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Antitumor Agents

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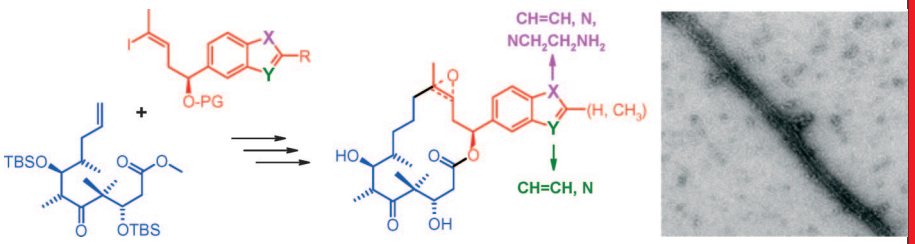

Where should the N atom be? A convergent synthesis has been developed for epothilone analogues with quinoline- or benzimidazole side chains. The microtubule-binding affinity (the figure on the right-hand side depicts a microtubule) of the quinoline-based derivatives depends on the position of the N atom in the quinoline system, but there is no general correlation with cellular potency.

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Epothilone Analogues with Benzimidazole and Quinoline Side Chains: Chemical Synthesis, Antiproliferative Activity, and Interactions with Tubulin

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Abstract: A series of epothilone B and D analogues bearing isomeric quinoline or functionalized benzimidazole side chains has been prepared by chemical synthesis in a highly convergent manner. All analogues have been found to interact with the tubulin/microtubule system and to inhibit human cancer cell proliferation in vitro, albeit with different potencies (IC_{50} values between 1 and 150 nM). The affinity of quinoline-based epothilone B and D analogues for stabilized microtubules clearly depends on the position of the N-atom in the quinoline system, while the induction of tubulin polymerization in vitro appears to be less sensitive to N-positioning. The potent inhibition of human cancer cell growth by epothilone analogues bearing functionalized benzimidazole side chains suggests that these systems might be conjugated with tumor-targeting moieties to form tumor-targeted prodrugs.

Keywords: antitumor agents · epothilone · microtubules · natural products · structure–activity relationships · total synthesis

Introduction

Epothilones comprise a family of bacterial natural products, the major representatives of which, epothilones A and B (Epo A and Epo B; Figure 1), were first isolated in 1986 by Höfle, Reichenbach, and co-workers from the cellulose-degrading myxobacterium *Sorangium cellulosum*.[1,2] These compounds were quickly recognized to exhibit potent antiproliferative activity against human cancer cells in vitro,[1] but it was not before 1995 that these growth inhibitory effects were demonstrated by Bollag et al. to arise from the ability of the epothilones to stabilize cellular microtubules (MTs).[3] Thus, epothilones inhibit human cancer cell growth through the same mechanism of action as the established clinical anticancer drugs taxol (paclitaxel; Taxol) and docetaxel (Taxotere), the interference of which with MT functionality is associated with cell cycle arrest in mitosis and the induction of apoptosis.[4] In contrast to taxol, however, epothilones are also active against various types of multidrug-resistant cell lines that overexpress the P-gp170 efflux pump (for which Epo A and B are very poor substrates)[3,5] or that have acquired taxol-resistant tubulin mutations.[6]

The discovery of their "taxol-like" mechanism of action led to an instantaneous surge of interest in the epothilones as important new lead structures for anticancer drug discovery[7] and as such they also became highly relevant targets for total synthesis.[8] Over the last 15 years, research into the structure–activity relationships (SARs) for epothilones has led to an exceptionally comprehensive knowledge base on the activity of structurally modified epothilone analogues (fully synthetic variants as well as semi-synthetic derivatives), ranging from modifications that lead to improved antiproliferative activity over the natural product leads to those that are completely detrimental to biological activity.[7] Most importantly, these efforts have also produced several compounds that have entered clinical evaluation in humans and are currently at different stages of clinical development.[9] In fact, the most advanced of these compounds, the Epo B lactam BMS-247550 (ixabepilone) was approved in 2007 by the US FDA for clinical use in breast cancer patients (under the trade name Ixempra).[10]

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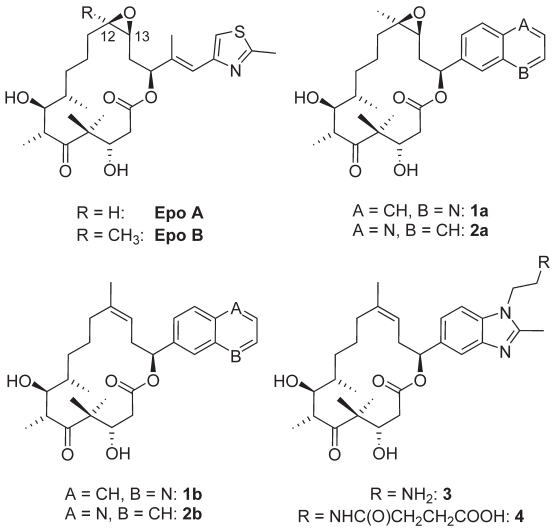


Figure 1.

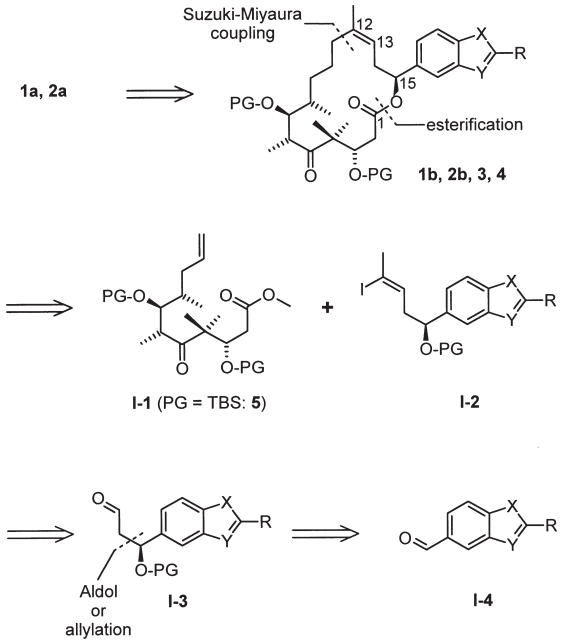
However, in spite of the vast body of SAR information available for epothilones today, our understanding of their detailed molecular interactions with their target protein β-tubulin is still limited by the lack of high-resolution structural data for complexes between tubulin/MTs and any epothilone analogue (or any other type of MT stabilizer for that matter). While the structure of a complex of Epo A with β-tubulin in Zn^{2+}-stabilized tubulin polymer sheets has been determined by a combination of electron crystallography (EC) at 2.89 Å resolution and NMR-based conformational analysis,[11] the tubulin-bound conformation of Epo A deduced from this analysis does not permit rationalization of a number of important features of the epothilone SAR. A more satisfactory explanation of the SAR data is provided by the structural model proposed by Carlomagno and coworkers on the basis of solution NMR studies on tubulin-bound Epo A.[12,13] This NMR-based model deviates from the EC-derived structure of the tubulin/Epo A complex in several respects, including the type of interaction between the side-chain thiazole moiety in epothilones and the side chain of His-227 in β-tubulin. The latter has been concluded to be hydrogen-bonded (in its protonated form) to the N-atom of the thiazole ring based on the EC data;[11] in contrast, no such H-bond was found in the solution NMR studies, but it is suggested that the thiazole ring is involved in a stacking interaction with the imidazole ring of the His-227 side chain.[13]

SAR studies on the importance of N-positioning in pyridine-based analogues of Epo B (with different isomeric pyridine moieties in place of the natural thiazole ring) have shown that antiproliferative activity similar to that of Epo B itself is only conserved if the pyridine nitrogen is located *ortho* to the attachment point of the vinyl linker between the heterocycle and the macrolactone ring.[14] Together with data on the effect of these compounds on tubulin polymerization (which are, however, not fully quantitative in nature), this led to the conclusion that N-positioning in the heterocycle has a significant influence on tubulin (MT) binding.[14] As an extension of this earlier work by Nicolaou et al., we have recently communicated the synthesis and biological evaluation of quinoline-based Epo B/D analogues **1** and **2** (Figure 1).[15] In accordance with Nicolaou's earlier data, analogue **1b** was found to be a significantly more potent antiproliferative agent than its isomer **2b**. At the same time, a preliminary assessment of the effects of **1b** and **2b** on tubulin assembly revealed similar EC_{50} values for the induction of tubulin polymerization in vitro, which suggested that the difference in cellular activity between **1b** and **2b** might not arise from differences in their interactions with the tubulin/MT system. No firm conclusions can be drawn from the polymerization data, however, as compounds with significantly different MT binding affinities may still exhibit similar EC_{50} values for the induction of tubulin polymerization, if they both exceed a certain affinity threshold. In contrast to **1b** and **2b**, the corresponding epoxides **1a** and **2a** (Figure 1) were found to be equally potent inhibitors of cancer cell growth in vitro and they showed similar EC_{50} values for tubulin polymerization induction.

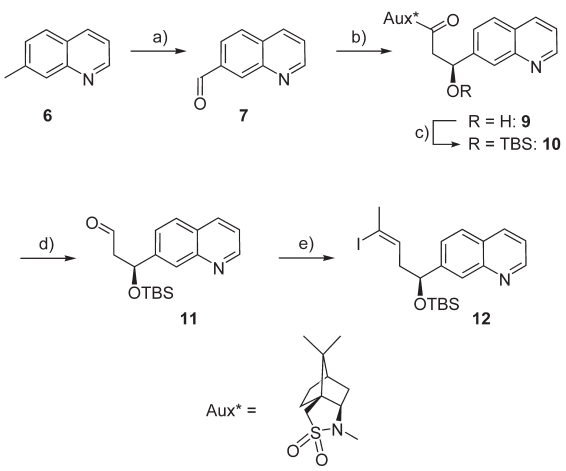
In this paper, we disclose full details of the synthesis of Epo B analogues **1a/b** and **2a/b**. In addition, we report on an extension of our previous studies on the interactions of these analogues with the tubulin/MT system to the investigation of their binding affinities to cross-linked MTs, in order to provide a direct measure of the stability of the corresponding protein/ligand complexes. Finally, we also describe the synthesis and preliminary biological assessment of the new epothilone analogues **3** and **4**, which belong to the same family of structures as **1a/b** and **2a/b**. Conformationally constrained analogues of this type exhibit enhanced cellular potency, especially in the Epo D[16] series (e.g., **1b** and **2b**), and of the various side chains investigated so far the activity-enhancing effect is clearly greatest for a dimethylbenzimidazole moiety.[17–19] Based on these previous findings, we have designed analogues **3** and **4**, which incorporate additional functional groups on the benzimidazole 3-substituent that are envisaged to be utilized for the reversible attachment of different types of tumor-targeting moieties. In a first step to determining the potential of these analogues to serve as active drug moieties in tumor-targeted prodrugs, we have assessed their intrinsic biological activities.

Results and Discussion

Chemistry: Our general retrosynthesis of analogues **1–4** is depicted in Scheme 1. In all cases, ring-closure was envisaged to be achieved through Yamaguchi-type macrolactonization of a suitably protected seco acid,[20] which was to be followed by removal of the protecting group and, in the cases of **1a** and **2a**, by epoxidation of the C12–C13 double bond. The requisite seco acid was to be obtained from the C1–C11 fragment **I-1** and vinyl iodides **I-2** by Suzuki–Miyaura coupling, a strategy that we had successfully utilized in our previous syntheses of *trans*-Epo A[21] and related



Scheme 1. Retrosynthesis of target structures **1**–**4**. X, Y, R = CH=CH, N, H (**1**); N, CH=CH, H (**2**); N($CH_2CH_2NH_2$), N, CH_3 (**3**); N($CH_2CH_2NHC(O)CH_2CH_2COOH$), N, CH_3 (**4**). PG = protecting group or H. Protecting groups could be varied independently.



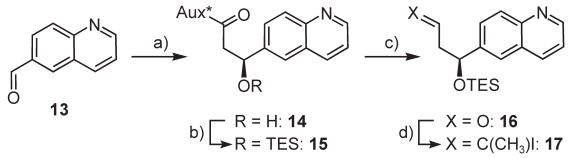
Scheme 2. a) SeO_2 (0.66 equiv), 155 °C, 18 h, 58 %. b) i) Et_3B (1.4 equiv), CF_3SO_3H (1.3 equiv), CH_2Cl_2, RT, 25 min; ii) + Ac-Aux* (**8**) (1 equiv), DIEA (1.3 equiv), CH_2Cl_2, 0 °C, 20 min; iii) + **7** (1.4 equiv), CH_2Cl_2, −78 °C, 3.5 h, 80 % (mixture of diastereoisomers). c) TBS-Cl (1.5 equiv), imidazole (3 equiv), DMF, 40 °C, 17 h, 63 % (single isomer). d) DIBAL-H (2.5 equiv), CH_2Cl_2, −78 °C, 3.5 h, 78 %. e) i) $[Ph_3PCH(CH_3)I]I$ (1.3 equiv), NaHMDS (1.2 equiv), THF, −78 °C, 45 min, −15 °C, 20 min; ii) + **11**, THF, −78 °C, 45 min, 41 %.

analogues[22,23] and that was first exploited for the synthesis of epothilones by Danishefsky and co-workers.[24]

While we have previously described the synthesis of ester **5** (i.e., intermediate **I-1** with PG = TBS),[21] vinyl iodides **I-2** were envisaged to be prepared aldehydes **I-3** through Zhao iodo-olefination.[25] The latter would be obtained from aldehydes **I-4** by stereoselective aldol or allylation reactions.

