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Ring-Closing Metathesis in the Synthesis of Biologically Active Peptidomimetics of Apicidin A

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Abstract: Syntheses of novel 16-membered macrocyclic peptidomimetics are reported, which employ iterative peptide coupling followed by high yielding ring-closing metathesis (RCM) as the key cyclization step. The target macrocyclic compounds include examples containing a (2*S*)-amino-8-oxodecanoic acid (Aoda) residue as analogues of apicidin A, a known potent histone deacetylase (HDAC) inhibitor. These showed modest levels of biological activity as HDAC inhibitors.

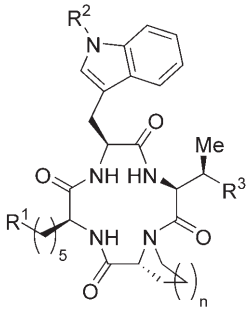
Keywords: cyclization; macrocycles; medicinal chemistry; metathesis; peptidomimetics

Introduction

The development of novel, potent, bioavailable inhibitors of histone deacetylase is of considerable current pharmaceutical importance. Such inhibitors may be of value as drugs for the treatment of parasite-mediated infections such as malaria, coccidiosis, cryptosporidiosis and toxoplasmosis, as well as cancer.[1] Cyclic tetrapeptides have shown particular promise, owing to their resistance to gut proteases, their structural rigidity and potent inhibition of the parasitic apicomplexan enzyme histone deacetylase (HDAC).[1,2] Among these, the apicidins **1a–g** (Table 1) show broad-spectrum antimalarial and coccidiostatic activity, due to their potent (nM range) reversible inhibition of HDAC.[2]

Owing to their interesting biological properties, these natural products have been subject to several synthetic investigations. Previous syntheses have exclusively relied upon macrolactam formation as the key step in building the tetrapeptide core.[3] These approaches have frequently been complicated by low efficiencies in peptide ring closures, caused by various side reactions upon cyclization of the open tetrapeptide, including epimerization of one or more stereocenters, as well as the preferential formation of dimeric octapeptides.[4] In general, macrolactam formation between the *C*-terminus of proline and the *N*-terminus of Aoda is crucial in such an approach, and all known synthetic studies have demonstrated that cyclization does not occur at any other position.[3,4]

Table 1. Known apicidin natural products.

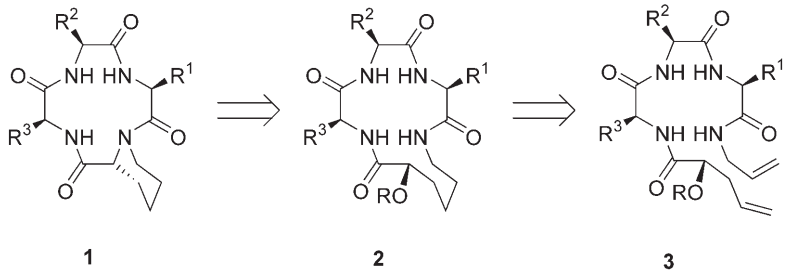


Name	R^1	R^2	R^3	n
apicidin (**1a**)	$COCH_2CH_3$	OMe	Et	2
apicidin A (**1b**)	$COCH_2CH_3$	H	Et	2
apicidin B (**1c**)	$COCH_2CH_3$	OMe	Et	1
apicidin C (**1d**)	$COCH_2CH_3$	OMe	Me	2
apicidin D_1 (**1e**)	$COCH(OH)CH_3$	OMe	Me	2
apicidin D_2 (**1f**)	$CH(OH)CH_2CH_3$	OMe	Me	2
apicidin D_3 (**1g**)	$CH_2CH(OH)CH_3$	OMe	Me	2

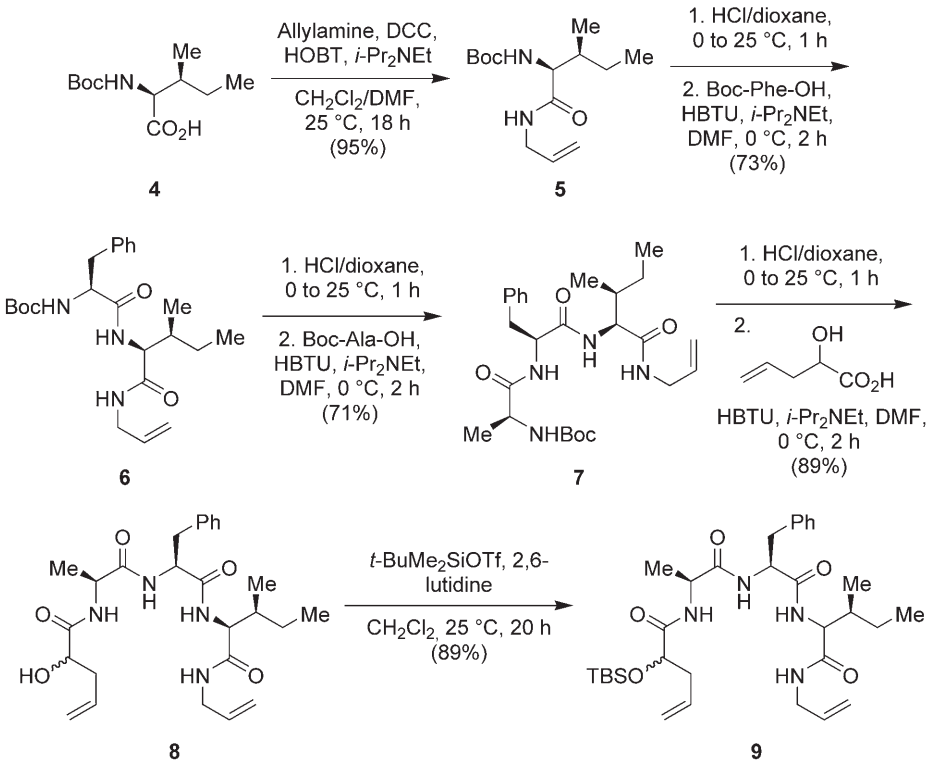
As part of our investigation into the synthesis of apicidin analogues, we became interested in the possibility of using macrocyclization by ring-closing metathesis (RCM)[5] and subsequent hydrogenation of a suitably protected diene **3**, followed by transannular ring closure[6] of intermediate **2** to give the target tetrapeptides **1** (Scheme 1). Herein, we report studies on this approach and the synthesis of peptoid analogues of apicidin using RCM and their biological evaluation.

