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Rubromycins: Structurally Intriguing, Biologically Valuable, Synthetically Challenging Antitumour Antibiotics

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Although known for more than 50 years the rubromycin family still constitutes a fascinating class of antitumour antibiotics. They are characterized by a challenging molecular architecture with the central spiroketal unit as the key feature and possess highly attractive biological properties. After a short treatment of the history of their isolation, structural elucidation and biosynthesis, their biological activities will briefly be summarized. This review strongly emphasizes the synthetic efforts aimed at these complex hexacyclic spiroketals. Reactions leading to simple spiroketal model compounds are described, followed by the synthetic approaches to the fully functionalized naphthalene and isocoumarin "wings". The coupling of these units and their transformations into more advanced spiroketals demonstrate "the state of the art" in this research field. Only Danishefsky and co-workers have so far completed the total synthesis of a fully functionalized rubromycin derivative; however, their product heliquinomycinone (**103**) is still only the aglycon of the natural product heliquinomycin (**7**), and it was prepared as the racemic compound. All these achievements and pitfalls reveal that increased engagement of synthetic organic chemists is required to develop new methods to make rubromycins and their analogues available by a modular approach and with reasonable efficacy.

1. Introduction

The rubromycins are a class of natural antibiotics which display particularly attractive biological activities. Apart from the well-known α-, β- and γ-rubromycins (**1**), (**2**) and (**4**), the structurally related pigments 3′-hydroxy-β-rubromycin (**3**), δ-rubromycin (**5**), purpuromycin (**6**), heliquinomycin (**7**) and the griseorhodins A (**9**), C (**10**) and G (**11**) are also members of this intriguing family of compounds (Figure 1). With the exception of compound **1** the basic structural motif of these natural products consists of an aliphatic 5,6-spiroketal core fused to aromatic naphthoquinone and isocoumarin moieties. In addition, heliquinomycin (**7**) constitutes a glycoside with the rare deoxypyranose L-cymarose (**8**).

Thorough structural investigations, detailed studies concerning their reactivity and their biosynthesis as well as the mode of their biological action have been undertaken. However, the relative and absolute configurations have so far only been established for β- and γ-rubromycin (**2**) and (**4**) and heliquinomycin (**7**). Various synthetic efforts towards the natural products have been reported, for example, studies on the synthesis of model compounds representing certain fragments of these structurally challenging molecules. Whereas a total synthesis of the racemic heliquinomycin agylcon (termed heliquinomycinone) was published by Danishefsky in 2001, completed syntheses of other rubromycins are still absent in the literature.

2. History of Rubromycins

In 1953, Brockmann and Renneberg reported the isolation of a novel red-coloured dye produced by *Streptomyces collinus* which was named rubromycin.[1a] A second metabolite produced by this strain, called collinomycin, was isolated shortly after.[1b] Reactivity studies revealed that treatment of rubromycin with refluxing pyridine transformed it quantitatively into collinomycin. On the other hand, heating rubromycin with dilute hydrochloric acid afforded yet another novel reddish pigment which in turn could also be isolated in traces from *Streptomyces* cultures. Due to the evident relationship between these three compounds,

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Brockmann et al. established the comprehensive class of rubromycins. In accordance with the R_f values of the three compounds, collinomycin was renamed α-rubromycin (**1**) and the former rubromycin became β-rubromycin (**2**). The third novel red pigment was termed γ-rubromycin (**4**).[1c] Consequently, structures were proposed on the basis of the chemical behaviour of these compounds, degradation experiments as well as early NMR studies (Scheme 1).[1a–1c]

At the time, the molecular architecture of β-rubromycin was assigned as an aliphatic 5,6-spiroketal core fused to aromatic naphthoquinone and isocoumarin nuclei. Treatment of β-rubromycin with base resulted in β-elimination of the tetrahydropyran ring and quinone rearrangement to give open-chain α-rubromycin (**1**). Whereas α-rubromycin (**1**) was assigned as a 1,4-quinone, it was plausible to assume a 1,2-quinoic structure for β-rubromycin: the acid-mediated rearrangement of β-rubromycin would furnish γ-rubromycin (**4**) and this isomerization could be understood as an analogy to the related β-lapachone→α-lapachone rearrangement of 1,2-quinoic systems. However, in 2000 Zeeck and co-workers revised the structure of β-rubromycin to be **2** with the aid of modern spectroscopic methods and a biosynthetic interpretation (see below).[2d] Thus, treatment of **2** with acid simply leads to the loss of a methyl group and quinone tautomerization to the thermodynamically favoured A-ring quinone of γ-rubromycin (**4**). In the course of this recent reinvestigation of β-rubromycin (**2**), two further co-metabolites were obtained from the fermentation of rubromycin-producing strains and named 3′-hydroxy-β-rubromycin (**3**) and δ-rubromycin (**5**).[2d]

In the 1970s, four additional rubromycin pigments were discovered, namely purpuromycin (**6**),[3a,3b] a bacterial metabolite of *Actinoplanes ianthinogenes*, and griseorhodins A (**9**), C (**10**) and G (**11**) from *Streptomyces californicus* and *griseus*, respectively (Figure 1).[4a–4c] Purpuromycin (**6**) is structurally closely related to γ-rubromycin (**4**), only differing in the additional hydroxy group at C-4 of the pyranoid ring. Griseorhodins A (**9**), C (**10**) and G (**11**) bear polyhydroxylated spiroketal cores and further differ from the other rubromycins in the substituent at C-7 of the isocoumarin moiety which is connected to a methyl instead of a methoxycarbonyl group. The structures of these four species were similarly deduced from NMR spectroscopic data and degradation studies but neither the relative nor the ab-

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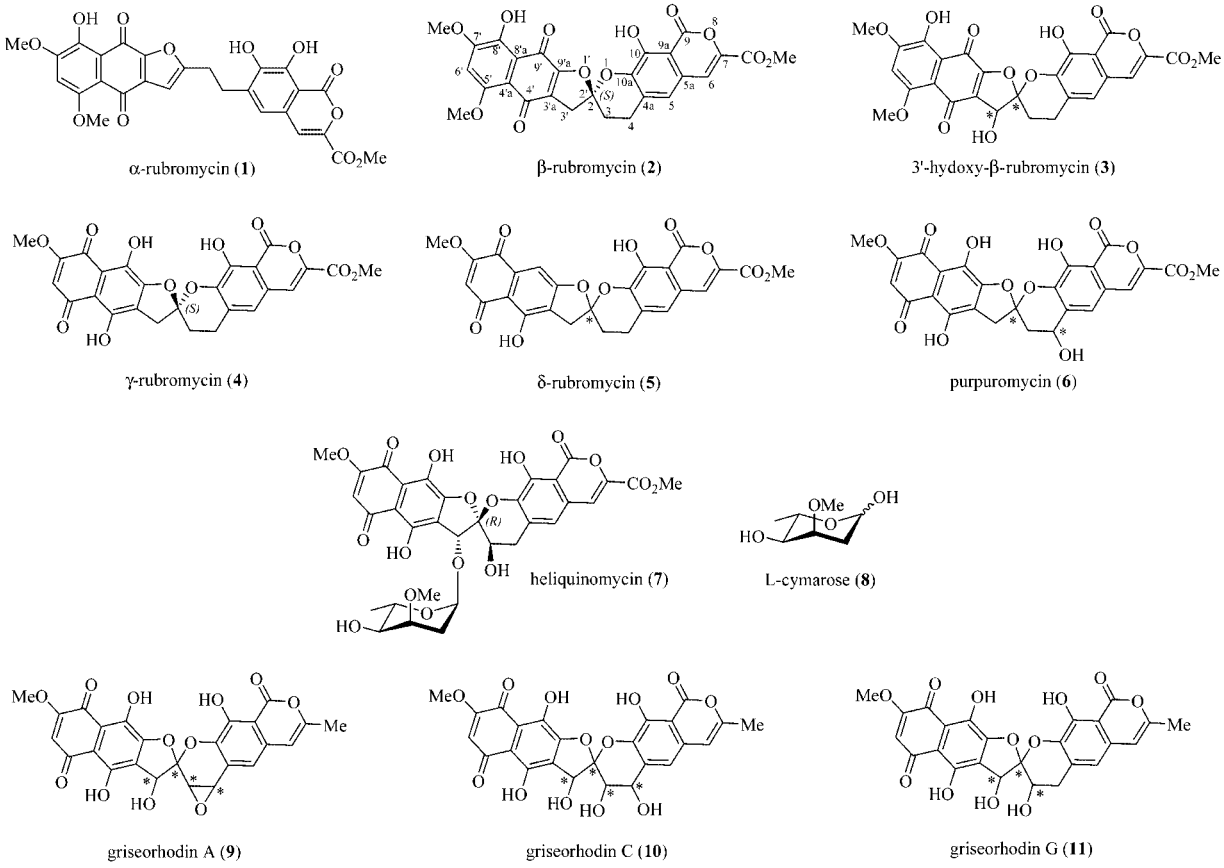
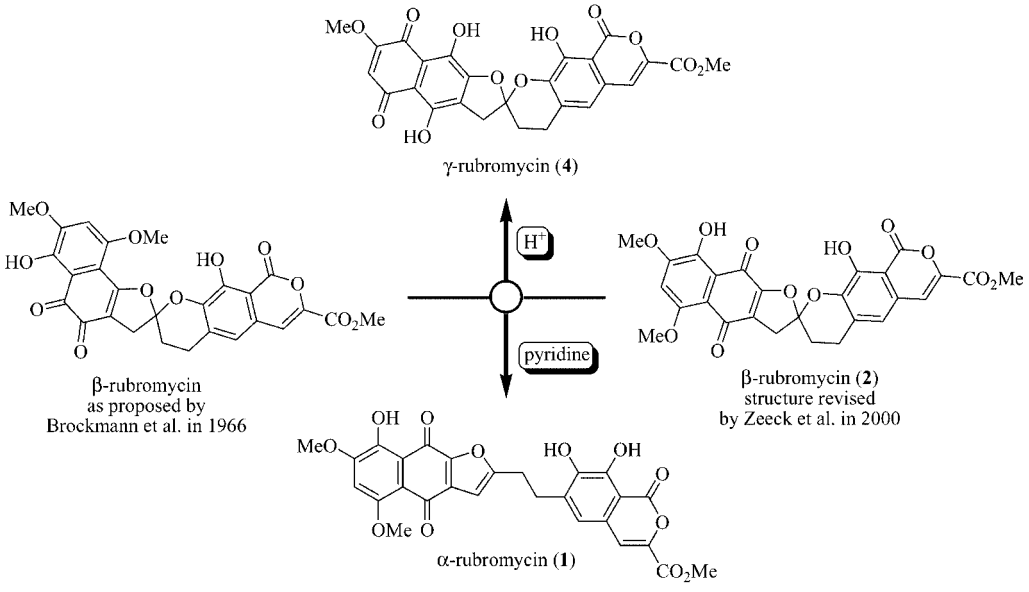


