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Total synthesis of (+)-A83586C, (+)-kettapeptin and (+)-azinothricin: powerful new inhibitors of β-catenin/TCF4- and E2F-mediated gene transcription

Karl J. Hale,*[a] Soraya Manaviazar[a] and Jonathan George[b]

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Herein we describe our asymmetric total syntheses of (+)-A83586C, (+)-kettapeptin and (+)-azinothricin. We also demonstrate that molecules of this class powerfully inhibit β-catenin/TCF4- and E2F-mediated gene transcription within malignant human colon cancer cells at low drug concentrations.

Introduction

The discovery of (+)-azinothricin in the culture filtrates of *Streptomyces* sp. X-14950 by Hubert Maehr and co-workers[1] at F. Hoffmann La Roche in 1986 heralded the community's first encounter with this new structural type of pyranylated cyclodepsipeptide (Fig. 1). Ever since that time, various other family members have periodically been found, the majority of which have been shown to have powerful antitumour effects *in vitro* and *in vivo*. Although (+)-azinothricin was itself never tested as an antitumour drug, it was documented as being one of the most potent Gram-positive antibiotics ever discovered, its MIC values ranging from 0.001–0.016 μg mL^{-1} against 51 different bacterial strains. Even so, because of a fairly poor toxicological profile in mice (LD_{50} = 10 mg kg^{-1} [intravenous] and 3.2 mg kg^{-1} [intraperitoneally]), (+)-azinothricin was never taken forward as a new antibiotic drug and, as a result, it initially looked set to rapidly fall from scientific view.

However, two years later in 1988, the closely-related natural product, (+)-A83586C, was identified in fermentation broths of the Guam soil microorganism, *Streptomyces karnatakensis* by Tim Smitka and his colleagues at Eli Lilly.[2] Like azinothricin, (+)-A83586C had its molecular structure determined by combined single-crystal X-ray analysis and chemical degradation. Together these techniques revealed significant differences in the *N*-hydroxamic acid components of both cyclodepsipeptides. In A83586C, an *N*-hydroxy-L-alanine unit resides within the peptidal array, whilst in (+)-azinothricin, an *N*-hydroxy-L-methoxyserine occupies this position. There is also significant dichotomy in the C(37) substituents (A83586C-numbering) that are present, with a methyl group being located at this site in (+)-A83586C, and an ethyl substituent in (+)-azinothricin.

Not unexpectedly, (+)-A83586C and (+)-azinothricin had quite similar antibiotic profiles, with (+)-A83586C also

[a] *The School of Chemistry and Chemical Engineering, and the Centre for Cancer Research and Cell Biology (CCRCB), Queen 's University Belfast, Stranmillis Road, Belfast, Northern Ireland, UK BT9 5AG. E-mail: k.j.hale@qub.ac.uk, s.manaviazar@qub.ac.uk; Fax: +44-(0)289097-4579; Tel: +44-(0)289097-5525*
[b] *Department of Chemistry, University of Oxford, Chemistry Research Laboratory, 12 Mansfield Road, Oxford, UK OX1 3TA*

Karl J. Hale

Karl J. Hale is currently Professor of Organic and Medicinal Chemistry and Chemical Biology at QUB. He came to Queen's in 2007 from UCL, where previously he served as Professor of Chemistry. His group's work has been recognised with a number of prestigious research prizes that have included the 1997 Zeneca Research Award in Organic Chemistry, the 1998 Pfizer Academic Award for Chemistry, and most recently, the 2007 Liebig Lectureship of the German Chemical Society. His laboratory has completed the total synthesis of many complex anti-tumour agents that include (−)-agelastatin A, bryostatin 7, (+)-eremantholide A and (+)-kettapeptin to name a few.

Soraya Manaviazar

Soraya Manaviazar obtained her PhD in organic chemistry from UCL in 1994 under the guidance of Professor Karl J. Hale. She then went on to work in the Medicinal Chemistry Department of Oxford Glyco-sciences before returning to UCL in 1998 as Principal Research Fellow. In August 2007, she moved to Queen's University Belfast where she became Senior Research Fellow in Organic and Bio-Medicinal Chemistry. Her research interests are in natural product total synthesis, medicinal chemistry, and chemical biology, and she has completed the synthesis of several complex natural products that include (+)-A83586C, (+)-azinothricin and bryostatin 7.

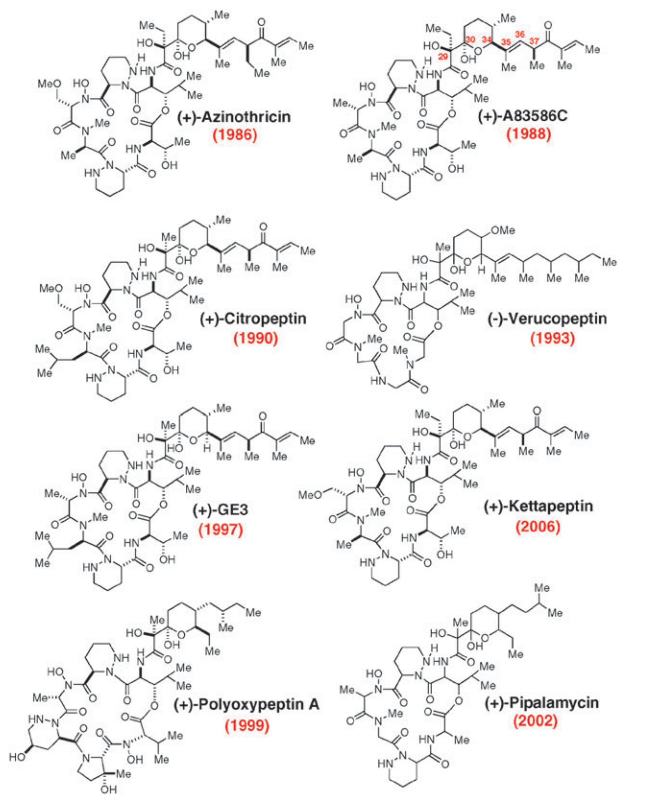
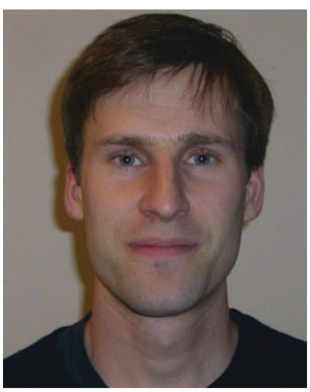


Fig. 1 The azinothricin/A83586C family of antitumour antibiotics.

potently inhibiting the growth of Gram-positive bacterial strains with MICs of 0.008–0.06 μg mL^{-1}. (+)-A83586C likewise induced toxic death in mice when given intravenously at 9.3 mg kg^{-1}.

Given these toxic liabilities, the Lilly team took the immediate decision not to develop A83586C clinically as a new antibacterial drug and, without any further delay, they simply published their findings in *J. Antibiotics*.[2] One of the more interesting observations that was made by this group regarded the very pronounced antitumour effects of (+)-A83586C against a CCRF-CEM human T-cell leukaemia cell line; its IC_{50} being 13.5 nM. This excellent antitumour potency notwithstanding, no further anticancer testing was done on (+)-A83586C because of its perceived toxicity *in vivo*. It thus appeared, at this point in time, as if molecules of the A83586C/azinothricin class would surely drift into the sea of chemical obscurity over the coming years.



Jonathan George

Jonathan George studied Chemistry at Oxford University (Exeter College), graduating with a First Class MChem degree in 2001. He then went on to study for a PhD in Synthetic Organic Chemistry at UCL under the supervision of Professor Karl J. Hale. In 2006, he returned to Oxford University to work as a post-doctoral fellow in the group of Professor Sir Jack Baldwin FRS and Dr Rob Adlington, on the biomimetic synthesis of natural products. He will shortly be moving to Australia to take up a Lectureship in Organic Chemistry at the University of Adelaide.

However, pharmacological interest was soon rekindled in 1990 when Nakagawa and co-workers,[3] at the Kirin Brewery in Japan, reported the results of their independent *in vivo* antitumour testing of (+)-citropeptin in mice, at doses well below the 4 mg kg^{-1} day^{-1} threshold needed to cause toxic death. Specifically, they observed that citropeptin could confer a 123% life-extension on mice with P388 lymphocytic leukaemia when administered at the low dosage of 2 mg kg^{-1} day^{-1}. This was a most significant result for it showed, for the first time ever, that molecules of the azinothricin-A83586C class could elicit a therapeutically beneficial anticancer effect in an established animal tumour model without serious toxic side-effects.

Although not outstanding in terms of antitumour potency, the data on (+)-citropeptin did nevertheless serve to spur many groups who were actively considering synthesising these molecules, for it signalled that natural products of this class might potentially be tractable as drug design leads, and that they might ultimately be capable of being further improved and exploited. Indeed, this was a view that only got further reinforced as later biological test data emerged on the sister molecules (−)-verucopeptin[4] and (+)-GE3.[5]

With regards to (−)-verucopeptin,[4] it showed pronounced broad-spectrum antitumour effects *in vitro* (IC_{50} = 0.004 μg mL^{-1} *vs.* B16 melanoma; IC_{50} = 0.08 μg mL^{-1} *vs.* P388 lymphocytic leukaemia), including against a HCT-116 human colon carcinoma cell line (IC_{50} = 0.04 μg mL^{-1}). However, subsequent *in vivo* assaying did later reveal that it was a quite specific and selective agent in its therapeutic window. For example, it did not increase the life-expectancy of mice xenografted with P388 lymphocytic leukaemia, although it did significantly extend the lives of mice with B16 melanoma, it conferring life-extensions of 146–162% in some instances; activity that was actually superior to mitomycin C at several of the dosages examined.

Of much greater significance, however, was Sakai's 1997 report[5] that the structurally more elaborate congener, (+)-GE3, could exert substantial antitumour effects against BALB/c-nu/nu nude mice xenografted with the currently incurable PSN1-human pancreatic carcinoma. Specifically, a *single* 2 mg kg^{-1} dosage of (+)-GE3 was found to produce a 47% reduction in tumour volume (11 days post-treatment) and, although this very substantial antitumour effect was associated with an 18.2% reduction in body weight, none of the treated animals died as a result of receiving the drug. To us, this substantial shrinkage of an incurable human tumour highlighted the great potential of these molecules as drug design leads. It also suggested that they might be useful new tools for deconvolutional biology and for new oncological target identification.

With regard to the mechanism of GE3 antitumour action, the Kyowa Hakko Kogyo group[5] suggested that it was functioning by preventing active E2F transcription factor complexes from binding to the promoter regions of target genes critically involved in cancer cell growth and proliferation. However, no firm details were ever provided of the precise experiments that were used to support these mechanistic assertions.

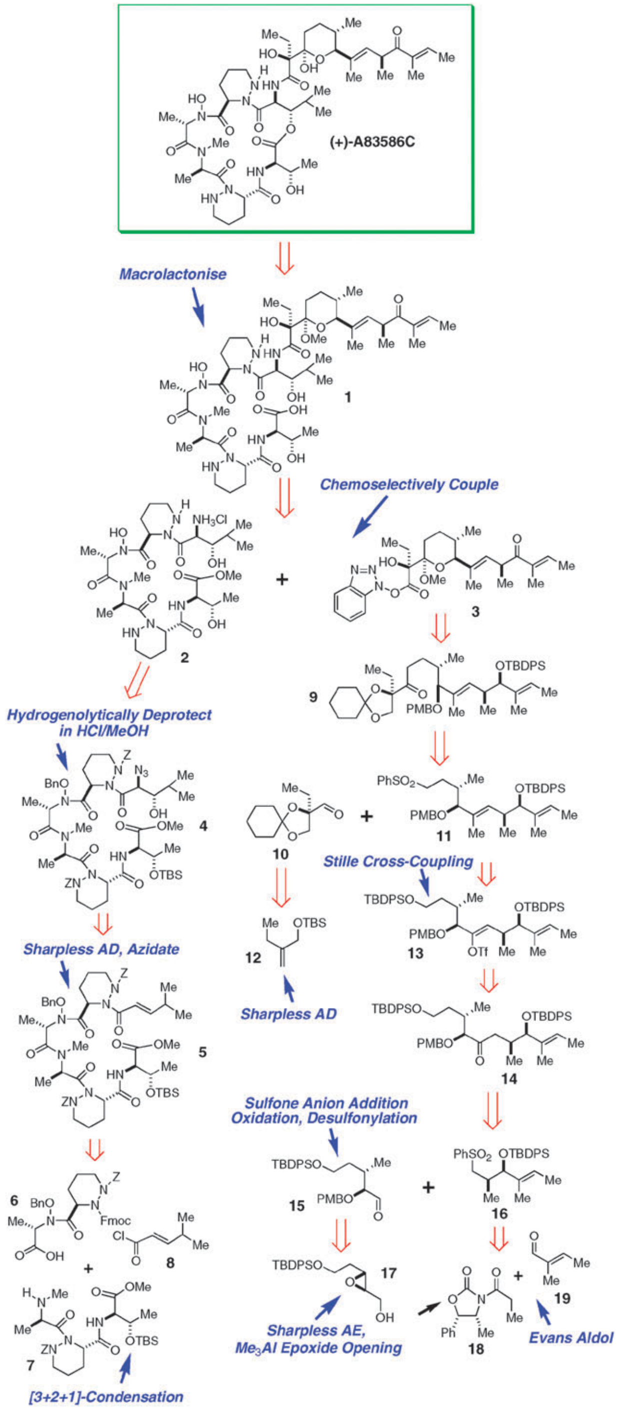
Since this 1997 report on (+)-GE3 by Sakai,[5] several closely related natural products have been isolated from various *Streptomyces* strains including (+)-polyoxypeptin A,[6] (+)-pipalamycin[7] and (+)-kettapeptin,[8] all of which have been found to have pronounced broad-spectrum *in vitro* antitumour effects. However, no additional communiques have appeared on the mechanism of antitumour action of this class.

In order to provide fresh insights into how these natural products are functioning as antitumour agents, we commenced asymmetric total syntheses of (+)-A83586C and (+)-azinothricin back in late 1991. We were particularly keen to devise syntheses that would be capable of providing both molecules in meaningful quantities for future chemical biology experiments and for more detailed *in vivo* antitumour screening. Herein, we now discuss our cumulative progress.

Our first-generation asymmetric total synthesis of antitumour antibiotic (+)-A83586C

Our original disconnective bond analysis of (+)-A83586C is shown in Scheme 1. It proposed acquisition of the natural product through a chemoselective macrolactonisation of the *seco*-acid **1** which itself would be obtained from the regioselective coupling of amine hydrochloride **2** with hydroxybenzotriazole activated ester **3**. Of special note in our planning was the fact that we would completely dispense with protecting groups at the final stages of our synthesis, to specifically avoid the many potential snares and pitfalls that could potentially arise when attempting to remove acid- or base- or redox-labile protecting groups from a complex and highly multifunctionalised molecule of this sort. Naturally, such a bold plan would greatly restrict the range of possible coupling reactions that could potentially be used to unify **2** with the pyran sector. Nevertheless, we considered this to be a risk worth taking given the extreme sensitivity of (+)-A83586C towards the majority of chemical reagents. To our way of thinking, the massively improved prospects for securing the natural product *via* such a daring approach far outweighed any possible operating constraints that we might have to work under. We also reasoned that a chemoselective coupling of this sort might gain some added advantage from the strong intermolecular hydrogen-bonding interactions that could potentially arise between both reaction partners, and from the greatly reduced steric hindrance that would exist around the two centres undergoing reaction.