Results and Discussion

Our initial model studies were focused on the synthesis and RCM of the model diene **9** (Scheme 2). Initially, we envisaged the requirement of protection at the α-hydroxy position to prevent the possible formation of stable ruthenium chelates during the RCM reaction.[7] Thus, starting with Boc-Ile-OH (**4**), dicyclohexylcarbodiimide coupling[8] with allylamine gave amide **5** in 95% yield. Deprotection under acidic conditions followed by direct HBTU coupling[9] with Boc-Phe-OH gave the dipeptide **6** in 73% yield. Further deprotection and peptide coupling subsequently gave the tripeptide **7** in 71% yield. Finally, this peptide was deprotected and coupled with (±)-2-hydroxy-4-pentenoic acid, synthesized using allylindium chemistry,[10] to give the diene **8**. This RCM precursor was isolated as an equimolar diastereoisomeric mixture of the epimers at the α-hydroxy position. Protection of the free alcohol as the silyl ether **9** required the use



Scheme 1. Retrosynthesis of apicidin analogues.



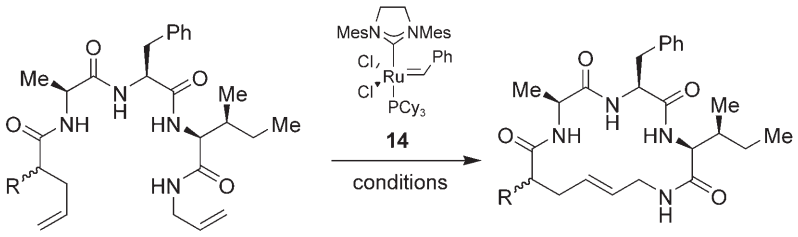
Scheme 2. Synthesis of peptide diene **9**. Boc = *tert*-butyloxycarbonyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, HOB*T* = 1-hydroxybenzotriazole, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, Tf = trifluoromethanesulfonate.

of a large excess (10 equivalents) of both *tert*-butyl(dimethyl)silyl triflate and 2,6-lutidine,[11] in order to provide the product in a satisfactory yield of 89%. We attribute the low reactivity of the hydroxy group to its position α to an amide, whose electron-withdrawing nature, hydrogen bonding ability and steric congestion render the α-hydroxy group particularly unreactive. The diene **9** was thus obtained in 39% overall yield starting from Boc-Ile-OH **4**. High temperature ^{1}H NMR spectra and LC-MS analysis were consistent with no detectable racemization having occurred during the peptide coupling steps.

Initial cyclization investigations showed that attempted RCM with the silylated diene **9** was unsuccessful with no conversion to the desired cyclic pseudopeptide **11**, using Grubbs second-generation catalyst **14**,[12] under a number of conditions (Table 2, entries 1–3). Instead, only unchanged starting material was observed by LCMS analysis of the crude reaction mixture. We considered that steric hindrance by the silyl ether residue was an impediment to cyclization. Therefore for comparison, peptide **10**, lacking the α-hydroxy functionality, was prepared. Diene **8**, bearing a free alcohol was also subjected to ring-closing metathesis conditions. Catalyst **14** (10 mol%) was used in dichloromethane at reflux, for the comparative study. Whilst the diene **10** underwent cyclization and gave at best a 55% yield after 18 h of reaction, with the mass balance accounted for by starting material, a much higher conversion was observed by LCMS analysis in the case of hydroxy-peptide **8**. This is in accordance with reports that the rate of metathesis reactions is enhanced by the presence of free hydroxy groups.[13] During the RCM studies, high dilution conditions (substrate concentration of 1 mM in CH_2Cl_2) were required. Complete dissolution of the peptide diene precursors, even at this high dilution, required the use of ultrasound agitation for 20 min, followed by reflux for 0.5 h, prior to the addition of the catalyst **14**. In the case of hydroxy-diene **8**, particularly low conversions were observed in the absence of this pre-treatment, owing to its poor substrate solubility. The use of alternative solvents, such as toluene or 1,2-dichloroethane at reflux was unsatisfactory with low conversions, whereas THF as solvent resulted in substantial double-bond migration, possibly through the action of ruthenium hydride species, as reported by Schmidt.[14]

Despite the high conversion in the cyclization of the open chain peptide **8** to its ring-closed counterpart **13**, purification of the desired product from small amounts of starting material, possibly higher oligomers and catalyst by-products was hampered by its poor solubility. Indeed, all attempts at recrystallization and chromatography failed to give satisfactory yields and purities of the desired macrocyclic product.

Table 2. RCM optimization studies for cyclization of dienes **8–10**.[a]



8 – 10 **11 – 13**

Entry	Substrate	Product	R	Solvent (conc.)	*T* [°C]/time	% conversion (LC-MS)[b]
1	**9**	**11**	OTBS	CH_2Cl_2 (10 mM)	25 °C/48 h	n.d.
2	**9**	**11**	OTBS	CH_2Cl_2 (10 mM)	40 °C/48 h	n.d.
3	**9**	**11**	OTBS	CH_2Cl_2 (1 mM)	40 °C/48 h	n.d.
4	**10**	**12**	H	CH_2Cl_2 (1 mM)	40 °C/18 h	55%[c]
5	**8**	**13**	OH	CH_2Cl_2 (1 mM)	25 °C/20 h	n.d.
6	**8**	**13**	**OH**	**CH_2Cl_2 (1 mM)**	**40 °C/16 h**	**>90%**
7	**8**	**13**	OH	CH_2Cl_2 (10 mM)	40 °C/20 h	40%
8	**8**	**13**	OH	$(CH_2)_2Cl_2$ (10 mM)	83 °C/20 h	40%
9	**8**	**13**	OH	PhMe (10 mM)	110 °C/20 h	n.d.
10	**8**	**13**	OH	THF (10 mM)	66 °C/20 h	75%

[a] Reactions were performed by addition of Grubbs' catalyst **14** (10 mol%) to substrate in the solvent. Substrate solutions prepared by sonication for 20 min, followed by heating the solution to reflux for 30 min, prior to the addition of catalyst **14**.

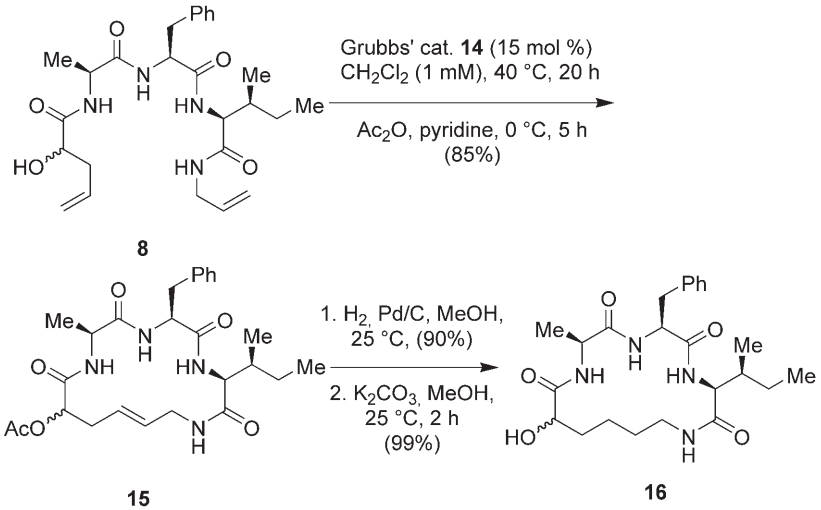
[b] n.d. = the conversion was not determined.

