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# Isolation, biological activity and synthetic studies towards the unique family of natural products, the rubromycins


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# Isolation, biological activity, biosynthesis and synthetic studies towards the rubromycin family of natural products.

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Covering from 1953 to 2014

The rubromycins are an ever growing family of natural products isolated from various Actinomycetes over the last 60 years. Exhibiting a highly attractive array of antimicrobial and enzyme activity, this unique family of compounds have attracted significant attention from many synthetic chemists. Investigations into the synthesis of the densely functionalised hexacyclic ring system have revealed many hidden synthetic challenges. This review covers the isolation, the reported biological activity and the detailed synthetic studies towards these complex natural products.



## 1. Introduction

The rubromycins are a family of structurally related compounds isolated from *Actinomycetes* that exhibit an attractive array of biological activity. This review covers their isolation, structure elucidation, a brief discussion of studies into the synthesis of the 5,6-spiroketal core and an in depth discussion of the total synthetic investigations towards this family of compounds by various chemistry research groups.

Accessing these compounds synthetically using the traditional method of acid-mediated spirocyclisation of a substituted dihydroxyketone precursor has been successful, however, with some limitations. Alternative, elegant, methods have also been developed and afforded successful results. However, a general synthetic method to access the vast rubromycin family is yet to be reported. The small intricacies and subtle functional group differences between each of the members of this family of natural products has been the main factor hindering the development of such a method.

The common structural motif of the rubromycins consists of a naphthazarin and isocoumarin framework linked through a 5,6-bisbenzannulated spiroketal. The only exception is α-rubromycin (**1**), which exhibits a furan based structure and is the open chain analogue of β-rubromycin (**2**). Each individual compound is distinguished by differing oxidation states at C-3′, C-3, C-4, and the functionality at C-7 (Fig. 1).

**Fig. 1** General structure of the rubromycins.

## 2. Isolation

Initially, the rubromycins were isolated due to their vivid colours. In 1953, Brockmann and Renneberg reported the isolation of a novel red pigment from a culture broth of *Streptomyces collinus* and named it rubromycin.[1] Shortly after, a second pigment was isolated from the same strain and named collinomycin.[2] Reactivity investigations undertaken in 1966 using isolates from the same bacterial strain showed that treatment of rubromycin with hydrochloric acid afforded a third novel red pigment.[3] This new pigment was also observed and isolated in trace amounts from the initial cultures. Further reactivity studies showed that exposure of rubromycin to refluxing pyridine led to complete conversion to collinomycin. This related reactivity led to the establishment of the rubromycin family: collinomycin renamed α-rubromycin (**1**), rubromycin became β-rubromycin (**2**) and the third pigment, γ-rubromycin (**3**), based on their respective $R_f$ values (Fig. 2).[3]

**Fig. 2** First compounds isolated establishing the rubromycin family.

Structurally, β-rubromycin was initially assigned as *ortho*-quinone **6**, despite both α-rubromycin (**1**) and γ-rubromycin (**3**) being assigned as *para*-quinone structures (Fig. 2). These structural assignments remained until 2000 when using modern spectroscopic techniques and biosynthetic isotope labelling experiments, Zeeck *et al.* reassigned β-rubromycin (**2**) to the *para*-quinone structure. Treatment of **2** with acid leads to cleavage of the methyl ether and quinone rearrangement to afford γ-rubromycin (**3**).[4] During the course of these structural investigations, an additional two compounds were also isolated from bacterial cultures: 3-hydroxy-β-rubromycin (**4**) and δ-rubromycin (**5**, Fig. 2).

In addition to these five compounds, a further 11 compounds have been isolated. In 1974, an *Actinoplanes ianthinogenes* culture broth extracted from soil samples collected from Blumenau Brazil yielded a bright purple compound, purpuromycin (**7**, Fig. 3).[5, 6] The exact sterochemical configuration of purpuromycin (**7**) remains undetermined, however, a *syn* relationship between the C-4 hydroxyl and O-1′ is proposed due to the presence of a hydrogen bond between O-1′ and the OH group which is further stabilised by the anomeric effect, inducing O1′ to adopt a pseudoaxial position.[7]

**Fig. 3** Structure of purpuromycin (**7**) and proposed stereochemistry.[7]

Heliquinomycin (**8**) was isolated in 1996 from a culture broth of *Streptomyces* sp. MJ929-SF2, which was extracted from a soil sample collected from Hachiohji, Tokyo, Japan (Fig 4). Heliquinomycin (**8**) is structurally unique, containing a glycosidic linkage at C-3′ to a rare deoxycymarose.[8, 9] Stereochemical assignment was achieved through X-ray structure analysis of heliquinomycinone (**9**), the aglycone resulting from acidic cleavage of the glycoside. The griseorhodins (**10-15**) are a smaller subgroup of compounds isolated from culture broths of various strains of *Streptomyces* collected from different parts of the world.[10-15] They are a unique subset of the rubromycins as they do not contain the same methyl ester functionality on C-7 of the isocoumarin compared to other members of the family. This smaller subset is distinguished by the dense oxygen functionality located on the spiroketal core. The DK-compounds (**16-18**) are another subclass of compounds that were isolated from the bacterium *Dactylosporangium purpureum* sp.[16] Interestingly, the DK-compounds share the same oxygen functionality patterns on the spiroketal core as the griseorhodins, but like the other rubromycin compounds contain the methyl ester functionality on the isocoumarin. The absolute stereochemical configuration of γ-rubromycin (**3**) and β-rubromycin (**2**) was assigned as (*S*) using quantum chemical circular dichroism (CD) and comparison with the known stereochemistry of heliquinomycin (**7**).[17] The stereochemical assignment of griseorhodin A (**10**), (*S*, *S*, *S*, *S*), was proposed upon analysis of quantum-chemical CD calculations and comparison to the experimental measurements.[18] The relative and absolute stereochemistry of all the other compounds currently remains unassigned.

**Fig. 4** Structures of other rubromycin compounds.

## 3. Biosynthesis

Biosynthetically, it is understood that all members of the rubromycin family are derived from a type-II polyketide synthase (PKS) pathway. This was uncovered by isotope feeding experiments. One experiment investigated the feeding of $^{13}C$ enriched malonic acid to *Streptomyces s*p. A1 and the incorporation of 12 labelled units affording enriched β-rubromycin (**2**).[4] While the feeding of $^{13}C$ labelled acetate to *Streptomyces* sp. MJ929-SF2 afforded the enriched heliquinomycin aglycone unit (**9**).[19] Further labelling experiments revealed that the methoxy groups are derived from *S*-methylmethionine and the cymarose unit unique to heliquinomycin (**8**) is delivered from glucose metabolites.[19]

Investigations to identify the intermediates during the post-PKS transformation of polyketide **19** to the rubromycin structures were undertaken in 2009 by Piel *et al.*(Scheme 1).[18] Their findings identified some of the gene clusters responsible for the biosynthetic tailoring of griseorhodin A (**10**) by *Streptomyces* sp. JP95. Their main focus was to investigate how the spirosystem of griseorhodin A (**10**) is formed. An array of gene-deletion experiments afforded biosynthetic intermediates: collinone (**22**) and lenticulone (**23**) from the resulting cultures.[18] The structures of many intermediates of each biosynthetic tailoring step still remain elusive. However, in 2014, Schmidt *et al*. proposed that the griseorhodins and therefore the rubromycin biosynthetic intermediates, might actually exist as dimeric compounds.[20] This hypothesis resulted after the isolation and characterisation of a rotameric

 

pentacyclic intermediate, griseorhodin D (**20a**/**20b**) (Scheme 1). This compound was initially isolated by Piel *et al*. during their studies, but its inherent instability and the fact that it was only isolated in a very small amount precluded full characterisation at the time.[20]

**Scheme 1** Proposed biosynthetic pathway of griseorhodin A (**10**) and mechanistic pathways for 5,6-spiroketal formation.[18, 20]

Key biosynthetic intermediate collinone (**22**) is the point at which the structural diversity of the rubromycin family is introduced.[21] A highly complex oxidative tailoring process converts collinone (**22**) to the spiroketal lenticulone (**23**) where two mechanistic pathways are proposed for further conversion to the 5,6-spiroketal core associated with the natural products (Scheme 1). Both pathways involve a Grh06 mediated decarboxylation step. Grh06 could react at the α-hydroxy position of **23** followed by ring opening, expulsion of $CO_2$ aiding re-aromatization and cyclisation affording the spiroketal moiety (Path A). Or alternatively, Grh06 may act as a Baeyer-Villigerase on the ester functionality, inducing ring expansion followed by expulsion of $CO_2$ and ring closure to afford the spiroketal (Path B).[18] The enzymes MtmOIV and CmmOIV are close homologues of Grh06 which both perform Baeyer-Villiger type reactions providing support to path B. However, Grh06 reacting at the ester of **27** in the presence of a ketone would be unusual for a monooxygenase.[18] These investigations shed light on the biosynthetic conversions required for formation of the rubromycins but further structural studies of the enzymes and reaction sites involved is required to determine the exact biosynthetic pathway involved.

## 4. Biological activity

### 4.1 Antimicrobial

The rubromycin family exhibit an array of antimicrobial activity. γ-Rubromycin (**3**) and β-rubromycin (**2**) exhibit potent activity against *S. aureus* and *B. subtilis,*[1, 3] while griseorhodins A (**10**), C (**11**), and G (**12**) are active against *B. subtilis, S. aureus and P. notatum.*[12] 8-Methoxygriseorhodin C (**13**) exhibited similar activity against Gram-positive bacteria, with notable activity against strains of methicillin-resistant *S. aureus* (MRSA) (MIC = 0.78 μg/mL).[13] Purpuromycin (**7**) and its semisynthetic derivatives exhibit potent activity against bacteria, fungi and protozoa, particularly those associated with vaginal infections.[22] The more structurally unique heliquinomycin (**8**) exhibits inhibition of Gram-positive bacteria including strains of MRSA but minimal activity against Gram-negative bacteria.[8]

### 4.2 Cytotoxicity

Interestingly, the rubromycin family also exhibit unique cytotoxic properties. 3-Hydroxy-β-rubromycin (**4**), β-rubromycin (**2**) and γ-rubromycin (**2**) exhibit activity against stomach, colon, breast and liver cancer cell lines at low concentrations.[4] The griseorhodins A (**10**), C (**11**) and G (**12**) all exhibit activity against KB-nasopharaynx cells,[12] while griseorhodin A (**10**) and 7,8-dideoxygriseorhodin C (**14**) exhibit activity against human leukocyte elastase at micromolar concentrations.[18] Heliquinomycin (**8**) inhibits an array of human tumour cell lines including HeLa S3, KB, LS180, K562 and HL60 at $IC_{50}$ values of 0.96-2.8 μg/mL. It was also observed to inhibit P388 murine leukemia cell lines which were resistant to both adriamycin and cisplatin, common chemotherapy drugs.[23]

### 4.3 Enzyme Inhibition

Enzyme inhibition studies performed on six of the rubromycin compounds showed varying activity. Activity against moloney murine leukemia virus reverse transcriptase (M-MLV RT) was measured and β- (**2**), γ-rubromycin (**3**) and purpuromycin (**7**) showed inhibition with $IC_{50}$ values of 3.42, 4.37 and 3.69 μM respectively. Griseorhodins A (**10**) ($IC_{50}$ = 7.38μM) and C (**11**) ($IC_{50}$ = 9.37 μM) and α-rubromycin (**1**) ($IC_{50}$ = 7.34 μM) showed slightly weaker activity. Interestingly, griseorhodins A (**10**) and C (**11**) show similar levels of activity against human immunodeficiency virus type 1 (HIV-1) RT but β-(**2**), γ-rubromycin (**3**) and purpuromycin (**7**) were weaker, with $IC_{50}$ values between 20-33 μM and α-rubromycin (**1**) was almost inactive ($IC_{50}$ >200 μM).[24] Further investigations into the antiviral properties of both β-(**2**) and γ-rubromycin (**3**) were inconclusive as they were cytotoxic to the non-infected host cells at higher concentrations (> 6 μM).[25] However, these results provide impetus for modification of the rubromycin scaffold to improve the potency and selectivity as a strategy to develop novel antiviral agents.

Heliquinomycin (**8**) has been shown to selectively inhibit DNA helicases prepared from HeLa S3 cells at concentrations between 5-10 μg/mL in a non-competitive manner ($K_i$ = 6.8 μM).[23] This unique selectivity highlights the potential for heliquinomycin (**8**) as a lead compound for further development of novel anti-tumour agents.[23]

### 4.4 Telomerase Inhibition

The standout biological property exhibited by the rubromycins is their potent ability to inhibit human telomerase, reported by Hayashi *et al.* in 2000.[24] During standard DNA replication, small portions of DNA are lost from chromosome termini with each successive replication event. This results in shortening of the chromosome ends until vital portions of DNA are lost or chromosome instability results and cellular senescence is triggered.[26] Nature's way of counteracting this problem is through the addition of telomeres to the ends of chromosomes and the expression and function of the telomerase enzyme. Telomerase is a unique ribonucleoprotein enzyme that maintains telomere length at the chromosome termini. Telomeres are small portions of sacrificial DNA that cap the ends of eukaryotic chromosomes preventing loss of vital portions of DNA and also help to stabilise the termini of chromosomes during replication. Telomerase is a dimeric enzyme consisting of two reverse transcriptase motifs (TERT) with two RNA components (TR) which function to direct the

addition of deoxynucleotide triphosphates (dNTPs) via an internal complementary template sequence and two dyskerin proteins which bind to the TR components to aid in stabilisation of the enzyme complex.[27-29]

Telomerase is highly expressed in 80-90% of cancer cells while almost completely absent in neighbouring healthy cells. This unique selective cellular expression makes telomerase a highly attractive target for the development of new anti-cancer agents.[30] Of the six compounds that were tested, all five that contain the spiroketal moiety (**2**, **3**, **7**, **10**, **11**) showed inhibition of the telomerase enzyme ($IC_{50}$ = 2.64-12.2 μM).[24]

Steady state kinetic analysis of inhibition by β-rubromycin (**2**) revealed a competitive interaction with the telomerase substrate primer, TS-A ($K_i$ 0.74 μM). The implication of this is that β-rubromycin (**2**) could interact with the human telomerase RNA (TR) and/or the catalytic subunit (TERT) since the binding site of TS-A should be found on both.[24] Studies of the structurally related purpuromycin (**7**) have shown inhibition of enzyme translation through inhibition of aminoacyl-*t*RNA formation. This suggests that other members of the rubromycin family might also exhibit a similar mode of inhibition.[31]

### 4.5 Structure-Activity Relationship

The diverse bioactivity exhibited by the rubromycin natural products has stimulated strong interest in the synthesis development and medicinal chemistry of these compounds. Biological evaluation of β-rubromycin (**2**) ($IC_{50}$ = 3.06 μM) compared to its open-chain variant α-rubromycin (**1**) ($IC_{50}$ > 200 μM), showed that the 5,6-spiroketal core is an essential pharmacophore for telomerase inhibition.[24] In 2007, Pettus *et al*. reported their investigations into the synthesis and bio-evaluation of six rubromycin analogues in an attempt to further understand the structural requirements for inhibition. Of the six compounds, only spiroketal **30** showed any inhibitory activity (Fig. 5). It was observed that removal of the spiroketal and/or quinone functionality resulted in loss of activity.[32] Bio-evaluation of spiroketal-containing compounds synthesised in the Brimble group laboratory for telomerase inhibition also showed that the isocoumarin functionality plays a key role in potent inhibition.[33] These studies highlight the fact that not one moiety (quinone, spiroketal, isocoumarin) is fully responsible for activity, therefore further in-depth SAR studies are required to fully evaluate the key pharmacophore responsible for interactions with the telomerase enzyme for inhibition.

**Fig. 5** Rubromycin-based structures prepared by Pettus *et al*.[32]

## 5. Fragment synthesis and synthesis of the 5,6-spiroketal moiety

In order to carry out detailed structural investigation to determine the key pharmacophore of the rubromycin family of antibiotics, methodology studies to develop effective, concise synthetic routes to construct the 5,6-spiroketal motif have been conducted (Scheme 2). Early work by Greul and Brockmann employed a Lewis acid-mediated demethylation-cyclisation sequence starting from a dimethylated dihydroxyketone precursor with moderate success.[34] New methods for construction of the 5,6-spiroketal substructure include: oxidative [3+2] dipolar cycloadditions,[32, 35] [4+2] hetero Diels-Alder cycloadditions,[36-38] metal-catalysed cycloadditions,[39-42] aromatic Pummerer-type reactions,[43] halo-etherification of benzofurans[44, 45] and acid-mediated ring closure of dihydroxyketones.[7, 46-56] The wide array of methods for the construction of this motif has provided a solid base for total synthesis investigations. However, the unforeseen complexity and hidden electronic nature of these natural products has caused many problems in applying these established methods to the total synthesis of the actual natural products. This is discussed in the following sections.

**Scheme 2** Synthetic methods investigated for the synthesis of the 5,6-spiroketal core of the rubromycins.

## 6. Investigations towards total synthesis

Although the structures of these compounds have been known for decades, biological studies have been undertaken and syntheses of various fragments of the compounds have been reported, a successful total synthesis of a natural product remained elusive for many years. Only three compounds have succumbed to total synthesis to date and it took until 2001 for Danishefsky *et al.* to report their synthesis of heliquinomycinone (**9**), the aglycone of heliquinomycin (**8**).[44, 45] This report was then followed by syntheses of (±)-γ-rubromycin (**3**) by Kita *et al.*, Brimble *et al.*, Pettus *et al.*, Li *et al.* and Reiβig *et al*.[43, 57-60] The only synthesis of (±)-δ-rubromycin (**5**) was reported by Li *et al*. in 2013.[61] Synthetic efforts from Kozlowski *et al*. have also been extensive.[47, 62-64] Although their final target, purpuromycin (**7**) has eluded them, the understanding and insight gained through their endeavours have been invaluable. Their investigations have established synthetic routes to the heavily substituted fragments that have been used in later investigations by other research groups for the synthesis of other rubromycin natural products. Discussion of the various synthetic methods to access the rubromycin family of antibiotics will be presented in the following sections in chronological order with respect to the total syntheses reported. Specific model and fragment syntheses will be referred to as required.

### 6.1 Synthesis of heliquinomycinone by Danishefsky *et al.*

The synthesis of heliquinomycinone (**9**) by Danishefsky *et al* centered on the anion addition of furan **37** to aldehyde **38** followed by electrophilic spiroketalisation to furnish the spiroketal core (Scheme 3).

**Scheme 3** Retrosynthesis of heliquinomycinone (**9**) by Danishefsky *et al*.[44]

Synthesis of naphthofuran **37** was completed in a relatively short three step sequence starting with the known nitrile **39**,

 

which was reacted with the dianion of 3-furoic acid **41** to give acid **40** (Scheme 4). Intramolecular Friedel-Crafts acylation followed by reductive methylation afforded target compound **37**. Synthesis of isocoumarin **45** began from opianic acid **42** where Horner-Wadsworth-Emmons (HWE) reaction with phosphonate **46** and treatment of the intermediate enol ether with acid afforded cyclised isocoumarin **43**. Demethylation and selective allylation followed by Claisen rearrangement completed installation of the required allyl aromatic compound **44**. Protection of both hydroxyl groups and a two-pot hydroxylation-oxidative cleavage procedure afforded aldehyde **45**.

**Scheme 4** Synthesis of furan **37** and aldehyde **45**.[44]

Unfortunately, attempts to add lithiated furan **37** to aldehyde **45** to furnish alcohol **47** as per model studies were unsuccessful (Scheme 5).[44] This lack of product formation was rationalized by the ability of aldehyde **45** to undergo enolisation forming an anionic species that is stabilised by resonance structures A and B.

Scheme 5 Attempted coupling of furan 37 and aldehyde 45.[44]

Modification of the strategy was then undertaken in an attempt to prevent the undesired proton transfer reaction. Installation of the lactone functionality of the isocoumarin was performed later in the synthesis, after union of the two fragments. This new strategy led to coupling partner **49** becoming the new target that was synthesised in six steps and 44% yield from aldehyde **48** (Scheme 6).[44] The anion of naphthofuran **37** added smoothly to aldehyde **49** affording desired adduct **50** after silyl protection of the resultant alcohol. Elaboration of the acetal-bromide functionality to afford required isocoumarin **51** in preparation for cyclisation was achieved using a similar strategy to that used to construct the initial isocoumarin **45**. Disappointingly, attempts to effect electrophilic spiroketalisation using the conditions established in model studies were unsuccessful. An extensive screen of conditions either resulted in oxidation of the naphthalene unit or the reagents were not reactive enough to activate the furan double bond to induce cyclisation.

