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Review - Lichen-associated bacteria as a hot spot of chemodiversity: focus on uncialamycin, a promising compound for future medicinal applications

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Abstract

This review presents the state of knowledge on the medicinal potential of bacteria associated with lichens. In fact, besides the classical symbiotic partners (photobiont and mycobiont) forming the lichen thallus, associated bacteria have been recently described as a third partner. Various studies demonstrated the diversity of these communities with a predominance of Alphaproteobacteria. Bacterial groups more relevant for secondary metabolite synthesis have also been highlighted. This article summarizes studies reporting the abilities of these communities to produce metabolites with relevant bioactivities. The biotechnological interest of these bacteria for drug discovery is highlighted regarding the production of compounds with therapeutic potential. Special focus is given to the synthesis of the most promising compound, uncialamycin, a potent enediyne isolated from a *Streptomyces* sp. associated with *Cladonia uncialis*.

Key words: Lichens - Bacterial communities - Chemodiversity - Drug discovery - Bioactive compounds - Uncialamycin

Introduction

Despite significant treatment advances, diseases such as cancer and microbial infections remain major public health issues due to the emergence of antibiotic resistance and the weak efficiency of current anticancer therapies. Therefore, biotechnological advances and the search of more specific and effective drugs are important challenges for pharmaceutical companies worldwide. The recent discovery of teixobactin from uncultivated soil bacteria highlights the unexplored microbial sources as

treasure chests for the access to new interesting bioactive drugs [1]. Among underexplored sources, lichens are unique and are classically described as a symbiotic association between a photobiont (green alga and/or cyanobacterium) and a mycobiont. Most lichens are outstanding in their capacities to produce specific secondary metabolites that present biological activities e.g. antioxidant, cytotoxic, antimicrobial activities [2–8]. These organisms were also known to represent habitats for diverse lichenicolous fungi, with a high biosynthetic potential [9,10]. Recent molecular approaches have also demonstrated the high diversity of lichen-associated bacterial communities which comprises millions of bacterial cells per gram of lichen thallus [11–21]. These associated organisms form stable and specific, communities and thus represent as a whole a third partner of the lichens symbiosis [22]. They particularly colonize hydrophilic surfaces of lichens and are also incorporated within the extracellular fungal matrix [13]. These bacteria could either live as biofilm-like structure or as individual colonies [23]. These communities were characterized by culture-independent methods (e.g. pyrosequencing and FISH/CLSM) [12–15], [17,18,20,21], [24–26] and culture-dependent approaches [11,13], [27–34], [35,36]. While proteobacteria are frequently the predominant bacterial group in the holobiont, interesting members of Actinobacteria, Bacteroidetes or Acidobacteria were also present. In a recent review, we focused on the diversity of these communities, particularly in the point of view of the presence of biotechnologically interesting bacteria [37].

These species-specific diversity patterns of bacteria associated with lichens are influenced by various parameters such as extrinsic factors (e.g. exposure, substrate type and location) and intrinsic factors (e.g. lichen species, age and part of lichen thallus and chemical composition of lichen) [16–18], [23,26], [38–40] (**Fig 1**). Indeed some studies highlighted the prevalence of Acetobacteraceae (Alphaproteobacteria) associated with lichens, proteobacteria families known to harbour nitrogen-fixing bacteria [13,17,18,20,25,39]. Their presence may be also correlated to their ability to use carbon sources furnished by lichen mycobiont such as mannitol [20]. Moreover, it was reported that secondary prevalent bioactive metabolites from lichens such as usnic acid, a secondary metabolite abundant in some lichen species and with multiple biological activities (such as antibacterial, cytotoxic and antifungal) [3–6], only influenced the bacterial diversity but not its abundance. Accordingly bacterial communities associated with lichens producing usnic acid were more specific and more resistant to this metabolite [23,26]. All these results suggest a closer relationship between the different partners.

The functional implications of associated bacteria in the lichen symbiosis remain to this day largely unexplained and unexplored. Nonetheless, some non exclusive hypotheses have been proposed and/or reported. Globally these hypotheses indicate that the symbiotic microbial communities in lichens might play an important role in (1) nutrient supply, especially nitrogen, phosphorus and sulfur, (2) recycling nutrients, (3) resistance against biotic stress factor by the production of bioactive metabolites (e.g. uncialamycin, aminocoumarins, angucyclines, butenolides), (4) resistance against abiotic factors, (5) support of photosynthesis by provision of vitamin B12, (6) fungal and algal growth

support by provision of hormones, (7) detoxification of metabolites, and (8) degradation of older parts of the lichen thallus [17,19], [21]–[25], [41,42] (**Fig 1**). Even if bacteria or their metabolites could be present in low amount in the holobiont, they could possibly play interesting ecological or biological roles. Further approaches to determine their localization inside the lichen thallus, the level of the activity of the produced metabolites and the ability of the latter ones to dissipate into the thallus must be explored. A better understanding of the roles of small chemical compounds (called by Davies the "parvome" [43]) in cell-to-cell communication, and particularly those produced by microbial organisms, will permit to develop rational approaches for the discovery of new drugs. In this context lichen-associated bacteria could be interesting sources of bioactive natural molecules.

We will describe herein the isolation and the chemical characterization associated to biological properties of compounds isolated from the cultivable bacteria [27–32], [34,44]. We will focus more precisely on uncialamycin, a compound isolated from a *Streptomyces* strain. Due to its potent DNA-breakage activity, this molecule with a reactive enediyne unit constitutes a valuable lead for pharmaceutical industries. As a result, intensive efforts had been implemented for the synthesis of this complex structure.

Biosynthetic potential of lichen-associated bacteria

The study of culturable lichen-associated bacterial communities led to discovery of new bacterial species [27–32], [34,36,45,46]. Six new strains of Actinobacteria were thus isolated from unidentified lichens collected in Japan e.g. *Schumannella luteola* sp. [28], nov. *Leifsonia lichenia*. sp. nov [27], *Actinomycetospora iriomotensis* sp. nov. [36], *Nocardioides exalbidus* sp. nov. [31], one from an unidentified lichen *Actinoplanes* sp. (MA7066 (ATCC 55532) from Spain [46], and one strain *Frondihabitans cladoniiphilus* sp. nov. from *Cladonia arbuscula* [45]. Two new *Streptomyces* strains were isolated from lichens belonging to *Cladonia* genus (*Cladonia gracilis*: strain L-44 [29] and *C. uncialis*: *Streptomyces uncialis* [30,34,44]) and four other *Streptomyces* sp from lichens collected in Japan (strains RI104-LiC106 and RI104-LiB101) [32] or in Spain (*Streptomyces cyaneofuscatus* M-27 and *S. carnosus* M40) [47]. At least, fourteen novel culturable bacterial strains associated with lichens were thus identified and gave new opportunities to discover bioactive metabolites of interest. To date, the bacterial communities of only few lichens have been studied as source of original metabolites: *Cladonia gracilis* and *Cladonia uncialis* both collected in Canada, one unidentified lichen from Japan. Additionally, one recent study reports the presence of various interesting metabolites from *S. cyaneofuscatus* M-27 and *S. carnosus* M-40 isolated from unidentified lichens (e.g. antifungal compound maltophilin, antitumor daunomycin or cosmomycin from the first strain or antibiotic and anti-inflammatory lodophorine B from the second one) but with the caveat that these identifications were only by HPLC [47] and production must eventually be confirmed by further chemical characterization. Notably all reports concerning the production of interesting chemicals by lichen

