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Structure–Activity Relationships

Efficient Total Synthesis of Bongkrekic Acid and Apoptosis Inhibitory Activity of Its Analogues

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Abstract: Bongkrekic acid (BKA), isolated from the bacterium *Burkholderia cocovenenans*, is an inhibitor of adenine nucleotide translocator, which inhibits apoptosis, and is thus an important tool for the mechanistic investigation of apoptosis. An efficient total synthesis of BKA has been achieved by employing a three-component convergent strategy based on Kocienski–Julia olefination and Suzuki–Miyaura coupling. It is noteworthy that segment B has been prepared as a new doubly functionalized coupling partner, which contributes to shortening of the number of steps. Torquoselective olefination with an ynolate has also been applied for the efficient construction of an unsaturated ester. Furthermore, it is revealed that 1-methyl-2-azaadamantane N-oxyl is an excellent reagent for final oxidation to afford BKA in high yield. Based on the total synthesis, several BKA analogues were prepared for structure–activity relationship studies, which indicated that the carboxylic acid moieties were essential for the apoptosis inhibitory activity of BKA. More easily available BKA analogues with potent apoptosis inhibitory activity were also developed.

Introduction

Bongkrekic acid (BKA; Figure 1) was discovered to be responsible for fatal food poisoning from the fermented coconut product, tempeh bongkrek.[1] This food poisoning has caused nearly 1000 deaths in Central Java (Indonesia) to date. The bacterium *Burkholderia cocovenenans* produce this natural toxin, BKA, which has also been found in fermented cornmeal in China.[2]

BKA binds to the adenine nucleotide translocator (ANT) from the matrix side in mitochondria to result in fixing its conformation in the M state. This strong binding also leads to inhibition of the permeability transition pore (MPT).[3] It has therefore been used as a biological tool to investigate the MPT or ANT, although BKA also suppresses mitochondria-dependent apoptosis, which is supposed to be due to inhibition of the formation of MPT.[4] Numerous types of bioactivity, such as a reduction in ischemic-induced neuronal death,[5] inhibition of phosphatidylserine exposure,[6] and inhibition of chloride channels,[7] are caused by the inhibition of apoptosis. Although it is now an important tool as an apoptosis inhibitor, the bioactivity of BKA has not been fully investigated, especially with respect to its in vivo activity and the relationship between ANT inhibition and the inhibition of apoptosis, probably because of its limited availability from fermentation or chemical synthesis.[8] To assess the use and potential contribution to apoptotic science of BKA and its analogues, these compounds must be synthesized on a large scale and in pure form.

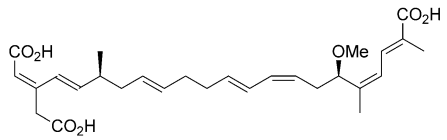

Figure 1. The structure of bongkrekic acid (BKA) described herein.

The proposed structure of BKA was reported in 1971,[9] and the corrected structure[10] and absolute configuration[11] were published in 1973. BKA is an unsaturated fatty acid with three conjugated dienes, two allylic chiral stereocenters, and three carboxylic acids. In 1984, the first enantioselective total synthesis of BKA was reported by Corey and Tramontano (Scheme 1)[12] who constructed the C17 asymmetric center with kinetic resolution. However, BKA itself could not be isolated in pure form because of its instability. In 2004, Shindo and co-workers reported the first-generation semiconvergent synthesis, which was too long (32 steps in the longest linear sequence) to supply a sufficient quantity of BKA.[13] Later, the groups of Shishido[14] and Shindo[15] published reports on the second-generation syntheses of BKA with improved overall efficiency. Recently, Ley and co-workers reported the shortest synthesis of 13 steps in the longest linear sequence and total of

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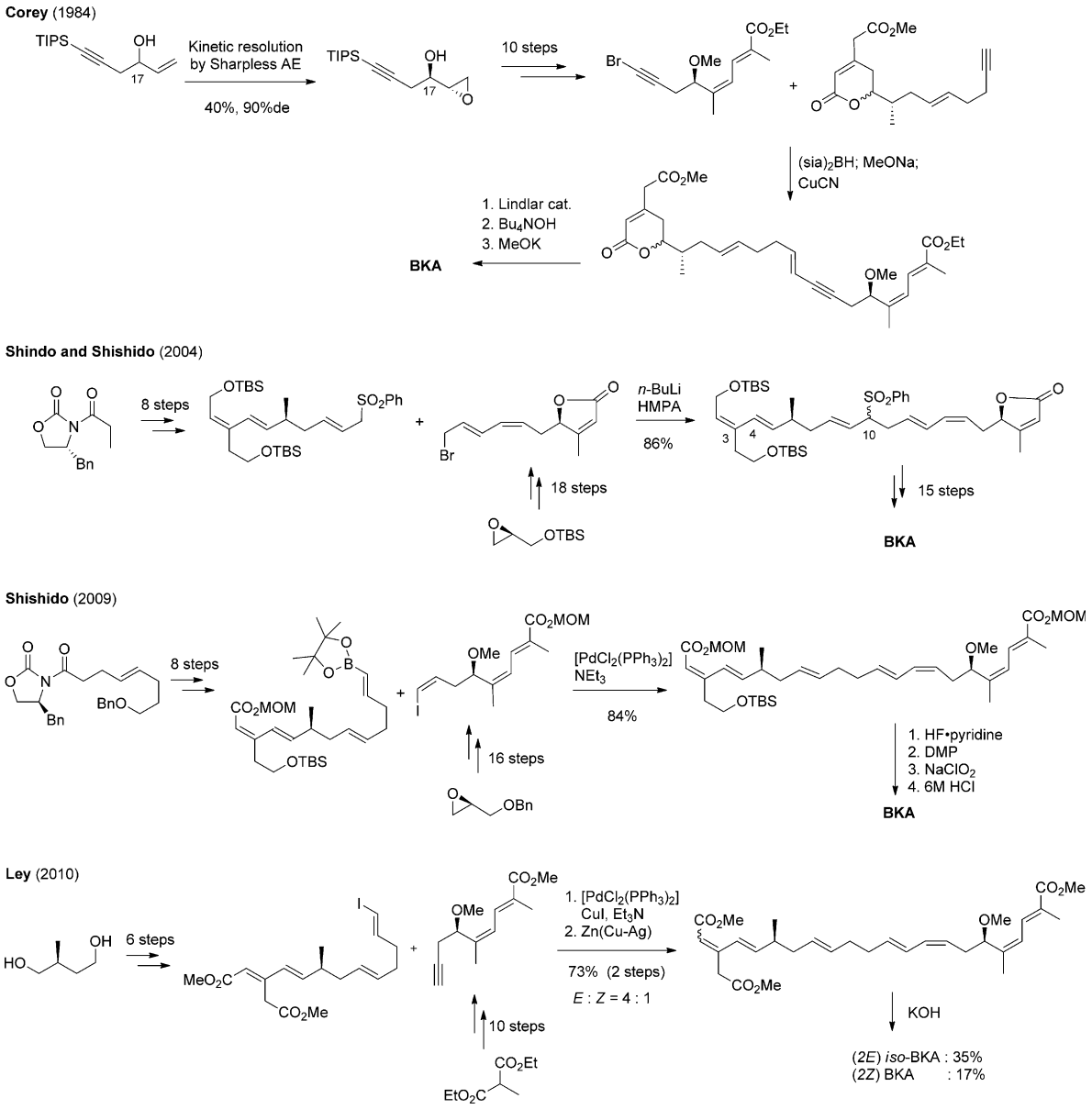


