CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.201102462

Total Synthesis of Bistramide A and Its 36(*Z*) Isomers: Differential Effect on Cell Division, Differentiation, and Apoptosis

Loïc Tomas,[a] Gustav Boije af Gennäs,[b] Marie Aude Hiebel,[c] Peter Hampson,[d] David Gueyrard,[a] Béatrice Pelotier,[c] Jari Yli-Kauhaluoma,[b] Olivier Piva,[c] Janet M. Lord,*[d] and Peter G. Goekjian*[a]

Dedicated to Professor Edwin Vedejs on the occasion of his 70th birthday

Abstract: The total synthesis of bistramide A and its 36(*Z*),39(*S*) and 36(*Z*),39(*R*) isomers shows that these compounds have different effects on cell division and apoptosis. The synthesis relies on a novel enol ether-forming reaction for the spiroketal fragment, a kinetic oxa-Michael cyclization reaction for the tetrahydropyran fragment, and an asymmetric crotonylation reaction for the amino acid fragment. Preliminary biological studies show a distinct pattern of influence of each of the three compounds on cell division, differentiation, and apoptosis in HL-60 cells, thus suggesting that these effects are independent activities of the natural product.

Keywords: alkenes · bistramide A · protein kinase C · total synthesis

Introduction

Bistramide A (bistratene A; Scheme 1) is a marine metabolite that was isolated from *Lissoclinum bistratum* in 1988 by Verbist and co-workers;[1] furthermore, four additional members of the family (bistramides B, C, D, and K) were isolated in 1994.[2] This class of compounds shows significant cytotoxicity and neurotoxicity and plays a complex role in cell-cycle regulation, differentiation, and apoptosis.[2–4] It was initially shown that bistramide A activates the δ isoform of protein kinase C (PKC) in human promyelocytic leukemia (HL-60) and human malignant melanoma (MM96E) cells, thus resulting in inhibition of cytokinesis and growth arrest.[5] However, Kozmin and co-workers more recently identified covalent modification of actin and disruption of filamentous actin as a primary mode of action for bistramide A in various cancer-cell lines.[6] There is currently little consensus on the activation of PKC-δ. It was determined early on that bistramide A does not activate PKC-δ in vitro, nor does it compete with phorbol esters in binding to the diacylglycerol activation site.[6,7] However, although bistramide A failed to translocate green fluorescent protein (GFP)/PKC-δ in rat basophilic leukemia (RBL) cells,[6] immunostaining and fractionation studies showed migration of PKC-δ to the perinuclear region rather than to the nuclear or cytoplasmic membrane.[5a] Furthermore, only certain substrates of PKC-δ were phosphorylated in whole cells, notably talin but not vimentin, vinculin, or tubulin,[5b] thus suggesting an atypical activation mode for this important family of second-messenger-activated protein kinases. Therefore, the synthesis of bistramide A analogues with modulated biological activities may serve to shed light on the biochemical basis of these different biological properties.

The first total synthesis of bistramide A was reported by Kozmin and co-workers 2004.[8] Later, Crimmins,[9] Panek,[10] and Yadav[11] and their respective co-workers also performed total syntheses of the natural compound, whereas the synthesis of bistramide C was realized by Wipf et al.[12] We report herein the synthesis of bistramide A and its 36(*Z*),39(*R*) and 36(*Z*),39(*S*) isomers. Inspection of the X-ray crystal structure of the bistramide A/actin complex shows a water-relayed hydrogen bond between the C39–OH unit and tyrosine143 (Tyr143) residue[13] (Figure 1). Inversion of the stereochemistry of the double bond might place the same hydroxy group in proximity to the Tyr143 carbonyl

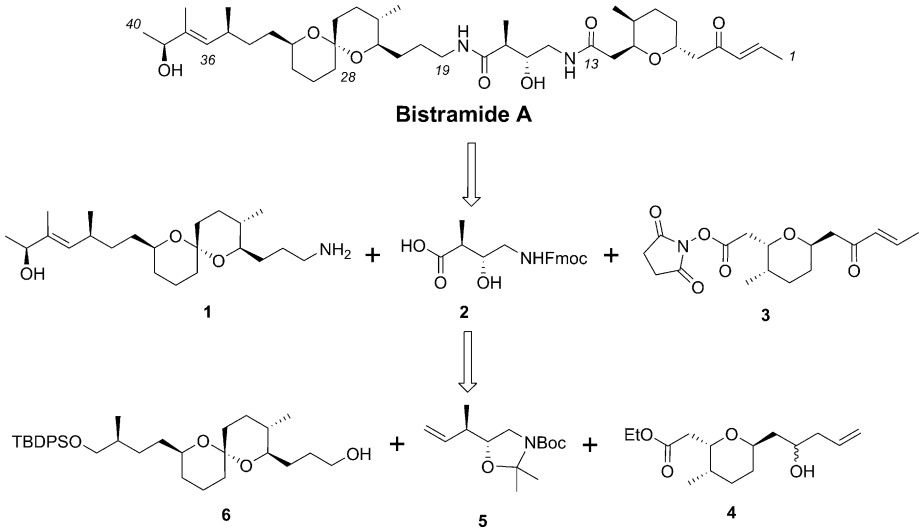
[a] Dr. L. Tomas, Dr. D. Gueyrard, Prof. P. G. Goekjian
Université de Lyon, ICBMS - UMR CNRS 5246
LCO2 - Glycochimie, Université Claude Bernard Lyon 1
Bat. 308 CPE Lyon, 43, bd du 11 Novembre 1918
69622 Villeurbanne Cedex (France)
Fax: (+33) 472 43 2752
E-mail: goekjian@univ-lyon1.fr

[b] Dr. G. Boije af Gennäs, Prof. J. Yli-Kauhaluoma
Division of Pharmaceutical Chemistry
Faculty of Pharmacy, University of Helsinki
PO Box 56 (Viikinkaari 5 E), 00014 Helsinki (Finland)

[c] Dr. M. A. Hiebel, Dr. B. Pelotier, Prof. O. Piva
Université de Lyon, ICBMS - UMR CNRS 5246
Surcoof, Université Claude Bernard Lyon 1
Bat. Raulin, 43, bd du 11 Novembre 1918
69622 Villeurbanne Cedex (France)

[d] Dr. P. Hampson, Prof. J. M. Lord
School of Immunity and Infection
The Medical School, University of Birmingham
Birmingham B15 2TT (UK)
E-mail: j.m.lord@bham.ac.uk

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201102462.



Scheme 1. Retrosynthetic analysis for the C19–C36 fragment. Boc = *tert*-butyloxycarbonyl, Fmoc = 9-fluorenylmethyloxycarbonyl, TBDPS = *tert*-butyldiphenylsilyl.

Synthesis of the spiroketal fragment: The spiroketal core of the C19–C40 fragment of the bistramides can be obtained through an exocyclic enol ether by extending our recent methodology for the synthesis of functionalized *exo*-glycals from sugar-derived lactones[16] to the coupling of a non-carbohydrate lactone **8** with the benzothiazolyl sulfone **9** (Scheme 2), which can be prepared from a common precursor, namely, 1,2:5,6-dicyclohexylidene-D-mannitol.[14] Such an approach might eventually allow for the synthesis of analogues substituted at the C28 position or kinetic spiroketal stereoisomers.

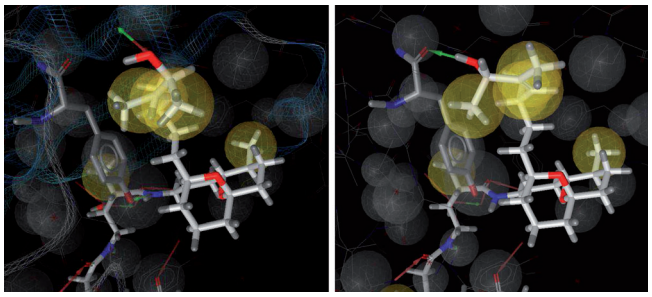
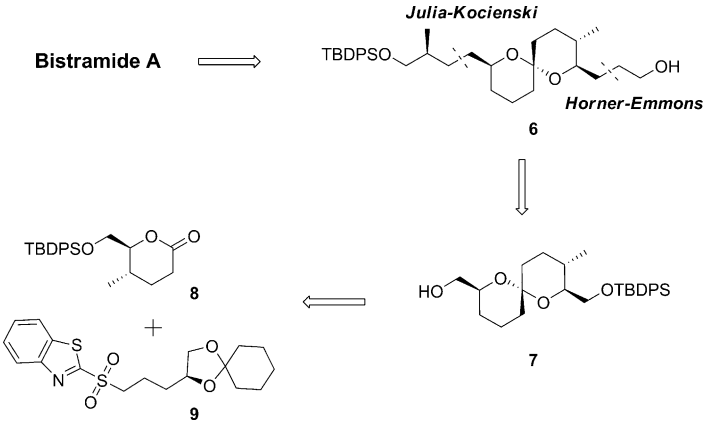


Figure 1. Left: X-ray structure of the bistramide A/actin complex that highlights the water-relayed hydrogen bond to the Tyr143 residue. Right: a model of the 36(*Z*),39(*S*) isomer obtained by substitution and MM2 energy minimization in the X-ray structure of the bistramide A/actin complex, thus showing the expected proximity of the hydroxy group to the Tyr143 carbonyl unit (Inte:ligand LigandScout).



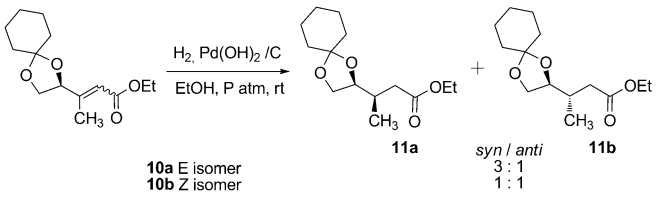
Scheme 2. Synthetic precursors of bistramide A.

group, thus allowing the formation of a direct hydrogen bond. Therefore, we sought to prepare these isomers to test this hypothesis.

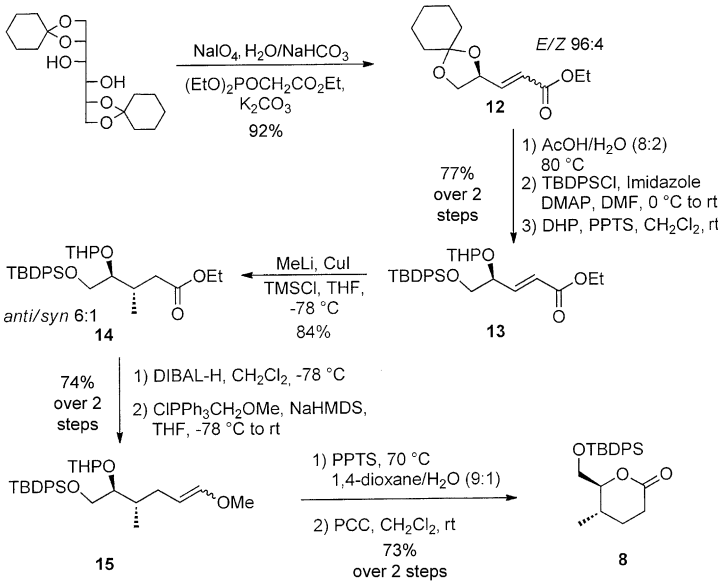
Results and Discussion

Bistramide A and its isomers were prepared by coupling spiroketal-containing amines (e.g., **1**) with the appropriately protected homothreonine central fragment **2** and the tetrahydropyran-containing carboxylic acid **3**. The spiroketal C19–C40 fragment **1** was obtained by using a novel synthesis of exocyclic enol ethers from lactones.[14] The amino acid fragment **2** was accessed by asymmetric crotyltitanation in a variation of the procedure developed by Kozmin and coworkers,[8] whereas the tetrahydropyran fragment **3** was prepared by a kinetically controlled oxa-Michael cyclization reaction (Scheme 1).[15]

The synthesis of lactone **8** is shown in Schemes 3 and 4. Initial studies focused on the stereoselective hydrogenation of the methyl-substituted unsaturated esters **10a**,**b**. In the case of the *E* isomer, the stereoselectivity is in favor of the undesired *syn* stereoisomer as reported, presumably due to 1,2-allylic strain.[17] Although the *Z* isomer might have been expected to favor the desired *anti* isomer if 1,3-allylic strain were to dominate, we obtained a disappointing 1:1 mixture with palladium on carbon as the catalyst. Although better



Scheme 3. Stereoselective hydrogenation.



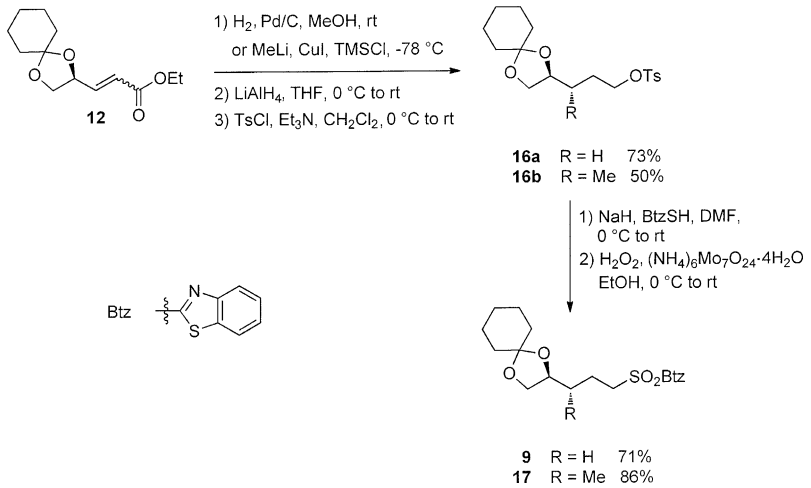
Scheme 4. Synthesis of lactone **8**. DHP = 3,4-dihydro-2*H*-pyran, DMAP = 4-dimethylaminopyridine, HMDS = hexamethyldisilazide, PCC = pyridinium chlorochromate, PPTS = pyridinium *para*-toluenesulfonate, THP = tetrahydropyran-2-yl, TMS = trimethylsilyl.

results might be obtained with other catalysts,[18] we did not pursue this approach further.

Therefore, the methyl group was introduced by using the approach of Hanessian and Sumi.[19] Ester **12**, obtained from dicyclohexylidene D-mannitol, was deprotected and then differentially protected with a silyl and tetrahydropyranyl group to yield **13** in 77% overall yield. The addition of lithium dimethylcuprate to unsaturated ester **13** in the presence of trimethylsilyl chloride provided the desired isomer **14**. Consistent with the report of Hanessian and Sumi,[19] a full six equivalents of the cuprate are required because a smaller excess proved ineffective, even under equal reaction concentrations. Homologation of the ester by reduction with diisobutylaluminum hydride (DIBAL-H) and the Wittig reaction furnished enol ether **15**. Finally, acidic hydrolysis and oxidation of the lactol intermediate provided the lactone **8**.

The synthesis of the benzothiazolyl sulfone reagents are shown in Scheme 5.[16] Ester **12** was hydrogenated, reduced, and converted into the tosylate derivative **16**, which provided the sulfone species upon substitution with 2-mercaptobenzothiazole and oxidation of the sulfur atom with ammonium molybdate and hydrogen peroxide.[20] The corresponding methyl-substituted sulfone **17** was obtained by cuprate addition rather than hydrogenation in the first step, followed by the same reaction sequence.

