Natural Product Synthesis

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Total Synthesis of Enzyme Inhibitor Spirastrellolide A—Stereochemical Confirmation

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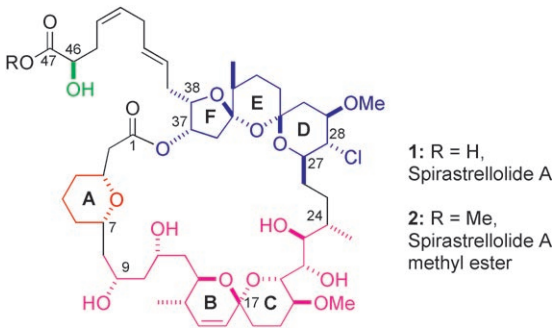
The search for novel compounds with activity against cancer cell-cycle progression in mitosis has led to the development of cell-based assays for the detection of mitotic arrest for the screening of natural product extracts. One such investigation by Andersen and co-workers in 2003[1] led to the isolation of spirastrellolide A (**1**) as its methyl ester **2** (Scheme 1) from the marine sponge *Spirastrella coccinea*. Spirastrellolide A (**1**) is a potent protein phosphatase 2A inhibitor ($IC_{50} = 1$ nM), but unlike other antimitotic sponge macrolides, such as spongistatin, it does not effect tubulin polymerization in vitro. Instead, it accelerates the entry of the cells into mitosis from other stages of the cell cycle, prior to bringing about mitotic arrest in a similar manner to the okadaic acid class of phosphatase inhibitors.[2]



Scheme 1. Revised structure of spirastrellolide A.

The structure initially proposed for spirastrellolide A (**1**) was lacking in stereochemical detail, but a revised structure was published in 2004.[2] From a structural perspective, spirastrellolide A (**1**) has a 47-carbon backbone containing some 21 stereocenters, of which all bar one are contained within a 38-membered macrocycle that contains three cyclic ether subunits (A, BC, and DEF rings). Ring A is a simple tetrahydropyran, while the BC ring system is a 6,6-spiroketal, and the DEF ring system comprises a unique chloro-substituted 5,6,6-trioxadispiroketal. It should be noted that in this revised structure the relative configuration within each of the three fragments C3–C7, C9–C24, and C27–C38 was determined in isolation, and the stereochemical relationship between them was not determined. The remote absolute configuration of the C46 stereocenter was unassigned and was still in question. These novel structural features, including the length of the polyketide chain, size of the macrolide ring, functionality of the side chain, and the unusual biological activity, set spirastrellolide A (**1**) apart from other antimitotic sponge macrolides.

From the perspective of a target-driven synthetic organic chemist, the small amount of spirastrellolide A which was isolated, together with its unusual biological activity and the potential for the development of novel therapeutic agents, makes this compound a very attractive target. The stereochemical uncertainty in spirastrellolide A (**1**) made the task of synthesis particularly difficult. The key to a successful approach was likely to be a flexible modular strategy in which each region of known relative configuration could be constructed independently. Subsequent union of these fragments and detailed comparisons of the NMR spectra with those of spirastrellolide would then hopefully enable the determination of the stereochemical interconnections between these regions. A strategy that allowed for the preparation of stereoisomers of the natural product could also provide material for biological testing that would give insight into the structure–activity relationship for the unusual bioactivity.

As soon as the structures of novel biologically active compounds such as spirastrellolide A (**1**) are published, synthetic chemists around the world begin the analysis of potential synthetic strategies and aim to be the first research group to complete the total synthesis. New synthetic methodologies provide novel means to prepare carbon–carbon bonds, but it is considered by many that only their application in the total synthesis of natural products is a true test of the effectiveness and worth of the new synthetic methodology, and this provides significant motivation for total synthesis programs.