We opted to use macrolactonisation to close the cyclodepsipeptide ring of (+)-A83586C to remove any possibility that O- to N-acyl rearrangement would occur in the hydroxyleucine moiety during the key fragment coupling step that would be used to join the pyran and peptide partners.[9] Such a problem might arise if an alternative union of a fully elaborated cyclodepsipeptide amine hydrochloride salt and the activated ester **3** was purveyed. Although activation of the carboxyl in **1** might potentially result in a β-lactone, this too could potentially macrocyclise in the desired way over time



Scheme 1 Our initial retrosynthetic analysis of A83586C.

and, given this possibility, we elected to pursue this approach with vigour, to see what would eventually come as a result.

For the construction of **2**, a sequential [3 + 2 + 1]-fragment condensation strategy was envisaged between **7**, **6** and **8**. This would lead to **5** whose hydroxyleucine residue would be elaborated by Sharpless asymmetric dihydroxylation (AD),[10] azidation and hydrogenolysis. Provided the hydrogenolytic deprotection of **4** could be conducted under carefully defined acidic conditions, this reaction sequence could be expected to yield the linear hexapeptide salt **2**. Although the aforementioned

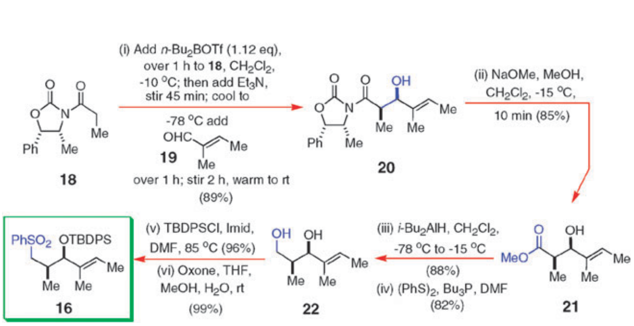
[3 + 2]-fragment coupling between **6** and **7** did risk a loss of stereochemical integrity at the α-methyl stereocentre of **6**, such a side-reaction might be totally avoidable through a judicious choice of carboxyl activating agent and a due optimisation of the fragment coupling conditions. Despite the fact that a Sharpless AD[10] had never previously been applied to a complex peptide such as **5** back in 1992, this lack of previous precedent only served to further heighten our desire to pursue this approach, for it could potentially establish the viability of using such a strategy to elaborate the hydroxyleucine residues of complex peptides. Certainly a plan of this sort would carry with it considerable economy of approach, when compared with other alternative strategies for installing this amino acid, which inevitably would involve us effecting a union with a more fully functionalised hydroxyleucine fragment. We therefore viewed the present investigation as an important new test case for Sharpless AD chemistry.

As for the activated ester **3**, it appeared derivable from the ketone **9**.[11] The latter would itself originate from a coupling between sulfone **11** and aldehyde **10**, if followed by alcohol oxidation and desulfonylation.

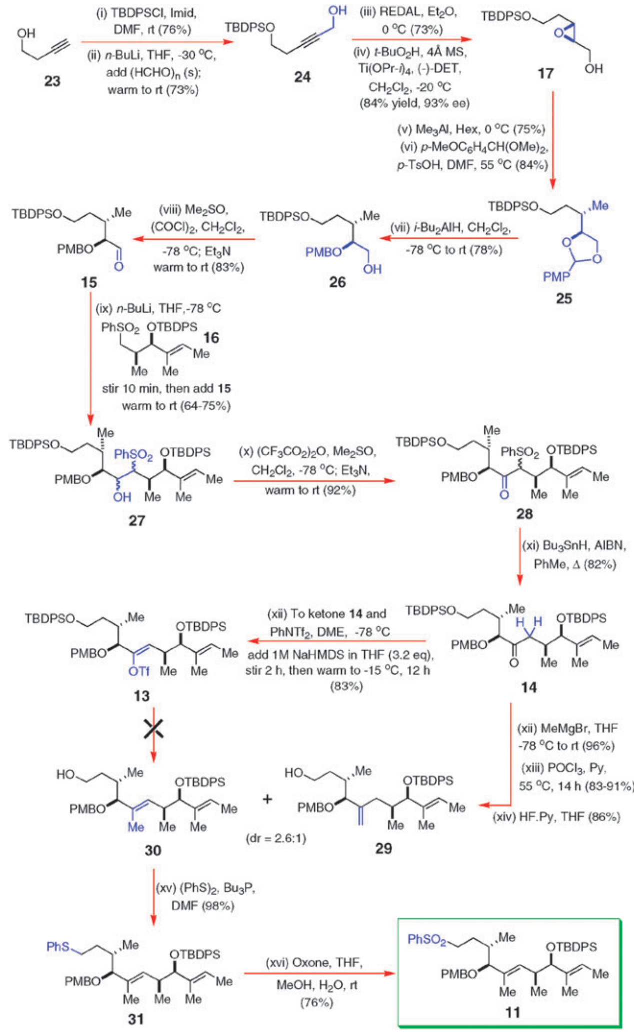
One possible way of setting the C(35)–C(36)-trisubstituted olefin in **11** would be to effect a Stille cross-coupling[12] between enol triflate **13** and tetramethylstannane, under Pd(0)-catalysis, or alternatively, to implement a McMurry-Scott cross-coupling between **13** and lithium dimethyl cuprate, in a manner similar to that used by Smith and co-workers in their successful synthesis of (−)-FK506.[13]

Ketone **14** therefore became a logical precursor of **13** and the former, in turn, appeared derivable from the anionic union of sulfone **16** with aldehyde **15**. With respect to **16**, its two stereocentres would be forged by an Evans asymmetric aldol reaction[14] between **18** and tiglic aldehyde **19** while aldehyde **15** would have its stereochemical arrangement secured by a C(3)-site-selective ring-opening of the 2,3-epoxy alcohol **17** with Me_3Al as first described by Masamune.[15]

With this synthetic blueprint in mind, our first objective in the route to **3** was to develop a synthesis of the phenylsulfone **16**.[11] This was fashioned according to the pathway shown in Scheme 2 which commenced with an Evans asymmetric *syn*-aldol reaction[14] for installation of the C(37)-stereocentre within **20**. The latter was then converted into ester **21** with NaOMe and the latter reduced with DIBAL. The resulting 1,3-diol **22** was regioselectively thioetherified with $Bu_3P/(PhS)_2$ and the thioether O-silylated prior to oxidation with oxone. The entire six step sequence to **16** proceeded in 52% overall yield and was fully amenable to large scale work.



Scheme 2 Synthesis of the A83586C phenylsulfone **16**.[11]



Scheme 3 First-generation route to the A83586C phenylsulfone **11**.[11]

The pivotal step used in the construction of aldehyde **15** (Scheme 3) was the site-selective C(3)-ring-opening of chiral 2,3-epoxy alcohol **17** with Me_3Al,[15,16] which proceeded with a 20 : 1 level of regiocontrol in favour of the desired ring-opened product, as first observed by Masamune.[15] In order to introduce the requisite PMB group onto the secondary-OH of this diol, recourse was made to reduction of the *p*-methoxybenzylidene acetal **25** with DIBAL,[17] which again proceeded with excellent regiocontrol. A Swern oxidation[18] of **26** then completed our synthesis of aldehyde **15**.

Not entirely unexpectedly, the anionic union of sulfone **16** with aldehyde **15** proceeded with reasonable efficiency (75%) to procure the β-hydroxysulfones **27** as a diastereoisomeric mixture. Without any purification, these were taken forward towards ketone **14** by Swern oxidation with trifluoroacetic anhydride and DMSO,[18] and by Smith/Hale/McCauley free radical-mediated desulfonylation with Bu_3SnH/AIBN.[19]

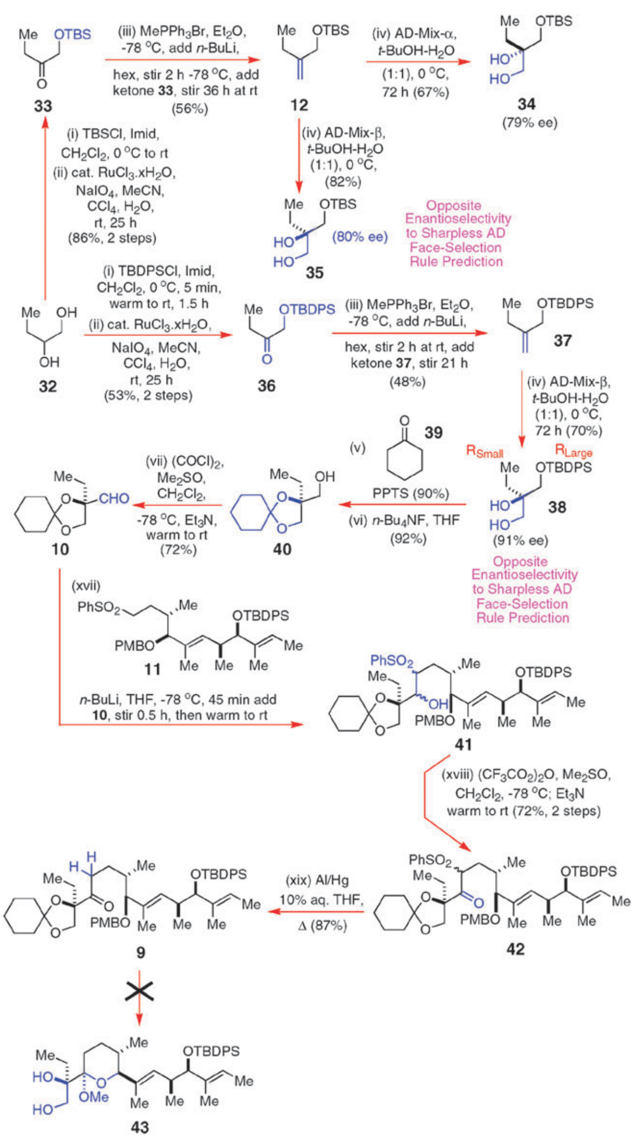
After significant experimentation, conditions were eventually identified for preparing the enol triflate **13** as a single geometric isomer. However, all of this effort proved futile for it subsequently emerged that enol triflate **13** was a most unwilling participant in transition metal-catalysed cross-coupling processes with various Me-carbanion sources. In this regard, various Pd(0)-, Cu(I)- and Ni(0)-catalysed cross coupling reactions were screened for their ability to elaborate the desired alkene **30** but none were successful.

Consequently, we resorted to a chelation-controlled addition of MeMgBr to ketone **14** to create a tertiary alcohol that was then dehydrated. After surveying a host of different dehydrating reagents to obtain **30** (including the Martin sulfurane), the combination of phosphorus oxychloride and pyridine proved optimal for our purposes, it delivering a 2.6 : 1 mixture of the tri- to 1,1-di-substituted alkenes that could be readily separated after the primary OTBDPS ether had been selectively cleaved with HF-pyridine complex to give **29** and **30**. Thiophenylation of **30** and sulfide oxidation with oxone then provided the desired phenylsulfone **11**.

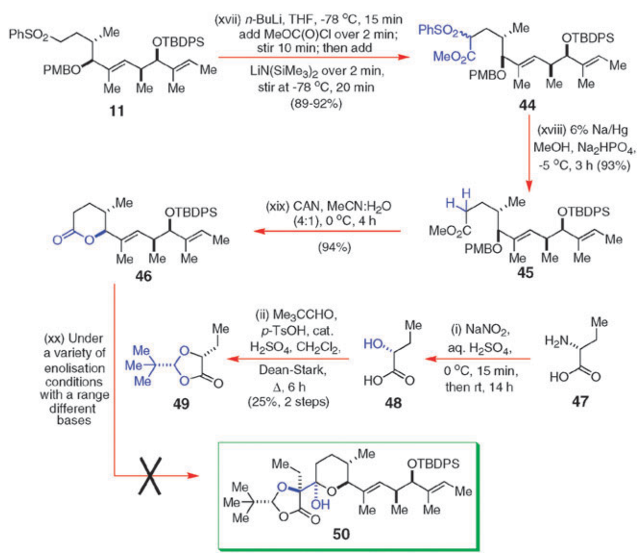
With the synthesis of **11** now complete, we turned our attention to the assembly of its aldehyde partner **10** (Scheme 4). For this, the 1,1-disubstituted alkene **12** had already been selected as a key intermediate. It was built up from commercially available butane-1,2-diol **32** *via* the chemistry outlined in Scheme 4. Although our application of the AD-mix-β reagent[10] to alkene **12** did actually provide the desired diol **35** in 80% ee and 82% yield (and it was successfully carried forward to **10**),[11] it was only after further detailed investigations into how O-protecting group size affects AD-facial selectivity in 1,1-disubstituted systems[20] that we successfully unearthed the result that would thereafter underpin our future A83586C synthesis studies over the coming years. Specifically, we observed that if we increased the size of the silicon protecting group on the allylic-oxygen to that of a TBDPS (as in **37**), we could dramatically improve the ee of our product diol **38** to a really quite impressive 91%, with minimal erosion in product yield.[20] Indeed, to this very day, this remains one of the great success stories of the Sharpless AD reaction in 1,1-disubstituted alkene systems,[20] which often do not perform that well when compared with other substituted olefin classes.

With diol **38** in hand we proceeded towards aldehyde **10** in two more steps. Even so, and notwithstanding us being able to successfully couple aldehyde **10** with sulfone **11**, and thereafter process **41** into ketone **9** by Swern oxidation[18] and Al/Hg-mediated desulfonylation,[21] we were unable to effect the subsequent successful conversion of **9** into methyl glycoside **43**. In fact, nothing resembling the desired product could ever be isolated from our many attempts at implementing this reaction under a range of conditions.[22]

Given this synthetic impasse, we decided to modify our tactics once more. Our intention now was to investigate whether a Seebach-style enantioretentive Claisen condensation[9b,23] between **46** and **49** would help us build up the pyran framework of **3** *via* **50** (Scheme 5).[22] The main advantage of following this approach to **3** would lie in its introduction of the C(28)-carbon at the correct acid oxidation state. It was hoped that Fischer glycosidation on **50** would subsequently allow a methyl glycoside to be positioned at C(30), to thereafter permit a cleavage of the carboxyl protecting group and enable a further elaboration into **3**.



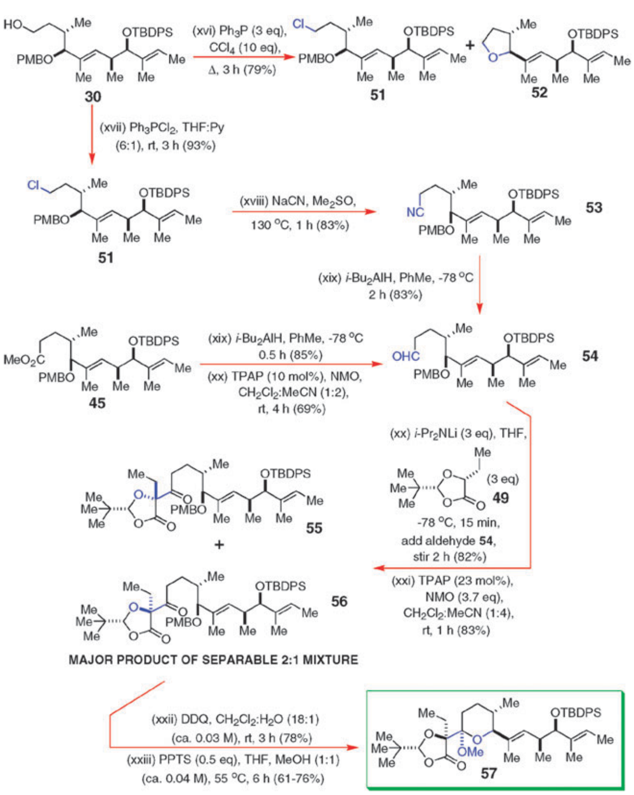
Scheme 4 Synthesis of ketone **9** and its attempted conversion into **43**.[11,20]



Scheme 5 Attempted implementation of a Seebach enantioretentive Claisen condensation route to the A83586C activated ester **3**.[22]

Accordingly, the δ-valerolactone **46** was prepared from phenylsulfone **11** by the route shown in Scheme 5.[22] The first step involved the C-acylation of the sulfone anion derived from **11** with methyl chloroformate. The α-phenylsulfonyl ester **44** was then reductively desulfonylated with 6% Na/Hg in MeOH. The desired lactone **46** was fashioned by deprotection of the C(34)-OPMB-ether in **45** with ceric ammonium nitrate; **46** was isolated in 94% yield. Unfortunately, the desired enantioretentive Claisen condensation[9b,23] of **46** with **49** failed to deliver even a small amount of **50** under the various reaction conditions that we examined. Invariably, the starting lactones **46** and **49** were always recovered unscathed from these coupling processes.