The key step of the construction of the spiroketal fragment is the coupling of lactone **8** and the Julia–Kocienski reagent **9** followed by *ipso* substitution and spontaneous reductive elimination in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) to afford the intermediate enol ether **19**. The pseudosymmetry of the spiroketal fragment also allows an alternative combination (e.g., **20**), in which the methyl group is attached to the sulfone **17** rather than the lactone. The extension of the enol ether synthesis to enol ethers from non-carbohydrate lactones required the development of newly optimized conditions (Table 1) because significant differences were observed in the reactivity of the lactone, the stability of the resulting enol ether, and the spirocyclization step. Indeed, under the conditions reported for sugar lactones,[16] the reaction gave disappointing yields in the region of 20% (Table 1, entries 1 and 2). The use of an alternative combination (e.g., sulfone **17** and lactone **18**),[21] did not improve the reaction (Table 1, entry 3), so subsequent studies focused on lactone **8** and sulfone **9**. As in the case of carbohydrates, the use of KHMDS (Table 1, entry 4) gave little or no *exo*-glycal and neither did the use of other lithium salts such as $LiClO_4$ (Table 1, entries 5 and 6). The use of a diethyl ether/THF solvent mixture (Table 1, entry 6) provided no improvement over THF alone. As the aliphatic lactone appeared to be significantly less reactive than the carbohydrate lactones, the addition of boron trifluoride etherate improved the degree of conversion considerably; although the resulting enol ether species was isolated almost exclusively in this case in its hydrated form **21** (Table 1, entry 7). Indeed, in addition to the lower reactivity of the lactone, the enol ether itself was considerably more sensitive than its carbohydrate counterparts. Therefore, we tried to isolate the enol ether moiety by chromatography on basic alumina (Table 1, entry 8) or to cyclize the crude enol ether moiety directly under thermodynamic conditions (Table 1, entry 9). This last option provided the differentially



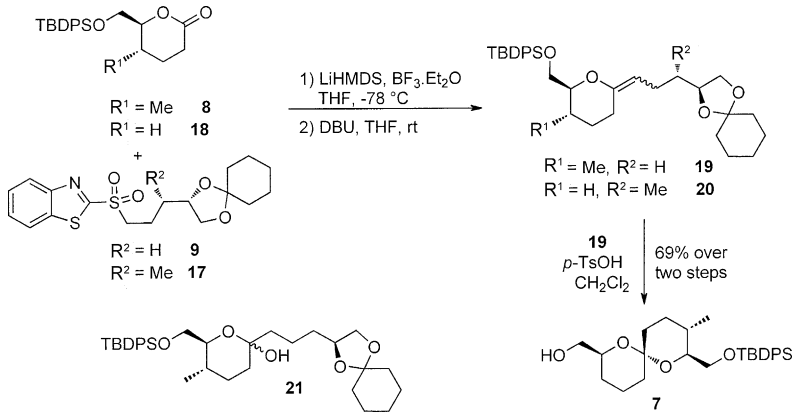
Scheme 5. Synthesis of the benzothiazolyl sulfones. Ts = tosyl.

Table 1. Optimization of the synthesis of the enol ether **19** and spiroketal **7**.

Entry	Reaction conditions[a]	Isolation	Product	Yield [%]
1	Barbier, LiHMDS (2.4 equiv), THF, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	20
2	Barbier, LiHMDS (1.2 equiv), LiCl, THF, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	23
3	Barbier, LiHMDS (1.2 equiv), LiCl, THF, −78 °C	chromatography on silica gel (5% Et_3N)	**20**	23
4	Barbier, KHMDS (2.4 equiv), THF, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	<5
5	premetallation, LiHMDS (1.5 equiv), $LiClO_4$ (2 equiv), THF, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	<5
6	Barbier, LiHMDS (2.0 equiv), LiCl (2 equiv), THF/Et_2O, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	14
7	premetallation, LiHMDS (2.0 equiv), BF_3 etherate (2.0 equiv), THF, −78 °C	chromatography on silica gel (5% Et_3N)	**19**	5
			21	70
8	premetallation, LiHMDS (2.0 equiv), BF_3 etherate (1.0 equiv), THF, −78 °C	chromatography on basic alumina	**19**	14
9	Barbier, LiHMDS (2.0 equiv), BF_3 etherate (1.0 equiv), THF, −78 °C	*p*-TSA, MeOH	**7**	43
10	Barbier, LiHMDS (2.0 equiv), BF_3 etherate (1.0 equiv), THF, −100 °C	*p*-TSA, MeOH	**7**	40
11	Barbier, LiHMDS (2.0 equiv), BF_3 etherate (1.0 equiv), THF, −50 °C	*p*-TSA, MeOH	**7**	40
12	Barbier, LiHMDS (2.0 equiv), BF_3 etherate (1.0 equiv), THF, −78 °C	*p*-TSA, CH_2Cl_2	**7**	69

[a] Conditions for the first step. The reaction was quenched at low temperature with acetic acid and, after aqueous workup, the crude product was treated with DBU (2 equiv) in THF at room temperature. *p*-TSA = *para*-toluenesulfonic acid.

protected spiroketal **7** in somewhat better yield (43%). Under the same cyclization conditions, changing the temperature of the enol ether-forming reaction did not improve the results (Table 1, entries 10 and 11). However, we noted that the spirocyclization step, which was systematically quantitative in the carbohydrate series under these conditions, was hampered in this case by sluggish methanolysis of the cyclohexylidene group, thus leading to the methyl ketal of **21**. Forcing conditions led to the loss of the silyl protective group and thus to a spiroketal product that cannot be desymmetrized efficiently. Finally, cyclization with *p*-TSA in dichloromethane instead of methanol prevented hydrolysis of the silyl group and provided the differentially protected spiroketal **7** as a single stereoisomer in 69% yield over the two steps on a gram scale (Scheme 6).



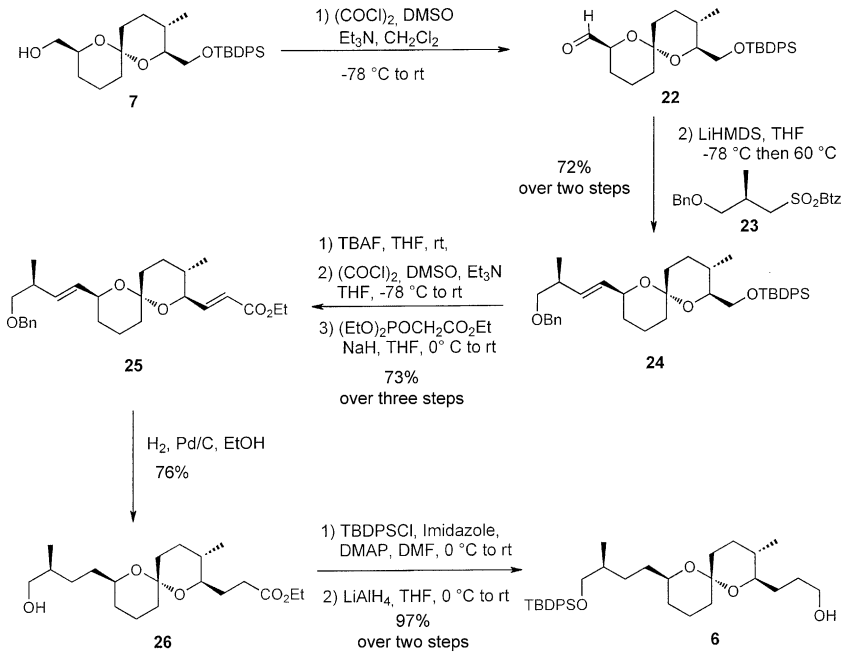
Scheme 6. Spiroketal synthesis. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Further elaboration of spiroketal **7** is shown in Scheme 7. Swern oxidation provided aldehyde **22**, which was subjected to a modified Julia reaction[22] with sulfone **23**[9] to provide alkene **24**. This traditional version of the reaction gave surprisingly poor results under the reported experimental conditions, and the reaction therefore also required optimization (see the Supporting Information). Finally, the best results were obtained by performing the coupling step at −78 °C and then heating directly to 60 °C, instead of warming progressively. The resulting olefin **24** was thus obtained in 85% yield.

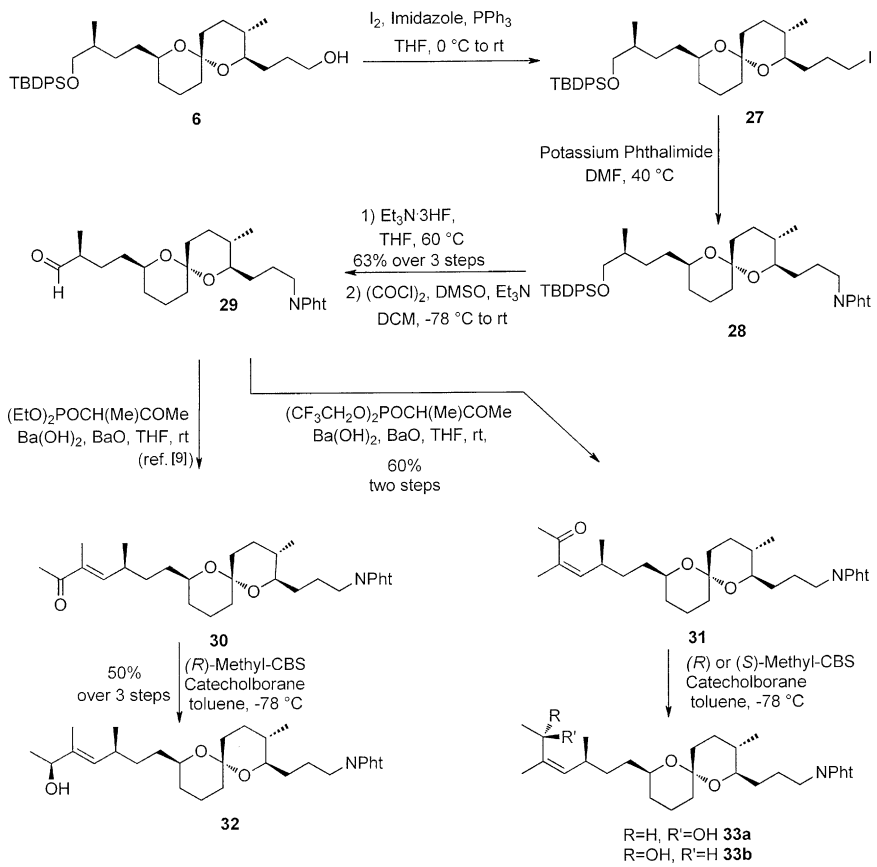
Compound **24** was deprotected to give the corresponding spiroketal alcohol, which was oxidized to undergo a Horner–Emmons olefination with triethylphosphonoacetate, thus providing the α,β-unsaturated ester **25** in good yield. The treatment of **25** with palladium on charcoal under a hydrogen atmosphere provided alcohol **26**, which was subsequently protected as the silyl ether by using TBDPSCl and submitted to reduction of the ester function to obtain **6**, which was previously described by Crimmins and DeBaillie.[9] The structure was confirmed by comparison of the NMR spectroscopic data.

The synthesis of the natural C19–C40 spiroketal fragment was completed from intermediate **6**[9] by generally following the route developed by Crimmin and DeBaillie (Scheme 8). A phthalimide group was introduced in two steps via the corresponding iodo derivative **27**. Deprotection of the *tert*-butyldiphenylsilyl protective group by using the $3HF{\cdot}Et_3N$ complex followed by oxidation of the alcohol under Swern conditions provided aldehyde **29**. At this stage, we decided to prepare the natural bistramide A fragment **32**, but also the C36(*Z*) analogues with both configurations at C39 via the *Z* enone **31**.

The *E* enone of the bistramide spiroketal system **30** (Scheme 8) was prepared from **29**, as described previously using $(EtO)_2POCH(CH_3)COCH_3$ and $Ba(OH)_2$ in THF/water,[9] although the mixture of BaO and $Ba(OH)_2$ was more robust and gave a slightly better selectivity in our



Scheme 7. Functionalization of the spiroketal **7**. DMSO = dimethyl sulfoxide, TBAF = tetra-*n*-butylammonium fluoride, Bn = benzyl.



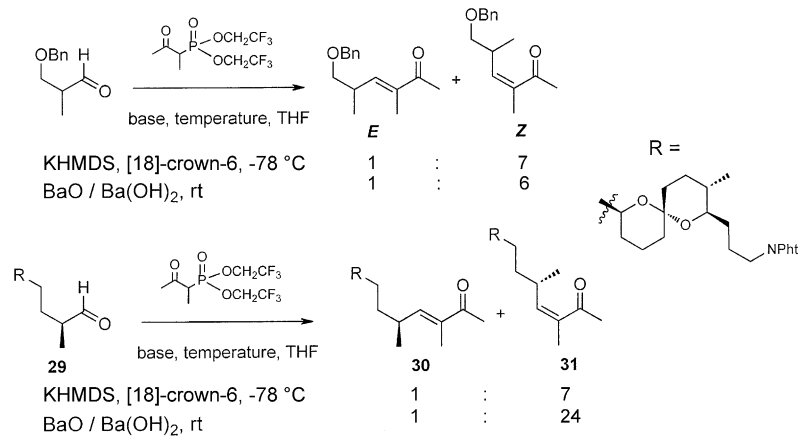
Scheme 8. Synthesis of the C19–C40 fragments. CBS = Corey–Bakshi–Shibata catalyst.

hands (see the Supporting Information). The Wittig–Horner phosphorane ($Ph_3P{=}C(CH_3)COCH_3$, toluene, reflux) also led to the *E* isomer **30** with good selectivity, but surprisingly the reaction was accompanied by racemization of the adjacent C35 methyl stereocenter, even at low conversion.

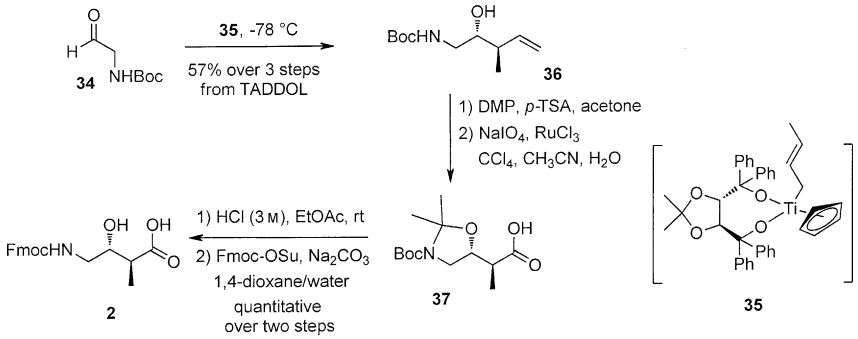
The *Z* isomer **31** was obtained selectively through a Still–Gennari reaction with $(CF_3CH_2O)_2POCH(CH_3)$-$COCH_3$.[23] Interestingly, the use of BaO/$Ba(OH)_2$ in THF gave significantly higher *Z* selectivities (24:1) and better yields in the authentic system than with the use of KHMDS, [18]-crown-6, and THF or *t*BuOK alone, which gave *Z* selectivities of 7:1 and 4:1, respectively (Scheme 9). Surprisingly, we did not observe the same trend in the stereoselectivity of the formation of the enone when using 3-benzyloxy-2-methylpropanal as a model (see the Supporting Information). A significant β-alkoxy effect is thus observed with barium, but not potassium bases, thereby reflecting differences either in the relative diastereoselectivity or in the degree of reversibility of the initial step.[24]

The *E* and *Z* unsaturated ketones **30** and **31** were reduced under Corey-Bakshi-Shibata conditions to give the C19–C40 spiroketal fragment with the natural 36(*E*),39(*S*) configuration in **32** and the unnatural configurations 36(*Z*),39(*R*) and 36(*Z*),39(*S*) in isomers **33a**,**b**, respectively. The 36(*E*),39(*R*) isomer has been reported previously by Panek and co-workers as part of an impressive library of stereoisomers and therefore was not prepared in this study.[10a]

Amino acid fragment: The amino acid fragment **2** was prepared in five steps from the *N*-Boc-protected aminoacetaldehyde by using a variation of the asymmetric crotonylation route developed by Kozmin et al.[8] by using TADDOL instead as a chiral ligand



Scheme 9. β-Alkoxy effect in the barium-catalyzed Still–Gennari reaction.



