Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1841–1860

BIOORGANIC & MEDICINAL CHEMISTRY

REVIEW ARTICLE

Chemistry and Clinical Biology of the Bryostatins

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Received 18 February 2000; accepted 10 May 2000

Abstract—Bryostatins are a class of antineoplastic compounds isolated from the bryozoans *Bugula neritina*. A wide range of scientific research is currently underway, studying different aspects of the bryostatins. In this review we try to summarize the latest findings, including all the topics involved, from marine biology to medicinal chemistry.

Contents

Introduction

The bryostatins are a class of highly oxygenated marine macrolides with a unique polyacetate backbone. Bryostatin 1 (**1a**) was first isolated and characterised by Pettit in 1982,[1] from the marine animal *Bugula neritina* (Linnaeus), after showing high activity against the murine P388 lymphocytic leukemia. Currently, there are 20 natural bryostatins (Fig. 1) known; their main difference being the nature of the substituents at C_7 and C_{20}.[2]

Since their discovery, bryostatins have created a wide interest in the scientific community. The antineoplastic activity, in combination with low toxicity, has made this class of compounds a chemotherapeutic candidate for the treatment of cancer. The specific interactions with protein kinase C (PKC), and other enzymes, led to a wide range of studies directed towards a better understanding of its specific activity as well as clarifying the mechanism of action of cellular signalling pathways in general. The limited supply and the low concentration in the natural source yielded an investigation of the marine animal and attempts to cultivate it. The unique chemical structure of the bryostatins posed a major synthetic challenge and its current market price at £261 (UK)/50 μg[3] has definitely created business interest. In this review we attempt to give an overview of the different aspects of research involving bryostatins and a summary of the latest results. Some topics are previously reviewed and mentioned in the appropriated section of this review.

Natural Sources and Biosynthesis

The first collection of *Bugula neritina* from the Gulf of Mexico in 1968 led to the isolation of bryostatin 1,[1] the major active substance in the animal. Further and larger

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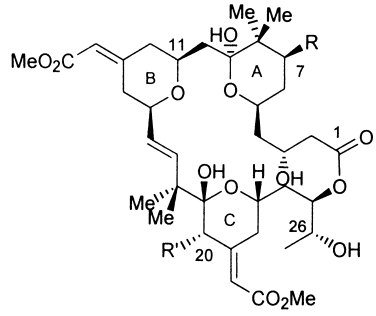
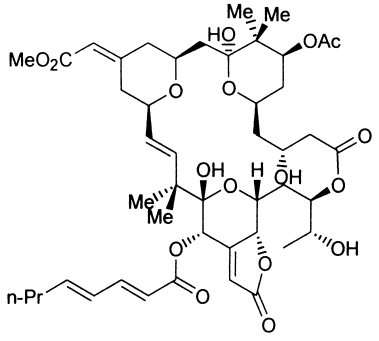
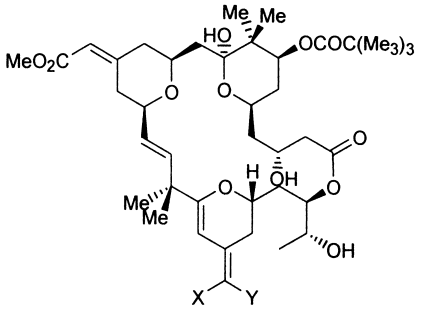
0968-0896/00/$ - see front matter
PII: S0968-0896(00)00150-4



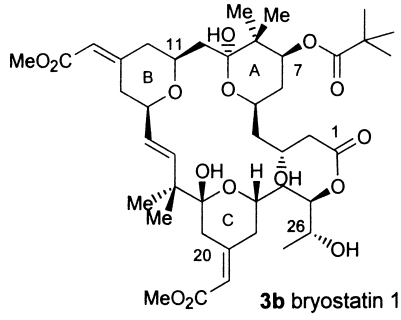
1a R = OAc, R' = $OCO(CH)_4$n-Pr bryostatin 1
1b R = OH, R' = $OCO(CH)_4$n-Pr bryostatin 2
1c R = $OCOC(CH_3)_3$, R' = OCOn-Pr bryostatin 4
1d R = $OCOC(CH_3)_3$, R' = OAc bryostatin 5
1e R = OCOn-Pr, R' = OAc bryostatin 6
1f R = OAc, R' = OAc bryostatin 7
1g R = OCOn-Pr, R' = OCOn-Pr bryostatin 8
1h R = OAc, R' = OCOn-Pr bryostatin 9
1i R = $OCOC(CH_3)_3$, R' = H bryostatin 10
1j R = OAc, R' = H bryostatin 11
1k R = $OCO(CH)_4$n-Pr, R' = OCOn-Pr bryostatin 12
1l R = OCOn-Pr, R' = H bryostatin 13
1m R = $OCOC(CH_3)_3$, R' = OH bryostatin 14
1n R = OAc, R' = $OCO(CH)_4CH(OH)Et$ bryostatin 15



2 bryostatin 3



3a X = H, Y = CO_2Me bryostatin 16
3b X = CO_2Me, Y = H bryostatin 17



3b bryostatin 18

Figure 1.

collections yielded in the isolation of bryostatins 2,[4] 3,[5] 4,[6] 5–7,[7] 8,[8] 9,[9] 10+11,[10] 12+13,[11] 14+15,[12] and 16–18.[13] The first published structure of bryostatin 3 was later revised.[14] Another collection from the same location led to the isolation of bryostatin 4 in 8.9×10^{-5}% wt yield. Collections from the Gulf of California and from the Gulf of Sangami yielded 9.8 and 2×10^{-5}% wt respectively,[6] indicating for the first time that the bryostatins are more likely to be a biosynthetic or symbiotic product rather than arising from a dietary source. The yield of the extraction is not only dependent on the geographical site, it also depends on the time of year and depths of the collection. Bryozoans collected from different sites also showed different bryostatin ratios. For example 1000 kg of damp *Bugula neritina* collected from the Gulf of Mexico gave: 306 mg bryostatin 4, 187 mg bryostatin 5, 32.5 mg bryostatin 6, 3.1 mg bryostatin 7, 23.5 mg bryostatin 8, 39 mg bryostatin 10 and 102 mg bryostatin 14. An approximately equal amount collected from the U.S. Southern California Coast gave: 1.5 g bryostatin 1, 2.0 g bryostatin 2, 5.6 mg bryostatin 8 and 8.6 mg bryostatin 15.[12] The highest yielding source reported so far produced 15 mg bryostatin 10 out of 1.5 kg wet animal (1.0×10^{-3}% wt) and originated from the Gulf of Aomori in Japan.[15] Bryostatins were also found in smaller quantities in the marine organisms *Lissodendoryx isodictyalis*, *Aplidium californium* and *Amathia convoluta*. Each organism was discovered to have *Bugula neritina* growing within its biomass. Whether these bryostatins are produced by the organism or transferred from *Bugula neritina* remains uncertain.[8] In the case of *Lissodendoryx isodictyalis*, two novel bryostatins (A + B) were found. Full characterisation was not possible because of the small amounts obtained but there is evidence that they are desoxybryostatin 5 and desoxybryostatin 4 respectively. It is thought that they may be

formed as metabolites by the organism,[16] whilst neristatin 1 is a possible biosynthetic precursor or degradation product obtained from *Bugula neritina*.[17]

The difference in bryostatin content in *Bugula neritina* led to the definition of two chemo types: chemo type O which includes bryozoas producing bryostatins with the octa-2,4-dienoatic acid ester chain and chemo type M in which the bryostatins do not have this ester chain.[18] Genetic studies showed that these two chemotypes differ in approximately 8% of their mitochondrial CO I sequences and are called genotype D (chemotype O) and S (chemotype M). They also contain different strains of symbionts, called '*Candidatus Endobugula neritina*'. The symbionts were first discovered in the larvae of the bryozoans[19] and identified as a novel species of γ-proteobacterium. Attempts to cultivate the bacterium are ongoing and it remains uncertain whether it is involved in the biosynthetic production of the bryostatins. The two genotypes appear to have different ecological environments and are not found immediately adjacent to one another. If they are found in the same geographical area, then genotype D (deep) and genotype S (shallow) live at different depths. This pattern suggests that the two types are separated by an environmental parameter such as temperature.

Currently there is only one report on the biosynthesis of bryostatins.[20] Freshly collected *Bugula neritina* was flash frozen and ground to a fine powder. This cell-free extract was incubated with a range of radiolabeled chemical building blocks to produce bryostatins. The biosynthetic precursors are found to be acetate, glycerol and S-adenosylmethionine, whereas propionate, *n*-butyrate, isobutyrate and succinate were not involved in the biosynthetic pathway.

Cultivation of Bryozoans

Bugula neritina is commonly used in the laboratory and in field studies because of its abundance and the ease with which the larvae can be collected and induced to 'settle'/adhere to a surface in the laboratory.[18] The larvae are nonfeeding and spend only a few hours in the water before settling. Studies on the effect of swimming duration, settling and growth of the larvae were carried out under laboratory conditions[21] as well as under field conditions.[22] The general outcome was that larvae swimming for less than 12 hours were more likely to settle, used less time to complete metamorphosis, were more likely to complete metamorphosis and were faster growing under both laboratory and field conditions. Colonies from larvae swimming for short duration, also showed an earlier reproduction time in their lifecycle. Independent of the swimming duration, colonies grew faster when facing down, rather than up. Freshly released larvae are strongly photopositive and after several hours become photonegative or alternate between positive and negative phototaxis.[23] This process seems to be regulated by dopamine; additional dopamine prevented larvae from a change of phototaxis whereas serotonin induced change. Whether this has any connection to the observation made in the studies on swimming duration, remains unknown.

The company CalBioMarine Technologies, from Carlsbad (California) has grown *Bugula neritina* in tanks on shore. Since early 1996 they have been moving their crop into the sea after initial colonisation by the larvae on plastic plates. After five months their growth is comparable to the natural colonies and they are ready for harvesting.[24]

Bioactivity

Bryostatin 1 has a wide range of activities including immune stimulation, growth inhibition, induction of differentiation, and enhancement of cytotoxicity of other drugs particularly towards target cells.[25]

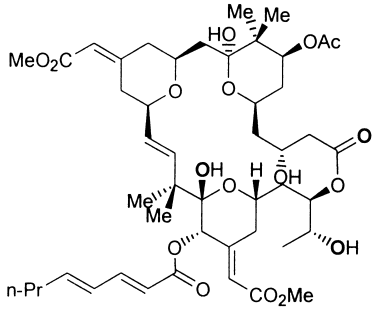
A wide range of studies on the molecular and cellular level was carried out involving bryostatins. They can be split into two general groups: studies designed to understand the action of bryostatins on enzymes and cell lines and studies designed to gain a better understanding of enzyme activities or cellular events, such as apoptosis. Although the first group deals specifically with bryostatins, the second, which has been the subject of more reports, contributes important information as well.

Molecular Interaction

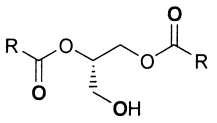
The family of protein kinase C isoenzymes are serine/threonine kinases comprising 12 isozymes that play crucial roles in various cell-signalling and other processes.

Diacylglycerols (DAG), phosphatidyl serine (PS), inositol triphosphate (IP_3) and calcium ions (Ca^{2+}) are the naturally occurring activators of these enzymes. The PKC group of kinases transfers the terminal phosphate from adenosine triphosphate (ATP) onto other proteins, and in some reactions self-phosphorylation occurs. To activate PKC DAG (produced by the action of phospholipase on phospholipid in a membrane) must be bound to the protein. Many PKCs also bind to PS so they are concentrated at the cytosolic surface of cell membranes. Under these conditions PKC is active at low physiological concentrations of Ca^{2+}-ions. When IP_3 is present in the cell cytoplasm, it releases Ca^{2+}-ions (mainly from the endoplasmic reticulum of the cell) and further activation of some PKCs occurs to produce a full cellular response. These events and some resulting implications are summarized in a recent review.[26]

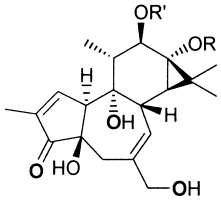
The PKCs can be classified into three groups: conventional PKCs (α, βI/βII, γ) which require calcium for activation, novel PKCs (δ, ε, θ, η/L) which are calcium-independent, and atypical PKCs (ξ, λ/ι) which do not bind to phorbol esters or bryostatins. The functions of the different isoforms are not fully understood so far, mainly because most activators and inhibitors lack isozyme selectivity. The conventional and novel PKCs consist of a regulatory and a catalytic domain. The regulatory domain can further be split in two cysteine-rich domains (CRD1 and CRD2). These two sites were shown to bind to different activators.[27]



1a bryostatin 1



4 1,2-diacyl-*sn*-glycerol



5 phorbol ester

Figure 2.

