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Synthetic studies toward shellfish toxins containing spiroacetal units*

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Abstract: The synthesis of the ABC spiroacetal-containing fragment of the marine biotoxins, the pectenotoxins (PTXs), is described. The synthetic strategy involves appendage of the highly substituted tetrahydofuran C ring to the AB spiroacetal unit via stereocontrolled cyclization of a γ-hydroxyepoxide. The bis-spiroacetal moiety of the spirolide family of shellfish toxins is also described, making use of an iterative radical oxidative cyclization strategy.

Keywords: spiroacetals; shellfish toxins; pectenotoxins; spirolides; oxidative cyclization.

INTRODUCTION

Algal blooms produce toxins that accumulate in shellfish, resulting in the death of fish and marine species [1]. Most of these shellfish toxins target ion channels, and molecules that can modulate the function of ion channels are useful for the rational design and development of drugs for clinical conditions such as pain, epilepsy, stroke, and cancer. These toxins have rich and diverse chemical structures, and their complex heterocyclic arrays pose significant synthetic challenges. We herein report summaries of our synthetic studies toward two families of shellfish toxins, the pectenotoxins (PTXs), and the spirolides.

SYNTHESIS OF THE ABC FRAGMENT OF THE PECTENOTOXINS

The PTXs are a family of polyether lactones that were first isolated in 1985 by Yasumoto et al. [2] and were originally produced by toxic dinoflagellate species of the genera *Dinophysis* (*D. acuta* and *D. fortii*). The absolute stereochemistry of PTX1 **1** was established by X-ray crystallography [2], and the structures of the remaining PTXs have been elucidated by comparison of NMR and mass spectrometry data [3–7]. The PTXs comprise a macrolide structure containing a spiroacetal, three substituted tetrahydrofurans and 19 (or 20 in the case of PTX11) stereocenters embedded within a 40-carbon chain (Fig. 1).

PTX2 **2** exhibited selective and potent cytotoxicity against several cancer cell lines at the nanomolar level [8]. PTX2 **2** and PTX6 **4** have also been shown to interact with the actin cytoskeleton at a unique site [9]. The architecturally complex structure of the PTXs, together with their potent biological activity, has attracted the attention of several research groups [8–13], and Evans et al. [14] have achieved the total synthesis of PTX4 and PTX8. In light of our research group's interest in the synthe-

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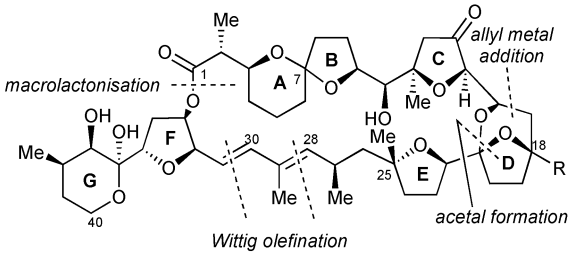
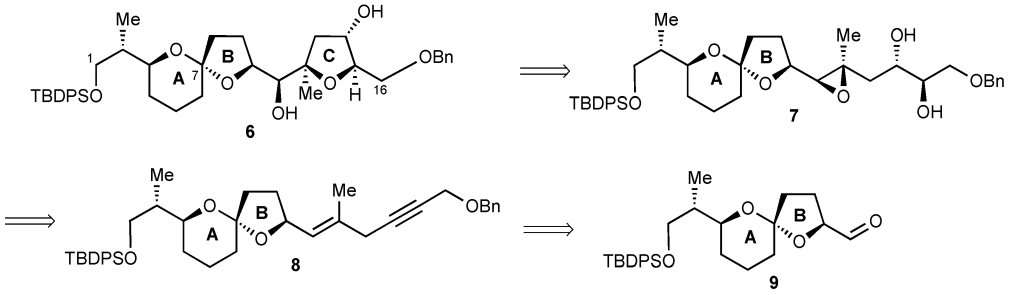
sis of spiroacetal-containing natural products, we herein describe our synthetic work focused on the synthesis of the ABC spiroacetal-containing tricyclic ring system of PTX2 **2** based on the retrosynthetic disconnections depicted in Fig. 1 [15].



