DISSERTATION

ASYMMETRIC TOTAL SYNTHESIS OF (-)-RENIERAMYCIN G AND STUDIES TOWARD THE TOTAL SYNTHESIS OF ECTEINASCIDIN-743

Submitted by
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WE HEREBY RECOMMENED THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY WEI JIN ENTITLED ASYMMETRIC TOTAL SYNTHESIS OF (-)-RENIERAMYCIN G AND STUDIES TOWARD THE TOTAL SYNTHESIS OF ECTEINASCIDIN-743 BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head

ABSTRACT OF DISSERTATION

ASYMMETRIC TOTAL SYNTHESIS OF (-)-RENIERAMYCIN G AND STUDIES TOWARD THE TOTAL SYNTHESIS OF ECTEINASCIDIN-743:

An efficient and asymmetric synthesis of a highly functionalized tetrahydroisoquinoline is presented, of which the key reactions are a sequential asymmetric Staudinger ketene-imine β-lactam-forming reaction and a Pictet-Spengler cyclization.

An efficient method to make a versatile chiral amino acid for tetrahydroisoquinoline antitumor alkaloids is also reported. The synthesis features an alkylation reaction with a chiral glycinate template that sets the stereocenter of the amino acid.

Additionally, the coupling of the tetrahydroisoquinoline and the amino acid leads to the asymmetric synthesis of a versatile pentacycle, which could potentially be used in the total synthesis of members of the ecteinascidin/saframycin/safracin/renieramycin family of antitumor alkaloids.

Finally, the approach to construct the pentacycle is applied to the first asymmetric total synthesis of (-)-renieramycin G.

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I am sure that there are others that I have slighted by not mentioning them in these pages, so I will give a broad thanks to all.

Contents

CHAPTER 1

Syntheses of Ecteinascidins and Saframycins

1.1 The tetrahydroisoquinoline antitumor antibiotics

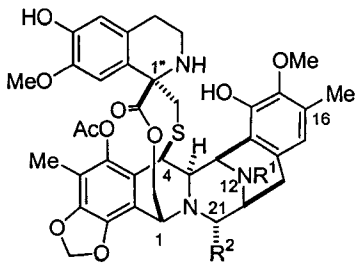
The antitumor antibiotics belonging to the tetrahydroisoquinoline family have been studied thoroughly over the past 30 years starting with the isolation of naphthyridinomycin in 1974. To date, numerous natural products in this family have been isolated. [1] The tetrahydroisoquinolines include potent cytotoxic agents that display a range of antitumor activities, antimicrobial activity, and other biological activities.

One member of the tetrahydroisoquinoline family is the saframycin class of natural products. Some representatives of this family are the ecteinascidins, saframycins, safracins and the renieramycins. In the following review, the syntheses as well as the semi-syntheses of the ecteinascidins and the saframycins are covered.

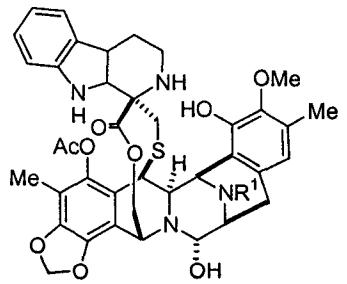
1.2 The ecteinascidins (Et's)

The isolation of the ecteinascidins (Et's) was first reported by Reinhart et al. in 1990.[2] In this report, the isolation of six ecteinascidins including Et-743 (**1**), 729 (**2**), 745 (**3**), 759A, 759B (**4**), and 770 (**5**) were reported (Figure 1). The structures for Et-743 (**1**) and 729 (**2**) with the correct relative stereochemistry were reported by the Reinhart and Wright[3] groups simultaneously. The structures were determined by extensive NMR and mass spectral studies. In 1992, Reinhart et al. published the isolation of Et-722 (**8**), 736 (**9**), and 734 N^{12}-oxide (**6**).[4] Crystal structures for **6** and **7** (a synthetic derivative of **11**) were also obtained to confirm the structures of the ecteinascidins.[4] Four putative biosynthetic precursors (Et-594 (**10**), 597 (**11**), 583 (**12**), and 596 (**13**)) were isolated in

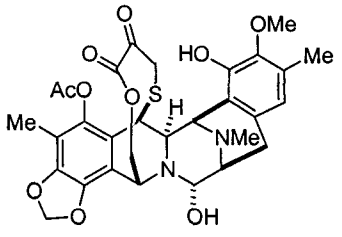
1996 by Reinhart et al.[5] In this report, the absolute stereochemistry of the ecteinascidins was determined via elucidation of the stereochemistry of the derivatized cysteine residue that was cleaved from **11**.



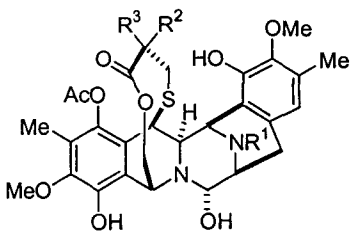
1 ET-743 R^1=Me, R^2=OH
2 ET-729 R^1=H, R^2=OH
3 ET-745 R^1=Me, R^2=H
4 ET-759 R^1=Me, R^2=OH, S-oxide
5 ET-770 R^1=Me, R^2=CN
6 Et-743 N^{12}-Oxide, R^1=(O)Me, R^2=OH
7 R^1=CHO, R^2=OMe



8 ET-722 R_1=H
9 ET-736 R_1=Me



10 ET-594



11 ET-597 R_1=Me, R_2=H, R_3=NH_2
12 ET-583 R_1=H, R_2=H, R_3=NH_2
13 ET-596 R_1=Me, R_2, R_3=O

Figure 1. The ecteinascidins

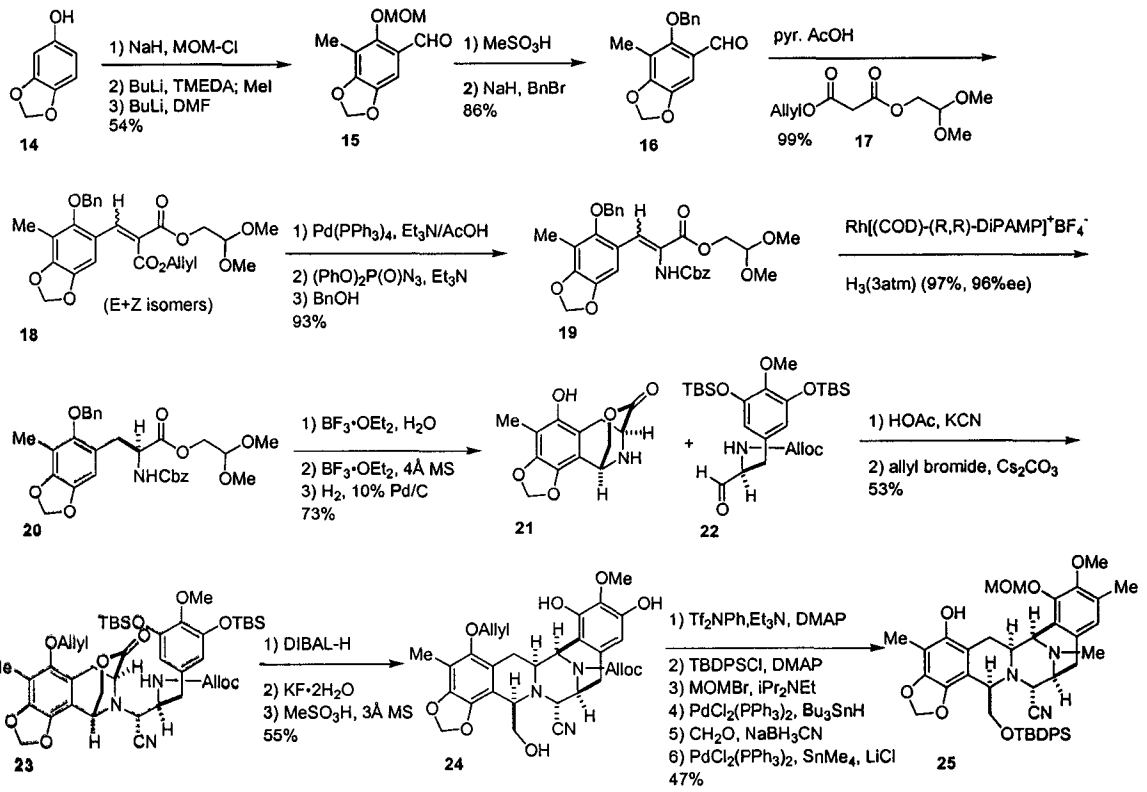
1.2.1 Total syntheses of the ecteinascidins

Currently, there have been three syntheses of ecteinascidin-743 (Et-743), including two total syntheses and one semi-synthesis. Corey et al. published the first total synthesis of Et-743 in 1996.[6] This was followed by a semi-synthetic route involving the conversion of cyanosafracin B to Et-743 by Cuevas et al. in 2000.[7] In 2002, Fukuyama et

al. reported another total synthesis of Et-743.[8] In 2003, Cuevas et al. reported the semi-synthesis of several ecteinascidins including Et-729, Et-745, Et-759B, Et-736, Et-637, and Et-594.[9]

1.2.1.1 Corey's total synthesis of ecteinascidin-743

In 1996, Corey and coworkers published the first total synthesis of ecteinascidin-743,[6] which was enantio- and stereo-controlled, convergent and concise.



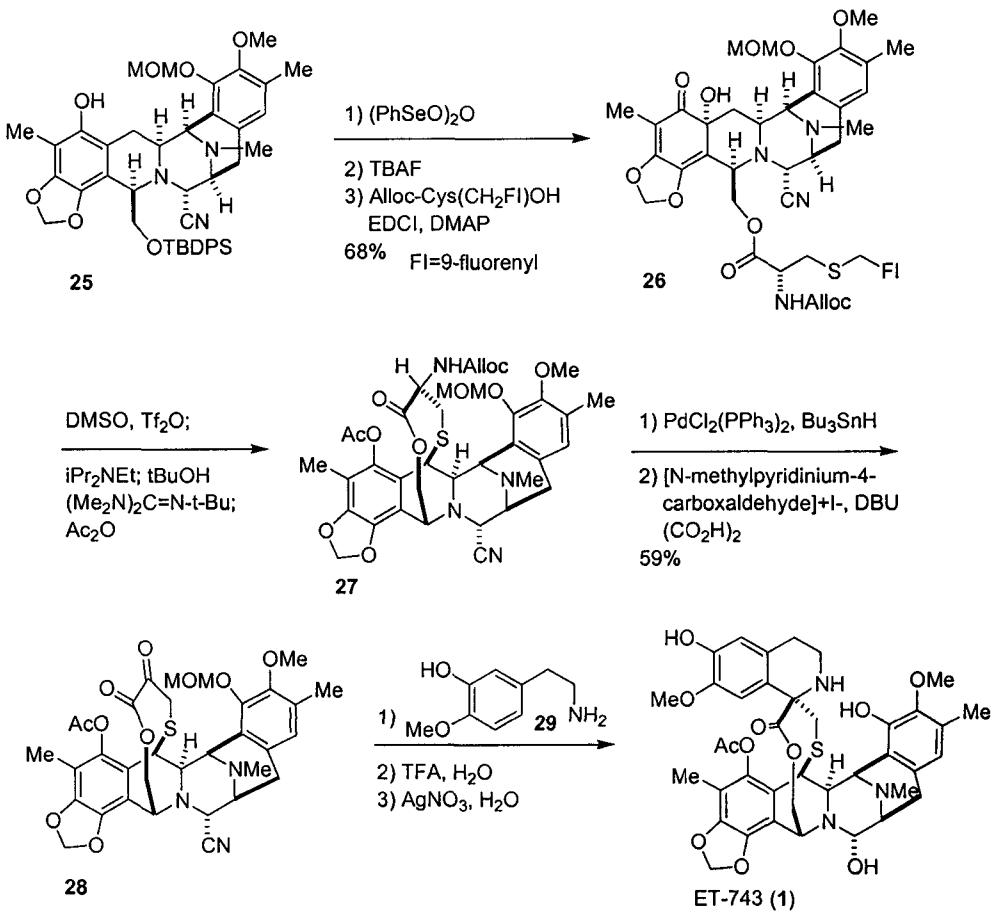
Scheme 1. Preparation of the pentacycle

As in Scheme 1, the synthesis commenced with commercial available sesamol **14**. The phenolic hydroxyl group of **14** was initially protected with the methoxymethyl protecting group. Subsequent methylation and formylation of the resulting product gave compound **15**. The methoxymethyl protecting group of **15** was then removed and

converted into corresponding benzyl protecting group. The α,β-unsaturated malonic ester **18**, prepared as a mixture of *E* and *Z* isomers from aldehyde **16** and allyl 2,2-dimethoxyethyl malonate **17**, was subjected to selective allyl ester cleavage, Curtius rearrangement, and reaction of the intermediate isocyanate with benzyl alcohol to form **19** stereospecifically. Hydrogenation of **19** with Rh[(COD)-(*R*,*R*)-DIPAMP]$^{+}BF_4^{-}$ as catalyst afforded **20** in 97% yield and 96% ee. Cleavage of the acetal protecting group in **20**, isolation, and exposure of the resulting aldehyde to $BF_3{\cdot}Et_2O$ and 4 Å molecular sieves gave a bridged lactone intermediate in 73% yield. Subsequent hydrogenolysis of this intermediate produced the free aminophenol **21**. The protected α-amino aldehyde **22** was synthesized through an analogous route, starting with 3,5-bis-((*tert*-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde and methyl hydrogen malonate.

The next stage of the synthesis, which involved the combination of the building blocks **21** and **22** and subsequent elaboration to construct the key monobridged pentacyclic intermediate **24**, commenced with the reaction of **21** and **22** in HOAc containing KCN to give a coupled phenolic α-amino nitrile intermediate. Subsequent *O*-allylation of this intermediate gave allyl ether **23** in 53% yield. Treatment of **23** with diisobutylaluminum hydride effected the selective conversion of the lactone functional group to a lactol. The lactol intermediate was then desilylated by exposure to excess $KF{\cdot}2H_2O$ and cyclized to pentacycle **24** through an internal Mannich bisannulation in the presence of 3 Å molecular sieves (55% overall from **23**). Selective trifluoromethanesulfonation of the least hindered phenolic hydroxyl group was followed by (1) selective silylation of the primary hydroxyl, (2) protection of the remaining phenolic hydroxyl group as the methoxymethyl ether, (3) double deallylation, (4)

reductive *N*-methylation, and (5) methylation through a Stille coupling to give **25** in 83% yield.



Scheme 2. The completion of Corey's total synthesis of ecteinascidin-743

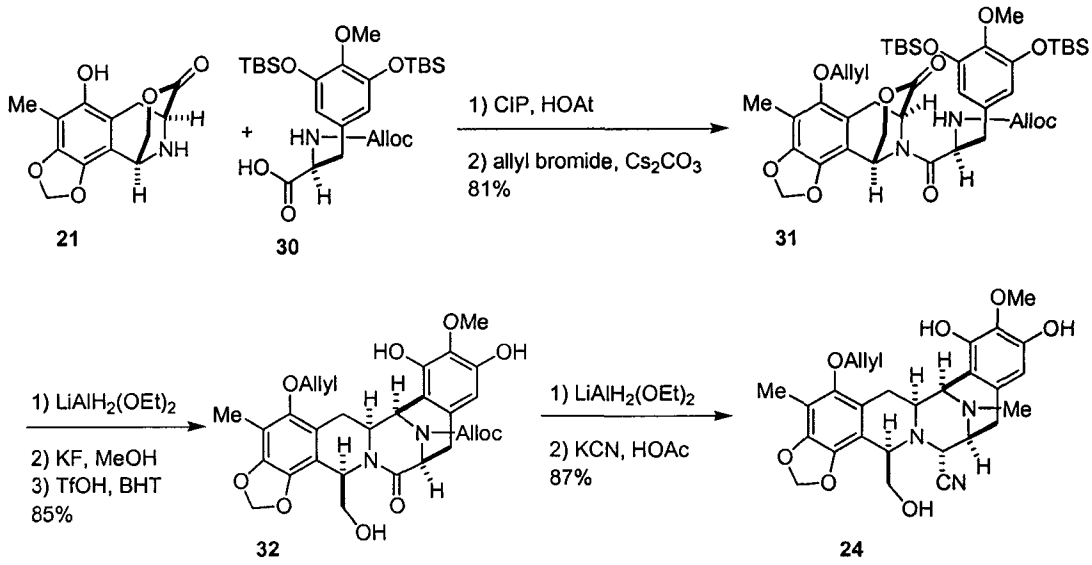
The last three rings of ecteinascidin 743, the 10-membered lactone bridge, and the spiro tetrahydroisoquinoline subunit were then added in the final stage of the synthesis of **1** by a novel sequence of reactions (Scheme 2). Oxidation of the phenol **25** effected position-selective angular hydroxylation to yield a dihydroxy dienone intermediate after desilylation. The primary hydroxyl functional group was esterified with (*S*)-*N*-((allyloxy)carbonyl)-*S*-(9-fluorenylmethyl)cysteine to form **26** (91%). Compound **26** was then transformed in one flask to the bridged lactone by the following operations: (1)

reaction of **26** with the *in situ*-generated Swern reagent from excess triflic anhydride and DMSO, (2) addition of *i*-Pr_2NEt and warming to 0 °C to form the exendo quinone methide, (3) quenching with *tert*-butyl alcohol to destroy excess Swern reagent, (4) addition of excess *N-tert*-butyl-*N'*,*N'*,*N''*,*N''*-tetramethylguanidine to convert the 9-fluorenylmethyl thioether to the thiolate ion and to promote nucleophilic addition of sulfur to the quinone methide to generate the 10-membered lactone bridge, and (5) addition of excess Ac_2O to acetylate the resulting phenoxide group. The *N*-((allyloxy)carbonyl) group of **27** was cleaved, and the resulting α-amino lactone intermediate was oxidized to the corresponding α-keto lactone **28**. This latter transformation was accomplished through transamination with the methiodide of pyridine-4-carboxaldehyde, 1,8-diazabicyclo[6.4.0]undec-7-ene (DBU). Reaction of **28** with 2-[3-hydroxy-4-methoxyphenyl]ethylamine (**29**) in the presence of silica gel generated the spiro tetrahydroisoquinoline unit stereospecifically. The resulting intermediate was then subjected to methoxymethyl cleavage and carbinolamine formation to afford ecteinascidin 743 (**1**).

1.2.1.2 Corey's improved synthesis of the pentacyclic intermediate

In 2000, Martinez and Corey reported an improved synthesis of intermediate **24**.[10] This synthesis improved the yield of **24** from 11% in 13 steps to 57% in six steps (Scheme 3). The amino lactone **21** was initially coupled with an acylating reagent prepared from acid **30**. The coupling product from this step was then allylated to give amide **31** in 81% overall yield from **21** and **30**. Selective reduction of the lactone functionality of **31** to the corresponding lactol was effected by reaction with lithium diethoxyaluminum hydride ($LiAlH_2(OEt)_2$) in ether in 95% yield. Desilylation and

cyclization (without purification) using triflic acid produced pentacyclic product **32** in 89% overall yield from **31**. Finally, the lactam of **32** could be reduced to the corresponding cyclic aminal through treatment with $LiAlH_2(OEt)_2$. Exposure of the resulting intermediate to HCN provided pentacyclic amino nitrile **24** in 87% overall yield from **32**.

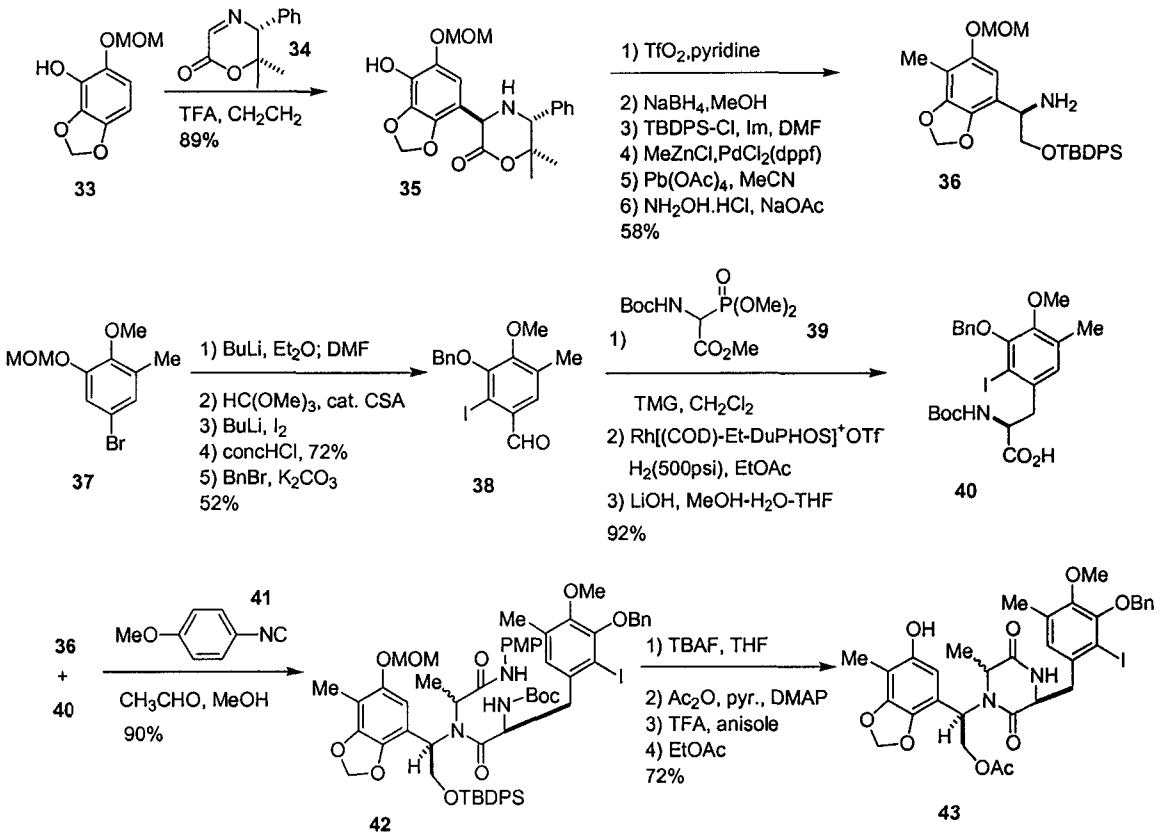


Scheme 3. Corey's improved synthesis of the intermediate **24**

1.2.1.3 Fukuyama's total synthesis of ecteinascidin-743

In 2002, Fukuyama et al reported their total synthesis of ecteinascidin-743.[8] The total synthesis commenced with phenol **33**. As in Scheme 4, the regio- and stereo-selective coupling of phenol **33** with iminolactone **34** (a chiral template that was developed recently in the Fukuyama laboratory) proceeded smoothly under acidic conditions to give the desired adduct **35** as a single product (89%). Conversion of the phenol of **35** to its corresponding triflate, reductive ring opening of the lactone, and subsequent silylation of the resulting primary alcohol afforded a protected intermediate. Subsequent introduction of a methyl group onto the aromatic ring, which was achieved

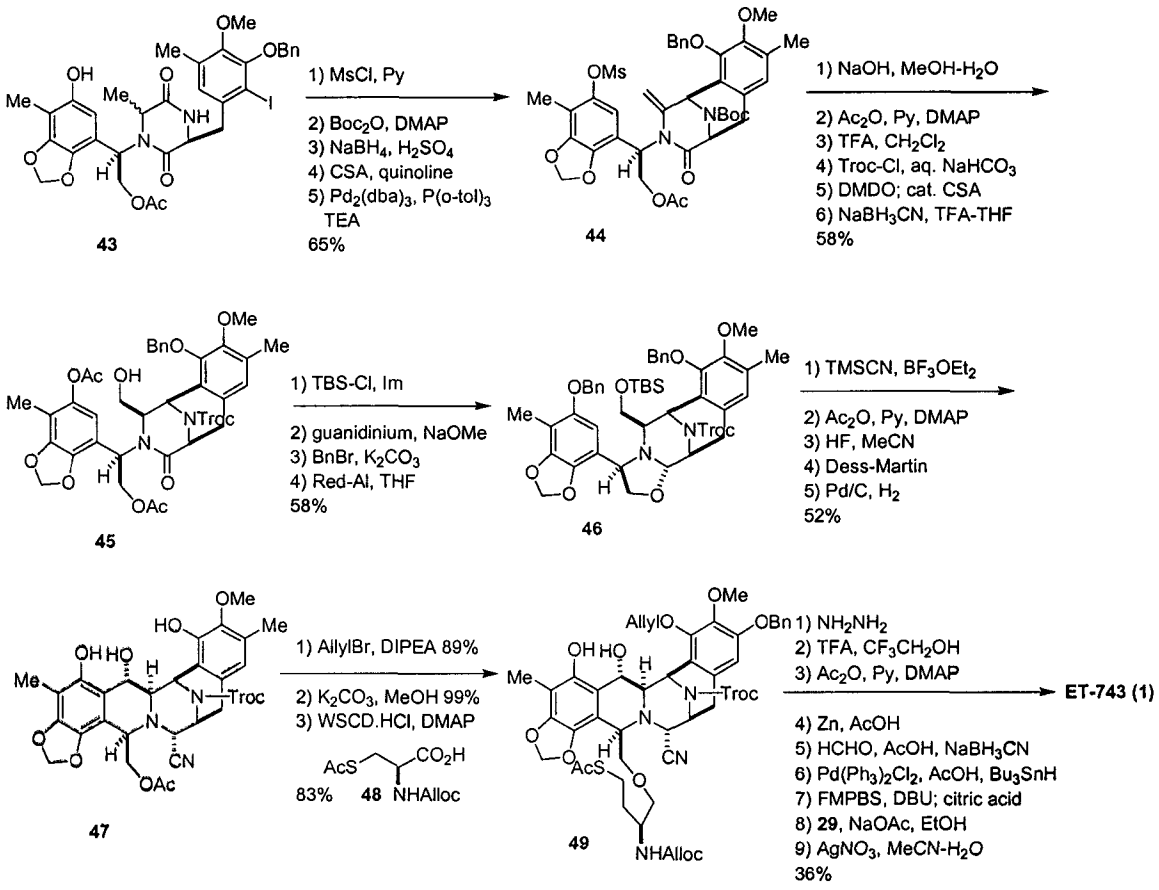
through a Pd-catalyzed cross-coupling reaction with MeZnCl, gave an amino alcohol intermediate. Oxidative cleavage of the amino alcohol moiety in this intermediate was effected with $Pb(OAc)_4$, and the resulting imine was converted to desired amine **36** by treatment with NH_2OH.



Scheme 4. Fukuyama's total synthesis of ecteinascidin-743-Ugi condensation

The reported bromide **37** was converted to the corresponding benzaldehyde by halogen-lithium exchange and subsequent treatment with DMF. Regioselective introduction of the iodide substitute was achieved by directed ortho-lithiation of the corresponding dimethylacetal intermediate followed by quenching with I_2. Simultaneous cleavage of the MOM ether and the dimethylacetal protecting groups from the resulting iodobenzene intermediate, followed by subsequent benzylation of the resulting phenol

afforded compound **38**. Subjecting of **38** to the Horner-Emmons reaction with phosphonate **39** gave a (*Z*)-dehydrophenylalanine intermediate. Catalytic asymmetric hydrogenation of this intermediate proceeded smoothly in the presence of Rh[(COD)-(*S,S*)-Et-DuPHOS]$^{+}$OTf under hydrogen (500 psi) to afford an aminoester intermediate without loss of the aromatic iodide (99%, 94% ee). Finally, basic hydrolysis of the methyl ester in the aminoester intermediate gave the desired carboxylic acid **40**.



Scheme 5 Fukuyama's completion of ecteinascidin-743

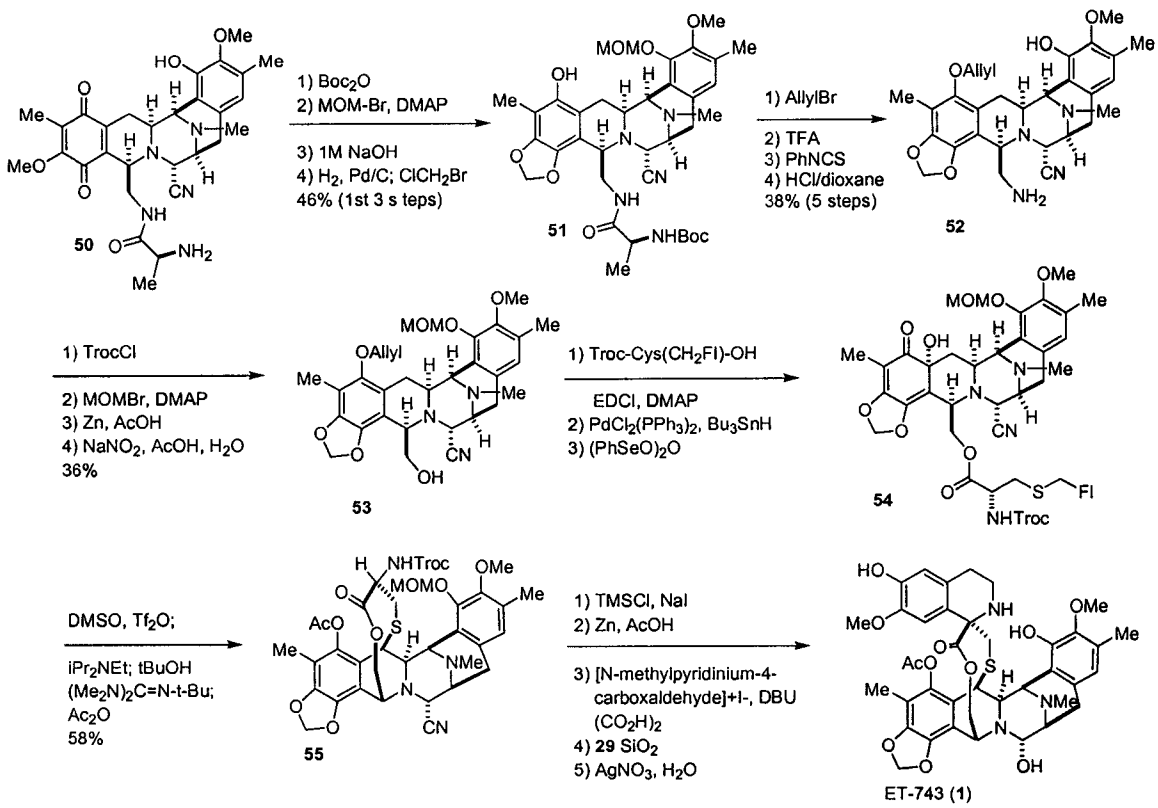
The two key segments, **36** and **40**, were incorporated into the diketopiperazine **42** by means of the powerful Ugi four-component condensation reaction. A mixture of amine (**36**), carboxylic acid (**40**), *p*-methoxyphenyl isocyanide (**41**), and acetaldehyde

was heated in MeOH to afford the dipeptide **42** in 90% yield. After switching from the TBDPS ether to the acetate protecting group at the primary alcohol position, simultaneous cleavage of the Boc group and the MOM ether gave an aminophenol intermediate. This compound cyclized to afford **43** upon gentle heating in EtOAc. Compound **43** was converted to a key coupling precursor through a four-step sequence involving (1) mesylation of the phenol, (2) introduction of a Boc group onto the lactam nitrogen, (3) partial reduction of the ring carbonyl with $NaBH_4$, and (4) dehydration of the resultant hemiaminal derivative by treatment with CSA and quinoline. The crucial Heck reaction was performed on the resulting intermediate in the presence of $Pd_2(dba)_3$ and P(*o*-tol)$_3$ to afford the desired tricycle **44**. After switching the protecting groups at the amine and phenol positions in compound **44** to produce the corresponding *N*-Troc-*O*-Ac compound, the enamide functional group in the resulting intermediate was oxidized with dimethyldioxirane in MeOH-acetone to generate an acid-sensitive epoxide. The epoxide intermediate was not isolated, but was immediately treated with CSA to afford the corresponding methoxyalcohol compound as a single isomer. Finally, subsequent acyliminium ion-mediated reduction under acidic conditions occurred from the less hindered *exo*-face of the molecule to afford alcohol **45** as a single isomer. Conversion of **45** to the oxazolidine **46** was achieved in a four-step sequence involving (1) silylation of the alcohol, (2) cleavages of the two acetyl groups, (3) selective benzylation of the phenolic hydroxyl group, and (4) partial reduction of the lactam carbonyl with Red-Al with concomitant formation of the oxazolidine ring. Cleavage of the oxazolidine functional group in **46** with TMSCN and $BF_3 \cdot OEt_2$ afforded an aminonitrile intermediate as a single stereoisomer. This compound was subsequently converted to an aldehyde

intermediate by a sequence involving (1) acetylation of the regenerated hydroxyl group, (2) cleavage of the TBS ether, and (3) oxidation of the resulting alcohol with the Dess-Martin reagent. Subsequent hydrogenolysis of the benzyl ethers invoked a spontaneous cyclization, giving the desired pentacycle **47**. Selective allylation of the phenols groups in compound **47**, cleavage of the acetyl group, and condensation of the resulting alcohol with L-cysteine derivative **48** furnished ester **49**. Chemoselective hydrogenolysis of the thioacetate group of **49** gave the corresponding thiol, which, upon exposure to TFA in 2,2,2-trifluoroethanol under high dilution conditions (0.009 M), underwent cyclization to give the ten-membered sulfide. Subsequent acetylation of the resulting phenol, cleavage of the Troc group, and reductive alkylation afforded a *N*-methyl amine intermediate. The alloc group and allyl ether in the resulting compound were simultaneously cleaved with palladium catalysis to give an aminophenol. The aminophenol intermediate was then subjected to the protocol reported by Corey. A biomimetic transamination reaction afforded the known α-ketolactone, and subsequent Pictet-Spengler reaction with amine **29** furnished ecteinascidin 770 (**5**). Finally, generation of the labile hemiaminal from the corresponding aminonitrile group in **5** was effected by treatment with $AgNO_3$ in CH_3CN-H_2O to give ecteinascidin-743 (**1**).

1.2.1.4 Cuevas's semi-synthesis of ecteinascidin-743

In 2000, Cuevas and coworkers in PharmMar SA reported the semi-synthesis of ecteinascidin-743 starting from cyanosafracin B (**50**),[7] which was available through fermentation of the bacteria *Pseudomonas fluorescens* on a kilogram scale.



Scheme 6. Cuevas' semi-synthesis of ecteinascidin 743

As in Scheme 6, the amino and phenol groups of **50** were initially protected as the BOC and MOM derivatives, respectively, and the methoxy-*p*-quinone was subsequently hydrolyzed. The quinone was the hydrogenated to give an unstable hydroquinone, which was immediately treated with bromochloromethane and Cs_2CO_3 to yield **51**. Alkylation of the phenol group in **51**, followed by removal of the MOM and BOC groups afforded an amide intermediate. Subsequent cleavage of the amide was accomplished by an Edman degradation to give **52**. Temporary protection of the primary amine of **52** as its TROC carbamate, followed by reaction with MOMBr and *i*-Pr_2NEt, and then removal of the TROC group with Zn/HOAc afforded the desired protected intermediate. Conversion of the free amine to the corresponding alcohol was accomplished with $NaNO_2$/HOAc to

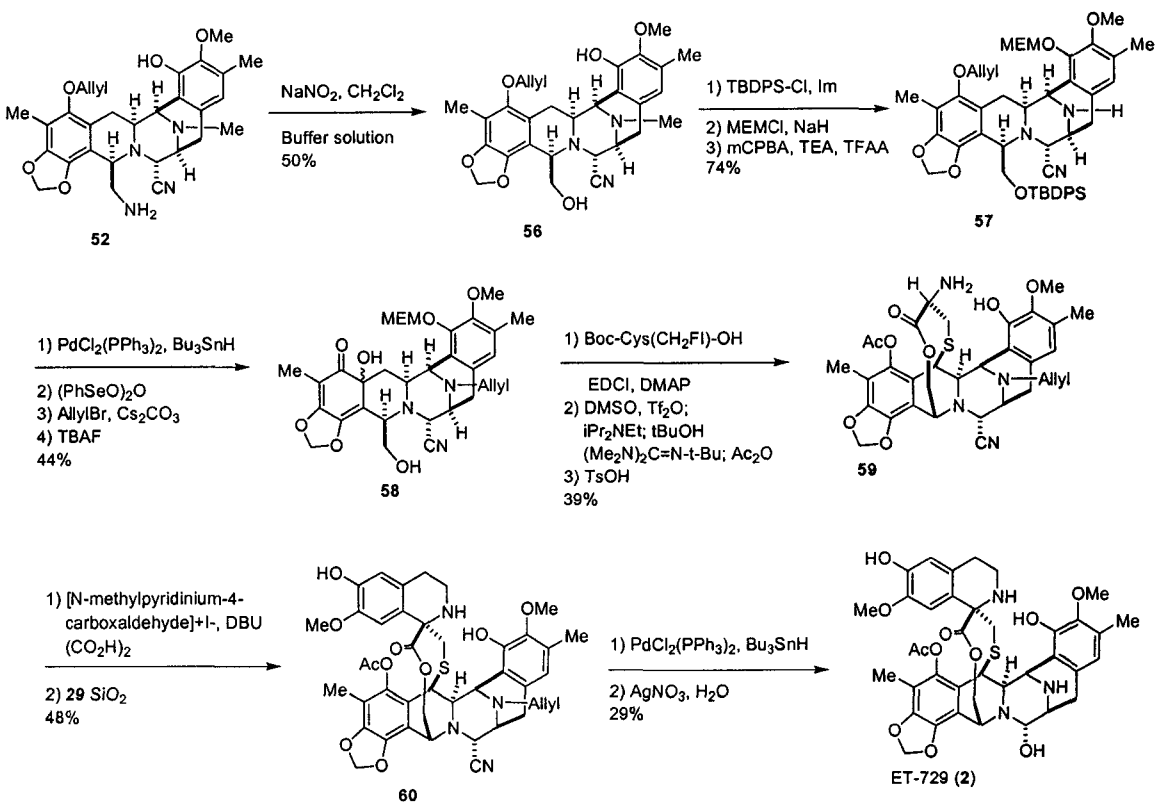
give compound **53**. The synthesis of Et-743 was completed using methods developed by Corey in their total synthesis of Et-743. A three-step sequence was employed to construct **54**. Dehydration under Swern conditions allowed for the requisite cyclization to afford **55**. Removal of the MOM and alloc protecting groups on compound **55** following by ketone formation provide an advanced intermediate. Finally, condensation with **29** and carbinolamine formation afforded Et-743.

1.2.1.5 Cuevas's semi-synthesis of ecteinascidins: Et-729, Et-745, Et-759B, Et-736, Et-637, and Et-594

In 2003, Cuevas et al extended the scope of the semi-synthetic route to other ecteinascidins: Et-729 (**2**), Et-745 (**3**), Et-759B (**4**), Et-736 (**9**), Et-637 (**63**), and Et-594 (**10**) using the key intermediate **56** (which was obtained through conversion of the free amine in **52** to the corresponding alcohol with $NaNO_2$) as shown in Scheme 7.

According to Scheme 7, silylation of the primary alcohol of intermediate **56** and protection of the corresponding phenol with MEMCl produced a protected intermediate. Demethylation at the bridgehead amine position using MCPBA, TEA, and TFAA afforded **57**. Removal of the allyl group in compound **57**, oxidation of the phenol, subsequent allyl protection of the bridgehead amine, and desilylation of the primary alcohol gave **58**. Esterification of the alcohol group in compound **58** with *(R)-N*-[(*tert*-butoxy)carbonyl-*S*-(9-fluorenylmethyl)]cysteine followed by subsequent cyclization gave a 10-membered lactone intermediate. The cyclization occurred through formation of the *exo* quinone methide followed by nucleophilic addition of the deprotected cysteine and final acetylation of the resulting phenoxide ion. Following by simultaneous removal of

the Boc and MEM protecting groups with *p*-TsOH, compound **59** was produced in 39% yield. Transamination followed by introduction of the dopamine moiety through a Pictet-Spengler reaction gave intermediate **60** in 48% yield. Removal of the allyl protecting group and carbinolamine formation using $AgNO_3$ gave Et-729 (**2**).

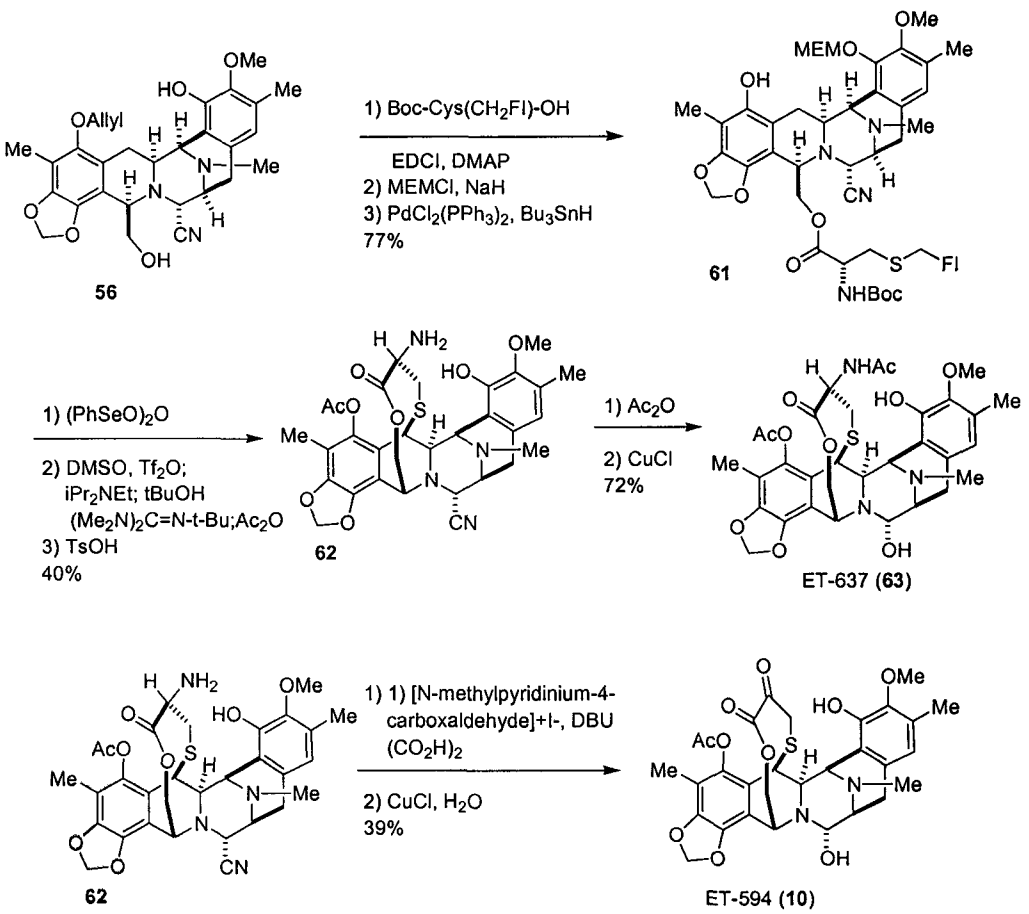


Scheme 7 Cuevas' semi-synthesis of Et-729

The syntheses of the other natural ecteinascidins that retained the *N*-Me group were achieved from the common intermediate **62** (Scheme 8, Scheme 9).

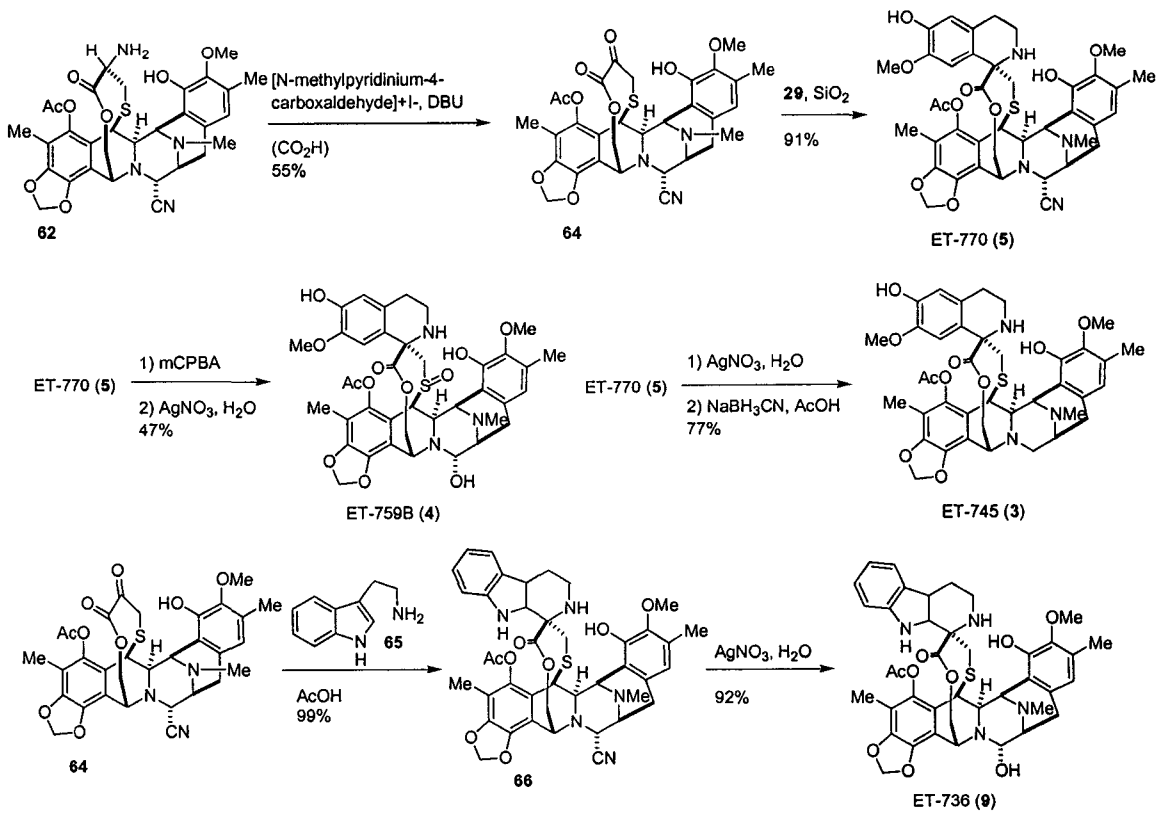
Initially, the common intermediate **62** was produced from compound **56**. This was accomplished through esterification of **56** with protected L-cysteine, followed by protection of the phenol with MEMCl. Final removal of the allyl group gave compound **61**. Compound **61** was initially oxidized and was then cyclized under the conditions

developed by Corey to produce a lactone intermediate. Treatment with acid effected simultaneous removal of the Boc and MEM protecting groups to produce compound **62** in 40% yield. Et-637 (**63**) was obtained in 72% yield through initial acetylation of **62** with Ac_2O in the absence of base, followed by carbinolamine formation, which was performed with CuCl in a mixture of THF-H_2O.



Scheme 8 Cuevas' semi-synthesis of Et-637, Et-594

Ecteinascidin-594 (**10**) was produced from **62** through an initial transamination protocol, followed by carbinolamine formation. The conditions employed in this two-step sequence were identical to those used to prepare Et-637 (**63**).



Scheme 9 Cuevas' semi-synthesis of Et-759B, Et-745, and Et-736

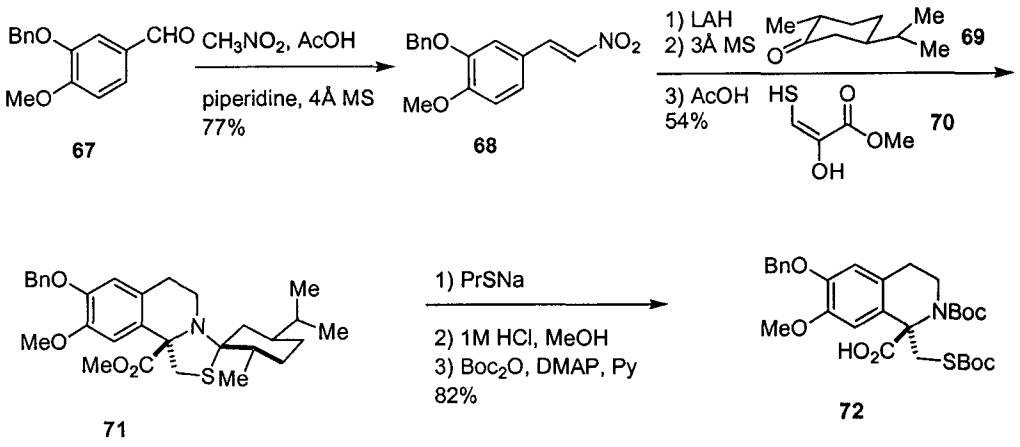
The synthesis of Et-759B (**4**), Et-745 (**3**), and Et-736 (**9**) (Scheme 9) followed the same sequence as was previous detailed during the synthesis of Et-743. The dopamine residue was introduced through a Pictet-Spengler reaction on compound **64** to give **5** (Et-770). Oxidation of the sulfide group in compound **5** with MCPBA and treatment with $AgNO_3$ afforded Et-759B (**4**). Initial conversion of Et-770 (**5**) to Et-743 (**1**) through carbinolamine formation, followed by reduction of Et-743 (**1**) with sodium cyanoborohydride gave Et-745 (**3**) in 77% yield.

Concerning the synthesis of Et-736 (**9**), the tetrahydro-β-carboline ring was initially introduced by treatment of **64** with the tryptamine (**65**) in acetic acid to afford compound **66** in good yield. Compound **66** was subsequently transformed into Et-736 (**9**) by reaction with $AgNO_3$ (Scheme 9).

1.2.2 Synthetic studies toward the ecteinascidins

In addition to the above syntheses, other research efforts have been directed toward the synthesis of Et-743. [11-17] The results from these studies are reported below.

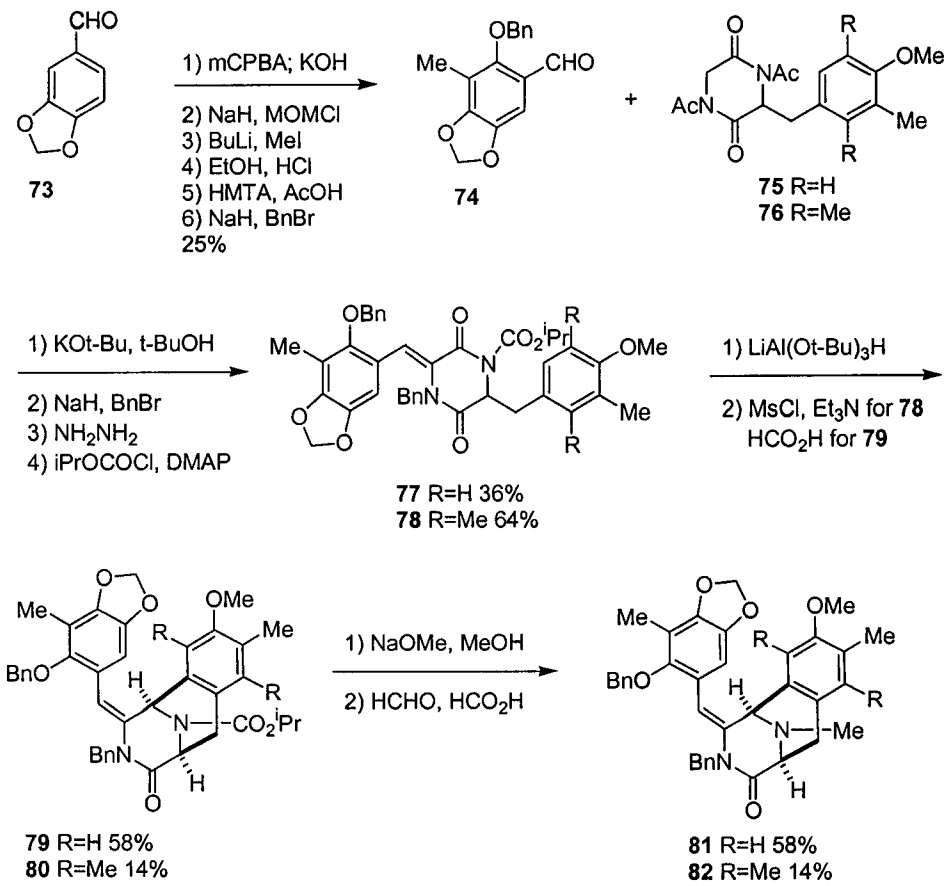
1.2.2.1 Corey's synthetic study of the tetrahydroisoquinoline C unit of Et-743



Scheme 10. Corey's synthetic studies toward Et-743

In 1996, Corey and Gin reported an efficient synthesis of the tetrahydroisoquinoline C unit of Et-743.[11] As shown in Scheme 10, aldehyde **67** was initially converted to the nitrostyrene **68** via a nitro-aldol condensation. Reduction of the olefin as well as the nitro group, followed by condensation of the resulted amine with (+)-tetrahydrocarvone (**69**) produced an imine intermediate. The resulting imine was subsequently treated with compound **70** to form compound **71** with a diastereoselectivity of 6.5:1. The inseparable mixture of diastereomers (**71**) was treated with sodium propylmercaptide, which allowed for a selective hydrolysis of the methyl ester of the major diastereomer. Acidic cleavage of the auxiliary in the resulting intermediate and subsequent protection at the amine and thiol positions afforded **72** in optically pure form.

1.2.2.2 Kubo's synthetic studies toward Et-743

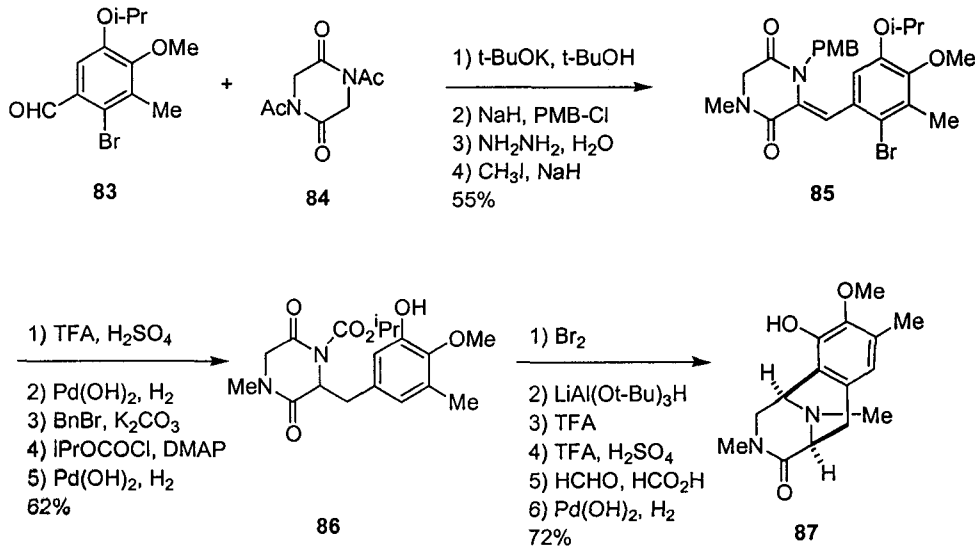


Scheme 11. Kubo's synthetic studies toward Et-743

In 1997, Kubo et al. published their synthetic studies toward the ecteinascidins, which employed chemistry similar to that deployed in their saframycin syntheses (Scheme 11).[12a] Aldehyde **73** was converted to aldehyde **74** in six steps featuring an initial Bayer-Villiger oxidation that was followed by formylation and phenol protection to produce **74**. An aldol condensation was then performed on diketopiperazine **75** or **76** affording **77** or **78**, respectively, after further manipulations. Partial reduction of the activated lactam in compound **77** or **78** followed by cyclization using the indicated conditions afforded the tricycles **79** and **80**. Carbamate cleavage was accomplished using

sodium methoxide in methanol and the secondary amine was methylated affording **81** and **82**. Compound **81** and **82** were presented as possible precursors to the ecteinascidins.

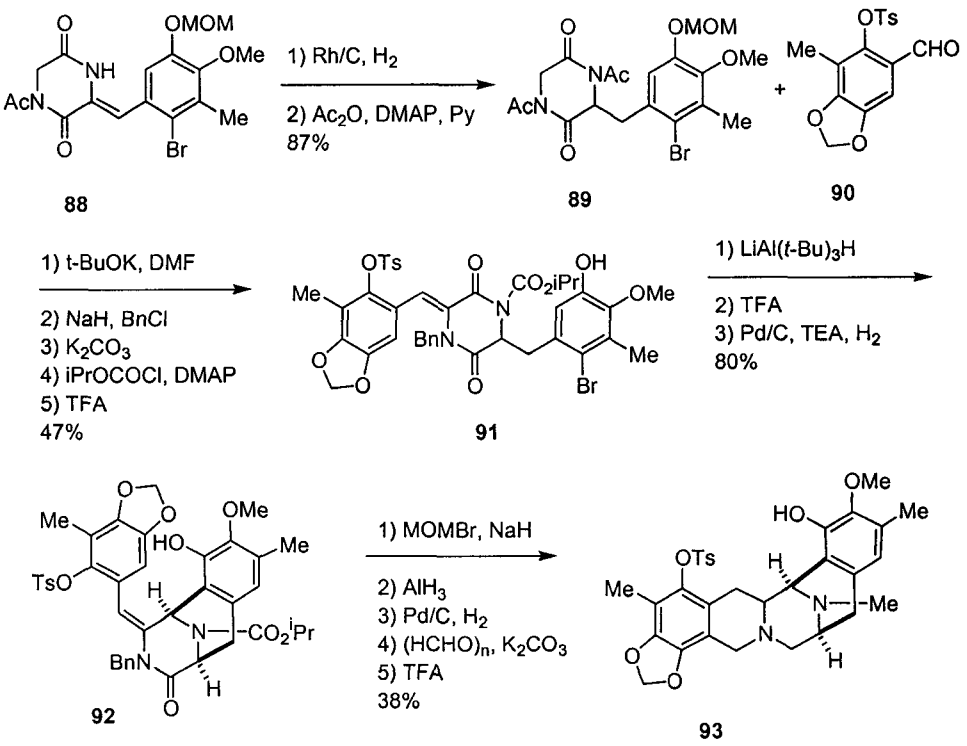
In 2000, Kubo et al. published a route to the ABC ring system of the ecteinascidins (Scheme 12).[12b,c] Aldol condensation of aldehyde **83** with diketopiperazine **84** afforded **85** after subsequent manipulations including protection of the lactam. The phenol protecting group was then switched, and the lactam was subsequently activated and reduced to afford **86**. Bromination of the aromatic ring allowed for the regioselective cyclization of the necessary intermediate to form the tricycle. Cleavage of the carbamate was followed by methylation and final removal of the bromide to give the tricycle **87**.



Scheme 12. Kubo's synthetic studies toward Et-743

In 2003, Kubo el al. reported the preparation of the ABCDE ring system of the ecteinascidins (Scheme 13).[12d] The double bond of diketopiperazine **88** was initially hydrogenated, and the amide was protected to afford **89**. Condensation with aldehyde **90**, followed by benzyl protection, acetyl group removal, introduction of the

propyloxycarbonyl group, and removal of MOM group gave **91**. Selective reduction of the lactam of **91**, followed by cyclization formed a tricyclic intermediate. Removal of the bromine substitute in the tricyclic intermediate produced compound **92**. The phenol group in **92** was subsequently protected as MOM ether. Reductive methylation followed by benzyl group removal and a cyclization protocol gave the pentacycle **93**.



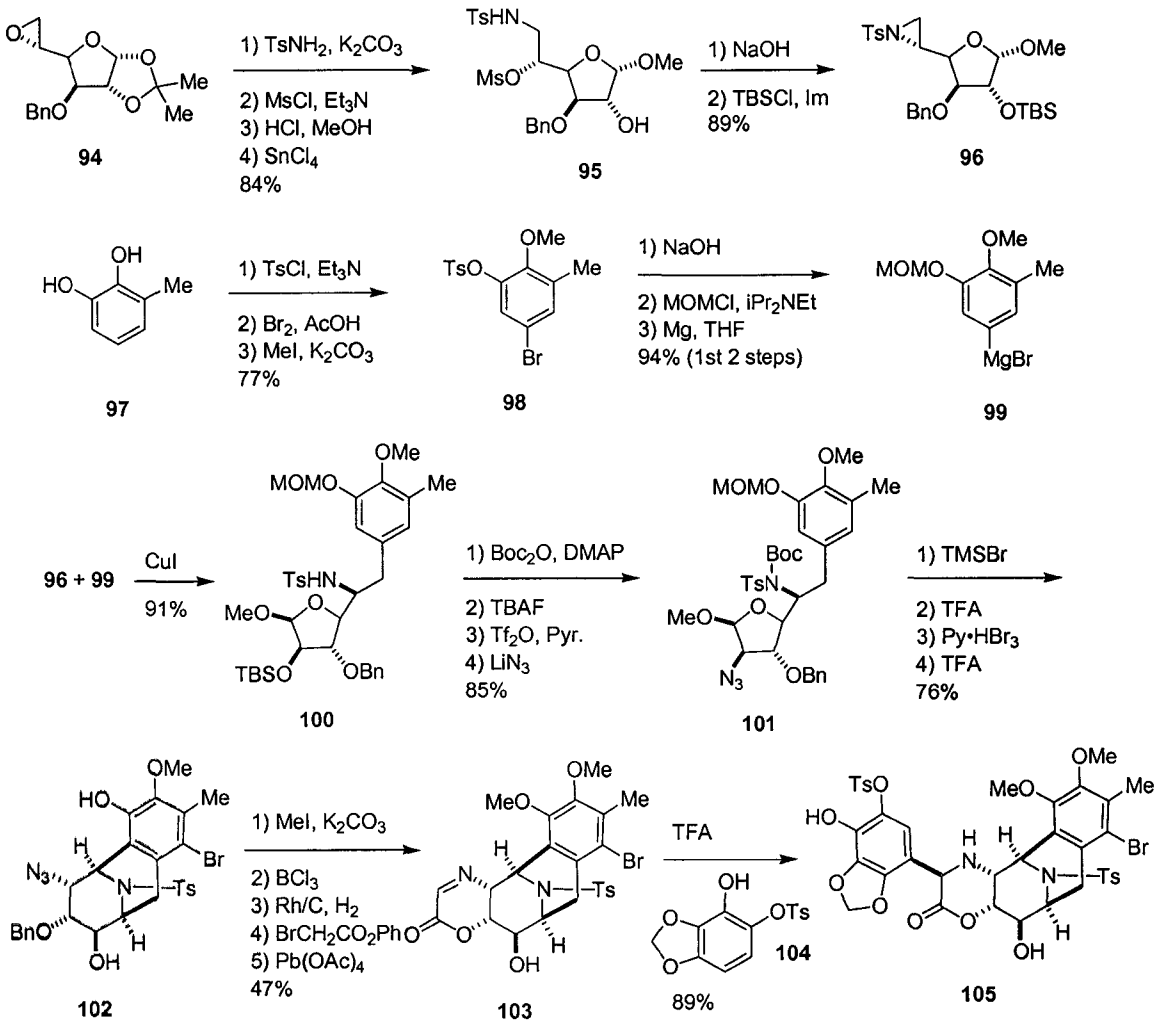
Scheme 13. Kubo's synthetic studies toward Et-743

1.2.2.3 Fukuyama's synthetic studies toward Et-743

In 1999, Fukuyama et al. published their synthetic studies toward Et-743 starting from D- glucose (Scheme 14).[13]

Epoxide **94**, available in five steps from D-glucose, was subjected to selective epoxide ring opening followed by mesylation and acetonide deprotection to afford a 3:2

mixture of diastereomeric methyl glycosides. Treatment of this mixture with stannous chloride furnished **95** as a single diastereomer. Aziridine formation was accomplished using sodium hydroxide, followed by silyl ether formation, to afford **96** in high yield.



Scheme 14. Fukuyama's synthetic studies toward Et-743

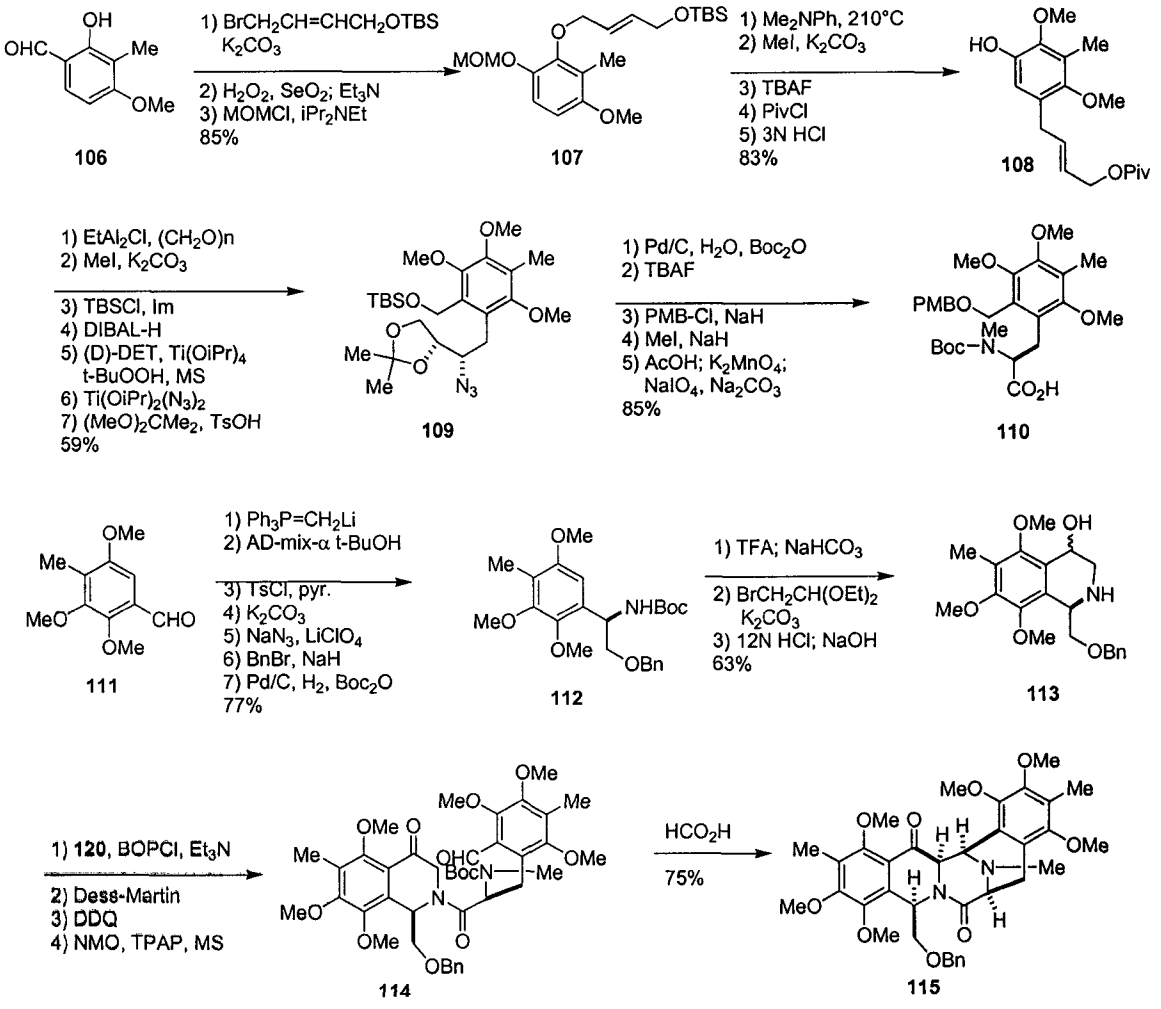
The E-ring of Et-743 was introduced via a Grignard addition to the aziridine in compound **96**. Initially, diphenol **97** was selectively protected with tosyl chloride. *Para*-bromination followed by methylation of the free phenol produced compound **98**. After switching protecting groups, the Grignard reagent **99** was produced for coupling to

aziridine **96**. Subsequent copper-catalyzed aziridine ring opening by **99** afforded **100** in 91% yield. Protection of the sulfonamide followed by alcohol deprotection yielded the corresponding free primary alcohol. Activation of the primary alcohol and azide displacement of the corresponding triflate furnished compound **101**. Removal of the MOM and Boc protecting groups in compound **101** followed by *para*-bromination provided a cyclization precursor. The subsequent cyclization reaction proceeded through an iminium ion intermediate affording the tricyclic compound **102** as a single stereoisomer. Protection of the phenol functional group on **102** was followed by benzyl ether cleavage and azide reduction to afford an amino diol. Amino lactonization was then followed by lead tetraacetate oxidation to form dehydrooxazinone **103**. Acidic alkylation of **103** with phenol **104** afforded **105** in 89% yield. The proposed completion of the synthesis from compound **105** involved the reduction of the lactone functional group followed by oxidative cleavage of the resulting diol of the C-ring to the corresponding dialdehyde. Closure of the B- and C-rings would then afford the pentacyclic core of the ecteinascidins.

1.2.2.4 Danishefsky's synthetic studies toward Et-743

In 2000, Danishefsky el al. reported their synthetic approach towards Et-743 utilizing a convergent intramolecular Mannich cyclization strategy (Scheme 15).[14a, b] The E-ring construction commenced with aldehyde **106**. The phenolic hydroxyl group of **106** was alkylated, and the aldehyde functional group was subjected to Bayer-Villiger oxidation to yield phenol **107**. Heating compound **107** in dimethylaniline at 210 °C effected Claisen rearrangement which, after protecting group manipulations, afforded compound **108**. Alkylation of the arene ring, protection of the hydroxyl groups, and

removal of the pivaloyl group gave an alcohol intermediate. Sharpless epoxidation, selective epoxide opening with azide, and diol protection lead to dioxolane **109**. Azide reduction in the presence of di-*tert*-butyl dicarbonate afforded the corresponding carbamate intermediate. Methylation of the resulting carbamate nitrogen, cleavage of the silyl ether, *p*-methoxyl benzyl ether formation, and oxidative cleavage of the diol intermediate afforded the *N*-Boc amino acid **110** in 85% from **109**.

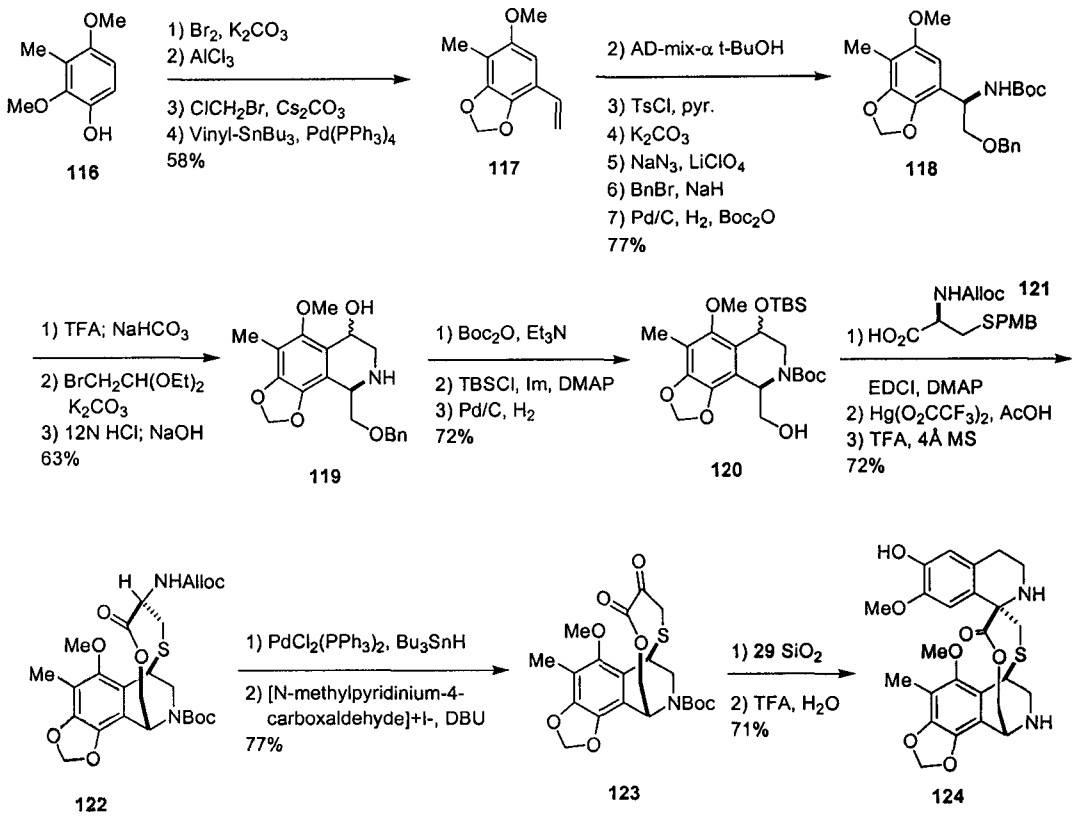


Scheme 15. Danishefsky's synthetic studies toward ecteinascidins

The A-ring system commenced with the olefination of aldehyde **111** followed by a Sharpless asymmetric dihydroxylation to furnish optically active intermediate. The diol intermediate was then converted to an optically pure epoxide via tosylation and base-mediated ring closure. The resulting epoxide was then opened with sodium azide followed by subsequent benzyl group protection, reducing the azide to the corresponding amine, and protection of the amine intermediate with Boc group to afford **112**. Removal of the Boc group in **112** was followed by alkylation of the resulting amine with bromoacetaldehyde diethyl acetal. The acetal intermediate was then cyclized under acidic condition to form the bicyclic substance **113**. The two fragments (**110** and **113**) were successfully coupled using BOPCl followed by a sequence of oxidations to furnish the precyclization substrate **114**. Treatment of compound **114** with formic acid effected removal of the *N*-Boc group to afford corresponding amine intermediate, which upon subsequent cyclization furnished the desired pentacyclic substances **115** in 75%. Curiously, efforts to complete the total synthesis of a natural compound from **115** have not been reported.

In 2002, Danishefsky el al. reported their synthetic approach towards the ABFGH ring of Et-743 (Scheme 16).[14c] This route commenced with phenol **116**, which, upon bromination, was converted to styrene **117**. Subsequent Sharpless asymmetric dihydroxylation furnished an optically active intermediate. The diol in this intermediate was then converted to the corresponding optically pure epoxide intermediate via tosylation and base-mediated ring closure. The epoxide was then opened with sodium azide, and the azide was subsequently reduced and protected yielding **118**. Removal of the Boc group was followed by alkylation of the resulting amine with bromoacetaldehyde

diethyl acetal. The resulting acetal intermediate was then cyclized under acidic conditions to form the bicyclic compound **119**. After Boc protection of the NH group and silylation of the secondary hydroxyl group, the primary ether was debenzylated to afford **120**.



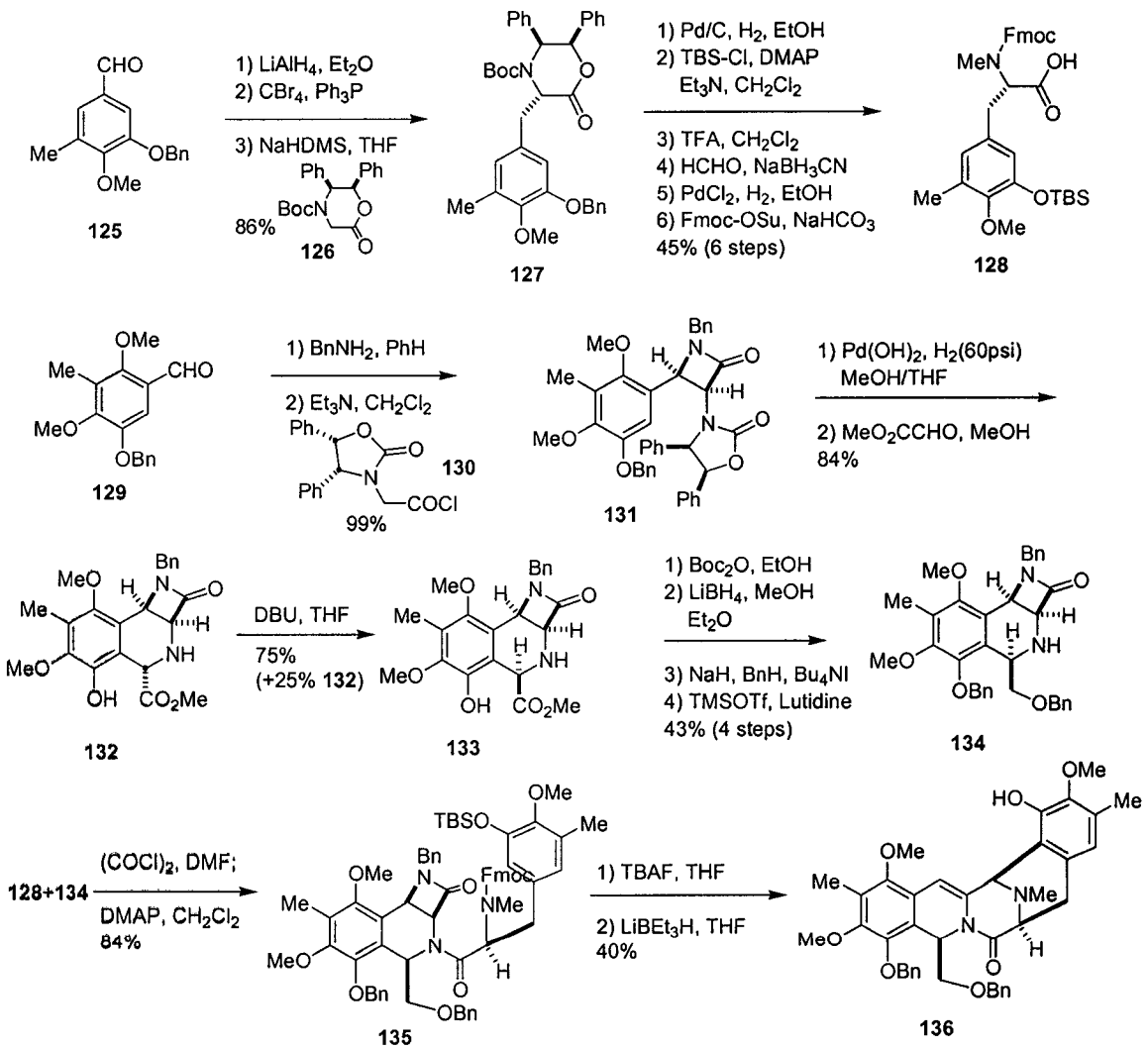
Scheme 16. Danishefsky's synthetic studies toward ecteinascidins

Following precedents of the Corey-Gin synthesis, the alcohols **120** were coupled to freshly prepared cysteine carboxylic acid **121**. Subsequent removal of the PMB group and treatment with TFA in the presence of activated 4 Å molecular sieves produced compound **122**. The *N*-allyloxycarbonyl group of **122** was cleaved by treatment with Bu_3SnH and $Pd(PPh_3)_2Cl_2$ and the resulting α-amino lactone was oxidized to the corresponding α-keto lactone **123** by transamination with the methiodide of pyridine-4-carboxaldehyde. The reaction of ketone **123** with amine **29** as described by Corey and

Gin, generated the spiro tetrahydroisoquinoline in a stereospecific fashion. Removal of the Boc protecting group afforded the amine **124**, which possessed the necessary orientation required for Et-743.

1.2.2.5 Williams' synthetic studies toward Et-743

In 2003, Williams el al. reported their synthetic studies toward Et-743 (Scheme 17). [15a, b]



Scheme 17. Williams' synthetic studies toward Et-743

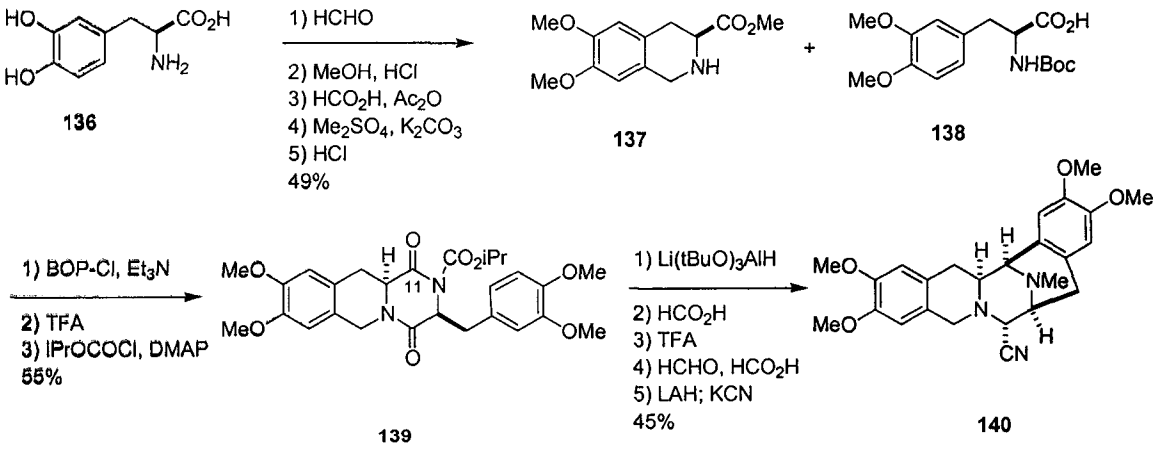
The aldehyde functional group in **125** was reduced to the corresponding alcohol and was then converted to the corresponding benzyl bromide derivative. The bromide intermediate was then condensed with the sodium enolate of oxazinone **126** to afford the alkylation product **127**. The *O*-benzyl group of **127** was removed by catalytic hydrogenation, followed by protection as the *O*-TBS ether. The *N-t*-Boc group was then removed by treatment with TFA. Subsequent *N*-methylation, removal of the chiral auxiliary, and protection of the secondary amine as the corresponding Fmoc derivative furnished amino acid **128**.