In light of this low reactivity, we reformulated our plan. On this pass through we would forge the C(29)–C(30) bond *via* an enantioretentive aldol addition between **49** and aldehyde **54** (Scheme 6).[22] It was hoped that this reaction would proceed under kinetically-controlled conditions to give a single aldol adduct having the correct stereochemistry at C(29). In the event, only low diastereocontrol was observed in this addition, presumably because of the reversibility of the aldol reaction and the occurrence of rapid product equilibration. In this regard, when we performed a subsequent TPAP oxidation, a very disappointing 1 : 2 mixture of the β-keto esters **55** and **56** was eventually encountered. Fortunately, the major component of this mixture was the desired product **56**, and it was separable in pure condition by SiO_2 flash chromatography. Compound **56** next had its PMB group oxidatively excised from O(34) with DDQ.[24] The resulting lactol was then subjected to a Fischer glycosidation to obtain **57**.[24] It was



Scheme 6 Attempted implementation a Seebach enantioretentive aldol pathway to **3**.[22]

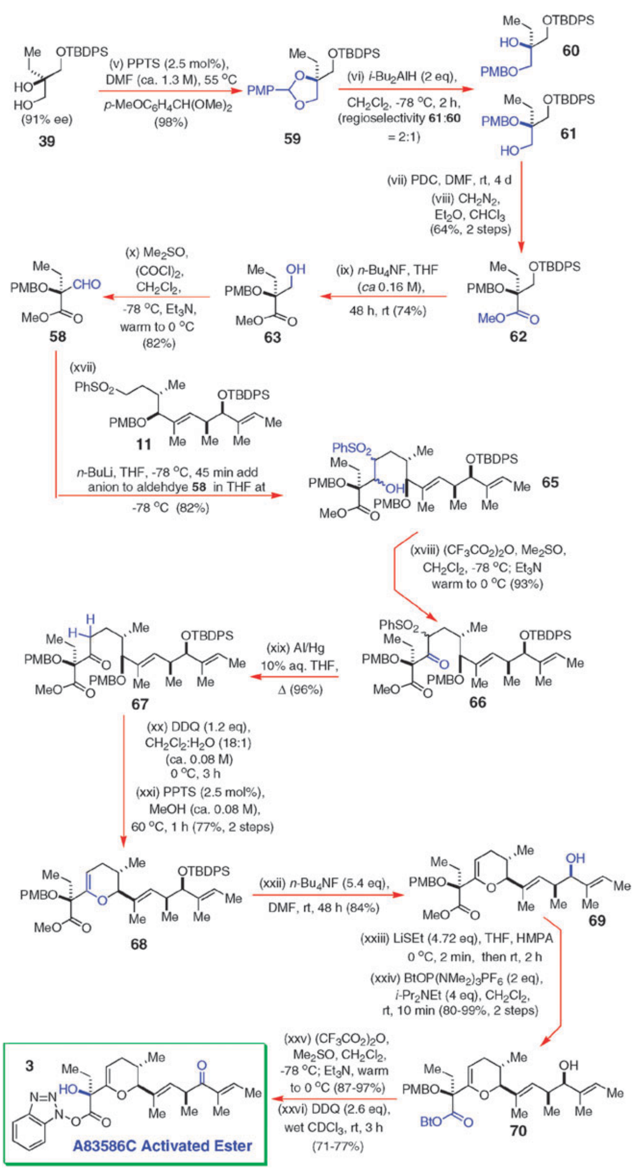
Scheme 7 Revised retrosynthetic planning for activated ester **3**.

hoped that these conditions would selectively transesterify the pivaldehyde acetal system but, unfortunately, this did not happen.[24] Difficulties were also encountered when we attempted to cleave the acetal ester from **57** with a variety of bases; invariably these conditions always inflicted damage on other regions of the molecule, most especially whenever we attempted to force the various reactions to proceed. Given all of these difficulties, we reluctantly decided to beat a retreat from this position, and modify our approach once more.

Our plan now was to couple the phenylsulfone **11** with aldehyde **58** (Scheme 7).[24,25] Although the presence of an ester group within **58** did carry with it the attendant risk of a competing sulfone anion addition to this carbonyl, we did not believe that this would be a particularly problematical side reaction at low temperatures, where the more electrophilic aldehyde would almost certainly react preferentially. Accordingly, we duly pursued the route in Scheme 8.

Of course now we had to find an effective way of introducing the requisite PMB-grouping onto the tertiary-OH of **39** without disrupting the remainder of the structure. Eventually, we found that reduction of the *p*-methoxybenzylidene acetal **59** with DIBAL accomplished this task successfully but, rather disappointingly, it only gave rise to a 2 : 1 mixture of regioisomeric products.[24] Still, we had to be grateful that the desired product **61** had predominated, and that it was readily separable from **60** by SiO_2 flash chromatography. We duly pressed forward and applied a PDC oxidation/CH_2N_2 esterification sequence to **61**. O-Desilylation of **62** then ensued with *n*-Bu_4NF/THF. The resulting alcohol **63** was oxidised to **58** under Swern conditions.[24]

We were now able to examine the critical coupling between **11** and **58** (Scheme 8). To our delight, this proceeded successfully, as did the subsequent Swern oxidation and Al/Hg amalgam reduction. Together these reactions furnished the desired ketone **67** in 73% overall yield. A delicate phase of the synthesis was now entered, where we had to chemoselectively remove the secondary PMB group from the O(34)-atom of **67** whilst retaining the more sterically hindered, less electron-rich, O(29)-PMB. Back in 1996, when this work was being done, nothing comparable to this had ever been accomplished. It was with some considerable trepidation therefore that we approached this reaction. In the event,[24] all our fears proved groundless, for the selective deprotection of **67** proceeded very straightforwardly when 1.2 equivalents of DDQ were used for the PMB cleavage.[26] This delivered a mixture of two ring-closed hemiketal anomers and the open-chain δ-hydroxy ketone, in addition to a small amount of the ring-closed glycal **68**. Because of the difficulties involved in processing this mixture, we had to identify conditions that could fully convert it into **68** in good yield such that we could progress our synthesis further. In the event, catalytic PPTS in methanol at 60 °C very nicely achieved our objective.[24]

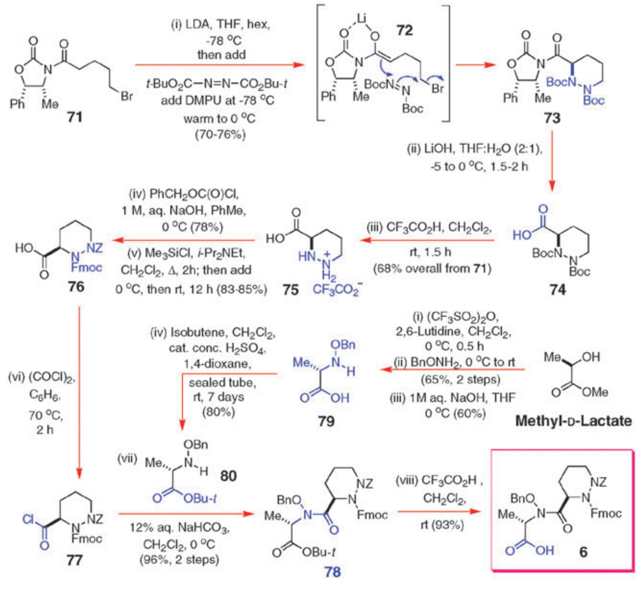


Scheme 8 Eventual successful route to the A83586C activated ester **3**.[24,25]

The O-desilylation of **68** with n-Bu$_4$NF in THF proved very slow, but as soon as we resorted to excess n-Bu$_4$NF in DMF our problems were quickly solved, with an 84% yield of **69** being obtained after 48 h.[25] The next obstacle that we faced was hydrolytic cleavage of the methyl ester group from **69** to access the acid. Unfortunately, every hydroxide source that we examined, over a range of different concentrations, failed to accomplish this task. Reasoning that severe steric hindrance from the O(29)-PMB group was responsible for this lack of reactivity, we duly modified our approach, examining the Bartlett–Johnson method[27] for methyl ester cleavage, which utilises lithium ethanethiolate in THF/HMPA at 0 °C. This worked very well indeed on **69**, providing the desired acid in good yield.[25] The latter was then treated with Castro's BOP reagent[28] to access the hydroxybenzotriazole activated ester **70**. Despite the fact that **70** had a pendant hydroxyl within its framework, it proved quite stable to prolonged storage. Indeed, as long as it was kept in the refrigerator at −20 °C under N_2, it remained intact and pure, even following 6 months of storage. This stability proved pivotal to us eventually performing the subsequent Swern oxidation to the α,β-enone in 87–97% yield. The final step in our route to the activated ester **3** was the DDQ-mediated cleavage[26] of the O(29)-PMB group from this enone which proceeded uneventfully when conducted in commercially available $CDCl_3$.[25] Whilst **3** was stable enough to undergo rapid SiO_2 flash chromatography, we generally found it best to use it for all subsequent couplings without delay, otherwise it started to decompose. It also proved necessary *to completely remove* all of the last traces of the DDQ residues from **3** prior to effecting any peptide couplings with unprotected A83586C hexapeptide fragments so as to prevent oxidation of the piperazic acid residues.

In parallel with these total synthesis efforts on the pyran sector, we also pursued a synthesis of the hexapeptide coupling fragment **2**.[29] Of massive importance to this endeavour was our development of the tandem asymmetric electrophilic hydrazination-nucleophilic cyclisation strategy for piperazic acid construction (Scheme 9),[30] which underpinned all subsequent synthetic progress on this peptide.

In this protocol, an Evans–Vederas asymmetric hydrazination[31] is effected on the Li-coordinated bromovaleryl enolate **72** with di-*tert*-butylazodicarboxylate (Scheme 9). This affords an N(1)-aza anion which rapidly cyclises after DMPU is added to the reaction mixture, and the reactants are allowed to warm to 0 °C. This tactic provides the cyclised adduct **73** alongside a small quantity of the hydrolysed acid **74**. In light of this, it was generally found best to submit the crude, worked-up, cyclisation mixture to LiOH-induced hydrolysis to fully convert it to **74**. The latter was then subjected to trifluoroacetic acid mediated Boc-removal. The crystalline (3R)-Piz TFA salt **75** so obtained was typically of >96% ee but recrystallisation rendered it completely pure both chemically and enantiomerically; the



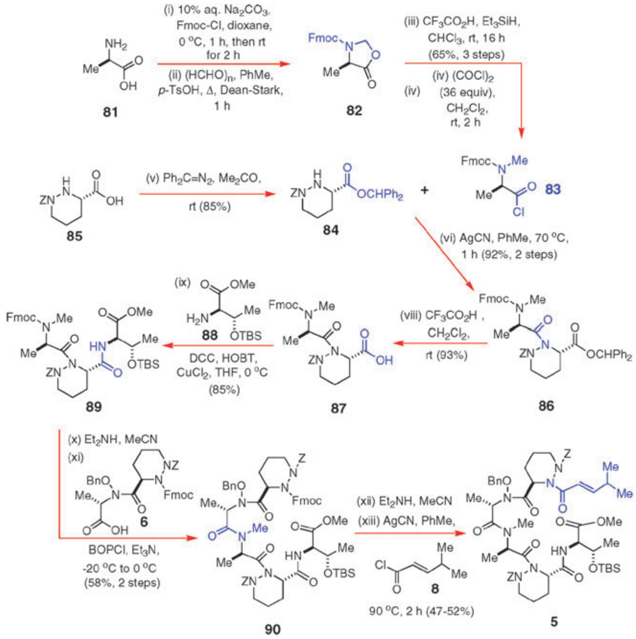
Scheme 9 Our tandem asymmetric electrophilic hydrazination–nucleophilic cyclisation strategy for building up enantiopure piperazic acid and its application in the synthesis of the northern dipeptide **6**.[9,29,30]

overall yield of pure **75** from **71** was 68% without resort to chromatography and even today, 18 years after its development,[30a,b] this remains the premier method for constructing this particular chiral α-hydrazino acid.

Compound **75** was then regioselectively N-acylated with benzyl chloroformate according to the procedure of Adams and co-workers at Roche, Nutley.[32] In our hands, this worked very well indeed. Although others have experienced difficulties in attaining high yields for this step, we have always found that the secret to success is to ensure that one properly acidifies the aqueous medium containing the *Z*-Piz, after the N-acylation has occurred. This must be done, however, ***after*** the excess ZCl has been completely extracted with ether. By adhering to this protocol, one can routinely obtain good yields of (3*R*)-*Z*-piperazic acid, which normally crystallises directly from the cooled, stirred, aqueous fraction. It transpires that dipeptide **6**[9,33] (Scheme 9) had previously been synthesised by the Durette/Caldwell team at Merck Rahway during their 1989 total synthesis of the pyranylated cyclodepsipeptide, L-156,602.[9,33] They prepared compound **78** from **76** by the pathway shown in Scheme 9 which exploited optically pure (3*R*)-*N*(1)-*Z*-piperazic acid to secure **6**. In their synthesis,[9,33] however, optically pure (3*R*)-*N*(1)-*Z*-Piz was obtained using Hassall's rather low yielding procedure of ephedrine-induced optical resolution of (±)-*N*(1)-*Z*-piperazic acid,[34] which itself had been prepared *via* a high-yielding Diels–Alder reaction between methyl 2,4-pentadienoate and DBAD in CCl_4 at reflux.[33] Our highly efficient tandem asymmetric hydrazination pathway to **75** and **76** thus greatly improved the prior art for obtaining **6** which utilised Carpino's Fmoc-amino acid chloride biphasic coupling technology[35] to connect the fragments **80** and **77** together. The hydroxamic acid partner **80** was itself obtained from methyl-D-lactate *via* Ottenheijm's triflate displacement technology[36] which again worked very well in our hands.

The creation of southern dipeptide **86** (Scheme 10) yet again required the use of Fmoc-amino acid chloride coupling technology for success in the N-acylation of the electronically deactivated (3*S*)-Piz residue **84**. In this instance, Durette and Caldwell's silver cyanide mediated N-acylation method[9a,33] was exploited to secure **86** in 92% yield over 2 steps. Dipeptide **86** then had its diphenylmethyl ester group[37] cleaved by CF_3CO_2H to give acid **87** which was coupled to the partially protected D-threonine derivative **88** with DCC[38]/ HOBt. It transpired that $CuCl_2$[39] was an essential additive for this reaction, if one wished to obtain an 85% yield of the diastereomerically pure tripeptide **89**, and one also wanted to avoid epimerisation[39] at the (3*S*)-piperazic acid centre, which was highly problematical when the $CuCl_2$ was missing.

