Synthesis and Chemistry of Isoprekinamycin and Analogues

by

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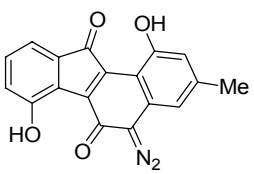
AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

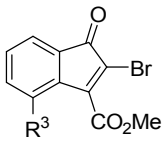
I understand that my thesis may be made electronically available to the public.

Abstract

A methodology was studied to set up the benzo[*a*]fluorene skeleton, which provides a potential route to the total synthesis of isoprekinamycin (IPK) (**1-112**) and analogues of this natural product that contains an unusual diazo group so as to study the relationship of the structure and bioactivities.



IPK



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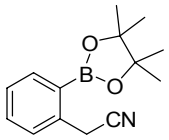

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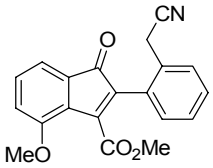

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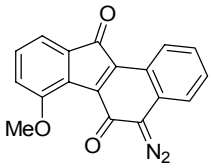
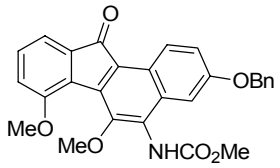


2-58



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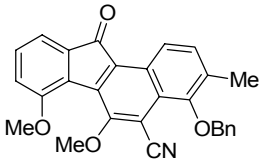

5-28

Basically the strategy involved a Suzuki coupling between the bromoindenone **2-64** as an AB ring building block and the pinacol boronate **2-89** as a D ring building block to generate a diaryl intermediate **2-90** that incorporates the A, B and D ring of isoprekinamycin. The diaryl intermediate was cyclized via a Dieckmann-like reaction to provide the full ABCD benzo[*a*]fluorene ring system that incorporates a cyano group at the carbon atom intended to become the carbon of the diazo group in the final structure. This intermediate was subjected to a sequence of reactions involving hydrolysis of the cyano group to the amide followed by a modified Hofmann rearrangement that provided the C-N bond at the correct position for creation of the diazo group in a three step sequence. This methodology provided a model of

IPK **2-58** with the longest linear path of the synthesis being thirteen steps and in a 7% yield overall.

A total synthesis of IPK which followed the method developed in the model synthesis was then attempted. In this IPK synthesis, the key Dieckmann-like cyclization that proceeded very selectively in the model synthesis proved to be problematic as a result of a competing aldol reaction involving the B-ring ketone group. This problem was eventually solved by reducing the ketone with LiAlH(*n*-Bu)(*i*-Bu)$_2$ to produce a reduced aluminate intermediate which served as a protecting group through several steps to the full benzo[*a*]fluorene system that provided a sample of IPK, after appropriate functional group modification. The synthetic compound was shown to be identical with a sample of the natural product. The synthesis represents the first total synthesis of IPK and was achieved with an overall yield of 6% and the longest linear path being fourteen steps.

An examination of the crystallographic data for the model compound **2-58** and IPK provided structure confirmation of the proposal that the diazonium ion character of the diazo group in IPK is enhanced by the presence of intramolecular H-bonding.

An analogue of IPK was designed, incorporating a side chain at C_3 of the D ring in order to improve solubility and potentially the affinity of the molecule for DNA. An intermediate **4-74** that contains a carbamate group intended to serve as the precursor of the diazo group of the IPK analogue, was synthesized using the same strategy as the model of IPK through eleven steps in 14% yield overall.

Another benzo[*a*]fluorene **5-28** was synthesized in 6% overall yield, via an eleven steps sequence initiated from commercial available 3-methylsalicylic acid. This benzo[*a*]fluorene

is intended to be a key intermediate for the total synthesis of fluorostatin A and for the synthesis of analogues of IPK with a side chain at the C_4 of the D ring.

A discussion of potential significance of the proposed analogues and how they might be best achieved synthetically from the intermediates prepared in this study is provided.

Acknowledgements

I would like to thank Professor Gary I. Dmitrienko for his time and guidance throughout the course of my research and in the preparation of this thesis. His input and support has been greatly appreciated.

I would also like to thank Mrs. Valerie Goodfellow for purification of the natural and synthetic compounds and biological study related my project, Dr. Nicholas Taylor for his X-ray crystallographic work and Mrs. Jan Venne for help with NMR experiments.

Finally I would like to thank my wife Xiaodan for her love and patience for the past several years.

Dedication

To my wife Xiaodan Zhang and my parents.

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Abbreviations

A	absorbance
[O]	oxidation
AIBN	azobisisobutyronitrile
atm	atmosphere
a.u.	atomic units (Hartree)
Ac	acetyl
Ar	aryl
B:	base
BTBSH	1,2-bis(*tert*-butyldimethylsilyl)hydrazine
Bn	benzyl
BINAP	2,2'-*bis*(diphenylphosphino)-1,1'-binaphthyl
Boc	*tert*-butoxycarbonyl
br	broad
t-Bu	butyl
CAN	ceric ammonium nitrate
m-CPBA	m-chloroperbenzoic acid
Cpd	compound
CSA	camphorsulfonic acid
Cy	cyclohexyl
d	doublet
1D	one dimension

2D	two dimension
DAIB	diacetoxyiodobenzene
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
D-DIPT	D-(-)-diisopropyl tartrate
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIEA	Diisopropylethylamine
DIBAl	diisobutylaluminium hydride
DMAP	4-*N*,*N*-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
eq.	equivalent
ESR	electron spin resonance
Et	ethyl
FGI	functional group interconversion
g	gram
GSH	glutathione
h	hour
HETCOSY	$^{1}H/^{13}C$ heteronuclear chemical shift correlation
HOMO	highest occupied molecular orbital

HMBC	heteronuclear multiple bond correlation
HMDS	hexamethyldisilazide
HMQC	heteronuclear correlation through multiple quantum coherence
HPLC	high-performance liquid chromatograph
HRMS	high resolution mass spectrum
Hz	Hertz
KAT I	kinamycin acetyltransferase I
IBX	2-Iodoxybenzoic acid
IPK	isoprekinamycin
IR	infra-red
LDA	lithium diisopropylamide
LTMP	lithium 2,2,6,6-tetramethylpiperidide
m	multiplet
mp	melting point
Me	methyl
mmol	millimole
MO	molecular orbital
MOM	methoxymethyl
Ms	methanesulfonyl, mesyl
MS	mass spectrum
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance

NMO	*N*-methylmorpholine *N*-oxide
nOe	nuclear Overhauser effect
Ph	phenyl
PPTS	pyridinium *p*-toluenesulfonate
i-Pr	*iso*-propyl
rt	room temperature
s	singlet
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBSHs	*N*-*tert*-butyldimethylsilylhydrazones
Tf	trifluoromethanesulfonyl
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
TMP	tetramethylpiperidide
TMS	tetramethylsilane or trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Ts	*p*-toluenesulfonyl, tosyl

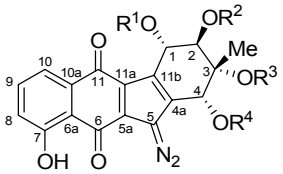
Chapter 1 General Introduction

1.1 Background

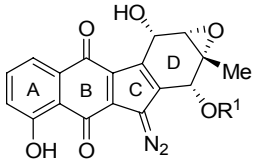
Benzo[*b*]fluorene quinones are really rare structures in natural products. The first four compounds with this structural motif, kinamycin A-D, were originally isolated by Omura and co-workers in 1970 from *Streptomyces murayamaensis* sp. Mov. Heta and Ohtani.[1] These bright golden-yellow compounds were shown to be strongly active against Gram-positive bacteria but less so against Gram-negative organisms.[1] Meanwhile, kinamycin C exhibited antitumor activity against Ehrlich ascites carcinoma and Sarcoma-180.[1]

Until 1989, *S. murayamaensis* was the only organism known to produce kinamycins. Since then, however, other kinamycin-producing microorganisms were reported. For example, in 1989, *Saccharothrix* species was found to produce **1-1h** and **1-1i**, and so was an unidentified actinomycete (produces **1-1j**, antibiotic A83016A) in 1992; In 1994, *S. chattanoogensis* was reported to produce kinamycin D and six new kinamycins, **1-1k** to **1-1n** (FL-120A, FL-120C, FL-120C', and FL-120D' respectively) and **1-2a** and **1-2b** (FL-120B and FL-120B').

Structural variations are confined to ring D, and typically are acetate, propionate and isobutyrate substitutions at the C-1 to C-4 positions (**1-1**). Furthermore, derivatives with other functionalities and different oxidation states of ring D have also been isolated (**1-2** to **1-4**).



1-1



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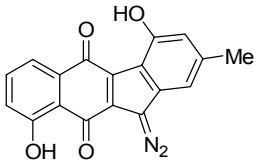

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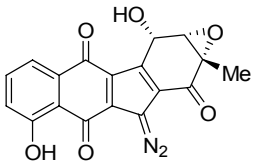

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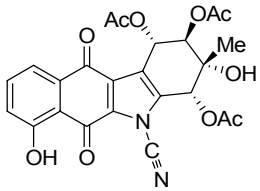


1-5

Table 1-1 Natural Kinamycins Derivatives

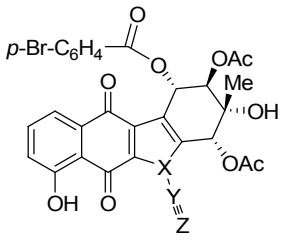
Name		R^1	R^2	R^3	R^4	Ref.
Kinamycin A[a]	**1-1a**	Ac	Ac	Ac	H	2
Kinamycin B[a]	**1-1b**	H	H	Ac	H	2
Kinamycin C[a]	**1-1c**	Ac	Ac	H	Ac	2
Kinamycin D[a]	**1-1d**	H	Ac	H	Ac	2
Kinamycin E[?]	**1-1e**	H	H	H	Ac	3
Kinamycin F[a]	**1-1f**	H	H	H	H	4
Kinamycin G[c]	**1-1g**	Ac	Ac	CO*i*-Pr	Ac	5
Kinamycin H[d]	**1-1h**	Ac	Ac	H	CO*i*-Pr	6
Kinamycin I[d]	**1-1i**	Ac	CO*i*-Pr	H	CO*i*-Pr	6
Kinamycin J[c]	**1-1j**	Ac	Ac	Ac	Ac	5
FL-120A[b]	**1-1k**	H	Ac	CO*i*-Pr	Ac	7
FL-120C[b]	**1-1l**	H	Ac	H	CO*i*-Pr	7
FL-120C'[b]	**1-1m**	H	Ac	H	CO*i*-Pr	7
FL-120D'[b]	**1-1n**	H	H	H	CO*i*-Pr	7
FL-120B[b]	**1-2a**	Ac	-	-	-	7
FL-120B'[b]	**1-2b**	CO*i*-Pr	-	-	-	7
Prekinmycin[a]	**1-3**		-	-	-	8
Ketoanhydrokinamycin[a]	**1-4**	-	-	-	-	4

Isolated from Streptomyces Source: [a] *murayamaensis*; [b] *chattanoogensis*; [c] *unidentified*; [d] *saccharothrix*

Until 1994 it had been believed that kinamycins were *N*-cyanobenzo[*b*]carbazoles such as structure **1-5** for kinamycin C. In 1994 the structures of kinamycins were altered to 5-diazobenzo[*b*]fluorenes **1-1**.The number system shown in **1-1**, consistent with the numbering of benzo[*a*]anthraquinone biosynthesis precursor[9] and with *Chemical Abstracts*, has been adopted for all benzo[*b*]fluorenes and benzo[*a*]fluorenes as suggested by Gould.[10]

1.2 Structure Elucidation of the Kinamycins

The kinamycins structures were originally determined in part by chemical, spectroscopic methods[2] as well as by X-ray study on the *p*-bromobenzoate of kinamycin C,[11] by which the absolute configuration and the tetracyclic skeleton were assigned except for the exact nature of the linear triatomic moiety, consisting of two nitrogen and one carbon atoms (X-Y-Z in **1-6**). The identification of this key functionality was based solely on chemical degradation experiments, which led to the conclusion that kinamycins must incorporate the *N*-cyano instead of *N*-isocyano group. The possibility of a diazo functionality was not considered because of likely instability.



1-6

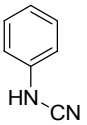
1-6a X=N, Y=C, Z=N

1-6b X=C$^{\ominus}$, Y=N$^{\oplus}$, Z=N

1-6c X=N, Y=N$^{\oplus}$, Z=C$^{\ominus}$

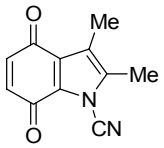
In 1985, the proton and carbon NMR of kinamycin D were fully assigned by Gould's group[12] except that the ^{13}C NMR signal of an *N*-cyano group was not observed. In 1988, an unexpected doublet of doublet at δ 78.5 ppm (J = 21.2 and 5.4 Hz) was observed in the ^{13}C

NMR of [$^{15}N_2$]kinamycin D which was prepared using $(^{15}NH_4)_2SO_4$ in the fermentation medium. Re-examination of previous spectra of natural kinamycin D led to assignment of this signal to the *N*-cyano group,[13] even though this signal was ca. 30 ppm upfield compared to known *N*-cyano compounds (e.g. **1-7**)[13] The large shift upfield was attributed to possible electronic effects of the indoloquinone subunit.



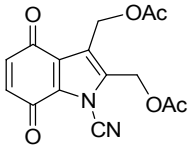
1-7

111.9 ppm



1-8

104.7 ppm



1-9

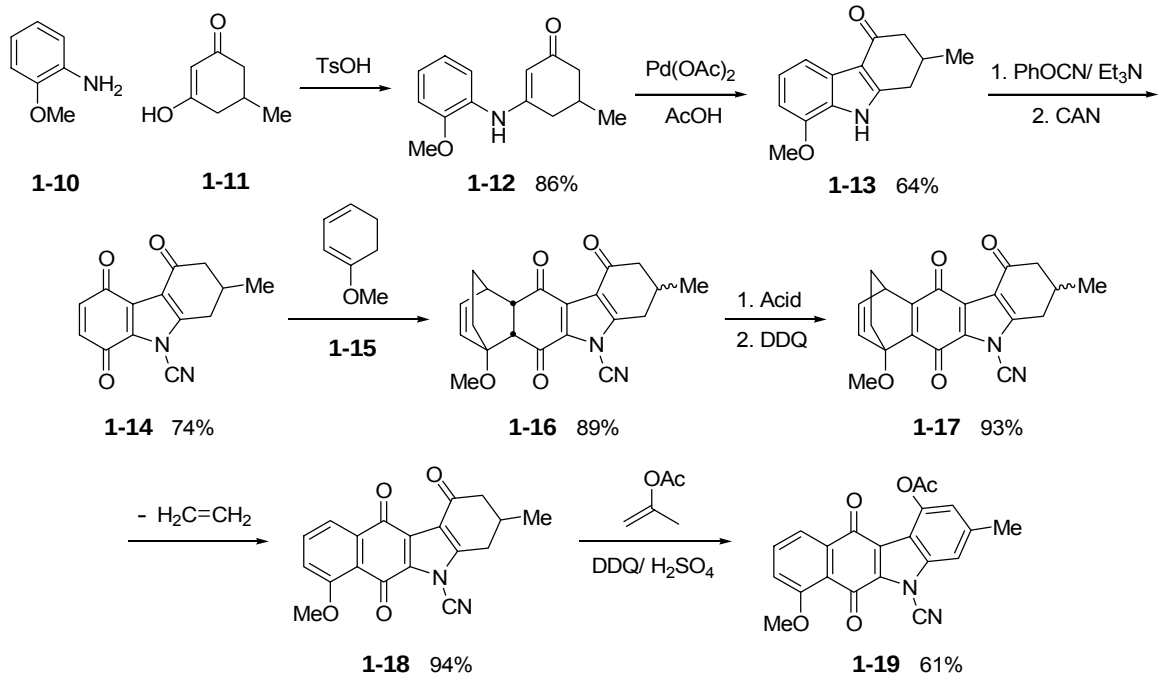
103.6 ppm

However, the ^{13}C NMR characteristics (δ 103–108 ppm) of model *N*-cyanoindoles (e.g. **1-8** and **1-9**) synthesized later in this laboratory agreed poorly with the data of kinamycins indicating that the unusual chemical shift observed from kinamycins could not be simply ascribed the *N*-cyano-indole-dione ring system.[14]

In 1993, Echavarren and co-workers reported the first synthesis of the *N*-cyano indole structure of prekinamycin[15] in which the spectroscopic properties of the synthetic compound did not match that of natural product.[4] Although the possibility of a questionable structure assignment of the synthetic product in this study was realized later,[16] this work raised concerns about the correctness of the assignment to prekinamycin and the kinamycins.

Meanwhile, S. Mithani in this laboratory synthesized the *N*-cyanocarbenzo system, compound **1-19**, using Pd (II)-mediated cyclization of **1-12** and regioselective Diels-Alder reaction between **1-14** and **1-15** in the key steps outlined in the Scheme 1-1.[16]

Scheme 1-1



1-10 **1-11** **1-12** 86% **1-13** 64%

1-14 74% **1-15** **1-16** 89% **1-17** 93%

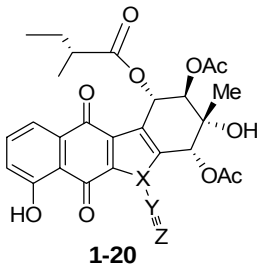
1-18 94% **1-19** 61%

Careful spectroscopic analysis of compound **1-19** indicated that this synthetic compound was not a derivative of prekinamycin. In the case of prekinamycin diacetate, a ^{13}C NMR signal at 83.7 ppm and an IR band at 2119 cm^{-1} were assigned to cyanamide group.[4] The apparent cyano group of compound **1-19**, on the other hand, gave rise to a ^{13}C NMR signal at 105.0 ppm and an IR band at 2253 cm^{-1}. In addition, comparison of the characteristics of all the *N*-cyanoindole derivatives prepared in this laboratory clearly revealed that the assignment of a cyanamide group to any of the kinamycins was incorrect and kinamycins should be assigned as diazobenzo[b]fluorene quinone structures instead. [16, 17]

Table 1-2 Comparison of Kinamycins and N-cyanoindole Derivatives

	^{13}C NMR	IR
N-cyanoindole derivative	104–109 ppm	2237–2259 cm^{-1}
Kinamycins	78.5 ppm for kinamycin D	2119–2170 cm^{-1}
Prekinamycin diacetate	83.7 ppm	2119 cm^{-1}

Gould and co-workers made the same conclusion independently by careful comparison of the statistical R factor for fitting of the three possible linear fragments (N-C-N, N-N-C and C-N-N) to the electron density map, based on the X-ray crystallographic data for the α-methylbutyrate ester of kinamycin D **1-20**.[10]



1-20

		R	R_w
1-20a	X=N, Y=C, Z=N	5.69%	6.35%
1-20b	X=N, Y=N$^{\oplus}$, Z=C$^{\ominus}$	5.73%	6.41%
1-20c	X=C$^{\ominus}$, Y=N$^{\oplus}$, Z=N	5.18%	5.63%

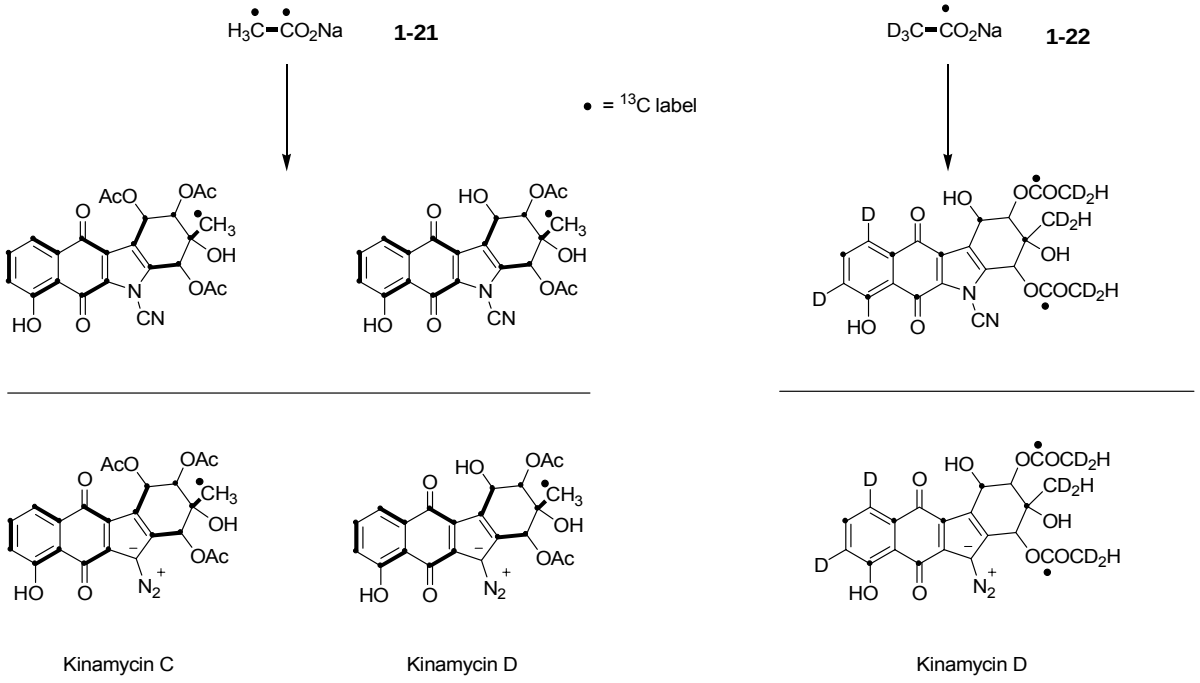
The reassignment of the structures of kinamycins had profound implications to various aspects of studies in chemistry and biochemistry in these natural products. In particular, Gould and co-workers carried out systematic studies on the biosynthesis of kinamycins since the 1980s as pioneers in this area. Their work defined the overall biosynthetic pathway to the kinamycins primarily at the structural level. However, the structural revision indicated above required a reinterpretation of their earlier observations.

1.3 Biosynthesis of Kinamycins

1.3.1 Determination of the Origin of the Ring System and Diazo Moiety

Incorporation of sodium [1, 2- $^{13}C_2$] acetate **1-21** and sodium [1-^{13}C, 2, 2, 2-D_3] acetate **1-22** to kinamycin D and C produced by feeding labelled sodium acetate to *S. murayaensis* revealed that the entire skeleton of kinamycins was derived from acetate, apparently through a decaketide.[18] However, the cyanamide resonance could not be found, so its origin was unknown.

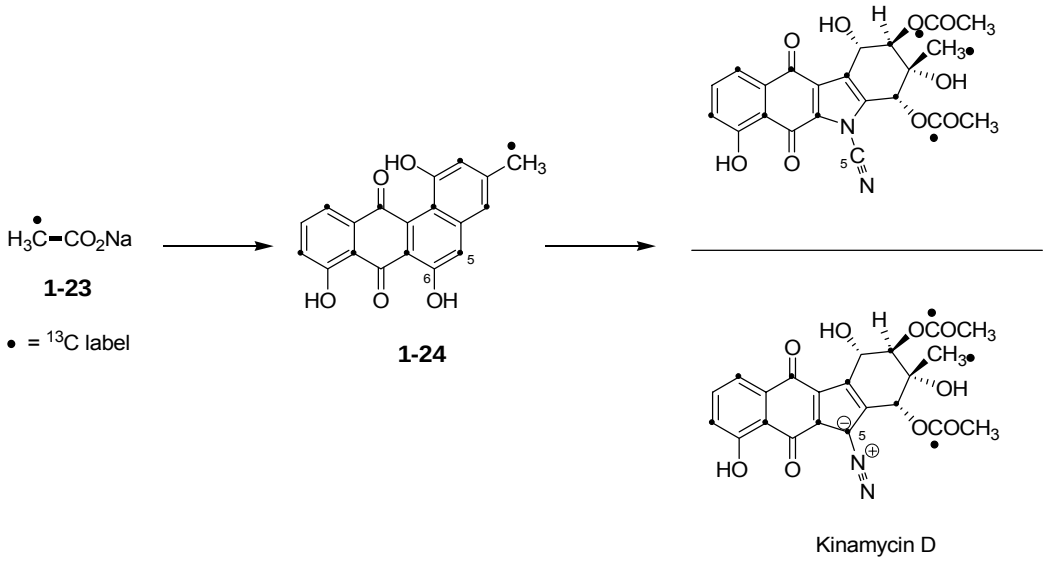
Scheme 1-2



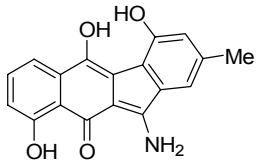
A green metabolite, dehydrorabelomycin **1-24**, isolated from *S. murayamaensis*, was confirmed to be a key intermediate in the biosynthetic pathway of the kinamycins.[9] Then the Gould groups assigned the ^{13}C NMR signal at δ 78.5 ppm to the putative cyanamide carbon, and they found this carbon was derived from C-2 of the acetate by separately feeding sodium

[1-^{13}C] acetate and [2-^{13}C] acetate to *S. murayamanensis*. The relatively uniform levels of isotope enrichment of the carbon of cyanamide moiety and the others of the skeleton led them to propose that this carbon was derived from C-5 of dehydrorabelomycin **1-24**.[13]

Scheme 1-3



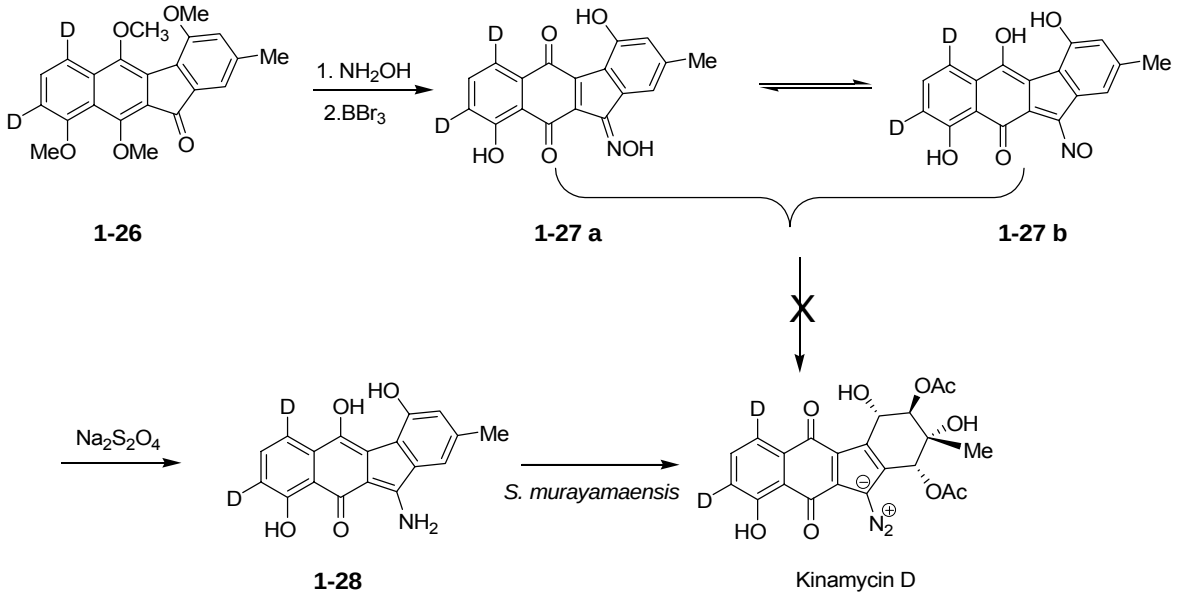
The 1994 landmark reassignment of structure to the kinamycins led to an extensive revision of the understanding of their biosynthesis in terms of the intermediate metabolites involved. Nevertheless, the new diazobenzo[*b*]fluorene structure was entirely consistent with labeling experiments.



1-25 Stealthin C

This structure correction greatly expanded the number of naturally occurring diazo compounds as well as the number of the benzo[*b*]fluorenes such as stealthin C (**1-25**), which was detected in 1997 in extracts of *S. murayamaensis* mutant strain MC2 and gained a new meaning for the biosynthetic studies.[19] Feeding deuterium-enriched stealthin C to *S. murayamensis* produced deuterated kinamycin D. However, the labeled oxime **1-27a**, which exists in equilibrium with its nitroso tautomer **1-27b**, was found to not be a precursor of the kinamycins. This result suggested that the diazo group of the kinamycins was formed by stepwise addition of the two individual nitrogens, which did not involve a nitroso benzo[*b*]fluorene intermediate. However, the details of the conversion of the amino group in stealthin C into the diazo group of the kinamycins have not yet been defined.

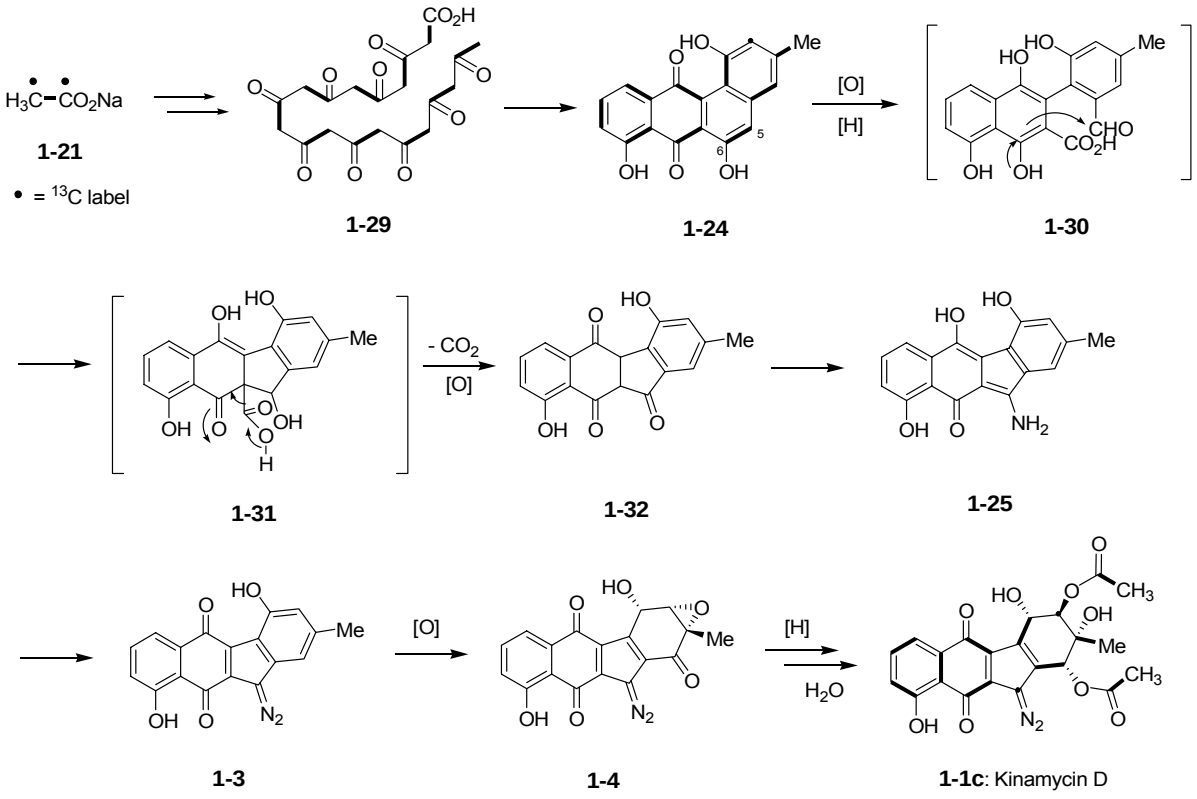
Scheme 1-4



The labeling studies along with identification of other minor metabolites from *S. murayamaensis* or its mutants (prekinamycin **1-3**,[4,8,20] kinobscurinone **1-32**,[21]

ketoanhydrokinamycin **1-4**)[4,22] allowed for a formulation of a hypothetical biosynthetic pathway for kinamycins as presented in Scheme 1-5. Dehydrorabelomycin **1-24**, which can be derived from a single decaketide precursor **1-29**, might undergo an oxidative ring opening at C-5 and C-6. An electrophilic cyclization of hydroquinone **1-30** followed by a decarboxylation would afford kinobscurinone **1-32**. A transformation equivalent to a reductive amination might lead to stealthin C **1-25** and then an oxidative incorporation of second nitrogen would provide prekinamycin **1-3**. Subsequent steps would alter the level of oxidation of ring D.

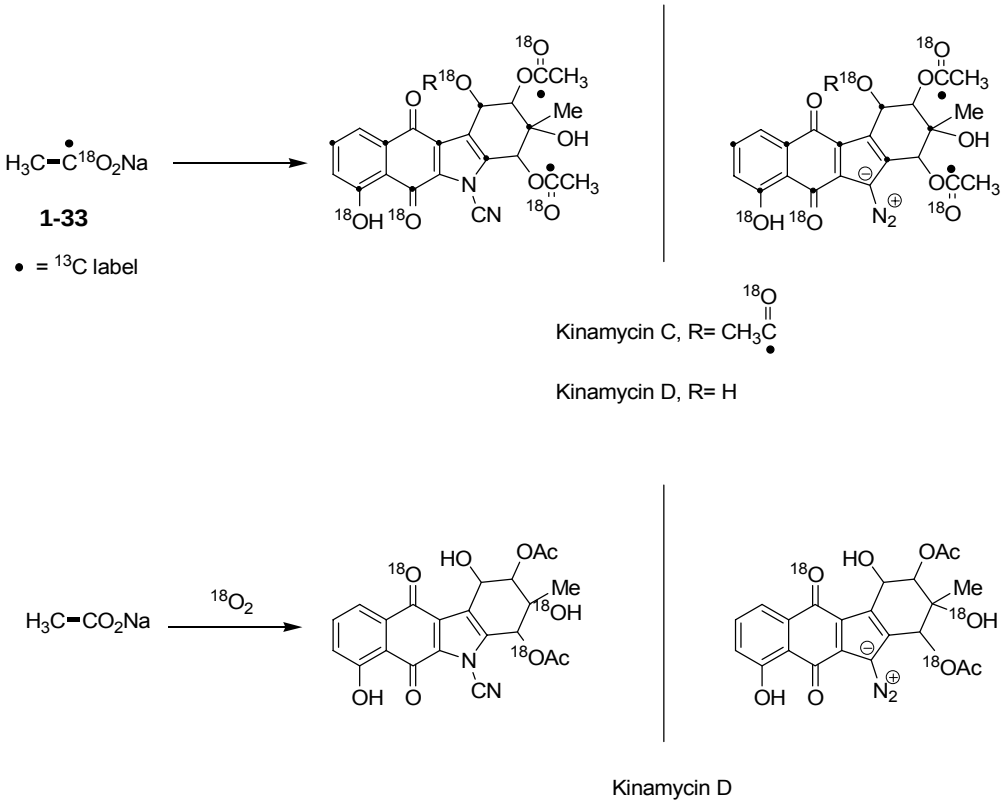
Scheme 1-5



1.3.2 Biosynthetic Elaboration of the Kinamycin D-ring

Incorporation of sodium [1-^{13}C, 1,1-$^{18}O_2$] acetate **1-33** into kinamycin C or D indicated that the oxygens of C_1, C_6, C_7 and the ester carbonyl were derived from sodium acetate through a polyketide intermediate. A fermentation experiment in the presence of $^{18}O_2$ was carried out and revealed that oxygens attached to C_3, C_4, and C_{11} of kinamycin D came from O_2. Thus the only oxygen unaccounted for, attached to C_2, was assumed to be derived from water.

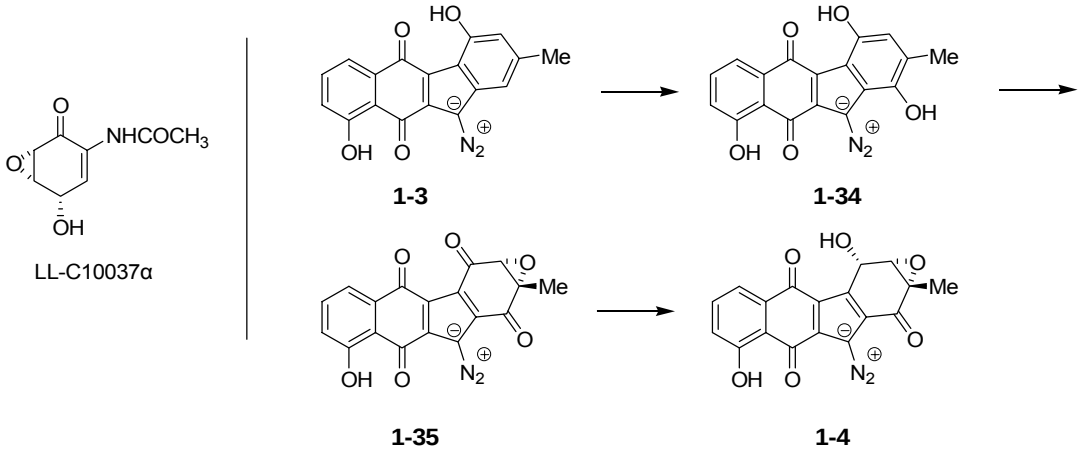
Scheme 1-6



The biosynthetic study of antibiotic LL-C10037α revealed a procedure involving oxidation of a phenol to a hydroquinone, followed by oxidation epoxidation directly to an

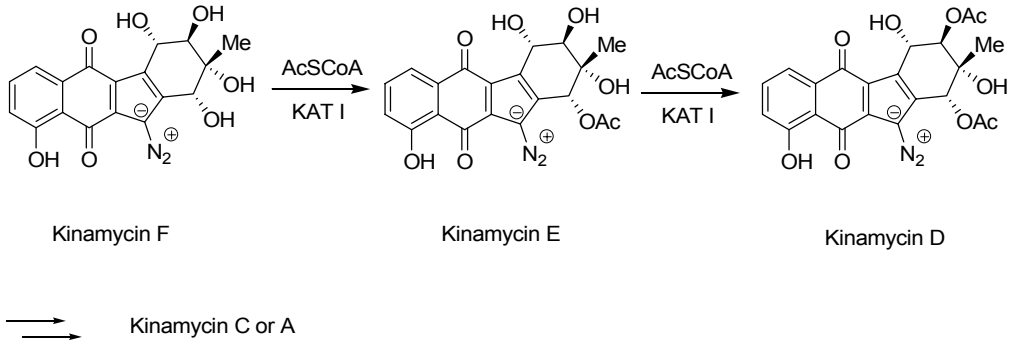
epoxyquinone, and then reduction to the epoxyquinol.[23, 24, 25] On the basis of this example, an analogous set of reactions was suggested for the conversion of prekinamycin to ketoanhydrokinamycin **1-4**, which contained an epoxyquinol moiety.

Scheme 1-7



The early biosynthetic study had identified kinamycin F, as a metabolite of *S. murayamaensis*, and it is apparently the branch point to the various *O*-acetylated kinamycins. The Gould group isolated kinamycin acetyltransferase I (KAT I) whose enzymatic activity was responsible for the two consecutive acetylations of kinamycin F via kinamycin E to kinamycin D.[26] Furthermore, in order to account for the rest of the ring-D acetylation at least two other KATs were required.

Scheme 1-8

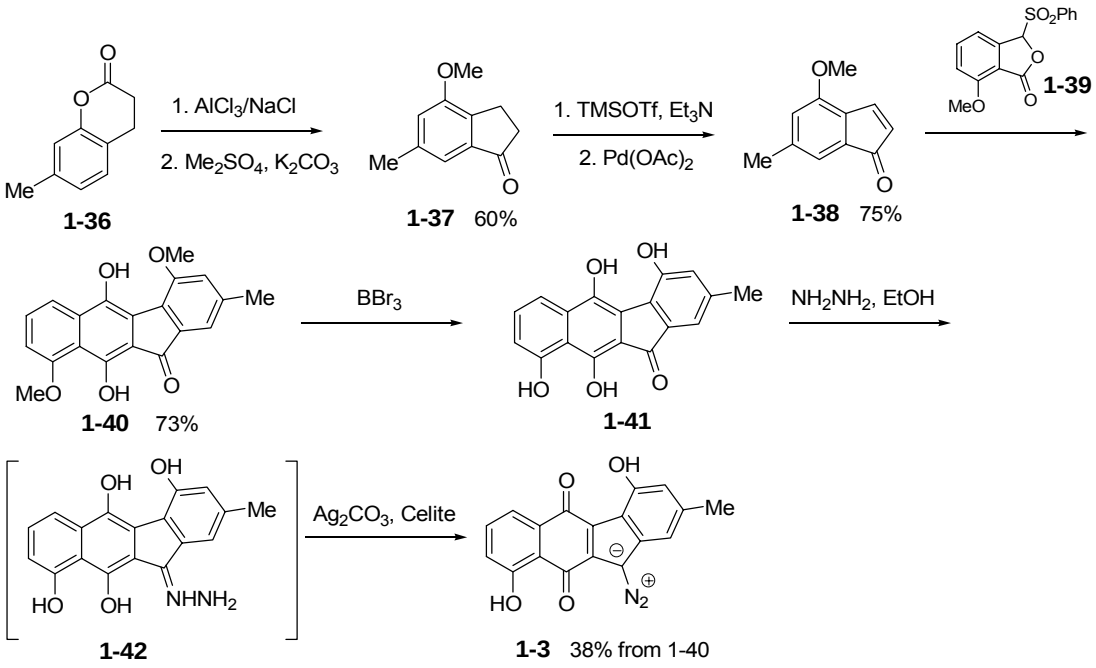


1.4 Synthetic Studies towards the Prekinamycin and Kinamycins

Since the reassignment of the structure of kinamycins to diazobenzo[*b*]fluorenes many groups have been working on the synthesis of these unusual natural products or the biosynthetic precursor prekinamycin **1-3**. A number of synthetic strategies have been disclosed, and are discussed below.

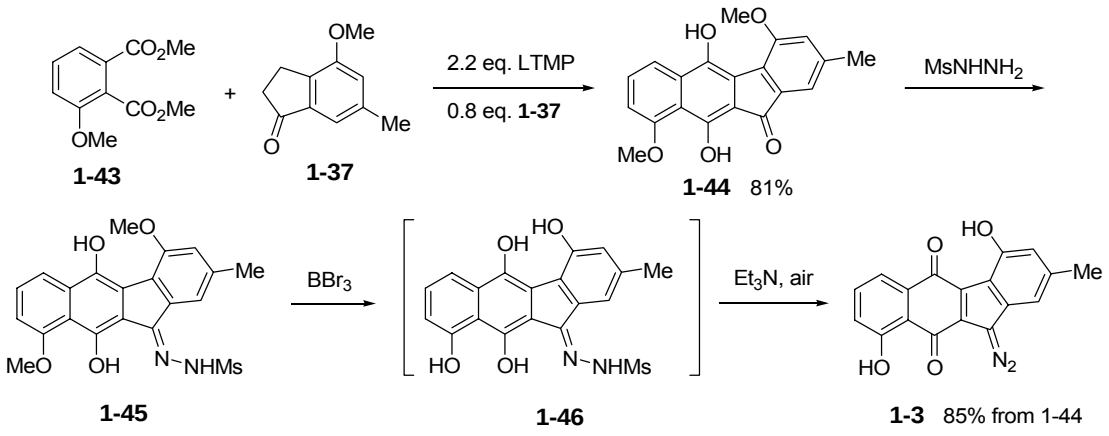
Hauser and Zhou reported the first total synthesis of the structure assigned to prekinamycin in 1996 as indicated in Scheme 1-9.[27] Starting from the dihydrocoumarin **1-36**, an intramolecular Friedel-Crafts reaction was carried out, followed by methylation to afford **1-37**, which was converted to a silyl enol ether, followed by treatment of $Pd(OAc)_2$ to give indenone **1-38**. Condensation of the anion of the phthalide sulfone **1-39** with the indenone **1-38** regiospecifically afforded the benzo[*b*]fluorene **1-40**. Treatment of **1-40** with BBr_3 gave **1-41**, which was sequentially reacted with hydrazine and Ag_2CO_3 on Celite to provide prekinamycin **1-3**.

Scheme 1-9



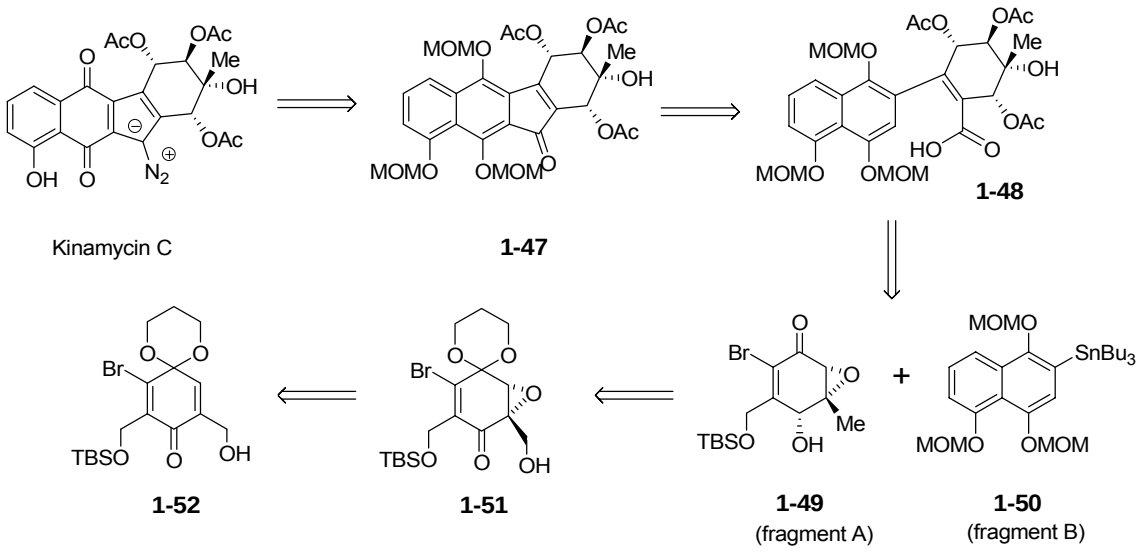
In 2007, Birman and co-workers reported a rapid construction of benzo[*b*]fluorenes via reaction of 1-indanone dianion with phthalate diesters, which was used in a concise synthesis of prekinamycin as presented in scheme 1-10.[28] Reaction of the phthalate diester **1-43** with a enolate of indanone **1-37**, using LTMP as a base, led to the desired product **1-44** regioslectively. Refluxing this benzo[*b*]fluorene **1-44** with $MsNHNH_2$ in ethanol gave crude mesylhydrazone **1-45**, which was demethylated with BBr_3 and then converted to prekinamycin by treatment with Et_3N under air.

Scheme 1-10



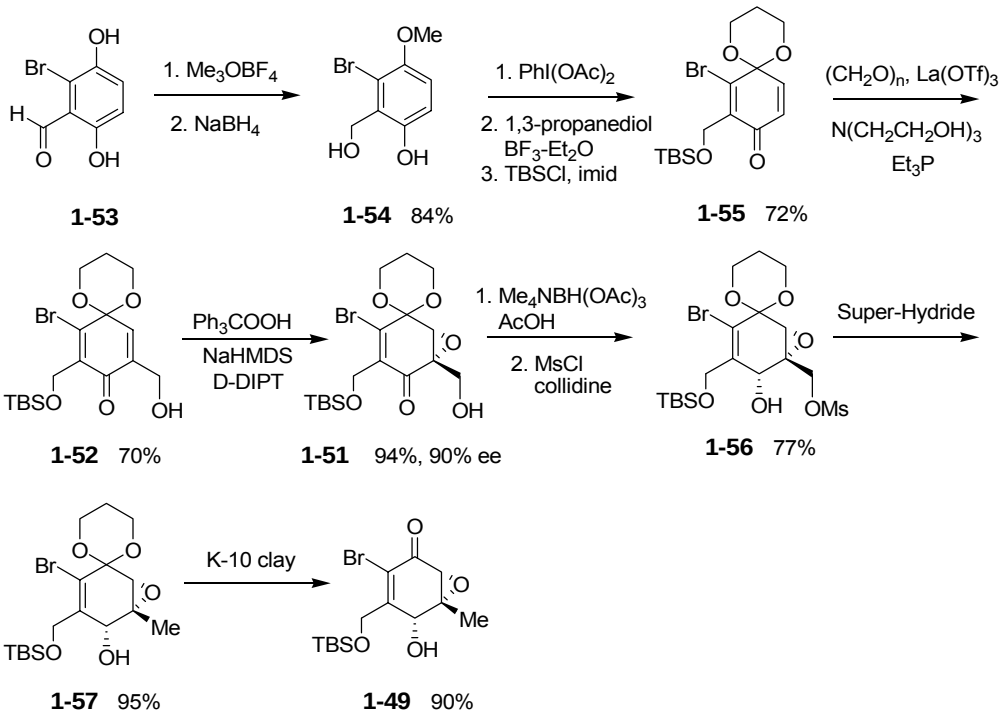
In 2006 Porco and Lei achieved the first enantioselective total synthesis of kinamycin C, and the retrosynthetic analysis is as presented in Scheme 1-11.[29] They reasoned that kinamycin C might be derived from oxidation and diazo group formation from MOM-protected benzofluorene ketone precursor **1-47**. Compound **1-47** might be obtained from intramolecular Friedel-Crafts cyclization of carboxylic acid **1-48**. Acid **1-48** should be available through Stille cross coupling of vinyl bromide **1-49** (fragment A) and MOM-protected arylstannane **1-50** (fragment B). Fragment A **1-49** may be derived from epoxyketone precursor **1-51**, which could be obtained from tartrate-mediated asymmetric nucleophilic epoxidation of quinone monoketal **1-52**. Fragment B **1-50** could be achieved from the commercially available 1, 5-naphthalenediol.

Scheme 1-11



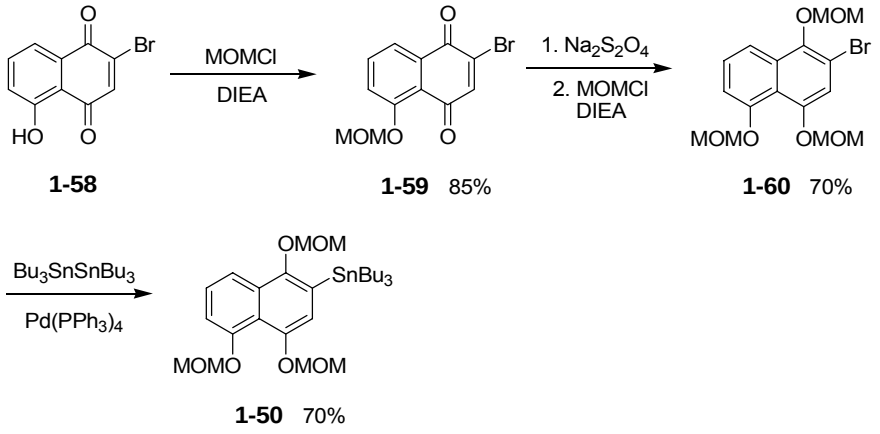
The synthesis of fragment A **1-49** started from the readily available 2-bromo-3,6-dihydroxy-benzaldehyde **1-53** as presented in Scheme 1-12. Selective methylation, followed by reduction, provided **1-54**. Hypervalent iodine oxidation of **1-54**, followed by transketalization and silyl protection, afforded quinone monoketal **1-55**, which was converted to the corresponding quinone monoketal **1-52** using Et_3P as a nucleophilic activator. These were conditions modified relative to those reported by Aggarwal.[30] Tartrate-mediated asymmetric nucleophilic epoxidation of compound **1-52** generated epoxide **1-51** with higher yield and enantioselectivity compared with Sharpless asymmetric epoxidation. This was followed by hydroxyl-directed reduction and selective mesylation of the primary alcohol which afforded **1-56**. Reductive demesylation cleanly produced **1-57**, and then selective removal of the cyclic ketal using K-10 clay provided the desired epoxyketone **1-49** (fragment A).

Scheme 1-12



The synthesis of fragment B **1-50** began with the readily available 1,5-naphthoquinone **1-58**. Protection of **1-58** with MOMCl/DIPEA generated naphthalenedione **1-59**, which was subsequently reduced with $Na_2S_2O_4$ and further protected to provide **1-60**. Stannylation of aryl bromide **1-60** afforded the desired arylstannane **1-50**.

Scheme 1-13

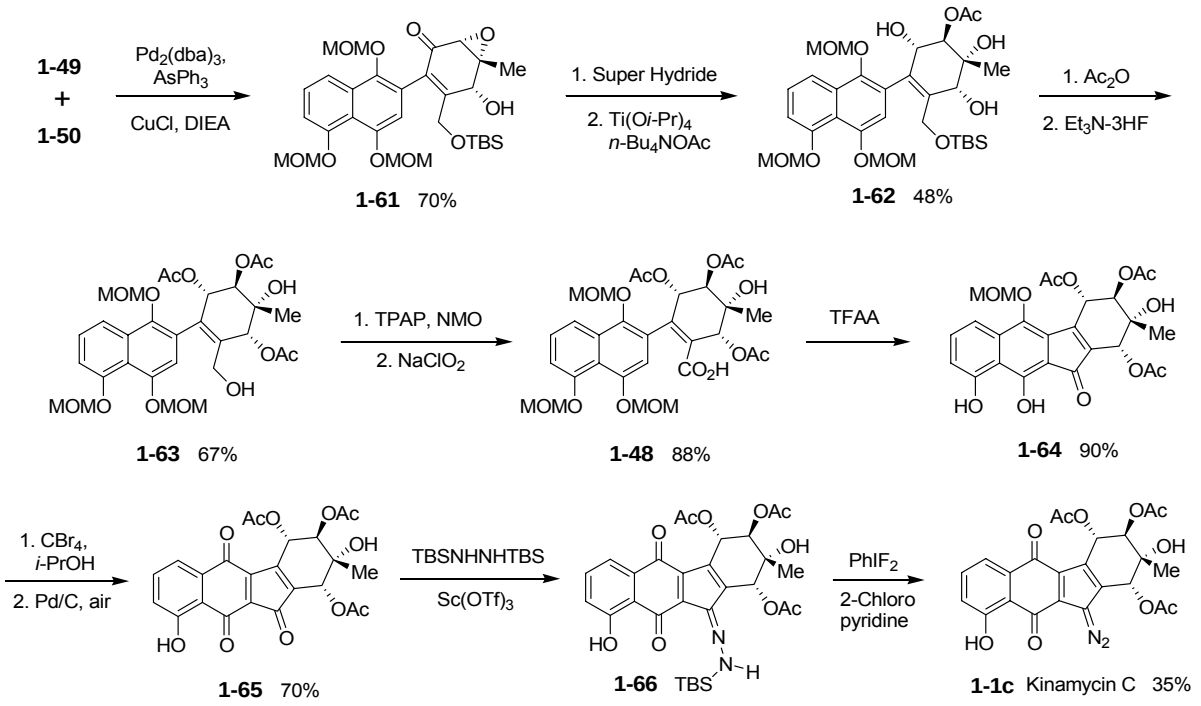


1-58 **1-59** 85% **1-60** 70%

1-50 70%

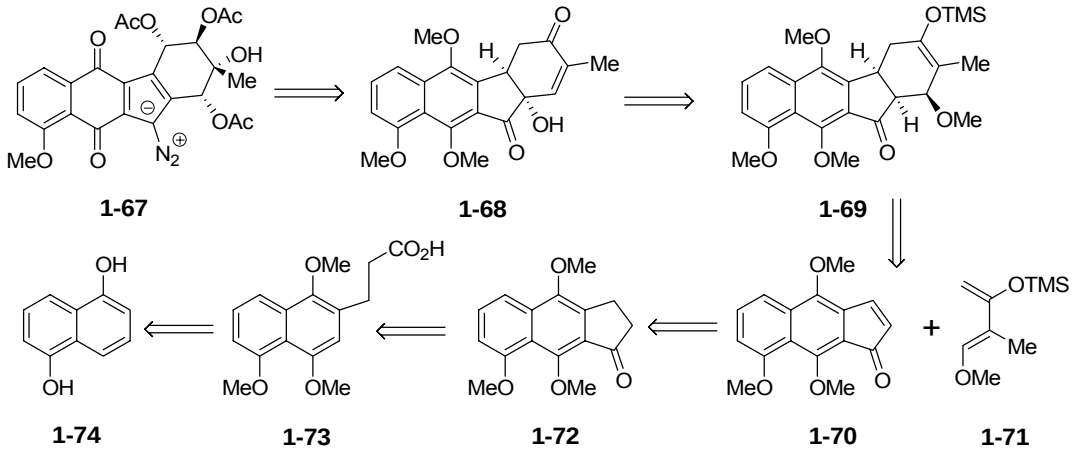
Cross coupling of fragment A **1-49** and fragment B **1-50** under suitable conditions ($Pd_2(dba)_3$, $AsPh_3$, CuCl, DIEA) generated desired product **1-61**. Further reduction of **1-61** with Super-Hydride® provided the *syn*-epoxyalcohol, which was subjected to the epoxide-opening using $Ti(O\textit{i}\text{-}Pr)_4$ and $\textit{n}\text{-}Bu_4NOAc$ to afford triol **1-62**. Acylation of **1-62** followed by deprotection of silyl ether produced alcohol **1-63**. Sequential oxidation of **1-63** using TPAP and $NaClO_2$ afforded carboxylic acid **1-48**, which was cyclized with intramolecular Friedel-Crafts cyclization to form C ring using TFAA. Meanwhile, two MOM protecting groups were also removed regioselectively. MOM deprotection, followed by oxidation using Pd/C and air, generated ketoquinone **1-65**. Condensation of **1-65** with 1,2-bis(*tert*-butyldimethylsilyl)hydrazine afforded **1-66**, which was treated with $PhIF_2$ to install the diazo functionality and complete the total synthesis of kinamycin C.

Scheme 1-14



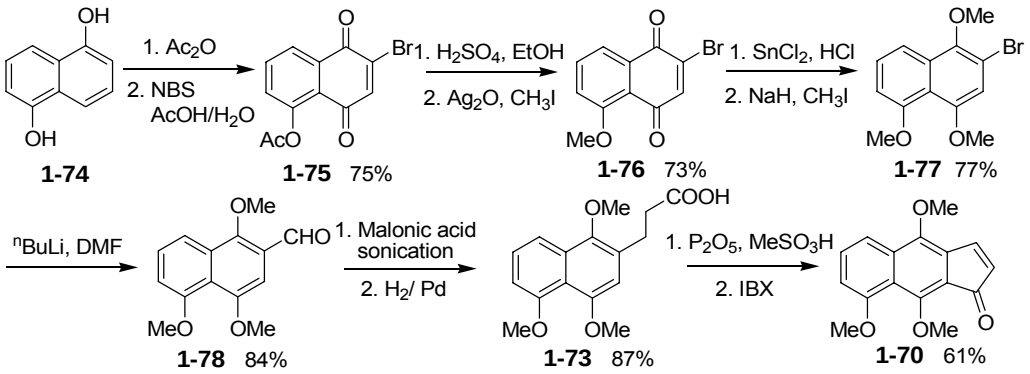
In 2007 Ishikawa and co-workers reported the total synthesis of racemic methyl-kinamycin C.[31] The strategy is as presented in Scheme 1-15: The tetracyclic ring structure **1-68** was constructed by Diels-Alder reaction of the benzo[*f*]indenone **1-70** and Danishefsky-type diene **1-71** followed by hydrolysis and oxygenation of the adduct **1-69**. Then the oxygen functionality was introduced on the D ring stereoselectively. The benzo[*f*]indenone **1-70** may be obtained from the corresponding indanone **1-72**, which was achieved from naphthalenepropanoic acid **1-73** via intramolecular Friedel-Crafts reaction. The acid **1-73** was accessed from naphthalene-1,5-diol **1-74**.

Scheme 1-15



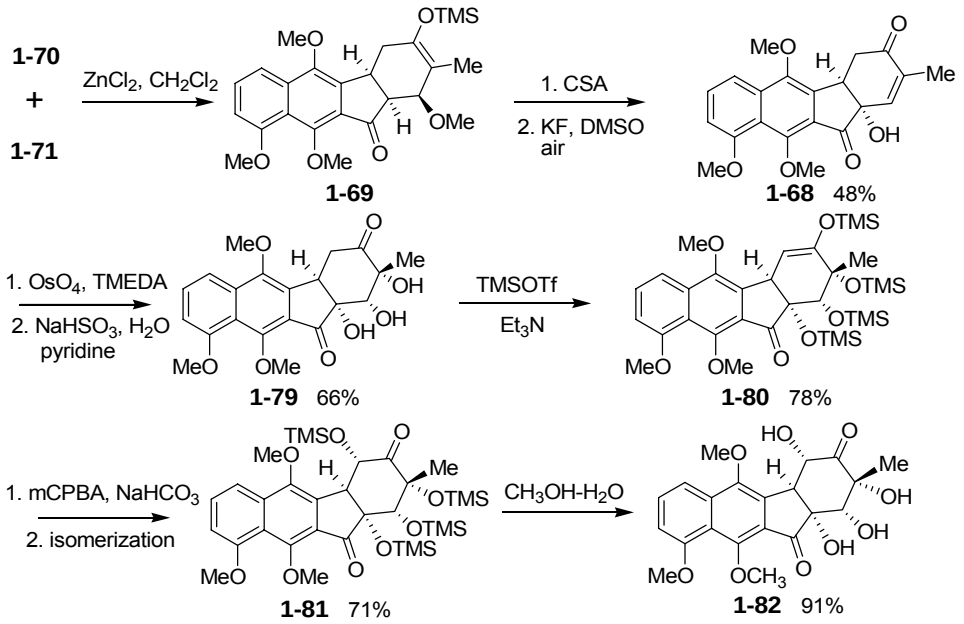
The synthesis of benzo[*f*]indenone **1-70** was initiated from naphthalene-1,5-diol **1-74**. Acetylation, followed by the oxidative bromination using NBS in AcOH provided bromo-5-*O*-acetyljuglone **1-75**, whose acetyl function was replaced by a methyl group to afford **1-76**. Reduction of **1-76** with $SnCl_2$ and HCl, followed by the methylation, produced 2-bromo-1,4,5-trimethoxylnaphthalene **1-77**. Then subsequent treatment of **1-77** with BuLi and DMF provided naphthaldehyde **1-78**. The Knoevenagel reaction of **1-78** with malonic acid under sonication followed by catalytic hydrogenation generated naphthalenepropanoic acid **1-73**. Then intramolecular Friedel-Crafts reaction of **1-73** using P_2O_5 and $MeSO_3H$ provided an indanone intermediate, which was subjected to oxidation with IBX to afford benzo[*f*]indenone **1-70**.

Scheme 1-16



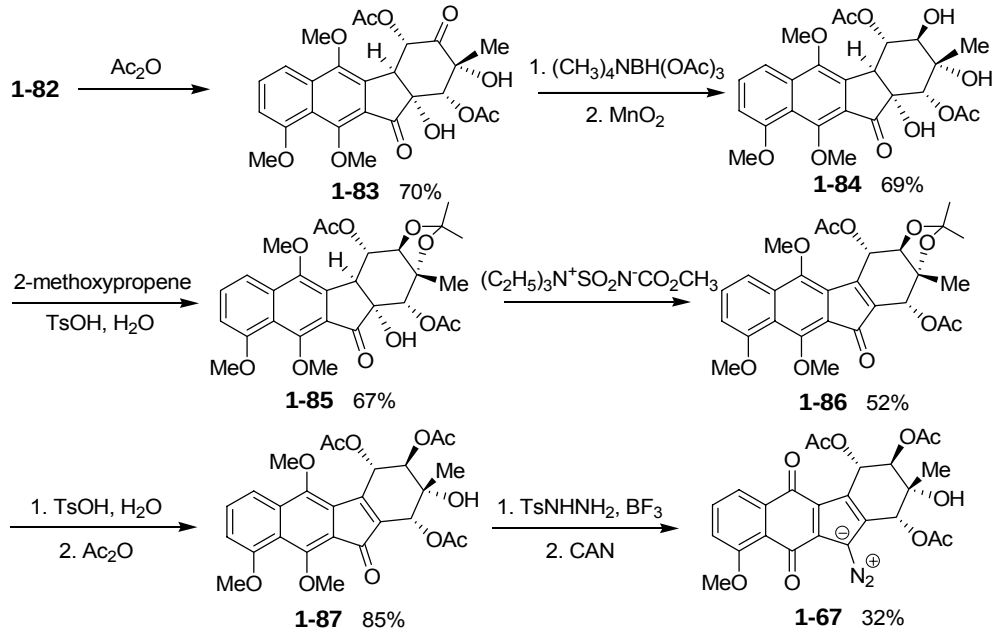
A Diels-Alder reaction in CH_2Cl_2 using a catalytic amount of $ZnCl_2$ gave smoothly the tetracyclic adduct **1-69**, which was treated with CSA and then oxidized to enone **1-68** using KF in the presence of air. *Syn*-selective dihydroxylation of enone **1-68** provided the desired *cis*, *cis*-triol **1-79**, which was converted to the corresponding silyl enol ether **1-80** using TMSOTf. Oxidation of **1-80** with *m*-CPBA afforded the undesired 1β-silyloxyketone, which was completely isomerized to the desired 1α-silyloxyketone **1-81**. Then cleavage of the TMS group using methanol-H_2O afforded tetraol **1-82**. (Scheme 1-17)

Scheme 1-17

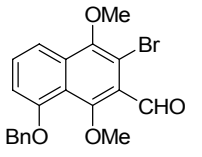


Acetylation of the secondary alcohols in tetraol **1-82** generated diacetate **1-83** selectively, which was subjected to diastereoselective reduction with $(CH_3)_4NBH(OAc)_3$ controlled via five-member ring chelation from the *o*-hydroxy group to afford triol **1-84**. Partial over-reduction was recovered with MnO_2. Then ketalization of triol **1-84**, followed by dehydration with methyl(carboxysulfamoyl)triethylammonium hydroxide inner salt, produced benzofluorene **1-86**. Then deketalization and partial acetylation provided triacetyl enone **1-87**. Condensation of **1-87** with $TsNHNH_2$ using BF_3-Et_2O followed by treatment with CAN, which led to oxidation to naphthoquinone as well as spontaneous desulfination of the tosyl hydrazone, completed the synthesis of racemic *O*-methyl-kinamycin C **1-67**. (Scheme 1-18)

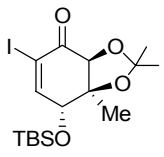
Scheme 1-18



Most recently Nicolaou and co-workers reported the enantioselective synthesis of kinamycin C, F and J.[32] The fluorenone skeleton of kinamycin was built up via an Ullmann-type coupling between the two key building blocks **1-88** and **1-89** followed by an intramolecular benzoin-like condensation and further elaboration. The diazo group was installed on the final stage of the synthetic sequence, considering the sensitivity of this group.



1-88

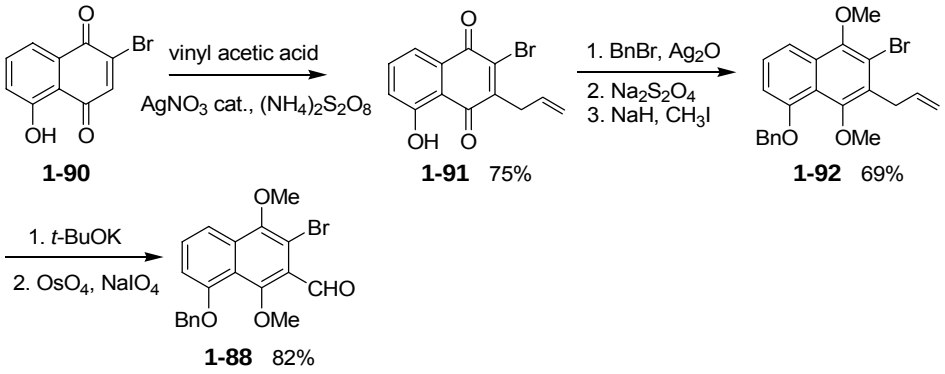


1-89

The synthesis of building block **1-88** was initiated from **1-90**, which was oxidatively allylated using Jacobsen-Torssell reaction with vinyl acetic acid in the presence of $(NH_4)_2S_2O_8$ and catalytic amount of $AgNO_3$ to generate naphthoquinone **1-91**.[33] This

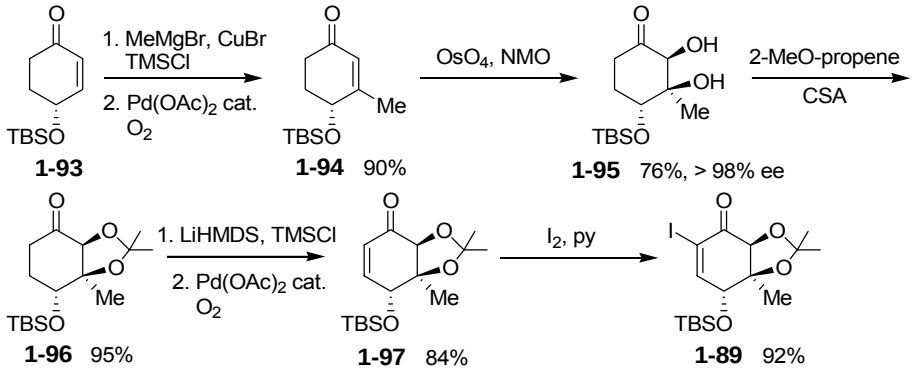
intermediate was converted to corresponding dimethoxy benzyl form **1-92**. Then conjugation of the olefinic bond of the latter compound, followed by cleavage using OsO_4 and $NaIO_4$, provided the bromoaldehyde **1-88**.

Scheme 1-19



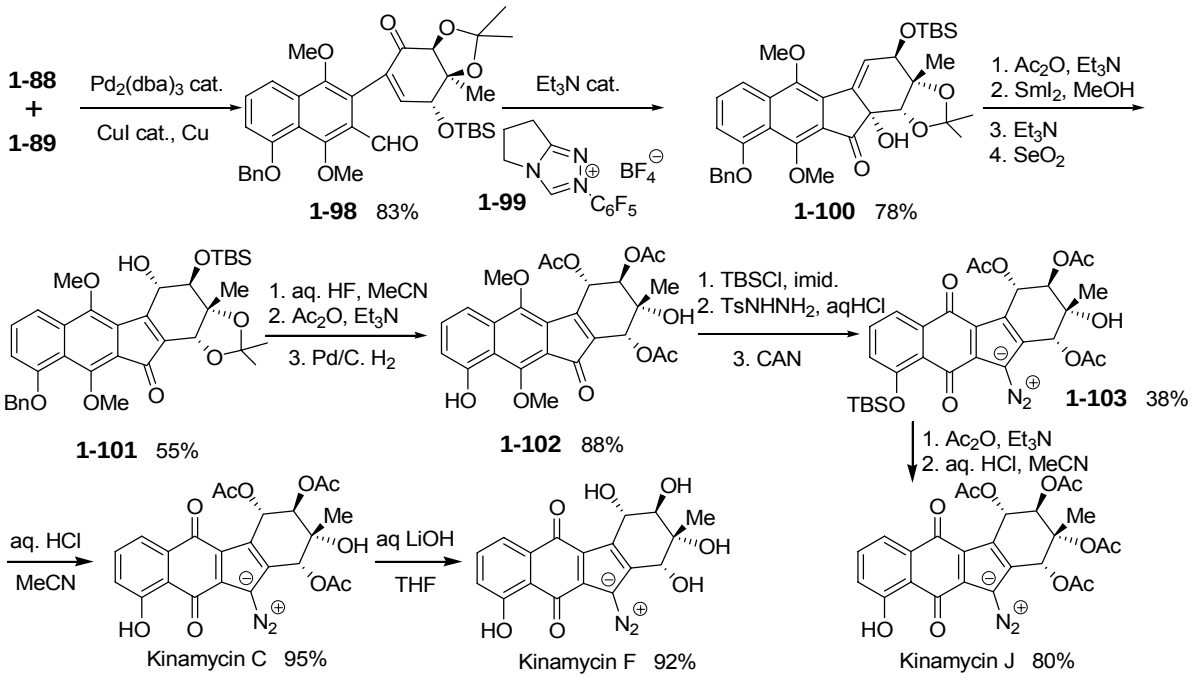
The construction of the other building block, iodo-enone **1-89**, in its enantiomerically pure form was carried out as summarized in Scheme 1-20. Thus, the readily available enone **1-93** was converted to its methylated derivative **1-94** via conjugate addition of MeMgBr and trapping with TMSCl, followed by Saegusa oxidation of the resulting silyl enol ether using catalytic $Pd(OAc)_2$ in the presence of O_2.[34] Then stereoselective dihydroxylation of the latter compound **1-94** with catalytic OsO_4 and NMO afforded a vicinal diol **1-95**, which was protected with 2-methoxypropene to generate an acetonide **1-96**. Treatment of ketone **1-96** with LiHMDS-TMSCl, followed by the Saegusa oxidation, led to an enone **1-97**. Iodination of **1-97** using I_2 and pyridine provided the iodo-enone **1-89**.

