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Synthesis and Biological Evaluation of Cruentaren A Analogues

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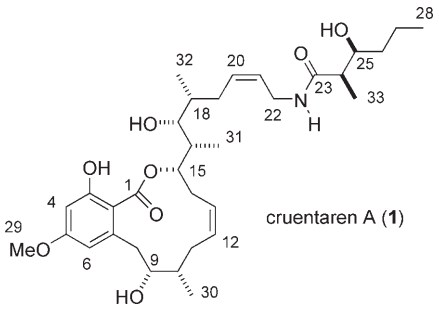
Abstract: The complex macrolide cruentaren A is a highly selective and potent inhibitor of F-ATPase (F-type adenosine triphosphatase). As it shows some resemblance to benzolactone enamides like apicularen A, it was of interest to perform some structure–activity studies to delineate the key functional groups that are responsible for the activity. Building upon our previously developed route to cruentaren A, which is based on a ring-closing alkyne metathesis reaction (RCAM), several cruentaren analogues were prepared. Replacement of the 3-hydroxy hexanoic part with acids that lack the hydroxy group function resulted in a significant drop in cytotoxicity and F-ATPase inhibition. Furthermore, two enamide analogues **23** and **50** were synthesized. However, these compounds were only cytotoxic in the micromolar range. Under the conditions for cleavage of the C3 aromatic methyl ether, the enamide function was transformed to the corresponding oxazinanone, resulting in analogues **25** and **52**.

Keywords: cruentaren · cyclization reactions · enzyme inhibitors · lactones · metathesis · Natural products

Introduction

Recently, Höfle and co-workers described the structure of the macrolide cruentaren A (**1**).[1,2] This unique natural product was isolated form the myxobacterium *Byssovorax cruenta* (Scheme 1). In a cellular assay with the L929 cell line, cruentaren A showed powerful cytotoxicity with an IC_{50} value of 1.2 ng mL^{-1}. Further studies revealed that on a molecular level, cruentaren A inhibits mitochondrial F-ATPase (F-ATPase = F-type adenosine triphosphatase).[3,4] These membrane-bound proteins are crucial for a living cell as they use a proton gradient to power the synthesis of ATP. Key structural features of cruentaren A include a 12-membered macrolactone with a *Z* double bond. The side chain extending from C15 contains a stereotetrad and an (*Z*)-allylamide terminus. Interestingly, cruentaren A does not show much similarity to other polyketide inhibitors of F-ATPase, such as apoptolidin or oligomycin.[5]



Scheme 1. Structure of cruentaren A.

Other natural products with an allylamide include leucascandrolide[6,7] (**2**) (Scheme 2), neopeltolide,[8] callipeltoside[9,10] and ajudazol A (**3**).[11] Except for ajudazol A, which was reported to be an inhibitor of mitochondrial electron transport, the mode of action of the other mentioned compounds still remains unclear. Furthermore, a similarity of cruentaren A to the benzolactone enamides,[12] like apicularen A (**4**) was noted.[2] However, cruentaren A does not inhibit V-ATPase (V-type ATPase),[3,13] the target of the ben-

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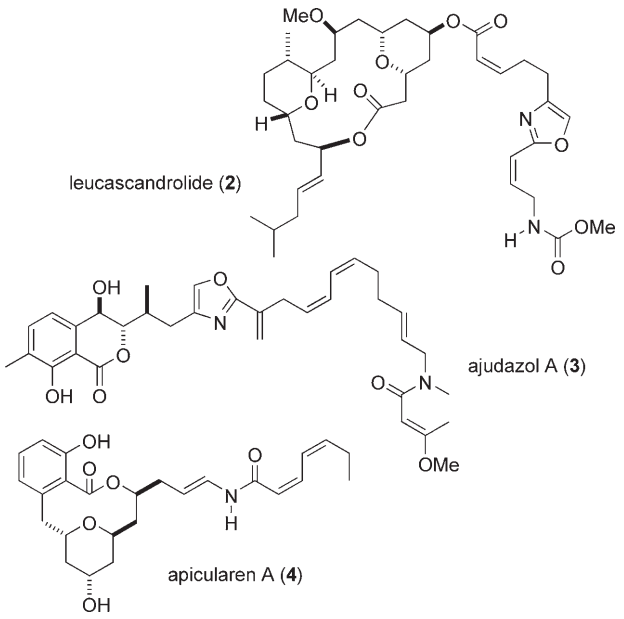


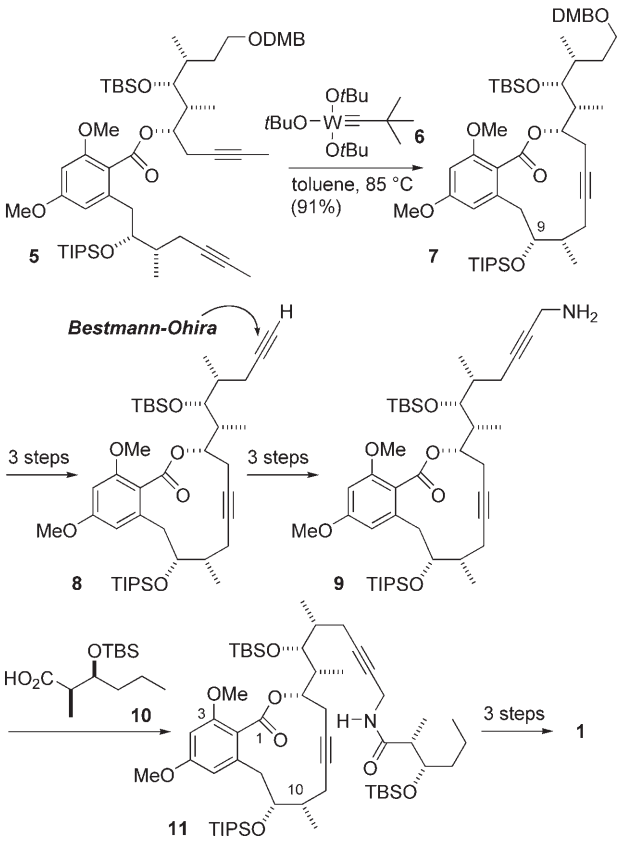
Scheme 2. Structures of some natural products that resemble cruentaren A.

zolactone enamides. Therefore, it would be of interest to identify some key structural elements that are decisive for the biological activity of cruentaren A.

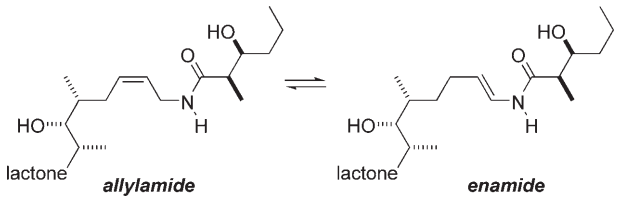
In previous papers, we outlined an efficient synthetic strategy towards cruentaren A.[14,15] The macrolactone ring was formed through a ring-closing alkyne metathesis reaction[16,17] on the ester **5** by using the Schrock catalyst **6** (Scheme 3). To prevent an unwanted translactonization to the six-membered lactone, extension of the side chain was done on the cyclic alkyne **7**. Thus, the aldehyde derived from **7** was converted to the alkyne **8** through the Bestmann–Ohira reaction. Extension of the terminal alkyne with formaldehyde allowed for the formation of the key propargyl amine **9** through the Mitsunobu reaction. Condensation of the amine **9** with the protected 3-hydroxy acid **10** led to the amide **11**. Finally, cleavage of the C3 OMe ether, the silicon protecting groups and Lindlar hydrogenation completed the total synthesis of cruentaren A.[15] Recently, another ring-closing alkyne metathesis (RCAM)-based synthesis of cruentaren A was achieved by Fürstner et al.[18]

With regard to the design of analogues, we wanted to use the available stereotetrad building blocks[14] and stick to the proven RCAM reaction. We intended to answer the following questions: How important is the carboxylic acid part of the amide? How important is the free OH at C3? Can we make enamides instead of (*Z*)-allylamides? Although quite speculative, it could be that the allylamide isomerizes to an enamide that then might form a highly electrophilic acyliminium ion upon protonation (Scheme 4).[19]

Herein, we describe the synthesis together with the biological evaluation of several cruentaren A analogues.



Scheme 3. Key steps in the synthesis of cruentaren A; DMB = 3,4-dimethoxybenzyl, TBS = *tert*-butyldimethylsilyl, TIPS = triisopropylsilyl.

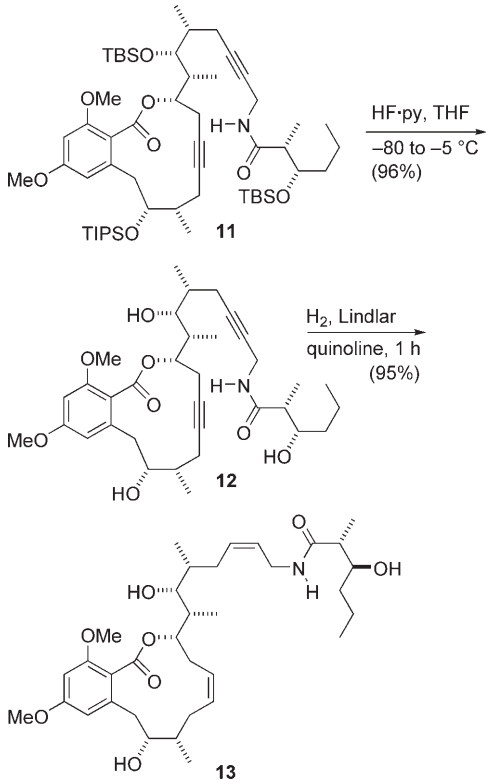


Scheme 4. Possible isomerization of the allyamide to an enamide.

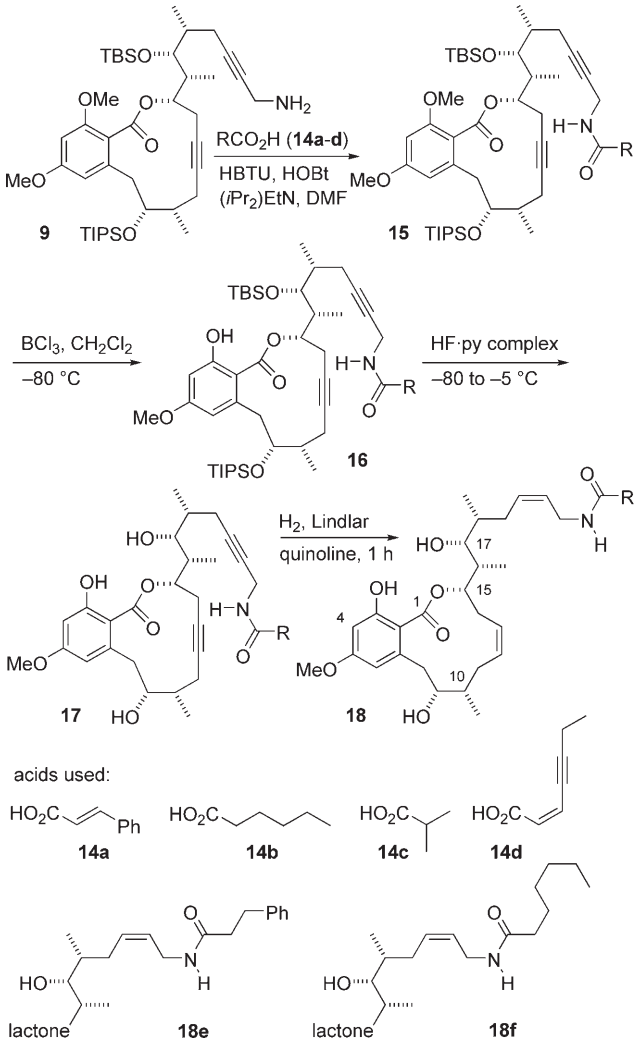
Results and Discussion

Synthesis: We began with the preparation of the 3-*O*-methyl ether of cruentaren. As outlined in Scheme 3, the synthesis of **1** passed through the diyne **11**. Omitting the cleavage of the C3 methyl ether and instead treating the lactone **11** with the HF·pyridine complex led to the triol **12** (Scheme 5). A final Lindlar reduction delivered 3-OMe cruentaren **13**.

