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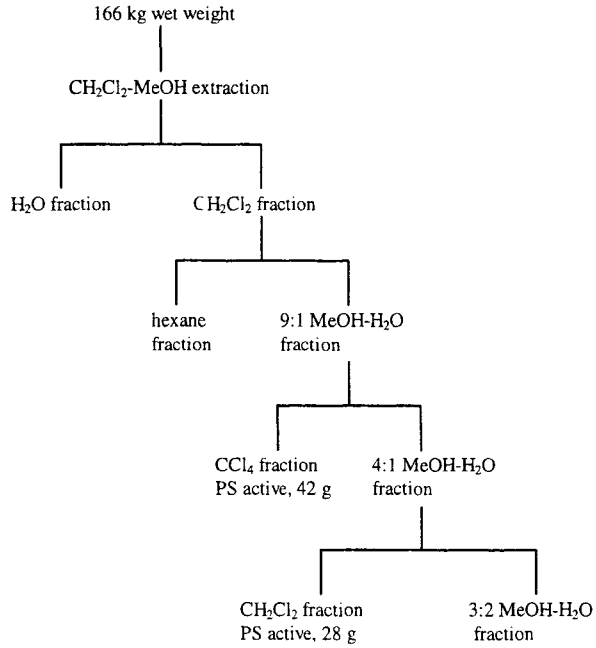
When Two Steroids are Better than One : The Dimeric Steroid-Pyrazine Marine Alkaloids

A. Ganesan

INTRODUCTION

This chapter reviews the history of a remarkable family of natural products first isolated from the marine tube-inhabiting invertebrate worm *Cephalodiscus gilchristi*. *C. gilchristi*, found in the temperate Southern Hemisphere, is often attached to bryozoans and sponges. This tiny worm (~ 5 mm in tube colonies) can exist independently of the coenicium (worm tube), and exposure to predators during such moments may have aided the evolution of chemical defence mechanisms.

In 1972, Pettit's group collected a sample of *C. gilchristi* at a depth of approximately 20 m in the Indian Ocean off southeast Africa. In 1974, methanol and aqueous extracts of *C. gilchristi* were confirmed active in the American National Cancer Institute's primary antitumour assay at that time, the murine lymphocytic leukemia P388 (PS system), with 32-41 % life extension at 25-37.5 mg/kg. Identification of the active components was a slow process hampered by the limited material, and required over ten years effort with recollections of fresh sample. Success was achieved with a 1981 batch of 166 kg (wet weight, including worm tubes) of *C. gilchristi*, from which PS bioassay-guided solvent partitioning[1] (Scheme 1) yielded active dichloromethane and carbon tetrachloride fractions.



Scheme 1. Solvent partitioning scheme for the extraction of cephalostatins.

The active fractions were further purified by extensive column chromatographic and HPLC separations (for details of the protocol, see next section) to obtain 138.8 mg ($8x10^{-4}$ % yield) of a pure compound, cephalostatin 1, mp 326 °C dec. Evidence from TLC staining, NMR, and mass

spectrometry indicated a steroidal alkaloid but the complete structural elucidation[2] required X-ray analysis of crystals carefully grown from a pyridine solvate.

As with many other compounds of marine origin, the structure of cephalostatin 1 (Figure 1) is unprecedented and has no analogy with terrestrial natural products. The compound is an unsymmetrical steroid dimer linked at the C-2,3 positions (for ease of reference, steroid numbering is used throughout; dimers are numbered from C-1 for the "right" half and C-1' for the "left" half) by an aromatic pyrazine ring. Oxygenation of the side chain results in a spiroketal ring system as in many saponin aglycones. Two other noteworthy features are the presence of a C-14,15 alkene in both halves, and the oxidation of the C-18' angular methyl to a hydroxymethyl group.

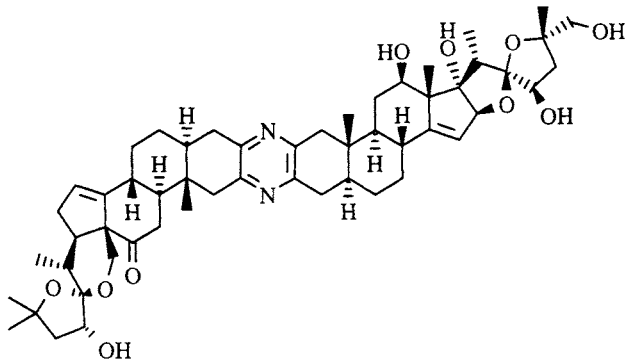


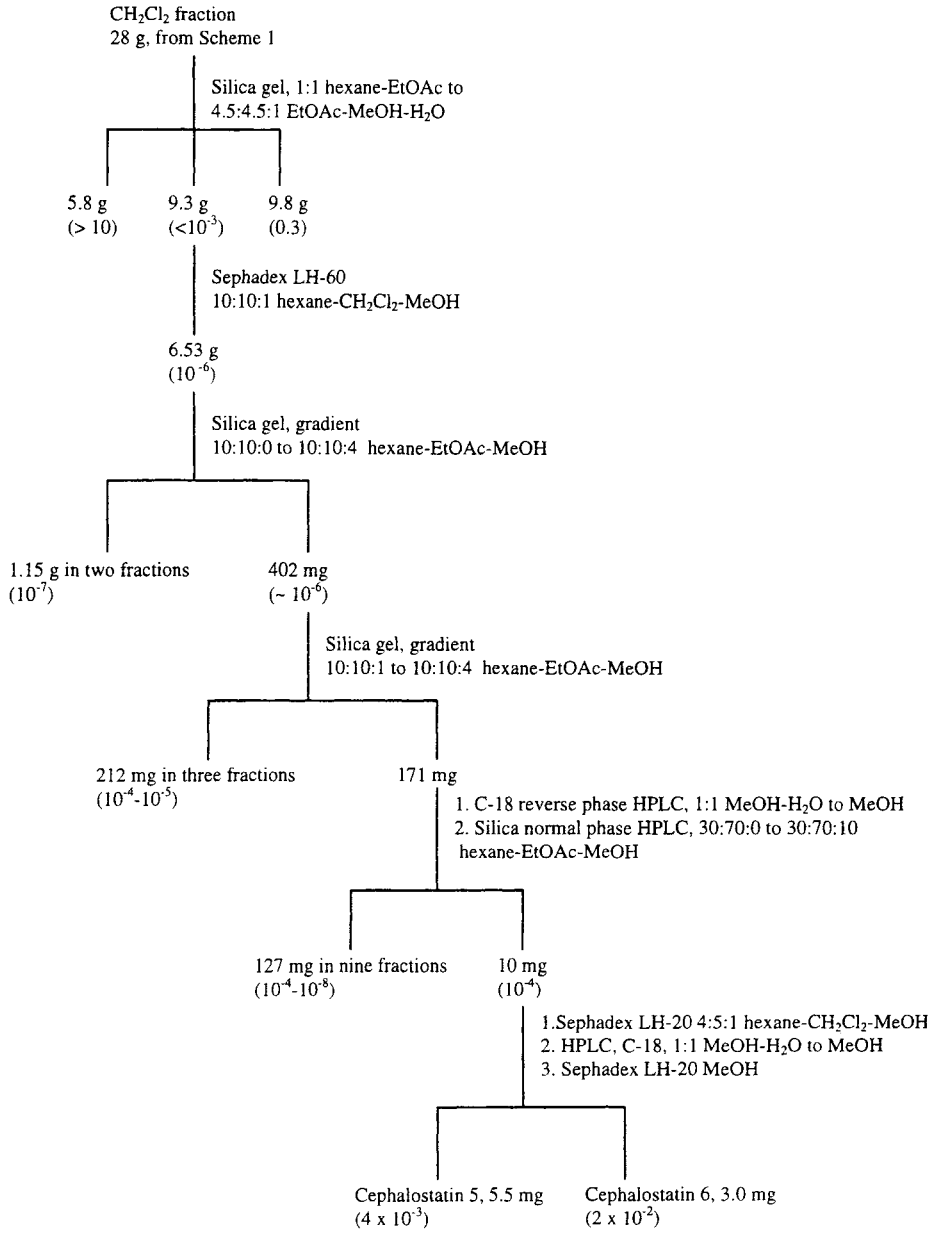
Figure 1. Cephalostatin 1.

ISOLATION OF OTHER CEPHALOSTATINS

Cephalostatin 1 has an ED_{50} of 10^{-7}-10^{-9} μg/ml against the PS cell line and is in fact among the most potent compounds ever recorded in the NCI's antitumour screening program. Over the next few years, the Pettit group solved the structures of several other cephalostatins purified from the 1981 batch of *C. gilchristi*. Compared to the parent compound cephalostatin 1, cephalostatins 2-4[3] (Figure 2) are hydroxylated at the C-9' ring juncture of the left half. Furthermore, cephalostatin 3 contains an additional methyl group in the side chain, while in cephalostatin 4 the C-14'-15' alkene is epoxidized. These three cephalostatins have similar PS activity to cephalostatin 1.

The isolation[4] of cephalostatins 5 and 6 (Scheme 2) is typical of the careful and lengthy separations performed for these compounds by the Pettit group. In these two cephalostatins, ring C of the left half is aromatized. The PS activity of cephalostatins 5 and 6 is dramatically reduced to an ED_{50} of 10^{-2} μg/ml, possibly due to the flattening out of the steroid and the loss of C-D ring stereochemistry.

Cephalostatin 6 can be derived from cephalostatin 4 by a plausible sequence of events. First, dehydration at C-9' would generate an enone (a similar enone was later isolated from *C. gilchristi*, see cephalostatin 14, *vide infra*). The epoxide ring can then undergo nucleophilic ring opening at C-14' by participation of the enone to yield a dienone. Finally, a retro-aldol reaction at C-18' would complete the aromatization. Alternatively, the angular hydroxymethyl group may be lost via oxidative fragmentation similar to that observed in the biosynthesis of cholesterol from lanosterol. Cephalostatin 5 is presumably related to the epoxy derivative of cephalostatin 3 by the same reaction sequence.



Scheme 2. Detailed chromatographic separation scheme for cephalostatins 5 and 6. Numbers in brackets refer to PS bioassay activity, in μg/ml.

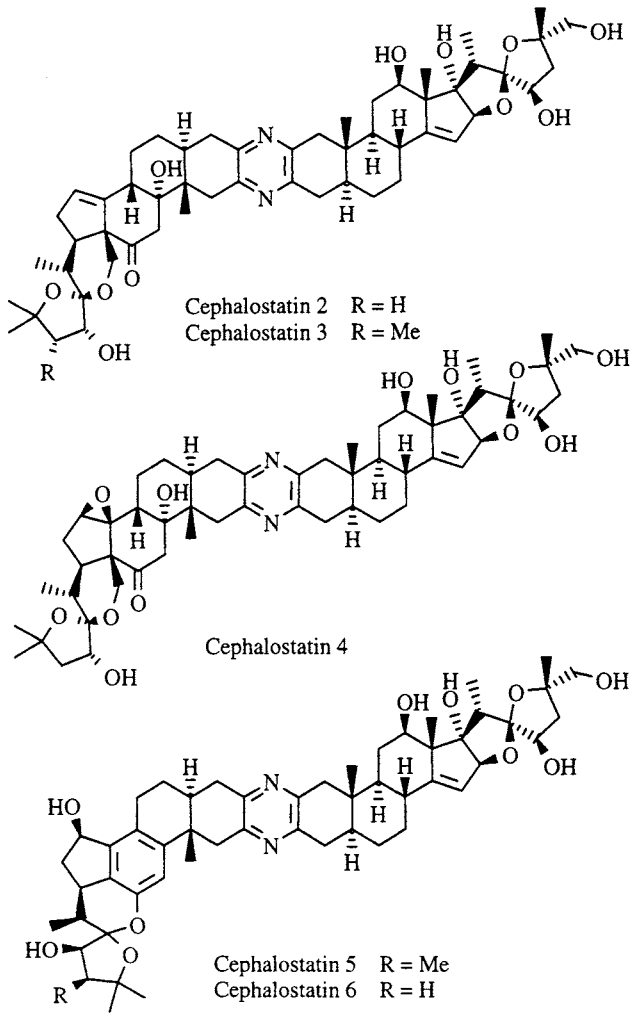


Figure 2. Cephalostatins 2-6.

Cephalostatins 7-9[5] (Figure 3) exhibit further structural variation in the left half. In cephalostatin 7, the C-18' angular methyl is no longer oxidized, and the side chain now forms a 6/5 spiroketal. This spiroketal can be derived from the right half's 5/5 spiroketal by ring opening followed by recyclization and removal of the C-23 alcohol. Thus, the two halves of cephalostatin 7 are almost identical. Cephalostatins 8 and 9 retain the C-18' hydroxymethyl group. Compared to the parent cephalostatin 1, cephalostatin 8 has one more methyl group and is less oxygenated in the side chain. Meanwhile, cephalostatin 9 is really the hemiketal form of cephalostatin 1.

By now, the NCI had switched from the PS bioassay to a panel of 60 human solid tumour cell lines. Against this panel, cephalostatins 7-9 are of similar potency to cephalostatin 1-4, with TI_{50} values of 10^{-9}-10^{-10} molar against a number of the cell lines. The cephalostatins also display a characteristic panel graph, and exhibit one of the most extreme cases of differential cytotoxicity encountered in the NCI assay. Cephalostatin 1, for example, has GI_{50} values in these cell lines ranging from $6x10^{-8}$ to $2.5x10^{-11}$ molar.

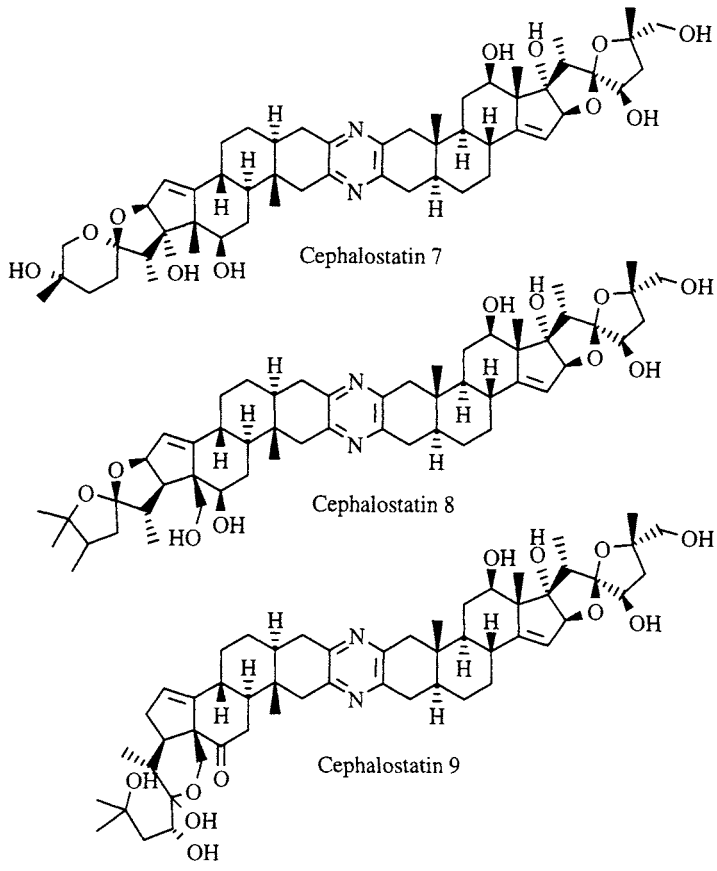


Figure 3. Cephalostatins 7-9.

