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The stereocontrolled total synthesis of altohyrtin A/spongistatin 1: fragment couplings, completion of the synthesis, analogue generation and biological evaluation†‡

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Received 22nd March 2005, Accepted 3rd May 2005
First published as an Advance Article on the web 24th May 2005

The antimitotic marine macrolide altohyrtin A/spongistatin 1 (**1**) has been synthesised in a highly convergent and stereocontrolled manner, thus contributing to the replenishment of the largely exhausted material from the initial isolation work. Coupling of the AB- and CD-spiroacetal subunits by a stereoselective aldol reaction was achieved by using either a lithium (67 : 33 dr) or boron enolate (90 : 10 dr). A highly (*Z*)-selective Wittig coupling was used to unite the northern hemisphere aldehyde **2** with the southern hemisphere phosphonium salt **3**. Deprotection and subsequent regioselective macrolactonisation on a triol *seco*-acid completed the synthesis of altohyrtin A. Two structural analogues were also prepared and evaluated as growth inhibitory agents against a range of human tumour cell lines, including Taxol-resistant strains, alongside altohyrtin A and paclitaxel (Taxol), revealing that dehydration in the E-ring is tolerated and results in enhanced cytotoxicity (at the low picomolar level), whereas the presence of the full C44–C51 side-chain appears to be crucial for biological activity.

Introduction

Altohyrtin A/spongistatin 1 (**1**, Scheme 1) is a remarkably cytotoxic macrolide that is in extremely limited supply from its natural marine sponge sources.[1–3] Our highly flexible, convergent synthetic approach[1,4] to the spongipyrans was designed to allow the generation of useful quantities of **1** for the resumption of biological studies, in addition to facilitating the synthesis of various altohyrtin congeners and wholly synthetic analogues for implementing structure–activity relationship (SAR) work. Furthermore, the endgame for our synthesis required careful planning and judicious choice of reagents and conditions in order to preserve the sensitive functionality present within late-stage compounds, *e.g.*, the acid-sensitive CD-spiroacetal and chlorotriene side-chain. The final stages of our synthetic strategy for altohyrtin A involve three key bond-forming steps

† Part 4 of a series of four papers.[1]

‡ Electronic supplementary information (ESI) available: general experimental information and procedures for the synthesis of compounds not detailed in the Experimental section of this paper. See http://www.rsc.org/suppdata/ob/b5/b504151a/

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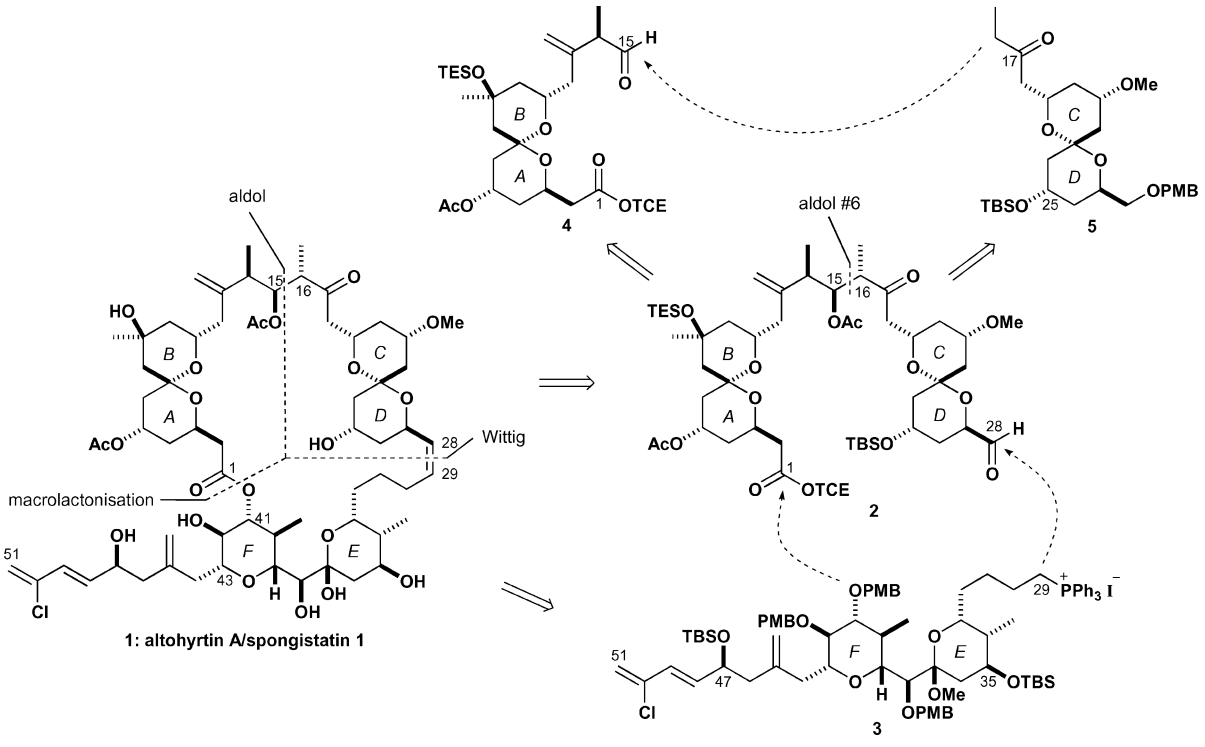
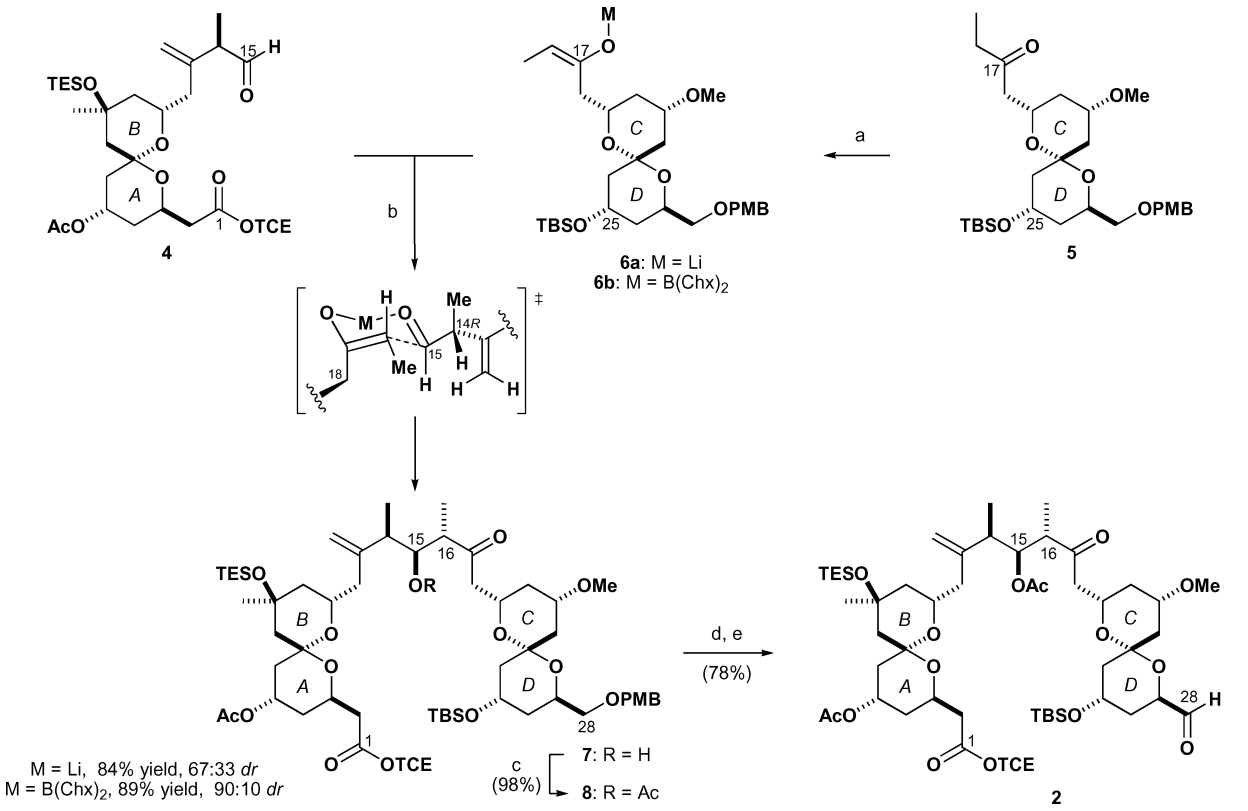


Scheme 1 Fragment couplings for the synthesis of altohyrtin A/spongistatin 1 (**1**).

DOI: 10.1039/b504151a



Scheme 2 *Reagents and conditions*: for M = Li: (a) LiTMP–LiBr, THF, −78 °C, 15 min; (b) **4**, −78 °C, 3 min; for M = $B(Chx)_2$: (a) Chx_2BCl, Et_3N, Et_2O, −78 → 0 °C, 20 min; (b) **4**, −78 → −20 °C, 17 h, then SiO_2, rt, 40 min; (c) Ac_2O, DMAP, pyr, rt, 2 h; (d) DDQ (portionwise), CH_2Cl_2–pH 7 buffer (10 : 1), 0 °C, 2 h; (e) cat. TPAP, NMO, 4 Å mol. sieves, CH_2Cl_2, rt, 15 min.

(Scheme 1). Macrocycle formation would be achieved by Yamaguchi macrolactonisation of a fully functionalised *seco*-acid, engaging the C41 alcohol selectively, as initially reported by Evans[2c,d] and Kishi[2f] on related systems. This advanced intermediate, in turn, would be derived, in the forward sense, by (*Z*)-selective Wittig coupling of the ABCD northern hemisphere aldehyde **2** and the EF southern hemisphere phosphonium salt **3**. An *anti*-aldol reaction between the AB-spiroacetal aldehyde **4** and the CD-spiroacetal ketone **5**, under Felkin–Anh control, would assemble the C1–C28 ABCD subunit. Herein, we provide a full account of the final fragment-coupling stages of our total synthesis of altohyrtin A, and the synthesis and biological evaluation {alongside **1** and paclitaxel (Taxol)} of two synthetic analogues of the natural product.

Results and discussion

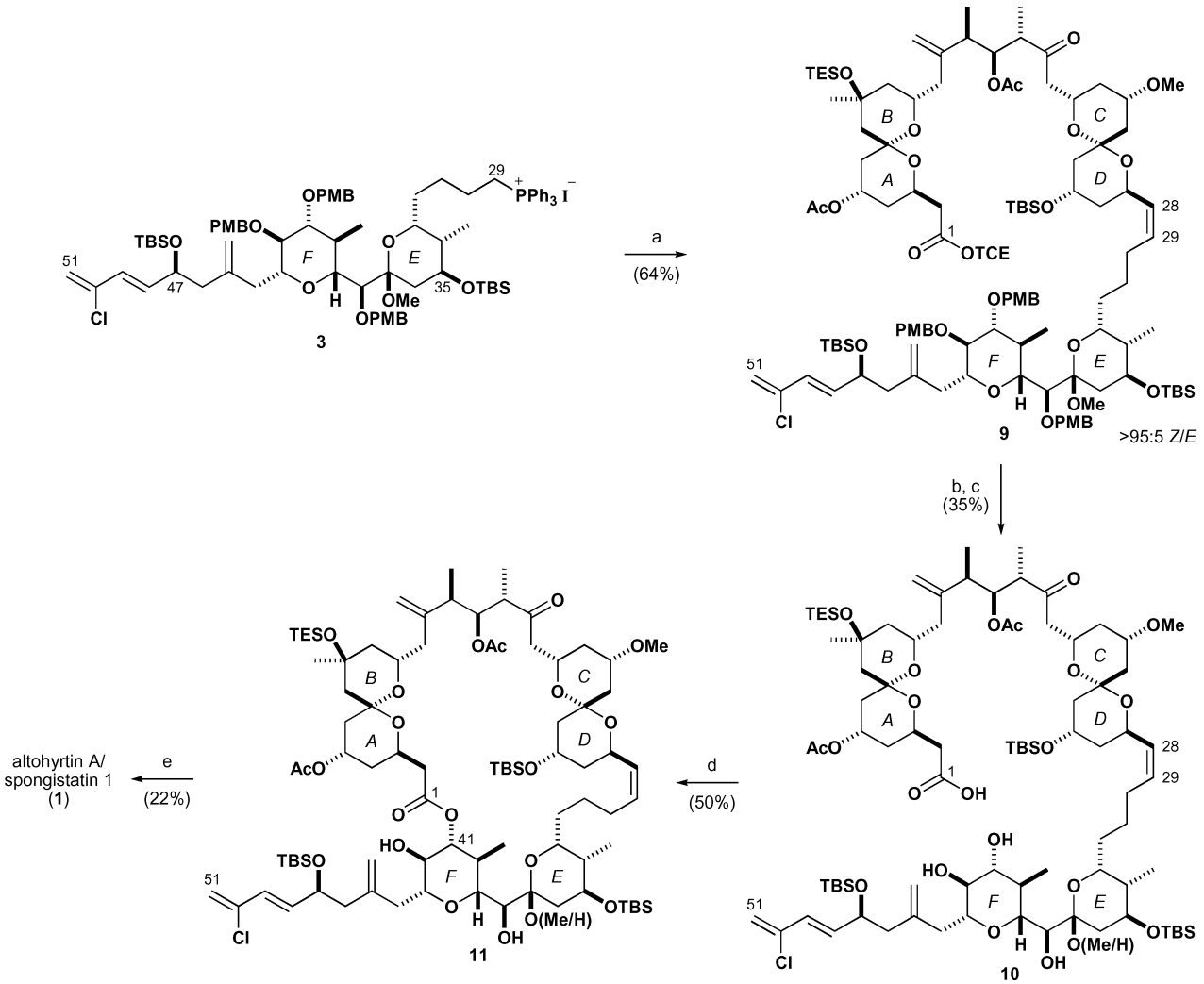
Aldol coupling of the AB- and CD-spiroacetal subunits