The left-hand fragment of the target commenced with aldehyde **129**. The necessary Staudinger reaction was accomplished by initially condensing benzylamine with aldehyde **129**, which afforded the corresponding imine intermediate. The requisite ketene was prepared from the optically pure acid chloride **130** using triethylamine. Reaction of the imine and ketene intermediates afforded, after workup, β-lactam **131** in excellent yield. Reductive removal of the chiral auxiliary and the benzyl ether, followed by treatment with methyl glyoxylate afforded tetrahydroisoquinoline **132** in 84% yield as a single stereoisomer. Epimerization of the *anti*-carbomethoxy group of **132** with DBU afforded a 75% yield of the desired *syn*-isomer **133** in addition to **132** (25%). Boc protection of the secondary amine in **133**, followed by the reduction of the carbomethoxy group afforded the corresponding alcohol intermediate. Benzyl protection of both the primary alcohol and the phenol group in this intermediate, followed by removal of the Boc group by treatment with TMSOTf and 2,6-lutidine afforded the tetrahydroisoquinoline **134**. The amino acid **128** was converted into the corresponding acid chloride and was then coupled to the amine **134** to afford the peptide **135** in 84%

yield. Both the Fmoc and TBS protecting groups were then removed by treatment with TBAF, and exposure to super-hydride ($LiBEt_3H$) remarkably furnished the desired pentacyclic compound **124** directly in 49% yield.

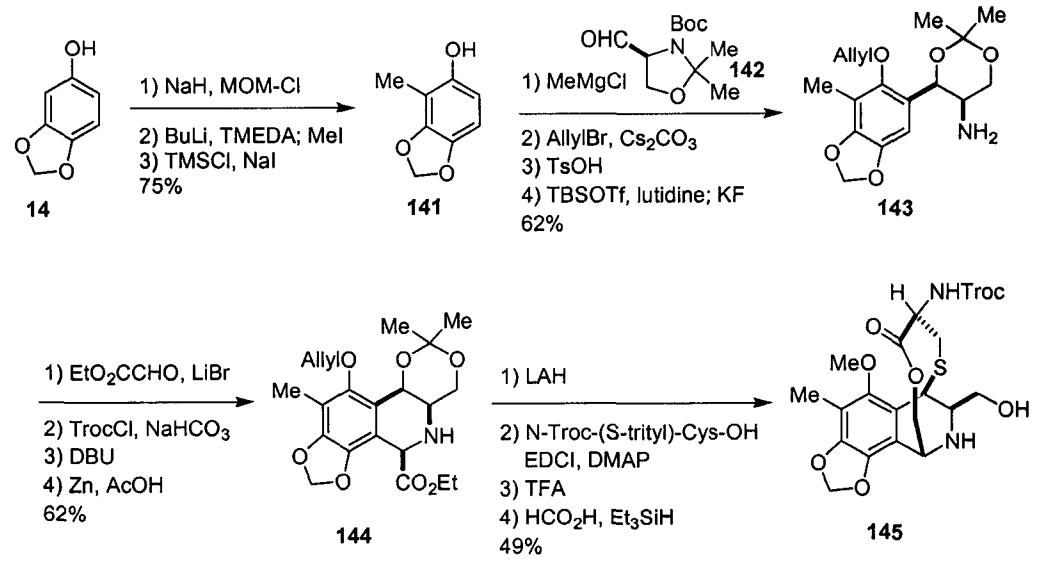
1.2.2.6 Liu's synthetic studies toward Et-743

In 2003, Liu et al. published their synthetic studies toward Et-743 (Scheme 18).[16a,b] The synthesis commenced with *L*-DOPA (**136**). Pictet–Spengler reaction of **136** with formaldehyde, subsequent esterification, protection of the nitrogen by a formyl group, methylation and cleavage of the formyl group afforded **137**. The coupling product of **137** and **138** was treated with TFA and then was converted to the carbamate **139**. Regioselective reduction of the C-11 carbonyl group in **139**, followed by treatment with HCO_2H, formed the pentacyclic framework of the ecteinascidins. Deprotection to the secondary amine, reductive methylation, and conversion of the lactam ring into then corresponding aminonitrile afforded **140** as an enantiomerically pure product.



Scheme 18. Liu's synthetic studies toward Et-743

1.2.2.7 Zhu's synthetic studies toward Et-743

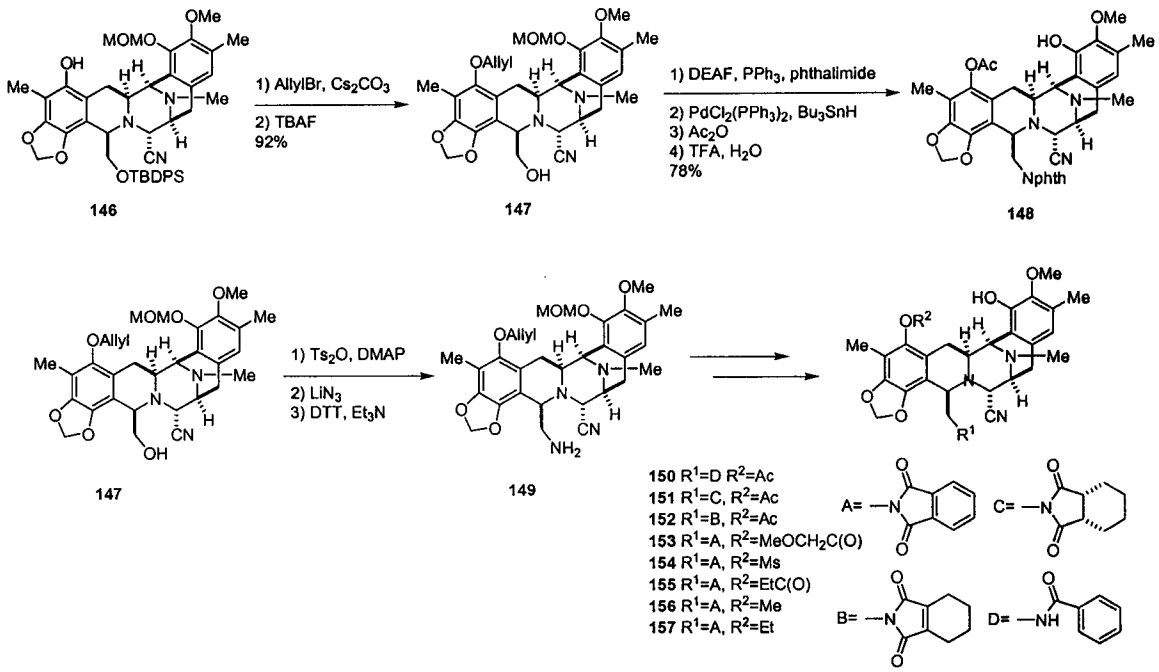


Scheme 19. Zhu's synthetic studies toward Et-743

In 2003, Zhu et al. published their synthetic studies toward Et-743 (Scheme 19).[17] Protection of sesamol **14**, followed by regioselective *ortho*-lithiation, methylation, and removal of the MOM ether, afforded phenol **141**. Phenolic aldol condensation between **141** and Garner's aldehyde (**142**) provided the protected *syn* amino diol, which was converted into the 1,3-dioxane **143** through a four-step sequence involving (1) allylation of the phenol, (2) hydrolysis of the oxazolidine, (3) selective ketalization of the 1,3-diol and (4) selective removal of Boc group. A Lewis acid promoted Pictet–Spengler cyclization on compound **143** provided the desired tetrahydroisoquinoline intermediate as a single diastereomer in 40% yield. Subsequent *N*-acylation, followed by DBU mediated epimerization and reductive removal of the *N*-Troc group, afforded **144** in a ratio of 2.5 :1 in favor of the desired isomer at the C-1 position. Reduction of the amino ester **144** with LAH afforded the corresponding aminoalcohol, which was chemoselectively *O*-acylated with (*R*)-*N*-Troc–(*S*-trityl)cysteine. Macrocyclization under acidic conditions and

reductive removal of trityl protecting group afforded **145**, which could be used as building block for Et-743.

1.2.2.8 Ecteinascidin analogs

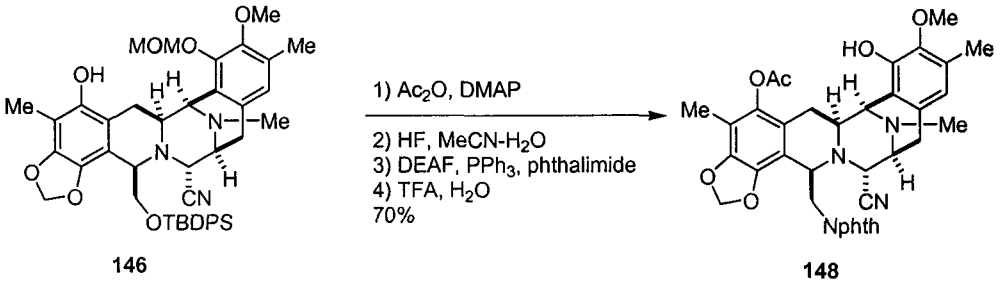


Scheme 20. Corey's synthesis of phthalascidin 650

Corey et al. reported the synthesis and biological activity of potent analogues of Et-743 as shown in Scheme 20.[18] In this study, the compound phthalascidin (**148** or Pt 650) was synthesized, which surprisingly displayed comparable biological activity to that of Et-743. The synthesis of phthalascidin commenced with compound **146**, which was initially allylated followed by removal of the silyl protecting group to afford **147**. A Mitsunobu reaction using phthalimide followed by removal of the allyl group, acylation of the phenol, and removal of the MOM group provided **148** in six steps and 72% overall yield from **146**.

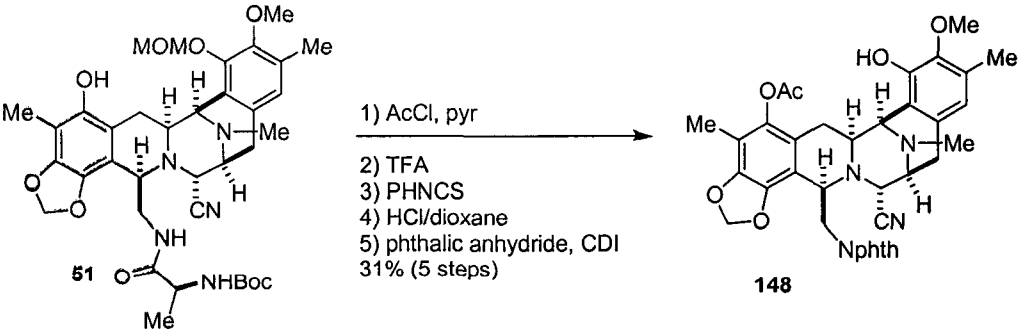
Several other ecteinascidin analogues were also prepared as described in Scheme 36. These analogues were formed by the conversion of alcohol **147** to amine **149** followed by amide or succinimide formation affording **150-157**.

In 2000, Corey and Martinez published a more concise synthesis of phthalascidin (**148**, Scheme 21).[10] In this new route, a protection step and deprotection step were omitted, shortening the synthesis to four overall steps with a slightly lower yield of 70%.



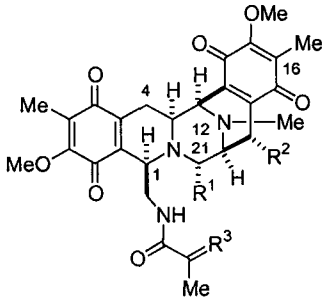
Scheme 21. Corey's improved synthesis of phthalascidin 650

In 2000, Cuevas et al. published a short synthesis of Pt-650 from an intermediate described in their Et 743 synthesis (Scheme 22).[7] Starting with compound **51**, Pt 650 was synthesized in five steps in 31% overall yield.

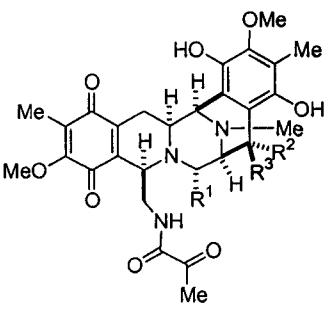


Scheme 22. Cuevas' synthesis of phthalascidin 650

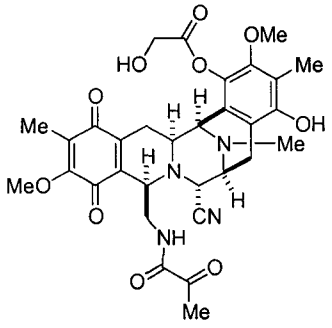
1.3 The saframycins



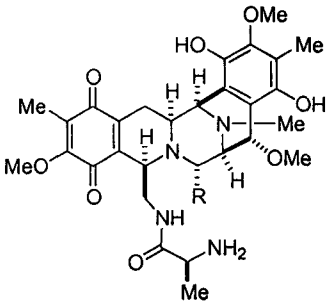
Saframycin A (**158**) R^1=CN, R^2=H, R^3=O
B (**159**) R^1=R^2=H, R^3=O
C (**160**) R^1=H, R^2=OMe, R^3=O
G (**161**) R^1=CN, R^2=OH, R^3=O
H (**162**) R^1=CN, R^2=H, R^3=OH, CH_2COMe
S (**163**) R^1=OH, R^2=H, R^3=O



Saframycin D (**164**) R^1=H, R^2,R^3=O
E (**165**) R^1=R^3=H, R^2=OH
F (**166**) R^1=CN, R^2,R^3=O



Saframycin R (167)

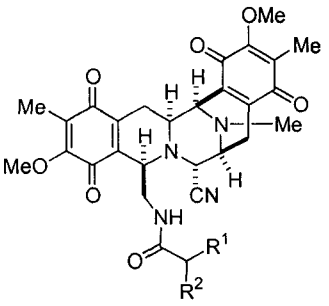


Saframycin Mx1 (**168**) R=OH
Mx2 (**169**) R=H

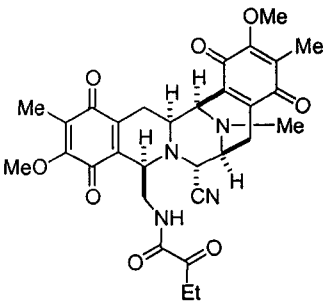
Figure 2. Saframycins

Saframycins A, B, C, D, and E (**158-160**, **164**, and **165**, respectively, Figure 2) were isolated from *Streptomyces lavendulae* in 1977 by Arai et al.[19] These were the first of many saframycins to be subsequently isolated from natural sources. The structure of saframycin C (**160**) was the first of this family to be determined. This was accomplished via X-ray crystallographic analysis.[20] From the comparison of the ^{13}C NMR data of saframycins B (**159**) and C (**160**), the structure of saframycin B (**159**) was determined. The structure of saframycin A (**158**), which contains a nitrile moiety at C-21, was

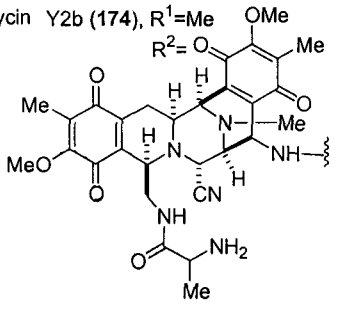
determined through various spectroscopic techniques including high-field ^{1}H NMR analyses of saframycins A (**158**) and C (**160**).[21] The structure of saframycin D (**164**) was the next to be determined, once again by extensive NMR studies.[22] Saframycin E (**165**) was found to be too unstable for spectroscopic studies, but it could be isolated and characterized as the corresponding triacetate.[19] The structure of saframycin E (**165**) was determined by Kubo et al. via an intermediate in their synthetic studies of the saframycins.[23] This intermediate had identical spectroscopic properties to that of the triacetate derivative of saframycin E (**165**).



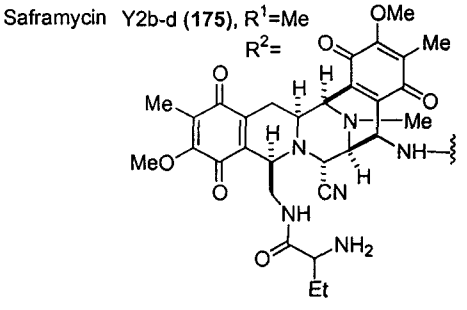
Saframycin Y3 (**170**) R^1=NH_2, R^2=Me
Yd-1 (**171**) R^1=NH_2, R^2=Et
Yd-2 (**172**) R^1=NH_2, R^2=H



Saframycin Ad-1 (**173**)



Saframycin Y2b (**174**), R^1=Me



Saframycin Y2b-d (**175**), R^1=Me

Figure 3. Saframycins obtained from directed biosynthesis.

During studies concerning the optimization of saframycin A (**158**) production, another saframycin was isolated, saframycin S (**163**).[24] Saframycin S (**163**) was believed

to be a biosynthetic precursor to saframycin A (**158**). It was found that treatment of saframycin S (**163**) with sodium cyanide leads to the formation of saframycin A (**158**) (Scheme 1). Treatment of saframycin A (**158**) with aqueous acid lead to the formation of saframycin S (**163**) and decyanosaframycin A.

Interestingly, the nitrile moiety of saframycin A (**158**) was not observable by infrared spectroscopy. It was hypothesized that the extensive oxygenation in this substance quenches the nitrile absorption intensity. This characteristic was observed in all of the saframycins that contain a nitrile moiety.

Saframycin R (**167**) was isolated in 1982 by Arai et al.[25] The structure was revised in 2000 by the use of HMQC and HMBC experiments on two acetate derivatives.[26] The main difference in structure between saframycin R (**167**) and the previously isolated saframycins was that the E-ring was in the form of a hydroquinone rather than a quinone.[26] The isolation and structures of saframycins F (**166**), G (**161**), and H (**162**) were determined in the study of the minor components of the saframycin mixture isolated from *Streptomyces lavendulae* No. 314.[27] The structures of saframycins F(**166**), G(**161**), and H (**162**) were determined by comparison of spectroscopic data with that of saframycins C (**160**) and D (**164**). In 1988, saframycins Mx1 (**169**) and Mx2 (**170**) were isolated.[28] Like saframycin R (**167**), one of the aromatic rings was in the hydroquinone form.

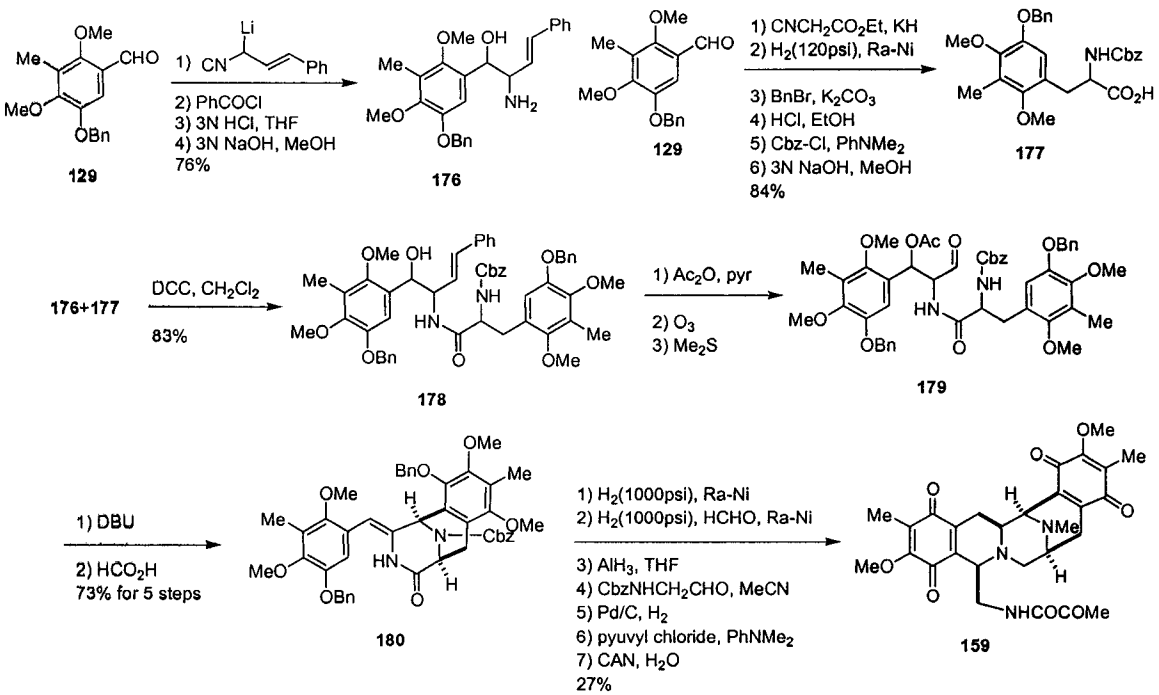
In the search for more biologically active saframycins, six new saframycins were produced by directed biosynthesis employing *Streptomyces lavendulae* No. 314 (Figure 3).[29] The supplementation of alanine and glycine or alanylglycine yielded saframycins Y3

(**170**) and the dimer Y2b (**174**). The addition of 2-amino-*n*-butyric acid and glycine or 2-amino-*n*-butyrylglycine produced saframycins Yd-1 (**171**), Ad-1 (**173**), and dimer Y2b-d (**175**). Saframycin Yd-2 (**172**) was produced by the supplementation of glycylglycine.

1.3.1 Total synthesis of the saframycins

Currently, several saframycins have been synthesized: (±)-saframycin B (Fukuyama, 1982),[30] (±)-saframycin A (Fukuyama, 1990),[31] (±)-saframycin B, C, D (Kubu, 1986),[32] (-)-saframycin A (Myers, 1999),[33] (-)-saframycin A (Corey, 1999),[34] (-)-saframycin A (Myers, 2002, solid phase).[35]

1.3.1.1 Fukuyama's total synthesis of (±)-saframycin B



Scheme 23. Fukuyama's total synthesis of (±)-saframycin B

The total synthesis of (±)-saframycin B, which was reported by Fukuyama and Sachleben[30] in 1982, constitutes the first total synthesis of a member of the saframycin family (Scheme 23). Starting with aldehyde **129**, treatment with the lithium anion of cinnamyl isocyanide afforded the benzylic alcohol, which was subsequently esterified with benzoyl chloride. Hydration of the isocyanide followed by hydrolysis of the formamide afforded amino alcohol **176** in good yield.

The A-ring of saframycin B was also synthesized from aldehyde **129**. Amino acid **177** was synthesized in six steps from aldehyde **129** in 84% overall yield. The synthesis commenced with initial formation of the necessary α,β-unsaturated isocyanide followed by subsequent reduction of the benzylic olefin. Coupling of amine **176** with the N-Cbz amino acid **177** yielded amide **178** in 83% yield. Acetylation of the secondary alcohol group in compound **178** followed by careful ozonolysis and reductive workup yielded a diastereomeric mixture of unstable aldehydes **179**. Elimination of the acetate group in compound **179** afforded a 1:1 diastereomeric mixture of olefin intermediates. Cyclization of these intermediates was accomplished using formic acid to form tetracycle **180** as a single diastereomer. A two-step sequence was used to reduce the benzylic olefin from the least hindered side, followed by removal of the Cbz protecting group and methylation of the amino group. Reduction of the lactam carbonyl using alane yielded the key Pictet-Spengler precursor. Upon treatment of the amine with N-Cbz-glycinal, the pentacycle was formed in a 6:1 diastereomeric ratio at C-1 with the desired diastereomer as the major product. Removal of the Cbz group, followed by coupling with pyruvyl chloride, produced an amide intermediate. The final step entailed the oxidation of the two

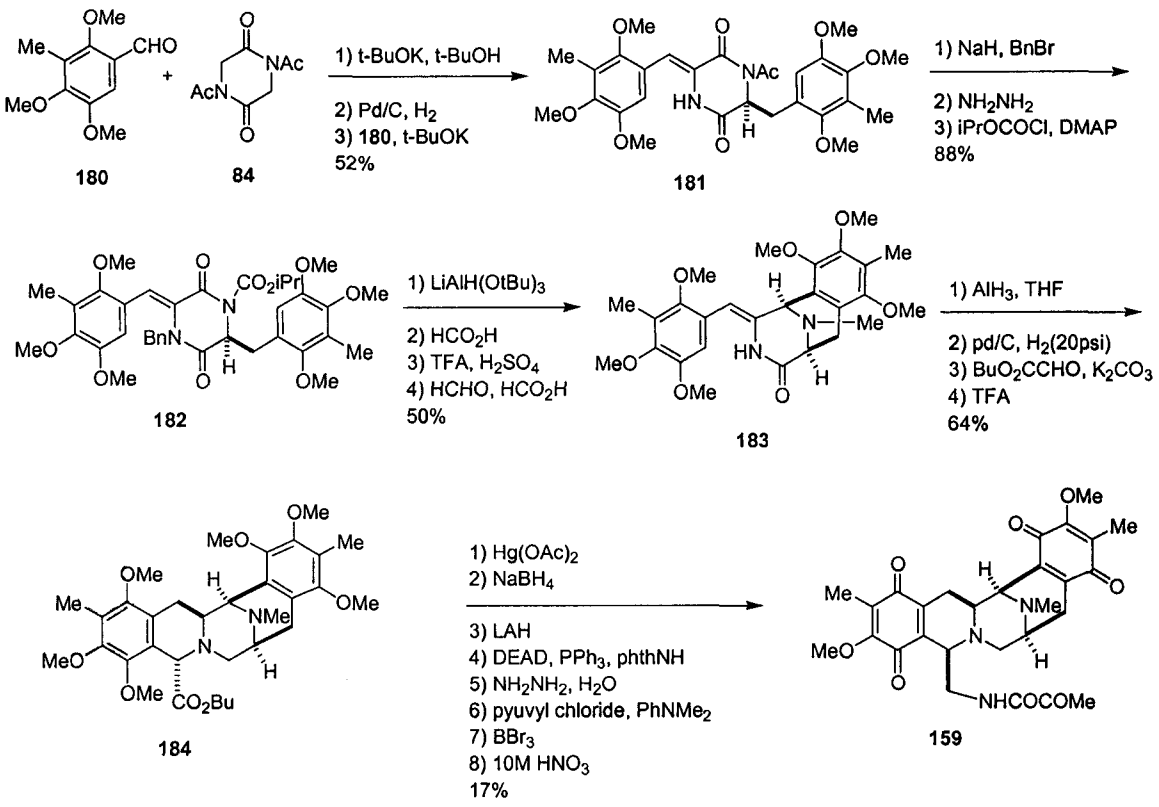
hydroquinones to quinones using ceric ammonium nitrate to afford (±)-saframycin B (**159**).

1.3.1.2 Kubo's total synthesis (±)-saframycin B

In 1987, Kubo et al. reported their synthesis of (±)-saframycin B (Scheme 4).[31] Aromatic aldehyde **180** was condensed with diketopiperazine **84**, followed by hydrogenation of the benzylic olefin in the resulting intermediate. A second aldol condensation provided **181** in 52% overall yield for the three steps. Selective activation of one of the lactam carbonyl groups was accomplished via the benzyl protection of the unprotected lactam group followed by acetate removal and subsequent carbamate formation to afford **182**. Partial reduction of the activated lactam group in **182** was accomplished using lithium aluminum tri-*tert*-butoxyhydride. Cyclization of the resulting carbinolamine intermediate was achieved using formic acid according to Fukuyama's syntheses.[30] Removal of the isopropyl carbamate group in the cyclization product followed by *N*-methylation yielded tricycle **183** in 50% yield.

Tricycle **183** was converted to pentacycle **184** via initial reduction of the amide to the amine using alane followed by hydrogenolysis of both the benzylic olefin as well as the benzylamine. A Pictet-Spengler cyclization on the resulting intermediate produced the necessary pentacycle. Unfortunately, the stereochemistry obtained at C-1 position corresponded to that of the undesired diastereomer. Subsequent epimerization of this center was accomplished by initial oxidation of the amine to the corresponding imine using mercury(II) acetate followed by selective reduction of the imine from the least hindered face using $NaBH_4$. The butyl ester was then reduced using LAH, and amination

of the alcohol was accomplished via a Mitsunobu reaction using phthalimide. Removal of the phthalimide protecting group, acylation of the primary amine with pyruvyl chloride, demethylation of the hydroquinones using boron tribromide, and oxidation to the diquinone using 10 M HNO_3 provided (±)-saframycin B.

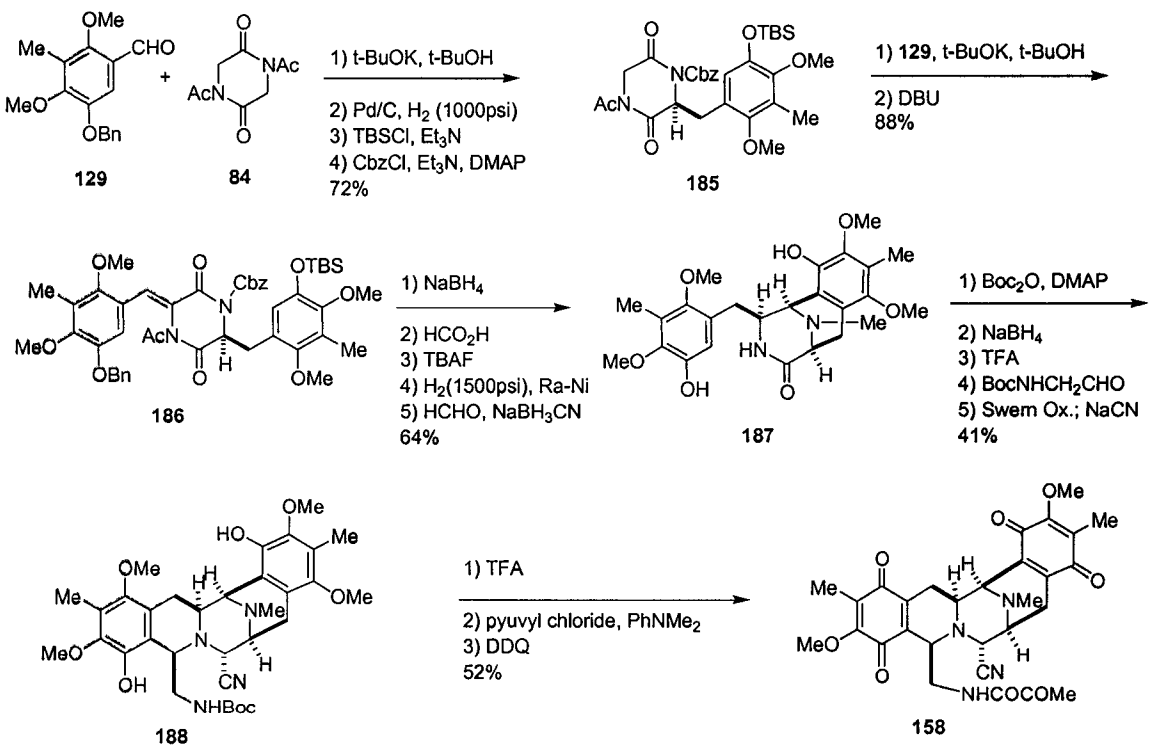


Scheme 24. Kubo's total synthesis of (±)-saframycin B

1.3.1.3 Fukuyama's total synthesis of (±)-saframycin A

In 1990, Fukuyama et al. reported the first synthesis of (±)-saframycin A as shown in Scheme 25.[32] Aromatic aldehyde **129** was treated with the potassium enolate of diketopiperazine **84**, followed by removal of one acetate group, and selective protection of the amide as a Cbz carbamate to afford diketopiperazine **185** in 72% yield. This aldol chemistry was first used by Kubo et al. in their saframycin B synthesis[31] (Scheme 24).

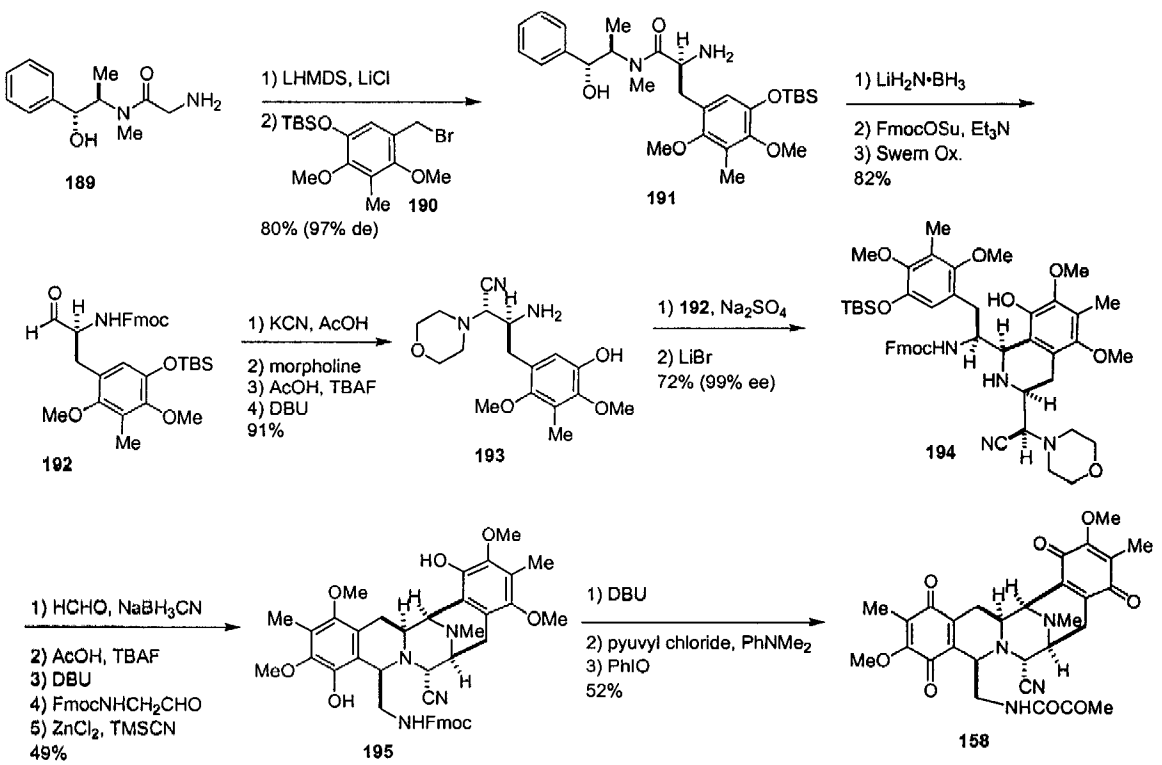
Following a second aldol condensation with aldehyde **129**, the N-Cbz-protected amide was selectively reduced to the carbinolamine using sodium borohydride. This allowed for a cyclization through the requisite iminium ion upon treatment with formic acid to afford a tricyclic intermediate. High-pressure hydrogenation over Raney-Ni followed by amine methylation yielded **187**. The lactam functional group in compound **187** was activated for ring opening through protection of the lactam nitrogen as the corresponding *tert*-butyl carbamate. The lactam carbonyl group in the resulting intermediate was then reduced under mild conditions to the corresponding amino alcohol. Removal of the Boc protecting group was followed by a Pictet-Spengler reaction on the resulting free amine, affording the pentacyclic core intermediate.



Scheme 25. Fukuyama's total synthesis of (±)-saframycin A

Swern oxidation of the primary alcohol in this intermediate afforded the corresponding aldehyde, which condensed with the amine to form an intermediate carbinolamine that was trapped with sodium cyanide to form the stable aminonitrile **188**. The final steps of the synthesis involved cleavage of the Boc group, amide formation using pyruvyl chloride, and oxidation of the hydroquinones to their corresponding quinones using DDQ, thus afforded (±)-saframycin A.

1.3.1.4 Myers' total synthesis of (-)-saframycin A



Scheme 26. Myers' total synthesis of (-)-saframycin A

The first asymmetric synthesis of (-)-saframycin A was accomplished in 1999 by Myers and Kung.[33] This elegant and convergent synthesis focused on the hidden symmetry of saframycin A (Scheme 10). Alkylation of pseudoephedrine **189** with

bromide **190** afforded the homobenzylic amine **191** in 80% yield. Cleavage of the auxiliary to form the amino alcohol was followed by amine protection and oxidation of the primary alcohol group to produce aldehyde **192**. Importantly, compound **192** aldehyde was used to form both halves of saframycin A. Treatment of the aldehydes group in **192** with HCN initially formed the corresponding cyanohydrin, which was subsequently treated with morpholine to yield the corresponding amino nitrile.[33b] This aminonitrile functional group served as a masked aldehyde. Removal of the TBS and Fmoc groups was accomplished in two steps in high yield to form amine **193**.

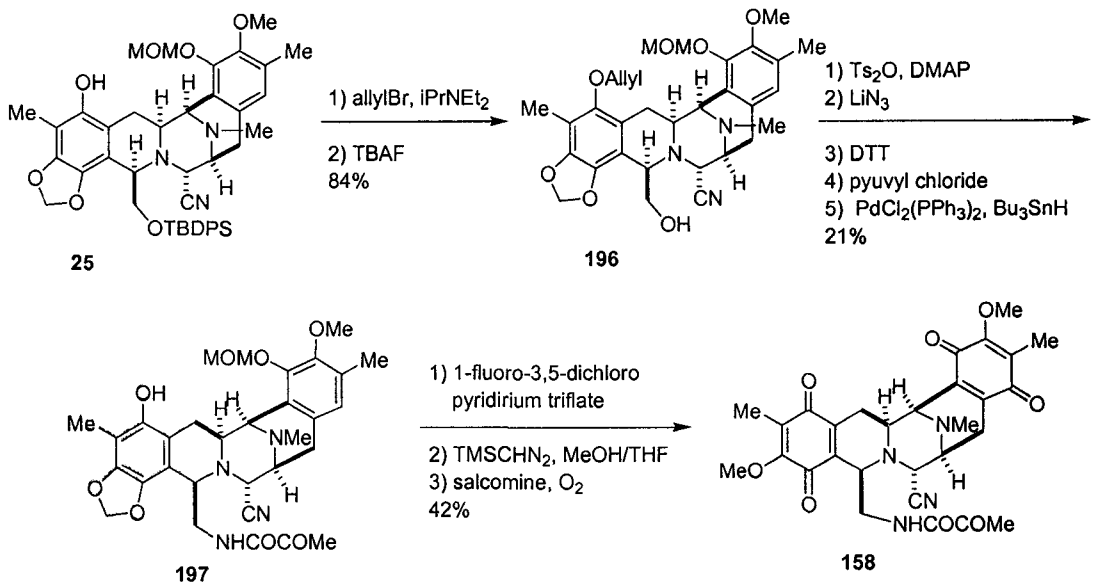
Pictet-Spengler cyclization of amine **193** with aldehyde **192** in the presence of Na_2SO_4 provided bicycle **194** in good yield and high enantiomeric excess. Reductive amination of compound **194** with formaldehyde followed by removal of the TBS and Fmoc groups afforded a *N*-methyl bicyclic intermediate. Performing a Pictet-Spengler cyclisation on the bicyclic intermediate with *N*-Fmoc glycinal in the presence of zinc chloride and TMSCN provided the pentacycle **195**. Removal of the Fmoc group in compound **195** was followed by acylation of the amine with pyruvyl chloride. Finally, treatment with iodosobenzene provided (-)-saframycin A in 52% yield for the final three steps.

1.3.1.5 Corey's total synthesis of (-)-saframycin A

In 1999, Corey and Martinez published the second asymmetric synthesis of (-)-saframycin A as illustrated in Scheme 27.[34] The synthesis commenced with hexacycle **25** (Scheme 27), an intermediate originally employed in their synthesis of ecteinascidin 743[8].

Allylation of phenol **25** followed by removal of the TBDPS groups provided the

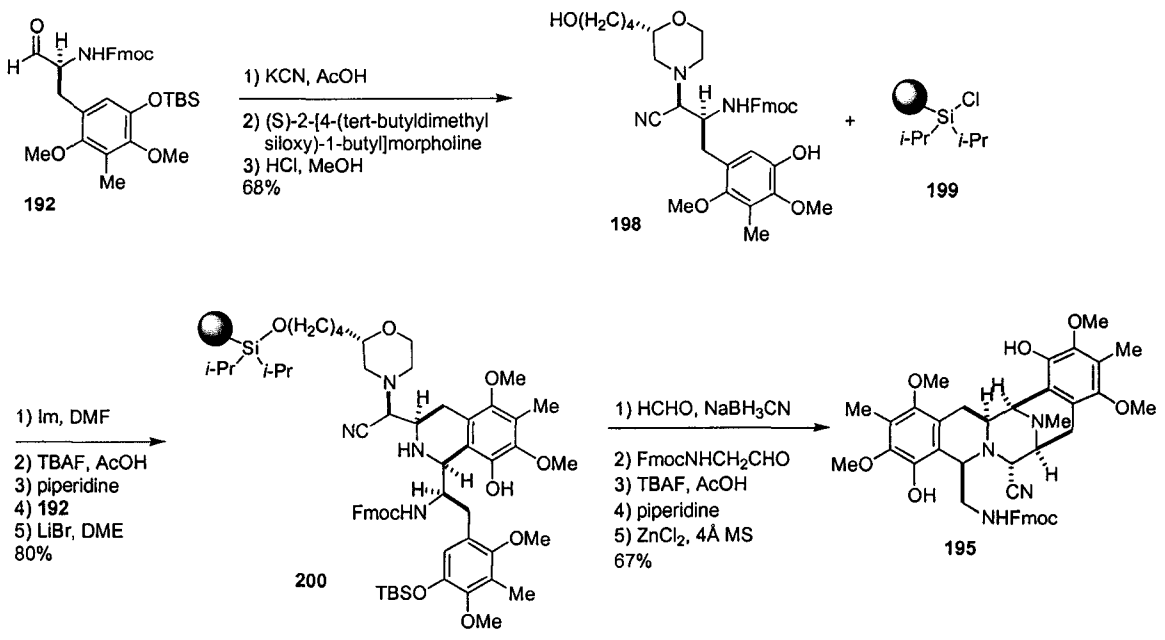
alcohol **196** in high yield (Scheme 27). The alcohol was then converted into the corresponding amine through a three step sequences. The amino group in the resulting intermediate was subsequently acylated with pyruvyl chloride. The phenol in the acylated intermediate was then deprotected to afford **197**. An efficient one-step oxidation of the E-ring and subsequent MOM group removal were both accomplished using 1-fluoro-3,5-dichloropyridinium triflate. Methylation of the free phenol followed by oxidation of the A-ring hydroquinone produced (-)-saframycin A.



Scheme 27. Corey's synthesis of (-)-saframycin A

1.3.1.6 Myers' total synthesis of (-)-saframycin A by solid phase synthesis

In 2002, Myers and Lanman reported the successful adaptation of their prior solution-phase synthesis of saframycin A[33] to a 10-step solid-supported synthesis suitable for the preparation of large numbers of diverse saframycin analogues with deep-seated structural modifications.[35]



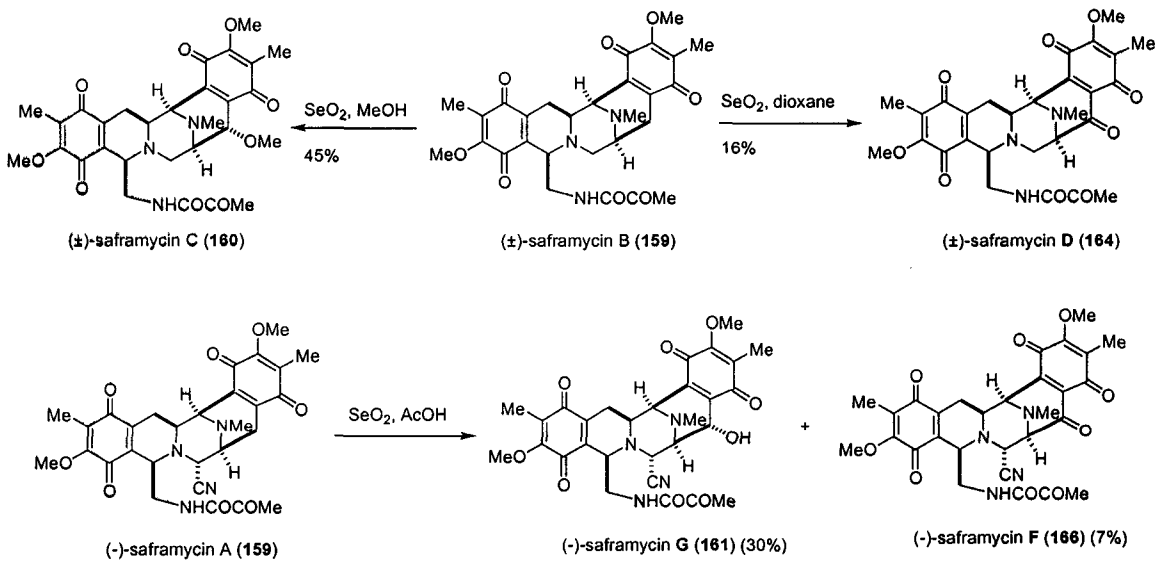
Scheme 28. Myers' solid phase synthesis of (-)-saframycin A

In this route, a novel dual linker was developed for resin immobilization of the first α-amino aldehyde component (compound **192**, Scheme 28). The dual linker was attached to the C-terminus of **192** through amino nitrile formation to form a mixture of *syn* and *anti* diastereomers (*dr* 1.2:1). Attachment of the *anti*-morpholino nitrile **198** to the requisite solid support was achieved through silyl ether formation with 4-(chlorodiisopropylsilyl)polystyrene, providing the corresponding resin-bound intermediate. Selective deprotection of the *tert*-butyldimethylsilyl ether group in this intermediate, followed by subsequent treatment with piperidine provided a free amine intermediate. Addition of a 3-fold excess of the N-protected α-amino aldehyde **192** to the free amine intermediate provided the corresponding resin-supported imine, which upon warming at 35 °C suffered a stereoselective Pictet-Spengler cyclization reaction to afford the *cis*-tetrahydroisoquinoline derivative **200** (cis:trans 7:1). The secondary amino group of the tetrahydroisoquinoline intermediate **200** was then methylated through reductive

amination. Subsequent deprotection of the phenol and primary amino groups of the resulting N-alkylation product produced the new amino-terminal resin-bound intermediate. The second Pictet-Spengler cyclization reaction occurred upon exposure of the intermediate to *N*-Fmoc glycinal to afford a bis-tetrahydroisoquinoline compound. A cyclization-autorelease sequence was executed in the single step by warming the bis-tetrahydroisoquinoline intermediate in the presence of zinc chloride to provide the saframycin intermediate **195** directly, in a remarkable yield of 53% for the 10-step sequence from **198**.

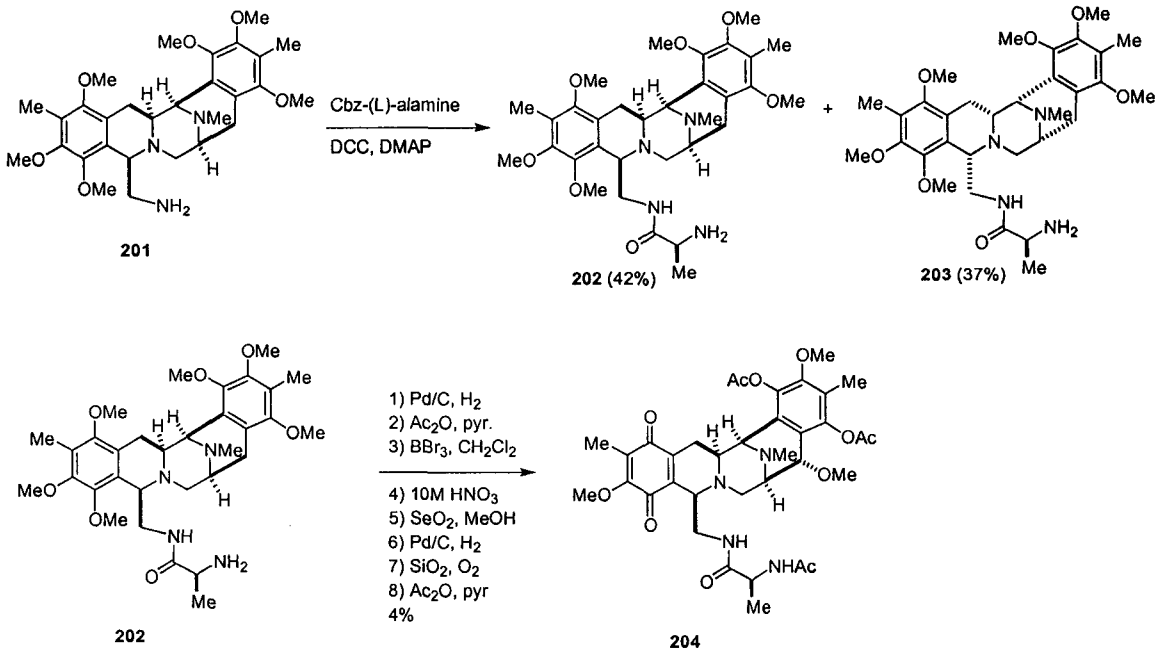
1.3.1.7 Conversion between members of the saframycin family

Kubo et al. showed that (±)-saframycin B could be converted to saframycins C (**160**) and D (**164**) via a selective oxidation using SeO_2 (Scheme 29).[36a] Using dioxane as the solvent in this transformation, (±)-saframycin D was synthesized in 16% yield. The use of methanol as the solvent yielded (±)-saframycin C in 45% yield.



Scheme 29. Selenium dioxide oxidation of saframycin B and saframycin A

Kubo et al. also showed that (-)-saframycin A could be oxidized with SeO_2 to yield saframycins (Scheme 29).[36b,c] The highest yielding product was (-)-saframycin G in 30% yield.



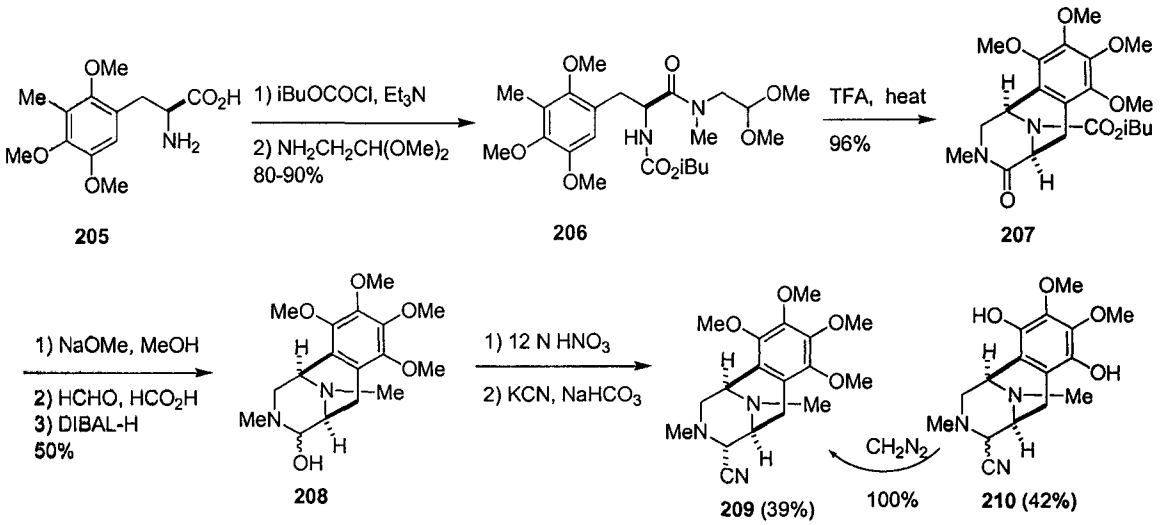
Scheme 30. Resolution and transformation of the racemic compound

Racemic pentacycle **201**, an intermediate in the Kubo's saframycin B synthesis, was used as a precursor for the synthesis of (-)-*N*-acetylsaframycin Mx2 (**204**) and *epi*-(+)-*N*-acetylsaframycin Mx2 by Kubo et al. (Scheme 30).[36d] The first step in the sequence involved the coupling of *N*-Cbz-L-alanine to the primary amine group in **201** produced the optically active amide **202** as well as its corresponding *epi*-enantiomer **203** in 42% and 37% isolated yields, respectively. Each diastereomer was subsequently carried on separately to the final Mx2-type compound. With respect to compound **202**, the *N*-Cbz group was initially removed, and the resulting amine was acylated to provide an intermediate. The hydroquinones were then deprotected and subsequently oxidized to the

corresponding quinones with SeO_2. This transformation also effected selective oxidation of the D-ring, furnishing the desired methyl ether intermediate. Reduction of the quinines in the methyl ether intermediate followed by regioselective oxidation of the A-ring hydroquinone yielded a saframycin Mx2 derivative that proved to be both light and air sensitive. Acetylation of the hydroquinone portion of this sensitive compound yielded the stable triacetate **204**. Similarly, pentacycle **203** was transformed into *epi*-(+)-*N*-acetylsaframycin Mx2 via the same sequence of transformations.

1.3.2 Synthetic studies of the saframycins

1.3.2.1 Kurihara's synthetic studies toward the saframycins

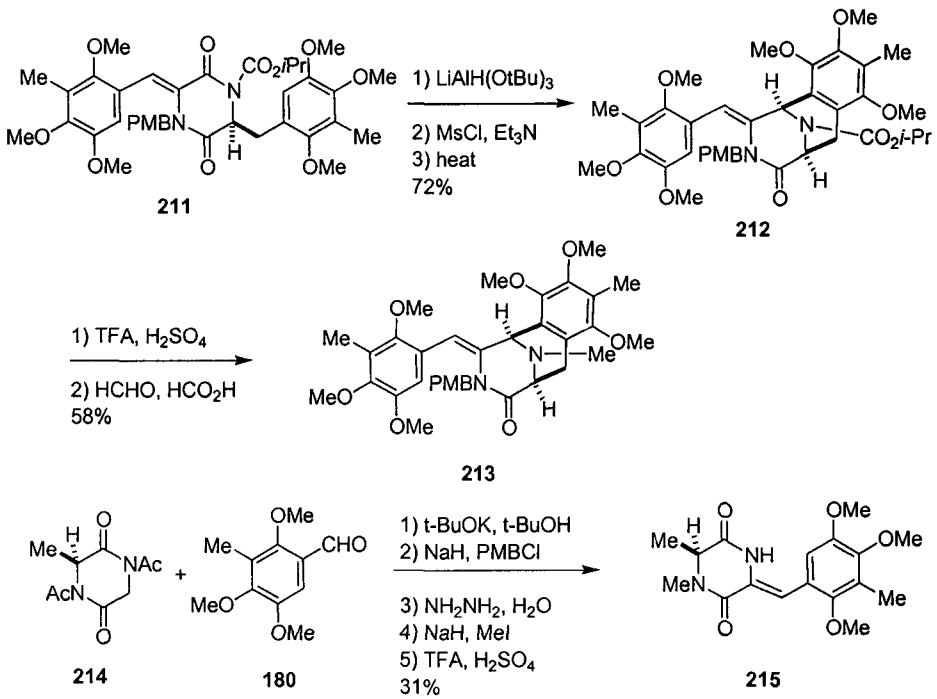


Scheme 31. Kurihara's synthetic studies toward the saframycins

In 1982, Kurihara et al. reported the first synthetic studies on the saframycins.[37] The synthesis commenced with condensation of compound **205** with aminoacetaldehyde dimethyl acetal through mixed anhydride methodology to afford **206** (Scheme 31). Heating compound **206** in trifluoroacetic acid afforded tricycle **207** via a double cyclization. Following partial reduction of the amide group in **207** using DIBALH,

oxidation of the hydroquinone, followed by treatment with potassium cyanide yielded a mixture of the desired tricycle **209** as well as **210** in 81% combined yield.

1.3.2.2 Kubo's synthetic studies toward the saframycins



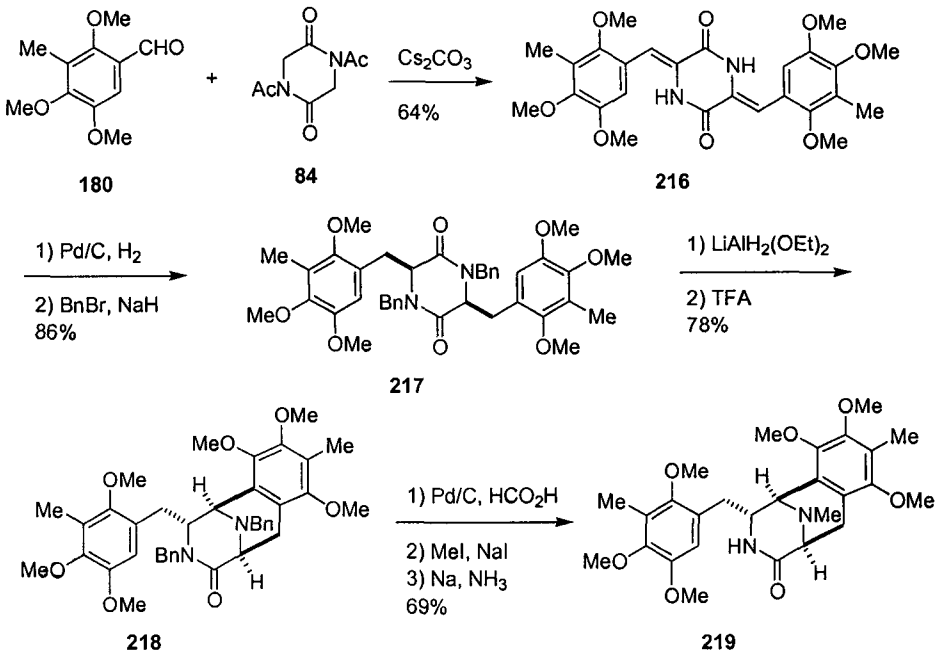
Scheme 32. Kubo's synthetic studies toward the saframycins

In 1989, Kubo et al. revealed in synthetic studies toward saframycin A that tetracycle **213** could be formed with the amide carbonyl group intact (Scheme 32).[38a] This strategy would allow for further functionalization to form the amino nitrile in saframycin A.

The first study on the asymmetric synthesis of the saframycins was published by Kubo et al. in 1997 (Scheme 16).[38b] Aldol condensation between the optically active diketopiperazine (+)-**214** and aldehyde **180** yielded (-)-**215** after further elaboration. It was envisioned that (-)-**215** could undergo a stereo-specific cyclization to form an

optically active tricyclic compound. However, on a racemic model system, little diastereoselectivity was observed in the cyclization.

1.3.2.3 Liebeskind's synthetic studies toward the saframycins

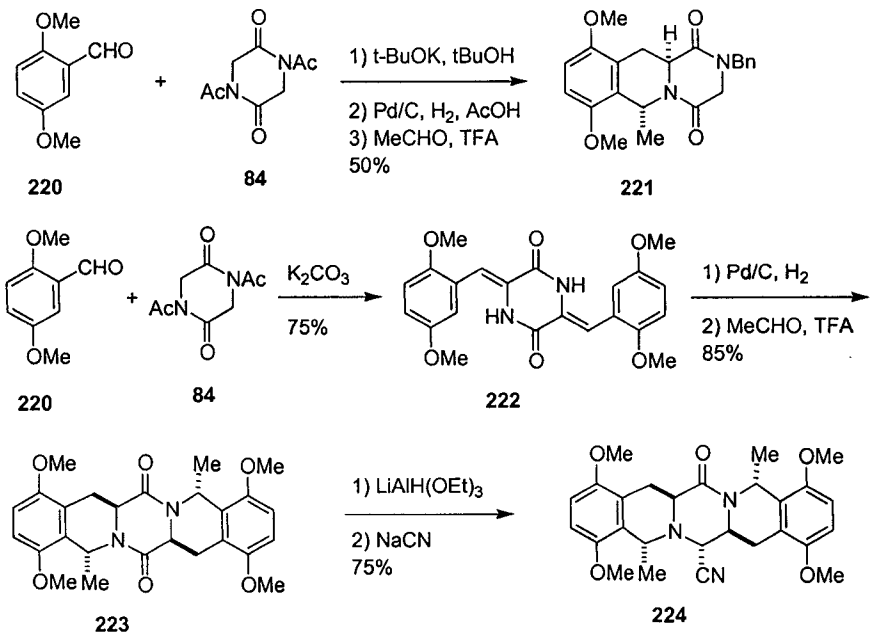


Scheme 33. Liebeskind's synthetic studies toward the saframycins

In 1991, Liebeskind and Shawe took advantage of the hidden symmetry of saframycin B in their synthetic study illustrated in Scheme 33.[39] Condensation of 2 equiv of aldehyde **180** with diketopiperazine **84** yielded the symmetrical diketopiperazine **216**. Following reduction of the olefin groups in **216** and subsequent protection of the amide nitrogens, a partial reduction of one of the amide carbonyl groups was accomplished using lithium diethoxyaluminum hydride. The carbinolamine intermediate was cyclized using TFA to form tricycle **218**. Unfortunately, the stereochemistry at C-3 was corresponded to the undesired configuration, and the authors noted that attempts to invert

the stereogenic center at C-3 of tricycle **219** were unsuccessful under a variety of conditions.

1.3.2.4 Ong's synthetic studies toward the saframycins

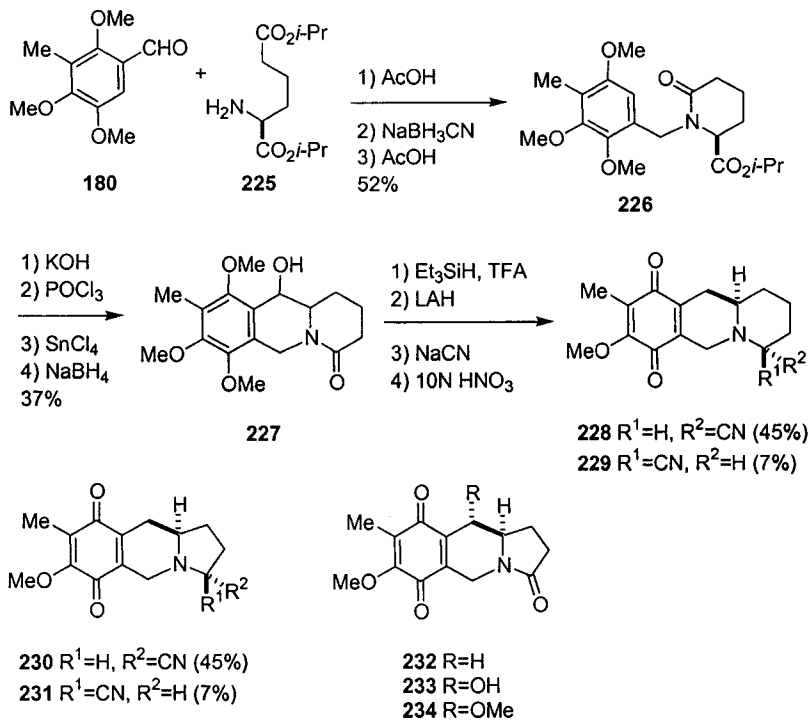


Scheme 34. Ong's synthetic studies towards the saframycins

In 1990, Ong and Lee synthesized the tricycle **221** via a Pictet-Spengler cyclization employing acetaldehyde and a diketopiperazine intermediate (Scheme 34).[40a] The major drawback to this approach was that the stereogenic center constructed in the Pictet-Spengler reaction possessed the unnatural relative configuration.

In 2003, Ong et al. reported a pentacycle **224**, which was synthesized through a double Pictet-Spengler cyclization using acetaldehyde and a different diketopiperazine intermediate (Scheme 34).[40b] Partial reduction of the amide in compound **223** and subsequent treatment of the resulting carbinolamine compound with cyanide afforded the aminonitrile **224**, which could be viewed as a structural mimic of saframycin A.

1.3.2.5 Saframycin analogs



Scheme 35. Saframycin A analogs

Two simple amino nitrile analogues of saframycin A were synthesized by Kubo et al.[41a] Scheme 35 illustrates the preparation of a diastereomeric pair of amino nitriles **228** and **229**. Condensation of aldehyde **180** with amine **225** yielded **226**. A four-step sequence featuring a Friedel-Crafts acylation afforded **227** from compound **226**. Deoxygenation of the secondary alcohol group in **227** was followed by reduction of the amide, in which the resulting carbinolamine intermediate was trapped with sodium cyanide. Finally, oxidation of the resulting aminonitrile intermediate to the corresponding quinone afforded diastereomers **228** and **229**.

A second set of amino nitriles were also synthesized by Kubo et al. that contained a five-membered C-ring as shown in Figure 6.[41b] The tricycles (**230-234**) were prepared utilizing the same chemistry as that previously described in Scheme 34.

Myers et al. reported the synthesis of a series of saframycin A analogues that were synthesized from pentacycle **195**. This was accomplished through initial removal of the Fmoc group in compound **195** followed by coupling of several carboxylic acids to the resulting primary amine (Scheme 26).[42] In addition to these studies, Myers et al. reported the synthesis of numerous analogs of saframycin A through a solid phase synthesis approach.[35]

CHAPTER 2

Biological Activities of the Ecteinascidins, Saframycins, Safracins and the Renieramycins

2.1 Biological activities of the ecteinascidins

2.1.1 Biological activities

The ecteinascidins (Figure 1) have the most potent biological activities by a significant margin relative to that of any of the tetrahydroisoquinoline antitumor antibiotics. The cytotoxic activity of Et-743 (**1**) is orders of magnitude more potent than saframycin A against B16 melanoma.[43] The exciting aspect of Et-743 (**1**) is that it appears to have a unique mode of action, thus constituting a new subclass of antitumor agent that could be active against drug-resistant cell lines. Et-743 (**1**) is currently in phase II human clinical trials in the United States.[43a,b] The *in vitro* activities of Et-743 (**1**) against several common tumor cell lines were exceedingly high and are summarized in Table 1.[43c]

Table 1. Activity of Et-743 against several tumor cell lines

tumor type	IC_{50} (nM)
P388 leukemia	0.00034
L1210 leukemia	0.00066
A549 lung cancer	0.00026
HT29 colon cancer	0.00046
MEL-28 melanoma	0.00050

Et-729 (**2**) exhibits higher *in vivo* activities against P 388 leukemia than Et-743 and Et-745 (Table 2).[44a] The IC_{50}'s for Et-729 against L1210 cells in the absence and presence of 2.5% murine plasma were 37 and 72 pM, respectively.[44b]

Table 2. Activities of Et's 729, 743, and 745 (**3**) against P388 Leukemia

Compound	Dose (μg/kg)	T/C[a]
Et-729	3.8	214
Et-743	15	167
Et-745	250	111

[a] T/C = is the increased lifespan of mice treated with the drug versus the control group.

Et's-722 (**8**) and Et-736 (**9**) were found to also have high *in vitro* activities against L1210 with IC_{90}'s of 2.5 and 5.0 ng/mL, respectively.[4a] Et-722 was also highly active *in vivo* against a variety of cell lines (Table 3).

Table 3. Activity of Et-722 against Several Tumor Cell Lines

tumor type	dose (μg/kg)	T/C[a]
P388 leukemia	25	>265
B16 melanoma	50	200
Lewis lung carcinoma	50	0.27
LX-1 lung carcinoma	75	0.00

[a] T/C = is the increased lifespan of mice treated with the drug versus the control group.

Valoti et al. treated several human ovarian carcinoma zenografts that were characterized by specific behavior and drug responsiveness versus *cis*-platinum (DDP) with Et-743.[44c] Et-743 was found to be very active against the HO22-S cell line (sensitive toward DDP). Et-743 also induced long-lasting regressions against HOC18 (marginally

sensitive to DDP). The HOC18 xenograft (nonresponsive to DPP) showed significant growth delay, but for MNB-PTX-1, a highly resistant tumor toward chemotherapy, Et-743 had no activity.

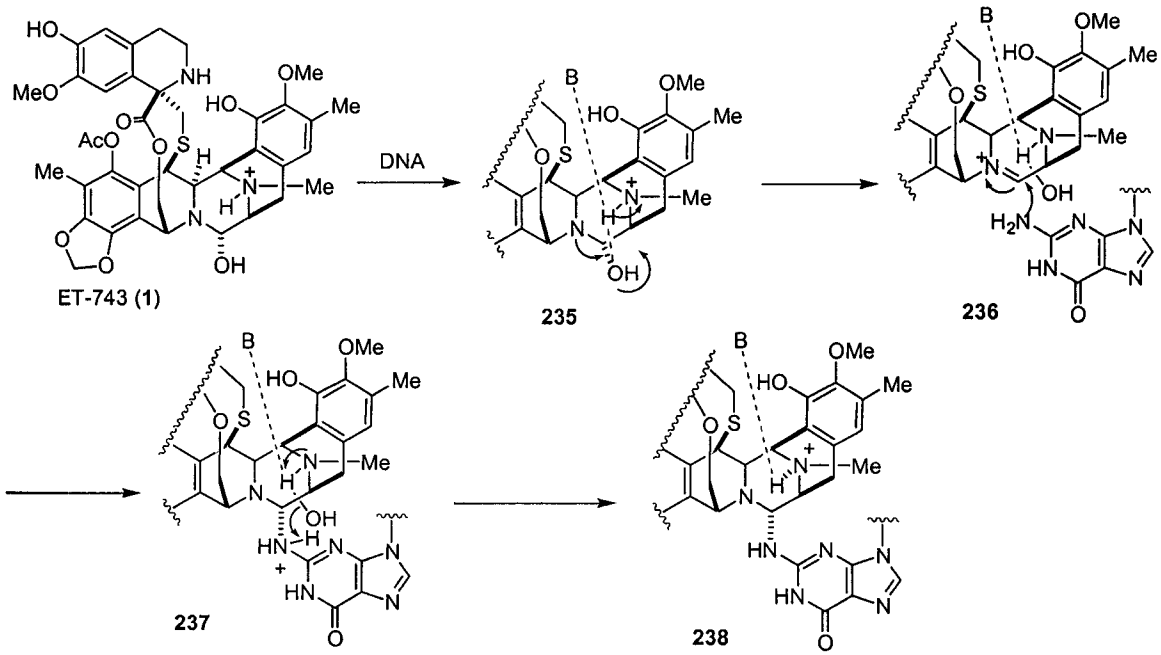
2.1.2 Mechanism of action

The mechanism of action of the ecteinascidins has been studied by several groups. It has been shown that Et-743 inhibits RNA, DNA, and protein synthesis with IC_{50} values of 8, 30, and 100 nM, respectively.[43c] Et-743 has a similar structure to that of saframycin S, indicating that DNA alkylation should indeed be possible. DNA alkylation has been studied by Pommier et al.[45a] and Hurley et al.[45b] The alkylation takes place in the minor groove, as does alkylation with the saframycins. The alkylated DNA substrate exhibits a bend or widening of the minor groove,[45b] presumably due to the C-subunit of the ecteinascidins. The C-subunit, which is perpendicular to the rest of the molecule, makes the ecteinascidins unique from the saframycins, which are fairly flat. It has been postulated that this bend in DNA disrupts DNA-protein binding and may be, in part, the source of the enhanced biological activities of the ecteinascidins.

It has been demonstrated that the ecteinascidins alkylate DNA at the N-2 residue of guanine in GC-rich regions.[45c] The unique sequence specificities of Et-743 have been shown to be 5'-GGG, 5'-GGC, and 5'AGC.

In 1998, Hurley et al. showed by NMR studies that the N-12 of Et-743 was protonated in the Et-743 DNA covalent adduct.[45d] From these data, a mechanism for DNA alkylation was suggested as illustrated in Scheme 36. The N-12 of Et-743 is protonated, which facilitates expulsion of the hydroxyl group in the form of water to form

the iminium species **236**. The exocyclic nitrogen of guanine is then envisioned to attack this electrophilic species resulting in covalent adduct formation (**238**). NMR studies support the contention that the final DNA-Et-743 adduct is protonated at N-12.



Scheme 36. Proposed mechanism of DNA alkylation by Et-743

The binding of DNA to the DNA base guanine at the minor groove causes the molecule to bend toward the DNA major groove. This process alters the normal structure of the DNA molecule.[45b] Of the three tetrahydroisoquinoline subunits of Et-743, subunit A and B bind to the DNA base guanine. Subunit C, which does not interact with guanine, appears to be important in Et-743's cytotoxic mechanism of action.[5]

After Et-743 binds to DNA, subunit C precluding out from the DNA molecule, may affect the binding of proteins required for regulating DNA activity, which, in turn, impairs the cell's ability to function, including the process of transcription.[46a,b] Additionally, Et-743 forms covalent DNA adducts, and the generated nucleotide prevents

the separation of the DNA strands. Also, those adducts stimulate the cell in attempting to repair the nucleotide excision repair defect, which will cause the DNA strand breaks and subsequently results in the cell's death.[47] The above newly described drug mechanism of action may only affect cells with transcribed genes.[46b]

In addition, Et-743 is unique in its ability to inhibit the overexpression of the multidrug resistance-1 gene (MDR-1) coding for the P-glycoprotein, a major factor responsible for cells developing resistance to cancer drugs.[48] Because of its activity in DNA transcription, Et-743 appears to be more effective in the G1 portion of the cell cycle and it blocks the G2 phase of the cell cycle. G2/mitosis also occurs and promotes apoptosis.[49]

Table 4. Activities of phthalascidin analogs *versus* various tumor cell lines

Compound	A-549 (nM)	A375 (nM)	PC-3 (nM)
148	0.95	0.17	0.55
150	3.2	0.35	0.64
151	1.5	0.27	1.1
152	1.2	0.35	0.75
153	1.6	0.31	0.90
154	1.7	0.29	0.86
155	2.1	0.51	2.9
156	3.1	0.55	3.1
157	3.0	0.97	2.4
1	1.0	0.15	0.70

The biological activity of several pthalascidin analogues were found to be similar to that of Et-743 (Table 4).[18] This was an important observation due to the fact that the phthalascidins are structurally less complex than the ecteinascidins and are also much easier to synthesize than the natural products.

The lethal biological target of Et-743 and the exact mechanism by which it kills cells at such extraordinarily low concentrations remains a partially unsolved and very fascinating problem despite the elucidation of the DNA repair inhibition mechanism. The intense interest in this clinical antitumor candidate is expected to continue to draw researchers to address the biological chemistry of Et 743.

2.2 Biological activities of the saframycins

2.2.1 Biological activities

Table 5. Antimicrobial activity of saframycins A and S

test organism	**3** MIC (μg/mL)	**8** MIC (μg/mL)
Staphylococcus aureus FDA 209P	0.1	0.025
Streptococcus faecalis	12.4	3.12
Bacillus subtilis PCI 219	0.1	0.025
Corynebacterium diphtheriae	0.003	0.004
Sarcina lutea	0.05	0.025

[a] T/C = is the increased lifespan of mice treated with the drug versus the control group.

All of the saframycins (Figure 2, 3) have been found to display antitumor and antimicrobial activity. Saframycin S displays the most potent antitumor activity, while saframycins R (**167**)[22] and A (**158**)[19] exhibited similar but less potent antitumor and antimicrobial activities (Table 5). These three saframycins have either a nitrile or

hydroxyl at C-21. Saframycins B (**159**) and D (**164**), which lack a leaving group at C-21, as expected, displayed the lowest antitumor activity.[19]

The ID_{50} (50% inhibition dose) activities against L1210 leukemia of several saframycins are listed in Table 6.[36c] Saframycins A, S (**158**, **163**, respectively) containing either a nitrile or hydroxyl group at C-21 possess the highest activities. Saframycins G, H, F (**161**, **162**, **166**, respectively) which contain a leaving group at C-21 also have sterically demanding side chains that apparently block the incipient iminium species from alkylating DNA. Saframycins B, C, D (**159**, **160**, **164**, respectively) which lack a leaving group at C-21 had much lower activities.

Table 6. Antitumor activity of saframycins and analogs versus L1210 leukemia

Compound	ID_{50} (μM)	compound	ID_{50} (μM)
158	0.0056	**166**	0.59
163	0.0053	**159**	0.80
161	0.030	**160**	3.9
162	0.033	**164**	4.8

Saframycin S (**163**) had very potent *in vivo* activity against Ehrlich ascites tumors. At the near optimum dose of 0.5 mg/kg/day the percentage of 40 day surviving mice was 80-90% *versus* all of the control mice that died within 18 days.

There was no difference in biological activity between saframycins Y3, Yd-1, Yd-2, and Ad-1 with respect to an amino group or a carbonyl at C-25. Also, the dimers Y2b and Y2b-d had similar activities to the corresponding monomers. In a study to examine side chain effects on biological activity, Arai et al. synthesized 15 acyl, 9 alkyl, and 3 carbonyl derivatives of the C-25 amino group of saframycin Y3. It was found that the

acyl derivatives had lower activity while the alkyl derivatives had similar activities to the natural product. Also, as the side chain became bulkier, the activity decreased.

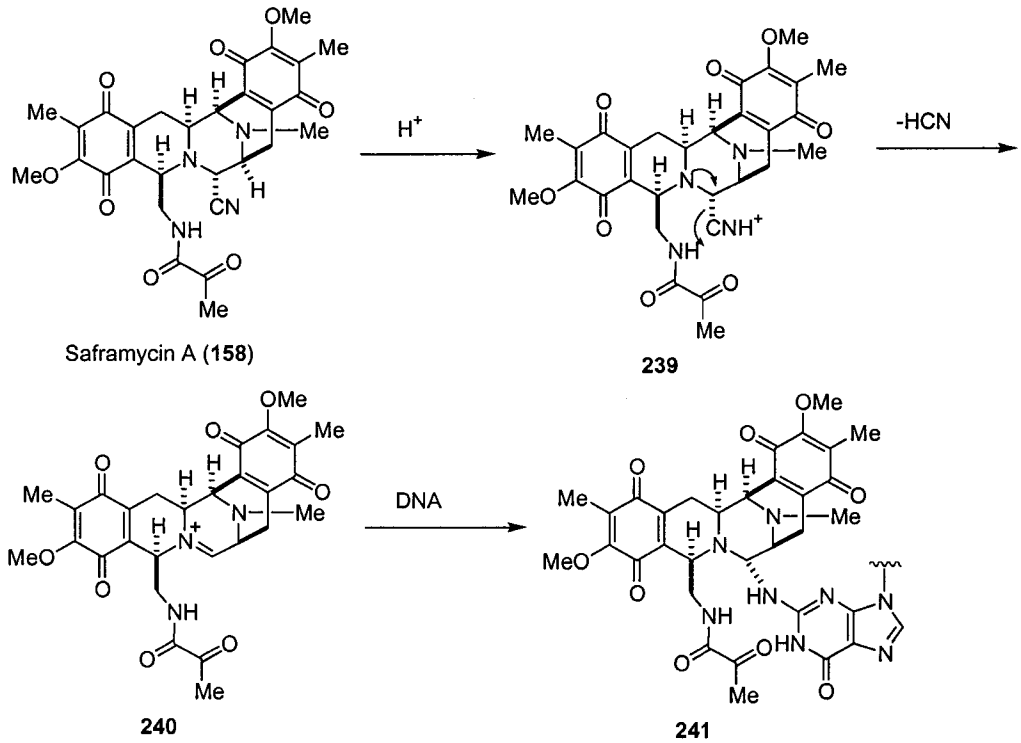
The simple saframycin A analogues **228-234** (Scheme 35) were also tested for biological activity;[41a] however, none of these compounds displayed significant cytotoxicity exhibiting 2.0-4.0 μg/kg ED_{50} values against L1210 murine leukemia. However, the amino nitriles **230** and **231** possessed good bioactivity against fungi in which saframycin A had little activity.[41b]

Saframycin A had been shown to inhibit RNA synthesis at 0.2 μg/mL, while DNA synthesis was inhibited at higher concentrations. Inhibition of nucleic acid biosynthesis was observed at lower concentrations when saframycin A was reduced to the corresponding hydroquinone prior to testing.[50a] Saframycin S does not need to be reduced to display antitumor activity, but the activity was enhanced when saframycin S was in the reduced form.[50b] Reductants such as dithiothreitol (DTT) reduce the quinone moieties to the corresponding dihydroquinones that activate these substances for iminium ion formation and subsequent DNA alkylation. For example, saframycin A in the presence of DTT has been shown to release cyanide, indicating that the iminium species is readily formed from this oxidation state.

