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Synthesis and Biological Properties of Cylindramide Derivatives: Evidence for Calcium-Dependent Cytotoxicity of Tetramic Acid Lactams

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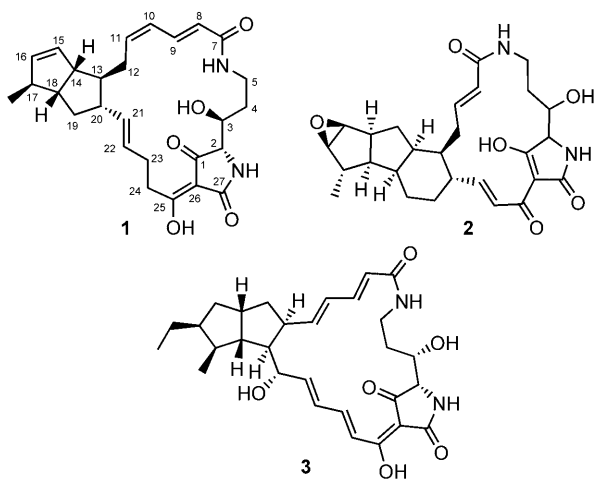
*To gain insight into the biological properties of tetramic acid lactam cylindramide **1**, the analogues **4a–d** bearing a cyclopentane ring instead of the pentalene unit were prepared by tandem conjugate addition/enolate trapping of cyclopentenone **10**; a Sonogashira or Stille coupling, followed by a Julia–Kocienski olefination, macrolactamisation and Lacey–Dieckmann cyclisation were the key steps. The previous NMR structure of cylindramide **1**, which was based on NOE and J coupling restraints, could be refined by including residual dipolar coupling data measured for a sample of cylindramide that was aligned in polyacrylonitrile (18%). Biological screening of cylindramide **1** and its analogues 2-epi-**1**, **20** and **4** revealed promising antiproliferative activity against several tumour cell lines. It turned out that the activity is strongly correlated to the functionalised pentalene system. The configuration of the cyclopentane ring and an intact tetramic acid lactam with the correct configuration seem to play an equal role in the cytotoxicity. The antiproliferative activity was found to be calcium dependent. Phenotypic characterisation of the mode of action showed vacuolisation and vesicle formation in the endoplasmic reticulum.*

Introduction

The 2,4-pyrrolidinedione (tetramic acid) moiety has been recognised as an important structural feature in many natural products.[1] Macrocyclic tetramic acid lactams such as cylindramide (**1**),[2] discodermide (**2**),[3] or alteramide A (**3**)[4] are particularly interesting because of their pronounced cytotoxicity (Scheme 1).

Surprisingly little, however, is known about the synthesis and biological mode of action of compounds **1**–**3**.[5] This motivated us to study the chemistry of cylindramide **1** in more detail, and resulted in the enantioselective total synthesis of **1** in 2005.[6,7] Having compound **1** and synthetic precursors in hand allowed us to investigate the biological properties with particular attention to the role of the tetramic acid and the pentalene moiety in **1**. The latter issue, however, required the synthesis of a pentalene-free analogue of **1**. We anticipated that 1,2-disubstituted cyclopentane might best serve as a pentalene surrogate because it keeps ring size and overall conformation of the macrocycle similar. For the preparation of the cyclopentane analogue **4d** of cylindramide two strategies were envisaged, proceeding via the precursors **7** or **10** (Scheme 2).



Scheme 1. Tetramic acid lactams cylindramide (**1**), discodermide (**2**) and alteramide A (**3**).

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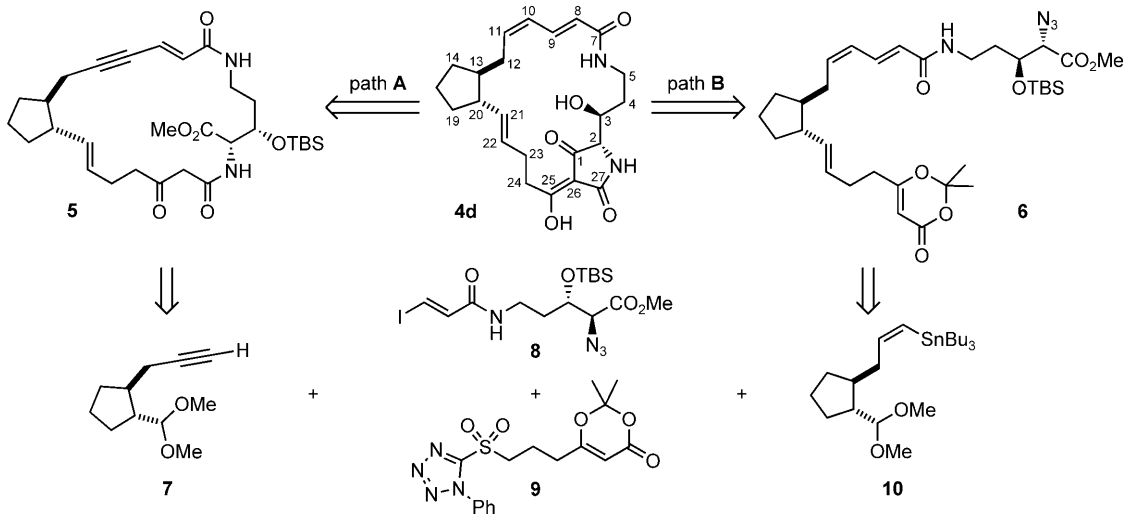


Scheme 2. Retrosynthetic pathways **A** and **B** to the cyclopentane analogue **4d** of cylindramide; **4d** is numbered according to cylindramide **1** for comparison.

Herein the synthetic routes to the cyclopentane analogue **4** as well as the biological properties of cylindramide (**1**), its synthetic precursors and non-natural analogues are reported.

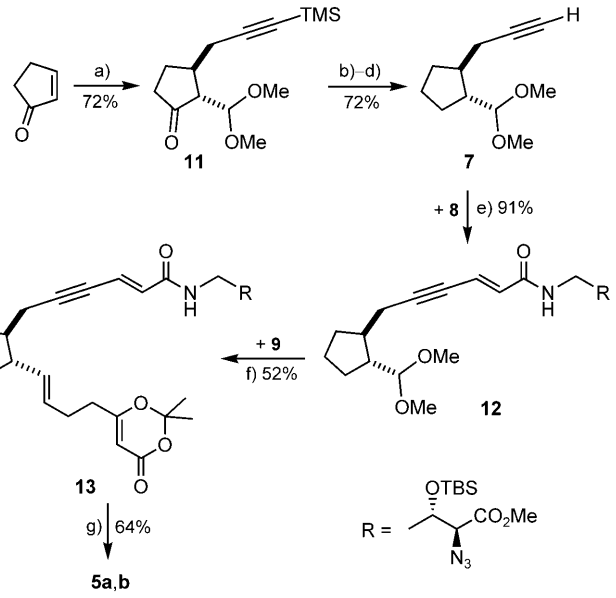
Results and Discussion

Synthesis of cyclopentane analogue 4 by route A

Route A follows the published procedure for the preparation of cylindramide (**1**). In order to synthesise alkyne **7**, cyclopentenone was sequentially treated with the Gilman cuprate from trimethylsilylpropyne in the presence of *t*BuLi, tetramethylethylenediamine (TMEDA) and CuI,[8] trimethylsilylchloride and trimethylorthoformate in the presence of $BF_3{\cdot}OEt_2$ (Scheme 3). Ketone **11** was reduced with $NaBH_4$ to give the corresponding diastereomeric alcohols, which were deoxygenated according to the method of Barton and McCombie by using 1,1-thiocarbonyldiimidazole in pyridine,[9] 2,2′-azobisisobutyronitrile (AIBN) and tris(trimethylsilyl)silane[10] instead of Bu_3SnH. In this manner, derivative **7** could be isolated in 53% overall yield.

Compound **7** was then submitted to a Sonogashira coupling[11] with the hydroxyornithine derivative **8**[6] to yield the enyne **12** in 91% (Scheme 3). Hydrolysis of the acetal group in **12** with acidic ion-exchange resin for 2 days set the aldehyde function free, which underwent a Julia–Kocienski olefination[12] with *N*-phenyltetrazolylsulfone **9**[6] to give the enyne **13** in 52% yield over both steps. After the Staudinger reduction of the azide group in **13** with PPh_3,[13] cyclisation was initiated by a thermally induced retro-Diels–Alder reaction of the trimethyldioxinone moiety to give the corresponding α-oxoketene. The latter was immediately trapped intramolecularly by the amino group, yielding the diastereomeric macrocycles **5a**,**b** in 64% (*dr* 1:1).

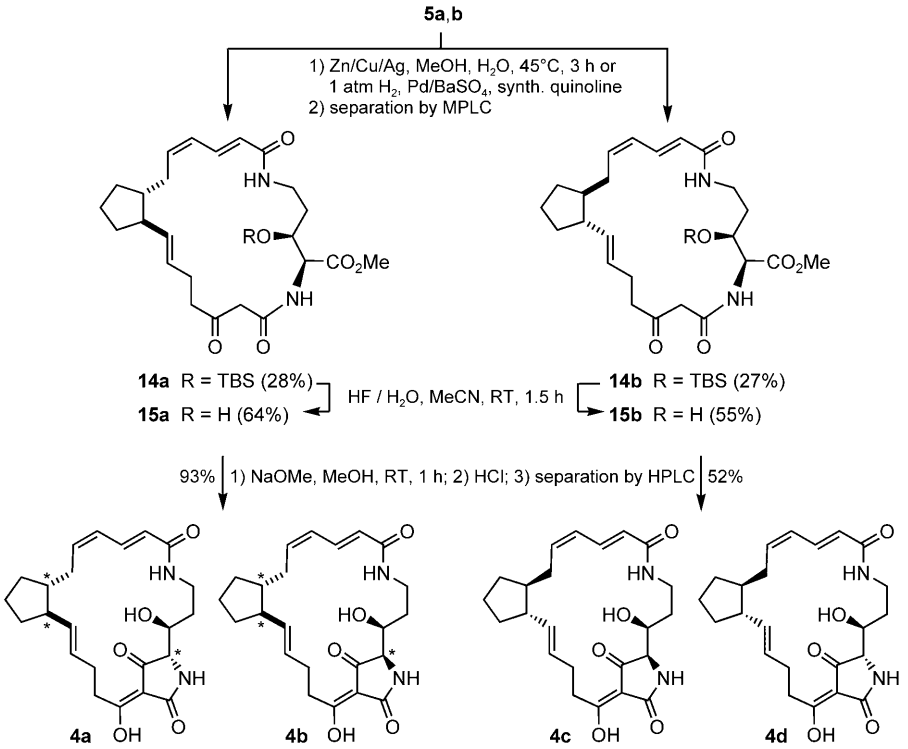
The *cis*-selective reduction of the enyne **5** was achieved either by using 1 atm H_2 and $Pd/BaSO_4$ as catalyst in the presence of synthetic quinoline, or by following the procedure of Boland,[14] in which zinc nanoparticles were activated with Cu-



Scheme 3. Preparation of precursor **5**. a) TMS-C≡CMe, *t*BuLi, TMEDA, THF, −78 °C, 1 h; CuI, THF, −40 °C→0 °C; TMSCl, THF, −78 °C→−40 °C, 3 h; $HC(OMe)_3$, $BF_3{\cdot}OEt_2$, CH_2Cl_2; b) $NaBH_4$, MeOH, 0 °C, 1 h; c) 1) $(Im)_2CS$, pyridine, CH_2Cl_2, 50 °C, 5 h, 2) $(Me_3Si)_3SiH$, AIBN, toluene, 80 °C, 30 min; d) K_2CO_3, EtOH, RT, 16 h; e) **8**, CuI, $[Pd(PPh_3)_4]$, NEt_3, THF; RT, 30 min; f) i: Amberlyst 15, acetone, H_2O, RT, 2 d; ii: **9**, NaHMDS, DME, −55 °C, 1 h; RT, 16 h; g) 1) PPh_3, THF, RT, 2 h; 2) H_2O, RT, 24 h; 3) toluene, 0.19 mM, 110 °C, 8 h.

$(OAc)_2$ and $AgNO_3$ in water (Scheme 4). The *Z*,*E*-dienes **14a**,**b** were isolated in 57% or 55% yield (*dr* 1:1). Both diastereomers could be separated by MPLC on a cyanopropyl phase with hexanes/ethyl acetate as eluent, to give **14a** in 28% and **14b** in 27% yield.

