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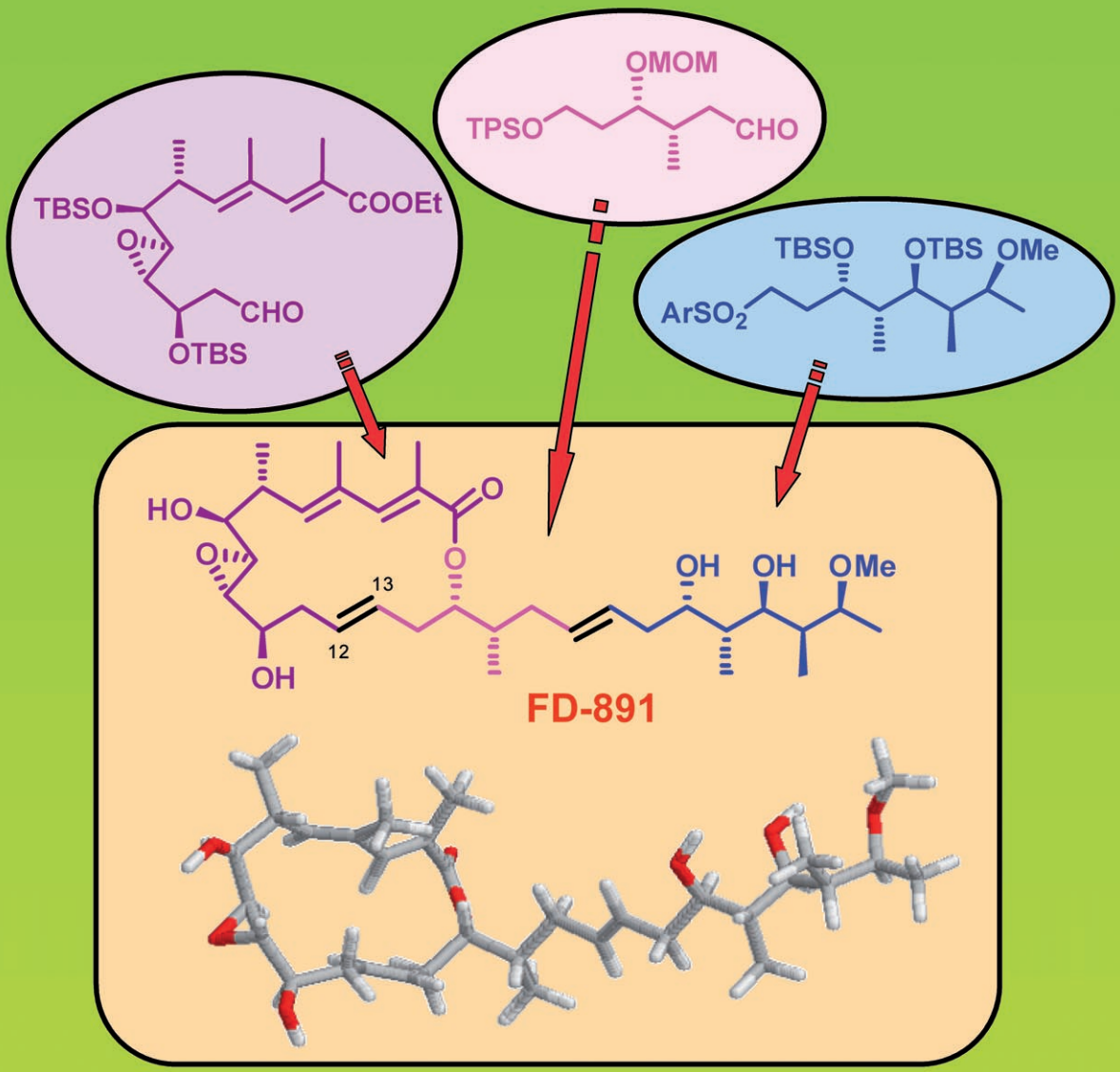

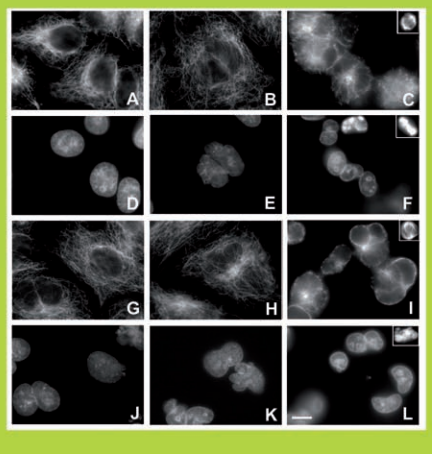

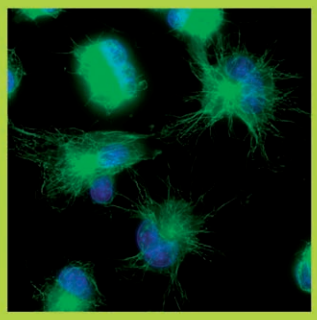

Effect of FD-891 and (*Z*)-FD-891 on a microtubule network

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The Total Synthesis and Biological Properties of the Cytotoxic Macrolide FD-891 and Its Non-Natural (*Z*)-C12 Isomer

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Dedicated to Professors J. Bosch and M. A. Yus on the occasion of their 60th birthdays and to Professor R. Mestres on his retirement.

Abstract: A total, stereoselective synthesis of the naturally occurring, cytotoxic macrolide FD-891 and of its non-natural (*Z*)-C12 isomer is described. Three fragments of the main carbon chain were stereoselectively prepared by using asymmetric aldol and allylation reactions as the key steps. The molecule was then assembled by using two Julia–Kocienski olefinations to connect the three fragments and a Yamaguchi reaction to close the macrolactone ring. Some specific biological properties (cytotoxicity, binding to tubulin) have been determined for both macrolides. The *E* configuration of the C12–C13 olefinic bond seems to be an important feature in determining the cytotoxicity but the precise biological mechanism of the latter still remains to be cleared.

Keywords: aldol reaction · allylation · antitumor agents · lactones · olefination · tubulin binding

Introduction

Natural products with novel structures and useful pharmacological activities have always constituted main targets for synthetic chemists. Among the former, macrocyclic lactones (macrolides) of polyketide origin with cytotoxic and other similarly valuable biological properties have attracted particular attention in the last three decades. A subclass within these lactones are the so-called plecomacrolides, produced by several *Streptomyces* species and characterized by their ability to specifically inhibit the vacuolar-type H^+-ATPase, that is, to disrupt the acidification of intracellular acidic organelles.[1] This property manifests itself in a range of various biological responses, such as antiviral, antifungal, antibacterial, anticancer and immunosuppressant activities.[2]

Several subtypes of compounds within the plecomacrolide group have been reported.[3] Particularly relevant are the bafilomycins, the hygrolidins and the concanamycins. Members of this group are characterized by displaying 16- or 18-membered lactone rings containing two diene units and a polyoxygenated side chain in which a hemiketal fragment is often present. Their structural complexity and potential pharmacological utility have aroused much interest in the chemical community and therefore given rise to a great deal of synthetic effort.[2] This has included not only the natural macrolides but also analogues thereof with the aim of improving their not always beneficial biological profiles.[4]

Macrolide FD-891, structurally related to the concanamycins even though it lacks a hemiketal moiety, was isolated in 1994 by a Japanese group from the fermentation broth of *S. graminofaciens* A-8890.[5] It shows cytotoxic activity against several tumour cell lines and, in addition, has been found to potently prevent both perforin- and FasL-dependent CTL-mediated killing pathways. In contrast to the structurally related concanamycin A, however, it is unable to inhibit va-

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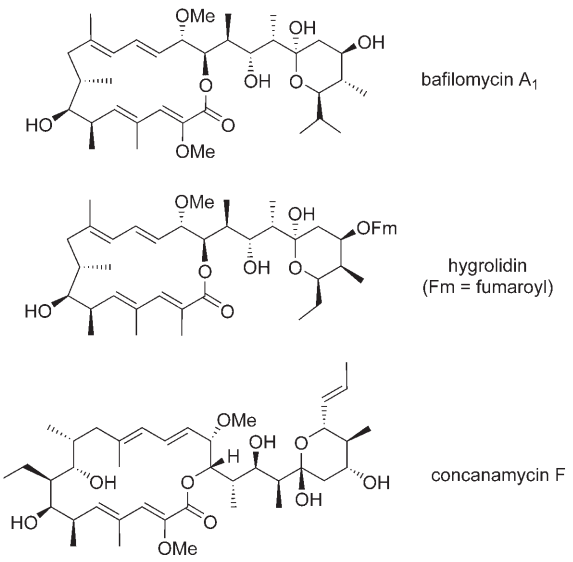
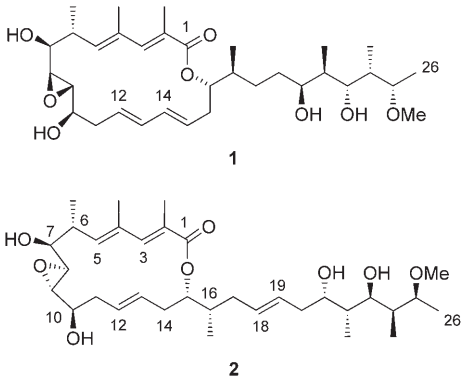


cuolar acidification.[6] More recently, it has been found to be the phytotoxic agent of infections by *Streptomyces* spp. causing potato russet scab.[7] According to the results of chemical degradations and X-ray diffraction analyses of some degradation products, the structure of FD-891 was initially reported in 2002 to be **1**. However, the same group published two years later a correction of its structure, which then turned out to be **2**.[8]



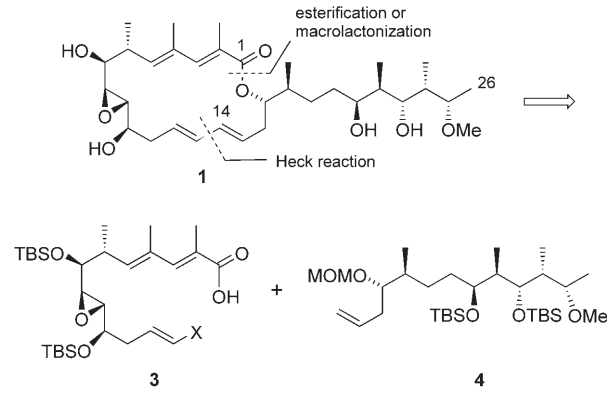
1

2

Results and Discussion

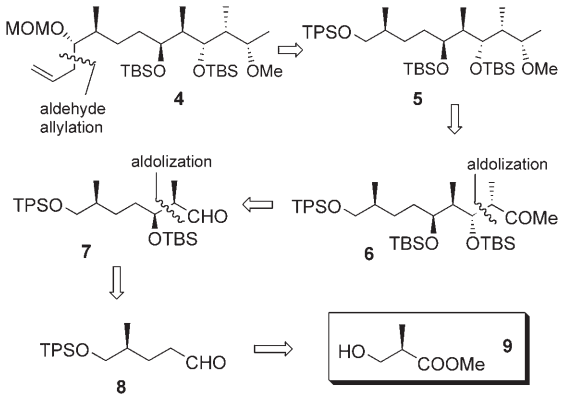
As only structure **1** was known when we started our synthetic work at the end of 2002, we proposed a retrosynthetic disconnection of molecule **1** to fragments **3** (C1–C13, X = Br, I or OSO_2CF_3) and **4** (C14–C26) by means of an esterification and a Heck coupling (Scheme 1). Which one of these two reaction types was to be the final macrocyclisation process would remain an open issue to be decided at a later stage. In any case, both macrolactonisations[9] and intramolecular Heck reactions[10] are amply represented in the literature.

To begin with, we performed a stereoselective synthesis of the whole side chain of molecule **1**. The C14–C26 fragment



Scheme 1. Retrosynthetic plan for macrolide **1**.

4, which displays seven stereocentres,[11] was retrosynthetically disconnected as depicted in Scheme 2. One key structural transformation in this retrosynthesis (**4**→**5**) is the stereoselective allylation of a chiral α-methyl aldehyde and was planned to be performed in an asymmetric way by using one of Brown's chiral allylboration reagents.[12] The protecting group TPS (*tert*-butyldiphenylsilyl) was selected with the idea of its later selective cleavage in the presence of two TBS (*tert*-butyldimethylsilyl) groups. Two other key retrosynthetic transformations, **6**→**7** and **7**→**8**, are aldol reactions conceived to create the C22–C25 dipropionate segment. In the actual synthesis, both aldol steps were executed with the aid of the chiral oxazolidinones developed by Evans and his group.[13] The ultimate chirality source was the commercially available ester **9**.



Scheme 2. Retrosynthetic plan for fragment **4**.

Scheme 3 depicts the synthetic sequence which led to **4**. Chiral ester **9** was converted into the known primary alcohol **10** by means of a literature procedure.[14] Swern oxidation of the latter to aldehyde **8** was followed by Evans asymmetric aldolisation by using the *Z* boron enolate of chiral oxazolidinone **21**.[13a] This provided aldol adduct **11** as a single stereoisomer. Conversion of **11** into the Weinreb amide **12**[15] and silylation afforded **13**, which was reduced (DIBAL) to alde-

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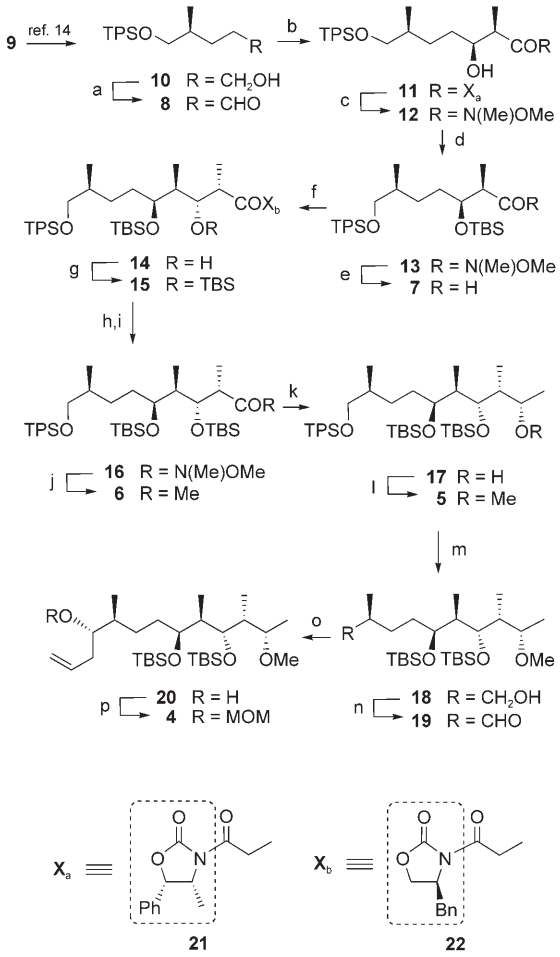
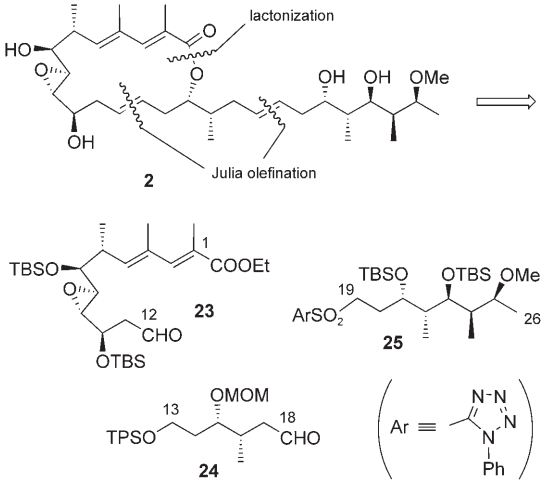