Another key intermediate of the total synthesis, the propargyl amine **9**, presented itself for derivatization reactions. Accordingly, the amine **9** was condensed with the acids **14a–d** by using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in the presence of 1-hydroxy-1*H*-benzotriazole (HOBt) and Hünig's base in *N*,*N*-dimethylformamide (DMF; Scheme 6). These acylation reactions proceeded in quite good yields (Table 1). For these derivatives, we chose to generate the original aromatic part



Scheme 5. Synthesis of 3-OMe cruentaren **13** from the amide **11**. Py = pyridine.



Scheme 6. Preparation of various amide analogues of cruentaren A. HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphates.

with the 3-OH group. Selective ether cleavage on the lactones **15a–d** by using boron trichloride furnished the corresponding 3-hydroxy compounds **16a–d**, again in excellent yields. The Lindlar reduction of the diynes **17b** and **17c** proceeded as expected to give the analogues **18b** and **18c**. From the reduction of diyne **17a**, the analogue **18a** was obtained, but we also isolated the dihydro compound **18e**, resulting from hydrogenation of the cinnamoyl double bond. In the case of the hept-2-en-4-ynamide **17d**, only the product **18f** resulting from complete hydrogenation was observed. The internal *Z* double bond survived as in the other amide analogues.

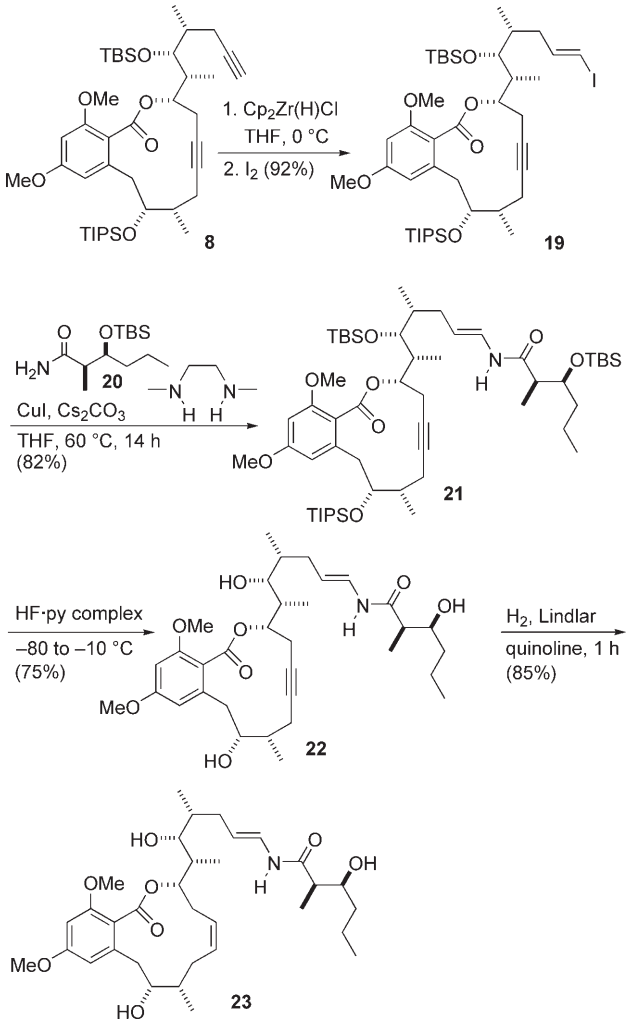
As a further branching point for the synthesis of the analogues, we identified the lactone **8** with a propynyl terminus. We thought that the derived vinyl iodide might be useful for the synthesis of enamide derivatives. With this in mind, diyne **8** was subjected to hydrozirconation with the Schwartz reagent followed by addition of iodine to the intermediate vinylmetal species (Scheme 7).[20] Thereafter, a copper-catalysed cross-coupling reaction of vinyl iodide **19** with the amide **20** under Buchwald conditions[21–26] was performed, resulting in enamide **21** in high yield. Owing to the expected sensitivity of the enamide to harsh acidic conditions, the demethylation step was omitted. Nevertheless, the enamide survived the conditions (HF·pyridine complex) for global deprotection of the silyl ethers. A Lindlar reduction on diyne **22** completed the synthesis of enamide analogue **23**.

Table 1. Yields for the various steps for the synthesis of amide analogues **18** of cruentaren A.

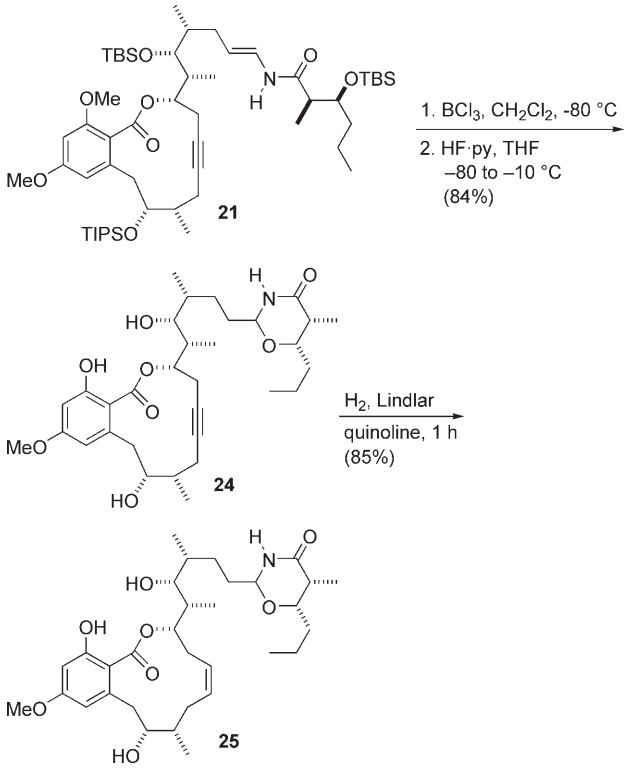
Acid used	Transformation			
	acylation [%]	BCl_3 [%]	HF·py [%]	Lindlar's catalyst [%]
14a	87	92	95	74[a]
14b	88	86	93	93
14c	91	83	85	87
14d	85	89	92	73[b]

[a] By-product dihydro derivative **18e**. [b] Only the saturated heptanoyl derivative **18f** was formed under the Lindlar conditions.

If however, the enamide **21** was treated with boron trichloride to cleave the C3 O-methyl ether, followed by global silyl removal with HF·pyridine complex, a compound (**24**), which lacked the enamide signals in the ^{1}H NMR spectrum was isolated (Scheme 8). According to LC–MS analysis, the mass was the same as expected for the enamide. The



Scheme 7. Preparation of the enamide analogue **23** of cruentaren A.

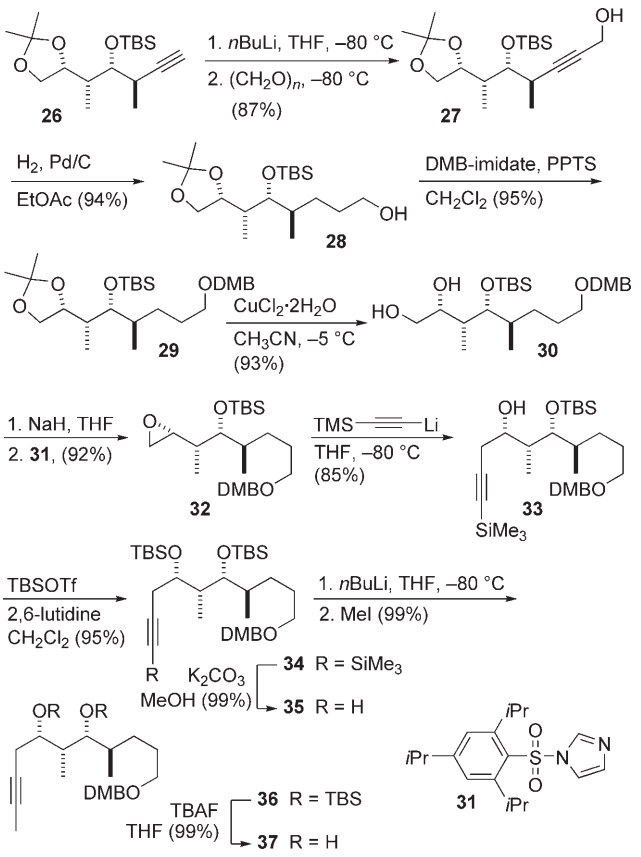


Scheme 8. Formation of the oxazinanone system during methyl ether cleavage on enamide **21** with BCl_3 resulting in analogue **25**.

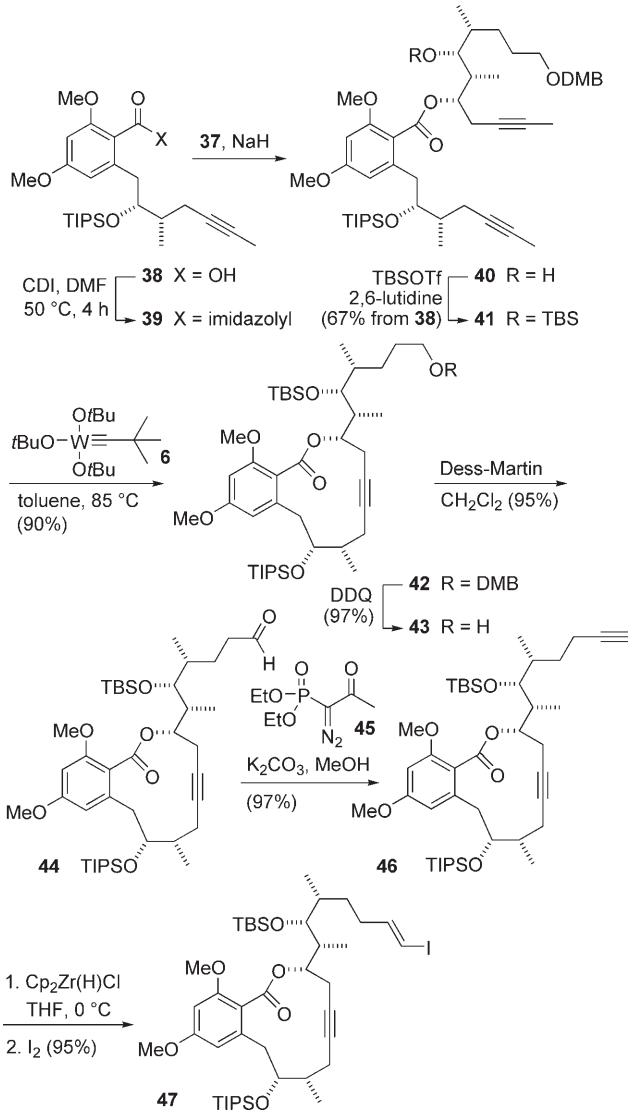
signal at $\delta = 5.02$ ppm in the ^{1}H NMR spectrum pointed to the presence of the 1,3-oxazinan-4-one. The formation of this heterocyclic ring system can easily be explained by the corresponding acyliminium ion. Although we were not able to unambiguously assign the stereochemistry at the aminal carbon, we assume a 2,6-*cis*-configuration (oxazinone-4-numbering). Force-field calculations using Chem3D on 2,5,6-trimethyl-1,3-oxazinan-4-one showed the *cis*-2,6-diastereomer to be 5.65 kJ mol^{-1} more stable than the corresponding 2,6-*trans* isomer. Lindlar reduction of the triple bond led to the oxazinan-4-one analogue **25**. It can be assumed that oxazinanone formation occurs upon treatment of the enamide **21** with BCl_3 as the HF·pyridine complex does not seem to affect the enamide as could be seen with the deprotection of **21** to enamide **22**.