Scheme 10. Synthesis of the C14–C18 fragment. DMP = 2,2-dimethoxypropane, Fmoc-OSu = N-(9-fluorenylmethoxycarbonyloxy)succinimide, TADDOL = (4*S*,5*S*)-2,2-dimethyl-α,α,α′,α′-tetraphenyldioxolane-4,5-dimethanol.

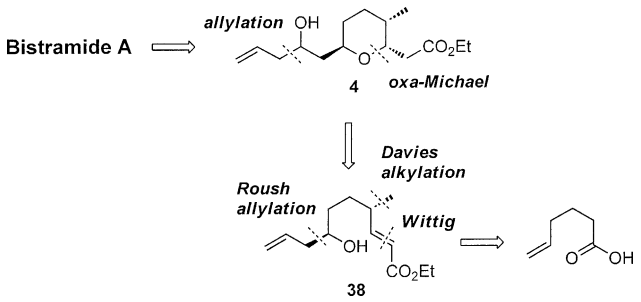
over two steps. A diastereoselective allylation of **40** was first attempted under the conditions developed by Brown and Jadhav with (−)-*B*-allyldiisopinocamphenylborane.[28] A high diastereoselectivity was observed, but homoallylic alcohol **41** could be isolated in only 46% yield. Therefore, we performed the allylation reaction with the Roush chiral diisopropyl tartrate allylboronate.[29] In this case, alcohol **41** was obtained in 91% yield as a 4:1 diastereomeric mixture, from which the major isomer was isolated in 73% yield.

The target Michael acceptor **38** was prepared from **41** as follows: The secondary alcohol was protected as a *tert*-butyldimethylsilyl (TBS) ether, and the primary alcohol was deprotected with DDQ and oxidized to the corresponding aldehyde, which was engaged in a Wittig reaction to provide the geometric *E* isomer **42** in 70% yield over four steps (Scheme 13). Removal of the TBS protecting group under acidic conditions furnished **38** in quantitative yield.

(Scheme 10). The first step consists of the formation of chiral amino alcohol **36** by asymmetric crotyltitanation.[25,26] The amino alcohol was protected as a 1,3-oxazolidine and cleavage of the double bond with ruthenium(III) chloride/sodium periodate provided amino acid **37**, which was fully deprotected under acidic conditions. Finally, the introduction of an Fmoc group gave the enantiomerically pure *N*-protected amino acid **2**.

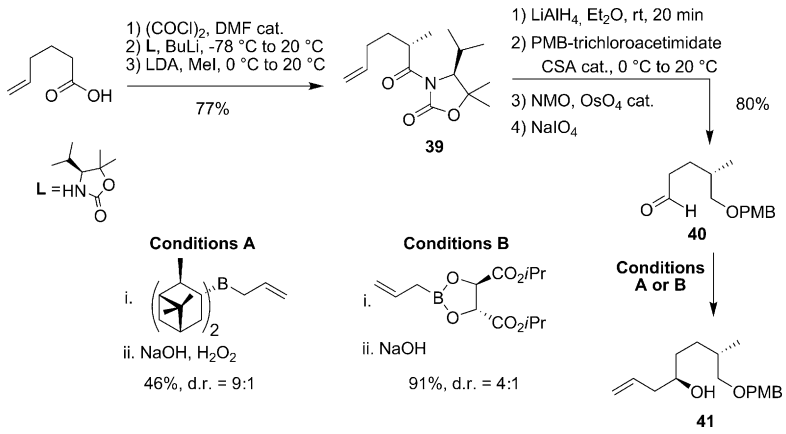
Synthesis of the tetrahydropyran fragment: The C1–C13 fragment of bistramides can be obtained from the core *trans*-2,6-disubstituted tetrahydropyran **4**, which can be prepared by a kinetically controlled intramolecular oxa-Michael cyclization of hydroxyester **38** (Scheme 11).[15] The oxa-Michael precursor was synthesized in a few steps from 5-hexenoic acid by using classical asymmetric methodologies.

By starting from 5-hexenoic acid, an asymmetric alkylation reaction that employed the Davies Superquat auxiliary[27] led to the methylated product **39**, which could be isolated as a single diastereoisomer in 86% yield after recrystallization (Scheme 12). After reductive removal of the chiral auxiliary, the resulting alcohol was protected as a *para*-methoxybenzyl ether, and aldehyde **40** was obtained by oxidative cleavage of the terminal alkene in 94% yield

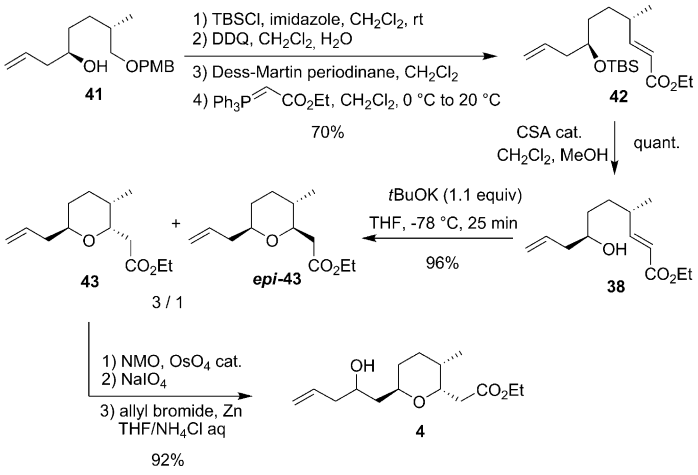


Scheme 11. Retrosynthetic analysis of the C1–C13 fragment.

The key step of the C1–C13 fragment synthesis is the intramolecular oxa-Michael cyclization reaction of **38**. Previous studies on nor-methyl derivatives have shown that the conjugate addition could lead to the major formation of the thermodynamically less-favoured 2,6-*trans*-disubstituted tetrahydropyran, when the reaction was performed under kinetic conditions.[30] In our case, the presence of a methyl substituent could be an issue, and indeed when the reaction was first attempted at −70 °C for 20 minutes with one equiv-



Scheme 12. Synthesis of chiral homoallylic alcohol intermediate **41**. CSA = camphorsulfonic acid, LDA = lithium diisopropylamide, PMB = *para*-methoxybenzyl, NMO = *N*-methylmorpholine-*N*-oxide.



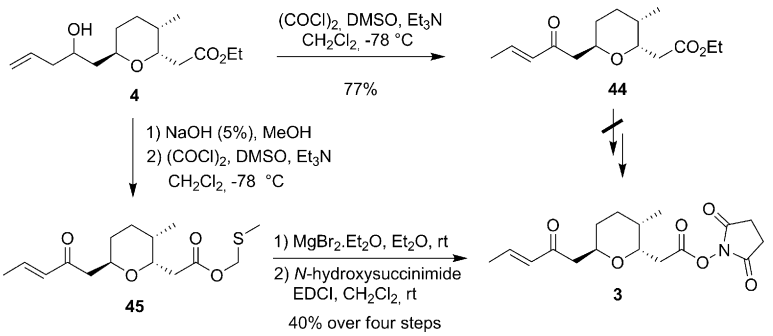
Scheme 13. Synthesis of key intermediate **4**. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TES = triethylsilyl.

alent of *t*BuOK, only the 2,6-*cis*-disubstituted tetrahydropyran epi-**43** (Scheme 13) was obtained. When the reaction was repeated at temperatures between −100 and −85 °C, the cyclization did not occur even after prolonged reaction times. Finally, the desired kinetic Michael adduct **43** could be obtained as the major isomer when the reaction was performed at −80/−78 °C with 1.1 equivalents of *t*BuOK in THF and was isolated in 72% yield. The C1–C13 fragment precursor **4** was prepared in three steps by oxidative cleavage of the terminal alkene and allylation of the resulting aldehyde under Barbier conditions in 92% overall yield.

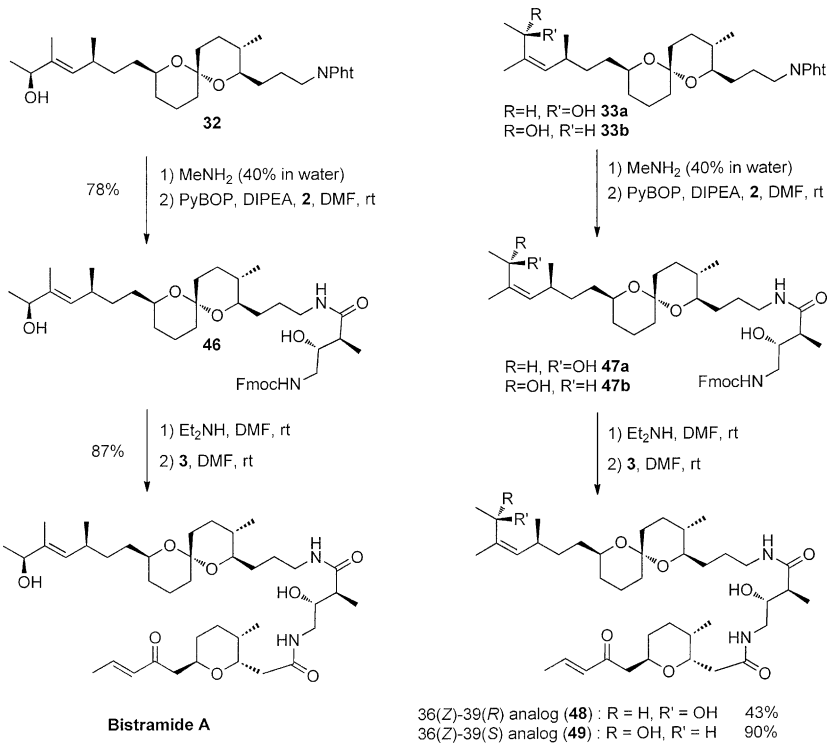
The ethyl ester of the natural tetrahydropyran fragment was next prepared under Swern conditions with an excess of triethylamine base to effectuate the oxidation/isomerization in one pot (Scheme 14). Unfortunately, deprotection of ester **44** was unsuccessful under a variety of conditions due to the instability of the α-(tetrahydropyranyl)enone system. The activated tetrahydropyran acid **3** was therefore prepared from the intermediate **4** in four steps. Saponification of the ester gave the corresponding carboxylic acid, which was oxidized under Swern conditions. At this stage, the α,β-unsaturated ketone moiety was successfully put in place, yet a Pummerer rearrangement of the Swern intermediate also led to the formation of the (methylthio)methyl (MTM) ester of the acid functional group to provide **45** in good yield.[31] The MTM ester facilitated the purification of the product and was subsequently deprotected with magnesium bromide, followed by EDCI-mediated coupling of *N*-hydroxysuccinimide to provide the activated ester **3** in 40% yield over four steps.

Synthesis of bistramide A and its *Z* isomers: To complete the synthesis of bistramide A (Scheme 15), cleavage of the phthalimide protecting group of **32** followed by benzotriazol-1-yl-oxytripyrrolidinophosphonium (PyBOP)-mediated condensation with acid **2** led us to the desired amide **46** in 78% yield. Removal of the Fmoc group and subsequent reaction of the amine with activated ester **3** provided bistramide A in excellent yield (87%).[8,9] The 36(*Z*),39(*R*) and 36(*Z*),39(*S*) analogues **48** and **49** (Scheme 14) were obtained by the same route from **47a**,**b**, respectively. The total synthesis of bistramide A and its 36(*Z*),39(*R*) and 36(*Z*),39(*S*) isomers was thus completed.

Preliminary biological studies of bistramide A and its *Z* isomers: We performed a preliminary biological evaluation of 36(*E*),39(*S*) bistramide A and the isomers 36(*Z*),39(*R*) **48**,and 36(*Z*),39(*S*) **49**. The overall antiproliferative effect was evaluated with the leukaemic cell line HL-



Scheme 14. Completion of the C1–C13 fragment. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.



Scheme 15. Completion of the synthesis of bistramide A and the analogues 36(*Z*),39(*R*) **48** and 36(*Z*),39(*S*) **49**.

60, as used in previous studies,[5a] by employing a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) assay to evaluate the number of viable cells (Figure 2). The natural bistramide A

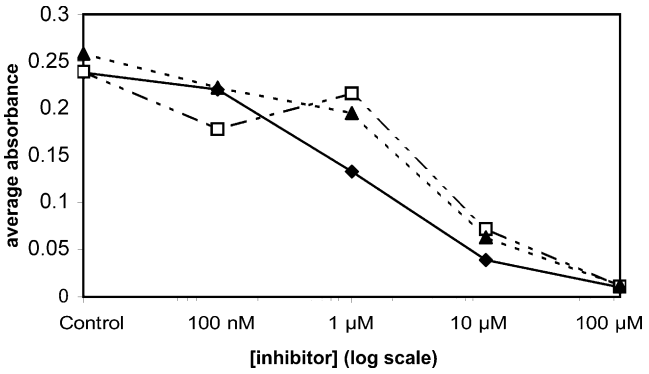


Figure 2. Evaluation of the antiproliferative effect in the leukaemic cell line HL-60 by using the MTS assay. ◆: bistramide A; □: 36(*Z*),39(*R*) isomer **48**; ▲: 36(*Z*),39(*S*) isomer **49**

isomer showed $IG_{50} = 1.6$ μM, which is generally in accord with that reported for this particular cell line with natural[33] and synthetic[6a] bistramide A. The *Z* isomers showed slightly lower potency, with IG_{50} values of 7.8 and 3.7 μM for the isomers 39(*R*) **48** and 39(*S*) **49**, respectively.

The observed growth inhibition could be due to a combination of factors, including inhibition of cell cycling (antimitotic effect), a proapoptotic effect, and/or the induction of differentiation. A more detailed analysis by flow cytometry was therefore undertaken to gain further insight into the relative contributions of these effects on the observed growth inhibition (Figure 3). The results suggest that although the overall antiproliferative potencies are similar, there are significant differences as to the origin of this activity for the three compounds. The results for bistramide A show accumulation in G2M that starts at a concentration of 1 μM, which is similar to that reported by Panek and co-workers[10] for bistramide A-treated renal carcinoma UO-31 cells. Previous reports for HL-60 cells treated with naturally derived bistramide A have also reported accumulation of cells in G2M, although at much lower concentrations (50 nM).[5a] These data are at odds with those reported by Verbist and co-workers, who saw irreversible accumulation in the G1 stage.[3b] This disparity most likely reflects a difference between the cell lines used, in that the nonsmall cell lung cancer (NSCLC) line used in the study of Verbist and coworkers is an adherent cell line, whereas HL-60 is not and bistramide A has actions upon the cytoskeleton that could influence the biological outcome. It may also reflect different sensitivities to differentiation and inhibition of cytokinesis between the two cell lines. Strong accumulation of 4*n* polyploid cells is seen at concentrations of 10 μM, which has been confirmed by differential staining and light microscopy (data not shown), and a corresponding decrease in G0/G1 cells, which is consistent with the inhibition of cytokinesis as reported by Kozmin and co-workers.[6] However, at higher concentrations (i.e., 100 μM), an accumulation of cells is observed in the sub-G0 region, which is consistent with the induction of apoptosis. The results for the isomer 36(*Z*),39(*S*) **49** show a similar strong accumulation in the 4*n* polyploid cells, with no corresponding accumulation in the sub-G0 region, and thus little apparent corresponding contribution of the proapoptotic activity. Interestingly, the isomer 36(*Z*),39(*R*) **48**, which has similar overall antiproliferative activity to **49**, shows a marked proapoptotic effect as evidenced by a strong accumulation in the sub-G0 region at 100 μM and thus has a biological profile more similar to bistramide A.