Scheme 1. Syntheses of BKA described in earlier publications.[12–16] TIPS = triisopropylsilyl, sia = siamyl, Bn = benzyl, TBS = *tert*-butyldimethylsilyl, HMPA = hexamethylphosphoric triamide, MOM = methoxymethyl, DMP = Dess–Martin periodinane.

29 steps, which gave a 4.7% overall yield with 7.0% of the less effective isomer, iso-BKA.[16]

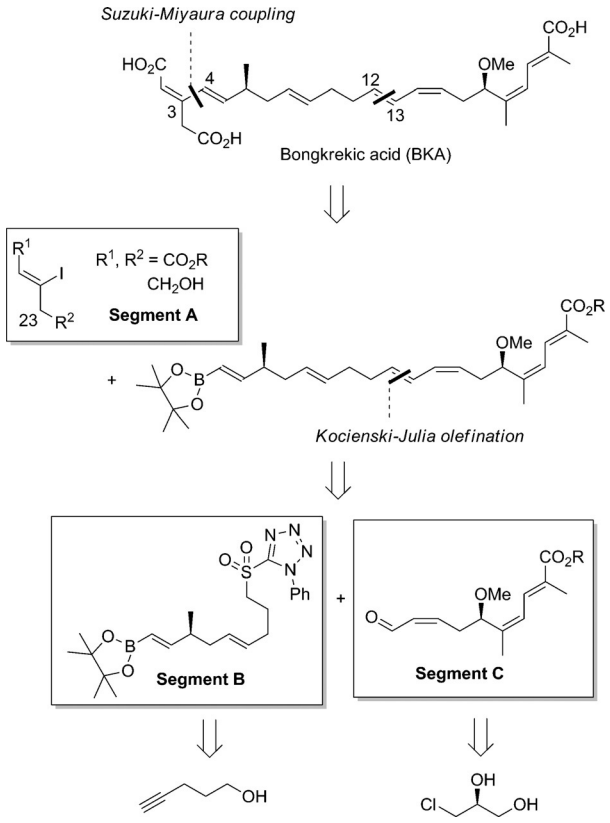
We herein describe the full details of the second-generation synthesis of BKA, which is improved from that described in the preliminary report,[15] a three-component convergent strategy by using a doubly functionalized middle segment, an improved method of final oxidation and purification, and the apoptosis inhibitory activity of BKA and its synthetic analogues.

Results and Discussion

Synthetic strategy

BKA is a long carbon chain compound with three carboxylic acids at the terminal positions. Disconnection of bonds in the retrosynthesis is very important for efficiency in the convergent strategy. In our first-generation synthesis (Scheme 1), BKA was divided into three segments, A, B, and C, at which the C3–C4 and the C10–C11 bonds were disconnected. The C3–C4 bond was constructed by Suzuki–Miyaura coupling, and the C10–C11 bond was formed by an allyl–allyl coupling of a sulfonyl anion. After combining the three segments, however, it took 15 steps to complete the total synthesis. The efficiency of the convergent strategy was reduced by the final steps.

To achieve an efficient synthesis by taking advantage of the convergent strategy, the coupling of each segment should be carried out in the last stage, and the number of the final transformations of functional groups after the coupling of each segment should be as low as possible. Therefore, each segment should have minimal protecting groups, ideally no protecting groups, and/or the same protecting groups for removal at one time. Based on this concept, a second-generation synthetic strategy was designed, as shown in Scheme 2. Segments A and B are coupled through Suzuki–Miyaura coupling at the C3–C4 bond. The disconnection of segments B and C is carried out at the C12–C13 *trans*-alkene, which is constructed by Kocienski–Julia olefination. In the first-generation synthesis, segment A had diol moieties, which were oxidized into dicarboxylic acids after the construction of the BKA carbon skeleton. This final oxidation sequence required many steps and had low yields, which reduced the total yield. Based on this result, it was considered necessary for the terminal functions at the C1 and C24 positions of segment A to be carboxylates to minimize the number of oxidation steps required after coupling.

