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Synthesis and evaluation of an Iejimalide-archazolid chimera

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ABSTRACT

Even though the macrolides of the iejimalide family are of marine origin, whereas those of the archazolid series derive from terrestrial myxobacteria, a comparison of their constitution, stereochemistry, and biological activity suggests that these natural products are close structural and functional relatives. Guided by this perception, compound **5** was prepared, which hybridizes the macrolactone core of iejimalide B (**2**) with the tail of archazolid A (**3**). The cytotoxicity profile of this chimera, as determined with a panel of 12 human cancer cell lines, corresponds to that of the parent compound **2**, although its potency is lower. This outcome may be interpreted on the basis of molecular dynamics calculations, which suggest that the low energy conformations of **2** and **5** are similar but the energetic barriers between the relevant conformers are distinctly higher for the hybrid structure. The synthesis of **5** hinged on a regioselective functionalization of 2,4-dibromothiazole **6**, a highly selective CBS-reduction of ketone **8**, a Suzuki cross coupling of vinyl boronate **17** with the elaborate alkenyl iodide **16**, and a productive closure of the macrocycle by RCM, which requires the selective activation of two out of eight double bonds present in the cyclization precursor **20** by the second-generation Grubbs catalyst **21**.

1. Introduction

As part of our ongoing program on the synthesis and evaluation of structurally novel anticancer agents,[1,2] we devoted considerable efforts to the iejimalide family of marine macrolides (Scheme 1). These polyunsaturated compounds were isolated by Kobayashi and co-workers from the tunicate *Eudistoma* cf. *rigida* and later from a *Cystodytes* sp. collected off Ie island in Japan.[3–5] Early studies had shown that **1** and **2** and their sulfated sister compounds[6] hold considerable promise because of their remarkable potency (average GI_{50} of **1** for the NCI 60 cell line cancer panel, 13 nM)[7] and promising selectivity.[4,8–10] Preliminary data also indicated in vivo activity upon intraperitoneal administration in mice.[4a]

Because of the extremely limited supply of the iejimalides from the natural sources, we developed the first total synthesis of these sensitive targets.[11–13] Since then, the original route was refined and up-scaled to provide gram amounts of iejimalide B (**2**) as needed for an in-depth preclinical evaluation.[14] In parallel work, deliberate digression from the total synthesis allowed us to prepare a set of more than 20 fully synthetic iejimalide analogs for biological testing, in which the entire skeleton of the parent natural product was subject to molecular editing.[11,14]

During this endeavor, our attention was caught by the structure and activity of archazolid A (**3**) and B (**4**), secondary metabolites derived from terrestrial myxobacteria of the strains *Archangium gephyra* and *Cystobacter* sp.[15,16] When drawn as shown in Scheme 1, a striking relationship to the iejimalides becomes apparent: both families are 24-membered macrolides comprising seven double bonds in the hydrophobic ring; their enoate moieties carry either a hydrogen atom (R^1=H, iejimalide A and archazolid B) or a methyl branch (R^1=Me, iejimalide B and archazolid A) at the C.2 position; in both series, the methylated compounds are somewhat more active. The lactone connects the enoate with a secondary alcohol at C.23 flanked by a methyl group in a 1,2-*anti* relationship. These chiral centers at C.22 and C.23 are (*S*,*S*) configured in the iejimalides and the archazolids alike. The structural similarity further extends to the C.18–C.21 diene and the adjacent 17*S*-configured methyl ether substituent displayed in both series. Moreover, it seems that even the 9*S*–OMe group with its neighboring *E*-configured trisubstituted alkene of the iejimalides has correspondence in the 7*S*–OH group of the archazolid located in the exact same environment. Overall, it only

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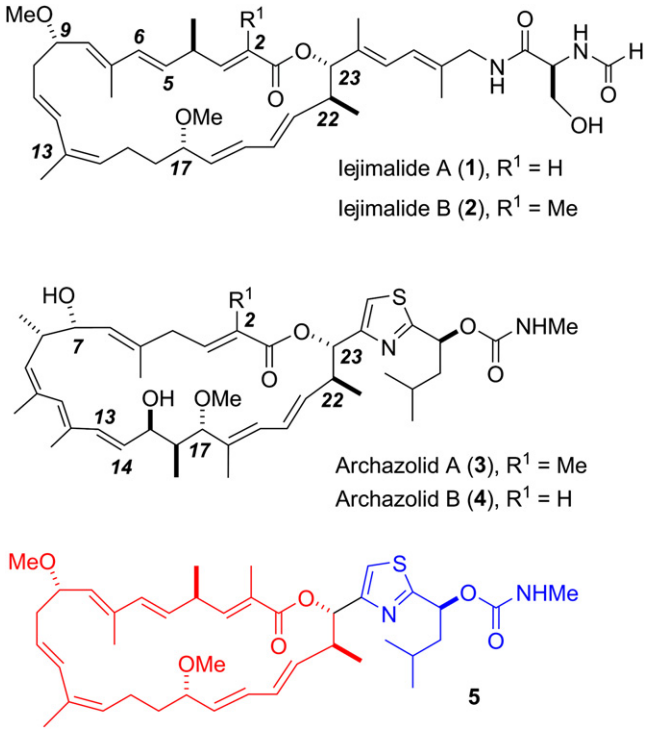


Scheme 1. Structures of the iejimalides and the archazolids, together with the targeted chimera **5**, which combines the iejimalide core and the archazolid side chain.

takes a formal relocation of the 5,6-alkene to the 13,14-position in the southern sector to convert the iejimalide framework into the archazolid scaffold, which, however, is slightly more adorned.

Even though the side chains of the iejimalides and archazolids are different, it is worth recognizing the common site of attachment to the macrolactone cores at C.23 in both series. These tails consist of a rigid hydrophobic spacer (1,3-diene in the iejimalides, thiazole in the archazolids) terminated by a polar head group (*N*-formylserine vs *N*-methyl-carbamate).

