2 The Pederin Family of Antitumor Agents: Structures, Synthesis and Biological Activity

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2.1 Structures

In 1775 the Danish entomologist Johann Christian Fabricius (1745–1808) first described the genus *Paederus*, which at that time included only two species. In the ensuing two centuries, over 600 species have been identified, including *Paederus fuscipes*, whose natural history deserves some mention (Frank and Kanamitsu 1987). *Paederus fuscipes* is about 8 mm long with a black head and abdominal apex, an orange thorax and abdominal base and iridescent blue elytra (wing case). It inhabits riverbanks, marshes, and irrigated fields, where it feeds mainly on insects, mites, soil nematodes, and decaying vegetable matter. Like most members of the genus, *Paederus fuscipes* is a predator of the fly population, but it is also a pest to man. The insect does not sting or bite, but a toxin in its hemolymph causes severe dermatitis when it is crushed on the skin, and the eyes are particularly sensitive though the palms of the hands and the soles of the feet are resistant. In addition to the lesions, severe symptoms such as fever, edema, neuralgia, arthralgia,

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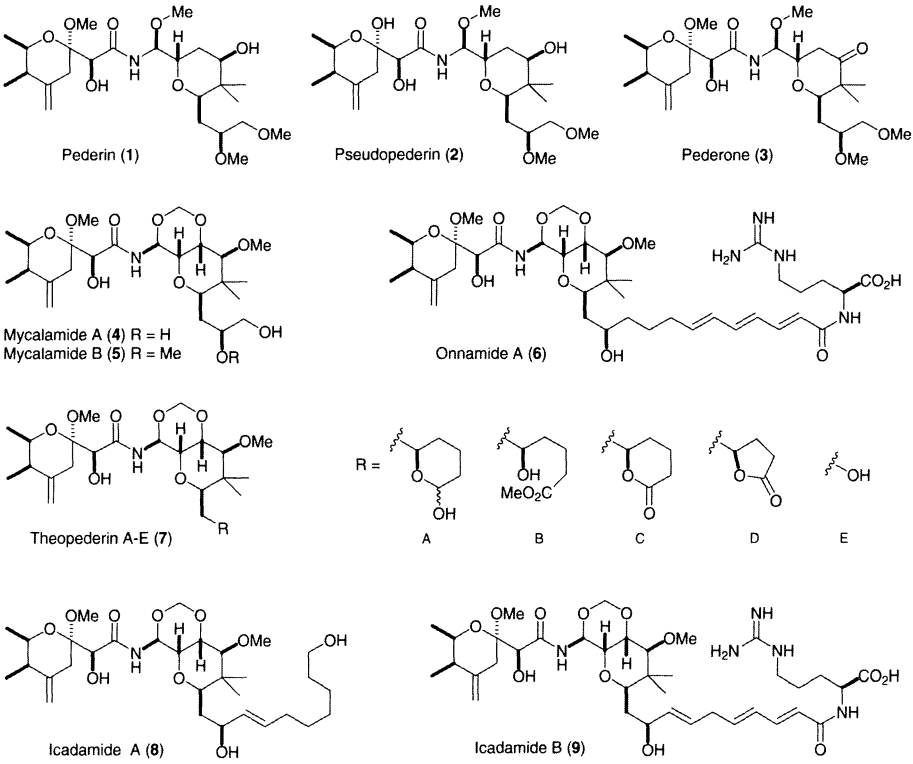
and vomiting are observed with erythema persisting for several months. It has been suggested (Frank and Kanamitsu 1987) that both the affliction and its causative agent were known to Chinese medicine over 1200 years earlier. An insect called *ch'ing yao ch'ung* was described by Ch'en in 739 A.D.: "It contains a strong poison and when it touches the skin it causes the skin to swell up. It will take the skin off one's face and remove tattoo marks completely. It is used as a caustic for toxic boils, nasal polypi, and ringworm."

The active chemical agent responsible for the dermatitis was first isolated in crystalline form by Netolitzky in 1919 (Netolitzky 1919). The research which eventually led to the correct structure began in 1952 with Pavan and Bo (1953), who named the toxic agent pederin and determined its melting point (112°C) on a sample derived from 25 million specimens (ca. 100 kg). The correct molecular formula ($C_{25}H_{45}O_9N$) established by Quilico et al. (1961) in 1961 led to detailed study of the chemical constitution of pederin and a structure, devoid of stereochemical definition, was proposed in 1965 (Cardani et al. 1965). An independent investigation by Matsumoto's team at Sapporo gave corroborating evidence (Matsumoto et al. 1964, 1968). With one minor exception (see below) all the conclusions drawn from the degradation and ^1H NMR studies of Quilico et al. (1966) and Matsumoto were later confirmed by an X-ray crystallographic analysis of pederin di-*p*-bromobenzoate (Furusaki et al. 1968; Bonamartini Corradi et al. 1971), which also established the absolute and relative stereochemistry.

Single *Paederus riparius* specimens reared from the egg and kept for prolonged periods of time show that only the females are able to biosynthesize pederin (Kellner 1998; Kellner and Dettner 1995, 1996). Preimaginal stages efficiently store pederin transferred by the females into their eggs, and the males' pederin content decreases slowly over time. Only males with access to eggs containing the substance moderately increase their pederin load. The females begin to accumulate the toxin a few weeks after imaginal eclosion and build up reserves for the egg-laying period within 60 days.

For many years, pederin (**1**), pseudopederin (**2**) (Cardani et al. 1965), and pederone (**3**) (Cardani et al. 1967) (also isolated from *Paederus fuscipes*) were structurally unique in the realm of natural products (Scheme 1). However, in 1988 routine screening for antiviral agents identified two marine natural products which bore a close structural



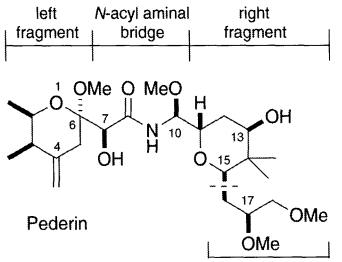
Scheme 1.

resemblance to pederin. Mycalamide A (**4**) was isolated from a sponge of the genus *Mycale*, found in the Otago harbor off New Zealand (Perry et al. 1988, 1990), while onnamide A (**6**) was isolated from a sponge of the genus *Theonella* found in Okinawan waters (Sakemi et al. 1988). The pederin family had grown to 24 members by 1995 with the isolation of mycalamide B (**5**) (Perry et al. 1990), a further 11 onnamides (Matsunaga et al. 1992; Kobayashi et al. 1993), and theopederins A–E (**7**) (Fusetani et al. 1992). The most recent additions to the pederin family are icadamides A (**8**) and B (**9**), isolated from a sponge of the genus *Leiosella* (Clardy and He 1995). The significance of the mycalamides, onnamides, theopederins, and icadamides in sponge physiology is un-

clear, although it has been suggested that the occurrence of closely related compounds in such taxonomically remote animals as sponges and terrestrial beetles may indicate connection by a common producer, possibly a symbiotic micro-organism (Fusetani and Sugawara 1993).

2.2 Synthetic Studies

All members of the pederin family are rare, difficult to isolate, and comparatively frail; many of them have potent and potentially useful activity as antiviral and antitumor agents (see below); this has stimulated considerable interest in their total synthesis. Total syntheses of pederin (Matsuda et al. 1982, 1983, 1988; Nakata et al. 1985a,b; Willson et al. 1987; Jarowicki et al. 1990; Kocienski et al. 1991, 1998a) , mycalamide A (Hong and Kishi 1990; Nakata et al. 1994, 1996), mycalamide B (Hong and Kishi 1990; Kocienski et al. 1998b), onnamide A (Hong and Kishi 1991), and theopederin D (Kocienski et al. 1998a) have been reported, as have significant syntheses of various fragments (Nakata et al. 1994; Isaac and Kocienski 1982; Isaac et al. 1983; Kocienski and Willson 1984; Matsumoto et al. 1984;Willson et al. 1990a,b; Hoffmann and Schlapbach 1992, 1993; Roush and Marron 1993; Toyota et al. 1995, 1998a,b; Marron and Roush 1995; Roush et al. 1997; Roush et al. 1998; Breitfelder et al. 1998; Trotter et al. 1999). The parent member of the family, pederin itself, is also the simplest. It is composed of two tetrahydropyran rings (a left fragment and a right fragment) connected by an *N*-acyl aminal bridge, as depicted in structure **10** (Scheme 2). The left fragment of all members of the pederin family is identical but all



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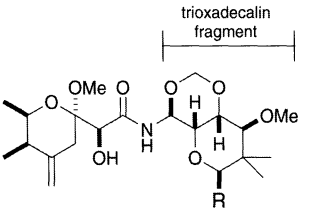