**Scheme 6** Synthesis of furan **52** and attempted electrophilic spirocyclisation.[44]

Alternative methods for the construction of the spiroketal unit were therefore investigated. An extensive screen of oxidants found that treatment of **51** with $OsO_4$ afforded diol **54** while leaving the naphthalene unit untouched (Scheme 7). However, further attempts to effect cyclisation at this stage under Mitsunobu conditions only afforded undesired regio-isomeric cyclised products.[45] Fortunately, it was observed that alcohol **54** underwent oxidation upon exposure to air in the presence of $NEt_3$ in MeOH and afforded ketone **55** upon cleavage of the benzyl protecting group. The use of Mitsunobu conditions successfully afforded spiroketal **56**. Spiroketal **56** was then converted to heliquinomycinone (**9**) in a further 4 steps completing the synthesis. This seminal report paved the way for future synthetic investigations and highlighted the potential difficulties in synthesising this family of natural products.

**Scheme 7** Synthesis of coupled ketone **56** and successful route to heliquinomycinone (**9**) by Danishefsky *et al.*[44, 45]

This investigation by Danishefsky paved the way for future synthetic investigations and provided a bench mark to work from. While a convergent synthetic route was desired, their attempts to unite elaborated coupling partners **37** and **45** revealed that the conjugation of the isocoumarin system was problematic. Extensive modification of the synthesis was required in that opening of the isocoumarin lactone was required for successful union with the naphthalene portion. Delicate functional group modification was then required to install compatible functionally for the Mitsunobu cyclisation reaction. The authors state that many of the difficulties that arose were unforeseen and proved challenging to overcome.

### 6.2 Synthesis of (±)-γ-rubromycin by Kita *et al.*

In 2007 Kita *et al.* reported the first total synthesis of (±)-γ-rubromycin (**3**). The synthesis used an elegant double aromatic Pummerer-type reaction to couple aryl sulfoxide **59** and exocyclic ether **60** to afford a 1,2-quinone spiroketal where upon treatment with acid led to a rearrangement affording the desired 1,4-quinone spiroketal **57** (Scheme 8). This unique method for construction of the 5,6-spiroketal core of (±)-γ-rubromycin (**3**) was initially investigated for the synthesis of model spiroketal systems and proved very successful.[43]

**Scheme 8** Retrosynthesis of (±)-γ-rubromycin (**3**) by Kita *et al.* using a double aromatic Pummerer type reaction.[43]

Mechanistically, the aromatic Pummerer reaction commences through acylation of the sulfoxide **61** followed by deprotonation of the phenol leading to rearrangement to sulfonium ion **63**. Nucleophilic attack of exocyclic ether followed by cyclisation and rearomatisation affords spiroketal **66**. Reoxidation of the sulfide prepares **68** for a second aromatic Pummerer reaction affording *ortho*-quinone spiroketal **71** completing the sequence. Further treatment of quinone **71** with acid induced rearrangement to afford the more stable *para*-quinone **72**.

**Scheme 9** Proposed mechanism of the aromatic Pummerer type reaction.[43, 65]

Synthesis of (±)-γ-rubromycin (**3**) began with synthesis of substituted sulfoxide **59** (Scheme 10). Known keto-ester **73** was subjected to protecting group manipulation and intramolecular condensation to afford phenol **74**. Regioselective instalment of the sulfide functionality, selective protection of the *ortho*-phenol as a carbonate ester and oxidation of sulfide **76** to the sulfoxide completed the synthesis of **59**.

**Scheme 10** Synthesis of sulfoxide **59** coupling partner.[43]

Exocyclic ether **60** was synthesised from bromophenol **77** in six steps (Scheme 11). The key step was the regioselective reaction of aryl bromide **79** with dimethyl malonate **82** to afford exocyclic ether **60** using a similar procedure to that of Danishefsky *et al*.[66] The regioselectivity of the reaction was proposed to be a consequence of a strong inductive effect caused by the C-8 methoxy substituent. This diester functionality was to be converted to the isocoumarin moiety after formation of the spiroketal.

**Scheme 11** Synthesis of exocyclic ether **60**.[43]

Coupling of fragments **59** and **60** proceeded upon *in situ* silylation of sulfoxide **59** and treatment with exocyclic ether **60** and triflic acid to furnish pentacyclic spiroketal **83** (Scheme 12). Deprotection of the phenol, and reoxidation of the sulfide to the sulfoxide afforded pentacyclic spiroketal **84**.

**Scheme 12** Synthesis of spiroketal sulfoxide **84**.[43]

Treatment of **84** with trifluoroacetic anhydride afforded *ortho*-quinone spiroketal **85** which underwent an acid-mediated rearrangement via ketal opening and recyclisation to the more stable 1,4-quinone **57** (Scheme 13). Functionalisation of the naphthalene fragment over five steps and selective ester saponification afforded acid **87**. Coupling with phosphorane **90** afforded intermediate cyanoketophosphorane **88** and treatment with DMDO in MeOH afforded the corresponding keto-ester **89** which spontaneously cyclised, thus completing construction of the isocoumarin ring. Finally, Lewis acid mediated demethylation completed the first total synthesis of (±)-γ-rubromycin (**3**).

**Scheme 13** Synthesis of (±)-γ-rubromycin (**3**) by Kita *et al*.[43]

This synthesis by Kita *et al.* employed an open chain isocoumarin precursor to ensure successful spiroketal formation using an elegant double aromatic Pummerer type reaction. Key to the success of this method was the facile nature in which *ortho*-quinone **85** underwent rearrangement to the desired *para*-quinone **57** upon treatment with acid. This synthesis required delicate modification of both the naphthalene and isocoumarin portions post formation of the spiroketal moiety which can be problematic with these highly functionalised systems. This report provided a new bench mark for future syntheses of γ-rubromycin (**3**).

### 6.3 Formal synthesis of (±)-γ-rubromycin by Brimble *et al.*

Following the success of Kita *et al*., investigations were undertaken in the Brimble group towards a total synthesis of (±)-γ-rubromycin (**3**). Attention focused on the use of an acid-mediated spirocyclisation of dihydroxyketone precursor **91** to construct the spiroketal core which in turn is accessed from union of alkynol **93** and iodide **94** via Sonogashira cross-coupling (Scheme 14).

**Scheme 14** Retrosynthesis of (±)-γ-rubromycin (**3**) by Brimble *et al*.[57]

Toward this end, synthesis of alkynol **93** was undertaken starting with 1,2,4-trimethoxybenzene (**95**) which was converted to bromoquinone **96** in five steps (Scheme 15). The key step in the synthesis was regioselective allyloxylation to afford allyl ether **98**. The electronic and steric effects of the leaving group were key to success in this reaction. Initial attempts using a bromide **96** afforded a mixture of regioisomeric products. The corresponding iodide afforded solely the undesired isomer. Pleasingly use of the uncommon azide provided the desired allyl ether **98** as the sole product in good yield. Microwave-assisted Claisen rearrangement and protection of the hydroxyl group as an EOM ether afforded alkene **99**. Reductive dimethylation of the quinone, Lemieux–Johnson oxidation and addition of ethynylmagnesium bromide to the intermediate aldehyde furnished alkynol **93**.

The Sonogashira coupling partner iodide **94** was synthesised from guaiacol (**101**), beginning with bromination and protection of the phenol as the EOM ether. Using a modified procedure developed by Danishefsky *et al*.[66] and also used by Kita *et al*.[43] regioselective addition of the anion of dimethyl malonate to the benzyne derived from bromide **102** afforded homophthalic ester **103** as a sole product. Deprotection of the phenol was required for the subsequent *ortho*-iodination. Reprotection of the phenol as an EOM ether completed the synthesis of iodide **94**.

**Scheme 15** Synthesis of coupling partners alkynol **93** and iodide **94**.[57]

With both coupling partners **93** and **94** in hand, their union by Sonogashira coupling was undertaken, affording coupled product **92** with only trace amounts of the Glaser-type coupling product detected (Scheme 16). Reduction of the triple bond and oxidation of the alcohol to afford ketone **91** and treatment with $NaHSO_4 \cdot SiO_2$ afforded spiroketal **105** in good yield. Selective hydrolysis of the aliphatic ester and oxidation of the naphthalene to afford quinone **87** completed the formal synthesis as conversion of quinone **87** to (±)-γ-rubromycin (**3**) could be achieved using the protocol developed by Kita *et al*.[43]

**Scheme 16** Formal synthesis of (±)-γ-rubromycin (**3**) by Brimble *et al*.[57]

This report provided a robust synthetic route to the desired alkynol **93** in 12 steps from commercially available starting material, which at the time was one of the most efficient methods for synthesis of the naphthalene unit. Regio-selectivity issues were solved in attempts to synthesise allyl ether **98** using the less common azide as a leaving group. The utilization of an open chain isocoumarin precursor facilitated the key double-deprotection/acid-mediated spirocyclisation affording the desired 5,6-spiroketal.

### 6.4 Synthetic investigations towards purpuromycin by Kozlowski *et al.*

Synthetic investigations towards purpuromycin (**7**) reported by Kozlowski *et al*. are extensive and detailed. Their synthetic

 

strategy centred on the [3+2] cycloaddition of substituted alkene **109** to the nitrile oxide derived from nitroalkane **108** to afford substituted isoxazole **107** which after reduction provided β-hydroxyketone **106** (Scheme 17). Further treatment with acid effected cyclisation affording a mono-hydroxylated 5,6-spiroketal product were cleavage of protecting groups and oxidation of the naphthalene moiety would afford purpuromycin (**7**).[7] Model studies were carried out using this synthetic method that also aided in the determination of the proposed relative stereochemistry of purpuromycin (**7**).[7]

**Scheme 17** Retrosynthesis of purpuromycin (**7**) by Kozlowski *et al*.[47]

Towards this end, an efficient and reliable synthetic route to the desired nitroalkane **108** and alkene **109** coupling partners was required. The first generation synthesis of the nitroalkane **108** began with vanillin (**110**) and centered on a Wulff-Dötz type reaction of chromium carbene **11** (accessed in five steps from **110**) to construct the basis of the naphthalene structure (Scheme 18). The two OTBS groups of **112** were differentiated through their differing oxidation potentials due to the electron donating nature of the methoxy group at C-6. DDQ was successfully used to effect oxidation at the C-2 $CH_2$OTBS group to afford aldehyde **113**. Reduction of the aldehyde and protection of the alcohol as a MOM ether afforded **114**. TBS cleavage and oxidation of the alcohol then afforded aldehyde **115**. Dakin oxidation of **115** and protection of the naphthol as a benzyl ether afforded ether **116**. While this synthetic route provided the desired intermediate **116** for elaboration to the desired nitroalkane, at this point the authors deemed the synthesis too lengthy to be feasible. The added fact that the naphthol afforded from Dakin oxidation of **115** was found to be unstable was also a contributing factor for the development of a new synthetic route.[67]

**Scheme 18** First generation synthesis of naphthalene **116**.[67]

The second synthesis of nitroalkane **108** started with furan **117** affording naphthalene **118** in six steps (Scheme 19). The differing oxidation potentials of the two $CH_2$OTBS groups were again exploited using DDQ to afford aldehyde **119**. Subsequent Henry reaction of aldehyde **119** with nitromethane followed by reduction of the intermediate alkene installed the required nitroalkane portion of **120**. Silyl deprotection and oxidation afforded aldehyde **121**. Dakin oxidation was then used to install the required naphthol functionality of **122**, however naphthol **122** was very unstable and proved difficult to protect as a TES ether.[47] Although this route was deemed inefficient, TES ether **123** was employed in 2006 in a key coupling with isocoumarin **136** (Scheme 21) in synthetic studies towards purpuromycin (**7**) and is discussed in scheme 22.

**Scheme 19** Second generation synthesis of nitroalkane **123**.[47]

To circumvent the issue of instability of the naphthol functionality that plagued the previous two syntheses, investigations towards a third synthesis of **108** were initiated in 2008 (Scheme 20). A series of condensation and cyclisation reactions followed by oxidation and tautomerization afforded naphthol **125** in four steps starting with aldehyde **124**. Oxidation of **125** with IBX afforded *ortho*-quinone **126** and quinone reduction and selective mono-benzylation as afforded benzyl ether **127**. Methylation of the remaining naphthol and a two-step reduction-oxidation of the ester afforded aldehyde **128**. Henry reaction of aldehyde **128** with nitromethane and oxidation of the intermediate alcohol provided eliminated product **129** in poor yield. Reduction of the double bond with sodium borohydride then afforded the desired nitroalkane **130**.[68] The authors deemed this route the most efficient to afford sufficient quantities of **130** upon optimisation for further investigations to complete the synthesis of purpuromycin (**7**).

**Scheme 20** Third generation nitroalkane synthesis of nitroalkane **130**.[68]

The isocoumarin coupling partner **136** was synthesised from terephthalic ester **132** available in four steps from catechol **131** (Scheme 21). Treatment of **131** with high pressure $CO_2$ at high temperature afforded a diaryl acid that upon global methylation afforded ester **132**. Nitration followed by Sandmeyer reaction installed the iodide of **133**. Selective ester hydrolysis and reduction of the newly afforded acid and protection of the alcohol as the TBS ether afforded silyl ether **124**. Heck coupling with enol ether **137** and acid-mediated cyclisation constructed the lactone unit. TBS deprotection afforded alcohol **135**. Oxidation with Dess-Martin periodinane and protecting group manipulation followed by a two-step homologation procedure and final treatment with acid afforded the desired alkene coupling partner **136**.

**Scheme 21** Synthesis of isocoumarin coupling partner **136**.[47, 69]

With initial access to coupling partners **123** and **136** established, *in situ* generation of the nitrile oxide from nitroalkane **123** followed by addition to isocoumarin **136** afforded isoxazole **138** (Scheme 22). Cleavage of the TES group, reduction of the isoxazole and debenzylation afforded ketone **140**. Unfortunately, all attempts to induce spiroketalisation of **140** upon exposure to acid failed to afford any spiroketal product **141**. Oxidation of the naphthalene unit to the quinone also did not facilitate spirocyclisation.[47]

**Scheme 22** Attempted cyclisation of naphthalene **140** and quinone **142** and potential reaction pathways of oxocarbenium **144**.[47]

This result prompted extensive investigations into the factors that were inhibiting cyclisation. Model studies revealed that the electronic nature of the isocoumarin fragment was the problem. Electron-donating groups (Y) promoted spirocyclisation (Path A), whereas electron-withdrawing substituents like the isocoumarin unit of purpuromycin (**7**), led to the formation of undesired benzofuran products (Path B) (Scheme 22). As the oxocarbenium **147** intermediate bears two acidic protons at C-

4′, irreversible elimination of one of the protons to afford furan **148** predominates if the hydroxyl group is not nucleophilic enough to cyclise (Scheme 23). The authors hypothesised that replacing these protons with a ketone would eliminate the potential for furan formation, while activating the adjacent carbonyl group towards electrophilic attack.[64]

**Scheme 23** Proposed solution to prevent benzofuran formation.[64]

Installation of the ketone functionality was therefore next attempted. However, it became apparent that the intermediates and synthetic protocol they had developed were problematic. Either functional groups were incompatible with the reaction conditions or the observed steric congestion around C-4′ prevented further functionalization. It was decided to alleviate the steric congestion around C-4′ by removal of the adjacent methoxy group at C-1 (Fig. 6). An additional requirement was that the two key hydroxyl groups that would form the spiroketal would have to have orthogonal protecting groups to the adjacent *ortho*-hydroxyl groups.[64]

**Fig. 6** Proposed functional group requirements for purpuromycin (**7**) spirocyclisation precursor.[64]

These changes to the synthetic protocol were made and nitroalkane **152** was prepared from intermediate quinone **151** (available from their latest reported naphthalene synthesis, scheme 20) in six steps (Scheme 24). Cycloaddition with alkene **153** followed by reduction of the isoxazole and protection of the alcohol as a silyl ether afforded ketone **154**. The reduced steric congestion provided by the removal of the C-1 methoxy facilitated successful installation of the desired ketone group using a selenium dioxide-mediated oxidation. Cleavage of the benzyl groups and treatment with tosic acid was finally successful in affording spiroketal product **156**. This result was the first time that a fully constructed isocoumarin fragment was present and that a spiroketal product was formed under acidic conditions. Despite this success, extensive efforts to elaborate spiroketal **156** to purpuromycin (**7**) were unsuccessful. Complete reduction of the ketone at C-4′ and instalment of the C-1 phenol could not be effected.[64]

**Scheme 24** Synthesis of advanced purpuromycin intermediate **156** by Kozlowski *et al.*[64]

These investigations by Kozlowski *et al.* show the complexity of the rubromycin compounds. Extensive efforts to afford a concise synthesis of the naphthalene portion and the determination to execute a convergent synthesis of purpuromycin (**7**) using a fully elaborated isocoumarin proved elusive. Employment of this strategy highlights the dramatic effect pendant functionality can have on the success of reactions. Results show that for a fully elaborated isocoumarin to undergo a classical acid-mediated spirocyclisation, the competing facile elimination pathways to benzofuran products must be prevented.

### 6.5 Total synthesis of (±)-γ-rubromycin by Pettus *et al.*

In 2006 Pettus *et al.* reported a new approach to construct rubromycin-based spiroketals using an oxidative [3+2] cyclisation reaction.[35] The full application of this method was put to the test in a total synthesis of (±)-γ-rubromycin (**3**) where construction of the spiroketal unit was to be undertaken using fully elaborated coupling partners, quinone **157** and methylene chroman **158** (Scheme 25).[58]

**Scheme 25** Retrosynthesis of (±)-γ-rubromycin (**3**) by Pettus *et al.*[58]

Synthesis of quinone **157** began with 1,2,4-trimethoxybenzene (**95**) and α-tetralone **159** was afforded in three steps (Scheme 26).[70] Using Nicolaou's protocol,[71] bromination of **159** was executed followed by oxidation with CAN to afford bromoquinone **96**. The unique leaving group potential of an azide was again utilised to ensure complete regioselective control upon reaction with methanol to afford ether **160**, a method previously demonstrated by the Brimble group.[57] Finally, hydrolysis of ether **160** with KOH completed the synthesis of quinone **157**.

**Scheme 26** Synthesis of quinone **157**.[58]

Synthesis of methylene chroman **158** began with iodide **161** which was available from vanillin using procedures reported by Reiβig *et al.* (Scheme 27)[72, 73] Heck reaction with methyl acrylate and reduction of the alkene product afforded ester **162**. Acid induced lactonisation of **162** and HWE reaction with phosphonate **165** gave silyl enol ether **164**. Selective methylenation and fluoride-induced lactonisation completed the synthesis of **158** in preparation for the key cycloaddition.

**Scheme 27** Synthesis of methylene chroman **158**.[58]

With both coupling partners in hand, treatment of quinone **157** and methylene chroman **158** with CAN and $NaHCO_3$ in THF gave a 1:2 mixture of spiroketal products **166** and **167** (Scheme 28). Lewis acid-mediated demethylation of **167** then afforded desired (±)-γ-rubromycin (**3**). Attempts to induce rearrangement of *ortho*-quinone **166** to the desired *para*-quinone **167** were initially unsuccessful using the protic conditions employed by Kita *et al.* for a similar rearrangement. Surprisingly, treatment of **166** with an excess $BBr_3$ cleanly afforded (±)-γ-rubromycin (**3**) in good yield.

**Scheme 28** Synthesis of (±)-γ-rubromycin (**3**) by Pettus *et al.*[58]

This synthesis of (±)-γ-rubromycin (**3**) has shown the robust nature of the [3+2] cycloaddition reaction to form the rubromycin spiroketal core using fully elaborated naphthoquinone and isocoumarin coupling partners. This key reaction facilitated a highly convergent synthesis requiring minimal synthetic steps after the construction of the delicate spiroketal core to afford (±)-γ-rubromycin (**3**).

### 6.6 Formal synthesis of (±)-γ-rubromycin by Li *et al.*

As the number of methods for the construction of the rubromycin spiroketal ring system increase, application of these methods in total synthesis endeavours has yielded successful results. Li *et al*. used their oxidative-hypoiodite cycloetherification method in a formal synthesis of (±)-γ-rubromycin (**3**) (Scheme 29).[59, 74] Their synthesis initially focused on the union of naphthalene **169** and aldehyde **170** and the cyclisation of ketone **168**.[59]

**Scheme 29** Retrosynthesis of (±)-γ-rubromycin (**3**) by Li *et al*.[59]

Aldehyde **170** was synthesised from known phenol **104**, prepared from guaiacol in four steps using reported procedures (Scheme 30).[57] Allylation of phenol **104** followed by Claisen rearrangement furnished allyl phenol **172**. Protection of the phenol as an EOM ether and ozonolysis of the terminal alkene afforded the desired aldehyde **170** in a short four step sequence from **104**.

**Scheme 30** Synthesis of aldehyde **170**.[59]

Synthesis of naphthalene coupling partner **169** began with Kozlowski's advanced intermediate, *ortho*-quinone **126** (Scheme 31).[68] Reduction of the quinone and selective alkylation gave diester **173**. Saponification of both esters and Dieckmann condensation afforded acetate **174**, where simple acidic hydrolysis provided the desired naphthalene **169**.