To further probe the potential biological relevance of a cruentaren A enamide, the homologated enamide derivative of analogue **25** was targeted. In this case, we could have used macrolactone **8**, with a propyne terminus, as the starting material, but instead we began with the stereotetrad-containing building block[14] **26** (Scheme 9). This compound, which originated from a Marshall–Tamaru reaction,[14,27] was extended to the propargyl alcohol **27**. A hydrogenation reaction provided the propanol derivative **28**. Protection of the hydroxyl group function with dimethoxybenzyl imidate[28] to give **29** was followed by cleavage of the isopropylidene group by using aqueous copper(II) chloride[29] to provide diol **30**. The 1,2-diol was converted to the epoxide **32** by using the arylsulfonyl derivative **31**.[30] The stage was now set for epoxide opening with lithium trimethylsilylacetylide in the presence of BF_3·OEt_2.[31,32] Silylation of **33** and removal of the acetylenic silyl group from **34** furnished alkyne **35**. In preparation for the RCAM reaction, the terminal alkyne **35** was converted to the inner alkyne **36** by using *n*BuLi followed by MeI. The subsequent treatment of bis-silyl ether **36** with tetrabutylammonium fluoride (TBAF) delivered diol **37**. A possible shortcut from epoxide **32** to the alkyne **36** was attempted by direct opening of the epoxides with propynyllithium. Unfortunately, the major product in this reaction by using propynyllithium prepared *in situ* from 1-bromopropene[33] turned out to be the corresponding bromohydrin.

As we had outlined in the synthesis of the core structure, esterification of benzoic acid **38** with building block **37** was best performed with the diol itself. After conversion of the acid to the carbonylimidazolide **39**, esterification with the sodium alcoholate of **37** went smoothly and in a regioselective manner (Scheme 10).[34] Silylation of the free hydroxy group function of **40** gave rise to the ester **41**, the substrate



Scheme 9. Synthesis of the alkynediol **37** from terminal alkyne **26**. CDI = *N,N'*-carbonyl diimidazole, PPTS = *p*-toluenesulfonate.



Scheme 10. Synthesis of the macrolactone **47** featuring a 4-iodobutenyl side chain. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

for the alkyne metathesis reaction. Based on the diol **37**, the yield for the ester **41** amounted to 67%. The RCAM[16,35] reaction of **41** with the Schrock catalyst[36] **6** proceeded in excellent chemical yield, furnishing lactone **42**. To set up an (*E*)-vinyl iodide at the side-chain terminus, the DMB protecting group was removed under oxidative conditions.[37] The resulting primary alcohol **43** was oxidized to the aldehyde **44**. Extension of the aldehyde **44** to the alkyne could be accomplished with the Bestmann–Ohira reagent[38] **45** in the presence of K_2CO_3. Finally, hydrozirconation and iodination provided the vinyl iodide **47**.

As outlined before, a cross-coupling reaction of vinyl iodide **47** with the amide **20** was used to set up the enamide functionality (Scheme 11). Global silicon ether cleavage on **48** and Lindlar reduction of the triple bond furnished analogue **50**.

If the enamide **48** was treated with BCl_3, cleavage of the C3 OMe ether was accompanied by the formation of the oxazinan-4-one **51** (Scheme 12). The analogue **52** was obtained through silyl group removal and the Lindlar reduction. Characteristic peaks for the oxazinanone part of **52** are as follows: $\delta = 4.71$ (2-H; 22-H), 6.40 ppm (N-H); ^{13}C NMR: $\delta = 83.8$ (C2; C22), 174.6 (C4; C23), 38.8 (C5; C24), 72.9 (C6; C25) ppm.

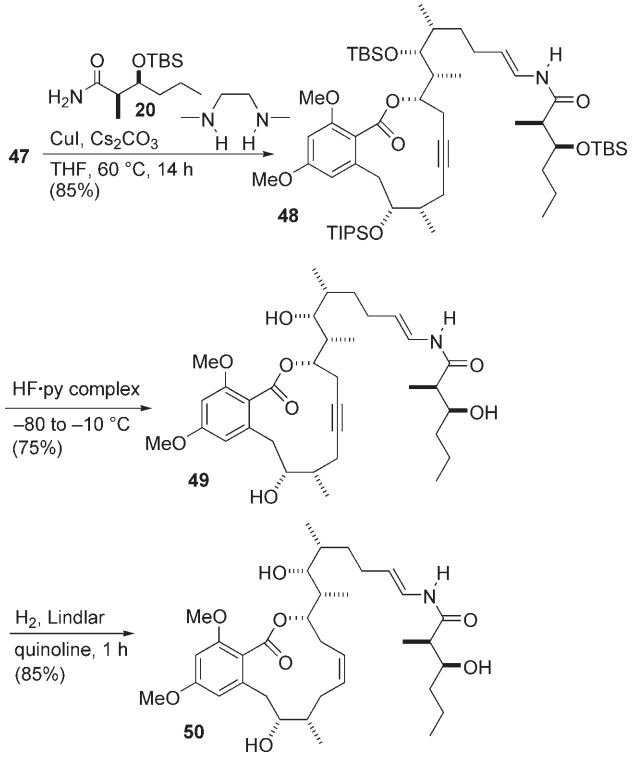
The formation of 1,3-oxazinan-4-ones from enamides containing a β-hydroxy acid seems to be unprecedented. Similar oxazinanones have been generated by condensation of aromatic aldehydes with alicyclic 2-hydroxy-1-carboxamides.[39] For such oxazinanone derivatives, a 2,6-*cis*-configuration was observed. The synthesis for 5-phenylthio-1,3-oxazinan-4-ones is based on the hetero Diels–Alder reaction between an azadiene and an aldehyde.[40] Other 1,3-oxazinanones are known as well.[41,42,43]

Biological testing: The described analogues as well as the diyne **53** were tested for cytotoxicity against the L929 cell line and the inhibitory efficacy on F-ATPase in mitochondrial preparations of bovine heart. The obtained IC_{50} values in the cell-culture assay as well as the percentage of F-ATPase inhibition of the compounds at a concentration of 0.1 and 1.0 μM, respectively, are listed in Table 2. The analogues are ordered according to increasing IC_{50} values against the L929 cell line.

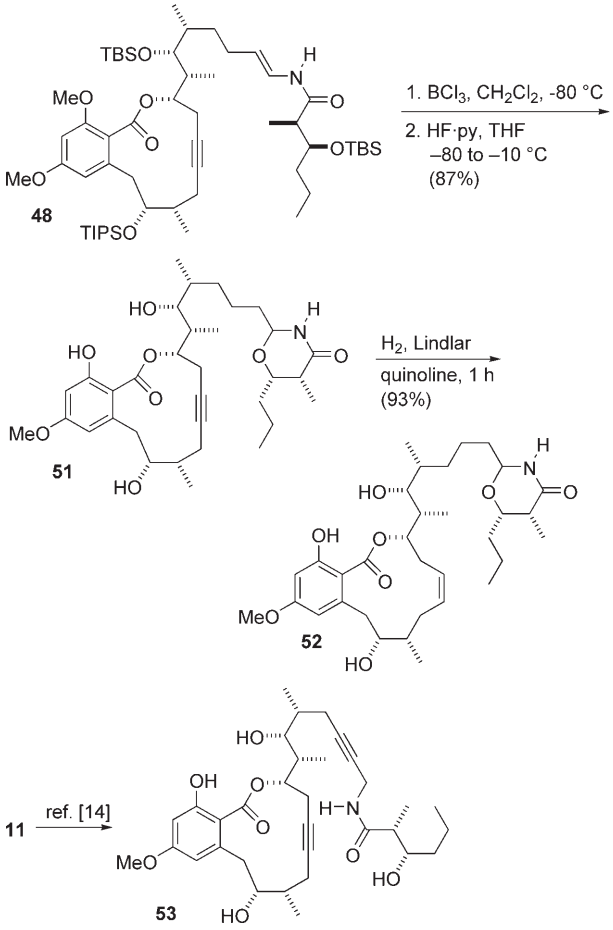
Table 2. Biological activity of cruentaren A (cru) and the analogues.

Entry	Compound	IC_{50} [$\mu g\,mL^{-1}$]	IC_{50} [μM]	Inhibition of F-ATPase activity [%][a] 1 μM	0.1 μM	Description
1	**1**	0.00042 ± 0.00005	0.00071 ± 0.00008	94	78	cru (synthetic)[b]
2	**13**	0.017 ± 0.004	0.028 ± 0.007	80	42	3-OMe-cru
3	**52**	0.085 ± 0.02	0.14 ± 0.03	34	2	7C-oxazinanone-cru
4	**18c**	0.3 ± 0.01	0.56 ± 0.02	44	30	isobutanoyl-cru
5	**18e**	2.4 ± 0.1	4.0 ± 0.2	47	32	dihydro-cinnamoyl-cru
6	**18b**	2.5 ± 0.1	4.5 ± 0.2	47	27	hexanoyl-cru
7	**18f**	2.9 ± 0.3	5.0 ± 0.5	67	48	heptanoyl-cru
8	**50**	3.0 ± 0.4	5.0 ± 0.7	47	8	7C-enamide-cru
9	**23**	3.0 ± 1.1	5.1 ± 1.9	51	15	6C-enamide-cru
10	**18a**	6.1 ± 0.7	10.3 ± 1.2	40	30	cinnamoyl-cru
11	**53**	6.5 ± 0.4	11.1 ± 0.7	21	10	diyne-cru
12	**25**	7.5 ± 0.9	13.0 ± 1.6	18	12	6C-oxazinanone-cru

[a] The inhibition values are the mean values of at least two independent assays. The deviations did not exceed a range of ±10% inhibition. [b] The natural cruentaren A displayed a slightly lower activity (IC_{50} = (0.002 ± 0.0019) μM, F-ATPase inhibition = 93% at 1 μM). This might be attributed to different purity values. The value for the synthetic product does lie within the deviation of the natural material.



Scheme 11. Synthesis of the enamide analogue **50**.



Scheme 12. Synthesis of the oxazinan-4-one analogue **52** and the diyne cruentaren **53**.

As can be seen in Table 2, there are some highly effective compounds. In most cases, cytotoxicity and inhibitory activity against F-ATPase in vitro run parallel. However, there are some exceptions in this regard. For example, **18c** and the three analogues **18e**, **18b** and **18f** differ in their cellular activity by a factor of almost 10, but display similar effects on the F-ATPase. This might be explained by differences in cellular uptake. The highest effective compound is cruentaren A itself (Table 2, entry 1), followed by 3-OMe cruentaren (**13**) (Table 2, entry 2). Furthermore, the oxazinanone derivative with a side chain of seven carbons can be considered as highly cytotoxic, but it showed only low inhibitory efficacy in the F-ATPase assay (seven-carbon, entry 3). Then there are compounds of intermediate cytotoxicity and F-ATPase inhibition, namely the cruentaren derivatives with a modified carboxylic part in the side chain (**18e**, **18b**, **18f**; Table 2, entries 5–7). In particular **18c** and **18b** make clear that the OH group of the carboxylic acid part is extremely important. Finally, there are compounds that are essentially nontoxic, starting with compound **18a**. Surprisingly, both enamides show neither a high cytotoxicity, nor significant inhibition of F-ATPase. One hypothesis in the design of the enamide analogues was that with a structural resemblance to typical V-

ATPase inhibitors like apicularen A or salicylihalamide A, these analogues would show corresponding activity. As it could be assumed that a V-ATPase inhibitor would be highly cytotoxic, this shows that the enamide side is not sufficient to convert the F-ATPase inhibitor cruentaren A into a V-ATPase inhibitor. The most puzzling observation is the relatively high cytotoxicity of the oxazinanone **52**, which shows only low inhibition of F-ATPase. We also checked for inhibitory effects on V-ATPase with PtK_2 potoroo cells. However, when we investigated treated cells by fluorescent techniques, we did not observe the characteristic changes in the endoplasmatic reticulum that are typical for V-ATPase inhibitors. One explanation for the cytotoxicity of **52** could be that the heterocyclic ring is opened to an electrophilic acyliminium ion when taken up by the cells. The lack of activity for diyne cruentaren **53** can be attributed to conformational effects.