Early attempts to effect the boron aldol[5] coupling of the AB- and CD-spiroacetal subunits by regio- and (*E*)-selective enolisation under standard conditions (Chx_2BCl, Et_3N, Et_2O)[6] of **5**,[1b] exposure to aldehyde **4**[1a] and oxidative workup (H_2O_2, MeOH, pH 7 buffer) afforded only trace quantities of the desired aldol adduct. Furthermore, none of the valuable starting materials were recovered. Given this initial set-back, efforts were directed to the lithium-mediated aldol reaction (Scheme 2). To this end, (*E*)-selective enolisation of **5** with lithium 2,2,6,6-tetramethylpiperidide–lithium bromide (LiTMP–LiBr)[7] to give enolate **6a**, and reaction with aldehyde **4** provided the desired aldol adduct **7** as the major diastereomer (67 : 33 dr) in 84% yield, where presumably the Felkin–Anh transition state is favoured (*i.e.*, stereoinduction from C14 as shown). At this point, it did not prove possible to form MTPA esters[8] of **7**, however, analysis of ^{1}H NMR coupling constants, extensive model studies[4c,e] and the excellent NMR spectroscopic agreement of the derived acetate **8**, *vide infra*, with the corresponding region for altohyrtin A/spongistatin 1 (**1**), allowed for the confident assignment of the major isomer as the desired species. While this lithium aldol procedure was successful at providing sufficient quantities of **7** to progress the synthesis, the boron aldol (as used with great effect by the Evans group[2c,d])¶ was re-investigated, in an effort to improve the scalability of the process. In the event, enolisation of **5** to give dicyclohexylboron enolate **6b**, *in situ*, and subsequent reaction with aldehyde **4**, was followed by a non-oxidative workup protocol, involving breakdown of the intermediate boron aldolate on silica gel, to afford **7** with improved diastereoselectivity (90 : 10 dr) in 89% yield. This boron aldol procedure proved to be highly scalable, providing >400 mg of **7** in a single reaction. Acetylation of the C15 hydroxyl (Ac_2O, DMAP, pyridine), provided **8** in 98% yield. Completion of the northern hemisphere aldehyde **2** was achieved in 78% yield from **8**, by removal of the C28 *p*-methoxybenzyl (PMB) ether under carefully controlled conditions (portionwise addition of excess of DDQ, 10 : 1 CH_2Cl_2–pH 7 buffer, 0 °C) and oxidation of the resultant primary alcohol (TPAP, NMO).[9]

Wittig coupling of the northern (ABCD) and southern (EF) hemisphere subunits and completion of the total synthesis of altohyrtin A/spongistatin 1

The crucial Wittig coupling to unite the northern hemisphere aldehyde **2** with the southern hemisphere phosphonium salt **3**[1c] was the final carbon–carbon bond-forming process in our synthesis, was also used in the Evans[2c,d] and Kishi[2f] routes, and represents one of the more challenging Wittig reactions to be performed in natural products synthesis. As such, significant effort was expended to study the Wittig coupling of model systems, such as E-ring phosphonium salts and a side-chain truncated C29–C46 EF phosphonium salt. Through these model

¶ Subsequently the Smith,[2i] Crimmins[2n] and Heathcock[2o] groups used boron aldol reactions to form the C15–C16 bond in a similar manner.



Scheme 3 *Reagents and conditions*: (a) LiHMDS, CaH_2, THF–HMPA (10 : 1), −78 °C, 10 min; then **2**, −78 °C → rt, 40 min; (b) DDQ (portionwise), CH_2Cl_2–pH 7 buffer (10 : 1), 0 °C, 1.5 h; (c) Zn, THF–1 M aq. NH_4OAc (10 : 1), rt, 0.5 h; (d) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, rt, 3 h; then DMAP, PhMe, 110 °C, 16 h; (e) HF, MeCN, H_2O, 0 °C, 4 h.

studies, the deleterious role of adventitious water and oxygen on the outcome of the Wittig reaction were demonstrated. While oxygen levels could be conveniently minimised by careful experimental setup, adventitious moisture was best removed by the use of *in situ* CaH_2. By stirring a 10 : 1 THF–HMPA solution of phosphonium salt **3** with CaH_2 prior to deprotonation with lithium hexamethyldisilazide (LiHMDS), subsequent Wittig reaction with aldehyde **2** reproducibly provided the desired (*Z*)-alkene **9** (Z/E > 97 : 3 by 800 MHz ^{1}H NMR) in 64–65% yield (Scheme 3).

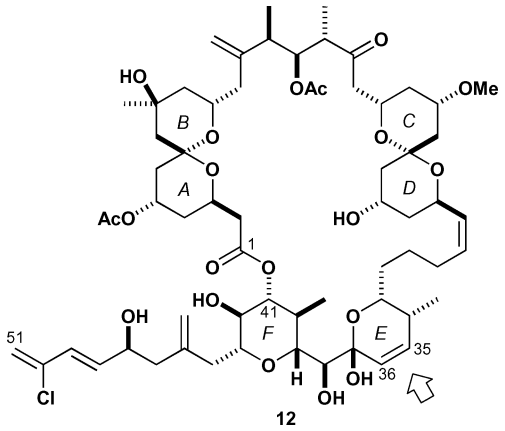
With the fully functionalised, protected *seco*-acid of **1** in hand, the closing stages of our synthesis were explored. It was considered that oxidative removal of the three PMB protecting groups in **9** could be accompanied by oxidation of the potentially labile unsaturated side-chain, since our group had previously shown that allylic dienol silyl ethers can be oxidised in good yields to the corresponding dienones with DDQ.[10] However, we were also guided by the successful PMB ether deprotections performed at a late stage in Kishi's synthesis of altohyrtin A.[2f] In the event, treatment of **9** with an excess of DDQ under carefully controlled conditions, afforded the *tris*-deprotected triol in 61% yield, with no indication of side-chain oxidation. However, partial hydrolysis of the C37 methyl acetal was unavoidable, resulting in formation of an *ca.* 1.1 : 1 inseparable mixture of the methyl acetal and the corresponding hemiacetal hydrolysis product, respectively.[11] Interestingly, Heathcock *et al.* subsequently reported that PMB deprotection of related compounds, with DDQ, *en route* to altohyrtin C/spongistatin 2 (lacking the C50 chlorine) led to competing oxidation of the diene segment to a dienone, attributing the difference in reactivity patterns to a retarding influence of the electron-withdrawing C50 halogen on the rate of side-chain oxidation.[2p]

In preparation for macrolactonisation, removal of the 2,2,2-trichloroethyl (TCE) ester protecting group with zinc dust in 10 : 1 THF–1 M NH_4OAc[12] provided *seco*-acid **10** in 58% yield, still as a methyl acetal–hemiacetal mixture. Surprisingly, significant quantities of partially dechlorinated compounds, the 2,2-dichloroethyl and 2-chloroethyl esters of **10** (24% combined yield), accompanied the formation of **10**.

Regioselective macrolactonisation of **10** under modified Yamaguchi conditions,[13] engaging the C41 hydroxy group in preference to those at C42 and C38, provided the 42-membered macrolide **11** in 50% yield. Finally, treatment of this compound with aqueous HF in MeCN effected deprotection of the four silyl ethers and hydrolysis of the remaining methyl acetal to provide (+)-altohyrtin A/spongistatin 1 (**1**) in an unoptimised 22% yield, after reverse-phase HPLC purification, along with an E-ring dehydrated analogue **12** in 13% yield, *vide infra*.* The spectroscopic data for synthetic **1** [^{1}H NMR (CD_3CN and CD_3OD, 500 and 800 MHz), ^{13}C NMR (CD_3CN), IR, HRMS],[14] along with specific rotation $[\alpha]_D^{20}$ = +21.0 (c = 0.44, MeOH); *c.f.* Kobayashi[15] $[\alpha]_D^{20}$ = +21.7 (c = 1.20, MeOH) and Pettit[16]

* This final deprotection has been optimised subsequently by the Heathcock group, both to enhance the yield of altohyrtin C/spongistatin 2 (92%) and circumvent the competing dehydration in the E-ring.[2p]

$[a]_D^{20}$ = +26.2 (c = 0.32, MeOH)] were in excellent agreement with that reported (and by comparison with the NMR spectra kindly provided by Professors Pettit and Kishi).[17] Moreover, our synthetic material was identical to natural spongistatin 1 in cytotoxicity bioassays performed by Professor Pettit's group. Altogether, we prepared 13 mg of altohyrtin A/spongistatin 1, which corresponds almost exactly to the amount of natural spongistatin isolated by the Pettit group from some 400 kg of the marine sponge *Spongia* sp.[16]

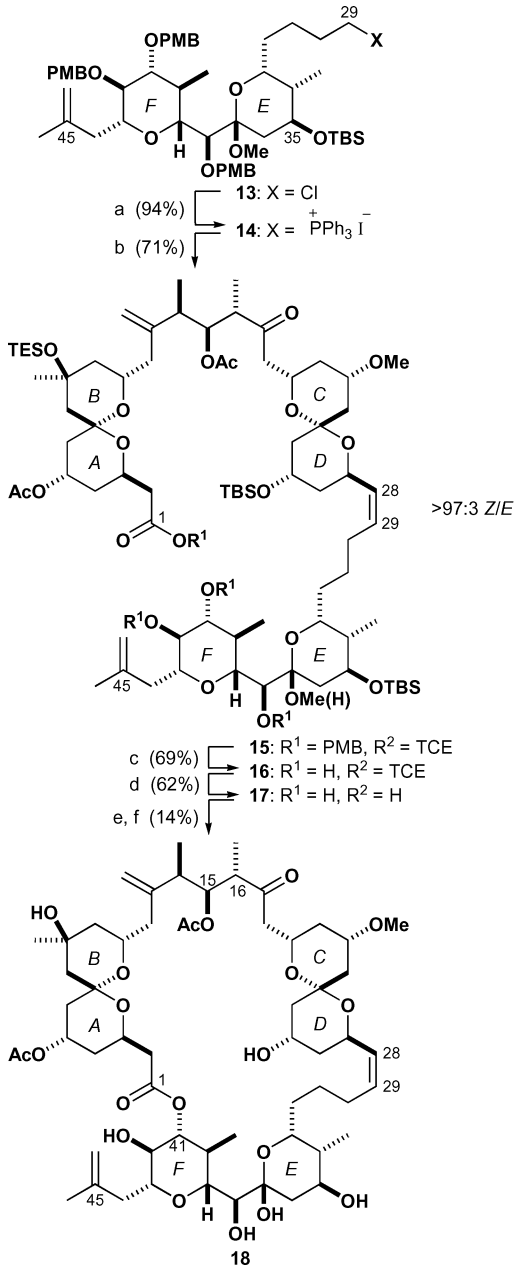


Synthesis and biological evaluation of analogues

Due to the extremely limited natural supply of the spongipyran natural products, lack of material has halted the preclinical development of these compounds in cancer chemotherapy. Despite the considerable synthetic interest that these compounds have garnered, relatively little is known regarding SAR for these architecturally complex natural products.[18–20] Smith *et al.* have described two simplified spongipyran analogues bearing a model F-ring and retaining the C44–C51 triene side-chain, both of which displayed growth inhibitory activity against several cancer cell lines, albeit only at the micromolar level.[18a] In contrast, the design, synthesis and surprisingly high level of biological activity of a greatly simplified analogue, based solely on the AB-spiroacetal motif, has been reported by Uckun and co-workers.[19] This claim has been questioned, as the Smith group prepared the same analogue, as well as another AB-spiroacetal analogue, and determined that neither had significant cytotoxic or anti-tubulin activity.[18b] With these contradictory results in mind, we decided to explore less extreme simplifications of **1**, in order to gather SAR data and help identify the spongipyran pharmacophore.