1: PTX1(7*R*, R=CH_2OH)
2: PTX2 (7*R*, R=Me)
3: PTX4 (7*S*, R=CH_2OH)
4: PTX6 (7*R*, R=COOH)
5: PTX7 (7*S*, R=COOH)

Fig. 1

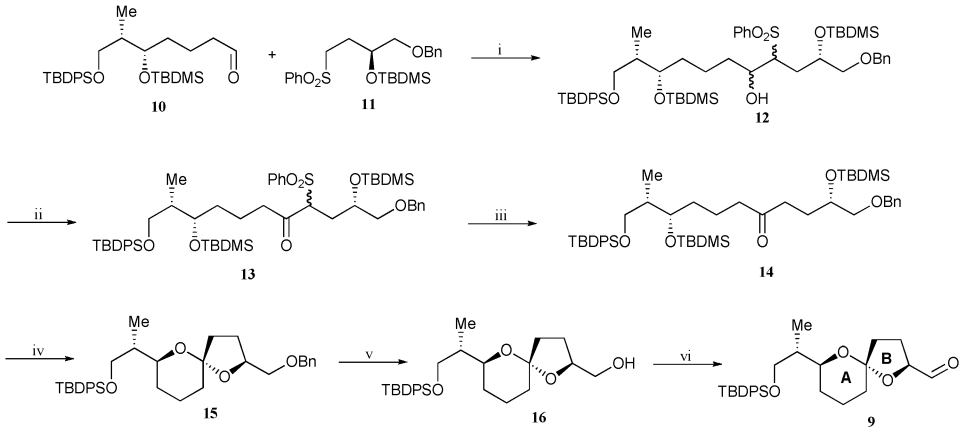
Our retrosynthetic analysis of the key spiroacetal-containing ABC tricyclic fragment **6** is depicted in Scheme 1. The ABC fragment **6** is constructed via a 5-*exo*-tet cyclization of epoxy-diol **7**, in which all the necessary stereogenic centers of the C ring are already installed. Epoxy-diol **7** in turn is obtained from enyne **8** by asymmetric epoxidation followed by semi-hydrogenation and asymmetric dihydroxylation (AD). Enyne **8** is then prepared from spiroacetal aldehyde **9**. The synthesis of PTX2 **2** requires establishment of the (7*R*)-configuration of the spirocenter. However, the (7*S*)-configuration as present in PTX4 **3** and PTX7 **5** is stabilized by the anomeric effect and is in fact the thermodynamically favored stereochemistry when the spiroacetal ring is not embedded in the macrocyclic structure. We therefore planned to obtain the natural (7*R*)-isomer of PTX2 **2** at a later stage in the synthesis after assembly of the macrolide ring. Our initial attention was therefore directed toward the synthesis of spiroacetal **9** with the (7*S*)-configuration as present in PTX7 **5**.



Scheme 1

The execution of our synthetic plan toward the synthesis of the ABC tricyclic system of PTX7 **5** commenced with the synthesis of the C1–C11 AB spiroacetal fragment starting from aldehyde **10** and sulfone **11** (Scheme 2). The *syn* stereochemistry in the aldehyde fragment **10** was installed using an asymmetric aldol reaction, and sulfone **11** was prepared from (*R*)-(+)-benzylglycidol [15]. The union of aldehyde **10** with sulfone **11** in THF using BuLi as base proceeded smoothly, giving a mixture of the four diastereomeric alcohols **12**. Oxidation of the resulting alcohols **12** to the two diastereomeric ke-

tones **13** was next effected using Dess–Martin reagent proceeding in 82 % yield over 2 steps. The mixture of sulfone diastereomers **13** was then exposed to sodium mercury amalgam in methanol to give ketone **14** as a single isomer in 68 % yield. Selective deprotection of the *t*-butyldimethylsilane (TBDMS) groups in the presence of the benzyl and *t*-butyldiphyenylsilyl (TBDPS) groups was achieved by heating ketone **14** at reflux with *p*-toluenesulfonic acid in CH_2Cl_2 for several hours. This method resulted in clean cyclization of the resulting diol to give the 5,6-spiroacetal **15** as a single isomer in 84 % yield. Removal of the terminal benzyl group using Raney nickel in ethanol at 35 °C for 48 h gave exclusively the desired 5,6-spiroacetal **16** with none of the more thermodynamically favored 6,6-spiroacetal being formed. Finally, Dess–Martin oxidation of alcohol **16** afforded the key spiroacetal aldehyde **9**.



