.7, 106.0, 101.9, 100.0, 77.2, 74.6, 68.4, 56.6, 52.9, 49.9, 26.0, 24.2; **HRMS (EI+)** calcd for $C_{26}H_{27}NO_9$: 497.1686, found: 497.1680; **LRMS (EI)** *m/z* (relative intensity) 497 (M^+, 48), 364 (97), 244 (50), 169 (50), 153 (100), 141 (61), 127 (73), 113 (69), 85 (71), 71 (53).

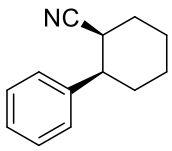


(1R,2S)-2-phenylcyclohexyl 4-methylbenzenesulfonate (226)

(1R,2S)-2-phenylcyclohexan-1-ol (1.70 mmol, 0.30 g, 1.00 eq) was charged to a 100-mL round-bottomed flask and subjected to high vacuum. Dry pyridine (10 mL) was added and the system was allowed to stir for 24 h, after which the cloudy white solution was poured into cold distilled water, forming a white precipitate. The precipitate was filtered with a Buchner funnel forming a white sticky powder that was washed many times with distilled water, then transferred to a 25-mL round-bottomed flask and placed under vacuum for 3 h. The product was recrystallized with hot ethanol, washed 3x with cold ethanol, and then placed under high vacuum overnight to afford 335 mg of tosylate **226** a white solid (60%).

226: The spectral and physical properties of the product match those reported in the literature.[60] **R_f** = 0.3 [EtOAc/hex (1:9)]; **mp** = 128-130 °C dec. (EtOH); **^{1}H NMR (300 MHz, $CDCl_3$) δ** 6.40 (d, *J* = 5.5 Hz, 1H), 5.94 (s, 2H), 5.53 (t, 1H), 5.42 (dd, *J* = 9.4 Hz, 1H), 4.70 (d, *J* = 4.9 Hz, 1H),

4.40 (s, 1H), 4.28 (s, 1H), 3.87 (s, 3H), 3.74 (d, *J* = 9.4 Hz, 1H), 3.56 (s, 3H), 3.20 (s, 1H), 2.15 (s, 3H), 1.85 (s, 3H), 1.60 (s, 3H), 1.37 (s, 3H); **^{13}C NMR (75 MHz, CDCl3) δ** 169.7, 156.4, 148.9, 143.6, 134.5, 132.5, 110.2, 107.6, 102.4, 101.6, 75.2, 71.7, 69.5, 56.7, 56.6, 52.3, 28.0, 25.8, 21.1, 20.8.

