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Total Synthesis and Biological Assessment of (−)-Exiguolide and Analogues

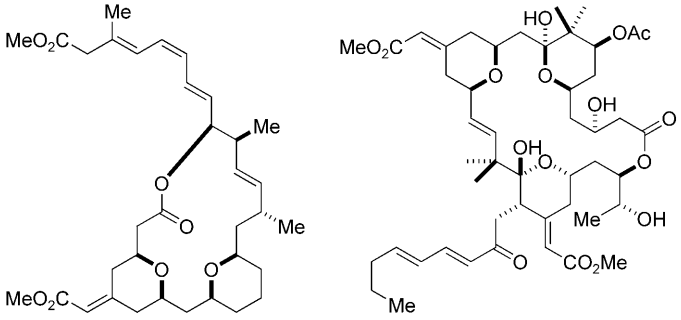
Haruhiko Fuwa,*[a] Takaya Suzuki,[b] Hiroshi Kubo,[b] Takao Yamori,[c] and Makoto Sasaki[a]

Abstract: We describe herein an enantioselective total synthesis of (−)-exiguolide, the natural enantiomer. The methylene bis(tetrahydropyran) substructure was efficiently synthesized by exploiting olefin cross-metathesis for the assembly of readily available acyclic segments and intramolecular oxa-conjugate cyclization and reductive etherification for the formation of the tetrahydropyran rings. The 20-membered macrocyclic framework was constructed in an efficient manner by means of Julia–Kocienski coupling and Yamaguchi macrolactonization. Finally, the (*E*,*Z*,*E*)-triene side chain was introduced stereoselectively via Suzuki–Miyaura coupling to complete the total synthesis. Assessment of the growth inhibitory activity of synthetic (−)-exiguolide against a panel of human cancer cell lines elucidated for the first time that this natural product is an effective antiproliferative agent against the NCI-H460 human lung large cell carcinoma and the A549 human lung adenocarcinoma cell lines. Moreover, we have investigated structure–activity relationships of (−)-exiguolide, which elucidated that the C5-methoxycarbonylmethylidene group and the length of the side chain are important for the potent activity.

Keywords: antiproliferative activity · macrolides · natural products · olefin metathesis · total synthesis

Introduction

Macrolide natural products originated from marine invertebrates and symbiotic bacteria represent a rich source of structurally novel and potent anticancer chemotherapeutic agents.[1,2] (−)-Exiguolide (**1**, see below) was isolated from the methanol extract of the marine sponge *Geodia exigua* Thiele (order Astrophorida, family Geodiidae), collected off Amami-Oshima, Japan, by Ohta, Ikegami, and co-workers.[3] The gross structure including relative stereochemistry of this naturally occurring substance was established by the combination of extensive 2D NMR studies and conformational analysis on the basis of $^{3}J_{H,H}$ values and NOE correlations. The assigned relative stereochemistry was also confirmed by *J*-based configurational analysis.[4] In 2008, Lee and co-workers reported the first total synthesis of the unnatural enantiomer, (+)-**1**, which unambiguously determined the absolute configuration of this natural product.[5] The molecular architecture of (−)-**1**, characterized by the 20-membered macrolide framework embedded with the methylene bis(tetrahydropyran) substructure, resembles to that of antineoplastic marine natural products, the bryostatins.[6] In addition, it has been reported that (−)-**1** specifically inhibits fertilization of sea urchin (*Hemicentrotus pulcherrimus*) gametes but not embryogenesis of the fertilized egg. These biological and structural aspects of (−)-**1** have led to an assumption that (−)-**1** represents a structurally simplified analogue of the bryostatins by Nature.[7] However, further details on the biological activity of (−)-**1** await elucidation.



(−)-exiguolide (**1**) bryostatin 1

Motivated by the elaborated structure and intriguing biological activity, we embarked on the enantioselective total synthesis of (−)-**1**, the full details of which are described herein.[8–10] Moreover, we report on assessment of the cell growth inhibitory activity and structure–activity relationships of (−)-**1** against a panel of human cancer cell lines.

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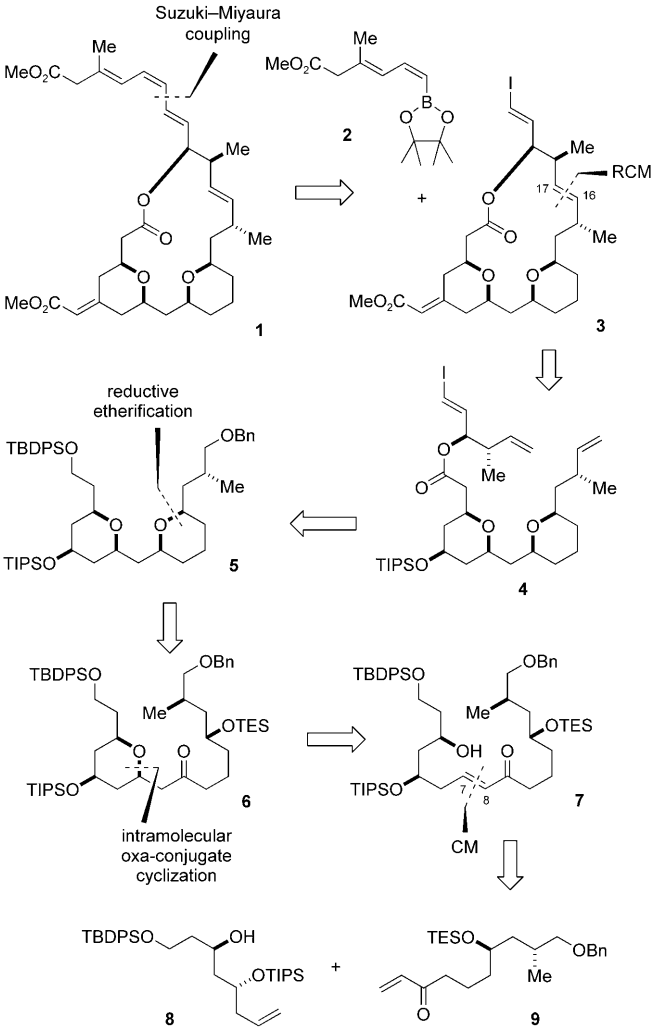
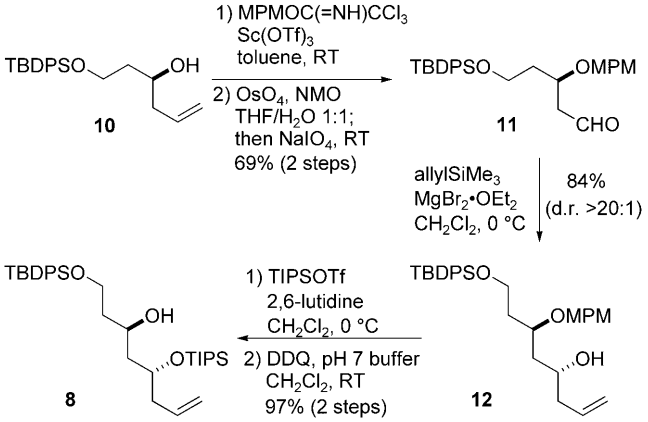
Results and Discussion