Scheme 2 *Reagents and conditions and yields*: (i) BuLi, THF, then **11**, –78 °C, 88 %; (ii) Dess–Martin periodinane, py, CH_2Cl_2, 93 %; (iii) 10 % Na/Hg, Na_2HPO_4, MeOH, 68 %; (iv) *p*-TsOH, toluene, 80 °C, 4 h, 84 %; (v) Raney Ni, EtOH, 35 °C, 2 days, **16**, 82 %; (vi) Dess–Martin periodinane, py, CH_2Cl_2, 95 %.

Nuclear Overhauser effect (nOe) correlations were observed for spiroacetal between H-2 and H-7 and also between H-2 and the methyl group, thus suggesting that O1 and O6 are axial to each other and that C4 adopts an equatorial position on the A ring **9** (Fig. 2). These observations established the (5*S*)-configuration of the 5,6-spiroacetal ring system as was expected due to anomeric stabilization dominating the thermodynamically controlled cyclization process. Furthermore, these nOe studies also established that no epimerization had occurred during the debenzylation and oxidation steps.

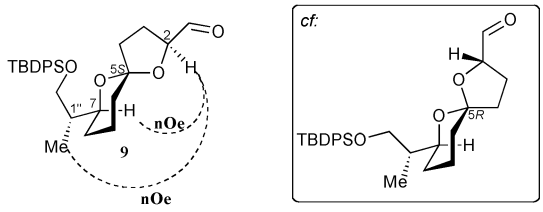
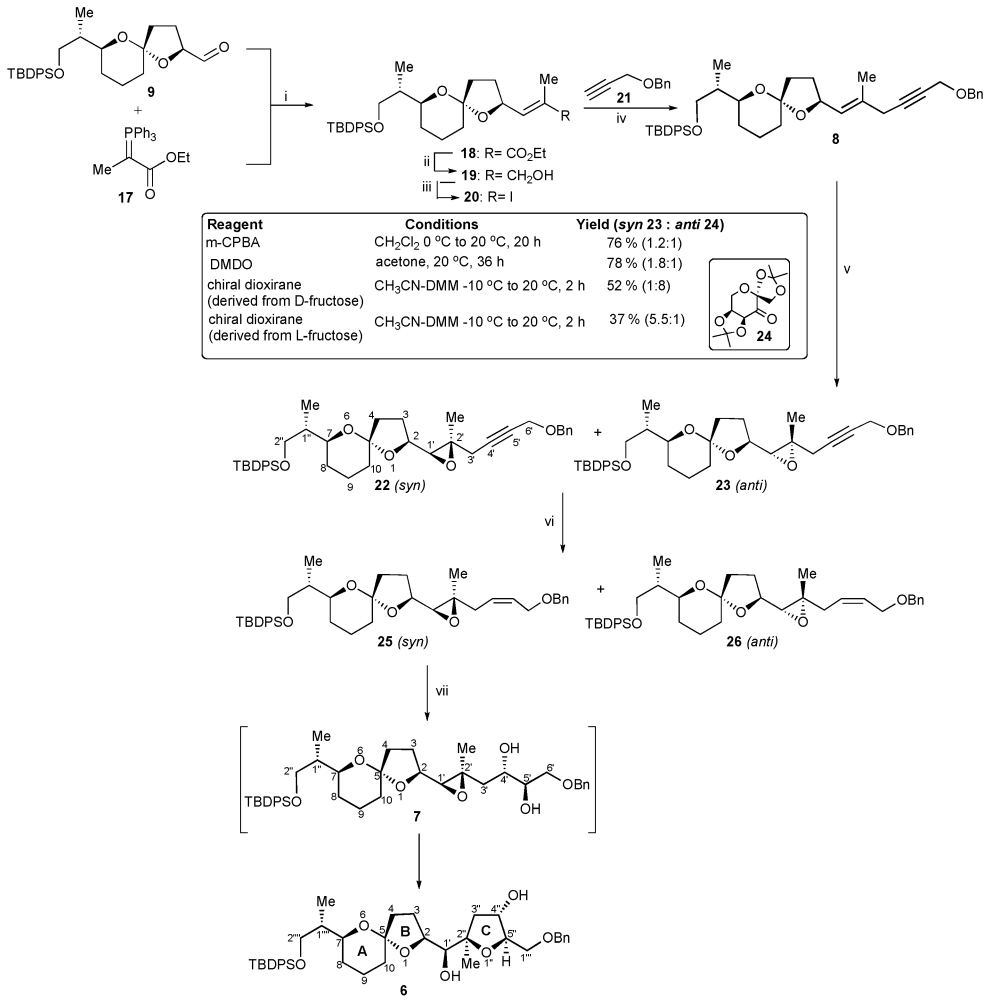


Fig. 2 nOe correlations for spiroacetal **9**.