The deprotection of **11** with aqueous HF–MeCN to afford **1**, as outlined above, was accompanied by the formation of a minor component in 13% yield. This byproduct was purified by reverse-phase HPLC and was readily identified as a close analogue of **1** by its ^{1}H NMR spectra, with differences only in the regions corresponding to resonances for the E-ring protons. The structure of this analogue was determined as **12**, corresponding to an E-ring dehydrated version of **1**, following mass spectrometry and ^{1}H NMR analysis (500 MHz, COSY).

A further analogue of **1** was synthesised in order to ascertain the importance of the C47–C51 chlorodienol side-chain for biological activity. Thus, side-chain truncated EF segment **13**[1c] was converted into the corresponding phosphonium salt **14** by exposure to PPh_3 in the presence of NaI (Scheme 4). Wittig coupling of this compound with northern hemisphere aldehyde **2**, under the optimised conditions (LiHMDS, CaH_2, 10 : 1 THF–HMPA), provided the side-chain truncated, protected *seco*-acid **15** in 71% yield (>97 : 3 Z : E). Removal of the PMB protecting groups with DDQ provided **16** in 69% yield, once again with partial hydrolysis of the C37 methyl acetal. Removal of the TCE protecting group (Zn, 10 : 1 THF–1 M NH_4OAc) gave the *seco*-acid **17** in 62% yield. Finally, macrolactonisation under modified Yamaguchi conditions,[13] and global deprotection (aq. HF–MeCN) provided the side-chain truncated spongipyran analogue **18** in 14% yield from **17**.



Scheme 4 *Reagents and conditions*: (a) PPh_3, NaI, *i*-Pr_2NEt, MeCN–MeOH (9 : 1), Δ, 20 h; (b) LiHMDS, CaH_2, THF–HMPA (10 : 1), −78 °C, 10 min; then **2**, −78 °C → rt, 40 min; (c) DDQ (portionwise), CH_2Cl_2–pH 7 buffer (10 : 1), 0 °C, 1.5 h; (d) Zn, THF–1 M aq. NH_4OAc (10 : 1), rt, 0.5 h; (e) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, rt, 3 h; then DMAP, PhMe, 110 °C, 16 h; (f) HF, MeCN, H_2O, 0 °C, 4 h.

With analogues **12** and **18** in hand, the effects of E-ring dehydration and removal of the C47–C51 chlorodienol segment on cytotoxicity were now investigated. The results of growth inhibition experiments[21] for paclitaxel/Taxol (**19**), altohyrtin A/spongistatin 1 (**1**), E-ring dehydrated analogue **12** and side-chain truncated analogue **18**, against a set of five different human cancer cell lines are listed in Table 1.

The paclitaxel-resistant strains, MIP101 colon and 1A9PTX22 ovarian cancer, are included in the cell lines that these compounds were tested against. In all cases examined, **1** was found to be significantly more active (6- to 2000-fold) than paclitaxel, and was particularly effective against the MIP101 colon cancer cell line, indicating that it is a poor substrate for the P-glycoprotein (Pgp) drug efflux pump. Given the already exceptional cytotoxicity displayed by **1**, we were surprised and delighted to observe that the E-ring dehydrated analogue **12**

Table 1 Growth inhibition against human cancer cell lines[a]

IC_{50} values (nM)	19	1	12	18
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

[a] Cells were plated in 96 well plates at 4×10^3 cells per well for MIP 101, HCT 116 and A549 cell lines, and at 2×10^4 cells per well for 1A9 and 1A9/PTX22 cell lines. Compounds dissolved in DMSO were added to the wells at 5 fold serial dilutions starting from 1 μg ml^{-1}. No compound was added to control wells. After 72 h incubation, the number of viable cells was assessed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay.[22] After processing, the plates were read in a Molecular Devices 96-well plate reader at 540 nm and IC_{50} values (concentrations of compounds in nM causing 50% inhibition of cell growth) were calculated.

was generally (2- to 4-fold) more potent than the parent natural product. Analogue **12** 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 **1** is unnecessary for biological activity, and that its removal leads to an increase in potency.†† In contrast, the dramatic attenuation of cytotoxicity for analogue **18**, 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 C47–C51 chlorodiene allylic alcohol moiety is an essential structural feature. These results suggest that the full C44–C51 triene side-chain is a crucial part of the spongipyran pharmacophore, which concurs with the findings of the Smith group[18] but differs from the SAR work of Uckun.[19]

Conclusions

Overall, this highly stereocontrolled total synthesis of altohyrtin A/spongistatin 1 (**1**) proceeds in 33 steps and *ca.* 0.5% overall yield for the longest linear sequence (based on the AB-spiroacetal subunit). This synthesis has served to demonstrate the power of boron-mediated aldol methodology for constructing structurally complex polyketides, having been used in 10 separate instances (Fig. 1), examples of which, such as the union of the AB- and CD-spiroacetal subunits and C47–C51 side-chain installation, constitute some of the most testing applications of this versatile carbon-carbon bond forming reaction in total synthesis.[5] To date, this total synthesis has provided useful quantities of altohyrtin A/spongistatin 1 (**1**) for further preclinical studies and has thus contributed to replenishing the largely exhausted natural material from the initial isolation work. Furthermore, two analogues of the altohyrtins/spongstatins, *i.e.*, **12** and **18**, have been synthesised and evaluated for their growth inhibitory activity against a panel of human cancer cell lines. The evaluation of these analogues has confirmed the crucial role of the full C44–C51 triene side-chain for biological activity and has also revealed an increase in potency can be achieved by dehydration in the E-ring. While E-ring modification can be tolerated but not side chain truncation, the design of simplified synthetic analogues of the spongipyrans, while retaining the exceptional antimitotic potency, constitutes an important goal and clearly requires a great deal more SAR work.

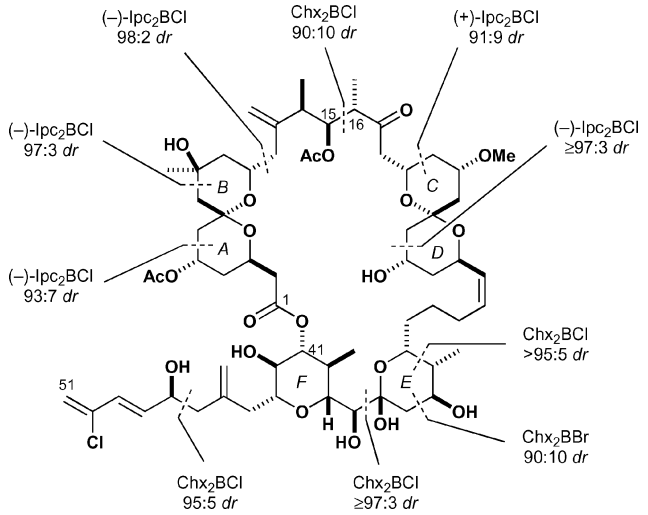


Fig. 1 Summary of aldol reactions employed in the total synthesis of altohyrtin A/spongistatin 1 (**1**), including boron Lewis acid reagents utilised and diastereoselectivities obtained.

†† A similar observation for the altohyrtin C/spongistatin 2 series has subsequently been made by Heathcock *et al.*[2p]

Experimental

ABCD aldol adduct (7)

To a cold (−78 °C) solution of ketone **5** (470 mg, 0.88 mmol, 2 eq.) in Et_2O (5.0 mL) was added Et_3N (195 μL, 1.40 mmol, 3.2 eq.) followed by Chx_2BCl (211 μL, 0.96 mmol, 2.2 eq.). The reaction mixture was stirred at −78 °C for 10 min and allowed to warm to 0 °C over a period of 10 min. The reaction mixture was then cooled to −78 °C and a solution of aldehyde **4** (281 mg, 0.44 mmol) in Et_2O (0.5 mL + 2 × 0.25 mL washings) was added *via* cannula. The reaction was stirred at −78 °C for 1 h and then at −20 °C for 16 h. The reaction was quenched by addition of silica gel (Merck Kieselgel 60, 230–400 mesh, *ca.* 2 g) and allowed to warm to rt and stirred for a further 40 min. The resultant slurry was filtered, eluting with EtOAc (30 mL) and concentrated *in vacuo*. Flash chromatography (5 : 95 → 60 : 40 EtOAc–light petroleum) afforded major aldol isomer **7** (415 mg, 80%), minor aldol isomers (45 mg, 9%) and recovered ketone **5** (231 mg, 98% recovery) as colourless oils.

Major diastereomer **7**: R_f: 0.17 (40 : 60 EtOAc–hexanes); $[\alpha]_D^{20}$ −31.8 (*c* 0.20, $CHCl_3$); IR (liquid film): 3500 (m, OH), 1758, 1729 (s, C=O), 1612 (w, C=C), 1513 (w, ArC=C); ^{1}H NMR: δ (500 MHz, $CDCl_3$) 7.26 (2H, d, J = 8.5 Hz, Ar*H*), 6.86 (2H, d, J = 8.5 Hz, Ar*H*), 5.17 (1H, s, C=CH_aH_b), 5.05 (1H, m, 5-C*H*), 4.95 (1H, s, C=CH_aH_b), 4.82 (1H, d, J = 12.0 Hz, OCH_aH_bCCl$_3$), 4.64 (1H, d, J = 12.0 Hz, OCH_aH_bCCl$_3$), 4.52 (3H, m, 27-C*H* + OCH_2Ar), 4.41 (1H, m, 3-C*H*), 4.30 (1H, m, 11-C*H*), 4.11 (1H, m, 25-C*H*), 3.97 (1H, m, 19-C*H*), 3.80 (3H, s, ArOCH_3), 3.63 (1H, m, 15-C*H*), 3.50–3.48 (3H, m, 21-C*H* + 28-CH_2), 3.32 (3H, s, OCH_3), 2.95 (1H, dd, J = 18.0, 3.9 Hz, 18-CH_aH_b), 2.86 (1H, dd, J = 18.0, 8.8 Hz, 18-CH$_a$$H_b$), 2.68 (1H, br quin., J = 7.4 Hz, 16-C*H*), 2.73 (1H, dd, J = 16.3, 5.9 Hz, 2-CH_aH_b), 2.52 (1H, dd, J = 16.3, 7.3 Hz, 2-CH$_a$$H_b$), 2.44 (1H, m, 14-C*H*), 2.29 (1H, dd, J = 14.4, 8.7 Hz, 12-CH_aH_b), 2.24 (1H, m, 20-CH_aH_b), 2.16 (1H, dd, J = 14.4, 3.5 Hz, 24-CH_aH_b), 2.01–2.08 (6H, m, 12-CH$_a$$H_b$ + 22-CH_aH_b + O*H* + COCH_3), 1.86–1.88 (2H, m, 4-CH_aH_b + 6-CH_aH_b), 1.77 (1H, d, J = 13.1 Hz, 8-CH_aH_b), 1.70 (1H, m, 26-CH_aH_b), 1.58–1.63 (4H, m, 4-CH$_a$$H_b$ + 6-CH$_a$$H_b$ + 10-CH_aH_b + 26-CH$_a$$H_b$), 1.51 (1H, dd, J = 14.4, 4.0 Hz, 24-CH$_a$$H_b$), 1.38 (1H, m, 22-CH$_a$$H_b$), 1.40 (1H, d, J = 13.1 Hz, 8-CH$_a$$H_b$), 1.21 (4H, m,

10-CH_aH_b + 9-CCH_3), 1.10 (1H, m, 20-CH_aH_b), 1.00–1.01 (6H, m, 14-$CHCH_3$ + 16-$CHCH_3$), 0.93 (9H, m, $Si(CH_2CH_3)_3$), 0.84 (9H, s, $SiC(CH_3)_3$), 0.54 (6H, m, $Si(CH_2CH_3)_3$), 0.03 (3H, s, $SiCH_3$), 0.02 (3H, s, $SiCH_3$); ^{13}C NMR: δ (100.6 MHz, $CDCl_3$) 213.0, 170.9, 169.0, 159.1, 148.0, 130.6, 129.3, 113.9, 113.7, 98.4, 97.1, 94.9, 74.0, 73.9, 73.3, 72.9, 70.3, 66.7, 66.4, 65.1, 64.5, 64.4, 60.7, 55.5, 55.3, 49.7, 48.4, 47.7, 45.2, 43.2, 41.4, 40.7, 40.0, 38.4, 36.9, 35.6, 35.2, 32.0, 25.9, 21.5, 18.1, 13.2, 11.3, 7.2, 6.8, −4.8, −4.9; HRMS: (+FAB) Calc. for $C_{57}H_{93}O_{15}Cl_3Si_2Na$ $[M + Na]^+$: 1201.5016, found: 1201.5030. *m/z*: (+FAB) 1204 ($[M + Na]^+$, 100), 587 (30).