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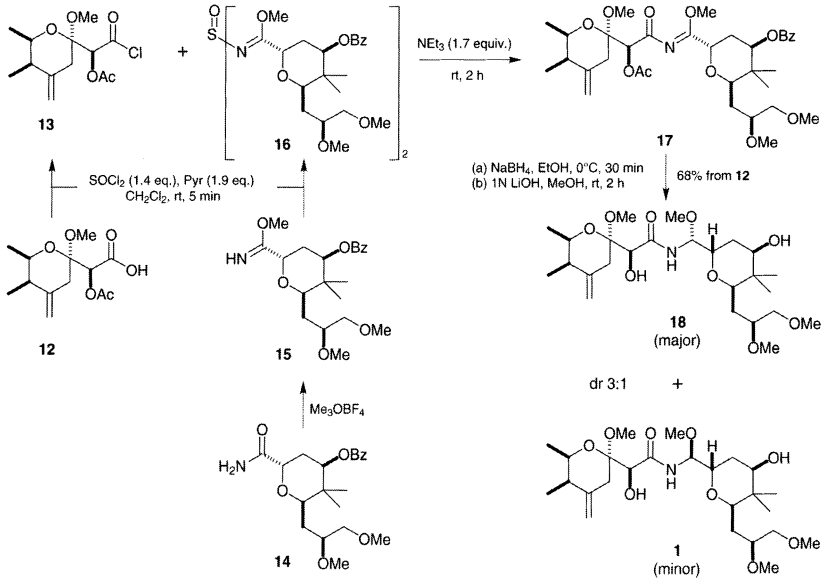
Scheme 2.

remaining members of the family (the mycalamides, onnamides, theopederins, and icadamides) have a trioxadecalin right fragment as shown in structure **11**. The principal site of structural variation is the side chain attached to C15. The common left fragment also imposes a common problem: the high acid lability associated with the alkene at C4, which activates the acetal function at C6. A comprehensive discussion of the syntheses of members of the pederin family is beyond the scope of this review, so we will focus on one of the most demanding challenges: the construction of the *N*-acyl aminal bridge linking the left and right fragments. The following discussion is arranged according to the bond formed in the fragment linkage strategy.

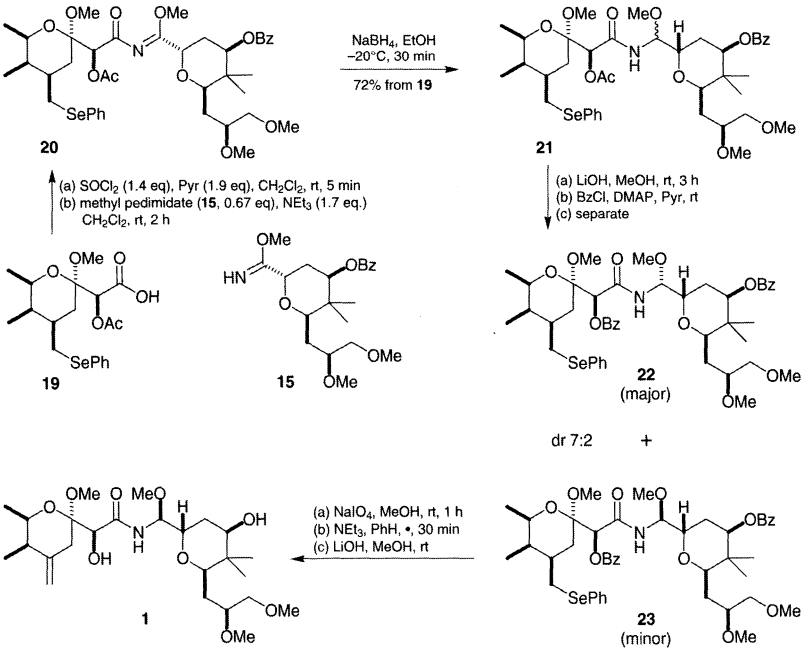
2.2.1 The C8–N9 Connection via Imidate Ester Acylation

Matsumoto and his team reported the first total synthesis of pederin in 1982, in which the left and right fragments were connected by the *N*-acylation of an imidate ester (Scheme 3) (Matsuda et al. 1982;



Scheme 3.

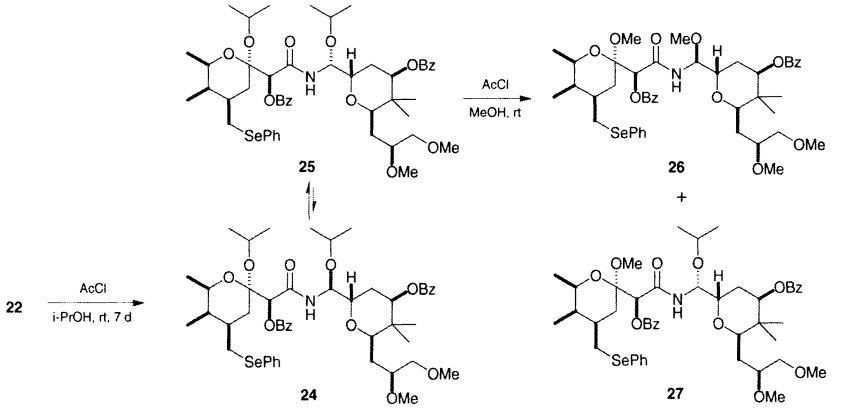
Yangiya et al. 1982). The crucial coupling reaction was accomplished by *brief* treatment of (+)-acetylpederic acid (**12**) with thionyl chloride in the presence of pyridine in dichloromethane, followed by addition of methyl pedimidate (**15**). The reaction time had to be minimized owing to the instability of the acid chloride intermediate **13**. Unfortunately, the highly hindered carboxylic acid was converted slowly to the acid chloride **13** under the reaction conditions, thereby allowing time for the reaction of imidate ester **15** with thionyl chloride to give *N,N'*-sulfinylbis(methyl pedimidate) (**16**), which can also serve as a substrate in the acylation of acid chloride **13**. Thus, the formation of *N*-acyl imidate intermediate **17** occurred by two different paths concurrently. The *N*-acyl aminal bridge was then constructed by reduction of **17** with sodium borohydride. The synthesis was completed by hydrolysis of the benzoate and acetate ester protecting groups to give a mixture of pederin (**1**) and 10-*epi*-pederin (**18**) in a ratio of 1:3 [68% overall from (+)-benzoylpedamide (**14**)], with pederin being the minor product.



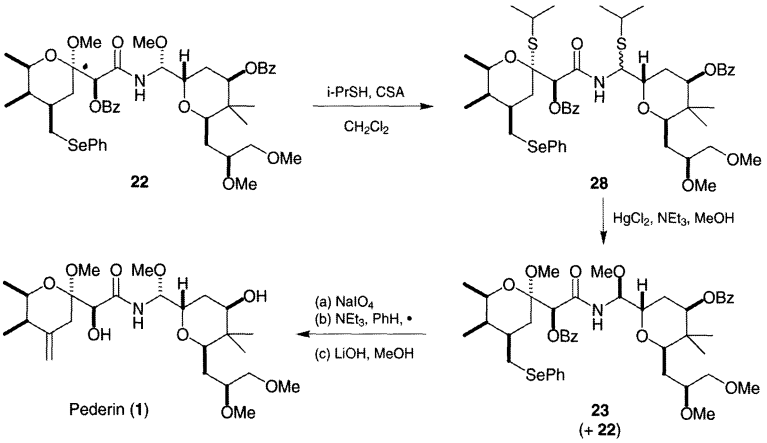
Scheme 4.

The high acid sensitivity of the homoallylic acetal in (+)-acetylpederic acid (**12**) was a major obstacle to Matsumoto's first synthesis. In a subsequent refinement, the C4 alkene was carried through the synthesis in latent form, with the troublesome alkene being introduced in the penultimate step of the synthesis as shown in Scheme 4 (Matsuda et al. 1983). However, the reduction of *N*-acyl imidate **20** once again afforded an unfavorable mixture of *N*-acyl aminals **21** in 72% overall yield (dr 7:2). After chromatographic separation, the requisite alkene was introduced by brief thermolysis of the selenoxide derived from oxidation of the minor selenoether **23**, whereupon hydrolysis of the C7 and C13 benzoates afforded pederin (**1**).

The poor stereoselectivity in the reduction of the *N*-acyl imidates **17** and **20** in the foregoing studies was beyond repair, and so isomerization of the *N*-acyl aminal in the 10-*epi*-pederin series was investigated. Transacetalization of 10-*epi*-pederin derivative **22** with acetyl chloride in methanol (rt, 3 h) gave an equilibrium mixture (**22**:**23** = 3:1), showing that the undesired 10-*epi* series was the thermodynamic product. The possibility of selective conversion of the 10-*epi*-pederin derivative **22** into pederin derivative **23** under *kinetically* controlled conditions was next examined, by taking into account the acceleration effect of the alkoxy-exchange reaction in methanol by a large alkoxy group (Matsuda et al. 1988). Thus, treatment of **22** with acetyl chloride (Scheme 5) in isopropanol gave **25** selectively after 7 days through initial formation of the kinetically controlled product **24**. Compound **25** was unstable and



Scheme 5.