Scheme 3. a) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, 1 h, 0 °C; b) **21**, Bu_2BOTf, Et_3N, 0 °C, then **8**, 2.5 h, 0 °C, 89% overall from **10**; c) MeNHOMe·HCl, $AlMe_3$, THF, 3 h, RT, 79%; d) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 92%; e) DIBAL, THF, −78 °C, 30 min; f) **22**, Bu_2BOTf, Et_3N, 0 °C, then **7**, 3 h, 0 °C, 75% overall from **13**; g) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 90%; h) 30% H_2O_2, aq LiOH, THF, 0 °C to RT, overnight; i) CDI, MeNHOMe·HCl, CH_2Cl_2, RT, 12 h, 80% overall from **15**; j) MeMgBr, THF, 0 °C, 1 h, 70%; k) Me_2AlCl, Bu_3SnH, CH_2Cl_2, −90 °C, 1 h, 91% (92:8 diastereoisomeric mixture); l) MeOTf, 2,6-di-*tert*-butylpyridine in $CHCl_3$, Δ, 4 h, 84%; m) 10% NaOH, MeOH, Δ, 30 h, 84%; n) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, 20 min, 0 °C; o) allylBIpc$_2$ (from (−)-IpcBCl and allylmagnesium bromide), Et_2O, 1 h, −90 °C, 55% overall from **18** as a single stereoisomer; p) MOMCl, *i*Pr_2NEt, CH_2Cl_2, RT, overnight, 79%. DMSO = dimethylsulfoxide, TBS = *tert*-butyldimethylsilyl, TPS = *tert*-butyldiphenylsilyl, DIBAL = diisobutylaluminum hydride, CDI = 1,1′-carbonyl-diimidazole, Tf = trifluoromethanesulfonyl, Ipc = diisopinocampheyl, MOM = methoxymethyl.

hyde **7**. The latter was submitted to a second aldolization with Evans oxazolidinone **22**,[13a] followed by silylation and amidation. This yielded Weinreb amide **16**,[16] which was then converted into methyl ketone **6** by treatment with methylmagnesium bromide. Stereoselective reduction of the carbonyl group of **6** under chelation control[17–20] to alcohol **17** and subsequent *O*-methylation with methyl triflate/2,6-di-*tert*-butylpyridine[21] afforded compound **5**. Selective cleavage of the TPS group in **5** under alkaline conditions[22] provided the primary alcohol **18**, which was oxidized to aldehyde **19**. Asymmetric allylation of the latter[12] afforded secondary alcohol **20** which, through protection of the secondary alcohol group as its MOM derivative,[23] afforded the desired compound **4**.

Just after having published our synthesis of fragment **4**,[11] macrolide FD-891 was reported to have structure **2**, as commented above.[8b] No changes in stereochemistry result from this structural modification, but one olefinic bond has now been moved from inside the ring to the side chain. In view of this, we saw ourselves in the need of carrying out a substantial modification of the initial synthetic plan. Fortunately, most of the ring part of the molecule has remained untouched by the structural amendment and so we have been able to use a part of our previous synthetic concept. For our modified synthesis of FD-891, now having structure **2**, we have chosen the retrosynthetic plan shown in Scheme 4. Ac-



Scheme 4. Retrosynthetic plan for macrolide **2**.

cording to this plan, the molecule of **2** is disconnected to fragments **23** (C1–C12), **24** (C13–C18) and **25** (C19–C26, Ar = 1-phenyl-1*H*-tetrazol-5-yl). The reactions planned to connect these three fragments are a macrolactonisation[9] and two *E*-selective Julia olefinations.[24]

Fragment **23** contains five of the twelve sp^3 stereocentres of the molecule[25] and was retrosynthetically disconnected as shown in Scheme 5. The reactions are basically the same as those we had already ideated for fragment **3** (Scheme 1) of the old structure of FD-891. One key retrotransformation (**26**→**27**) is the stereoselective allylation of an α,β-epoxyaldehyde, while the other (**29**→**30**) is an asymmetric aldol reaction intended to add the Me–C6–C7 propionate segment. The other two propionate segments are added by means of Wittig olefinations (**28**→**29**).

Scheme 6 depicts the synthesis of **23**.[26] The commercially available (*Z*)-2-butene-1,4-diol was first converted into its monoprotected derivative **31**,[27] oxidation of which afforded the (*E*)-2-butenal **30**.[28] The aldol reaction which generates the initial chirality was performed with the aid of Evans

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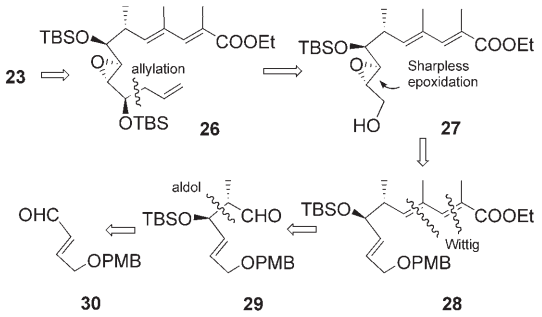
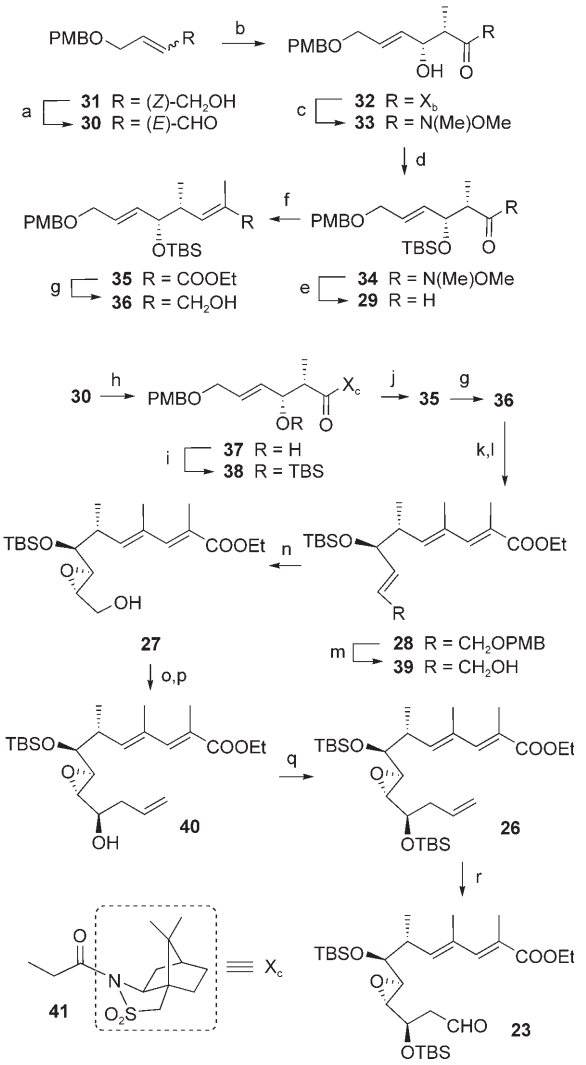


Scheme 5. Retrosynthetic plan for fragment **23**.

chiral oxazolidinone **22** as described in Scheme 3. This yielded aldol **32**, which was converted into Weinreb amide **33** and then silylated to **34**. DIBAL reduction of **34** gave aldehyde **29** which, without chromatographic purification, was taken to the Wittig olefination step to afford the conjugated enoate **35**,[29] subsequently reduced to allylic alcohol **36**. At this stage, we found that the yield of the initial sequence[26] could be markedly improved when the Evans chiral oxazolidinones were replaced by Oppolzer's chiral sultams.[30] Thus, aldehyde **30** was allowed to react with the *Z* boron enolate of chiral sultam **41**. This furnished aldol **37**, which was silylated to **38**. DIBAL reduction of **38** to **29** and subsequent Wittig olefination provided ester **35** with a much better yield than with the previous procedure.[31] Oxidation of **36** to the corresponding aldehyde and Horner–Wadsworth–Emmons olefination yielded the conjugated dienoate **28**. Cleavage of the PMB protecting group with DDQ[32] in wet CH_2Cl_2 to yield **39** was followed by an asymmetric Sharpless epoxidation.[33] The resulting epoxy alcohol **27** was oxidized to the corresponding aldehyde and the latter, in crude form, underwent asymmetric allylation at −110 °C.[12] This procedure provided in 60 % overall yield homoallyl alcohol **40** as a 92:8 mixture of diastereoisomers. Subsequent silylation of the mixture and chromatographic purification gave **26**. Selective oxidative cleavage of the terminal olefinic bond proved problematic due to the presence of the conjugated diene unit. After some experimentation, the problem was solved with the use of Sharpless's reagent AD-mix-β, which permits the dihydroxylation of monosubstituted C=C bonds in the presence of others with a higher substitution degree.[34] This gave a mixture of diastereoisomeric 1,2-diols, which were then oxidatively cleaved with silica-gel-supported $NaIO_4$[35] to yield aldehyde **23**.

Scheme 7 shows the synthesis of fragment **24**.[36] The known aldehyde **42**[37] underwent an asymmetric aldol reaction by using the *Z*-enol borane derived from Oppolzer's chiral propionate equivalent *ent*-**41**.[30] This provided aldol adduct **43** as a single stereoisomer (dr ≥ 98 %, as the minor stereoisomer was not detected by means of high-field $^1H/^{13}C$ NMR spectroscopy). Introduction of the MOM protecting group[23] and functional manipulation gave nitrile **47**, reduction of which afforded aldehyde **24**, which was used directly in crude form in the reaction with **25**.



Scheme 6. a) PCC, CH_2Cl_2, RT, 4 d, 52 %. b) **22**, Bu_2BOTf, Et_3N, CH_2Cl_2, −78 °C, 30 min, then **30**, −40 °C, 12 h, 86 %. c) *N*,*O*-dimethylhydroxylamine hydrochloride, Me_3Al, THF, RT, 1 h, then **32**; d) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 77 % overall yield for the two steps; e) DIBAL, THF, −78 °C, 30 min; f) Ph_3P=C(Me)COOEt, 1,2-dichloroethane, 60 °C, 12 h, 55 % overall yield for the two steps; g) DIBAL, hexane, RT, 1 h, 94 %; h) **41**, Bu_2BOTf, *i*Pr_2NEt, CH_2Cl_2, −5 °C, 30 min, then **30**, −78 °C, 16 h, 95 %; i) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 88 %; j) DIBAL, CH_2Cl_2, −78 °C, 1 h, then Ph_3P=C(Me)COOEt, 1,2-dichloroethane, 60 °C, 12 h, 88 % overall yield for the two steps; k) MnO_2, CH_2Cl_2, Δ, 2 h; l) $(EtO)_2P(O)CH(Me)COOEt$, *n*BuLi, THF, 0 °C, then addition of the crude aldehyde from step **k**, 16 h, 84 % overall yield for the two steps; m) DDQ, wet CH_2Cl_2, RT, 2 h, 89 %. n) diethyl L-tartrate, Ti(O*i*Pr)$_4$, *t*BuO_2H, powdered 4 Å MS, CH_2Cl_2, −23 °C, 24 h, 90 %; o) Swern oxidation; p) allylBIpc$_2$ (from (+)-Ipc$_2$BCl and allylmagnesium bromide), Et_2O, 1 h, −110 °C, 60 % overall yield for the two steps (dr 92:8); q) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 95 %; r) 1) AD-mix-β, aq *t*BuOH, RT, 16 h, 81 % based on recovered starting material; 2) $NaIO_4$ on silica gel, CH_2Cl_2, RT, 30 min. PMB = *p*-methoxybenzyl; PCC = pyridinium chlorochromate; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; MS = molecular sieves.

For the synthesis of fragment **25**, depicted in Scheme 8, we made use of much of the chemistry developed for the preparation of fragment **4**[11] of macrolide **1**, the "old" struc-

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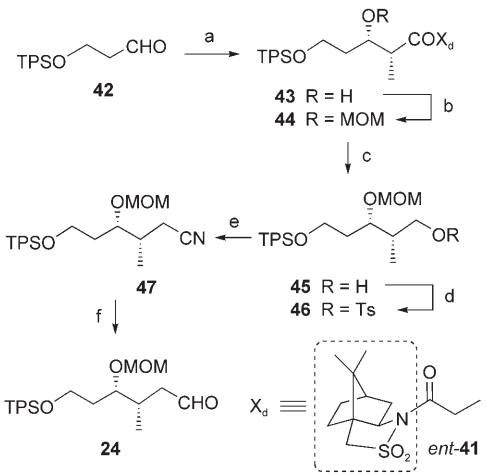
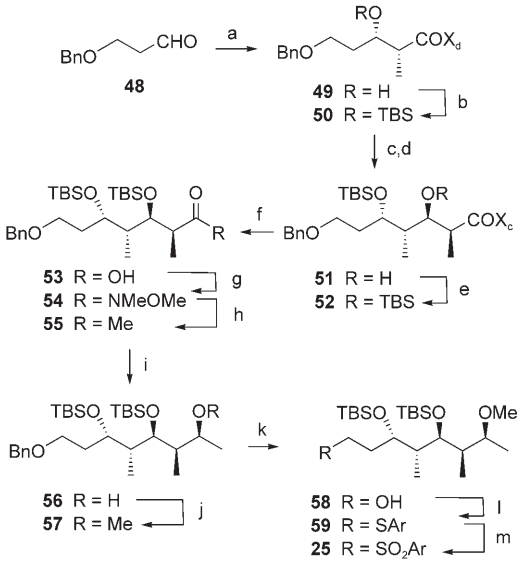


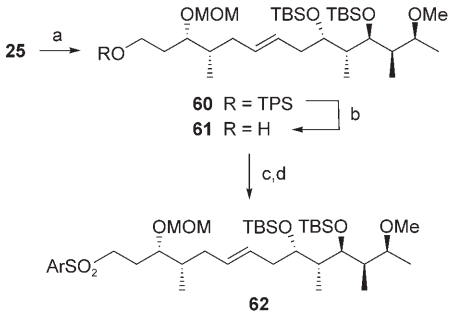
Scheme 7. a) *ent*-**41**, Bu_2BOTf, iPr_2NEt, CH_2Cl_2, −5 °C, 30 min, then **42**, −78 °C, 16 h, 94 %; b) MOMCl, iPr_2NEt, CH_2Cl_2, Δ, 4 h, 91 %; c) $LiAlH_4$, Et_2O, 0 °C, 2 h, 94 %; d) TsCl, Et_3N, CH_2Cl_2, RT, 16 h, 87 %; e) NaCN, DMSO, 80 °C, 2 h, 97 %; f) DIBAL, THF, RT, 3 h. Ts = *p*-toluenesulfonyl.