Scheme 1-20



The fragments **1-88** and **1-89** were coupled under modified Ullmann condition using Cu, catalytic amount of CuI and $Pd_2(dba)_3$ to generate coupling product **1-98**, which was subjected to benzoin-type reaction in the presence of the Rovis catalyst **1-99** to provide alcohol **1-100**. Acetylation of the latter, followed by the reductive cleavage of the acetate and conjugation of the double bond, generated a fluorenone, which was oxidized to an allylic alcohol **1-101** stereoselectively. The TBS and acetonide were removed from **1-101** using aqueous HF in MeCN, and the resulting tetrol was selectively tri-acetylated and debenzylated to afford an advanced fluorenone **1-102**. The fluorenone **1-102** was temporarily protected with TBSCl and then sequentially treated with $TsNHNH_2$ and CAN to generate TBS-protected kinamycin C, which could be converted to kinamycin J by acylation and deprotection of TBS silyl ether, on one hand, as well as converted to kinamycin C using aqueous HCl in MeCN and kinamycin F by treatment of kinamycin C with aqueous LiOH in THF sequentially on the other hand. (Scheme 1-21)

Scheme 1-21



1.5 Identification of Isoprekinamycin

As indicated above, Hauser and Zhou reported the first total synthesis of prekinamycin in 1996.[27] However, the synthetic compound was found not to match the spectral properties of the prekinamycin (also known in Gould's group as Cpd A). The synthetic product was found to be indistinguishable from a previous structurally uncharacterized metabolite, Cpd B, discovered by the Gould group by screening the library of components isolated from *S. murayamaensis*.[8] Thus, the Gould group assigned the prekinamycin structure to Cpd B, which left Cpd A without an assigned structure.

Cpd A, originally also referred to as prekinamycin, was first isolated in 1989 by Gould and co-workers from a fermentation medium of *S. murayamaensisi* sp. Nov. Heta et Ohtani.[4, 22]

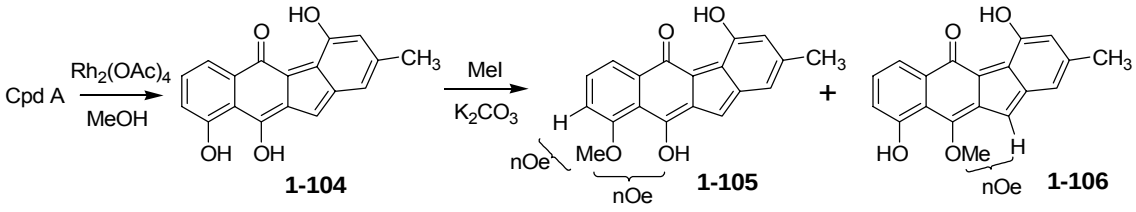
This purple solid was found to be moderately active against Gram-positive organisms such as *Staphylococcus aureus* ATCC 25923 and *Streptococcus faecalis* ATCC 29212, which is important since these organisms have developed substantial resistance to the more common β-lactam antibiotics.[22, 35]

This group at the University of Waterloo cooperated with Proteau's group at Oregon State University to reassign the diazobenzo[*a*]fluorene structure to Cpd A based on detailed re-examination of the available chemical and spectroscopic data of Cpd A as well as results of synthetic and spectroscopic studies done by R. S. Laufer in this laboratory.[36]

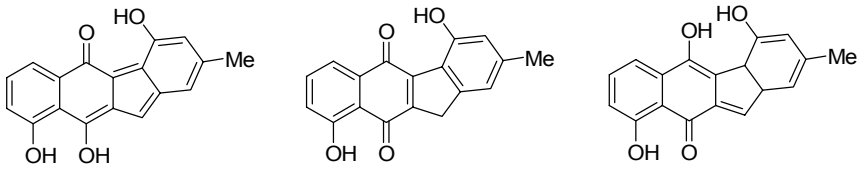
The elemental and mass spectrometric analysis of the Cpd A led to the assignment of $C_{18}H_{10}N_2O_4$ as the molecular formula so that it is isomeric with prekinamycin (Cpd B). The IR spectrum included a band at 2162 cm^{-1}, which was attributed to the diazo group. The proton NMR spectrum revealed a pattern of three adjacent hydrogens, just as in the A-ring of the kinamycins: δ 7.04 (1H, dd, J = 0.9, 7.2 Hz), 7.17 (1H, dd, J = 7.0, 7.2 Hz), 7.24 (1H, dd, J = 0.9, 7.0 Hz). The two other resonances at 6.60 and 6.69 ppm indicated the presence of two *meta*-related protons in a tetra-substituted aromatic ring, which were related to the methyl resonance at 2.39 ppm as indicated by HMBC NMR experiments. The two remaining resonances at 11.60 and 12.32 ppm could belong to two hydrogen-bonded, exchangeable hydrogens, likely phenols in this case. Although this compound was not suitable for ^{13}C NMR analysis because of its very poor solubility in any desired solvent, the diacetate of it did show resonances at δ 174.14, 192.48, 170.28, 170.64 and a signal at 83.71 ppm, the latter of which belonged to the carbon bearing a diazo group.

One particular chemical evidence reported by Gould and co-workers involving the deazotization of Cpd A as presented in Scheme 1-22, led them to assign **1-104** to the structure of the deazotization product of Cpd A. The monomethylated products **1-105** and **1-106**, which were founded from the deazotization of Cpd A using $Rh_2(OAc)_4$ in MeOH followed by methylation with MeI/K_2CO_3, showed nOe enhancements which were compatible with the assigned structure **1-105** and **1-106**.[8]

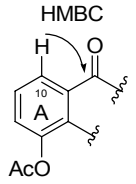
Scheme 1-22



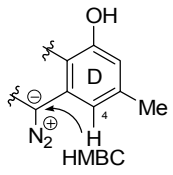
However, in the independent work at Waterloo, a comparison of **1-104** and its two tautomers **1-107** and **1-108** using ab initio MO calculation led to the conclusion that **1-104** was expected to be much less stable than the tautomers **1-107** and **1-108**. This led to a rejection of structure **1-104** for product of the hydrodediazotization of Cpd A. The structures **1-107** and **1-108** were rejected as possible structures because they were incompatible with nOe and HMBC NMR results. These revealed that the deazotization product of Cpd A could not be benzo[*b*]fluorene structure, nor could Cpd A.



The HMBC spectrum of Cpd A diacetate showed a weak three bond correlation between H-10 and carbonyl carbon at 192.48 ppm in addition to the known correlation of H-4 and the diazo carbon (82.64 ppm). This indicated that Cpd A incorporated partial structure **1-109** and **1-110**, but how these fragments combined in the structure of Cpd A was unclear. Any combination of these partial structure had to fit the criteria that both phenolic hydroxyls are capable of intramolecular hydrogen bonding and a carbonyl must be in the ring adjacent to the ring A (based on a weak HMBC correlation between H-10 and the carbonyl at 192.48 ppm). Because the ^{13}C NMR shift of 192.48 ppm suggested that this carbonyl is in a five-membered ring, the possible structures might have a 6, 5, 6, 6 ring system, rather than the 6, 6, 5, 6 rings system observed in the kinamycins. Distinguishing between the various possibilities by direct NMR experiments, however, was hampered by the lack of protons in rings B and C as well as by the challenge of poor solubility of this compound.



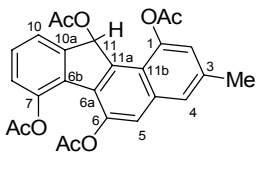
1-109



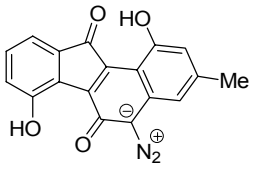
1-110

Fortunately, Proteau and co-workers treated Cpd A with sodium borohydride, which reduced the carbonyl and deazotized Cpd A to generate an alcohol in which the necessary connectivities suitable for both 1D and 2D NMR analysis (HMQC, HMBC) could be established. The two new introduced protons appeared at 7.16 and 7.50 ppm, and turned out to be critical for assembling the structure of Cpd A. The proton at 7.16 ppm was attached to a carbon at 76.08 ppm, which was the site of ketone reduction. This hydrogen showed HMBC

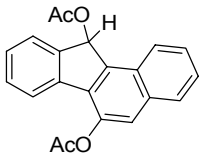
correlation to ring A carbons 6b, 10, 10a, indicating the placement of the secondary alcohol center in ring B. Meanwhile, the HMBC correlation from H-10 to the carbon at 76.08 ppm confirmed the placement of the alcohol at C-11. The 7.50 ppm proton had HMBC correlation with C-4, C-4a, and C-11b, indicating that this hydrogen, H-5, had replaced the diazo group. Further HMBC correlations from H-5 to a carbon resonance at 144.2 ppm and an acetate carbonyl carbon at 169.92 ppm established C-6 as a phenolic carbon Additional correlations in the HMBC spectrum allowed for complete assignment of the tetraacetate which was determined to have structure **1-111**.



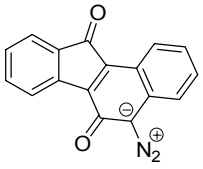
1-111



1-112



1-113



1-114

The structure of this reduction product is compatible only with the structure **1-112** from among several possibilities considered for Cpd A. This structure assignment was further confirmed by the comparison of the spectroscopic properties of **1-111** and Cpd A with the model benzo[*a*]fluorene systems **1-113** and **1-114** synthesized in this laboratory.

Table 1-3 Comparison of 1-111 and Cpd A Diacetate with Model 1-113 and 1-114

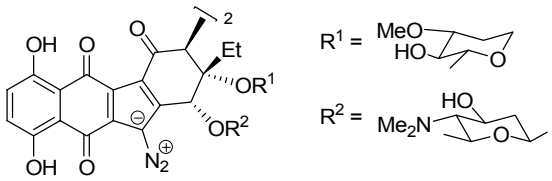
	1-111	**1-113**
^{13}C NMR of C-11	76.08 ppm	74.37 ppm
^{1}H NMR of H-11	7.16 ppm	7.23 ppm

	Cpd A diacetate	**1-114**
^{13}C NMR of C-diazo	83.71 ppm	82.64 ppm
^{13}C NMR of C-11	192.48 ppm	196.77 ppm
IR band of diazo group	2119 cm^{-1}	2105 cm^{-1}

Thus, Cpd A was shown to have the structure **1-112**, and was renamed isoprekinamycin.

1.6 Studies Related to the Mechanism of Action of Kinamycins and Isoprekinamycin

The kinamycins, shown to exhibit good activity against Gram-positive but not Gram-negative bacteria, are of additional current interest because of recently reported in vitro cytotoxicity profiles against cancer cells which are suggestive of a mode of action different than that of anticancer agents in current clinical use.[37] The discovery of lomaiviticin **1-115**, which is a dimeric analogue of the kinamycins possessing potent cytotoxicity against a range of cancer cell lines, that is higher than that observed for monomeric kinamycins, has heightened interest in these unusual natural products.[38]



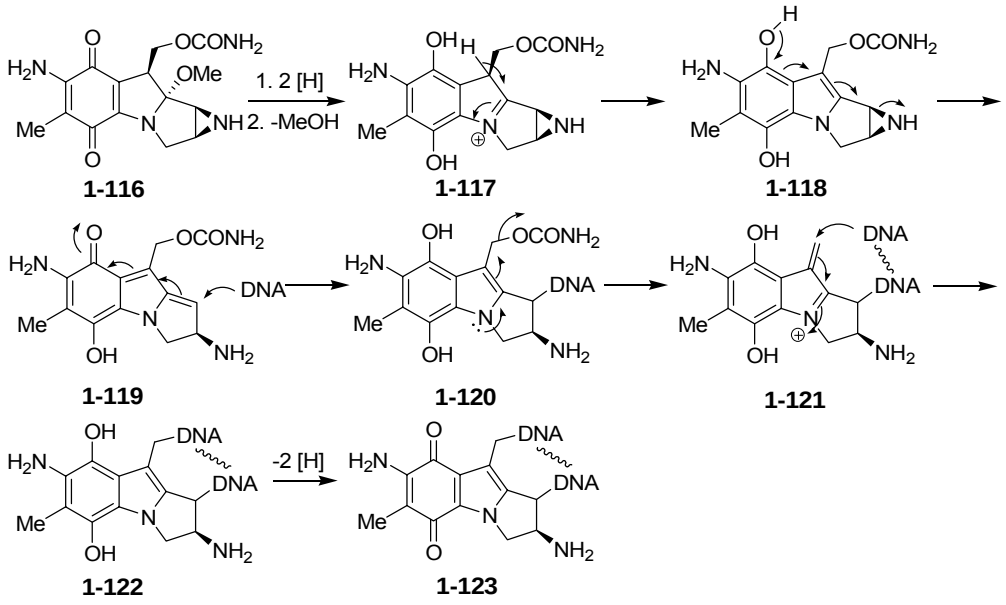
1-115 Lomaiviticin A

1.6.1 Proposed Mechanism of Action for Cyanobenzo[*b*]carbazole Structure

Although much is known about the biosynthesis of the kinamycin[39], surprisingly little information exists concerning the chemical basis for their biological activity.

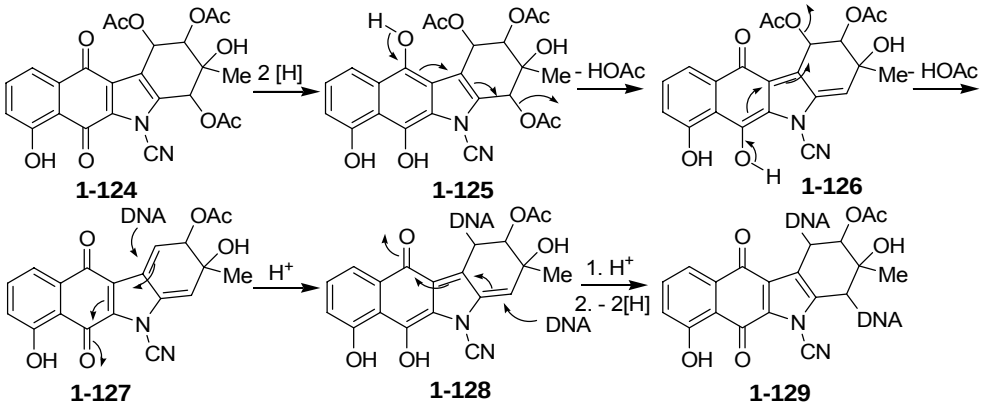
In 1977, Moore speculated that kinamycin C might be a potential reductive alkylation agent same as mitomycin C **1-116** based on the fact that both of them had structures containing indoloquinones incorporating suitably positioned leaving groups.[40] Mitomycin C had been known to be a DNA cross-linking agent under reductive conditions as presented in Scheme 1-23.[41] Thus, a reduction to the hydroquinone, followed sequentially by an expulsion of the angular methoxy group then tautomerization, provided a vinylogous quinone methide **1-119**, which was activated to nucleophilic attack (e.g. by deoxyguanosine of DNA[42,43]). A subsequent expulsion of the carbamate group in **1-120** generates a Michael acceptor **1-121**. The DNA bis-alkylation might be reversible for both strands of DNA,[44] but reversal might be prevented by reoxidation of the hydroquinone **1-122** to the quinone **1-123**.

Scheme 1-23



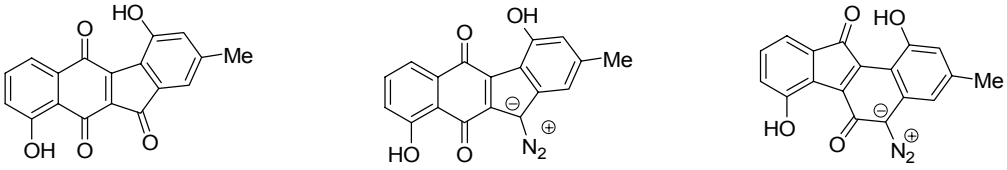
For kinamycin C (with the unrevised structure **1-124**), an in vivo reduction to the hydroquinone **1-125** followed by elimination of two acetate groups might generate a Michael acceptor **1-127** as presented in Scheme 1-24. This bioreductive alkylation proposal by Moore can not be disregarded even after the reassignment of the structure of kinamycins from *N*-cyanocarbazoles to diazobenzo[*b*]fluorenes since the diazo group is isomeric and isoelectronic with the cyanamide group.

Scheme 1-24



1.6.2 Suggested Mechanism for Diazobenzofluorenes

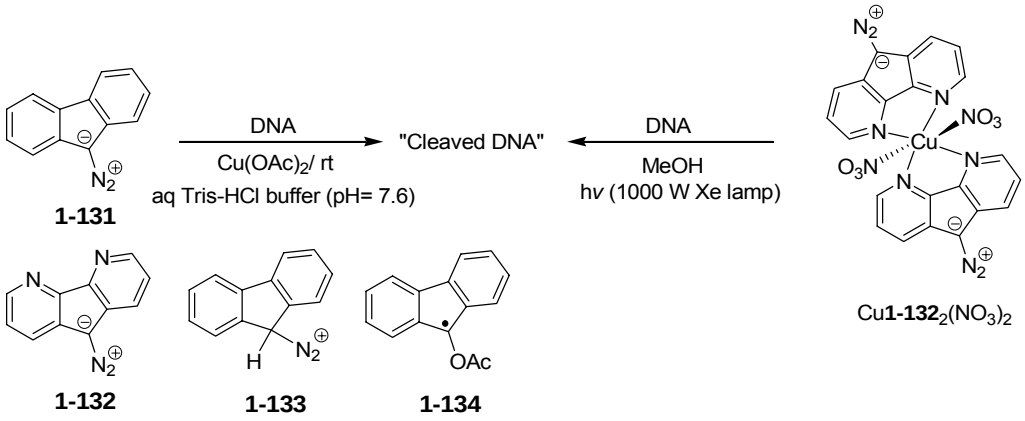
Moore's proposal did not involve the *N*-cyano or the isoelectronic diazo group, which might be relevant to the biological activity of the kinamycins. In addition, kinafluorenone **1-130**, which lacks a diazo moiety, shows no detectable antibiotic activity against *B. stubtilis* ATCC 663, which is very sensitive to the kinamycins.[21] When the project began neither prekinamycin and isoprekinamycin (IPK) **1-112** had been known to present anticancer activity. IPK, however, had been shown to have moderate activity against Gram positive bacteria.[22]



Since the structural revision of the kinamycins, there have been considerable speculations concerning the mode of action based on the assumption that the kinamycins are DNA

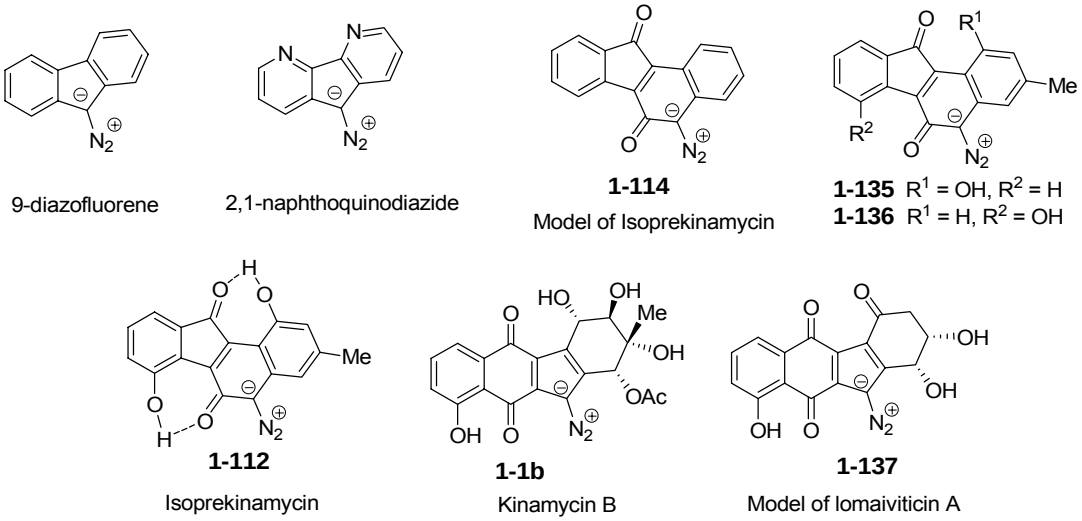
cleaving agents in vivo. Jebaratnam and Arya suggested that the diazo group in the kinamycins may serve as a precursor for carbene or radical intermediate in vivo, which are involved in the mode of action for these antitumor antibiotics.[45] As presented in Scheme 1-25, *9H*-fluorene-9-diazoniun ion or 9-diazofluorene alone in the presence of the reductive regents such as Cu_2Cl_2 did not induce any cleavage of DNA, but oxidation of 9-diazofluorene with cupric acetate led to a cleavage of DNA, which was speculated to involve either copper-carbenoids or acetoxy radical.[46]

Scheme 1-25



Meanwhile, Zaleski and co-workers used photoreactions of *bis*(9-diazo-4,5-diazofluorene)copper(II) nitrate Cu**1-132**$_2(NO_3)_2$ as a model for a possible mode of action of the kinamycin antibiotics (scheme 1-22), in which Cu^{II} again was found important for the DNA cleavage.[45] The metal-ligand photoredox was suggested to play a critical role in the oxidative formation of damaging cation radicals from Cu**1-132**$_2(NO_3)_2$.[47, 48]

The significance of Zaleski's work is not clear since there is no experimental evidence for photoactivation for the kinamycins to exhibit antibacterial or anticancer activity.



Model compound **1-114** was synthesized in this laboratory. Comparison of its susceptibility to nucleophilic attack with that of isoprekinamycin indicated that this natural product is rendered more diazonium ion-like because of the intramolecular H-bonding network present. Furthermore ab intio MO calculations supported this and revealed that even greater diazonium ion-like character should be expected from kinamycins and lomaiviticin A. All these led to the proposal that the enhanced reactivity of the diazo group in these natural products may play a role in their antitumor and antibiotic activity.[47]

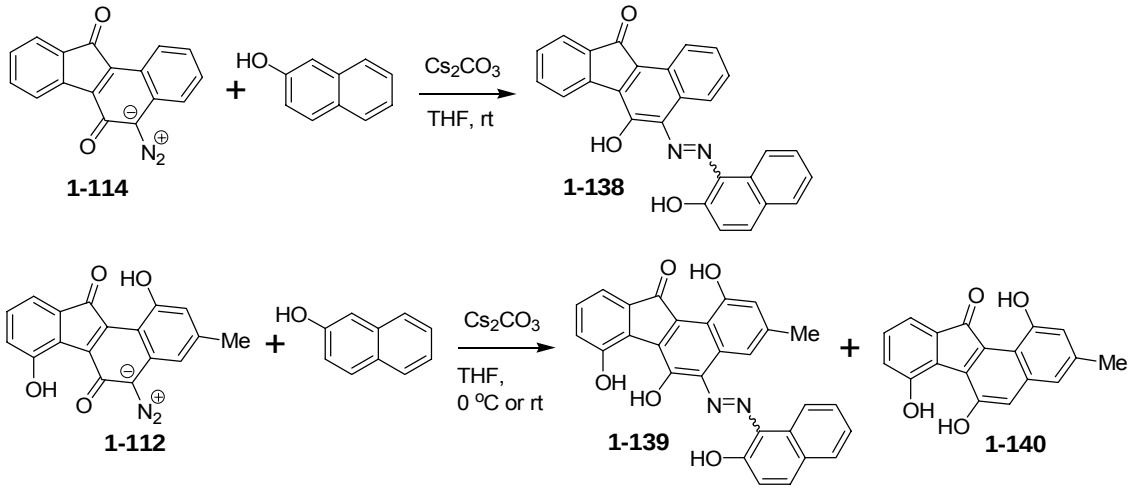
Table 1-4 Calculated C-N_2 Frequencies and N-N Bond Lengths

Compound	Calcd ν (cm^{-1})	Calcd N-N (Å)
9-diazofluorene	1906	1.133
2,1-naphthoquinodiazide	2056	1.111
1-114	2087	1.108
1-135	2101	1.107
1-136	2125	1.105
1-112	2139	1.103
1-1b	2188	1.099
1-137	2212	1.097
Ph-N≡N^+Cl^-	2212	1.100

The IR stretching frequency assigned to the diazo group in the model compound (**1-114**, 2105 cm^{-1}) was 57 cm^{-1} lower than that of isoprekinamycin **1-112** (2162 cm^{-1}), which matched quite well with the calculation ($\Delta\nu$ = 52 cm^{-1}). Meanwhile the calculation revealed that the N-N bond of model **1-114** was shorter relative to that of isoprekinamycin **1-112**. Furthermore, the N-N bond lengths and stretching frequencies calculated for the related systems indicated a trend of increasing the diazonium ion character in the order of 9-diazofluorene < 2,1-naphthoquinodiazide < **1-114** < **1-335** <**1-336** < isoprekinamycin **1-112** (table 1-4), which indicated that the diazonium ion character of the diazo group in isoprekinamycin was enhanced by both the carbonyl group in B ring and the H-bonding network. Similar calculations showed that an even higher degree of diazonium ion character in the N-N bond in the kinamycins and the simplified model of lomaiviticin A **1-137** paralleling the trend in antitumor and antibiotic activities (**1-112** < **1-1** < **1-115**). The

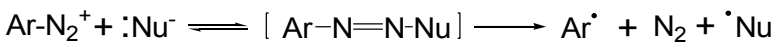
enhancement of the reactivity of isoprekinamycin compared to the key model **1-114** was confirmed in the experiment as presented in Scheme 1-26. The model **1-114** and isoprekinamycin **1-112** were reacted with β-naphthol in the presence of Cs_2CO_3. At room temperature, the model compound gave azo adduct **1-138** whereas isoprekinamycin provided a 1:2 mixture of adduct **1-139** and hydrodeazonization product **1-140**. However, at 0 °C in the same condition, isoprekinamycin **1-112** was completely converted to a mixture of **1-139** and **1-140** in 9 h, but negligible reaction of **1-114** was observed after 17 h.

Scheme 1-26



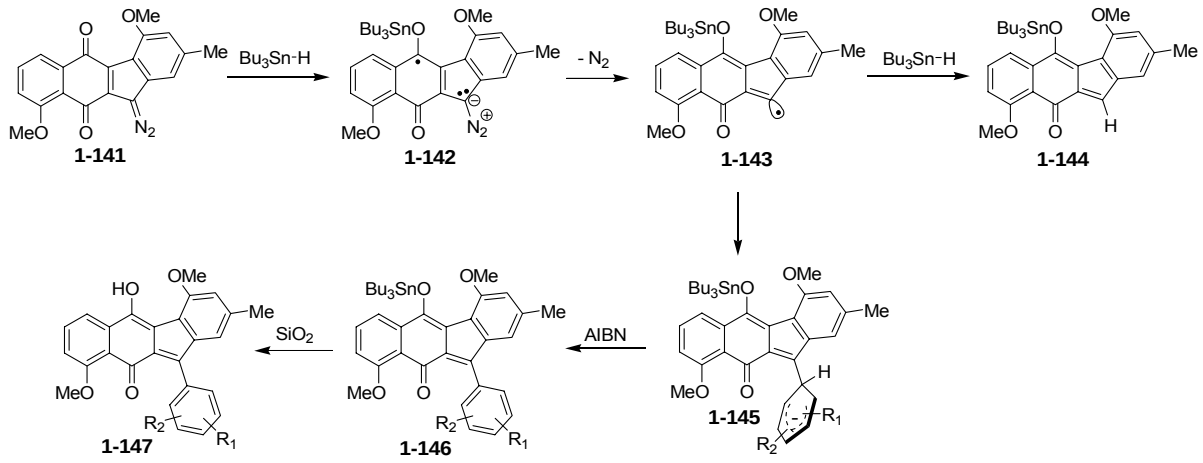
Thus, isoprekinamycin, the kinamycins and the lomaiviticins might be activated toward attack by nucleophiles at the diazo group. In the case of aryl diazonium ions, nucleophilic attack at the terminal nitrogen of the diazonium ion group is an obligatory step prior to radical formation by loss of N_2.[50]

Scheme 1-27



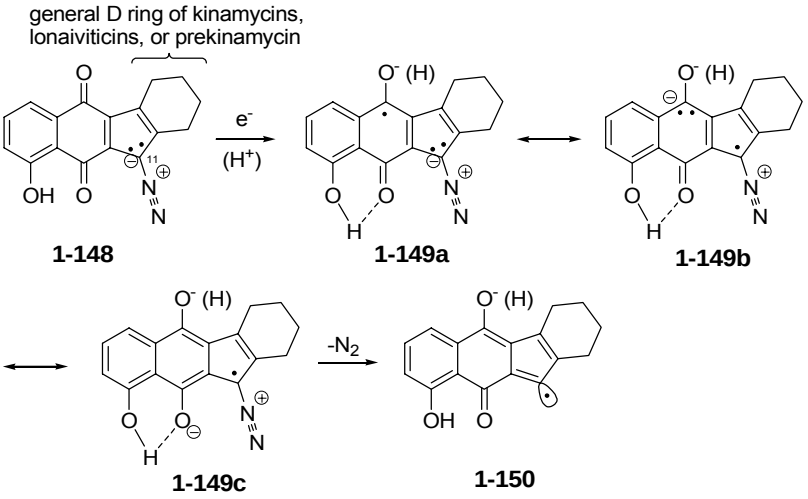
Feldman and Eastman have explored reactions of prekinamycin and its *O,O*-diacetyl and *O,O*-dimethyl derivatives with tributyltin hydride and AIBN at 80 °C in various aromatic solvents. In benzene, the major product is the apparent result of addition of the radical **1-143** to the benzene ring followed by an undefined oxidative process to give the 11-phenylbenzo[*b*]fluorene **1-147**.[51,52]

Scheme 1-28



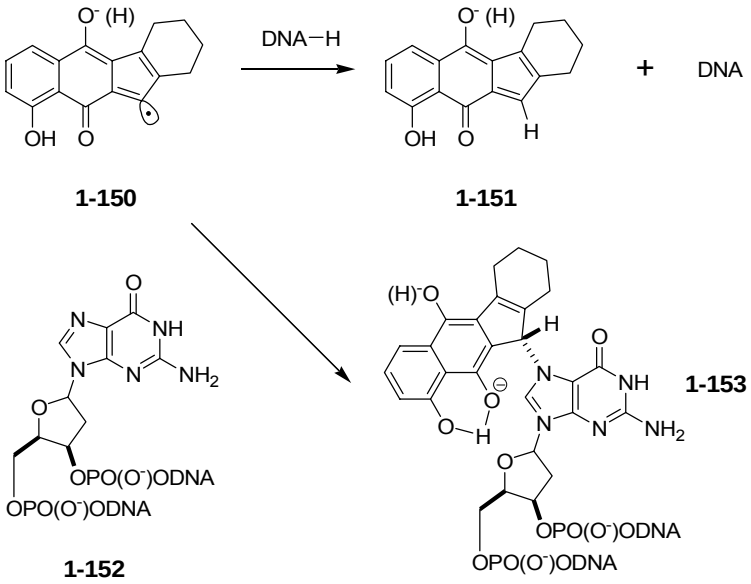
It was suggested that this chemistry serves as a model for a single electron transfer mechanism for activation of kinamycins as DNA cleaving agents in vivo. In particular it was suggested that the single electron reduction would lead to formation of sp^2 radical **1-150** at C-11 of the benzo[*b*]fluorene system. (Scheme 1-29)

Scheme 1-29



It was further hypothesized by Feldman and Eastman that the radical **1-150** might abstract a hydrogen atom from a deoxyribose unit of DNA to initiate DNA strand cleavage and to produce an *o*-quinodiazide intermediate **1-153** which itself might contribute to DNA cleavage by acting as a potent electrophilic Michael acceptor.

Scheme 1-30



Although they provided a convincing argument in support of the formation of a sp^2 radical at C-11 as an intermediate in their chemistry it is not clear how relevant these observations are to the true mode of action of the kinamycins given the highly non-physiological conditions of the model reactions that Feldman and Eastman have reported.

1.6.3 Studies of Mechanism of the Cytotoxicity of the Kinamycins

Recently, this group established the collaboration with a cell biology group headed by Professor Brian Hasinoff in the Faculty of Pharmacy at the University of Manitoba in order to probe the mode of action of the kinamycins and IPK in whole cells.

Hasinoff, Dmitrienko, and coworkers investigated the cell growth and cell cycle inhibitory effects of the kinamycin A and kinamycin C in attempt to determine their mechanism of action.[37] Both these kinamycins were shown to have very potent cell growth inhibitory effects on both Chinese hamster ovary and K562 human leukemia cells. Kinamycin C was found to induce a rapid apoptotic response in K562 human leukemia cells. In cell cycle analysis results of synchronized Chinese hamster ovary cells, kinamycin A was found to induce a G_1/S phase block upon entry to the second cycle. Both kinamycins inhibited the catalytic decatenation activity of DNA topoismerase IIα, but neither kinamycin acted as a topoisomerase II poison. IPK and IPK diacetate were also found to inhibit topoisomerase IIα.[53] Their inhibition of catalytic activity did not, however, correlate with cell growth inhibitory effects. Pretreatment of kinamycins with dithiothreitol protected the topoisomerase IIα activity in vitro, which indicated that inhibition of topoisomerase IIα was perhaps as a result of reaction with the activated electrophilic diazo group. Although the cellular target(s)

of the kinamycins has (have) yet to be identified, the cluster map analysis of the anticancer activity of kinamycin C against the 60-cell line panel at the National Cancer Institute, and the cell cycle and pro-apoptotic effects suggested that kinamycin C has a target different than other established anticancer compounds.[37]

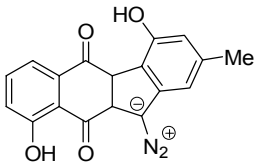
More recently, kinamycin F was investigated as a potent antitumor agent by Hasinoff, Dmitrienko and coworkers.[54] Kinamycin F was shown to react with glutathione (GSH) in a complex series of reactions which suggested that kinamycin F may have its cytotoxicity modulated by GSH. Kinamycin F was found to bind weakly to DNA and induced DNA damage in K562 cells which was independent of GSH level. In vitro studies showed that kinamycin F was capable of causing DNA nicking and that this effect was enhanced in the presence of GSH at approximately intracellular concentration.

Still more recent unpublished studies by Hasinoff and Dmitrienko groups[55] indicate that kinamycin F causes down-regulation of Cyclin D3 in human myelogenous leukemia K562 cells, suggesting a mode of action that may be unique relative to other known anticancer agents.

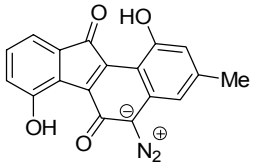
Chapter 2 Model Studies for the Synthesis of Diazobenzo[*a*]fluorenes

2.1 Introduction

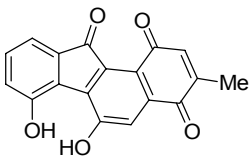
As indicated in Chapter 1, isoprekinamycin (IPK), first assigned structure **2-1**,[1] is now recognized as the diazobenzo[*a*]fluorene **2-2**.[2] The previously rare benzo[*a*]fluorene class of natural products now boasts several other members (fluostatins A–E) with various degree of oxygenation in D ring but lacking a diazo group.[3,4,5,6] In this group, studies suggesting a substantial diazonium ion character in the diazo group of IPK, which might play a role in its bioactivity, have inspired us to design a practical synthesis of IPK. This work was initiated from the model study of the synthesis of IPK system, which will be discussed in this chapter.



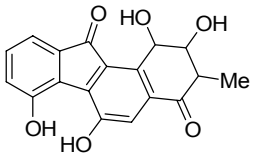
2-1



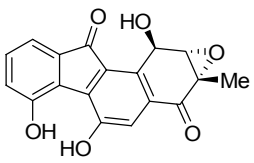
2-2



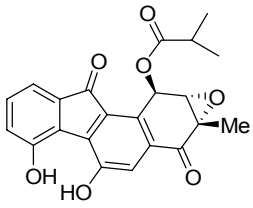
Fluostatin A



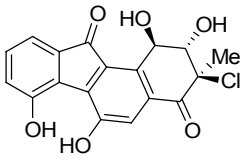
Fluostatin B



Fluostatin C



Fluostatin D

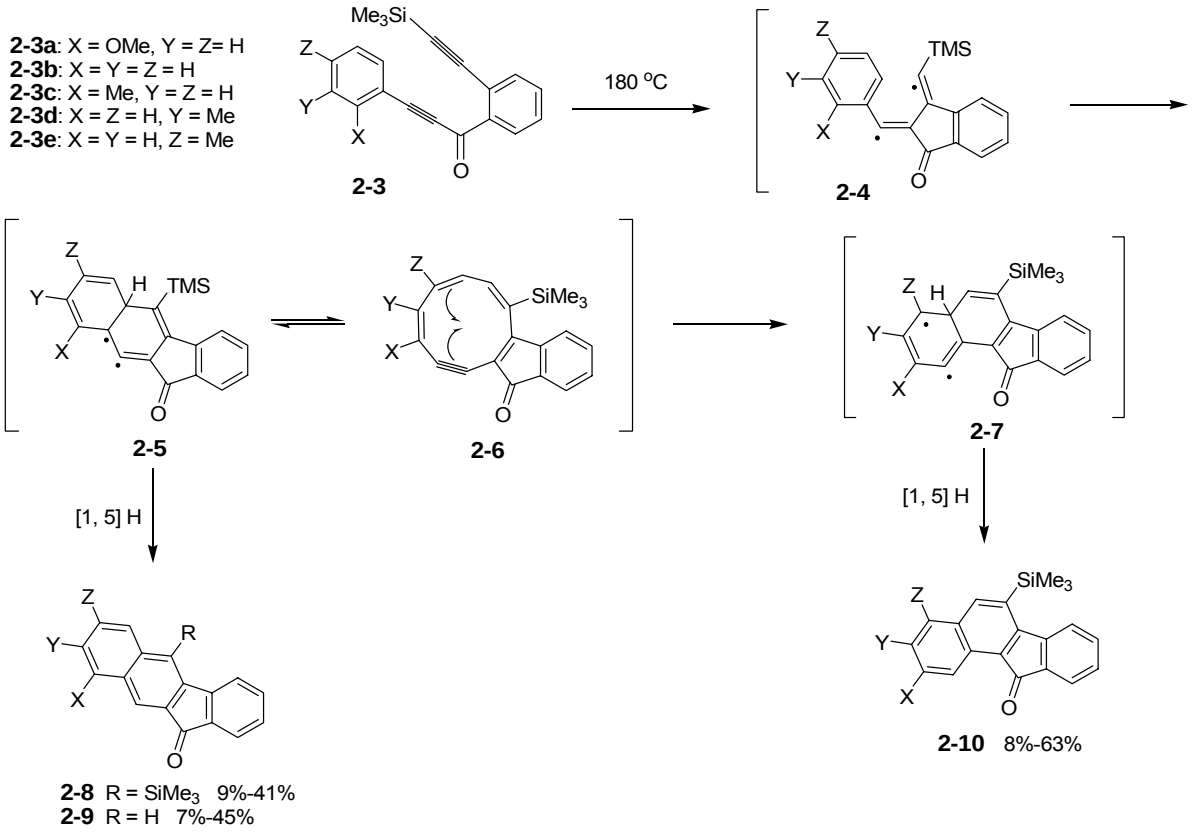


Fluostatin E

Besides the formal work in this laboratory there are a number of reports related to the synthesis of the benzo[*a*]fluorenes. Echavarren and coworkers reported that the thermal

cyclization of diaryldiynones **2-3** generated not only the benzo[*b*]fluorenes (**2-8** and **2-9**), but also the unanticipated benzo[*a*]fluorenes (**2-10**). The formation of the latter products possibly occurs via electrocyclic ring opening-ring closing sequence from **2-5** as presented in Scheme 2-1.[7]

Scheme 2-1

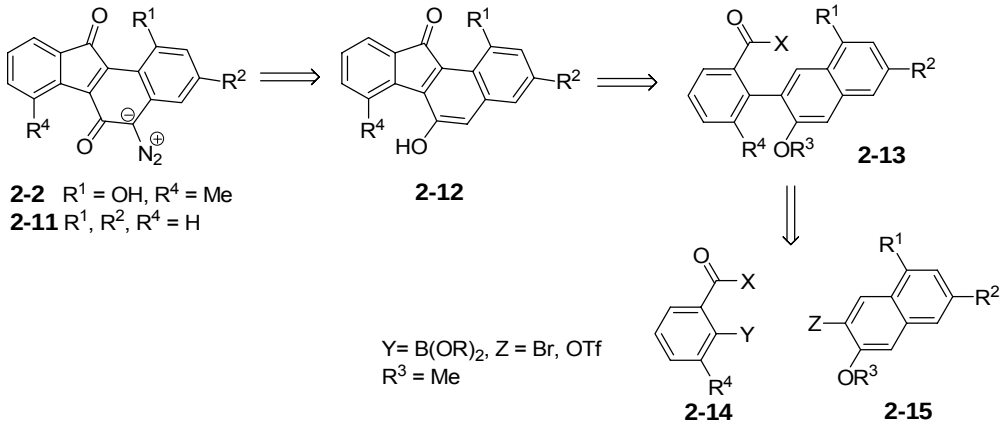


2.2 Previous Works in This laboratory

Radoslaw S. Laufer in this laboratory developed a methodology for building up the diazobenzo[*a*]fluorene, and a model compound **2-11** of isoprekinamycin was synthesized successfully.[8] A retrosynthetic analysis is presented in Scheme 2-2. It was reasoned that the diazo group was introduced at final stage via the phenol **2-12**. The entire ring system might

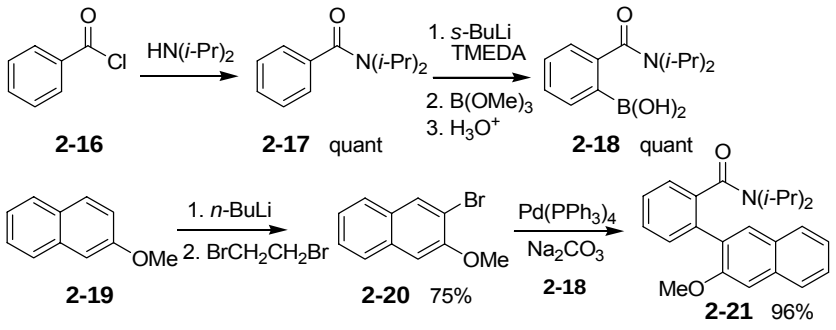
be completed in a Friedel-Crafts cyclization of a biaryl compound such as **2-13**, derived from a cross coupling reaction of fragments **2-14** and **2-15**.

Scheme 2-2



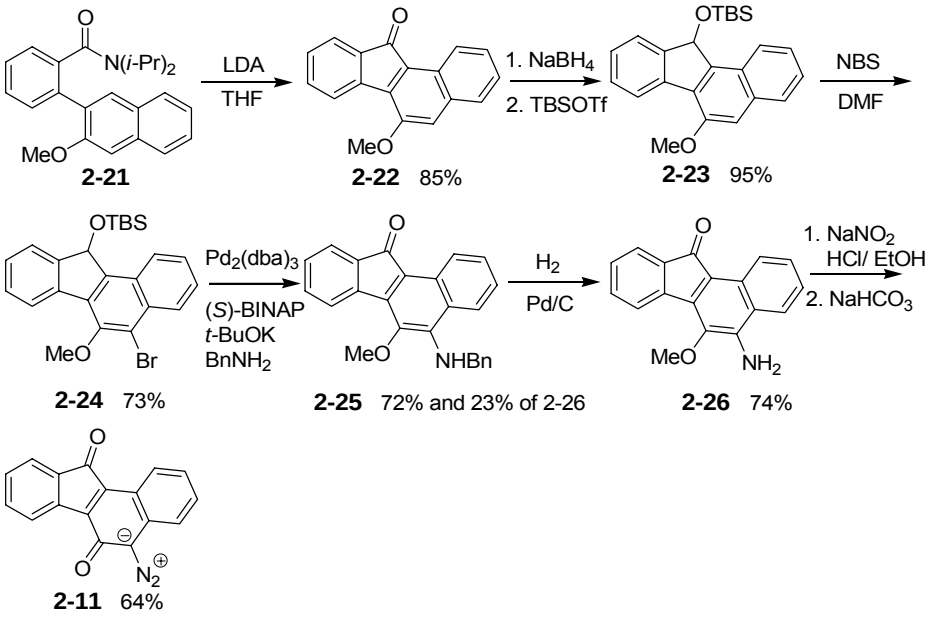
The boronic acid fragment **2-18** was synthesized by sequential treatment of benzamide **2-17** with *s*-BuLi and $B(OMe)_3$. The benzamide was derived from benzoyl chloride **2-16**. Orthometalation of 2-methoxynaphthalene **2-19** with *n*-BuLi, followed by treatment with $BrCH_2CH_2Br$ provided fragment **2-20**, which was coupled with **2-18** via Pd catalyzed Suzuki coupling to produce diaryl compound **2-21**.

Scheme 2-3



The anionic cyclization of **2-21** produced benzo[*a*]fluorenone **2-22**, which was inert towards electrophilic bromination presumably because of deactivation by the carbonyl group of the B ring. Thus, **2-22** was reduced with $NaBH_4$ then protected with TBSOTf to form the silyl ether **2-23**, which could be brominated smoothly with NBS in DMF. The palladium-catalyzed amination reaction of aryl bromide **2-24** with benzylamine, via the Buchwald procedure with $Pd_2(dba)_3$, (*S*)-BINAP and *t*-BuOK, resulted in the desired amination as well as in situ desilylation and oxidation to form ketone **2-25**. Palladium-catalyzed hydrogenolysis of **2-25** provided the aniline **2-26**, which was transformed to diazo compound **2-11** by sequential treatments with $NaNO_2$/HCl and $NaHCO_3$.

Scheme 2-4



2-21 **2-22** 85% **2-23** 95%

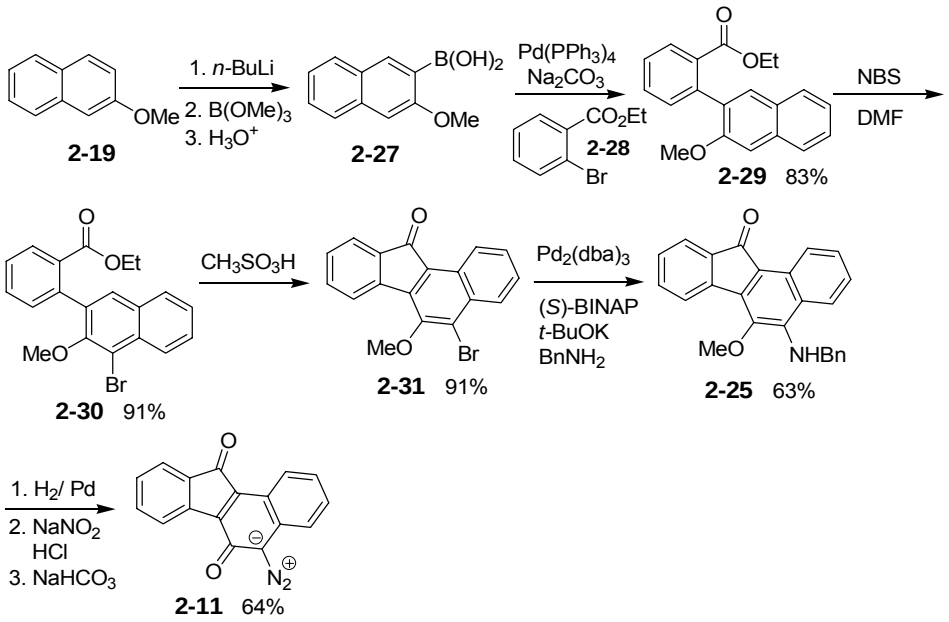
2-24 73% **2-25** 72% and 23% of 2-26 **2-26** 74%

2-11 64%

Given the difficulties encountered in the introduction of functionalities *ortho* to the oxygen substituent in the benzo[*a*]fluorenone system described above, an alternative strategy was carried out to improve the efficiency of the synthesis (Scheme 2-5). Thus, *ortho*-metalation

of 2-methoxynaphthalene **2-19** with *n*-BuLi, followed by treatment with $B(OMe)_3$ afforded the desired aryl boronic acid **2-27**, which was cross-coupled with **2-28** by Suzuki coupling reaction to form diaryl adduct **2-29**. The **2-29** was brominated then cyclized via intramolecular Friedel-Crafts reaction with methanesulfonic acid to provide aryl bromide **2-31**. Buchwald benzylamination of **2-31** produced the secondary amine **2-25** which could be converted into *o*-quinodiazide **2-21**, as done previously, by debenzylation followed by demethylative diazotization steps.

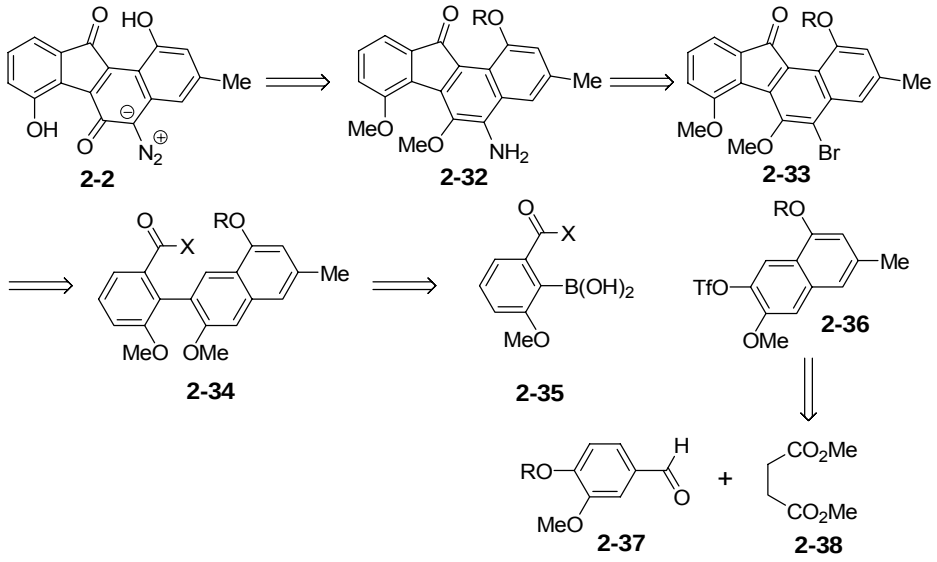
Scheme 2-5



After the synthesis of **2-11**, an attempt was made to extend the methodology to the total synthesis of isoprekinamycin as presented as the retrosynthetic analysis in Scheme 2-6. The *o*-quinodiazide motif in the isoprekinamycin would be established by demethylative diazotization as in the synthesis of the model **2-11**. The required aniline **2-32** might be derived by subjecting biaryl **2-34** to a sequence of bromination, Friedel-Crafts cyclization

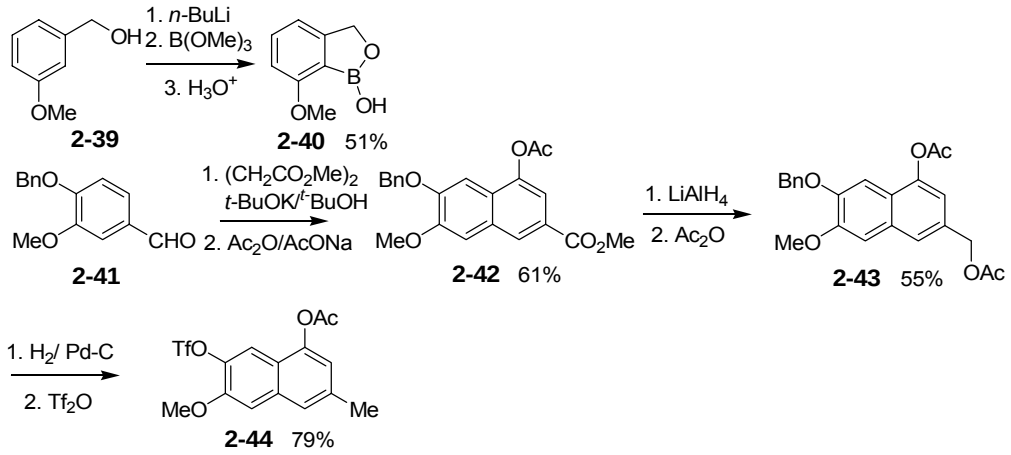
and palladium-catalyzed amination. The biaryl **2-34** might be synthesized by a cross-coupling between fragments **2-35** and **2-36**, and **2-36** might possibly be derived from the Stobbe reaction between vanillin **2-37** and dimethyl succinate **2-38**.

Scheme 2-6



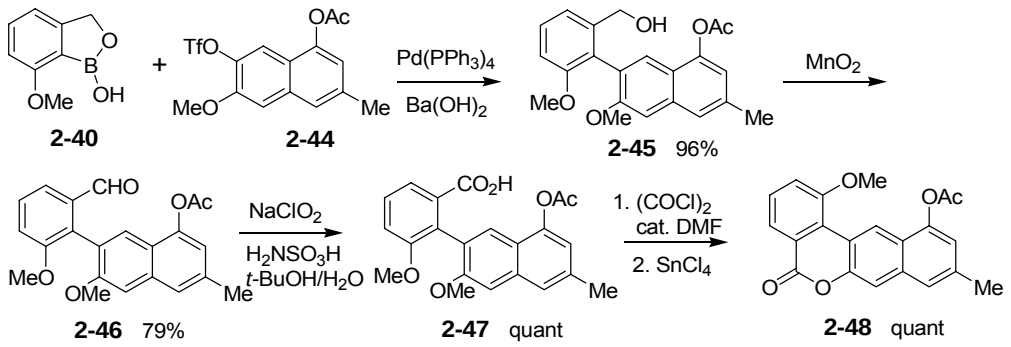
In practice, the A ring precursor boronate **2-40** was synthesized by *ortho* metalation of 3-methoxybenzyl alcohol **2-39** followed by an electrophilic boronation with $B(OMe)_3$ and acidic aqueous workup. The synthesis of the suitable naphthalene **2-44** was initiated from an anionic condensation between dimethyl succcinate with the commercially available benzaldehyde **2-41**, which was heated with sodium acetate in acetic anhydride to provide an aromatic ester **2-42**. The **2-42** was reduced with lithium aluminium hydride and subsequently diacetylated with acetic anhydride to afford intermediate **2-43**, which was transformed to triflate **2-44** as the CD ring component of isoprekinamycin by sequential treatment with H_2/Pd and then triflic anhydride. (Scheme 2-7)

Scheme 2-7



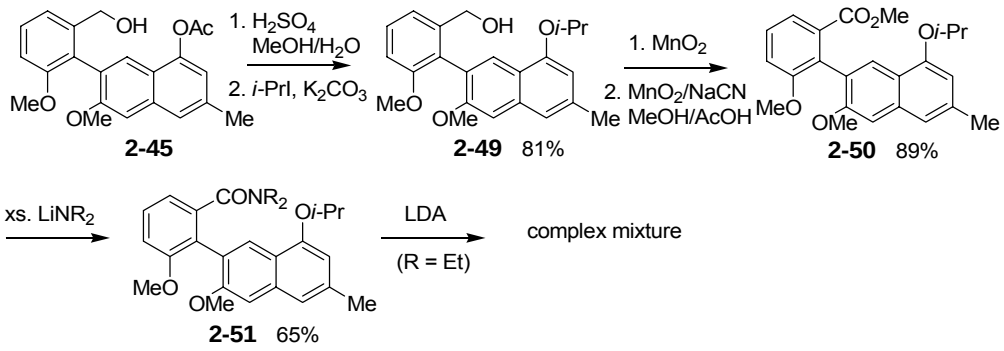
The Suzuki coupling between fragment **2-40** and **2-44** with $Pd(PPh_3)_4$ in the presence of $Ba(OH)_2$ afforded benzylic alcohol **2-45**, which was doubly oxidized subsequently by MnO_2, then $NaClO_2$ to produce carboxylic acid **2-47**. However, the cyclization of the **2-47** via Friedel-Crafts reaction in the presence of methanesulfonic acid which was quite efficient in the synthesis of model compound was unattainable. Attempts to use the mild Lewis acidic $SnCl_4$ to effect cyclization of the acyl chloride, prepared by a treatment of **2-47** with oxalyl chloride and DMF, resulted in clean formation of the lactone **2-48** instead.

Scheme 2-8



In order to investigate the possibility of the anionic approach to the synthesis of the target benzo[*a*]fluorenone ring system, the *O*-1 acetate functionality was exchanged to a robust protected group, isopropyl group. Replacement of the acetate in **2-45** was achieved by a sequence involving acidolysis of the protecting group in aqueous methanol and isopropylation with *i*-PrI and K_2CO_3. Two consecutive oxidations afforded a methyl ester **2-50**, which was transformed to the corresponding secondary amide **2-51**. Unfortunately, anionic cyclization of the amide **2-51** with LDA resulted in a complex mixture instead of the desired product.

Scheme 2-9

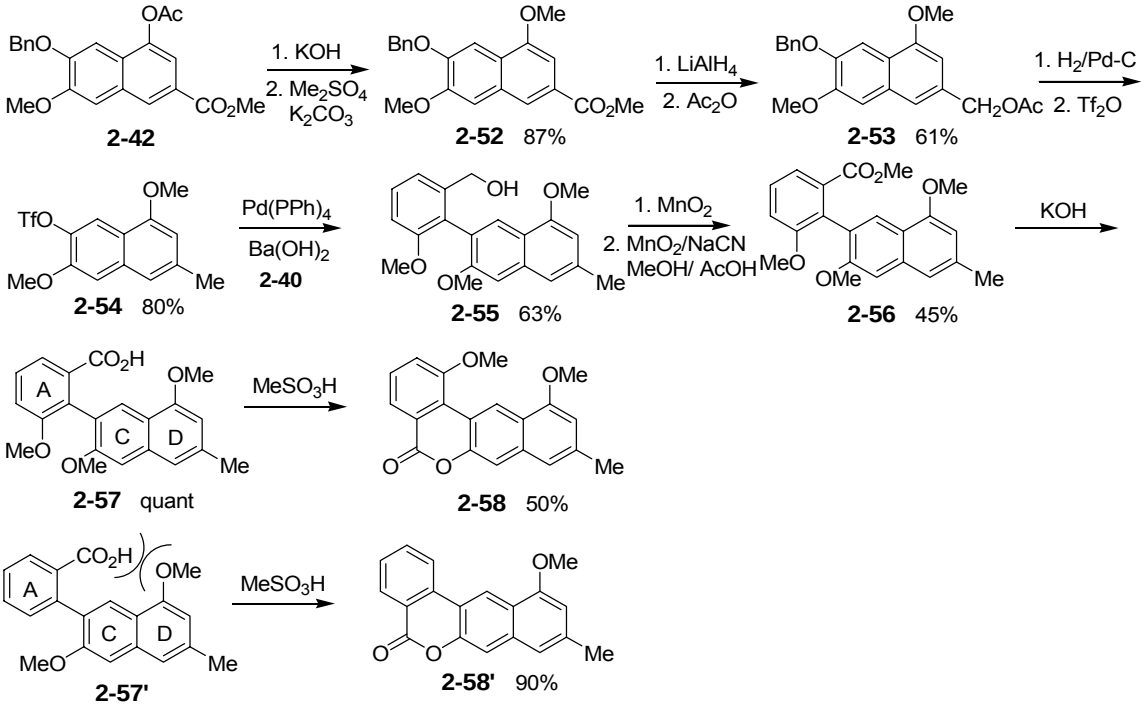


Then the possibility of cyclization using the less labile *O*-methyl protecting group was explored. The carboxylic acid **2-57** was synthesized by only minor modification of the route used before, in which the intermediate **2-42** was saponified and doubly methylated to provide methyl ether **2-52** which was subjected to the following steps as shown in Scheme 2-10. Heating of the carboxylic acid **2-57** in methanesulfonic acid led to lactonization to **2-58**.

Matthew Buck in this laboratory synthesized the carboxylic acid **2-57'** without a methoxyl group in A ring, and found that **2-57'** was still converted to the lactone **2-58'** even without the

interaction between the methoxyl groups in the A and C rings, presumably because of the bad interaction between the carboxylic acid group in the A ring and the methoxyl group in the D ring.

Scheme 2-10

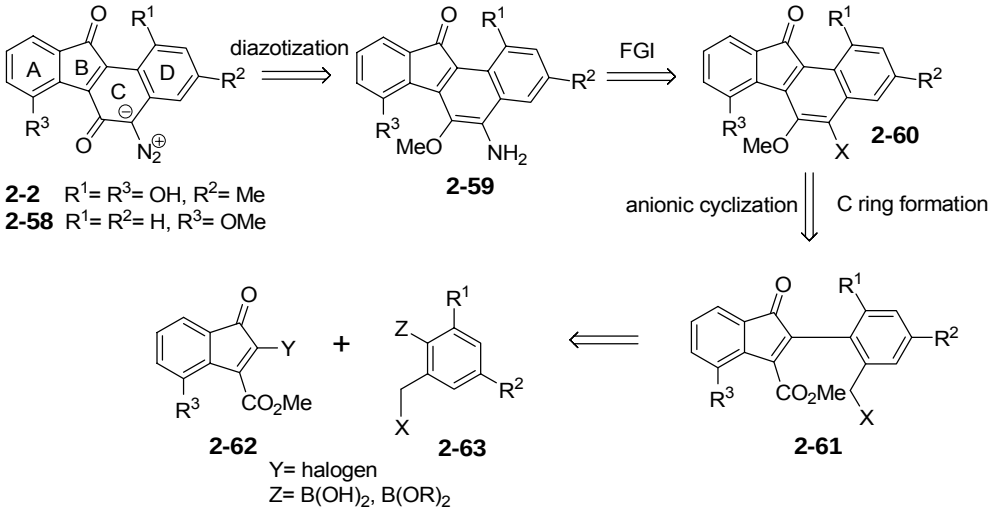


2.3 A New Route to Diazobenzo[*a*]fluorenes

Facing the fact that the formal methodology, although it was successful for the synthesis of the model **2-11**, but finally failed to achieve the total synthesis of isoprekinamycin, a new route had to be found, which is general and practical for the synthesis of isoprekinamycin and its analogues. An alternative strategy was explored as shown in the Scheme 2-11. The diazo functionality would be installed late in the sequence by diazo transformation from aniline **2-59**, which might be derived from a suitable benzo[*a*]fluorene intermediate **2-60**. This **2-60**

could be obtained by an anionic cyclization forming C ring of the system from a diaryl precursor **2-61**, which might be formed by the cross coupling between fragment **2-62** and **2-63** (as AB ring and D ring building block, respectively).

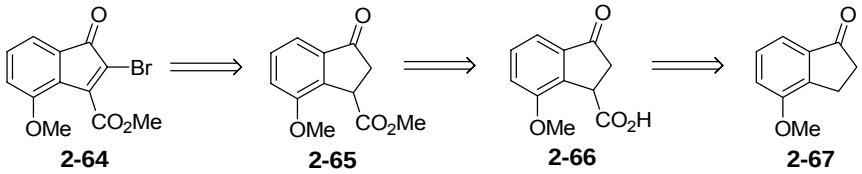
Scheme 2-11



2.3.1 Synthesis of the AB Ring of Diazobenzo[*a*]fluorenes

In practice, as the retrosynthetic analysis in Scheme 2-12 shows, the AB ring building block bromoindenone **2-64** would be obtained from the indanone **2-65**, which might be converted from available indanone **2-67**.

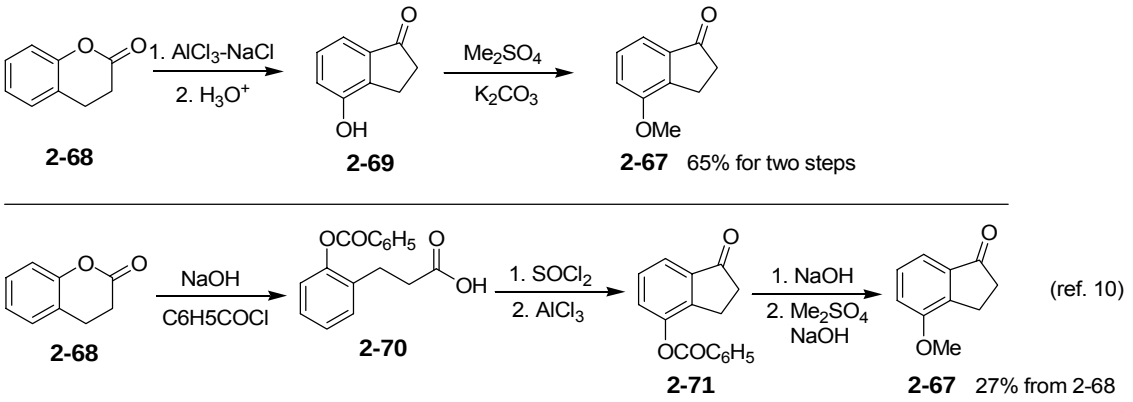
Scheme 2-12



The indanone **2-67** was synthesized via ring opening/Friedel-Crafts cyclization of dihydrocoumarin (**2-68**) followed by methylation with Me_2SO_4. Although the condition of the cyclization is harsh (180-200 ^oC, $AlCl_3$),[9] the reaction proceeded smoothly with 65%

yield after methylation. An alternative procedure was reported by Cannon and coworkers.[10] Ring opening of **2-68** with NaOH followed by protection with benzoyl chloride afforded phenyl propanoic acid **2-70**, which was converted to an acid chloride then cyclized to provide indanone **2-71**. Then replacement of the protecting group led to the desired indanone **2-67**. The latter procedure avoided the former harsh condition, the yield was decreased significantly (over all yield 27% versus 65%).

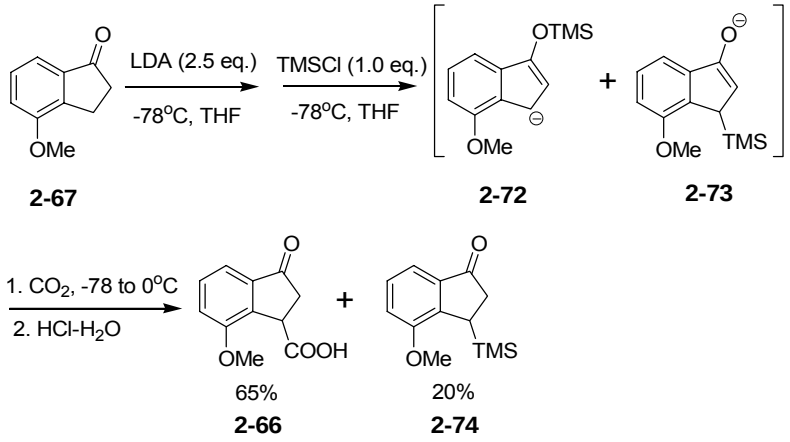
Scheme 2-13



The indanone **2-67** was converted to a carboxylic acid **2-66** by the β-carboxylation procedure reported by Kozikowski and coworkers.[11] The indanone **2-67** was treated with 2.5 equivalents LDA at -78^{o}C in THF as presented in Scheme 2-14. It was believed that only one equivalent LDA was consumed at this stage to generate the enolate anion. The other equivalent of LDA remained unreacted. After adding TMSCl, the enolate anion was converted to an enol silyl ether, and at this stage the benzylic proton is acidic enough to be deprotonated by the remaining one equivalent LDA to afford a homoenolate intermediate **2-72**, which was converted to the carboxylic acid **2-66** by sequential treatment with CO_2 and HCl. Matthew Buck in this laboratory found that treatment of **2-67** with 2.5 equivalents of

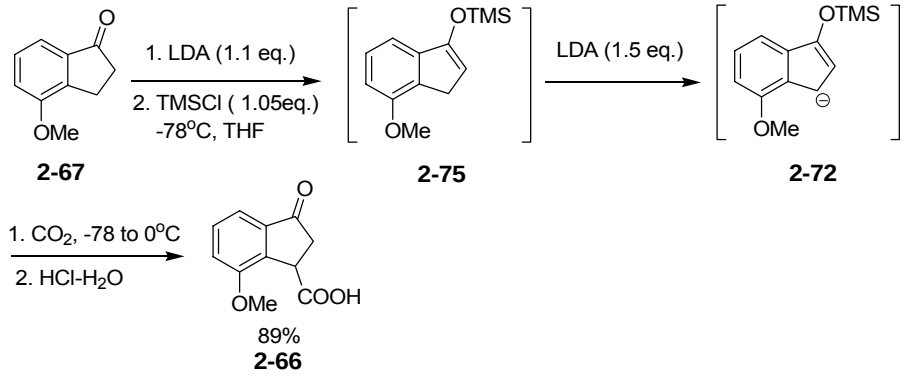
LDA, followed by trapping with TMSCl at –78 °C in THF, generated a mixture of **2-72** and **2-73**. Then the anion mixture of **2-72** and **2-73** was carboxylated with solid carbon dioxide then quenched with aqueous HCl to provide the mixture of **2-66** and **2-74** in 65% and 20% yield, respectively.

Scheme 2-14



Because of the selectivity of the procedure above was not as good as expected, this procedure was modified as shown in Scheme 2-15. Thus, enolization of indanone **2-67** with 1.1 equivalents LDA followed by treatment with 1.05 equivalents TMSCl provided TMS-protected silyl enolate **2-75**. Deprotonation of **2-75** generated anion **2-72**, which was transformed into the carboxylic acid just as in the same sequence above. In this reaction, several carboxylating reagents ($BrCO_2Et$, $(MeO)_2CO$ and CO_2) were examined in order to optimize the yield, and maximum yields were obtained by use of CO_2, solid carbon dioxide.[11] In order to allow for the water in the solid carbon dioxide, more than one equivalent of the second amount of LDA was added to optimize the yield as 89% over all.