In the last two years, Pettit's group has reported the structure of eight more cephalostatins isolated from a 450 kg collection of *C. gilchristi* made in 1990. Cephalostatins 10 and 11[6] (Figure 4) are the C-1 and C-1' methoxy derivatives of cephalostatin 2 respectively. Cephalostatin 10 thus represents the first member with a structural change in the right half, which has remained constant in cephalostatins 1 through 9. In their cytotoxic effects, both these cephalostatins are of similar activity to cephalostatin 1.

The disymmetry of the cephalostatin halves can be considered a biosynthetic puzzle - was the enzymatic machinery selectively fusing two non-identical steroids only, or was it differentiating the two halves of a homodimer? The latter possibility sounds more likely, and is supported by the similarity between the two halves of cephalostatin 7 (*vide supra*). The structures[7] of cephalostatins 12 and 13 (Figure 4) provide further corroborating evidence. For the first time, the two halves of cephalostatin 12 are identical and correspond to the right half of earlier cephalostatins. Cephalostatin 13 differs from the symmetrical dimer only by C-1' hydroxylation. Interestingly, these compounds were found in the more polar *n*-butanol fraction during solvent partitioning, while previous cephalostatins were retained in the dichloromethane layer. The symmetrical compounds are also much less active against the NCI panel; while cephalostatin 1 has a mean panel GI_{50} of 1 nmolar, cephalostatin 12 and 13 were 400 nmolar and >1 μmolar respectively. The implications of this are unclear. Possibly, the increased polarity of the left half in these compounds is responsible for the decreased activity.

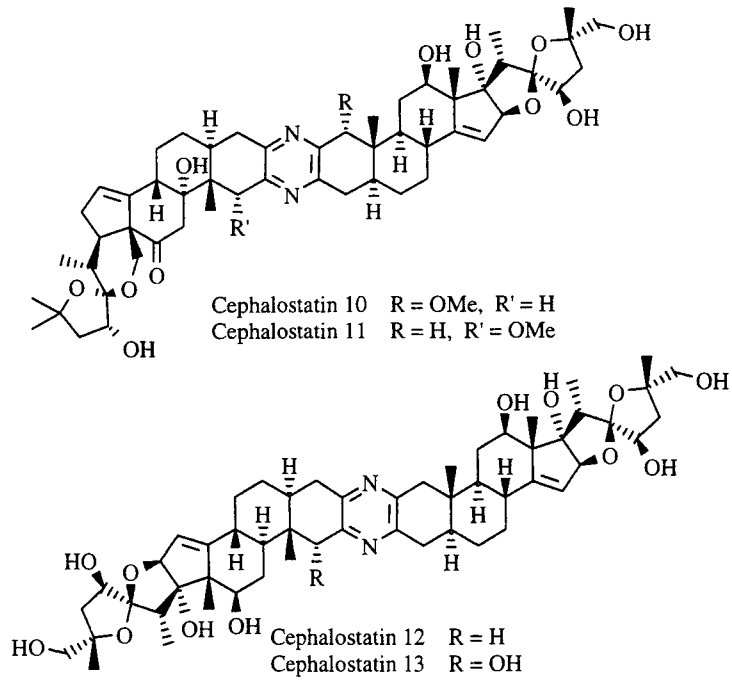


Figure 4. Cephalostatins 10-13.

Cephalostatins 14 and 15[8] (Figure 5) are related to cephalostatins 2 and 3 respectively by α-epoxidation at C-14'-15', dehydration of the C-9' alcohol, and hydroxylation at C-8'. Cephalostatins 14 and 15 display reduced activity, with mean panel GI_{50} of 100 and 68 nmolar respectively, perhaps due to the epoxide orientation - with steroidal bufadienolides, the β-epoxides are more cytotoxic.

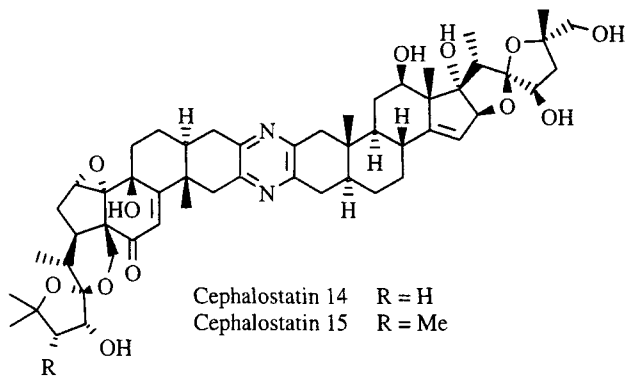
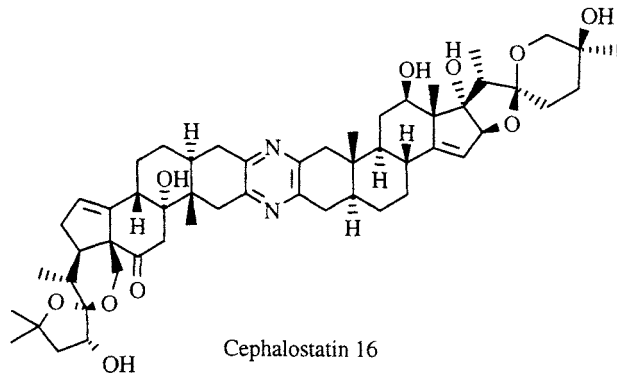


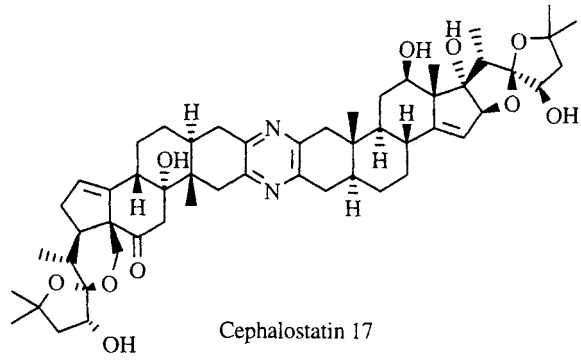
Figure 5. Cephalostatins 14 and 15.

The most recent cephalostatins to be reported are cephalostatin 16 and 17[9] (Figure 6). Cephalostatin 16 is composed of the left half of cephalostatin 2 coupled to the left half of cephalostatin 7. Cephalostatin 17 also contains the left half of cephalostatin 2, while the other half is identical to the typical right half of cephalostatins 1-11 except for one less hydroxyl group. The

mean panel values for cephalostatins 16 and 17, at 1 nmolar and 4 nmolar respectively, are comparable to cephalostatin 1.



Cephalostatin 16



Cephalostatin 17

Figure 6. Cephalostatins 16 and 17.

In general, the cephalostatins isolated recently are in relatively minor abundance compared to cephalostatin 1 (for example, only 3.8 mg, $8x10^{-7}$ % yield, of cephalostatin 17 was obtained), and of lower activity except for cephalostatins 16 and 17. The Pettit group has also detected[10] other new cephalostatins in very small quantities (approximate yield of 10^{-8} %) with promising activity against brain cancer xenografts.

THE RITTERAZINES

It remains to be seen what structural surprises are in store with future compounds isolated from *C. gilchristi*, and also if similar compounds are produced by Antarctic members of the *Cephalodiscus* genus. Recently, exciting developments have been disclosed[11] from an unexpected quarter by Fusetani's group, working with the tunicate *Ritterella tokioka* Kott 1992 collected off the coast of Japan. The lipophilic extract of the tunicate showed promising cytotoxicity. PS bioassay-directed fractionation of 5.5 kg of tunicate yielded 2.9 mg of ritterazine A, with an ED_{50} of 10^{-3} µg/ml in the PS assay. The structure of ritterazine A (Figure 7) was solved based on ^{1}H and ^{13}C NMR data, and it bears an obvious resemblance to the cephalostatins. Prior to the extraction of the tunicate, colonies were first washed free of macroepibionts and sands. No attached hemichordates were observed.

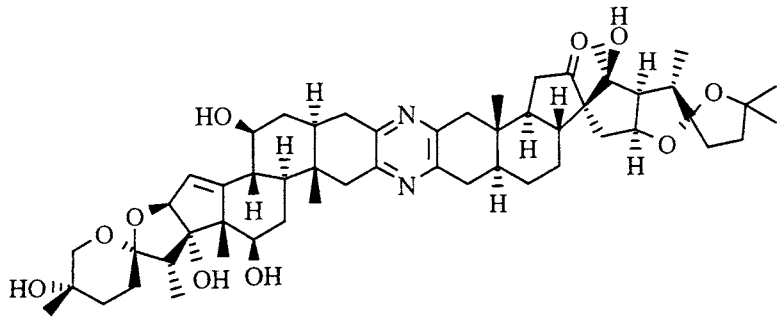
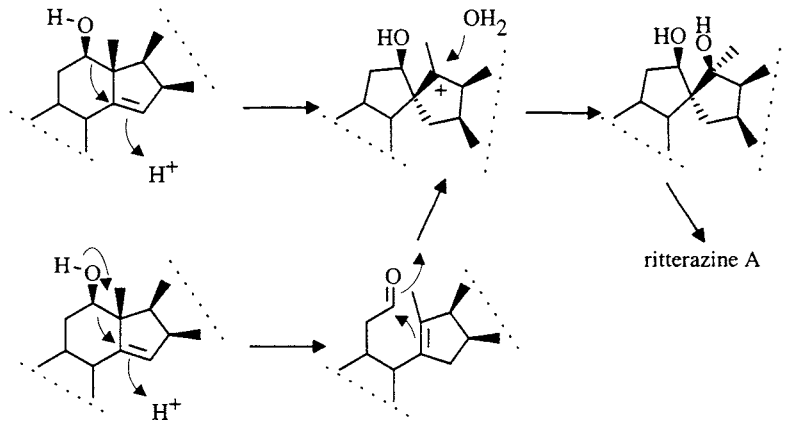


Figure 7. Ritterazine A.

The left half of ritterazine A is identical to that of cephalostatin 7, except for an additional C-7' hydroxyl group. The right half comprises a rearranged steroid nucleus. A reasonable biogenetic pathway for the right half is shown in Scheme 3 (In this and later schemes, hydrogens at steroid trans ring-junctures and portions of the steroid nucleus that are not participating in the reaction are usually omitted). Protonation of the C-14,15 alkene is followed by a 1,2-Wagner-Meerwein shift and trapping of the resulting carbocation by water to give the observed skeleton. The intermediate carbocation can also be derived by an alternative mechanism via protonation at C-15, pinacol-like rearrangement, and Prins reaction between the aldehyde and alkene.



Scheme 3. Possible biogenesis of the ritterazine A right half.

Subsequently, two other ritterazines with an unrearranged steroid nucleus as in the cephalostatins were isolated[12] from the same collection of *R. tokioka* (Figure 8). Ritterazine B has the same left half as ritterazine A. The right half is comparable to that of cephalostatin 1, except for the absence of hydroxylation at C-17, C-23, and C-26. Ritterazine B is also the first of these dimeric steroids where C-14 is not part of an alkene or epoxide. Instead, a β-hydrogen giving rise to cis C-D ring fusion is present. In ritterazine C, one half is identical to the right half of ritterazine B, while the other half is identical to the right half of cephalostatin 1 (this half is chosen as the "right" half in Figure 8 to emphasize this relationship) except for additional C-7 hydroxylation. Ritterazines B and C have an IC_{50} in the PS assay of 1.8×10^{-4} μg/ml and 9.4×10^{-3} μg/ml respectively.

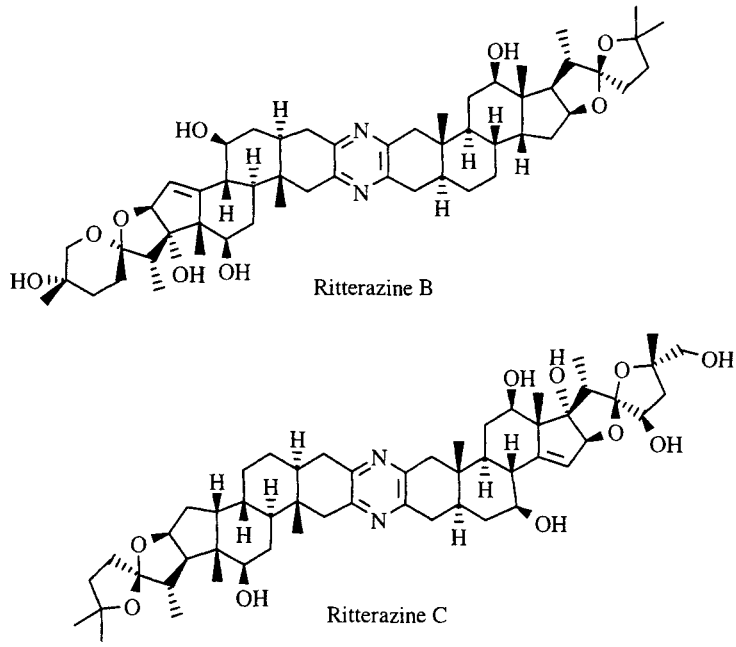


Figure 8. Ritterazines B and C.

Further work with an 8.2 kg collection of the tunicate led to the characterization[13] of ten new ritterazines (Figure 9). Ritterazine D is the C-22 epimer of ritterazine A. Ritterazine E has one additional methyl group compared to ritterazine D. The biological activity of these two compounds is similar to that of ritterazine A.

In ritterazines F and G, C-22 is again epimeric to the configuration observed in ritterazine B; ritterazine G has the additional modification of C-14,15 unsaturation. In the PS bioassay, ritterazines F and G have IC_{50} values of $7.3x10^{-4}$ μg/ml - the highest activity seen among the ritterazines apart from ritterazine B (note that this is still much lower than the activity of cephalostatin 1).