Figure 1. Natural products of the rubromycin family (numbering for compounds **3**–**7** and **9**–**11** is as shown for **2**. Asterisk *: configuration unknown!).



Scheme 1. Brockmann's structural assignments according to the chemical transformations of β-rubromycin and Zeeck's reassignment (only constitutions given).

solute configurations of these compounds are known to date. Finally, in 1996 Chino and co-workers isolated heliquinomycin (**7**) from *Streptomyces sp.* MJ 929-SF2[5a] and the structure of this dye could unambiguously be assigned by X-ray crystallography. Most strikingly, **7** features a glycosidic linkage at C-3′ of the tetrahydrofuran ring to the rare deoxysugar cymarose (**8**). The absolute configuration of **7** was soon elucidated by acidic hydrolysis which

furnished the aglycon itself along with (–)-cymarose (**8**), which apparently belongs to the L series.[5b]

The absence of stereochemical assignments within this family of compounds is an intriguing issue. For example, compounds **2** and **4** have, if any, very small optical rotations,[2b] but optical rotatory dispersion (ORD) measurements indicated at least enantioenrichment in these isolates. Based on quantum chemical simulation of their chiroptical properties and comparison with heliquinomycin (**7**), Zeeck and co-workers were recently able to deduce the absolute configurations of β- and γ-rubromycin (**2**) and (**4**) and it is thus proven that these compounds are isolated, in contrast to heliquinomycin (**7**), with (*S*)-configured spiro centres. It follows from this investigation that different *actinomyces* strains are actually capable of producing different spiroketal configurations.[6]

Biosynthetic investigations clearly proved the polyketide origin of the rubromycins. Feeding experiments on *Streptomyces* sp. A1[2d] and *Streptomyces* sp. MJ929-SF2[5c] showed the incorporation of 12 acetate units into β-rubromycin (**2**) and the heliquinomycin aglycon, respectively (Figure 2). The labelling patterns of the polycyclic aromatic skeletons were found to be identical in these two independent studies. Further, it was demonstrated that the methoxy groups present in the natural products derive from L-methionine and, finally, feeding of D[U-^{13}C]-glucose to *Streptomyces* sp. MJ929-SF2 resulted in label incorporation at all six ring carbon atoms of the L-cymarose portion of heliquinomycin (**7**).

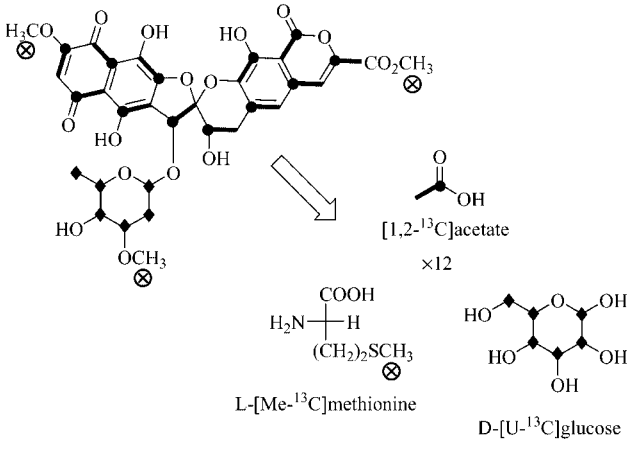
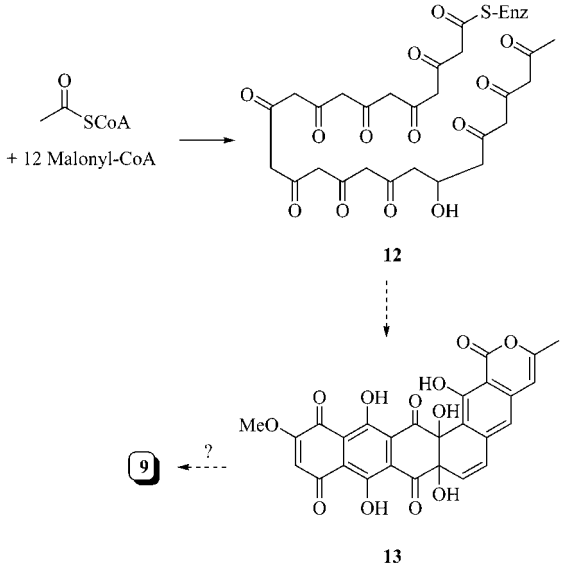


Figure 2. Labelling pattern in heliquinomycin (**7**) obtained after feeding experiments on *Streptomyces* sp. MJ929-SF2.

The actual post-PKS modification of the polyketide precursors to the rubromycin spiroketal system has been the subject of discussion. One might conceive that either two polyketide chains merge in this process (e.g., a decaketide and a diketide) or a dodecaketide undergoes an oxidative C–C bond cleavage.[2d] To this end, Li and Piel could identify and sequence the gene cluster of *Streptomyces* sp. JP95, which encodes the biosynthesis of griseorhodin A (**9**), and proposed that the post-PKS tailoring of a tridecaketide **12** proceeds via a hypothetical didehydrocollinone intermediate **13** (Scheme 2).[7]



Scheme 2. Proposed pathway for the biosynthesis of griseorhodin A (**9**).

3. Biological Activity of Rubromycins

The rubromycins are potent antimicrobial agents. β- and γ-Rubromycin (**2**) and (**4**) inhibit growth of various bacteria and fungi at micromolar to nanomolar concentrations, most markedly *St. aureus* and *Bact. subtilis*.[1a,1c] Likewise, a high activity of purpuromycin (**6**) against *Staphylococcus* strains was detected in the course of its isolation.[3b] Further, semisynthetic derivatives of purpuromycin (**6**) have been considered as potential agents for vaginal infections.[8] More interestingly, highly attractive cytostatic and enzyme inhibitory properties of some members of the compound family were found more recently. Hayashi and co-workers have demonstrated that β- and γ-rubromycins (**2**) and (**4**) as well as purpuromycin (**6**) and griseorhodins A (**9**) and C (**10**) inhibit human telomerase at IC_{50} values ranging from 3 to 12 μM. In the cell cycle, telomerase enzymes play a crucial role by reconstructing the chromosome termini (the telomeres) during cell division. If this activity is suppressed, telomeres shorten upon every single cell cycle and, ultimately, apoptosis occurs. The high telomerase activity in cancer cells thus makes these enzymes potential targets in cancer therapy. It has also been demonstrated that the spiroketal moiety of the rubromycins is an important pharmacophore in the interaction with human telomerase since open-chain α-rubromycin (**1**) displays a dramatically decreased activity ($IC_{50} > 200$ μM). Goldman, Hayashi, Sakaguchi and their co-workers additionally revealed that β- and γ-rubromycins (**2**) and (**4**) also inhibit mammalian DNA-polymerase, a reverse transcriptase of HI virus type 1, thus indicating again the relevance of these compounds in medicinal research.[2d,9]

Heliquinomycin (**7**) displays moderate antitumour and antimicrobial activity but acts as a selective inhibitor of DNA helicases.[5d] These enzymes play an essential role in numerous cell processes since they unwind the double-

stranded DNA to provide single-stranded DNA, which is crucial for replication, recombination or the repair of DNA.[10] Since cancer cells have an increased tendency to reproduce, the inhibitory activity of heliquinomycin (**7**) may allow for the development of a new class of anticancer drugs. Notably, heliquinomycin (**7**) is unique since it is the only selective DNA helicase inhibitor known to date and therefore its exploration could lead to a better understanding of the mode of action of these important enzymes.[5d]