Our initial synthetic blueprint toward (−)-**1** is summarized in Scheme 1. We envisioned that the apparently sensitive (*E*,*Z*,*E*)-triene side chain should be introduced at a late stage of the total synthesis and this would be achieved by means of Suzuki–Miyaura coupling[11] of vinyl boronate **2** and vinyl iodide **3**. We thought that it would be possible to forge the C16–C17[12] double bond via chemoselective ring-closing olefin metathesis (RCM)[5,13] of triene **4**, which could be accessible from methylene bis(tetrahydropyran) **5** through esterification. We planned to build the methylene bis(tetrahydropyran) substructure of (−)-**1** in a convergent manner from readily available acyclic segments. Thus, olefin cross-metathesis[14] of hydroxy olefin **8** and enone **9** would produce hydroxy enone **7**, which would undergo intramolecular oxa-conjugate cyclization[15,16] to deliver silyloxy ketone **6** under thermodynamic control. Subsequent reductive etherification[17] of **6** would then afford methylene bis(tetrahydropyran) **5** stereoselectively.



Scheme 1. Initial synthetic blueprint toward (−)-**1**. Bn = benzyl, TBDPS = *tert*-butyldiphenylsilyl, TIPS = triisopropylsilyl, TES = triethylsilyl.

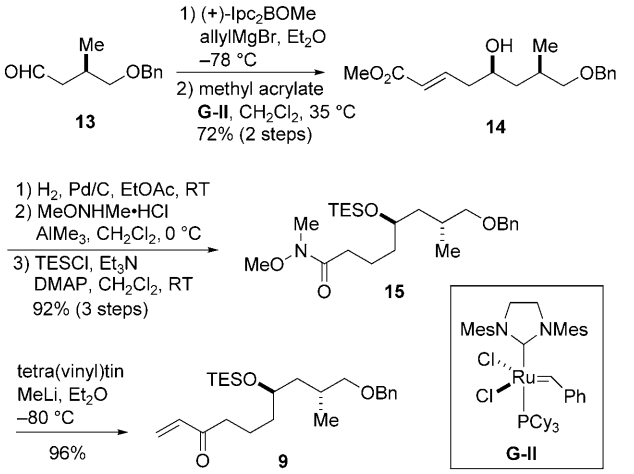
The synthesis of hydroxy olefin **8**, illustrated in Scheme 2, commenced with MPM protection of the known homoallylic alcohol **10**,[18] readily prepared in multi-gram quantities. Oxidative cleavage of the double bond then gave aldehyde **11** in 69% yield for the two steps. Chelation-controlled diastereoselective allylation of **11** (allylSiMe$_3$, MgBr$_2$·OEt$_2$, CH$_2$Cl$_2$, 0°C) produced homoallylic alcohol **12** in 84% yield as a single stereoisomer (judged by 600 MHz ^{1}H NMR). Silylation of the resultant alcohol with TIPSOTf/2,6-lutidine followed by oxidative removal of the MPM group delivered hydroxy olefin **8** in 97% yield for the two steps.



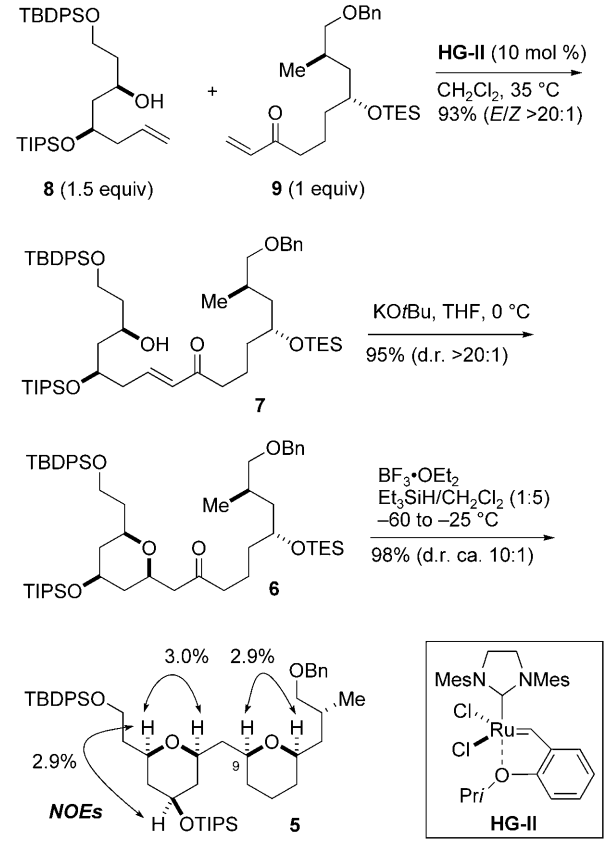
Scheme 2. Synthesis of hydroxy olefin **8**. MPM = *p*-methoxyphenylmethyl, Tf = trifluoromethanesulfonyl, RT = room temperature, NMO = *N*-methylmorpholine-*N*-oxide, d.r. = diastereomer ratio, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

The synthesis of enone **9** started with Brown asymmetric allylation[19] of the known aldehyde **13**,[20] prepared from (*S*)-Roche ester in five steps (Scheme 3). The resultant homoallylic alcohol was homologated via olefin cross-metathesis with methyl acrylate using the Grubbs second-generation catalyst (**G-II**)[21] to give enoate **14** in 72% yield for the two steps. The double bond within **14** was saturated by hydrogenation and the ester functionality was transformed into Weinreb amide (Me$_3$Al, MeONHMe·HCl).[22] Subsequent silylation of the hydroxy group afforded **15** in 92% yield (three steps). Treatment of **15** with vinyl lithium,[23] generated in situ from tetra(vinyl)tin and MeLi, furnished enone **9** in 96% yield.

With the requisite segments available, we focused our attention to the construction of methylene bis(tetrahydropyran) **5** (Scheme 4). After several preliminary experiments, we found that hydroxy olefin **8** and enone **9** could be assembled efficiently through olefin cross-metathesis using 10 mol% of the Hoveyda–Grubbs second-generation catalyst (**HG-II**)[24] in CH$_2$Cl$_2$ at 35°C, affording hydroxy enone **7** in 93% yield as a single stereoisomer. Exposure of **7** to 0.2 equiv of KO*t*Bu in THF at 0°C for 1 h cleanly effected intramolecular oxa-conjugate cyclization to deliver silyloxy ketone **6** in 95% yield as a single stereoisomer (judged by 600 MHz



Scheme 3. Synthesis of enone **9**. Ipc = isopinocampheyl, DMAP = 4-dimethylaminopyridine.



Scheme 4. Construction of methylene bis(tetrahydropyran) **5**.