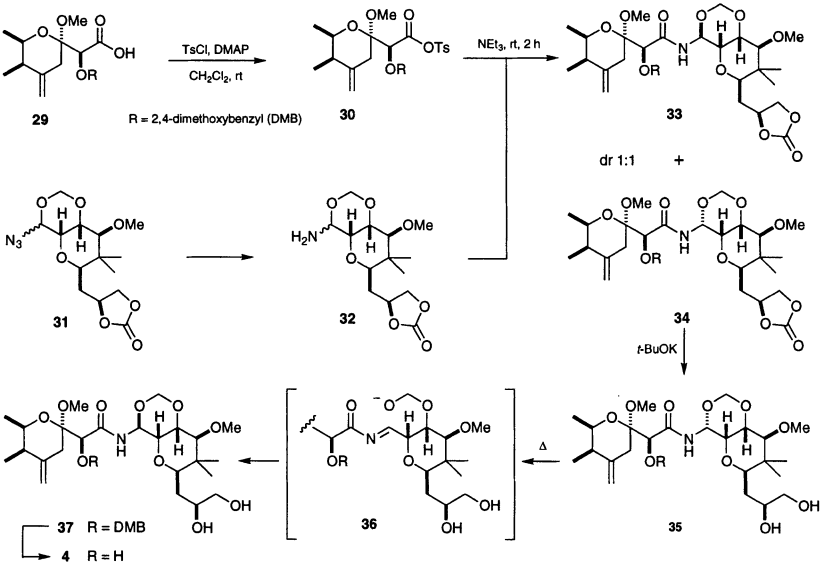
Scheme 6.

could not be isolated in a pure state, but kinetically controlled transacetalization of **25** with acetyl chloride in methanol (rt, 4.5 h, 50% conversion) proceeded in a stereoselective manner to give a 60% isolated yield (based on consumed **25**) of **22** and a 14% yield of **26** (**26:22** = 4:1). The 10α-isopropoxy compound was recovered in 42% yield in the form of a 6α-methoxy compound (**27**).

Nakata and his associates (1985a,b) discovered that reduction of the 7-*O*-benzoyl analogue of *N*-acyl imidate **20** with $NaBH_4$ in a mixture of isopropanol and CH_2Cl_2 gave **23** (28%) and **22** (30%) (Scheme 6). The 10-*epi* derivative **22** was converted into **23** by reaction of **22** with 2-propanethiol and camphorsulfonic acid (CSA) in CH_2Cl_2 to give thioacetal **28**, which was then treated with $HgCl_2$ in MeOH in the presence of NEt_3 to give **23** in 47% yield (from **22**) along with epimer **22** (36%). The completion of the synthesis was performed as previously described by Matsumoto.

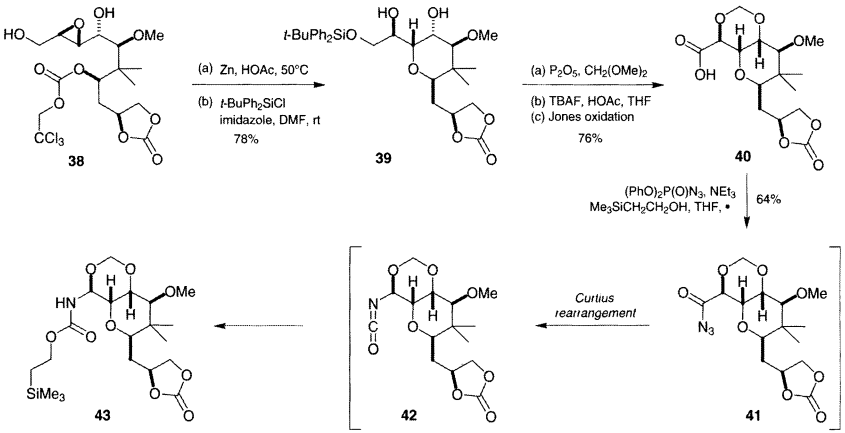
2.2.2 The C8–N9 Connection via Aminal Acylation

In the Matsumoto-Nakata approaches to pederin, the *N*-acyl aminal bridge was constructed by first condensing an activated carboxylic acid



Scheme 7.

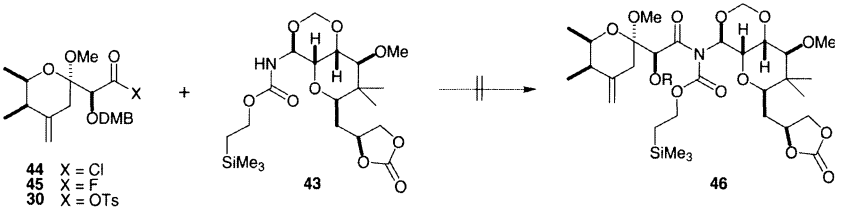
(e.g., acid chloride **13**) with an imidate ester to give an *N*-acyl imidate, which was reduced with sodium borohydride to generate the *N*-acyl aminal – a reaction which gave poor stereoselectivity. In the mycalamides, onnamides, and theopederins, the incorporation of the oxygen atom of the aminal into a dioxane ring makes the Matsumoto-Nakata protocol less attractive, owing to the difficulty associated with the synthesis of the appropriate imidate ester. Therefore, the first syntheses of mycalamide A, mycalamide B (Hong and Kishi 1990), and onnamide A (Hong and Kishi 1991) by Kishi and Hong adapted the Matsumoto-Nakata protocol by condensing an aminal **32** with the activated carboxylic acid derivative **30**, as illustrated in Scheme 7. The C10 aminal unit of **32** is configurationally unstable under acidic, basic, and neutral conditions, and consequently a 1:1 mixture of adducts **33** and **34** was obtained. However, treatment of **34** with potassium *tert*-butoxide at room temperature first accomplished the transesterification of the carbonate group of **34** to the corresponding diol **35**, which then epimerized on heating to yield **37**. Owing to competing decomposition, the reaction



Scheme 8.

was stopped at approximately 60% completion to yield the epimerized natural diastereoisomer **37** in 42% yield along with the unnatural diastereoisomer **35** (33% yield). Nakata has also described a synthesis of mycalamide A in which the *N*-acyl aminal bridge is constructed by the Kishi procedure (Nakata et al. 1996).

In 1993 Roush and Marron described a route to the mycalamides which incorporates two significant advances. First, the stereochemistry of the aminal was assured by a Curtius rearrangement, and second, the configuration of the aminal center was stabilized as a temporary carbamate appendage. The steps leading up to the Curtius rearrangement are noteworthy. Reductive cleavage of the 2,2,2-trichloroethyl carbonate **38** (Scheme 8) with Zn released an alkoxide that performed a nucleophilic attack on the neighboring oxirane to give a cyclic diol, which was selectively protected as its mono-*tert*-butyldiphenylsilyl ether **39** (Marron and Roush 1995). After closure of the 1,3-dioxane ring, the *tert*-butyldiphenylsilyl ether was cleaved and the resultant alcohol oxidized to the corresponding carboxylic acid **40**. The key Curtius rearrangement was performed by treatment of acid **40** with diphenylphosphoryl azide at 65°C. The intermediate acyl azide **41** underwent the desired Curtius rearrangement with clean retention of configuration to the isocyanate **42**, which was trapped with 2-trimethylsilylethanol to give the car-



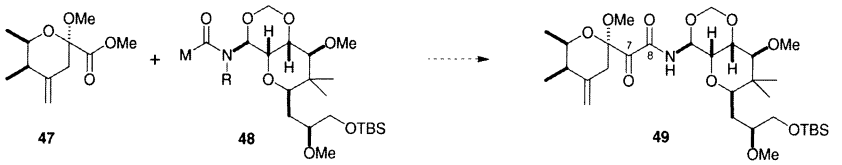
Scheme 9.

bamate **43** as a single diastereoisomer. Hoffmann published an approach to the trioxadecalin ring system of the mycalamides in 1993; it was similar to that of Roush, in that it included a Curtius rearrangement to form the C10-aminal diastereoselectively (Hoffmann and Schlapbach 1993).

