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Total synthesis and biological evaluation of (−)-atrop–abyssomicin C†

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Enantioselective synthesis of a marine antibiotic (−)-atrop–abyssomicin C was accomplished in 21 steps, in 1.8% overall yield (4%, based on the recovered starting material). The key steps of the synthesis are the formation of the functionalized cyclohexane core by an organocatalyzed Tsuji–Trost reaction, the formation of a tricyclic spirotetronate unit by a gold-catalyzed reaction sequence and the highly efficient eleven-membered ring closure by a Nozaki–Hiyama–Kishi reaction. Biological tests showed all abyssomicin derivatives to possess strong antibacterial activity against methicillin resistant *S. aureus* strains; however, they also proved to be cytotoxic, both to malignant and to normal somatic cells.

Introduction

The discovery of antibiotics is considered as one of the greatest contributions to human welfare, but unfortunately, not an everlasting one, as their excessive use, coupled with the unparalleled ability of natural organisms to adapt to external threat, brought about new generations of pathogenic microorganisms, resistant even to the last line therapeutic agents.[1] Therefore, the search for new antibacterials remains an important aspect of medicinal chemistry. Natural products have traditionally been a rich source of biologically active compounds, with a vast diversity of molecular structures and modes of action.[2] In 2004, one such discovery was reported by Süssmuth *et al.*:[3] the rare actinomycete strain *Verrucosispora* AB-18-032, collected at a depth of 289 m in the Japanese sea, was found to produce a secondary metabolite named abyssomicin C, which immediately attracted the attention of the scientific community, for several reasons. This spirotetronate-polyketide,[4] structurally related to several topologically complex natural products,[5] contains four interwoven ring systems (including an eleven-membered one), and seven stereogenic centers (including a quaternary one). A salient feature of this molecule is its ability to inhibit the synthesis of tetrahydrofolate; as this coenzyme is synthesized in microorganisms, but not in humans, it offers a possibility for a selective antibacterial activity. Remarkably, abyssomicin C performs this by inhibiting the biosynthesis of *p*-aminobenzoic acid, which is a novel, unprecedented mechanism of action that distinguishes it from other tetrahydrofolate synthesis inhibitors (*i.e.* sulfonamides), and it is active against methicillin- and vancomycin-resistant *Staphylococcus aureus* strains.[6] In addition to abyssomicin C, several other congeners have been found (Fig. 1); while abyssomicins B,[3] D,[3] G and H,[7] originating from the aforementioned marine strain, are believed to be the products of post-synthetic transformations of abyssomicin C, abyssomicins E[8] and I,[9] isolated from terrestrial *Streptomyces* strains, are produced by a genuine polyketide biosynthetic pathway. Recently, *ent*-homoabyssomicins A and B have been reported,[10] as well as abyssomicins J, K and L;[11] the last three do justify the name of this family of compounds, as they were isolated from the marine *Verrucosispora* strain MS100128, collected at a depth of 2733 m. The combination of strong antibacterial activity and structural complexity made abyssomicin C an attractive synthetic target.[12] Two total syntheses have been reported,[13,14] as well as one formal synthesis[15] and several synthetic studies.[16] A common feature of all these retrosynthetic concepts is the recognition of the central cyclohexane core as a partial retron for the application of the Diels–Alder transform.[17] While in Sorensen's synthesis the crucial bond forming step was effected by an intramolecular

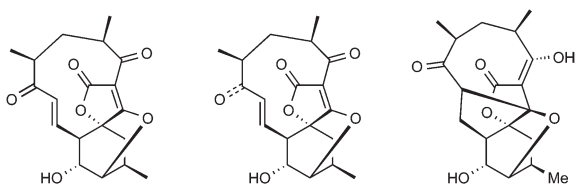

Fig. 1 Some members of the abyssomicin family.

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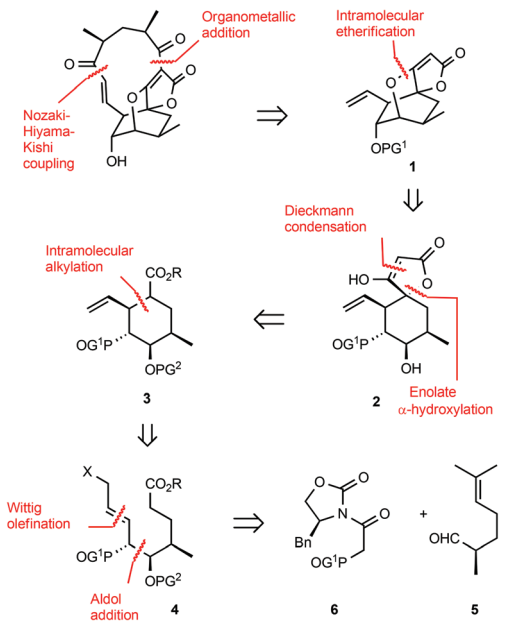
Diels–Alder reaction, in line with the presumed biosynthetic pathway, the stereoselective assembly of the bicyclic core in Nicolaou's synthesis was achieved through an intermolecular, template-controlled Diels–Alder reaction. The second pivotal step – the formation of the oxygen bridge – has been invariably performed by an intramolecular epoxide ring opening with a tetronate nucleophile, in a presumed biomimetic fashion. An interesting finding disclosed by Nicolaou is the fact that the restricted rotation around one of the bonds in the eleven-membered ring gives rise to atropisomerism.[14] Thus, the compound named atrop–abyssomicin C turned out to be the genuine secondary metabolite and the most active bactericide, while the initially described abyssomicin C was a conformational artefact. Recently, in a preliminary communication,[18] we reported an enantioselective synthesis of atrop–abyssomicin C; we now wish to provide the full account of this research, along with some observations on the chemical reactivity and biological activity of the intermediates and products.

Results and discussion

We set out to develop an enantioselective synthesis of atrop–abyssomicin C, which would rely on an alternative strategy, as compared to those previously described,[13,14] and which would be complementary to the Diels–Alder based approach, in terms of a flexible synthetic access to the analogues for structure–activity relationship (SAR) studies. The retrosynthetic blueprint is represented in Scheme 1. The initial disconnection of the eleven-membered ring by a combination of a Nozaki–Hiyama–Kishi transform and the organometallic addition reveals the key tricyclic intermediate **1**. The introduction of the oxygen bridge in **1** was considered as one of the key steps in the synthesis: we planned to create this structural subunit by the nucleophilic attack of a β-hydroxy group to the tetronate structural unit. This "casting", which confers on the reacting centres the opposite roles, as compared to the previous approaches,[13,14] was motivated by the need to develop a complementary synthetic method, which would be feasible for the syntheses of other polycyclic tetronate ethers, where the epoxide ring opening cannot provide a solution.