Additional studies were performed to evaluate the proapoptotic effect of the three compounds more accurately (Figure 4). Consistent with the above results, annexin V staining experiments showed a mild apoptotic effect for bis-

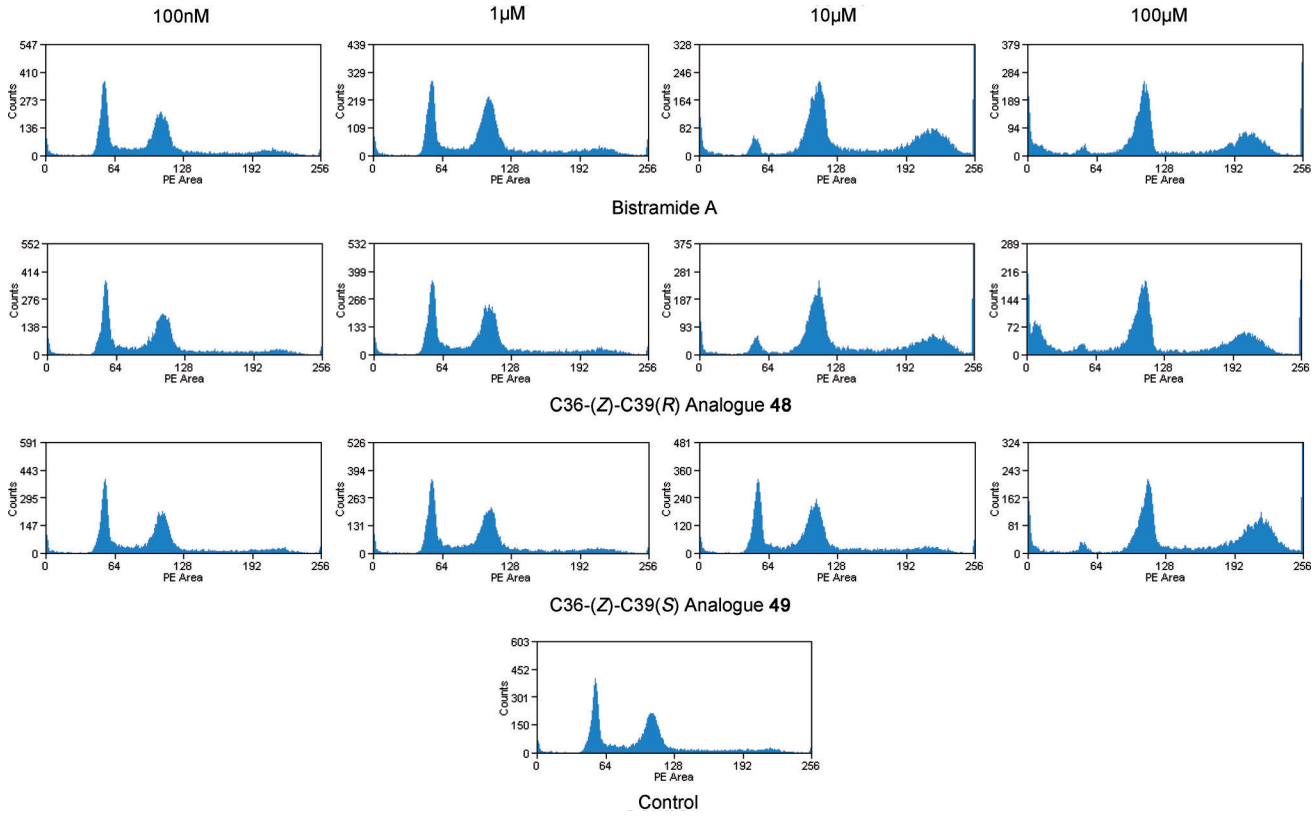


Figure 3. Cell-cycle analysis for HL-60 cells treated with synthetic bistramide A and its Z isomers **48** and **49**. The HL-60 cells were incubated with these compounds at the concentrations shown, stained with propidium iodide, and analyzed by flow cytometry to assess the proportion of cells at each stage of the cell cycle (G1, S, G2M) or in the sub-G0 (apoptotic) phase.

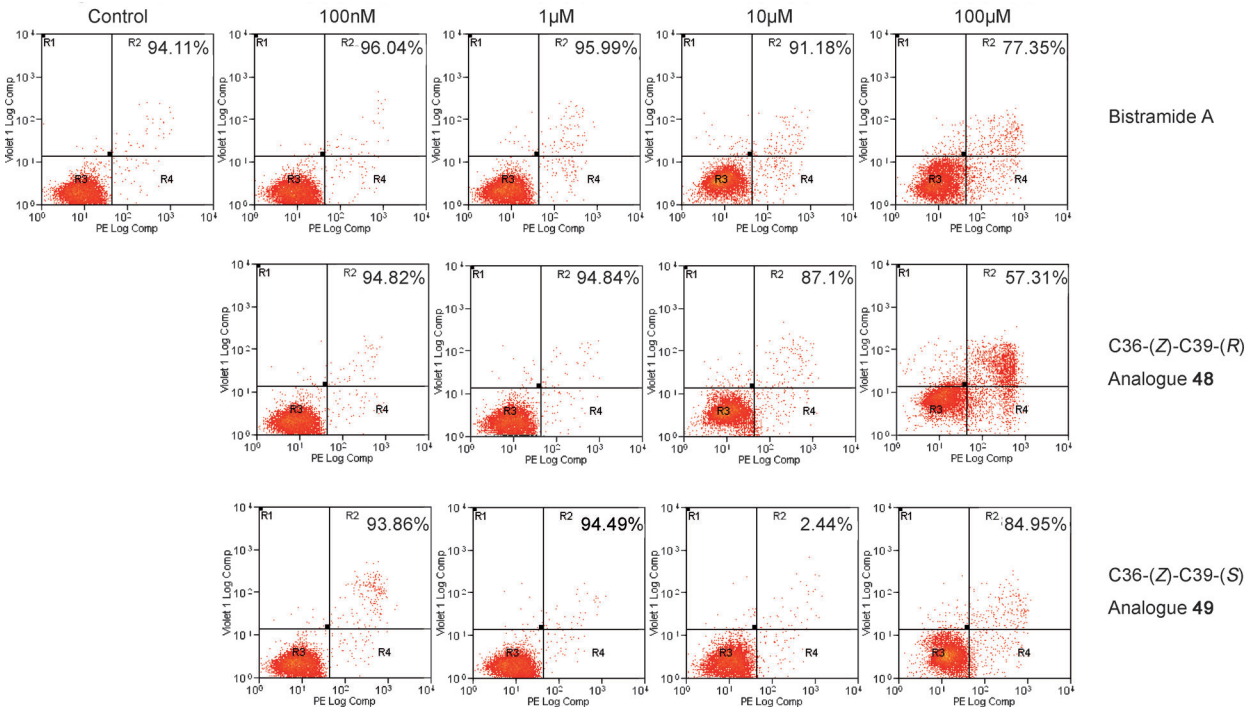


Figure 4. Annexin V/sytox analysis of apoptosis on synthetic bistramide A and its Z isomers **48** and **49**. The HL-60 cells were treated with these compounds at the concentrations shown and stained with annexin V/phycoerythrin (PE) and sytox green and analyzed by flow cytometry. The cells that were positive for annexin V and negative for sytox green were taken as early apoptotic cells, and double positive cells were in late apoptosis. The percentage of viable cells is shown in the top right-hand corner of each flow plot.

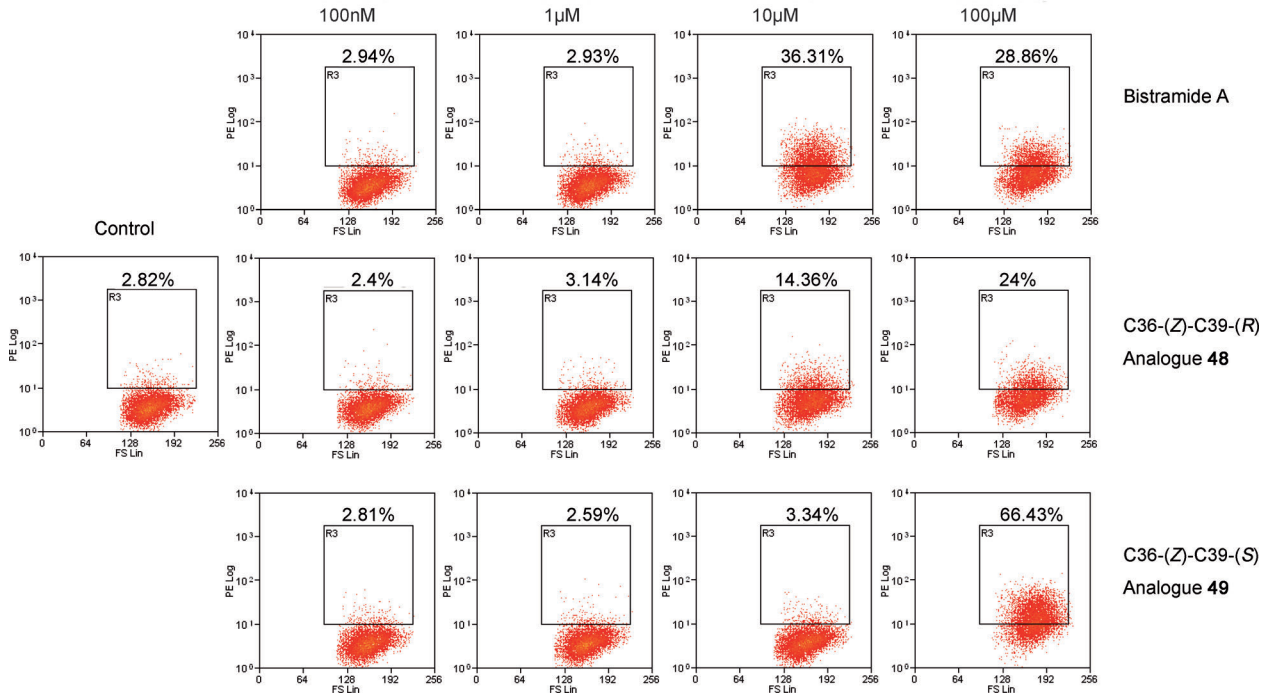


Figure 5. CD11b expression in cells treated with synthetic bistramide A and isomers **48** and **49**. The HL-60 cells were treated with these compounds at the concentrations shown and stained with an antibody to CD11b. The immunostaining was analyzed by flow cytometry. The percentage of CD11b positive cells is shown in each flow plot.

tramide A (77% viable cells at 100 μM), whereas little or no apoptosis was induced by the isomer 36(*Z*),39(*S*) **49** at the highest concentration tested (85% viable cells at 100 μM). On the other hand, a definite proapoptotic effect was seen for the isomer 36(*Z*),39(*R*) **48** (57% viable cells at 100 μM), with cells seen in both early and late apoptosis.

Finally, CD11b immunostaining studies were performed to evaluate CD11b expression as an early marker of HL-60 cell differentiation (Figure 5). Bistramide A showed a mild induction of CD11b expression at lower concentrations (36% of cells were positive for CD11b at 10 μM), which is consistent with a mild prodifferentiation activity reported previously for the natural product.[5a] Interestingly, the isomer 36(*Z*),39(*S*) **49**, which showed little or no proapoptotic effect, induced a marked expression of CD11b (66% CD11b were positive cells at 100 μM). Conversely, the isomer 36(*Z*),39(*R*) **48** showed only a weak induction of CD11b expression (24% CD11b were positive cells at 100 μM). CD11b expression should not be overinterpreted, and additional experiments are necessary to correlate these results to the potential contribution of cell differentiation to the antiproliferative effects of the compounds.

Conclusion

We have reported the total synthesis of bistramide A and its 36(*Z*),39(*R*) and 36(*Z*),39(*S*) isomers. Although our synthesis is slightly more linear than previous ones (29 steps, 2% overall yield), it is convenient and robust. The use of new methodologies, for example, the synthesis of enol ethers from lactones, provides the impetus for new insight and can provide access to alternative analogues of the spiroketal moiety. In addition, we have shown that the three synthetic stereoisomers have similar overall antiproliferative activities, yet each shows a distinct distribution of antimitotic, proapoptotic, and prodifferentiation effects. These results confirm the existence of all three reported effects and confirm that accumulation of 4*n* polyploid cells represents the primary mode of action of the natural product. Our results also suggest that the antimitotic and proapoptotic activities are independent effects of the natural product, as opposed to common effects of actin binding, because the activity profiles would be parallel in the latter case. Additional analogues will be needed to determine whether induction of differentiation is dependent or independent of actin binding. As the effects of bistramide A and isomers **48** and **49** were seen at high concentrations in HL-60 cells, it is not possible to state categorically whether they could be achieved in vivo, and, if so, whether different profiles (polyploidy, apoptosis, and differentiation) would be observable with these compounds in vivo. A more detailed report of the biological studies on these and related compounds will be reported in due course.

Experimental Section

Experimental procedures are provided only for selected key steps. The general experimental procedures; detailed descriptions of biological

assays; synthesis of compounds **2**, **6**, **8**, **9**, **10a**, **10b**, **11a**, **11b**, **13**–**15**, **22**, **25**–**30**, **32**, **37**, **39**, **40**, **46**, and other synthetic intermediates; and the details of the optimization of various olefination reactions are provided in the Supporting Information.

{(2*S*,6*S*,8*S*,9*S*)-8-[(*tert*-Butyldiphenylsilyloxy)methyl]-9-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}methanol (7): Boron trifluoride etherate (400 μL, 3.16 mmol, 1 equiv) and benzothiazolyl sulfone **9** (1.37 g, 3.8 mmol, 1.2 equiv) were added to a solution of **8** (1.21 g, 3.2 mmol, 1 equiv; see the Supporting Information) in THF (5.3 mL). The mixture was cooled to −78 °C and lithium hexamethyldisilazide (1 M in THF, 6.32 mL, 6.3 mmol, 2 equiv) was added dropwise. The reaction mixture was stirred at −78 °C for 30 min, hydrolyzed at −78 °C with acetic acid (542 μL, 9.48 mmol, 3 equiv), stirred at room temperature for 15 min, and extracted with ethyl acetate. The organic layers were combined, washed with brine, and dried over anhydrous sodium sulfate. After filtration and evaporation in vacuo, the residue was dissolved in THF (30 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (943 μL, 3.32 mmol, 2 equiv) was added to the reaction mixture over 10 min. The reaction mixture was stirred at room temperature for 30 min, quenched by the addition of aqueous NH_4Cl solution, and diluted and extracted with ethyl acetate. The organic layers were combined, washed with brine, and dried over anhydrous sodium sulfate. Filtration and evaporation in vacuo provided a mixture of diastereomeric enol ethers **19** as a pale yellow oil, which was used without further purification. *p*-TSA (300 mg, 1.6 mmol) was added to the mixture of enol ethers **19** in dichloromethane (45 mL). The reaction mixture was stirred at room temperature for 25 min, hydrolyzed by the addition of aqueous $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous magnesium sulfate, and filtered. The solvent was evaporated in vacuo and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 85:15) to afford spiroketal **7** as a colorless oil (1.03 g, 2.21 mmol, 69% over 2 steps). $[\alpha]_D^{25} = +26.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, tetramethylsilane (TMS)): $\delta = 0.84$ (d, $J = 6.2$ Hz, 3H), 1.05 (s, 9H), 1.22–2.05 (m, 12H), 3.33 (ddd, $J = 3.1$, 4.4, 9.4 Hz, 1H), 3.48 (dd, $J = 6.9$, 11.2 Hz, 1H), 3.58 (dd, $J = 3.4$, 11.3 Hz, 1H), 3.73–3.84 (m, 3H), 7.35–7.42 (m, 6H), 7.73–7.78 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 135.8$, 135.7, 134.1, 134.0, 129.6, 127.7, 127.6, 96.0, 76.1, 69.5, 66.3, 65.2, 35.7, 35.4, 30.7, 28.0, 26.8, 26.6, 19.4, 18.5, 17.7 ppm; IR (film): $\tilde{\nu} = 2931$, 2858, 1456, 1428, 1382, 1225, 1105, 984, 738 cm^{-1}; MS (ESI): *m/z*: 491 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{28}H_{40}O_4SiNa$: 491.2594 $[M+Na]^+$; found: 491.2593.

