Chapter XX

TOTAL SYNTHESIS OF SPONGISTATIN 1 (ALTOHYRTIN A): A TALE OF TEN ALDOLS

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I. Introduction

The opening of Charles Dickens' classic novel, *A Tale of Two Cities* – "It was the best of times, it was the worst of times" – will sound familiar to those who work on the total synthesis of complex natural products. Many emotional highs and lows are experienced during ambitious total synthesis projects. There can be periods when it seems that nature is contriving against the bench chemist, cruelly preventing all attempts to achieve the desired transformation, while at other times a string of successes may leave one feeling they have the Midas touch.

This chapter attempts to recount some of the highs and lows encountered during the Paterson group's total synthesis of spongistatin 1/altohyrtin A, an extremely rare marine macrolide with a seductively complex structure in combination with displaying promising anticancer properties. We provide the reader with an emotional roller coaster ride through a project that spanned some 7 years, with early studies aimed at

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developing the methodology needed to tackle this "hot target", leading on to the tense final stages, involving intricate coupling reactions of densely functionalised subunits, and the final push to solve the many problems encountered and complete the total synthesis. The perspective taken is one of a PhD student (MJC) involved in the later phases of the synthetic effort, leading right up to the exhilarating final stages. As such, and due to the need for brevity, a great deal of the initial pioneering work by the first "spongi" students and postdocs is not covered in equal detail. We emphasise that the eventual success of this challenging project was due to the hard work, enthusiasm and determination of all in the "spongi team", who are listed in the acknowledgements.

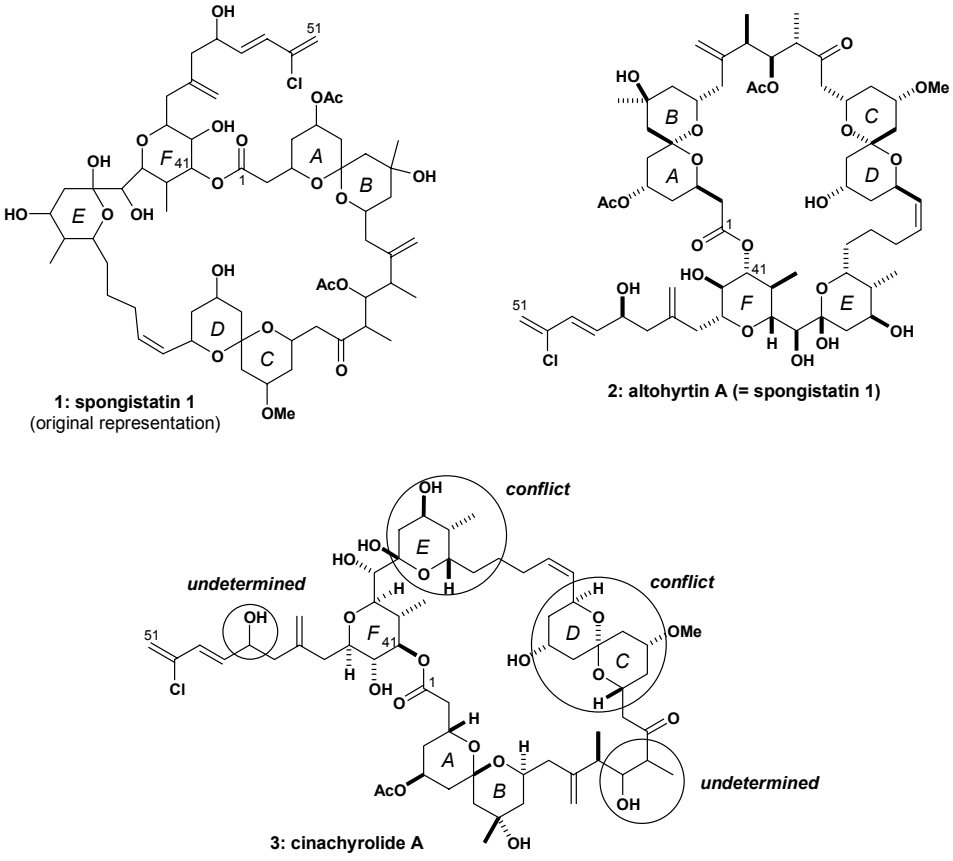


FIGURE 1. ORIGINALLY PROPOSED STRUCTURES FOR SPONGISTATIN 1 (**1**), ALTOHYRTIN A (**2**) AND CINACHYROLIDE A (**3**), SHOWING THAT NO STEREOCHEMISTRY WAS DETAILED IN THE ORIGINAL REPRESENTATION OF **1**, THE PARTIAL STEREOCHEMISTRY PROPOSED FOR **3** CONFLICTED WITH THAT FOR **2** AND LATER REPRESENTATIONS OF **1** BY PETTIT.

In 1993, Pettit *et al*. reported the isolation and gross structure of spongistatin 1 (**1**, original representation), the first of an unprecedented family of extremely potent antimitotic macrolides of sponge origin (Figure 1).[1] Also in 1993, Kobayashi/Kitagawa and the group of Fusetani reported the isolation from different sponge sources of the altohyrtins (as in **2**)[2] and cinachyrolide A (**3**),[3] respectively, having the same gross structures as those reported for the spongistatins. Initially, there were some notable discrepancies apparent in the relative stereochemical relationships attributed to these unprecedented 42-membered macrolides, where the Kitagawa/Kobayashi group was the only one to propose the full relative and absolute configuration, as shown in structure **2** for altohyrtin A.[2d] Thus, initiating our total synthesis programme required a best guess at the target's stereostructure and the design of a flexible approach to allow later corrections if required. As it turned out, the Evans and Kishi groups were the first past the post in the "spongi race", reporting their completed total syntheses of altohyrtin C and altohyrtin A in late 1997 and early 1998, respectively.[4,5] This served to confirm the full stereostructure in **2** and established that the spongistatins indeed had identical structures to the altohyrtins.

The spongipyran family[6] of marine macrolides are among the most potent cancer cell growth inhibitory antimitotic agents tested by the National Cancer Institute (NCI), with spongistatin 1/altohyrtin A (**2**) being one of the most active members of the class. The exceptional level of cytotoxicity displayed by this compound in the NCI 60 human carcinoma cell line screen (mean panel GI_{50} 1.3 x 10^{-10} M) is complemented by potency against a subset of highly chemoresistant tumour types (GI_{50} 2.5-3.5 x 10^{-11} M) and promising preliminary *in vivo* results in xenograft experiments. However, despite this enticing biological profile, further testing has been severely hampered by the paltry supply from the natural sponge source (*e.g*. 13.8 mg from 400 kg wet sponge in the original collection by Pettit).

Due to the promising anticancer properties exhibited by the spongistatins, the lack of material available from the sponge sources, the uncertain stereochemical assignment (when we started out in 1994) and the unprecedented molecular architecture, these intriguing compounds represent compelling targets for total synthesis. Within our group, we aimed to establish a highly flexible synthetic route, allowing access to significant quantities of spongistatin 1/altohyrtin A (**2**) (the most potent congener), along with novel structural analogues, to enable preclinical development to resume. We were regularly spurred on in our quest by the constant encouragement of Prof. Pettit. In addition, we had a great deal of

company from many other synthetic groups around the world that were also attracted to the spongistatin problem.[7,8] We now start our adventure…..

II. Retrosynthetic Analysis

The spongipyran natural products present a bewildering array of functionality for the organic chemist to ponder. In the case of spongistatin 1/altohyrtin A (**2**), there are 24 stereogenic centres to be dealt with; a 42-membered macrolactone (longest carbon chain); two spiroacetals, only one of which benefits from two stabilising anomeric effects; a *bis*-tetrahydropyran segment and a highly sensitive chlorotrienol side-chain. Our retrosynthetic analysis for spongistatin 1/altohyrtin A (**2**) involved the disconnection of the molecule into three portions of roughly similar complexity (Figure 2).

The principal fragment couplings envisaged were an aldol reaction to unite the AB- and CD-spiroacetal subunits (**4** and **5**) *via* formation of the C_{15}-C_{16} bond and the accompanying stereocentres, a Wittig coupling between an ABCD aldehyde subunit and EF phosphonium salt **6**, followed by a regioselective macrolactonisation at the C_{41} hydroxyl. The dissection of the target into three fragments to be assembled in late-stage coupling reactions allows for a high degree of convergency, with no major functional group manipulations required following the fragment coupling steps.

Ten key aldol disconnections were identified as part of our overall strategy for assembling this highly oxygenated polyketide. As shown in Figure 2, three of these are present in the AB-spiroacetal subunit, two in the CD-spiroacetal subunit, one to join these two fragments together and four aldol disconnections are apparent in the C_{29}-C_{51}, EF-containing subunit. The mixture of acetate- and propionate-derived portions in the various subunits **4**-**6** would require the development of efficient and highly stereoselective aldol reactions of methyl and ethyl ketones, respectively, with appropriate aldehyde partners. Altogether, the total synthesis of spongistatin 1 provided an unparalleled opportunity to showcase the versatility and practicality of asymmetric aldol methodology based on the use of ketone-derived boron enolates, as developed extensively in our group. For a comprehensive literature survey, experimental details, and transition state models, rationalising the stereoinduction for these synthetically important C–C bond forming reactions, the reader is directed to two recent reviews.[9a,b] Additionally,

such a complex and demanding natural product provided the exciting challenge of developing new synthetic methodologies, both with regard to asymmetric aldol reactions and in other areas, as well as (hopefully) solving the many unanticipated chemical and logistical problems that would inevitably be encountered along the route.

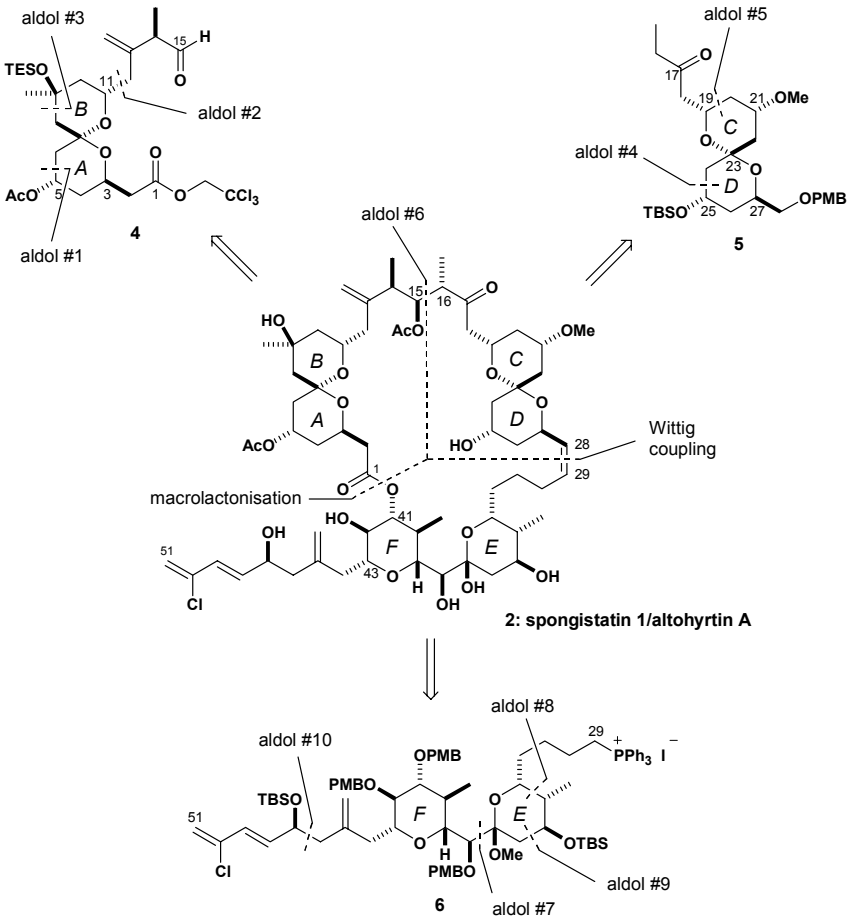


FIGURE 2. RETROSYNTHETIC ANALYSIS.