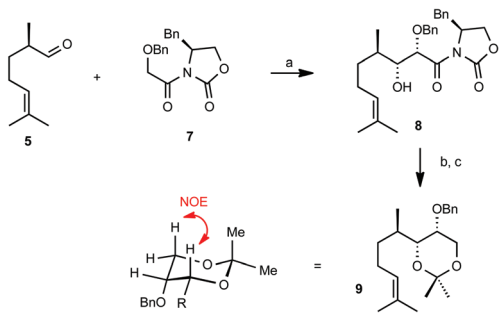
Due to the considerable rigidity of the bicyclic core in **2**, it was anticipated that this step may be difficult to achieve, and that some new chemistry would have to be developed, in order to solve this problem. Retrosynthetic simplification of the spirotetronate **2** relies on a Dieckmann condensation, followed by a stereoselective oxidation of the enolate corresponding to **3**, which is in turn obtainable by the cyclisation of the suitable precursor **4**. The installation of three contiguous stereocenters in this intermediate was conferred to the reliable Evans aldol methodology:[19] the absolute stereochemistry of the oxygen-bearing carbon atoms would be established in the reagent-controlled reaction, whereas the stereocenter bearing a methyl group would be imported from a chiral synthon – (*R*)-2,6-dimethylhept-5-enal **5** (also known as (−)-norcitronellal).



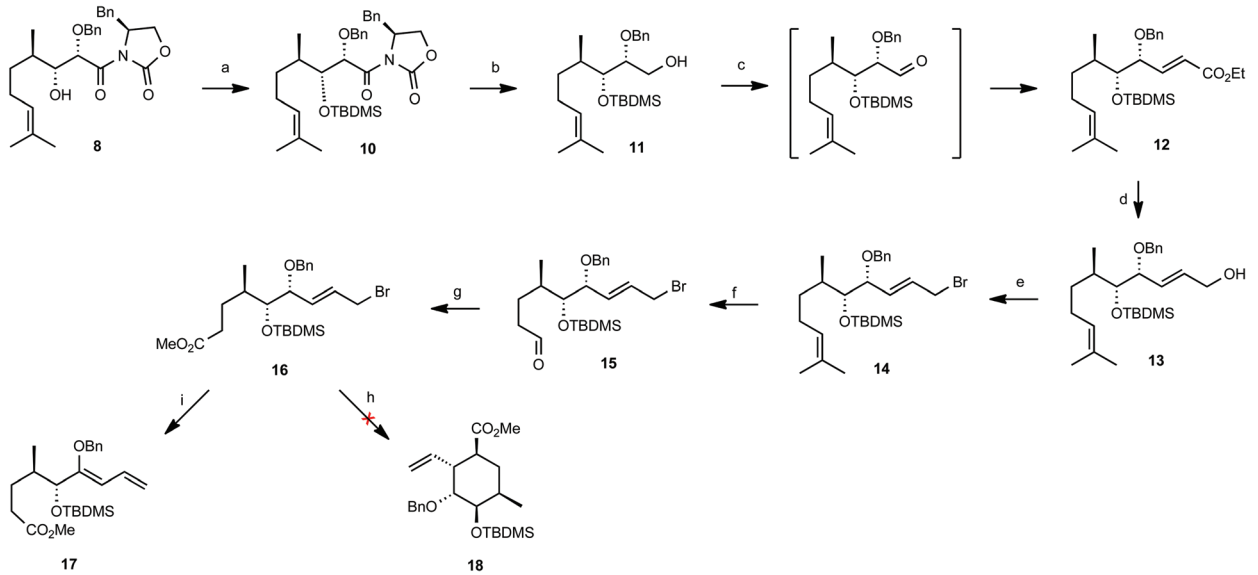
Scheme 1 Retrosynthetic analysis of abyssomicin C.

Stereoselective formation of the cyclohexane core

The synthesis commenced with a boron enolate-mediated diastereoselective aldol addition of α-benzyloxyacetyloxazolidinone **7**[20] to (*R*)-2,6-dimethylhept-5-enal **5**,[21] which furnished the expected adduct **8** in 77% yield (90% based on the recovered starting material (BRSM) **7**), as a single diastereoisomer. The stereochemical outcome of the reaction was unambiguously established by the NOESY analysis of the dioxane **9**, which was derived from the aldol product **8**, as represented in Scheme 2. The 6-*exo*-cyclisation leading to **18** was planned to be effected as an intramolecular allylation of an ester enolate. The aldol adduct **8** contains all three stereogenic centres and the proenolate functionality (in a latent form, as an isopropylidene moiety), but the nucleofugic part of the cyclisation precursor remained to be elaborated. To this end, the secondary hydroxy group was protected as the TBDMS-ether **10**,[22] and the oxazolidinone auxiliary was reductively removed with sodium borohydride (Scheme 3). The alcohol **11** thus obtained was



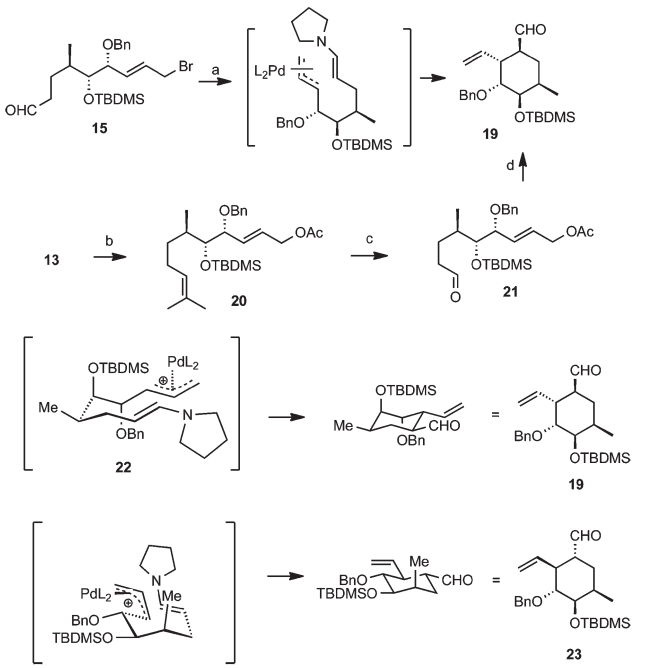
Scheme 2 Reagents and conditions: (a) nBu$_2$BOTf, Et$_3$N, CH$_2$Cl$_2$, −78 °C; then H$_2$O$_2$, MeOH, phosphate buffer, 77% (90% BRSM); (b) NaBH$_4$, THF, H$_2$O; (c) 2,2-dimethoxypropane, *p*TsOH (cat.), 64% from **8**.