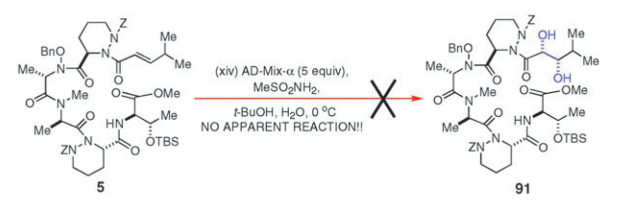
Following Fmoc-group removal from **89** we explored the [3 + 2]-union of the two peptide fragments **89** and **6**. A range of carboxyl activation methods were surveyed unsuccessfully before BOPCl[40] was eventually identified as the optimal coupling reagent for effecting this union. It performed admirably in this condensation, it delivering a 58% overall yield of the desired pentapeptide in diastereomerically pure condition. After Fmoc detachment, we were now able to focus on N-acylating the (3*R*)-piperazic acid residue with acid chloride **8**. Again a high temperature silver cyanide coupling fashioned the desired pentapeptide **5** in an unoptimised 47–52% yield.



Scheme 10 The route developed to pentapeptide **5**.[29]

We next addressed whether Sharpless AD chemistry[10] could be used to elaborate the hydroxyleucine side chain of the target (Scheme 11). Unfortunately, even when a massive excess of super-reinforced AD-mix-α (which contains extra potassium osmate) was employed for this AD, we were never successful in coaxing any reaction out of the alkene **5**. The substrate simply sat there untouched in the reaction mixture. We now recognised that we had to abandon our originally formulated plan and focus on connecting a fully elaborated (2*S*,3*S*)-hydroxyleucine side chain in which appropriate protecting groups were now present.

When we first confronted this problem back in 1994, the best pathway to (2*S*,3*S*)-hydroxyleucine was that of Bondy and Caldwell.[41] They exploited a C(2)-site-selective ring-opening of a Sharpless asymmetric epoxidation (AE)-derived chiral 2,3-epoxy acid for installation of the requisite *anti*-amino alcohol motif. We sought a much more convenient method for obtaining this amino acid on large scale. The pathway that was eventually devised[42] set off with a Wittig reaction on isobutyraldehyde **92** to obtain enoate **93** (Scheme 12) which thereafter was subjected to a Sharpless AD with AD-mix-α. We then used Sharpless′ excellent cyclic sulfate ester-nucleophilic displacement technology[43] for introducing the required α-azido group in **96**. Ester hydrolysis and azide reduction completed this very convenient pathway to optically pure **97** which requires no column chromatographic purifications of the intermediates at any stage, and which proceeds in 73% overall yield.[42]



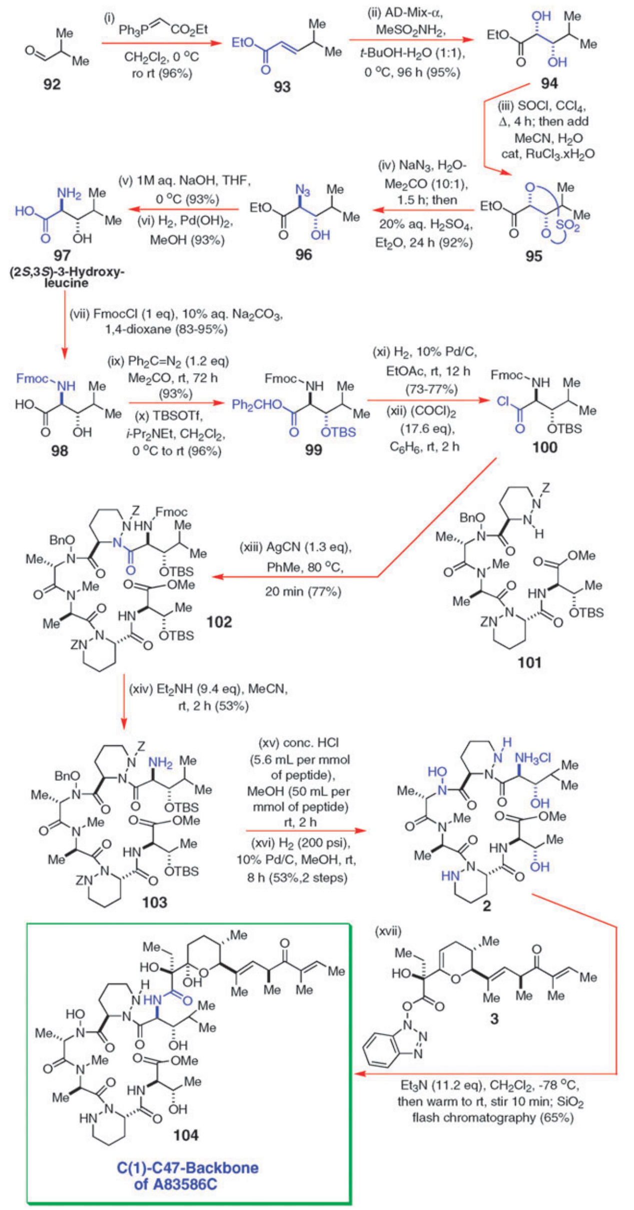
Scheme 11 Outcome of attempted Sharpless AD on pentapeptide **5**.

Given that amino acid chloride coupling technology would be a prerequisite for acylation of the (3*R*)-piperazic acid N(2)-atom in **101**, we had to make an appropriate choice of amine protecting group for the hydroxyleucine coupling partner. After giving all viable options due consideration (α-Boc- and α-*Z*- groups excluded, of course), we opted to employ an α-Fmoc group for our purposes. It was introduced into **97** in 83–95% yield. Diphenyldiazomethane treatment of **98** then installed a diphenylmethyl ester[37] which, after O-silylation with TBSOTf, provided **99**. Hydrogenolysis of the diphenylmethyl ester group was then accomplished in EtOAc and the resulting acid was converted into acid chloride **100** by exposure to oxalyl chloride in benzene. Silver cyanide-induced N-acylation[9a,33] of **101** now provided hexapeptide **102** in 77% yield. Having served its purpose, the Fmoc unit was detached from **102** with diethylamine in acetonitrile. The two TBS protecting groups were then removed by exposing **103** to concentrated HCl in methanol. The resulting amine hydrochloride was concurrently O- and N-debenzylated by catalytic hydrogenation in methanol in the presence of 10% Pd/C at 200 psi. After 8 h, the fully deprotected hexapeptide methyl ester **2** was obtained in 53% yield for these last two steps.[25]

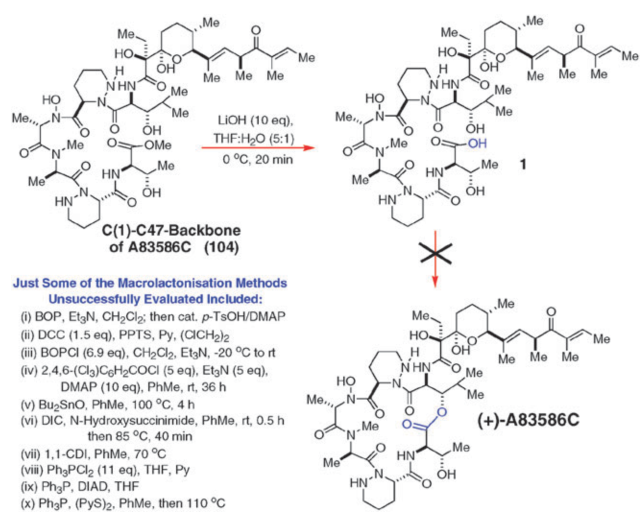
The activated ester **3** and the hexapeptide amine hydrochloride **2** were now mixed together in a flask and dichloromethane was added. After cooling to −78 °C, excess Et_3N was introduced. The mixture was briefly stirred at this temperature for several minutes before being warmed to room temperature where it was maintained for a further 10 min. TLC analysis then revealed that a very clean and smooth coupling reaction had taken place. Following chromatographic purification on SiO_2, the hydrated product **104** was isolated in a noteworthy 65% yield.[25]

It was now time to hydrolyse the methyl ester from **104**, and LiOH appeared to do this successfully. However, despite our repeated attempts to macrolactonise the crude *seco*-acid **1**, we were never successful in securing (+)-A83586C (Scheme 13).[44] Confronted with this massive breakdown in our strategy at the very final step, we had no other choice but to completely redesign our synthesis or face defeat.

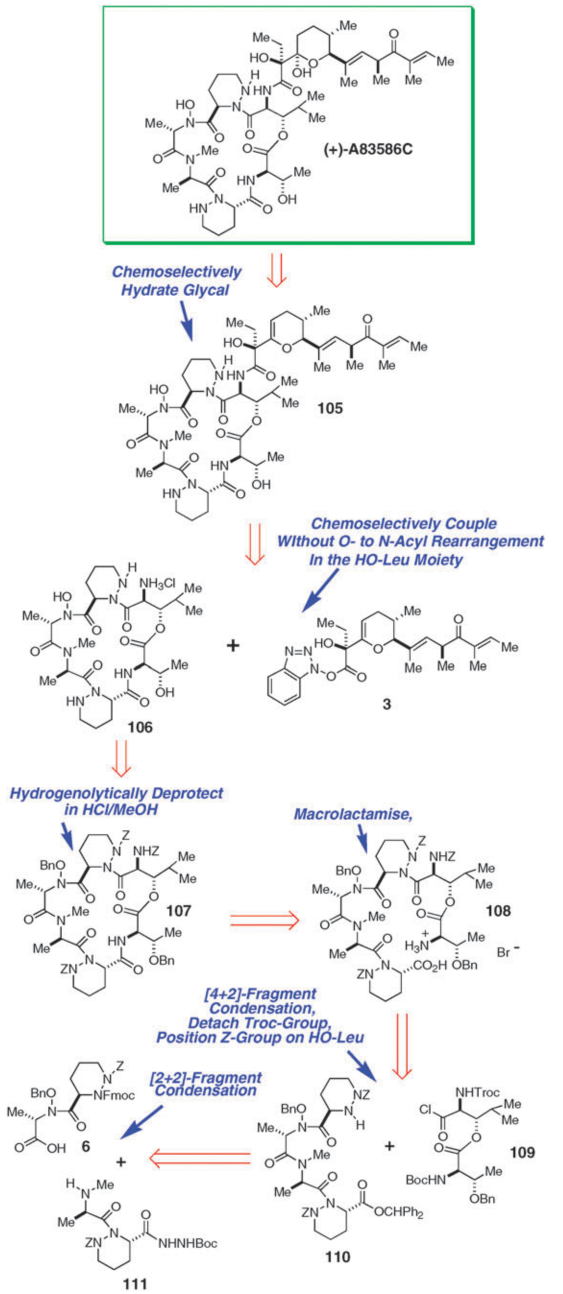
In our new plan for (+)-A83586C (Scheme 14),[44] we would now attempt the high-risk chemoselective coupling of cyclised hexadepsipeptide salt **106** with activated ester **3**. Buoyed by the success and rapidity of our previous chemoselective coupling of **2** with **3**,[25] which was complete after only 10 min at room temperature, and which provided **104** in 65% yield, we reasoned that we might able to effect a similar rapid coupling between **106** and **3** and avoid the potential problem of us encountering a deleterious O- to N-acyl rearrangement in the hydroxyleucine moiety.[9a] The latter would, of course, give rise to a ring-contracted macrolactam which would be totally unmanipulable. Although rearrangements of this sort can, on occasion, be quite facile, they typically do not proceed with great rapidity; often taking many hours before rearrangement is anywhere near fully complete.[9a,45] Cogniscent of this fact, we reasoned that if rearrangement only took place slowly,[45] and our coupling reaction proceeded rapidly, we might be able to overcome this potential snag.



Scheme 12 Synthesis of the C(1)–C(47) backbone of A83586C (**104**).[25,42]



Scheme 13 Attempted macrolactonisation of **1** to obtain A83586C.[44]



Scheme 14 Revised retrosynthetic analysis of A83586C.[44]

Accordingly, we now made the acquisition of **107** our top priority, and this required us to completely reconsider how we would assemble the peptide sector. Given that many of the potential macrolactamisation sites in this sequence would necessitate the N-acylation of an inductively-deactivated or a sterically-hindered nitrogen atom there was, in the end, only one viable option for effecting ring closure, and that was at the amide bond which connected the D-threonine and (3*S*)-piperazic acid residues. However, even this particular choice could potentially cause our downfall, since this now presented us with the highly daunting (and synthetically challenging) prospect of having to close the cyclodepsipeptide ring at an N(2)-acylated (3*S*)-piperazic acid residue which we knew would be highly prone to undergoing significant epimerisation once its carboxyl was activated.[29] Indeed, as we shall see later, in the synthesis of 4-*epi*-A83586C, we had every reason to be fearful of this occurrence, even though we had previously shown[29] that the inclusion of $CuCl_2$ could very powerfully suppress epimerisation[39] in the intermolecular coupling of **87** with **88** with DCC[38]/HOBt. Nevertheless, it was not at all clear whether these conditions would again lead to a successful outcome in the context of macrolactamisation. Even so, given that all of the other ring-closure options looked completely untenable, we elected to pursue this synthetic analysis further. Our plan for assembling the linear hexapeptide **108** would first effect a [2 + 2]-fragment union between **111** and **6** to secure a tetrapeptide whose Fmoc-group would be removed to give **110**; the latter would then be N-acylated with the acid chloride **109** and the resulting linear hexapeptide subjected to a series of protecting group interchanges to arrive at **108**. Clearly the build up of a complex amino-acid chloride such as **109** might not be technically feasible given the potentially acid-labile Boc-carbamate protecting group that was also going to be present within its structure. However, given our desire to unmask the two reactive centres of the *seco*-amino acid **108** concurrently, we felt that we had very little choice in our selection of this strategy. There was, as well, the issue of whether a Troc group would survive the silver cyanide mediated N-acylation process.[9a,33] This was yet another obstacle that could potentially smite us, but given the need for amino acid chloride coupling chemistry to solve this problem, and our fear that use of an Fmoc-alternative might trigger an O- to N-acyl rearrangement in the subsequent Fmoc-deprotection step, after macrocyclisation had occurred, we stuck firm with our protecting group choice for **109**.

Quite early on in our synthetic forays on A83586C we had observed that if one attempted to remove the Fmoc-protecting group from ester-protected dipeptides such as **86** (Scheme 10) with Et_2NH in MeCN, one always forms the diketopiperazine instead of the desired dipeptide amine.[44] Such internal cyclisations are often problematical in dipeptide esters that contain an *N*-methyl amino acid residue in the sequence, but this tendency towards cyclisation is even more pronounced when a (3*S*)-piperazic acid residue is also present in the chain; the latter appears to induce considerable ''turn-like'' character within the dipeptide to further favour the ring-closure process. The increased propensity of Piz-containing dipeptide esters to form diketopiperazines is most beautifully illustrated by the fact that even when a *t*-butyl ester analogue of **86** is subjected to the same conditions, it cyclises in exactly the same way, which is most unusual, since *tert*-butyl ester dipeptides are usually stable under such circumstances! In light of this, we were forced to devise a new protecting group solution to overcome the diketopiperazine-forming problem.[44]

Recognising that removal of the Fmoc-group from tripeptide **89** was facile and successful (Scheme 10),[29] we sought to install a removable amide-type protecting group into the (3*S*)-Piz residue of **87** to perform a similar cyclisation-deterring role in an analogous Fmoc-protected dipeptide. However, the protecting group installed would have to be cleavable at a later point in the synthesis, under conditions that would leave the remainder of the peptide intact.

After giving the problem some due thought, we eventually decided to employ the seldom-used *tert*-butyloxycarbonyl-hydrazide group in this role.[46] We were attracted to this particular option by the excellent 1981 report of Meienhofer and Walho[47] at Roche Nutley who demonstrated that complex peptidal acyl hydrazides could be readily converted into acids using aqueous NBS.[46,47] Encouraged by their results, we sought to apply this technology to overcome the diketopiperazine ring-forming problem in systems such as **86**.