[c] Isolated yield quoted.

Additionally, hydrogenation of the crude product, which was required in the subsequent step, did not alleviate the problem of purification, since the hydrogenated product had a similar solubility to the crude metathesis product. Purification was therefore best achieved by conversion of the crude metathesis product to the corresponding acetate **15**, which was readily purified by chromatography, giving the pure macrocycle in 85% yield. Hydrogenation of **15** then gave the saturated acetate, which following purification and hydrolysis using potassium carbonate in methanol gave pure RCM product **16** (Scheme 3) Additionally, this synthesis and purification process was reproducible on a multi-gram scale.

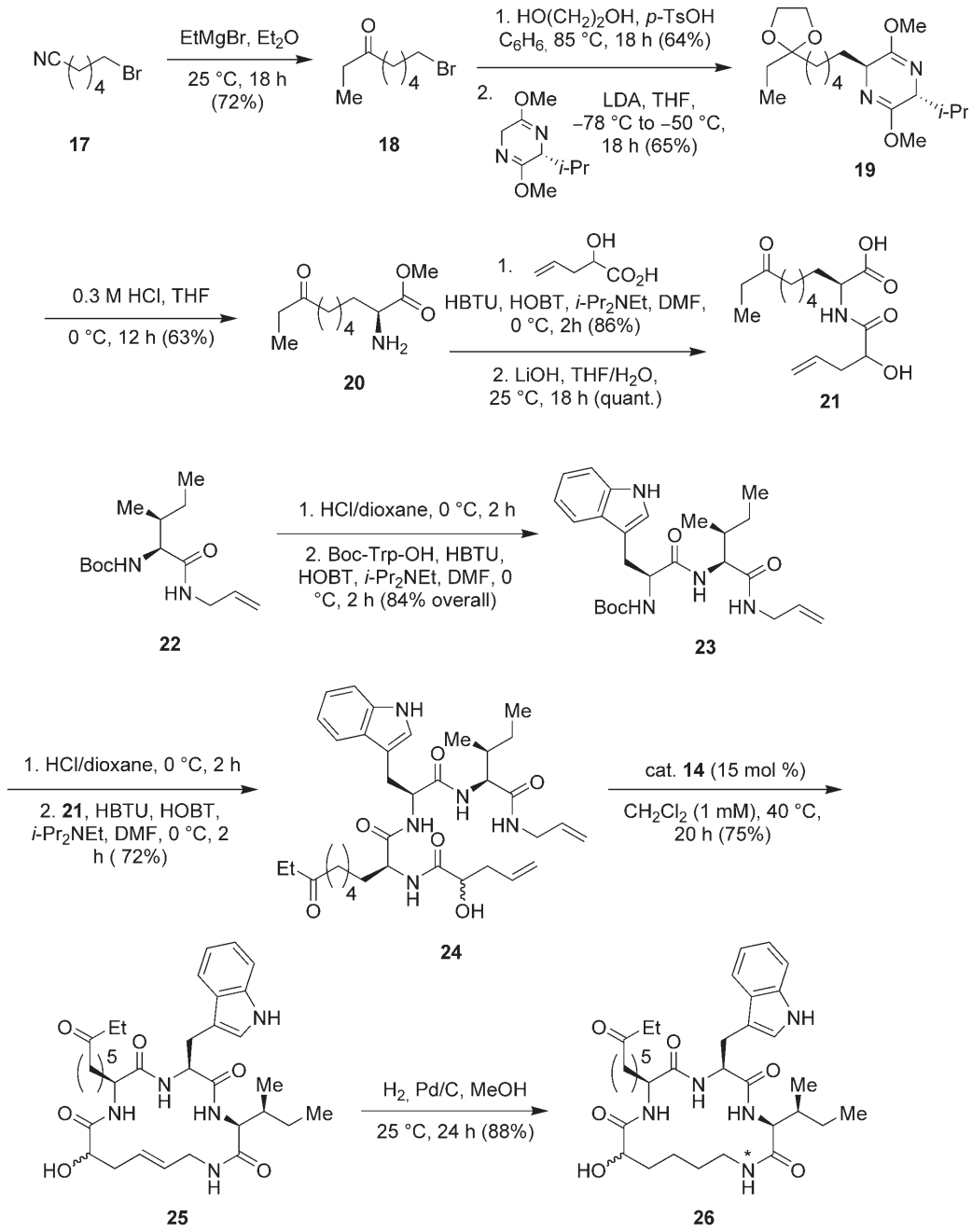
We reasoned that since RCM was now optimized for the model system, it could similarly be applied to a tetrapeptide backbone bearing the correct functionality of apicidin A. Thus, the macrocyclic peptoid alkene **25** and its hydrogenated derivative **26** were prepared, bearing the (2*S*)-amino-8-oxo-decanoic acid side chain, to ascertain whether such a compound could mimic the HDAC activity of apicidin (Scheme 4). Reaction of bromonitrile **17** with ethylmagnesium bromide at 25 °C gave the ethyl ketone **18** in 72% yield. The previous reported synthesis of ketone **18** involved the addition of ethylmagnesium bromide to cyclohexanone,[15] followed by retro-Barbier fragmentation.[16] However, in our hands this method proved to be capricious, and led to the formation of aldol products. Following the procedure by Kim,[17] ketal protection of the bromo-ketone **18** followed by Schöllkopf alkylation gave the *trans*-2,5-dihydropyrazine **19**, which was isolated in 65% yield. Hydrolysis of the pyrazine was achieved using dilute hydrochloric acid in THF, and gave the free amino ester **20** (63%).[17] The key peptide fragment **21** was prepared by HBTU-mediated peptide coupling with (±)-2-hydroxy-4-pentenoic acid[10] and hydrolysis of the methyl ester. The second peptide fragment **23** was synthesized from Boc-Ile-NHAll **22** by Boc-deprotection and HBTU peptide coupling with Boc-Trp-OH. The peptide **23** was again deprotected and coupled with the side chain-containing peptide **21** to give the metathesis precursor diene **24**. RCM was repeated on this diene under our previously optimized conditions, and the resulting macrocycle **25** was obtained in 75% isolated yield, without the need for derivatization prior to purification. A further macrocycle **26** was obtained by the hydrogenation of the alkene unit. Attempted transannular ring-closure of macrocycle **26** by activation of the secondary alcohol as its derived tosylate or mesylate and S_N2 displacement by the starred amide nitrogen under basic conditions was unsuccessful and resulted only in unchanged starting material or degradation.