Scheme 8. a) *ent*-**41**, Bu_2BOTf, iPr_2NEt, CH_2Cl_2, −5 °C, 30 min, then **48**, −78 °C, 16 h, 95 %; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, RT, 1 h, 88 %; c) DIBAL, CH_2Cl_2, −78 °C, 1 h; d) **41**, Bu_2BOTf, iPr_2NEt, CH_2Cl_2, −5 °C, 30 min, then aldehyde, −78 °C, 16 h, 80 % overall for the two steps; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, Δ, 1 h; f) aq LiOH, THF, H_2O_2, RT, 1 d; g) MeNHOMe·HCl, CDI, CH_2Cl_2, RT, 16 h, 65 % overall from **51**; h) MeMgBr, THF, 0 °C, 1 h, 78 %; i) Me_2AlCl, Bu_3SnH, CH_2Cl_2, −90 °C, 1 h, 91 % (dr 92:8); j) MeOTf, proton sponge, $CHCl_3$, Δ, 16 h, 85 %; k) H_2, $Pd(OH)_2$, EtOH, RT, 3 h, 98 %; l) 1-phenyl-1*H*-tetrazol-5-thiol, nBu_3P, DIAD, THF, 0 °C, 90 min, 91 %; m) H_2O_2, $Mo_7O_{24}(NH_4)_6$, EtOH, RT, 16 h, 86 %. Bn = benzyl; DIAD = diisopropyl azodicarboxylate; Ar = 1-phenyl-1*H*-tetrazol-5-yl.

ture of FD-891. Thus, the known aldehyde **48**[38] was subjected to an asymmetric aldol reaction with Oppolzer's chiral reagent *ent*-**41**[30,31] to yield aldol **49** as a single stereoisomer. Hydroxyl silylation to **50** followed by reductive cleavage of the chiral auxiliary gave an intermediate aldehyde which was submitted to an asymmetric aldol reaction with **41**.[30] This yielded a single crystalline aldol **51**,[39] which was converted into the silyl derivative **52**. The latter was transformed into methyl ketone **55** by the intermediate acid **53** and the Weinreb[15] amide **54**.[40] Reduction of **55** under chelation control[17] to **56** and *O*-methylation by using methyl triflate and proton sponge[41] stereoselectively afforded **57**. Hydrogenolytic *O*-debenzylation to **58**, introduction of the tetrazolylthio[42] moiety via Mitsunobu reaction by using nBu_3P[43] and Mo(VI)-catalysed sulfide–sulfone oxidation[44] gave rise to the desired aryl sulphone **25**.

With all key fragments in hand, the synthesis proceeded as shown in Scheme 9. Connection between **24** and **25** was performed by using the Julia–Kocienski[24] olefination protocol, which yielded olefin **60** in good yield (75 %, based on recovered **25**) as a single *E* stereoisomer. Selective cleavage of the TPS group[22] gave alcohol **61**, which was converted into aryl sulphone **62** by means of a Mitsunobu reaction (Ph_3P performed better here than nBu_3P) and Mo(VI)-catalyzed oxidation.



Scheme 9. a) NaHMDS, −78 °C, DME-HMPA 9:1, then addition of freshly prepared **24** (1.5 equiv), 2 h, 75 % (>95 % *E*); b) NaOH, MeOH, Δ, 4 h, 89 %; c) 1-phenyl-1*H*-tetrazol-5-thiol, Ph_3P, DIAD, THF, RT, 1 h, 93 %; d) H_2O_2, $Mo_7O_{24}(NH_4)_6$, EtOH, RT, 16 h, 95 %. NaHMDS = sodium hexamethyldisilazide; DME = 1,2-dimethoxyethane; HMPA, hexamethylphosphoramide.

The final attack towards macrolide **2** was carried out as depicted in Scheme 10. Connection of fragments **23** and **62** was performed as above with the aid of the Julia–Kocienski olefination protocol and gave **63**. The yield was, however, not as high as in Scheme 9 and the reaction was not stereoselective. Changes in various reaction conditions did not lead to improvements.[45] Separation of the *E* and *Z* stereoisomers of **63** was not feasible, but could be done after selective cleavage of the MOM group,[46] which yielded (*E*)-**64** and (*Z*)-**64**.[47] Hydrolysis of the ethyl ester group of (*E*)-**64** was achieved under mild, anhydrous conditions by using TMSOK.[48] The macrolactonisation of the resulting hydroxy acid was performed at high dilution (0.006 M) by using the Yamaguchi procedure[49] and yielded lactone (*E*)-**65**. Cleavage of all silyl groups with TASF[50] finally gave macrolide (*E*)-**2** (FD-891).[51,52] Following the same series of reactions, lactone (*Z*)-**64** was transformed into (*Z*)-**2**, a stereoisomer of the natural macrolide at the C12–C13 double bond.

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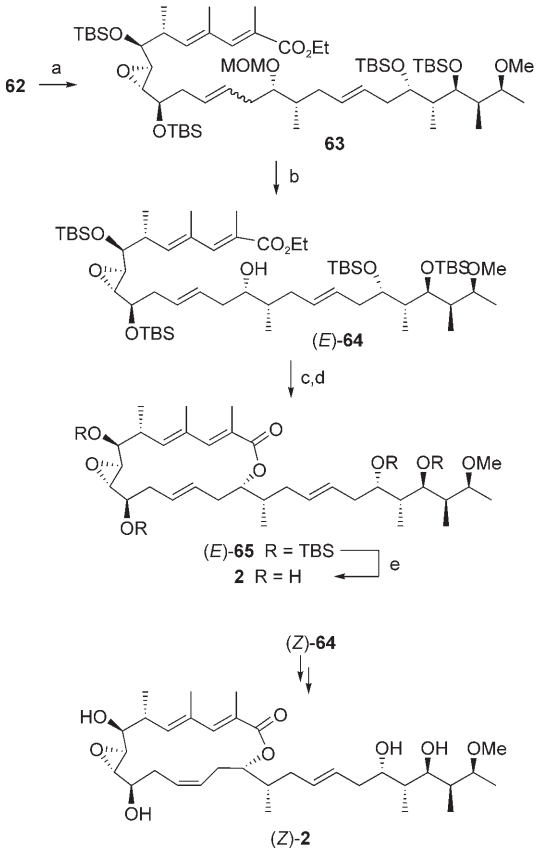

Scheme 10. a) NaHMDS, −78 °C, DME-HMPA 9:1, then addition of freshly prepared **23** (1.5 equiv), 3 h, 60 % (1:1 *E*/*Z* mixture); b) Me_2BBr, CH_2Cl_2, −78 °C, 1 h, 96 %, then isomer separation; c) Me_3SiOK, THF, RT, 10 h; d) 2,4,6-$Cl_3C_6H_2COCl$, iPr_2NEt, CH_2Cl_2, RT, 1 h, 50 % overall for the two steps; e) TASF, DMF, RT, 4 d, 32 %. TASF = tris(dimethylamino) sulfonium difluorotrimethylsilicate.

Biological and biochemical assays: After finishing the total synthesis of the natural macrolide FD-891 ((*E*)-**2**) and its (*Z*)-isomer ((*Z*)-**2**), we investigated some of its biological properties. We first checked the cytotoxicity of compounds (*E*)-**2** and (*Z*)-**2** against two tumoral cell lines. Thus, the IC_{50} values in the case of the ovarian carcinoma A2780 and A2780-AD-MDR (multidrug resistant) cell lines were determined and compared with those of a classical clinical drug, paclitaxel (Table 1).

Table 1. Effect of macrolides (*E*)-**2** and (*Z*)-**2** as compared with paclitaxel on the growth of two human carcinoma cell lines.[a]

Cell line	Paclitaxel[b]	(*E*)-**2**[b]	(*Z*)-**2**[b]
A2780	2.1 ± 0.28	590 ± 174	1916 ± 500
A2780-AD-MDR	660 ± 28 (314)[c]	500 ± 140 (0.85)[c]	2600 ± 450 (1.4)[c]

[a] IC_{50} (50 % inhibition of cell proliferation) of the ligands determined in the parental ovarian carcinoma A2780 cell line and the MDR P-glycoprotein overexpressing ovarian carcinoma A2780 AD. [b] IC_{50} values (nM) are the mean ± standard error of four independent experiments. [c] The numbers in parentheses are the relative resistance of the A2780 AD cell line obtained dividing the IC_{50} of the resistant cell line by the IC_{50} of the parental A2780 cell line.

The results show that the two macrolides were cytotoxic towards both the resistant and the non-resistant cells, even though at a concentration higher than paclitaxel. In addition, (*E*)-**2** appears to be 3–5 times more cytotoxic than (*Z*)-**2**, a fact which suggests that the geometry of the C12−C13 bond plays a relevant role in determining the cytotoxicity. In all likelihood, this is related to the appreciable modification in molecular shape associated to such a stereochemical change. Interestingly, the multidrug-resistant cells showed no resistance to both compounds, indicating that the latter are not substrates for the P-glycoprotein (P-gp) which these cells overexpress in order to pump out the cytotoxic compounds.[53]

In order to study the possible cellular mechanisms that impart cytotoxicity to (*E*)-**2** and (*Z*)-**2**, lung carcinoma A549 cells were treated with either compound at concentrations of 10.0 to 0.2 μM for twenty four hours. Subsequently, their microtubule cytoskeleton and DNA were immunostained. Two hours after addition of the drug, the cells became rounded at the concentrations at which we see the effect. Untreated cells (Figure 1A and D) and cells treated with 0.2 μM of (*Z*)-**2** (G and F) had a typical microtubule cytoskeleton and nucleus. In cells treated with 0.2 μM of (*E*)-**2** (B and E) and 2.5 μM of (*Z*)-**2** (H and K), the microtubule cytoskeleton seems sparser and the nucleus lobulated and in a few cells multinucleated. In cells treated with 1–10 μM of (*E*)-**2** (C and F) and in those treated with 5–10 μM of (*Z*)-**2** (I and L), the cytoplasmic microtubule cytoskeleton is disorganized with fewer and shorter microtubules as compared to PBS-EDTA-treated detached control cells. In some treated cells, the microtubule cytoskeleton is not clearly seen and only a green fluorescence under the cytoplasmic membrane is observed. There is no increase in the number of mitotic cells; most cells have a small nucleus with chromatin that is less dense than that of controls and a few apoptotic cells are observed.

In cell cycle experiments, non-small cell lung carcinoma A549 cells were incubated as before with serial dilutions of the compounds (0.2–10 μM). There is an accumulation in the G2/M phase at 0.2 μM of (*E*)-**2** or at 0.5 μM of (*Z*)-**2** (50 and 55 %, respectively), as compared to 20 % of the control cells. This is accompanied by a reduction of the G0/G1 cells from 64 % in the control cells to 21 and 20 % in the cells treated with (*E*)-**2** and (*Z*)-**2**, respectively. A sub G1 population of 9 and 8 %, respectively, appeared in the treated cells.

In view of the fact that both macrolides showed a visible effect on the cytoplasmic microtubules, we investigated their effect on the in vitro tubulin assembly, as well as the possible binding to microtubules and tubulin. When we checked the influence of the compounds on the in vitro assembly of purified tubulin, however, only negative results were observed, with neither (*E*)-**2** nor (*Z*)-**2** being able to modify the amount of assembled tubulin. To check whether the in vivo effect may be caused through microtubule-associated proteins, we performed similar experiments with microtubular protein, that is, in the presence of microtubule-associated proteins (MAPs). No effect on the amount of polymer pel-

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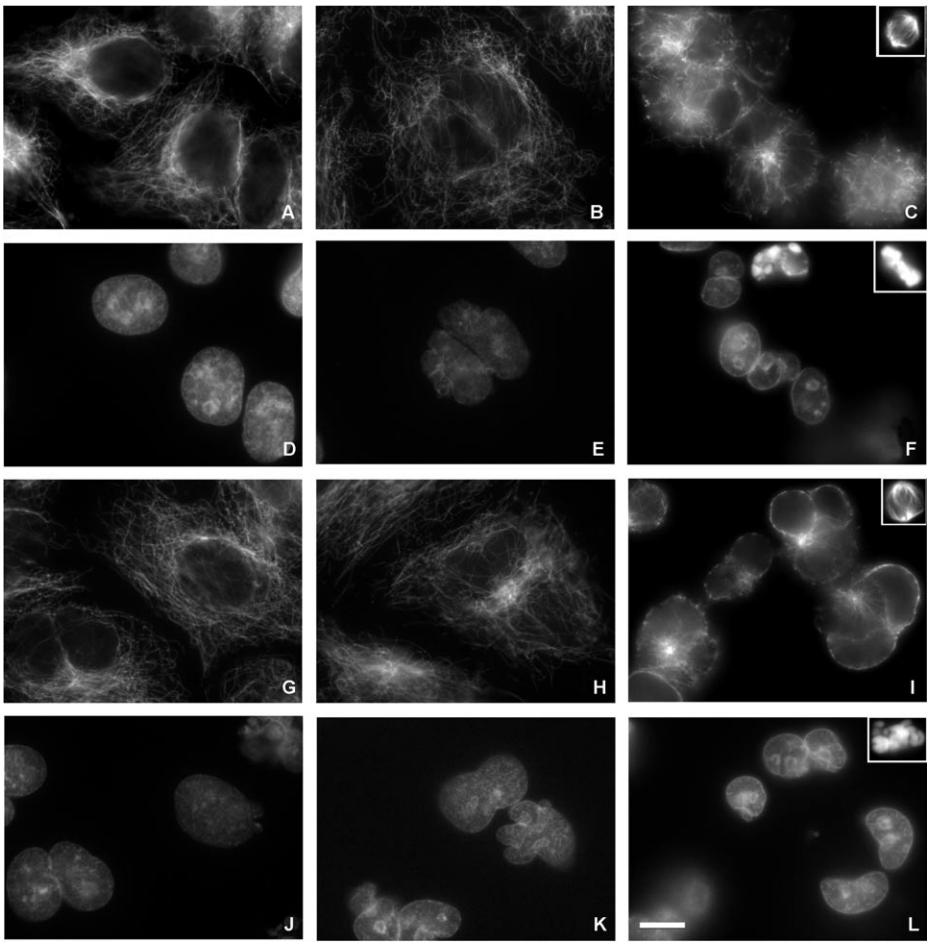

Figure 1. Effect of (*E*)-**2** and (*Z*)-**2** on microtubule network (A–C, G–I) and nucleus morphology (D–F, J–L). A549 cells were incubated for 24 h with DMSO (A,D), (*E*)-**2** 0.2 μM (B,E) or 1 μM (C,F), *Z*-2 0.2 μM (G,J), 2,5 μM (H,K) or 5 μM (I,L). Microtubules were immunostained with α-tubulin monoclonal antibodies and DNA was stained with Hoechst 33342. Insets are mitotic spindles from the same preparation. The scale bar represents 10 μm. All panels and insets have the same magnification.

leted was observed. Electron microscopy was performed on all the polymers and these were normal microtubules.