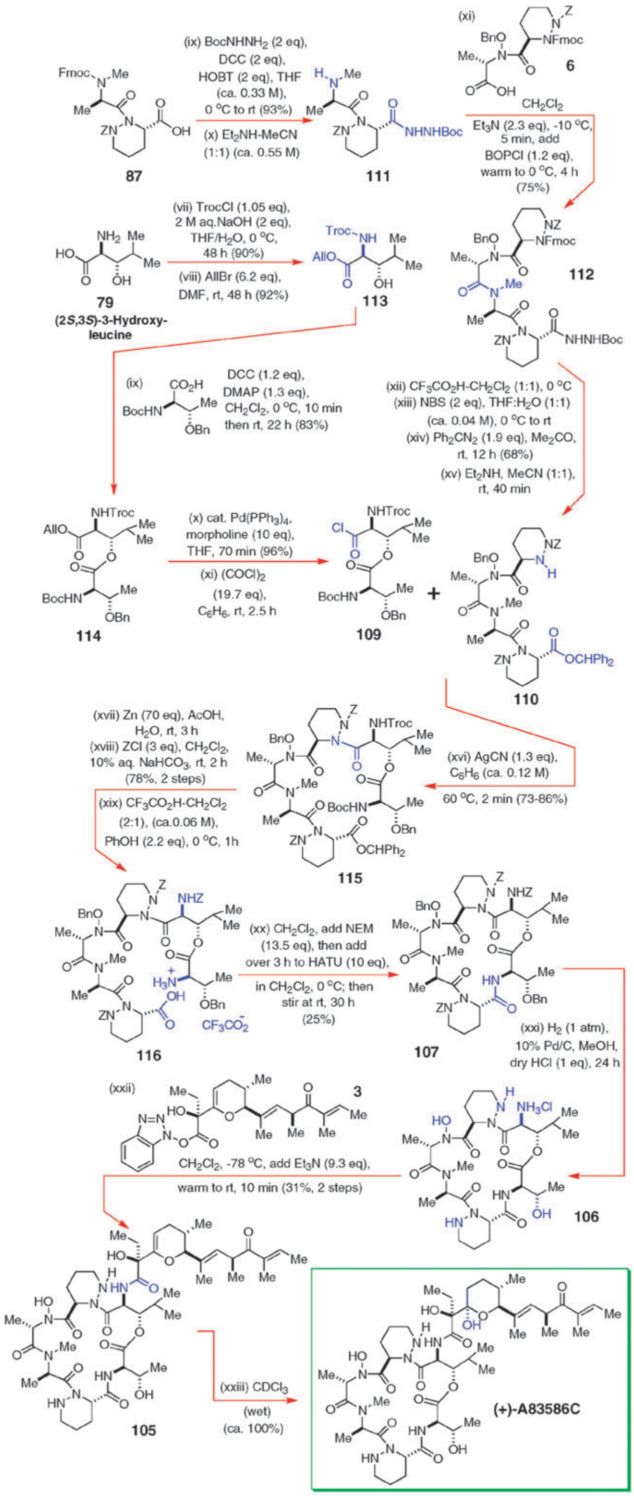
Accordingly, we converted[44] our previously prepared dipeptide acid **87**[29] into the amine **111** (Scheme 15) and, most notably, we found that this device completely overcame the internal cyclisation issue. Amine **111** was then used for a BOPCl-mediated[40] coupling with acid **6**. After 4 h at 0 °C, a 75% overall yield of tetrapeptide **112** was obtained over two steps.

Given the potential risks of simultaneously N-acylating the Boc-hydrazide unit of the Fmoc-deprotected tetrapeptide with acid chloride **109** and the possible difficulties that we might encounter on converting the acyl *t*-Boc-hydrazide unit into an acid in a more complex hexapeptide substrate, we elected to perform this conversion at this stage. This was accomplished by cleaving the Boc group from **112** with trifluoroacetic acid, oxidising the acyl hydrazide to the carboxylic acid with NBS in aqueous THF,[46,47] and then esterifying this acid with diphenyldiazomethane.[37] The Fmoc-group was then detached from the product to obtain **110**.

Its acid chloride coupling partner **109** was prepared by a DCC[38]/DMAP mediated union between the Troc-protected hydroxyleucine allyl ester **113** and *N*-Boc-*O*-benzyl-D-threonine.[48] This proceeded in 83% yield, but was always accompanied by 5–10% epimerisation at the α-carbon of the D-threonine unit. The unwanted diastereoisomer was readily separated from **114** by SiO_2 flash chromatography. Following cleavage of the O-allyl ester from **114** with Pd(0) and morpholine, according to the method of Kunz and Waldmann,[49] we duly converted the resulting acid into acid chloride **109** by treating it with excess oxalyl chloride in benzene. After removing the excess chlorinating agent *in vacuo* the crude **109** was used for the silver cyanide-assisted N-acylation[9a,33] of **110** which proceeded in 73–86% yield after heating the reactants at 60 °C in benzene for two minutes.

We now decided to remove the Troc-group from **115** and to temporarily cap this position with a *Z*-group to allow purification of the hexapeptide fragment **116** to very high standards and to help us remove all of the zinc residues from the initially liberated amine salt. We were concerned that if we delayed this purification to a later stage of the synthesis (after cyclisation), we might encounter O- to N-acyl rearrangement[9a,45] during the purification step. In the event, this protecting group swap worked out very well indeed. Exposure to trifluoroacetic acid and phenol thereafter fashioned the *seco*-amino-acid **116**.

Much effort went into identifying conditions that could successfully macrolactamise **116** without also causing significant epimerisation of the (3*S*)-piperazic acid residue undergoing activation. The DCC/HOBT/$CuCl_2$ method[29,39] failed in this instance. Eventually, we found that Carpino's excellent HATU reagent[50] accomplished this cyclisation in



Scheme 15 First total synthesis of (+)-A83586C.[44]

25% overall yield over two steps, provided high-dilution conditions and a large excess of HATU[50] were employed.

The highly pure macrolactam **107** was now hydrogenated for 24 h at 1 atmosphere pressure in the presence of 10% Pd/C and 1 equivalent of anhydrous HCl in methanol. The latter was generated by adding 1 equivalent of acetyl chloride to the anhydrous methanol that was used for the reaction, and allowing the solution to stand for several hours under a dry N_2 atmosphere prior to use. This protocol nicely delivered the desired cyclodepsipeptide hydrochloride salt in essentially pure condition without O- to N-acyl transfer[9a,33,45] in the hydroxyleucine component. Compound **106** was combined with a freshly prepared sample of pure activated ester **3**, and dichloromethane was added. The reaction flask was then cooled to −78 °C and excess Et_3N was then added dropwise. After the Et_3N addition was complete, the cooling bath was removed and the reactants were allowed to warm to room temperature, whereafter stirring was continued for a further 10 min. TLC analysis at this stage revealed that a single coupled product **105** had formed along with some of the hydrated activated ester. The desired glycal **105** was obtained in 31% overall yield from **107**. Following chromatographic purification, **105** was then completely hydrated in essentially quantitative yield by storing the sample in commercial $CDCl_3$ (Aldrich) over 2–3 days. At long last, we had completed the first asymmetric total synthesis of (+)-A83586C[44] and we had done so *via* the most high-risk end-game that one could have possibly imagined! The result was clearly very satisfying.

New insights into the mechanism of antitumour action of A83586C

With our synthetic sample of (+)-A83586C in hand, we could now examine its antitumour effects in more detail in collaboration with Drs Alexander Wood and Ying-Nan Chen of Novartis.[51]

At first we jointly evaluated whether A83586C had broad spectrum antitumour activity against a range of different human tumour cell lines. It transpired that it did, its IC_{50} values ranging from 18–90 nM.[51]

Because Sakai and co-workers[5] had stated that "..GE3 was shown to prevent the E2F transcriptional factor, the intracellular target of retinoblastoma susceptibility gene product, from binding to its recognition sequence", we duly examined whether A83586C, over a range of drug concentrations, could interfere with E2F-1/DP-1/DNA binding, using E2F-1 response element DNA and HeLa lysate in a gel-shift assay. At concentrations of 2 and 10 μM, A83586C did not inhibit the gel-shift, suggesting that it was not inhibiting E2F/DP transcription factor activity by this mechanism as was originally proposed by Sakai for GE3.[51]

There are eight E2F proteins (E2Fs 1–8) and two DP proteins (DPs 1 and 2) so far characterised in human cells. Only E2Fs 1-6 form functionally active heterodimeric E2F/DP transcription factor complexes with one of two DP proteins.[52] The ability of these complexes to modulate gene transcription is generally tightly controlled in normal cells by several "pocket" proteins that include the retinoblastoma protein (pRb).[53] The latter functions as a tumour-suppressor protein when it is in its dephosphorylated state. Functionally-active dephosphorylated pRb controls the G1-S boundary in cell cycle by binding to, and repressing, the transcriptional-activating capabilities of E2F1-3/DP complexes. There are a number of genes critically involved in cell growth and proliferation that are switched on by functionally active E2F-DP transcription factor complexes. These include: cyclins A and E, cdc2, thymidylate synthase, dihydrofolate reductase, DNA polymerase α and ORC1, and their overexpression in cancer patients is typically associated with poor disease outcome. Mutations to the pRb or other pocket proteins, or upregulated cyclin/cdk activity leading to pRb hyperphosphorylation, are now known to increase aberrant E2F transcriptional activity and contribute to the onset of many cancers. E2F transcription factor inhibitors such as A83586C are thus of considerable pharmaceutical interest.

E2F7 and E2F8 lack this requirement for a specific DP partner to elicit their biological effects,[52a] and they primarily serve as transcriptional repressors or delayers of cell cycle progression. E2Fs 4–6 can also act as repressors in G0 or G1.

Given that upregulated E2F 1–3/DP transcription factor activity is thought to be a major contributor to the onset of human malignancy, we sort to examine whether A83586C might be inhibiting E2F through a disruption of the E2F-DP protein-protein interaction. For this, we performed pull-down experiments with full length GST-E2F1 and ^{35}S-labelled DP1 in the presence of various concentrations of A83586C (2, 10 and 50 μM, in fact). Our results indicated that A83586C does not perturb this protein-protein interaction.[51]

Since cyclin/cyclin-dependent kinase activity is known to be upregulated in many human cancers, and this maintains pRb in its oncogenic (tumour-promoting) hyper-phosphorylated state, we next examined whether A83586C had the ability to induce pRb hypophosphorylation (dephosphorylation) to produce the tumour-suppressing form of the protein in HCT-116-human colon carcinoma cells. Indeed, at 0.3 μM drug concentration in DMSO/RPMI1640 culture medium, A83586C does this very effectively after just 24 h of cell exposure to the drug.[51]

We also evaluated whether A83586C had the ability to downregulate E2F1 expression in HCT-116 colon carcinoma cells at 0.3 μM drug concentration, and significantly, the Western blots revealed that it did this very markedly.[51]

As a result of this combined work, we have now shown that molecules of the A83586C/GE3 class probably do not disrupt or prevent active E2F-DP transcription factors from binding to the target promoters of E2F regulated genes. Rather, our evidence suggests that they most likely operate as E2F inhibitors by other indirect means, such as through E2F1 protein downregulation and through induction of pRb hypophosphorylation.[51] Indeed, provided the tumour in question has a functionally active pRb, which can often be the case, this would appear to be one of the main mechanisms by which tumour growth and progression are halted by A83586C.

Yet another novel mechanism that we have jointly identified by which A83586C and its congeners can exert their powerful antitumour effects is through a potent disruption/blockade of upregulated β-catenin/TCF4 transcriptional activity and Wnt signalling within human cancer cells.[51] Upregulated β-catenin signalling is often a major contributor to cancer onset in many human tumours.[54] Since the β-catenin/TCF4 protein-protein interaction is known to initiate transcription from a number of genes that are centrally involved in cancer cell growth and metastatic spread, our discovery that A83586C is the most potent inhibitor of this β-catenin/TCF4 transcriptional activating interaction so far identified is of major biological significance.

We established[51] this property for A83586C *via* TOP-FLASH/FOP-FLASH TCF4-luciferase reporter assaying in HCT-116 human colon carcinoma cells,[54] which indicated that A83586C has an IC_{50} of 3 nM in this capacity. As such, *A83586C currently holds the world-record in terms of its potency for inhibiting β-catenin-TCF4-mediated Wnt-signalling*.[51,54]

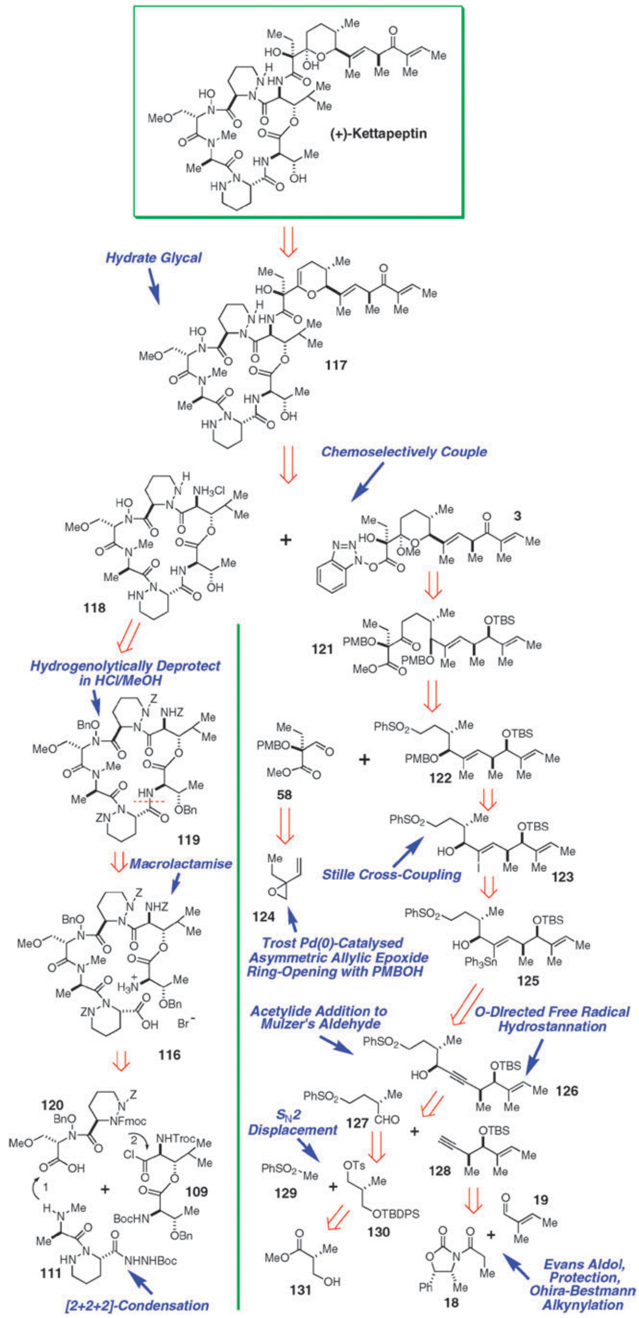
A new second-generation synthesis of (+)-A83586C, (+)-kettapeptin and (+)-azinothricin

Based upon these exceptional biological findings, Novartis sponsored our group to develop a new and improved second-generation synthesis of molecules of this class and, in this section, we now illustrate our latest strategy for securing various family members in the context of us completing the first ever total synthesis of (+)-kettapeptin (Scheme 16);[55] a natural product that has the pyran side-chain of (+)-A83586C grafted on to the cyclodepsipeptide sector of (+)-azinothricin.[8]

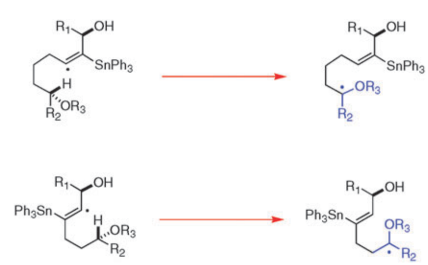
At the time we were attempting to devise our new route to the A83586C/kettapeptin activated ester **3**,[55] our group was heavily involved in developing the O-directed free radical hydrostannation reaction of propargylically oxygenated alkylacetylenes with Ph_3SnH and catalytic Et_3B.[56] When applied in acetylenic systems whose stereocentres cannot be jeopardised *via* 1,5-H-atom abstraction reactions by the intermediary vinyl radicals, this reaction can give superb results in terms of offering a highly stereocontrolled entry into various stereodefined trisubstituted alkenes.[56] However, because the complex mechanistic course of this reaction involves vinylic radicals being reversibly generated from stannyl radical addition to ***either*** acetylenic carbon,[56c] it is important to examine any potential substrate very carefully before deploying this reaction in synthesis, so as to ensure that ***either*** of the vinyl radicals that are generated cannot engage in stereocentre-compromising 1,5-H-atom abstraction processes (Scheme 17).