For spirastrellolide A (**1**) the race to determine the complete absolute and relative configuration of the natural product through total and partial synthesis had started even before the revised structure was published in 2004. A number

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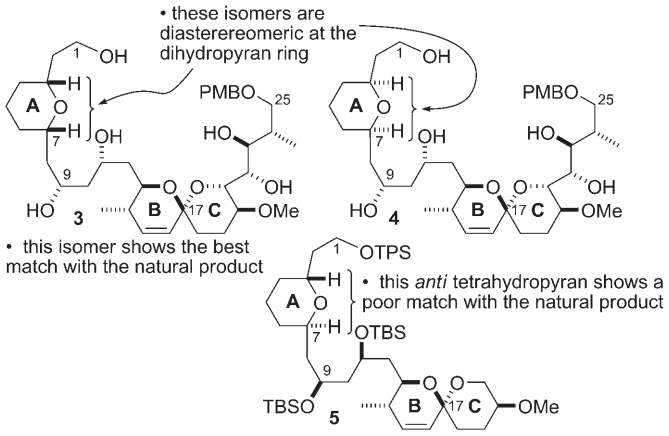
of research groups began the synthesis of fragments with the aim of comparing these to the natural product. Thus, when the revised structure was published in 2004 it was quickly followed by reports of the synthesis of a number of fragments. Most notably, the studies of Paterson et al.[3,4] in 2005 detailed the synthesis of a tetracyclic C26–C40 subunit containing the DEF spiroacetal and the construction of two C1–C25 diastereomers containing the tetrahydropyran A ring and the BC spiroketal (Scheme 2). While the results were not

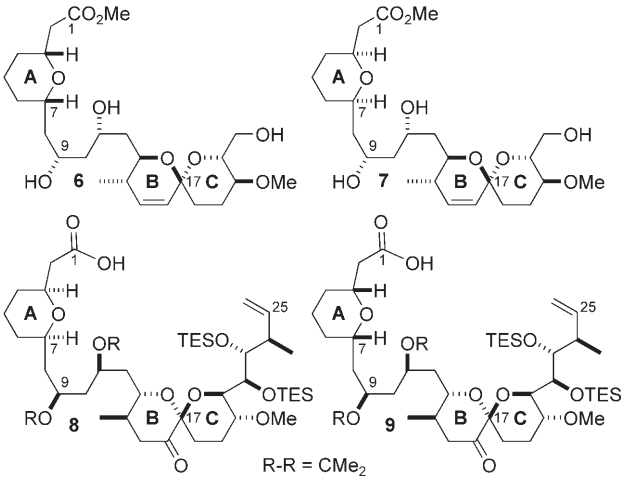


Scheme 2. Key fragments synthesized by Paterson et al.[3–5] in the structure determination of spirastrellolide A. PMB = *para*-methoxybenzyl, TBS = *tert*-butyldimethylsilyl, TPS = triphenylsilyl.

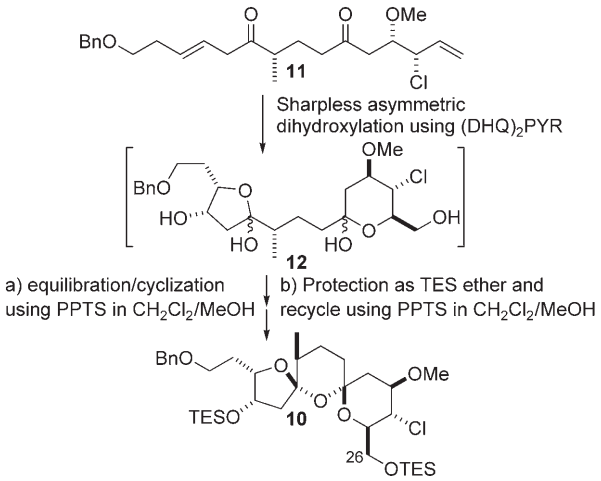
conclusive, the study found a better match for compound **3** than for the diastereomeric tetrahydropyran **4**. Both these compounds had a *syn* tetrahydropyran ring as indicated in the revised structure. The spectroscopic data of the 3,7-*anti*-substituted fragment **5** with the opposite configuration at C9 and C11 showed a poor correlation with that of the natural product.[5]