**Scheme 31** Synthesis of naphthalene **169**.[59]

Coupling of naphthalene **169** and aldehyde **170** was successful upon treatment of naphthalene **169** with LiHMDS and then addition of aldehyde **170** to afford alcohol **175** (Scheme 32). It was also found that treatment of acetate **174** with LDA then aldehyde **170** also afforded alcohol **175** in good yield. Removal of the newly formed hydroxyl group through a two-step elimination-reduction sequence followed by acidic cleavage of the EOM group set the stage for the key cycloetherification reaction. Treatment of **168** with *m*CPBA, TBAF and TBAI in THF afforded the desired spiroketal product **176** in good yield. A two-step procedure was required to effect complete reduction of the ketone to afford spiroketal intermediate **105** thus completing a formal synthesis of (±)-γ-rubromycin (**3**). Using procedures reported by both, Brimble *et al.* and Kita *et al*., **105** can be converted to the natural product.[43, 57]

**Scheme 32** Formal synthesis of (±)-γ-rubromycin (**3**) by Li *et al*.[59]

This synthesis of (±)-γ-rubromycin (**3**) further shows that alternative methods for construction of spiroketals can lead to successful total syntheses of the rubromycins. Interestingly, the ketone and hydroxyl functionality at positions 3′ and 2 were removed to access spiroketal **176** en route to (±)-γ-rubromycin (**3**). However, retention of this functionality could enable access to other members of the rubromycin family that contain oxygen functionality around the spiroketal core, namely the griseorhodins and DK compounds.

### 6.7 Total synthesis of (±)-δ-rubromycin by Li *et al.*

As an extension of their oxidative-hypoiodite cycloetherification chemistry, Li *et al.* also pursued an alternative synthetic route to construct the 5,6-spiroketal moiety of the rubromycins. The gold-catalysed double intramolecular alkyne hydroxylation reaction has provided access to substituted spiroketal substrates[39] and was implemented by Li *et al.* in a synthesis of (±)-δ-rubromycin (**5**) (Scheme 33).[61] This new synthesis initially centered on the union of alkyne **179** and iodide **178** via a Sonogashira coupling and a gold-catalysed cyclisation to construct the spiroketal core.

**Scheme 33** Retrosynthetic route of (±)-δ-rubromycin (**5**) by Li *et al*.[61]

Towards this end, iodide **178** was synthesised from bromoquinone **180** (Scheme 34). Cycloaddition of bromide **180** with diene **183** afforded quinone **181**. Protecting group exchange, iodination and methylation of the remaining free hydroxyl group completed the synthesis of **178** in preparation for coupling.

**Scheme 34** Synthesis of iodide **178** coupling partner.[61]

Alkyne **179** was synthesised from advanced alkene intermediate **184**, obtained from their previous synthesis of (±)-γ-rubromycin (**3**, Scheme 35).[59] Hydroboration/oxidation of the alkene and oxidation of the alcohol afforded aldehyde **185** which underwent Seyferth-Gilbert homologation with phosphonate **187** to give alkyne **186**. Following a protocol developed by Kita *et al*. homophthalic ester **186** was converted to isocoumarin **179** via a cyanoketophosphorane intermediate.[43] Acidic cleavage of the EOM protecting group afforded alkyne **179** thus completing the synthesis.

**Scheme 35** Synthesis of alkyne **179** coupling partner.[61]

Union of iodide **178** and alkyne **179** via Sonogashira coupling afforded two products: the initially desired alkyne **177** and the unexpected pyran **188** (Scheme 36). However, attempts to cyclise alkyne **177** to afford pyran **188** using the established gold-catalysts conditions were unsuccessful. As pyran **188** was afforded from the initial Pd-mediated coupling, exposure of alkyne **177** to $PdCl_2(PPh_3)_2$ and base afforded pyran **188** in good yield. Spiroketal formation was induced upon treatment of pyran **188** with acid. Final $BBr_3$-mediated demethylation completed the first total synthesis of (±)-δ-rubromycin (**5**). This synthesis of (±)-δ-rubromycin (**5**) provides insight into the complexity of the rubromycin natural products. The fact that a substrate bearing a fully substituted isocoumarin was nucleophilic enough to form pyran **188** suggests that nucleophilic reactions to construct the spiroketal core with fully substituted naphthalene and isocoumarin moieties might

actually still be viable. This is notable despite the initial reports from Kozlowski *et al*. and Reiβig *et al*. on their disappointing attempts to effect acid-mediated cyclisation reactions.[47, 54]

**Scheme 36** Synthesis of (±)-δ-rubromycin (**5**) by Li *et al*.[61]

### 6.8 General synthetic investigations towards the rubromycins and total synthesis of (±)-γ-rubromycin by Reiβig *et al*.

Reiβig *et al*. have contributed greatly to the synthetic efforts towards the rubromycins. Their initial focus was to synthesise heliquinomycin (**8**) with the overall goal to develop a flexible synthetic method to also access other members of the natural product family (Scheme 37). Initial investigations and model studies focused on addition of lithiated allene **194** to a substituted naphthaldehyde **193** to afford hydroxyenone **191** after hydrolysis of the intermediate enol ether. Heck reaction with isocoumarin **192** and reduction of the double bond, followed by treatment with acid was envisioned to construct the spiroketal core of the rubromycins.

**Scheme 37** Retrosynthesis of the rubromycin structure **189** by Reiβig *et al*.[75]

Synthesis of the isocoumarin **197** proceeded smoothly starting with standard protection of the phenol and aldehyde functional groups of vanillin (**110**, Scheme 38). Regioselective lithiation and reaction with methyl chloroformate followed by global deprotection afforded aldehyde **195**. Regioselective iodination and HWE reaction with phosphonate **46** afforded a mixture of E-Z (1:1.9) enol ethers **196**. HBr-induced cyclisation and benzyl protection of the phenol completed the synthesis of isocoumarin **197**.[72]

**Scheme 38** Synthesis of isocoumarin **197**.[72]

Synthesis of enone **204** was more problematic. Initial attempts using 1,2,4-trimethoxybenzene as a starting material (ring A) and then constructing ring B led to difficulty in selective functionalization at C-2 (Scheme 39). To circumvent this problem, the authors endeavoured to reverse the way in which the naphthalene structure was constructed, beginning with aldehyde **198** (ring B) and constructing ring A. Thus, bromination of **198** followed by protection of the aldehyde as an acetal afforded bromide **199**. Diels-Alder reaction with furan followed by careful ring opening of the intermediate ether provided naphthol **200**. *Ortho*-bromination with pyridinium tribromide followed by oxidation to the quinone and reductive dimethylation afforded bromide **201**. Lithiation of the bromide and quenching of the anion with $B(OMe)_3$ afforded boronic acid **203**. Oxidation with $H_2O_2$ and NaOH afforded the corresponding naphthol which was immediately methylated. Unmasking of the aldehyde and reaction with lithiated allene **194** followed by acid hydrolysis and TES protection afforded enone **204** thus completing the synthesis.[75]

**Scheme 39** Synthesis of enone **204**.[75]

With routes established to both the coupling partners, investigations to unite enone **204** with isocoumarin **197** and elaborate to the spiroketal ring system began (Scheme 40). Heck reaction of **204** and **197** afforded coupled product **206** in good yield. Reduction of the alkene and cleavage of the benzyl ethers unmasked the phenols followed by treatment with HCl in isopropanol afforded spiroketal **207** in a disappointing 7% yield.

**Scheme 40** Synthesis of quinone **207**.[46]

This lack of reactivity was puzzling and called for further investigation. Extensive model studies afforded a similar hypothesis to that of Kozlowski *et al.* namely that the isocoumarin pendant functionality was too electron withdrawing, thereby reducing the nucleophilicity of the phenol inhibiting its ability to cyclise to afford the desired spiroketal.[54] The fact that an oxidised quinone product was isolated suggests the extensive substitution on the naphthalene structure and the added electron density results in these systems being very prone to oxidative demethylation and further degradation. To circumvent this issue, Reiβig *et al*. chose to revise their synthetic strategy and utilise an open chain isocoumarin precursor and acid-labile hydroxyl protecting groups to help promote spirocyclisation (Scheme 41). To simplify this undertaking, (±)-γ-rubromycin (**3**) became the new target. Access to enone **209** was to be effected through addition of lithiated allene **194** to bromide **208**. Attempts to effect this transformation only led to the decomposition of the starting material as bromide **208** was deemed too labile.

**Scheme 41** Attempted addition of allene **194** to bromide **208** en route to (±)-γ-rubromycin (**3**).[60]

Undeterred by this initial set back, an alternative route was sought utilizing chemistry established by Murray *et al*.[76] to install the enone functionality of **209** in preparation for coupling with iodide **210** (Scheme 47). Murray *et al.* reported that reaction of phosphonate **214** with aldehyde substrates afforded HWE product, enol ether **215**. Hydrolysis of the enol ether **215** then afforded the desired enone **216**. As two reaction sites are available for anion **213**, blocking of the γ position of the phosphonate first with a bulky silyl group promotes reaction with the aldehyde at the desired α position. To this end, synthesis of silyl phosphonate **218** was undertaken and completed in two steps from acetal **217** (Scheme 42). Treatment of **218** with KHMDS and naphthaldehyde **219** (afforded from aldehyde **124** in 11 steps) afforded silyl alkene **220** after acidic hydrolysis of the enol ether intermediate.

**Scheme 42** Application of Murray's protocol to synthesise silyl enone **220**.[60]

Synthesis of iodide coupling partner **210** began with conversion of vanillin to aldehyde **161** in four steps.[72] Protection of the phenol as a MOM ether and HWE coupling with phosphonate

 

**222** afforded the required iodide **210** in a short sequence (Scheme 43).

**Scheme 43** Synthesis of iodide **210** coupling partner.[60]

Formation of the Grignard reagent **223** was required to effect a copper (I)-mediated 1,4-addition to silyl alkene **220** (Scheme 44). Treatment of the intermediate silyl enol ether with acid afforded ketone **224** in good yield. Next it was decided to adjust the oxidation state of the naphthalene and quinone **225** spirocyclised upon treatment with triflic acid affording **226**. A novel procedure for formation of the isocoumarin using $HBF_4{\cdot}Et_2O$ also induced desilylation. Treatment of the resulting fluorosilane intermediate with peracid afforded a protonated product in favour of a Tamao-Fleming type oxidation product. A one-pot procedure for isocoumarin formation and desilylation was developed whereupon treatment of **226** with $HBF_4{\cdot}Et_2O$ with a MeOH-$NEt_3$ work-up simplified the protocol to afford **227**. Final demethylation of the methyl ethers afforded (±)-γ-rubromycin (**3**) and completed the synthesis.

**Scheme 44** Synthesis of (±)-γ-rubromycin (**3**) by Reiβig *et al.*[60]

The initial synthetic work by Reiβig *et al*.[49, 54] in conjunction with reports from Kozlowski *et al.*[47] established the understanding behind the influence that the isocoumarin fragment has on acid–mediated spirocyclisation reactions. While initial attempts were unrewarding with regards to a total synthesis, the initial problems encountered were solved through the use of an open chain isocoumarin precursor. The development of the unique transformation of silane **226** to **227** using $HBF_4{\cdot}Et_2O$ and base demonstrates the strong influence that the extensive conjugation of the isocoumarin moiety can have on functional group transformations.[77]

### 6.9 Synthetic investigations towards the griseorhodins by Brimble *et al.*

Interest in the synthesis of this natural product family has led the Brimble group to apply their synthetic strategy developed for the formal synthesis of (±)-γ-rubromycin (**3**)[57] to the griseorhodins.[78] Unlike γ-rubromycin (**3**) the griseorhodins contain a methyl group at C-7 of the isocoumarin as opposed to a methyl ester and they endeavoured to exploit this functionality difference to establish a synthetic route to this subclass of rubromycin–based natural products (Scheme 45). Their previous synthesis employed an acid-mediated cyclisation to construct the spiroketal core. They hypothesised that exchange of the methyl ester for a methyl group on C-7 would alleviate the negative electron-withdrawing mesomeric effect,[47, 54] thus facilitating successful spirocyclisation using a fully formed isocoumarin moiety.

Scheme 45 Comparing reaction pathways for isocoumarins under acidic spirocyclisation conditions.[78]

It was envisioned that a synthesis of the model griseorhodin structure **228**, accessed from union of alkynol **93** and isocoumarin **230**, would provide a good platform upon which future synthetic investigations towards the griseorhodin natural products can be based (Scheme 46).

**Scheme 46** Retrosynthesis of griseorhodin model **228** by Brimble *et al.*[78]

Synthesis of isocoumarin **230** started with known phenol **104** (Scheme 47).[57] *Ortho*-bromination and protection of the phenol as a benzyl ether afforded homophthalic ester **213**. Saponification of both esters followed by Dakin-West oxidation afforded keto-acid **232** which cyclised upon treatment with catalytic $HClO_4$ to give the desired isocoumarin **230**.

**Scheme 47** Synthesis of isocoumarin **230**.[78]

Sonogashira coupling of alkynol **93** (available from their (±)-γ-rubromycin (**3**) synthesis)[57] and isocoumarin **230** afforded alkyne **233** in moderate yield (Scheme 48). IBX oxidation of the 2° alcohol and treatment of the corresponding ketone with Pd/C under a hydrogen atmosphere afforded ketone **229** in preparation for cyclisation. Initial attempts to effect spirocyclisation of ketone **229** using protic acidic conditions were unsuccessful. Exposure of **229** to PPTS in $CH_2Cl_2$ afforded dihydroxyketone **234** but further attempts to effect spirocyclisation of **234** with both protic and Lewis acids did not afford any spirocyclised products.

**Scheme 48** Synthesis of ketone **234** and attempted spirocyclisation.[78]

In light of these investigations, it was concluded that the exchange of the methyl ester for a methyl group was not sufficient to facilitate spirocyclisation. To further probe this reaction, three isocoumarin precursors namely, **236 - 238** were synthesised from acid **232** to reveal the key functionality that is compatible with the spirocyclisation step (Scheme 49).

**Scheme 49** Synthesis of isocoumarin precursors **236** - **238**.[78]

All three precursors were then coupled with alkynol **93** and elaborated to the corresponding spirocyclisation ketone precursors **240**, **243** and **247** (Scheme 50). Upon treatment with a range of acids, ketone **240** containing a lactone group did not form a spiroketal product. However, treatment of ketones **243** and **247** with $NaHSO_4{\cdot}SiO_2$ in $CH_2Cl_2$ afforded spiroketal products **244** and **248** from the respective reaction mixtures. Further elaboration of spiroketals **244** and **248** to furnish the desired griseorhodin model was then undertaken. Attempts to cleave the silyl ether of **224** with various sources of $F^-$ were unsuccessful. However, treatment of spiroketal **248** with NaH afforded isocoumarin **235** and subsequent oxidation of the naphthalene moiety using DDQ followed by $BCl_3$ mediated demethylation afforded the desired griseorhodin model compound **228**.

**Scheme 50** Synthesis of griseorhodin based spiroketals and griseorhodin model **228** by Brimble *et al.*[78]

Throughout this systematic investigation of the functionality of the isocoumarin moiety of the griseorhodins, it was determined that the lactone functional group was the key structure that prevented successful acid-mediated spirocyclisation of these substrates. These investigations further demonstrated that use of an open-chain isocoumarin precursor is required to enable successful acid-mediated spirocyclisation to construct a rubromycin-based 5,6-spiroketal.

## 7. Concluding Remarks

The common strategy for the synthesis of the rubromycin family of natural products is to effect a convergent synthesis whereby the naphthalene and isocoumarin moieties are synthesised then united in preparation for construction of the spiroketal. However, the extensive conjugation of the aromatic portions of these molecules renders them sensitive and challenging synthetic targets. Subtle changes in pendant functionality have been shown to have a great effect on the successful outcome of subsequent of reactions. This effect has been observed in the work reported by Kozlowski *et al.*, Reiβig *et al.* and Brimble *et al.* during efforts to employ an acid-mediated spirocyclisation reaction on precursors that contain an intact isocoumarin ring system. The synthetic routes to access the rubromycin compounds are long and often rely on complex transformations. While this makes further biological evaluation of the parent natural products difficult, large numbers of structurally-related intermediates are synthesised en route offering the potential to create libraries of compounds that can undergo biological screening and enable potential construction of a structure-activity relationship profile.

Biosynthetic methods for the synthesis of these structures revolve around the late stage oxidation at specific positions on the spiroketal core. However, in synthetic studies a majority of the functionality is installed at an early stage of the synthesis, often prior to complete construction of the carbon framework. Performing selective oxidation reactions on the heavily substituted systems related to the rubromycin structure have been shown to be problematic. This is apparent from Kozlowski *et al*.'s efforts towards purpuromycin (**7**), where repeated fine-tuning of the reaction sequence had to be undertaken due to the incompatibility of the substrates with the reaction conditions. Unforeseen steric issues arose further complicating realisation of their desired synthetic targets.

The rubromycin family of natural products continue to invoke interest due to their extensive biological activity and what has now become a uniquely challenging chemical structure to synthesise. After isolation and structure elucidation of the first compounds, it took over 50 years before a total synthesis of (±)-γ-rubromycin (**3**) was reported. The only other notable synthesis prior to this report was the synthesis of the racemic aglycone unit of heliquinomycin (**8**), prepared six years earlier by Danishefsky *et al*.[45] The flurry of reported syntheses of (±)-γ-rubromycin (**3**) after the initial report by Kita *et al.* resulted from significant advances in the development of synthetic methods to synthesise the spiroketal substrate. The impetus for these intensive investigations into formation of the spiroketal results from the structural studies showing that the central 5,6-spiroketal core is key for potent inhibition of the telomerase enzyme. Of the members of the natural product family that contain oxygen functionality on the spiroketal core, only Danishefsky's heliquinomycinone (**9**) and Kozlowski's advanced purpuromycin-based spiroketal intermediate **156** have been synthesised. Efficient routes towards the heavily oxygen substituted spiroketals of the griseorhodins and the DK-compounds currently remain unreported and still require further advancement in synthetic methods for their synthesis.

With more interesting biological activity to be discovered, further determination of the exact functional groups to inhibit the telomerase enzyme being required, together with the limited synthetic routes available to access members of this natural product family, the rubromycins will continue to attract attention from both the synthetic chemistry and biological communities.