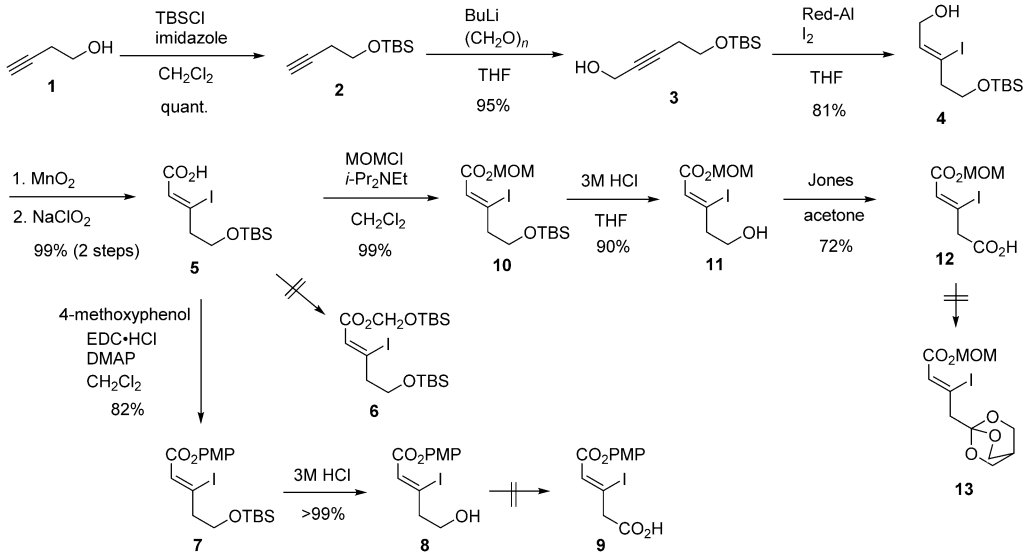


Scheme 2. Synthetic strategy for the second-generation synthesis of BKA.

Synthesis of segment A

The issues for the molecular design of segment A were the terminal oxygen functionalities and their protecting groups. Ideally, carboxylic acids or their esters would have been preferred as the oxygen functionalities, R^1 and R^2, to avoid an oxidation step at the final conversion. The ester moiety had to be carefully selected because the final deprotection of the ester to give the carboxylic acid was complicated. Although published studies reported that the methyl ester was hydrolyzed to the carboxylic acid,[12] we were unsuccessful in achieving basic hydrolysis of the trimethyl esters of BKA. The diesters (R^1 and R^2) of the segment A moiety are easily isomerized to the *E* form, which is not easily separable, leading to less bioactive iso-BKA with even weak bases because of the high acidity of an allylic proton at C23. The ester must be converted into a carboxylic acid under neutral or acidic conditions. Based on these findings, we attempted to prepare several types of segment A (**6**, **9**, **12**) as substrates for Suzuki–Miyaura coupling (Scheme 3).

3-Butyn-1-ol (**1**) was silylated and the lithium acetylide of **2** was then homologated with paraformaldehyde to form **3**, which had the carbon skeleton of segment A. Hydroalumination of **3** with Red-Al, followed by iodination, afforded (*Z*)-allyl-



Scheme 3. Synthesis of segment A. Red-Al = sodium bis(2-methoxyethoxy)aluminum hydride, PMP = *p*-methoxyphenyl, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP = 4-dimethylaminopyridine.

ic alcohol **4** without any isomers. Oxidation of allylic alcohol **4** with MnO_2, followed by Pinnick oxidation[17] of the resulting aldehyde, provided (*Z*)-carboxylic acid **5** in excellent yield.[14] In the first-generation synthesis of BKA, we used a *tert*-butyldimethylsiloxymethyl ester at the C1 moiety, which was easily hydrolyzed by treatment with acids in reasonable yield. However, we could not prepare the corresponding ester **6** in this case, probably due to its instability. We then tried to prepare the PMP ester of **5**, and successfully obtained ester **7** in good yield without isomerization. The TBS group on C24 was removed with 3 M HCl to afford **8** in high yield, although fluoride reagents, such as tetra-*n*-butylammonium fluoride (TBAF) and HF–pyridine gave not **8**, but an alkynyl compound in low yield through elimination of iodide. Attempts at mild oxidation of **8** to form carboxylic acid **9** via an aldehyde were not successful. We also prepared MOM ester **10** from **5** quantitatively without isomerization. Careful treatment of **10** with 3 M HCl gave alcohol **11** in high yield. Successive oxidations of **11** were attempted with various mild oxidizing reagents, such as the Dess–Martin reagent, pyridinium dichromate (PDC), and Swern oxidation, but were unsuccessful, probably because of the instability of the aldehyde intermediate. However, Jones oxidation successfully afforded carboxylic acid **12** in acceptable yield. Further protection of the carboxylic acid as *ortho*-ester **13** was unsuccessful. Thus, we decided to use the MOM esters **11** and **12** as candidate for segment A that were expected to be hydrolyzed under mildly acidic conditions.