Assembly of the ABC tricyclic ring system began with Wittig olefination of spiroacetal aldehyde **9** with ylide **17** (Scheme 3) affording olefin **18** (*E*:*Z* = 100:1 by ^{1}H NMR) in quantitative yield. Reduction of ester **18** to alcohol **19** was then achieved in 91 % yield using di*iso*butylaluminum hydride in toluene at –78 °C. The allylic alcohol **19** was converted to the corresponding iodide **20** via the



Scheme 3 *Reagents and conditions and yields*: (i) CH_2Cl_2, 0–20 °C, 20 h, 99 %; (ii) 1 M DIBAL-H, CH_2Cl_2, –78 °C, 91 %; (iii) MsCl, Et_3N, THF, 0 °C, 20 min, then Nal, THF, 20 °C, 2 h, filter; (iv) acetylene **21**, BuLi, THF, –78 °C then iodide **20**, THF, –78 to 0 °C, 20 h, 56 and 18 % (*Z*)-isomer; (v) see Table in Scheme; (vi) H_2, $Pd/CaCO_3$ (5 % Pb), Et_3N, hexane, 50 min, **25**, 88 %; (vii) DHQ-IND, $K_3Fe(CN)_6$, $MeSO_2NH_2$, OsO_4, *t*BuOH–H_2O (1:1), 20 h, **6**, 38 %; or OsO_4, acetone-H_2O (5:1), 18 h, **6**, 70 %.

mesylate and immediately used in the next step. Addition of the iodide **20** in THF to the acetylide formed from acetylene **21** with BuLi in THF at –78 °C followed by warming the mixture to 0 °C afforded (*E*)-enyne **8** in 56 % yield together with the (*Z*)-enyne in 18 % yield.

Having finally fully assembled the C1–C16 carbon chain fragment of PTX7 **5**, in the form of spiroacetal enyne **8**, our attention next focused on the conversion of the enyne unit to the required epoxy diol fragment **7**, which could be transformed into the target ABC ring fragment **6** by acid-catalyzed cyclization. The *syn*-epoxide was envisaged to be formed via asymmetric epoxidation and the diol by AD of the olefin formed upon subsequent semi-hydrogenation of the triple bond. The first attempts to effect epoxidation of alkene **8** were carried out using achiral epoxidation reagents *m*-CPBA and dimethyldioxirane (DMDO), hoping that the neighboring C–O bond on the adjacent chiral center may influence the stereochemical outcome of epoxidation. Epoxidation of (*E*)-enyne **8** using *m*-CPBA in CH_2Cl_2 afforded a 1.2:1 mixture of the *syn*-epoxide **22** and *anti*-epoxide **23** in 76 % yield. Epoxidation

of (*E*)-enyne **8** using freshly prepared DMDO in acetone for 36 h afforded a 1.8:1 mixture of the *syn*-epoxide **22** and *anti*-epoxide **23** in 78 % yield.

Disapppointed by the lack of selectivity in the epoxidation of (*E*)-enyne **8** using achiral epoxidation agents, it was decided to use a chiral dioxirane generated in situ from potassium peroxomonosulfate (Oxone®) and a chiral fructose-derived ketone, a method reported by Shi et al. [16] to effect epoxidation of unfunctionalized (*E*)-olefins in a highly enantioselective fashion. Invoking the spiro transition state predictive model [16], it was envisaged that use of ketone **24**, derived from L-fructose, would generate the desired *syn*-epoxide **22** whilst use of the enantiomeric D-fructose-derived ketone ent-**24** would result in predominant formation of the undesired *anti*-epoxide **23**. Reaction of (*E*)-enyne **8** with the more readily available chiral dioxirane prepared in situ from D-fructose-derived ketone ent-**24** (3 equiv) and Oxone (3 equiv) at –10 to 20 °C in acetonitrile and dimethoxymethane (DMM) (1:2 v/v) for 2 h afforded an inseparable 1:8 mixture of the *syn*-epoxide **22**:*anti*-epoxide **23** in 52 % yield (see Table in Scheme 3). In this case, the major epoxide formed had the opposite configuration to the major epoxide formed using *m*-CBPA and DMDO. Epoxidation of (*E*)-enyne **8** with the chiral dioxirane formed from L-fructose-derived ketone **24** (prepared from L-sorbose [17]) was then attempted in an effort to produce more of the desired *syn*-epoxide **22**. However, in this case, conversion to the epoxide proceeded in a lower 37 % yield. Encouragingly, the stereoselectivity observed was promising with a 5.5:1 ratio of the desired *syn*-epoxide **22** to *anti*-epoxide **23** being observed.