Interactions of bryostatins with PKC

Bryostatins, phorbol esters (a family of tumour promoters) and DAG all compete for the same binding sites, as can be shown by displacement binding assays. A previously determined pharmacophore model for DAG and phorbol esters[28] could be applied successfully to the bryostatins[29] and other activators[30] of PKCs. The main binding sites in bryostatins include the C_1, C_{19} and C_{26} oxygen atoms (Fig. 2). This was proven by testing chemically modified derivatives which, depending on the modification, lost some of their binding affinities (Table 1). It remains unexplained why tumour-promoting compounds such as phorbol esters appear to have the same binding sites in PKCs as the antineoplastic bryostatins. Results of studies on cell lines indicated that the CRD1 and CRD2 sites do bind the activators with different affinities and that PKCδ seems to be a key enzyme, which shows different behaviour towards phorbol esters and bryostatins. Studies were designed to look more closely at the binding sites in the PKCα CRD2,[31] the PKCδ CRD2[32] and others,[33,34] and at the differences in their binding affinities.[35] Although these studies showed remarkable results and indicate that activators can dock onto different binding sites of PKCs, no explanations were found why the biological response between bryostatins and phorbol esters is so different.

Table 1. PKC (isoform mix) binding affinities

Entry	Bryostatin	K_i ($\times 10^{-9}$ M)
1	1	1.35±0.17
2	2	5.86±1.13
3	3	2.75±0.05
4	4	1.30±0.19
5	5	1.04±0.10
6	6	1.18±0.29
7	7	0.84±0.07
8	8	1.72±0.10
9	9	1.31±0.00
10	10	1.56±0.16
11	1 (26-*epi*)	32.6±6.6
12	2 (13,30,2′,3′,4′,5′-hexahydro)	9.61±0.94
13	2 (13,30,21,34,2′,3′,4′,5′-octahydro)	473±96
14	4 (26-acetate)	>> 100
15	4 (13,30-epoxide)	0.54±0.07
16	16	118±2
17	17	188±7
18	18	4.82±0.06

The solid-phase synthesis of the CRD1 and CRD2 sites of the different isoforms of PKC was recently reported and it was shown that they could mimic the binding properties of the PKCs towards phorbol esters.[36] Further studies of the PKC isozyme selectivity towards bryostatins are underway and should offer some clarification of this matter.

Cellular Interaction

In almost all studies the action of bryostatin 1 on PKC-isoforms was investigated. The general outcome is that bryostatin 1 induces an initial short lasting activation and self-phosphorylation of PKCs leading to its translocation to the membrane and subsequent down-regulation by induced proteolysis by a proteasome. Beside this commonly reported observation, studies focus on diverse range of aspects and effects on different proteins. Many contradictory results have been reported and it seems that the responses caused by bryostatin 1 are concentration dependent and cell-line specific.

Only one study compared the bryostatins 5 and 8 to bryostatin 1.[37] It appears that they have identical anticancer potential but indications were found that the former two compounds might have fewer or different side effects.

Interactions of bryostatin 1 with PKC

Initial studies focused on the observed down-regulation of PKCs by bryostatin 1. It was found that the expression of PKCs is not directly affected by bryostatin 1.[38] It was later discovered that PKCα[39] and PKCε[40] is ubiquitinated before degradation through the proteasome takes place. Down-regulation of PKCs with phorbol esters leads to this degradation pathway as well. PKCδ seems to be the exception, not being affected by high levels of bryostatin 1.[41] Further studies showed that the catalytic domain of PKCδ confers protection from down-regulation and that this happens in a biphasic manner.[42] The regulatory domains (CRD1 and CRD2) were found to

be involved in the translocation upon exposure to bryostatin.[43] Interestingly, PKCδ is down-regulated by phorbol 12-myristate 13-acetate (PMA) but high levels of bryostatin inhibits this down-regulation, even when co-administrated with PMA.[44] It was suggested that this difference in modulating PKCδ could explain the different response between the tumour promoter PMA and bryostatin 1.

However, these findings alone cannot explain the growth inhibition caused by bryostatin 1. A study with 26-*epi*-bryostatin 1 using a melanoma cell-line showed a 60-fold reduced affinity and a similar reduced down-regulation of PKCs whereas the growth inhibition caused by 26-*epi*-bryostatin 1 was nearly the same as that induced by bryostatin 1.[45] This suggests an alternative mechanism of growth inhibition operates at least in this cell-line.

Bcl-2 and related proteins

The Bcl-2 (B-cell lymphoma gene 2) family plays a crucial role in early apoptotic events. It is thought that this family of genes regulate programmed cell death (apoptosis) and the family consists of anti-apoptotic (Bcl-2, Bcl-X_L, Bcl-W, Mcl-1, A1, and NR-13) and pro-apoptotic (BAX, BAK, BOK, and Bcl-X_S) members.[46,47] The ratio of BAX:Bcl-2 determines the response to apoptotic signals and many drug-resistant cell lines express high levels of Bcl-2. It is reported that Bcl-2 phosphorylation is required for its anti-apoptotic function.[48]

Mitochondrial PKCα is needed for phosphorylation and if REH cell-line is treated with bryostatin 1, translocation of PKCα to the membrane leads to Bcl-2 phosphorylation and increased REH cell resistance to drug-induced apoptosis.[49] In contradiction, bryostatin 1 induced down-regulation and ubiquitination of Bcl-2 in REH cell-lines, followed by degradation of Bcl-2 was reported.[50,51] The dephosphorylation is mediated by protein phosphatase 2A (PP2A),[52] which is activated by C2-ceramide.[53] A significant increase in the BAX:Bcl-2 ratio could be observed after treatment with bryostatin 1 in WSU-CLL cells[54] but no change was observed in HL-60 cells,[55] although a synergistic interaction between bryostatin 1 and 1-[β-D-arabinofuranosyl]cytosine (ara-C) was observed.

Other observed interactions

A biphasic activation of caspase-3, similar to the one observed for PKCδ, was found in HeLa cells. This correlates well with the biphasic response in potentiating cell death by *cis*-diamminedichloroplatinum(II) (cDDP).[56] High levels of bryostatin 1 decreases P-glycoprotein (Pgp) phosphorylation in the MCF-7 cell line through down-regulation of PKCs but it cannot reverse Pgp-mediated multidrug resistance.[57] Later it was found that even low levels of bryostatin 1 down-regulate the E_S nucleoside transporter in WSU-CLL cells and increase 2-CdA influx into cells.[58] Long-term treatment with bryostatin 1 results in inhibited levels of desmoglein 1 and 3 but not desmoglein 2 in normal human epidermal keratinocyte cells.[59] It was also shown that bryostatin 1 can induce differentiation in NB4[60] and other[61] cell lines but there are cell lines, such as HL-60[62] which do not differentiate after treatment. The effect of bryostatin 1 on human monocytes and their production of cytokines was studied and it was found that bryostatin induces interleukin-1β (IL-1β), IL-6, IL-8 and tumour necrosis factor-α (TNF-α) mRNA expression and protein secretion. It also acts synergistically with IL-2 in triggering monocyte activation.[63] This activation could be applied in an adoptive cellular immunotherapy, as preliminary studies have shown.[64]

Synergistic interactions of bryostatin 1

Recently, much attention has been given to the study of synergistic interaction between bryostatin 1 and other cytotoxic compounds. The benefits sought are an improvement in the outcome of treatment (i.e., increase in apoptotic cells) and a lowering of dosage of cytotoxic compounds to avoid possible side effects.

Table 2. Synergistic interactions between bryostatin 1 and other cytotoxic compounds

Cell line[a]	Schedule[b]	Cytotoxic compound[c]	Remarks	Ref.
HL-60	b24h/cc6h	ara-C[65]	doubled the amount of apoptotic cells	55,62,66
U937	Cc6h/b15h	taxol	doubled the amount of apoptotic cells	67,68
P388	cc/b	tamoxifen staurosporine	the growth inhibition was 200 times enhanced in the case of tamoxifen	69
Reh	B24h/cc	Dolastatin 10 Auristatin PE Vincristine	the enhancement of apoptotic cells was greater with auristatin PE and vincristine than for dolastatin 10	70
WSU-CLL	b/cc	2-CdA	animal study, 5 days bryostatin followed by 5 days 2-CdA extended tumour growth delay from 37 days to 76 days	54
WSU-DLCL2	B24h/cc	Vincristine ara-C	animal study, vincristine extended tumour growth delay from 16 days to 38 days; none was seen for ara-C	71

[a]L-60, human myeloid leukemia; P388, murine lymphocytic leukemia; Reh, acute lymphoblastic leukemia; U937, human leukemia; WUS-CLL, chronic lymphocytic leukemia; WUS-DLCL2, diffuse large cell lymphoma.
[b]b, bryostatin 1; cc, cytotoxic compound; (i.e. b24h/cc1h = bryostatin 1 incubation for 24 h followed by cytotoxic compound for 1 h).
[c]Ara-C, 1-[β-D-arabinofuranosyl]cytosine; 2-CdA, 2-chlorodeoxyadenosine.

Almost all of the cell-lines studied revealed a strong schedule-dependent response (Table 2). The reasons for this could be differences in PKC isozyme expression and/or the cell-line-specific interactions with the Bcl-2 protein family.

The observed sensitisation of cell-lines and improved results are now being evaluated in clinical trials.

Results of Preclinical Studies and of Clinical Trials

The preclinical and clinical aspects of bryostatin 1 was recently reviewed by Zonder and Philip.[72]

Several preclinical studies were undertaken to understand how bryostatin 1 might be best used as an anticancer drug. The pharmacokinetics, tissue distribution, metabolism, and elimination of bryostatin 1 in CD1/F2 mice were studied,[73] using [C26-^{3}H]-labelled bryostatin 1. The half-lives after iv administration were 1.05 h and 22.97 h. Similar results were obtained after ip administration. There was only one major unidentified metabolite detected in the plasma after 12 h following injection, but the majority of the drug remained unmodified. Bryostatin 1 had a wide tissue distribution and could be found in all the tissue probes examined. The highest concentrations were found in the liver, lungs, and bone marrow. Elimination occurs in the first 12 h after administration mainly through urinary excretion. However, within 72 h faecal excretion became the major pathway. The inhibitory effects of bryostatin 1 on the growth of rabbit papillomas were proven to be dose independent,[74] but no cure was observed. Weekly or daily administration was most successful for iv administration of 0.1–2.5 μg/kg doses. Combination treatment studies were carried out with different anticancer drugs and bryostatin 1. One study, involving WSU-CLL-bearing SCID mice, showed that the anticancer activity is highly dose and schedule specific.[75] The best results were obtained when 2-chlorodeoxyadenosine was given at 30 mg/kg/injection/day after bryostatin 1 at 75 μg/kg/injection/day. Reversal of administration or 2-chlorodeoxyadenosine alone was not active at all. Bryostatin 1 on its own was active, but not as good as the combination. The only reported cure of WSU-CLL-bearing SCID mice (5/5) was achieved with auristatin PE[76] (1.5 mg/kg iv) followed by bryostatin 1 (75 μg/kg ip) every second day, repeated three times.