2.2.2 Mechanism of action

The presence of either a nitrile or hydroxyl group at C-21 allows for the formation of an electrophilic iminium species that alkylates DNA in the minor groove. The mechanism originally proposed by Lown et al. (Scheme 37) for alkylation invokes protonation of the nitrile (**239**) with expulsion of HCN to form the iminium ion species

240.[51a] The N-2 residue of guanine subsequently forms a covalent bond to the drug resulting in an adduct such as **241**.

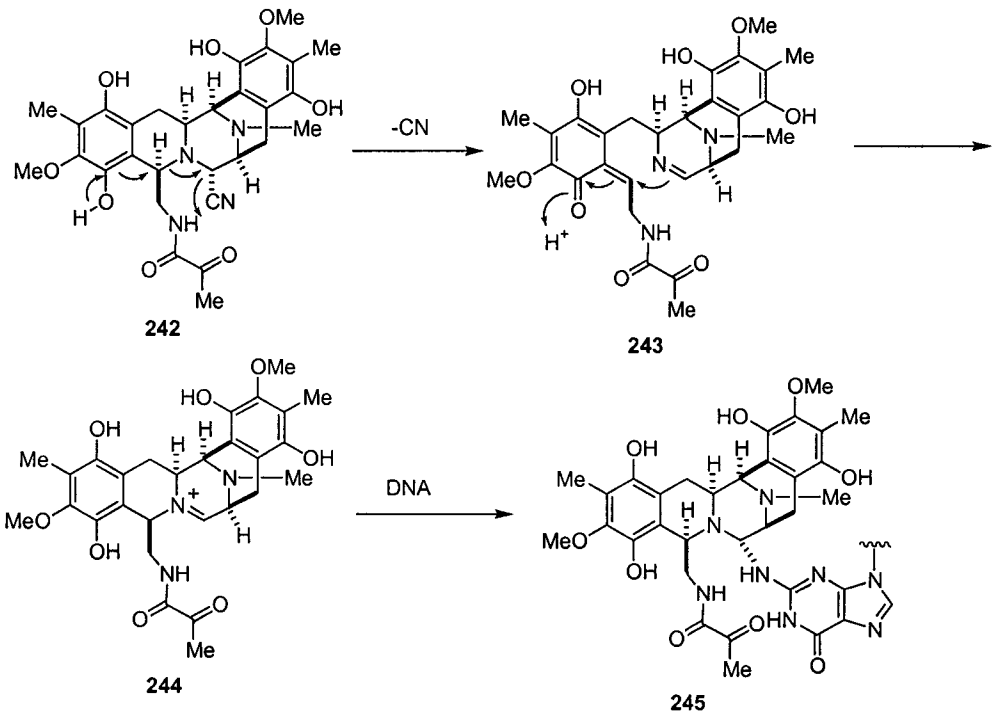


Scheme 37. Proposed mechanism of DNA alkylation by saframycin A

Evidence to support the alkylation hypothesis was obtained by radiolabeling experiments in which ^{14}C-labeled tyrosine was biosynthetically converted to saframycin A.[50b] Upon exposure of the ^{14}C-labeled saframycin to DNA in the presence of DTT, it was found that the DNA retained the ^{14}C label. When ^{14}CN was used to label the C-21 nitrile, under the same set of conditions, it was found that the ^{14}C label was not incorporated into DNA. Furthermore, footprinting studies on saframycin-treated DNA also provide direct experimental evidence for alkylation of DNA by the saframycins.

Another mechanism was proposed by Hill and Remers, which was based on the fact that saframycin A does not alkylate DNA unless it was converted into the

corresponding hydroquinone form (**242**) (Scheme 38).[51b] These workers speculate that the phenol facilitates scission of the B-ring C-N bond, which in turn leads to the expulsion of cyanide. The resulting imine **243** subsequently re-attacks the *o*-quinone methide to form the iminium species **244**, which subsequently alkylates DNA to form the adduct **245**.



Scheme 38. Alternate mechanism of DNA alkylation by saframycin A

The characteristics of DNA binding by the saframycins thus appears to be a simple two-step process whereby: (1) reversible noncovalent binding of the drug to the minor groove of DNA is immediately followed by, (2) the formation of a covalent bond to DNA within the minor groove. Being a diamino aminal, this linkage is subject to thermal reversal. There is a second type of covalent binding that is promoted by a reducing agent, and the binding presumably proceeds through the more reactive dihydroquinones form that more readily form the iminium ion species.

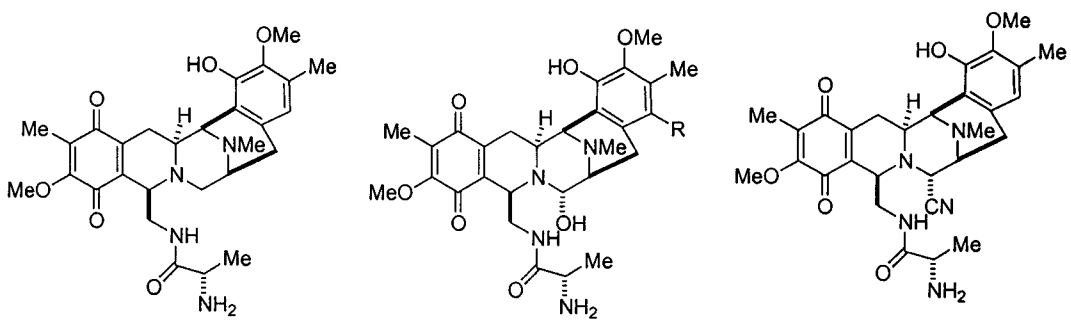
The bishydroquinone saframycin A analogues synthesized by Myers and Plowright were used to investigate if there would be increased activity in the reduced form of this natural product.[42] These analogues showed very potent activity against the A375 melanoma and A549 lung carcinoma tumor cell lines with some analogues having a 20-fold increase in activity over saframycin A.

Saframycins A and S were found to be modestly sequence specific with respect to DNA alkylation, exhibiting a preference for 5'-GGG and 5'-GGC sequences by the use of MPE (methidium propyl EDTA) Fe(II) footprinting studies. Saframycin S also displayed a specificity for 5'-CGG, while saframycin A did not. Saframycins Mx1 and Mx3, which both contain the hydroxyl group at C-21, showed the same selectivity as saframycin S.[51c] It has been reasoned that the moderate sequence specificity observed is due to the molecular recognition of the saframycins for specific DNA sequences prior to iminium ion formation.

It has been argued that the cytotoxicity of the saframycins is not exclusively due to DNA alkylation, and it has also been demonstrated, for instance, that the saframycins cause oxidative damage to DNA under aerobic conditions.[51a] Mechanistic studies have provided evidence that superoxide and hydroxyl radical species are formed in the presence of saframycin A in the hydroquinone form while DNA cleavage was not observed in the presence of saframycin A in the quinone form. This is consistent with the well-known capacity of quinones to reduce molecular oxygen to superoxide.[52] Saframycin R, which has an acyl group on the phenol, caused much less DNA cleavage than saframycin A, making it much less toxic without any loss in biological activity.[26]

Recently, Myers et al.[53] identified a new strain of yeast: *S. cerevisiae* strain (CCY-333), which is sensitive to treatment with saframycin A and a saframycin analog. This new strain allowed the comparison of the genetic responses to drug treatment by whole-genome transcription. Of the many genetic response pathways that was identified, they found that known DNA-damage response pathways may not be transcriptionally modified, suggesting that the primary mechanism of action of saframycin A may not involve known DNA-damage pathways.

2.3 Biological activities of the safracins



246 Safracin A

247 Safracin B R=H
248 R=Br

50 Cynosafrasin B

Figure 4. The safracins

Ikeda et al. isolated safracins A and B (**246** and **247**, respectively) from *Pseudomonas fluorescens* A2-2 in 1983 (Figure 8).[54] The safracins have structures very similar to that of the saframycins with the exception that the E-ring is a phenol instead of a quinone or hydroquinone as in the saframycins. The structures were determined by comparison to spectral data for saframycin B.[54b] Soon after that, the absolute stereochemistry was determined by X-raycrystallography using **248** (C-15 bromosafracin A).[54c]

In 1985, Ikeda found that addition of Fe(II) and Fe(III) to the fermentation broth increased the production of safracin B at a concentration of 0.01%.[55] Safracin A production was increased at higher iron concentration (0.1%). The cyano derivative of safracin B (**50**) was isolated on a multikilogram scale by Cuevas et al. for use in the semisynthetic synthesis of ecteinascidin 743 as discussed before.[7]

Safracin B was a more potent antibiotic than safracin A.[56] Interestingly, both safracins have antimicrobial activity against *Pseudomonas aeruginosa* and *Serratia marcencens* in which saframycin A was ineffective. Safracin B, possessing a C-21 carbinolamine, was much more active than safracin A against P388 and L1210 leukemia cell lines *in vitro*.

2.4 Biological activities of the renieramycins

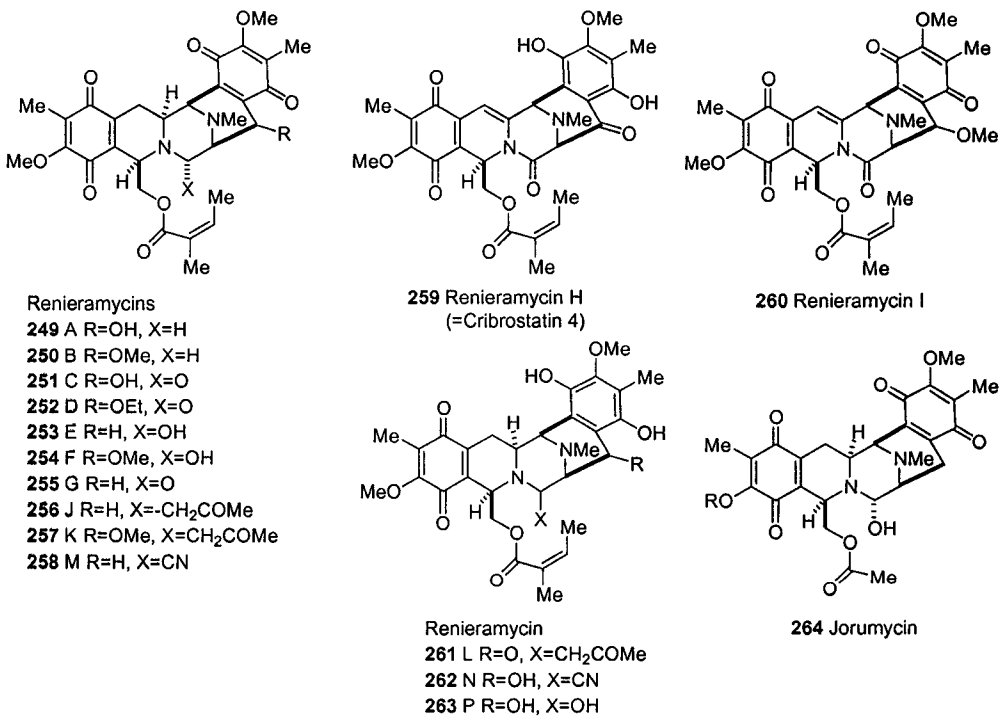


Figure 5. The renieramycins

In 1982, Frincke and Faulkner isolated four new natural products from the sponge *Reniera* sp.[57a] that possess structures similar to that of the saframycins. These compounds were named renieramycin A-D, **249-252,** respectively (Figure 5). The main difference between the saframycins and renieramycins is that the side chain is an angelate ester instead of a pyruvamide. Seven years later, He and Faulkner isolated renieramycins E and F, **253** and **254,** respectively;[57b] both compounds proved to be unstable. Renieramycin G (**255**) was isolated in 1992 by Davidson from the Fijian sponge *Xestospongia caycedoi,*[57c] and this renieramycin was also found to be unstable. Two different renieramycins were isolated in 1998 by Parameswaran et al. from the sponge *Haliclona cribricutis,*[57d,e,f] which were named as renieramycins H and I (**259** and **260,** respectively). Compound **260** was also isolated from *Cribrochalina* sp. and given the name cribrostatin 4.[57f] The structure of cribrostatin 4 (**260**) was determined by X-ray crystal analysis. Renieramycin P (**263**), which was originally named Renieramycin J, was isolated in 2003.[57g] Satio et al. isolated five renieramycins (J, K, M, L and N, **256, 257, 258, 261** and **262,** respectively) from a blue sponge, *Xestospongia sp,* which is pretreated with potassium cyanide to increase the yield of renieramycins approximately 100-fold.[57h] Fontana et al. isolated jorumycin (**263**) from *Jorunna funebris.*[58] The structure of jorumycin is most similar to that of renieramycin F with exception of the acetate group on the alcohol versus the angelate ester on the renieramycins.

Renieramycins A-D,[57a] H, and I[57d] have moderate antimicrobial activities, while renieramycin G has shown moderate activity against KB and LoVo cell lines with MIC values of 0.5 and 1.0 μg/mL, respectively.[57c] Cytotoxicities of renieramycin P against 3Y1, HeLa and P388 cell (IC_{50}, nM) were 5.3, 12.3, and 0.53, respectively.[57g]

The IC_{50} values of renieramycin M (**258**), N (**261**) were almost equal to those of saframycin A (**158**) and ecteinascidin-770 (**5**) (Table 7).

Table 7. Cytotoxicity of renieramycins M, N against several cancer lines[a]

Compound	IC50 (nM)			
	HCT116	QG56	NCI-H460	DLD1
Renieramycin M	7.9	19	5.9	9.6
Renieramycin N	5.6	11	6.7	5.7
Saframycin A	0.4	5.5	2.1	0.6
Ecteinascidin-770	1.2	3.9	0.64	2.4

[a]HCT116=human colon carcinoma; QC56=human lung carcinoma; NCI-H460=human lung carcinoma; DLD1=human colon carcinoma

Table 8. Cytotoxicity of renieramycins against cancer lines

Compound	IC50 (nM)		
	HCT116	QG56	DU145
Renieramycin M	7.9	11.0	NT[a]
Renieramycin E	0.38>	1.0	0.38>
Renieramycin J	730.0	510.0	370.0
Jorumycin	0.57	0.76	0.49

Note: HCT116=human colon carcinoma; QC56=human lung carcinoma; DU145=human prostate cancer. [a] NT: not tested.

Renieramycin E (**253**) and jorumycin (**263**) have very similar cytotoxic activities against human cancer cell lines, which were similar to those of the saframycins (Table 8).[59] However renieramycin J, which lacks a leaving group at the C-21 position, exhibits lower cytotoxic activity. To realize good cytotoxic activity, a cyano or a hydroxyl group at the C-21 position is essential, suggesting that the elimination of this functional group

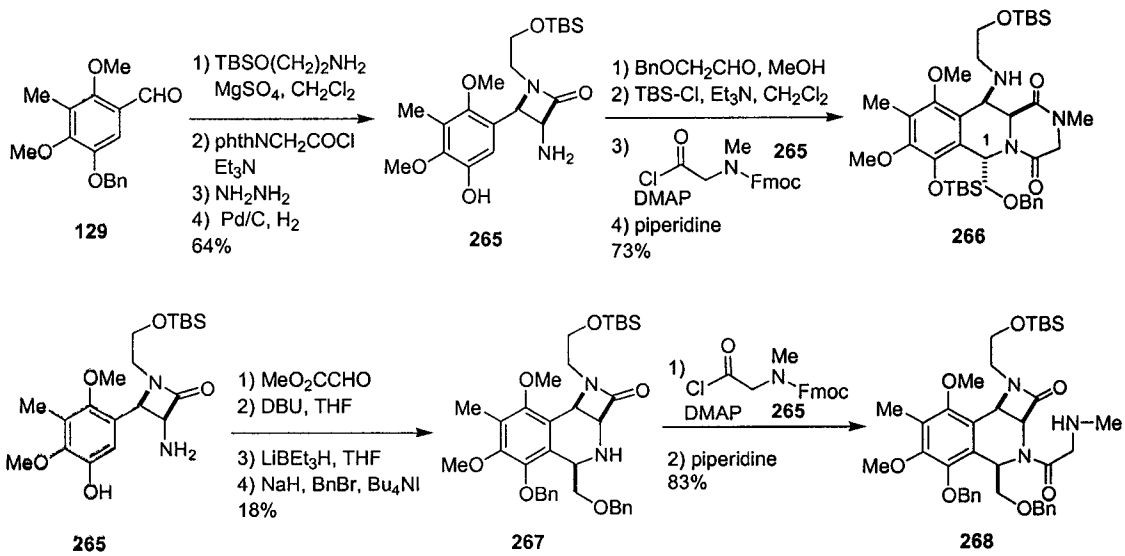
under physiological conditions results in the formation of a reactive iminium species that is responsible for covalent bond formation with DNA.

CHAPTER 3

Asymmetric Total Synthesis of (-)-Renieramycin G and Studies toward the Total Synthesis of Ecteinascidin-743

3.1 Background

In 2001, Williams et al. reported the synthesis of a tricyclic tetrahydroisoquinoline **266** (Scheme 39), which is currently being utilized in an approach toward the total synthesis of bioxalomycin α_2.[60] The approach involves a sequential Staudinger/Pictet-Spengler reaction strategy, which produces a key β-lactam unit.



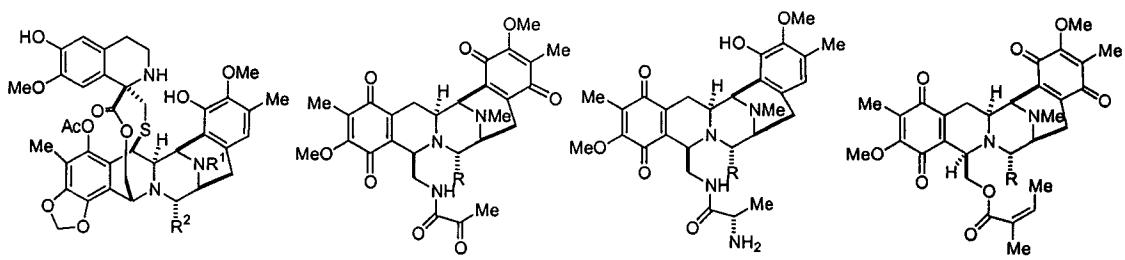
Scheme 39. Williams' synthetic approach towards bioxalomycin α_2

The efficient synthesis of this β-lactam commenced with the formation of an imine derived from aldehyde **129** and *O*-TBS-protected ethanolamine. The requisite ketene, produced from phthalimidoacetyl chloride, subsequently treated with the imine to

form a β-lactam intermediate in high yield. Cleavage of the phthalimide and benzyl ether groups afforded **265** in 64% overall yield. Pictet-Spengler cyclization of compound **265** with benzyloxyacetaldehyde afforded a single diastereomer; however, following amide coupling with Fmoc-sarcosine and subsequent cyclization, it was discovered that the tricyclic diketopiperazine **266** had the undesired *anti*-configuration at C-1.

A modified Pictet-Spengler cyclization was then performed on compound **265** using methyl glycoxylate to afford a single *anti*-diastereomer that could undergo epimerization in the presence of DBU to afford the desired *cis*-diastereomer. Reduction of the carbomethoxy group followed by protection of the resulting alcohol afforded **267**. Peptide coupling was followed by cleavage of the Fmoc carbamate; however, cyclization did not occur as in the case of the *anti*-diastereomer intermediate.

Based on the above results, the goal of this project is to design a general approach for the syntheses of the following biologically active *bis*-tetrahydroisoquinoline antitumor alkaloids (Figure 6), of which Et-743 is the primary target.



Ecteinascidins
1 743 R^1=Me, R^2=OH
2 729 R^1=H, R^2=OH
3 745 R^1=Me, R_2=H
5 770 R^1=Me, R^2=CN

Saframycins
158 A R=CN
159 B R=H
163 S R=OH

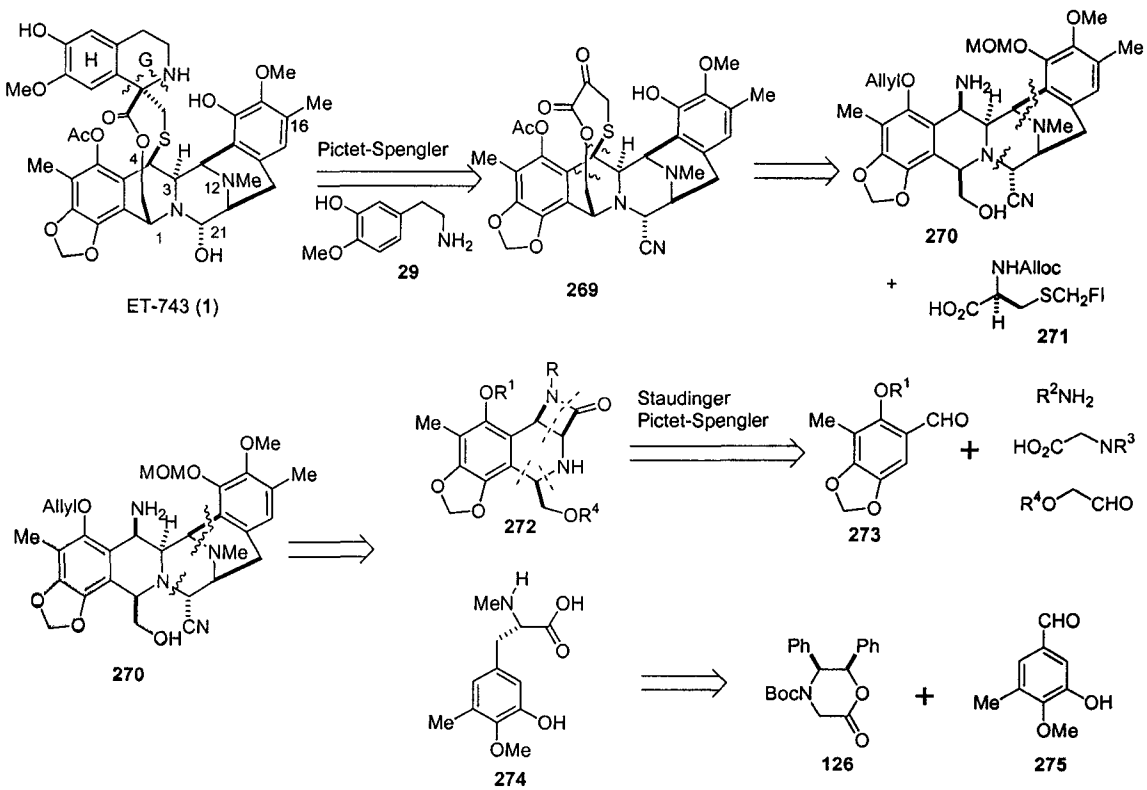
Safracins
246 A R=H
247 B R=OH
Cynosafrasin B
50 R=CN

Renieramycins
253 E R=OH
255 G R=O
258 M R=CN

Figure 6. Potential targets

3.2 Retrosynthetic analysis

With respect to synthetic strategy, the G and H ring of ecteinascidin 743 could arise from the coupling of β-keto lactone **269** with amine **29** by a Pictet-Spengler reaction. The β-keto lactone could, in turn, be produced through the coupling of pentacycle **270** with amino acid **271**. The pentacycle **270** could be made from tetrahydroisoquinoline **272** and amino acid **274**. And both **272** and **274** could be made through the methodologies that developed in our group, which are a sequential Staudinger reaction followed by a Pictet-Spengler reaction[61] and alkylation of enolate of Williams' chiral glycinate (**126**).[62]



Scheme 40. Retrosynthetic analysis of Et-743

Since one of our goals is to design a convergent approach for the total synthesis of Et-743, we embedded the C-4 chiral center at early stage: during synthesis of the tetrahydroisoquinoline ring **272**. Also by comparing to Corey's synthesis,[6] during the

amino acid synthesis, we will introduce the C-16 and N-12 methyl groups to make the designed synthesis of Et-743 more concise.

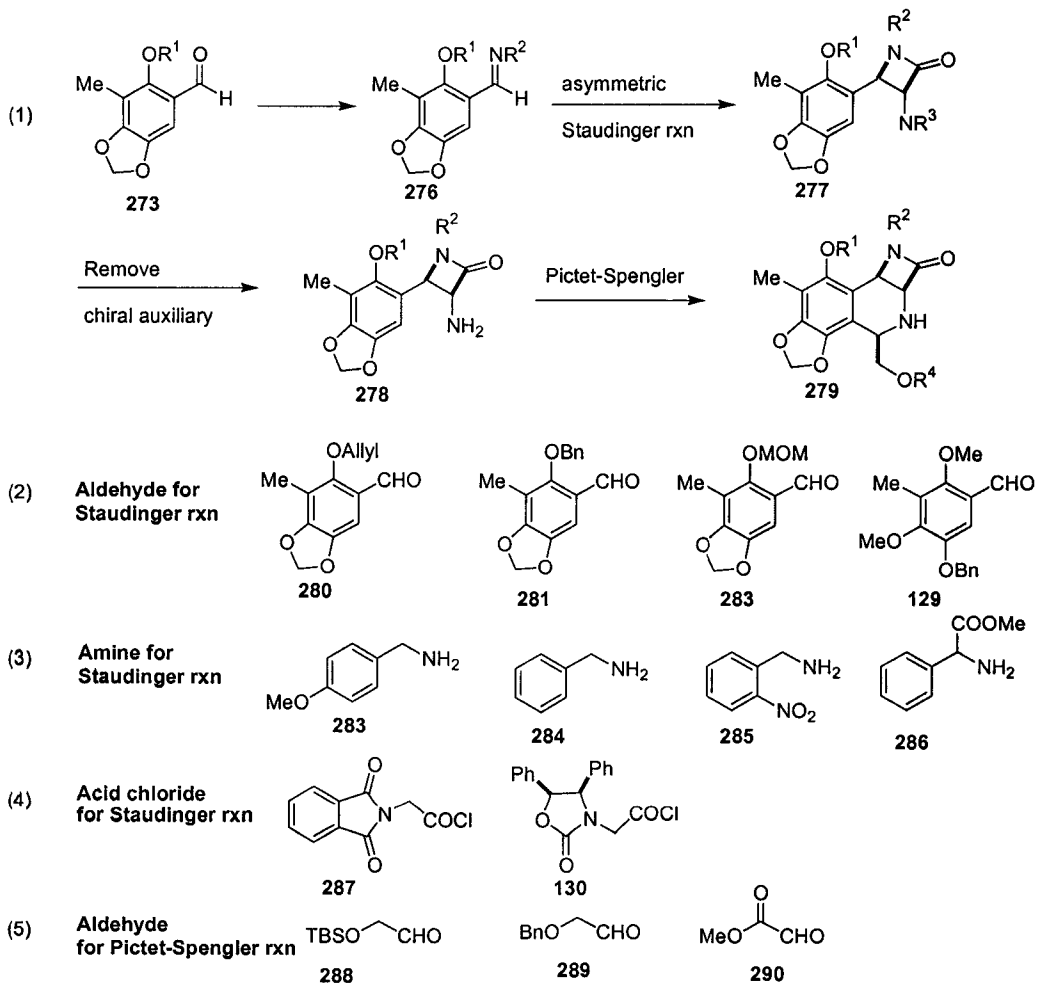
3.3 Synthesis of the tetrahydroisoquinoline ring system

3.3.1 Choosing the starting materials for the tetrahydroisoquinoline synthesis

The initial goal of this project was to develop a general approach to synthesize the chiral tetrahydroisoquinoline ring system (**272**). Through previous work,[60] compound **272** was synthesized in racemic form; however, several shortcomings existed with this non-selective approach. First, an asymmetric synthesis was desired which could not be realized through the previous route. Second, the key Pictet-Spengler reaction of the compound **265** with methyl glycoxylate was problematic with respect to reproducibility. Finally, several other transformations including the carbomethoxy group reduction and protecting group installation were low-yielding. In an effort to overcome the difficulties associated with the racemic strategy, several modifications were necessary. First, an appropriate chiral auxiliary would be incorporated into the synthesis to induce high stereoselectivity. Second, a method would be devised to control the selectivity of the Pictet-Spengler reaction in an effort to achieve the correct relative stereochemistry at C-1. Finally, compatible protecting group strategy would necessarily be developed.

In light of the requirements of the modified strategy (Scheme 41 (1)) and the readily available starting materials (Scheme 41 (2-5)), a new route was constructed for the synthesis of Et-743. Compound **273** would necessarily bear a protecting group at the phenol position, which would be labile, yet stable under hydrogenation conditions. Compound **282** met these requirements (Scheme 41 (2)). Alternatively, compound **129** would be an appropriate choice with respect to the synthesis of other targets of interest in

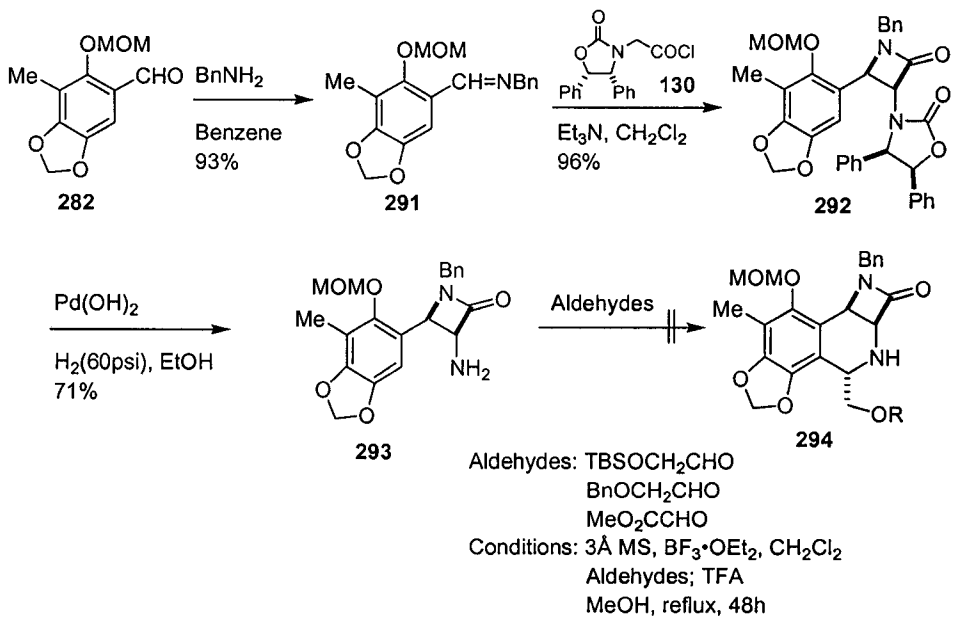
the tetrahydroisoquinoline family. In order to transform compound **273** to its corresponding imine (**276**), an appropriate amine starting material was necessary as well. The primary amine candidate would be labile, yet stable under hydrogenation conditions. Upon consideration of the available options (Scheme 41 (3)), compound **284** was selected for condensation onto aldehyde **282**. Finally, a ketene precursor was chosen for the asymmetric Staudinger reaction, which would convert intermediate **276** to β-lactam **277**. Acid chloride **130** was chosen as the appropriate chiral partner for this transformation and was predicted to afford excellent stereoselectivity for this process, Compound **130** was available through a convenient three-step sequence from 1,2-diphenylaminoalcohol.[45]



Scheme 41. Starting materials for asymmetric synthesis of the tetrahydroisoquinoline

Following the selection of the necessary components for the asymmetric Staudinger reaction, an appropriate aldehyde compound was selected for use in the subsequent Pictet-Spengler reaction (**278**→**279**, Scheme 41 (1)). Based on our previous result, compound **290** was selected for the Pictet-Spengler transformation. The ester functional group in compound **290** would allow for a subsequent epimerization step necessary to establish the desired configuration at C-1.

3.3.2 A first-generation Pictet-Spengler approach to the tetrahydroisoquinoline ring



Scheme 42. First generation Pictet-Spengler approach to the tetrahydroisoquinoline ring

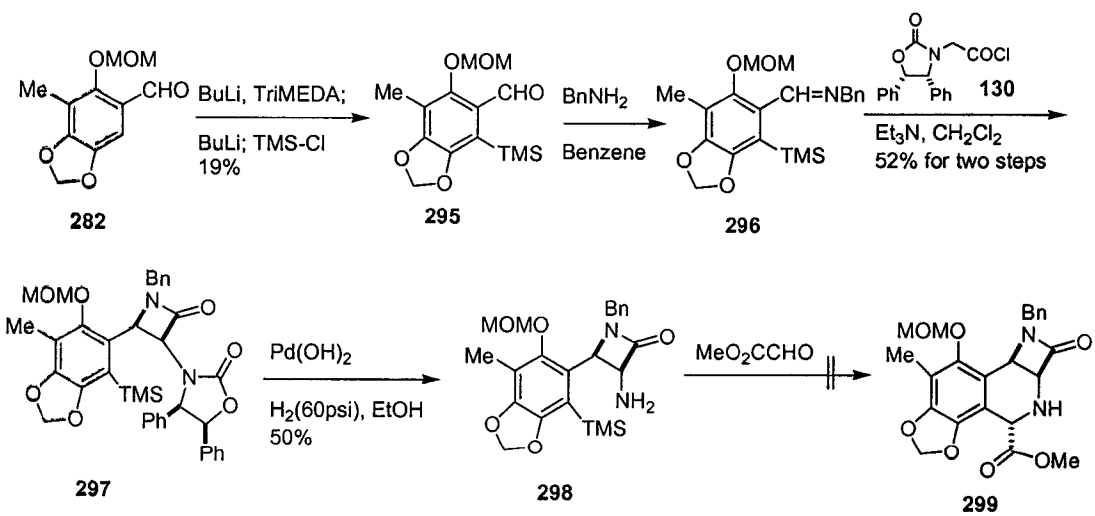
The initial approach commenced with aldehyde **282**, which was synthesized in three steps from commercial available sesamol (**11**) as described in Corey's total synthesis of Et-743.[6] The aldehyde **282** was condensed with benzylamine by refluxing in benzene with a Dean-Stark trap to afford imine **291** in 93% yield. The imine **291** was then reacted with the ketene generated from the chiral acid chloride **130** through an asymmetric Staudinger reaction. This transformation provided the β-lactam **292** in 96% yield as a single diastereomer.[63,64] The chiral auxiliary of compound **292** was

subsequently removed by hydrogenolysis in the presence of $Pd(OH)_2$ (Pearlman's catalyst) to provide amino alcohol **293**. Unfortunately, although compound **269** was readily available, any attempts to convert **293** to **294** through the Pictet-Spengler sequence were unsuccessful.[6,17]

It was reasoned that the lack of reactivity of compound **293** could be attributed to the methylenedioxy group. Specifically, alkyl groups are known for be poor *ortho* directors in Pictet-Spengler reactions without proper activation.

3.3.3 A second-generation Pictet-Spengler approach-with TMS activation

Previous research had established that appropriate halogen or silyl group substitution could effect an increase in the reactivity as well as regio-selectivity of the Pictet-Spengler reaction,[64] Therefore, an alternate approach would entail the synthesis of a modified Pictet-Spengler precursor bearing TMS-substitution. Specifically, the TMS group would be installed on the aromatic portion of the target and would act as an ipso-director in the cyclization (Scheme 43).

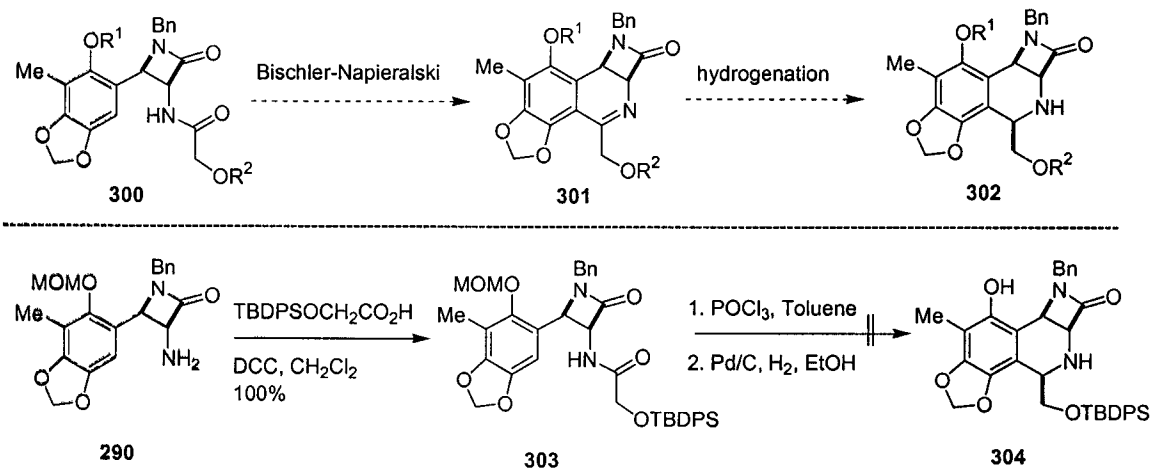


Scheme 43. Secondary generation Pictet-Spengler approach with TMS activation

The TMS group was introduced into aldehyde **282** through initial lithiation followed by treatment with TMS-Cl to afford aldehyde **295**. Aldehyde **295** was then reacted with benzylamine to produce imine **296**. Reaction of **296** compound **130** through the asymmetric Staudinger process afforded β-lactam **297**. The chiral auxiliary in compound **297** was removed to afford Pictet-Spengler precursor **298**. However, as before, any attempts to execute the cyclization were unsuccessful. Substrate reactivity was again presumed to be the likely reason.

3.3.4 A Bischler-Napieralski approach to the tetrahydroisoquinoline ring

In addition to the Pictet-Spengler reaction, the Bischler-Napieralski reaction offers an alternative ring-forming strategy (Scheme 44).[65] With respect to compound **300**, treatment with $P(O)Cl_3$ would produce dihydroisoquinoline **301**, which would undergo subsequent hydrogenation to afford the desired tetrahydroisoquinoline **302**. Importantly, the hydrogenation reaction was predicted to afford the desired *cis*-diastereomer through reduction at the least-hindered bottom face of the compound.



Scheme 44. Bischler-Napieralski approach to the tetrahydroisoquinoline ring

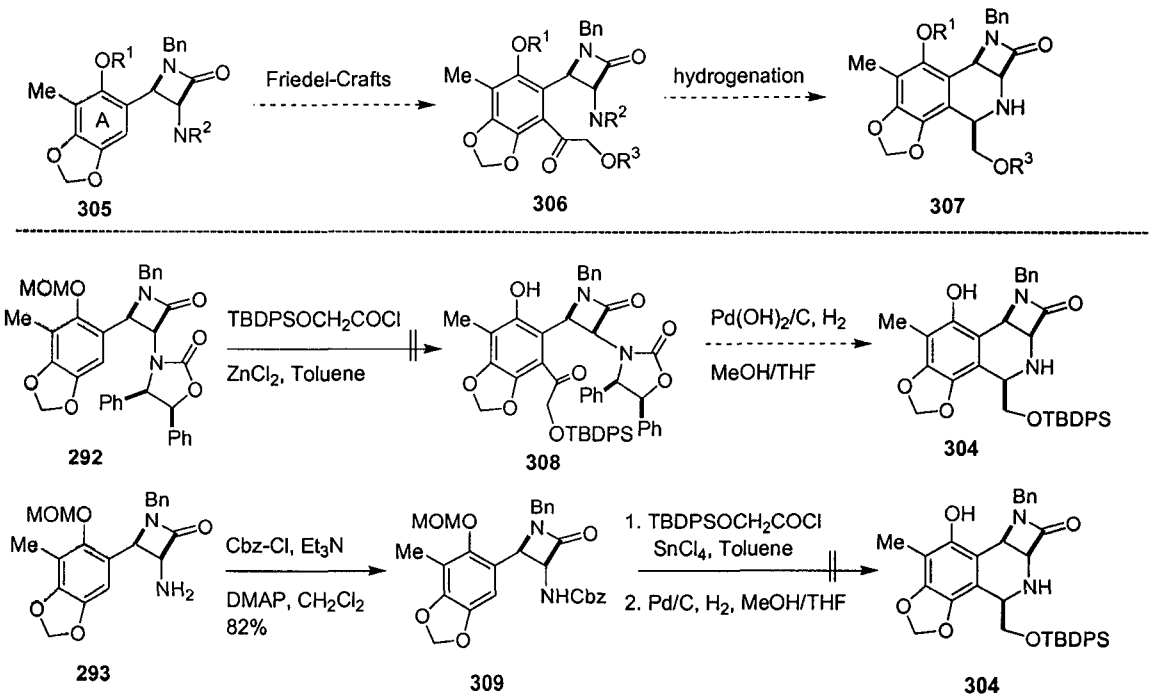
As in Scheme 44, the amine **290** was coupled with the appropriate carboxylic acid ($TBDPSOCH_2CO_2H$) using DCC to afford amide **303** in quantitative yield. Unfortunately,

treatment of amide **303** with various reagents and conditions (Table 9) did not produce the desired intermediate bearing the tetrahydroisoquinoline ring.

Table 9. Conditions for Bischler-Napieralski reaction

Reaction conditions	Results
P_2O_5, toluene, 110°C, 3h	Decomposed
PCl_5, toluene, 110°C, 3h	Decomposed
$POCl_3$, toluene, 110°C, 3h	Decomposed
$POCl_3$, toluene, RT, 12h	Decomposed
$POCl_3$, refluxed, 3h	Decomposed

3.3.5 A Friedel-Crafts approach to the tetrahydroisoquinoline ring



Scheme 45. Friedel-Crafts approach to tetrahydroisoquinoline ring

Upon consideration of the electronic properties of aromatic ring A in compound **305** (Scheme 45), it was determined that a Friedel-Crafts reaction might be useful for the introduction of a carbonyl group at this position. Following acylation of **305** to produce

intermediate **306**, subsequent deprotection of the amine group, presumably through hydrogenation followed by condensation onto the carbonyl group, would provide an imine intermediate. The imine would suffer reduction under the reaction conditions to afford compound **307**.

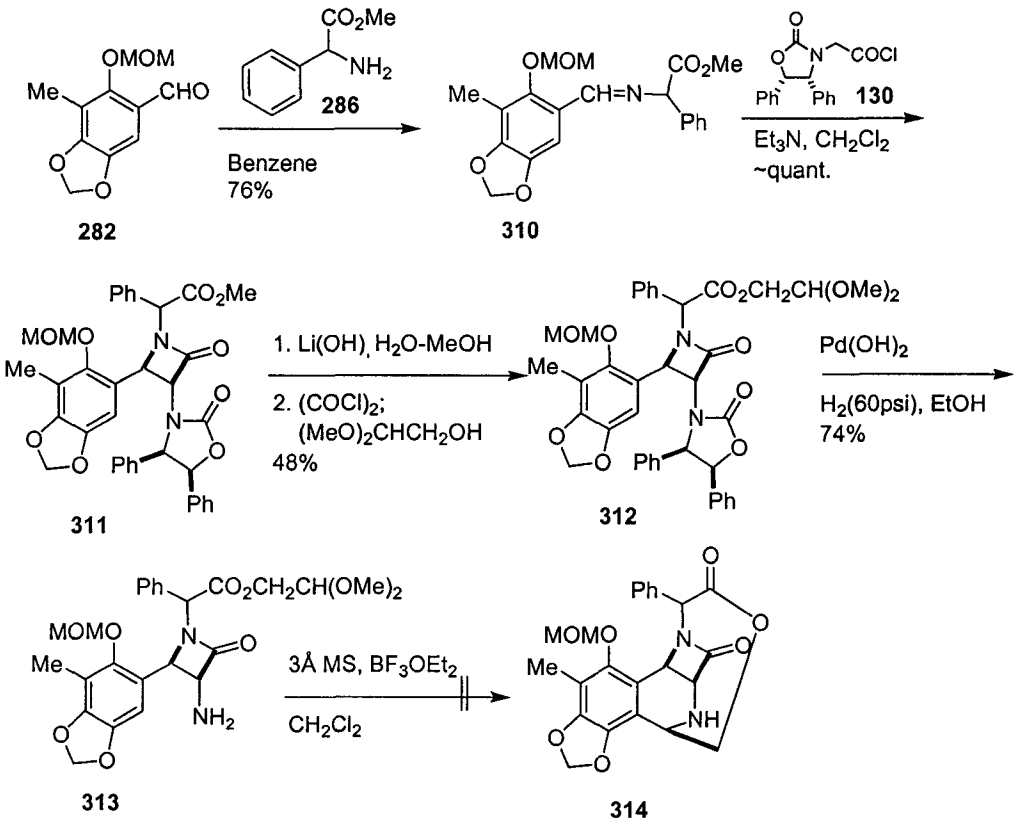
Two substrates, β-lactam **292** and **309** were investigated with respect to the Friedel-Crafts transformation (Scheme 45). Unfortunately, neither substrate proved reactive under standard conditions. It was postulated that steric hindrance imparted by other substituents on the respective aromatic positions of these substrates was precluding the introduction of a sixth substituent.

3.3.6 A third-generation intramolecular Pictet-Spengler approach

In light of Corey's successful use of an intramolecular Pictet-Spengler reaction in their synthesis of Et-743.[6] We endeavored to implement a similar approach into our strategy.

In effort to synthesize an appropriate precursor for the intramolecular transformation, amine **286** was condensed with aldehyde **282** to give imine **310** (Scheme 46). Staudinger reaction with compound **130** under the usual conditions produced β-lactam **311**. The methyl ester in compound **311** was then hydrolyzed by lithium hydroxide to the corresponding carboxylic acid, which was subsequently coupled with dimethoxylglycol to afford ester **312**. The chiral auxiliary on compound **312** was removed by hydrogenolysis to give amine **313**. However, subsequent treatment of **313** with the conditions that were used in Corey's total synthesis did not give the desired tetrahydroisoquinoline. The intramolecular reaction in Corey's synthesis produced a six-membered lactone. Therefore, it was determined that our intramolecular approach, which

would produce a nine-membered lactone, was perhaps energetically unfavorable relative to the six-membered precedent.



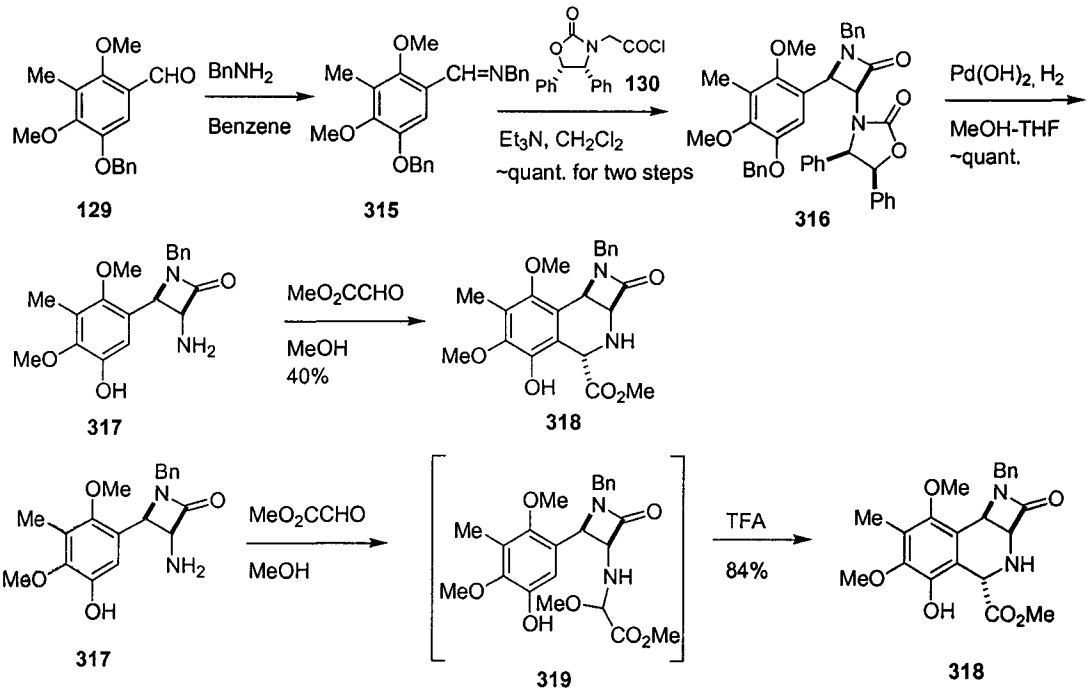
Scheme 46. Third generation Pictet-Spengler approach-intramolecular reaction

3.3.7 A forth-generation Pictet-Spengler approach using a free phenol

Based on our previous successes with the Pictet-Spengler transformation, which utilized a substrate processing a free phenol group on the aromatic component (Scheme 36), approaches incorporating aldehyde **282** were abandoned in favor of compound **129**. Specifically, compound **129** possessed the appropriate functional array necessary for the synthesis of a new class of Pictet-Spengler substrate.

As shown in Scheme 47, the route commenced with aldehyde **129**, which is available in 7 steps from 2,6-dimethoxyltoluene.[60] Condensation of aldehyde **129** with benzyl amine generated imine **315**, which was then subjected to the asymmetric

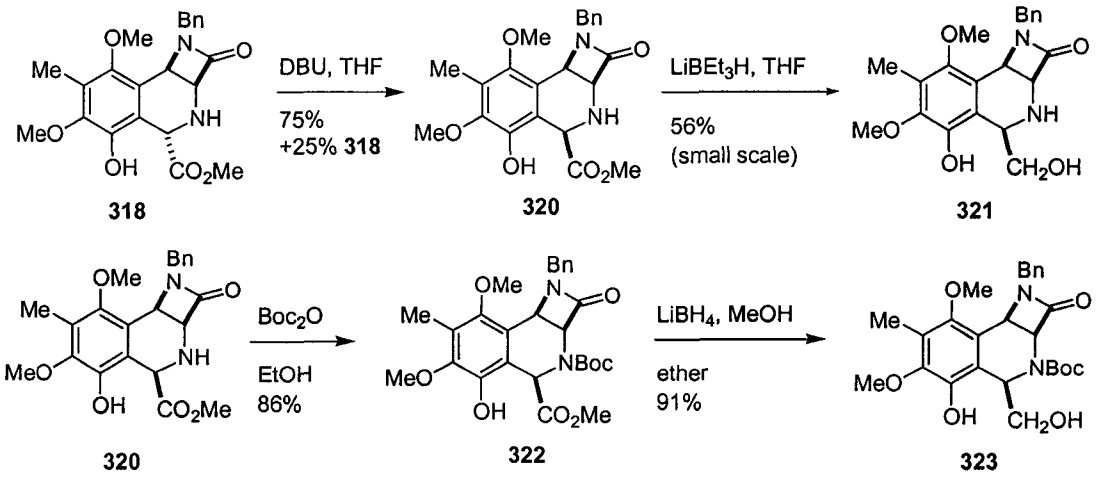
Staudinger reaction with acid chloride **130** in the presence of triethylamine to afford β-lactam **316**. Compound **316** was afforded as a single diastereomer in quantitative yield for the two steps. The chiral auxiliary and benzyl protecting group of β-lactam **316** were removed by hydrogenolysis to give amine **317**.



Scheme 47. Pictet-Spengler reaction using a free phenol

Following the previous procedure,[66] refluxing with methyl glycoxylate in methanol for 48 hours, provided the desired product **318** in 40% yield.[60] The major byproduct in the Pictet-Spengler transformation of **317** was determined to be compound **319**, which arises from the addition of methanol to the imine intermediate generated in the course of the reaction. Compound **319** could be successfully converted to compound **318** upon treatment with trifluoroacetic acid. However, in addition to compound **318**, bearing the *trans*-orientation with respect to the β-lactam and ester functional groups, a small quantity of the corresponding *cis*-isomer was isolated as well. The configuration of

the *trans*-isomer was verified by X-ray analysis of a similar compound in a subsequent model study (Scheme 60).



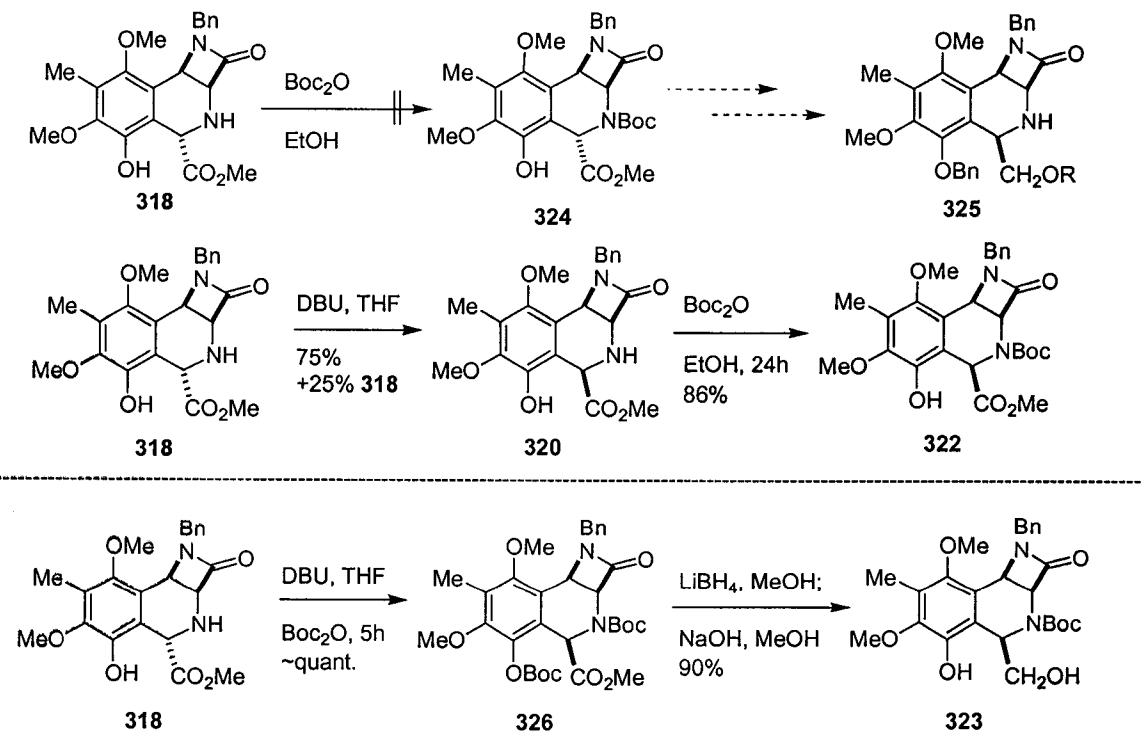
Scheme 48. Reduction of the carbomethoxy group

In accords with the previously developed protocol (Scheme 39), the *trans*-isomer **318** was treated with DBU to afford a mixture of compound **318** and **320** (75:25 *cis*: *trans* isomers) in quantitative yield, which could be easily separated by flash column chromatography (Scheme 48). The next step in the modified approach was to reduce the ester functional group. Treatment of compound **320** with super-hydride afforded compound **321** in modest yield on small scale (ca. 20 mg). Attempts to perform the reduction on larger scale led to lower yields. The low yields were attributed to the possibility of forming a complex between the aminoalcohol substrate and the reducing agent. In addition to the problematic reduction step, subsequent differentiation of the various functional groups in the corresponding product (**321**) appeared nontrivial. Therefore, it was determined that protecting the amine group in **320** prior to the reduction would be advantageous. First, a protected amino group would be less likely to complex to the reducing reagent, which would perhaps improve the yields in this transformation.

Second, subsequent differentiation of the free phenol and primary alcohol groups should prove more straightforward.

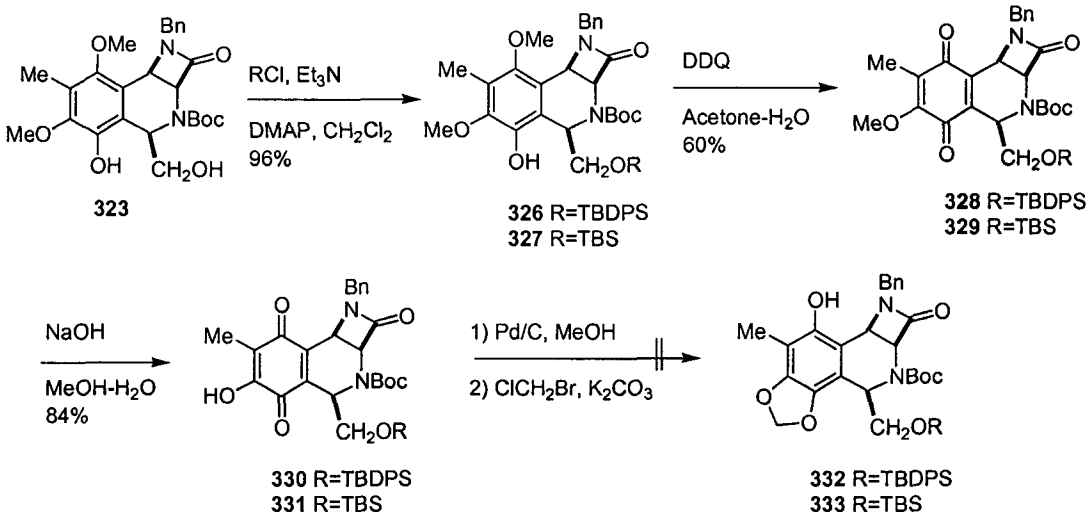
Protection of the secondary amine in compound **320** afforded compound **322**. Subsequent reduction of the carbomethoxy group in **322** with lithium borohydride in the presence of methanol produced diol **323** in excellent 91% yield.[67]

Importantly, through additional studies it was determined that the *trans*-diastereomer **318** did not react with Boc_2O at a significant rate (Scheme 49). Therefore, in an effort to profit from this observation, a one-pot epimerization/selective protection transformation was developed. Treatment of compound **318** with DBU in the presence of Boc_2O afforded intermediate **326** in quantitative yield. Although protection at the phenol group in **318** was unavoidable, the carbonate group was subsequently removed from the compound **326** following the usual ester reduction to afford **323**.



Scheme 49. Optimized condition for the epimerization

3.3.8 Attempts to install the methylenedioxy group



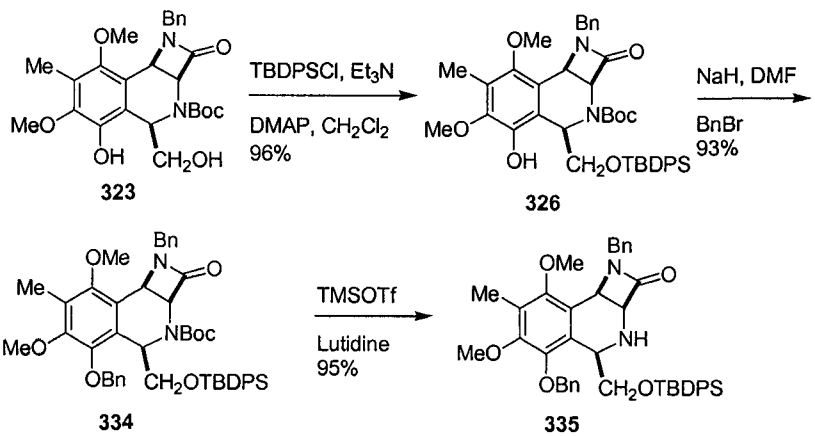
Scheme 50. Attempts to install the methylenedioxy unit

Previous studies were unsuccessful in incorporating the methylenedioxy group, through compound **282**, into the synthesis prior to the Pictet-Spengler transformation. However, with compound **323** successfully in-hand, attempts were now made to incorporate this necessary functional unit (Scheme 50). In accords with this goal, the primary alcohol of **323** was selectively protected as the corresponding TBDPS or TBS ether, compound **326** or **327**, respectively, in very good yield. Subsequent DDQ oxidation of compounds **326** and **327** afforded quinones **328** and **329**, respectively. The methoxyl groups of quinones **328/329** were converted into the corresponding hydroxyl groups in the presence of sodium hydroxide in methanol affording hydroxyl quinone **330/331**. Successful catalytic hydrogenation of the quinone units in the compound **330/331** furnished the desired hydroquinone intermediate. However, any attempts to install the requisite methylenedioxy group through treatment of these intermediates with bromochloromethane in the presence of Cs_2CO_3[7,68] failed to afford either of the desired products. It was reasoned that the steric hindrance of the β-lactam or silyl protecting

groups was possibly responsible for the failure of this strategy. Therefore, the approach was necessarily modified to incorporate the methylenedioxy unit at a later stage as in Cuevas' semi-synthesis.[7]

3.3.9 Synthesis of the tetrahydroisoquinoline component

As in Scheme 51, the primary alcohol of diol **323** was selectively protected with the TBDPS group affording compound **326**. Subsequent benzyl protection of the phenol afforded **334** in 93% yield. The Boc protecting group was removed by treatment of **334** with TMSOTf and 2,6-lutidine to afford the tetrahydroisoquinoline **335**, which embodies all of the requisite functionality to tackle the planned asymmetric synthesis of Et-743.

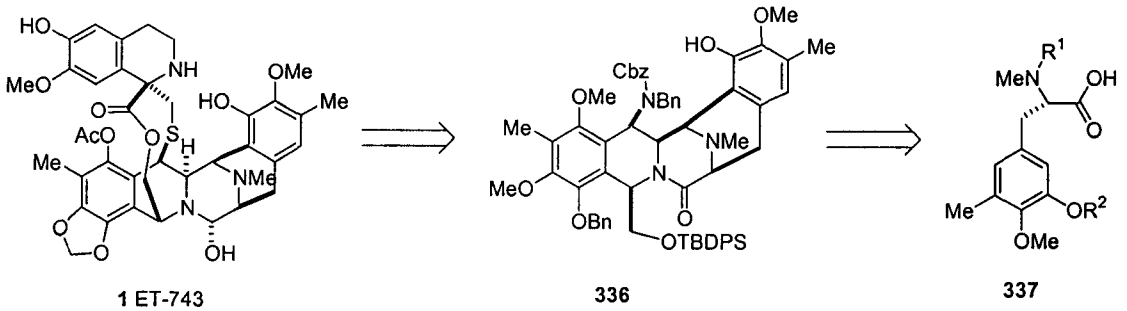


Scheme 51. Synthesis of the tetrahydroisoquinoline component

3.4 Synthesis of the amino acid component

With the tetrahydroisoquinoline ring **335** in hand, the next stage of the planned synthesis was to synthesize the necessary amino acid component. Initially, an appropriate protecting group strategy for compound **337** (Scheme 52) had to be determined. From a previous model study (Scheme 60), it was found that the protecting group of the secondary amine must be removed under acidic or neutral conditions, and the protecting group of the phenol must be removed prior to removal of the amine protecting group.

After carefully consideration, the Boc group was selected as the amine protecting group and the TBS group was chosen as the phenol protecting group (Scheme 52).



Scheme 52. The protecting groups of the amino acid

3.4.1 Synthesizing the starting materials

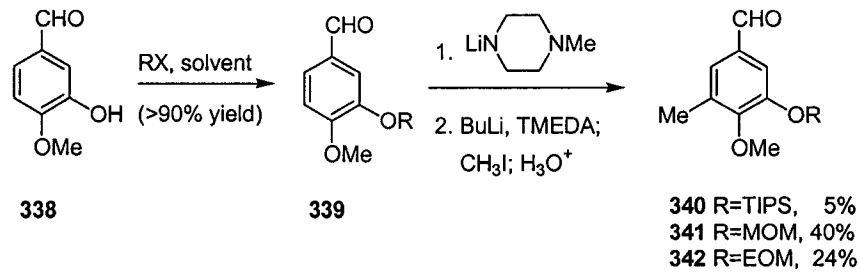
After selection of the appropriate amino acid protecting groups, 3-hydroxyl-4-methoxy-5-methylbenzylaldehdye (**251**) was selected as the starting material for the amino acid synthesis for the following reasons. First, from compound **251** it was possible to access to a number of the tetrahydroisoquinoline antitumor alkaloids. Second, the C-16 methyl group will be introduced at a very early stage, thereby rendering the synthesis more convergent. The importance of introducing the C-16 methyl group at an early stage cannot be overemphasized. Indeed, installation of this group into our aromatic system proved very difficult, and many early attempts (ie. hydrolysis of the diazo salt, regio-selective bromination followed by hydrolysis, etc.) had failed.

3.4.1.1 *A meta*-methylation approach

The first successful synthesis of compound aldehyde **275** was realized through a direct *meta*-methylation approach (Scheme 53).

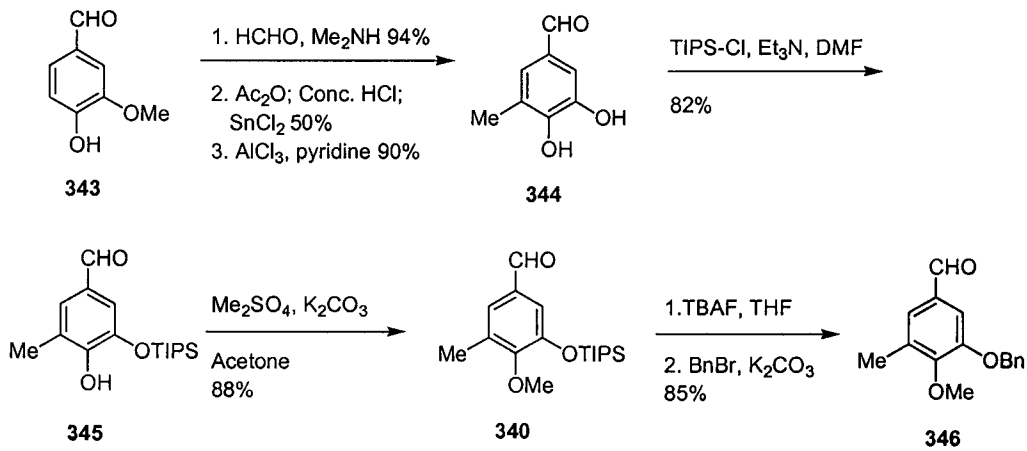
As in Scheme 53, starting from *iso*-Vanillin (**338**), the phenol group was protected in excellent yield to give the intermediate **339**. Protection of the aldehyde in **339** with lithium N-methylpiperizium, subsequent metalation at the *meta*-position and methylation

afforded the protected aldehydes (**340-342**).[69] Unfortunately, the yields for the regioselective methylation were too low to be practical for the synthesis. The low yields could possibly be attributed due to the lack of regioselectivity for direct methylation of aldehyde **339**.



Scheme 53. *A meta*-methylation approach

3.4.1.2 An approach with protection of the 3-hydroxy group

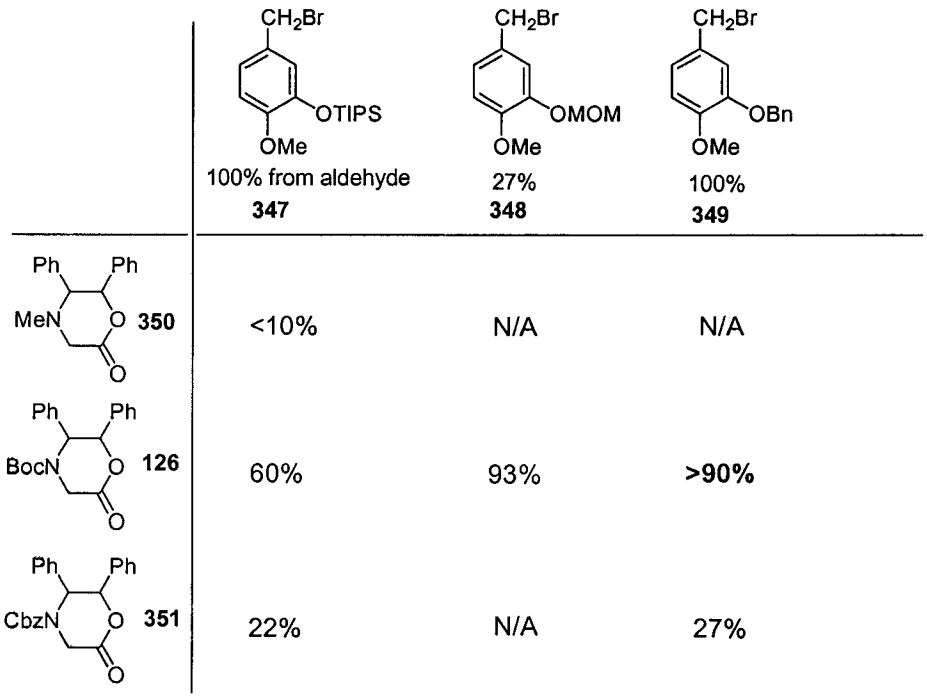


Scheme 54. An approach of protection of the 3-hydroxy group

In an effort to improve the strategy, a more practical, but somewhat more lengthy approach was devised for the synthesis of the necessary starting material (Scheme 54).[70] Starting from vanillin, the aldehyde (**344**) was synthesized in a three-step procedure: 1) Mannich reaction to introduce the 2-(N,N)-dimethylaminomethyl group; 2) conversion of the dimethylaminomethyl group into the corresponding methyl group; 3) demethylation of the methoxyl group. Fortunately, the less hindered 3-hydroxyl group was able to be

selectively protected with the TIPS group to give aldehyde **344** in 82% yield. The 4-hydroxyl group of **344** was then methylated with methyl sulfate in the presence of potassium carbonate to afford **340** in 88% yield. Removal of the silyl group from intermediate **340** and subsequent protection the phenol with the benzyl group gave aldehyde **346** in 85% for two steps. Through this new approach, aldehyde **346** could be synthesized on a multi-gram scale.

3.4.2 Coupling of the benzyl bromide derivatives with the Williams' lactone

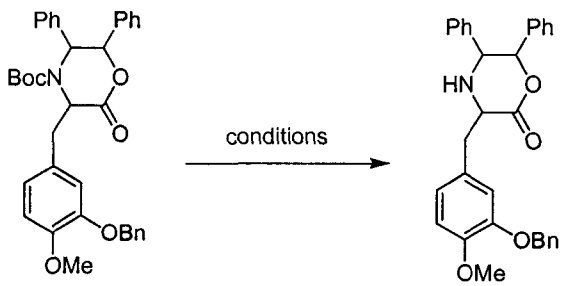


Scheme 55. Coupling of the benzyl bromide derivatives with Williams' lactones

The choice of a benzyl protecting strategy for compound **346** was the result of a previous model study (Scheme 55). The study was conducted in an effort to determine the optimal coupling partners for the alkylation reaction between the electrophilic aromatic component and the nucleophilic lactone enolate.[71] Therefore, benzyl bromides (**347-349**) were synthesized from *iso*-vanillin in two steps, and were subsequently reacted with the corresponding lactone enolate of compound **350**, **126**, and **351**. The best results,

both in terms of substrate preparation and coupling yields, were obtained with compounds **349** and **126**, thereby securing the choice of a benzyl protecting group for the relevant phenol position.

3.4.3 Removal of the Boc protecting group



352 **353**

Scheme 56. Removal of the Boc protecting group

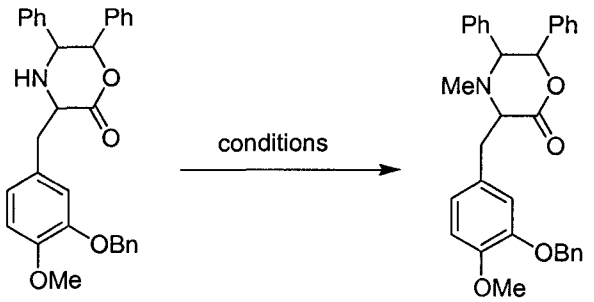
Table 10. Conditions for removal of the Boc group

Reaction conditions	Results
1) TFA, CH_2Cl_2	No desired product
2) $ZnBr_2$, CH_2Cl_2	No desired product
3) TFA, thioanisole, CH_2Cl_2	48%
4) TMS-Cl, phenol (1:3), 10equiv	39%
5) TMS-Cl, phenol (1:1), 3equiv	80% (30% conversion)
6) TMS-I, MeCN	No desired product
7) NaI, TMS-Cl, phenol, CH_3CN, CH_2Cl_2	53%
8) TMS-I (2equiv), phenol, CH_2Cl_2	77%

In addition to studies directed toward optimizing the coupling reaction, a model study was conducted to determining the appropriate conditions for removal of the Boc group from coupling product **330** (Scheme 56). Our efforts revealed that some standard deprotection conditions (ie. TFA, $ZnBr_2$, TFA/thioanisole, etc.)[72] were unsuccessful and

produced low yields of the desired product **353** (Table 10). Conditions 4 and 5, employed in peptide synthesis, produced low yields or suffered from poor substrate conversion. However, although TMS-I alone (conditions 6) produced no desired result, at best, conditions employing TMS-I in the presence of phenol (conditions 7 and 8) afforded the desired product **353** in good yield.

3.4.4 Methylation of the secondary amine



353 **354**

Scheme 57. Methylation of secondary amine

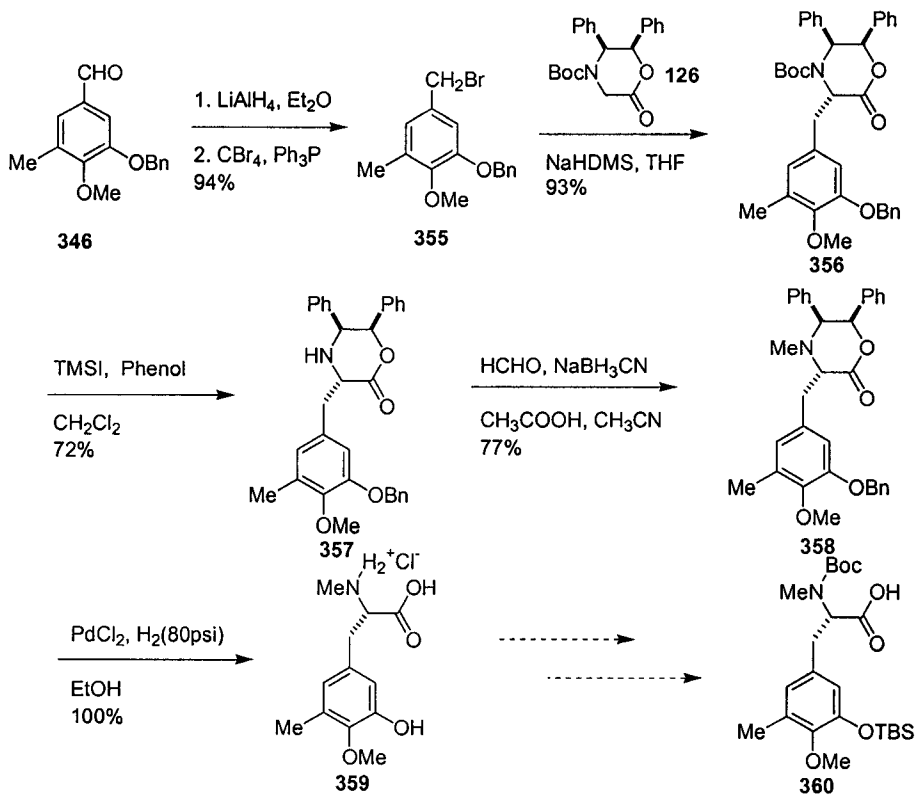
Table 11. Methylation of the secondary amine

Reaction conditions	Results
1) NaH, DMF; MeI	40% (hard to separate)
2) NaH_2PO_4, HCHO, dioxane	23%
3) nBuLi, THF; MeI	No desired product
4) HCHO, $NaBH_4CN$, MeCN	50%
5) HCHO, $NaBH_4CN$, MeCN, AcOH	65%

Finally, a further investigation into the proposed synthetic strategy was conducted in order to determine the optimal conditions for the methylation of the secondary amine group in the lactone portion of the addition product. Therefore, in a model study, the methylation of compound **353** (Scheme 57) was studied using a variety of conditions

(Table 11).[73] While the direct methylation (conditions 1 and 3) or the modified Leuckart reaction (conditions 2) produced low yields of the desired product **354**, reductive amination (conditions 4 and 5) afforded **354** in acceptable yields.

3.4.5 A first approach to the amino acid component

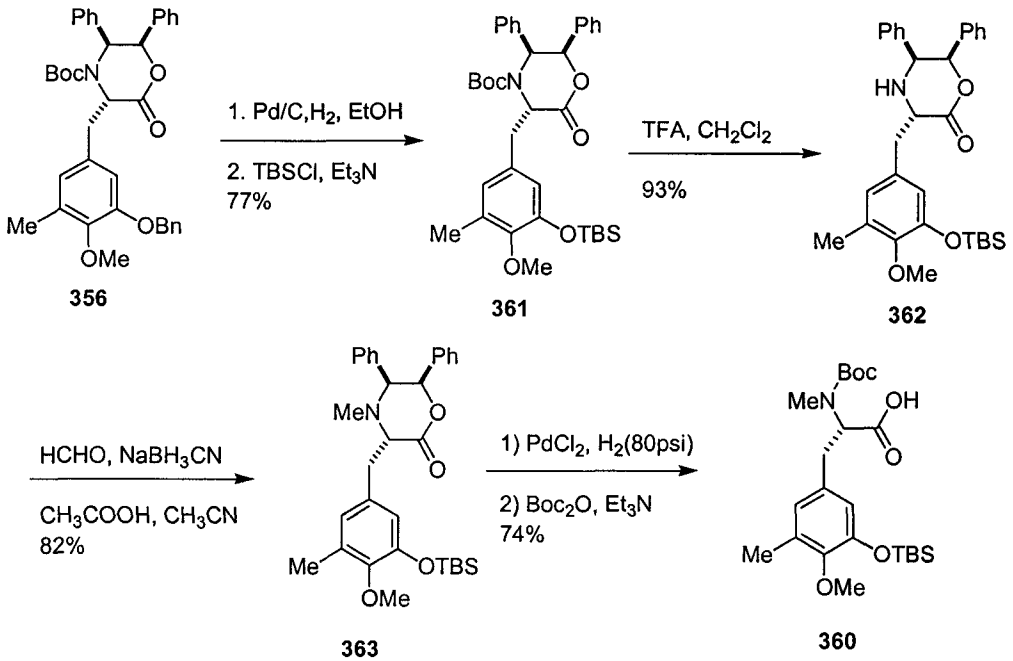


Scheme 58. A first approach to the amino acid component

As shown in Scheme 58, the aldehyde of **346** was reduced to the corresponding alcohol intermediate in quantitative yield by treatment with LAH. The alcohol was then converted into the benzyl bromide derivative **355** through the agency of CBr_4, Ph_3P in 94% yield. Next, compound **355** was condensed with the sodium enolate (NaHMDS, THF, -78°C) of **126** to afford the alkylation product **356** in 93% yield. The Boc protecting group of **356** was then removed (TMSI and phenol) to produce compound **357**. Reductive amination of **357** using formaldehyde furnished **358** ($NaBH_3CN$, HCHO, CH_3CN, 77%

yield). The chiral auxiliary and benzyl protecting groups of **358** were subsequently removed by catalytic hydrogenation to provide the amino acid hydrochloride salt **359**. Unfortunately, although compound **359** was readily available, subsequent efforts to introduce the necessary protecting groups at the amine and phenol positions were unsuccessful. These findings justified the need to install the phenol protecting group at an earlier stage in the synthesis of the amino acid component.

3.4.6 A second approach to the amino acid component



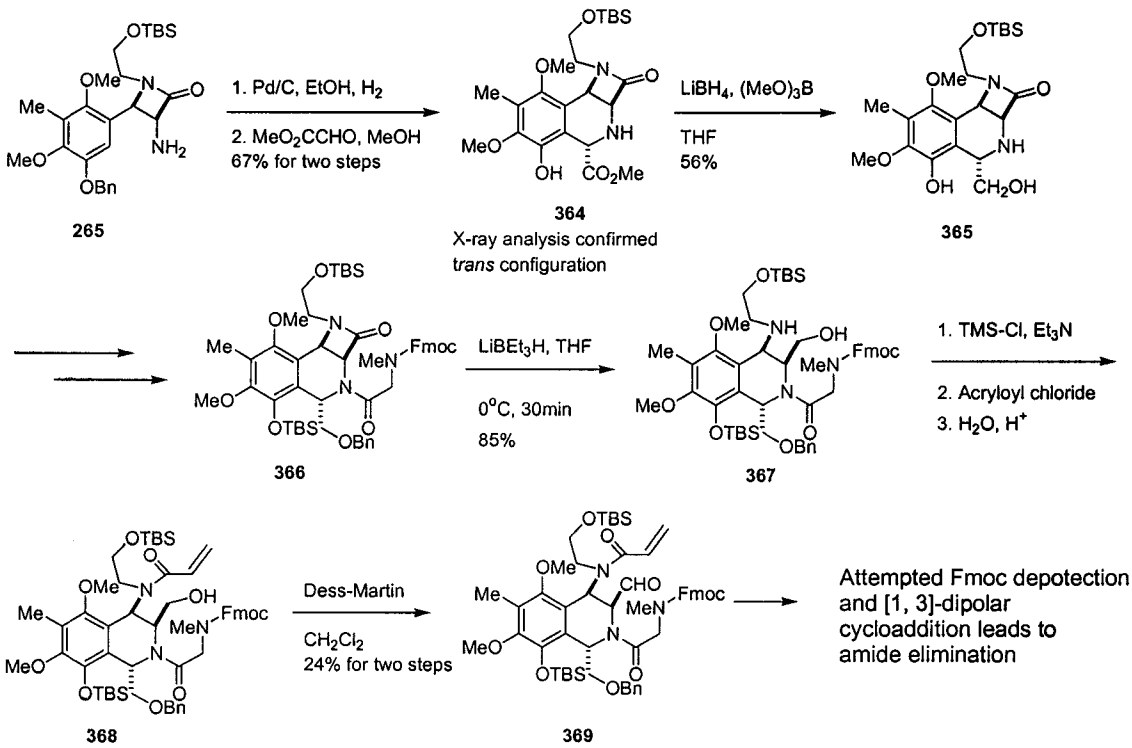
Scheme 59. A second approach to the amino acid component

Therefore, the amino acid synthesis approach was further optimized as illustrated in Scheme 59. The *O*-benzyl group of **356** was initially removed by catalytic hydrogenation to give the corresponding phenol, followed by subsequent protection as the *O*-TBS ether under standard conditions providing **361** in 77% yield for two steps. The *N*-*t*-Boc group of compound **361** was then removed by treatment with TFA in 93% yield to afford amine **362**. Reductive amination of the secondary amine of **362** using

formaldehyde furnished **363** ($NaBH_3CN$, HCHO, AcOH, CH_3CN, 82% yield). The chiral auxiliary of **363** was then removed by catalytic hydrogenation to provide the corresponding amino acid hydrochloride salt. Finally, the secondary amine was protected with Boc_2O furnishing amino acid **360** in 74% yield for the two steps.