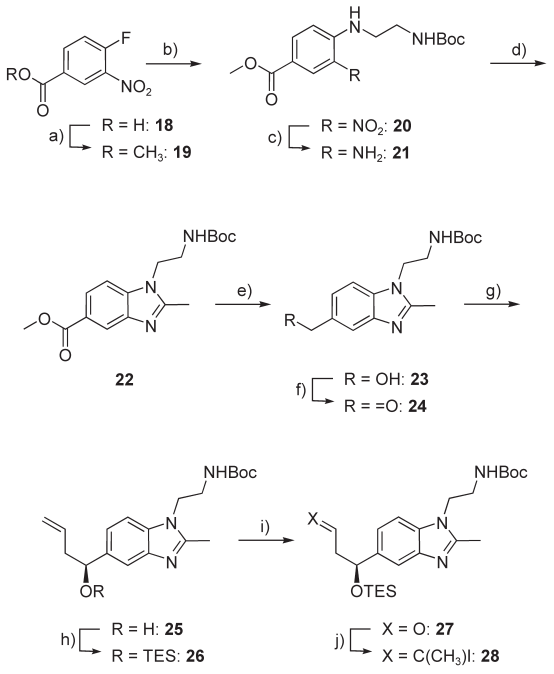
Scheme 2 summarizes the synthesis of vinyl iodide **12** as the requisite C13–C15 building block for the elaboration of analogues **1a** and **1b**. Oxidation of commercially available 7-methylquinoline (**6**) with SeO_2 gave quinoline-7-carboxaldehyde (**7**),[26] which was then submitted to aldol reaction with the boron-enolate of the acetylated Oppolzer sultam **8**.[27,28] The aldol product **9** was obtained in 80 % yield as a 5:1 mixture of isomers, which were not separated, but directly converted to the corresponding C15-O-TBS ethers (epothilone numbering). At this stage, the isomers were easily separable and the desired product **10** could be isolated in 50 % overall yield for the two-step sequence from aldehyde **7**.[29] While the conversion of **10** to aldehyde **11** with DIBAL-H was straightforward, the subsequent Zhao olefination[25] provided the desired vinyl iodide **12** only in moderate (but still acceptable) yield. Yields not exceeding 50 % have been consistently reported in the literature for this type of iodo-olefination reaction for a variety of substrates and appear to be an intrinsic feature of this transformation.[30]

Following an analogous sequence of reactions to that described above, the isomeric vinyl iodide **17** was prepared from quinoline-6-carboxaldehyde (**13**) (obtained from 6-methylquinoline by oxidation with SeO_2 in 59 % yield) (Scheme 3). In keeping with our own experience and previous literature reports (see above), the lowest yielding step of the sequence was once again the formation of the vinyl iodide (**17**), which was obtained from aldehyde **16** in 30 % yield under virtually identical conditions to those employed for the transformation of **11** to **12**.



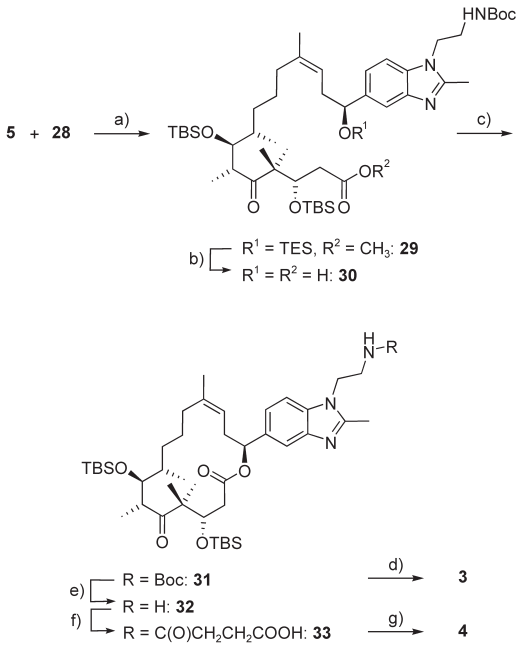
Scheme 3. a) i) Et_3B (1.4 equiv), CF_3SO_3H (1.3 equiv), CH_2Cl_2, RT, 25 min; ii) + Ac-Aux* (**8**) (1.3 equiv), DIEA (1.3 equiv), CH_2Cl_2, 0 °C, 20 min; iii) + **13** (1 equiv), CH_2Cl_2, −78 °C, 3.5 h, 81 % (mixture of diastereoisomers). b) TES-Cl (1.5 equiv), imidazole (3 equiv), DMF, 45 °C, 18 h, 37 % (2 steps, single isomer). c) DIBAL-H (4 equiv), CH_2Cl_2, −78 °C, 5.5 h, 74 %. d) i) $[Ph_3PCH(CH_3)I]I$ (1.3 equiv), NaHMDS (1.2 equiv), THF, −78 °C, 65 min, −15 °C, 20 min; ii) + **16**, THF, −78 °C, 45 min, 30 %.

Compared to the synthesis of quinoline-derived building blocks **12** and **17**, the preparation of benzimidazole-containing vinyl iodide **28** was significantly more elaborate, as in this case the required aldehyde **I-4** (Scheme 1) could not be simply obtained in one step from a commercially available starting material. As illustrated in Scheme 4, this aldehyde (i.e., **24** in Scheme 4) had to be prepared in a multistep sequence from 4-fluoro-3-nitrobenzoic acid (**18**), starting with the conversion of the latter to its methyl ester **19** (Scheme 4). Nucleophilic displacement of the aromatic



Scheme 4. a) MeOH, H_2SO_4, reflux, 98%. b) $BocNHCH_2CH_2NH_2$, Et_3N, CH_2Cl_2, RT, 96%. c) 1 atm H_2, Pd/C, MeOH, RT, 99%. d) $(EtO)_3CCH_3$ (6 equiv), EtOH, reflux, 96%. e) DIBAL-H, CH_2Cl_2, −78 °C→RT, 78%. f) MnO_2 (10 equiv), $CHCl_3$, reflux, 1 h, 98%. g) i) C_3H_5MgBr (1.5 equiv), (−)-Ipc_2BCl (1.5 equiv), Et_2O, 0 °C→RT, 1 h (solution A); ii. slow addition of solution A to a solution of **24** (1 equiv) in Et_2O, −100 °C, 3 h, 89%, 94% *ee*. h) TES-Cl, imidazole, DMAP, RT, 4 h, 98%. i) OsO_4, $NaIO_4$, 2,6-lutidine, dioxane/water/*t*BuOH, RT, 23 h, 74%. j) i) $[Ph_3PCH(CH_3)I]I$ (1.3 equiv), NaHMDS (1.2 equiv), THF, −78 °C, 45 min, −15 °C, 20 min; ii) **+27**, THF, −78 °C, 45 min, 42%.

As shown in Scheme 5, Suzuki–Miyaura coupling of the borane derived in situ from alkene **5** and 9-BBN with vinyl iodide **28** produced the fully protected seco acid **29** in excellent yield (83%). The conditions for this coupling had been optimized during our previous work on *trans*-Epo A and related analogues,[21–23] but it is worth emphasizing that the success of the reaction critically depends on the exclusion of moisture in the hydroboration step and thus requires careful and extensive drying of ester **5**. The coupling product **29** was directly converted to the immediate cyclization precursor **30** through ester saponification with LiOH and exposure of the resulting C15-O-TES derivative to aqueous acid during work-up in 85% yield. Seco acid **30** was then cyclized at RT according to the Yamaguchi protocol[20] to provide the fully protected macrolactone **31** in 57% yield. Finally, treatment of **31** with TFA gave target structure **3** in 44% yield after HPLC purification. Alternatively, the Boc group in **31** could be selectively removed by treatment with $ZnBr_2$ in CH_2Cl_2[33] and the resulting free amine was reacted with succinic anhydride to give **33**, which was converted to target structure **4** by deprotection with TFA. The latter structure **4** was obtained in 21% overall yield for the two-step sequence from amine **32**. The low yield in the transformation of **31** to **4** is largely a consequence of the difficulties associated with the purification of the very polar products.

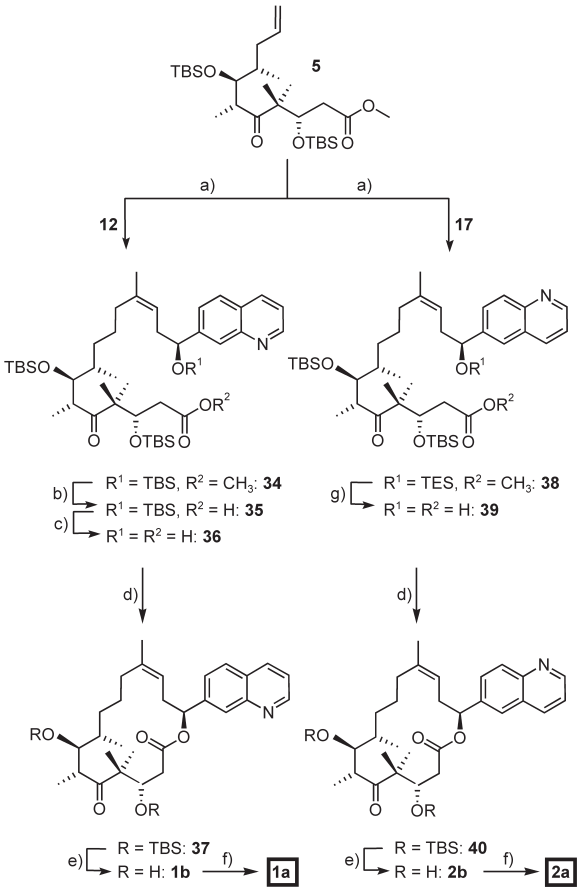


Scheme 5. a) i) **5**, 9-BBN, THF, RT; ii) Cs_2CO_3, $[PdCl_2(dppf)_2]$, Ph_3As, **28**, −5 °C→RT, 83%. b) LiOH, dioxane/water 4:1, 60 °C, 11.5 h, acidic work-up, 85%. c) i) Et_3N, 2,4,6-trichlorobenzoyl chloride, THF, 10 °C; ii) DMAP, toluene, RT, 57%. d) CF_3COOH, CH_2Cl_2, RT, 2 h, 44%. e) $ZnBr_2$, CH_2Cl_2, 0 °C, 72 h, 57%. f) Succinic anhydride, DIEA, DMF, RT, 2 h, 92% (crude). g) CF_3COOH, CH_2Cl_2, RT, 5 h, 23%.

fluoro substituent in **19** with mono-Boc-protected ethylenediamine gave nitroaniline **20**, which was reduced with H_2/Pd-C to provide the phenylenediamine derivative **21** in essentially quantitative yield. The latter was cyclized to benzimidazole **22**, the ester group of which was reduced with DIBAL-H followed by oxidation of the resulting alcohol **23** with MnO_2. Aldehyde **24** was thus obtained from **18** in excellent overall yield (70% over eight steps). While **24** could be successfully elaborated into the desired vinyl iodide **28** following the aldol-based approach that had been developed for the synthesis of **12** and **17**, the selectivity of the aldol reaction in the case of aldehyde **24** proved to be very moderate (2:1 vs. 5:1 and 3:1 for aldehydes **11** and **15**, respectively; see above), thus leading to unsatisfactory overall yields for the elaboration of **24** into **28** (not shown). As a consequence, an alternative approach for the stereoselective two-carbon extension of **24** was developed, which was based on Brown allylation of **24** with (−)-Ipc_2B(allyl) (Ipc = isopinocampheyl) at −100 °C and dihydroxylation/periodate cleavage of the double bond in the resulting homoallylic alcohol (after protection). Aldehyde **27** was thus obtained in 66% overall yield and with >90% *ee* for the three-step sequence from **24**.[31,32] Reaction of **27** with $Ph_3P{=}C(CH_3)I$ finally provided vinyl iodide **28**; as for **12** and **17**, the latter could be obtained only in moderate yield (42%).

As for the reaction of **5** with vinyl iodide **28**, the corresponding Suzuki–Miyaura couplings with **12** and **17** proceed-

ed with excellent efficiencies and provided the respective coupling products **34** and **38** in yields of 91 and 94%, respectively (Scheme 6).



Scheme 6. a) i) **5**, 9-BBN, THF, RT; ii) Cs_2CO_3, $[PdCl_2(dppf)_2]$, Ph_3As, **12** or **17**, −10 °C→RT, 91% (**34**) and 94% (**38**). b) LiOH, *i*PrOH/water 4:1, 52 °C, 15 h, 85%. c) TBAF (3 equiv), THF, RT, 18 h, 82%. d) i) Et_3N, 2,4,6-trichlorobenzoyl chloride, THF, 0 °C; ii) DMAP, toluene, 70–75 °C, 83% (**37**) and 75% (**40**). e) HF·pyridine, THF, RT, 22 h, 89% (**1b**) and 89% (**2b**). f) i) $MeReO_3$, H_2O_2, pyridine, CH_2Cl_2, RT, 46 h; ii) Raney Ni, MeOH, RT, 3 h, 39% (**1a**) and 39% (**2a**) (2 steps). g) LiOH, *i*PrOH/H_2O 4:1, 55 °C, 15 h, acidic work-up, 74%.

Fully protected seco acid **38**, which bears a C15-O-TES protecting group, was converted into Epo D analogue **2b** following the same sequence of transformations as described above for the elaboration of **29** into **31**. In particular, **38** could be converted into **40** in one step (74% yield) owing to the selective cleavage of the C15-TES ether during work-up. In contrast, the conversion of the coupling product **34** to the macrolactonization substrate **36** involved the removal of the C15-O-TBS group in a discrete step (after ester saponification); **36** was obtained in 70% overall yield from **34**. Compared to the cyclization of benzimidazole-containing seco acid **30**, macrolactonization was more efficient for the quinoline-derived intermediates **36** and **39**, which could be cyclized in yields of 83 and 75%, respectively (compared to 57% for **30**). We have not investigated the formation of side products in the macrolactonization of **30** in this study, but it should be noted that epothilone analogues incorporating 2,3-dimethylbenzimidazole-derived side chains have been obtained in yields comparable to those for quinoline-bearing macrocycles **37** and **40** (for the macrolactonization step).[22,23]

The Epo D analogues **1b** and **2b** were converted to the corresponding epoxide-based Epo B analogues **1a** and **2a** using the ReO_3/pyridine/H_2O_2 system developed by Sharpless[34] and Hermann.[35] In both cases, the epoxidation produced the desired epoxide isomer with about 6:1 selectivity, but it was also accompanied by N-oxidation of the quinoline side chain (which was, in fact, faster than the epoxidation of the C12/C13 double bond). However, conditions could be identified that allowed selective reduction of the *N*-oxide without affecting the epoxide moiety (and, in particular, without reductive cleavage of the benzylic ester moiety). Thus, careful catalytic hydrogenation of the *N*-oxides of **1a** and **2a** over Raney Ni gave the desired target compounds **1a** and **2a**, which were finally obtained in 39% yield in each case for the two-step sequence from **37** and **40**, respectively.

