Studies on the Biomimetic Synthesis of the Manzamine Alkaloids

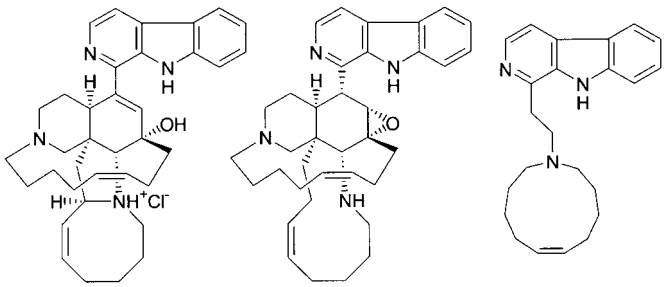
Jack E. Baldwin,* Timothy D. W. Claridge, Andrew J. Culshaw, Florian A. Heupel, Victor Lee, David R. Spring, and Roger C. Whitehead[a]

Abstract: The biomimetic synthesis of the manzamine-related alkaloid keramaphidin B is described. The key intramolecular Diels–Alder reaction proposed in the Baldwin and Whitehead hypothesis is demonstrated. An investigation of the modified biomimetic hypothesis proposed by Marazano et al. is also reported. Finally, an alternative synthesis of keramaphidin B by Grubbs methathesis is presented.

Keywords: biomimetic synthesis · Diels–Alder reaction · keramaphidin B · manzamine · natural products

Introduction

Structurally, the manzamines are a unique class of alkaloids,[1] which were first isolated from the marine sponge *Haliclona sp.* collected off Manzamo, Okinawa in Japan.[2] Manzamine A (**1**) was found to possess an in vitro activity against P388 mouse leukaemia cells ($IC_{50} = 0.07\ \mu g\,mL^{-1}$[2] or $2.4\ \mu g\,mL^{-1}$[3]) and its structure was established by X-ray crystallography (Figure 1). Later, the same group reported[4]



1 2 3

Figure 1. Manzamine A (**1**), B (**2**) and C (**3**).

the isolation of manzamines B (**2**) and C (**3**) from the same sponge. The complex structures of the manzamines were unprecedented in nature, which prompted the authors to write "the provenance of manzamines B and C, like that of A, is biogenetically problematical."[2]

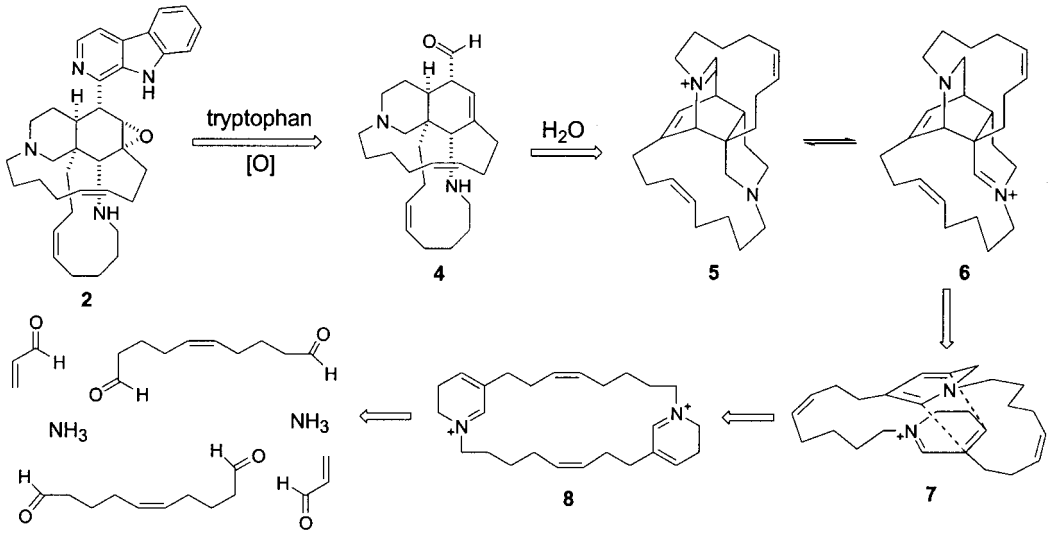
[a] Prof. J. E. Baldwin, Dr. T. D. W. Claridge, Dr. A. J. Culshaw, Dr. F. A. Heupel, Dr. V. Lee, D. R. Spring, Dr. R. C. Whitehead
The Dyson Perrins Laboratory, University of Oxford
South Parks Road, Oxford, OX1 3QY (UK)
Fax: (+441) 865-275674
E-mail: jack.baldwin@chem.ox.ac.uk

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/chemistry/ or from the author.

The manzamine biosynthetic mystery posed an intellectual challenge to organic chemists. It is conceivable that a solution to this problem could provide an efficient biomimetic pathway towards the synthesis of these complex molecules. The biosynthetic investigation of marine sponges is notoriously difficult,[5] and to date, no biosynthetic work has been published on the manzamines. However, in 1992 a hypothesis was proposed by the present authors in which the manzamine alkaloids could be formed from just four building blocks: tryptophan, ammonia, a C_{10} unit (a symmetrical dialdehyde) and a C_3 unit (an acrolein equivalent).[6] Careful analysis of the structure of manzamine B (**2**), for example, reveals the hidden symmetry (Scheme 1). Retrosynthetically, removal of the epoxide and the tryptophan unit from **2** gives aminoaldehyde **4**. The aldehyde **4** may be derived from the hydrolysis of iminium ion **5**. Manzamine precursors **5** and **6** could be related by a redox equilibrium. Disconnection of **6** by an intramolecular *endo* Diels–Alder reaction gives macrocycle **7**, which is the conjugate base of **8**. The symmetrical macrocycle **8** may be derived from the other three building blocks (two equivalents of ammonia, and the C_3 and C_{10} units). Thus, a simple explanation is provided for the biosynthesis of a very complex molecule.

Since the publication of this hypothesis, there has been a great increase in the number of discoveries of manzamine-related alkaloids. Indeed, our hypothesis has been adapted and modified on numerous occasions to explain the possible biosynthetic origin of these new alkaloids. Many of these natural products bear a striking resemblance to intermediates encompassed by the hypothesis, such as ircinal A[7] (**9**, the aldehyde precursor to **1**) and keramaphidin B[8] (**10**, the reduced form of **5** and **6**) (Figure 2). Concurrent with the publication of the manzamine biosynthetic hypothesis, a programme to investigate the validity of the hypothesis was initiated. Herein, the full account of our investigation is provided which led to the biomimetic synthesis of keramaphidin B (**10**).[9]

0947-6539/99/0511-3154 $ 17.50+.50/0



Scheme 1. The Baldwin–Whitehead hypothesis for the biosynthesis of the manzamine alkaloids.

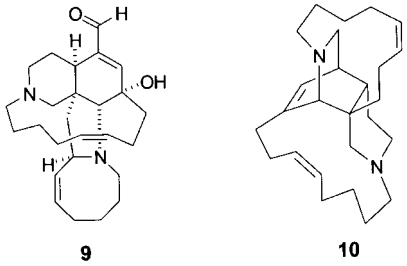
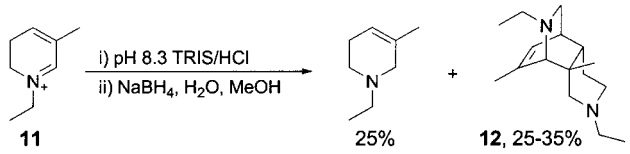


Figure 2. Ircinal A (**9**) and keramaphidin B (**10**).



Scheme 2. Formation of tricyclic core structure **12**.

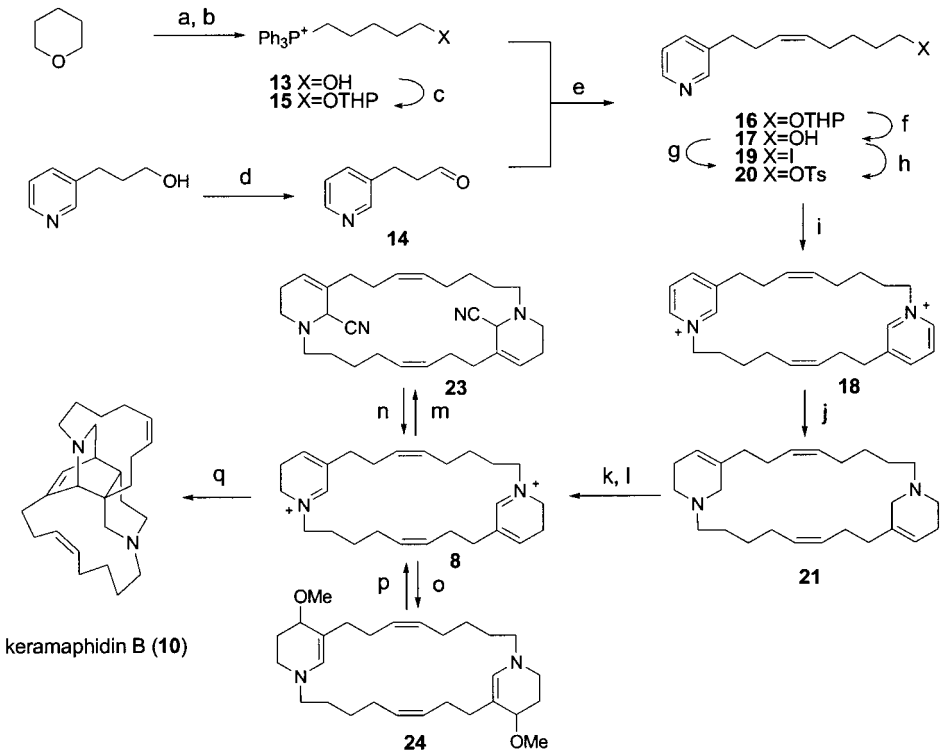
Keramaphidin B (**10**) is a pentacyclic alkaloid isolated independently by Kobayashi et al.[8a–c] and Andersen and Kong[8d] from *Amphimedon sp.* and *Xestospongia ingens*, respectively. The structure of **10** was elucidated through a combination of NMR spectroscopy and X-ray crystallography. Interestingly, **10** crystallised as a racemate even though there was approximately 97% of the (+)-form in the sponge. The hypothesis of a Diels–Alder cycloaddition with an achiral precursor **7** proposed in the manzamine biosynthesis is strengthened by this observation, since both enantiomers of the reduced cycloadduct **10** were found to occur naturally.[10]

Results and Discussion

Biomimetic synthesis: Previous model studies conducted by the Baldwin group and others confirmed the feasibility of the proposed key Diels–Alder reaction.[11] Treatment of **11** in buffer solution followed by quenching with sodium borohydride resulted in the formation of **12**, the core structure of keramaphidin B (Scheme 2).[11a] Armed with this knowledge the group set out to verify whether the same Diels–Alder reaction could be performed intramolecularly.