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Figures and Schemes

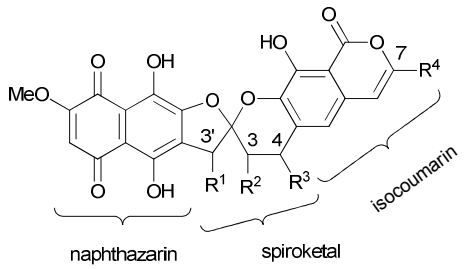



Fig. 1

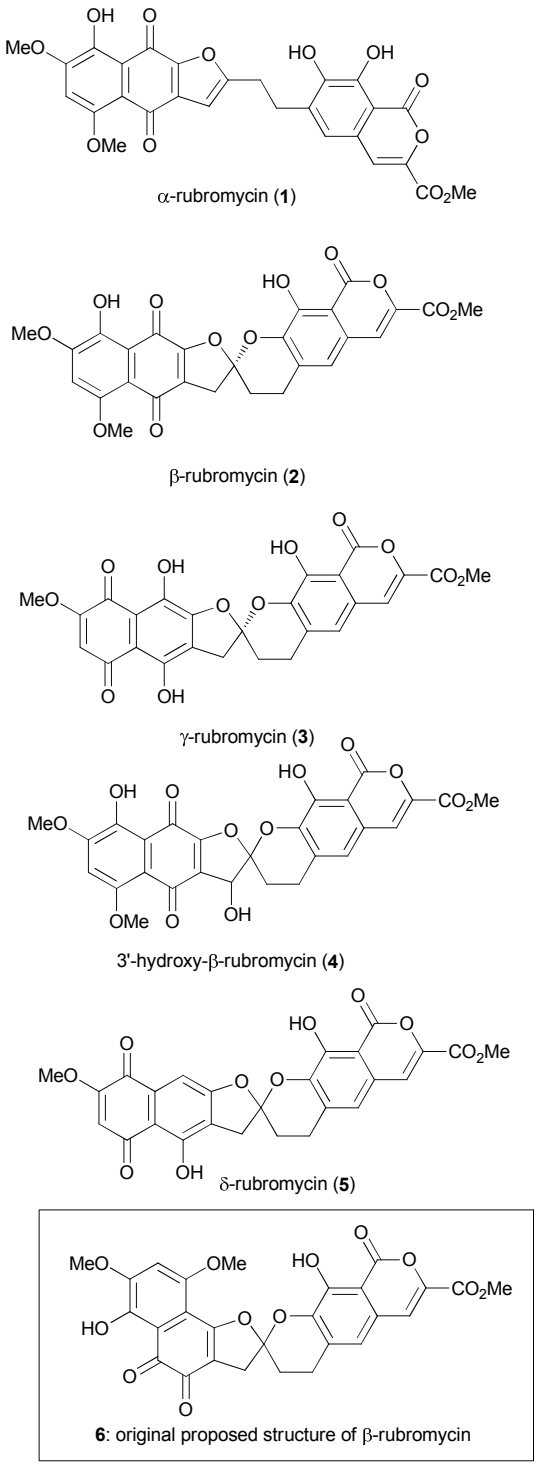
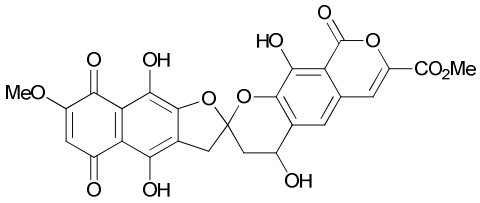



Fig. 2



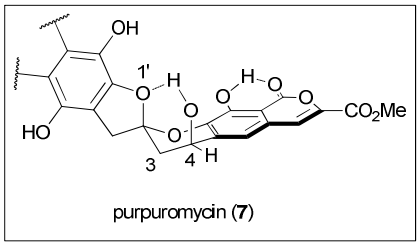
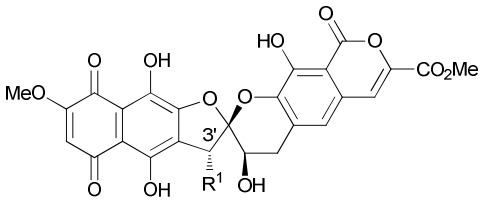
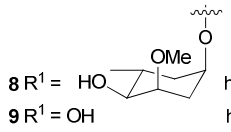
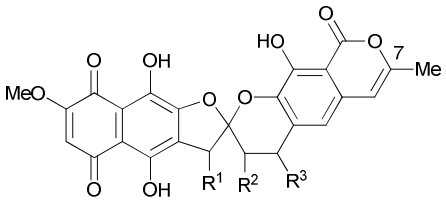




Fig. 3

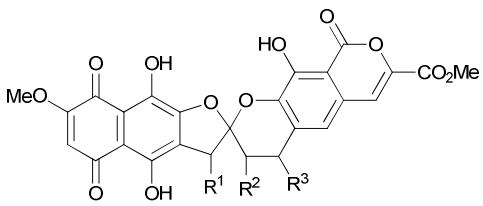




**8** $R^1$ = heliquinomycin
**9** $R^1$ = OH heliquinomycinone

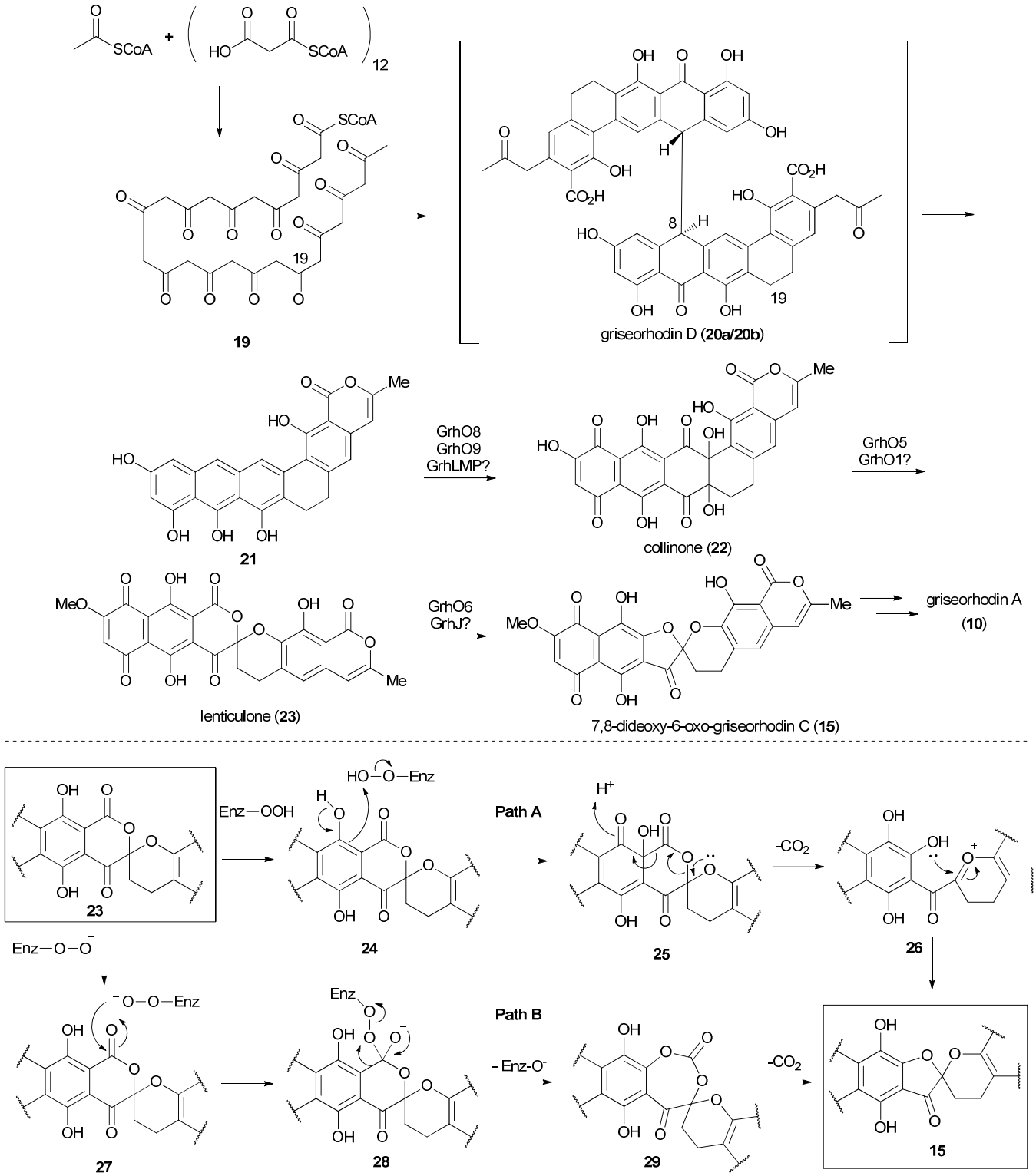


griseorhodin A (**10**) $R^1$ = OH, $R^2$, $R^3$ =
griseorhodin C (**11**) $R^1$, $R^2$, $R^3$ = OH
griseorhodin G (**12**) $R^1$, $R^2$ = OH, $R^3$ = H
8-methoxygriseorhodin C (**13**) $R^1$, $R^2$ = OH, $R^3$ = OMe
7,8-dideoxygriseorhodin C (**14**) $R^1$ = OH, $R^2$, $R^3$ = H
7,8-dideoxy-6-oxogriseorhodin C (**15**) $R^1$ = ketone, $R^2$, $R^3$ = H



DK-7814-A (**16**) $R^1$, $R^2$, $R^3$ = OH
DK-7814-B (**17**) $R^1$, $R^2$ = OH, $R^3$ = H
DK-7814-C (**18**) $R^1$= OH $R^2$, $R^3$ =

Fig. 4




Scheme 1

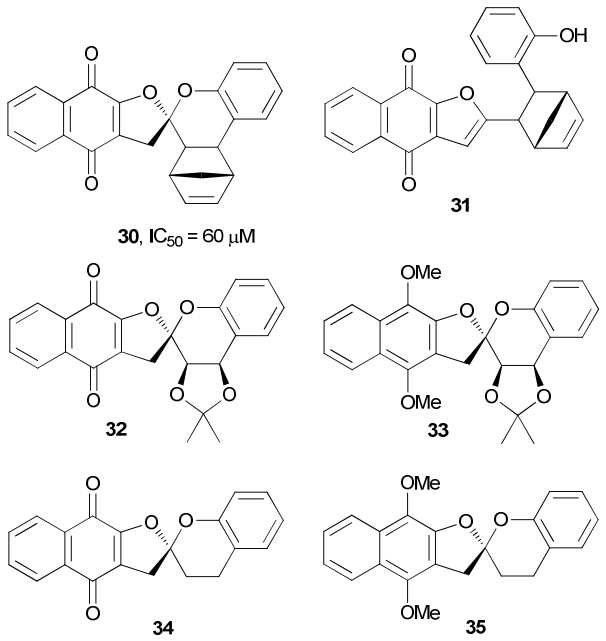
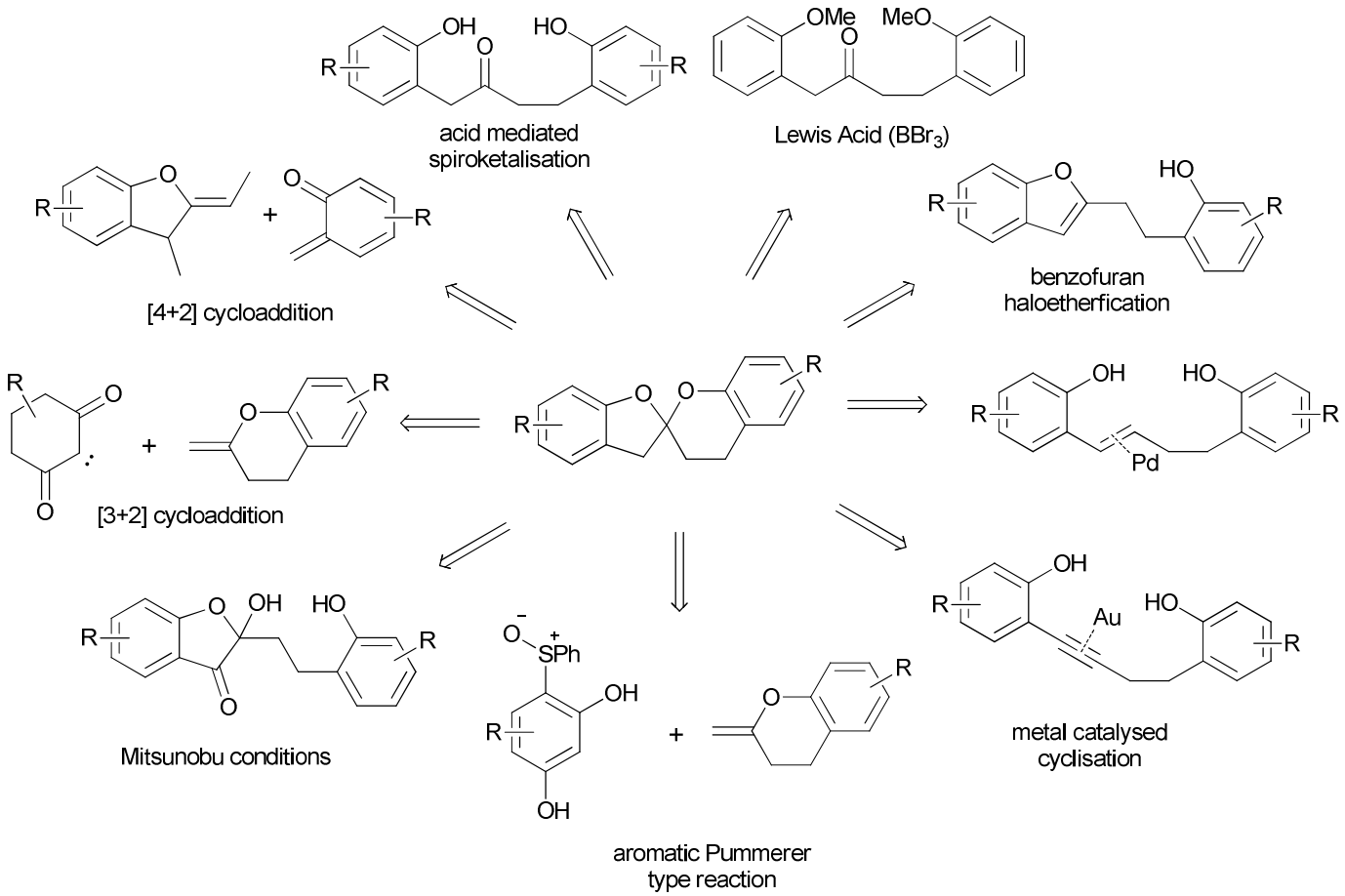
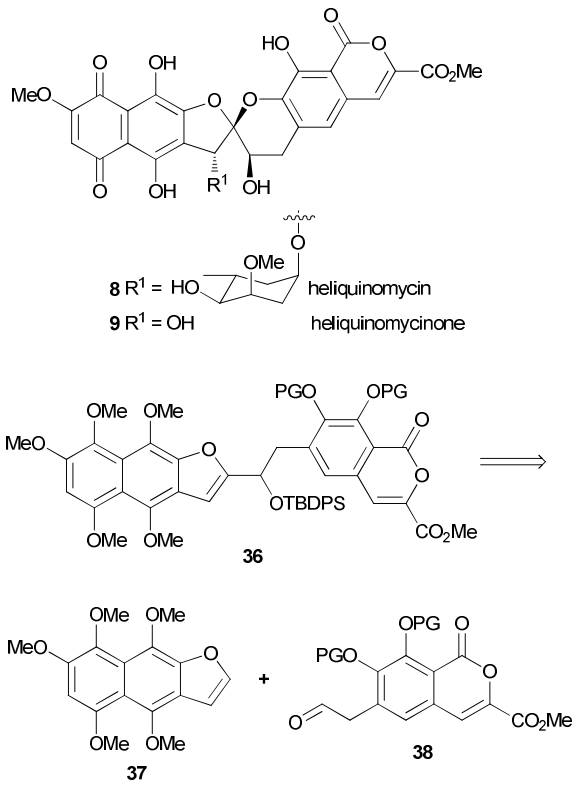



Fig. 5




Scheme 2

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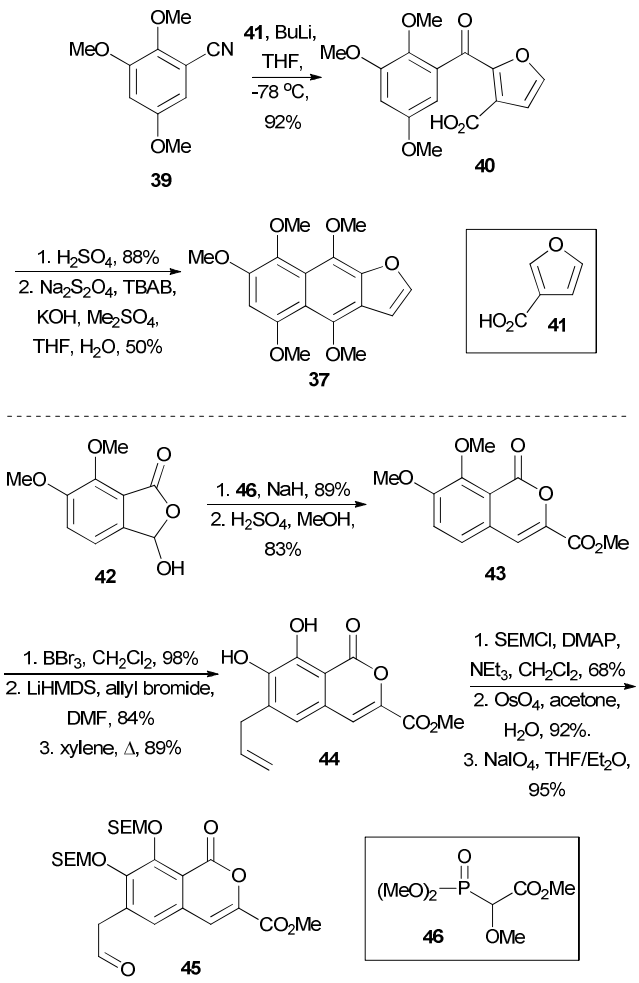
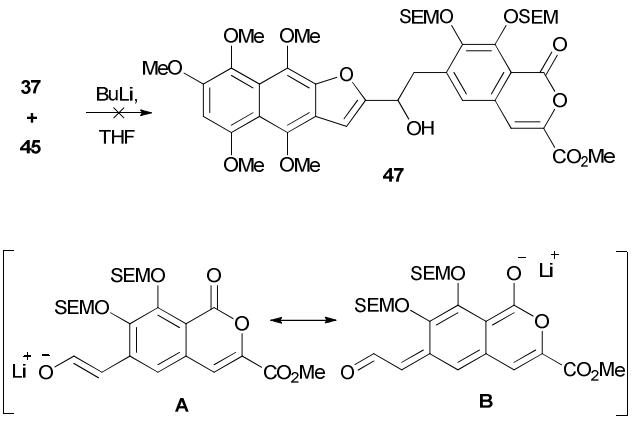
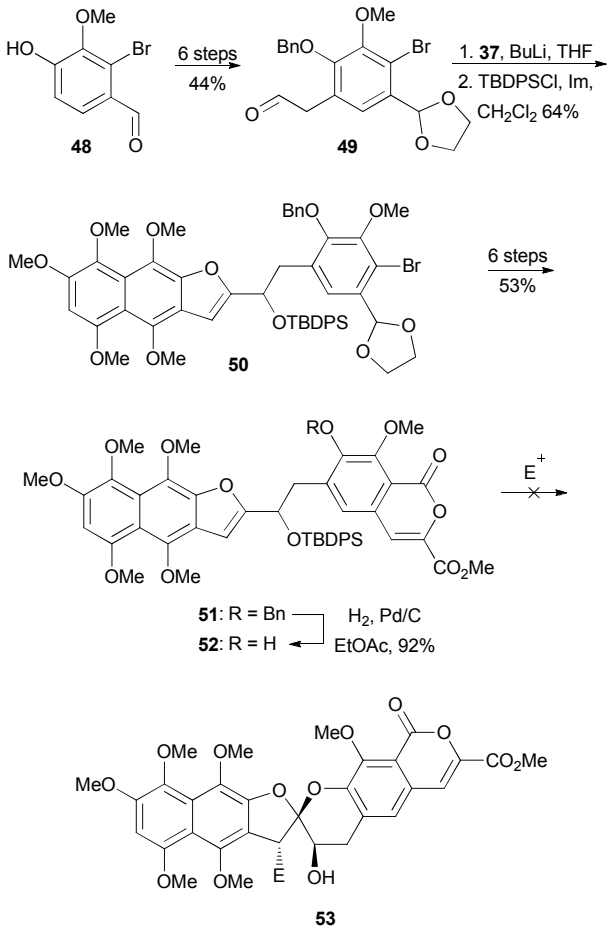