Scheme 2-15



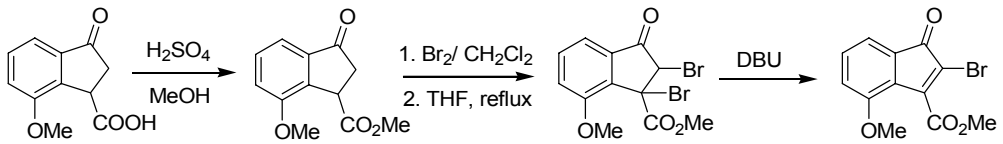
2-67 **2-75** **2-72**

89%

2-66

Matthew Buck in this laboratory found that dibromination of the methyl ester **2-65** with 2.0 equivalents of Br_2 in CH_2Cl_2 for two days generated a dibromo compound, which was believed to be isomerized in refluxed THF for another two days to afford dibromo compound **2-76**. The elimination of **2-76** with DBU provided bromo-indenone **2-64**. When this procedure was followed in the present study the result was a complex mixture.

Scheme 2-16 (Procedure reported by M. Buck)

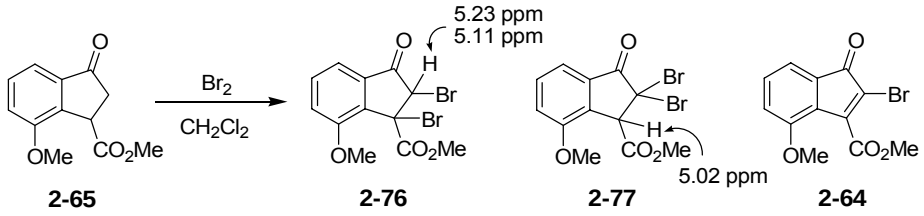


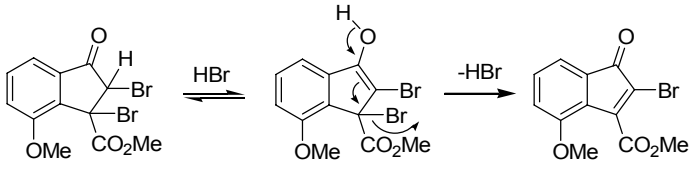
2-66 **2-65** **2-76** **2-64**

The unexpected long time and the difficulty of duplication of this procedure made us explore the reaction in more detail. After treatment of **2-65** with 2.0 equivalents of Br_2 in CH_2Cl_2 at room temperature for three days, the major product was unexpectedly the desired bromoindenone **2-64** along with dibromoindanone **2-76** and **2-77**, in whose proton NMR the shifts 5.23 ppm or 5.11 ppm referred to the proton α- to the ketone group in the dibromoindanone **2-76**, and the shift 5.02 ppm might correspond to the proton β- to the

ketone group in the dibromoindanone **2-77**. Following the time course of the reaction by proton NMR of the crude product it was found that after the first day of the reaction all starting material was consumed and no monobromo products appeared. The dibromoindanone **2-76** was the major product with a minor amount of dibromoindanone **2-77**. After the second day, the ratio of **2-76** to **2-77** was lower and some bromoindenone **2-64** was observed. After the third day, the ratio of **2-76** to **2-77** kept going down and more **2-64** appeared compared with the second day. We believed that dibromoindanones were the major products of this reaction in the first day. Meanwhile the reaction resulted in HBr formation in situ, which catalyzed the β-elimination of the dibromoindanone to generate bromoindenone **2-64**.

Scheme 2-17 (NMR Study)

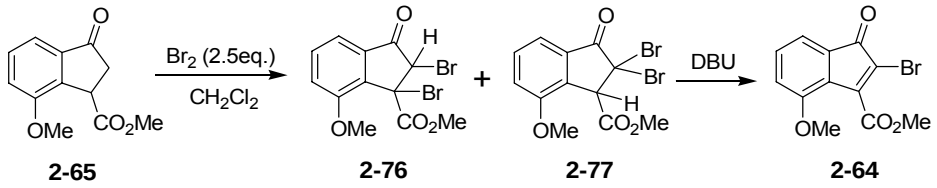




This reaction was found to be sensitive to the ratio of Br_2 to starting material, the concentration of the Br_2 in the reaction and the time of the reaction. Using 2.5 equivalents Br_2 in CH_2Cl_2 (0.5 M in reaction solution) at room temperature for 24 hours, **2-65** was

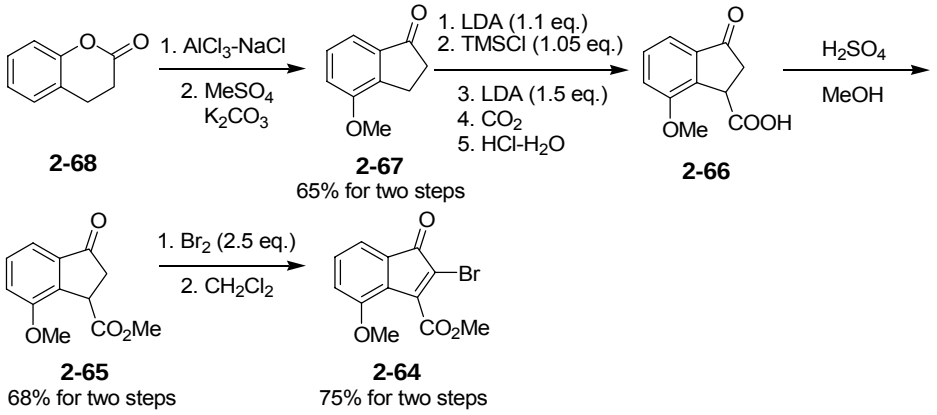
converted to the mixture of **2-76** and **2-77**. Treatment with DBU provided the desired bromoindenone **2-64** in 75% yield over two steps.

Scheme 2-18 (Present Study)



Thus, in the present project the synthesis of the AB ring synthon bromoindenone **2-64** was initiated from the dihydrocoumarin **2-70** via the sequence as presented in Scheme 2-19 and the yield is 33% for six linear steps overall.

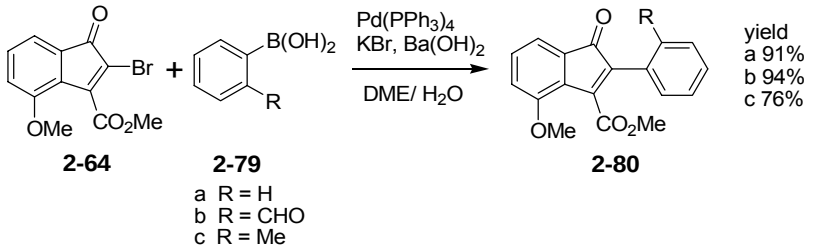
Scheme 2-19



2.3.2 Synthesis of a Model of Isoprekinamycin

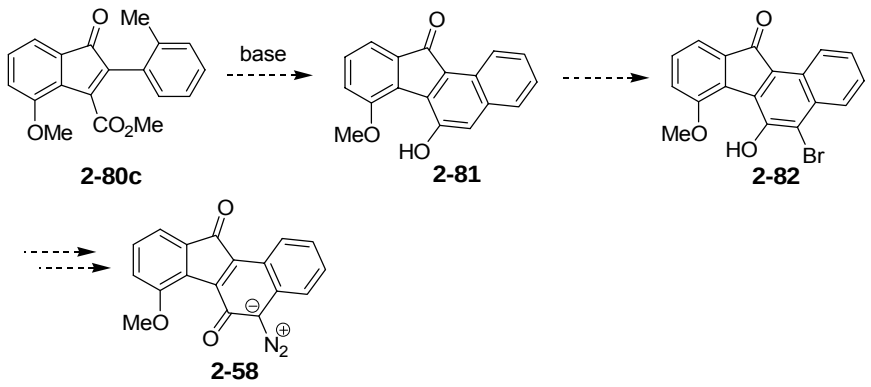
With the AB ring building block **2-64** in hand, the palladium catalyzed Suzuki coupling between **2-64** and **2-79a-c** using $Pd(PPh_3)_4$, KBr, $Ba(OH)_2$ in DME and water was carried out by Matthew Buck in this laboratory, which generated diaryl compounds **2-80a-c** smoothly with quite high yield.

Scheme 2-20



The biaryl compound **2-80c** could be converted to benzo[*a*]fluorene **2-81** by an anionic cyclization using suitable base, which might be transformed to bromo precursor **2-82**. Then the precursor **2-82** should be converted to the model of isoprekinamycin **2-58** via the same sequence as the previous work by R. S. Laufer in this laboratory.

Scheme 2-21

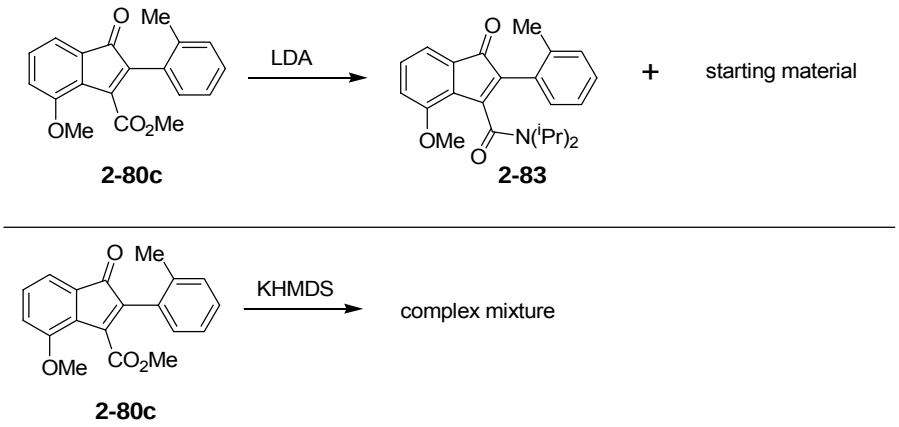


Matthew Buck found that if LDA was used as a base for the anionic cyclization at 40 °C for 12 hours, only diisopropyl amide **2-83** was generated with some starting material remaining.

In the present project, the cyclization of **2-80c** was investigated using potassium hexamethyldisilazide (KHMDS) as base in THF with or without 18-crown-6 from 0 °C to

room temperature. However, the reaction always provided a complex mixture. Hence, it was felt that a stronger base was needed to force this reaction.

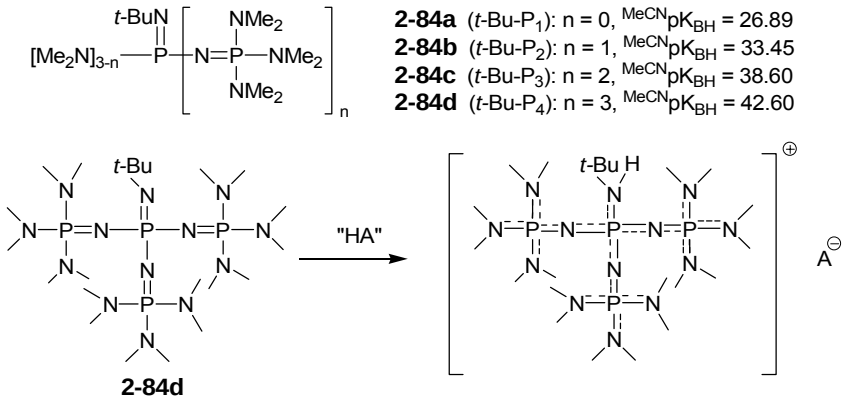
Scheme 2-22



2-80c **2-83** **2-80c**

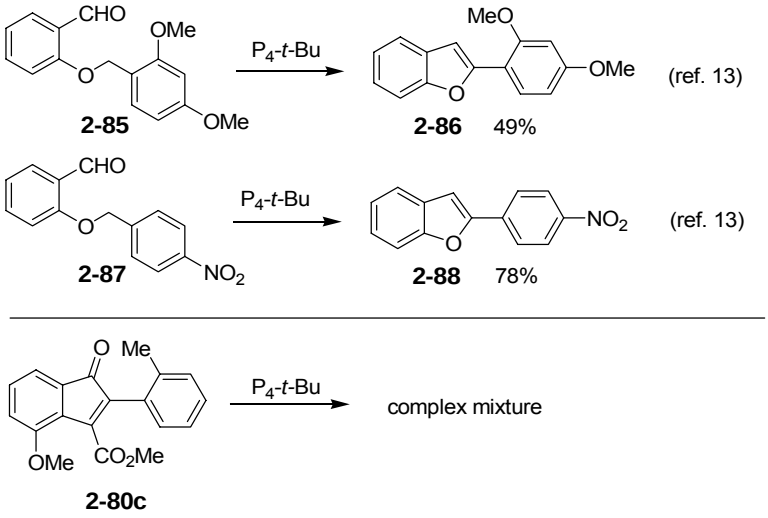
In 1987 Schwesinger and coworkers reported the extraordinarily basic uncharged polyaminophosphazenes **2-84a-d** exhibiting $^{MeCN}pK_{BH}$ values of up to 42.6.[12] These polyaminophosphazenes were such strong bases because of the better charge delocalization of their cationic conjugated acids. With the exception of "proton sponges," uncharged bases are kinetically highly active even towards weak acids, and naked anions thus generated are often extremely reactive. In reaction of such substrates with electrophiles, a low equilibrium concentration of the anion often suffices as long as the base is inert to the electrophile. Thus both an increase in basicity and steric bulk of phosphazene bases should extend their range of application.

Scheme 2-23



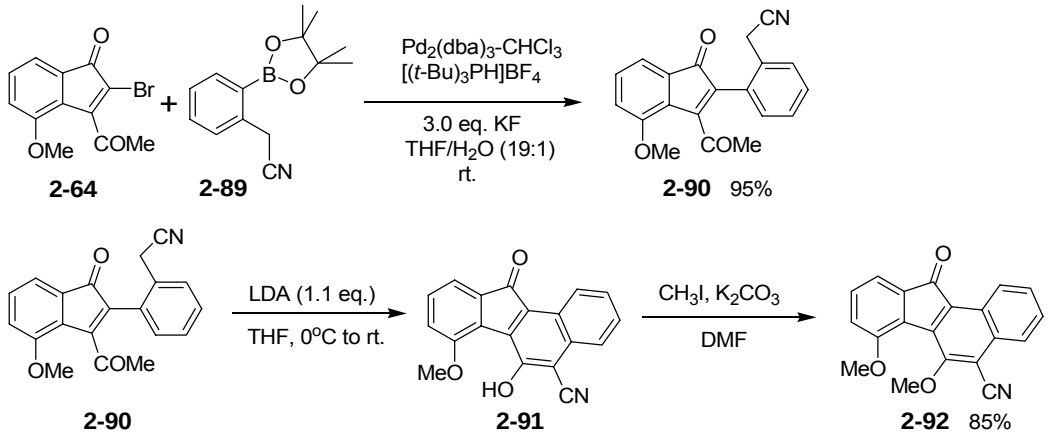
Kraus and coworkers reported that the base **2-84d** (P_4-*t*-Bu) could efficiently deprotonate *o*-arylmethoxy benzaldehydes leading to a direct synthesis of benzofuranes.[13] Treatment of **2-85** with LDA in THF from -78 °C to 25 °C returned recovered starting material. The reaction of **2-85** with lithium tetramethylpiperidide (LiTMP) in THF from 0 °C to 25 °C afforded mostly recovered starting material. The **2-85** did not react with sodium hydride or potassium hydride in THF or DMF. However, the reaction of **2-85** with 1.1 equiv of **2-84d** in pivalonitrile at 90–100 °C generated 2-(2-methoxylphenyl)benzofurane **2-86** in 49% isolated yield. Introducing an electron-withdrawing group in the system improved the cyclization such as the case of **2-87**. Unfortunately with the present study, the cyclization of **2-80c** with 1.1 equiv of **2-84d** in refluxing benzene for 15 hours afforded a complex mixture. (Scheme 2-24)

Scheme 2-24



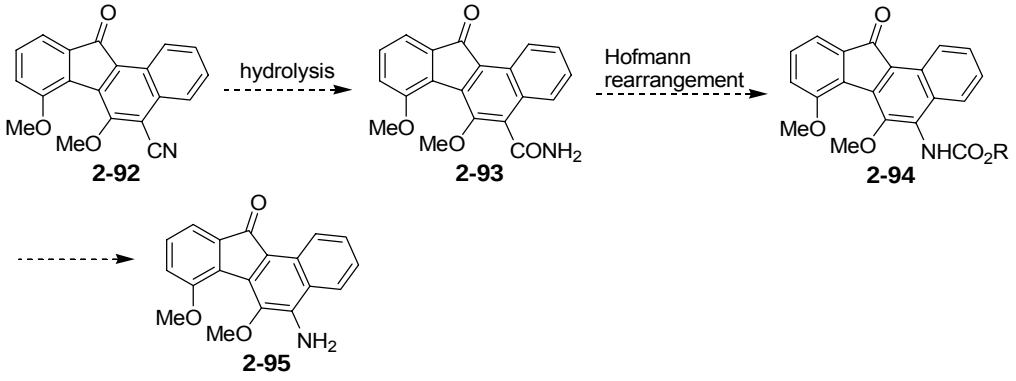
Matthew Buck in this laboratory developed the sequence for the synthesis of **2-92** as shown in Scheme 2-25. Suzuki coupling between bromoindenone **2-64** and commercially available pinacol boronate **2-89** using $Pd_2(dba)_3$ as a catalyst, $[(t\text{-}Bu)_3PH]BF_4$ as a ligand with three equiv of KF as reported by Fu and coworkers[14,15] provided diaryl compound **2-90** smoothly. Cyclization of **2-90** with LDA, followed by methylation with CH_3I/K_2CO_3, produced benzo[*a*]fluorene **2-92**.

Scheme 2-25



In this study, it proved possible to replicate the formation of **2-92** as achieved by M. Buck. With the benzofluorene **2-92** in hand, a path was investigated as presented in Scheme 2-26: It was felt that compound **2-92** might be hydrolyzed to the carboxamide **2-93**, which might be converted to the carbamate **2-94** as the precursor of the aniline **2-95**.

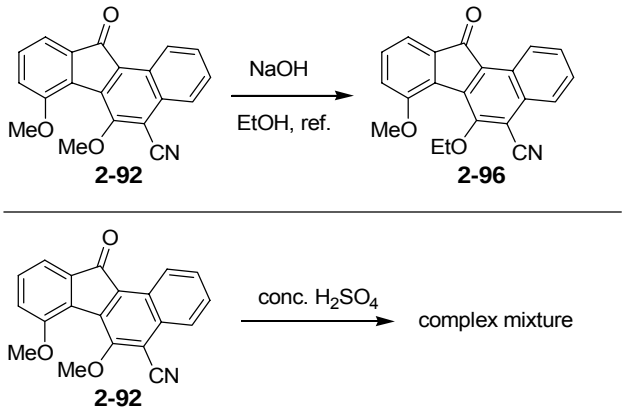
Scheme 2-26



In the previous study, Matthew Buck found that heating **2-92** in ethanol with NaOH generated ethyl phenolate **2-96** resulting from the addition-elimination of the *o*-methoxyl group instead of the hydrolysis of the cyano group.

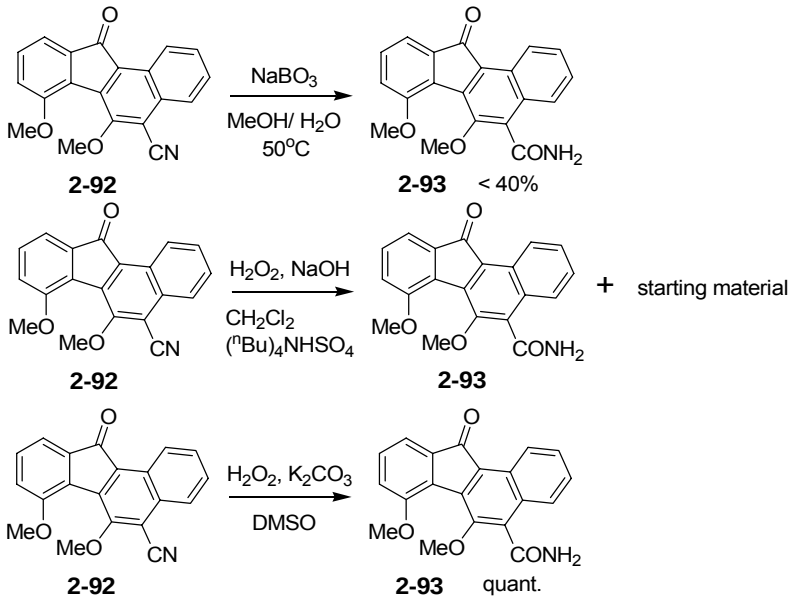
In the present work an attempt to hydrolyze **2-92** with concentrated H_2SO_4 at 0 °C only afforded a complex mixture. (Scheme 2-27)

Scheme 2-27



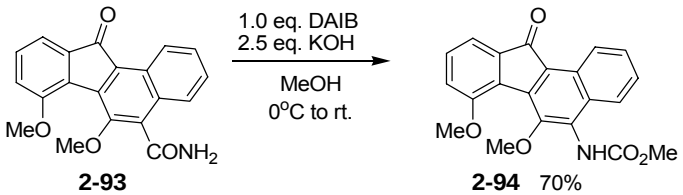
Hence, milder conditions were further explored. McKillop and Kemp reported a method for conversion aromatic nitriles to the corresponding amides using sodium perborate in aqueous methanol at 50 °C.[16] Use of these conditions generated desired the amide **2-93**, but in a low yield. Then treatment of **2-92** with hydrogen peroxide in the presence of aqueous NaOH in CH_2Cl_2 with phase transfer catalyst $(n\text{-Bu})_4NHSO_4$ at room temperature overnight provided amide **2-93** with some starting material remaining. It seemed the reaction was clean but slow because of the two phase system. Changing these conditions by using H_2O_2 with K_2CO_3 in DMSO (a polar solvent) led to the conversion of **2-92** to **2-93** smoothly and quantitatively. (Scheme 2-28)

Scheme 2-28



Hofmann rearrangements typically employ Br_2 with NaOH or heavy metal reagents such as $Pb(OAc)_4$ with NaOH or $Hg(OAc)_2$ or AgOAc with NBS. Moriarty and coworkers, however, have reported the use of diacetoxyiodobenzene (DAIB), a stable and commercial available iodine (III) reagent, in methanolic KOH at 2–10 °C to generate methyl carbamates in good yields from the corresponding primary carboxamide.[17] In our case, the carboxamide **2-93** was transformed into the desired carbamate **2-94** using 1.0 equiv DAIB, 2.5 equiv KOH in methanol from 0 °C to room temperature in 70% yield.

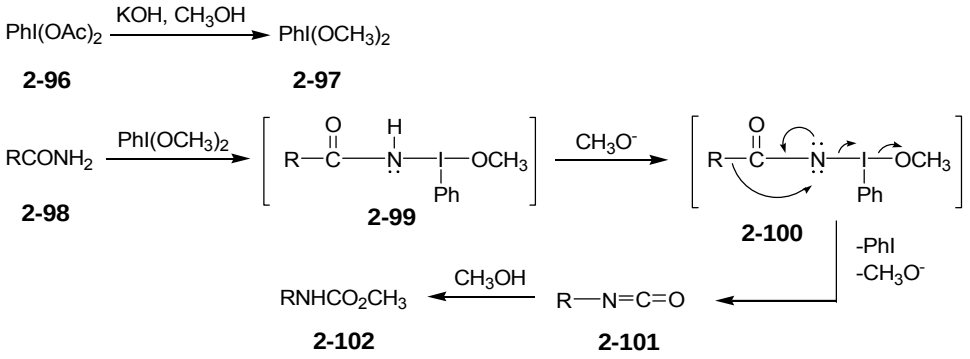
Scheme 2-29



The mechanism proposed by Moriarty is shown in Scheme 2-30.[17] Addition of DAIB **2-96**

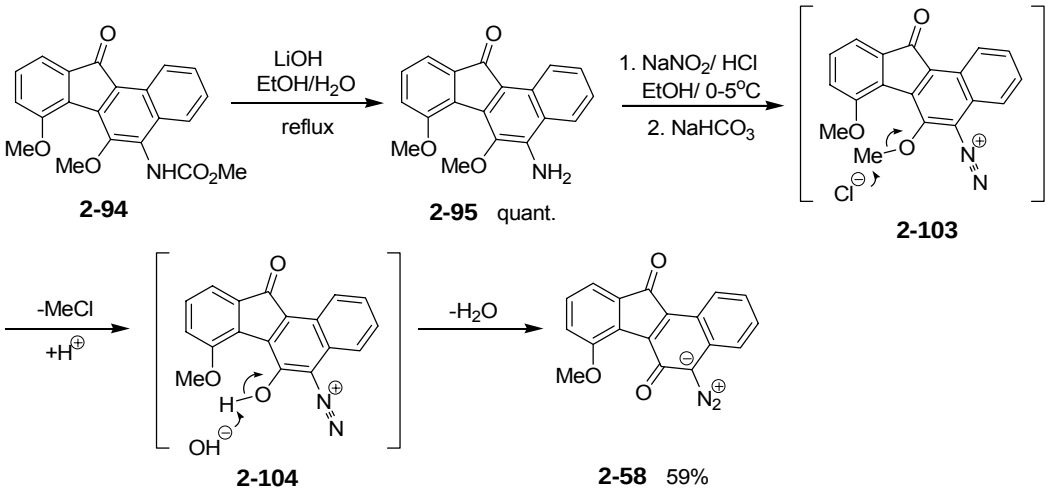
to methanolic KOH leads to the formation of $PhI(OMe)_2$ **2-97**. Reaction of the amide **2-98** with **2-97** probably provides *N*-(phenyliodonio) intermediate **2-99**, which is subjected to rearrangement to generate isocyanate **2-101**. Under acidic condition the isocyanate is readily hydrolyzed to amine but with basic condition in the presence of methanol it is converted to corresponding methyl carbamate **2-102**.

Scheme 2-30



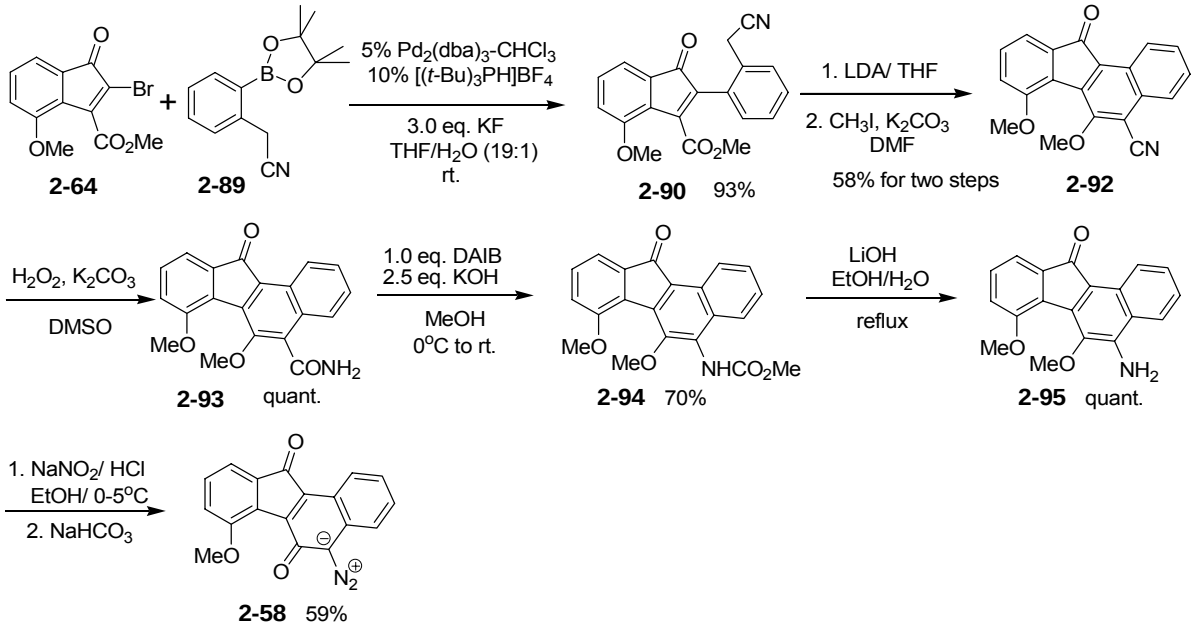
Hydrolysis of methyl carbamate **2-104** using LiOH in refluxing ethanol and water provided the aniline **2-95** quantitatively. The reaction of aniline **2-95** with nitrous acid gave the corresponding diazonium salt which underwent demethylation in situ to generate *o*-quinodiazde **2-58** in 59% yield, hence finishing the synthesis of the model of isoprekinamycin. The facile demethylation was believed to probably occur via a nucleophilic attack at the methyl ether group in the intermediate **2-103** resulting from the activating effect of the diazonium group. (Scheme 2-31)

Scheme 2-31



In summary the Suzuki coupling of bromoindenone **2-64** and commercial available pinacol borate **2-89** afforded diaryl intermediate **2-90**, which followed the sequence as presented in Scheme 2-32, seven steps, to achieve the synthesis of model compound **2-58** in 22% yield. If including the synthesis of bromoindenone **2-64**, the longest linear path of this synthesis is thirteen steps in 7% yield overall.

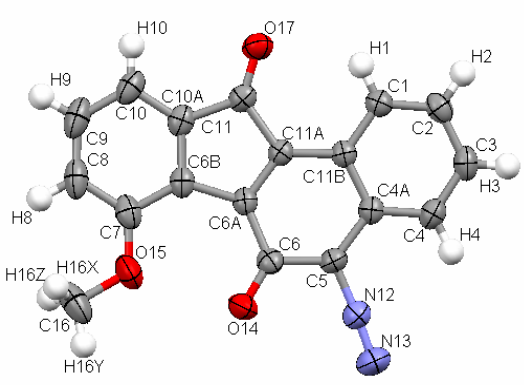
Scheme 2-32



2.3.3 Spectroscopic Comparison of the Two Models and Isoprekinamycin

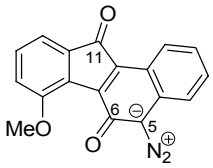
The model compound **2-58** crystallized as fine dark red needles from CH_2Cl_2 by slowly evaporating the solvent during two weeks. The crystals were good enough quality to allow for X-ray diffraction analysis. A comparison of this structure with that of IPK is presented in Chapter 3.

Figure 2-1 X-ray Crystal Structure of Model Compound 2-58

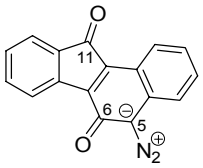


The compound was found to be stable in dilute acids and moderately inert to mildly alkaline conditions but basic conditions cause hydrodediazotization at higher temperature. High resolution mass spectrometry data for the compound **2-58** supports a molecular formula of $C_{18}H_{10}\ N_2O_3$ as the observed exact mass is 302.0691. The ^{1}H NMR spectrum of **2-58** exhibits four doublets (δ 8.86 (*J* = 8.1 Hz), 7.40 (*J* = 8.0 Hz), 7.25 (*J* = 6.7 Hz), 7.13 (*J* = 8.1 Hz)), one triplet (δ 7.51 (*J* = 7.4 Hz)), two protons that form a multiplet at δ 7.33, and a singlet at δ 3.96 representing the methoxy group. These signals can be observed as eight cross peaks in HMQC experiment at δ 8.86 (126.5), 7.51 (124.9), 7.40 (119.5), 7.33 (131.4), 7.33 (125.7), 7.25 (117.0), 7.13 (121.2) and 3.96 (56.5). Because of the low solubility of **2-58**, the ^{13}C NMR signal corresponding to the carbon of the diazo group was not observable. However, the HMBC experiment indicated a three bond correlation from H-4 at δ 7.40 ppm to a ^{13}C atom with a resonance at δ 90.0 ppm, assigned to the diazo group carbon atom. This result agrees with the value of δ 83.7 ppm for the isoprekinamycin diacetate.

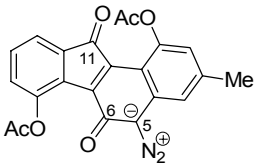
Other ^{13}C NMR characteristics of model **2-58** are also in excellent agreement with the corresponding structural features of model **2-105** and IPK diacetate **2-106** (table 2-1). In particular, the carbonyl resonance at 196.2 ppm in spectrum of **2-58**, assigned to the five member ring ketone, has counterparts at 196.77 ppm in model **2-105** and 192.48 ppm in IPK diacetate **2-106**. In addition, the diazo group gives rise to a band at 2095 cm^{-1} in the IR spectrum of **2-58** as compared with a band at 2105 cm^{-1} in the spectrum of model **2-105** and at 2119 cm^{-1} for the IPK diacetate **2-106**.



2-58



2-105



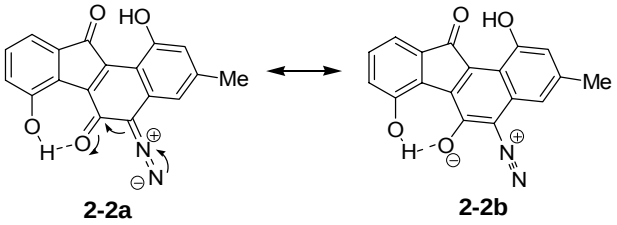
2-106

Table 2-1 Spectroscopic Data for 2-58, 2-105 and 2-106

		2-58	**2-105**	**2-106**
^{13}C NMR	C-11	196.2 ppm	196.77 ppm	192.48 ppm
	C-6	170.5 ppm	176.33 ppm	174.14 ppm
	C-5	90.0 ppm	82.64 ppm	83.17 ppm
IR	ν (C-N_2)	2095.7 cm^{-1}	2105 cm^{-1}	2119 cm^{-1}
	ν (CO)	1710.8 cm^{-1}	1727 cm^{-1}	1727 cm^{-1}
	ν (CC)	1620.1 cm^{-1}	1613 cm^{-1}	1613 cm^{-1}

Further examination of the available data revealed an interesting inconsistency. That is, whereas the IR bands for the diazo groups in model **2-58** and **2-105** (2095.7 cm^{-1} and 2105 cm^{-1}, respectively) are in good agreement with that of the diacetate of isoprekinamycin **2-106** (2119 cm^{-1}), all these values differ considerably from that observed for isoprekinamycin **2-2** (2162 $^{-1}$cm). Ab intio calculations carried out previously in this group by Laufer for model **2-105** and IPK **2-2** revealed that these discrepancies can be explained on the basis of intramolecular H-bonding to the *ortho*-quinodiazide oxygen in **2-2**,[8] which is absent in **2-105** and also absent in the model **2-58**.

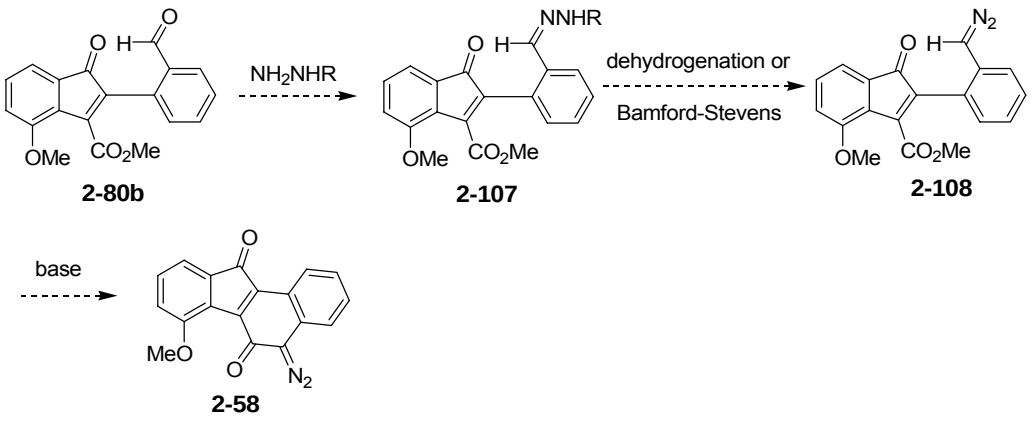
Figure 2-2 Effect of the H-bonding in IPK (2-2) on the Diazonium Character of the Diazo Group



2.4 Another Route to Diazobenzo[*a*]fluorenes

With diaryl aldehyde **2-80b** in hand, an alternative strategy as shown in Scheme 2-33 was explored briefly. The aldehyde **2-80b** might be converted to the corresponding hydrazone **2-107**, which might be transferred to diazo compound **2-108** via hydrazone dehydrogenation or Bamford-Stevens reaction. The cyclization of diazo compound **2-108** could generate model **2-58**.

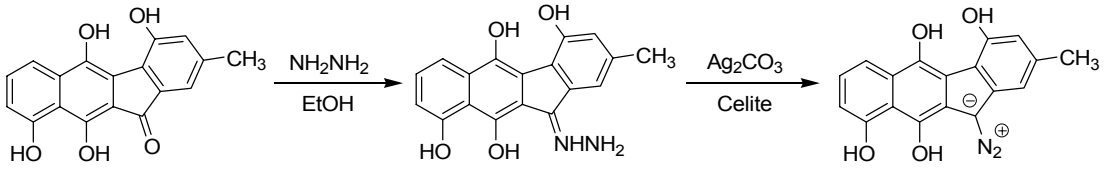
Scheme 2-33



Dehydrogenation of hydrazones is one of the oldest methods for the preparation of diazo compounds. For a long time, mercuric oxide was used almost exclusively as the oxidation agent. Since the 1960s, however, silver oxide, manganese dioxide, and lead(IV) acetate have been found to be efficient alternatives. This discovery has led to a renaissance of the method.

The limiting factor of this preparative method is, in some cases, the synthesis of the required hydrazone from a carbonyl compound and hydrazine. Hauser and coworkers reported the diazo transformation following the sequence as shown in Scheme 2-34: the ketone **2-109** was converted to a hydrazone **2-110**, which was dehydrogenated using Ag_2CO_3 on Celite to afford prekinamycin **2-1**.[18]

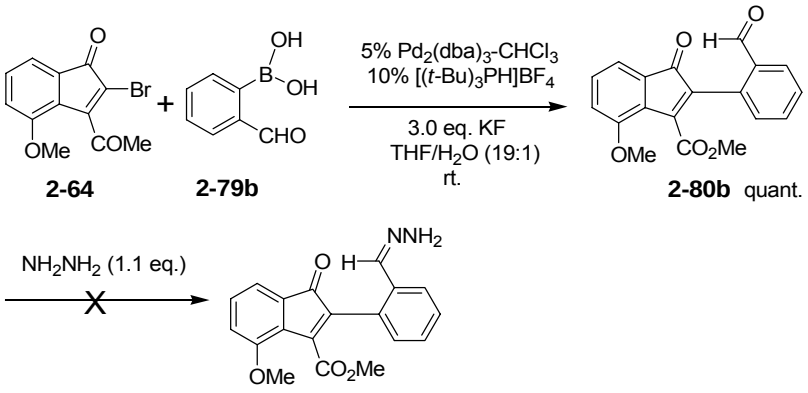
Scheme 2-34



2-109 **2-110** **2-1**

In our case, the Suzuki coupling between bromoindenone and boronic acid **2-79b** using Fu's conditions generated the diaryl aldehyde **2-80b** quantitatively. However, all the attempts to achieve **2-111** using 1.1 equiv of hydrazine in various conditions finally failed.

Scheme 2-35



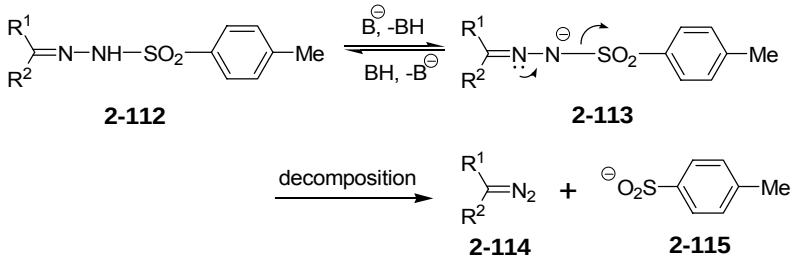
2-64 **2-79b** **2-80b** quant.

2-111

The Bamford-Stevens reaction is, next to hydrazone dehydrogenation, one of the most versatile methods to convert carbonyl compounds to diazo compounds. Starting compounds

are the tosyl hydrazine of aldehydes or ketones, which are then cleaved by base to give the diazoalkane and *p*-tolyl sulfinate. Kinetic investigations in the case of cyclohexanone hydrazone and camphor tosyl hydrazone support the E1 mechanism for the reaction with the fragmentation of the hydrazone anion being rate determining.

Scheme 2-36

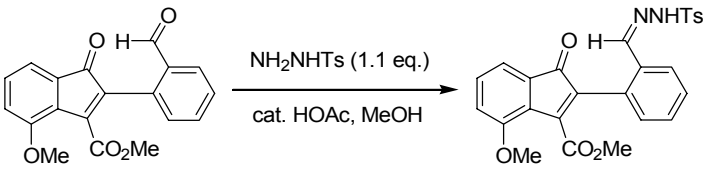


2-112 **2-113** **2-114** **2-115**

The synthesis of tosyl hydrazone **2-116** from **2-80b** using 1.1 equivalents of tosyl hydrazine was investigated. Almost no observable reaction was found in methylene chloride. In ethanol, the reaction was quite slow at room temperature, and provided a complex mixture at higher temperature (refluxing). In methanol, the reaction was very slow at room temperature, but was much faster after adding catalytic amount of AcOH to afford **2-116** in 50% yield. Electrospray mass spectrometric analysis indicated that the product possessed the mass expected for the hydrazone **2-116**. The ^{1}H NMR spectrum, however, was complicated by the fact that several key signals such as those from the methyl ether, the methyl ester and the tosyl group methyl were doubled up in approximately a 1:1 ratio. This complexity likely arises from the fact that there is hindered rotation about the C-C bond linking the indenone to the aromatic hydrazone-bearing ring, giving rise to a chiral axis, and the fact that the tosyl hydrazone nitrogen is pyramidal, giving rise to a chiral center. Thus the system consists of a

mixture of diastereomers. In principle, the hydrazone might also exist as an *E/Z* mixture of stereoisomers about the C=N bond, but it seems likely that in that case the *E* isomers would be much favoured over the *Z* isomer.

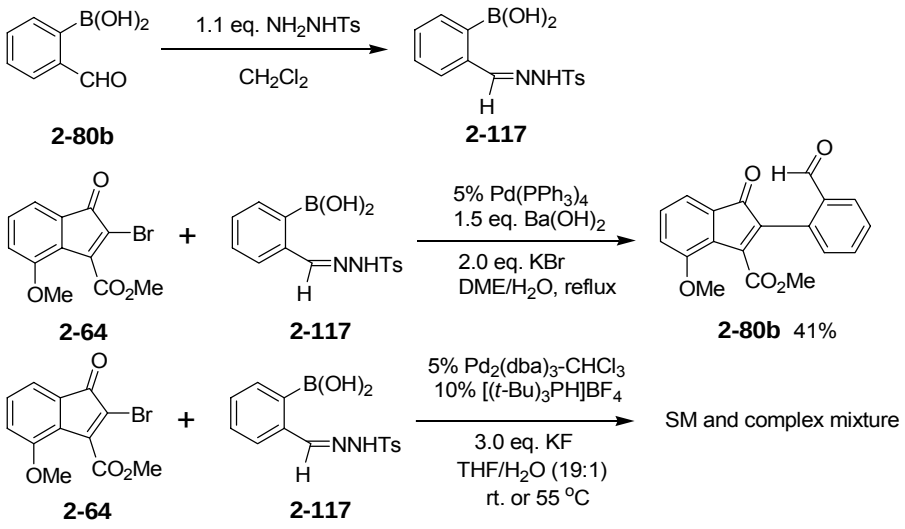
Scheme 2-37



2-80b **2-116** 50%

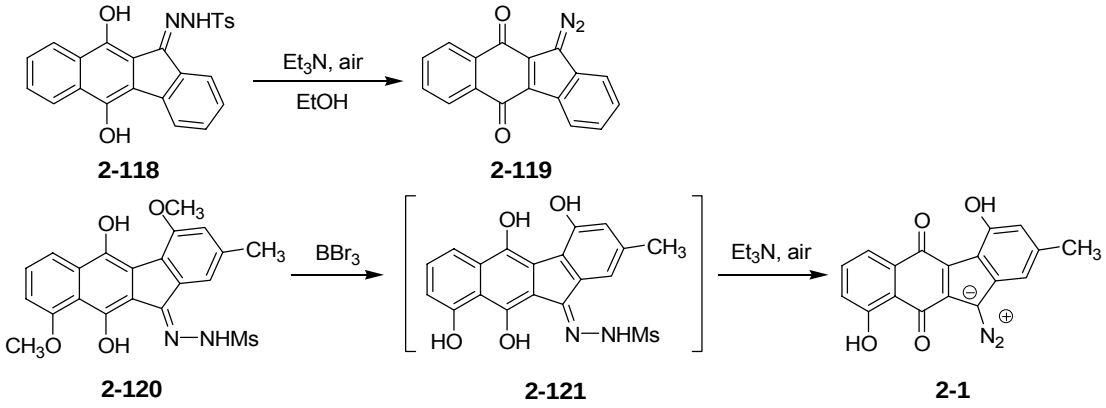
An alternative procedure to synthesize tosyl hydrazone **2-116** was explored. The boronic acid **2-79b** was converted to the tosyl hydrazone **2-117** with 1.1 equivalents of tosyl hydrazine in CH_2Cl_2 at room temperature quantitatively. The Suzuki coupling between **2-117** and bromoindenone **2-64** using $Pd(PPh_3)_4$, $Ba(OH)_2$, KBr, in refluxed DME and water afforded diaryl aldehyde **2-80b** in 41%. It was concerned that, with a strong base $Ba(OH)_2$ under the elevated temperature, the tosyl hydrazone **2-117** was hydrolyzed to the corresponding aldehyde then coupled with **2-64**. Further more, in the coupling using Fu's condition at room temperature, almost all starting material remained. With heating at 55 °C for 24 hours, a complex mixture with some starting material was observed.

Scheme 2-38



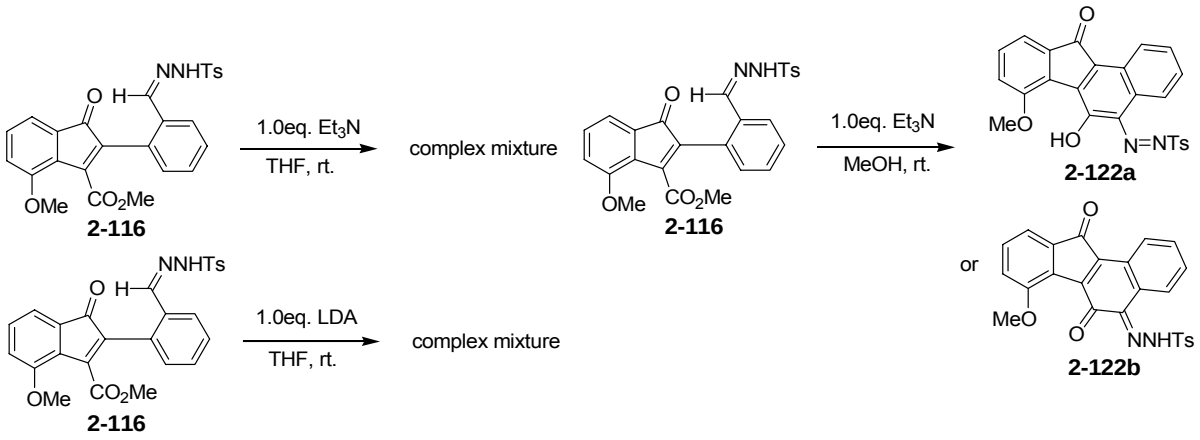
In their recent synthesis of prekinamycin, Birman and coworkers reported that a tosyl hydrazone **2-118** was converted to a diazo compound **2-119** using triethylamine in ethanol in the presence of air, in which the hydroquinone was oxidized in situ. Furhter more, a tosyl hydrazone **2-120** was demethylated to provide the intermediate **2-121**, which was converted to the prekinamycin **2-1** using the same conditions as above.[19]

Scheme 2-39



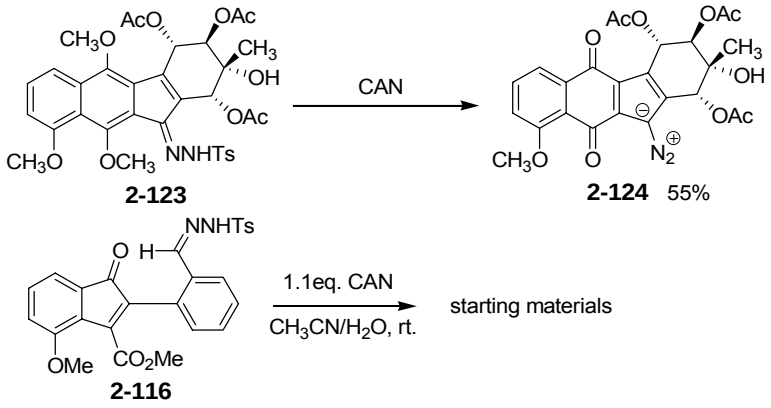
For our case, treatment of tosylhydrazone **2-116** with 1.0 equivalent of Et_3N in THF at room temperature provided a complex mixture. A similar result was obtained upon changing the base to LDA. However, treatment of **2-116** on a small scale with Et_3N in methanol at room temperature provided a crude product with ^{1}NMR characterization compatible with a tosyldiazene **2-122a** or a tosylhydrazone **2-122b** rather than the desired diazo compound. This preliminary observation was not pursued further.

Scheme 2-40



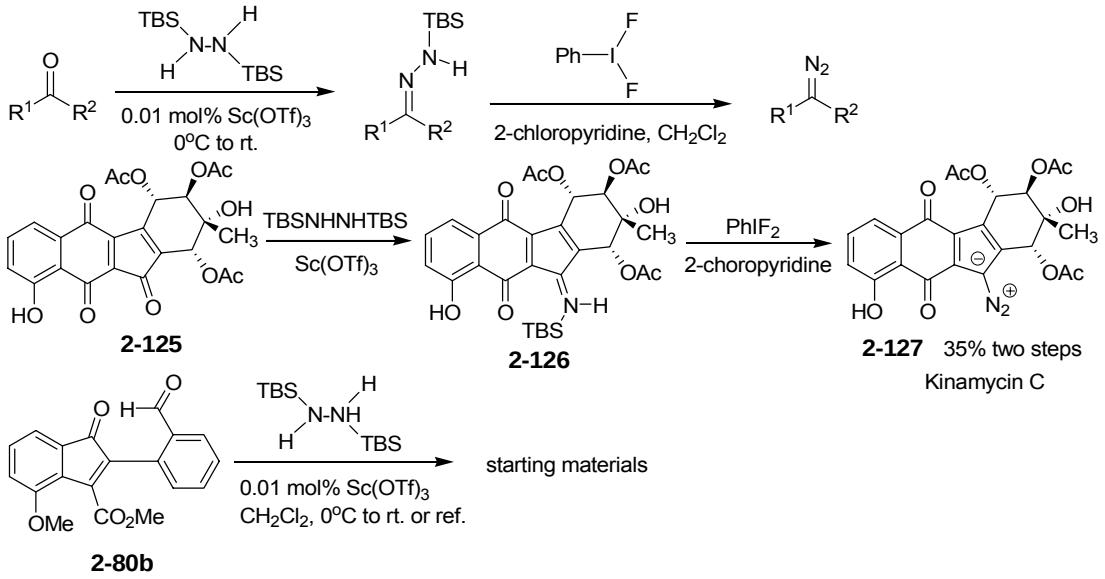
Kumamoto-Ishikawa and coworkers found that treatment of tosylhydrazone **2-123** with cerium ammonium nitrate (CAN) afforded methyl kinamycin C **2-124** , in which not only the expected oxidation to a naphthoquinone but also spontaneous desulfination leading to diazoalkane occurred.[20] However, no reaction was observed when tosylhydrazone **2-116** was treated with 1.1 equivalents of CAN in CH_3CN and water at room temperature.

Scheme 2-41



Myers and co-workers developed a new method for the synthesis of diazoalkanes by oxidation of *N*-*tert*-butyldimethylsilylhydrazones (TBSHs) with difluoroiodobenzene.[21,22] The TBSHs was converted by the condensation of the corresponding ketone or aldehyde with 1,2-bis(*tert*-butyldimethylsilyl)hydrazine (BTBSH) in the presence of a catalytic amount of scandium triflate. This method was used for the synthesis of kinamycin C **2-127** by Porco and coworkers.[23] Unfortunately, treatment of diarylaldehyde **2-80b** with BTBSH and $Sc(OTf)_3$ in CH_2Cl_2 at room temperature or higher temperature gave no reaction.

Scheme 2-42



The alternative route to IPK model via the route in Scheme 2-33 was abandoned at this point.

2.5 Experimental

2.5.1 General Information

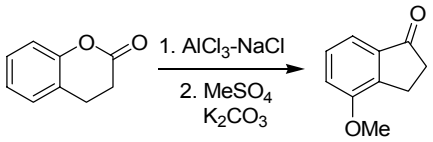
^{1}H NMR spectra were recorded on a Brüker AVANCE500 (500 MHz), Brüker AC300 (300 MHz) or Brüker AVANCE300 (300 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The following abbreviations are used for NMR peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplet; m, multiplet; b, broad; w, weak. ^{13}C NMR spectra were broad band decoupled and recorded on a Brüker AVANCE500 (125.8 MHz), Brüker AC300 (75.5 MHz) and Brüker AVANCE300 (75.5 MHz) NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. HMQC and HMBC experiments

were performed on a Brüker AVANCE500 spectrometer. IR spectra were determined on a Perkin-Elmer RX I FT-IR spectrometer. High/low resolution electron impact (EI or ESI) mass spectra (MS) were measured by the WATSPEC Mass Spectrometry Facility (Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada) and the McMaster Regional Center for Mass Spectrometry (Department of Chemistry, McMaster University, Hamilton, Ontario, Canada). Gas chromatography-mass spectrometry (GC-MS) was performed on a HP GCD 1800 with a column (HP5) length of 30.0 m and diameter of 0.25 mm. The following temperature program was applied: initial temperature 70 °C, rising rate 10 °C/minute, final temperature 265 °C held for 20.0 minutes. Elemental analyses were performed by the M-H-W Laboratories (Phoenix, Arizona, USA).

Anhydrous THF and Et_2O were freshly distilled from sodium/benzophenone under nitrogen prior to use. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen prior to use. All commercial reagents were purchased from Aldrich Chemical Co., Strem Chemicals Inc., Alfa Aesar, Lancaster Synthesis Ltd. or BDH Inc. and were used as received unless otherwise indicated.

The –78 °C and 0 °C designations refer to solid carbon dioxide/acetone and ice/water slush, respectively. The room temperature refers to 22 °C to 25 °C. Flash column chromatography was carried out using the Merck silica gel (230–400 mesh) and SiliCycle silica gel (60 Å). Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with Merck pre-coated silica gel plates (silica gel 60 F_{254} on aluminum sheet). All reported yields are isolated yields.

2.5.2 Detailed Experimental Procedures



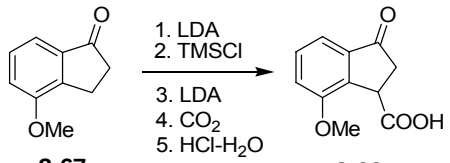
2-68 **2-67**

4-Methoxy-2,3-dihydroindan-1-one (2-67)

$AlCl_3$ (58.19 g, 0.436 mol) and NaCl (12.03 g, 0.256 mol) were mixed and heated in an oil bath. When the bath temperature was about 150 ºC, dihydrocoumarin (10 mL, 0.079 mol) was added slowly while the bath temperature was maintained between 150 ºC and 180 ºC. The bath temperature was then raised to 200 °C and the mixture was stirred for 1 h. The mixture was cooled to room temperature and quenched with 100 g crushed ice and 50 mL conc. HCl at 0 °C. The suspension was stirred at room temperature for 30 min and the crude product (9.688 g) was obtained as a gray solid upon filtration.

To a solution of the crude indanone (9.688 g, 0.065 mol) and K_2CO_3 (56.94 g, 0.411 mol) in acetone (667 mL) was added Me_2SO_4 (45.36 g, 0.360 mol) dropwise at room temperature and the reaction mixture was then refluxed for 2.5 h. The solution was cooled to room temperature, filtered and concentrated. The residue was mixed with water (100 mL) and triethylamine (100 mL), followed by stirring at room temperature for 1 h. The resulting solution was extracted with EtOAc (3 × 200 mL) and the organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:9, v/v) to obtain the title compound as a white solid (8.317 g, 65%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.34 (m, 2H), 6.96–7.01 (m, 1H), 2.97–3.01 (m, 2H), 2.62–

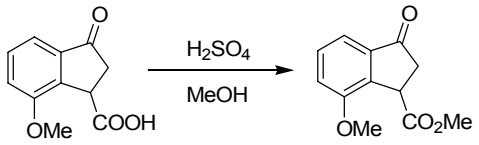
2.66 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 207.2, 156.9, 143.9, 138.5, 128.7, 115.1, 114.6, 55.3, 36.0. 22.4; MS (GC-MS): 13.34 minutes, *m/z* 162 (M^+).



2-67 **2-66**

7-Methoxy-3-oxo-2, 3-dihydro-1H-indene-1-carboxylic acid (2-66)

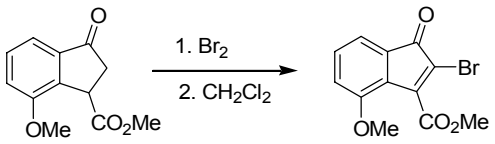
To a solution of 4-methoxy-1-indanone (2.000 g, 12.33 mmol) in anhydrous THF (25 mL) at –78 ºC was added freshly prepared LDA in THF (12.95 mmol in 65 mL) dropwise, and the reaction mixture was stirred for 1 h. TMSCl (1.64 mL, 12.95 mmol) was then added slowly at –78 °C and the reaction mixture was further stirred for 1 hour. The second batch of freshly prepared LDA in THF (18.50 mmol in 65 mL) was added at –78 °C and the reaction mixture was stirred for one more hour. Solid carbon dioxide (100 g) was added and the reaction mixture was stirred at room temperature for 1.5 hours. The reaction was quenched with 2 M HCl in an ice bath and then extracted with ether (3 × 150 mL). The ether phase was concentrated and the residue was redissolved in enough 2 M aqueous NaOH solution until the pH was ca. 12. The basic aqueous solution was washed with ether (3 × 20 mL) and then acidified with conc. HCl until the pH reached ca. 1. The resulting acidic aqueous solution was then extracted with a mixture of ether and THF (9:1, v/v, 4 × 100 mL). The resulting organic phase was dried over Na_2SO_4 and concentrated to give the crude product as a yellow solid (2.270 g).



2-66 **2-65**

Methyl 7-methoxy-3-oxo-2,3-dihydro-1*H*-indene-1-carboxylate (2-65)

To a solution of the crude carboxylic acid (2.270 g) in methanol (50 mL) was added conc. H_2SO_4 (10 drops) and the reaction mixture was refluxed for 48 h. The solution was concentrated to ca. 5 mL, followed by addition of saturated aqueous $NaHCO_3$ solution to adjust the pH to ca. 8. The solution was extracted with EtOAc (3 × 80 mL) and the organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:4, v/v) to obtain the ester as a yellow solid (1.845 g, 68% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (t, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 4.19 (dd, *J* = 8.2, 3.5 Hz, 1 H), 3.85 (s, 3 H), 3.69 (s, 3 H), 2.95 (dd, *J* = 11.7, 8.2 Hz, 1 H), 2.73 (dd, *J* = 11.7, 3.5 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 204.0, 173.4, 157.0, 140.5, 138.4, 130.6, 115.8, 115.5, 55.8, 52.4, 41.1, 41.0; Elemental analysis: calculated for $C_{12}H_{12}O_4$: C, 65.45, H, 5.49; found: C, 65.22, H, 5.49.



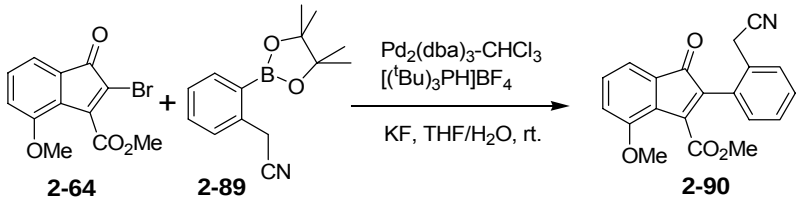
2-65 **2-64**

Methyl 2-bromo-7-methoxy-3-oxo-3*H*-indene-1-carboxylate (2-64)

To a solution of compound **2-65** (2.330 g, 10.59 mmol) in freshly distilled CH_2Cl_2 (46 mL) was added Br_2 in CH_2Cl_2 (1 M, 26.5 mL) at room temperature and the solution was stirred

for 24 h. The solution was diluted with CH_2Cl_2 (300 mL) and washed with water (3 × 25 mL). The CH_2Cl_2 phase was dried over Na_2SO_4 and concentrated. The crude product was obtained as a red solid.

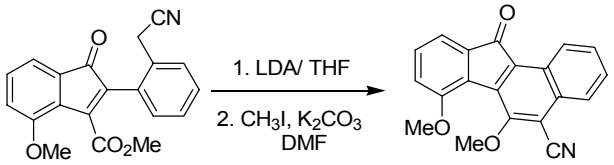
To a solution of the above crude product in CH_2Cl_2 (100 mL) at 0 °C was added DBU (1.59 mL, 10.59 mmol) slowly and the reaction mixture was stirred at this temperature for 1 h. The reaction solution was washed with water (3 × 15 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:5, v/v) to obtain the title compound as a red solid (2.365 g, 75% for two steps). Mp: 139–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (t, *J* = 4.5 Hz, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 3.98 (s, 3H), 3.85(s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 188.9, 164.9, 152.1, 148.4, 131.7, 129.7, 128.0, 119.6, 117.8, 116.8, 56.4, 52.9; IR (KBr): 1737, 1723 cm^{-1}; MS (GC-MS): 18.52 minutes, *m/z* 296 (M^+ containing ^{79}Br) 298 (M^+ containing ^{81}Br) (1:1); Elemental analysis: calculated for $C_{12}H_9BrO_4$: C, 48.51, H, 3.05; found: C, 48.70, H 3.02; HRMS: calculated for $C_{12}H_9{}^{79}BrO_4$: 295.9684, found: 295.9689.



Methyl 2-(2-(cyanomethyl)phenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (2-90)

A mixture of compound **2-64** (273 mg, 0.916 mmol), KF (160 mg, 2.49 mmol), $Pd_2(dba)_3$•$CHCl_3$ (51 mg, 0.049 mmol) and [(*t*-Bu)$_3$PH]BF_4 (28 mg, 0.099 mmol) was

deoxygenated five times with an argon balloon and a vacuum pump. Pinacolboronate **2-89** (200 mg, 0.824 mmol) was dissolved in a mixture of THF and water (19:1, v/v, 8 mL) and the solution was deoxygenated three times by a freeze-thaw process. The deoxygenated solution was then added to the solid mixture and stirred for 24 h at room temperature under an argon atmosphere. The solution was diluted with ether (100 mL) and washed with water (3 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (Et_2O:Hexanes = 1:1, v/v) to obtain the product as a red-orange solid (255 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, *J* = 7.7 Hz, 1H), 7.4 (dt, *J* = 1.1 Hz, *J* = 7.5 Hz, 1H), 7.28 (m, 2H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 6.80 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 3.85 (s, 3H), 3.76 (s, 3H), 3.75 (s, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ 194.76, 165.76, 152.95, 148.06, 132.24, 132.10, 130.46, 129.63, 129.49, 128.96, 128.56, 127.93, 127.61, 119.12, 117.58, 117.11, 56.15, 52.40, 22.49; IR (KBr): 2246, 1735, 1711 cm^{-1}; Elemental analysis: calculated for $C_{20}H_{15}NO_4$: C, 72.06, H, 4.54; found: C, 71.88, H, 4.72; HRMS: calculated for $C_{20}H_{15}NO_4$: 333.1001, found: 333.0985.



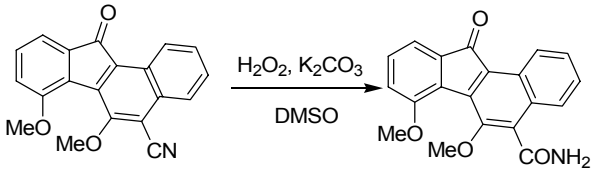
2-90 **2-92**

6,7-Dimethoxy-11-oxo-11H-benzo[*a*]fluorene-5-carbonitrile (2-92)

To a solution of compound **2-90** (255 mg, 0.766 mmol) in anhydrous THF (15 mL) at room temperature was added LDA in THF (0.781 mmol in 6 mL) slowly and the solution was stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution (15 mL)

leading to the formation of some red-orange precipitate. The precipitate was dissolved in EtOAc (600 mL) and then washed with H_2O (3 × 30 mL). The organic phase was dried over Na_2SO_4 and concentrated to obtain the crude product as a red orange solid.

To a solution of the above crude product in DMF (100 mL) was added K_2CO_3 (0.529 g, 3.83 mmol) at room temperature and the mixture was stirred for 15 minutes, followed by addition of CH_3I (0.48 mL, 7.66 mmol). The solution was heated at 80 °C for 45 min then filtered and concentrated. The solution residue was diluted with $CHCl_3$ (300 mL) and washed with H_2O (3 × 30 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography ($CHCl_3$:Hexanes = 3:1, v/v) to give the product as an orange solid (140 mg, 58% for two steps). ^{1}H NMR (300MHz, $CDCl_3$): δ 9.05 (m, 1H), 8.03 (m, 1H), 7.58 (m, 2H), 7.32 (m, 2H), 7.15 (d, J = 8.4 Hz, 1H), 4.04 (s, 3H), 4.00 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 194.1, 157.8, 154.8, 139.2, 135.6, 135.0, 132.0, 129.2, 129.1, 127.5, 126.8, 124.7, 124.6, 120.2, 117.0, 115.1, 110.8, 63.9, 56.3; HRMS: calculated for $C_{20}H_{13}NO_3$: 315.0895, found: 315.0896.

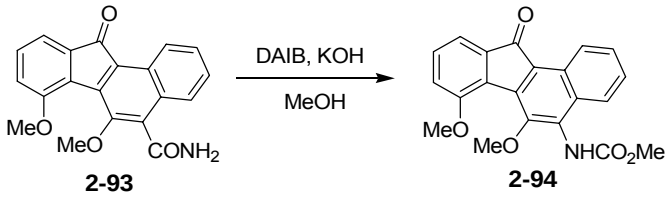


2-92 **2-93**

6,7-Dimethoxy-11-oxo-11H-benzo[*a*]fluorene-5-carboxamide (2-93)

To a solution of compound **2-92** (140 mg, 0.443 mmol) and K_2CO_3 (95 mg, 0.687 mmol) in DMSO (55 mL) at 0 °C was added 30% H_2O_2 (10 mL) slowly, then the mixture was stirred at

room temperature for 21 h. The reaction was quenched with water (60 mL), followed by extraction with EtOAc (300 mL). The organic phase was washed with water (3 × 30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 3:1, v/v) to give the product as an orange solid (151 mg, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.88 (d, J = 8.3 Hz, 1H), 8.12 (s, br, 1H), 7.88(s, br, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.52 (m, 2H), 7.34 (m, 2H), 7.20 (d, J = 6.6 Hz, 1H), 3.93 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 194.4, 168.2, 154.8, 149.2, 140.7, 138.7, 135.9, 132.4, 131.7, 129.1, 127.7, 127.6, 127.4, 127.3, 125.5, 123.7, 121.2, 116.7, 63.9, 56.5; IR (film): 3389.8, 1692.6, 1654.5, 1272.1, 1046.8 cm^{-1}; MS (EI): *m/z* 333.1 (100, M^+), 316.1 (44).

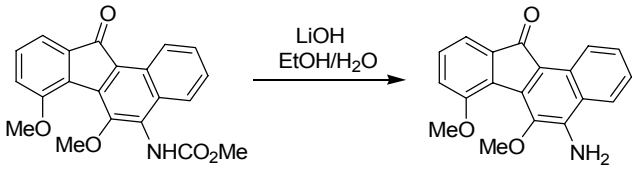


2-93 **2-94**

Methyl 6,7-dimethoxy-11-oxo-11H-benzo[*a*]fluoren-5-ylcarbamate (2-94)

To a solution of compound **2-93** (151 mg, 0.453 mmol) in methanol (160 mL) was added KOH (64 mg, 1.13 mmol) at rt. The solution was cooled to 0 °C and stirred for 10 minutes. $PhI(OAc)_2$ (146 mg, 0.453 mmol) was added and the mixture was stirred at 0 °C for additional 15 min. The solution was then warmed to rt and further stirred for 7 h. The solution was concentrated and the residue was mixed with CH_2Cl_2 (200 mL) and water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash

chromatography (EtOAc:Hexanes = 3:1, v/v) to give the title compound as an orange solid (115 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.05 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.29 (m, 2H), 7.07 (m, 1H), 6.81 (s, br, 1H), 3.98 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 194.2, 155.6, 154,3, 147.5, 139.8, 136.7, 133.6, 131.3, 130.8, 128.6, 128.3, 128.0, 126.6, 124.4, 123.9, 119.5, 116.8, 62.6, 56.3, 53.1; IR (film): 3275.8, 1730.0, 1690.0, 1283.6, 1261.9, 1238.0 cm^{-1}; MS (EI): m/z 363.1 (80, M^+), 331.1 (100), 316.1 (60), 288.1 (19).

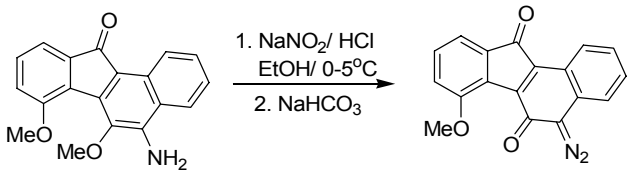


2-94 **2-95**

5-Amino-6, 7-dimethoxy-11H-benzo[*a*]fluoren-11-one (2-95)

To a solution of compound **2-94** (115 mg, 0.317 mmol) in ethanol (125 mL) was added aqueous LiOH solution (2 M, 0.79 mL) slowly at room temperature and the reaction mixture was refluxed for 21 h. The solution was concentrated and the residue was dissolved in EtOAc (300 mL) and washed with water (3 × 30 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 2:3, v/v) to give the product as a red solid (99 mg, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.6 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.27 (m, 2H), 7.18 (s, 1H), 7.15 (m, 2H), 7.06 (d, *J* = 6.9 Hz, 1H), 3.91 (s, 3H), 3.66 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 190.8, 154.7, 148.0, 140.5, 139.1, 136.7, 131.9, 130.2, 129.4, 126.6, 124.2, 123.9, 123.6, 121.3, 119.3, 115.2, 112.9, 61.7, 56.5; IR (film): 3315.8, 3216.1, 1654.0,

1613.2, 1546.9, 1519.9, 1285.9, 1212.8, 1097.7, 1055.4 cm^{-1}; MS (EI): *m/z* 305.1 (100, M^+), 290.1 (81).