Ritterazines H and I form another pair of C-22 epimers, with C-12 now at the ketone oxidation state and biological activity reduced by approximately twenty-fold. The presence of both 5/5 spiroketal diastereomers for a number of ritterazines implies that epimerization at C-22 is not energetically prohibitive. Interestingly, such spiroketal epimers have yet to be observed among the cephalostatins.

Ritterazines J-M are most closely related to the left half of cephalostatin 7. Ritterazine K is the symmetrical dimer formed from this half, while ritterazine J has one additional hydroxyl group. Ritterazines L and M are a pair of C-22 spiroketal epimers in which the C-17 hydroxyl group is lost. These ritterazines all have IC_{50} values around 10^{-2} μg/ml in the PS assay.

The biological profile of the ritterazines, with ritterazines B, F, and G the most potent, seem to indicate the importance of the 5/5 and 6/5 spiroketals, while the C14,15 alkene is not crucial. Oxidation at C-12 to a ketone results in decreased activity, as does the rearrangement of the steroid nucleus as in ritterazines A, D, and E. However, further data on related compounds will be needed before these hypotheses can be confirmed. Meanwhile, the difference in nomenclature between the cephalostatin and ritterazine families is rather confusing. Regardless of their origin, these compounds clearly belong together and perhaps they should be reclassified under a single family of steroidal alkaloids.

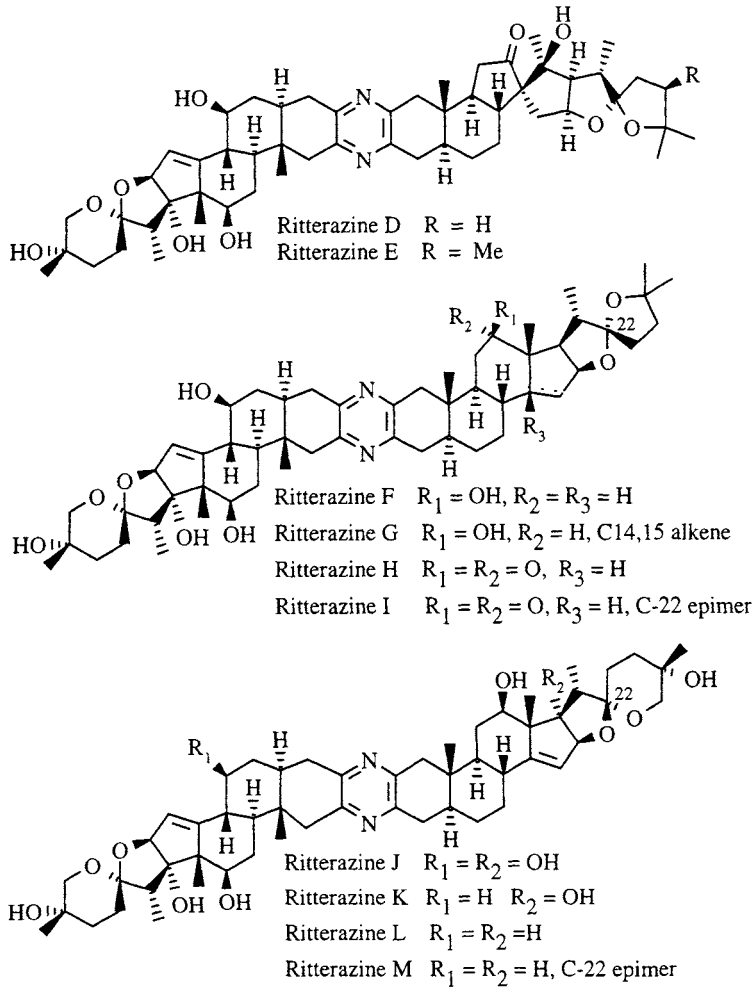


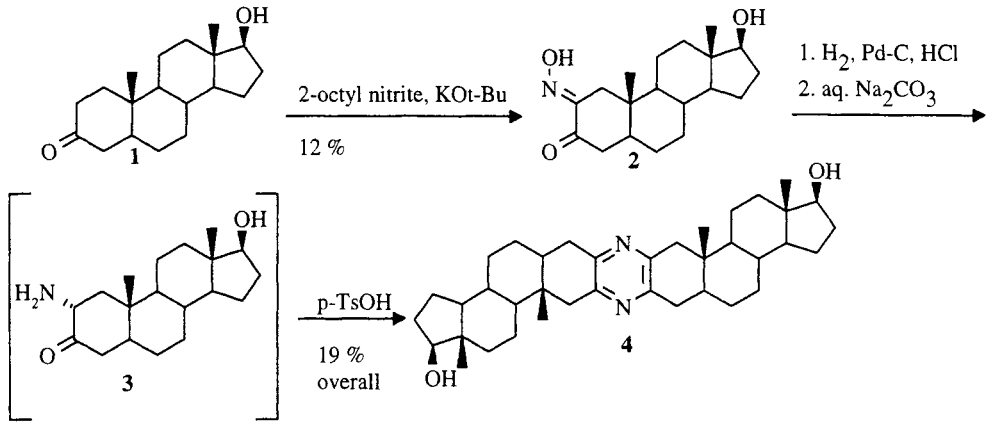
Figure 9. Ritterazines D-M.

The isolation of the ritterazines from a phylum unrelated to the hemichordates leaves the true source of these dimers unclear. With certain natural products isolated from marine macroorganisms it is now established[14] that they are actually produced by symbiotic microflora, and this may be case here. If a microorganism that produces these dimers can be identified and grown in laboratory conditions, it raises the exciting prospect of obtaining these highly potent steroids in greater quantities by large-scale fermentation.

The biosynthesis of these compounds seems to occur in two phases: (1) coupling of two steroids via a pyrazine linker, and (2) relatively unselective oxidation at various positions. Some of these compounds are related to others by simple processes: the hydration of cephalostatin 1 to its hemiketal form cephalostatin 9; the dehydration of cephalostatin 2 to an enone, which in turn may be an intermediate to cephalostatin 6; the skeletal rearrangement of ritterazine B to ritterazine A, which may be acid catalyzed; and the pairs of ritterazines epimeric at C-22. One can speculate whether such reactions are non-enzymatic transformations occurring in the organism or even during the isolation procedure.

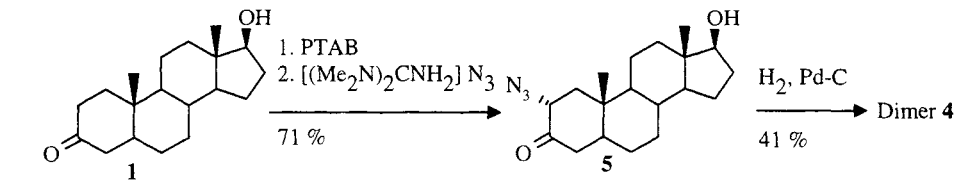
SYNTHETIC STEROID-PYRAZINE DIMERS VIA α-AMINO KETONES

The intriguing structure of these steroids coupled with their potent biological activity and limited availability makes them an attractive challenge for the synthetic organic chemist. One of the key features of any attempted synthesis is the central heterocyclic ring. The classical method of pyrazine synthesis involves the dimerization of α-amino ketones. A steroidal example relevant to the cephalostatin problem was reported[15] as early as 1968 (Scheme 4): androstanolone (**1**) was converted to α-oximino ketone **2**, which was hydrogenated to afford the hydrochloride salt of α-amino ketone **3**. The salt was neutralized to the free base, and condensed in situ to yield symmetrical dimer **4** in modest overall yields.



Scheme 4. First preparation of a pyrazine linked steroid dimer.

Subsequent to the isolation of the cephalostatins, there was renewed interest in such steroid dimers. An improved procedure for the preparation of **4** was reported[16] by the Fuchs group (Scheme 5), in which the intermediate α-amino ketone was produced in higher yield by the sequence of bromination with phenyltrimethylammonium bromide, displacement by azide to give α-azido ketone **5**, and hydrogenation (an identical route was independently developed[17] in the Heathcock group).

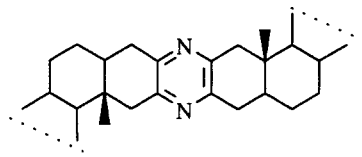


Scheme 5. Synthesis of **4** via azido ketone **5**.

The Fuchs group also prepared dimers **6-11** (Figure 10) by analogous procedures. These compounds were tested by the MTT cytotoxicity method in a panel of five human tumour cell lines. The dimer **6** with the cholestanyl side chain had little activity, with an ED_{50} > 100 μg/ml. However, all the other dimers displayed some degree of cytotoxicity. For example, dimer **4** had an ED_{50} of 7 μg/ml against the colon adenocarcinoma cell line HT-29, and values around 30 μg/ml against the others.

Although the activity is low, it is interesting given the huge simplification in structure compared to the natural products. The results suggest that more sophisticated cephalostatin analogues with improved biological activity can be prepared synthetically. Such compounds would be more accessible than the scarce natural products; moreover, their cytotoxicity can be modulated, whereas the natural products may be too toxic for direct therapeutic use.

Dimers **4** and **10** were also tested in two murine epithelial tumour xenografts transformed by mutations in the low-molecular weight guanine nucleotide binding protein Ras. Ras is an important protein in cellular signal transduction, cycling between an inactive GDP-bound state and an active GTP-bound form. The protein also has intrinsic GTPase enzymatic capability, thus preventing permanent activation. In the mutated Ras, GTP hydrolysis is greatly diminished, leading to signals that cause cell proliferation. At the maximally tolerated dose of 150 mg/kg/day, **4** inhibited the tumours by 28 and 59 %, without any deaths due to cytotoxicity.



Common pyrazine and A-B ring core of dimers **6-11**.

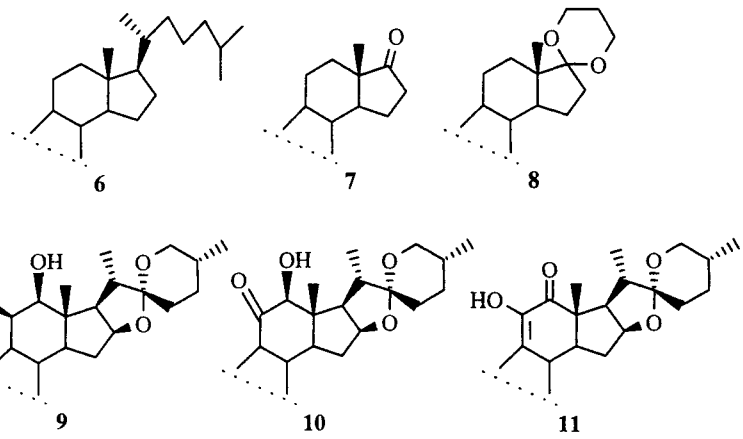
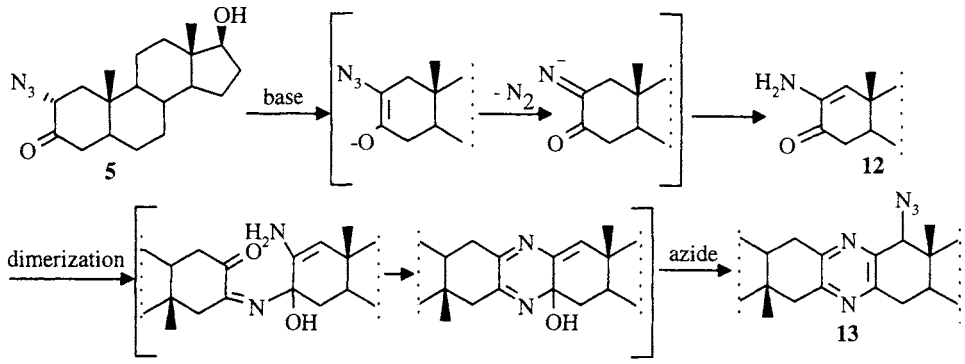


Figure 10. Symmetrical dimers prepared from azido ketones by Fuchs and coworkers.

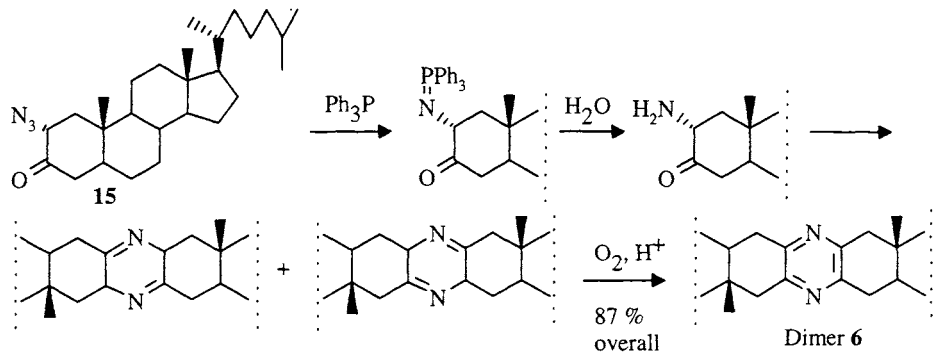
While preparing large quantities of **4** for animal testing, the Fuchs group isolated 5-10 % of a byproduct, identified as its C1-azido derivative. Control studies determined that this compound was produced by the presence of excess azide during the displacement reaction, and the suggested mechanism is shown in Scheme 6. Thus, azide acting as a base[18] enolizes α-azido ketone **5**, which then loses nitrogen to yield α-amino enone **12**. This process can also be recreated by treating **5** with DBU. Dimerization of **12** followed by reaction with azide completes the process. It is possible that similar generation of an electrophilic centre adjacent to the pyrazine occurs during cephalostatin biosynthesis, as some of the natural products also show C-1 substitution.

Interestingly, dimer **13** has increased cytotoxicity compared to **4** and **6-11**, with an ED_{50} in the 0.2-0.4 μg/ml range against the same tumour cell lines. Presumably, this increase is a nonspecific effect caused by the presence of the toxic azide functional group.



Scheme 6. Proposed mechanism for formation of the C-1 azido dimer **13**.

Further improvement was achieved[19] in the α-amino ketone dimerization process by Smith and Heathcock. Cholestanone (**14**) was converted to its 2α-azido derivative **15**. The azide (Scheme 7) was reacted with aqueous triphenylphosphine, rather than reduction by hydrogenation, and the crude dihydropyrazine dimers aromatized by air oxidation in the presence of p-toluenesulfonic acid. The product (**6**) was then isolated in high yield by simple filtration. The triphenylphosphine reaction proceeds via an imino phosphorane, which in principle can undergo dimerization by a Staudinger-like reaction. However, no dimerization was observed under anhydrous conditions, implying that hydrolysis of the phosophorane ylide to the α-amino ketone occurs first. The rate of phosphorane hydrolysis in NMR experiments was relatively slow (several hours), and it may be possible to produce unsymmetrical dimers by trapping the α-amino ketone as it is being formed by a more reactive keto steroid.