Samples of the two dienes **25** and **26** were purified further using preparative reverse-phase HPLC, and their biological activities as HDAC inhibitors in mammalian HeLa cell extracts determined. IC_{50} values were determined by measuring the ^3H decay of [^3H]acetate released by the action of HDAC enzyme on [^3H]acetyl histone H_4 peptide in an assay standardized by trichostatin A, a known potent HDAC inhibitor.[18] Plots of HDAC activity, expressed as a percentage of the control against $\log_{10}$[substrate] gave IC_{50} values, following extrapolation at 50% of control (50% HDAC activity). In our assay, trichostatin A gave an IC_{50} value of 1.0 nM, with 95% chemical inhibition within the range 0.86–1.2 nM, which is in accordance with the reported values.[18] Apicidin A displays an IC_{50} in the single nanomolar range for numerous cell types.[2] Our substrate **25**, containing a *trans* alkene, displayed an IC_{50} of 0.59 μM, with 95% chemical inhibition in the range 0.34–1.0 μM, giving a



8 **15** **16**

Scheme 3. Purification of macrocycle **16** *via* the acetate **15**.



Scheme 4. Synthesis of apicidin analogues **25** and **26**.

500-fold drop in activity. The saturated peptide **26** showed a greater loss in activity compared to apicidin A (IC_{50} 3.3 μM, 95% chemical inhibition at 1.4–7.6 μM).

The loss of biological activity of these analogues compared to apicidin A is not surprising, since for compounds **25** and **26**, the heterocyclic core is no longer a rigid cyclic tetrapeptide but is composed instead of a more conformationally mobile 16-membered ring. In the case of the saturated compound **26**, a more pronounced ring flexibility may explain the weaker binding. Despite the attenuated activity of these analogues compared to the apicidins, these represent biologically active HDAC inhibitors that are readily synthesized with a high yield in the key macrocyclization step.

Conclusions

In summary, we have investigated the RCM macrocyclization reaction as a high-yielding strategy for the synthesis of analogues of apicidin. In particular, diene precursors bearing a free hydroxy group gave the

most efficient conversion to the *trans* macrocyclic alkenes. The optimized conditions for RCM were applied to a synthesis of two analogues, bearing an 8-oxo side-chain, which both displayed biological activity as histone deacetylase inhibitors, and represent novel macrocyclic analogues of apicidin. Moreover, this work demonstrates the wide-ranging utility of metathesis chemistry in the synthesis of polyfunctional biologically active compounds, and as a means of providing rapid access to structures which otherwise would be difficult to synthesize.

Experimental Section

General Remarks

All reactions were carried out in oven or flame dried glassware under an inert atmosphere of N_2 or Ar using standard Schlenk techniques when required. Reaction temperatures other than 25 °C were recorded as bath temperatures except in cases of solvents at reflux at 1 atm pressure, when the boiling point of the solvent was recorded. Prolonged periods of vessel cooling were attained using a CryoCool apparatus. The following reaction solvents were distilled under N_2: THF and Et_2O from K/Ph_2CO or Na/Ph_2CO, respectively; CH_2Cl_2, from CaH_2. The following organic reagents were purified by distillation under N_2: $SOCl_2$, Et_3N, $(i\text{-}Pr)_2NEt$ and pyridine from CaH_2; Et_2NH from KOH. All other solvents and reagents were obtained from commercial sources and used without further purification unless stated otherwise. Chromatography was carried out on silica gel 60, particle size 20–63 μm using flash chromatographic techniques[19] (eluants are given in parenthesis).

Melting points were obtained on a hot-stage apparatus and are uncorrected. Infra-red spectra were recorded as thin films on sodium chloride plates with absorptions reported in wave numbers (cm^{-1}). Proton magnetic resonance spectra (^1H NMR) were recorded at 270 MHz, 300 MHz or 400 MHz. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak, 7.26 for $CDCl_3$, 4.79 for D_2O or 2.52 for DMSO-d_6. Carbon magnetic resonance spectra (^{13}C NMR) were recorded at 75 MHz with chemical shifts (δ) quoted in parts per million (ppm) and are referenced to the residual solvent peak 77.0 for $CDCl_3$ or 39.7 for DMSO-d_6. Coupling constants (J) are quoted in Hertz (Hz) for both ^1H and ^{13}C NMR. Mass spectrometry (EI, CI, FAB) was recorded by the Imperial College Department of Chemistry Mass Spectrometry service using a Micromass Platform II spectrometer (low resolution) and a Micromass Autospec Q spectrometer (low and high resolution). LC-MS analysis of reaction mixtures and purified compounds was performed using a Micromass ZMD quadropole spectrometer using electrospray ionization (positive and negative mode). UV detection was performed using a Waters 600 Diode Array detector, and all analyses were performed using MassLynx 3.1. All LCMS runs used a linear MeCN/H_2O solvent gradient in a 10 min run on a C-18 column, starting from 5% MeCN at $t=0$ min, increasing to 95% MeCN at $t=6$ min, and maintaining this composition until $t=7$ min, at which point 5% MeCN was used.

Boc-Ile-NHAll (5)

$i\text{-}Pr_2NEt$ (9.7 mL, 55.9 mmol), allylamine (6.5 mL, 86 mmol), HOBT (7.6 g, 56 mmol) and DCC (12 g, 56 mmol) were sequentially added at 0 °C to a solution of Boc-Ile-OH (10 g, 43 mmol) in CH_2Cl_2 (50 mL) and DMF (50 mL) at 25 °C. The mixture was stirred at 25 °C for 20 h and filtered. The filtrate was evaporated and the residue dissolved in EtOAc (50 mL), washed with 0.2 M aqueous citric acid (2 × 30 mL), saturated aqueous $NaHCO_3$ (30 mL), saturated aqueous NaCl (30 mL), dried ($MgSO_4$) and evaporated. Chromatography (gradient hexanes:EtOAc, 9:1 to 5:1) gave **5** as a white solid; yield: 12 g (95%).

Boc-Phe-Ile-NHAll (6)

HCl (4.0 M in dioxane; 200 mL, 800 mmol) was added with stirring directly to Boc-peptide **5** (10 g, 38 mmol) at 0 °C. After 2 h, the solvent was evaporated and the crude hydrochloride salt was dried under high vacuum for 18 h. DMF (380 mL), $i\text{-}Pr_2NEt$ (13 mL, 77 mmol), Boc-Phe-OH (10 g, 38 mmol), HOBT (6.2 g, 46 mmol) and HBTU (18 g, 46 mmol) were added to the crude salt. The mixture was stirred at 0 °C for 2 h, prior to dilution in EtOAc (400 mL), washing sequentially with saturated aqueous $NaHCO_3$ (200 mL), saturated aqueous NaCl (300 mL), 0.2 M citric acid (200 mL), H_2O (200 mL) and saturated aqueous NaCl (200 mL) and dried ($MgSO_4$). Solvent evaporation and chromatography (CH_2Cl_2:EtOAc, 1:1) gave **6** as a white solid; yield: 12 g (73%).