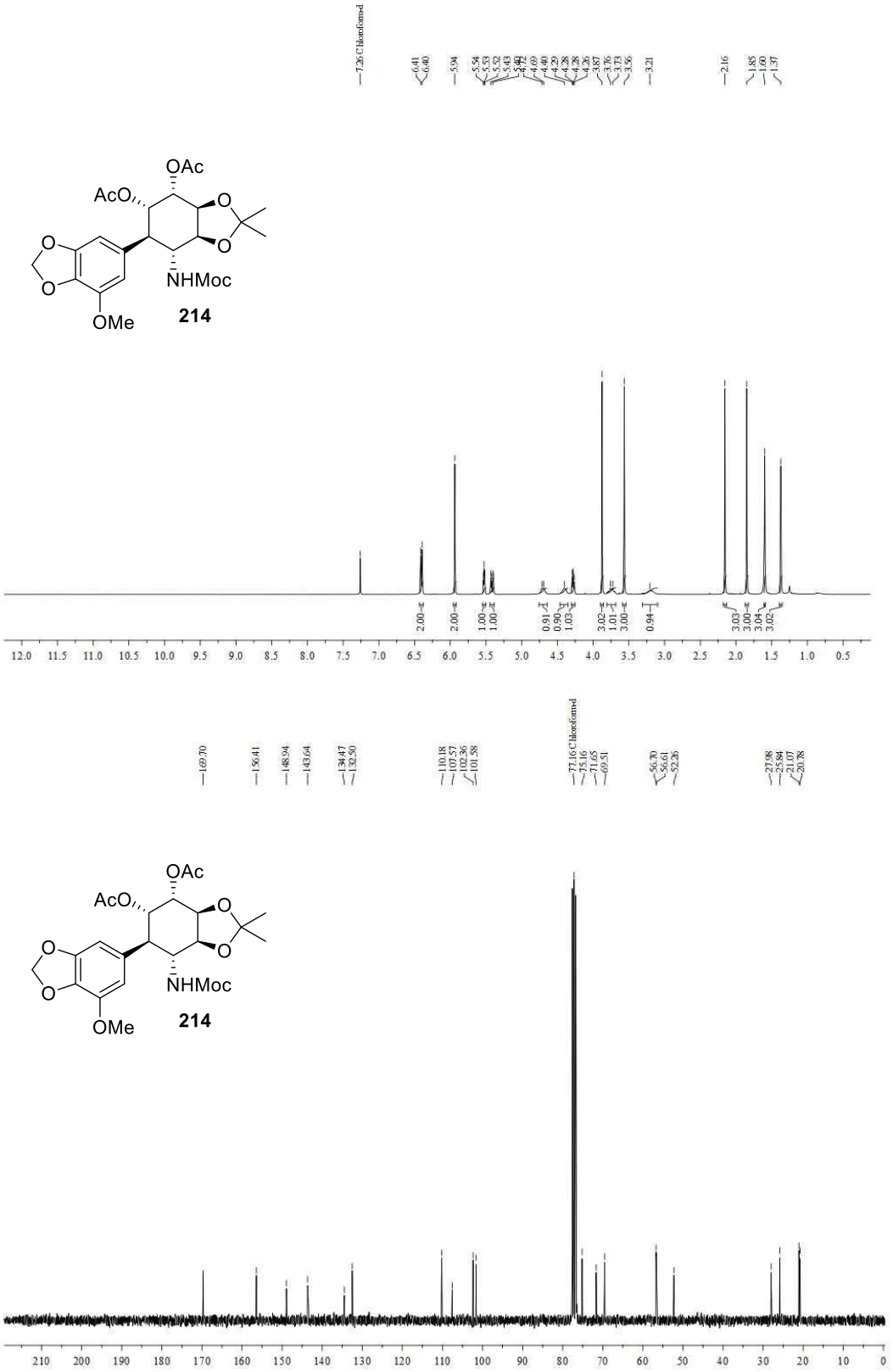


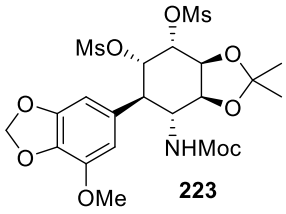
(1S,2R)-2-phenylcyclohexane-1-carbonitrile (227)

(1R,2S)-2-phenylcyclohexyl 4-methylbenzenesulfonate (0.15 mmol, 0.05 g, 1.00 eq) was subjected to alternating vacuum/argon cycles. Distilled DMF (2 mL) was added, forming a white suspension. NaCN (0.23 mmol, 0.037 g, 5.00 eq) was added in one portion, turning the suspension yellow. The system was stirred at room temperature for 1 h, followed by stirring at 100 °C overnight without reflux. TLC in 1:9 EA/hex indicated consumption of starting material. The solution was quenched using 3 mL H_2O, then extracted with ethyl acetate 5 times. The organic layer was dried under sodium sulfate, filtered, and concentrated under reduced pressure to obtain 58 mg of the crude product. This was applied to prep TLC to purify. The plate was eluted twice with 1:9 EA/hex, and the band containing product was collected, filtered using a fritted funnel, and placed under high vacuum to obtain 18 mg of pure nitrile **227** as a light-yellow oil (65%).

227: The spectral and physical properties of the product match those reported in the literature.[61] **R_f** = 0.3 [EtOAc/hex (1:9)]; **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.40 – 7.27 (m, 5H), 3.07 (br, 1H), 2.78 (m, 1H), 2.18 – 1.20 (m, 8H); **^{13}C NMR (75 MHz, $CDCl_3$) δ** 142.5, 128.7, 127.3, 45.0, 36.3, 30.0, 27.5, 25.9, 21.7.

6. SELECTED SPECTRA

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O
O
O
O
NHMoc
OMe

223

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5.09
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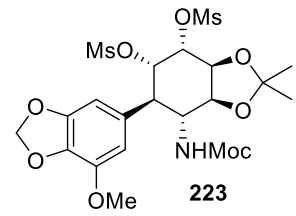
5.04
4.78
4.58
4.57
4.56
4.55
4.37
4.34
4.32
4.18
4.15
3.60
3.20
2.46
1.63
1.40
2.00
2.06
1.99
0.96
1.04
0.98
0.93
2.98
2.93
2.89
1.06
2.90
2.97
3.05
f1 (ppm)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O
O
O
NHMoc
OMe

223

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101.73
98.89
96.50
94.53
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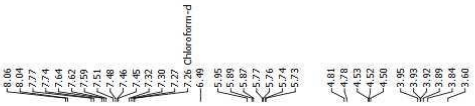
f1 (ppm)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7.46
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7.30
7.27
Chloroform-d
6.49
5.95
5.89
5.87
5.77
5.76
5.74
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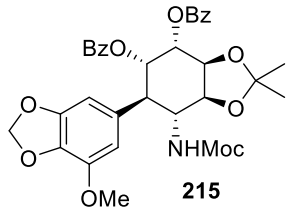
3.84
3.60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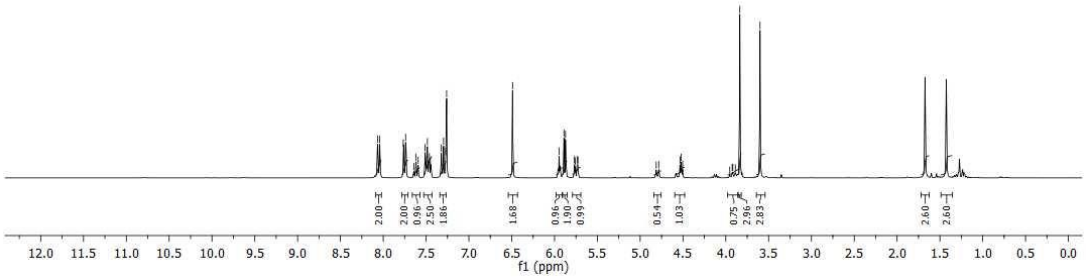
O
O
O
O
NHMoc
OMe