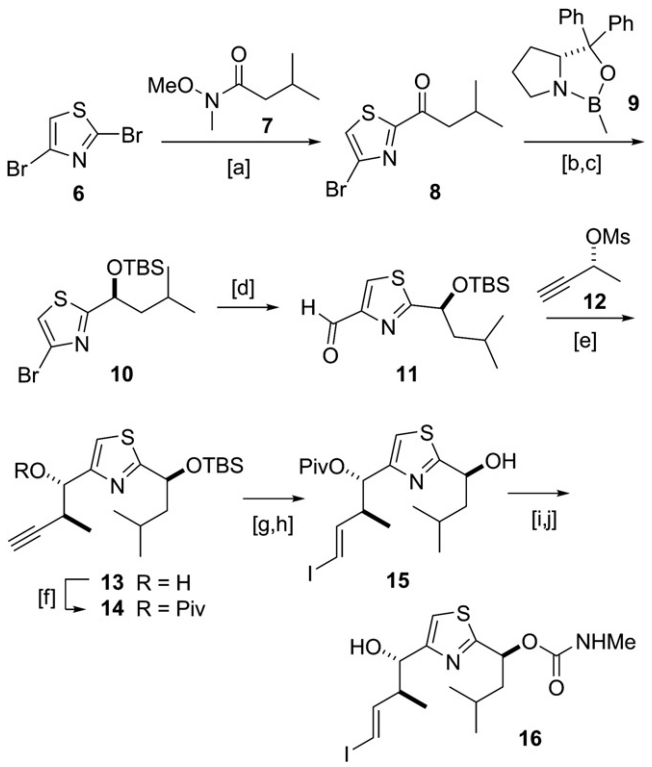
In addition to this constitutional and stereochemical similarity, a comparison of the currently known activity data is also quite instructive. Although one has to be careful in drawing conclusions from a preliminary set of biochemical and biological results, it is tempting to see more than a mere coincidence in the fact that the iejimalides as well as the archazolids are potent and selective inhibitors of vacuolar-type ATPases (V-ATPases).[8,15] Whether interference with these vital proton pumps is the only and/or the decisive reason for the impressive cytotoxicity of these macrolides, however, remains yet to be investigated in more detail.[17]

The analogies outlined above encouraged us to prepare a chimera, which merges the macrolactone of the iejimalides with the side chain of the archazolids. If such a hybrid retains the bioactivity profile common to both parent compounds, further credence is lent to the supposed relationship between these natural products of distinctly different origin.[18] The design of compound **5** (Scheme 1) was guided by the perception that the side chain of the iejimalides is more amenable to structural changes than the core region.[14] The synthesis and evaluation of this 'designer natural product' are outlined below.

2. Results and discussion

2.1. Synthesis

The preparation of the required building block representing the side chain of the targeted chimera **5** commenced with 2,4-dibromothiazole (**6**), which was subjected to a regioselective metal/halogen exchange with *n*-BuLi in ether at low temperature (Scheme 2).[19] In contrast to literature recommendations, which suggested trapping of the resulting heteroaryl lithium intermediate with isovaleric acid ethyl ester or the corresponding nitrile,[20] the use of the corresponding Weinreb amide **7** was found to give ketone **8** in much better yield and significantly higher purity.[21,22] A subsequent CBS-reduction [23] furnished the required secondary alcohol with an ee of 95%, the *S*-configuration of which was confirmed by Mosher-ester analysis. *O*-Silylation followed by lithium/halogen exchange of the remaining bromide in **10** (Fig. 1) with *t*-BuLi and quenching of the reactive species with DMF gave aldehyde **11** in good overall yield.



Scheme 2. (a) *n*-BuLi, Et_2O, −78 °C, then **7**, 88%; (b) **9** (50 mol %), $BH_3 \cdot SMe_2$, THF, −30 °C, 95%, ee 95%; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 84%; (d) *t*-BuLi, Et_2O, −78 °C, then DMF, 70%; (e) $Pd(OAc)_2$ (20 mol %), PPh_3 (20 mol %), Et_2Zn, THF, −78 °C→−20 °C, 71%, dr=1:1 (only one isomer shown); (f) pivaloyl chloride, DMAP cat., pyridine, 67%; (g) $Cp_2Zr(H)Cl$, THF, then I_2, 83%; (h) TBAF, THF, 0 °C, 77%; (i) 1,1′-carbonyldiimidazole, CH_2Cl_2, 0 °C, then $MeNH_2$; (j) $LiBHEt_3$, CH_2Cl_2, 0 °C, 91% over both steps.

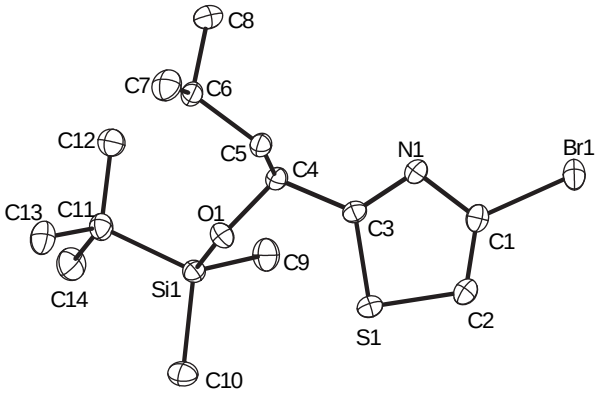
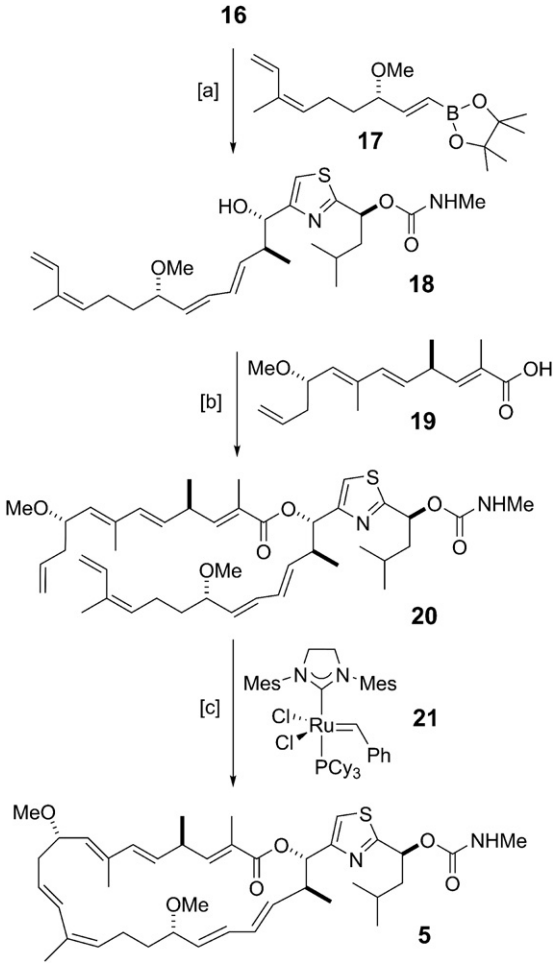


Figure 1. Structure of compound **10** in the solid state. Anisotropic displacement parameters are drawn at the 50% probability level and hydrogen atoms are omitted for clarity.

The reaction of **11** with propargyl mesylate **12** under the conditions developed by Marshall and co-workers gave a 1:1 mixture of diastereomeric alcohols.[24–26] Although the isomers are separable by careful flash chromatography (cf. Experimental section), it is also possible to process the mixture by temporary protection of the alcohol as the corresponding pivalate **14** in preparation for a hydrozirconation/iodination sequence and subsequent cleavage of the TBS ether. At this stage, isomerically pure **15** could be readily attained and further elaborated to the targeted iejimalide/archazolid hybrid.