Acetylated ABCD aldol adduct (8)

To a solution of aldol product **7** (179 mg, 0.15 mmol) in pyridine (3.2 mL) was added DMAP (cat.) and Ac_2O (0.97 mL). The reaction mixture was stirred at rt for 2 h then quenched by slow addition to a stirred solution of sat. aq. $NaHCO_3$ (50 mL). EtOAc (30 mL) was added and the layers were separated. The aqueous phase was extracted with EtOAc (3 × 50 mL), combined organics were washed with sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (5 : 95 → 60 : 40 EtOAc–light petroleum) afforded acetate **8** (180 mg, 98%) as a colourless oil: R_f: 0.25 (40 : 60 EtOAc–hexanes); $[\alpha]_D^{20}$ −15.0 (*c* 1.60, $CHCl_3$); ^{1}H NMR: δ (400 MHz, CD_3CN) 7.26 (2H, d, J = 8.4 Hz, Ar*H*), 6.86 (2H, d, J = 8.4 Hz, Ar*H*), 5.15 (1H, dd, J = 10.0, 2.5 Hz, 15-C*H*), 4.95 (1H, s, C=CH_aH_b), 4.93 (1H, m, 5-C*H*), 4.86 (1H, s, C=CH_aH_b), 4.84 (1H, d, J = 12.2 Hz, OC$H_aH_bCCl_3$), 4.74 (1H, d, J = 12.2 Hz, OC$H_aH_bCCl_3$), 4.38–4.48 (3H, m, 27-C*H* + OCH_2Ar), 4.31 (1H, m, 3-C*H*), 4.23 (1H, m, 11-C*H*), 4.14 (1H, m, 25-C*H*), 3.88 (1H, m, 19-C*H*), 3.78 (3H, s, ArOCH_3), 3.46 (1H, br tt, J = 11.3, 4.4 Hz, 21-C*H*), 3.38 (2H, m, 28-CH_2), 3.24 (3H, s, OCH_3), 3.00 (1H, dq, J = 10.0, 7.1 Hz, 16-C*H*), 2.80 (1H, dd, J = 17.5, 3.8 Hz, 18-CH_aH_b), 2.72 (1H, dd, J = 16.6, 5.5 Hz, 2-CH_aH_b), 2.71 (1H, dd, J = 17.5, 8.8 Hz, 18-CH_aH_b), 2.54 (1H, dd, J = 16.6, 7.9 Hz, 2-CH_aH_b), 2.57 (1H, m, 14-C*H*), 2.28 (1H, dd, J = 14.4, 3.7 Hz, 12-CH_aH_b), 2.10–2.18 (3H, m, 12-CH_aH_b + 20-CH_aH_b + 24-CH_aH_b), 2.00 (1H, m, 22-CH_aH_b), 1.96 (3H, s, $COCH_3$), 1.86 (3H, s, $COCH_3$), 1.85 (1H, m, 6-CH_aH_b), 1.71–1.76 (2H, m, 4-CH_aH_b + 6-CH_aH_b), 1.44–1.63 (6H, m, 4-CH_aH_b + 8-CH_aH_b + 10-CH_aH_b + 24-CH_aH_b + 26-CH_2), 1.34 (1H, d, J = 14.2 Hz, 8-CH_aH_b), 1.20–1.24 (4H, m, 9-CCH_3 + 10-CH_aH_b), 1.12 (1H, br t, J = 11.8 Hz, 22-CH_aH_b), 1.06 (3H, d, J = 7.0 Hz, 16-$CHCH_3$), 1.03 (3H, d, J = 7.1 Hz, 14-$CHCH_3$), 0.93 (9H, m, $Si(CH_2CH_3)_3$), 0.88 (1H, m, 20-CH_aH_b), 0.86 (9H, s, $SiC(CH_3)_3$), 0.58 (6H, m, $Si(CH_2CH_3)_3$), 0.03 (3H, s, $SiCH_3$), 0.02 (3H, s, $SiCH_3$); ^{1}H NMR: δ (800 MHz, $CDCl_3$) 7.26 (2H, d, J = 8.5 Hz, Ar*H*), 6.86 (2H, d, J = 8.5 Hz, Ar*H*), 5.22 (1H, dd, J = 9.7, 2.6 Hz, 15-C*H*), 5.04 (1H, m, 5-C*H*), 5.01 (1H, s, C=CH_aH_b), 4.83 (1H, s, C=CH_aH_b), 4.82 (1H, d, J = 12.0 Hz, OC$H_aH_bCCl_3$), 4.63 (1H, d, J = 12.0 Hz, OC$H_aH_bCCl_3$), 4.51 (3H, m, 27-C*H* + OCH_2Ar), 4.34 (1H, m, 3-C*H*), 4.21 (1H, m, 11-C*H*), 4.10 (1H, m, 25-C*H*), 3.93 (1H, m, 19-C*H*), 3.80 (3H, s, ArOCH_3), 3.44–3.51 (3H, m, 21-C*H* + 28-CH_2), 3.30 (3H, s, OCH_3), 2.86–2.91 (2H, m, 16-C*H* + 18-CH_aH_b), 2.75 (1H, dd, J = 16.4, 9.3 Hz, 2-CH_aH_b), 2.71 (1H, m, 18-CH_aH_b), 2.56 (1H, m, 14-C*H*), 2.51 (1H, dd, J = 16.4, 7.5 Hz, 2-CH_aH_b), 2.27 (2H, m, 12-CH_2), 2.14 (2H, m, 20-CH_aH_b + 24-CH_aH_b), 2.06 (1H, m, 22-CH_aH_b), 2.03 (3H, s, $COCH_3$), 1.91 (3H, s, $COCH_3$), 1.85–1.88 (2H, m, 4-CH_aH_b + 6-CH_aH_b), 1.76 (1H, d, J = 14.1 Hz, 8-CH_aH_b), 1.70 (1H, m, 26-CH_aH_b), 1.62–1.53 (3H, m, 4-CH_aH_b + 6-CH_aH_b + 10-CH_aH_b), 1.58 (1H, br dd, J = 14.4, 3.6 Hz, 26-CH_aH_b), 1.34 (1H, br t, J = 12.0 Hz, 22-CH_aH_b), 1.29 (1H, d, J = 14.1 Hz, 8-CH_aH_b), 1.19–1.25 (5H, m, 9-CCH_3 + 10-CH_aH_b + 24-CH_aH_b), 1.08 (6H, m, 14-$CHCH_3$ + 16-$CHCH_3$), 0.93 (9H, m, $Si(CH_2CH_3)_3$), 0.88 (1H, m, 20-CH_aH_b), 0.85 (9H, s, $SiC(CH_3)_3$), 0.54 (6H, m, $Si(CH_2CH_3)_3$), 0.02 (3H, s, $SiCH_3$), 0.01 (3H, s, $SiCH_3$); ^{13}C NMR: δ (100.6 MHz, CD_3CN) 210.1, 170.5, 169.1, 169.0, 147.3, 130.7, 129.4, 113.6, 113.3, 98.2, 97.0, 95.1, 74.0, 73.5, 73.1, 72.4, 70.5, 66.8, 66.3, 64.8, 64.4, 63.9, 60.6, 54.9, 54.6, 49.2, 47.6, 47.4, 45.0, 43.3, 42.4, 39.7, 38.1, 37.4, 36.9, 34.9, 34.8, 33.5, 31.3, 25.4, 20.8, 20.1, 18.7, 17.8, 13.0, 11.2, 10.7, 6.8, 6.5, −5.6, −5.7; HRMS: (+FAB) Calc. for $C_{59}H_{95}O_{16}Cl_3Si_2Na$ $[M + Na]^+$: 1243.5134, found: 1243.5122.

PMB deprotection of 8 – ABCD 1° alcohol

To a cold (0 °C) solution of *p*-methoxybenzyl ether **8** (877 mg, 0.72 mmol) in CH_2Cl_2–pH7 buffer (10 : 1, 20 mL) was added DDQ (1.96 g, 8.62 mmol, 12 eq.) over a period of 2 h (1 eq. every 10 min). The reaction was quenched by pouring into sat. aq. $NaHCO_3$ (100 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 100 mL), combined organics were washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (50 : 50 → 100 : 0 EtOAc–light petroleum) afforded the 1° alcohol (743 mg, 94%) as a colourless oil: R_f: 0.31 (50 : 50 EtOAc–hexanes); $[\alpha]_D^{20}$ −6.2 (*c* 1.20, $CHCl_3$); IR (liquid film): 3503, 1758, 1732 (C=O), 1612; ^{1}H NMR: δ (400 MHz, $CDCl_3$) 5.23 (1H, dd, J = 10.0, 2.8 Hz, 15-C*H*), 5.04 (1H, m, 5-C*H*), 5.01 (1H, s, C=CH_aH_b), 4.83 (1H, s, C=CH_aH_b), 4.82 (1H, d, J = 12.0 Hz, OC$H_aH_bCCl_3$), 4.62 (1H, d, J = 12.0 Hz, OC$H_aH_bCCl_3$), 4.42 (1H, m, 27-C*H*), 4.34 (1H, m, 3-C*H*), 4.23 (1H, m, 11-C*H*), 4.10 (1H, m, 25-C*H*), 3.93 (1H, m, 19-C*H*), 3.68 (1H, m, 28-CH_aH_b), 3.51 (1H, m, 28-CH_aH_b), 3.46 (1H, m, 21-C*H*), 3.30 (3H, s, 21-OCH_3), 2.92 (1H, dq, J = 10.0, 7.1 Hz, 16-C*H*), 2.88 (1H, dd, J = 17.6, 3.7 Hz, 18-CH_aH_b), 2.73 (1H, dd, J = 17.6, 6.9 Hz, 18-CH_aH_b), 2.68 (1H, dd, J = 16.4, 5.9 Hz, 2-CH_aH_b), 2.57 (1H, m, 14-C*H*), 2.53 (1H, dd, J = 16.4, 7.5 Hz, 2-CH_aH_b), 2.27 (2H, m, 12-CH_2), 2.13 (2H, m, 20-CH_aH_b + 24-CH_aH_b), 2.02 (3H, s, $COCH_3$), 2.00 (1H, m, 22-CH_aH_b), 1.92 (3H, s, $COCH_3$), 1.84 (2H, m, 6-CH_aH_b + 4-CH_aH_b), 1.74 (1H, dd, J = 14.2, 1.7 Hz, 8-CH_aH_b), 1.66 (1H, m, 26-CH_aH_b), 1.60–1.53 (3H, m, 10-CH_aH_b + 4-CH_aH_b + 6-CH_aH_b), 1.48 (1H, dd, J = 14.2, 3.3 Hz, 26-CH_aH_b), 1.38–1.20 (4H, m, 22-CH_aH_b + 8-CH_aH_b + 24-CH_aH_b + 10-CH_aH_b), 1.22 (3H, s, 9-CCH_3), 1.06 (6H, m, 16-$CHCH_3$ + 14-$CHCH_3$), 0.94 (9H, m, $Si(CH_2CH_3)_3$), 0.88 (1H, m, 20-CH_aH_b), 0.85 (9H, s, $SiC(CH_3)_3$), 0.54 (6H, m, $Si(CH_2CH_3)_3$), 0.03 (3H, s, $SiCH_3$), 0.01 (3H, s, $SiCH_3$); ^{13}C NMR: δ (62.5 MHz, $CDCl_3$) 209.8, 171.0, 169.4, 169.0, 147.0, 113.7, 98.3, 97.0, 94.9, 74.1, 73.9, 73.8, 70.4, 66.6, 66.3, 66.3, 65.9, 64.3, 64.3, 60.6, 55.6, 49.4, 47.9, 47.7, 45.3, 43.2, 42.1, 40.1, 38.5, 37.9, 36.8, 35.7, 34.2, 33.9, 32.0, 25.9, 21.5, 20.8, 18.1, 13.3, 12.0, 7.3, 6.8, −4.9, −4.9; *m/z*: (+ESI) 1123 ($[M + Na]^+$, 100).