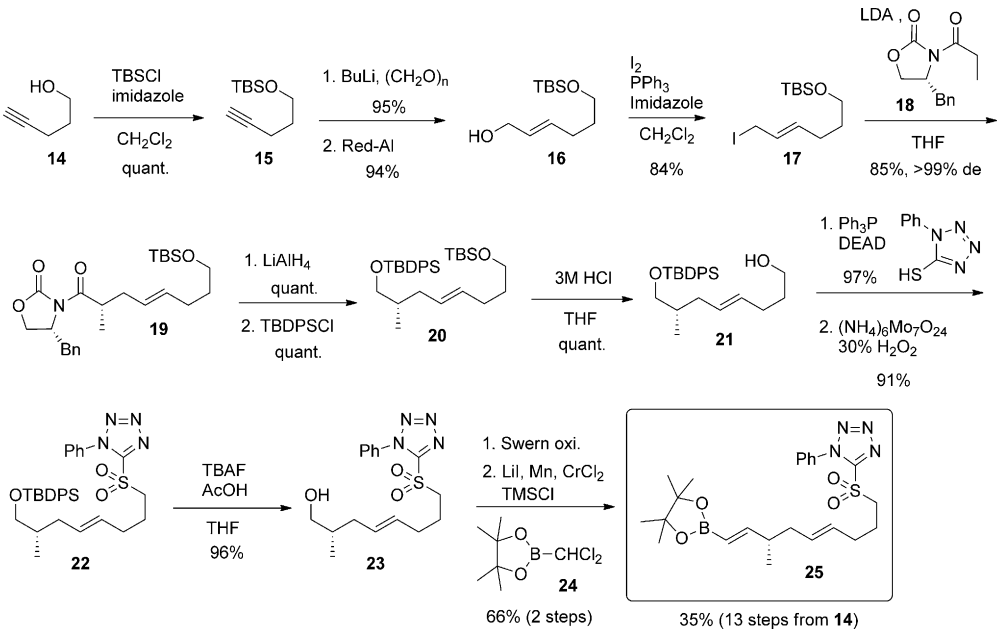
Synthesis of segment B

The key aspect of the design and preparation of segment B was the introduction of functional groups into both ends of segment B for coupling with segments C and A (Scheme 4). Alkenyl boronate was used for the Suzuki–Miyaura coupling[18] and sulfonyl tetrazole for Kocienski–Julia olefination[19] (or their precursors); these moieties were embedded in segment B at each terminal. 6-*tert*-Butyldimethylsiloxy-2-hexenol (**16**), prepared from 4-pentyn-1-ol (**14**) by three steps, was converted into iodide **17**, which was subjected to Evans asymmetric alkylation[20] with oxazolidinone **18** to afford **19** as a single isomer. After reduction with $LiAlH_4$, the primary alcohol was protected with a TBDPS group to give **20** in excellent yield. The selective removal of the TBS group, followed by the Mitsunobu reaction and oxidation, afforded sulfonyl tetrazole **22** in good yield. Compound **22** was a candidate for segment B containing a sulfonyl tetrazole and a precursor of a boronate. Another segment B candidate, which was expected to lead to a more efficient synthetic pathway, was compound **25**, which had a boronic ester in addition to the sulfonyl tetrazole at each end. Doubly terminally functionalized compound **25** was prepared from **22** through desilylation, Swern oxidation, and the Takai reaction with boronic ester **24**.[21] Although there had been concerns regarding the stability and functional compatibility, compound **25** was obtained in excellent yield without isomerization and was stable.

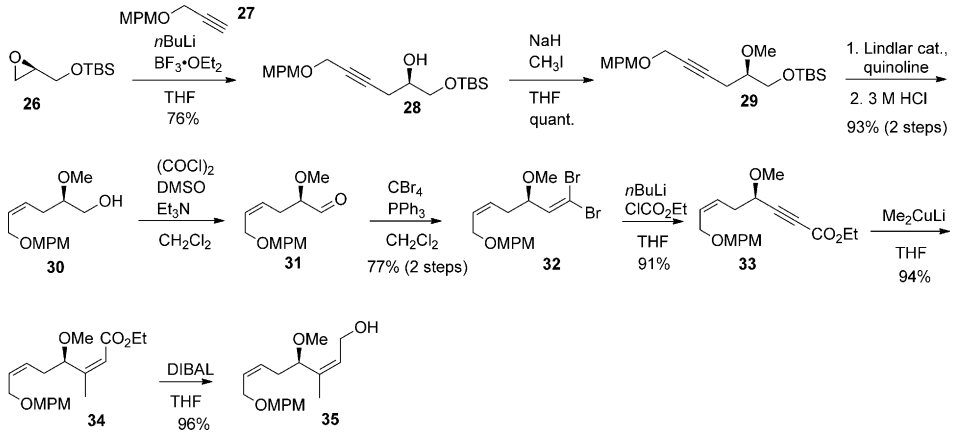
Synthesis of segment C

Segment C has a multisubstituted conjugated diene coupled with the MOM ester, a *cis*-alkene, and an asymmetric center. Alkynylation of readily available (*S*)-glycidyl ether **26**, prepared from enantiomerically pure (*S*)-3-chloropropane-1,2-diol, with the lithium acetylide of *p*-methoxyphenylmethyl (MPM)-protected propargyl alcohol **27**, followed by methylation, afforded **29**, which was subjected to Lindlar reduction to give *cis*-alkene **30** after removal of the TBS group under acidic conditions (Scheme 5). This partial reduction of **29** should be carried out carefully and monitored with TLC to avoid over-reduction. Alcohol **30** was oxidized by means of the Swern protocol to provide aldehyde **31**. Although the Ohira–Bestmann reagent[22] led to alkynylation in moderate yield, the Corey–Fuchs alkynylation,[23] followed by in situ carboxylation, afforded alkyne **33** in excellent yield. The *cis*-selective conjugate addition[24] of a cuprate, and then successive treatment with DIBAL, afforded the requisite trisubstituted alkene **35**.

The next crucial step was construction of the unsaturated MOM ester. Attempts to prepare Wittig reagents with MOM ester **36** or carboxylic acid **37** were unsuccessful, presumably because of their instability (Figure 2).



Scheme 4. Synthesis of segment B. LDA = lithium diisopropylamide, TBDPS = *tert*-butyldiphenylsilyl, DEAD = diethyl azodicarboxylate.