Conclusion

By using the RCAM strategy that led to the total synthesis of cruentaren A (**1**), a range of cruentaren analogues were prepared. Replacing the 3-hydroxy-hexanoic acid gave analogues **18a**, **18b**, **18c**, **18e**, **18f**, however, with the exception of the truncated isobutanoyl analogue **18c**, none of the analogues were highly active. Furthermore, the two enamide analogues **23** and **50** were prepared via cross-coupling (amination) of the corresponding vinyl iodides **19** and **47**, respectively. As the enamides did not survive the conditions (BCl_3) of the cleavage of the aromatic methyl ether, we prepared the 3-OMe derivatives. Even though this methyl ether is important (see **1** and **13**), the complete lack of activity for the two enamides **23** and **50** is somewhat surprising. Upon cleavage of the 3-OMe ether with BCl_3, the enamide function of **21** and **49** reacted with the hydroxyl group function of the carboxylic acid to give an unusual oxazinanone heterocycle. Among the two oxazinanone analogues, compound **52**, which might be a metabolite of **1**, was quite active and showed an IC_{50} value of 140 nM. This work also constitutes a novel synthesis of 1,3-oxazinan-4-ones from enamides.

Experimental Section

General details are included in the Supporting Information. The experimental details for Scheme 5, part of Scheme 6, Scheme 8 and Scheme 9 are covered in the Supporting Information as well. The pH 7 buffer was prepared by dissolving KH_2PO_4 (85 g, 0.625 mol) and NaOH (14.5 g, 0.3625 mol) in water (1 L).

Cinnamic acid amide (15a): (*E*)-cinnamic acid (6.4 mg, 0.043 mmol, 1.6 equiv), HBTU (20.5 mg, 0.054 mmol, 2 equiv), HOBt (7.3 mg, 0.054 mmol, 2 equiv), and N,N-diisopropylethylamine (48 μL, 0.27 mmol, 10 equiv) were added to a solution of amine[15] **9** (20 mg, 0.027 mmol, 1 equiv) in dry DMF (2 mL). After the mixture was stirred at room temperature for 4 h, H_2O (5 mL) was added and the obtained emulsion was extracted with Et_2O (3×15 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc, 10:1→4:1) to give 20.5 mg (87%) of amide **15a** as a colourless amorphous solid. TLC (petroleum ether/EtOAc, 4:1): $R_f = 0.39$; $[\alpha]_D^{20} = -13.1$ ($c = 1.4$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.04$ (s, 3H, $Si(CH_3)_2$), 0.06 (s, 3H, $Si(CH_3)_2$), 0.87–1.02 (m, 39H, 16-CH_3, 18-CH_3, 10-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.72–1.83 (m, 1H, 16-H), 1.91–2.11 (m, 3H, 19-H, 18-H), 2.12–2.20 (m, 1H, 11-H), 2.22–2.30 (m, 1H, 10-H), 2.33–2.55 (m, 4H, 8-H, 14-H, 11-H), 3.60–3.65 (m, 1H, 17-H), 3.73 (s, 3H, OCH_3), 3.75–3.83 (m, 4H, OCH_3, 8-H), 4.00–4.18 (m, 3H, 9-H, 22-H), 5.47–5.59 (m, 1H, 15-H), 6.02 (br s, 1H, NH), 6.31 (d, $J = 2.0$ Hz, 1H, 6-H), 6.40–6.46 (m, 2H, 4-H, 25-H), 7.29–7.34 (m, 3H, *o*-, *p*-CH of Ph), 7.41–7.47 (m, 2H, *m*-CH of Ph), 7.62 ppm (d, $J = 15.7$ Hz, 1H, 24-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -3.9$ ($Si(CH_3)_2$), 11.4 (16-CH_3), 13.0 ($CH(CH_3)_2$), 17.2 (10-CH_3), 17.9 ($CH(CH_3)_2$), 18.1 ($CH(CH_3)_2$), 18.4 (18-CH_3), 21.7 (C19), 23.1 (C14), 23.7 (C11), 26.1 ($Si(C(CH_3)_3)$), 30.0 (C22), 37.2 (C18), 37.3 (C16), 38.6 (C8), 40.3 (C10), 55.2 (OCH_3), 55.7 (OCH_3), 75.0 (C17), 76.6 (C9), 77.2 (C15), 79.6 (C≡C), 81.7 (C≡C), 83.2 (C≡C), 96.6 (C4), 108.2 (C6), 118.0 (C2), 120.2 (C24), 127.8 (C4′), 128.7 (C3′), 129.6 (C2′), 134.8 (C1′), 139.4 (C7), 141.2 (C25), 157.4 (C5), 160.4 (C3), 165.4 (C1), 167.6 ppm (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{51}H_{77}NNaO_7Si_2$ 894.51308, found 894.51309.

2-Hydroxy-4-methoxybenzoate (16a): A solution of amide **15a** (18 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) was treated with BCl_3 (80 μL, 1.0 M in CH_2Cl_2, 0.08 mmol, 4 equiv) at −80 °C. The reaction was stirred for 2 h at −80 °C before a saturated solution of NaOAc (3 mL) was added. After separation of the layers, the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed with H_2O followed by saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (petroleum ether/EtOAc, 4:1) afforded phenol **16a** (15.8 mg, 92%) as a slightly yellow oil. TLC (petroleum ether/EtOAc, 4:1): $R_f = 0.4$; $[\alpha]_D^{20} = +17.0$ ($c = 0.7$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.05$–0.08 (m, 6H, $Si(CH_3)_2$), 0.85–1.05 (m, 39H, 16-CH_3, 18-CH_3, 10-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.67–1.91 (m, 2H, 16-H, 19-H), 1.94–2.20 (m, 4H, 19-H, 18-H, 11-H), 2.23–2.35 (m, 2H, 10-H, 14-H), 2.44–2.52 (m, 1H, 8-H), 2.55–2.67 (m, 1H, 14-H), 2.85–2.97 (m, 1H, 8-H), 3.56–3.62 (m, 1H, 17-H), 3.77 (s, 3H, OCH_3), 4.10–4.15 (m, 2H, 22-H), 4.17–4.25 (m, 1H, 9-H), 5.19–5.28 (m, 1H, 15-H), 5.81 (br s, 1H, NH), 6.33–6.42 (m, 3H, 6-H, 4-H, 25-H), 7.32–7.39 (m, 3H, *o*-, *p*-CH of Ph), 7.47–7.53 (m, 2H, *m*-CH of Ph), 7.64 (d, $J = 15.7$ Hz, 1H, 24-H), 11.22 ppm (br s, 1H, 3-OH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.0$ ($Si(CH_3)_2$), 11.1 (16-CH_3), 13.0 ($CH(CH_3)_2$), 16.6 (10-CH_3), 18.2 ($CH(CH_3)_2$), 18.2 ($CH(CH_3)_2$), 18.4 (18-CH_3), 22.1 (C19), 22.7 (C14), 26.0 ($Si(C(CH_3)_3)$), 30.0 (C22), 36.7 (C18), 37.4 (C16), 55.2 (OCH_3), 74.6 (C17), 75.6 (C9), 76.9 (C≡C), 77.2 (C15), 82.7 (C≡C), 83.2 (C≡C), 98.9 (C4), 104.2 (C6), 119.7 (C2), 120.0 (C24), 127.8 (C4′), 128.8 (C3′), 129.8 (C2′), 134.7 (C1′), 141.6 (C7), 143.3 (C25), 163.4 (C3), 164.6 (C5), 165.3 (C1), 171.1 ppm (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{50}H_{75}NO_7Si_2Na$ 880.49743, found 880.49810.

Deprotected macrolactone (17a): HF·pyridine complex (70% HF, 0.3 mL) was added dropwise to a stirred solution of the phenol **16a** (14 mg, 0.016 mmol) in THF (0.4 mL, in a plastic test tube) at −80 °C. The reaction mixture was allowed to warm to −5 °C. After 2 h, the mixture was partioned between an ice-cooled mixture of EtOAc (20 mL) and a saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated and the H_2O layer extracted with EtOAc (2×20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give 9.0 mg (95%) of triol **17a**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.56$; $[\alpha]_D^{20} = +11.7$ ($c = 0.8$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.91$–0.96 (m, 6H, 16-CH_3, 18-CH_3), 1.02 (d, $J = 6.8$ Hz, 3H, 10-CH_3), 1.74–1.85 (m, 2H, 16-H, 18-H), 2.00–2.10 (m, 2H, 19-H, 18-H), 2.15–2.27 (m, 4H, 19-H, 11-H, OH), 2.28–2.37 (m, 2H, 11-H, 8-H), 2.38–2.46 (m, 1H), 2.57–2.65 (m, 1H, 14-H), 2.77–2.85 (m, 1H, 14-H), 2.88–2.98 (m, 1H, 10-H), 3.63–3.69 (m, 1H, 17-H), 3.72–3.76 (m, 1H, 8-H), 3.91–3.99 (m, 1H, 9-H), 4.11–4.16 (m, 2H, 22-H), 5.32–5.39 (m, 1H, 15-H), 5.97 (br s, 1H, NH), 6.34–6.43 (m, 3H, 6-H, 4-H, 25-H), 7.33–7.40 (m, 3H, *o*-, *p*-CH of Ph), 7.46–7.52 (m, 2H, *m*-CH of Ph), 7.63 (d, $J = 15.7$ Hz, 1H, 24-H), 11.00 ppm (br s, 1H, 3-OH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 8.5$ (16-CH_3), 14.1 (24-CH_3), 16.2 (18-CH_3), 16.5 (10-CH_3), 21.1 (C19), 22.5 (C14), 23.0 (C11), 29.9 (C22), 35.7 (C26), 36.7

(C18), 37.3 (C16), 38.4 (C8), 55.4 (OCH_3), 73.8 (C17), 75.3 (C9), 77.2 (C15), 77.6 (*C*≡C), 79.3 (*C*≡C), 81.8 (*C*≡C), 83.3 (*C*≡C), 99.5 (C4), 106.5 (C2), 111.5 (C6), 120.0 (C24), 127.8 (C4′), 128.8 (C3′), 129.8 (C2′), 134.7 (C1′), 141.7 (C7), 143.2 (C25), 163.7 (C5), 164.5 (C3), 165.5 (C1), 170.7 ppm (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{35}H_{41}NaNO_7$ 610.27752, found 610.27802.

Cinnamoyl cruentaren (18a) and diyhdrocinnamoyl cruentaren (18e): Lindlar's catalyst (5 wt% Pd on $CaCO_3$, poisoned with lead, 4.2 mg, 100 wt%) was added to a stirred solution of diyne **17a** (4.2 mg, 0.007 mmol) in EtOAc (2 mL) containing quinoline (1.5 mg, 0.01 mmol). The reaction was placed under H_2 atmosphere and stirred for 1 h. The mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH, 95:5→9:1) afforded cinnamide **18a** (3.1 mg, 74%) and phenylpropionamide **18e** (1.0 mg, 24%).