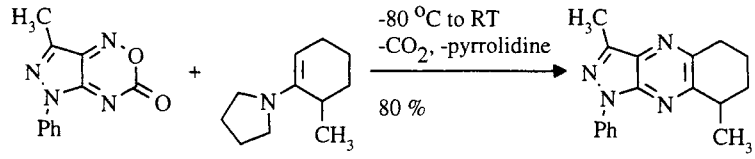


Scheme 7. Preparation of pyrazine dimers using triphenylphosphine for azide reduction.

UNSYMMETRICAL PYRAZINES

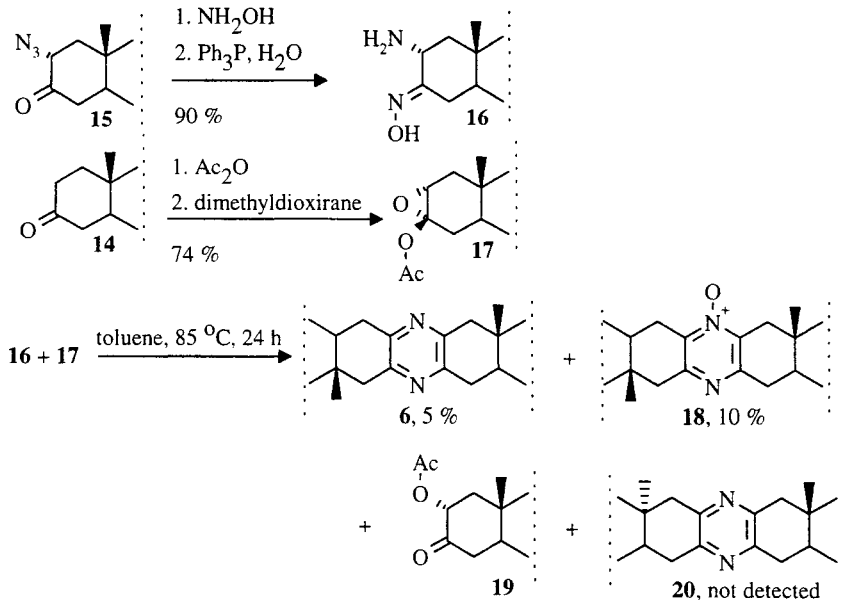
An obvious disadvantage of α-amino ketone dimerizations is their unsuitability for cross-coupling. The alternative of condensing a 1,2-dicarbonyl component with a 1,2-amine is also inappropriate for most cephalostatins and ritterazines, which are unsymmetrical both from right to left and top to bottom. It is possible that unsymmetrical condensation could be carried out in a stepwise manner using two α-amino ketones protected in different ways. However, this would

decrease the efficiency of pyrazine formation, which would be a late step in a total synthesis with highly functionalized and precious monomers. In the Heathcock group, a number of solutions to this problem were explored. One discovery[20] was the hetero-Diels-Alder-like reaction between oxadiazinones and enamines (Scheme 8). Although this reaction has the required regiospecificity and takes place under very mild conditions, it proved impossible to prepare an oxadiazinone fused to a cycloalkane, as required in a cephalostatin synthesis.



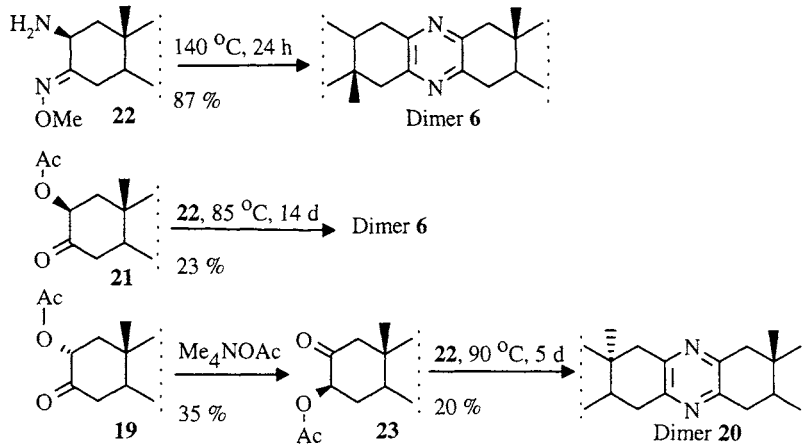
Scheme 8. Synthesis of an unsymmetrical pyrazine via a Diels-Alder like reaction.

Later,[19] Smith and Heathcock studied dimerizations with α-amino oximes as one of the components. Amino oxime **16**, derived from **15** in two steps (Scheme 9), was reacted with epoxy acetate **17**. The product mixture contained "trans" pyrazine dimer **6** and its *N*-oxide derivative **18** in low yield, together with 2α-acetoxycholestanone (**19**). However, the expected "cis" dimer **20** was produced in too small a quantity for isolation. Heating **16** alone yielded **6** together with a trace of *N*-oxide **18**. Epoxy acetate **17** was also unstable to the reaction conditions, slowly rearranging to 2β-acetoxycholestanone (**21**) which then epimerized to the equatorial acetate **19**. Heating amino oxime **16** together with 2β-acetoxycholestanone gave dimers **6** and **18** with the latter predominating, as observed in the initial experiment. Furthermore, the ratio of **6** to **18** increased with higher initial concentrations of **21**, suggesting that dimer **18** was the product of cross-coupling.



Scheme 9. Dimerization of the cholestanyl amino oxime **16** and epoxy acetate **17**.

For improved yields, an analogue of amino oxime **16** less prone to self-dimerization was needed. Towards this end, the *O*-methyl derivative **22** was prepared, which undergoes complete dimerization only at 140 ^{0}C. Heating acetoxy ketone **21** with **22** at 85 ^{0}C gave dimer **6** in 3.5 % yield after 1 day, together with unidentified compounds which may be intermediates in its formation. Extending the reaction time to 14 days increased the yield to 23 % (Scheme 10). A protocol was worked out involving initial heating at 85-90 ^{0}C during which the acetoxy ketone and amino oxime ether preferentially react with each other, followed by heating at 140 ^{0}C to complete the process. Acetoxy ketone **19** was also isomerized to the 3β-acetoxy-2-one **23**, which then dimerizes with **22** to give "cis" pyrazine **20**.



Scheme 10. Dimerizations involving amino oxime ether **22**.

A complication in these reactions is the similar properties for "trans" dimer **6** and the "cis" dimer **20**. The only noticeable difference in the NMR spectrum is the shift of one ^{1}H resonance by 0.01 ppm, and a shift in the pyrazine ^{13}C resonances by about 0.1 ppm, while the optical rotations are identical ($[\alpha]_D = +82^0$). However, the compounds have very different solubilities - trituration of the mixture with ethanol yields the trans dimer upon filtration, while the crude cis dimer is obtained by evaporation of the filtrate. This separation procedure enabled purification of the mixture obtained from reaction of 2α,3α-diaminocholestane (obtained by borane reduction of **22**) and 2,3-diketocholestane (obtained by oxidation of 3-cholestanone with potassium t-butoxide and oxygen) (Figure 11).

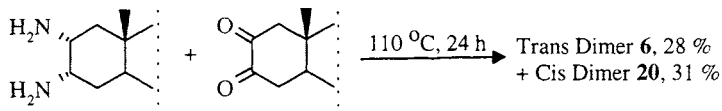
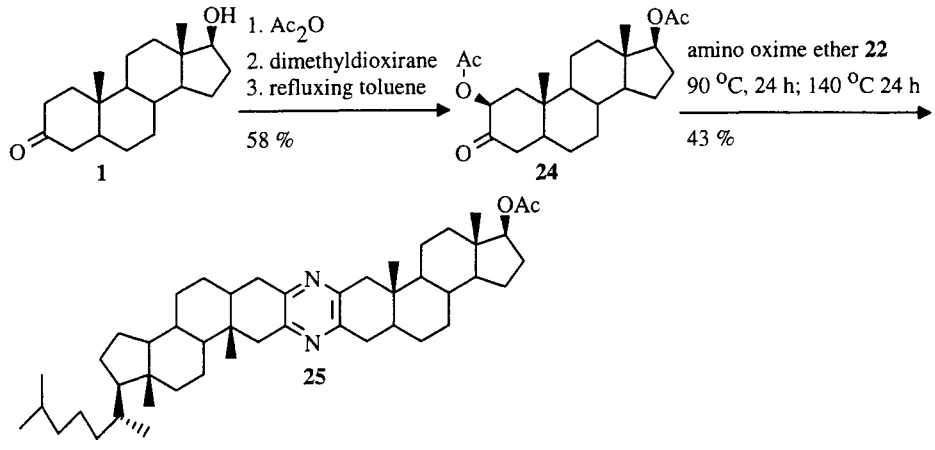


Figure 11. Pyrazine dimers via reaction between 1,2-diamines and 1,2-diketones.

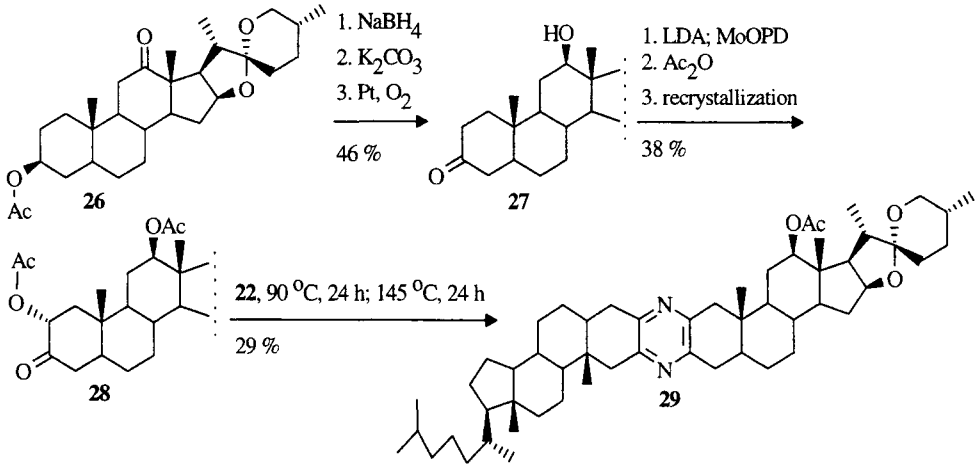
The utility of the amino oxime ether - acetoxy ketone combination was shown using two different steroid monomers. Androstanolone was converted to the 2β,17β-diacetate **24** (Scheme 11), which was then reacted with amino oxime ether **22** to afford unsymmetrical dimer **25** in reasonable yield. In this dimerization, replacement of **24** by its 2α-acetoxy epimer gave similar results, while the 2α-bromoketone instead led to messy reaction mixtures. Reactions between **22** and 2,3-epoxy acetates were also tried, which produced an approximately 1:1 ratio of cis and trans pyrazine dimers.

Compound **25** was also hydrolyzed to give the corresponding dimer with the free C-17 hydroxyl group.



Scheme 11. Smith and Heathcock's synthesis of an unsymmetrical pyrazine.

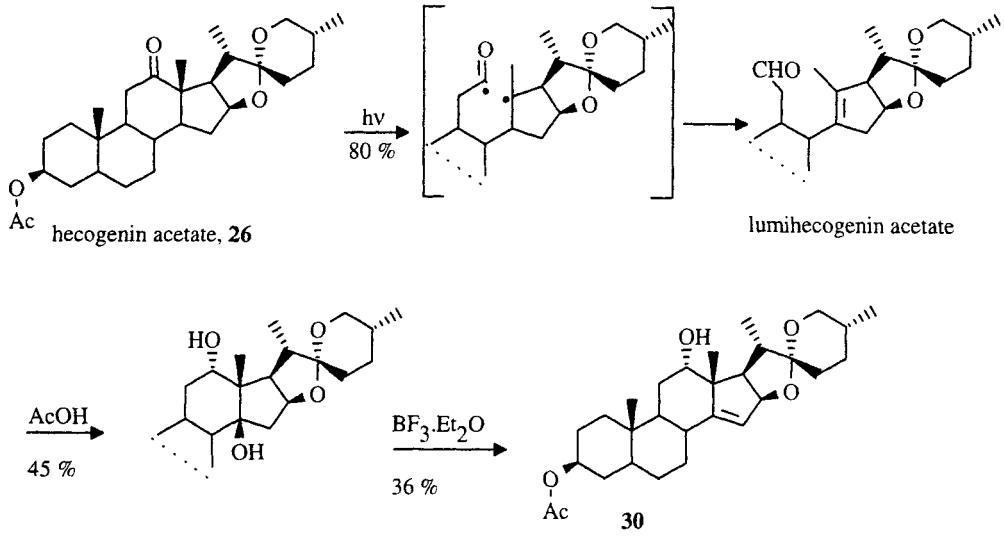
Starting from hecogenin acetate (**26**), an inexpensive commercially available sapogenin whose side chain resembles that of the cephalostatins, keto alcohol **27** was prepared (Scheme 12). This was carried forward to acetoxy ketone **28**, the recrystallization step in this sequence being necessary as the MoOPD oxidation yields an inseparable mixture of the 2-hydroxy-3-one, 3-hydroxy-2-one, and 4-hydroxy-3-one. Reaction of **28** with amino oxime ether **22** under the usual conditions provided 29 % of unsymmetrical dimer **29**. The acetate group in **29** was also hydrolyzed to give a more crystalline dimer. Dimers **6**, **25**, and **29**, together with the deacetoxy derivatives of **25** and **29**, were all submitted for testing in the NCI's solid tumour panel. None of the dimers were sufficiently active to warrant further investigation.



Scheme 12. Synthesis of a unsymmetrical dimer with a spiroketal side chain.

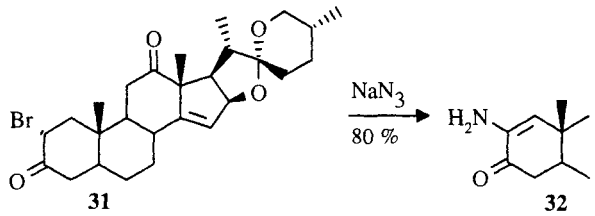
Another route to unsymmetrical steroidal pyrazines is the likely biosynthetic process of differentiation of a symmetrical dimer, as accomplished[21] by Winterfeldt's group. Starting from

hecogenin acetate, compound **30** containing the C-14,15 alkene present in the cephalostatins was synthesized by a literature procedure[22] involving the interesting sequence of photochemical isomerization and Prins reaction (Scheme 13).