The syntheses of other fragments and model studies towards the spirastrellolides were reported.[6–9] In one such study, the spectra of the diastereomers **6** and **7** (Scheme 3) synthesized by Pan and De Brabander[8] were compared with the natural product. It was noted that the spectroscopic data for neither **6** nor **7** correlated to the natural product, but of the two **6** (which has analogous stereochemistry to the Paterson fragment **3**) showed a closer match. Diastereomers **8** and **9** from the enantiomeric series were prepared by Fürstner et al.,[7] but no conclusion as to which was the better match was postulated.

The chloro-substituted 5,6,6-trioxadispiroketal fragment **10** was also prepared by Paterson et al. in 2005,[3] and a good correlation between the spectra of this compound and that of the natural product was found. An improved synthesis was subsequently reported[10] in which diene **11** was dihydroxylated twice using the $(DHQ)_2PYR$ ligand to give a complex mixture of isomeric hemiacetals **12** that could not be purified (Scheme 4). This hemiacetal mixture was treated with PPTS in CH_2Cl_2/MeOH (1:1) to give a spiroketal with the desired configuration, as well as other isomers. This highly sensitive intermediate was purified after conversion into the bis(triethylsilyl) ether **10**. The other isomers were readily recycled



Scheme 3. Key fragments synthesized by Pan and De Brabander[8] as well as by Fürstner et al.[7] in the structure determination of spirastrellolide A. TES = triethylsilyl.