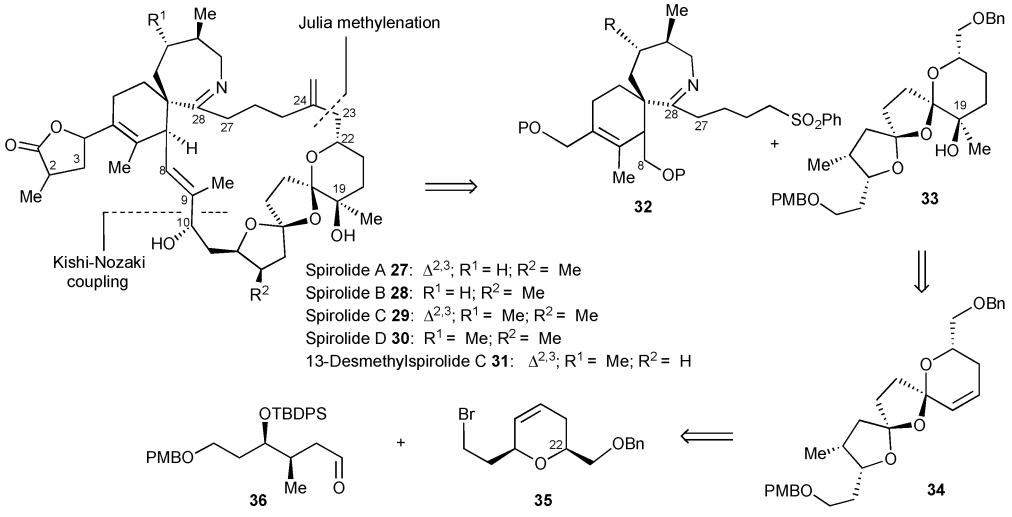
The 5.5:1 mixture of *syn*-epoxide **22**:*anti*-epoxide **23** was subjected to semi-hydrogenation over Lindlar catalyst, affording a 5.5:1 mixture of (*Z*)-olefin **25**:(*Z*)-olefin **26** in 88 % yield in preparation for the subsequent AD step. High enantioselectivity in the AD of (*Z*)-olefins is usually observed when the size of the two olefinic substituents is significantly different as is the case for (*Z*)-olefin **25**. Application of the mnemonic developed for predicting stereoselectivity in AD reactions predicts that using DHQ-IND, the hydroxyl group would be predominantly delivered to (*Z*)-olefin **25** from the α-face [18], affording diol **7** that would undergo cyclization to the desired spiroacetal-containing tetrahydrofuran **6**. AD reaction of the 5.5:1 mixture of (*Z*)-olefins **25**:**26** using DHQ-IND as the chiral ligand afforded the ABC ring fragment **6** in 38 % yield together with a complex diastereomeric mixture of diols (43 % yield). The exact stereochemistry of the diol mixture obtained was not established, and the lack of diastereoselectivity observed in this reaction was disappointing.

The low yield of the desired tricyclic fragment **6** obtained using DHQ-IND as the chiral ligand prompted us to investigate the use of OsO_4 without the chiral catalyst to see whether the neighboring chiral centers in the olefin substrate might influence the stereoselectivity in the dihydroxylation step. Somewhat surprisingly, treatment of the 5.5:1 mixture of (*Z*)-olefins **25**:**26** with OsO_4 afforded the desired tricyclic fragment **6** as the major product in 70 % yield together with a mixture of diols (<10 % yield). Thus, the neighboring chiral centers in this system clearly play a role in the hydroxylation, which may contribute to the lower diastereoselectivity being observed in the above reaction using DHQ-IND as the chiral ligand.