associated bacteria deal with Actinobacteria, likely due to their known biotechnological potential, and even though it has been shown that Alphaproteobacteria are the predominant bacterial group on most lichens. Actinobacteria isolated from natural sources are widely recognized for their production of secondary metabolites with unusual structures and potent biological activities [48]. Among them, many known antimicrobials of major interest have been reported (e.g. erythromycin, streptomycin, and tetracycline). Moreover relatively few interesting compounds were reported from Proteobacteria (including Alphaproteobacteria) in comparison with Actinobacteria [49]. As lichens hosts a significant number of Actinobacteria [35,37], efforts have been made to isolate and study such bacteria from lichens, especially *Streptomyces*, as promising resource for new lead compounds in drug development [37,48]. This section provides the state of knowledge of chemical potential of lichen-associated bacteria and demonstrates the real chemodiversity that these organisms could offer for medicinal applications. Secondary metabolites like angucyclin, the butenolide derivative JBIR-89, coumabiocins, uncialamycin, unciaphenol and cladoniamides, have been isolated from *Streptomyces* inhabiting lichens. Even though the ecological role of these compounds is still unclear, they have demonstrated remarkable biological activities (e.g. anticancer and antibacterial). Considering the complexity to identify them and their wide spectrum of biological activities, several research groups have achieved their total syntheses. Focus will be made on total synthesis of uncialamycin and analogues, as this molecule presents interesting cytotoxic activity associated with a specific mechanism of action.

Isolation and bioactivity of metabolites produced by culturable bacteria

During their investigations, Motohashi and co-workers have focused their attention on the isolation and the chemical characterization of *Streptomyces* strains from a lichen collected at Risiri Island, Hokkaido prefecture, Japan [32]. By searching homology of their 16S rRNA gene sequence with other bacteria, they identified strains RI104-LiC106 and RI104-LiB101 as new species of the genus *Streptomyces*. Partition using ethyl acetate was performed on an acetone crude extract of the mycelium produced during the fermentation (180 r.p.m., 27°C, pH 7.2, 5 d). Chemical studies of the dried residues using reverse phase chromatography led to the discovery of a new 1,1-dichlorocyclopropane-containing angucycline (**1**) from the culture broth of RI104-LiC106, and a new butenolide, designated as JBIR-89 (**2**) from RI104-LiB101 (**Fig. 2**). The isolation of angucycline **1** constituted the first report of a tetraphene containing a dichlorocyclopropane ring [32].

Bioactivity studies on angucycline **1** showed antibacterial activity against on *Micrococcus luteus* (diameter of inhibition zone: 11 mm, c = 25 μg on 6 mm disk) and a lack of activity against *Candida albicans* and *Escherichia coli*. While angucycline **1** exhibited weak cytotoxic activity against HeLa (IC_{50}= 36 μM) and ACC-MESO-1 (IC_{50}=52 μM) cells, the butenolide derivative (**2**) was not cytotoxic against either of the two cell lines [32].

Another Streptomycetaceae strain, *Streptomyces* L-4-4, sharing 98% identity with the strain *Streptomyces caeruleus* based on 16S rRNA analysis, has been isolated from the surface of the lichen *Cladonia gracilis* from Mount Fromme at British Columbia using ISP4 (International Streptomyces Project 4) media plates supplemented with 50 µg/ml of cycloheximide and 20 µg/ml of nalidixic acid [29]. Studies of the butanol-soluble extract of fermented *Streptomyces* L-4-4 (250 r.p.m., 30°C, pH 7.0, 7d) highlighted an activity in hyphae-formation inhibition (HFI) assays of prokaryotic whole cells [50,51]. This analysis indicated the presence of protein kinase inhibitors in this extract. Further investigations of this extract led to the isolation of new aminocoumarins named coumabiocins A-F (**3-8**), along with two known compounds, novobiocin (**9**) and isonovobiocin (**10**). Coumabiocins A-E (**3-7**) contain three structural elements: a 3-amino-7-hydroxycoumarin core linked at the 3-amino group *via* a prenylated 4-hydroxybenzoic acid moiety and at the 7-position to an *L*-noviosyl sugar. Only coumabiocin F (**8**) lacks the sugar moiety (**Fig 3**).

The antimicrobial efficacy of novobiocin (**9**) had been confirmed in several clinical trials during the 1960s. After approval, it had been used as antibiotic for the treatment of human infections [52,53]. However, novobiocin is no longer used due to its toxicity. Significant inhibitory activities against *Streptomyces* 85E have been observed for coumabiocins A-E (**3-7**) (10-15 mm clear zone of inhibition at 20 µg/disk), while moderate activities were detected at lower concentrations (10 mm bald zone of inhibition at 2.5 µg/disk). Coumabiocin F (**8**), where the *L*-noviosyl sugar group is absent, was inactive, suggesting that the *L*-noviose group is essential for the activity in this class of molecules.

Studies of the bacterial communities of the lichen *Cladonia uncialis* (Pitt River, British Columbia) by J. Andersen and co-workers, revealed a *Streptomyces* isolate putatively belonging to a new species named "*S. uncialis*" [30]. Based on its 16S rRNA sequences, this strain is related (98.4%) to *Streptomyces rubrogriseus*. Laboratory cultures on ISP4 solid agar medium (30°C, 14-21 d) followed by extractions with EtOAc and purification steps of the crude extract by flash C_{18} reverse phase chromatography and reverse phase HPLC yielded uncialamycin (**11**), a new enediyne antibiotic. The structure of uncialamycin (**11**), similar to that of dynemicin A (**12**) isolated from *Micromonospora chersina* [54], combines a ten-membered enediyne with an anthraquinone substructure (**Fig. 4**).
Structural similarities between uncialamycin (**11**) and dynemicin A (**12**) strongly suggest that they share the same biosynthetic pathway. Uncialamycin (**11**) could result from a degradation of a dynemicin-like precursor in which the C-5, C-6, and C-30 skeletal carbon atoms have been truncated. This metabolite showed *in vitro* antibacterial activities against Gram-positive and Gram-negative human pathogens, including *Burkholderia cepacia* (MIC=0.001 µg/mL), a major cause of morbidity and mortality in patients with cystic fibrosis, methicillin-resistant *Staphylococcus aureus* (MIC=0.0000064 µg /mL), and *Escherichia coli* (MIC=0.002 µg/mL) [30]. Later, the synthesis of this compound and isomers confirmed the activities against *Burkholderia cepacia* (MIC=0.0004 µg/mL),

Staphylococcus aureus (MIC=0.0002 µg/mL), and allowed the detection of further bioactivities on *Staphylococcus epidermidis* (MIC=0.00009 µg/mL), *Streptococcus pneumoniae* (MIC=0.0004 µg/mL), and *Enterococcus faecalis* (MIC=0.002 µg/mL) [55].