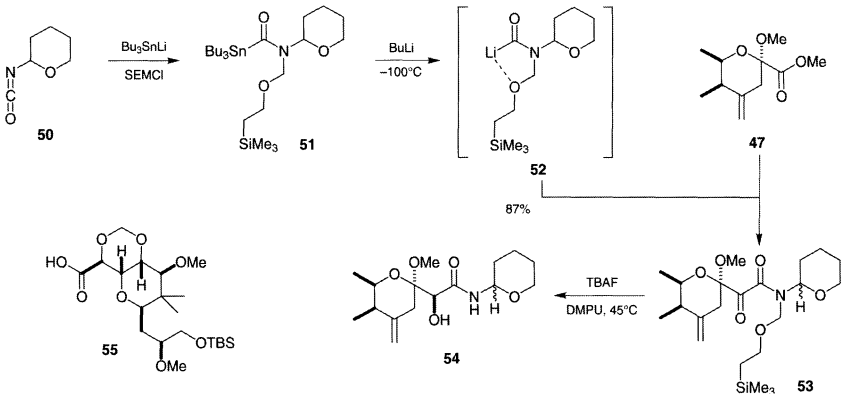
Roush and co-workers later published a very brief route to the pederic acid derivatives **44**, **45**, and **30** and they achieved some success in the construction of *N*-acyl aminal bridges with model systems, but the high steric hindrance of the carboxyl group in the left fragments once again thwarted condensation with the carbamate **43** (Scheme 9) under a wide variety of conditions, and none of the desired adduct **46** was observed (Roush et al. 1997, 1998; Roush and Pfeifer 1998).

2.2.3 The C7–C8 Connection

In 1996 Hoffmann and co-workers proposed an original strategy to the construction of the *N*-acyl aminal bridge of mycalamide B based on union of the ester **47** with the acyl anion **48** to forge the C7–C8 bond (Scheme 10). Once again, the stereochemistry of the aminal is to be controlled by a Curtius rearrangement.

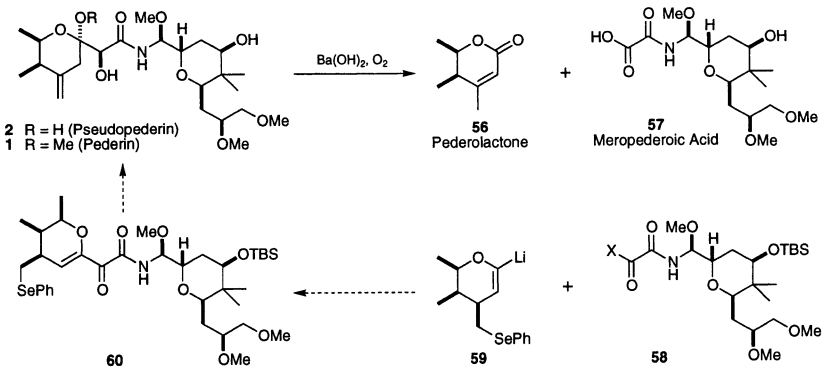


Scheme 10.



Scheme 11.

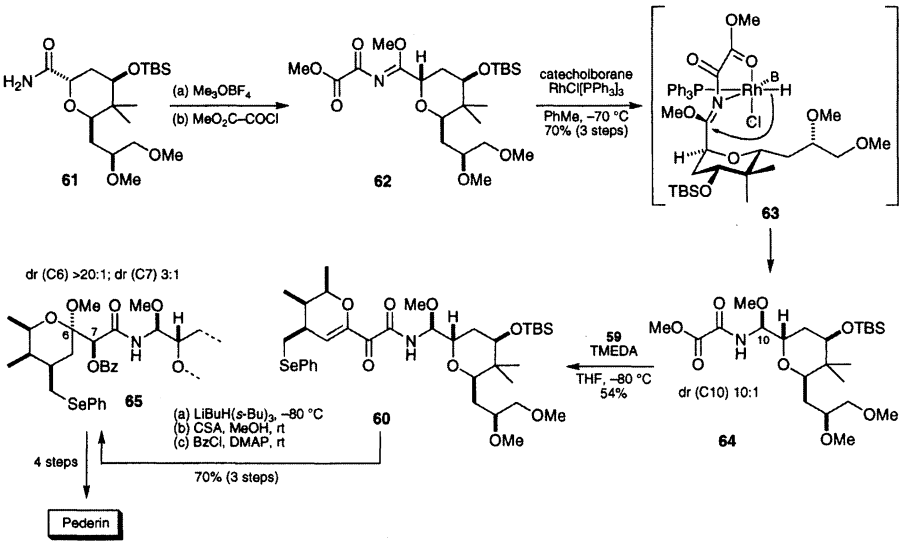
Model studies for the novel coupling step were carried out with the commercially available isocyanate **50** as a surrogate for the right fragment. The acylstannane **51** (Scheme 11), protected as its 2-(trimethylsilyl)ethoxymethoxy (SEM) derivative, was easily prepared by addition of Bu_3SnLi to the isocyanate **50** followed by *N*-alkylation with 2-(trimethylsilyl)ethoxymethyl chloride. The use of SEM protection for the N-atom was a judicious choice, the purpose being to stabilize the highly labile acyllithium **52** which was generated and trapped in situ by transmetallation with BuLi at –100°C. The desired model adduct **53** was obtained in 87% yield. A fortuitous and unprecedented reduction of the α-oxo ester accompanied the deprotection of the SEM group in adduct **53** with TBAF in *N,N′*-dimethylpropyleneurea (DMPU) to give the desired α-hydroxyamide **54** as a mixture of diastereoisomers (dr 3:1). The origin of the hydride for the reduction of the keto function is unknown. Progress towards implementation of the strategy has been reported: a synthesis of **55** from D-arabinose has been accomplished (Breitfelder et al. 1998), but union of the fragments remains elusive.



Scheme 12.

2.2.4 The C6–C7 Connection: The Metallated Dihydropyran Approach

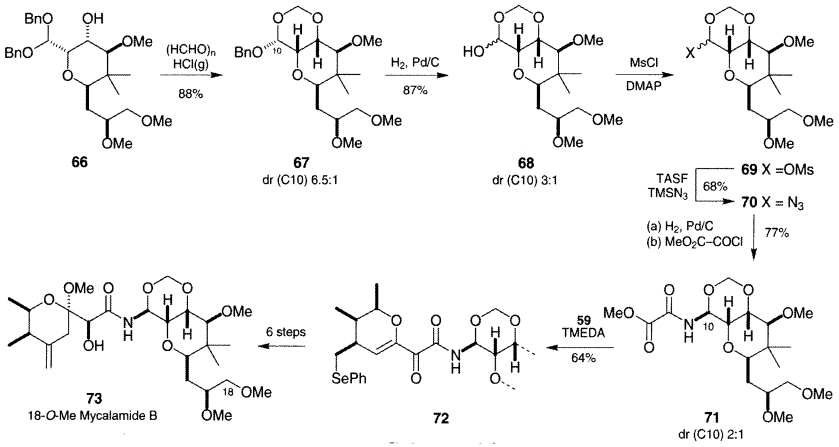
In our early approaches to the synthesis of pederin, we chose the well-used Matsumoto-Nakata protocol to construct the *N*-acyl aminal bridge (Willson et al. 1987; Isaac and Kocienski 1982; Isaac et al. 1983; Kocienski and Willson 1984; Willson et al. 1990a,b). We were similarly chastened by the difficulty of reconciling slow reactions with unstable reagents, which has been a persistent feature of nearly all of the accounts reported to date. The problem was compounded by the poor stereoselectivity of the subsequent reaction of the *N*-acyl imidate adducts. In the mid 1980s we turned to a new strategy which attempted to alleviate the problems associated with the high steric hindrance surrounding the acetal center at C6. Our new strategy was inspired by some of the elegant degradation studies conducted by Quilico and co-workers during their structure elucidation of pederin (Cardani et al. 1966). Pseudopederin (**2**, Scheme 12), the hydrolysis product of pederin, undergoes an easy retroaldol reaction on heating in base in the presence of air to give pederolactone (**56**) and meropederoic acid (**57**), wherein the *N*-acyl aminal group is still intact. These transformations suggested an alternative disconnection between C6 and C7 that circumvented the cascade of problems which beset the closing stages of the previous syntheses. The new strategy required a metallated dihydropyran **59**



Scheme 13.

functioning as an acyl anion equivalent in reaction with a suitably activated meropederoic acid derivative **58** (Jarowicki et al. 1990; Kocienski et al. 1991).