To this end, the methyl carbamate moiety was installed by sequential treatment of **15** with carbonyldiimidazole and methylamine. Cleavage of the remaining pivalate ester with $LiBHEt_3$ did not touch the carbamate and hence provided the fully functional side chain building block **16** in readiness for fragment coupling. Under previously optimized conditions,[11] this alkenyl iodide was first reacted with the known boronate **17**[11] under palladium catalysis before the somewhat labile acid **19**[11] was attached. The resulting compound **20** was subjected to ring-closing olefin metathesis (RCM).[27] It is remarkable that exposure of this substrate—containing no less than eight double bonds—to catalytic amounts of **21**[28] resulted in selective activation of the terminal alkenes, even though second-generation Grubbs catalysts are well known for their ability to react with more highly substituted olefins under mild conditions.[29,30] In any case, the desired 24-membered polyene **5** representing the targeted 'hybrid natural product' was isolated in respectable yield as the *E*-isomer at the newly formed double bond (Scheme 3).



Scheme 3. (a) [(dppf)$PdCl_2$] (12 mol %), $Ba(OH)_2 \cdot 8H_2O$, DMF, 70%; (b) EDC·HCl, 4-pyrrolidinylpyridine, CH_2Cl_2, 75%; (c) complex **21** (20 mol %), CH_2Cl_2, 68%.

2.2. Evaluation and structural investigations

The cytotoxicity of the chimera **5** was evaluated in vitro in an assay comprising 12 selected human cancer cell lines (Table 1).[31,32] An average IC_{50} of 2.02 μM was determined. It is of note, however, that the lung adeno cancer cell lines LXFA 629L (IC_{50} 0.32 μM) and LXFL 529L (IC_{50} 0.63 μM), as well as the colorectal cancer cell line CXF HT29 (IC_{50} 0.49 μM) were found to be significantly more sensitive than average. Least sensitive were the pancreas-(PAXF 1657L), the melanoma-(MEXF 462NL), and the renal (RXF 486L) tumor cell lines. Overall, **5** retains a selectivity profile similar to that of iejimalide B (**2**), although it is clearly less potent.[14,33]

Table 1
Antitumor activity of compound **5** against selected human tumor cell lines

Cell line	Type[a]	IC_{50} (μM)	IC_{70} (μM)
CXF HT29	Colon adeno ca., pd	0.49	0.94
GXF 251L	Gastric adeno ca., pd	1.30	6.21
LXFL 529L	Lung large cell ca., pd	0.63	2.15
LXFA 629L	Lung adeno ca., pd	0.32	0.71
MAXF 401NL	Mammary adeno ca., wd	2.49	6.49
MEXF 462NL	Amelanotic melanoma	5.99	14.07
OVXF 899L	Ovarian adeno ca., wd	3.49	6.17
PAXF 1657L	Pancreatic adeno ca., md	5.34	15.20
PRXF 22RV1	Prostate ca., pd	2.46	5.05
PXF 1752L	Pleuramesothelioma	3.27	13.22
RXF 486L	Hypernephroma, pd, clear cell	6.56	14.13
UXF 1138L	Endometrium carcino sarcoma, pd	2.40	4.99

[a] ca=carcinoma, pd=poorly differentiated, wd=well differentiated, md=moderately differentiated.

Extensive NMR investigations indicate that the common macrolactone rings of **2** and **5** adopt similar but not identical conformations, at least in CD_2Cl_2.[34] In both compounds, the 17-OMe group resonates at significantly higher field (<3 ppm) than the 9-OMe substituent (ca. 3.3 ppm). This distinctive shift difference has previously been attributed to the orientation of the C.17-OMe group toward the inside of the macrolactone ring of **2**.[4,35] Likewise, the diastereomeric protons of the C.10 methylene group in **5** shows a characteristic pattern signature similar to that observed for **2**. The comparison of the recorded NOESY/ROESY data further advocates a high degree of conformational homology, even though these spectra also reveal subtle differences: thus, the reported ROESY correlations between H.10a,b and the protons of the adjacent methyl ether at C.9 in **2**[4] are missing for **5**; in contrast, distinct cross peaks in the NOESY spectra of **5** indicate a proximity between H.20 and the methyl group branching off C.22, as well as between H.23 and H.21; no such contacts were described for iejimalide **2**.[4]

Molecular dynamic simulations with the MMFF94 force field[36] in the CHARMM program[37] were carried out to further assess the level of homology between **2** and **5** (for details, see the Experimental section). The lowest energy conformations of both molecules were found to be very similar, with the lateral chain being folded back over the macrocycle. For iejimalide, however, a second conformational cluster of only slightly (0.16 kcal mol^{-1}) higher energy is available, in which the side chain points away from the macrolactone, the C.2 methyl branch, and the ester carbonyl are synclinal to each other, and the C.17 methoxy group is directed toward the interior of the ring, which adopts a puckered conformation (Fig. 2). This second conformational cluster nicely corresponds to the 3D-structure of **2** in solution as deduced from the NMR spectra.

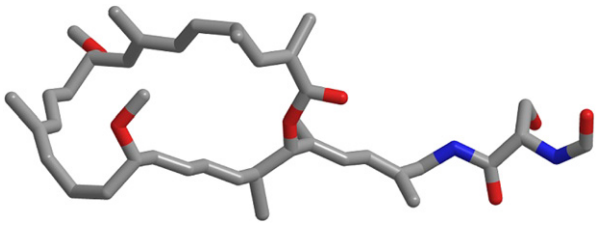


Figure 2. Representative of the computed conformational cluster of iejimalide B (**2**), which matches the solution structure most closely.

The third conformational cluster of **2**, which lies 1.55 kcal mol^{-1} above the global minimum, also orients the C.17 methoxy group inside the macrocycle; however, the torsional angle for the enoate part C.3–C.2–C.1–O.24 is increased from +51° in cluster 2 to +161°, which brings the C.2 methyl branch in a roughly antiperiplanar orientation relative to the carbonyl group. The large ring accommodates this change by adopting a rather flat shape.

The calculated conformers of the chimera **5** can be grouped into four clusters. Although their gross features closely resemble those computed for iejimalide, the energetic barriers between them are clearly more pronounced. Specifically, the second most stable cluster is already 2.1 kcal mol^{-1} higher in energy than the global minimum; the conformers, in which the macrocycle of **5** matches the NMR structure of iejimalide in solution (C.17–OMe inward, C.2–Me, and ester carbonyl synclinal to each other, puckered macrocycle), are even 2.8 kcal mol^{-1} higher (Fig. 3). One may therefore conclude that the parent natural product **2** and the 'hybrid natural product' **5** populate a qualitatively similar conformational space, but the energetic barriers are clearly more important in the latter case. Although the lack of information about the conformation of these macrolides, when bound to their biological receptor(s), makes any firm conclusion impossible at this stage, the computational results outlined above may explain why the selectivity profiles of **2** and **5** are similar, whereas their potencies are quite distinct.