215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0.99
0.54
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2.96
2.83
2.60
2.60
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


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165.17
152.09
149.01
143.65
133.63
133.17
132.30
130.33
130.05
129.70
129.66
129.63
128.78
128.63
128.40
128.41
107.81
102.44
101.55
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70.15
56.74
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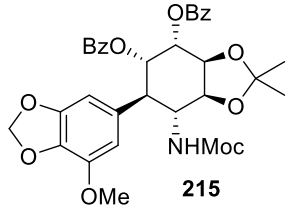
28.14
25.98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O
O
O
NHMoc
OMe

215

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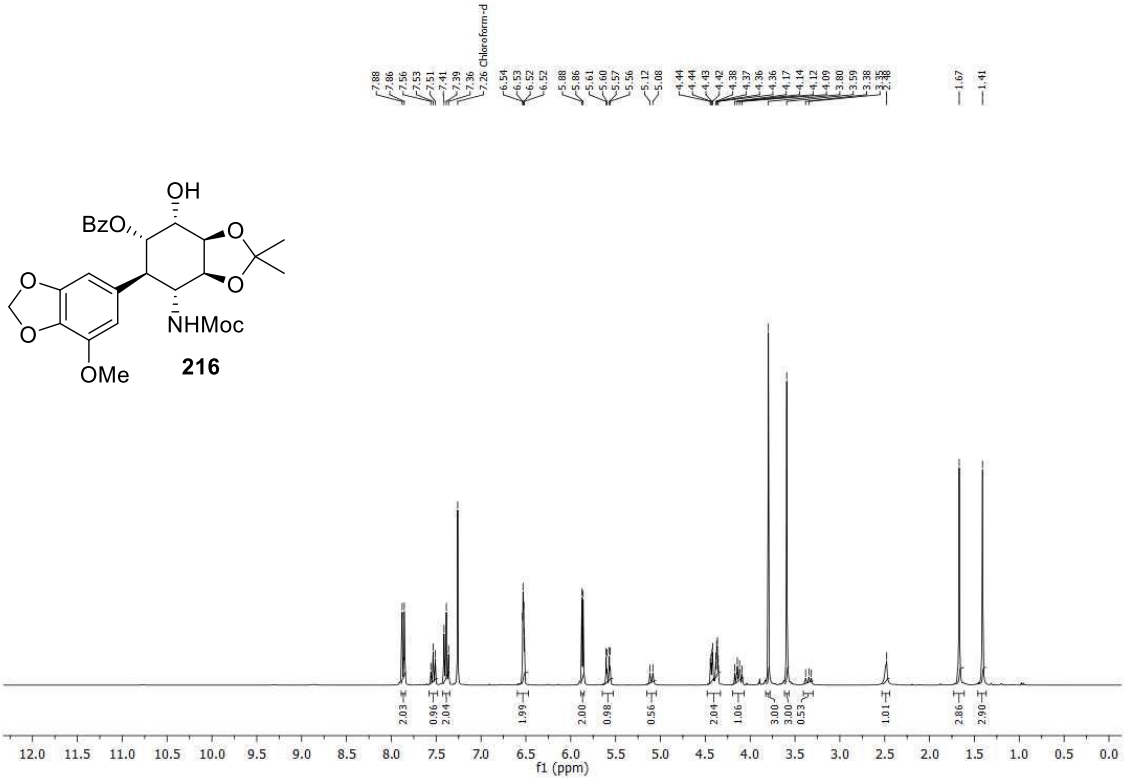
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BzO
O
O
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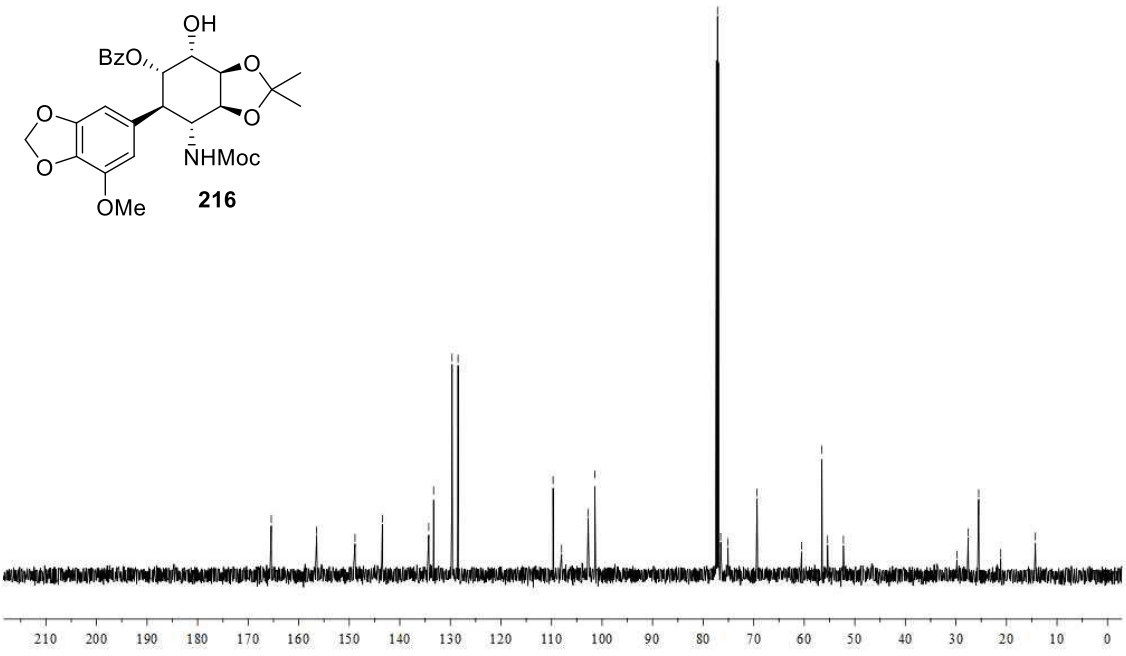
NHMoc
OMe
216
f1 (ppm)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O
O
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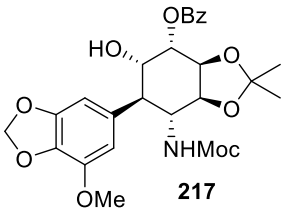
NHMoc
OMe
216