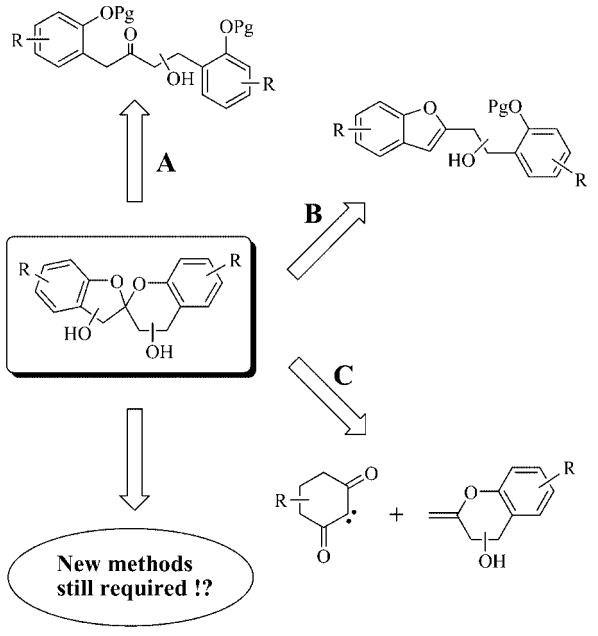
4. Efforts towards the Total Synthesis of Rubromycin-Type Compounds

Even though the biological properties of the rubromycins have been thoroughly investigated, reports on synthetic routes to these compounds are surprisingly scarce. Just a single total synthesis, namely Danishefsky's synthesis of the heliquinomycin aglycon, has yet been accomplished (see below, Scheme 26). Ideally, the different members of the rubromycin family should be synthesized in a modular fashion and, apparently, the different research groups contributing to this area favour this strategy. In this section we will present an overview of the efforts directed towards the synthesis of rubromycins published up to November 2006.

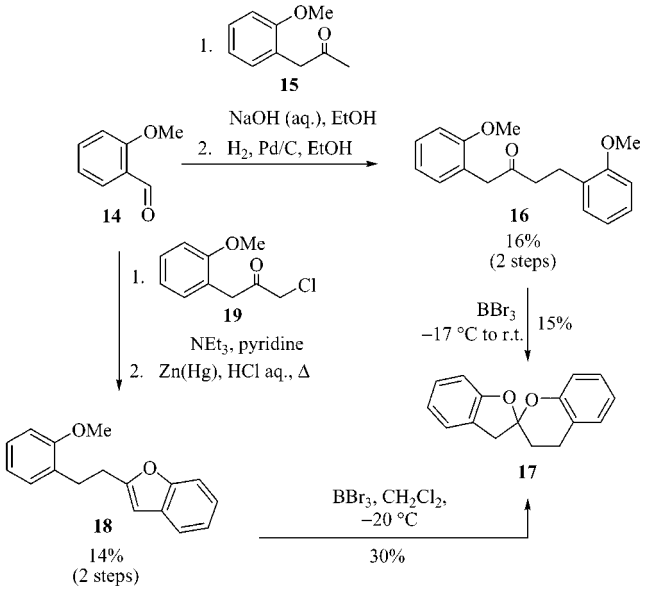
4.1 Model 5,6-Spiroketal Compounds

Synthetic endeavours towards the rubromycins began with the assembly of diverse model 5,6-spiroketal compounds. The most obvious retrosynthetic disconnections lead back to bis-phenolic ketones (path **A**, Scheme 3) and 2-(2-aryl)ethylbenzofuran precursors (path **B**). However one must state that there is still a demand for the development of new methodologies in the construction of this central structural motif, as can be seen from the various attempts to apply these established protocols to more complex advanced intermediates (see below). As an example, the Pettus group addressed this synthetic problem by conceiving an approach based on cycloaddition chemistry (path **C**).[18a]

The first studies on the assembly of rubromycin-type model compounds were performed by Greul and Brockmann in 1971.[2c] Two alternative methods for the construction of the benzannulated 5,6-spiroketal core had already been evaluated at that time: the spirocyclization of a suitable α,β′-bis(hydroxyaryl)-substituted ketone and, less obvious, the employment of a 2-(2-aryl)ethylbenzofuran precursor (Scheme 4). Thus, bis(*o*-methoxyphenyl)-substituted ketone **16** was prepared by aldol condensation of *O*-methylated salicyl aldehyde **14** with methyl ketone **15** followed by hydrogenation. Exposure of **16** to boron tribromide furnished spiro compound **17** with very moderate overall efficacy. Alternatively, **17** could be prepared from the benzofuran **18**, obtained by condensation of aldehyde **14** with chloromethyl ketone **19** and subsequent Clemmensen reduction. Lewis acid promoted demethylation and cyclization delivered the unfunctionalized 5,6-spiroketal **17**.

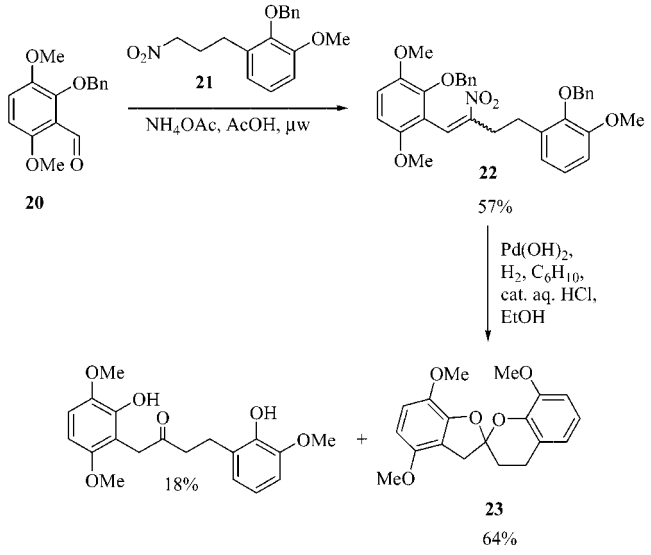


Scheme 3. Retrosynthesis of benzannulated 5,6-spiroketals.



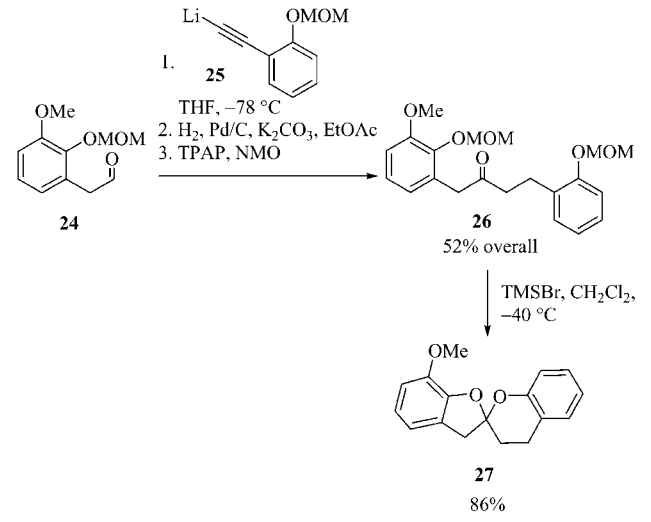
Scheme 4. Synthesis of 5,6-spiroketal model compound **17** according to Greul and Brockmann.

The initial work of Greul and Brockmann remained the only study in this area for more than two decades. Presumably stimulated by Chino's discovery of heliquinomycin (**7**) in 1996, investigations were performed in this field by de Koning's group and others. de Koning's approach involved a microwave-assisted Henry condensation of aldehyde **20** with nitroalkane **21** to afford nitroalkene **22** (Scheme 5). This intermediate was elegantly converted into spiro compound **23** in good yield by a Nef-type reaction in the presence of $Pd(OH)_2$ (Pearlman's catalyst) and catalytic amounts of aqueous hydrochloric acid.[11]



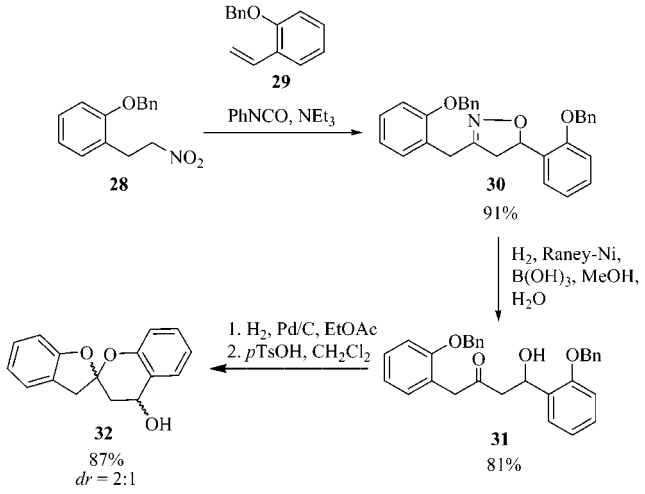
Scheme 5. Synthesis of 5,6-spiroketal model compound **23** according to de Koning and co-workers.