Enediyne compounds are remarkable active natural products, which own a unique and versatile core [56]. They are potent antitumor agents and have been studied extensively for use in the form of targeted antibody complexes [57–59]. To date, all natural enediynes exhibit cytotoxic effects as they act on duplex DNA and cause single- and double-stranded breaks. It is the result of the action of benzenoid diradicals formed by Bergmann rearrangement of the antibiotic molecule within the minor groove of the target DNA [60–62] (**Fig. 5**).

Initial studies indicate that uncialamycin reacts with plasmid DNA leading to its degradation, and it is thus not surprising it presents potent activities against a broad panel of cancer cells, including Taxol-resistant ovarian cells (1A9/PTX10; $IC_{50}=6x10^{-11}$ M) and epothilone B-resistant cells (1A9/A8; $IC_{50}=9x10^{-12}$ M) [55]. Interestingly, an additional chemical study on this *Streptomyces* strain recently described the isolation of unciaphenol (**13**) by a supplementary purification using a LH-20 Sephadex column [44]. This compound was described as putative naturally occurring even if it corresponds to a Bergman cyclization product analogue of uncialamycin (**Fig 6**). This compound was devoid of cytotoxicity but exhibited an interesting *in vitro* anti-VIH activity against various strains resistant to antiretroviral compounds with a IC_{50} range of 6.5 to 14.1 µM [44].

Later, investigations of the EtOAc extracts of culture of *S. uncialis* have resulted in the isolation of new alkaloids named cladoniamides A-G (**16-22**) (**Fig. 7**). These metabolites are derived of rearrangement and degradation of indolocarbazole precursors. The indolocarbazole alkaloids are a family of natural products isolated from marine invertebrates and cultures of diverse microorganisms [63].

During bioassays, only cladoniamide G (**20**) demonstrated cytotoxic activities on human breast cancer MCF-7 cells *in vitro* at 10 µg/mL. Indolocarbazole alkaloid derivatives as cladoniamides are new aglycons which could be used to obtain new staurosporine/rebeccamycin (**21**/**22**) analogues (**Fig. 8**) by chemical and biotechnological approaches. Staurosporine and rebeccamycin have shown high potential for the development of anticancer drugs [64–69], as these two molecules are potent inhibitors of protein kinases and topoisomerase-1, respectively. Several analogues of staurosporine (**21**) and rebeccamycin (**22**) have entered clinical trials.

Besides the study of various *Streptomyces*, one patent from Singh and coauthors, reports the production of actinoplanic acids A and B (**Fig. 9**) from *Actinoplanes* sp. isolated from a Spanish lichen [46]. These 20-membered macrocyclic polycarboxylic acids exhibited potent and selective inhibition of farnesyl protein transferase with IC_{50} of 230 nM and 50 nM respectively [46,70,71] and thus

inhibited the farnesylation of the oncogene protein Ras, one interesting target for anticancer therapies [72].

After the overview of the different metabolites isolated from lichen-associated bacteria, it appeared that these bacteria represent a very promising source of structurally diverse bioactive compounds. Reports of secondary metabolites from lichen-associated bacteria mention the use of various culture conditions of the target bacteria depending on their intrinsic characteristics. Parameters like temperature, pH, media (solid or liquid), considerably impact the production of these metabolites. However, these culture conditions are very far from the *in situ* environment of the bacterial strains on the lichen, which are confronted to other biotic and abiotic factors. As a consequence, the metabolomic potential of these microorganisms are still poorly understood and thus, underexploited. Despite of these difficulties, the isolated metabolites belong to important classes of molecules (e.g. aminocoumarins, indolocarbazoles, alkaloids, enediynes). Uncialamycin is one of these examples as it presents a broad spectrum of antibacterial activities and interesting cytotoxic properties associated with an original mechanism of action. This atypical structure jointly with these impressive bioactivities has therefore generated interest for chemical synthesis.

Synthesis of uncialamycin

Considering the small production yields of uncialamycin by *S. uncialis* and its high biological potential, different research groups have attempted the total synthesis of this metabolite. In the remainder of this section we will discuss two major synthetic strategies.

The first strategy, developed by Nicolaou and co-workers is based on the use of the building blocks **25**-**27** and is centered around three key reactions for the formation of uncialamycin: i) an addition of an acetylide (**26**) to a pyridinium species followed by ii) an intramolecular addition of **26** to form the enediyne core and iii) a Hauser annulation with fragment **27** to form the anthraquinone moiety (**Fig. 10**) [73].

Fragment **25** was formed by starting with a two-step Friedländer quinoline synthesis [74]: condensation of 5-methoxyisatin (**28**) and methoxyenone (**29**) led to the ketocarboxylate **31** *via* intermediate **30**; then **31** was reduced *in situ* to furnish the tricyclic lactone **32** with 86% overall yield (**Fig. 11**). Thereafter, a deprotection/protection step of the phenol moiety yielded compound **33** with 50% yield. After reduction of the lactone moiety and protection of the obtained lactol, the quinoline system **25** was achieved with 86% overall yield for the two steps and approximately a 1:1 mixture of diastereoisomers.

The pyridine moiety of fragment **25** was activated by the formation of a pyridinium species, which was trapped by the acetylide derivative (**26**), generated from EtMgBr to afford the intermediate **34** with 92% yield (**Fig. 12**). After removal of the TES group, reduction of the lactol followed by a selective epoxidation and a monoacetylation yielded the hydroxyepoxide **35** (66% overall yield for the

three steps). After a protection/deprotection sequence leading to the formation of compound **36** (78% overall yield for two steps), this latter one was submitted to an oxidation step of the hydroxyl group (87% yield) and the deprotection of the phenol (87% yield) to obtain the aldehyde **37**. The cyclisation, *via* an intramolecular acetylide addition, afforded the desired 10-membered ring enediyne **38** (61% yield, 70% ee). Compound **38** was then oxidized to a semiquinone system (80% yield), and subsequent removal of the *N*-protective group furnished the iminoquinone **39** (74% yield based on 70% conversion). At last, Hauser annulation of **39** with nitrile **27** in the presence of LiHMDS (63% yield) and desilylation ($3HF \cdot Et_3N$, 92% yield) gave (26*S*)-uncialamycin (**11a**).