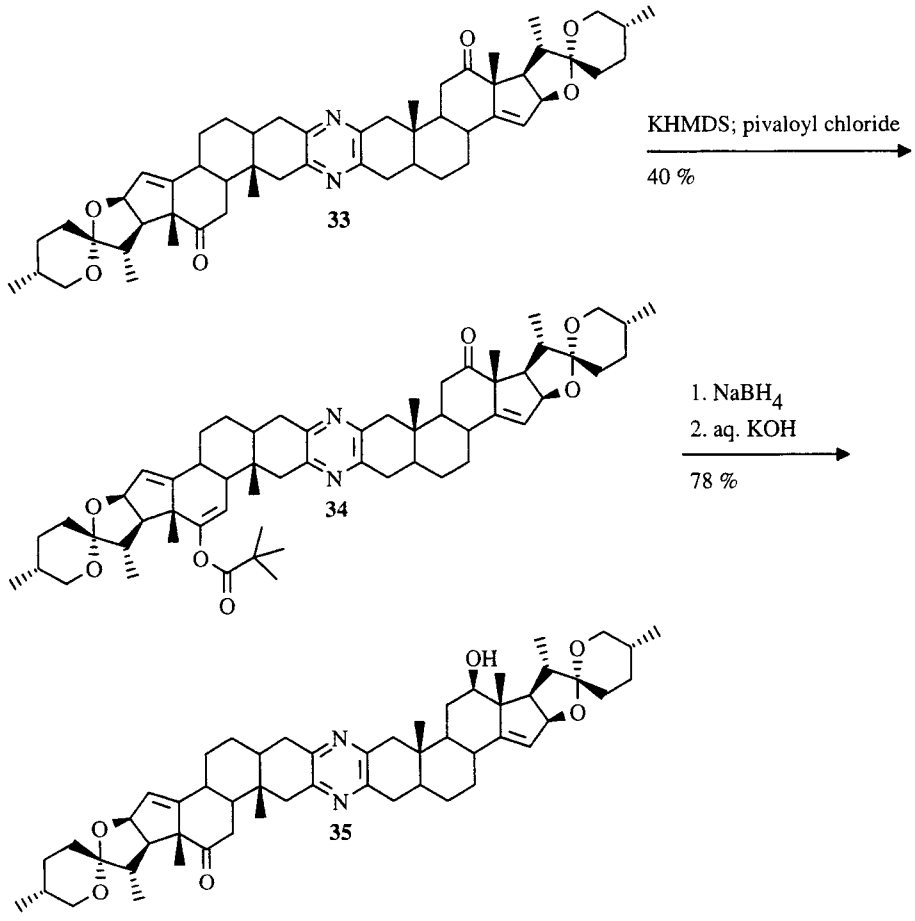
Scheme 13. Introduction of the C-14,15 alkene into hecogenin acetate.

Compound **30** was carried forward to α-bromoketone **31** which was displaced by azide to yield α-amino enone **32** directly in good yield (Scheme 14). The formation of **32** is probably due to the use of excess azide (as discussed in Scheme 6, *vide supra*).



Scheme 14. Direct formation of an enamino ketone by azide displacement.

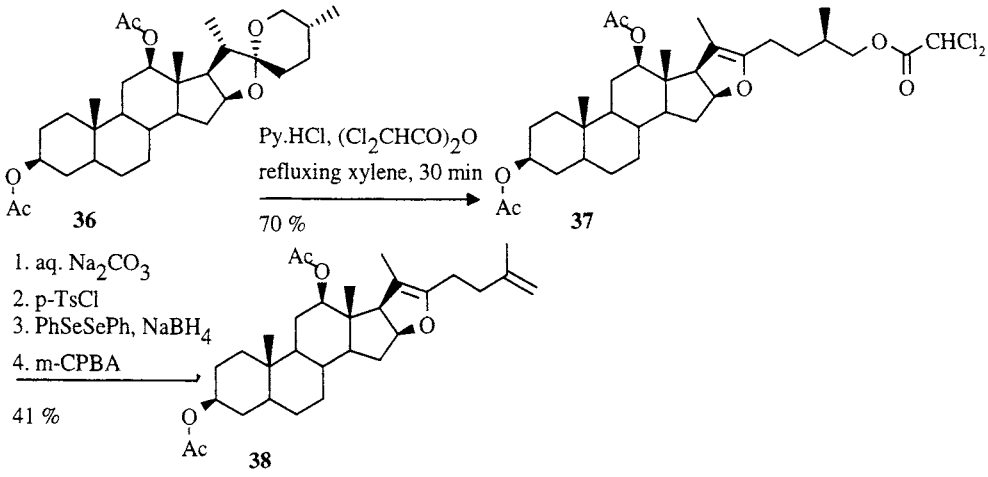
The amino enone was hydrogenated to directly afford symmetrical pyrazine dimer **33** (Scheme 15) in 64 % yield. The dimer was enolized with an excess (3.2 equivalents) of base, and trapped with pivaloyl chloride to give a statistical 1:2 mixture of the bis-enol pivalate and the unsymmetrical monopivalate **34**. The two compounds were readily separable by column chromatography. The bis-pivalate can be recycled by hydrolysis back to the ketone, while reduction of monopivalate **34** followed by hydrolysis yielded hydroxy ketone **35**, in which the C-ring oxygenation pattern is similar to that in cephalostatin 1. It would be interesting to see if **35** can undergo acid catalyzed rearrangement to the ritterazine skeleton, although such experiments may be complicated by the known high sensitivity of ritterazine A to acid.



Scheme 15. Synthesis of an unsymmetrical pyrazine dimer by Winterfeldt and coworkers.

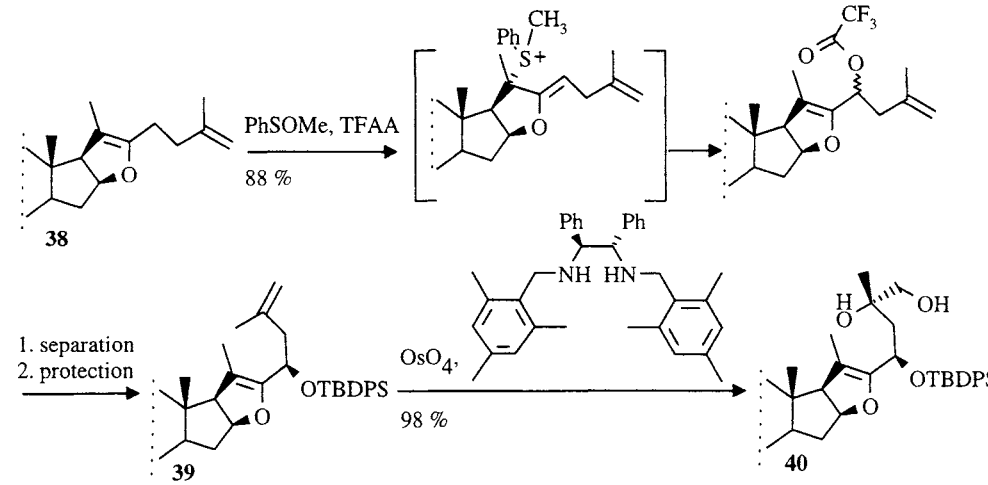
THE FUCHS SYNTHESIS OF TETRAHYDROCEPHALOSTATIN 12

The successful synthesis of symmetrical and unsymmetrical steroid-pyrazine dimers outlined in the previous sections has solved one of the key issues for a total synthesis of the cephalostatins. However, the preparation of suitably functionalized steroid monomers is still a daunting challenge, and it is Fuchs and coworkers who have accomplished the most progress in this endeavour. In 1994, Jeong and Fuchs reported[23] the preparation of a homodimer corresponding to the right half of cephalostatin 1, except for the C-14,15 alkene. In fact, this dimer corresponds to the saturated version of cephalostatin 12, but the existence of this symmetrical cephalostatin had yet to be reported at the time of this work. Diacetate **36** (Scheme 16), prepared from hecogenin acetate **26** in two steps, was subjected to a modified Marker sapogenin ring-opening procedure[24] to give dihydrofuran **37**. The ester was hydrolyzed, and the free alcohol dehydrated by the sequence of tosylate formation, displacement by selenide, and selenoxide elimination to yield diene **38**. This compound already contains all the necessary carbons for the cephalostatin right half, thus obviating the need for any carbon-carbon bond forming reactions.



Scheme 16. Jeong and Fuchs' synthesis of diene **38** from hecogenin acetate.

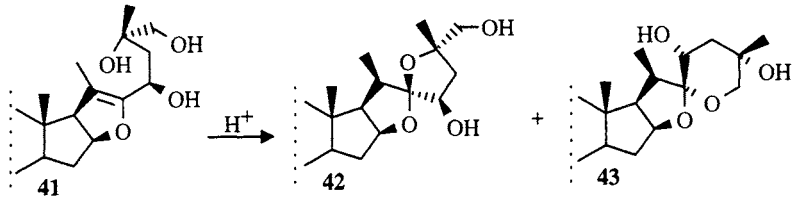
Treatment of **38** with phenyl methyl sulfoxide activated[25] by trifluoroacetic anhydride produced the separable C-23 diastereomeric trifluoroacetates in a ratio of 2.2:1 (Scheme 17). Following hydrolysis, the correct diastereomer (stereochemistry was assigned by X-ray analysis) for the cephalostatin synthesis was protected as its silyl ether **39**, while the wrong epimer was recycled by Mitsunobu inversion in 80 % yield. Stereospecific osmylation of **39** was performed with the use of Corey's chiral diamine,[26] and gave the required diastereomer **40** in a ratio of 7.7:1.



Scheme 17. Hydroxylation of the steroid side chain of **38**.

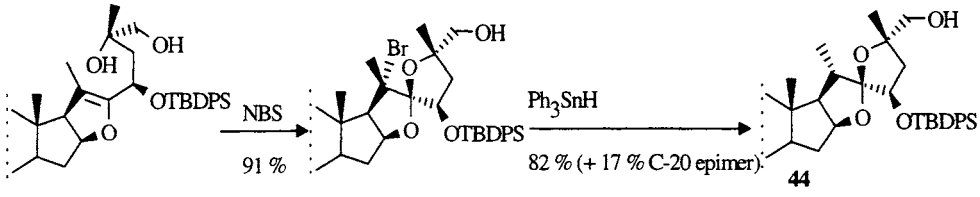
Spiroketalization of **40** under acid catalysis was unproductive. Upon prior deprotection of the silyl ether to give triol **41**, the spiroketalization was successfully accomplished, providing a mixture of the 5/5 and 6/5 spiroketals **42** and **43** respectively (Scheme 18). While the 6/5 spiroketal **43** could be quantitatively isomerized to **42** under more vigorous conditions, both these spiroketals unfortunately have the "unnatural" configuration at C-20. Presumably, electrophilic attack on the

alkene takes place from the less hindered α-face to give the β-methyl group. Under equilibrating conditions, epimerization at this position to the more favourable α-methyl orientation as in the natural products can be anticipated, but was not observed.



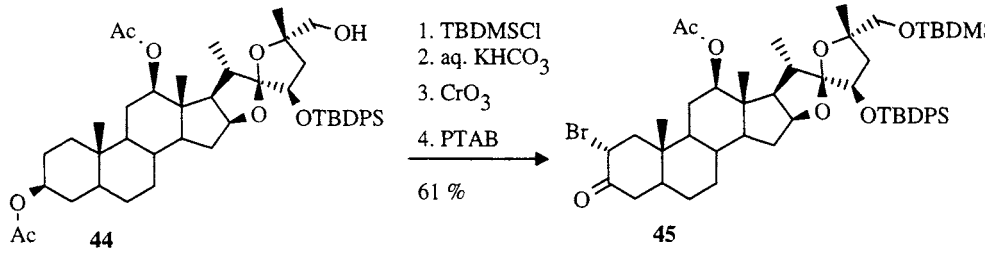
Scheme 18. Formation of 5/5 and 6/5 spiroketals.

The inability to equilibrate C-20 required a detour involving electrophilic spiroketalization via bromination. In this case, only the 5/5 spiroketal was formed from **41**, but reduction of the halogen with triphenyltin hydride only gave **42**, the undesired C-20 epimer. However, the corresponding spiroketalization product of the silyl ether **40** was reduced by triphenyltin hydride with a diastereomeric ratio of 4.2:1 in favour of **44** with the desired C-20 stereochemistry (Scheme 19).



Scheme 19. Adjustment of C-20 stereochemistry via reductive dehalogenation.

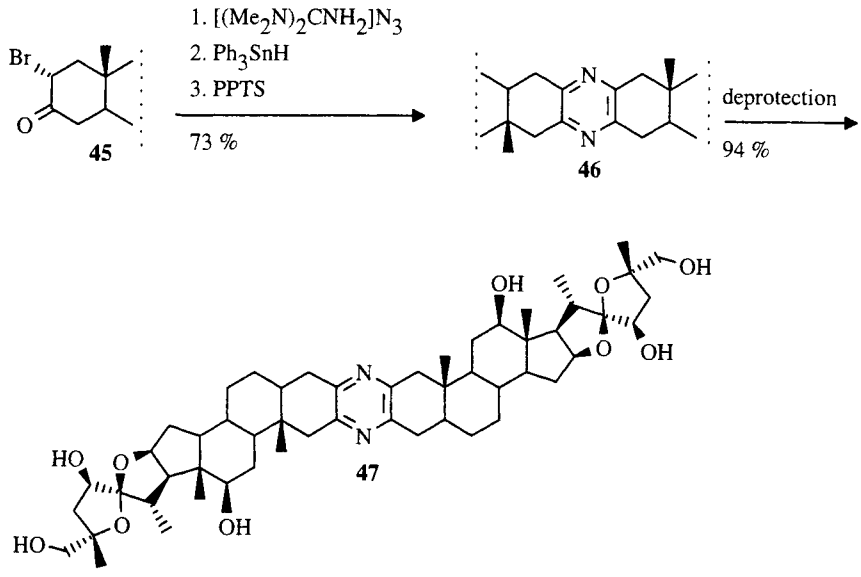
The free alcohol in **44** was protected as the silyl ether, and the less hindered C-3 acetate selectively hydrolysed and subjected to Jones oxidation (Scheme 20). The ketone was α-brominated to give **45** in 76 % yield, together with 14 % of the C-26 desilylated monobromide and 7 % of the 2,2-dibrominated product.



Scheme 20. Preparation of an α-bromo ketone intermediate for dimerization.