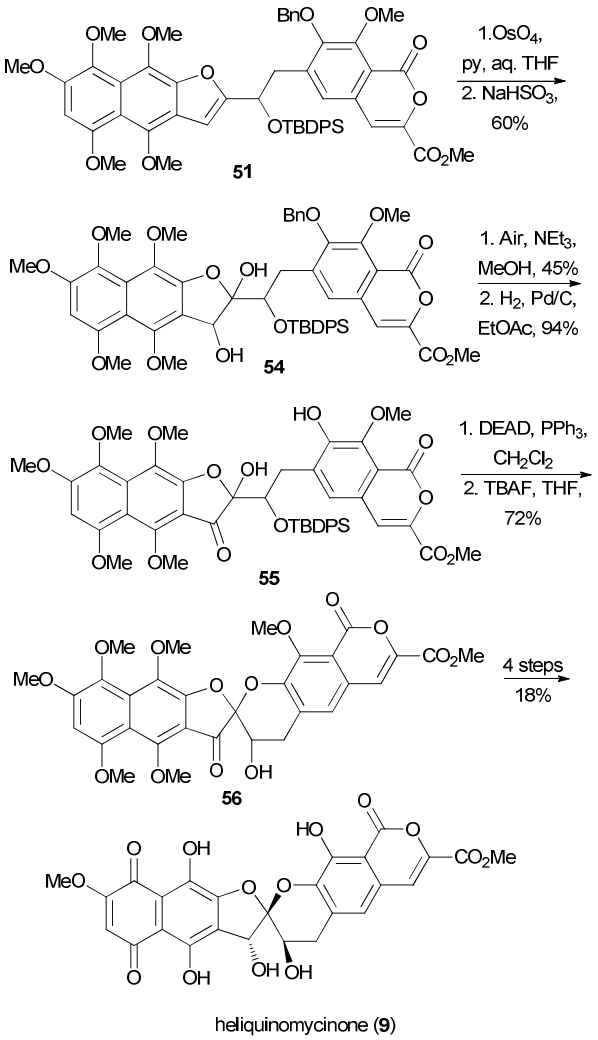

Scheme 4




Scheme 5




Scheme 6

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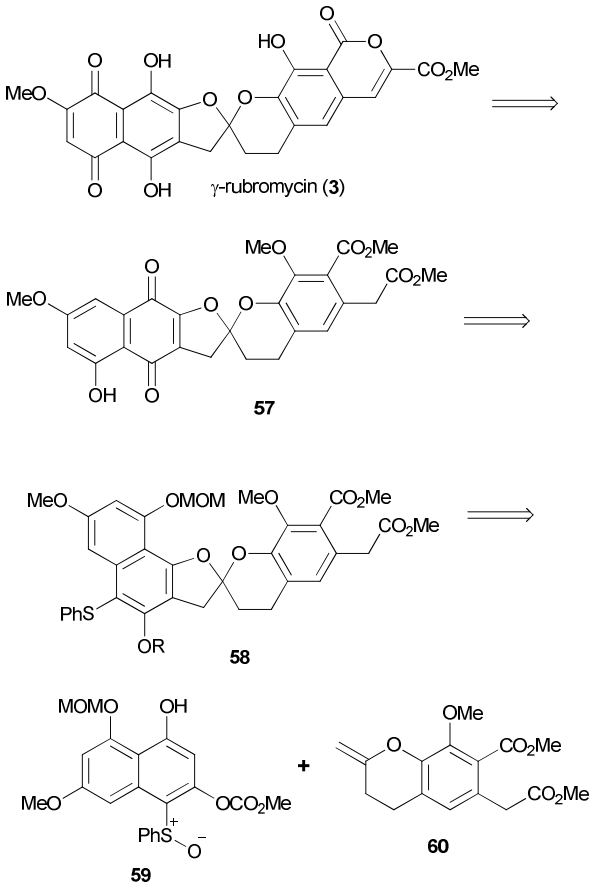
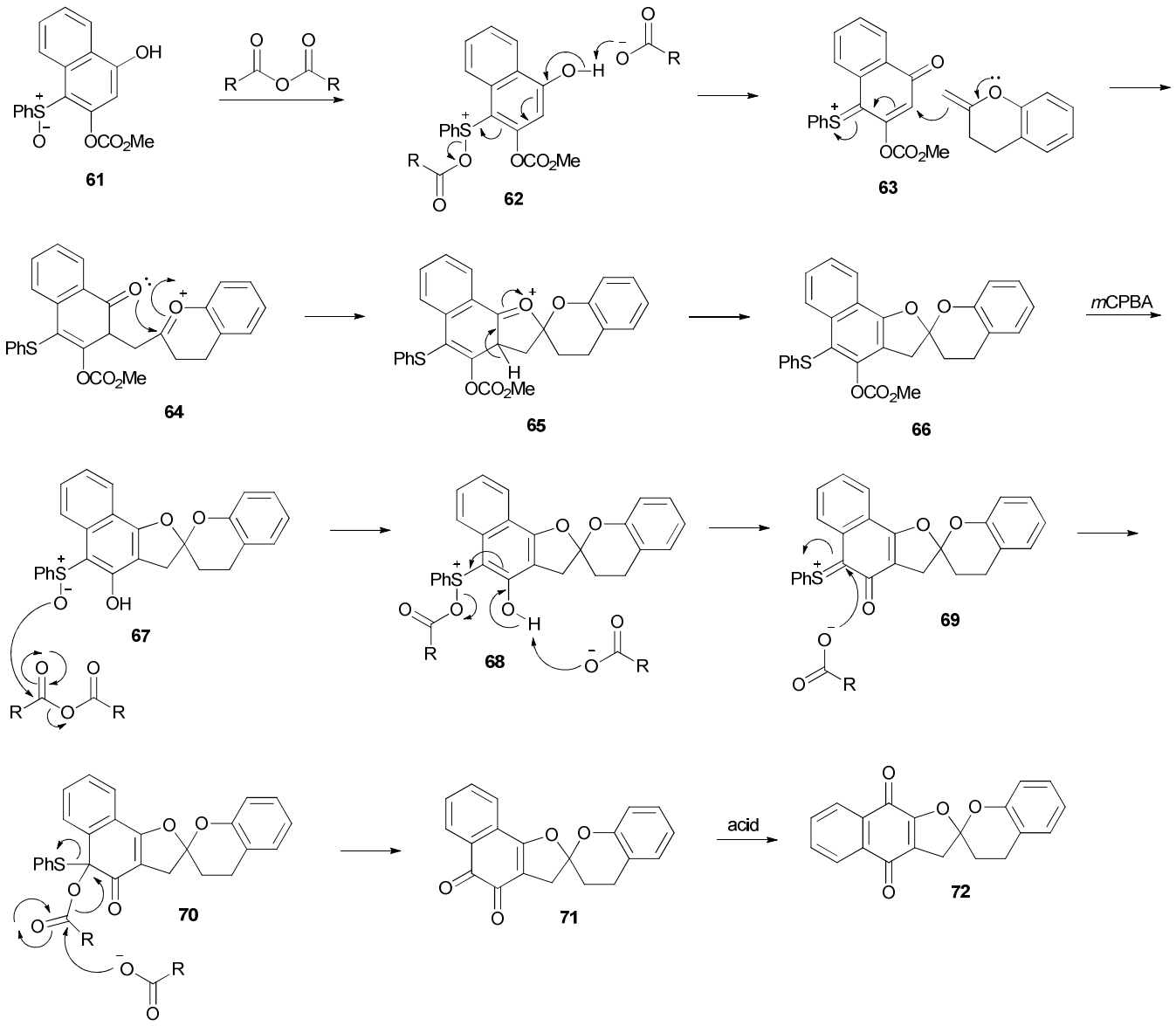
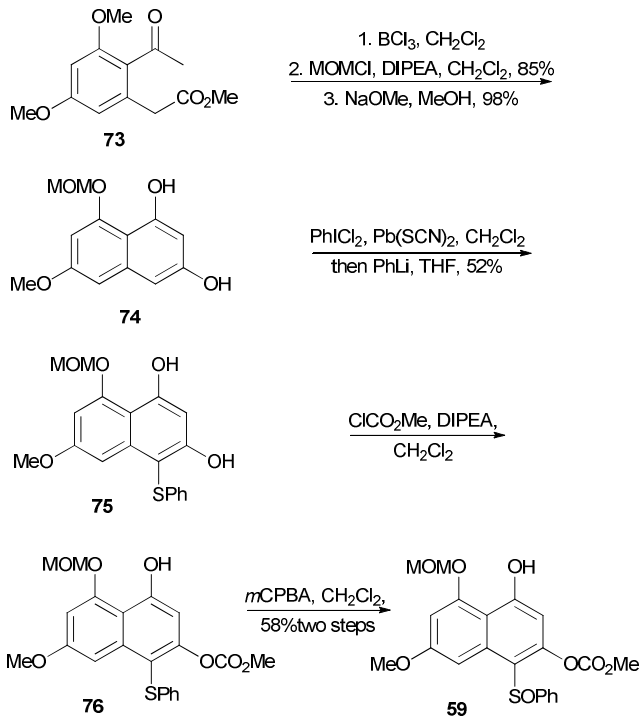