Brimble and co-workers have strongly been involved in the synthesis of aromatic spiroketals found in pyranonaphthoquinone antibiotics and also contributed to the chemistry of rubromycin in 2003. They added lithiated aryl acetylide **25** to aryl-substituted acetaldehyde **24** (Scheme 6) and by subsequent hydrogenation and oxidation of the intermediate propargylic alcohol the desired spiroketal precursor **26** with MOM-protected phenolic hydroxy functions was obtained. In this case, cyclization affording target compound **27** was initiated using bromotrimethylsilane as promoter.[12]



Scheme 6. Synthesis of 5,6-spiroketal model compound **27** according to Brimble and co-workers.

More recently, the Kozlowski group introduced isoxazoline derivatives, readily obtained by 1,3-dipolar cycloadditions, as precursors to 5,6-spiroketals (Scheme 7). Hence, nitroalkane **28** was converted into the corresponding nitrile oxide in the presence of styrene derivative **29**. The [3+2] cycloaddition regioselectively furnished isoxazoline derivative **30** in excellent yield, which was reduced to dibenzyl-protected phenolic ketone **31**. Hydrogenolysis followed by acid-promoted ketalization of **31** led to 5,6-spiroketal **32** as a 2:1 mixture of diastereoisomers, mimicking the central portion of purpuromycin (**6**).[13]

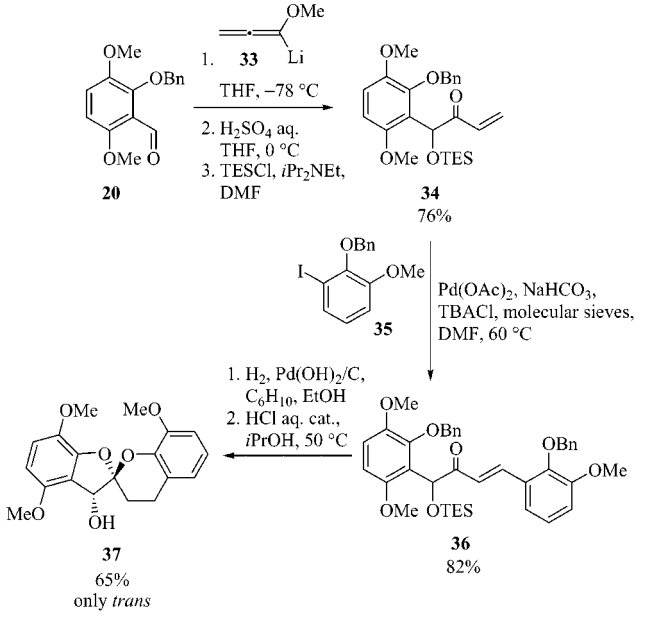


Scheme 7. Synthesis of 5,6-spiroketal model compound **32** according to Kozlowski and co-workers.

Our research in the area of rubromycin syntheses also began with the assembly of spiroketal model compounds. In our strategy, we focused on the utility of lithiated methoxyallene **33** as a synthetic equivalent of an α,β-unsaturated acyl anion synthon (Scheme 8).[14] Nucleophilic addition of **33** to de Koning's aryl aldehyde **20** followed by acidic hydrolysis and silylation gave TES-protected α-hydroxy enone **34**. The Heck reaction of **34** with aryl iodide **35** furnished intermediate **36** which underwent a highly diastereoselective spiroketalization to model compound **37** upon hydrogenation and subsequent treatment with catalytic amounts of acid. Here, the observed exclusive formation of the *trans* diastereoisomer is very likely the result of thermodynamic control.[15]

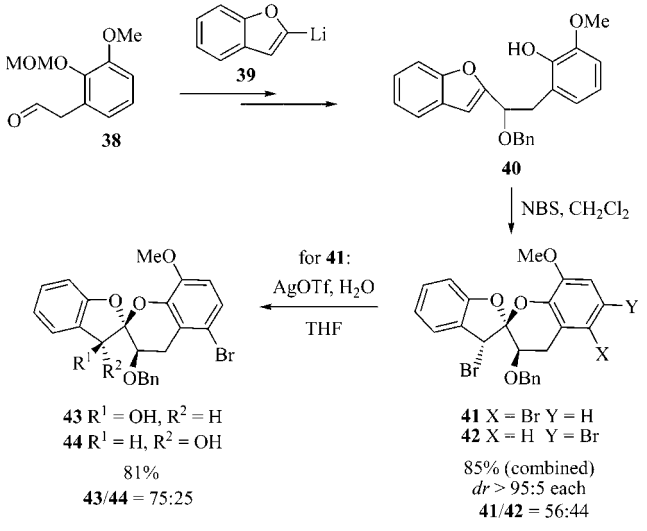
Following this synthetic concept, a variety of model compounds were prepared in good yields and with high *trans* selectivity. Importantly, our approach allows for the functionalization of the enone double bond present in compounds such as **36** in order to introduce further hydroxy groups. It also has the option to furnish enantiomerically enriched compounds by using chiral alkoxyallenes. First experiments indicated that at least good levels of asymmetric induction can be achieved.[16]

Interesting model studies were also conducted by the Danishefsky group (Scheme 9). For the intended total synthesis of heliquinomycin (**7**) an electrophile-assisted cyclization of benzofuran derivative **40** was investigated. Intermediate **40** was easily prepared by addition of lithiated benzofuran **39** to aryl acetaldehyde **38**.[17a] Activation of the furan



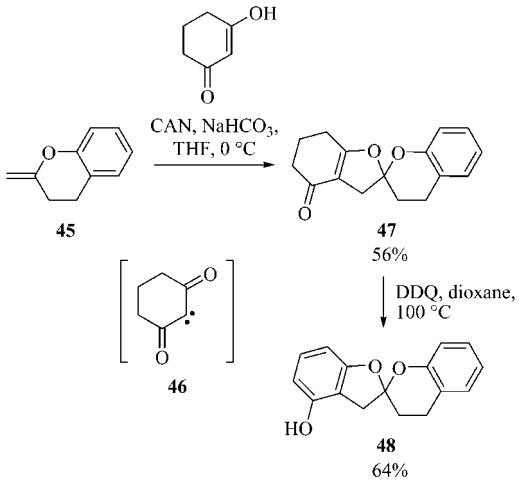
Scheme 8. Alkoxyallene-based approach to 5,6-spiroketal precursor **37** by Reißig and co-workers.

double bond of **40** with NBS and bromonium ion opening by the neighbouring phenolic hydroxy group led to spiroketal formation in good yield. Although the cyclization proceeded with high diastereoselectivity, bromination of the electron-rich aryl group could not be avoided, providing compounds **41** and **42** as a 56:44 mixture of constitutional isomers. The benzylic bromine of isomer **41** was subsequently replaced in a silver(I)-promoted nucleophilic substitution furnishing epimers **43** and **44**.



Scheme 9. Synthesis of 5,6-spiroketal model compounds **43** and **44** according to Danishefsky and co-workers.

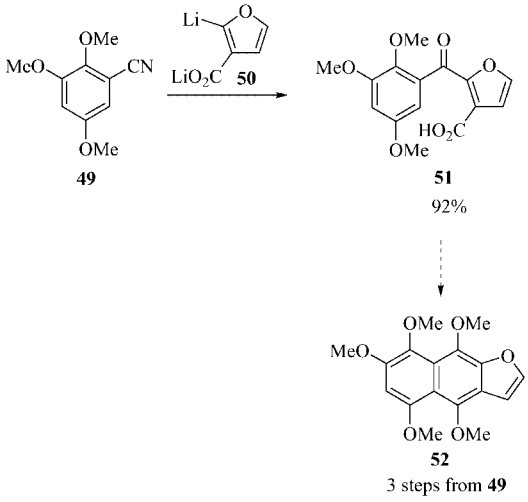
A distinctively new strategy for spiroketal formation was conceived by Pettus and co-workers who directly linked two functionalized units to form the central spiro core by a [3+2] cycloaddition, thus avoiding any acid-promoted ketalization processes. The [3+2] cycloaddition of enol ether **45** with an oxidatively generated carbonyl carbene **46** provided enone **47** which could be aromatized with DDQ to furnish spiro compound **48** in reasonable yield (see Scheme 3 and Scheme 10).[18]



Scheme 10. Synthesis of 5,6-spiroketal model compound **48** according to Pettus and co-workers.

4.2 Syntheses of Naphthalene and Isocoumarin Modules and Their Coupling to Advanced Intermediates

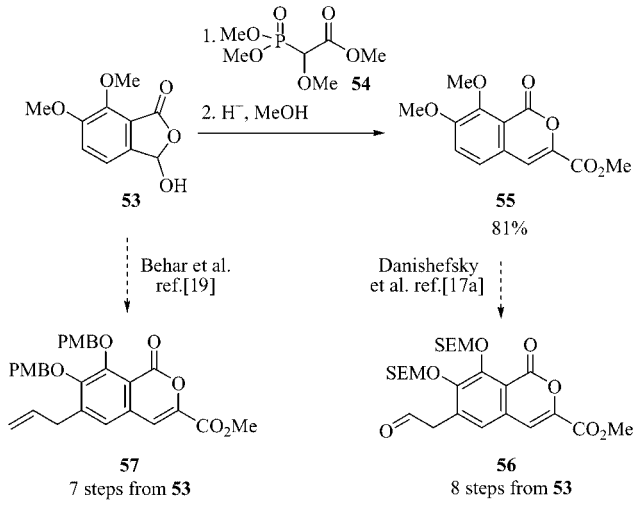
As a second stage, efficient routes to naphthalene and isocoumarin fragments needed to be established, delivering building blocks with the required substitution pattern for assembly of advanced intermediates. In the course of their pioneering total synthesis of heliquinomycinone (**103**) (see Scheme 26), the Danishefsky group prepared pentamethoxy-substituted naphthofuran **52** starting from benzonitrile derivative **49** (Scheme 11). Addition of the dianion of 3-furoic acid **50** to this nitrile followed by intramolecular



Scheme 11. Preparation of naphthofuran derivative **52** (Danishefsky and co-workers.).