Scheme 3 Reagents and conditions: (a) TBDMSOTf, *sym*-collidine, CH_2Cl_2, 0 °C, 99%; (b) $NaBH_4$, THF, H_2O, 86%; (c) DMP, CH_2Cl_2, rt, then $Ph_3P{=}CHCO_2Et$, 82%; (d) DIBAL, Et_2O, −40 °C, 85%; (e) CBr_4, Ph_3P, CH_2Cl_2, 0 °C, 95%; (f) *m*CPBA, CH_2Cl_2, rt; then: H_5IO_6, Et_2O, 100%; (g) Oxone, DMF; then CH_2N_2, 75%; (h) LDA, THF, −78 °C; (i) KHMDS, $Pd(PPh_3)_4$, THF, −78 °C to rt, 87%.

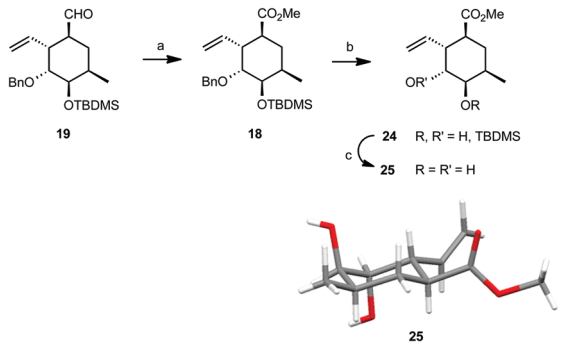
homologated into conjugated ester **12** *via* a two-step, one pot procedure involving Dess–Martin periodinane (DMP) oxidation/Wittig olefination.[23] To transform the conjugated ester into a leaving group, **12** was reduced with DIBAL, and the resulting alcohol **13** was treated with PPh_3/CBr_4 reagent, to afford allylic bromide **14** in excellent yield. Oxidative cleavage of the isopropylidene group in **14**, effected by a combination of regioselective epoxidation and the treatment of the resulting epoxide with the etheral periodic acid,[24] revealed aldehyde **15**. Oxidation of the aldehyde with Oxone®, followed by esterification, furnished cyclisation precursor **16**.[25] Disappointingly, cyclisation of an enolate derived from ester **16** turned out to be unfeasible, under a variety of reaction conditions, even with modifications of protecting groups in the molecule.[26] An attempt to increase the reactivity of the allylic part of the molecule by performing the reaction in the presence of tetrakis-(triphenylphosphine)palladium gave rise to the elimination product **17**; apparently, the ester enolate is a too hard nucleophile, which acts rather as a base.

We then turned our attention to aldehyde **15**, in the hope that the corresponding enamine intermediates would be softer, better nucleophiles, with respect to the ester enolate. However, pyrrolidine did not promote the cyclisation of aldehyde **15**, and proline catalyzed reaction afforded the cyclized product only in a low yield.[27] Therefore, a new cyclisation method was developed,[28] based on the concept of dual catalysis,[29] *i.e.* the combination of organotransition metal catalysis and organocatalysis.[30] Thus, when submitted to the simultaneous action of catalytic amounts of $Pd(PPh_3)_4$ and pyrrolidine, aldehyde **15** was smoothly converted into a cyclohexane carbaldehyde derivative **19** (83% from **15**; Scheme 4). The cyclisation could be effected *via* the corresponding allylic acetate **21** as well, but in inferior yield (43%).



Scheme 4 Reagents and conditions: (a) $Pd(PPh_3)_4$, pyrrolidine, THF, rt, 83%, dr = 5 : 1; (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 91%; (c) *m*CPBA, CH_2Cl_2, rt; then: H_5IO_6, Et_2O, 96%; (d) $Pd(PPh_3)_4$, pyrrolidine, DMSO, rt, 43%.

There were some ambiguities concerning the stereochemical outcome of the reaction. The product was obtained as a mixture of diastereoisomers in a 5 : 1 ratio,[31] and initially the configuration of the major isomer was wrongly assigned as **23**. To determine the stereochemistry of the product without any uncertainty, it was converted into crystalline diol **25**



Scheme 5 Reagents and conditions: (a) oxone, DMF; then CH_2N_2, 65%; (b) BBr_3, CH_2Cl_2, −78 °C, 85%, (c) HF, 91%.

(Scheme 5). Single crystal X-ray diffraction analysis of this compound confirmed that the absolute configuration of the cyclic aldehyde corresponds to **19**.[32] This stereochemical outcome indicates that the reaction proceeds *via* transition state **22**, in which two oxygen substituents occupy pseudoaxial positions (Scheme 4). The reasons for this preference are currently not clear, although dipole moment and steric hindrance of the protecting groups certainly play a role.

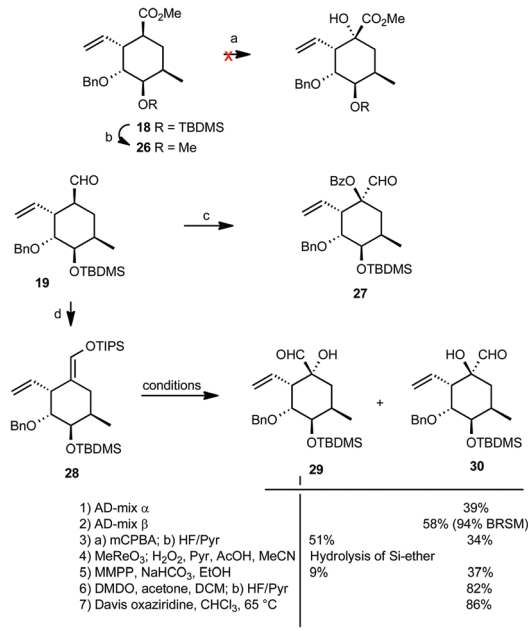
Formation of the tricyclic spirotetronate

The first step towards the elaboration of spirotetronate **1** was stereoselective α-hydroxylation of aldehyde **19**. The accomplishment of this task turned out to be surprisingly difficult. Some of the problems encountered are delineated in Scheme 6. Ester **18** could not be hydroxylated under standard conditions (KHMDS, THF, −78 °C, followed by O_2 and $P(OMe)_3$, either in the presence or absence of HMPA).[5f,33]