III. The AB-Spiroacetal Subunit

The C_1-C_{15} fragment of spongistatin 1, comprising the AB-spiroacetal **4**,[10] exhibits an "axial-axial" disposition of the two acetal oxygen atoms at C_7 and hence benefits from two stabilising anomeric effects (Figure 3). Based on this, it was expected that the correct spiroacetal would be

provided under thermodynamic conditions, such as acid-catalysed acetal formation from a linear precursor.

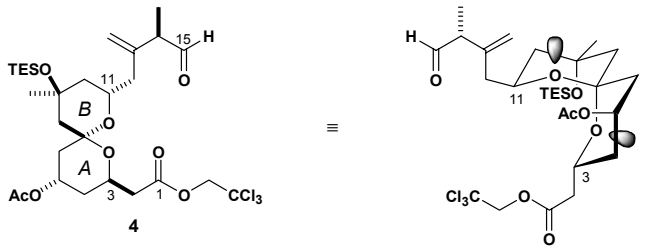
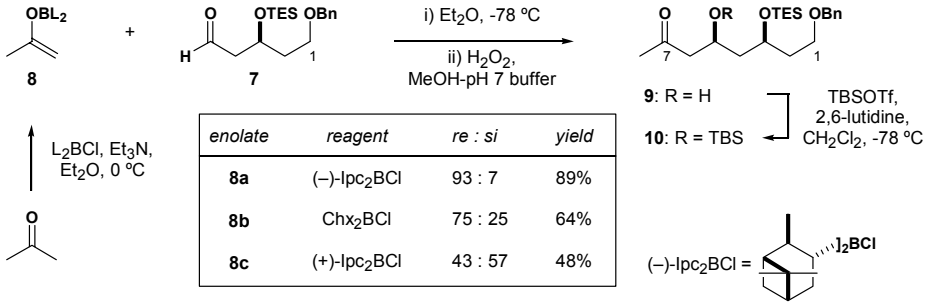


FIGURE 3. THE AB-SPIROACETAL SUBUNIT.

A. ALDOL #1

The first instance of an asymmetric aldol reaction in our spongistatin synthesis involved the reaction of aldehyde **7**, readily available in excellent enantiopurity (97% ee) *via* Brown's allylation methodology,[11] with the enol borinate **8a**, generated *in situ* from the reaction of acetone with (–)-Ipc_2BCl (Et_3N, Et_2O, 0 °C).[10a,b] Following an oxidative workup, this matched reaction gave predominantly the desired 1,3-*syn* isomer **9** (93:7 ds) in 89% yield (Scheme 1). By comparison, the dicyclohexylboron enolate **8b** delivered **9** with reduced selectivity (75:25 ds), while using enolate **8c**, prepared from (+)-Ipc_2BCl, overturned the inherent 1,3-*syn* induction from the aldehyde to give a modest preference for the 1,3-*anti* diastereomer. Subjection of **9** to TBSOTf and 2,6-lutidine provided the corresponding TBS ether **10**.



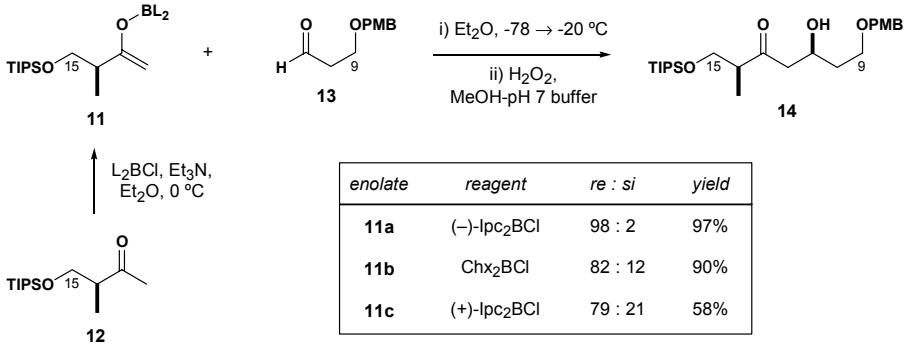
enolate	*reagent*	*re : si*	*yield*
8a	(–)-Ipc_2BCl	93 : 7	89%
8b	Chx_2BCl	75 : 25	64%
8c	(+)-Ipc_2BCl	43 : 57	48%

SCHEME 1

B. ALDOL #2

Previous work by our group had demonstrated that high levels of 1,4-*syn* diastereoselection were possible in boron-mediated aldol reactions of

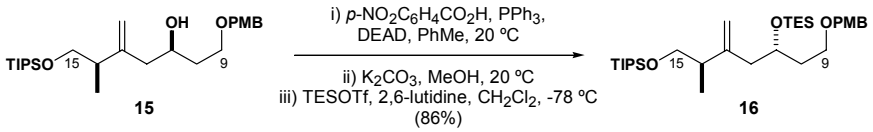
certain α-chiral methyl ketones, and could be enhanced by appropriate choice of Ipc ligand chirality.[12] In the present case, the 1,4-*anti* relationship between the C_{11} and C_{14} stereocentres was best realised by combining a highly 1,4-*syn* selective, matched aldol reaction with a subsequent Mitsunobu inversion at C_{11}.[10c] Enol borinate **11a**, derived from methyl ketone **12** by reaction with (–)-Ipc_2BCl and Et_3N, underwent the desired aldol addition with aldehyde **13** to provide a 97% yield of the 1,4-*syn* product **14** (98:2 ds). Once again, use of Chx_2BCl (enolate **11b**) or the mismatched (+)-Ipc_2BCl (enolate **11c**) led to lower levels of 1,4-*syn* selectivity, 82:18 ds and 79:21 ds, respectively (Scheme 2).



enolate	*reagent*	*re : si*	*yield*
11a	(–)-Ipc_2BCl	98 : 2	97%
11b	Chx_2BCl	82 : 12	90%
11c	(+)-Ipc_2BCl	79 : 21	58%

SCHEME 2

Not surprisingly, an attempt at direct Mitsunobu inversion of β-hydroxyketone **14** led only to elimination, yielding the corresponding α,β-unsaturated ketone. To circumvent this problem, **14** was converted to homoallylic alcohol **15** by Petasis methylenation *via* the corresponding TES ether. Attempts to methylenate β-hydroxyketone **14** directly under Petasis conditions led to substantial decomposition via elimination and retro-aldol pathways. Alcohol **15** underwent smooth Mitsunobu inversion to give, following methanolysis and TES ether formation, the desired 1,4-*anti* compound **16** (Scheme 3). This was then converted in three straightforward steps to aldehyde **17**, ready for the proposed aldol union with ketone **10**.

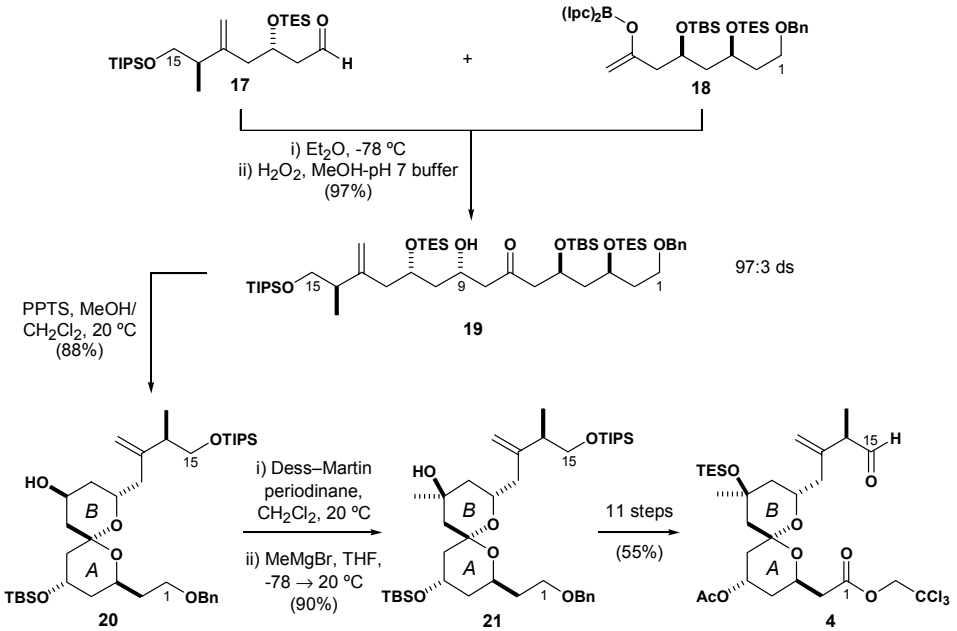


SCHEME 3

C. ALDOL #3

The third and final aldol reaction utilised in our synthesis of the AB-spiroacetal subunit exploited triple asymmetric induction, wherein the influence of all three chiral components (aldehyde, ketone and boron reagent) were matched (Scheme 4). As such, the reaction of aldehyde **17** with enolate **18**, prepared using (–)-Ipc_2BCl, provided the desired aldol adduct **19** in 81% yield (97:3 ds). The 1,3-*syn* preference of aldehyde **17**, the 1,5-*anti* preference of ketone **10** (see following section) and the stereodirecting influence of the boron reagent act in a synergistic fashion, facilitating the excellent level of stereocontrol observed.

Selective desilylation of **19** was accompanied by spiroacetal formation to provide the thermodynamically favoured AB-spiroacetal **20**. At this stage, stereoselective introduction of the C_9 methyl substituent was achieved by oxidation of the secondary alcohol moiety to the corresponding ketone, followed by equatorial addition of MeMgBr to provide spiroacetal **21**, bearing all of the necessary stereogenic centres contained in the C_1-C_{15} portion of the spongistatins. From this point, a series of functional group interconversions were all that was needed to arrive at the fully elaborated AB-spiroacetal aldehyde **4**, ready for the challenging aldol coupling with the CD-spiroacetal ketone **5**.[10d]



SCHEME 4

IV. The CD-Spiroacetal Subunit

The C_{16}-C_{28} fragment of spongistatin 1, comprising the CD-spiroacetal **5**, exhibits an "axial-equatorial" disposition of the two acetal oxygen atoms and hence, unlike the AB-spiroacetal system, benefits from only one stabilising anomeric effect (Figure 4).