Subsequent deprotection of the silyl ethers **14a**,**b** by aqueous HF afforded the hydroxylactams **15a**,**b** in 64% and 55% yield, respectively. Finally, a Lacey–Dieckmann cyclisation[15] of **15** with NaOMe in MeOH provided the diastereomeric tetramic acid lactams **4a**,**b** and **4c**,**d** in 93% and 52% yield. The four

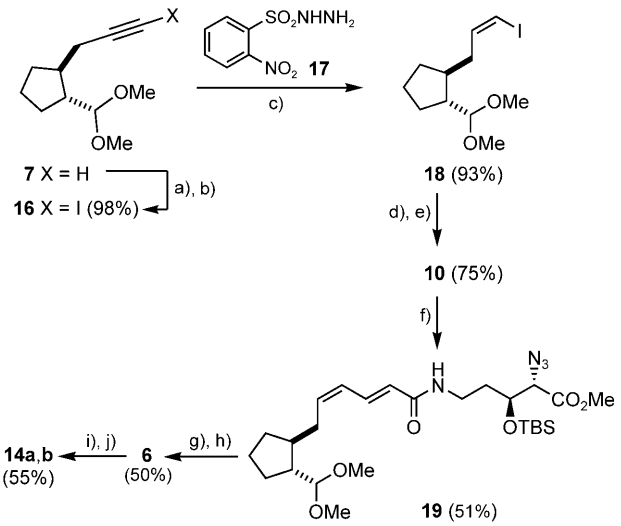


Scheme 4. Preparation of cylindramide analogues **4**. Stereogenic centres with an asterisk are arbitrarily drawn. The configuration of **4c** and **4d** was derived from NMR spectroscopy experiments.

diastereomers could be separated by preparative reversed-phase HPLC.

Synthesis of cyclopentane analogue 4 by route B

As depicted in Scheme 5, alkyne **7** was deprotonated with butyllithium and treated with iodine[16] to give the iodoalkyne **16** almost quantitatively. A *cis*-selective reduction of the triple bond in **16** was achieved with a diimine that was generated in situ from *o*-nitrobenzenesulfonylhydrazide **17**[17] in THF/*i*PrOH in the presence of NEt_3 under strict exclusion of light.[18] A subsequent halogen–lithium exchange and quenching with Bu_3SnCl gave the target precursor **10** in 75% yield. The Stille coupling[19] of **10** with hydroxyornithine **8** provided compound **19** in 51% yield. Hydrolysis of the acetal group and Julia–Kocienski olefination of the intermediate aldehyde gave the cyclisation precursor **6**, which was subjected to a Staudinger reduction, followed by macrolactamisation as described above. The desired diastereomeric lactams **14a,b** were isolated in 55% yield (*dr* 1:1).



Scheme 5. Preparation of macrolactams **14** by route B. a) BuLi, THF, −78 °C; b) I_2; c) THF, *i*PrOH, NEt_3, RT, 24 h; d) *t*BuLi, Et_2O, pentane, −78 °C; e) $ClSnBu_3$, −78 °C→RT, 16 h; f) **8**, $Pd(PhCN)_2Cl_2$, DMF, RT, 10 min; g) Amberlyst 15, acetone, H_2O, 3 d; h) NaHMDS, **9**, DME −78 °C→RT; i) PPh_3, THF, H_2O, 1 d; j) toluene, reflux, 7 h.

Structure refinement of cylindramide

The structure of cylindramide **1** has been previously determined by using 71 distance restraints that were calculated from cross peak intensities from the 400 ms mixing time ROESY spectra.[6a] The calculations did not converge to a single structure, but the data were compatible with two different conformations. The ten lowest-energy structures for each of the two structures showed an RMSD of 0.87 Å. We recalculated the structure of cylindramide **1** on the basis of newly measured residual dipolar couplings (RDCs) that provide information about the long-range structure of the cyclic structure.[20] For cylindramide **1**, the overall curvature of the macrolactam ring can especially be improved. For the measurement of RDCs, cylindramide **1** was dissolved in DMSO and the solution was placed on top of a polyacrylonitrile gel that had been swollen in DMSO.[21] The deuterium signal that was recorded under these gel conditions showed a deuterium quadrupolar splitting of 13.9 Hz. After one week of diffusion, residual dipolar couplings from −20 to +25 Hz for cylindramide could be observed. We determined the ^1D(CH) couplings for all 15 methine groups in the molecule and the two amide sites in t_1-coupled ^{13}C HSQC, constant-time ^{13}C HMQC and ^{15}N HSQC spectra. In addition, we developed a general new NMR pulse sequence to measure ^1D(CH) couplings in two methylene groups.

^1D(C,H1) and ^1D(C,H2) couplings for the two diastereotopic methylene protons H1 and H2 cannot easily be measured from a t_1-coupled HSQC experiment, because in the case of almost equal scalar couplings of ^1J(C,H1) and ^1J(C,H2), the middle component of the expected triplet will be cancelled. The new constant-time HMQC experiment that is depicted in Figure 1 has been developed to determine ^1J(C,H1) and ^1J(C,H2) couplings in methylene groups.

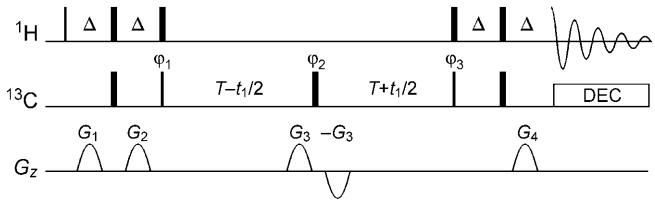


Figure 1. Coupled constant-time HMQC pulse sequence. $\Delta = 1/[^2J(CH)]$. $\varphi_1 = x, -x$; $\varphi_2 = 2(x), 2(-x)$; $\varphi_3 = 4(x), 4(-x)$; $\varphi_{rec} = x, -x, x, -x, -x, x, -x, x$. Gradient strength with a duration of 1000 ms each: $G_1 = 5.5$ G cm^{-1}; $G_2 = -2.8$ G cm^{-1}; $G_3 = 44$ G cm^{-1}; $G_4 = 30.3$ G cm^{-1}. Coherence selection was obtained by inversion of G_3 and incrementation of φ_2 in echo–antiecho manner. The constant time period was set to 8.8 ms. The spectrum has been processed by using magnitude calculation along t_2.

In the HMQC experiment that is depicted in Figure 1, double and zero quantum coherence of the type $C^-H1^{+/-}$ or $C^-H2^{+/-}$ evolve for the CH1 and CH2 cross peak, whereas the scalar couplings ^1J(C,H1) and ^1J(C,H2) do not evolve. Thus, only one of the two ^1J(CH) couplings will evolve during t_1 (Figure 2). The evolution of proton chemical shift is suppressed by applying constant time in t_1, whereas J(H,H) evolve during $(4\Delta + 2T + t_2)$, therefore the magnitude calculation is applied during t_2.

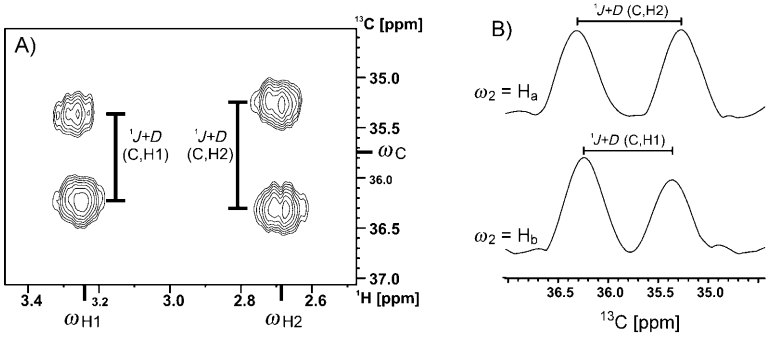


Figure 2. Selected region of a t_1-coupled CT-HMQC spectrum of cylindramide **1** (A) and the ^{13}C NMR signal columns of the shown peaks (B).

The constant time delay T is set to 8.8 ms, which would suppress homonuclear carbon–carbon couplings during t_1 in the case of isotope-labelled substances. In contrast to other pulse sequences like the SPITZE-HSQC,[22] which are able to measure ^1J(CH) couplings in methylene goups, the pulse sequence presented here offers the possibility of measuring the desired couplings in the indirect dimension. This is usually favoured because of the less-efficient spin relaxation in the indirect dimension and because of the fact that proton–proton dipolar couplings can lead to peak distortion in the direct dimension.[23]

All structural calculations have been performed by using XPLOR-NIH.[24] By starting from a temperature of 8000 K, 15 000 conjugate steps have been calculated to a final temperature of 150 K.

By using additional residual dipolar couplings, the structure could be improved to a RMSD of 0.48 Å and converged to a single conformation (Figure 3). The bundle that showed the ten lowest-energy structures includes one outlier. The conformation of the part reaching from C22 to C25 is not clearly defined; this indicates flexibility. No NOE contacts could be observed for this region, the RDCs could not be measured due to overlap with the broad signals of the aligning media.

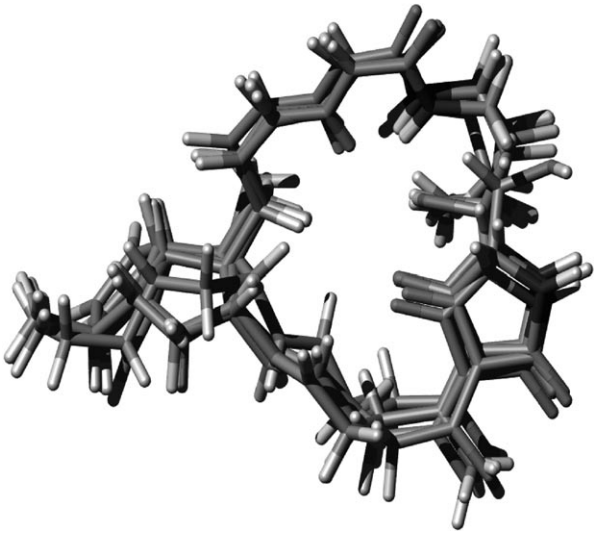


Figure 3. Ten lowest-energy structures of cylindramide **1** that were calculated by using 71 NOE-derived distances and 21 RDCs.

The resulting structures are in good agreement with the measured data. Back-calculated RDCs fit to the measured ones very well ($R > 0.99$) (see Figure S1 in the Supporting Information) and only two NOE restraints are violated by more than 0.3 Å. A comparison of the resulting structures with the previously calculated structures from NOE data reveals minor differences, the differences are mainly in the bend of the macrocyclic ring.

Configuration of cylindramide analogues 4a–d

The configuration of the cylindramide analogues **4** (for atom numbering see Scheme 2) was determined by qualitative cross peak inspection in DQF-COSY spectra[25] and comparison with cylindramide **1** of known configuration. To compare the configuration of the five-membered rings, the COSY cross peaks H21–H20 were analysed (Figure 4) because the H13–H20 cross

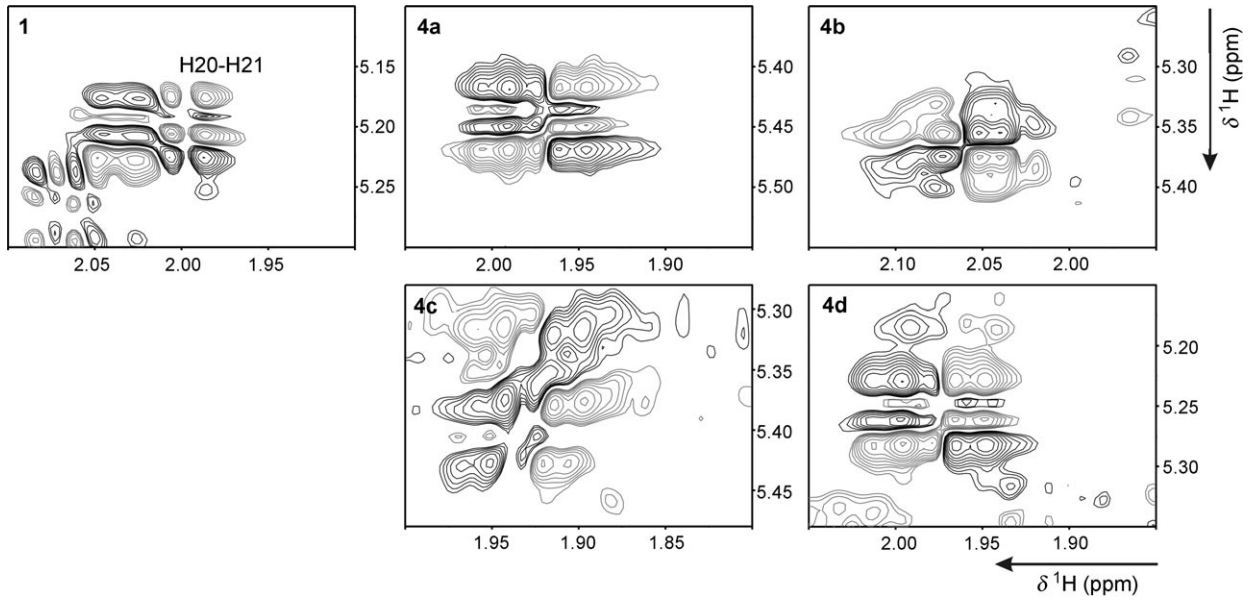


Figure 4. H21–H20 DQF-COSY cross peak in the spectrum of cylindramide **1** and its analogues **4a**–**d**.

peak could not be resolved, and H20 has a different chemical environment when comparing cylindramide **1** and its analogues **4**. We use the size of the active and passive coupling constants that were revealed in the cross peak to assign the configuration of one of the analogues **4** to the configuration of cylindramide **1**. All measurements were carried out on a 600 MHz Bruker Avance II spectrometer that was equipped with a $^{1}H\{^{13}C,^{15}N\}$ cryogenic triple resonance probe with z gradients. Only the analogues **4a** and **4d** showed a similar cross peak pattern compared to cylindramide; this indicates that both compounds have the same relative configuration.