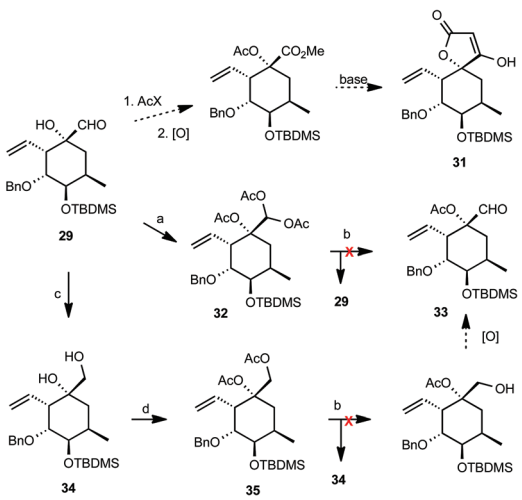
However, the reaction worked well with the model compound – methyl cyclohexanecarboxylate – indicating that the problem is in a hindered approach of a base to the axially positioned acidic hydrogen in **18**. Substituting Me for the TBDMS protecting group in **26** did not resolve the problem. α-Hydroxylation of aldehyde **19** with *N*-methyl-*O*-benzoylhydroxylamine afforded exclusively the undesired stereoisomer **27**.[34] As these attempts at direct hydroxylation of the ester and aldehyde derivatives failed, TIPS-enol ether **28** was prepared from **19** in quantitative yield, and submitted to several oxidation protocols, such as the Sharpless asymmetric dihydroxylation,[35] or reactions with modified Davis reagent,[36] dimethyldioxirane (DMDO),[37] methyl trioxorhenium(VII)[38] and peroxyacids.[39] While most of these reagents produced stereoselectively again the undesired stereoisomer **30**, *m*CPBA provided an unseparable diastereoisomeric mixture (with a predominance of the desired diastereoisomer in a 1.5 : 1 ratio) in a 85% combined yield.[40] Although this result was not satisfactory, it allowed us to investigate the reactivity of hydroxyaldehyde **29** and the possibility of its conversion to spirotetronate **31**.[41]

The tertiary hydroxy group in **29** could not be acetylated under a variety of conditions;[42] under forcing conditions (Ac_2O, $Sc(OTf)_3$)[43] triacetate **32** was obtained, and could not be selectively hydrolyzed into **33** (Scheme 7). An indirect approach to **33** involved the reduction of aldehyde **29** into diol **34**, followed by acetylation and selective hydrolysis; however, this last step could not be accomplished, as the hydrolysis of diacetate **35** provided only diol **34** (most probably due to the rapid migration of the tertiary acetate in the monoacetylated intermediate).

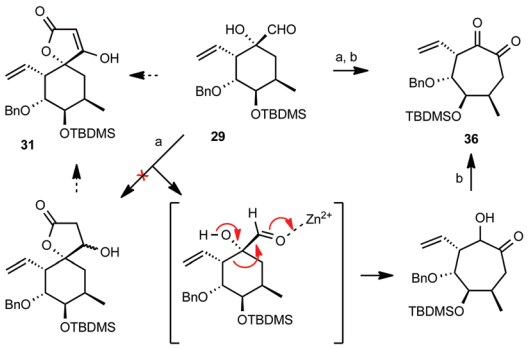
Based on a successful model-study with α-hydroxycyclohexane carbaldehyde, an attempt was made to proceed with the Reformatsky reaction on hydroxyaldehyde **29**; lactonization of the expected product, followed by oxidation, should give



Scheme 6 Reagents and conditions: (a) KHMDS, O_2, THF, −78 °C; then $P(OMe)_3$; (b) 1. HF, MeCN, Δ, 90%; 2. NaH, MeI, THF, 64%; (c) *N*-methyl-*O*-benzoylhydroxylamine hydrochloride, DMSO, 50 °C, 33%; (d) TIPSOTf, Et_3N, CH_2Cl_2, 50 °C, 99%. TIPSOTf = triisopropylsilyl triflate; MMPP = magnesium monoperphthalate.



Scheme 7 Reagents and conditions: (a) $Sc(OTf)_3$, Ac_2O, 0 °C, 76%; (b) K_2CO_3, MeOH, H_2O; (c) $NaBH_4$, THF, H_2O, 100%; (d) Ac_2O, Et_3N, DMAP, 70 °C, 50%.

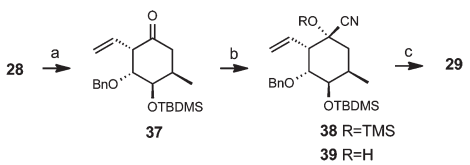


Scheme 8 Reagents and conditions: (a) $BrZnCH_2CO_2Et$; (b) DMP, CH_2Cl_2, 49% from **29**.

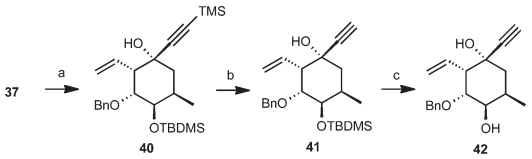
spirotetronate **31** (Scheme 8). However, the exposure of aldehyde **29** to a Reformatsky reagent and subsequent treatment of the initial product with DMP did not result in a two carbon atom chain elongation: instead, the product of α-ketol rearrangement – cycloheptadione derivative **36** – was obtained as a single product.[44]

These disappointing findings thwarted the approach to the spirotetronate *via* hydroxyaldehyde **29**. As all of the attempted procedures worked well on structurally simpler model systems, it was clear that the specific stereoelectronic profile of **19** and the related structures is responsible for the unfavorable reaction outcomes. Analysis of NMR spectra of **18** and **19**, and their comparison with the spectra of **25**, for which the X-ray structural analysis was obtained, revealed that these cyclohexane derivatives (be it the two hydroxy groups protected, or free) adopt chair-like conformations with the oxygen substituents in the axial positions.[45] Thus, these compounds have a pronounced stereochemical bias and exert substrate control in their reactions, which can hardly be overridden by the reagent. In addition, aldehyde **29** shows proclivity towards rearrangements and fragmentation, under the reaction conditions designed for its homologization. Evidently, a modification of the approach was necessary, and we next considered the attack of a carbon nucleophile (instead of oxygen electrophiles used in the preceding examples) on a cyclohexanone-type precursor.