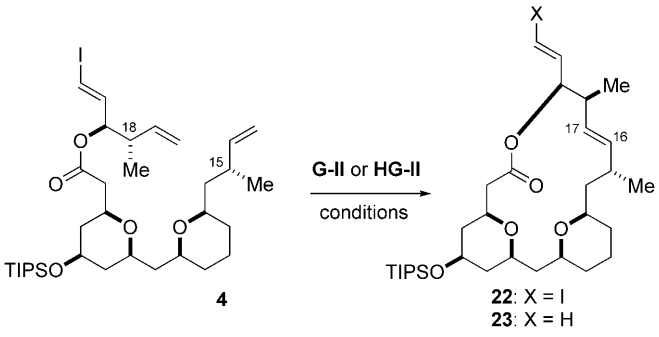
^{1}H NMR), presumably under thermodynamic control.[25] Finally, treatment of **6** with $BF_3{\cdot}OEt_2$ in Et_3SiH/CH_2Cl_2 1:5 at −60 to −25 °C furnished methylene bis(tetrahydropyran) **5** in 98% yield as an approximately 10:1 mixture of diastereomers at the C9 stereogenic center. At this stage, we established the newly generated stereogenic centers of the major diastereomer by NOE experiments as shown.

Moreover, as illustrated in Scheme 5, we have examined one-pot synthesis of **5** based on our recently developed domino olefin cross-metathesis/intramolecular oxa-conjugate cyclization.[26] Thus, microwave heating of a mixture of **8** (1.5 equiv) and **9** in the presence of **HG-II** (10 mol %) in CH_2Cl_2 at 100 °C for 30 min generated silyloxy ketone **6**, which without isolation was reacted with $BF_3{\cdot}OEt_2$ and Et_3SiH (−60 to −15 °C over 50 min) to afford **5** in 89% yield as an approximately 10:1 mixture of diastereomers at the C9 stereogenic center. Thus, by taking advantage of the superb chemoselectivity and bond-forming ability of olefin metathesis, we were able to build up the complex methylene bis(tetrahydropyran) substructure of (−)-**1** from readily available acyclic segments **8** and **9** in a very rapid and efficient manner.

Having completed the methylene bis(tetrahydropyran) substructure, triene **4** was next synthesized as depicted in Scheme 6. Deprotection of the benzyl group within **5** by hydrogenolysis gave alcohol **16** in 90% yield. At this stage, the minor C9 epimer could be removed by flash chromatography on silica gel. Oxidation of **16** with Dess–Martin periodinane[27] followed by Wittig methylenation of the derived aldehyde **17** provided olefin **18** in 87% yield for the two steps. Selective cleavage of the TBDPS group under basic conditions led to alcohol **19** in 90% yield. A two-stage oxidation of **19** followed by esterification of the resultant carboxylic acid **20** with alcohol **21** under Yamaguchi conditions[28] gave rise to triene **4** in 95% yield for the three steps.

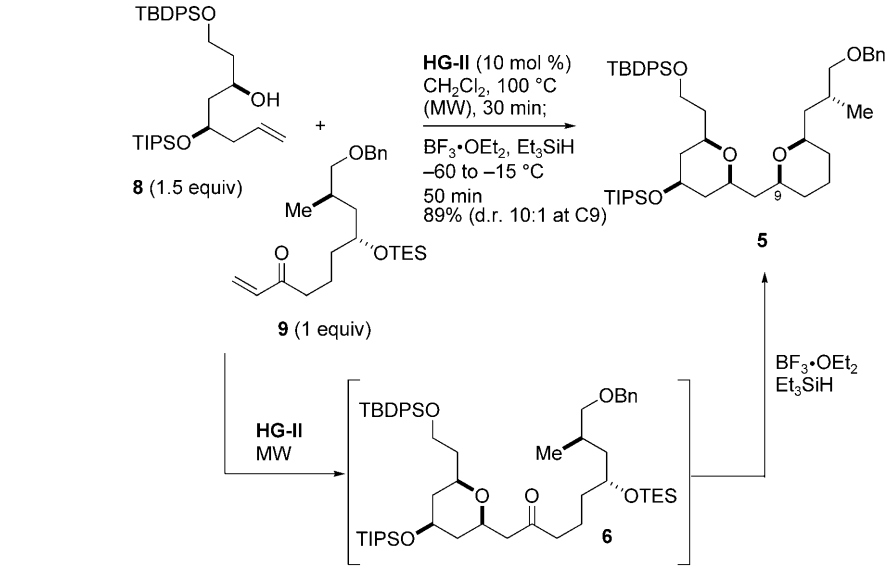
We investigated RCM of triene **4** under a number of conditions (Table 1). We quickly recognized that triene **4** is a challenging substrate for RCM; the presence of the C15 and C18 allylic methyl groups renders **4** less reactive toward RCM,[29] while the vinyl iodide moiety reacts with ruthenium methylidene species, the actual active catalyst of the

Table 1. Screening of the reaction conditions for the RCM of triene **4**.

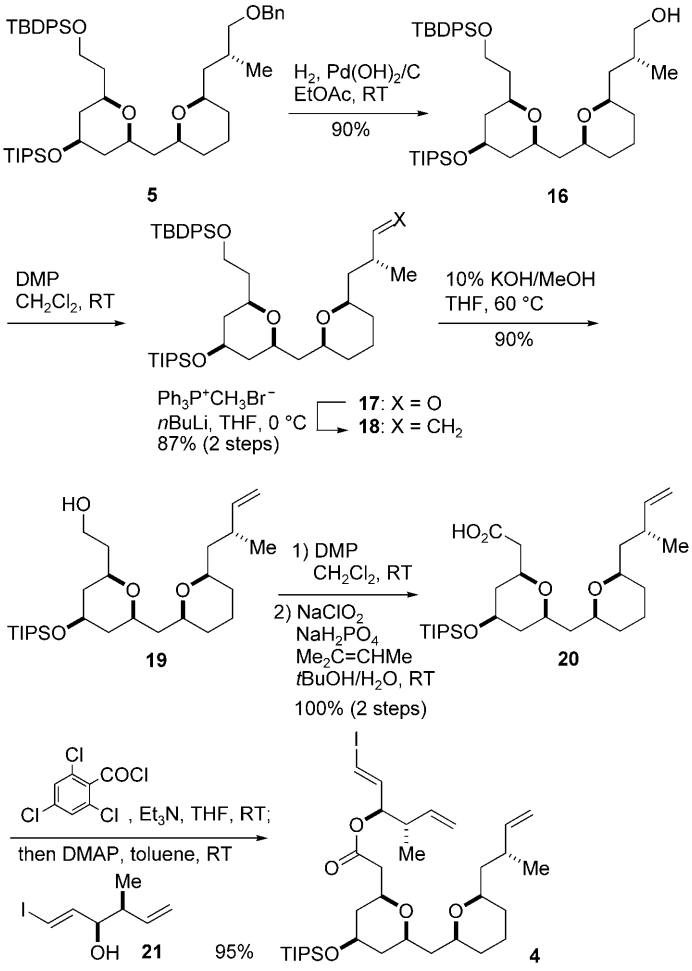


Entry	Catalyst [mol %]	Conditions	Yield [%] 22	23	Recovered 4 [%]
1	**G-II** [30]	CH_2Cl_2 (2.5 mM), reflux, 2 d	trace	n.d.[a]	n.d.[a]
2	**G-II** [60]	toluene (5 mM), 80 °C, 2 d	trace	n.d.[a]	n.d.[a]
3	**HG-II** [60]	toluene (5 mM), 80 °C, 1 d	30	trace	18
4	**HG-II** [90]	toluene (5 mM), 95 °C, 2 d	31	19	0
5	**HG-II** [60]	$(CH_2Cl)_2$ (5 mM), 75 °C, 2 d	28–52	trace	24–27

[a] n.d. = not determined.