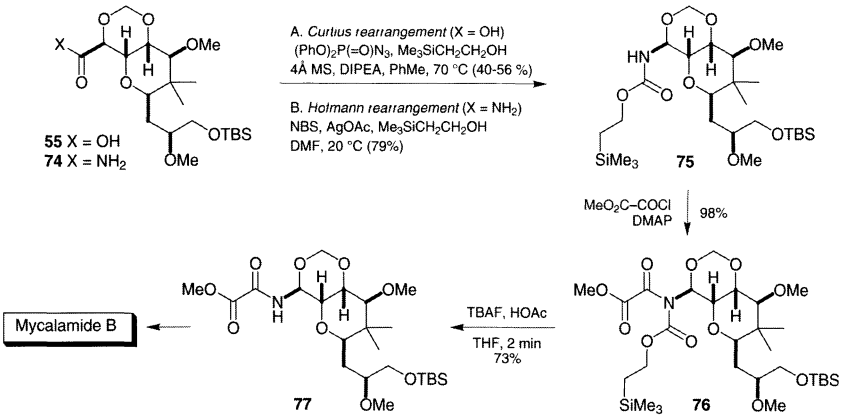
Amide **61** was converted to the *N*-acylimidate **62** in two steps using standard transformations. The imidate ester intermediate **62** was prone to hydrolysis, but good yields were obtained by working fast and with minimal purification. Reduction of the *N*-acylimidate **62** was achieved by using an unprecedented reaction – reduction with catecholborane in the presence of a catalytic amount of $[Ph_3P]_3RhCl$. Under these conditions a 70% yield of a mixture of diastereoisomeric *N*-acyl aminals was obtained in which the desired isomer **64** predominated (10:1). Thus, for the first time, a metal hydride reduction of an *N*-acyl imidate in the pederin series afforded appreciable selectivity in favor of the desired diastereoisomer at C10. The stereochemistry of the reduction was interpreted in terms of an intermediate **63**, in which an octahedral Rh complex delivers a hydride intramolecularly as indicated in Scheme 13.



Scheme 14.

The key reaction of the sequence entailed addition of the metallated dihydropyran **59** to the methyl ester **64** in the presence of TMEDA at low temperature to give a 54% yield of the adduct **60**. With the bulk of the pederin skeleton now constructed, completion of the synthesis required merely the introduction of the two adjacent stereogenic centers at C6 and C7 and a few functional group transformations. The stereogenic center at C7 was introduced by metal hydride reduction of the enone function in **60**. Use of the bulky reducing agent LiBH(*s*-Bu)$_3$ afforded the desired diastereoisomer in modest diastereoselectivity (3:1). Addition of methanol to the dihydropyran occurred with excellent diastereoselectivity (dr=20:1). Completion of the synthesis required four further steps which were well precedented.

The success of the metallated dihydropyran approach in the synthesis of pederin suggested an easy adaptation of the strategy to syntheses in the mycalamide-theopederin series. An initial foray published in 1996 was superseded by tactical improvements, depicted in Scheme 14, for the synthesis of 18-*O*-methyl mycalamide B (Kocienski et al. 1996), which had been identified as the most potent of the mycalamide derivatives in assays against a series of human tumors (Richter et al. 1997; Thompson et al. 1992) (see below). The construction of the 1,3-dioxane ring was accomplished by treatment of **66** with paraformaldehyde in the



Scheme 15.

presence of HCl to give a mixture of diastereoisomeric 1,3-dioxane acetals **67** (dr=6.5:1) in 88% yield. Separation of acetals **67** by column chromatography was possible but useless, since hydrogenolysis of the benzyl group gave the same mixture of hemiacetals **68** (dr 3:1).

Replacement of the hydroxyl group in **68** by an azido group via substitution of the mesylate **69** by Bu_4NN_3 had been reported by Hong and Kishi (1990), but in our hands the yields ranged from 20% (typically) to 72% (rarely). We therefore developed a new method which, to our knowledge, is novel: the crude mesylate **69** derived from the mixture of hemiacetals **68** was treated with trimethylsilyl azide in the presence of tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) to give the azides **70** as a mixture of diastereoisomers in the ratio 1:1 to 2:1, depending on the reaction conditions. The isomers could be separated for purposes of characterization but in practice it was best to carry the mixture of azides forward to the next stage of the synthesis. Catalytic reduction of the azides **71** gave a sensitive mixture of aminals which were acylated with methyl oxalyl chloride in the presence of DMAP to afford the diastereoisomeric methyl oxalamides (1:2) in 77% yield. The diastereoisomers were separated by column chromatography and the minor crystalline diastereoisomer **71**, having the correct stereochemistry at C10, was added to a solution of the dihydro-2*H*-pyranyllithium reagent **59** in the presence of excess TMEDA to give the acylated dihydro-

2*H*-pyran derivative **72** in 64% yield. The remainder of the synthesis was conducted as described above for the synthesis of pederin (Kocienski et al. 1991).

The successful implementation of the metallated dihydropyran approach to 18-*O*-methyl mycalamide B (Scheme 14) was gratifying, but the unfavorable stereochemistry at the C10 aminal center was a blemish which eluded correction. Therefore, we examined the Roush-Hoffmann-Curtius protocol as part of a synthesis of mycalamide B (Kocienski et al. 1998b). Treatment of the carboxylic acid **55** (Scheme 15) with diphenylphosphoryl azide afforded an acyl azide intermediate that rearranged to an isocyanate, which was trapped by 2-(trimethylsilyl)ethanol to give the carbamate **75**. However, the elevated temperatures (70°C) required for the rearrangement resulted in some decomposition with an overall reduction in yield to 56% at best, with typical yields being more like 40%. A much better alternative was a classical Hofmann rearrangement using Ag(I)-assisted rearrangement of the *N*-bromoamide derived from amide **74** (Kocienski et al. 2000). The reaction occurred at room temperature with clean retention of configuration to give the carbamate **75** in 79% overall yield. The remaining 2-carbon fragment was installed by reaction of carbamate **75** with methyl oxalyl chloride in the presence of DMAP to yield the imide derivative **76**. To complete the sequence, the carbamate function was expunged using TBAF buffered with acetic acid to give the *N*-acyl aminal intermediate **77** of mycalamide B. A similar strategy was used in the first synthesis of a member of the theopederin family, theopederin D (**7D**) Kocienski et al. 1998a).

2.3 Biological Activity

2.3.1 The Natural Products

Pederin is a very weak antibacterial agent but it is highly toxic to eukaryotic cells. Ingestion can cause severe internal damage and intravenous injection causes death at levels which suggest that it is more potent than cobra venom. The toxicity of pederin appears to be related to its inhibition of protein biosynthesis and cell division. Using human tonsil ribosomes, Vazquez (1979; Carrasco et al. 1976) showed that pederin binds irreversibly to the ribosome, preventing translation of

mRNA. Inhibition occurred at the translocation step during the elongation cycle. The irreversible binding of pederin to ribosomes and its vesicant activity suggest it may function as an alkylating agent with the homoallylic acetal or *N*-acyl aminal as sites of potential reactivity. The biochemical and pharmacological activities are probably not related, however, since the hydrogenated derivative dihydropederin is not a vesicant though it remains a potent inhibitor of protein biosynthesis. Unfortunately, there have been very few attempts to evaluate the clinical potential of pederin. Pavan (1982) demonstrated that elderly patients with chronic necrotic and purulent sores completely recovered in some cases after treatment with minute amounts of pederin. A Russian study has also revealed a therapeutic effect on eczema and neurodermatitis with no complications (Mikhailova 1967). Potential use as an anticancer agent has been suggested based on the ability of pederin to block mitosis in normal and tumor cells at doses of 1 ng/ml and on reports that it inhibited sarcoma-180 tumors in mice (Soldati 1966).

Like pederin, the mycalamides and their derivatives induce severe dermatitis. Mycalamides A and B reveal potent in vitro cytotoxicity and in vivo antitumor efficacy against several leukemia and solid tumor model systems, as well as antiviral activity. They inhibit in vitro replication of murine lymphoma P388 cells (IC_{50} 3.0±1.3 and 0.7±0.3 ng/ml, respectively, and human promyelocytic (HL-60), colon (HT-29), and lung (A549) cells (IC_{50} <5 n*M*) (Burres and Clement 1989). Mycalamide A was also active against B16 melanoma, Lewis lung carcinoma, M5076 ovarian carcinoma, colon 26 carcinoma, and the human MX-1 (mammary), CX-1 (colon), LX-1 (lung), and Burkitt's lymphoma tumor xenografts (Burres and Clement 1989). Mode of action studies confirm that the mycalamides, like pederin, are protein synthesis inhibitors. Mycalamide A also disrupts DNA metabolism but does not intercalate into DNA itself. A correlation between their relative ability to inhibit protein synthesis, their cytotoxicity, and their in vivo efficacy suggests that their antitumor activity is a consequence of protein synthesis inhibition (Burres and Clement 1989).