Boc-Ala-Phe-Ile-NHAll (7)

HCl (4.0 M in dioxane; 75 mL, 300 mmol) was added with stirring directly to dipeptide **6** (6.3 g, 15 mmol) at 0 °C. After 2 h, the solvent was evaporated and the residue dissolved in DMF (150 mL). $i\text{-}Pr_2NEt$ (2.6 mL, 15 mmol), Boc-Ala-OH (2.8 g, 15 mmol) and HOBT (2.4 g, 18 mmol) were added with stirring at 0 °C. The mixture was cooled to 0 °C, prior to addition of HBTU (6.7 g, 18 mmol). After 2 h, the mixture was partitioned between saturated aqueous $NaHCO_3$ (70 mL) and EtOAc (100 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 50 mL), brine (2 × 40 mL), 0.2 M citric acid (3 × 50 mL), and brine (3 × 30 mL), dried ($MgSO_4$), filtered and evaporated. Chromatography (CH_2Cl_2:MeOH, 19:1 to 9:1) gave tripeptide **7** as a white solid; yield: 5.2 g (71%).

(2*R*,*S*)-2-Hydroxy-4-pentenoyl-Ala-Phe-Ile-NHAll (8)

HCl (4.0 M in dioxane; 7.0 mL, 28 mmol) was added with stirring directly to Boc-Ala-Phe-Ile-NHAll (**7**) (360 mg, 0.73 mmol) at 0 °C. After 2 h, the solvent evaporated, the residue further dried under high vacuum for 18 h and dissolved in DMF (10 mL). $i\text{-}Pr_2NEt$ (380 μL, 2.2 mmol), 2-hydroxy-4-pentenoic acid (170 mg, 1.5 mmol), HOBT (200 mg, 1.5 mmol), and HBTU (560 mg, 1.5 mmol) were added. The mixture was stirred at 0 °C for 2 h, prior to dilution in EtOAc (20 mL), washing sequentially with saturated aqueous $NaHCO_3$ (10 mL), saturated aqueous NaCl (10 mL), 0.2 M citric acid (10 mL), H_2O (10 mL) and saturated aqueous NaCl (15 mL). The organic phase was dried ($MgSO_4$), evaporated and chromatographed (CH_2Cl_2:EtOAc, 1:1) to

give diene **8** as a white solid, and a 1:1 mixture of epimers at C-2; yield: 280 mg (80%).

(2*R*,*S*)-2-(*tert*-Butyl(dimethyl)silyloxy)-4-pentenoyl-Ala-Phe-Ile-NHAll (9)

2,6-Lutidine (0.9 mL, 7.8 mmol) and *t*-BuMe$_2$SiOTf (1.8 mL, 7.8 mmol) were added with stirring to a suspension of alcohol **8** (370 mg, 0.75 mmol) in CH_2Cl_2 (25 mL) at 25°C. After 20 h at 25°C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL), and concentrated to one-third volume. The mixture was dissolved in EtOAc (50 mL) and washed sequentially with 1M HCl (20 mL), H_2O (20 mL) and saturated aqueous NaCl (20 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. Chromatography (CH_2Cl_2:MeOH, 97:3) gave diene **9** as a clear viscous oil, which solidified upon standing, as a 1:1 mixture of epimers at C-2; yield: 400 mg (89%).

4-Pentenoyl-Ala-Phe-Ile-NHAll (10)

HCl (4.0M in dioxane; 2.6 mL, 10 mmol) was added with stirring directly to Boc-Ala-Phe-Ile-NHAll (**7**) (510 mg, 1.0 mmol), at 0°C. After 20 min at 0°C and 25°C for 1 h, the solvent was evaporated, the residue further dried under high vacuum for 18 h and dissolved in DMF (5.2 mL). *i*-Pr$_2$NEt (400 L, 2.3 mmol), HOBT (170 mg, 1.2 mmol) and HBTU (470 mg, 1.2 mmol) in DMF (6.2 mL) were added dropwise with stirring at 0°C. After 2 h, the mixture was partitioned between saturated aqueous $NaHCO_3$ (20 mL) and EtOAc (20 mL). The organic layer was washed sequentially with saturated aqueous $NaHCO_3$ (3×20 mL), saturated aqueous NaCl (2×30 mL), 0.2M citric acid (3×20 mL), and saturated aqueous NaCl (3×20 mL), dried ($MgSO_4$) and the solvent evaporated. Chromatography (gradient CH_2Cl_2:MeOH, 19:1 to 9:1) gave diene **10** as a white crystalline solid; yield: 282 mg (60%) .

(6*S*)-Benzyl-(9*S*)-methyl-(3*S*)-[(1*S*)-methylpropyl]-1,4,7,10-tetraazacyclohexadec-14-ene-2,5,8,11-tetraone (12)

Diene **10** (400 mg, 0.85 mmol) was added to CH_2Cl_2 (800 mL) and the mixture was immersed in an ultrasound bath for 25 min until the solid formed a finely dispersed suspension. The suspension was stirred under reflux at 40°C for 30 min until complete dissolution was observed, at which point catalyst **14** (100 mg, 0.12 mmol) was added. The mixture was stirred under reflux at 40°C for 18 h, allowed to cool to 25°C and quenched with ethyl vinyl ether (2 mL) and was stirred for 20 min at 25°C. Solvent evaporation and chromatography (gradient CH_2Cl_2 to CH_2Cl_2:MeOH, 94:6) gave the alkene **12** as a light brown amorphous solid containing a mixture of rotamers; yield: 207 mg (55%).

(6*S*)-Benzyl-(9*S*)-methyl-(3*S*)-[(1*S*)-methylbutyl]-2,5,8,11-tetraoxo-1,4,7,10-tetraazacyclohexadec-14-en-12-yl Acetate (15)

Diene **8** (380 mg, 0.78 mmol) was suspended in CH_2Cl_2 (780 mL) and immersed in an ultrasound bath for 30 min until the suspension became homogenous. The mixture was stirred under reflux at 40°C for 25 min, by which time complete dissolution was observed and catalyst **14** (100 mg, 0.12 mmol) was added. The mixture was stirred at 40°C for 14 h, allowed to cool to 25°C, prior to quenching with ethyl vinyl ether (2 mL). The mixture was stirred for 20 min at 25°C and the solvents evaporated. The residue was dissolved in pyridine (3.5 mL) and cooled to 0°C prior to addition of Ac_2O (3.5 mL). The mixture was stirred at 0°C for 5 h, diluted in EtOAc (30 mL), and washed with saturated aqueous $CuSO_4$ (3×20 mL) (until no dark blue washings were obtained), saturated aqueous $NaHCO_3$ (20 mL), saturated aqueous NaCl, dried ($MgSO_4$) and the solvent evaporated. Chromatography (gradient elution, CH_2Cl_2:MeOH, 97:3 to 93:7) gave the acetate **15** as a light brown solid; yield: 330 mg (85%).