18a: TLC (CH_2Cl_2/MeOH, 9:1): $R_f=0.64$; $[\alpha]_D^{20}=+8.3$ ($c=0.3$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.80$ (d, $J=6.8$ Hz, 3H, 18-CH_3), 0.92 (d, $J=7.1$ Hz, 3H, 16-CH_3), 0.97 (d, $J=6.8$ Hz, 3H, 10-CH_3), 1.70–1.79 (m, 2H, 18-H, OH), 1.93–2.06 (m, 3H, 10-H, 11-H, 16-H), 2.16–2.26 (m, 2H, 14-H, 19-H), 2.28–2.40 (m, 3H, 11-H, 8-H, 19-H), 2.79–2.96 (m, 1H, 14-H), 3.51 (dd, $J=9.4$ Hz, 1.8 Hz, 1H, 17-H), 3.61–3.67 (m, 1H, 9-H), 3.72–3.74 (m, 1H, 8-H), 3.75–3.80 (m, 4H, OCH_3, OH), 3.87–3.95 (m, 1H, 22-H), 4.05–4.14 (m, 1H, 22-H), 5.27–5.35 (m, 1H, 15-H), 5.42–5.52 (m, 3H, 21-H, 12-H, 13-H), 5.53–5.63 (m, 1H, 20-H), 6.02 (br s, 1H, NH), 6.30 (d, $J=2.6$ Hz, 1H, 6-H), 6.35–6.41 (m, 2H, 4-H, 25-H), 7.32–7.39 (m, 3H, *o*-, *p*-CH of Ph), 7.46–7.51 (m, 2H, *m*-CH of Ph), 7.62 (d, $J=15.7$ Hz, 1H, 24-H), 11.50 ppm (br s, 1H, 3-OH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{35}H_{45}NaNO_7$ 614.30882, found 614.30923.

18e: TLC (CH_2Cl_2/MeOH, 9:1): $R_f=0.58$; $[\alpha]_D^{20}=+6.4$ ($c=0.1$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.77$ (d, $J=6.8$ Hz, 3H, 18-CH_3), 0.90 (d, $J=7.1$ Hz, 3H, 16-CH_3), 1.00 (d, $J=6.6$ Hz, 3H, 10-CH_3), 1.63–1.77 (m, 1H, 18-H), 1.90–2.50 (m, 12H, 10-H, 11-H, 16-H, 14-H, 19-H, 8-H, 25-H, OH), 2.78–2.90 (m, 1H, 14-H), 2.91–2.99 (m, 2H, 24-H), 3.43–3.50 (m, 1H, 17-H), 3.61–3.67 (m, 1H, 9-H), 3.72–3.82 (m, 5H, 8-H, 22-H, OCH_3), 3.85–3.97 (m, 1H, 22-H), 5.25–5.36 (m, 2H, 15-H, 21-H), 5.40–5.59 (m, 3H, 12-H, 13-H, 20-H), 5.7 (br s, 1H, NH), 6.28–6.32 (m, 1H, 6-H), 6.34–6.39 (m, 1H, 4-H), 7.15–7.22 (m, 3H, *m*-, *p*-CH of Ph), 7.24–7.31 (m, 2H, *o*-CH of Ph), 11.50 ppm (br s, 1H, 3-OH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{33}H_{47}NO_8Na$ 616.32447, found 616.32467.

(*E*)-Vinyl iodide (19): $[Cp_2Zr(H)Cl]$ (31 mg, 0.12 mmol) was added to a solution of alkyne[15] **8** (44 mg, 0.06 mmol) in THF (1.5 mL) at 0°C and the resulting mixture was stirred for 2 h at 0°C. A solution of I_2 (0.24 mL, 0.5 M in THF, 0.12 mmol) was then added dropwise and stirring was continued for 2 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (5 mL). The mixture was repeatedly extracted with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and evaporated, and the residue was purified by flash chromatography (petroleum ether/EtOAc, 4:1) to give (*E*)-vinyl iodide **19** as an amorphous solid (52 mg, 92%), which was used directly in the next step. TLC (petroleum ether/EtOAc, 4:1): $R_f=0.74$.

Enamide 21: A Schlenk tube was charged with CuI (10.5 mg, 0.055 mmol, 1 equiv), amide[44] **20** (28.5 mg, 0.11 mmol, 2 equiv) and Cs_2CO_3 (46 mg, 0.14 mmol, 2.5 equiv). The tube was evacuated and backfilled with argon. *N*,*N*′-Dimethylethylenediamine (12.0 μL, 0.11 mmol, 2 equiv), vinyl iodide **19** (52 mg, 0.055 mmol) and THF (1.0 mL) were added under argon. The Schlenk tube was closed and immersed in an oil bath, which was preheated to 60°C. The mixture was stirred for 14 h. After the resulting pale-blue suspension was allowed to reach room temperature, ethyl acetate (5 mL) was added. The reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/EtOAc, 10:1→4:1) to give enamide **21** as an amorphous solid (44 mg, 82%). TLC (petroleum ether/EtOAc, 4:1): $R_f=0.66$; $[\alpha]_D^{20}=-23.5$ ($c=1.6$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.03$–0.07 (m, 6H, $Si(CH_3)_2$), 0.08–0.11 (m, 6H, $Si(CH_3)_2$), 0.84–0.98 (m, 48H, 24-CH_3, 27-H, 16-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.02–1.10 (m, 6H, 18-CH_3, 10-CH_3), 1.16–1.26 (m, 1H, 26-H), 1.31–1.50 (m, 3H, 25-H, 26-H), 1.65–1.75 (m, 2H, 18-H, 16-H), 1.76–1.84 (m, 1H, 11-H), 1.90–1.99 (m, 1H, 10-H), 2.10–2.27 (m, 2H, 19-H), 2.35–2.54 (m, 5H, 8-H, 14-H, 23-H, 11-H), 3.45–3.49 (m, 1H, 17-H), 3.72–3.78 (m, 8H, OCH_3, 24-H, 8-H), 3.97–4.03 (m, 1H, 9-H), 4.92–5.01 (m, 1H, 20-H), 5.38–5.51 (m, 1H, 15-H), 6.31 (d, $J=2.0$ Hz, 1H, 6-H), 6.41 (d, $J=2.0$ Hz, 1H, 4-H), 6.73 (dd, $J=14.0$ Hz, 10.6 Hz, 1H, 21-H), 8.08 ppm (br d, $J=10.6$ Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ −4.6 ($Si(CH_3)_2$), −4.6 ($Si(CH_3)_2$), −3.8 ($Si(CH_3)_2$), −3.7 ($Si(CH_3)_2$), 11.3 (16-CH_3), 12.4 (23-CH_3), 13.0 ($CH(CH_3)_2$), 14.1 (C27), 16.7 (10-CH_3), 17.9 ($CH(CH_3)_2$), 17.9 ($Si(C(CH_3)_3)$), 18.1 ($CH(CH_3)_2$), 18.4 (18-CH_3), 19.4 (C26), 23.3 (C14), 23.7 (C11), 25.9 ($Si(C(CH_3)_3)$), 26.1 ($Si(C(CH_3)_3)$), 32.6 (C19), 34.7 (C25), 37.6 (C18), 38.1 (C16), 38.6 (C8), 40.7 (C10), 45.6 (C23), 55.2 (OCH_3), 55.7 (OCH_3), 74.9 (C17), 76.2 (C9), 77.2 (C24), 79.7 (*C*≡C), 81.2 (*C*≡C), 96.6 (C4), 108.3 (C6), 110.9 (C20), 118.2 (C2), 123.4 (C21), 139.4 (C7), 157.2 (C5), 160.2 (C3), 167.4 (C1), 170.9 ppm (C22); HRMS (ESI): $[M+Na]^+$ calcd for $C_{54}H_{97}NaNO_8Si_3$ 994.64142, found 994.64053.

Deprotected enamide macrolactone (22): The HF·pyridine complex (70% HF, 0.3 mL) was added dropwise to a stirred solution of the enamide **21** (10 mg, 0.01 mmol) in THF (0.4 mL, in a plastic test tube) at −80°C. The reaction mixture was allowed to warm to −10°C. After 2 h, the mixture was partioned between an ice-cooled mixture of EtOAc (20 mL) and saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated and the H_2O layer extracted with EtOAc (2× 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give triol **22** (4.4 mg; 75%). TLC (CH_2Cl_2/MeOH, 9:1): $R_f=0.47$; $[\alpha]_D^{20}=-3.3$ ($c=0.4$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.81$ (d, $J=6.8$ Hz, 3H, 18-CH_3), 0.90–0.98 (m, 6H, 27-H, 10-CH_3), 1.10 (d, $J=7.1$ Hz, 3H, 16-CH_3), 1.16 (d, $J=7.3$ Hz, 3H, 23-CH_3), 1.28–1.39 (m, 2H, 25-H, 26-H), 1.41–1.51 (m, 2H, 25-H, 26-H), 1.58–1.67 (m, 2H, 19-H, OH), 1.90–2.02 (m, 4H, 18-H, 19-H, 11-H, OH), 2.05–2.14 (m, 1H, 16-H), 2.29–2.40 (m, 2H, 8-H, 10-H), 2.46–2.63 (m, 3H, 14-H, 11-H), 2.71–2.79 (m, 1H, 23-H), 2.85 (br s, 1H, OH), 3.29–3.26 (m, 1H, 8-H), 3.45–3.49 (m, 1H, 17-H), 3.78 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.83–3.90 (m, 2H, 9-H, 24-H), 5.10–5.19 (m, 1H, 20-H), 5.46–5.52 (m, 1H, 15-H), 6.35 (d, $J=2.0$ Hz,1H, 6-H), 6.41 (d, $J=$ 2.0 Hz,1H, 4-H), 6.75 (dd, $J=14.2$, 10.4 Hz, 1H, 21-H), 7.52 ppm (d, $J=$ 10.4 Hz, 1H, NH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{33}H_{49}NaNO_8$ 610.33504, found 610.33433.

Enamide analogue (23): Lindlar's catalyst (5 wt% Pd on $CaCO_3$, poisoned with lead, 4.0 mg, 100 wt%) was added to a stirred solution of diyne **22** (4.0 mg, 0.007 mmol) in EtOAc (2 mL) containing quinoline (1.4 mg, 0.011 mmol). The reaction was placed under H_2 atmosphere and stirred for 1 h. The reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give enamide **23** as a colourless oil (3.4 mg, 85%). TLC (CH_2Cl_2/MeOH, 9:1): $R_f=0.52$; $[\alpha]_D^{20}=$ −4.3 ($c=0.3$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.83$ (d, $J=$ 6.8 Hz, 3H, 18-CH_3), 0.93 (t, $J=6.8$ Hz, 3H, 27-H), 0.98 (d, $J=6.8$ Hz, 3H, 16-CH_3), 1.05 (d, $J=6.8$ Hz, 3H, 10-CH_3), 1.16 (d, $J=7.3$ Hz, 3H, 23-CH_3), 1.28–1.40 (m, 2H, 25-H, 26-H), 1.42–1.71 (m, 4H, 25-H, 26-H, 19-H, OH), 1.88–1.99 (m, 4H, 18-H, 19-H, 11-H, 14-H), 2.07–2.21 (m, 1H, 16-H), 2.27–2.37 (m, 2H, 8-H, 10-H), 2.37–2.50 (m, 1H, 11-H), 2.70–3.00 (m, 4H, 23-H, 14-H, 8-H, OH), 3.45–3.52 (m, 1H, 17-H), 3.71–3.81 (m, 7H, OCH_3, 24-H), 3.85–3.91 (m, 1H, 9-H), 5.09–5.19 (m, 1H, 20-H), 5.40 (dd, $J=9.9$, 4.0 Hz, 1H, 15-H), 5.48–5.54 (m, 2H, 12-H, 13-H), 6.33–6.36 (m, 2H, 6-H, 4-H), 6.74 (dd, $J=14.2$, 10.4 Hz, 1H, 21-H), 7.52 ppm (d, $J=10.4$ Hz, 1H, NH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{33}H_{51}NaNO_8$ 612.35069, found 612.35104.