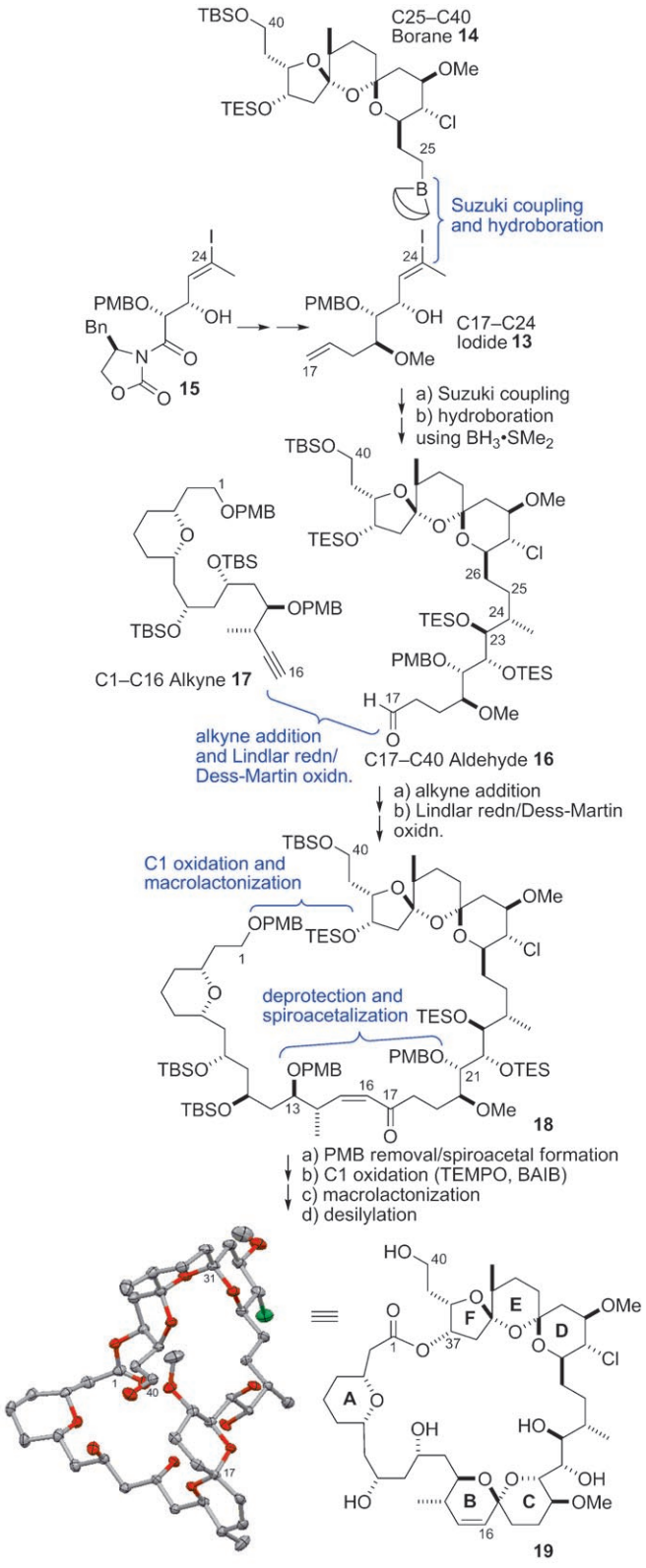


Scheme 4. Preparation of key fragment **10** using a double Sharpless asymmetric dihydroxylation. Bn = benzyl, $(DHQ)_2PYR$ = 1,4-bis(dihydroquininyl)pyridine, PPTS = pyridinium *p*-toluenesulfonate.

through desilylation and re-equilibration to the desired trioxadispiroketal using PPTS in CH_2Cl_2/MeOH (1:1).

A significant breakthrough in the structure determination of the spirastrellolides came when a crystalline derivative of a related natural product, spirastrellolide B, was published by Andersen and co-workers[11] in 2007, which revealed both the absolute and relative configuration of the macrocyclic core. Late in 2007,[12] the configuration of the remote C46 stereocenter in methylspirastrellolide D was also determined, thus giving the complete structure of spirastrellolide A as shown in Scheme 1. This final structure determined for the spirastrellolides was a reassuring result for the Paterson research group as it was consistent with the structure of the better-matching fragment **3** which they had prepared during their syntheses of the fragments. Indeed, the finale to this story is the total synthesis of spirastrellolide A methyl ester **2** by Paterson et al. which is reported[13] in this issue of *Angewandte Chemie*.

The total synthesis reported in these two publications[13] is summarized in Schemes 5 and 7. In the first paper, an improved preparation of the diene **11** is reported, and the 5,6,6-trioxadispiroketal **10** is prepared as described previously (Scheme 4). A Julia coupling reaction of the C26 aldehyde derived from **10** is reported, but suffers from low yields as a result of steric hindrance in the aldehyde. An alternative approach, which was described by Paterson et al. as "an adventurous sp^2-sp^3 Suzuki cross-coupling" between iodide **13** and the borane **14** (derived in four steps from **10**), was highly successful and gave the product in 83% yield (Scheme 5). Iodide **13** was readily prepared from the Evans aldol product **15** by a stereoselective reduction of a triethylsilyl-protected intermediate by using zinc borohydride. Simultaneous hydroboration of both the C17–C18 and C23–C24 double bonds was carried out to generate the C23 and C24 stereocenters, with moderate selectivity (3:1) in favor of the desired isomer. Manipulation of the protecting groups followed by oxidation gave the key aldehyde **16**.