Scheme 5. Synthesis of segment C. DMSO = dimethyl sulfoxide, DIBAL = diisobutylaluminum hydride.

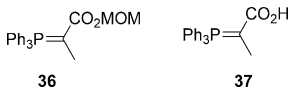
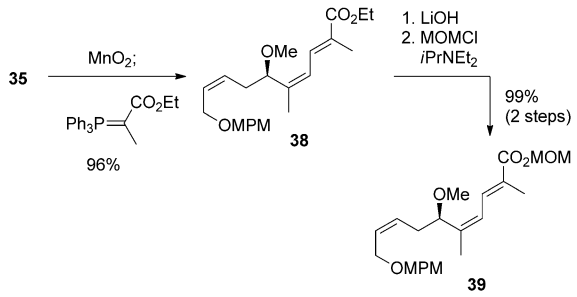
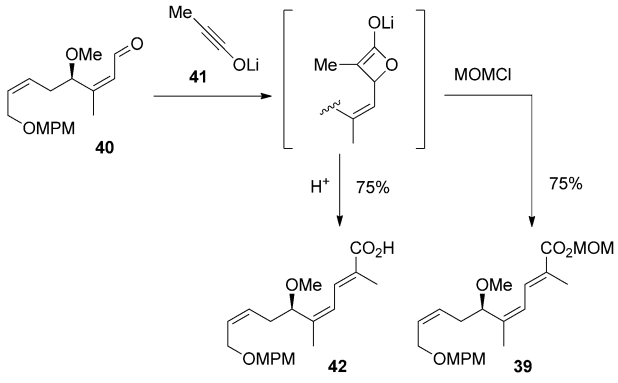


Figure 2. Attempted synthesis of the Wittig reagents **36** and **37**.



Scheme 6. Synthesis of MOM ester **39**.

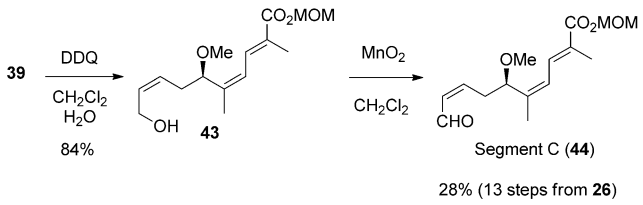


Scheme 7. Torquoselective olefination with lithium ynolate.

Thus, allylic alcohol **35** was subjected to MnO_2 oxidation, followed by an in situ Wittig reaction, to give ethyl ester **38**, which was then hydrolyzed and esterified with MOMCl to afford MOM ester **39** (Scheme 6).

Although the two-step transesterification resulted in a good yield of the product, direct formation of the MOM ester along with olefination would be considerably more efficient. As an alternative method, torquoselective olefination via ynolates[25] was employed for the one-pot esterification (Scheme 7). This method can directly provide unsaturated esters from aldehydes. Isolated aldehyde **40** was reacted with lithium ynolate **41**, prepared by treatment of ethyl 2,2-dibromopropanoate with *t*BuLi,[26] to give the unsaturated carboxylic acid **42** after protonation in 75% yield. Direct in situ esterification with MOMCl successfully gave conjugated MOM ester **39** in similar yield to that of a single isomer. This procedure would be also useful for constructing unsaturated esters, which are not directly available from the Wittig reaction.

Elimination of the MPM group on **39** was performed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in good yield after careful workup, whereas ceric ammonium nitrate (CAN) oxidation did not work well (Scheme 8). Subsequent MnO_2 oxidation of *cis*-allylic alcohol **43** was also carefully employed in anhydrous CH_2Cl_2 at room temperature, because it was labile to lead to isomerization to give the *trans*-alkenyl aldehyde, to afford segment C (**44**) in good yield.

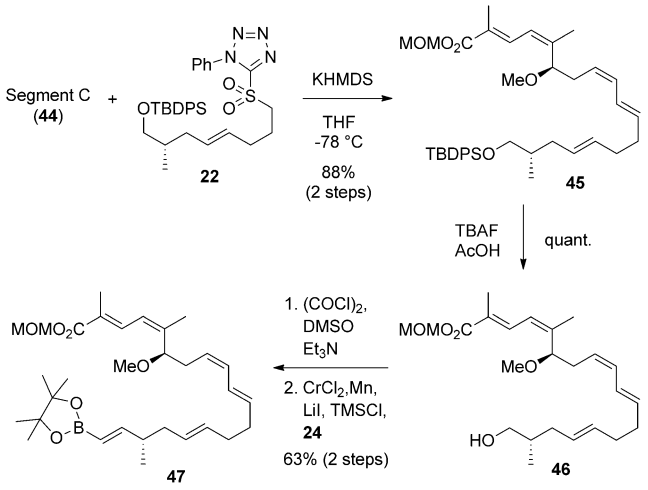


Scheme 8. Synthesis of segment C.

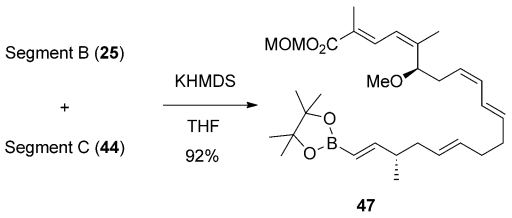
Three-segment coupling

With the three segments in hand, we addressed the final challenge of the three-component coupling (Scheme 9). The Kocienski–Julia olefination of segment C (**44**) with segment B candidate **22** in the presence of KHMDS successfully gave coupled product **45** with excellent *E* selectivity in high yield. After removal of the TBDPS group with TBAF/AcOH, followed by Swern oxidation, the resulting aldehyde was subjected to $CrCl_2$-catalyzed borylalkenylation to give alkenyl boronic ester **47**, which corresponded to segment B–C.