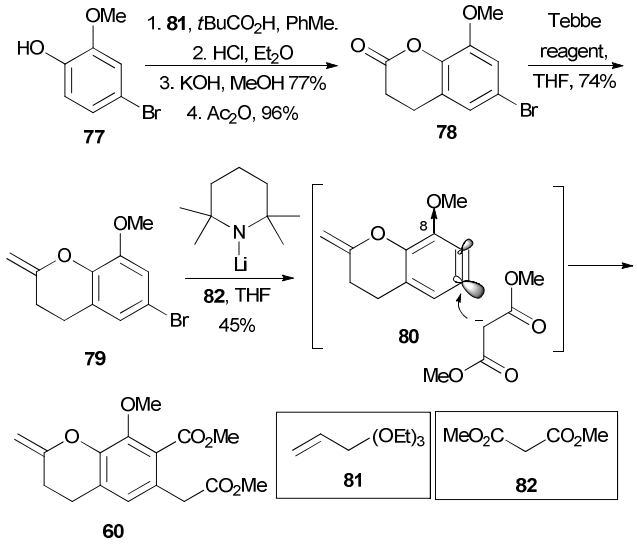

Scheme 8



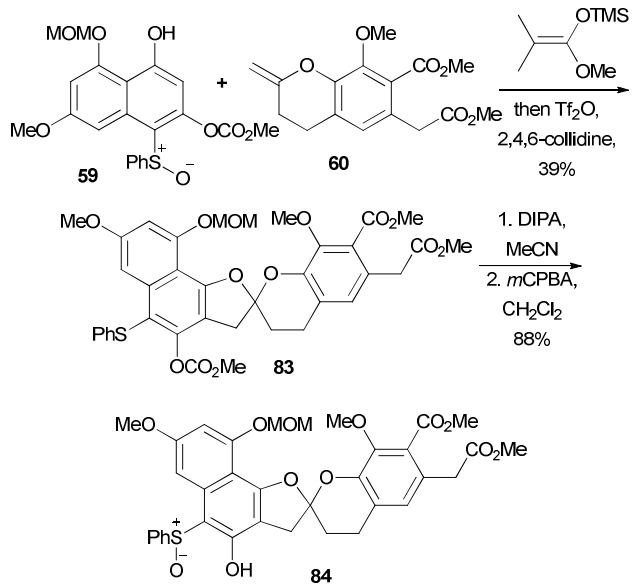

Scheme 9



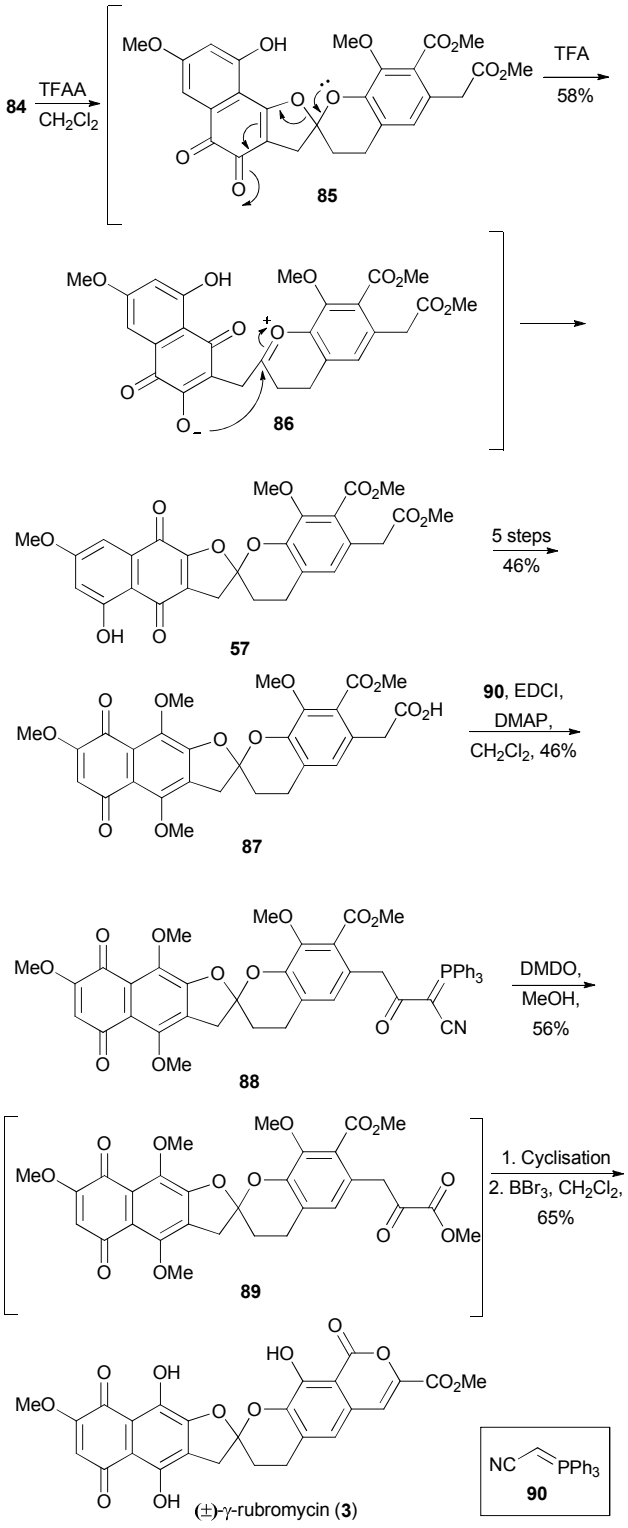

Scheme 10



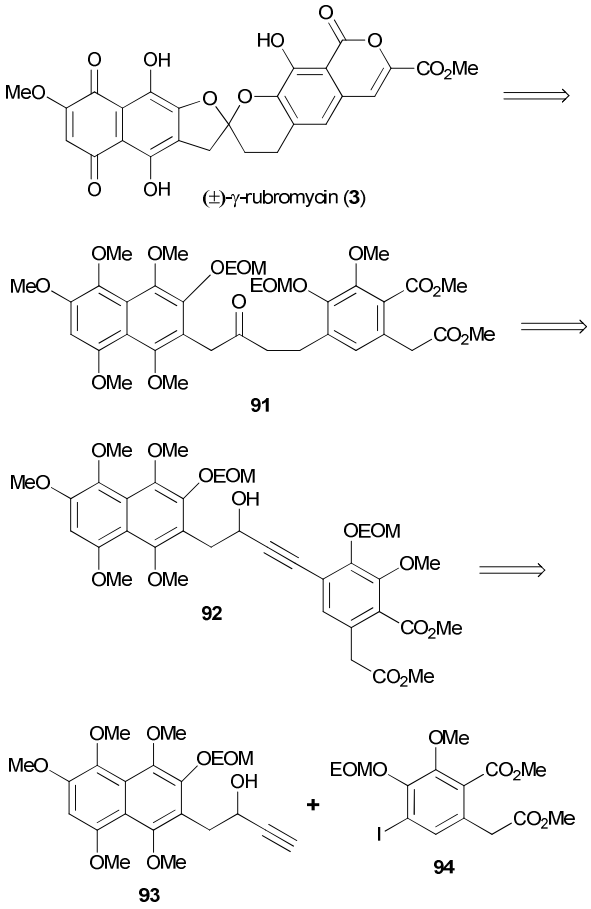

Scheme 11



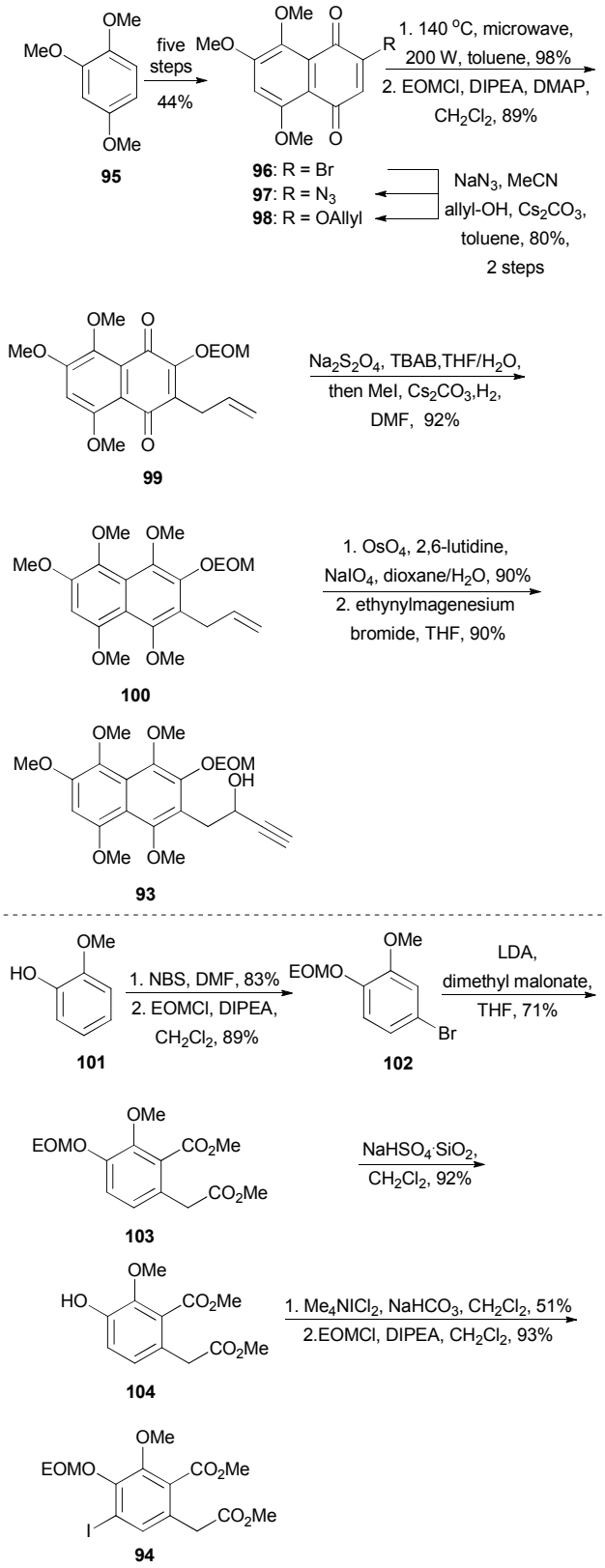

Scheme 12



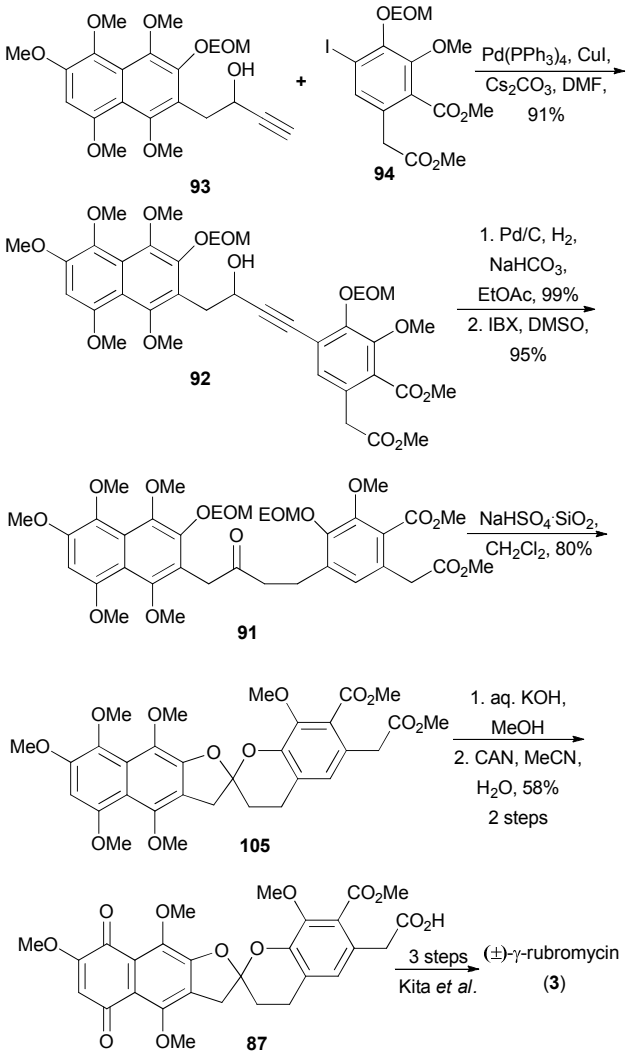

Scheme 13



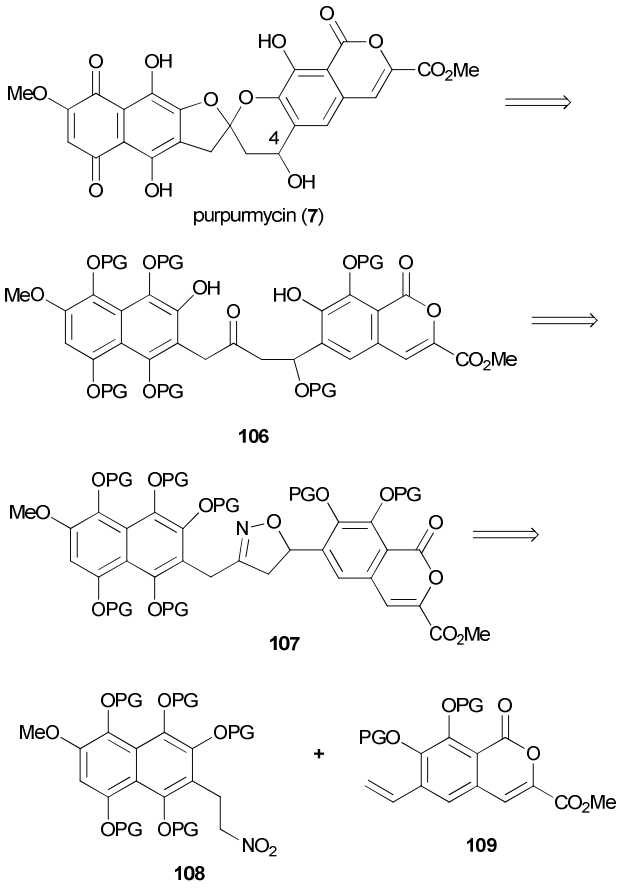

Scheme 14



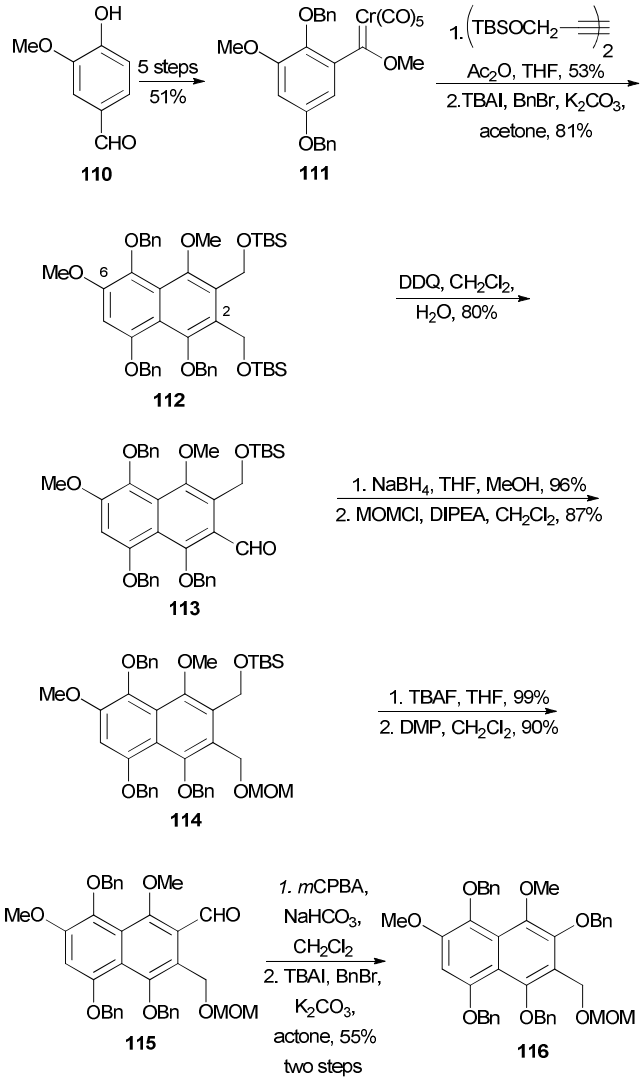

Scheme 15



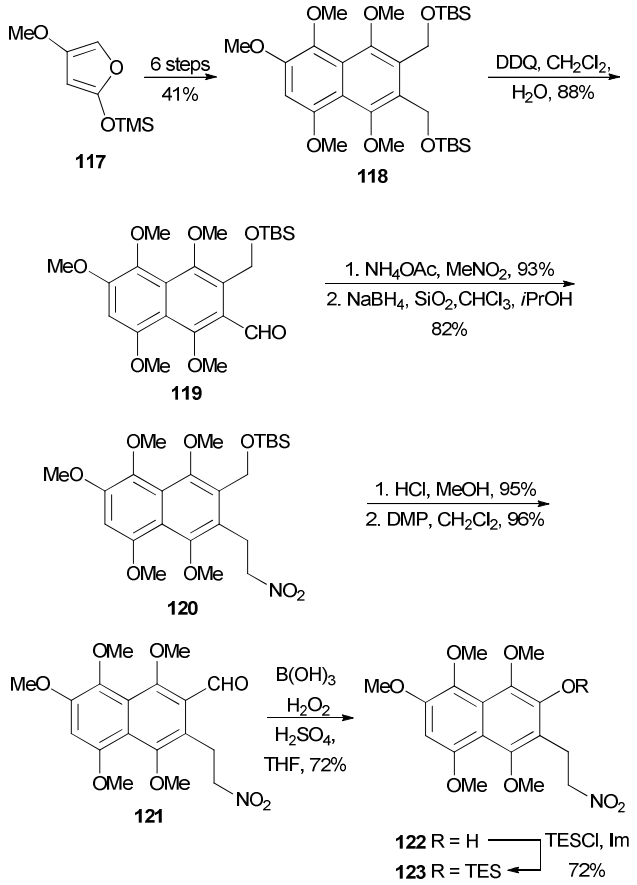

Scheme 16



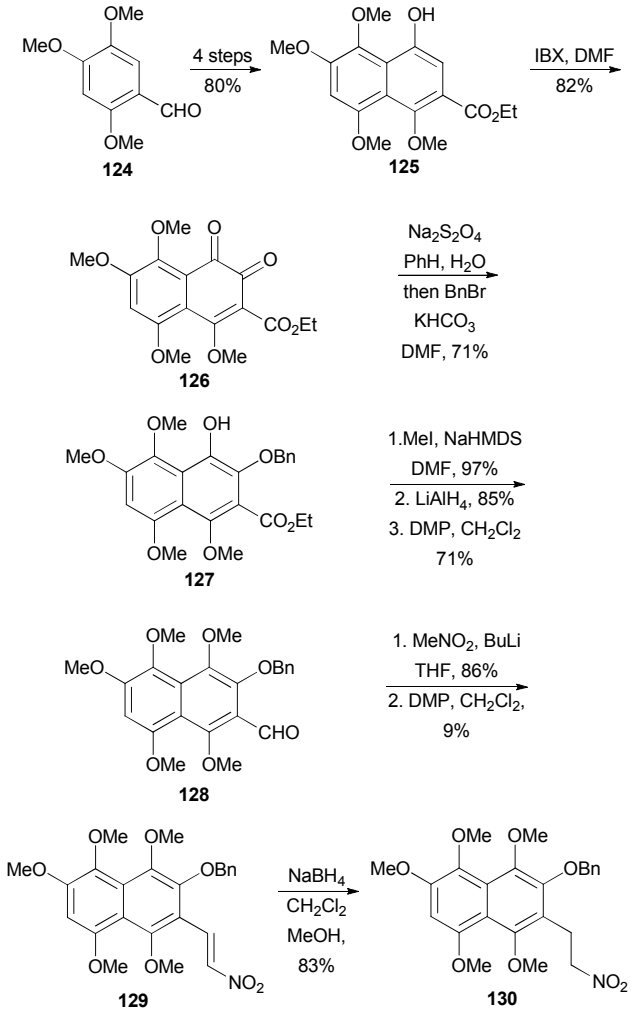

Scheme 17



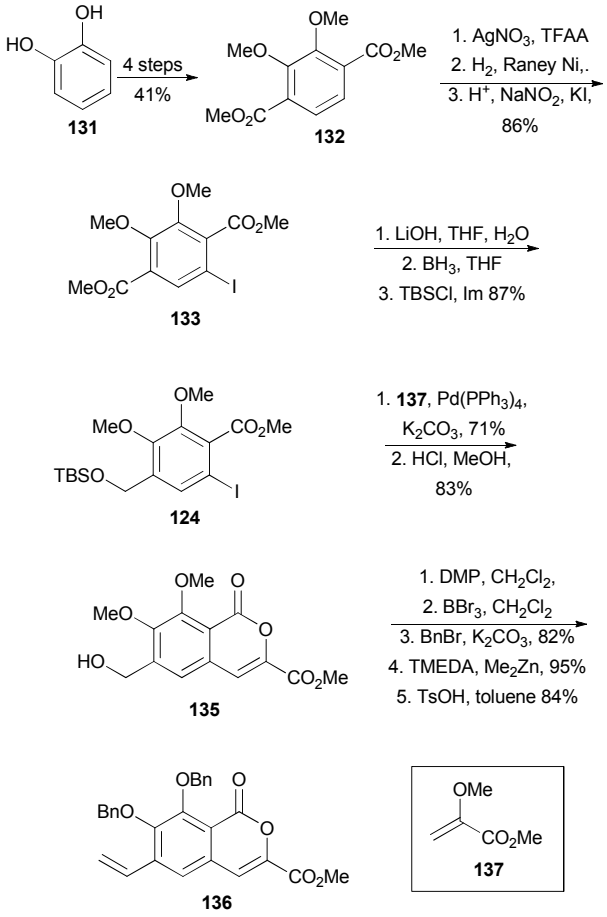

Scheme 18



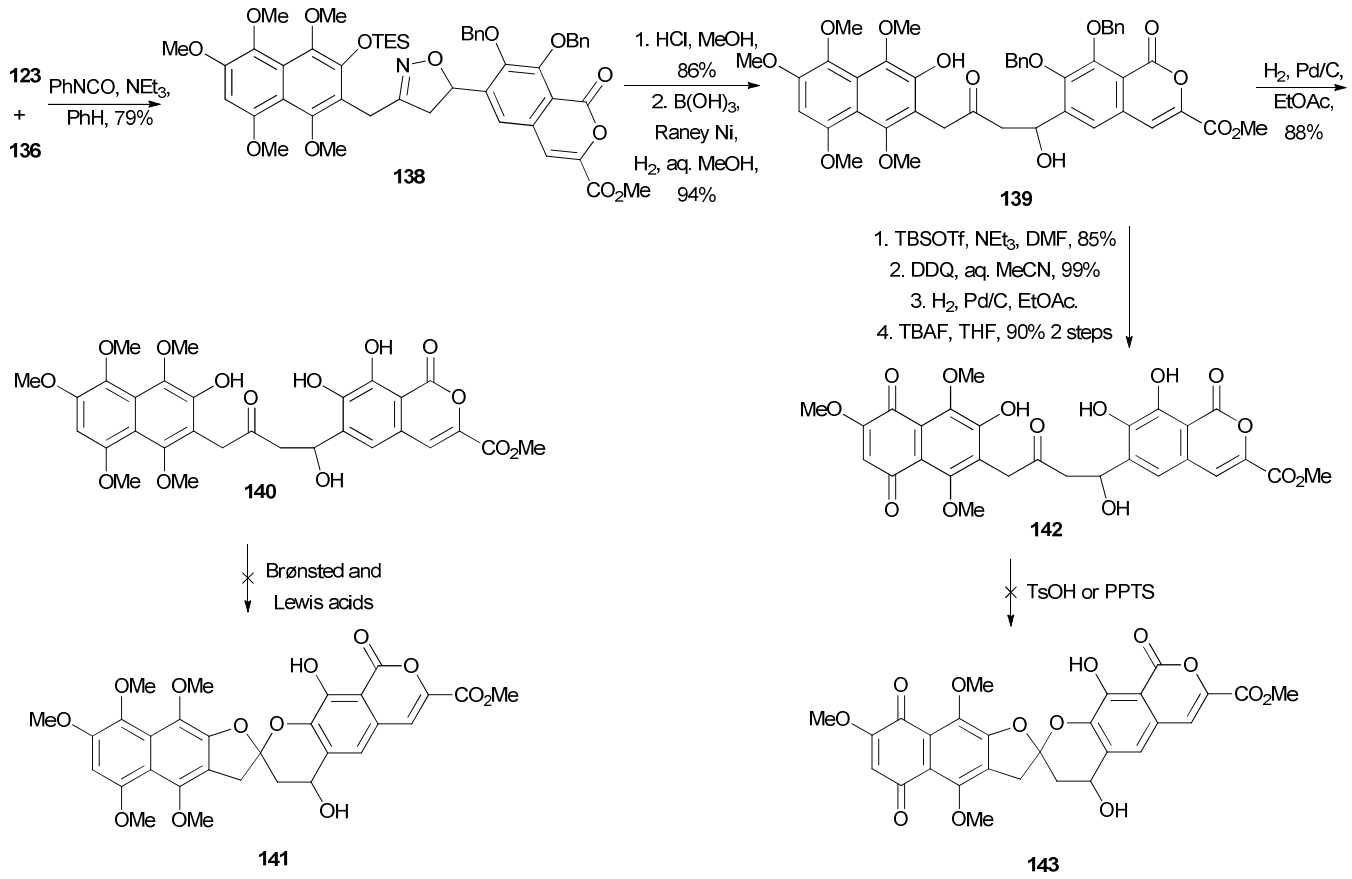

Scheme 19

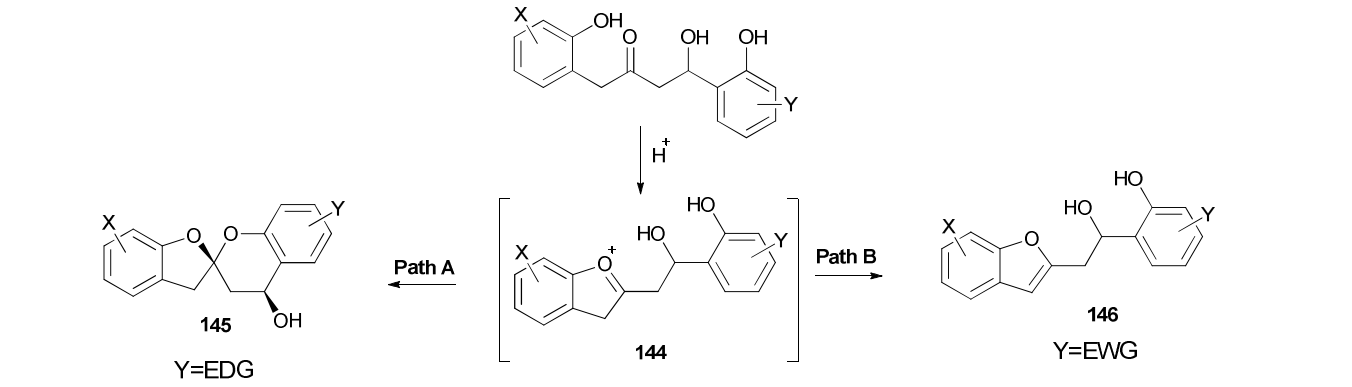



Scheme 20

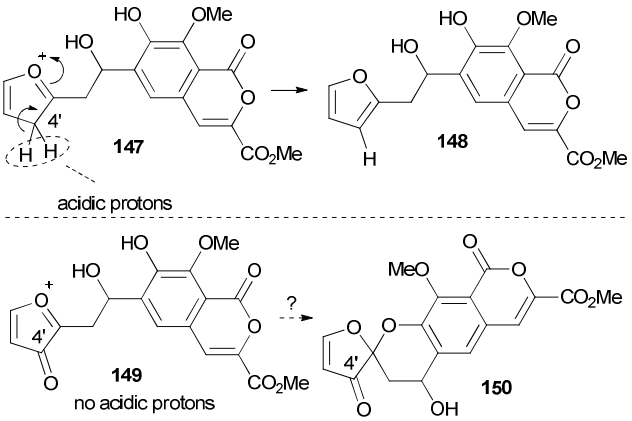

OH
HO
4 steps
41%
131
OMe
MeO
CO2Me
MeO2C
132
1. AgNO3, TFAA
2. H2, Raney Ni,.
3. H+, NaNO2, KI,
86%
OMe
MeO
CO2Me
MeO2C
I
133
1. LiOH, THF, H2O
2. BH3, THF
3. TBSCl, Im 87%
OMe
MeO
CO2Me
TBSO
I
124
1. 137, Pd(PPh3)4,
K2CO3, 71%
2. HCl, MeOH,
83%
OMe
O
MeO
O
HO
CO2Me
135
1. DMP, CH2Cl2,
2. BBr3, CH2Cl2
3. BnBr, K2CO3, 82%
4. TMEDA, Me2Zn, 95%
5. TsOH, toluene 84%
OBn
O
BnO
O
CO2Me
136
OMe
CO2Me
137

Scheme 21




Scheme 22




Scheme 23

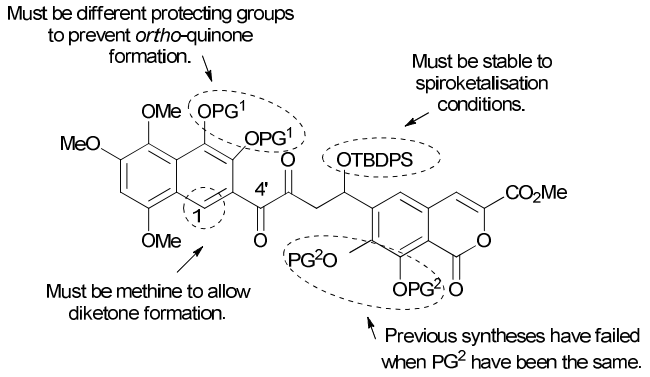
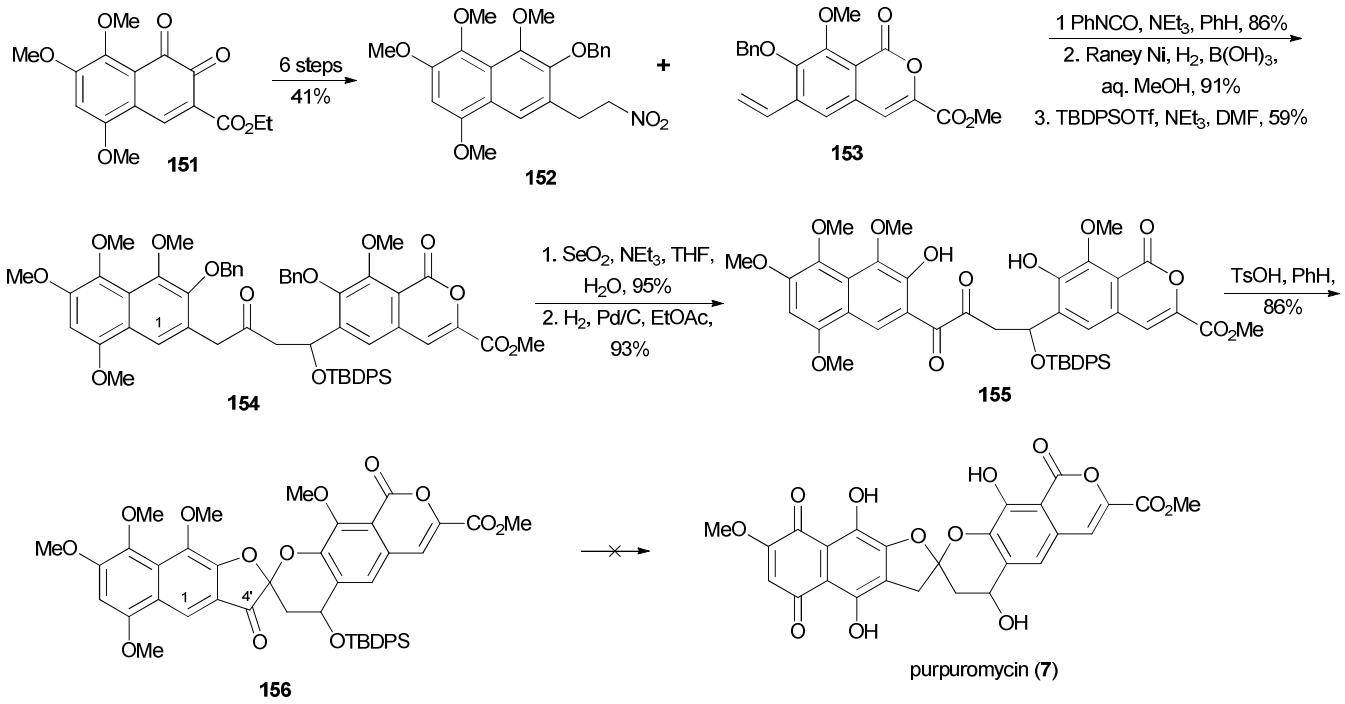
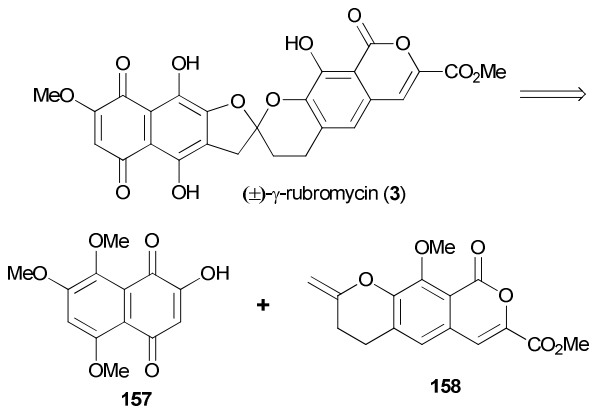