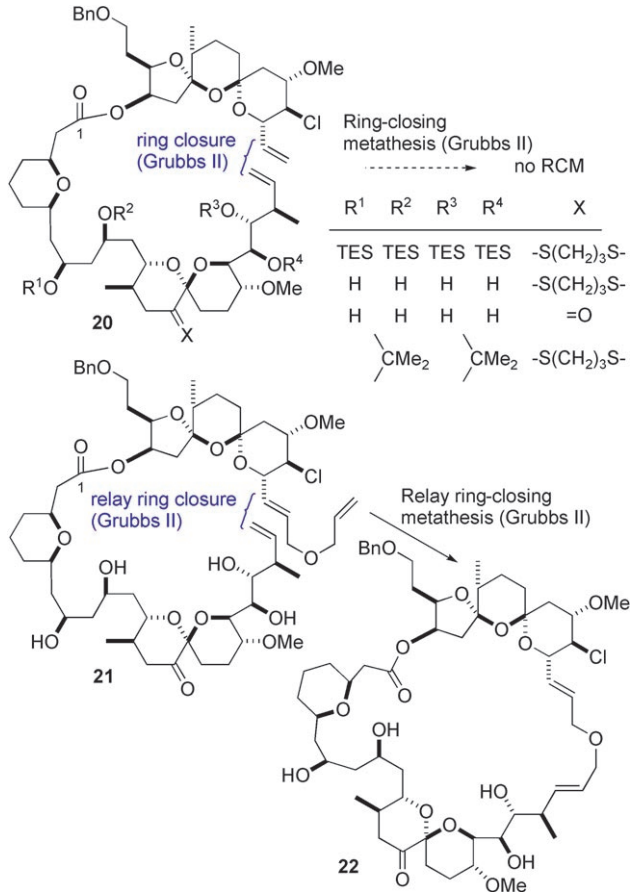


Scheme 5. Synthesis of crystalline macrolide **19** by Paterson et al. BAIB = [bis(acetoxy)iodo]benzene, TEMPO = 2,2,6,6-tetramethyl-1-piperidinoxyl (free radical):

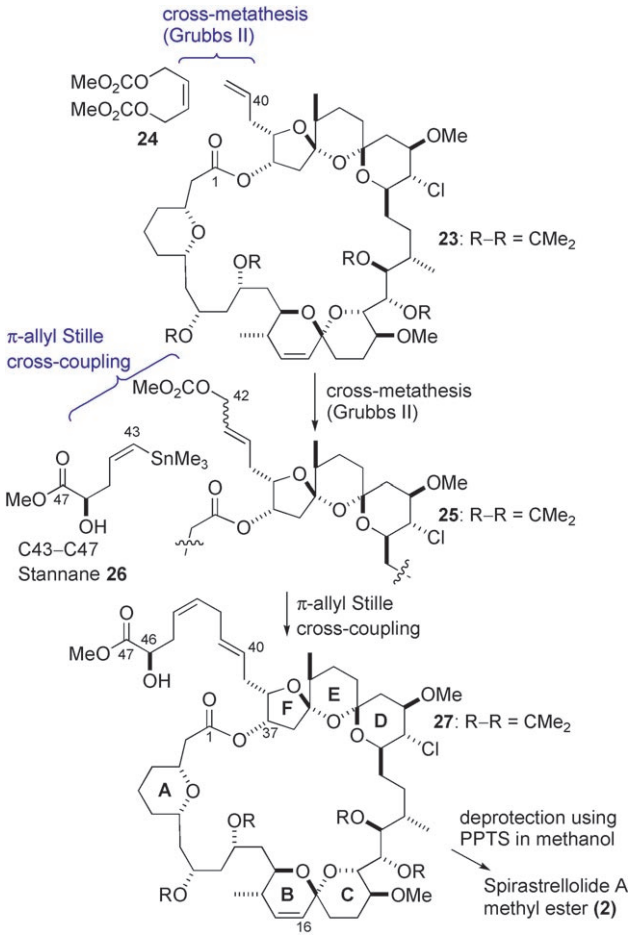
Aldehyde **16** was coupled with the lithium anion of alkyne **17** by using an approach developed previously.[14] Lindlar reduction of the alkyne and subsequent Dess–Martin oxidation then gave the desired (*Z*)-enone **18**. Removal of the C1, C13, and C21 PMB protecting groups facilitated selective formation of the BC spiroacetal, with complete control over the C17 acetal stereocenter. Oxidation of the primary alcohol to the C1 carboxylic acid and selective removal of the C37 TES ether using tetrabutylammonium fluoride (TBAF) set the scene for the crucial macrolactonization step. Macrolactonization, by using the Yamaguchi protocol, gave the macrolide in 79% yield, thereby suggesting a favorable conformational preorganization of the *seco* acid. At this stage, selective removal of the C40 TBS protecting group was not possible. Hence, global deprotection was effected using HF·pyridine in a mixture of pyridine and THF. Fortuitously, this procedure gave a crystalline product **19** which was of sufficient quality to yield an X-ray crystal structure. This solid-state structure confirmed the stereochemical assignment of the synthetic macrocyclic ring to be the same as that found for the crystalline derivative of spirastrellolide B.[11] Surprisingly, despite this structural similarity, the ^{1}H NMR spectrum of this compound was found to be significantly different from that of the macrolide region of spirastrellolide A. Paterson et al. proposed that these differences were the result of a well-defined structure for spirastrellolide A in which the side chain is in proximity to the macrolide core.