(1*S*)-1-{(1*R*,2*R*,3*R*)-2-{[*tert*-Butyl(dimethyl)silyl]oxy}-6-[(3,4-dimethoxybenzyl)oxy]-1,3-dimethylhexyl}pent-3-ynyl 2,4-dimethoxy-6-{(2*R*,3*S*)-3-methyl-2-[(triisopropylsilyl)oxy]hept-5-ynyl}benzoate (41): A solution of diol **37** (470 mg, 1.24 mmol) in anhydrous DMF (2.5 mL) was stirred in the presence of sodium hydride (60% wt in mineral oil, 124 mg, 3.1 mmol, 2.5 equiv) at 0°C for 10 min and for a further 1 h at room temperature. CDI (390 mg, 2.4 mmol) was added to a solution of acid **38** (917 mg, 2.0 mmol) in anhydrous DMF (3.5 mL) in a separate flask and the reaction mixture was then allowed to stir for 4 h at 50°C. Then, the solution of the imidazolide derivative **39** (analysed by LC–MS) was

cooled to 0°C and added to the above solution of the disodium salt of diol **37** at 0°C. The mixture was allowed to warm to room temperature and stirred for three days. After the addition of saturated NH_4Cl solution, the mixture was extracted with EtOAc (3×30 mL). The combined organic extracts were washed with 1M HCl, saturated $NaHCO_3$ and saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated in vacuo to provide 1.01 g of crude hydroxyester **40**, which was used in the next step without further purification.

A solution of the crude hydroxyester **40** (1.01 g, 1.23 mmol) in CH_2Cl_2 (10 mL) was cooled to −50°C, then 2,6-lutidine (0.58 mL, 4.9 mmol) followed by *tert*-butyldimethylsilyltriflate (TBSOTf; 0.46 mL, 2.0 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred for 2 h before it was treated with water. After separation of the layers, the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with 1M HCl, saturated $NaHCO_3$ and saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (petroleum ether/EtOAc, 10:1→4:1) afforded ester **41** (0.778 g, 67% for 2 steps, based on diol **37**) as a colourless oil. Besides ester **41**, some unreacted imidazolide derivative **39** (295 mg, 30%) was isolated. TLC (petroleum ether/EtOAc, 4:1): R_f= 0.46, $[\alpha]_D^{20}$=+24.8 (*c* 2.4, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ= −0.01, 0.02 (2 s, 6H, $Si(CH_3)_2$), 0.83–0.89 (m, 12H, $Si(C(CH_3)_3)$, 3‴-CH_3), 0.89 −0.97 (m, 27H, 1‴-CH_3, 3′-CH_3, $Si(CH(CH_3)_2)_3$), 1.04–1.11 (m, 1H, 5‴-H), 1.45–1.58 (m, 3H, 4‴-H, 5‴-H), 1.66 (t, *J*=2.3 Hz, 3H, C≡CCH_3), 1.71 (t, *J*=2.3 Hz, 3H, C≡CCH_3), 1.80–1.91 (m, 1H, 3′-H), 1.96–2.05 (m, 2H, 3‴-H, 4′-H), 2.07–2.20 (m, 2H, 1‴-H, 4′-H), 2.49–2.71 (m, 4H, 2″-H, 1′-H), 3.34–3.40 (m, 2H, CH_2ODMB), 3.50–3.54 (m, 1H, CH(OTBS)), 3.69 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.80–3.84 (m, 6H, OCH_3 of DMB), 4.21–4.36 (m, 1H, CH(OTIPS)), 4.37 (s, 2H, CH_2 of DMB), 4.93–5.00 (m, 1H, 1″-H), 6.24 (d, *J*=2.3 Hz, 1H, 5-H), 6.43 (d, *J*=2.3 Hz, 3-H), 6.74–6.85 ppm (m, 3H, aryl H of DMB); ^{13}C NMR (100 MHz, $CDCl_3$): δ= −3.7 ($Si(CH_3)_2$), −3.6 ($Si(CH_3)_2$), 3.5 (C≡CCH_3), 3.5 (C≡CCH_3), 10.3 (1‴-CH_3), 12.9 ($CH(CH_3)_2$), 14.6 (3′-CH_3), 16.5 (Si*C*-$(CH_3)_3$), 18.0 ($CH(CH_3)_2$), 18.2 ($CH(CH_3)_2$), 18.5 (3‴-CH_3), 22.1 (C≡CCH_2), 22.1 (C≡CCH_2), 26.1 ($Si(C(CH_3)_3)$), 28.0 (C5‴), 28.7 (C4‴), 36.2 (C1′), 37.5 (C3‴), 37.8 (C1‴), 38.8 (C3′), 55.2 (OCH_3), 55.6 (OCH_3), 55.8 (OCH_3), 55.9 (OCH_3), 70.7 (C6‴), 72.8 (CH_2 of DMB), 74.6 (CH_3C≡C), 74.9 (CH_3C≡C), 75.2 (C2′), 76.0 (CH_2C≡C), 76.6 (CH_2C≡C), 77.9 (C2‴), 78.1 (C1″), 96.7 (C3), 107.0 (C5), 110.8 (Ar of DMB), 111.0 (Ar of DMB), 117.9 (C1), 120.2 (Ar of DMB), 131.2 (Ar of DMB), 139.0 (C6), 148.4 (Ar of DMB), 148.9 (Ar of DMB), 157.8 (C4), 160.7 (C2), 167.7 ppm (CO_2R); HRMS (ESI): $[M+Na]^+$ calcd for $C_{54}H_{88}NaO_9Si_2$ 959.58591, found 959.58513.

(3*S*,8*S*,9*R*)-3-{(1*R*,2*R*,3*R*)-2-{[*tert*-Butyl(dimethyl)silyl]oxy}-6-[(3,4-dimethoxybenzyl)oxy]-1,3-dimethylhexyl}-12,14-dimethoxy-8-methyl-9-[(triisopropylsilyl)oxy]-5,6-didehydro-3,4,7,8,9,10-hexahydro-1*H*-2-benzoxacyclododecin-1-one (42): A solution of $(tBuO)_3W{\equiv}CCMe_3$ (**6**) (32.8 mg, 0.069 mmol) in toluene (1.0 mL) was added to a solution of ester **41** (650 mg, 0.69 mmol) in toluene (81 mL) and the mixture was stirred at 85°C for 3 h. For workup, the solvent was evaporated and the residue purified by flash chromatography (petroleum ether/EtOAc, 10:1) to give macrolactone **42** as an amorphous solid (553 mg, 90%). TLC (petroleum ether/EtOAc, 4:1): R_f=0.63; $[\alpha]_D^{20}$=−19.0 (*c*=2.4, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.01–0.06 (m, 6H, $Si(CH_3)_2$), 0.87–0.97 (m, 36H, 18-CH_3, 10-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.03 (d, *J*= 6.8 Hz, 3H, 16-CH_3), 1.40–1.52 (m, 2H, 20-H), 1.60–1.85 (m, 4H, 19-H, 11-H, 16-H), 1.89–1.98 (m, 1H, 18-H), 2.09–2.19 (m, 1H, 11-H), 2.35–2.51 (m, 4H, 14-H, 8-H, 10-H), 3.37 (dd, *J*=6.4, 6.4 Hz, 2H, 21-H), 3.48 (dd, *J*=4.3, 4.3 Hz, 1H, 17-H), 3.71–3.79 (m, 7H, 8-H, OCH_3), 3.84–3.87 (m, 6H, OCH_3 of DMB), 3.98–4.02 (m, 1H, 9-H), 4.40 (s, 2H, CH_2 of DMB), 5.35–5.50 (m, 1H, 15-H), 6.30 (d, *J*=2.0 Hz, 1H, 6-H), 6.40 (d, *J*=2.0 Hz, 1H, 4-H), 6.78–6.88 ppm (m, 3H, Ar of DMB); ^{13}C NMR (100 MHz, $CDCl_3$): δ=−4.0 ($Si(CH_3)_2$), −3.7 ($Si(CH_3)_2$), 11.2 (16-CH_3), 12.9 (*C*H-$(CH_3)_2$), 16.7 (10-CH_3), 17.8 ($CH(CH_3)_2$), 18.0 ($CH(CH_3)_2$), 18.3 (18-CH_3), 23.3 (C≡CCH_2), 23.5 (C≡CCH_2), 26.1 ($Si(C(CH_3)_3)$), 27.7 (C20), 28.4 (C19), 37.4 (2C, C16, C18), 38.5 (C8), 40.3 (C10), 55.1 (OCH_3), 55.7 (OCH_3), 55.7 (OCH_3), 55.8 (OCH_3), 70.3 (C21), 72.7 (CH_2 of DMB), 76.0 (C15), 77.2 (C17), 79.6 (C9), 81.2 (CH_2C≡C), 96.6 (C3), 108.2 (C6), 110.8 (Ar of DMB), 110.9 (Ar of DMB), 118.1 (C2), 120.1 (Ar of DMB), 131.1 (Ar of DMB), 139.3 (C7), 148.4 (Ar of DMB), 148.9 (Ar of DMB), 157.2 (C5), 160.2 (C3), 167.3 ppm (CO_2R); HRMS (ESI): $[M+Na]^+$ calcd for $C_{50}H_{82}NaO_9Si_2$ 905.53896, found 905.53829.

Alcohol 43: DDQ (194 mg, 0.85 mmol, 1.4 equiv) was added to a cooled (0°C) solution of DMB ether **42** (540 mg, 0.61 mmol) in a mixture of CH_2Cl_2/pH 7 phosphate buffer solution (20:1, 32 mL). The mixture was allowed to warm to room temperature and stirred for 40 min. Then it was treated with saturated $NaHCO_3$ solution and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$ and saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (petroleum ether/EtOAc, 4:1) afforded alcohol **43** (435 mg, 97%) as an amorphous solid. TLC (petroleum ether/EtOAc, 4:1): R_f=0.42; $[\alpha]_D^{20}$=−28.0 (*c*=4.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.02, 0.06 (2 s, 6H, $Si(CH_3)_2$), 0.86–0.96 (m, 36H, 18-CH_3, 16-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.02 (d, *J*=7.1 Hz, 3H, 10-CH_3), 1.05–1.10 (m, 1H, 20-H), 1.38–1.51 (m, 2H, 20-H, 19-H), 1.55–1.73 (m, 3H, 19-H, 11-H, OH), 1.76–1.82 (m, 1H, 11-H), 1.86–1.97 (m, 1H, 16-H), 2.10–2.18 (m, 1H, 18-H), 2.34–2.51 (m, 4H, 8-H, 14-H, 10-H), 3.50 (dd, *J*=4.3, 4.3 Hz, 1H, 17-H), 3.55 (dd, *J*=6.3, 6.3 Hz, 2H, 21-H), 3.70–3.78 (m, 7H, 8-H, OCH_3), 3.76 (s, 3H, OCH_3), 3.96–4.02 (m, 1H, 9-H), 5.37–5.50 (m, 1H, 15-H), 6.31 (d, *J*=2.3 Hz, 1H, 6-H), 6.40 ppm (s, *J*=2.3 Hz, 1H, 4-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=−4.0 ($Si(CH_3)_2$), −3.7 ($Si(CH_3)_2$), 11.4 (16-CH_3), 12.9 (*C*H$(CH_3)_2$), 16.7 (10-CH_3), 17.8 ($Si(C(CH_3)_3)$), 18.0 ($CH(CH_3)_2$), 18.3 (18-CH_3), 23.2 (C14), 23.4 (C11), 26.1 ($Si(C(CH_3)_3)$), 28.0 (C20), 30.7 (C19), 37.4 (C16), 37.4 (C18), 38.6 (C8), 40.3 (C10), 55.1 (OCH_3), 55.8 (OCH_3), 62.9 (C21), 76.0 (C15), 76.7 (C17), 77.3 (C≡C), 79.6 (C9), 81.2 (C12), 96.7 (C4), 108.3 (C2), 118.1 (C6), 139.4 (C7), 157.2 (C5), 160.2 (C3), 167.4 ppm (C1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{41}H_{72}NaO_7Si_2$ 755.47088, found 755.47080.