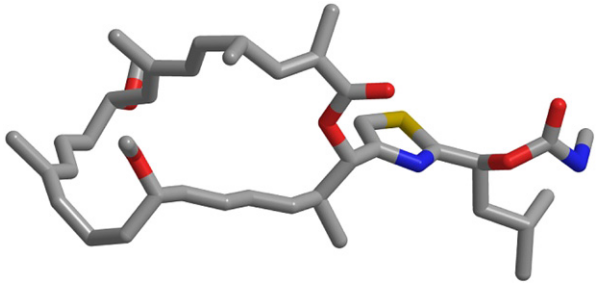


Figure 3. Representative conformer of the chimera **5** belonging to the cluster, in which the macrocycle closely resembles the solution structure of iejimalide. This cluster, however, lies ca. 2.8 kcal mol^{-1} above the global minimum.

3. Experimental

3.1. General

All reactions were carried out under Ar in flame-dried glassware. The solvents used were purified by distillation over the drying agents indicated and transferred under Ar: THF, Et_2O, 1,4-dioxane (Mg/anthracene), CH_2Cl_2, pyridine (CaH_2), hexane, toluene, (Na/K), DMF (Desmodur 15, dibutyl tin dilaurate), EtOH (Mg). Flash chromatography (FC): Merck silica gel 60 (230–400 mesh) or CombiFlash (Teledyne Isco). NMR: spectra were recorded on Bruker DPX 300, AMX 300, AV 400, and DMX 600 spectrometers in the solvents indicated; chemical shifts (δ) are given in parts per million relative to TMS, coupling constants (J) in hertz. The solvent signals were used as references and the chemical shifts converted to the TMS scale ($CDCl_3$: $\delta_C \equiv 77.0$ ppm; residual $CHCl_3$: $\delta_H \equiv 7.26$ ppm; CD_2Cl_2: $\delta_C \equiv 53.8$ ppm; residual $CHDCl_2$: $\delta_H \equiv 5.32$ ppm); IR: Magna IR750 (Nicolet) or Spectrum One (Perkin/Elmer) spectrometer, wavenumbers ($\tilde{V}$) in cm^{-1}; ESIMS: ESQ3000 (Bruker), accurate mass determinations: Bruker APEX III FT-MS (7 T magnet) or Mat 95 (Finnigan). Elemental analyses: Kolbe, Mülheim/Ruhr. All commercially available compounds (Fluka, Lancaster, Aldrich) were used as received.

*3.1.1. Weinreb amide **7**.* A solution of isopropylmagnesium chloride (2 M in THF, 15 mL) was slowly added to a solution of ethyl isovalerate (1.51 mL, 10 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (1.51 g, 15.5 mmol) in THF (20 mL) at −20 °C. After stirring for 40 min at this temperature, the reaction was quenched with satd aq NH_4Cl (10 mL) and warmed to room temperature. The resulting mixture was extracted with EtOAc (3×10 mL), the combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated to give amide **7**, which was pure enough for direct use in the next reaction (1.41 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.67 (s, 3H), 3.17 (s, 3H), 2.29 (d, J=7.0 Hz, 2H), 2.20–2.13 (m, 1H), 0.96 (d, J=6.6 Hz, 6H); IR (film): 2958, 2872, 1659, 1465, 1414, 1378, 1168, 1004 cm^{-1}; HRMS (ESI) calcd for $C_7H_{15}O_2N$ [M$^+$] 145.11028; found: 145.11012.

*3.1.2. Compound **8**.* A solution of *n*-BuLi (6.1 mL, 9.72 mmol) was slowly added to a solution of 2,5-dibromothiazole (1.97 g, 8.1 mmol) in Et_2O (40.5 mL) at −78 °C. After stirring for 1 h at this temperature, Weinreb amide **7** (1.41 g, 9.72 mmol) was introduced and stirring continued for 1 h before the mixture was allowed to reach ambient temperature over the course of 1 h. The reaction was quenched with satd aq NH_4Cl (20 mL), the aqueous layer was extracted with Et_2O (3×20 mL), the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. Purification of the residue by flash chromatography (SiO_2, hexanes/EtOAc 80:1) afforded compound **8** as a colorless oil (1.77 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.56 (s, 1H), 3.02 (d, J=7.0 Hz, 2H), 2.38–2.29 (m, 1H), 1.00 (d, J=6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=192.5, 167.4, 126.8, 124.7, 46.8, 24.9, 22.6; IR (film): 3119, 2959, 2924, 2868, 1689, 1457, 1387, 1300, 1262, 1210, 986, 930, 890, 836, 746 cm^{-1}; MS (EI) m/z (%): 249 (43, M$^+$), 247 (42, M$^+$), 207 (46), 205 (46), 192 (25), 190 (24), 179 (32), 177 (31), 165 (32), 163 (32), 83 (32), 57 (100); HRMS (ESI) calcd for $C_8H_{10}ONSBr$ [M+Na$^+$] 269.95588; found: 269.95573.

*3.1.3. Compound **10**.* Solutions of (*R*)-2-methyl-oxazaborolidine **9** (1 M in toluene, 3.5 mL) and $BH_3 \cdot SMe_2$ (3.3 mL, 34.9 mmol) were slowly added via two dropping funnels to a solution of ketone **8** (1.73 g, 6.97 mmol) in THF (42 mL) at −30 °C. After stirring for 1.5 h at this temperature, the reaction was carefully quenched with EtOH (12 mL) and warmed to room temperature. A standard extractive work up followed by purification of the crude material by flash chromatography (SiO_2, hexanes/EtOAc 50:1→8:1) afforded the corresponding alcohol as a crystalline compound (1.65 g, 95%), ee=95% (HPLC: Chiracel OD-H 250 mm, ∅ 4.6 mm, *n*-heptane/2-propanol=95:5, 0.5 mL min^{-1}, 298 K, UV detection (220 nm), t_R (minor)=13.74 min, t_R (major)=15.09 min), which analyzed as follows: $[\alpha]_D^{20}$ −18.3 (c 0.42, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.34 (s, 1H), 5.15–5.08 (m, 1H), 2.06–1.90 (m, 1H), 1.88–1.78 (m, 2H), 1.08 (d, J=6.5 Hz, 3H), 1.07 (d, J=2.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=176.9, 124.4, 116.7, 70.5, 47.0, 24.6, 23.3, 21.6; IR (film): 3359, 2956, 2926, 2870, 1738, 1480, 1367, 1250, 1183, 1081, 1067, 888, 838, 733 cm^{-1}; MS (EI) m/z (%): 251 (10, M$^+$), 249 (10, M$^+$), 207 (16), 205 (16), 194 (100), 192 (97), 166 (40), 164 (40), 139 (13), 137 (14), 57 (31); HRMS (EI) calcd for $C_8H_{12}ONSBr$ [M$^+$] 248.98231; found: 248.98260.