To this end, silyl enol ether **28** was oxidatively fragmented into cyclohexanone derivative **37** using the aforementioned two step procedure (Scheme 9; 87% overall yield). Ketone **37** was then submitted to the action of trimethylsilyl cyanide/zinc iodide to give the corresponding cyanohydrin in good yield and with correct stereoselectivity.[46] The stereochemistry of



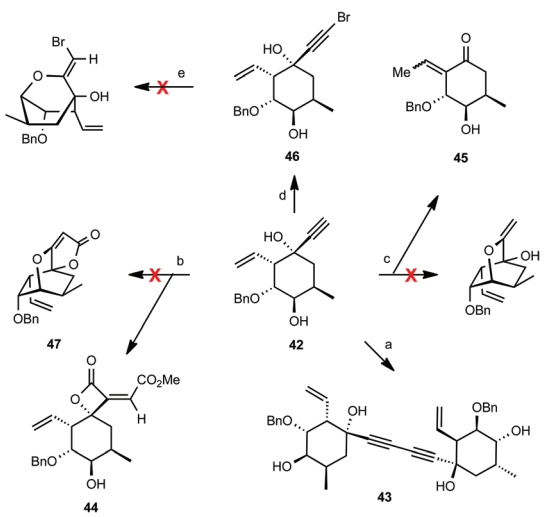
Scheme 9 Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, rt; then: H_5IO_6, Et_2O, 87%; (b) TMSCN, ZnI_2, CH_2Cl_2, rt, 90%; on purification on SiO_2 **38** was partially deprotected to give **39**; (c) DIBAL, CH_2Cl_2, −78 °C; then HCl, 50%.



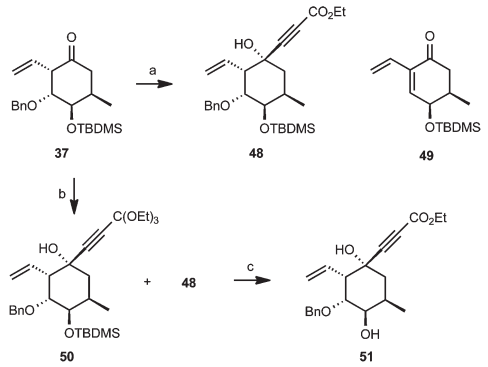
Scheme 10 Reagents and conditions: (a) TMS-acetylene, *n*-BuLi, THF, −78 °C, 83%; (b) K_2CO_3, MeOH, 88%; (c) HF, MeCN, 63%.

cyanohydrin **39** was confirmed by its conversion into aldehyde **29**. Unfortunately, cyanohydrin **39** could not be acetylated, nor did it undergo the Blaise reaction:[47] under both reaction conditions it underwent fragmentation into ketone **37**.

Therefore, we focused our attention on acetylide-based synthons. Addition of lithium trimethylsilylacetylide to ketone **37** gave propargylic alcohol **40** which, after consecutive deprotection of two silyl groups, gave rise to alkyne diol **42** – a precursor for the intramolecular alkyne etherification (Scheme 10). In principle, this type of reaction could be promoted by transition metals, which would allow us to intercept vinyl metal intermediates with carbon monoxide and effect a one-pot intramolecular etherification/alkoxycarbonylation reaction (*i.e.* transformation **42** → **47** in Scheme 11). An attempt to effect this reaction sequence with a palladium catalyst in THF, in the presence of $CuCl_2$ as a stoichiometric oxidant, resulted in Glaser coupling to give dimer **43**.[48] When the reaction was performed in methanol, a spiro β-lactone **44** was formed, by a mechanism which involves a combination of intra- and intermolecular alcoxycarbonylation;[49] apparently, carbonylation of tertiary alcohol was faster than the desired etherification. Treatment of diol **42** with silver carbonate in refluxing benzene resulted in the fragmentation of the alkyne moiety and the isomerization of the vinylic double bond, to give enone **45**.[50] Mercuric acetate, or perchlorate, was also ineffective. Diol **42**



Scheme 11 Reagents and conditions: (a) $Pd(OAc)_2$, TMTU, $CuCl_2$, NH_4OAc, propene oxide, CO, THF, 55 °C, 50%; (b) $Pd(MeCN)_2Cl_2$, CO, MeOH, Δ, 77%; (c) Ag_2CO_3, benzene, Δ, 39%; (d) NBS, $AgNO_3$, acetone, 85%; (e) NaOMe, MeOH, Δ.

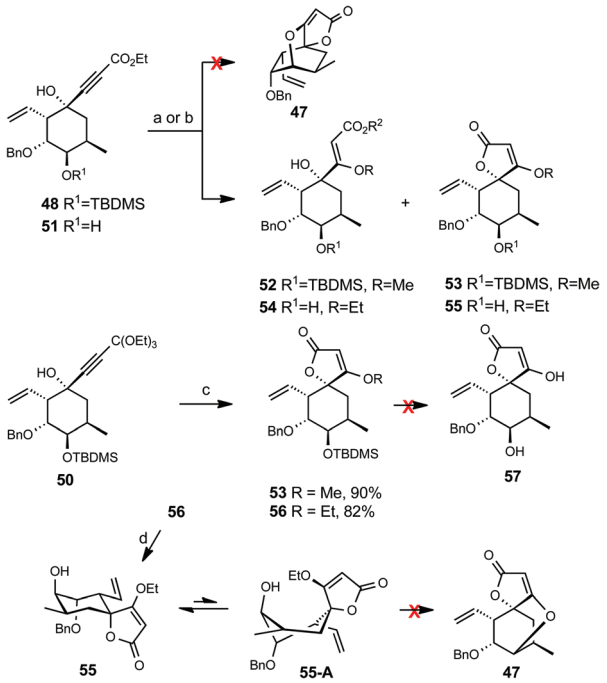


Scheme 12 Reagents and conditions: (a) $LiCCCO_2Et$, THF, −78 °C, 33% (89% BRSM); (b) $LiCCC(OEt)_3$, toluene, −78 °C to rt, 88%; (c) HF, MeCN, 60 °C, 91%.

was converted into bromoalkyne **46**; the latter, however, proved inert under the reaction conditions previously reported as suitable for the cyclisation of structurally related, but conformationally more flexible sugar derivatives.[51]

As the etherification/alkoxycarbonylation sequence proved unfeasible, we decided to introduce a three-carbon unit into a cyclohexane core, and to obtain a cyclisation precursor with an alkoxycarbonyl group already in place, activating the alkyne for etherification. Addition of the ethyl propiolate anion to ketone **37** under standard conditions provided the desired product **48** stereoselectively, but in low yield (Scheme 12). In the presence of HMPA, elimination of the benzyloxy group gave enone **49** (52%). Low yield in this reversible reaction was a consequence of the enolization of ketone **37**, as well as of the unfavorable equilibrium between the starting compounds and the product. Literature methods to circumvent this obstacle were not helpful.[52] We observed that the precursor/product ratio is more favorable at higher reaction temperatures, but we could not exploit this observation, as ethyl propiolate rapidly decomposes at temperatures above −78 °C (methyl propiolate at >−100 °C).[53] Therefore, a thermally more stable synthetic equivalent of a propiolate anion was needed. The lithium anion of ethyl orthopropiolate was such a reactive species, but its reaction with ketone **37** at 0 °C produced adduct **50** in only 36% yield, indicating that the reversibility of the reaction was still a problem. We surmised that the coordination of the metal ion (of the corresponding propargylic alkoxide) with the solvent (THF) increases the basicity of the alkoxide and favors the elimination; in this case, performing the reaction in a non-coordinating solvent should be beneficial. Indeed, when the reaction was performed in toluene, the desired adducts (**48** + **50**) were obtained in 88% yield.