The potential difficulties in closing the macrocyclic ring is seen in the attempted cyclization of the advanced intermediate **20** (in the enantiomeric series) by Fürstner et al. (Scheme 6).[9] Ring-closing metathesis on a number of differently protected compounds **20** failed to give any of the desired ring-closed product. This low reactivity was attributed to steric hindrance at the C26 carbon atom adjacent to the chlorinated bis(spiroketal) core. Attempts to overcome this problem by using relay ring-closing metathesis of **21** yielded only the ring-expanded product **22** in 64% yield. An evaluation of the phosphatase inhibitory activity of the ring-expanded product **22** is underway.[9]

The crystalline macrolide **19** generated in the synthesis by Paterson et al. was shown to be a suitable intermediate for the completion of the total synthesis of spirastrellolide A methyl ester (**2**), as indicated in Scheme 7. The pentaol **19** was



Scheme 6. Attempted ring-closing metathesis (RCM) of **20** and attempted relay ring-closing metathesis of **21** by Fürstner et al.[9]



Scheme 7. Completion of the synthesis of spirastrellolide A methyl ester (**2**) by Paterson et al.

converted into its bis(acetonide), oxidized, and subsequently methylenated using $Ph_3P{=}CH_2$ to give alkene **23**. A Grubbs II catalyzed cross-metathesis of **23** with dimeric carbonate **24** gave the required carbonate **25** in good yield, despite the requirement for elevated temperatures (80 °C). Carbonate **25** underwent the crucial π-allyl Stille cross-coupling reaction with the (*R*)-stannane **26** to give the bis(acetonide) **27**. This compound was found to be identical in all respects (^{1}H, ^{13}C NMR, MS, optical rotation) with the compound formed by Andersen and co-workers[12] from spirastrellolide A itself, thereby confirming the relative and absolute configuration (including the remote C46 stereocenter) of the natural product. Finally, the total synthesis of the methyl ester (**2**) was completed by removal of the acetonide groups by using PPTS in methanol.

The total synthesis of spirastrellolide A methyl ester (**2**) was achieved by the Paterson research group in 36 linear steps. The identity of the synthetic material with the methyl ester of the natural product was shown through comparison of the NMR spectra recorded in several solvents, IR and mass spectra, optical rotation, CD spectra, and HPLC retention time, and thus also confirming the structural assignment of spirastrellolide A.[2,11,12]

We noted at the start the interplay of biology and chemistry in which a specific cell-based assay was used for the discovery of a novel molecular structure, spirastrellolide A (**1**). The successful completion of the total synthesis of this natural product, as reported in this issue, is notable for the speed with which it was achieved in the face of a number of initial stereochemical uncertainties. In addition, the comparatively concise synthetic route employed should allow the production of material for further biological testing. Finally, the crystal structure of the macrolide core should aid with docking studies against protein phosphatase 2A, thus allowing the rational design of analogues with improved efficacy. As such, this synthesis provides an outstanding example of the application of modern synthetic methods.

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