Antiproliferative activity: The effects of epothilone analogues **1**–**4** on human cancer cell growth in vitro were investigated for three different cell lines and the corresponding IC_{50} values are summarized in Table 1. Epoxide-containing analogues **1a** and **2a** were both found to exhibit highly potent antiproliferative activity, with IC_{50} values in the sub-nM range against all three cell lines. Compared to Epo B, the activities of these analogues are reduced only slightly and they are more active than Epo A. Most significantly, however, the growth inhibitory activities of **1a** and **2a** are independent of the position of the N-atom in the quinoline side chain. This finding clearly contrasts with the results of previous studies on pyridine-based Epo B analogues, which showed a 3-pyridyl derivative (corresponding to quinoline derivative **2a**) to be substantially less active than the corresponding 2-isomer (corresponding to **1a**).[14] In contrast to epoxide-containing analogues **1a** and **2a**, a significant difference in potency was observed between the (side-chain) isomeric Epo D derivatives **1b** and **2b**. The antiproliferative activity of **1b** is, quite remarkably, almost comparable to that of **1a**, in spite of the lack of an epoxide moiety, thus making this analogue significantly more potent than the corresponding parent compound Epo D (Table 1). Evidently, the activity-enhancing effect of the 7-quinolyl moiety (relative to the natural side chain) is more pronounced in combination with the epoxide-free Epo D macrocycle than with the epoxide-containing Epo B core structure; this is in line with previous observations on the increase in antiproliferative activity associated with a 2,3-dimethylbenzimidazole side chain, which was also found to be more pronounced in the Epo D series than in the Epo B series.[22,23] Analogues **1a** and **1b** were also investigated in the highly P-gp-overexpressing, multidrug-resistant human cervix carcinoma cell line KB-8511.[36] No loss in activity was observed against this cell line compared to the drug-sensitive KB-31 parental line

Table 1. Cancer cell growth inhibition by epothilone analogues **1–4** (IC_{50} [nM])[a]

	HCT-116 (colon)	A549 (lung)	MCF-7 (breast)
1a	0.22 ± 0.04	0.46 ± 0.08	0.59 ± 0.08
1b	0.82 ± 0.07	0.91 ± 0.09	1.21 ± 0.16
2a	0.57 ± 0.07	0.49 ± 0.01	0.74 ± 0.14
2b	112 ± 8	107 ± 5	134 ± 11
3	–[b]	13.0 ± 4.8	10.5 ± 3.0
4	–[b]	108 ± 14	65 ± 12
Epo A	2.8 ± 0.4	–[b]	2.9 ± 0.3
Epo B	0.16 ± 0.01	0.34 ± 0.03	0.33 ± 0.01
Epo D[c]	4.48 ± 0.47	4.62 ± 2.19	2.31 ± 0.55

[a] For compound structures, see Figure 1. Cells were exposed to the test compounds for 72 h. Cell numbers were estimated by quantification of the protein content of fixed cells by methylene blue staining (cf. Supporting Information). Values shown are the means of three independent experiments (± standard deviation). For compounds **3** and **4**, the bis- and mono-TFA salts were used, respectively. Data for Epo D are from ref. [15]. [b] Not determined. [c] Epo D = 12,13-deoxyEpo B; see also ref. [16].

(IC_{50} values of **1a** and **1b** against the KB-31/KB-8511 lines were 0.1 nM/0.1 nM and 0.59 nM/0.38 nM, respectively), thus indicating that neither compound is a substrate for the P-gp efflux pump.

Compared to **1b**, the antiproliferative activity of its side-chain isomer **2b** is >100-fold lower, which is in line with expectation based on the data previously reported for pyridine-based Epo B analogues[14] (see above). As will be shown below, the difference in cellular activity between **1b** and **2b** is paralleled by a significant difference in MT-binding affinity. However, a similar difference in MT binding also exists for epoxide-containing analogues **1a** and **2a** and this does not translate into significantly different growth inhibitory activities for the two compounds. In addition, the >100-fold activity increase observed upon incorporation of a C12,C13-epoxide moiety into **2b** (**2b** → **2a**) is substantially higher than what is usually observed for transitions from the Epo D to the Epo B series (5–30-fold; cf., e.g., the activities of Epo B and Epo D in Table 1).[7] Thus, the very potent antiproliferative activity of **2a** is very surprising and it cannot be readily accommodated within the general SAR landscape that has been delineated for epothilones over the last few years.[7]

Benzimidazole-based analogues **3** and **4** exhibit IC_{50} values for cancer cell growth inhibition in the range 10–100 nM, which makes them significantly less potent than the corresponding 2,3-dimethylbenzimidazole-based Epo B analogue (IC_{50} values in the sub-nM range have been observed for the latter against a variety of human cancer cell lines in vitro[17,37]). While these findings might be simply attributed to the increased size of the benzimidazole 3-substituent in **3** and **4** (compared to a simple methyl group), it should be noted that Nicolaou and co-workers have recently demonstrated a significant tolerance to steric bulk in the northeastern quadrant of the epothilone structure,[38] which should also apply to the modified side chains present in analogues **3** and **4**. Alternatively, the presence of ionizable groups in **3** and **4** (possibly in addition to the benzimidazole moiety, the pK_a of which in **3** and **4** is unknown) could lead to changes in cellular uptake and/or intracellular distribution of the compounds that could adversely affect their cellular activity. These issues have not been investigated, but it should be noted that, based on preliminary data in one cell line, the *N*-Boc derivative of **3** (which was obtained from fully protected macrolactone **31** (Scheme 5) by partial deprotection with HF·pyridine) appears to be equally as potent as **3**. This finding would indicate that the free primary amino group in **3** does not have a negative impact on cellular potency; in addition, it also suggests that the reduction in activity associated with the succinylation of **3** (**3** → **4**) is not simply a consequence of the increased size of the substituent on the benzimidazole moiety.

The design of analogues **3** and **4** was driven by the idea of providing functionalized epothilone analogues that could be readily converted to tumor-targeted prodrugs by the attachment of appropriate targeting moieties to the amino or carboxyl groups, respectively. In principle, tumor-targeted conjugates could also be prepared from unmodified natural epothilones, utilizing one of the two hydroxyl groups at C3 and C7 as an anchoring point; however, the hindered nature of these nucleophiles may render such an approach less than straightforward. While we had anticipated **3** and **4** to be somewhat more potent inhibitors of cancer cell growth than was eventually observed experimentally, it should be emphasized that both compounds are still potent antiproliferative agents and thus represent interesting candidates for the development of tumor-targeted prodrugs. The synthesis of such analogues is currently in progress in our laboratory and the results of these efforts will be reported in future publications.

Interactions with tubulin/microtubules: The interactions of epothilone analogues **1–4** with the tubulin/MT system were first assessed through a tubulin polymerization assay, which provides a measure of the ability of a compound to induce the assembly of soluble αβ-tubulin heterodimers into MT-like polymers.[39,40] The extent of tubulin polymerization is determined for different ligand concentrations at a fixed concentration of soluble tubulin (in our case 10 μM), with the concentration leading to 50% of the maximum polymerization observed (EC_{50}) serving as a comparator for the tubulin-polymerizing capacity of different ligands. While this approach allows the rapid identification of compounds that are devoid of any MT-stabilizing properties, due to the often small differences in EC_{50} values the unambiguous ranking of agents with measurable tubulin-polymerizing activity is more difficult. In addition, it has been demonstrated by Hamel and co-workers that the relative rank order of tubulin-polymerizing activity within a series of compounds may vary depending on the exact experimental conditions employed in the polymerization experiments.[41]

Within these limitations, the EC_{50} values summarized in Table 2 for epothilone analogues **1–4** suggest the following rank order of tubulin-polymerizing activities: **1a** ~ **1b** > **2a** ~ **3** ~ **4** > **2b**. For **1a**/**b** and **2b**/**3**/**4**, this rank order is

(qualitatively) consistent with the pronounced difference in antiproliferative activities between the two groups of compounds, while compound **2a** might have been expected to be a less active inhibitor of cancer cell growth than was actually observed (Table 1). It should be emphasized, however, that differences between the EC_{50} values shown in Table 2 are small (with overlapping standard deviations for several compound pairs) and should not be overinterpreted.

Table 2. Interactions of epothilone analogues **1**–**4** with tubulin and stabilized MTs.

	EC_{50} TubPol.[a] [μM]	Cr[b] [μM]	K_{el} TubPol.[c] [$10^5\,M^{-1}$]	$-\Delta G_{el}$ MTs[d] [kJ mol^{-1}]	K_b MTs[e] [$10^7\,M^{-1}$]	$-\Delta G$ MTs[f] [kJ mol^{-1}]
1a	3.2±0.4	0.28±0.03	37.4±0.3	38.7±0.2	91.8±13.2	52.8±0.3
1b	3.4±0.5	0.29±0.12	34.3±9.9	38.5±0.6	88.0±3.7	52.7±0.1
2a	4.3±0.8	0.34±0.02	29.3±1.3	38.1±0.1	6.92±0.22	46.2±0.3
2b	5.2±0.5	0.40±0.05	25.2±2.8	37.7±0.3	6.12±0.23	45.9±0.1
3	4.3±0.8	0.53±0.12	18.8±3.4	37.2±0.5	23.4±0.8	49.3±0.1
4	4.1±0.5	0.51±0.10	19.6±3.2	37.3±0.4	20.7±1.0	49.0±0.1
Epo A	3.9±0.6	0.41±0.19	24.7±7.9	37.7±0.7	3.63±0.5[g]	44.5±0.3[g]
Epo B	3.0±0.3	0.26±0.12	38.7±1.2	38.8±0.7	75.0±7.4[g]	52.6±0.5[g]
Epo D	–[h]	0.29±0.04	34.5±4.2	38.8±0.4	14.2±3.8	48.0±0.7

[a] Concentration required to induce 50% of maximal tubulin polymerization at 25°C (10 μM of porcine brain α/β tubulin). For compounds **3** and **4**, the bis- and mono-TFA salts were used, respectively. [b] Critical tubulin concentration in the presence of the respective ligand at 37°C. *Cr* in the absence of ligand was 3.30±1.16 μM, corresponding to a K_{el} of $3.0\pm0.8\times10^5\,M^{-1}$ and a ΔG_{el} of -32.3 ± 0.6 kJ mol^{-1}. [c] Equilibrium constant for the MT growth reaction, that is, for the addition of a new tubulin heterodimer to a pre-existing MT polymer at 37°C. [d] Binding free energy for the MT growth reaction. [e] Association constant with glutaraldehyde-stabilized MTs at 35°C, as determined by the Epo B displacement method of Matesanz et al.[46] [f] Binding free energy for the association with glutaraldehyde-stabilized MTs. Errors are standard deviations for EC_{50} values and standard errors of the mean for all other parameters. [g] Data are from ref. [43]. [h] Not determined.

An alternative measure of the ability of tubulin-interacting agents to promote the tubulin assembly reaction is the apparent critical tubulin concentration, *Cr*, in the presence of an MT-stabilizing agent (*Cr* being the concentration below which no significant formation of large polymers occurs).[42–44] Assuming the assembly process to be ligand-mediated (i.e., with ligand binding preceding MT assembly), the inverse of the *Cr* represents a good approximation of the equilibrium constant K_{el} for the growth reaction, that is, for the addition of a new tubulin heterodimer to a pre-existing polymer [$1/Cr = K_{el}$; Eqs. (1) and (2)].[42,43] Two thermodynamically equivalent pathways can be envisaged for the ligand-mediated assembly reaction, with the ligand ("L") binding to either unassembled tubulin ("Tub") [Eq. (1)] or to the ends of the MT [Eq. (2)].[42,43]

$$MT + L + Tub \rightleftharpoons MT + L\cdot Tub \overset{K_{el}}{\rightleftharpoons} Tub\text{-}MT\cdot L \qquad (1)$$

$$MT + L + Tub \rightleftharpoons MT\cdot L + Tub \overset{K_{el}}{\rightleftharpoons} Tub\text{-}MT\cdot L \qquad (2)$$

The results of *Cr* measurements in the presence of epothilone analogues **1** and **2** are in excellent agreement with the data obtained from the tubulin polymerization assay, with the rank order of apparent *Cr* values in glycerol assembly buffer (GAB) at 37°C (Table 2) being identical to the rank order of EC_{50} values from the polymerization assay. Thus, both methods for the assessment of tubulin-polymerizing capacity suggest that the assembly reaction is promoted most efficiently by analogues **1a** and **1b**, while analogue **2b** is clearly least active and the activity of **2a** is intermediate between those of **1a/b** and **2b**. As pointed out above, this rank order does not reflect the rank order of the antiproliferative activities of the compounds, thus indicating that the induction of tubulin polymerization in vitro may not be a very reliable predictor of growth inhibitory activity in cells. At the same time, both EC_{50} values for the induction of tubulin polymerization as well as apparent *Cr* values for tubulin assembly are spread only over a narrow numerical range, which hampers the detection of possible correlations with IC_{50} values for growth inhibition, at least for a limited number of compounds.

In an attempt to achieve a better resolution of the MT-interaction potential of the quinoline-based epothilone analogues **1** and **2**, and thus to gain a better understanding of the relationship between N-positioning in the side chain and MT-binding affinity, we also investigated the binding of **1a/b** and **2a/b** to stabilized MTs.[45] These binding studies were performed according to a protocol that was recently developed by Matesanz et al.[46] and utilizes Epo B as a reference ligand, the displacement of which from MTs by a given test compound is quantified by HPLC. As illustrated by the data shown in Table 2, this approach revealed a clear difference between the MT-binding affinities of analogues **1a/1b** and **2a/2b**.