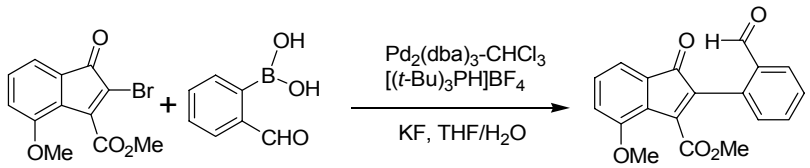


2-95 **2-58**

5-Diazo-5*H*-7-methoxy-11*H*-benzo[*a*]fluoren-6, 11-dione (2-58)

To a solution of compound **2-95** (99 mg, 0.33 mmol) in ethanol (150 mL) was added conc. HCl (2.5 mL) at room temperature. The solution was cooled to 0 ºC, and an aqueous $NaNO_2$ solution (28 mg, 0.41 mmol in 5 mL) was added slowly. The solution was stirred at 0 °C for 4 h, followed by addition of $NaHCO_3$ powder (2.5 g), and the mixture was stirred for additional 20 min. The remained $NaHCO_3$ was filtered and the filtrate was concentrated. The residue was dissolved in EtOAc (300 mL) and washed with water (3 × 30 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:1, v/v) to obtain the title compound as a purple solid (58 mg, 59%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ 8.86 (d, *J* = 8.1 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.33 (m, 2H), 7.25 (d, *J* = 6.7 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (125.8 MHz, CD_2Cl_2)*: δ 196.2, 170.5, 154.8, 133.7, 133.6, 131.4, 129.4, 128.6, 127.5, 126.5, 125.7, 122.2, 121.2, 119.5, 117.0, 56.5; IR (CH_2Cl_2 solution): 3061.0, 2945.0, 2838.1, 2095.7, 1710.8, 1620.1, 1480.1 1336.0 cm^{-1}; MS (EI): *m/z* 302.1 (30, M^+), 274.1 (96), 259.1 (100), 231.1 (25), 187.1 (24), 175.1 (23); HRMS (EI): calculated for $C_{18}H_{10}N_2O_3$: 302.0691, found: 302.0691.

*As a result of low solubility of the title compound in CD_2Cl_2, no ^{13}C NMR signal was observed for the quaternary carbon attached to the diazo group. A cross peak at 90.0 ppm, assignable to the carbon atom attached to the diazo group, is a clear evidence, however, in the HMBC experiment.

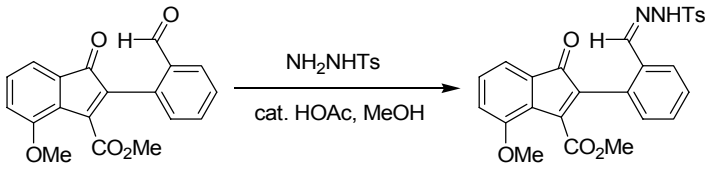


2-64 **2-79b** **2-80b**

Methyl 2-(2-formylphenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (2-80b)

A mixture of compound **2-64** (230.7 mg, 0.774 mmol), KF (135 mg, 2.32 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (40.1 mg, 0.039 mmol) and $[(t\text{-}Bu)_3PH]BF_4$ (22.5 mg, 0.077 mmol) was deoxygenated five times with an argon balloon and a vacuum pump. 2-formylphenylboronic acid **2-79b** (200 mg, 0.697 mmol) was dissolved in a mixture of THF and water (19:1, v/v, 6 mL) and the solution was deoxygenated three times by the thaw-freeze process. The deoxygenated solution was then added to the solid mixture and stirred for 24 h at rt under argon atmosphere. The solution was diluted with ether (150 mL) and washed with water (3 × 15 mL). The water phase was then extracted by Et_2O (2 ×50 mL), and the ether phase was combined. The ether phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the product as a red-orange solid (238.2 mg, 100%). ^1H NMR (500 MHz, $CDCl_3$): δ 9.97 (s, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.32 (t, J = 8.37 Hz, 1H), 7.24 (d, J = 7.1 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 3.89 (s, 3H), 3.78 (s, 3H);

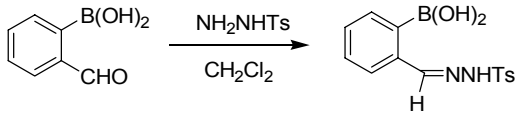
^{13}C NMR (125.8 MHz, $CDCl_3$): δ 194.42, 190.83, 165.99, 153.04, 146.11, 135.37, 133.61, 132.52, 131.92, 131.35, 130.87, 130.82, 130.35, 129.31, 128.13, 119.11, 117.39, 56.29, 52.42; HRMS (EI): calculated for $C_{19}H_{14}O_5$: 322.0841, found: 322.0831.



2-80b **2-116**

***p*-Tolunesulfonylhydrazone of methyl 2-(2-formylphenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (2-116)**

To a solution of compound **2-80b** (68.4 mg, 0.212 mmol) in anhydrous methanol (20 mL) was added $TsNHNH_2$ (43.5 mg, 0.234 mmol) at room temperature, and then two drops AcOH. The reaction was stirred at room temperature for 24 hours. The solution was concentrated, and the residue was purified by flash chromatography (EtOAc:Hexanes = 1:2, v/v) to obtain a red orange solid (51.8 mg, 50%). MS (ESI): Calcd for $C_{26}H_{23}N_2O_6^+$ 491.13; Found 491.12 (100, $(M+H)^+$). ^1H NMR (300 MHz, $CDCl_3$): The proton NMR was complex and exhibited an approximate doubling up of key signals suggesting the presence of isomers or conformers: δ 6.8 – 7.8 (m, 11H), 3.95 (s, approx. 1.5H), 3.85 (s, approx. 1.5H), 3.69 (s, approx. 1.5H), 3.58 (s, approx. 1.5H), 2.37 (s, approx. 1.5H), 2.32 (s, approx. 1.5H).



2-80b **2-117**

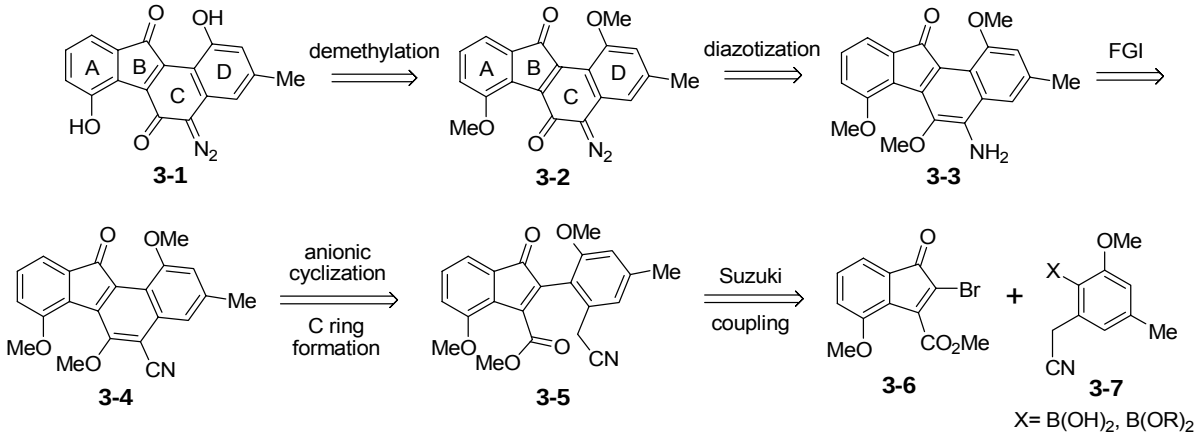
***p*-Toluenesulfonylhydrazone of 2-formylphenylboronic acid (2-117)**

To a solution of 2-formylphenylboronic acid (100 mg, 0.667 mmol) in CH_2Cl_2 (20 mL) was added $TsNHNH_2$ (136.6 mg, 0.734 mmol) at room temperature. The reaction was stirred at room temperature for 3 hours. The solution was concentrated, and the residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as a white solid (193.2 mg, 100%).^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (d, *J* = 7.2 Hz, 1H), 7.99 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.65 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.42 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 2.39 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 145.1, 143.6, 134.7, 134.0, 132.7, 132.0, 130.7, 129.6, 128.2, 127.5, 21.6.

Chapter 3 Total Synthesis of Isoprekinamycin

With the successful synthesis of an isoprekinamycin model, the same strategy was explored for the total synthesis of isoprekinamycin **3-1** (Scheme 3-1). Thus, it was hoped that isoprekinamycin could be obtained from the *O,O*-dimethyl isoprekinamycin **3-2** by demethylation, and the diazo group could be installed via demethylative diazotization of the aniline **3-3**. The formation of the aniline could be achieved by the functional group interconversion from the corresponding carbonitrile **3-4**, and this benzo[*a*]fluorene **3-4** might be formed from an anionic cyclization of **3-5**. The diaryl compound **3-5** could be constructed by a Suzuki coupling involving bromoindenone **3-6** and the substituted phenyl acetonitrile **3-7**, which would function as AB ring and D ring building blocks, respectively.

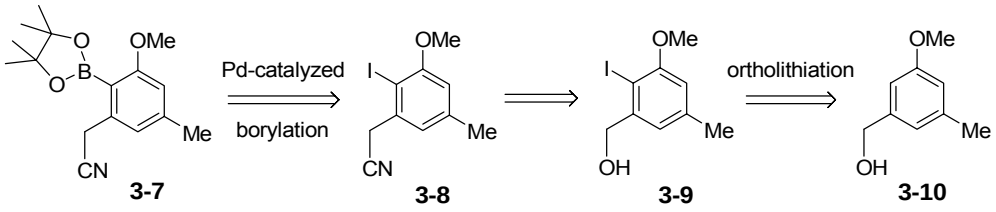
Scheme 3-1



3.1 Synthesis of a Precursor to the D Ring of Isoprekinamycin

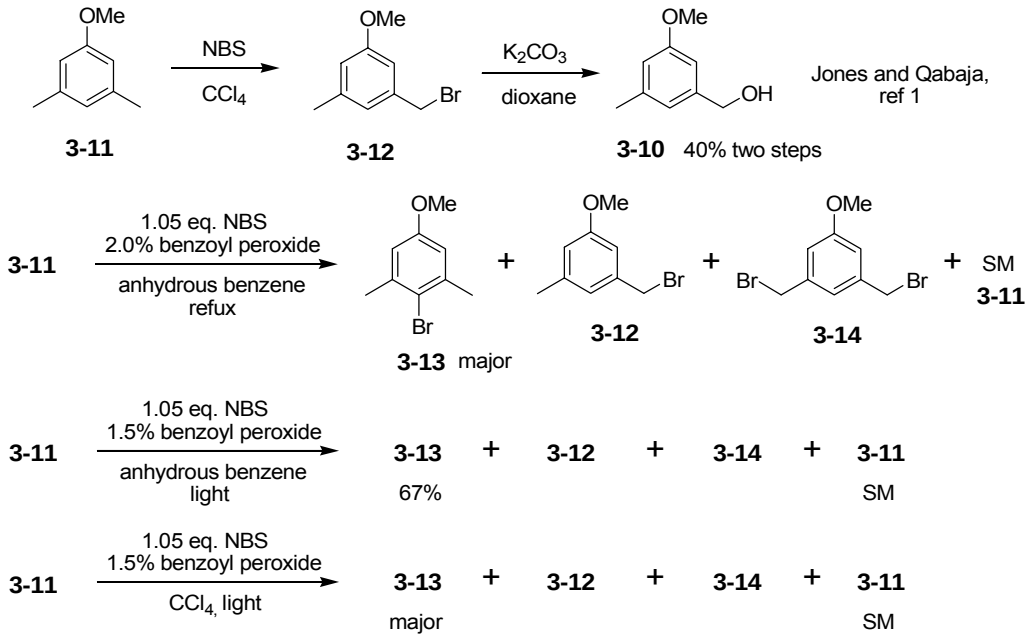
It was intended that target boronate **3-7** could be produced from iodophenyl acetonitrile **3-8** and it was thought that **3-8** could be generated from the known *o*-iodobenzylic alcohol **3-9**, which could be derived from the benzyl alcohol **3-10**. A retrosynthetic analysis for preparation of the target boronate **3-7** is shown in Scheme 3-2.

Scheme 3-2



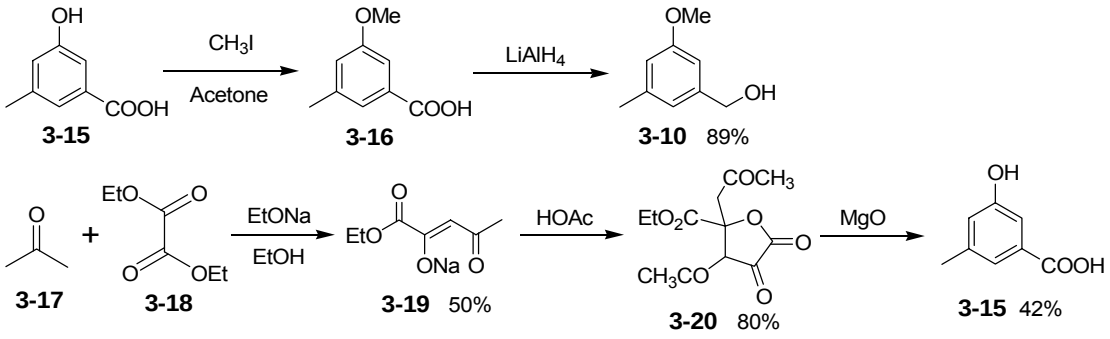
Jones and coworkers reported that the bromination of dimethylanisole using NBS in CCl_4 provided the benzyl bromide **3-12**, which was hydrolyzed to generate alcohol **3-10** (Scheme 3-3).[1] However, all attempts in this laboratory at duplication and modification of the procedure to obtain benzyl bromide **3-12** had failed. Treatment of dimethylanisole **3-11** with 1.05 equivalent of NBS, 2.0% of benzoyl peroxide in refluxing anhydrous benzene provided 2-bromo-5-methoxy-1,3-dimethylbenzene (**3-13**) as a major product, as well as minor amounts of mono and dibromo compounds **3-12** and **3-14** and remaining starting material based on by NMR analysis; Treatment of the same system with light generated *p*-bromo compound **3-13** in 67% yield and a small amount of **3-12**, **3-14** and starting material. Even upon changing the solvent to CCl_4, the reaction still afforded **3-13** as a major product. It was concluded that since the dimethylanisole is quite electron-rich, the reaction went mostly through electrophilic aromatic substitution rather than substitution at the benzylic position through a radical chain reaction.

Scheme 3-3



Jung and coworkers reported that methylation of available 3-hydroxy-5-methylbenzoic acid (**3-15**) with MeI in acetone provided the methyl protected acid **3-16**, which was reduced by lithium aluminum hydride to provide benzyl alcohol **3-10** (Scheme 3-4).[2] In that study, the synthesis of **3-15** was accomplished according to Gearien's procedure.[3] Claisen condensation of acetone and diethyl oxalate **3-18** generated ethyl sodium acetopyruvate (**3-19**) in 50% yield. Treatment of **3-19** with AcOH provided **3-20** in 80% yield, which was then converted to 3-hydroxy-5-methylbenzoic acid (**3-15**) in 42% yield. The whole procedure involved three steps with 17% yield overall, but this was considered to be inadequate for preparing the starting materials in this project.

Scheme 3-4

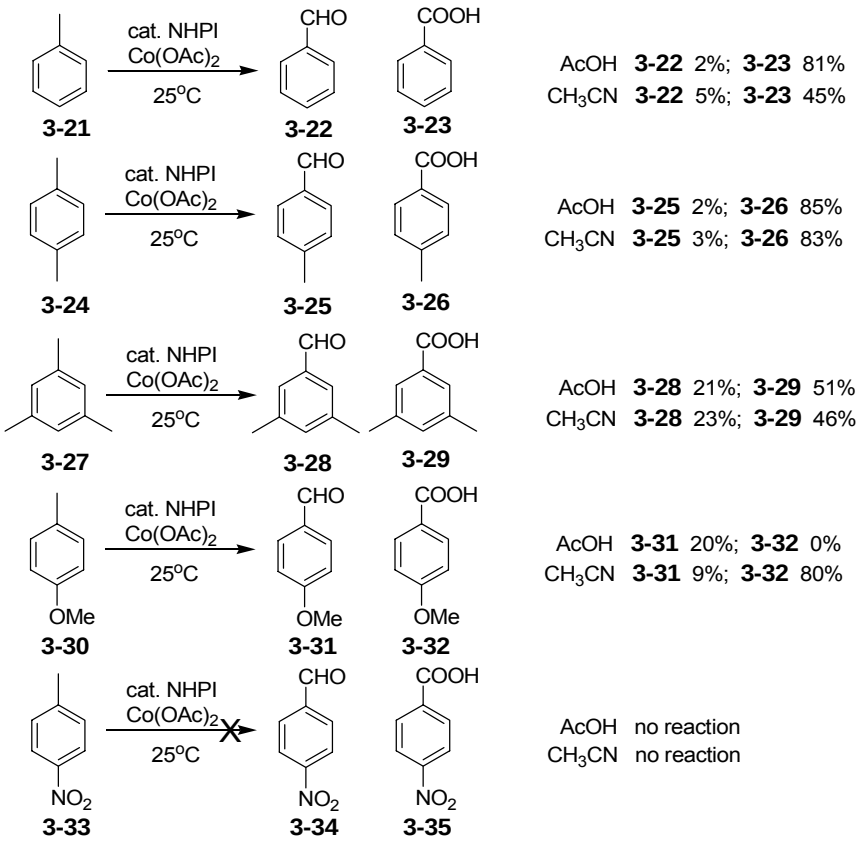


The selective catalytic oxidation of alkylbenzenes with O_2 is a very important reaction for the production of bulk chemicals such as benzoic acids and terephthalic acids. For example, the oxidation of toluene is currently carried out in the presence of a catalytic amount of cobalt(II) 2-ethylhexanoate under a pressure of 10 atm of air at 140–190 °C.[4] However, examples of the direct aerobic oxidation of alkylbenzenes under normal pressure and temperature are relatively rare.

Sakaguchi and coworkers developed a new strategy for alkylbenzene oxidations with O_2 using *N*-hydroxyphthalimide (NHPI) as a catalyst.[5] They found that the oxidation of toluene **3-21** in the presence of 10 mol% of NHPI and 0.5 mol% of $Co(OAc)_2$ in acetic acid under an atmosphere of O_2 at 25 °C for 20 hours generated benzaldehyde (**3-22**) and benzoic acid (**3-23**) in 2% and 81% yield, respectively. The oxidation of **3-21** in acetonitrile resulted in lower yield. Various alkylbenzenes were investigated and some representative cases were shown in Scheme 3-5. Both *p*-xylene (**3-24**) and mesitylene (**3-27**) were oxidized to the corresponding aldehyde and benzoic acid smoothly in either acetic acid or acetonitrile. Conversion of 4-methylanisole (**3-30**) into the benzoic acid proceeded in 80% yield in acetonitrile but did not

proceed as well in acetic acid. *p*-Nitrotoluene (**3-33**), which contains a strong electron-withdrawing group, was not oxidized under these conditions.

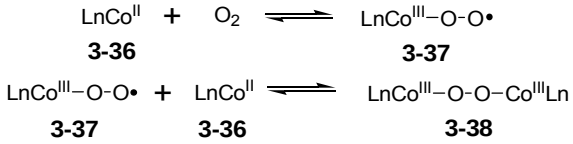
Scheme 3-5 (ref. 5)



In order to obtain further information on the role of Co(II) species in the reaction, the Sakaguchi group carried out ESR measurements under the same conditions as those for the oxidation of **3-21**.[5] When an acetonitrile solution of NHPI and **3-21** was exposed to an oxygen atmosphere, no ESR signal was observed over 10 hours. However, when a very small amount of $Co(OAc)_2$ was added to the solution, an ESR signal of the phthalimido-*N*-oxy (PINO) radical was observed. These observations suggested that the complexation of Co(II) with O_2 to generate a labile dioxygen complex such as superoxocobalt(III) **3-37** or μ-

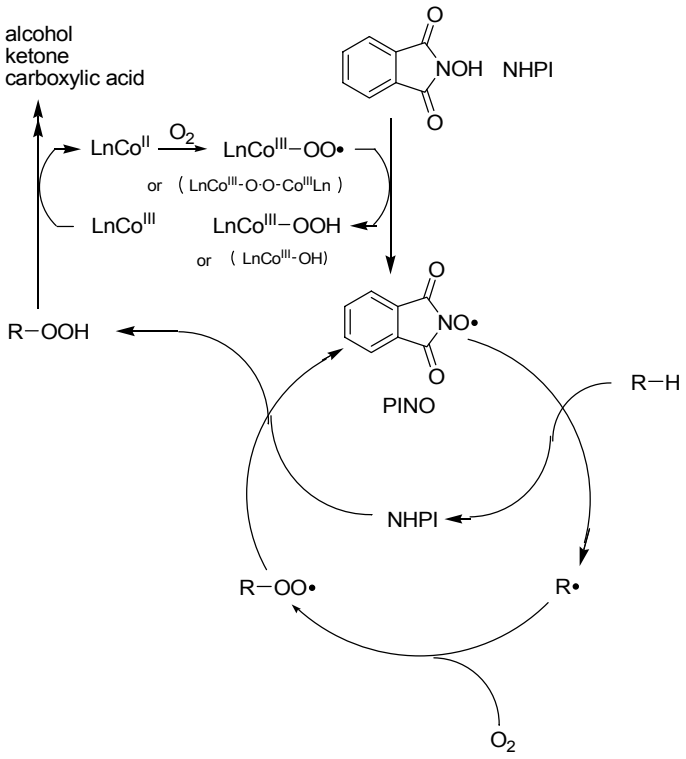
peroxocobalt(III) complex **3-38**, which had been reported to be easily formed by the one electron reduction of O_2 by Co(II) species,[6–9] is present during the oxidation step (Scheme 3-6).

Scheme 3-6



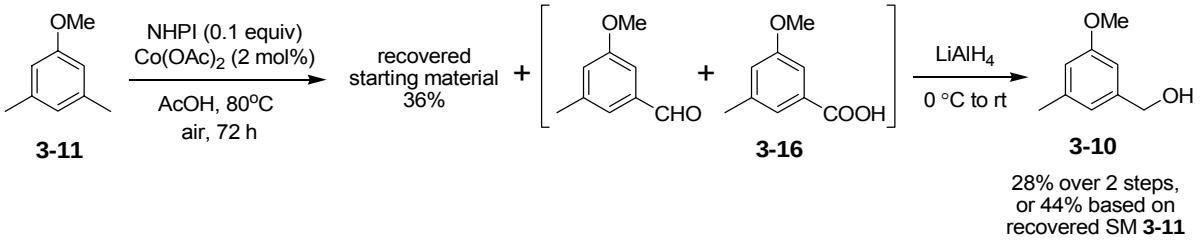
A plausible reaction pathway for the aerobic oxidation of alkanes is as presented in Figure 3-1. The generation of PINO by the reaction of the NHPI with the cobalt(III)-oxygen complex would be the most important step in the present oxidation. The next step in the oxidation involves the hydrogen abstraction from RH by PINO to form R·, which is readily trapped by dioxygen to provide ROO· followed by ROOH and eventually the formation of alcohols, ketones and/or carboxylic acids.

Figure 3-1 Catalytic Cycle of the Oxidation using NHPI and $Co(OAc)_2$



In this laboratory, the oxidation of 1,3-dimethylanisole (**3-11**) with NHPI and $Co(OAc)_2$ was investigated. Treatment of **3-11** with 0.1 equivalent of NHPI and 0.5 mol % of $Co(OAc)_2$ in acetonitrile under one atm of O_2 at room temperature for 24 hours provided mostly starting material with small amounts of the aldehyde and benzoic acid **3-16**. It was found that the conversion ratio was very low after one day under these conditions, and increased catalyst loading and elevation of the reaction temperature did not significantly improve the rate of reaction. Improvement in the conversion ratio was observed, however, when the reaction was conducted in acetic acid at 80 °C.

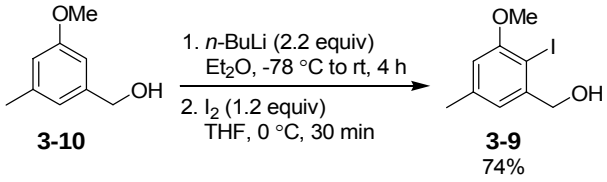
Scheme 3-7



The reaction, which was optimized by increasing the amount of $Co(OAc)_2$ to 2.0 mol% and reaction time to 72 hours, generated the mixture of aldehyde and benzoic acid **3-16**, which could be cleanly reduced to the corresponding benzyl alcohol **3-10** with an excess of $LiAlH_4$. Since this two-step procedure proved to be relatively clean and the unreacted starting material **3-11** could be easily recovered after the first step (36%), it was felt that this process was suitable for the large-scale preparation of starting materials for the proposed synthetic project (Scheme 3-7).

Treatment of **3-10** with 2.2 equivalents of *n*-BuLi from –78 °C to room temperature for 4 hours, followed by treatment with 1.2 equivalents of iodine in THF at 0 °C for 30 minutes, generated the desired iodobenzyl alcohol **3-9** in 74% yield and high regioselectively. As intended, the regioselectivity likely arises from the inductive effect of the *o*-methoxy group and the coordination of the Li^+ of the base with benzylic alkoxide oxygen, and the structure of **3-9** was later confirmed indirectly through further transformations (vide infra). The addition of TMEDA was not helpful as it gave rise to a complex mixture and did not improve the yield of the reaction.

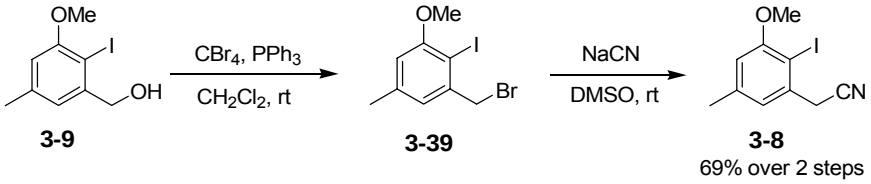
Scheme 3-8



3-10 **3-9** 74%

Treatment of the iodobenzyl alcohol **3-9** with 1.05 equivalents of CBr_4 and 1.05 equivalents of PPh_3 in CH_2Cl_2 at room temperature smoothly provided the benzylic bromide **3-39**, which was subjected to an S_N2 reaction with 1.5 equivalents of NaCN in DMSO to afford the iodophenylacetonitrile **3-8** in 69% overall yield.

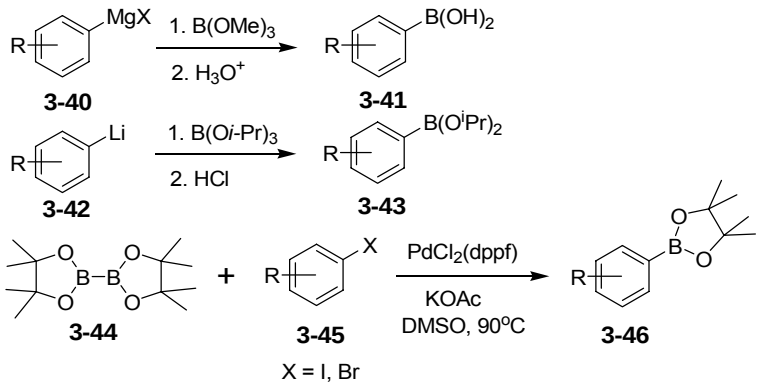
Scheme 3-9



3-9 **3-39** **3-8** 69% over 2 steps

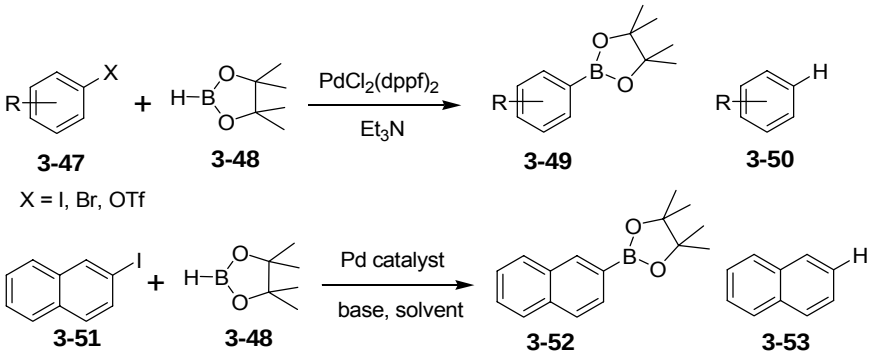
While classical methods for the synthesis of aryl boronic acids or their esters involve the use of Grignard reagents **3-40** or lithium reagents **3-42**, these conditions could not be used for the borylation of **3-8**, due to the acidic benzylic protons activated by the cyano group. Miyaura and co-workers reported a palladium-catalyzed cross-coupling reaction of the tetraalkoxydiboron **3-44**, which provides a one-step procedure for generating organoboronates from aryl halides or triflates.[10–13] (Scheme 3-10)

Scheme 3-10



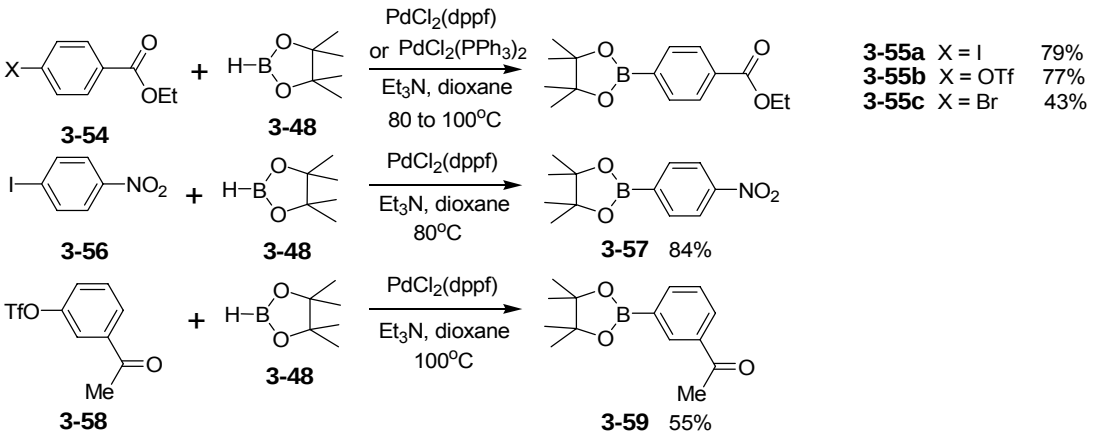
Masuda and coworkers reported that a palladium-catalyzed coupling reaction of the dialkoxyhydroborane **3-48** with aryl halides or aryl triflates **3-47** afforded the corresponding arylboronates (e.g. **3-49 or 3-52**) in high yield as presented in Scheme 3-11.[14] To explore optimal borylation conditions, the reaction of 1-iodonaphthalene **3-51** with pinacolborane (**3-48**) was carried out under various conditions. The reaction was efficiently catalyzed by the palladium(II) complexes with 2 equivalents of phosphine ligands, such as $PdCl_2$(dppf) and $PdCl_2(PPh_3)_2$. However, an additional phosphine ligand tended to retard the reaction. In general, tertiary amines are known to not contribute to coupling reactions with boron compounds, and triethylamine was found to be most effective for the selective formation of **3-52**. In the presence of other bases such as pyridine, DBU and KOAc, the formation of the undesirable reduction product **3-53** predominated. The reaction was not sensitive to different solvents (dioxane, toluene, MeCN, and $ClCH_2CH_2Cl$), but the use of the polar solvent DMF did result in both lower yields and poorer selectivity due to a decomposition of the dialkoxyborane to diborane (B_2H_6).[15]

Scheme 3-11 (ref. 14)



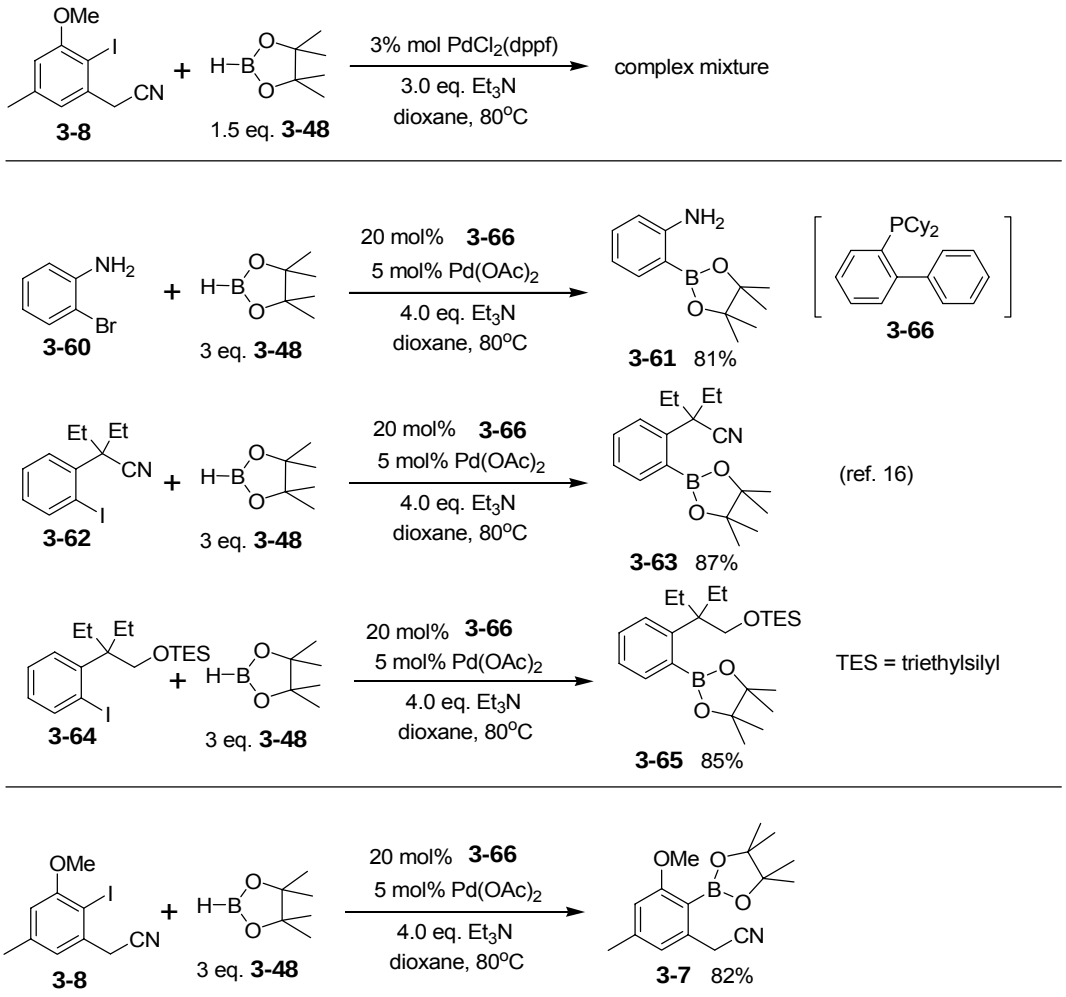
The order of relative reactivity for this reaction was aryl iodide > aryl triflate > aryl bromide. The differences in the yields and in the selectivity among aryl iodides having electron-donating or electron-withdrawing groups were not particularly large such as in the case of **3-56**. The product yields of the borylation of aryl triflates also were not substantially affected by substituents, but the reaction of those having electron-withdrawing groups was sluggish at 80 °C. A more elevated temperature (100 °C) was needed in the case of **3-58**. In addition, the borylation of aryl bromides with electron-donating groups proceeded smoothly at 100 °C; however, the presence of electron-withdrawing substituents decreased the yield such as **3-55c**.

Scheme 3-12 (ref. 14)



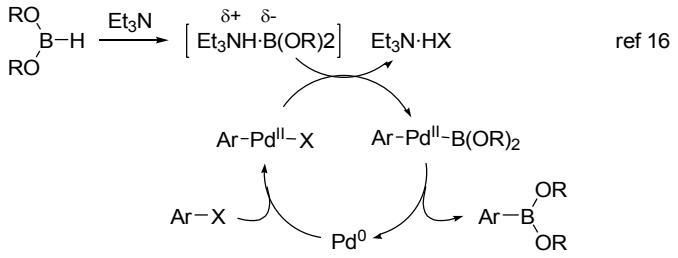
In this laboratory, the borylation of iodophenylacetonitrile **3-8** with 1.5 equivalents of pinacol borane (**3-48**), 3 mol% of $PdCl_2$(dppf), and 3.0 equivalents of Et_3N in dioxane at 80 °C, unfortunately, provided a complex mixture. A possible problem was that, although the **3-8** is an electron-rich species, it did include a sterically hindered di-*ortho*-substituted structure, which might hinder the reaction. Guéritte and coworkers extended this reaction to *ortho*-substituted aryl bromides or iodides.[16] For example, treatment of 2-bromoaniline (**3-60**) with 3 equivalents of pinacolborane **3-48**, 4.0 equivalents of Et_3N using $Pd(OAc)_2$ as a catalyst in the presence of the 20 mol % of biphenylphosphine ligand **3-66** in dioxane at 80 °C generated piancol boronate **3-61** in much higher yield (81%) than that of the original condition. For the aryl iodide, even for the more sterically hindered species such as **3-62** and **3-64**, the borylation proceeded smoothly under same conditions. For our case, the borylation of **3-8** afforded the desired pinacol boronate **3-7** in 82%. (Scheme 3-13)

Scheme 3-13



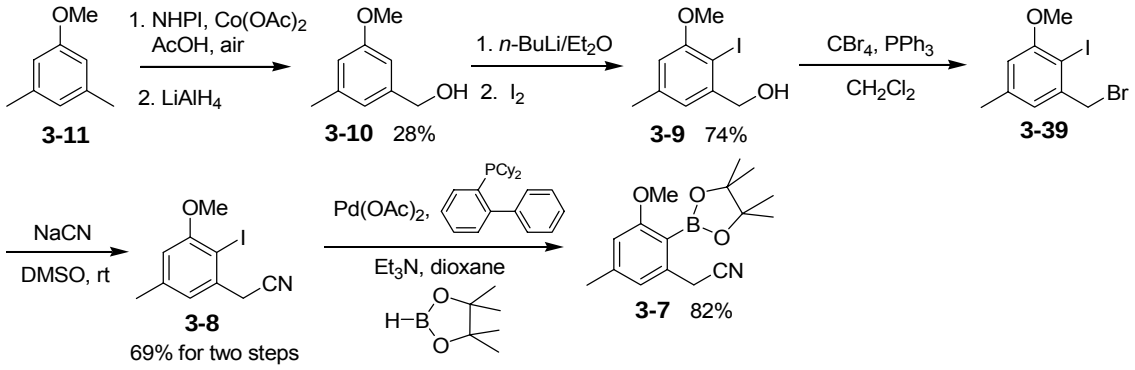
Guéritte et al. proposed a possible mechanism and catalytic cycle for the borylation that involves a boryl anion, generated by the combination of Et_3N and the dialkoxyborane **3-48** (Figure 3-2).[16] Initially, the aryl electrophile would add oxidatively to the palladium catalyst to give arylpalladium(II) species $Ar–Pd^{II}–X$. A subsequent ligand exchange between X of $Ar–Pd^{II}–X$ and the boryl anion would produce the $Ar–Pd^{II}–B(OR)_2$ intermediate and $Et_3NH{\cdot}X$, followed by reductive elimination that would give the arylboronate $ArB(OR)_2$ and regenerate the palladium catalyst.

Figure 3-2 Catalytic Cycle for Palladium-Catalyzed Borylation with Et_3N



Overall, the synthesis of pinacol boronate **3-7**, the D-ring building block for the synthesis of isoprekinamycin, was achieved in seven steps from commercial available 1,3-dimethylanisole **3-11** following the sequence as shown in Scheme 3-14, was achieved in six steps in 12% yield.

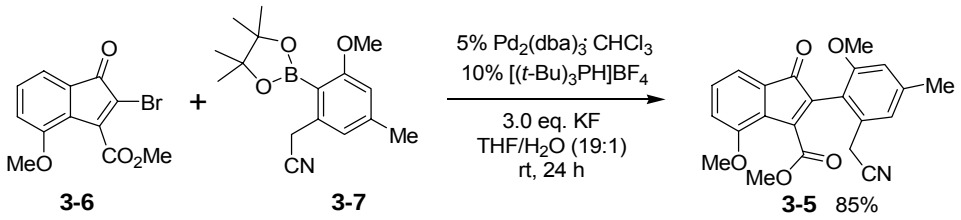
Scheme 3-14



3.2 The Total Synthesis of Isoprekinamycin

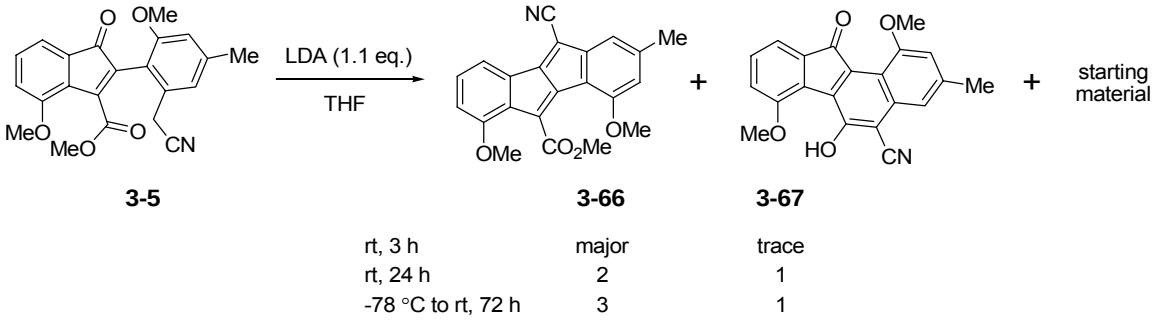
With the D ring synthon pinacol boronate **3-7** in hand, Suzuki coupling between **3-7** and bromoindenone **3-6** was explored as shown in Scheme 3-15. The desired diaryl compound **3-5** was obtained in 85% by using 5 mol% of $Pd_2(dba)_3{\cdot}CH_3Cl$, 10 mol% of $[(t\text{-}Bu)_3PH]BF_4$ and 3 equivalents of potassium fluoride in THF and water at room temperature for one day.

Scheme 3-15



Cyclization of diaryl compound **3-5** using 1.1 equivalents of LDA in THF unfortunately provided aldol-dehydration product **3-66** as a major product as presented in Scheme 3-16.

Scheme 3-16

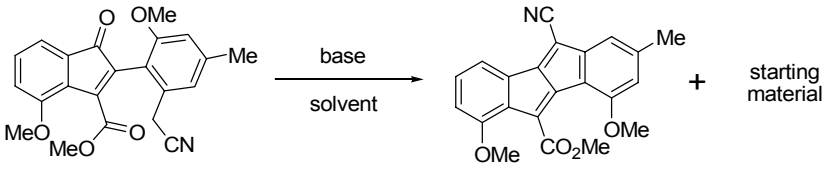


Treatment of **3-5** with 1.1 equivalents of LDA in THF at room temperature for 3 hours, the conditions under which the cyclization of the model compound was completed, generated only trace amounts of the desired phenol **3-67**, but more of the aldol product **3-66** and a substantial amount of un-reacted starting material. Increasing the reaction time to 24 hours led to a higher conversion of starting material, but **3-66** remained the major product. Cyclization of diaryl compound **3-5** was clearly much slower than the simpler model compound and the aldol reaction was the favoured reaction, in comparison to the desired Dieckmann-like cyclization. Since the aldol reaction is reversible, it was hoped that the conjugate base of phenol **3-67** (phenolic anion) is the thermodynamically more stable product

so that the yield of phenol **3-67** might be improved by increasing the reaction time. However, even increasing the reaction time to three days, the ratio of the desired Dieckmann product **3-67** to aldol product **3-66** was not improved (**3-66**:**3-67** ≈ 3:1), and small amounts of starting material remained.

Cyclization of the diaryl compound **3-5** using bases other than LDA was investigated as shown in Scheme 3-17. Treatment of **3-5** with two equivalents of DBU in refluxing THF afforded aldol product **3-66** in 60% yield with some remaining starting material. A reaction carried out in DMF at room temperature overnight generated the aldol product **3-66** cleanly in 66% yield with some starting material left. However, at 80 °C the reaction provided a complex mixture possibly resulting from the decomposition of **3-66** at elevated temperature in a polar solvent such as DMF. A similar result was obtained when using 1.1 equivalents of sodium hydride. Meanwhile, treatment of **3-5** with 1.1 equivalents of sodium methoxide in methanol at room temperature also provided the aldol product **3-66** as a major product with some starting material.

Scheme 3-17



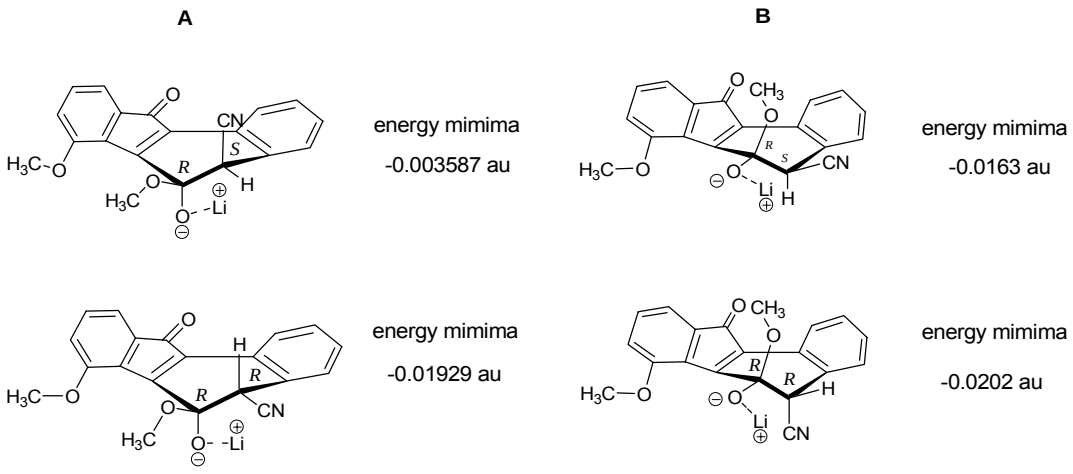
3-5 **3-66**

DBU (2.0 eq.), THF, reflux	60%
DBU (2.0 eq.), DMF, rt	66%
DBU (2.0 eq.), DMF, 80 °C	complex mixture
NaH (1.1 eq.), DMF, 80 °C	complex mixture
NaOMe (1.1 eq.), MeOH, rt	major

Hence, a computational analysis of the aldol versus Dieckmann cyclizations was carried out.

It was decided that the analysis would be restricted to a ground state calculation of the initial tetrahedral addition product between the cyano-stabilized anion and the ester group. In considering a computational analysis of the Dieckmann cyclization a number of issues had to be considered. To begin with, two conformations of the C-ring had to be considered as well as the relative configurations of the two chiral centers created in the initial reaction. The two conformations are labeled **A** and **B** in Figure 3-3. Calculations were done for the two ring conformations of the two relative configurations (*RR* and *RS)* as shown in Figure 3-3.

Figure 3-3 Energy Optimization of Model-Dieckmann Intermediate



Additionally, the ring-A methoxy group has some conformational options. Two starting geometries with the methyl group of the methyl ether oriented at 90° relative to the aromatic ring (up and down) as well as one starting geometry with the O-Me bond in the aromatic plane and away from the *ortho*-substituent were considered. The other planar geometry was clearly too sterically strained and was not minimized. Given the substantial number of

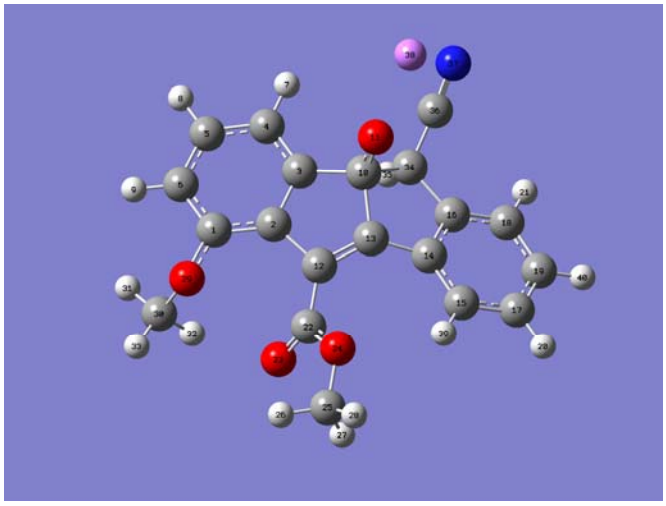
starting geometries that had to be probed, the computational study was restricted to the AM1 semi-empirical method. In each case, a full geometry optimization was performed and frequencies were calculated. All structures obtained had no imaginary frequencies suggesting that they were true energy minima. The lowest energy minima were found for the *RR* isomer in the **B**-type conformation. With total electronic energy of –0.0202 a.u. = –12.7 kcal/mol.

In the case of the aldol condensation, the most stable initial adduct has the alkoxide and the cyano group *syn* such that the lithium ion is shared between the alkoxide oxygen and the cyano nitrogen and has a total electronic energy of –0.01470649 a.u. = –9.22 kcal/mol.

If one assumes that the reaction proceeds in each case via the most stable initial adduct then the above analysis predicts that the Dieckmann cyclization should be favoured by approximately 3.5 kcal/mol. This would be consistent with the observation that in this model series the Dieckmann-like cyclization appears to occur essentially to the exclusion of the intramolecular aldol condensation. Models of the computed lowest energy structures for the aldol and Dieckmann cyclizations are shown in Figure 3-4.

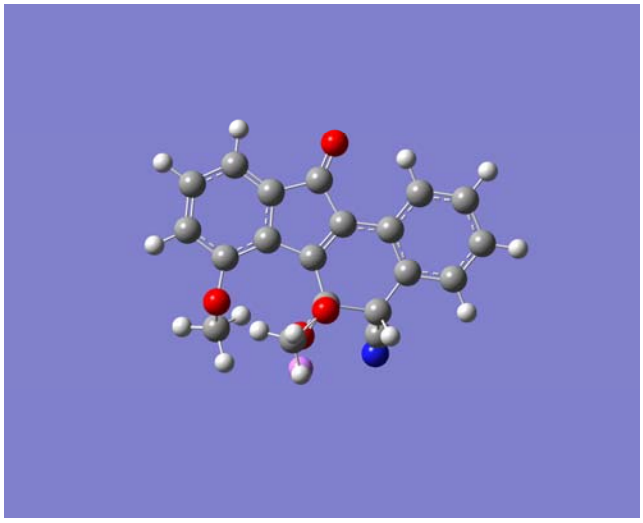
Figure 3-4 Comparison of the Minimum Energy with Aldol and Dieckmann Intermediates for the Model

Model –Aldol Intermediate



Total Electronic Energy: –9.22 kcal/mol

Model – Dieckmann Intermediate



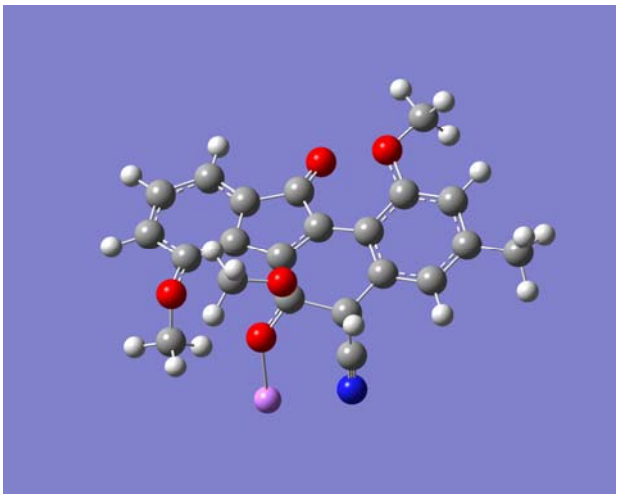
Total Electronic Energy = –12.80 kcal/mol

For computational analysis of the intermediates in the IPK synthesis, the starting geometries were constructed by modifying the lowest energy structures shown above for the

model series. The computed optimized geometries for the Dieckmann and aldol intermediates are shown in Figure 3-5.

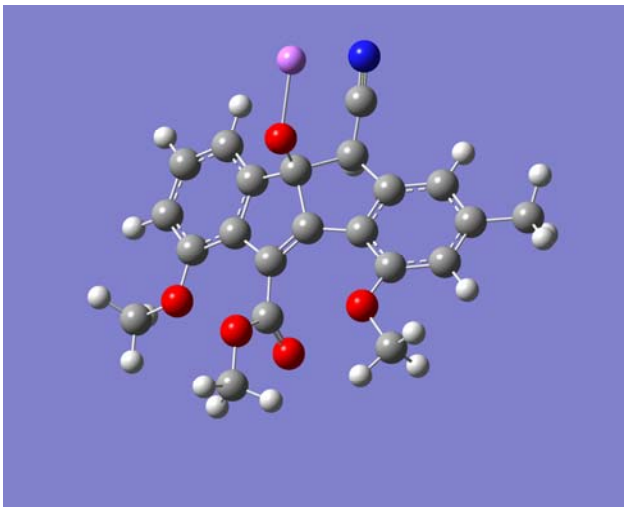
Figure 3-5 Comparison of the Minimum Energy with Aldol and Dieckmann Intermediates for the IPK

IPK-Dieckmann Intermediate



Total Electronic Energy = -51.47 kcal/mol

IPK –Aldol Intermediate



Total Electronic Energy = –51.0 kcal/mol

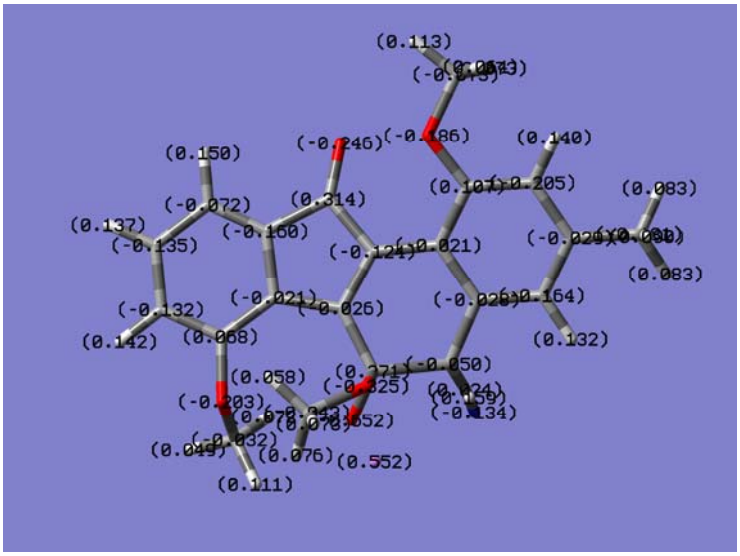
These semi-empirical calculations do predict that the degree to which the Dieckmann condensation is favoured over the intramolecular aldol is substantially diminished (less that 0.5 kcal/mol) in the case of the IPK synthesis versus the case in the model synthesis, where the Dieckmann condensation intermediate is favoured by about 3.5 kcal/mol.

These calculations are in the gas phase and clearly could be influenced by solvent effects.

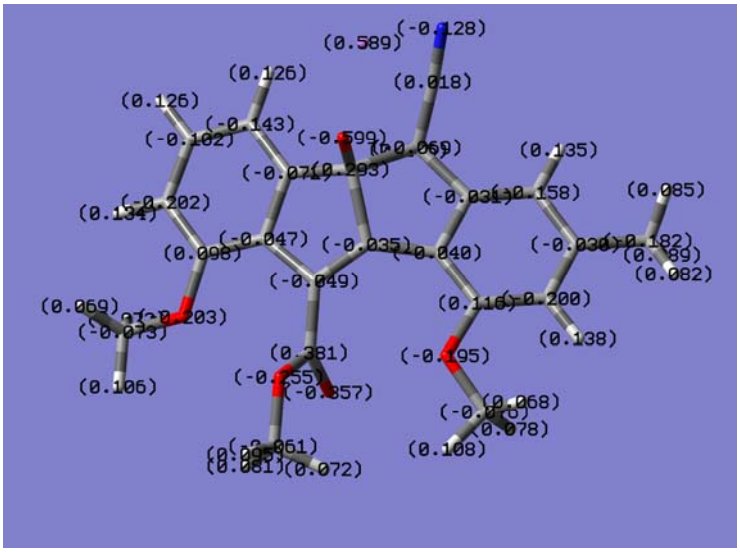
Examination of the computed models of the intermediates reveals that some steric interaction is expected between the ring D OMe group and the ester group in forming the aldol intermediate. Likewise, some steric interactions are expected between the ketone oxygen atom and the ring D OMe group in forming the initial Dieckmann intermediate. However, in the initial Dieckmann product the steric clash between the oxygen atoms of the ketone and the methyl ether is at an O–O distance of 2.38 Å whereas the O–C clash in the aldol intermediate is at a distance of 3.14 Å. Furthermore, as indicated in the figure below, the partial charges on the atoms involved in the in the steric contacts in the Dieckmann intermediate are both partially negative (–0.25 and –0.19) whereas the partial charges on the ester C and ether O atoms that clash are of opposite sign (+0.38 and –0.195) in the aldol intermediate.

Figure 3-6 Comparison of the Steric and Electronic Effects with Aldol and Dieckmann Intermediates for the IPK

Dieckmann intermediate



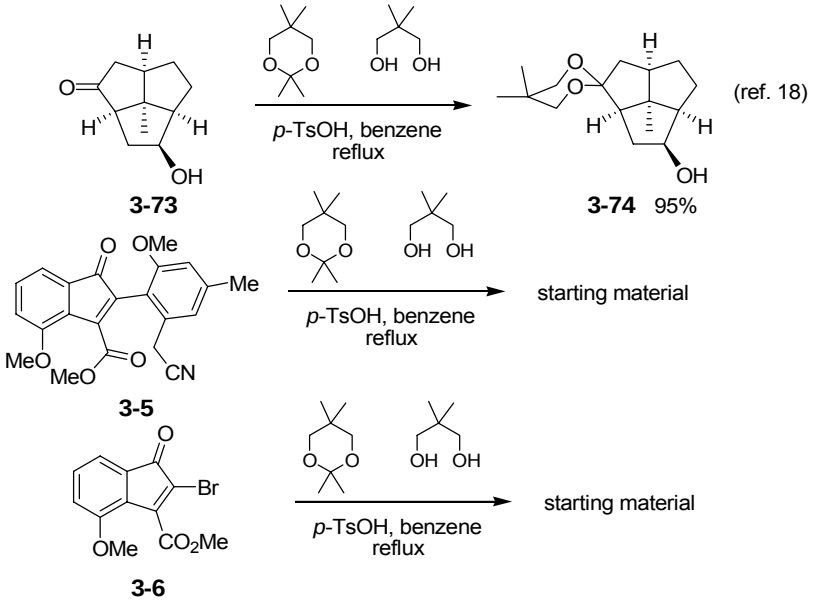
Aldol intermediate



See Appendix **3-10** for a more detailed description of the computational method and a reference for the Gaussian 03 program.

Since the cyclization of the diaryl compound with various bases generated the aldol product, the conversion of **3-5** to the corresponding cyclic ketal was explored as shown in Scheme 3-18, in order to control the regioselectivity of the cyclization. It has been observed previously in this group that ketalization of a similar diaryl ketone is difficult (R. S. Laufer Ph.D thesis).[17]

Scheme 3-18

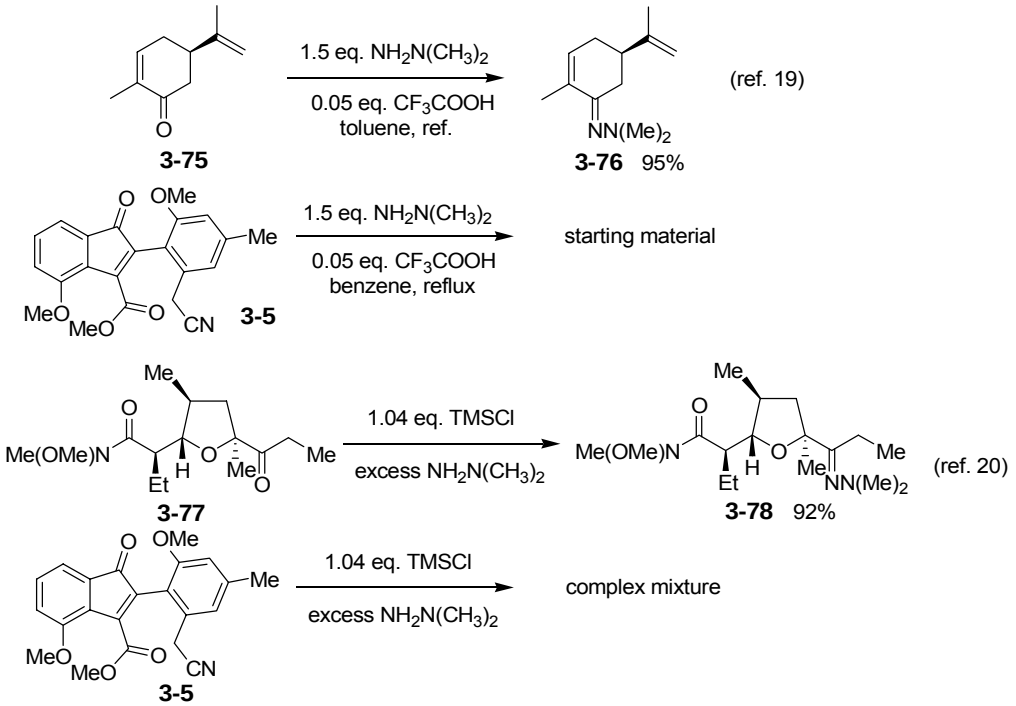


In practice, heating diaryl compound **3-5** with ethylene glycol in benzene in the presence of *p*-toluenesulfonic acid (*p*-TsOH) led to no reaction. Paquette and coworkers reported an essentially quantitative conversion to **3-74** under transacetalization conditions, in which the ketone **3-73** was unresponsive to normal ketalization involving ethylene glycol.[18] For our case, heating the **3-5** with 0.1 equivalent of 2,2-dimethyl-1,3-propanediol and 3 equivalents of 2,2,5,5-tetramethyl-1,3-dioxane in the presence of *p*-TsOH in benzene provided only the starting materials. Attempted ketalization of the bromoindenone **3-6** (i.e. prior to Suzuki

coupling) also failed.

Attempts were also made to protect the ketone as an *N*,*N*-dimethyl hydrazone derivative. Corey and coworkers reported that (–)-carvone was converted to the dimethylhydrazone **3-76** in 95% yield using 1.5 equivalents of dimethyhydrazine in the presence of 0.05 equivalent of trifluoroacetic acid in refluxing toluene.[19] No reaction was observed under such conditions applied to **3-5**. Evans and coworkers reported a mild condition for converting ketones to *N*,*N*-dimethyl hydrazones[20] using an excess of dimethylhydrazine with TMSCl to synthesize hydrazone **3-78**. Unfortunately, applying Evans conditions to **3-5** gave a complex mixture of products.

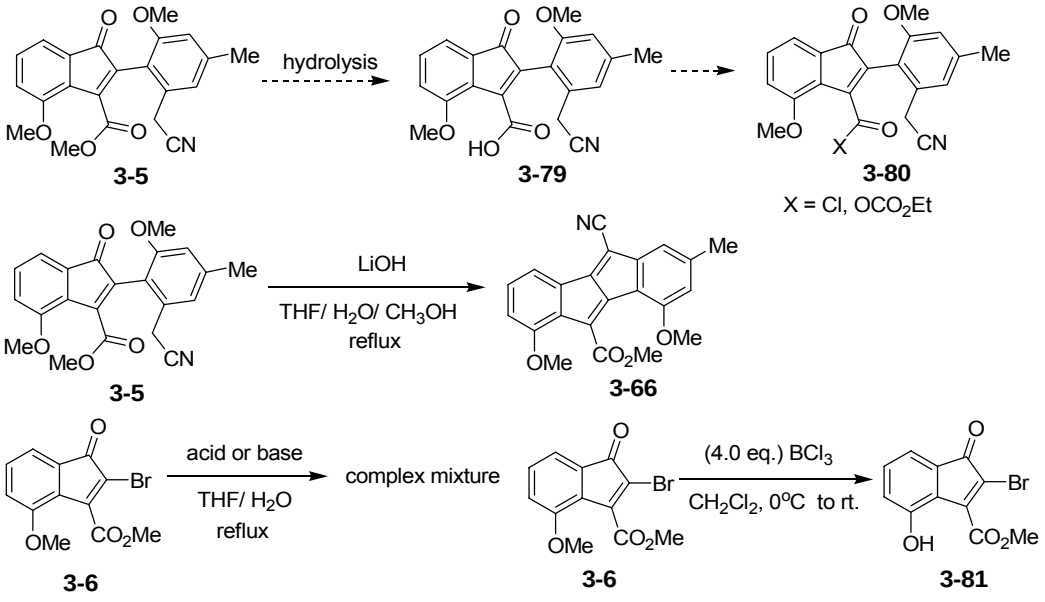
Scheme 3-19



An alternative way of improving the regioselectivity of the cyclization was considered, and involved conversion of the diaryl compound **3-5** to the corresponding carboxylic acid **3-**

79. In this way, the system could be activated by introducing a good leaving group to the carbonyl group, and was investigated as presented in Scheme 3-20. Attempted hydrolysis of diaryl compound **3-5** with lithium hydroxide in refluxing THF, water and methanol provided aldol compound **3-66**. On the other hand, attempted hydrolysis of the bromoindenone **3-6** using different bases such as LiOH and NaOH led to complex mixtures of products. Heating **3-6** with HCl in refluxing THF and water still afforded a complex mixture. Meanwhile, treatment of **3-6** with 4.0 equivalents of BCl_3 in methylene chloride only provided phenol **3-81**.

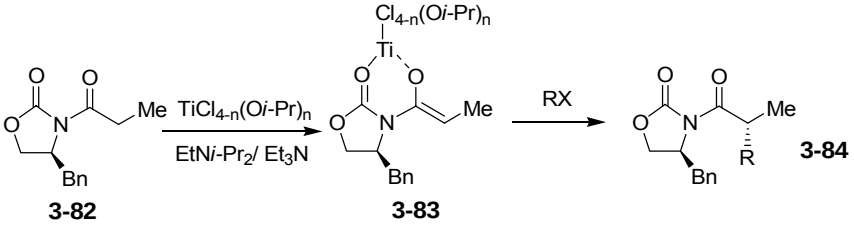
Scheme 3-20



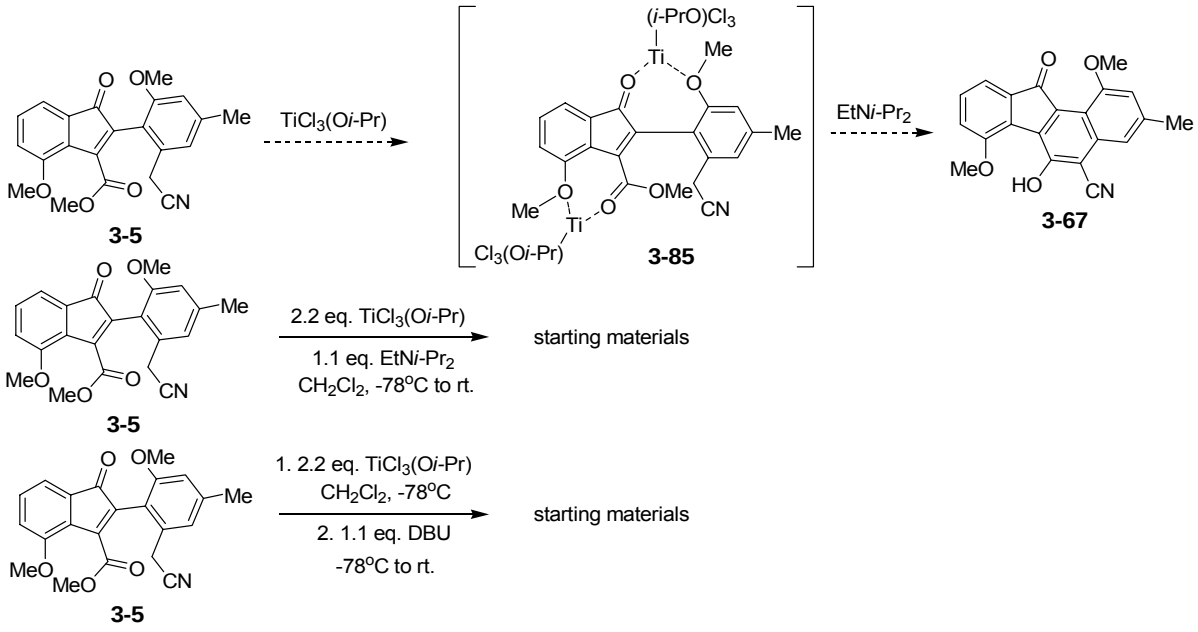
Evans and coworkers developed a procedure to generate titanium enolates from the β-ketone imides such as **3-82**, which reacted with electrophiles diastereoselectively.[21–23] $TiCl_4$ and *i*-Pr_2NEt or Et_3N were used for this system and isopropoxytitanium trichloride Ti(O*i*-Pr)Cl_3 could be used in place of $TiCl_4$. Increasing the number of the alkoxy substituents on

the titanium reagent decreases its enolization potential, but the coordination between the oxygenated titanium reagent and *i*-Pr_2NEt or Et_3N is reversible, which means that the sequence of the addition of reagents does not need to be controlled.

Scheme 3-21 (ref. 21-23)



Scheme 3-22

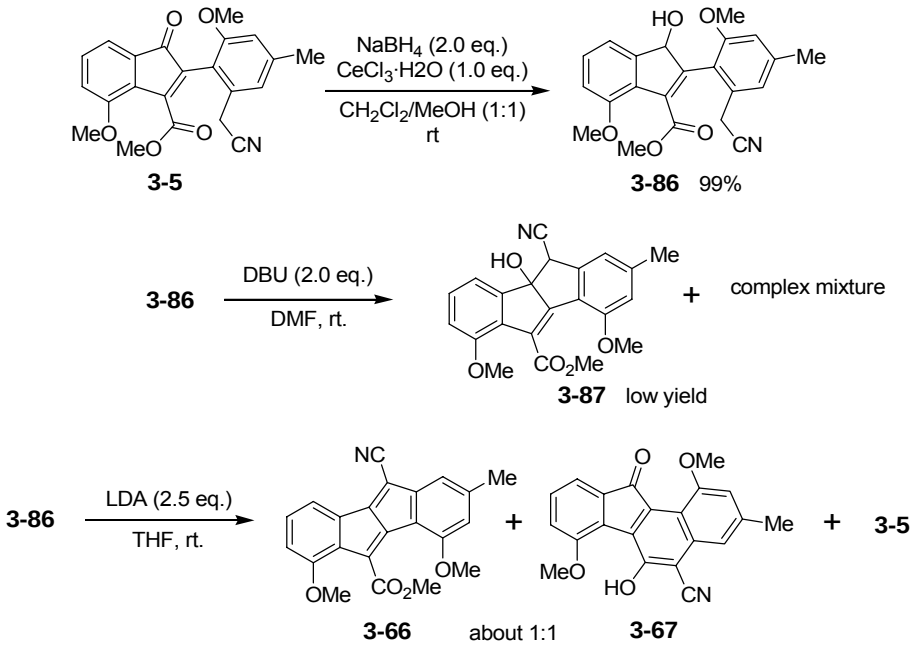


The possibility was considered that such conditions might be applied to the cyclization of **3-5** such that the titanium reagent would complex to the carbonyl group of the B ring and the nearest methoxy group and also to the carbonyl group of the ester to its nearest methoxy

group to form an intermediate such as **3-85**. It was hoped that such a species could undergo deprotonation and then cyclized selectively by reaction with the titanium-complexed ester group. In practice, no reaction was observed when **3-5** was treated with 2.2 equivalents of $Ti(O\textit{i}\text{-}Pr)Cl_3$, 1.1 equivalents of $\textit{i}\text{-}Pr_2NEt$ in CH_2Cl_2, or when DBU, a stronger base, was used (Scheme 3-22).