3.5 Construction of the pentacyclic ring system

3.5.1 A model study to open the β-lactam

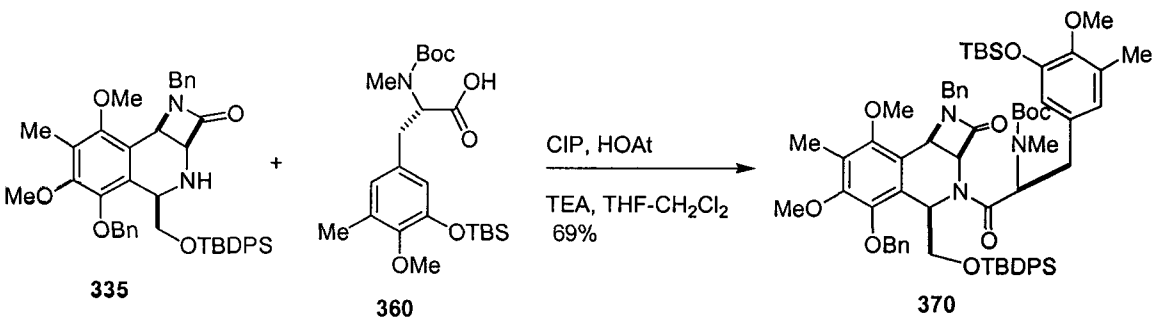


Scheme 60. A model study to open the β-lactam

With the appropriate intermediates in hand, representing the two portions of Et-743, model studies were then conducted in order to investigate the various transformation that would be compatible with the overall synthetic strategy. Several important results were obtained from the studies (Scheme 60). First, as previously mentioned, X-ray analysis of Pictet-Spengler product **364** confirmed the expected *trans*-configuration with respect to the β-lactam and the carbomethoxy functional groups. Second, the reduction of

the carbomethoxy group in **364** to the corresponding alcohol **365** was improved through the use of $LiBH_4$/methanol as a reductant. Third, conditions for the reduction of the β-lactam unit in **366** to the amino alcohol **367** were optimized using super-hydride. Finally, it was determined that compound **369** was unstable under the basic conditions typically employed in the removal of the Fmoc protecting group. Indeed, treatment of **369** with base produced an α,β-unsaturated aldehyde product, resulting from elimination of the amine group. Therefore, it was postulated that the amine protecting group would necessarily be removed under acidic or neutral conditions.

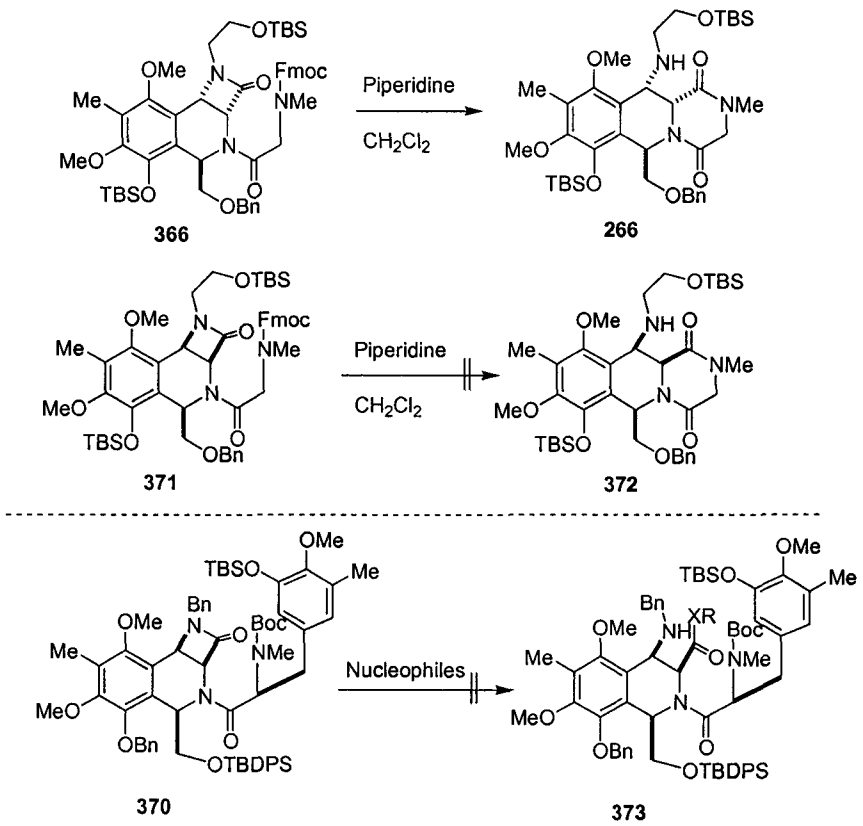
3.5.2 Coupling of the amino acid and the amine



Scheme 61. Coupling of the amino acid and the amine

The use of a Boc protecting group in compound **360**, precluded the incorporation of acid chloride methodology into the peptide coupling. Therefore, methods utilizing activated esters were employed in this transformation. Amine **335** and carboxylic acid **360** amino acid were effectively coupled using HOAt and CIP[10,74] to afford the desired product **370** (Scheme 61). Attempts to use the coupling reagents such as BOP, EDCI and BOP-Cl were unsuccessful.

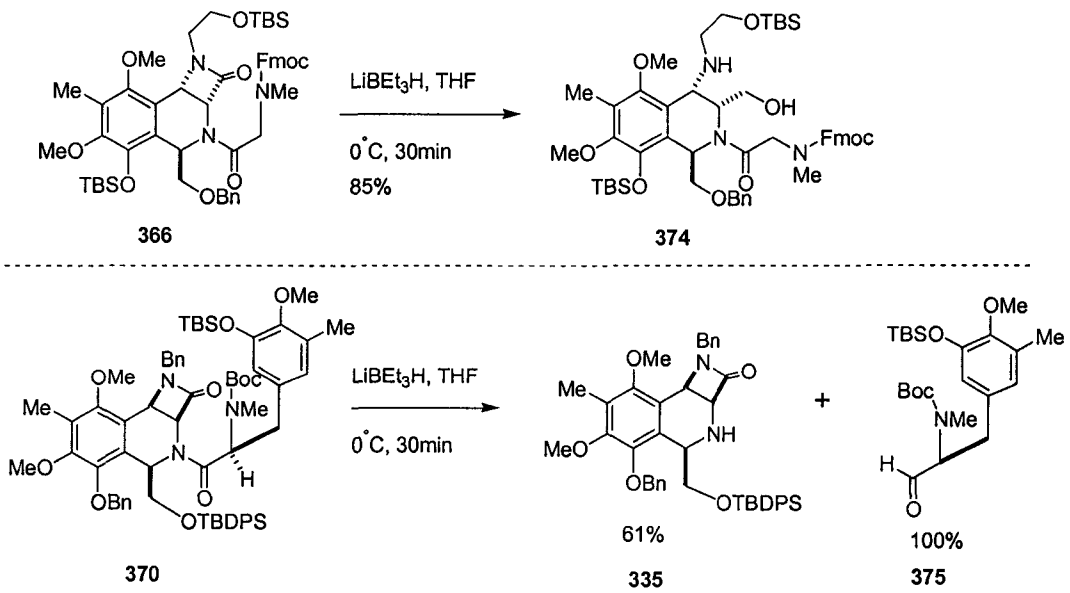
3.5.3 Attempts to open the β-lactam



Scheme 62. Attempts to open the β-lactam by nucleophiles

In our previous work,[60] it was discovered that upon removing the amine protecting group in compound **366** (Scheme 62), the resulting free amine would undergo a transformation reaction with the β-lactam, forming compound **266**. However, this pathway was not observed for the corresponding *cis*-isomer, compound **371**. Following this observation, it was subsequently reasoned that the free amine group, resulting from deprotection of compound **371**, would not reside in close proximity to the β-lactam unit, and could therefore not participate in a transamidation process. Ultimately, it would be necessary to open the β-lactam unit in order to form the pentacyclic backbone of Et-743. Therefore, a study was conducted in an effort to open the β-lactam unit in **370** with a variety of agents through an intermolecular process. Unfortunately, however, the β-

lactam proved unreactive toward a variety of common nucleophiles (MeONa, EtSNa, NH_2Na et al.).



Scheme 63. Attempts to open the β-lactam

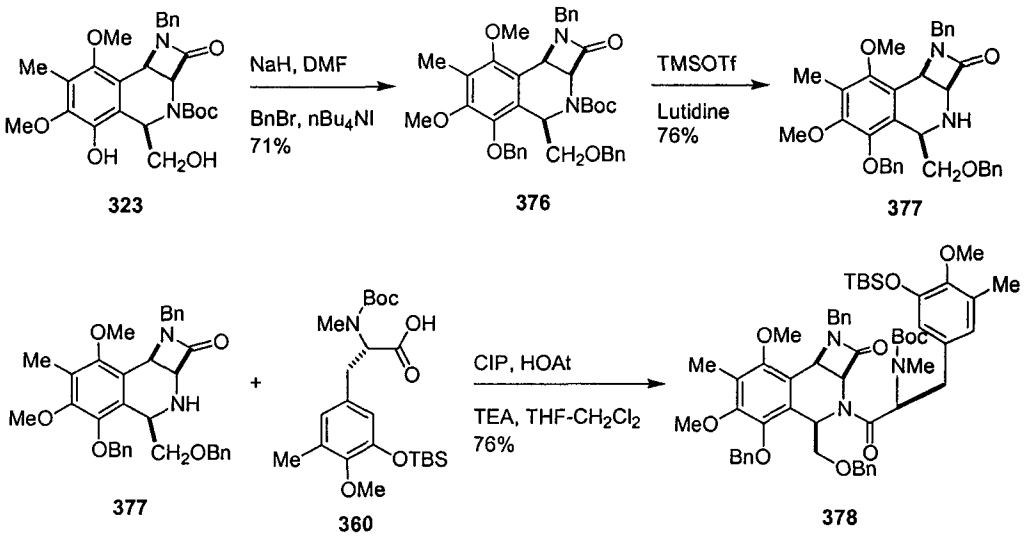
In addition to exploring the use of nucleophiles to open the β-lactam functional unit, an attempt was made to open the β-lactam in compound **370** using super-hydride (Scheme 63). However, despite our previous success in accomplishing this transformation using compound **366**, treatment of **370** with super-hydride led to cleavage of the amide group producing compound **335** and **375**.

Upon consideration of the structural difference between compounds **370** and **366**, it was postulated that the TBDPS group in compound **370** was sterically shielding the β-lactam group, precluding its reduction. Therefore, one possible solution would entail switching the TBDPS group in favor of a smaller benzyl protecting group.

3.5.4 Switching to smaller protecting groups

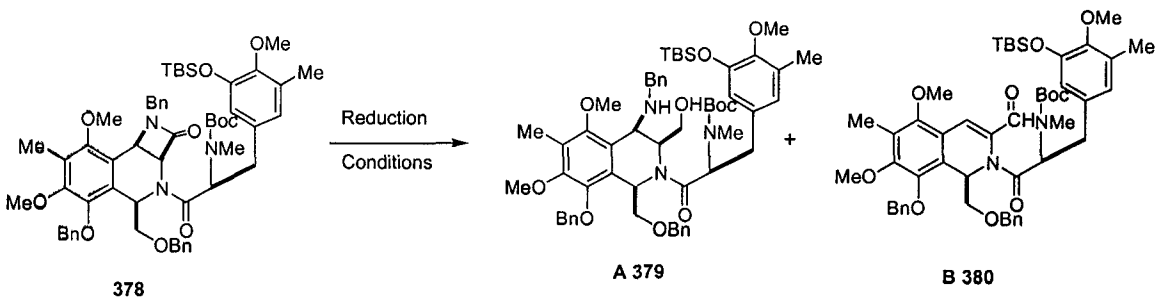
As in Scheme 64, the primary alcohol and the phenolic hydroxyl groups of diol **323** were protected with benzyl groups in one step to give dibenzyl protected **376** in 71%

yield. Then treatment of compound **376** with TMSOTf and 2,6-lutidine afforded amine **377**. Following the usual coupling protocol (Scheme 61), amine **377** and amino acid **360** were coupled with CIP and HOAt to provide peptide **378** in 76% yield.



Scheme 64. Switch to smaller protective groups

3.5.5 Reduction of the β-lactam



Scheme 65. Reduction of the β-lactam

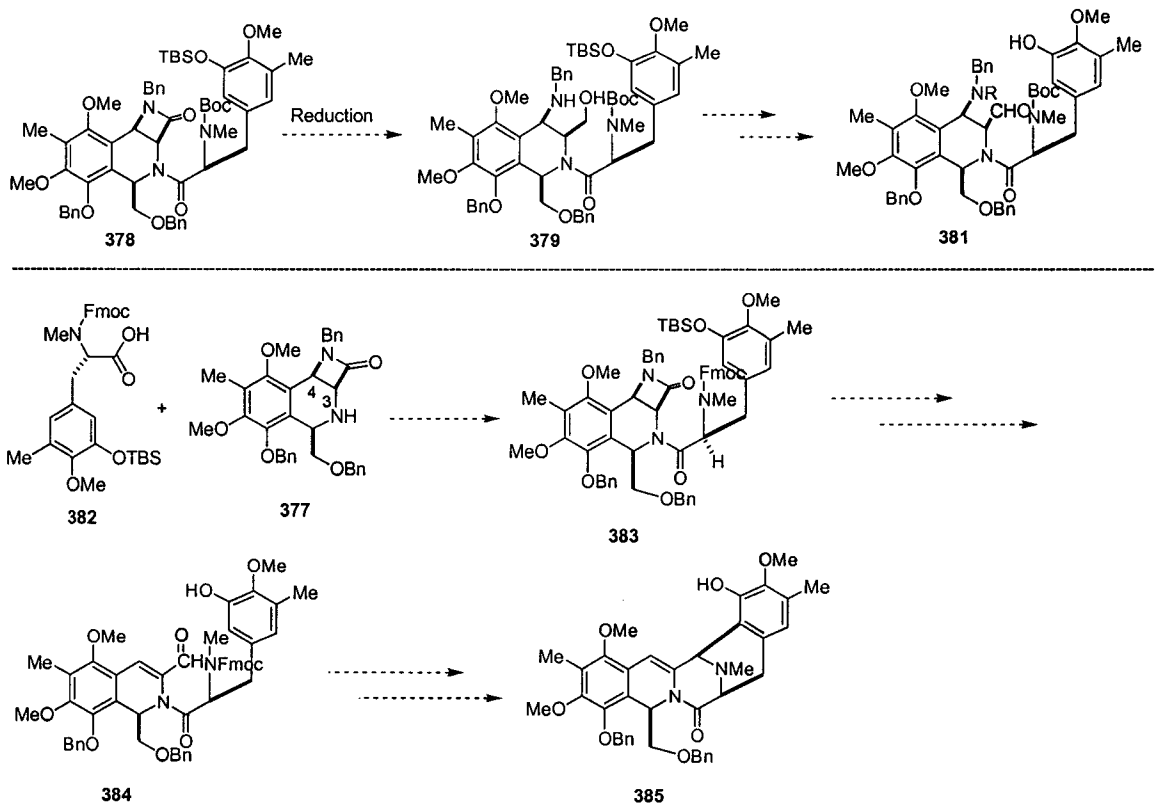
Table 12. Reduction of the β-lactam

Reaction conditions	Results
BH_3, THF	No reaction
BH_3NH_3, *n*-BuLi, 30min	No reaction
Super-Hydride, RT, 30min	20% **380**
Super-Hydride, 0°C, 30min	53% **380**

With compound **378** in hand, the stage was set for studies concerning the reduction of the β-lactam group in this sterically less-encumbered substrate (Scheme 65). Several conditions were investigated with respect to this reductive transformation (Table 12).[75] Although trials with borane were unsuccessful, treatment of **378** with super-hydride provided an unexpected product. Indeed, α,β-unsaturated aldehyde **380**, resulting from initial reductive opening of the β-lactam followed by elimination of the alkylamine group in the aldehyde intermediate, was produced as the sole product over the anticipated amino alcohol **379**. Reasoning suggested that the amino aldehyde intermediate, resulting from the partial reduction of the β-lactam group in **378**, could not undergo complete reduction to the corresponding amino alcohol due to possible steric factors. Upon quenching the reaction, the benzylamine group suffered elimination to form the corresponding conjugated system in compound **380**. Despite the unexpected result, compound **380** was determined to be a useful intermediate in the overall synthetic plan, and its production was subsequently optimized to 57% yield.

Importantly, extensive experience on related compounds in our hands and a search of the literature has revealed that the reduction of β-lactams directly to aldehydes is a synthetically challenging.[15a] A search of the literature did not reveal any general methods for the reduction of β-lactams to aldehydes. The general reagents used to reduce β-lactam included LAH, B_2H_6,[76b] Ra-Ni, AlH_3, and $NaBH_4$-$AlCl_3$. Usually, reduction of N-substituted β-lactam would result in the cleavage of 1,2-bond to give the N-substituted 3-aminopropanol[76b] or azitidines.[76a, c]

3.5.6 An optimized approach



Scheme 66. An optimized approach

In the original synthetic design, it was supposed that the reduction of β-lactam group in compound **378** would provide the amino alcohol **379**. Compound **379** could subsequently be converted to compound **381** through oxidation of the primary alcohol (Scheme 66). The intrinsic instability of the amino aldehyde functional unit in compound **381** toward base had been previously addressed in our planning. Therefore, the acid labile Boc group had been selected to protect the secondary amine group in the right-hand portion of compound **378**. In light of the new evidence that reduction of the β-lactam in compound **378** produced compound **380** (Scheme 65), changes were made to the original approach. Since elimination of the alkylamine group was unavoidable in reduction process, the Boc group originally used for protecting of the secondary amine was exchanged for the Fmoc protecting group. This modification would allow for the use of

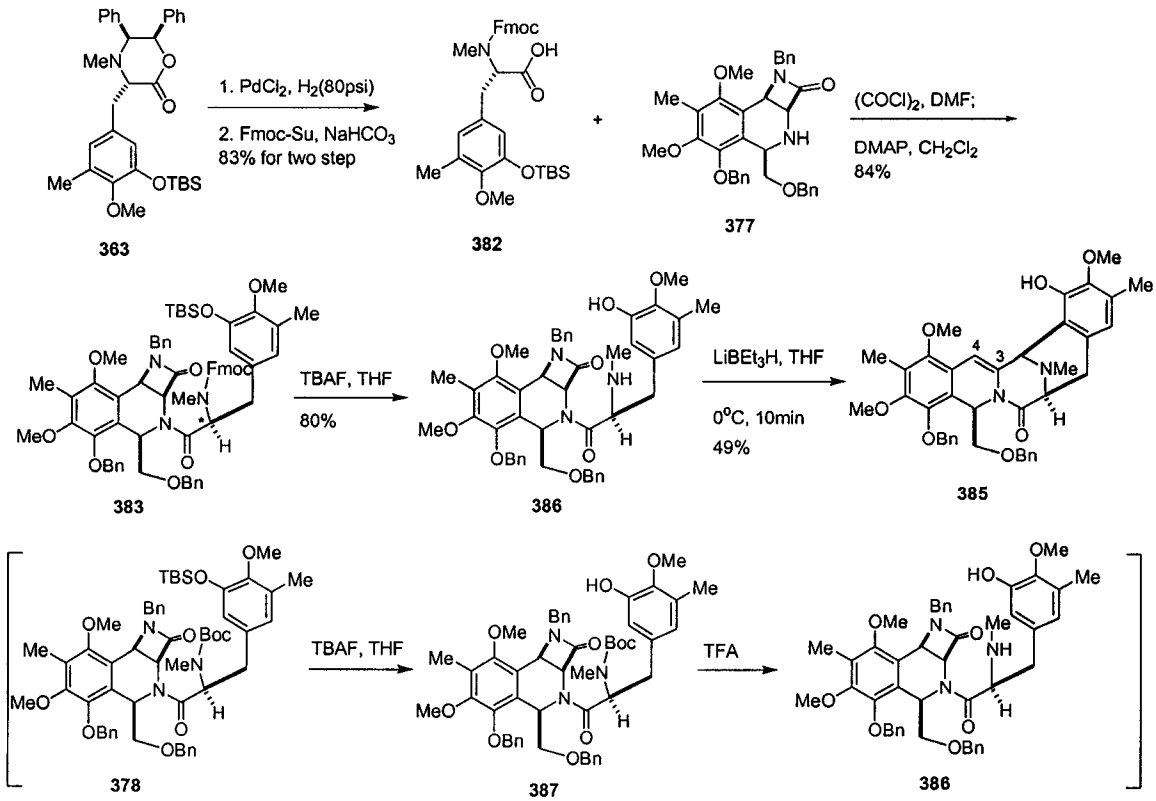
convenient and high-yielding acid chloride-based methods for the requisite peptide coupling reaction necessary to join the left and right-hind portions of the Et-743 core. Additionally, attempts were made to incorporate an intermediate bearing the readily accessible *trans*-orientation with respect to the β-lactam and primary alcohol functional groups. Use of this type of left-hind fragment would constitute a more concise and stereospecific approach, and would be logical since the stereochemistry at the β-lactam group is lost during the elimination process. Unfortunately, any attempts to perform a peptide coupling with the *trans*-β-lactam isomer were unsuccessful.

3.5.7 Construction of the pentacyclic ring system

In an effort to incorporate the modified strategy, compound **363** was initially converted to the Fmoc protected amino acid **382** (Scheme 67). This was accomplished by reductive removal of the chiral auxiliary in compound **363** followed by carbamate protection of the resulting intermediate to provide **382** in 83% yield for two steps. Importantly, sodium bicarbonate was used as base in the protection step in an effort to avoid undesired removal of the TBS protecting group.

The amino acid **382** was converted into the corresponding acid chloride by treatment with oxalyl chloride and was then coupled to the amine **377** in the presence of DMAP to afford the peptide **383** in 84% yield. Importantly, polarimetry studies confirmed that there was no detectable equilibration at the amino acid stereocenter during the peptide coupling. These studies were performed on compound **386,** available form coupling product **383** through removal of the Fmoc and TBS groups and the same product (**386**) generated through a prior sequence via intermediate **378**. Compound **378**

was synthesized through a different peptide coupling employing activated ester methods (ie. CIP and HOAt).



Scheme 67. Construction of the pentacyclic ring system

Both the Fmoc and TBS protecting groups of **383** were removed in a single operation by treatment with TBAF, which afforded the phenolic amine **386** in 80% isolated yield. Exposure of **386** to $LiBEt_3H$ in THF at 0 °C remarkably furnished the desired pentacyclic compound **385** directly in one step in 49% yield. The conversion of **386** into **385** is envisioned to proceed by initial partial reduction of the β-lactam to the amine-coordinated borane complex that obviates over-reduction of the incipient aldehyde. Elimination of benzylamine occurs spontaneously under the reaction conditions to afford a α,β-unsaturated aldehyde that subsequently suffers cyclization of the secondary amine on the aldehyde intermediate to generate an iminium ion species. Regioselective

intramolecular Pictet-Spengler cyclization finally affords the pentacyclic compound **385** without contamination of the alternative regioisomer. The pentacyclic compound **385** contains the olefinic moiety at C3-C4 (saframycin numbering) that is flexibly poised for either saturation to the saframycins and related compounds or functionalization at C-4 for closure of the sulfur-containing macrocyclic ring of the ecteinascidins. Unfortunately, although an intermediate had been secured which possessed a versatile functional handle, the olefin group in compound **385** proved extremely unreactive.

The regioselective intramolecular Pictet-Spengler cyclization to **385** is a key transformation in our synthesis. Indeed, condensations of dopamine, L-dopa, and their congeners with formaldehyde and other aldehydes normally proceed with preferential formation of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines over their 7,8-dihydroxy isomers,[77a,b,c] so the case of isolation of **385** as the sole product suggested a fast and regiochemical formation of the six-membered ring, possible due to the electron properties of the aromatic ring of the Pictet-Spengler precursor.[77d,e] It was postulated that the total charge distributions of the Pictet-Spengler precursor caused the position *ortho* to the activating OH group as the most reactive toward electrophilic agents.[77e]

3.5.8 The double bond problem and switching target

Attempts to perform nucleophilic additions as well as reducing on the double bond were unsuccessful. Additionally, the amide group was unable to be converted to the corresponding aminonitrile (Scheme 68) (Table 13).

In an attempt to rationalize the resistance of the olefin group in **385** toward reduction, ^{1}H NMR chemical shifts were carefully analysed. Indeed, the chemical shift of

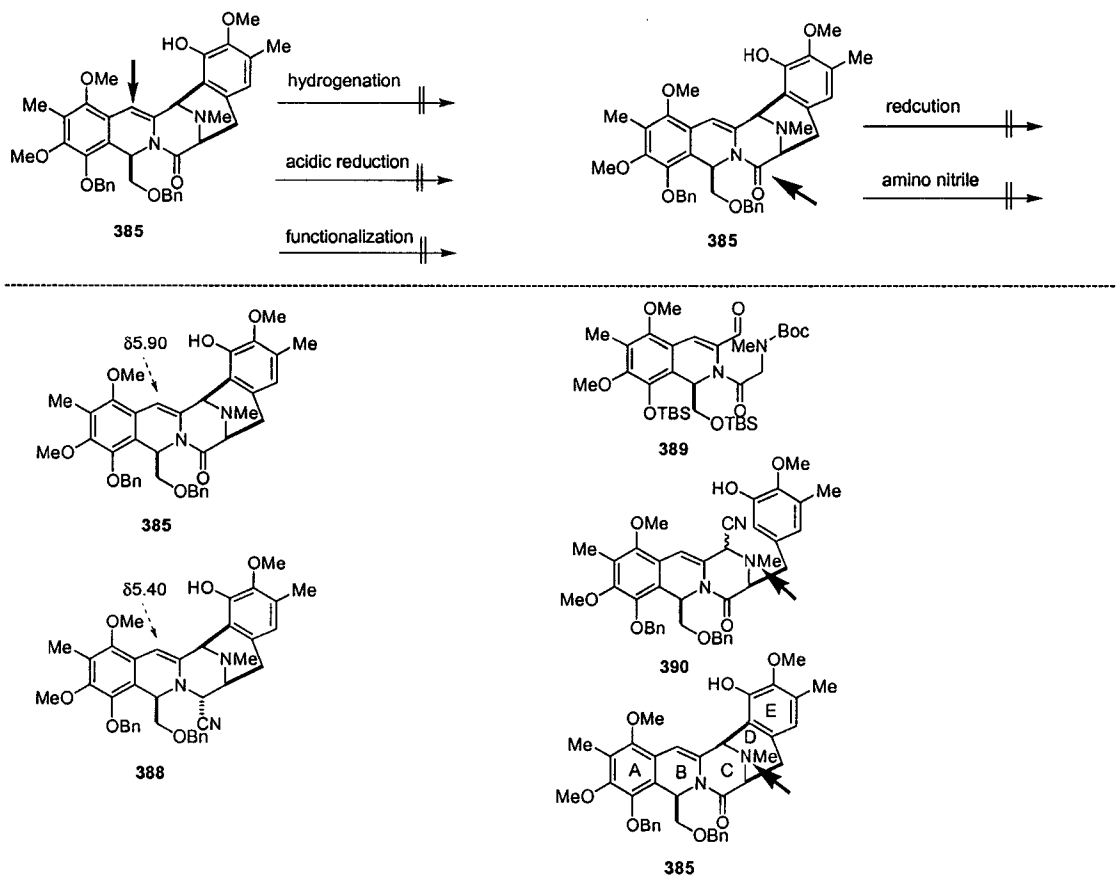
the olefin proton in compound **385** more closely resembled that of a corresponding aromatic proton, suggesting an unusual level of stability for the group.

Table 13. Attempted transformations on pentacycle **385**

Conditions	Results
RaNi, EtOH, 100-1500 psi H_2	No reaction
Pd-Black, AcOH, 100 °C, 100 psi H_2	No reaction
$TsNHNH_2$, NaOAc, DME, water	Decomposed
PhSeBr, TEA, CH_2Cl_2; Bu_3SnH, AIBN, toluene	Decomposed
Et_3SiH(5 equiv), TFA, reflux	No reaction
$BH_3.SMe_2$, THF ; AcOH	No reaction
HCOOH, $NaBH_3CN$, 105 °C	No reaction
$Pyr.HBr_3$	Decomposed
MCPBPA, $CHCl_3$, NaOH, reflux	Decomposed
$HOCH_2CH_2SH$, THF	No reaction
$NaBH_4$, TFA, Toluene	No reaction
LAH, THF, rt.	No reaction
LAH, THF, reflux	Decomposed
$LiAl(OEt)H_3$; NaCN; $NaBH_4$, TFA	Decomposed
$LiAl(OEt)H_3$; NaCN; Ra-Ni, H_2	Decomposed
Na, NH_3	Decomposed

Additional studies demonstrated that the olefin group in compound **389** could be successfully reduced. Conversely, the olefin group in compound **390** could not be reduced. Through molecular modelling (Chem 3D MM2), it was determined that steric hindrance could also be influencing the success of the reduction. Specifically, the benzyloxyethyl group as well as the D and E ring system in compound **385** effectively

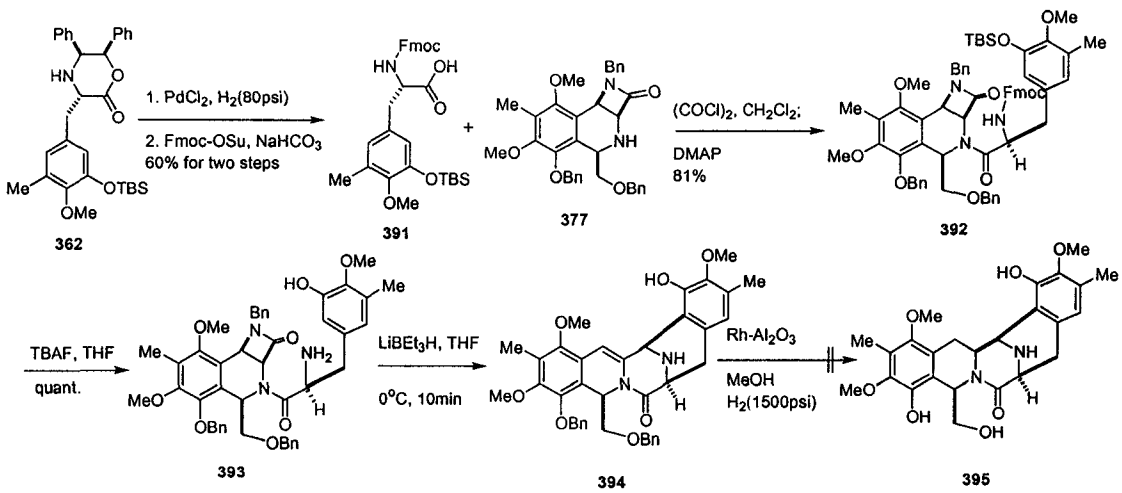
shielded the top face of the olefin in an energy-minimized structure. The bottom face of the olefin was shielded by the N^{12}-methyl and adjacent O-methyl groups.



Scheme 68. Double bond problem

Therefore, in an effort to relieve some of the postulated steric hindrance at the olefin group in compound **385**, a modified substrate was designed that lacked the N-methyl group. This methyl group could then be incorporated at a later stage in the synthesis following successful reduction. Another possible solution to the reduction scenario entailed an early-stage reduction of the olefin group in an intermediate prior to the formation of the pentacycle. This particular strategy was encouraged by the successful reduction of the olefin group in compound **389**.

3.5.9 Attempts to reduce the double bond

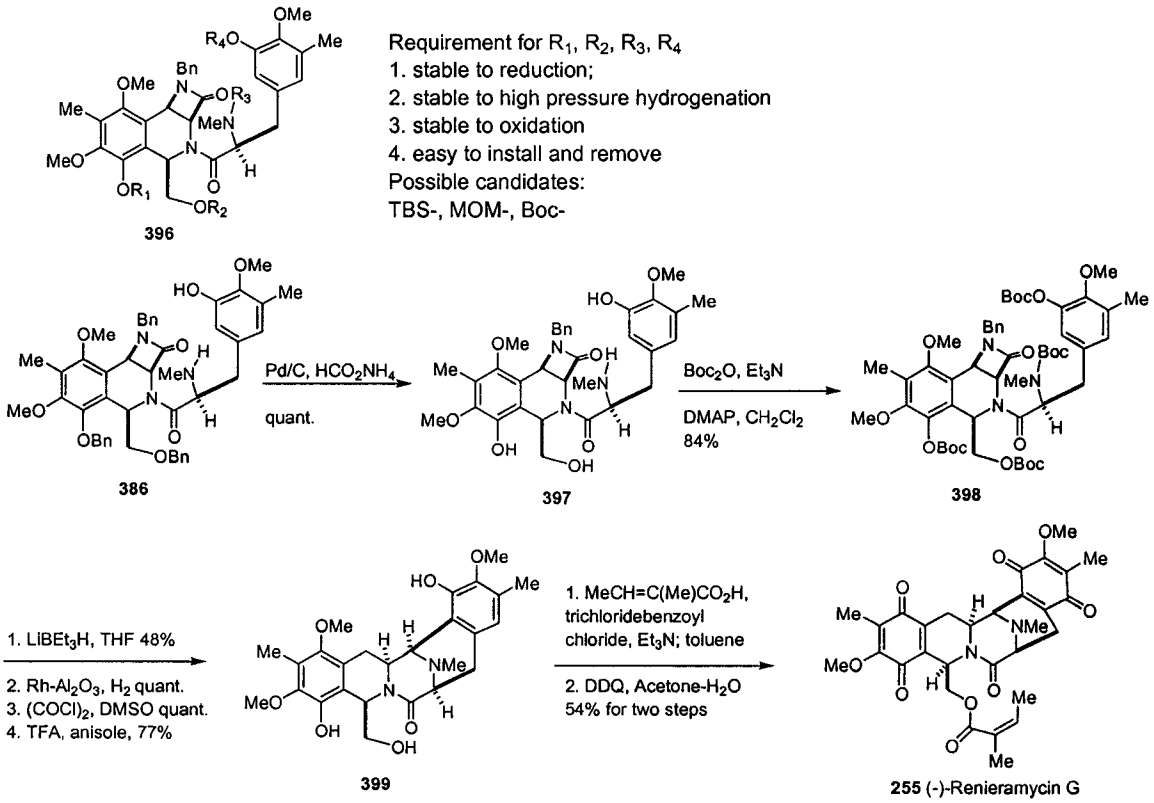


Scheme 69. Attempts to reduce the double bond

In an effort to produce a less sterically encumbered substrate for the necessary olefin reduction reaction, compound **362**, lacking a methyl group at the nitrogen position, was converted to amino acid **391** in two steps. The amino acid **391** was then converted into the corresponding acid chloride by treatment with oxalyl chloride and then subsequently coupled to the amine **377** in the presence of DMAP to afford the peptide **392** in 81% yield. Both the Fmoc and TBS protecting groups of **392** were removed in a single operation by treatment with TBAF, which afforded the phenolic amine **393** in quantitative yield. Exposure of **393** to $LiBEt_3H$ in THF at 0 °C furnished the desired pentacyclic compound **394** directly. Unfortunately, attempts to reduce compound **394** were unsuccessful.

Also, since the introduction of functional groups onto the double bond failed on various conditions (Table 13), it was thought the best way to overcome this problem was to reduce the double bond before the cyclization into pentacycle. Also the best way to test this approach to use is on the total synthesis of a simpler natural product: renieramycin G.

3.6 Completion of the renieramycin G total synthesis



Scheme 70. Completion of the renieramycin G total synthesis

Initially, the protecting group strategy for crucial intermediate prior to installation of the pentacycle was closely examined and tuned for the synthesis of the new target, renieramycin G. The following requirements were necessary for success of the synthetic strategy: 1) the protecting groups had to be stable to conditions employed in the reduction of the β-lactam unit; 2) the groups must be stable to high-pressure catalytic hydrogenation conditions necessary to convert the α,β-unsaturated aldehyde unit to the corresponding saturated primary alcohol; 3) stability to oxidative conditions would be required for the conversion of the primary alcohol to the requisite aldehyde; 4) the groups had to be easily installed and subsequent removed using conditions amenable to the

synthesis. Although a number of protecting groups met these requirements, the Boc group was selected to protect all four of the requisite functional groups.

Perhaps most attractive was the fact that four of the Boc groups could be removed under the same conditions used to promote formation of the pentacycle.

As in Scheme 70, the benzyl groups of compound **386** were removed under transfer hydrogenation conditions, affording triol **397** in quantitative yield. Global Boc protection of all four necessary functional groups (two phenolic hydroxyl groups, one primary alcohol and one secondary amine) afforded peptide **399**. The β-lactam of compound **399** was reduced to the corresponding unsaturated aldehyde intermediate with super-hydride in 48% yield. The unsaturated aldehyde intermediate, which upon hydrogenation with Rh on alumnia (1500psi) reduced both the aldehyde and the alkene, furnished a single diastereomer with the desired relative configuration. The primary alcohol was oxidized to the corresponding aldehyde *via* Swern oxidation, followed by treatment with TFA to afford pentacycle **399** in 77% yield for three steps. The primary alcohol of pentacycle **399** was acylated with angelic acid under modified Yamaguchi conditions[78] to provide the corresponding angelate ester. Finally, DDQ[32,79] oxidation of both phenols to the *para*-quinones afforded (-)-renieramycin G in 54% yield. The synthetic renieramycin G ($[\alpha]_D^{25}$=-30.5 (c = 0.12, CH_2Cl_2)) had spectral data consistent with that reported for the natural substance.

Thus, (-)-renieramycin G was prepared in 26 steps (longest linear steps)(42 steps total) from commercially available 2,6-dimethoxyltoluene in an overall yield of 4.3% from known compound **129**.

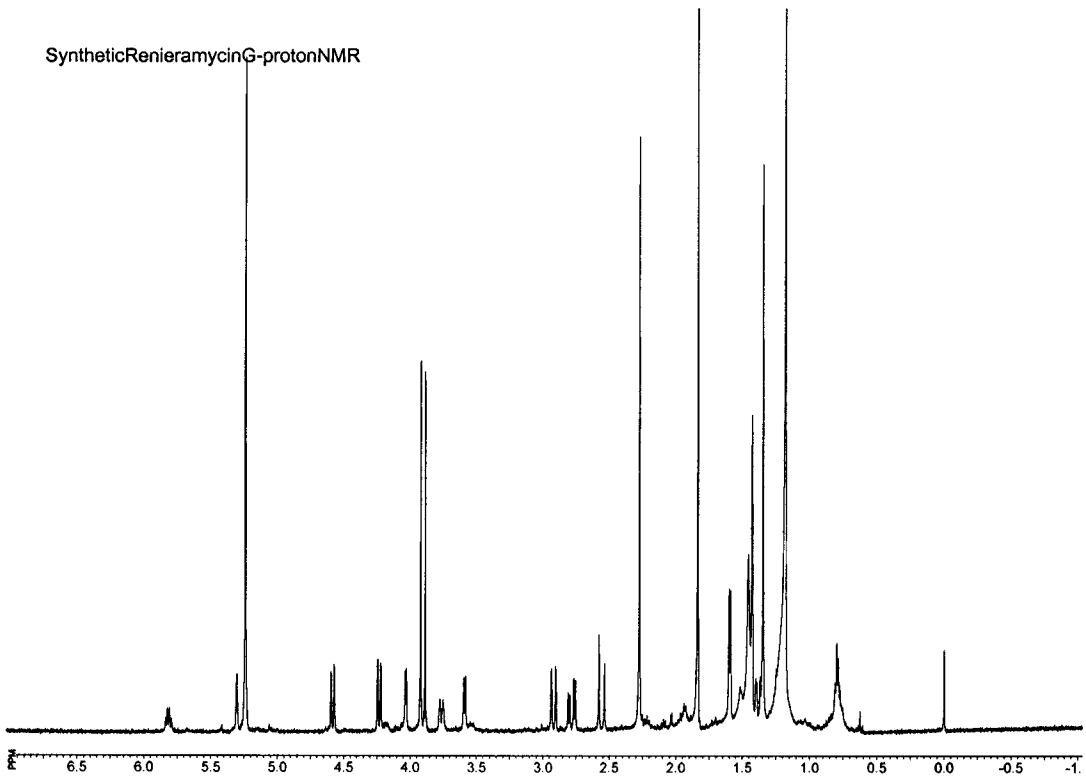


Figure 7. 500 MHz ^{1}H nmr spectra of synthetic (-)-renieramycin G (CD_2Cl_2)

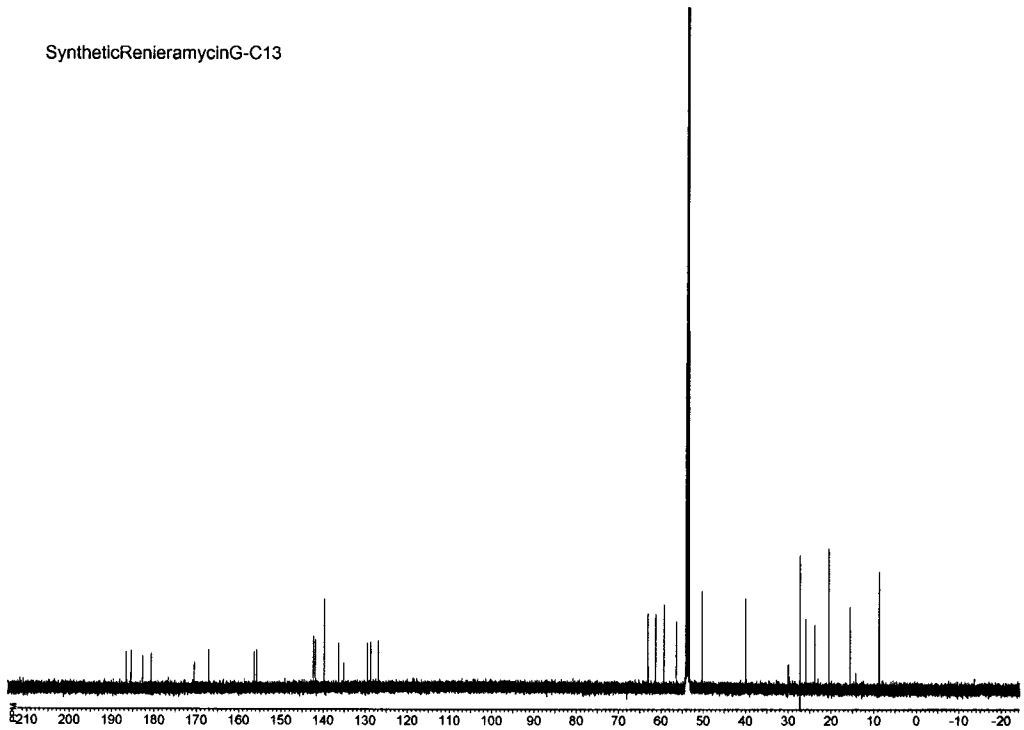


Figure 8. 125 MHz ^{13}C nmr spectra of synthetic (-)-renieramycin G (CD_2Cl_2)

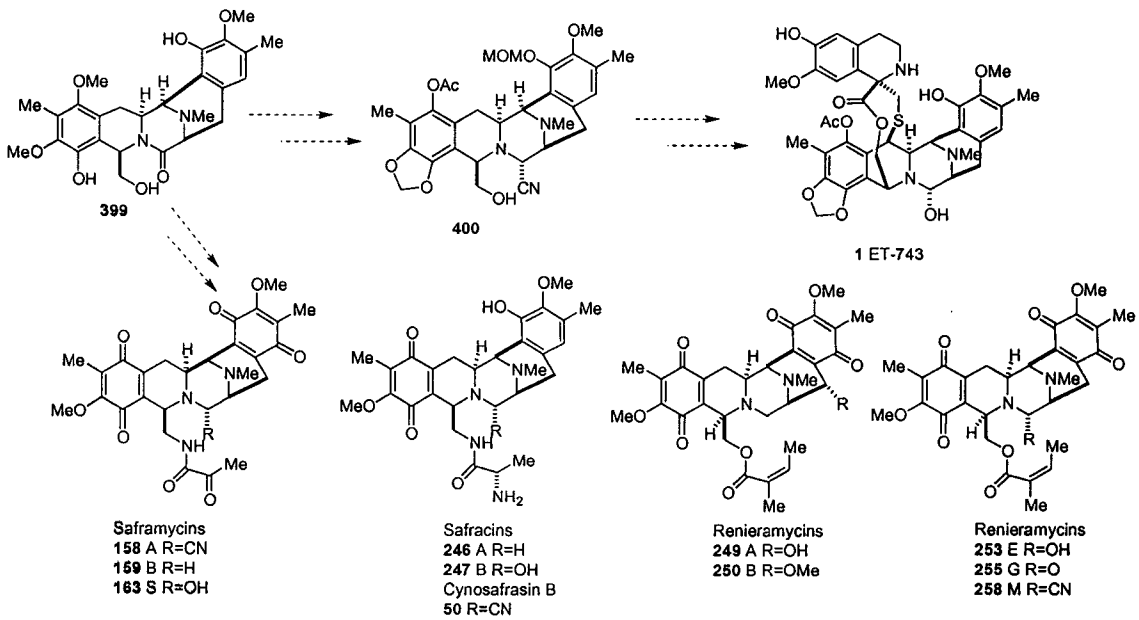
Table 14. Comparison of ^{13}C NMR data of synthetic and natural renieramycin G

	13C NMR (synthetic)[a]	13C NMR (natural)[a]
1	50.38	50.52
3	56.44	56.63
4	25.89	26.09
5	141.92	142.15
6	185.49	185.58
7	129.57	129.63
8	156.37	155.99
9	180.67	180.84
10	136.36	136.59
11	53.34	53.2
13	59.33	59.60
14	23.76	24.00
15	142.36	142.56
16	186.57	186.64
17	128.74	128.79
18	155.81	156.56
19	182.76	182.90
20	135.19	135.45
21	170.54	170.71
22	63.05	63.34
24	167.15	167.30
25	126.94	127.26
26	139.80	139.50
Me(25)	15.58	15.60
Me(26)	20.53	20.51
ArMe	8.76	8.76
OMe	61.30	61.28
OMe	61.26	61.24
NMe	39.97	40.06

[a] All data were recorded in CD_2Cl_2 and to the solvent signal (53.8 ppm), and measured at 125 MHz

2.7 Other possible targets and future plans for total synthesis of Et-743

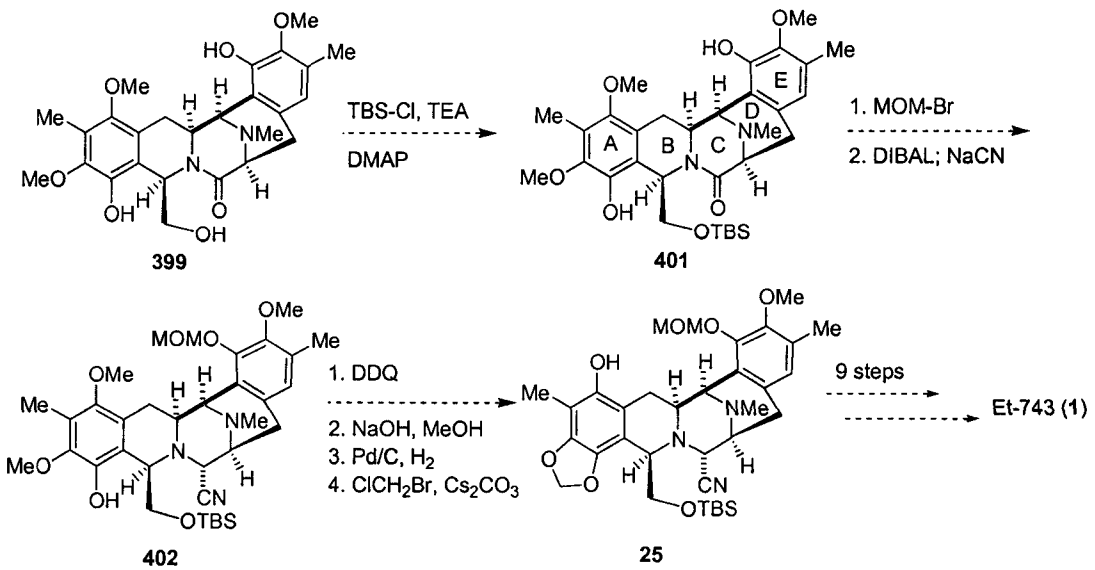
The approach developed for renieramycin G is versatile and could be used in the synthesis of other tetrahydroisoquinoline antitumor alkaloids (Scheme 71). Installation of a methylenedioxy group in intermediate **399**, as in the Cuevas' semi-synthesis,[7] would lead to the total synthesis of Et-743. Alternatively, a formal synthesis of Et-743 could be achieved through the interception of the intermediates published by Corey or Cuevas.[6,7] Finally, minor modifications to compound **399** could produce an intermediate poised for incorporation into the saframycins, safracins, as well as other members of the renieramycin family.



Scheme 71. Other possible targets

As in Scheme 72, a synthetic plan is proposed that commenced with the triol **399**. Once the primary alcohol of triol **399** is protected with TBS group, the less-hindered phenol group on aromatic ring E of intermediate **401** could be selectively protected with MOM group, and subsequent conversion of the amide in **401** to aminonitrile would afford intermediate **402**. DDQ oxidation of the phenol group in compound **402** affords to

quinone intermediate, and subsequently, followed the protocol of Cuevas,[7] the methylenedioxy unit is installed to give intermediate **25**, which is the same intermediate as in Corey's total synthesis (9 steps from Et-743).[6]



Scheme 72. Future plan for the formal synthesis of ecteinascidin-743

Comparing with the total syntheses that reported by Corey or Fukuyama groups, our proposed approach is more concise and convergent (Corey's total synthesis: 31 steps in longest linear pathway and 42 steps in total steps; Fukuyama's total synthesis: 45 steps in longest linear pathway and 52 steps in total steps; our proposed synthetic plan: 33 steps in longest linear pathway and 41 steps in total steps). Other advantages of our proposed synthesis include: 1) our synthesis could be a general method to synthesize all four family members of the ecteinascidins, saframycins, safracins and the renieramycins; 2) in our synthesis, the tetrahydroisoquinoline ring is a versatile, it could be used in the asymmetric total synthesis of bioxalomycin or other tetrahydroisoquinoline antitumor antibiotics; 3) Our methodology for the asymmetric synthesis of the amino acid

component is general and versatile, without major change to the approach, several unnatural amino acids are accessible in grams scale.

In summary, we have developed the asymmetric methodology for constructing the *cis*-tetrahydroisoquinoline system by a sequential asymmetric Staudinger reaction followed by a Pictet-Spengler reaction in multi-gram scale. Additionally, a synthesis was developed for the multigram scale preparation of 3-hydroxy-4-methoxy-5-methylbenzaldehyde, a crucial intermediate in the synthesis of the amino acid component necessary to construct Et-743. Furthermore, an asymmetric approach for the synthesis of the aforementioned amino acid component was successfully developed. Finally, methods were developed and utilized to construct a key pentacyclic intermediate, which led to the first asymmetric total synthesis of (-)-renieramycin G.

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Experimental section:

General procedure

Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Toluene, diethyl ether, THF and dimethyl formamide were degassed with argon and passed through a solvent system (J.C. Meyer of glass Contour). The molecular sieves were activated by heating at 150 °C at 1 mm Hg for 3h in a vacuum oven.

All reactions involving hydroscopic substances were conducted with flames or oven dried glassware under an inert atmosphere (Ar) dried by passage of atmospheric gases through a column packed with $CaSO_4$.

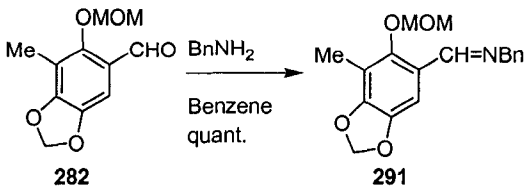
Chromatographic separations were performed with EM Science TLC plates (silica gel 60, F254, 20 x 20cm x 250μm) or with EM Science 230-240 mesh silica gel under positive air pressure. Reactions and chromatographic fractions were monitored and analyzed with EM Science TLC plates. Visualizations on TLC were achieved with ultraviolet light and heating of TLC plates submerged in a 5% solution of phosphomolybic acid in 95% ethanol.

Infrared spectra were recorded on a Perkin-Elmer 1600 series FT-IR as thin films from methylene chloride and are reported as λ_{max} in wavenumbers (cm^{-1}).

Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AC-300, Varian 300 or 400 spectrometers. NMR chemical shifts are given in parts per million (ppm) relative to internal $CHCl_3$, DMSO, or methanol. Proton (^1H) NMRs are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q,

quartet; and m, multiplet) and coupling constant in hertz. When appropriate, the multiplicity of a signal in denoted as "br" to indicate that the signal was broad.

Mass spectra were obtained on Fisons VG Autospec. Optical rotations were obtained on a Rudolph Research automatic polarimeter Autopol III.



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Imine 291 (Benzyl-(6-methoxymethoxy-7-methyl-benzo[1,3]dioxol-5-ylmethylene)-amine):

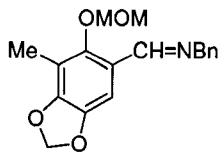
To a solution of aldehyde **282** (2.13 g, 9.51 mmol, 1.0 equiv) in benzene (100 mL) was added benzylamine (1.02 g, 9.51 mmol, 1.0 equiv) at room temp. The resulting solution was stirred at room temp. for 30 min. and then refluxed for 3 h (1H NMR analysis revealed that the reaction was complete). The solvent was removed under reduced pressure to afforded the imine **291** 2.99 g (quant.). The resulting imine was pure enough to be used for next step without further purification.

^{1}H NMR (300MHz, $CDCl_3$, 298K) δ 8.65 (1H, s); 7.30-7.40(6H, m); 6.01(2H, s); 4.99(2H, s); 4.85(2H, s); 3.60(3H, s); 2.23(3H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 158.33, 144.08, 139.58, 128.51, 128.03, 126.93, 122.93, 113.26, 103.37, 101.64, 100.95, 65.26, 58.02, 9.90;

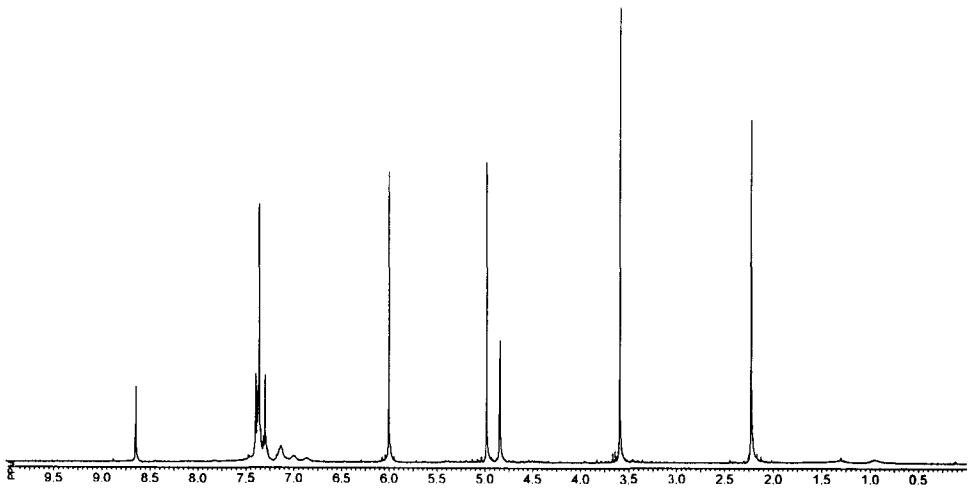
IR (neat, film): 2956, 2930, 2872, 1640, 1626, 1496, 1472, 1427, 1279, 1159, 1071, 960, 700 cm^{-1};

HRMS (FAB+) calcd for $C_{18}H_{20}NO_4$ 314.1392, found 314.1395.

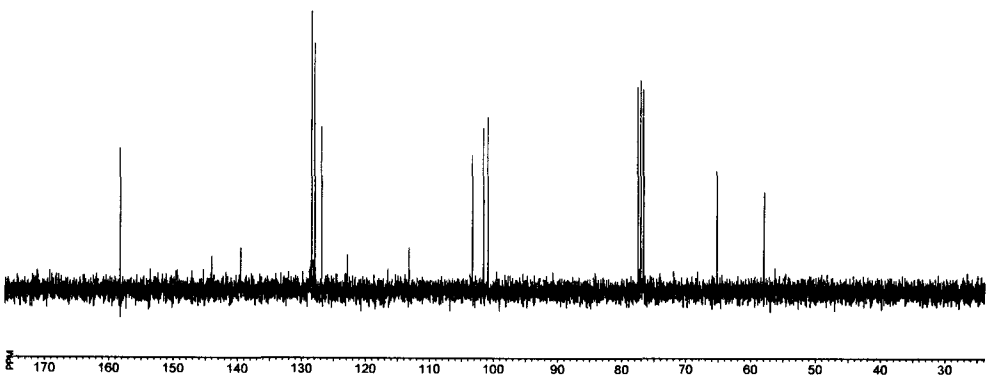


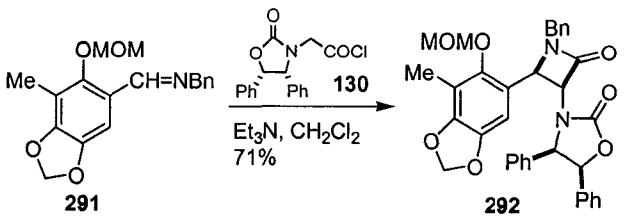
291

wj-l-252-2



wj-l-252-2C





β-Lactam 292 (3-[1-Benzyl-2-(6-methoxymethoxy-7-methyl-benzo[1,3]dioxol-5-yl)-4-oxo-azetidin-3-yl]-4,5-diphenyl-oxazolidin-2-one):

To a solution of (2-oxo-4(R),5(S)-diphenyl-oxazolidin-3-yl)-acetic acid (2.97 g, 9.27 mmol, 1.1 equiv) and DMF (120 μL, 1.39 mmol, 0.15 mmol) in CH_2Cl_2 (50 mL) at room temp. was added $(COCl)_2$ (1.21 mL, 13.90 mmol, 1.5 equiv) dropwise, the resulting solution was stirred at room temp. for 1 h. The solvent and excess $(COCl)_2$ were removed under reduced pressure. The resulting crude acid chloride **130** was dissolved in CH_2Cl_2 (50 mL) and cooled to –78°C, then Et_3N (1.94 mL, 13.9 mmol, 1.5 equiv) was added dropwise. After the solution was stirred for 15 min., a solution of imine **291** (2.9 g, 9.27 mmol, 1.0 equiv) was added and after stirring 10 min., the mixture was allowed to warm to 0°C and stirred for another 2.5 h. After the reaction was quenched with sat. NH_4Cl solution (50 mL), it was extracted with CH_2Cl_2 (100 mL x3) and the combined organic layer was dried over anhydrous Sodium sulfate. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 50% EtOAc in hexanes) to gave β-lactam **292** (3.88 g, 71%)

^1H NMR (300MHz, $CDCl_3$, 298K) δ 7.26-7.30(3H, m); 7.19-7.22(2H, m); 7.11-7.13(3H, m); 7.02-7.06(4H, m); 6.94-6.96(2H, m); 6.86-6.89(2H, m); 5.99-6.00(2H, dd, *J*=1.5Hz, 6.2Hz); 5.35-5.38(1H, d, *J*=8.4Hz); 5.02-5.07(1H, d, *J*=14.4Hz); 4.85-4.90(3H, m); 4.79-

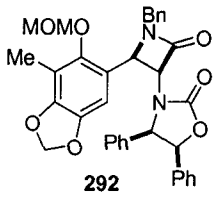
7.81(1H, d, *J*=6.3Hz); 4.35-4.37(1H, d, *J*=5.1Hz); 4.00-4.05(1H, d, *J*=14.7Hz); 3.25(3H, s); 2.25(3H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 170.96, 164.11, 156.38, 149.61, 143.65, 134.91, 134.13, 133.67, 128.68, 128.55, 128.25, 127.79, 127.73, 127.68, 125.95, 119.42, 112.85, 105.58, 101.51, 99.93, 99.81, 79.80, 64.96, 64.72, 60.40, 57.33, 56.65, 45.19, 21.14, 14.31, 10.11;

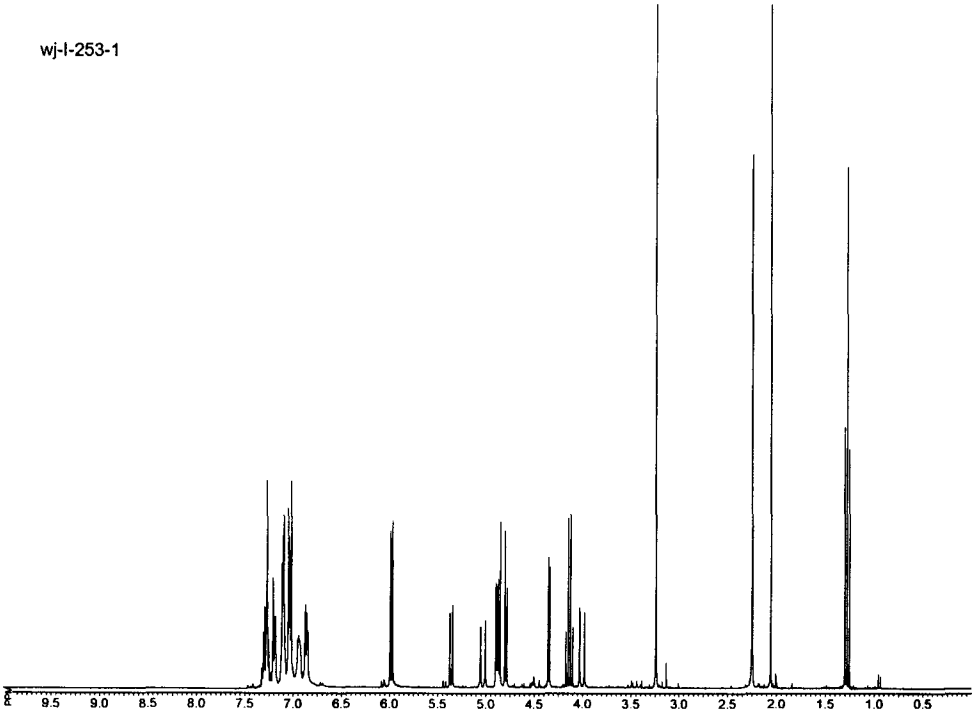
IR (neat, film) 3064, 3032, 2905, 1767, 1498, 1478, 1413, 1219, 1183, 1100, 1068, 1002, 963, 699cm^{-1};

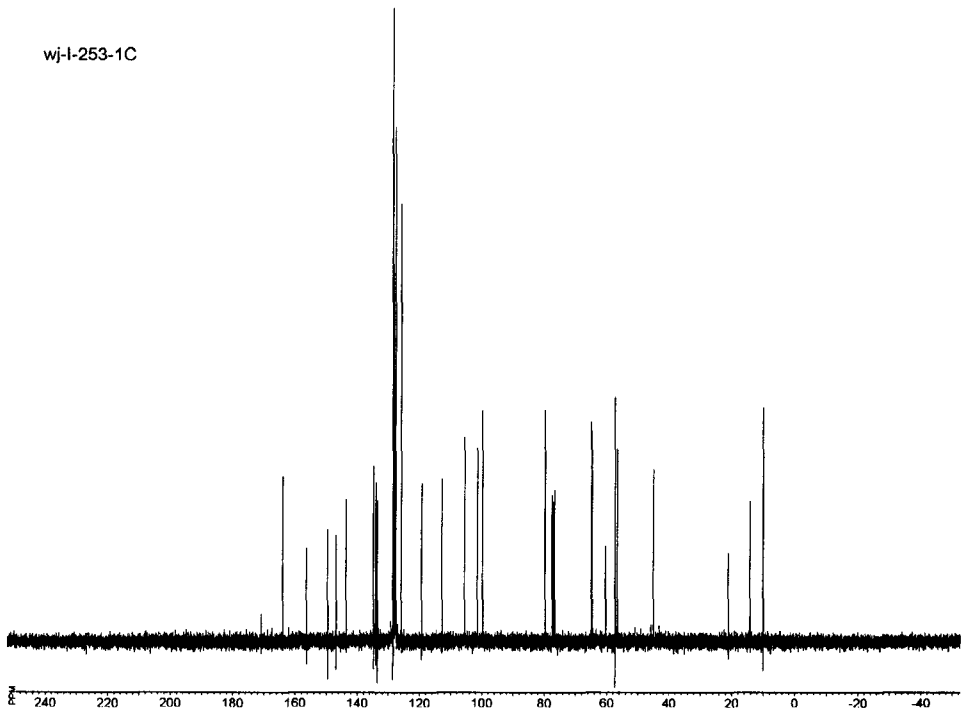
HRMS(FAB+) calcd for $C_{35}H_{33}N_2O_7$ 593.2288, found: 593.2305;

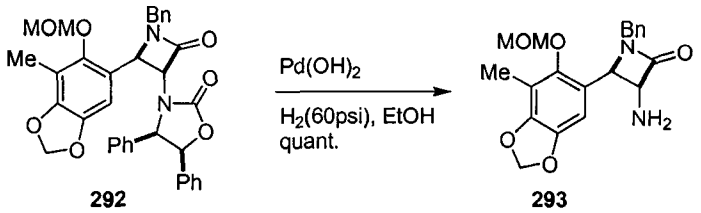
$[\alpha]_D^{25}$ =–0.91 (c1.32, CH_2Cl_2).



292







Amine 293 (3-Amino-1-benzyl-4-(6-methoxymethoxy-7-methyl-benzo[1,3]dioxol-5-yl)-azetidin-2-one):

To a solution of β-Lactam **292** (254 mg, 0.433 mmol, 1.0 equiv.) in MeOH/THF (1:1) (10 mL) was added $Pd(OH)_2$ (303 mg, 0.433 mmol, 1.0 equiv). The resulting mixture was hydrogenated at 1atm H_2 for 18 h. The catalyst was then filtered off through Celite, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluted with EtOAc) to give β-Lactam **293** (160 mg, quant.).

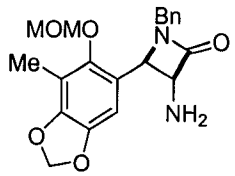
^{1}H NMR (300MHz, $CDCl_3$, 298K) δ 7.30-7.36(3H, m); 7.21-7.24(2H, m); 6.55(1H, s); 6.00-6.04(2H, dd, *J*=1.2, 7.8Hz); 5.02-5.03(1H, d, *J*=4.8Hz); 4.89-4.94(1H, d, *J*=23.7Hz); 4.78-4.85(2H, dd, *J*=6.0, 9.0Hz), 4.40-4.42(1H, d, *J*=5.1Hz); 3.87-3.92(1H, d, *J*=15.0Hz); 3.47(3H, s); 2.20(3H, s); 1.40(2H, broad);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 170.59, 150.39, 146.72, 143.90, 135.34, 128.83, 128.55, 128.46, 127.83, 120.37, 144.55, 103.44, 101.60, 100.11, 65.03, 57.56, 57.37, 44.41, 10.20;

IR (neat, film) 3391, 3328, 2913, 1752, 1477, 1404, 1276, 1158, 1067, 960, 700cm^{-1};

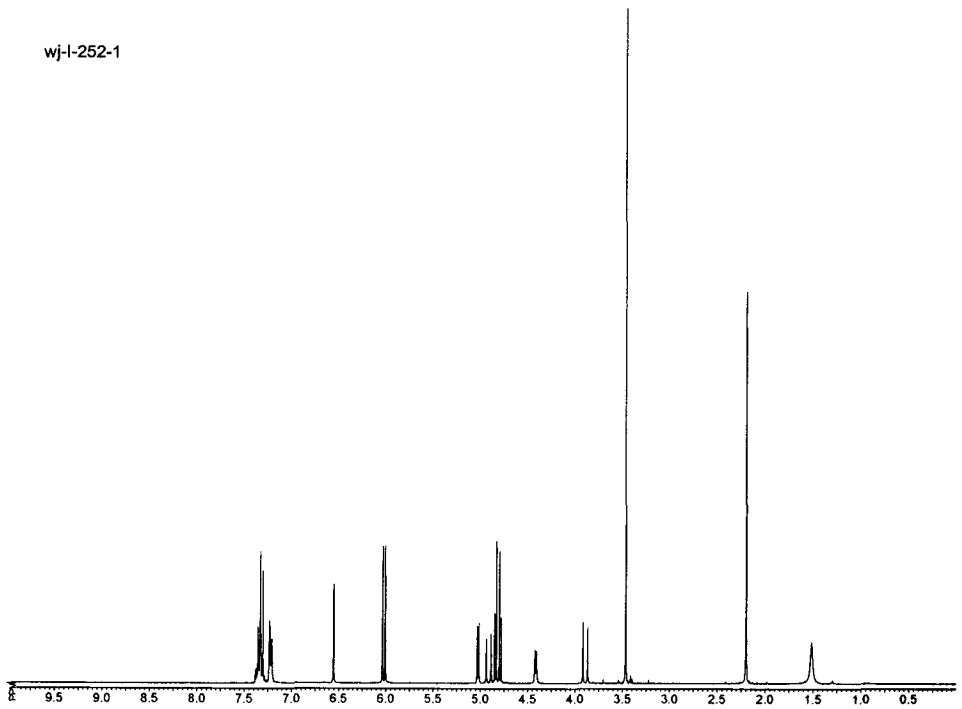
HRMS (FAB+) calcd for $C_{20}H_{22}N_2O_5$ 370.1529, found: 370.1519;

$[\alpha]_D^{25}$ =+16.87 (c 0.664, CH_2Cl_2).

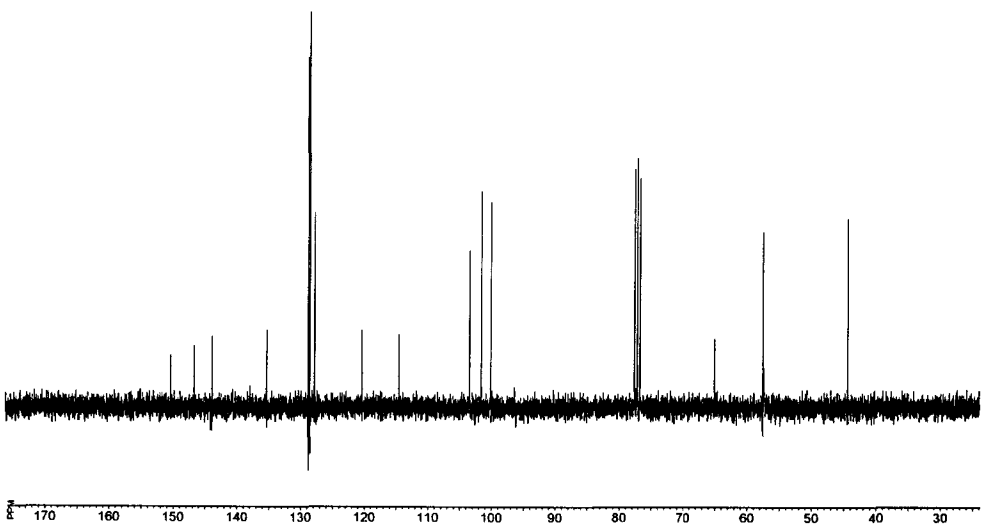


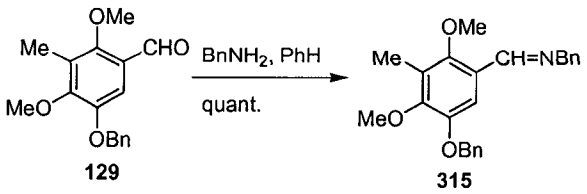
293

wj-l-252-1



wj-l-252-1C





Imine 315 (Benzyl-(5-benzyloxy-2,4-dimethoxy-3-methyl-benzylidene)-amine):

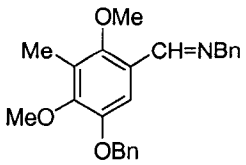
To a solution of aldehyde **129** (1.0 g, 3.50 mmol, 1.0 equiv) in benzene (50 mL) was added benzyl amine (0.41 g, 3.85 mmol, 1.1 equiv) at room temp. The resulting solution was refluxed for 3 h (^{1}H NMR analysis revealed that the reaction was complete). The solvent was removed under reduced pressure to afford imine **315** 1.31 g (quant.). The resulting imine was pure enough to be used for next step without further purification.

^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ 8.75 (1H, s), 7.36-7.63 (11H, m), 5.18 (2H, s), 4.92 (2H, s), 3.94 (3H, s), 3.81 (3H, s) 2.31 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 157.82, 153.66, 150.98, 148.81, 139.52, 137.01, 128.45, 127.92, 127.84, 127.47, 126.91, 125.58, 124.24, 108.63, 99.96, 70.89, 65.41, 62.67, 60.53, 9.50;

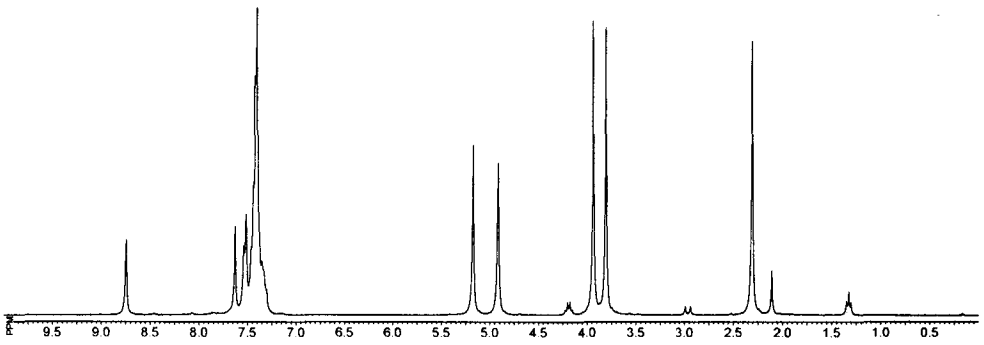
IR (neat, film): 3086,3062, 3029, 2935, 2826, 1681, 1634, 1591, 1453, 1415, 1375, 1126, 1074, 1009, 735 cm^{-1};

HRMS (FAB+) calcd for $C_{24}H_{26}N_1O_3$ 376.1913, found 376.1904.



315

wj-II-54-1



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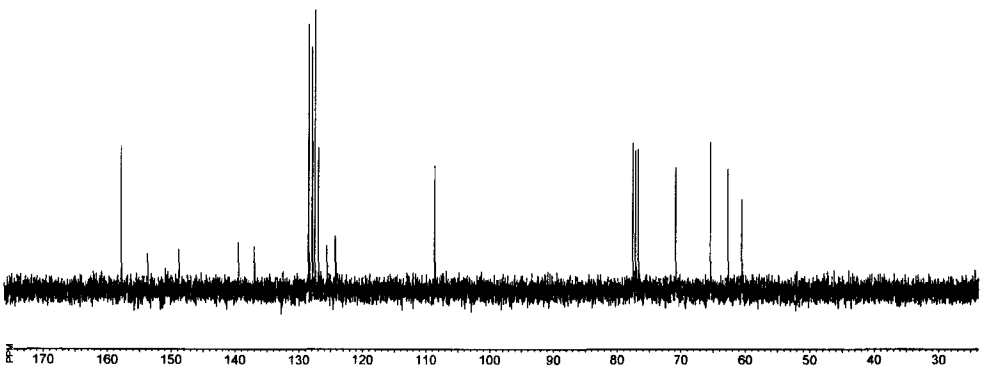
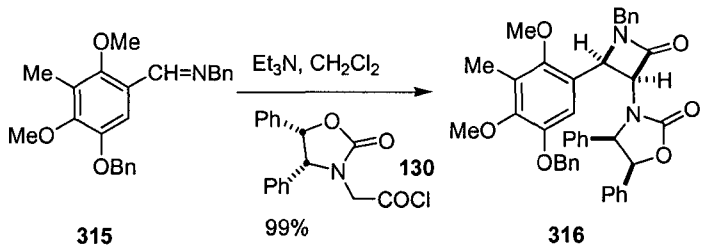




β-Lactam 316 (3-[1-Benzyl-2-(5-benzyloxy-2,4-dimethoxy-3-methyl-phenyl)-4-oxo-azetidin-3-yl]-4,5-diphenyl-oxazolidin-2-one):

To a solution of (2-oxo-4(R),5(S)-diphenyl-oxazolidin-3-yl)-acetic acid (1.12 g, 3.85 mmol, 1.1 equiv) and DMF (46 μL, 0.53 mmol, 0.15 mmol) in CH_2Cl_2 (25 mL) at room temp. was added $(COCl)_2$ (485 μL, 5.25 mmol, 1.5 equiv) dropwise, the resulting solution was stirred at room temp. for 1 h. The solvent and excess $(COCl)_2$ were removed under reduced pressure. The resulting crude acid chloride **130** was dissolved in CH_2Cl_2 (25 mL) and cooled to –78°C, then Et_3N (732 μL, 5.25 mmol, 1.5 equiv) was added dropwise. After the solution was stirred for 15 min., a solution of imine **315** (1.31 g, 3.50 mmol, 1.0 equiv) was added and stirring for 10min., the mixture was allowed to warm to 0°C and stirred for another 2.5 h. After the reaction was quenched with sat. NH_4Cl solution (25 mL), it was extracted with CH_2Cl_2 (50 mL x3) and the combined organic layer was dried with anhydrous Sodium sulfate. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 50% EtOAc in hexanes) to give β-lactam **316** (2.51 g, 84%) along with the recovery of aldehyde **129** (157 mg, 16%).