The investigation required an efficient synthesis of the macrocycle **8**. Thus, tetrahydropyran was treated with acetyl chloride to give 5-bromopentylacetate,[12] which was heated with triphenylphosphane followed by hydrolysis to give the phosphonium salt **13** (Scheme 3).[12e] All three steps were performed without chromatography and 100 grams of **13** were prepared routinely. 3-Pyridin-3-yl-1-propanol, commercially available, was converted into aldehyde **14** by Swern oxidation.[13] Wittig olefination of **14** with the ylide, generated from **13**; *t*BuOK gave a disappointing result. The yield of the product was moderate (43–66%) and the *cis*/*trans* selectivity was around 85:15. In addition, as a result of the presence of moisture in the reactions a substantial amount of diphenylphosphane oxide was isolated (10–39%).[14] The use of oxidoylides in the Wittig reaction has been studied in detail by Maryanoff and co-workers, including studies with **13** as a substrate.[15] The reduced selectivity as compared with aliphatic ylides was attributed to the ability of the oxido group to facilitate the reversible dissociation of oxaphosphetanes to the ylide and aldehyde **14**, introducing a degree of thermodynamic control.[15] Protection of the alcohol group in **13** was sought to eliminate the effect of the alkoxide group on the *Z*/*E* ratio. Hydroxyphosphonium salt **13** was masked as its tetrahydropyranyl (THP) derivative **15** in 93% yield.[16] When the Wittig reaction was carried out with the ylide generated from **15** and potassium hexamethyldisilazide (KHMDS) a superior *Z*/*E* ratio of 99:1 and a yield of isolated alkene **16** of 83% was observed, along with 5% diphenylphosphane oxide. The THP protecting group was removed by dilute hydrochloric acid in methanol to give **17** (94%).[17] The incorporation of the additional protection and deprotection step in the synthesis was offset by the significant improvement in yield and quality of material.

With alcohol **17** in hand, the attention was turned to its activation and cyclodimerisation. An investigation into the cyclodimerisation found that an iodide leaving group was



Scheme 3. Synthesis of bis-dihydropyridine **8**: a) AcBr, cat. Zn, Δ, 95%; b) Ph_3P, 100 °C; then K_2CO_3, H_2O, MeOH, 76%; c) 3,4-dihydro-2*H*-pyran, PPTS, CH_2Cl_2, 93%; d) $(COCl)_2$, DMSO, TEA, −65 to 25 °C, 90%; e) KHMDS, THF, −78 °C to RT; then **14**, −78 °C to RT, 83% (*Z*:*E* ∼ 99:1); f) 3 M HCl, MeOH, 94%; g) Ph_3P, I_2, imidazole, MeCN, 56–90%; h) TsCl, Et_3N, CH_2Cl_2, 0 °C, 95%; i) NaI, butan-2-one, Δ, 192 h; j) $NaBH_4$, MeOH, −78 °C to RT, 56% over two steps; k) *m*CPBA, CH_2Cl_2, 0 °C, 98%; l) TFAA, CH_2Cl_2, 100%; m) KCN, H_2O, pH 3–4, 92%; n) CF_3CO_2Ag, quant.; o) MeONa, MeOH, 86%; p) camphorsulfonic acid (CSA), quant.; q) MeOH/1 M aq. buffer (1:1 pH 7.3); then $NaBH_4$, MeOH, −78 °C to RT, 0.2–0.3%.

optimum, providing the desired bis-pyridinium salt **18** in 40–44% yield.[11b] Alcohol **17** was converted into iodide **19** with triphenylphosphane/iodine/imidazole. However, it was discovered that the yield of iodination varied from 56–90% and that **19** was difficult to handle. In a condensed state, **19** polymerised at room temperature over a period of hours. The instability of **19**, coupled with the moderate yield of cyclodimerisation, prompted the search for an alternative protocol. The tosylate **20** was prepared easily from alcohol **17** with TsCl and NEt_3 (95%),[18] and in condensed state decomposed only slowly over a week upon standing at room temperature. It may be stored conveniently as a solution in CH_2Cl_2 at −80 °C for many weeks without any observable decomposition as detected by ^{1}H NMR spectra. By generating the iodide **19** in situ from tosylate **20**, it was possible to avoid handling the unstable intermediate.

Next, we focused on optimising the cyclodimerisation reaction; this reaction consists of two separate S_N2 reactions. The first, the intermolecular reaction, is favoured by a high concentration, but so is the nonproductive polymerisation. The second, the ring-closing reaction, is favoured by low concentration. It was expected that operating at a low concentration should be beneficial on balance to the formation of dimer. This assumption was tested in practice. Slow addition of a solution of **20** in butan-2-one to a refluxing solution of NaI in the same solvent effected a one-pot Finkelstein/dimerisation/macrocyclisation reaction. The crude product obtained was reduced with $NaBH_4$ in MeOH to give bis-tetrahydropyridine **21** in 56% yield over two steps. Flash chromatography with base-washed silica and a basic eluent facilitated the easy purification of dimeric **21** from any other oligomers.

Partial desaturation of **21** was required to obtain the precursor for the study of the Diels–Alder reaction. The modified Polonovski reaction (also known as the Polonovski–Potier reaction)[19] was chosen for this purpose. Treatment of **21** with *m*CPBA (*m*-chloroperbenzoic acid) gave two separable diastereomeric *N*-oxides **22** (not shown). Either diastereomer could be treated with trifluoroacetic anhydride (TFAA) to give the proposed manzamine biosynthetic precursor **8**. Compound **8** could be stored either as its bis-cyano derivative **23** (by treatment **8** with KCN) or bis-methoxy derivative **24** (by treatment of **8** with NaOMe).

With a reliable route to **8** in hand, the investigation of the proposed Diels–Alder reaction was undertaken. Initial NMR studies with **8**, made from either **23** (with silver trifluoroacetate) or **24** (with camphor-10-sulfonic acid), conducted in a 1:1 mixture of CD_3OD and D_2O revealed several interesting observations:

1) As in the model studies disproportionation of the iminium ion was observed; however, this occurred to a far greater extent for **8**.
2) The rate of disproportionation was approximately the same when the reaction was conducted at 30 mM and at 3 mM concentrations of **8**.
3) Signals from iminium ion **8** virtually disappeared after one hour in pH 7.3 buffer.

Extensive studies were conducted in the hope of optimising the biomimetic Diels–Alder reaction. The reaction time was varied prior to quenching. A variety of solvents was tested (mixture of 1 M aq. buffer with MeOH, EtOH, CF_3CH_2OH, *i*PrOH, THF or CH_3CN), the buffer was varied (TRIS/HCl or K_2HPO_4/HCl) and the effect of changes of pH on the reaction was investigated (pH 7.3 to 7.5); the ionic strength and the use of additives (SDS, CHAPS, Triton X-100 and α- and β-cyclodextrin) were also investigated.[20] The best result was obtained by dissolution of **8** in a 1:1 mixture of 1 M aq. TRIS/HCl (pH 7.3) and MeOH, followed by reduction of the reaction after one hour with $NaBH_4$ at low temperature. With the best protocol, a small amount of keramaphidin B (**10**) was observed in the ^{1}H NMR spectrum of the crude product when the reaction was conducted on a preparative scale. The

0947-6539/99/0511-3156 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 11

problem of isolation of the desired product from the complex reaction mixture was far from trivial, as a result of the lack of a good UV chromophore. After extensive chromatographic purification (flash chromatography and repeated HPLC), **10** was obtained in 0.2–0.3% yield.[21] The major product of reduction was the recyclable bis-tetrahydropyridine **21**. This product arose from the disproportionation of **8** to give a mixture of tetrahydropyridine and pyridinium salt,[22] which was reduced by the sodium borohydride quench affording **21**.

While the result demonstrated clearly the validity of the group's proposal, the yield of keramaphidin B (**10**) was far from satisfactory. In order to shed light on the kinetic barrier of this reaction, a molecular modelling study was conducted on the Diels–Alder precursor **7** (Figure 3). The search

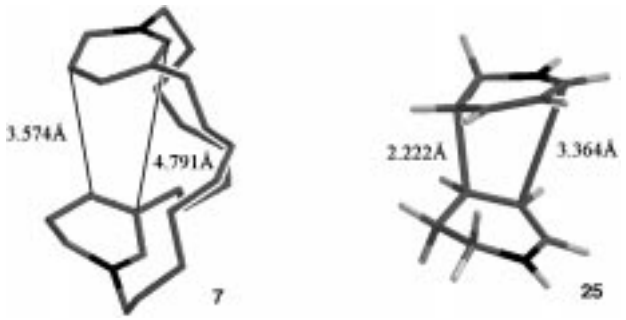


Figure 3. Transition states of **7** and **25**.[24]

revealed that there are conformations[23] available to the macrocycle which are close to the required transition state.[24] The result suggested that the low yield in the Diels–Alder reaction is not a result of the inaccessibility of the two reactive moieties, but rather to the kinetic preference of **7** to disproportionate.