(6*S*)-Benzyl-(12*R*,*S*)-hydroxy-(9*S*)-methyl-(3*S*)-[(1*S*)-methylpropyl]-1,4,7,10-tetraazacyclohexadecan-2,5,8,11-tetraone (16)

Pd/C (10 mol%) was added to a solution of alkene **15** (1.0 g, 2.1 mmol) in MeOH (50 mL) at 25°C. The mixture was purged three times with a balloon of H_2 and allowed to stir for 18 h at 25°C under H_2. Evaporation and chromatography (CH_2Cl_2:MeOH, 94:6) gave the corresponding dihydro-derivative (950 mg, 90%) as a cream-colored, amorphous, diastereoisomeric mixture at C-2. K_2CO_3 (240 mg, 1.8 mmol) was added to a solution of this dihydro-derivative (890 mg, 1.8 mmol) in MeOH (60 mL) at 25°C. The mixture was stirred at 25°C for 3 h following which the mixture was shaken with excess Amberlyst resin (3 g) for 1 h. The resin was filtered under reduced pressure and the filtrate evaporated to give **16** as a white amorphous solid containing a 4 : 1 mixture of diastereoisomers; yield: 810 mg (99%).

8-Bromo-3-octanone (18)

EtMgBr in Et_2O (3.0M; 4.8 mL, 14.5 mmol) was added gradually to a solution of 6-bromohexanenitrile (**17**) (1.7 g, 9.7 mmol) in Et_2O (32 mL) at 25°C. The mixture was stirred for 18 h, and stirred rapidly with hydrochloric acid (6M, 30 mL) for 1 h. The separated aqueous layer was extracted with Et_2O (2×20 mL) and the combined organic phases were washed with saturated aqueous $NaHCO_3$ (50 mL), H_2O (2×40 mL), dried ($MgSO_4$) and evaporated. Chromatography (hexanes:Et_2O, 8:2) gave the ketone **18**[16] as a clear, colorless oil; yield: 1.5 g (72%).

Methyl (2*S*)-Amino-8-oxodecanoate (20)

Ester **20** was prepared from bromide **18** *via* ketal protection, Schöllkopf alkylation and acid catalyzed hydrolysis using exactly the same methods as described by Kim et al.[17]

Methyl (2*S*)-[(2*R*,*S*)-Hydroxypent-4-enoylamino]-8-oxodecanoate

i-Pr$_2$NEt (303 μL, 1.7 mmol), 2-hydroxy-4-pentenoic acid (202 mg, 1.7 mmol), HOBT (235 mg, 1.7 mmol) and HBTU (662 mg, 1.7 mmol) were added with stirring to a solution of amine **20**[17] (312 mg, 1.5 mmol) in anhydrous DMF (14.5 mL) at 0°C. After 16 h, the mixture was diluted with EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL), 0.2M citric acid (20 mL), H_2O (30 mL),

and saturated aqueous NaCl. The organic phase was dried ($MgSO_4$) and the solvent was evaporated. Chromatography (CH_2Cl_2:MeOH, 95:5) gave the title amide as a pale yellow gum containing an equimolar mixture of diastereoisomers; yield: 393 mg (86%) .

(2*S*)-[(2*R*,*S*)-Hydroxy-4-pentenoylamino]-8-oxodecanoic Acid (21)

LiOH (200 mg, 8.3 mmol) was added to the preceding ester (2.35 g, 7.5 mmol) in THF (25 mL) and H_2O (7.5 mL) and the mixture stirred at 25 °C for 18 h. The mixture was evaporated to one-third of its volume, acidified to pH 0.5, saturated with NaCl and extracted with EtOAc (5 × 20 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give acid **21** as a viscous oil, which gave an amorphous solid upon standing and was used directly in the subsequent step without further purification; yield: 2.2 g (100%).

Boc-Trp-Ile-NHAll (23)

HCl (4.0 M in dioxane; 180 mL) was added to Boc-Ile-NHAll (**22**) (9.6 g, 36 mmol) at 0 °C and the mixture stirred for 2 h prior to warming to 25 °C. The solvent was evaporated, the residue further dried under high vacuum for 18 h and dissolved in DMF (350 mL). *i*-Pr_2NEt (12.4 mL, 71 mmol), HOBT (5.77 g, 43 mmol), Boc-Trp-OH (10.8 g, 36 mmol) and HBTU (16.2 g, 43 mmol) were sequentially added with stirring at 0 °C. After 2 h, EtOAc (200 mL) was added and the mixture was washed successively with saturated aqueous $NaHCO_3$ (200 mL), the aqueous phase extracted with EtOAc (3 × 75 mL) and the combined organic phases washed with 0.2 M citric acid (150 mL), H_2O (200 mL), saturated aqueous NaCl (150 mL), dried ($MgSO_4$) and evaporated. Chromatography (95:5, CH_2Cl_2:MeOH) gave peptide **23** as a white solid; yield: 13.6 g (84%).

(2*R*,*S*)-Hydroxy-4-pentenoyl-Aoda-Trp-Ile-NHAll (24)

HCl in dioxane (4.0 M; 40 mL) was added at 0 °C to peptide **23** (3.7 g, 8.0 mmol) and the mixture stirred for 2 h. The solvent was evaporated, and the residue left under high vacuum for 16 h. *i*-Pr_2NEt (2.5 mL, 14 mmol), acid **21** (1.9 g, 6.4 mmol), HOBT (1.0 g, 7.7 mmol) and HBTU (2.9 g, 7.7 mmol) were added with stirring to a portion of the residue (2.5 g, 6.4 mmol) in DMF (64 mL) at 0 °C. After 16 h, the mixture was diluted with EtOAc (60 mL), and the solution washed sequentially with saturated aqueous $NaHCO_3$ (30 mL), saturated aqueous NaCl (30 mL), 0.2 M citric acid (30 mL) and saturated aqueous NaCl (30 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated to give diene **24** as an equimolar mixture of epimers at C-2; yield: 3.7 g (72%).