To determine the configuration at C2, the H3–H4 cross peak was analysed, again because the H2–H3 cross peak could not be resolved for cylindramide **1** due to overlap of H2 and H3 (Figure 5).

Only analogue **4d** shows the same COSY cross peak pattern as cylindramide **1**, when comparing the ratio between active and passive couplings; this indicates that **4d** has the same configuration as **1**. We therefore can propose with certainty the relative stereochemistry of **4d** by comparing the COSY spectrum of **1** and **4d**, but we cannot assign the other three stereoisomers to their relative stereochemistry. Due to the fact that epimerisation at C2 in the synthetic route occurs during Lacey–Dieckmann cyclisation, however, the configuration of **4c** is also fixed. The relative stereochemistry and orientations of cylindramide **1** according to couplings and structure calcula-

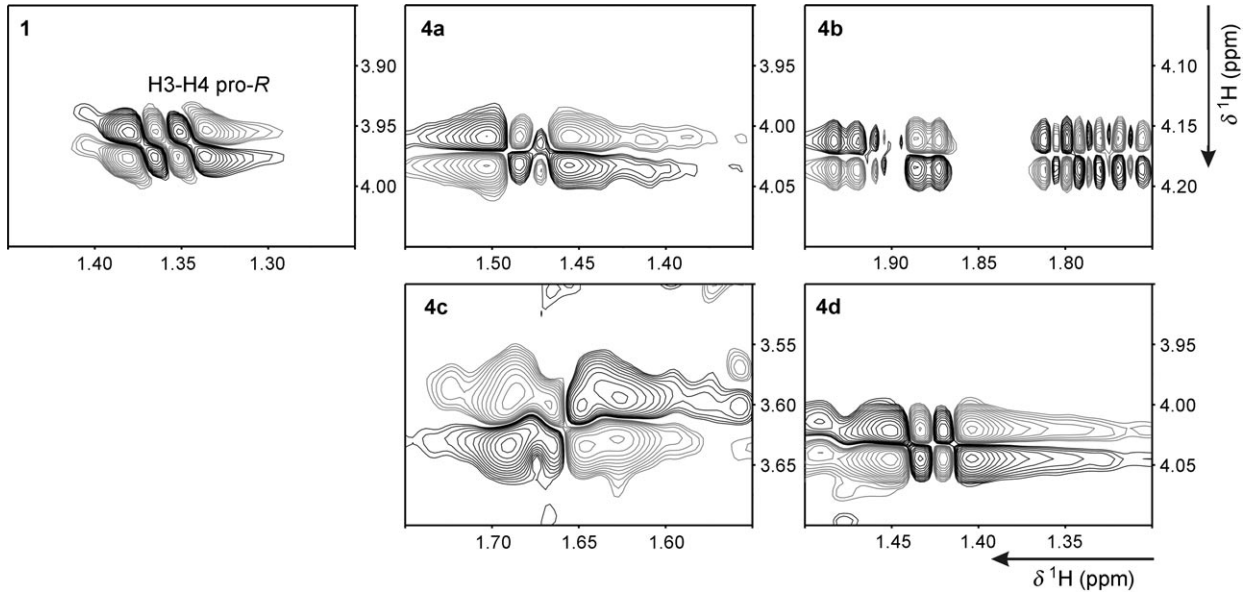


Figure 5. H3–H4 DQF-COSY cross peak in the spectrum of cylindramide **1** and its analogues **4a**–**d**.

tions are shown in Figure 6. The assignment of protons $H4^{proR}$ and $H4^{proS}$ was based on the structure. The lowest-energy structure shows a dihedral angle H3–C3–C4–$H4^{proS}$ of 87°,

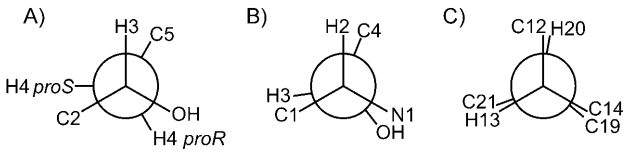
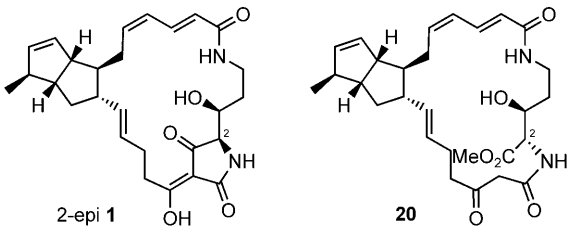


Figure 6. Newman projections along the A) 3C4 bond, B) 2C3 bond and the C) 13C20 bond.

which results in a coupling of about 0 Hz according to the Karplus equation.[26] Analysis of the ten lowest-energy structures results in a dihedral angle of 86 ± 15°, which can still explain the missing COSY cross peak due to a very small coupling. Therefore, only one H3–H4 cross peak is observed for cylindramide **1** and its analogue **4d**.

Biological studies

A broad screening of cylindramide derivatives 2-epi-**1** and **20** (Scheme 6) revealed only moderate activity against microorganisms, and proliferation tests with the mouse fibroblast cell line L929 indicated high cytotoxicity.[27]



Scheme 6. Cylindramide derivatives 2-epi-**1** and **20**.[6a]

Thus, this issue was explored in more detail. Compounds **1**, 2-epi-**1**, **20**, and **4a–d** were tested against various tumour cell lines (Table 1). It is evident from the data that cylindramide **1** displayed the highest cytotoxicity regardless of the cell line. Moreover, the tetramic acid moiety might be more important for activity than the correct stereochemistry at C2. For example, the IC_{50} values in the L929 series are much higher for precursor macrocycle **20** without the tetramic acid moiety compared to the corresponding tetramic acids **1**, 2-epi-**1**. In the series of cyclopentane analogues **4a–d**, diastereomer **4d** displayed the highest cytotoxicity against the investigated cell lines. This result can be taken as further support for the NMR spectroscopic assignments, that is, analogue **4d** has the same configuration both at the cyclopentane unit and at the tetramic acid lactam as compared to cylindramide **1**. The results of tests with the multidrug-resistant cell line KB-V1 showed that cylindramide **1** and 2-epi-**1** have no affinity to the overexpressed glycoprotein efflux pump (P-gp) of the cells. The values with or without verapamil, which inhibits P-gp transport, are the same.

Only in the FAB mass spectra of cylindramide **1** did we find $[M+Cu]^+$ adducts. Obviously, complexation of Cu^{2+} from the metal FAB tip of the mass spectrometer took place. This observation showed that cylindramide **1** is a good ligand especially for divalent metal cations. Therefore, cylindramide **1** could exert its cytotoxic effect by binding of Ca^{2+} or Mg^{2+}, and decreasing their intracellular concentrations. To examine this issue, IC_{50} values of L929 cell line at various Ca^{2+} and Mg^{2+} concentrations were measured. For this purpose a culture medium with reduced Ca^{2+} and Mg^{2+} concentrations was used to which different amounts of $CaCl_2$ and $MgSO_4$ were added. As shown in Figure 7, the IC_{50} values significantly increased with increasing Ca^{2+} ions in the culture medium up to 1.6 mM, that is, cylindramide **1** has lower cytotoxic effects in presence of higher Ca^{2+} concentration. The IC_{50} values are generally higher than the values given in Table 1. This is probably mainly due to shorter incubation time. A positive effect of Ca^{2+} could not be observed with U-937 human lymphoma cells. Adding 1 mM $CaCl_2$ to normal RPMI 1640 medium (final concentration 1.4 mM then) had rather a negative effect on

Table 1. Cytotoxicity of cylindramide and analogues against different mammalian cell lines.[a]

Cell line	Origin/remarks	1	epi-1	20	4a	4b	4c	4d
L929	mouse, connective tissue	0.067 ± 0.022	1.2 ± 0.4	6.8 ± 0.4	17 ± 4.2	12 ± 0.7	22 ± 0.7	2.3 ± 0.1
L-R118	epothilone-dependent L929[b]	0.072 ± 0.008	1.2 ± 0.1	3.6 ± 0.0	–	–	–	–
PtK_2	potoroo, kidney	0.13 ± 0.01	3.2 ± 0.3	14 ± 2.8	–	–	–	–
A-431	human epidermoid carcinoma	0.24 ± 0.00	6.6 ± 0.6	24 ± 0.7	–	–	–	–
PC-3	human prostate carcinoma	0.28 ± 0.02	7.4 ± 0.0	15 ± 0.0	19 ± 0.0	4.5 ± 0.0	11 ± 0.0	4.7 ± 0.4
SKOV-3	human ovary carcinoma	0.27 ± 0.05	–	–	> 40	> 40	> 40	6.5 ± 0.0
KB-3-1	human cervix carcinoma	0.16 ± 0.05	2.4 ± 0.0	21 ± 0.7	> 40	3.1 ± 0.4	> 40	8.0 ± 0.0
KB-V1	mdr KB line	0.089 ± 0.00	2.4 ± 0.0	–	–	–	–	–
KB-V1	in the presence of verapamil (5 μg mL^{-1})	0.078 ± 0.00	2.2 ± 0.0	–	–	–	–	–
K-562	human leukemia	0.19 ± 0.16	6.6 ± 0.6	18 ± 0.0	–	–	–	–
U-937	human lymphoma	0.52 ± 0.15	7.9 ± 0.8	–	–	–	–	–

[a] Cell proliferation was measured by an MTT or WST-1 assay after 5 d of incubation. IC_{50} values are given in μg mL^{-1}. [b] The cell line was kept in the presence of epothilone A (40 ng mL^{-1}).

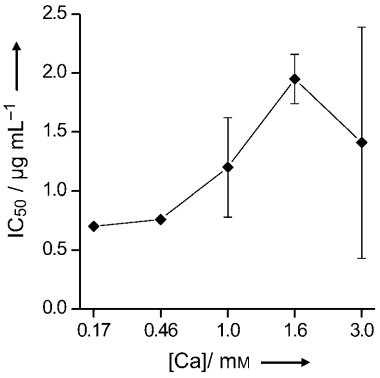


Figure 7. Ca^{2+}-dependent cytotoxicity of cylindramide **1** against the L929 mouse fibroblast cell line. The IC_{50} of an inhibitory effect of cylindramide on cell proliferation was measured by an MTT assay after two days of incubation in DME-based media that contained different concentrations of $CaCl_2$. The $MgSO_4$ concentration was 0.08 mM.

the viability of this cell line, which was generally eight times less sensitive to cylindramide **1** than L929 (Table 1). In contrast to the fibroblasts, the U-937 cells grow nonadherently. Upon addition of Mg^{2+}, no significant beneficial effects with both cell lines, L929 and U-937 were observed.

When we examined the Ca^{2+} concentrations in PtK_2 (potoroo kidney) and U-937 cells with calcium indicators like Fura-2 and Fluo-4 we found a reduction of fluorescence in the presence of cylindramide **1** after short incubation times (30 min). When we checked the inner membrane system of the PtK_2 cells that were incubated with cylindramide **1** overnight by labelling an indicator protein of the endoplasmic reticulum (ER), we observed vacuolisation and vesicle formation (Figure 8).

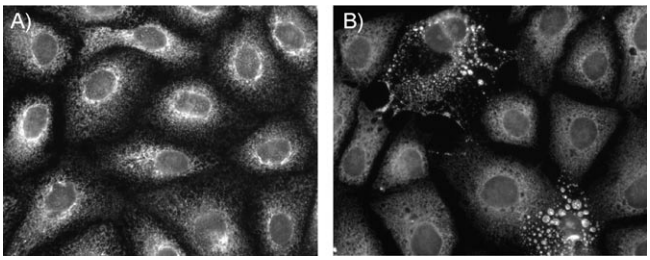


Figure 8. Cultured cells of the potoroo kidney cell line PtK_2 were incubated with cylindramide **1** (0.5 $\mu g\,mL^{-1}$) overnight and stained for endoplastic reticulum. A) Control cells and B) cylindramide-treated cells that show vesicle formation.

Thus, we have two phenomena, a short-term reduction of free Ca^{2+} in the cytosol and a long-term interference with the integrity of the ER. Normally the concentration of free Ca^{2+} in the cytosol is low, whereas its concentration in the endoplasmic reticulum is 10000 times higher. It could be that cylindramide **1** is accumulated in the ER and thereby affects the membrane integrity. We have the first hints that Ca^{2+} complexation could be the basis of the toxicity of cylindramide **1**, but further investigations are needed to elucidate the mode of action in more detail.

Conclusions

Based on the original total synthesis of cylindramide **1**,[6] four analogues **4a**–**d** have been prepared in which the substituted pentalene unit of **1** was replaced with a cyclopentane moiety. Detailed NMR studies with residual dipolar couplings not only resulted in a refined conformation of cylindramide **1**, but allowed assignment of the configuration of analogue **4d**. The measurement of residual dipolar coupling has significant impact on the determination of the concave shape of the macrolactam ring. In order to improve the RDC measurement in methylene groups, we developed a new HMQC-type correlation experiment to decouple the second passive spin in the ω_1-evolution period. The RDCs for methylene groups are important because the fixed geometry for the H–C–H moiety dissolves the up-to-fourfold degeneracy within a single alignment medium.