2,6-Lutidine (1.92 mL, 16.5 mmol) and TBSOTf (3.0 mL, 13.2 mmol) were added to a solution of this alcohol (1.65 g,

6.6 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the resulting mixture was stirred for 1.5 h. The reaction was quenched with satd aq $NaHCO_3$ (10 mL) and extracted with Et_2O (3×15 mL), the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. Purification of the residue by flash chromatography (SiO_2, pentanes/Et_2O 200:1→80:1) gave compound **10** as a colorless solid (2.03 g, 84%). $[\alpha]_D^{20}$ −43.7 (*c* 1.15, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.13 (s, 1H), 5.03 (dd, *J*=7.8, 4.5 Hz, 1H), 1.84–1.68 (m, 2H), 1.65–1.57 (m, 1H), 0.94 (d, *J*=6.6 Hz, 3H), 0.90 (s, 9H), 0.92 (d, *J*=6.6 Hz, 3H), 0.18 (s, 3H), −0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=178.8, 123.8, 116.4, 71.6, 48.9, 25.7, 24.0, 23.4, 22.2, 18.1, −4.80, −4.86; IR (film): 2957, 2926, 2855, 1481, 1471, 1252, 1188, 1090, 901, 832, 778 cm^{-1}; MS (EI) *m*/*z* (%): 308 (100, M^+−*t*-Bu), 306 (94, M^+−*t*-Bu), 251 (6), 249 (6), 222 (5), 220 (5), 192 (2), 190 (2), 139 (13), 137 (14), 57 (31); HRMS (ESI) calcd for $C_{14}H_{26}ONSSiBr$ [M+Na^+] 386.05801; found: 386.05796.

3.1.4. Compound ***11***. A solution of compound **10** (1.96 g, 5.38 mmol) in Et_2O (50 mL) was added dropwise at −78 °C to a solution of *t*-BuLi (2.1 M in pentane, 7.7 mL) in Et_2O (50 mL). After stirring for 5 min, carefully dried DMF (1.25 mL, 16.14 mmol) was introduced and the mixture stirred for 1.5 h before the reaction was quenched with satd aq NH_4Cl (30 mL) and the mixture allowed to reach ambient temperature. A standard extractive work up followed by purification of the crude product by flash chromatography (SiO_2, pentanes/Et_2O 80:1→10:1) furnished compound **11** as a colorless oil (1.17 g, 70%). $[\alpha]_D^{20}$ −44.2 (*c* 0.43, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ=9.98 (s, 1H), 8.11 (s, 1H), 5.10 (dd, *J*=7.6, 4.7 Hz, 1H), 1.88–1.71 (m, 2H), 1.70–1.60 (m, 1H), 0.96 (d, *J*=6.5 Hz, 3H), 0.93 (s, 9H), 0.93 (d, *J*=6.4 Hz, 3H), 0.12 (s, 3H), −0.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=185.1, 179.6, 154.5, 128.0, 71.5, 48.8, 25.7, 24.0, 23.4, 22.3, 18.1, −4.9; IR (film): 2956, 2926, 2855, 1708, 1470, 1362, 1252, 1189, 1089, 1001, 900, 837, 808, 777 cm^{-1}; HRMS (ESI) calcd for $C_{15}H_{27}O_2NSSi$ [M+Na^+] 336.14240; found: 336.14235.

3.1.5. Compound ***13***. $Pd(OAc)_2$ (42 mg, 0.19 mmol) and PPh_3 (48.2 mg, 0.19 mmol) were added at −78 °C to a solution of mesylate **12** (664 mg, 4.5 mmol)[24] in THF (27.2 mL). After stirring for 5 min, a solution of aldehyde **11** (1.17 g, 3.7 mmol) in THF (12 mL) was introduced, followed by the dropwise addition of $ZnEt_2$ (1 M in hexane, 11.2 mL). After stirring for 30 min at this temperature, the solution was warmed to −20 °C over a period of 30 min and stirred overnight. The mixture was carefully quenched with satd aq NH_4Cl (20 mL) before it was allowed to reach ambient temperature. The aqueous phase was extracted with EtOAc (3×20 mL), the combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated. Purification of the crude product by flash chromatography (SiO_2, pentanes/Et_2O 100:1→7:1) afforded a 1:1 mixture of diastereomers **13** as a colorless oil (976 mg, 71%). A second flash chromatography with the same eluent enriched this mixture in the desired *anti*-isomer (dr≥7.5:1), which showed the following characteristic data: ^1H NMR (300 MHz, $CDCl_3$): δ=7.14 (d, *J*=0.6 Hz, 1H), 5.00 (dd, *J*=7.3, 4.9 Hz, 1H), 4.65 (d, *J*=5.0 Hz, 1H), 3.12–3.02 (m, 1H), 2.88 (br s, 1H), 2.09 (d, *J*=2.4 Hz, 1H), 1.78–1.69 (m, 2H), 1.62–1.55 (m, 1H), 1.16 (d, *J*=7.1 Hz, 3H), 0.91–0.86 (m, 6H), 0.89 (s, 9H), 0.07 (s, 3H), −0.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=177.4, 155.9, 114.4, 84.9, 73.3, 71.6, 71.1, 48.9, 33.6, 25.7, 24.0, 23.3, 22.4, 18.1, 17.0, −4.8, −4.9; IR (film): 3312, 2956, 2931, 2855, 1471, 1360, 1256, 1087, 1001, 902, 838, 778 cm^{-1}; MS (EI) *m*/*z* (%): 310 (100, M^+−*t*-Bu), 256 (24), 224 (6), 249 (6), 182 (22), 140 (6); HRMS (ESI) calcd for $C_{19}H_{33}O_2NSSi$ [M+Na^+] 390.18935; found: 390.18919.

3.1.6. Compound ***14***. Pivaloyl chloride (84 μL, 0.68 mmol) was added to a solution of alcohol **13** (83 mg, 0.23 mmol, dr≥7.5:1) and catalytic amounts of DMAP in pyridine (1.0 mL) at 0 °C. The mixture was stirred overnight at ambient temperature before the reaction was quenched with aq 1 M HCl. A standard extractive work up followed by purification of the residue by flash chromatography (SiO_2, hexanes/EtOAc 100:1→50:1) yielded product **14** as a yellow oil (68 mg, 67%). $[\alpha]_D^{20}$ −80.5 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ=7.15 (s, 1H), 5.84 (d, *J*=6.6 Hz, 1H), 5.02 (dd, *J*=7.7, 4.8 Hz, 1H), 3.36–3.23 (m, 1H), 1.99 (d, *J*=2.4 Hz, 1H), 1.82–1.68 (m, 2H), 1.64–1.50 (m, 1H), 1.24 (s, 9H), 1.14 (d, *J*=7.1 Hz, 3H), 0.95–0.86 (m, 15H), 0.07 (s, 3H), −0.08 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=177.5, 174.0, 152.4, 116.3, 84.6, 73.6, 71.6, 69.8, 49.0, 40.2, 30.8, 27.1, 25.7, 24.1, 23.3, 22.3, 18.1, 17.4, −4.88, −4.96; IR (film): 2957, 2926, 2855, 1737, 1463, 1365, 1217, 1150, 1086, 838, 778 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{41}O_3NSSi$ [M+Na^+] 474.24686; found: 474.24667.