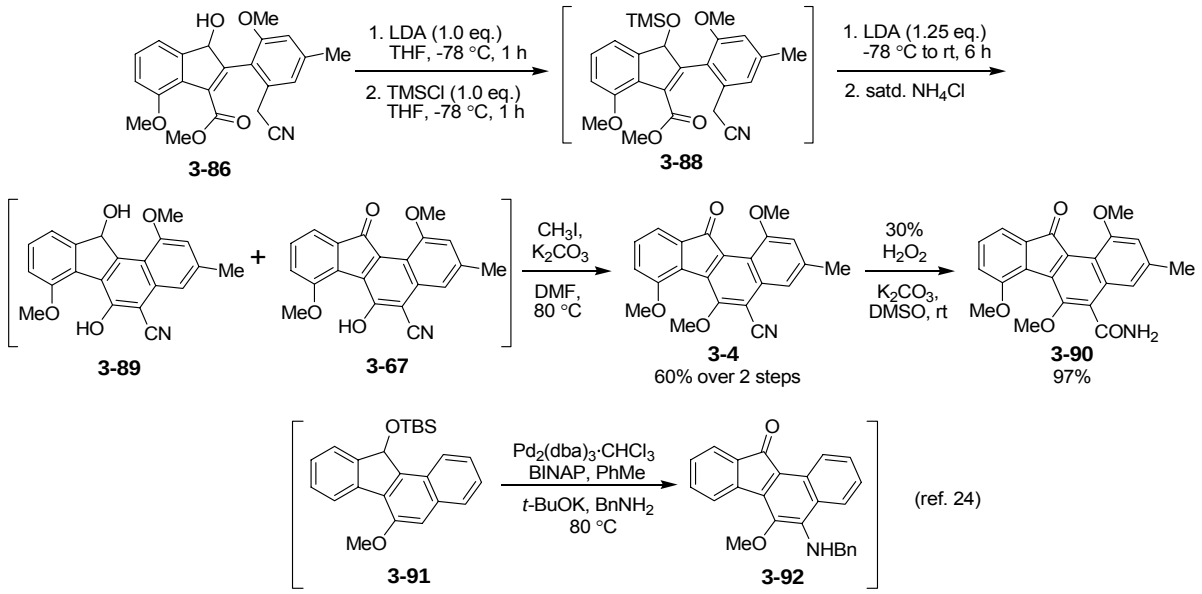
Since it was difficult to directly protect the keto group of **3-5** as either a ketal or a hydrazone derivative, the reduction of it was investigated. Treatment of ketone **3-5** with 2.0 equivalents of $NaBH_4$ in the presence of 1.0 equivalent of cerium (III) chloride hydrate to favour 1,2-reduction of the unsaturated ketone in a 1:1 mixture of methylene chloride and methanol at room temperature generated the corresponding alcohol **3-86** in 99% yield. However, treatment of alcohol **3-86** with 2.0 equivalents of DBU in DMF at room temperature provided the aldol product **3-87** accompanied by a complex mixture of products. Replacement of DBU with 2.5 equivalents of LDA in THF at room temperature led to a mixture of the aldol-dehydration product **3-66**, the desired phenol **3-67**, and the ketone **3-5**. It is not clear what the oxidant is in this procedure. Perhaps the oxidation involves traces of desolved oxygen or, perhaps the oxidation occures at the expense of reduction of another molecule of **3-86** which leads to products as the complex mixture that accompanies **3-87**. (Scheme 3-23)

Scheme 3-23



It was then thought that the alcohol **3-86** could be protected prior to cyclization, as presented in Scheme 3-24. Treatment of **3-86** with one equivalent of LDA, followed by one equivalent of TMSCl, generated the TMS-protected ether **3-88**, which was treated in situ with 1.25 equivalents of LDA, and worked up with saturated aqueous ammonium chloride to afford a mixture of the phenols **3-89** and **3-67**. Methylation of this mixture provided **3-4** in 60% yield in two steps, and with the benzo[*a*]fluorene skeleton built up, the carbonitrile functionality was converted to the corresponding carboxamide **3-90** smoothly in 97% yield. Similar to the attempted cyclizations mentioned above, it was noticed that the benzylic alcohol was oxidized in situ by air during the methylation. A similar sensitivity to oxidation of the related TBS-protected alcohol **3-91** was previously observed in this group in a Buchwald benzylamination reaction.[24]

Scheme 3-24



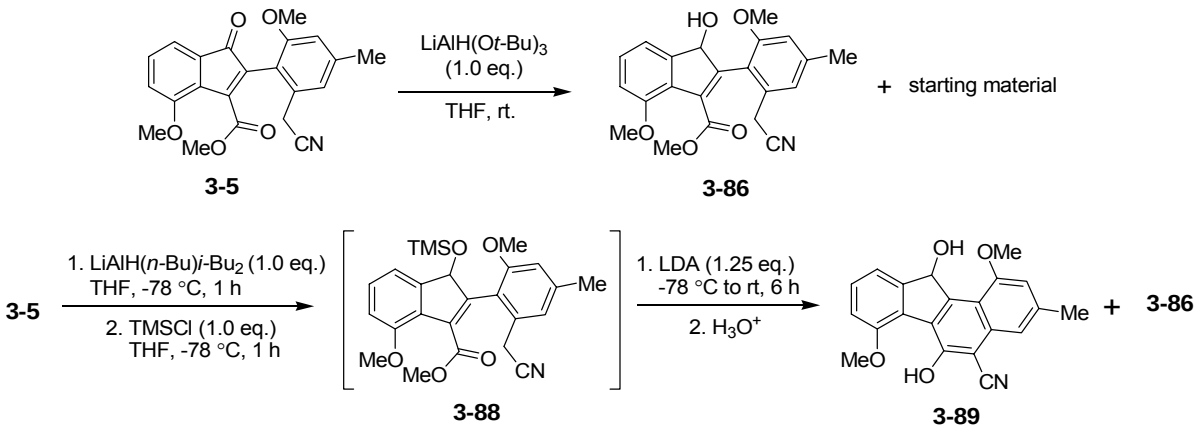
It was found later that the yield of the sequence from **3-86** to **3-4** was difficult to achieve reproducibly on different scales, perhaps because of instability of the TMS ether or the tendency of the alkoxide derived from **3-86** to undergo oxidation.

Hence, treatment of ketone **3-5** with one equivalent of lithium tri-*tert*-butoxyaluminum hydride, which resembles sodium borohydride in terms of its selectivity for functional groups, in THF at room temperature overnight led to alcohol **3-86** and a small amount of starting material. Although the reaction seemed clean, it seemed too slow to be practical for large-scale preparations. (Scheme 3-25)

Exploration of the literature revealed that Kim and coworkers reported that an ate complex, LiAlH(*n*-Bu)*i*-Bu_2, generated from diisobutylaluminum hydride and *n*-butyllithium in an equimolar ratio in tetrahydrofuran-hexane could reduce the keto group in an enone selectively while tolerating ester or cyano groups.[25] Reduction of **3-5** with one equivalent of

LiAlH(*n*-Bu)*i*-Bu_2 in THF at –78 °C for one hour, followed by treatment with one equivalent of TMSCl in THF at –78 °C for one hour, and treatment with 1.25 equivalents of LDA in THF from –78 °C to room temperature for six hours provided a mixture of alcohol **3-86** and the desired cyclization product **3-89**. It was considered that the trialkylaluminate **3-93** (in Scheme 3-26), generated from the reduction of **3-5** using LiAlH(*n*-Bu)*i*-Bu_2, was relatively stable and that its reaction with TMSCl was slower than expected. However, elevation of the reaction temperature to room temperature after the addition of TMSCl did not improve the ratio of **3-89** to **3-86** in the mixture.

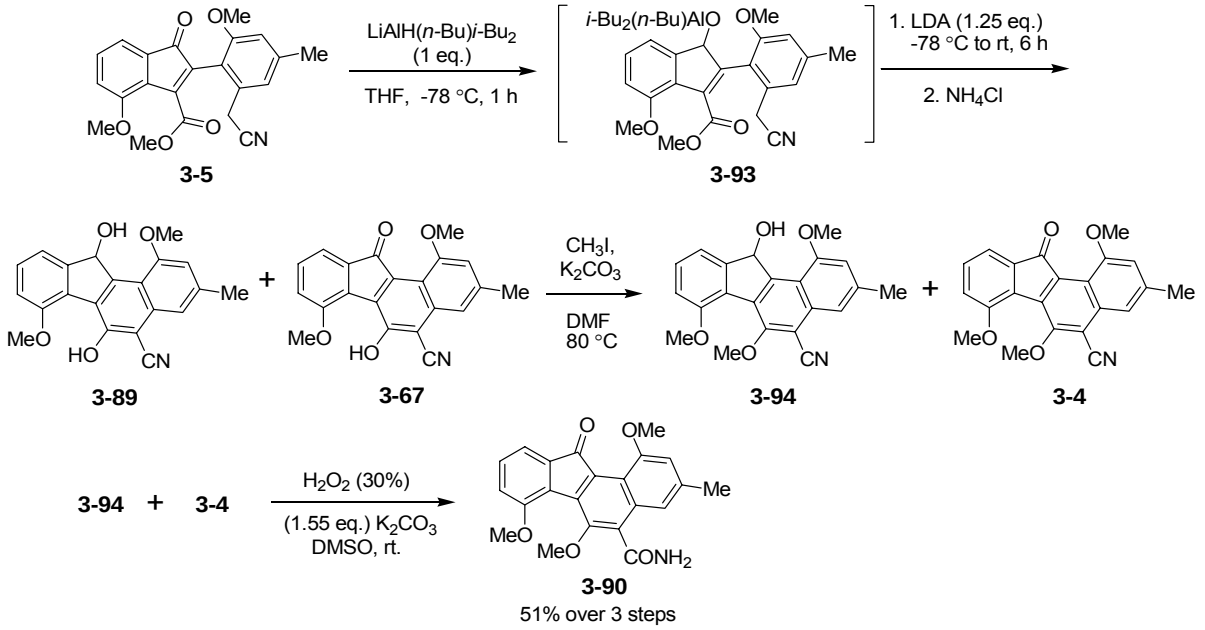
Scheme 3-25



It was decided that, since the trialkylaluminate **3-93** is bulky and relatively stable, it could be viewed as a protecting group for the alcohol **3-86**. Thus, treatment of **3-5** with the same sequence as above, without the TMSCl, afforded a mixture of the desired phenol **3-89** and its oxidized product **3-67** (as expected), which were methylated to provide the mixture of **3-94** and **3-4**. Since the next step in the planned sequence was hydrolysis of the cyano group by H_2O_2/K_2CO_3/DMSO, it was assumed that the alcohol **3-94** would be oxidized in this step. In practice, the mixture of **3-94** and **3-4** gave the desired amide **3-90** in 51% yield overall from

3-5.

Scheme 3-26

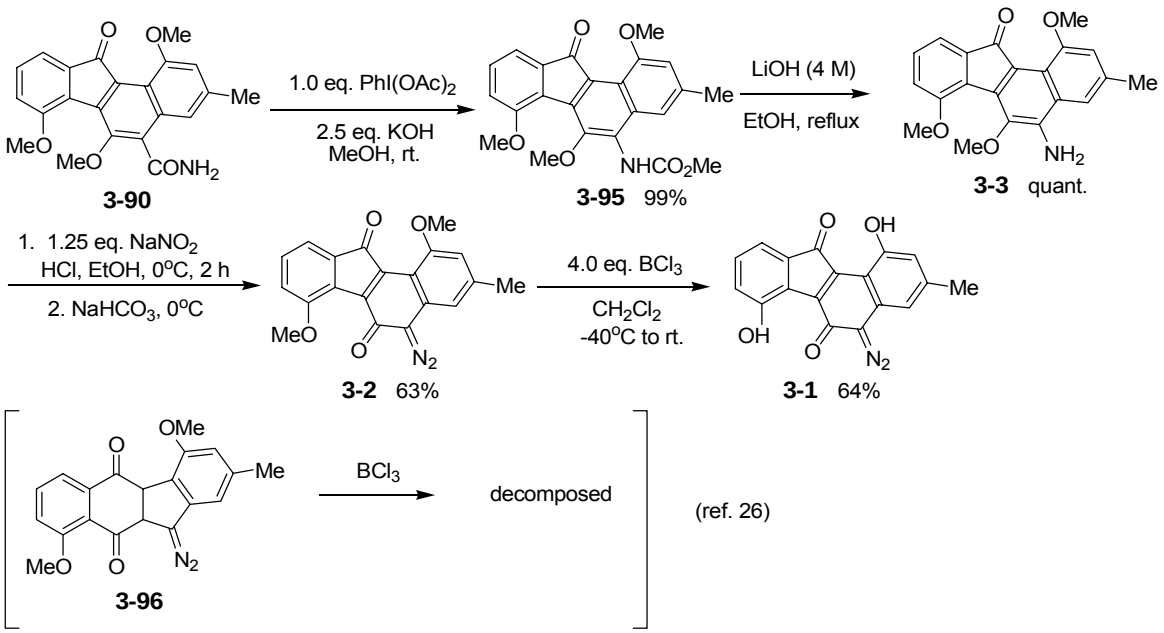


Thus, this sequence provides **3-90** via a convenient and reproducible protocol. To our knowledge, the use of an aluminate intermediate such as **3-93** for protection of an alcohol is new and this way offers an advantage over trialkylsilyl protection when the alcohol is very base sensitive.

With carboxamide **3-90** in hand, the modified Hofmann rearrangement of **3-90** using 1.0 equivalent of $PhI(OAc)_2$ and 2.5 equivalents of KOH in methanol at room temperature provided the carbamate **3-95** in 99% yield. This reaction is sensitive to the amount of $PhI(OAc)_2$, and more than one equivalent of this oxidative reagent led to complex mixtures of byproducts, perhaps resulting from the oxidation of the electron rich D ring of **3-90**. Hydrolysis of the **3-95** afforded an aniline **3-3** quantitatively, which was subjected to the demethylative diazotization conditions to generate dimethyl isoprekinamycin **3-2** in a yield

of 63%. Fortunately, the demethylation of dimethyl IPK **3-2** using four equivalents of BCl_3 in methylene chloride proceeded well and provided IPK **3-1** in 64% yield. This contrasts with reported conversion of the dimethyl prekinamycin **3-96**, which was decomposed with BCl_3 and other Lewis acids such as BBr_3, TMSI, and $MgI_2 \cdot Et_2O$ during the attempted demethylation to prekinamycin.[26]

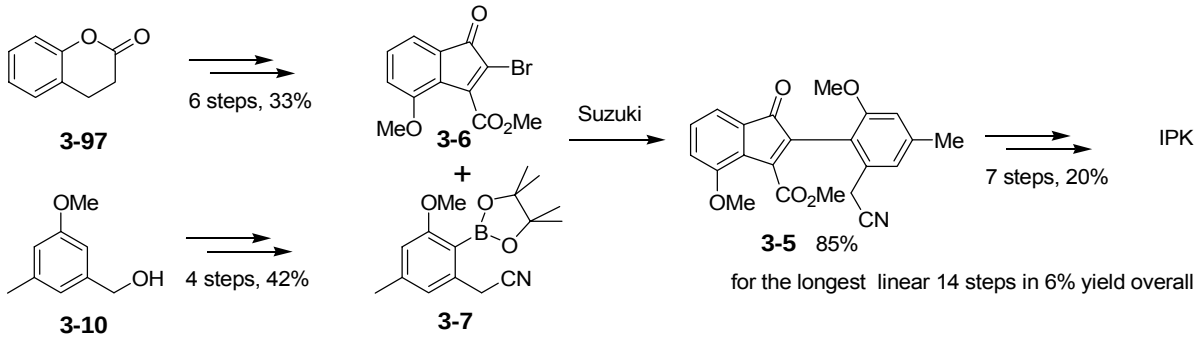
Scheme 3-27



Thus, the first total synthesis of IPK was achieved via the sequence as presented in Scheme 3-28. The synthesis was initiated from the commercially available dihydrocoumarin **3-97** through six steps to generate the AB ring synthon, bromoindenone **3-6,** in 33% yield. Suzuki coupling between **3-6** and a D ring building block pinacol boronate **3-7**, which was obtained from the known benzyl alcohol **3-10** through four steps in 42% yield, gave **3-5** in

85% yield. IPK was obtained in another seven steps, and overall, the longest linear sequence is fourteen steps with an overall yield of 6% from **3-97**.

Scheme 3-28

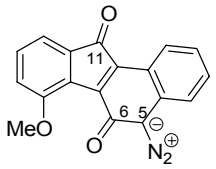


3.3 Comparison between the Synthetic Compounds and Natural IPK

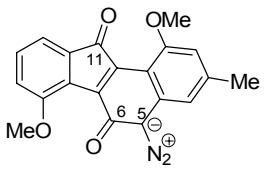
Although the synthetic route that was followed and the detailed characterization of the intermediates gave us confirmation that we had indeed synthesized the structure assigned to IPK, it was felt that a rigorous comparison to establish that the synthetic material was identical to the natural product remained important.

High resolution mass spectrometry data for the synthetic dimethylisoprekinamycin **3-2** supports the molecular formula as $C_{20}H_{14}O_4N_2$ with an exact mass of 346.0953. The proton NMR spectrum of **3-2** exhibits one multiplet at δ 7.33, two doublets at δ 7.27 (J = 6.5 Hz) and 7.16 (J = 8.2 Hz) and two singlets at δ 6.86, 6.69 representing the aromatic protons and three singlets at δ 3.99, 3.98, 2.48 representing the two methoxy groups and one methyl group respectively. 2-Dimensional HMQC NMR experiments showed cross peaks at δ 7.33 (130.9), 7.27 (120.5), 7.16 (116.7), 6.86 (112.5), 6.69 (109.1), 3.99 (56.7), 3.98 (55.8) and 2.48 (21.7). As a result of the low solubility of **3-2**, the ^{13}C NMR signal corresponding to the

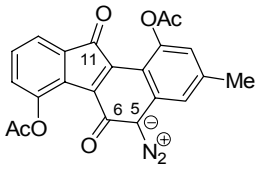
carbon of the diazo group was not observable directly. However, the HMBC experiment indicated a three bond correlation from H-4 at 6.86 ppm to a ^{13}C atom with a resonance at δ 88.0 ppm, which was then assigned to the carbon attached to the diazo group. This result agrees with the value of δ 83.7 ppm reported for isoprekinamycin.[27]



3-98



3-2



3-99

Table 3-1 Spectroscopic Data for Compounds 3-98, 3-2, and 3-99

		3-98	**3-2**	**3-99**
^{13}C NMR	C-11	196.2 ppm	192.2 ppm	192.48 ppm
	C-6	170.5 ppm	171.2 ppm	174.14 ppm
	C-5	90.0 ppm	88.0 ppm	83.17 ppm
IR	*ν* (C-N_2)	2095.7 cm^{-1}	2112.6 cm^{-1}	2119 cm^{-1}
	ν (CO)	1710.8 cm^{-1}	1722.3cm^{-1}	1727 cm^{-1}
	ν (CC)	1620.1 cm^{-1}	1608.6 cm^{-1}	1613 cm^{-1}

Other ^{13}C NMR characteristics of dimethyl IPK **3-2** are also in excellent agreement with the corresponding structural features of model **3-98** and IPK diacetate **3-99** (Table 3-1). In particular, the carbonyl resonance at 192.2 ppm in the spectrum of **3-2**, assigned to the five-membered ring ketone, has counterparts at 196.2 ppm in the model **3-98** and 192.48 ppm in IPK diacetate **3-99**. In addition, the diazo group gives rise to a band at 2112.6 cm^{-1} in the IR spectrum of **3-2** as compared with a band at 2095.7 cm^{-1} in the spectrum of the model **3-98** and at 2119 cm^{-1} for IPK diacetate **3-99**.

Spectroscopic comparison of the synthetic and natural IPK was then carried out in the following manner: (1) for ^{1}H NMR spectral comparison, saturated CD_2Cl_2 solutions of the synthetic and natural IPK were examined on a 500 MHz NMR spectrometer; (2) for IR spectral comparison, saturated CH_2Cl_2 solutions of the synthetic and natural IPK were examined on an FT-IR spectrometer using a demountable liquid-cell IR kit (Aldrich Z11200-3) with two CaF_2 windows (32 mm × 3 mm) and a light path of 0.1 mm, and the solvent and air backgrounds were deducted from the recorded spectra; (3) for HPLC retention time and UV-vis spectra comparison, the synthetic and natural IPK and also a mixture of both compounds were analyzed with a Waters HPLC system equipped with a photodiode array detector.

Table 3-2 Comparison of ^{1}H NMR with Natural and Synthetic IPK (500 MHz in CD_2Cl_2)

Natural IPK (ppm)	Synthetic IPK (ppm)
12.32 (s, 1H)	12.32 (s, 1H)
11.61 (s, 1H)	11.61 (s, 1H)
7.26 (dd, *J* = 7.0 Hz, 0.7 Hz, 1H)	7.26 (d, *J* = 6.7 Hz, 1H)
7.18 (t, *J* = 7.6 Hz, 1H)	7.18 (t, *J* = 7.6 Hz, 1H)
7.06 (dd, *J* = 8.3 Hz, 0.8 Hz, 1H)	7.06 (d, *J* = 8.3 Hz, 1H)
6.71 (s, 1H)	6.72 (s, 1H)
6.69 (s, 1H)	6.69 (s, 1H)
2.41 (s, 1H)	2.41 (s, 1H)

Table 3-3 Comparison of IR Frequencies of Natural and Synthetic IPK (saturated CH_2Cl_2 solution)

Natural IPK (cm^{-1})	Synthetic IPK (cm^{-1})
3688.5	3686.1
3600.9	3600.7
2926.6	2926.6
2855.3	2855.0
2126.0*	2126.1*
1733.8	1733.9
1686.4	1686.8
1611.6	1611.5
1559.9	1560.5
1466.4	1466.5

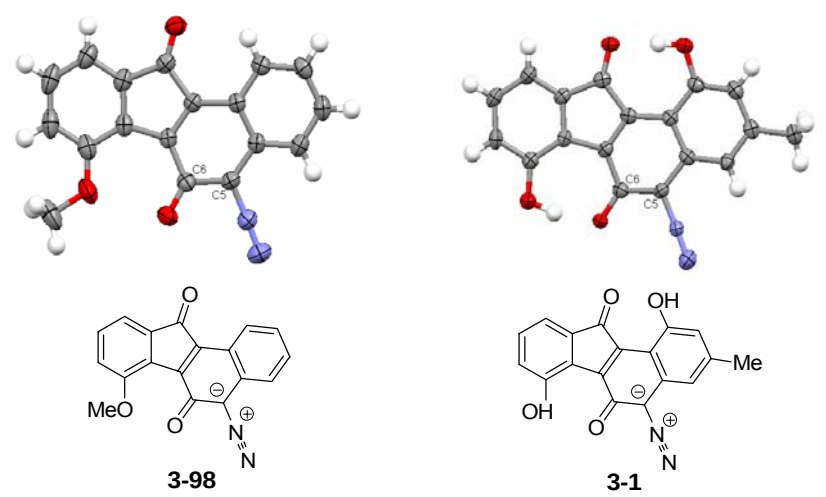
* Absorption of the diazo group

Table 3-4 Comparison of UV-vis with Natural and Synthetic IPK (HPLC)

Natural IPK (nm)	Synthetic IPK (nm)
218.1	211.0
247.4	247.4
284.0	284.0
560.1	561.3

Synthetic and natural isoprekinamycin exhibited the same R_f values in the following three different TLC solvent systems: R_f = 0.80 (EtOAc:Hexanes = 3:2), R_f = 0.38 (Et_2O:Hexanes = 1:1), R_f = 0.14 (CH_2Cl_2:Hexanes = 2:1).

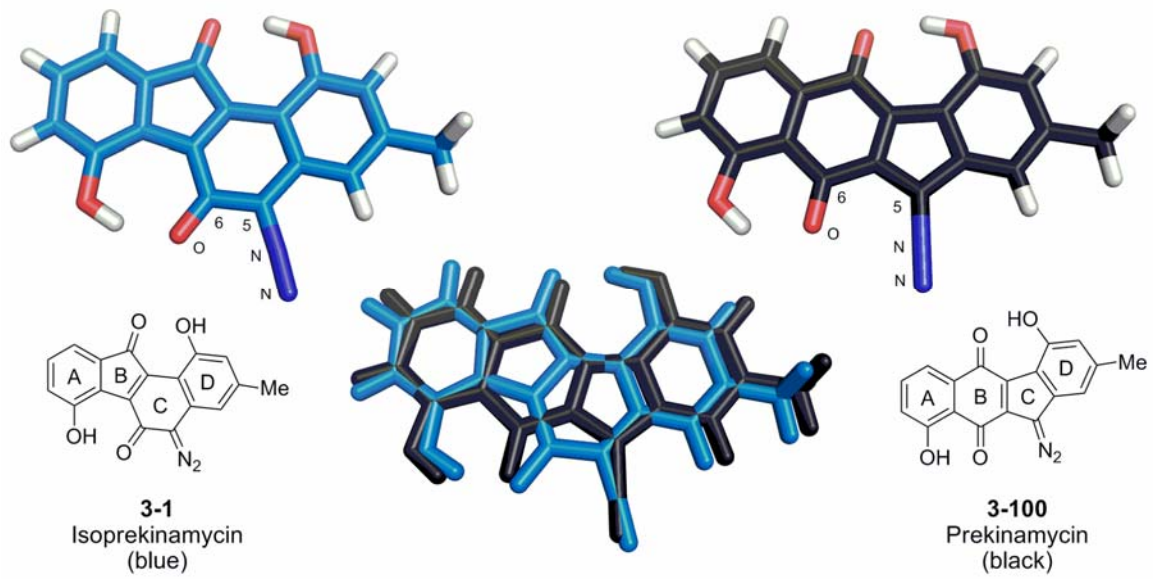
Figure 3-7 X-ray Structures of Model Compound 3-98 and IPK (3-1)



Slow crystallization of IPK by Nan Chen in this group, provided crystals for an X-ray diffraction study that confirmed the assigned structure **3-1**. Comparison of structures of the synthetic model compound **3-98** and IPK **3-1** reveals increased diazonium ion character in IPK as evidenced by the alternating shorter N–N (1.104 versus 1.116 Å), longer C5–N (1.362 versus 1.334 Å), shorter C5–C6 (1.424 versus 1.457 Å) , and longer C6–O (1.246 versus 1.227 Å) bond lengths in the IPK structure which are entirely consistent with an increased diazonium ion contribution to the resonance hybrid representing the *o*-quinodiazide functionality in IPK relative to that **3-98**.

The increased availability of IPK opens the door to more extensive biological studies. We have now found that IPK, previously shown to exhibit modest antibacterial activity, significantly inhibits the growth of CHO (IC_{50} = 5.8 μM) and K562 human leukemia cells (IC_{50} = 6.4 μM).[28]

Figure 3-8 Structural Comparison of the Diazobenzo[*a*]fluorene and Diazobenzo[*b*]fluorene Systems



Superimposition of a computed model of isoprekinamycin (RHF/6-31G) on a model of prekinamycin(RHF/6-31G) reveals that the oxygen and nitrogen atoms on the periphery align well despite the rearranged carbon skeleton, suggesting that the diazobenzo[*a*]- and diazobenzo[*b*]fluorenes are variants of the same pharmacophore. Consistent with this hypothesis is our finding that IPK-diacetate **3-99**, which is more soluble than IPK, inhibits the topoisomerase IIα-catalyzed decatenation of kDNA (IC_{50} = 9.7 μM) just as was observed recently for kinamycin A and kinamycin C (IC_{50} = 3.2 and 43 μM, respectively) in the laboratory of our collaborators in the Faculty of Pharmacy at the University of Manitoba.[29]

The ease of synthesis of IPK relative to the kinamycins and lomaiviticins makes the diazobenzo[*a*]fluorene system attractive for generation of synthetic analogues. Efforts to create other benzo[*a*]fluorenes and congeners of IPK with improved solubility and drug-like properties will be discussed in next two chapters.

3.4 Experimental

3.4.1 General Information

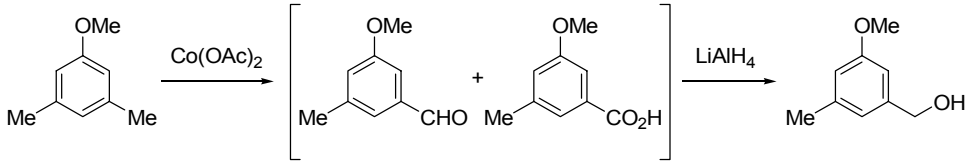
^{1}H NMR spectra were recorded on a Brüker AVANCE 500 (500 MHz), Brüker AC 300 (300 MHz) and Brüker AVANCE 300 (300 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The following abbreviations are used for NMR peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; b, broad; w, weak. ^{13}C NMR spectra were broad band decoupled and recorded on a Brüker AVANCE 500 (125.8 MHz), Brüker AC 300 (75.5 MHz) and Brüker AVANCE 300 (75.5 MHz) NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. HMQC and HMBC experiments were performed on a Brüker AVANCE 500 spectrometer. IR spectra were determined on a Perkin-Elmer RX I FT-IR spectrometer as KBr discs unless otherwise indicated. High- and low resolution electron impact or electrospray ionization (EI or ESI) mass spectra (MS) were measured by the WATSPEC Mass Spectrometry Facility (Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada). Elemental analyses were performed by the M-H-W Laboratories (Phoenix, Arizona, USA).

Anhydrous THF and Et_2O were freshly distilled from sodium/benzophenone under nitrogen prior to use. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen prior to use. All commercial reagents were purchased from Aldrich Chemical Co., Strem Chemicals Inc., Alfa Aesar, Lancaster Synthesis Ltd. or BDH Inc. and were used as received unless

otherwise indicated. Deionized water was supplied by a Biolab vertical series reverse osmosis system.

The –78 °C and 0 °C designations refer to solid carbon dioxide/acetone and ice/water slush respectively. The room temperature refers to 22 °C to 25 °C. Flash column chromatography was carried out using the Merck silica gel (230–400 mesh) and SiliCycle silica gel (60 Å). Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with Merck pre-coated silica gel plates (silica gel 60 F_{254} on aluminum sheet). All reported yields are isolated yields.

3.4.2 Detailed Experimental Procedures



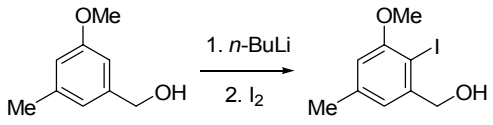
3-11 **3-16** **3-10**

(3-Methoxy-5-methylphenyl)methanol (3-10)

To a solution of 3,5-dimethylanisole **3-11** (4.752 g, 34.90 mmol) in HOAc (50 mL) were added *N*-hydroxyphthalimide (0.569 g, 3.490 mmol) and $Co(OAc)_2$•4 H_2O (0.174 g, 0.698 mmol). The solution was stirred at 100 °C for 72 h. The solution was concentrated, and 1 M aqueous NaOH solution was added to the residue to adjust the pH to ca. 12. The resulting solution was extracted with CH_2Cl_2 (3 × 100 mL), and the organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to recover the starting material 3,5-dimethylanisole (1.712 g,

36%) and meanwhile to obtain the crude aldehyde. The pH of the basic aqueous phase was adjusted to ca. 1 with conc. HCl and the resulting solution was extracted with EtOAc (4 × 100 mL). The EtOAc phase was dried over Na_2SO_4 and concentrated to obtain the crude carboxylic acid **3-16**. The crude aldehyde and carboxylic acid **3-16** were combined (3.425 g) and subjected to the next reduction step.

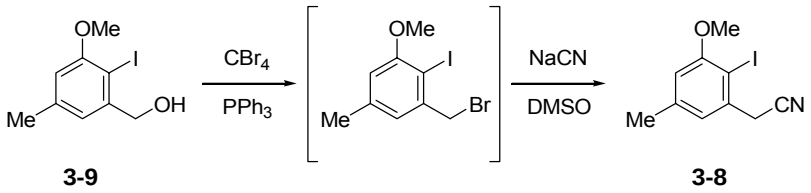
To a suspension of $LiAlH_4$ (1.299 g, 34.25 mmol) in anhydrous THF (75 mL) at 0 °C was added slowly a solution of the crude aldehyde and acid in anhydrous THF (3.425 g in 9 mL), and the reaction mixture was further stirred at 0 °C for 15 min. The solution was then warmed to room temperature and stirred for 20 h. Excess $LiAlH_4$ was quenched with the addition of saturated aqueous NH_4Cl solution at 0 ºC and the suspension was filtered, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:5, v/v) to obtain the title compound as a white solid (1.487 g, 28% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 1H), 6.68 (s, 1H), 6.62 (s, 1H), 4.53 (s, 2H), 3.74 (s, 3H), 3.08 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 159.7, 142.4, 139.5, 120.0, 113.9, 109.2, 64.9, 55.1, 21.4; IR (film): 3366.1, 2999.8, 2940.0, 2871.3, 2839.1, 1613.5, 1462.7, 1325.3, 1295.3, 1193.6, 1152.5, 1068.2 cm^{-1}; MS (EI): *m/z* 152.09 (100, M^+), 137.07 (23), 123.09 (39), 109.07 (22), 91.05 (18), 77.02 (11).



3-10 **3-9**

(2-Iodo-3-methoxy-5-methylphenyl)methanol (3-9)

To a solution of compound **3-10** (3.743 g, 24.62 mmol) in anhydrous diethyl ether (160 mL) was added *n*-BuLi (1.6 M in hexane, 33.86 mL) slowly, and the solution was warmed to room temperature and stirred for 4 hours, after which the solution was again cooled to 0 °C. Anhydrous THF (80 ml) was then added to the solution and the reaction mixture was stirred for 1 h, followed by slow addition of I_2 (7.687 g, 30.29 mmol) dissolved in minimum amount of THF and the mixture was further stirred for 30 min at 0 ºC. The reaction mixture was washed with 10% aqueous $Na_2S_2O_3$ solution and then mixed with saturated aqueous NH_4Cl solution (50 mL), followed by extraction with Et_2O (3 × 100 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:5, v/v) to obtain the title compound as a white solid (5.068 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86 (s, 1H), 6.54 (s, 1H), 4.59 (s, 2H), 3.83 (s, 3H), 2.60 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 157.7, 144.0, 139.7, 121.7, 111.1, 85.2, 69.4, 56.5, 21.4; IR (film): 3262.1, 2902.6, 1651.8, 1575.5, 1457.7, 1433.9, 1409.8, 1356.2, 1307.6, 1168.2, 1099.7, 1041.8, 1009.6, 839.7, 582.0 cm^{-1}; MS (EI): *m/z* 278.0 (100, M^+), 263.0 (5).

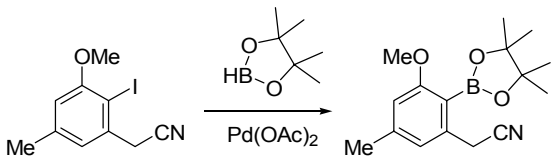


3-9 **3-8**

2-(2-Iodo-3-methoxy-5-methylphenyl)acetonitrile (3-8)

To a solution of compound **3-9** (5.068 g, 18.23 mmol) in anhydrous CH_2Cl_2 (100 mL) was added CBr_4 (6.348 g, 19.14 mmol) at room temperature. The solution was cooled to 0 ºC and PPh_3 (5.021 g, 19.14 mmol) was added slowly. The solution was then warmed to room temperature and stirred for 6 h. The solution was concentrated and the residue was purified by flash chromatography (EtOAc:Hexanes = 1:50, v/v) to obtain the intermediate benzyl bromide as a white solid.

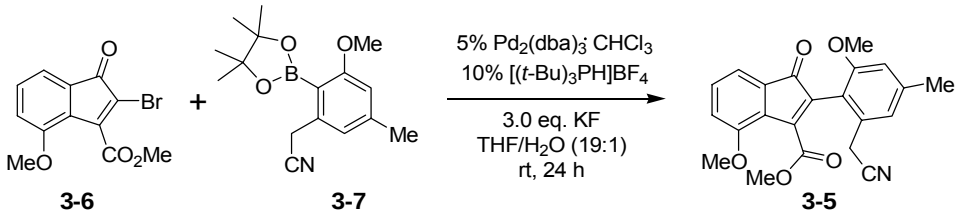
To a solution of the above white solid in anhydrous DMSO (30 mL) was added NaCN (1.340 g, 27.35 mmol) and the mixture was stirred for 36 h. The reaction mixture was diluted with water (150 ml) and extracted with CH_2Cl_2 (2 x 100mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to obtain the title compound as a white solid (3.606 g, 69% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.91 (s, 1H), 6.55 (s, 1H), 3.82 (s, 3H), 3.73 (s, 2H), 2.30 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 158.4, 140.3, 134.5, 122.2, 117.6, 111.4, 87.4, 56.6, 30.2, 21.4; IR (film): 3020.3, 2968.7, 2942.4, 2920.1, 2250.9, 1572.7, 1458.9, 1408.0, 1391.9, 1319.7, 1298.3, 1243.0, 1184.4, 1081.5, 1015.7 cm^{-1}; MS (EI): *m/z* 287.0 (100, M^+), 272.0 (9); HRMS (EI): calculated for $C_{10}H_{10}INO$: 286.9807, found: 286.9816.



3-8 **3-7**

2-(3-Methoxy-5-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)aceto-nitrile (3-7)

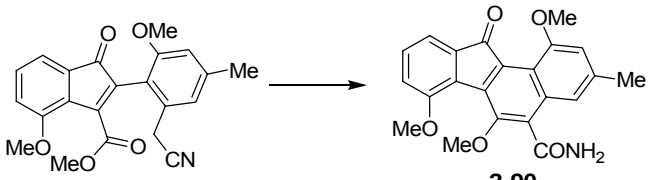
To a solution of compound **3-8** (1.500 g, 5.226 mmol) in anhydrous dioxane (18 mL) were added pinacolborane (2.28 mL, 15.98 mmol) and Et_3N (2.91 mL, 20.91 mmol), and the mixture was degassed three times by the thaw-freeze process. $Pd(OAc)_2$ (59 mg, 0.2613 mmol) and (2-biphenyl)dicyclohexylphosphine (366 mg, 1.045 mmol) were added to the mixture then the reaction mixture was degassed again by a freeze-thaw process. The mixture was stirred at 100 ºC for 4 h. Saturated aqueous NH_4Cl solution (20 mL) was added to the solution at 0 ºC followed by extraction with CH_2Cl_2 (2 × 100 mL). The organic phase was dried over Na_2SO_4 and concentrated, and the residue was purified by flash chromatography (EtOAc:Hexanes = 1:10, v/v) to obtain the title compound as a white solid (1.229 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.77 (s, 1H), 6.56 (s, 1H), 3.76 (s, 2H), 3.71 (s, 3H), 2.28 (s, 3H), 1.31 (s, 12H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 164.2, 142.3, 135.7, 121.6, 118.5, 110.8, 83.8, 55.8, 24.7, 23.4, 21.7; IR (film): 3458.1, 2978.7, 2935.6, 2838.5, 2249.0, 1612.1, 1567.3, 1144.2, 1084.9, 1063.8 cm^{-1}; MS (EI): *m/z* 287.2 (100, M^+), 286.2 (27), 272.2 (26), 228.2 (38), 214.2 (25), 200.2 (37), 187.1 (99), 186.1 (32), 161.1 (18), 131.1 (26), 130.1 (12); HRMS (EI): calculated for $C_{16}H_{22}{}^{10}BNO_3$: 286.1729, found: 286.1722.



Methyl 2-(2-(cyanomethyl)-6-methoxy-4-methylphenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (3-5)

Compound **3-6** (132 mg, 0.443 mmol) was mixed with KF (77 mg, 1.329 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$ (23 mg, 0.022 mmol) and $[(t\text{-}Bu)_3PH]BF_4$ (13 mg, 0.044 mmol) and the mixture was deoxygenated five times with an argon balloon and vacuum pump. Compound **3-7** (114 mg, 0.339 mmol) was dissolved in a mixture of THF and water (19:1, v/v, 4 mL) and the solution was deoxygenated three times using a freeze-thaw process. The deoxygenated solution was then added to the mixture of solids and the reaction mixture was stirred at room temperature for 24 hours under an argon atmosphere. The solution was then diluted with Et_2O (100 mL) and washed with H_2O (3 × 10 mL). The aqueous phase was extracted with Et_2O (2 × 50 mL) and all Et_2O phases were combined. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as an orange solid (127 mg, 85%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.27 (m, 1H), 7.16 (d, J = 6.8 Hz, 1H), 7.01 (d, J = 8.3 Hz, 1H), 6.93 (s, 1H), 6.67 (s, 1H), 3.83 (s, 3H), 3.75 (s, 3H), 3.73 (d, J = 19.0 Hz, 1H), 3.69 (s, 3H), 3.64 (d, J = 19.0 Hz, 1H), 2.35 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 195.1, 166.0, 158.1, 152.8, 148.3, 141.0, 131.8, 131.1, 130.8, 130.4, 128.0, 121.2, 119.0, 118.0, 117.0, 115.1, 111.4, 56.2, 55.8, 52.4, 22.0, 21.8; IR (film): 2950.0, 2842.3, 2251.6, 1733.1, 1717.1,

1610.0, 1575.8, 1479.4, 1464.0, 1337.9, 1274.2, 1242.6, 1202.1, 1173.8, 1092.5, 1055.1 cm^{-1}; MS (EI): *m/z* 377.2 (100, M^+), 345.1 (25), 318.1 (40), 286.1 (65), 59.2 (12); HRMS (EI): calculated for $C_{22}H_{19}NO_5$: 377.1263, found: 377.1263.



1, 6, 7-Trimethoxy-3-methyl-11-oxo-11*H*-benzo[*a*]fluorene-5-carboxamide (3-90)

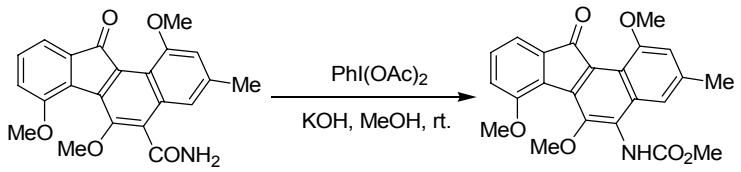
Preparation of lithium complex solution: To a solution of *i*-Bu_2AlH (1.75 mL of a 1.0 M solution, 1.75 mmol) in anhydrous THF (2.05 mL) at –78 °C was added *n*-BuLi in hexane (1.20 mL, 1.75 mmol) slowly, the mixture was further stirred for 1 hour.

Preparation of LDA: To a solution of *i*-Pr_2NH (0.26 mL, 1.834 mmol) in anhydrous THF (8.54 mL) at 0 ºC was added *n*-BuLi in hexane (1.20 mL, 1.75 mmol) slowly, and the mixture was further stirred for 30 minutes.

To a solution of compound **3-5** (66 mg, 0.175 mmol) in anhydrous THF (4 mL) at –78 ºC was added the above lithium complex solution (0.5 mL) slowly, and the reaction mixture was further stirred for 1 hour, followed by slow addition of the freshly prepared LDA solution (1.25 mL). The solution was stirred for 1 hour at –78 ºC and then warmed to room temperature to be further stirred for 5 hours. Saturated aqueous NH_4Cl solution (10 mL) was added to quench the reaction and the mixture was stirred for 20 minutes at 0 ºC. The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated to obtain the crude product as a red-orange solid (75 mg).

To a solution of the above crude product (75 mg) in anhydrous DMF (10 mL) was added K_2CO_3 (121 mg) and the reaction mixture was stirred at room temperature for 10 min, followed by addition of CH_3I (0.11 mL). The reaction mixture was heated and stirred at 80 ºC for 45 minutes. The solution was cooled to room temperature and diluted with H_2O (100 mL), followed by extraction with Et_2O (3 × 100 mL). The organic phase was washed with H_2O (3 × 30 mL), dried over Na_2SO_4 and concentrated to obtain the crude product as a red-orange solid (63 mg).

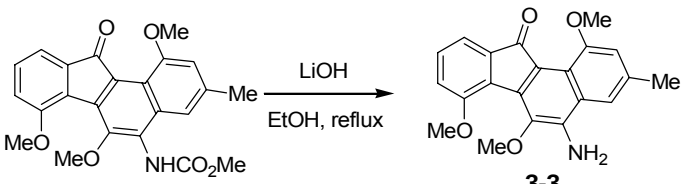
To a solution of the above crude product (63 mg) and K_2CO_3 (37 mg) in DMSO (25 ml) at 0 ºC was added slowly 30% H_2O_2 (5 mL). The solution was warmed to room temperature and stirred for 24 h. The reaction mixture was diluted with H_2O (100 mL) and then extracted with EtOAc (3 × 100 mL). The EtOAc phase was washed with H_2O (3 × 30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 2:1, v/v) to obtain the title compound as a red orange solid (34 mg, 51% for three steps). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.78 (s, 1H), 7.35 (m, 1H), 7.26 (d, J = 8.2 Hz, 1H), 7.15 (d, J = 6.8 Hz, 1H), 7.00 (s, 1H), 6.83 (s, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.70 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 190.4, 168.5, 156.2, 154.4, 149.5, 140.1, 138.0, 137.3, 135.8, 133.6, 131.8, 131.6, 126.9, 120.2, 118.4, 116.5, 116.4, 110.1, 63.5, 56.1, 55.6, 22.2; IR (film): 3425.0, 3337.5, 2937.5, 2837.5, 1706.3, 1670.9, 1605.8, 1554.6, 1483.8, 1355.5, 1266.3, 1047.6 cm^{-1}; MS (EI): *m/z* 377.2 (100, M^+); HRMS (EI): Calc for $C_{22}H_{19}NO_5$: 377.1263, found: 377.1268.



3-90 **3-95**

Methyl 1, 6, 7-trimethoxy-3-methyl-11-oxo-11*H*-benzo[*a*]fluoren-5-ylcarbamate (3-95)

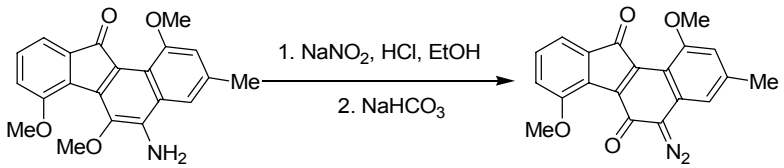
To a solution of compound **3-90** (24 mg, 0.065 mmol) and KOH (9 mg, 0.16 mmol) in anhydrous methanol (14 mL) at 0 ºC was added $PhI(OAc)_2$ (21 mg, 0.065 mmol). The reaction mixture was stirred for 30 minutes at 0 ºC, then warmed to rt and stirred for 3 h. The solution was diluted with EtOAc (150 mL) and washed with brine (3 x 15 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:1, v/v) to obtain the title compound as a red orange solid (26 mg, 99%). ^{1}H NMR (300MHz, CD_2Cl_2): δ 7.24 (m, 2H), 7.12 (s, 1H), 7.06 (d, J = 7.9 Hz, 1H), 6.82 (s, 1H), 6.66 (s, 1H), 3.94 (s, 6H), 3.78 (s, 3H), 3.73 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (75.5 MHz, CD_2Cl_2): δ 190.3, 156.3, 155.6, 153.9, 149.0, 139.8, 137.6, 136.4, 133.7, 132.1, 130.7, 130.5, 127. 3, 118.9, 118.6, 116.1, 114.8, 109.6, 61.8, 56.0, 55.6, 52.7, 21.8; IR (film): 3304.4, 2939.3, 1706.2, 1605.5, 1559.8, 1483.9, 1273.5, 1045.8 cm^{-1}; MS (EI): *m/z* 407.1 (100, M^+), 375.1 (15); HRMS (EI): calculated for $C_{23}H_{21}NO_6$: 407.1369, found: 407.1377.



3-95 **3-3**

5-Amino-1,6,7-trimethoxy-3-methyl-11*H*-benzo[*a*]fluoren-11-one (3-3)

To a solution of compound **3-95** (26 mg, 0.065 mmol) in ethanol (16 mL) was added aqueous LiOH solution (4 M, 0.16 mL, 0.65 mmol) and the mixture was refluxed for 24 h. The solution was concentrated to ca. 5 mL and then diluted with CH_2Cl_2 (100 mL). The resulting solution was washed with H_2O (3 × 10 mL), and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:1, v/v) to obtain the title compound as a dark red solid (23 mg, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (s, 1H), 7.26 (m, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.02 (d, J = 6.9 Hz, 1H), 6.75 (m, br, 3H), 3.88 (s, 3H), 3.82 (s, 3H), 3.62 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 187.5, 156.6, 154.2, 146.1, 140.8, 138.9, 136.8, 134.6, 131.3, 126.6, 123.8, 120.7, 118.7, 115.9, 115.1, 114.6, 110.7, 61.1, 56.6, 55.8, 22.0; IR (film): 3394.6, 1627.8, 1513.1, 1457.0, 1269.9 cm^{-1}; MS (EI): *m/z* 349.1 (100, M^+), 334.1 (27), 319.1 (18); HRMS (EI): calculated for $C_{21}H_{19}NO_4$: 349.1314, found: 349.1323.



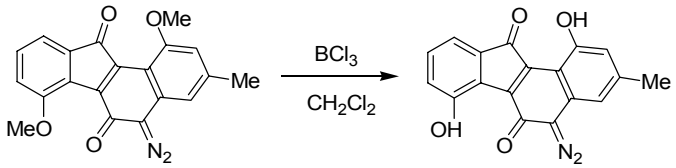
3-3 **3-2**

Dimethylisoprekinamycin (3-2)

To a solution of compound **3-3** (15 mg, 0.044 mmol) in ethanol and water (5:1, v/v, 12 mL) at 0 ºC was added dilute HCl (1.2 M, 0.91 mL) slowly and the mixture was stirred for 10 minutes. An aqueous solution of $NaNO_2$ (4 mg/0.044 mmol in 0.5 mL) was added and the reaction was stirred at 0 ºC for 1 h, followed by addition of aqueous $NaHCO_3$ solution (150 mg in 2.0 mL), and the mixture was stirred at 0 ºC for additional 20 minutes. The reaction mixture was diluted with EtOAc (150 mL) and washed with H_2O (3 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:2, v/v) to obtain the title compound as a dark purple solid (10 mg, 63%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ 7.33 (m, 1H), 7.27 (d, J = 6.5 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.86 (s, 1H), 6.69 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125.8 MHz, CD_2Cl_2)*: δ 192.2, 171.2, 158.3, 154.4, 142.1, 140.9, 139.2, 133.9, 130.9, 130.4, 127.4, 120.5, 116.7, 112.5, 110.7, 109.1, 56.7, 55.8, 21.7; IR (CH_2Cl_2 solution): 2996.6, 2926.9, 2852.8, 2112.6, 1722.3, 1608.6, 1563.4, 1483.9, 1468.0, 1361.4 cm^{-1}; MS (EI): *m/z* 346.1 (78, M^+), 318.1 (100), 303.1 (97), 275.1 (27), 247.1 (22), 219.1 (15), 189.1 (23), 187.1 (12); HRMS (EI): calculated for $C_{20}H_{14}N_2O_4$: 346.0954, found: 346.0953.

*As the result of low solubility of the title compound in CD_2Cl_2, no ^{13}C NMR signal was observed for the quaternary carbon attached to the diazo group. A cross peak at 88.0 ppm,

assignable to the carbon atom attached diazo group, is clear evidence, however, in the HMBC experiment.



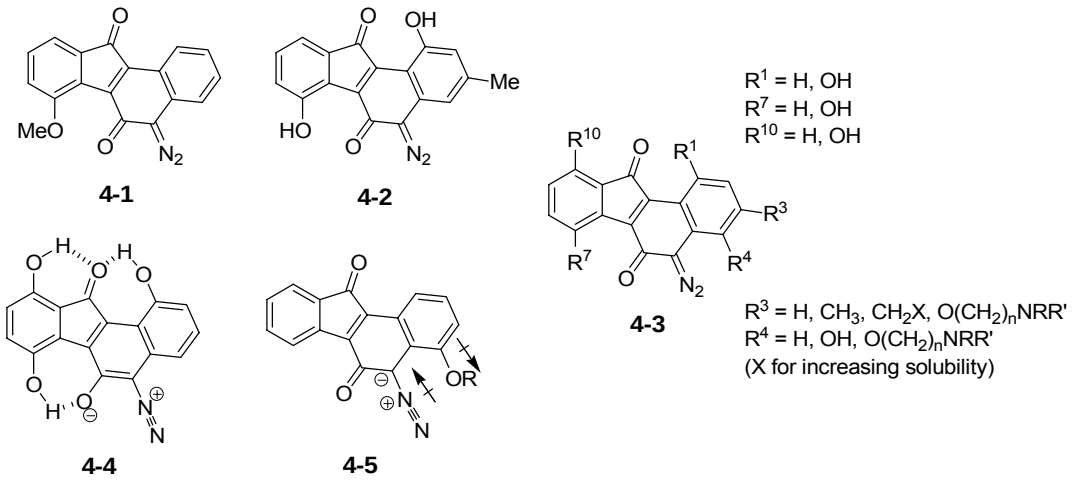
3-2 **3-1**

Isoprekinamycin (3-1)

To a solution of compound **3-2** (8 mg, 0.022 mmol) in anhydrous CH_2Cl_2 (2 mL) at –78 °C was added slowly a CH_2Cl_2 solution of BCl_3 (1 M, 0.09 mL, 0.09 mmol), and the reaction mixture was stirred for 30 minutes. The reaction mixture was warmed to rt and further stirred for 90 minutes, followed by addition of ice (1 g) and CH_2Cl_2 (100 mL). The CH_2Cl_2 solution was washed with water (3 × 10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (Et_2O:CH_2Cl_2 = 1:200, v/v) to obtain the title compound as a dark purple solid (5 mg, 64%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ 12.32 (s, 1H), 11.61 (s, 1H), 7.26 (d, J = 6.7 Hz, 1H), 7.1 8 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 6.72 (s, 1H), 6.69 (s, 1H), 2.41 (s, 1H); IR (CH_2Cl_2 solution): 3686.1, 3600.7, 2926.6, 2855.0, 2126.1, 1733.9, 1686.8, 1611.5, 1560.5, 1466.5 cm^{-1}; UV-vis (HPLC): 211.0, 247.4, 284.0, 561.3 nm.

Chapter 4 Design and Synthesis of Analogues of Isoprekinamycin

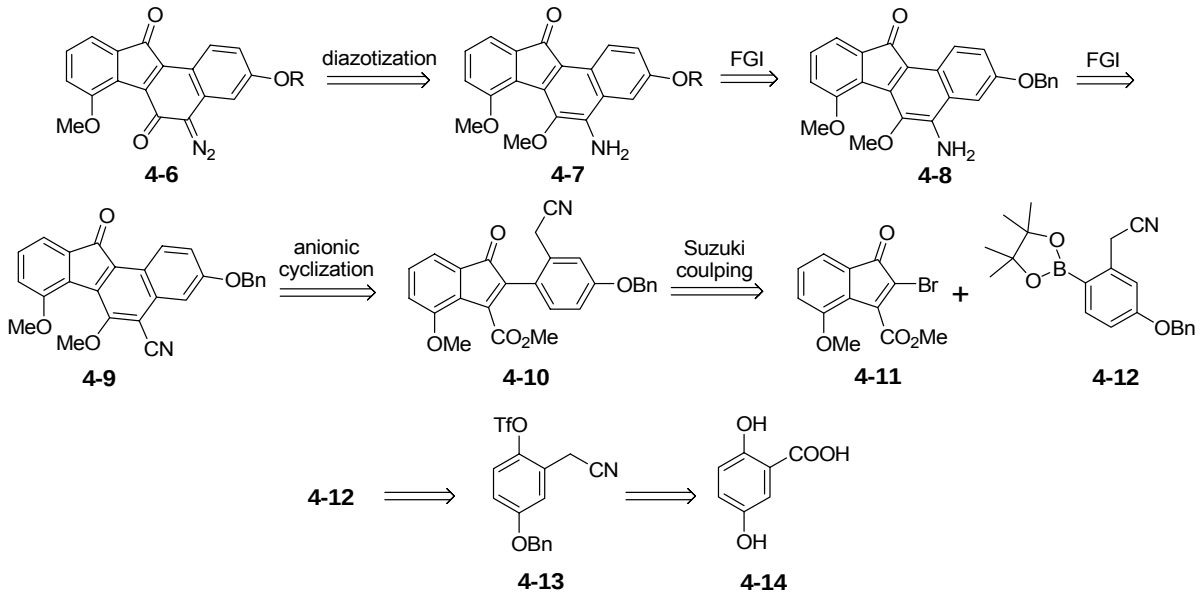
The general methodology that had been established for the synthesis of diazobenzo[*a*]fluorenes systems, including the model compound **4-1** and isoprekinamycin (**4-2**), allowed the design of analogues of isoprekinamycin. Functionalities that were considered for analoging of the core structure of **4-3** included OH groups, for example, which could be introduced at C_1, C_7, or C_{10} in order to increase the diazonium ion character of the diazo group as a result of intramolecular hydrogen bonding. The solubility of analogues might also be improved with the introduction of polar groups such as carboxylic acids, amides, or amines in a side chain, at the R^3 or R^4 positions of **4-3**. An amino group could also possibly increase the affinity of the analogues for DNA. The introduction of a polar group such as a phenol at C_4 could potentially increase the diazonium ion characteristics of the diazo group in the analogues because of the dipole-dipole interactions as shown in **4-5**. Ab initio molecular orbital calculations by R.S. Laufer in this group support the existence of such an interaction.[1] Thus a study of the design and synthesis of the analogues was initiated in this laboratory, and is discussed in this and the following chapters. It was hoped that this work would open the door for further exploration of the structure and activity relationship of these compounds and would lead to more clues concerning the mode of action of isoprekinamycin and the related kinamycins and lomaiviticins.



4.1 Studies Toward the Synthesis of the Analogues from the Corresponding Phenol Precursor

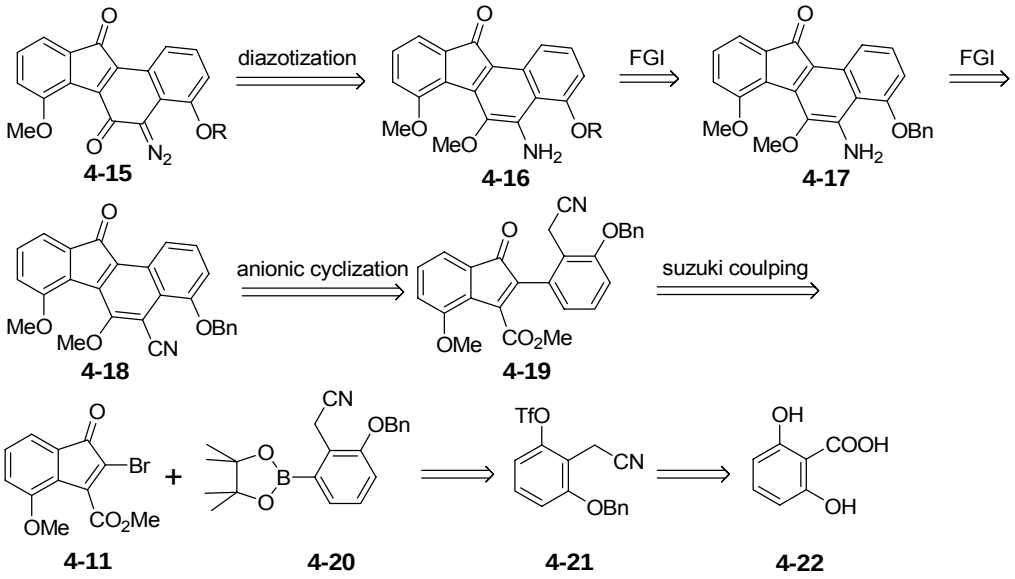
It was felt that one series of analogues might be constructed by *O*-alkylation of a phenol group present at C_3 of the D ring (e.g. **4-6**, R = H). Retrosynthetic reasoning suggested that such system might be prepared from the aniline **4-7** via demethylative diazotization. The aniline **4-7** might be transformed from the corresponding benzyl group protected phenol ether **4-8**, which might be obtained from benzo[*a*]fluorene **4-9**. The intermediate **4-9** might be formed via anionic cyclization of diaryl compound **4-10**, which could be provided by the Suzuki coupling between bromoindenone **4-11** and pinacolboronate **4-12**. The boronate **4-12** might be formed from the triflate **4-13**, which might be synthesized from the commercially available 2,5-dihydroxybenzoic acid **4-14**. (Scheme 4-1)

Scheme 4-1



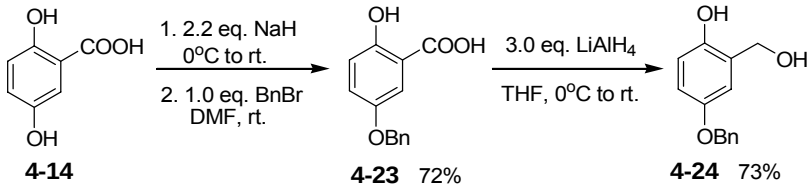
Another analogue of interest was **4-15**, in which a side chain OR is present at C_4. This might be synthesized by a sequence similar to **4-6** as presented in Scheme 4-2. The synthesis might start from the isomer of **4-14**, 2,6-dihydroxybenzoic acid **4-22**, which is also commercially available.

Scheme 4-2



Salvadori and coworkers reported that treatment of 2,5-dihydroxybenzoic acid **4-14** with 2.2 equivalents of sodium hydride, followed by treatment of one equivalent of benzyl bromide, provided mono-benzyl protected phenol ether **4-23** regioslectively.[2] In this laboratory, this procedure was duplicated to convert **4-14** to **4-23** in 72% yield, which was reduced by three equivalents of lithium aluminium hydride in THF from 0 °C to room temperature to give the corresponding benzyl alcohol **4-24** in 73%

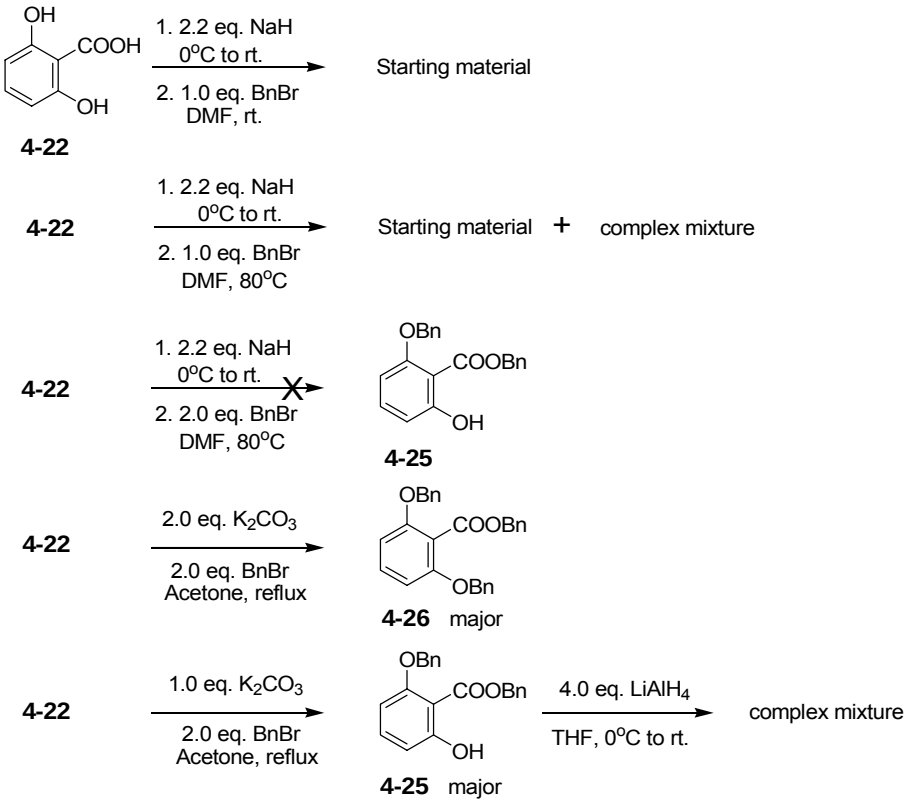
Scheme 4-3



Unfortunately, Salvadori's procedure was unsuccessful for the analogous transformation with 2,6-dihydroxybenzoic acid **4-22**, and no obvious reaction occurred under these

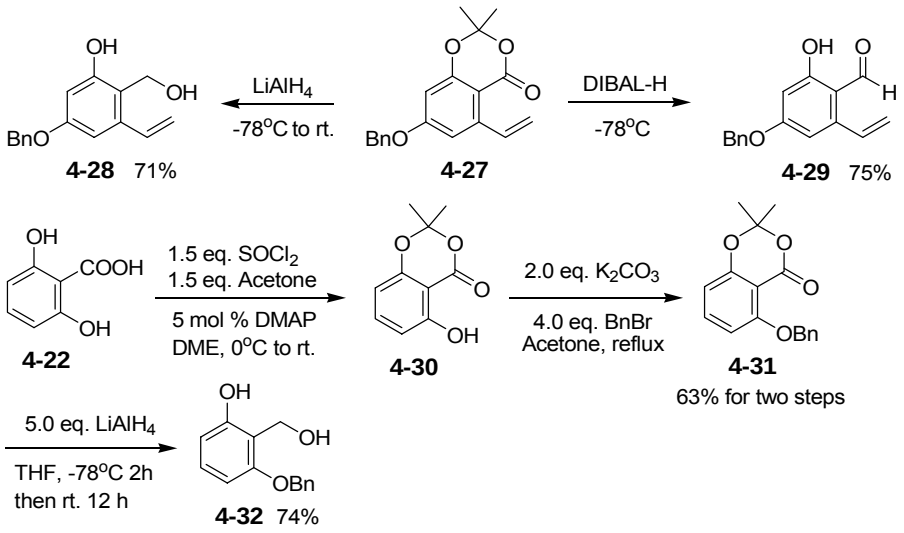
conditions. Increasing the temperature of the reaction from room temperature to 80 °C provided a complex mixture with small amounts of starting material. Increasing the amount of BnBr to two equivalents did not lead to the desired dibenzyl protected product **4-25**. Treatment of **4-22** with two equivalents of K_2CO_3 and two equivalents of benzyl bromide in refluxing acetone led to a tribenzyl-protected product **4-26** as the major product. However, decreasing the amount of K_2CO_3 to one equivalent provided the desired dibenzyl protected product **4-25**. Unfortunately, attempted reduction using four equivalents of lithium aluminium hydride in THF from 0 °C to room temperature afforded a complex mixture.

Scheme 4-4



Jennings and coworkers developed a general method which allowed for the synthesis of either substituted salicylaldehydes or the corresponding 2-hydroxybenzyl alcohols upon treatment of the 2,2-dimethyl-1,3-benzo-dioxan-4-one functional group with DIBAL-H or LAH, respectively.[3] Treatment of 2,6-dihydroxybenzoic acid **4-22** with 1.5 equivalents of $SOCl_2$ and acetone in the presence of 5 mol% of DMAP in DME from 0 °C to room temperature afforded the 5-hydroxy-2,2-dimethyl-1,3-benzo-dioxan-4-one **4-30**, which was protected with benzyl bromide to provide benzyl protected phenol ether **4-31** in 63% yield overall. Then **4-31** was reduced smoothly to the corresponding benzyl alcohol **4-32** in 74% yield, which was somewhat higher than that in the report by Jennings. (Scheme 4-5)

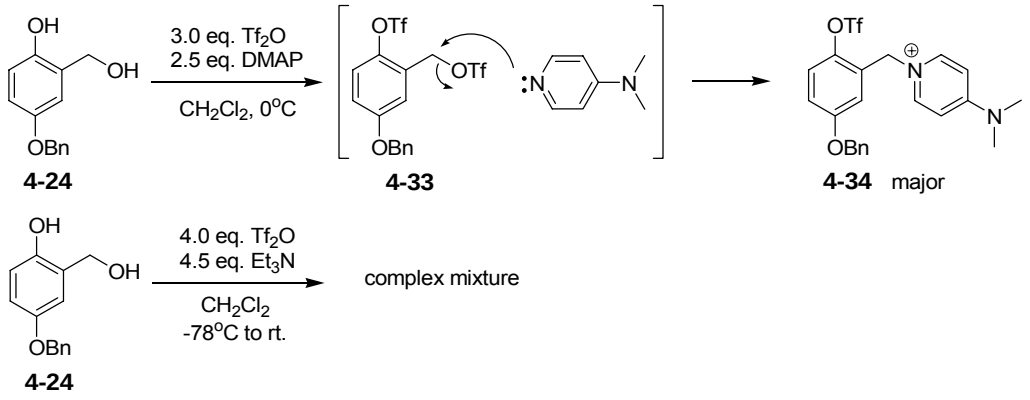
Scheme 4-5



Treatment of diol **4-24** with three equivalents of triflic anhydride in the presence of 2.5 equivalents of 4-dimethylaminopyridine (DMAP) in methylene chloride at 0 °C provided the quaternary ammonium salt **4-34,** suggesting that the desired ditriflate **4-33** was unstable and

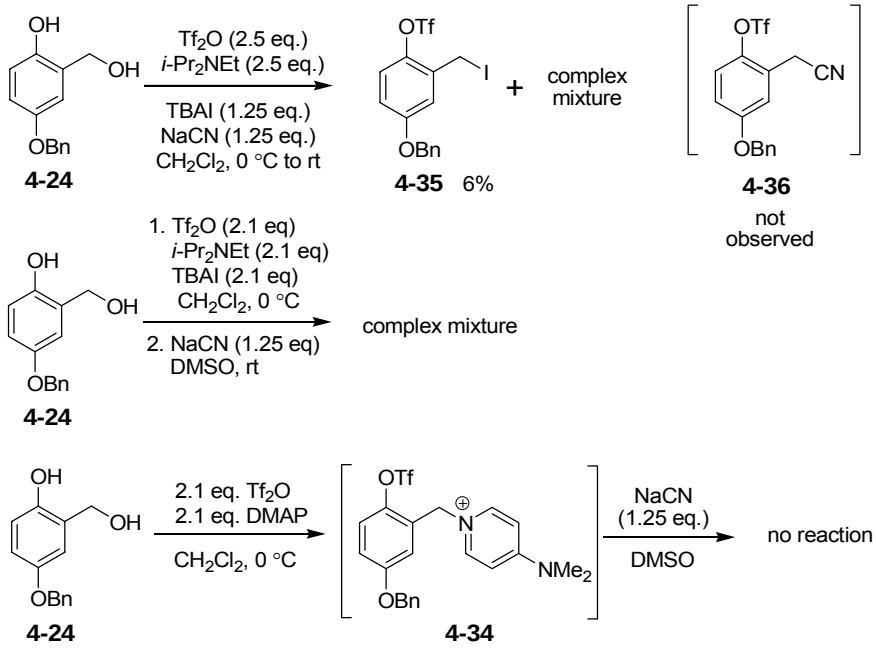
underwent substitution by DMAP. Replacement of the base of this reaction with triethylamine showed no improvement and generated a complex mixture.