({(2*S*,3*S*,6*S*,8*S*)-8-[(*S*,*E* or *Z*)-4-(Benzyloxy)-3-methylbut-1-enyl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}methoxy)(*tert*-butyl)diphenylsilane (24): Lithium hexamethyldisilazide (1 M in THF, 1.84 mL, 1.84 mmol, 1.2 equiv) was added dropwise to a solution of sulfone **23** (665 mg, 1.84 mmol, 1.2 equiv) in THF (4 mL) at −78 °C for 30 min. A solution of **22** (1.53 mmol, 1 equiv; see the Supporting Information) in THF (4 mL) was slowly added to the reaction mixture, which was heated directly to 60 °C for 1.5 h. The reaction was quenched by pouring the mixture into an aqueous solution of ammonium chloride, which was then extracted with ethyl acetate. The organic layers were combined, washed with brine, and dried over anhydrous sodium sulfate. After filtration and evaporation in vacuo, the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 97:3) to afford a diastereomeric mixture (*E*/*Z* = 7:3) of **24** as a colorless oil (584 mg, 0.95 mmol, 62% over 2 steps). Analytical samples of the two isomers were obtained by repeated chromatography on silica gel.

({(2*S*,3*S*,6*S*,8*S*)-8-[(*S*,*E*)-4-(Benzyloxy)-3-methylbut-1-enyl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}methoxy)(*tert*-butyl)diphenylsilane ((*E*)-24): $[\alpha]_D^{25} = +9.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.81$ (d, $J = 6.2$ Hz, 3H), 1.03–1.05 (m, 12H), 1.20–1.68 (m, 10H), 1.98–2.08 (m, 1H), 2.45–2.55 (m, 1H), 3.26 (dd, $J = 7.5$, 9.1 Hz, 1H), 3.32–3.36 (m, 1H), 3.39 (dd, $J = 6.0$, 9.1 Hz, 1H), 3.74 (dd, $J = 5.5$, 10.9 Hz, 1H), 3.79 (dd, $J = 2.5$, 10.9 Hz, 1H), 4.17 (ddd, $J = 2.1$, 4.9, 11.4 Hz, 1H), 4.50 (d, $J = 2.9$ Hz, 2H), 5.50 (dd, $J = 5.2$, 15.8 Hz, 1H), 5.56 (dd, $J = 5.7$, 15.8 Hz, 1H), 7.32–7.43 (m, 11H), 7.74–7.76 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 135.9$, 135.8, 134.2, 134.1, 133.4, 131.4, 129.6, 128.5, 127.7, 127.7, 127.6, 96.2, 76.1, 75.4, 73.1, 69.6, 65.4, 36.6, 36.0, 35.2, 31.2, 31.0, 28.0, 26.9, 19.5, 19.1, 17.7, 17.0 ppm; IR (film): $\tilde{\nu} = 2930$, 2857, 1455, 1428, 1380, 1360, 1272, 1224, 1111, 983, 822, 738 cm^{-1}; MS (ESI): *m/z*: 613 $[M+H]^+$, 635.4 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{39}H_{52}O_4SiNa$: 635.3531 $[M+Na]^+$; found: 635.3531.

({(2*S*,3*S*,6*S*,8*S*)-8-[(*S*,*Z*)-4-(Benzyloxy)-3-methylbut-1-enyl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}methoxy)(*tert*-butyl)diphenylsilane ((*Z*)-24): $[\alpha]_D^{25} = +43.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.86$ (d, $J = 5.9$ Hz, 3H), 0.99 (d, $J = 6.7$ Hz, 3H), 1.05 (s, 9H), 1.22–1.69 (m, 10H), 1.99–2.10 (m, 1H), 2.79–2.90 (m, 1H), 3.22 (d, 6.5 Hz, 2H), 3.40 (ddd, $J = 2.1$, 5.0, 8.8 Hz, 1H), 3.74 (dd, $J = 5.5$, 10.9 Hz, 1H), 3.83 (dd, $J = 2.2$, 10.9 Hz, 1H), 4.41 (s, 2H), 4.48 (ddd, $J = 1.8$, 8.1, 10.6 Hz, 1H), 5.33 (pdd, $J = 10.3$, 11.1 Hz, 1H), 5.42 (dd, $J = 8.2$, 11.1 Hz, 1H), 7.23–7.28 (m 5H), 7.25–7.40 (m, 6H), 7.69–7.79 ppm (m, 4H).

2-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(*S*,*Z*)-3,5-Dimethyl-6-oxohept-4-en-1-yl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)isoindoline-1,3-dione (31): DMSO (10 μL, 0.11 mmol, 4 equiv) in dichloromethane (0.1 mL) was added to a solution of oxalyl chloride (30 μL, 0.57 mmol, 2 equiv) in dichloromethane (0.2 mL) at −78 °C over 15 min and then stirred for an additional 15 min. 2-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(*S*)-4-Hydroxy-3-methylbutyl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)isoindoline-1,3-dione (11 mg, 0.03 mmol, 1 equiv; see the Supporting Information for the preparation from **26**) was dissolved in dichloromethane (0.6 mL) was added dropwise to the reaction mixture, which was stirred for 30 min. Dry triethylamine (25 μL, 0.17 mmol, 6 equiv) was added dropwise to the reaction mixture, which was allowed to warm to room temperature for 30 min, brine was added, and the aqueous phase was extracted with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The resulting oil was used without further purification. $Ba(OH)_2 \cdot 8H_2O$ (21 mg, 0.068 mmol, 2 equiv) and BaO (21 mg, 0.136 mmol, 4 equiv) were added to a solution of $MeCOCH(Me)P(O)(OCH_2CF_3)_2$ (16 mg, 0.041 mmol, 1.4 equiv) in THF at 0 °C under argon and the reaction mixture was stirred at 0 °C for 45 min. A solution of aldehyde **29** (0.034 mmol, 1 equiv) in THF was added slowly to the reaction mixture, which was left for 1 h at 0 °C and aged at room temperature for 1 h. The reaction was quenched with saturated sodium bicarbonate solution and diluted with ethyl acetate. The aqueous phase was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (toluene/diethyl ether 9:1) to afford the corresponding enone (*Z*)-**31** as a colorless oil (9.5 mg, 0.019 mmol, 60%). $[\alpha]_D^{25} = +12.4$ ($c = 0.2$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.82$ (d, $J = 6.3$ Hz, 3H), 0.98 (d, $J = 6.2$ Hz, 3H), 1.10–1.75 (m, 17H), 1.78–1.87 (m, 1H), 1.90 (br d, 3H), 1.97–2.03 (m, 1H), 2.22 (s, 3H), 2.74–2.78 (m, 1H), 3.15 (ddd, $J = 1.4$, 9.7, 9.7 Hz, 1H), 3.45–3.50 (m, 1H), 3.66–3.79 (m, 2H), 5.42 (dd, $J = 1.0$, 9.9 Hz, 1H), 7.71 (dd, $J = 3.0$, 5.4 Hz, 2H), 7.85 ppm (dd, $J = 3.0$, 5.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 204.3$, 168.6, 144.3, 135.1, 134.0, 132.4, 123.3, 95.6, 74.2, 69.3, 38.6, 36.2, 35.6, 35.2, 34.4, 33.8, 33.7, 31.5, 30.8, 30.3, 28.0, 25.3, 21.2, 21.1, 19.3, 18.2 ppm; IR (film): $\tilde{\nu} = 2925$, 2855, 1773, 1714, 1456, 1396, 885, 720 cm^{-1}; MS (ESI): *m/z*: 518 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{30}H_{41}NO_5Na$: 518.2877 $[M+Na]^+$; found: 518.2873.

2-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*R*,*Z*)-6-Hydroxy-3,5-dimethylhept-4-en-1-yl)-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)isoindoline-1,3-dione (33a): (*S*)-CBS (1.0 M in toluene, 60 μL, 0.060 mmol, 2 equiv) was added to a solution of ketone **31** (11 mg, 0.022 mmol, 1 equiv) in distilled toluene (0.4 mL). The reaction mixture was cooled under argon at −78 °C and stirred for 30 min. A solution of catecholborane (1.0 M in THF, 40 μL, 0.040 mmol, 2 equiv) was added to the reaction mixture, which was stirred for 12 h at −78 °C, quenched with methanol (0.1 mL), and allowed to warm to room temperature. The reaction mixture was diluted with diethyl ether and washed with 1 M NaOH saturated with $NaHCO_3$ until the aqueous washings were colorless. The aqueous phase was extracted three times with diethyl ether and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/diethyl ether 6:4) to

afford the corresponding reduced ketone (*Z*)-**33a** as a colorless oil (9.7 mg, 0.019 mmol, 85%). $[\alpha]_D^{25} = +23.3$ ($c = 0.18$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): $\delta = 0.81$ (d, $J = 6.5$ Hz, 3H), 0.94 (d, $J = 6.6$ Hz, 3H), 1.09–1.14 (m, 1H), 1.22 (d, $J = 6.4$ Hz, 3H), 1.31–1.66 (m, 16H), 1.71 (d, $J = 1.1$ Hz, 3H), 1.78–1.89 (m, 1H), 1.97–2.06 (m, 1H), 2.42–2.50 (m, 1H), 3.14 (ddd, $J = 1.8$, 9.8, 9.8 Hz, 1H), 3.45–3.48 (m, 1H), 3.67–3.80 (m, 2H), 4.76 (q, $J = 6.5$ Hz, 1H), 4.95 (br d, $J = 9.6$ Hz, 1H), 5.37 (br s, 1H), 7.71 (dd, $J = 3.0$, 5.4 Hz, 2H), 7.85 ppm (dd, $J = 3.0$, 5.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): $\delta = 168.7$, 136.7, 134.0, 133.7, 132.3, 123.4, 95.6, 74.3, 68.6, 65.9, 38.7, 36.2, 35.6, 35.3, 34.2, 33.5, 31.6, 31.4, 30.9, 28.0, 25.5, 22.0, 21.52, 19.3, 18.2, 17.2 ppm; IR (film): $\tilde{\nu} = 3464$, 2926, 2855, 1772, 1713, 1438, 1267, 1072, 984, 720 cm^{-1}; MS (ESI): *m/z*: 520 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{30}H_{43}NO_5Na$: 520.3033 $[M+Na]^+$; found: 520.3019.

2-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*S*,*Z*)-6-Hydroxy-3,5-dimethylhept-4-en-1-yl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)isoindoline-1,3-dione (33b): (*R*)-CBS (1.0 M in toluene, 60 μL, 0.051 mmol, 2.2 equiv) was added to a solution of ketone **31** (11.6 mg, 0.023 mmol, 1 equiv) in distilled toluene (0.80 mL). The reaction mixture was cooled under argon at −78°C and stirred for 30 min. A solution of catecholborane (1.0 M in THF, 46 μL, 0.046 mmol, 2 equiv) was added. The reaction mixture was stirred for 12 h at −78°C, quenched with methanol (100 μL), and allowed to warm to room temperature. The reaction mixture was diluted with diethyl ether and washed with 1 M NaOH solution saturated with $NaHCO_3$ until the aqueous washings were colorless. The aqueous phase was extracted three times with diethyl ether and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/diethyl ether 6:4) to afford the corresponding reduced ketone (*Z*)-**33b** as a colorless oil (9.8 mg, 0.020 mmol, 85%). $[\alpha]_D^{25} = +18.0$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): $\delta = 0.82$ (d, $J = 6.5$ Hz, 3H), 0.92 (d, $J = 6.5$ Hz, 3H), 1.09–1.15 (m, 1H), 1.23 (d, $J = 6.5$ Hz, 3H; H_{20}), 1.24–1.62 (m, 17H), 1.71 (d, $J = 1.3$ Hz, 3H; H_{18}), 1.71–1.85 (m, 1H), 1.94–2.04 (m, 1H), 2.41–2.47 (m, 1H), 3.17 (ddd, $J = 2.0$, 9.9, 9.9 Hz, 1H), 3.46–3.50 (m, 1H), 3.66–3.81 (m, 2H), 4.78 (q, $J = 6.4$ Hz, 1H), 4.96 (br d, $J = 9.6$ Hz, 1H), 7.71 (dd, $J = 3.0$, 5.5 Hz, 2H), 7.85 ppm (dd, $J = 3.0$, 5.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): $\delta = 168.7$, 136.5, 134.0, 133.5, 132.3, 123.4, 95.6, 74.3, 69.2, 66.0, 38.6, 36.2, 35.6, 35.3, 34.3, 33.8, 31.7, 31.5, 30.8, 28.0, 25.4, 21.74, 21.70, 19.3, 18.2, 17.2 ppm; IR (film): $\tilde{\nu} = 3464$, 2926, 2855, 1772, 1713, 1438, 1267, 1072, 984, 720 cm^{-1}; MS (ESI): *m/z*: 520 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{30}H_{43}NO_5Na$: 520.3038 $[M+Na]^+$; found: 520.3038.

***tert*-Butyl [(2*R*,3*R*)-2-hydroxy-3-methylpent-4-enyl]carbamate (36)**: A solution of (4*S*,5*S*)-2,2-dimethyl-α,α,α′,α′-tetraphenyldioxolane-4,5-dimethanol (TADDOL; 2.0 g, 4.29 mmol, 1 equiv) in diethyl ether (10 mL) was added to a mixture of cyclopentadienyltitanium(IV) trichloride (0.94 g, 4.29 mmol, 1 equiv) and diethyl ether (20 mL). An additional portion of diethyl ether (4 mL) was used to rinse all the diol into the reaction mixture. The resulting mixture was stirred for 2 min at room temperature and a solution of Et_3N (1.5 mL) in diethyl ether (5 mL) was added dropwise to the reaction mixture over 55 min. An additional portion of diethyl ether (5 mL) was used to rinse all the Et_3N into the reaction mixture. The resulting mixture was stirred for 23 h protected from light at room temperature and was filtered under argon. The solid was washed with diethyl ether (4×5 mL), the yellow filtrate was cooled to 0°C, and a solution of 2-butenylmagnesium chloride in THF (0.5 M, 7.72 mL, 3.89 mmol, 0.9 equiv) was added dropwise to the filtrate over 20 min. The resulting red mixture was stirred for 60 min at 0°C and cooled to −78°C. *N*-Boc-2-aminoacetaldehyde (0.55 g, 3.43 mmol, 0.8 equiv) was added to the reaction mixture, which was stirred at −78°C for 20 h. A 40% aqueous solution of NH_4F (8.71 mL, 94.1 mmol, 22 equiv) was added dropwise to the resulting orange reaction mixture, which was left to warm to room temperature and stirred for 24 h. The white reaction mixture was filtered through celite 545, the pad was washed with THF and dichloromethane, and the filtrate was evaporated in vacuo. Water (40 mL) was added to the solid residue, the aqueous phase was extracted with EtOAc (3× 40 mL), and the combined organic layers were washed with brine (2× 40 mL), dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/EtOAc/dichloromethane 16:4:1) to give **36** as a clear oil (0.42 g, 1.97 mmol, 57%). $[\alpha]_D^{25}$ −2.93 ($c = 5.8$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS) $\delta = 1.05$ (d, $J = 6.8$ Hz, 3H), 1.44 (s, 9H), 2.23 (sext, $J = 7.0$ Hz, 2H), 3.06 (app ddd, $J = 13.8$, 8.2, 4.9 Hz, 1H), 3.42–3.28 (m, 1H), 3.48 (ddd, $J = 7.9$, 6.7, 2.9 Hz, 1H), 4.94 (br s, 1H), 5.16–5.09 (m, 2H), 5.81–5.69 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS) $\delta = 156.8$, 140.0, 116.6, 79.6, 74.3, 44.4, 42.5, 28.5, 16.3 ppm.