Comparison of NMR data of isolated uncialamycin and the (26*S*)-isomer showed differences, which proved that the natural compound was not the 26*S*-isomer. To confirm these observations, Nicolaou and co-workers synthesized the 26*R*-epimer **11b**. An oxidation/reduction sequence afforded total inversion of the configuration at the C26 position (compound **41**, 90% overall yield, 96% stereoselectivity; **Fig. 4**). The 26*R*-epimer (**41**) was then submitted to the previously described routine (**Fig. 13**) to yield (26*R*)-uncialamycin (**11b**). The comparison of the spectroscopic data of this compound with the isolated one confirmed the configuration of the natural uncialamycin as the 26*R*-epimer.

If this synthetic strategy provided an answer to the question of the relative configuration at C26, it did not solve the issue of the absolute configuration of the natural product. To obtain more stereochemistry information, Nicolaou *et al*. developed a catalytic asymmetric synthesis of uncialamycin (**11**) (**Fig. 14**) [55]. The prochiral quinoline carboxylic acid **42** was converted to its methyl ester **43** with 65% overall yield. Noyori reduction [75,76] of the methyl ketone moiety within **43** resulted in the formation of γ-lactone **32**, presumably *via* intermediate hydroxy ester **45**, in 95% yield and 93% ee. Conversion to compound **33**, necessary for the pursuit of the synthesis [73]**,** under acidic conditions led to difficulties in maintaining the configurational integrity of the generated asymmetric center. To reach compound **33**, another route has been envisaged starting from compound **42**. Methyl ether carboxylic acid **42** was converted to DMB ether **46** (55% overall yield). Noyori reduction of **46** under the same conditions as those described previously for **43** furnished lactone **33** *via* intermediate **47**, in 95% yield and 98% ee. The intermediate **33** was then used to obtain (+)-uncialamycin (**11**, natural) and (+)-26-epi-uncialamycin as described for the synthesis of the racemic forms of these compounds [73].

One year after the realisation of the first asymmetric synthesis of uncialamycin by Nicolaou's group, a second research group focused their interest on the synthesis of this metabolite. While Nicolaou and its team introduced the asymmetry in their molecule almost at the end of their total synthesis, van de Weghe and co-workers based their approach on the preparation of a chiral quinoline by introducing the chirality from the second step of their synthetic strategy. They developed an intramolecular imino Diels-Alder reaction, based on the Povarov reaction, coupled to an oxidative aromatization that

allowed the formation of substituted quinolones [77]. They used these reaction conditions to prepare the chiral quinoline moiety as the chiral center C26 was fixed by an enantioselective reduction of an α-alkyne ketone **48** affording the alcohol **49** with 80% ee. The enantiomeric excess has been determined by ^{1}H NMR after derivatization into its corresponding *O*-methylmandelic ester **56**. The alkynol **49** was converted in acryloyl ester **51** and the diol **52** was isolated as a diastereomeric mixture after osmylation reaction with 55% yield. The diol **52** was converted into its glyoxylic acid derivative then transformed into the imine **53** (**Fig. 15**). After imino Diels-Alder cycloaddition the quinoline **54** was isolated in 65% yield overall from the diol **52** with 89% ee as determined by HPLC.

Later, van de Weghe's group developed a second approach for the total synthesis of uncialamycin [78]. **Fig. 16** shows the retrosynthetic analysis of the construction of the quinoline core. Fragment **58** was obtained from a Michael-aldolisation-crotonisation reaction cascade starting from compound **60** and **61**. The acetylide addition to the activated quinoline moiety followed by ring closure reaction led to the synthon **57**.

The synthetic procedure started by the formation of the aniline derivative **63** using the Sugasawa reaction conditions [79]. This *ortho*-acylation has been achieved by condensation of chloroacetonitrile with compound **62**, followed by a substantial HCl expulsion (**Fig. 17**). The aniline derivative **63** was submitted to a Michael addition with methyl vinyl ketone **61**, which led to the Michael adduct **64**. The aldolisation of compound **64** has been successfully realized in the presence of Cs_2CO_3 under an oxygen atmosphere. The hemiketal **65** led to the ketone **66** in 63% yield over two steps [80], which was reduced into the racemic alcohol **67**. At last, a protection step of the secondary alcohol of **67** afforded compound **68** as the quinoline key fragment. The quinoline core was activated using methyl chloroformate [81] and trapped by the addition of the acetylide derived from enediyne **69** to furnish **70** in good yield as a mixture of cis/trans isomers in an unchanged ratio 6:4 which were separated by flash chromatography. Each isomer was then submitted to a deprotection step followed by an epoxidation. Compound **70**-cis gave an unique isomer of the corresponding oxirane, while **70**-trans led to a mixture. Further experiments were performed using the epoxide from **70**-cis. After oxidation of the primary alcohol, the cyclization step was carried out and yielded a mixture of **72** and **73** in a ratio 7:3. After isolation NMR spectroscopic data of **72** showed consistent similitude with the quinoline/enediyne moiety of the uncialamycin **11**.

Conclusion

Lichens represent interesting microenvironments where a number of different organisms closely interact and with a rich bacterial diversity. Therefore the lichen holobiont have also a very large potential for natural product discovery. So far, the bacterial diversity has been studied by various culture–dependent and culture-independent molecular and microscopic approaches which highlighted in almost all of the studies the prevalence of Alphaproteobacteria, Firmicutes and Actinobacteria. Various abiotic and biotic factors affect this diversity, and as a consequence, structure and localization

of the bacterial microbiome may be correlated with chemical patterns of metabolites released by all symbionts in the lichen thallus. Even if many roles of these bacteria still need to be experimentally confirmed, it seems now clearer that they contribute to various functions such as protection against biotic and abiotic stresses, nutrient cycling, reallocation of resources, detoxification, and other functions. The lichen-associated culturable bacteria have become a promising source of interesting bioactive metabolites with most of them exhibiting potent activities. To date, uncialamycin constitutes one of the most exciting compounds as it presents a broad variety of biological activities associated with a unique and specific mechanism of action. Two main approaches in the design of this compound have been undertaken and highlight the structurally complex enediyne derivatives as challenging target for chemists. One of the strategies allowed the determination of the configuration of the natural compound. These major breakthroughs paved the way for the synthesis of analogues and new related enediynes which could become new pharmaceutical leads. Finally, these data confirm the real biotechnological interest of lichen-associated bacteria as untapped source of interesting metabolites with useful activity. The next challenges will be to explore the whole metabolome of these bacteria by activating silent biosynthetic pathways. One of the approaches could be based on co-culture studies.

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Conflict of interest

The authors declare that they have no conflict of interest

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Figures and Tables Legends

Fig. 1: Model of relationships between lichen symbiosis partners. The model includes relevant functions of the colonizing bacteria, which are derived from –omics analysis, as well as culture-dependent approaches (adapted from Grube et *al.*, 2015) [22].

Fig. 2 Structures of angucycline **1** and the butenolide JBIR-89 (**2**).

Fig. 3 Structures of coumabiocins A-F (**3-8**), novobiocin (**9**) and isonovobiocin (**10**).