To reduce the number of functional group transformations after coupling, we then carried out Kocienski–Julia olefination by using **25** as segment B to successfully obtain (*E*)-olefin **47** in better yield (Scheme 10), because the alkenyl boronic ester group was stable enough under basic conditions. The improved version of segment B reduced the number of steps.



Scheme 9. Kocienski–Julia olefination of **22** with segment C (**44**). KHMDS = potassium hexamethyldisilazide, TMS = trimethylsilyl.



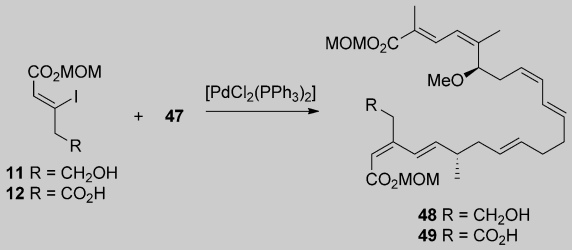
Scheme 10. Kocienski–Julia olefination of segment B (**25**) with segment C (**44**).

The Suzuki–Miyaura coupling of **47** was attempted by using dicarboxylic acid **12** as segment A, but **49** was not obtained (Table 1, entries 1 and 2). Elimination of the highly acidic proton on C23 in **12** might lead to an allene or alkyne. Alternatively, coupling with **11** as segment A successfully afforded desired product **48**, which had the entire BKA skeleton, in excellent yield (Table 1, entry 3).

Completion of synthesis

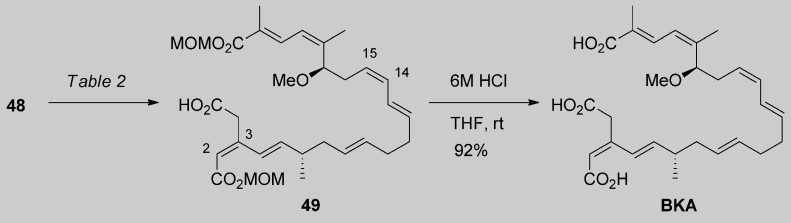
The final stage, including oxidation, deprotection, and purification of BKA, had issues to be solved. Successive oxidations of alcohol **48** under mild conditions with Dess–Martin oxidation, followed by Pinnick oxidation, gave carboxylic acid **49** in modest yield, with several side products, such as the C2–C3 *E* isomer and C14–C15 *E* isomer (Table 2, entry 1). We could not easily separate some side products from BKA, and the yields were not reproducible. Therefore, upon consideration of the instability of the aldehyde intermediate, we examined the one-pot oxidation of alcohol **48** by a modified Altmann protocol[27] (Table 2, entry 2). This approach improved the yield by up to 95%, but isomerized side products were still generated. The ^{1}H NMR spectrum of the aldehyde intermediate trapped after Dess–Martin oxidation revealed that the alkenes of the intermediate were not isomerized under the conditions of this oxidation, which suggested that isomerization occurred during Pinnick oxidation. Hence, we attempted the direct oxidation of **48** to give carboxylic acid **49** under various conditions (Table 2, entries 3–7). TEMPO-catalyzed $NaClO_2$/NaOCl oxidation, CuCl-catalyzed *tert*-butyl hydroperoxide oxidation, and CrO_3-catalyzed oxidation[28] were unsuitable for the oxidation of **48**, but Jones oxidation at 0 °C under highly diluted conditions afforded desired carboxylic acid **49** in moderate yield, along with isomers. Careful extraction from an aqueous solution at pH 5 after quenching with isopropanol was essential for a reproducible yield because more acidic conditions (pH 1) during extraction caused decomposition of the product. Finally, we success-

Table 1. Suzuki–Miyaura coupling of alkenyl boronic ester **47**.



Entry	Segment A	Additive	Conditions	Product
1	**12**	Cs_2CO_3	DMF, RT	complex mixture
2	**12**	NEt_3	MeOH, RT	complex mixture
3	**11**	NEt_3	MeOH, RT	**48**: 86%

Table 2. Oxidation of alcohol **48**.



Entry	Conditions	Results	**49**/Isomers
1	1) DMP; 2) $NaClO_2$, NaH_2PO_4	35%	1:0–1:6
2	DMP then $NaClO_2$, NaH_2PO_4 (one pot)	95%	2.5:1–1:1
3	$NaClO_2$, NaOCl, TEMPO	complex mixture	–
4	CuCl, *t*BuOOH	complex mixture	–
5	CrO_3, H_5IO_6	trace	–
6	Jones reagent, acetone (0.1 M), 0 °C	24%	–
7	Jones reagent, acetone (6 mM), 0 °C	54%	6:1–1:1
8	1-Me-AZADO (0.2 equiv), $PhI(OAc)_2$[a]	99%	>20:1

[a] In acetate buffer (pH 4.0). TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl, 1-Me-AZADO = 1-methyl-2-azaadamantane-*N*-oxyl.

fully found that one-step oxidation of **48** was successfully achieved with 1-Me-AZADO to afford **49** in excellent yield, without detectable isomers being formed (Table 2, entry 8).[29] Although this reaction was very sluggish under phosphate buffer (pH 6.8) conditions, with the use of more acidic acetate buffer (pH 4.0) it was dramatically accelerated to give carboxylic acid **49** in the presence of a catalytic amount of 1-Me-AZADO with $PhI(OAc)_2$ as a co-oxidant.[30] Because of its instability and high polarity, BKA must be carefully purified and the side products should be removed from MOM ester **49** by column chromatography at this stage.