In order to discard any other possible tubulin-mediated effects, binding of the compounds to polymeric and non-polymeric tubulin was investigated by centrifugation techniques. Native and glutaraldehyde-stabilized microtubules incubated in the presence of the ligand ((*E*)-**2** or (*Z*)-**2**) were pelleted and analysed by means of HPLC. No bound ligand was found in the microtubule pellets, indicating that none of the compounds bind to microtubules with significant affinity.

In order to discard any other site in tubulin that may be occluded by microtubule assembly, non-assembled tubulin was incubated with the ligands and the solution was then centrifuged. All the tubulin was found in the lower part of the tube with none being present in the upper part. HPLC analyses showed no significant differences in compound concentrations between the upper and lower part of the tube indicating that the compounds do not bind with high affinity to tubulin.

From the aforementioned results we can conclude that, at the concentrations at which the drugs produce the effect in cells, they do not significantly bind to tubulin nor do they induce a significant effect in microtubule polymerisation. This indicates that the effects observed in the cellular cytoskeleton take place through other microtubule stabilization/destabilization mechanisms such as, for example, microtubule acetylation/deacetylation,[54] or else through other proteins of the eukaryotic cytoskeleton. This conclusion is further supported by the fact that these drugs do not act in cells as other inhibitors of tubulin polymerisation, such as vinblastin. Thus, while vinblastin does not rapidly affect cell morphology and vinblastin-treated preparations present many mitotic cells, (*E*)-**2** and (*Z*)-**2** exert a rapid effect on cell morphology detaching cells from the plates and show an effect on cytoplasmic microtubules. In contrast, they show no effect on mitotic spindle microtubules at high drug concentrations, as typical with microtubule depolymerisers, and do not give rise to an increase in the number of mitotic cells, even though there is an accumulation on the G2/M phase of the cell cycle.

Experimental Section

General methods: NMR spectra were recorded at 500 (^{1}H NMR spectroscopy) and 125 MHz (^{13}C NMR spectroscopy) in $CDCl_3$ solution at 25 °C. The residual solvent signals were taken as the reference (7.25 ppm for ^{1}H NMR spectroscopy and 77 ppm for ^{13}C NMR spectroscopy). ^{13}C NMR signal multiplicities were determined with the DEPT pulse sequence. Mass spectra were run in the EI (70 eV) or the FAB (*m*-nitrobenzyl alcohol matrix) mode. IR data, which were measured as films on NaCl plates (oils) or as KBr pellets (solids), are given only when relevant functions (C=O, OH, etc.) are present. Optical rotations were measured at 25 °C. Reactions requiring an inert atmosphere (all except those involving water or hydroxylic solvents in the reaction medium) were carried out under dry N_2 with flame-dried glassware. Commercial reagents were used as received. THF and Et_2O were freshly distilled from sodium-benzophenone ketyl. Dichloromethane was freshly distilled from CaH_2. Tertiary amines were freshly distilled from KOH. Unless detailed otherwise, "work-up" means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parenthesis. If the reaction

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medium was acidic (basic), an additional washing with 5% aq $NaHCO_3$ (aq NH_4Cl) was performed, followed by washing with brine, drying over anhydrous $MgSO_4$ and elimination of the solvent under reduced pressure. This was followed by chromatography of the residue on a silica-gel column (60–200 μm) with the indicated solvent. Where solutions were filtered through a Celite pad, the pad was additionally rinsed with the same solvent used and the washing liquids incorporated to the main organic layer.

(4*R*,5*S*)-4-Methyl-5-phenyl-3-[(2*R*,3*S*,6*S*)-7-(*tert*-butyldiphenylsilyloxy)-3-hydroxy-2,6-dimethylheptanoyl]-1,3-oxazolidin-2-one (11): Oxalyl chloride (1.3 mL, 15 mmol) was added dropwise at −78 °C to a solution of DMSO (2.1 mL, 30 mmol) in dry CH_2Cl_2 (30 mL). The mixture was stirred for 5 min at this temperature. A solution of alcohol **10**[14] (4.28 g, 12 mmol) in dry CH_2Cl_2 (10 mL) was then added via syringe. The reaction mixture was stirred for 15 min at −78 °C. After addition of Et_3N (8.4 mL, 60 mmol), the mixture was stirred for 15 min. at −78 °C and then for 1 h at 0 °C. Workup (extraction with CH_2Cl_2) gave crude aldehyde **8** which was used as such in the next reaction.

A solution of oxazolidinone **21** (3.5 g, 15 mmol) in dry CH_2Cl_2 (60 mL) was cooled to −78 °C and treated with nBu_2BOTf (1 M solution in CH_2Cl_2, 27 mL, 27 mmol) and Et_3N (4.2 mL, 30 mmol). After stirring for 30 min at −78 °C and then for 1 h at 0 °C, the mixture was treated dropwise with a solution of crude **8** from above in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at 0 °C for 2.5 h, quenched by addition of a pH 7 buffer solution (60 mL), MeOH (60 mL) and 30% H_2O_2 (30 mL). The resulting mixture was then stirred at room temperature for 30 min. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 90:10) afforded aldol **11** as a single diastereoisomer (5.23 g, 89% overall, based on **10**). Colourless oil; $[\alpha]_D = +7.8$ ($c = 1$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.75$–7.70 (m, 4H), 7.45–7.30 (br m, 11H), 5.65 (d, $J = 7.3$ Hz, 1H), 4.80 (quint, $J = 6.8$ Hz, 1H), 3.96 (m, 1H), 3.82 (qd, $J = 7.0$, 2.5 Hz, 1H), 3.58 (dd, $J = 9.8$, 5.8 Hz, 1H), 3.52 (dd, $J = 9.8$, 6.5 Hz, 1H), 3.00 (br s, 1H; OH), 1.73 (m, 1H), 1.65–1.50 (br m, 2H), 1.50–1.35 (br m, 2H), 1.26 (d, $J = 7.0$ Hz, 3H), 1.11 (s, 9H), 0.98 (d, $J = 6.5$ Hz, 3H), 0.90 ppm (d, $J = 7$ Hz, 3H); ^{13}C NMR: $\delta = 177.1$, 152.5, 133.9, 133.8, 133.1, 19.2 (C), 135.5 (×4), 129.4 (×2), 128.7, 128.6 (×2), 127.5 (×4), 125.5 (×2), 78.7, 71.7, 54.6, 42.3, 35.6 (CH), 68.7, 31.4, 29.4 (CH_2), 26.9 (×3), 16.8, 14.2, 10.3 ppm (CH_3); IR: $\tilde{\nu} = 3530$ (br; OH), 1783, 1699 cm^{-1} (C=O); HR-EIMS *m*/*z* (%): calcd for $C_{35}H_{45}NO_5Si$–*t*Bu: 530.2362; found: 530.2334 (5) $[M-t\mathrm{Bu}]^+$, 468 (8), 408 (4), 297 (44), 199 (100); elemental analysis: calcd (%) for $C_{35}H_{45}NO_5Si$: C 71.51, H 7.72; found: C 71.39, H 7.60.

(2*R*,3*S*,6*S*)-7-(*tert*-Butyldiphenylsilyloxy)-3-hydroxy-*N*-methoxy-2,6-*N*-trimethylheptanamide (12): A solution of *N*,*O*-dimethylhydroxylamine hydrochloride (2.54 g, 26 mmol) in dry THF (50 mL) was treated dropwise at 0 °C with Me_3Al (2.0 M solution in toluene, 13 mL, 26 mmol). The mixture was stirred for 1 h at room temperature. A solution of aldol **11** from above (5.11 g, 8.7 mmol) in dry THF (15 mL) was then added dropwise by syringe. The reaction mixture was stirred for 3 h at room temperature and then quenched through addition of a saturated aq solution of potassium sodium tartrate (60 mL). Stirring at room temperature for 30 min and workup (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexane/EtOAc 70:30) to yield the Weinreb amide **12** (3.24 g, 79%). Colourless oil; $[\alpha]_D = -12.6$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.70$–7.65 (m, 4H), 7.45–7.30 (br m, 6H), 3.84 (m, 1H), 3.80 (br s, 1H; OH), 3.68 (s, 3H), 3.54 (dd, $J = 9.8$, 5.8 Hz, 1H), 3.52 (dd, $J = 9.8$, 6.2 Hz, 1H), 3.20 (s, 3H), 2.90 (m, 1H), 1.70 (m, 1H), 1.60 (m, 1H), 1.50 (m, 1H), 1.40–1.25 (br m, 2H), 1.18 (d, $J = 7.0$ Hz, 3H), 1.08 (s, 9H), 0.96 ppm (d, $J = 6.8$ Hz, 3H); ^{13}C NMR: $\delta = 178.3$*, 134.0 (×2), 19.3 (C), 135.5 (×4), 129.4 (×2), 127.5 (×4), 71.7, 38.5, 35.8 (CH), 68.8, 31.4, 29.4 (CH_2), 61.4, 31.8*, 26.9 (×3), 16.8, 10.0 ppm (CH_3) (starred peaks are very low and broad); IR: $\tilde{\nu} = 3460$ (br, OH), 1640 cm^{-1} (C=O); HR-EIMS *m*/*z* (%): calcd for $C_{27}H_{41}NO_4Si$–*t*Bu: 414.2100; found: 414.2097 (2) $[M-t\mathrm{Bu}]^+$, 336 (9), 297 (14), 199 (100); elemental analysis: calcd (%) for $C_{27}H_{41}NO_4Si$: C 68.75, H 8.76; found: C 68.68, H 8.61.

(2*R*,3*S*,6*S*)-3-(*tert*-Butyldimethylsilyloxy)-7-(*tert*-butyldiphenylsilyloxy)-*N*-methoxy-2,6-*N*-trimethylheptanamide (13): A solution of alcohol **12** (3.21 g, 6.8 mmol) in dry CH_2Cl_2 (40 mL) was treated dropwise at RT with 2,6-lutidine (1.2 mL, approximately 10.2 mmol) and TBSOTf (2 mL, approximately 8.5 mmol). The mixture was stirred for 1 h at room temperature. Workup (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexane/EtOAc 90:10) to yield **13** (3.66 g, 92%). Colourless oil; $[\alpha]_D = +4.3$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.70$–7.65 (m, 4H), 7.45–7.35 (br m, 6H), 3.98 (m, 1H), 3.65 (s, 3H), 3.54 (dd, $J = 9.8$, 5.5 Hz, 1H), 3.49 (dd, $J = 9.8$, 6.0 Hz, 1H), 3.17 (s, 3H), 3.00 (m, 1H), 1.70–1.45 (br m, 5H), 1.19 (d, $J = 7.0$ Hz, 3H), 1.09 (s, 9H), 0.95 (d, 3H, overlapped), 0.94 (s, 9H), 0.09 (s, 3H), 0.08 ppm (s, 3H); ^{13}C NMR: $\delta = 176.5$*, 134.0 (×2), 19.3, 18.1 (C), 135.5 (×4), 129.4 (×2), 127.5 (×4), 73.7, 40.4, 36.2 (CH), 69.2, 33.2, 27.7 (CH_2), 61.2, 32.1*, 26.9 (×3), 26.0 (×3), 16.7, 14.4, −4.2, −4.6 ppm (CH_3) (starred peaks are very low and broad); IR: $\tilde{\nu} = 1666$ cm^{-1} (C=O); HR-EIMS *m*/*z* (%): calcd for $C_{33}H_{55}NO_4Si_2$–Me: 570.3435; found: 570.3403 (3) $[M-\mathrm{Me}]^+$, 528 (100) $[M-t\mathrm{Bu}]^+$; elemental analysis: calcd (%) for $C_{33}H_{55}NO_4Si_2$: C 67.64, H 9.46; found: C 67.68, H 9.59.

(4*S*)-4-Benzyl-3-[(2*S*,3*R*,4*S*,5*S*,8*S*)-5-(*tert*-butyldimethylsilyloxy)-9-(*tert*-butyldiphenylsilyloxy)-3-hydroxy-2,4,8-trimethylnonanoyl]-1,3-oxazolidin-2-one (14): A solution of **13** (3.63 g, 6.2 mmol) in dry THF (60 mL) was treated dropwise at −78 °C with DIBAL (1 M solution in hexane, 31 mL, 31 mmol). The mixture was stirred for 30 min at the same temperature and quenched through addition of saturated aqueous NH_4Cl (2 mL). The mixture was then stirred at room temperature until formation of a persistent gel. Filtration through Celite (washing with EtOAc) and solvent removal under reduced pressure gave crude aldehyde **7** which was used as such in the next reaction.

A solution of oxazolidinone **22** (1.75 g, 7.5 mmol) in dry CH_2Cl_2 (40 mL) was cooled to −78 °C and treated with nBu_2BOTf (1 M solution in CH_2Cl_2, 13.5 mL, 13.5 mmol) and Et_3N (2.1 mL, 15 mmol). After stirring for 30 min at −78 °C and then for 1 h at 0 °C, the mixture was treated dropwise with a solution of crude **7** from above in dry CH_2Cl_2 (15 mL). The reaction mixture was stirred at 0 °C for 3 h, quenched by addition of a pH 7 buffer solution (40 mL), MeOH (40 mL) and 30% H_2O_2 (20 mL) and stirred at room temperature for 30 min Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 90:10) afforded aldol **14** as a single diastereoisomer (3.53 g, 75% overall from **13**). Colourless oil; $[\alpha]_D = +8.6$ ($c = 1$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.75$–7.70 (m, 4H), 7.45–7.25 (br m, 11H), 4.70 (m, 1H), 4.20–4.15 (m, 2H), 4.00 (br d, $J = 9.7$ Hz, 1H), 3.90–3.85 (m, 2H), 3.50 (2H; AB system), 3.33 (dd, $J = 13.4$, 3.0 Hz, 1H), 2.78 (dd, $J = 13.4$, 9.7 Hz, 1H), 1.80 (m, 1H), 1.70 (br s, 1H; OH), 1.65–1.55 (br m, 2H), 1.50–1.35 (br m, 3H), 1.24 (d, $J = 6.8$ Hz, 3H), 1.07 (s, 9H), 0.92 (d, $J = 6.8$ Hz, 3H), 0.90 (s, 9H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.11 (s, 3H), 0.08 ppm (s, 3H); ^{13}C NMR: $\delta = 176.3$, 153.2, 135.4, 134.0, 133.9, 19.3, 18.0 (C), 135.5 (×4), 129.5 (×2), 129.4, (×2), 128.9 (×2), 127.5 (×4), 127.3, 76.4, 73.0, 55.7, 40.5, 39.6, 35.9 (CH), 68.9, 66.1, 37.8, 30.3, 30.0 (CH_2), 26.9 (×3), 25.9 (×3), 16.8, 11.8, 8.8, −4.4, −4.5 ppm (CH_3); IR: $\tilde{\nu} = 3530$ (br, OH), 1783, 1690, 1680 cm^{-1} (C=O); HR-EIMS *m*/*z* (%): calcd. for $C_{44}H_{65}NO_6Si_2$: 759.4350; found: 759.4367 (1) $[M]^+$, 702 (2), 199 (100); elemental analysis: calcd (%) for $C_{44}H_{65}NO_6Si_2$: C 69.52, H 8.62; found: C 69.67, H 8.60.