In the final stages of the synthesis (Scheme 21), the dimerization protocol developed earlier (Scheme 5, *vide supra*) was followed, with the slight modification that triphenyltin hydride was used for reduction of the azide functional group. Cyclization of the α-aminoketone with PPTS afforded dimer **46** in 79 % yield, accompanied by 17 % of deazidoketone monomer. Removal of the

protecting groups yielded tetrahydrocephalostatin 12 (**47**). Unfortunately, the biological activity of this compound was not disclosed.



Scheme 21. The synthesis of tetrahydrocephalostatin 12.

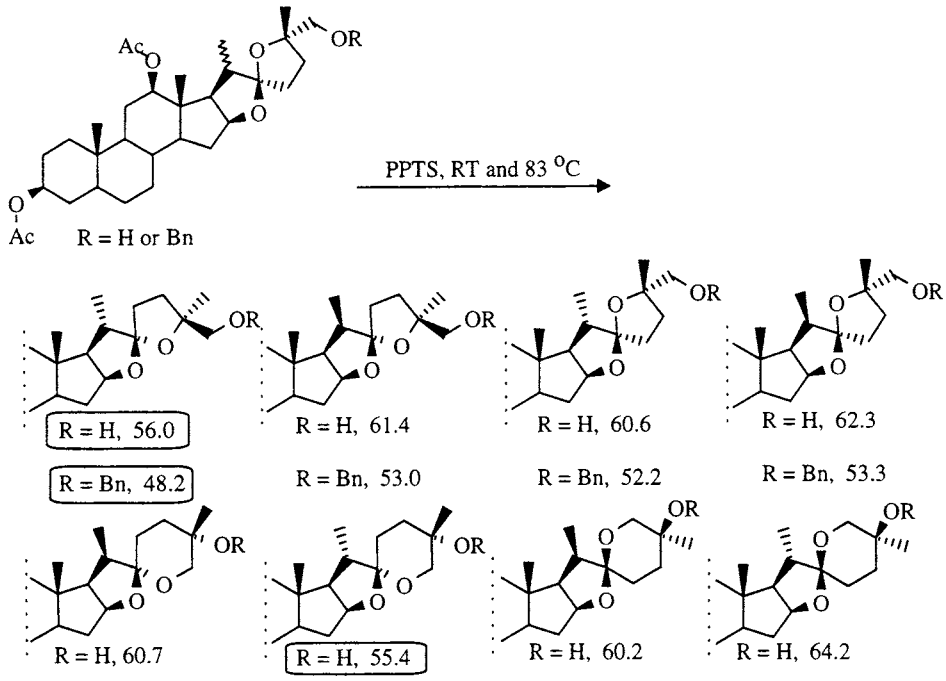
A COMMON INTERMEDIATE FOR CEPHALOSTATIN 7 MONOMERS

The next results reported by the Fuchs group concerned cephalostatin 7. The ability to form the 5/5 spiroketal of the right half and the 6/5 spiroketal of the left half from a common advanced intermediate would be advantageous from a practical standpoint. The feasibility of this approach can be seen in the work on tetrahydrocephalostatin 12 (Scheme 18, *vide supra*).

A more detailed study[27] was carried out of the spiroketalization reaction. Compounds obtained by C-23 deoxygenation of intermediates in the tetrahydrocephalostatin 12 synthesis were used, with various permutations differing in their C-20 stereochemistry and also the presence or absence of benzylation at C-26. These compounds were subjected to a series of acid-catalyzed cyclizations.

In the case of the C-26 alcohol, eight products are possible (Scheme 22). Depending on the starting point, four of these products were observed upon overnight treatment with PPTS at room temperature (structural assignments were aided by X-ray analysis of two of the compounds). However, when heated overnight at 83 °C, the product mixtures collapse to only one 5/5 spiroketal and one 6/5 spiroketal in a 1.5:1 ratio. Molecular mechanics calculations performed using the CAChe v3.5 program are consistent with the experimental observations. The two products detected in the high-temperature equilibration are only approximately 0.5 kcal/mol apart, while the six other possibilities are significantly higher in energy, by at least 4 kcal/mol.

With the C-26 benzyl derivative, four different 5/5 spiroketals are possible (Scheme 22). All of these were observed in room temperature cyclizations, while equilibration at higher temperatures gave a single 5/5 spiroketal. Again, this is in agreement with calculations, where one product is at least 4 kcal/mol lower in energy than the others.



Scheme 22. Possible products of spiroketalization experiments, together with their energies in kcal/mol by molecular mechanics calculations. Highlighted diastereomers indicate those of lowest energy.

Molecular mechanics calculations were also performed on compounds with the C-14,15 alkene and the C-17 alcohol present as in the natural product (Figure 12). The results indicate that preparation of the left half's 6/5 spiroketal is best achieved before deoxygenation at C-23, as the 6/5 spiroketal is then 2.5 kcal/mol lower in energy than the 5/5 spiroketal. In the deoxygenated series, both 5/5 and 6/5 spiroketals are within 1 kcal/mol of each other.

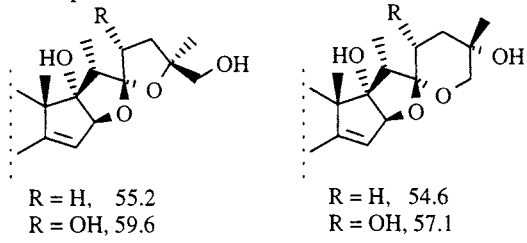
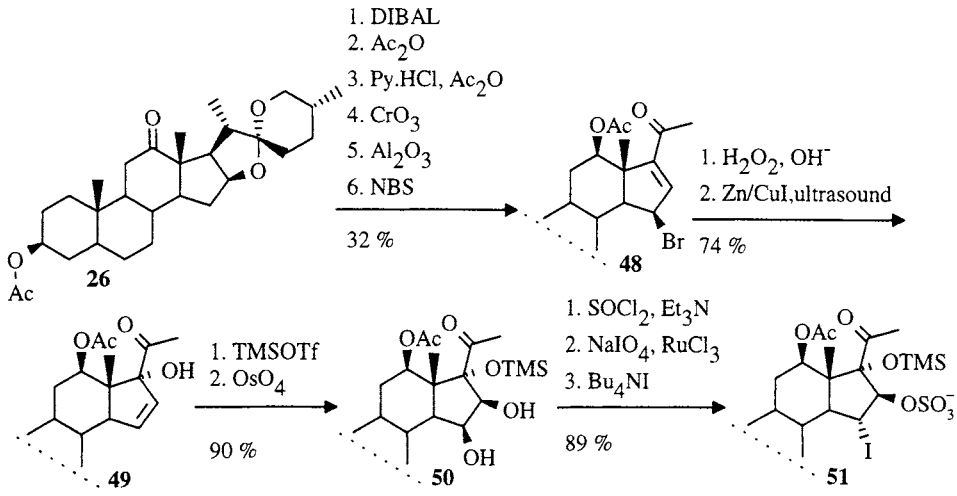


Figure 12. Molecular mechanics calculations on spiroketals. Numbers indicate energies in kcal/mol.

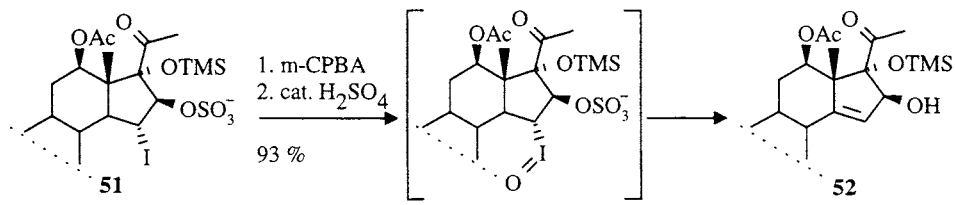
Subsequent to this study, the preparation of a suitable common intermediate for cephalostatin 7 was reported[28] by Kim and Fuchs. The need to introduce the C-14,15 alkene and the C-17 alcohol necessitated a substantially different synthetic route from that earlier employed for tetrahydrocephalostatin 12. Marker side chain degradation of hecogenin acetate was followed by allylic bromination to yield **48** (Scheme 23). This compound was epoxidized and the halogen reductively cleaved with concomitant epoxide ring opening using a zinc/copper couple to give intermediate **49**.

The alcohol was protected as its TMS ether, and the C-15,16 alkene stereospecifically dihydroxylated to give compound **50**. The diol was then converted to its cyclic sulfate derivative according to the Sharpless protocol.[29] Attempted base-catalyzed elimination of the sulfate to introduce the C-14,15 alkene was plagued by side-reactions involving epoxide formation by displacement of the sulfate by the adjacent TMS ether, perhaps aided by enolization of the methyl ketone. Instead, displacement of the sulfate by iodide ion occurred uneventfully to provide **51** as its tetrabutylammonium salt.



Scheme 23. Introduction of the C-17 alcohol into a precursor for cephalostatin 7.

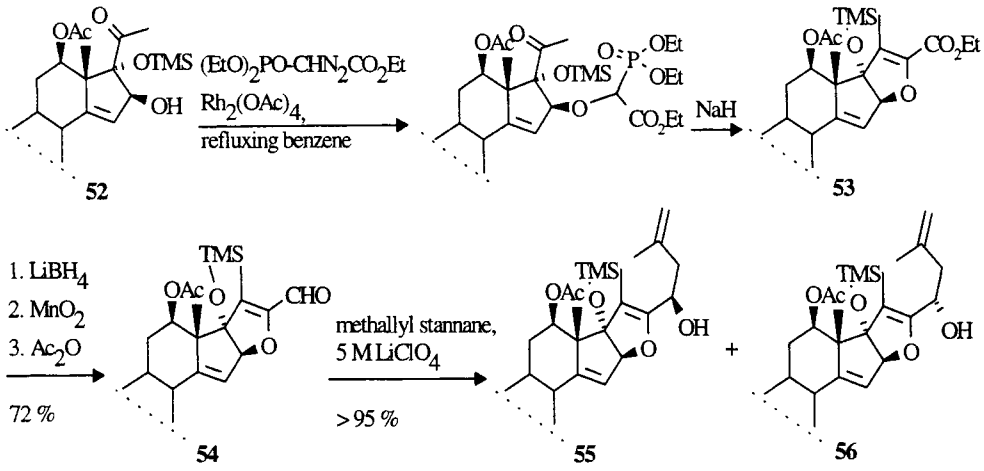
The successful formation of the C-14,15 alkene was accomplished by oxidation of **51** with m-CPBA (Scheme 24), probably via an iodoso intermediate which undergoes syn-elimination of hypoiodous acid and hydrolysis of the sulfate to yield **52**. The oxidative elimination is a procedure originally developed[30] by Reich with obvious potential for complex molecule synthesis, although it is presently less popular than related sequences involving sulfoxides or selenoxides.



Scheme 24. Introduction of the C-14,15 alkene by the Reich protocol.

With the alkene in place, the E-ring dihydrofuran was constructed by an intramolecular Wadsworth-Emmons reaction to yield ester **53** (Scheme 25). Adjustment of the oxidation state afforded aldehyde **54**, and completion of the synthesis involved extension of the side-chain by addition of methallylstannane. Various conditions for this reaction were investigated, with the best results obtained with the use of 5 M lithium perchlorate[31]. This gave a 1.3:1 mixture of separable alcohols **55** and **56** in quantitative yield. The production of both diastereomers in nearly equal

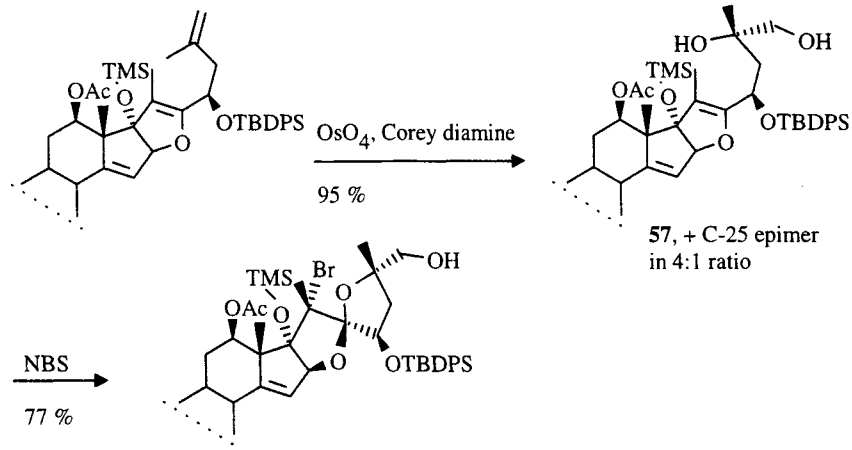
proportions is not a disadvantage in this case, as the compounds are suitable for processing into the two halves of cephalostatin 7 separately (see next section).



Scheme 25. Preparation of a common advanced intermediate for cephalostatin 7.

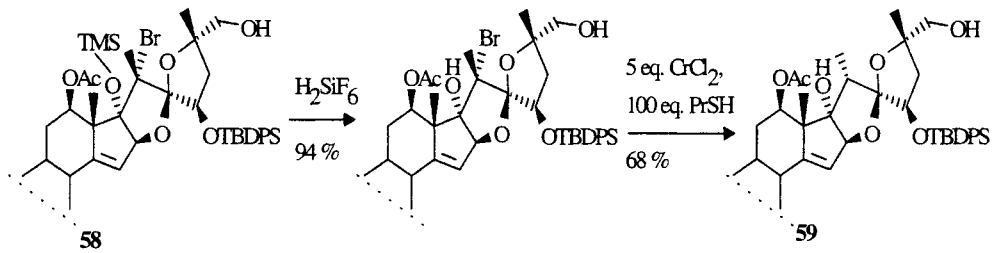
THE FUCHS SYNTHESIS OF THE TWO HALVES OF CEPHALOSTATIN 7

The right half of cephalostatin 7 was synthesized[32] in an analogous manner to that of tetrahydrocephalostatin 12. The silyl ether of **55** was dihydroxylated to give a pair of inseparable diastereomers in a 4:1 ratio, the major product **57** corresponding to the natural C-25 stereochemistry (Scheme 26). However, no reaction was observed with conditions used for spiroketalization (Scheme 22, *vide supra*), while harsher conditions led to undesired products. As in the earlier work, the brominated derivative **58** of the desired 5/5 spiroketal was formed by treatment with NBS. A diastereomeric byproduct was also obtained, occurring from cyclization of the minor epimer of **57**.