Bryostatin 1 is currently being evaluated alone and in combination with other chemotherapeutic agents in over 43 separate phase I and II human clinical trials (23 closed and 20 open trials) for melanoma,[77,78] myeloma, acute myeloid leukaemia,[79] chronic lymphocytic leukaemia (CCL),[80–82] AIDS-related lymphoma, non-Hodgkin's lymphoma,[83] colorectal, renal, prostate, head and neck, cervix, ovarian, breast, peritoneal, stomach, oesophagus, anus and non-small cell lung cancer.[84–86]

In all the cases myalgia (muscle pain) was the dose-limiting toxicity (DLT). It was observed that some patients suffered from myalgia for weeks, even when they were removed from the study.[83] In a study on children regular analgesics were used to treat this side effect.[86] The occurrence of myalgia was studied to gain a better understanding of its causes.[87] The observed reduction in mitochondrial function and proton efflux from the cell could be explained through muscle vasoconstriction. However, administration of Nifedipine did not reduce the clinical toxicity of bryostatin 1,[88] although it counteracted the vasoconstrictive effect. It seems that the myalgia is a direct toxicity of bryostatin 1. Other side effects observed were photophobia, eye pain, headache, fatigue, phlebitis, vomiting, anorexia, anaemia, fever and flu-like symptoms.

The maximum-tolerated dose reported is 120 μg/m^2 per course (72-h continuous infusion).[83] In phase II clinical trials doses between 25–35 μg/m^2 per course were administered[77] as a weekly 1 h or 24 h infusion three times every 4 weeks.[85]

So far no cures have been measured in the clinical trials of bryostatin 1 alone, but some patients had a stable disease state for up to 19 months. In combination phase I trials with cisplatin[89] or vincristine,[90] partial responses in one and two patients respectively have been observed.

Synthesis

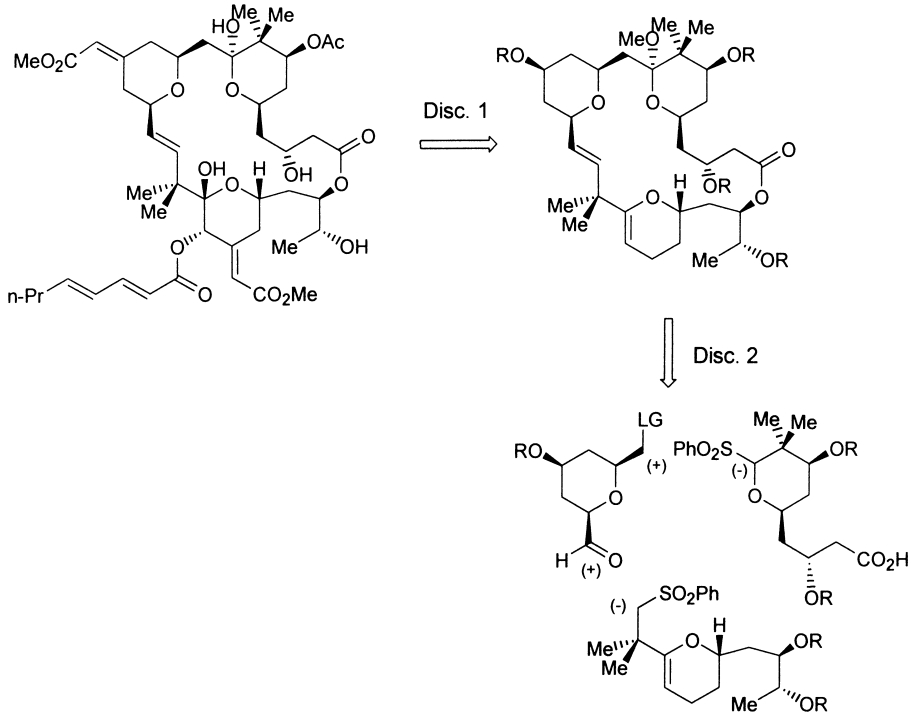
Several groups have contributed towards the synthesis of bryostatin and some work remains to be published. The structure has led to the development of a number of new reactions and many different disconnection strategies have been applied. Often the strategies have had to be modified during the work because of unexpected problems arising from unstable intermediates or from problems with the final coupling steps.

This part of the review describes the latest developments in the synthetic work on the total and partial synthesis of various bryostatins published after 1995. The earlier work is already included in an excellent review published by Norcross and Paterson.[91]

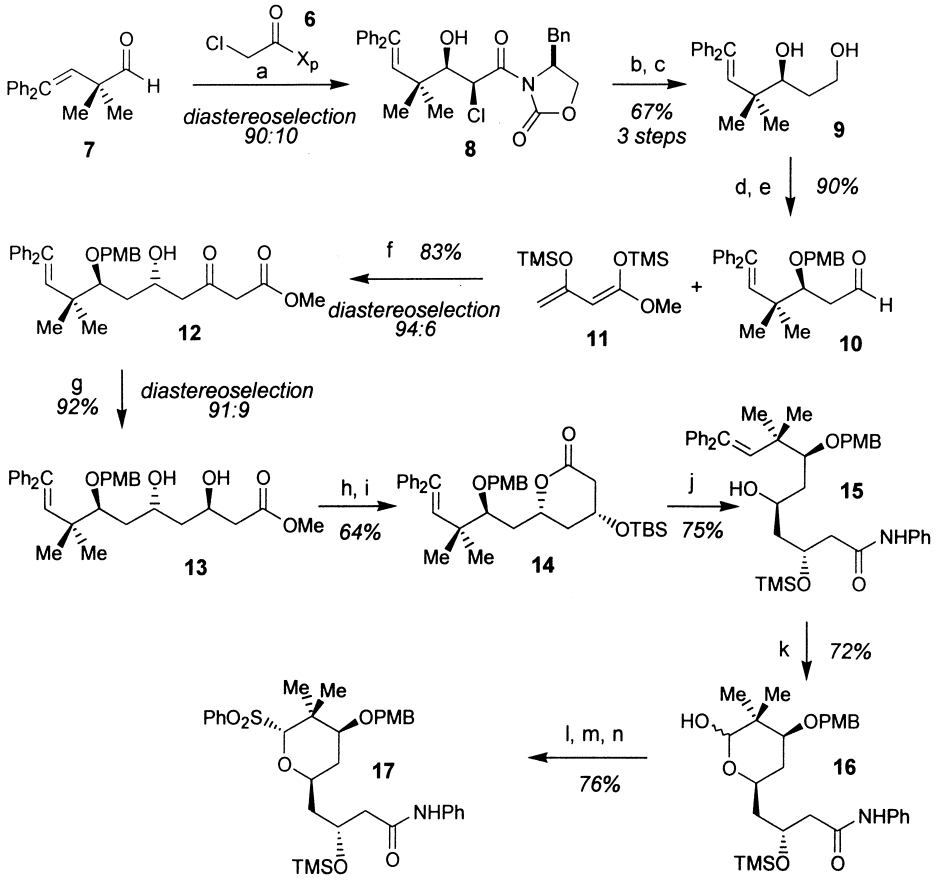
Total Synthesis

Evans' total synthesis of bryostatin 2.[92,93] The disconnection that was employed for the total synthesis of bryostatin 2 (Scheme 1) features the introduction of some of the functional groups as well as the removal of the protecting groups (Disconnection 1). The macrocyclic intermediate was formed from three cyclic segments (Disconnection 2). Using more complex segments, which would simplify the first disconnection, proved to be unsatisfactory because of low yielding coupling reactions.

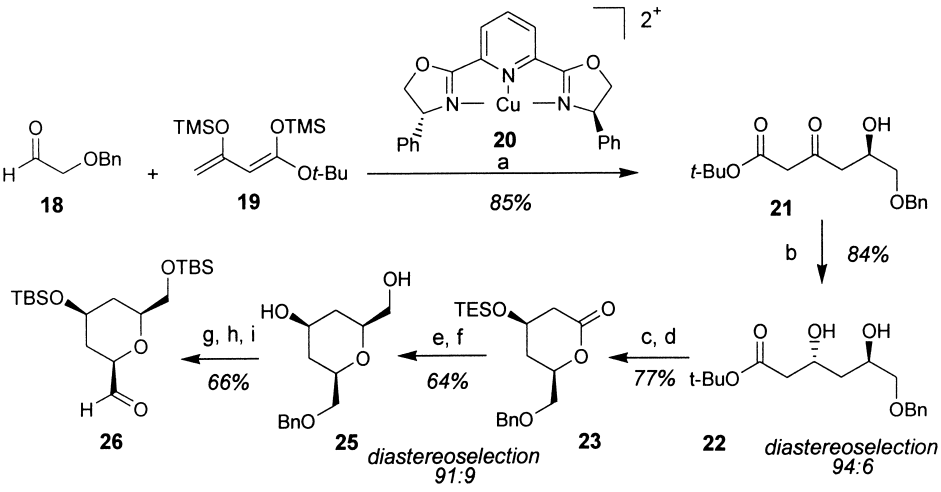
C_1–C_9 segment (Scheme 2). The dibutylboron triflate-mediated aldol reaction between the oxazolidinone (**6**) and butenal (**7**) yielded aldol (**8**) as a 9:1 mixture of diastereomers. Removal of the halide auxiliary and reduction gave diol (**9**). Protection and regioselective



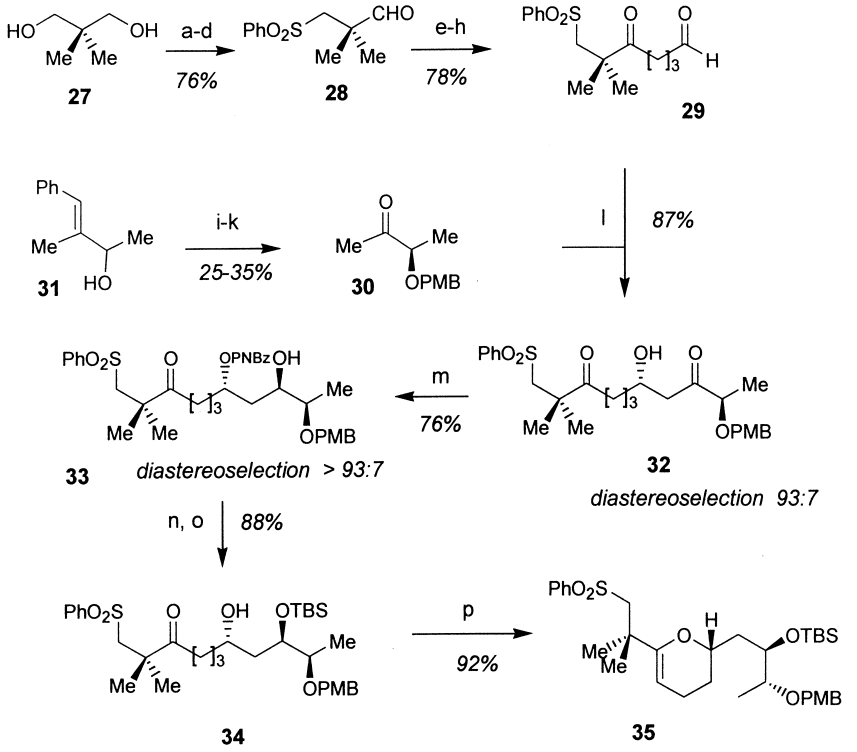
Scheme 1. Evans' disconnections.



Scheme 2. Evans' C_1–C_9 synthesis. (a) 1.9 equiv of **6**, Bu_2BOTf, *i*-Pr_2NEt, then **7**, CH_2Cl_2, −78 to 0°C, 20 h; (b) Zn, 2:1 THF/AcOH; (c) $LiBH_4$, MeOH, THF, 0 °C; (d) i. $PMPCH(OMe)_2$, 10 mol% PPTS, CH_2Cl_2, ii. DIBAL-H, CH_2Cl_2, 0 °C; (e) Swern oxidation; (f) $TiCl_2(O$*i*-$Pr)_2$, $PhCH_3$, −78 °C, then **11**, −78°C; (g) $Me_4NHB(OAc)_3$, AcOH/MeCN, −35 °C; (h) PPTS, PhH, 80 °C; (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, −10 °C; (j) Me_3Al, $HCl.H_2NPh$, CH_2Cl_2, 30 °C, then **14**, 0 °C; (k) O_3, 10:1 CH_2Cl_2/MeOH, −78 °C, then Me_2S; (l) Ac_2O, pyr; (m) PhSTMS, ZnI_2, n-Bu_4NI, CH_2Cl_2; (n) *m*-CPBA, $NaHCO_3$, EtOAc.