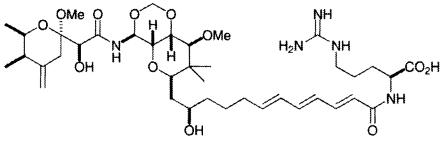
Antiviral assays on mycalamide A by the Munro group (Perry et al. 1988, 1990) indicated that the minimum dose that inhibited the cytopathic effect of the test viruses (herpes simplex type-1 and polio type-1 viruses) over a whole (17 mm) well was 5 ng/disk. No in vivo antiviral results on pure mycalamide A were available from the initial screen, but

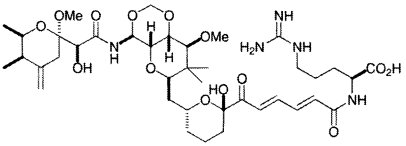
Table 1. IC_{50} values (ng/ml) in vitro for members of the pederin family against murine P388 leukemia cells

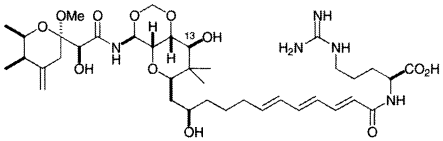
Compound[a]	Munro[b]	Fusetani[c]	Fusetani[d]	Kobayashi[e]
Pederin (**1**)	0.07		–	–
Mycalamide A (**4**)	0.5		–	–
Mycalamide B (**5**)	0.1	–	–	–
Theopederin A (**7A**)	–	0.05	–	–
Theopederin B (**7B**)	–	0.1	–	–
Theopederin C (**7C**)	–	0.7	–	–
Theopederin D (**7D**)	–	1.0	–	–
Theopederin E (**7E**)	–	9.0	–	–
Onnamide A (**6**)	0.4	–	10	2.0
13-des-*O*-Methyl-onnamide A (**78**)	–	–	150	–
21,22-Dihydo-onnamide A (**79**)	–	–	40	4.6
Pseudo-onnamide A (**80**)	–	–	130	–
Onnamide B (**81**)	–	–	130	–
17-Oxo-onnamide B (**82**)	–	–	100	–
Onnamide C (**83**)	–	–	70	–
Onnamide D (**84**)	–	–	20	–
Onnamide E (**85**)	–	–	inactive	–
21,22-Dihydro-17-oxo-onnamide A (**86**)	–	–		16.0
17-Oxo-onnamide A (**87**)	–	–		9.2
4(Z)-Onnamide A (**88**)	–	–		1.5

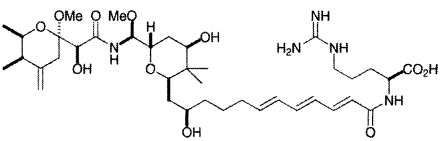
[a]For structures see Schemes 1 and 16.
[b]Perry et al. 1990; Thompson et al. 1992.
[c]Fusetani et al. 1992.
[d]Matsunaga et al. 1992.
[e]Kobayashi et al. 1993. Murine L1210 lymphoma cells were used.

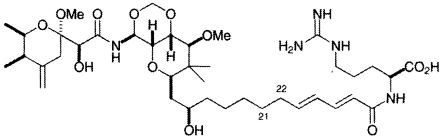
in vitro assays showed that it was responsible for the in vitro activity of the crude sponge extract and thus probably the in vivo activity as well. A crude extract (ca. 2% mycalamide A) was tested in mice infected with A59 coronavirus. Four mice dosed with virus and the extract at 0.1 mg/kg survived 14 days, while eight mice dosed with the virus only all died within 8 days. For further data relevant to the antiviral activity of mycalamide A see Sect. 3.4.

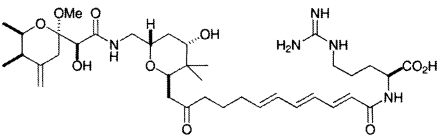

Onnamide A (**6**)

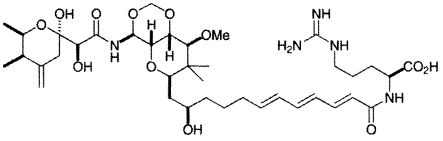

Onnamide C (**83**)

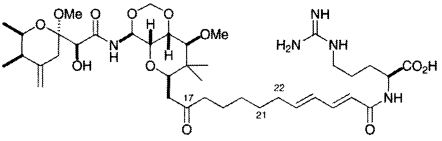

13-Des-*O*-methyl onnamide A (**78**)

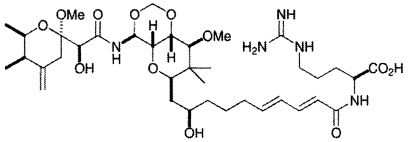

Onnamide D (**84**)

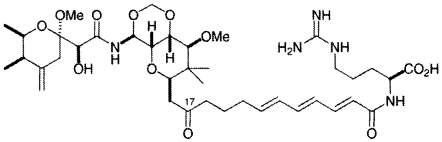

21,22-Dihydro-onnamide A (**79**)

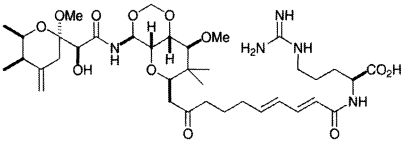

Onnamide E (**85**)

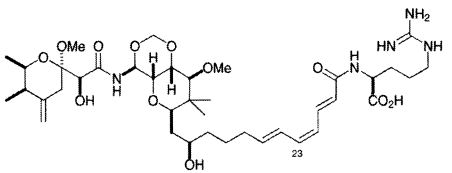

Pseudo-onnamide A (**80**)


21,22-Dihydro-17-oxo-onnamide A (**86**)


Onnamide B (**81**)


17-Oxo-onnamide A (**87**)


17-Oxo-onnamide B (**82**)


23(*Z*)-Onnamide A (**88**)

Scheme 16.

Onnamide A is approximately equivalent in potency to mycalamides A and B against murine P388 leukemia cells (IC_{50} 2.4±0.3 n*M*) in vitro but it was inactive against P388 cells in vivo (15% increase in life span at 40 μg/kg). Onnamide A is about 70 times less active against HL-60, HT-29, and A549, in line with its reduced potency as an inhibitor of protein synthesis (Burres and Clement 1989). Biological data for the remaining members of the onnamide subfamily are limited to in vitro studies against P388, and the relevant data are collected in Table 1. Onnamide A and 23(*Z*)-onnamide A (**88**, Scheme 16) are the most potent members of the family, while onnamide E (**85**), lacking the *N*-acyl aminal functionality, is inactive. All members of the onnamide subfamily were vesicants (Matsunaga et al. 1992). There are also very few published data regarding the antiviral activity of the onnamides. In their original report on the isolation and structural determination of onnamide A, Sakemi et al. (1988) claimed potent antiviral activity against herpes simplex type-1, vesicular stomatitis virus, and coronavirus A-59.

Theopederins A–E (**7A–E**) were markedly cytotoxic against P388 leukemia cells (see Table 1; Fusetani et al. 1992). Theopederins A and B also showed promising antitumor activity against P388 (i.p.): T/C = 205% (0.1 mg/kg/day, treated on days 1, 2, and 4–6, i.p.) and T/C = 173% (0.4 mg/kg/day, treated on days 1, 2, and 4–6, i.p.), respectively.

2.3.2 Simple Derivatives of the Natural Mycalamides

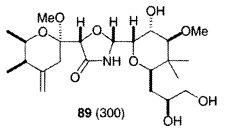
In 1992, Thompson et al. (1992) prepared 34 simple acyl, alkyl, and silyl derivatives of the C7, C17, and C18 hydroxyl groups and the *N*-amido group of mycalamides A and B and their relative potency was assayed in vitro against P388 cells. The most noteworthy conclusions from this study are (a) methylation of the amide nitrogen together with the C7 hydroxyl group causes at least a 10^3-fold reduction of activity; (b) derivatization of the C7 hydroxyl group causes a 10-to 100-fold reduction in activity; and (c) methylation of both the C17 and C18 hydroxyl groups (as found in pederin) renders the mycalamides as active as pederin. From these observations, Munro concluded that the intact *N*-acyl aminal bridge is vitally important for the biological activity of the mycalamides.

In 1997 we studied the biological activities of 18-*O*-methyl mycalamide B (**73**), 10-*epi*-18-*O*-methyl mycalamide B, and pederin, all prepared by total synthesis (Richter et al. 1997). The activities of 18-*O*-methyl mycalamide B and pederin were virtually indistinguishable when evaluated in DNA or protein synthesis assays and in cytotoxicity assays using human carcinoma cell lines (IC_{50}s 0.2–0.6 n*M*). In the assays, 10-*epi*-18-*O*-methyl mycalamide B was 10^3 times less toxic than its diastereoisomer, demonstrating that the cytotoxicity of 18-*O*-methyl mycalamide B is inseparable from its ability to inhibit protein synthesis. Short-term exposure of squamous carcinoma cells to 18-*O*-methyl mycalamide B or pederin caused an irreversible inhibition of cellular proliferation and induced cellular necrosis. In contrast, the antiproliferative effects of the compounds on human fibroblasts were reversible and there was no evidence of necrosis.