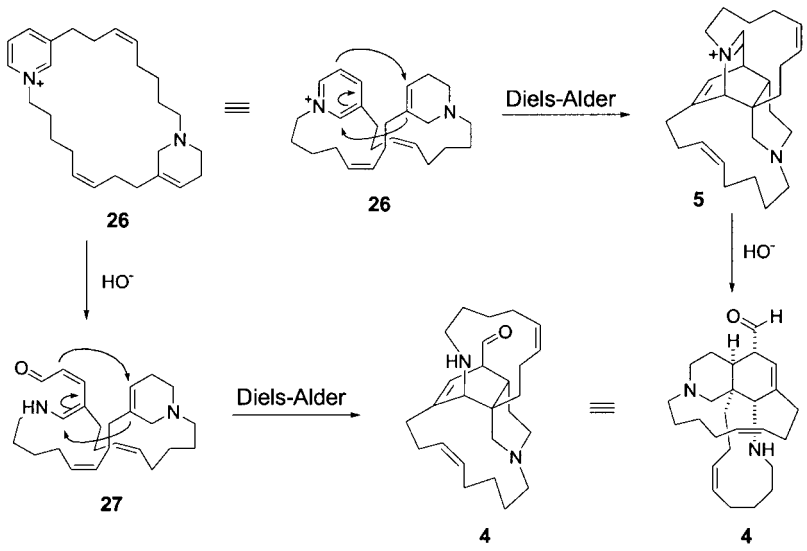
It is feasible to envisage a in vivo Diels–Alderase[25] which would mediate the conversion of bis-iminium salt **8** to keramaphidin B (**10**). The putative enzyme may catalyse the reaction by limiting the conformational mobility of the substrate, thereby minimising the change in entropy to the transition state.[26] Furthermore, the enzyme must avoid the problem of disproportionation, which is clearly the preferred reaction of **7**.

Alternative biosynthetic hypotheses: In the light of the unfavourable disproportionation in our bis-dihydropyridine route the group wondered whether the hypothesis was using the correct oxidation state of pyridine. For instance, if a pyridinium salt was used as the diene and a tetrahydropyridine as the dienophile in **26**, the [4+2] cycloaddition would directly form pentacyclic **5** (Scheme 4). This route exhibits two advantages over the original bis-dihydropyridine route: i) the pyridine moieties are at the tetrahydropyridine and pyridine oxidation states, thus avoiding the problem of disproportionation; and ii) there is now no need to postulate the redox equilibrium between the cycloadduct **6** and the ircinal precursor **5** shown in Scheme 1. However, at no time were cycloadducts observed (NMR/MS) by using model systems even under enforced reaction conditions (90 °C, 19 kbar).[27]

Marazano and co-workers have proposed recently that the biomimetic Diels–Alder reaction could involve substituted 5-amino-2,4-pentadienals as the diene.[28] This pathway would avoid not only the problems with disproportionation but would have the added advantage of less ring strain during the Diels–Alder cycloaddition. Ring-opening of pyridinium salt **26** gives amino-aldehyde **27** which could undergo a Diels–Alder reaction to give directly the ircinal core **4** (Scheme 4).[29]

The chemical feasibility of generating directly the substituted 5-amino-2,4-pentadienals (required for Marazano's modified hypothesis) from ring-opening of the pyridinium salt **26** was tested.[30] This was investigated by using an NMR experiment with 1-ethyl-3-methylpyridinium iodide (**28**) in a 2 M solution of NaOD in D_2O. No ring-opened products were observed. Only the deuterium exchange of the hydrogens at the pyridine C2 and C6 positions was noted followed by a slower exchange at the C4 and C5 positions.[31] Furthermore, the generation of **27** from **26** is not expected to be advantageous because the regiospecificity of ring-opening 3-substituted pyridinium salts (with strong electron-withdrawing groups on nitrogen) with the hydroxide ion has been reported to occur exclusively at the pyridine C2 position.[32]

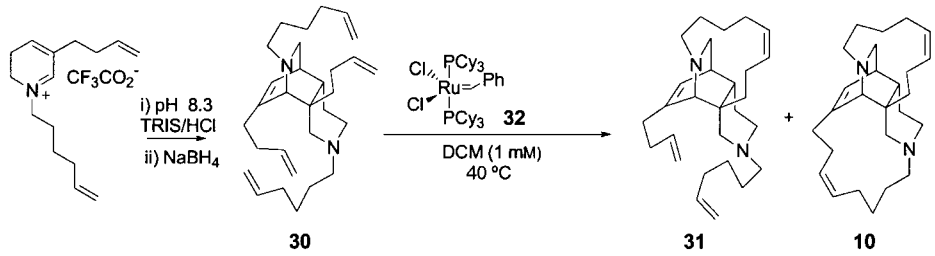
Marazano's hypothesis uses a 5-amino-2,4-pentadienal as the Diels–Alder diene.[28] However, such dienes have been reported to be poor participants in the Diels–Alder reaction.[33] (2*E*,4*E*)-5-Morpholino-2,4-pentadienal (**29**) and other analogues were prepared by published procedures,[30] and their utility in the Diels–Alder reaction was investigated. Heating **29** in a sealed tube for 24 hours without or with various dienophiles (1-ethyl-3-methyl-1,2,5,6-tetrahydropyri-



Scheme 4. Alternative biosynthetic hypotheses.

dine, maleic anhydride and 4-phenyl-1,2,4-triazoline-3,5-dione) failed to produce any cycloadducts (NMR/MS). The failure of the model systems to undergo the proposed cycloaddition, in this case and in those not at the dihydropyridine oxidation state, cast doubt over a modification of our hypothesis.

A semi-biomimetic approach—application of metathesis: As a result of the low yields of keramaphidin B (**10**) obtained from the intramolecular Diels–Alder cycloaddition, we investigated a two-step synthesis by an intermolecular cycloaddition followed by two ring-closing metathesis reactions (Scheme 5).[34] The biomimetic cycloaddition to give the keramaphidin B core (**30**) was achieved in a reproducible



Scheme 5. Synthesis of Keramaphidin B (**10**) by ring-closing metathesis.

22% yield.[11c] The ring-closing metathesis of **30** could give theoretically six "mono-cyclised" products (such as **31**) and three "bi-cyclised" products (one of which is keramaphidin B), along with oligomers and *E*-double bond isomers. However, we hoped that there would be a preference for the naturally occurring product **10**. Experiments were carried out to investigate both the Schrock molybdenum catalyst and the Grubbs ruthenium catalyst **32**. The Grubbs catalyst **32** gave a better profile (LCMS).[35] When the reaction was conducted on a preparative scale followed by HPLC, mono-cyclised **31** (10–20%)[36] and keramaphidin B (1–2%) were obtained. It appeared that there was indeed a bias towards the natural product **10**, but the difficulty of forming 11- and 13-membered rings by ring-closing metathesis was evident.

Conclusion

The key step in the Baldwin–Whitehead manzamine biosynthetic hypothesis is a Diels–Alder reaction between the dihydropyridine and dihydropyridinium ion in **7**. We have synthesised the bis-dihydropyridinium macrocycle **8** over eleven steps in 37% yield. Treatment of **8** with buffer, followed by reduction, yielded a small quantity of keramaphidin B (**10**) in 0.2–0.3% yield. This experiment showed that the proposed Diels–Alder reaction is feasible chemically, thus providing the first in vitro evidence for the Baldwin–Whitehead manzamine biosynthetic hypothesis. The major product was the bis-tetrahydropyridine (**21**), illustrating the kinetic preference of **7** to disproportionate. If the Baldwin–Whitehead hypothesis is correct then the biosynthetic Diels–Alder reaction would benefit from enzymatic mediation, because the enzyme would limit the conformational mobility of the substrate, thereby minimising the entropy change of activation ($\Delta S^{\neq}$). Furthermore, the enzyme would need to avoid the problem of disproportionation, which is clearly the preferred reaction of **7**.

Intermolecular biomimetic cycloadditions have been accomplished successfully in model systems (20–35% yields). The reduced cycloadduct **30** has been transformed into keramaphidin B (**10**) by ring-closing metathesis.