The purified MOM ester **49** could be deprotected with 6 M HCl in THF without the formation of geometrical isomers. In this case, the pH value of the aqueous solution during workup must be around five to seven or the yield will be low. The spectra of synthetic BKA were identical to that of the natural product reported by Berends et al.,[10] except for the optical rotation.[31,32] This synthesis contains 17 steps in the longest linear sequences (total of 37 steps) with 20% overall yield. We have already synthesized more than 10 mg of BKA with this procedure, and continue to supply BKA and its derivatives for biochemical research.

Preparation of BKA analogues

After the efficient total synthesis of BKA was established, we investigated the identification of the pharmacophore and the development of more easily available BKA analogues with potent apoptosis inhibitory activity. However, there are few reports of structure–activity relationship (SAR) studies of BKA to date,[33] because of synthetic intractability, although several total syntheses of BKA have been reported. Because we were initially interested in the role of the carboxylic acid groups in BKA, four analogues, **50**–**53**, in which some of the three carboxylic acids were transformed into hydroxyl or trityl ether groups, and the structurally simplified analogue **54**, in which a methyl group on the C17 asymmetric center was removed and the alkene (C14–C15) was reduced, were designed to gain a preliminary insight into the structural features that influenced the apoptosis inhibitory activity of BKA (Figure 3).

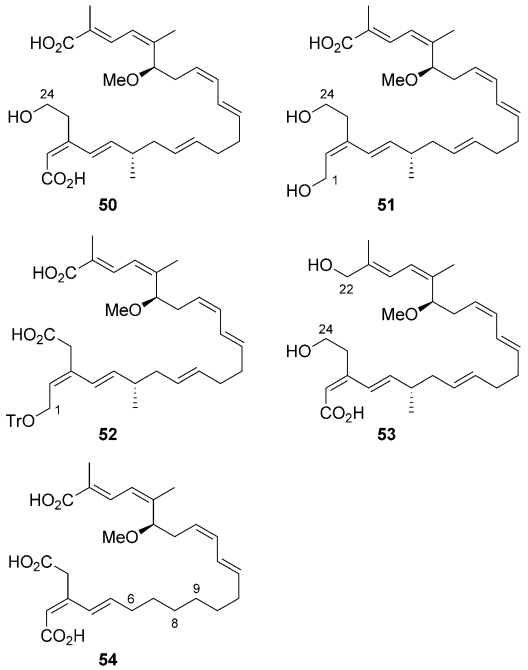
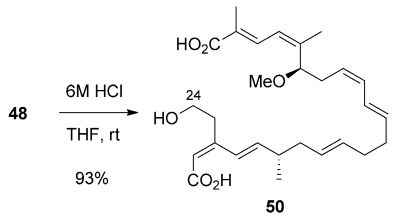


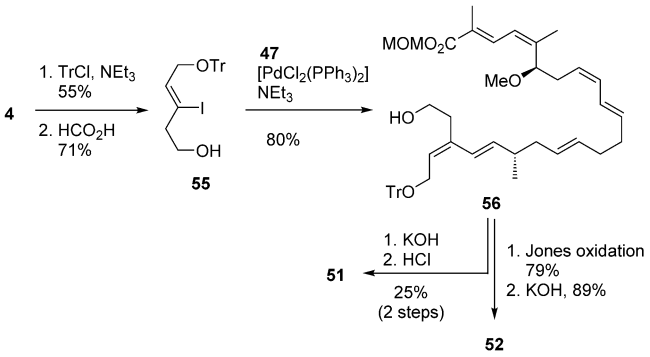
Figure 3. Structures of some synthetic BKA analogues.

The C24–hydroxyl analogue **50** was prepared in good yield through removal of the MOM ester in **48** under acidic conditions (Scheme 11).

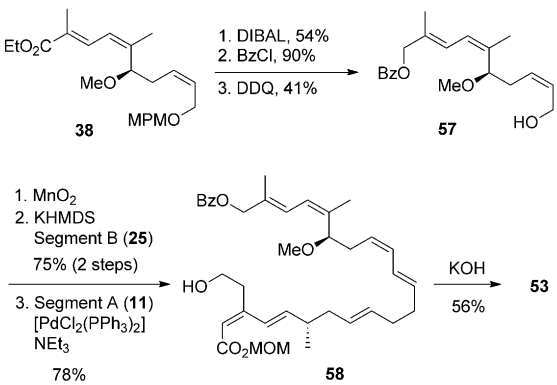
The synthesis of analogues **51** and **52** commenced with the tritylation of alcohol **4** and selective removal of the TBS group with formic acid to afford trityl ether **55** (Scheme 12). According to our synthesis of BKA, iodide **55** was coupled with alkenyl boronic ester **47** under palladium-catalyzed Suzuki–Miyaura conditions to give desired product **56** in good yield. Because various attempts at the simultaneous removal of both the



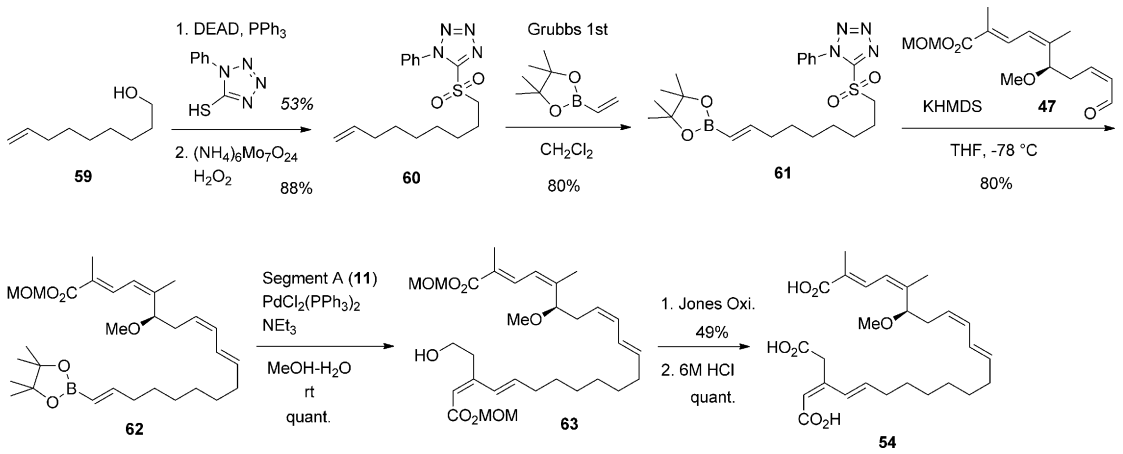
Scheme 11. Synthesis of BKA analogue **50**.