While **1a** and **1b** bind to MTs with similar affinities and K_b values of the order of 10^9, the binding affinity of the respective isomers **2a** and **2b** are 13- and 14-fold lower, respectively. Interestingly, the difference in MT-binding affinities between **1a/b** and **2b** mirrors the separation of these analogues in the tubulin assembly experiments, although the differences in K_b values are clearly more pronounced than those between the EC_{50} values for polymerization induction or the *Cr* values for tubulin assembly. For analogue **2a**, the correlation between MT-binding and the promotion of tubulin assembly is less obvious; while **2a** binds to MTs with similar affinity as **2b**, and thus one order of magnitude less tightly than **1a/b**, its ability to promote tubulin assembly appeared to be intermediate between those of **1a/b** and **2b** (Table 2). Based on the MT-binding data, the position of the N-atom in the quinoline side chain seemingly has a significant impact on the interactions of analogues **1** and **2** with MTs, with the location of the N-atom in the "natural" position leading to higher-affinity binding. While this may be taken to indicate that the side chain N-atom in **1a/b** (and, by inference, the thiazole nitrogen in the natural side chain)

is involved in hydrogen bonding to tubulin,[11] this conclusion is not inevitable. Recent NMR-based structural studies on the tubulin-bound conformations of **1a** and **2a** indicate that the quinoline side chains in these ligands adopt distinctly different conformations, thus affecting their stacking interactions with His-227[47] (which have been suggested by Carlomagno and co-workers to be more relevant for the tubulin binding of epothilones than hydrogen bonding[13] (see above)).

Based on the critical tubulin concentrations for benzimidazole derivatives **3** and **4**, these compounds are less potent promoters of tubulin assembly than quinoline-based analogues **1a/b** and **2a/b**. With regard to **1a** and **1b**, this conclusion is also supported by the EC_{50} values obtained in the tubulin polymerization assay (which are lower for **1a** and **1b** than for **3** or **4**), although the latter would also suggest compounds **3** and **4** to possess similar tubulin-polymerizing activities as analogue **2a** and to be more potent inducers of tubulin polymerization than **2b**. The MT-binding affinities of **3** and **4** are higher than those of **2a** and **2b**, but still substantially lower than those of **1a** and **1b**; in addition, and somewhat surprisingly, **3** and **4** also bind to MTs with comparable affinity to Epo D, which contrasts with the substantially different activities of **4** and Epo D at the cellular level (Table 1). It may be speculated that the reduced cellular potency of **4**, relative to Epo D, is related to reduced cellular uptake due to the presence of the negatively charged N3-substituent on the benzimidazole moiety, although we do not have any experimental data that would directly support this hypothesis.

Conclusions

We have accomplished the stereoselective synthesis of a series of side-chain-modified epothilone analogues displaying varying antiproliferative activity, tubulin-assembling potential, and MT-binding affinity. Based on the results of the MT-binding studies, the position of the side chain N-atom in quinoline-based epothilone analogues **1** and **2** is an important determinant of MT-binding affinity. For **1a/b** and **2b**, the binding data are consistent with the relative effects of these analogues on tubulin polymerization, while the correlation is less clear for **2a**. For **1b** and **2b**, the difference in MT-binding affinity is clearly reflected in their cellular potencies, whereas **1a** and **2a** show virtually identical antiproliferative activity, in spite of a more than ten-fold difference in MT-binding affinity. The reasons for this discrepancy remain to be elucidated. Benzimidazole-based analogues **3** and **4**, although less potent than quinoline derivatives **1a/b** and **2a**, are still potent antiproliferative agents in vitro. Due to the presence of a free amino or carboxyl group, these analogues may be readily conjugated with appropriately functionalized tumor-targeting moieties and are thus attractive building blocks for the preparation of tumor-targeted prodrugs. Experiments along these lines are currently ongoing in our laboratory.

Experimental Section

General: All solvents used for reactions were purchased as anhydrous grade from Fluka and were used without further processing. Solvents for extractions, column chromatography, and TLC were commercial grade and were distilled before use. TLC was performed on Merck TLC aluminum sheets (silica gel 60 F_{254}). Spots were visualized with UV light ($\lambda =$ 254 nm) or through staining with phosphomolybdic acid or $KMnO_4$. Flash column chromatography (FC) was performed using Fluka silica gel 60 for preparative column chromatography (40–63 μm), unless specifically noted otherwise. NMR spectra were recorded on a Bruker AMX-300 (300 MHz), a Bruker AV-400 (400 MHz), or a Bruker DRX-500 (500 MHz) spectrometer at room temperature (298 K). Infrared spectra (IR) were recorded on a Jasco FT/IR-6200 instrument. Optical rotations were measured on a Jasco P-1020 polarimeter. Melting points were measured on a Büchi B-540 apparatus and are uncorrected. RP-HPLC analyses were carried out on a Waters Symmetry column (C18, 3.5 μm, 4.6 × 100 mm) at a flow rate of 1 mL min^{-1} and with a detection wavelength of 254 nm. For the purification of analogues **1a/b** and **2a/b**, elution was performed with water/CH_3CN gradients without addition of TFA to the mobile phase. For **3** and **4**, the TFA-free system produced only broad peaks and therefore compounds were eluted with 0.1% aqueous TFA/0.1% TFA in CH_3CN. Preparative RP-HPLC was carried out using a Waters Symmetry column (C18, 5 μm, 19 × 100 mm) at a flow rate of 25 mL min^{-1}, using the same solvent systems as for the respective analytical separations.

Quinoline-7-carboxaldehyde (7): 7-Methylquinoline (**6**; 10.0 g, 69.84 mmol) was heated to 160 °C and SeO_2 (5.1 g, 45.96 mmol) was added portionwise at this temperature (evolution of gas). The mixture was then maintained at 150–161 °C for 18 h. After cooling to RT, CH_2Cl_2 (60 mL) was added, leading to the formation of a dark precipitate, which was filtered off. After concentration of the filtrate to a volume of about 25 mL, hexane (100 mL) was added, which produced a second viscous, dark-red to brownish precipitate. Filtration of this mixture and concentration of the filtrate gave the crude product, which was purified by FC eluting with AcOEt/hexane 1:1 to give the target compound **7** as light-yellow crystals (4.22 g, 58%). M.p. 81–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta = 10.26$ (d, $J = 0.6$ Hz, 1H), 9.05 (dd, $J = 4.3$, 1.7 Hz, 1H), 8.62 (s, 1H), 8.27 (d, $J = 8.4$ Hz, 1H), 8.07 (dd, $J = 8.5$, 1.6 Hz, 1H), 7.96 (d, $J = 8.5$ Hz, 1H), 7.58 ppm (dd, $J = 8.3$, 4.2 Hz, 1H); IR (film): $\tilde{\nu} = 1695$, 1116, 840, 800, 775, 758, 754 cm^{-1}; MS (ESI): m/z (%): 158 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{10}H_7NO$: 157.0528 [M^+]; found: 157.0522.

Acetylsultam 8: AcCl (8.25 mL, 116.13 mmol) was added to a solution of (−)-10,2-camphorsultam (10.0 g, 46.44 mmol) in dry CH_3CN (200 mL) and the solution was heated under reflux for 19 h. After cooling to RT, K_2CO_3 (12.83 g, 92.84 mmol) was added and the mixture was stirred for 2 h at RT. The solvent was then evaporated and the residue was partitioned between CH_2Cl_2 (50 mL) and water (150 mL). The organic layer was separated and the aqueous solution was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were dried over $MgSO_4$, the solvent was evaporated, and the residue was recrystallized from EtOH to provide **8** as white needles (10.98 g, 84%). M.p. 129–131 °C; $[\alpha]_D^{RT} = -104.07°$ ($c = 2.32$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): $\delta =$ 3.87–3.81 (m, 1H), 3.46 (dd, $J = 28.4$ Hz, $J = 13.9$ Hz, 2H), 2.40 (s, 3H), 2.19–2.02 (m, 2H), 1.97–1.83 (m, 3H), 1.46–1.30 (m, 2H), 1.15 (s, 3H), 0.97 ppm (s, 3H); IR (film): $\tilde{\nu} = 1691$, 1330, 1283, 1166, 1137, 983, 746, 668 cm^{-1}; MS (ESI): m/z (%): 258.64 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{12}H_{19}NO_3S$: 257.1086 [M^+]; found: 257.1082.

Aldol product 9: CF_3SO_3H (1.8 mL, 20.63 mmol) was added to a solution of Et_3B in hexane (15%, 20.8 mL, 21.55 mmol) and the mixture was stirred under Ar for 10 min at 40 °C. After cooling to RT, CH_2Cl_2 (6 mL) was added and stirring was continued for an additional 15 min. The mixture was then cooled to 0 °C and a solution of **8** (3.95 g, 15.34 mmol) in CH_2Cl_2 (8 mL) was added over 10 min. Thereafter, a solution of diisopropylethylamine (3.4 mL, 19.85 mmol) in CH_2Cl_2 (4 mL) at −5 °C was added dropwise over a period of 10 min. After cooling this mixture to −78 °C, a solution of aldehyde **7** (3.37 g, 21.44 mmol) in CH_2Cl_2 (17 mL) was finally added dropwise over 35 min and the reaction mixture was

stirred at −78 °C for 3 h. The mixture was then allowed to warm to −10 °C, saturated aqueous NH_4Cl solution (50 mL) was added, and the solution was extracted with CH_2Cl_2 (3×140 mL). The combined organic extracts were washed with water (140 mL), dried over $MgSO_4$, and concentrated. The residue was purified by FC eluting with AcOEt/hexane 3:2 to yield **9** (5.97 g, 80 %) as a 5:1 mixture of diastereoisomers (white-yellow foam). This mixture was separated after conversion to the TBS ethers **10** (see below). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.91 (dd, J = 4.3, 1.7 Hz, 1H), 8.18–8.12 (m, 2H), 7.85–7.80 (m, 1H), 7.68–7.60 (m, 1H), 7.42–7.38 (m, 1H), 5.43 (dd, J = 8.3, 4.1 Hz, 1H), 3.90 (dd, J = 7.6, 4.9 Hz, 1H), 3.52–3.41 (m, 2H), 3.32–3.16 (m, 2H), 2.22–2.02 (m, 2H), 1.97–1.80 (m, 3H), 1.45–1.30 (m, 2H), 1.13–1.02 (m, 3H), 0.98–0.91 ppm (m, 3H); IR (film): $\tilde{\nu}$ = 2958, 2880, 1691, 1330, 1137, 1116, 840, 771 cm^{-1}; MS (ESI): m/z (%): 414.52 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{22}H_{26}N_2O_4S$+H: 415.1692 [M+H$^+$]; found: 415.1677.

TBS-protected alcohol 10: Imidazole (2.51 g, 36.87 mmol) and TBSCl (2.82 g, 18.71 mmol) were added to a solution of aldol product **9** (5.07 g, 12.23 mmol) in DMF (80 mL) and the reaction mixture was stirred at 40 °C for 17 h. The solution was then concentrated, CH_2Cl_2 (50 mL) and water (300 mL) were added, and the organic layer was separated. The aqueous solution was additionally extracted with CH_2Cl_2 (300 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated. The residue was purified by FC (two columns; hexane/AcOEt 3:2) to give the protected alcohol **10** (4.06 g, 63 %) as a light-yellow oil (single isomer). $[\alpha]_D^{RT}$ = −54.70° (c = 3.34 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.89 (dd, J = 4.3, 1.8 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 7.98 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.5, 1.7 Hz, 1H), 7.37 (dd, J = 8.2, 4.3 Hz, 1H), 5.46 (t, J = 6.7 Hz, 1H), 3.78 (dd, J = 7.8, 4.9 Hz, 1H), 3.35 (s, 2H), 3.19 (dd, J = 6.7, 2.5 Hz, 2H), 2.06–1.95 (m, 1H), 1.88–1.74 (m, 3H), 1.68 (t, J = 3.9 Hz, 1H), 1.38–1.23 (m, 2H), 0.86 (s, 9H), 0.82 (s, 3H), 0.54 (s, 3H), 0.07 (s, 3H), −0.14 ppm (s, 3H); IR (film): $\tilde{\nu}$ = 2958, 2930, 2858, 1695, 1334, 1133, 1091, 1076, 840, 775 cm^{-1}; MS (ESI): m/z (%): 529.84 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{28}H_{40}N_2O_4SSi$+H: 529.2556 [M+H$^+$]; found: 529.2542.

Aldehyde 11: A 1 M solution of DIBAL-H in CH_2Cl_2 (19.00 mL, 19.00 mmol) was added dropwise to a solution of **10** (3.96 g, 7.49 mmol) in CH_2Cl_2 (30 mL) over a period of 20 min at −78 °C under Ar and the mixture was stirred at this temperature for 3 h. Water (20 mL) was then added to quench the reaction, the mixture was diluted with additional water (400 mL) and CH_2Cl_2 (400 mL), and the pH was adjusted to basic with 1 N NaOH (40 mL). The organic layer was then separated and the aqueous solution was additionally extracted with CH_2Cl_2 (2×300 mL). The combined organic extracts were washed with water (300 mL), dried over $MgSO_4$, and the solvent was evaporated. Purification of the residue by FC eluting with hexane/AcOEt 1:1 (two columns) gave aldehyde **11** (1.84 g, 78 %) as a light-yellow, transparent oil. $[\alpha]_D^{RT}$ = −58.22° (c = 2.72 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.84 (dd, J = 2.7, 1.9 Hz, 1H), 8.93 (dd, J = 4.3, 1.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.05 (s, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.59 (dd, J = 8.5, 1.7 Hz, 1H), 7.41 (dd, J = 8.2, 4.3 Hz, 1H), 5.45 (dd, J = 8.1, 4.2 Hz, 1H), 2.99–2.91 (m, 1H), 2.76–2.68 (m, 1H), 0.89 (s, 9H), 0.09 (s, 3H), −0.12 ppm (s, 3H); IR (film): $\tilde{\nu}$ = 2955, 2930, 2858, 1724, 1255, 1219, 1091, 833, 775 cm^{-1}; MS (ESI): m/z (%): 316.65 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{18}H_{25}NO_2Si$+H: 316.1733 [M+H$^+$]; found: 316.1720.