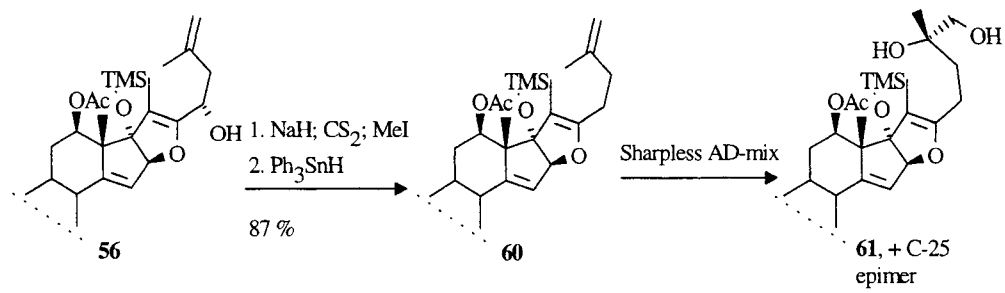
Scheme 26. Spiroketalization of the cephalostatin 7 right half.

Removal of the halogen in **58** using triphenyltin hydride as in the tetrahydrocephalostatin 12 synthesis led to complex product mixtures, possibly due to the bulky TMS ether at C-17 which can hinder quenching of the radical as well as form a reactive site for radical fragmentation. Instead, treatment of **58** with chromium[II] diacetate in the presence of a thiol gave 15 % of the undesired C-20 α-methyl compound and 60 % recovered starting material, in a very slow reaction (48 hours). These conditions were based on analogy[33] to Barton's chromium[II]-mediated halohydrin reductions. The speed of the reduction was improved by adding ethylenediamine; however, the product was the C-20 exo-methylene compound. Better results were obtained with chromium[II] chloride, provided a large excess of thiol (~ 100 molar equivalents) was used, suggesting that the thiol not only serves as the source of the hydrogen but also as an activating ligand for chromium[II]. The optimum conditions (Scheme 27) involved removal of the TMS protecting group prior to the reduction, and yielded 68 % of the desired compound **59** together with 19 % of the C-20 epimer.



Scheme 27. Stereoselective removal of the C-20 halogen.

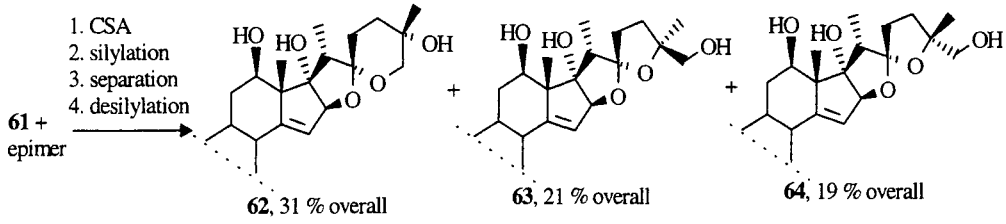
The preparation[34] of the left half of cephalostatin 7 was more straightforward. Compound **56**, the minor product of the stannane addition (Scheme 25, *vide supra*) was deoxygenated by Barton's xanthate procedure (Scheme 28) to give **60**, which was stereoselectively dihydroxylated using the Sharpless AD-mix-α reagent[35] to yield **61** and its inseparable C-25 epimer in a 2.5:1 ratio.



Scheme 28. Elaboration of the left half of cephalostatin 7.

Treatment of the mixture of **61** and its epimer with (+)-camphorsulfonic acid yielded an inseparable mixture of three spiroketals, which were purified by the sequence of silylation, chromatographic separation, and desilylation (Scheme 29). The major product **62** corresponds to the desired left half, while **63** is the alternative 5/5 spiroketal product. The formation of **62** and **63** in nearly equal amounts is consistent with molecular mechanics calculations, which indicate the two compounds are within 0.1 kcal/mol of each other. The third product, **64**, is derived from the C-25 epimer of **61**. This epimer can also produce a 6/5 spiroketal, although it was not detected (molecular mechanics calculations predict the 6,5 spiroketal to be 0.8 kcal/mol less stable than **64**). The structures of all three products were established by X-ray analysis. Interestingly, in all three products,

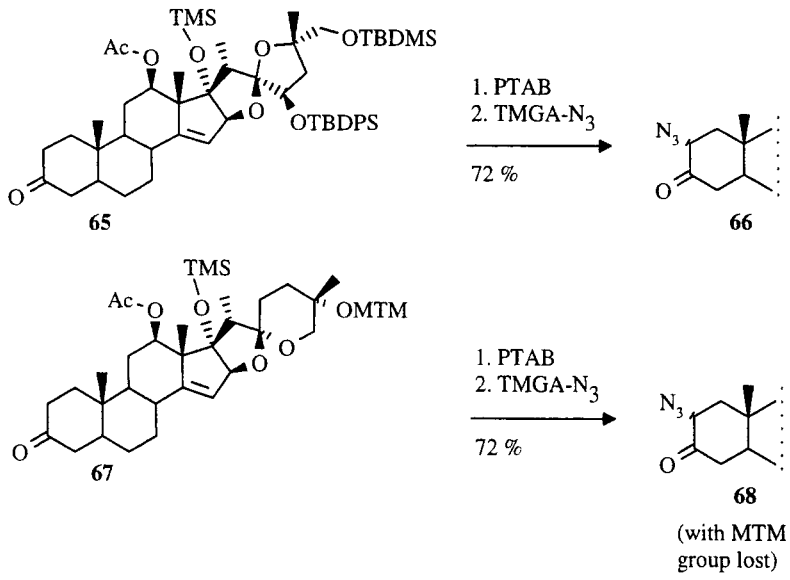
the desilylation step also hydrolyzed the C-12 acetate, which is normally less reactive than the C-3 acetate. The accelerated hydrolysis at C-12 is probably due to neighbouring group participation by the C-17 alcohol.



Scheme 29. Spiroketalization of the left half of cephalostatin 7.

SYNTHESIS OF CEPHALOSTATINS 7, 12 AND RITTERAZINE K

While this manuscript was in preparation, the Fuchs group completed[36] the total synthesis of cephalostatins 7. Right half ketone **65** (Scheme 30), obtained from **59** (*vide supra*) was brominated and displaced by tertramethylguanidinum (TMGA) azide to afford **66**. In methylene chloride, the azide displacement was plagued by formation of the α-amino enone (Scheme 6, *vide supra*) while the use of nitromethane[37] as solvent resulted in a quantitative yield. Similarly, left half ketone **67**, obtained from **62**, was brominated and displaced by TMGA azide in nitromethane to give **68**. The MTM protecting group was lost during the bromination step, but bromination of an unprotected ketone gave a byproduct with a rearranged 5/5 spiroketal.

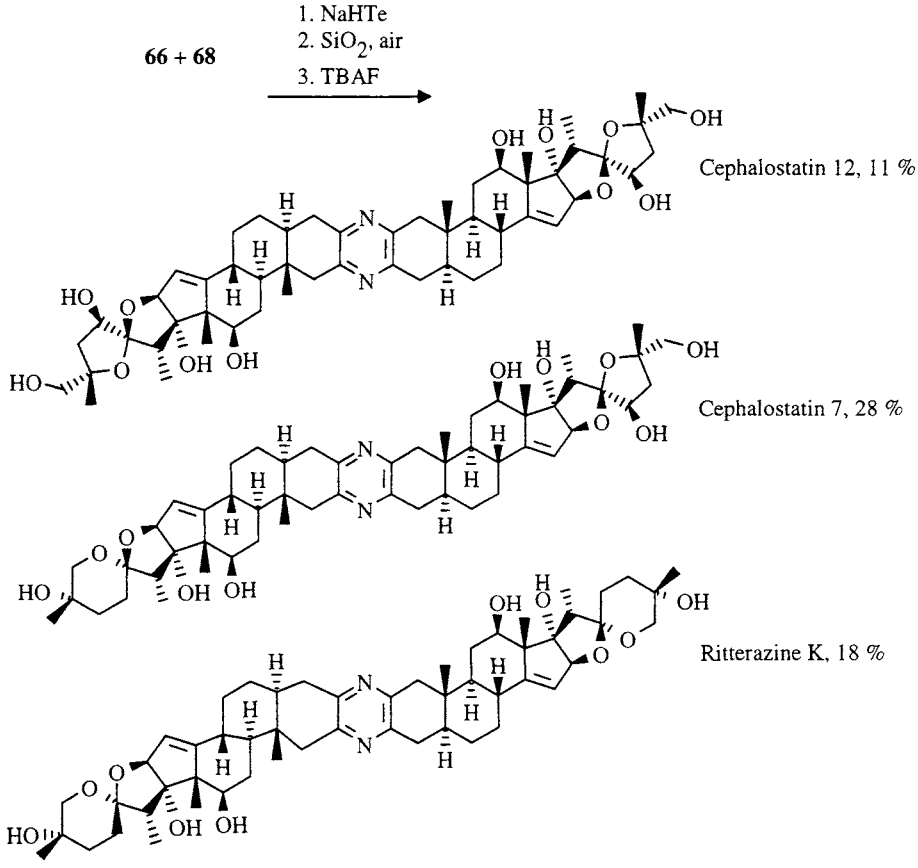


Scheme 30. Preparation of α-azido ketones for the two halves of cephalostatin 7.

The final step involved reduction of azido ketones **66** and **68** to amino ketones followed by statistical pyrazine formation. In this reaction, the product of cross-coupling is a protected form of

cephalostatin 7, while self-dimerization yields precursors to the symmetrical natural products cephalostatin 12 and ritterazine K. It is possible that such coupling occurs biosynthetically. If the rates of coupling are assumed to be similar, the fact that cephalostatin 12 (product of right half dimerization) is isolated in 10-fold higher quantity than cephalostatin 7 (cross-coupling product) from *C. gilchristi* implies that the right half monomer is present in greater concentration. There may be a downstream biosynthetic step in which the symmetrical cephalostatin 12 or ritterazine K are converted to the more abundant unsymmetrical cephalostatin 1.

In the laboratory, a 1:1 mixture of **66** and **68** was reduced with an excess of ethanolic NaHTe (Scheme 31). Silica gel was then added as a mild acid catalyst, and the reaction mixture stirred under exposure to air to complete the aromatization. Chromatography afforded protected cephalostatin 7 in 35 % yield, while the cephalostatin 12 and ritterazine K precursors were produced in 14 % and 23 % yield respectively. The deazido derivatives of **66** and **68** were also found in 36 % and 15 % respectively. As this side-reaction perturbs the initial 1:1 ratio of reactants, it helps explain why the pyrazine product distribution is not strictly 1:2:1 but biased towards formation of ritterazine K. Each of the three protected pyrazines was individually treated with excess tetrabutylammonium fluoride to give the first synthetic samples of cephalostatin 7, cephalostatin 12, and ritterazine K.

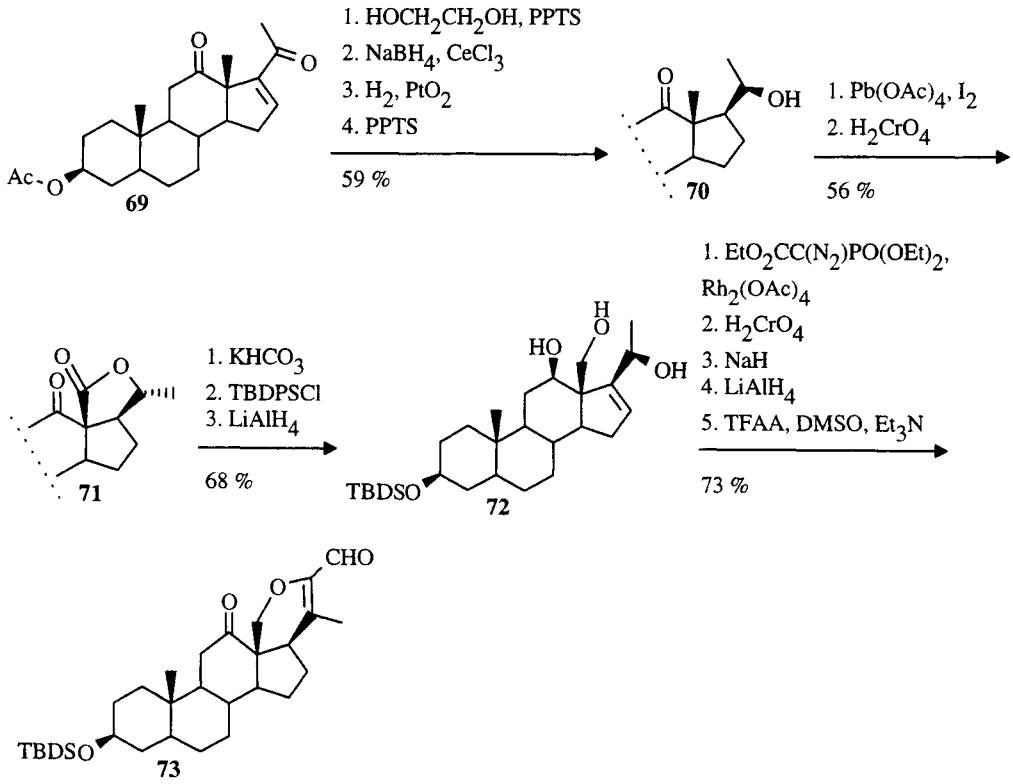


Scheme 31. Total synthesis of cephalostatin 7, cephalostatin 12, and ritterazine K.

Samples of the synthetic compounds were provided to Professor Pettit's group, who confirmed the identity of cephalostatins 7 and 12 based on NMR and chromatographic comparison. If this mechanism for pyrazine formation occurs in *C. gilchristi*, one would expect it to also produce ritterazine K. A search among the currently unidentified residual *Cephalodiscus* extracts revealed a substance with identical chromatographic profile to ritterazine K. However, it was present in only microgram quantities, and its identity could not be confirmed by NMR.