(12*R*,*S*)-Hydroxy-(6*S*)-(1*H*-indol-3-ylmethyl)-(3*S*)-[(1*S*)-methylpropyl]-(9*S*)-(6-oxooctyl)-1,4,7,10-tetraazacyclohexadec-14-ene-2,5,8,11-tetraone (25)

Diene **24** (500 mg, 0.78 mmol) was added to CH_2Cl_2 (780 mL) and immersed in an ultrasound bath for 20 min until a finely dispersed suspension was formed. The suspension was stirred at 40 °C under reflux for 30 min until complete dissolution was observed, at which point catalyst **14** (100 mg, 0.12 mmol) was added and the mixture stirred under reflux for 20 h at 40 °C. The mixture was allowed to cool to 25 °C and ethyl vinyl ether (5 mL, 67 mmol) was added and the mixture stirred at 25 °C for 20 min. Solvent evaporation and chromatography (gradient CH_2Cl_2 to CH_2Cl_2:MeOH, 93:7) gave the diene **25** as a brown amorphous solid containing a mixture of diastereoisomers at C-12; yield: 357 mg (75%).

(12*R*,*S*)-Hydroxy-(6*S*)-(1*H*-indol-3-yl-methyl)-(3*S*)-[(1*S*)-methylpropyl]-(9*S*)-(6-oxooctyl)-1,4,7,10-tetraazacyclohexadecan-2,5,8,11-tetraone (26)

Pd/C (13 mg, 10 mol%) was added to a solution of alkene **25** (134 mg, 0.22 mmol) in MeOH (2 mL) at 25 °C. The mixture was purged three times with gaseous H_2 and stirred for 24 h under H_2. Solvent evaporation and chromatography (gradient CH_2Cl_2 to CH_2Cl_2:MeOH, 93:7) gave the peptoid **26** as a white amorphous powder containing a mixture of diastereoisomers at C-12; yield: 118 mg (88%).

HDAC Assay[20]

The standard assay (200 μL) contained HDAC buffer (40 μL) [from a stock solution containing 50 mM tris (pH 8.0), 750 mM NaCl, 50% glycerol (332.5 μL total) and 100 mM PMSF ($PhCH_2SO_2F$) (17.5 μL)], either 30 μg HeLa cell nuclear extract (4 μL of 7.5 mg mL^{-1} extract) with H_2O (153 μL), or only H_2O (157 μL) for the zero enzyme control assay. Three zero substrate control assays were prepared as above, containing HeLa extract, and diluted with DMSO (2 μL), while substrate assays, also containing HeLa extract, were charged with 2 μL of substrate solutions with varying concentrations. All mixtures were incubated for 30 min at 25 °C and charged with 1 μL (approx. 10^6 counts per min, CPM) aqueous [^{3}H]acetylhistone H4 peptide fragment, and incubated further for 1 h at 25 °C. The mixtures were then quenched using 50 μL of a quenching solution (259 μL concentrated HCl and 28 μL glacial AcOH in 2713 μL H_2O). The released [^{3}H]acetate was extracted with EtOAc (600 μL), and 2 × 200 μL aliquots of the organic phase were each mixed with 5 mL Hionic-Fluor and the radioactivity was measured in counts per minute (CPM). Substrate assays were first run for a known inhibitor (trichostatin A, TCA) at dilutions of 1 μM, 100 nM, 10 nM and 1 nM, to test for correlation of assay results with reported values for the known inhibitor. Assays for substrates **24** or **25** were run at dilutions of 100 μM, 10 μM, 1 μM, 100 nM, 10 nM and 1 nM. HDAC enzyme activity (expressed in CPM) was measured by subtracting the radioactivity for the zero enzyme control assay from the radioactivity of each assay. Percentage HDAC activity was calculated by division of HDAC activity for each assay by the mean HDAC activity of the three zero substrate control assays.

References

[1] For reviews on HDAC inhibitors, see: a) A. Villar-Garea, M. Estheller, *Int. J. Cancer* **2004**, *112*, 171; b) T. A. Miller, D. J. Witter, S. Belvedere, *J. Med.*

Chem. **2003**, *46*, 5097; for HDAC and cancer, see: c) H.-Y. Lin, C.-S. Chen, S.-P. Lin, J.-R. Weng, C.-S. Chen, *Med. Res. Rev.* **2006**, *26*, 397; d) T. Liu, S. Kuljaca, A. Tee, G. M. Marshall, *Cancer Treatment Rev.* **2006**, *32*, 157; e) O. H. Krämer, M. Göttlicher, T. Heinzel, *Trends in Edocrin. & Metab.* **2001**, *12*, 294.

[2] a) S. B. Singh, D L. Zink, J. M. Liesch, R. T. Mosley, A. W. Dombrowski, G. F. Bills, S. J. Darkin-Rattray, D. M. Schmatz, M. A. Goetz; *J. Org. Chem.* **2002**, *67*, 815; b) S. L. Colletti, R. W. Myers, S. J. Darkin-Rattray, A. M. Gurnett, P. M. Dulski, S. Galuska, J. J. Allocco, M. B. Ayer, C. Li, J. Lim, T. M. Crumley, C. Cannova, D. M. Schmatz, M. J. Wyvratt, M. H. Fisher, P. T. Meinke, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 107; c) S. L. Colletti, R. W. Myers, S. J. Darkin-Rattray, A. M. Gurnett, P. M. Dulski, S. Galuska, J. J. Allocco, M. B. Ayer, C. Li, J. Lim, T. M. Crumley, C. Cannova, D. M. Schmatz, M. J. Wyvratt, M. H. Fisher, P. T. Meinke, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 113; d) S. J. Darkin-Rattray, A. M. Gurnett, R. W. Myers, P. M. Dulski, T. M. Crumley, J. J. Allocco, C. Cannova, P. T. Meinke, S. L. Colletti, M. A. Bednarek, S. B. Singh, M. A. Goetz, A. W. Dombrowski, J. D. Polishook, D. M. Schmatz, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13143; e) M. Kijima, M. Yoshida, K. Sugita, S. Horinouchi, T. Beppu, *J. Biol. Chem.* **1993**, *268*, 22429.

[3] a) M. Ladlow, F. Berst, A. B. Holmes, *Chem. Commun.* **2002**, *5*, 508; b) L. Mou, G. Singh, *Tetrahedron Lett.* **2001**, *42*, 6603; c) S. L. Schreiber, J. Taunton, C. A. Hassig, *Science* **1996**, *272*, 408; d) J. E. Baldwin, R. M. Adlington, C. R. A. Godfrey, V. K. Patel, *Tetrahedron* **1993**, *49*, 7837; e) R. Jacquier, R. Lazaro, H. Ranirihiseheno, P. Viallefont, *Tetrahedron Lett.* **1986**, *27*, 4735; f) M. Kawai, J. H. Gardner, D. H. Rich, *Tetrahedron Lett.* **1986**, *27*, 1877; g) U. Schmidt, A. Lieberknecht, H. Griesser, F. Bartkowiak, *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 318.