^1H NMR (300 MHz, $CDCl_3$, 273K) δ 7.56-7.59 (2H, d, *J*=7.2Hz), 7.44-7.47 (2H, m), 7.25-7.42 (3H, m), 7.06-7.17 (10H, m), 6.82-6.93 (4H, m), 5.20-5.25 (1H, d, *J*=12.3 Hz),

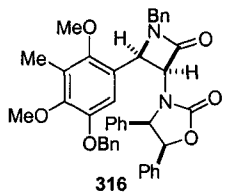
5.16-5.19 (1H, d, *J*=8.4Hz), 5.05-5.10 (1H, d, *J*=12.3 Hz), 4.86-4.91 (1H, d, *J*=14.7 Hz), 4.73-4.75 (1H, d, *J*=5.1 Hz), 4.66-4.69 (1H, d, *J*=6.6Hz), 4.39-4.41 (1H, d, *J*=5.1Hz), 3.95 (3H, s), 3.76-3.81 (1H, d, *J*=14.7Hz), 3.46 (3H, s), 2.34 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 273K) δ 163.91, 163.87, 156.51, 150.54, 148.29, 137.57, 135.02, 134.26, 134.21, 133.41, 128.83, 128.76, 128.55, 128.51, 128.46, 128.38, 128.05, 127.96, 127.86, 127.81, 127.68, 127.42, 126.08, 121.00, 111.83, 100.02, 79.82, 70.78, 64.96, 64.40, 60.94, 60.63, 56.14, 45.24, 9.89;

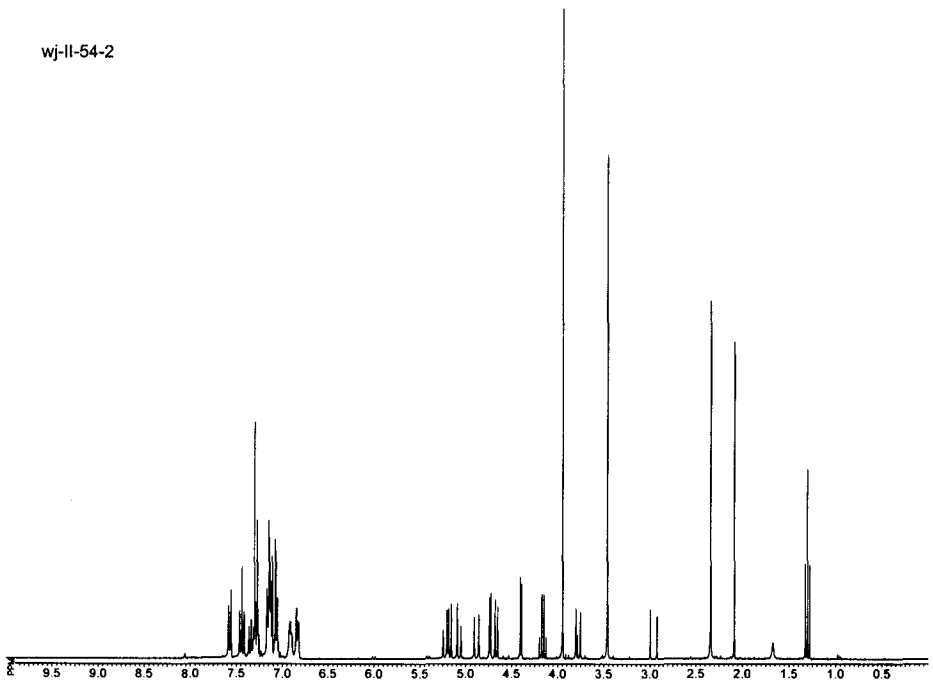
IR (neat, film): 3063, 3.32, 2935, 2836, 1755, 1677, 1597, 1414, 1230, 1075, 970, 909 cm^{-1};

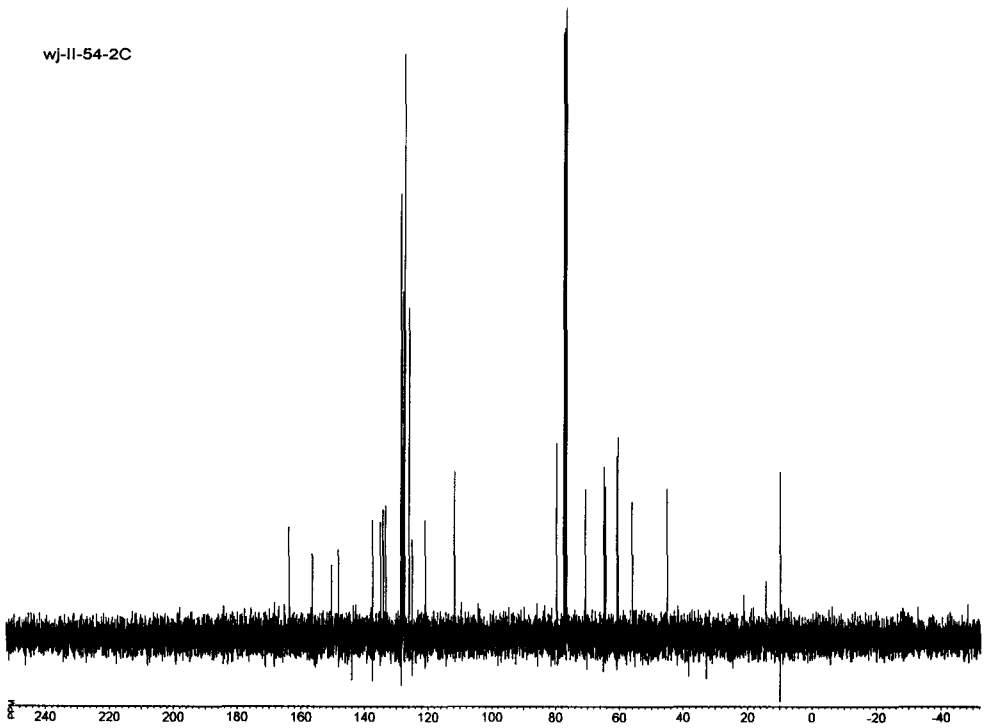
HRMS (FAB+) calcd for $C_{41}H_{39}N_2O_6$ 655.2808, found 655.2807;

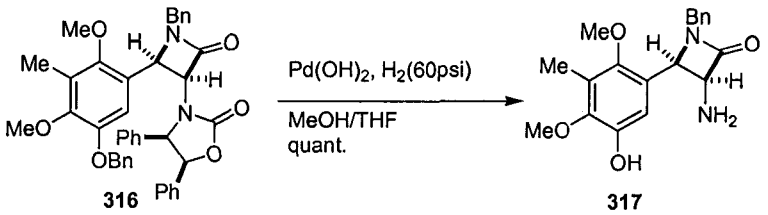
$[\alpha]_D^{25}$ = +37.8 (*c* 0.97, CH_2Cl_2).



316







Amine 317 (3-Amino-1-benzyl-4-(5-hydroxy-2,4-dimethoxy-3-methyl-phenyl)-azetidin-2-one):

To a solution of β-Lactam **316** (1.51 g, 2.31 mmol, 1.0equiv) in MeOH/THF(1:1) (46 mL) was added $Pd(OH)_2$ (1.77 g, 2.54 mmol, 1.1 equiv). The resulting mixture was hydrogenated at 60psi H_2 for 12 h. The catalyst was the filtered off through Celite, the solvent was removed under reduced pressure and residue was purified by flash column chromatography (eluted with 5% methanol in EtOAc) to give amine **317** (786 mg, quant.).

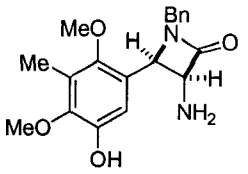
^{1}H NMR (300 MHz, $CDCl_3$, 273K) δ 7.21-7.35 (5H, m), 6.70 (1H, s), 4.94-4.99 (1H, d, *J*=14.7 Hz), 4.96-4.98 (1H, d, *J*=5.1Hz), 4.46-4.48 (1H, d, *J*=5.1Hz), 3.91-3.95 (1H, d, *J*=14.7Hz), 3.8 (3H, s), 3.54 (3H, s), 3.2 (2H, broad), 2.26 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 273K) δ 150.83, 145.96, 135.17, 128.83, 128.59, 127.86, 125.65, 123.25, 110.99, 64.33, 61.09, 60.74, 56.63, 44.39, 9.98;

IR (neat, film): 3332, 2936, 1744, 1666, 1454, 1421, 1229, 1122, 1007 cm^{-1};

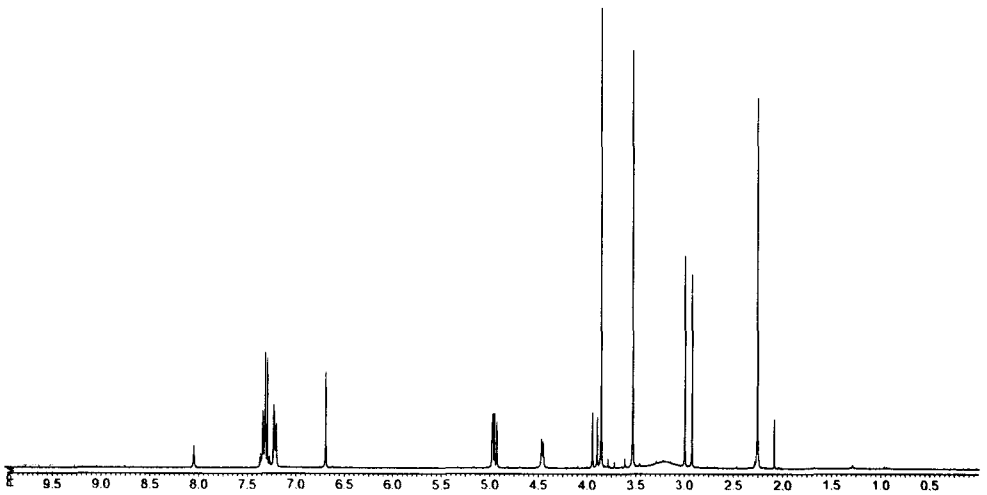
HRMS (FAB+) calcd for $C_{19}H_{22}N_2O_4$ 342.1580, found 342.1582;

$[\alpha]_D^{25} = -146.1$ (*c* 0.68, CH_2Cl_2).



317

wj-II-55-2



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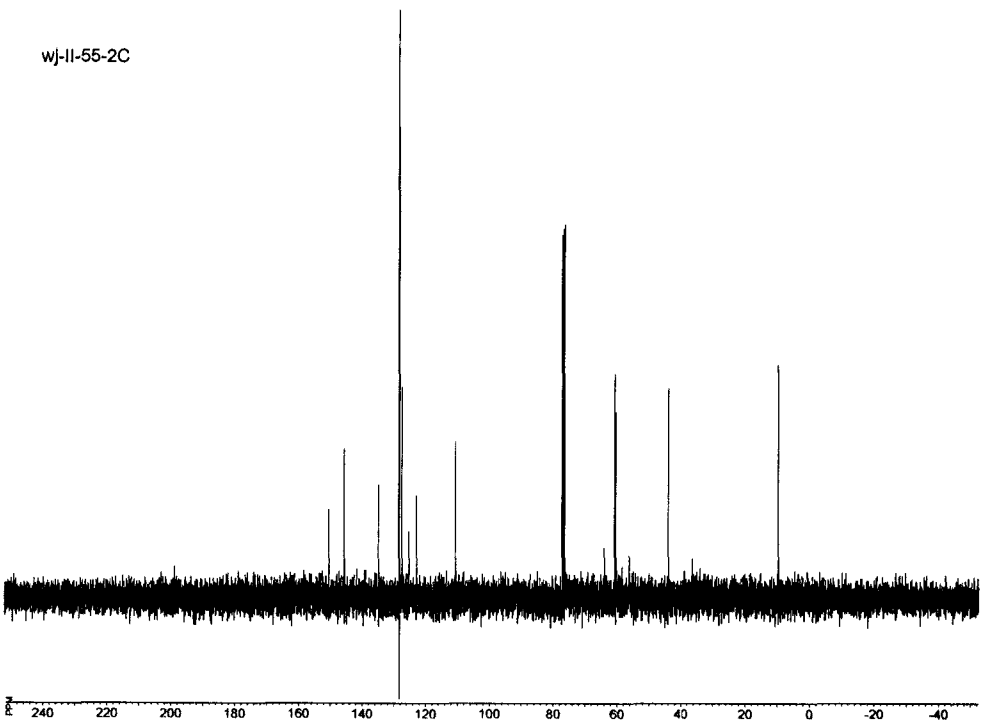
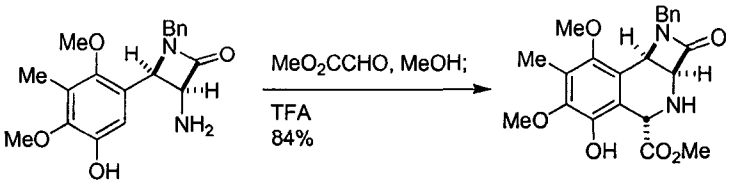




317 318

***Trans*-Tetrahydroisoquinoline (318) (1-Benzyl-5-hydroxy-6,8-dimethoxy-7-methyl-2-oxo-1,2,2a,3,4,8b-hexahydro-1,3-diaza-cyclobuta[a]naphthalene-4-carboxylic acid methyl ester):**

To a solution of amine **317** (780 mg, 2.28 mmol, 1.0 equiv) in methanol (40 mL) was added MeO_2CCHO (241 mg, 2.73 mmol, 1.2 equiv). The resulting solution was stirred at room temp. for 30min., and then heated to reflux for 96 h. After removal of the solvent under reduced pressure, the residue was treated with TFA (15 mL), and the mixture was stirred for 1 h. The excess TFA was removed under reduced pressure and quenched with Sat. $NaHCO_3$ solution (15 mL). The mixture was extracted with EtOAc (50 mL x3) and washed with water (50 mL), sat. NaCl solution (50 mL) and dried with anhydrous Sodium sulfate. After the solvent was removed under reduced pressure, the residue was purified by flash column chromatography (eluted with 25% hexanes in EtOAc) to afforded β-lactam **318** (788 mg, 84%)

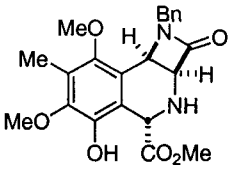
^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ 7.22-7.32 (5H, m), 5.80 (1H, s), 4.77-4.79 (1H, d, *J*=4.8Hz), 4.74-4.75 (2H, d, *J*=4.5Hz), 4.65 (1H, s), 4.50-4.55 (1H, d, *J*=15Hz), 4.08-4.03 (1H, d, *J*=15Hz), 3.83 (3H, s), 3.78 (3H, s), 3.57 (3H, s), 2.24 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 172.65, 167.93, 151.78, 145.72, 141.79, 136.55, 128.27, 127.32, 123.58, 121.61, 119.05, 63.14, 61.24, 60.98, 53.74, 52.70, 46.58, 44.09, 10.03;

IR (neat, film): 3369, 3309, 2949, 2935, 1750, 1727, 1468, 1415, 1291, 1217, 1069, 998 cm^{-1};

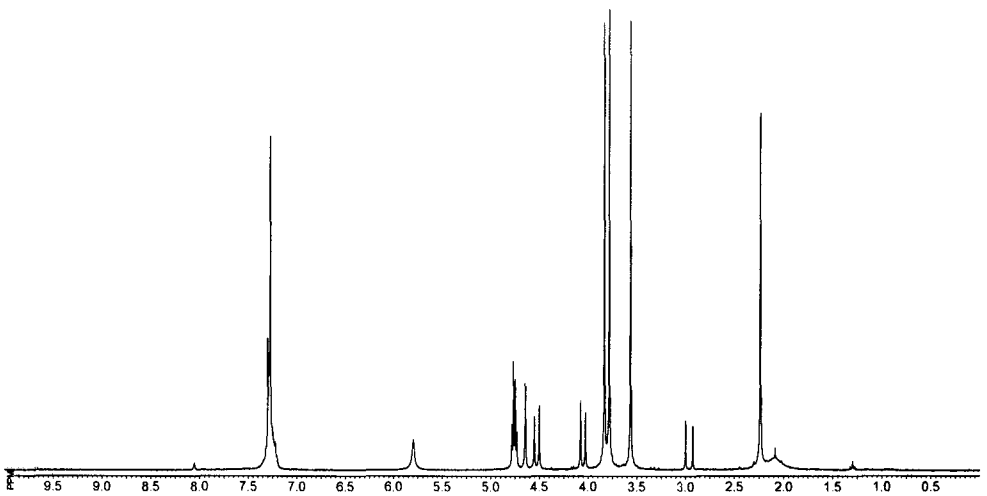
HRMS (FAB+) calcd for $C_{22}H_{25}N_2O_6$ 413.1713, found 413.1717;

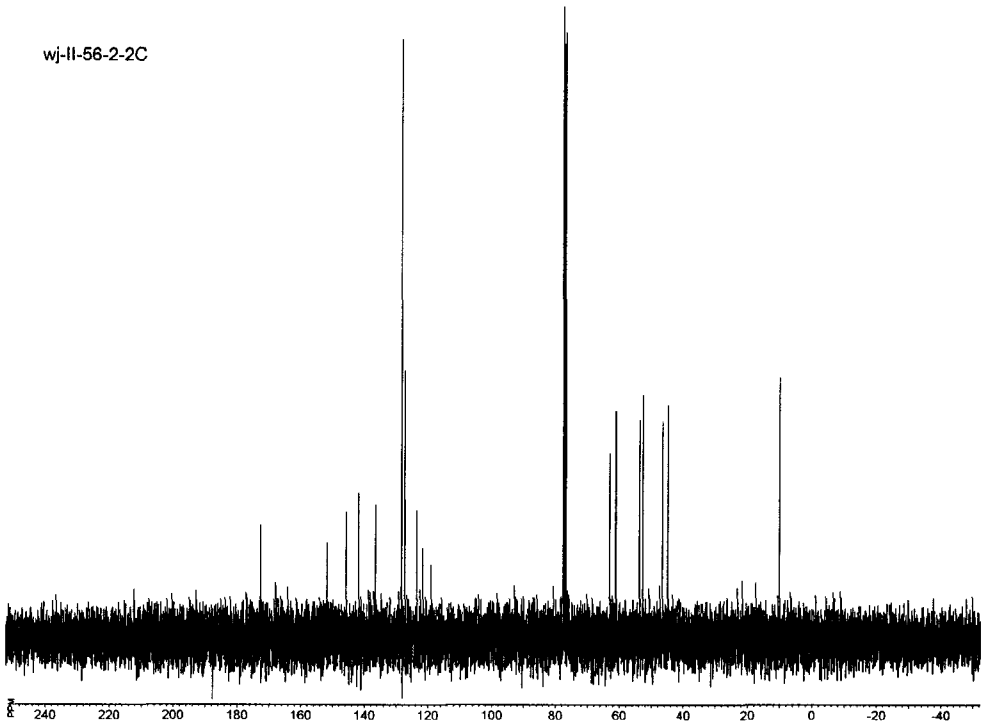
$[\alpha]_D^{25} = +133.7$ (*c* 0.69, CH_2Cl_2).

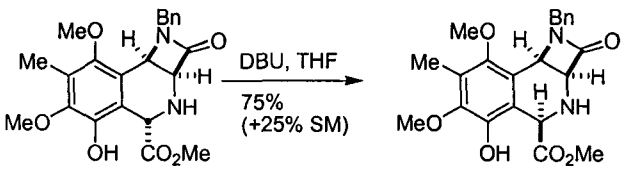


318

wj-II-56-2-2







***Cis*-Tetrahydroisoquinoline (320) (1-Benzyl-5-hydroxy-6,8-dimethoxy-7-methyl-2-oxo-1,2,2a,3,4,8b-hexahydro-1,3-diaza-cyclobuta[a]naphthalene-4-carboxylic acid methyl ester):**

To a stirred solution of β-lactam **318** (327 mg, 0.793 mmol, 1.0 equiv) in THF (8 mL) was added DBU (59.3 μL, 0.396 mmol, 0.5 equiv). The resulting solution was stirred at room temp. for 2 h. and the reaction was quenched with 10% aq. NH_4Cl (8 mL). The mixture was extracted with EtOAc (15 mL x3) and the organic layers were combined, dried and concentrated. The residue was purified by flash column chromatography (eluted with 25% hexanes in EtOAc; then EtOAc; then 5% MeOH in EtOAc) to afford β-lactam **320** (271 mg, 75%) along with β-lactam **318** (71 mg, 25%).

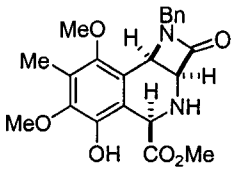
^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ 7.22-7.34 (5H, m), 5.92 (1H, broad), 5.10(1H, s), 4.84-4.86 (1H, d, *J*=5.1Hz), 4.59-4.61 (1H, d, *J*=5.7Hz), 4.49-4.54 (1H, d, *J*=15Hz), 3.82-3.87 (1H, d, *J*=15Hz), 3.85 (3H, s), 3.79 (3H, s), 3.60 (3H, s), 2.29 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 172.46, 168.43, 151.54, 145.90, 142.93, 136.57, 128.59, 128.35, 127.30, 123.68, 120.38, 118.36, 63.51, 61.20, 60.98, 52.98, 52.81, 46.92, 44.41, 10.15;

IR (neat, film): 3341, 2949, 1740, 1468, 1414, 1303, 1257, 1200, 1121, 1066, 1003 cm^{-1};

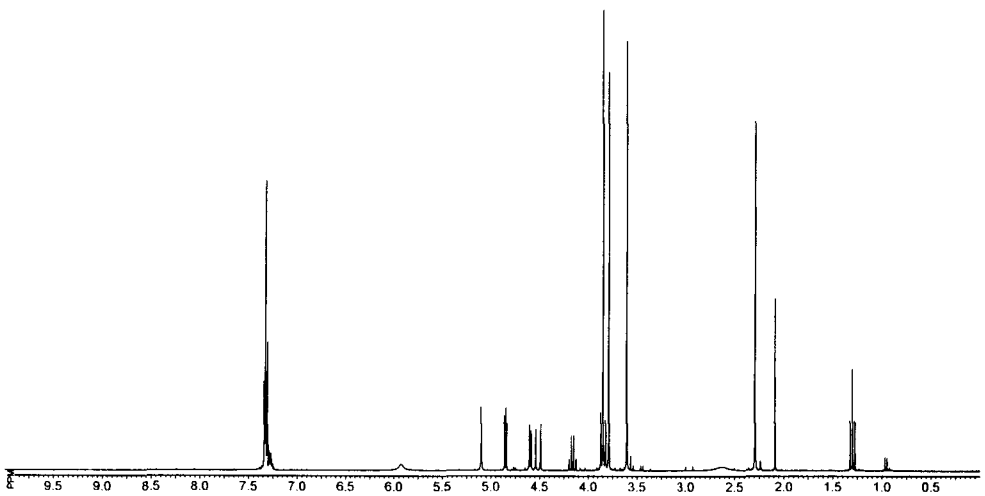
HRMS (FAB+) calcd for $C_{22}H_{25}N_2O_6$ 413.1713, found 413.1716;

$[\alpha]_D^{25}$ = +65.9 (*c* 0.54, CH_2Cl_2).



320

wj-II-58-1-3



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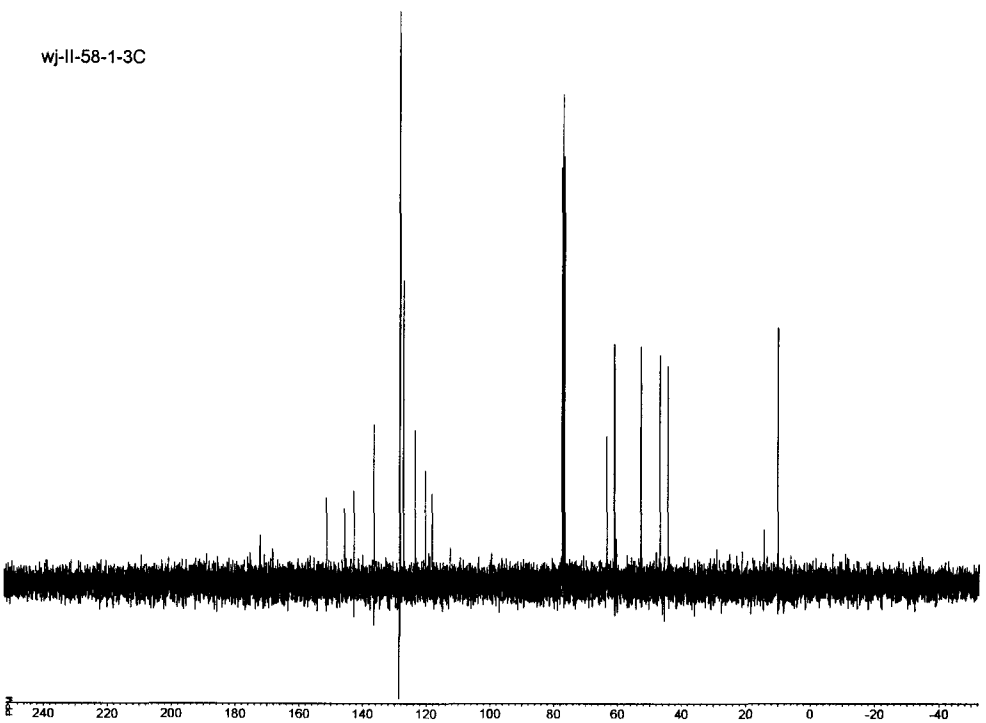
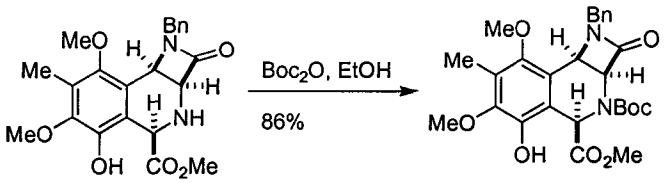




320 322

***N-t*-Boc-*Cis*-Tetrahydroisoquinoline (322) (1-Benzyl-5-hydroxy-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3,4-dicarboxylic acid 3-tert-butyl ester 4-methyl ester):**

To a solution of β-lactam **320** (254 mg, 0.616 mmol, 1.0 equiv) in ethanol (15 mL) was added Boc_2O (172 mg, 0.788 mmol, 1.2 equiv). The resulting solution was stirred at room temp. for 24 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 50% EtOAc in hexanes) to afford **322** (242 mg, 86%).

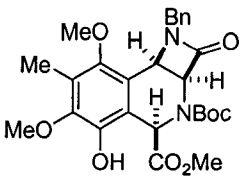
^{1}H NMR (300 MHz, DMSO-d_6, 373K) δ 8.62 (1H, s), 7.19-7.28 (5H, m), 6.24 (1H, s), 5.63-5.65 (1H, d, *J*=5.1Hz), 5.00-5.02 (1H, d, *J*=5.4Hz), 4.29-4.34 (1H, d, *J*=15.9Hz), 3.89-3.94 (1H, d, *J*=15.3Hz), 3.73 (3H, s), 3.55 (6H, s), 2.16 (3H, s), 1.49 (9H, s);

^{13}C NMR (75 MHz, DMSO-d_6, 373K) δ 169.03, 165.33, 152.91, 146.70, 143.01, 136.14, 127.65, 127.17, 126.48, 123.26, 118.79, 117.85, 80.70, 60.41, 60.16, 59.65, 51.46, 51.00, 47.41, 43.81, 27.54, 8.99;

IR (neat, film): 3376, 2978, 2951, 1755, 1704, 1470, 1415, 1317, 1259, 1163, 1112, 1066, 1006 cm^{-1};

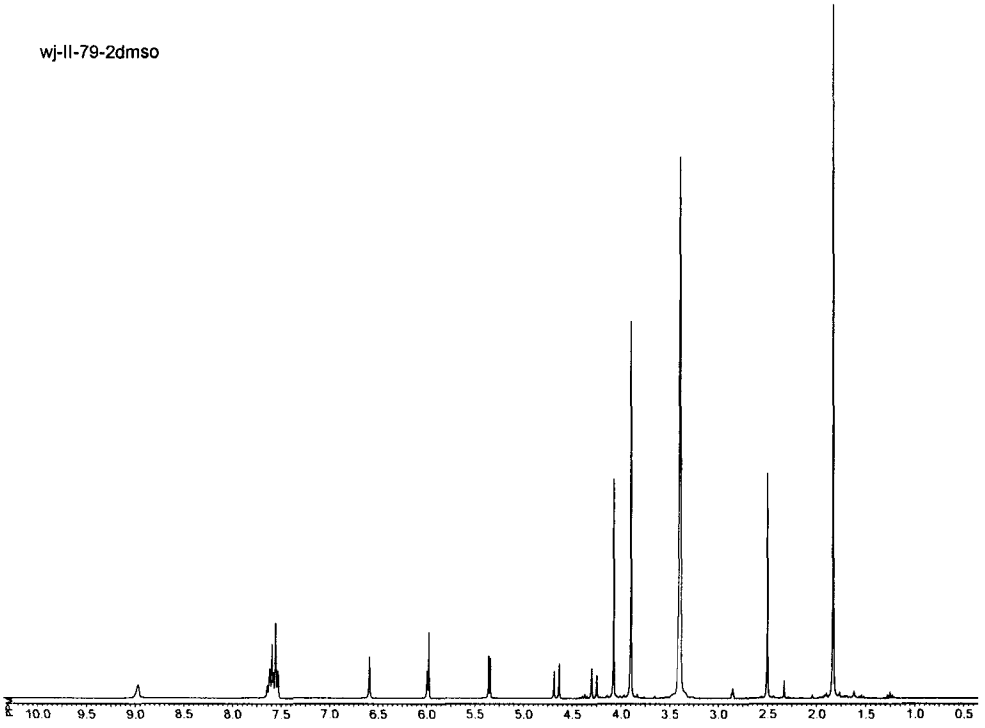
HRMS (FAB+) calcd for $C_{27}H_{33}N_2O_8$ 513.2237, found 513.2236;

$[\alpha]_D^{25}$ = +73.0 (*c* 0.68, CH_2Cl_2).



322

wj-II-79-2dmso



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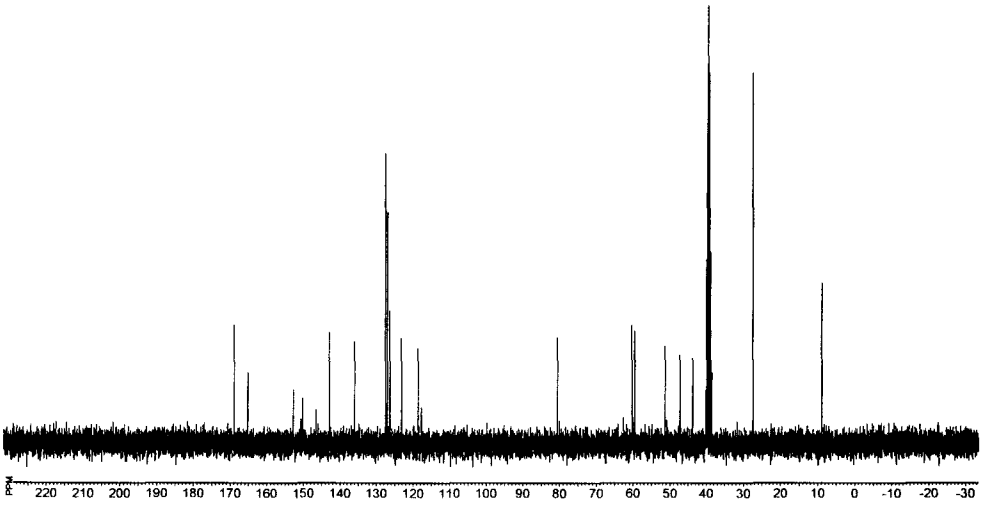
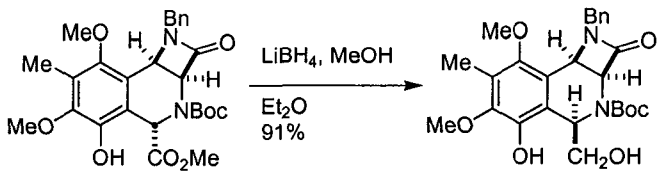




322 323

Diol 323 (1-Benzyl-5-hydroxy-4-hydroxymethyl-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester):

To a solution of phenol **322** (226 mg, 0.441 mmol, 1.0equiv), MeOH (107.2 μL, 2.64 mmol, 6 equiv) in ether (40 mL) was added $LiBH_4$ (60.7 mg, 2.64 mmol, 6 equiv) at room temp. The resulting mixture was heated to reflux for 60 min. The reaction was quenched with 10% aq. NH_4Cl solution (20 mL) and the mixture was extracted with EtOAc (20 mL x2). The organic layers were combined, dried and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluted with 50% EtOAc in hexanes) to afford alcohol **323** (188 mg, 91%).

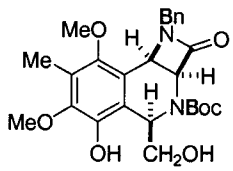
^{1}H NMR (400 MHz, DMSO-d_6, 373K) δ 8.55 (1H, s), 7.17-7.29 (5H, m), 5.64-5.65 (1H, d, J=6.0Hz), 5.60-5.62 (1H, d, J=5.6Hz), 4.98-4.99 (1H, d, J=5.2Hz), 4.54 (1H, broad), 4.36-4.39 (1H, d, J=15.2Hz), 4.02-4.06 (1H, d, J=14.0Hz), 3.72 (3H, s), 3.58 (3H, s), 3.53-3.54 (2H, d, J=5.6Hz), 2.15 (3H, s), 1.47 (9H, s);

^{13}C NMR (100 MHz, DMSO-d_6, 373K) δ 167.85, 153.47, 150.50, 146.85, 142.39, 136.26, 127.64, 127.20, 126.49, 123.40, 122.40, 120.70, 119.06, 79.78, 62.79, 60.20, 59.47, 58.91, 51.84, 47.56, 43.90, 27.58, 8.90;

IR (neat, film): 3371, 2977, 2938, 1742, 1693, 1466, 1456, 1416, 1368, 1344, 1320, 1258, 1166, 1114, 1070, 991 cm^{-1};

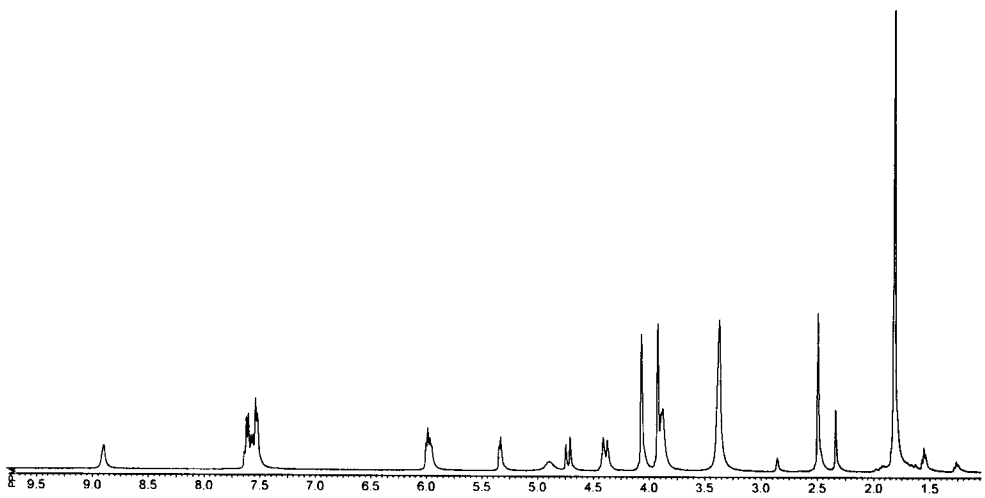
HRMS (FAB+) calcd for $C_{26}H_{33}N_2O_7$ 485.2288, found 485.2280;

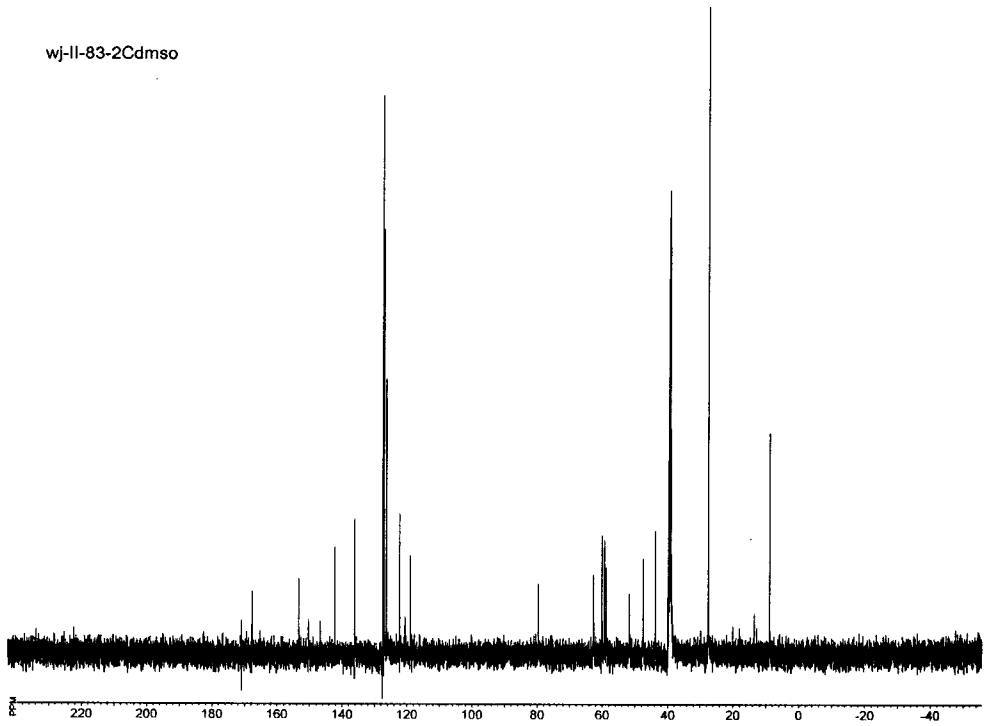
$[\alpha]_D$ +68.0 (c = 0.49, CH_2Cl_2).

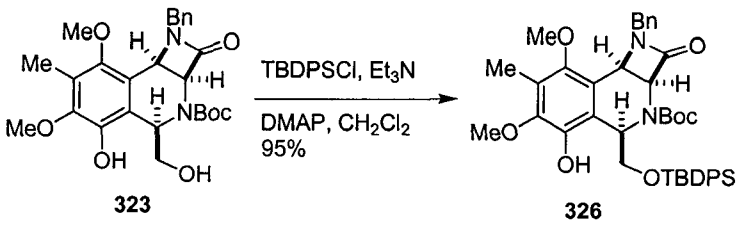


323

wj-II-83-2dmso







323 326

Phenol 326 (1-Benzyl-4-(tert-butyl-diphenyl-silanyloxymethyl)-5-hydroxy-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester):

To a solution of diol **323** (176 mg, 0.388 mmol, 1.0 equiv), Et_3N (59.5 μL, 0.427 mmol, 1.1 equiv) and DMAP (2 mg, 0.0155 mmol. 0.04 equiv) in methylene chloride (10 mL) was added TBDPS-Cl (111 μL, 0.427 mmol, 1.1 equiv). The resulting solution was stirred at room temp. for 18 h. The reaction was diluted with EtOAc (50 mL) and washed with water (20 mL x2) and sat. NaCl solution (20 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluted with 25% EtOAc in hexanes) to give phenol **326** (233 mg, 95%).

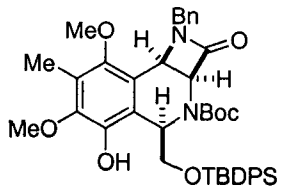
^{1}H NMR (400MHz, DMSO-d_6, 373K) 8.47 (1H, s), 7.70-7.74 (4H, m), 7.35-7.43 (6H, m), 7.11 (5H, s), 5.95-5.99 (1H, dd, *J*=4Hz, 6.8Hz), 5.74-5.75 (1H, d, *J*=4.8Hz), 4.97-4.99 (1H, d, *J*=5.6Hz), 4.22-4.26 (1H, d, *J*=15.6Hz), 4.05-4.08 (1H, d, *J*=14.0Hz), 3.60-3.75 (2H, m), 3.63 (3H, s), 3.58 (3H, s), 2.12 (3H, s), 1.44 (9H, s), 1.04 (9H, s);

^{13}C NMR (100MHz, DMSO-d_6, 373K) δ 167.34, 153.45, 150.30, 146.42, 142.19, 136.22, 134.76, 134.67, 133.29, 133.21, 128.93, 127.46, 127.28, 127.06, 126.99, 126.38, 122.50, 119.65, 119.50, 79.54, 63.97, 60.20, 59.53, 59.25, 51.59, 47.45, 44.03, 27.60, 26.27, 20.08, 18.40, 13.50, 8.90;

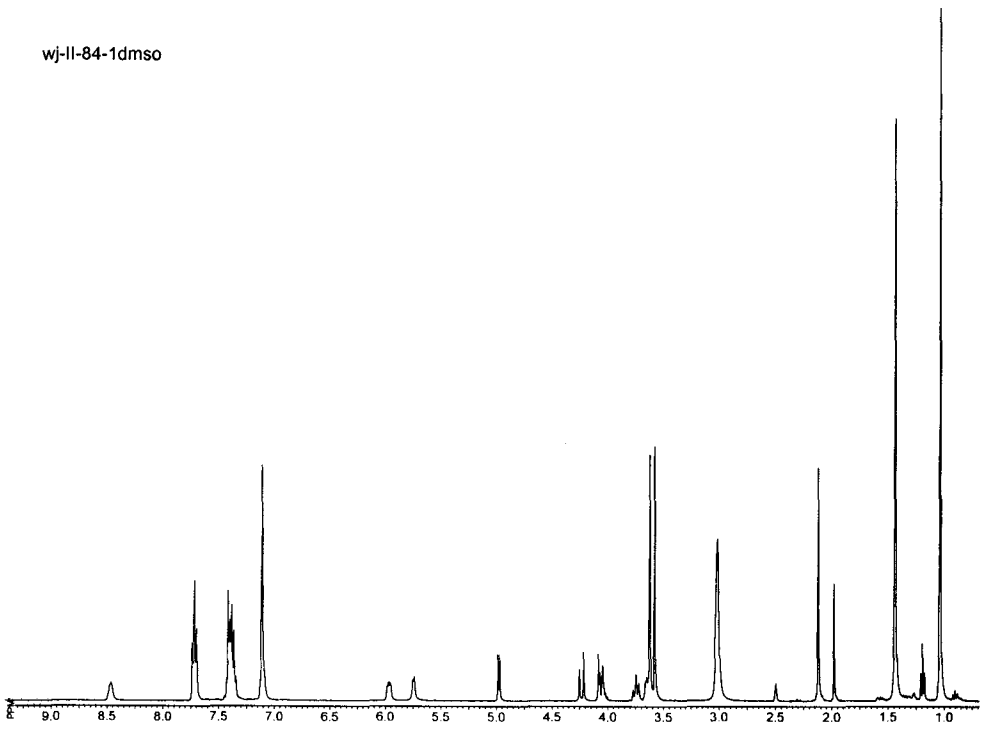
IR (neat, film): 3338, 2933, 2857, 1755, 1704, 1468, 1416, 1367, 1344, 1256, 1167, 1113, 1009 cm^{-1};

HRMS calcd for $C_{42}H_{51}N_2O_7Si$ 723.3466, found 723.3470;

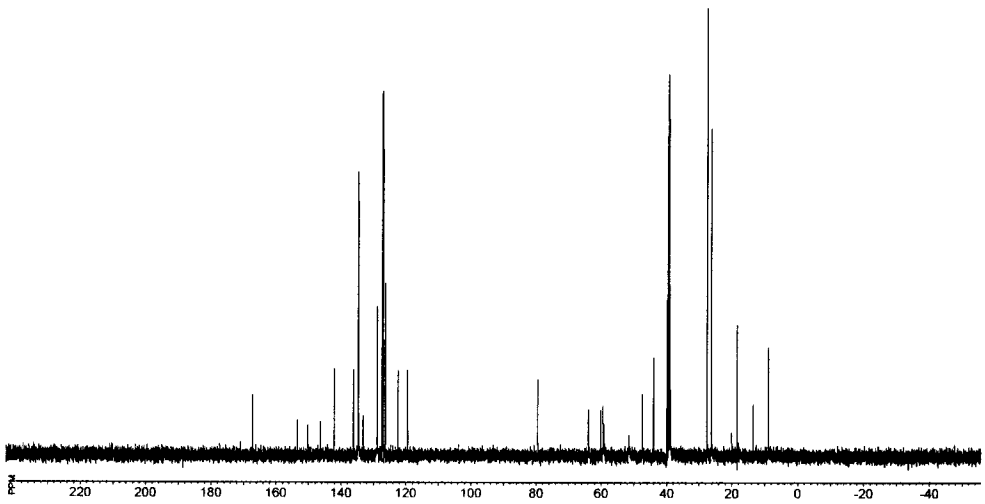
$[\alpha]_D^{25}$ =+49.8 (c = 0.92, CH_2Cl_2).

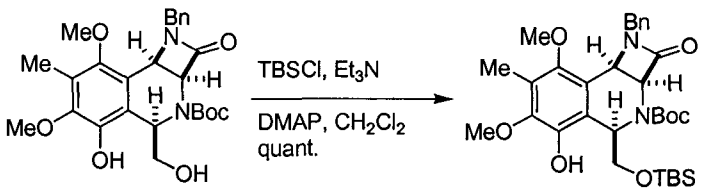


326



wj-II-84-1Cdmso





323 **327**

Phenol 327 (1-Benzyl-4-(tert-butyl-dimethyl-silanyloxymethyl)-5-hydroxy-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester)

To a solution of alcohol **323** (174 mg, 0.359 mmol, 1.0 equiv), Et_3N (76 μL, 0.539 mmol, 1.5 equiv) and DMAP (1.7 mg, 0.0144 mmol, 0.04 equiv) in methylene chloride (15 mL) was added TBS-Cl (81 mg, 0.539mmol, 1.5equiv). The resulting solution was stirred at room temp. for 24 h. The reaction was diluted with EtOAc (50 mL) and washed with water (20 mL x2) and sat. NaCl solution (20 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give Phenol **327** (237 mg, quant.).

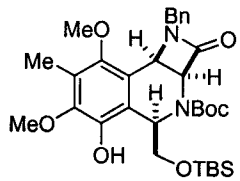
^{1}H NMR (400MHz, DMSO-d_6, 373K) 8.43 (1H, s), 7.14-7.24 (5H, m), 5.65-5.69 (1H, dd, J=4.0, 9.2Hz), 4.94-4.96 (1H, d, J=6.0Hz), 4.32-4.36 (1H, d, J=15.2Hz), 4.08-4.12 (1H, d, J=15.6Hz), 3.70 (3H, s), 3.57-3.63 (2H, m), 3.58 (3H, s), 2.15 (3H, s), 1.46 (9H, s), 0.88 (9H, s), 0.04 (3H, s), 0.03 (3H, s);

^{13}C NMR (100MHz, DMSO-d_6, 373K) δ 167.33, 153.28, 150.29, 146.49, 142.16, 136.31, 127.51, 127.24, 126.42, 122.38, 119.75, 119.57, 79.38, 63.50, 60.11, 59.54, 59.00, 51.89, 47.27, 44.01, 27.54, 25.32, 17.47, 8.88, -6.02;

IR (neat, film): 3338, 2928, 2855, 1759, 1697, 1464, 1416, 1367, 1318, 1256, 1168, 1115, 1073, 1020, 837, 781 cm^{-1};

HRMS calcd for $C_{32}H_{47}N_2O_7Si$ 599.3153, found 599.3153;

$[\alpha]_D^{25}$ =+64.66 (c = 0.52, CH_2Cl_2).



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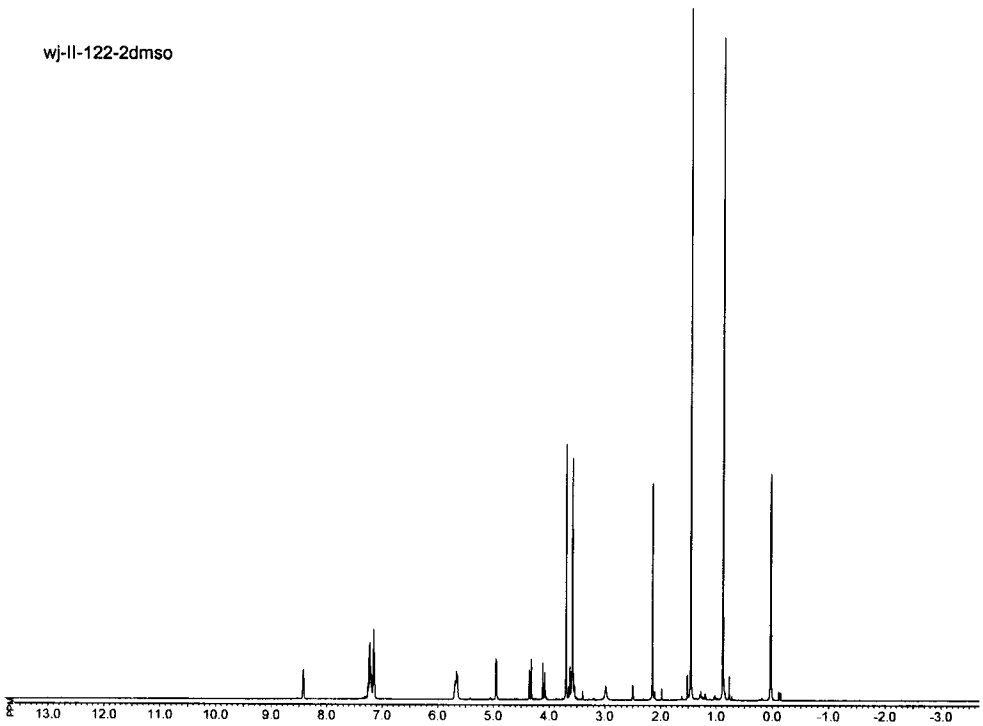
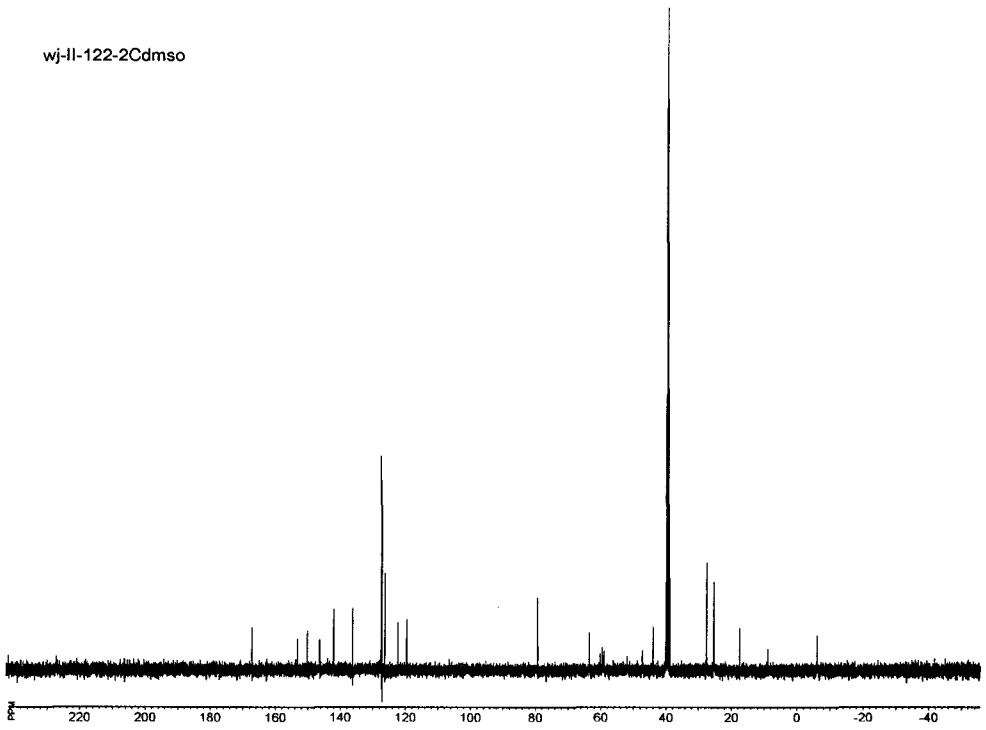
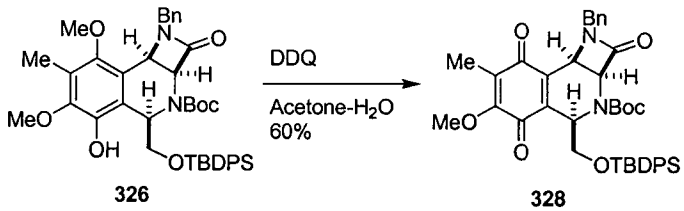






326 328

***Para*-Quinone 328 (1-Benzyl-4-(tert-butyl-diphenyl-silanyloxymethyl)-6-methoxy-7-methyl-2,5,8-trioxo-2,2a,4,5,8,8b-hexahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester):**

To solution of phenol **326** (214 mg, 0.296 mmol, 1.0 equiv) in acetone-H_2O (9:1) (15 mL) was added DDQ (134 mg, 0.592 mmol, 2.0 equiv), the resulting solution was stirred at room temp. for 1.5 h. The reaction was quenched with sat. NaCl solution (10 mL) and extracted with EtOAc (20 mL x3). The organic layers were combined, dried and concentrated under reduced pressure. The residue was purified with flash column chromatography (eluted with 30% EtOAc in hexanes, then 50% EtOAc in hexanes) to afford quinone **328** (142 mg, 60%) along with dihydroquinone (100 mg, 40%).

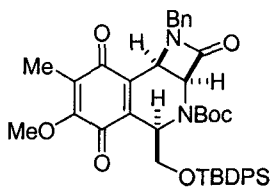
^{1}H NMR (400MHz, DMSO-d_6, 398K) 7.69-7.70 (4H, m), 7.38-7.47 (6H, m), 7.08-7.18 (5H, m), 5.71-5.72 (1H, d, J=5.6Hz), 5.64-5.67 (1H, dd, J=5.2Hz, 9.6Hz), 4.80-4.82 (1H, d, J=5.6Hz), 4.27-4.31 (1H, d, J=15.6Hz), 4.13-4.16 (1H, d, J=15.2Hz), 3.83-3.85 (1H, d, J=9.6Hz), 3.81 (3H, s), 3.59-3.63 (1H, dd, J=5.2Hz, 10.4Hz), 1.81 (3H, s), 1.43 (9H, s), 1.04 (9H, s);

^{13}C NMR (100Hz, DMSO-d_6, 378K) δ 185.74, 179.93, 166.93, 154.89, 152.77, 138.46, 136.98, 135.80, 134.65, 134.54, 132.61, 129.17, 128.99, 127.68, 127.29, 127.09, 127.00, 126.71, 80.36, 64.17, 59.87, 59.23, 50.57, 45.99, 44.54, 27.44, 26.16, 18.31, 13.47, 7.71;

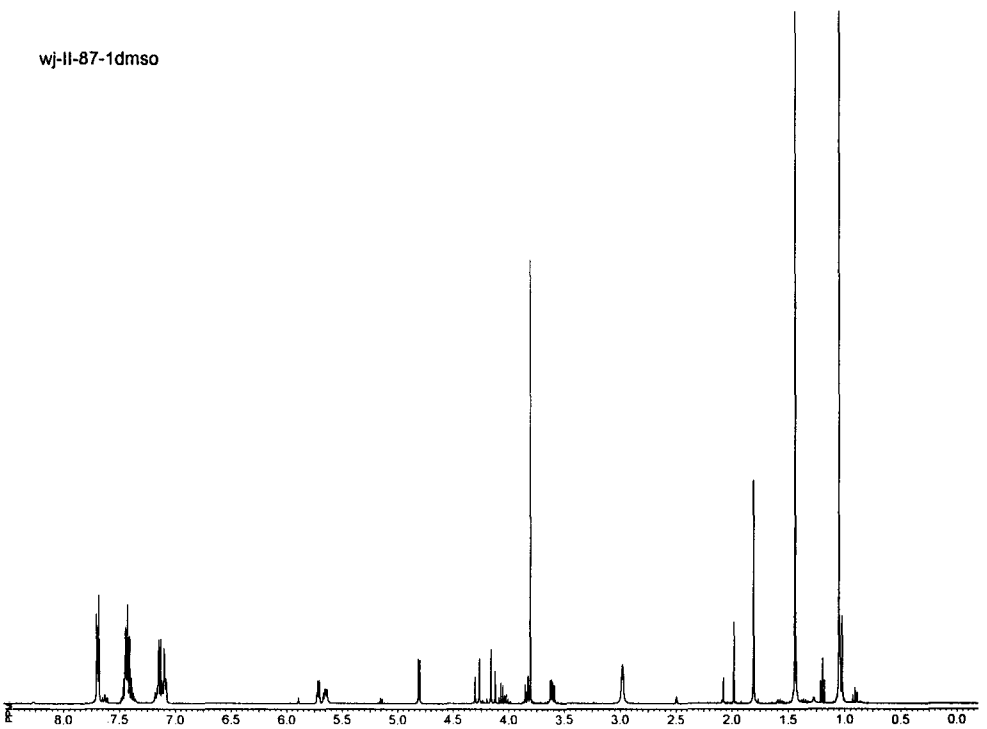
IR (neat, film): 2931, 2857, 1764, 1704, 1658, 1455, 1427, 1340, 1314, 1207, 1164, 1113 cm^{-1};

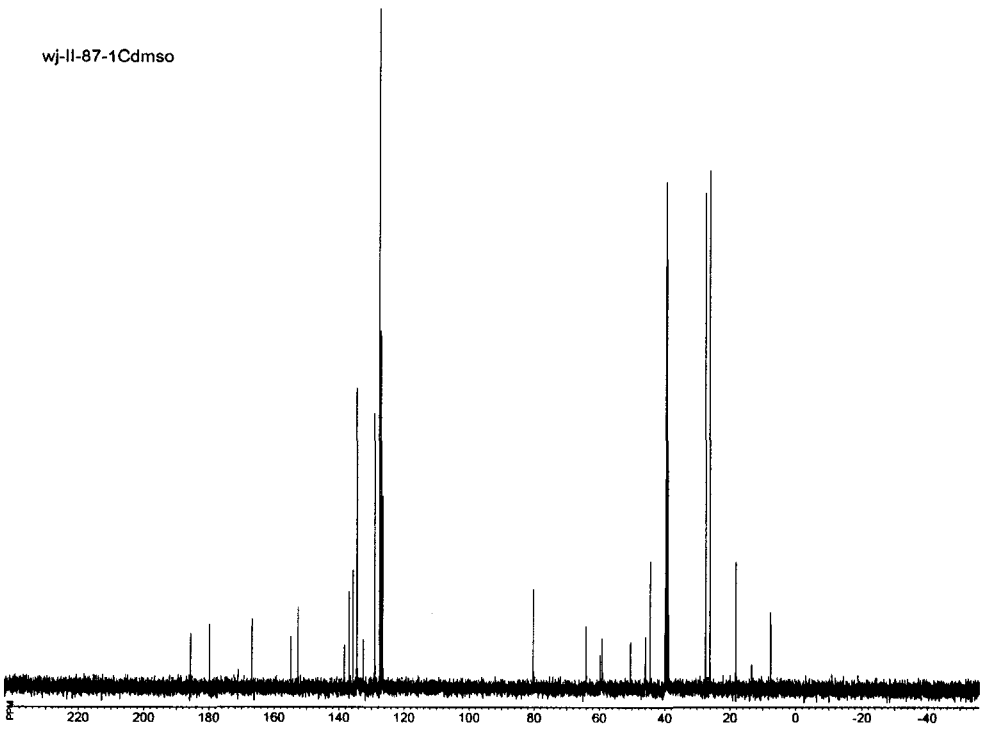
HRMS calcd for $C_{41}H_{47}N_2O_7Si$ 707.3153, found 707.3144;

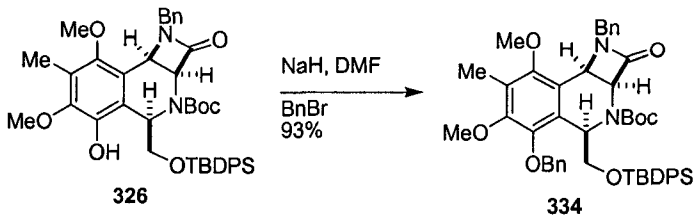
$[\alpha]_D^{25}$ =+70.8 (c = 0.72, CH_2Cl_2).



328







326 334

***Cis*-Tetrahydroisoquinoline 334 (1-Benzyl-5-benzyloxy-4-(tert-butyl-diphenyl-silanyloxymethyl)-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester):**

To a solution of phenol **326** (76 mg, 0.105 mmol, 1.0 equiv) in DMF (2 mL) was added NaH (6.6 mg, 0.137 mmol, 1.3 equiv) at 0°C, the resulting mixture was stirred at 0°C for 30 min.. Benzyl bromide (16.2 μL, 0.137 mmol, 1.3 equiv) was added, and the mixture was stirred at 0°C for 3 h. The reaction was quenched with water (2 mL) and diluted with EtOAc (20 mL). The organic layer was washed with water (10 mL), and sat. NaCl solution (10 mL). After removal of the solvent under reduced pressure, the residue was purified flash column chromatography (eluted with 10 % EtOAc in hexanes; then 30% EtOAc in hexanes) to afford **334** (69 mg, 93%).

^{1}H NMR (400MHZ, DMSO-d_6, 373K) 7.58-7.64 (4H, m), 7.45-7.47 (2H, m), 7.28-7.39 (7H, m), 7.20-7.24 (2H, m), 7.09-7.13 (5H, m), 5.97-6.00(1H, dd, J=5.2Hz, 8.4Hz), 5.75-5.76 (1H, d, J=5.6Hz), 4.99-5.02(2H, m), 4.82-4.84 (1H, d, J=6.8Hz), 4.20-4.24 (1H, d, J=15.6Hz), 4.04-4.09 (2H, m), 3.75-3.80 (1H, t, J=10.0Hz), 3.66 (3H, s), 3.61 (3H. s), 3.54-3.58 (1H, dd, J=4.8Hz, 10.4Hz), 2.14 (3H, s), 1.44 (9H, s), 0.99 (9H, s);

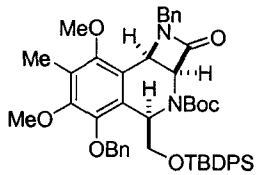
^{13}C NMR (100MHz, DMSO-d_6, 373K) δ 167.11, 153.88, 153.35, 151.41, 143.88, 136.60, 136.02, 134.74, 134.60, 132.96, 132.89, 128.84, 128.79, 127.71, 127.42, 127.28, 127.22,

126.86, 126.37, 125.53, 123.78, 119.88, 79.78, 74.19, 64.88, 60.11, 59.45, 59.14, 51.91, 47.25, 44.01, 27.54, 26.22, 18.31, 13.47, 8.90;

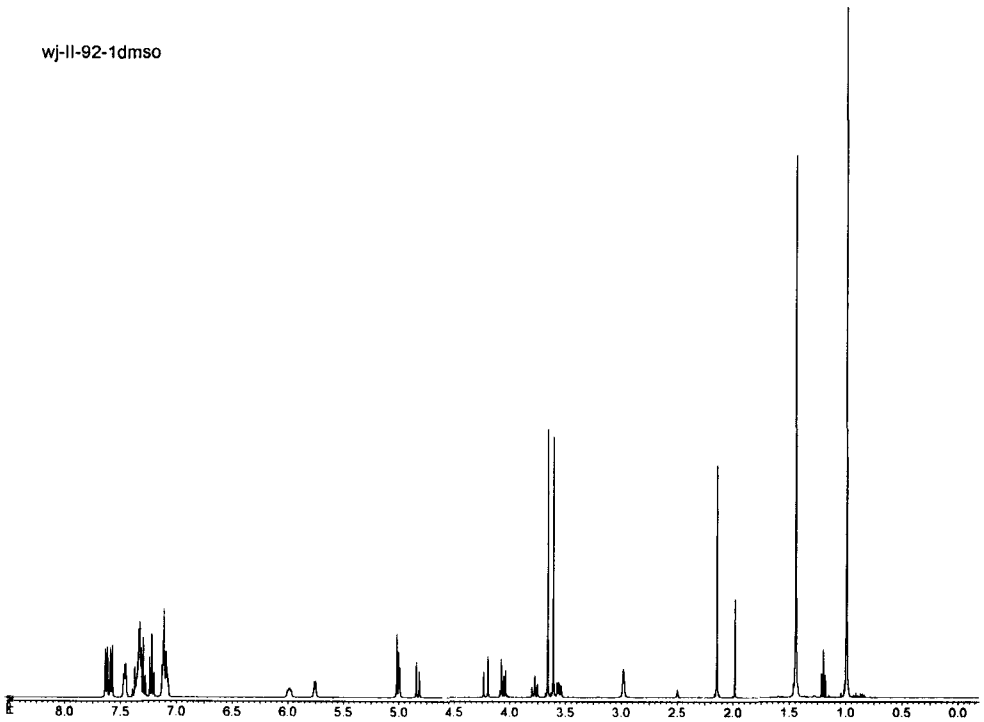
IR (neat, film): 2932, 2857, 1759, 1693, 1588, 1455, 1415, 1352, 1331, 1258, 1167, 1113 cm^{-1};

HRMS calcd for $C_{49}H_{57}N_2O_7Si$ 813.3935, found 813.3956;

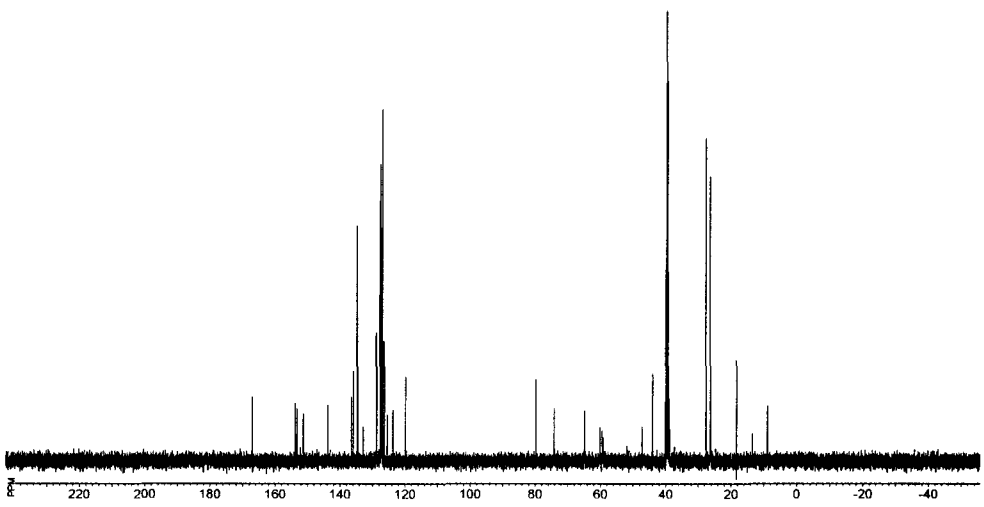
$[\alpha]_D^{25}$ =+28.3 (*c* = 0.57, CH_2Cl_2).

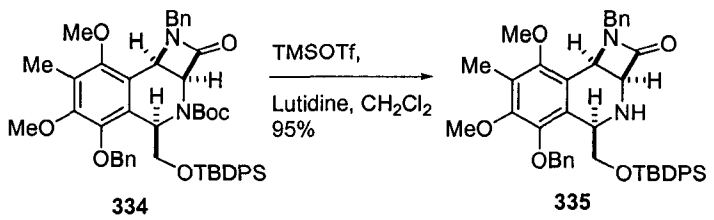


334



wj-II-92-1Cdmso





334 335

***Cis*-Tetrahydroisoquinoline 335 (1-Benzyl-5-benzyloxy-4-(tert-butyl-diphenyl-silanyloxymethyl)-6,8-dimethoxy-7-methyl-2a,3,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalen-2-one):**

To a solution of compound **334** (67 mg, 0.0787 mmol, 1.0 equiv) and 2,6-lutidine (57.6 μL, 0.494 mmol, 6.0 equiv) in methylene chloride (2.5 mL) was added TMSOTf (71 μL, 0.394 mmol, 5.0 equiv) dropwise. The resulting solution was stirred at room temp. for 30 min. The reaction was quenched with sat. aq. NH_4Cl solution (5 mL) and extracted with EtOAc (10 mL x2). The organic layers were combined, dried and concentrated under reduced pressure, and the residue was purified flash column chromatography (eluted with 50% EtOAc in hexanes) to afford compound **335** (55 mg, 95%).

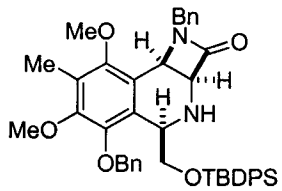
^1H NMR (300MHz, $CDCl_3$, 298K) 7.75-7.78 (2H, m), 7.61-7.64 (2H, m), 7.30-7.37 (9H, m), 7.13-7.26 (7H, m), 4.96-5.00(1H, d, *J*=11.1Hz), 4.79-4.81 (1H, d, *J*=5.4Hz), 4.74-4.78(1H, d, *J*=8.1Hz), 4.56-4.57 (1H, d, *J*=5.1Hz), 4.50-4.54 (1H, t, *J*=5.9Hz), 4.37-4.42 (1H, d, *J*=15Hz), 3.92-3.96 (1H, d, *J*=14.7Hz), 3.68 (3H, s), 3.59 (3H. s), 3.56-3.58 (1H, d, *J*=3.3Hz), 2.19 (3H, s), 1.09 (9H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 171.16, 154.27, 151.74, 145.48, 137.42, 136.81, 135.97, 135.51, 133.76, 133.31, 129.50, 129.31, 128.66, 128.50, 128.20, 128.14, 127.97, 127.53, 127.16, 124.33, 120.96, 75.17, 65.54, 62.30, 60.74, 60.37, 54.39, 47.22, 44.72, 27.09, 19.59, 9.83;

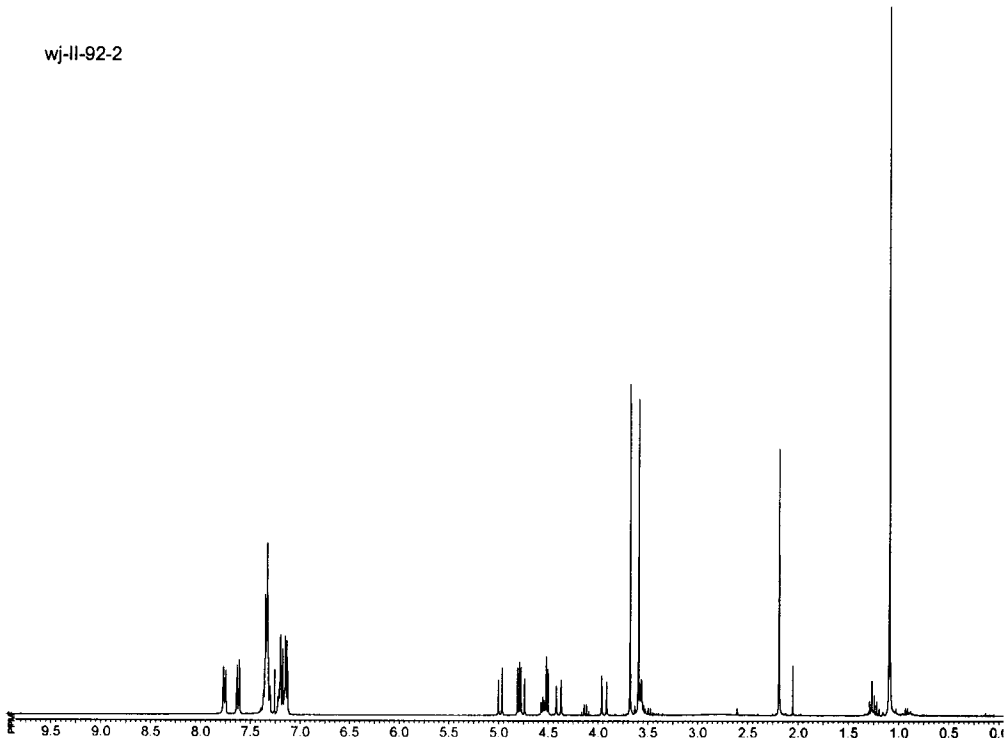
IR (neat, film): 3348, 3031, 2932, 2856, 1748, 1588, 1462, 1428, 1341, 1258, 1113 cm^{-1};

HRMS calcd for $C_{44}H_{49}N_2O_5Si$ 713.3411, found 713.3411;

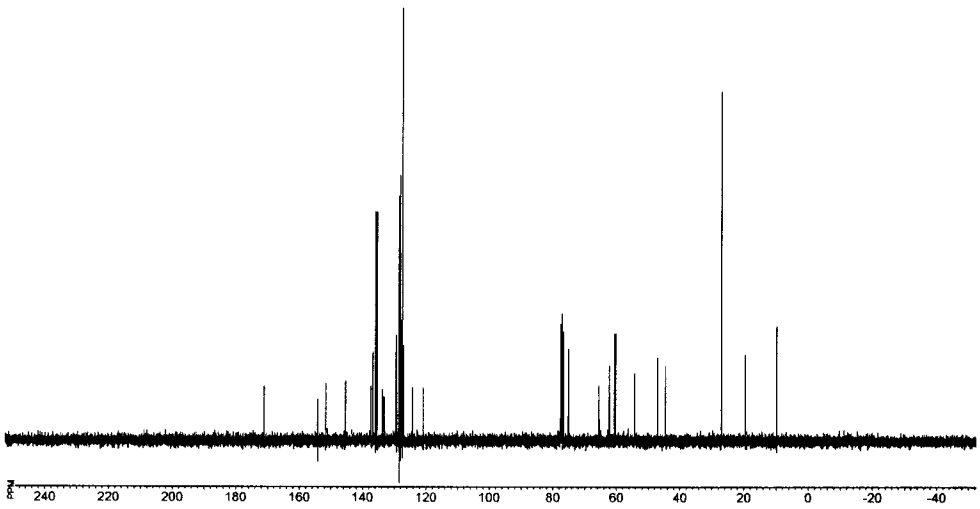
$[\alpha]_D^{25}$ =+50.2 (c = 0.52, CH_2Cl_2).

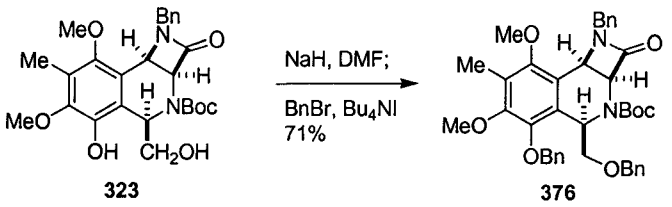


335



wj-II-92-2C





323 376

***Bis-O*-benzylation of *Cis*-Tetrahydroisoquinoline (376) (1-Benzyl-5-benzyloxy-4-benzyloxymethyl-6,8-dimethoxy-7-methyl-2-oxo-2,2a,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalene-3-carboxylic acid tert-butyl ester):**

To a solution of diol **323** (214 mg, 0.442 mmol, 1.0 equiv) in DMF (10 mL) was added NaH (55.1 mg, 1.148 mmol, 2.6 equiv) at 0°C, the resulting mixture was stirred at 0°C for 15 min. Next, benzyl bromide (137 μL, 1.148 mmol, 2.6 equiv) and nBu$_4$NI (16.3 mg, 0.0441 mmol, 0.1 equiv) were added. The solution was warmed to room temp., and stirred for 3 h. The reaction was diluted with EtOAc (50 mL), washed with water (20 mL x2) and sat. NaCl solution (20 mL). The organic layer was dried and concentrated under reduced pressure, and the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give **376** (204 mg, 71%).

^{1}H NMR (400 MHz, DMSO-d_6, 373K) δ 7.15-7.51 (15H, m), 5.87-5.90 (1H, dd, J=4.0, 9.2Hz), 5.69-5.70 (1H, d, J=5.6Hz), 5.07-5.09 (1H, d, J=11.2Hz), 5.02-5.04 (1H, d, J=5.6Hz), 4.92-4.95 (1H, d, J=11.2Hz), 4.31-4.42 (3H, m), 4.09-4.13 (1H, d, J=15.2Hz), 3.78 (3H, s), 3.63 (3H, s), 3.54-3.59 (1H, m), 3.37-3.41 (1H, dd, J=4.4, 10.8Hz), 2.18 (3H, s), 1.38 (9H, s);

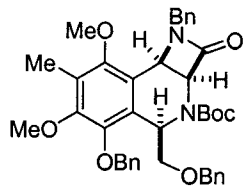
^{13}C NMR (100 MHz, DMSO-d_6, 373K) δ 167.44, 161.93, 161.83, 153.33, 153.23, 153.19, 151.44, 154.41, 143.72, 143.69, 138.11, 136.80, 136.77, 136.13, 136.11, 127.77, 127.73, 127.55, 127.51, 127.35, 127.29, 127.20, 127.06, 126.62, 126.60, 126.48, 126.42, 125.29,

123.76, 119.97, 79.71, 73.79, 71.42, 70.85, 60.16, 60.09, 59.56. 59.49, 58.91, 49.89, 47.34, 47.25, 44.03, 27.40, 18.91, 8.84;

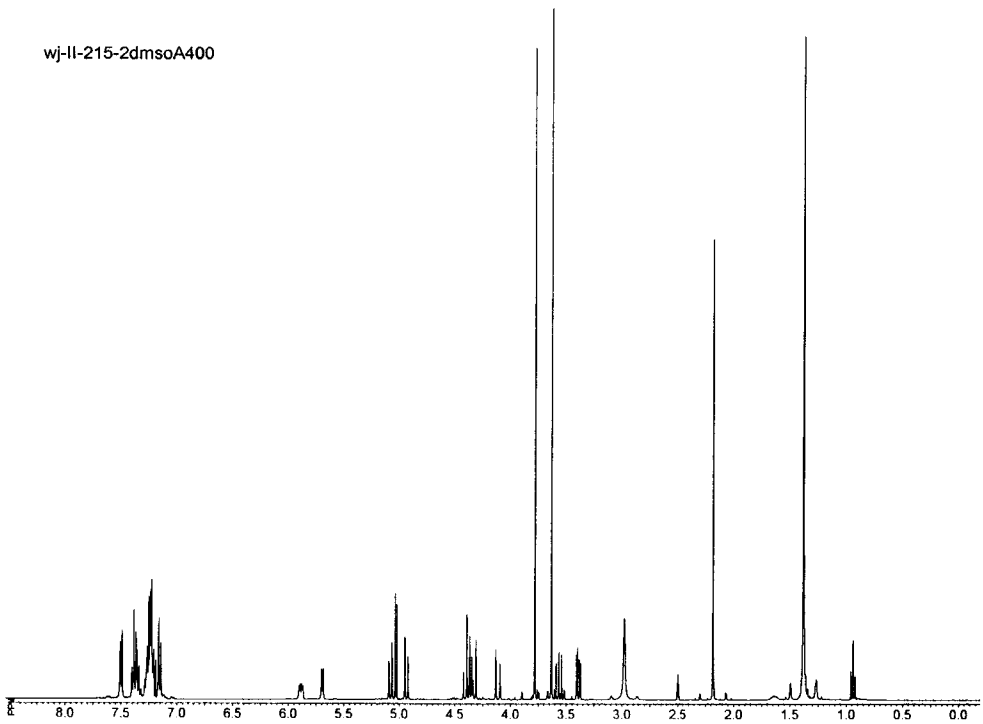
IR (neat, film): 3030, 2934, 2868, 1759, 1696, 1455, 1414, 1350, 1328, 1167, 1114, 1075, 1024, 698. cm^{-1};

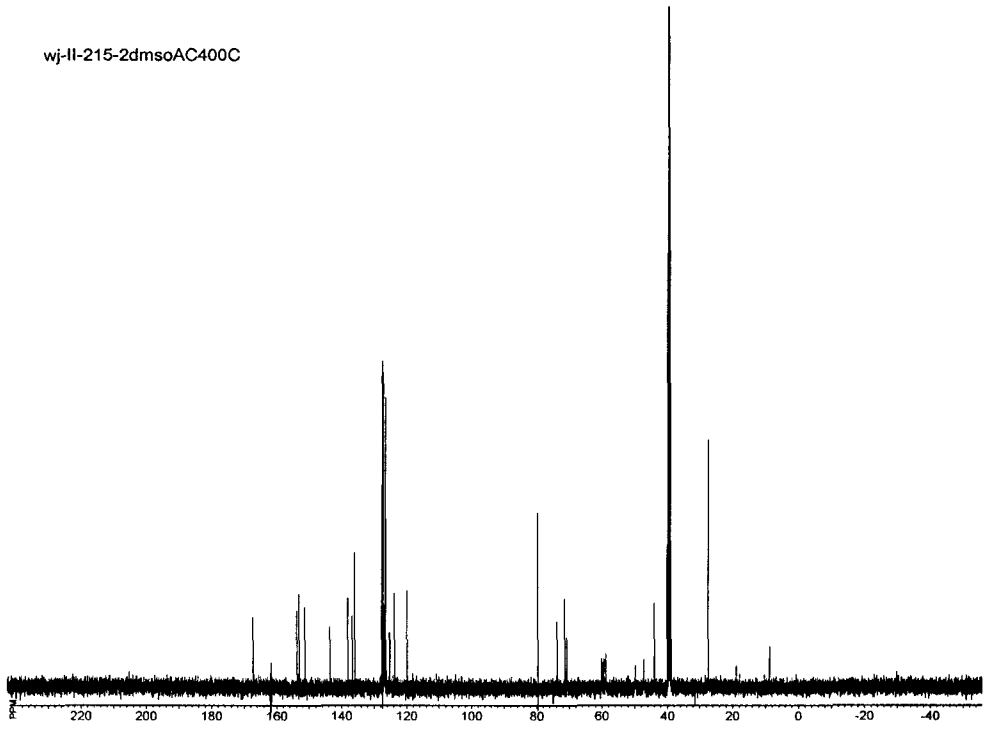
HRMS (FAB+) calcd for $C_{40}H_{45}N_2O_7$ 665.3227, found 665.3244;

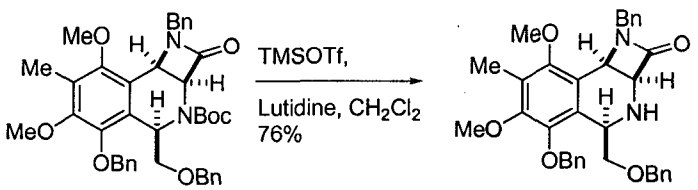
$[\alpha]_D^{25}$ = +51.9 (*c* 0.64, CH_2Cl_2).