Vinyl iodide 12: A 1 M solution of Na-bis(trimethylsilyl)amide (7.00 mmol) in THF (7 mL) was added dropwise over a period of 15 min to a stirred mixture of $[Ph_3PCH(CH_3)I]I$ (3.97 g, 7.30 mmol) and THF (180 mL) at −78 °C under Ar. The mixture was then stirred for an additional 35 min at −78 °C and thereafter for 20 min at −15 °C. After cooling to −78 °C once more, a solution of **11** (1.84 g, 5.83 mmol) in THF (10 mL) was added dropwise over 5 min and the mixture was stirred at −78 °C for an additional 40 min. The reaction was then quenched by the addition of saturated aqueous NH_4Cl solution (40 mL) and the resulting mixture was poured into a mixture of Et_2O (400 mL) and water (200 mL). The organic layer was separated, washed with water (2× 100 mL), dried over $MgSO_4$, and concentrated. Purification of the residue by FC, eluting with hexane/Et_2O 1:1, gave the target compound **12** as a yellow oil (1.07 g, 41 %). $[\alpha]_D^{RT}$ = −9.38° (c = 0.96 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 8.92 (dd, J = 4.3, 1.8 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 8.02 (s, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.4, 1.7 Hz, 1H), 7.39 (dd, J = 8.3, 4.3 Hz, 1H), 5.52–5.47 (m, 1H), 4.89 (dd, J = 7.2, 5.3 Hz, 1H), 2.65–2.49 (m, 2H), 2.49–2.46 (m, 3H), 0.91 (s, 9H), 0.07 (s, 3H), −0.09 ppm (s, 3H); IR (film): $\tilde{\nu}$ = 2955, 2930, 2855, 2360, 2332, 1473, 1455, 1252, 1093, 947, 940, 836, 668 cm^{-1}; MS (ESI): m/z (%): 454.72 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{20}H_{28}INOS$+H: 454.1063 [M+H$^+$]; found: 454.1051.

Ester 34: 9-BBN (90 mg, 0.74 mmol) was added to a solution of alkene **5** (156 mg, 0.29 mmol) in THF (1.8 mL) and the mixture was stirred under Ar at RT for 2.5 h (solution A). In a separate flask, a solution of vinyl iodide **12** (0.100 g, 0.22 mmol) in THF (1 mL, 0.684 g, 2 mmol) was added to a mixture of H_2O (48 μL, 2.67 mmol), DMF (1.5 mL), Cs_2CO_3 (144 mg, 0.44 mmol), $AsPh_3$ (18 mg, 0.06 mmol), and [Pd-(dppf)$_2$Cl$_2$]·CH_2Cl_2 (22 mg, 0.03 mmol) (solution B). Solution B was cooled to −10 °C and solution A was added dropwise over a period of 10 min. The mixture was allowed to warm to RT and then stirred for 2 h. It was then diluted with AcOEt (17 mL) and water (9 mL), the layers were separated, and the aqueous solution was further extracted with AcOEt (2×9 mL). The combined organic extracts were washed with water (3×5 mL), dried over $MgSO_4$, and the solvent was evaporated. Purification of the residue by FC eluting with hexane/Et_2O 3:2 gave **34** (170.5 mg, 91 %) as a dark-yellow oil. $[\alpha]_D^{RT}$ = −19.75° (c = 3.06 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.91 (dd, J = 4.3, 1.7 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.00 (s, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.40 (dd, J = 8.1, 4.3 Hz, 1H), 5.17 (t, J = 7.1 Hz, 1H), 4.83 (t, J = 6.4 Hz, 1H), 4.37 (dd, J = 6.9, 3.2 Hz, 1H), 3.73 (dd, J = 7.1, 1.9 Hz, 1H), 3.65 (s, 3H), 3.14–3.05 (m, 1H), 2.70–2.33 (m, 4H), 2.25 (dd, J = 16.1, 6.9 Hz, 1H), 1.95–1.81 (m, 3H), 1.64 (s, 3H), 1.34–1.21 (m, 3H), 1.19 (s, 3H), 1.03 (s, 3H), 1.02 (d, J = 7.7 Hz, 3H), 0.91–0.88 (m, 3H), 0.89 (s, 9H), 0.87 (s, 9H), 0.85 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H), 0.00 (s, 3H), −0.11 ppm (s, 3H); IR (film): $\tilde{\nu}$ = 2952, 2930, 2858, 1741, 1695, 1469, 1255, 1083, 986, 940, 833, 775, 671 cm^{-1}; MS (ESI): m/z (%): 857.26 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{48}H_{85}NO_6Si_3$+H: 856.5763 [M+H$^+$]; found: 856.5742.

Carboxylic acid 35: LiOH (57 mg, 2.38 mmol) was added to a solution of the coupling product **34** (333 mg, 0.39 mmol) in *i*PrOH/H_2O 4:1 (10 mL) and the mixture was maintained at 52 °C for 15 h. After cooling to RT, water (3.3 mL) was added and most of the *i*PrOH was removed by evaporation. The remaining solution was treated with CH_2Cl_2 (41 mL), water (33 mL), and 1 N HCl (2.5 mL). The layers were separated and the aqueous solution was extracted with CH_2Cl_2 (2×25 mL). The combined organic extracts were washed with water (4 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by FC eluting with CH_2Cl_2/MeOH 97:3 to yield the target compound **35** as a light-yellow oil (287 mg, 85 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.86 (s, 1H), 8.49 (s, 1H), 8.27 (s, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.47 (s, 1H), 5.36–5.27 (m, 1H), 4.92 (dd, J = 9.5, 3.0 Hz, 1H), 4.55 (dd, J = 7.4, 2.6 Hz, 1H), 3.68 (t, J = 3.3 Hz, 1H), 3.26–3.15 (m, 1H), 2.47–2.29 (m, 5H), 1.91–1.80 (m, 1H), 1.73 (s, 3H), 1.65–1.50 (m, 4H), 1.25 (s, 3H), 1.24–1.18 (m, 1H), 1.10 (s, 3H), 1.09 (d, J = 6.0 Hz, 3H), 1.07–1.04 (m, 1H), 0.91 (d, J = 7.0 Hz, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.87 (s, 9H), 0.17 (s, 3H), 0.13 (s, 3H), 0.09 (s, 3H), 0.02 (s, 3H), −0.01 (s, 3H), −0.10 ppm (s, 3H); IR (film): $\tilde{\nu}$ = 2955, 2930, 2858, 1702, 1466, 1252, 1087, 986, 836, 775 cm^{-1}; MS (ESI): m/z (%): 842.43 (100) [M+H$^+$]; HRMS: m/z: calcd for $C_{47}H_{83}NO_6Si_3$+H: 842.5606 [M+H$^+$]; found: 842.5585.

Seco acid 36: A 1 M solution of TBAF in THF (1.4 mL, 1.4 mmol) was added to a solution of **35** (375 mg, 0.45 mmol) in THF (7 mL) and the mixture was stirred at RT for 18 h. Water and AcOEt were then added and the layers were separated. The pH of the aqueous solution was adjusted to 4.5 and it was re-extracted with the AcOEt phase. The layers were separated once more and the above process was repeated. The aqueous solution was then additionally extracted with AcOEt (2× 42 mL), and the combined organic extracts were washed with water (28 mL), dried over $MgSO_4$, and concentrated. The residue was purified by FC eluting with CH_2Cl_2/MeOH 94:6 to provide the target compound **36** as a transparent, slightly yellow oil (264 mg, 82 %) . $[\alpha]_D^{RT}$ = −5.40° (c = 0.5 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.86 (dd, J = 4.4,

1.6 Hz, 1H), 8.52 (s, 1H), 8.25 (d, J=7.5 Hz, 1H), 7.85 (d, J=8.4 Hz, 1H), 7.69 (dd, J=8.4, 1.5 Hz, 1H), 7.45 (dd, J=8.2, 4.4 Hz, 1H), 5.33–5.30 (m, 1H), 4.89 (dd, J=8.3, 4.8 Hz, 1H), 4.53 (t, J=5.6 Hz, 1H), 3.74 (dd, J=4.2, 2.8 Hz, 1H), 3.21–3.18 (m, 1H), 2.55–2.49 (m, 2H), 2.48–2.44 (m, 2H), 2.43–2.34 (m, 1H), 1.95–1.85 (m, 1H), 1.78 (s, 3H), 1.65–1.50 (m, 4H), 1.21 (s, 3H), 1.20–1.07 (m, 2H), 1.12 (s, 3H), 1.10 (d, J=6.2 Hz, 3H), 0.94 (d, J=6.5 Hz, 3H), 0.89 (s, 9H), 0.86 (s, 9H), 0.13 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H), 0.02 ppm (s, 3H); IR (film): $\tilde{\nu}$=2955, 2926, 2858, 1713, 1695, 1465, 1255, 1219, 1080, 986, 836, 768 cm^{-1}; MS (ESI): m/z (%): 729.18 (100) [M+H^+]; HRMS: m/z: calcd for $C_{41}H_{70}NO_6Si_2$+H: 728.4742 [M+H^+]; found: 728.4723.

Protected macrolactone 37: Et_3N (0.271 mL, 1.95 mmol) was added to a solution of seco acid **36** (235 mg, 0.32 mmol) in THF (8 mL) at 0 °C under Ar, and then 2,4,6-trichlorobenzoyl chloride (0.254 mL, 1.63 mmol) was added. After stirring at 0 °C for 30 min, the solution was diluted with THF (25 mL) and then added over a period of 2.5 h to a previously prepared solution of 4-dimethylaminopyridine (0.401 g, 3.29 mmol) in toluene (325 mL) at 70 °C under vigorous stirring. The reaction mixture was then evaporated to dryness and the residue was treated with Et_2O (60 mL). Insoluble material was removed by filtration and the filtrate was concentrated. The crude product was purified by FC (hexane/AcOEt 4:1; two columns) to afford bis-TBS-protected macrolactone **37** as a light-yellow oil (190 mg, 83%). $[\alpha]_D^{RT}=-26.54°$ (c=1.8 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.92 (dd, J=4.3, 1.7 Hz, 1H), 8.16 (d, J=8.2 Hz, 1H), 8.11 (s, 1H), 7.83 (d, J=8.5 Hz, 1H), 7.56 (dd, J=8.4, 1.6 Hz, 1H), 7.40 (dd, J=8.2, 4.4 Hz, 1H), 5.66 (d, J=10.1 Hz, 1H), 5.26 (t, J=8.3 Hz, 1H), 3.97 (dd, J=9.3, 2.8 Hz, 1H), 3.91 (d, J=8.9 Hz, 1H), 3.10–3.00 (m, 1H), 3.00–2.88 (m, 1H), 2.86–2.72 (m, 2H), 2.67–2.54 (m, 1H), 2.49–2.12 (m, 1H), 1.85–1.75 (m, 1H), 1.72 (s, 3H), 1.68–1.53 (m, 3H), 1.25–1.07 (m, 8H), 1.15 (s, 3H), 1.01 (d, J=6.6 Hz, 3H), 0.97 (s, 9H), 0.85 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H), −0.09 ppm (s, 3H); IR (film): $\tilde{\nu}$=2952, 2930, 2858, 1741, 1695, 1466, 1380, 1252, 1155, 1097, 1019, 983, 833, 775 cm^{-1}; MS (ESI): m/z (%): 711.20 (100) [M+H^+]; HRMS: m/z: calcd for $C_{41}H_{67}NO_5Si_2$+H: 710.4636 [M+H^+]; found: 710.4618.

Macrolactone 1b: HF·pyridine (14 mL in total) was added in five portions over a period of 21.5 h to a solution of bis-TBS-protected **37** (180 mg, 0.25 mmol) in THF (21 mL) in a Teflon tube at 0 °C. The mixture was stirred at RT between additions. The reaction mixture was then added dropwise at 0 °C to saturated aqueous $NaHCO_3$ solution (400 mL). Additional solid $NaHCO_3$ (10 g) was then added (pH 8–9) and the aqueous solution was extracted with AcOEt (3×160 mL). The combined organic extracts were washed with water (200 mL), dried, and concentrated in vacuo. The crude product was purified by FC eluting with CH_2Cl_2/MeOH 97:3 to provide **1b** (108 mg, 89%) as a colorless, viscous foam. A portion of this material (56 mg) was additionally purified by RP-HPLC (20% CH_3CN/water for 2 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min) to give **1b** as a white lyophilisate (33 mg). $[\alpha]_D^{RT}=-44.00°$ (c=0.7 in $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.91 (dd, J=4.1, 1.7 Hz, 1H), 8.35 (dd, J=8.4, 1.0 Hz, 1H), 8.09 (s, 1H), 7.97 (d, J=8.4 Hz, 1H), 7.70 (dd, J=8.5, 1.6 Hz, 1H), 7.53 (dd, J=8.2, 4.3 Hz, 1H), 5.88 (d, J=9.4 Hz, 1H), 5.24 (t, J=7.6 Hz, 1H), 5.18 (d, J=6.9 Hz, 1H), 4.49 (d, J=6.1 Hz, 1H), 4.29–4.18 (m, 1H), 3.55 (t, J=7 Hz, 1H), 3.29–3.16 (m, 1H), 2.93–2.78 (m, 1H), 2.58–2.47 (m, 1H), 2.47–2.29 (m, 3H), 1.92–1.77 (m, 1H), 1.76–1.62 (m, 1H), 1.67 (s, 3H), 1.56–1.44 (m, 1H), 1.43–1.33 (m, 1H), 1.30–1.06 (m, 2H), 1.19 (s, 3H), 1.11 (d, J=6.7 Hz, 3H), 0.94 (d, J=6.8 Hz, 3H), 0.92 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=217.6, 170.4, 150.7, 147.6, 143.0, 138.7, 135.7, 128.1, 127.1, 125.4, 124.8, 121.3, 120.2, 75.7, 75.5, 70.8, 53.1, 44.7, 38.9, 36.6, 35.3, 31.7, 30.1, 26.1, 23.1, 22.3, 20.6, 17.8, 16.3 ppm; IR (film): $\tilde{\nu}$=1735, 1688, 1463, 1377, 1302, 1252, 1040, 936, 836, 750, 668, 607 cm^{-1}; MS (ESI): m/z (%): 482.95 (100) [M+H^+]; HRMS: m/z: calcd for $C_{29}H_{39}NO_5$+H: 482.2906 [M+H^+]; found: 482.2892; RP-HPLC: t_R=8.25 min (20% CH_3CN/water for 1 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min).