The stereochemical assignment agreed well with the biological screening of cylindramide **1** and the analogues 2-epi-**1**, **20** as well as cyclopentane derivatives **4a**–**d**. Thus, analogue **4d**, which is assumed to have the same configuration as **1**, has a higher cytotoxicity than diastereomers **4a**–**c**. Furthermore, comparative screening revealed that the pentalene seems to be of major importance for the biological activity, whereas the correct configuration at the cyclopentane ring and the presence of an intact tetramic acid lactam contribute equally to the cytotoxicity. We have hints that cylindramide **1** is a good ligand for divalent cations. Biological investigations with cell cultures sustained the hypothesis that cylindramide could exert its cytotoxic effect through Ca^{2+} complexation.

Experimental Section

General methods: HPLC was performed using Nucleosil C_{18} AB (5 µm; Macherey–Nagel). For further details see ref. [6a].

2-(Dimethoxymethyl)-3-[3-(trimethylsilyl)prop-2-ynyl]cyclopentanone 11: The title compound was prepared according to ref. [6a] from TMEDA (1.52 g, 15.0 mmol), trimethylsilylpropyne (2.2 mL, 15.0 mmol) in THF (25 mL), *t*BuLi (10 mL, 15.0 mmol, 1.5 M in pentane), CuI (1.43 g, 7.50 mmol) in THF (5 mL), cyclopentenone (420 µL, 5.00 mmol), TMSCl (1.39 mL, 10.4 mmol), $HC(OMe)_3$ (3.6 mL, 33.0 mmol), and $BF_3{\cdot}OEt_2$ (3.25 mL, 26.0 mmol), yield: 0.96 g (3.58 mmol, 72%), yellow oil; R_f (hexanes/EtOAc, 3:1) = 0.35; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.13 (s, 9H; Me_3Si), 1.65–1.74 (m, 1H; H3), 2.08–2.23 (m, 2H; H4, H5), 2.34 (dd, J = 17.9, 8.1 Hz, 1H, H4), 2.43 (dd, J = 16.8, 4.9 Hz, 1H; H5), 2.52 (dd, J = 17.0, 6.4 Hz, 1H; H6), 2.57 (dd, J = 17.0, 4.4 Hz, 1H; H6), 2.59–2.76 (m, 1H; H2), 3.40 (s, 6H; MeO), 4.60 ppm (d, J = 2.9 Hz, 1H; C*H*$(OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 0.1 (Me_3Si), 24.7 (C6), 26.2 (C4), 35.0 (C3), 38.7 (C5), 55.1 (OMe), 55.7 (OMe), 57.2 (C2), 86.8 (C8), 104.6 (C7), 106.0 (*C*H$(OMe)_2$), 217.6 ppm (C1); FTIR (ATR): $\tilde{\nu}$ = 2958 (s), 2900 (m), 2833 (m), 2173 (s), 1741 (vs), 1459 (m), 1408 (m), 1353 (m), 1250 (s), 1216 (m), 1185 (m), 1131 (m), 1093 (m), 1067 (vs), 1029 (m), 1000 (m), 967 (m), 837 (vs), 759 cm^{-1} (s); GC–MS (CI, CH_4): *m/z* (%) = 267 (2) $[M-H]^+$, 253 (11) $[M-CH_3]^+$, 237 (22) $[M-CH_3O]^+$, 221 (5), 207 (1), 193 (5) $[M-HC(OMe)_2]^+$, 179 (1), 165 (15), 133 (8), 121 (100) $[M-HC(OMe)_2, -SiMe_3]^+$, 75 (60) $[HC(OMe)_2]$; elemental analysis calcd (%) for $C_{14}H_{24}O_3Si$ (268.42): C 62.64, H 9.01; found: C 62.67, H 9.00.

1-(Dimethoxymethyl)-2-prop-2-ynylcyclopentane 7: Was prepared analogously to ref. [6a] a) from **11** (268 mg, 1.00 mmol) in absolute MeOH (3 mL) and $NaBH_4$ (57 mg, 1.50 mmol), yield 259 mg (96%) of 2-(dimethoxymethyl)-3-[3-(trimethylsilyl)prop-2-ynyl]cyclopentanol as a light-yellow oil (diastereomeric ratio: 55:45); R_f (hexanes/EtOAc, 3:1) = 0.35; diastereomer 1: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.14 (s, 9H; Me_3Si), 1.45–2.49 (m, 7H; H2, H3, H4, H5, H1′), 2.57 (d, J = 2.3 Hz, 1H; H5), 3.39 (s, 3H; MeO), 3.41 (s, 3H; MeO), 4.07 (dt, J = 6.5, 6.0 Hz, 1H; H1), 4.55 ppm (d, J = 6.7 Hz, 1H; C*H*($OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 0.1 (Me_3Si), 24.8 (C1′), 27.8 (C4), 32.5 (C5), 37.5 (C3), 50.2 (OMe), 53.5 (C2), 54.2 (OMe), 74.6 (C1), 85.2 (C3′), 106.1 (*C*H($OMe)_2$), 106.2 ppm (C2′); diastereomer 2: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.15 (s, 9H; Me_3Si), 1.45–2.49 (m, 7H; H2, H3, H4, H5, H6), 2.61 (d, J = 2.4 Hz, 1H; H5), 3.37 (s, 3H; MeO), 3.43 (s, 3H; *Me*O), 4.33–4.39 (m, 1H; H1), 4.35 ppm (d, J = 6.8 Hz, 1H; C*H*($OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 0.1 (Me_3Si), 25.1 (C1′), 28.8 (C4), 33.5 (C5), 38.2 (C3), 53.3 (C2), 55.2 (OMe), 75.1 (C1), 85.4 (C3′), 106.1 (C2′), 108.0 ppm (*C*H($OMe)_2$); FTIR (ATR): $\tilde{\nu}$ = 3453 (m), 2956 (s), 2831 (m), 2172 (m), 1249 (s), 1130 (s), 1051 (s), 964 (s), 841 (vs), 759 (s), 698 cm^{-1} (m); MS (CI, CH_4): *m/z* (%) = 271 (0.5) $[M+H]^+$, 270 (1) $[M]^+$, 269 (2) $[M-H]^+$, 255 (5), 253 (10), 239 (30), 238 (10), 223 (40), 221 (45), 209 (20), 207 (20), 193 (25), 191 (15), 167 (10), 157 (25), 149 (15), 127 (12), 119 (16), 75 (100); HRMS (EI): *m/z* calcd for $C_{14}H_{26}O_3Si$: 270.1651; found: 270.1637 $[M]^+$. b) A solution of cyclopentanol (84 mg, 0.31 mmol), 1,1-thiocarbonyldiimidazole (83 mg, 0.47 mmol), and *N*,*N*′-dimethylaminopyridine (DMAP; 10 mg, 81.9 μmol) in CH_2Cl_2 (5 mL) was stirred at 50 °C for 16 h. After removal of the solvent under vacuum, the residue was filtered through SiO_2 with Et_2O. The filtrate was concentrated, taken up in toluene (2 mL) and tris(trimethylsilyl)silane (143 μL, 0.47 mmol) and 2,2′-azobisisobutyronitrile (AIBN; 10 mg, 61.0 μmol) were added. The mixture was heated at 80 °C for 30 min, cooled to RT and concentrated under vacuum. The residue was chromatographed on SiO_2 with hexanes/EtOAc (20:1) to give {3-[2-(dimethoxymethyl)cyclopentyl]prop-1-ynyl}(trimethyl)silane (60 mg, 0.24 mmol, 76%) as a colourless oil; R_f (hexanes/EtOAc, 25:1) = 0.40; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.14 (s, 9H; Me_3Si), 1.42–1.64 (m, 4H; H1, H4, H5), 1.68–1.88 (m, 2H; H1, H4), 1.89–2.07 (m, 2H; H2, H3), 2.25 (dd, J = 16.8, 6.9 Hz, 1H; H_a6′), 2.43 (dd, J = 16.8, 4.9 Hz, 1H; H_b6′), 3.33 (s, 3H; MeO), 3.35 (s, 3H; MeO), 4.18 ppm (d, J = 6.9 Hz, 1H; C*H*($OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 0.2 (Me_3Si), 24.6 (C1), 25.2 (C6′), 28.1 (C5), 32.3 (C4), 40.5 (C3), 46.1 (C2), 53.3 (OMe), 54.1 (OMe), 84.8 (C8′), 107.0 (C7′), 108.2 ppm (*C*H($OMe)_2$); FTIR (ATR): $\tilde{\nu}$ = 2954 (s), 2871 (m), 2829 (m), 2172 (s), 1491 (m), 1451 (m), 1373 (m), 1249 (s), 1182 (m), 1123 (s), 1055 (s), 1022 (m), 965 (m), 841 cm^{-1} (vs); GC–MS (CI, CH_4): *m/z* (%) = 253 (1) $[M-H]^+$, 239 (20) $[M-CH_3]^+$, 224 (22) $[M-2CH_3]^+$, 223 (100) $[M-OCH_3]^+$, 207 (10), 192 (30), 151 (20), 141 (10), 111 (20), 75 (70) $[HC(OCH_3)_2]$; HRMS (CI): *m/z* calcd for $C_{14}H_{26}O_2Si$: 254.1624; found: 253.1627 $[M-H]^+$. c) Compound **7** was prepared according to ref. [6a] from a solution of silane (53 mg, 0.21 mmol), and K_2CO_3 (30 mg, 0.22 mmol) in MeOH (1.5 mL) to yield **7** (38 mg, 99%) as a colourless oil. R_f (hexanes/Et_2O 3:1) = 0.65; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45–1.65 (m, 4H; H1, H4, H5), 1.71–1.78 (m, 1H; H1), 1.80–1.88 (m, 1H; H4), 1.92 (t, J = 2.6 Hz, 1H; H8), 1.89–1.96 (m, 1H; H3), 1.96–2.03 (m, 1H; H2), 2.23 (ddd, J = 16.7, 7.5, 2.6 Hz, 1H; H6), 2.42 (ddd, J = 16.7, 4.6, 2.6 Hz, 1H; H6), 3.32 (s, 3H; MeO), 3.36 (s, 3H; MeO), 4.17 ppm (d, J = 7.1 Hz, 1H; C*H*($OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.6 (C6), 24.4 (C5), 28.1 (C4), 32.1 (C1), 40.4 (C3), 45.9 (C2), 52.8 (MeO), 54.2 (MeO), 68.4 (C7), 83.8 (C8), 108.2 ppm (*C*H($OMe)_2$); FTIR (ATR): $\tilde{\nu}$ = 3296 (m), 2947 (s), 2870 (s), 2830 (s), 2115 (m), 1449 (m), 1383 (m), 1190 (s), 1121 (vs), 1050 (s), 968 (s), 900 (s), 623 cm^{-1} (vs) ; MS (CI, CH_4): *m/z* (%) = 181 (3) $[M-H]^+$, 151 (100), 121 (4), 119 (10), 111 (20), 91 (5), 75 (90); HRMS (CI): *m/z* calcd for $C_{11}H_{17}O_2$: 181.1229; found: 181.1229 $[M-H]^+$.

Methyl (2*S*,3*S*)-2-azido-3-[*tert*-butyl(dimethyl)silanyloxy]-5-((2*E*)-{6-[2-(dimethoxymethyl)cyclopentyl]hex-2-en-4-ynoyl}amino)-pentanoate 12: The title compound was prepared according to ref. [6a] from tetraphenylpalladium (about 8 mg), CuI (3.8 mg, 20 μmol) in NEt_3 (1.2 mL), **8** (215 mg, 0.63 mmol) and **7** (80 mg, 0.44 mmol) in THF (1 mL), yield: 216 mg (91%), yellow oil; R_f (hexanes/EtOAc, 5:1) = 0.17; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.07 (s, 6H; Me_2Si), 0.86 (s, 9H; Me_3CSi), 1.37–1.60 (m, 4H; CH_2), 1.67–1.75 (m, 2H; CH_2), 1.74–1.83 (m, 1H; H_a13), 1.83–1.88 (m, 1H; H_b13), 1.89–1.96 (m, 2H; H2, H3), 2.35 (ddd, J = 16.8, 6.9, 2.2 Hz, 1H; H6), 2.54 (ddd, J = 16.8, 4.0, 2.2 Hz, 1H; H6), 3.28–3.45 (m, 2H; H12), 3.29 (s, 3H; MeO), 3.31 (s, 3H; MeO), 3.74 (s, 3H; CO_2Me), 4.06 (d, J = 4.5 Hz, 1H; 15H), 4.12 (d, J = 6.8 Hz, 1H; C*H*($OMe)_2$), 4.16 (dt, J = 6.7, 4.5 Hz, 1H; H14), 6.03 (br s, 1H; N*H*), 6.08 (d, J = 15.5 Hz, 1H; H10), 6.65 ppm (dt, J = 15.5, 2.2 Hz, 1H; H9); ^{13}C NMR (125 MHz, $CDCl_3$): δ = −4.8 (Me_2Si), −4.6 (Me_2Si),17.8 (Me_3CSi), 24.4 (C4), 24.9 (C6), 25.5 (Me_3CSi), 28.1 (C5), 32.3 (C1), 32.4 (C13), 35.8 (C12), 40.4 (C3), 46.1 (C2), 52.5 (CO_2Me), 52.9 (MeO), 54.2 (MeO), 66.4 (C15), 71.4 (C14), 78.4 (C8), 97.9 (C7), 108.2 (*C*H($OMe)_2$), 122.3 (C10), 131.3 (C9), 164.8 (C11), 168.4 ppm (C16); FTIR (ATR): $\tilde{\nu}$ = 3286 (m), 2932 (m), 2928 (m), 2856 (m), 2832 (m), 2245 (m), 2109 (vs), 1744 (m), 1647 (m), 1614 (m), 1548 (m), 1360 (s), 1327 (s), 1257 (vs), 1203 (vs), 1118 (vs), 1052 (m), 958 (m), 908 (m), 836 cm^{-1} (s); MS (EI): *m/z* (%) = 536 (3) $[M]^+$, 505 (10), $[M-CH_3O]^+$, 479 (30), 449 (5), 436 (8), 219 (20), 171 (12), 75 (100) $[HC(OCH_3)_2]$; HRMS (EI): *m/z* calcd for $C_{26}H_{44}N_4O_6Si$: 536.3125; found: 536.3035 $[M]^+$.