ABCD aldehyde (2)

To a solution of ABCD 1° alcohol from the above procedure (229 mg, 0.21 mmol) in CH_2Cl_2 (2.0 mL) was added activated (heated under vacuum) powdered 4 Å molecular sieves (200 mg), NMO (73 mg, 0.63 mmol, 3.0 eq.) and TPAP (15 mg, 0.042 mmol, 20 mol%). The reaction mixture was stirred at rt for 15 min, and loaded directly onto a silica column. Flash chromatography (50 : 50 EtOAc–light petroleum) afforded aldehyde **2** (190 mg, 83%) as a colourless oil: R_f: 0.24 (50 : 50 EtOAc–hexanes); $[\alpha]_D^{20}$ −8.3 (*c* 0.60, $CHCl_3$); IR (liquid film): 1734 (C=O), 1647; ^{1}H NMR: δ (400 MHz, $CDCl_3$) 9.72 (1H, s, C*H*O), 5.22 (1H, dd, J = 9.7, 2.8 Hz, 15-C*H*), 5.04 (1H, m, 5-C*H*), 5.01 (1H, s, C=CH_aH_b), 4.80–4.88 (3H, m, C=CH_aH_b + OC$H_aH_bCCl_3$ + 27-C*H*), 4.62 (1H, d, J = 12.0 Hz, OC$H_aH_bCCl_3$), 4.35 (1H, m, 3-C*H*), 4.25 (1H, m, 11-C*H*), 4.17 (1H, m, 25-C*H*), 3.93 (1H, m, 19-C*H*), 3.49 (1H, m, 21-C*H*), 3.31 (3H, s, 21-OCH_3), 2.95–2.87 (2H, m, 16-C*H* + 18-CH_aH_b), 2.76 (1H, dd, J = 18.2, 9.5 Hz, 18-CH_aH_b), 2.74 (1H, dd, J = 16.1, 6.0 Hz, 2-CH_aH_b), 2.59 (1H, m, 14-C*H*), 2.53 (1H, dd, J = 16.1, 7.5 Hz, 2-CH_aH_b), 2.26 (2H, m, 12-CH_2), 2.20 (2H, m, 20-CH_aH_b + 24-CH_aH_b), 2.09 (1H, dd, J = 12.2, 3.1 Hz, 22-CH_aH_b), 2.02 (3H, s, $COCH_3$), 1.92 (3H, s, $COCH_3$), 1.86–1.83 (3H, m, 6-CH_aH_b + 4-CH_aH_b + 26-CH_aH_b), 1.73 (1H, dd,

J = 14.2, 1.76 Hz, 8-CH_aH$_b$), 1.66–1.49 (5H, m, 10-CH_aH$_b$ + 4-CH$_a$$H_b$, + 6-CH$_a$$H_b$ + 26-CH$_a$$H_b$ + 24-CH$_a$$H_b$), 1.44 (1H, br t, J = 12.0 Hz, 22-CH$_a$$H_b$), 1.28 (2H, m, 8-CH$_a$$H_b$ + 10-CH$_a$$H_b$), 1.20 (3H, s, 9-CC$H_3$), 1.07 (6H, m, 16-CHC$H_3$ + 14-CHCH_3), 0.93 (9H, m, Si(CH$_2$CH_3)$_3$), 0.88 (1H, m, 20-CH$_a$$H_b$), 0.85 (9H, s, SiC(C$H_3$)$_3$), 0.54 (6H, m, Si(C$H_2CH_3$)$_3$), 0.05 (3H, s, SiC$H_3$), 0.03 (3H, s, SiC$H_3$); ^{13}C NMR: δ (100.6 MHz, CDCl$_3$); 209.6, 202.6, 171.0, 169.3, 169.0, 147.0, 113.8, 110.1, 98.6, 96.9, 94.9, 74.1, 73.9, 73.5, 71.1, 70.3, 66.6, 64.3, 63.6, 60.6, 55.6, 49.4, 47.8, 47.6, 45.3, 43.3, 42.1, 40.1, 38.5, 37.8, 36.9, 35.2, 33.9, 33.1, 32.0, 25.9, 21.5, 20.8, 18.1, 13.3, 11.9, 7.3, 6.8, −4.9, −5.0; HRMS: (+FAB) Calc. for $C_{51}H_{85}O_{15}Si_2Cl_3Na$ [M + Na]$^+$: 1121.4385, found: 1121.4319.

The protected *seco*-acid of spongistatin 1 (9)

CaH_2 (spatula tip) was added to phosphonium salt **3** (195 mg, 0.129 mmol) in a glove box and the system was evacuated and recharged with Ar. O_2-free THF (1.5 mL) was added, followed by HMPA (150 μL) and the mixture was stirred at rt for 2 h. The phosphonium salt–CaH_2 suspension was cooled to −78 °C and a freshly prepared solution of LiHMDS (0.253 M in THF, 816 μL, 0.206 μmol, 1.6 eq.) was added, upon which the solution developed an intense yellow–orange colour. The reaction mixture was stirred at −78 °C for a further 10 min. A 500 μL gas-tight syringe was flushed with a solution of aldehyde **2** (190 mg, 0.173 mmol, 1.3 eq.) in THF (0.5 mL) and then the aldehyde solution was added to the ylide solution *via* this syringe. The resultant mixture was removed from the cooling bath and allowed to warm to rt. The mixture was stirred at rt for 40 min by which time a deep red coloration had developed. The reaction was quenched by transferring it, by pipette, to a cold (0 °C), vigorously stirred mixture of sat. aq. NH_4Cl–20% aq. $Na_2S_2O_3$ (2 : 1, 30 mL), and washing the flask with Et_2O (3 × 10 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 30 mL), the combined organic extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (10 : 90 → 60 : 40 EtOAc–hexanes) afforded the desired Wittig product **9** (181 mg, 64%) as a white amorphous solid: R_f: 0.18 (40 : 60 EtOAc–hexanes); $[\alpha]_D^{20}$ −8.7 (c 0.60, $CHCl_3$); IR (liquid film): 2932, 1732, 1514 cm^{-1}; ^{1}H NMR: δ (800 MHz, C_6D_6) 7.44 (2H, d, J = 8.5 Hz, ArH), 7.36 (2H, d, J = 8.4 Hz, ArH), 7.29 (2H, d, J = 8.4 Hz, ArH), 6.84–6.85 (4H, m, ArH), 6.83 (2H, d, J = 8.5 Hz, ArH), 6.46 (1H, dd, J = 14.9, 5.2 Hz, 48-CH), 6.39 (1H, d, J = 14.9 Hz, 49-CH), 5.76 (1H, br t, J = 9.7 Hz, 28-CH), 5.65 (1H, br t, J = 10.3 Hz, 27-CH), 5.63 (1H, dd, J = 11.6, 2.3 Hz, 15-CH), 5.55 (1H, br dt, J = 10.8, 7.3 Hz, 29-CH), 5.22 (1H, s, 13-C=CH_aH$_b$), 5.15 (1H, s, 51-CH_aH$_b$), 5.15 (1H, s, 45-C=CH_aH$_b$), 5.13 (1H, s, 45-C=CH$_a$$H_b$), 5.04 (1H, d, J = 11.2 Hz, OCH_aH$_b$Ar), 5.03 (1H, s, 51-CH$_a$$H_b$), 5.02 (1H, br s, 5-C$H$), 4.98 (1H, s, 13-C=CH$_a$$H_b$), 4.98 (1H, d, J = 10.7 Hz, OCH_aH$_b$Ar), 4.94 (1H, d, J = 11.1 Hz, OCH_aH$_b$Ar), 4.77 (1H, d, J = 12.0 Hz, OCH_aH$_b$CCl$_3$), 4.76 (1H, d, J = 11.2 Hz, OCH$_a$$H_b$Ar), 4.63 (1H, d, J = 10.7 Hz, OCH$_a$$H_b$Ar), 4.57 (1H, d, J = 11.1 Hz, OCH$_a$$H_b$Ar), 4.48–4.52 (2H, m, 3-CH + 47-CH), 4.47 (1H, d, J = 12.0 Hz, OCH$_a$$H_bCCl_3$), 4.35–4.38 (1H, m, 33-CH), 4.34 (1H, m, 11-CH), 4.19 (1H, br t, J = 10.6 Hz, 19-CH), 3.96 (1H, m, 35-CH), 3.91 (1H, br s, 25-CH), 3.71 (1H, s, 38-CH), 3.58 (1H, app t, J = 9.0 Hz, 43-CH), 3.38 (1H, m, 42-CH), 3.37 (1H, d, J = 10.0 Hz, 39-CH), 3.32 (3H, s, ArOCH_3), 3.32 (3H, s, ArOCH_3), 3.32 (3H, s, ArOCH_3), 3.25 (1H, tt, J = 10.9, 4.6 Hz, 21-CH), 3.23 (3H, s, 37-COCH_3), 3.19 (1H, dd, J = 10.4, 9.8 Hz, 41-CH), 3.10 (1H, dd, J = 17.7, 2.5 Hz, 18-CH_aH$_b$), 3.05 (3H, s, 21-COCH_3), 2.91 (1H, dq, J = 9.7, 6.9 Hz, 16-CH), 2.77 (1H, br t, J = 9.7 Hz, 44-CH_aH$_b$), 2.74–2.79 (2H, m, 14-CH + 18-CH$_a$$H_b$), 2.68 (1H, dd, J = 13.6, 6.4 Hz, 46-CH_aH$_b$), 2.66 (1H, dd, J = 16.6, 6.4 Hz, 2-CH_aH$_b$), 2.57 (1H, dd, J = 15.5, 3.7 Hz, 36-CH_aH$_b$), 2.53 (1H, dd, J = 13.4, 6.7 Hz, 46-CH$_a$$H_b$), 2.48 (2H, app d, J = 6.6 Hz, 12-CH_2), 2.40 (1H, br d, J = 12.5 Hz, 20-CH_aH$_b$), 2.37 (2H, dt, J = 7.3, 7.0 Hz, 30-CH_2), 2.31–2.33 (1H, m, 44-CH$_a$$H_b$), 2.30 (1H, dd, J = 16.6, 6.7 Hz, 2-CH$_a$$H_b$), 2.20 (1H, m, 40-C$H$), 2.15 (1H, br d, J = 12.1 Hz, 22-CH_aH$_b$), 2.10 (1H, d, J = 14.0 Hz, 24-CH_aH$_b$), 2.01 (1H, d, J = 15.5 Hz, 36-CH$_a$$H_b$), 1.95 (3H, s, COC$H_3$), 1.92 (1H, d, J = 14.7 Hz, 6-CH_aH$_b$), 1.82–1.86 (1H, m, 32-CH_aH$_b$), 1.79 (3H, s, COCH_3), 1.77–1.80 (1H, m, 31-CH_aH$_b$), 1.76 (1H, app t, J = 12.1 Hz, 22-CH$_a$$H_b$), 1.71 (1H, d, J = 13.4 Hz, 4-CH_aH$_b$), 1.67 (1H, d, J = 13.7 Hz, 26-CH_aH$_b$), 1.61–1.63 (1H, m, 8-CH_aH$_b$), 1.60 (1H, m, 26-CH$_a$$H_b$), 1.57–1.60 (1H, m, 34-CH), 1.49–1.53 (1H, m, 31-CH$_a$$H_b$), 1.48–1.52 (1H, m, 32-CH$_a$$H_b$), 1.41 (1H, d, J = 12.9 Hz, 10-CH_aH$_b$), 1.23 (3H, d, J = 7.0 Hz, 14-CHCH_3), 1.22–1.24 (1H, m, 6-CH$_a$$H_b$), 1.18 (3H, d, J = 6.9 Hz, 16-CHCH_3), 1.17 (1H, m, 4-CH$_a$$H_b$), 1.13 (9H, s, SiC(C$H_3$)$_3$), 1.12–1.14 (1H, m, 24-CH$_a$$H_b$), 1.02–1.06 (1H, m, 20-CH$_a$$H_b$), 1.02 (18H, s, 2 × SiC(CH_3)$_3$), 1.01–1.04 (9H, m, Si(CH$_2$CH_3)$_3$), 1.00–1.03 (1H, m, 8-CH$_a$$H_b$), 0.99 (3H, s, 9-CC$H_3$), 0.97–1.00 (1H, m, 10-CH$_a$$H_b$), 0.97–0.99 (3H, m, 34-CHCH_3), 0.81 (3H, d, J = 6.4 Hz, 40-CHCH_3), 0.57–0.60 (6H, m, Si(CH_2CH$_3$)$_3$), 0.25 (3H, m, SiCH_3), 0.16 (3H, m, SiCH_3), 0.14 (3H, m, SiCH_3), 0.10 (3H, m, SiCH_3), 0.08 (6H, m, 2 × SiCH_3); ^{13}C NMR: δ (100.6 MHz, C_6D_6) 209.6, 170.2, 169.2, 168.8, 159.9, 159.8, 148.0, 143.3, 138.9, 138.7, 132.3, 131.7, 131.6, 131.5, 131.0, 130.8, 129.5, 129.4, 128.7, 126.5, 115.7, 115.1, 114.2, 114.1, 113.8, 103.0, 98.4, 97.2, 95.6, 87.2, 83.4, 80.0, 78.7, 76.2, 74.9, 74.8, 74.7, 74.6, 74.1, 73.9, 71.4, 71.1, 70.7, 67.6, 66.9, 66.7, 65.3, 64.6, 62.3, 60.9, 55.2, 54.8, 54.8, 54.8, 51.2, 48.0, 47.9, 47.3, 46.2, 45.5, 44.6, 43.0, 40.4, 39.5, 39.2, 39.0, 38.7, 38.6, 38.0, 34.9, 34.2, 33.3, 32.2, 31.7, 28.8, 26.9, 26.2, 26.2, 21.4, 20.6, 18.5, 18.4, 18.4, 13.7, 13.4, 12.3, 10.6, 7.6, 7.2, −4.1, −4.2, −4.4, −4.7, −4.8; HRMS: (+ESI) Calc. for $C_{114}H_{180}O_{25}Cl_4Si_4Na$ [M + Na]$^+$: 2224.0537, found: 2224.0346.