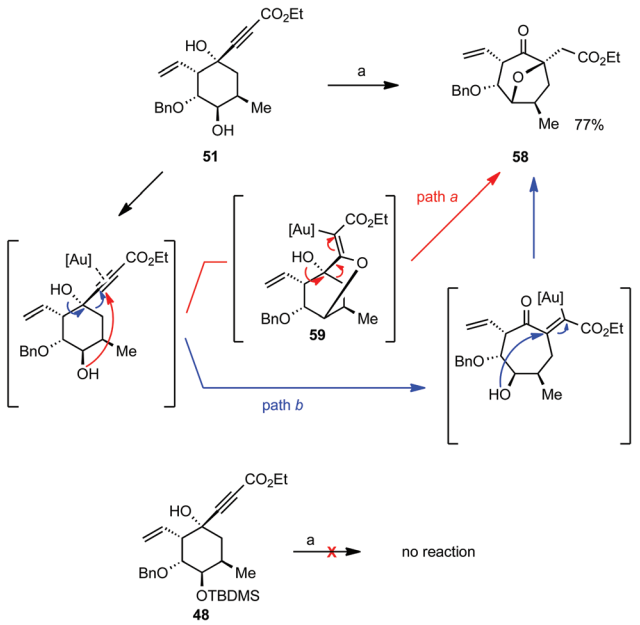
We first tried to convert propargylic diol **51** into tricyclic spirotetronate **47** by a hetero-Michael addition/lactonization sequence. Treatment of diol **51** with sodium methoxide induced only transesterification of the starting compound.[16b,54] Magnesium based reagents were more effective,[55] due to the enhanced coordinating ability of the Mg^{2+} cation, which activates propiolate fragment towards nucleophilic addition: both magnesium methoxide and bromomagnesium



Scheme 13 Reagents and conditions: (a) From **48**: $Mg(OMe)_2$, MeOH, 65%, **52** : **53** = 5 : 4, R = R^2 = Me; (b) from **51**: iPr_2NMgBr, THF, Δ, 65%, **54** : **55** = 4 : 3, R = R^2 = Et; (c) Nafion-Hg, ROH, H_2O; (d) HF, MeCN, 40 °C, 91%.

diisopropylamide promoted the formation of nearly equimolar mixture of spirotetronates **53** (**55**), and the hetero-Michael adducts **52** (**54**) (Scheme 13). The spirotetronate structures **53** and **56** could be accessed in high yields by treatment of propargylic alcohol **50** with mercury-modified Nafion resin;[56] however, **56** proved to be a dead end intermediate, as its conversion to tricyclic ether **47** proved impossible. Neither transetherification nor hydrolysis into tetronic acid **57**, which was expected to be a better cyclisation precursor than the tetronic ether, could be accomplished under a variety of conditions (BBr_3,[54] HCl,[54] HBr,[54] NaSPr/HMPA,[57] and PhSTMS/ZnI_2[58]).[59] In retrospect, the failure of **55** to cyclize should not be surprising, given that an extremely unfavorable, distorted conformation of the rigid spirobicycle is required in the transition state **55-A** for the intramolecular hetero-Michael addition (*i.e.* to allow the approach of the hydroxy group to the alkene acceptor at a Burgi–Dunitz angle of 105°). Therefore, an inversion of steps was required; *i.e.* intramolecular etherification should be performed on a conformationally more flexible alkyne derivative, prior to the spirotetronate formation.

For this purpose, we considered the application of gold catalysis – a rapidly emerging field where significant advances have been made, notably in the domain of activation of carbon–carbon multiple bonds towards nucleophilic addition.[60] Initial experiments with $AuCl_3$, either alone or in the presence of silver triflate, were unsuccessful, so we turned our attention to the highly active Au(I) catalyst described by Gagosz.[61] While no reaction occurred in boiling THF, heating a CH_2Cl_2 solution of alkyne diol **51** and a catalytic amount of Gagosz's catalyst, PPh_3AuNTf_2, at 120 °C (in a sealed tube)



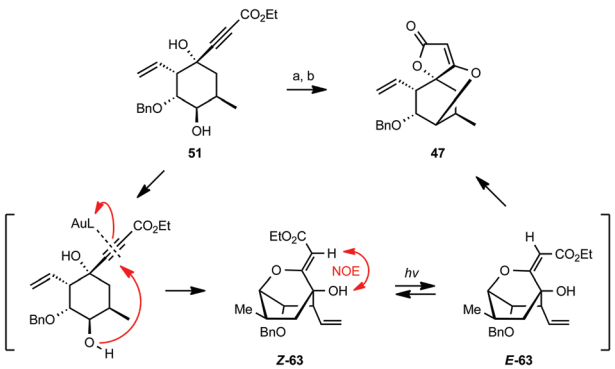
Scheme 14 Reagents and conditions: (a) $Ph_3PAuNTf_2$, CH_2Cl_2, 120 °C, 77%.

resulted in the formation of a bicyclic ketone **58** (Scheme 14). This was an encouraging result, as the presumed mechanism of formation of **58** might involve the initial formation of the desired bicyclic ether **59**, followed by its subsequent rearrangement into **58** (path a). A less favorable mechanistic possibility would involve Au-catalyzed rearrangement as the first step (path b). As the silylated derivative **48** did not react under the same conditions, we excluded path b and focused our efforts on finding the reaction conditions that would preserve structure **59** and secure a synthetically fruitful outcome of the reaction sequence. After a screening of solvents and reaction conditions, we found that the intramolecular etherification could be effected by heating a solution of **51** and a catalytic amount of PPh_3AuNTf_2 in methanol (Scheme 15). Yet, the yield of **Z-63** was low and the product was accompanied by several side products, arising from a nucleophilic attack of methanol to the reaction intermediates. Substituting sterically more demanding isopropanol for methanol substantially diminished side reactions and improved the yield of **Z-63** (60%). Interestingly, a catalytic cocktail that was described in the literature as more active – $[P(C_6F_6)_3AuCl]/AgSbF_6$ – proved unsuitable for this type of transformation, due to its thermal instability.[62]