Fig. 4 Structures of uncialamycin (**11**) and dynemicin A (**12**).

Fig. 5 Cycloaromatization of a (Z)-1,5-diyn-3-ene systeme.

Fig. 6 Structures of unciaphenol and its putative obtention from uncialamycin (**13**).

Fig. 7 Structures of cladoniamides A-G (**14-20**).

Fig. 8 Structures of staurosporine (**21**) and rebeccamycin (**22**).

Fig. 9 Structures of actinoplanic acids A (**23**) and B (**24**).

Fig. 10 Structures and retrosynthetic analysis of (26*S*)- and (26*R*)-uncialamycin (**11a**, **11b**). DMB: 3,4-dimethoxybenzyl, TES: triethylsilyl, TMS: Trimethylsilyl.

Fig. 11 Synthesis of quinoline **25.**

Fig. 12 Synthesis of (26*S*)-uncialamycin (**11a**). DDQ=2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMP=Dess-Martin periodinane; KHMDS= potassium hexamethyldisilazide, *m*CPBA=meta-chloroperoxybenzoic acid.

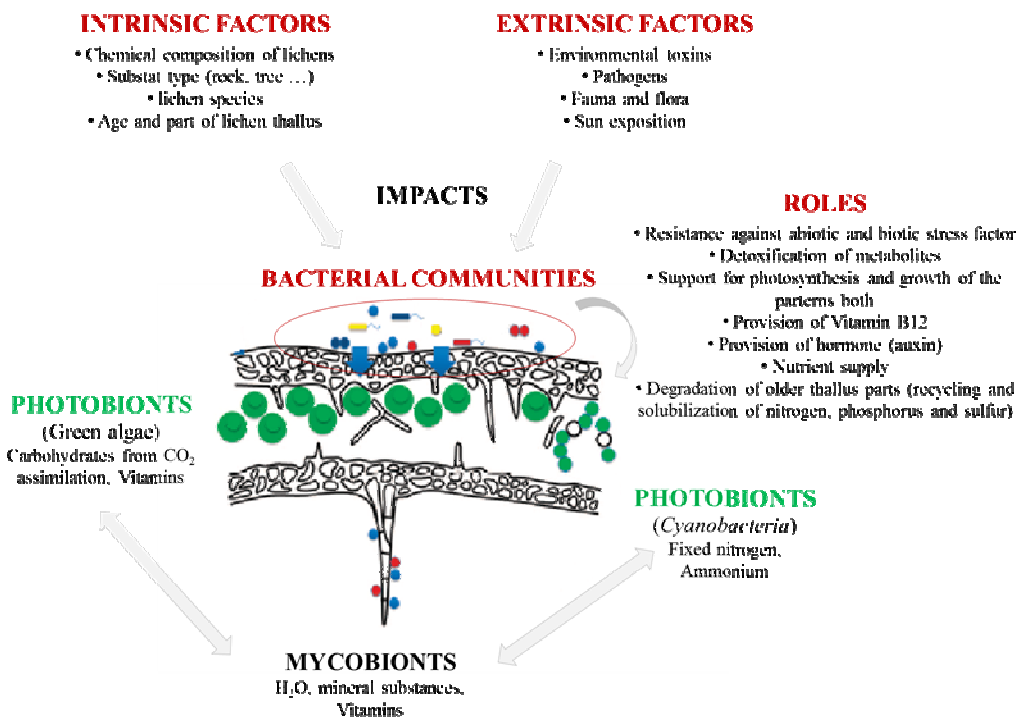
Fig. 13 Synthesis of (26*R*)-uncialamycin (**11b**).

Fig. 14 Catalytic asymmetric synthesis of chiral quinoline (**11**). DMAP=4-(*N*,*N*-dimethylamino)pyridine, DMB=3,4-dimethoxybenzyl, DMF=*N*,*N*-dimethylformamide, Ts=toluenesulfonyl.

Fig. 15 Preparation of the chiral quinoline by P. van de Weghe and colleagues.

Fig. 16 Retrosynthetic approach developed by P. van de Weghe and colleagues.

Fig. 17 Synthesis of the synthon **72**.



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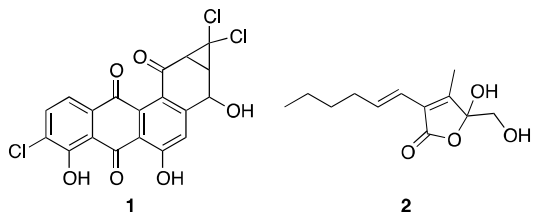

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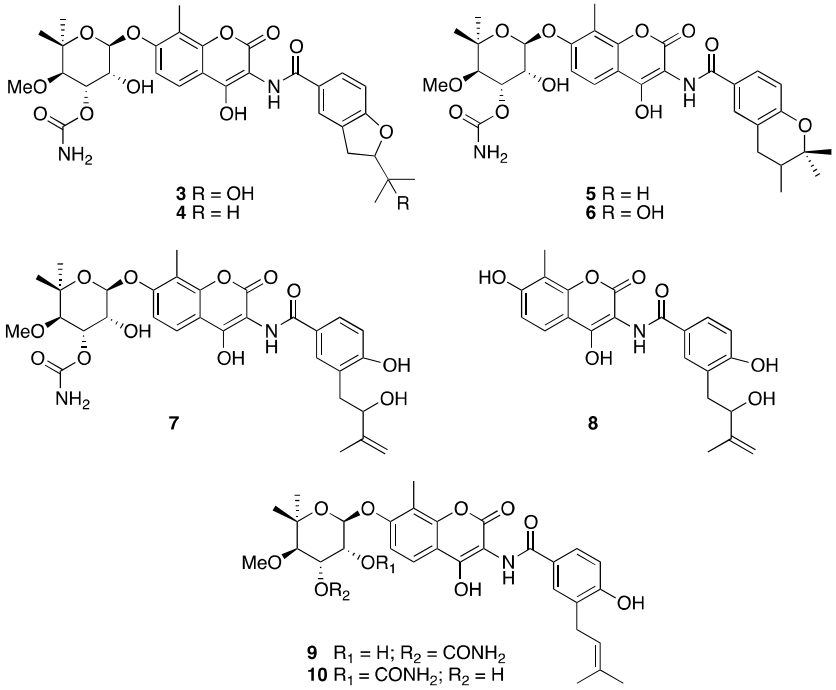

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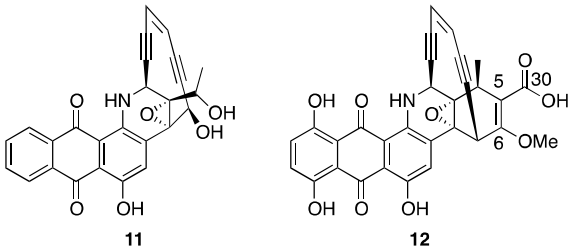