Experimental Section[37]

5-Hydroxypentyltriphenylphosphonium bromide (13): An intimate mixture of triphenylphosphane (102.3 g, 390 mmol) and 5-bromopentyl acetate (67.95 g, 325 mmol) was heated at 100 °C for 24 hours under N_2 with a large, egg-shaped stirrer bar to agitate the mixture. The mixture was allowed to cool to room temperature and the resulting glassy solid was dissolved in MeOH (700 mL). H_2O (60 mL) and K_2CO_3 (3.0 g, 21.7 mmol) were added and the mixture was stirred vigorously for 24 hours. The mixture was evaporated to dryness and washed with Et_2O to recover unreacted starting materials. The solid residue was recrystallised from EtOH and Et_2O to give **13** (138.04 g, 99%) as a white powder; m.p. 190-191 °C (lit.[11e] m.p. 190–191 °C); ^{1}H NMR (200 MHz, CD_3OD): $\delta = 1.47-1.86$ (m, 6H, C(2)H_2, C(3)H_2, C(4)H_2), 3.40–3.54 (m, 4H, C(1)H_2, C(5)H_2), 3.98 (br s, 1H, O*H*), 7.61–7.74 (m, 15H, phenyl C*H*); ^{31}P NMR (101.3 MHz, CD_3OD): $\delta = 24.66$ (s, Ph_3P^+); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 21.8$, 22.3 (d, $J = 50$ Hz), 26.5 (d, $J = 16.5$ Hz), 31.1, 60.8, 117.9 (d, $J = 86$ Hz), 130.2, 130.5, 133.2, 133.4, 134.9; IR (KBr disc): $\tilde{\nu} = 3313$, 2879, 1587, 1436, 1116, 1044, 724, 692 cm^{-1}; MS (APCI): *m*/*z* (%): 349 (100) $[M - Br]^+$, 289 (16), 279 (40), 273 (26), 263 (86) $[Ph_3PH]^+$; $C_{23}H_{26}BrOP$ (429.33): calcd C 64.3, H 6.1; found: C 64.5, H 6.15.

5-Tetrahydropyran-2-yloxypentyltriphenylphosphonium bromide (15): Pyridinium toluene-*p*-sulfonate (PPTS, 11.7 mg, 0.047 mmol) under an argon atmosphere was added to a mixture of **13** (2.00 g, 4.66 mmol) and 3,4-dihydro-2*H*-pyran (588 mg, 6.99 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature for 15 hours (monitored by TLC), and then concentrated in vacuo to yield a colourless oil. Flash chromatography yielded **15** (2.22 g, 93%) as a colourless glassy solid; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.39-1.79$ (m, 12H, 6 × CH_2), 3.31–3.92 (m, 6H, PCH_2, OCH_2), 4.48 (br s, 1H, OC*H*O), 7.65–7.91 (m, 15H, aromatic C*H*); ^{31}P NMR (101.3 MHz, $CDCl_3$): $\delta = 25.05$ (s, Ph_3P^+); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 19.4$, 22.1 (d, $J = 51$ Hz), 22.1 (d, $J = 4$ Hz), 25.0, 27.0 (d, $J = 16.5$ Hz), 28.8, 30.4, 62.3, 66.8, 98.8, 118.1 (d, $J = 86.5$ Hz), 130.4, 130.7, 133.4, 133.7, 135.1; IR (thin film): $\tilde{\nu} = 2940$, 1587, 1485, 1440, 1200, 1114, 1075, 1033, 996, 724, 692 cm^{-1}; MS (APCI): *m*/*z* (%): 433 (100) $[M - Br]^+$, 349 (6) $[M H - Br - THP]^+$, 289 (10), 279 (11), 263 (29) $[Ph_3PH]^+$; HRMS calcd for $C_{28}H_{34}O_2P$ ($[M - Br]^+$) 433.2296, found 433.2296.

(*Z*)-3-(8-Tetrahydropyran-2-yloxyocta-3-enyl)pyridine (16): A solution of **15** (545 mg, 1.06 mmol) in THF (10 mL) under argon was cooled to −78 °C, and a solution of 0.35 M KHMDS in toluene (3.03 mL, 1.06 mmol) was added dropwise. The mixture was allowed to warm to room temperature over one hour to give an orange solution. The mixture was cooled again to −78 °C and a solution of **14** (120 mg, 0.88 mmol) in THF (5 mL) was added through a cannula. The mixture was stirred at −78 °C for 5 min and then allowed to warm to room temperature over two hours. H_2O (15 mL) was added to the black solution, and the two yellow layers which formed were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), dried with Na_2SO_4, filtered and concentrated in vacuo to give a yellow oil. Flash chromatography yielded **16** (212.1 mg, 83%, *Z*:*E* ∼ 99:1 by ^{1}H NMR) as a colourless oil; $R_f = 0.46$ (SiO_2, PE 40–60/EtOAc/NEt_3 73:23:4, ammonium molybdate); ^{1}H NMR (500 MHz, C_6D_6): $\delta = 1.22-1.39$ (m, 6H, C=CCH_2CH_2CH_2, OCH_2CH_2CH_2CH_2CH_2OH_2), 1.45–1.62 (m, 3H,

0947-6539/99/0511-3158 $ 17.50+.50/0 *Chem. Eur. J.* **1999**, *5*, No. 11

C=C(CH$_2$)$_2$CH$_2$, O(CH$_2$)$_3$CHH′CHO$_2$), 1.70–1.79 (m, 1H, O(CH$_2$)$_3$CHH′-CHO$_2$), 1.87 (pseudo q, 2H, J = 7.5 Hz, C=CCH$_2$(CH$_2$)$_3$), 2.11 (pseudo q, 2H, J = 7.5 Hz, pyCH$_2$CH$_2$), 2.29 (t, 2H, J = 7.5 Hz, pyCH$_2$CH$_2$), 3.26–3.32 (m, 1H, THPOCHH′), 3.37–3.41 (m, 1H, OCHH′), 3.77–3.82 (m, 2H, THPOCHH′, OCHH′), 4.57 (pseudo t, 1H, J = 3.5 Hz, OCHO), 5.25 (dtt, 1H, J = 11, 7, 1.5 Hz, py(CH$_2$)$_2$CH=C), 5.35 (dtt, 1H, J = 11, 7, 1 Hz, py(CH$_2$)$_2$C=CH), 6.73 (dd, 1H, J = 7.5, 4.5 Hz, C(5)H), 6.98 (d, 1H, J = 7.5 Hz, C(4)H), 8.46 (dd, 1H, J = 4.5, 1.5 Hz, C(6)H), 8.51 (d, 1H, J = 2 Hz, C(2)H); ^{13}C NMR (125 MHz, C$_6$D$_6$): δ = 19.70, 25.93, 26.64, 27.27, 28.96, 29.72, 31.07, 33.08, 61.63, 67.23, 98.63, 123.06, 128.44, 131.08, 135.31, 136.97, 147.90, 150.71; IR (thin film): $\tilde{\nu}$ = 3006, 2940, 2864, 1575, 1478, 1453, 1441, 1424, 1352, 1201, 1137, 1120, 1077, 1034, 715 cm^{-1}; MS (APCI) m/z (%): 290 (13) [MH]$^+$, 206 (100) [MH$_2$ – THP]$^+$; HRMS calcd for C$_{18}$H$_{28}$NO$_2$ [MH]$^+$ 290.2120, found 290.2120.

(*Z*)-8-Pyridin-3-yloct-5-en-1-ol (17): A solution of **16** (194 mg, 0.669 mmol) and 3M aq. HCl (0.5 mL) in MeOH (15 mL) was stirred at room temperature for three hours. The mixture was concentrated in vacuo and chromatographed to give **17** (129 mg, 94%) as a colourless oil; R_f = 0.30 (SiO$_2$, PE 40–60/EtOAc/NEt$_3$ 40:50:10, ammonium molybdate); ^{1}H NMR (400 MHz, C$_6$D$_6$): δ = 1.33 (tt, 2H, J = 7.5, 7 Hz, CH$_2$(CH$_2$)$_2$OH), 1.53 (pseudo qui, 2H, J = 7 Hz, CH$_2$CH$_2$OH), 1.88 (pseudo q, 2H, J = 7.5 Hz, CH$_2$(CH$_2$)$_3$OH), 2.14 (pseudo q, 2H, J = 7.5 Hz, pyCH$_2$CH$_2$), 2.29 (t, 2H, J = 7.5 Hz, pyCH$_2$), 3.65 (t, 2H, J = 6.5 Hz, CH$_2$OH), 4.54 (br s, 1H, OH), 5.23 (dtt, 1H, J = 11, 7, 1.5 Hz, py(CH$_2$)$_2$CH), 5.36 (dtt, 1H, J = 11, 7, 1 Hz, py(CH$_2$)$_2$C=CH), 6.75 (dd, 1H, J = 7.5, 5 Hz, C(5)H), 7.01 (dd, 1H, J = 8, 1.5 Hz, C(4)H), 8.35 (dd, 1H, J = 5, 1.5 Hz, C(6)H), 8.41 (d, 1H, J = 2 Hz, C(2)H); ^{13}C NMR (100 MHz, C$_6$D$_6$): δ = 26.26, 27.31, 28.85, 32.87, 32.98, 62.12, 123.36, 128.19, 131.36, 136.03, 137.38, 147.36, 150.16; IR (thin film): $\tilde{\nu}$ = 3326, 2931, 2859, 1578, 1424, 1030, 714 cm^{-1}; MS (APCI): m/z (%): 206 (100) [MH]$^+$, 188 (6); C$_{13}$H$_{19}$NO (205.30): calcd C 76.1, H 9.35, N 6.8, found: C 75.9, H 9.35, N 6.8; HRMS calcd for C$_{13}$H$_{20}$NO [MH]$^+$ 206.1545, found 206.1545.