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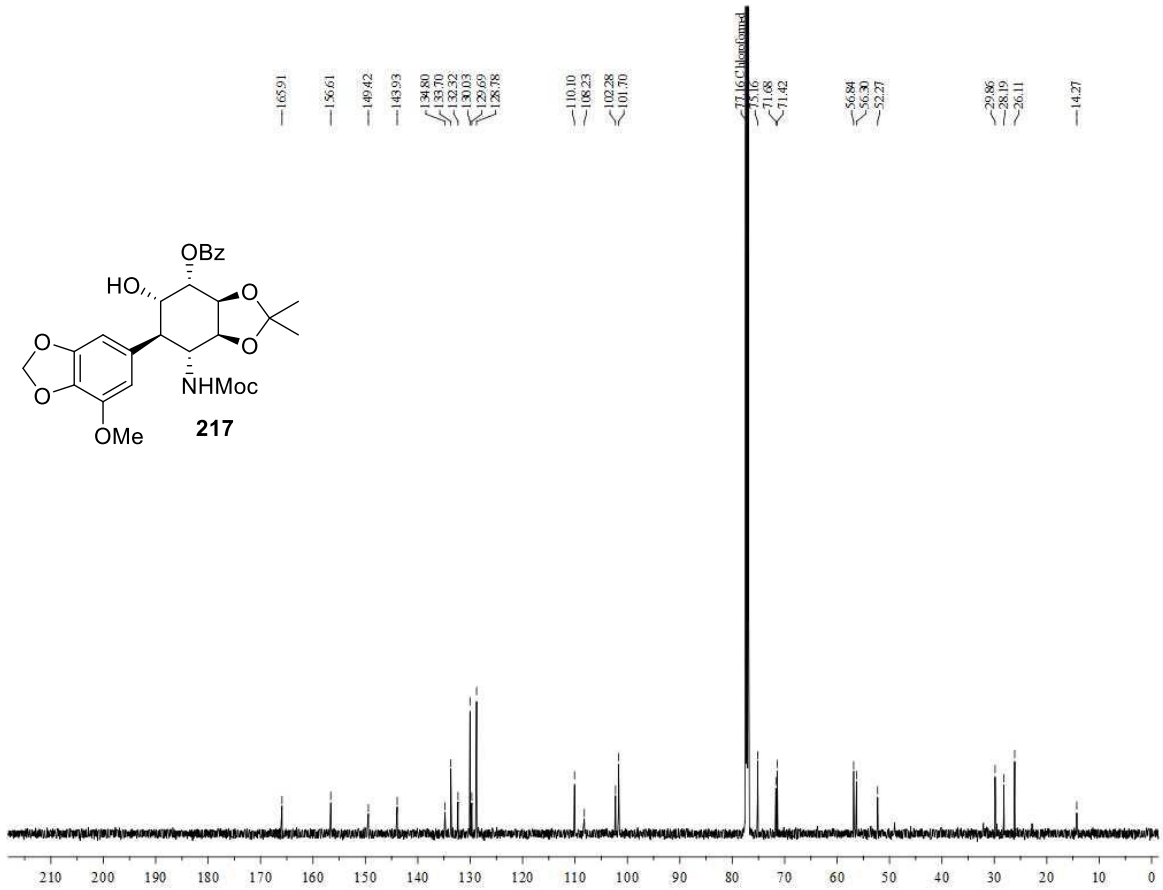
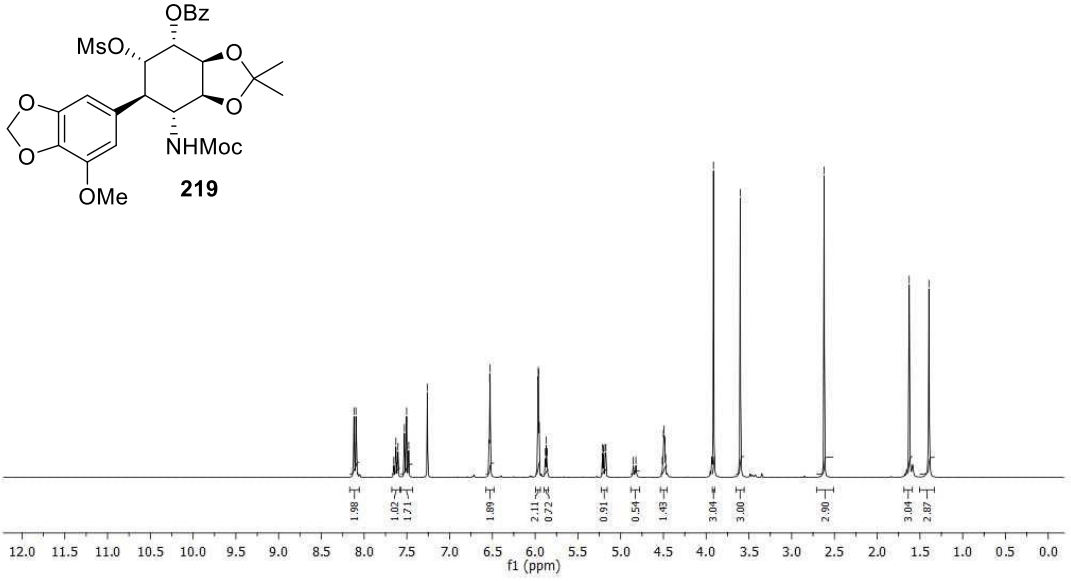