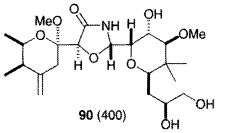
2.3.3 Degradation Products of the Natural Mycalamides

An extensive program on the chemistry of the mycalamides by the New Zealand group of Munro and Blunt involved treatment of mycalamide A and some of its alkyl derivatives with alkoxide, hydroxide, oxide bases, sodium borohydride, and azide (Thompson et al. 1994). The study was extended to acid-catalyzed degradations, acetal exchange reactions, catalytic hydrogenation, epoxidation, and oxidation reactions (Thompson et al. 1995). The numerous derivatives and degradation products were then tested in vitro against P388 cells. The IC_{50} values in ng/ml are given together with the relevant structures in Scheme 17.

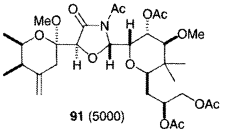
Oxazolidinones **89–91** and the 7-*O-benzyl* derivatives **92** and **93** displayed poor activity, as was expected from the previous studies which had established the need for a free hydroxyl group at C7. The relative inactivity of the cleavage fragments **97–103** demonstrates further that both segments of the mycalamide structure are essential to the biological activity. Only the reduction product **96** (40-fold deactivation) has a significant biological activity, showing that the C10 configuration was crucial for activity to be displayed. The moderate activity of the reduction product **96** is surprising, since the aminal function is generally considered a crucial structural motif for biological activity. The importance of the C6 acetal is shown by the retention of activity with a C6



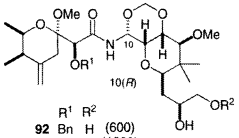
89 (300)



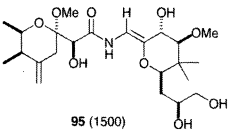
90 (400)



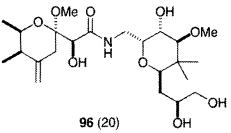
91 (5000)



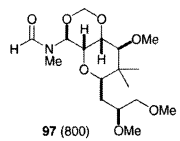
	R^1	R^2	
92	Bn	H	(600)
93	Bn	Bn	(1300)
94	H	H	(300)



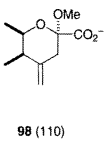
95 (1500)



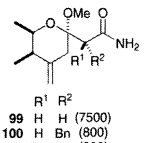
96 (20)



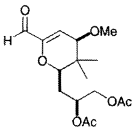
97 (800)



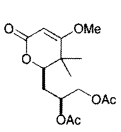
98 (110)



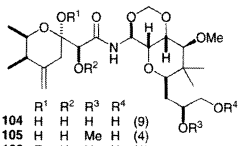
	R^1	R^2	
99	H	H	(7500)
100	H	Bn	(800)
101	Bn	H	(800)



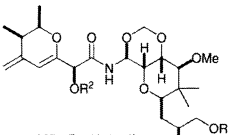
102 (500)



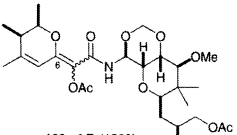
103 (125)



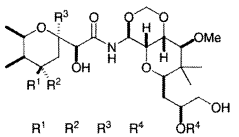
	R^1	R^2	R^3	R^4	
104	H	H	H	H	(9)
105	H	H	Me	H	(4)
106	Et	H	H	H	(1)



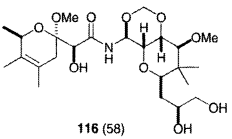
107 R = H (120)
108 R = Ac (270)



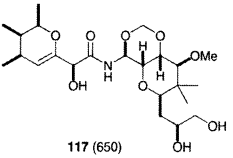
109 6*E* (1300)
110 6*Z* (220)



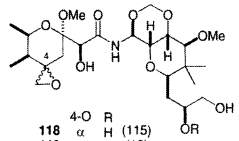
	R^1	R^2	R^3	R^4	
111	H	Me	OMe	H	(0.2)
112	Me	H	OMe	H	(0.8)
113	H	Me	OMe	Me	(0.1)
114	Me	H	OMe	Me	(0.8)
115	Me	H	H	H	(250)



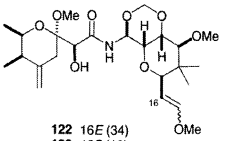
116 (58)



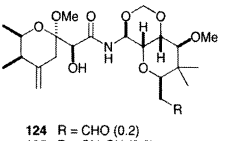
117 (650)



	4-O	R	
118	α	H	(115)
119	β	H	(16)
120	α	Me	(90)
121	β	Me	(4)

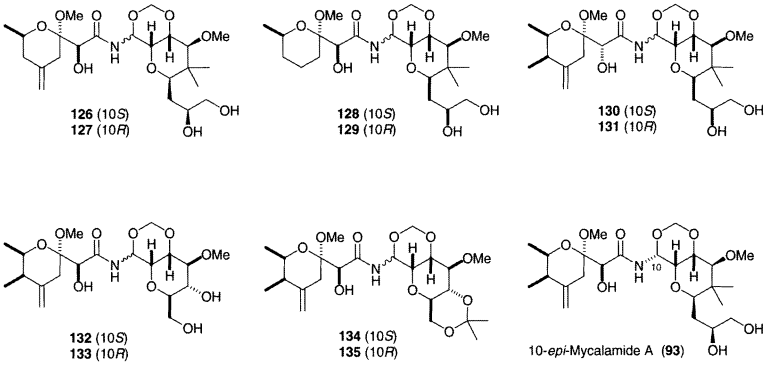


122 16*E* (34)
123 16*Z* (10)



124 R = CHO (0.2)
125 R = CH_2OH (0.2)

Scheme 17.



Scheme 18.

Table 2. Biological activity of mycalamide A analogues[a,b]

Compound	Cytotoxicity against HeLa cells	Antiviral activity against HSV-1		Antiviral activity against VZV	
	IC_{50}	MIC[c]	IC_{50}[d]	MIC[e]	IC_{50}[f]
5-Fluorouracil	3.0	–	–	–	–
Acyclovir	–	1.6	1.6	6.25	>50.0
Mycalamide A (**4**)	<0.03	<0.4	<0.4	<0.4	<0.4
10-epi-Mycalamide A (**93**)	3.0	12.5	12.5	1.6	12.5
126	<0.03	<0.4	<0.4	<0.4	<0.4
127	3.0	50.0	50.0	1.6	>50.0
128	0.03	3.1	3.9	<0.4	1.6
129	>10.0	50.0	>50.0	3.1	>50.0
130	>10.0	50.0	12.5	<0.4	12.5
131	>10.0	50.0	>50.0	12.5	>50.0
132	1.0	25.0	25.0	3.1	>50.0
133	>10.0	>50.0	>50.0	25.0	>50.0
134	3.0	50.0	>50.0	12.0	>50.0
135	10.0	50.0	50.0	12.5	>50.0

[a]Data taken from Fukui et al. 1997.
[b]IC_{50} (μg/ml), MIC (μg/ml).
[c]Against HSV-1 cells.
[d]Against vero cells.
[e]Against VZV.
[f]Against HEL cells.

ethoxy derivative **106**, a 20-to 40-fold drop in activity for a C6 hydroxy substituent as in compounds **104** and **105**, and further losses (>100-fold) with elimination or hydrogenolysis at C6 (compounds **107–110**, **115, 117**). Compounds involving derivatization or transposition of the exocyclic double bond retained significant activity: thus, 4β-dihydromycalamide A **111** was significantly more active than mycalamide A but 4β-dihydromycalamide B **113** had the same activity as mycalamide B and Δ^3-mycalamide A **116** was 100-fold less active than mycalamide A. The epoxide derivatives **118–121** were also much less active, although there was an even more pronounced isomer effect.

Modifications at the C16 side chain were expected to be well tolerated, so it was surprising that both Δ^{16}-normycalamide B isomers (**122** and **123**) were approximately 100 times less active than mycalamide B. However, the C17 aldehyde and alcohol derivatives (**124** and **125**) were significantly more active than both mycalamide A and theopederin E (IC_{50} = 9 ng/ml against P388).

2.3.4 Advanced Synthetic Analogues of Mycalamide A

In 1997 the Nakata group (Fukui et al. 1997) synthesized a set of ten advanced analogues of mycalamide A (Scheme 18) in order to probe the minimum structural requirements of the left fragment for biological activity and to explore the possibility of replacing the right fragment with glucose derivatives. The cytotoxicity against HeLa cells and antiviral activity against herpes simplex type 1 (HSV-1) and varicella-zoster virus (VZV) were tested in vitro, along with 5-fluorouracil and acyclovir as standards. The data are summarized in Table 2. For comparison, parallel tests were run on mycalamide A itself and its 10-epimer (**95**).

Mycalamide A, **125** and **127**, showed very potent cytotoxicity against HeLa cells but their corresponding 10-epimers (i.e., 10-*epi*-mycalamide A, **127** and **129**) were 100 times less active, suggesting that the C10 configuration is a crucial determinant for high cytotoxicity. As expected from the results described in the previous section, the high activity of compound **127** verifies that the presence of the C4-*exo*-methylene and C3-methyl groups is not an important factor for the potent cytotoxicity. The unnatural C7 hydroxyl isomer **130** and its C10-epimer **131** de-

creased the activity, which suggests that the configuration of the C7-hydroxy group is also essential for potent cytotoxicity. It is noteworthy that **132** and **134**, in which the right fragment is replaced by glucose derivatives, showed nearly the same activity as 5-fluorouracil.