Scheme 5. One-pot synthesis of methylene bis(tetrahydropyran) **5**.

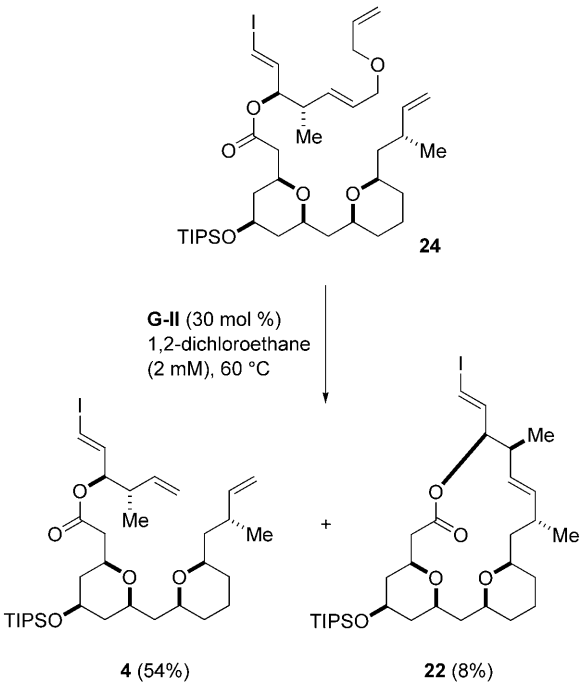


Scheme 6. Synthesis of triene **4**. DMP = Dess–Martin periodinane.

propagation step of RCM, to lose the iodine atom under forcing conditions. Our initial attempts at the RCM of **4** using **G-II** (30–60 mol %) in CH_2Cl_2 (2.5 mM) at reflux or in toluene (5 mM) at 80 °C resulted in decomposition of the catalyst at an early stage of the reaction, and very low conversion (<5 %) was observed under these conditions (entries 1 and 2). By contrast, when **4** was treated with more robust and powerful **HG-II** (60 mol %) in toluene (5 mM) at 80 °C for one day, the desired macrocycle **22** was isolated in 30 % yield along with recovered **4** in 18 % yield (entry 3). The geometry of the newly formed double bond was confirmed to be *E* on the basis of the large coupling constant between H-16 and H-17 protons ($^3J_{16,17} = 15.0$ Hz). Under more forcing the reaction conditions (90 mol % of **HG-II**, toluene (5 mM), 95 °C), a significant amount of **23** was observed as a byproduct (entry 4). Switching the solvent to 1,2-dichloroethane was beneficial to suppress the undesired collapse of the vinyl iodide moiety. Thus, when the reaction was carried out in the presence of **HG-II** (60 mol %) in 1,2-dichloroethane (5 mM) at 75 °C for two days, macrocycle **22** was isolated in 52 % yield and **4** was recovered in 24 % yield (entry 5). Unfortunately, this reaction was found to be capricious; our attempts to reproduce the result under the optimized conditions sometimes ended up in low mass recovery and, for example, gave **22** in only 28 % yield (**4** was recovered in 27 % yield). We have also evaluated the use of Ti(O*i*Pr)$_4$[30] or 2,6-dichloro-1,4-benzoquinone,[31] but these additives did not improve the outcome of the present RCM.

We have also explored relay RCM[32] of triene **24** as shown in Scheme 7. However, we found that triene **24** was rapidly converted to triene **4** in 54 % yield upon exposure to **G-II** catalyst (30 mol %) in 1,2-dichloroethane (2 mM) at 60 °C, and only 8 % of macrocycle **22** was isolated alongside. Changing the catalyst to **HG-II** did not have any impact on the outcome. These disappointing results could be explained as summarized in Scheme 8. Thus, exposure of **24** to **G-II** or **HG-II** would give ruthenium alkylidene **A**, which would undergo RCM to deliver ruthenium alkylidene **B** via extrusion of 3,4-dihydrofuran. Finally, RCM of the generated **B** would afford macrocycle **22**. However, the low reactivity of the C16 double bond precluded this successful scenario at the final stage. Actually, ruthenium alkylidene species **B** reacted with triene **24** as a propagation catalyst itself to generate ruthenium alkylidene **A** and triene **4**, even though the reaction was carried out under high-dilution conditions.[33]

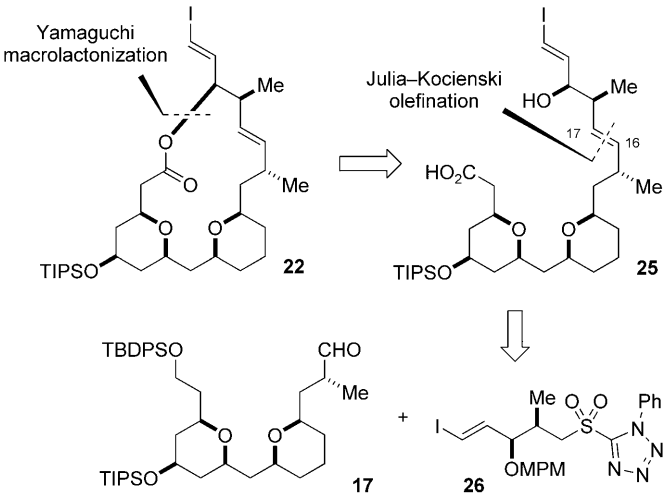
The unsatisfactory results of the RCM of **4** and **24** led us to seek for an alternative scenario toward the macrocyclic framework of (−)-**1**. We envisioned that the 20-membered macrolactone **22** would be forged by means of macrolactonization of the corresponding hydroxy acid **25** (Scheme 9).