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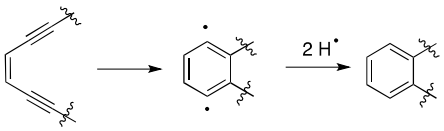

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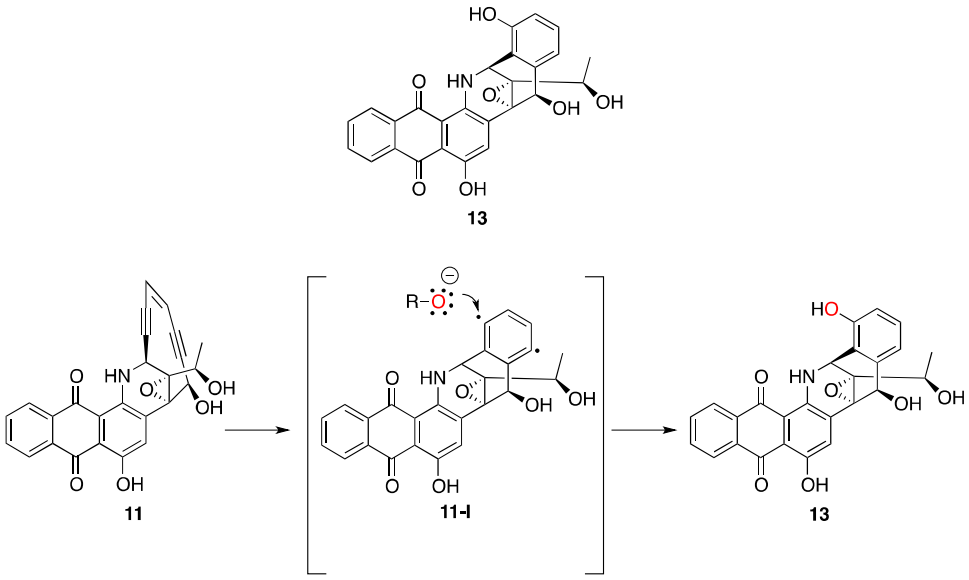

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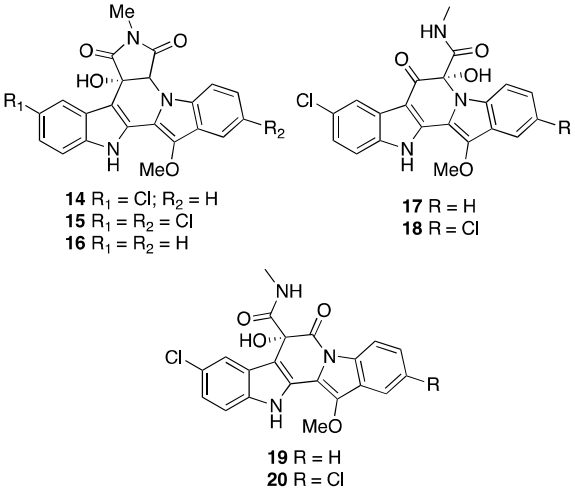

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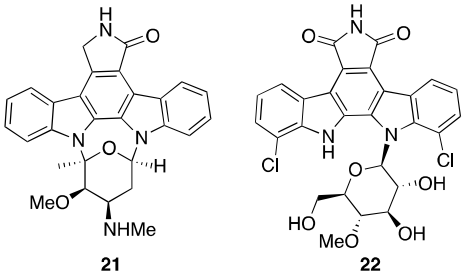

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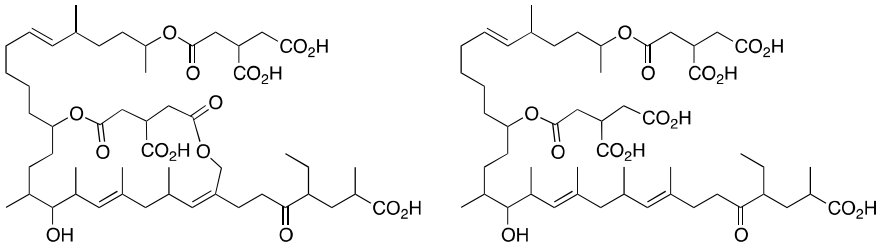
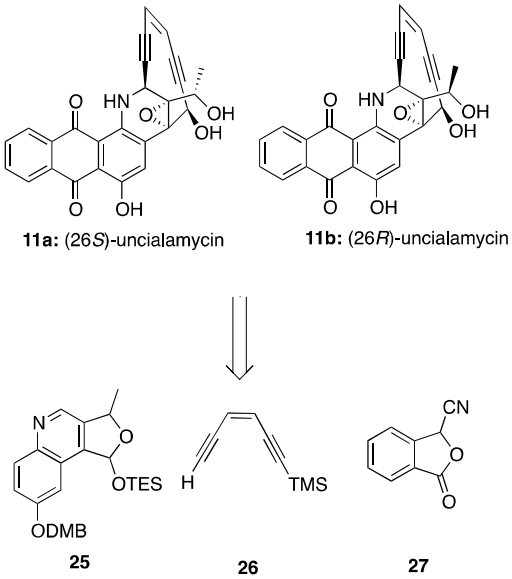