Methyl (2*S*,3*S*)-2-azido-3-*O*-[*tert*-butyl(dimethyl)silyl]-5-((2*E*)-6-{2-[(1*E*)-4-(2,2-dimethyl-4-oxo-4*H*-1,3-dioxin-6-yl)but-1-enyl]cyclopentyl}hex-2-en-ynyl)amidopentanoate 13: 1) Amberlyst 15 (110 mg) was added to a solution of **12** (331 mg, 0.73 mmol) in acetone/4% H_2O (10 mL) and the mixture was stirred for 2 d. The ion exchange resin was filtered off, the filtrate was concentrated under vacuum, and the residue was dried azeotropically with toluene. R_f (hexanes/EtOAc, 3:1) = 0.26; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.11 (s, 6H; Me_2Si), 0.91 (s, 9H; Me_3CSi), 1.43–1.52 (m, 1H; CH*H*), 1.57–1.66 (m, 1H; CH*H*), 1.71–1.79 (m, 2H; H13), 1.86–1.96 (m, 4H; CH_2), 2.39–2.46 (m, 1H; H3), 2.47–2.52 (m, 2H; H2, H6), 2.57 (ddd, J = 15.7, 7.9, 2.3 Hz, 1H; H6), 3.34–3.43 (m, 1H; H12), 3.43–3.51 (m, 1H; H12), 3.79 (s, 3H; CO_2Me), 4.10 (d, J = 4.7 Hz, 1H; H15), 4.21 (dt, J = 6.6, 4.4 Hz, 1H; H14), 5.70 (br s, 1H; N*H*), 6.10 (d, J = 15.4 Hz, 1H; H10), 6.68 (dt, J = 15.4, 2.2 Hz, 1H; H9), 9.67 ppm (d, J = 2.5 Hz, 1H; C*H*O). 2) The compound was prepared according to ref. [6a] from **9** (680 mg, 1.80 mmol) in dry DME (4 mL), NaHMDS (3.7 mL, 0.5 M in DME), and a solution of methyl (2*S*,3*S*)-2-azido-3-*O*-[*tert*-butyl(dimethyl)silyl]-5-{(2*E*)-6-[2-formylcyclopentyl]hex-2-en-4-ynyl}-amidopentanoate in DME (4 mL), yield 250 mg (52%) of **13** as a viscous colourless oil; R_f (hexanes/EtOAc, 2:1) = 0.22; $[\alpha]_D^{20}$ = +7.1 (c = 1.00 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.10 (s, 6H; Me_2Si), 0.88 (s, 9H; Me_3CSi), 1.31–1.46 (m, 2H; CH_2), 1.58–1.68 (m, 3H; H3, CH_2), 1.67 (s, 6H; H25), 1.70–1.78 (m, 1H; H13), 1.79–1.93 (m, 3H; CH_2, H13), 2.06–2.13 (m, 1H; H2), 2.20–2.31 (m, 5H; H6, H19, H20), 2.46 (ddd, J = 17.0, 4.4, 2.3 Hz, 1H; H6), 3.31–3.47 (m, 2H; H12), 3.77 (s, 3H; CO_2Me), 4.09 (d, J = 4.5 Hz, 1H; H15), 4.19 (dt, J = 6.8, 4.5 Hz, 1H; H14), 5.22 (s, 1H; H22), 5.96 (t, J = 5.4 Hz, 1H; N*H*), 6.10 (d, J = 15.4 Hz, 1H; H10), 6.68 ppm (dt, J = 15.4, 2.3 Hz, 1H; H9); ^{13}C NMR (125 MHz, $CDCl_3$): δ = −4.7 (Me_2Si), −4.6 (Me_2Si), 17.8 (Me_3CSi), 23.0 (C6), 23.3 (C4), 25.0 (C25), 25.6 (Me_3CSi), 28.5 (C19), 31.0 (C5), 32.5 (C20), 32.9 (C1), 33.6 (C13), 35.9 (C12), 44.6 (C3), 48.4 (C2), 52.6 (CO_2Me), 66.5 (C15), 71.5 (C14), 78.8 (C8), 93.4 (C22), 97.2 (C7), 106.3 (C24), 122.2 (C10), 127.4 (C17), 131.5 (C9), 135.4 (C18),

161.3 (C21), 164.8 (C11), 168.5 (C16), 171.3 ppm (C23); FTIR (ATR): $\tilde{\nu}$ = 3302 (m), 3068 (m), 2952 (s), 2930 (s), 2885 (m), 2857 (s), 2213 (m), 2108 (vs), 1730 (vs), 1666 (vs), 1647 (vs), 1632 (vs), 1614 (vs), 1542 (vs), 1471 (s), 1462 (s), 1436 (s), 1389 (vs), 1375 (vs), 1327 (s), 1271 (vs), 1252 (vs), 1201 (vs), 1176 (s), 1113 (vs), 1011 (vs), 961 (vs), 901 (s), 836 (vs), 777 cm^{-1} (vs); MS (FAB): *m/z* (%) = 665 (10) $[M+Na]^+$, 643 (100) $[M+H]^+$, 585 (40), 528 (5), 171 (20), 73 (40); HRMS (FAB): *m/z* calcd for $C_{33}H_{51}N_4O_7Si$: 643.3522; found: 643.3539 $[M+H]^+$.

Methyl (10*S*,11*S*)-10-{[*tert*-butyl(dimethyl)silyl]oxy}-6,13,15-trioxo-2,3-didehydro-1,6,7,8,9,10,11,12,13,14,15,16,17,19a,20,21,22,22a-octadecahydrocyclopenta[*m*][1,6]diazacyclohenicosin-11-carboxylate 5: The compound was prepared according to ref. [6a] from PPh_3 (157 mg, 0.60 mmol) and **13** (370 mg, 0.56 mmol) in THF (5 mL), toluene (3 L), yield 201 mg (0.36 mmol, 64%) of **5** in a diastereomeric ratio 1:1; R_f (hexanes/EtOAc, 1:1) = 0.32; $[\alpha]_D^{20} = +31$ (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -0.03$ (s, 3H; Me_2Si*), −0.01 (s, 6H; Me_2Si), 0.00 (s, 3H; Me_2Si*), 0.82 (s, 9H; Me_3CSi), 1.20–1.34 (m, 1H; CH*H*), 1.33–1.44 (m, 1H; CH*H*), 1.54–1.64 (m, 2H; CH_2), 1.75–1.92 (m, 4H; CH_2, H3, H13), 1.95–2.04 (m, 1H; H13), 2.16–2.36 (m, 4H; H2, H6, H20), 2.55–2.62 (m, 1H; H6), 2.65–2.79 (m, 2H; H19), 3.12–3.22 (m, 1H; H12), 3.34 (d, *J* = 13.5 Hz, 1H; H22*), 3.36 (d, *J* = 13.6 Hz, 1H; H22), 3.47 (d, *J* = 13.6 Hz, 1H; H22), 3.52 (d, *J* = 13.5 Hz, 1H; H22*), 3.78 (s, 3H; CO_2Me), 3.89–3.98 (m, 2H; H12, H14), 4.59–4.63 (m, 1H; H15), 5.43–5.46 (m, 2H; H17, H18), 6.22 (d, *J* = 15.7 Hz, 1H; H10), 6.24 (d, *J* = 15.6 Hz, 1H; H10*), 6.62 (dt, *J* = 15.6, 2.3 Hz, 1H; H9*), 6.62 (dt, *J* = 15.7, 2.3 Hz, 1H; H9), 6.96 (t, *J* = 6.1 Hz, 1H; N*H*), 7.47–7.54 ppm (m, 1H; N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.1$, −5.1* (Me_2Si), −4.6, −4.5* (Me_2Si), 17.7, 17.7* (Me_3CSi), 23.5, 23.7* (C20), 24.6, 24.8* (C1), 25.5 (Me_3CSi), 26.4, 26.5* (C6), 32.7, 32.8* (C4), 33.4, 33.5* (C13), 33.6, 33.6* (C5), 35.5, 35.6* (C12), 43.3, 43.7* (C19), 44.5, 44.5* (C3), 49.1, 49.3* (C2), 50.6, 51.0* (C22), 52.5 (CO_2Me), 56.1, 56.2* (C15), 73.3, 73.4* (C14), 79.0, 79.0* (C8), 97.0, 97.1* (C7), 121.2, 121.2* (C9), 127.0, 127.3* (C18), 132.7, 132.9* (C17), 135.4, 135.4* (C10), 165.0, 165.0* (C11), 166.4, 166.6* (C23), 169.1, 169.1* (C16), 203.1, 203.2* ppm (C21) (*signals of the second diastereomer); FTIR (ATR): $\tilde{\nu}$ = 3289 (s), 3059 (m), 2951 (s), 2929 (s), 2897 (s), 2857 (s), 2214 (m), 1746 (vs), 1719 (vs), 1644 (vs), 1615 (vs), 1541 (vs), 1502 (s), 1471 (s), 1462 (s), 1436 (s), 1361 (s), 1326 (s), 1252 (vs), 1205 (vs), 1179 (s), 1111 (vs), 961 (vs), 911 (s), 837 (vs), 778 cm^{-1} (vs); MS (DCI, NH_3): *m/z* (%) = 576 (5) $[M+NH_4]^+$, 559 (100) $[M+H]^+$, 558 (40) $[M]^+$, 501 (60), 164 (20), 147 (60), 118 (10), 92 (10), 75 (20), 74 (20); HRMS (EI): *m/z* calcd for $C_{30}H_{46}N_2O_6Si$: 558.3125; found: 558.3115 $[M]^+$.

Methyl (10*S*,11*S*)-10-{[*tert*-butyl(dimethyl)silyl]oxy}-6,13,15-trioxo-1,6,7,8,9,10,11,12,13,14,15,16,17,19a,22,21,20,22a-octadecahydrocyclopenta[*m*][1,6]diazacyclohenicosin-11-carboxylate 14: a) The compound was prepared according to ref. [6a] from Pd/$BaSO_4$ (7 mg, 5% Pd) in EtOH (1 mL), synthetic quinoline (5 μL, 42.2 μmol) and **5** (35 mg, 62 μmol) in EtOH (1.5 mL), yield 20 mg (57%), colourless resin. b) A suspension of zinc nanoparticles (700 mg, 10.7 mmol) in H_2O (4 mL) was degassed with N_2 for 15 min. Then $Cu(OAc)_2 \cdot H_2O$ (73 mg, 0.36 mmol) and after 15 min, $AgNO_3$ (73 mg, 0.43 mmol) were added and the mixture stirred for 30 min. Activated zinc was then washed successively with H_2O, MeOH, acetone and Et_2O (15 mL each), taken up in H_2O/MeOH (1:1), and added to a solution of **5** (181 mg, 0.32 mmol) in MeOH (2 mL). After stirring at 45 °C for 3 h, the diastereomers were separated by MPLC on a CN-phase with hexanes/EtOAc (2:1; flow rate 100 mL min^{-1}) to give **14a** (50 mg, 89.3 μmol, 28%) and **14b** (48 mg, 5.7 μmol, 27%). **14a**: $[\alpha]_D^{20} = -9.0$ (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.02$ (s, 3H; Me_2Si), 0.04 (s, 3H; Me_2Si), 0.85 (s, 9H; Me_3CSi), 1.25–1.36 (m, 3H; CH_2), 1.38–1.46 (m, 1H; CH_2), 1.56–1.62 (m, 2H; CH_2), 1.75–1.82 (m, 2H; CH_2), 1.85–1.92 (m, 4H; CH_2), 2.26–2.42 (m, 2H; H6), 2.64–2.74 (m, 1H; H_a19), 2.78 (dt, *J* = 2.1, 7.0 Hz, 1H; H_b19), 3.43 (d, *J* = 15.1 Hz, 1H; H22), 3.57 (m, 1H; CH_2), 3.72 (s, 3H; CO_2Me), 4.08–4.12 (m, 1H; H14), 4.65 (dd, *J* = 6.3, 15.1 Hz, 1H; H17), 5.43 (ddd, *J* = 15.1, 6.3, 6.0 Hz, 1H; H18), 5.82 (d, *J* = 15.2 Hz, 1H; H10), 5.85–5.90 (ddd, *J* = 6.7, 10.1, 10.1 Hz, 1H; H7), 6.06 (dd, *J* = 11.0, 10.8 Hz, 1H; H8), 6.61 (br s, 1H; NH), 7.43 (dd, *J* = 11.5, 15.2 Hz, 1H; H9), 8.19 ppm (br s, 1H; NH); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.1$ (Me_2Si), −4.7 (Me_2Si), 17.8 (Me_3CSi), 23.5 (C1), 25.6 (Me_3CSi), 26.4 (C6), 31.2 (C4), 31.4 (C20), 32.7 (C5), 34.1 (C13), 35.9 (C12), 43.7 (C19), 46.9 (C3), 50.0 (C2), 50.3 (C22), 52.3 (CO_2Me), 57.2 (C15), 71.0 (C14), 123.9 (C9), 126.7 (C18), 128.8 (C8), 135.3 (C17), 135.4 (C10), 138.7 (C7), 166.0 (C11), 167.2 (C23), 170.7 (C16), 204.8 ppm (C21); FTIR (ATR): $\tilde{\nu}$ = 3253 (br, s), 3030 (s), 2951 (vs), 2929 (vs), 2857 (s), 1745 (vs), 1721 (vs), 1652 (vs), 1616 (s), 1547 (vs), 1436 (m), 1334 (m), 1255 (s), 1200 (m), 1116 (s), 837 cm^{-1} (vs).