The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum for tricyclic fragment **6** showed a clear correlation between C2''-Me and H-5'', thus establishing the desired *syn* relationship between these two groups on the tetrahydrofuran C ring system. The ^{13}C NMR data for the ABC tricyclic fragment **6** was also in agreement with the ^{13}C NMR data reported for the ABC fragment of PTX7 **5**, which also contains the (*S*)-configuration at the spiroacetal center. Thus, the successful synthesis of the ABC tricyclic fragment **6** of PTX7 **5** has been achieved and synthetic efforts are now directed toward the synthesis of the E and FG fragments of the PTXs in preparation for union with the ABC fragment **6**.

SYNTHESIS OF THE BIS-SPIROACETAL MOIETY OF THE SPIROLIDES

The spirolides A–D **27**–**31** (Scheme 4) comprise a novel family of pharmacologically active macrocyclic imines found in the polar lipid fraction obtained from the digestive glands of contaminated mussels (*Mytilus edulis*), scallops (*Placopecten magellanicus*), and toxic plankton from the eastern coast of Nova Scotia, Canada [19,20]. The spirolides contain an unusual 5,5,6-bis-spiroacetal moiety together with a rare 6,7-spirocyclic imine. Spirolides E and F are keto amine hydrolysis derivatives resulting from ring opening of the cyclic imine, suggesting that this functionality is the pharmacophore responsible for toxicity [21]. The spirolides A–D **27–31** cause potent and characteristic symptoms in the mouse bioassay and are activators of L-type calcium channels. Preliminary pharmacological research into the mode of action of the spirolides suggests they are antagonists of the muscarinic acetylcholine receptor [22]. The absolute stereochemistry of the spirolide family of toxins has not been established to date, however, a computer-generated relative assignment of 13-desmethyl spirolide C **31** showing the same relative stereochemistry as the related toxin pinnatoxin A [23] in the region of their common structure has been reported [24].