217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7.48
7.26 Chloroform-d
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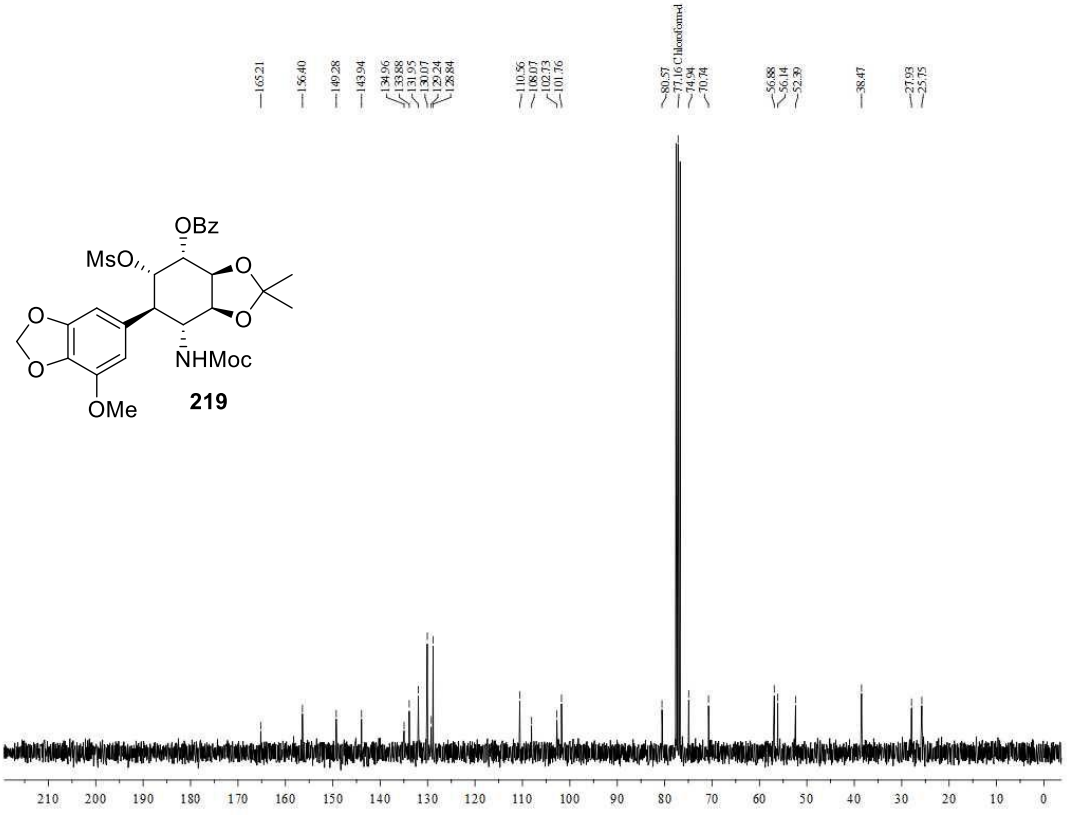
5.96
5.96
5.88
5.87
5.86
5.21
5.18
5.17
4.82
4.51
4.50
4.49
4.48
3.60
2.62
1.63
1.39
OBz
MsO
O
O
O
O
NHMoc
OMe
219
1.98
1.02
1.71
1.89
2.11
0.72
0.91
0.54
1.43
3.04
3.00
2.90
3.04
2.87
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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131.95
130.07
129.24
128.84
110.56
108.07
102.73
101.76
80.57
77.16 Chloroformd
74.94
70.74
56.88
56.14
52.39
38.47
27.93
25.75
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NHMoc
OMe
219
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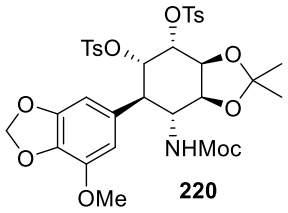
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OMe