(4*S*)-4-Benzyl-3-[(2*S*,3*R*,4*R*,5*S*,8*S*)-3,5-bis(*tert*-butyldimethylsilyloxy)-9-(*tert*-butyldiphenylsilyloxy)-2,4,8-trimethylnonanoyl]-1,3-oxazolidin-2-one (15): Alcohol **14** (3.50 g, 4.6 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and treated dropwise at room temperature with 2,6-lutidine (4.8 mL, 41 mmol) and TBSOTf (6.3 mL, approximately 27.6 mmol). The mixture was stirred for 1 h at room temperature. Workup (extraction with CH_2Cl_2) was followed by *careful* column chromatography of the residue on silica gel (hexane/EtOAc 90:10) to yield **15** (3.62 g, 90%). Colourless oil; $[\alpha]_D = +35.4$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.70$–7.65 (m, 4H), 7.45–7.25 (br m, 11H), 4.60 (m, 1H), 4.20–4.10 (br m, 3H), 4.00 (m, 1H), 3.75 (m, 1H), 3.54 (dd, $J = 9.8$, 5.7 Hz, 1H), 3.50 (dd, $J = 9.8$, 6.3 Hz, 1H), 3.30 (dd, $J = 13.4$, 3.0 Hz, 1H), 2.80 (dd, $J = 13.4$, 9.6 Hz, 1H), 1.75 (m, 1H), 1.65 (m, 1H), 1.60–1.45 (br m, 4H), 1.24 (d, $J = 6.8$ Hz, 3H), 1.09 (s, 9H), 0.95 (br s, 9H, overlapping two methyl doublets), 0.90 (s, 9H), 0.12 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H), 0.05 ppm (s, 3H); ^{13}C NMR: $\delta = 175.8$, 152.7, 135.4, 134.0, 133.9, 19.3, 18.4, 18.3 (C), 135.6 (×4), 129.5 (×4), 128.9 (×2), 127.6 (×4), 127.3, 73.3, 72.7, 55.7, 43.3, 41.1, 33.2 (CH), 68.9, 65.8, 37.6, 36.3, 28.4 (CH_2), 26.9 (×3), 26.0 (×3), 25.9 (×3), 16.9, 11.7,

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10.2, −3.0, −3.4, −3.5, −3.9 ppm (CH_3); IR: $\tilde{\nu}$ = 1786, 1704 cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{50}H_{79}NO_6Si_3$–*t*Bu: 816.4611; found: 816.4601 (100) $[M-t\mathrm{Bu}]^+$, 684 (12), 639 (15), 290 (42); elemental analysis: calcd (%) for $C_{50}H_{79}NO_6Si_3$: C 68.68, H 9.11; found: C 68.60, H 9.01.

(2*S*,3*R*,4*R*,5*S*,8*S*)-3,5-bis(*tert*-Butyldimethylsilyloxy)-9-(*tert*-butyldiphenylsilyloxy)-*N*-methoxy-2,4,8-*N*-tetramethylnonanamide (16): A solution of compound **15** (3.59 g, 4.1 mmol) in THF/H_2O 3:1 (20 mL) was cooled to 0 °C and treated with 30% H_2O_2 (2.5 mL, approximately 22 mmol) and LiOH monohydrate (345 mg, 8.2 mmol). The mixture was stirred for 2 h at 0 °C and then overnight at room temperature. After addition of Na_2SO_3 (2.8 g dissolved in 20 mL of water), most of the THF was removed under reduced pressure and the residue was extracted with CH_2Cl_2. This gave a crude acid which was used as such in the next reaction.

A solution of the crude acid from above in dry CH_2Cl_2 (40 mL) was cooled to 0 °C and treated with CDI (650 mg, 4 mmol). After stirring for 30 min at 0 °C, *N*,*O*-dimethylhydroxylamine hydrochloride (780 mg, 8 mmol) was added. The reaction mixture was then stirred overnight at room temperature. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 80:20) furnished Weinreb amide **16** (2.49 g, 80% overall from **15**). Colourless oil; $[\alpha]_D = -0.7$ (c = 1.8 in $CHCl_3$); ^{1}H NMR: δ = 7.70–7.65 (m, 4H), 7.45–7.35 (br m, 6H), 4.10 (t, J = 5.3 Hz, 1H), 3.69 (s, 3H), 3.63 (m, 1H), 3.55 (dd, J = 9.8, 5.7 Hz, 1H), 3.48 (dd, J = 9.8, 6.4 Hz, 1H), 3.19 (s, 3H), 3.05 (m, 1H), 1.77 (m, 1H), 1.70–1.55 (br m, 4H), 1.45 (m, 1H), 1.16 (d, J = 7 Hz, 3H), 1.09 (s, 9H), 0.97 (d, J = 6.6 Hz, 3H), 0.94 ppm (br s, 9H, overlapping one methyl doublet), 0.91 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.05 ppm (s, 3H); ^{13}C NMR: δ = 176.6*, 134.1, 134.0, 19.3, 18.3, 18.2 (C), 135.6 (×4), 129.5 (×2), 127.6 (×4), 73.2, 72.7, 43.7, 37.8, 36.4 (CH), 69.3, 32.8, 27.7 (CH_2), 61.0, 32.4*, 26.9 (×3), 26.1 (×3), 26.0 (×3), 16.9, 13.4, 11.1, −3.5, −3.8, −4.0, −4.4 ppm (CH_3) (starred peaks are very low and broad); IR: $\tilde{\nu}$ = 1670 cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{42}H_{75}NO_5Si_3$–*t*Bu, 700.4248; found: 700.4213 (4) $[M-t\mathrm{Bu}]^+$, 670 (100), 199 (58); elemental analysis: calcd (%) for $C_{42}H_{75}NO_5Si_3$: C 66.52, H 9.97; found: C 66.39, H 9.82.

(3*S*,4*R*,5*R*,6*S*,9*S*)-4,6-Bis(*tert*-butyldimethylsilyloxy)-10-(*tert*-butyldiphenylsilyloxy)-3,5,9-trimethyldecan-2-one (6): A solution of Weinreb amide **16** (2.43 g, 3.2 mmol) in dry THF (30 mL) was cooled under N_2 at −20 °C and treated dropwise with MeMgBr (3 M solution in Et_2O, 3.7 mL, 11.2 mmol). The reaction mixture was then stirred at 0 °C for 1 h and worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexane/EtOAc 95:5) afforded ketone **6** (1.6 g, 70%). Colourless oil; $[\alpha]_D = +14.6$ (c = 1.2 in $CHCl_3$); ^{1}H NMR: δ = 7.70–7.65 (m, 4H), 7.45–7.35 (br m, 6H), 4.27 (dd, J = 6.7, 2.3 Hz, 1H), 3.80 (dt, J = 7.2, 4.0 Hz, 1H), 3.52 (dd, J = 9.8, 5.8 Hz, 1H), 3.49 (dd, J = 9.8, 6.0 Hz, 1H), 2.60 (qd, J = 7.0, 2.3 Hz, 1H), 2.17 (s, 3H), 1.70 (m, 1H), 1.65–1.40 (br m, 5H), 1.13 (d, J = 7.0 Hz, 3H), 1.08 (s, 9H), 0.96 (d, J = 6.8 Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.87 (d, J = 7.0 Hz, 3H), 0.10 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H), 0.01 ppm (s, 3H); ^{13}C NMR: δ = 210.8, 134.0 (×2), 19.3, 18.3, 18.2 (C), 135.6 (×4), 129.5 (×2), 127.6 (×4), 73.1, 72.7, 50.2, 43.0, 36.3 (CH), 68.9, 33.3, 28.5 (CH_2), 28.6, 26.9 (×3), 26.1 (×3), 26.0 (×3), 16.9, 10.3, 10.2, −3.3, −3.9, −4.0 (×2) (CH_3); IR: $\tilde{\nu}$ = 1716 cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{41}H_{72}O_4Si_3$–*t*Bu, 655.4034; found: 655.4038 (12) $[M-t\mathrm{Bu}]^+$, 483 (27), 469 (33), 199 (44), 135 (73), 73 (100); elemental analysis: calcd (%) for $C_{41}H_{72}O_4Si_3$: C 69.04, H 10.17; found: C 69.19, H 10.00.

(2*S*,3*R*,4*S*,5*R*,6*S*,9*S*)-4,6-Bis(*tert*-butyldimethylsilyloxy)-10-(*tert*-butyldiphenylsilyloxy)-3,5,9-trimethyldecan-2-ol (17): Ketone **6** (1.57 g, 2.2 mmol) was dissolved at −78 °C in dry CH_2Cl_2 (25 mL) and treated with Me_2AlCl (2 M solution in hexane, 5.5 mL, 5.5 mmol). The reaction mixture was stirred for 5 min at −78 °C, then cooled to −90 °C and treated with nBu$_3$SnH (675 μL, 2.5 mmol). After stirring for 1 h at −90 °C, the cooling bath was removed, saturated aq $NaHCO_3$ (10 mL) was added and the mixture was further stirred at room temperature for 30 min. Workup (extraction with CH_2Cl_2) provided a 92:8 mixture of diastereoisomeric alcohols (1.43 g, 91%), which was carefully purified by means of flash column chromatography on silica gel (hexane/EtOAc 95:5) to yield pure **17**. Colourless oil; $[\alpha]_D = -2.6$ (c = 2.2 in $CHCl_3$); ^{1}H NMR: δ = 7.70–7.65 (m, 4H), 7.45–7.35 (br m, 6H), 3.89 (dd, J = 5.5, 1.5 Hz, 1H), 3.84 (quint, $J \approx$ 6.0 Hz, 1H), 3.68 (m, 1H), 3.53 (dd, J = 9.8, 5.7 Hz, 1H), 3.48 (dd, J = 9.8, 6.0 Hz, 1H), 2.20 (br s, 1H; OH), 1.80 (m, 1H), 1.65–1.45 (br m, 6H), 1.18 (d, J = 6.0 Hz, 3H), 1.08 (s, 9H), 0.98 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.93 (s, 9H, overlapping one methyl doublet), 0.90 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 ppm (s, 3H); ^{13}C NMR: δ = 134.0 (×2), 19.3, 18.3, 18.2 (C), 135.6 (×4), 129.5 (×2), 127.6 (×4), 76.9, 73.8, 72.2, 43.0, 41.6, 36.3 (CH), 69.0, 33.0, 28.2 (CH_2), 26.9 (×3), 26.1
(×3), 26.0 (×3), 21.2, 16.9, 10.6, 8.4, −3.2, −3.3, −4.1 −4.4 ppm (CH_3); IR: $\tilde{\nu}$ = 3450 cm^{-1} (br, OH); HR-EIMS *m/z* (%): calcd for $C_{41}H_{74}O_4Si_3$–*t*Bu: 657.4190; found: 657.4168 (1) $[M-t\mathrm{Bu}]^+$, 525 (2), 469 (7), 199 (14), 135 (18), 73 (100); elemental analysis: calcd (%) for $C_{41}H_{74}O_4Si_3$: C 68.85, H 10.43; found: C 68.98, H 10.25.

(2*S*,3*R*,4*S*,5*R*,6*S*,9*S*)-4,6-Bis(*tert*-butyldimethylsilyloxy)-10-(*tert*-butyldiphenylsilyloxy)-2-methoxy-3,5,9-trimethyldecane (5): A solution of alcohol **17** (1.29 g, 1.8 mmol) in dry $CHCl_3$ (80 mL) was treated at room temperature with 2,6-di-*tert*-butylpyridine (8 mL, 36 mmol) and MeOTf (2 mL, 18 mmol). Both reagents were added in two portions with an interval of 2 h. The reaction mixture was then stirred at reflux until consumption of the starting material (about 2 h, TLC monitoring), and then quenched by addition of methanol (2 mL) and saturated aqueous $NaHCO_3$ (6 mL), followed by further stirring for 30 min at room temperature. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 95:5) gave **5** (1.10 g, 84%). Colourless oil; $[\alpha]_D = +4.1$ (c = 1.7 in $CHCl_3$); ^{1}H NMR: δ = 7.70–7.65 (m, 4H), 7.45–7.35 (br m, 6H), 3.82 (br d, J = 6.0 Hz, 1H), 3.74 (m, 1H), 3.53 (dd, J = 9.8, 5.7 Hz, 1H), 3.47 (dd, J = 9.8, 6.2 Hz, 1H), 3.30 (s, 3H), 3.15 (quint, J = 6.5 Hz, 1H), 1.73 (m, 1H), 1.65–1.50 (br m, 5H), 1.45 (m, 1H), 1.13 (d, J = 6.3 Hz, 3H), 1.08 (s, 9H), 0.95 (d, J = 6.5 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.86 (d, J = 7.0 Hz, 3H), 0.10 (s, 3H), 0.07 (s, 6H), 0.05 ppm (s, 3H); ^{13}C NMR: δ = 134.1, 134.0, 19.3, 18.5, 18.3 (C), 135.6 (×4), 129.5 (×2), 127.6 (×4), 79.8, 73.3, 72.8, 43.3, 41.4, 36.5 (CH), 69.2, 33.0, 28.1 (CH_2), 56.5, 26.9 (×3), 26.1 (×3), 26.0 (×3), 16.9, 16.5, 11.0, 10.5, −3.2, −3.4, −3.9 −4.0 ppm (CH_3); HR-EIMS *m/z* (%): calcd for $C_{42}H_{76}O_4Si_3$–*t*Bu: 671.4347; found: 671.4293 (7) $[M-t\mathrm{Bu}]^+$, 539 (6), 469 (23), 199 (18), 135 (22), 73 (51), 59 (100); elemental analysis: calcd (%) for $C_{42}H_{76}O_4Si_3$: C 69.17, H 10.50; found: C 69.00, H 10.65.