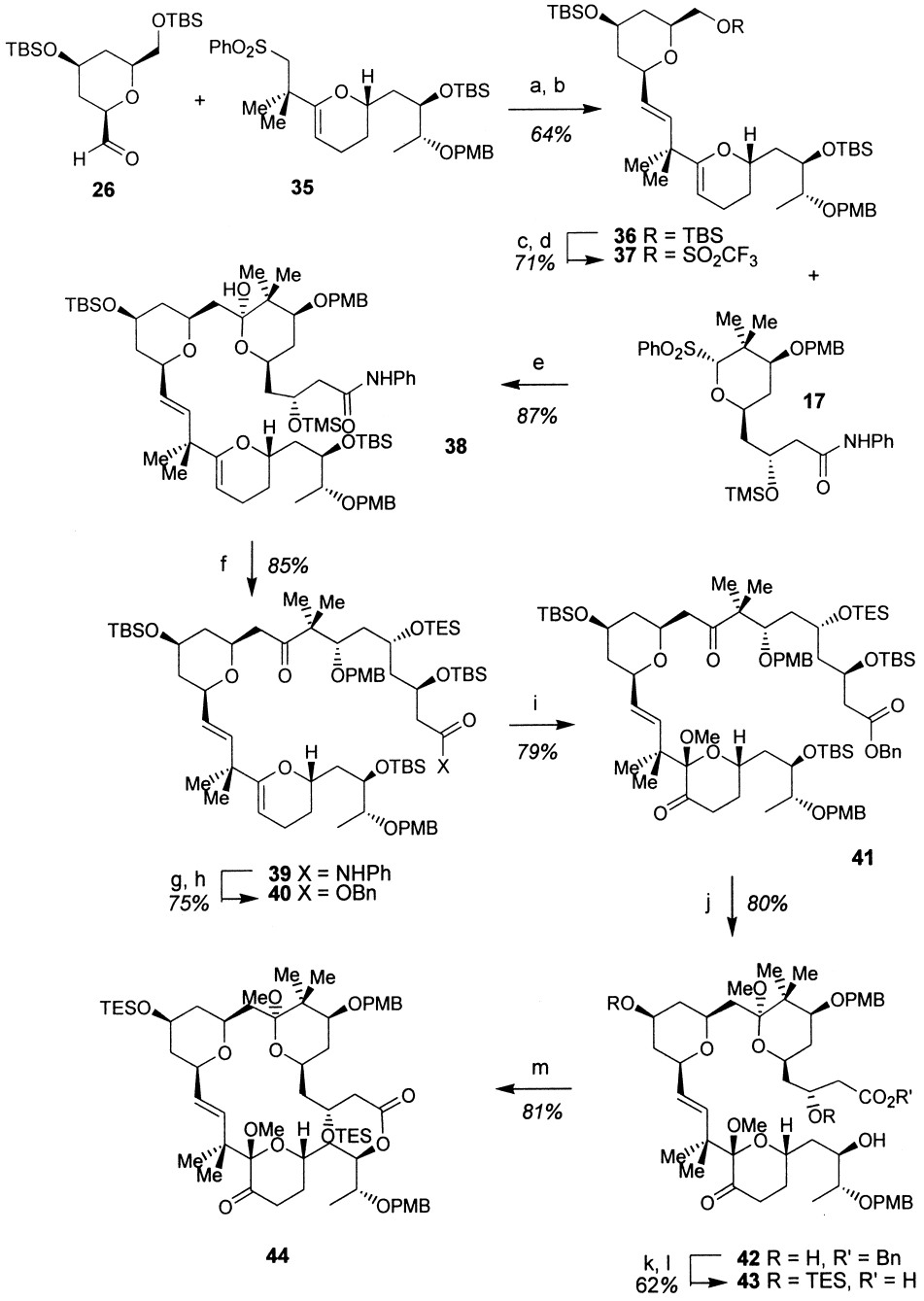
Scheme 3. Evans' C_{10}–C_{16} synthesis. (a) 5 mol% **20**, then **19**, CH_2Cl_2, −90 °C, then **18**, −90 °C; (b) $Me_4NHB(OAc)_3$, AcOH/MeCN, −35 °C; (c) F_3CCO_2H, CH_2Cl_2; (d) TESCl, imidazole, CH_2Cl_2, 0 °C; (e) $PMBOCH_2Li$, THF, −78 °C to −50 °C; (f) $BF_3.OEt_2$, Et_3SiH, CH_2Cl_2, −20 °C; (g) TBSCl, imidazole, DMAP, CH_2Cl_2; (h) 1 atm H_2, catalytic 10% Pd/C, AcOH, EtOAc; (i) Swern oxidation.



Scheme 4. Evans' C_{17}–C_{27} synthesis. (a) 0.2 equiv of TosCl, pyr, CH_2Cl_2, 0 °C to rt; (b) PhSH, NaH, DMF, 80 °C; (c) *m*-CPBA, CH_2Cl_2, 0 °C to rt; (d) Swern oxidation; (e) $BrMg(CH_2)_3CH=CH_2$, 1:1 Et_2O/CH_2Cl_2, 0 °C to rt; (f) Swern oxidation; (g) 2 mol% $K_2OsO_4(OH_2)_2$, 2 mol% quinuclidine, $K_3Fe(CN)_6$, K_2CO_3, 1:1 *t*-BuOH/H_2O; (h) $NaIO_4$, $NaHCO_3$, 2:2:1 *t*-BuOH/H_2O/THF; (i) 15 mol% L-(+)-DIPT, 10 mol% Ti(O*i*-Pr)$_4$, 0.7 equiv of *t*-BuO_2H, CH_2Cl_2, −20 °C; (j) NaH, PMBBr, catalytic n-Bu_4NI, THF, 0 °C to rt; (k) O_3, CH_2Cl_2/MeOH, −78 °C, then Me_2S, −78 °C to rt; (l) **30**, (−)-DIPCl, NEt_3, CH_2Cl_2, −78 °C, then **29**, −70 °C; (m) 20 mol% SmI_2, *p*-NO_2PhCHO, THF, 0 °C; (n) TBSOTf, 2,6-lutidine, CH_2Cl_2, −15 °C; (o) LiOH, 2:2:1 HF/MeOH/H_2O; (p) 5 mol% CSA, PhH, 80 °C.

deprotection followed by a Swern oxidation led to aldehyde (**10**). This was connected to silyldienol ether (**11**) through a Ti-mediated aldol reaction yielding aldol (**12**) in 15:1 diastereoselection. The subsequent hydroxyl-directed *anti* reduction gave diol (**13**) which was transformed into lactone (**14**) under acidic conditions followed by silyl protection. Lactone (**14**) was then converted to anilide (**15**) and treatment with ozone provided the cyclized lactol (**16**) as a 1.5:1 (β:α) mixture of anomers. Acetylation and conversion of the product to the α-sulfide followed by oxidation led to the sulfone (**17**), the final C_1–C_9 segment.

C_{10}–C_{16} segment (Scheme 3). An aldol reaction between aldehyde (**18**) and silyldienol ether (**19**) catalysed by the chiral copper (II) complex (**20**) led to aldol (**21**). A



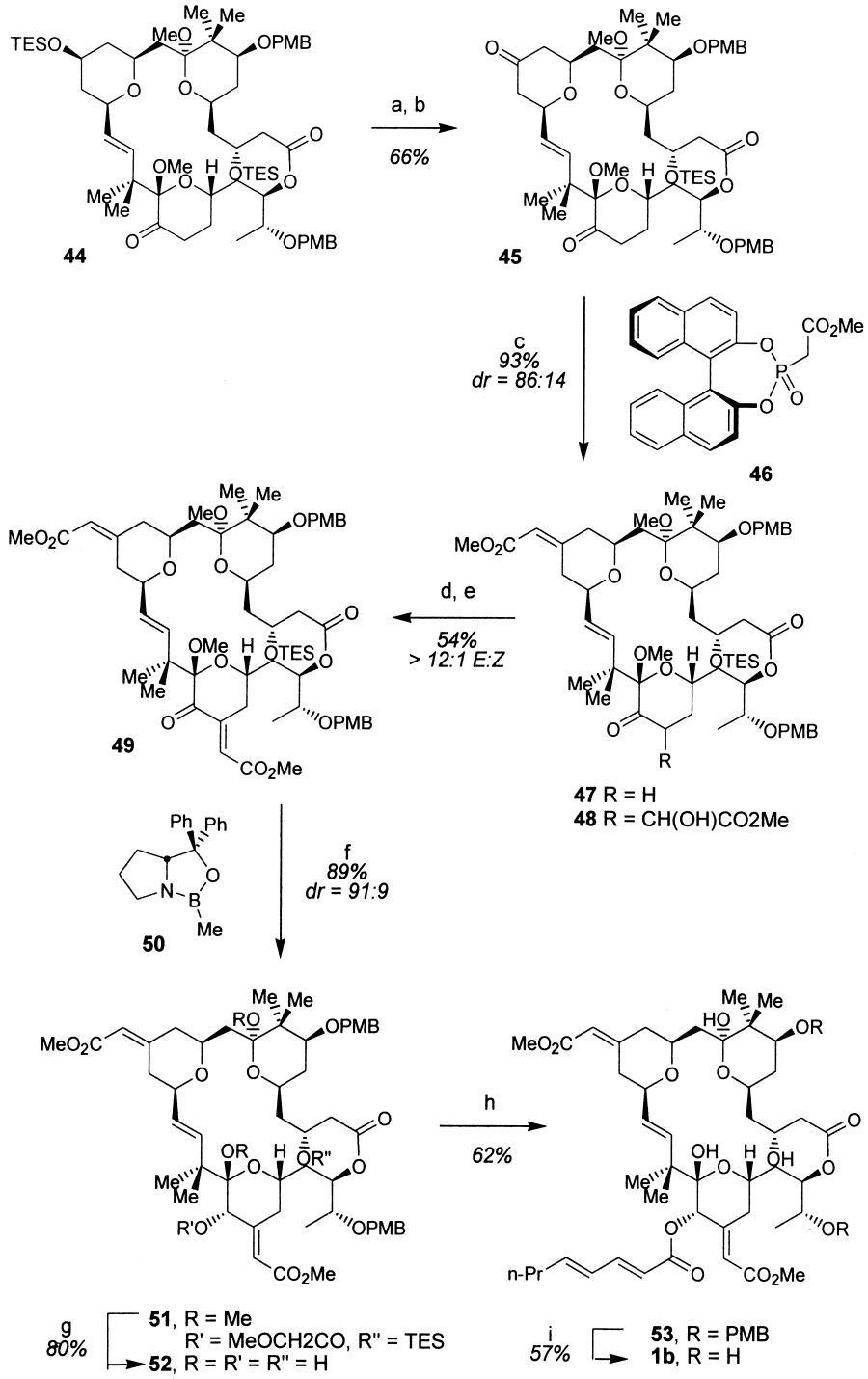
Scheme 5. Evans' fragment coupling. (a) i. *n*-BuLi, THF, −78 °C, then **26**, −78 to −50 °C; ii. Ac_2O, DMAP, CH_2Cl_2; (b) Mg, 20 mol% $HgCl_2$, EtOH; (c) TBAF, THF, −15 °C; (d) Tf_2O, 2,6-lutidine, CH_2Cl_2, −10 °C; (e) **17**, 2 equiv of *n*-BuLi, THF, −78 °C, then HMPA, then **37**, −78 °C; (f) TESCl, imidazole, MeCN; (g) Boc_2O, DMAP, MeCN; (h) BnOLi, 1:1 THF/DMF, −30 °C; (i) i. *m*-CPBA, MeOH, −20 °C; ii. $ClCH_2CO_2H$, MeOH, 0 °C; iii. Dess–Martin periodinane, pyr, CH_2Cl_2; (j) HF.pyr, 4:4:1 THF/MeOH/pyr; (k) TESCl, DMAP, CH_2Cl_2, 10 °C (65% + 15% each of the mono- and tris-silylether); (l) 1,4-cyclo-hexadiene, 10% Pd/C (50 mol%), EtOAc; (m) 2,4,6-trichlorobenzoyl chloride, *i*-$PrNEt_2$, PhH, then DMAP, 1.0 mM PhH.

hydroxyl-directed *anti* reduction gave diol (**22**) which was transformed into lactone (**23**) under acidic conditions followed by silyl protection. One-carbon homologation of (**23**) proceeded smoothly to afford the α-lactol (**24**) as a single diastereomer. The subsequent reduction of the lactol using the Kishi conditions was accompanied by a desilylation to form diol (**25**). Silylation, hydrogenolysis of the benzyl ether and Swern oxidation led to aldehyde (**26**), the final C_{10}–C_{16} segment.