3.1.7. Compound ***15***. A solution of compound **14** (68 mg, 0.15 mmol) in THF (1.25 mL) was slowly added to a suspension of $Cp_2Zr(H)Cl$ (58.2 mg, 0.23 mmol) in THF (950 μL) and the mixture stirred in the dark for 1 h before it was cooled to 0 °C. A solution of I_2 (63 mg, 0.25 mmol) in THF (1.25 mL) was added dropwise until a pale yellow color persisted. After stirring for 5 min, the reaction was quenched with satd aq $Na_2S_2O_3$ (1 mL), the aqueous phase was extracted with EtOAc (3×1 mL), the combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 100:1→60:1) to give the corresponding alkenyl iodide as a yellow oil (73 mg, 83%), which analyzed as follows: $[\alpha]_D^{20}$ −75.1 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, CD_2Cl_2): δ=7.06 (s, 1H), 6.46 (dd, *J*=14.4, 8.8 Hz, 1H), 6.05 (dd, *J*=14.4, 0.7 Hz, 1H), 5.72 (d, *J*=6.6 Hz, 1H), 5.02 (dd, *J*=7.5, 5.1 Hz, 1H), 3.04–2.95 (m, 1H), 1.80–1.71 (m, 2H), 1.64–1.56 (m, 1H), 1.21 (s, 9H), 0.97–0.84 (m, 18H), 0.10 (s, 3H), −0.06 (s, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ=177.8, 177.4, 153.4, 147.4, 116.2, 76.6, 74.4, 72.1, 49.5, 45.1, 39.2, 27.3, 25.9, 24.5, 23.4, 22.5, 18.4, 16.3, −4.67, −4.78; IR (film): 2956, 2930, 2855, 1735, 1462, 1364, 1279, 1257, 1148, 1086, 1003, 837, 777 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{42}O_3NSSiI$ [M+Na^+] 602.15916; found: 602.15943.

TBAF (1 M in THF, 250 μL) was added to a solution of this alkenyl iodide (73 mg, 0.13 mmol) in THF (2 mL) at 0 °C and the resulting mixture stirred for 20 min before the reaction was quenched with satd aq NH_4Cl (2 mL) and extracted with EtOAc (3×2 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 50:1→8:1) to give compound **15** as a colorless oil (45 mg, 77%). Alternatively, isomerically pure **15** can be obtained at this stage by flash chromatography of the crude product mixture resulting from the elaboration of *syn*/*anti*-**13** primarily formed in the Marshall reaction. $[\alpha]_D^{20}$ −83.2 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.06 (s, 1H), 6.44 (dd, *J*=14.4, 8.6 Hz, 1H), 6.04 (d, *J*=14.4 Hz, 1H), 5.78 (d, *J*=6.5 Hz, 1H), 5.01 (dd, *J*=8.1, 5.1 Hz, 1H), 3.05–2.95 (m, 1H), 2.70 (br s, 1H), 1.92–1.82 (m, 1H), 1.78–1.71 (m, 2H), 1.22 (s, 9H), 1.02–0.96 (m, 9H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ=177.5, 176.2, 153.9, 147.3, 116.1, 76.7, 74.4, 70.7, 47.7, 45.1, 39.2, 27.3, 25.0, 23.4, 22.0, 16.2; IR (film): 3451, 2958, 2931, 2870, 1732, 1479, 1464, 1280, 1149, 1069, 947 cm^{-1}; MS (EI) *m*/*z* (%): 465 (33, M^+), 422 (68), 409 (15), 364 (3), 338 (7), 284 (10), 236 (22), 200 (19), 182 (95), 57 (100); HRMS (ESI) calcd for $C_{18}H_{28}O_3NSI$ [M+Na^+] 488.07269; found: 488.07303.

3.1.8. Compound ***16***. A solution of compound **15** (19.5 mg, 0.042 mmol) and 1,1′-carbonyldiimidazole (13.6 mg, 0.084 mmol) in CH_2Cl_2 (500 μL) was stirred for 40 min before the mixture was cooled to 0 °C. A solution of methylamine (2 M in THF, 84 μL) was added and stirring continued for 2 h, before the mixture was acidified with aq HCl (1 M, 1 mL) and extracted with Et_2O (3×1 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated, and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 20:1→5:1) to give the

corresponding carbamate as a colorless oil (20 mg), which analyzed as follows: $[\alpha]_D^{20}$ −112.2 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, CD_2Cl_2): δ=7.07 (s, 1H), 6.45 (dd, *J*=14.4, 8.7 Hz, 1H), 6.05 (d, *J*=14.4 Hz, 1H), 5.97 (dd, *J*=8.4, 5.6 Hz, 1H), 5.75 (d, *J*=6.3 Hz, 1H), 4.84 (br s, 1H), 3.01–2.95 (m, 1H), 2.78 (d, *J*=4.8 Hz, 3H), 1.88–1.82 (m, 2H), 1.73–1.67 (m, 1H), 1.22 (s, 9H), 0.99–0.95 (m, 9H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ=177.5, 171.6, 156.2, 154.4, 147.2, 116.1, 76.7, 74.5, 72.2, 45.2, 44.6, 39.2, 27.8, 27.3, 25.0, 23.1, 22.3, 16.2; IR (film): 3441, 2956, 2931, 2870, 1728, 1524, 1251, 1149, 971 cm^{-1}; MS (EI) *m*/*z* (%): 522 (6, M^+), 465 (53), 448 (21), 422 (30), 409 (18), 395 (6), 257 (21), 182 (100), 140 (9); HRMS (ESI) calcd for $C_{20}H_{31}O_4N_2SI$ [M+Na^+] 545.09415; found: 545.09449.