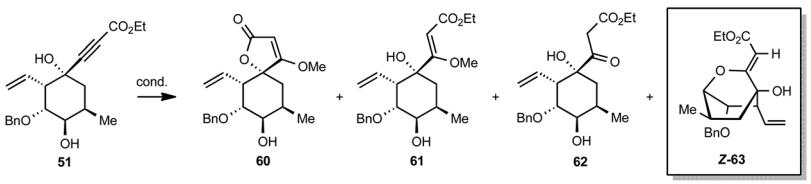
However, the stereospecific reaction produced exclusively the *Z*-isomer **Z-63**, while only the corresponding *E*-isomer could cyclize into spirotetronate **47**. Whereas the radical-based methods failed,[63] isomerization could be effected by irradiation with UV light in a quartz vessel (isomerization does not occur in Pyrex glassware), and the *E*-isomer could be cyclized into spirotetronate by the action of sodium hydride. After some experimentation we found that this three-step transformation could be accomplished more elegantly and much more efficiently as a one-pot sequence, which involved the initial heating of **51** in the presence of PPh_3AuNTf_2, followed by irradiation in the presence of a catalytic amount of sodium isopropoxide (Scheme 16): in this way, although the *Z*-isomer **Z-63** predominated in the photostationary state, it was funneled into spirotetronate **47**, thus shifting the *Z*/*E* equilibrium, obviating the need for the separation of isomers and recycling, and affording spirotetronate **47** in a 60% yield (over three steps).

Macrocyclisation and the completion of the synthesis

With spirotetronate **47** in hand, the stage was set for the attachment of the side chain and completion of the synthesis. We anticipated that the closure of the eleven-membered ring might be difficult; in line with this presumption, the work of Nicolaou has shown that the ring closing metathesis on related systems can be capricious, highly dependent on the substitution pattern.[14] Therefore our first choice was to effect this step by a Nozaki–Hiyama–Kishi reaction[64] – a powerful cyclisation method that has given good results in some



Scheme 16 Reagents and conditions: (a) PPh_3AuNTf_2, iPrOH, 70 °C; (b) *hv*, iPrONa, 60% from **51**.

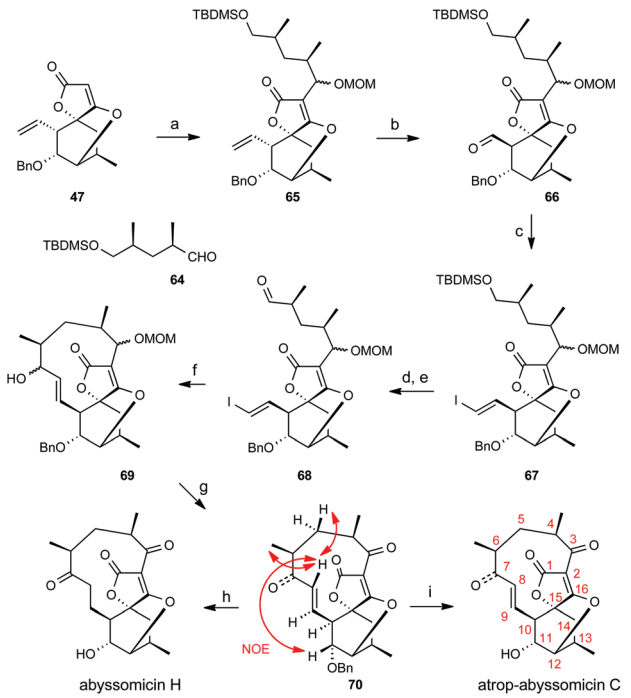


Scheme 15

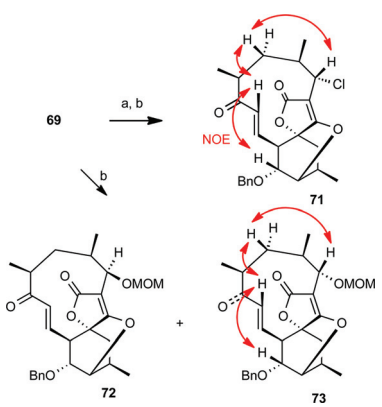
difficult cases.[65] The side-chain was attached in the reaction of lithiated **47**[14] with optically pure aldehyde **64**;[66] quenching the reaction with methoxymethyl bromide gave MOM-protected allylic alcohol **65** as a mixture of diastereoisomers (ratio of isomers = 1.8 : 1) (Scheme 17). The conversion of the vinyl group into a vinyl iodide was effected *via* the corresponding aldehyde, whose intermediacy was exploited to rectify the stereochemistry of the vinyl bearing stereocenter through base-catalyzed epimerization. Thus, a one-pot procedure, comprising ozonolysis, followed by the addition of a catalytic amount of DBU, furnished the required aldehyde **66** as a single epimer, in 81% yield (over two steps). After Takai olefination of **66**,[67] the deprotection of the primary hydroxy group in **67**, followed by oxidation with DMP,[68] furnished the precursor for macrocyclisation, **68**. The intramolecular Nozaki–Hiyama–Kishi reaction proceeded with remarkable efficiency, affording tetracyclic intermediate **69** in 90% yield (96% BRSM), as a mixture of four diastereoisomers, which was without consequence for the success of the synthesis, as the two stereocenters in question would be destroyed in the penultimate oxidation step. After trying several Bronsted and Lewis acids with limited success, the deprotection of the secondary alcohol was accomplished with methanolic hydrogen chloride. Without purification, upon treatment with freshly prepared DMP,[69] the crude diol was converted into diketone **70** (78% from **69**), whose NOESY spectrum showed correlations corresponding to the atrop–abyssomicin derivative. Attempts were made to remove the benzyl group in **70** by hydrogenolysis: however, in these experiments the reduction of the alkene preceded the cleavage of the benzyl group, which resulted in the formation of abyssomicin H. After some experimentation, the ultimate deprotection step was accomplished by treatment of **70** with boron tribromide at room temperature, which provided atrop–abyssomicin C identical to the natural compound in all respects. This total synthesis also constitutes the formal synthesis of abyssomicins D,[14] J, K and L.[11]

With the target compound in hand, we undertook an SAR study of abyssomicin analogues. Our first goal was to clarify the roles of the C-11 hydroxy group and of the C-3 carbonyl group for the antibacterial activity – two issues that have remained ambiguous so far. An earlier study has indicated the presence of C-11 oxygen substituent to be essential for the antibacterial activity.[70] Abyssomicin C-11 acetate was also strongly active, but it remained unclear whether its antibacterial activity is an inherent property of the acetate, or it acts as a prodrug.[14b] In another study on the mechanism of inhibition of 4-amino-4-deoxychorismate synthase PabB by atrop–abyssomicin, some importance was attributed to the propensity of atrop–abyssomicin C to undergo sequential reaction: thiol addition/cyclisation, with the formation of pentacyclic, abyssomicin D type product.[6b] Therefore, we wanted to investigate the antibiotic activity of derivatives which cannot undergo this sequential cyclisation reaction, or can only perform it by a different mechanism.