tris-PMB removal of 9

To a cooled (0 °C) solution of *tris*-PMB ether **9** (188 mg, 0.085 mmol) in CH_2Cl_2–pH7 buffer (10 : 1, 11 mL) was added DDQ (1.74 g, 7.67 mmol, 90 eq.) over a period of 1.5 h (10 eq. every 10 min). The reaction was quenched by pouring into sat. aq. $NaHCO_3$ (50 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 100 mL), combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (20 : 80 → 100 : 0 EtOAc–hexanes) afforded the desired *tris*-PMB deprotected material (96 mg, 61%) as a white amorphous solid, consisting of an inseparable *ca.* 1.1 : 1 mixture of methyl acetal and hemiacetal at C37: R_f: 0.67 (80 : 20 EtOAc–hexanes); IR (liquid film): 3448, 2952, 1734 (C=O), 1459 cm^{-1}; ^{1}H NMR: δ (500 MHz, C_6D_6) 6.44–6.51 (2H, m, 48-CH + 49-CH), 5.81 (1H, m, 28-CH), 5.71 (1H, br d, J = 9.6 Hz, 15-CH), 5.68 (1H, m, 27-CH), 5.60 (1H, m, 29-CH), 5.38 (0.5H, s, 37-COH), 5.31 (1H, s, 13-C=CH_aH$_b$), 5.28, 5.27, 5.20, 5.15, 5.13 (4H, 5 × s, 45-C=CH_2 + 51-CH_2), 5.10 (1H, m, 5-CH), 5.09 (1H, 13-C=CH$_a$$H_b$), 4.87 (0.5H, d, J = 12.0 Hz, CH_aH$_b$CCl$_3$), 4.87 (0.5H, J = 12.0 Hz, CH_aH$_b$CCl$_3$), 4.58 (1H, d, J = 12.0 Hz, CH$_a$$H_bCCl_3$), 4.58 (2H, m, 3-C$H$ + 47-CH), 4.41 (2H, m, 33-CH + 11-CH), 4.26 (1H, m, 19-CH), 4.01, (2H, br s, 25-CH + 35-CH), 3.89 (0.5H, d, J = 6.2 Hz), 3.85 (0.5H, d, J = 10.3 Hz), 3.76 (0.5H, d, J = 8.0 Hz), 3.56 (0.5H, br t, J = 9.0 Hz, 43-CH), 3.44 (0.5H, br t, J = 9.0 Hz, 43-CH), 3.28–3.41 (1.5H, m), 3.26 (1.5 H, s, 37-OCH_3), 3.04–3.22 (3H, m), 3.15 (3H, 2 × s, 21-OCH_3), 2.97 (0.5H, m), 2.84–2.92 (3.5H, m), 2.76 (1H, dd, J = 16.4, 6.5 Hz), 2.60–2.67 (2H, m), 2.57 (2H, m), 2.49–2.53 (2H, m), 2.41 (2H, m), 2.33 (2H, m), 2.11–2.21 (3H, m), 2.05 (3H, 2 × s, COCH_3), 2.02 (2H, m), 1.92 (3H, 2 × s, COCH_3), 1.81–1.88 (3H, m), 1.70 (5H, m), 1.50 (3H, m), 1.08–1.22 (51H, m), 1.02 (3H, m, 34-CHCH_3), 0.96 (3H, m, 40-CHCH_3), 0.68 (6H, q, J = 7.6 Hz, Si(CH_2CH$_3$)$_3$), 0.13–0.32 (18H, m, 3 × Si(CH_3)$_2$); HRMS: (+ESI) Calc. for desired methyl acetal, $C_{90}H_{156}O_{22}Cl_4Si_4Na$ [M + Na]$^+$: 1863.8812, found: 1863.8779, Calc. for desired hemiacetal, $C_{89}H_{154}O_{22}Cl_4Si_4Na$ [M + Na]$^+$: 1849.8655, found: 1849.8575.

Spongistatin 1 *seco*-acid (10)

To a solution of the PMB deprotected material from the above procedure (30.6 mg, max. 0.017 mmol) in THF (1.0 mL) was added Zn dust (1.0 g, excess). The resultant suspension was stirred at rt for 2 min and aq. NH_4OAc (1 M, 100 μL) was added. The reaction mixture was stirred at rt for 30 min and then diluted with EtOAc (10 mL). The supernatant was separated with a pipette and the resultant Zn residue was washed with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered through sinter, and concentrated *in vacuo*. Flash column chromatography (30 : 70 → 100 : 0 EtOAc–hexanes, then 1 : 99 AcOH–EtOAc) afforded *seco*-acid **10** (16.5 mg, 58%) and partially dechlorinated products (7.1 mg, 24%) as an amorphous solid and a colourless oil respectively.

seco-Acid **10**: R_f: 0.48 (80 : 20 EtOAc–hexanes); IR (liquid film): 3583, 2952, 1734 (C=O), 1459 cm^{-1}; ^1H NMR: δ (500 MHz, C_6D_6) 6.50–6.55 (2H, m, 48-C*H* + 49-C*H*), 5.80 (2H, m, 15-C*H* +28-C*H*), 5.69 (1H, m, 27-C*H*), 5.56 (1H, m, 29-C*H*), 5.09–5.28 (7.5H, m, 37-O*H* + 5-C*H* + 13-C=CH_2, 45-C=CH_2 + 51-C=CH_2), 4.53–4.62, 4.30–4.38 (5H, 2 × m, 3-C*H* + 11-C*H* + 19-C*H* + 33-C*H* + 47-C*H*), 3.85–4.01 (4H, m, 25-C*H* + 35-C*H* + 38-C*H* +39-C*H*), 3.39–3.60 (1.5H, m), 3.27–3.36 (1.5H, m), 3.27 (1.5H, s, 37-OCH_3), 3.15 (3H, s, 21-OCH_3), 2.99–3.13 (3H, m), 2.76–2.97 (4H, m), 2.67 (2H, m), 2.50 (6H, m), 2.33 (2H, m), 2.20 (3H, m), 2.07, 2.05 (3H, 2 × s, COCH_3), 1.99–2.12 (2H, m), 1.97, 1.95 (3H, 2 × s, COCH_3), 1.88–2.02 (3H, m), 1.83 (3H, m), 1.68 (5H, m), 1.01–1.51 (57H, m), 0.71 (6H, m, Si(CH_2CH$_3$)$_3$), 0.09–0.38 (18H, m, 3 × Si(CH_3)$_2$). The absence of doublet peaks at 4.58 and 4.87 indicated the loss of the 2,2,2-trichloroethyl ester moiety; HRMS: (+ESI) Calc. for methyl acetal **10**Me, $C_{88}H_{155}O_{22}ClSi_4Na$ $[M + Na]^+$: 1733.9668, found: 1733.9790, Calc. for hemiacetal **10**H, $C_{87}H_{153}O_{22}ClSi_4Na$ $[M + Na]^+$: 1719.9511, found: 1719.9625.

Protected macrocycle of spongistatin 1 (11)

To a solution of *seco*-acid **10** (28.4 mg, max. 0.017 mmol) in THF (200 μL) was added a freshly prepared solution of Et_3N (0.5 M in THF, 100 μL, 0.050 mmol, 3 eq.) and a solution of 2,4,6-trichlorobenzoyl chloride (0.5 M in THF, 166 μL, 0.083 mmol, 5 eq.). The resultant solution was left to stir at rt for 1 h before a further aliquot of reagents (Et_3N and 2,4,6-trichlorobenzoyl chloride, same stoichiometry as above) were added. This process was repeated for a third time such that a total of Et_3N (15 eq.) and 2,4,6-trichlorobenzoyl chloride (9 eq.) were added in three portions over a period of 3 h. The reaction mixture was diluted with PhMe (2 mL) and this solution was then added to a refluxing solution of DMAP (41 mg, 0.33 mmol, 20 eq.) in PhMe (12 mL) over a period of 3 min. The anhydride flask was rinsed with PhMe (2 × 1 mL) and added to the refluxing reaction. The resultant cloudy white suspension was heated at reflux for 16 h and then cooled to rt before being quenched with sat. $NaHCO_3$ (30 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine (30 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (20 : 80 → 80 : 20 EtOAc–hexanes) afforded macrolactone **11** (14.1 mg, 50%) as a colourless oil consisting of an inseparable mixture of methyl acetal and hemiacetal at C37: R_f: 0.45 (40 : 60 EtOAc–hexanes); IR (liquid film): 2926, 1734 (C=O), 1458 cm^{-1}; ^1H NMR: δ (500 MHz, CD_3CN) 6.37 (1H, d, $J = 14.8$ Hz, 49-C*H*), 6.11 (1H, m, 48-C*H*), 4.44 (1H, s, 51-C=CH_aH$_b$), 5.40 (1H, m, 28-C*H*), 5.38 (1H, s, 51-C=CH$_a$$H_b$), 5.36 (1H, m, 29-C*H*), 5.10–5.20 (2H, m, 15-C*H* + 27-C*H*), 4.85–4.92 (5H, m, 5-C*H* + 13-C=CH_2 + 45-C=CH_2), 4.72, 4.68 (1H, m, dd, $J = 10.6$, 9.0 Hz, 41-C*H*), 4.45 (1H, m, 47-C*H*), 4.30 (2H, m, 3-C*H* + 11-C*H*), 4.13 (2H, m), 4.06 (1H, m), 3.98 (0.5H, m), 3.89 (1.5H, m), 3.79 (1H, m), 3.60–3.63 (1H, m), 3.48 (1H, m, 21-C*H*), 3.30–3.43 (2H, m, 38-C*H* + 43-C*H*), 3.24, 3.24 (3H, 2 × s, 21-OCH_3), 3.16 (1.5H, s, 37-OCH_3), 3.14 (1H, m, 42-C*H*), 3.06 (1H, m, 16-C*H*), 2.89 (1.5H, m), 2.58–2.82 (4.5H, m), 2.44 (1H, br d, $J = 13.8$ Hz), 2.24–2.38 (4H, m), 1.99–2.18 (6H, m), 1.95 (3H, m, COCH_3), 1.87, 1.87 (3H, 2 × s, COCH_3), 1.82–1.87 (2H, m), 1.71–1.76 (2H, m), 1.29–1.65 (10H, m), 1.05–1.21 (13H, m), 0.87–0.98 (43H, m), 0.60 (6H, m, Si(CH_2CH$_3$)$_3$), 0.02–0.11 (18H, m, 3 × Si(CH_3)$_2$). The appearance of double-doublet signal at δ 4.68 ($J = 10.6$, 9.0 Hz) indicated the formation of the macrolactones; *m/z*: (+ESI) 1684 (55), 1151 (17), 907 (23), 862 (100), 734 (49).