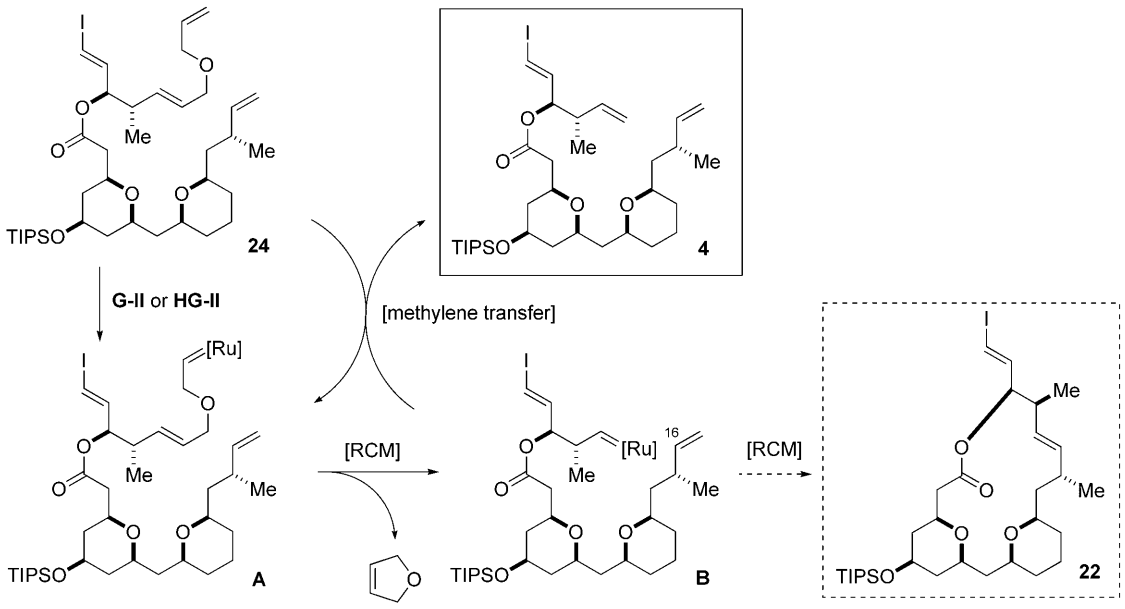
Scheme 7. Unsuccessful relay RCM of triene **24**.

The sterically encumbered C16–C17 double bond would be formed via Julia–Kocienski olefination[34] of aldehyde **17** and an anion derived from sulfone **26**.

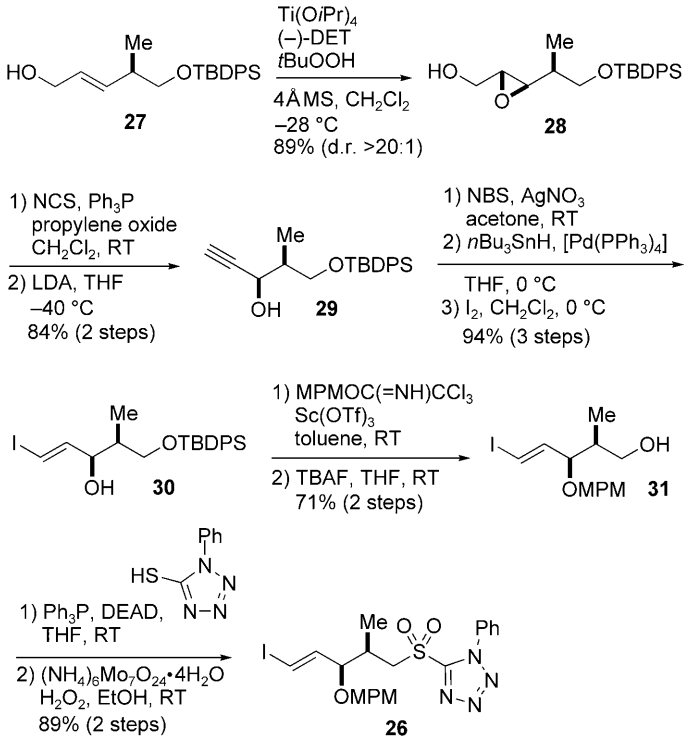
The synthesis of sulfone **26** commenced with Sharpless asymmetric epoxidation of the known allylic alcohol **27**,[35] available in five steps from (*S*)-Roche ester in multi-gram quantities (Scheme 10). This led to epoxy alcohol **28** in 89% yield as a single stereoisomer (judged by 600 MHz ^{1}H NMR). Chlorination of **28** with NCS/Ph_3P in the presence of propylene oxide[36] followed by exposure of the resultant chloro-epoxide to LDA[37] provided propargylic alcohol **29** in 84% yield for the two steps. After conversion of **29** to the corresponding bromoalkyne with NBS in the presence of $AgNO_3$, palladium-catalyzed hydrostannylation (nBu$_3$SnH, [Pd(PPh$_3$)$_4$])[38] and subsequent iodolysis of the derived (*E*)-vinylstannane delivered (*E*)-vinyl iodide **30** as a sole product in 94% yield for the three steps. Protection of the hydroxy group within **30** as its MPM ether followed by desilylation with TBAF gave alcohol **31** in 71% yield for the two steps. Finally, Mitsunobu reaction[39] of **31** with 1-phenyl-1*H*-tetrazole-5-thiol and subsequent molybdenum-catalyzed peroxide oxidation[40] afforded sulfone **26** in 89% yield (two steps).



Scheme 9. Revised synthesis plan toward macrolactone **22**.

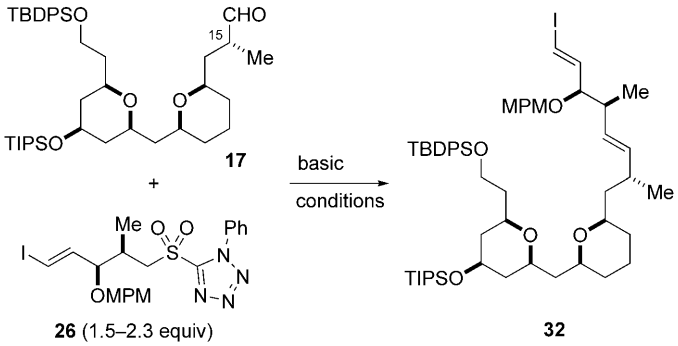


Scheme 8. Plausible rationale for the outcome of the relay RCM of **24**.



Scheme 10. Synthesis of sulfone **26**. DET = diethyl tartrate, MS = molecular sieves, NCS = *N*-chlorosuccinimide, LDA = lithium diisopropylamide, NBS = *N*-bromosuccinimide, TBAF = tetra-*n*-butylammonium fluoride, DEAD = diethyl azodicarboxylate.

Table 2. Optimization of Julia–Kocienski olefination of aldehyde **17** and sulfone **26**.



Entry	Base	Conditions	Yield [%]	*E*/*Z*[a]	Recovery of **17** [%]
1	KHMDS	THF, −78 °C (1 h) to RT (21 h)	14	7:1	39[b]
2	KHMDS	DME, −55 °C (1 h) to RT (1.5 h)	11	13:1	62
3	KHMDS	THF/HMPA 4:1, −78 °C (2.5 h) to RT (3.5 h)	15	>20:1	80
4	LHMDS	THF/HMPA 4:1, −78 °C (2.5 h) to RT (3.5 h)	63	>20:1	23
5	LHMDS	THF/HMPA 4:1, −78 °C (0.5 h) to 0 °C (2 h)	80	15:1	<5

[a] Estimated by ^{1}H NMR analysis (600 MHz) of a purified mixture of *E*/*Z* isomers. [b] Recovered as a 1:1 mixture of diastereomers at the C15 stereogenic center. KHMDS = potassium bis(trimethylsilyl)amide, DME = 1,2-dimethoxyethane, HMPA = hexamethylphosphoramide, LHMDS = lithium bis(trimethylsilyl)amide.