Scheme 4-6



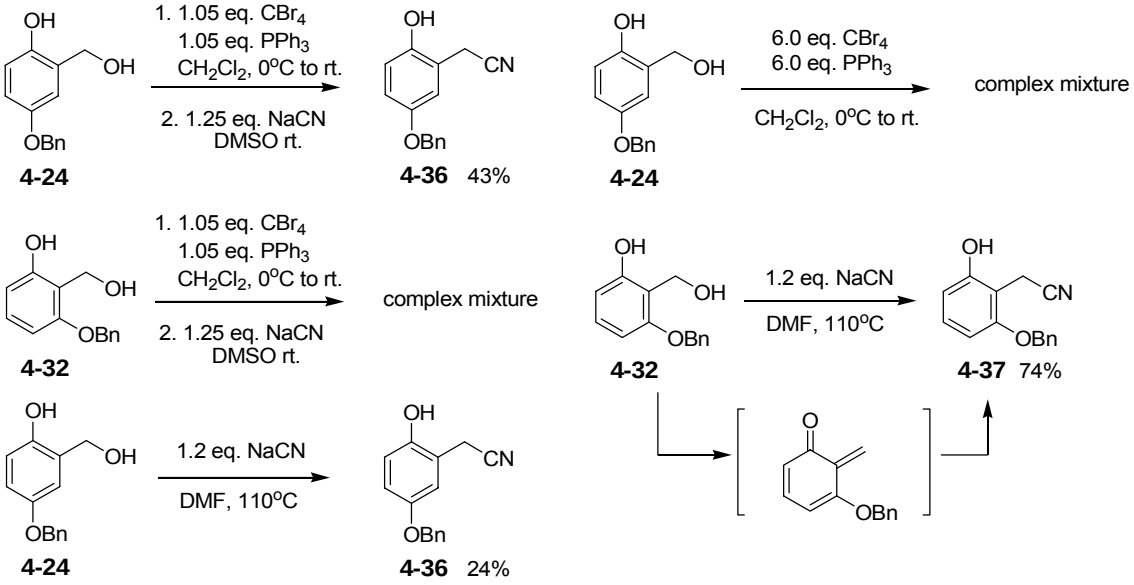
Treatment of diol **4-24** with 2.5 equivalents of triflic anhydride and diisopropylethylamine (DIPEA) in the presence of 1.25 equivalents of tetrabutylammonium iodide (TBAI) and sodium cyanide in methylene chloride at 0 °C generated benzyl iodide **4-35** in 6% yield from a complex mixture, but none of the desired product **4-36** was observed. This was possibly the result of the low stability of **4-35** and low solubility of NaCN in CH_2Cl_2. Attempts were then made to achieve the transformation stepwise, such that the triflate could be prepared in CH_2Cl_2 and NaCN could be used in DMSO in the second step, but this also resulted in a complex mixture. Since the instability of the benzyl triflate intermediate was thought to be problematic, the conversion of the diol **4-24** to the salt **4-34** was considered since DMAP could also potentially act as a leaving group. The salt **4-34** was treated with NaCN in DMSO, but no obvious substitution occurred over 24 hours. (Scheme 4-7)

Scheme 4-7



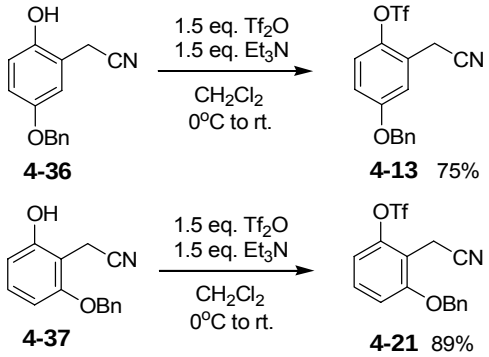
Since it was difficult to obtain the ditriflate from diol **4-24**, the substitution was investigated from the phenol **4-36**. The alcohol **4-24** was treated with 1.05 equivalents of CBr_4 and PPh_3 in CH_2Cl_2 from 0 °C to room temperature to generate benzyl bromide as an intermediate. Conversion to the corresponding phenyl acetonitrile **4-36** was accomplished using 1.25 equivalents of NaCN in DMSO in 43% overall yield. However, increasing the amount of CBr_4 and PPh_3 did not improve the bromination and led to a complex mixture. Unfortunately, this procedure was unsuccessful with the diol **4-32**. Schwartz and coworkers, however, reported the conversion of phenolic benzyl alcohols to the corresponding phenolic phenylacetonitriles by direct treatment with cyanide ion, presumably by way of a quinone methide intermediate.[4] This method proved successful for diol **4-32** and the corresponding phenylacetonitrile **4-37** was obtained in 74% yield by using 1.2 equivalents of NaCN in DMF at 110 °C. The yield of the reaction was much poorer with its isomer **4-24** (24%).

Scheme 4-8



The phenols **4-36** or **4-37** could then be protected with 1.5 equivalents of triflic anhydride in the presence of triethylamine in CH_2Cl_2 from 0 °C to room temperature to provide the triflates **4-13** and **4-21** in 75% and 89% yield, respectively (Scheme 4-9).

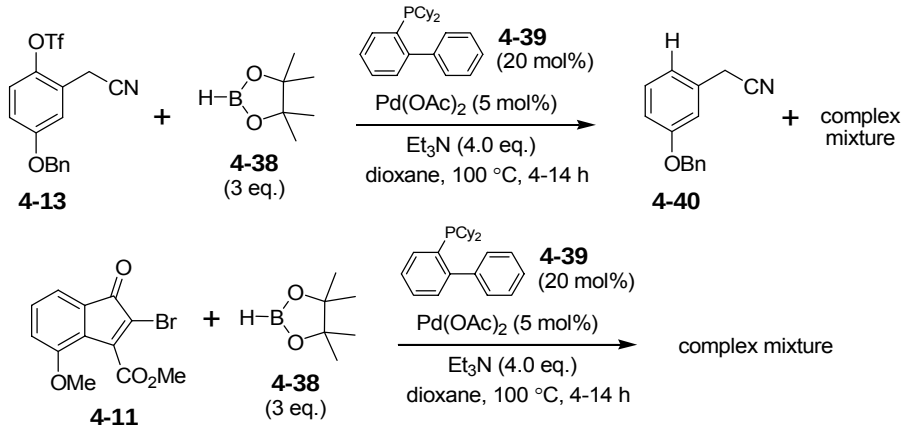
Scheme 4-9



Unfortunately, the palladium-catalyzed borylation of triflates **4-13** and **4-21** did not proceed well. Treatment of **4-13** with 3 equivalents of pinacolborane (**4-38)**, 4.0 equivalents of Et_3N, and $Pd(OAc)_2$ as a catalyst, in the presence of the 20 mol% of biphenylphosphine

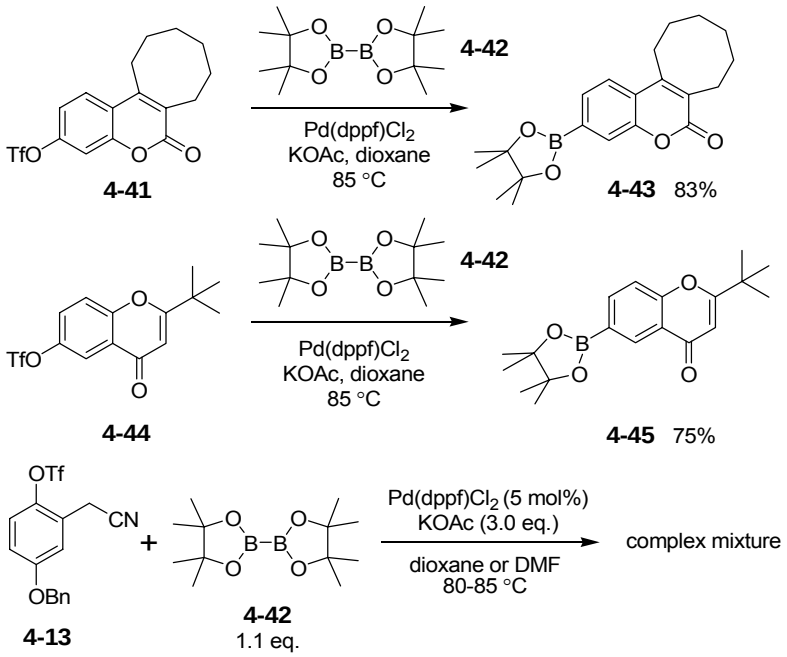
ligand **4-39** in dioxane at 110 °C for four hours led to hydrogenated product **4-40** along with starting material and a complex mixture. Increasing the reaction time improved the formation of **4-40** according to the ^{1}H NMR of the crude product. Furthermore, the borylation of the bromoindenone **4-11** under these conditions was also unsuccessful, but this is not surprising in retrospect since similar reactions are known to be ineffective with substrates that possess strong electron-withdrawing groups.[5]

Scheme 4-10



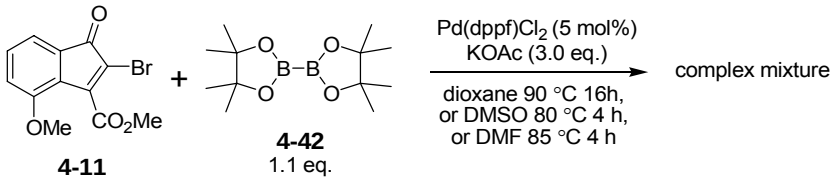
Difficulties with the borylation of triflates **4-41** and **4-44** was reported by Taylor et al. with pinacolborane (**4-38**), but conversion to the corresponding pinacol boronates occurred smoothly with the use of diborane **4-42**.[6,7] However, treatment of **4-13** with 1.1 equivalents of **4-42** and three equivalents of KOAc in the presence of 5 mol% of Pd(dppf)Cl_2 in dioxane at 85 °C for 24 hours generated a complex mixture that did not include the desired product. The use of DMF instead of dioxane was also unproductive.

Scheme 4-11



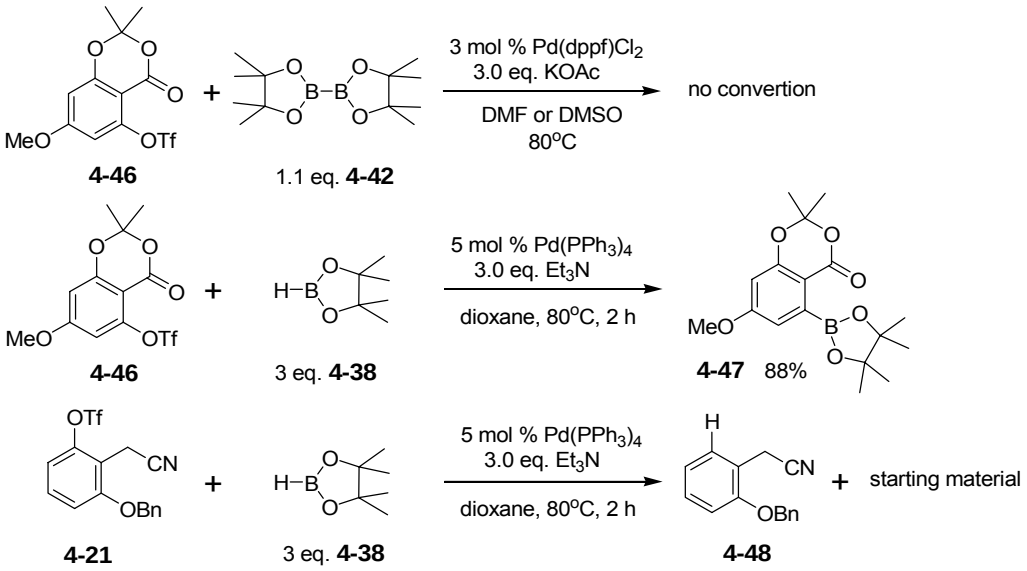
It has been reported that the cross-coupling reaction between haloarenes and **4-42** can be enhanced by electron-withdrawing substituents sufficiently so as to permit the use of less reactive aryl bromides.[6] However, treatment of bromoindenone **4-11** with 1.1 equivalents of **4-42** and 3.0 equivalents of KOAc in the presence of 5 mol% of $Pd(dppf)Cl_2$ in dioxane at 90 °C over 16 hours provided a complex mixture without the desired product. The reaction was also attempted in DMSO and DMF since polar solvents have been known to enhance the rate of borylation reactions,[6] but the desired product was not observed in the resulting complex mixtures.

Scheme 4-12



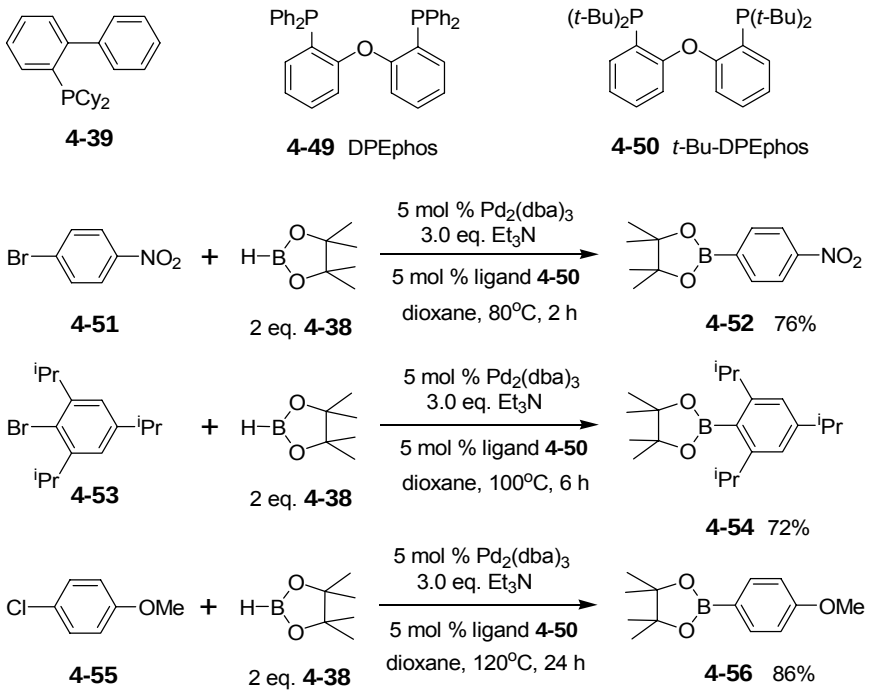
In contrast to that described above for the borylations of **4-41** and **4-44**, Joachim and coworkers reported that no reaction was observed upon treatment of triflate **4-46** with diborane **4-42** (1.1 eq. **4-42**, 3 eq. KOAc, 3 mol% $Pd(dppf)Cl_2$ in DMF or DMSO at 80 °C), but the cross-coupling was achieved in 88% yield when using pinacol borane **4-38** (3 eq. pinacol borane, 3 eq. Et_3N, 5 mol% $Pd(PPh_3)_4$, dioxane, 80 °C, 2 h).[8] Unfortunately, attempts in our laboratory with **4-21** led to the hydrogenated product **4-48** rather than the desired pinacol boronate. (Scheme 4-13)

Scheme 4-13



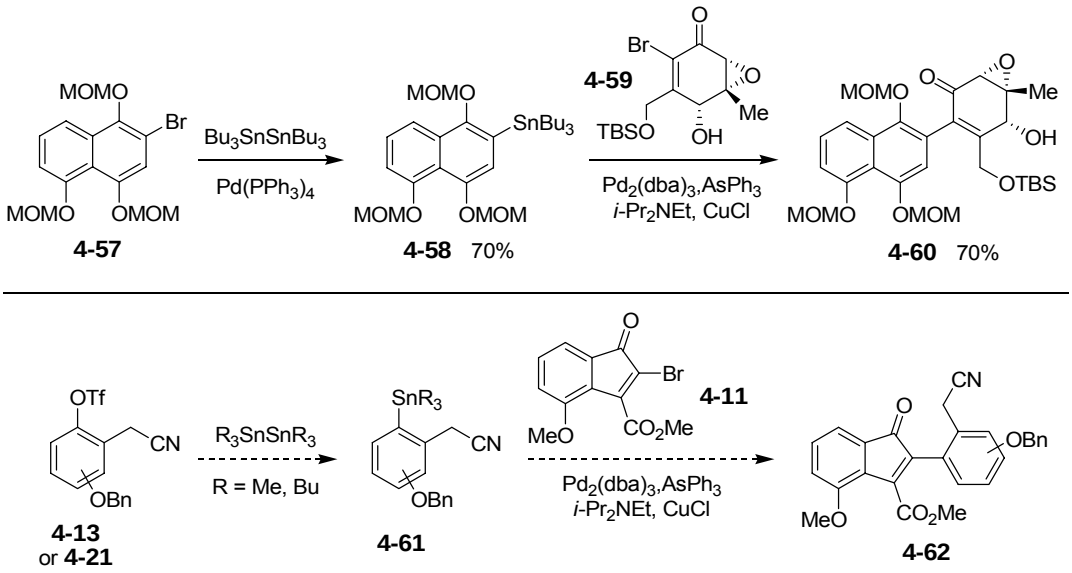
The Buchwald phosphine ligand[9] **4-39** and DPEphos[10] **4-49** have been used as efficient supporting ligands for the palladium-catalyzed borylation of aryl bromides. Although the scope of the aryl halides in the palladium-catalyzed borylation has been improved, these catalyst systems still have some drawbacks. For example, reactions of electron-deficient or *ortho, ortho'*-substituted bromides produce relatively poor yields, and no examples of the borylation of aryl chlorides have been provided. Murata and coworkers developed a catalyst system using $Pd_2(dba)_3$ and the electron-rich bis-phosphine ligand bis(2-di-*tert*-butylphosphinophenyl)ether **4-50**, which overcame many limitations of the previous methods. It is felt that this system could possibly be used for the borylation of triflate **4-13** and **4-21** or even the bromoindenone **4-11**, but these reactions have not yet been explored in this laboratory because of time limitations. (Scheme 4-14)

Scheme 4-14



Porco and coworkers reported[11] that stannylation of aryl bromide **4-57** provided the arylstannane **4-58**,[12] which was subjected to the cross-coupling with vinyl bromide **4-59** to generate **4-60**.[13] Thus, the triflate **4-13** or **4-21** could potentially be converted to the corresponding arylstannane **4-61**, and **4-61** might be suitable for the cross-coupling with bromoindenone **4-11** to produce the diaryl **4-62**. While this strategy was acknowledged as a potential solution, an alternative approach was considered (vide infra). (Scheme 4-15)

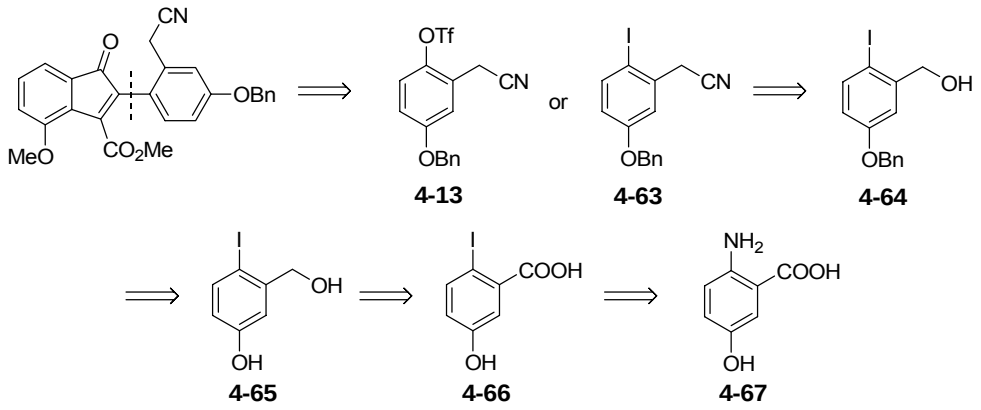
Scheme 4-15



4.2 Studies Toward the Synthesis of Analogues from an Aniline Precursor

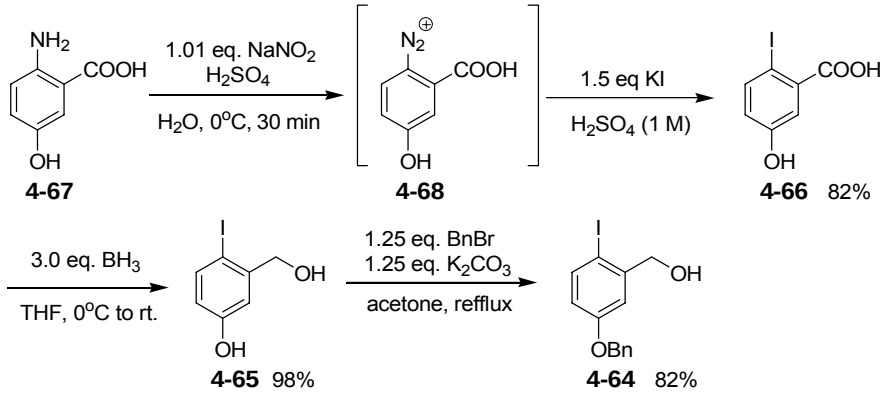
Since the borylation of triflate **4-13** proved difficult, the synthesis of the same synthon but in the form of more active iodophenylacetonitrile **4-63** was considered (Scheme 4-16). Thus, it was felt that **4-63** might be formed from the corresponding iodobenzyl alcohol **4-64** by substitution, and that **4-64** might be achieved by selective protection of the phenol group of diol **4-65**, which might come from the iodobenzoic acid **4-66** by reduction. The acid **4-66** might be produced from the commercially available 2-amino-5-hydroxybenzoic acid **4-67**.

Scheme 4-16



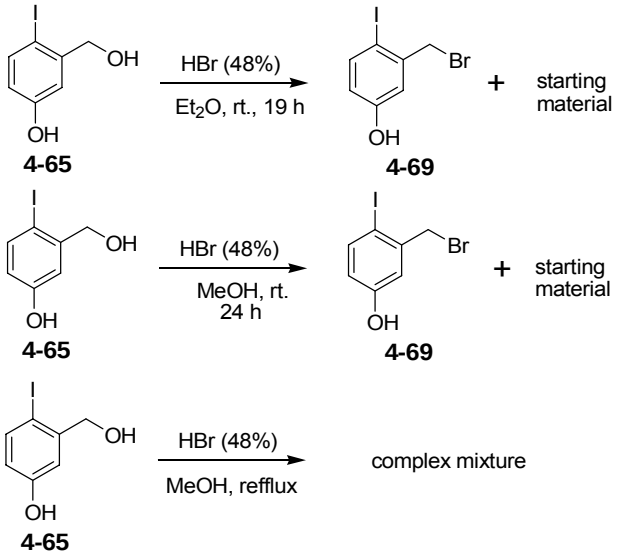
In practice, treatment of 2-amino-5-hydroxybenzoic acid (**4-67)** with 1.01 equivalents of sodium nitrite and concentrated H_2SO_4 in water at 0 °C for 30 minutes generated diazonium salt **4-68**, which was subjected to the substitution with 1.5 equivalents of potassium iodide and 1 M H_2SO_4 at 100 °C for one hour to produce the aryl iodide **4-66** in 82% yield.[14] The benzoic acid **4-66** was then reduced with borane in THF to give the diol **4-65**,[15] and the phenolic group of the diol was selectively protected with benzyl bromide to afford the iodobenzyl alcohol **4-64** in 82% yield.

Scheme 4-17



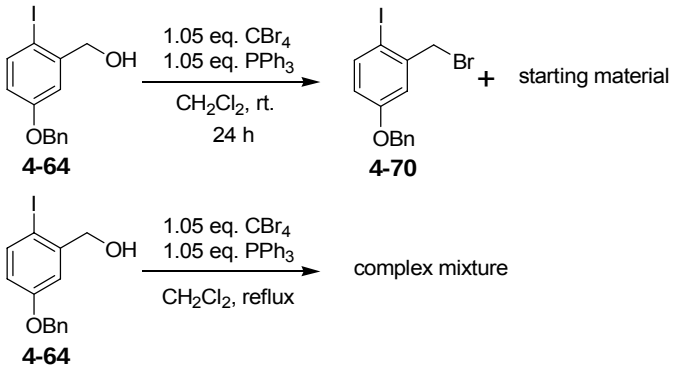
All attempts to convert the **4-65** to the benzylic bromide **4-69** with 48% aqueous HBr failed. The reaction in ether at room temperature over 19 hours led to a small amount of the desired bromo compound **4-69,** but much starting material remained. It was considered that the reaction was slow possibly because of the two-phase system. However, replacement of the ether by methanol to give a one-phase system made no difference. Elevating the temperature led to a complex mixture.

Scheme 4-18



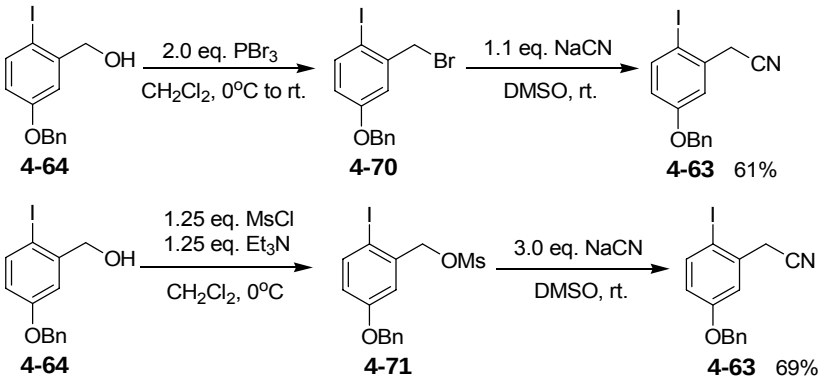
It was found that treatment of benzyl alcohol **4-64** with 1.05 equivalents of CBr_4 and PPh_3 in CH_2Cl_2 at room temperature for one day led to a low ratio of conversion from **4-64** to desired bromo product **4-70**. Meanwhile, increasing the temperature led to a more complicated mixture.

Scheme 4-19



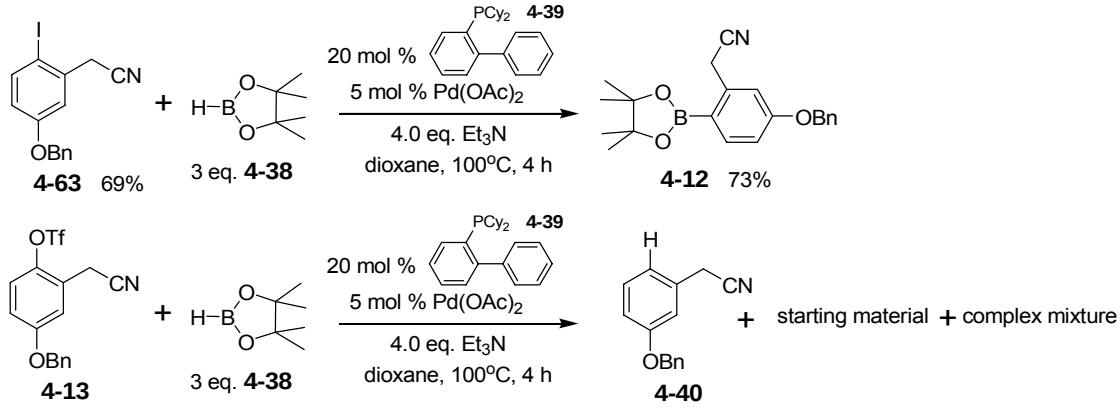
An alternative procedure[16] for conversion of the alcohol to the bromide using PBr_3 was investigated after all previous failure. Treatment of the iodobenzyl alcohol **4-64** with two equivalents of PBr_3 in anhydrous methylene chloride from 0 °C to room temperature over night provided benzyl bromide **4-70**, which was subjected directly to the substitution by NaCN to generate the desired iodophenyl acetonitrile **4-63** in 64% yield over all. This reaction was not perfect, partially because of the instability of the bromo intermediate **4-70**. Meanwhile, treatment of **4-64** with 1.25 equivalents of mesyl chloride and triethylamine in CH_2Cl_2 at 0 °C provided the corresponding mesylate **4-71**, which was unexpectedly stable and was substituted by NaCN smoothly in DMSO in 69% yield overall.

Scheme 4-20



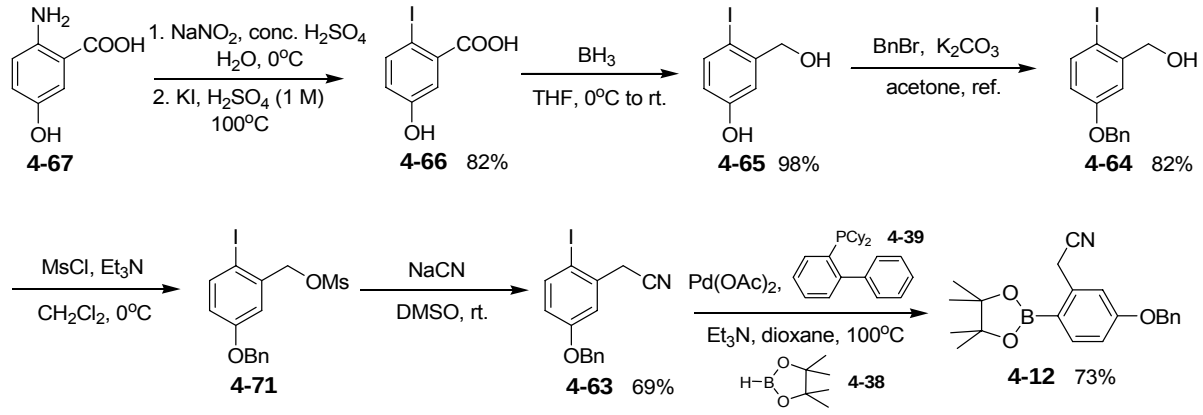
Treatment of iodophenylacetonitrile **4-63** with three equivalents of pinacol borane **4-38** and four equivalents of triethylamine and 20 mol% of ligand **4-39** in the presence of 5 mol % of $Pd(OAc)_2$ in dioxane for 4 hours provided the desired pinacol boronate **4-12** in 73% yield. However the same procedure did not work for the triflate **4-13** possibly because of a lower activity of the triflate **4-13** than that of the iodo compound **4-63**.

Scheme 4-21



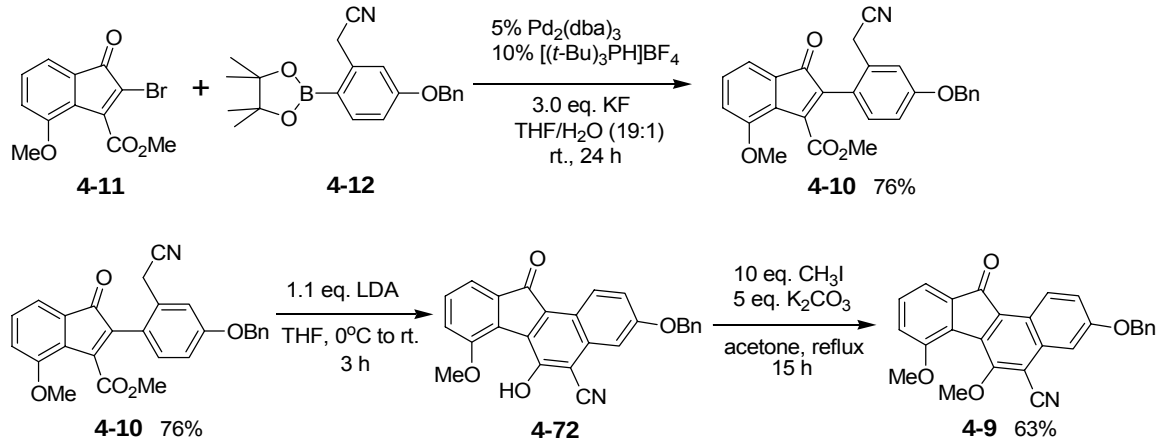
Hence, the synthesis of the D ring building block pinacol boronate **4-12** initiated from 2-amino-5-hydroxybenzoic acid **4-67**, following the sequence as shown in Scheme 4-22 was achieved in six steps in 33% yield overall.

Scheme 4-22



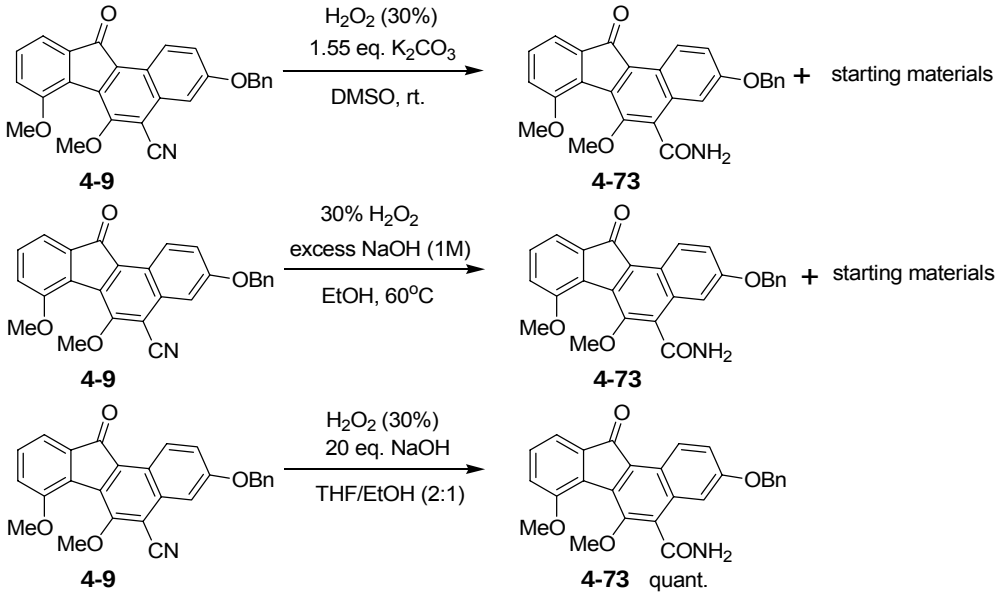
With the AB ring and D ring building blocks **4-11** and **4-12** of the analogue of isoprekinamycin in hand, the Suzuki cross coupling between them using the same conditions as before provided the diaryl **4-10** compound in 76% yield. Without any group at C_1 of the D ring in **4-10**, the cyclization of it with 1.1 equivalents of LDA in THF from 0 °C to room temperature for three hours afforded the desired phenol **4-72** smoothly without competition from the aldol reaction. The product was methylated to generate the carbonitrile **4-9** in 63% yield over two steps. Here the solvent of the methylation was changed from DMF to acetone without affecting the yield. (Scheme 4-23)

Scheme 4-23



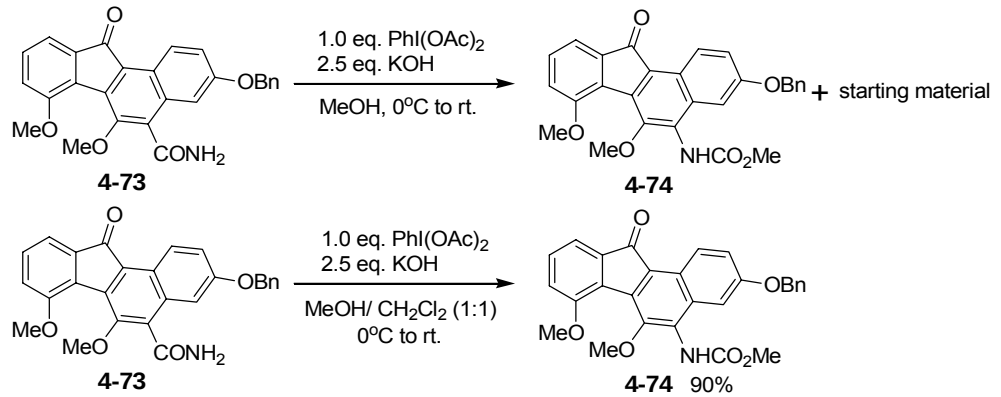
Hydrolysis of carbonitrile **4-9** with 30% aqueous hydrogen peroxide and 1.55 equivalents of potassium carbonate in DMSO, as used in the synthesis of IPK, did not proceed well in this case. Since the conversion of **4-9** to the desired carboxamide **4-73** was low, it was thought that the low solubility of the starting materials in DMSO was the major cause. Conducting the reaction in ethanol and increasing the temperature led to no improvement, but it was found that treatment of **4-9** with excess sodium hydroxide in the presence of the 30% H_2O_2 in a 2:1 mixture of THF and ethanol was effective, and afforded **4-73** quantitatively. (Scheme 4-24)

Scheme 4-24



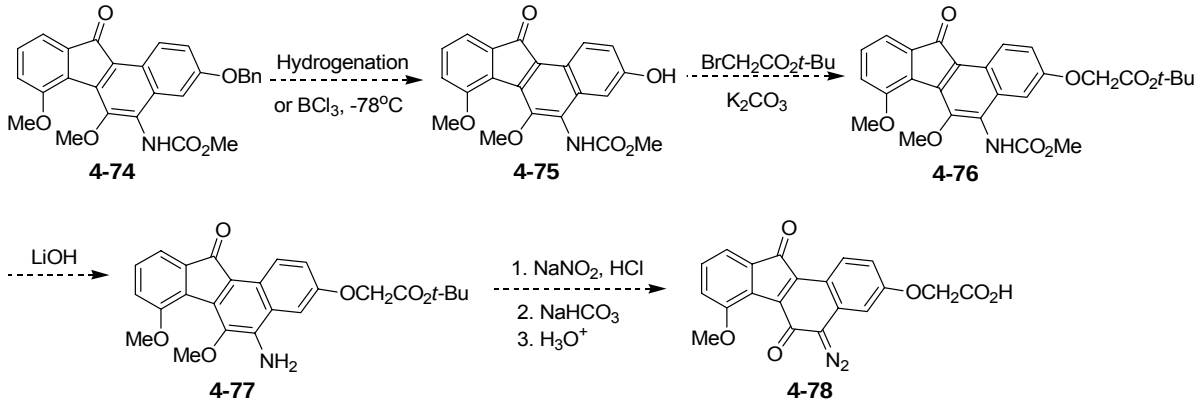
Solubility of the starting materials was also problematic in the modified Hofmann rearrangement of **4-73**, in which one equivalent of $PhI(OAc)_2$ and 2.5 equivalents of KOH was used in methanol. Since **4-73** seemed to have low solubility in methanol, the reaction was attempted with a 1:1 mixture of methanol and methylene chloride, and the desired carbamate **4-74** was obtained in 90% yield.

Scheme 4-25



The synthesis of the analogues in which a group was introduced at C_3 of the D ring was stopped at this stage because of time limitations. However, the planned sequence for the synthesis to follow is shown in Scheme **4-26**. Cleavage of the benzyl group of **4-74** via hydrogenation or treatment with BCl_3 at low temperature would provide phenol **4-75**, which might be alkylated with $BrCH_2CO_2t$-Bu to generate ether **4-76**. Hydrolysis of **4-76** would afford corresponding aniline, which might be subjected to demethylative diazotization conditions that simultaneously cleave the *tert*-butyl group in situ. Thus, this represents a potential route for the synthesis of an analogue of isoprekinamycin such as **4-78**, which possesses a carboxylic acid-containing side chain at C_3.

Scheme 4-26



The acid group in **4-78** could then be used as a handle for the introduction of a variety of other functionalities, including those discussed at the beginning of this chapter.

4.3 Experimental

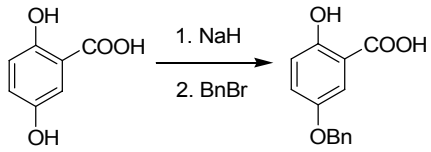
4.3.1 General information

^{1}H NMR spectra were recorded on a Brüker AVANCE 500 (500 MHz), Brüker AC 300 (300 MHz) or Brüker AVANCE 300 (300 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The following abbreviations are used for NMR peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplet; m, multiplet; b, broad; w, weak. ^{13}C NMR spectra were broad band decoupled and recorded on a Brüker AVANCE 500 (125.8 MHz), Brüker AC 300 (75.5 MHz) and Brüker AVANCE 300 (75.5 MHz) NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. HMQC and HMBC experiments were performed on a Brüker AVANCE 500 spectrometer. IR spectra were determined on a Perkin-Elmer RX I FT-IR spectrometer as KBr discs unless otherwise indicated. High/low resolution electron impact or electrospray ionization (EI or ESI) mass spectra (MS) were measured by the WATSPEC Mass Spectrometry Facility (Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada). Elemental analyses were performed by the M-H-W Laboratories (Phoenix, Arizona, USA).

Anhydrous THF and Et_2O were freshly distilled from sodium/benzophenone under nitrogen prior to use. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen prior to use. All commercial reagents were purchased from Aldrich Chemical Co., Strem Chemicals Inc., Alfa Aesar, Lancaster Synthesis Ltd. or BDH Inc. and were used as received unless otherwise indicated. Deionized water was supplied by a Biolab vertical series reverse osmosis system.

The –78 ºC and 0 ºC designations refer to solid carbon dioxide/acetone and ice/water slush respectively. The room temperature refers to 22 °C to 25 °C. Flash column chromatography was carried out using the Merck silica gel (230-400 mesh) and SiliCycle silica gel (60 Å). Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with Merck pre-coated silica gel plates (silica gel 60 F_{254} on aluminum sheet). All reported yields are isolated yields.

4.3.2 Detailed Experimental Procedures

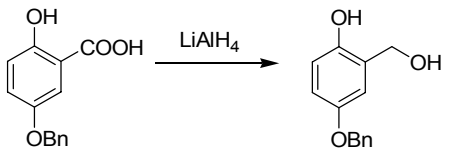


4-14 **4-23**

5-(Benzyloxy)-2-hydroxybenzoic acid (4-23)

To a suspension of NaH (0.528 g, 13.20 mmol) in DMF (5 mL) was added 2,5-dihydroxybezoic acid (0.924 g, 6.0 mmol) at 0 °C dropwise. The reaction was then warmed up to room temperature and stirring was continued at this temperature for another 2 hours. Benzyl bromide (0.71 mL, 6.0 mmol) in DMF (1.5 mL) was added, and the reaction was continued 23 hours at room temperature. Water (10 mL) was added at 0 °C and the pH value of the solution was adjusted to 1 with 1 M HCl. Then the resulting solution was extracted with Et_2O (3 × 100 mL) and the organic phase was washed with H_2O (3 × 30 mL). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by recrystallization from chloroform to obtain the title compound as a white solid (1.061 g,

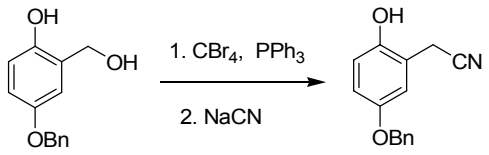
72%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.01 (s, 1H), 7.38 (m, 6H), 7.21 (m, 1H), 6.93 (d, J = 9.1 Hz, 1H), 5.03 (s, 2H).



4-23 **4-24**

4-(Benzyloxy)-2-(hydroxymethyl)phenol (4-24)

To a suspension of $LiAlH_4$ (0.494 g, 13.23 mmol) in anhydrous THF (30 mL) was added 5-(benzyloxy)-2-hydroxybenzoic acid (1.061 g, 4.34 mmol) slowly at 0 °C. The reaction was warmed up to room temperature and stirring was continued at this temperature for 20 hours. The solution was diluted with ether (20 mL). Saturated aqueous ammonium chloride solution was added until no bubbling was observed. The solution was filtered through Celite. The filtrate was then extracted with CH_2Cl_2 (3 × 100 mL), and the organic phase was washed with water (3 × 30 mL). Then the resulting CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:2, v/v) to obtain the title compound as a white solid (0.726 g, 73%).^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H), 6.79 (m, 3H), 6.67 (s, 1 H), 4.97 (s, 2H), 4.79(d, J = 4.0 Hz, 2H), 2.15 (s, 1H); MS (EI): *m/z* 230.2 (29, M^+), 212.2 (7), 139.1 (4), 121.1 (5), 91.1 (100), 84.0 (3), 65.1 (9); HRMS (EI): calculated for $C_{14}H_{14}O_3$: 230.0943, found: 230.0941.

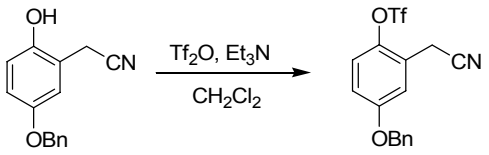


4-24 **4-36**

2-(5-(Benzyloxy)-2-hydroxyphenyl)acetonitrile (4-36)

To a colled (ice-bath) solution of 4-(benzyloxy)-2-(hydroxymethyl)phenol (100 mg, 0.43 mmol) and CBr_4 (151.2 mg, 0.46 mmol) in CH_2Cl_2 (10 mL) was added PPh_3 (119.6mg, 0.46 mmol) under ice-bath dropwise. Then the reaction was warmed up to room temperature and stirring was continued at this temperature over 7 hours.

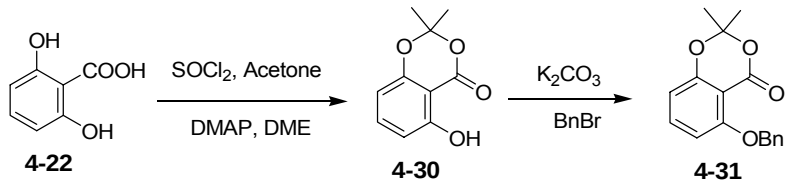
The solvent was evaporated and the residue was dissolved in DMSO (5 mL). NaCN (26.6 mg, 0.54 mmol) was added and the solution was stirred at room temperature for 17 hours. Saturated aqueous NH_4Cl (10 mL) and water (40 mL) were added sequentially, and the resulting solution was extracted with EtOAc (3 × 50 mL). Then the organic phase was washed with water (3 × 20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as a white solid (45.0 mg, 43%).^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 6H), 6.99 (s, 1H), 6.79 (dd, J = 8.6, 2.9 Hz, 1H), 6.68 (d, J = 8.7 Hz, 1H), 5.00 (s, 2 H), 3.68 (s, 2H).



4-36 **4-13**

4-(Benzyloxy)-2-(cyanomethyl)phenyl trifluoromethanesulfonate (4-13)

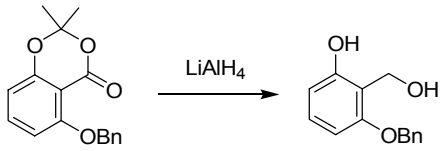
To a stirred solution of 2-(5-(benzyloxy)-2-hydroxyphenyl)acetonitrile (67.6 mg, 0.28 mmol) and Et_3N (0.059 ml, 0.42 mmol) in anhydrous CH_2Cl_2 (4 mL) cooled by an ice-bath was added Tf_2O (0.071 ml, 0.42 mmol) dropwise under ice-bath. The reaction was stirred at 0 °C for 2 hours. Then saturated aqueous NH_4Cl (10 mL) was added. The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL) and the organic phase was dried and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as a white solid (79.1 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (m, 5H), 7.26 (d, J = 9.1 Hz, 1H), 7.21 (d, J = 2.7 Hz, 1H), 6.98 (dd, J = 9.1, 2.9 Hz, 1H), 5.08 (s, 2H), 3.78 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 158.6, 140.1, 135.8, 128.8, 128.4, 127.6, 124.8, 123.1, 118.6 (q, J = 320.5 Hz, CF_3), 116.5, 116.1, 115.9, 70.7, 19.1; IR (film): 3035.6, 2937.6, 2261.9, 1591.1, 1495.2, 1423.9, 1250.2, 1216.9, 1171.6, 1138.7, 1025.4, 871.1, 612.0 cm^{-1}; MS (EI): *m/z* 371.0 (8, M^+), 91.1 (100) 65.1 (7).



5-(Benzyloxy)-2,2-dimethyl-4Hbenzo[*d*][1,3]dioxin-4-one (4-31)

To a stirred solution of 2,6-dihydroxybenzoic acid (1.54 g, 10 mmol), DMAP (61.1 mg, 0.5 mmol) and acetone (1.1 ml, 15 mmol) in DME (8 mL) cooled by an ice-bath was added $SOCl_2$ (1.1 ml, 15 mmol) dropwise. The reaction was stirred at 0 °C for 1 hour and then warmed up to room temperature. Stirring was continued at this temperature for 23 hours. Water (10 mL) was added and the resulting solution was extracted with ether (3 × 50 mL). The organic phase was washed with water (3 × 15 mL) and dried over Na_2SO_4. Concentration of this organic phase afforded a crude product as a white solid.

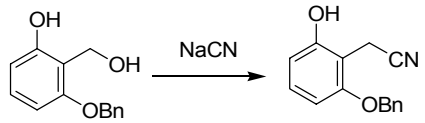
The a solution of the crude product in acetone (20 mL) was added solid K_2CO_3 (2.764 g, 20 mmol). The reaction mixture was stirred at room temperature for 10 minutes. Then benzyl bromide (4.76 ml, 40 mmol) was added and the temperature was increased to reflux and the reaction was continued at this temperature for 19 hours. The resulting solution was filtered and the precipitate was washed with EtOAc. The combined organic phase was dried over Na2SO4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as a white solid (1.781 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, 7.4 Hz, 2H), 7.37 (m, 3H), 7.29 (d, *J* = 7.2 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.53 (d, *J* = 8.2 Hz, 1H), 5.23 (s, 2H), 1.69 (s, 6H).



4-31 **4-32**

3-(Benzyloxy)-2-(hydroxymethyl)phenol (4-32)

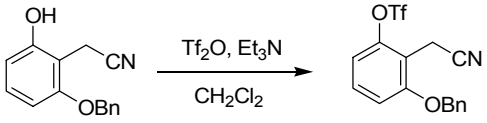
To a suspension of $LiAlH_4$ (676.1 mg, 17.82 mmol) in anhydrous THF (7 mL) was added **5-**(benzyloxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (1.013 g, 3.56 mmol) in anhydrous THF (14 mL) slowly at –78 °C. Then the reaction was warm up to room temperature and stirred at this temperature for 14 hours. The solution was diluted with ether (25 mL). Saturated aqueous NH_4Cl was added until bubbling ceased. Then the pH of the solution was adjusted to 1 and the resulting solution was filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the title compound as a white solid (607.3 mg, 74%). ^1H NMR (300 MHz, $CDCl_3$): δ 8.31 (s, 1 H), 7.37 (m, 5H), 7.07 (t, J = 8.2 Hz, 1H), 6.50 (t, J = 8.7 Hz, 1H), 5.01 (s, 2H), 4.98 (s, 2H), 3.43 (s, 1H); MS (EI): *m/z* 230.1 (23, M^+), 212.1 (15), 122.1 (15), 91.1 (100), 84.0 (13), 65.1 (8), 51.1 (4); HRMS (EI): calculated for $C_{14}H_{14}O_3$: 230.0943, found: 230.0950.



4-32 4-37

2-(2-(Benzyloxy)-6-hydroxyphenyl)acetonitrile (4-37)

The 3-(benzyloxy)-2-(hydroxymethyl)phenol (100 mg, 0.43 mmol) was dissolved in DMF (5 mL). NaCN (25.5 mg, 0.52 mmol) was added and the reaction was stirred at 110 °C for 15 hours. The resulting solution was cooled by an ice-bath and the pH was adjusted to 5 with HCl (1 M). The solution was extracted with EtOAc (3 × 50 mL). The organic phase was separated, washed with brine (3 × 10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:4, v/v) to obtain the title compound as a white solid (76.9 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (m, 5H), 7.08 (t, *J* = 8.3 Hz, 1H), 6.53 (d, *J* = 8.3 Hz, 1H), 6.44 (d, *J* = 8.2 Hz, 1H), 5.94 (s, 1H), 5.10 (s, 2H), 3.73 (s, 2H).

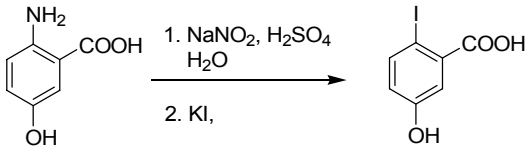


4-37 4-21

3-(Benzyloxy)-2-(cyanomethyl)phenyl trifluoromethanesulfonate (4-21)

To a solution of 2-(2-(benzyloxy)-6-hydroxyphenyl)acetonitrile (76.9 mg, 0.32 mmol) and Et_3N (0.067 ml, 0.48 mmol) in anhydrous CH_2Cl_2 (4 mL) cooled by an ice-bath was added Tf_2O (0.081 ml, 0.48 mmol) dropwise and the reaction was stirred with cooling for 2 hours.

Saturated aqueous NH_4Cl (10 mL) was added. The resulting solution was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phase was dried and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:6, v/v) to obtain the title compound as a white solid (105.8 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (m, 2H), 7.40 (m, 4H), 7.03 (d, *J* = 4.0 Hz, 1H), 7.00 (d, *J* = 4.2 Hz, 1H), 5.17 (s, 2H), 3.77 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 157.8, 147.7, 135.6, 130.6, 128.8, 128.5, 127.5, 118.6 (q, *J* = 320.5 Hz, CF_3), 116.3, 113.9, 112.9, 112.1, 71.2, 13.0; IR (film): 3035.3, 2938.6, 2258.6, 1613.9, 1585.0, 1466.1, 1454.3, 1424.2, 1276.9, 1216.2, 1138.3, 1047.4, 887.4, 810.7, 605.8 cm^{-1};MS (EI): *m/z* 371.0 (13, M^+), 91.1 (100) 65.1 (7).

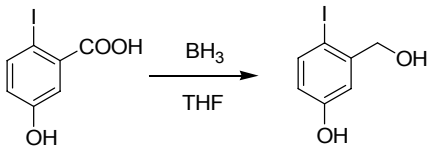


4-67 **4-66**

5-Hydroxy-2-iodobenzoic acid (4-66)

To a solution of 2-amino-5-hydroxybenzoic acid (1.53 g, 10 mmol) in water (10 mL) was added conc. H_2SO_4 (1.4 mL) slowly. Then $NaNO_2$ (0.697 g, 10.1 mmol) in water (3 mL) was added dropwise over 15 minutes and the reaction mixture was stirred with cooling for another 30 minutes. The patasium iodide (2.49 g, 15 mmol) in H_2SO_4 (5 mL, 1 M) was added slowly with ice-bath cooling. The reaction was heated to 100 °C and was continued at this temperature for 1 hour. The resulting solution was cooled to room temperature and left over night. A lot of precipitate came out, which was filtered and washed with water to obtain the

title compound as a brown solid (2.159 g, 82%). Mp: 188–199 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.1 (s, br, 1H), 9.93 (s, br, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.13 (d, J = 2.9 Hz, 1H), 6.66 (dd, J = 8.6, 2.9 Hz, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 168.3, 157.8, 141.7, 137.9, 120.6, 117.8, 80.5; IR (KBr): 3254.9, 1695.5, 1594.3, 1439.0, 1285.6, 1253.8, 1233.3, 1143.4, 1014.5, 827.8, 759.8, 659.5 cm^{-1}; MS (EI): *m/z* 263.9 (100, M^{+}), 246.9 (35), 218.9 (8), 81.0 (5), 63.1 (7); HRMS (EI): calculated for $C_7H_5IO_3$: 263.9283, found: 263.9290.

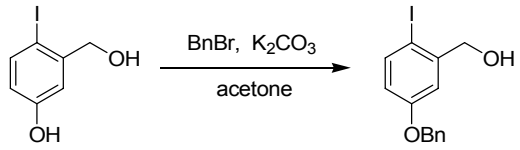


4-66 **4-65**

3-(Hydroxymethyl)-4-iodophenol (4-65)

To a ice-bath cooled solution of 5-hydroxy-2-iodobenzoic acid (500 mg, 1.89 mmol) in anhydrous THF (5 mL) was added a THF solution of BH_3 (5.7 ml, 1 M, 5.7 mmol) dropwise. The reaction was warmed up to room temperature and stirred at this temperature for 20 hours. The reaction was quenched with THF and H_2O (1:1, 20 mL) with ice-bath cooling. The resulting solution was extracted with EtOAc (100 mL). The organic phase washed with brine (3 × 10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc) to obtain the title compound as a white solid (462.8 mg, 98%). Mp: 145–146 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.56 (s, 1H), 7.49 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 2.9 Hz, 1H), 6.45 (dd, J = 8.4, 2.9 Hz, 1H), 5.32 (s, br, 1H), 4.29 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 158.3, 145.1, 139.3, 116.6, 115.7, 82.9, 67.6; IR (KBr): 3359.9,

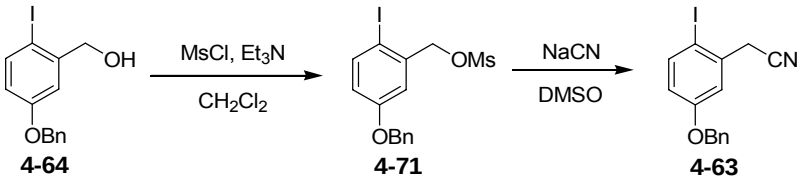
3164.9, 1590.2, 1572.9, 1463.6, 1437.1, 1321.9, 1283.0, 1235.8, 1162.6, 1042.6, 1009.8, 851.7, 800.1, 613.9 cm^{-1}; MS (EI): *m/z* 249.9 (100, M^+), 121.0 (13), 95.0 (15), 94.0 (12), 77.0 (11), 65.1 (7); HRMS (EI): calculated for $C_7H_7IO_2$: 249.9491, found: 249.9494.



4-65 **4-64**

(5-(Benzyloxy)-2-iodophenyl)methanol (4-64)

To a solution of 3-(hydroxymethyl)-4-iodophenol (462.8 mg, 1.85 mmol) in acetone (10 mL) was added solid K_2CO_3 (319.8 mg, 2.31 mmol). The reaction mixture was stirred at room temperature for 10 minutes. Benzyl bromide (0.28 mL, 2.31 mmol) was added and the temperature was heated to reflux and was continued at this temperature for 18 hours. The resulting solution was filtered and the precipitate was washed with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:4, v/v) to obtain the title compound as a white solid (515.3 mg, 82%). Mp: 103–104 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, *J* = 8.6 Hz, 1 H), 7.36 (m, 5H), 7.13 (d, *J* = 2.4 Hz, 1H), 6.65 (dd, *J* = 8.6, 2.5 Hz, 1H), 5.04 (s, 2H), 4.59 (s, 2H), 2.13 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 159.5, 143.8, 139.7, 136.6, 128.8, 128.2, 127.6, 116.2, 115.3, 85.8, 70.2, 69.3; IR (film): 3305.5, 2869.0, 1587.8, 1567.3, 1461.8, 1292.9, 1233.8, 1165.2, 1005.1, 794.1, 732.8, 695.5 cm^{-1}; MS (EI): *m/z* 340.0 (36, M^+), 91.1 (100), 65.1 (8); HRMS (EI): calculated for $C_{14}H_{13}IO_2$: 339.9960, found: 339.9967.

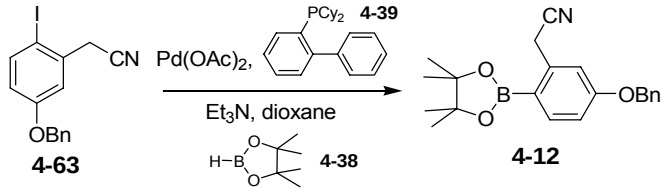


4-64 **4-71** **4-63**

2-(5-(Benzyloxy)-2-iodophenyl)acetonitrile (4-63)

To a ice-bath cooled solution of (5-(benzyloxy)-2-iodophenyl)methanol (8.84g, 25.99 mmol) and Et_3N (4.6 ml, 32.49 mmol) in anhydrous CH_2Cl_2 (200 mL) was added MsCl (2.6 mL, 32.49 mmol) slowly at 0 °C and the reaction was stirred with cooling for 3 hours.

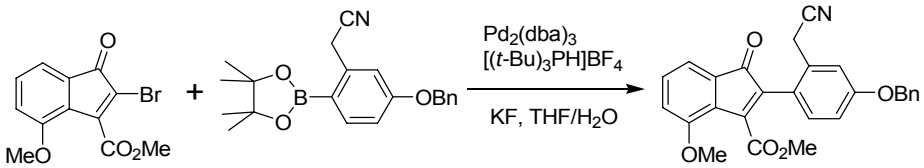
The solution was concentrated and the residue was dissolved in DMSO (80 mL). Solid NaCN (3.821 g, 77.97 mmol) was added and the reaction was stirred at room temperature for 16 hours. Water (20 mL) was added to the ice-bath cooled solution which was then extracted with EtOAc (3 × 300 mL). The combined organic phase was washed with water (3 × 90 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to obtain the title compound as a white solid (6.256 g, 69%). Mp: 114–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, *J* = 8.7 Hz, 1H), 7.36 (m, 5H), 7.18 (d, *J* = 2.4 Hz, 1H), 6.68 (dd, *J* = 8.7, 2.5 Hz, 1H), 5.06 (s, 2H), 3.74 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 159.6, 140.3, 136.2, 134.3, 128.8, 128.4, 127.6, 117.2, 116.8, 116.3, 87.5, 70.3, 30.0; IR (film): 3032.4, 2924.1, 2249.0, 1590.1, 1568.6, 1467.1, 1411.8, 1316.7, 1292.4, 1237.3, 1171.6, 1027.8, 1007.4, 806.0, 736.4, 696.9 cm^{-1}; MS (EI): *m/z* 349.0 (44, M^+), 91.1 (100), 65.1 (5); HRMS (EI): calculated for $C_{15}H_{12}INO$: 348.9964, found: 348.9968.



2-(5-(Benzyloxy)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetonitrile (4-12)

A mixture of $Pd(OAc)_2$ (19.4 mg, 0.086 mmol) and (2-biphenyl)dicyclohexyl-phosphine (120.8 mg, 0.344 mmol) was deoxygenated five times with an argon balloon and a vacuum pump. To a solution of 2-(5-(benzyloxy)-2-iodophenyl)acetonitrile (601.3 mg, 1.722 mmol) in dioxane (8 mL) was added pinacol borane (0.75 mL, 5.166 mmol) and Et_3N (0.96 mL, 6.888 mmol) and the solution was deoxygenated three times by a freeze-thaw process. The deoxygenated solution was then added to the solid mixture of the catalyst and ligand and stirred at 100 °C for 4 hours in an argon atmosphere. The reaction was quenched with saturated aqueous NH_4Cl (10 ml) and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to obtain the title compound as a white solid (437.7 mg, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.3 Hz, 1H), 7.38 (m, 5H), 7.09 (d, *J* = 2.3 Hz, 1H), 6.91 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.10 (s, 2H), 4.08 (s, 2H), 1.34 (s, 12H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 161.6, 138.8, 138.7, 136.5, 128.7, 128.2, 127.5, 118.9, 115.5, 113.4, 83.9, 69.9, 24.9, 23.7; IR (film): 3011.9, 2978.4, 2261.9, 1603.2, 1566.5, 1381.4, 1350.4, 1322.9, 1292.1, 1232.3, 1168.1, 1144.5, 1125.4, 1025.20, 857.9, 668.2 cm^{-1}; MS (EI): *m/z* 349.2 (33,

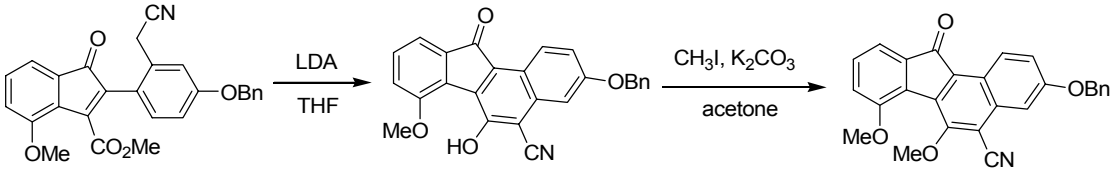
M^+), 348.2 (9), 249.1 (3), 91.1 (100), 65.1 (3); HRMS (EI): calculated for $C_{21}H_{24}BNO_3$: 349.1849, found: 349.1891.



Methyl 2-(4-(benzyloxy)-2-(cyanomethyl)phenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (4-10)

A mixture of bromoindenone **4-11** (2.73 g, 9.16 mmol), KF (1.597 g, 27.49 mmol), $Pd_2(dba)_3$ (419.5 mg, 0.458 mmol) and $[(t\text{-}Bu)_3PH]BF_4$ (265.9 mg, 0.916 mmol) was deoxygenated five times with an argon balloon and a vacuum pump. Pinacolboronate **4-12** (2.880 g, 8.246 mmol) was dissolved in a mixture (19:1) of THF and water (100 mL) and the solution was deoxygenated three times by a freeze-thaw process. The deoxygenated solution was then added to the solid mixture and stirring was continued for 24 hours at room temperature in an argon atmosphere. The solution was concentrated and the residue was dissolved in CH_2Cl_2 (150 mL). The organic phase was washed with water (3 × 20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the product as a red orange solid (2.75 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 6H), 7.17 (m, 3H), 7.01 (d, J = 8.4 Hz, 1H); 6.92 (dd, J = 8.5, 2.4 Hz, 1H), 5.08 (s, 2H), 3.84 (s, 3H), 3.77 (s, 3H) 3.73 (s, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 195.4, 166.2, 159.9, 153.0, 147.9, 136.4, 132.3, 132.1, 132.0, 131.3, 130.7, 128.7, 128.3, 128.0, 127.6,

121.3, 119.3, 117.7, 117.3, 115.5, 114.3, 70.2, 56.4, 52.6, 22.7; IR (film): 3035.7, 2950.2, 2261.9, 1734.8, 1711.4, 1610.3, 1498.0, 1479.7, 1274.1, 1241.4, 1202.1, 1171.0, 1055.3, 932.6, 766.5, 738.2, 698.0 cm^{-1}; MS (EI): *m/z* 439.2 (94, M^+), 408.2 (8), 348.2 (38), 320.2 (10), 91.1 (100); HRMS (EI): calculated for $C_{27}H_{21}NO_5$: 439.1420, found: 439.1416.



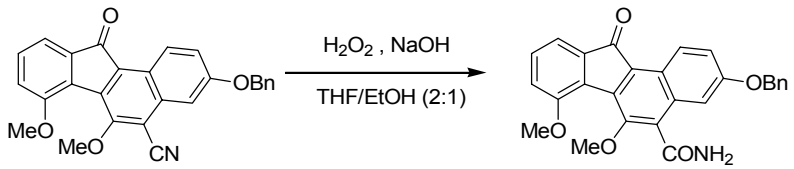
4-10 **4-72** **4-9**

3-(Benzyloxy)-6,7-dimethoxy-11-oxo-11H-benzo[a]fluorene-5-carbonitrile (4-9)

To a solution of methyl 2-(4-(benzyloxy)-2-(cyanomethyl)phenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (348.1 mg, 0.792 mmol) in anhydrous THF (10 mL) was added LDA (0.871 mmol) in THF (2 mL) slowly with ice-bath cooling. The solution was warmed up to room temperature and stirring was continued for 2 hour. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) solution leading to the formation of some red-orange precipitate. The suspension was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic phase was washed with H_2O (3 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated to obtain the crude product as a red-orange solid.

To a solution of the crude product in acetone (100 mL) was added solid K_2CO_3 (547.4 mg, 3.96 mmol) at room temperature and the mixture was stirred for 15 minutes. CH_3I (0.50 mL, 7.92 mmol) was added and the reaction mixture was heated to reflux and stirred at this temperature for 13 hours. The solution was filtered and the precipitate was washed with

CH_2Cl_2. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (CH_2Cl_2: Hexanes = 1:1, v/v) to give the product as an orange solid (211.2 mg, 63% for two steps). Mp: 187–188 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (d, J = 9.3 Hz, 1 H), 7.50 (d, J = 6.9 Hz, 2H), 7.39 (m, 4H), 7.29 (m, 3H), 7.10 (dd, J = 7.3, 1.8 Hz, 1H), 5.20 (s, 2H), 4.04 (s, 3H), 3.99 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 194.4, 159.6, 158.2, 154.6, 137.5, 136.0, 135.7, 132.3, 131.5, 128.9, 128.5, 128.4, 128.0, 126.7, 123.1, 122.8, 120.4, 117.5, 115.7, 109.2, 103.9, 70.5, 64.1, 56.6 (missing one peak); IR (film): 3023.8, 2940.5, 2219.8, 1705.4, 1621.1, 1602.4, 1484.8, 1442.0, 1376.7, 1281.6, 1226.3, 1202.2, 1052.6, 1023.9, 967.8, 833.4, 750.7, 694.1 cm^{-1}; MS (EI): *m/z* 421.1 (100, M^+), 330.1 (15), 302.1 (7), 229.1 (7), 201.1 (6), 91.1 (84); HRMS (EI): calculated for $C_{27}H_{19}NO_4$: 421.1314, found: 421.1320.

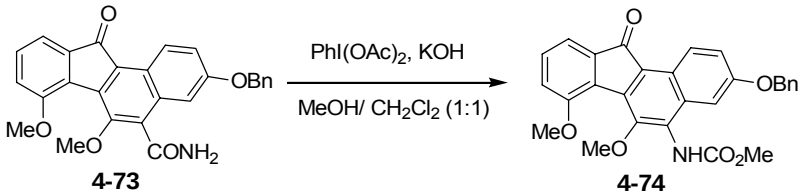


4-9 **4-73**

3-(Benzyloxy)-6,7-dimethoxy-11-oxo-11H-benzo[a]fluorene-5-carboxamide (4-73)

To a solution of 3-(benzyloxy)-6,7-dimethoxy-11-oxo-11H-benzo[a]fluorene-5-carbonitrile (46.5 mg, 0.11 mmol) and NaOH (91.3 mg, 1.10 mmol) in a mixture of EtOH (10 mL) and THF (20 mL) was added 30% H_2O_2 (10 mL) slowly at room temperature, then the mixture was stirred this temperature for 72 hours. The solution was concentrated to recover the organic solvents and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was

washed with water (3 × 15 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatograph (EtOAc:Hexanes = 3:1, v/v) to give the product as an orange solid (51.1 mg, quant.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (d, J = 9.3 Hz, 1 H), 8.06 (s, 1H), 7.82 (s, 1H), 7.37 (m, 8H), 7.17 (d, J = 6.7 Hz, 1 H), 7.07 (d, J = 2.1 Hz, 1H), 5.12 (s, 2H), 3.91 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 194.1, 167.8, 156.9, 154.2, 149.5, 137.3, 136.8, 136.4, 135.3, 132.9, 131.4, 128.5, 128.0, 127.9, 127.5, 127.4, 124.9, 122.6, 121.5, 120.8, 116.3, 104.7, 69.3, 63.4, 56.1; IR (film): 3416.7, 3333.3, 3178.6, 2916.8, 1700.0, 1660.7, 1602.9, 1482.9, 1275.6, 1228.0, 1049.7, 754.4 cm^{-1}; MS (EI): *m/z* 439.2 (100, M^+), 422.2 (17), 394.2 (7), 348.2 (57), 320.2 (18), 277.2 (6), 91.1 (53); HRMS (EI): calculated for $C_{27}H_{21}NO_5$: 439.1420, found: 439.1406.