A compound can be judged to have significant antiviral activity if its therapeutic ratio (TR = IC_{50}/MIC) is higher than that of acyclovir. The antiviral activity of mycalamide A **126** and **128** against HSV-1 is very strong. However, their cytotoxicity (IC_{50}) against vero cells is also very strong: TRs of all synthetic compounds tested are less than 1 (TR of acyclovir = 32). Although mycalamide A, **44** and **46** showed strong activity against VZV, their potent cytotoxicity (IC_{50}) against HEL cells was also observed. 10-*epi*-Mycalamide A (**94**), **127**, **129**, **130**, and **132** showed potent antiviral activity against VZV and low cytotoxicity against HEL cells: TRs of 10-*epi*-mycalamide A (**94**), **127**, **129**, **130,** and **132** are 8, >32, >16, >32, and >16, respectively (cf. TR of acyclovir >8). Thus 7- or 10-epimeric compounds showed significant antiviral activity against VZV.

2.3.5 Simple Synthetic Derivatives

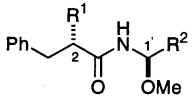
None of the biological data presented thus far identify the *minimum* structural requirements for cytotoxicity or antiviral activity. In 1997, Abell et al. (1997) evaluated the cytotoxicity of simple analogues (Table 3) of the *N*-acyl aminal bridge against the P388 leukemia cell line in vitro. In general, compounds **136a–i** with a (1'*R*,2*S*) configuration (equivalent to C7 and C10 in the natural products) show significantly greater in vitro cytotoxicity than the corresponding (1'*S*,2*S*) derivatives **137a–i**. Notable exceptions were the parent natural products (pederin, mycalamides A and B) for which the equivalent C10 position is *S*. A preference for a (1'*R*) configuration over a (1'*S*) configuration does not seem to be evident within the cyclic oxazolidinone series **138–143** (Scheme 19), where the (1'*S*) and (1'*R*) compounds show similar in vitro cytotoxicity.

A variety of R^1 groups appear to be accommodated for the induction of in vitro cytotoxicity. For example, the corresponding acetates of **136a** and **137a**, compounds **136e** and **137e**, show comparable activity. By comparison, acylation of the C7-hydroxyl group of mycalamide A and

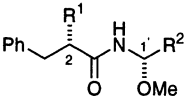
Table 3. IC_{50} values of **136** and **137** against P388 cells

	R^1	R^2	IC_{50} (μg/ml) **136** (1'*R*,2*S*)	**137** (1'*S*,2*S*)
a	OH	Ph	52	>340
b	OH	Me	>125	>188[a]
c	NHZ	Ph	14	>188[b]
d	NH-Ala-Z	Ph	36	>125
e	OAc	Ph	101	>313
f	OH	Et	176	43
g	NHZ	Me	>375	>375
h	$OCOC_6H_4Br$	i-Pr	105[c]	105
i	O-camphanyl	Ph	42[d]	78[d]

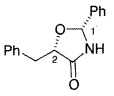
[a] 1:1 mixture of epimers.
[b] 3:1 mixture of epimers.
[c] 17:3 mixture of epimers.
[d] 9:1 mixture of epimers.



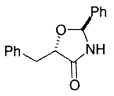
136 (1'*R*,2*S*)



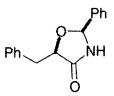
137 (1'*S*,2*S*)



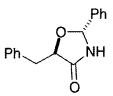
138 (57)



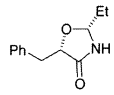
139 (37)



140 (12)



141 (8)



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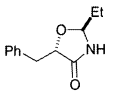
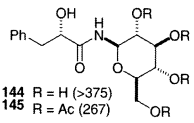


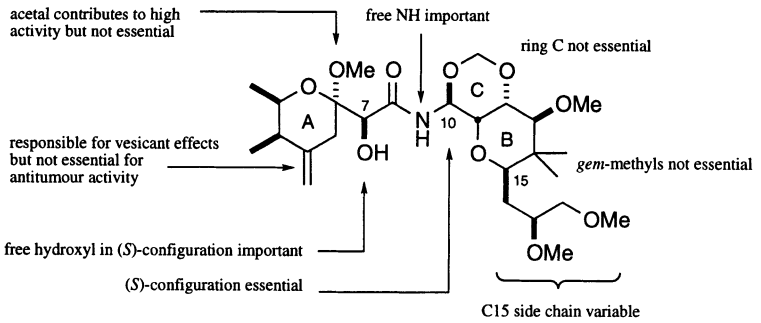
143 (47)



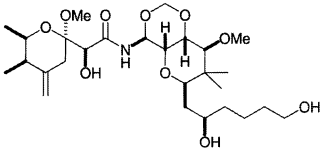
144 R = H (>375)
145 R = Ac (267)

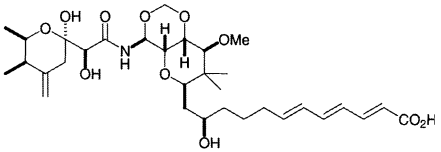
Scheme 19.

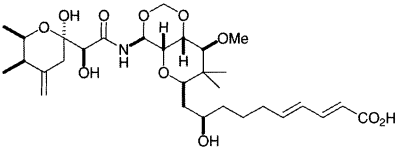
B (analogous to C2 in **136/137**) results in compounds with significantly decreased activity. The (1')-epimeric pairs **136c,d,g** and **137c,d,g** were designed to give the derivatives more peptide character. The most active compounds in this series, compounds **136c,d** show activities comparable to, or better than, that of **136a**. Again a preference for a (1'*R*) configuration is noted (**136c/137c** and **136d/137d**). A change from R^2=Ph to Et appears to be tolerated, although in this case, contrary to the other compounds, a (1'*S*) configuration seems to give the most potent in vitro bioactivity (**136f >137f**). It should be noted that **137f** and the

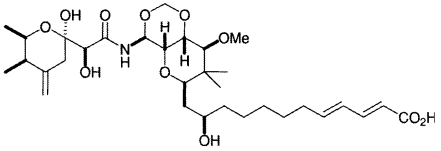


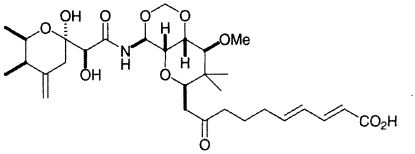
Scheme 20.


Theopederin F (**146F**)


Theopederin I (**146I**)


Theopederin G (**146G**)


Theopederin J (**146J**)


Theopederin H (**146H**)

Scheme 21.

parent natural products possess the same relative configuration at this center (i.e., *S*). The introduction of a methyl group at the R^2 position resulted in compounds with significantly reduced activity (see compounds **136b,g**, **137b,g**). Finally, the glucosyl derivatives **143** and **144** show less activity than the corresponding acyclic analogues **136** and **137**, where R^2=Et and Ph.

In conclusion, the foregoing structure-activity studies on the pederin family of antitumor agents established that the *N*-acyl aminal bridge is the pharmacophore. The homoallylic acetal array encompassing C4–C6, which is responsible for the acid lability of the natural products as well as their vesicant effects, is not necessary for antitumor or antiviral activity. The C6 acetal function contributes to the high activity of the natural products, though simpler analogue studies reveal that it is not essential. The presence of a free hydroxyl group at C7 with the (*S*) configuration is important for high activity. The configuration of the aminal center is also very important, with the (*S*) configuration at C10 being significantly more active as an antitumor agent than the (*R*) epimer; however, compounds with the (*R*) configuration at C10 remain potent antiviral agents. The complex trioxadecalin ring system characteristic of the mycalamides, onnamides, and theopederins is not essential for high activity since pederin, with its simpler monocyclic right half, is one of the most active of the natural products, followed closely by 18-*O*-methyl-mycalamide B, a simple synthetic derivative of natural mycalamide B. Finally, the side chain at C15 tolerates considerable variation with comparatively little impact on activity. A summary of the SAR data is given in Scheme 20.

Addendum. Theopederins F–J (**145F–J**, Scheme 21) have recently been isolated from *Theonella swinhoei* (Tsukamoto et al. 1999). Theopederin F was antifungal against an *erg*6 mutant of *Saccharomyces cerevisiae* deficient in (*S*)-adenosylmethionine-Δ^{24}-methyltransferase involved in the biosynthesis of ergosterol. Theopederin F was also cytotoxic against murine P388 cells with an IC_{50} of 0.15 ng/ml. No biological data were reported for theopederins G–J. The close structural kinship between theopederins G–J and the onnamides suggests that the theopederins may be biosynthetic precursors of the onnamides.

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