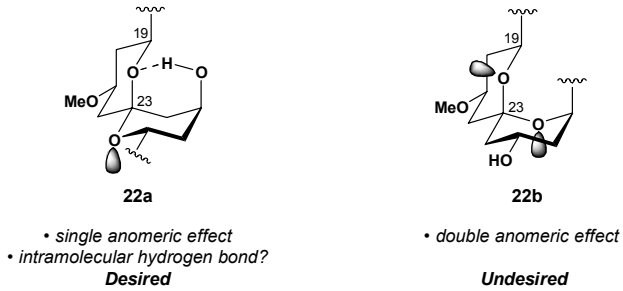
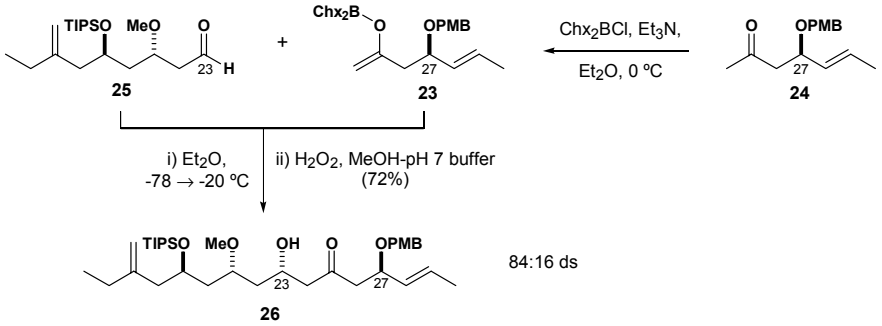


FIGURE 4. THE CD-SPIROACETAL SUBUNIT.

At the outset of our work on this section of the spongistatin structure,[13] it was not known whether an internal acetalisation procedure, analogous to that employed for the AB subunit, would lead to the desired spiroacetal **22a** rather than the potentially more stable **22b**. However, the axial C_{25} hydroxyl may hydrogen bond to an acetal oxygen, providing structure **22a** additional stability not available to **22b**, and this might be exploited in our synthetic endeavours.

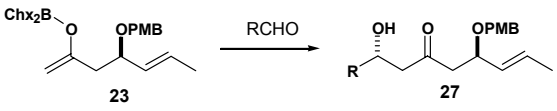
A. A SURPRISING RESULT: REMOTE 1,5-*ANTI* STEREOINDUCTION

Initially, two distinct approaches to this portion of the spongistatins were pursued. The first relied on *bis*-desilylation of a linear precursor and concomitant spiroacetal formation. A complementary strategy was based on the stepwise construction of the C- and D-rings. This latter route (Scheme 5) utilised the boron-mediated aldol reaction between enolate **23**, derived from ketone **24**, and aldehyde **25**, yielding **26** in 72% yield with a high degree of 1,5-*anti* selectivity (84:16 ds). Considering that the stereocentre created in this step (C_{23}) is subsequently destroyed in the next by oxidation to the corresponding ketone, it is to the credit of Karl Gibson that the stereoselectivity of this reaction was paid such close attention.



SCHEME 5

This work resulted in the discovery of high levels of substrate-based, 1,5-*anti* stereoinduction in the boron-mediated aldol reactions of a variety of chiral β-alkoxy methyl ketones with prochiral aldehydes.[14] A typical example is shown in the reaction of methyl ketone enolate **23** with simple aldehydes, leading to the 1,5-*anti* adducts **27** with remarkably high levels (>95:5) of long-range acyclic stereoinduction (Scheme 6). While the mechanistic basis of this remarkable effect must lie in a subtle balance of electronic and steric effects operating in the highly ordered cyclic transition state, the finer details still need to be determined.[9b] This new method for remote stereocontrol has been applied not only in several aspects of our spongistatin total synthesis, but also in other 1,3-polyol syntheses,[15] and a similar effect has been independently observed by the Evans group.[14b]

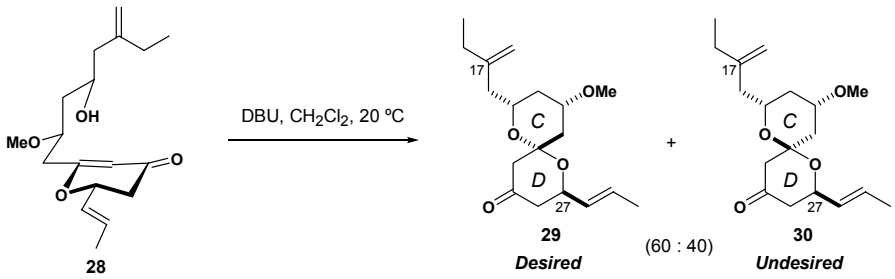


compound	*R*	*anti : syn*	*yield*
27a	*i*-Pr	97 : 3	79%
27b	C(Me)=CH$_2$	97 : 3	82%
27c	CH=CHMe	98 : 2	80%

SCHEME 6

The kinetic control approach to constructing the CD-spiroacetal segment envisaged a hetero-Michael cyclisation of the dihydropyrone **28** (derived from **26** by sequential oxidation to the β-diketone, PMB removal and cyclisation to provide the D-ring), where axial attack might be favoured (Scheme 7). In practice, treatment of **28** with DBU led to installation of the C-ring *via* hetero-Michael reaction, with a small preference (60:40) for formation of the desired spiroacetal **29** over **30**. Despite the modest selectivity observed in this mode of spiroacetal formation, the endeavour highlighted an important new means for

achieving remote acyclic stereoinduction in a 1,5-*anti* sense, which would prove invaluable to our ongoing spongistatin work.



SCHEME 7

B. ALDOL #4

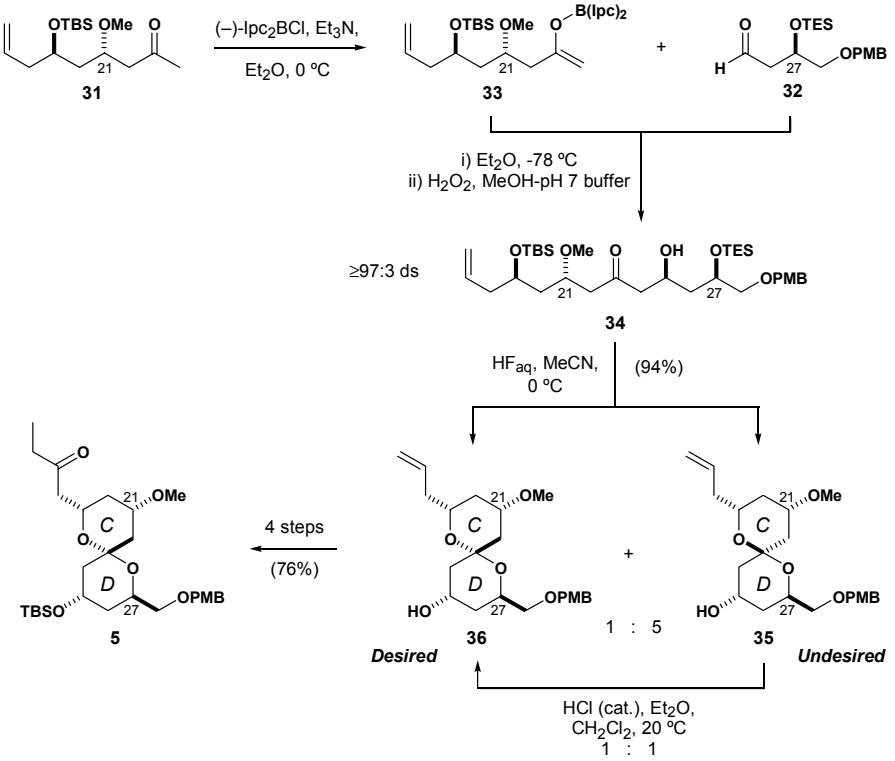
The alternative thermodynamic approach to establishing the CD-spiroacetal subunit relied on the aldol union of ketone **31** with aldehyde **32**, both available in high enantiomeric purity *via* Brown allylation methodology. Enolisation of ketone **31** with (–)-Ipc_2BCl/Et_3N in Et_2O led to regioselective formation of enol borinate **33**, which underwent smooth aldol reaction with aldehyde **32** to provide the adduct **34** in 85% yield, with excellent selectivity for the desired diastereomer (≥97:3 ds). Notably, once again, this boron-mediated aldol reaction exploits triple asymmetric induction, *i.e*. the influence of all three chiral components (aldehyde, ketone and boron reagent) are matched (Scheme 8).

Treatment of **34** with aqueous HF in MeCN solution led to desilylation with concomitant acetal formation favouring the undesired spiroacetal **35** over **36** (*ca*. 5:1). This was not a welcome result! At this juncture, we surveyed some equilibration conditions to yield more favourable quantities of the desired spiroacetal isomer **36**. Bearing in mind the possibility of an intramolecular hydrogen bond between the axial C_{25} hydroxyl and an acetal oxygen in **36**, which is not possible in **35**, we rapidly established that acid catalysed (HCl) equilibration in an aprotic solvent ($CDCl_3$, CH_2Cl_2, Et_2O) provided *ca*. 1:1 mixtures of **35** and **36**.

Separation of these spiroacetal isomers was initially achieved using normal phase semi-preparative HPLC, which allowed re-equilibration of the undesired spiroacetal **35** and thereby a method to convert essentially all of the material to the desired isomer **36** after several cycles. The scale-up of this procedure was greatly facilitated by the determination of a simple method for separating the spiroacetal isomers by flash column

chromatography. Thus, a few hours spent running TLCs in a number of eluent mixtures saved a great deal of labour later on.

The desired spiroacetal **36** was converted to the TBS ether and the terminal alkene moiety was elaborated to the corresponding ethyl ketone in four steps, to provide the fully-functionalised CD-spiroacetal ketone **5**, now ready for aldol union with the AB-spiroacetal aldehyde **4**. This route was found to be highly scalable, enabling production of multi-gram quantities of the desired C_{16}-C_{28} fragment **5** with little need to repeat the synthetic sequence.