Spongistatin 1 (1)[14]

To a cold (0 °C) solution of macrolactones **11** (27.5 mg, max. 0.016 mmol) in MeCN (4.9 mL) was added a freshly prepared HF solution (40% $HF_{(aq)}$–H_2O–MeCN, 0.5 : 0.9 : 8.6, 405 μL) and the reaction mixture was stirred at 0 °C for 4 h before being quenched with sat. aq. $NaHCO_3$ (20 mL). The resultant mixture was diluted with CH_2Cl_2 (20 mL) and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL), combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (5 : 95 → 10 : 90 MeOH–CH_2Cl_2) afforded spongistatin 1 (**1**) and dehydrated product **12** as an inseparable mixture (11.1 mg, 55%). Further purification by reverse phase HPLC (Prodigy C_{18} 4.6 × 250 mm, 5 μm analytical column; 27.5% H_2O–MeOH; 1 mL min^{-1}) afforded spongistatin 1 (**1**) (4.4 mg, 22%) and dehydrated product **12** (2.7 mg, 13%) as white solids.

Spongistatin 1 (**1**): R_f: 0.20 (5 : 95 MeOH–CH_2Cl_2); Reverse phase HPLC: R_t 12.5 min (27.5 : 72.5 H_2O–MeOH); $[a]_D^{20}$ +21.0 (*c* 0.44, MeOH); IR (liquid film): 3430, 2940, 1733, 1386, 1259, 1176, 1086, 893 cm^{-1}; ^1H NMR: δ (500 MHz, CD_3CN) 6.41 (1H, dd, $J = 15.0$, 1.1 Hz, 49-C*H*), 6.11 (1H, dd, $J = 15.0$, 5.7 Hz, 48-C*H*), 5.48 (1H, ddd, $J = 10.6$, 9.9, 9.9 Hz, 29-C*H*), 5.45 (1H, br s, 51-CH_aH$_b$), 5.35 (1H, br s, 51-CH$_a$$H_b$), 5.32 (1H, br t, $J = 10.8$ Hz, 28-C*H*), 5.11 (1H, dd, $J = 10.7$, 1.8 Hz, 15-C*H*), 5.00 (1H, ddd, $J = 9.9$, 9.9, 3.8 Hz, 27-C*H*), 4.92 (1H, br s, 5-C*H*), 4.89 (1H, br s, 45-C=CH_aH$_b$), 4.86 (1H, br s, 45-C=CH$_a$$H_b$), 4.83 (2H, br s, 13-C=CH_2), 4.75 (1H, dd, $J = 11.0$, 9.2 Hz, 41-C*H*), 4.72 (1H, d, $J = 2.5$ Hz, 37-O*H*), 4.39 (1H, d, $J = 10.0$ Hz, 25-O*H*), 4.36 (1H, m, 47-C*H*), 4.35 (1H, br d, $J = 5.3$ Hz, 42-O*H*), 4.31 (1H, br s, 9-O*H*), 4.24 (2H, br t, $J = 10,7$ Hz, 3-C*H*, 11-C*H*), 4.13 (1H, dt, $J = 9.0$, 3.0 Hz, 33-C*H*), 3.99 (1H, br t, $J = 11.1$ Hz, 19-C*H*), 3.94 (1H, br m, 25-C*H*), 3.82 (1H, d, $J = 9.4$ Hz, 35-O*H*), 3.72 (1H, br d, $J = 10.2$ Hz, 39-C*H*), 3.65 (1H, m, 35-C*H*), 3.51 (1H, d, $J = 2.5$ Hz, 47-O*H*), 3.46 (1H, tt, $J = 11.6$, 4.2 Hz, 21-C*H*), 3.38 (1H, td, $J = 8.6$, 2.2 Hz, 43-C*H*), 3.34 (1H, d, $J = 10.7$ Hz, 38-C*H*), 3.24 (3H, s, 21-OCH_3), 3.11 (1H, td, $J = 9.1$, 5.4 Hz, 42-C*H*), 3.04 (1H, dq, $J = 10.7$, 6.9 Hz, 16-C*H*), 2.86 (1H, dd, $J = 19.4$, 10.1 Hz, 18-CH_aH$_b$), 2.86 (1H, br d, $J = 10.1$ Hz, 38-O*H*), 2.78 (1H, m, 14-C*H*), 2.75 (1H, br d, $J = 13.9$ Hz, 44-CH_aH$_b$), 2.61 (1H, br d, $J = 18.2$ Hz, 18-CH$_a$$H_b$), 2.52 (1H, dd, $J = 16.2$, 1.8 Hz, 2-CH_aH$_b$), 2.45 (1H, dd, $J = 16.2$, 10.5 Hz, 2-CH$_a$$H_b$), 2.33 (1H, br dd, $J = 13.6$, 6.3 Hz, 46-CH_aH$_b$), 2.27 (1H, m, 24-CH_aH$_b$), 2.26 (1H, br d, $J = 14.0$ Hz, 12-CH_aH$_b$), 2.18 (2H, m, 30-CH_aH$_b$ + 46-CH$_a$$H_b$), 2.08 (1H, m, 44-CH$_a$$H_b$), 2.00 (1H, m, 30-CH$_a$$H_b$), 1.99 (2H, m, 12-CH$_a$$H_b$ + 22-CH_aH$_b$), 1.98 (1H, m, 20-CH_aH$_b$), 1.94 (3H, s, COCH_3), 1.91 (1H, m, 40-C*H*), 1.89 (1H, m, 36-CH_aH$_b$), 1.84 (3H, s, COCH_3), 1.78 (1H, d, $J = 15.1$ Hz, 6-CH_aH$_b$), 1.68 (1H, m, 4-CH_aH$_b$), 1.66 (1H, dd, $J = 15.2$, 4.4 Hz, 6-CH$_a$$H_b$), 1.61 (1H, m, 36-CH$_a$$H_b$), 1.60 (2H, m, 8-C$H_aH_b$ + 31-CH_aH$_b$), 1.57 (3H, m, 26-CH_2 + 34-C*H*), 1.55 (3H, m, 4-CH$_a$$H_b$ + 10-CH_aH$_b$ + 24-CH$_a$$H_b$), 1.46 (1H, d, $J = 14.0$ Hz, 8-CH$_a$$H_b$), 1.42 (1H, m, 32-C$H_aH_b$), 1.30 (1H, m, 32-CH$_a$$H_b$), 1.28 (1H, m, 10-CH$_a$$H_b$), 1.23 (1H, m, 31-CH$_a$$H_b$), 1.15 (3H, d, $J = 6.9$ Hz, 16-CHCH_3), 1.08 (1H, t, $J = 12.1$ Hz, 22-CH$_a$$H_b$), 1.06 (3H, s, 9-CC$H_3$), 1.04 (3H, d, $J = 6.9$ Hz, 14-CHCH_3), 0.96 (1H, ddd, $J = 12.0$, 12.0, 12.0 Hz, 20-CH$_a$$H_b$), 0.81 (3H, d, $J = 7.2$ Hz, 34-CHCH_3), 0.74 (3H, d, $J = 6.6$ Hz, 40-CHCH_3); ^{13}C NMR: δ (125 MHz, CD_3CN) 213.5, 173.1, 171.6, 170.2, 148.0, 144.0, 139.2, 139.2, 133.4, 131.2, 127.0, 116.6, 116.5, 114.9, 99.9, 99.4,

99.3, 81.3, 80.6, 78.7, 75.3, 74.0, 73.1, 73.1, 71.5, 70.1, 69.6, 67.2, 67.1, 66.2, 64.7, 64.4, 63.6, 61.2, 55.7, 52.0, 47.6, 46.8, 45.0, 44.2, 44.2, 43.9, 40.9, 40.2, 39.3, 39.1, 38.2, 37.7, 37.3, 36.6, 34.9, 34.7, 33.8, 32.8, 30.2, 28.1, 27.8, 27.0, 21.0, 13.7, 12.7, 12.1, 11.6; HRMS: (+ESI) Calc. for $C_{63}H_{95}O_{21}ClNa$ $[M + Na]^+$: 1245.5946, found: 1245.5895.

Dehydrated product **12**: R_f: 0.20 (5 : 95 MeOH–CH_2Cl_2); Reverse phase HPLC: R_t 19.4 min (27.5 : 72.5 H_2O–MeOH); $[\alpha]_D^{20}$ +5.8 (*c* 0.22, MeOH); IR (liquid film): 3450, 2936, 1733, 1649, 1382, 1256, 1169, 1088 cm^{-1}; ^1H NMR: δ (500 MHz, CD_3CN) 6.42 (1H, dd, $J = 14.9$, 1.1 Hz, 49-C*H*), 6.14 (1H, dd, $J = 14.9$, 5.4 Hz, 48-C*H*), 5.92 (1H, dd, $J = 10.0$, 5.5 Hz, 35-CH) 5.86 (1H, dd, $J = 10.1$, 0.8 Hz, 34-C*H*), 5.46 (1H, br s, 51-CH_aH_b), 5.41 (1H, m, 29-C*H*), 5.36 (1H, br s, 51-CH$_a$$H_b$) 5.35 (1H, br t, $J = 11.0$ Hz, 28-C*H*), 5.19 (1H, dd, $J = 10.6$, 1.6 Hz, 15-C*H*), 4.99 (1H, m, 27-C*H*), 4.94 (1H, br s, 5-C*H*), 4.90 (1H, br s, 45-C=CH_aH_b), 4.89 (1H, br s, 45-C=CH$_a$$H_b$), 4.84 (2H, br s, 13-C=CH_2), 4.79 (1H, dd, $J = 11.0$, 9.2 Hz, 41-C*H*), 4.48 (1H, br t, $J = 11.3$ Hz, 11-C*H*), 4.41 (1H, d, $J = 10.5$ Hz, 25-O*H*), 4.35 (1H, m, 47-C*H*), 4.27 (1H, br t, $J = 11.3$ Hz, 3-C*H*), 4.02 (1H, br t, $J = 11.1$ Hz, 19-C*H*), 3.94 (1H, br m, 25-C*H*), 3.90 (1H, d, $J = 5.0$ Hz, 42-O*H*), 3.88 (1H, br d, $J = 10.4$ Hz, 33-C*H*), 3.82 (1H, d, $J = 10.3$ Hz, 39-C*H*), 3.49 (2H, m, 43-C*H* + 21-C*H*), 3.30 (1H, d, $J = 10.5$ Hz, 38-C*H*), 3.27 (3H, s, 21-OCH_3), 3.23 (1H, d, $J = 5.0$ Hz, 47-O*H*), 3.17 (1H, td, $J = 9.0$, 4.9 Hz, 42-C*H*), 3.04 (1H, dq, $J = 10.8$, 7.0 Hz, 16-C*H*), 2.91 (1H, d, $J = 10.8$ Hz, 38-O*H*), 2.89 (1H, m, 14-C*H*), 2.83 (1H, dd, $J = 19.4$, 10.0 Hz, 18-CH_aH_b), 2.77 (1H, br d, $J = 13.9$ Hz, 44-CH_aH_b), 2.62 (1H, br d, $J = 18.1$ Hz, 18-CH$_a$$H_b$), 2.59 (1H, dd, $J = 16.5$, 1.6 Hz, 2-CH_aH_b), 2.51 (1H, dd, $J = 16.5$, 10.5 Hz, 2-CH$_a$$H_b$), 2.31 (3H, m, 46-C$H_aH_b$ + 24-CH_aH_b + 12-CH_aH_b), 2.20 (1H, dd, $J = 13.7$, 6.1 Hz, 46-CH$_a$$H_b$), 2.03–2.12 (5H, m, 12-CH$_a$$H_b$ + 20-CH_aH_b + 22-CH_aH_b + 30-CH_aH_b + 44-CH$_a$$H_b$), 1.98 (3H, s, COC$H_3$), 1.94 (2H, m, 34-C*H* + 40-C*H*), 1.85 (3H, s, COCH_3), 1.80 (2H, m, 4-CH_aH_b + 30-CH$_a$$H_b$), 1.67 (2H, m, 4-CH$_a$$H_b$ + 6-CH_aH_b), 1.54–1.59 (7H, m, 6-CH$_a$$H_b$ + 8-CH_aH_b + 10-CH_aH_b + 24-CH$_a$$H_b$ + 26-CH_2 + 31-CH_aH_b), 1.47 (2H, m, 8-CH$_a$$H_b$ + 32-CH_aH_b), 1.28 (1H, m, 10-CH$_a$$H_b$), 1.21 (2H, m, 31-CH$_a$$H_b$ + 32-CH$_a$$H_b$), 1.18 (3H, d, $J = 6.9$ Hz, 16-CHCH_3), 1.09 (1H, t, $J = 12.1$ Hz, 22-CH$_a$$H_b$), 1.06 (3H, s, 9-CC$H_3$), 1.00 (3H, d, $J = 6.9$ Hz, 14-CHCH_3), 0.91 (1H, ddd, $J = 11.9$, 11.9, 11.9 Hz, 20-CH$_a$$H_b$), 0.91 (3H, d, $J = 7.0$ Hz, 34-CHCH_3), 0.77 (3H, d, $J = 6.7$ Hz, 40-CHCH_3); ^{13}C NMR: δ (125 MHz, CD_3CN) 213.0, 172.9, 171.5, 170.0, 148.1, 143.9, 139.1, 139.0, 134.4, 133.0, 131.7, 127.6, 126.8, 116.3, 116.3, 114.6, 99.8, 99.2, 95.8, 81.3, 80.2, 78.6, 75.1, 73.8, 73.2, 72.6, 71.1, 70.3, 69.0, 67.0, 66.0, 64.5, 64.1, 62.4, 61.3, 55.6, 51.7, 47.3, 46.9, 45.0, 44.3, 44.1, 43.9, 40.6, 40.0, 39.3, 38.2, 37.6, 37.2, 36.4, 34.7, 34.4, 33.4, 32.3, 30.1, 27.9, 26.5, 21.7, 20.9, 13.7, 13.7, 12.6, 12.0; *m/z*: (+ESI) 1227 ($[M + Na]^+$, 100), 734 (26), 576 (20), 445 (31), 413 (38), 381 (26).