Friedel–Crafts acylation and reductive methylation furnished the target compound. Although this reported sequence is fairly short and highly efficient the preparation of **49** and that of 3-furoic acid has to be taken into account.[17a]

Isocoumarin derivative **56** was accessed in eight steps starting from opianic acid (**53**). Horner–Wadsworth–Emmons olefination with phosphonate reagent **54** and subsequent condensation gave isocoumarin **55**. This was converted into target compound **56** by further manipulations.[17a] Similarly starting from **53**, Behar and co-workers prepared 6-allyl-isocoumarin-3-carboxylate **57**[19] (Scheme 12).

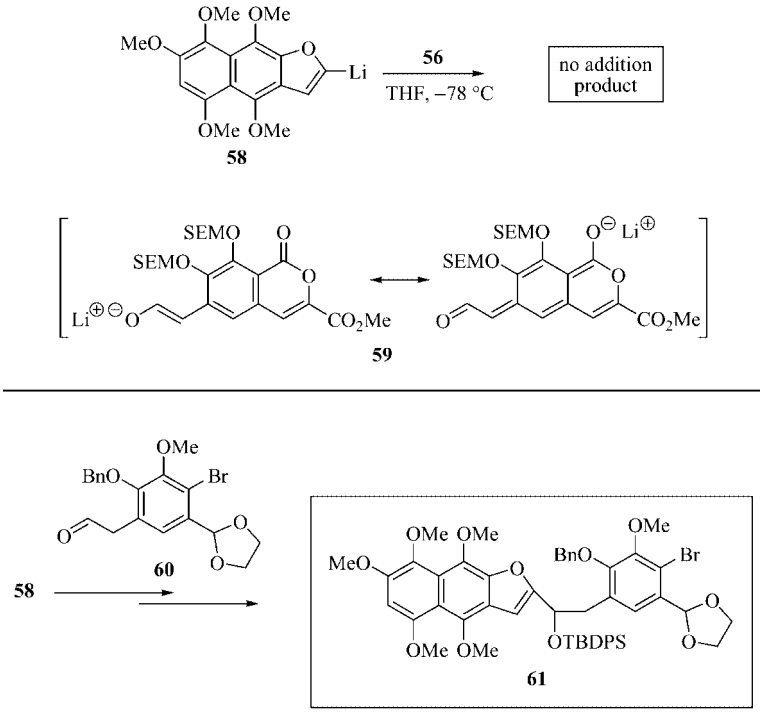


Scheme 12. Preparation of isocoumarin derivatives **56** (Danishefsky and co-workers) and **57** (Behar and co-workers).

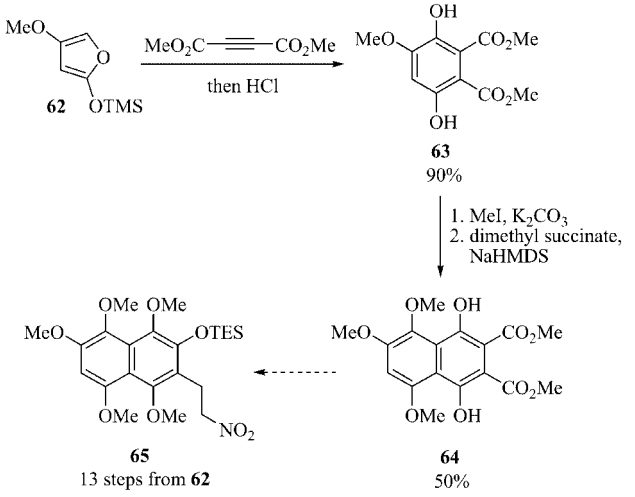
The two modules thus prepared by the Danishefsky group required a coupling analogous to the reaction of lithiated benzofuran **39** with aldehyde **38** (see Scheme 9). Unexpectedly, this model reaction could not be applied to aldehyde **56** (Scheme 13). When reacted with lithiated naphthofuran **58**, no addition but only deprotonation in the benzylic position α to the formyl group occurred. Due to the electron-withdrawing carbonyl functions present in **56**, it was rationalized that the highly stabilized enolate **59** was formed in favour of any addition to the carbonyl group. This unforeseen behaviour of aldehyde **56** led to a modification of plans such that the isocoumarin unit would be generated at a much later stage of the synthesis. The addition of **58** to aryl acetaldehyde **60**, prepared in 11 steps starting from vanillin, cleanly furnished coupling product **61** which indeed was a suitable precursor to the racemic heliquinomycin aglycon (see below).[17]

The Kozlowski group also synthesized highly substituted naphthalene building blocks. Whereas a first generation synthesis relied on a Dötz reaction as the key step,[20] a recently published more concise second generation approach started from furan derivative **62** (Scheme 14). Diels–Alder reaction of **62** with dimethyl acetylenedicarboxylate followed by acidic hydrolysis furnished phenol **63** which was converted into naphthalenedicarboxylate **64** by methylation and a two-fold Claisen condensation with dimethyl succinate. Further manipulations gave nitroalkyl-substituted pentamethoxynaphthalene derivative **65**, prepared within 13 steps.[21]

This group also prepared 6-vinylisocoumarin-3-carboxylate **70** (Scheme 15). Catechol (**66**) was converted into iodoarene **67** which was coupled with enol ether **68** in a

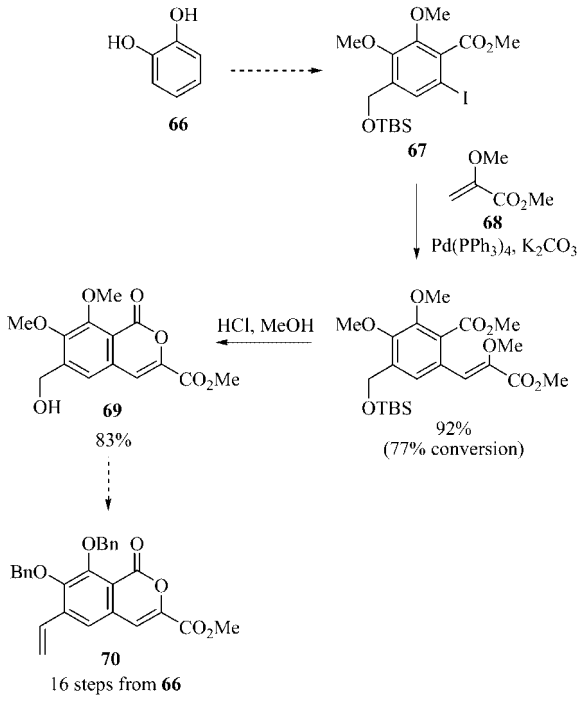


Scheme 13. Preparation of advanced intermediate **61** (Danishefsky and co-workers).



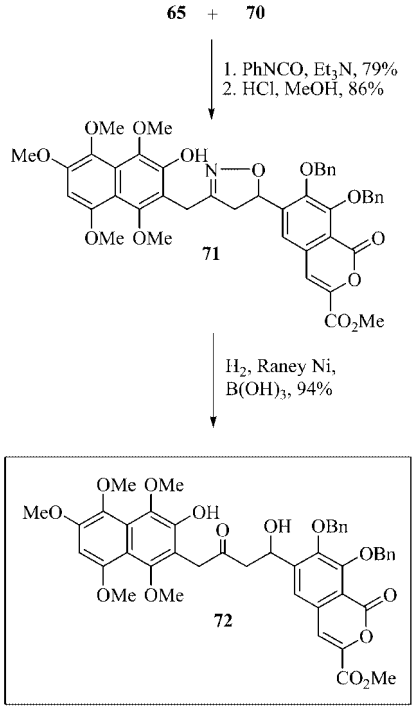
Scheme 14. Synthesis of naphthalene derivative **65** (Xie and Kozlowski).