Epoxylactone 1a: 1.05 mL of a solution of pyridine (48 μL) and 30% H_2O_2 (760 μL) in water (6.84 mL) and $MeReO_3$ (19 mg, 0.019 mmol) were each simultaneously added in seven equal portions over a period of 46 h to a solution of **1b** (51 mg, 0.11 mmol) in CH_2Cl_2 (0.500 mL). Thereafter, excess MnO_2 was added to the mixture and stirring was continued for a further 1 h. The mixture was then diluted with CH_2Cl_2 (45 mL) and water (45 mL), the aqueous layer was removed, and the organic solution was dried over $MgSO_4$. The solvent was evaporated and the residue was purified by FC eluting with CH_2Cl_2/acetone 1:1 to provide the *N*-oxide of **1a** (59.3 mg). A portion of this material (44.3 mg, 0.09 mmol) was hydrogenated over Raney Ni in MeOH (8 mL) at atmospheric pressure for 3 h. The catalyst was then filtered off, the filtrate was evaporated to dryness, and the residue was purified by FC eluting with CH_2Cl_2/acetone 3:1, to provide **1a** (19.7 mg, 39%) as a colorless, glassy resin. For biological experiments, this material was further purified by preparative HPLC (20% CH_3CN/water for 2 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min) to provide **1a** (7.2 mg) as a white lyophilized powder. $[\alpha]_D^{RT}=-57.40°$ (c=0.5 in $CHCl_3$); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=8.94 (dd, J=4.2, 1.6 Hz, 1H), 8.39 (d, J=8.2 Hz, 1H), 8.11 (s, 1H), 8.01 (d, J=8.5 Hz, 1H), 7.74 (dd, J=8.5, 1.3 Hz, 1H), 7.56 (dd, J=8.3, 4.2 Hz, 1H), 6.08 (d, J=8.9 Hz, 1H), 5.19 (d, J=6.9 Hz, 1H), 4.56 (d, J=6.3 Hz, 1H), 4.28–4.15 (m, 1H), 3.56 (t, J=7.0 Hz, 1H), 3.29–3.20 (m, 1H), 3.02 (dd, J=9.2, 3.6 Hz, 1H), 2.61–2.50 (m, 2H), 2.45 (dd, J=15.7, 10.5 Hz, 1H), 2.30–2.21 (m, 1H), 2.20–2.08 (m, 2H), 1.77–1.65 (m, 1H), 1.62–1.43 (m, 2H), 1.42–1.25 (m, 2H), 1.27 (s, 3H), 1.21 (s, 3H), 1.12 (d, J=6.7 Hz, 3H), 0.97 (d, J=6.7 Hz, 3H), 0.95 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=217.7, 170.5, 150.8, 147.5, 142.9, 135.8, 128.3, 127.2, 125.5, 124.6, 121.4, 75.7, 73.4, 70.6, 61.5, 61.1, 53.0, 45.2, 38.8, 36.2, 35.8, 32.1, 29.7, 23.2, 22.2, 22.1, 20.5, 18.6, 16.5 ppm; IR (film): $\tilde{\nu}$=2955, 2930, 1735, 1691, 1459, 1377, 1252, 836, 758 cm^{-1}; MS (ESI): m/z (%): 499.01 (100) [M+H^+]; HRMS: m/z: calcd for $C_{29}H_{39}NO_6$+H: 498.2856 [M+H^+]; found: 498.2840; RP-HPLC: t_R=6.47 min (20% CH_3CN/water for 1 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min).

Protected macrolactone 40: Et_3N (0.825 mL, 5.93 mmol) was added to a solution of seco acid **39** (760 mg, 1.04 mmol) in THF (2 mL) at 0 °C, and then 2,4,6-trichlorobenzoyl chloride (0.813 mL, 5.20 mmol) was added. After stirring at 0 °C for 15 min, the solution was diluted with THF (100 mL) and then added over a period of 3 h to a previously prepared solution of 4-dimethylaminopyridine (1.27 g, 10.4 mmol) in toluene (700 mL) at 75 °C under vigorous stirring. The reaction mixture was then evaporated to dryness and the residue was treated with Et_2O (200 mL). Insoluble material was filtered off and the filtrate was concentrated. The crude product was purified by FC (hexane/AcOEt 4:1) to afford bis-TBS-protected macrolactone **40** as white crystals (550 mg, 75%). M.p. 80–82 °C; $[\alpha]_D^{RT}=-4.8°$ (c=0.01 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.89 (d, J=4.1 Hz, 1H), 8.16 (d, J=8.2 Hz, 1H), 8.11 (d, J=8.8 Hz, 1H), 7.81–7.74 (m, 2H), 7.39 (dd, J=8.3, 4.2 Hz, 1H), 5.67 (d, J=10.1 Hz, 1H), 5.24 (t, J=8.0 Hz, 1H), 3.98 (t, J=5.6 Hz, 1H), 3.92 (d, J=8.9 Hz, 1H), 3.07–2.92 (m, 1H), 2.74–2.56 (m, 2H), 2.21–2.14 (m, 1H), 1.83–1.55 (m, 5H), 1.72 (s, 3H) 1.36–0.99 (m, 15H), 0.95 (s, 9H), 0.85 (s, 9H), 0.12–0.08 (m, 9H), −0.10 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=215.0, 171.4, 150.6, 147.9, 141.1, 139.6, 136.1, 130.1, 128.0, 127.4, 125.1, 121.4, 118.8, 79.5, 77.0, 76.2, 53.3, 48.1, 39.3, 37.7, 35.2, 32.1, 31.4, 27.5, 26.4, 26.1, 24.4, 24.3, 23.2, 19.3, 18.7, 18.6, 17.8, −3.4, −3.6, −3.7, −5.6 ppm; MS (ESI): m/z: 711.15 [M+H^+].

Macrolactone 2b: HF·pyridine (9 mL, 344 mmol in total) was added in three portions over a period of 15 min to a solution of bis-TBS-protected **41** (150 mg, 0.21 mmol) in THF (20 mL) in a Teflon tube at 0 °C and the reaction mixture was stirred at RT for 22 h. It was then added dropwise at 0 °C to saturated aqueous $NaHCO_3$ solution (200 mL). Additional solid $NaHCO_3$ (10 g) was then added to the mixture. After stirring for 1 h, the aqueous solution was extracted with AcOEt (3×80 mL). The combined organic extracts were then washed with water (100 mL), dried, and concentrated in vacuo. The crude product was purified by FC eluting with CH_2Cl_2/MeOH 98:2 to provide **2b** as white crystals (90 mg, 89%). M.p. 188.5–189.5 °C; $[\alpha]_D^{RT}=-11.7°$ (c=0.90 in AcOEt); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=8.89 (dd, J=4.1, 1.6 Hz, 1H), 8.35 (dd, J=8.2, 1.6 Hz, 1H), 8.02 (d, J=1.9 Hz, 1H), 7.99 (d, J=8.8 Hz, 1H), 7.83 (dd, J=8.8, 1.9 Hz, 1H), 7.54 (dd, J=8.5, 4.4 Hz, 1H), 5.84 (dd, J=9.1, 1.3 Hz, 1H), 5.21 (t, J=7.6 Hz, 1H), 5.16 (d, J=6.6 Hz, 1 Hz, 1H), 4.46 (d, J=6.3 Hz, 1H), 4.26–4.19 (m, 1H), 3.56–3.50 (m, 1H), 3.21–3.13 (m (quint), 1H), 2.87–2.76 (m, 1H), 2.46 (dd, J=15.5, 3.2 Hz, 1H), 2.41–2.30

(m, 3H), 1.89–1.77 (m, 1H), 1.74–1.60 (m, 1H), 1.66 (s, 3H), 1.50–1.32 (m, 2H), 1.28–1.04 (m, 2H), 1.21 (s, 3H), 1.11 (d, J=6.6 Hz, 3H), 0.93 (d, J=6.6 Hz, 3H), 0.91 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=217.51, 170.34, 150.42, 147.11, 139.67, 138.71, 136.04, 128.89, 127.74, 127.51, 124.44, 121.57, 120.18, 75.55, 75.47, 70.77, 53.21, 44.62, 38.85, 36.50, 35.21, 31.63, 30.01, 26.00, 23.07, 22.63, 20.03, 17.74, 16.28 ppm; MS (ESI): m/z: 482.9 [M+H$^+$]; RP-HPLC: t_R = 7.49 min (20% CH_3CN/water for 1 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min).

Epoxylactone 2a: A solution of pyridine (48 μL) and 30% H_2O_2 (760 μL) in water (6.84 mL) and $MeReO_3$ (81 mg, 0.08 mmol) were each simultaneously added in six equal portions over a period of 25 h to a solution of **2b** (62.9 mg, 0.13 mmol) in CH_2Cl_2 (0.82 mL). Thereafter, excess MnO_2 was added to the mixture and stirring was continued for a further 1 h. The mixture was then diluted with CH_2Cl_2 (110 mL), the aqueous layer was removed, and the organic solution was dried over $MgSO_4$. The solvent was evaporated and the residue was purified by FC eluting with CH_2Cl_2/acetone 5:4 to provide the *N*-oxide of **2a** as a glassy resin (54.5 mg). A portion of this material (45.1 mg) was hydrogenated over Raney Ni in MeOH (8 mL) at atmospheric pressure for 4.5 h. The catalyst was then filtered off, the filtrate was evaporated to dryness, and the residue was purified by FC eluting with CH_2Cl_2/acetone 3:1, which gave **2a** (26.4 mg, 39%) as a colorless, glassy resin. For biological experiments, this material was further purified by preparative HPLC (20% CH_3CN/water for 2 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min) to provide **2a** (14.1 mg, 20%) as a white lyophilized powder. $[\alpha]_D^{25}=-45.79°$ (c=1.34 in $CHCl_3$); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=8.93 (dd, J=4.2, 1.7 Hz, 1H), 8.39 (dd, J=8.3, 1.9 Hz 1H), 8.08 (d, J=1.4 Hz, 1H), 8.04 (d, J=8.8 Hz, 1H), 7.89 (dd, J=8.8, 1.8 Hz, 1H), 7.58 (dd, J=8.3, 4.3 Hz, 1H), 6.07 (d, J=9.1 Hz, 1H), 5.19 (d, J=5.5 Hz, 1H), 4.58 (d, J=6.1 Hz, 1H), 4.27–4.19 (m, 1H), 3.56 (t, J=6.8 Hz, 1H), 3.29–3.20 (m, 1H), 3.01 (dd, J=9.2, 3.2 Hz, 1H), 2.59–2.49 (m, 1H), 2.44 (dd, J=15.5, 10.7 Hz, 1H), 2.27 (d, J=14.9 Hz, 1H), 2.16–2.06 (m, 1H), 1.75–1.65 (m, 1H), 1.60–1.17 (m, 6H), 1.27 (s, 3H), 1.23 (s, 3H), 1.13 (d, J=6.6 Hz, 3H), 0.98 (d, J=6.6 Hz, 3H), 0.95 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=218.1, 170.9, 151.0, 147.6, 140.1, 136.5, 129.5, 128.1, 127.9, 125.0, 122.1, 76.1, 73.7, 71.1, 61.9, 61.5, 53.5, 45.6, 39.3, 36.7, 36.1, 32.5, 30.1, 23.6, 22.7, 22.6, 20.7, 19.0, 17.0 ppm; IR (film): $\tilde{\nu}$=2966, 2933, 1735, 1688, 1252, 1147, 1051, 1008, 980, 840, 750 cm^{-1}; MS (ESI): m/z: 499.07 [M+H$^+$]; HRMS: m/z: calcd for $C_{29}H_{39}NO_6$+H: 498.2856 [M+H$^+$]; found: 498.2841; RP-HPLC: t_R=6.17 min (20% CH_3CN/water for 1 min; then 20% CH_3CN/water → 95% CH_3CN/water in 8 min).

Methyl 4-fluoro-3-nitrobenzoate 19: Concentrated H_2SO_4 (10 mL, 178 mmol) was added to a solution of 4-fluoro-3-nitrobenzoic acid (**18**) (15 g, 108 mmol) in absolute MeOH (500 mL) and the mixture was heated to reflux for 6 h. After cooling to RT, AcOEt (300 mL) was added and MeOH was removed under reduced pressure. The mixture was then diluted with water (300 mL) and the aqueous phase was neutralized with 2 N aqueous sodium hydroxide. The organic layer was separated and the aqueous solution was back-extracted with CH_2Cl_2 (2× 200 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$. Evaporation of the solvent afforded **19** as a yellow solid (15.7 g, 97%). M.p. 60.5–61.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.74 (dd, J=7.4, 2.2 Hz, 1H), 8.32 (ddd, J=8.8, 4.2, 2.0 Hz, 1H), 7.39 (dd, J=10.2, 8.6 Hz, 1H), 3.98 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=164.24, 159.57, 156.86, 136.66 (d, J=10.1 Hz), 128.00 (d, J=2.2 Hz), 127.38 (d, J=4.2 Hz), 118.95 (d, J=21.2 Hz), 53.06 ppm; IR (film): $\tilde{\nu}$=3076, 2956, 1729, 1615, 1540, 1439, 1351, 1276, 1233, 1197, 1116 cm^{-1}; MS (ESI): m/z: 199.75 [M+H$^+$]; HRMS (EI): m/z: calcd for $C_8H_6FNO_4$+H: 199.0276 [M+H$^+$]; found: 199.0274.