Julia–Kocienski olefination of aldehyde **17** and an anion generated from sulfone **26** was investigated under several conditions, as summarized in Table 2. Our initial attempts involving the use of KHMDS as a base gave only poor yield of the desired (*E*)-olefin **32**. Thus, deprotonation of sulfone **26** with KHMDS in THF at −78 °C followed by addition of aldehyde **17** and warming the reaction mixture to room temperature over 22 h gave olefin **32** in 14% yield as an approximately 7:1 mixture of *E*/*Z* isomers (entry 1). Although aldehyde **17** was recovered in 39% yield alongside, complete epimerization of the C15 stereogenic center was observed by ^{1}H NMR analysis. Monitoring the reaction by TLC analysis suggested that the reaction stalled after initial several hours and extended reaction time only resulted in decomposition of unreacted materials. When the reaction was performed in DME (−55 °C for 1 h then warmed to room temperature over 1.5 h), the *E*/*Z* selectivity was improved to ca. 13:1, while the product yield and mass recovery remained poor (entry 2). The stereoselectivity and mass recovery could be improved by running the reaction in THF/HMPA 4:1 (−78 °C for 2.5 h then warmed to room temperature over 3.5 h), giving olefin **32** as a sole *E* isomer in 15% yield along with 80% recovery of aldehyde **17** with no sign of epimerization at the C15 stereogenic center (entry 3). Gratifyingly, the product yield was significantly enhanced upon switching the base to LHMDS (entry 4). Thus, reaction of a sulfone anion, generated in situ from sulfone **26** and LHMDS, with aldehyde **17** in THF/HMPA 4:1 (−78 °C for 2.5 h then warmed to room temperature over 3.5 h) furnished (*E*)-olefin **32** in 63% yield as a single stereoisomer, along with recovered **17** (23%). Although the yield could be further improved to 80% under the reaction conditions described for entry 5, a slightly declined stereoselectivity was observed in this case (*E*/*Z* ca. 15:1).

With the sterically encumbered C16–C17 double bond secured, we proceeded to forge the 20-membered macrocycle and complete the total synthesis of (−)-**1** (Scheme 11). Selective deprotection of the TBDPS group within **32** under basic conditions gave alcohol **33** in 94% yield, which was oxidized to the corresponding carboxylic acid via a two-stage oxidation and then esterified with $TMSCHN_2$ to provide methyl ester **34** in 94% yield for the three steps. Cleavage of the MPM group within **34** was best achieved by treatment of **34** with $BF_3{\cdot}OEt_2$ in Et_3SiH/CH_2Cl_2 1:2 at 0 °C, giving alcohol **35** in 89% yield.[41] After saponification of **35** with TMSOK,[42] macrolactonization of the resultant hydroxy acid under Yamaguchi conditions (2,4,6-$Cl_3C_6H_2COCl$, Et_3N, THF, room temperature; then added to DMAP, toluene (final concentration = 0.5 mM), 80 °C over 2 h) smoothly proceeded to afford macrolactone **22** in 94% yield for the two steps. We observed competitive formation of the corresponding dimer under higher concentrations (above 1 mM). Deprotection of the TIPS group of **22** with HF·pyridine followed by oxidation of the resultant alcohol **36** with Dess–Martin periodinane provided ketone **37** in quantitative yield. Horner–Wadsworth–Emmons reaction of

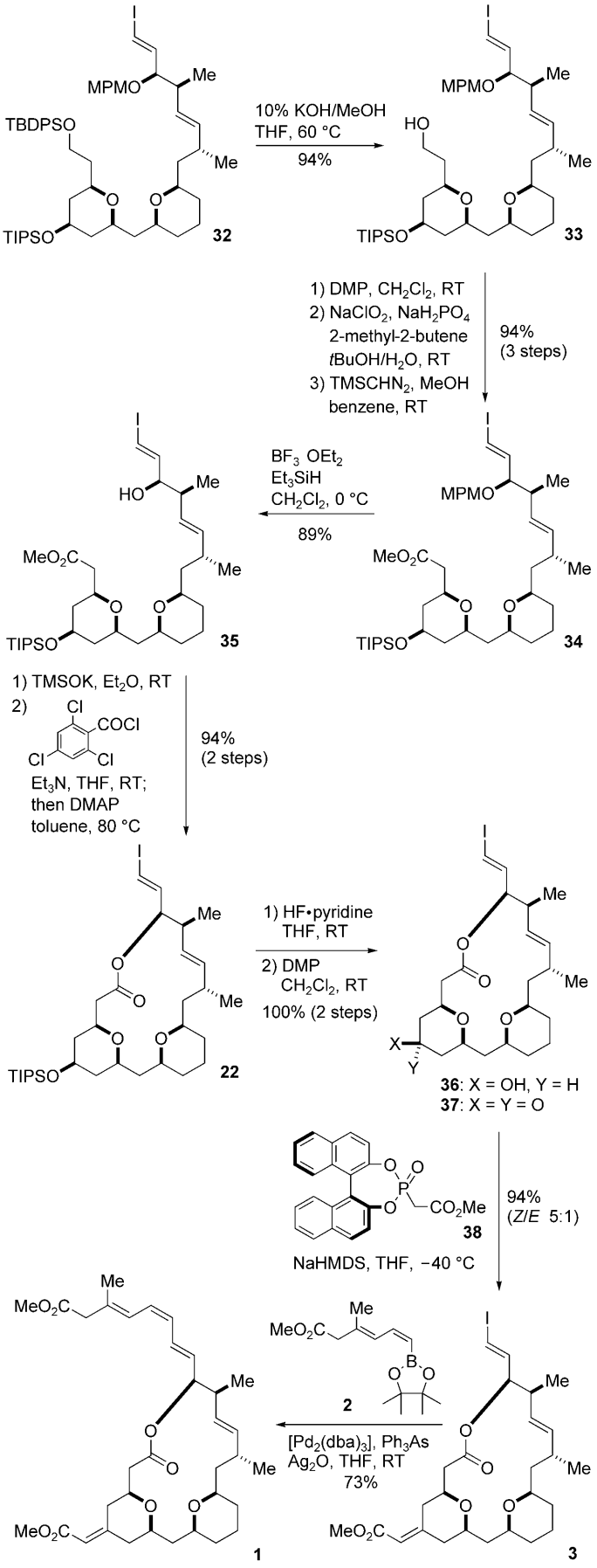
37 with chiral phosphonate **38** developed by Fuji and coworkers[5,43] led to enoate **3** in 94% yield as an approximately 5:1 mixture of *Z*/*E* isomers. Fortunately, the desired (*Z*)-isomer **3** could be isolated in a geometrically pure form in 75% yield after flash chromatography on silica gel. Finally, stereoselective introduction of the (*E*,*Z*,*E*)-triene side chain was accomplished by means of Suzuki–Miyaura coupling of **3** with (*Z*)-vinyl boronate **2**[44] under the influence of a $[Pd_2(dba)_3]/Ph_3As$ catalyst system in the presence of Ag_2O[45] in THF at room temperature.[46] Under these exceptionally mild conditions, (−)-exiguolide (**1**) was isolated in 73% yield as a single stereoisomer (judged by 600 MHz ^1H NMR). The spectroscopic data (^1H, ^{13}C NMR, HRMS) were in full accordance with those of the natural product. The specific rotation value of the synthetic (−)-exiguolide ($[\alpha]_D^{24} = -121.5$ ($c = 0.22$ in $CHCl_3$)) slightly differed from that of the natural product (lit.[3] $[\alpha]_D^{25} = -92.5$ ($c = 0.069$ in $CHCl_3$)) but matched that of the synthetic (+)-exiguolide, except for the sign of the rotation (lit.[5] $[\alpha]_D^{25} = +119$ ($c = 0.11$ in $CHCl_3$)). The present total synthesis proceeded in 23 longest linear steps from the known aldehyde **13** (28 longest linear steps from commercially available (*S*)-Roche ester).