Aldehyde 44: A solution of Dess–Martin periodinane (15% wt, 1.02 mL, 0.49 mmol) was added to a cooled (0°C) solution of alcohol **43** (198 mg, 0.27 mmol) in CH_2Cl_2 (6 mL). After stirring for 0.5 h at 0°C and for 2 h at room temperature, the reaction mixture was concentrated, loaded on a flash column and eluted with petroleum ether/EtOAc (4:1) to give 188 mg (95%) of aldehyde **44**, which was used directly in the next reaction. TLC (petroleum ether/EtOAc, 4:1): R_f=0.69.

Alkyne 46: Diethyl-1-diazo-2-oxopropylphosphonate[38] (**45**) (124 mg, 0.52 mmol, 2 equiv) was added to a solution of aldehyde **45**, which was obtained in the previous step (188 mg, 0.26 mmol), and K_2CO_3 (122 mg, 0.88 mmol, 3.4 equiv) in MeOH (5 mL) followed by stirring of the mixture for 12 h at room temperature. The reaction mixture was diluted with Et_2O (50 mL) and washed with an aqueous solution (5%) of $NaHCO_3$ (20 mL). The layers were separated and the organic layer dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by flash chromatography (EtOAc/petroleum ether, 1:10) to give 179 mg (97%) of alkyne **46** as an amorphous solid. TLC (petroleum ether/EtOAc, 4:1): R_f=0.78; $[\alpha]_D^{20}$=−32.2 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.03–0.07 (m, 6H, $Si(CH_3)_2$), 0.86–0.99 (m, 36H, 16-CH_3, 18-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.04 (d, *J*=7.1 Hz, 3H, 10-CH_3), 1.20–1.32 (m, 1H, 20-H), 1.61–1.71 (m, 1H, 20-H), 1.75–1.84 (m, 1H, 16-H), 1.87–1.98 (m, 3H, 19-H, C≡CH), 2.02–2.28 (m, 3H, 10-H, 11-H, 18-H), 2.36–2.47 (m, 4H, 8-H, 14-H, 11-H), 3.52 (dd, *J*=4.3, 4.3 Hz, 1H, 17-H), 3.71–3.79 (m, 7H, 8-H, OCH_3), 3.78 (s, 3H, OCH_3), 3.97–4.02 (m, 1H, 9-H), 5.40–5.52 (m, 1H, 15-H), 6.31 (d, *J*=2.0 Hz, 1H, 6-H), 6.41 ppm (s, *J*=2.0 Hz, 1H, 4-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= −4.0 ($Si(CH_3)_2$), −3.6 ($Si(CH_3)_2$), 11.3 (16-CH_3), 13.0 (*C*H$(CH_3)_2$), 16.4 (10-CH_3), 16.5 (C20), 17.8 ($CH(CH_3)_2$), 18.0 ($CH(CH_3)_2$), 18.4 (18-CH_3), 23.2 (C14), 23.3 (C11), 26.1 ($Si(C(CH_3)_3)$), 30.8 (C19), 36.5 (C18), 37.5 (C16), 38.6 (C8), 40.4 (C10), 55.1 (OCH_3), 55.7 (OCH_3), 68.4 (C22), 74.8 (*C*≡C), 75.6 (C15), 77.2 (C17), 79.6 (*C*≡C), 81.2 (*C*≡C), 84.5 (C9), 96.6 (C4), 108.2 (C2), 118.1 (C6), 139.4 (C7), 157.2 (C5), 160.2 (C3), 167.3 ppm (C1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{42}H_{70}NaO_6Si_2$ 749.46031, found 749.46039.

(*E*)-Vinyl iodide 47: $[Cp_2Zr(H)Cl]$ (38 mg, 0.14 mmol) was added to a solution of alkyne **46** (51 mg, 0.07 mmol) in THF (1.5 mL) at 0°C and the resulting mixture was stirred for 2 h at that temperature. A solution of I_2 (0.28 mL, 0.5M in THF, 0.14 mmol) was then added dropwise and stirring

was continued for 2 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (5 mL), and the mixture was repeatedly extracted with Et_2O. The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 4:1) to give (*E*)-vinyl iodide **47** as an amorphous solid (66 mg, 95%). TLC (petroleum ether/EtOAc, 4:1): $R_f = 0.77$; $[\alpha]_D^{20} = -21.0$ ($c = 3.6$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.03$–0.07 (m, 6H, $Si(CH_3)_2$), 0.84–0.97 (m, 36H, 16-CH_3, 18-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.04 (d, $J = 6.8$ Hz, 3H, 10-CH_3), 1.20–1.26 (m, 1H, 20-H), 1.43–1.54 (m, 1H, 19-H), 1.64–1.72 (m, 1H, 20-H), 1.74–1.82 (m, 1H, 16-H), 1.87–1.98 (m, 2H, 19-H, 18-H), 2.02–2.20 (m, 2H, 10-H, 11-H), 2.37–2.48 (m, 4H, 8-H, 14-H, 11-H), 3.52 (dd, $J = 4.3$, 4.3 Hz, 1H, 17-H), 3.72–3.81 (m, 7H, 8-H, OCH_3), 3.97–4.02 (m, 1H, 9-H), 5.39–5.44 (m, 1H, 15-H), 5.90 (d, $J = 14.4$ Hz, 1H, 22-H), 6.33 (d, $J = 2.0$ Hz, 1H, 6-H), 6.36–6.45 ppm (m, 2H, 4-H, 21-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.0$ ($Si(CH_3)_2$), −3.6 ($Si(CH_3)_2$), 11.5 (16-CH_3), 13.0 ($CH(CH_3)_2$), 16.4 (10-CH_3), 16.5 (C20), 17.9 ($CH(CH_3)_2$), 18.1 ($CH(CH_3)_2$), 18.4 (18-CH_3), 23.2 (C14), 23.2 (C11), 26.1 ($Si(C(CH_3)_3)$), 30.8 (C19), 34.1 (C20), 37.4 (C18), 37.5 (C16), 38.6 (C8), 40.0 (C10), 55.2 (OCH_3), 55.8 (OCH_3), 74.5 (C22), 75.4 (C15), 77.2 (C17), 79.6 (C≡C), 81.4 (C9), 96.6 (C4), 108.4 (C2), 118.0 (C6), 139.4 (C7), 146.6 (C21), 157.3 (C5), 160.3 (C3), 167.3 ppm (C1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{42}H_{71}INaO_6Si_2$ 877.37261, found 877.37329.

Enamide 48: A Schlenk tube was charged with CuI (14.0 mg, 0.075 mmol, 1 equiv), amide **20** (39 mg, 0.15 mmol, 2 equiv) and Cs_2CO_3 (62 mg, 0.19 mmol, 2.5 equiv). The tube was evacuated and backfilled with argon. *N*,*N*′-Dimethylethylenediamine (16.0 μL, 0.15 mmol, 2 equiv), vinyl iodide **47** (64 mg, 0.075 mmol) and THF (1.0 mL) were added under argon. The Schlenk tube was closed with a glass stopper, immersed in a preheated to 60 °C oil bath and the reaction mixture was stirred for 14 h. After the resulting pale-blue suspension was allowed to reach room temperature, ethyl acetate (5 mL) was added and the reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 10:1→4:1) to give enamide **48** as an amorphous solid (63 mg, 85%). TLC (petroleum ether/EtOAc, 4:1): $R_f = 0.72$; $[\alpha]_D^{20} = -23.9$ ($c = 1.3$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.02$–0.07 (m, 6H, $Si(CH_3)_2$), 0.08–0.10 (m, 6H, $Si(CH_3)_2$), 0.85–1.01 (m, 48H, 24-CH_3, 28-H, 16-CH_3, $Si(C(CH_3)_3)$, $Si(CH(CH_3)_2)_3$), 1.02–1.10 (m, 6H, 18-CH_3, 10-CH_3), 1.16–1.27 (m, 3H, 20-H, 27-H), 1.30–1.52 (m, 5H, 26-H, 27-H, 18-H, 19-H), 1.74–1.83 (m, 1H, 16-H), 1.86–1.99 (m, 2H, 10-H, 11-H), 2.04–2.19 (m, 2H, 19-H, 11-H), 2.36–2.55 (m, 5H, 8-H, 14-H, 24-H), 3.43–3.48 (m, 1H, 17-H), 3.71–3.79 (m, 7H, OCH_3, 25-H), 3.97–4.03 (m, 1H, 9-H), 4.94–5.03 (m, 1H, 21-H), 5.35–5.49 (m, 1H, 15-H), 6.31 (d, $J = 2.0$ Hz, 1H, 6-H), 6.41 (d, $J = 2.0$ Hz, 1H, 4-H), 6.73 (dd, $J = 14.2$, 10.6 Hz, 1H, 22-H), 8.08 ppm (br d, $J = 10.6$ Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.6$ ($Si(CH_3)_2$), −4.6 ($Si(CH_3)_2$), −3.8 ($Si(CH_3)_2$), −3.7 ($Si(CH_3)_2$), 11.2 (16-CH_3), 12.6 (24-CH_3), 13.0 ($CH(CH_3)_2$), 14.1 (C28), 16.5 (10-CH_3), 17.9 ($Si(C(CH_3)_3)$), 17.9($CH(CH_3)_2$), 18.1 ($CH(CH_3)_2$), 18.4 (18-CH_3), 19.3 (C27), 23.3 (C14), 23.6 (C11), 25.9 ($Si(C(CH_3)_3)$), 26.2 ($Si(C(CH_3)_3)$), 27.7 (C20), 32.5 (C19), 34.7 (C26), 37.0 (C18), 37.6 (C16), 38.6 (C8), 40.6 (C10), 45.6 (C24), 55.2 (OCH_3), 55.7 (OCH_3), 75.0 (C17), 76.1 (C9), 76.8 (C15), 77.2 (C25), 79.6 (C≡C), 81.2 (C≡C), 96.7 (C4), 108.3 (C6), 112.2 (C21), 118.2 (C2), 122.6 (C22), 139.4 (C7), 157.2 (C5), 160.2 (C3), 167.4 (C1), 171.0 ppm (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{55}H_{99}NNaO_8Si_3$ 1008.65707, found 1008.65733.