A solution of $LiBHEt_3$ (1 M in THF, 134 μL) was added to a solution of this product in CH_2Cl_2 (2.2 mL) at 0 °C and the resulting mixture stirred for 1 h before the reaction was quenched with EtOAc (1 mL). A standard extractive work up followed by flash chromatography of the crude material (SiO_2, hexanes/EtOAc, 10:1→2:1) furnished product **16** as a colorless oil (16.5 mg, 91% over both steps). $[\alpha]_D^{20}$ −83.5 (*c* 1.65, CH_2Cl_2); ^1H NMR (300 MHz, CD_2Cl_2): δ=7.09 (d, *J*=0.5 Hz, 1H), 6.54 (dd, *J*=14.5, 8.1 Hz, 1H), 6.06 (dd, *J*=14.5, 0.9 Hz, 1H), 5.97 (dd, *J*=8.6, 5.3 Hz, 1H), 4.85 (br s, 1H), 4.59 (dd, *J*=5.8, 5.8 Hz, 1H), 2.80–2.71 (m, 1H), 2.77 (d, *J*=4.8 Hz, 3H), 2.63 (d, *J*=6.0 Hz, 1H), 1.89–1.80 (m, 2H), 1.75–1.68 (m, 1H), 0.99 (d, *J*=6.9 Hz, 3H), 0.96 (d, *J*=6.5 Hz, 6H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ=171.8, 157.9, 148.0, 125.8, 114.8, 76.4, 74.0, 72.4, 47.2, 44.8, 30.5, 25.0, 23.1, 22.3, 15.5; IR (film): 3337, 2958, 2870, 1706, 1525, 1467, 1369, 1255, 1130, 950 cm^{-1}; HRMS (ESI) calcd for $C_{15}H_{23}O_3N_2SI$ [M+Na^+] 461.03663; found: 461.03702.

3.1.9. Compound ***18***. $Ba(OH)_2 \cdot 8H_2O$ (17.5 mg, 0.055 mmol) and (dppf)$PdCl_2$ (4.0 mg, 0.0055 mmol) were added to a degassed solution of compound **16** (16.2 mg, 0.037 mmol) and boronate **17** (13.0 mg, 0.044 mmol) in DMF (810 μL). The mixture was stirred for 6 h at ambient temperature before it was poured on ice-cold aq HCl (1 M, 2 mL). The aqueous phase was extracted with EtOAc (3×3 mL) and the combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 10:1→1:1) to give product **18** as a colorless oil (12.3 mg, 70%). $[\alpha]_D^{20}$ −32.8 (*c*=1.0, CH_2Cl_2); ^1H NMR (400 MHz, CD_2Cl_2): δ=7.09 (s, 1H), 6.78 (dd, *J*=17.3, 10.8 Hz, 1H), 6.17–6.07 (m, 2H), 5.98 (dd, *J*=8.7, 5.2 Hz, 1H), 5.65 (dd, *J*=14.3, 7.8 Hz, 1H), 5.42 (dd, *J*=14.2, 7.9 Hz, 1H), 5.38 (t, *J*=7.8 Hz, 1H), 5.19 (d, *J*=17.2 Hz, 1H), 5.07 (dd, *J*=11.0, 1.6 Hz, 1H), 4.84 (br s, 1H), 4.57 (t, *J*=5.7 Hz, 1H), 3.51 (dd, *J*=7.3, 6.1 Hz, 1H), 3.20 (s, 3H), 2.78 (d, *J*=5.0 Hz, 3H), 2.78–2.73 (m, 1H), 2.48 (d, *J*=5.7 Hz, 1H), 2.25–2.14 (m, 2H), 1.94–1.77 (m, 2H), 1.80 (d, *J*=1.0 Hz, 3H), 1.74–1.69 (m, 1H), 1.67–1.44 (m, 2H), 0.99 (d, *J*=6.9 Hz, 3H), 0.96 (d, *J*=6.5 Hz, 6H); IR (film): 3358, 2956, 2916, 2870, 1717, 1523, 1459, 1369, 1254, 1127, 1096, 991 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{40}O_4N_2S$ [M+Na^+] 499.26010; found: 499.25999.

3.1.10. Compound ***20***. EDC·HCl (2.9 mg, 0.015 mmol) and 4-pyrrolidino-pyridine (0.3 mg, 0.0023 mmol) were added to a solution of acid **19** (4.5 mg, 0.017 mmol) in CH_2Cl_2 (120 μL) at 0 °C. The mixture was warmed to ambient temperature for 15 min and re-cooled to 0 °C before alcohol **18** (7.3 mg, 0.015 mmol) was introduced. After stirring for 70 h at ambient temperature, EtOAc (2 mL) was added, the organic phase was washed with brine (1 mL), dried over $MgSO_4$, and evaporated, and the residue purified by flash chromatography (SiO_2, hexanes/EtOAc, 10:1→5:1) to yield product **20** as a colorless oil (8.15 mg, 75%). $[\alpha]_D^{20}$ −30.7 (*c* 0.51, CH_2Cl_2); ^1H NMR (600 MHz, CD_2Cl_2): δ=7.13 (s, 1H), 6.76 (ddd, *J*=17.4, 10.9, 0.8 Hz, 1H), 6.61 (dd, *J*=9.8, 1.3 Hz, 1H), 6.13–6.05 (m, 3H), 5.98 (dd, *J*=8.9, 5.2 Hz, 1H), 5.77 (ddt, *J*=17.2, 10.3, 7.0 Hz, 1H), 5.76 (d, *J*=7.1 Hz, 1H), 5.61 (dd, *J*=14.4, 8.4 Hz, 1H), 5.59 (dd, *J*=15.5, 7.1 Hz, 1H), 5.40–5.35 (m, 2H), 5.26 (d, *J*=9.2 Hz, 1H), 5.18 (dd, *J*=17.3, 1.0 Hz, 1H), 5.07–4.99 (m, 3H), 4.84 (br d, *J*=4.7 Hz, 1H), 4.03 (td, *J*=9.1, 6.4 Hz, 1H), 3.48 (dt, *J*=7.4, 6.1 Hz, 2H), 3.30–3.23 (m, 1H), 3.20 (s, 3H), 3.16 (s, 3H), 3.03 (dt, *J*=7.6, 7.2 Hz, 1H), 2.77 (d, *J*=4.9 Hz, 3H), 2.36–2.31 (m, 1H), 2.23–2.13 (m, 2H), 1.90–1.81 (m, 2H), 1.85 (d, *J*=1.4 Hz, 3H), 1.79 (d, *J*=1.1 Hz, 3H), 1.77 (d, *J*=1.2 Hz, 3H), 1.72–1.66 (m, 1H), 1.63–1.58 (m, 1H), 1.49–1.43 (m, 1H), 1.13 (d, *J*=6.8 Hz, 3H), 0.96–0.94 (m, 9H); ^{13}C NMR (150 MHz, CD_2Cl_2): δ=171.6, 167.5, 156.2, 154.6, 145.7, 136.8, 135.3, 135.1, 134.0, 133.8, 133.0, 132.9, 132.8, 132.0, 131.6, 131.0, 130.9, 126.7, 116.8, 116.8, 113.4, 81.5, 77.0, 75.6, 72.3, 56.1, 56.0, 44.7, 41.4, 40.3, 36.8, 35.9, 27.8, 24.9, 23.5, 23.0, 22.2, 20.4, 19.8, 16.7, 13.1, 12.6; IR (film): 2956, 2926, 2870, 1732, 1717, 1522, 1448, 1367, 1248, 1125, 1097, 991, 968 cm^{-1}; HRMS (ESI) calcd for $C_{42}H_{62}O_6N_2S$ [M+Na^+] 745.42208; found: 745.42210.