For the problem at hand, we considered this situation very carefully, and saw an excellent opportunity to apply the O-directed hydrostannation process to the acetylenic alcohol **126** with a view to controlling the C(35)–C(36)-double bond geometry of **122** (Scheme 16). Of course, given all of the knowledge that we had accrued on this reaction over the preceeding years, we predicted that it would lead to the vinylstannane **125** at high stannane concentration, and that this would thereafter be manipulable into the iodide **123** and thence into **122**. Although our first-generation pathway to the A83586C pyran sector[11,24,25] had indeed encountered a very notable failure when it attempted to elaborate a very similar enol triflate **13** by transition metal catalysed cross-coupling (see Scheme 3), we believed that we might attain success with the more reactive vinyl iodide **123**, particularly now that the OPMB group and the two large TBDPS groups had been deleted and, in the latter case, where they had been replaced by two smaller substituents.

The desired alkynol **126** would be prepared by a union between alkyne **128** and aldehyde **127**. Mulzer[57] had previously prepared aldehyde **127** and noted good *anti*-selectivity in an aldol reaction that it subsequently underwent. We reasoned, however, that even if poor stereocontrol was obtained in this addition, it might still be correctable through a



Scheme 16 Retrosynthetic strategy for (+)-kettapeptin.[55]



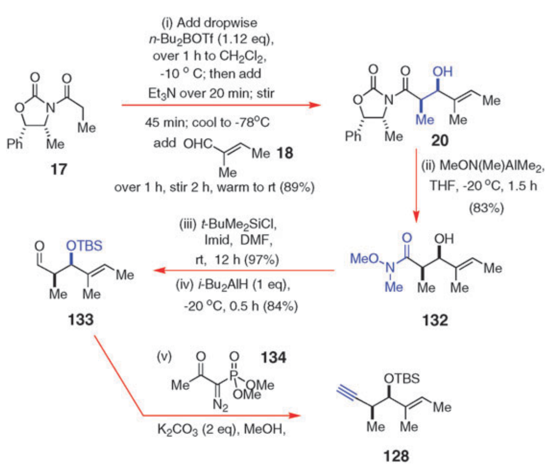
Scheme 17 Some examples of stereocentre-compromising 1,5-H-atom abstraction processes that need to be considered when planning to use the O-directed free radical hydrostannation reaction.

successive oxidation and a Noyori reduction[58] on the corresponding alkynone which should deliver **126**.

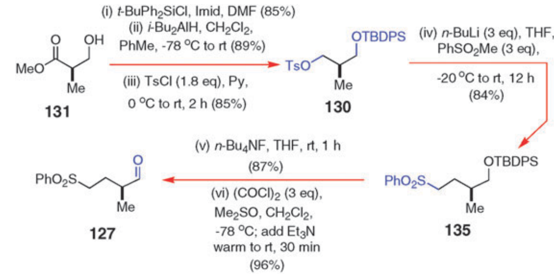
With regards to aldehyde **58**, we planned to use a Trost asymmetric allylic O-alkylation reaction[59] on the racemic epoxide **124** to introduce its tertiary OPMB group. Trost has had considerable success in his use of this reaction in a number of total synthesis settings[59] and given these great triumphs, we thought that a strategy of this sort might nicely overcome the 2 : 1 regioisomer problem that we had unexpectedly encountered in our previous synthesis of **58**.[24,25]

As for **119**, it would again be prepared by macrolactamisation and a [2 + 2 + 2]-fragment union, on this occasion, involving **111**, **120**, and **109**. The only difference this time through would be that we would try to retain the *t*-Boc-hydrazide protecting group[44,46,47] in the (3*S*)-Piz residue throughout the synthesis, including for the [4 + 2]-fragment coupling with acid chloride **109**. We also planned to build **111** directly, rather than indirectly from the dipeptide acid **87**, as had been done previously. These various modifications promised to cut quite a few steps off the forward synthesis. As for dipeptide acid **120**, we reckoned that a different protecting group strategy would almost certainly be required for its construction, given its potentially β-eliminatable OMe group. To our mind, its presence would necessitate protection of the L-hydroxamic acid residue with an *O*-allyl ester, which would be cleavable at the final step under neutral conditions.

An early objective in new our route to the A83586C activated ester **3**[55] was alkyne **128** (Scheme 18). It was prepared[56c] from the aldol adduct **20** by Weinreb amidation, O-silylation, DIBAL reduction, and Ohira-Bestmann alkynylation. For the synthesis of aldehyde **127** we followed the excellent six step protocol of Mulzer and co-workers[57] which worked very well in our hands (Scheme 19). Aldehyde **127** was thereafter condensed with the lithium acetylide derived from **128** at low temperature to obtain a 1 : 1 mixture of alcohol epimers **136** (Scheme 20).[60] Given the disappointing selectivity observed in this addition, we immediately oxidised **136** and attempted the asymmetric reduction of **137** with the Noyori-Ru catalyst **138**.[60] This proved to be a very clean reaction, and it did achieve its overall objective of enriching the dr in favour of the *anti*-configured product **126**. However,



Scheme 18 Synthesis of alkyne **128**.[56c]

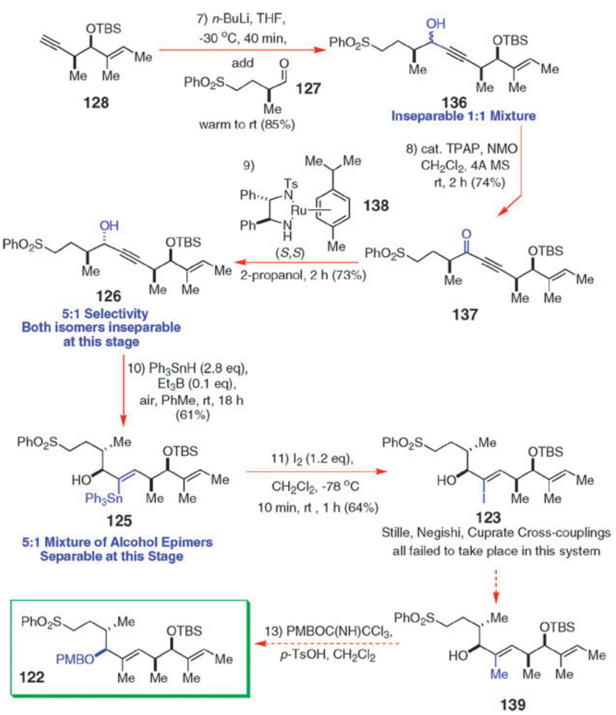


Scheme 19 Our repetition of Mulzer's route[57] to aldehyde **127**.[60]

it did not do this to particularly high standards, it furnishing **126** as the major product of an inseparable 5 : 1 mixture of epimers at this stage.

To our great delight, the O-directed free radical hydrostannation of **126** worked with virtually complete regio- and stereo-control.[60] It now proved possible to separate the resulting 5 : 1 mixture by SiO_2 flash chromatography and, with diastereomerically pure **125** in hand we pressed forward towards vinyl iodide **123** by subjecting it to iodine-tin exchange. Again, this worked very nicely indeed, but sadly, all manner of subsequent transition metal-catalysed cross coupling reactions on **123** failed to deliver the desired alkene **139**.[60] Reluctantly, we again decided to abandon this approach having been vanquished once again.

We next decided to forge the C(35)–C(36)-trisubstituted alkene of phenylsulfone **122** *via* a Kishi-Nozaki-Hiyama[61] Ni/Cr coupling sequence (Scheme 21) which involved us in converting the vinyl iodide **141** into a vinylchromium species which rather disappointingly underwent a poorly stereo-controlled addition to **127** to give a mixture of allylic alcohols, in which the desired product predominated only slightly



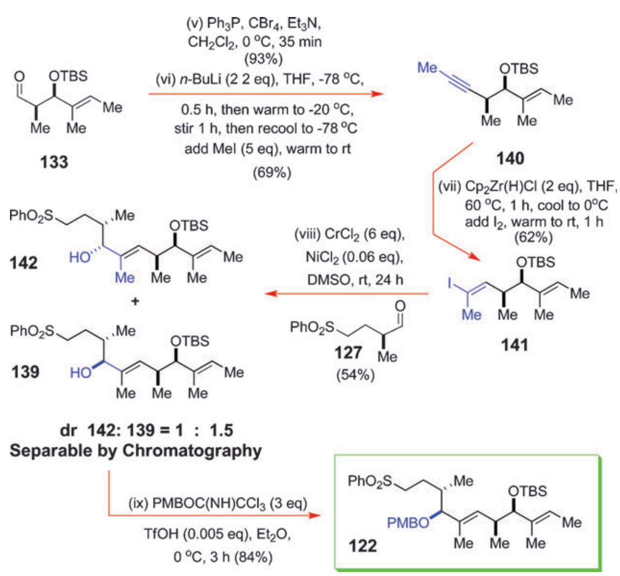
Scheme 20 Attempted synthesis of phenylsulfone **122** *via* O-directed free radical hydrostannation.[60]

(dr **142** : **139** = 1 : 1.5). Fortunately, the two alcohol epimers could be readily separated by SiO_2 flash chromatography, which allowed **139** to be readily converted into the desired phenylsulfone **122** in 84% yield. Whilst this approach did set the C(35)–C(36)-alkene with complete stereocontrol, it did so at a price, that being a significant loss of stereocontrol in the setting of the C(34)-hydroxyl. Although this new route[60] to **122** did, in essence, cut seven steps off our first-generation pathway, its low stereocontrol, high cost, and poor scaleability[60] mandated that we continue to seek an alternative solution to the problem at hand.

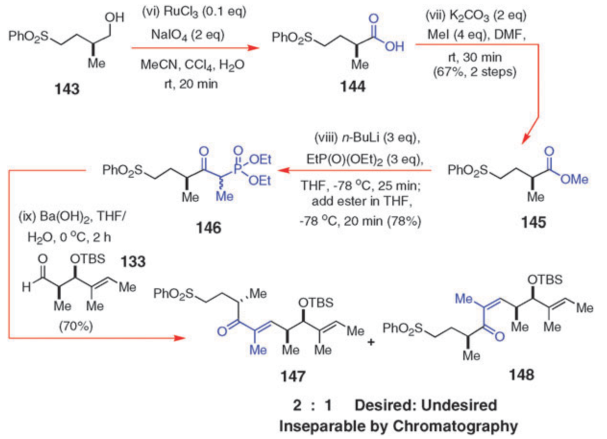
Because of the need to move forward in our analogue programme with Novartis, we had to quickly find an improved and considerably shortened pathway to the phenylsulfone **122** that could rapidly deliver the tens of gram quantities of this material that we needed to make progress in these efforts. After examining several unsuccessful approaches, including the poorly selective Wadsworth–Horner–Emmons process[62] shown in Scheme 22 (which ultimately was abandoned),[60] we eventually devised the route presented in Scheme 23, which actually turned out to be quite effective.

It implemented a stereocontrolled Wittig olefination on aldehyde **133**, a DIBAL reduction and an MnO_2 oxidation on the primary allylic alcohol to secure enal **150**. A Roush crotylboration[63] on **150** then ensued to install the C(33) and C(34) stereocentres of **152** with a 2.3 : 1 level of stereoselectivity. Fortunately, the two diastereoisomers that were formed were readily separable by SiO_2 flash chromatography and, following purification, **152** was isolated in 65% yield. Interestingly, our application of Brown's asymmetric crotylboration protocol[64] to aldehyde **150** using (*E*)-crotyldiisopinocampheylborane derived from (−)-Ipc_2BOMe, actually gave rise to inferior selectivity, which is somewhat surprising given that the reaction of unsaturated aldehydes with such chiral boronates is generally known to deliver homoallylic alcohols with good stereocontrol.[64]

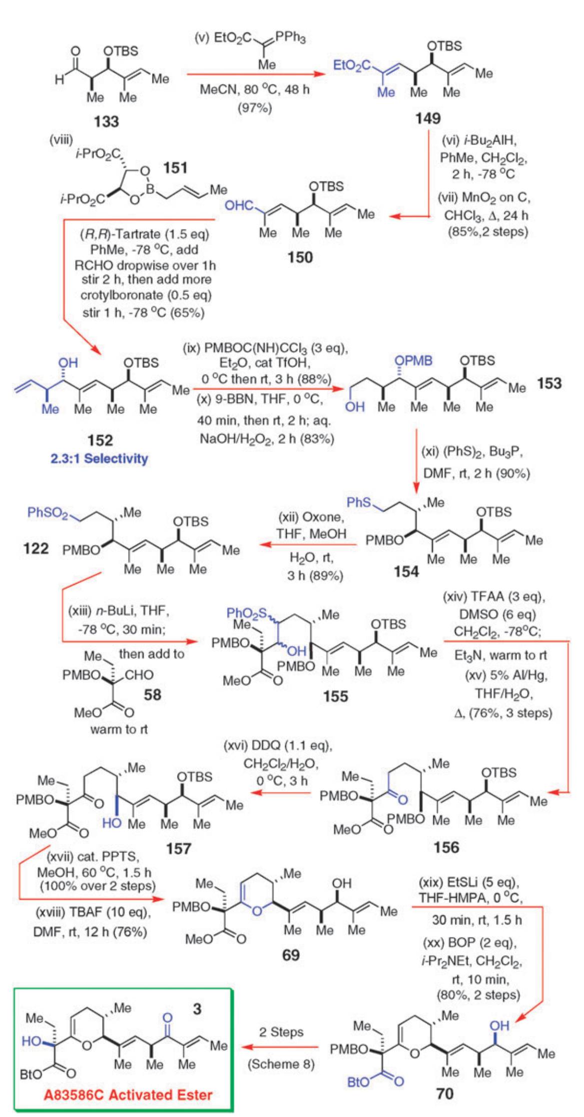
Alcohol **152** was now O-benzylated with *p*-methoxybenzyl trichloroacetimidate and the product regioselectively



Scheme 21 Our first abridged pathway to phenylsulfone **122**.[60]



Scheme 22 Our unsuccessful WHE route to phenylsulfone **122**.[60]



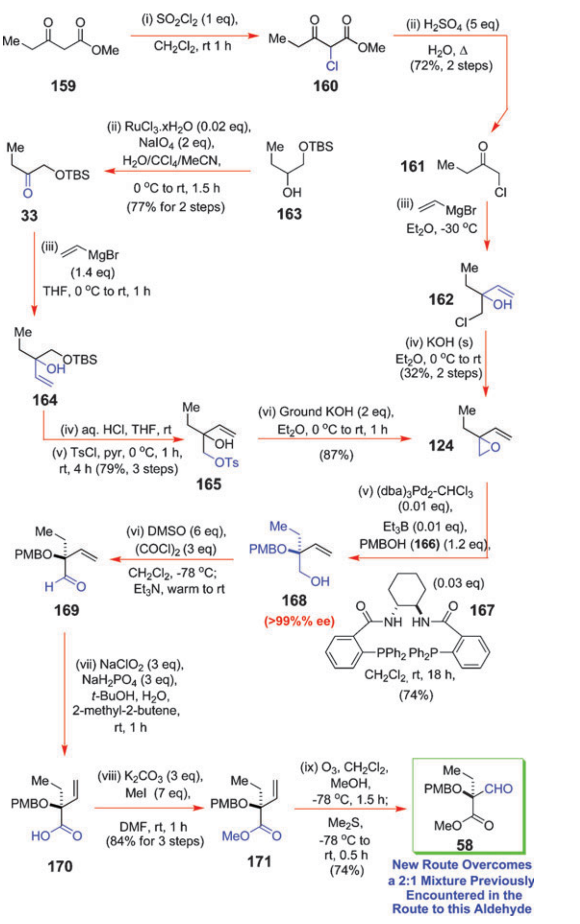
Scheme 23 Our new second-generation synthesis of the A83586C/ kettapeptin activated ester **3**.[55]

hydroborated with 9-BBN to obtain alcohol **153**, following basic H_2O_2 work-up. Thiophenylation and sulfide oxidation with oxone then provided the desired phenylsulfone **122** by a pathway which now shaved ten steps off the original route to the analogous sulfone **11**, and which now only required twelve steps as opposed to the previous twenty-two! Although the new pathway to **122** did significantly shorten our overall synthesis, it did nevertheless deliver a 2.3 : 1 mixture at one point in the synthesis and, in this respect, it was marginally worse than the original route to phenylsulfone **11**! However, on the plus side, the new pathway now allowed us to make 50 gram batches of **122** in half of the time that was originally required to access a similar quantity of **11** which, from an operational perspective, was a massive improvement on what we previously had in place.