(*Z*)-8-Pyridin-3-yloct-5-enyl-4-toluenesulfonate (20): NEt$_3$ (2.85 mL, 20.3 mmol) and freshly recrystallised *para*-toluenesulfonyl chloride (2.90 g, 15.2 mmol) were added under an atmosphere of argon to a solution of **17** (2.09 g, 10.2 mmol) in dry, alcohol-free CH$_2$Cl$_2$. The mixture was stirred at 0°C for three hours before 2M aq. Na$_2$CO$_3$ (100 mL) was added. The two-phase mixture was separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 100 mL). The combined CH$_2$Cl$_2$ extracts were dried with MgSO$_4$, filtered and concentrated in vacuo to afford a bright red oil. Flash chromatography yielded **20** (3.48 g, 95%) as a pale orange oil; R_f = 0.37 (SiO$_2$, EtOAc/CHCl$_3$ 1:1); ^{1}H NMR (200 MHz, CDCl$_3$): δ = 1.13–1.24 (m, 2H, CH$_2$(CH$_2$)$_2$OTs), 1.39–1.49 (m, 2H, CH$_2$CH$_2$OTs), 1.79 (pseudo q, 2H, J = 7.5 Hz, CH$_2$(CH$_2$)$_3$OTs), 2.22 (pseudo q, 2H, J = 7.5 Hz, pyCH$_2$CH$_2$), 2.33 (s, 3H, CH$_3$), 2.54 (t, 2H, J = 7.5 Hz, pyCH$_2$), 3.88 (t, 2H, J = 6.5 Hz, CH$_2$OTs), 5.19–5.29 (m, 2H, CH=CH), 7.07–7.13 (m, 1H, C(5)H), 7.25 (br d, 2H, J = 7 Hz, *meta*-Ts CH), 7.40 (d, 1H, J = 7.5 Hz, C(4)H), 7.69 (d, 2H, J = 8.5 Hz, *ortho*-Ts CH), 8.31–8.34 (m, 2H, C(2)H, C(6)H); ^{13}C NMR (50 MHz, CDCl$_3$): δ = 21.5, 25.1, 26.3, 28.1, 28.6, 32.8, 70.4, 123.2, 127.7, 128.5, 129.8, 130.1, 133.0, 135.9, 137.0, 144.7, 147.2, 149.9; IR (thin film): $\tilde{\nu}$ = 3006, 2930, 2860, 1598, 1357, 1189, 1176, 1098, 935, 816, 715, 664 cm^{-1}; MS (APCI): m/z (%): 360 (100) [MH]$^+$; HRMS calcd for C$_{20}$H$_{26}$NO$_3$S [MH]$^+$ 360.1633, found 360.1633.

(6*Z*,19*Z*)-1,14-Diazatricyclo[21.3.1.1^{10,14}]octacosa-6,10,19,23-tetraene (21): A solution of **20** (185 mg, 0.51 mmol) in butan-2-one (2 × 10 mL) was added at a rate of 0.01 mL per minute over 48 hours to a solution of NaI (92 mg, 0.62 mmol) in butan-2-one (50 mL) heated at reflux. The orange solution was heated at reflux for one week, cooled to room temperature, then concentrated in vacuo to give an off-white powder. The powder was triturated with Et$_2$O (2 × 50 mL) to remove any unreacted iodide, then dissolved in a MeOH (15 mL) and CH$_2$Cl$_2$ (15 mL) mixture and cooled to –78°C. NaBH$_4$ (58 mg, 1.54 mmol) was added in one portion to the vigorously stirred solution. The solution was allowed to warm to 0°C over 30 minutes [**Caution**: hydrogen gas evolved at –30°C], then AcOH (10 mL) was added and it was left to stir for 16 hours at room temperature. Then the reaction mixture was concentrated in vacuo and partitioned between EtOAc (15 mL) and sat. aq. NaHCO$_3$ (to pH 9). The two-phase solution was separated and the aqueous phase extracted with EtOAc (3 × 15 mL). The combined EtOAc solutions were dried with K$_2$CO$_3$, filtered and concentrated in vacuo to give a pale yellow oil. Flash chromatography yielded **21** (55 mg, 56%) as a white solid; R_f = 0.26 (base-washed SiO$_2$, hexane/EtOAc/*i*Pr$_2$NH 80:20:2, visualised with Dragendorff reagent); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.39 (pseudo qui, 4H, J = 7.5 Hz, N(CH$_2$)$_2$CH$_2$), 1.52–1.58 (m, 4H, NCH$_2$CH$_2$CH$_2$), 1.99 (t, 4H, J = 8 Hz, C(3)CH$_2$), 2.07 (pseudo q, 4H, J = 7 Hz, N(CH$_2$)$_3$CH$_2$), 2.11–2.17 (m, 8H, C(5)H$_2$, C(3)CH$_2$CH$_2$), 2.42 (t, 4H, J = 8 Hz, NCH$_2$(CH$_2$)$_2$), 2.50 (t, 4H, J = 6 Hz, C(6)H$_2$), 2.86 (br s, 4H, C(2)H$_2$), 5.32–5.43 (m, 4H, CH=CH), 5.47 (br s, 2H, C(4)H); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 25.94, 26.40, 26.98, 27.34, 35.91, 50.96, 55.03, 58.91, 119.33, 129.50, 129.91, 135.63; IR (KBr disc): $\tilde{\nu}$ = 3001, 2906, 2855, 1631, 1460, 1449, 1176, 1055 cm^{-1}; MS(APCI): m/z (%): 383 (100) [MH]$^+$; HRMS calcd for C$_{26}$H$_{43}$N$_2$ [MH]$^+$ 383.3426, found 383.3426.

(6*Z*,19*Z*)-1,14-diazatricyclo[21.3.1.1^{10,14}]octacosa-6,10,19,23-tetraene-1,14-dioxide (22): Dried *m*CPBA (91% active, 131 mg, 0.69 mmol) was added in one portion to a cooled (0°C) solution of **21** (132 mg, 0.35 mmol) in CH$_2$Cl$_2$ (60 mL). The reaction was followed by TLC and, after one hour, concentrated in vacuo, behind a safety screen, to give a colourless oil. Flash chromatography yielded two isomers of **22** (overall 140 mg, 98%) both as white solids. Less polar isomer (104 mg, 73%): m.p. 161–163°C (dec.); R_f = 0.33 (Al$_2$O$_3$; CH$_2$Cl$_2$/MeOH 95:5, visualised with Dragendorff reagent); ^{1}H NMR (500 MHz, CDCl$_3$/CD$_3$OD 9:1): δ = 1.27–1.35 (m, 2H, N(CH$_2$)$_2$CH$_2$), 1.35–1.42 (m, 2H, N(CH$_2$)$_2$CH$_2$), 1.71–1.80 (m, 2H, NCH$_2$CH$_2$CH$_2$), 1.81–1.90 (m, 4H, NCH$_2$CH$_2$CH$_2$, C(3)CH$_2$ or C(3)CH$_2$CH$_2$), 1.92–2.01 (m, 4H, N(CH$_2$)$_3$CH$_2$), 2.01–2.11 (m, 6H, C(3)CH$_2$ or C(3)CH$_2$CH$_2$), 2.22–2.26 (br m, 2H, C(5)H), 2.45 (br d, 2H, J = 18 Hz, C(5)H′), 3.00–3.10 (m, 4H, NCH$_2$(CH$_2$)$_2$), 3.15–3.21 (m, 2H, C(6)H), 3.28–3.34 (m, 2H, C(6)H′), 3.58–3.67 (AB q, 4H, J_{AB} = 17 Hz, C(2)H$_2$), 5.25–5.33 (m, 4H, CH=CH), 5.53 (s, 2H, C(4)H); ^{13}C NMR (125 MHz, CDCl$_3$/CD$_3$OD 9:1): δ = 21.47, 23.31, 25.38, 26.31, 26.60, 33.74, 61.61, 64.61, 66.61, 118.54, 128.70, 129.70, 131.91; IR (KBr disc): $\tilde{\nu}$ = 2902, 2855, 1652, 1450, 922, 826 cm^{-1}; MS (APCI): m/z (%): 415 (100) [MH]$^+$, 399 (39) [MH – O]$^+$, 381 (52); HRMS calcd for C$_{26}$H$_{43}$N$_2$O$_2$ [MH]$^+$ 415.3324, found 415.3324. More polar isomer (36 mg, 25%): m.p. 154–156°C (dec.); R_f = 0.14 (Al$_2$O$_3$, CH$_2$Cl$_2$/MeOH 95:5, visualised with Dragendorff reagent); ^{1}H NMR (500 MHz, CDCl$_3$/CD$_3$OD 9:1): δ = 1.27–1.35 (m, 2H, N(CH$_2$)$_2$CH$_2$), 1.37–1.45 (m, 2H, N(CH$_2$)$_2$CH$_2$), 1.65–1.74 (m, 2H, NCH$_2$CH$_2$CH$_2$), 1.89–2.16 (m, 14H, NCH$_2$CH$_2$CH$_2$, C(3)CH$_2$, C(3)CH$_2$CH$_2$, N(CH$_2$)$_3$CH$_2$), 2.23–2.27 (m, 2H, C(5)H), 2.44 (br d, 2H, J = 17 Hz, C(5)H′), 3.02 (pseudo td, 2H, J = 12, 4.5 Hz, NCH$_2$(CH$_2$)$_2$), 3.13 (pseudo td, 2H, J = 12, 4.5 Hz, NCH$_2$(CH$_2$)$_2$), 3.20–3.26 (m, 2H, C(6)H), 3.31–3.35 (m, 2H, C(6)H′), 3.60–3.74 (AB q, 4H, $J_{A,B}$ = 6.5 Hz, C(2)H$_2$), 5.24–5.33 (m, 4H, CH=CH), 5.52 (s, 2H, C(4)H); ^{13}C NMR (125 MHz, CDCl$_3$/CD$_3$OD 9:1): δ = 21.48, 23.37, 25.16, 26.10, 26.63, 33.79, 62.10, 64.28, 65.68, 118.63, 128.82, 129.79, 131.97; IR (KBr disc): $\tilde{\nu}$ = 2900, 2854, 1652, 1454, 909, 826 cm^{-1}; MS (APCI): m/z (%): 415 (100) [MH]$^+$, 398 (68), 381 (58); HRMS calcd for C$_{26}$H$_{43}$N$_2$O$_2$ [MH]$^+$ 415.3324, found 415.3324.