(2*S*,5*S*,6*R*,7*S*,8*R*,9*S*)-5,7-Bis(*tert*-butyldimethylsilyloxy)-9-methoxy-2,6,8-trimethyldecanol (18): A solution of compound **5** (1.1 g, approximately 1.5 mmol) in 10% NaOH/MeOH (40 mL) was stirred at reflux for 30 h. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 90:10) gave **18** (618 mg, 84%). Colourless oil; $[\alpha]_D = +4.9$ (c = 1.7 in $CHCl_3$); ^{1}H NMR: δ = 3.82 (dd, J = 5.8, 1.5 Hz, 1H), 3.68 (br q, $J \approx$ 5.0 Hz, 1H), 3.51 (dd, J = 10.5, 5.8 Hz, 1H), 3.47 (dd, J = 10.5, 6.6 Hz, 1H), 3.30 (s, 3H), 3.14 (quint, J = 6.5 Hz, 1H), 1.73 (m, 1H), 1.70–1.55 (br m, 4H), 1.50–1.45 (m, 2H), 1.40 (br s, 1H; OH), 1.13 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.88 (d, J = 7.0 Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.06 ppm (s, 6H); ^{13}C NMR: δ = 18.5, 18.3 (C), 80.1, 73.3, 72.4, 43.4, 41.0, 36.2 (CH), 68.2, 32.3, 27.5 (CH_2), 56.5, 26.1 (×3), 26.0 (×3), 16.6, 16.3, 11.3, 10.7, −3.3, −3.6, −4.1 ppm (×2) (CH_3); IR: $\tilde{\nu}$ = 3360 cm^{-1} (br, OH); HR-EIMS *m/z* (%): calcd for $C_{26}H_{58}O_4Si_2$–MeCHOMe: 431.3371; found: 431.3321 (2) $[M-\mathrm{MeCHOMe}]^+$, 231 (48), 75 (100), 59 (65); elemental analysis: calcd (%) for $C_{26}H_{58}O_4Si_2$: C 63.61, H 11.91; found: C 63.74, H 11.75.

(4*S*,5*S*,8*S*,9*R*,10*S*,11*R*,12*S*)-8,10-Bis(*tert*-butyldimethylsilyloxy)-12-methoxy-5,9,11-trimethyltridec-1-en-4-ol (20): Oxalyl chloride (130 μL, 1.5 mmol) was added dropwise at −78 °C to a solution of DMSO (210 μL, 3 mmol) in dry CH_2Cl_2 (3 mL). The mixture was stirred for 5 min at this temperature. A solution of alcohol **18** (614 mg, 1.25 mmol) in dry CH_2Cl_2 (1 mL) was then added via syringe. The reaction mixture was stirred for 15 min at −78 °C. After addition of Et_3N (840 μL, 6 mmol), the mixture was stirred for 15 min at −78 °C and then for 20 min at 0 °C. Workup (extraction with CH_2Cl_2) gave crude aldehyde **19** which was used as such in the next reaction.

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Allylmagnesium bromide (commercial 1 M solution in Et_2O, 1.5 mL, 1.5 mmol) was added dropwise at 0 °C via syringe to a solution of (−)-Ipc_2BCl (580 mg, 1.8 mmol) in dry Et_2O (8 mL). The mixture was further stirred for 1 h at 0 °C. The solution was then allowed to stand, which caused precipitation of magnesium chloride. The supernatant solution was then carefully transferred to another flask by means of a cannula. After cooling this flask at −90 °C, a solution of crude **19** from above in dry Et_2O (5 mL) was added dropwise by syringe. The resulting solution was further stirred at the same temperature for 1 h. The reaction mixture was then quenched through addition of phosphate pH 7 buffer solution (8 mL), MeOH (8 mL) and 30 % H_2O_2 (4 mL). After stirring for 30 min, the mixture was poured onto saturated aqueous $NaHCO_3$ and worked up (extraction with Et_2O). Careful column chromatography on silica gel (hexane/EtOAc 95:5) afforded **20** (365 mg, 55 % overall from **18**). Colourless oil; $[\alpha]_D = +2.7$ ($c = 1.4$ in $CHCl_3$); ^{1}H NMR: $\delta = 5.84$ (m, 1H), 5.15–5.10 (m, 2H), 3.82 (dd, $J = 5.7$, 1.5 Hz, 1H), 3.68 (br q, $J \approx 5.5$ Hz, 1H), 3.52 (dt, $J = 8.8$, 4.2 Hz, 1H), 3.30 (s, 3H), 3.14 (quint, $J = 6.5$ Hz, 1H), 2.30 (m, 1H), 2.17 (dt, $J = 14$, 8.5 Hz, 1H), 1.73 (m, 1H), 1.70–1.60 (br m, 2H), 1.60–1.40 (m, 3H), 1.15 (m, 1H), 1.12 (d, $J = 6.3$ Hz, 3H), 0.93 (d, $J = 6.5$ Hz, 3H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.06 ppm (s, 6H) (hydroxyl proton not detected); ^{13}C NMR: $\delta = 18.5$, 18.3 (C), 135.5, 80.0, 74.1, 73.4, 72.5, 43.5, 41.1, 38.6 (CH), 117.8, 39.1, 33.0, 27.6 (CH_2), 56.4, 26.1 (×3), 26.0 (×3), 16.4, 14.2, 11.3, 10.7, −3.3, −3.6, −4.1 ppm (×2) (CH_3); IR: $\tilde{\nu} = 3490$ cm^{-1} (br, OH); HR-EIMS *m/z* (%): calcd for $C_{29}H_{62}O_4Si_2-t$Bu: 473.3482; found: 473.3458 (1) $[M-t\text{Bu}]^+$, 455 (3), 271 (22), 253 (43), 231 (55), 139 (54), 59 (100); elemental analysis: calcd (%) for $C_{29}H_{62}O_4Si_2$: C 65.60, H 11.77; found: C 65.49, H 11.60.

(4*S*,5*S*,8*S*,9*R*,10*S*,11*R*,12*S*)-8,10-Bis(*tert*-butyldimethylsilyloxy)-12-methoxy-4-methoxymethoxy-5,9,11-trimethyltridec-1-ene (4): Alcohol **20** (345 mg, 0.65 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and treated with iPr$_2$NEt (350 μL, 2 mmol) and MOMCl (100 μL, 1.3 mmol). The reaction mixture was then stirred overnight at room temperature. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 95:5) furnished **4** (295 mg, 79 %). Colourless oil; $[\alpha]_D = -3.1$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR: $\delta = 5.82$ (m, 1H), 5.10 (br dd, $J = 17.0$, 1.5 Hz, 1H), 5.04 (br d, $J = 10.0$ Hz, 1H), 4.65 (d, $J = 7.0$ Hz, 1H), 4.63 (d, $J = 7.0$ Hz, 1H), 3.82 (dd, $J = 5.8$, 1.5 Hz, 1H), 3.70 (br q, $J \approx 5.5$ Hz, 1H), 3.47 (m, 1H), 3.37 (s, 3H), 3.30 (s, 3H), 3.14 (quint, $J = 6.5$ Hz, 1H), 2.35–2.25 (m, 2H), 1.75–1.50 (br m, 6H), 1.40 (m, 1H), 1.12 (d, $J = 6.3$ Hz, 3H), 0.94 (d, $J = 6.8$ Hz, 3H), 0.91 (d, 3H, overlapped), 0.90 (s, 9H), 0.89 (s, 9H), 0.86 (d, $J = 7$ Hz, 3H), 0.09 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H), 0.05 ppm (s, 3H); ^{13}C NMR: $\delta = 18.5$, 18.3 (C), 135.6, 81.4, 80.0, 73.4, 72.7, 43.5, 41.2, 36.6 (CH), 116.7, 96.1, 36.0, 33.4, 27.2 (CH_2), 56.4, 55.6, 26.1 (×3), 26.0 (×3), 16.4, 14.7, 11.1, 10.6, −3.3, −3.5, −4.0 ppm (×2, CH_3); HR-EIMS *m/z* (%): calcd for $C_{31}H_{66}O_5Si_2-t$Bu: 517.3744; found: 517.3756 (3) $[M-t\text{Bu}]^+$, 283 (22), 253 (6), 231 (55), 139 (34), 59 (100); elemental analysis: calcd for $C_{31}H_{66}O_5Si_2$: C 64.75, H 11.57; found: C 64.54, H 11.63.

(*E*)-4-(4-Methoxybenzyloxy)but-2-enal (30): Alcohol **31**[27] (4.16 g, 20 mmol) was dissolved in dry CH_2Cl_2 (100 mL) and treated with PCC (6.50 g, approximately 30 mmol). The reaction mixture was stirred for four days at room temperature and then filtered through Celite (washing with CH_2Cl_2). After removal of the solvent under reduced pressure, the residue was purified by means of column chromatography on silica gel (hexane/EtOAc 80:20) to yield aldehyde **30** (2.15 g, 52 %). Colourless oil; ^{1}H NMR: $\delta = 9.46$ (d, $J = 8.0$ Hz, 1H), 7.20 (m, 2H), 6.82 (m, 2H), 6.74 (dt, $J = 15.5$, 4.0 Hz, 1H), 6.30 (ddt, $J = 15.5$, 8.0, 2.0 Hz, 1H), 4.42 (s, 2H), 4.14 (dd, $J = 4.0$, 2.0 Hz, 2H), 3.70 ppm (s, 3H); ^{13}C NMR: $\delta = 159.0$, 129.2 (C), 192.7, 153.0, 131.1, 128.9 (×2), 113.4 (×2) (CH), 72.1, 67.8 (CH_2), 54.7 ppm (CH_3).

(4*S*)-4-Benzyl-3-[(2*S*,3*R*,4*E*)-3-hydroxy-6-(4-methoxybenzyloxy)-2-methylhex-4-enoyl]-1,3-oxazolidin-2-one (32): A solution of oxazolidinone **22** (700 mg, 3 mmol) in dry CH_2Cl_2 (20 mL) was cooled to 0 °C and treated sequentially with nBu$_2$BOTf (1 M solution in CH_2Cl_2, 5.4 mL, 5.4 mmol) and Et_3N (840 μL, 6 mmol). After stirring for 1 h, the mixture was treated dropwise with a solution of freshly prepared aldehyde **30** (720 mg, 3.5 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at −40 °C for 12 h, quenched by addition of a pH 7 buffer solution (20 mL), MeOH (20 mL) and 30 % H_2O_2 (10 mL) and then stirred at room temperature for 30 min. Workup (extraction with EtOAc) and column chromatography on silica gel (hexane/EtOAc 70:30) afforded aldol adduct **32** as a single diastereoisomer (1.13 g, 86 % based on **22**). Colourless oil; $[\alpha]_D = +47.6$ ($c = 1.3$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.35$–7.15 (br m, 7H), 6.85 (m, 2H), 5.90 (dt, $J = 15.5$, 5.5 Hz, 1H), 5.78 (dd, $J = 15.5$, 5.5 Hz, 1H), 4.66 (m, 1H), 4.49 (t, $J = 5.5$ Hz, 1H), 4.43 (s, 2H), 4.15–4.10 (m, 2H), 4.00 (d, $J = 5.5$ Hz, 2H), 3.90 (dq, $J = 5.5$, 7.0 Hz, 1H), 3.77 (s, 3H), 3.21 (dd, $J = 13.5$, 3 Hz, 1H), 2.90 (br s, 1H, OH), 2.79 (dd, $J = 13.5$, 9.5 Hz, 1H), 1.26 ppm (d, $J = 7.0$ Hz, 3H); ^{13}C NMR: $\delta = 176.1$, 159.1, 153.0, 135.0, 130.2 (C), 132.1, 129.3 (×2), 129.2 (×2), 128.8 (×2), 128.5, 127.2, 113.7 (×2), 72.1, 55.1, 42.7 (CH), 71.7, 69.6, 66.0, 37.6 (CH_2), 55.0, 11.3 ppm (CH_3); IR: $\tilde{\nu} = 3500$ (br, OH), 1775, 1695 cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{25}H_{29}NO_6-H_2O$: 421.1889; found: 421.1881 (1) $[M-H_2O]^+$, 285 (8), 233 (24), 121 (100); elemental analysis: calcd (%) for $C_{25}H_{29}NO_6$: C 68.32, H 6.65; found: C 68.21, H 6.77.

(2*S*,3*R*,4*E*)-3-Hydroxy-*N*-methoxy-6-(4-methoxybenzyloxy)-2-*N*-dimethylhex-4-enamide (33): A solution of *N*,*O*-dimethylhydroxylamine hydrochloride (730 mg, approximately 7.5 mmol) in dry THF (15 mL) was treated dropwise at 0 °C with Me_3Al (2 M solution in toluene, 3.7 mL, approximately 7.5 mmol). The mixture was stirred for 1 h at room temperature. A solution of aldol **32** (1.10 g, 2.5 mmol) in dry THF (6 mL) was then added dropwise via syringe. The reaction mixture was stirred for 3 h at room temperature and then quenched through addition of a saturated aqueous solution of potassium sodium tartrate (50 mL). Stirring at room temperature for 30 min and workup (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexane/EtOAc 1:1) to yield impure **33** (contaminated with the chiral auxiliary), which was used as such in the next step. An aliquot was carefully purified for analytical purposes. Colourless oil; $[\alpha]_D = +12$ ($c = 1$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.85 (m, 2H), 5.90 (dt, $J = 15.5$, 5.5 Hz, 1H), 5.72 (dd, $J = 15.5$, 5.5 Hz, 1H), 4.45 (m, 1H), 4.44 (s, 2H), 4.01 (d, $J = 5.5$ Hz, 2H), 3.90 (br s, 1H; OH), 3.79 (s, 3H), 3.69 (s, 3H), 3.19 (s, 3H), 2.95 (m, 1H), 1.16 ppm (d, $J = 7$ Hz, 3H); ^{13}C NMR: $\delta = 177.6$*, 159.1, 130.4 (C), 132.2, 129.3 (×2), 128.2, 113.7 (×2), 71.8, 39.5 (CH), 71.7, 69.8 (CH_2), 61.5, 55.2, 31.9*, 10.7 ppm (CH_3) (starred peaks are very low and broad); IR: $\tilde{\nu} = 3440$ (br, OH), 1651 cm^{-1} (C=O); elemental analysis: calcd for $C_{17}H_{25}NO_5$: C 63.14, H 7.79; found: C 63.21, H 7.77.

(2*S*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-6-(4-methoxybenzyloxy)-2-*N*-dimethylhex-4-enamide (34): Weinreb amide **33** from above was dissolved in dry CH_2Cl_2 (20 mL) and treated dropwise at room temperature with 2,6-lutidine (410 μL, 3.5 mmol) and TBSOTf (690 μL, 3 mmol). The mixture was stirred for 1 h at room temperature. Workup (extraction with CH_2Cl_2) was followed by *careful* column chromatography of the residue on silica gel (hexane/EtOAc 70:30) to yield **34** (842 mg, 77 % overall yield for the two steps). Colourless oil; $[\alpha]_D = -6.1$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.88 (m, 2H), 5.80 (m, 2H), 4.42 (s, 2H), 4.33 (m, 1H), 4.00 (br d, $J \approx 4.5$ Hz, 2H), 3.79 (s, 3H), 3.66 (s, 3H), 3.15 (s, 3H), 3.02 (m, 1H), 1.23 (d, $J = 7$ Hz, 3H), 0.95 (s, 9H), 0.11 (s, 3H), 0.10 ppm (s, 3H); ^{13}C NMR: $\delta = 175.3$*, 158.9, 130.3, 17.9 (C), 134.4, 128.8 (×2), 127.4, 113.4 (×2), 74.6, 42.6 (CH), 71.0, 69.3 (CH_2), 61.0, 54.8, 31.7*, 25.6 (×3), 14.0, −4.4, −5.1 (CH_3) (starred peaks are very low and broad); IR: $\tilde{\nu} = 1657$ cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{23}H_{39}NO_5Si-Me$: 422.2362; found: 422.2317 (4) $[M-Me]^+$, 380 (100), 350 (14), 121 (55); elemental analysis: calcd for $C_{23}H_{39}NO_5Si$: C 63.12, H 8.98; found: C 63.24, H 8.79.