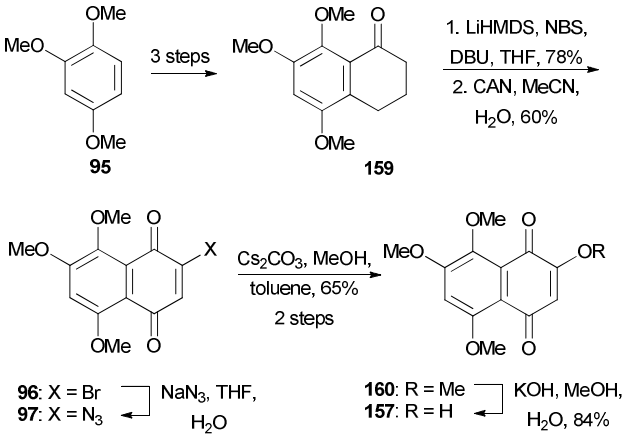

Fig 6



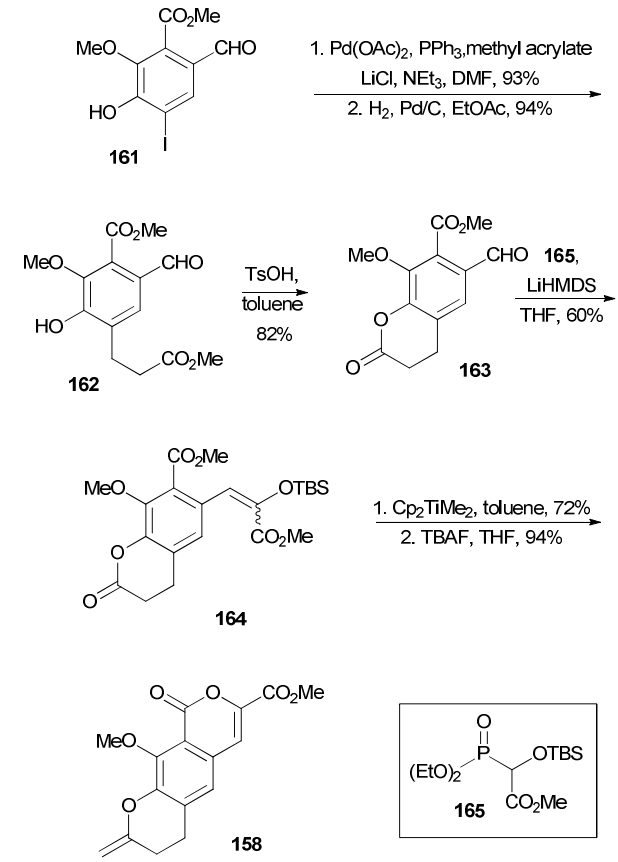

Scheme 24



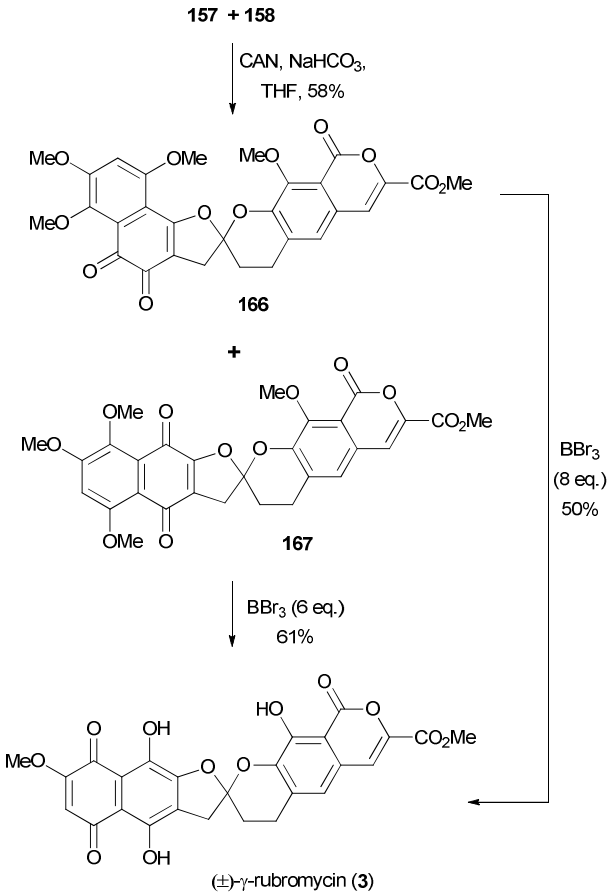

Scheme 25



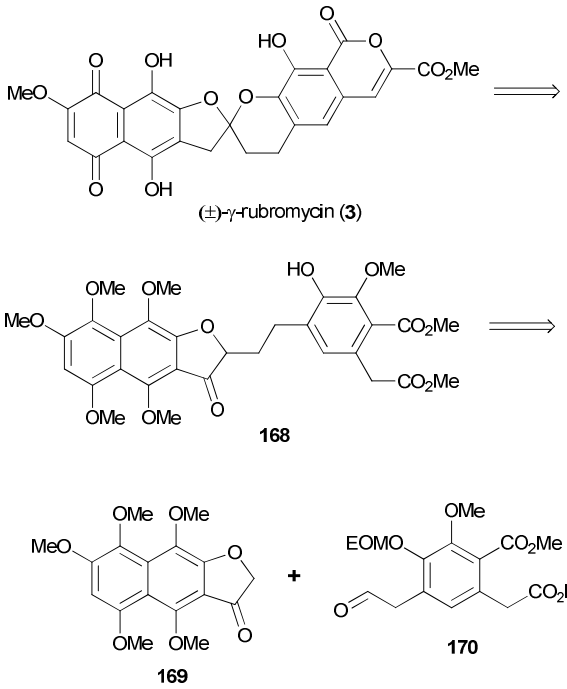

Scheme 26



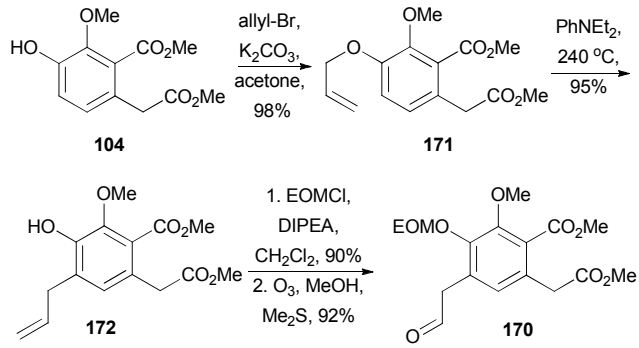

Scheme 27



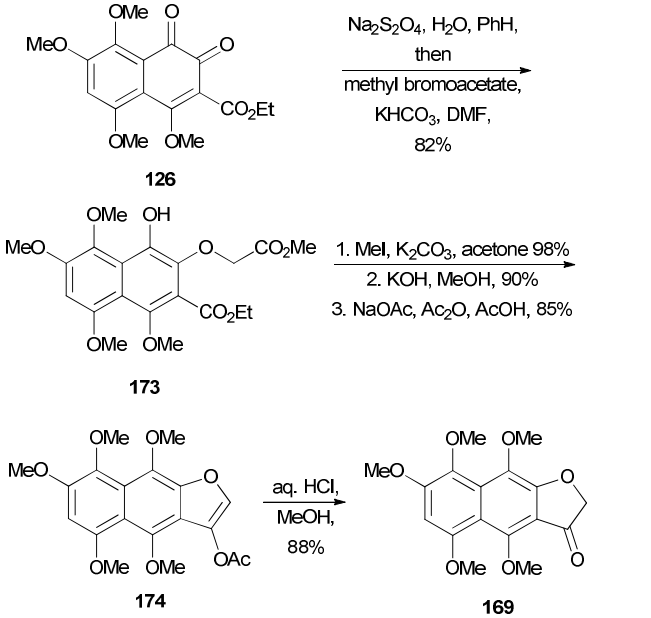

Scheme 28



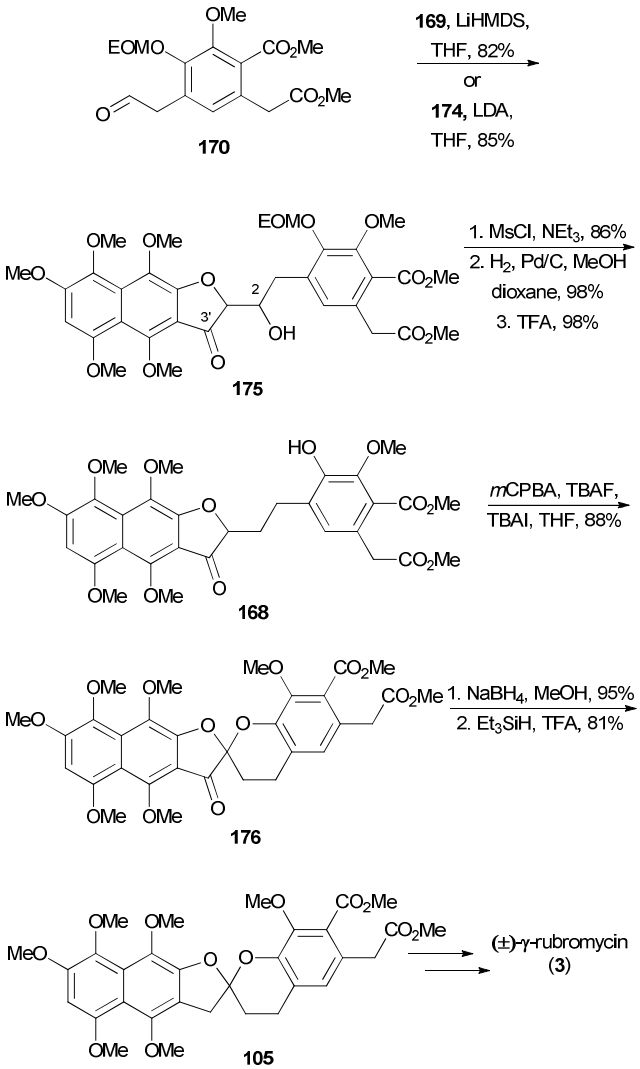

Scheme 29



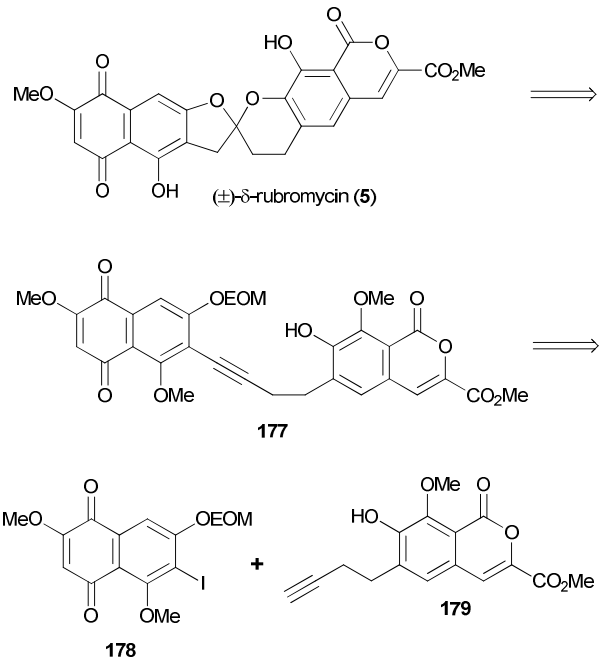

Scheme 30



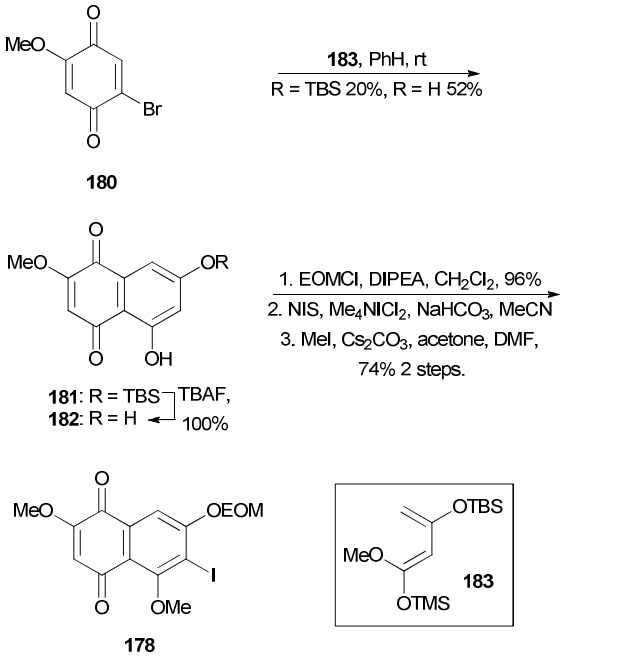

Scheme 31



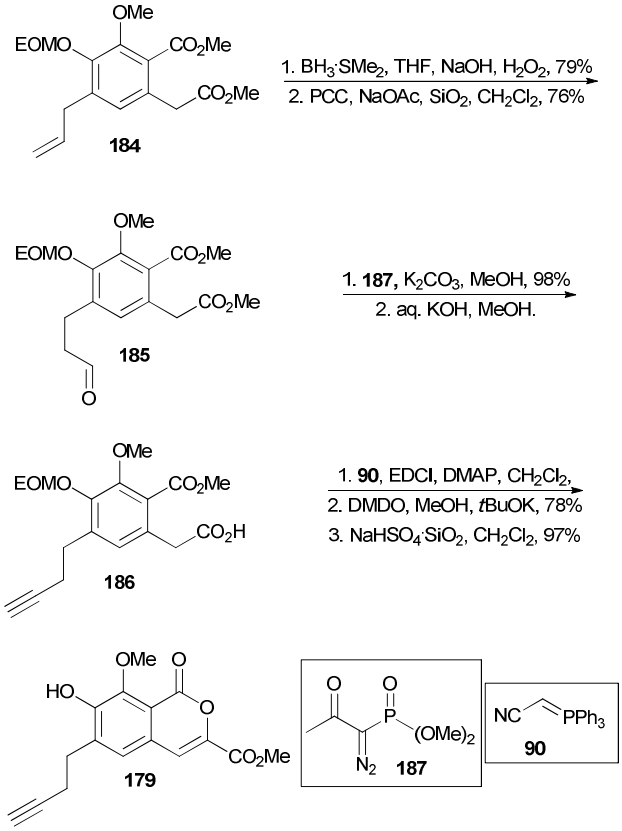

Scheme 32



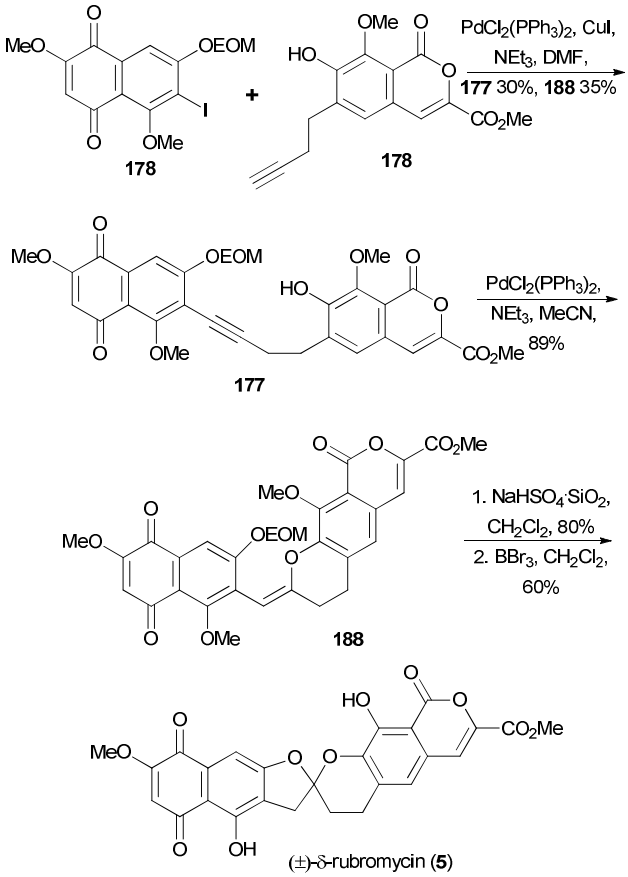

Scheme 33



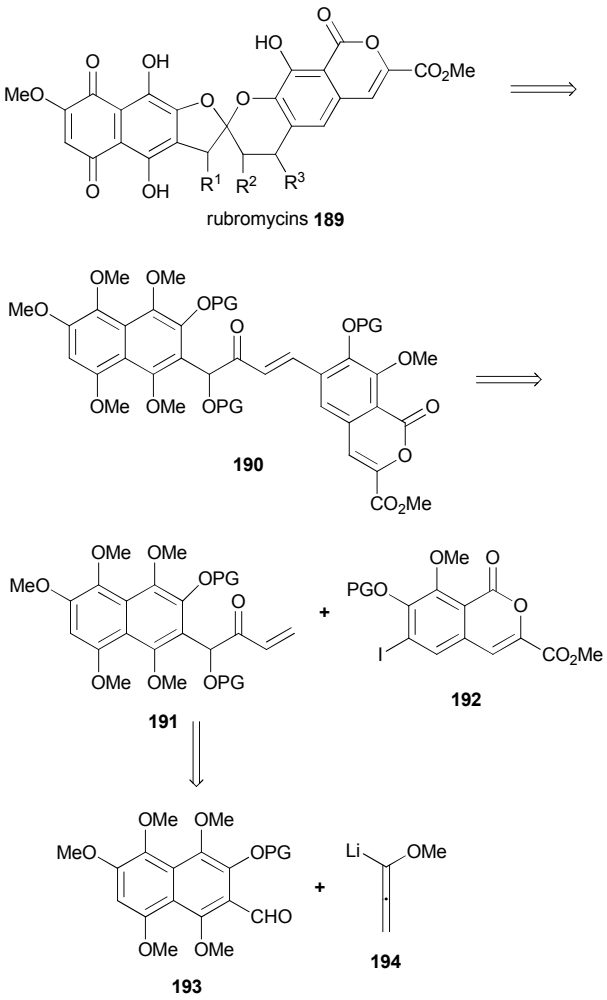

Scheme 34



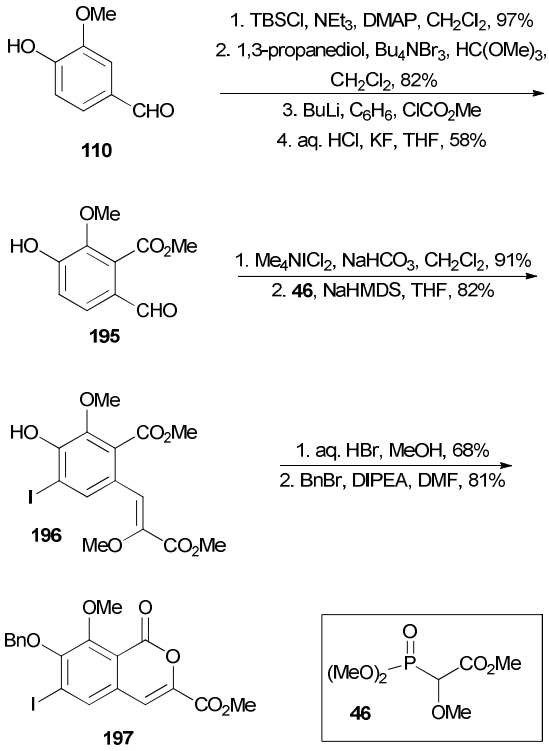

Scheme 35



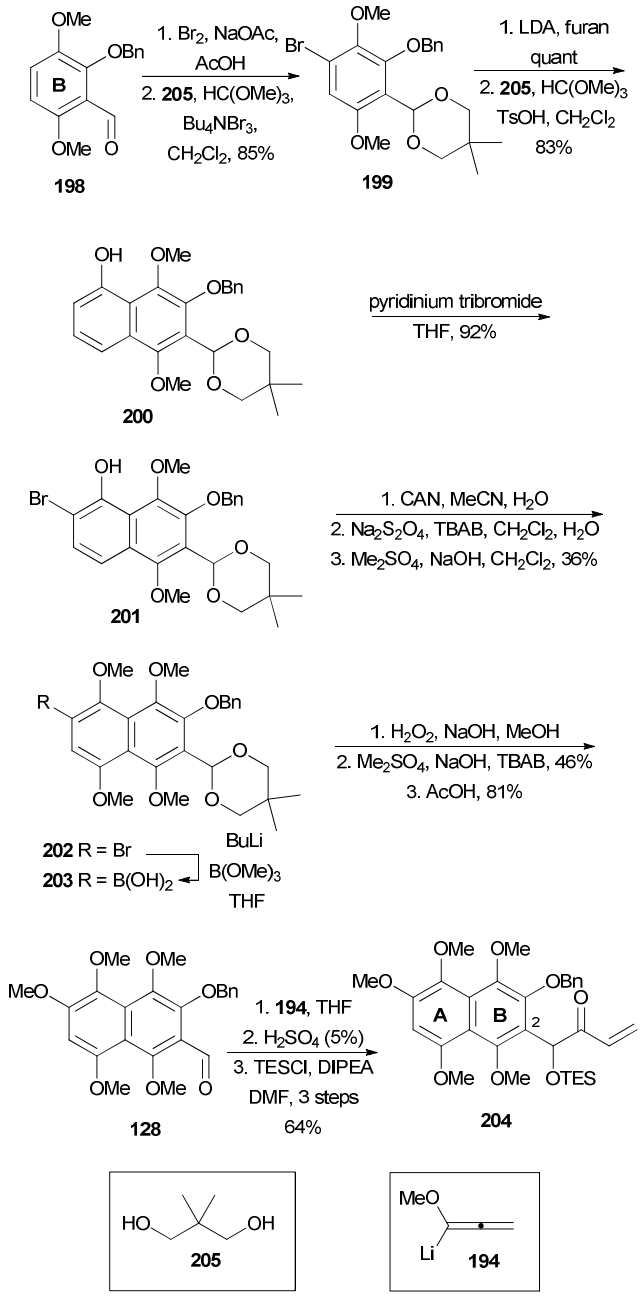

Scheme 36



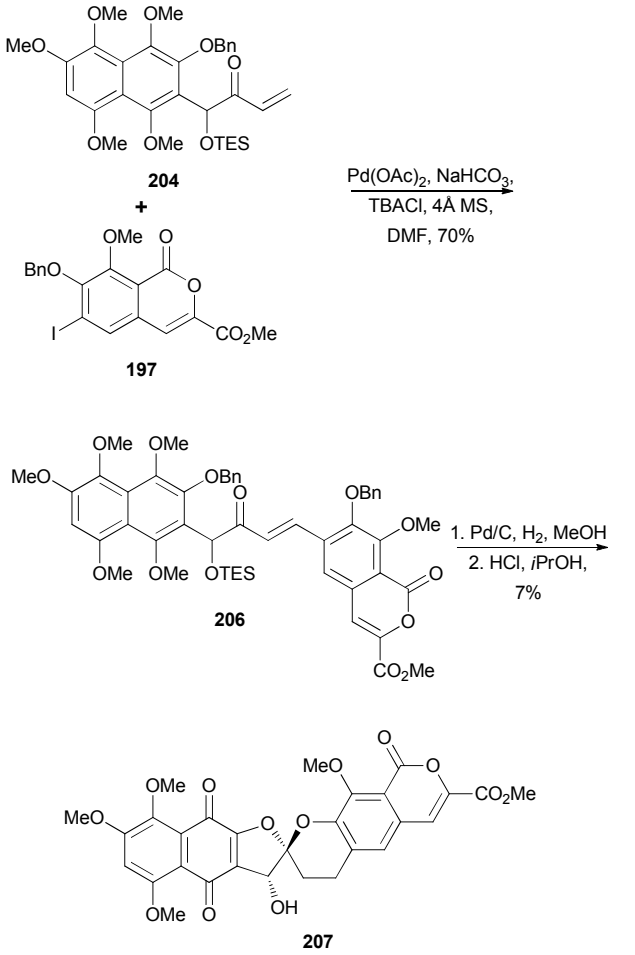

Scheme 37



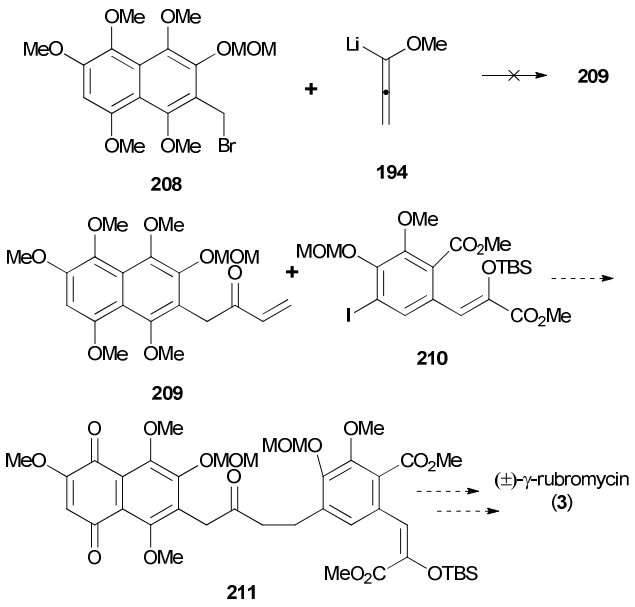

Scheme 38



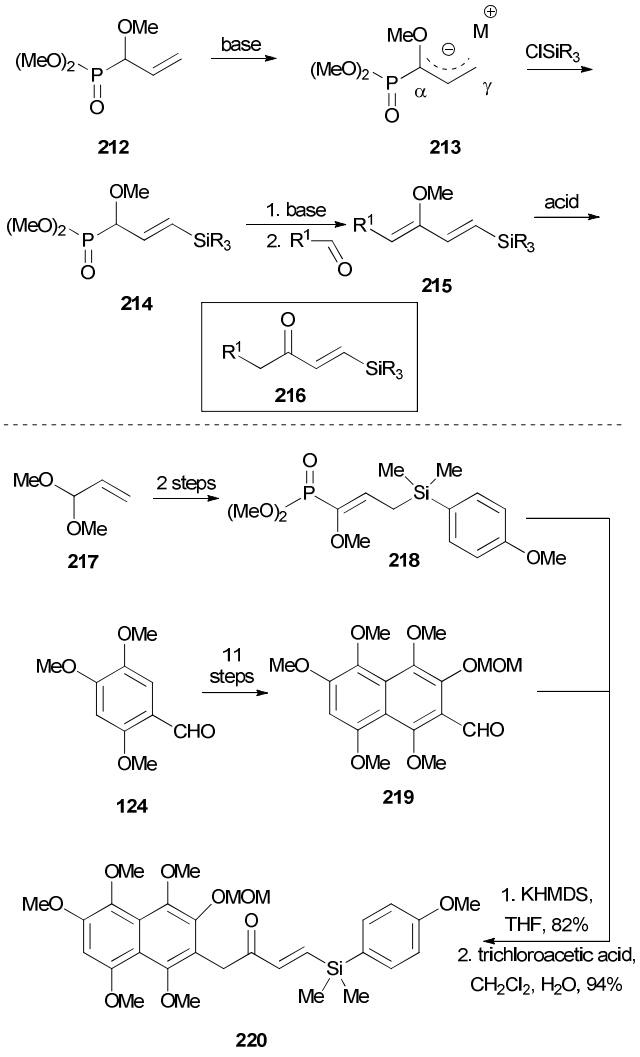

Scheme 39