With sufficient quantities of the synthetic material available, we evaluated the growth inhibitory activity of (−)-**1** against a panel of 39 human cancer cell lines,[47,48] and a part of the results is shown in Table 3 (for full details, see the Supporting Information). Importantly, (−)-**1** effectively inhibited in vitro proliferation of the NCI-H460 human lung large cell carcinoma, the A549 human lung adenocarcinoma, the SK-OV-3 human ovarian carcinoma, and the MKN-74 human gastric carcinoma cell lines, with GI_{50} values below submicromolar concentrations. Importantly, higher concentrations of (−)-**1** did not completely abolish cell viability of these sensitive cell lines ($LC_{50} > 100$ μM). Other cancer cell lines were found to be less sensitive to (−)-**1**. Significantly, (−)-**1** exhibited antiproliferative activity against several human cancer cell lines with approximately 10 to 1000-fold greater potency than that of bryostatin 1; the log GI_{50} values of bryostatin 1 against the NCI-H460, A549, and SK-OV-3 cells are −5.6, −5.4, and −5.3, respectively.[49] On the basis

Table 3. The growth inhibitory activity of (−)-**1** against selected human cancer cell lines.[a]

Cell line	log GI_{50}[b]	GI_{50}[b] [μM]	LC_{50}[c] [μM]
NCI-H460	−8.00	0.01	>100
A549	−6.19	0.65	>100
SK-OV-3	−6.15	0.70	>100
MKN74	−6.16	0.69	>100

[a] For details of the assay procedure, see reference [47]. [b] GI_{50} = concentration that induces 50% growth inhibition. [c] LC_{50} = concentration that induces 50% cell death.



Scheme 11. Completion of the total synthesis of (−)-**1**. TMS = trimethylsilyl, NaHMDS = sodium bis(trimethylsilyl)amide, dba = dibenzylideneacetone.

of the COMPARE analysis,[47,48] the fingerprint of (−)-**1** showed only marginal similarities with those of DNA-related agents listed in Table 4 (see below) and did not show any significant correlation with those of more than 100 anticancer agents, implying the possibility that (−)-**1** may have a unique biological mode-of-action.

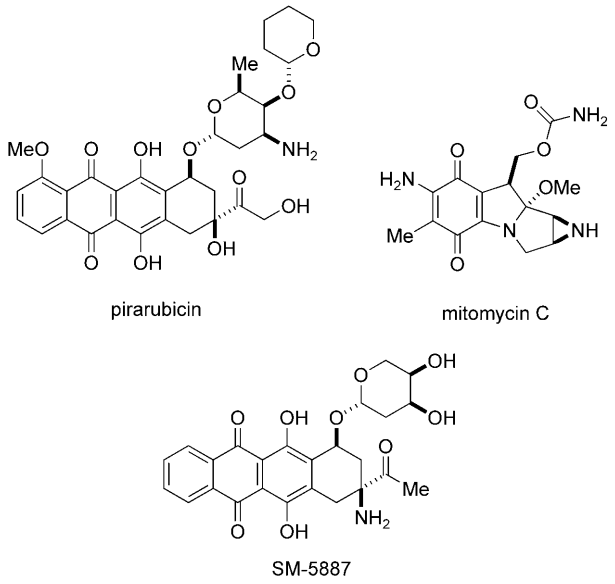


Table 4. COMPARE analysis on (−)-**1**.

Rank	Compound	r[a]	Molecular targets/Drug type
1	pirarubicin	0.561	DNA intercalater
2	mitomycin C	0.556	DNA alkylating drugs
3	SM-5887	0.537	DNA topoisomerase II inhibitors

[a] r = correlation efficiency.

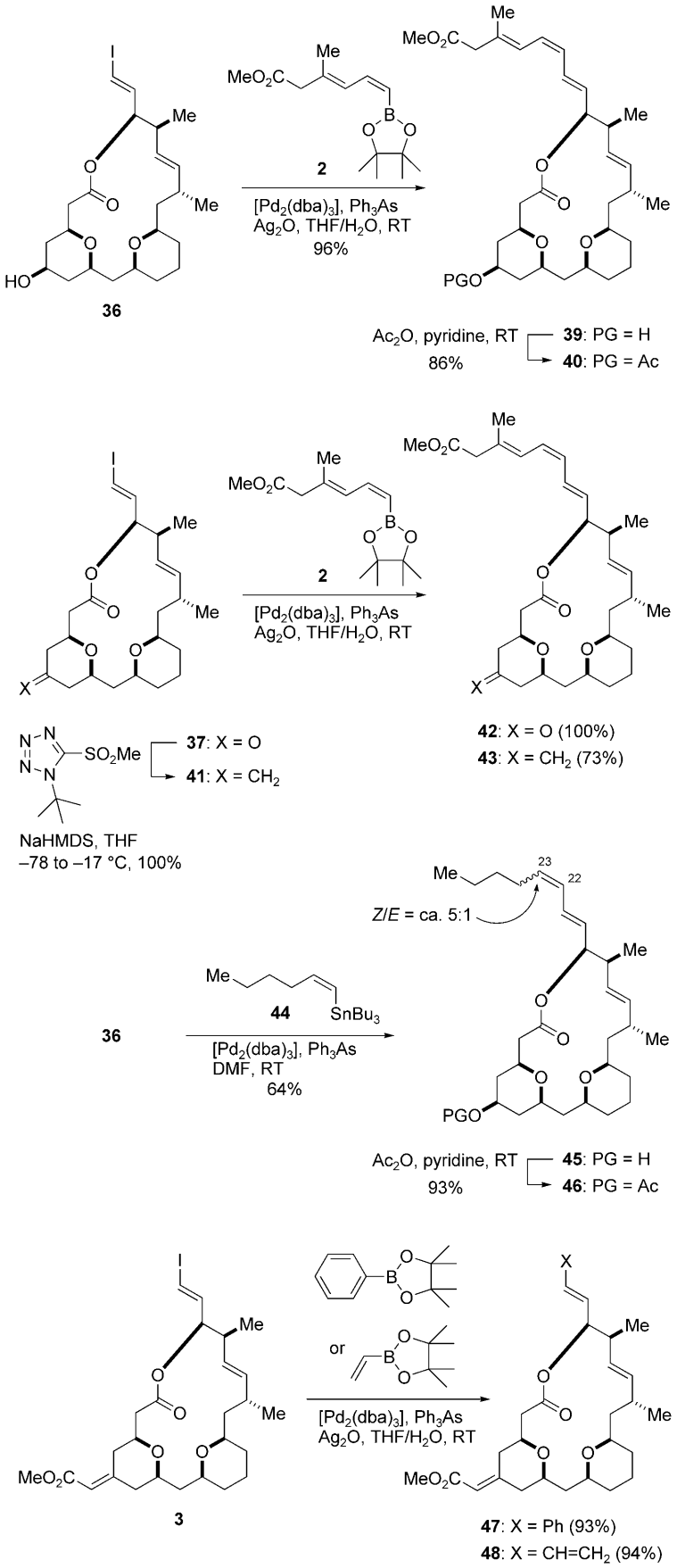
Encouraged by the results of the panel screening, we next explored the structure–activity relationships of (−)-exiguolide. Specifically, we focused our attention to the modification of the C5 methoxycarbonylmethylidene group and the triene side chain, because omission of these functionalities would reduce the complexity of the molecule and the side chain was found to be somewhat labile under acidic conditions.[50] Thus, we designed and synthesized analogues **39**, **40**, **42**, **43**, and **45**–**48** as summarized in Scheme 12.