14b: $[\alpha]_D^{20} = -4.2$ (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.01 (s, 3H; Me_2Si), 0.05 (s, 3H; Me_2Si), 0.84 (s, 9H; Me_3CSi), 1.20–1.34 (m, 4H; CH_2), 1.56–1.62 (m, 2H; CH_2), 1.75–1.82 (m, 2H; CH_2), 1.85–1.92 (m, 4H; CH_2), 2.18–2.28 (m, 1H; H_a6), 2.30–2.36 (m, 1H; H_b6), 2.61–2.67 (m, 1H; H_a19), 2.74 (dt, *J* = 3.3, 7.1 Hz, 1H; H_b19), 3.27–3.43 (m, 1H; H12), 3.38–3.43 (m, 1H; CH_2), 3.61–3.67 (m, 1H; CH_2), 3.75 (s, 3H; CO_2Me), 3.90–3.92 (m, 1H; CH_2), 4.09–4.12 (m, 1H; H14), 4.68 (dd, *J* = 7.4, 4.6 Hz, 1H; H15), 5.29 (dd, *J* = 15.3, 8.5 Hz, 1H; H17), 5.38 (ddd, *J* = 15.1, 7.8, 4.9 Hz, 1H; H18), 5.81–5.86 (m, 1H; H7), 5.83 (d, *J* = 14.8 Hz, 1H; H10), 6.06 (dd, *J* = 11.0, 11.0 Hz, 1H; H8), 6.48 (t, *J* = 6.1 Hz, 1H; NH), 7.42 (dd, *J* = 15.0, 11.5 Hz, 1H; H9), 8.03 ppm (d, *J* = 7.3 Hz, 1H; NH); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.1$ (Me_2Si), −4.6 (Me_2Si), 17.9 (Me_3CSi), 23.3 (C1), 25.5 (Me_3CSi), 26.3 (C6), 31.5 (C4), 31.7 (C20), 33.1 (C5), 33.9 (C13), 35.7 (C12), 43.2 (C19), 46.3 (C3), 50.1 (C2), 50.2 (C22), 52.3 (CO_2Me), 56.7 (C15), 71.5 (C14), 124.0 (C9), 126.9 (C18), 128.2 (C8), 135.3 (C17), 135.5 (C10), 138.7 (C7), 166.1 (C11), 167.1 (C23), 170.3 (C16), 204.5 ppm (C21); FTIR (ATR): $\tilde{\nu}$ = 3336 (br, s), 3030 (m), 2951 (vs), 2929 (vs), 2859 (s), 1745 (m), 1721 (vs), 1653 (vs), 1618 (s), 1546 (vs), 1436 (m), 1372 (m), 1334 (m), 1255 (s), 1215 (s), 1119 (s), 1040 (vs), 990 (m), 921 (m), 837 cm^{-1} (vs); MS (DCI, NH_3): *m/z* (%) = 561 (100) $[M+H]^+$, 560 (60) $[M]^+$, 503 (85), 171 (12), 92 (16), 75 (40); HRMS (EI): *m/z* calcd for $C_{30}H_{48}N_2O_6Si$: 560.3282; found: 560.3298 $[M]^+$.

Methyl (10*S*,11*S*)-10-hydroxy-6,13,15-trioxo-1,6,7,8,9,10,11,12,13,14,15,16,17,19a,22,21,20,22a-octadecahydrocyclopenta[*m*][1,6]-diazacyclohenicosine-11-carboxylate 15: HF/H_2O (48%, 400 μL) was added to a solution of **14a** (50 mg, 89.3 μmol) in MeCN (4 mL) in a Teflon vessel, and the mixture was stirred at RT for 1.5 h. After quenching with H_2O (5 mL), the mixture was extracted with $CHCl_3$ (20 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by MPLC on a CN-phase ($CHCl_3$/MeOH, 100:1; flow rate 100 mL min^{-1}; R_f = 0.53) to give **15a** (28 mg, 62.8 μmol, 64%). $[\alpha]_D^{20} = +4.5$ (*c* = 1.00 in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23–1.35 (m, 2H; CH_2), 1.37–1.49 (m, 1H; CH_2), 1.51–1.72 (m, 4H; H2, CH_2), 1.70–1.85 (m, 4H; H13, H20), 1.87–1.97 (m, 1H; H_a3), 2.20–2.40 (m, 2H; H6), 2.43–2.54 (m, 1H; H_a19), 2.60–2.74 (m, 2H; H_b3, H_b19), 3.07–3.19 (m, 1H; H12), 3.35 (d, *J* = 14.1 Hz, 1H; H_a22), 3.45 (d, *J* = 14.2 Hz, 1H; H_b22), 3.78 (s, 3H; CO_2Me), 4.60 (dd, *J* = 8.5, 3.6 Hz, 1H; H15), 4.88 (d, *J* = 4.2 Hz, 1H; OH), 5.15–5.32 (m, 2H; H17, H18), 5.80 (d, *J* = 14.8 Hz, 1H; H10), 5.90 (ddd, *J* = 10.4, 10.4, 6.2 Hz, 1H; H7), 6.01 (t, *J* = 5.8 Hz 1H; N*H*), 6.16 (t, *J* = 11.2 Hz, 1H;

H8), 7.24 (d, $J=8.5$ Hz, 1H; NH), 7.51 ppm (dd, $J=14.8$, 11.2 Hz, 1H; 9H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=23.5$ (C1), 26.8 (C6), 29.0 (C4), 31.7 (C20), 32.9 (C5), 34.4 (C13), 36.1 (C12), 42.8 (C19), 45.4 (C3), 47.8 (C2), 51.7 (C22), 52.4 (CO_2Me), 56.4 (C15), 69.4 (C14), 122.6 (C9), 127.5 (C18), 128.8 (C8), 134.8 (C17), 137.2 (C10), 137.8 (C7), 165.1 (C11), 168.2 (C23), 169.7 (C16), 204.4 ppm (C21); FTIR (ATR): $\tilde{\nu}=3322$ (m), 2952 (m), 2869 (m), 1736 (s), 1659 (s), 1616 (vs), 1519 (m), 906 (vs), 746 (vs), 731 (vs), 632 cm^{-1} (vs); MS (EI): *m/z* (%) = 446 (100) $[M]^+$, 428 (20) $[M-OH, -H]^+$, 415 (7) $[M-CH_3O]^+$, 256 (15), 200 (15), 173 (15), 146 (15), 128 (20), 107 (16), 94 (30), 67 (20), 30 (35); HRMS (EI): *m/z* calcd for $C_{30}H_{46}N_2O_6Si$: 446.2417; found: 446.2420 $[M]^+$.

Compound 15b: The title compound was prepared analogously to **15a**, from **14b** (48 mg, 85.7 μmol) in MeCN (2 mL) and HF/H_2O (48%, 100 μL), yield 21 mg (55%); $[\alpha]_D^{20}=+20.5$ ($c=1.00$ in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=1.20$–1.31 (m, 2H; CH_2), 1.31–1.35 (m, 1H; CH_2), 1.51–1.72 (m, 4H; H2, CH_2), 1.70–1.85 (m, 4H; H13, H20), 1.87–1.97 (m, 1H; H_a3), 2.15–2.28 (m, 2H; H6), 2.52–2.66 (m, 3H; H_b3, H19), 3.04–3.20 (m, 1H; H12), 3.32 (d, $J=14.9$ Hz, 1H; H_a22), 3.40 (d, $J=14.9$ Hz, 1H; H_b22), 3.72 (s, 3H; CO_2Me), 4.53 (dd, $J=$ 8.3, 3.7 Hz, 1H; H15), 4.66 (d, $J=4.7$ Hz, 1H; OH), 5.19–5.26 (m, 1H; H18), 5.33–5.38 (m, 1H; H17), 5.74 (d, $J=14.8$ Hz, 1H; H10), 5.90 (ddd, $J=10.6$, 10.6, 5.8 Hz, 1H; H7), 6.01 (t, $J=5.8$ Hz, 1H; N*H*), 5.99 (t, $J=11.1$ Hz, 1H; H8), 7.22 (d, $J=8.3$ Hz, 1H; NH), 7.43 ppm (dd, $J=14.7$, 11.4 Hz, 1H; H9); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=23.6$ (C1), 26.0 (C6), 29.6 (C4), 32.2 (C20), 32.9 (C5), 34.3 (C13), 36.0 (C12), 42.5 (C19), 46.3 (C3), 50.0 (C2), 50.8 (C22), 52.5 (CO_2Me), 56.5 (C15), 69.5 (C14), 122.7 (C9), 126.1 (C18), 127.8 (C8), 134.4 (C17), 136.9 (C10), 139.7 (C7), 165.3 (C11), 168.4 (C23), 169.9 (C16), 204.2 ppm (C21); FTIR (ATR): $\tilde{\nu}=3298$ (m), 2948 (m), 2866 (m), 1740 (s), 1719 (s), 1653 (s), 1618 (m), 1542 (s), 1437 (m), 1332 (w), 1276 (w), 1204 (w), 1108 (w), 911 (w), 868 (w), 731 (w), 632 cm^{-1} (s); MS (EI): *m/z* (%) = 446 (100) $[M]^+$, 428 (20) $[M-OH, -H]^+$, 415 (7) $[M-CH_3O]^+$, 256 (15), 200 (15), 173 (15), 146 (15), 128 (20), 107 (16), 94 (30), 67 (20), 44 (15), 30 (35); HRMS (EI): *m/z* calcd for $C_{30}H_{46}N_2O_6Si$: 446.2417; found: 446.2429 $[M]^+$.

10,15-Dihydroxy-1,7,8,9,10,11,12,16,17,19a,20,21,22,22a-tetradecahydro-11,14-methanocyclopenta[*m*][1,6]diazacyclohenicosine-6,13,23-triones 4: A solution of NaOMe in MeOH (62 μL, 0.18 mmol, 2.88 mol L^{-1}) was added to a solution of **15a** (16 mg, 35.9 μmol) in MeOH (2 mL), and the mixture was stirred at RT for 1 h. The reaction mixture was adjusted to pH 2–3 with HCl (180 μL in 10 mL H_2O) and extracted with *n*BuOH. The organic layer was washed with brine and concentrated under vacuum. The residue was purified by HPLC (CH_3CN/H_2O 50:50, 0.05% TFA, flow rate: 1.5 mL min^{-1}) to give in the first fraction ($t_R=5.13$ min) **4a** (6.13 mg, 14.8 μmol) and in the second fraction ($t_R=5.88$ min) **4b** (7.66, 18.5 mmol) in 93% total yield.