Heck reaction. Acidic intramolecular condensation of the coupling product gave 6-hydroxymethylisocoumarin **69** and this compound was transformed into target compound **70**, thus prepared within 16 linear steps.[21,22]



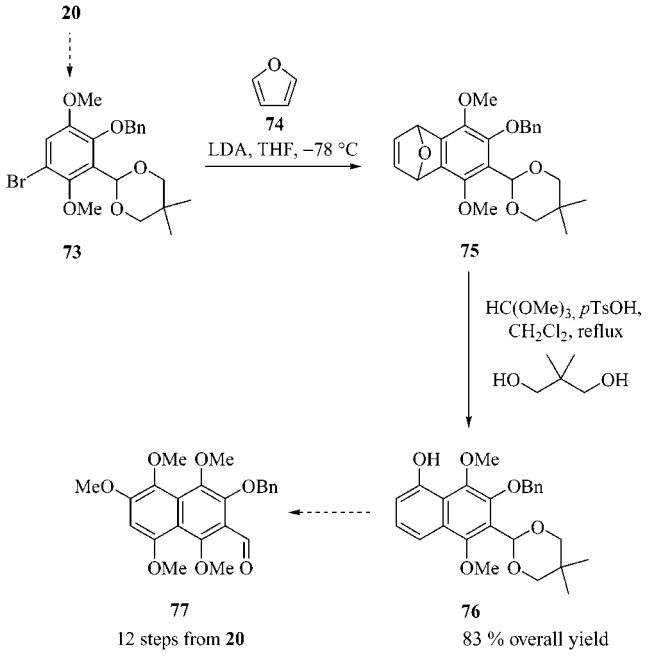
Scheme 15. Synthesis of isocoumarin derivative **70** (Kozlowski and co-workers).

The envisaged coupling of building blocks **65** and **70** proceeded uneventfully, as observed for model fragments **28** and **29**. The advanced spiroketalization precursor **72** was obtained in high overall yield via cycloadduct **71** (Scheme 16).[21] Unfortunately, this compound was not a suitable precursor for the rubromycins (see below, Scheme 20).



Scheme 16. Preparation of advanced intermediate **72** (Kozlowski and co-workers).

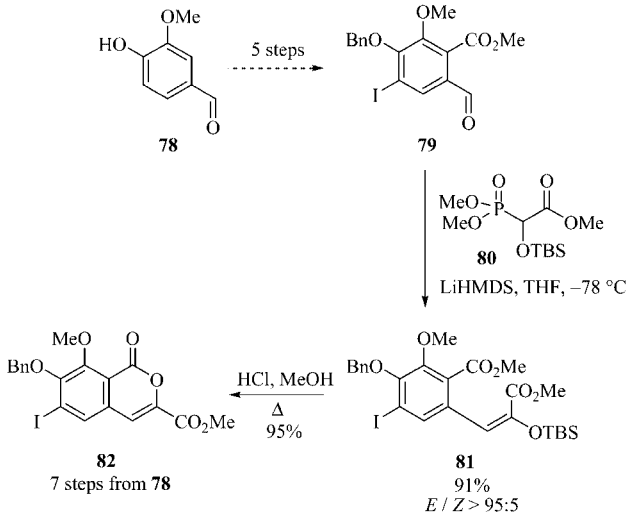
The synthetic efforts of our group were first aimed at pentamethoxynaphthaldehyde **77** (Scheme 17). Aryl acetal **73**, derived from de Koning's aldehyde **20**, was treated with LDA in the presence of furan (**74**) furnishing cycloadduct **75**. Regioselective cleavage of the ether bridge under well-balanced acidic conditions gave naphthalene derivative **76**



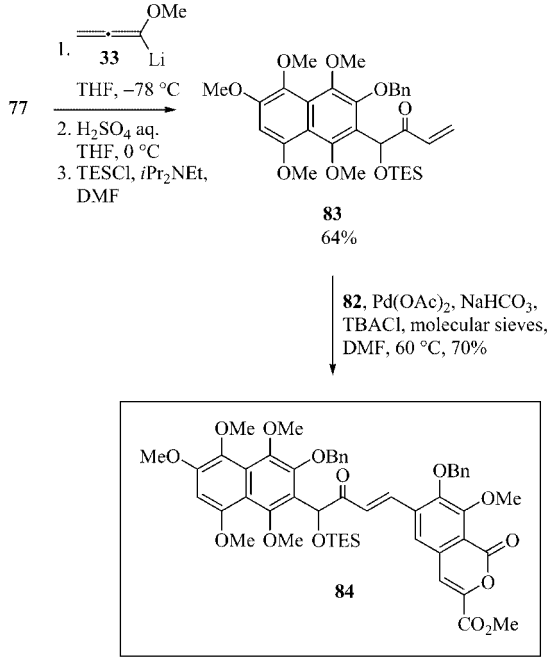
Scheme 17. Synthesis of naphthaldehyde **77** (Reißig and co-workers).

which was converted into target aldehyde **77** by further transformations. Starting from **20** we required 12 steps to obtain the crucial precursor compound **77** in 9% overall yield (16 steps with respect to commercially available starting material).[23]

For the synthesis of 6-iodoisocoumarin-3-carboxylate derivative **82** we converted vanillin (**78**) into ester **79** within five steps. Lactone formation was achieved by olefination of **79** with phosphonate **80** and intramolecular condensation of silyl enol ether **81**. Thus, the desired building block **82** was obtained in an efficient seven-step sequence. Palladium-catalyzed couplings of **82** served as model reactions for the planned rubromycin synthesis (Scheme 18).[24]



Scheme 18. Synthesis of 6-iodo-isocoumarin derivative **82** (Reißig and co-workers).

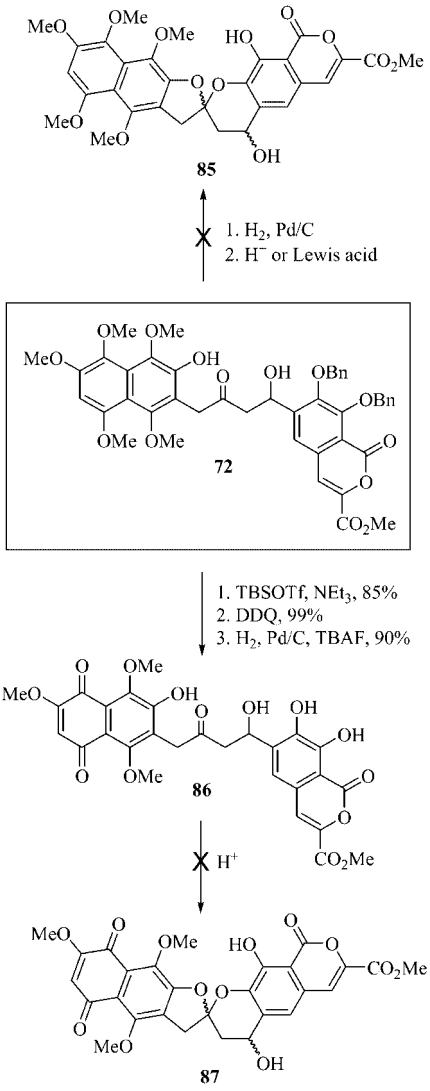


Scheme 19. Preparation of advanced intermediate **84** (Reißig and co-workers).

Aldehyde **77** was then elaborated to α-siloxy enone **83** by the method already applied to the preparation of model compound **37** (Scheme 19). Heck coupling of enone **83** with 6-iodoisocoumarin derivative **82** cleanly led to advanced intermediate **84** in good yield.[16]

4.3 Spirocyclizations of Advanced Intermediates: So Close and Yet so Far Away!

As a general rule in synthesis, the ultimate confirmation of a synthetic concept can only be gained during the terminal synthetic stages. This proved true at least in rubromycin syntheses! The various attempts at converting the different advanced intermediates **61**, **72** and **84** into fully mature spiroketal compounds – painfully capricious, but only partially successful – will be discussed in this final paragraph.

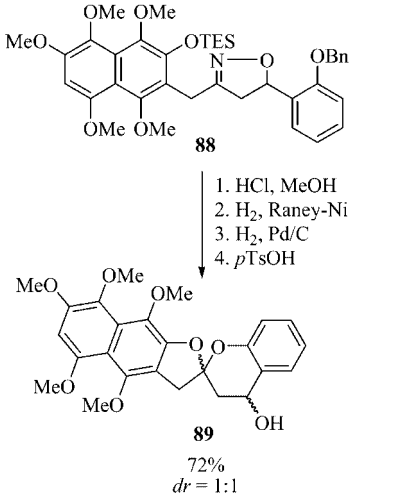


Scheme 20. Attempted spirocyclizations of advanced intermediates **72** and **86** (Kozlowski and co-workers).

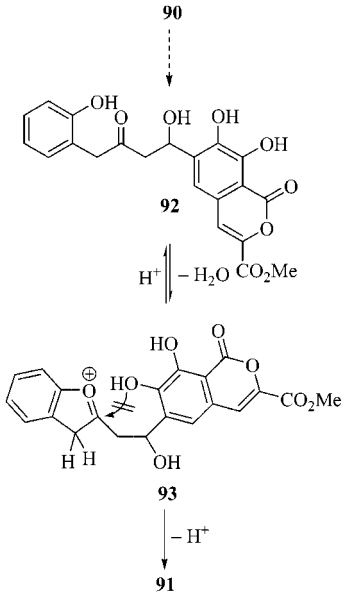
Kozlowski and co-workers planned to carry out ketal formation on bis-phenolic ketone **72** as the key step in the synthesis of purpuromycin (**6**).[21] It turned out that **72** did not ketalize cleanly upon treatment with various Brønsted and Lewis acids; instead complex mixtures of products were produced (Scheme 20). One hypothesis for the reason of this failure was that the electron-rich naphthalene portion in **72** may undergo undesired oxidative side-reactions. Thus, compound **72** was converted into naphthoquinone derivative **86** prior to the ketalization step. Likewise, **86** also gave complex mixtures when treated with different Brønsted acids.