(4*R*,5*R*,2*E*,6*E*)-5-(*tert*-Butyldimethylsilyloxy)-8-(4-methoxybenzyloxy)-2,4-dimethylocta-2,6-dienoic acid ethyl ester (35): A solution of Weinreb amide **34** (438 mg, 1 mmol) in dry THF (8 mL) was treated at −78 °C with DIBAL (1 M solution in hexane, 2.5 mL, 2.5 mmol). The reaction was then stirred for 30 min at −78 °C and quenched through addition of saturated aq NH_4Cl (1 mL). The mixture was then stirred at room temperature until formation of a persistent gel. Filtration through Celite (washing with CH_2Cl_2) and solvent removal under reduced pressure gave crude aldehyde **29** which was used as such in the next reaction.

Crude **29** from above was dissolved in dry 1,2-dichloroethane (8 mL) and treated with $Ph_3P{=}C(Me)CO_2Et$ (725 mg, 2 mmol). The reaction was

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then heated at 60 °C for 12 h. Removal of all volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane/EtOAc 90:10) furnished conjugated enoate **35** (255 mg, 55% overall yield for the two steps). Colourless oil; $[\alpha]_D = +6.7$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.88 (m, 2H), 6.67 (dq, $J = 10$, 1.5 Hz, 1H), 5.75–5.65 (br m, 2H), 4.42 (s, 2H), 4.17 (m, 2H), 4.05 (br t, $J \approx 5.5$ Hz, 1H), 4.00 (m, 2H), 3.81 (s, 3H), 2.60 (m, 1H), 1.84 (d, $J = 1.5$ Hz, 3H), 1.27 (d, $J = 7$ Hz, 3H), 1.01 (d, $J = 6.8$ Hz, 3H), 0.92 (s, 9H), 0.06 (s, 3H), 0.03 ppm (s, 3H); ^{13}C NMR: $\delta = 168.2$, 159.2, 130.4, 127.2, 18.2 (C), 144.4, 134.4, 129.2 (×2), 127.6, 113.8 (×2), 75.8, 40.3 (CH), 71.4, 69.7, 60.4 (CH_2), 55.3, 25.9 (×3), 14.8, 14.2, 12.7, −4.2, −5.0 ppm (CH_3); IR: $\tilde{\nu} = 1710$ cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{26}H_{42}O_5Si-Me$: 447.2567; found: 447.2576 $[M-Me]^+$ (1), 405 (2), 121 (100); elemental analysis: calcd (%) for $C_{26}H_{42}O_5Si$: C 67.49, H 9.15; found: C 67.57, H 9.29.

Sultam 37: A solution of sultam **41** (1.36 g, 5 mmol) in dry CH_2Cl_2 (35 mL) was cooled to −5 °C and treated sequentially with nBu$_2$BOTf (1 M solution in CH_2Cl_2, 5.5 mL, 5.5 mmol) and iPr$_2$NEt (1 mL, approximately 5.5 mmol). After stirring for 30 min at −5 °C, the mixture was cooled to −78 °C and treated dropwise with a solution of aldehyde **30** from above in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at −78 °C for 16 h, quenched by addition of a pH 7 buffer solution (15 mL) and stirred at room temperature for 30 min. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 70:30) afforded aldol adduct **37** as a single diastereoisomer (2.27 g, 95% based on **41**). White solid, m.p. 97–98 °C; $[\alpha]_D = +85$ ($c = 1.6$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.88 (m, 2H), 5.92 (dtd, $J = 15.5$, 5.6, 1.3 Hz, 1H), 5.72 (br dd, $J = 15.5$, 5 Hz, 1H), 4.56 (m, 1H), 4.44 (s, 2H), 4.01 (br d, $J = 5.6$ Hz, 2H), 3.88 (t, $J = 6.3$ Hz, 1H), 3.80 (s, 3H), 3.50 (d, $J = 14.0$ Hz, 1H), 3.44 (dd, $J = 14.0$ Hz, 1H), 3.20 (br s, 1H; OH), 3.16 (qd, $J = 7.0$, 3.3 Hz, 1H), 2.05 (m, 2H), 1.95–1.85 (m, 3H), 1.40–1.30 (m, 2H), 1.26 (d, $J = 7.0$ Hz, 3H), 1.15 (s, 3H), 0.97 ppm (s, 3H); ^{13}C NMR: $\delta = 176.5$, 159.2, 130.5, 48.4, 47.8 (C), 131.8, 129.4 (×2), 128.7, 113.8 (×2), 70.7, 65.0, 44.6, 44.4 (CH), 71.7, 69.8, 53.1, 38.3, 32.8, 26.4 (CH_2), 55.3, 20.8, 19.8, 11.8 ppm (CH_3); IR: $\tilde{\nu} = 3520$ (br, OH), 1689 cm^{-1} (br, C=O); HR-FABMS *m/z* (%): calcd for $C_{25}H_{36}NO_6S$: 478.2263; found 478.2215 $[M+H]^+$; elemental analysis: calcd (%) for $C_{25}H_{35}NO_6S$: C 62.89, H 7.44; found: C 62.98, H 7.50.

Sultam 38: Alcohol **37** (2.25 g, 4.7 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and treated dropwise with 2,6-lutidine (815 μL, 7 mmol) and TBSOTf (1.35 mL, 5.9 mmol). The mixture was stirred for 1 h at room temperature. Workup (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexane/EtOAc 90:10) to yield **38** (2.45 g, 88%). Colourless oil; $[\alpha]_D = +60$ ($c = 4.7$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.86 (m, 2H), 5.73 (m, 2H), 4.40 (m, 3H), 4.00 (dd, $J = 13.0$, 4.0 Hz, 1H), 3.93 (dd, $J = 13.0$, 4.0 Hz, 1H), 3.78 (s, 3H), 3.73 (m, 1H), 3.44 (d, $J = 14.0$ Hz, 1H), 3.33 (dd, $J = 14.0$ Hz, 1H), 3.19 (quint, $J \approx 7.0$ Hz, 1H), 2.05–2.00 (m, 2H), 1.95–1.85 (m, 3H), 1.30–1.20 (m, 2H), 1.27 (d, $J = 7.0$ Hz, 3H), 1.12 (s, 3H), 0.93 (s, 3H), 0.90 (s, 9H), 0.06 (s, 3H), 0.03 ppm (s, 3H); ^{13}C NMR: $\delta = 173.8$, 159.0, 130.7, 48.1, 47.6, 18.1 (C), 135.1, 129.2 (×2), 128.6, 113.6 (×2), 70.8, 64.8, 47.6, 44.5 (CH), 73.8, 69.8, 53.0, 38.3, 32.6, 26.3 (CH_2), 55.2, 25.8 (×3), 20.7, 19.8, 15.6, −4.2, −4.9 ppm (CH_3); IR: $\tilde{\nu} = 1693$ cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{31}H_{49}NO_6SSi-Me$: 576.2815; found: 576.2803 (2) $[M-Me]^+$, 534 (26), 328 (56), 121 (100); elemental analysis: calcd (%) for $C_{31}H_{49}NO_6SSi$: C 62.91, H 8.34; found: C 63.03, H 8.49.

Conversion of sultam 38 into ester 35: A solution of sultam **38** (2.37 g, approximately 4 mmol) in dry CH_2Cl_2 (50 mL) was treated at −78 °C with DIBAL (1 M solution in hexane, 10 mL, 10 mmol). The reaction was then stirred for 30 min at −78 °C and quenched through addition of saturated aqueous NH_4Cl (2 mL). The mixture was then stirred at room temperature until formation of a persistent gel. Filtration through Celite (washing with CH_2Cl_2) and solvent removal under reduced pressure gave crude aldehyde **29**, which was used as such in the next reaction.

Crude **29** from above was dissolved in dry 1,2-dichloroethane (30 mL) and treated with $Ph_3P=C(Me)CO_2Et$ (2.9 g, 8 mmol). The reaction was then heated at 60 °C for 16 h. Removal of all volatiles under reduced pressure and column chromatography of the residue on silica gel (hexane/EtOAc 90:10) furnished conjugated enoate **35** (1.63 g, 88% overall yield for the two steps).

(4*R*,5*R*,2*E*,6*E*)-5-(*tert*-Butyldimethylsilyloxy)-8-(4-methoxybenzyloxy)-2,4-dimethylocta-2,6-dienol (36): A solution of ethyl ester **35** (1.4 g, approximately 3 mmol) in dry hexane (20 mL) was treated at 0 °C with DIBAL (1 M solution in hexane, 6.5 mL, 6.5 mmol). The reaction was stirred for 1 h at 0 °C and quenched through addition of saturated aqueous NH_4Cl (2 mL). The mixture was then stirred at room temperature until formation of a persistent gel. Filtration through Celite (washing with EtOAc) and solvent removal under reduced pressure, followed by column chromatography of the residue on silica gel (hexane/EtOAc 90:10) afforded alcohol **36** (1.19 g, 94%). Colourless oil; $[\alpha]_D = -19.4$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.26$ (m, 2H), 6.88 (m, 2H), 5.65 (m, 2H), 5.25 (br d, $J = 10$ Hz, 1H), 4.42 (s, 2H), 4.00–3.90 (br m, 5H), 3.81 (s, 3H), 2.48 (m, 1H), 1.70 (br s, 1H; OH), 1.66 (br s, 3H), 0.97 (d, $J = 6.8$ Hz, 3H), 0.91 (s, 9H), 0.05 (s, 3H), 0.03 ppm (s, 3H); ^{13}C NMR: $\delta = 159.2$, 134.6, 130.4, 18.2 (C), 135.5, 129.3 (×2), 129.1, 126.9, 113.8 (×2), 77.0, 39.2 (CH), 71.4, 69.9, 69.1 (CH_2), 55.3, 25.9 (×3), 16.3, 14.2, −4.2, −4.9 ppm (CH_3); IR: $\tilde{\nu} = 3450$ cm^{-1} (br, OH); HR-EIMS *m/z* (%): calcd for $C_{24}H_{40}O_4Si$: 420.2696 $[M]^+$; found: 420.2674 (1) $[M]^+$, 402 (1) $[M-H_2O]^+$, 121 (100); elemental analysis: calcd for $C_{24}H_{40}O_4Si$: C 68.53, H 9.58; found: C 68.57, H 9.69.

(6*R*,7*R*,2*E*,4*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-10-(4-methoxybenzyloxy)-2,4,6-trimethyldeca-2,4,8-trienoic acid ethyl ester (28): A solution of alcohol **36** (1.18 g, 2.8 mmol) in dry CH_2Cl_2 (40 mL) was treated with activated MnO_2 (3.5 g, approximately 40 mmol) and heated at reflux for 2 h. Filtration through Celite (washing with CH_2Cl_2) and solvent removal under reduced pressure afforded a crude aldehyde which was used as such in the next step.

An ice-cooled solution of phosphonate $(EtO)_2P(O)CH(Me)COOEt$ (1.2 mL, 5.6 mmol) in dry THF (20 mL) was treated with BuLi (1.6 M solution in hexane, 3 mL, 4.8 mmol). The mixture was stirred for 15 min at 0 °C and treated dropwise with the crude aldehyde from above dissolved in dry THF (20 mL). The stirring was continued for 16 h at 0 °C. Workup (extraction with Et_2O) and column chromatography on silica gel (hexane/EtOAc 80:20) provided **28** (1.18 g, 84% overall yield for the two steps). Colourless oil; $[\alpha]_D = +26.1$ ($c = 2.2$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.25$ (m, 2H), 7.09 (br s, 1H), 6.86 (m, 2H), 5.69 (m, 2H), 5.43 (br d, $J = 9.8$ Hz, 1H), 4.41 (s, 2H), 4.19 (q, $J = 7$ Hz, 2H), 4.05–3.95 (br m, 3H), 3.79 (s, 3H), 2.59 (m, 1H), 1.97 (br s, 3H), 1.81 (br s, 3H), 1.29 (t, $J = 7.0$ Hz, 3H), 0.99 (d, $J = 6.8$ Hz, 3H), 0.90 (s, 9H), 0.05 (s, 3H), 0.03 ppm (s, 3H); ^{13}C NMR: $\delta = 169.1$, 159.2, 131.7, 130.4, 125.7, 18.2 (C), 142.9, 138.1, 134.6, 129.2 (×2), 127.5, 113.8 (×2), 76.8, 40.1 (CH), 71.4, 69.8, 60.5 (CH_2), 55.2, 25.9 (×3), 16.7, 16.3, 14.3, 14.0, −4.2, −4.9 ppm (CH_3); IR: $\tilde{\nu} = 1703$ cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{29}H_{46}O_5Si-t$Bu: 445.2410; found: 445.2392 (1) $[M-t\text{Bu}]^+$, 121 (100); elemental analysis: calcd (%) for $C_{29}H_{46}O_5Si$: C 69.28, H 9.22; found: C 69.20, H 9.34.

(6*R*,7*R*,2*E*,4*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-10-hydroxy-2,4,6-trimethyldeca-2,4,8-trienoic acid ethyl ester (39): A solution of compound **28** (1.16 g, approximately 2.3 mmol) in wet CH_2Cl_2 (30 mL mixed with 1 mL water) was treated with DDQ (570 mg, 2.5 mmol). The mixture was stirred at room temperature until consumption of the starting material (approximately 2 h, TLC monitoring). Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc 95:5) provided **39** (783 mg, 89%). Colourless oil; $[\alpha]_D = +30.7$ ($c = 1.7$ in $CHCl_3$); ^{1}H NMR: $\delta = 7.08$ (br s, 1H), 5.74 (dt, $J = 15.6$, 5.3 Hz, 1H), 5.67 (dd, $J = 15.6$, 6.2 Hz, 1H), 5.41 (br d, $J = 9.8$ Hz, 1H), 4.19 (q, $J = 7.0$ Hz, 2H), 4.15–4.10 (m, 2H), 3.98 (t, $J = 6.2$ Hz, 1H), 2.58 (m, 1H), 1.96 (br s, 3H), 1.80 (br s, 3H), 1.50 (br s, 1H; OH), 1.29 (t, $J = 7$ Hz, 3H), 0.97 (d, $J = 6.8$ Hz, 3H), 0.89 (s, 9H), 0.03 (s, 3H), 0.00 ppm (s, 3H); ^{13}C NMR: $\delta = 169.2$, 131.7, 125.7, 18.2 (C), 143.0, 138.0, 133.1, 129.9, 76.6, 40.1 (CH), 63.1, 60.5 (CH_2), 25.9 (×3), 16.7, 16.2, 14.3, 14.0, −4.1, −4.9 ppm (CH_3); IR: $\tilde{\nu} = 3460$ (br, OH), 1708 cm^{-1} (C=O); HR-EIMS *m/z* (%): calcd for $C_{21}H_{38}O_4Si$: 382.2539; found: 382.2513 (1) $[M]^+$, 201 (85), 107 (33), 73 (100); elemental analysis: calcd (%) for $C_{21}H_{38}O_4Si$: C 65.92, H 10.01; found: C 66.10, H 10.14.