23: Actinoplanic acid A

24; Actinoplanic acid B

Figure 9

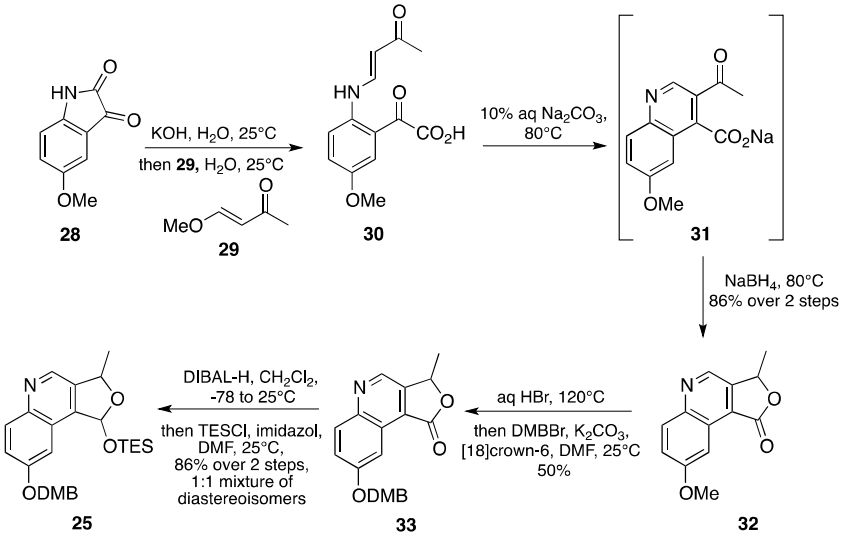


11a: (26*S*)-uncialamycin

11b: (26*R*)-uncialamycin

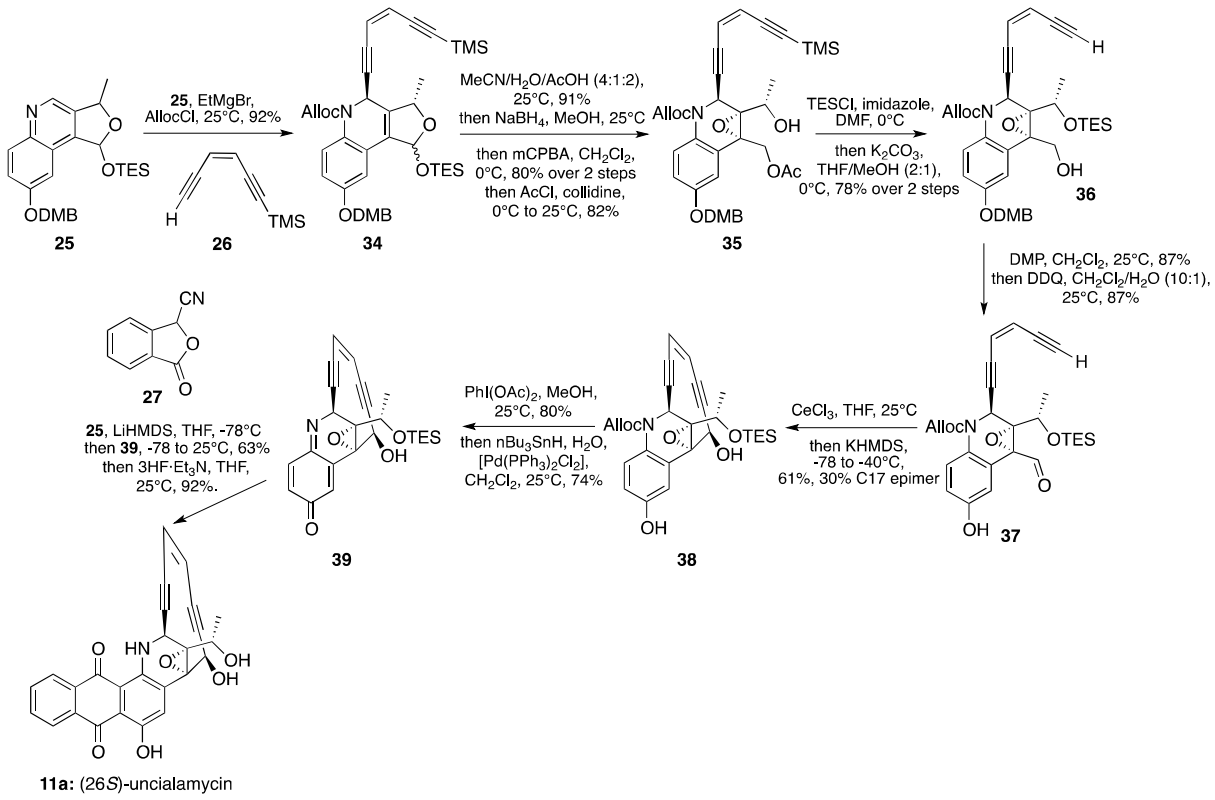
25 **26** **27**

Figure 10



28 **29** **30** **31** **32** **33** **25**

Figure 11



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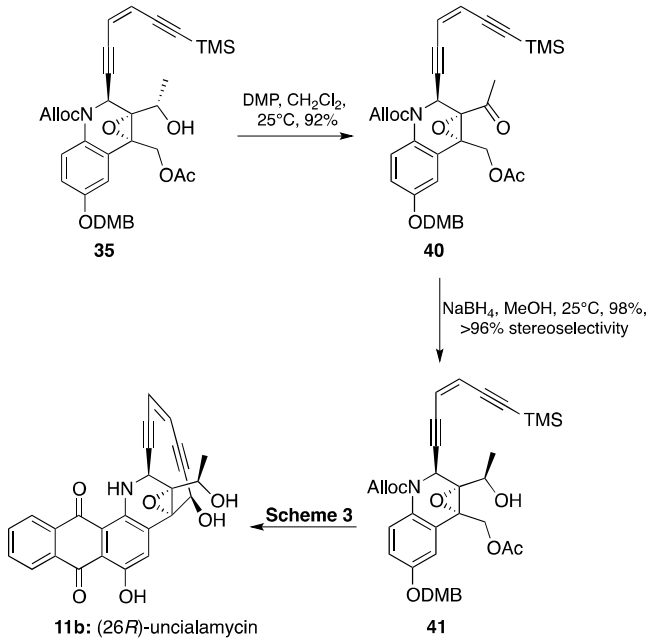

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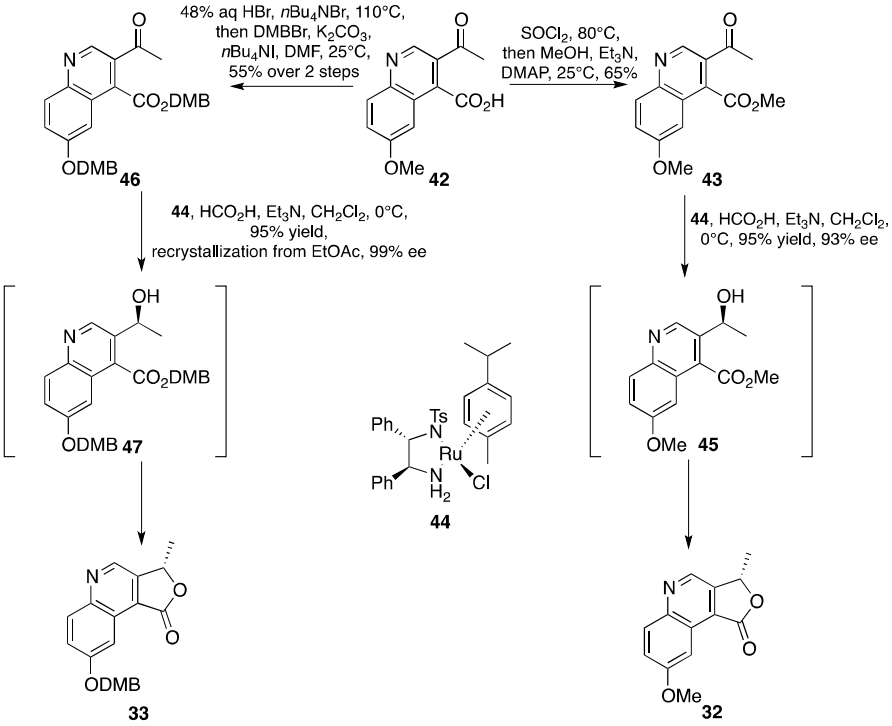