In order to obtain detailed information on the origins of these difficulties, spiroketalization reactions were conducted in a combinatorial fashion with precursors derived from isoxazolines (Scheme 21). Whereas compound **88** bearing the pentamethoxynaphthyl substituent could smoothly be converted into spiroketal **89** according to the established procedure, isocoumarin-substituted compound **90** led to the benzofuran derivative **91** exclusively. Notably, no background oxidation of the naphthalene system occurred in the transformation of **88** to **89**. On the other hand, no cyclization to the spiroketal system took place in the case of precursor **90** and only irreversible formation of benzofuran derivative **91** was observed.[21]

The authors attributed these findings to the markedly diminished nucleophilicity of the isocoumarin phenolic oxygen atom (Scheme 22). Deprotected ketone **92**, obtained by hydrogenolysis of **90**, would cyclize in the presence of acid to intermediate oxonium ion **93**. In this intermediate, deprotonation to furnish benzofuran derivative **91** would be



Scheme 21. Attempted spirocyclizations of model compounds **88** and **90** (Kozlowski and co-workers).



Scheme 22. Rationale for the formation of benzofuran derivative **91**.

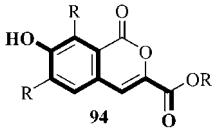
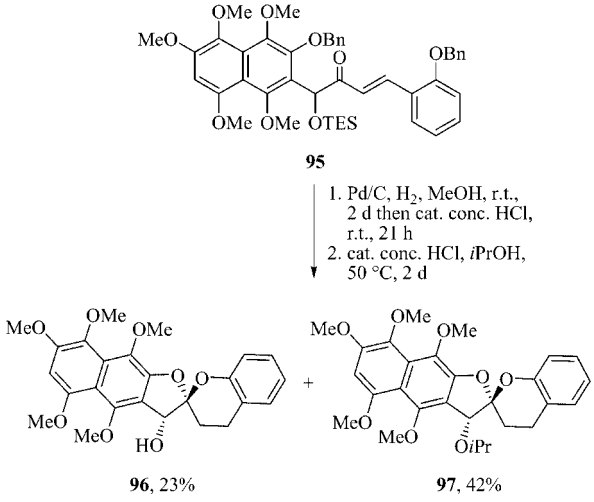


Figure 3. Conjugation in isocoumarin derivative **94**.



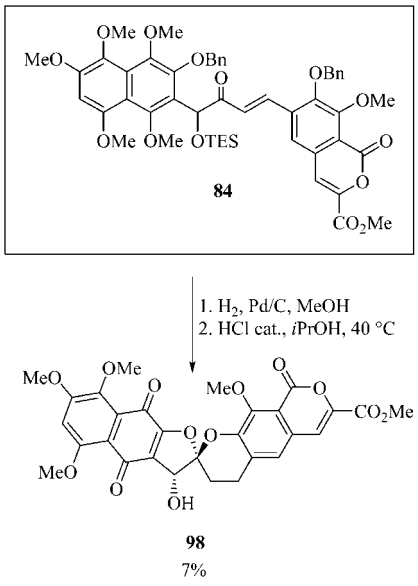
Scheme 23. Spirocyclization of model compound **95** (Reißig and co-workers).

much faster than nucleophilic attack by the isocoumarin hydroxy function. Indeed, nucleophilic attack of this group may be strongly hampered by the extended conjugation present in this system (see sketch **94**, Figure 3).

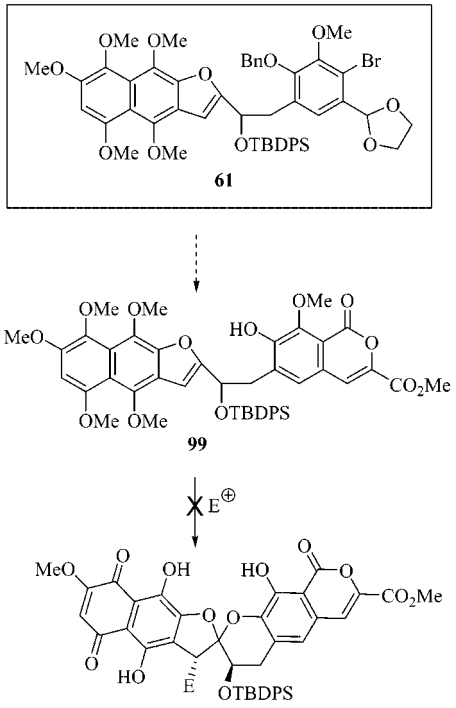
Preliminary results from our laboratory are supportive of these findings. Model compound **95**, with a fully functionalized naphthalene moiety, cleanly gave *trans*-configured spiroketal products **96** and **97** upon hydrogenolysis of the benzyl groups and double bond reduction followed by treatment with catalytic amounts of hydrochloric acid. Ketal formation was partially accompanied by solvolytic displacement of the benzylic hydroxy group at C-3′ with 2-propanol (Scheme 23).[15]

In contrast, when advanced intermediate **84** was subjected to identical conditions, a mixture of products was produced. As one component, we could isolate dimethylated 3′-hydroxy-β-rubromycin **98** in small quantities (Scheme 24).[16]

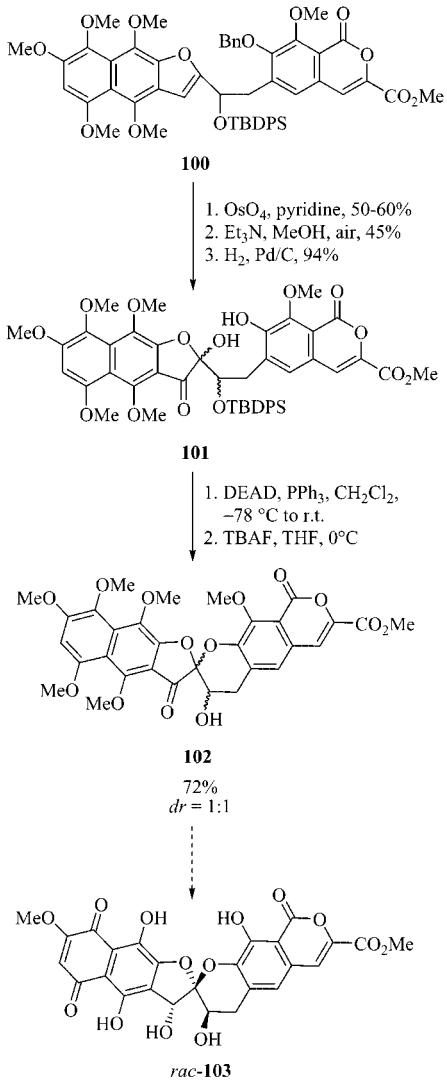
It can be learned from the formation of compound **98** that acid-promoted ketalizations of compounds such as **84**, **72** and **86** are indeed delicate but not impossible. Electronic complications are caused by both the electron-rich naphthalene and the electron-deficient isocoumarin moieties. It seems that no straightforward conclusion can be drawn from the observations made in these cases. We are convinced that electronically well-balanced aromatic units on



Scheme 24. Attempted spirocyclization of advanced intermediate **84** (Reißig and co-workers).



Scheme 25. Attempted spirocyclization reactions with precursor **61** (Danishefsky and co-workers).



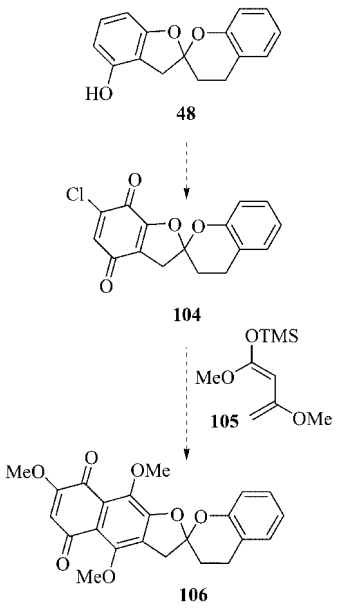
Scheme 26. Final stages of Danishefsky's total synthesis of racemic heliquinomycinone (**103**).

either side of the precursor will finally allow a successful acid-promoted ketal formation from bis-phenolic ketone precursors.

In the final stages of Danishefsky's total synthesis of heliquinomycinone (**103**) severe complications also had to be overcome.[17b] Bromo acetal **61** was elaborated to isocoumarin-containing spiroketal precursor **99** within a number of routine steps. Most disappointingly, the electrophile-assisted cyclization, elegantly achieved with the reaction of NBS on model compound **40** (Scheme 9), did not proceed with **99**. Subsequent screening for alternative conditions showed that some reagents were primarily attacking the electron-rich naphthalene portion leading to A- or B-ring 1,4-quinones, while others were not sufficiently reactive for an activation of the furan double bond (Scheme 25).

As a final alternative the authors were able to utilize the dihydroxylation of the benzofuran double bond in benzyl-protected compound **100** to reach their target by a minor detour. The vicinal diol thus obtained was very prone to aerobic oxidation and subsequent debenzylation provided α-keto hemiketal **101**. This intermediate could be transformed into full ketal **102** under Mitsunobu conditions, finally paving the way to racemic heliquinomycinone (**103**) (Scheme 26).[17b]

Regarding the major obstacles and synthetic detours outlined in this final paragraph, one must conclude that there is a remarkable lack of novel approaches to the rubromycins. Work recently published by Pettus meets this demand. As depicted in Scheme 27, simple model 5,6-spiroketal **48** could be elaborated all the way to fully mature naphthoquinone derivative **106** by very elegant oxidation and cycloaddition processes.[18] However, it has still to be demonstrated that this strategy is successful with compounds bearing isocoumarin moieties.