For this purpose, in addition to atrop–abyssomicin C (AA) and its benzyl ether **70**, three more derivatives were prepared from the late stage intermediate **69**, according to Scheme 18, namely chloro derivative **71** and two diastereoisomeric MOM-ethers – **72** and **73**. The first estimation of the antibacterial activity of these compounds was obtained by the agar plate diffusion assay, which has shown all compounds to be active against ATCC 25923, as well as against four other methicillin resistant *Staphylococcus aureus* strains (Table 1). Determination of MIC values against two MRSA strains showed all compounds to be active at a nanomolar level, benzyl ether **70** of atrop–abyssomicin C being the most active (Table 2). From these results several conclusions can be drawn: (1) for the



Scheme 17 Reagents and conditions: (a) *t*-BuLi, THF, −78 °C; then aldehyde **64**, −78 °C to −40 °C; then MOMBr, −40 °C to −25 °C, 44% (79% BRSM). (b) O_3, CH_2Cl_2, −78 °C; then Me_2S; then DBU (30 mol%), 81%. (c) $CrCl_2$, CHI_3, THF, rt to 50 °C, 71%. (d) HCl, MeOH, 94%. (e) DMP, CH_2Cl_2, rt, 88%). (f) $CrCl_2$, $NiCl_2$, DMF, rt to 45 °C, 90% (96% BRSM). (g) HCl_{aq}, MeOH, rt; then DMP, CH_2Cl_2, 78%. (h) H_2, Pd/C, EtOAc, 74%. (i) BBr_3, CH_2Cl_2, rt, 81%.



Scheme 18 Reagents and conditions: (a) HCl, MeOH; (b) DMP, CH_2Cl_2.

Table 1 Preliminary screening of antibiotic activity of selected compounds[a]

	Compound, 20 μg mL^{-1}				
Microorganism	AA	70	71	72	73
Staphylococcus aureus ATCC 25923	16	32	18	20	21
MRSA, 100b	17	27	18	21	23
MRSA, 2775	16	28	18	17	19
MRSA, 106	13	28	16	15	18
MRSA, 58	11	26	15	21	21

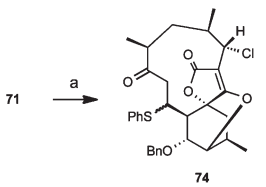
[a] Zones of inhibition (mm) determined by the agar plate diffusion assay (well diameter 8 mm), according to the literature procedure.[6a,71]

Table 2 MIC values of selected compounds against two types of MRSA[a]

	MIC against methicillin resistant *S. aureus* (MRSA), μg mL^{-1}	
Compound	MRSA, 100b	MRSA, 2775
AA	20	20
70	8	10
71	15	15
72	12	15
73	12	15

[a] Determined according to the literature procedure.[6a,72]

antibacterial activity, it is not necessary that the oxygen functionality at C-11 be present as a free hydroxy group (as in the natural product). The fact that benzyl ether **70** has superior activity, as compared to the free alcohol (*i.e.* atrop–abyssomicin C), indicates that the C-11 probably serves as a hydrogen bond acceptor, but also that hydrophobic interactions may play a role. (2) While the hetero-Michael addition is the necessary first step for the covalent binding of abyssomicin derivatives to PabB, the second addition (*i.e.* transannular cyclisation) is not necessary. This is confirmed by the fact that both MOM-diastereoisomers **72** and **73**, as well as chloride **71**, show only a minor decrease of activity, with respect to the parent compound. Neither **72** nor **73** is expected to cyclize, as the tetronate unit is only singly activated. Compound **71** allows for the cyclisation to occur, as the chlorine atom is properly oriented for an S_N2 substitution with allylic transposition; such a reaction would give rise to a 3-deoxy abyssomicin D type derivative. A model study on this compound, however, has shown that it does not undergo phenylthio anion induced cyclisation, but only a simple addition (Scheme 19). The fact that all three compounds retain antibacterial activity indicates



Scheme 19 Reagents and conditions: PhSH, Et_3N, THF, 48% (obtained as a 1 : 1 mixture of diastereoisomers separable by HPLC).

Table 3 Cytotoxicities of selected abyssomicin analogues on HeLa cells and peripheral blood mononuclear cells[a]

	HeLa		PBC	
Compound	IC_{50}	IC_{90}	IC_{50}	IC_{90}
AA	31.8	68.3	7.48	23.0
70	18.4	45.5	6.21	15.1
71	18.4	40.1	6.16	17.4
72	18.4	50.7	5.07	28.1
73	10.7	80.5	5.01	13.5

[a] Cytotoxicities determined by the MTT assay; concentrations are expressed in nM.[73]

that cyclisation is not essential for the activity and neither is the presence of the C-3 carbonyl group.

The presence of a reactive enone moiety in organic molecules can give rise to cytotoxicity. Therefore, abyssomicin derivatives were probed for cytotoxicity on HeLa cells. Indeed, this assay showed that all analogues investigated possess strong cytotoxic activity at a nanomolar level (Table 3). However, and unfortunately, they also proved to be even more cytotoxic on normal somatic cells, as shown by the test on peripheral blood mononuclear cells. Therefore, if the development of novel, abyssomicin based antibiotics is considered, the reduction of these unwanted effects should be a *sine qua non* for any therapeutic perspective.

Conclusions

Enantioselective total synthesis of (−)-atrop–abyssomicin C (which also constitutes formal syntheses of abyssomicins B, D, G, J, K and L, as well as the total synthesis of abyssomicin H) was accomplished in 21 steps, in 1.8% overall yield (4.0% based on the recovered starting compounds). The pivotal steps in the synthesis were stereoselective formation of the cyclohexane core **19**, formation of tricyclic spirotetronate intermediate **47** and the eleven-membered ring closure (*i.e.* formation of **69**). Much attention has been paid to the flexibility of the synthetic sequence, *i.e.* in providing several mechanistic alternatives for effecting the envisaged key structural transformations. The importance of this precautionary aspect of synthetic planning was fully confirmed during the execution of the synthesis: two of the aforementioned key transformations could not be accomplished according to the initial plan, and new protocols, namely cyclisation by dual catalysis (**15** → **19**) and gold catalyzed cyclisation cascade (**51** → **47**), were developed to overcome the encountered difficulties. The SAR study on abyssomicin derivatives showed that neither the free C-11 hydroxy group nor the C-3 carbonyl group is needed for antibiotic activity and that C-11 benzyl ether shows superior activity with respect to the natural product. However, all compounds tested also showed strong cytotoxic activity on both HeLa and normal somatic cells, which may prove to be a serious obstacle in further development of this class of antibiotics.

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