(4*R*,7*S*)-8-(4-Methoxybenzyloxy)-7-methyloct-1-en-4-ol (41): A solution of Roush allylboronate[28] (0.12 M in toluene, 15 mL, 1.8 mmol) was added to activated powdered 4 Å molecular sieves under argon. The mixture was stirred for 30 min at room temperature and cooled to −78°C. A solution of aldehyde **40** (344 mg, 1.45 mmol) in dry toluene (2 mL) was added dropwise to the reaction mixture, which was stirred for 2.5 h at −78°C. After hydrolysis with an aqueous solution of NaOH (2.5 M, 25 mL), the mixture was stirred for 2 h at room temperature. Diethyl ether (15 mL) was added to the reaction mixture, the phases were separated, and the aqueous phase was extracted with diethyl ether (3× 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 80:20) to allow isolation of alcohol **41** (295 mg, 1.06 mmol, 73%) and its diastereoisomer (73 mg 0.26 mmol, 18%) in 91% total yield. $R_f = 0.6$ (hexanes/EtOAc 70:30). ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta = 0.92$ (d, $J = 6.6$ Hz, 3H), 1.15–1.30 (m, 1H), 1.40–1.70 (m, 3H), 1.71–1.79 (m, 1H), 2.10–2.18 (m, 1H), 2.28–2.34 (m, 1H), 3.23 (dd, $J_{AB=}$9.1 Hz, $J = 6.4$ Hz, 1H), 3.30 (dd, $J_{AB=}$9.1 Hz, $J = 6.2$ Hz, 1H), 3.58–3.65 (m, 1H), 3.80 (s, 3H), 4.44 (s, 2H), 5.11–5.16 (m, 2H), 5.82 (dddd, $J = 17.5$, 9.4, 7.9, 6.6 Hz, 1H), 6.88 (d, $J = 8.7$ Hz, 2H), 7.25 ppm (d, $J = 8.6$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): $\delta = 17.3$, 29.7, 33.6, 34.2, 41.9, 55.4, 71.1, 72.8, 76.7, 113.9, 118.2, 129.3, 129.4, 135.0, 159.2 ppm; IR (film): $\nu = 3700$–3090, 3077, 2933, 2893, 1641, 1614, 1587, 1514, 1463, 1361, 1302, 1249, 1172, 1090, 1036, 914 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{17}H_{26}O_3Na^+$: 301.1780 $[M+Na]^+$; found: 301.1780.

(4*S*,7*R*)-Ethyl 7-(*tert*-butyldimethylsilyloxy)-4-methyldeca-2,9-dienoate (42): (Carbethoxymethylene)triphenylphosphorane (711 mg, 2.04 mmol) was added to a solution of (2*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyloct-7-enal (39 mg, 0.144 mmol) in dichloromethane (15 mL) cooled to 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. Water (15 mL) was added to the reaction mixture, and the aqueous phase was extracted with dichloromethane (4×8 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. Ester **42** (46 mg, 0.135 mmol) was isolated after flash column chromatography on silica gel (hexanes/EtOAc 97:3) in 94% yield as a colorless oil. $R_f = 0.5$ (hexanes/EtOAc 95:5); $[\alpha]_D^{25} = +20.8$ ($c = 0.92$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta = 0.03$ (s, 6H), 0.87 (s, 9H), 1.03 (d, $J = 6.6$ Hz, 3H), 1.28 (t, $J = 7.5$ Hz, 3H), 1.32–1.55 (m, 4H), 2.16–2.26 (m, 3H), 3.65–3.69 (m, 1H), 4.17 (q, $J = 7.2$ Hz, 2H), 4.99–5.04 (m, 2H), 5.70–5.84 (m, 2H), 6.84 ppm (dd, $J = 15.6$, 7.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): $\delta = -4.4$, −4.2, 14.4, 18.2, 19.6, 26.0, 31.5, 34.2, 36.7, 41.9, 60.3, 71.9, 116.9, 119.9, 135.3, 154.5, 167.0 ppm; IR (film): $\nu = 3078$, 2954, 2931, 2858, 1723, 1652, 1463, 1652, 1464, 1368, 1257, 1177, 1145, 1046, 987, 914, 836, 775 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{19}H_{36}O_3SiNa^+$: 363.2331 $[M+Na^+]$; found: 363.2331.

(4*S*,7*R*)-Ethyl 7-hydroxy-4-methyldeca-2,9-dienoate (38): A catalytic amount of CSA (6 mg, 0.026 mmol) was added to a solution of **42** (44 mg, 0.135 mmol) in methanol/dichloromethane (3:1, 4 mL). The reaction mixture were stirred for 3 h at room temperature, the solvents were evaporated, and the residue purified by flash column chromatography on silica gel (hexanes/EtOAc 80:20). Alcohol **38** was isolated in quantitative yield as a colorless oil (30 mg, 0.135 mmol). $R_f = 0.6$ (hexanes/EtOAc 80:20); $[\alpha]_D^{25} = +27.0$ ($c = 0.6$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.05$ (d, $J = 6.6$ Hz, 3H), 1.28 (t, $J = 7.2$ Hz, 3H), 1.40–1.55 (m, 4H), 2.07–2.17 (m, 1H), 2.26–2.31 (m, 2H), 3.55–3.65 (m, 1H), 4.17 (q, $J = 7.2$ Hz, 2H), 5.10–5.15 (m, 2H), 5.74–5.85 (m, 1H), 5.77 (br d, $J = 15.2$ Hz, 1H), 6.84 ppm (dd, $J = 15.6$, 7.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.4$, 19.6, 32.0, 34.4, 36.6, 42.1, 60.3, 70.6, 118.5, 120.1, 134.7, 154.2, 167.0 ppm; IR (film): $\nu = 3700$–3090, 3077, 2972, 2957,

2933, 2881, 1716, 1652, 1456, 1369, 1269, 1198, 1037, 987, 914 cm^{-1}; HRMS (CI): m/z calcd for $C_{13}H_{22}O_3H^+$: 227.1647 [$M+H^+$]; found: 227.1647.

(2′*S*,3′*S*,6′*R*)-Ethyl 2-(6′-allyl-3′-methyltetrahydro-2*H*-pyran-2′-yl)acetate (43): *t*BuOK (33 mg, 0.29 mmol) was added to a solution of alcohol **38** (60 mg, 0.27 mmol) in THF (2 mL) at −78 °C, and the reaction mixture was stirred at −78 °C over 25 min. A saturated solution of NH_4Cl (3 mL) was added to the reaction mixture, which was warmed to room temperature and extracted with diethyl ether (3×3 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The purification of the residue by flash column chromatography on silica gel (hexanes/EtOAc 98:2) furnished the Michael adduct **43** (43 mg, 0.19 mmol, 72%) as a colorless oil. R_f=0.7 (hexanes/EtOAc 90:10); $[\alpha]_D^{25}=-60.4$ (c=0.98 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ=0.81 (d, J=7.0 Hz, 3H), 1.26 (t, J=7.1 Hz, 3H), 1.27–1.40 (m, 2H), 1.61–1.67 (m, 2H), 1.86–1.98 (m, 1H), 2.07–2.26 (m, 2H), 2.31 (dd, $J_{AB=}$14.0 Hz, J=4.5 Hz, 1H), 2.71 (dd, $J_{AB=}$14.0 Hz, J=10.6 Hz, 1H), 3.60–3.68 (m, 1H), 4.14 (qt, J=7.1, 1.1 Hz, 2H), 4.30 (dt, J=10.7, 4.7 Hz, 1H), 4.98 (br d, J=10,4 Hz, 1H), 5.03 (br d, J=17.5 Hz, 1H), 5.78 ppm (ddt, J=17.1, 10.2, 7.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ=14.3, 16.7, 26.7, 30.3, 32.9, 33.2, 40.2, 60.5, 69.2, 74.2, 116.4, 135.3, 172.1 ppm; IR (film): ν=3071, 2976, 2952, 2932, 1737, 1438, 1370, 1285, 1191, 1711, 1037, 911 cm^{-1}; HRMS (ESI) calcd for $C_{13}H_{22}O_3Na^+$: 249.1467 [$M+Na^+$]; found: 249.1468.

(2′*S*,3′*S*,6′*R*)-Ethyl 2-[6′-(2-hydroxypent-4-enyl)-3′-methyltetrahydro-2*H*-pyran-2-yl]acetate (4): A solution of NMO (12 mg, 0.092 mmol) in water (0.5 mL) followed by OsO_4 (4% in water, 0.0008 mmol, 1 drop) was added to cycloadduct **43** (19 mg, 0.084 mmol) dissolved in acetone (2 mL). The reaction mixture was stirred for 24 h at room temperature, water (5 mL) was added, and the aqueous phase was extracted with ethyl acetate (5×5 mL). The organic layer was washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. $NaIO_4$ (19 mg, 0.088 mmol) was added to the crude residue (23 mg) dissolved in acetonitrile/water (5:1, 3 mL). After sthe had been stirred at room temperature for 2 h, the solvents were evaporated and the residue dissolved in ethyl acetate/water (1:1, 10 mL). The organic layer was separated and the aqueous phase was further extracted with ethyl acetate (5×5 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Allyl bromide (0.18 mmol, 15 μL) and zinc powder (0.18 mmol, 11 mg) were added to the crude residue (20 mg) in THF/saturated NH_4Cl solution (1.5:1, 2.5 mL). The reaction mixture was stirred for 21 h at room temperature, diethyl ether (5 mL) was added, the phases were separated, and the aqueous phase was further extracted with diethyl ether (5×3 mL). The combined organic phases were washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 80:20) to give alcohol **4** as a colorless oil (21 mg, 0.078 mmol, 92% over 2 steps). R_f=0.7 (hexanes/EtOAc 70:30); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): (mixture of isomers) δ=0.81 (d, J=7.0 Hz, 3H), 1.24–1.60 (m, 7H), 1.90–2.04 (m, 2H), 2.14–2.37 (m, 4H), 2.74–2.84 (m, 1H), 3.42–3.91 (m, 2H), 4.09–4.35 (m, 3H), 5.01–5.10 (m, 2H), 5.75–5.92 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): (mixture of isomers) δ=14.3 (2C), 17.4, 17.5, 26.6, 27.1, 29.9, 30.5, 31.9, 32.1, 32.5, 32.9, 33.3, 41.7, 41.9, 42.9, 61.0, 61.1, 65.7, 66.7, 70.9, 71.4, 74.5, 74.9, 116.8, 117.1, 135.3, 135.7, 172.1, 173.3 ppm; HRMS (ESI) calcd for $C_{15}H_{27}O_4^+$: 271.1909 [$M+H^+$]; found: 271.1909.

(2′*S*,3′*S*,6′*R*,3″*E*)-Ethyl 2-[3′-methyl-6′-(2-oxopent-3-enyl)tetrahydro-2*H*-pyran-2′-yl]acetate (44): Dry DMSO (13 μL, 0.19 mmol) was added to a solution of freshly distilled oxalyl chloride (8 μL, 0.09 mmol) in dry dichloromethane (1.2 mL) cooled at −78 °C, and the reaction mixture was stirred for 15 min. A solution of alcohol **4** (21 mg, 0.078 mmol) in dry dichloromethane (0.8 mL) was added to the reaction mixture at −78 °C, which was stirred for a further 20 min at that temperature. A solution of freshly distilled Et_3N (550 μL, 4 mmol) in dichloromethane (2 mL) was added dropwise to the reaction mixture, which was allowed to warm to room temperature and stirred for 24 h. The mixture was diluted with dichloromethane (10 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (10 mL), NH_4Cl (10 mL), and NaCl (5 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 85:15) to yield enone **44** (16 mg, 0.060 mmol, 77%) as a colorless oil. R_f=0.5 (hexanes/EtOAc 80:20); $[\alpha]_D^{25}=-50.0$ (c=0.4 in $CHCl_3$; $[\alpha]_D^{25}=-54.0$ (c=0.1 in $CHCl_3$) for the acid derivative);[10a] ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ=0.81 (d, J=7.0 Hz, 3H), 1.25 (t, J=7.1 Hz, 3H), 1.27–1.43 (m, 2H), 1.59–1.78 (m, 2H), 1.87–1.95 (m, 1H), 1.89 (dd, J=7.0, 1.7 Hz, 3H), 2.34 (dd, $J_{AB=}$14.5 Hz, J=4.7 Hz, 1H), 2.51 (dd, $J_{AB=}$15.2 Hz, J=6.2 Hz, 1H), 2.71 (dd, $J_{AB=}$14.5 Hz, J=10.0 Hz, 1H), 2.78 (dd, $J_{AB=}$15.2 Hz, J=6.4 Hz, 1H), 4.05–4.32 (m, 3H), 4.28 (bdt, J=9.8, 4.9 Hz, 1H), 6.11 (dq, J=15.7, 1.7 Hz, 1H), 6.83 ppm (dq, J=15.7, 6.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ=14.3, 16.7, 18.4, 26.6, 30.6, 32.8, 33.1, 45.9, 60.6, 66.7, 74.3, 132.6, 143.3, 172.0, 198.4 ppm; HRMS (ESI) calcd for $C_{15}H_{24}O_4Na^+$: 291.1572 [$M+Na^+$]; found: 291.1572.