376







376 377

***Cis*-Tetrahydroisoquinoline 377 (1-Benzyl-5-benzyloxy-4-benzyloxymethyl-6,8-dimethoxy-7-methyl-2a,3,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalen-2-one):**

To a solution of compound **376** (81 mg, 0.122 mmol, 1.0 equiv) and 2,6-lutidine (85 μL, 0.731 mmol, 6.0 equiv) in methylene chloride (4 mL) was added TMSOTf (110 μL, 0.609 mmol, 5.0 equiv) dropwise. The resulting solution was stirred at room temp. for 30 min. The reaction was quenched with saturated NH_4Cl solution (10 mL) and extracted with EtOAc (20 mL x2). The organic layers were combined, dried and concentrated under reduced pressure, and the residue was purified by flash column chromatography (eluted with EtOAc) to afford compound **377** (52.3 mg, 76%).

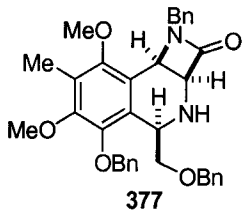
^{1}H NMR (300 MHz, $CDCl_3$, 273K) 7.20-7.42 (15H, m), 5.03-5.07(1H, d, *J*=11.1Hz), 4.87-4.91 (1H, d, *J*=11.4Hz), 4.83-4.85(1H, d, *J*=8.1Hz), 4.66-4.70 (1H, d, *J*=11.7Hz), 4.59-4.66 (1H, dd, *J*=3.9, 10.5Hz), 4.50-4.55 (1H, t, *J*=6.6Hz), 4.41-4.45 (1H, d, *J*=11.4Hz), 4.01-4.06 (1H, d, *J*=14.7Hz), 3.80 (3H, s), 3.63 (3H. s), 3.42-3.49 (2H, m) 3.0-3.1 (1H, broad), 2.24 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 273K) δ 190.08, 171.20, 154.60, 152.16, 145.48, 138.54, 137.60, 136.64, 128.80, 128.65, 128.38, 128.15, 128.10, 127.89, 127.62, 127.45, 126.81, 124.72, 121.05, 117.49, 100.12, 74.75, 73.26, 71.14, 61.94, 60.85, 60.35, 52.15, 47.18, 44.89, 9.73;

IR (neat, film): 3030, 2937, 2866, 1747, 1453, 1411, 1340, 1259, 1119, 1077, 699 cm^{-1};

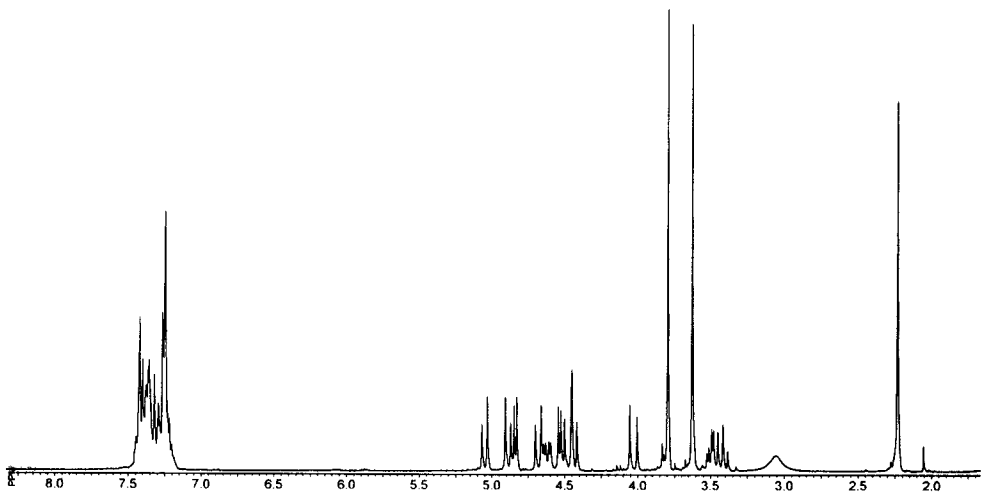
HRMS (FAB+) calcd for $C_{35}H_{37}N_2O_5$ 565.2702, found 565.2722;

$[\alpha]_D^{25} = +72.1$ (*c* 0.97, CH_2Cl_2).

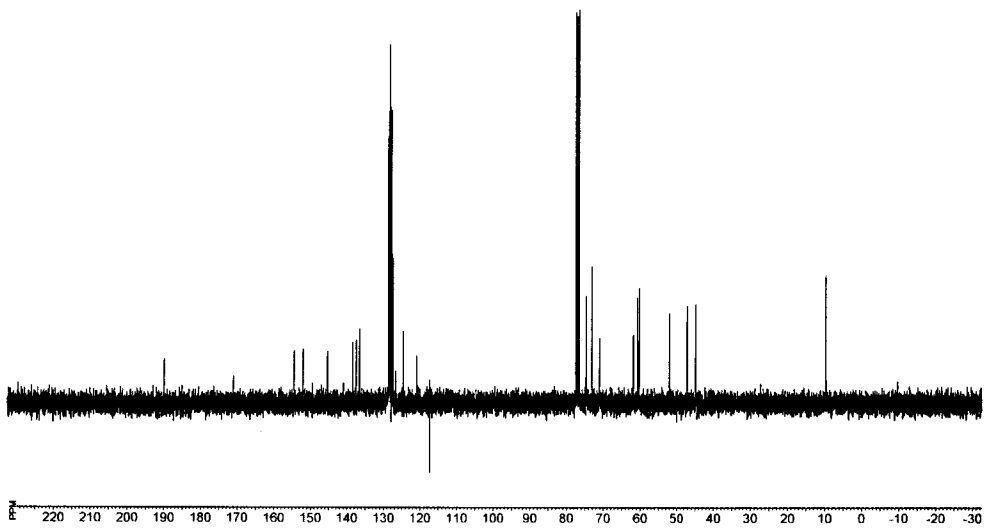


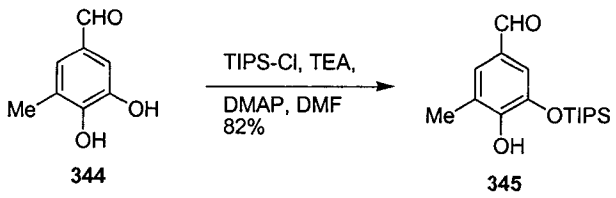
377

wj-II-216-1



wj-II-216-1C





3-(O-tripropylsilyl)-4-hydroxy-5-methylbenzylaldehyde:

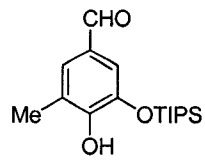
To a solution of **344** (16.86 g, 110.9 mmol, 1.0 equiv), TEA (23.20 mL, 166.38 mmol, 1.5 equiv) in DMF (150 mL) was added TIPS-Cl (26.11 mL, 122.01 mmol, 1.1 equiv) dropwise at room temp. The resulting solution was stirred at room temp. for 24 h. Then the reaction mixture was diluted with EtOAc (250 mL), and washed with water (100 mL x2), saturated NaCl solution (150 mL) and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give **345** (28.1 g, 82%).

^{1}H NMR (300MHz, $CDCl_3$, 298K) 9.74 (1H, s), 7.28 (1H, s), 7.22-7.23 (1H, d, *J*=1.2Hz), 6.3 (1H, s), 2.30 (3H, s), 1.32-1.40 (3H, m), 1.10-1.13 (18H, m);

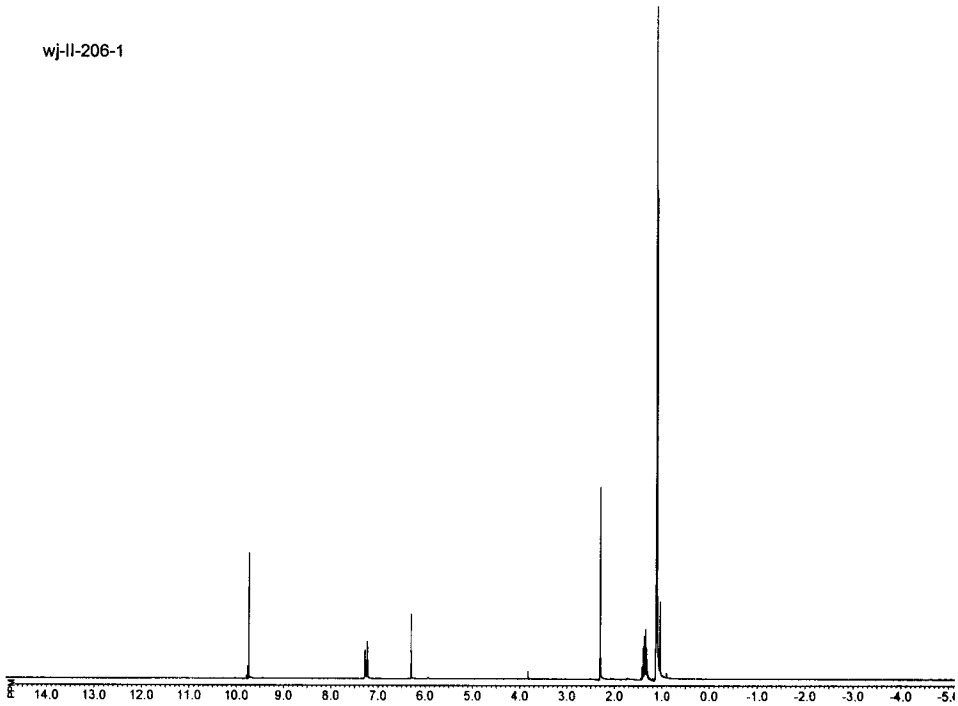
^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 190.93, 151.33, 142.89, 128.74, 128.31, 124.44, 113.82, 18.13, 17.94, 15.75, 14.18, 12.94;

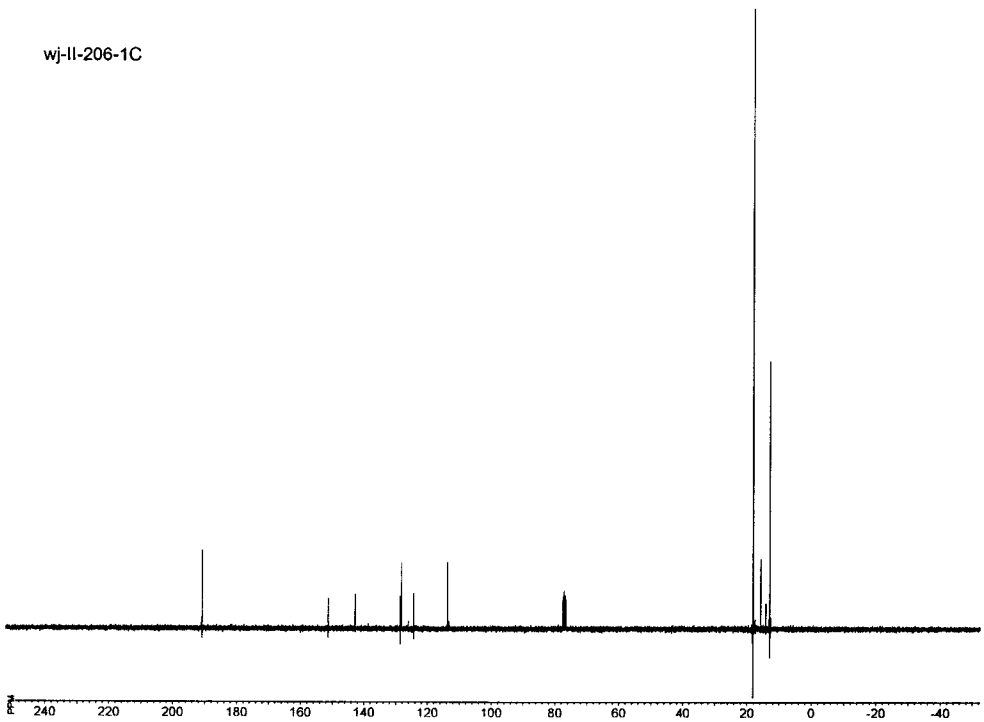
IR (neat, film): 3320, 2947, 2891, 2867, 1667, 1591, 1495, 1455, 1406, 1313, 1147, 1058, 980, 885, 680 cm^{-1};

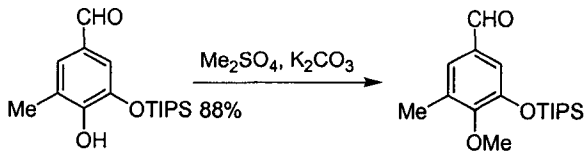
HRMS calcd for $C_{17}H_{29}O_3Si$ 309.1886, found 309.1882.



345







345 340

Aldehyde 340 (4-Methoxy-3-methyl-5-triisopropylsilanyloxy-benzaldehyde):

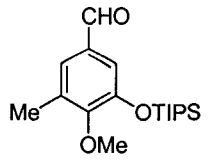
To a mixture of **345** (15.0 g, 48.62 mmol, 1.0 equiv), K_2CO_3 (33.6 g, 243.12 mmol, 5.0 equiv) in acetone (200 mL) was added Me_2SO_4 (9.2 mL, 97.25 mmol, 2.0 equiv) at room temp. The resulting mixture was heated to reflux for 12 h. After the mixture was cooled, the solid was filtered and washed with acetone (50 mL x2). The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes) to afford compound **340** (13.7 g, 88%).

^{1}H NMR (300MHz, $CDCl_3$, 298K) 9.81 (1H, s), 7.29 (1H, s), 7.22-7.23 (1H, d, *J*=1.8Hz), 3.86 (3H, s), 2.31 (3H, s), 1.28-1.34 (3H, m), 1.03-1.13 (18H, m);

^{13}C NMR ($CDCl_3$) δ 191.24, 154.96, 149.75, 133.16, 132.20, 126.47, 117.76, 60.20, 18.14, 16.38, 13.10;

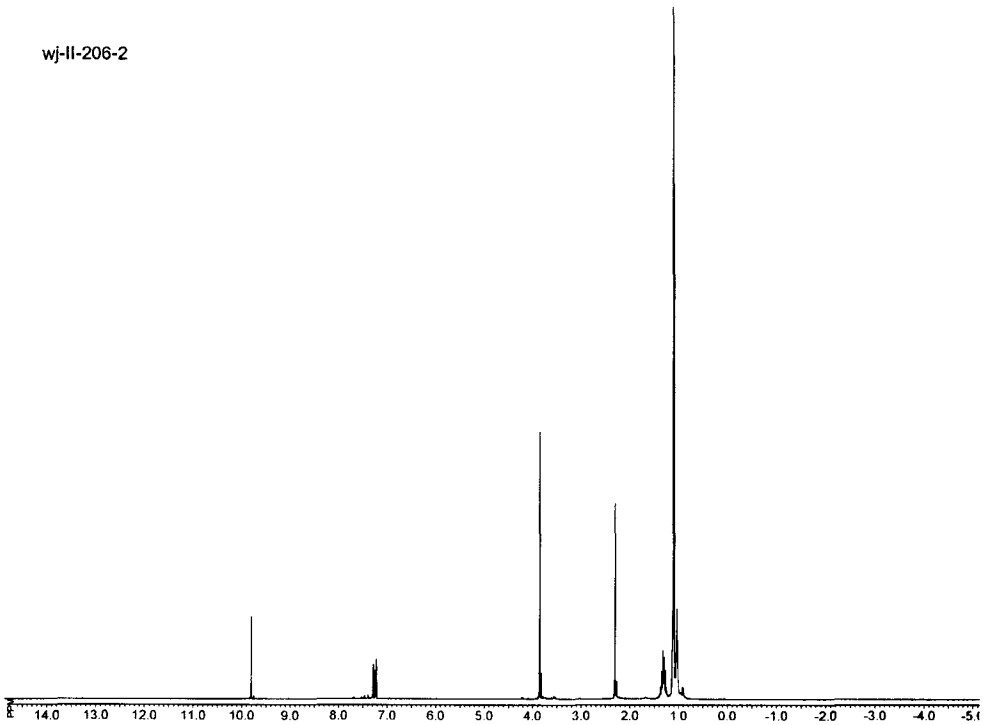
IR (neat, film): 2946, 2893, 2868, 1697, 1584, 1487, 1438, 1387, 1237, 1140, 1078, 1009, 883, 798, 685 cm^{-1};

HRMS calcd for $C_{18}H_{21}O_3Si$ 323.2042, found 3323.2045.



340

wj-II-206-2



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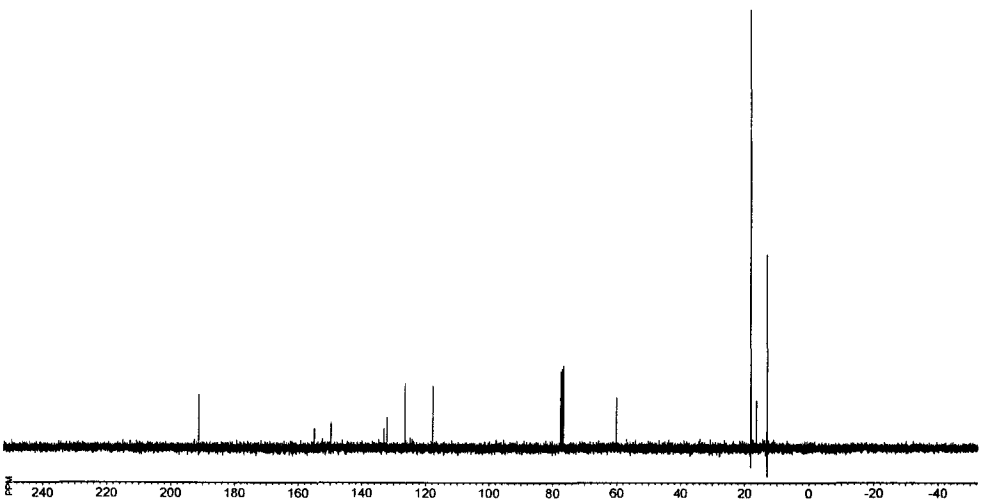
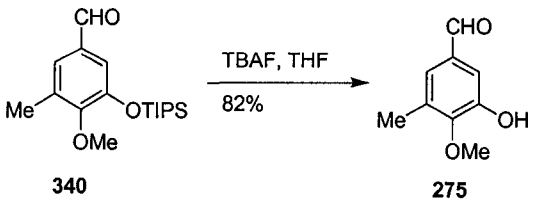




340 275

Phenol 275 (3-Hydroxy-4-methoxy-5-methyl-benzaldehyde):

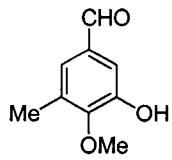
To a solution of **340** (13.3 g, 41.30 mmol, 1.0 equiv) in THF (120 mL) was added TBAF (1M solution in THF)(82 mL, 82.6 mmol, 2.0 equiv) at room temp. The resulting mixture was stirred at room temp. for 60 min.. The reaction was quenched with sat. NH_4Cl solution (50 mL) and extracted with EtOAc (100 mL x2). The organic layers were combined and washed with water (100 mL x2), saturated NaCl solution (150 mL) and dried with anhydrous Sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes, then 50% EtOAc in hexanes) to afford compound **275** (5.6 g, 82%).

^{1}H NMR (300Mhz, $CDCl_3$, 298K) 9.84 (1H, s), 7.32-7.33 (1H, d, *J*=1.8Hz), 7.27-7.27 (1H, d, *J*=1.5Hz), 6.06 (1H, s), 3.88 (3H, s), 2.37 (3H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 191.47, 150.81, 149.55, 132.92, 131.66, 125.22, 113.92, 60.83, 16.28;

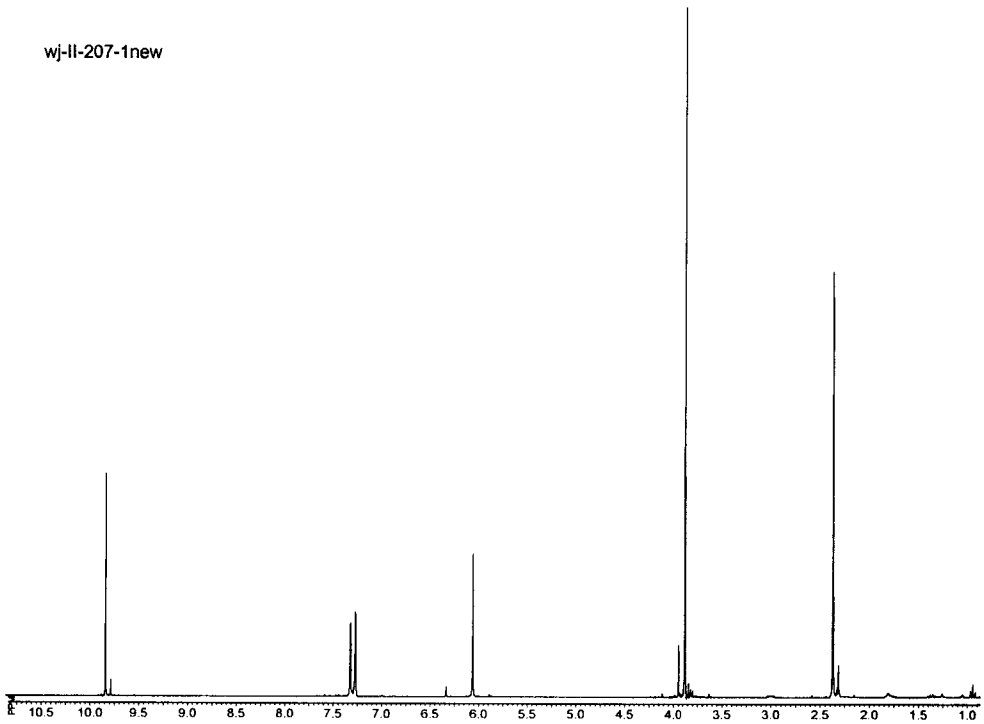
IR (neat, film): 3380, 2937, 2840, 1688, 1589, 1493, 1455, 1315, 1135, 998, 861, 688cm^{-1};

HRMS calcd for $C_9H_{11}O_3$ 167.0708, found 167.0708.

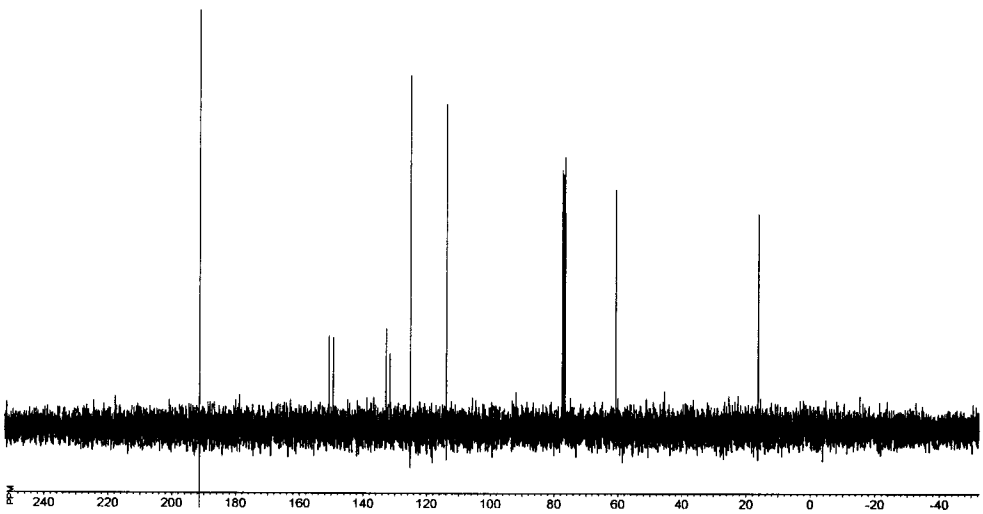


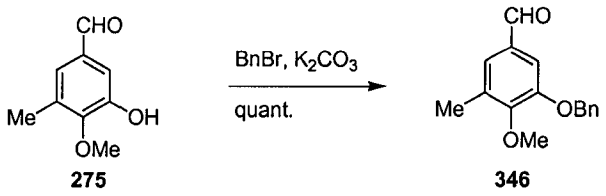
275

wj-II-207-1new



wj-II-207-1newC





Aldehyde 346 (3-Benzyloxy-4-methoxy-5-methyl-benzaldehyde):

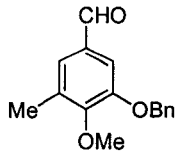
To a solution of aldehyde **275** (5.0 g, 27.78 mmol, 1.0 equiv), K_2CO_3 (11.52 g, 83.33 mmol, 3 equiv) in acetone (134 mL) was added benzyl bromide (4.3 mL, 36.11 mmol, 1.3 equiv) at room temp. The resulting mixture was heated to reflux for 1.5 h. The solid was filtered and washed with acetone (50 mL x2). The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes) to afford aldehyde **346** (7.1 g, quant.).

^{1}H NMR (300Mhz, $CDCl_3$, 298K) 9.87 (1H, s), 7.37-7.52 (7H, m), 5.20 (2H, s), 3.98 (2H, s), 2.38 (3H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 191.25, 153.21, 152.14, 136.40, 132.61, 131.95, 128.66, 128.14, 127.44, 127.38, 110.58, 70.84, 60.53, 16.27;

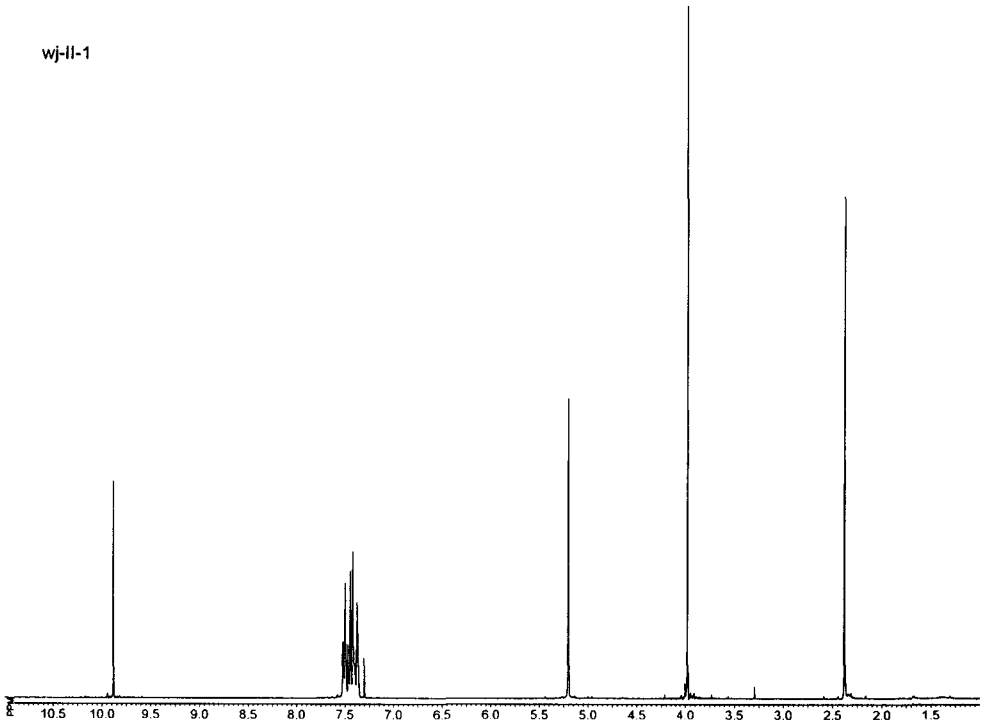
IR (neat, film): 3033, 2935, 2831, 1692, 1584, 1489, 1382, 1296, 1139, 1089, 1004, 740, 704 cm^{-1};

HRMS calcd for $C_{16}H_{17}O_3$ 257.1178, found 257.1176.

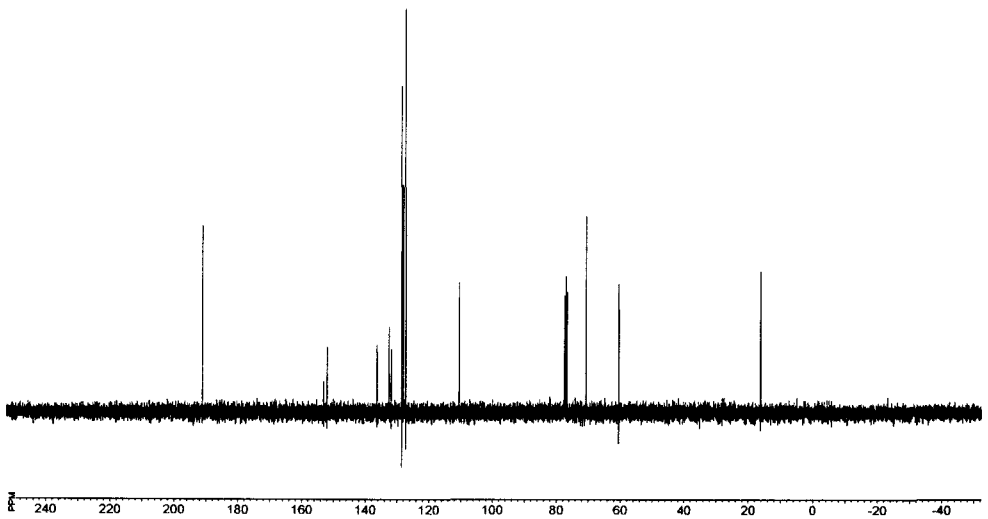


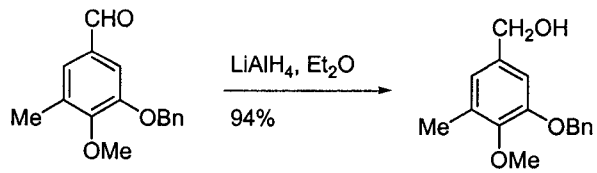
346

wj-II-1



wj-II-1C





346

Alcohol ((3-Benzyloxy-4-methoxy-5-methyl-phenyl)-methanol):

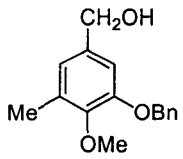
To a stirred suspension of $LiAlH_4$ (24 mg, 0.62 mmol, 0.5 equiv) in dry ether (2.5 mL) was added aldehyde **346** (318 mg, 1.24 mmol, 1.0 equiv) in dry ether (2.5 mL) at room temp.. The resulting suspension was stirred at room temp. for 30 min, and then the excess $LiAlH_4$ was quenched with addition of water (1 mL). To this mixture was added 2N HCl solution (5 mL) and the mixture was extracted with ether (5 mL x3). The combined extracts were washed with water (5 mL x2), saturated NaCl solution (5 mL x2), and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) afford 300 mg (94%) of alcohol compound as colorless oil.

^1H NMR (300 MHz, $CDCl_3$, 298K) δ 7.37-7.52 (5H, m), 6.89-6.90 (1H, d, *J*=1.8Hz), 6.82 (1H, s), 5.15 (2H, s), 4.60 (2H, s), 3.88 (3H, s), 2.32 (3H, s), 1.95(1H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 151.8, 147.1, 137.1, 136.4, 132.1, 128.6, 127.9, 127.3, 121.8, 110.7, 70.7, 65.3, 60.4, 16.1;

IR (neat, film) 3396, 2932, 2872, 2827, 1591, 1495, 1433, 1325, 1230, 1143, 1089, 1008, 737, 697 cm^{-1};

HRMS (FAB^+) Calcd for $C_{16}H_{18}O_3$ 258.1256; found 258.1249.



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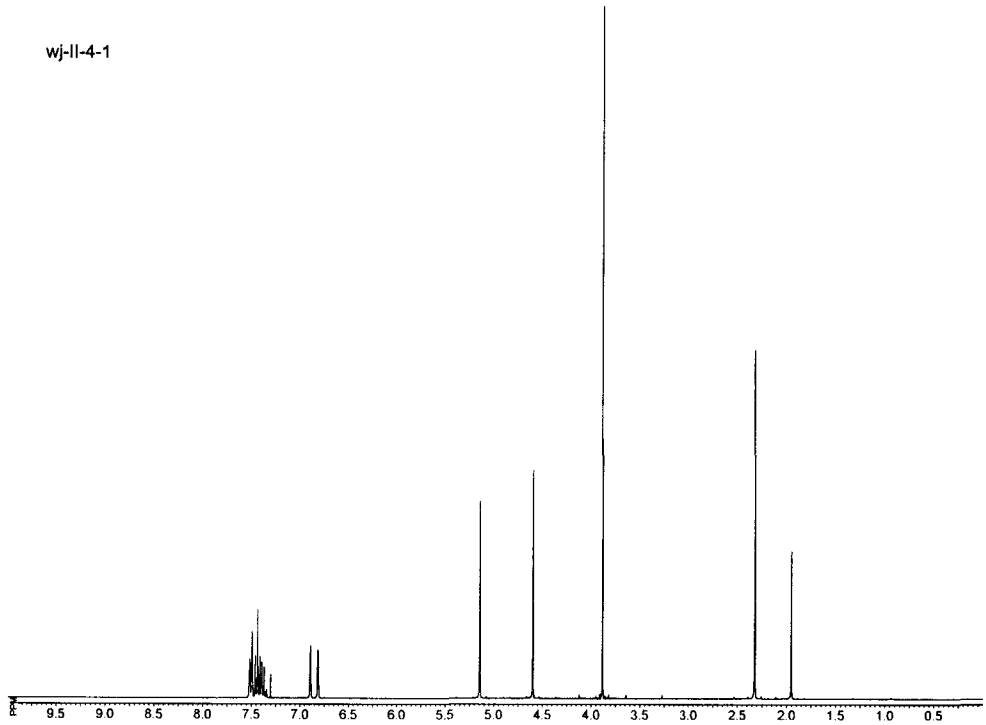
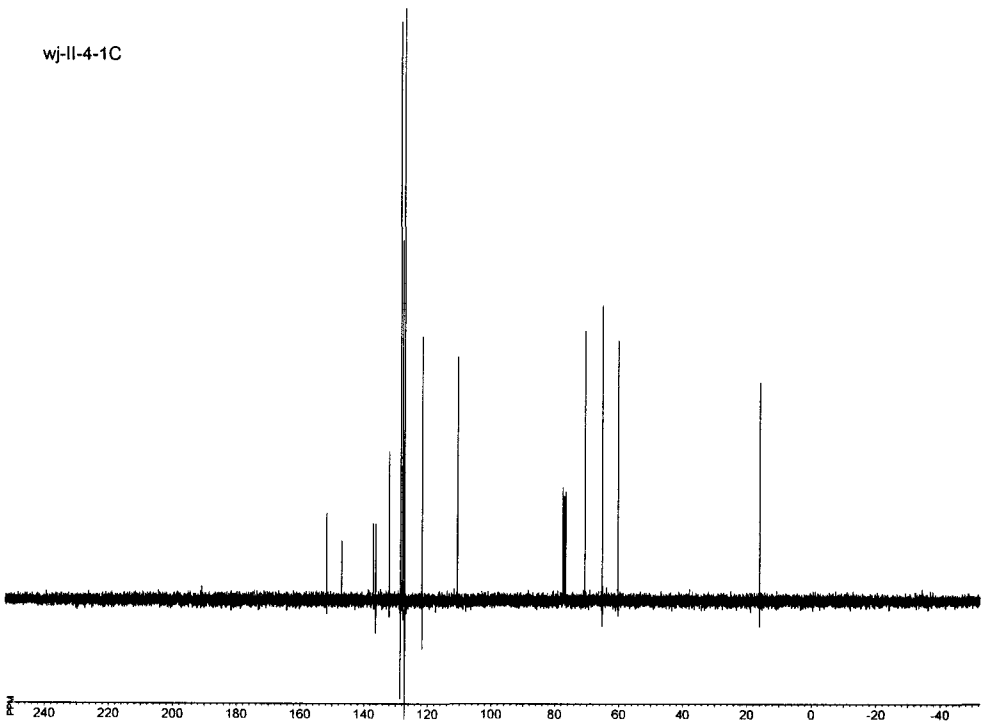
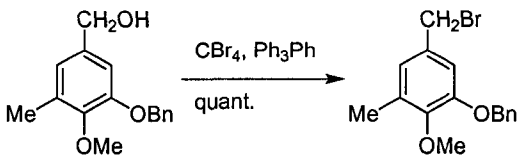


wj-II-4-1C





355

Bromide 355:

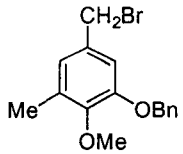
To a solution of alcohol obtained above (300 mg, 1.16 mmol, 1.0 equiv), CBr_4 (424 mg, 1.28 mmol, 1.1 equiv) in THF (7 mL) was added PPh_3 (335 mg, 1.28 mmol, 1.1 equiv) at room temp.. The resulting mixture was stirred at room temp. for 3.5 h. The white solid was filtered and washed with THF (10 mL x2). The solvent was removed under reduced pressure the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes) to afford bromide **355** (380 mg, qunat.).

^{1}H NMR (300 MHz, $CDCl_3$, 298K) δ 7.39-7.54 (5H, m), 6.92-6.93 (1H, d, *J*=2.4Hz), 6.89-6.90 (1H, d, *J*=2.1Hz), 5.17 (2H, s), 4.49 (2H, s), 3.91 (3H, s), 2.33 (3H, s);

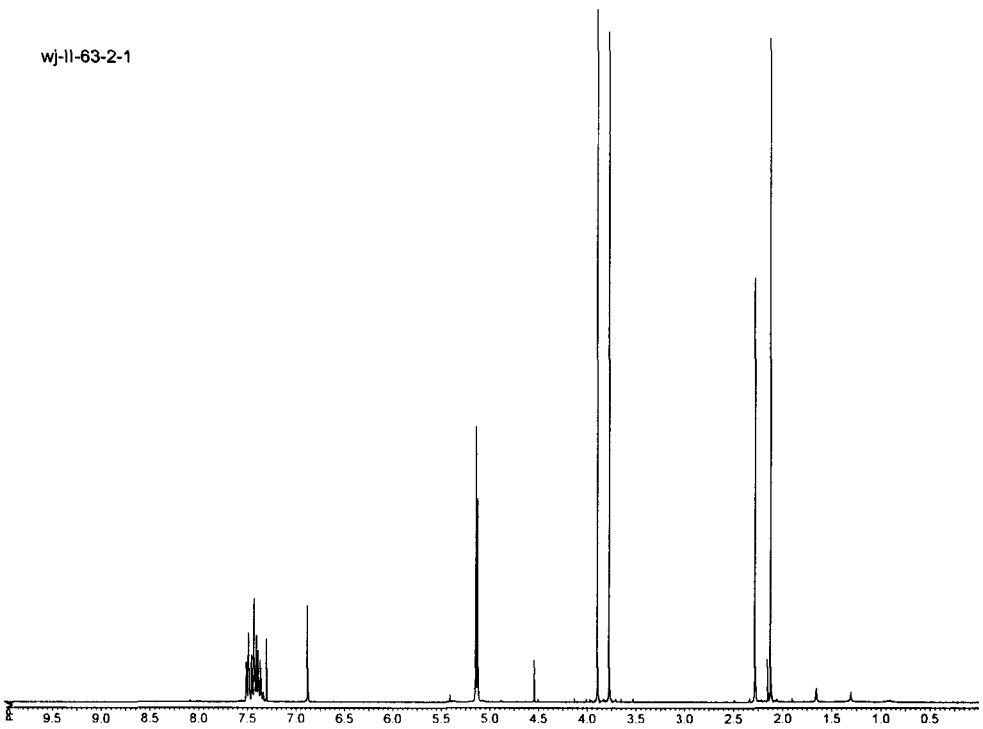
^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 150.77, 147.0, 135.9, 131.9, 131.4, 127.6, 127.0, 126.4, 123.0, 111.7, 69.8, 59.3, 33.3, 15.1;

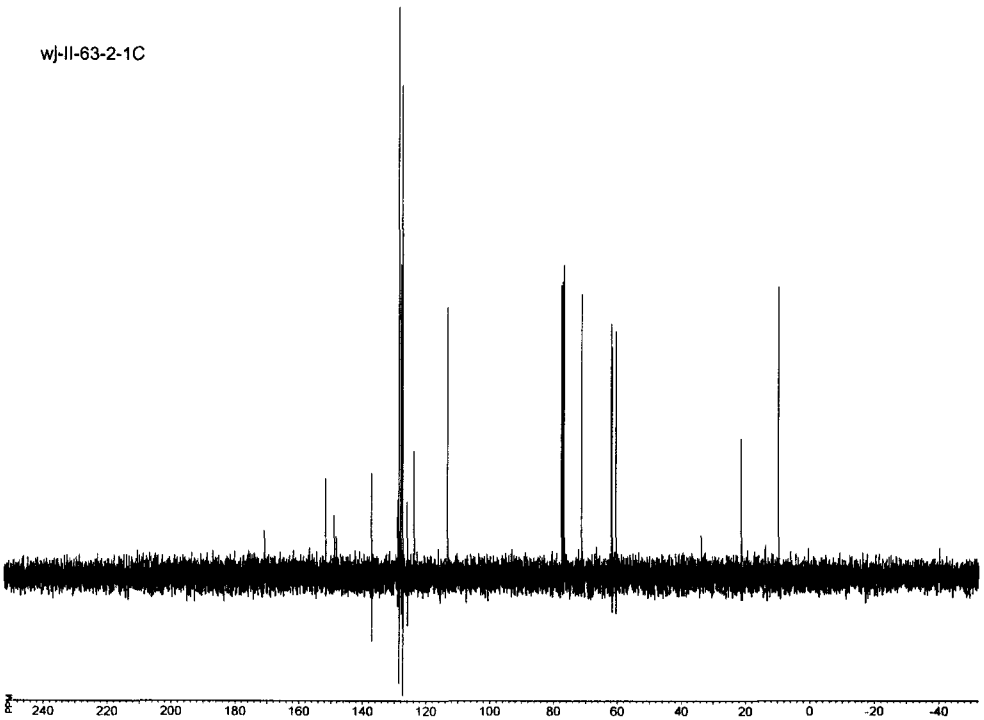
IR (neat, film): 3031, 2932, 2828, 1589, 1493, 1434, 1333, 1287, 1211, 1150, 1090, 1008, 738, 698 cm^{-1};

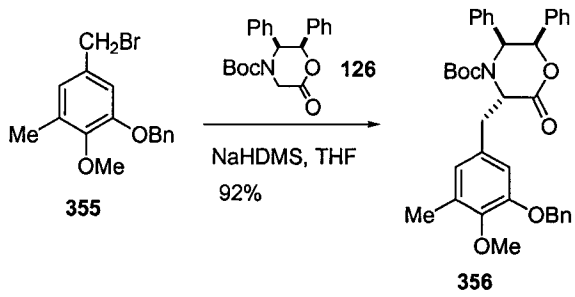
HRMS (FAB^+) Calcd for $C_{16}H_{17}O_2Br$ 320.0419; found 320.0418.



355







Lactone 356 (3-(3-Benzyloxy-4-methoxy-5-methyl-benzyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylic acid tert-butyl ester):

To a solution of lactone **126** (683 mg, 1.93 mmol, 1.2 equiv) in THF (20 mL) at –78°C was added NaHMDS (1M in THF) (2.41 mL, 2.41 mmol, 1.5 equiv). The resulting solution was stirred at –78°C for 15 min. The solution was transferred to the solution of benzylbromide derivative **355** (516 mg, 1.61 mmol, 1.0 equiv) in THF (10 mL) at –78°C in 5 min. period. The resulting solution was stirred at –78°C for an additional 4 h. The solution was diluted with EtOAc (150 mL) and the organic layer was washed with saturated NaCl solution (50 mL x2) and dried over anhydrous Sodium sulfate. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes; then 30% EtOAc in hexanes) to give lactone **356** (855 mg, 90%).

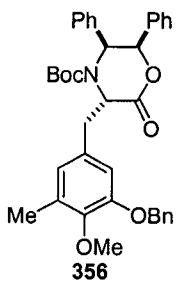
^{1}H NMR (400 MHz, DMSO-d_6, 373K) δ 7.09-7.49 (9H, m), 6.93 (1H, s), 6.86-6.87 (2H, d, J=5.6Hz), 6.59-6.61 (2H, d, J=7.2Hz), 6.78 (1H, s), 5.4 (1H, broad), 5.14(3H, s), 5.04 (1H, broad), 3.77 (3H, s), 3.41-3.46 (1H, m), 3.27-3.30 (3H, d, J=16.0Hz), 2.23 (3H, s), 1.16-1.41 (9H, broad);

^{13}C NMR (100 MHz, DMSO-d_6, 373K) δ 169.5, 167.8, 151.0, 146.1, 136.7, 142.2, 131.2, 130.8, 127.8, 127.6, 127.3, 127.2, 126.9, 126.7, 125.8, 123.8, 113.9, 80.0, 77.5, 70.3, 59.7, 59.2, 57.9, 27.2, 15.0, 13.5;

IR (neat, film): 3032, 2931, 1754, 1697, 1588, 1494, 1453, 1382, 1331, 1233, 1161, 1085, 1010, 738, 702 cm^{-1};

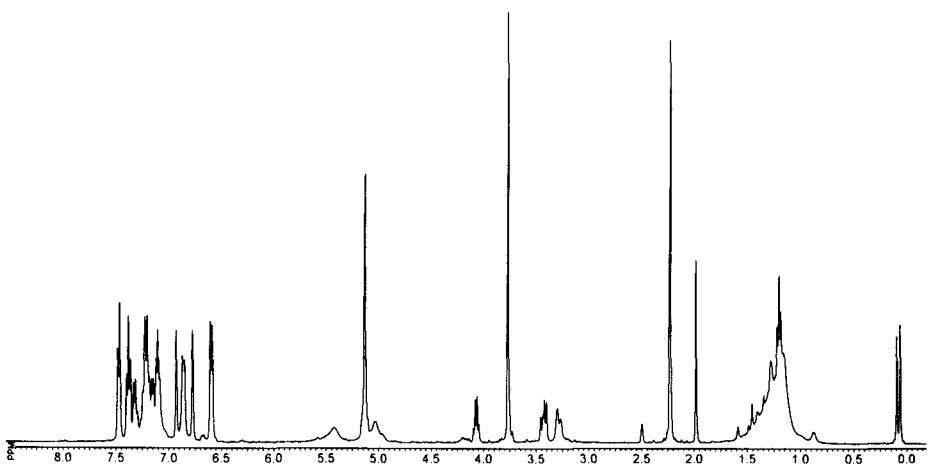
HRMS (FAB$^+$) Calcd for $C_{37}H_{39}NO_6$ 593.2777; found 593.2781;

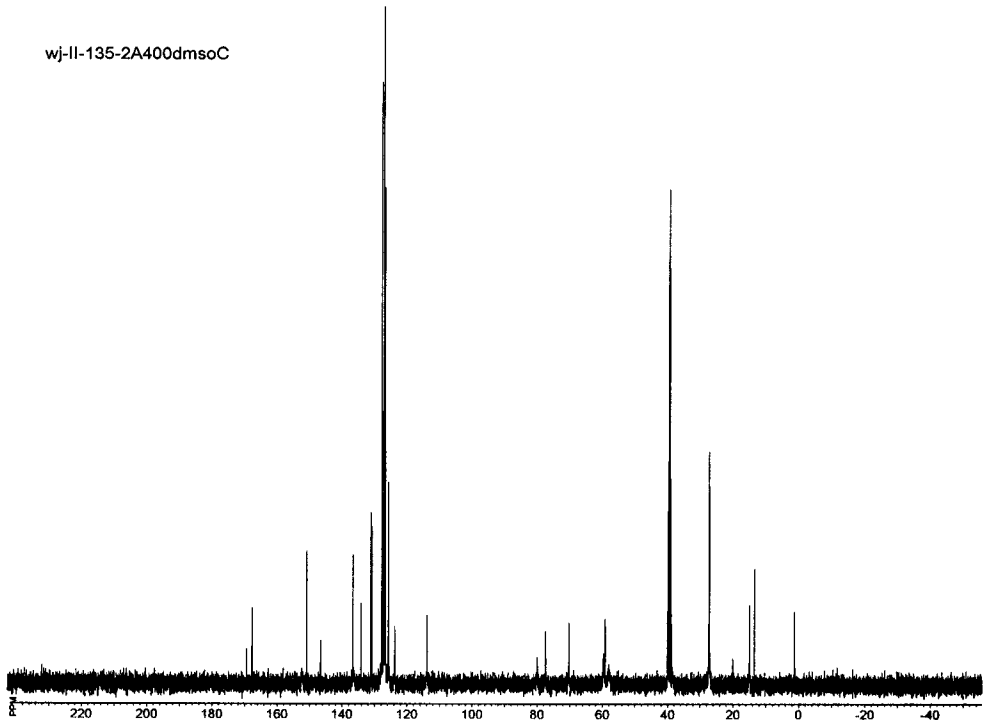
$[\alpha]_D^{25}$ = -22.5 (c = 0.71, CH_2Cl_2).

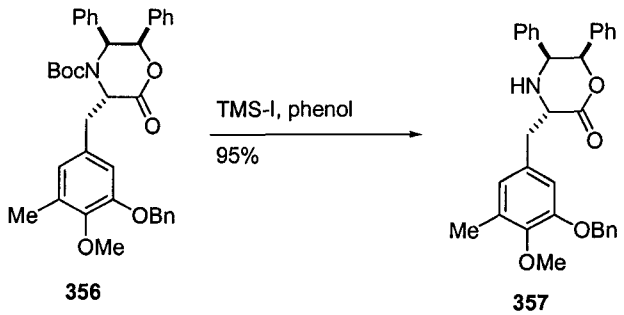


356

wj-II-135-2A400dmso







356 357

Amine 357 (3-(3-Benzyloxy-4-methoxy-5-methyl-benzyl)-5,6-diphenyl-morpholin-2-one):

To a solution of lactone **356** (430 mg, 0.724 mmol, 1.0 equiv) and phenol (136 mg, 1.448 mmol, 2.0 equiv) in CH_2Cl_2 (6 mL) was added TMS-I (206 μL, 1.448 mmol, 2.0 equiv) dropwise. The resulting solution was stirred at room temp. for 30 min, and the reaction was quenched with methanol (5 equiv MeOH per TMS-I), and stirred for an additional 5 min.. The reaction mixture was diluted with EtOAc (50 mL), washed with water (20 mL), sat. NaCl solution (20 mL) and dried with anhydrous $NaSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give lactone **357** (344 mg, 95%).

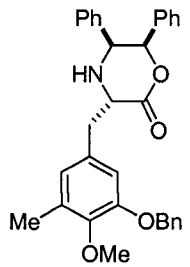
^{1}H NMR (300MHz, $CDCl_3$, 298K) 7.15-7.36 (11H, m), 6.93 (1H, d, *J*=1.5Hz), 6.91 (2H, s), 6.88 (1H, d, *J*=1.2Hz), 6.66-6.67 (2H, d, *J*=1.8Hz), 5.59-5.60 (1H, d, *J*=3.9Hz), 4.90 (2H, d, *J*=2.1Hz), 4.57-4.58 (1H, d, *J*=3.6Hz), 4.18-4.22 (1H, t, *J*=6.6Hz), 3.81 (3H, s), 3.20-3.22 (2H, d, *J*=6.9Hz), 2.20 (3H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 170.47, 151.82, 146.83, 136.98, 134.93, 132.48, 132.29, 128.51, 128.37, 128.16, 128.10, 127.86, 127.66, 127.29, 127.16, 124.18, 112.95, 85.25, 70.65, 60.37, 58.19, 56.85, 38.92, 16.10;

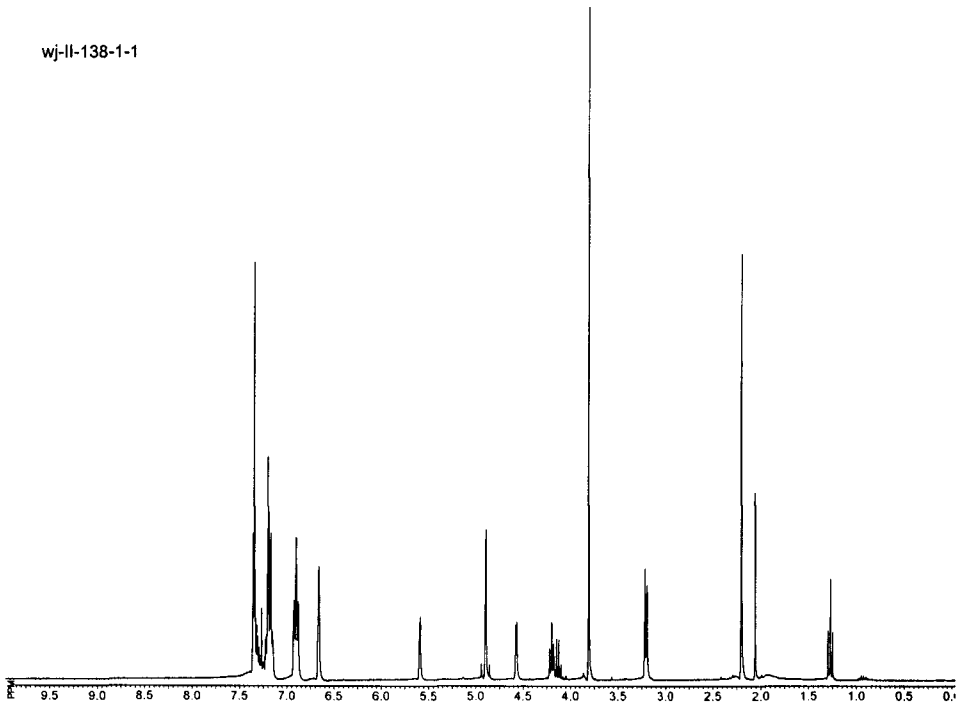
IR (neat, film): 3327, 3063, 3032, 2931, 1736, 1587, 1497, 1453, 1337, 1231, 1181, 1086, 1011, 736, 699 cm^{-1};

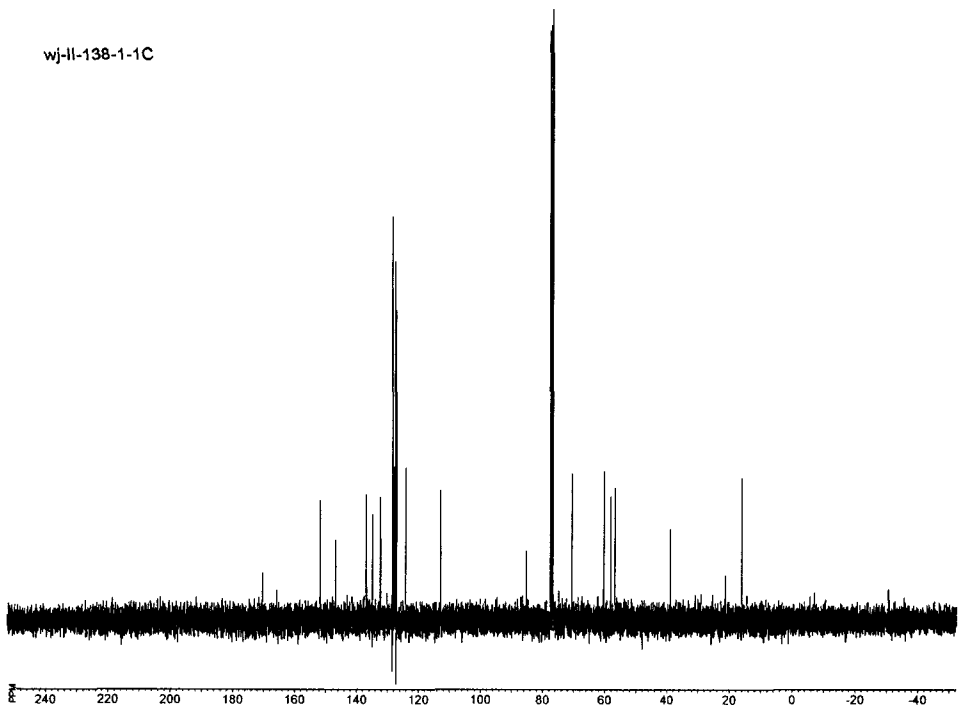
HRMS calcd for $C_{32}H_{32}NO_4$ 494.2331, found 494.2327;

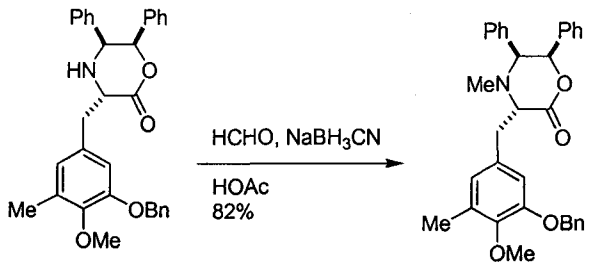
$[\alpha]_D^{25}$ =–50.49 (c = 0.61, CH_2Cl_2).



357







357 358

***N*-methyl lactone 358 (3-(3-Benzyloxy-4-methoxy-5-methyl-benzyl)-4-methyl-5,6-diphenyl-morpholin-2-one):**

To a solution of lactone **357** (76 mg, 0.154 mmol, 1.0 equiv) in CH_3CN (2 mL) was added formalin (37% aqueous solution) (57.6 μL, 0.769 mmol, 5.0 equiv) at room temp. The resulting mixture was stirred for 10 min, then, $NaBH_3CN$ (20 mg, 0.308 mmol, 2.0 equiv) was added. The mixture was stirred for 15 min, acetic acid (20μL, 0.323 mmol, 2.1 equiv) was added, and the reaction mixture was stirred at room temp. for 45 min. The mixture was diluted with EtOAc (20 mL) and the solution was washed with water (5 mL), sat. NaCl solution (5 mL) and dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give Lactone **358** (64 mg, 82%).

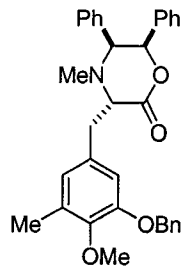
^{1}H NMR (300MHz, $CDCl_3$, 298K) 7.06-7.49 (11H, m), 6.71-6.81 (6H, m), 5.10-5.21 (2H, dd, *J*=6.9, 12.5), 4.91 (1H, s), 3.93-3.96 (1H, t, *J*=4.2Hz), 3.84 (3H, s), 3.82 (1H, s), 3.01-3.23 (2H, ddd, *J*=3.3Hz, 4.5Hz, 13.5, 50.1Hz), 2.27 (6H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 171.65, 151.17, 146.89, 137.59, 135.77, 134.17, 132.51, 131.79, 129.28, 128.63, 128.12, 127.86, 127.77, 127.70, 127.23, 127.14, 126.17, 124.92, 114.23, 81.64, 70.71, 67.04, 64.70, 60.48, 39.65, 37.88, 16.21;

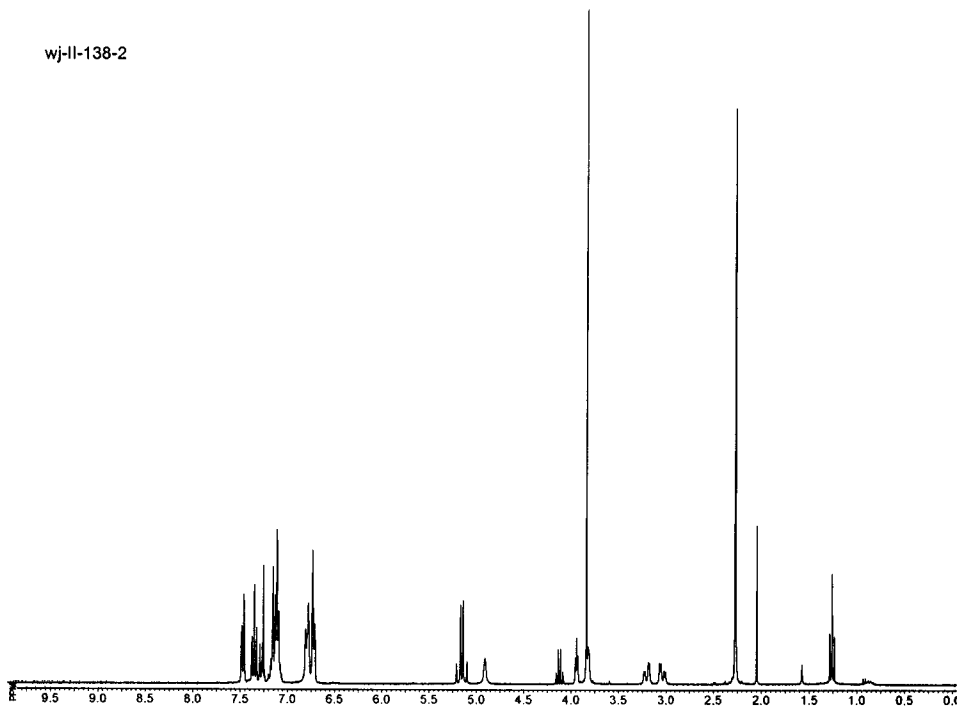
IR (neat, film): 3031, 2931, 2825, 1740, 1588, 1495, 1453, 1347, 1232, 1147, 1090, 1010, 738, 713, 697 cm^{-1};

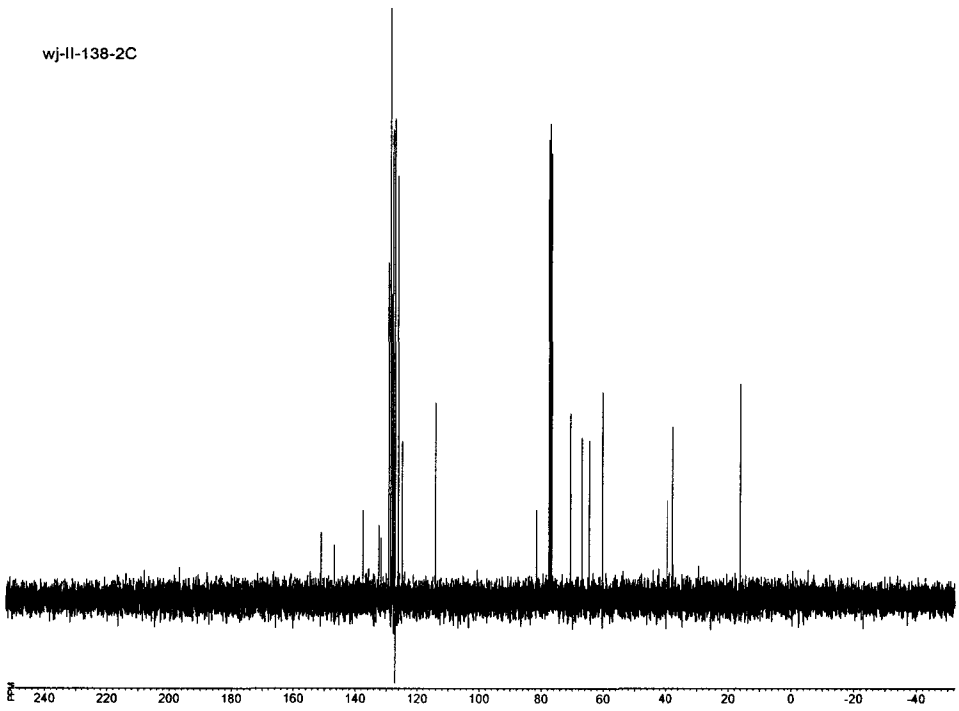
HRMS calcd for $C_{33}H_{34}NO_4$ 508.2488, found 508.2489;

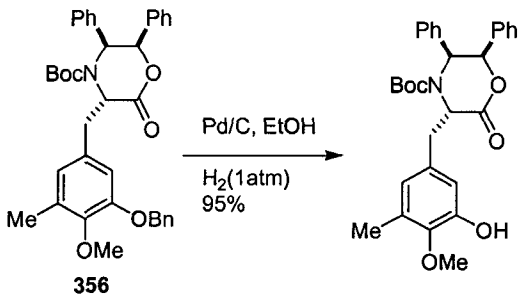
$[\alpha]_D^{25}$ =+56.15 (c = 1.09, CH_2Cl_2).



358







356

Phenol lactone (3-(3-Hydroxy-4-methoxy-5-methyl-benzyl)-2-oxo-5,6-diphenyl-morpholine-4-carboxylic acid tert-butyl ester):

To a solution of lactone **356** (1.09 g, 1.80 mmol, 1.0 equiv) in ethanol (30 mL) was added Pd/C (10% on C) (383 mg, 0.36 mmol, 0.2 equiv). The resulting mixture was hydrogenated at 1 atm H_2 for 16 h. The catalyst was filtered off through Celite and the solvent was removed under reduced pressure. The residue was pure enough to be used directly for next step without further purification. Crude yield: 858 mg (95%).

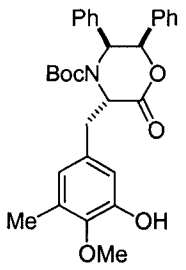
^{1}H NMR (400 MHz, DMSO-d_6, 373K) δ 7.08-7.26 (6H, m), 6.85-6.86 (2H, d, J=6.4Hz), 6.69 (1H, s), 6.56-6.59 (3H, m), 5.38 (1H, broad), 5.08 (1H, broad), 5.01 (1H, broad), 3.73 (3H, s), 3.47-3.51 (1H, m), 3.34-3.39 (1H, m), 3.16-3.19 (1H, broad), 2.20 (3H, s), 1.08-1.47 (9H, broad);

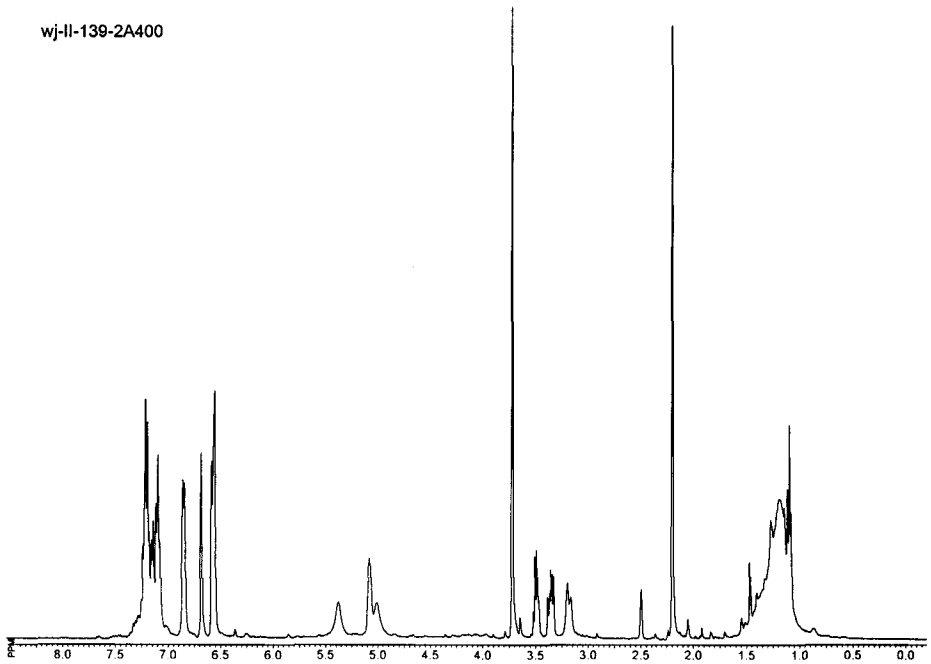
^{13}C NMR (100 MHz, DMSO-d_6, 373K) δ 167.9, 149.4, 145.1, 134.2, 131.0, 130.4, 127.6, 127.2, 126.9, 126.7, 125.7, 121.8, 115.4, 115.3, 50.0, 77.4, 59.6, 58.8, 55.6, 27.2, 17.8, 15.0;

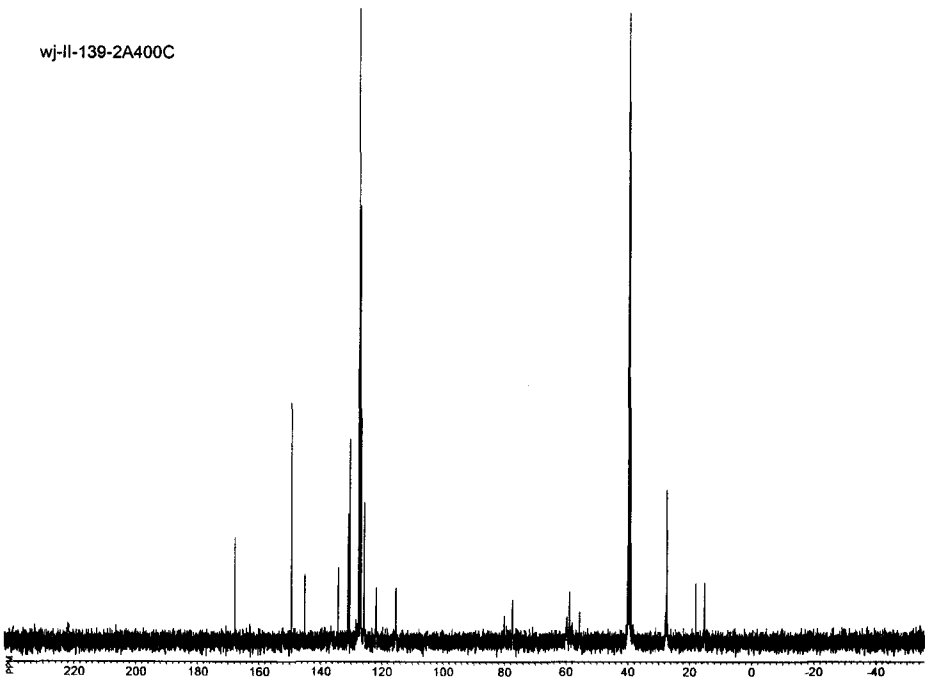
IR (neat, film): 3399, 2977, 2933, 1754, 1697, 1590, 1497, 1454, 1386, 1357, 1301, 1161, 1119, 1060, 1003, 736, 702 cm^{-1};

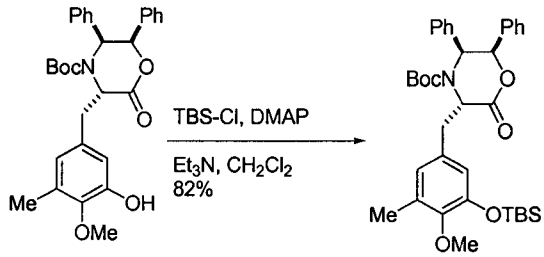
HRMS (FAB$^+$) Calcd for $C_{30}H_{33}NO_6$ 503.2308, found 503.2307;

$[\alpha]_D^{25}$ =-37.8 (c =1.27, CH_2Cl_2).









361

***O*-TBS lactone 361 (3-[3-(tert-Butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-benzyl]-2-oxo-5,6-diphenyl-morpholine-4-carboxylic acid tert-butyl ester):**

To a solution of lactone obtained above (340 mg, 0.67 mmol, 1.0 equiv), Et_3N (141 μL, 1.01 mmol, 1.5 equiv), DMAP (12.3 mg, 0.10 mmol, 0.15 equiv) in methylene chloride (9 mL) was added TBS-Cl (112 mg, 0.74 mmol, 1.1 equiv) at room temp. The resulting solution was stirred at room temp. for 8 h. Then the reaction was diluted with EtOAc (100 mL) and washed with water (20 mL x2) and saturated NaCl solution (20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure, and the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes; then 20% EtOAc in hexanes) to give lactone **361** in 82% yield (277 mg).

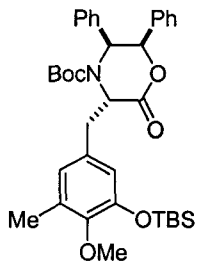
^{1}H NMR (400 MHz, DMSO-d_6, 373K) δ 7.10-7.25 (6H, m), 6.83-6.84 (2H, d, *J*=4.8Hz), 6.73 (1H, s), 6.66 (1H, s), 6.56-6.58 (2H, d, *J*=6.8Hz), 5.30 (1H, broad), 5.08 (1H, broad), 4.99 (1H, broad), 3.73 (3H, s), 3.37-3.42 (1H, broad), 3.19-3.22 (1H, broad), 2.22 (3H, s), 1.05-1.50 (9H, broad), 1.00 (9H, s), 0.17-0.18 (6H, s);

^{13}C NMR (100 MHz, DMSO-d_6, 373K) δ 167.8, 148.2, 147.7, 134.1, 131.3, 127.7, 127.3, 127.0, 1525.7, 124.2, 119.5, 119.4, 119.4, 80.1, 77.5, 58.9, 27.2, 25.1, 17.4, 15.1, -5.2;

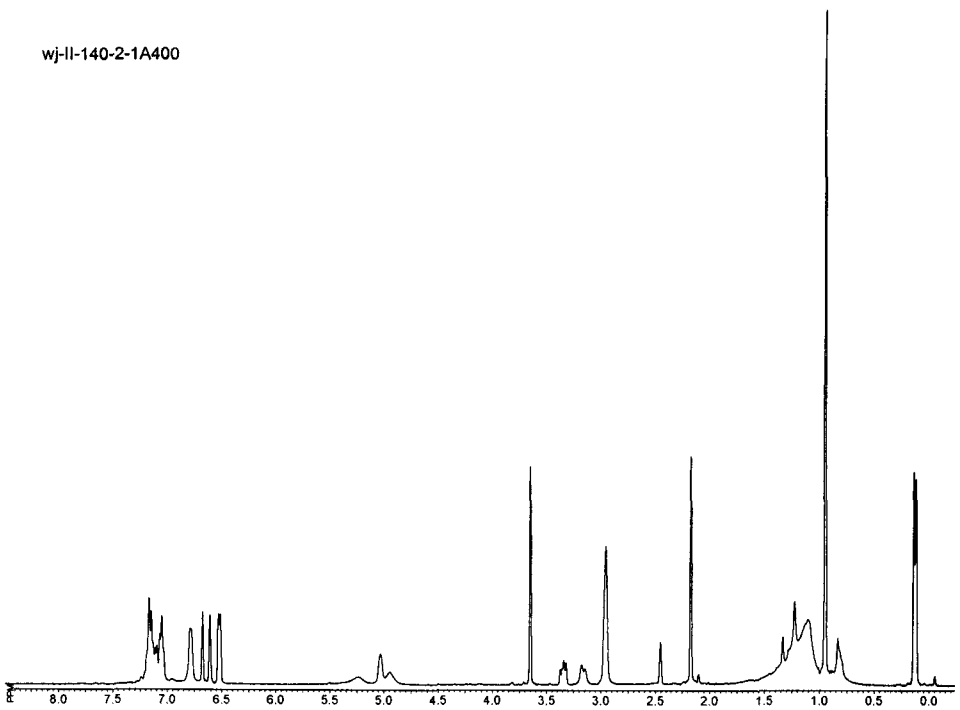
IR (neat, film): 3032, 2927, 2856, 1757, 1702, 1585, 488, 1454, 1380, 1357, 1254, 1162, 1116, 1067, 1011, 838, 784, 701 cm^{-1};

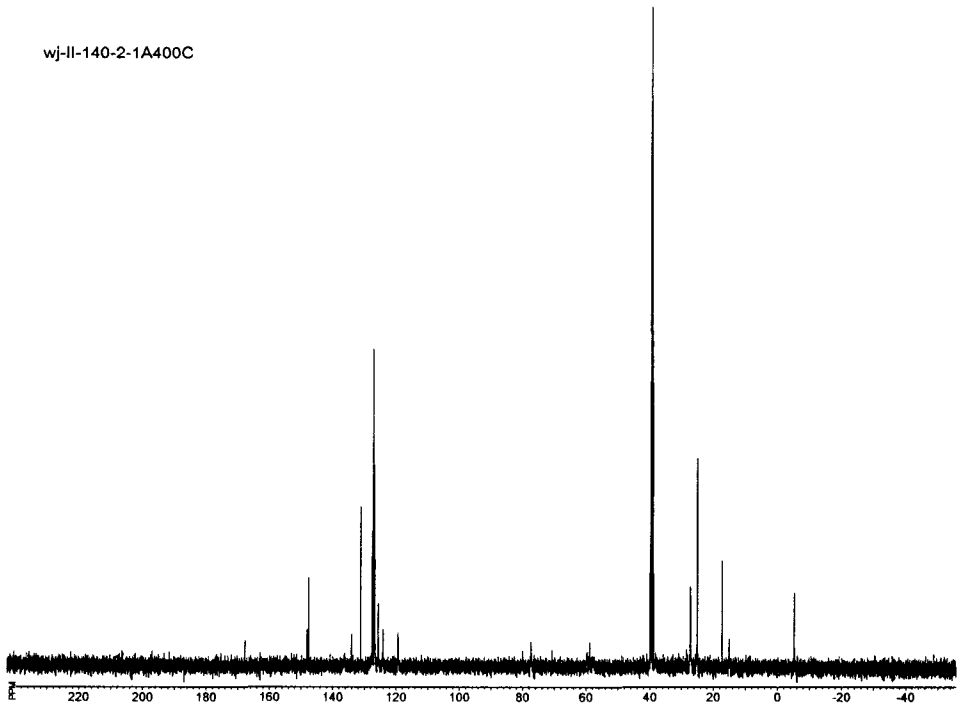
HRMS (FAB^+) Calcd for $C_{36}H_{47}NO_6Si$ 617.3173; found 617.3169;

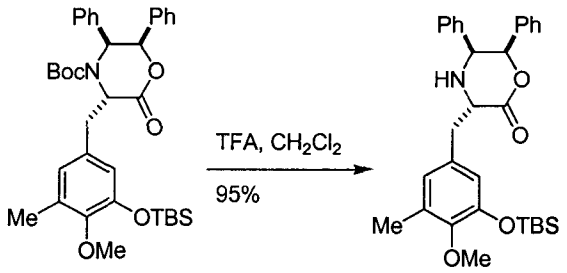
$[\alpha]_D^{25}$ =-22.1 (c =1.09, CH_2Cl_2).



361







361 **362**

Amine 362 (3-[3-(tert-Butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-benzyl]-5,6-diphenyl-morpholin-2-one):

To a solution of lactone **361** (95 mg, 0.15 mmol, 1.0 equiv) in CH_2Cl_2 (3 mL) was added TFA (3 mL) at room temp. The resulting solution was stirred at room temp. for 30 min. The solvent and TFA were removed under reduced pressure, the residue was diluted with EtOAc (20 mL) and the organic layer was washed with saturated $NaHCO_3$ solution till pH >7. The organic layer was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes) to give lactone **362** in 93% yield (73.6 mg).