3.1.11. Compound ***5***. Complex **21** (0.6 mg, 0.0007 mmol) was added to a solution of compound **20** (5.1 mg, 0.007 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred at ambient temperature for 24 h. At this point, a second portion of **21** (0.6 mg, 0.0007 mmol) was introduced and stirring continued for another 24 h before the reaction was quenched with ethyl vinyl ether (100 μL). After stirring for 1 h, all volatile materials were evaporated and the residue purified by flash chromatography (hexanes/EtOAc, 10:1→4:1) to give product **5** as a pale yellow oil (3.3 mg, 68%). $[\alpha]_D^{20}$ −78.7 (*c* 0.15, CH_2Cl_2); ^1H NMR (600 MHz, CD_2Cl_2): δ=7.24 (s, 1H), 6.60 (dd, *J*=10.3, 1.4 Hz, 1H), 6.46 (d, *J*=15.5 Hz, 1H), 6.14 (dd, *J*=15.0, 10.5 Hz, 1H), 6.03–5.98 (m, 2H), 5.90 (d, *J*=15.5 Hz, 1H), 5.75 (d, *J*=9.8 Hz, 1H), 5.53 (ddd, *J*=15.2, 10.1, 4.9 Hz, 1H), 5.50–5.39 (m, 3H), 5.18 (dd, *J*=10.3, 5.8 Hz, 1H), 5.09 (d, *J*=9.6 Hz, 1H), 4.85 (br q, *J*=4.7 Hz, 1H), 4.11 (td, *J*=9.8, 2.8 Hz, 1H), 3.30–3.26 (m, 1H), 3.20 (s, 3H), 3.18–3.12 (m, 1H), 3.02–2.97 (m, 1H), 2.97 (s, 3H), 2.77 (d, *J*=4.8 Hz, 3H), 2.61 (br d, *J*=13.8 Hz, 1H), 2.54–2.47 (m, 1H), 2.31 (ddd, *J*=13.8, 10.0, 10.0 Hz, 1H), 1.91–1.85 (m, 2H), 1.82–1.79 (m, 1H), 1.79 (d, *J*=1.5 Hz, 3H), 1.77 (s, 3H), 1.74 (d, *J*=1.2 Hz, 3H), 1.72–1.68 (m, 1H), 1.62–1.57 (m, 1H), 1.34–1.27 (m, 1H), 1.03 (d, *J*=6.7 Hz, 3H), 0.96 (d, *J*=6.5 Hz, 3H), 0.95 (d, *J*=6.5 Hz, 3H), 0.89 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (150 MHz, CD_2Cl_2): δ=172.1, 167.7, 156.2, 154.1, 146.1, 137.1, 135.7, 133.8, 133.7, 133.2, 132.4, 132.3, 131.8, 131.5, 129.7, 128.8, 125.7, 125.3, 117.9, 80.0, 76.9, 74.8, 72.5, 56.4, 55.8, 44.8, 42.8, 40.9, 38.2, 35.2, 27.8, 24.9, 23.2, 23.1, 22.1, 21.4, 20.7, 16.6, 13.1, 12.2; IR (film): 2956, 2931, 1732, 1721, 1522, 1448, 1367, 1254, 1125, 1094, 993 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{58}O_6N_2S$ [M+Na^+] 717.39078; found: 717.39095.

3.1.12. X-ray crystal structure analysis of compound ***10***. $C_{14}H_{26}BrNOSSi$, M_r=364.42 $gmol^{-1}$, colorless plate, crystal size 0.35×0.31×0.16 mm, monoclinic, space group $P2_1$, *a*=8.9105(13) Å, *b*=10.4868(16) Å, *c*=9.6494(14) Å, β=94.209(4)°, *V*=899.2(2) $Å^3$, *T*=100 K, *Z*=2, D_{calcd}=1.346 gcm^3, λ=1.54178 Å, μ(*Cu*-K_α)= 4.780 mm^{-1}, Gaussian absorption correction (T_{min}=0.35, T_{max}=0.55), Proteum X8 diffractometer, 4.59<θ<68.82°, 20,675 measured reflections, 3223 independent reflections, 3211 reflections with $I>2\sigma(I)$, Structure solved by direct methods and refined by full-matrix least-squares against F^2 to R_1=0.024 [$I>2\sigma(I)$], wR_2=0.060, 179 parameters, Absolute structure parameter=0.020 (14), H atoms riding, *S*=1.108, residual electron density 0.3/ −0.3 e $Å^{-3}$. CCDC 763567 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.ca.ac.uk/data_request/cif.

3.1.13. Computational methods. The molecules were simulated by molecular dynamics with the Merck Molecular Force Field (MMFF94)[36] in the CHARMM program,[37] applying a dielectric constant of 80 to simulate the effect of solvent. The simulations were carried out at 1000 K to ensure that conformational barriers are readily crossed. Possible trajectories starting from five conformationally different starting points were calculated and 500 frames were extracted from each trajectory (0.002 ps per step and

5×100 steps=10 ns). For each conformer, an energy minimization was performed by 750 steps of the steepest descent (SD) algorithm in CHARM, and their MMFF energy was calculated. The 2500 conformers (500 conformers per trajectory) were then clustered to determine the dominant conformational space. The lowest energy conformation (LEC) was taken as representative of the first cluster, and all conformations having a root-mean-square deviation (RMSD) lower than 3 Å were compiled into that cluster. Then, the LEC of the remaining conformations was taken as starting point for the second cluster, and this process was iterated until all 2500 conformers were clustered.

3.1.14. Cytotoxicity assays. Ten cell lines were established from patient-derived tumor xenografts passaged subcutaneously in nude mice; the origin of the donor xenografts has already been described.[31] The other cell lines were obtained from American Type Culture Collection (PRXF 22RV1), Rockville, MD, USA or the National Cancer Institute (CXF HT29), Bethesda, MD, USA. Authenticity of all cell lines was proven by STR (short tandem repeat) analysis. All cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO2) in RPMI 1640 medium (PAA, Cölbe, Germany) supplemented with 10% fetal calf serum (PAA) and 0.1 mg mL^{-1} gentamicin (PAA). A modified propidium iodide assay was used to assess the effects of the compounds.[31] Tumor derived cell lines were incubated in 96 multi-well plates. After one day, the compounds under test were added to the plates at five concentrations in the range from 0.001 μg up to 10 μg mL^{-1} and left for further four days. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 μg mL^{-1}). Fluorescence was measured using the Cytofluor 4000. In each experiment, all data points were determined in triplicate.

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