(Methylthio)methyl-2-{(2*S*,3*S*,6*R*)-3-methyl-6-[(*E*)-2-oxopent-3-enyl]-tetrahydropyran-2-yl}acetic acid (45): A solution of NaOH (5 wt%, 0.2 mL) was added to a solution of pyran **4** (8 mg, 0.0296 mmol, 1 equiv) in methanol (0.3 mL), and the reaction mixture was stirred and monitored by TLC. The reaction mixture was diluted with water and washed with diethyl ether. The aqueous layer was then acidified until pH 3 with 3 M HCl and extracted with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was used without further purification. DMSO (9 μL, 0.12 mmol, 4 equiv) in dichloromethane (0.1 mL) was added to a solution of oxalyl chloride (30 μL, 0.06 mmol, 2 equiv) in dichloromethane (0.2 mL) at −78 °C over 15 min and stirred for 15 min. The crude alcohol (0.03 mmol, 1 equiv) dissolved in dichloromethane (0.5 mL) was added dropwise to this mixture, which was stirred for 30 min before dry triethylamine (200 μL, 1.5 mmol, 50 equiv) was added dropwise. The reaction mixture was allowed to warm to room temperature overnight and diluted with brine. The aqueous phase was extracted with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 95:5→7:3) to afford **45** as a colorless oil (5.9 mg, 0.0197 mmol, 66%). $[\alpha]_D^{25}=-64.3$ (c=0.14 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ=0.81 (d, 9.3 Hz, 3H), 1.19–1.42 (m, 2H), 1.62–1.67 (m, 1H), 1.72–1.77 (m, 1H), 1.89 (dd, J=1.6, 6.8 Hz, 3H), 1.88–2.00 (m, 1H), 2.23 (bs, 3H), 2.40 (dd, J=4.7, 14.7 Hz, 1H), 2.52 (dd, J=6.3, 15.3 Hz, 1H), 2.78 (dd, J=10.4, 14.7 Hz, 1H), 2.80 (dd, J=6.5, 15.3 Hz, 1H), 4.06–4.12 (m, 1H), 4.30 (ddd, J=4.8, 4.8, 9.9 Hz, 1H), 5.15 (dd, J=11.8, 24.9 Hz, 2H), 6.11 (ddd, J=1.6, 3.2, 15.7 Hz, 1H), 6.83 ppm (dddd, J=6.8, 6.8, 6.8, 15.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): δ=198.3, 171.7, 143.3, 132.6, 74.3, 68.4, 66.9, 45.9, 33.1, 32.8, 30.6, 26.6, 18.5, 16.8, 15.6 ppm; IR (film): $\tilde{\nu}$=2924, 2875, 1739, 1693, 1670, 1629, 1437, 1396, 1146, 1073, 972, 949, 916 cm^{-1}; MS (ESI): m/z: 323 [$M+Na]^+$; HRMS (ESI): m/z calcd for $C_{15}H_{24}O_4SNa$: 323.1293 [$M+Na]^+$; found: 323.1292.

2,5-Dioxopyrrolidin-1-yl 2-{(2*S*,3*S*,6*R*)-3-methyl-6-[(*E*)-2-oxopent-3-enyl]tetrahydropyran-2-yl}-acetate (3): $MgBr_2{\cdot}Et_2O$ (30 mg, 0.12 mmol, 6 equiv) was added to a solution of the unsaturated enone **45** (5.9 mg, 0.0197 mmol, 1 equiv) in dry diethyl ether at room temperature under argon. The reaction mixture was stirred for 3 h, diluted with water and diethyl ether, and stirred for a further 15 min. The aqueous layer was extracted with diethyl ether and the organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was used without further purification. *N*-Hydroxysuccinimide (4 mg, 0.03 mmol, 1.5 equiv) and 1-ethyl 3-(3-dimethylaminopropyl) carbodiimide hydrochloride (6 mg, 0.03 mmol, 1 equiv) were added to a solution of the crude acid (0.0197 mmol, 1 equiv) in freshly distilled dichloromethane (0.6 mL). The reaction mixture was stirred at room temperature overnight under argon and quenched with a solution of saturated $NaHCO_3$. The mixture was extracted with dichloromethane, and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 6:4) to afford *N*-hydroxysuccinimide ester **3** as a colorless oil (3.6 mg, 0.011 mmol, 55%). $[\alpha]_D^{25}=-26.3$ (c=0.9 in

$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.87$ (d, 7.0 Hz, 3H), 1.28–1.42 (m, 2H), 1.64–1.72 (m, 1H), 1.79–1.86 (m, 1H), 1.89 (dd, $J = 1.6$, 6.8 Hz, 3H), 1.98–2.03 (m, 1H), 2.63 (dd, $J = 7.9$, 15.8 Hz, 1H), 2.69 (dd, $J = 5.2$, 15.0 Hz, 1H), 2.82 (s, 4H), 2.98 (dd, $J = 4.9$, 15.8 Hz, 1H), 3.03 (dd, $J = 9.5$, 15.0 Hz, 1H), 4.06–4.15 (m, 1H), 4.30 (ddd, $J = 5.0$, 5.0, 9.7 Hz, 1H), 6.11 (ddd, $J = 1.6$, 3.2, 15.8 Hz, 1H), 6.86 ppm (dddd, $J = 6.8$, 6.8, 6.8, 15.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 198.5$, 169.0, 167.2, 143.5, 132.6, 73.9, 67.2, 45.5, 32.7, 30.5, 29.9, 26.5, 25.8, 18.5, 16.6 ppm; IR (film): $\tilde{\nu} = 2923$, 2852, 1812, 1785, 1741, 1691, 1633, 1463, 1377, 1208, 1067, 803 cm^{-1}; MS (ESI): *m/z*: 360 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{17}H_{23}NO_6Na$: 360.1423 $[M+Na]^+$; found: 360.1423.

(2*S*,3*R*)-3-Hydroxy-*N*-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*S*,*E*)-6-hydroxy-3,5-dimethylhept-4-enyl]-3-methyl-1,7-dioxaspiro[5.5]undec-2-yl}propyl)-2-methyl-4-(2-{(2*S*,3*S*,6*R*)-3-methyl-6-[(*E*)-2-oxopent-3-enyl]-tetrahydro-2*H*-pyran-2-yl}-ethanamido)butanamide (bistramide A): Diethylamine (68 μL) was added to a solution of **46** (3.2 mg, 0.005 mmol, 1 equiv) in dry DMF (0.25 mL), and the reaction mixture was stirred at room temperature until total consumption of the starting material. The reaction mixture was concentrated in vacuo, and DMF was removed by coevaporation with toluene. A solution of the crude amine and the *N*-hydroxysuccinimide ester fragment **3** (3.0 mg, 0.010 mmol, 1.4 equiv) in dry DMF (0.25 mL) was stirred for 24 h at room temperature, and the reaction mixture was concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (chloroform/methanol 88:2) to afford bistramide A as a colorless oil (2.8 mg, 0.004 mmol, 87%). $[\alpha]_D^{25} = +5.3$ ($c = 0.32$ in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.81$ (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 7.0$ Hz, 3H), 0.95 (d, $J = 6.7$ Hz, 3H), 1.07–1.75 (m, 31H), 1.78–1.83 (m, 2H), 1.92 (dd, $J = 1.6$, 6.8 Hz, 3H), 1.98–2.06 (m, 1H), 2.14 (dd, $J = 1.5$, 15.2 Hz, 1H), 2.31–2.40 (m, 2H), 2.52 (dd, $J = 2.9$, 17.0 Hz, 1H), 2.75 (dd, $J = 11.7$, 15.2 Hz, 1H), 2.90 (dd, $J = 9.0$, 17.0 Hz, 1H), 3.15 (dt, $J = 2.2$, 9.6 Hz, 1H), 3.23 (dt, $J = 5.7$, 13.8 Hz, 1H), 3.30 (dt, $J = 6.6$, 12.8 Hz, 2H), 3.41–3.47 (m, 1H), 3.50 (ddd, $J = 5.3$, 6.5, 13.9 Hz, 1H), 3.71 (dt, $J = 5.1$, 10.4 Hz, 1H), 4.06 (dd, $J = 4.6$, 11.0 Hz, 1H), 4.17–4.22 (m, 2H), 4.61 (br s, 1H), 5.18 (d, $J = 9.4$ Hz, 1H), 6.12 (dd, $J = 1.6$, 15.8 Hz, 1H), 6.90 (dq, $J = 6.8$, 15.5 Hz, 1H), 6.94 (br t, $J = 5.5$ Hz, 1H), 7.30–7.31 ppm (br t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 199.1$, 175.3, 173.7, 144.7, 137.3, 132.3, 131.6, 95.6, 75.0, 74.4, 74.1, 73.5, 69.2, 64.9, 45.4, 44.9, 43.5, 39.6, 36.3, 35.6, 35.0, 34.3, 33.6, 33.5, 32.5, 32.0, 31.5, 30.9, 30.6, 28.1, 26.7, 26.0, 21.9, 21.1, 19.4, 18.6, 18.2, 17.3, 15.7, 12.0 ppm; IR (film): $\tilde{\nu} = 3361$, 2921, 2852, 1740,1658, 1464, 1378, 1261, 1097, 887, 720 cm^{-1}; MS (ESI): *m/z*: 727 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{68}N_2O_8Na$: 727.4868 $[M+Na]^+$; found: 727.4834.

(9*H*-Fluoren-9-yl)methyl {(2*R*,3*S*)-2-hydroxy-4-[(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*R*,*Z*)-6-hydroxy-3,5-dimethylhept-4-en-1-yl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)amino]-3-methyl-4-oxobutyl}carbamate (47a): A solution of methylamine in water (40% wt, 6 μL) was added to a solution of **33a** (9.7 mg, 0.019 mmol, 1 equiv) in ethanol (0.6 mL). The reaction mixture was stirred at 50 °C for 3 h and then concentrated in vacuo. A solution of PyBOP (9.9 mg, 0.019 mmol, 1 equiv) and diisopropylethylamine (7 μL) were added to a solution of the crude amine and the amino acid fragment **2** (7.5 mg, 0.020 mmol, 1 equiv) in dry DMF (0.4 mL) at room temperature. The reaction mixture was stirred overnight at room temperature and diluted with diethyl ether. The organic layer was washed with a saturated $NaHCO_3$ solution. The aqueous phase was extracted with diethyl ether (3×), and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 8:2) to afford **47a** as a colorless oil (5.1 mg, 0.007 mmol, 40%). $[\alpha]_D^{25} = +21.8$ ($c = 0.11$ in $CHCl_3$); ^1H NMR (400 MHz, CD_3CN, 25 °C, TMS): $\delta = 0.80$ (d, $J = 6.5$ Hz, 3H), 0.94 (d, $J = 6.6$ Hz, 3H), 0.91 (d, $J = 6.6$ Hz, 3H), 1.14 (d, $J = 6.4$ Hz, 3H), 1.20–1.53 (m, 15H), 1.64 (d, $J = 1.3$ Hz, 3H), 1.70–1.85 (m, 4H), 2.23–2.33 (m, 1H), 2.43–2.52 (m, 1H), 2.94 (d, $J = 3.1$ Hz, 1H), 3.02–3.17 (m, 3H), 3.20–3.30 (m, 2H), 3.44–3.50 (m, 1H), 3.52–3.59 (m, 1H), 4.00 (d, $J = 6.4$ Hz, 1H), 4.23 (t, $J = 6.8$ Hz, 1H), 4.34 (dd, $J = 2.6$, 6.8 Hz, 2H), 4.62–4.69 (m, 1H), 4.84 (d, $J = 9.2$ Hz, 1H), 5.75 (br t, 1H), 6.72 (br t, 1H), 7.34 (t, $J = 7.1$ Hz, 2H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.66 (d, $J = 7.6$ Hz, 2H), 7.84 ppm (d, $J = 7.5$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3CN, 25 °C, TMS): $\delta = 176.3$, 157.7, 145.2, 142.1, 138.3, 133.2, 128.7, 128.1, 126.1, 121.0, 96.0, 75.2, 73.8, 68.8, 67.0, 65.7, 48.1, 45.9, 43.7, 40.0, 36.8, 35.9, 34.8, 33.9, 32.3, 31.7, 31.4, 30.4, 28.7, 26.9, 22.16, 22.10, 20.0, 18.3, 17.3, 15.6 ppm; IR (film): $\tilde{\nu} = 3316$, 2926, 2856, 1705, 1645, 1547, 1451, 1377, 1255, 1225, 1099, 1073, 1033, 985, 740 cm^{-1}; MS (ESI): *m/z*: 727 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{42}H_{60}N_2O_7Na$: 727.4293 $[M+Na]^+$; found: 727.4296.

(2*S*,3*R*)-3-Hydroxy-*N*-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*R*,*Z*)-6-hydroxy-3,5-dimethylhept-4-enyl]-3-methyl-1,7-dioxaspiro[5.5]undec-2-yl}-propyl)-2-methyl-4-(-{(2*S*,3*S*,6*R*)-3-methyl-6-[(*E*)-2-oxopent-3-enyl]tetrahydro-2*H*-pyran-2-yl}-ethanamido)butanamide (48): Diethylamine (90 μL) was added to a solution of **47a** (5.1 mg, 0.007 mmol, 1 equiv) in dry DMF (0.4 mL), and the reaction mixture was stirred at room temperature until total consumption of the starting material. The reaction mixture was concentrated in vacuo and DMF was removed by coevaporation with toluene. A solution of the crude amine and the *N*-hydroxysuccinimide ester fragment **3** (3.5 mg, 0.010 mmol, 1.4 equiv) in dry DMF (0.1 mL) was stirred 24 h at room temperature. The reaction mixture was concentrated in vacuo, and the crude compound was purified by column chromatography on silica gel (chloroform/methanol 88:12) to afford **48** (2.5 mg, 0.003 mmol, 43%). $[\alpha]_D^{25} = +9.2$ ($c = 0.13$ in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.82$ (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.9$ Hz, 3H), 0.96 (d, $J = 6.9$ Hz, 3H), 1.11–1.72 (m, 32H), 1.92 (dd, $J = 1.1$, 6.8 Hz, 3H), 1.99–2.05 (m, 1H), 2.14 (dd, $J = 1.3$, 15.2 Hz, 1H), 2.35–2.38 (m, 1H), 2.48–2.57 (m, 2H), 2.70–2.77 (m, 2H), 2.92 (dd, $J = 8.9$, 17.1 Hz, 1H), 3.10–3.31 (m, 3H), 3.39–3.53 (m, 3H), 3.70–3.75 (m, 1H), 4.06–4.10 (m, 1H), 4.18–4.21 (m, 1H), 4.58–4.66 (bs, 1H), 4.76 (q, $J = 6.3$ Hz, 1H), 4.91 (d, $J = 9.6$ Hz, 1H), 6.12 (dd, $J = 1.6$, 15.8 Hz, 1H), 6.90 (dq, $J = 6.8$, 15.6 Hz, 1H), 7.16 (br t, $J = 5.5$ Hz, 1H), 7.26 ppm (br t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 199.3$, 175.4, 173.6, 144.9, 136.7, 133.5, 132.3, 95.6, 74.89, 74.85, 73.9, 67.8, 65.7, 65.0, 45.3, 44.6, 43.4, 39.8, 36.2, 35.7, 35.2, 33.8, 33.4, 33.0, 32.6, 31.8, 31.0, 30.81, 30.75, 28.1, 26.7, 25.7, 22.1, 21.6, 19.4, 18.6, 18.2, 17.21, 17.19, 15.7 ppm; IR (film): $\tilde{\nu} = 3335$, 2925, 2855, 1697, 1649, 1554, 1452, 1378, 1260, 1097, 1026, 798, 711 cm^{-1}; MS (ESI): *m/z*: 727 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{68}N_2O_8Na$: 727.4868 $[M+Na]^+$; found: 727.4859.