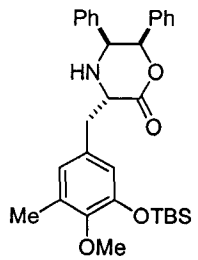
^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ 7.11-7.23 (6H, m), 6.85-6.88 (4H, m), 6.62 (1H, d, *J*=1.6Hz), 6.49-6.50 (1H, d, *J*=2.0Hz), 5.55-5.56 (1H, d, *J*=3.6Hz), 4.60 (1H, d, *J*=3.6Hz), 4.15-4.18 (1H, dd, *J*=4.0Hz, 9.2Hz), 3.68 (3H, s), 3.12-3.17 (2H, m), 2.15 (3H, s), 1.23 (1H, broad), 0.90 (9H, s), 0.02 (3H,s), -0.01 (3H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 170.6, 149.0, 148.8, 136.8, 135.1, 132.9, 132.4, 128.4, 128.2, 127.8, 127.7, 127.3, 124.6, 120.0, 85.3, 59.9, 58.3, 56.7, 38.5, 29.8, 25.8, 18.3, 16.1, -4.6;

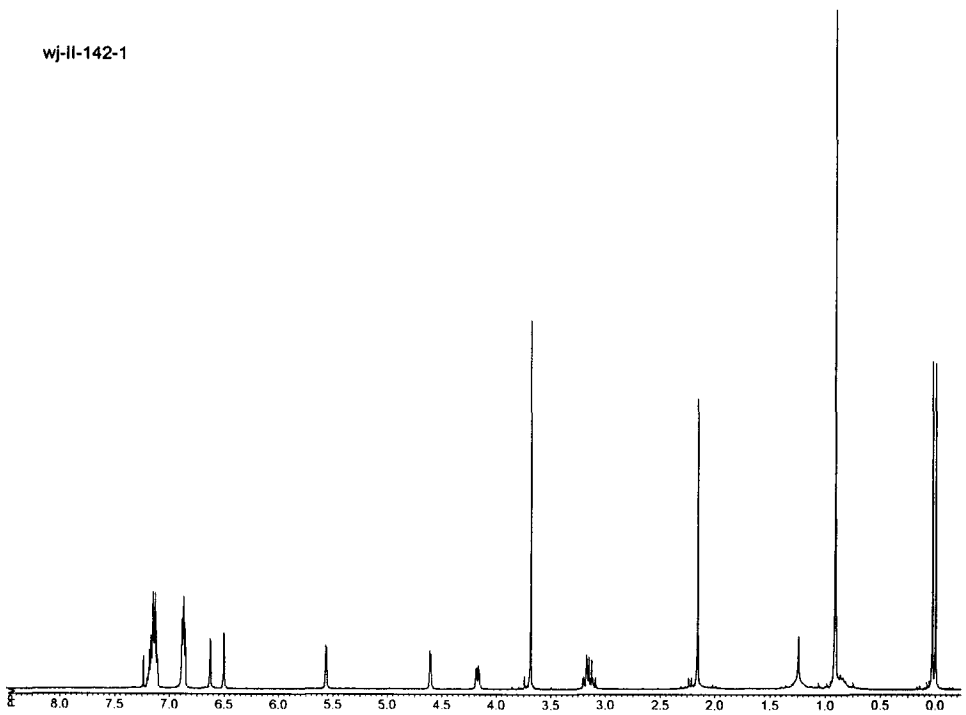
IR (neat, film): 3325, 2955, 2929, 2857, 1738, 1584, 1489, 1336, 1253, 1226, 1180, 1070, 1012, 838, 783, 699 cm^{-1};

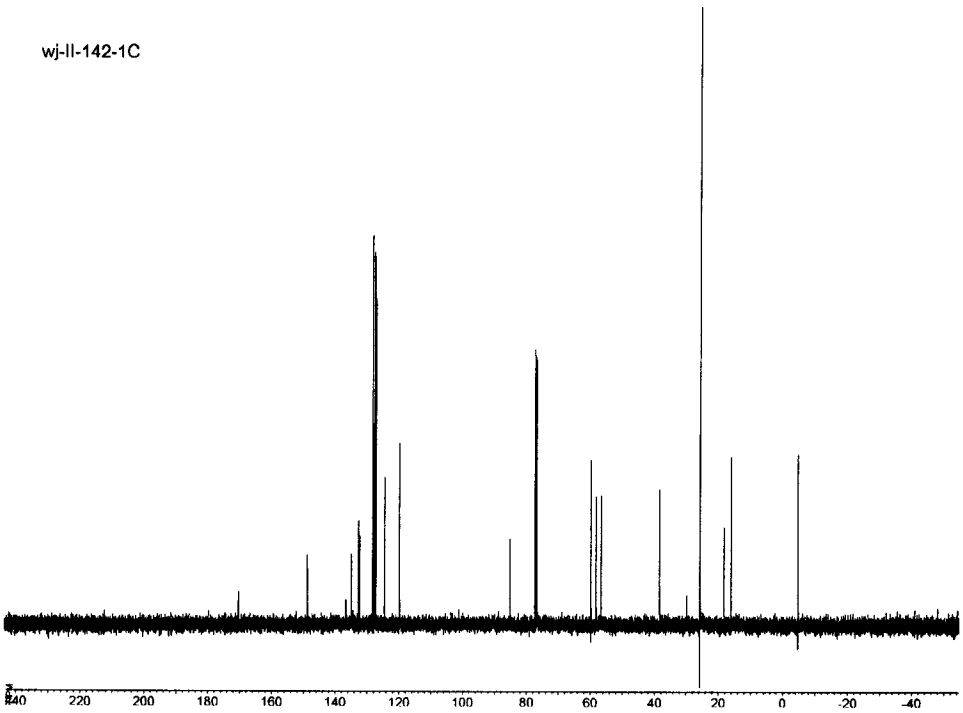
HRMS (FAB^+) calcd for $C_{31}H_{40}NO_4Si$ 518.2727; found 518.2719;

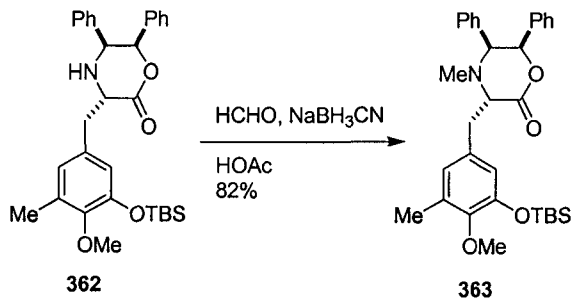
$[\alpha]_D^{25}$ = -55.4 (*c* = 0.70, CH_2Cl_2).



362







_N_-Methyl lactone 363 (3-[3-(tert-Butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-benzyl]-4-methyl-5,6-diphenyl-morpholin-2-one):

To a solution of lactone **362** (343 mg, 0.66 mmol, 1.0 equiv) in CH_3CN (7 mL) was added formalin (37% aqueous solution) (248 μL, 3.31 mmol, 5.0 equiv) at room temp. and the resulting mixture was stirred for 10 min, then, $NaBH_3CN$ (93.3 mg, 1.33 mmol, 2.0 equiv) was added. The mixture was stirred for 15 min, acetic acid (79.6 μL, 1.39 mmol, 2.1 equiv) was added, and the reaction mixture was stirred at room temp. for 45 min. The mixture was diluted with EtOAc (100 mL), and the solution was washed with water (20 mL), saturated NaCl solution (20 mL) and dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 10% EtOAc in hexanes) to give lactone **362** (288 mg, 82%).

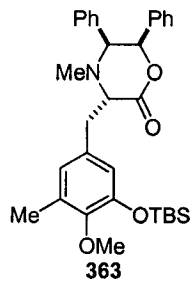
^1H NMR (400 MHz, $CDCl_3$, 298K) δ 7.10-7.19 (6H, m), 6.86-6.87 (2H, d, J=5.2Hz), 6.78-6.79 (2H, d, J=7.2Hz), 6.74 (1H, s), 6.71 (1H, s), 5.06 (1H, s), 3.97-4.01 (2H, m), 3.76 (3H, s), 3.03-3.23 (2H, dd, J=4.0Hz, 13.2Hz), 2.39 (3H, s), 2.27 (3H, s), 1.03 (9H, s), 0.20 (3H,s), 0.18 (3H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 171.7, 148.8, 148.4, 135.9, 134.3, 132.6, 132.0, 129.4, 128.2, 128.0, 127.9, 127.8, 126.1, 125.5, 121.0, 81.7, 67.4, 64.7, 60.0, 39.7, 37.4, 29.8, 25.9, 18.4, 16.2, -4.4, -4.5;

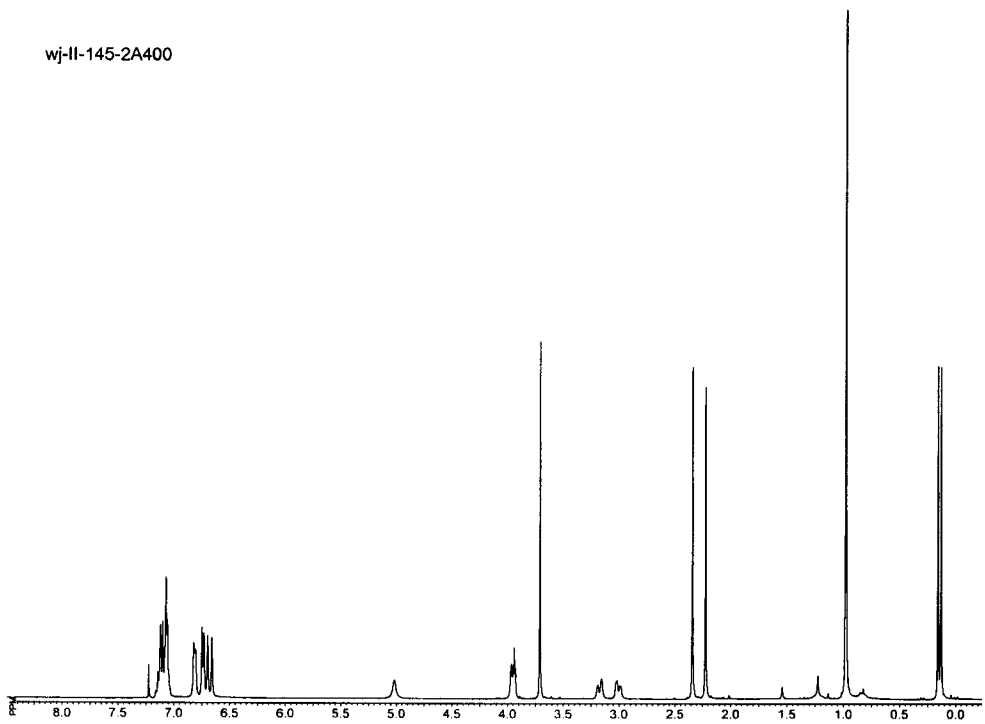
IR (neat, film): 2930, 2857, 1744, 1585, 1489, 1351, 1315, 1254, 1176, 1146, 1070, 1012, 839, 783, 712, 697 cm^{-1};

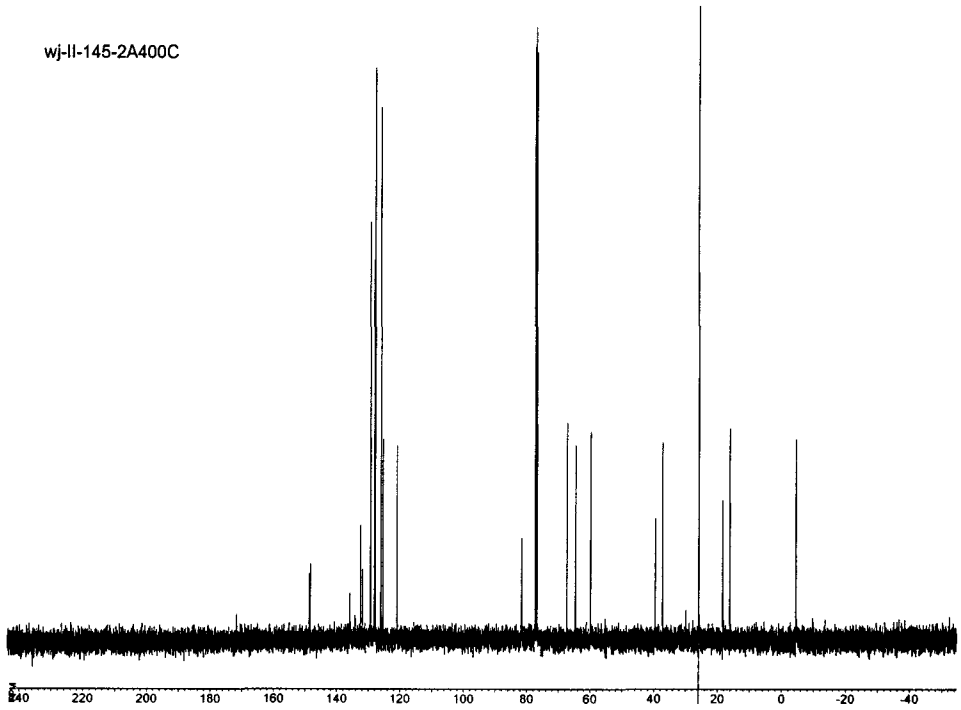
HRMS (FAB^+) calcd for $C_{32}H_{42}NO_4Si$ 532.2883; found 532.2861;

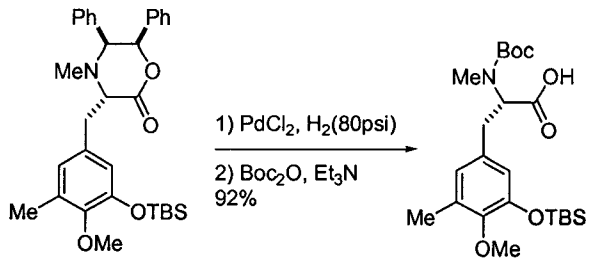
$[\alpha]_D^{25}$ =+40.5 (*c* = 1.11, CH_2Cl_2).



363







363 360

***N*-*t*-Boc-Amino acid 360 (2-(tert-Butoxycarbonyl-methyl-amino)-3-[3-(tert-butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-phenyl]-propionic acid):**

To a solution of lactone **363** (44 mg, 0.083 mmol, 1.0 equiv) in absolute ethanol (6 mL) was added $PdCl_2$ (8.8 mg, 0.050 mmol, 0.6 equiv), the resulting mixture was charged with hydrogen (80psi) and stirred for 18 h. The catalyst was filtered off through Celite, the solvent of the filtrate was removed under reduced pressure to give the crude amino acid hydrochloride salt (32 mg crude), which was used directly for next step without purification.

The amino acid salt obtained above (32 mg, 0.082 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (4 mL), and to this mixture was added TEA (34.3 μL, 0.25 mmol, 3.0 equiv) at room temp. The resulting solution was stirred for 5 min., Boc_2O (26.8 mg, 0.12 mmol, 1.5 equiv) was added and the resulting solution was stirred at room temp. for an additional 24 h. The reaction mixture was loaded directly onto the column, and purified by flash column chromatography (eluted with 10% methanol in CH_2Cl_2) to afford amino acid **360** (34.5 mg, 92% for two steps).

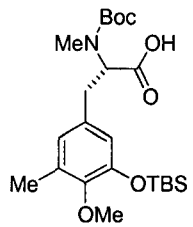
^1H NMR (300MHz, $CDCl_3$, 298K) δ 6.59 (1H, s), 6.51-6.53 (1H, d, J=6.6Hz), 5.57-5.62 (1H, dd, J=4.4Hz, 10.5Hz), 3.72 (3H, s), 3.13-3.19 (1H, dd, J=4.4 Hz, 14.4Hz), 2.81-3.04 (1H, m), 2.68-2.77 (3H, d, J=22.5Hz), 1.31-1.42 (9H, m), 1.07 (9H, s), 0.17 (6H, s);

^{13}C NMR (100MHz, $CDCl_3$, 298K) δ 148.6, 132.3, 124.3, 119.6, 59.9, 29.9, 28.4, 25.9, 48.4, 16.2, -4.4;

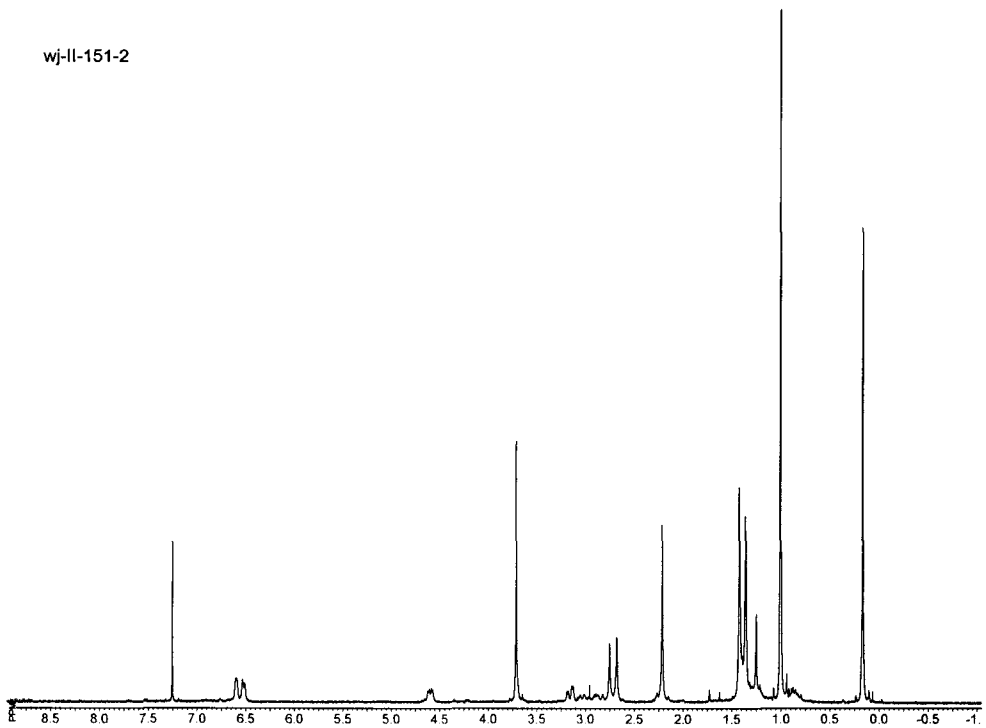
IR (neat, film): 2931, 2858, 1744, 1704, 1489, 1391, 1320, 1145, 839cm^{-1};

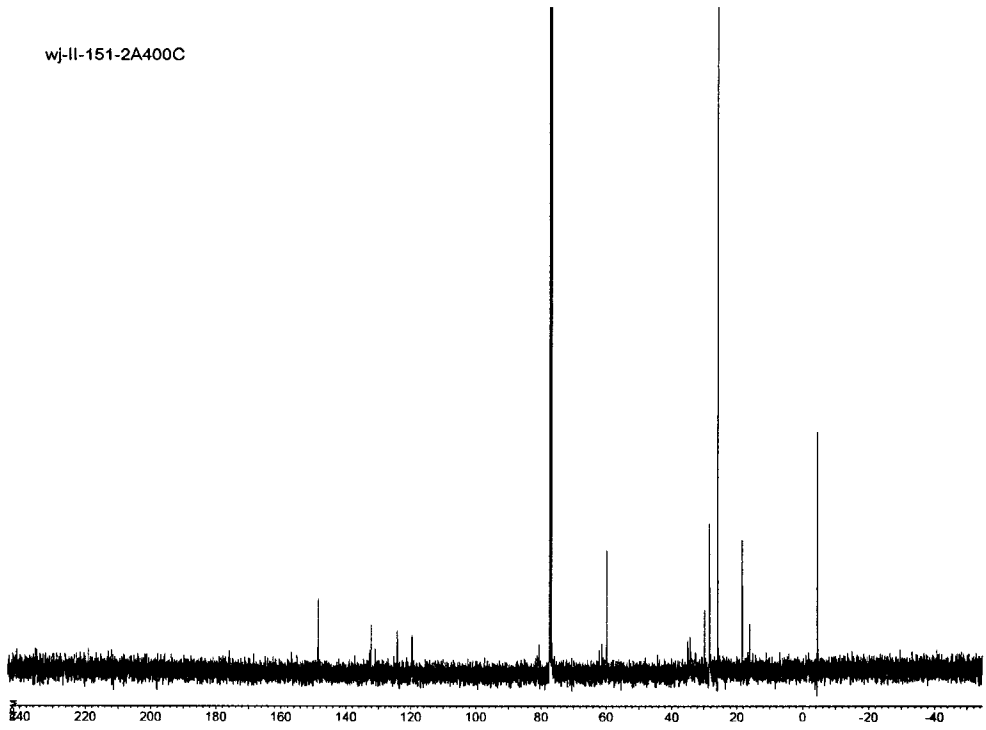
HRMS (FAB^+) calcd for $C_{23}H_{39}NO_3Si$ 453.2547, found 453.2548;

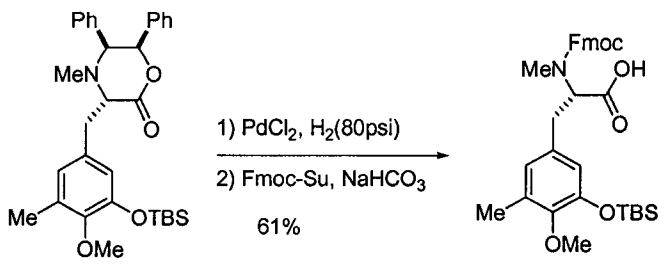
$[\alpha]_D^{25}$ =+39.2 (*c* = 0.50, CH_2Cl_2).



360







363 **382**

***N*-Fmoc-Amino acid 382 (3-[3-(tert-Butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-phenyl]-2-[(9H-fluoren-9-ylmethoxycarbonyl)-methyl-amino]-propionic acid):**

To a solution of lactone **363** (650 mg, 1.22 mmol, 1.0 equiv) in absolute ethanol (62 mL) was added $PdCl_2$ (130 mg, 0.73 mmol, 0.6 equiv), the resulting mixture was charged with hydrogen (80psi) and stirred for 18 h. The catalyst was filtered off through Celite, the solvent of the filtrate was removed under reduced pressure to give crude amino acid hydrochloride salt (500 mg crude), which was used directly for next step without purification.

The amino acid salt obtained above (477 mg, 1.22 mmol, 1.0 equiv) was dissolved in chloroform: sat. sodium bicarbonate (1:1) (10 mL) and to this mixture was added Fmoc-OSu (495 mg, 1.47 mmol, 1.2 equiv). The resulting solution was stirred at room temp. for 30 mim, then cooled to 0°C. The reaction mixture was adjusted to pH=2 with conc. HCl and then diluted with EtOAc (50 mL). The organic layer was washed with water (25 mL), sat. NaCl solution (25 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (eluted with 10% methanol in CH_2Cl_2) to afford amino acid **382** (423mg, 61% for two steps).

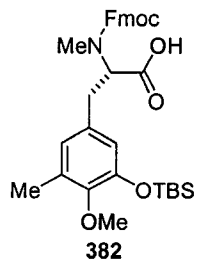
^{1}H NMR (300MHz, $CDCl_3$, 298K) δ 8.04 (1H, s), 7.72-7.76 (2H, dd, J=3Hz, 7.5Hz), 7.25-7.54 (6H, m), 6.59-6.64 (1H, s), 6.48 (1H, s), 4.64-4.93(1H, m), 4.14-4.37 (3H, m), 3.66 (3H, s), 2.6-3.3 (4H, m), 2.87 (3H, s), 2.18 (3H, s), 0.99 (9H, s), 0.14 (6H, s);

^{13}C NMR (75MHz, $CDCl_3$, 298K) δ 174.77, 163.03, 148.60, 143.97, 143.80, 141.27, 132.42, 132.29, 127.71, 127.10, 125.11, 124.92, 124.01, 120.01, 119.33, 68.07, 60.92, 59.86, 47.29, 36.93, 34.38, 32.58, 31.87, 25.92, 18.46, 16.27, -4.3;

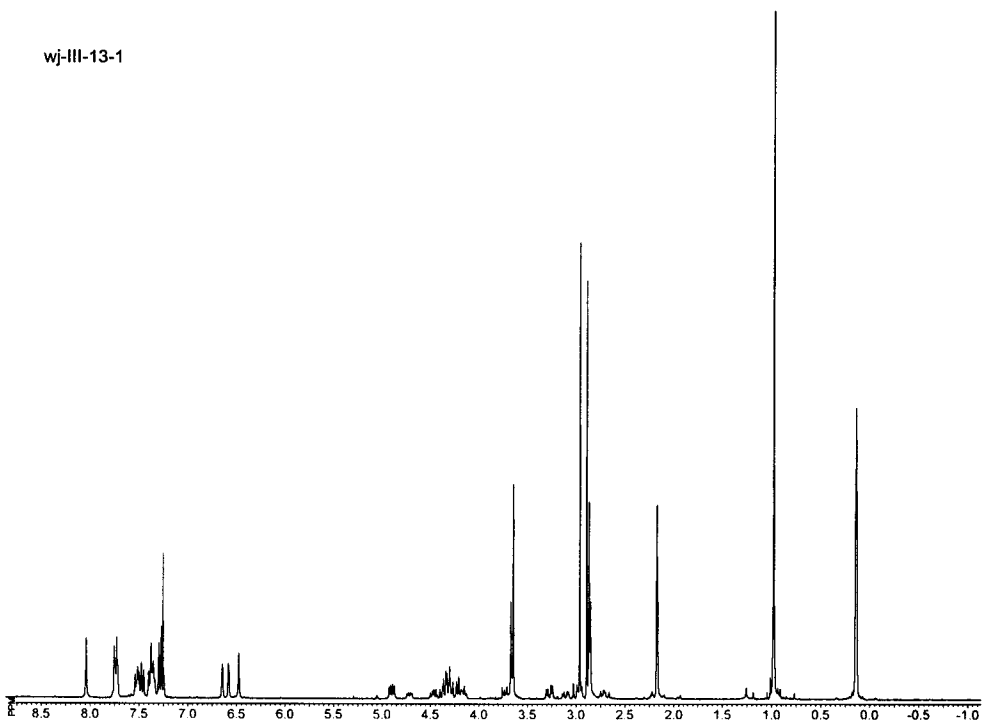
HRMS calcd for $C_{33}H_{41}NO_6Si$ 575.2703, found 575.2697;

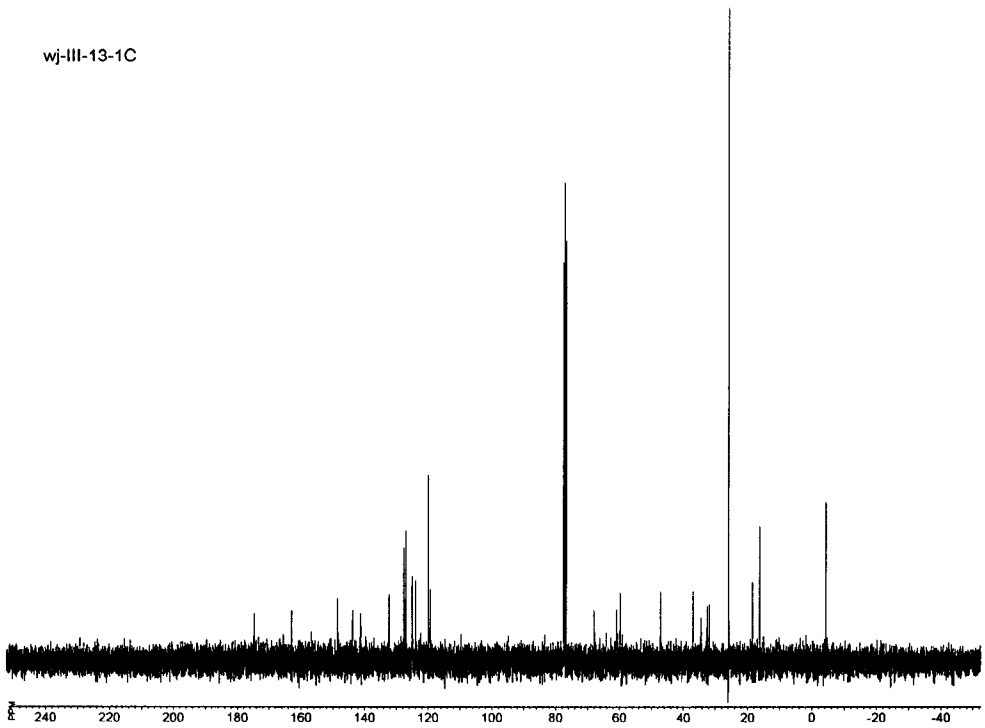
IR (neat, film): 2954, 2929, 2857, 1682, 1598, 1451, 1322, 1142, 839 cm^{-1};

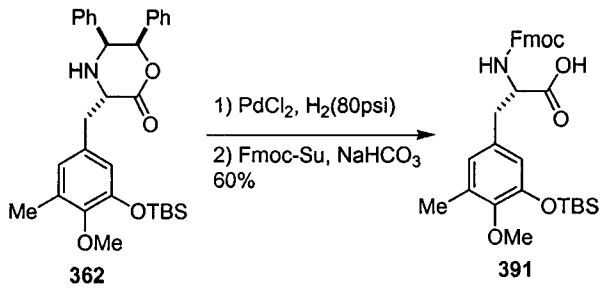
$[\alpha]_D^{25}$ = -40.8 (c = 0.5, CH_2Cl_2).



382







_N_-Fmoc-Amino acid 391 (3-[3-(tert-Butyl-dimethyl-silanyloxy)-4-methoxy-5-methyl-phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid):

To a solution of lactone **362** (127 mg, 0.245 mmol, 1.0 equiv) in absolute ethanol (62 mL) was added $PdCl_2$ (26 mg, 0.147 mmol, 0.6equiv), the resulting mixture was charged with hydrogen (80 psi) and stirred for 18 h. The catalyst was filtered off through Celite, the solvent of the filtrate was removed under reduced pressure to give crude amino acid hydrochloride salt (120 mg crude), which was used directly for next step without purification.

The amino acid salt obtained above (120mg, 0.245 mmol, 1.0equiv) was dissolved in methylene chloride: saturated sodium bicarbonate (1:1) (10 mL) and to this mixture was added Fmoc-OSu (99 mg, 0.294mmol, 1.2equiv). The resulting mixture was stirred at room temp. for 120 mim., then cooled to 0°C. The reaction mixture was adjusted to pH=2 with conc. HCl and then extracted with methylene chloride (5 mL x2). The organic layer was washed with water (10 mL), sat. NaCl solution (10 mL) and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluted with 10% methanol in CH_2Cl_2) to afford amino acid **391** (83 mg, 60% for two steps).

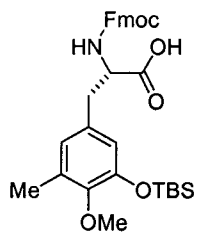
^{1}H NMR (300MH$_Z$, $CDCl_3$, 298K) (broad peak because of rotamer) δ 7.31-7.72 (8H, br), 6.54 (2H, s), 4.08-4.52 (3H, br), 3.68 (3H, s), 2.86-3.14 (2H, br), 2.13 (3H, s), 0.98 (9H, s), 0.08 (6H, s);

^{13}C NMR (75MHz, $CDCl_3$) δ 148.50, 143.75, 141.10, 132.23, 127.62, 127.08, 125.19, 124.46, 119.86, 67.58, 59.77, 50.92, 47.07, 29.94, 25.88, 18.39, 16.17, -4.3;

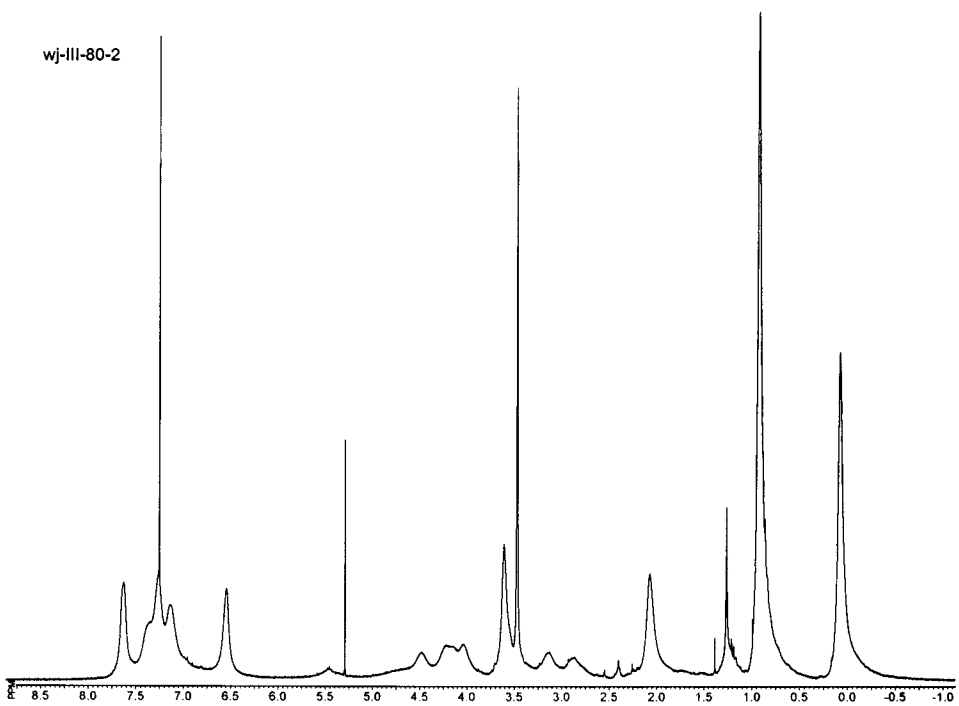
IR (neat, film): 3326, 2955, 2928, 2856, 1713, 1489, 1450, 1253, 1075, 839 cm^{-1};

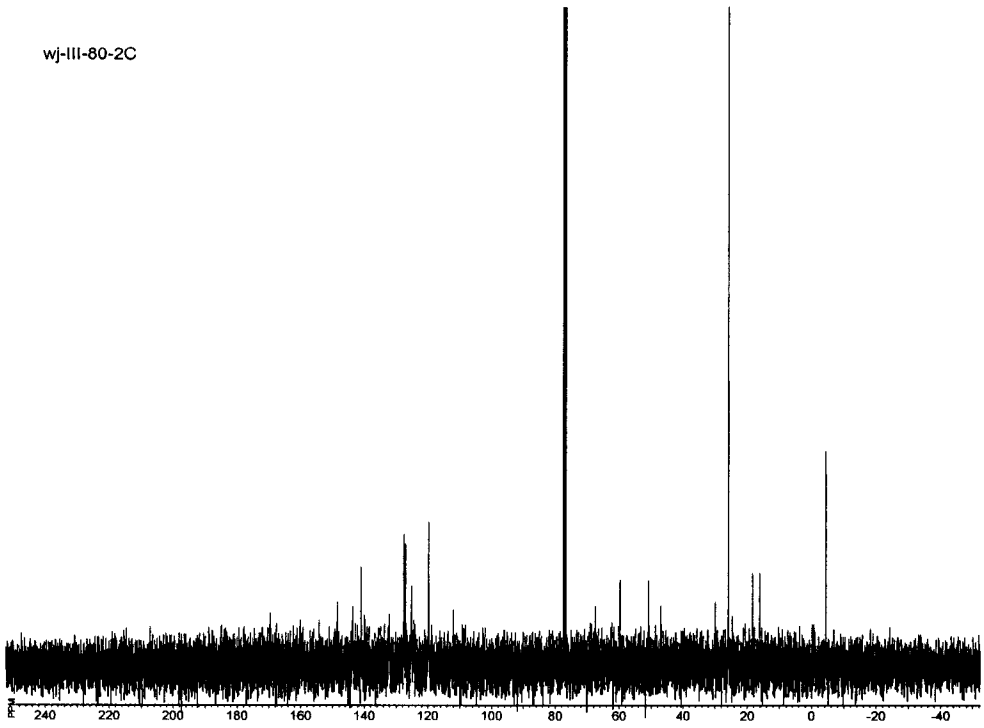
HRMS for sodium salt calcd for $C_{32}H_{39}NO_6SiNa$ 584.2444, found 584.2429;

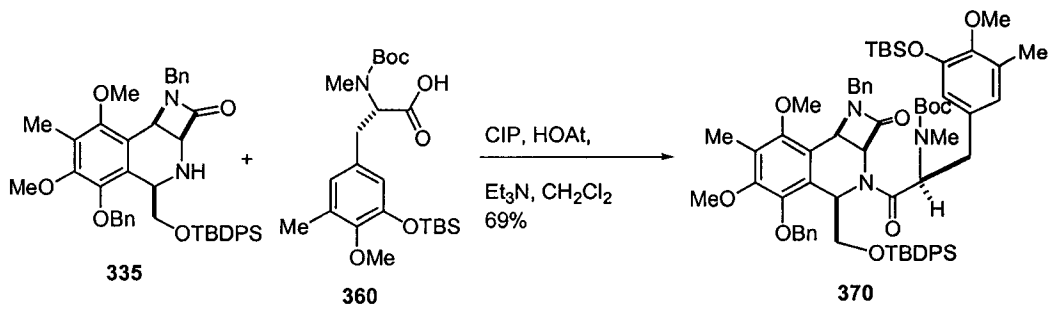
$[\alpha]_D^{25}$ =+34.0 (c = 0.6, CH_2Cl_2).



391







Peptide 370:

(Note: Both starting materials were azeotropically dried with toluene three times before use). To a solution of amino acid **360** (63 mg, 0.139 mmol, 1.0 equiv), HOAT (19.5 mg, 0.143 mmol, 1.03 equiv) in methylene chloride (5 mL) was added Et_3N (39.8 μL, 0.286 mmol, 2.06 equiv) at 0°C, then to this mixture was added CIP reagent (39.8 mg, 0.143 mmol, 1.03 equiv). The above solution was added to the solution of amine **335** (149 mg, 0.208 mmol, 1.5 equiv) in THF (5 mL) dropwise at 0°C, and the resulting solution was stirred at 0°C for 18 h., and stirred at room temp. for an additional 6 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (20 mL), sat. NaCl solution (20 mL) and dried with anhydrous sodium sulfate. After removal of the solvent the residue was purified by PTLC (eluted with 50 % EtOAc in hexanes) to afford peptide **370** (110 mg, 69%) along with 118 mg amine **335**.

^{1}H-NMR (400MHz, $CDCl_3$, 298K) δ 7.08-7.68 (20H, m), 6.46-6.67 (3H, m), 5.41 (1H, d), 5.23 (1H, d), 4.92 (1H, d), 4.84 (1H, d), 4.63 (1H, d), 4.45-4.49 (1H, d, *J*=14.8Hz), 3.73-3.84 (1H, d), 3.66 (3H, s), 3.64 (3H, s), 3.53 (3H, s), 3.00-3.06 (1H, t, *J*=14.0Hz), 2.89 (1H, t), 2.63 (3H, s), 2.20 (3H, s), 2.15 (3H, s), 1.23-1.38 (9H, m), 0.85-1.05 (18H, m), 0.15 (6H, s);

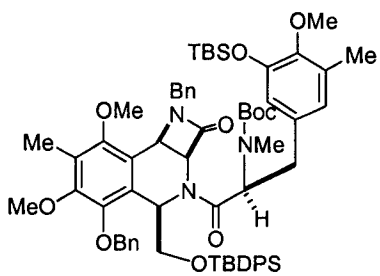
^{13}C NMR (100MHz, $CDCl_3$, 298K) δ 169.87, 166.89, 154.37, 154.28, 148.38, 145.3, 137.07, 136.53, 135.83, 133.65, 133.28, 132.01, 129.57, 129.42, 129.00, 128.93, 128.68,

128.62, 128.46, 128.26, 128.13, 127.64, 126.60, 125.02, 124.67, 120.46, 119.79, 80.41, 75.26, 65.53, 60.78, 60.36, 59.87, 58.16, 51.04, 47.85, 44.95, 34.90, 29.89, 28.87, 28.34, 28.00, 26.87, 19.52, 19.42, 18.40, 16.23, 9.45, -4.39;

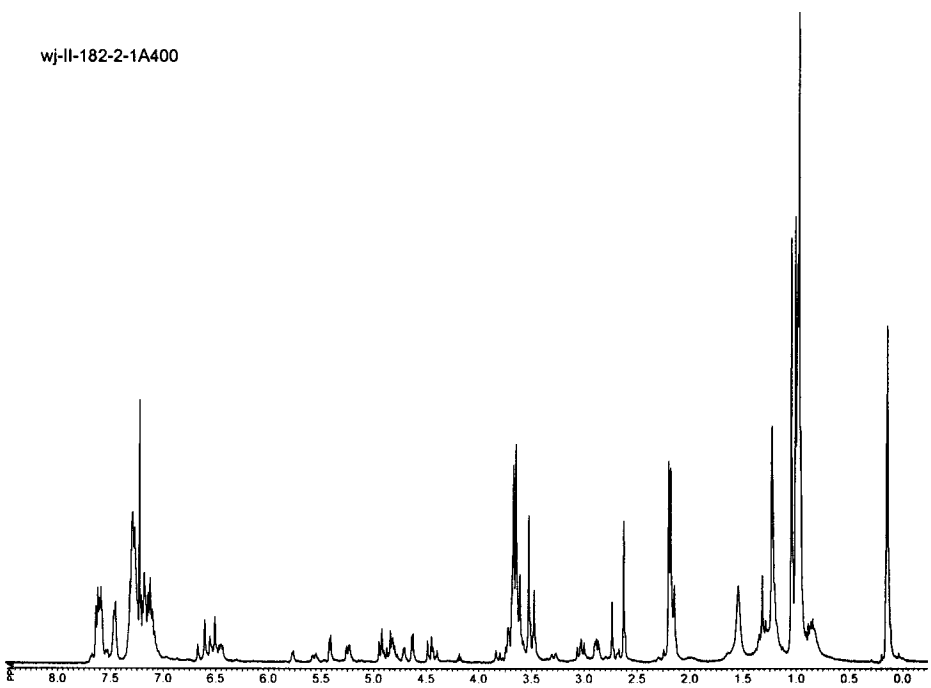
IR (neat, film): 2956, 2930, 2857, 1764, 1702, 1692, 1666, 1428, 1332, 1259, 1113, 838, 701 cm^{-1};

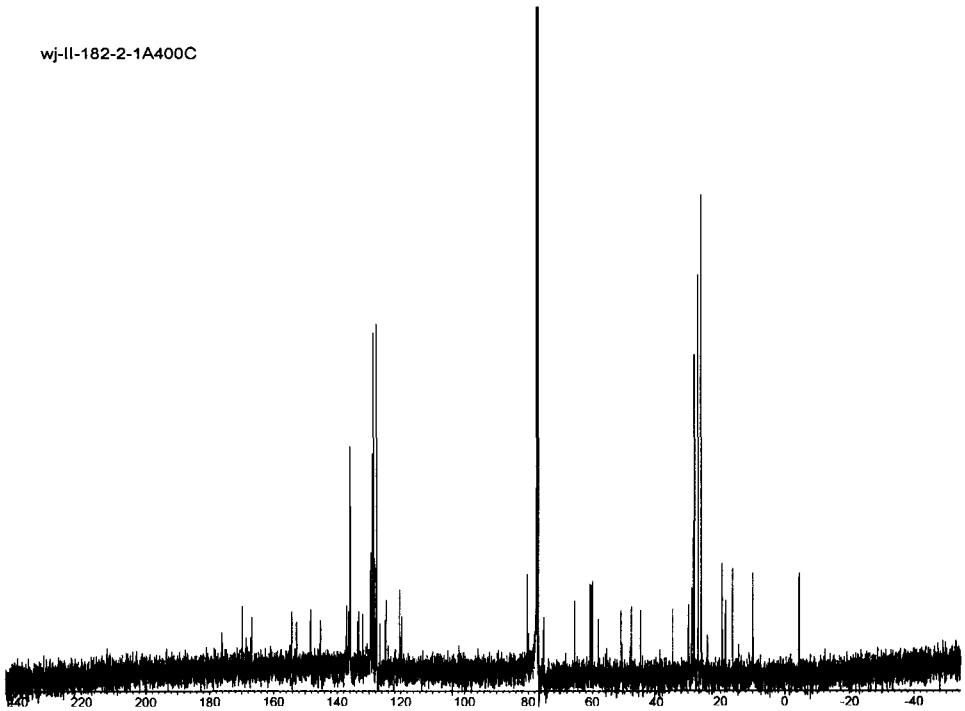
HRMS calcd for $C_{67}H_{86}N_3O_{10}Si_2$ 1148.5852, found 1148.5843;

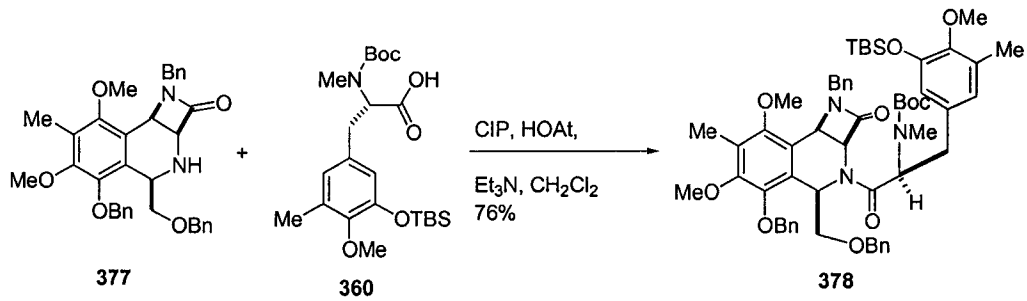
$[\alpha]_D^{25}$ =+65.57 (c = 0.61, CH_2Cl_2).



370







Peptide 378:

(Noted: Both starting materials were azeotropically dried with toluene three times before use). To a solution of amino acid **360** (57.2 mg, 0.126 mmol, 1.0 equiv), HOAT (17.7 mg, 0.130 mmol, 1.03 equiv) in methylene chloride (4.6 mL) was added DIPEA (45.2 μL, 0.260 mmol, 2.06 equiv) at 0°C, then to this mixture was added CIP reagent (36.2 mg, 0.130 mmol, 1.03 equiv). The above solution was added to the solution of amine **377** (107 mg, 0.189 mmol, 1.5equiv) in THF (4.6 mL) dropwise at 0°C, and the resulting solution was stirred at 0°C for 18 h. and then stirred at room temp. for an additional 6 h. The reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (20 mL), sat. NaCl solution (20 mL) and dried with anhydrous sodium sulfate. After removal of the solvent the residue was purified by PTLC (eluted with 50% EtOAc in hexanes) to afford peptide **378** (96 mg, 76%).

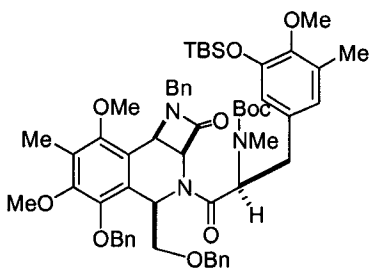
^{1}H-NMR (400MHz, $CDCl_3$, 298K) δ 7.21-7.58 (15H, m), 6.60 (1H, s), 6.52 (1H, s), 5.34-5.55 (1H, d, *J*=7.6Hz), 5.22-5.25 (1H, m), 5.02-5.05 (1H, d, *J*=14.0Hz), 4.93-4.96 (1H, d, *J*=14.0Hz), 4.80-4.82 (1H, d, *J*=7.2Hz), 4.47-4.56 (3H, m), 3.82 (3H, s), 3.67 (3H, s), 3.57 (3H, s), 3.50-3.84 (3H, m), 2.83 (3H, s), 2.24 (3H, s), 2.18 (3H, s), 1.22-1.38 (9H, m), 0.85-1.09 (9H, m), 0.16 (6H, s);

^{13}C NMR (100MHz, $CDCl_3$, 298K) δ 170.32, 167.09, 155.75, 154.45, 152.63, 148.15, 145.28, 138.76, 137.30, 136.52, 134.95, 133.31, 131.63, 128.76, 128.59, 128.50, 128.30, 128.12, 128.01, 127.86, 127.77, 127.59, 127.17, 126.37, 124.55, 120.51, 80.0, 75.12, 72.52, 72.41, 71.30, 60.79, 60.46, 59.82, 57.62, 48.33, 48.02, 44.67, 34.96, 29.83, 28.22, 27.90, 26.69, 25.86, 18.33, 16.11, 14.27, 9.77, -4.34, -4.40;

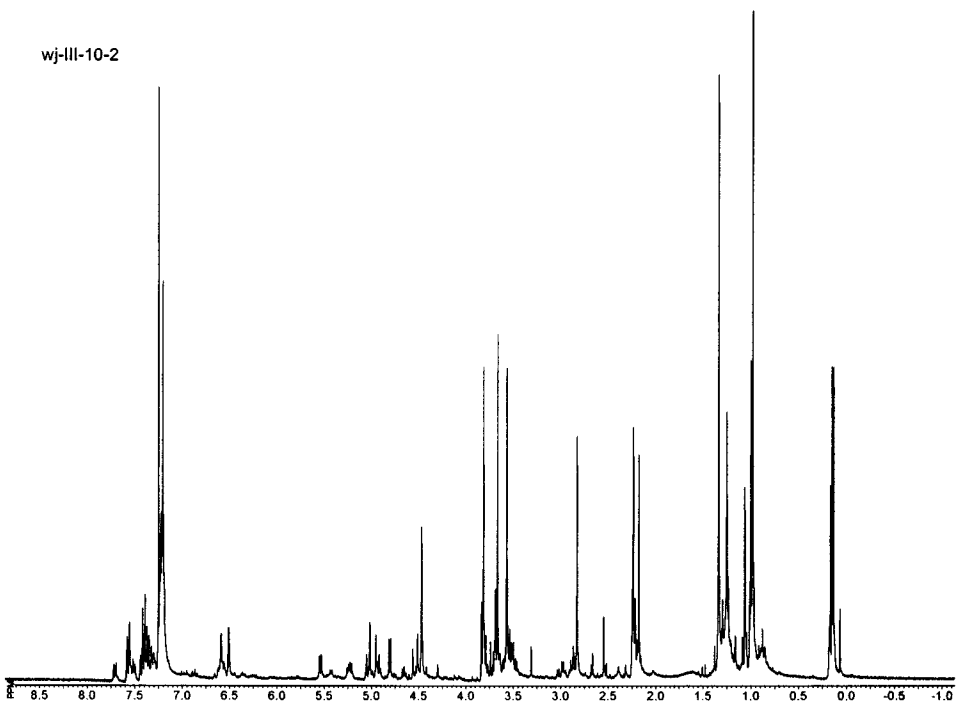
IR (neat, film): 2926, 2854, 1761, 1668, 1455, 1325, 1259, 1145, 1074, 839, 698 cm^{-1};

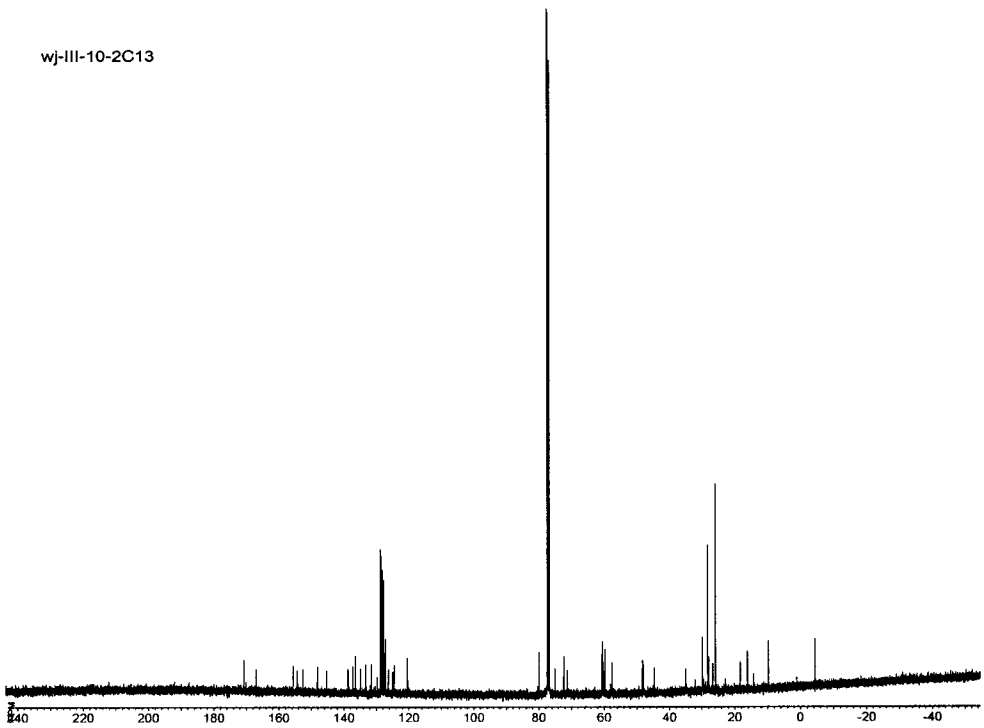
HRMS calcd for $C_{58}H_{74}N_3O_{10}Si$ 1000.5144, found 1000.5181;

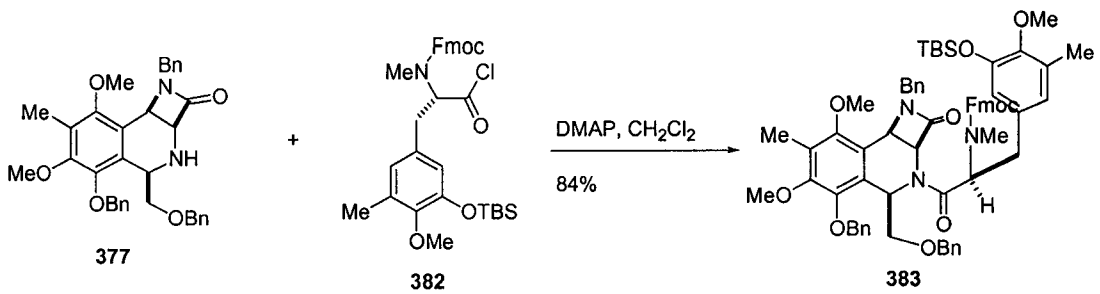
$[\alpha]_D$ +26.9 (c = 0.72, CH_2Cl_2).



378







Peptide 383

(Note: Both starting materials were azeotropically dried with toluene three times before use). To a solution of the amino acid **382** (60 mg, 0.104 mmol, 1.0 equiv), DMF (1.4 μL, 0.0156 mmol, 0.15 equiv) in methylene chloride (1.5 mL) was added $(COCl)_2$ (14 μL, 0.156 mmol, 1.5 equiv) at room temp. The resulting solution was stirred at room temp. for 60 min, and the solvent and excess $(COCl)_2$ was removed under reduced pressure. The resulting crude acid chloride was then dissolved in CH_2Cl_2 (1.5 mL) and was added to the solution of amine **377** (88.3 mg, 0.156 mmol, 1.5 equiv) and DMAP (14 mg, 0.115 mmol, 1.1 equiv) in CH_2Cl_2 (1.5 mL). The resulting solution was stirred at room temp. for 2 h, then quenched by addition of water, and the mixture was extracted with EtOAc (20 mL x2) and washed with H_2O (10 mL), sat. NaCl solution (10 mL) and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was purified by PTLC (eluted with 50% EtOAc in hexanes) to afford peptide **383** (97 mg, 84%).

^1H NMR (300 MHz, $CDCl_3$, 298K) 7.09-7.79 (23H, m), 6.69 (1H, s), 6.66 (1H, s), 6.56-7.03 (2H, m), 5.60-5.63 (1H, d, *J*=7.5Hz), 4.84-5.08 (5H, m), 4.37-4.52 (6H, m), 4.14-4.20 (4H, m), 3.89-3.94 (2H, d, *J*=15.0Hz), 3.83 (3H, s), 3.64 (3H, s), 3.54 (3H, s), 3.46-

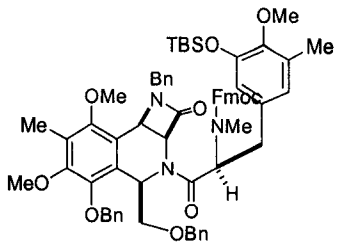
3.60 (3H, m), 3.20-3.49 (2H, m), 3.05 (3H, s), 2.80-2.90 (3H, m), 2.24 (3H, s), 2.12 (3H, s), 0.95 (9H, s), 0.13 (3H, s), 0.10 (3H, s);

^{13}C NMR (75 MHz, $CDCl_3$, 298K) δ 190.09, 172.33, 170.11, 167.03, 156.78, 154.41, 152.64, 148.32, 148.16, 145.28, 144.99, 144.17, 141.42, 141.24, 138.68, 137.26, 136.47, 133.17, 131.82, 128.77, 128.62, 128.44, 128.34, 128.17, 127.94, 127.65, 127.54, 127.24, 126.13, 125.82, 125.46, 125.09, 124.69, 123.52, 120.42, 119.95, 76.74, 75.14, 72.55, 71.34, 68.47, 60.89, 60.53, 60.45, 59.69, 58.28, 48.49, 48.35, 47.54, 45.07, 35.33, 29.91, 25.83, 18.29, 16.06, 9.78, -4.44;

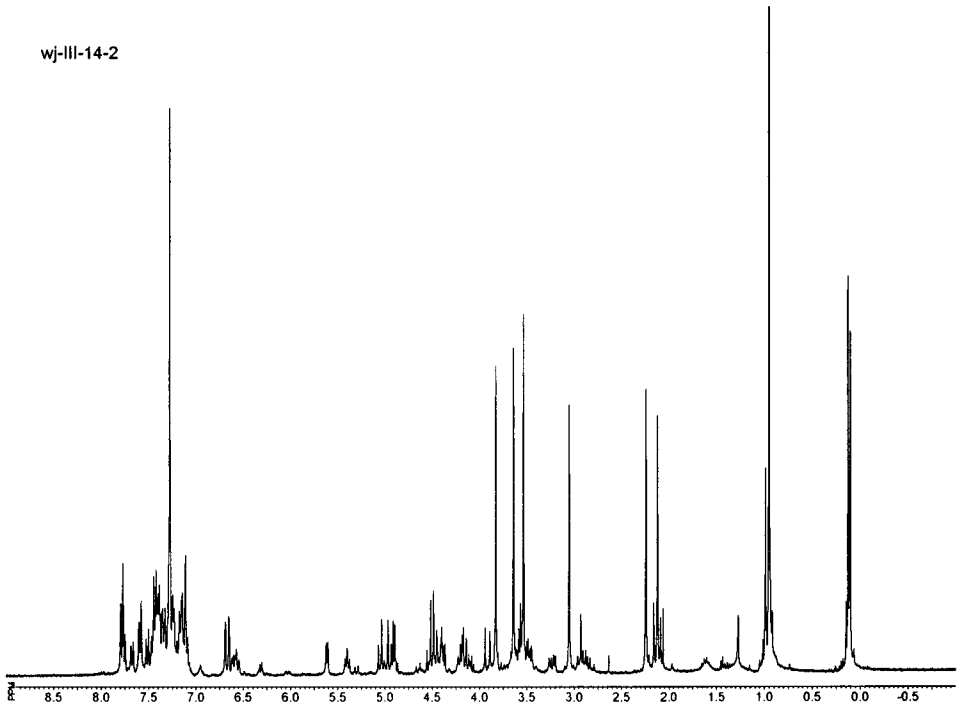
IR (neat, film): 2929, 2856, 1759, 1687, 1663, 1488, 1452, 1428, 1336, 1316, 1262, 1075, 839, 740, 698 cm^{-1};

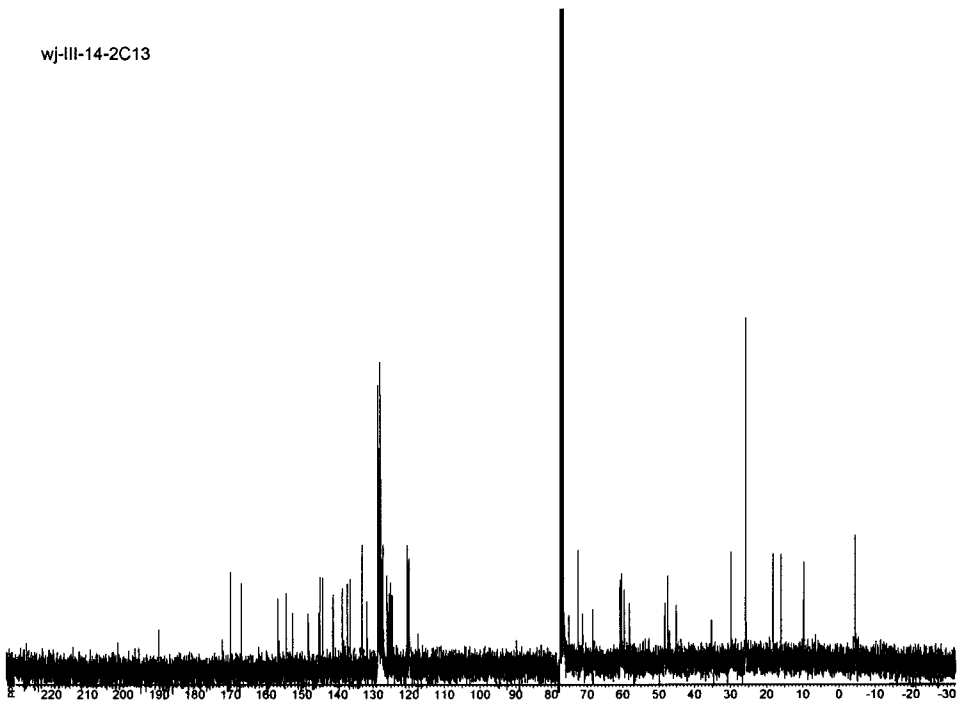
HRMS (FAB+) calcd for $C_{68}H_{76}N_3O_{10}Si$ 1122.5300, found 1122.5321;

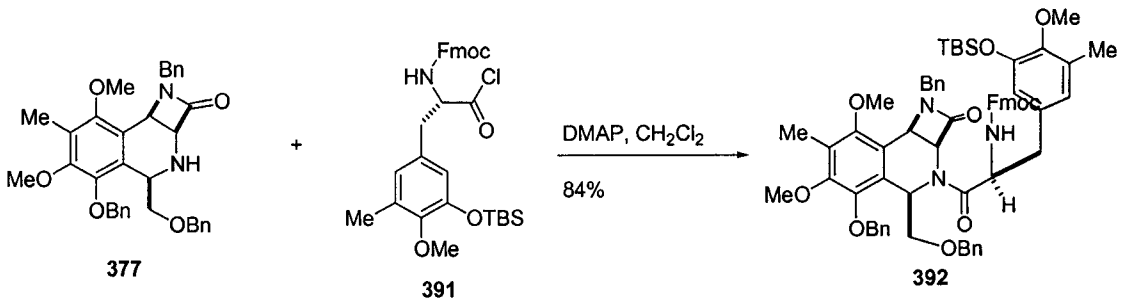
$[\alpha]_D^{25}$ = -10.0 (*c* 0.54, CH_2Cl_2).



383







Peptide 392:

(Note: Both starting materials were azeotropically dried with toluene three times before use). To a solution of the amino acid **391** (53 mg, 0.094 mmol, 1.0 equiv), DMF (2.0 μL, 0.0141 mmol, 0.15 equiv) in methylene chloride (2.5 mL) was added $(COCl)_2$ (20 μL, 00141 mmol, 1.5 equiv) at room temp. The resulting solution was stirred at room temp. for 30 min., and the solvent and excess $(COCl)_2$ was removed under reduced pressure. The resulting crude acid chloride was then dissolved in CH_2Cl_2 (2.5 mL) and was added to the solution of amine **377** (53 mg, 0.141 mmol, 1.0 equiv) and DMAP (13 mg, 0.110 mmol, 1.1 equiv) in CH_2Cl_2 (2.5 mL). The resulting solution was stirred at room temp. for 2 h, then quenched by the addition of water, and the mixture was extracted with EtOAc (20 mL x2) and washed with H_2O (10 mL), sat. NaCl solution (10 mL) and dried with anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was purified by flash column chromatography (eluted with 50 % EtOAc in hexanes) to afford peptide **392** (85 mg, 84%).

^1H NMR (300 MHz, $CDCl_3$, 298K) 7.18-7.77 (23H, m), 6.54 (2H, d, *J*=2.5Hz), 6.35-6.40 (2H, m), 6.08-6.09 (1H, d, *J*=8.4Hz), 4.88-5.20 (6H, m), 4.69-4.73 (1H, d, *J*=15.0Hz), 4.50-4.55 (1H, d, *J*=14.4Hz), 4.34-4.47 (3H, m), 3.96-4.13 (3H, m), 3.77 (3H, s), 3.71

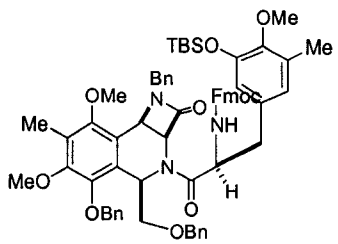
(3H, s), 3.57 (3H, s), 3.54 (3H, s), 3.71-3.84 (3H, m), 3.46-3.63 (2H, m), 3.11-3.16 (1H, d, *J*=13.2Hz), 2.83-2.86 (2H, m), 2.25 (3H, s), 2.16 (3H, s), 0.99 (9H, s), 0.16 (6H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 173.72, 168.19, 155.73, 154.63, 152.59, 148.32, 145.24, 144.13, 143.71, 141.18, 137.95, 136.39, 132.76, 132.05, 128.72, 128.41, 128.12, 127.74, 127.08, 125.09, 125.13, 124.62, 124.24, 120.38, 119.96, 75.12, 74.76, 73.52, 71.39, 71.13, 67.20, 60.82, 60.53, 60.33, 59.86, 57.25, 53.74, 53.01, 51.90, 48.88, 48.07, 47.84, 47.40, 47.00, 45.27, 44.71, 39.95, 38.42, 37.89, 29.84, 25.87, 18.37, 16.26, 15.55, 9.76, -4.43;

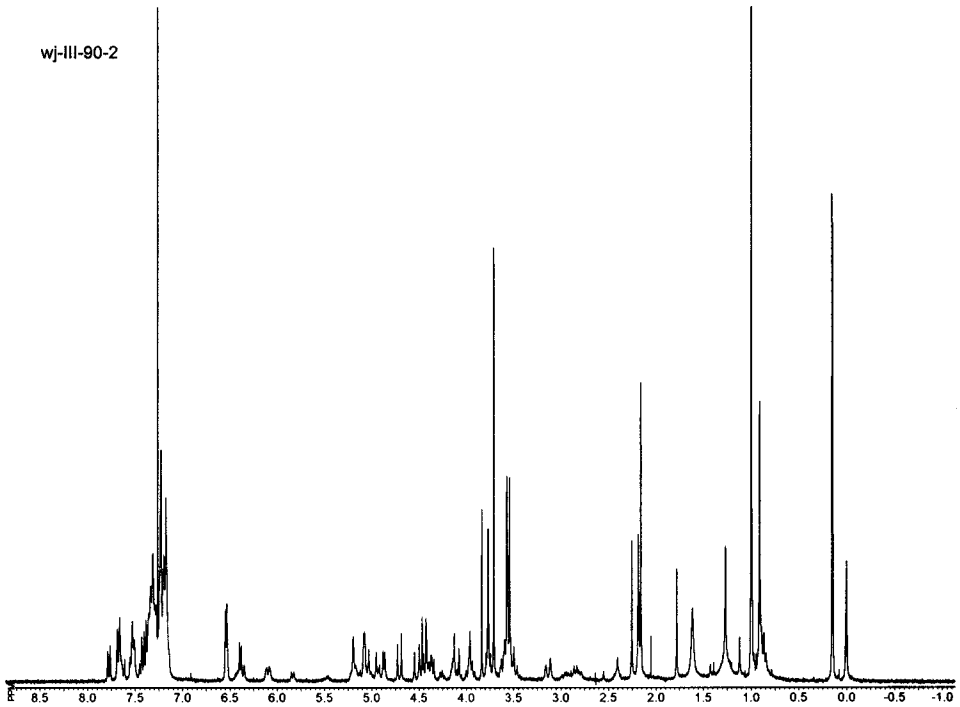
IR (neat, film): 3290, 2929, 2857, 1759, 1711, 1633, 1489, 1449, 1428, 1339, 1249, 1076, 839, 739 cm^{-1};

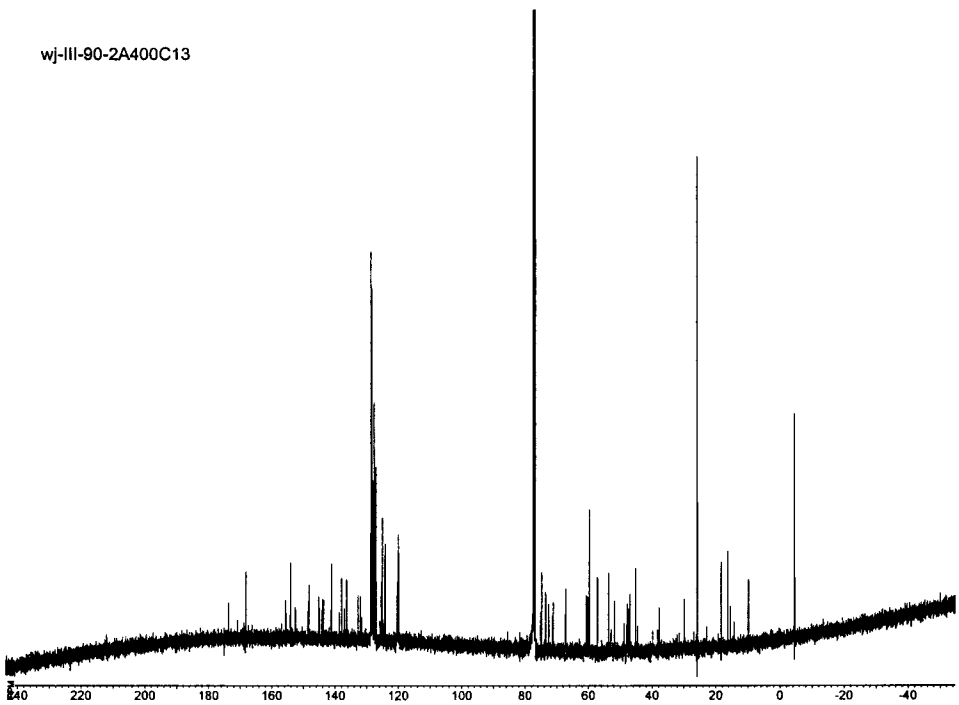
HRMS (FAB+) calcd for $C_{67}H_{74}N_3O_{10}Si$ 1108.5144, found 1108.5124;

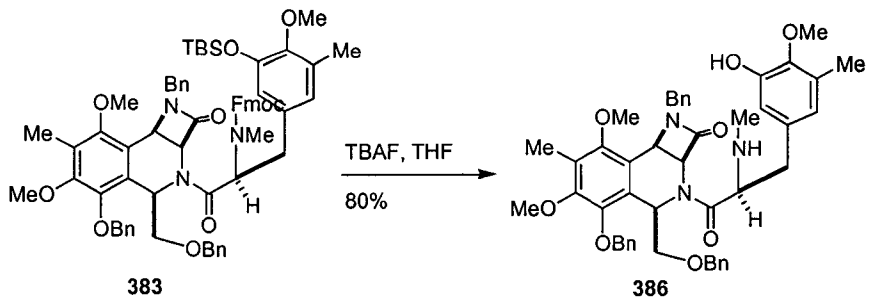
$[\alpha]_D^{25}$ = +42.7 (*c* 0.48, CH_2Cl_2).



392







383 386

Amine 386 (1-Benzyl-5-benzyloxy-4-benzyloxymethyl-3-[3-(3-hydroxy-4-methoxy-5-methyl-phenyl)-2-methylamino-propionyl]-6,8-dimethoxy-7-methyl-2a,3,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalen-2-one):

To a solution of **383** (10 mg, 0.0089 mmol, 1.0 equiv) in THF (1 mL) was added TBAF (1M in THF) (44.5 μL, 0.0445 mmol, 5.0 equiv). The resulting solution was stirred at room temp. for 30 min. The reaction was quenched with sat. NH_4Cl solution, and extracted with EtOAc (5 mL x2). The combined organic layers were washed with water (5 mL), sat. NaCl solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by PTLC (eluted with 15% methanol in EtOAc) to afford **386** (5.6 mg, 80%).

^{1}H NMR (300 MHz, $CDCl_3$, 273K) 7.56-7.58 (2H, d, *J*=6.9Hz), 7.18-7.45 (13H, m), 6.55 (1H, s), 6.37-6.53 (1H, m), 6.35 (1H, s), 5.00-5.04 (2H, m), 4.82-4.86 (2H, m), 4.33-4.60 (4H, m), 4.22-4.24 (1H, d, *J*=4.8Hz), 4.00-4.20 (2H, m), 3.83 (3H, s), 3.71-3.78 (5H, m), 3.59 (3H, s), 3.51 (3H, s), 3.41-3.49 (3H, m), 2.70-2.96 (3H, m), 2.35 (3H, s), 2.22 (3H, s), 2.20 (3H, s);

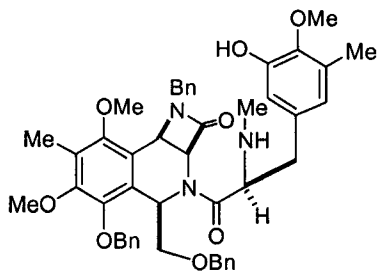
^{13}C NMR (75 MHz, $CDCl_3$, 273K) δ 190.09, 174.46, 168.94, 166.81, 154.26, 152.71, 152.42, 148.92, 145.12, 144.80, 144.05, 138.49, 137.66, 137.11, 136.43, 135.03, 133.15, 130.87, 128.81, 128.52, 128.12, 127.57, 127.31, 125.80, 124.77, 123.30, 120.74, 119.61,

114.24, 76.74, 76.51, 75.15, 74.83, 73.43, 72.71, 71.50, 61.92, 60.58, 60.14, 59.90, 57.26, 52.86, 48.51, 48.09, 47.75, 45.23, 45.02, 39.45, 36.82, 34.02, 32.75, 29.89, 16.03, 15.43, 9.93;

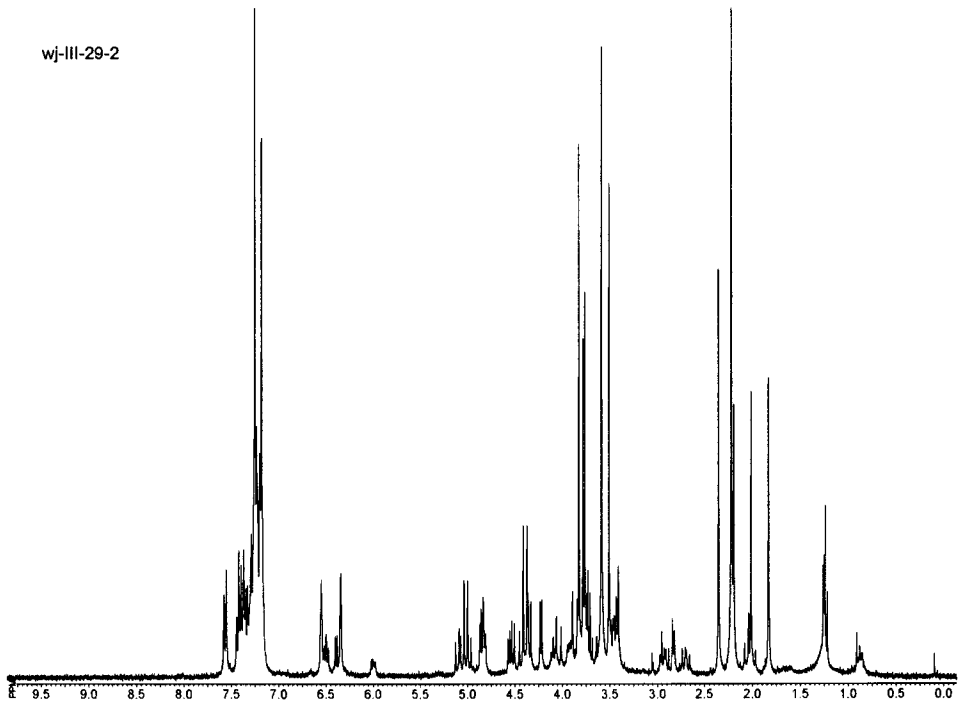
IR (neat, film): 3306, 3031, 2928, 2858, 1758, 1652, 1496, 1454, 1416, 1346, 1263, 1106, 1077, 1009, 735, 699 cm^{-1};

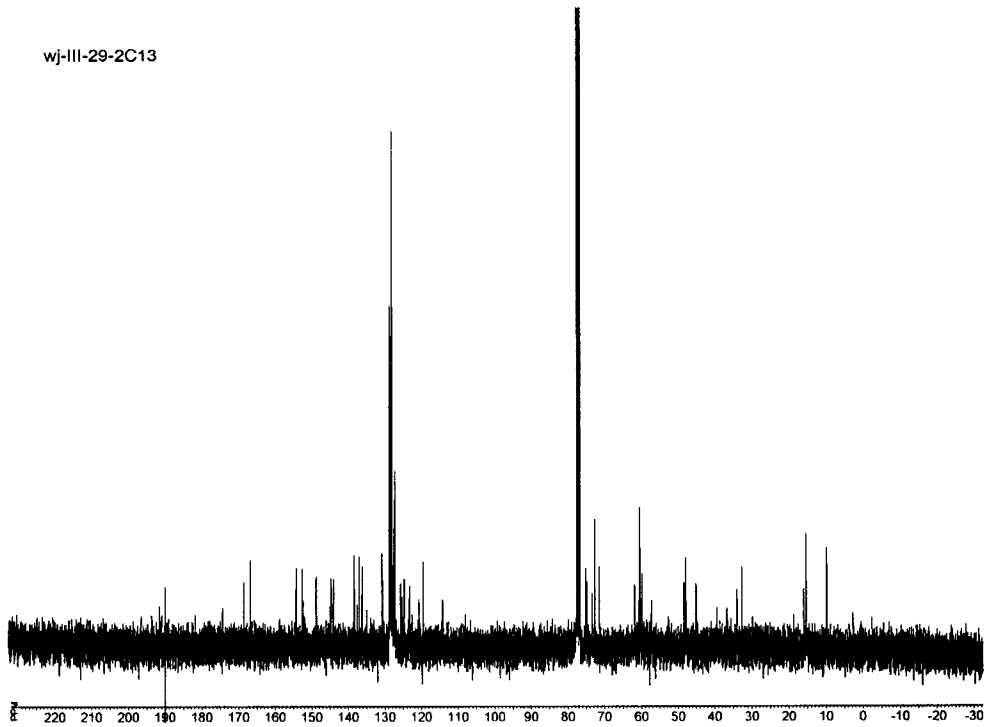
HRMS (FAB+) calcd for $C_{47}H_{52}N_3O_8$ 786.3754, found 786.3780;

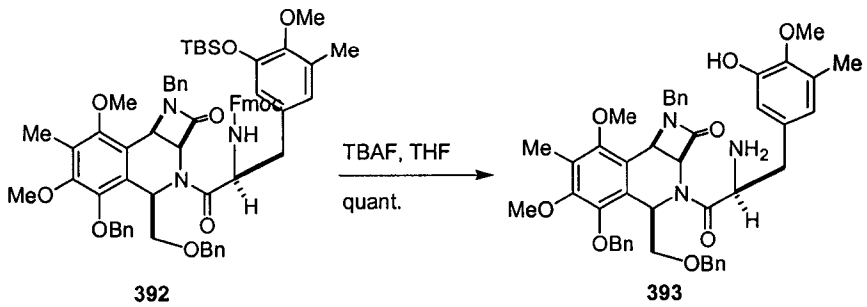
$[\alpha]_D^{25}$ = +41.5 (*c* 0.61, CH_2Cl_2).



386







392 393

Amine 393 (3-[2-Amino-3-(3-hydroxy-4-methoxy-5-methyl-phenyl)-propionyl]-1-benzyl-5-benzyloxy-4-benzyloxymethyl-6,8-dimethoxy-7-methyl-2a,3,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalen-2-one)

To a solution of **392** (20 mg, 0.018 mmol, 1.0 equiv) in THF (1 mL) was added TBAF (1M in THF) (180 μL, 0.180 mmol, 10.0 equiv). The resulting solution was stirred at room temp. for 30 min. The reaction was quenched with sat. NH_4Cl solution, and extracted with EtOAc (10 mL x2). The combined organic layers was washed with water (10 mL), sat. NaCl solution (10 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by PTLC (eluted with 15% methanol in EtOAc) to afford **393** (13.9 mg, quant.).