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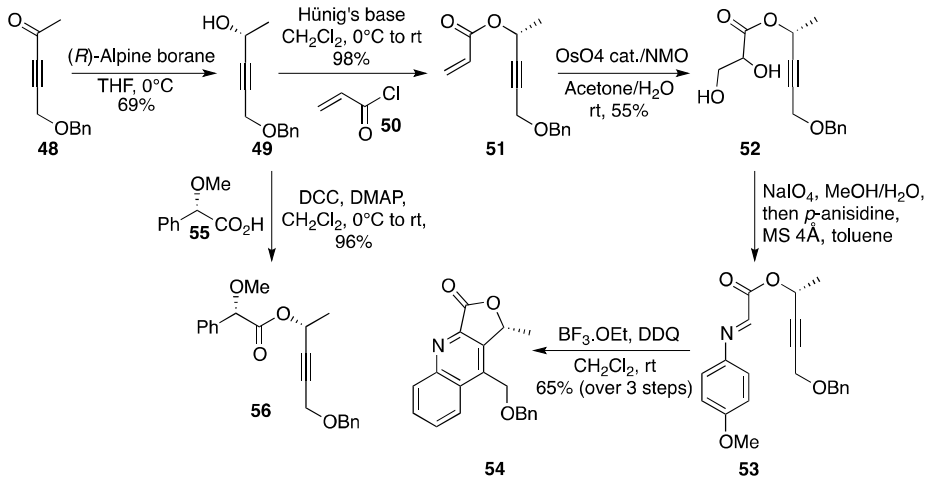

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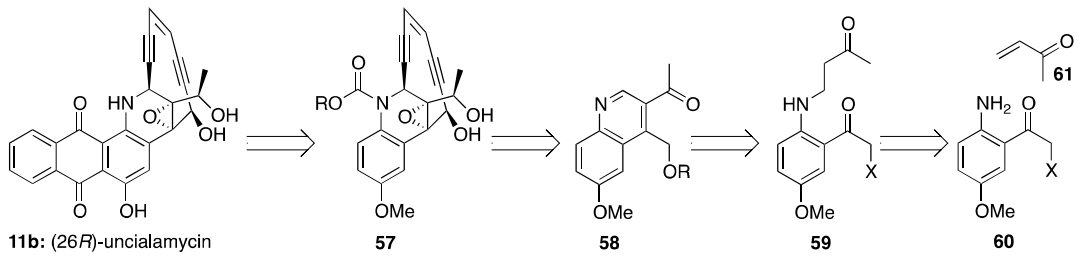

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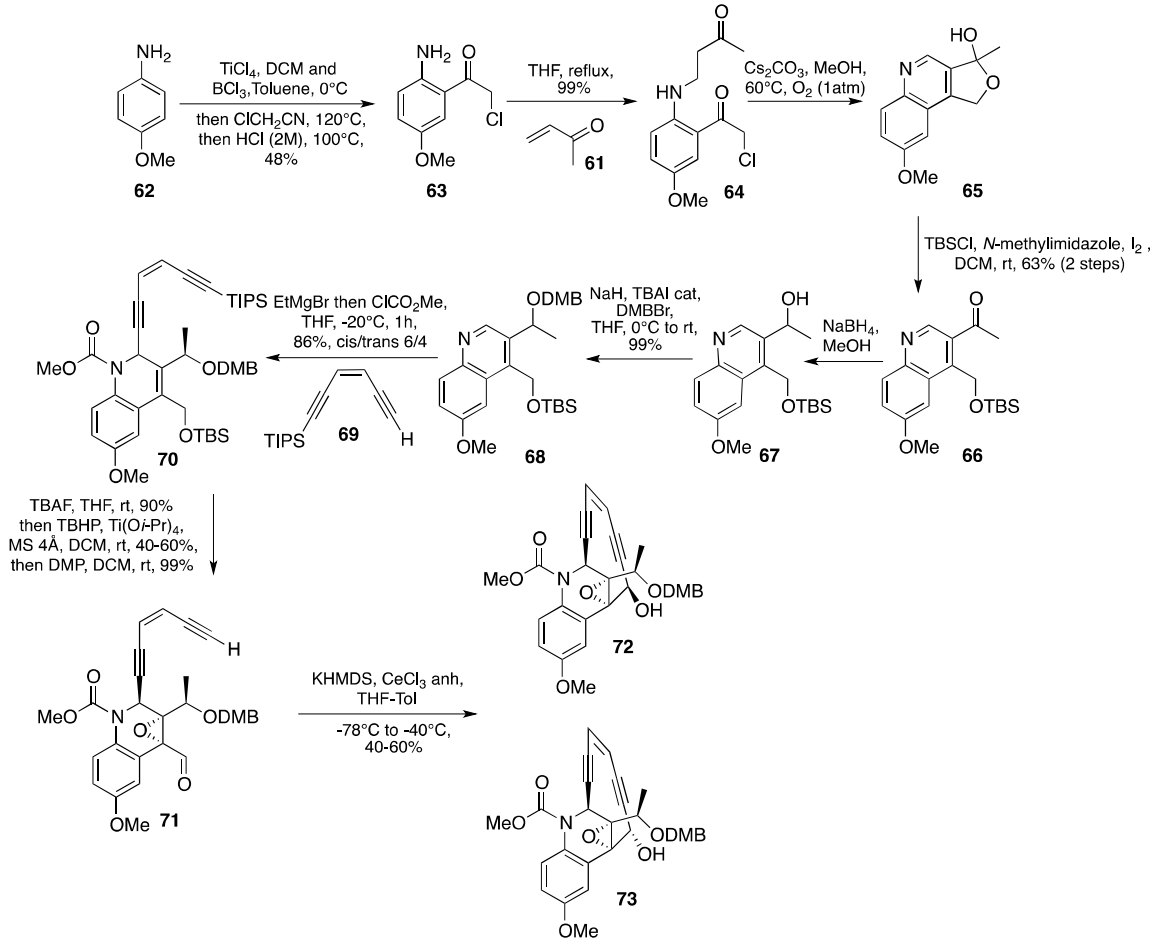
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62
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then HCl (2M), 100°C,
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Cl
OMe
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THF, reflux,
99%
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61
O
HN
O
Cl
OMe
64
Cs2CO3, MeOH,
60°C, O2 (1atm)
HO
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OMe
65
TBSCl, N-methylimidazole, I2 ,
DCM, rt, 63% (2 steps)
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OTBS
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NaBH4,
MeOH
OH
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OTBS
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67
NaH, TBAI cat,
DMBBr,
THF, 0°C to rt,
99%
ODMB
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OTBS
OMe
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EtMgBr then ClCO2Me,
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TBAF, THF, rt, 90%
then TBHP, Ti(Oi-Pr)4,
MS 4Å, DCM, rt, 40-60%,
then DMP, DCM, rt, 99%
H
O
MeO
N
O
ODMB
O
OMe
71
KHMDS, CeCl3 anh,
THF-Tol
-78°C to -40°C,
40-60%
O
MeO
N
O
ODMB
OH
OMe
72
O
MeO
N
O
ODMB
OH
OMe
73

Figure 17