(9*H*-Fluoren-9-yl)methyl {(2*R*,3*S*)-2-hydroxy-4-[(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*S*,*Z*)-6-hydroxy-3,5-dimethylhept-4-en-1-yl]-3-methyl-1,7-dioxaspiro[5.5]undecan-2-yl}propyl)amino]-3-methyl-4-oxobutyl}carbamate (47b): A solution methylamine in water (40% wt, 70 μL) was added to a solution of **33b** (9.8 mg, 0.020 mmol, 1 equiv) in ethanol (0.6 mL). The reaction mixture was stirred at 50 °C for 3 h and then concentrated in vacuo. A solution of the amino acid fragment **2** (10.0 mg, 0.03 mmol, 1.5 equiv) in DMF (0.4 mL), followed by PyBOP (11.4 mg, 0.022 mmol, 1.5 equiv) and diisopropylethylamine (7 μL), was added to a solution of the crude amine in dry DMF (0.4 mL) at room temperature. The reaction mixture was stirred overnight at room temperature and diluted with diethyl ether. The organic layer was washed with a saturated $NaHCO_3$ solution. The aqueous phase was extracted with diethyl ether (3×), and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 8:2) to afford **47b** as a colorless oil (8.5 mg, 0.012 mmol, 60%). $[\alpha]_D^{25} = +14.2$ ($c = 0.1$ in $CHCl_3$); ^1H NMR (400 MHz, CD_3CN, 25 °C, TMS): $\delta = 0.79$ (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.8$ Hz, 3H), 1.12–1.14 (m, 6H), 1.17–1.56 (m, 15H), 1.63 (d, $J = 1.3$ Hz, 3H), 1.66–1.83 (m, 4H), 2.22–2.30 (m, 1H), 2.39–2.48 (m, 1H), 2.86 (d, $J = 3.4$ Hz, 1H), 3.03–3.08 (m, 1H), 3.11–3.22 (m, 4H), 3.45–3.50 (m, 1H), 3.52–3.56 (m, 1H), 4.01 (d, $J = 6.9$ Hz, 1H), 4.22 (t, $J = 6.9$ Hz, 1H), 4.33 (dd, $J = 2.6$, 6.8 Hz, 2H), 4.61–4.67 (m, 1H), 4.85 (d, $J = 9.7$ Hz, 1H), 5.74–5.78 (br t, 1H), 6.66–6.68 (br t, 1H), 7.33 (t, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.4$ Hz, 2H), 7.65 (d, $J = 7.5$ Hz, 2H), 7.83 ppm (d, $J = 7.5$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3CN, 25 °C, TMS): $\delta = 176.2$, 157.7, 145.2, 142.1, 138.5, 132.8, 128.6, 128.1, 126.1, 120.9, 96.0, 75.0, 73.7, 69.3, 67.0, 66.0, 48.1, 46.0, 43.6, 40.0, 36.8, 35.9, 34.8, 34.3, 32.2, 31.9, 31.2, 30.3, 28.8, 26.6, 22.4, 22.0, 19.9, 18.2, 17.4, 15.6 ppm; IR (film): $\tilde{\nu} = 2922$, 2853, 1708, 1647, 1544, 1462, 1260, 1099, 984, 740 cm^{-1}; MS (ESI): *m/z*: 727 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{42}H_{60}N_2O_7Na$: 727.4293 $[M+Na]^+$; found: 727.4285.

(2*S*,3*R*)-3-Hydroxy-*N*-(3-{(2*R*,3*S*,6*S*,8*S*)-8-[(3*S*,6*S*,*Z*)-6-hydroxy-3,5-dimethylhept-4-enyl]-3-methyl-1,7-dioxaspiro[5.5]undec-2-yl}propyl)-2-methyl-4-(2-{(2*S*,3*S*,6*R*)-3-methyl-6-[(*E*)-2-oxopent-3-enyl]-tetrahydro-2*H*-pyran-2-yl}ethanamido)butanamide (49): Diethylamine (110 μL) was added to a solution of **47b** (5.1 mg, 0.007 mmol, 1 equiv) in dry DMF (0.35 mL), and the reaction mixture was stirred at room temperature until total consumption of the starting material. The reaction mixture was concentrated in vacuo and DMF was removed by coevaporation with toluene. A solution of the crude amine and the *N*-hydroxysuccinimide ester fragment **3** (4.7 mg, 0.014 mmol, 2 equiv) in dry DMF (0.2 mL) was stirred 24 h at room temperature. The reaction mixture was concentrated in vacuo, and the crude compound was purified by column chromatography on silica gel (chloroform/methanol 88:12) to afford **49** (4.6 mg, 0.0065 mmol, 90%). $[\alpha]_D^{25} = -3.0$ ($c = 0.1$ in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 0.82$ (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.9$ Hz, 3H), 0.92 (d, $J = 6.9$ Hz, 3H), 1.08–1.69 (m, 28H), 1.71 (d, $J = 1.2$ Hz, 3H), 1.75–1.87 (m, 2H), 1.92 (dd, $J = 1.5$, 6.8 Hz, 3H), 2.12 (dd, $J = 1.4$, 15.3 Hz, 1H), 2.34–2.41 (m, 1H), 2.48–2.56 (m, 2H), 2.70–2.80 (m, 2H), 2.89 (dd, $J = 8.9$, 17.0 Hz, 1H), 3.12–3.38 (m, 3H), 3.45–3.55 (m, 3H), 3.68–3.75 (m, 1H), 4.01–4.08 (m, 1H), 4.15–4.23 (m, 1H), 4.64–4.71 (b s, 1H), 4.78 (q, $J = 6.3$ Hz, 1H), 4.96 (d, $J = 9.7$ Hz, 1H), 6.12 (dd, $J = 1.6$, 15.8 Hz, 1H), 6.90 (dq, $J = 6.8$, 15.6 Hz, 1H), 7.08 (br t, $J = 5.5$ Hz, 1H), 7.26 ppm (br t, 1H); ^{13}C NMR (100 MHz $CDCl_3$), $\delta = 199.3$, 175.4, 173.6, 144.7, 136.7, 133.8, 133.2, 95.6, 75.0, 74.4, 73.9, 68.6, 65.7, 64.9, 45.5, 44.8, 43.4, 40.1, 36.2, 35.7, 35.2, 34.0, 33.5, 33.2, 32.0, 31.6, 31.0, 30.45, 30.34, 28.1, 26.7, 26.3, 22.3, 21.8, 19.3, 18.6, 18.1, 17.35, 17.27, 15.8 ppm; IR (film): $\tilde{\nu} = 3333$, 2924, 2854, 1724, 1654, 1550, 1455, 1378, 1261, 1225, 1098, 985, 740 cm^{-1}; MS (ESI): *m/z*: 727 $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{40}H_{68}N_2O_8Na$: 727.4868 $[M+Na]^+$; found: 727.4885.

Acknowledgements

Financial support from the European Commission (contract No. LSHB-CT-2004–503467, research fellowship for M.A.H. and G.B.aG) and from the Ministère de la Recherche et de la Technologie (research fellowship for L.T.) is gratefully acknowledged. We thank Inte:Ligand Software-Entwicklungs- und Consulting GmbH (Maria Enzersdorf, Austria) for providing a test copy of the LigandScout 2.03 software. We also thank Prof. M. Crimmins and Prof. W. Kitching for kindly providing the spectroscopic data.

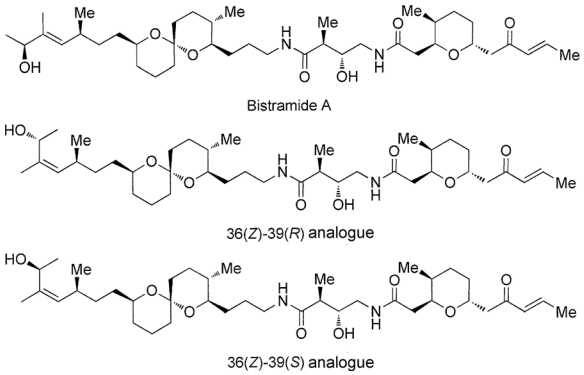
[1] D. Gouiffès, S. Moreau, N. Helbecque, J. L. Bernier, J. P. Hénichart, Y. Barbin, D. Laurent, J. F. Verbist, *Tetrahedron* **1988**, *44*, 451–459.
[2] J. F. Biard, C. Roussakis, J. M. Kornprobst, D. Gouiffes-Barbin, J. F. Verbist, P. Cotelle, M. P. Foster, C. M. Ireland, C. Debitus, *J. Nat. Prod.* **1994**, *57*, 1336–1345.
[3] a) D. Riou, C. Roussakis, N. Robillard, J. F. Biard, J. F. Verbist, *Biol. Cell* **1993**, *77*, 261–264; b) C. Roussakis, N. Robillard, D. Riou, J. F. Biard, G. Pradal, P. Piloquet, C. Debitus, J. F. Verbist, *Cancer Chemother Pharmacol.* **1991**, *28*, 283–292.
[4] J. Pusset, B. Maillere, C. Debitus, *J. Nat. Toxins* **1996**, *5*, 1–6.
[5] a) G. Griffiths, B. Garrone, E. Deacon, P. Owen, J. Pongracz, G. Mead, A. Bradwell, D. Watters, J. Lord, *Biochem. Biophys. Res. Commun.* **1996**, *222*, 802–808; b) D. Watters, B. Garrone, G. Gobert, S. Williams, R. Gardiner, M. Lavin, *Exp. Cell Res.* **1996**, *229*, 327–335; c) D. Watters, B. Garrone, J. Coomer, W. E. Johnson, G. Brown, P. Parsons, *Biochem. Pharmacol.* **1998**, *55*, 1691–1699.
[6] a) A. V. Statsuk, R. Bai, J. L. Baryza, V. A. Verma, E. Hamel, P. A. Wender, S. A. Kozmin, *Nat. Chem. Biol.* **2005**, *1*, 383–388; b) S. A. Rizvi, D. S. Courson, V. A. Keller, R. S. Rock, S. A. Kozmin, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 4088–4092.
[7] C. Stanwell, A. Gescher, D. Watters, *Biochem. Pharmacol.* **1993**, *45*, 1753–1761.
[8] A. V. Statsuk, D. Liu, S. A. Kozmin, *J. Am. Chem. Soc.* **2004**, *126*, 9546–9547.
[9] M. T. Crimmins, A. C. DeBaillie, *J. Am. Chem. Soc.* **2006**, *128*, 4936–1937.
[10] a) I. E. Wrona, J. T. Lowe, T. J. Turbyville, T. R. Johnson, J. Beignet, J. A. Beutler, J. S. Panek, *J. Org. Chem.* **2009**, *74*, 1897–1916; b) J. T. Lowe, I. E. Wrona, J. S. Panek, *Org. Lett.* **2007**, *9*, 327–330.
[11] J. S. Yadav, L. Chetia, *Org. Lett.* **2007**, *9*, 4587–4589.
[12] a) P. Wipf, Y. Uto, S. Yoshimura, *Chem. Eur. J.* **2002**, *8*, 1670–1681; b) P. Wipf, T. D. Hopkins, *Chem. Commun.* **2005**, 3421–3423.
[13] S. A. Rizvi, V. Tereshko, A. A. Kossiakoff, S. A. Kozmin, *J. Am. Chem. Soc.* **2006**, *128*, 3882–3883.
[14] L. Tomas, D. Gueyrard, P. G. Goekjian, *Tetrahedron Lett.* **2010**, *51*, 4599–4601.
[15] M. A. Hiebel, B. Pelotier, O. Piva, *Tetrahedron Lett.* **2010**, *51*, 5091–5093.
[16] a) D. Gueyrard, R. Haddoub, A. Salem, N. Said Bacar, P. G. Goekjian, *Synlett* **2005**, 520–522; b) B. Bourdon, M. Corbet, P. Fontaine, P. G. Goekjian, D. Gueyrard, *Tetrahedron Lett.* **2008**, *49*, 747–749; c) M. Corbet, B. Bourdon, D. Gueyrard, P. G. Goekjian, *Tetrahedron Lett.* **2008**, *49*, 750–754.
[17] M. Kabeya, Y. Hamada, T. Shioiri, *Tetrahedron* **1997**, *53*, 9769–9776.
[18] J. Zhao, K. Burgess, *Org. Lett.* **2009**, *11*, 2053–2056.
[19] S. Hanessian, K. Sumi, *Synthesis* **1991**, 1083–1089.
[20] P. R. Blakemore, D. K. H. Ho, W. Mieke Nap, *Org. Biomol. Chem.* **2005**, *3*, 1365–1368.
[21] P. Wipf, J. T. Reeves, *Chem. Commun.* **2002**, 2066–2067; E. Bourque, P. J. Kocienski, M. Stokes, J. Yuen, *Synthesis* **2005**, 3219–3224.
[22] J. B. Baudin, G. Hareau, S. A. Julia, O. Ruel, *Tetrahedron Lett.* **1991**, *32*, 1175–1178.
[23] W. C. Still, C. Gennari, *Tetrahedron Lett.* **1983**, *24*, 4405–4408.
[24] a) K. Ando, *J. Org. Chem.* **1999**, *64*, 6815–6821; b) E. Vedejs, M. J. Peterson, *Top. Stereochem.* **1994**, *21*, 1–157.
[25] D. Amans, V. Bellosta, J. Cossy, *Angew. Chem.* **2006**, *118*, 6002–6006; *Angew. Chem. Int. Ed.* **2006**, *45*, 5870–5874.
[26] a) A. Hafner, R. O. Duthaler, R. Marti, J. Rihs, P. Rhote-Streit, F. Schwarzenbach, *J. Am. Chem. Soc.* **1992**, *114*, 2321–2336; b) H. Pellissier, *Tetrahedron* **2008**, *64*, 10279–10317.
[27] S. D. Bull, S. G. Davies, S. Jones, H. J. Sanganee, *J. Chem. Soc. Perkin Trans. 1*, **1999**, 387–398.
[28] H. C. Brown, P. K. Jadhav, *J. Am. Chem. Soc.* **1983**, *105*, 2092–2093.
[29] W. R. Roush, L. K. Hoong, M. A. J. Palmer, J. C. Park, *J. Org. Chem.* **1990**, *55*, 4109–4117.
[30] a) M. G. Banwell, C. T. Bui, H. T. T. Pham, G. W. Simpson, *J. Chem. Soc. Perkin Trans. 1* **1996**, 967–969; b) A. Bhattacharjee, O. Soltani, J. K. De Brabander, *Org. Lett.* **2002**, *4*, 481–484; c) M.-A. Hiebel, B. Pelotier, P. Lhoste, O. Piva, *Synlett* **2008**, 1202–1204; d) R. W. Bates, K. Palani, *Tetrahedron Lett.* **2008**, *49*, 2832–2834.
[31] S. B. Jahdav, U. Ghosh, *Tetrahedron Lett.* **2007**, *48*, 2485–2487.
[32] D. Watters, K. Marshall, S. Hamilton, J. Michael, M. McArthur, G. Brown, G. Seymour, C. Hawkins, R. Gardiner, M. Lavin, *Biochem. Pharmacol.* **1990**, *39*, 1609–1614; Reported values are of 424 nM and > 1000 nM in the supplementary material of reference [6a], respectively.
[33] K. Hori, N. Hikage, A. Inagaki, S. Mori, K. Nomura, E. Yoshii, *J. Org. Chem.* **1992**, *57*, 2888–2902.

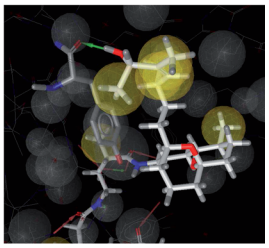
Received: August 8, 2011
Revised: December 7, 2011
Published online: ■■ ■■, 2012

Natural Products

L. Tomas, G. Boije af Gennäs,
M. A. Hiebel, P. Hampson,
D. Gueyrard, B. Pelotier,
J. Yli-Kauhaluoma, O. Piva,
*J. M. Lord,**
*P. G. Goekjian** ..... ▮▮▮▮–▮▮▮▮

Total Synthesis of Bistramide A and Its 36(*Z*) Isomers: Differential Effect on Cell Division, Differentiation, and Apoptosis





Actin versus apoptosis–not so EZ: The total synthesis of bistramide A and its 36(*Z*),39(*S*) and 36(*Z*),39(*R*) isomers (see figure) relies on a novel synthesis of exocyclic enol ethers for the spiroketal fragment, a kinetic oxa-Michael cyclization for the tetrahydropyran fragment, and an asymmetric crotonylation for the amino acid fragment. These compounds show distinctly different effects on cell division, differentiation, and apoptosis, thus suggesting that there are multiple independent biological activities of the natural product.