One of the special highlights of our new second-generation route to **3** was the considerably improved synthesis that we devised for the β-aldehydo-ester **58** (Scheme 24).[55] The key step in this new sequence to **58** was the stereocontrolled Trost asymmetric epoxide ring-opening[59] of racemic epoxide **124** with *p*-methoxybenzyl alcohol **166** conducted in the presence of the chiral phosphine ligand **167**, Pd(0), and catalytic Et_3B, which delivered the desired product **168** as essentially a single enantiomer in 74% yield. The superb performance of the Trost ligand in this reaction is highly noteworthy for it completely overcame the 2 : 1 regioisomer mixture problem that plagued our original route to **58**; it also improved our product ee to > 99%. With a good pathway to alcohol **168** now secure, we duly advanced towards β-aldehydo ester **58** (Scheme 24).

Aldehyde **58** condensed readily with the α-phenylsulfonyl anion derived from **122** to give a β-hydroxysulfone mixture **155** (Scheme 23) which underwent facile Swern oxidation and Al/Hg reduction to provide β-ketoester **157** in 76% overall yield for the three steps. Given that Paterson had previously reported that allylic OTBS ethers can be readily cleaved and oxidised to enones by the action of DDQ in CH_2Cl_2–H_2O,[65] we were somewhat apprehensive that our selective deprotection of the PMB group from O(34) might simultaneously dislodge the allylic TBS group from O(38), to give a newly liberated hydroxyl/ketone that might now complicate the following step. In the event, these concerns turned out to be unfounded, for the DDQ-promoted cleavage proceeded uneventfully, as did the subsequent glycal-forming step. Now, it was just a simple matter of cleaving the allylic OTBS group with n-Bu_4NF in DMF to obtain our previously synthesised alcohol **69** in 76% yield. An intersection[55] with the original A83586C synthesis[24,25,44] had thus been achieved, and the remaining steps towards the A83586C/kettapeptin activated ester **3** were carried out as had been done previously.[25]

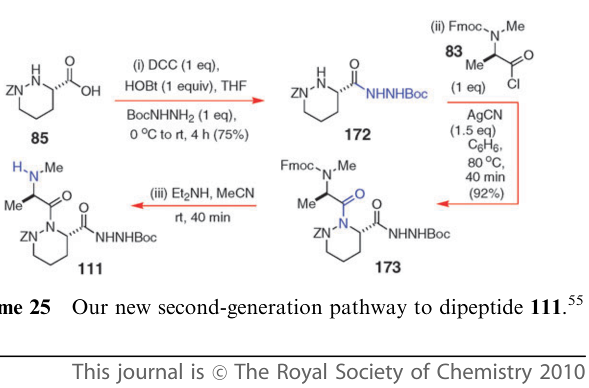
Whilst we were endeavouring to reduce the overall length of our synthesis of **3**, we became engaged in developing a new improved pathway to the cyclodepsipeptide region of these molecules,[55] and we will illustrate our new approach to the peptide sectors of this family in the context of our (+)-kettapeptin synthesis.[55] This effort began with the attempted conversion of (3*S*)-*Z*-piperazic acid **85** into **172** (Scheme 25) without the use of a protecting group on the N(2)-atom of **85**. Such a coupling had never previously attempted in this synthetic arena and there were clearly concerns about the enantiomeric purity of the resulting product **172**. All of these fears were soon allayed however when **172** was produced in optically pure condition in 75% yield from the DCC[38]/HOBt mediated hydrazidation of



Scheme 24 Our new second-generation pathway to aldehyde **58**.[55]

85 with *tert*-buylcarbazate. Moreover, **172** underwent a very smooth AgCN-promoted coupling[9a,33] with the acid chloride **83** without perturbation of the *t*-Boc acyl hydrazide unit, it producing the dipeptide **173** in a superb 92% yield. Removal of the Fmoc group was accomplished as it had been done previously to afford **111** in good yield.

Our need to access the dipeptide acid coupling partner **120** for **111** mandated a synthesis of the protected hydroxamic acid derivative **182** (Scheme 26). For this we set off from Jager's 2-*O*-benzyl-D-threitol **174** which was prepared in two steps from diethyl D-tartrate.[66] The 1,2-diol unit of **174** was regioselectively protected as an O-isopropylidene acetal by treatment with 2,2-dimethoxypropane and catalytic *p*-TsOH to allow introduction of the methoxy grouping onto the remaining hydroxyl by NaH mediated O-alkylation with MeI in DMF.

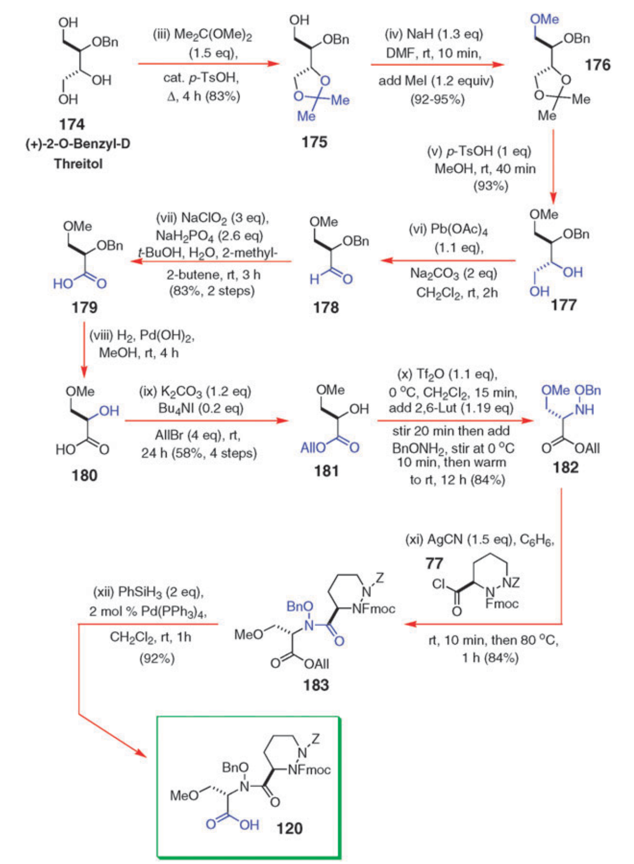


Scheme 25 Our new second-generation pathway to dipeptide **111**.[55]

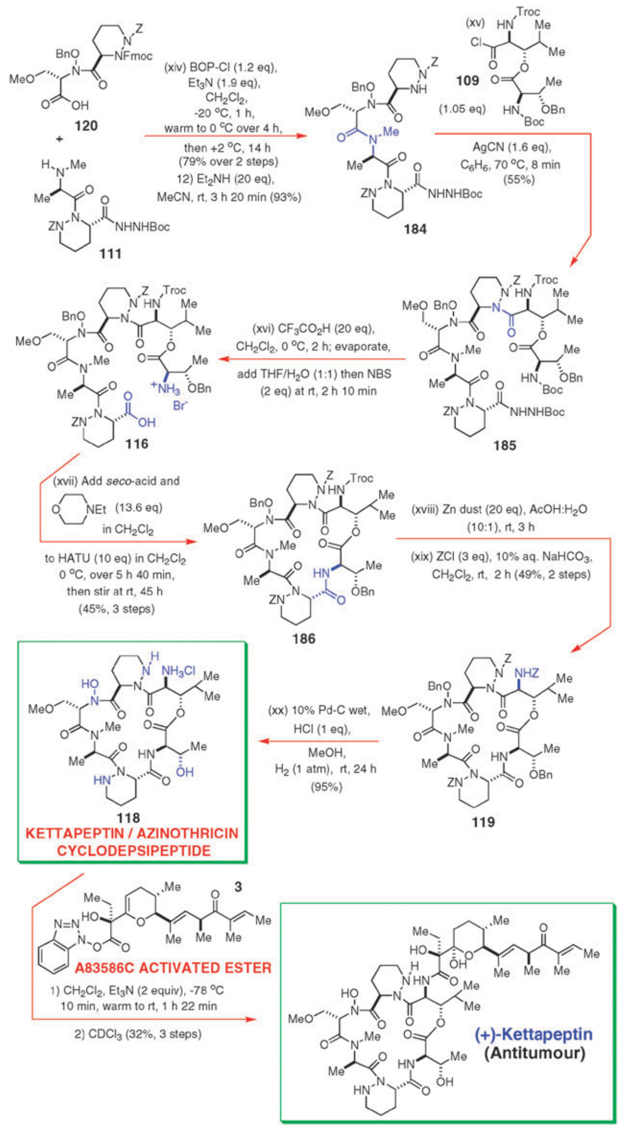
Having served its purpose in installing this feature, the O-isopropylidene group was detached from **176** and the liberated 1,2-diol **177** oxidatively cleaved to the acid by successive lead tetraacetate glycol fission and Pinnick oxidation[67] which gave the acid **179**. The latter thereafter had its O-benzyl ether group detached by catalytic hydrogenolysis and, following this, potassium carbonate promoted esterification of **180** with allyl iodide furnished ester **181** ready for O-triflation and S_N2 displacement with O-benzylhydroxylamine.[36] The desired substitution proceeded in 84% yield to provide **182** in optically pure condition. The northern kettapeptin/azinothricin dipeptide **120** was elaborated by a high-temperature AgCN-mediated coupling between **182** and acid chloride **77** in benzene which again occurred in excellent yield (84%). The final step needed to access acid **120** was the Pd(0)-mediated O-deallylation of **183** with phenylsilane in CH_2Cl_2,[68] which worked well on a problematical substrate where many other O-deallylation protocols had failed.

We could now investigate the critical [2 + 2]-fragment union between **111** and **120** (Scheme 27) which proceeded in a truly remarkable 79% yield when mediated by BOPCl[40] and Et_3N at low temperature over 19 h with rigorous exclusion of moisture. Diethylamine then excised the Fmoc group from this product to give the tetrapeptide **184** which was subjected to a AgCN-promoted N-acylation[9a,33] with acid chloride **109** to obtain **185**. As we had anticipated, this proved quite a tricky reaction to work out. However, reaction conditions were eventually devised that could reproducibly deliver **185** in 55% yield. Although this direct coupling protocol worked less efficiently than our original A83586C [4 + 2]-coupling,[44] it did carry with it the significant advantage that it cut a further two steps from the overall forward synthesis.

At this juncture, the two *N*-Boc-groups were removed from **185** and the newly exposed acyl hydrazide unit of the (3*S*)-Piz residue was oxidatively hydrolysed to the carboxylic acid with NBS in aqueous THF[46,47] without any apparent loss of stereochemical integrity within this residue, and without damage to the D-threonine amine. The crude *seco*-amino acid salt **116** was then macrolactamised with excess HATU[50] and *N*-ethylmorpholine in CH_2Cl_2 at high dilution. The product cyclodepsipeptide **186**, which had been obtained in 45% overall yield from **185**, now had its Troc-group reductively removed with Zn dust in aqueous acetic acid, and the crude amine salt was temporarily N-acylated with benzyl chloroformate to allow the cyclodepsipeptide **119** to be completely freed of all zinc residues and purified to very high standards by SiO_2 flash chromatography. The purified cyclodepsipeptide

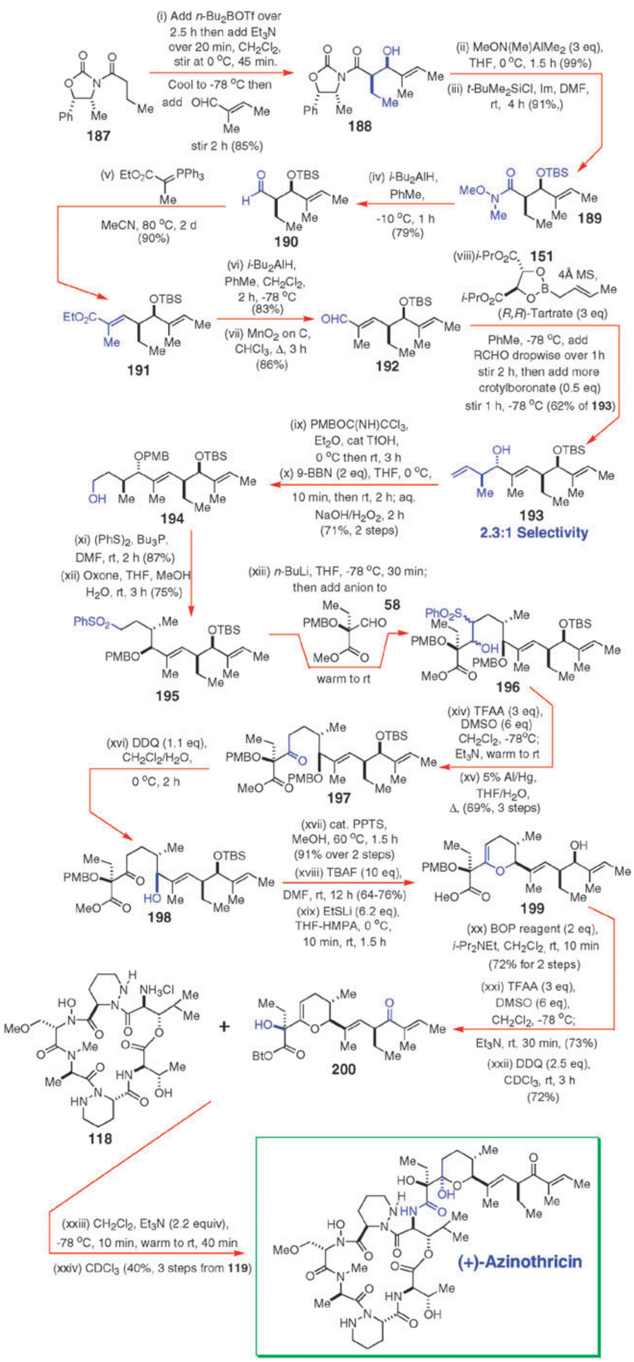


Scheme 26 Our route to the kettapeptin/azinothricin dipeptide **120**.[55]



Scheme 27 Completion of the total synthesis of (+)-kettapeptin.[55]

119 was then catalytically hydrogenolysed at atmospheric pressure, over a 10% Pd/C catalyst, in anhydrous methanolic HCl. The crude hydrochloride salt **118** was then coupled with the A83586C activated ester **3** in CH_2Cl_2 to give, after hydration of the coupled glycal in $CDCl_3$, a 32% overall yield of (+)-kettapeptin for the three steps from **119**, completing the first ever total synthesis[51] of this recently discovered anticancer natural product,[8] and simultaneously providing a new abridged second-generation total synthesis of (+)-A83586C with the new routes to **3** and **111** that it had developed.[51]



Scheme 28 Completion of the total synthesis of (+)-azinothricin.[55]

Given our success in this venture, and our possession of the azinothricin cyclodepsipeptide **118**, we now wished to forge ahead with a synthesis of the long elusive (+)-azinothricin.