Acknowledgements

Financial support was provided by the EPSRC (GR/L41646), EC (Marie Curie Fellowship to J.L.A.), Churchill College (Research Fellowship to D.J.W.), Cambridge Commonwealth Trust (M.J.C.), King's College and Sim's Fund, Cambridge (D.Y.-K.C.). We thank Merck, AstraZeneca and Novartis Pharmaceuticals for generous support, Dr Kenneth W. Bair, Dr Frederick R. Kinder, Jr., Dr Peter T. Lassota and Dr Erik F. Sorensen at Novartis for providing the biological data,[21] and Dr Anne Butlin (AZ) and Dr Nick Bampos (Cambridge) for valuable assistance.

References

1 For a full introduction, background and the synthesis of the AB-, CD- and EF-subunits of altohyrtin A, see the preceding papers in this series: (*a*) I. Paterson, M. J. Coster, D. Y.-K. Chen, R. M. Oballa, D. J. Wallace and R. D. Norcross, *Org. Biomol. Chem.*, 2005, **3**, DOI: 10.1039/b504146e; (*b*) I. Paterson, M. J. Coster, D. Y.-K. Chen, K. R. Gibson and D. J. Wallace, *Org. Biomol. Chem.*, 2005, **3**, DOI: 10.1039/b504148a; (*c*) I. Paterson, M. J. Coster, D. Y.-K. Chen, J. L. Aceña, J. Bach, L. E. Keown and T. Trieselmann, *Org. Biomol. Chem.*, 2005, **3**, DOI: 10.1039/b504149j.

2 Completed altohyrtin/spongistatin total syntheses: (*a*) D. A. Evans, P. J. Coleman and L. C. Dias, *Angew. Chem., Int. Ed.*, 1997, **36**, 2738–2741; (*b*) D. A. Evans, B. W. Trotter, B. Cote and P. J. Coleman, *Angew. Chem., Int. Ed.*, 1997, **36**, 2741–2744; (*c*) D. A. Evans, B. W. Trotter, B. Cote, P. J. Coleman, L. C. Dias and A. N. Tyler, *Angew. Chem., Int. Ed.*, 1997, **36**, 2744–2747; (*d*) D. A. Evans, B. W. Trotter, P. J. Coleman, B. Cote, L. C. Dias, H. A. Rajapakse and A. N. Tyler, *Tetrahedron*, 1999, **55**, 8671–8726; (*e*) J. Guo, K. J. Duffy, K. L. Stevens, P. I. Dalko, R. M. Roth, M. M. Hayward and Y. Kishi, *Angew. Chem., Int. Ed.*, 1998, **37**, 187–192; (*f*) M. M. Hayward, R. M. Roth, K. J. Duffy, P. I. Dalko, K. L. Stevens, J. Guo and Y. Kishi, *Angew. Chem., Int. Ed.*, 1998, **37**, 192–196; (*g*) A. B. Smith, III, V. A. Doughty, Q. Lin, L. Zhuang, M. D. McBriar, A. M. Boldi, W. H. Moser, N. Murase, K. Nakayama and M. Sobukawa, *Angew. Chem., Int. Ed.*, 2001, **40**, 191–195; (*h*) A. B. Smith, III, Q. Lin, V. A. Doughty, L. Zhuang, M. D. McBriar, J. K. Kerns, C. S. Brook, N. Murase and K. Nakayama, *Angew. Chem., Int. Ed.*, 2001, **40**, 196–199; (*i*) A. B. Smith, III, V. A. Doughty, C. Sfouggatakis, C. S. Bennett, J. Koyanagi and M. Takeuchi, *Org. Lett.*, 2002, **4**, 783–786; (*j*) A. B. Smith, III, W. Zhu, S. Shirakami, C. Sfouggatakis, V. A. Doughty, C. S. Bennett and Y. Sakamoto, *Org. Lett.*, 2003, **5**, 761–764; (*k*) M. T. Crimmins and D. G. Washburn, *Tetrahedron Lett.*, 1998, **39**, 7487–7490; (*l*) M. T. Crimmins, J. D. Katz, L. C. McAtee, E. A. Tabet and S. J. Kirincich, *Org. Lett.*, 2001, **3**, 949–952; (*m*) M. T. Crimmins and J. D. Katz, *Org. Lett.*, 2000, **2**, 957–960; (*n*) M. T. Crimmins, J. D. Katz, D. G. Washburn, S. P. Allwein and L. F. McAtee, *J. Am. Chem. Soc.*, 2002, **124**, 5661–5663; (*o*) J. L. Hubbs and C. H. Heathcock, *J. Am. Chem. Soc.*, 2003, **125**, 12836–12843; (*p*) C. H. Heathcock, M. McLaughlin, J. Medina, J. L. Hubbs, G. A. Wallace, R. Scott, M. M. Claffey, C. J. Hayes and G. R. Ott, *J. Am. Chem. Soc.*, 2003, **125**, 12844–12849.

3 Formal total synthesis of altohyrtin C/spongistatin 2 by Nakata and co-workers: T. Terauchi, T. Terauchi, I. Sato, W. Shoji, T. Tsukada, T. Tsunoda, N. Kanoh and M. Nakata, *Tetrahedron Lett.*, 2003, **44**, 7741–7745; T. Terauchi, T. Tanaka, T. Terauchi, M. Morita, K. Kimijima, I. Sato, W. Shoji, Y. Nakamura, T. Tsukada, T. Tsunoda, G. Hayashi, N. Kanoh and M. Nakata, *Tetrahedron Lett.*, 2003, **44**, 7747–7751.

4 For previous communications of our work, see: (*a*) I. Paterson, R. M. Oballa and R. D. Norcross, *Tetrahedron Lett.*, 1996, **37**, 8581–8584; (*b*) I. Paterson and L. E. Keown, *Tetrahedron Lett.*, 1997, **38**, 5727–5730; (*c*) I. Paterson and R. M. Oballa, *Tetrahedron Lett.*, 1997, **38**, 8241–8244; (*d*) I. Paterson, D. J. Wallace and K. R. Gibson, *Tetrahedron Lett.*, 1997, **38**, 8911–8914; (*e*) I. Paterson, D. J. Wallace and R. M. Oballa, *Tetrahedron Lett.*, 1998, **39**, 8545–8548; (*f*) I. Paterson, D. Y.-K. Chen, M. J. Coster, J. L. Acena, J. Bach, K. R. Gibson, L. E. Keown, R. M. Oballa, T. Trieselmann, D. J. Wallace, A. P. Hodgson and R. D. Norcross, *Angew. Chem., Int. Ed.*, 2001, **40**, 4055–4060; (*g*) I. Paterson and M. J. Coster, *Tetrahedron Lett.*, 2002, **43**, 3285–3289; (*h*) I. Paterson, J. L. Acena, J. Bach, D. Y.-K. Chen and M. J. Coster, *Chem. Commun.*, 2003, 462–463; (*i*) I. Paterson and M. J. Coster, in *Strategy and Tactics in Organic Synthesis*, ed. M. Harmata, Elsevier, Oxford, 2004, vol. 4, ch. 8, pp. 211–245.

5 C. J. Cowden and I. Paterson, *Org. React.*, 1997, **51**, 1–200; I. Paterson, C. J. Cowden, and D. J. Wallace, in *Modern Carbonyl Chemistry*, ed. J. Otera, Wiley-VCH, Weinheim, 2000, pp. 249–297.

6 H. C. Brown, R. K. Dhar, K. Ganesan and B. Singaram, *J. Org. Chem.*, 1992, **57**, 499–504.

7 P. L. Hall, J. H. Gilchrist and D. B. Collum, *J. Am. Chem. Soc.*, 1991, **113**, 9571–9574.

8 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543–2549; J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512–519; I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092–4096.

9 S. V. Ley, J. Norman, W. P. Griffith and S. P. Marsden, *Synthesis*, 1994, 639–666.

10 I. Paterson, C. J. Cowden, V. S. Rahn and M. D. Woodrow, *Synlett*, 1998, 915–917.

11 A similar result was observed by the Kishi group (ref. 2*f*).

12 R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbrüggen, *J. Am. Chem. Soc.*, 1966, **88**, 852–853; G. Jou, I. Gonzalez, F. Albericio, P. Lloyd-Williams and E. Giralt, *J. Org. Chem.*, 1997, **62**, 354–366.

13 J. Inanaga, K. Hirata, H. Saeki, T. Katsuki and M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1989–1993.

14 See the electronic supplementary information for tabulated ^1H and ^{13}C NMR data and copies of spectra.

15 M. Kobayashi, S. Aoki, H. Sakai, K. Kawazoe, N. Kihara, T. Sasaki and I. Kitagawa, *Tetrahedron Lett.*, 1993, **34**, 2795–2798.
16 G. R. Pettit, Z. A. Chicacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt and J. N. A. Hooper, *J. Org. Chem.*, 1993, **58**, 1302–1304.
17 We thank Professors Pettit and Kishi for kindly providing comparison NMR spectra.
18 (*a*) A. B. Smith and Q. Lin, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 567–568; (*b*) A. B. Smith, R. M. Corbett, G. R. Pettit, J.-C. Chapuis, J. M. Schmidt, E. Hamel and M. K. Jung, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2039–2042.
19 F. M. Uckun, C. Mao, A. O. Vassilev, H. Huang and S.-T. Jan, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 541–545; H. Huang, C. Mao, S.-T. Jan and F. M. Uckun, *Tetrahedron Lett.*, 2000, **41**, 1699–1702.
20 A C50 chlorine substituent (as in altohyrtin A/spongistatin 1) confers somewhat greater cell growth inhibitory activity than the des-chloro version (altohyrtin C/spongistatin 2), see: J. Pietruszka, *Angew. Chem., Int. Ed.*, 1998, **37**, 2629–2636.
21 The biological assays were performed by Novartis Pharmaceuticals Corporation, Summit, New Jersey.
22 M. C. Alley, C. M. Pacula-Cox, M. L. Hursey, L. R. Rubenstein and M. R. Boyd, *Cancer Res.*, 1991, **51**, 1247–1256.