Boc-protected amino ester 20: A solution of **19** (0.53 g, 2.7 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of $BocNHCH_2CH_2NH_2$ (0.48 g, 3 mmol) in CH_2Cl_2 (5 mL) at RT; a yellow precipitate formed immediately. Et_3N was then added to the mixture in four portions over a period of 25 h (0.8 mL, 5.7 mmol in total). CH_2Cl_2 (5 mL) was then added and the solution was washed once with 2% aqueous $KHSO_4$ solution (10 mL). The aqueous layer was back-extracted with CH_2Cl_2 (3× 10 mL) and the combined organic extracts were dried over $MgSO_4$. Evaporation of the solvent afforded **20** as a yellow solid (0.88 g, 96%). M.p. 116.5–117.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.89 (d, J=2.4 Hz, 1H), 8.50 (br, 1H), 8.07 (dd, J=9.2, 2.0 Hz, 1H), 6.96 (d, J=9.2 Hz, 1H), 4.85 (br, 1H), 3.91 (s, 3H), 3.56–3.44 (m, 4H), 1.46 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.57, 147.76, 136.44, 129.54, 117.55, 113.46, 80.12, 52.12, 43.23, 39.56, 28.34 ppm; IR (film): $\tilde{\nu}$=3363, 2976, 1707, 1623, 1566, 1524, 1440, 1362, 1287, 1227, 1163 cm^{-1}; MS (ESI): m/z: 362.73 [M+Na$^+$]; HRMS (ESI): m/z: calcd for $C_{15}H_{21}N_3O_6$+Na: 362.1323 [M+Na$^+$]; found: 362.1327.

Anilino ester 21: Nitrobenzene **20** (16.7 g, 49 mmol) was hydrogenated over Pd/C (0.8 g) at RT and atmospheric pressure in MeOH (300 mL) for 17 h. Filtration of the mixture through a pad of Celite, evaporation of the filtrate, and purification of the residue by FC eluting with hexane/AcOEt 2:1 → 1:2 gave **21** as a red oil (15.1 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.53 (dd, J=8.4 Hz, 2 Hz, 1H), 7.36 (d, J=1.6 Hz, 1H), 6.52 (d, J=8.4 Hz, 1H), 5.04 (br, 1H), 3.83 (s, 3H), 3.47–3.41 (m, 2H), 3.28–3.25 (m, 2H), 1.46 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.71, 157.09, 142.46, 132.43, 123.98, 118.8, 117.61, 108.94, 79.86, 51.67, 44.77, 40.03, 28.45 ppm; IR (film): $\tilde{\nu}$=3371, 2976, 2948, 1686, 1600, 1524, 1442, 1393, 1365, 1296, 1253, 1221, 1158, 1113 cm^{-1}; MS (ESI): m/z: 310.32 [M+H$^+$]; HRMS (ESI): m/z: calcd for $C_{15}H_{21}N_3O_6$+H: 310.1761 [M+H$^+$]; found: 310.1759.

Ester 22: A solution of **21** (100 mg, 0.33 mmol) and triethyl orthoacetate (0.35 mL, 1.94 mmol) in EtOH (6 mL) was heated to reflux for 19.5 h. Evaporation of the solvent gave a dark-brown solid, which was purified by FC eluting with AcOEt/MeOH 95:5 to provide **22** as a white solid (104 mg, 96%). M.p. 180.1–181.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.32 (s, 1H), 7.98 (dd, J=8.4, 1.2 Hz, 1H), 7.35 (d, J=8.4 Hz, 1H), 4.96 (br, 1H), 4.33 (t, J=5.4 Hz, 2H), 3.94 (s, 3H), 3.51 (q, J=5.7 Hz, 2H), 2.67 (s, 3H), 1.43 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.54, 156.32, 153.44, 141.82, 138.37, 124.15, 123.93, 120.99, 108.68, 80.08, 52.08, 43.56, 40.29, 28.51, 13.92 ppm; IR (film): $\tilde{\nu}$=3223, 2977, 2952, 1705, 1619, 1522, 1435, 1396, 1366, 1336, 1285, 1253, 1209, 1165, 1082 cm^{-1}; MS (ESI): m/z: 334.13 [M+H$^+$]; HRMS (ESI): m/z: calcd for $C_{17}H_{23}N_3O_4$+H: 334.1761 [M+H$^+$]; found: 334.1759.

Alcohol 23: A 1 M solution of DIBAL-H in CH_2Cl_2 (159 mL, 159 mmol) was added dropwise to a solution of **22** (13.23 g, 39.7 mmol) in CH_2Cl_2 (170 mL) at −78 °C. The mixture was allowed to warm to RT and stirred for 17 h. After re-cooling to −30 °C, MeOH (200 mL) was added and the precipitate formed was removed by paper filtration. Concentration of the filtrate and recrystallization of the residue from MeOH afforded **23** as a beige-colored solid (10.03 g, 78%). M.p. 130 °C (decomposition); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.50 (m, 1H), 7.24 (m, 2H), 5.12 (br, 1H), 4.74 (s, 2H), 4.25 (t, J=5.4 Hz, 2H), 3.50–3.44 (m, 2H), 2.57 (s, 3H), 1.44 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=156.16, 153.29, 142.63, 135.41, 134.57, 121.95, 117.80, 109.17, 80.25, 65.79, 43.62, 40.17, 28.50, 13.84 ppm; IR (film): $\tilde{\nu}$=3310, 3218, 2976, 2932, 2869, 1689, 1623, 1519, 1436, 1404, 1356, 1332, 1277, 1251, 1164, 1084, 1035, 1014 cm^{-1}; MS (ESI): m/z: 306.1 [M+H$^+$]; HRMS (ESI): m/z: calcd for $C_{16}H_{23}N_3O_3$+H: 306.1812 [M+H$^+$]; found: 306.1807.

Aldehyde 24: MnO_2 (704 mg, 7.8 mmol) was added to a solution of **23** (207 mg, 0.66 mmol) in $CHCl_3$ (1.5 mL) and the mixture was refluxed for 1 h. After cooling to RT, the mixture was filtered through a pad of Celite and the filtrate was concentrated to yield **24** as a white solid (193 mg, 96%). m.p. 186 °C (decomposition); ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.04 (s, 1H), 8.16 (d, J=1.2 Hz, 1H), 7.88 (dd, J=5.2, 1.2 Hz, 1H), 7.49 (d, J=5.0 Hz, 1H), 4.87 (br, 1H), 4.38 (m, 2H), 3.54 (m, 2H), 2.75 (s, 3H), 1.42 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.03, 156.21, 154.31, 142.24, 139.55, 131.53, 123.47, 122.15, 109.63, 80.20, 43.66, 40.24, 28.49, 13.96 ppm; IR (film): $\tilde{\nu}$=3339, 3226, 2974, 2929, 2854, 2736, 1687, 1613, 1586, 1522, 1422, 1395, 1366, 1335, 1285, 1253, 1165 cm^{-1}; MS (ESI): m/z: 304.38 [M+H$^+$]; HRMS (MALDI): m/z: calcd for $C_{16}H_{21}N_3O_3$+H: 304.1656 [M+H$^+$]; found: 304.1654.

Homoallylic alcohol 25: A 1 M solution of allylmagnesium bromide in Et_2O (60 mL, 60 mmol) was added to a solution of (−)-diisopinocampheyl chloride (16.17 g, 50.3 mmol) in Et_2O (100 mL) at 0 °C under Ar and the mixture was stirred for 1 h. Dry pentane (80 mL) was then added, the mixture was cooled to −30 °C and filtered under Ar through a sintered

glass septum, and the residue was washed with dry pentane (20 mL). The clear filtrate was cooled to −78 °C (solution A). In a separate flask, **24** (8.99 g, 29.6 mmol) was suspended in Et_2O (200 mL) and the mixture was cooled to −100 °C (suspension B). Solution A was then added dropwise to suspension B over a period of 1 h, while keeping the temperature at −100 °C, and then the mixture was stirred for a further 1 h at the same temperature. Dry MeOH (15 mL) was then added and the temperature was allowed to rise to −15 °C, whereupon ethanolamine (20 mL, 0.33 mol) was added and the mixture was stirred for 18 h at RT. Filtration of the suspension and concentration of the filtrate afforded 41.87 g of a yellow oil, which was purified by FC eluting with CH_2Cl_2/MeOH 20:1 (three runs) to afford **25** as a white solid (9.15 g, 89 %). The absolute configuration of **25** was confirmed by Mosher ester analysis; *ee* 94 %, as estimated from the ^{19}F NMR spectrum of the Mosher ester. M.p. 132.8–137.4 °C; $[\alpha]_D^{20} = -28.2°$ ($c = 1.23$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.29$ (m, 1H), 7.19 (m, 2H), 5.84–5.70 (m, 2H), 5.14–5.04 (m, 2H), 4.73 (t, $J = 6.4$ Hz, 1H), 4.25–4.16 (m, 2H), 3.56–3.38 (m, 2H), 3.18–2.75 (br, 1H), 2.52 (s, 3H), 2.48 (t, $J = 7.2$ Hz, 2H), 1.45 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 156.25$, 152.36, 142.31, 138.35, 134.94, 134.35, 120.07, 118.02, 116.36, 108.85, 79.97, 73.74, 44.40, 43.47, 40.16, 28.55, 13.76 ppm; IR (film): $\tilde{\nu} = 3318$, 3215, 2980, 2930, 1688, 1518, 1433, 1403, 1365, 1275, 1252, 1165, 1039 cm^{-1}; MS (ESI): m/z: 345.97 $[M+H^+]$, 367.92 $[M+Na^+]$; HRMS (ESI): m/z: calcd for $C_{19}H_{27}N_3O_3$ 345.2057 $[M^+]$; found: 345.2050.

TES-protected alcohol 26: TES-Cl (3 mL, 17.8 mmol), imidazole (1.3 g, 19.4 mmol), and DMAP (0.2 g, 1.6 mmol) were added to a solution of alcohol **25** (5.6 g, 16.2 mmol) in DMF (40 mL) and the mixture was stirred at RT for 4 h. It was then diluted with saturated aqueous $NaHCO_3$ solution (200 mL) and the resulting mixture was extracted with AcOEt (3 × 200 mL). The combined organic extracts were dried over $MgSO_4$, the solvent was evaporated, and the oily residue was purified by FC (AcOEt/hexane 6:4 → AcOEt → AcOEt/MeOH 10:1) to afford **26** as a yellow oil (7.3 g, 98 %). M.p. 99.5–101.1 °C; $[\alpha]_D^{20} = -25.7°$ ($c = 1.06$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.58$ (s, 1H), 7.23–7.20 (m, 2H), 5.81–5.69 (m, 1H), 5.03–4.93 (m, 2H), 4.78 (m, 2H), 4.30–4.20 (m, 2H), 3.47 (q, $J = 6.0$ Hz, 2H), 2.58–2.37 (m, 5H), 1.43 (s, 9H), 0.86 (t, $J = 7.8$ Hz, 9H), 0.58–0.44 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 156.05$, 152.14, 142.59, 139.63, 135.53, 134.45, 120.63, 116.78, 116.59, 108.66, 80.04, 75.29, 46.07, 43.34, 40.13, 28.45, 13.87, 6.96, 4.98 ppm; IR (film): $\tilde{\nu} = 3293$, 2954, 2875, 1701, 1521, 1453, 1433, 1404, 1365, 1326, 1276, 1254, 1170, 1166, 1082, 1036, 1004 cm^{-1}; MS (ESI): m/z: 459.98 $[M+H^+]$; HRMS (ESI): m/z: calcd for $C_{25}H_{41}N_3O_3Si+H$: 460.2990 $[M+H^+]$; found: 460.2990.

Aldehyde 27: 2,6-Lutidine (510 μL, 4.4 mmol), a solution of OsO_4 in *t*BuOH (2.5 % w/w, 0.6 mL, 0.1 mmol), and $NaIO_4$ (1.9 g, 8.8 mmol) were added to a solution of **26** (1.0 g, 2.2 mmol) in dioxane/water 3:1 (20 mL) at RT. The mixture was stirred at RT for 23 h, and then brine (30 mL) and Et_2O (30 mL) were added, the layers were separated, and the aqueous layer was further extracted with Et_2O (2 × 30 mL). The combined organic extracts were dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by FC eluting with hexane/AcOEt/MeOH 6:4:0.1 to provide **27** as a brown oil (0.77 g, 74 %). $[\alpha]_D^{20} = -44.5°$ ($c = 1.06$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.80$ (t, $J = 2.2$ Hz, 1H), 7.66 (s, 1H), 7.28–7.23 (m, 2H), 5.34 (dd, $J = 8.0$, 4.4 Hz, 1H), 4.65 (br, 1H), 4.27 (t, $J = 5.6$ Hz, 2H), 3.51–3.46 (m, 2H), 2.93–2.86 (m, 1H), 2.69–2.85 (m, 1H), 2.59 (s, 3H), 1.44 (s, 9H), 0.86 (t, $J = 8.0$ Hz, 9H), 0.58–0.48 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 201.66$, 152.5, 142.71, 138.20, 134.62, 120.09, 116.27, 108.99, 80.04, 70.86, 54.47, 43.25, 40.01, 28.31, 13.80, 6.71, 4.77 ppm; IR (film): $\tilde{\nu} = 3731$, 3624, 2957, 2882, 1705, 1515, 1455, 1399, 1363, 1247, 1165, 1088, 1005 cm^{-1}; MS (ESI): m/z: 462.06 $[M+H^+]$; HRMS (ESI): m/z: calcd for $C_{24}H_{39}N_3O_4Si+CH_3OH+H$: 494.3042 $[M+H+MeOH^+]$; found: 494.3041.