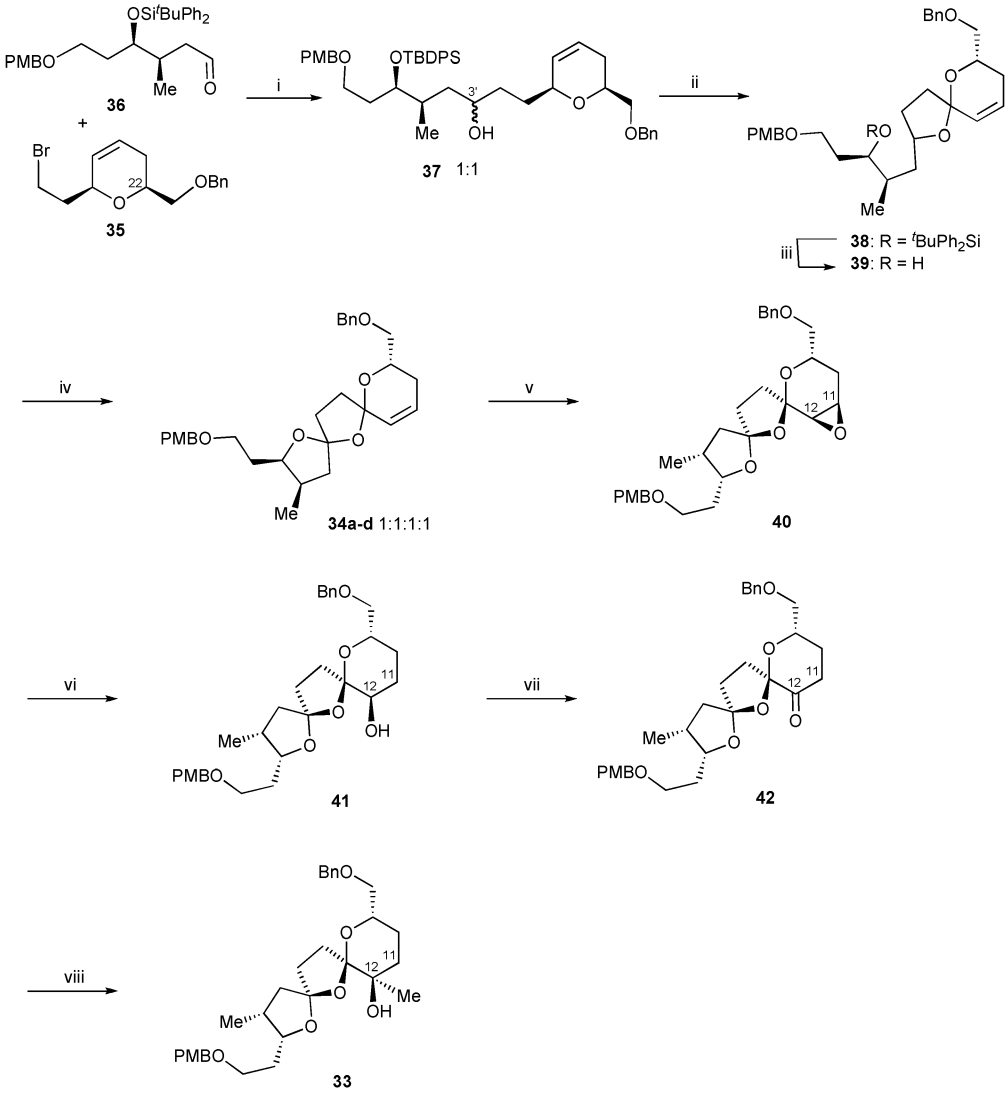
Scheme 4

A total synthesis of the spirolides has not been reported to date, however, a synthesis of the bis-spiroacetal core of spirolide B **28** via acid-catalyzed cyclization of an acyclic triketone has been communicated [25]. Our interest in the synthesis of natural products containing bis-spiroacetal ring systems led us to pursue the synthesis of the bis-spiroacetal ring system present in the spirolides using an oxidative radical cyclization to construct the two five-membered rings in the 5,5,6-bis-spiroacetal unit of the spirolides.

The key disconnection in our proposed retrosynthesis of spirolides B **28** and D **30** (Scheme 4) involves Ni^{II}/Cr^{II}-mediated Kishi–Nozaki coupling between an aldehyde and a vinyl iodide to form the C9–C10 bond of the macrocyclic ring in a similar fashion to that used by Kishi et al. [26] in the synthesis of pinnatoxin A. Our revised synthetic plan [27] (Scheme 4) relied on disconnection of the C23–C24 bond focusing on the synthesis of bis-spiroacetal **33**, making use of a Julia methylenation [28] for subsequent union with spiroimine sulfone **32**. This new approach required access to dihydropyran **35** with the required (*S*)-configuration at C22 using a silyl-modified Prins cyclization [29]. The two spiroacetal centers in unsaturated spiroacetal **34** are then formed by oxidative radical cyclization of the alcohol resulting from the Barbier coupling of this dihydropyran **35** with aldehyde **36**, followed by de-

protection of the TBDPS ether and execution of a second oxidative radical cyclization. The *syn* stereochemistry in aldehyde **36** is assembled using an enantioselective crotylation [29]. The alkene in bis-spiroacetal **34** provides functionality for subsequent installation of the tertiary alcohol. It was also envisaged that the *cis* stereochemistry between the terminal rings of the bis-spiroacetal will be established by equilibration after incorporation into the macrocyclic ring. Thus, initial synthesis of *trans* bis-spiroacetals **34** and **33** was required.

Union of bromide **35** with aldehyde **36** proceeded using a Grignard reaction (Scheme 5), affording the coupled product **37** in 88 % yield as a ~1:1 mixture of diastereomers at C3'. The two diastereomers could be easily separated by flash chromatography, but usually the mixture was used throughout

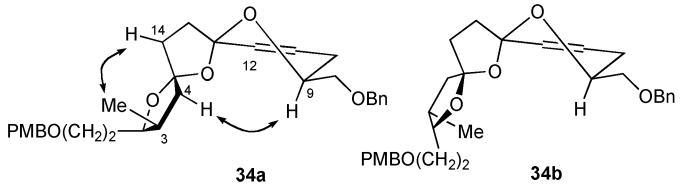


Scheme 5 *Reagents and conditions and yields*: (i) **35**, Mg, $Br(CH_2)_2Br$, I_2, Et_2O, room temp. then aldehyde **36**, 88 %; (ii) $PhI(OAc)_2$, I_2, hν, cyclohexane, room temp., 86 %; (iii) Bu_4NF, DMF, 80 °C, 82 %; (iv) $PhI(OAC)_2$, I_2 hν, cyclohexane, room temp., 81 %; *m*-CPBA, CH_2Cl_2, 0 °C to room temp., 63 %; (vi) DIBALH, hexane, 0 °C, 54 %; (vii) Dess–Martin periodinane, CH_2Cl_2, room temp., 88 %; (viii) MeMgBr, Et_2O, –78 °C, 86 %.