4a: $[\alpha]_D^{20}=+109.3$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (600 MHz, CD_3OD): $\delta=1.28$–1.42 (m, 3H; H2, CH_2), 1.44–1.50 (m, 1H; H_a13),1.62–1.69 (m, 2H; H_a3, CH_2), 1.71–1.88 (m, 3H; H_a6, H_b13, CH_2), 1.91–2.03 (m, 2H; CH_2), 2.21–2.38 (m, 2H; H19), 2.61–2.72 (m, 1H; H_a20), 2.81–2.91 (m, 1H; H_b6), 2.96–3.09 (m, 1H; H_b20), 3.09–3.22 (m, 1H; H_a12), 3.35–3.45 (m, 1H; H_b12), 3.87–3.94 (m, 1H; H15), 3.98–4.05 (m, 1H; H14), 5.47 (dd, $J=14.5$, 7.7 Hz, 1H; H17), 5.52–5.60 (m, 1H; H18), 5.89–5.94 (m, 1H; H7), 5.96 (d, $J=15.0$ Hz, 1H; H10), 6.13 (t, $J=10.9$ Hz, 1H; H8), 7.43 ppm (dd, $J=15.0$, 10.7 Hz, 1H; H9); ^{13}C NMR (125 MHz, CD_3OD): $\delta=24.7$ (CH_2), 29.4 (C19), 31.0 (C13), 32.6 (CH_2), 32.9 (C6), 33.7 (CH_2), 36.9 (CH_2), 37.7 (C12), 49.0 (C2), 51.0 (C1), 68.8 (C15), 70.5 (C14), 102.5 (C22), 125.2 (C7), 127.6 (C8), 130.0 (C18), 135.7 (C17), 136.2 (C9), 139.4 (C10), 169.5 (C11), 178.1 (C23), 192.9 (C21), 195.2 ppm (C16); FTIR (ATR): $\tilde{\nu}=3260$ (m), 2955 (m), 2937 (s), 2855 (m), 1740 (m), 1658 (m), 1602 (s), 1535 (m), 1478 (m), 1445 (w), 1361 (w), 1331 (w), 1310 (w), 1280 (w), 1206 (s), 1182 (w), 1139 (m), 1084 (w), 1016 (w), 992 (w), 965 (w), 864 (w), 839 (m), 801 (w), 758 (m), 722 cm^{-1} (w); MS (FAB, glycerine): *m/z* (%) = 867 (7) $[2M+K]^+$, 849 (4) $[2M+Na-2H]^+$, 545 (48) $[M+C_3H_8O_3+K]^+$, 453 (70) $[M+K]^+$, 437 (25) $[M+Na]^+$, 223 (50), 207 (30), 179 (27), 133 (85) $[Cs]^+$, 115 (100) $[C_3H_8O_3+Na]$; HRMS (FAB): *m/z* calcd for $C_{23}H_{30}N_2O_5$: 414.2155; found: 459.1888 $[M+2Na-H]^+$.

4b: $[\alpha]_D^{20}=-21.4$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): $\delta=1.25$–1.39 (m, 3H; H3, H13, CH_2), 1.41–1.51 (m, 1H; H2), 1.55–1.67 (m, 3H; CH_2), 1.68–1.81 (m, 1H; CH_2), 1.86–2.04 (m, 3H; H_a6, H1, H19), 2.06–2.32 (m, 2H; H19, H20), 2.53–267 (m, 1H; H_b6), 2.92–3.06 (m, 1H; H20), 3.40–3.49 (m, 2H; H12), 3.58–3.69 (m, 2H; H15, H14), 5.20–5.35 (m, 1H; H17), 5.39–5.54 (m, 1H; H18), 5.77–5.97 (m, 2H; H10, H7), 6.05 (dd, $J=11.0$, 11.0 Hz, 1H; H8), 7.26–7.42 ppm (m, 1H; H9); ^{13}C NMR (150 MHz, CD_3OD): $\delta=24.7$ (CH_2), 29.4 (CH_2), 30.9 (CH_2), 32.6 (CH_2), 32.9 (CH_2), 33.7 (CH_2), 37.6 (C12), 44.7 (C2), 51.0 (C1), 64.9 (C14), 70.5 (C15), 102.5 (C22), 125.3 (C10), 127.6 (C8), 130.0 (C18), 135.7 (C17), 136.2 (C9), 197.3 (C7), 147.1 (Cq), 162.4 (Cq), 169.5 (C21), 195.1 ppm (C16); FTIR (ATR): $\tilde{\nu}=3260$ (m), 2955 (m), 2926 (s), 2855 (m), 1740 (m), 1647 (s), 1611 (s), 1546 (m), 1473 (m), 1435 (w), 1381 (w), 1361 (w), 1330 (w), 1280 (w), 1257 (m), 1203 (m), 1176 (w), 1122 (m), 1054 (w), 1006 (w), 959 (w), 837 (m), 778 cm^{-1} (m); MS (FAB): *m/z* (%) = 873 (19) $[2M+2Na-H]^+$, 459 (100) $[M+2Na-H]^+$, 437 (21) $[M+Na]^+$, 381 (10), 264 (20), 172 (40), 91 (37), 73 (80), 41 (70); HRMS (FAB): *m/z* calcd for $C_{23}H_{30}N_2O_5$: 414.2155; found: 459.1868 $[M+2Na-H]^+$.

Lactams 4c,d: These were prepared analogously to **4a**, **b** from **15b** (17.2 mg, 38.6 μmol) and separated by HPLC (CH_3CN/H_2O, 70:30, 0.05% TFA, flow rate: 0.7 mL min^{-1}) to give **4c** ($t_R=$ 17.88 min) and **4d** ($t_R=21.99$ min) (8.35 mg, 20.2 mmol, 52%), diastereomeric ratio: 65:35.

4c: ^{1}H NMR (600 MHz, CD_3OD): $\delta=1.26$–1.45 (m, 3H; H_a3, CH_2), 1.48–1.71 (m, 3H; H2, CH_2), 1.71–1.81 (m, 1H; H_a13), 1.87–1.98 (m, 3H; H1, H_b3, H_b13), 2.02–2.23 (m, 2H; H_a6), 2.24–2.43 (m, 2H; H19), 2.45–2.63 (m, 2H; H_b6, H_a20), 3.15–3.25 (m, 1H; H_b20), 3.36–3.66 (m, 4H; H12, H14, H15), 5.30–5.50 (m, 2H; H17, H18), 5.82–5.95 (m, 2H; H7, H10), 6.12 (dd, $J=10.7$, 10.7 Hz, 1H; H8), 7.26–7.35 ppm (m, 1H; H9); ^{13}C NMR (150 MHz, CD_3OD): $\delta=23.0$ (CH_2), 28.1 (C6), 29.3 (CH_2), 30.4 (CH_2), 31.9 (C19), 32.6 (CH_2), 35.5 (C12), 46.3 (C2), 49.0 (C1), 64.3 (C14), 70.4 (C15), 100.9 (C22), 124.3 (C10), 126.9 (C8), 129.7 (C18), 133.8 (C17), 134.6 (C9), 137.3 (C7), 161.8 (Cq), 168.8 (Cq), 169.3 (C21), 177.9 ppm (Cq); FTIR (ATR): $\tilde{\nu}=3260$ (m), 2957 (m), 2923 (s), 2852 (m), 1740 (m), 1659 (m), 1608 (s), 1539 (m), 1483 (m), 1377 (w), 1283 (w), 1206 (s), 1180 (w), 1137 (m), 993 (w), 965 (w), 864 (w), 840 (m), 801 (w), 722 cm^{-1} (w); MS (FAB, glycerine): *m/z* (%) = 851 (1) $[2M+Na]^+$, 505 (10) $[M+C_3H_8O_3-H]^+$, 437 (5) $[M+Na]^+$, 258 (10), 207 (18), 171 (20), 75 (75), 73 (100); HRMS (FAB): *m/z* calcd for $C_{23}H_{30}N_2O_5$: 414.2155; found: 459.1880 $[M+2Na-H]^+$.

4d: $[\alpha]_D^{20}=+202.1$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (600 MHz, CD_3OD): $\delta=1.26$–1.45 (m, 3H; H2, CH_2), 1.53–1.71 (m, 3H; CH_2), 1.72–1.87 (m, 3H; CH_2), 1.94–2.01 (m, 2H; H1, CH_2), 1.98–2.34 (m, 3H; CH_2), 2.45–2.52 (m, 1H; H19), 2.51–2.58 (m, 1H; H_a6), 2.58–2.73 (m, 1H; H_b6), 2.87–3.01 (m, 1H; H12), 3.13–3.27 (m, 2H; H20), 3.41–3.64 (m, 1H; H_b12), 3.98–4.01 (m, 1H; H15), 4.02–4.05 (m, 1H; H14), 5.18–5.30 (m, 1H; H17), 5.31–5.41 (m, 1H; H18), 5.90–6.00 (m, 2H; H7, H10), 6.23 (dd, $J=11.4$, 12.2 Hz, 1H; H8), 7.49 ppm (dd, $J=12.2$, 14.3 Hz, 1H; H9); ^{13}C NMR (125 MHz, CD_3OD): $\delta=24.7$, 29.4 (CH_2), 31.0 (CH_2), 32.6 (CH_2), 32.9 (C6), 33.7 (CH_2), 36.9 (C20), 37.7 (CH_2), 49.0 (CH_2), 51.0 (C1), 68.8 (C15), 70.5 (C14), 102.5 (C22), 125.2 (C10),

127.6 (C8), 130.0 (C18), 135.7 (C17), 136.2 (C9), 139.4 (C7), 169.5 (C11), 178.1 (C23), 192.9 (C21), 195.2 ppm (C16); FTIR (ATR): $\tilde{\nu}$ = 3292 (m), 2956 (m), 2923 (s), 2852 (m), 1781 (m), 1680 (m), 1655 (w), 1603 (s), 1548 (w), 1516 (w), 1478 (m), 1377 (w), 1329 (w), 1280 (w), 1207 (s), 1180 (w), 1141 (m), 1012 (m), 967 (w), 889 (w), 838 (m), 801 (w), 757 (w), 722 cm^{-1} (w); MS (FAB): *m/z* (%) = 867 (8), $[2M+K]^+$, 545 (10) $[M+C_3H_8O_3+K]^+$, 453 (65) $[M+K]^+$, 397 (20), 179, 102 (100); HRMS (FAB): *m/z* calcd for $C_{23}H_{30}N_2O_5$: 414.2155; found: 459.1903 $[M+2Na-H]^+$.

***trans*-1-(Dimethoxymethyl)-3-(3-iodoprop-2-ynyl)cyclopentane 16**: A solution of BuLi in hexane (1.6 M, 0.4 mL, 0.64 mmol) was added to a solution of **7** (165 mg, 0.64 mmol) in dry THF (1 mL) at −78 °C and the mixture stirred for 30 min. Then a solution of I_2 (163 mg, 0.64 mmol) in THF (0.5 mL) was added and the mixture allowed to warm to RT. After addition of Et_2O (15 mL), the mixture was washed with a sat. solution of $Na_2S_2O_3$ (5 mL) and brine, dried ($MgSO_4$) and concentrated under vacuum to give **16** (194 mg, 98%) as a colourless oil, R_f (hexanes/Et_2O, 9:1) = 0.36; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.40–1.63 (m, 4H; CH_2), 1.70–1.77 (m, 1H; CH*H*), 1.79–1.86 (m, 1H; CH*H*), 1.87–1.98 (m, 2H; H2, H3), 2.39 (dd, *J* = 16.7, 7.3 Hz, 1H; H6), 2.58 (dd, *J* = 16.7, 4.6 Hz, 1H; H6), 3.31 (s, 3H; MeO), 3.34 (s, 3H; MeO), 4.15 ppm (d, *J* = 6.9 Hz, 1H; C*H*-$(OMe)_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 7.4 (C8), 24.4 (C1), 26.1 (C6), 28.1 (C5), 32.3 (C4), 40.5 (C3), 46.0 (C2), 52.9 (O*Me*), 54.2 (O*Me*), 94.1 (C7), 108.1 ppm (C*H*$(OMe)_2$); FTIR (ATR): $\tilde{\nu}$ = 2947 (vs), 2869 (s), 2829 (s), 1448 (s), 1382 (m), 1360 (m), 1310 (m), 1189 (m), 1122 (vs), 1051 (vs), 960 (m), 912 cm^{-1} (m); MS (NCI): *m/z* (%) = 308 (100) $[M]^-$, 181 (20), 149 (20), 127 (80) $[I]^-$; HRMS (EI): *m/z* calcd for $C_{11}H_{16}IO_2$: 307.0195; found: 307.0194 $[M-H]^+$.

***trans*-1-(Dimethoxymethyl)-2-[(2*Z*)-3-iodoprop-2-enyl]cyclopentane 18**: Compound **17** (93 mg, 0.43 mmol) was added to a solution of **16** (101 mg, 0.33 mmol) and NEt_3 (69 μL, 0.50 mmol) in THF/*i*PrOH (1 mL each) and the mixture stirred in the dark for 16 h. Further NEt_3 (25 μL, 0.18 mmol) and **17** (30 mg, 0.097 mmol) were added, and the mixture was stirred for 30 min. After quenching with H_2O, the mixture was diluted with EtOAc (10 mL), washed with brine, dried ($MgSO_4$) and concentrated under vacuum to give **18** (95 mg, 0.31 mmol, 93%) as a colourless oil. R_f (hexanes/Et_2O, 9:1) = 0.40; ^1H NMR (500 MHz, CD_2Cl_2): δ = 1.23–1.31 (m, 1H; C*H*H), 1.41–1.60 (m, 3H; C*H*H, CH_2), 1.65–1.76 (m, 2H; CH_2), 1.84–1.93 (m, 2H; H2, H3), 2.01–2.09 (m, 1H; H6), 2.34–2.41 (m, 1H; H6), 3.27 (s, 3H; OMe), 3.33 (s, 3H; OMe), 4.11 (d, *J* = 6.7 Hz, 1H; C*H*$(OMe)_2$), 6.17–6.25 ppm (m, 2H; H7, H8); ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 24.4 (C1), 27.6 (C5), 32.1 (C4), 40.1 (C3), 40.4 (C6), 46.8 (C2), 52.6 (OMe), 54.2 (OMe), 82.1 (C8), 108.0 (C*H*$(OMe)_2$), 140.7 ppm (C7); FTIR (ATR): $\tilde{\nu}$ = 2945 (s), 2868 (s), 2827 (s), 1448 (m), 1381 (m), 1318 (m), 1275 (m), 1189 (m), 1120 (vs), 1054 (vs), 964 (s), 911 (m), 710 cm^{-1} (m); MS (CI): *m/z* (%) = 311 (1) $[M+H]^+$, 309 (2) $[M-H]^+$, 279 (85), 247 (100), 151 (50), 121 (30), 120 (50), 119 (80), 111 (30), 75 (50); HRMS (EI): *m/z* calcd for $C_{11}H_{18}IO_2$: 309.0351; found: 309.0360 $[M-H]^+$.