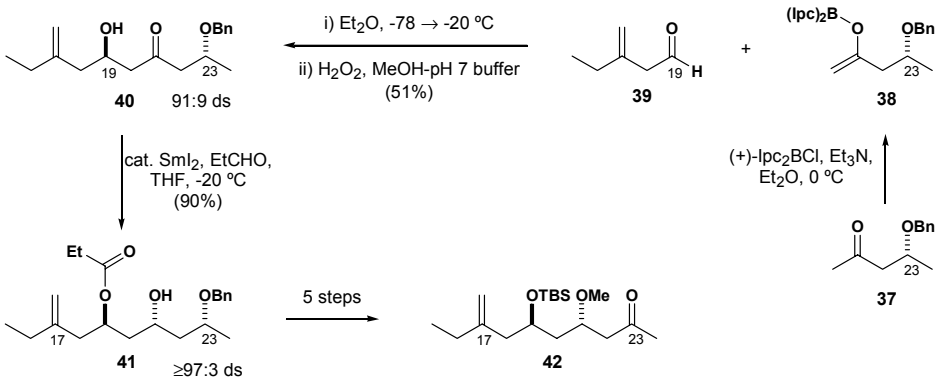
SCHEME 8

C. ALDOL #5

Whereas the thermodynamic route described above relied on reagent control to establish the spongistatin C_{19} and C_{21} stereocentres, the discovery of highly stereoselective 1,5-*anti* aldol reactions of methyl ketones enabled us to examine an alternative,[16] substrate-based stereocontrol route to **5**. Regioselective enolisation of enantiomerically pure ketone **37**, derived from a readily available biopolymer, gave enol

borinate **38** *in situ* (Scheme 9). Reaction of this species with the volatile and readily isomerisable aldehyde **39** provided aldol adduct **40** (91:9 ds) in an unoptimised yield of 51%. The high stereoselectivity of this reaction was the result of the matched 1,5-*anti* preference of ketone **37** and appropriate choice of Ipc ligands on the boron atom. That the acid- and base-sensitive aldehyde **39** undergoes this reaction without any sign of double bond migration is testimony to the mild nature of the boron-mediated aldol reaction.

With the required configuration established at C_{19}, a 1,3-*anti* selective reduction was pursued. The Evans-Tishchenko reduction, utilising catalytic SmI_2 and an aldehyde (in this case EtCHO), was found to be ideal in this instance, providing the mono-acylated diol **41** with complete 1,3-*anti* selectivity (≥97:3 ds). The C_{23} stereocentre, having served its purpose in the remote stereocontrol at C_{19}, is no longer necessary. Thus, after some manipulation of protecting groups, the C23 position was converted to the corresponding carbonyl function providing **42** and thereby enabling the aldol union with aldehyde **32**. From this point, the synthesis proceeded without incident in an analogous manner to that described above.



SCHEME 9

The CD-spiroacetal subunit of the spongistatins proved to be of an appropriate level of complexity that several different synthetic strategies were evaluated. Access to the desired spiroacetal **5** was readily achieved by acid catalysed equilibration of the mixture of spiroacetals in aprotic solvents, followed by separation and recycling of the undesired isomer. Furthermore, the 1,5-*anti* aldol reaction of methyl ketones proved invaluable for construction of certain portions of the target molecule.

V. The Northern Hemisphere

The spiroacetal subunits **4** and **5** were two of the most complex partners to be used in an aldol coupling by our group. Model studies[10b] had confirmed our belief that α-methyl-β-methylene aldehydes of type **4** would undergo aldol reaction with (*E*)-enolates to deliver the *anti*- aldol adducts with high levels of Felkin-Anh induction, corresponding to the desired spongistatin (15*S*,16*S*)-configuration. Furthermore, these model studies had shown that the potentially precarious (14*R*)-stereocentre survived unscathed from this chemistry.

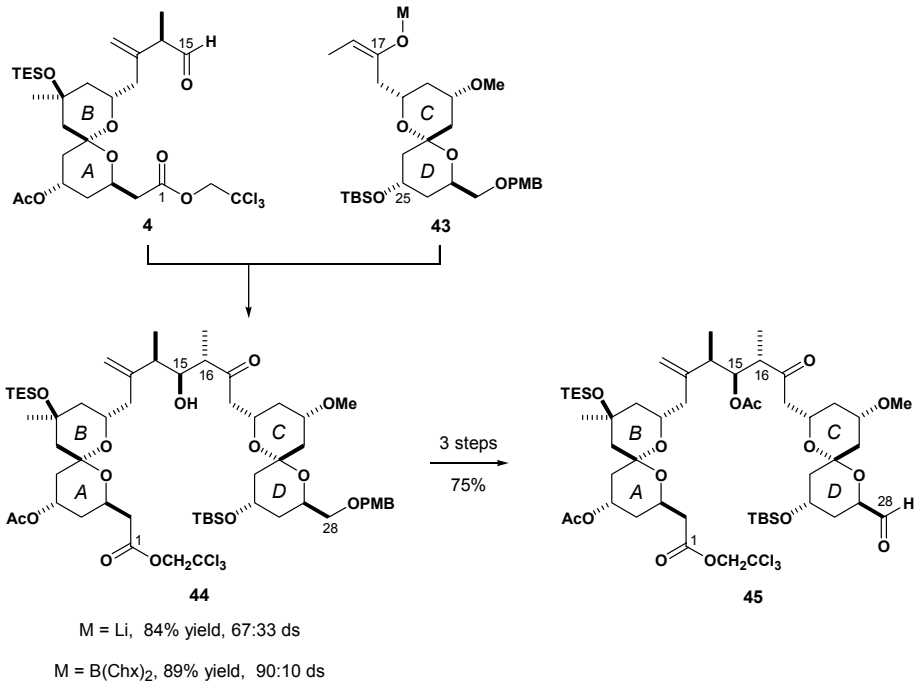
A. ALDOL #6

The first attempts to accomplish aldol coupling of the AB- and CD-spiroacetal subunits involved selective enolisation (Chx_2BCl) of **5** followed by exposure to aldehyde **4**. Following oxidative workup, only trace quantities of the desired aldol adduct were obtained, and furthermore none of the valuable starting materials were recovered! As with most total synthesis endeavours, this key coupling step was first investigated on a very small scale. After establishing successful routes to the two spiroacetal subunits, and fully characterising all relevant compounds, the frontline material was now available to scout out the exciting next steps in the total synthesis. Often it is the case that the first methods investigated are not wholly successful and so valuable advanced material is lost to exploring different ideas and optimising conditions. When the first few trials of the boron-mediated aldol coupling of **4** and **5** had failed to deliver the desired product in synthetically useful quantities and had destroyed valuable materials, attention was quickly turned to the lithium-mediated variant.

Formation of the lithium (*E*)-enolate **43** (2 equiv.) from ketone **5** was readily achieved using LiTMP•LiBr (Scheme 10). Reaction with aldehyde **4** was rapid (< 2 min) at –78 ºC, providing the aldol adduct **44** as the major diastereomer (84%, 67:33 ds).[10c] Additionally, the excess ketone could be recovered quantitatively. This fragment coupling reaction was found to be fairly robust, providing sufficient quantities of **44** to enable further investigation of our proposed synthetic route. However, the possibility of using the boron-mediated variant remained in our minds, and around this time the Evans group reported on a similar coupling step.[4c] If this pivotal coupling reaction were coerced to proceed, the diastereoselectivity was expected to be superior relative to the lithium aldol reaction and a stoichiometry close to 1:1 seemed more feasible in

the case of boron, particularly upon scaling up to larger quantities. For these reasons, the boron-mediated aldol coupling of **4** and **5** was re-investigated intermittently amongst the work being done to carry through material *via* the lithium aldol reaction. It was also around this time that we received a highly encouraging letter from Prof. Pettit, expressing his admiration for the synthetic work which had been carried out thus far within our group, and re-iterating just how exciting the biological properties of the spongistatins were. This was a timely morale boost for us, and a greatly appreciated gesture of support.

Our persistence with the boron-mediated aldol reaction of **4** and **5** was rewarded when the reaction was conducted without recourse to the usual oxidative workup. Other work conducted by our group had shown that oxidative cleavage of certain aldol borinates under standard conditions (H_2O_2, pH 7 buffer, H_2O/MeOH) led to poor yields of the aldol products. In the case of **44,** the oxidative step was omitted and the reaction mixture was placed directly on silica gel and then eluted to afford aldol adduct **44** in excellent yield (89%) and with improved diastereoselectivity (90:10 ds) relative to the corresponding lithium conditions.[17]



SCHEME 10

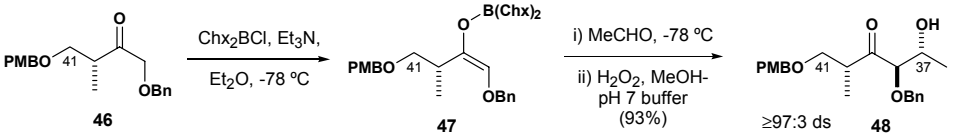
This coupling procedure proved highly efficient upon scaling up and also operationally straightforward, requiring simply that the reaction mixture be placed at the top of a silica gel flash column to break down the intermediate boron aldolate for *ca*. 20 min prior to elution. The remaining three steps required to convert **44** into the fully-functionalised northern hemisphere aldehyde **45** were readily achieved, providing material for Wittig coupling with the southern hemisphere ylide. While we first prepared the ABCD aldehyde **45** in early 1997, we were sadly lacking the southern hemisphere to go forward to complete the total synthesis. This logistical problem required a sustained effort by the "spongi team" to tackle the problems posed by the highly challenging C_{29}-C_{51} fragment.

VI. The Southern Hemisphere

The C_{29}-C_{51} fragment **6** of spongistatin 1 incorporates two tetrahydropyran moieties, the E- and F-rings, along with an array of 11 stereogenic centres. Owing largely to the dense oxygenation of the C_{35}-C_{43} segment and the sensitive nature of the chlorotrienol side-chain, construction of subunit **6** represents one of the more challenging aspects of the spongistatin venture, particularly with regard to choice of appropriate protecting groups. The target fragment **6**, which we selected for our synthetic efforts, is a triphenylphosphonium salt, as required for a (*Z*)-selective Wittig coupling with the northern hemisphere aldehyde **45**. Protection of the hydroxyl groups at C_{38}, C_{41} and C_{42} as the corresponding PMB ethers would allow for late-stage selective deprotection, prior to a regioselective macrolactonisation engaging the C_{41} hydroxyl. The hydroxyl groups at C_{35} and C_{47} would be protected as TBS ethers and the hemiacetal hydroxyl at C_{37} would be masked as the corresponding methyl acetal. It was planned that these silicon protecting groups and the methyl acetal would be removed under mild conditions in the final step of the synthesis.

A. ALDOL #7

Early work on this region of the spongistatins had established a concise route to the C_{36}-C_{46} segment, incorporating the F-ring tetrahydropyran.[18] The synthesis began in earnest with enolisation of enantiopure glycolate ketone **46**, to afford (*E*)-enolate **47** *in situ*, followed by a highly diastereoselective aldol reaction with MeCHO to provide **48** in 93% yield as the sole adduct (≥97:3 ds) by ^{1}H NMR analysis (Scheme 11).



SCHEME 11

This aldol reaction is believed to proceed *via* a chair-like transition state ***TS*-1**, in which the α-hydrogen of the boron enolate eclipses the double bond, in order to minimise A(1,3) strain (Figure 5).[19] The choice then is whether the methyl (***TS*-2**) or the *p*-methoxybenzyloxymethyl ($PMBOCH_2$-) group (***TS*-1**) is directed into the transition state, towards the aldehyde component. On steric grounds, the methyl group may be considered the more likely candidate. However, the stereochemical outcome of this aldol reaction, and many related cases, suggests that it is the $PMBOCH_2$- group which faces inwards. This contra-steric preference has been rationalised on electronic grounds, *i.e*. the disfavoured transition state ***TS*-2** may experience an unfavourable repulsion between the oxygen lone pairs of the boron enolate and the PMB ether. This unfavourable interaction would not be present in the transition state ***TS*-1** leading to the observed product **48**. More recently, Corey and co-workers have proposed the involvement of formyl hydrogen bonds to rationalise the observed stereoselectivity of processes utilising boron and non-boron Lewis acids.[20] Following these suggestions, an intramolecular hydrogen bond between the MeCHO formyl hydrogen and the β-oxygen atom of the enolate, thereby stabilising transition state ***TS*-1**, may then be invoked to rationalise the observed selectivity in the present aldol reaction.