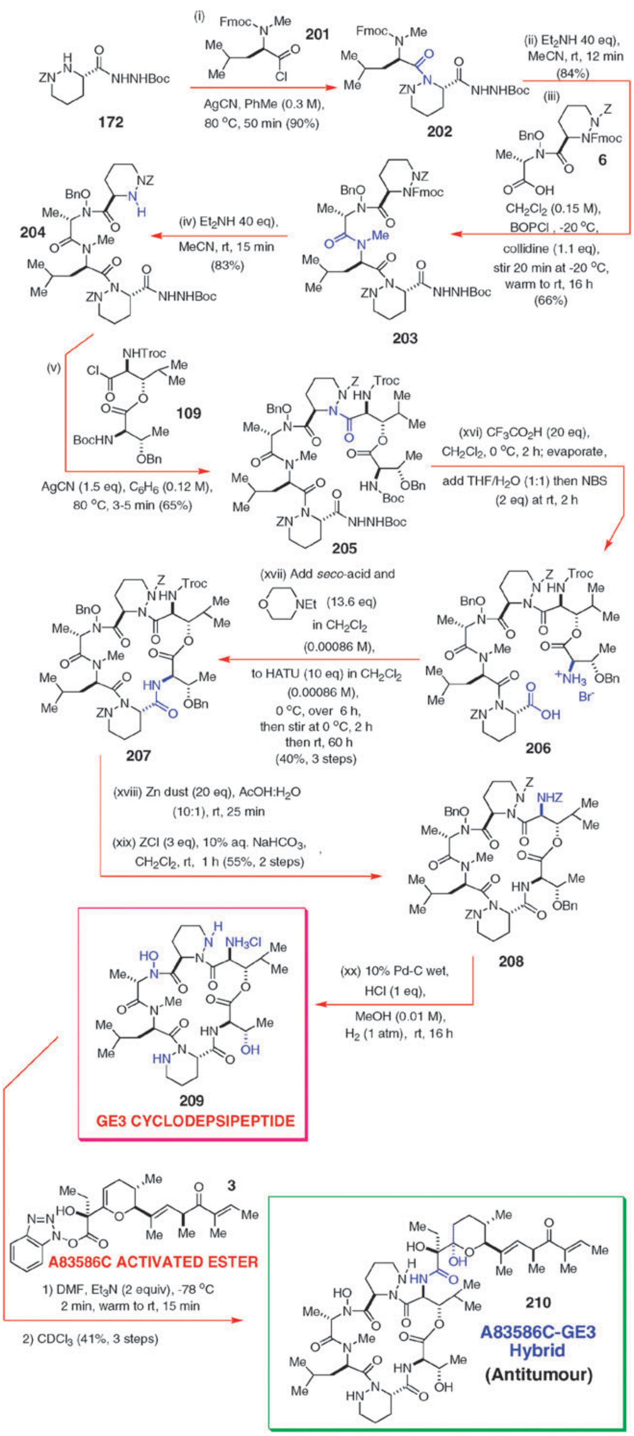
We therefore adapted our synthetic strategy for **3** to the synthesis of the azinothricin activated ester **200** (Scheme 28).[55] The results essentially mirrored those of the previous synthesis of **3** including for the stereoselectivity of the Roush asymmetric crotylboration[63] of enal **192** (2.3 : 1 selectivity in favour of **193** and 62% yield). The final coupling of **200** with **118** also proceeded in good yield. In fact, this particular union was one of the very best that we have so far encountered, it delivering (+)-azinothricin in 40% overall yield from the protected cyclodepsipeptide **119**,[55] further vindicating the high-risk planning that we had implemented at the very outset of our programme.

Some highlights of our A83586C analogue work and evidence that molecules of the A83586C class can potently disrupt/blockade transcriptional activation from the β-catenin/TCF4 promoter to thereby inhibit expression of metastasis-inducing osteopontin (Opn)

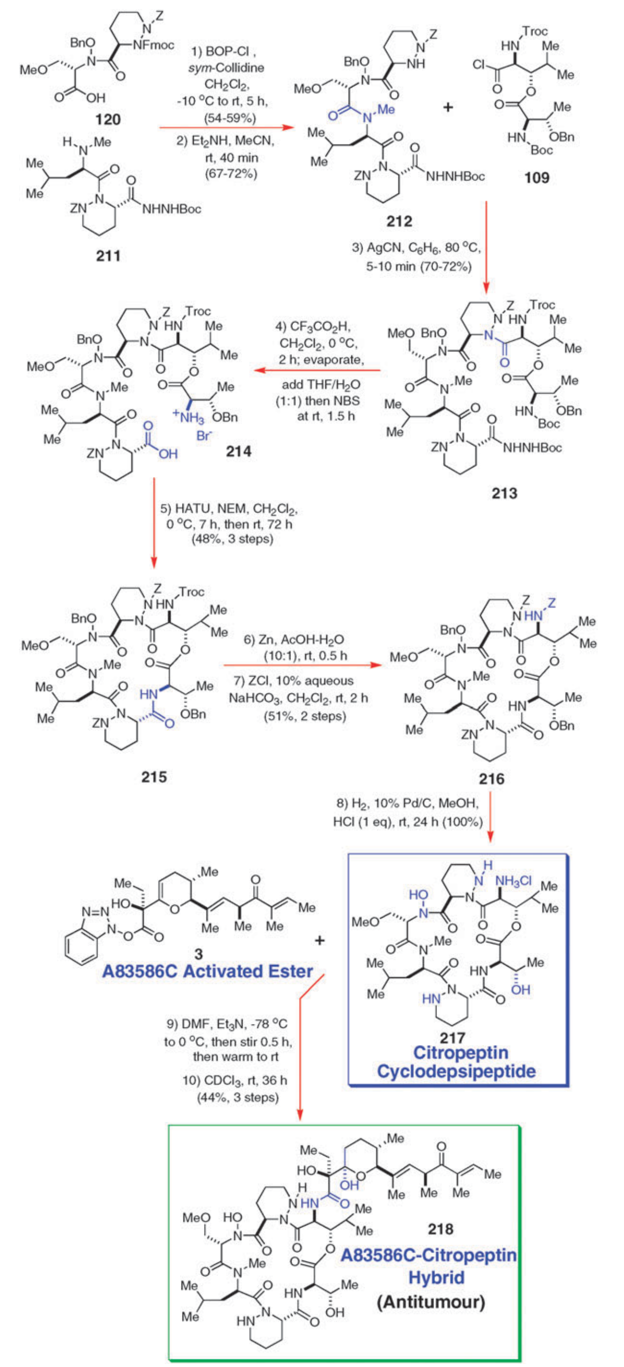
With our two synthetic strategies in place for building up this family, we duly adapted our routes to allow the construction of a wide range of synthetic analogues that have included the A83586C-GE3 hybrid (Scheme 29),[51,69,70] the A83586C-citropeptin hybrid (Scheme 30),[51] L-Pro-A83586C (Fig. 2),[51] and 4-*epi*-A83586C[72] (Fig. 2) to name but a few.

Of the various synthetic analogues that we have so far screened (Fig. 2), the A83586C-citropeptin hybrid **218** appears to have the most potent tumour growth inhibitory effects that we have so far seen, it being roughly of the same order of potency as (+)-A83586C itself against various different human colon tumour cell lines. The A83586C-GE3 hybrid showed potent but less pronounced growth inhibitory effects, while L-Pro-A83586C[51,71] was a much less potent tumour growth inhibitor. Significantly, all four of these molecules were powerful inhibitors of β-catenin/TCF4-mediated transactivation from the TCF4 promoter in HCT116 human colon cells after 24 h of exposure (IC_{50}s = 3–5 nM), but L-Pro-A83586C[51,71] was approximately 100-fold less active as an inhibitor of transcription.[51]

In the case of the A83586C-citropeptin and A83586C-GE3 hybrid molecules **218** and **210**, our colleague at Queen's University Belfast, Dr Mohamed El-Tanani, very kindly validated the β-catenin/TCF4 inhibition results for us by examining the simultaneous expression of β-catenin and a relevant known β-catenin/TCF4 downstream target gene, osteopontin (Opn), in Rama-37-Opn cells. The latter is a highly metastatic rat mammary epithelial cell line that has been genetically engineered to specifically overexpress Opn. Opn is a tumour- and metastasis-promoting protein that is generally significantly overexpressed in the most aggressive and highly metastatic of human tumours; its overexpression is normally correlated with patient demise and mortality.[73] Significantly, the El-Tanani group demonstrated,[51] by quantitative real-time PCR, that while **218** and **210** can markedly downregulate over-expressed Opn at 10 nM drug concentration,



Scheme 29 Synthesis of the GE3 cyclodepsipeptide and its subsequent use in the synthesis of the A83586C-GE3 hybrid **210**.[51,69]



Scheme 30 Synthesis of the citropeptin cyclodepsipeptide **217** and its subsequent use in the synthesis of the A83586C-citropeptin hybrid **218**.[51]

β-catenin levels are always maintained, confirming that blockade of this protein-protein interaction with target DNA must be occurring. We have thus jointly demonstrated that small molecule disruption of the β-catenin-TCF4 protein-protein transcription-activating interaction is a viable drug intervention strategy for downregulating Opn in metastatic tumours.[51]

By way of contrast, 4-*epi*-A83586C[72] only had very weak antitumour properties against different human tumour cell lines; it having an IC_{50} = 46 μM *vs.* the WI-38 VA13 (SV40 virus transformed) human lung fibroblast cell line and an IC_{50} = 28 μM *vs.* HCT-116 and HT-29 human colon carcinoma cell lines. TOP-FLASH/FOP-FLASH TCF4-luciferase assaying[54] of 4-*epi*-A83586C likewise demonstrated that high micromolar concentrations were required for this molecule to inhibit β-catenin/TCF4 protein-protein-mediated transactivation. The latter result reinforces the idea that significant changes to the southern hemisphere of A83586C-type cyclodepsipeptides are not beneficial to antitumour potency nor their β-catenin/TCF4/Opn inhibiting ability.

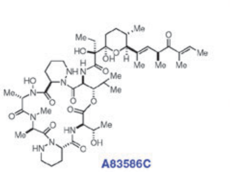
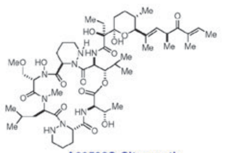
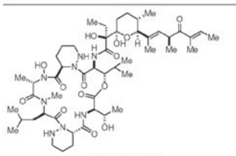
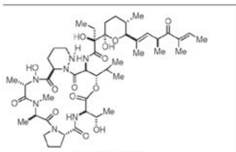
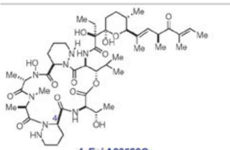
	A549 (Lung)	MDA-MB-435 (Breast)	RKO (Colon)	HCT116 (Colon)	HT29 (Colon)	TOP/FOP β-Cat/TCF4 Luciferase HCT116 (Colon)
 A83586C	60 nM	90 nM	18 nM	40 nM	46 nM	3 nM
 A83586C-Citropeptin Hybrid			18 ± 4 nM	47 ± 4 nM	50 ± 13 nM	5 nM
 A83586C-GE3 Hybrid			67± 5 nM	139 ± 3 nM	107 ± 9 nM	3 nM
 L-Pro-A83586C				880 ± 7 nM		430 ± 150 nM
 4-*Epi*-A83586C	25 ± 6 μM			28 ± 12 μM	28 ± 12 μM	> 5 μM

Fig. 2 IC_{50} values of A83586C and its analogues at inhibiting the growth of various human tumour cell lines, and β-catenin/TCF4- mediated transcription from the TCF4 promoter in HCT116 colon carcinoma cells.[51]

In this regard, our NMR and molecular modelling studies on 4-*epi*-A83586C have indicated that it contains a *cis*-amide linkage in the southern hemisphere (3*R*)-Piz residue, which is quite different from its (3*S*)-Piz-configured natural product congeners, which each have a *trans*-amide linkage in this region.[72] No doubt as further analogues are prepared, even more detailed SAR data will be built up. For now, however, our group is concentrating its efforts on the synthesis of various biotinylated probes of this class for use in future oncological target isolation work. It seems likely that other biological targets will also be modulated by molecules of the A83586C/kettapeptin/GE3 class and, hopefully, such affinity chromatography work will, in the future, lead to the discovery of new oncologically relevant proteins of similar significance to the cyclins and their associated cyclin-dependent kinases in cancer onset and progression.

Conclusions

In this article, we have given a broad overview of our group's synthetic efforts on molecules of the A83586C, kettapeptin and azinothricin class over the period 1991–2009. Not only have we demonstrated that all three natural products can be readily synthesised through a novel chemoselective coupling

Cell Line	β-Catenin mRNA Expression	OPN mRNA Expression
Rama 37 (Benign)	28.0 ± 1.2	**38.0 ± 4.9 (Base Level)**
Rama 37 (Benign) + **218**	29.0 ± 0.4	**3.6 ± 0.43 (Downregulated)**
Rama 37 (Benign) + **210**	28.1 ± 0.5	**6.2 ± 0.49 (Downregulated)**
Rama 37-OPN (Overexpresses OPN) (Metastatic)	26.4 ± 0.9	**89.2 ± 3.4 (Base Level)**
Rama 37-OPN (Metastatic) + **218**	26.2 ± 0.4	**15.2 ± 1.5 (Downregulated)**
Rama 37-OPN (Metastatic) + **210**	25.9 ± 0.2	**21.7 ± 1.31 (Downregulated)**

Fig. 3 Real-time PCR data for two rat breast cell lines treated with the A83586C-citropeptin and A83586C-GE3 hybrids **218** and **210** at 10 nM concentration in DMSO/DMEM. The differential expression of the OPN gene *vs.* the S18 ribosomal housekeeping gene was calculated for every individual cell line.[51]

strategy that merges unprotected pyran and cyclodepsipeptide fragments at the final stages, we have also proven that our approach can be used to build up all sorts of non-natural A83586C congeners. Moreover, with the molecules that we have fashioned to date, we have provided fascinating new insights into the mechanism of antitumour action of this class. Specifically we have shown that A83586C-type molecules are able to function as powerful nM disrupters of β-catenin/TCF4 protein/protein-mediated Wnt signalling without causing downregulation of β-catenin expression, which is quite distinct behaviour from other highly potent β-catenin/TCF4 inhibitory natural products such as (−)-agelastatin A.[54c] In so doing, we have also demonstrated that they switch off the expression of β-catenin/TCF4-regulated metastasis- and tumour-promoting genes such as Opn. We have also provided new insights into how members of this family function as potent E2F/DP transcription factor inhibitors. Specifically, we have produced evidence which suggests that they do this by an indirect mechanism wherein downregulation of E2F1 protein expression occurs alongside induction of pRb-dephosphorylation. No doubt A83586C/ kettapeptin/azinothricin-type molecules will emerge as important new chemobiological tools over the coming years now that these significant biological discoveries have been made (Fig. 3).

Acknowledgements

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