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Chloroform-d
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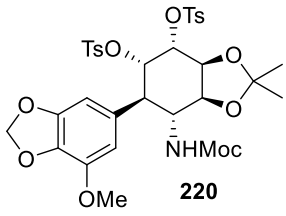
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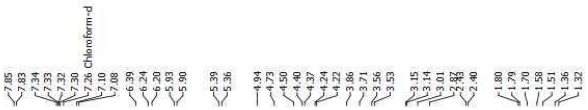

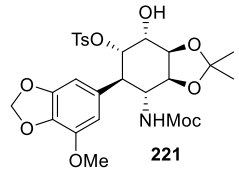

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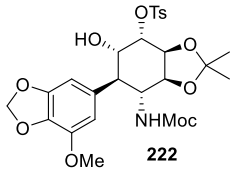

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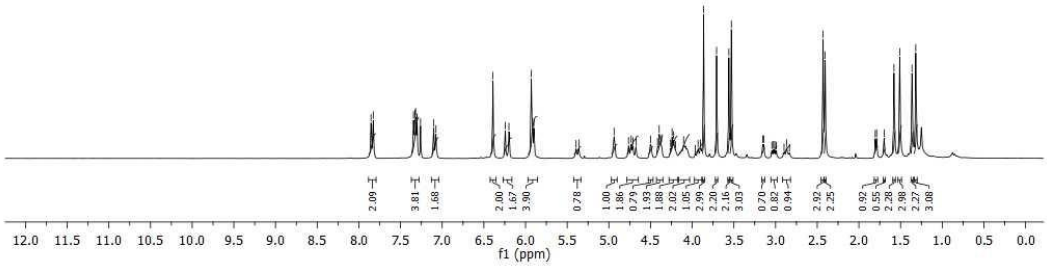

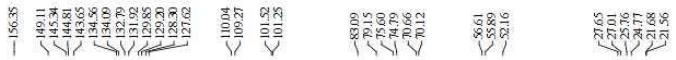

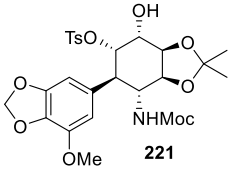

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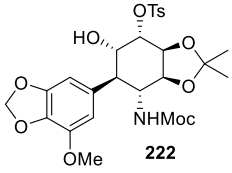

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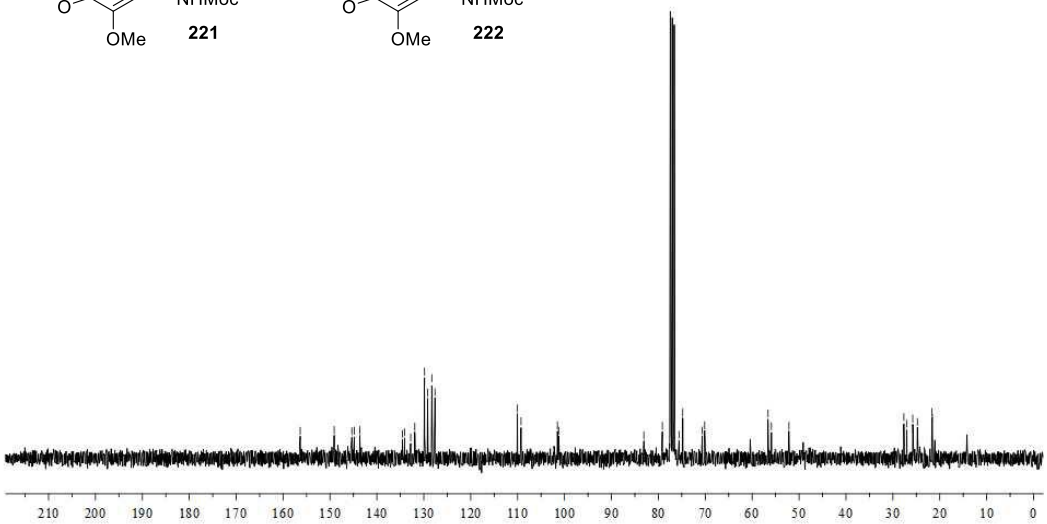