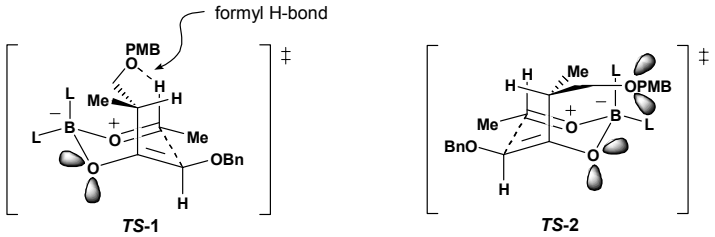


FIGURE 5. DIASTEREOMERIC TRANSITION STATES FOR GLYCOLATE ALDOL REACTION.

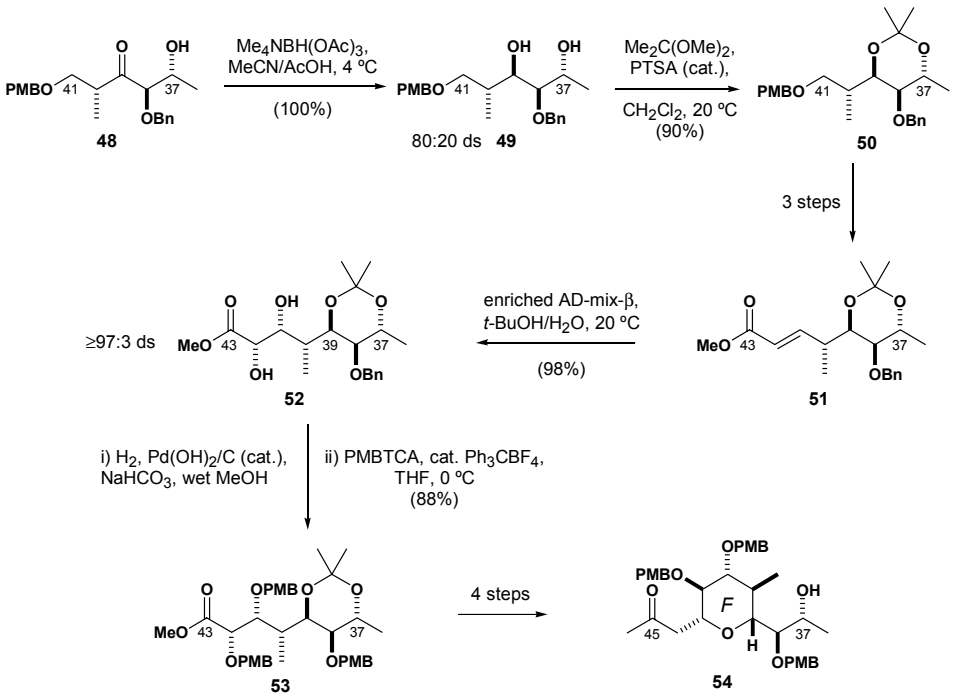
A stereoselective reduction of **48** with $Me_4NBH(OAc)_3$ provided the corresponding 1,3-*anti* diol **49** (80:20 ds), which was then converted to acetonide **50** (Scheme 12). Although these compounds were separable, this was more conveniently achieved at a later stage. Attempts to utilise the samarium promoted Evans-Tishchenko reduction on **48** failed to

provide useful quantities of product, presumably due to unproductive sequestration of the catalytic samarium species *via* chelation between the C_{37} alcohol and C_{38} benzyl ether moieties. Conversion of **50** to α,β-unsaturated ester **51** was achieved in three steps, involving DDQ-mediated removal of the PMB ether, oxidation with the Dess–Martin periodinane and Horner–Wadsworth–Emmons (HWE) olefination. Through synthetic manipulations on compounds in this sequence, the acid-lability of the 1,3-*trans* acetonide became apparent. Hence, transformations performed on compounds bearing this functionality had to be performed using conditions as close to neutral as possible, especially in the presence of protic solvents. Thus, removal of the PMB group from **50** was best achieved in the presence of pH 7 buffer at 0 ºC and oxidation of the resultant alcohol under Swern conditions was found to be unreliable (destroying valuable material on several occasions).

B. ELABORATION TO THE F-RING

The Sharpless asymmetric dihydroxylation protocol, using enriched AD-mix-β, gave solely the desired (41*R*,42*S*)-diol **52** in 98% yield. The importance of the choice of benzyl protecting group at C_{38} is illustrated by the fact that the analogous TBS ether underwent dihydroxylation under the same conditions with markedly reduced facial selectivity (*ca*. 67:33 ds). However, the benzyl ether was an unsuitable protecting group to be carried through to the final stages of the synthesis and hence a protecting group swap was undertaken. Our synthetic strategy called for a regioselective macrolactonisation engaging the C_{41} hydroxyl of a triol *seco*-acid as the penultimate step. This allowed for a relatively simple protecting group plan, involving the use of one mode of protection for the C_{38}, C_{41} and C_{42} oxygens and the application of silyl protecting groups in other regions to be removed in the final step by global deprotection. The implementation of PMB ethers at C_{38}, C_{41} and C_{42} had a number of advantages, including their stability to acid and base used in subsequent steps and orthogonality to the silyl ethers employed elsewhere in the molecule. It was envisaged that the three-fold removal of the PMB groups would be readily effected by treatment with DDQ at a late stage, shortly prior to macrolactonisation. Potential problems we foresaw with this strategy were (i) oxidation of the silyl protected allylic alcohol moiety of the side-chain and (ii) acid-catalysed epimerisation of the sensitive CD-spiroacetal during prolonged exposure to the DDQ conditions. However, we had confidence that suitable conditions could be developed which avoided these prospective pitfalls.

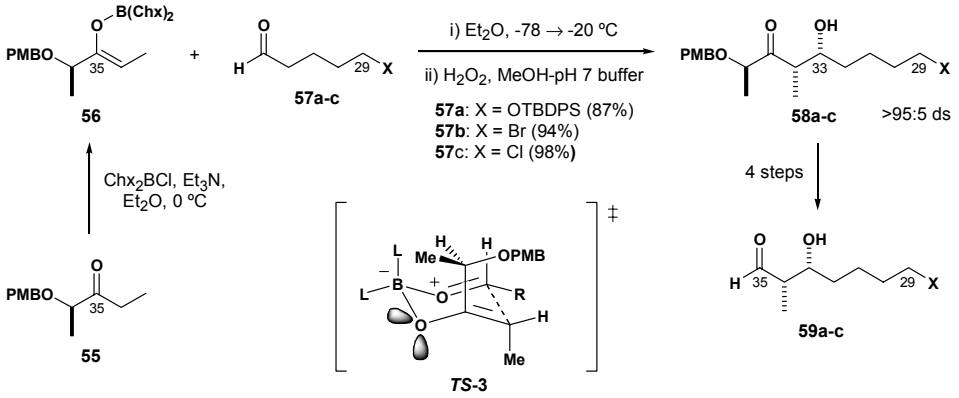
Removal of the C_{38} benzyl ether of **52** was best achieved by hydrogenolysis ($Pd(OH)_2/C$, H_2) in wet MeOH in the presence of $NaHCO_3$.[17] This added mild base was found to be necessary to avoid removal of the acetonide moiety to deliver a highly polar and useless pentaol. In one disastrous early experiment, we sacrificed a substantial amount of advanced material by premature cleavage of the acetonide due to traces of acid – this was a black day for the project indeed! Three-fold PMB protection of the resultant triol was achieved by treatment with *p*-(methoxybenzyl)-trichloroacetimidate (PMBTCA) under mild catalysis with Ph_3CBF_4, providing the *tris*-PMB ether **53** in 88% yield for the two steps. From this point, reduction to the corresponding aldehyde and HWE chain extension with dimethyl (2-oxopropyl)-phosphonate were followed by treatment with acetic acid in aqueous THF and equilibration with KOH in MeOH to yield solely the desired F-ring tetrahydropyran **54**, having all five substituents equatorially arranged.



SCHEME 12

C. ALDOL #8

At this juncture, the C_{36}-C_{46} segment **54** could, in principle, be elaborated to append the C_{47}-C_{51} side-chain or the C_{29}-C_{35} E-ring fragment, in either order. Indeed, both of these options were explored, however, due to the labile nature of the C_{47}-C_{51} side-chain, the most successful strategy involved first appending the F-ring segment then the sensitive chlorodienol side-chain. The C_{29}-C_{35} segment was rapidly constructed by utilising some suitable *syn*-aldol chemistry developed by our group (Scheme 13).[21] Treatment of lactate-derived, PMB-protected, α-hydroxy ketone **55** with Chx_2BCl/Et_3N led to selective formation of the (*Z*)-enol borinate **56**, in contrast to the (*E*)-selective enolisation obtained with the analogous benzoyl protected ketone. Reaction of **56** with aldehyde **57**, followed by oxidative workup, provided the *syn*-aldol adduct **58** in good yield (>95:5 ds). This reaction is believed to proceed *via* transition state ***TS*-3**, where the PMB ether and enolate oxygens are directed away from each other and the methyl group is outside. Four further steps were then required to arrive at the desired aldehyde **59**, ready for aldol coupling with the F-ring subunit.



SCHEME 13

Initial work involved the functionalisation of C_{29} as a TBDPS ether (as in **59a**). However, this proved to be incompatible with our overall approach. An alternative, and somewhat more direct strategy involved the incorporation of a halogen at C_{29} from the outset. This was expected to be entirely compatible with subsequent steps, conveniently undergoing transformation into the corresponding triphenylphosphonium salt at a later stage. Initially, the bromide **59b** was chosen and indeed was found to be admirably compatible with the ensuing chemistry. However, during

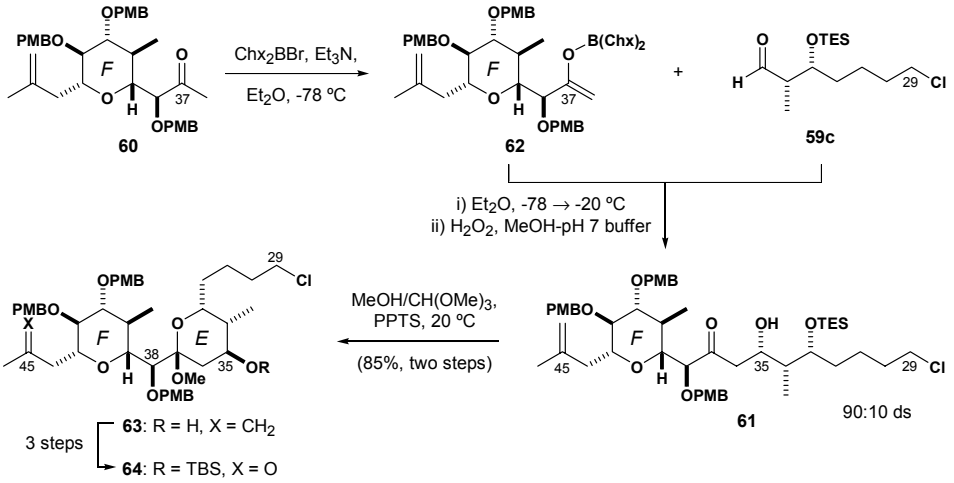
prolonged exposure of a subsequent intermediate to conditions involving TBSCl and imidazole in DMF, significant halide exchange was observed to yield inseparable Br/Cl mixtures. Although this was not a terminal problem for the synthesis plan, it did unnecessarily complicate the interpretation of characterisation data. Utilisation of the corresponding chloride **59c** from the beginning, removed these complications and was readily converted into the desired phosphonium salt, *via* the iodide, at a later stage.