Protected macrolactone 31: Et_3N (16 μL, 0.137 mmol) and 2,4,6-trichlorobenzoyl chloride (18 μL, 0.114 mmol) were added to a solution of dry **30** (20 mg, 0.023 mmol) in THF (250 μL) at 10 °C under Ar. The mixture was stirred at 10 °C for 1 h and then diluted at 0 °C with toluene (2 mL). This solution was added by means of a syringe pump over a period of 3 h at RT to a solution of DMAP (29 mg, 0.23 mmol) in toluene (8 mL). After stirring for a further 2 h at RT, the solvent was evaporated and the residue was purified by FC eluting with hexane/AcOEt/MeOH 25:75:0 → 15:85:0 → 0:1:0 → 0:10:1 to give **31** as a white solid (11.4 mg, 57 %). M.p. 108.4–109.5 °C; $[\alpha]_D^{20} = -41.3°$ ($c = 1.00$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.70$ (s, 1H), 7.26 (m, 2H), 5.58 (d, $J = 9.2$ Hz, 1H), 5.24 (t, $J = 8.0$ Hz, 1H), 4.66 (br, 1H), 4.25 (br, 2H), 3.95 (t, $J = 5.8$ Hz, 1H), 3.90 (d, $J = 8.8$ Hz, 1H), 3.46 (q, $J = 5.7$ Hz, 2H), 3.08–2.98 (m, 1H), 2.98–2.88 (m, 1H), 2.71 (d, $J = 6$ Hz, 2H), 2.67–2.55 (m, 1H), 2.58 (s, 3H), 2.18–2.08 (m, 1H), 1.84–1.72 (m, 2H), 1.70 (s, 3H), 1.68–1.52 (m, 2H), 1.43 (s, 9H), 1.24–1.14 (m, 1H), 1.14–1.06 (m, 10H), 0.99 (d, $J = 6.8$ Hz, 3H), 0.96 (s, 9H), 0.85 (s, 9H), 0.14–0.05 (m, 9H), −0.09 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 215.08$, 171.40, 155.87, 152.63, 142.85, 140.82, 136.04, 134.63, 120.85, 119.24, 116.24, 108.91, 80.07, 79.57, 77.52, 76.35, 53.37, 48.15, 43.28, 39.96, 39.36, 37.73, 36.03, 32.10, 31.55, 28.34, 27.53, 26.41, 26.16, 24.61, 24.14, 23.09, 19.36, 18.71, 18.60, 17.76, 13.74, −3.27, −3.68, −3.70, −5.68 ppm; IR (film): $\tilde{\nu} = 2955$, 2932, 2888, 2857, 1737, 1699, 1518, 1468, 1388, 1365, 1253, 1162, 1096, 1066, 1018, 985 cm^{-1}; MS (ESI): m/z: 856.62 $[M+H^+]$; HRMS (MALDI): m/z: calcd for $C_{47}H_{81}N_3O_7Si_2+H$: 856.5686 $[M+H^+]$; found: 856.5681.

Macrolactone 3: Protected macrolactone **31** (20 mg, 0.023 mmol) was added at 0 °C to a solution of CF_3COOH (100 μL, 0.135 mmol) in CH_2Cl_2 (200 μL). The mixture was stirred at RT for 2 h and then the solvent was evaporated to leave a yellow oil (29 mg). FC of this material eluting with $CHCl_3$/MeOH/water/AcOH 85:13:1.5:0.5 afforded a pale-yellow oil (16.8 mg, 64 % HPLC purity), which was further purified by preparative RP-HPLC (A/B 80:20 → 50:50 over 6 min; A = 0.1 % aqueous TFA, B = 0.1 % TFA in CH_3CN) to afford pure **3** (bis-TFA salt) as a white solid (7.6 mg, 44 %; HPLC purity > 99 %). $[\alpha]_D^{20} = -36.9°$ ($c = 0.97$ in $CHCl_3$); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 8.26$–8.09 (br, 3H), 7.89–7.82 (m, 2H), 7.61 (d, $J = 9.0$ Hz, 1H), 5.78 (d, $J = 10.5$ Hz, 1H), 5.20 (t, $J = 5.8$ Hz, 1H), 5.14 (br, 1H), 4.59 (t, $J = 6.2$ Hz, 2H), 4.19 (d, $J = 10.5$ Hz, 1H), 3.52 (d, $J = 8.1$ Hz, 1H), 3.38–3.29 (m, 2H), 3.17 (quint, $J = 7.2$ Hz, 1H), 2.84–2.72 (m, 4H), 2.48–2.42 (m, 1H), 2.41–2.30 (m, 2H), 2.27–2.20 (m, 1H), 1.85–1.76 (m, 1H), 1.73–1.62 (m, 4H), 1.49–1.40 (m, 1H), 1.39–1.31 (m, 1H), 1.27–1.14 (m, 4H), 1.13–1.03 (m, 1H), 1.09 (d, $J = 6.5$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H), 0.90 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 217.55$, 170.36, 158.49 (q, $J = 32$ Hz), 152.74, 139.36, 138.87, 131.64, 122.85, 120.15, 116.92 (q, $J = 297$ Hz), 112.03, 111.76, 75.77, 75.55, 70.75, 53.14, 44.75, 41.99, 38.74, 37.53, 36.49, 35.80, 31.75, 30.12, 26.18, 23.11, 22.43, 20.38, 17.86, 16.35, 12.03 ppm; IR (film): $\tilde{\nu} = 3417$, 2928, 1733, 1659, 1502, 1460, 1414, 1388, 1255, 1198, 1177, 1132, 1096 cm^{-1}; MS (ESI): m/z: 528.26 $[M+H^+]$; HRMS (ESI): m/z: calcd for $C_{30}H_{46}N_3O_5+H$: 528.3432 $[M+H^+]$; found: 528.3429; RP-HPLC: $t_R = 4.4$ min (A/B 80:20 for 2 min; then A/B 80:20 → 50:50 over 6 min; A = 0.1 % aqueous TFA, B = 0.1 % TFA in CH_3CN).

Partially protected macrolactone 32: $ZnBr_2$ (93 mg, 0.35 mmol) was added to a solution of protected macrolactone **31** (101 mg, 0.12 mmol) in CH_2Cl_2 (2.5 mL) at 0–4 °C under Ar and the mixture was stirred at 0 °C for 72 h. It was then diluted with CH_2Cl_2 (5 mL) and washed with saturated aqueous $NaHCO_3$ solution (5 mL). The aqueous layer was back-extracted with CH_2Cl_2 (2 × 6 mL). The combined organic extracts were dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by FC eluting with $CHCl_3$/MeOH/water/AcOH 85:13:1.5:0.5 to give **32** as a yellow oil (57 mg, 57 %). In addition, 18 mg of **3** and 7 mg of the starting material **31** were recovered. $[\alpha]_D^{20} = -41.6°$ ($c = 0.52$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.68$ (m, 1H), 7.42 (d, $J = 8.1$ Hz, 1H), 7.29 (d, $J = 8.1$, 1H), 5.52 (d, $J = 9.9$ Hz, 1H), 5.21 (t, $J = 7.6$ Hz, 1H), 4.54–4.40 (m, 2H), 3.97–3.86 (m, 2H), 3.39–3.22 (m, 2H), 3.03 (t, $J = 7.4$ Hz, 1H), 2.95–2.82 (m, 1H), 2.80–2.50 (m, 6H), 2.15–2.02 (m, 1H), 1.83–1.70 (m, 2H), 1.70 (s, 3H), 1.69–1.50 (m, 2H), 1.20–1.00 (m, 10H), 0.99 (d, $J = 6.1$ Hz, 3H), 0.94 (s, 9H), 0.84 (s, 9H), 0.14–0.01 (m, 9H), −0.06–0.13 ppm (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 215.16$, 171.79, 152.70, 141.08, 140.97, 137.08, 134.03, 121.67, 119.13, 115.79, 109.66, 79.80, 77.37, 76.53, 53.47, 48.18, 42.51, 39.45, 39.20, 37.91, 36.01, 32.27, 31.71, 27.73, 26.53, 26.27, 24.71, 24.24, 23.23, 19.40, 18.81, 18.71, 7.92, 13.57, −3.21, −3.53, −5.51, −3.53 ppm; IR (film): $\tilde{\nu} = 2953$, 2928, 2856, 1737, 1697, 1578, 1545, 1522, 1467, 1442, 1403, 1386, 1364,

1253 cm^{-1}; MS (ESI): *m/z*: 756.25 [M^+]; HRMS (ESI): *m/z*: calcd for $C_{42}H_{74}N_3O_5Si_2$+H: 756.5162 [M+H^+]; found: 756.5181.

Macrolactone 33: Diisopropylethylamine (8 μL, 0.05 mmol) and a solution of succinic anhydride (5 mg, 0.05 mmol) in DMF (0.2 mL) were added to a solution of **32** (15 mg, 0.020 mmol) in DMF (0.2 mL) at RT. After stirring at RT for 2 h, saturated aqueous NH_4Cl solution (4 mL) was added and the resulting mixture was extracted with AcOEt (3× 4 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was evaporated to provide **33** as a colorless oil (15.4 mg, 92%), which was used in the next step without further purification. $[\alpha]_D^{20}=-31.2°$ ($c=0.9$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.68$ (m, 1H), 7.34 (m, 2H), 6.82 (br, 1H), 5.53 (d, $J=10.7$ Hz, 1H), 5.22 (m, 1H), 4.33 (t, $J=5.3$ Hz, 2H), 3.94–3.87 (m, 2H), 3.69–3.61 (m, 2H), 3.08–2.99 (m, 1H), 2.96–2.86 (m, 1H), 2.81–2.65 (m, 2H), 2.64–2.56 (m, 5H), 2.56–2.48 (m, 2H), 2.43–2.32 (m, 2H), 2.16–2.05 (m, 1H), 1.86–1.72 (m, 2H), 1.70 (s, 3H), 1.64–1.50 (m, 2H), 1.33–1.24 (m, 1H), 1.16–1.05 (m, 9H), 0.99 (d, $J=6.9$ Hz, 3H), 0.95 (s, 9H), 0.84 (s, 9H), 0.14–0.04 (m, 9H), −0.09–0.12 ppm (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=215.15$, 173.62, 173.05, 171.80, 153.03, 141.15, 139.91, 137.54, 133.68, 122.12, 119.13, 115.28, 109.98, 79.72, 77.37, 76.99, 53.48, 48.31, 43.28, 39.37, 38.76, 37.82, 36.70, 35.88, 32.27, 31.27, 30.03, 29.05, 26.56, 26.29, 24.85, 24.25, 23.23, 19.43, 18.84, 18.75, 17.94, 13.01, −3.18, −3.49, −3.54, −5.54 ppm; IR (film): $\tilde{\nu}=2932$, 2857, 1735, 1697, 1668, 1545, 1468, 1436, 1408, 1384, 1364, 1253, 1201, 1161, 1096, 1066, 1020, 984 cm^{-1}; MS (ESI): *m/z*: 856.28 [M^+]; HRMS (ESI): *m/z*: calcd for $C_{46}H_{77}N_3O_8Si_2$+H: 856.5322 [M+H^+]; found: 856.5317.

Macrolactone 4: Protected macrolactone **33** (21 mg, 0.025 mmol) was added at RT to a solution of CF_3COOH (30 μL, 0.40 mmol) in CH_2Cl_2 (300 μL) and the mixture was stirred for 5 h. Evaporation of the solvent and purification of the residue by FC eluting with $CHCl_3$/MeOH/water/AcOH 85:13:1.5:0.5 afforded two batches of material with 84% (9.1 mg) and 74% (7.4 mg) HPLC purities. HPLC purification (A/B 75:25 → 55:45 over 6 min; A = 0.1% aqueous TFA, B = 0.1% TFA in CH_3CN) of the combined materials gave 3.4 mg of **4** with >99% HPLC purity and a second fraction that was 88% pure (0.9 mg), corresponding to a total yield of 23% (calculated for the mono-TFA salt of **4**). All analytical data are for the HPLC-purified material with >99% purity. This material was also used in the tubulin polymerization, microtubule binding, and proliferation experiments. $[\alpha]_D^{20}=-46.4°$ ($c=1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.05$ (br, 1H), 8.09 (t, $J=5.8$ Hz, 1H), 7.86–7.79 (m, 2H), 7.62 (d, $J=8.9$ Hz, 1H), 5.79 (d, $J=9.4$ Hz, 1H), 5.20 (t, $J=7.5$ Hz, 1H), 5.13 (br, 1H), 4.56–4.39 (m, 3H), 4.20 (d, $J=10.6$ Hz, 1H), 3.60–3.30 (m, 3H), 3.17 (quint, $J=7.2$ Hz, 1H), 2.83–2.73 (m, 4H), 2.48–2.42 (m, 1H), 2.41–2.31 (m, 2H), 2.30–2.21 (m, 3H), 2.20–2.13 (m, 2H), 1.86–1.76 (m, 1H), 1.74–1.62 (m, 4H), 1.50–1.40 (m, 1H), 1.39–1.30 (m, 1H), 1.27–1.15 (m, 4H), 1.14–1.04 (m, 4H), 0.93 (d, $J=6.6$ Hz, 3H), 0.90 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=217.60$, 173.72, 171.77, 170.35, 158.03 (q, $J=31$ Hz), 152.12, 139.54, 138.83, 131.58, 122.97, 120.19, 117.04 (q, $J=298$ Hz), 112.15, 111.53, 75.71, 75.54, 70.69, 53.14, 44.72, 44.26, 38.74, 37.42, 36.52, 35.76, 31.74, 30.11, 29.74, 28.68, 26.18, 23.12, 22.44, 20.35, 17.84, 16.35, 13.68 ppm; IR (film): $\tilde{\nu}=3338$, 2927, 2858, 1729, 1671, 1555, 1539, 1526, 1461, 1424, 1376, 1337, 1299, 1256, 1198, 1180, 1141 cm^{-1}; MS (ESI): *m/z*: 628.14 [M+H^+]; HRMS (ESI): *m/z*: calcd for $C_{34}H_{49}N_3O_8$+H: 628.3592 [M+H^+]; found: 628.3588; RP-HPLC: $t_R=5.2$ min (A/B 75:25 for 2 min; then A/B 75:25 → 55:45 over 6 min; A = 0.1% aqueous TFA, B = 0.1% TFA in CH_3CN).

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