4-73 **4-74**

Methyl 3-(benzyloxy)-6,7-dimethoxy-11-oxo-11H-benzo[a]fluoren-5-ylcarbamate (4-74)

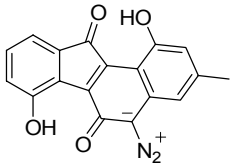
To a solution of 3-(benzyloxy)-6,7-dimethoxy-11-oxo-11H-benzo[a]fluorene-5-carboxamide (32.7 mg, 0.074 mmol) in a mixture of methanol (70 mL) and CH_2Cl_2 (30 mL) in an ice-bath was added solid KOH (10.4 mg, 0.19 mmol) at room temperature. The solution was cooled to 0 ºC and stirred for 10 minutes. Solid $PhI(OAc)_2$ (24 mg, 0.074 mmol) was added and the mixture was stirred with cooling for an additional 15 minutes. The solution was then warmed to room temperature and further stirred for 20 hours. The solution was concentrated and the

residue was dissolved in CH_2Cl_2 (150 mL) which was washed with water (3 × 15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 2:1, v/v) to give the title compound as an orange solid (31.3 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H), 8.82 (d, J = 9.0 Hz, 1H), 7.48 (m, 2 H), 7.33 (m, 6H), 7.19 (m, 2H), 5.19 (s, 2H), 3.93 (s, 3H), 3.69 (s, 3H), 3.64 (s, 2H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 194.4, 157.4, 155.8, 154.7, 150.6, 138.1, 137.0, 136.0, 134.2, 133.8, 131.8, 129.0, 128.5, 128.3, 128.0, 126.2, 125.5, 123.2, 122.1, 121.3, 116.7, 104.0, 69.9, 62.4, 56.6, 52.6; IR (film): 3250.0, 2904.8, 1697.5, 1624.7, 1528.3, 1257.7, 1050.7, 755.0 cm^{-1}; MS (EI): *m/z* 469.1 (100, M^+), 378.0 (76), 346.0 (43), 318.0 (30), 91.1 (30).

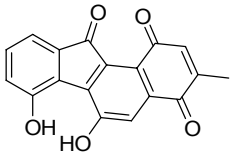
Chapter 5 Studies Toward the Total Synthesis of Fluostatin A and C4 Substituted IPK

5.1 Introduction

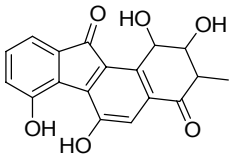
As indicated in the introduction, isoprekinamycin (IPK), first assigned a diazobenzo[*b*]fluorene,[1] is now recognized to be the diazobenzo[*a*]fluorene **5-1**.[2] Actually, IPK was the only known member of the benzo[*a*]fluorene class of natural products until recently, when the fluostatin class of natural products was discovered in 1998.[3] Fluostatins A–E (**5-2**–**5-6**)[3–6] possess various degrees of oxygenation in ring D but lack the diazo group，which is characteristic of the kinamycin class of natural products.



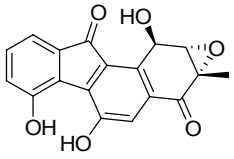
5-1
Isoprekinamycin



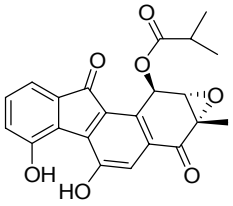
5-2
Fluostatin A



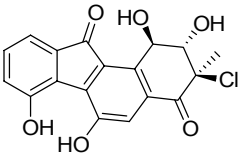
5-3
Fluostatin B



5-4
Fluostatin C



5-5
Fluostatin D

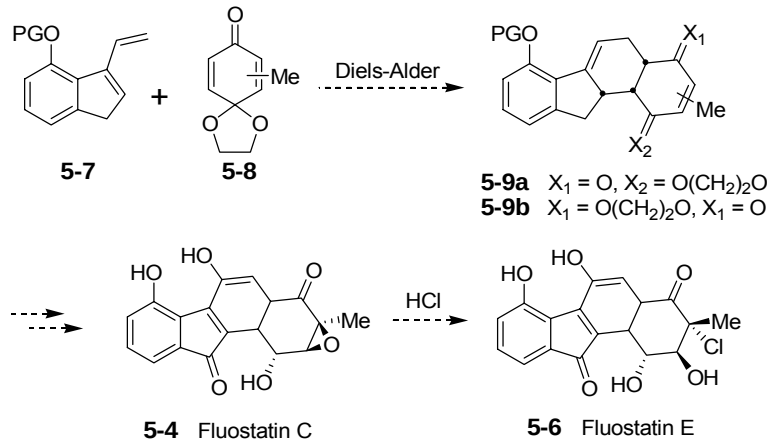


5-6
Fluostatin E

When this project began, there were no reports related to the total synthesis of any of the fluostatins. Very recently, Danishefsky and coworkers achieved a concise total synthesis of fluostatin C and E.[7] The strategy of Danishefsky's synthesis is shown in Scheme 5-1. The Diels-Alder cycloaddition between diene **5-7** and dienophile quinoneketal **5-8** provided

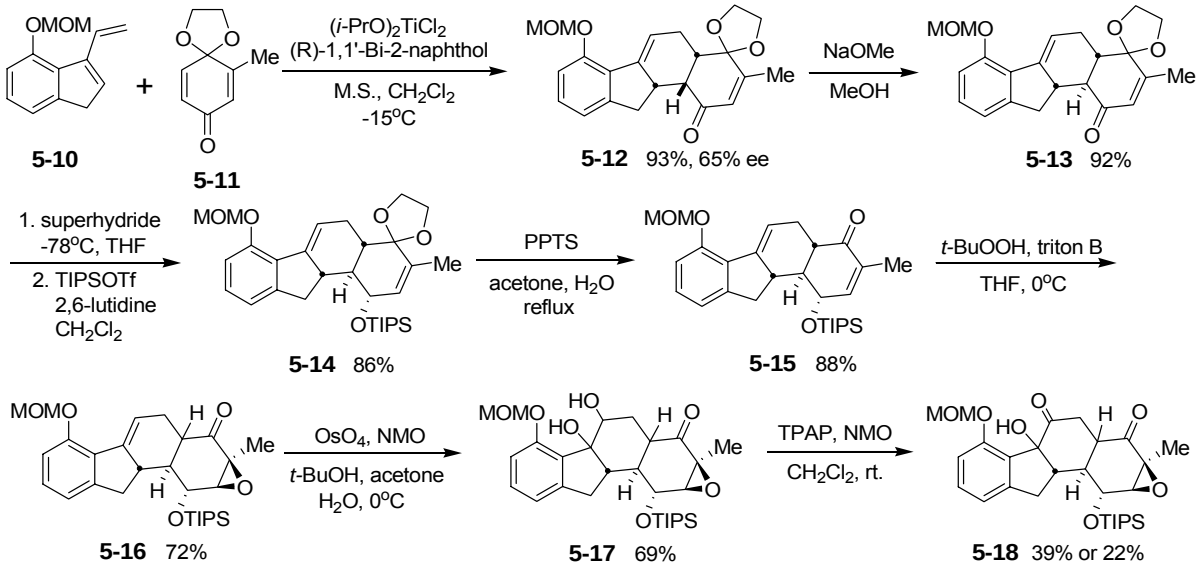
cycloadduct **5-9a** or **5-9b**. Regardless of the regioselectivity of the cycloaddition, either product was suitable for further functionalization to give fluostatin C and fluostatin E.

Scheme 5-1



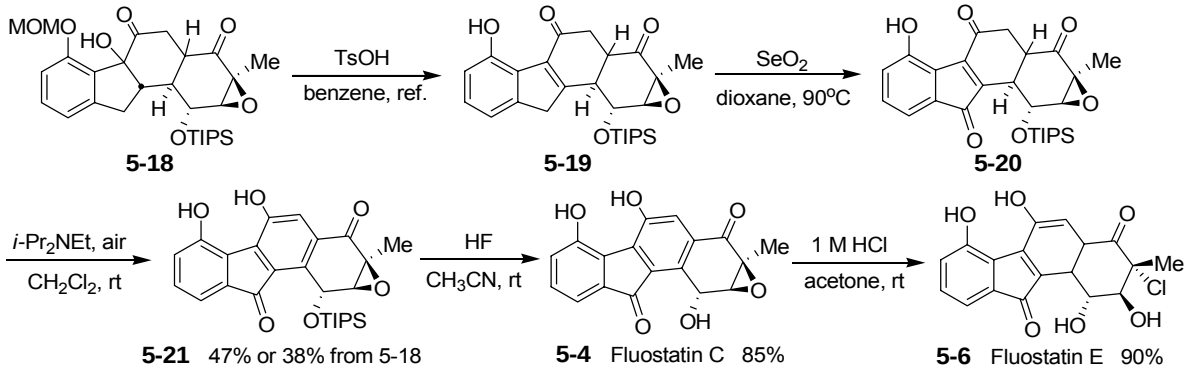
The Diels-Alder reaction between **5-10** and **5-11** under Mikami-Corey protocols[8-10] provided **5-12**, in which the CD junction was isomerized with NaOMe in methanol to generate **5-13**. The keto group of **5-13** was reduced with superhydride then protected with TIPSOTf to produce triisopropyl silyl derivative **5-14**, which was transformed to the corresponding α,β-unsaturated ketone **5-15** with PPTS in acetone. The nucleophilic epoxidation of **5-15** with triton B and *t*-BuOOH in THF generated epoxide **5-16** stereospecifically, in which the *trans*-junction was partially isomerized. Oxidation of **5-16** with osmium tetroxide afforded diol **5-17**, which was further oxidized to hydroxylketone **5-18** with TPAP/NMO.

Scheme 5-2



Removal of the MOM protecting group and dehydration of **5-18** with TsOH in benzene generated **5-19**, which was converted to **5-20** via benzylic oxidation with SeO_2. The dehydrogenation-tautomerization of **5-20** with Hünig's base in the presence of air afforded **5-21**. Cleavage of the TIPS protecting group in **5-21** with HF produced fluostatin C (**5-4**), which was converted to fluostatin E (**5-6**) by treatment with aqueous HCl.

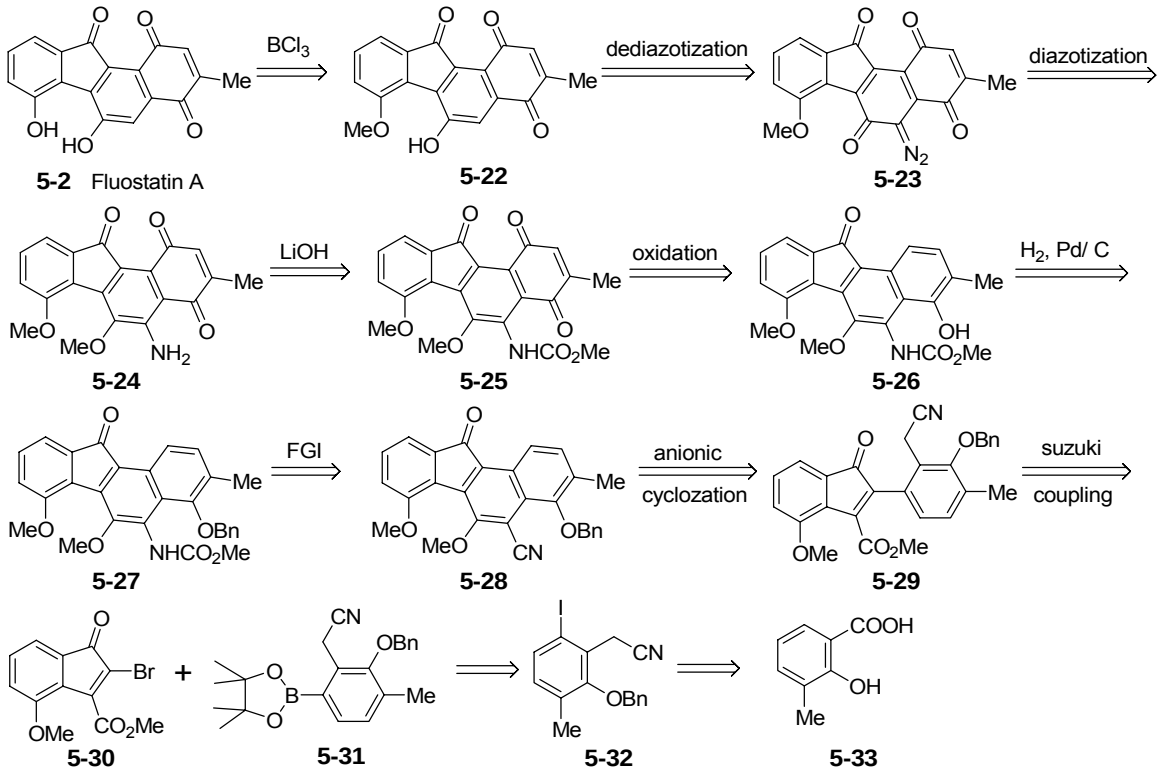
Scheme 5-3



5.2 Studies Toward the Total Synthesis of Fluostatin A

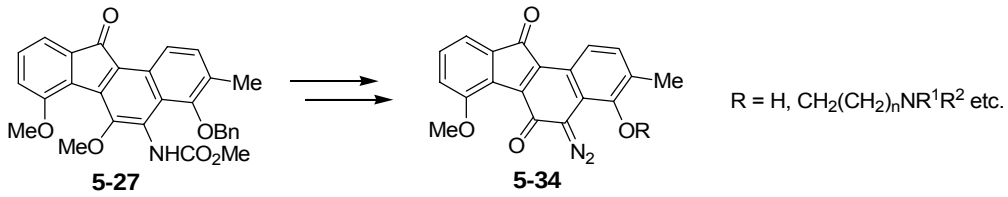
Given the similarities in the structures of the fluostatins and isoprekinamycin, it was considered that fluostatin A (**5-2**) might be synthesized with the same strategy used for the synthesis of IPK (**5-1**) and its model compound (Scheme 5-5). In this plan, fluostatin A would be formed via demethylation of **5-22**, which might be obtained from the diazo compound **5-23** by hydrodediazotization. The *o*-quinodiazide **5-23** might be achieved via demethylative diazotization of the aniline **5-24**, which would come from the corresponding carbamate **5-25** via hydrolysis. The quinone **5-25** would be obtained by oxidation of the phenol **5-26**, which might come from the benzylic ether **5-27** via hydrogenolysis. The compound **5-27** might be obtained from the carbonitrile **5-28**, which would be achieved from the anionic cyclization of diaryl compound **5-29**. Compound **5-29** would be obtained from the Suzuki coupling between bromoindenone **5-30** and pinacol boronate **5-31**, which might be formed from the iodophenylacetonitrile **5-32** by palladium-catalyzed borylation. Compound **5-32** might be synthesized from the commercially available 3-methylsalicylic acid (**5-33**).

Scheme 5-4



It was also felt that the key intermediate **5-27** might serve as a precursor to a series of IPK analogues in which substituents could be attached to the oxygen atom at C_4. For example, a compound such as **5-34** might be generated with side chain incorporating amino group which might lead to tightened binding to DNA.

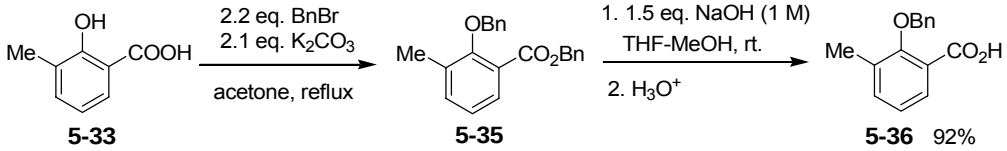
Scheme 5-5



In practice, treatment of 3-methylsalicylic acid **5-33** with 2.2 equivalents of benzyl bromide in the presence of 2.1 equivalents of K_2CO_3 in refluxing acetone gave the di-benzyl

adduct **5-35**, which was hydrolyzed with 1 M aqueous NaOH in THF and methanol to provide benzoic acid **5-36** in 92% overall.

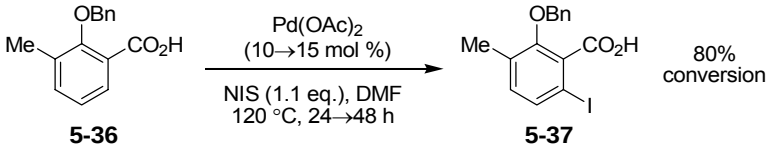
Scheme 5-6



5-33 **5-35** **5-36** 92%

Palladium-catalyzed iodination of **5-44** using the protocol of Kodama and coworkers[11] (10 mol% of $Pd(OAc)_2$ and 1.1 equivalents of *N*-iodosuccinimide (NIS) in DMF at 120 °C for 24 hours) generated a mixture of the desired iodobenzoic acid **5-45** and starting materials in 4:1 ratio. The reaction seemed clean, but various attempts to improve the conversion ratio of the starting material to product, either by increasing the reaction time or the amount of the catalyst, were unsuccessful (Scheme 5-7).

Scheme 5-7

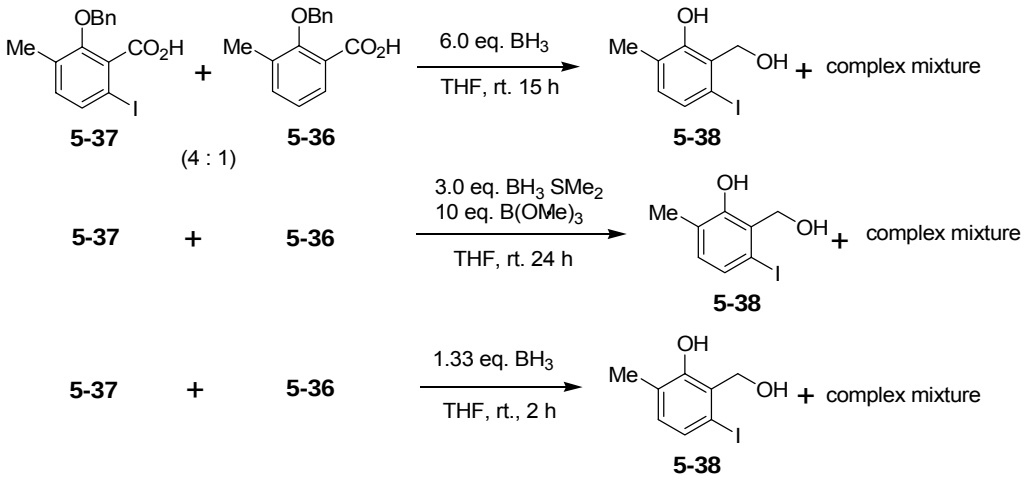


5-36 **5-37**

Since it was difficult to separate **5-37** and **5-36**, the reduction of this mixture was investigated. Treatment of the mixture of **5-37** and **5-36** with six equivalents of borane in THF from 0 °C to room temperature provided unexpectedly the diol **5-38** in a low yield, in which the cleavage of the benzyl group in the presence of large excess of borane had occurred. Replacement of the reducing agent by a less active borane species $BH_3{\cdot}SMe_2$ did

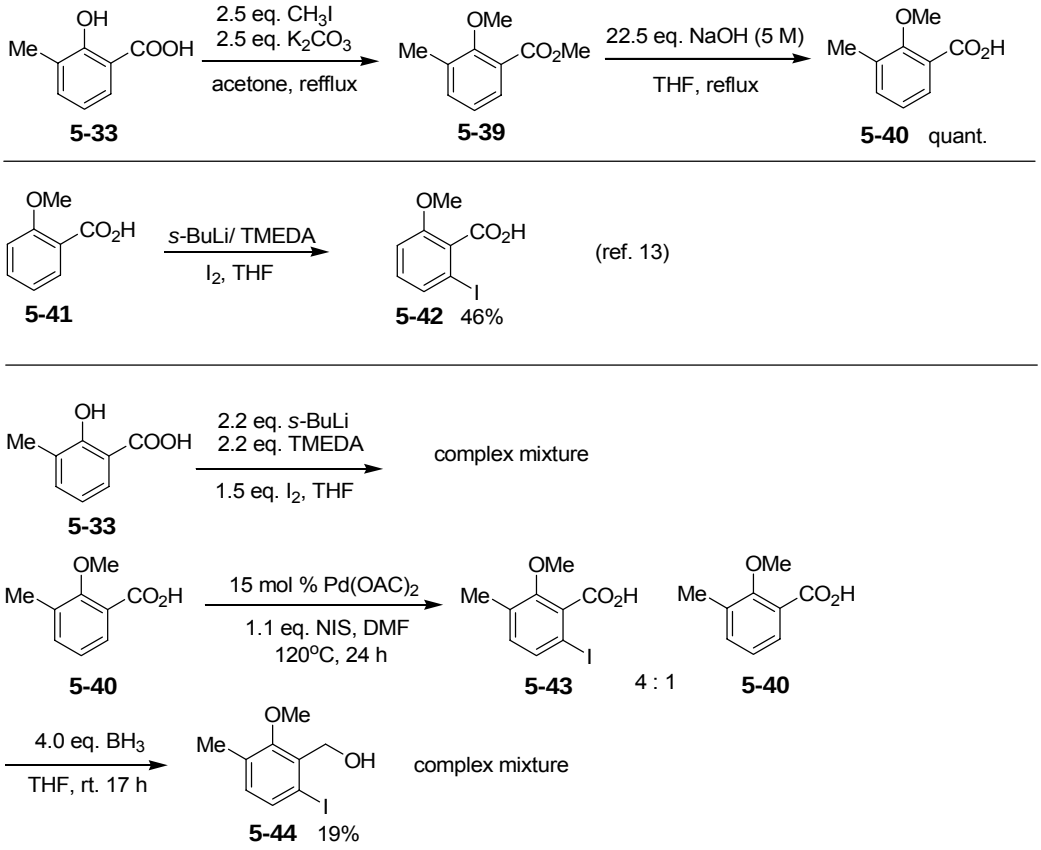
not improve the selectivity of the reaction. Furthermore, controlling the amount of borane in a shorter reaction time did not help.

Scheme 5-8



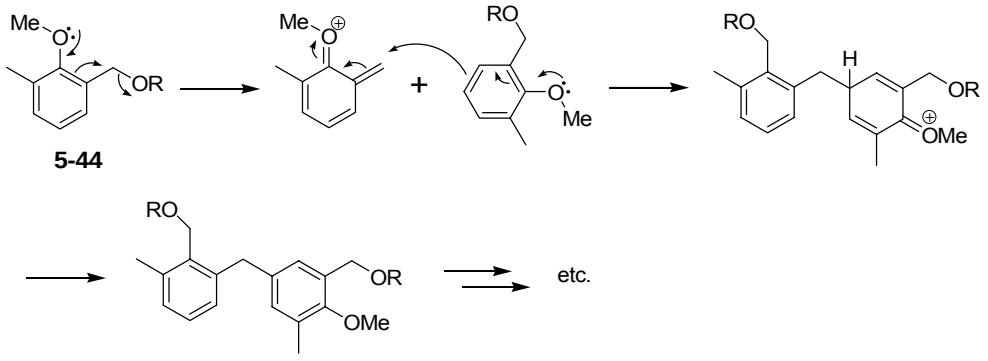
Since the benzyl ether was sensitive to borane, the methyl ether **5-40** was synthesized from **5-33** by methylation and hydrolysis. Nguyen and coworkers reported that orthometalation of benzoic acid **5-41** followed by treatment with I_2 generated *o*-iodobenzoic acid **5-42** regioselectively.[12] However, treatment of **5-33** with 2.2 equivalents of *s*-BuLi and TMEDA followed by trapping with I_2 provided a complex mixture. Meanwhile, palladium-catalyzed iodination of **5-40** produced a mixture of the desired iodobenzoic acid **5-43** along with starting material. Reduction of the mixture by borane without purification afforded the iodophenyl methanol **5-44** but only in 19% yield overall.

Scheme 5-9



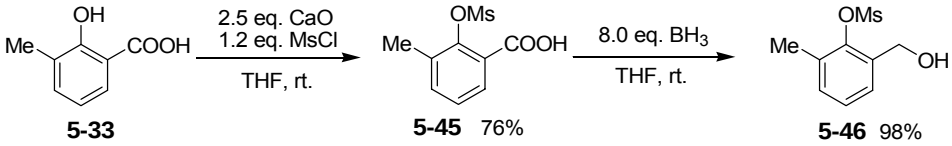
It was decided that the system **5-44** might be too electron rich and possibly prone to undergo polymerization as suggested in Scheme 5-10 below.

Scheme 5-10



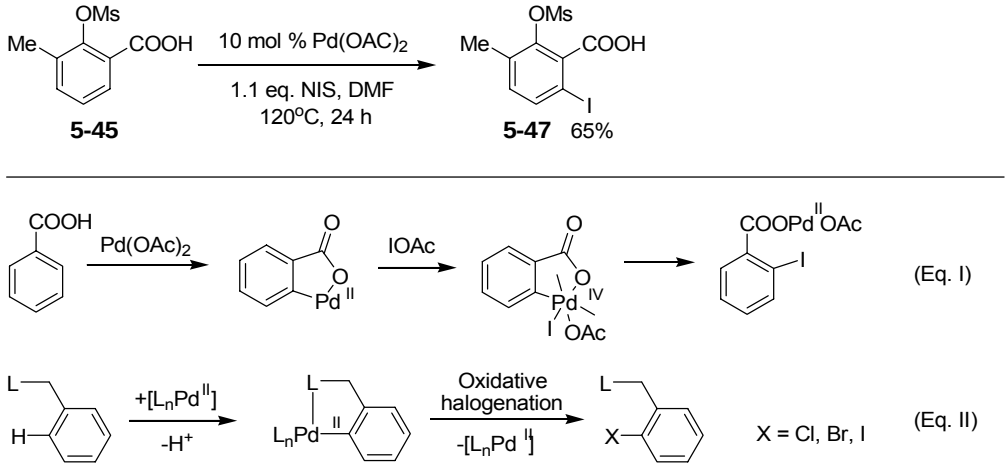
Thus, the mesyl protected phenol **5-45** was synthesized from **5-33** by treatment with 2.5 equivalents of CaO and 1.2 equivalent of mesyl chloride in THF at room temperature in 76% yield. Reduction of **5-53** with borane generated benzylic alcohol **5-46** in 98% yield.

Scheme 5-11



Palladium-catalyzed iodination of **5-45** with 10 mol % of $Pd(OAc)_2$ and 1.1 equivalents of NIS in DMF at 120 °C for 24 hours provided iodobenzoic acid **5-47** in 65% yield. More recently, Yu and coworkers observed the carboxylate-directed C-H iodination using $Pd(OAc)_2$ and IOAc (Eq. I, Scheme 5-12),[13] which was a special case of the palladium-catalyzed ligand-directed C-H bond halogenation (Eq. II).[14]

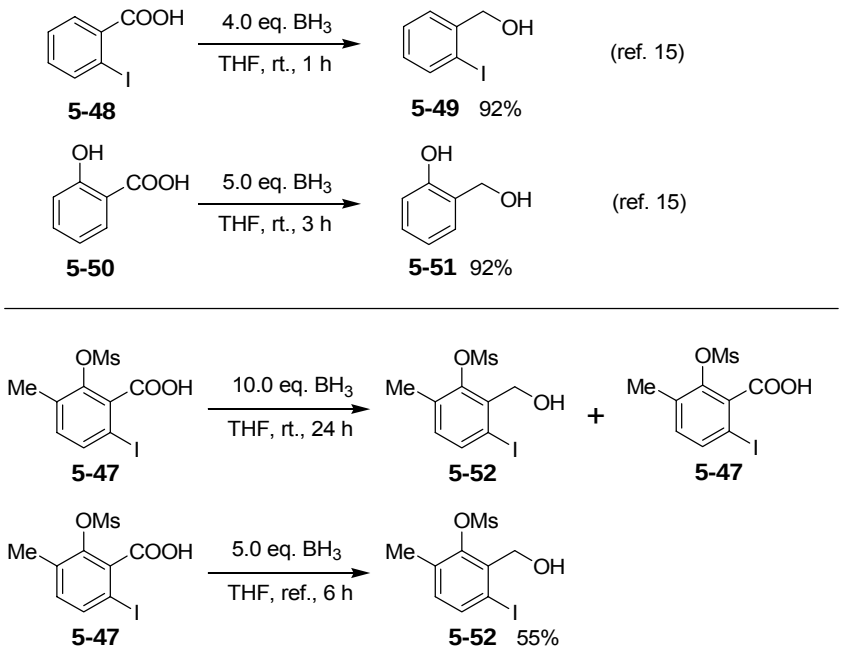
Scheme 5-12



Palladium Catalyzed Ligand-Directed C-H Bond Halogenation

Brown and coworkers reported many years ago that the *o*-iodobenzoic acid (**5-48)** was reduced with four equivalents of borane in THF smoothly to the corresponding alcohol **5-49**. Salicylic acid **5-50** was also reduced but required more borane and longer reaction time (Scheme 5-13).[15] However, treatment of iodobenzoic acid **5-47** with ten equivalents of borane in THF at room temperature led only to partial conversion of the starting material to the desired iodobenzyl alcohol **5-52**, even though a similar reaction of **5-45** worked quite well. Apparently, the di-*o*-substituted benzoic acid is a much more sterically hindered species than the other benzoic acids above. Thus, the reaction was run at a higher temperature with five equivalents of borane to provide the desired iodobenzyl alcohol **5-52** in 55% yield.

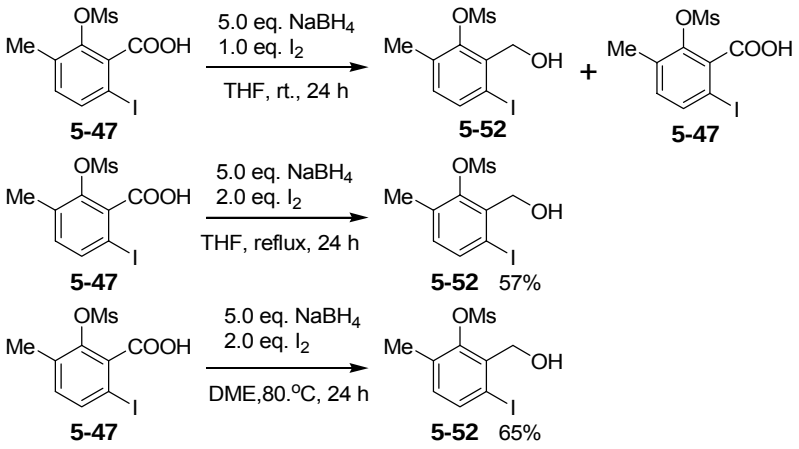
Scheme 5-13



Since it was intended that **5-52** would be produced eventually on large scale, a less expensive route was sought. Periasamy and coworkers developed a general method by which a carboxylic acid could be selectively reduced to an alcohol by $NaBH_4$ and I_2.[16] The

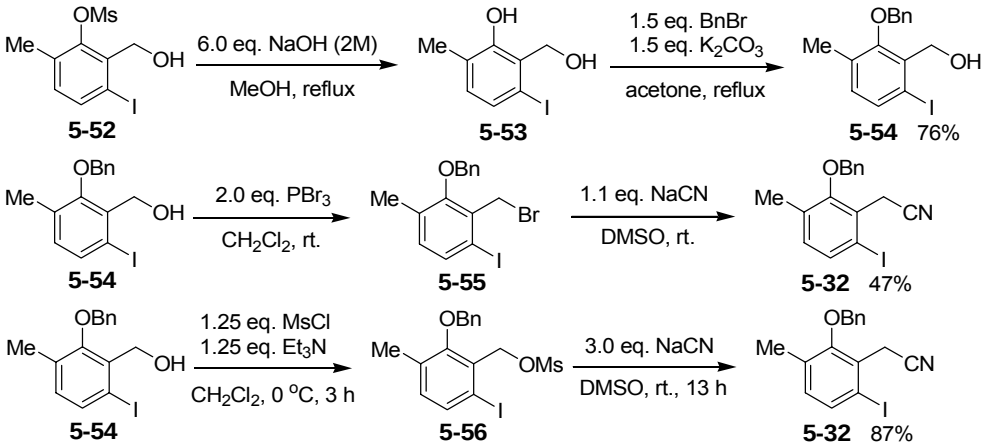
conversion ratio of the starting material was low when **5-47** was treated with five equivalents of $NaBH_4$ and one equivalent of iodine in THF at room temperature over one day. Hence, the amount of iodine was increased and was run at an elevated temperature, leading to **5-52** in 57% yield. Since both the reaction with borane and $NaBH_4$ in THF in a high temperature led to contamination by byproducts, derived by decomposition of THF, that was difficult to separate from the desired product, THF was replaced by DME leading to **5-52** in 65% yield.

Scheme 5-14



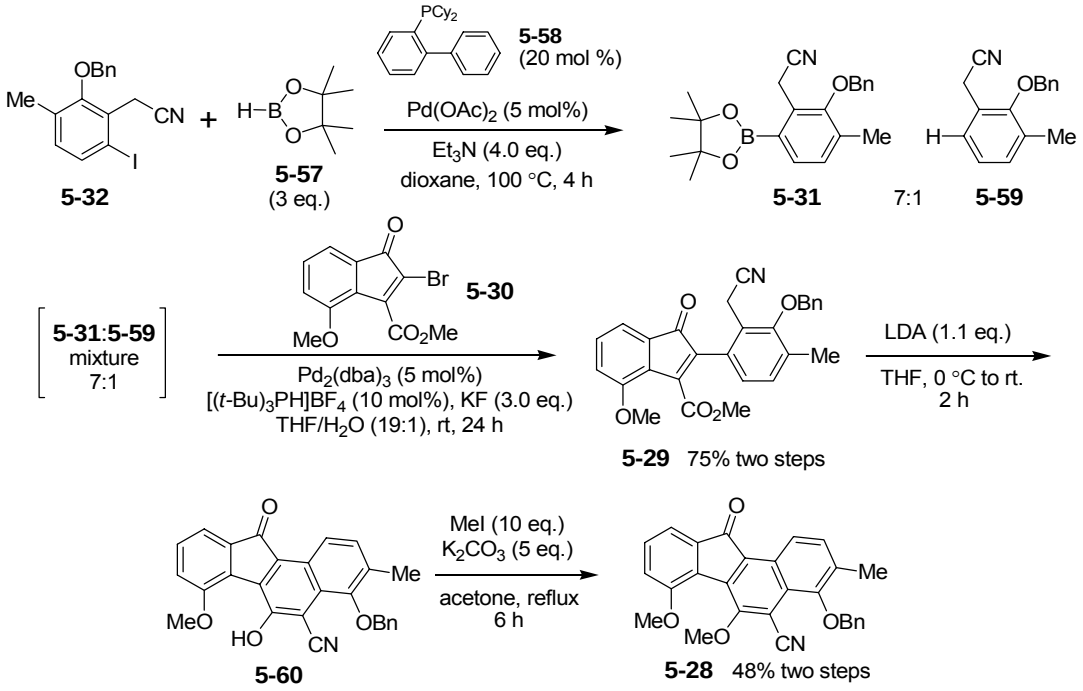
Hydrolysis of mesylate **5-52** with aqueous NaOH in refluxing methanol led to diol **5-53**, which was converted to the benzyl ether **5-54** in 76% yield over two steps. Treatment of iodobenzyl alcohol **5-54** with two equivalents of PBr_3 in methylene chloride provided the corresponding bromo compound **5-55**, which was subjected to a substitution with NaCN in DMSO to generate iodophenyl acetonitrile **5-32** in 47% yield over all. Alternatively, mesylation of the benzylic alcohol **5-54** with mesyl chloride and triethylamine produced the corresponding mesylate **5-56**, which was converted to the desired product **5-32** in 87% yield over two steps.

Scheme 5-15



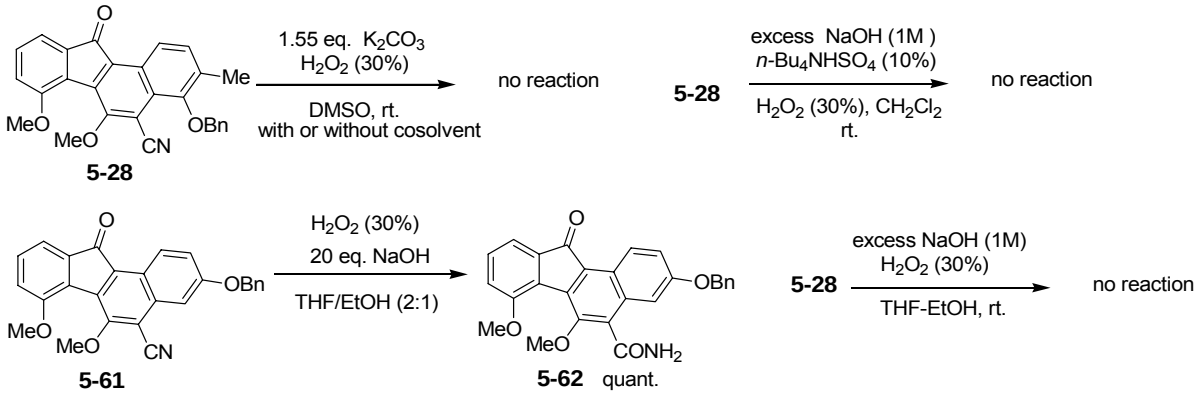
With **5-32** in hand, the palladium-catalyzed borylation of **5-32** with pinacolborane (**5-57**) using 20 mol% ligand **5-58** and 5 mol% $Pd(OAc)_2$ and four equivalents of triethylamine in dioxane at 100 °C for 4 hours provided a mixture of the desired pinacol boronate **5-31** and the reduction product **5-59** (7:1, respectively). Since the separation of **5-31** and **5-59** was difficult, the mixture was subjected to the Suzuki coupling with bromoindenone **5-30** using 5 mol% $Pd_2(dba)_3$, 10 mol% $[(t\text{-}Bu)_3PH]BF_4$, and three equivalents of KF in THF and water (19:1) at room temperature over one day to generate the diaryl adduct **5-29** in 75% yield for two steps. The diaryl compound **5-29** was cyclized with LDA then protected by CH_3I to produce the carbonitrile **5-28** in 48% yield overall.

Scheme 5-16



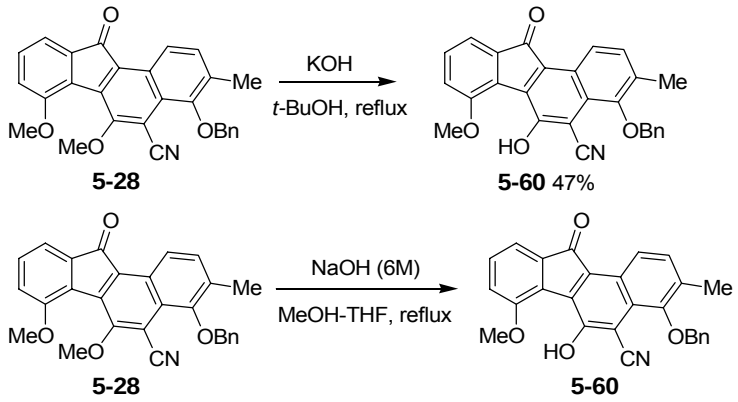
However, the hydrolysis of carbonitrile **5-28** to the corresponding carboxamide did not work well. Treatment of **5-28** with 1.55 equivalents of K_2CO_3 and excess 30% H_2O_2 in DMSO at room temperature provided nothing but starting material over one day. The failure of the reaction likely arose, at least partially, because of the poor solubility of **5-28** in DMSO. Thus, co-solvent THF or CH_2Cl_2 was used, but no obvious reaction was observed. Although methylene chloride is the best solvent for **5-28**, but the two phase condition using 10% *n*-Bu_4NHSO_4 as a phase transfer catalyst did not work. Even though hydrolysis of **5-61** with excess NaOH and H_2O_2 in THF and ethanol afforded the carboxamide **5-62** smoothly as described in chapter 4, this condition did not work for **5-28**. The OBn group at C_4 of D ring appeared to be blocking the reaction.

Scheme 5-17



Alternatively, heating of **5-28** with KOH in refluxing *t*-BuOH, common conditions for the hydrolysis of nitriles, unexpectedly generated phenol **5-60**, likely, resulting from an addition-elimination reaction rather than the desired hydrolysis of the nitrile. Treatment of **5-28** with 6 M NaOH in refluxing methanol and THF also led to the phenol **5-60** as major product.

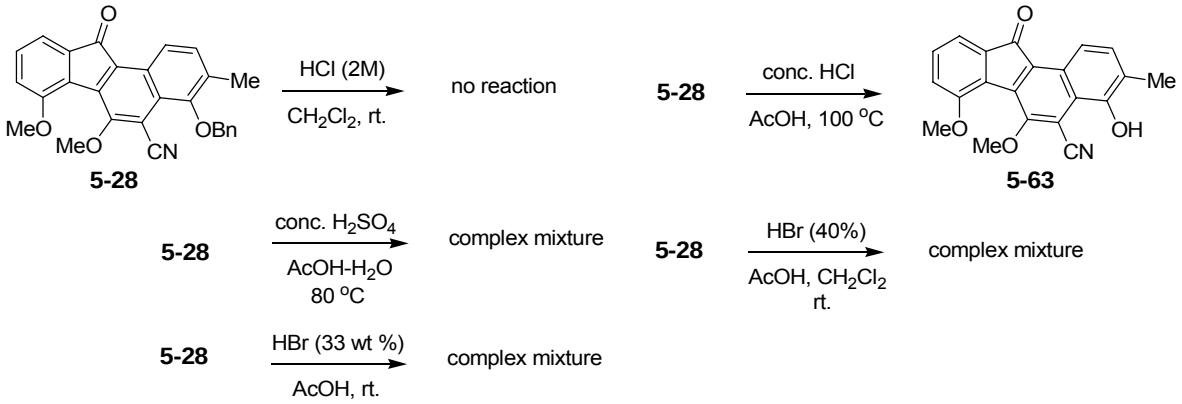
Scheme 5-18



Ruano and coworkers reported hydrolysis of cyanohydrins to the corresponding hydroxyl amides with HCl in ether.[17] However, no reaction was observed when a solution of **5-28** in methylene chloride was treated with a 2 M solution of HCl in ether. Alternative methods for the acid-catalyzed hydrolysis of **5-28** were considered, but the use of concentrated HCl and

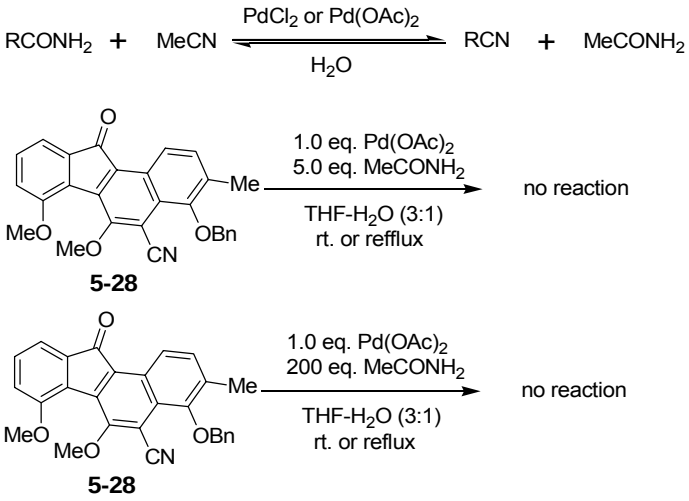
AcOH (1:1) at 100 °C led to cleavage of the benzyl group, with no detectable hydrolysis of the nitrile, and the use of concentrated H_2SO_4 provided a complex mixture. Treatment of **5-28** with either 40% aqueous HBr or 33 wt % HBr in acetic acid also generated complex mixtures.

Scheme 5-19



Maffioli and coworkers reported the palladium-catalyzed reversible dehydration of primary amides in aqueous organic solvents under mild conditions (Scheme 5-20).[18] However, attempts to apply these conditions to the desired hydrolysis were not successful as no reaction was observed when **5-36** was treated with one equivalent of $Pd(OAc)_2$ and five equivalents of acetamide in THF and water (3:1) at room temperature or at elevated temperature. The use of excess acetamide was also ineffective for pushing the equilibrium in the desired direction.

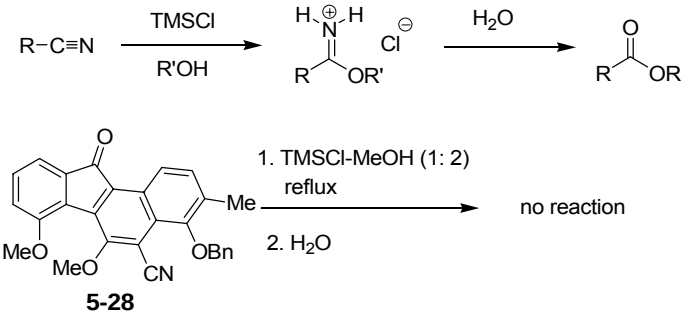
Scheme 5-20



5-28 **5-28**

Luo and Jeevanandam[19] reported a method for transformation of nitriles into esters by heating with a 1:2 mixture of TMSCl and alcohol to give an *O*-alkyl imidate intermediate, which then reacted with water upon aqueous workup. However, no reaction was observed when **5-28** was heated to reflux in a 1:2 mixture of TMSCl and methanol.

Scheme 21

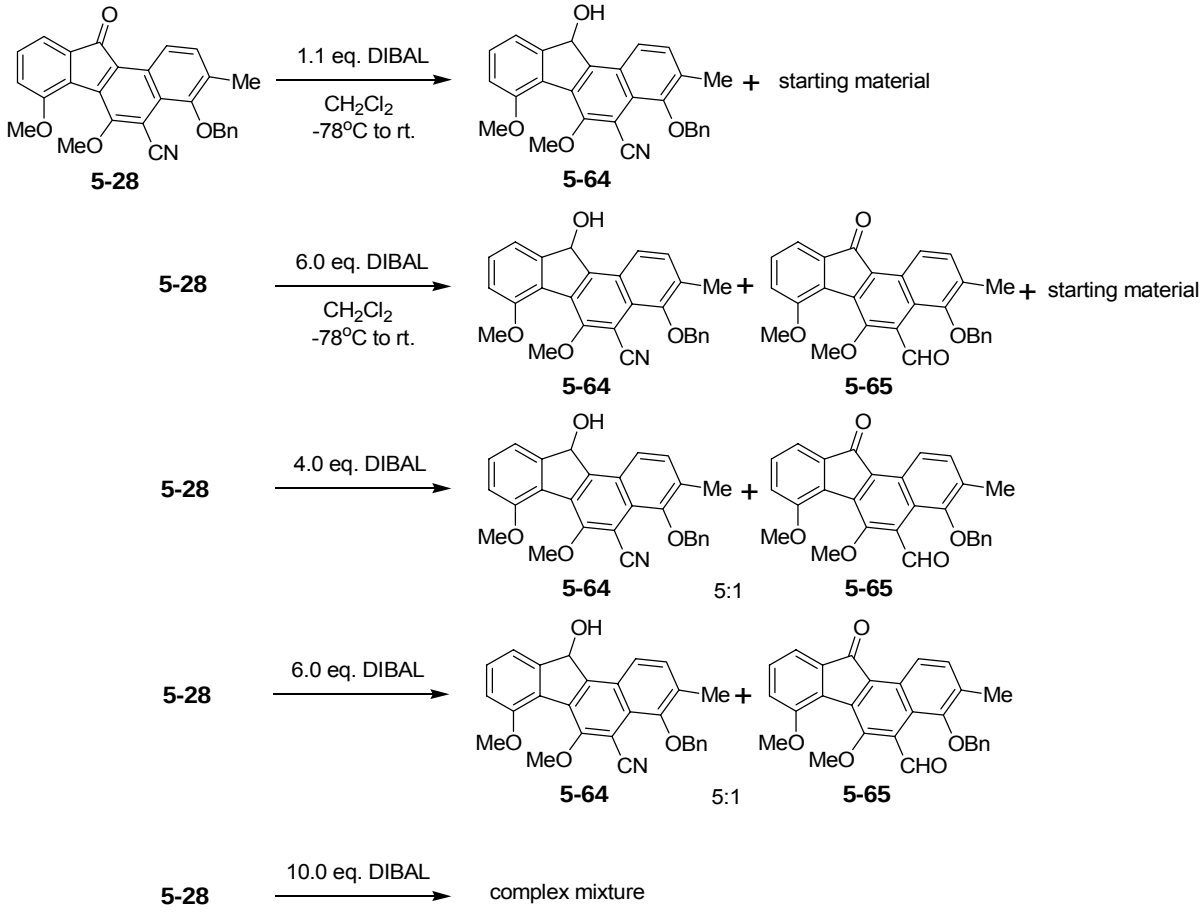


5-28

Since it was difficult to hydrolyze the carbonitrile **5-28** to the corresponding carboxamide, the reduction of **5-28** to an aldehyde, which might possibly be further functionalized, was investigated. Treatment of **5-28** with 1.1 equivalents of DIBAL in methylene chloride from –78 °C to room temperature led to partial reduction of **5-28** to the secondary alcohol **5-64**.

Increasing the amount of DIBAL to six equivalents, the reaction provided some desired aldehyde **5-65** along with alcohol **5-64** and starting material. It was felt that, on the small scale being studied, dissolved O_2 might oxidize the alcohol **5-64** back to **5-36**, leading to consumption of the DIBAL.

Scheme 5-22

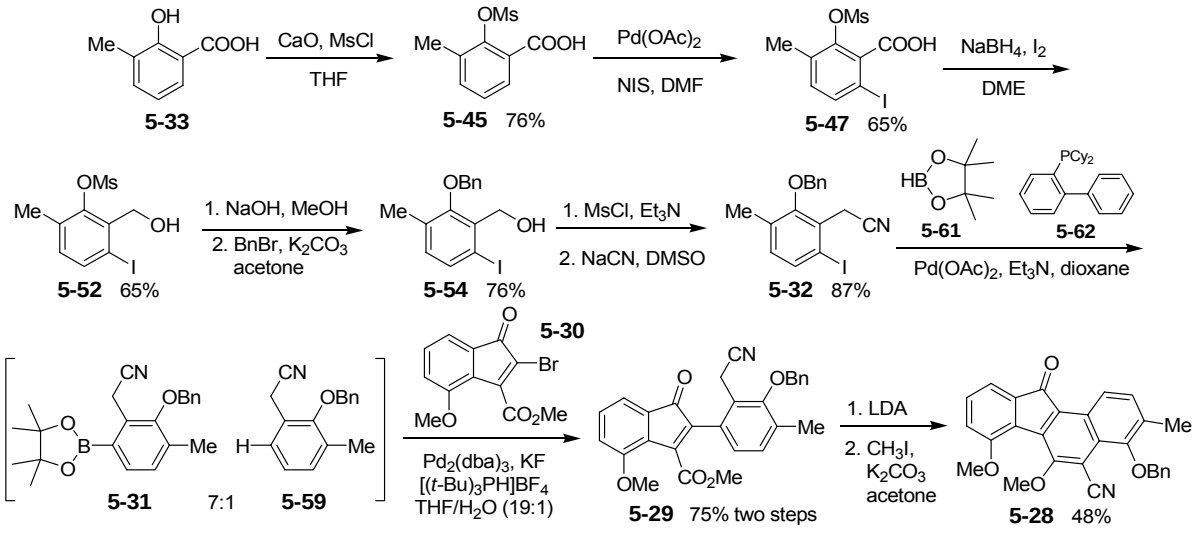


Hence, **5-28** was degassed and then treated with four equivalents of DIBAL. In this case, all starting material was consumed and the reaction generated a mixture of **5-64** and **5-65** (5:1, respectively). Increasing the amount of DIBAL to six equivalents did not improve the ratio of

5-65 to **5-64**. Furthermore, increasing the amount of DIBAL to ten equivalents led to a complex mixture.

The synthesis was stopped at this stage because of the limitation of time. In summary, the benzo[*a*]fluorene **5-36**, which is a potential intermediate for the total synthesis of fluostatin A and analogs of isoprekinamycin, was synthesized from commercial available 3-methylsalicylic acid (**5-33**) as the sequence shown in scheme 5-23 through eleven steps in 8% overall yield.

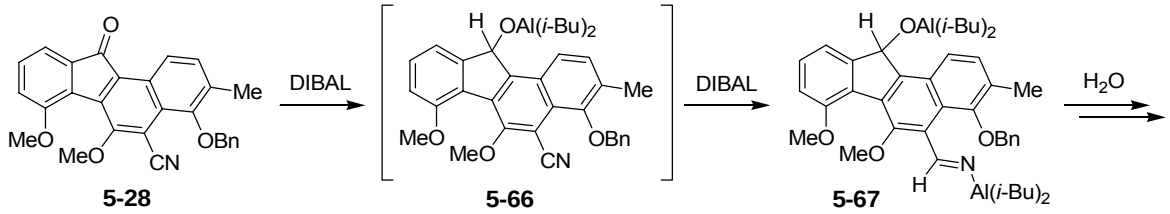
Scheme 5-23



Future work beyond **5-28** may involve removal of the *o*-benzyl group which interferes with further reactions of the cyano group sterically and which also creates problems associated with poor solubility.

Additionally, some variations during the DIBAL reduction of the cyano group in **5-28** might be explored. It is possible that in the DIBAL reduction both the ketone and nitrile group react as shown in Scheme 5-24.

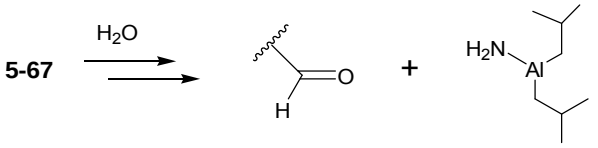
Scheme 5-24



5-28 **5-66** **5-67**

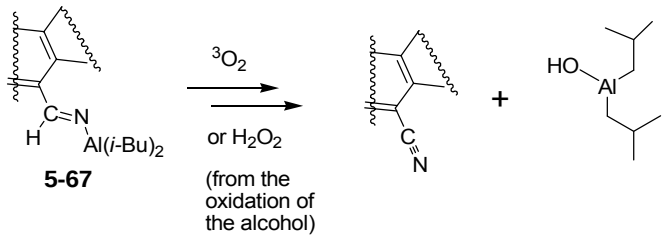
Conversion of **5-67** to the desired aldehyde requires hydrolysis of the hindered imine group in **5-67**.

Scheme 5-25



It was possible that this process is very slow and that a competing air oxidation may occur in the workup leading to conversion not only the alcohol to ketone but also perhaps the oxidation of the imine-aluminate in **5-67** back to nitrile.

Scheme 5-26



The precise mechanism for this process is unknown.

If this is true, then future work might involve reduction with DIBAL in O_2-free medium as done above, but followed by aqueous workup also under air free condition so that hydrolysis might compete with air oxidation.

5.3 Experimental

5.3.1 General Information

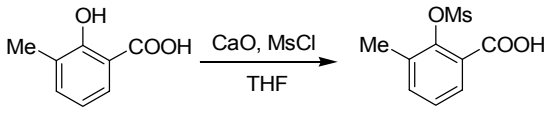
^{1}H NMR spectra were recorded on a Brüker AVANCE500 (500 MHz), Brüker AC300 (300 MHz) and Brüker AVANCE300 (300 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). The following abbreviations are used for NMR peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplet; m, multiplet; b, broad; w, weak. ^{13}C NMR spectra were broad band decoupled and recorded on a Brüker AVANCE500 (125.8 MHz), Brüker AC300 (75.5 MHz) and Brüker AVANCE300 (75.5 MHz) NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. HMQC and HMBC experiments were performed on a Brüker AVANCE500 spectrometer. IR spectra were determined on a Perkin-Elmer RX I FT-IR spectrometer as KBr discs unless otherwise indicated. High/low resolution electron impact or electrospray ionization (EI or ESI) mass spectra (MS) were measured by the WATSPEC Mass Spectrometry Facility (Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada). Elemental analyses were performed by the M-H-W Laboratories (Phoenix, Arizona, USA).

Anhydrous THF and Et_2O were freshly distilled from sodium/benzophenone under nitrogen prior to use. Anhydrous CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen prior to use.

All commercial reagents were purchased from Aldrich Chemical Co., Strem Chemicals Inc., Alfa Aesar, Lancaster Synthesis Ltd. or BDH Inc. and were used as received unless otherwise indicated. Deionized water was supplied by a Biolab vertical series reverse osmosis system.

The –78 ºC and 0 ºC designations refer to solid carbon dioxide/acetone and ice/water slush respectively. The room temperature refers to 22 °C to 25 °C. Flash column chromatography was carried out using the Merck silica gel (230-400 mesh) and SiliCycle silica gel (60 Å). Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with Merck pre-coated silica gel plates (silica gel 60 F_{254} on aluminum sheet). All reported yields are isolated yields.

5.3.2 Detailed Experimental Procedures

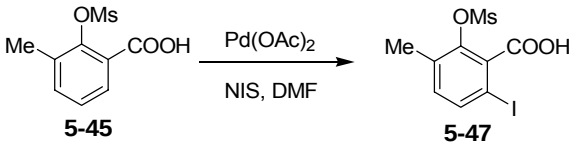


5-33 **5-45**

3-Methyl-2-(methylsulfonyloxy)benzoic acid (5-45)

To a mixture of 3-methylsalicylic acid (15.52 g, 100 mmol) and CaO (14.02, 250 mmol) in THF (150 mL) was added MsCl (9.4 ml, 120 mmol) at room temperature slowly, and the reaction was stirred at this temperature for 48 hours. With ice-bath cooling, conc. HCl was added until the pH value of the solution was near 1 (pH page). Then the resulting solution was extracted by ether (3 × 200 mL), and the combined organic phase was dried over Na_2SO_4

and concentrated. The residue was washed with ether several times to provide the title compound as a white solid (17.48 g, 76%). Mp: 147–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.29 (t, *J* = 7.7 Hz, 1H), 3.34 (s, 3 H), 2.47 (s, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.2, 146.2, 136.8, 134.3, 130.1, 126.9, 124.4, 39.4, 17.4; IR (film): 3027.5, 1688.2, 1606.4, 1586.0, 1465.3, 1419.0, 1335.5, 1298.8, 1206.9, 1189.2, 1169.9, 1142.7, 1087.5, 975.0, 868.1, 768.7, 701.6 cm^{-1}; MS (EI): *m/z* 230.05 (13, M^+), 151.05 (9), 134.05 (100), 133.04 (27), 106.05 (36), 105.04 (13), 77.01 (13), 51.19 (7); HRMS (EI): calculated for $C_9H_{10}O_5S$: 230.0249, found: 230.0254.

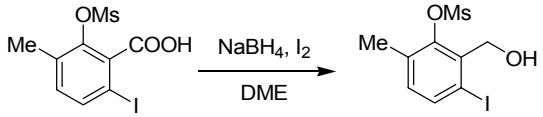


5-45 **5-47**

6-Iodo-3-methyl-2-(methylsulfonyloxy)benzoic acid (5-47)

To a solution of 3-methyl-2-(methylsulfonyloxy)benzoic acid (4.0 g, 17.37 mmol) and NIS (4.30 g, 19.11 mmol) in 12 ml DMF was added $Pd(OAc)_2$ (0.39 g, 1.74 mmol) and the reaction was stirred at 120 °C for 24 hours. The reaction mixture was filtered through a plug of silica gel, and washed with ether (2 L). The filtrate was concentrated to 500 mL and then washed with water (6 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was recrystallized from THF to obtain the title compound as a white solid (4.025 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.32 (s, 1H), 7.69 (d, 8.1 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 3.29 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.4, 144.7, 138.2,

134.7, 134.0, 133.5, 88.9, 39.6, 17.6; IR (film): 3037.5, 2939.0, 1711.9, 1593.1, 1562.6, 1454.7, 1359.5, 1250.9, 1190.0, 1150.1, 1116.7, 972.6, 886.5, 815.7, 792.8, 727.8 cm^{-1}; MS (EI): *m/z* 355.9 (56, M^+), 337.9 (7), 276.9 (18), 259.9 (100), 231.9 (34), 230.9 (13), 134.0 (12), 105.0 (18), 77.0 (8), 51.2 (7); HRMS (EI): calculated for $C_9H_9O_5IS$: 355.9215, found: 355.9218.

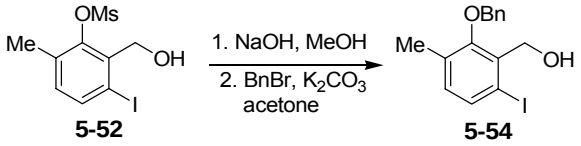


5-47 **5-52**

2-(Hydroxymethyl)-3-iodo-6-methylphenyl methanesulfonate (5-52)

To a solution of 6-iodo-3-methyl-2-(methylsulfonyloxy)benzoic acid (435.2 mg, 1.22 mmol) in anhydrous DME (12 mL) was added $NaBH_4$ (232.2 mg, 6.11 mmol) followed by adding I_2 (620.3 mg, 2.44 mmol) in anhydrous DME (12 mL) slowly at room temperature. The reaction was stirred at 80 °C for 24 hours. At 0 °C, 1 M HCl (20 mL) was added, and the resulting solution was extracted with EtOAc (3 × 50 mL). The organic phase was washed with water (3 × 15 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:2, v/v) to obtain the title compound as a white solid (273.6 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (d, *J* = 8.1 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 4.75 (d, *J* = 6.3 Hz, 2H), 3.38 (s, 3 H), 2.65 (t, *J* = 6.8 Hz, 1H), 2.35 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 145.8, 138.6, 136.7, 133.6, 133.2, 98.3, 64.0, 38.8, 17.6; IR (film): 3341.5, 3021.9, 2952.4, 1563.4, 1454.6, 1386.6, 1360.5, 1184.3, 1153.4, 1118.0,

974.4, 878.5, 793.0 cm^{-1}; MS (EI): *m/z* 342.0 (55, M^+), 324.0 (5), 264.0 (10), 263.0 (100), 246.0 (36), 136.1 (7), 108.1 (34), 107.1 (13), 77.0 (8), 51.2 (5); HRMS (EI): calculated for $C_9H_{11}IO_4S$: 341.9423, found: 341.9426.



5-52 **5-54**

(2-(Benzyloxy)-6-iodo-3-methylphenyl)methanol (5-54)

To a solution of 2-(hydroxymethyl)-3-iodo-6-methylphenyl methanesulfonate (219.3 mg, 0.64 mmol) in MeOH (6 mL) was added aqueous NaOH (2.0 mL, 2 M) slowly at room temperature. The reaction mixture was stirred and heated to reflux for 3 hours. With ice-bath cooling, 2 M HCl was added to adjust the pH to about 1 (pH page), and the resulting solution was extracted with EtOAc (3 × 50 mL). The organic phase was washed with water (3 × 15 mL), dried over Na_2SO_4 and concentrated to obtain the crude diol as a white solid (164.0 mg).

The crude diol (164.0 mg) was dissolved in acetone (10 mL). Solid K_2CO_3 (128.8 mg, 0.93 mmol) was added and the mixture was stirred at room temperature for 10 minutes. Benzyl bromide (0.12 ml, 0.93 mmol) was added and the temperature was increased to reflux and stirring was continued at this temperature for 5 hours. The resulting solution was filtered and the precipitate was washed with CH_2Cl_2. Then the combined organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:10, v/v) to obtain the title compound as a white solid (171.9 mg, 76%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, J = 8.0 Hz, 1H), 7.48 (m, 2H), 7.40 (m, 3H), 6.88 (d, J = 8.0 Hz, 1H), 4.92 (s, 2H), 4.80 (s, 2H), 2.38 (s, 1H), 2.28 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 156.5, 136.8, 136.6, 135.2, 132.8, 132.4, 128.7, 128.4, 128.1, 97.5, 76.5, 64.6, 16.3; IR (film): 3357.1, 3272.2, 3035.7, 2952.4, 2928.6, 2881.0, 1572.4, 1454.7, 1367.5, 1259.0, 1216.2, 1037.6, 975.0, 746.4, 694.1 cm^{-1}; MS (EI): *m/z* 354.0 (55, M^+), 336.0 (17), 324.0 (4), 278.1 (3), 246.0 (90), 209.1 (4), 119.1 (5), 91.1 (100), 65.1 (7), 51.2 (3); HRMS (EI): calculated for $C_{15}H_{15}IO_2$: 354.0117, found: 354.0118.

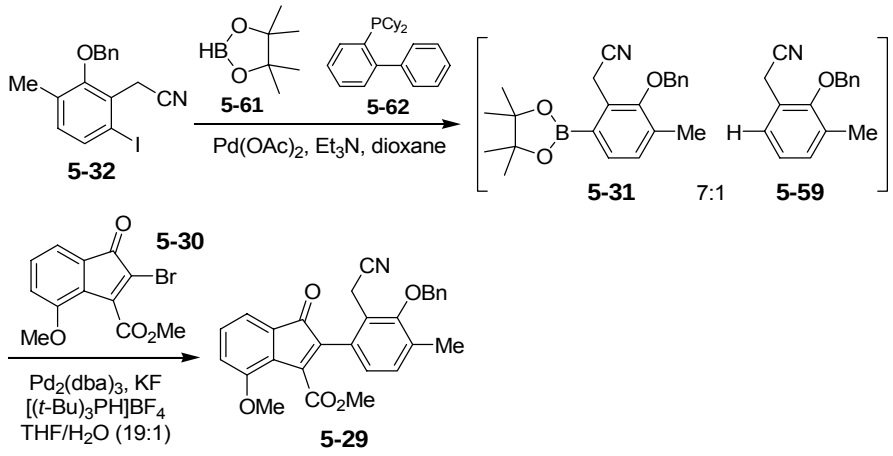
5-54 **5-32**

2-(2-(Benzyloxy)-6-iodo-3-methylphenyl)acetonitrile (5-32)

To a solution of (2-(benzyloxy)-6-iodo-3-methylphenyl)methanol (1.0 g, 2.82 mmol) and Et_3N (0.5 mL, 3.52 mmol) in anhydrous CH_2Cl_2 (25 mL) was added MsCl (0.28 mL, 3.52 mmol) slowly with ice-bath cooling and the reaction was stirred for 3 hours.

The solution was concentrated and the residue was dissolved in DMSO (10 mL) containing NaCN (415.1 mg, 8.47 mmol), and the reaction was stirred at room temperature for 15 hours. Water (50 mL) was added to the ice-bath solution, which was extracted with EtOAc (3 × 100 mL). The organic phase was washed with water (2 × 30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to obtain the title compound as a white solid (892.9 mg, 87%).

Mp: 95–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55 (d, J = 8.1 Hz, 1H), 7.43 (m, 5H), 6.94 (d, J = 8.1 Hz, 1H), 4.92 (s, 2H), 3.83 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 156.0, 136.2, 135.4, 133.3, 132.3, 128.8, 128.6, 128.1, 127.9, 117.2, 96.9, 75.5, 24.2, 16.4; IR (film): 3064.0, 3032.2, 2925.1, 2878.0, 2247.6, 1569.7 1497.2, 1454.9, 1405.9, 1370.1, 1261.8, 1214.7, 1201.0, 1125.1, 1004.8, 974.9, 920.3, 808.6, 743.8, 697.4 cm^{-1}; MS (EI): *m/z* 363.0 (45, M^+), 91.0 (100), 83.9 (8), 65.1 (5); HRMS (EI): calculated for $C_{16}H_{14}INO$: 363.0120, found: 363.0132.

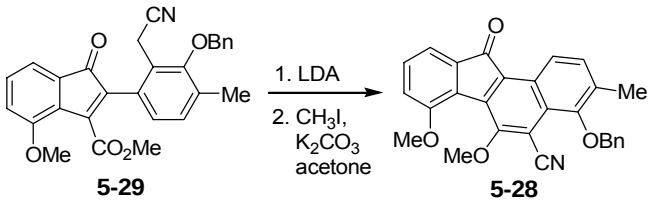


Methyl 2-(3-(benzyloxy)-2-(cyanomethyl)-4-methylphenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (5-29)

A mixture of $Pd(OAc)_2$ (61.9 mg, 0.28 mmol) and (2-biphenyl)dicyclohexyl-phosphine (386.0 mg, 1.10 mmol) was deoxygenated five times with an argon balloon and a vacuum pump. The 2-(2-(benzyloxy)-6-iodo-3-methylphenyl)acetonitrile (2.0 g, 5.51 mmol) was dissolved in dioxane 935 mL). Pinacolborane (2.4 mL, 16.52 mmol) and Et_3N (3.1 mL, 22.03

mmol) were added and the solution was deoxygenated three times by a freeze-thaw process. The deoxygenated solution was then added to the solid mixture of catalyst and ligand and stirred at 100 °C for 4 hours under argon atmosphere. The reaction was quenched with 20 mL saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:20, v/v) to obtain the crude as a white solid (1.64 g).

A mixture of bromoindenone (1.50 g, 5.03 mmol), KF (876.7 mg, 15.09 mmol), $Pd_2(dba)_3$ (230.3 mg, 0.25 mmol) and $[(t\text{-Bu})_3PH]BF_4$ (146.0 mg, 0.50 mmol) was deoxygenated five times with an argon balloon and a vacuum pump. The crude pinacolboronate (1.64 g) was dissolved in a mixture (19:1) of THF and water (60 mL) and the solution was deoxygenated three times by the thaw-freeze process. The deoxygenated solution was then added to the solid mixture and stirred for 24 hours at room temperature under an argon atmosphere. The solution was concentrated to remove THF and the residue was dissolved in CH_2Cl_2 (200 mL). The organic phase washed with water (3 × 20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc:Hexanes = 1:3, v/v) to obtain the product as a red-orange solid (1.88 g, 75% for two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.49 (m, 2H), 7.39 (m, 3H), 7.29 (dd, J = 8.3, 7.1 Hz, 1H), 7.20 (d, J = 7.2 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 4.95 (s, 2H), 3.85 (s, 3H), 3.78 (s, 3H), 3.64 (s, br, 2H), 2.37 (s, 3H).



5-29 **5-28**

4-(Benzyloxy)-6,7-dimethoxy-3-methyl-11-oxo-11H-benzo[a]fluorene-5-carbonitrile (5-28)

To a solution of methyl 2-(3-(benzyloxy)-2-(cyanomethyl)-4-methylphenyl)-7-methoxy-3-oxo-3H-indene-1-carboxylate (137.9 mg, 0.304 mmol) in 5 mL anhydrous THF was added LDA in THF (0.335 mmol in 1 mL) slowly under ice-bath cooling and the solution was warmed up to room temperature and continued to stir for 2 hours. The reaction was quenched with 10 mL saturated aqueous NH_4Cl solution leading to the formation of some red-orange precipitate. The suspension was extracted with CH_2Cl_2 (3 × 50 mL) and then washed with H_2O (3 ×15 mL). The organic phase was dried over Na_2SO_4 and concentrated to obtain the crude product as a red orange solid (147.5 mg).

To a solution of the above crude product in acetone (30 mL) was added solid K_2CO_3 (126.1 mg, 0.91 mmol) at room temperature and the mixture was stirred for 15 minutes, followed by addition of CH_3I (0.06 mL, 0.91 mmol). The reaction was heated to reflux and stirred at this temperature for 5 hours. The solution was filtered and the precipitate was washed with CH_2Cl_2. The filtrate was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (CH_2Cl_2: Hexanes = 3:1, v/v) to give the product as an orange solid (63.5 mg, 48 % for two steps). Mp: 208–209 °C;^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.81 (d, J = 8.7 Hz, 1H), 7.60 (d, J = 6.6 Hz, 2H), 7.36 (m, 6H), 7.16 (dd, J = 8.0, 0.8 Hz, 1H), 5.06 (s,

2H), 4.01 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (75.5 MHz, CD_2Cl_2): δ 193.9, 159.4, 154.8, 150.8, 138.2, 136.7, 135.7, 133.1, 131.9, 131.7, 130.9, 130.3, 128.5, 128.3, 128.1, 127.4, 127.3, 120.5, 120.2, 116.9, 116.6, 106.9, 76.4, 63.4, 56.2, 16.6; IR (film): 3041.7, 2958.3, 2875.0, 2220.1, 1706.6, 1606.1, 1552.5, 1483.6, 1369.8, 1280.2, 1258.0, 1215.9, 1063.3, 948.3, 737.9 cm^{-1}; MS (EI): *m/z* 435.2 (21, M^+); 406.2 (54), 344.1 (100), 327.1 (28), 301.1 (12), 286.1 (7), 214.1 (9), 91.1 (65); HRMS (EI): Calc for $C_{28}H_{21}NO_4$: 435.1471, found: 435.1461.