D. ALDOL #9

The aldol reaction to unite ketone **60**, available from **54** by Petasis olefination and oxidation at $C_{37,}$ with aldehyde **59c** proved a highly challenging task (Scheme 14). Earlier model studies of the Mukaiyama aldol reaction of aldehyde **59c** with simple lactate-derived silyl enol ethers had demonstrated a strong preference for Felkin-Anh induction from the aldehyde. This preference was also exhibited to varying extents in the reaction of aldehyde **59c** with boron, tin and lithium enolates. The application of these methods to the coupling of the highly oxygenated ketone **60** and aldehyde **59c** failed to deliver any of the desired compound **61**. The lithium-mediated aldol reaction was the only variant to deliver any aldol adduct, and this was found to be the undesired diastereomer, in the opposite sense to Felkin-Anh attack on aldehyde **59c**. Given the numerous successes of boron enolates in the coupling of complex fragments, we were disappointed by the failure of Chx_2BCl/Et_3N conditions to provide any desired aldol adduct, resulting solely in recovery of starting materials. Several attempts were made to effect the desired transformation using Chx_2BCl under a variety of conditions, none of which led to the formation of the desired aldol adduct. However, when recourse was made to the use of the more reactive Chx_2BBr in this procedure, success was had at last!

Treatment of ketone **60** with Chx_2BBr/Et_3N in Et_2O at –78 ºC led to smooth enolisation to give **62**, which was followed by reaction with aldehyde **59c** to provide aldol adduct **61** as the major diastereomer (90:10 ds). Provided that recently prepared (<2 weeks old), high quality Chx_2BBr was used, this procedure reproducibly delivered the desired aldol adduct at a variety of scales. Acid-promoted desilylation (PPTS, MeOH, $(MeO)_3CH$) was accompanied by formation of the E-ring as a methyl acetal **63**, and at this stage separation of the small amount of C_{35} epimeric compound was readily achieved. Three straightforward steps then provided the C_{29}-C_{46} methyl ketone **64**, ready for introduction of the

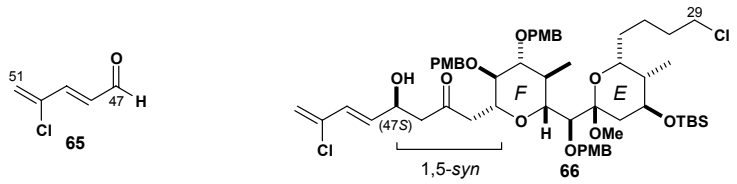
remaining elements of the side-chain. It took considerable time, effort and persistence to achieve the synthesis of the C_{29}-C_{46} subunit **64**, but once a path had been cleared, the chemistry was found to be robust and readily allowed for the production of significant quantities of advanced material for the southern hemisphere.



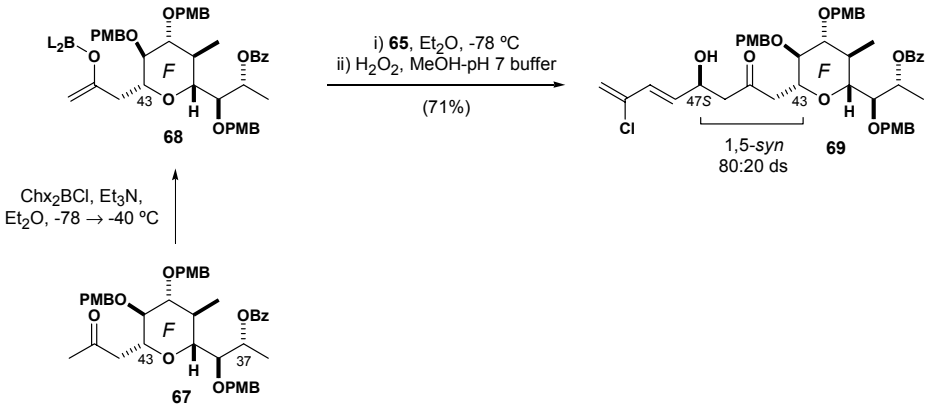
SCHEME 14

E. ALDOL #10

The final aldol reaction used in our synthesis of spongistatin 1 was one of the more remarkable reactions of this type our group has witnessed over the years. The aldol union of ketone **64** with (*E*)-4-chloro-2,4-pentadienal **65** required the creation of the (47*S*) stereochemistry in the resultant alcohol **66**. Formally, this would require 1,5-*syn* induction from the ketone **64**, which is opposite to that observed previously for boron aldol reactions with simple β-alkoxy methyl ketones. However, ketone **64** is densely packed with stereocentres, and predicting the influence of these remote centres on the reaction outcome was not possible with any degree of certainty. It was hoped that should **64** display undesirable 1,5-*anti* bias, this may be overturned by appropriate choice of Ipc ligands on boron.



Model studies related to this aldol coupling involved the reaction of F-ring ketone **67** with aldehyde **65**. Enolisation with Chx_2BCl to produce **68**, and aldol reaction with **65** provided adduct **69** in 71% yield, following oxidative workup, as the major diastereomer (80:20 ds) by ^1H NMR analysis (Scheme 15). Furthermore, this substrate-based stereocontrol could be reinforced by the use of (+)-Ipc_2BCl to increase the reaction diastereoselectivity to 91:9 ds. These results were very encouraging, not only were we able to effect aldol reaction with the sensitive aldehyde **65**, but we appeared to have a good chance for inducing the desired configuration at C_{47} for the spongistatin side-chain. Once the full C_{29}-C_{46} fragment **64** became available, it was time to move away from model studies and onto the "real thing!"

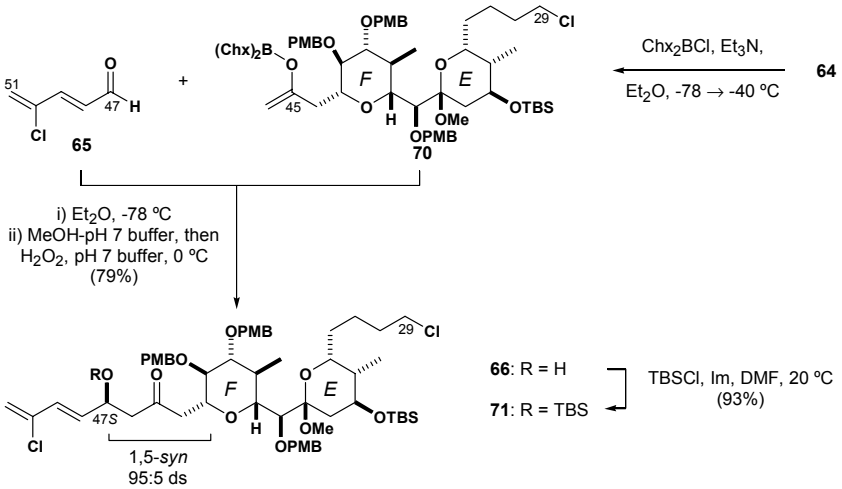


SCHEME 15

The lithium aldol reaction between ketone **64** and aldehyde **65** was successful at providing moderate quantities of a *ca*. 1:1 mixture of **66** and its undesired C_{47} epimer, which were readily separable by TLC and flash chromatography. With TLC samples of these two aldol adducts at hand, ketone **64** was subjected to Chx_2BCl/Et_3N resulting in clean formation of enol borinate **70** which was exposed to an excess of **65** (Scheme 16). Analysis of the reaction mixture by TLC clearly showed the disappearance of ketone **64** accompanied by appearance of aldol adduct **66** and minute amounts of undesired C_{47} epimer. Such a clearly efficient and selective reaction caused great excitement! However, elation turned to despair when, after a standard oxidative workup, none of the desired aldol adduct could be isolated. Clearly the oxidative workup was at fault once again and was to be avoided. The reaction between **64** and **65** was repeated, this time placing the reaction mixture directly on a silica column. In this instance, the aldol adduct **66** was isolated in 73% yield

with excellent selectivity (95:5 ds) for the 1,5-*syn* product. The level and direction of stereocontrol observed in this reaction was a very welcome surprise, and could not have been planned beforehand, and reflects how great an influence remote stereocentres can have in boron-mediated aldol reactions.

The material provided by this initial reaction was sufficient to explore some further chemistry, but soon there was a need for more. Repetition of the procedure resulted in highly variable results. In some cases, the aldol adduct was afforded in significant yield, while in several other cases little or none of **66** was isolated. On one of these anguishing occasions, the column fractions seen to contain **66** were noticed to visibly darken and become cloudy as solvent was removed *in vacuo*. Analysis of these samples by ^{1}H NMR showed broad signals related to regions of **66** but no sign of the desired product itself. In another instance, an evaporated column fraction thought to contain **66** was seen to slightly fume when bench-grade CH_2Cl_2 was added to it! In despairing of ever persuading this key reaction to reproducibly yield **66**, it was tempting to dismiss these observations as illusory, conjured up by a weary mind. However, it seemed most likely that the silica workup of the reaction was proving insufficient at breaking down the intermediate boron aldolate and/or boronic acid related impurities were co-eluting with the desired product and then destroying it upon concentration.



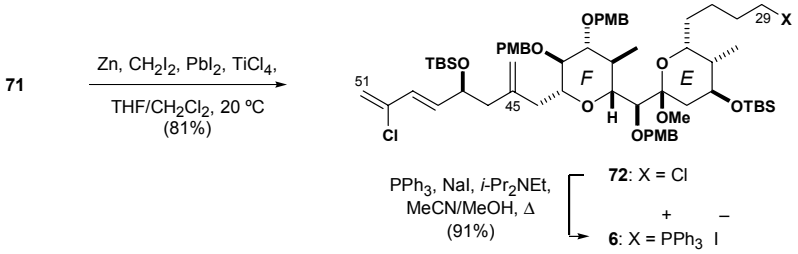
SCHEME 16

A re-evaluation of the oxidative workup procedure was warranted in the current instance. Although this method had failed previously, it was reasoned that a carefully controlled oxidative workup may result in a

more robust and reliable procedure. Gratifyingly, a protocol was rapidly developed that provided a consistently high yield of the desired aldol adduct **66**. This involved very careful control of the quantity of H_2O_2 used, and the way in which it was introduced to the reaction mixture. Utilising this procedure, it was possible to reproducibly obtain the desired aldol adduct in good yield (79%), and maintaining excellent 1,5-*syn* selectivity (95:5 ds). Protection of allylic alcohol **66** as the corresponding TBS ether was best performed by brief exposure to TBSCl and imidazole in DMF, providing **71**, which was much more stable towards storage than **66**.