Deprotected macrolactone 49: HF·pyridine complex (70% HF, 0.3 mL) was added dropwise to a stirred solution of the enamide **48** (12 mg, 0.012 mmol) in THF (0.4 mL, in a plastic test tube) at −80 °C. The reaction mixture was allowed to warm to −10 °C. After 2 h the mixture was partioned between an ice-cooled mixture of EtOAc (20 mL) and saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated and the H_2O layer extracted with EtOAc (2×20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5 → 9:1) to give 6.1 mg (85%) of triol **49**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.38$; $[\alpha]_D^{20} = -4.2$ ($c = 1.2$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.84$ (d, $J = 6.8$ Hz, 3H, 18-CH_3), 0.91 (t, $J = 7.1$ Hz, 3H, 28-H), 0.99 (d, $J = 7.1$ Hz, 3H,10-CH_3), 1.10 (d, $J = 7.1$ Hz, 3H, 16-CH_3), 1.14 (d, $J = 7.1$ Hz, 3H, 24-CH_3), 1.24–1.50 (m, 5H, 26-H, 27-H, 20-H), 1.55–1.77 (m, 4H, 19-H, 20-H, OH), 1.89–2.02 (m, 3H, 18-H, 19-H, 11-H), 2.05–2.20 (m, 3H, 16-H, 24-H, 11-H), 2.24–2.33 (m, 1H, 8-H), 2.46–2.62 (m, 3H, 14-H, 10-H, OH), 2.73–2.82 (m, 1H, 14-H), 3.09 (br s, 1H, OH), 3.27 (dd, $J = 13.8$ Hz, 2.7 Hz, 1H, 8-H), 3.38–3.44 (m, 1H, 17-H), 3.75–3.89 (m, 8H, OCH_3, 9-H, 25-H), 5.01–5.10 (m, 1H, 21-H), 5.42–5.50 (m, 1H, 15-H), 6.34 (d, $J = 2.0$ Hz,1H, 6-H), 6.40 (d, $J = 2.0$ Hz,1H, 4-H), 6.70 (dd, $J = 14.2$, 10.4 Hz, 1H, 22-H), 7.68 ppm (d, $J = 10.4$ Hz, 1H, NH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{34}H_{51}NNaO_8$ 624.35069, found 624.35092.

Enamide analogue 50: A 5-mL round-bottom flask was charged with diyne **49** (3.0 mg, 0.005 mmol) and stirred with a stir bar. EtOAc (2 mL) and quinoline (1.0 mg, 0.08 mmol) were added with stirring. This was followed by the addition of Lindlar's catalyst (5 wt% Pd on $CaCO_3$, poisoned with lead, 3 mg, 100 wt%). The reaction was placed under H_2 atmosphere and stirred for 1 h. The reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give enamide **50** as a colourless oil (2.7 mg, 90%). TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.41$; $[\alpha]_D^{20} = -5.6$ ($c = 0.2$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.84$–0.89 (m, 6H, 28-H, 18-CH_3), 1.01–1.06 (m, 6H, 10-CH_3, 16-CH_3), 1.14 (d, $J = 7.1$ Hz, 3H, 24-CH_3), 1.21–1.31 (m, 4H, 26-H, 27-H, 20-H), 1.34–1.49 (m, 2H, 26-H, 27-H), 1.57 (br s, 1H, OH), 1.63–1.75 (m, 2H, 18-H, 19-H), 1.81–2.07 (m, 5H, 10-H, 11-H, 16-H, 19-H, 8-H), 2.15–2.24 (m, 1H, 14-H), 2.25–2.40 (m, 1H, 24-H), 2.58 (br s, 1H, OH), 2.74–2.86 (m, 1H, 11-H), 2.92–3.00 (m, 1H, 8-H), 2.82 (dt, $J = 14.3$, 11.5 Hz, 1H, 14-H), 3.29–3.39 (m, 2H, 17-H, OH), 3.69–3.76 (m, 4H, 25-H, OCH_3), 3.77–3.83 (m, 4H, 9-H, OCH_3), 5.10–5.19 (m, 1H, 21-H), 5.36 (dd, $J = 10.2$, 3.2 Hz, 1H, 15-H), 5.41–5.56 (m, 2H, 12-H, 13-H), 6.32–6.36 (m, 2H, 6-H, 4-H), 6.76 (dd, $J = 14.2$ Hz, 10.6 Hz, 1H, 22-H), 7.69 ppm (d, $J = 10.4$ Hz, 1H, NH); HRMS (ESI): $[M+Na]^+$ calcd for $C_{34}H_{53}NO_8Na$ 626.36689, found 626.36672.

Oxazinane-4-one 51: a) *ortho*-Demethylation: A solution of enamide **49** (18 mg, 0.018 mmol) in CH_2Cl_2 (2 mL) was treated with BCl_3 (72 μL, 1.0 M in CH_2Cl_2, 0.072 mmol, 4 equiv) at −80 °C. The reaction was stirred for 2 h at −80 °C before a saturated solution of NaOAc (5 mL) was added. After separation of the layers, the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed with H_2O, saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated in vacuo to give 17 mg of crude 2-hydroxy-4-methoxybenzoate which was used in the next step without further purification.

b) Global deprotection: HF·pyridine complex (70% HF, 0.6 mL) was added dropwise to a stirred solution of the crude 2-hydroxy-4-methoxybenzoate (17 mg) in THF (0.8 mL, in a plastic test tube) at −80 °C dropwise. The reaction mixture was allowed to warm to −10 °C. After 2 h, the mixture was partioned between an ice-cooled mixture of EtOAc (20 mL) and saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated and the H_2O layer extracted with EtOAc (2×20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give 9.2 mg (87% for 2 steps) of triol **51**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.38$; $[\alpha]_D^{20} = +14.0$ ($c = 0.4$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.82$ (d, $J = 6.6$ Hz, 3H, 18-CH_3), 0.91–0.97 (m, 6H, 28-H, 16-CH_3), 1.04 (d, $J = 7.1$ Hz, 3H, 10-CH_3), 1.19 (d, $J = 7.3$ Hz, 3H, 24-CH_3), 1.27–1.75 (m, 10H, 19-H, 26-H, 27-H, 20-H, 21-H), 1.95–2.21 (m, 4H, 18-H, 16-H, 11-H, OH), 2.28–2.36 (m, 3H, 8-H, 24-H, OH), 2.54–2.73 (m, 3H, 14-H, 10-H, 11-H), 2.78–2.86 (m, 1H, 14-H), 3.53 (d, $J = 8.8$ Hz, 1H, 17-H), 3.69–3.78 (m, 2H, 25-H, 8-H), 3.80 (s, 3H, OCH_3), 3.91–3.99 (m, 1H, 9-H), 4.79 (t, $J = 5.6$ Hz, 1H, 22-H), 5.37–5.43 (m, 1H, 15-H), 6.38–6.40 (m, 2H, 4-H, 6-H), 6.76 (br s, 1H, NH), 11.1 ppm (br s, 1H, 3-OH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 7.9$ (16-CH_3), 12.0 (24-CH_3), 14.0 (C28), 16.4 (18-CH_3), 16.7 (10-CH_3), 18.8 (C27), 20.9, 21.7 (C14), 22.3 (C11), 29.7 (C20), 32.5 (C19), 33.3 (C21), 35.6 (C26), 36.0, 36.3 (C18), 37.8 (C16), 38.2 (C8), 40.0 (C24), 55.4 (OCH_3), 71.0 (C25), 75.1 (C9), 76.2 (C15), 77.2 (C≡C), 79.0 (C≡C), 83.0 (C22), 84.0, 99.4 (C4), 110.2 (C6), 142.0 (C7), 162.0 (C5), 163.0 (C3), 170.2 (C1), 175.0 (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{33}H_{49}NaNO_8$ 610.33504, found 610.33543.

Oxazinan-4-one 52: A 10-mL round-bottom flask was charged with alkyne **51** (9.0 mg, 0.015 mmol) and stirred with a stir bar. EtOAc (5 mL) and quinoline (3.0 mg, 0.024 mmol) were added with stirring. This was followed by the addition of Lindlar's catalyst (5 wt % Pd on $CaCO_3$, poisoned with lead, 9 mg, 100 wt %). The reaction was placed under H_2 atmosphere and stirred for 1 h. The reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5→9:1) to give analogue **52** as a colourless oil (8.4 mg, 93 %). TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.38$; $[\alpha]_D^{20} = -9.4$ ($c = 0.8$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 0.78 (d, $J = 6.6$ Hz, 3H, 18-CH_3), 0.89–0.96 (m, 6H, 28-H, 16-CH_3), 1.01 (d, $J = 6.8$ Hz, 3H, 10-CH_3), 1.17 (d, $J = 7.3$ Hz, 3H, 24-CH_3), 1.27–1.60 (m, 10H, 19-H, 26-H, 27-H, 20-H, 21-H), 1.82 (br s, 1H, OH), 1.93–2.10 (m, 3H, 18-H, 11-H, 16-H), 2.17–2.42 (m, 5H, 10-H, 14-H, 11-H, 8-H), 2.78–2.89 (m, 1H, 24-H), 3.38–3.47 (m, 1H, 17-H), 3.62–3.75 (m, 3H, 9-H, 8-H, 25-H), 3.79 (s, 3H, OCH_3), 4.71 (t, $J = 4.6$ Hz, 1H, 22-H), 5.23 (dd, $J = 11.0$, 4.2 Hz, 1H, 15-H), 5.38–5.54 (m, 2H, 12-H, 13-H), 6.30 (d, $J = 2.5$ Hz, 1H, 6-H), 6.36 (d, $J = 2.5$ Hz, 1H, 4-H), 6.40 (br s, 1H, NH), 11.6 ppm (br s, 1H, 3-OH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 9.0$ (16-CH_3), 12.0 (24-CH_3), 14.0 (C28), 14.2 (18-CH_3), 15.8 (10-CH_3), 18.9 (C27), 20.8 (C20), 29.4 (C14), 29.7 (C11), 31.6 (C19), 32.0 (C21), 33.3 (C26), 36.3, 36.7, 37.1 (C18), 37.8 (C16), 38.3 (C8), 38.8 (C24), 40.1 (C10), 55.4 (OCH_3), 72.9 (C25), 75.5 (C9), 78.0 (C15), 83.8 (C22), 99.7 (C4), 104.8 (C2), 112.4 (C6), 125.6 (C13), 132.3 (C12), 143.6 (C7), 163.6 (C5), 165.9 (C3), 171.5 (C1), 174.6 (C23); HRMS (ESI): $[M+Na]^+$ calcd for $C_{33}H_{51}NaNO_8$ 612.35069, found 612.35076.

Biological assays: The biological activity of the compounds was tested by a growth-inhibition assay with fibroblast cells of the mouse cell line L929 (ACC2, DSMZ). The cells were cultivated in Dulbeco's modified Eagle medium with high glucose and 10 % fetal calf serum at 37 °C and 10 % CO_2. Aliquots of 120 μL of suspended cells (50,000 mL^{-1}) were given to 60 μL of serial dilutions of the compounds in 96-well microplates. After five days, the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MMT) was measured as a parameter of growth and metabolic activity of the cells and related to control cells that were incubated with the solvent only.

Selected compounds were checked for special phenotypic effects with PtK_2 potoroo cells (ATCC CCL-56). Cells grown on glass coverslips (13 mm diameter) in four-well plates were incubated with the compounds overnight, fixed with cold (−20 °C) acetone-methanol (1:1) for 10 min and labelled for endoplasmatic reticulum (ER) with a primary antibody against GRP-94 (1:1000; Affinity BioReagents) and a secondary goat anti-rat IgG antibody conjugated with Alexa Fluor 488 (10 μg mL^{-1}; Molecular Probes).

ATPase assays with submitochondrial particles from beef heart were performed in a final volume of 1000 μL and a pH of 8.0 at room temperature as described previously.[1] The samples contained 150 μg of bovine protein, 50 mM Tris-HCl (Tris = tris(hydroxymethyl)aminomethane), 50 mM KCl and 2.5 mM $MgCl_2$. The specific ATPase activity without inhibitor was (0.5 ± 0.13 μ)mol mg^{-1} min^{-1}.

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