(6*Z*,19*Z*)-1,14-Diazoniatricyclo[21.3.1.1^{10,14}]octacosa-1(27),6,10,14(28),19,23-hexaene ditrifluoroacetate (8): TFAA (8.2 μL, 58 μmol) was added under an atmosphere of argon to a cooled (0°C) solution of **22** (6.0 mg, 14 μmol) in CH$_2$Cl$_2$ (2 mL). The reaction mixture was stirred for one hour at 0°C before concentrated in vacuo to give **8** (quant.) as a pale yellow oil; ^{1}H NMR (200 MHz, CDCl$_3$): δ = 1.39–1.50 (m, 4H, N(CH$_2$)$_2$CH$_2$), 1.82–2.09 (m, 8H, NCH$_2$CH$_2$CH$_2$, N(CH$_2$)$_3$CH$_2$), 2.22–2.45 (m, 8H, C(3)CH$_2$, C(3)CH$_2$CH$_2$), 2.71–2.82 (m, 4H, C(5)H$_2$), 3.79–4.03 (m, 8H, C(6)H$_2$, NCH$_2$(CH$_2$)$_2$), 5.33–5.49 (m, 4H, CH=CH), 6.71 (s, 2H, C(4)H), 8.98 (s, 2H, C(2)H); IR (thin film): $\tilde{\nu}$ = 2938, 1732, 1675, 1199, 797 cm^{-1}; MS (ES): m/z (%): 190 (100) [C$_{26}$H$_{40}$N$_2$]$^{2+}$.

Biomimetic synthesis of keramaphidin B (10): TFAA (89 μL, 0.61 mmol) was added under an atmosphere of argon to a cooled (0°C) solution of **22** (63.0 mg, 0.15 mmol) in CH$_2$Cl$_2$ (5 mL). The reaction mixture was stirred for one hour at 0°C before concentrated in vacuo to give a pale yellow oil. Then the residue was dissolved in a mixture of MeOH (30 mL) and 1M aqueous TRIS/HCl (pH 7.30, 30 mL). The pH of this solution was checked with a calibrated pH meter and adjusted to pH 7.30 by the dropwise addition of 1M aq. TRIS/HCl (pH 8.30). The solution was stirred under an atmosphere of argon at room temperature (22°C) for one hour and was then diluted with MeOH (30 mL) and cooled to –78°C quickly. The reaction mixture was reduced by the addition of NaBH$_4$ (28.7 mg, 0.76 mmol) followed by allowing the solution to warm to 0°C over one hour. The reduced solution was concentrated in vacuo to remove the MeOH. CH$_2$Cl$_2$ (25 mL) was added to the resulting liquid. The two-phase

mixture was separated and the aqueous layer was extracted with CH_2Cl_2 (3×25 mL). The combined organic extracts were dried with K_2CO_3, filtered and concentrated in vacuo to give a colourless oil. Flash chromatography (base-washed SiO_2, toluene/EtOAc/iPr$_2$NH 198:2:3) followed by normal-phase HPLC (toluene/EtOAc/iPr$_2$NH 198:2:3, then $CHCl_3$/MeOH 95:5) yielded **10** (150 μg, 0.3%) as a colourless oil; synthetic material was identified by NMR, TLC, LCMS and by doping with authentic **10**; $R_f = 0.29$ (SiO_2, $CHCl_3$/MeOH 95:5, visualised with Dragendorff reagent); ^{1}H NMR (750 MHz, CD_3OD, 50 °C): $\delta = 0.93$ (ddd, 1H, $J = 12$, 4.5, 1.5 Hz, C(7)*H*), 1.21 (qd, 1H, $J = 14$, 4 Hz, C(6)*H*), 1.36–1.40 (m, 1H, N(10)CH_2CHH'), 1.42–1.63 (m, 8H, N(10)$CH_2CHH'CH_2CHH'$, N(4)$CH_2CHH'CH_2$, C(6)*H*′), 1.67 (dd, 1H, $J = 9$, 2.5 Hz, C(9)*H*), 1.69–1.76 (m, 4H, C(2)CH_2CHH', N(4)CH_2CHH'), 1.99 (br d, 1H, $J = 13$ Hz, N(4)$(CH_2)_3CHH'$), 2.08 (d, 1H, $J = 11.5$ Hz, C(3)*H*), 2.09–2.12 (m, 1H, C(11)CH_2CHH'), 2.20 (ddd, 1H, $J = 12.5$, 5.5, 1.5 Hz, N(10)$CHH'CH_2$), 2.20–2.28 (m, 2H, C(8)*H* and N(4)$(CH_2)_3CHH'$), 2.30–2.42 (m, 7H, C(11)CH_2CHH', C(2)CH_2CHH', N(10)$(CH_2)_3CHH'$, N(4)$CHH'(CH_2)_2$, C(3)*H*′), 2.69–2.71 (m, 1H, C(5)*H*), 2.78 (br t, 1H, $J = 13$ Hz, C(5)*H*′), 2.87 (dd, 1H, $J = 9$, 2 Hz, C(9)*H*′), 2.95 (td, 1H, $J = 12.5$, 5 Hz, N(10)$CHH'CH_2$), 3.03–3.06 (m, 1H, N(4)$CHH'(CH_2)_2$), 3.11 (s, 1H, C(1)*H*), 5.25 (pseudo t, 1H, $J = 10.5$ Hz, C(11)$(CH_2)_2CH{=}CH$), 5.38 (pseudo t, 1H, $J = 10$ Hz, C(11)$(CH_2)_2CH{=}CH$), 5.60–5.66 (m, 2H, C(2)$(CH_2)_2CH{=}CH$), 5.86 (d, 1H, $J = 6$ Hz, C(12)*H*); ^{13}C NMR (190 MHz, CD_3OD): (only signals which can be observed clearly above the noise or which can be traced back with HMQC experiments are reported) $\delta = 21.32$ (N(4)CH_2CH_2), 21.68 (C(2)CH_2CH_2), 23.80 (N(10)$(CH_2)_3CH_2$), 26.10 (N(4)$(CH_2)_3CH_2$), 26.53 (C(11)CH_2CH_2), 27.12 and 27.50 (C6, N(10)$CH_2CH_2CH_2$, N(4)$(CH_2)_2CH_2$), 37.81 (C(11)CH_2), 38.99 (C8), 42.10 (C(2)CH_2), 44.72 (C7), 48.77 (C5), 50.94 (C3), 54.48 (C9), 55.15 (N(10)CH_2CH_2), 57.04 (N(4)$CH_2(CH_2)_2$), 65.04 (C1), 124.52 (C12), 131.27 (C(2)$(CH_2)_2CH{=}CH$), 132.59 (C(11)$(CH_2)_2CH{=}CH$), 132.60 (C(2)$(CH_2)_2CH{=}CH$), 133.33 (C(11)$(CH_2)_2CH{=}CH$), 142.94 (C11); MS (APCI): *m/z* (%): 381 (100) $[MH]^+$; HRMS calcd for $C_{26}H_{41}N_2$ $[MH]^+$ 381.3270, found 381.3270.

Ring-closing metathesis synthesis of keramaphidin B (10): Compound **32** (9.4 mg, 11.7 μmol) was added under an atmosphere of dry argon to a stirred solution of **30** (50.0 mg, 117 μmol) in CH_2Cl_2 (100 mL) at 40 °C. The mixture was stirred for six hours with argon being bubbled through the solution, after which time another portion of **32** (4.7 mg, 5.9 μmol) was added and the mixture stirred for a further 12 hours. The orange solution was exposed to air and 1M aqueous NaOH (5 mL) was added. The solution was left standing at room temperature for 12 hours, and over this time it turned dark green, after which it was concentrated in vacuo. The black residue was taken up in Et_2O (15 mL), filtered and the filtrate was extracted with 1M HCl (3×15 mL). The combined aqueous extracts were basified carefully to pH 11 with 1M NaOH and the resultant cloudy aqueous solution was extracted with EtOAc (3×15 mL). The organic extracts were combined, washed with sat. aq. NaCl (15 mL), dried over K_2CO_3, filtered and concentrated in vacuo to give a colourless oil (71.1 mg). The oil was purified by reversed-phase HPLC (5 micron Supelcosil LC-ABZ Plus ODS; H_2O (0.1% TFA): MeCN (0.1% TFA); stepped gradient from 100–5% aq.), normal-phase HPLC (5 micron Hypersil normal-phase silica; hexane/acetone/Et_2NH 80:20:2) and by a pipette flash column (SiO_2, CH_2Cl_2/MeOH 97:3) to give **10** (0.71 mg, 2%) as a white solid; synthetic material was identified by NMR, TLC, LCMS and by doping with authentic keramaphidin B; $R_f = 0.50$ (SiO_2, CH_2Cl_2/MeOH 95:5, visualised with Dragendorff reagent); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.01–1.09$ (m, 1H, C(7)*H*), 1.11–1.30 (m, 2H, C(6)*H*, N(10)CH_2CHH'), 1.40–1.78 (m, 11H, N(10)$CH_2CHH'CH_2CHH'$, N(4)$CH_2CH_2CH_2$, C(6)*H*′, C(9)*H*, C(2)CHH'), 2.02–2.46 (m, 13H, C(8)*H*, C(3)*H*, N(10)$CHH'(CH_2)_2CHH'$, C(2)$CHH'CH_2$, N(4)$(CH_2)_3CH_2$, C(11)CH_2CH_2), 2.68 (ddd, 1H, $J = 13.5$, 7.5, 2.5 Hz, N(4)CHH'), 2.84–2.91 (m, 4H, C(9)*H*′, C(5)*H*, C(3)*H*′, N(10)CHH'), 3.10 (s, 1H, C(1)*H*), 3.39 (t, 1H, $J = 13.5$ Hz, C(5)*H*′), 3.48–3.52 (m, 1H, N(4)CHH'), 5.29 (br t, 1H, $J = 11$ Hz, C(11)$(CH_2)_2CH{=}CH$), 5.41 (br t, 1H, $J = 10.5$ Hz, C(11)$(CH_2)_2CH{=}CH$), 5.66 (ddd, 1H, $J = 13.5$, 10, 5 Hz, C(2)$(CH_2)_2CH{=}CH$), 5.76 (br dd, 1H, $J = 10$, 5.5 Hz, C(2)$(CH_2)_2CH{=}CH$), 5.93 (d, 1H, $J = 6.5$ Hz, C(12)*H*); MS (APCI): *m/z* (%): 381 (100) $[MH]^+$; HRMS calcd for $C_{26}H_{41}N_2$ $[MH]^+$ 381.3270, found 381.3270.