The synthesis of analogues lacking the C5 methoxycarbonylmethylidene group started from intermediate **36** or **37**. C5-Hydroxy analogue **39** was synthesized by Suzuki–Miyaura coupling of alcohol **36** with (*Z*)-vinylboronate **2** ($[Pd_2(dba)_3]$, Ph_3As, Ag_2O, THF/H_2O 10:1, room temperature) in 96% yield. Acetylation (Ac_2O, pyridine) of **39** afforded C5-acetoxy analogue **40** in 86% yield. Two additional C5-modified analogues, **42** and **43**, were synthesized. Suzuki–Miyaura coupling of **37** with **2** gave C5-keto analogue **42** in quantitative yield. Methylenation of **37** under the modified Julia–Kocienski olefination conditions (1-*tert*-butyl-5-methanesulfonyl-1*H*-tetrazole, NaHMDS, THF, −78 to −17 °C)[51] provided exomethylene **41** quantitatively, which was coupled with **2** under Suzuki–Miyaura conditions to afford C5-methylene analogue **43** in 73% yield. Analogues **45** and **46** with a simple alkyl side chain were prepared from **36**. Stille coupling[52] of **36** with the known (*Z*)-vinylstannane **44**[53] in the presence of a $[Pd_2(dba)_3]$/Ph_3As catalyst system in DMF at room temperature proceeded with partial erosion of the olefin geometry to give analogue **45** as an inseparable 5:1 mixture of *Z*/*E* isomers in 64% yield. Acetylation of **45** afforded analogue **46** in 93% yield. We have also prepared analogues with a truncated side chain to probe the role of the triene side chain of (−)-**1**. Suzuki–Miyaura coupling of **3** with commercially available phenyl or vinyl pinacolboronate provided analogues **47** (93%) or **48** (94%), respectively.

The antiproliferative activity of (−)-exiguolide (**1**) and analogues **39**, **40**, **42**, **43**, and **45**–**48** against the NCI-H460, A549, and A172 human glioblastoma cell lines was evaluated in detail, and the results are summarized in Table 5. (−)-**1** displayed potent antiproliferative activity with submicromolar IC_{50} values (0.28, 0.59, and 0.47 μM against NCI-H460, A549, and A172 cells, respectively). We found that C5-hydroxy analogue **39** showed about 10-fold less activity than (−)-**1**. On the other hand, C5-acetoxy analogue **40** was inactive at 10 μM against the A549 and A172 cell lines, indicating that masking of the C5 hydroxy group of **39** was detrimental for the activity. C5-Keto analogue **42** showed 10- to 100-fold less potency than (−)-**1**. Importantly, the fact that C5-methylene analogue **43** was inactive indicated the striking effect of the C5 methoxycarbonylmethylidene group on the potent antiproliferative activity of (−)-**1**. Thus, only a limited repertoire of functionalities would be able to replace the C5 methoxycarbonylmethylidene group of (−)-**1**. Interestingly, analogue **45** was almost equipotent to analogue **39**, implying that the triene side chain of the natural product could be replaced with a simple alkyl chain without losing potency. However, analogues with a truncated side chain displayed diminished activity; phenyl analogue **47** was inactive and vinyl analogue **48** was only marginally active compared to (−)-**1**. These results suggested that the length of the side chain of (−)-**1** is important for exerting potent antiproliferative activity, while the terminal C27 methyl ester group would not be essential.

Conclusion

We have accomplished the total synthesis of (−)-exiguolide (**1**), the naturally occurring enantiomer, for the first time. Our strategy for the construction of the methylene bis(tetrahydropyran) substructure **5** of (−)-**1** exploited the superb chemoselectivity and bond-forming ability of olefin metathesis reactions, which allowed for direct utilization of the preexisting functionalities within acyclic segments **8** and **9** in subsequent ring-forming events. Thus, the readily available segments **8** and **9** were assembled through olefin cross-metathesis reaction, and the two tetrahydropyran rings were suc-



Scheme 12. Synthesis of structural analogues of (−)-exiguolide. DMF = *N*,*N*-dimethylformamide.

cessively forged in a stereocontrolled manner via intramolecular oxa-conjugate cyclization and reductive etherification. Although our initial efforts on the construction of the 20-membered macrocyclic framework of (−)-**1** via RCM ultimately met with a limited success, we eventually developed an efficient strategy that hinges on Julia–Kocienski olefination for the stereoselective formation of the C16–C17 double bond and Yamaguchi macrolactonization for the construction of the 20-membered macrocycle. Finally, the (*E*,*Z*,*E*)-triene side chain was introduced in a highly stereoselective manner via Suzuki–Miyaura coupling under exceptionally mild conditions to complete the total synthesis of (−)-**1**. Assessment of the growth inhibitory activity of synthetic (−)-**1** against a panel of 39 human cancer cell lines elucidated for the first time that (−)-**1** exhibits potent antiproliferative activity against the NCI-H460 human lung large cell carcinoma, the A549 human lung adenocarcinoma, the SK-OV-3 human ovarian carcinoma, and the MKN-74 human gastric carcinoma cells. The COMPARE analysis suggested the possibility that this natural product may have a unique biological mode-of-action, which would be of worth investigating. In addition, we have synthesized a series of structural analogues and explored structure–activity relationships of (−)-**1**, which laid the foundation for further structure optimization study. Future studies will include synthesis and evaluation of designed analogues, mouse xenograft studies, and target identification.

Table 5. Detailed evaluation of antiproliferative activity of (−)-**1** and its structural analogues against selected human lung cancer cell lines (IC_{50} values in μM).[a]

Compound	NCI-H460	A549	A172
1	0.28	0.59	0.47
39	3.6	2.9	1.9
40	3.0	>100	>100
42	110	40	46
43	>100	>100	>100
45	6.5	2.7	1.7
46	>100	>100	>100
47	>100	>100	>100
48	24	>100	2.9

[a] For details of the assay procedure, see the Supporting Information.

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