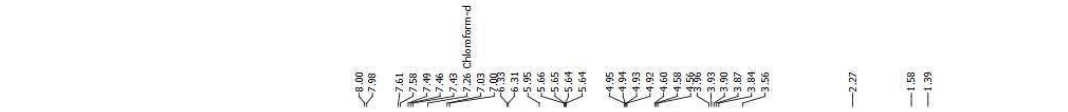
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5.64
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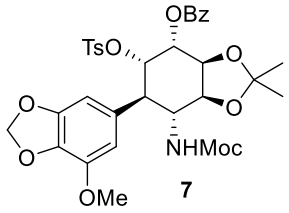
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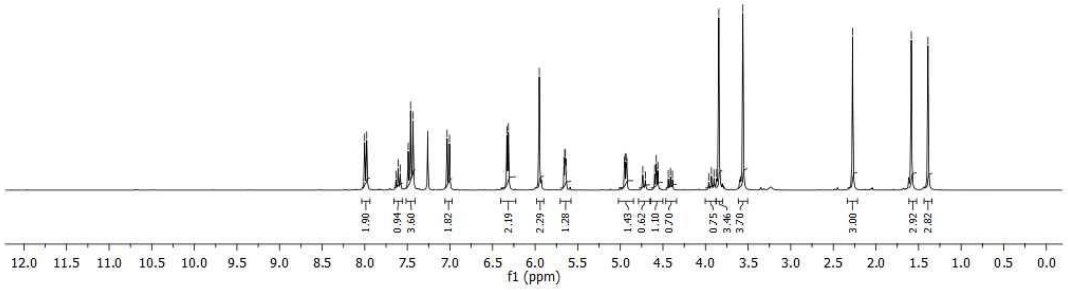
NHMoc
OMe

7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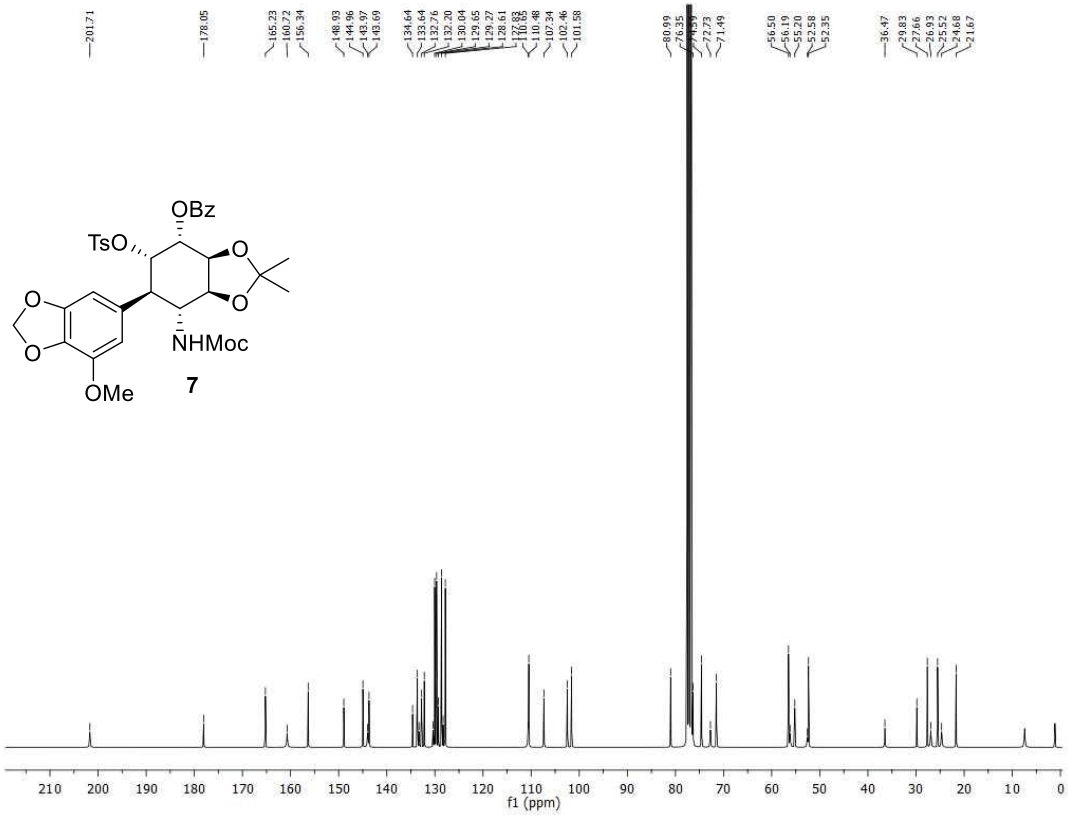
3.46
3.70
3.00
2.92
2.82
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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110.48
107.34
102.46
101.58
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76.35
72.73
71.49
56.50
56.19
55.20
52.58
52.35
36.47
29.83
27.66
26.93
25.52
24.68
21.67
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