(11*Z*,15*Z*)-11-But-3-enyl-4,10-diaza-4-hex-5-enyltetracyclo-[6.2.2.0^{2,7}.8^{2,10}]icosa-11,15-diene (31): The above reaction also yields **31** (ca. 10–20% when isolated) as a colourless oil; $R_f = 0.22$ (SiO_2, CH_2Cl_2/MeOH 95:5, visualised with Dragendorff reagent); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD 4:1, referenced at 7.27 ppm): $\delta = 0.84$ (ddd, 1H, $J = 11.5$, 6.5, 2 Hz, C(7)*H*), 1.07–1.12 (m, 2H, C(6)*H*, N(10)CH_2CHH'), 1.21–1.45 (m, 9H, C(6)*H*′, N(10)$CH_2CHH'CH_2CHH'$, N(4)$CH_2CH_2CH_2$), 1.51–1.58 (m, 3H, C(2)$CHH'CHH'$, C(9)*H*), 1.64–1.69 (m, 1H, C(2)CHH'), 1.78 (d, 1H, $J = 12$ Hz, C(3)*H*), 1.90 (pseudo q, 2H, $J = 7.5$ Hz, N(4)$(CH_2)_3CH_2$), 2.00–2.18 (m, 8H, C(11)CH_2CH_2, C(2)CH_2CHH', C(8)*H*, N(10)$CHH'(CH_2)_2CHH'$), 2.19 (d, 1H, $J = 12$ Hz, C(3)*H*′), 2.30 (t, 2H, $J = 7.5$ Hz, N(4)CH_2), 2.42–2.50 (m, 2H, C(5)H_2), 2.69 (dd, 1H, $J = 12.5$, 5 Hz, N(10)CHH'), 2.73 (dd, 1H, $J = 9$, 1.5 Hz, C(9)*H*′), 2.76 (s, 1H, C(1)*H*), 4.77–4.91 (m, 4H, CH=CH_2), 5.48–5.52 (m, 2H, C(2)$(CH_2)_2CH{=}CH$), 5.63 (ddt, 1H, $J = 17$, 10, 7 Hz, N(4)$(CH_2)_4CH{=}CH_2$), 5.68–5.73 (m, 2H, C(11)$(CH_2)_2CH{=}CH_2$, C(12)*H*); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD 4:1, referenced at 77.00 ppm): $\delta = 20.59$ (C(2)CH_2CH_2), 22.41 (N(10)$(CH_2)_3CH_2$), 25.58 (N(10)CH_2CH_2), 25.97 (N(10)$(CH_2)_2CH_2$), 26.04 (C6), 26.20 (N(4)CH_2CH_2), 26.45 (N(4)$(CH_2)_2CH_2$), 31.08 (C(11)CH_2CH_2), 33.25 (N(4)$(CH_2)_3CH_2$), 34.91 (C(11)CH_2), 37.45 (C8), 40.99 (C(2)CH_2), 43.47 (C7), 46.06 (C2), 49.41 (C5), 49.84 (C3), 53.57 (C9), 53.88 (N(10)CH_2), 58.96 (N(4)CH_2), 63.25 (C1), 114.07 (N(4)$(CH_2)_4CH{=}CH_2$), 114.17 (C(11)-$(CH_2)_2CH{=}CH_2$), 121.69 (C12), 129.99 and 131.16 (*CH*=*CH*), 138.15 (C(11)$(CH_2)_2CH{=}CH_2$), 138.32 (N(4)$(CH_2)_4CH{=}CH_2$), 142.31 (C11); IR (thin film): $\tilde{\nu} = 2922$, 2852, 1682, 1642, 1612, 1445, 1362, 1172, 1131, 908, 822 cm^{-1}; MS (APCI): *m/z* (%): 409 (100) $[MH]^+$; HRMS calcd for $C_{28}H_{45}N_2$ $[MH]^+$ 409.3583, found 409.3583.

Acknowledgment

This work was supported financially by the EPSRC (A.J.C. and D.R.S.) and the Rhodes Trust (F.A.H.). We are indebted to Prof. Raymond J. Andersen (Department of Chemistry, University of British Columbia) and Prof. Jun′ichi Kobayashi (Faculty of Pharmaceutical Sciences, Hokkaido University, Japan), for their generous gifts of keramaphidin B. We thank Mr. Robert J. Boughtflower, Mr. Ian M. Mutton and Dr. Richard J. Upton for their help with HPLC, LCMS and 750 MHz NMR. We also thank Prof. Vernon C. Gibson (Department of Chemistry, Imperial College) for his cooperation and advice regarding the Schrock catalyst.

[1] Subsequent reports have described another twenty five related alkaloids, including manzamines D, E, F, G, H, J, K (K = G), L, M, X and Y, and hydroxy, dihydro, tetrahydro and other derivatives. Reviews: a) R. J. Andersen, R. W. M. Van Soest, F. Kong, *Alkaloids: Chemical and Biological Perspectives*, *Vol. 10* (Ed.: S. W. Pelletier), Pergamon, London, **1996**, pp. 301–355; b) M. Tsuda, J. Kobayashi, *Heterocycles* **1997**, *46*, 765–794; c) N. Matzanke, R. J. Gregg, S. M. Weinreb, *Org. Prep. Proceed. Int.* **1998**, *30*, 1–52; some recent additions include: d) J. Kobayashi, D. Watanabe, N. Kawasaki, M. Tsuda, *J. Org. Chem.* **1997**, *62*, 9236–9239; e) Y. Guo, E. Trivellone, G. Scognamiglio, G. Cimino, *Tetrahedron* **1998**, *54*, 541–550; f) F. Kong, E. I. Graziani, R. J. Andersen, *J. Nat. Prod.* **1998**, *61*, 267–271; g) M.Tsuda, D. Watanabe, J. Kobayashi, *Tetrahedron Lett.* **1998**, *39*, 1207–1210; h) D.Watanabe, M. Tsuda, J. Kobayashi, *J. Nat. Prod.* **1998**, *61*, 689–692; i) R. J. Clark, K. L. Field, R. D. Charan, M. J. Garson, I. M. Brereton, A. C. Willis, *Tetrahedron* **1998**, *54*, 8811–8826; j) M. Tsuda, D. Watanbe, J. Kobayashi, *Heterocycles* **1999**, *50*, 485–488; for a review on the synthesis of the manzamines see: E. Magnier, Y. Langlois, *Tetrahedron* **1998**, *54*, 6201–6258. Two total synthesis of manzamine A (**1**) have recently been disclosed: k) J. D. Winkler, J. M. Axten, *J. Am. Chem. Soc.* **1998**, *120*, 6425–6426; l) S. F. Martin, J. M. Humphery, A. Ali, M. C. Hillier, *J. Am. Chem. Soc.* **1999**, *121*, 866–867.

[2] R. Sakai, T. Higa, C. W. Jefford, G. Bernardinelli, *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405.

[3] R. E. Longley, O. J. McConnell, E. Essich, D. Harmody, *J. Nat. Prod.* **1993**, *56*, 915–920.

[4] R. Sakai, S. Kohmoto, T. Higa, C. W. Jefford, G. Bernardinelli, *Tetrahedron Lett.* **1987**, *28*, 5493–5496.

[5] M. J. Garson, *Nat. Prod. Rep.* **1989**, *6*, 143–170.

[6] J. E. Baldwin, R. C. Whitehead, *Tetrahedron Lett.* **1992**, *33*, 2059–2062.