***trans*-Tributyl{(1*Z*)-3-[2-(dimethoxymethyl)cyclopentyl]prop-1-enyl}stannane 10**: A solution of *t*BuLi in pentane (1.5 M, 850 μL, 1.27 mmol) was added to a solution of **18** (164 mg, 0.53 mmol) in dry pentane/Et_2O (1:1, 4 mL) at −78 °C, and the mixture was stirred for 1 h. Then chlorotributylstannane (200 μL, 0.74 mmol) was added, and the reaction mixture was warmed to RT over 16 h. The reaction was terminated with phosphate buffer (pH 7.0), and the mixture was extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated under vacuum. The residue was chromatographed on SiO_2 (hexanes/NEt_3 100:1→hexanes/Et_2O/NEt_3, 100:10:1) to give **10** (180 mg, 76%) as a colourless oil, which was used without further purification. R_f (hexanes/Et_2O/NEt_3 100:10:1) = 0.39; MS (CI): *m/z* (%) = 473 (0.2) $[M-H]^+$, 471 $[M-H]^+$, 443 (1), 441 (1), 415 (1.2), 385 (90), 383 (60), 381 (40), 291 (30), 289 (20), 287 (10), 153 (40), 121 (95), 111 (45), 75 (40), 57 (100).

Methyl (2*S*,3*S*)-2-azido-3-*O*-[*tert*-butyl(dimethyl)silyl]-5-{(2*E*,4*Z*)-6-[2-(dimethoxymethyl)cyclopentyl]hexa-2,4-dienyl}amidopentanoate 19: A solution of **8** (250 mg, 0.52 mmol) and **10** (165 mg, 0.35 mmol) in dry DMF (3 mL) was degassed with argon for 5 min. A solution of bis(benzonitrile)palladium(II) dichloride (31 mg, 0.08 mmol) in dry DMF (0.5 mL) was added and the reaction was stirred for 10 min. The reaction was quenched with a sat. solution of NH_4Cl and extracted with Et_2O. The organic layer was washed with H_2O and brine, dried ($MgSO_4$) and concentrated under vacuum. The residue was chromatographed on SiO_2 (hexanes/EtOAc, 2:1) to give **19** (95 mg, 51%) as a light-yellow oil. R_f (hexanes/EtOAc, 1:1) = 0.61; $[\alpha]_D^{20} = +13.5$ (*c* = 1.00 in $CHCl_3$); ^1H NMR (300 MHz, CD_2Cl_2): δ = 0.10 (s, 3H; *Me*Si), 0.11 (s, 3H; *Me*Si), 0.89 (s, 9H; Me_3CSi), 1.15–1.91 (m, 10H; H1, H2, H3, H4, H5, H13), 2.16–2.28 (m, 1H; H6), 2.49–2.60 (m, 1H; H6), 3.28 (s, 3H; O*Me*), 3.32–3.40 (m, 2H; H12), 3.33 (s, 3H; O*Me*), 3.75 (s, 3H; CO_2Me), 4.09–4.22 (m, 3H; H14, H15, C*H*$(OMe)_2$), 5.82 (d, *J* = 14.8 Hz, 1H; H10), 5.87 (dt, *J* = 11.4, 5.4 Hz, 1H; H7), 6.11 (t, *J* = 11.4 Hz, 1H; H8), 7.48 ppm (ddd, *J* = 14.8, 11.4, 0.9 Hz, 1H; H9); ^{13}C NMR (75 MHz, CD_2Cl_2): δ = −5.1 (*Me*Si), −5.0 (*Me*Si), 17.6 (Me_3CSi), 24.4 (C1), 25.3 (Me_3CSi), 27.6 (C5), 32.1 (C4), 32.6 (C13), 33.6 (C3), 35.6 (C12), 41.5 (C6), 46.6 (C2), 52.3 (CO_2Me), 52.5 (OMe), 54.1 (OMe), 66.2 (C15), 71.3 (C14), 108.1 (C*H*$(OMe)_2$), 123.8 (C10), 126.7 (C8), 135.4 (C7), 138.9 (C9), 165.6 (C11), 168.4 ppm (C16); FTIR (ATR): $\tilde{\nu}$ = 3284 (m), 2951 (vs), 2930 (vs), 2858 (s), 2830 (m), 2108 (vs), 1740 (vs), 1654 (vs), 1621 (vs), 1542 (vs), 1462 (s), 1437 (s), 1410 (m), 1380 (m), 1360 (m), 1327 (m), 1258 (vs), 1201 (s), 1175 (s), 1119 (vs), 1054 (vs), 996 (s), 962 (s), 913 (m), 867 (m), 837 (vs), 778 (vs), 742 (m), 666 cm^{-1} (m); MS (FAB): *m/z* (%) = 561 (100) $[M+Na]^+$, 518 (5), 507 (35), 481 (4), 441 (60), 149 (20), 111 (20); HRMS (FAB): *m/z* calcd for $C_{26}H_{46}N_4NaO_6Si$: 561.3079; found: 561.3060 $[M+Na]^+$.

Methyl (2*S*,3*S*)-2-azido-3-*O*-[*tert*-butyl(dimethyl)silyl]-5-[((2*E*,4*Z*)-6-{2-[(1*E*)-4-(2,2-dimethyl-4-oxo-4*H*-1,3-dioxin-6-yl)but-1enyl]cyclopentyl}hexa-2,4-dienyl)amido]pentanoate 6: The compound was prepared as described above for **13**, a) from **19** (90 mg, 167 μmol) in acetone (4 mL), Amberlyst 15 (70 mg) and H_2O (0.3 mL); b) aldehyde from a), **9** (227 mg, 0.60 mmol) in dry DME (3 mL), NaHMDS (0.65 mL, 1 M in DME), yield 54 mg (50% over both steps) as a colourless resin; R_f (hexanes/EtOAc, 2:1) = 0.2; $[\alpha]_D^{20} = +9.0$ (*c* = 1.00 in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 0.10 (s, 3H; *Me*Si), 0.11 (s, 3H; *Me*Si), 0.89 (s, 9H; Me_3CSi), 1.12–1.39 (m, 10H; H1, H2, H3, H4, H5, H13), 1.65 (s, 6H; H25), 2.07–2.46 (m, 6H; H6, H19, H20), 3.30–3.50 (m, 2H; H12), 3.76 (s, 3H; CO_2Me), 4.09 (d, *J* = 4.7 Hz, 1H; H15), 4.16–4.24 (m, 1H; H14), 5.23 (s, 1H; H22), 5.30 (dd, *J* = 14.9 Hz, 4.7 Hz, 2H; H17, H18), 5.78 (d, *J* = 15.9 Hz, 1H; H10), 5.68–5.83 (m, 1H; H7), 5.97 (br s, 1H; N*H*), 6.07 (t, *J* = 11.9 Hz, 1H; H8), 7.51 ppm (dd, *J* = 15.1, 11.9 Hz, 1H; H9); ^{13}C NMR (75 MHz, $CDCl_3$): δ = −4.8 (*Me*Si), −4.6 (*Me*Si), 17.8 (Me_3CSi), 23.3 (C1), 24.9 (C25 A), 25.0 (C25B), 25.5 (Me_3CSi), 28.5 (C19), 31.4 (C20), 32.1 (C4), 32.4 (C13), 33.0 (C5), 33.5 (C3), 35.8 (C12), 45.8 (C6), 49.5 (C2), 52.6 (CO_2Me), 66.3 (C15), 71.5 (C14), 93.3 (C22), 106.2 (C24), 123.5 (C10), 126.7 (C8), 127.2 (C17), 135.8 (C7), 135.9 (C18), 139.0 (C9), 161.3 (C21), 166.2 (C11), 168.5 (C16), 171.4 ppm (C23); FTIR (ATR): $\tilde{\nu}$ = 3308 (m), 2952 (s), 2932 (s), 2858 (m), 2109 (vs), 1731 (vs), 1656 (s), 1630 (s), 1539 (s), 1471 (m), 1462 (m), 1436 (s), 1389 (s), 1376 (s), 1329 (m), 1272 (vs), 1254 (vs), 1204 (vs), 1177 (m), 1115 (s), 1065 (m), 1037 (m), 1012 (s), 966 (m), 837 (vs), 807 (s), 778 (s), 667 cm^{-1}

(m); MS (FAB): *m/z* (%) = 667 (10) $[M+Na]^+$, 645 (100) $[M+H]^+$, 602 (5), 587 (40), 530 (8), 171 (20), 73 (55); HRMS (FAB): *m/z* calcd for $C_{33}H_{53}N_4O_7Si$: 645.3678; found: 645.3654 $[M+H]^+$.

Methyl (10*S*,11*S*)-10-{[*tert*-butyl(dimethyl)silyl]oxy}-6,13,15-trioxo-1,6,7,8,9,10,11,12,13,14,15,16,17,19a,20,21,22,22a-octadecahydrocyclopenta[*m*][1,6]diazacyclohenicosin-11-carboxylate 14: The compound was prepared as described for **5**, from **6** (50 mg, 77.6 μmol), PPh_3 (24 mg, 91.0 μmol), H_2O/THF (30 μL:1 mL), toluene (160 mL), yield 11 mg (25%) and 13 mg (30%) of the diastereomers.

Antimicrobial assays: Antimicrobial activities were determined by agar diffusion tests by using paper discs (6 mm diameter) that had been soaked with a methanolic solution of the test compound (20 μL, 1 mg mL^{-1}). Microorganisms were from the HZI collection and grown on standard medium and seeded into liquid agar medium to a final O.D. of 0.01 (bacteria) or 0.1 (yeasts). Spores of fungi were seeded according to experience. Plates were incubated at 30 °C and the diameter of the resulting inhibition zones were measured after one or two days.

Cell lines and culture conditions: Cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) or American Type Culture Collection (ATCC) and cultivated in the media that was recommended by the supplier at 37 °C and 10% CO_2. L-R118 is a epothilone-resistant and dependent cell line that was selected by adding increasing amounts of epothilone A to the culture medium and was continuously grown in the presence of epothilone A (40 ng mL^{-1}).

Cytotoxicity assay: In most cases MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to measure growth and viability of cells, which were capable of reducing it to a violet formazan product. Serial dilutions of the test compounds (60 μL) were added to aliquots of a cell suspension (120 μL, 50000 mL^{-1}) in 96-well microplates. Blank and solvent controls were incubated under identical conditions. After five days, MTT in phosphate buffered saline (PBS) (20 μL) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h, the precipitate of the formazan crystals was centrifuged, and the supernatant was discarded. The precipitate was washed with PBS (100 μL) and dissolved in *i*PrOH (100 μL) that contained 0.4% HCl. The microplates were gently shaken for 20 min to ensure a complete dissolution of the formazan, and finally measured at 595 nm by using an ELISA plate reader. All experiments were carried out in two parallel experiments. Activity values were calculated as the mean with respect to the controls, which were set to 100%. In the case of U-937 cells, which grow in suspension, we used WST-1 from Roche (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) instead of MTT. WST-1 was reduced to produce a soluble red colour that could directly be measured at 450 nm. To check the influence of Ca^{2+} and Mg^{2+}, a DME medium with a reduced concentration of these divalent cations was prepared (0.17 mM $CaCl_2$, 0.08 mM $MgSO_4$), and $CaCl_2$ and $MgSO_4$ were added as indicated. In this case, L929 cells were seeded at a density of 24000 in a final volume of 200 μL, and incubated for 2 d only.

Cell staining: PtK_2 cells (ATCC CCL-56) were grown in medium (750 μL) in 4-well plates (Nunc) on glass coverslips, and incubated with cylindramide **1** (0.5 μg mL^{-1}). For calcium staining, cells were loaded with Pluronic (16 μM) and Fura-2-AM or Fluo-4-AM (5 μg mL^{-1}; Molecular Probes) after 30 min. For ER staining, cells were fixed with cold (−20 °C) MeOH/acetone (1:1) for 10 min after 18 h, incubated with a primary antibody against GRP-94 (1:1000; Affinity Bioreagents, Golden, CO, USA), and then with a secondary Alexa Fluor 488 goat anti-rat IgG antibody (1 μg mL^{-1}; Molecular Probes), and mounted in ProLong Antifade Gold (Molecular Probes), which included DAPI to stain the nuclei.

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[27] For further results see the Supporting Information.

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