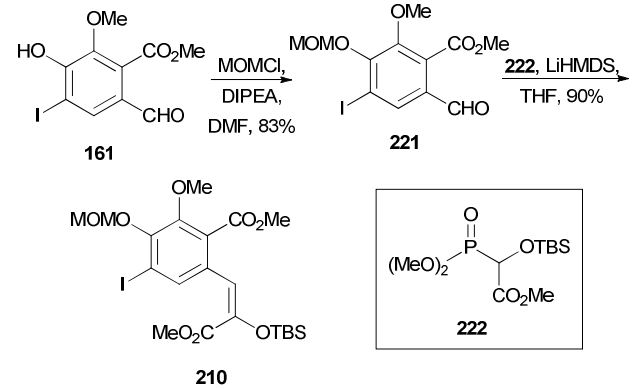

Scheme 40



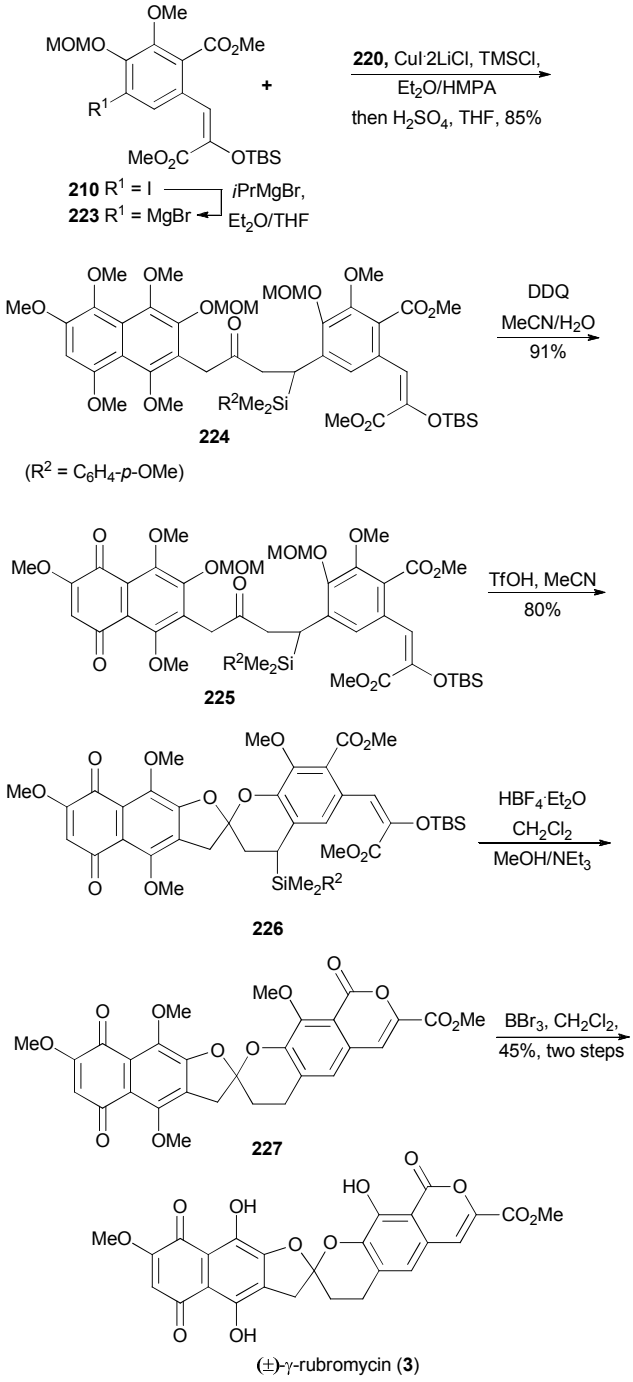

Scheme 41



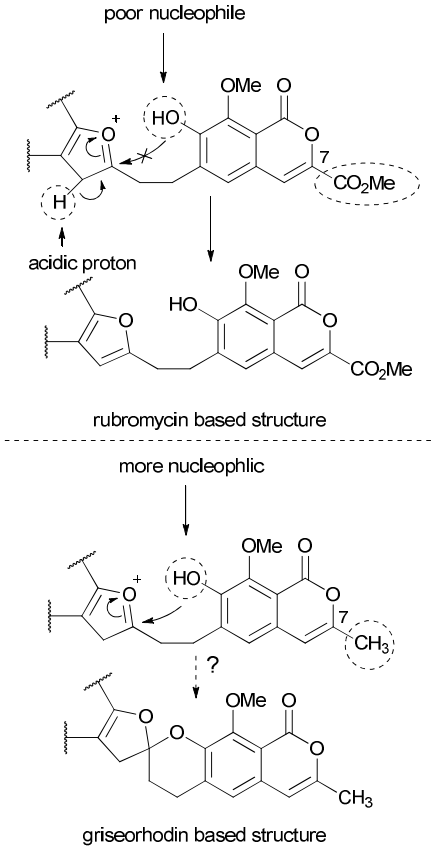

Scheme 42



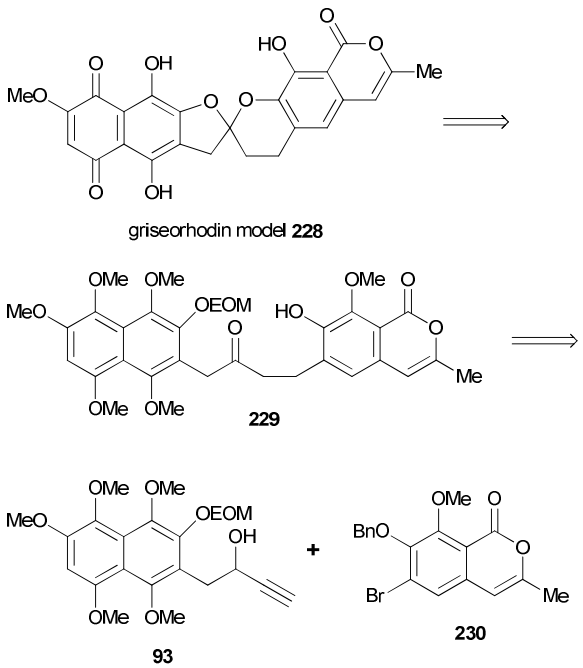

Scheme 43



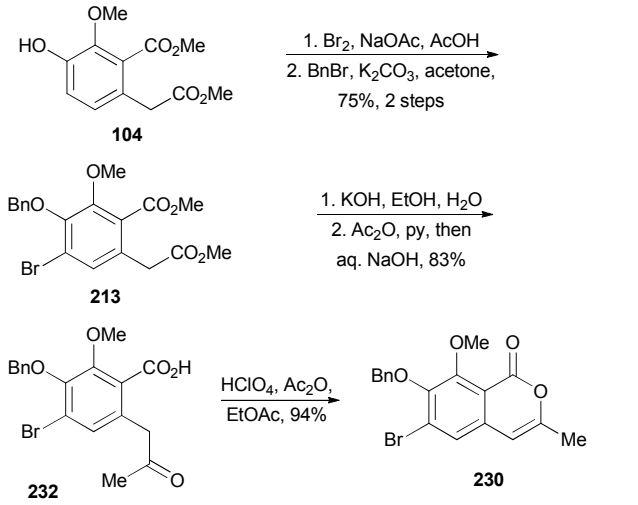

Scheme 44



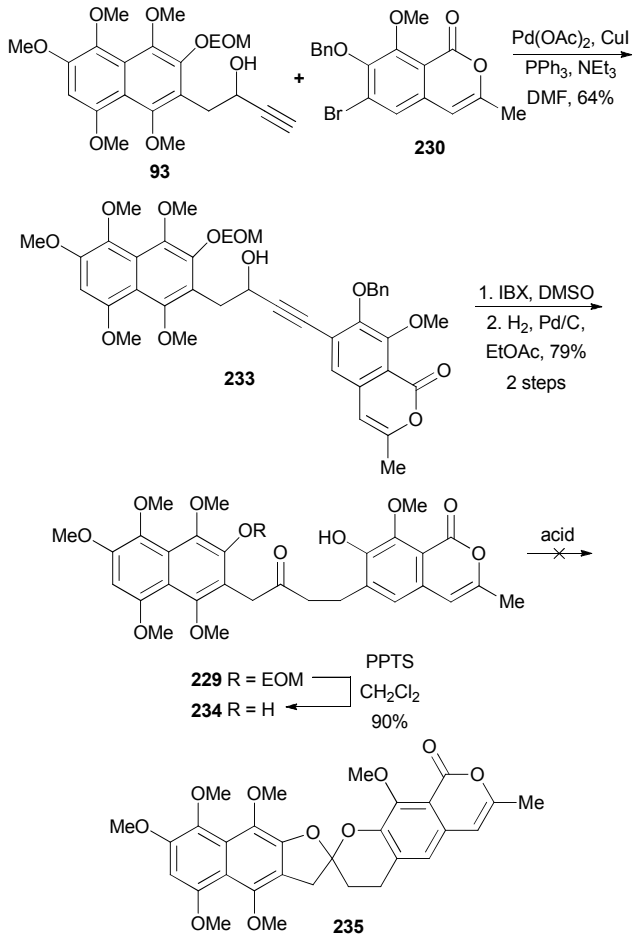

Scheme 45



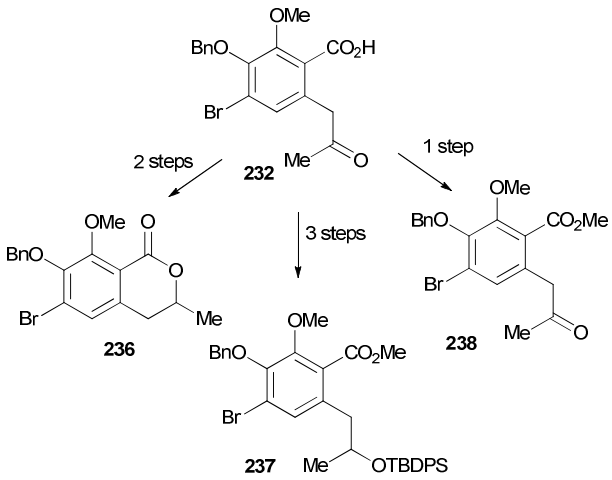

Scheme 46



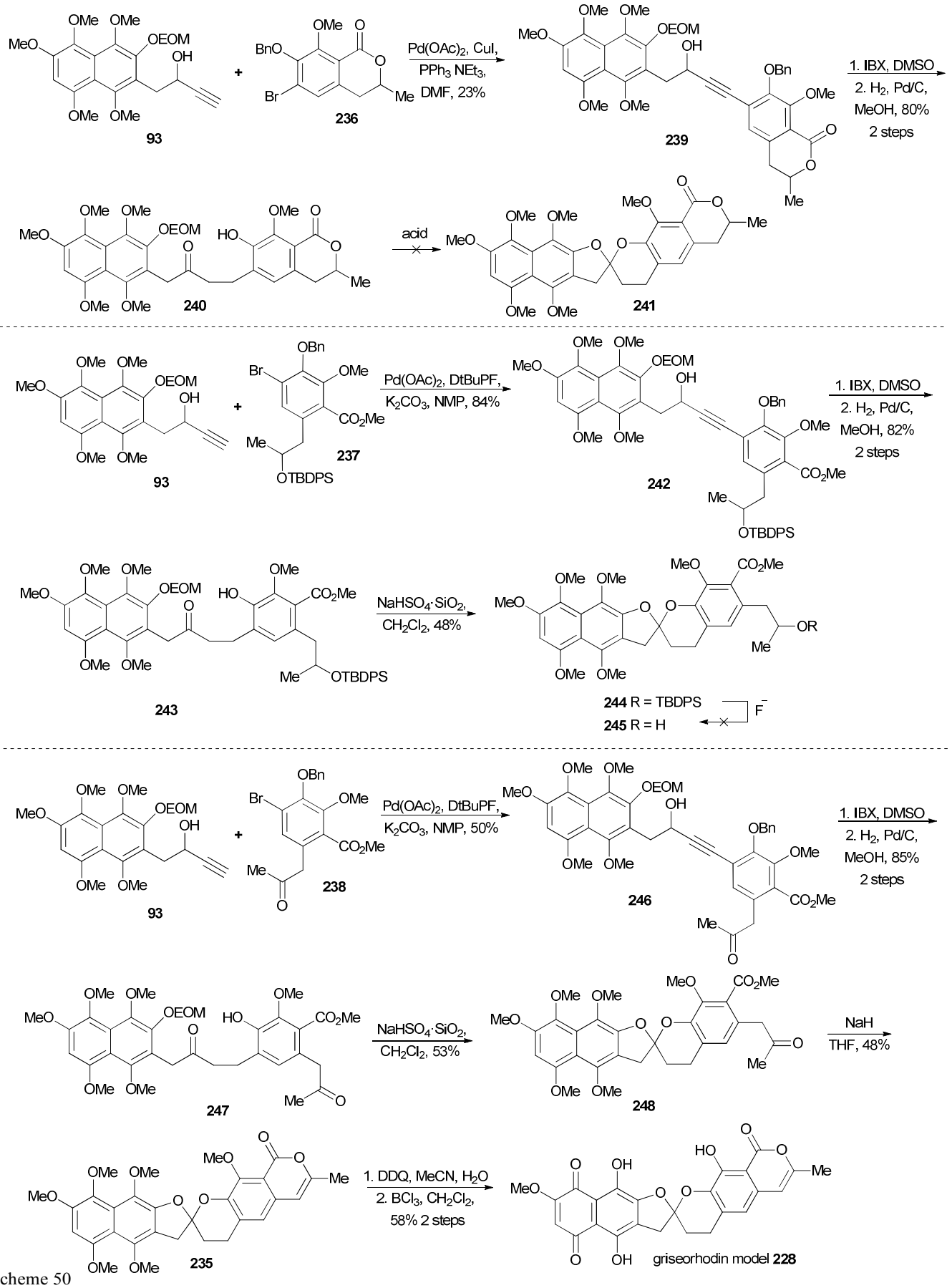

Scheme 47




Scheme 48




Scheme 49


93
236
Pd(OAc)2, CuI, PPh3 NEt3, DMF, 23%
239
1. IBX, DMSO
2. H2, Pd/C, MeOH, 80%
2 steps
240
acid
241
93
237
Pd(OAc)2, DtBuPF, K2CO3, NMP, 84%
242
1. IBX, DMSO
2. H2, Pd/C, MeOH, 82%
2 steps
243
NaHSO4·SiO2, CH2Cl2, 48%
244 R = TBDPS
245 R = H
F−
93
238
Pd(OAc)2, DtBuPF, K2CO3, NMP, 50%
246
1. IBX, DMSO
2. H2, Pd/C, MeOH, 85%
2 steps
247
NaHSO4·SiO2, CH2Cl2, 53%
248
NaH
THF, 48%
235
1. DDQ, MeCN, H2O
2. BCl3, CH2Cl2, 58% 2 steps
griseorhodin model 228

Scheme 50