C_{17}–C_{27} segment (Scheme 4). The diol (**27**) was converted into sulfonylaldehyde (**28**) through a monotosylation, sulfide displacement, sulfide oxidation, and Swern oxidation. Reaction with the Grignard reagent derived from 5-bromo-1-pentene followed by a Swern oxidation, a dihydroxylation and a diol cleavage gave keto aldehyde (**29**). The required ketone partner (**30**) for the aldol reaction was prepared through resolution of alcohol (**31**), silylprotection and ozonolysis. The obtained

aldol (**32**) was transformed to the diol monoester (**33**) through a Tishchenko reduction. Silyl protection and ester cleavage led to hydroxyketone (**34**). Acidic conditions gave the dehydrated glycal (**35**), the final C_{17}–C_{27} segment.

Fragment coupling and macrocyclisation (Scheme 5). The aldehyde (**26**) was coupled with the deprotonated sulfone (**35**). Acetylation of the products followed by elimination according to the Pak method led to the (*E*) olefin (**36**). Selective desilylation and sulfonylation gave the unstable triflate (**37**), which was directly coupled to the dianion of sulfonylamide (**17**) to deliver the lactol (**38**). Problems arising from the acidity of the anilide N–H proton and the acid lability of the glycal (C_{19}–C_{20}) led to functionalisation of the A-ring through silylation to yield the open-chain hydroxyketone ether (**39**). This was transesterified to the benzylester (**40**) and oxidised to oxoketal (**41**). Complete desilylation and cyclisation of



Scheme 6. Evans' synthesis of bryostatin 2. (a) 20 mol% PPTS, 2:1 MeOH/$(MeO)_3CH$, CH_2Cl_2, −30 °C; (b) Dess–Martin periodinane, pyr, CH_2Cl_2; (c) **46**, NaHMDS, THF, −78 °C, then **45**, −15 °C; (d) KHMDS, THF, −78 °C, then $OHCCO_2Me$, −78 °C; (e) $Et_3NSO_2NCO_2Me$, PhH; (f) **50**, $BH_3.SMe_2$, CH_2Cl_2, then MeOH, then Ac_2O, pyr, DMAP; (g) i. PPTS, 3:1 THF/H_2O, ii. Na_2CO_3, MeOH, iii. pTsOH, 4:1 MeCN/H_2O; (h) (E,E)-2,4-octadienoic acid, DIC, DMAP, CH_2Cl_2; (i) DDQ, 10:1 CH_2Cl_2/pH 7 buffer.

the A-ring led to triol (**42**). Selective disilylation followed by debenzylation gave hydroxyacid (**43**), which was cyclised under modified Yamaguchi conditions to afford macrocycle (**44**).

Synthesis of bryostatin 2 (Scheme 6). Selective removal of the silyl group from the macrocycle (**44**) and a Dess–Martin oxidation of the alcohol led to diketone (**45**). For reasons of steric hindrance and electronic effects only one carbonyl group reacted with the sodium anion of Fuji's chiral phosphonate (**46**) to provide an 86:14 mixture of diastereomers (**47**). Selective enolisation of the C_{20} ketone and aldol addition to (**48**) followed by elimination mediated by the Burgess reagent led to ketone (**49**). The ketone was reduced employing the CBS reagent (**50**). The reaction mixture was quenched with isobutyraldehyde and addition of methoxyacetic anhydride afforded compound (**51**). Methoxyacetate (**51**) was then transformed to tetraol (**52**) via a three-step sequence: (i) desilylation and hydrolysis of C_9; (ii) saponification of the methoxyacetate; (iii) hydrolysis of C_{19}. Monoacylation was achieved by using carbodiimide activation giving compound (**53**). A DDQ oxidative deprotection finally yielded the bryostatin 2 (**1b**).

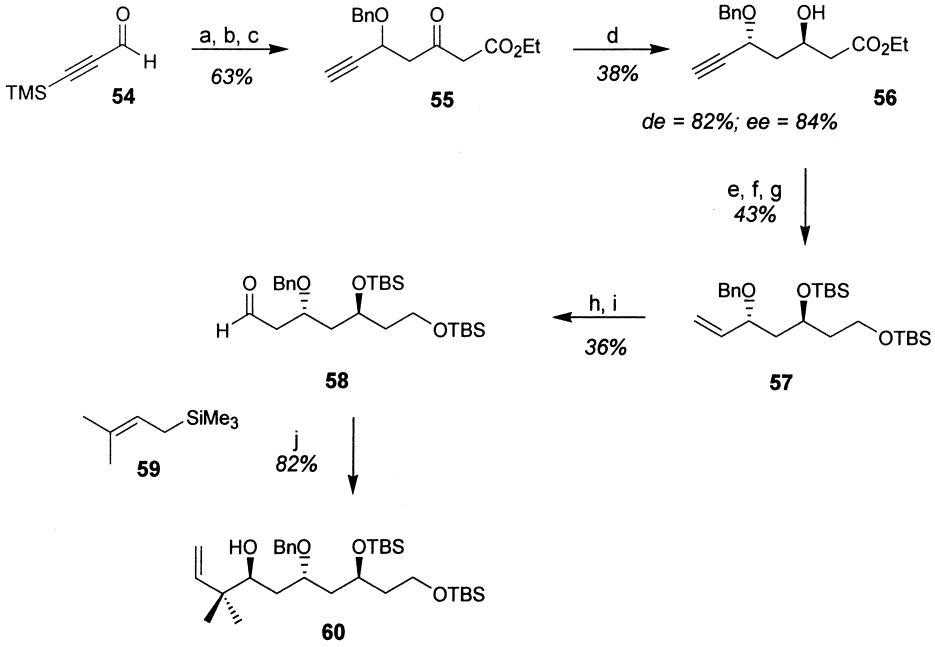
Partial Synthesis

Kalesse's C_1-C_9 segment synthesis[94] (Scheme 7). Addition of ethyl acetoacetate to propargylic aldehyde (**54**) gave racemic hydroxy ketone, which was benzylated and desilylated to β-keto ester (**55**). Bakers' yeast kinetic resolution led to β-hydroxy ester (**56**) in a de of 82% and an ee of 84%. Better de and ee's were obtained with different protecting groups, but the benzyl-protecting group was chosen because of its stability under the Sakurai conditions. Hydrogenation of the acetylene moiety followed by reduction and silylation led to the protected triol (**57**). Hydroboration and successive Dess–Martin oxidation gave aldehyde (**58**). The Sakurai reaction gave the partially protected tetraol (**60**), the final C_1–C_9 segment, as a 6:1 mixture of two diastereomers with the desired *anti* product as major isomer.

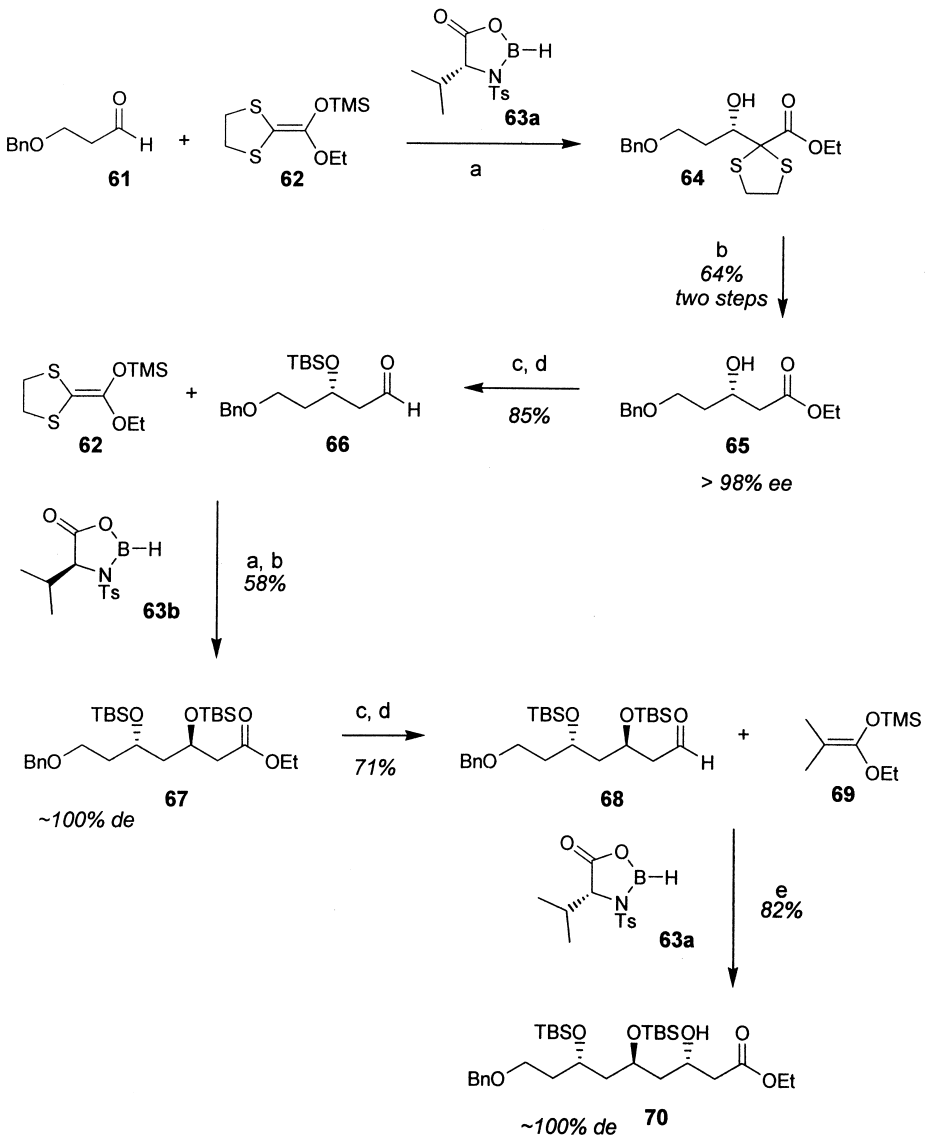
Kiyooka's C_1–C_9 segment synthesis[95] (Scheme 8). The oxazaborolidinone (**63a**) mediated aldol reaction of aldehyde (**61**) with thiosilylenolate (**62**) yielded thiohydroxy ester (**64**) with an ee of 98%. Desulfurisation led to hydroxy ester (**65**), which was protected and reduced to aldehyde (**66**). A second aldol reaction with thiosilylenolate (**62**) mediated through oxazaborolidinone (**63b**) and subsequent desulfurization gave *anti* diol (**67**) as one single diastereoisomer. Silyl protection and reduction yielded aldehyde (**68**). A third aldol reaction with silylenolate (**69**) mediated with oxazaborolidinone (**63a**) gave *anti, anti* triol (**70**), the final C_1–C_9 segment, as one single diastereoisomer.

Vandewalle's C_{27}–C_{34} segment synthesis[96] (Scheme 9). Vandewalle and co-workers published the synthesis of C_1–C_9, C_{11}–C_{16} and C_{17}–C_{27} segments previously and this work is included in the review of Norcross and Paterson.[89] In their more recent publication they describe an improved C_{27}–C_{34} segment synthesis, an intermediate in their own approach as well as in the work of Masamune, Roy, Evans and Hale.