Scheme 27. Transformation of model compound **48** into spiroketal **106** (Pettus and co-workers).

5. Conclusions

The rubromycins represent an intriguing class of natural products which display diverse biological properties ranging from antimicrobial to enzyme inhibitory activities. They bear three potent pharmacophore moieties, namely the naphthoquinone, the spiroketal and the isocoumarin units. First isolated in the 1950s, neither structural elucidation nor mode of activity are fully resolved with the exception of heliquinomycin. For this member of the rubromycin family not only has the absolute configuration been determined, but several studies have demonstrated the DNA helicase inhibitory effect of heliquinomycin. Rubromycin-type compounds as well as their derivatives are potential drug candidates for certain diseases and cancer therapy. Although numerous attempts have been made towards the construction of building blocks and model compounds, no total syntheses of these natural products have been achieved so far. Only the racemic aglycon of heliquinomycin has successfully been prepared in a fairly long sequence by the Danishefsky group. This lack of synthetic progress is certainly due to the complex substitution pattern of these compounds and the reactivity of the final stage intermediates, which makes handling increasingly difficult. Taking these facts into account an elegant and modular strategy for the construction of rubromycins and their derivatives is still a challenging task.

Acknowledgments

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[1] a) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 59–60; b) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 166–167; c) H. Brockmann, W. Lenk, G. Schwantje, A. Zeeck, *Tetrahedron Lett.* **1966**, *7*, 3525–3530.

[2] a) H. Brockmann, W. Lenk, G. Schwantje, A. Zeeck, *Chem. Ber.* **1969**, *102*, 126–151; b) H. Brockmann, A. Zeeck, *Chem. Ber.* **1970**, *103*, 1709–1726; c) V. Greul, Dissertation, Georg-August Universität Göttingen, **1971**; d) C. Puder, S. Loya, A. Hizi, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 729–735.

[3] a) C. Coronelli, H. Pagani, M. R. Bardone, G. C. Lancini, *J. Antibiot.* **1974**, *27*, 161–168; b) C. Coronelli, L. F. Zerilli, M. R. Bardone, E. Martinelli, *Tetrahedron* **1974**, *30*, 2747–2754.

[4] a) D. Tresselt, K. Eckardt, W. Ihn, *Tetrahedron* **1978**, *34*, 2693–2699; b) K. Eckardt, D. Tresselt, W. Ihn, *J. Antibiot.* **1978**, *31*, 970–973; c) R. M. Stroshane, J. A. Chan, E. A. Rubalcaba, A. L. Garretson, A. A. Aszalos, P. P. Roller, *J. Antibiot.* **1979**, *32*, 197–204.

[5] a) M. Chino, K. Nishikawa, M. Umekita, C. Hayashi, T. Yamazaki, T. Tsuchida, T. Sawa, M. Hamada, T. Takeuchi, *J. Antibiot.* **1996**, *49*, 752–757; b) M. Chino, K. Nishikawa, T. Tsuchida, R. Sawa, H. Nakamura, K. Nakamura, Y. Muraoka, D. Ikeda, H. Naganawa, T. Sawa, T. Takeuchi, *J. Antibiot.* **1997**, *50*, 143–146; c) M. Chino, K. Nishikawa, R. Sawa, M. Hamada, H. Naganawa, T. Sawa, T. Takeuchi, *J. Antibiot.* **1997**, *50*, 781–784; d) M. Chino, K. Nishikawa, A. Yamada, M. Ohsono, T. Sawa, F. Hanaoka, M. Ishizuka, T. Takeuchi, *J. Antibiot.* **1998**, *51*, 480–486.

[6] G. Bringmann, J. Kraus, U. Schmitt, C. Puder, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 2729–2734.
[7] A. Li, J. Piel, *Chem. Biol.* **2002**, *9*, 1017–1026.
[8] A. Trani, C. Dallanoce, G. Panzone, F. Ripamonti, B. P. Goldstein, R. Ciabatti, *J. Med. Chem.* **1997**, *40*, 967–971.
[9] a) M. E. Goldman, G. S. Salituro, J. A. Bowen, J. M. Williamson, D. L. Zink, W. A. Schleif, E. A. Emini, *Mol. Pharmacol.* **1990**, *38*, 20–25; b) T. Ueno, H. Takahashi, M. Oda, M. Mizunuma, A. Yokoyama, Y. Goto, Y. Mizushina, K. Sakaguchi, H. Hayashi, *Biochemistry* **2000**, *39*, 5995–6002; c) Y. Mizushina, T. Uena, M. Oda, T. Yamaguchi, M. Saneyoshi, K. Sakaguchi, *Biochim. Biophys. Acta* **2000**, *1523*, 172–181.
[10] a) D. Röleke, H. Hoier, C. Bartsch, P. Umbach, E. Scherzinger, R. Lurz, W. Saenger, *Acta Crystallogr., Sect. D* **1997**, *53*, 213–216; b) H. Xu, J. Frank, T. Niedenzu, W. Saenger, *Biochemistry* **2000**, *39*, 12225–12233; c) T. Niedenzu, D. Röleke, G. Bains, E. Scherzinger, W. Saenger, *J. Mol. Biol.* **2001**, *306*, 479–487; d) H. Xu, G. Ziegelin, W. Schröder, J. Frank, S. Ayora, J. C. Alonso, E. Lanka, W. Saenger, *Nucleic Acids Res.* **2001**, *29*, 5058–5066; e) H. Xu, J. Frank, U. Trier, S. Hammer, W. Schröder, J. Behlke, M. Schäfer-Korting, J. F. Holzwarth, W. Saenger, *Biochemistry* **2001**, *40*, 7211–7218; f) H. Xu, N. Sträter, W. Schröder, C. Böttcher, K. Ludwig, W. Saenger, *Acta Crystallogr., Sect. D* **2003**, *59*, 815–822; g) G. Ziegelin, T. Niedenzu, R. Lurz, W. Saenger, E. Lanka, *Nucleic Acids Res.* **2003**, *31*, 5917–5929.
[11] a) T. Capecchi, C. B. de Koning, J. P. Michael, *Tetrahedron Lett.* **1998**, *39*, 5429–5432; b) T. Capecchi, C. B. de Koning, J. P. Michael, *J. Chem. Soc., Perkin Trans. 1* **2000**, 2681–2688.
[12] a) K. Y. Tsang, M. A. Brimble, J. B. Bremner, *Org. Lett.* **2003**, *5*, 4425–4427; b) for the preparation of 6,6-spiroketals by similar means, see: M. A. Brimble, C. L. Flowers, M. Trzoss, K. Y. Tsang, *Tetrahedron* **2006**, *62*, 5883–5896.
[13] S. P. Waters, M. W. Fennie, M. C. Kozlowski, *Tetrahedron Lett.* **2006**, *47*, 5409–5413.
[14] For reviews, see: a) R. Zimmer, *Synthesis* **1993**, 165–178; b) R. Zimmer, H.-U. Reißig in *Modern Allene Chemistry* (Eds.: N. Krause, A. S. K. Hashmi), Wiley-VCH, Weinheim, **2004**, vol. 1, pp. 425–492.
[15] S. Sörgel, C. Azap, H.-U. Reißig, *Org. Lett.* **2006**, *8*, 4875–4878.
[16] S. Sörgel, Dissertation, Freie Universität Berlin, Germany, **2006**.
[17] a) D. Qin, R. X. Ren, T. Siu, C. Zheng, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4845–4849; *Angew. Chem. Int. Ed.* **2001**, *40*, 4709–4713; b) T. Siu, D. Qin, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4849–4852; *Angew. Chem. Int. Ed.* **2001**, *40*, 4713–4716.
[18] a) C. C. Lindsey, K. L. Wu, T. R. R. Pettus, *Org. Lett.* **2006**, *8*, 2365–2367; b) for the construction of 6,6-spiroketals by hetero-Diels–Alder reactions, see: G. Zhou, D. Zheng, S. Da, Z. Xie, Y. Li, *Tetrahedron Lett.* **2006**, *47*, 3349–3352.
[19] T. P. Thrash, T. D. Welton, V. Behar, *Tetrahedron Lett.* **2000**, *41*, 29–31.
[20] X. Xie, M. C. Kozlowski, *Org. Lett.* **2001**, *3*, 2661–2663.
[21] S. P. Waters, M. W. Fennie, M. C. Kozlowski, *Org. Lett.* **2006**, *8*, 3243–3246.
[22] S. P. Waters, M. C. Kozlowski, *Tetrahedron Lett.* **2001**, *42*, 3567–3570.
[23] S. Sörgel, C. Azap, H.-U. Reißig, *Eur. J. Org. Chem.* **2006**, 4405–4418.
[24] a) M. Brasholz, H.-U. Reißig, *Synlett* **2004**, 2736–2738; b) M. Brasholz, X. Luan, H.-U. Reißig, *Synthesis* **2005**, 3571–3580.

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