[4] U. Schmidt, J. Langner, *J. Peptide Res.* **1997**, *49*, 67.

[5] RCM in cyclopeptoid synthesis: a) P. W. R. Harris, M. A. Brimble, *Org. Biomol. Chem.* **2006**, *4*, 2696; b) M. Poirier, N. Aubry, C. Boucher, J. M. Ferland, S. LaPlante, Y. S. Tsantrizos, *J. Org. Chem.* **2005**, *70*, 10765; c) P. Van de Weghe, J. Eustache, *Curr. Top. Med. Chem.* **2005**, *5*, 1495; d) W. H. C. Martin, S. Blechert, *Curr. Top. Med. Chem.* **2005**, *5*, 1521; e) G. Dimartino, D. Y. Wang, R. N. Chapman, P. S. Arora, *Org. Lett.* **2005**, *7*, 2389; f) S. Oishi, Z. D. Shi, K. M. Worthy, L. K. Bindu, R. J. Fisher, T. R. Burke, *Chem. Biochem.* **2005**, *6*, 668; g) J. H. Chen, C. J. Forsyth, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12067; h) X. Z. Wang, T. R. Burke, *Synlett* **2004**, 469; i) A. G. M. Barrett, A. J. Hennessy, R. Le Vezouet, P. A. Procopiou, P. W. Seale, S. Stefaniak, R. J. Upton, A. J. P. White, D. J. Williams, *J. Org. Chem.* **2004**, *69*, 1028; j) Z. D. Shi, K. Lee, C. Q. Wei, L. R. Roberts, K. M. Worthy, R. J. Fisher, T. R. Burke, *J. Med. Chem.* **2004**, *47*, 788; k) F. J. Dekker, N. J. de Mol, M. J. E. Fischer, J. Kemmink, R. M. J. Liskamp, *Org. Biomol. Chem.* **2003**, *1*, 3297; l) C. Q. Wei, Y. Gao, K. Lee, R. Guo, B. H. Li, M. C. Zhang, D. J. Yang, T. R. Burke, *J. Med. Chem.* **2003**, *46*, 244; m) S. Rajesh, B. Banerji, J. Iqbal, *J. Org. Chem.* **2002**, *67*, 7852; n) M. Saviano, E. Benedetti, R. M. Vitale, B. Kaptein, Q. B. Broxterman, M. Crisma, F. Formaggio, C. Toniolo, *Macromolecules* **2002**, *35*, 4204; o) H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary, R. H. Grubbs, *J. Org. Chem.* **2001**, *66*, 5291; p) Y. Gao, C. Q. Wei, T. R. Burke, *Org. Lett.* **2001**, *3*, 1617; q) J. N. Lambert, J. P. Mitchell, K. D. Roberts, *J. Chem. Soc., Perkin. Trans. 1* **2001**, 471; r) H. E. Blackwell, R. H. Grubbs, *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3281; s) J. Pernerstorfer, M. Schuster, S. Blechert, *Chem. Commun.* **1997**, 1949; t) S. J. Miller, H. E. Blackwell, R. H. Grubbs, *J. Am. Chem. Soc.* **1996**, *118*, 9606.

[6] Transannular reactions in peptoid arrays: a) S. Surprenant, W. D. Lubell, *Org. Lett.* **2006**, *8*, 2851; b) K. A. Carpenter, C. Weltrowska, B. C. Wilkes, R. Schmidt, P. W. Schiller, *J. Am. Chem. Soc.* **1994**, *116*, 8450.

[7] The formation of unproductive chelated 5- and 6-membered ruthenacyclic intermediates has previously been reported, see: A. Fürstner, K. Langemann, *J. Am. Chem. Soc.* **1997**, *119*, 9130.

[8] J. C. Sheenan, G. P. Hess, *J. Am. Chem. Soc.* **1955**, *77*, 1067.

[9] V. Dourtogiou, J. C. Ziegler, B. Gross, *Tetrahedron Lett.* **1978**, *19*, 1269.

[10] a) P. Kaur, P. Singh, S. Kumar, *Tetrahedron* **2005**, *61*, 8231; b) J. Lu, S.-J. Ji, R. Qian, J.-P. Chen, Y. Liu T.-P. Loh, *Synlett* **2004**, *5*, 534; c) J. G. Lee, K. I. Choi, A. N. Pae, H. Y. Koh, Y. Kang, Y. S. Cho, *J. Chem. Soc., Perkin Trans. 1* **2002**, 1314; d) S. Kumar, P. Kaur, S. S. Chimni, *Synlett* **2002**, 573.

[11] E. J. Corey, H. Cho, C. Rücker, D. H. Hua, *Tetrahedron Lett.* **1981**, *22*, 3455.

[12] a) M. Scholl, C. Ding. C. W. Lee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 953; b) P. Schwab, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100; c) P. Schwab, M. B. France, J. W. Ziller, R. H. Grubbs, *Angew. Chem.* **1995**, *107*, 2179, *Angew. Chem. Int. Ed.* **1995**, *34*, 2039; d) S. T. Nguyen, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1993**, *115*, 9858.

[13] a) J. Cossy, S. BouzBouz, A. H. Hoveyda, *J. Organomet. Chem.* **2001**, *624*, 327; b) T. R. Hoye, H. Zhao, *Org. Lett.* **1999**, *1*, 1123.

[14] B. Schmidt, *Eur. J. Org. Chem.* **2003**, *5*, 816.

[15] R. E. Flannery, K. G. Hampton, *J. Org. Chem.* **1972**, *37*, 2806.

[16] W.-C. Zang, C.-J. Li, *Ibid.* **2000**, *65*, 5831.

[17] S. Kim, E.-Y. Kim, H. Ko, Y. H. Jung, *Synthesis* **2003**, *14*, 2194.

[18] a) M. Yoshida, M. Kijima, M. Akita, T. Beppu, *J. Biol. Chem.* **1990**, *265*, 17174; b) M. Yoshida, Y. Hoshikawa, K. Koseki, K. Mori, T. Beppu, *J. Antibiotics* **1990**, *43*, 1101; c) Y. Hoshikawa, M. Kijima, T. Beppu, *Agricult. Biol. Chem.* **1991**, *55*, 1491.

[19] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923.

[20] a) S. Emiliani, W. Fischle, C. Van Lint, Y. Al-Abed, E. Verdin, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2795; b) J. Taunton, C. A. Hassig, S. L. Schreiber, *Science* **1996**, *272*, 408; c) A. Kervabon, J. Mery, J. Parello, *FEBS Lett.* **1979**, *106*, 93.