(6*R*,7*S*,8*R*,9*S*,2*E*,4*E*)-7-(*tert*-Butyldimethylsilyloxy)-8,9-epoxy-10-hydroxy-2,4,6-trimethyldeca-2,4-dienoic acid ethyl ester (27): Powdered 4 Å

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MS (100 mg) were suspended in dry CH_2Cl_2 (15 mL) and the suspension was cooled to −23 °C. Then, titanium tetra*iso*propoxide (600 μL, approximately 2 mmol) and diethyl L-(+)-tartrate (343 μL, 2 mmol) were added with stirring, followed by a solution of compound **39** (766 mg, 2 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at −23 °C for 20 min followed by addition of *tert*-butylhydroperoxide (5 mL of a freshly prepared ≈4.1 M solution in toluene, approximately 20 mmol) and further stirring at −23 °C for 24 h. Reaction quenching was performed through addition of water (10 mL), followed by stirring for 30 min at room temperature, addition of 30% aq NaOH (1 mL) and further stirring for 30 min. Workup (extraction with CH_2Cl_2) and column chromatography on silica gel (hexane/EtOAc, first 80:20 then 70:30) provided **27** (718 mg, 90%) as a single stereoisomer. Colourless oil; ^{1}H NMR: δ = 7.06 (brs, 1H), 5.50 (brd, J = 9.8 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 3.90 (brs, 1H, OH), 3.60–3.55 (m, 3H), 3.10 (dt, J = 4.0, 2.0 Hz, 1H), 2.97 (dd, J = 4.0, 2.3 Hz, 1H), 2.67 (m, 1H), 1.95 (brs, 3H), 1.80 (brs, 3H), 1.26 (t, J = 7.0 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H), 0.85 (s, 9H), 0.01 (s, 3H), 0.00 ppm (s, 3H); ^{13}C NMR: δ = 169.1, 131.9, 125.9, 18.2 (C), 142.6, 137.7, 73,5, 56.5, 55.7, 37.6 (CH), 61.3, 60.6 (CH_2), 25.8 (x 3), 16.5, 15.5, 14.3, 14.0, −4.3, −5.0 ppm (CH_3); IR: $\tilde{\nu}$ = 3450 (br, OH), 1708 cm^{-1} (C=O); elemental analysis: calcd for $C_{21}H_{38}O_5Si$: C 63.28, H 9.61; found: C 63.15, H 9.79.

(6*R*,7*S*,8*R*,9*S*,10*R*,2*E*,4*E*)-7-(*tert*-Butyldimethylsilyloxy)-8,9-epoxy-10-hydroxy-2,4,6-trimethyltrideca-2,4,12-trienoic acid ethyl ester (40): Oxalyl chloride (390 μL, 4.5 mmol) was added dropwise at −78 °C to a solution of DMSO (630 μL, 9 mmol) in dry CH_2Cl_2 (10 mL). The mixture was stirred for 5 min at this temperature. A solution of alcohol **27** (718 mg, 1.8 mmol) in dry CH_2Cl_2 (3 mL) was then added by syringe. The reaction mixture was stirred for 15 min at −78 °C. After addition of Et_3N (2.5 mL, 18 mmol), the mixture was stirred for 15 min at −78 °C and then for 20 min at 0 °C. Workup (extraction with CH_2Cl_2) gave a crude aldehyde which was used as such in the next reaction.

Allylmagnesium bromide (commercial 1 M solution in Et_2O, 2.3 mL, 2.3 mmol) was added dropwise at 0 °C via syringe to a solution of (−)-Ipc_2BCl (870 mg, 2.7 mmol) in dry Et_2O (20 mL). The mixture was further stirred for 1 h at 0 °C. The solution was then allowed to stand, which caused precipitation of magnesium chloride. The supernatant solution was then carefully transferred to another flask via cannula. After cooling this flask at −110 °C, a solution of the crude aldehyde from above in dry Et_2O (10 mL) was added dropwise via syringe. The resulting solution was further stirred at the same temperature for 1 h. The reaction mixture was then quenched through addition of phosphate pH 7 buffer solution (20 mL), MeOH (20 mL) and 30% H_2O_2 (10 mL). After stirring for 30 min, the mixture was poured onto saturated aq $NaHCO_3$ and worked up (extraction with EtOAc). Column chromatography on silica gel (hexane/EtOAc 95:5) afforded **40** as a 92:8 diastereomeric mixture. Repeated column flash chromatography afforded pure **40** (473 mg, 60% overall from **27**). Colourless oil; $[\alpha]_D$ = +9.4 (c = 0.9 in $CHCl_3$); ^{1}H NMR: δ = 7.08 (brs, 1H), 5.84 (ddt, J = 17.5, 10.0, 7.0 Hz, 1H), 5.51 (brd, J = 9.8 Hz, 1H), 5.20–5.10 (m, 2H), 4.18 (q, J = 7.0 Hz, 2H), 3.65 (m, 1H), 3.62 (m, 1H), 3.00 (m, 2H), 2.67 (m, 1H), 2.37 (t, J = 7.0 Hz, 2H), 1.96 (brs, 3H), 1.90 (brd, J = 8.0 Hz, 1H; OH), 1.82 (brs, 3H), 1.27 (t, J = 7.0 Hz, 3H), 1.02 (d, J = 7.0 Hz, 3H), 0.85 (s, 9H), 0.01 (s, 3H), 0.00 ppm (s, 3H); ^{13}C NMR: δ = 169.0, 132.0, 126.0, 18.3 (C), 142.6, 137.7, 133.5, 73.4, 69.0, 57.5, 56.9, 37.6 (CH), 118.4, 60.6, 39.5 (CH_2), 25.9 (×3), 16.6, 15.7, 14.3, 14.0, −4.3, −4.9 ppm (CH_3); IR: $\tilde{\nu}$3490 (br, OH), 1707 cm^{-1} (C=O); HR-FABMS m/z (%): calcd for $C_{24}H_{43}O_5Si$: 439.2879; found: 439.2905 $[M+H]^+$; elemental analysis: calcd (%) for $C_{24}H_{42}O_5Si$: C 65.71, H 9.65; found: C 65.59, H 9.60.

(6*R*,7*S*,8*R*,9*R*,10*R*,2*E*,4*E*)-7,10-Bis(*tert*-butyldimethylsilyloxy)-8,9-epoxy-2,4,6-trimethyltrideca-2,4,12-trienoic acid ethyl ester (26): Alcohol **40** (439 mg, 1 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and treated dropwise with 2,6-lutidine (175 μL, 1.5 mmol) and TBSOTf (290 μL, 1.25 mmol). The mixture was stirred for 1 h at room temperature. Workup (extraction with CH_2Cl_2) was followed by column chromatography of the residue on silica gel (hexane/EtOAc 90:10) to yield **38** (525 mg, 95%). Colourless oil; $[\alpha]_D$ = +11.5 (c = 0.6 in $CHCl_3$); ^{1}H NMR: δ = 7.10 (brs, 1H), 5.84 (ddt, J = 17.5, 10.0, 7.0 Hz, 1H), 5.55 (brd, J = 10.0 Hz, 1H), 5.10–5.05 (m, 2H), 4.19 (q, J = 7.0 Hz, 2H), 3.61 (dd, J = 5.0, 4.0 Hz, 1H), 3.48 (td, J = 6.5, 5.5 Hz, 1H), 2.96 (dd, J = 5.5, 2.2 Hz, 1H), 2.88 (dd, J = 4.0, 2.2 Hz, 1H), 2.68 (ddq, J = 10.0, 5.0, 6.8 Hz, 1H), 2.28 (t, J = 6.5 Hz, 2H), 2.00 (d, J = 1.3 Hz, 3H), 1.85 (d, J = 1.0 Hz, 3H), 1.29 (t, J = 7.0 Hz, 3H), 1.06 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.04 (s, 6H), 0.01 ppm (s, 3H); ^{13}C NMR: δ = 169.1, 131.9, 125.9, 18.3, 18.2 (C), 142.6, 138.1, 134.5, 73.7, 73.0, 58.5, 57.2, 37.7 (CH), 117.3, 60.6, 39.5 (CH_2), 25.9 (×3), 25.8 (×3), 16.6, 15.7, 14.3, 14.0, −4.2, −4.5, −4.8, −4.9 ppm (CH_3); IR: $\tilde{\nu}$ = 1710 cm^{-1} (C=O); HR-EIMS m/z (%): calcd for $C_{30}H_{56}O_5Si_2$: 552.3666 $[M]^+$; found: 552.3666 (2) $[M]^+$, 511 (10), 371 (26), 107 (63), 73 (100); elemental analysis: calcd (%) for $C_{30}H_{56}O_5Si_2$: C 65.17, H 10.21; found: C 65.29, H 10.02.

The remaining experimental procedures and spectral data can be taken from the supplementary material in reference [36a].

Materials and methods for the biological and biochemical work

Cell culture: Human A549 non-small lung carcinoma cells were continuously maintained in RPMI-1640 supplemented with 10% fetal calf serum, 2 mM L glutamine, 40 μg mL^{-1} gentamycin, 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Human ovarian carcinoma A2780 and A2780AD (MDR overexpressing P-glycoproteins, P-gp) were cultured as above with the addition of 0.25 units/mL of bovine insuline.

Indirect immunofluorescence: A549 cells were plated at a density of 150,000 cells/mL onto 24 well tissue culture plates containing 12 mm round coverslips, cultured overnight and then treated with ligands at different concentrations or with drug vehicle (DMSO) for 24 h. Residual DMSO was less than 0.5%. Attached cells were permeabilised with Triton X100 and fixed with 3.7% formaldehyde, as previously described.[55] Cytoskeletons were incubated with DM1A monoclonal antibody reacting with α-tubulin, washed twice and incubated with FITC goat antimouse immunoglobulins. The coverslips were incubated with 1 μg mL^{-1} Hoechst 33342 in order to stain the chromatin. After washing, the samples were examined and photographed by using a Zeiss Axioplan epifluorescence microscope. The images were recorded with a Hamamatsu 4742–95 cooled CCD camera.

Cytotoxicity assay: Human ovarian carcinomas A2780 and A2780AD were seeded in 96 well plates at a density of 15,000 cells in 0.08 mL per well. The following day, the cells were exposed to 0.02 mL serial dilutions of ligands for 48 h, after which time an MTT assay was performed in order to determine viable cells with some modifications.[56] Briefly, 20 μL of 2.5 mg mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, incubated 4 h at 37 °C, then treated with 0.1 mL MTT solubilizer (10% SDS, 45% dimethylformamide pH 5.5). Plates were again incubated overnight at 37 °C in order to solubilise the blue formazan precipitate before measuring the absorbance at 595/690 nm in an automated Multiscan microplate reader. Control wells containing medium without cells were used as blanks. MTT response is expressed as a percentage of the control (untreated) cells. The IC50 was calculated from the log-dose response curves.

Cell cycle analysis: Progression through the cell cycle was assessed by flow cytometry DNA determination with propidium iodide. Cells (150,000 per ml) were incubated with several concentrations of the drugs for 24 h. The cells were fixed with 70% ethanol, treated with RNase and stained with propidium iodide as previously described.[57] The analysis was performed with a Coulter Epics XL flow cytometer.

Effects of ligands on microtubule assembly and stability: The effects of the ligands on tubulin assembly were monitorised by incubating concentrations from 10 to 20 μM tubulin in buffer GAB-1 mM GTP in the presence of 11 or 22 μM ligand. In this buffer tubulin can assemble without ligand with a critical concentration of 3.3 μM.[58] The polymers were sedimented at 90,000 g for 20 min in a TLA 100 rotor, preequilibrated at 37 °C, in a Beckman Optima TLX ultracentrifuge. The supernatants were carefully removed by pipetting, and the pellets resuspended in 10 mM phosphate, 1% SDS, pH 7.0. The pellets and the supernatants were diluted 1:10 in the same buffer, and their concentrations were fluorimetrically measured employing a Fluorolog 3 spectrofluorimeter (excitation wavelength 285 nm, emission wavelength 320 nm using slits of 2 and 5 nm, respectively). Tubulin concentration standard curves were constructed for

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each experiment, by using spectrophotometrically measured concentrations of purified tubulin.

The effect of the ligand on polymerisation was also tested in a MAP containing system; microtubular protein (1 mg/mL) was incubated in buffer AB-1 mM GTP, for 30 min at 37 °C in the presence of 11 μM ligand. The polymers were then pelleted at 90,000 g for 20 min in a TLA 100 rotor, preequilibrated at 37 °C, in a Beckman Optima TLX ultracentrifuge. The supernatants were taken, the pellets resuspended in 10 mM sodium phosphate pH 7.0, and the microtubular protein concentrations measured by the method described by Bradford[59] by using BSA (bovine serum albumin) as standard.

Binding of ligands to tubulin microtubules: Samples containing ligand (11 μM) and stabilized crosslinked microtubules (10 μM taxoid binding sites) were prepared as described[60] and incubated in GAB buffer for 30 min at 37 °C in polycarbonate centrifuge tubes (Beckman) (DMSO concentration was always kept under 2%). The samples were then centrifuged at 90,000 g for 10 min at 25 °C in a TLA100 rotor. The supernatants were collected by pipetting and the pellets were resuspended in 10 mM phosphate pH 7.0. Both pellets and supernatants were extracted three times with an excess volume of dichloromethane, dried in vacuum, and dissolved in 25 μL of a methanol/water (v/v 75:25) mixture. Ligands (both bound to pelleted polymers and free in the supernatant), were determined by HPLC. The HPLC analyses of the samples were performed in a C18 column (Supercosil, LC18 DB, 250×4.6 mm, 5 μM bead size) developed in a gradient from 60 to 90% of methanol in water (v/v) at a flow rate of 1 mL min^{-1}.

Samples containing ligand (5 μM) and non-polimerised tubulin (5 μM) were incubated for 30 min at 37 °C in 10 mM phosphate, 1 mM EDTA, 1.5 mM $MgCl_2$, 1 mM GTP, pH 7.0 buffer in polycarbonate centrifuge tubes (Beckman) (DMSO concentration was always kept under 2%). The samples were then centrifuged at 380,000 g for 2 h at 25 °C in a TLA100 rotor. The 100 μL upper and lower fractions of the tube were carefully collected, extracted and analysed as described above.

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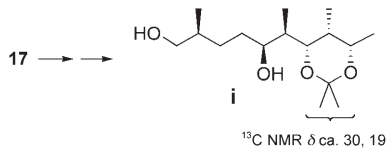

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