OBz
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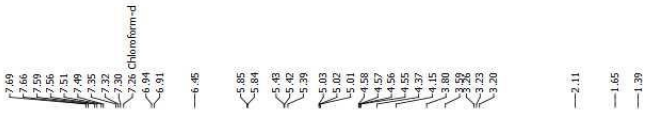
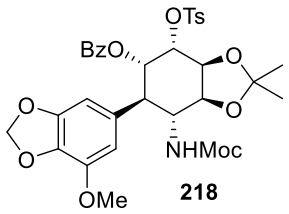
7





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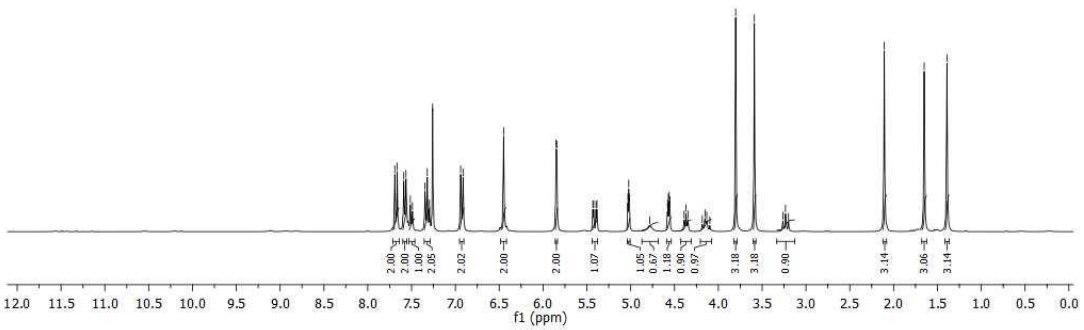

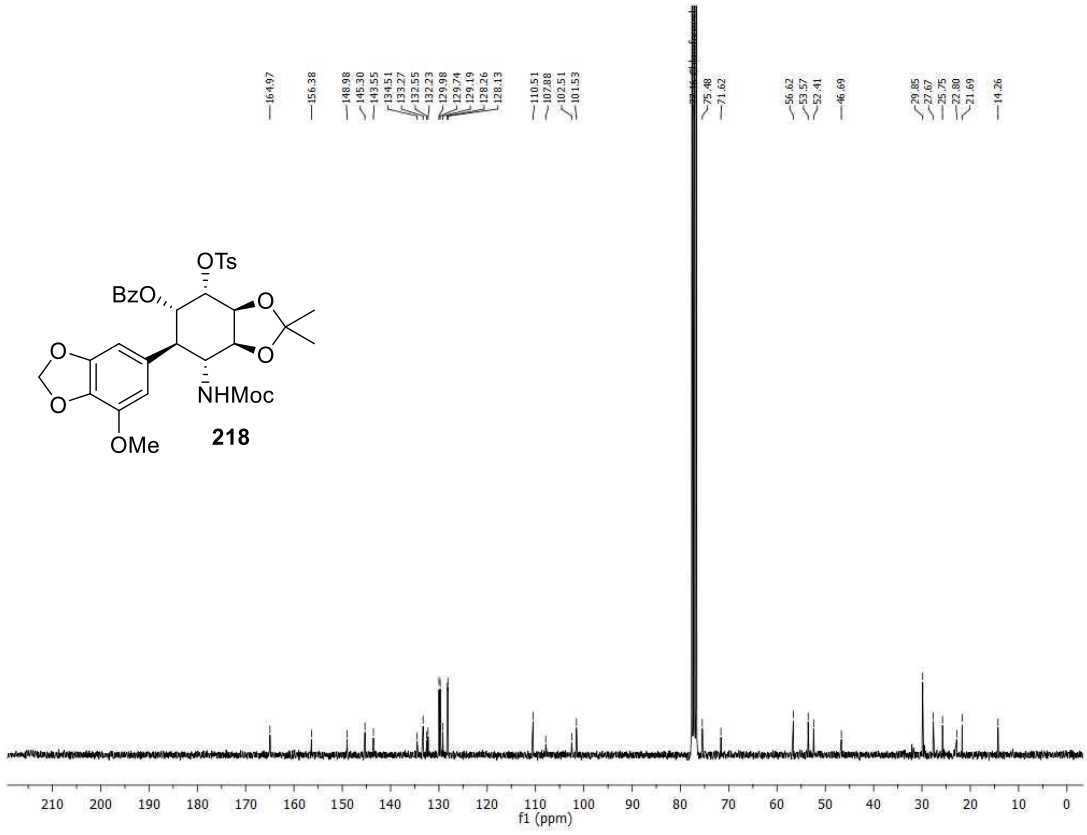

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8. VITA

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