F. EF-SUBUNIT: THE FINAL STEPS

Methylenation of the carbonyl function within **71** was required and model studies on related compounds had suggested that the Petasis olefination was unsuitable in this case. The modified methylenation procedure of Takai[22] was found to be more productive. However, once again we encountered problems of reproducibility. In this case, the quality of the zinc metal used was found to be absolutely crucial to the success of the reaction. Zinc powder which had been purified according to standard procedures was found to be unsatisfactory. The most reliable method for activating the zinc, prior to formation of the Takai methylenating reagent was treatment of a suspension of the zinc in THF with an aliquot of TMSCl, followed by removal of the THF solution and successive washes of the zinc with fresh THF. When this procedure was adhered to the desired methylenation product **72** was obtained in reproducibly high yields (81%). The final step, in preparation for the Wittig coupling with the northern hemisphere aldehyde **45**, involved the direct conversion of **72** into phosphonium salt **6** by heating with PPh_3 in the presence of NaI (Scheme 17).[17]



SCHEME 17

VII. Wittig Coupling of the Northern and Southern Hemispheres

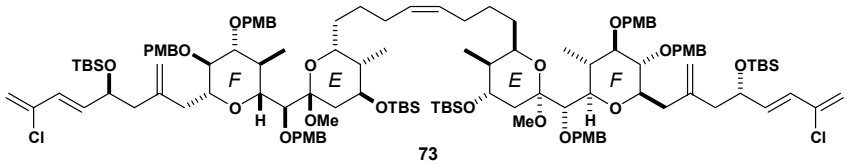
Significant effort was expended on model studies of the crucial ABCD + EF Wittig coupling step. This work not only provided a means for testing this strategy with regard to effectiveness and level of (*Z*)- double bond selectivity, but equally important was the development of practical methods for conducting this step. Initial model studies involving the Wittig coupling of E-ring phosphonium salts with simple aldehydes failed to give synthetically viable quantities of the desired alkene products under standard conditions (LHMDS, THF). The weak colourations observed in the ylide formation step suggested that adventitious water was having a deleterious effect on the reaction, despite best efforts to avoid any traces of moisture in the system. This may have been largely due to the hygroscopic nature of the phosphonium salt, but was likely exacerbated by the extremely small scale at which these reactions were being performed. Two possible desiccants were examined as *in situ* scavengers of water. Perhaps unsurprisingly, 4Å molecular sieves were ineffective in this role, whereas powdered calcium hydride was found to be uniquely effective. A model Wittig coupling between an E-ring phosphonium salt and EtCHO which proved unproductive under standard conditions, provided the desired alkene in 80% yield when CaH_2 was incorporated into the reaction medium. From this point, our confidence in completing the total synthesis grew steadily.

A. DELAYED DEPARTURE

Having spent a productive three year period of laboratory work associated with this project, it was nearing time for MJC to write-up his PhD thesis and move on to other things. It was proposed that the major component of this thesis writing should be performed in Australia, and so a non-refundable, no date changes, airline ticket had been purchased for the trip well ahead of time, it being a difficult thing to procure flights of this type at an affordable student price. However, there was such momentum with the work with recent breakthroughs, that at this point all parties agreed that more time was needed to crack the last few problems. So as the original airline ticket passed its date and became void, another one was secured for a later date and the toughest leg of the spongistatin journey was undertaken.

Extension of the *in situ* CaH_2 method to the Wittig reaction of more complex model compounds, on ever smaller quantities of material, led progressively to the formation of C_{16}-C_{39} (CDE), C_1-C_{39} (ABCDE) and

C_1-C_{46} (ABCDEF) spongistatin substructures, all accompanied by excellent selectivity (>95:5) for the required (*Z*)-alkene. Despite the psychological boost afforded by producing these large structures, the yields obtained in these more complex situations were lower than desired (14-32%). Nevertheless, with great anticipation the Wittig coupling of the fully-functionalised, C_{29}-C_{51} phosphonium salt **6** with aldehyde **45** was attempted. To our great disappointment, utilisation of the above Wittig coupling procedure, with *in situ* CaH_2, failed to provide any of the desired product. Indeed, a significant quantity (24%) of an undesired by-product **73** was obtained. Ylides which do not contain stabilising α-substituents are well known to react rapidly with molecular oxygen, to afford symmetrical alkenes by autoxidative self-condensation, presumably *via* the intermediacy of the corresponding aldehyde. Hence, the presence of adventitious oxygen appeared to be the latest evil to be combated.

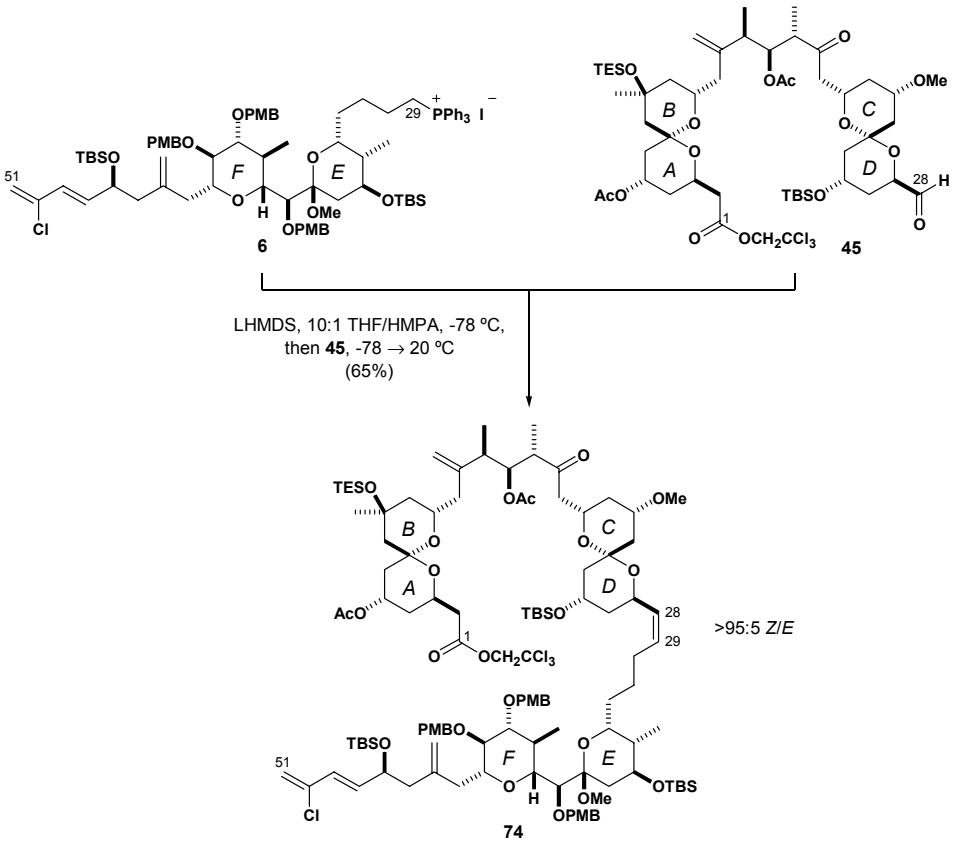


73

In order to conserve precious quantities of the full C_{29}-C_{51} phosphonium salt **6**, a model E-ring compound was once again utilised. A freeze-pump-thaw protocol was utilised in an effort to degas the phosphonium salt solution and aldehyde **45** prior to their attempted union. Under these conditions, exasperatingly, the corresponding E-ring alkene dimer, resulting from oxygen-induced autoxidative dimerisation, was produced in near quantitative yield!

At this point, so much effort had been expended on exploring the use of this Wittig coupling methodology that supplies of the model phosphonium salts had been exhausted. In a brave (or perhaps foolish) move, a modification to the previous Wittig procedure was attempted on the fully-functionalised components, phosphonium salt **6** and aldehyde **45** (Scheme 18). Firstly, an elaborate attempt was made to prevent the introduction of oxygen into the reaction medium. A second modification to the Wittig procedure was the use of HMPA as a co-solvent for the reaction. The decision to use HMPA was based upon the success of Nicolaou and co-workers in their coupling of two complex fragments, *via* Wittig reaction with HMPA as co-solvent, in their synthesis of brevetoxin B.[23] Despite the risks associated with changing two variables at the same time and performing the reaction on valuable "front-end" material, we

were confident we had a much greater understanding of the important factors in this crucial Wittig coupling step.



SCHEME 18

Encouragingly, under these new conditions (LHMDS, THF/HMPA), phosphonium salt **6** yielded an intensely orange-coloured ylide solution. After introduction of aldehyde **45**, the resultant mixture was warmed to 0 ºC whereupon it turned blood red in hue. These heartening signs were first observed on a wintry Friday evening in Cambridge and were soon reinforced by promising TLC evidence. After a rapid workup and a column conducted with bated breath and barely contained excitement, a quick trip to the NMR spectrometer uncovered those two joyous, greatly anticipated alkene signals at *ca*. 5.5-5.8 ppm, along with all the other complexities associated with the ABCD and EF portions of the molecule brought into intimate association. As was customary for the Paterson group on a Friday night, most of them, including the leader, were relaxing in the local pub (The Panton Arms). So the single page A3 printout of the

^{1}H NMR spectrum for **74** was proudly taken directly to the Panton, resulting in a spontaneous celebration. Although somewhat stained by its use as a beer mat that night, this long sought-after NMR spectrum is still preserved as a memento of that important breakthrough. It may not yet have been the natural product, but **74**, which represented the fully protected *seco*-acid of spongistatin 1, could now be reliably produced in 60-65% yield (>95:5 *Z*/*E*). Given the efforts we had made to ensure a concise endgame we were now truly on the home stretch.