the synthesis, as equilibration of the bis-spiroacetal ring system was carried out at a later stage. With the basic carbon skeleton fully assembled, use of iterative radical oxidative cyclizations then allowed formation of the bis-spiroacetal. Irradiation of the mixture of alcohols **37** with a 60 W standard desk lamp in the presence of iodobenzene diacetate and iodine [30] in cyclohexane afforded spiroacetal **38** as a mixture of diastereomers in 86 % yield. After deprotection of the TBDPS ether in spiroacetal **38**, the final bis-spiroacetal ring system was then formed upon execution of a second oxidative radical cyclization of alcohol **39**, providing bis-spiroacetals **34a–d** in 81 % yield as a 1:1:1:1 mixture of diastereomers.

Acid-catalyzed spiroacetalization of the 1:1:1:1 mixture of **34a–d** gave a ~4:1 mixture of two major isomers (**34a** and **34b**) together with trace quantities (<5 %) of two other minor isomers (Table 1). Interestingly, use of indium trichloride gave better results than the commonly used reagents such as HF·py, PPTS, $ZnBr_2$, or $ZnCl_2$, affording a 87:13 mixture of the thermodynamically favored isomers **34a** and **34b** (entry 5). This is the first example of the use of indium trichloride as a Lewis acid to effect equilibration of a mixture of spiroacetals allowing convergence to one major diastereomer. The absolute configuration at C5 and C7 in isomer **34a** was assigned unambiguously using 2D NMR NOESY experiments, which showed clear correlations between H-9 and H-4, and between 3-C$\underline{H}_3$ and H-14, respectively (Table 1).

Table 1 Equilibration of bis-spiroacetals **34a** and **34b**.



34a **34b**

Entry	Conditions	**34a:34b** (yield)
1	HF·Pyr, MeCN, room temp., 12 h	76:24 (81 %)
2	PPTS (0.2 equiv), MeCN, room temp., 18 h	~81:19 (89 %)
3	$ZnBr_2$ (0.2 equiv),CH_2Cl_2, room temp., 19 h	76:24 (95 %)
4	$ZnCl_2$ (0.2 equiv),CH_2Cl_2, room temp., 24 h	~83:17 (88 %)
5	$InCl_3$ (0.2 equiv), MeCN, room temp., 1 h	87:13 (85 %)

Finally, treatment of the 1:1:1:1 mixture of bis-spiroacetals **34** with *m*CPBA afforded β-epoxide **40** as a single diastereomer together with recovered starting material. Remarkably, the presence of *meta*-chlorobenzoic acid and water in the *m*CPBA (*m*CPBA purchased from Fluka contains ~10 % *m*-chlorobenzoic acid and ~20 % H_2O) effected equilibration of the mixture of bis-spiroacetals **34a–d** to the most thermodynamically favored isomer **34a**, which then underwent stereoselective epoxidation from the β-face presumably due to the involvement of the neighboring oxygens in hydrogen bonding to the *m*CPBA. Epoxide **40** underwent regioselective reductive opening with DIBALH in hexane and the resultant alcohol **41** was oxidized to ketone **42** using Dess–Martin periodinane. Addition of methylmagnesium bromide to ketone **42** proceeded stereoselectively from the axial direction, affording the desired tertiary alcohol **33** with the same stereochemistry as that present in the spirolides. Ishihara and coworkers have similarly reported [25] the use of MeLi in THF to effect stereoselective axial introduction of the methyl group. The *trans* stereochemistry of the bis-spiroacetal ring system adopted by tertiary alcohol **34** represents the thermodynamically favored isomer, and it is hoped that re-equilibration of the bis-spiroacetal to the desired *cis* stereochemistry as found in the spirolides will take place upon incorporation of this moiety into the larger macrocyclic system. Further synthetic work toward the synthesis of spirolides B **28** and D **30** awaits the synthesis of the spiroimine unit of these marine biotoxins.

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