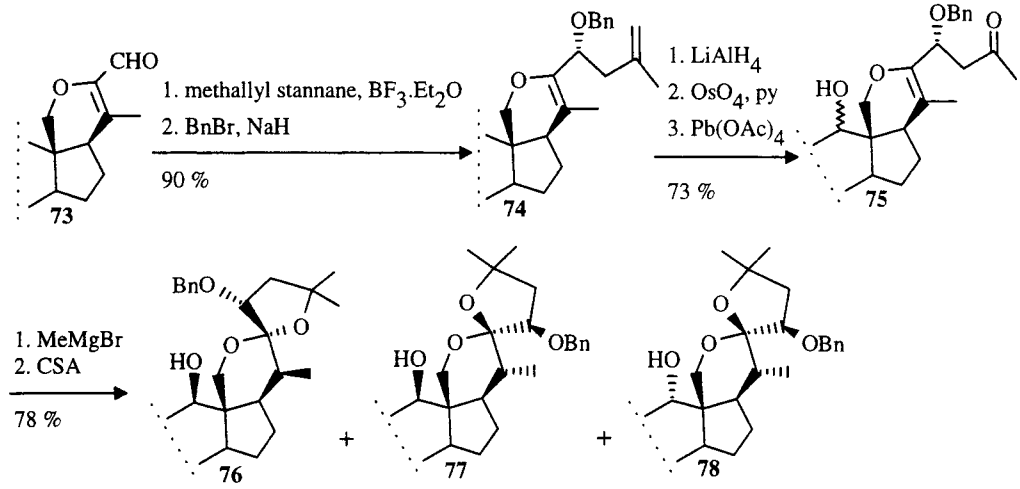
THE FUCHS APPROACH TO DIHYRDOCEPHALOSTATIN 1

The Fuchs group has also prepared a monomer for the left half of dihydrocephalostatin 1. The dihydro compound was chosen in order to investigate the spiroketalization process and also the importance of the C-14,15 alkene for biological activity. The route begins[38] with keto aldehyde **69** (Scheme 32), available in 60 % yield from Marker degradation of hecogenin. This was reduced to keto alcohol **70**, and the C-18 methyl group functionalized by Meystre's hypoiodite method,[39] after which Jones oxidation provided lactone **71**. The C-3 acetate was hydrolyzed, and the free alcohol reprotected as a silyl ether, followed by reduction to give triol **72**. Regioselective carbenoid insertion into a neopentyl alcohol set the stage an intramolecular Wadsworth-Emmons reaction (see Scheme 25, *vide supra*), after which the oxidation state was adjusted to yield **73**.



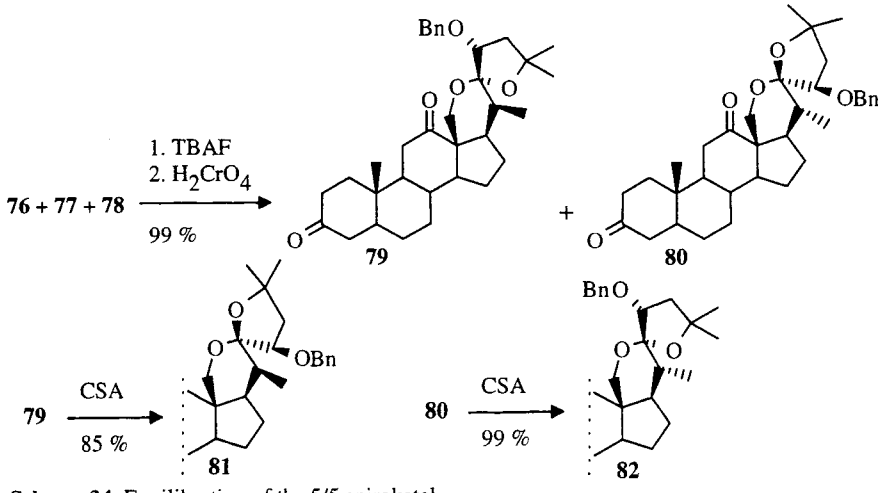
Scheme 32. Preparation of a keto aldehyde precursor for dihydrocephalostatin 1.

Compound **73** was reacted with methallylstannane to give a separable pair of diastereomeric alcohols (Scheme 33) in a 1:2.7 ratio favouring the desired product. The unwanted diastereomer was recycled in 79 % yield by Mitsunobu inversion. The alcohol was then benzylated to afford intermediate **74**, after which reduction of the C-12 ketone yielded a 1:9 ratio of α– and β–C-12 epimeric alcohols. The alcohols were osmylated and subjected to periodate cleavage to provide **75**. This was reacted with methyl Grignard, followed by acid catalyzed cyclization with (+)-camphorsulfonic acid. Three spiroketal products **76**, **77**, and **78** were isolated in a 1:15:1 ratio.



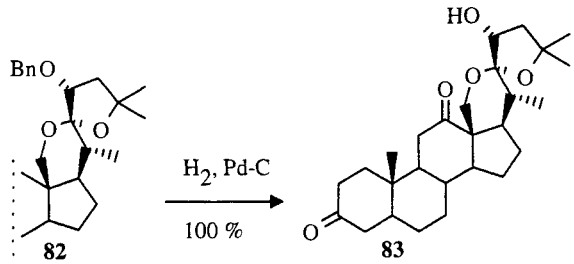
Scheme 33. Elaboration of 5/5 spiroketal of dihydrocephalostatin 1.

Attempts at producing X-ray quality crystals of the spiroketals were unsuccessful. The compounds were treated with fluoride to effect C-3 deprotection, and nOe effects used to assign C-12 stereochemistry. Oxidation of the diols gave ketones **79** and **80** (Scheme 34), whose structures were determined by 2D-NMR experiments.



Scheme 34. Equilibration of the 5/5 spiroketal.

According to molecular mechanics calculations, the C-23 benzyl group favours equatorial attack of the alcohol on the oxonium ion intermediate during spiroketalization, hence explaining the kinetic preference for product **77**. The calculations also revealed that **79** and **80** should be less stable than their diastereomeric spiroketals by approximately 2 kcal/mol. Indeed, heating **79** with camphorsulfonic acid gave a new ketone **81**, while similar treatment of **80** produced **82** (Scheme 34). Upon extended reaction times, **81** was converted to 30 % of **82** (estimated to be approximately 3 kcal/mol lower in energy) along with decomposition products. This last reaction requires epimerization at C-20 through an oxonium ion-enol ether equilibration. Deprotection of the benzyl group in diketone **82** yielded **83** (Scheme 35), a dihydrocephalostatin 1 left half intermediate suitable for coupling with the right half.



Scheme 35. Preparation of a dihydrocephalostatin 1 left half monomer.

SUMMARY AND FUTURE PROSPECTS

The family of dimeric steroid-pyrazine alkaloids isolated from *Cephalodiscus* and *Ritteria* now stands at thirty members. There are undoubtedly other examples of this group of steroidal alkaloids that have yet to be discovered, and it is probable that members common to both sources will be found.

On the synthetic front, the discovery of these alkaloids has sparked interest in the construction of unsymmetrical pyrazines, and the methods developed will be useful in other settings as well. The Fuchs group has successfully accomplished landmark syntheses of tetrahydrocephalostatin 12, cephalostatin 7, cephalostatin 12, and ritterazine K and is clearly close to a synthesis of dihydrocephalostatin 1. These efforts have added significantly to the areas of steroid and spiroketal chemistry.

The ability to make unnatural cephalostatins will greatly aid our understanding of the biological potency of these compounds. For example, one half could be kept identical to a natural product, while varying the other with synthetic steroids prepared from commercially available materials. In this sense, the cephalostatins provide a unique opportunity for such experiments, as the steroid skeleton is a readily accessible and well understood scaffold in which the effects of particular substituents can be determined. The situation here is in stark contrast to certain other potent biologically active compounds such as taxol (which promotes microtubule assembly) and bryostatin (a protein kinase C inhibitor). In the latter cases, construction of the skeletal framework is a formidable enterprise, hindering further dissection of the biological activity.

The availability of larger quantities of biologically active material, whether from natural or synthetic sources, will unravel the site of action of these steroids, about which nothing is known at present. These alkaloids do not contain functional groups commonly assisted with antitumour agents e.g. alkylation and Michael acceptor sites, intercalators, redox-active quinonoid groups, and radical generators. The range of biological activity among the various natural products contains some tantalizing structure/activity clues which are difficult to fully decipher.

Soon after the 1988 communication on cephalostatin 1, it was predicted[41] that the compound acts on the cell membrane. Steroids are components of eukaryotic cell membranes, where they incorporate into one half of the phospholipid bilayer and provide rigidity. Taking into account the dimeric nature of the cephalostatins, these steroids may now traverse the full length of the bilayer (for example, cephalostatin 1 is 30 Å x 9 Å x 5 Å) and adversely affect its properties. A number of other highly oxygenated marine natural products (e.g. brevetoxin, palytoxin) are also membrane active agents.

Fuchs has made two suggestions on the cephalostatins' mechanism of action. First,[16] the oxygenated functional groups of these compounds may form a network of hydrogen bond donors and acceptors that interacts with a specific enzyme target. More recently,[38] Fuchs has implicated the C-14,15 alkene as being important in biological activity, and there is some evidence[42] supporting this hypothesis. He points out that the C-14,15 alkene may be susceptible to electrophilic attack *in vivo*, either by protonation or epoxidation, followed by a rearrangement similar to that postulated in Scheme 3 (*vide supra*) which would generate a number of reactive centres in the molecule.

It will be interesting to see if the CD-ring skeletal rearrangement can be achieved in the laboratory, and if the reactive intermediates can alkylate DNA, for example. Another potentially reactive site in these molecules is the spiroketal ring system. Perhaps this undergoes ring opening to generate reactive carbonyl and free alcohol groups.

Finally, returning to the title of this chapter, the necessity for these compounds to be dimeric remains unclear. It is not known if any of the advanced synthetic mono-steroid intermediates prepared also exhibit high cytotoxicity. Furthermore, whether the central pyrazine ring is simply a linker or serves some additional function is also a mystery. This could be tested by examining the biological activity of unnatural dimers with other linkages e.g. benzene rings, other heterocyclic systems, or even acyclic tethers.

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REFERENCES

[1] Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985-994.

[2] Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006-2007.

[3] Pettit, G. R.; Inoue, M.; Kamano, Y.; Dufresne, C.; Christie, N.; Niven, M. L.; Herald, D. L.; *J. Chem. Soc., Chem. Commun.* **1988**, 865-867. Also see *J. Chem. Soc., Chem. Commun.* **1988**, 1440 for correction of a minor typographical error in the structures.

[4] Pettit, G. R.; Kamano, Y.; Dufresne, C.; Inoue, M.; Christie, N.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *Can. J. Chem.* **1989**, *67*, 1509-1513.

[5] Pettit, G. R.; Kamano, Y.; Inoue, M.; Dufresne, C.; Boyd, M. R.; Herald, C. L.; Schmidt, J. M.; Doubek, D. L.; Christie, N. D. *J. Org. Chem.* **1992**, *57*, 429-431.

[6] Pettit, G. R.; Xu, J.; Williams, M. D.; Christie, N. D.; Doubek, D. L.; Schmidt, J. M.; Boyd, M. R. *J. Nat. Prod.* **1994**, *57*, 52-63.

[7] Pettit, G. R.; Ichihara, Y.; Xu, J.; Boyd, M. R.; Williams, M. D. *Bioorg. & Med. Chem. Lett.* **1994**, *4*, 1507-1512.

[8] Pettit, G. R.; Xu, J.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *Can. J. Chem.* **1994**, *72*, 2260-2267.

[9] Pettit, G. R.; Xu, J.-P.; Schmidt, J. M. *Bioorg. & Med. Chem. Lett.* **1995**, *5*, 2027-2032.
[10] Pettit, G. R. *Pure & Appl. Chem.* **1994**, *66*, 2271-2281.
[11] Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1994**, *59*, 6164-6166.
[12] Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 608-614.
[13] Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707-6716.
[14] For example, see: Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753-1769.
[15] (a) Ohta, G.; Koshi, K.; Obata, K. *Chem. Pharm. Bull.* **1968**, *16*, 1487-1497. (b) Smith, H. E.; Hicks, A. A. *J. Org. Chem.* **1971**, *36*, 3659-3668.
[16] Pan, Y.; Merriman, R. L.; Tanzer, L. R.; Fuchs, P. L. *Bioorg. & Med. Chem. Lett.* **1992**, *2*, 967-972.
[17] Ganesan, A. Ph.D. dissertation, University of California-Berkeley, 1992.
[18] Edwards, O. E.; Purushothaman, K. K. *Can. J. Chem.* **1964**, *42*, 712-716.
[19] (a) Smith, S. C.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 6379-6380. (b) Full paper: Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828-6839.
[20] Ganesan, A.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 6155-6157.
[21] Kramer, A.; Ullmann, U.; Winterfeldt, E. *J. Chem. Soc., Perkin Trans. I* **1993**, 2865-2867.
[22] Welzel, P.; Janssen, B.; Duddeck, H. *Leibigs Ann. Chem.* **1981**, 546-564.
[23] Jeong, J. U.; Fuchs, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 773-774.
[24] Marker, R. E.; Wagner, R. B.; Ulshafer, P. R.; Wittbecker, E. L.; Goldsmith, D. P. J.; Ruof, C. H. *J. Am. Chem. Soc.* **1947**, *69*, 2167-2230. For modifications, see: (a) Dauben, W. G.; Fonken, G. J. *J. Am. Chem. Soc.* **1954**, *76*, 4618-4619. (b) Micovic, I. V.; Ivanovic, M. D.; Piatak, D. M. *Synthesis* **1990**, 591-592.
[25] (a) Suryawanshi, S. N.; Fuchs, P. L. *J. Org. Chem.* **1986**, *51*, 902-921. (b) Jain, S.; Shukla, K.; Mukhopadhyay, A.; Suryawanshi, S. N.; Bhakuni, D. S. *Synth. Commun.* **1990**, *20*, 1315-1320.
[26] Corey, E. J.; Da Silva Jardine, P.; Virgil, S.; Yuen, P.-W.; Connell, R. D. *J. Am. Chem. Soc.* **1989**, *111*, 9243-9244.
[27] Jeong, J. U.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 5385-5388.
[28] Kim, S.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 7163-7166.
[29] Sharpless, K. B.; Gao, Y. *J. Am. Chem. Soc.* **1988**, *110*, 7538-7539.
[30] Reich, H. J.; Peake, S. L. *J. Am. Chem. Soc.* **1978**, *100*, 4888-4889.
[31] Henry, Jr. K. J.; Grieco, P. A.; Jagoe, C. T. *Tetrahedron Lett.* **1992**, *33*, 1817-1820.
[32] Kim, S.; Sutton, S. C.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 2427-2430.
[33] Barton, D. H. R.; Basu, N. K.; Hesse, R. H.; Morehouse, F. S.; Pechet, M. M. *J. Am. Chem. Soc.* **1966**, *88*, 3016-3021.
[34] Jeong, J. U.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *36*, 2431-2434.
[35] Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768-2771.
[36] Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157-10158.
[37] Li, C.; Shih, T.-L.; Jeong, J. U.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 2645-2646.
[38] Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *46*, 8347-8350.
[39] Heusler, K.; Wieland, P.; Meystre, C. H. *Organic Synthesis* **1965**, *45*, 57-63.
[40] Bhandaru, S.; Fuchs, P. L. *Tetrahedron Lett.* **1995**, *46*, 8351-8354.
[41] Ganesan, A.; Heathcock, C. H. *Chemtracts-Org. Chem.* **1988**, *1*, 311-312.
[42] Unpublished results from E. Winterfeldt's group, quoted in reference 19(b).