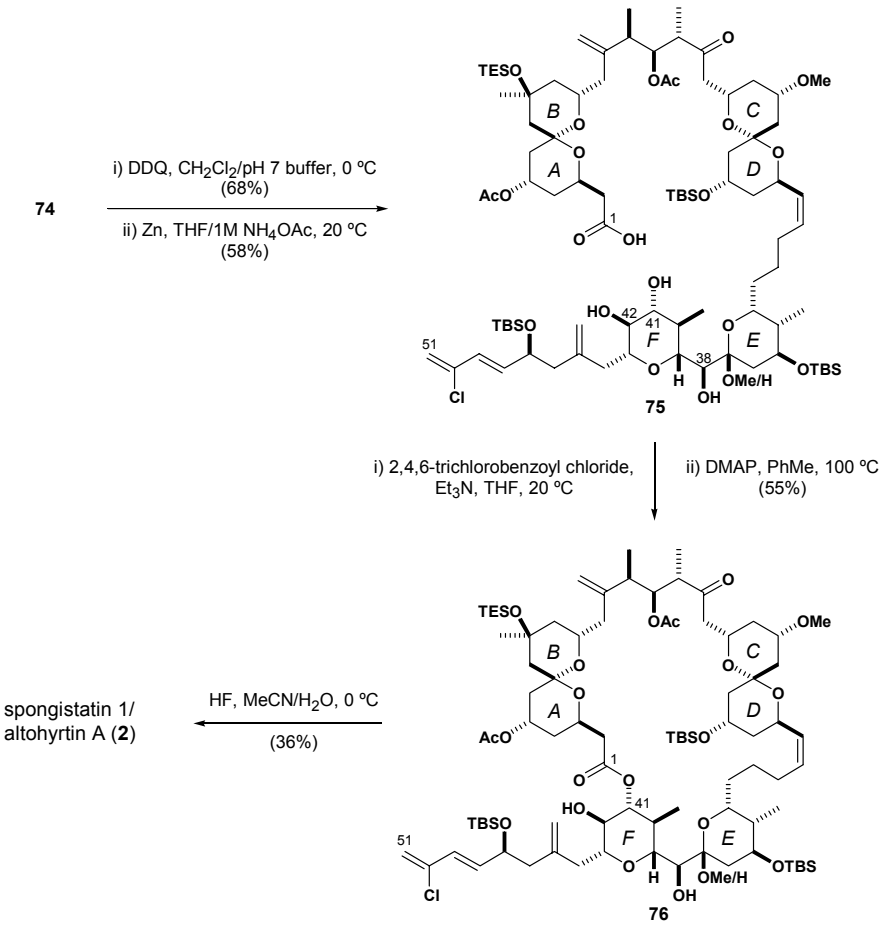
VIII. Final Steps

With the fully protected *seco*-acid **74** of spongistatin 1 in hand, all that remained to complete the total synthesis was removal of the PMB ether and trichloroethyl ester protecting groups, regioselective macro-lactonisation and global deprotection. The DDQ-mediated removal of the three PMB groups in **74** turned out to be relatively trouble-free, involving brief exposure to an excess of DDQ in the presence of pH 7 buffer (Scheme 19). This accomplished the smooth removal of all three PMB groups without effecting the sensitive chlorodienol side-chain or CD-spiroacetal subunits. Partial hydrolysis of the E-ring methyl acetal to the corresponding hemiacetal was unavoidable, but of little consequence as the mixture was able to be taken through the remaining steps of the synthesis. Exposing the C_1 carboxylic acid was achieved by treatment with zinc powder in THF/1M NH_4OAc to yield the *seco*-acid **75**.

A. THE FINAL COUNTDOWN

With the clock ticking down to a flight from Heathrow (in time for MJC to celebrate Christmas 2000 in Australia) and very little material available (<1 mg), the macrolactonisation step was pursued. Due to the extremely small scale involved, monitoring the reaction progress by TLC was found to be impossible. After a slow (7 hour) addition of the anhydride to the refluxing DMAP solution (in C_6H_6), TLC analysis of the concentrated mixture was not encouraging. Attempts to purify the crude mixture by micro-scale flash chromatography were made difficult due to the large excess of 2,4,6-trichlorobenzoyl chloride related by-products. Exhaustive analysis of column fractions by ^{1}H NMR (500 MHz, C_6D_6) revealed a component which appeared to correspond with the desired macrocycle **76**. Further purification of this heavily contaminated macrocyclic component by HPLC was found to be difficult. The

compound did not appreciably absorb at 254 nm, the typical wavelength used for HPLC analysis. Presumably the UV spectral characteristics of **76** are similar to that of spongistatin 1, λ_{max} (MeOH) = 216 nm. Detection at these shorter wavelengths prohibits the use of EtOAc mixtures as eluent. In the event, purification of the crude macrocyclic component by HPLC (10:90 *i*-PrOH/hexanes, detection at 220 nm) was not successful. Regardless, the mixture was subjected to global deprotection conditions in the hope that a small sample of spongistatin 1 could be obtained.



SCHEME 19

Exposure of **76** to HF in H_2O/MeCN for 20 hours was followed by a short microscale flash column. Analysis by ^{1}H NMR spectroscopy (500 MHz, CD_3CN, 16 hours) of the global deprotection product showed it to be a complex mixture, however there were some encouraging signals. Purification of this mixture was attempted by HPLC-MS (25:75

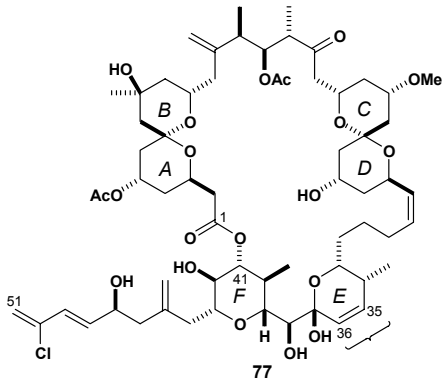
H_2O/MeOH). It was with great excitement that a peak was observed (R_t 13 minutes) with strong ions at m/z 1245.6 ($[M + Na]^+$, 100) and 1240.1 ($[M + NH_4]^+$, 23). This component was collected and analysed by high field ^1H NMR, once again requiring >16 hours of acquisition to observe the important signals, heavily swamped by impurities. Unfortunately, the HPLC had not been overly successful, probably due to overloading of the analytical scale column, but some characteristic signals were evident. Although not conclusive at the time, this close match of partial data from ^1H NMR spectra provided tantalising evidence that the preceding steps resulted in the formation of a small quantity (50 micrograms at best) of our first crop of synthetic spongistatin 1 (**2**).

At this point, the revised departure date for MJC had arrived, and although it was tempting for him to remain longer and resolutely tie down the total synthesis, it was time to move on, and write up his thesis. The well-poised project passed into the capable hands of David Chen, as the final member of the "spongi team". A typically impressive solo effort from David at this late stage resulted in many improvements in the final steps of the synthesis, accomplished over the first few months of 2001. Together with the prior development of robust, highly scaleable routes to the individual subunits, this now allowed the generation of significant quantities (ca. 15 mg) of spongistatin 1, allowing its complete characterisation (including a stunning 800 MHz NMR spectrum, as well as the first pristine ^{13}C NMR to be obtained on synthetic material), and enabling the biological testing of this much sought after anticancer agent to be resumed. Indeed, some of our synthetic spongistatin was provided to Prof. Pettit at the Cancer Research Institute in Arizona to allow further preclinical evaluation. However, this was not quite the end of the "spongi" tale of ten aldols.

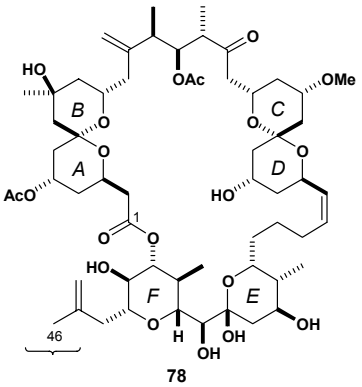
B. SYNTHETIC SPONGISTATIN ANALOGUES - “SPONGILOGUES”

An important advantage afforded by the synthesis of natural products is the access this provides to structurally diverse analogues, enabling the establishment of structure-activity relationships (SAR). Little is known about the spongistatins in this regard. Our synthesis rapidly provided access to two novel "spongilogues" which have proven valuable as SAR probes.[24] The E-ring dehydrated compound **77** was afforded as a minor (and, at the time, unwanted) by-product of the final aqueous HF induced deprotection step. The side-chain truncated compound **78** was obtained from Wittig coupling of a C_{29}-C_{46} phosphonium salt with northern hemisphere aldehyde **45**, followed by deprotection, macrolactonisation

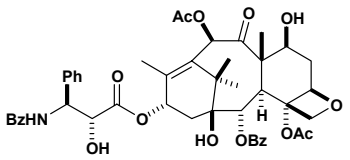
and global deprotection steps. Illustrated in Table 1 are the results of growth inhibition experiments for paclitaxel/Taxol (**79**), synthetic spongistatin 1 (**2**), E-ring dehydrated analogue **77** and side-chain truncated analogue **78**.



77



78



79: paclitaxel/Taxol

In all cases, **2** was found to be substantially more active (6- to 2000-fold) than paclitaxel (**79**), and was particularly effective against the MIP101 cell line, indicating that it is a poor substrate for the P-glycoprotein (Pgp) drug efflux pump. Given the already exceptional cytotoxicity displayed by **2**, we were surprised and delighted to observe that the E-ring dehydrated analogue **77** was generally (2- to 4-fold) more potent than the parent natural product. Analogue **77** had low picomolar IC_{50} values, in the range 0.007-0.08 nM, against this set of cancer cell lines. This indicates that the C35 hydroxyl of **2** is unnecessary for biological activity, and that its removal leads to an increase in potency. In contrast, the dramatic attenuation of cytotoxicity for analogue **78**, against all cell lines employed in these assays (*e.g.* 0.587 and 0.407 μM against the MIP101 and HCT116 colon carcinoma cell lines), reveals that the C_{47}-C_{51} chlorodiene allylic alcohol moiety is an essential structural feature. These results suggest that the full C_{44}–C_{51} triene side-chain is a crucial part of the spongipyran pharmacophore.

TABLE I
GROWTH INHIBITION AGAINST HUMAN CANCER CELL LINES

IC_{50} values (nM)	**79**	**2**	**77**	**78**
MIP101 colon (Pgp-1 overexpressing)	200	0.1	0.08	587
HCT116 colon	0.3	0.05	0.02	407
1A9PTX22 ovarian (mutation in β-tubulin)	47	0.03	0.007	> 632
1A9 ovarian (parental)	1	0.03	0.007	> 632
A549 non-small cell lung	6	0.07	0.04	> 632

IX. Conclusions

This total synthesis adventure advanced our knowledge of factors influencing the stereochemical outcome of complex aldol coupling steps and other reactions, and also resulted in better understanding of the criteria for selecting appropriate tactics and strategies for complex natural product synthesis. Overall, our synthetic route to **2** proceeds in 33 steps and 1.0% yield for the longest linear sequence. With respect to material output, valuable quantities of synthetic spongistatin 1/altohyrtin A were obtained (approximating to the 2.4 tons of the initial sponge collection required for isolating the natural product), enabling further biological evaluation of these remarkable anti-cancer compounds. Additionally, by using the developed methodology, two novel spongistatin analogues were synthesised and tested for growth inhibition against human tumour cell lines, providing invaluable SAR data to help define the pharmacophore.

The "spongi" journey was at times a bumpy and frustrating one, but nevertheless wholly rewarding to all involved. Once more, Charles Dickens' *A Tale of Two Cities* has a poignant passage in this regard:

"Unsettled weather, a long journey, uncertain means of travelling, a disorganised country, a city that may not be even safe for you."

"My dear Charles," said Mr. Lorry, with cheerful confidence, "you touch some of the reasons for my going: not for my staying away."

Acknowledgements

The work described in this chapter would not have been possible without the tireless efforts and experimental skill of the following people (the "spongi" team): José Luis Aceña, Jordi Bach, David Chen, Karl Gibson, Andrew Hodgson, Linda Keown, Roger Norcross, Renata Oballa, Thomas Trieselmann, Debra Wallace.

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