Epoxidation of carvone (**71**) led to epoxide (**72**). Reductive epoxide opening gave alcohol (**73**) as a 4:1 mixture of diastereoisomers. Silyl protection yielded ether (**74**), which was oxidized to ketone (**75**). Through a double Baeyer–Villiger oxidation the lactone (**76**) was then obtained. Enzyme catalysed hydrolysis afforded alcohol



Scheme 7. Kalesse's C_1–C_9 synthesis. (a) Ethyl acetonate, NaH, 0 °C, LDA, −78 °C, THF, then **54**; (b) $C_6H_5CH_2OC(=NH)CCl_3$, CH_2Cl_2; (c) KF, 18-crown-6, DMF, H_2O; (d) baker's yeast, H_2O, sucrose, 3d; (e) Lindlar catalyst, H_2, EtOH; (f) $LiAlH_4$, Et_2O; (g) TBDMSTf, THF; (h) BH_3.THF; (i) Dess–Martin periodinane; (j) $TiCl_4$, CH_2Cl_2, **59**.



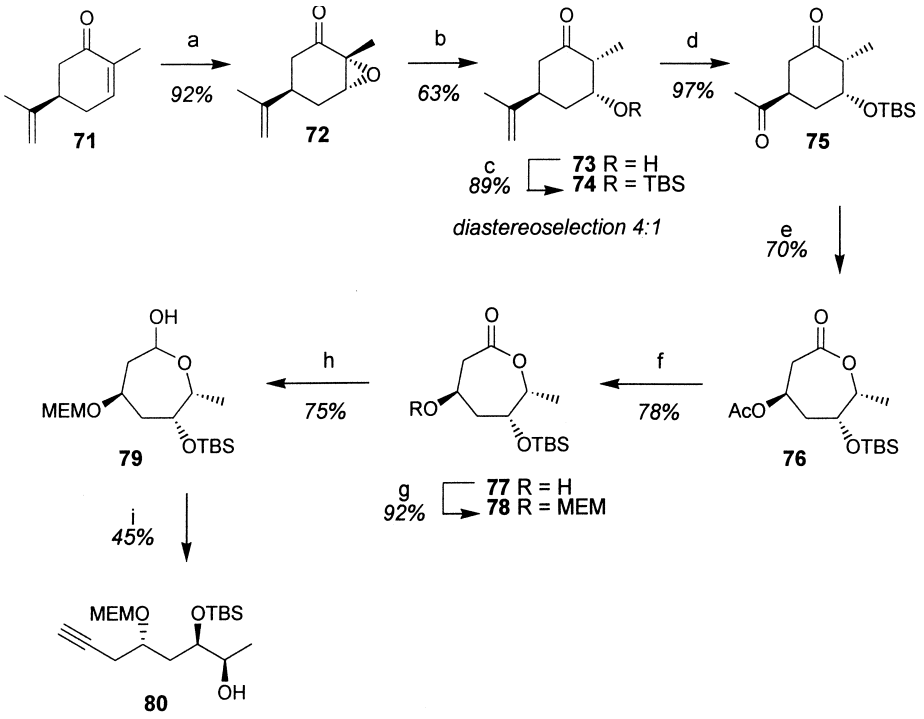
Scheme 8. Kiyooka's C_1–C_9 synthesis. (a) 1 equiv of **63**, CH_2Cl_2, −78 °C, 8 h; (b) Ni_2B-H_2, EtOH, rt, 16 h; (c) TBSCl, imidazole, DMF, rt, 16 h; (d) DIBAL-H, CH_2Cl_2, −78 °C, 2 h; (e) 1 equiv of **63**, CH_2Cl_2, −78 °C, 3 h.

(**77**), which was MEM-protected (**78**) and reduced to lactol (**79**). Treatment with dimethyl(diazomethyl)-phosphonate yielded alkyne (**80**), the final C_{27}–C_{34} segment.

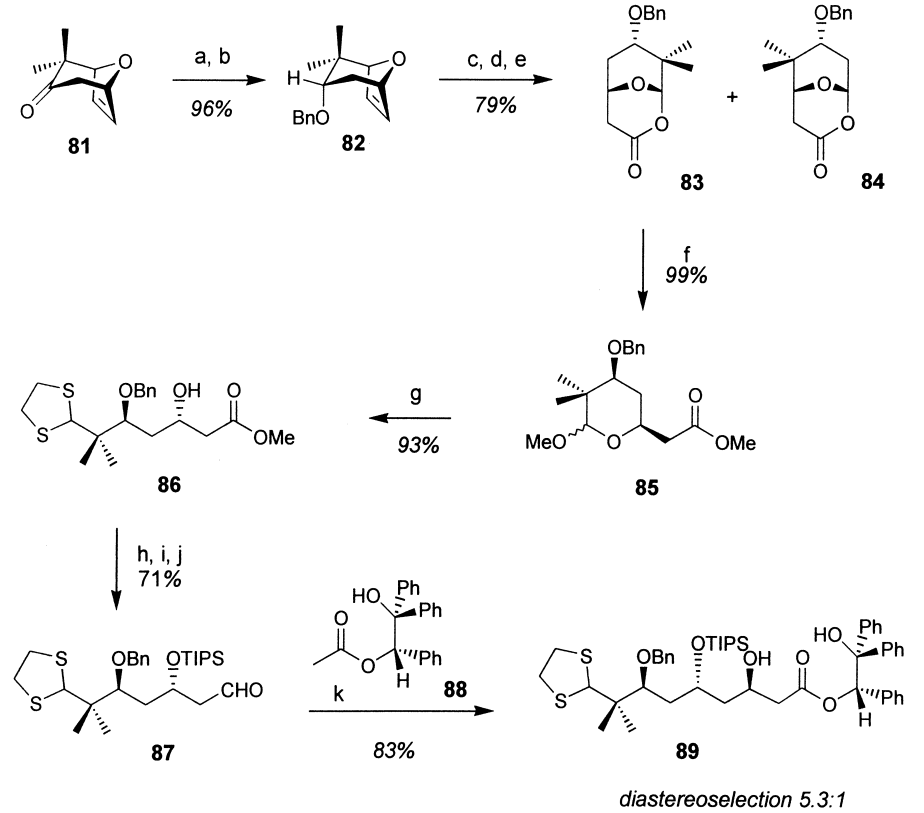
Hoffmann's C_1–C_9 segment synthesis[97] (Scheme 10). Racemic bicyclo ketone (**81**) was reduced and the benzyl ether (**82**) was obtained after protection. An asymmetric hydroboration, followed by PCC and Baeyer–Villiger oxidation led to the two enantiomers of lactones (**83**+**84**) as a 1:1 mixture with ee's of >98% for (**83**) and 96% for (**84**). The products could be easily separated by standard flash chromatography. The desired enantiomer (**83**) was hydrolysed to yield acetal (**85**). Transformation to the thioacetal (**86**) led to the open chain form. Silyl protection followed by reduction and reoxidation gave aldehyde (**87**). The last chiral centre was introduced with a aldol reaction following the procedure of Braun–Devant, yielding alcohol (**89**), the final C_1–C_9 segment, as 5.3:1 mixture of two diastereoisomers with the desired *anti* product in excess.

Yamamura's C_{17}–C_{27} segment synthesis of bryostatin 3[98] (Scheme 11). Yamamura and Nishiyama already published a C_1–C_{16} segment synthesis. This work is included in Norcross and Paterson's review.[91] In this later publication the C_{17}–C_{27} segment synthesis of bryostatin 3 is described.

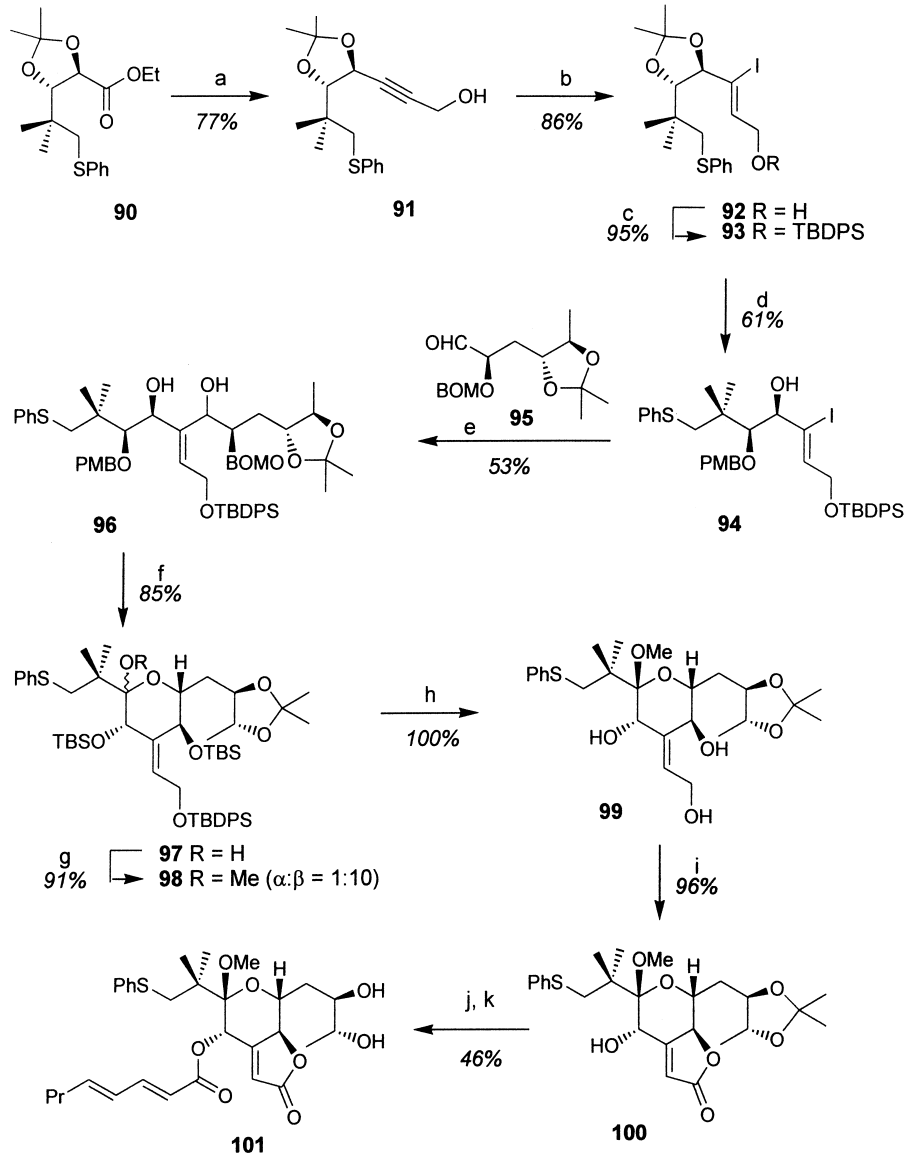
Known ester (**90**)[99] was reduced and a triple bond introduced to give propargyl alcohol (**91**). Hydroalumination and iodination led to vinyl iodide (**92**), which was transformed into silyl ether (**93**). A number of deprotection/protection manipulations yielded vinyl iodide (**94**). A halogen-metal exchange procedure followed by coupling to known aldehyde (**95**)[100] gave diol (**96**) as a 3:1 mixture of the *syn*:*anti* diastereoisomers. The *syn* isomer was converted into lactol (**97**), which on acetalization



Scheme 9. Vanderwalle's C_{27}–C_{34} synthesis. (a) H_2O_2, NaOH, MeOH, −10 °C, 3 h; (b) Ph_2Se_2, $NaBH_4$, EtOH, AcOH, 0 °C, 15 min; (c) TBSOTf, CH_2Cl_2, 2,6-lutidine, 0 °C, 20 min; (d) KIO_4, OsO_4, 1:1 THF/H_2O, 12 h; (e) 20 equiv of *m*-CPBA, CH_2Cl_2, rt, 6d, then Me_2S; (f) PLE (EC 3.1.1.1), phosphate buffer pH 7, Me_2CO, 35 °C; (g) MEMCl, DIPEA, CH_2Cl_2, rt, 16 h; (h) DIBAL-H, CH_2Cl_2, −78 °C, 1 h; (i) $(MeO)_2P(O)CHN_2$, *t*-BuOK, THF, −78 to −30 °C, 12 h.