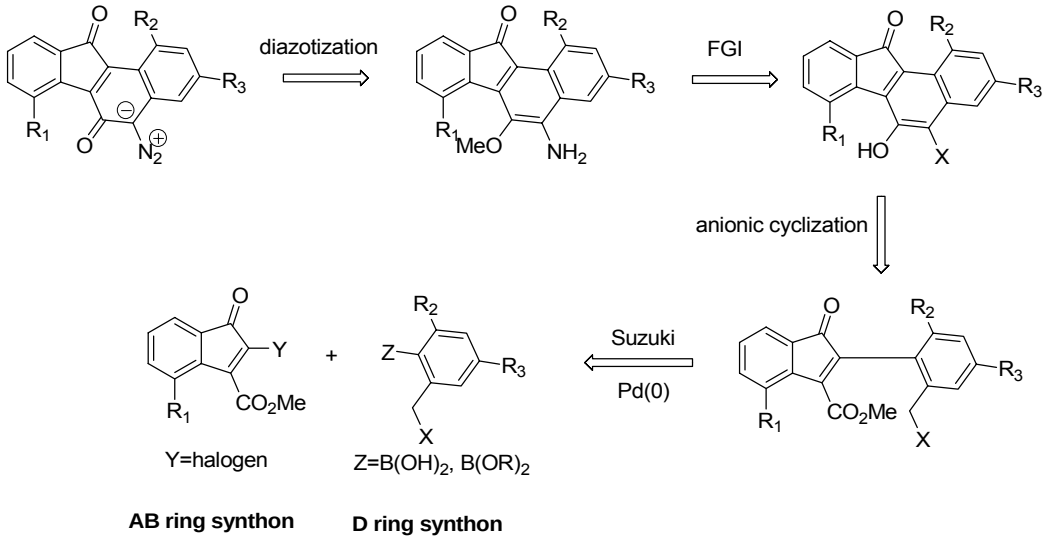
Chapter 6 Conclusions and Future Work

6.1 Conclusions

This research thesis is mainly focused on developing methodology to set up the benzo[*a*]fluorene skeleton, which provides a potential route to the total synthesis of isoprekinamycin and analogues of this natural product so as to study the relationship of the structure and bioactivities.

The synthetic strategy to IPK is based on the retrosynthetic analysis shown in scheme 6-1. Basically the strategy assumed that a suitably substituted precursor, which incorporates the AB and D rings of the natural product, might be induced to cyclize to provide the full benzo[*a*]fluorene system followed by selective functional group interconversion to provide the diazo group.

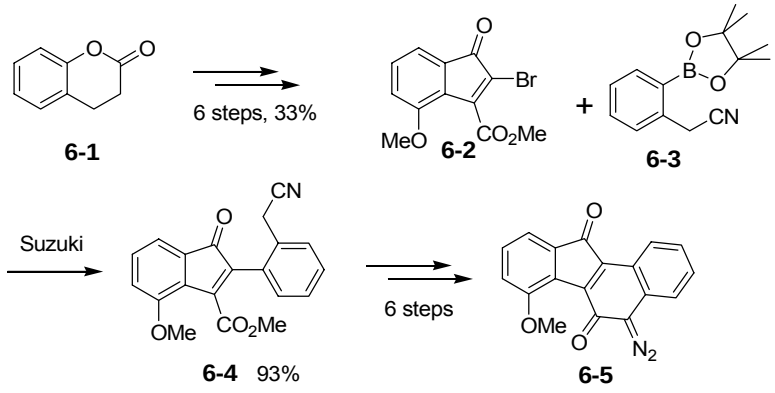
Scheme 6-1



In summary, the model of IPK **6-5** was synthesized as the sequence shown in Scheme 6-2.

The synthesis was initiated from the commercially available dihydrocoumarin **6-1** through six steps to generate AB ring synthon, bromoindenone **6-2,** in 33% yield. The Suzuki coupling of bromoindenone **6-2** and commercial available pinacol boronate **6-3** afforded diaryl intermediate **6-4**, which was followed by another six steps to achieve the synthesis of model compound **6-5**. Including the synthesis of bromoindenone **2-63**, the longest linear path of this synthesis is thirteen steps in 7% yield over all.

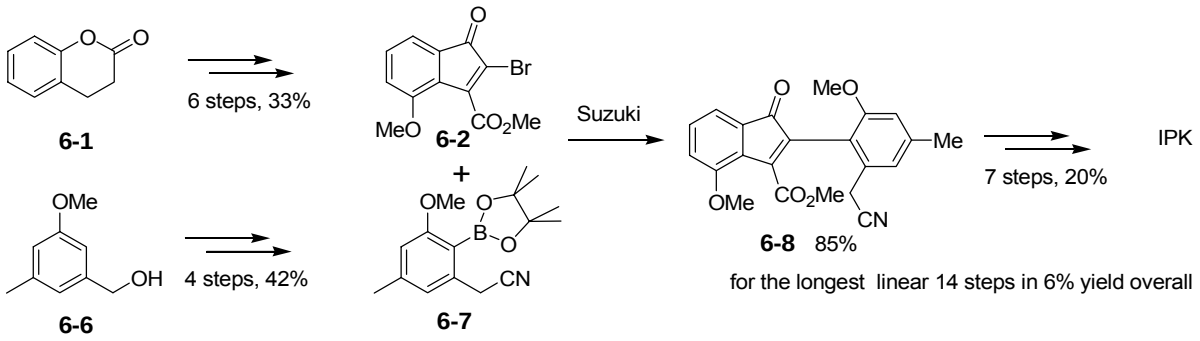
Scheme 6-2



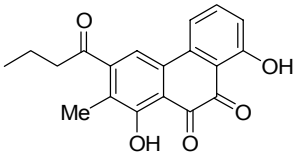
for the longest linear 13 steps in 7% yield overall

The first total synthesis of IPK was achieved via the sequence as presented in scheme 6-3. Suzuki coupling between **6-2** and the D ring building block pinacol boronate **6-7**, which was obtained from the known benzyl alcohol **6-6** through four steps in 42% yield, gave **6-8** in 85% yield. Another seven steps generated IPK. Overall, the longest linear path of this synthesis is fourteen steps and the overall yield from **6-1** is 6%.

Scheme 6-3



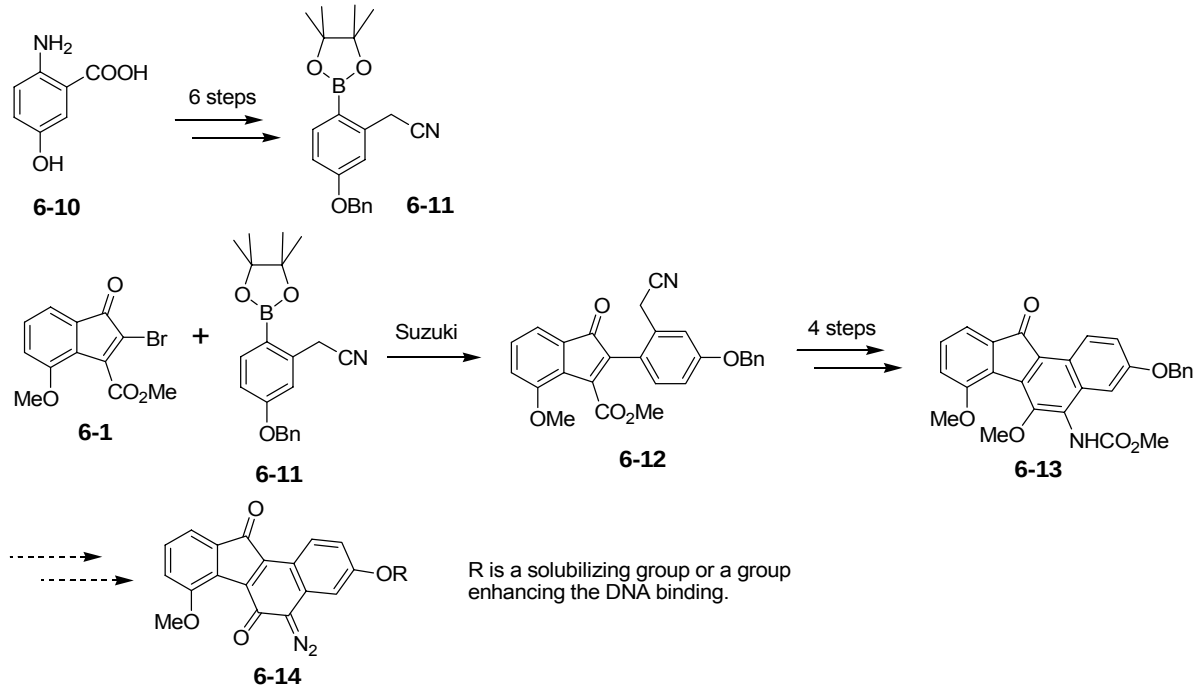
This method now provides a reliable method for producing IPK for biological study. The fermentation approach was found, in this laboratory, to be problematic since IPK production was not observed in some fermentation experiments while the red polyketide-derived compound called murayaquinone[1,2] **6-6** was found instead.



6-9

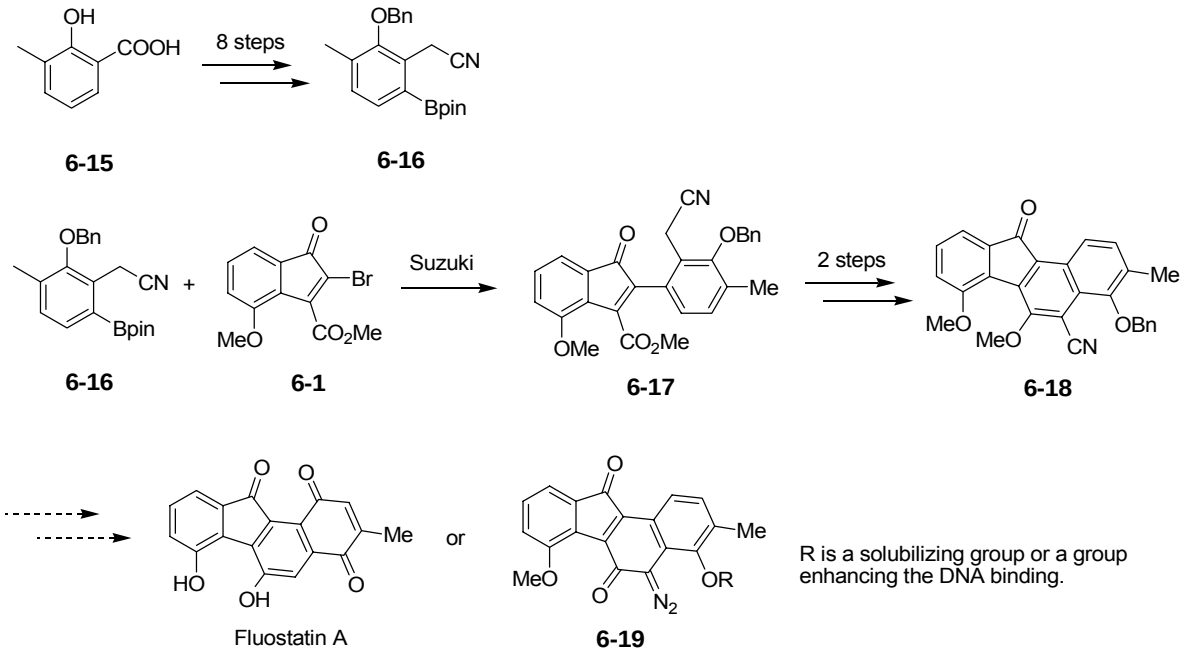
An analogue of IPK **6-14** was designed, incorporating a side chain at C_3 of D ring in order to improve solubility and potentially the affinity of the molecule with DNA. The carbamate **6-13**, a precursor of **6-14**, was synthesized as shown in scheme 6-4 using the same strategy as the model of IPK through eleven steps in 14% yield overall.

Scheme 6-4



The benzo[*a*]fluorene **6-18** was synthesized in 6% overall yield, via an eleven step sequence initiated from 3-methyl salicylic acid (**6-15**) shown in the scheme 6-5. The benzo[*a*]fluorene **6-18** is intended to be the key intermediate for the total synthesis of fluostatin A and the synthesis of analogues of IPK **6-19** with a side chain at the C_4 of the D ring.

Scheme 6-5



6.2 Future Work

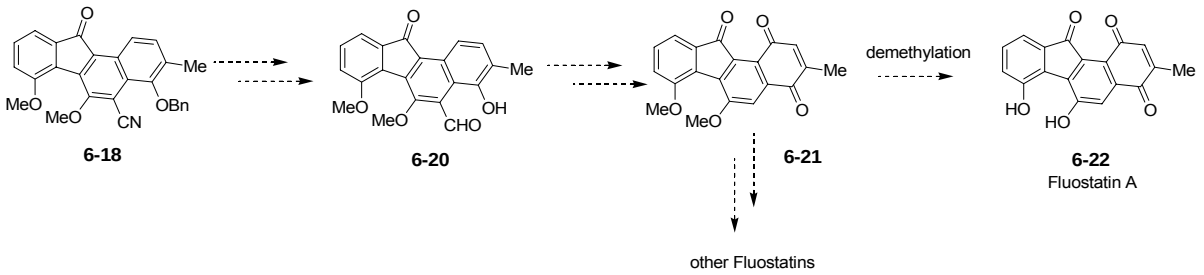
For the future work, the methodology for the synthesis of model **6-5** and IPK will be used for the synthesis of various analogues to study the mode of action of IPK and kinamycins systematically. The goals of this work should be to:

1. Introduce side chains at D ring to improve the solubility of the analogues of IPK (e.g. **6-14** and **6-19**).
2. Use handles at D ring to attach group to increase DNA binding ability of the analogues of IPK (e.g. **6-14** and **6-19**).

In principle, the above two goals might be achieved via either C_3 or C_4 substituted analogues discussed in this thesis. However, it is suggested that the C_3 substituted variations will be the most readily achievable via **6-13**, since the chemistry of C_4-benzyloxy substituted intermediate **6-18** was found to be problematic in the present study.

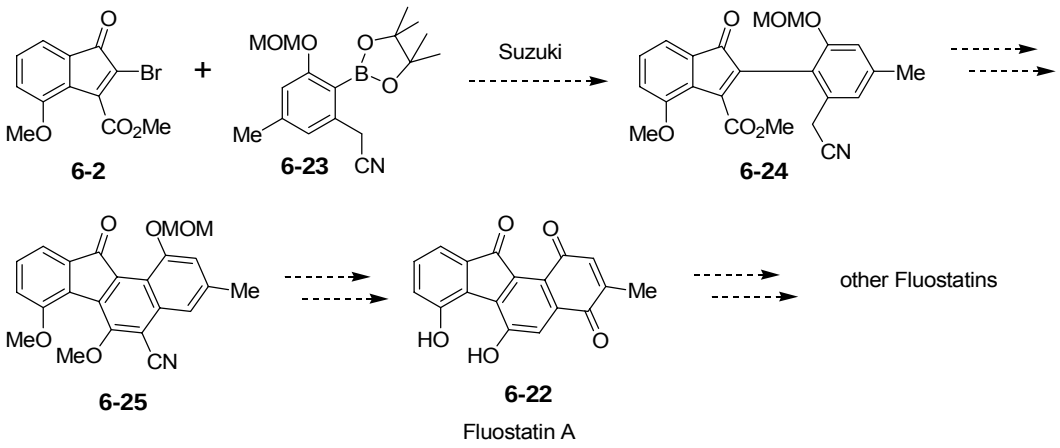
Furthermore, benzo[*a*]fluorene **6-18** should be studied as a precursor for the total synthesis of fluostatin A and other fluostatins. As shown below, debenzylation of **6-18**, followed by reduction to give the aldehyde **6-20**, is recommended. This should relieve some of the steric effect which made the modification of the cyano group in **6-18** very difficult in the present study. Reduction of the cyano group to the aldehyde using completely oxygen-free conditions for the DIBAL reduction is also recommended. Decarbonylation might then be accomplished with Wilkinson's catalyst,[3] which is followed by oxidation to eventually generate **6-21**.

Scheme 6-6



However, an alternative approach to fluostatins would involve the use of a strategy very similar to that used in the total synthesis of IPK (chapter 3). In this approach, different protection of the A and D ring oxygenation and a reduction-reoxidation sequence to avoid the competing intramolecular aldol condensation in the ring-C forming step would be required.

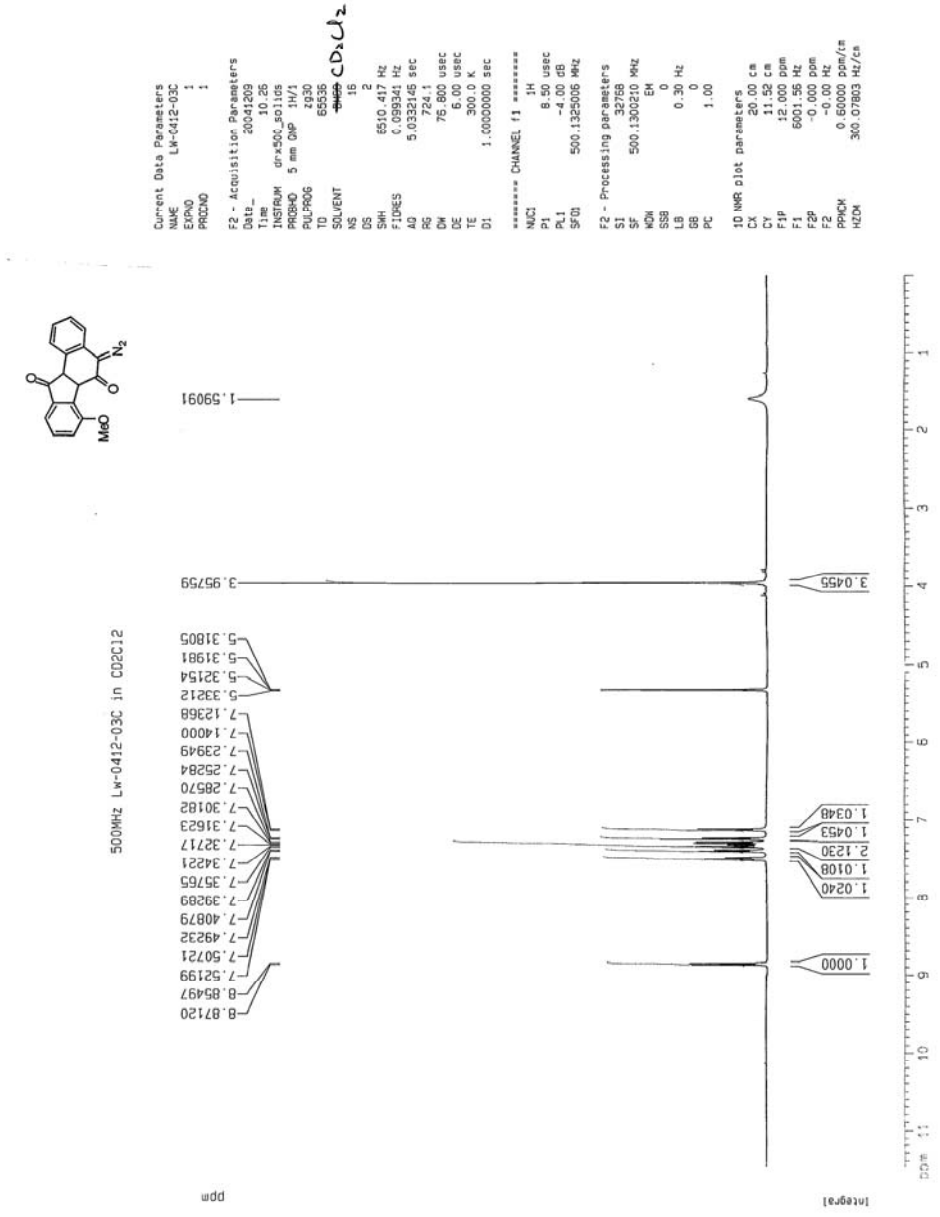
Scheme 6-7


O
Br
MeO
CO2Me
6-2
+
MOMO
O
B
O
Me
CN
6-23
Suzuki
O
MOMO
Me
MeO
CO2Me
CN
6-24
O
OMOM
Me
MeO
MeO
CN
6-25
O
O
Me
HO
HO
O
6-22
Fluostatin A
other Fluostatins

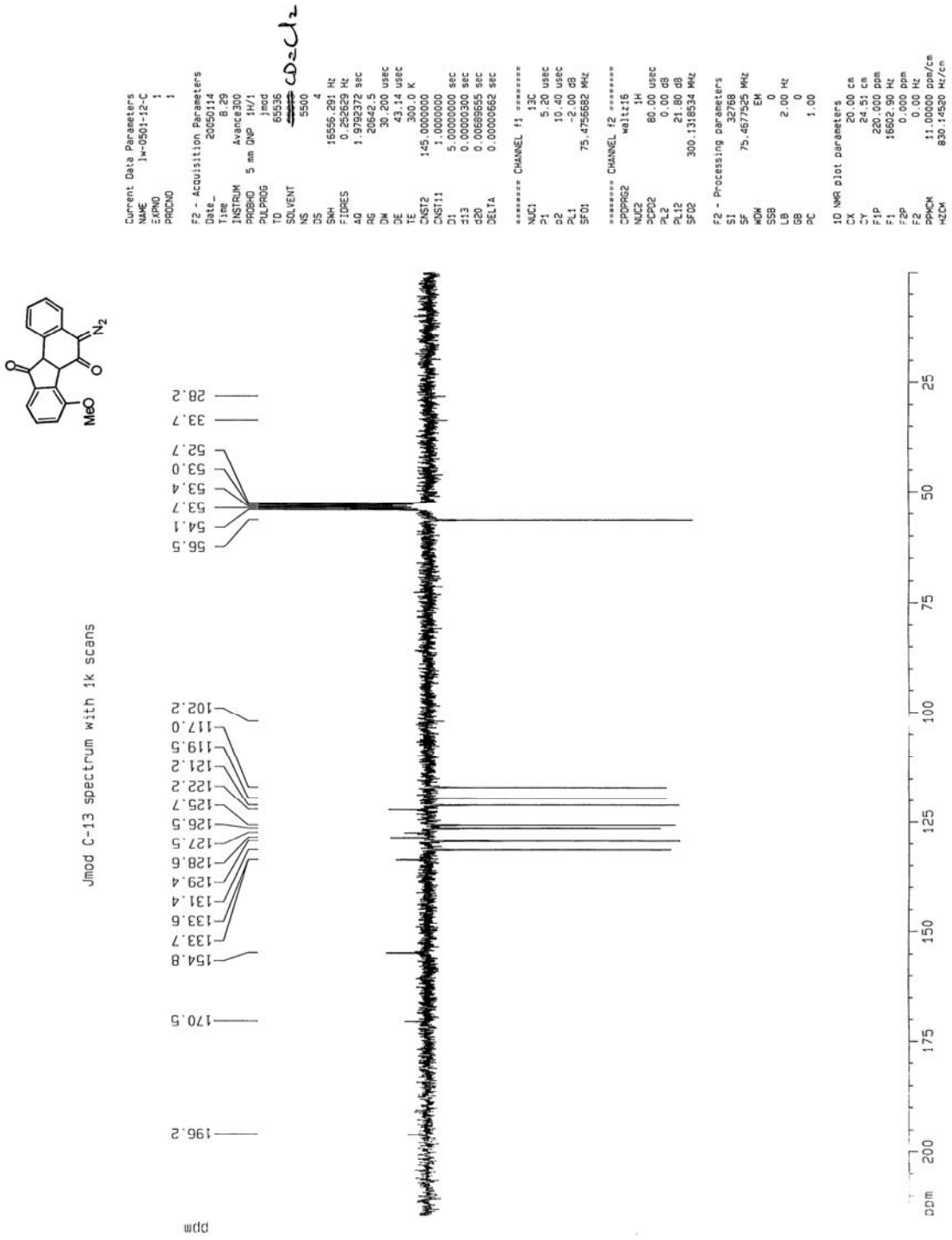
Appendix

Appendix for Chapter 2

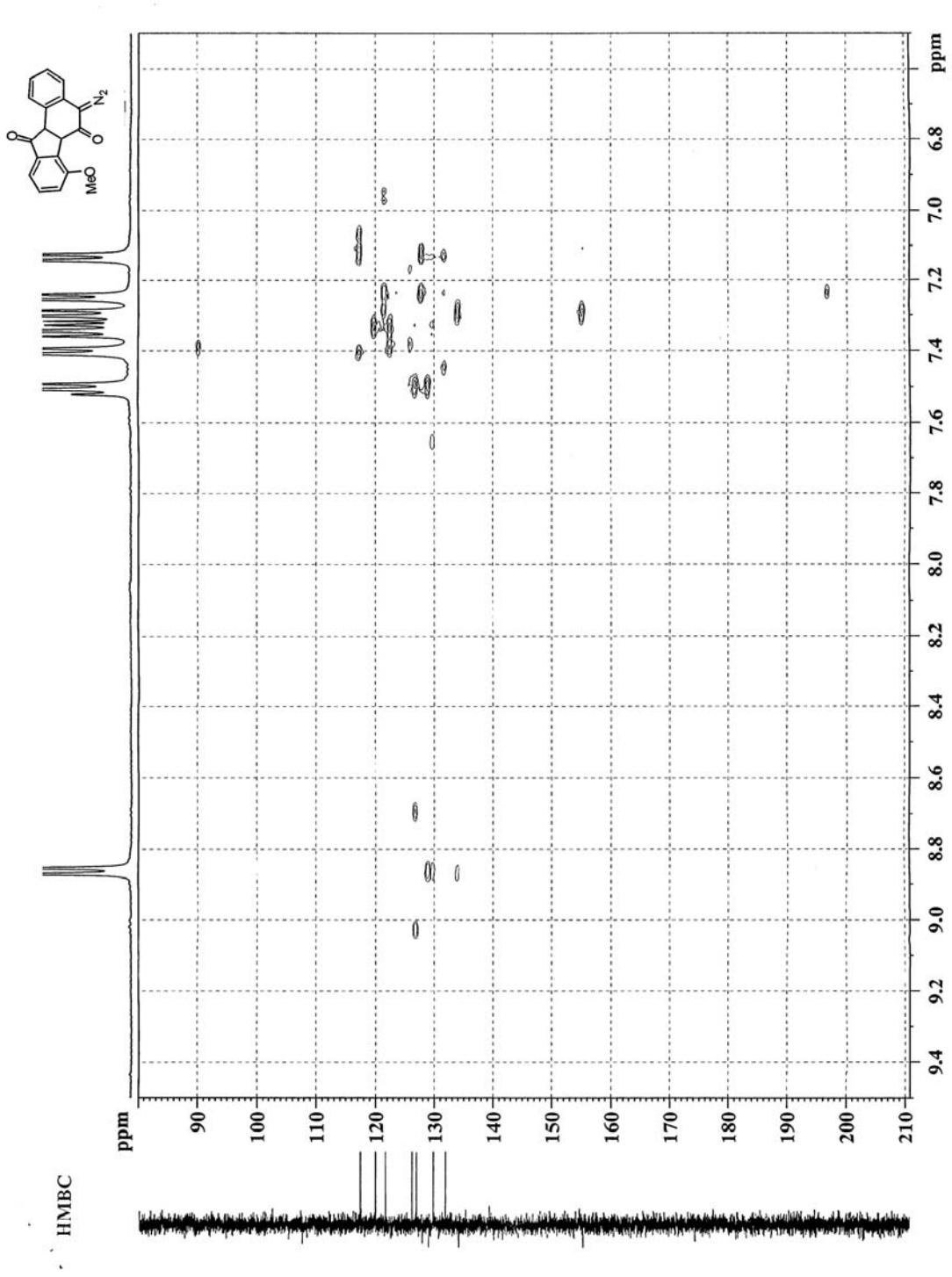
Appendix 2-1 ^{1}H NMR Spectrum of Model Compound 2-58



Appendix 2-2 ^{13}C NMR Spectrum of Model Compound 2-58



Appendix 2-3 HMBC Spectrum of Model Compound 2-58



Appendix 2-4 X-ray Crystallographic Structure of Compound 2-58

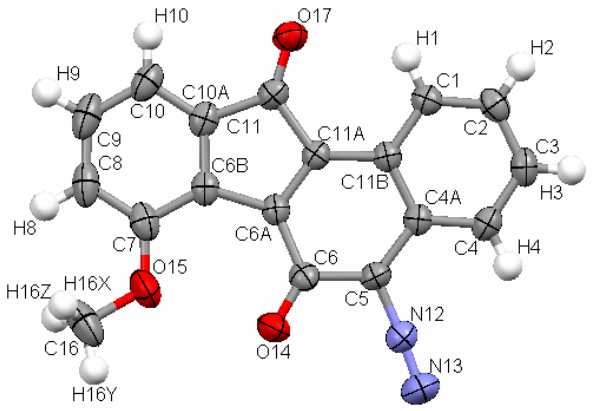


Table 1 Crystal Data and Structure Refinement of Compound **2-58**

Empirical formula	C18 H10 N2 O3
Formula weight	302.28
Cell measurement temperature	180(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, $Pna2_1$
Unit cell dimensions	a = 19.6350(16) Å, b = 4.6545(4) Å, c = 14.8606(12) Å
Volume, Z	1358.13(19) $Å^3$, 4
Density	1.478 g/cm^3
Absorption coefficient	0.103 mm^{-1}
F(000)	624
Crystal size	0.24 mm × 0.11 mm × 0.11 mm
2θ range for data collection	2.07 to 28.02°
Limiting indices	-24 < h < 25, -6 < k < 6, -19 < l < 19
Reflections collected / unique	9029 / 1707 [$R_{(int)}$ = 0.0464]
Completeness to θ = 28.02	100.0 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1707 / 1 / 210
Goodness-of-fit on F^2	1.044
Final R indices [I>2σ(I)]	R1 = 0.0344, wR2 = 0.0656
R indices (all data)	R1 = 0.0414, wR2 = 0.0673
Largest diff. peak and hole	0.158 and -0.162 e-.$Å^{-3}$

Table 2 Atomic Coordinates and Equivalent Isotropic Displacement Parameters

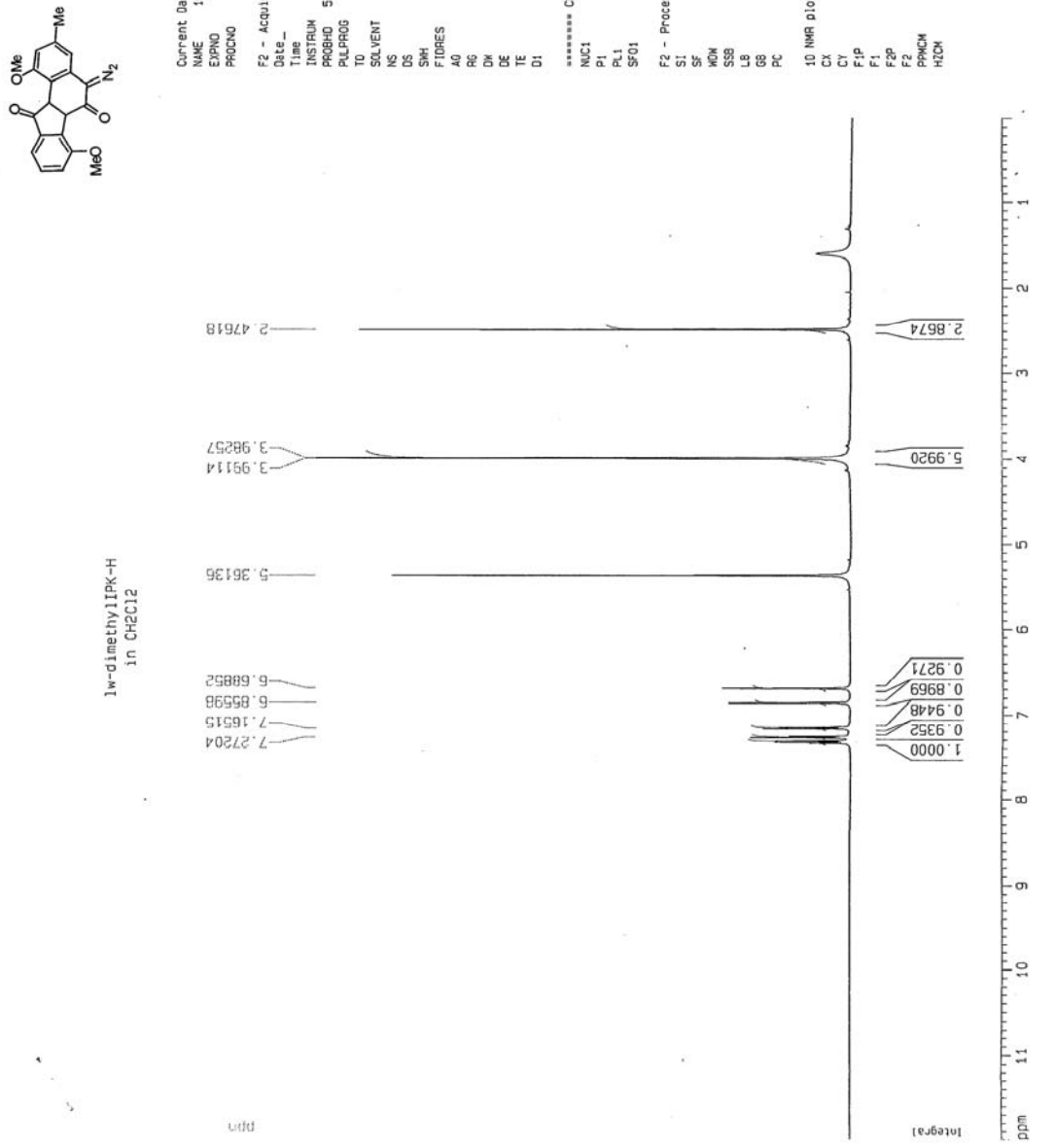
	x/a	y/b	z/c	U_{eq}
C(1)	-246(1)	805(4)	2184(2)	32(1)
C(2)	-620(1)	-1272(4)	1748(2)	38(1)
C(3)	-470(1)	-2009(4)	866(2)	39(1)
C(4)	51(1)	-672(4)	410(2)	36(1)
C(4A)	438(1)	1458(4)	840(2)	30(1)
C(5)	984(1)	2979(4)	413(1)	33(1)
C(6)	1386(1)	5326(4)	785(1)	33(1)
C(6A)	1236(1)	5799(4)	1740(2)	29(1)
C(6B)	1573(1)	7761(4)	2400(2)	33(1)
C(7)	2131(1)	9627(5)	2362(2)	41(1)
C(8)	2317(1)	11128(5)	3144(2)	49(1)
C(9)	1964(1)	10809(5)	3934(2)	52(1)
C(10)	1406(1)	8988(5)	3984(2)	44(1)
C(10A)	1225(1)	7525(4)	3214(2)	34(1)
C(11)	658(1)	5458(4)	3104(1)	32(1)
C(11A)	712(1)	4365(4)	2154(1)	28(1)
C(11B)	289(1)	2214(4)	1741(1)	28(1)
N(12)	1154(1)	2327(4)	-432(1)	41(1)
N(13)	1330(1)	1849(5)	-1130(2)	60(1)
O(14)	1790(1)	6697(3)	323(1)	48(1)
O(15)	2470(1)	9880(4)	1567(1)	60(1)
C(16)	2976(1)	12049(6)	1477(2)	65(1)
O(17)	232(1)	4848(3)	3667(1)	43(1)
H(1)	-350	1287	2790	39
H(2)	-984	-2204	2053	45
H(3)	-730	-3454	573	46
H(4)	148	-1189	-194	43
H(8)	2696	12396	3125	59
H(9)	2104	11844	4454	62
H(10)	1157	8760	4528	53
H(16X)	2781	13917	1639	97
H(16Y)	3136	12108	852	97
H(16Z)	3360	11615	1876	97

Table 3 Bond Lengths and Bond Angles

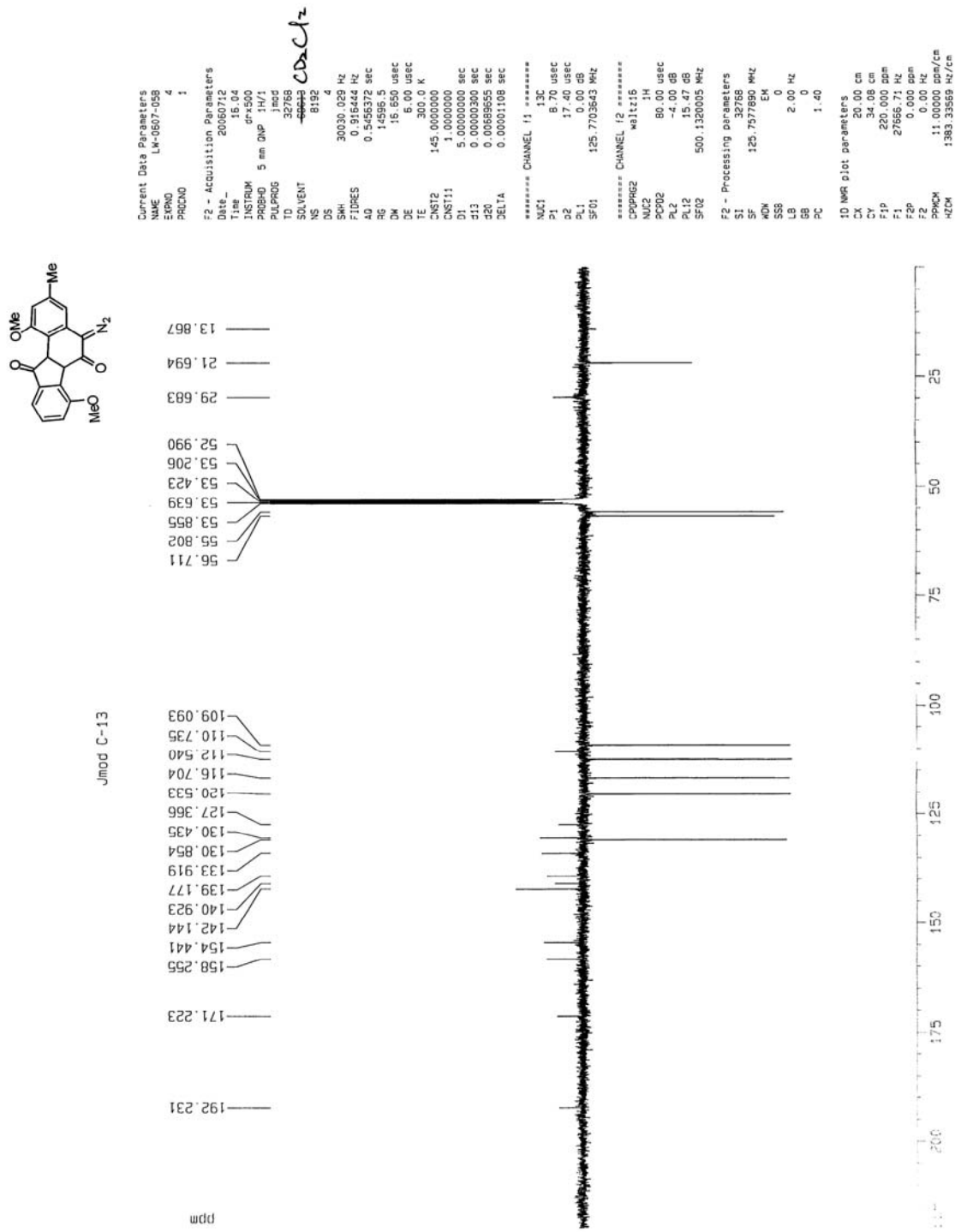
	Length [Å]		Angle [°]
C1-C2	1.377(3)	C2-C1-C11B	120.5(2)
C1-C11B	1.403(3)	C1-C2-C3	120.3(2)
C2-C3	1.387(3)	C4-C3-C2	120.8(2)
C3-C4	1.375(3)	C3-C4-C4A	119.9(2)
C4-C4A	1.403(3)	C4-C4A-C11B	119.68(19)
C4A-C11B	1.414(3)	C4-C4A-C5	123.5(2)
C4A-C5	1.434(3)	C11B-C4A-C5	116.77(18)
C5-N12	1.334(3)	N12-C5-C4A	119.46(19)
C5-C6	1.457(3)	N12-C5-C6	113.06(19)
C6-O14	1.227(2)	C4A-C5-C6	127.44(18)
C6-C6A	1.466(3)	O14-C6-C5	121.83(19)
C6A-C11A	1.374(3)	O14-C6-C6A	126.4(2)
C6A-C6B	1.495(3)	C5-C6-C6A	111.77(18)
C6B-C10A	1.392(3)	C11A-C6A-C6	120.79(19)
C6B-C7	1.400(3)	C11A-C6A-C6B	109.49(18)
C7-O15	1.360(3)	C6-C6A-C6B	129.72(19)
C7-C8	1.403(4)	C10A-C6B-C7	117.9(2)
C8-C9	1.372(4)	C10A-C6B-C6A	107.80(18)
C9-C10	1.389(4)	C7-C6B-C6A	134.3(2)
C10-C10A	1.378(3)	O15-C7-C6B	118.1(2)
C10A-C11	1.481(3)	O15-C7-C8	123.3(2)
C11-O17	1.216(2)	C6B-C7-C8	118.6(2)
C11-C11A	1.504(3)	C9-C8-C7	121.6(2)
C11A-C11B	1.438(3)	C8-C9-C10	120.7(2)
N12-N13	1.116(3)	C10A-C10-C9	117.4(2)
O15-C16	1.423(3)	C10-C10A-C6B	123.8(2)
		C10-C10A-C11	127.3(2)
		C6B-C10A-C11	108.93(19)
		O17-C11-C10A	126.4(2)
		O17-C11-C11A	128.0(2)
		C10A-C11-C11A	105.68(19)
		C6A-C11A-C11B	125.40(19)
		C6A-C11A-C11	108.00(18)
		C11B-C11A-C11	126.60(19)
		C1-C11B-C4A	118.88(19)
		C1-C11B-C11A	123.80(19)
		C4A-C11B-C11A	117.31(18)
		N13-N12-C5	176.3(2)
		C7-O15-C16	119.0(2)

Appendix for Chapter 3

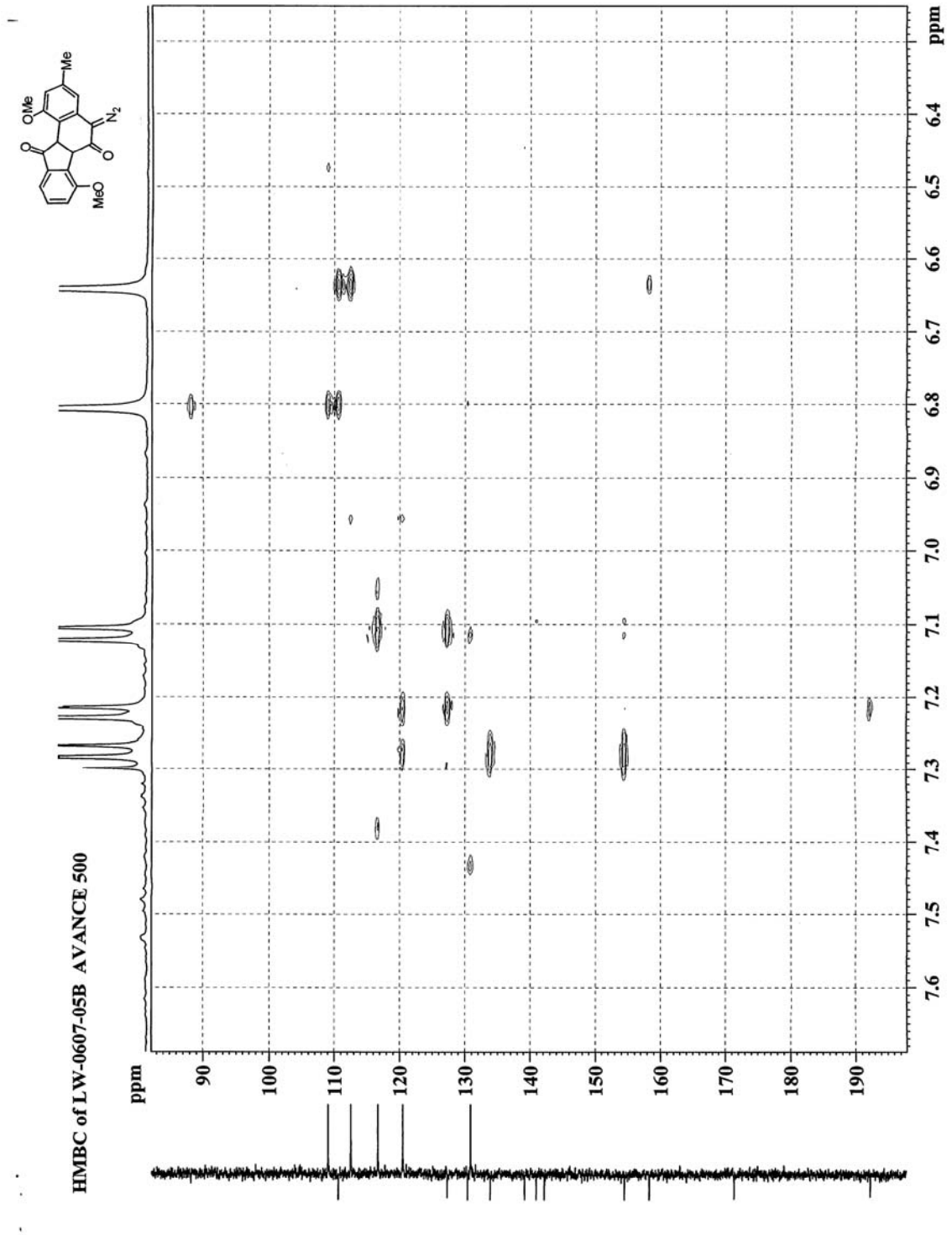
Appendix 3-1 ^{1}H NMR Spectrum of dimethylisoprekinamycin (3-2)



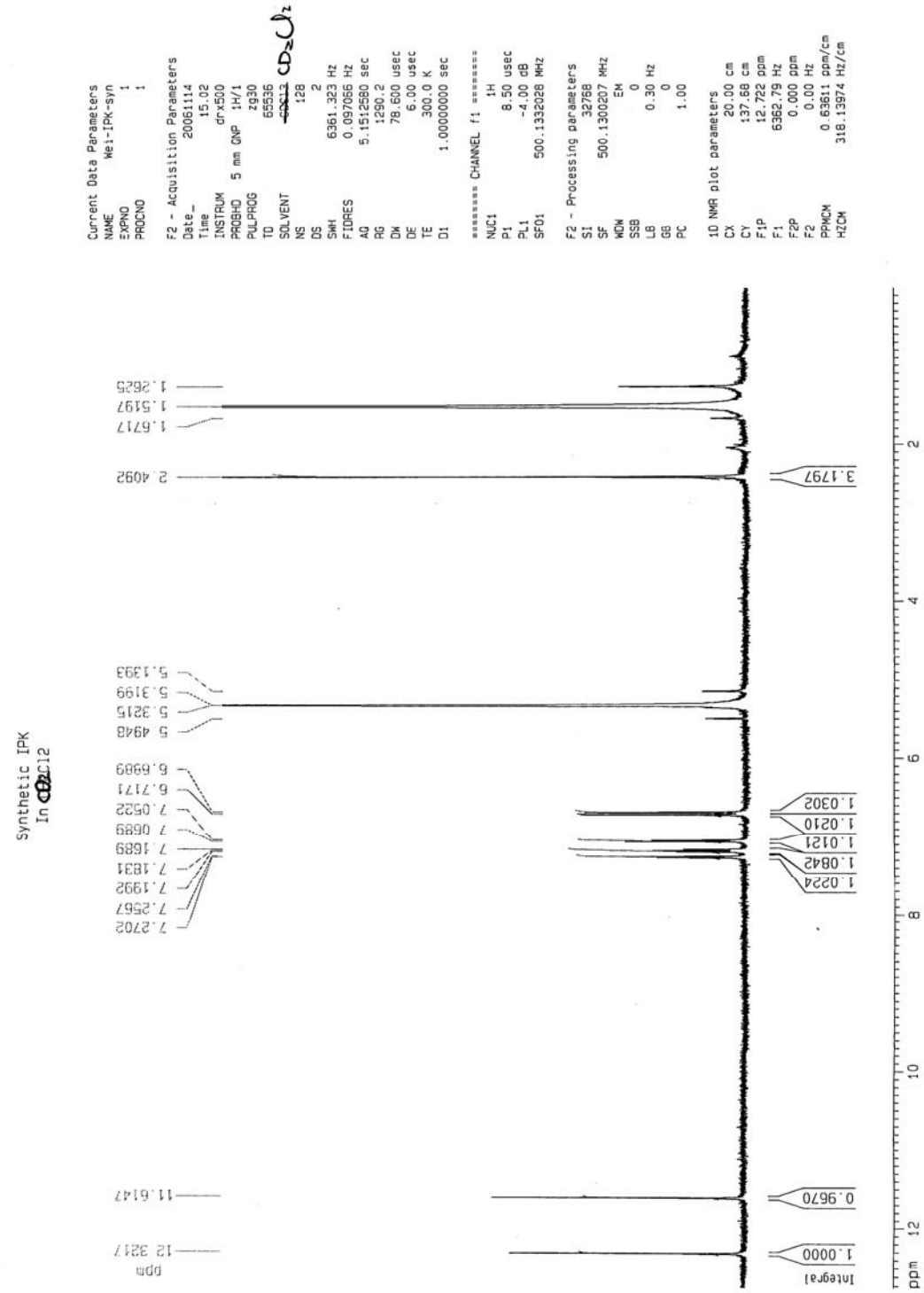
Appendix 3-2 ^{13}C NMR Spectrum of dimethylisoprekinamycin (3-2)



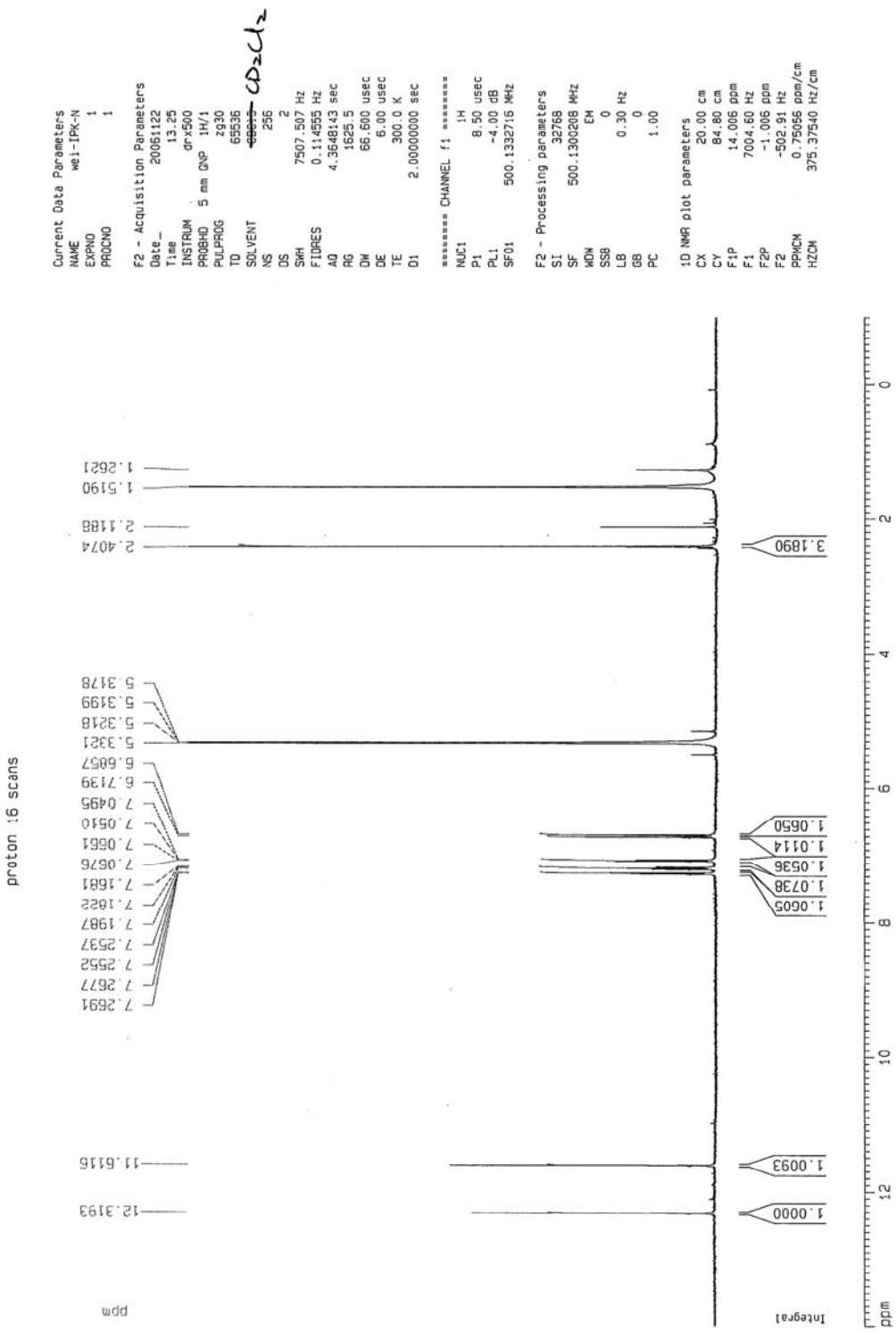
Appendix 3-3 HMBC Spectrum of dimethylisoprekinamycin (3-2)



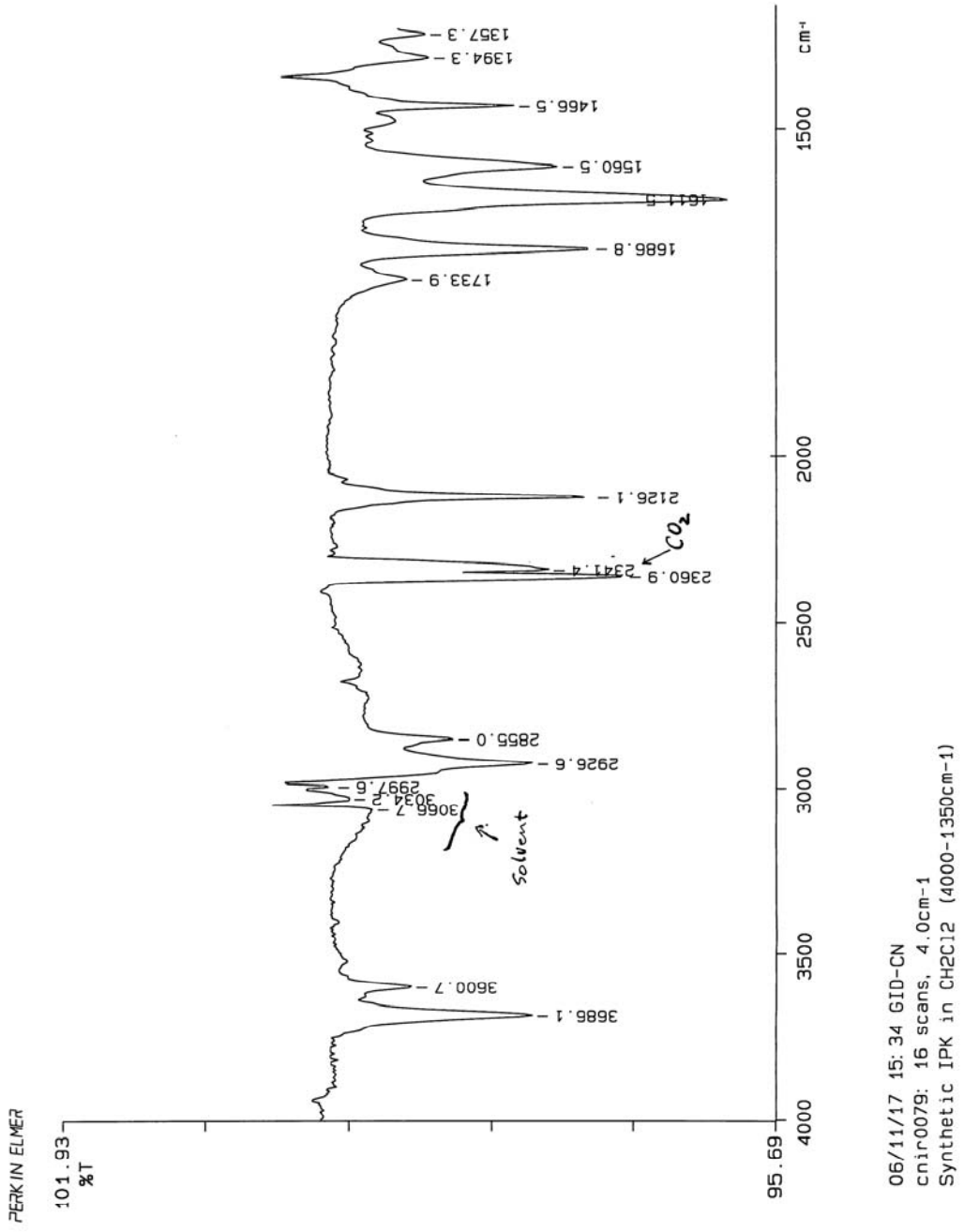
Appendix 3-4 ^{1}H NMR Spectrum of Synthetic Isoprekinamycin (3-1)



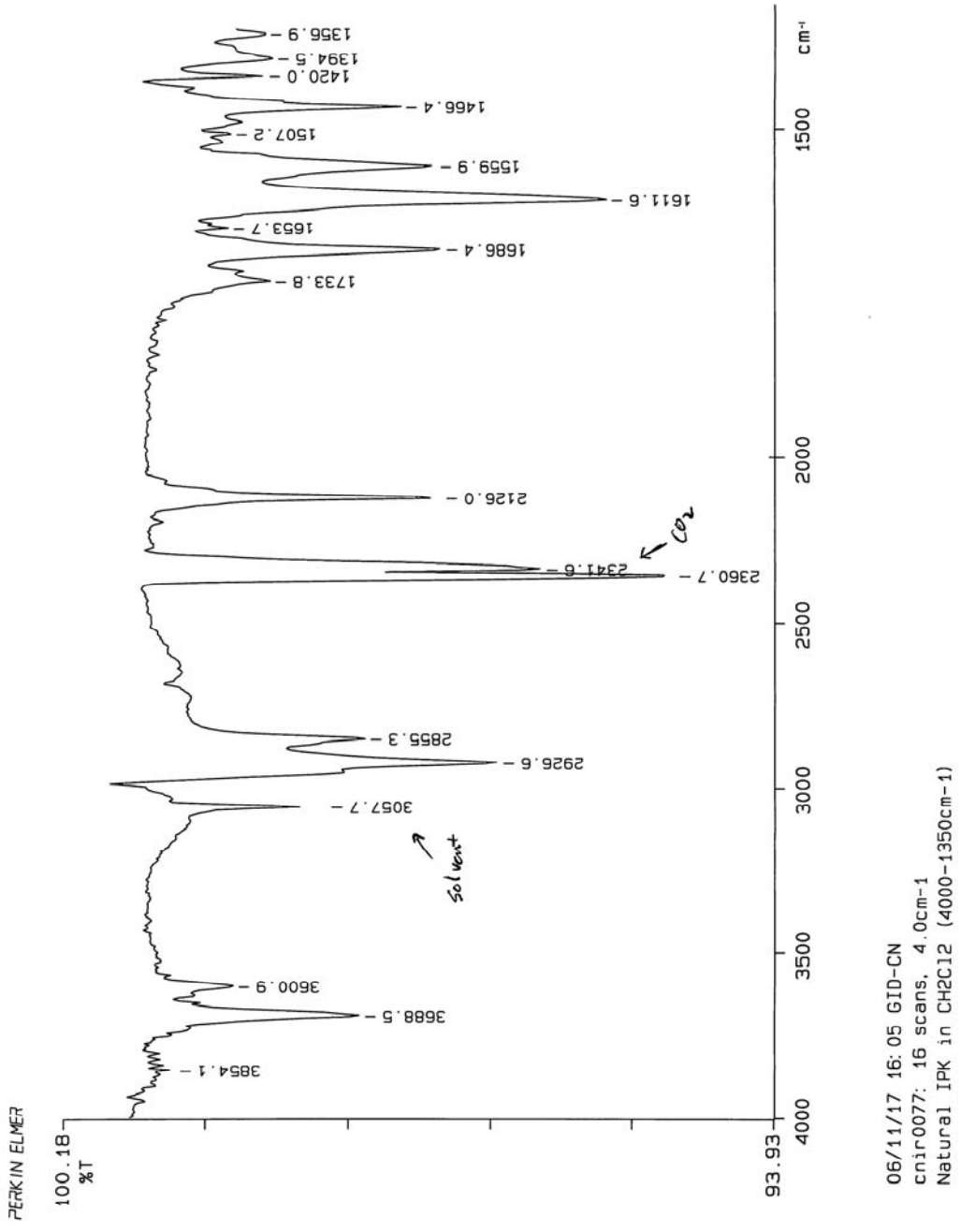
Appendix 3-5 ^{1}H NMR Spectrum of Natural Isoprekinamycin (3-1)



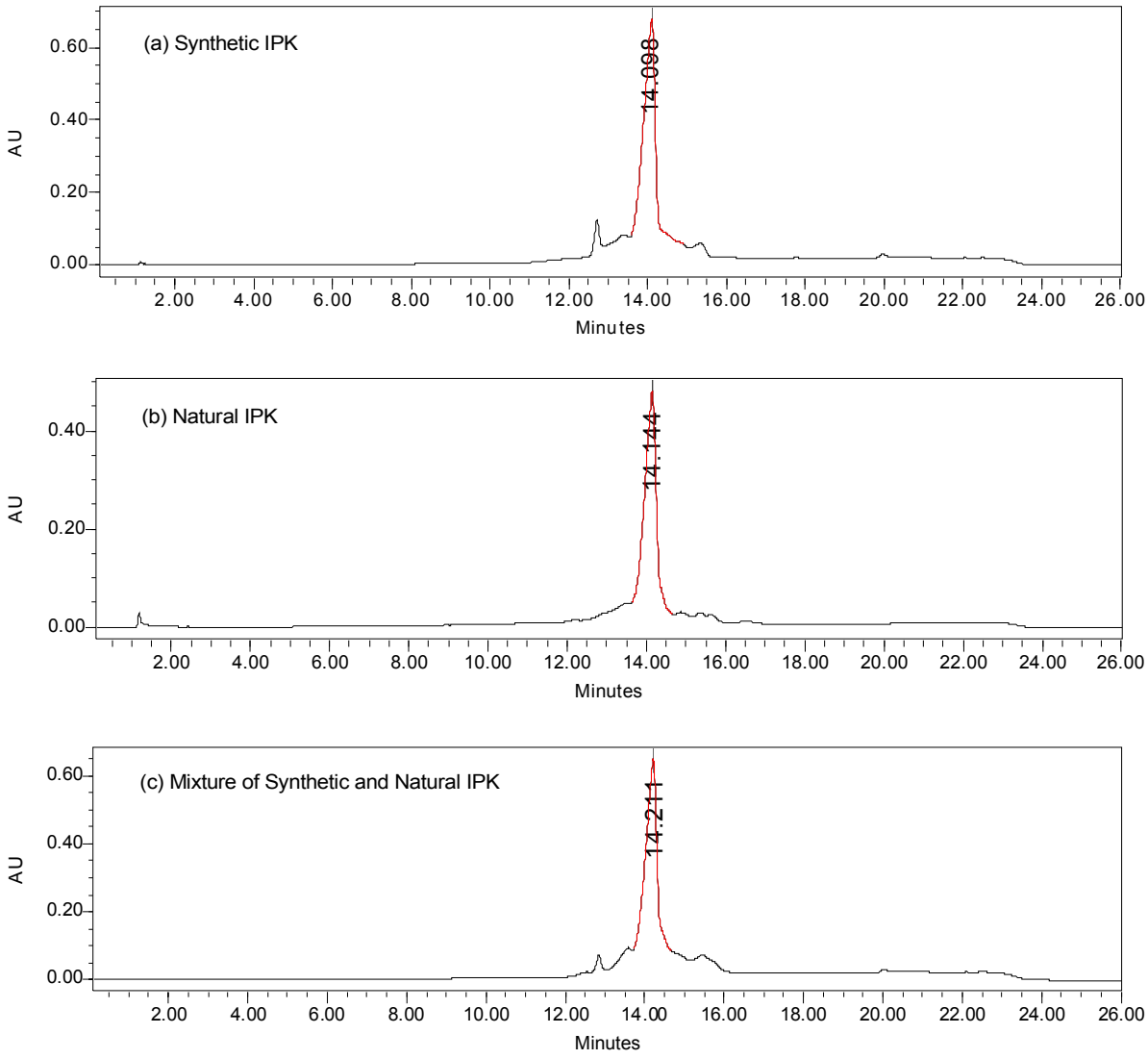
Appendix 3-6 IR Spectrum of Synthetic Isoprekinamycin (3-1)



Appendix 3-7 IR Spectrum of Natural Isoprekinamycin (3-1)



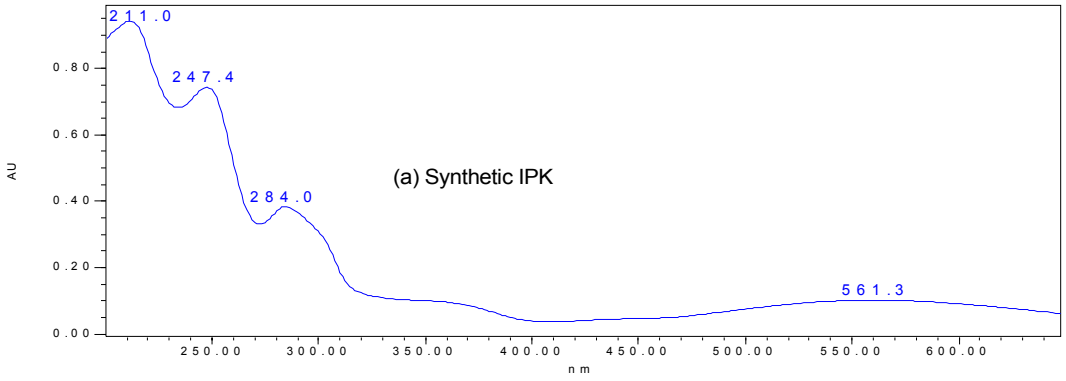
Appendix 3-8 HPLC Comparison of Synthetic and Natural Isoprekinamycin (3-1)

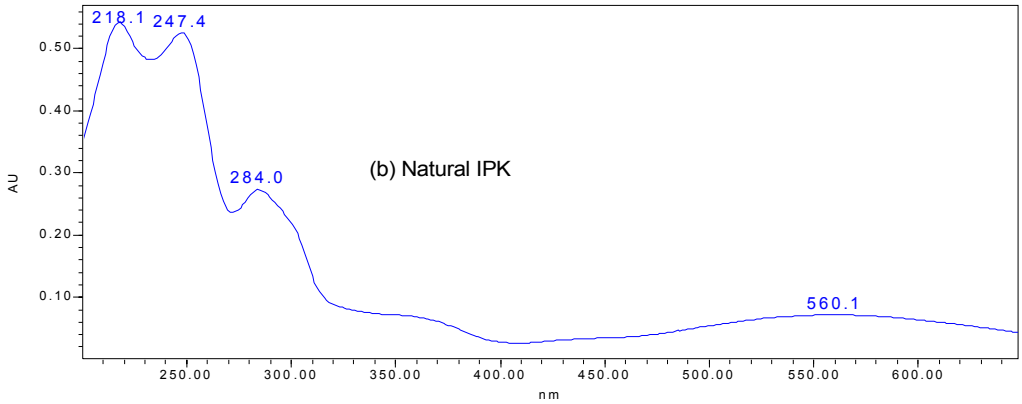


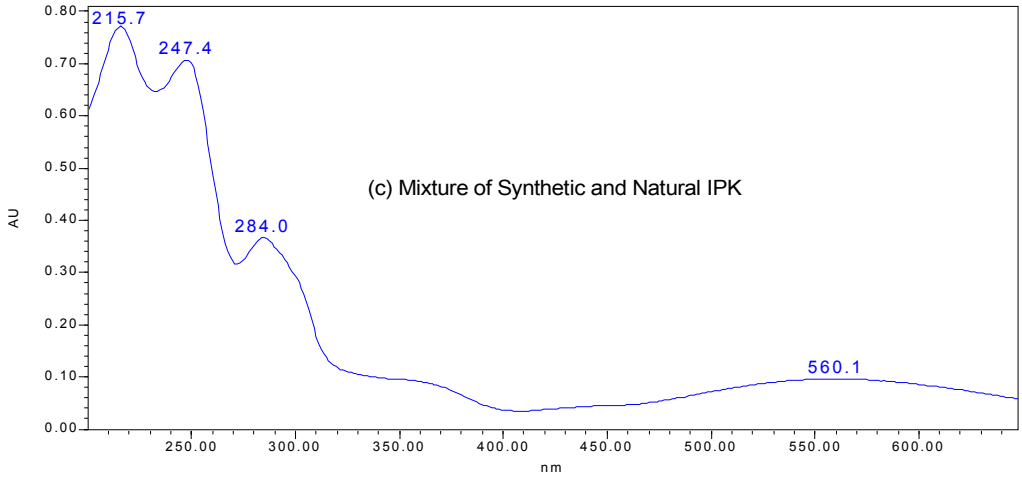
Comparison of the HPLC retention times of the synthetic and natural isoprekinamycin (IPK) with the following experimental setup and conditions: (1) HPLC system: Waters 600 controller, Waters 996 photodiode array detector, Waters Millennium® software; (2) Column: Nova-Pak® C18 60 Å 4 μm, 3.9 × 150 mm; (3) linear gradient (20 minutes): 94% H_2O, 5%

CH_3CN and 0.1% AcOH to 5% H_2O, 94% CH_3CN and 0.1% AcOH at a flow rate of 1.5 mL/minute at room temperature.

Appendix 3-9 UV-vis Spectra Comparison of Synthetic and Natural Isoprekinamycin (3-1)







Appendix 3-10 Description of the molecular orbital computational methods used

All molecular orbital calculations were carried out using the Gaussian 03[30] software package. All input geometries were constructed using the Gaussview 3.08 graphical interface.[31] The electronic energies that are quoted were computed using the AM1 semi-empirical molecular orbital method within Gaussian 03 with the OPTFREQ options which effects a full geometry optimization and a full calculation of the vibrational frequencies. All of the computed results quoted in Chapter 3 correspond to minimized structures for which all vibrational frequencies are positive.

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