  0947-6539/99/0511-3160 $ 17.50+.50/0

[7] K. Kondo, H. Shigemori, Y. Kikuchi, M. Ishibashi, T. Sasaki, J. Kobayashi, *J. Org. Chem.* **1992**, *57*, 2480–2483.
[8] a) J. Kobayashi, M. Tsuda, N. Kawasaki, K. Matsumoto, T. Adachi, *Tetrahedron Lett.* **1994**, *35*, 4383–4386; b) M. Tsuda, K. Inaba, N. Kawasaki, K. Honma, J. Kobayashi, *Tetrahedron* **1996**, *52*, 2319–2324; c) J. Kobayashi, N. Kawasaki, M. Tsuda, *Tetrahedron Lett.* **1996**, *37*, 8203–8204; d) F. Kong, R. J. Andersen, *Tetrahedron* **1995**, *51*, 2895–2906. (+)-Keramaphidin B has the opposite absolute stereochemistry as that shown for **10** in Figure 2.
[9] For a preliminary report see: J. E. Baldwin, T. D. W. Claridge, A. J. Culshaw, F. A Heupel, V. Lee, D. R. Spring, R. C. Whitehead, R. J. Boughtflower, I. M. Mutton, R. J. Upton, *Angew. Chem.* **1998**, *110*, 2806–2808; *Angew. Chem. Int. Ed.* **1998**, *37*, 2661–2663.
[10] If the cycloaddition occurs in the absence of enzyme catalysis, a racemic mixture will result. Alternatively: i) an enzyme may catalyse the cycloaddition, but with limited enantiomeric differentiation since both enantiomers are present; or ii) two enzymes catalyse the cycloaddition with antipodal selectivity. To achieve the unequal enantiomeric mixture a kinetic resolution may occur, the (−)-antipode being processed to the ircinals and manzamines faster than the (+)-antipode is processed to its products.
[11] Model studies: a) J. E. Baldwin, T. D. W. Claridge, F. A. Heupel, R. C. Whitehead, *Tetrahedron Lett.* **1994**, *35*, 7829–7832; b) J. E. Baldwin, T. D. W. Claridge, A. J. Culshaw, F. A. Heupel, S. Smrckova, R. C. Whitehead, *Tetrahedron Lett.* **1996**, *37*, 6919–6922; c) J. E. Baldwin, L. Bischoff, T. D. W. Claridge, F. A. Heupel, D. R. Spring, R. C. Whitehead, *Tetrahedron* **1997**, *53*, 2271–2290; d) L. Gil, A. Gateau-Olesker, C. Marazano, B. C. Das, *Tetrahedron Lett.* **1995**, *36*, 707–710; e) L. Gil, A. Gateau-Olesker, Y.-S. Wong, L. Chernatova, C. Marazano, B. C. Das,*Tetrahedron Lett.* **1995**, *36*, 2059–2062; f) L. Gil, X. Baucherel, M.-T. Martin, C. Marazano, B. C. Das, *Tetrahedron Lett.* **1995**, *36*, 6231–6234.
[12] a) J. B. Cloke, F. J. Pilgrim, *J. Am. Chem. Soc.* **1939**, *61*, 2667–2669; b) I. J. Borowitz, G. J. Williams, L. Gross, R. Rapp, *J. Org. Chem.* **1968**, *33*, 2013–2020; c) J. F. Oughton, *Chem. Abstr.* **1964**, *60*, P4011h; d) P. S. Manchand, R. A. Micheli, S. J. Saposnik, *Tetrahedron* **1992**, *48*, 9391–9398; e) A. I. Meyers, E. W. Collington, *Tetrahedron* **1971**, *27*, 5979–5985.
[13] a) A. J. Mancuso, D. Swern, *Synthesis* **1981**, 165–185; b) T. T. Tidwell, *Synthesis* **1990**, 857–870.
[14] a) A. D. Buss, S. Warren, *J. Chem. Soc. Perkin Trans. I* **1985**, 2307–2325; b) P. M. Ayrey, M. A. Bolton, A. D. Buss, N. Greeves, D. Levin, P. Wallace, S. Warren, *J. Chem. Soc. Perkin Trans. I* **1992**, 3407–3417.
[15] a) B. E. Maryanoff, A. B. Reitz, B. A. Duhl-Emswiler, *J. Am. Chem. Soc.* **1985**, *107*, 217–226; b) B. E. Maryanoff, A. B. Reitz, *Chem. Rev.* **1989**, *89*, 863–927. The interconversion of *cis* and *trans* oxaphosphetanes by reversal to ylide and aldehyde **14** was proposed to be promoted by the anionic moiety "biting back" onto phosphorus to generate a transient hexacoordinate phosphate intermediate or a hexacoordinate transition state during a reaction reminiscent of an intramolecular S_N2 step.
[16] N. Miyashita, A. Yoshikoshi, P. A. Grieco, *J. Org. Chem.* **1977**, *42*, 3772–3774.
[17] A. V. R. Rao, G. R. Reddy, B. V. Rao, *J. Org. Chem.* **1991**, *56*, 4545–4547.
[18] G. W. Kabalka, M. Varma, R. S. Varma, *J. Org. Chem.* **1986**, *51*, 2386–2388.
[19] a) A. Ahond, A. Cave, C. Kan-Fan, H.-P. Husson, J. de-Rostolan, P. Potier, *J. Am. Chem. Soc.* **1968**, *90*, 5622–5623; b) M. Lounasmaa, A. Koskinen, *Heterocycles* **1984**, *22*, 1591–1612.
[20] The reactions were all worked up and analysed by 500 MHz ^{1}H NMR and LCMS to detect the presence and approximate quantity of keramaphidin B (**10**); SDS: sodium dodecyl sulfate; CHAPS: 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate.
[21] The synthetic material was identified by TLC, ^{1}H and ^{13}C NMR, LCMS and by doping the synthetic sample with authentic material (see Supporting Information);
[22] R. J. Sundberg, D. S. Grierson, H.-P. Husson, *J. Org. Chem.* **1984**, *49*, 2400–2404.
[23] The conformational search was carried out with 10000 Monte Carlo steps in MacroModel V5.5. Each conformer was minimised with the MM2* force field in water; F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[24] Structure **25** was calculated from an ab initio transition state search at the RHF/6–31G* level. Note that the transition state structure is highly asynchronous, where the formation of one bond is far advanced and the other is in the initial stages.
[25] The most compelling evidence for a Diels–Alderase comes from work published by Oikawa and co-workers; however, the enzyme still has to be fully purified and characterised; see: a) A. Ichihara, H. Oikawa, *Biosci. Biotech. Biochem.* **1997**, *61*, 12–18; b) K. Katayama, T. Kobayashi, H. Oikawa, Honma, A. Ichihara, *Biochim. Biophys. Acta* **1998**, *1384*, 387–395; c) H. Oikawa, T. Kobayashi, K. Katayma, Y. Suzuki, A. Ichihara, *J. Org. Chem.* **1998**, *63*, 8748–8756; d) H. Oikawa, Y. Suzuki, K. Katayama, A. Naga, C. Sakano, A. Ichihara, *J. Chem. Soc. Perkin Trans. I* **1999**, 1225–1232.
[26] F. H. Westheimer, *Adv. Enzymol.* **1962**, *24*, 441–482.
[27] All other routes, such as those involving pyridin-2-ones as dienes, were unsuccessful in model systems.
[28] A. Kaiser, X. Billot, A. Gateau-Olesker, C. Marazano, B. C. Das, *J. Am. Chem. Soc.* **1998**, *120*, 8026–8034.
[29] 5-Amino-2,4-pentadienals are formally ring-opened pyridines, although Marazano and co-workers proposed that they would be biosynthesised from the condensation of malondialdehyde, an aldehyde and an amino group. For convenience, the dienophile illustrated in Scheme 4 is a tetrahydropyridine. However, Marazano suggested that the dienophile is likely to be a 2,3-dihydropyridinium ion or another 5-amino-2,4-pentadienal.
[30] J. Becher, *Synthesis* **1980**, 589.
[31] 1,3-Dimethylpyridinium salts have been treated with alkaline ferricyanide in the Decker oxidation to provide the pyridone in 70–82% yield (H. Decker, *Chem. Ber.* **1892**, *25*, 443; H. Weber, *Adv. Heterocycl. Chem.* **1987**, *41*, 275–317). It is presumed that the mechanism involves the formation of the "pseudo base", that is a hydroxide–pyridinium adduct. Moreover, the pseudo base is deprotonated before the rate determining complex formation with ferricyanide. If the mechanism is correct, then the formation of the deprotonated pseudo base can occur reversibly with **28**, but ring-opening is not favoured due to the lack of an electron-withdrawing group on the nitrogen.
[32] J. Becher, L. Finsen, I. Winckelmann, *Tetrahedron* **1981**, *37*, 2375–2378. To form the correct 5-amino-2,4-pentadienal **27** hydroxide is required to attack the pyridine C6 position.
[33] Other glutaconaldehyde derivatives have been investigated and used as dienes in the Diels–Alder reaction; see: a) E. J. J. Grabowski, R. L. Autrey, *Tetrahedron* **1969**, *25*, 4315–4330; b) J. Becher, H. C. Nielsen, J. P. Jacobsen, O. Simonsen, H. Clausen, *J. Org. Chem.* **1988**, *53*, 1862–1871; c) L. Berthon, A. Tahri, D. Uguen, *Tetrahedron Lett.* **1994**, *35*, 3937–3940.
[34] For reviews on ring-closing metathesis see: a) R. H. Grubbs, S. J. Miller, G. C. Fu, *Acc. Chem. Res.* **1995**, *28*, 446–452; b) H.-G. Schmalz, *Angew. Chem.* **1995**, *107*, 1981–1984; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1833–1836; c) A. Fürstner, K. Langemann, *Synthesis* **1997**, 792–803; d) S. K. Armstrong, *J. Chem. Soc. Perkin Trans. I* **1998**, 371–388.
[35] Since the Grubbs catalyst **32** is not compatible completely with tertiary amines, the cycloadduct was protected as its bis-hydrochloride salt (G. C. Fu, S. T. Nguyen, R. H. Grubbs, *J. Am. Chem. Soc.* **1993**, *115*, 9856–9857). However, when the salt was treated with **32** then the quantity of keramaphidin B (**10**) was reduced (LCMS).
[36] Attempts to close the 13-membered ring in tetracyclic **31** with the Schrock catalyst mainly resulted in decomposition. Similar results were observed when the Grubbs catalyst was used with **31** and its bis-hydrochloride salt.
[37] General experimental procedures are described in the Supplementary Information. Note that a consistent numbering system has been employed for NMR assignments of the pyridine and the 4,10-diazatricyclo[6.2.2.0^{2,7}]dodec-11-ene ring systems.

Received: May 3, 1999 [F 1763]