Scheme 10. Hoffmann's C_1–C_9 synthesis. (a) i. L-selectride, −78 °C; ii. NaOH, H_2O_2; (b) BnBr, NaH; (c) i. (–)-Ipc_2BH, −15 °C, 3d; ii. NaOH, H_2O; (d) PCC; (e) *m*-CPBA; (f) MeOH, H^+ (cat), $MgSO_4$, reflux; (g) 1,2-ethanedithiol, $BF_3.Et_2O$; (h) TIPSOTf; (i) DIBAL-H; (j) PCC; (k) **88**, LDA, −110 to −78 °C.



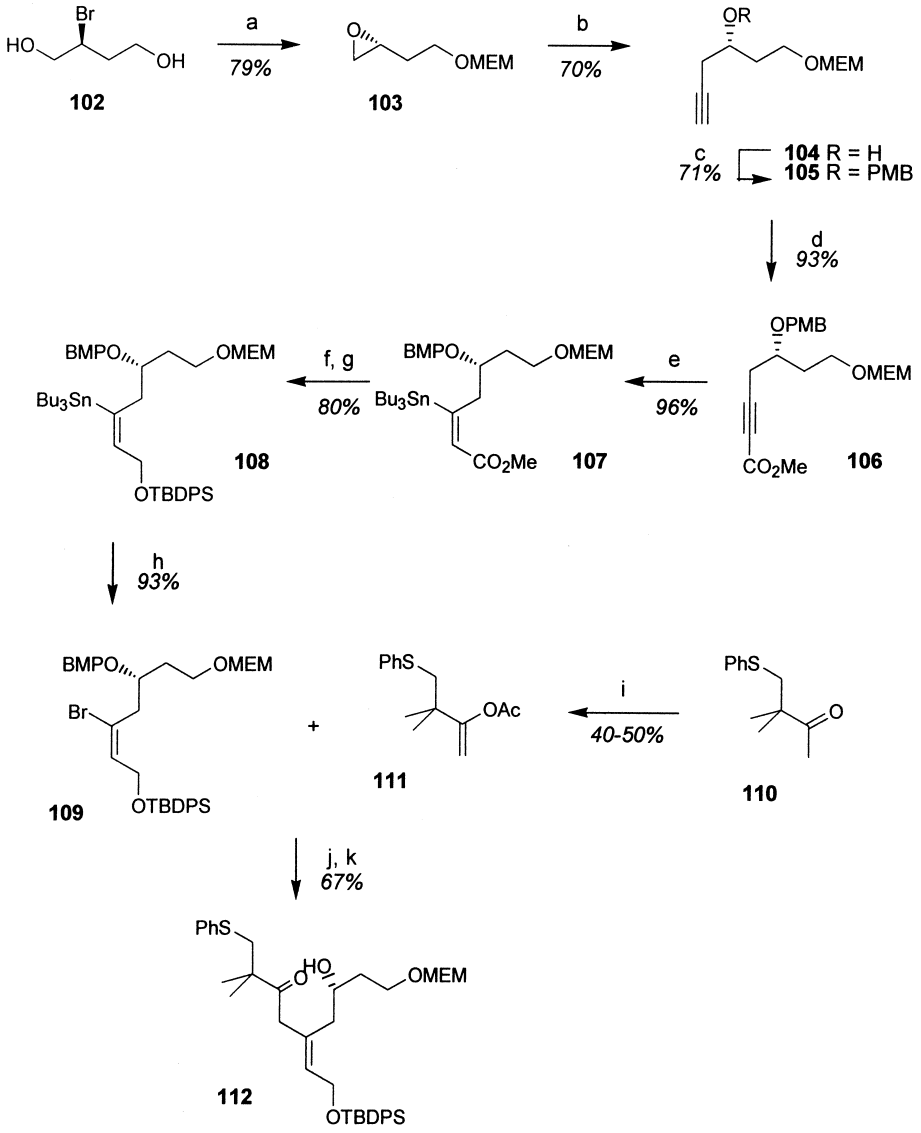
Scheme 11. Yamamura's C_{17}–C_{27} synthesis. (a) i. DIBAL-H, PhMe, −78 °C; ii. Ph_3P-CBr_4, CH_2Cl_2, 0 °C; iii. n-BuLi, THF, then $(CH_2O)_n$; (b) Red-Al, THF, then I_2; (c) TBDPSCl, imidazole; (d) i. TFA-H_2O, THF; ii. TBDPSCl, imidazole; iii. $MeOC_6H_4CH(OMe)_2$, PPTS, CH_2Cl_2; iv. DIBAL-H, PhMe, 0 °C; (e) MeLi, Et_2O, −30 °C, *t*-BuLi, −90 °C, then **95**; (f) i. TBSOTf, 2,6-lutidine; ii. *m*-CPBA, Na_2HPO_4; iii. DDQ, 10% aq CH_2Cl_2; iv. Dess–Martin oxidation; v. H_2, $Pd(OH)_2$-C, EtOH, then $Me_2C(OMe)_2$, PPTS, acetone; (g) TBSOTf, TMSOMe, $Me_2C(OMe)_2$, MS4A, CH_2Cl_2; (h) TBAF, THF; (i) TPAP, NMO, MS4A, CH_2Cl_2; (j) octa-2,4-dienoic acid, EDCI, DMAP, CH_2Cl_2; (k) CSA, MeOH.

afforded (**98**) as a 10:1 mixture of *cis*:*trans* cycles. Desilylation led to triol (**99**) and selective oxidation yielded in γ-lactone (**100**). Esterification with (2*E*,4*E*)-octa-2,4-dienoic acid followed by deprotection gave (**101**), the final C_{17}–C_{27} segment.

Thomas' C_{17}–C_{25} segment synthesis of bryostatin 11[101] (Scheme 12). Diol (**102**) was converted into protected epoxide (**103**) in one step. Epoxide opening with lithium acetylide gave alcohol (**104**), which was PMB-protected and C-acylated to yield ester (**106**). Addition of tributyltin following a procedure by Piers gave vinyl stannane (**107**) in a stereoselective manner. Reduction of the ester and silyl protection led to ether (**108**). Subsequent tin-bromine exchange yielded vinyl bromide (**109**). This was reacted with enol acetate (**111**), which was available from ketone (**110**), through a palladium-mediated coupling. Deprotection of the coupling product gave hydroxy-ketone (**112**), the final C_{17}–C_{25} segment.

Studies Towards Analogues

Bryostatins have a limited availability, the extraction from the natural sources is complex and there is no commercially viable total synthesis of any of the molecules. Therefore, methods to make simplified analogues are an attractive way to solve the supply problem. Knowing the general pharmacophore model of the PKC enzyme and the groups in bryostatin, to which it binds, Wender and co-workers started to study the matter closely. Recently, he reviewed his work,[102] and therefore we present only a brief summary in this report.



Scheme 12. Thomas' C_{17}-C_{27} synthesis. (a) NaH, MEMCl, THF; (b) LiCCH, ethylendiamine, DMSO, THF; (c) NaH, PMBCl, NBu_4I, DMF, THF, 20 °C, 16 h; (d) n-BuLi, MeOCOCl, 1 h, −75 °C; (e) $Bu_3SnLi.CuBr$, Me_2S, THF, −78 °C, 3 h, then MeOH; (f) DIBAL-H, hexane; (g) TBDPSCl, imidazole, DMF; (h) *N*-bromosuccinimide, CH_2Cl_2, 20 °C; (i) isopropenyl acetate, PTSA (cat.), reflux, 48 h; (j) $PdCl_2[P(o\text{-}tolyl)_3]_2$, Bu_3SnOMe, PhMe, 100 °C; (k) DDQ, CH_2Cl_2, H_2O.

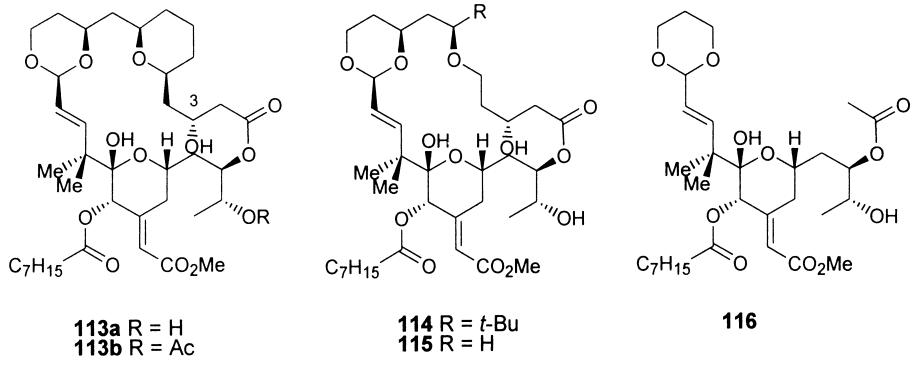
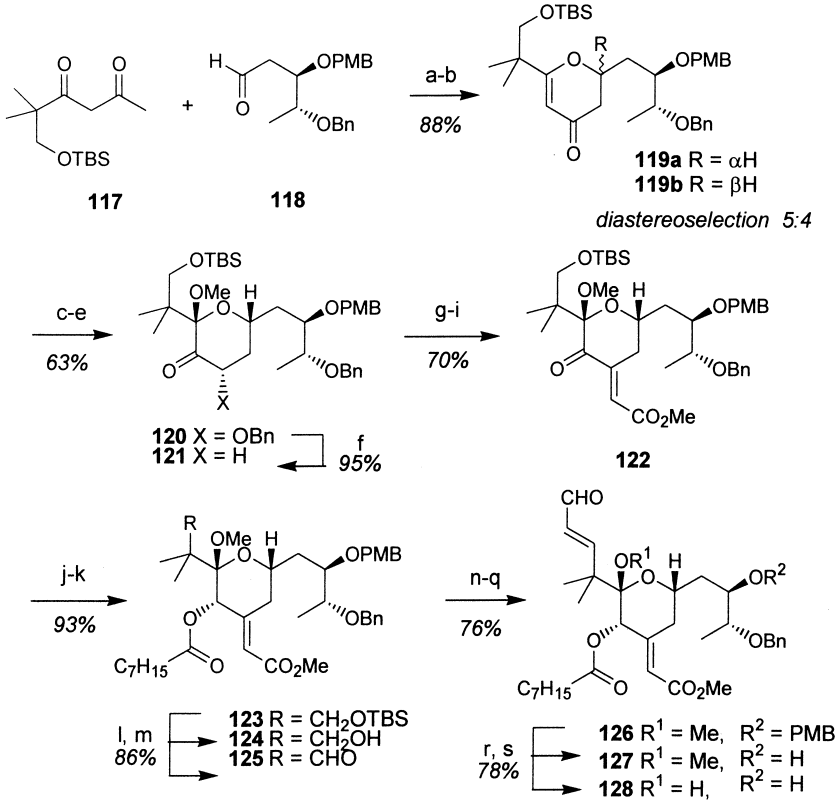


Figure 3.