^{1}H NMR (300 MHz, $CDCl_3$, 273K) 7.07-7.52 (15H, m), 6.42 (1H, s), 6.32-6.34 (1H, d, *J*=4.8Hz), 6.23 (1H, s), 5.83-5.84 (1H, t, *J*=5.2Hz), 5.01-5.13 (2H, m), 4.82-4.95 (2H, m), 4.62-4.66 (1H, d, *J*=11.4Hz), 4.51-4.56 (1H, d, *J*=15.0Hz), 4.38-4.41 (1H, d, *J*=11.4Hz), 4.04-4.09 (1H, d, *J*=14.7Hz), 3.83 (3H, s), 3.77 (3H, s), 3.34-3.63 (2H, m), 3.52 (3H, s), 3.31-3.36 (5H, m), 2.93-2.99 (3H, m), 2.22 (3H, s), 2.20 (3H, s);

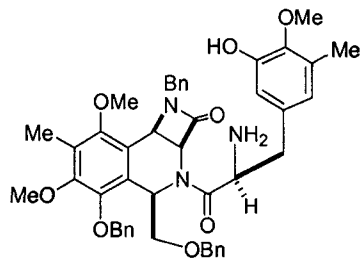
^{13}C NMR (75 MHz, $CDCl_3$, 273K) δ 168.59, 154.28, 152.29, 148.78, 145.31, 138.34, 137.73, 136.40, 130.67, 128.73, 128.68, 128.42, 128.38, 128.07, 127.76, 127.54, 125.32,

123.57, 114.55, 74.99, 73.62, 71.40, 60.82, 60.60, 60.27, 59.12, 57.17, 53.14, 52.20, 47.77, 45.19, 25.23, 24.28, 20.30, 19.88, 15.98, 13.81, 13.68, 9.75;

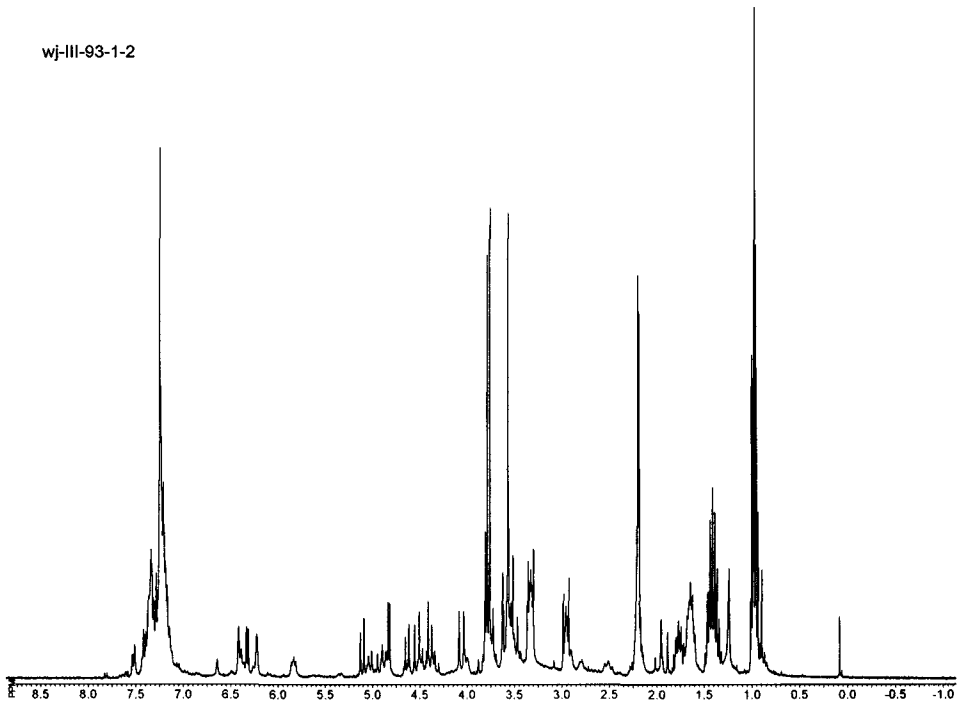
IR (neat, film): 3306, 3031, 2960, 2874, 1754, 1652, 1496, 1454, 1415, 1338, 1266, 1122, 1076, 1022, 735, 699 cm^{-1};

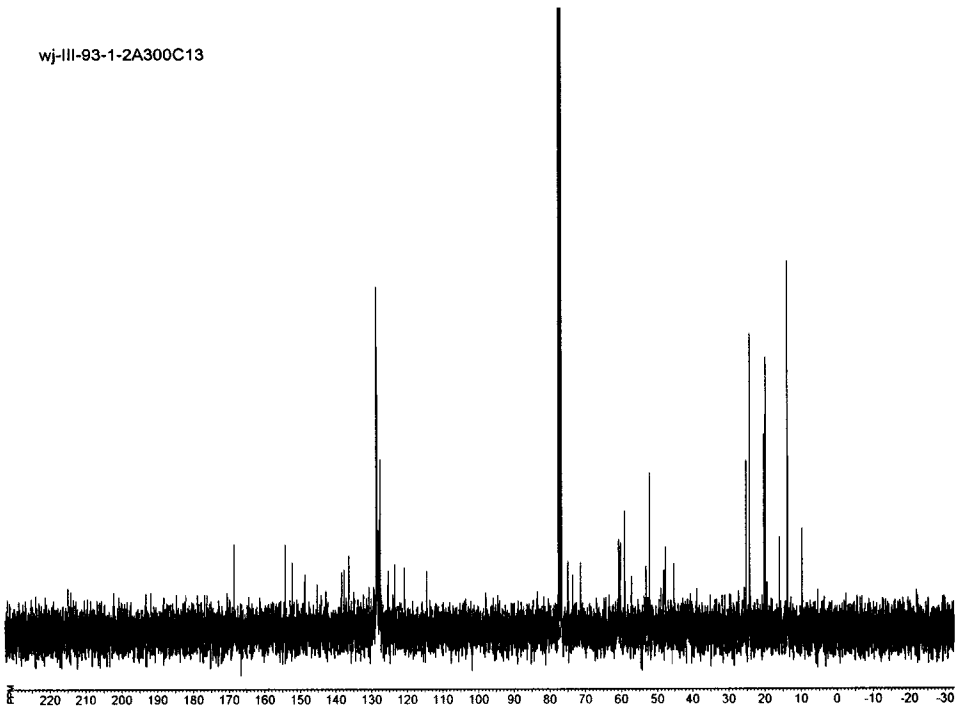
HRMS (FAB+) calcd for $C_{46}H_{50}N_3O_8$ 772.3598, found 772.3589;

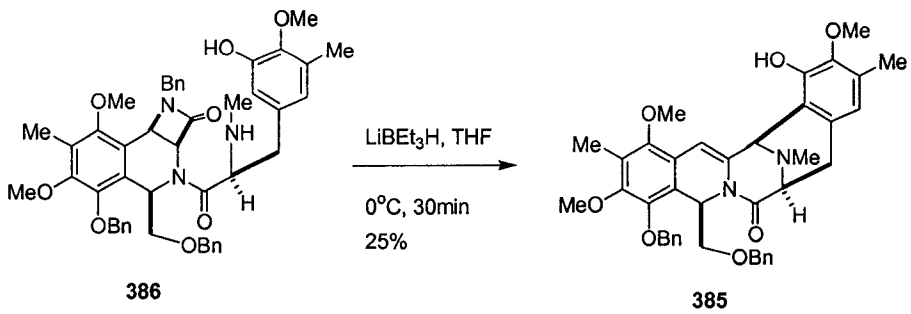
$[\alpha]_D^{25}$ = +44.3 (*c* 0.42, CH_2Cl_2).



393







386 385

Pentacycle 385:

To a solution of **386** (5.6 mg, 0.00713 mol, 1.0 equiv) in THF (0.5 mL) was added $LiBEt_3H$ (1M in THF) (42.8 μL, 0.0428 mol, 6.0 equiv) at 0°C, and the reaction was stirred at 0°C for 10 min. and then quenched by addition of sat. NH_4Cl solution. The resulting mixture was stirred at room temp. for 10 min., and diluted with EtOAc (10 mL). The organic layer was washed with water (5 mL), sat. NaCl solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by PTLC (eluted with EtOAc) to afford **385** (1.6 mg, 49%).

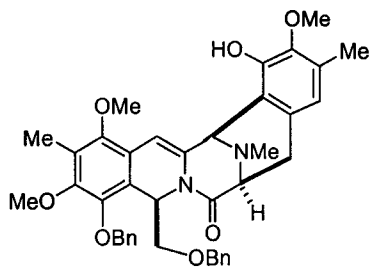
^1H NMR (400 MHz, $CDCl_3$, 298K) 7.20-7.46 (8H, m), 6.91-6.94 (2H, m), 6.36-6.50 (1H, br), 6.24-6.30 (1H, m), 6.19 (1H, s), 5.84 (1H, br), 5.34 (1H, s), 5.02 (2H, s), 3.78 (3H, s), 3.77 (3H, s), 3.68 (3H, s), 2.96-3.07 (3H, m), 2.30-2.37 (1H, t, *J*=9.4Hz), 2.21 (3H, s), 2.10 (3H, s), 2.03 (3H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 182.81, 176.33, 144.62, 137.17, 128.70, 128.52, 128.30, 128.06, 127.15, 126.68, 122.44, 112.91, 105.19, 95.39, 75.19, 72.21, 70.19, 60.95, 60.53, 50.83, 36.95, 33.54, 32.07, 29.84, 29.50, 29.39, 29.21, 24.87, 22.83, 17.77, 15.95, 14.25, 9.46;

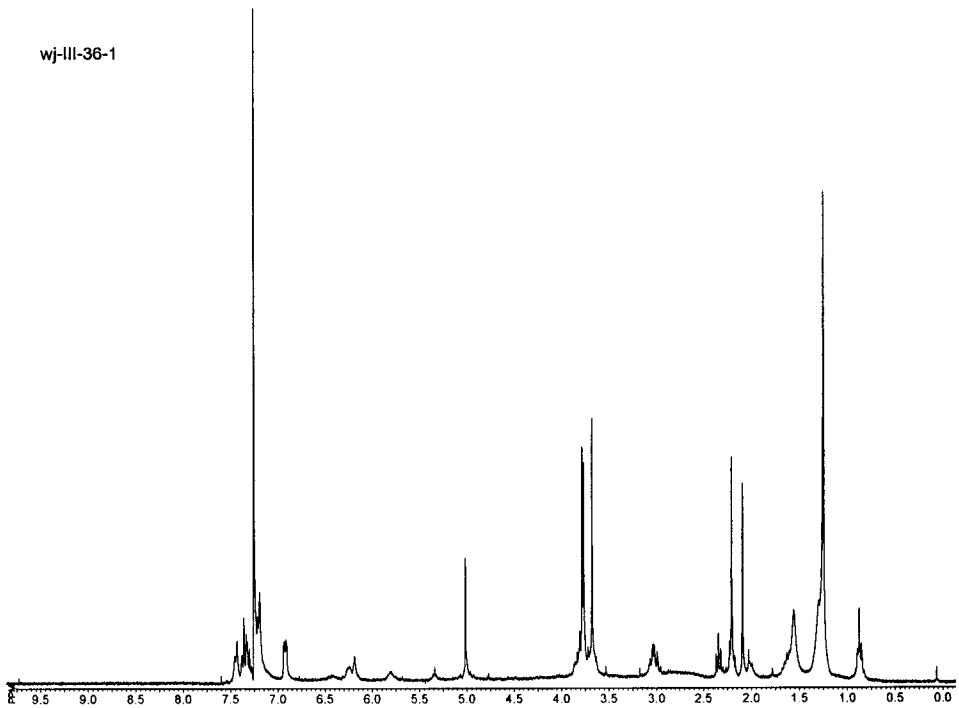
IR (neat, film): 3328, 3029, 2926, 2853, 1675, 1636, 1455, 1416, 1339, 1237, 1124, 1060, 1004, 735, 698 cm^{-1};

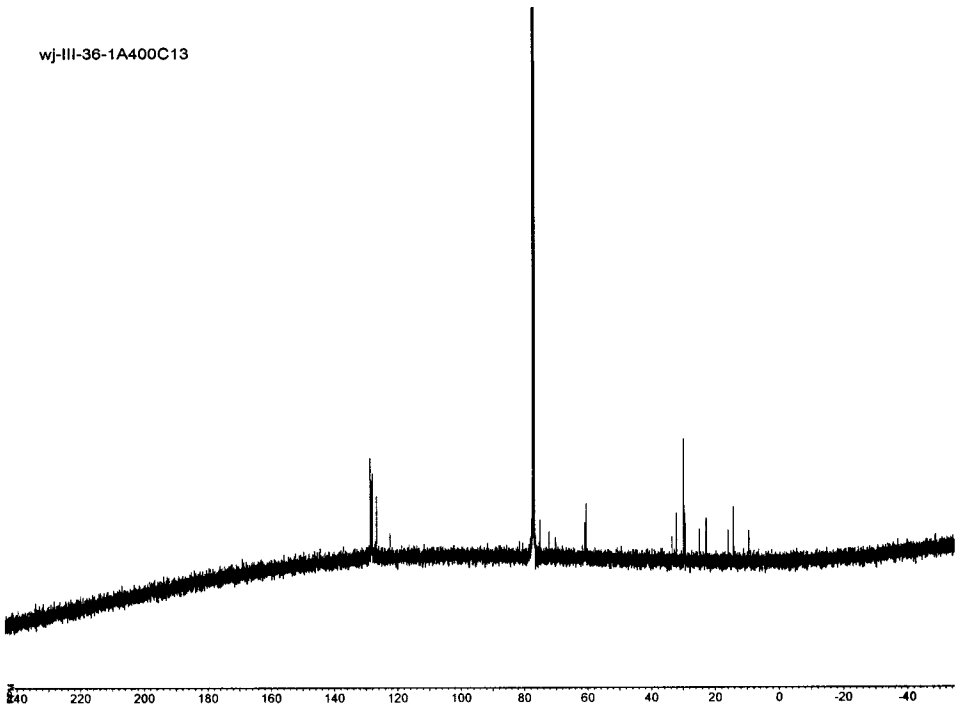
HRMS (FAB+) calcd for $C_{40}H_{43}N_2O_7$ 663.3070, found 663.3066;

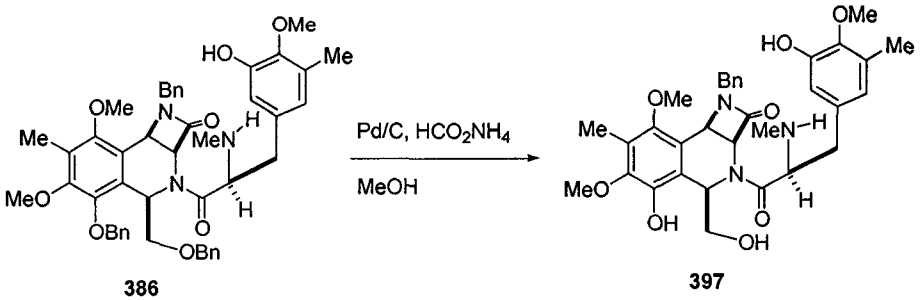
$[\alpha]_D^{25}$ = -5.5 (*c* 0.44, CH_2Cl_2).



385







386 397

Triol 397 ((-Benzyl-5-hydroxy-3-[3-(3-hydroxy-4-methoxy-5-methyl-phenyl)-2-methylamino-propionyl]-4-hydroxymethyl-6,8-dimethoxy-7-methyl-2a,3,4,8b-tetrahydro-1H-1,3-diaza-cyclobuta[a]naphthalen-2-one)

To a solution of peptide **386** (63 mg, 0.08 mmol, 1.0 equiv) in methanol (6 mL) was added HCO_2NH_4 (500 mg, 8.90 mmol, 100.0 equiv) and Pd/C (10%) (29 mg, 0.024 mmol, 0.3 equiv). The resulting mixture was heated to reflux for 6 h. The solid was filtered off through Celite. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (eluted with 10% methanol in CH_2Cl_2) to afford **397** (49 mg, quant.).

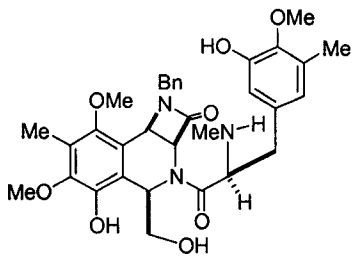
^{1}H NMR (300 MHz, $CDCl_3$, 273K) 7.25-7.27 (5H, m), 6.60 (1H, s), 6.33-6.34 (1H, d, *J*=4.2Hz), 5.76 (1H, m), 5.23 (1H, s), 4.86-4.88 (1H, d, *J*=5.7Hz), 4.52-4.57 (1H, d, *J*=15.0Hz), 4.26 (1H, m), 3.96-4.01 (1H, d, *J*=15.0Hz), 3.78 (3H, s), 3.72 (3H, s), 3.61-3.62 (1H, m), 3.55 (3H, s), 3.47 (1H, m), 2.95 (3H, s), 2.23 (3H, s), 2.07 (3H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 273K) δ 173.67, 169.68, 151.14, 147.32, 146.59, 144.09, 142.27, 135.91, 130.43, 128.70, 128.57, 127.77, 127.26, 124.88, 123.77, 119.89, 118.85, 113.73, 62.24, 61.08, 56.73, 53.72, 53.08, 52.21, 48.28, 45.42, 31.72, 29.84, 25.27, 20.38, 13.94, 11.23, 10.01;

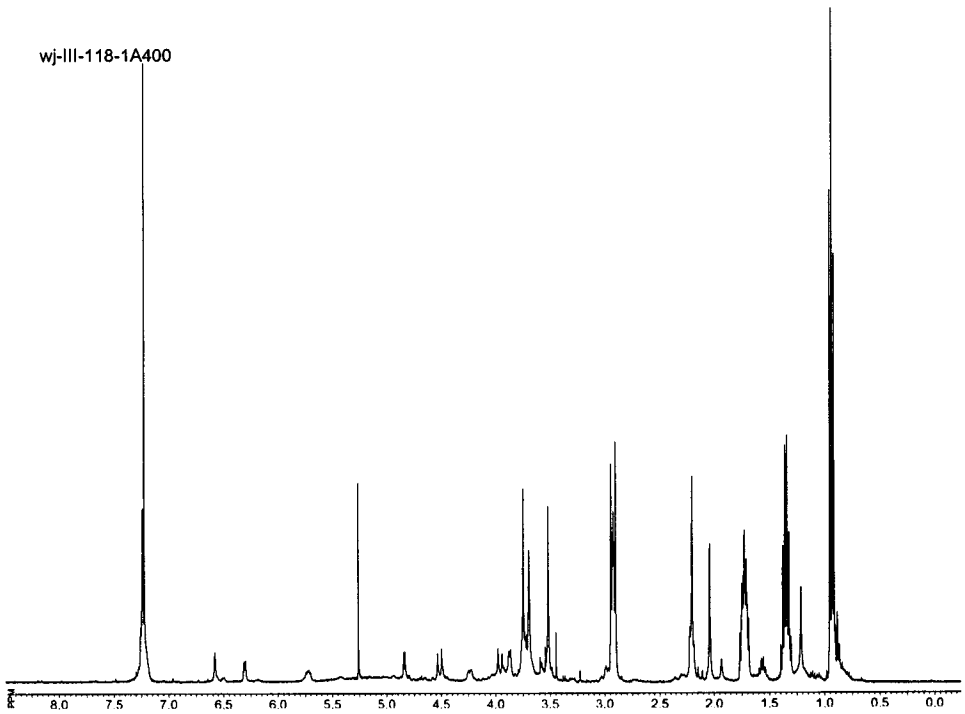
IR (neat, film): 3111, 2959, 2933, 2873, 1740, 1654, 1456, 1415, 1344, 1263, 1120, 1069, 1018 cm^{-1};

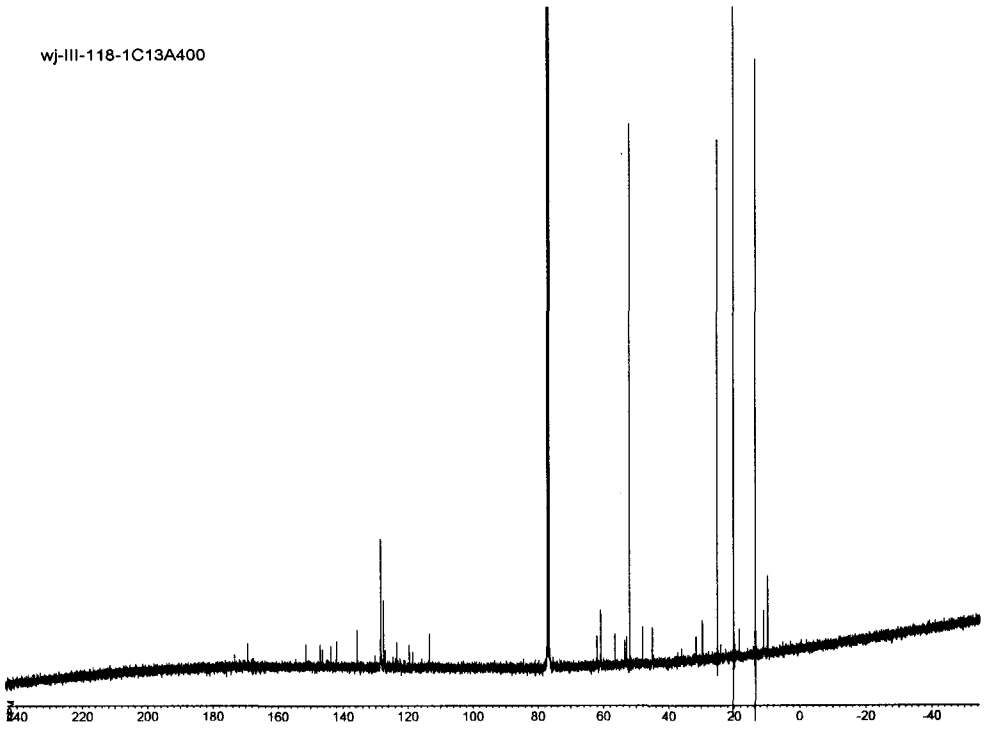
HRMS (FAB+) calcd for $C_{33}H_{38}N_3O_8$ 604.2659, found 604.2654;

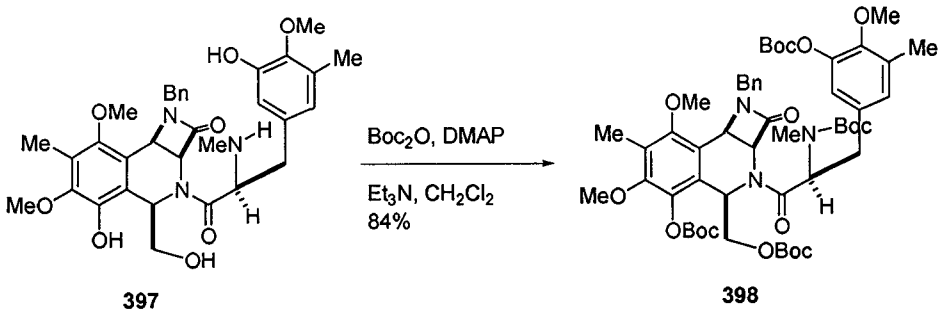
$[\alpha]_D^{25}$ = +14.4 (*c* 0.52, CH_2Cl_2).



397







397 398

Peptide 398:

To a solution of triol **397** (49 mg, 0.081 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added Boc_2O (177 mg, 0.81 mmol, 10.0 equiv). The resulting solution was stirred at room temp. for 30 min. Next, Et_3N (135 μL, 0.97 mmol, 12.0 equiv) and DMAP (1.0 mg, 0.008 mmol, 0.1 equiv) were added and the solution was stirred at room temp. for 8 h. The mixture was diluted with EtOAc (50 mL) and washed with water (10 mL x2), saturated NaCl solution (20 mL), dried over anhydrous sodium sulfite. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (eluted with 30% EtOAc in hexanes) to give **398** 68 mg (84%).

^1H NMR (300 MHz, $CDCl_3$, 273K) 7.27-7.28 (5H, m), 6.83 (1H, s), 6.68-6.37 (1H, m), 5.67-5.68 (1H, d, *J*=5.4Hz), 5.37-5.39 (1H, d, *J*=6.3Hz), 5.07-5.13 (1H, d, *J*=18.0Hz), 4.91-4.93 (1H, d, *J*=5.7Hz), 4.58-4.63 (1H, d, *J*=15.0Hz), 4.27 (1H, s), 4.22-4.23 (1H, d, *J*=5.7Hz), 3.85-3.96 (2H, m), 3.79 (3H, s), 3.74 (3H, s), 3.60 (3H, s), 3.15-3.20 (1H, m), 2.98-3.00 (1H, m), 2.25 (3H, s), 2.19 (3H, s), 1.56 (18H, br), 1.26 (18, br);

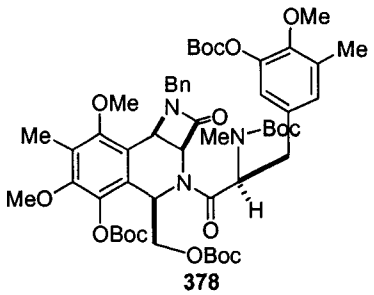
^{13}C NMR (100 MHz, $CDCl_3$, 273K) δ 168.75, 166.66, 156.24, 153.26, 152.16, 152.00, 151.36, 147.64, 142.42, 138.48, 136.52, 130.72, 129.59, 128.74, 128.54, 127.65, 125.66, 124.51, 120.49, 120.31, 84.15, 83.39, 81.79, 81.22, 66.56, 60.99, 60.91, 60.81, 60.53,

60.33, 52.25, 50.66, 48.06, 47.42, 44.80, 41.48, 37.53, 32.07, 30.17, 29.84, 29.50, 28.42, 27.84, 27.77, 27.70, 22.83, 20.34, 14.35, 14.27, 13.71, 11.61, 10.01;

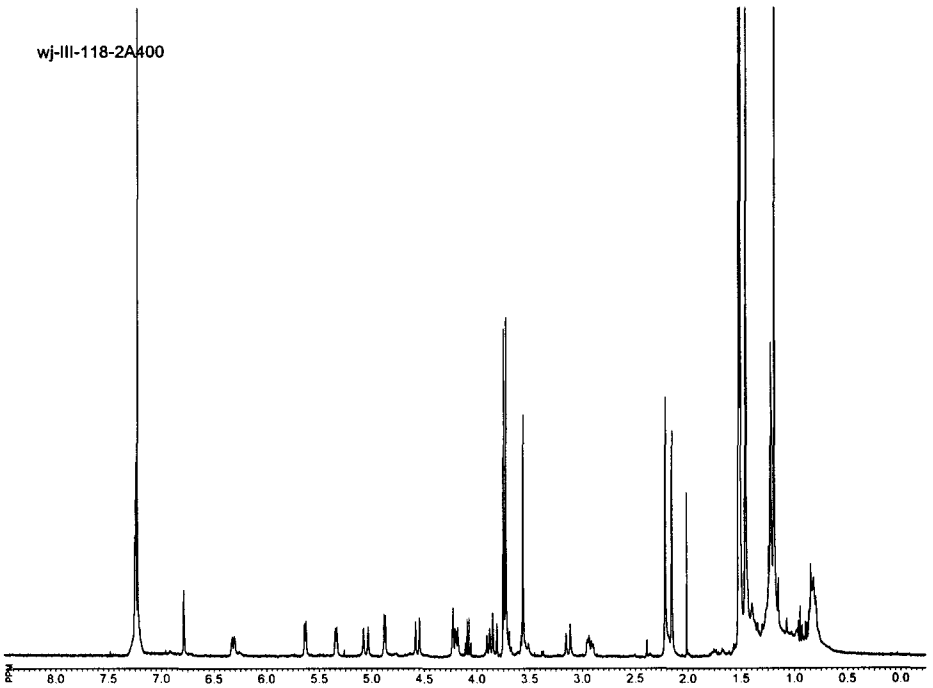
IR (neat, film): 2928, 2855, 1763, 1675, 1417, 1370, 1276, 1256, 1158, 1099cm^{-1};

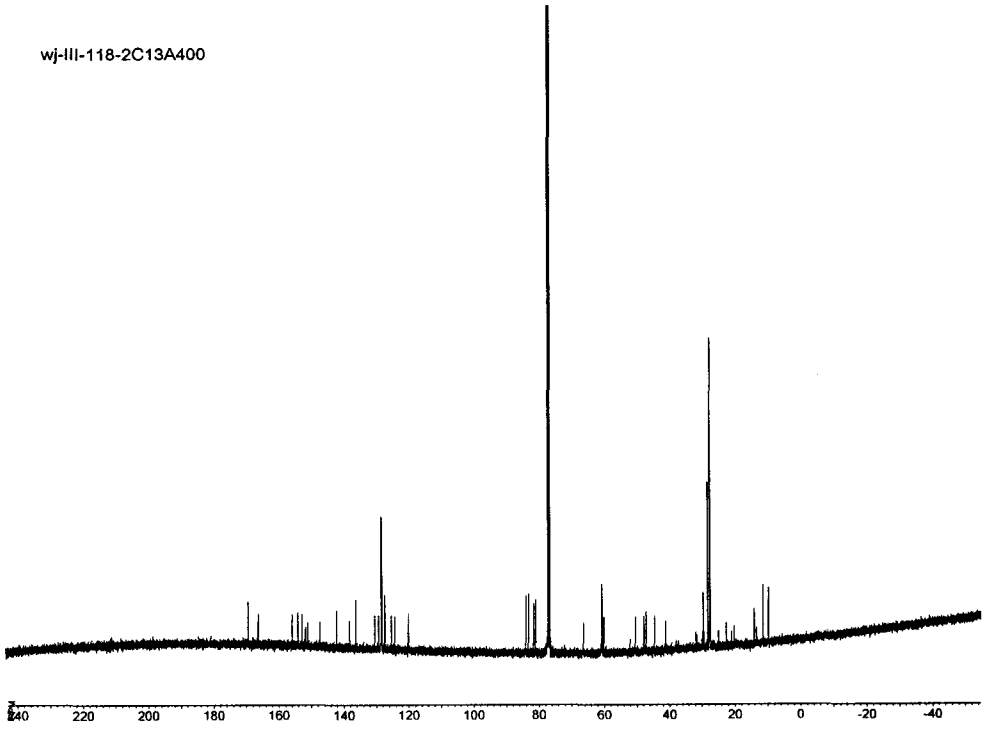
HRMS (FAB+) calcd for $C_{53}H_{71}N_3O_{16}Na$ 1028.4732, found 1028.4712;

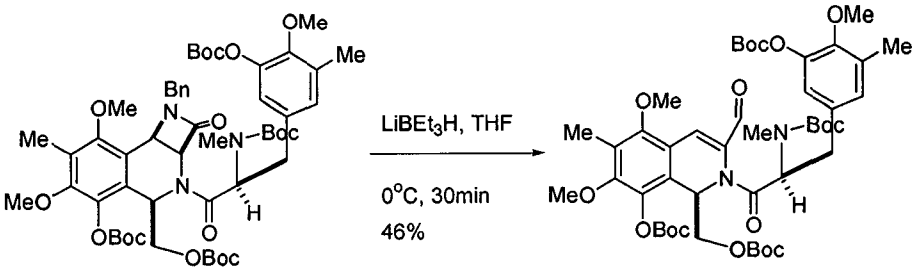
$[\alpha]_D^{25}$ = +23.2 (*c* 0.50, CH_2Cl_2).



378







398

Aldehyde:

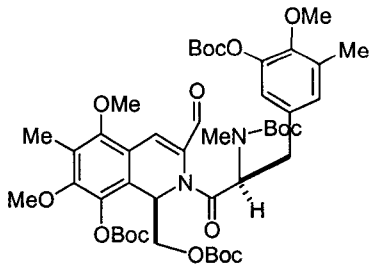
To a solution of **398** (20 mg, 0.0198 mmol, 1.0 equiv) in THF (2 mL) was added $LiBEt_3H$ (1M in THF) (119 μL, 0.119 mol, 6.0 equiv) at 0°C.The reaction was stirred at 0°C for 30 min. and then quenched with sat. NH_4Cl solution (10 mL). The resulting mixture was stirred at room temp. for 10 min. and then diluted with EtOAc (20 mL). The organic layer was washed with water (10 mL), sat. NaCl solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by PTLC to afford aldehyde compound (8.2 mg, 46%).

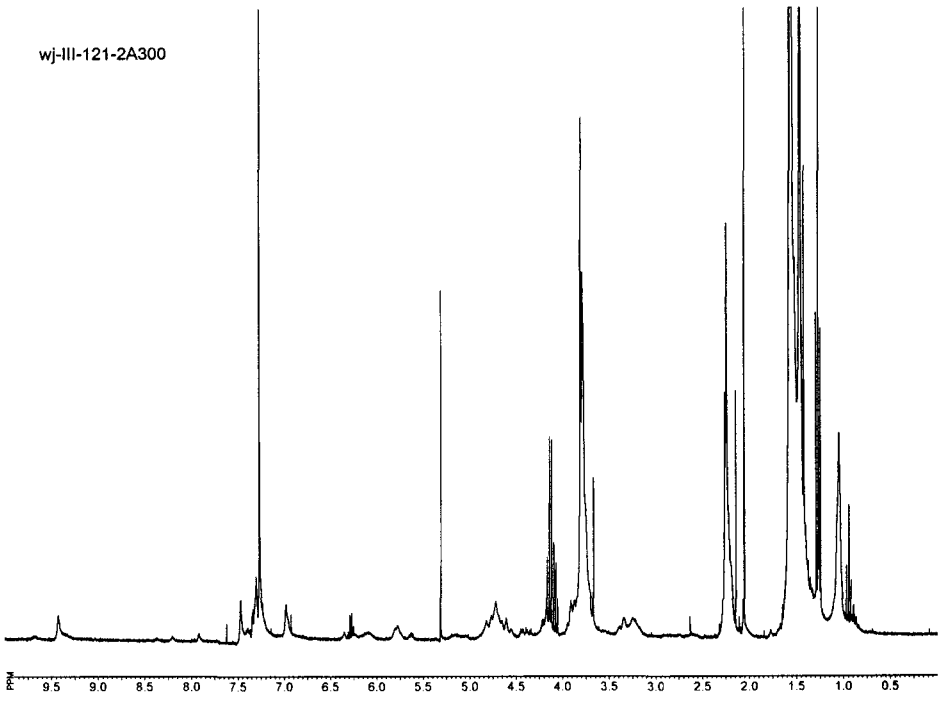
^1H NMR (300 MHz, $CDCl_3$, 298K) δ 9.43 (1H, s), 7.44 (1H, s), 6.96 (1H, s), 6.00-6.40 (1H, m), 5.60-5.90 (1H, br), 4.50-4.90 (3H, br), 4.05-4.25 (2H, br), 3.80 (3H, s), 3.79 (3H, s), 3.78 (3H, s), 3.10-3.45 (3H, br), 2.26 (3H, s), 2.24 (3H, s), 1.30-1.75 (36H, br);

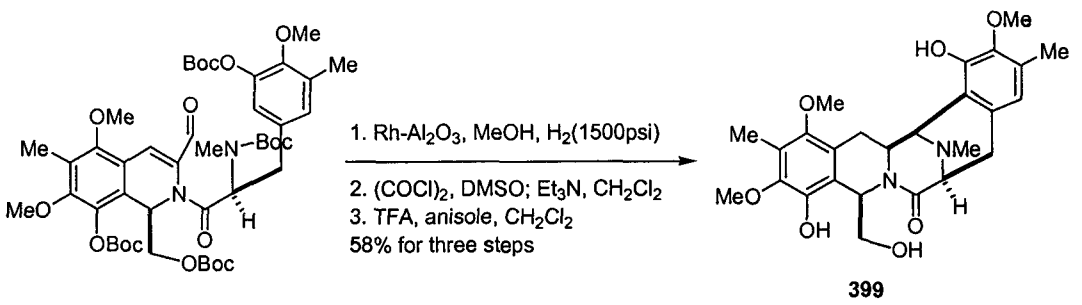
IR (neat, film): 2979, 2936, 1760, 1701, 1370, 1274, 1255, 1156, 1004, 1079cm^{-1};

LRMS (FAB+) calcd for $C_{46}H_{64}N_2O_{16}$ 900.43, found 900.51;

$[\alpha]_D^{25}$ =+101.33 (*c* 0.60, CH_2Cl_2).







399

Pentacycle 399:

To a solution of the aldehyde obtained above (3.5 mg, 0.0038 mmol, 1.0 equiv) in methanol (1 mL) was added Rh on Al_2O_3 (5%)(1.1 mg, 0.00038 mmol, 0.1 equiv). The resulting mixture was hydrogenated at 1500psi H_2 for 12 h. The solids were filtered off through Celite. The solvent was removed under reduced pressure and the residue was used for next step without purification.

To a solution of $(COCl)_2$ (1.1 μL, 0.0114 mmol, 3.0 equiv) in CH_2Cl_2 (0.5 mL) at –78°C was added DMSO (1.2 μL, 0.0152 mmol, 4.0 equiv). The resulting solution was stirred at –78°C for 15 min. To this solution was added the residue obtained above (3.6 mg, 0.0038 mmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL) and the mixture was stirred at –78°C for 30 min. Et_3N (5.4 μL, 0.038 mmol, 10 equiv) was added dropwise, and the mixture was stirred at –78°C for 5 min, warmed to 0°C and stirred for an additional 20 min. The reaction was quenched with saturated NH_4Cl solution (5 mL) and extracted with EtOAc (5 mL x3). The organic layers were combined and dried over anhydrous sodium sulfate. After solvent was removed under reduced pressure, the residue was used for next step without additional purification.

To a solution of crude amino aldehyde obtained above (3.8 mg, 0.0038 mmol, 1.0 equiv) and anisole (4 μL, 0.038 mmol, 10 equiv) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL)

at 0°C. The solution was stirred at 0°C for 30 min. and then stirred at room temp. for an additional 2.5 h. The solvent and TFA was removed under reduced pressure and the residue was purified with PTLC (eluted with EtOAc) to afford **399** (1.5 mg, 77% for three steps).

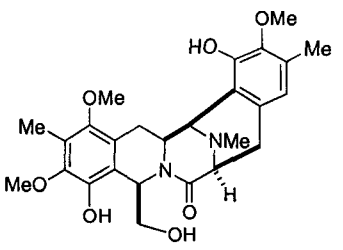
^{1}H NMR (300 MHz, $CDCl_3$, 298K) 6.67 (1H, s), 6.35 (1H, s), 6.29-6.33 (1H, t, *J*=6.6 Hz), 5.81 (1H, s), 5.48 (1H, s), 3.92-3.99 (1H, dd, *J*=7.5Hz, 15.0 Hz), 3.80 (3H, s), 3.75 (3H, s), 3.69 (3H, s), 3.40-3.59 (3H, m), 3.16-3.36 (6H, m), 2.33-2.38 (1H, t, *J*=7.2Hz), 2.21 (3H, s), 2.14 (3H, s);

^{13}C NMR (100 MHz, $CDCl_3$, 298K) δ 175.94, 147.37, 146.64, 144.07, 127.08, 124.11, 119.81, 116.41, 115.46, 112.84, 73.55, 72.32, 69.15, 61.59, 61.22, 60.84, 58.62, 48.11, 38.04, 33.45, 32.08, 30.03, 29.86, 29.59, 29.52, 29.39, 29.22, 26.66, 25.93, 24.89, 22.85, 14.27, 11.45, 9.61;

IR (neat, film): 3358, 2923, 2851, 1674, 1639, 1464, 1418, 1337, 1250, 1118, 1058, 1004cm^{-1};

LRMS (FAB+) [M+TFA-H_2O] calcd for $C_{26}H_{31}N_2O_8F_3$ 580.55, found 580.49;

$[\alpha]_D^{25}$ = -10.9 (*c* 0.22, CH_2Cl_2).



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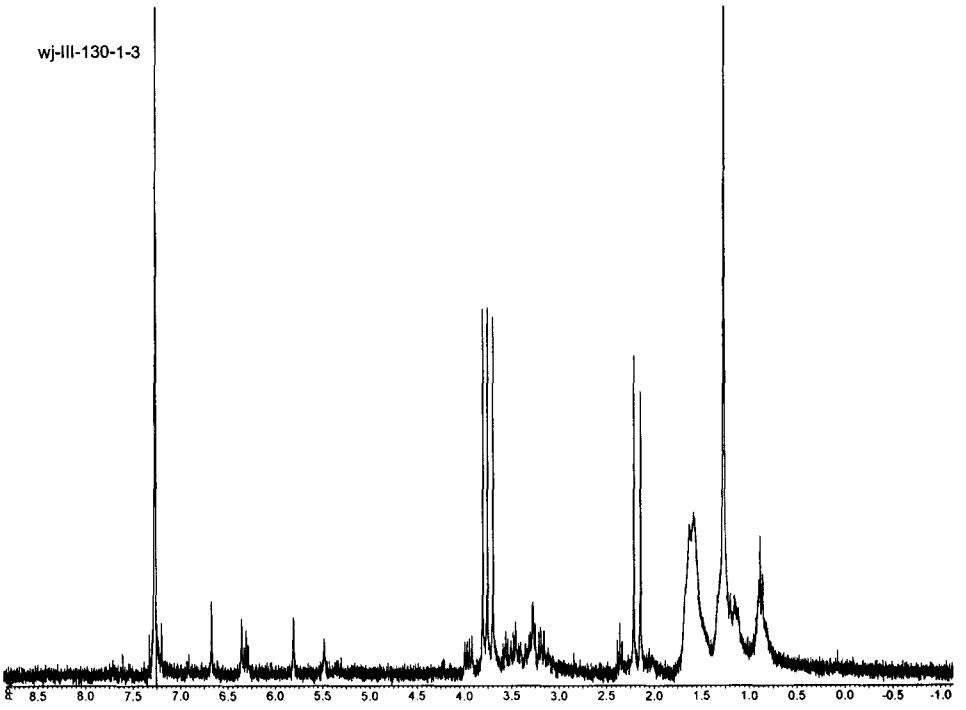
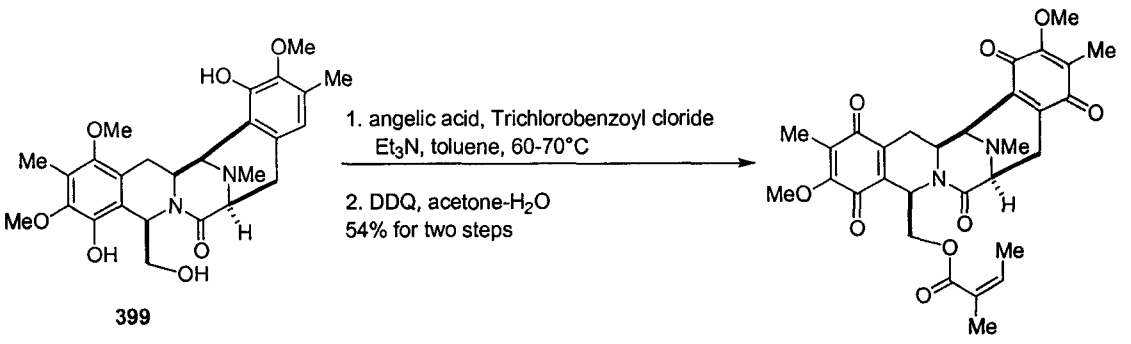




399

(-)-renieramycin G

(-)-Renieramycin G:

To a solution of angelic acid (0.23 mg, 0.0046 mmol, 2.0 equiv) in toluene (0.5 mL) was added 2,4,6-trichlorobenzoyl chloride (0.7 μL, 0.0046 mmol, 2.0 equiv) and Et_3N (0.6 μL, 0.0046 mmol, 2.0 equiv) at 0°C and the resulting solution was stirred at room temp. for 2 h. To this solution was added the solution of triol **399** (1.1 mg, 0.0023 mmol, 1.0 equiv) in toluene (0.5 mL). The resulting mixture was heated at 80°C for 20 h. The mixture was diluted with EtOAc (10 mL) and washed with saturated sodium bicarbonate (3 mL), water (3 mL), saturated NaCl solution (3 mL) and dried over anhydrous sodium sulfate. After solvent was removed under reduced pressure, the residue was used directly for next step without additional purification.

To a solution of the crude angelate obtained above (1.2 mg, 0.0046 mmol, 1.0 equiv.) in acetone-water (9:1)(0.5 mL) was added DDQ (2.3 mg, 0.010 mmol, 4.4 equiv) at room temp. The resulting mixture was stirred for 60 min at room temp. The mixture was quenched with saturated sodium bicarbonate (1 mL) and extracted with EtOAc (3 mL x3). The organic layers were combined and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by PTLC (eluted with 50% EtOAc in hexanes) to give (-)-renieramycin G 0.7 mg (54% for two steps).

^{1}H NMR (500 MHz, CD_2Cl_2, 273K) 5.89-5.90 (1H, m), 5.38 (1H, br), 4.65-4.68 (1H, dd, *J*=2.5, 12.0Hz), 4.30-4.33 (1H, dd, *J*=2.5, 11.5Hz), 4.11-4.12 (1H, d, *J*=4.5Hz), 4.00 (3H, s), 3.97 (3H, s), 3.83-3.85 (1H, d, *J*=12.0Hz), 3.66-3.67 (1H, d, *J*=6.5Hz), 2.99-3.02 (1H, dd, *J*=3.0, 16.5Hz), 2.83-2.89 (1H, dd, *J*=6.0, 20.5Hz), 2.62-2.66 (1H, d, *J*=20.5Hz), 2.35 (3H, s), 1.93 (6H, s), 1.67-1.68 (3H, dq, *J*=2.0, 7.0Hz), 1.54 (3H, t, *J*=2.0Hz), 1.47 (1H, m);

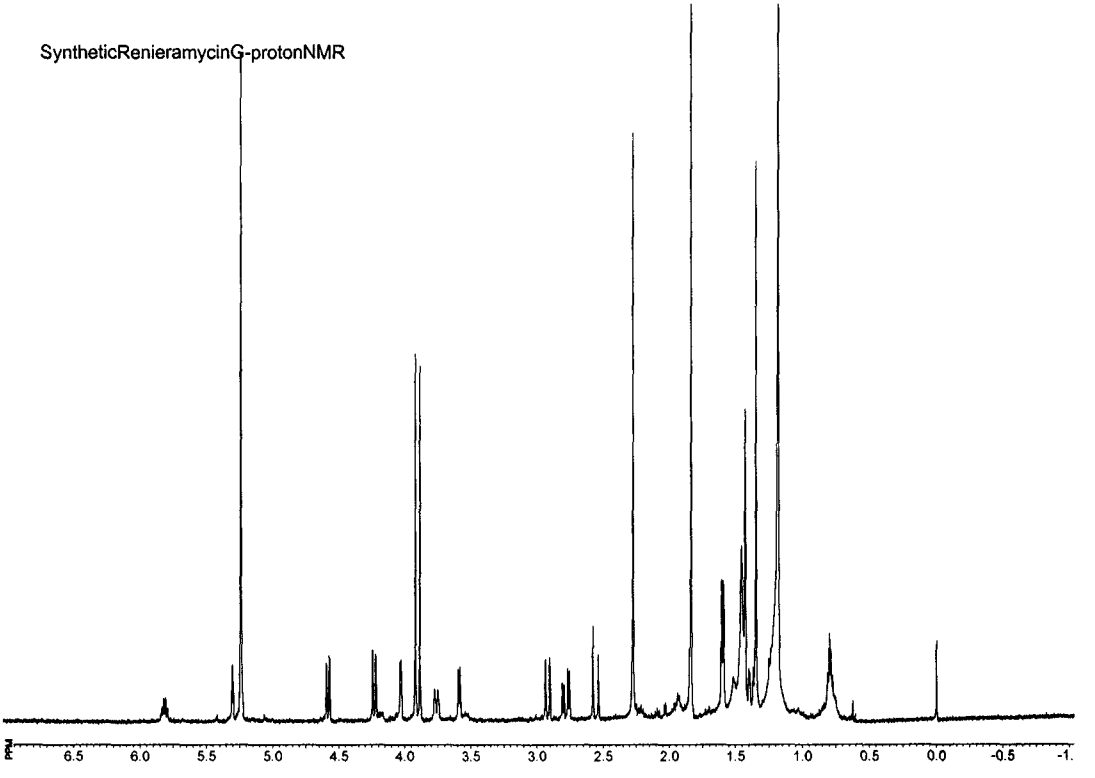
^{13}C NMR (125 MHz, $CDCl_3$, 298K) δ 186.56, 185.48 182.75, 180.67, 170.53, 167.15, 156.37, 155.81, 142.36, 141.92, 139.79, 136.36, 135.18, 129.57, 128.74, 126.94, 63.05, 61.30, 61.25, 59.33, 56.44, 53.34, 50.38, 39.97, 25.88, 23.76, 20.53, 15.58, 8.76;

IR (neat, film): 2926, 2854, 1718, 1655, 1615, 1456, 1420, 1351, 1307, 1229, 1150cm^{-1};

HRMS (FAB+) $[M+H]^+$calcd for $C_{30}H_{33}N_2O_9$ 565.2186, found 565.2161;

$[M+3H]^+$calcd for $C_{30}H_{35}N_2O_9$ 567.2343, found 567.2336;

$[\alpha]_D^{25}$ = -30.8 (*c* 0.12, CH_2Cl_2).



Appendix I

Publications

Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 4635–4639

TETRAHEDRON LETTERS

Synthetic studies on ecteinascidin 743: asymmetric synthesis of the versatile amino acid component

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Received 10 April 2003; revised 26 April 2003; accepted 29 April 2003

Abstract—The asymmetric synthesis of the modified tyrosine derivative as a basic building block for the ecteinascidin and safracin family of antitumor alkaloids has been achieved in nine steps and 39% overall yield.

Ecteinascidin (ET)-743 (**1**), is a natural product isolated from the marine tunicate *Ecteinascidia turbinata*,[1] and has been demonstrated to be a highly promising, exceedingly potent antitumor agent currently in phase II/III clinical trials.[2] The novel structure of ET-743 combined with its meagre availability from natural sources, and the unique mechanism of action[3] have made this drug a very attractive synthetic target. The first total synthesis of ET-743 was accomplished by Corey and co-workers.[4] Later Corey, Schreiber and co-workers prepared a synthetic analogue of ET-743 (phthalascidin, Pt-650) that has virtually the same cytotoxicity as the natural product.[5] In 2000, a semi-synthesis of Et-743 from cyanosfracin B was described.[6] Very recently, another total synthesis of ET-743 was reported by Fukuyama and co-workers.[7]

In addition to Et-743 (Fig. 1), the structurally related safracins,[8] saframycins[9] and renieramycins[10] are also potent antitumor antibiotics that contain structurally related amino acid components. We report here, a potentially general method to synthesize a highly functionalized tyrosine derivative that represents the 'eastern' sector of Et-743 that may be of potential use for the asymmetric total synthesis of several members of this family of natural products and congeners.[11] Our approach is based on the use of the optically pure oxazinone **12** as template.[12,13] Our retrosynthetic strategy for Et-743 is illustrated in Scheme 1.

As shown in Scheme 1, the key amino acid **9** was envisioned to arise via the coupling of the sodium enolate of optically active (>99:1 er) oxazinone **12**[12,13] with the benzyl bromide derivative of aldehyde **11** to furnish the alkylation product **10**. Further manipulation would involve *N*-methylation of the amine group and the phenol protecting group to give amino acid **9**. We have reported separately, the construction of optically pure β-lactams corresponding to **8** which has been converted into a pentacyclic intermediate such as **7**.[14]

The synthesis commenced with 3-(benzyloxy)-4-methoxy-5-methylbenzaldehyde (**13**),[15] which could be conveniently made in ~10 gram scale batches from

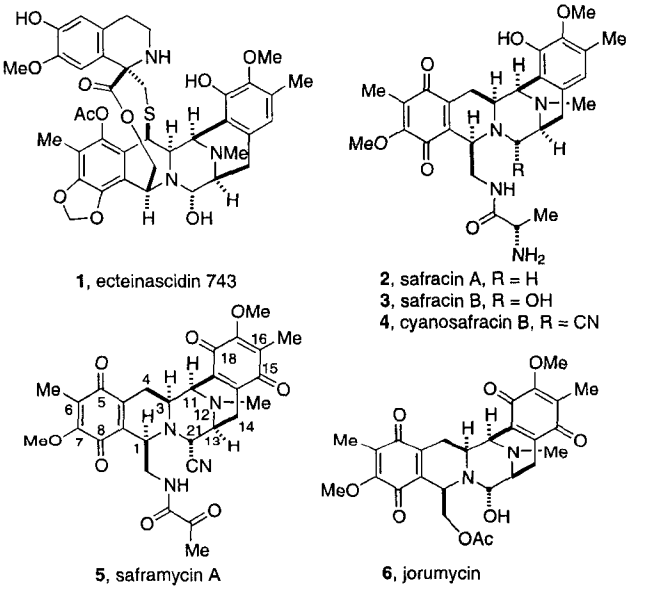

Figure 1.

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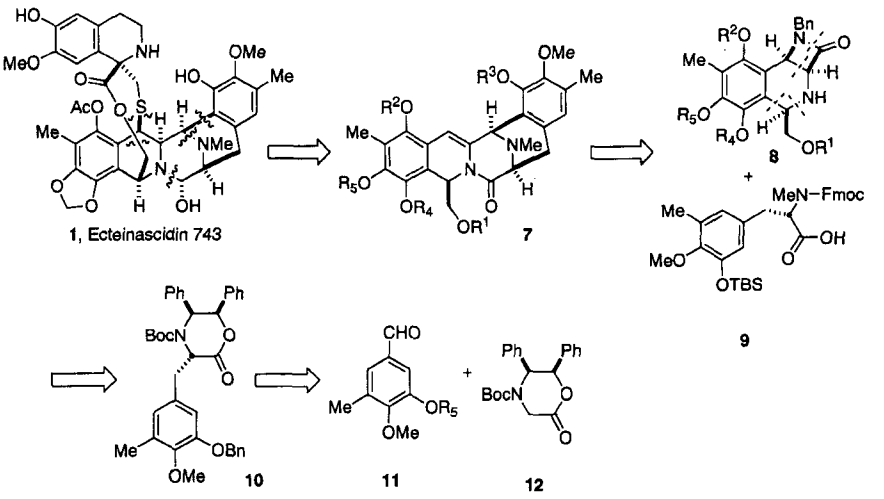
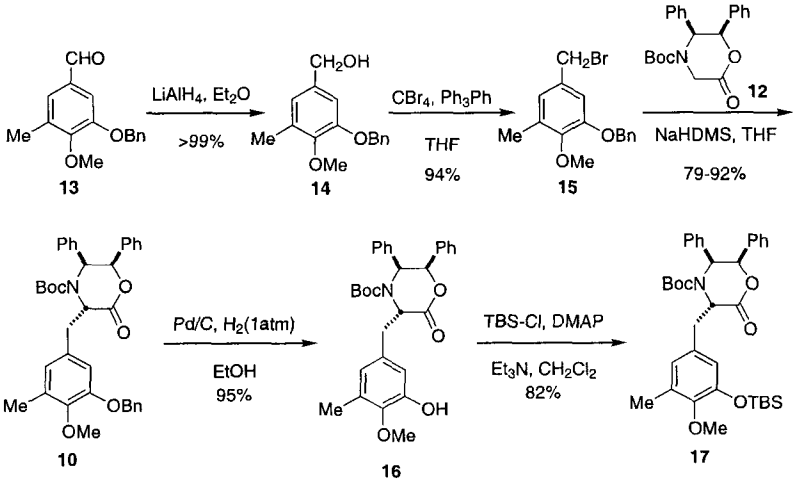


Scheme 1.

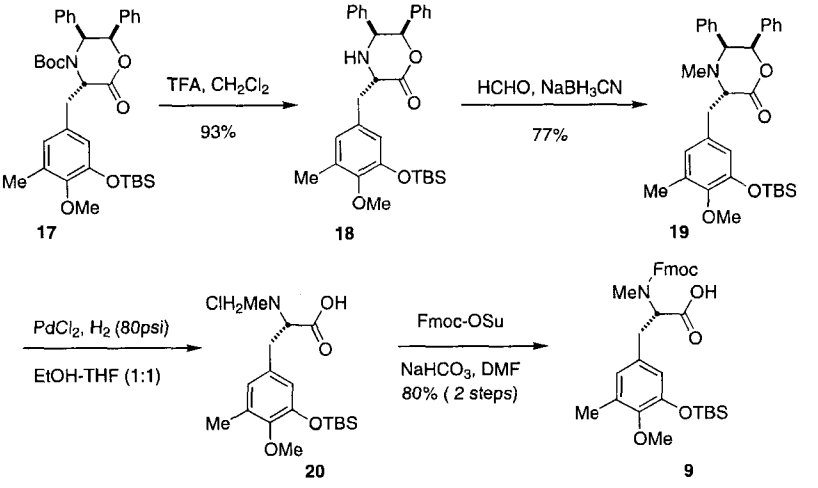
vanillin in seven steps: (i) HCHO, Me_2NH, ethanol (94%); (ii) Ac_2O; conc. HCl; $SnCl_2$ (78%); (iii) $AlCl_3$, pyridine, CH_2Cl_2 90%; (iv) TIPS-Cl, Et_3N, DMF (82%); (v) Me_2SO_4, K_2CO_3, acetone (88%); (vi) TBAF, THF (82%); (vii) BnBr, K_2CO_3, acetone (86%). As shown in Scheme 2, the aldehyde of **13** was reduced to the corresponding alcohol (**14**) by treatment with LAH in quantitative yield. Alcohol **14** was converted into benzyl bromide derivative **15** through the agency of CBr_4, Ph_3P in 94% yield. Next, **15** was condensed with the sodium enolate (NaHMDS, THF, −78°C) of oxazinone **12** to afford the alkylation product **10** in 79–92% yield. The *O*-benzyl group of **10** was removed by catalytic hydrogenation to give the corresponding phenol **16** in 95% yield, followed by protection as the *O*-TBS ether under standard conditions providing **17** in 82% yield.

The final conversion of **17** into **9** is illustrated in Scheme 3. The *N*-*t*-Boc group of compound **17** was removed by treatment with TFA in 93% yield affording the secondary amine **18**. In practice, we found it quite difficult to effect the efficient *N*-methylation of **18** and after extensive studies, it was found that the best conditions for this transformation was via reductive amination using formaldehyde furnishing **19** ($NaBH_3CN$, HCHO, CH_3CN, 77% yield). The chiral auxiliary of **19** was removed by catalytic hydrogenation to provide the amino acid hydrochloride salt **20**.[16] Finally, the secondary amine was protected as the corresponding Fmoc derivative furnishing **9** in 80% yield for the two steps.[17]

Compound **9** should prove to be a versatile amino acid, which contains the requisite functionality and desired absolute stereochemistry, for the synthesis of the ecteinascidins and related alkaloids. Efforts to utilize this intermediate for the concise asymmetric synthesis of the ecteinascidin, saframycin as well as safracin family are currently under study in these laboratories.



Scheme 2.



Scheme 3.

Acknowledgements

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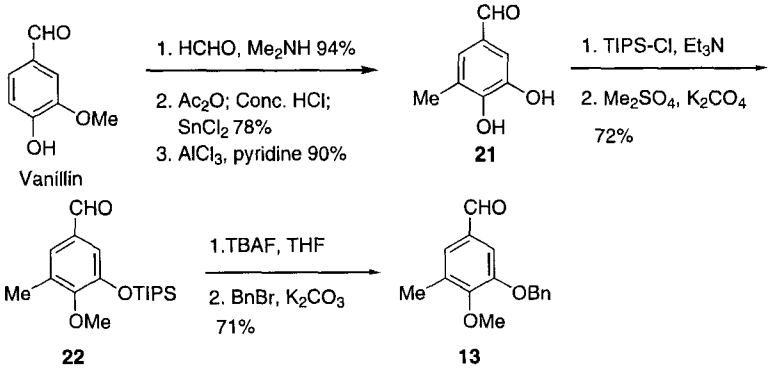
15. (a) Synthesis of this aldehyde could also be achieved in four steps for small scale (1 ~2 g) from commercial available isovanillin: (i) NaH, DMF; MOM-Br 95%; (ii) *n*BuLi, *N*-methylpiperazine, THF; *n*BuLi; MeI 40%; (iii) MsOH, CH_2Cl_2/H_2O; (iv) BnBr, K_2CO_3, acetone 81% for two steps; (b) Sinhababu, A. K.; Borchardt, R. T. *Synth. Commun.* **1983**, *13*, 677–683. The synthesis of **13** as described in the text is prepared according to the following scheme.



16. This amino acid salt is soluble in ether, and it thus proved expedient to take the crude salt on directly for the next step without further purification.
17. All new compounds gave satisfactory spectroscopic and analytical data consistent with the assigned structures.
Alcohol **14**: ^{1}H NMR (300 MHz) δ $CDCl_3$: 1.95 (1H, s), 2.32 (3H, s), 3.88 (3H, s), 4.60 (2H, s), 5.15 (2H, s), 6.82 (1H, s), 6.89–6.90 (1H, d, J=1.8 Hz), 7.37–7.52 (5H, m). ^{13}C NMR (75 Hz) δ $CDCl_3$: 16.1, 60.4, 65.3, 70.7, 110.7, 121.8, 127.3, 127.9, 128.6, 132.1, 136.4, 137.1, 147.1, 151.8. IR (NaCl, neat) 3396, 2932, 2872, 2827, 1591, 1495, 1433, 1325, 1230, 1143, 1089, 1008, 737, 697 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{16}H_{18}O_3$: 258.1256; found: 258.1249.
Bromide **15**: ^{1}H NMR (300 MHz) δ $CDCl_3$: 2.33 (3H, s), 3.91 (3H, s), 4.49 (2H, s), 5.17 (2H, s), 6.89–6.90 (1H, d, J=2.1 Hz), 6.92–6.93 (1H, d, J=2.4 Hz), 7.39–7.54 (5H, m). ^{13}C NMR δ $CDCl_3$: 15.1, 33.3, 59.3, 69.8, 111.7, 123.0, 126.4, 127.0, 127.6, 131.4, 131.9, 135.9, 147.0, 150.77. IR (NaCl, neat): 3031, 2932, 2828, 1589, 1493, 1434, 1333, 1287, 1211, 1150, 1090, 1008, 738, 698 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{16}H_{17}O_2Br$: 320.0419; found: 320.0418.
Compound **10**: ^{1}H NMR (400 MHz) (DMSO-d_6, 373 K) δ DMSO: 1.16–1.41 (9H, broad), 2.23 (3H, s), 3.27–3.30 (3H, d, J=16 Hz), 3.41–3.46 (1H, m), 3.77 (3H, s), 5.04 (1H, broad), 5.14 (3H, s), 5.4 (1H, broad), 6.59–6.61 (2H, d, J=7.2 Hz), 6.78 (1H, s), 6.86–6.87 (2H, d, J=5.6 Hz), 6.93 (1H, s), 7.09–7.49 (9H, m). ^{13}C NMR (100 MHz) (DMSO-d_6, 373 K) δ DMSO: 13.5, 15.0, 27.2, 57.9, 59.2, 59.7, 70.3, 77.5, 80.0, 113.9, 123.8, 125.8, 126.7, 126.9, 127.2, 127.3, 127.6, 127.8, 130.8, 131.2, 134.2, 136.7, 146.1, 151.0, 167.8, 169.5. IR (NaCl, neat): 3032, 2931, 1754, 1697, 1588, 1494, 1453, 1382, 1331, 1233, 1161, 1085, 1010, 738, 702 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{37}H_{39}NO_6$: 593.2777; found: 593.2781. $[\alpha]_D^{25}$ +22.5 (c 0.71, CH_2Cl_2).
Compound **16**: ^{1}H NMR (400 MHz) (DMSO-d_6, 373 K) δ DMSO: 1.08–1.47 (9H, broad), 2.20 (3H, s), 3.16–3.19 (1H, broad), 3.34–3.39 (1H, m), 3.47–3.51 (1H, m), 3.73 (3H, s), 5.01 (1H, broad), 5.08 (1H, broad), 5.38 (1H, broad), 6.56–6.59 (3H, m), 6.69 (1H, s), 6.85–6.86 (2H, d, J=6.4 Hz), 7.08–7.26 (6H, m). ^{13}C NMR (100 MHz) (DMSO-d_6, 373 K) δ DMSO: 15.0, 17.8, 27.2, 55.6, 58.8, 59.6, 77.4, 80.0, 115.3, 115.4, 121.8, 125.7, 126.7, 126.9, 127.2, 127.6, 130.4, 131.0, 134.2, 145.1, 149.4, 167.9. IR (NaCl, neat): 3399, 2977, 2933, 1754, 1697, 1590, 1497, 1454, 1386, 1357, 1301, 1161, 1119, 1060, 1003, 736, 702 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{30}H_{33}NO_6$: 503.2308, found: 503.2307. $[\alpha]_D^{25}$ +37.8 (c 1.27, CH_2Cl_2).
Compound **17**: ^{1}H NMR (400 MHz) (DMSO-d_6, 373 K) δ DMSO: 0.17–0.18 (6H, s), 1.00 (9H, s), 1.05–1.50 (9H, broad), 2.22 (3H, s), 3.19–3.22 (1H, broad), 3.37–3.42 (1H, broad), 3.73 (3H, s), 4.99 (1H, broad), 5.08 (1H, broad), 5.30 (1H, broad), 6.56–6.58 (2H, d, J=6.8 Hz), 6.66 (1H, s), 6.73 (1H, s), 6.83–6.84 (2H, d, J=4.8 Hz), 7.10–7.25 (6H, m). ^{13}C NMR (100 MHz) (DMSO-d_6, 373 K) δ DMSO: −5.2, 15.1, 17.4, 25.1, 27.2, 58.9, 77.5, 80.1, 119.4, 119.5, 124.2, 125.7, 126.8, 127.0, 127.3, 127.7, 131.3, 134.1, 147.7, 148.2, 167.82. IR (NaCl, neat): 3032, 2927, 2856, 1757, 1702, 1585, 488, 1454, 1380, 1357, 1254, 1162, 1116, 1067, 1011, 838, 784, 701 cm^{-1}. HRMS (FAB$^+$) calcd for $C_{36}H_{47}NO_6Si$: 617.3173; found: 617.3169. $[\alpha]_D^{25}$ +22.1 (c 1.09, CH_2Cl_2).
Compound **18**: ^{1}H NMR (400 MHz) δ $CDCl_3$: −0.01 (3H, s), 0.02 (3H, s), 0.90 (9H, s), 1.23 (1H, broad), 2.15 (3H, s), 3.12–3.17 (2H, m), 3.68 (3H, s), 4.15–4.18 (1H, dd, J=4.0 Hz, 9.2 Hz), 4.60 (1H, d, J=3.6 Hz), 5.55–5.56 (1H, d, J=3.6 Hz), 6.49–6.50 (1H, d, J=2.0 Hz), 6.62 (1H, d, J=1.6 Hz), 6.85–6.88 (4H, m), 7.11–7.23 (6H, m). ^{13}C NMR (100 MHz) δ $CDCl_3$: −4.6, 16.1, 18.3, 25.8, 29.8, 38.5, 56.7, 58.3, 59.9, 85.3, 120.0, 124.6, 127.3, 127.7, 127.8, 128.2, 128.4, 132.4, 132.9, 135.1, 136.8, 148.8, 149.0, 170.6. IR (NaCl, neat): 3325, 2955, 2929,

2857, 1738, 1584, 1489, 1336, 1253, 1226, 1180, 1070, 1012, 838, 783, 699 cm^{-1}. HRMS (FAB^+) calcd for $C_{31}H_{40}NO_4Si$: 518.2727; found: 518.2719. $[\alpha]_D^{25}$ +55.4 (*c* 0.70, CH_2Cl_2).

Compound **19**: ^1H NMR (400 MHz) δ $CDCl_3$: 0.18 (3H, s), 0.20 (3H, s), 1.03 (9H, s), 2.27 (3H, s), 2.39 (3H, s), 3.03–3.23 (2H, dd, J=4.0 Hz, 13.2 Hz), 3.76 (3H, s), 3.97–4.01 (2H, m), 5.06 (1H, s), 6.71 (1H, s) 6.74 (1H, s), 6.78–6.79 (2H, d, J=7.2 Hz), 6.86–6.87 (2H, d, J=5.2 Hz), 7.10–7.19 (6H, m). ^{13}C NMR (100 MHz) δ $CDCl_3$: −4.5, −4.4, 16.2, 18.4, 25.9, 29.8, 37.4, 39.7, 60.0, 64.7, 67.4, 81.7, 121.0, 125.5, 126.1, 127.8, 127.9, 128.0, 128.2, 129.4, 132.0, 132.6, 134.3, 135.9, 148.4, 148.8, 171.7. IR (NaCl, neat): 2930, 2857, 1744, 1585, 1489, 1351, 1315, 1254, 1176, 1146, 1070, 1012, 839, 783, 712, 697 cm^{-1}. HRMS (FAB^+) calcd for $C_{32}H_{42}NO_4Si$: 532.2883; found: 532.2861. $[\alpha]_D^{25}$ −40.5 (*c* 1.11, CH_2Cl_2).

Compound **9**: ^1H NMR (300 Hz) δ $CDCl_3$ 0.14 (6H, s), 0.99 (9H, s), 2.18 (3H, s), 2.87 (3H, s), 2.6–3.3 (4H, m), 3.66 (3H, s), 4.14–4.37 (3H, m), 4.64–4.93 (1H, m), 6.48 (1H, s), 6.59–6.64 (1H, s), 7.25–7.54 (6H, m), 7.72–7.76 (2H, dd, J=3.0 Hz, 7.5 Hz), 8.04 (1H, s); ^{13}C NMR (75 MHz) δ −4.3, 16.3, 18.5, 25.9, 31.9, 32.6, 34.4, 36.9, 47.3, 59.9, 60.9, 68.1, 119.3, 120.0, 124.0, 124.9, 125.1, 127.1, 127.7, 132.3, 132.4, 141.3, 144.0, 148.6, 163.0, 174.8; IR (neat, film): 2954, 2929, 2857, 1682, 1598, 1451, 1322, 1142, 839 cm^{-1}; HRMS calcd for $C_{33}H_{41}NO_6Si$: 575.2703, found: 575.2697. $[\alpha]_D$ −40.8 (*c* 0.5, CH_2Cl_2).

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Synthetic Studies on Ecteinascidin-743: Constructing a Versatile Pentacyclic Intermediate for the Synthesis of Ecteinascidins and Saframycins

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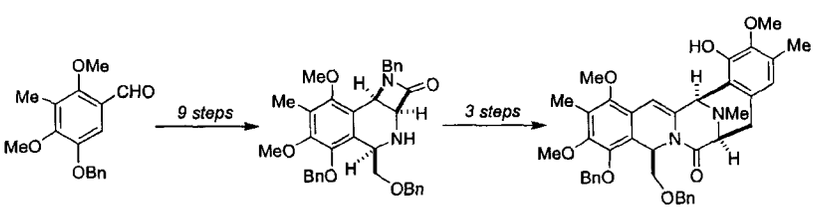
ABSTRACT



The asymmetric synthesis of a highly functionalized pentacyclic tetrahydroisoquinoline relevant to the ecteinascidin, saframycin, safracin, and renieramycin family of antitumor alkaloids is described.

Ecteinascidin (ET)-743 (**1**) is a natural product isolated from the marine tunicate *Ecteinascidia turbinata*,[1] which has been demonstrated to be a highly promising, exceedingly potent antitumor agent currently in phase II/III clinical trials.[2] The novel structure of ET-743 combined with the meager availability from natural sources and the unique mechanism of action of this drug[3] have made this substance a very attractive and important synthetic target.

The first total synthesis of ET-743 was accomplished by Corey and co-workers.[4] Later, Corey, Schreiber, and co-workers prepared a simpler synthetic analogue of ET-743 (phthalascidin, Pt-650) that exhibited virtually the same cytotoxity as the natural product.[5] In 2000, a semisynthesis of Et-743 from cyanosfracin B was described,[6] and more recently, a total synthesis of ET-743 was accomplished by Fukuyama and co-workers.[7]

In addition to Et-743, the structurally related safracins,[8] saframycins,[9] and renieramycins[10] are also potent antitumor antibiotics that contain densely functionalized tetrahydroiso-

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quinoline ring systems constituted from similar amino acid components. As part of a program directed toward efficient, asymmetric total syntheses of these substances and mechanistically inspired analogues for biochemical and biological evaluation,[11] we report here a potentially general and concise

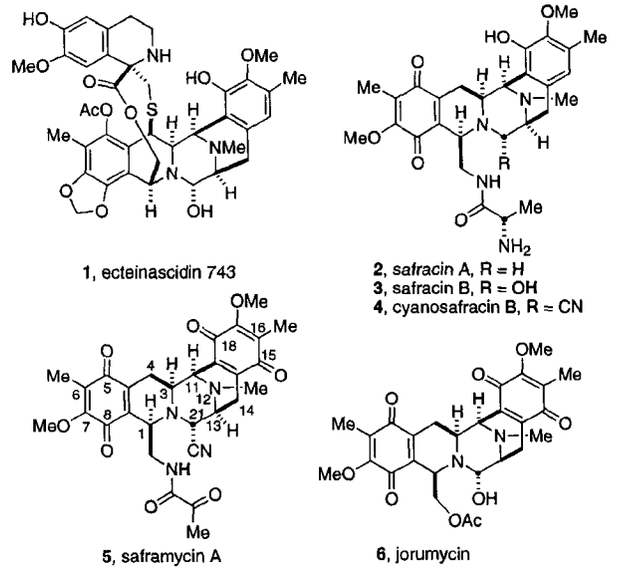
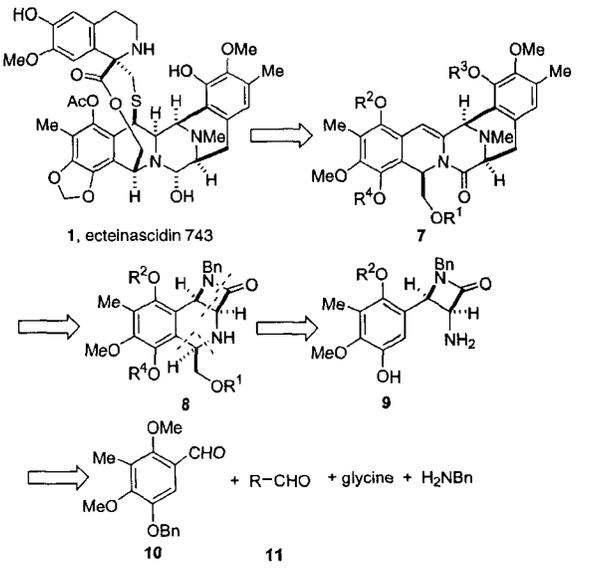


Figure 1. Structures of Et-743, safracins, saframycin A, and jorumycin.

method to construct densely functionalized pentacyclic tetrahydroisoquinoline ring systems that represent the "western" sector of Et-743 that should prove useful for the asymmetric total synthesis of several members of this family of natural products and congeners. Our approach is based on the use of sequential asymmetric Staudinger and Pictet–Spengler cyclization reactions.[11]

Our retrosynthetic strategy for Et-743 is illustrated in Scheme 1. It was anticipated that the Staudinger reaction between an imine derived from aldehyde **10** and a chiral *N*-protected ketene would afford the *cis*-relationship at C-3 and C-4 (ecteinascidin numbering). After removal of the chiral auxiliary and deprotection of the phenolic residue, the

Scheme 1. Retrosynthetic Analysis of Et-743



chiral amino phenol **9** would be subjected to a Pictet–Spengler reaction to form tetrahydroisoquinoline **8** that embodies all the requisite functionality to tackle the planned asymmetric synthesis of Et-743 and bioxalomycin. We have previously reported a racemic model study along these lines[11] and now describe an asymmetric approach that specifically provides the western half of Et-743 in the optically pure form.

In a separate report, we described the asymmetric synthesis of the amino acid component (**18**).[12] Herein, we describe an asymmetric synthesis of the tetrahydroisoquinoline **8** that represents the "Western" sector of Et-743 and the coupling of these two fragments culminating in the construction of the pentacyclic core of the ecteinascidin, saframycin, and related alkaloids.

As shown in Scheme 2, the Staudinger reaction[13] was accomplished by condensing benzylamine with aldehyde **10** in refluxing benzene to afford the corresponding imine in quantitative yield. The ketene of the optically pure acid chloride **12** was prepared at −78 °C by the addition of triethylamine and the benzyl imine obtained from **10** was added and the reaction warmed to 0 °C, which afforded, after workup, β-lactam **13** in excellent yield.

Reductive removal of the chiral auxiliary and the benzyl ether was accomplished by hydrogenolysis over $Pd(OH)_2$ to afford the corresponding amino phenol, which upon treatment with methylglyoxylate afforded **14** in 84% yield as a single stereoisomer.[14] The relative stereochemistry of this substance was determined by comparison of ^1H NMR coupling constants to a related racemic substance for which an X-ray crystal structural analysis on a Pictet–Spengler cyclization

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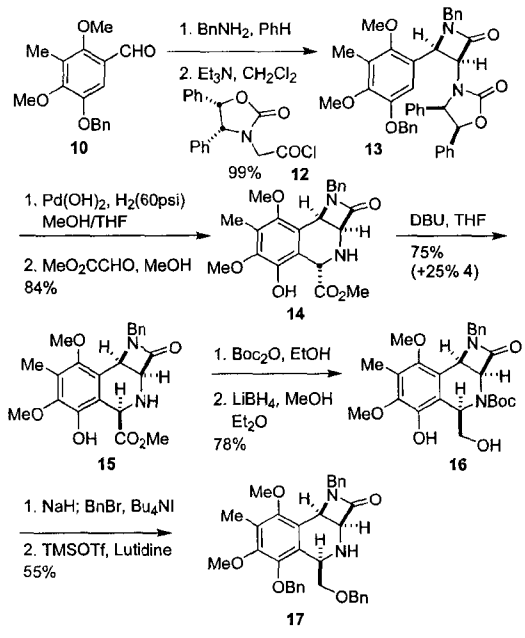
(14) It should be noted that the free phenol was found to be essential for a successful Pictet–Spengler cyclization reaction to proceed. Related substrates containing the methylenedioxy moiety or simple aryl methyl ethers failed to afford the corresponding Pictet–Spengler products.

Scheme 2. Asymmetric Staudinger Sequence

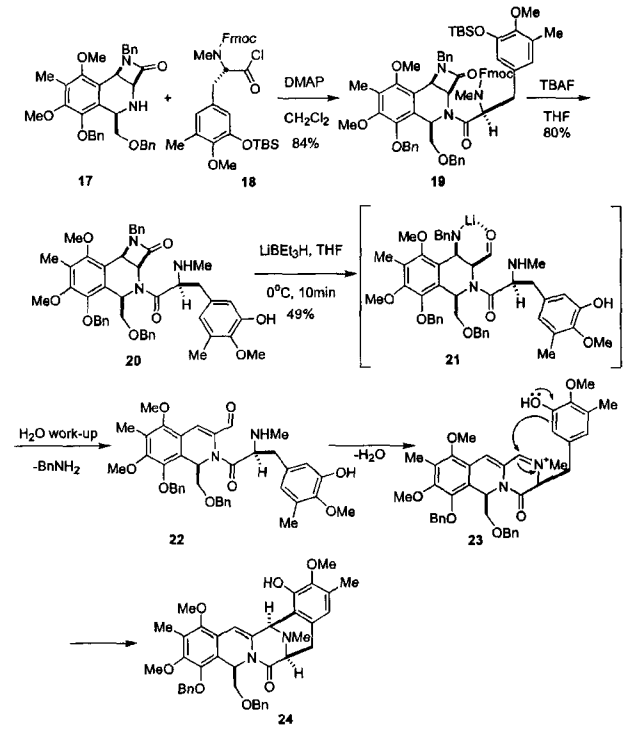


product has been secured.[15] As expected, on the basis of our previous racemic study, the relative stereochemistry of **14** at C-1 possessed the undesired *anti*-configuration.

Epimerization of the *anti*-carbomethoxy group of **14** with DBU in THF at room temperature afforded a 75% yield of the desired *syn*-isomer **15** plus 25% of recovered **14**, which could be readily separated and recycled. The secondary amine of **15** was selectively protected by treatment with Boc_2O in ethanol and reduction of the carbomethoxy group to the corresponding alcohol was accomplished by treatment with $LiBH_4$–MeOH in refluxing ether in excellent yield to afforded **16** in 78% yield. Benzyl protection of both the primary alcohol and the phenol was achieved by treatment with NaH and BnBr in the presence of a catalytic amount nBu$_4$NI. Next, the Boc group was removed by treatment with TMSOTf and lutidine to afford the tetrahydroisoquinoline **17** in 55% isolated yield.

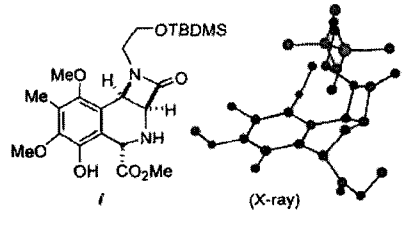
The amino acid was converted into the corresponding acid chloride by treatment with oxalyl chloride and then coupled to the amine in the presence of DMAP to afford the peptide **19** in 84% yield.[16] Both the Fmoc and TBS protecting groups were removed in a single operation by treatment with TBAF, which afforded the phenolic amine **20** in 80% isolated yield. Exposure of **20** to $LiBEt_3H$ in THF at 0 °C remarkably furnished the desired pentacyclic compound **24** directly in one step in 49% yield.

Scheme 3



The conversion of **20** into **24** is envisioned to proceed by initial partial reduction of the β-lactam to the amine-coordinated lithium complex **21** that obviates over-reduction of the incipient aldehyde. Extensive experience on related compounds in our hands and a search of the literature has revealed that the reduction of β-lactams directly to aldehydes is a synthetically challenging reaction for which few good solutions exist.[17] Elimination of benzylamine occurs spontaneously under the reaction conditions to afford the α,β-unsaturated aldehyde **22** that subsequently suffers cyclization of the secondary amine on the aldehyde to generate the key iminium ion species **23**. Regioselective intramolecular Pictet–Spengler cyclization finally affords the pentacyclic compound **24** without contamination of the alternative regioisomer.[18] The pentacyclic compound **24** contains the olefinic moiety at C3–C4 (saframycin numbering) that is flexibly poised for either saturation to the saframycins and related compounds or functionalization at C-4 for closure of the sulfur-containing macrocyclic ring of the ecteinasci-

(15) The relative stereochemistry of model compound ***i*** was secured by X-ray analysis, confirming the *trans*-configuration at the C-1 position. Comparison of ^{1}H NMR data of the pre-Pictet–Spengler β-lactam used to prepare ***i*** to that of **21** was used to establish the relative stereochemistry of **21**.



(16) The coupling with the acid chloride **18** in the presence of DMAP did not lead to detectable racemization.

(17) A search of the literature did not reveal any general methods for the reduction of β-lactams to aldehydes. For a pertinent reference, see: Ojima, I.; Zhao, M.; Yamato, T.; Nakahashi, K.; Yamashita, M.; Abe, R. *J. Org. Chem.* **1991**, *56*, 5263–5277.

(18) In the Pictet–Spengler reaction, the regioselectivity of the observed product was secured by ^{1}H NMR nOe studies.

dins.[19] The retention of the oxidation state of the C-4 carbon atom (saframycin numbering) from the initial tricyclic β-lactam should prove to be very versatile, particularly for the ecteinascidins, since this was a difficult problem in the Corey total synthesis approach,[4] as well as the semisynthetic approach to the ecteinascidins that was based on benzylic C-4 oxidation of saframycin.[6]

In summary, the optically pure, densely functionalized pentacyclic tetrahydroisoquinoline **24** was prepared in 12 steps, with an overall yield of 12%. Efforts to utilize this intermediate and the approach outlined for the concise asymmetric synthesis of the ecteinascidin, saframycin families, and related alkaloids are currently under study in these laboratories.

(19) All new compounds gave satisfactory spectroscopic and analytical data consistent with the assigned structures (see Supporting Information).

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Supporting Information Available: Complete spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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