The PKC binding oxygen atoms in the bryostatins are all allocated in the bottom part of the molecule (C_{15}–C_{27}). Therefore, this area was not changed in the first analogues that were designed. The top half of bryostatin seemed to be important to hold the bottom part in place but was not directly involved in the binding interactions. It was therefore simplified and molecular modelling of **113a** (Fig. 3) gave a good fit when compared to the

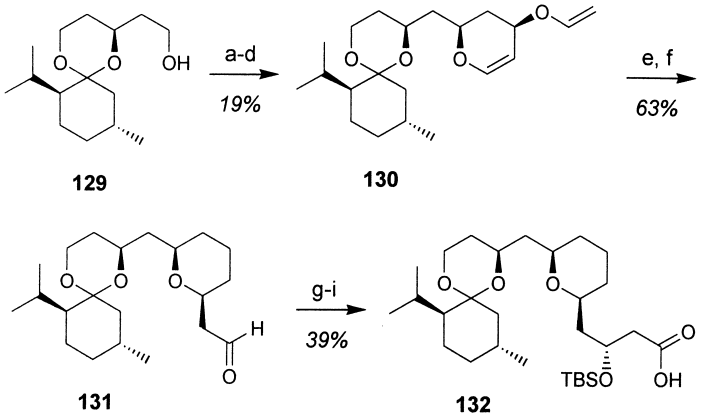


Scheme 13. Wenders' C_{17}–C_{27} synthesis. (a) **117**, 2 equiv LDA, THF, −78 °C, 30 min; (b) cat *p*TsOH, PhMe, rt; (c) **119b**, $NaBH_4$, $CeCl_3.7H_2O$, MeOH, −20 °C; (d) *m*-CPBA, $NaHCO_3$, 2:1 CH_2Cl_2/MeOH; (e) i. PhCOCl, DMAP, CH_2Cl_2, −10 °C; ii. Dess–Martin periodinane, rt; (f) SmI_2, THF, MeOH, −78 °C; (g) LDA, $OHCCO_2Me$, THF, −78 °C; (h) $ClSO_2Me$, Et_3N, CH_2Cl_2, −10 °C; (i) DBU, THF, rt; (j) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, −20 °C; (k) $C_7H_{15}CO_2H$, 2,4,6-trichlorobenzoylchloride, Et_3N, PhMe, rt; (l) HF.pyr, THF, rt; (m) Dess–Martin periodinane, CH_2Cl_2, rt; (n) allylBEt_2, Et_2O, −10 °C; (o) Ac_2O, DMAP, CH_2Cl_2; (p) cat OsO_4, NMO, THF/H_2O; (q) $Pb(OAc)_4$, Et_3N, PhH, DBU, rt; (r) DDQ, CH_2Cl_2, H_2O; (s) HF, CH_3CN, H_2O, rt.

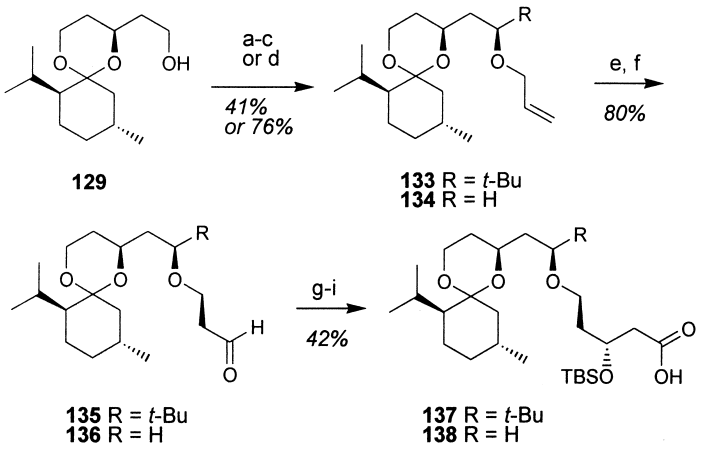
Table 3. PKC (isoform mix) binding affinities

Analogue	K_i ($\times 10^{-9}$ M)
113a	3.4
113b	> 10000
114	8.3
115	47
116	> 10000
epi-C_3 **113a**	285
Desoxy-C_3 **113a**	297

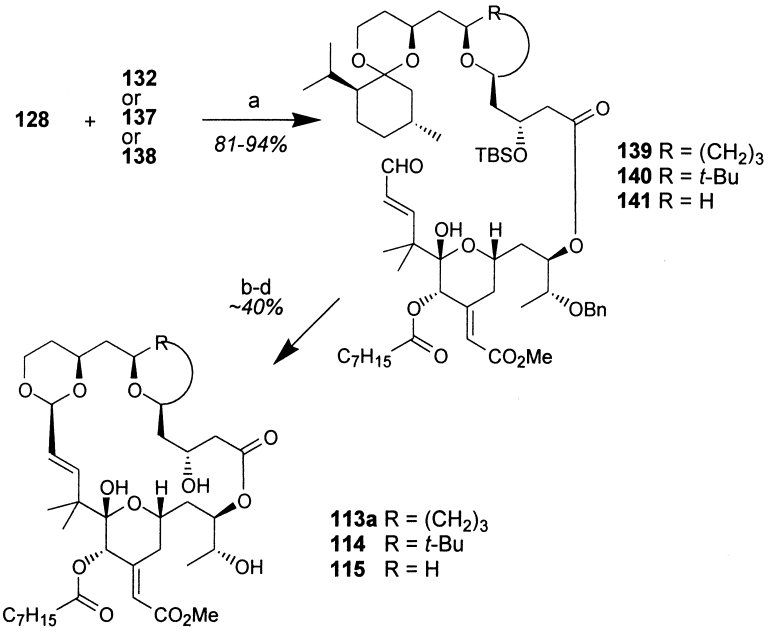
bryostatins. The binding affinity of analogue **113a** was indeed comparable to those of bryostatins 1–10 (Table 3). Acetylation of the C_{26} alcohol gave **113b**, and as with the bryostatins the binding affinity dropped drastically. Further reducing the top half of the molecule led to analogues **114** and **115**, which both bound strongly to PKC, although to a lesser extent than **113a**. Cutting out the whole top half of the molecule led to compound **116**, which lost the ability to bind PKCs.



Scheme 14. Wenders' C_1–C_{16} synthesis. (a) Dess–Martin periodinane, CH_2Cl_2, rt; (b) Danishefsky's diene, (+)-Eu(hfc)$_3$, CH_2Cl_2, rt, then TFA; (c) $NaBH_4$, $CeCl_3.7H_2O$, −40 °C; (d) 10 mol% $Hg(TFA)_2$, ethylvinylether, −5 °C; (e) n-nonane, 150 °C; (f) H_2, Pd/C, EtOAc, 1 atm; (g) (−)-$(Ipc)_2$BOMe, allylmagnesium bromide, CH_2Cl_2, −78 °C to rt; (h) TBSCl, imidazole, THF, rt; (i) cat $KMnO_4$, $NaIO_4$, rt.



Scheme 15. Wenders' C_1–C_{16} synthesis. (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (b) *t*-BuLi, Et_2O, −78 °C, (1:1 mixture of diasteromers which can be recycled: Dess–Martin periodinane, CH_2Cl_2, rt then $NaBH_4$, $CeCl_3.7H_2O$, −40 °C); (c) *t*-BuOK, allylbromide, THF, rt; (d) NaH, allylbromide, THF, rt; (e) 9-BBN, THF, 65 °C, then NaOH, H_2O_2; (f) Dess–Martin periodinane, CH_2Cl_2, rt (g) (−)-$(Ipc)_2$BOMe, allylmagnesium bromide, CH_2Cl_2, −78 °C to rt; (h) TBSCl, imidazole, THF, rt; (i) cat $KMnO_4$, $NaIO_4$, rt.



Scheme 16. Wenders' assembly of analogues. (a) **132** or **137** or **138**, 2,4,6-trichlorobenzoylchloride, Et_3N, DMAP, then **128**, CH_2Cl_2, rt; (b) HF.pyr, CH_3CN, rt; (c) Amberlist-15 resin, CH_2Cl_2, rt; (d) $Pd(OH)_2$, H_2, EtOAc, 1 atm.

The role of the alcohol group on C_3 in analogue **113a** was further investigated. Epi-C_3 and desoxy-C_3 analogues of **113a** showed less binding activity. Whether this is due to direct interaction with the protein or because of intermolecular hydrogen bonding to the oxygen in the B-ring of the molecule has not yet been established, although NMR and molecular modelling studies suggest the latter.

Analogues **113a** and **114** were also tested against several human cancer cell lines. Both showed remarkable abilities to inhibit growth and in some cases they were even superior to bryostatin 1.

Synthesis of Analogues

C_{17}–C_{27} segment synthesis (Scheme 13). Aldol condensation of the dieneolate of (**117**) and aldehyde (**118**) followed by acid-catalysed cyclodehydration gave a 1:1 mixture of dihydropyranones (**119a,b**). The β-isomer was reduced using Luche conditions, the resulting glycal was deoxidised and the ring opened followed by selective benzoylation and oxidation to afford ketone (**120**). Deoxygenation gave ketone (**121**), which was transformed to E-enone (**122**) via aldol addition, mesylation and elimination. Selective reduction using Luche condition and esterification of the labile alcohol under Yamaguchi's

condition led to bisester (**123**). This ester was deprotected to alcohol (**124**) and oxidized to aldehyde (**125**). Allylation of the hindered aldehyde followed by the acylation of the formed hydroxy function and oxidation of the installed double bond followed by the elimination of the acetate yielded enal (**126**). Deprotection with DDQ gave alcohol (**127**) and aqueous HF afforded the hemiketal (**128**), the final C_{17}–C_{27} segment.

C_1–C_{16} segment synthesis (Scheme 14). The menthone derived partly protected triol (**129**) was oxidised, followed by a $Eu(hfc)_2$ promoted heteroatom Diels–Alder with Danishefsky's diene to afford the desired dihydropyranone in high yield but low diastereoselectivity. Reduction under Luche condition followed by vinylation gave vinyl ether (**130**). Claisen rearrangement followed by reduction of the double bond led to aldehyde (**131**) which was subsequently allylated using Brown's condition, silylated and oxidised to the acid (**132**), the final C_1–C_{16} segment.

C_1–C_{16} segment synthesis (Scheme 15). The menthone derived partly protected triol (**129**) was allylated to give ether (**134**) or was oxidised, followed by treatment with *t*-butyllithium to yield a 1:1 mixtures of diastereoisomers. The undesired separated isomers was recycled and the desired allylated to give ether (**133**). Hydroboration followed by oxidation led to aldehydes (**135**, **136**). These were allylated using Brown's conditions, silylated and oxidised to the acids (**137**, **138**), the final C_1–C_{16} segments.

Assembly of analogue (Scheme 16). The spacer subunits (**132**, **137** and **138**) were esterified with acid (**128**) using Yamaguchi conditions yielding esters (**139**, **140** and **141**). These were desilylated and underwent macrocyclisation in dilute solution of Amberlist-15 and molecular sieves. Deprotection then led to the final analogues (**113a**, **114** and **115**).

Acknowledgements

We would like to thank Sandra Van Meurs, Professor Timothy D. H. Bugg and Dr. Peter Critchley for their help and assistance.

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Biographies

Martin Wills was born in Swansea in 1964, and grew up in Swansea, Cardiff and Reading. He completed a BSc at Imperial College, London in 1982 and a DPhil at Oxford University under the supervision of S. G. Davies in 1985. Following a year of postdoctoral research with the late W. Oppolzer at Geneva he was appointed to a lectureship in organic chemistry at Bath University. In 1995 he moved to a readership at Warwick University, where he is currently working on the development of novel asymmetric catalysts and synthetic methodology. Dr. Wills was the winner of the 1993 Meldola Medal and Prize of the Royal Society of Chemistry and the 1999 GlaxoWellcome Prize of innovative synthesis. He is honoured to have been associated with so many talented current and former postdoctoral reseachers and postgraduate students with whom a vigorous programme of research into asymmetric catalysts and total synthesis is flourishing at Warwick.

Roger Mutter originates from Niederwald (Switzerland) and was born in 1971 in Sion. After completing an apprenticeship as laboratory assistant at Lonza Ltd in Visp he moved on to the Institute of Technology in Sion, where he obtained a BSc in chemical engineering. He then rejoined Lonza Ltd on a temporary basis as assistant to the team leader, validating an in-house built LIMS-program. In 1997 he joined the research group of Martin Wills as a self-funded student. He was working on the synthesis of bryostatin analogues and obtained an ORS Award for his second and third year, During his study he was given the opportunity to enjoy an industrial placement at Norvartis Ltd Basel, where he was working under supervision of Dr. Karl-Heinz Altmann on the synthesis of epothilone analogues. A second placement at GlaxoWellcome Ltd in Stevenage under supervision of Dr. Andy Merritt led him into the field of parallel synthesis. He will finish his PhD in summer 2000. His main research interests are medicinal chemistry.