Scheme 12. Synthesis of BKA analogues **51** and **52**.



Scheme 13. Synthesis of BKA analogue **53**.



Scheme 14. Synthesis of BKA analogue **54**.

MOM ester and trityl ether of **56** under acidic conditions were unsuccessful, we developed a two-step protocol, in which the MOM ester was first hydrolyzed under basic conditions and the trityl ether was then carefully removed with 6 M HCl to afford the C1·C24–diol analogue **51**. Jones oxidation of alcohol **56** and subsequent basic hydrolysis of the MOM ester gave the C1–trityl ether analogue **52**.

The synthesis of analogue **53** is depicted in Scheme 13. The α,β-unsaturated ester **38** was converted into alcohol **57** through DIBAL reduction, benzoylation of the resulting primary alcohol, and removal of the MPM group with DDQ. MnO_2 oxidation of **57**, followed by Kocienski–Julia olefination with segment B (**25**), and successive Suzuki–Miyaura cross-coupling with segment A (**11**) led to three-component-coupled **58** in good yield. Basic hydrolysis of the benzoate and MOM ester in **58** afforded the C22·C24–diol analogue **53** in 56% yield after purification by HPLC.

The Mitsunobu reaction of 8-nonen-1-ol (**59**) with 1-phenyltetrazole-5-thiol, followed by oxidation, afforded the sulfonyl tetrazole **60** (Scheme 14). Cross metathesis with pinacol vinylboronate in the presence of Grubbs first-generation catalyst afforded segment B2 (**61**) in good yield. Segment B2 (**61**) was deprotonated with KHMDS, followed by reaction with aldehyde **47** to afford olefinated compound **62** in 80% yield. Suzuki–Miyaura coupling of **62** with segment A (**11**) under standard conditions afforded alcohol **63** in excellent yield. Careful oxidation of **63** with Jones reagent and removal of the MOM groups of the resulting carboxylic acid gave analogue **54**.

Apoptosis inhibitory activities of synthetic BKA and its analogues

A SAR study was conducted by using the synthesized BKA and its five analogues. BKA is known to inhibit apoptosis induced by various reagents in many different cell types.[4,34] We estimated the apoptosis inhibitory activity by the WST-1 assay by measuring the viability of HeLa cells treated with staurosporine (STS), which is a well-known apoptosis inducer.[35] As previously reported,[36,37] pretreatment of the synthesized BKA dose-dependently increased cell viability compared with treatment with 100 nM of STS only (Figure 4a).[38] We evaluated the bioactivities of BKA analogues to identify the biologically important structural attributes of BKA. We recently demonstrated that the structurally simple tricarboxylic acids, which were not equipped with the olefin, two conjugated alkenes, three methyl groups, and methoxy group of BKA, suppressed STS-induced cell death and reduced the mitochondrial inner membrane potential in comparison with BKA (Figure 5).[36] Further-

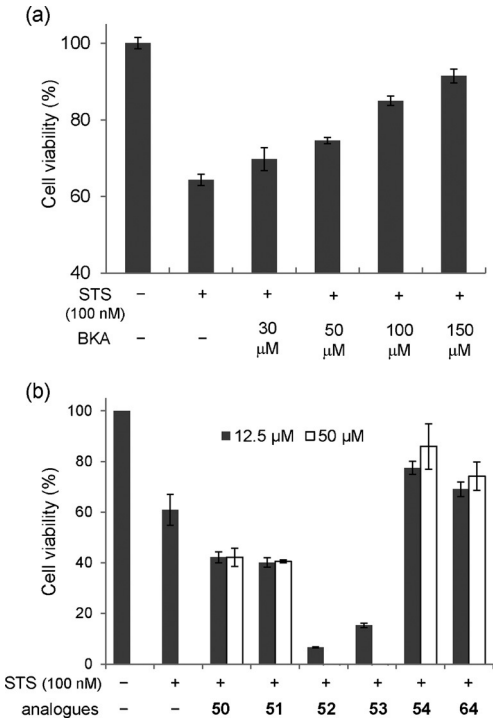


Figure 4. Apoptosis inhibitory activity. Cultured HeLa cells were pretreated with BKA or BKA analogues for 2 h. Apoptosis was induced in cells by exposure to 100 nM STS for 22 h together with BKA or BKA analogues. Cell viability was determined by the WST-1 assay. Each value represents the mean ± standard deviation of three experiments. a) Synthetic BKA, b) BKA analogues. Black and white columns show the results observed at 12.5 or 50 μM, respectively.

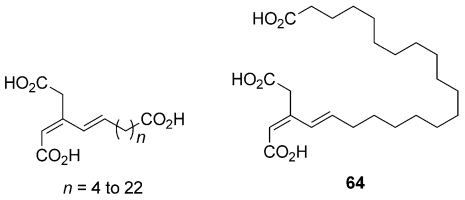


Figure 5. Structures of apoptosis inhibitors.[36]

more, because we found that the length of the carbon chain was critical for apoptosis inhibition, and compound **64**, which had the same length of chain as that of BKA, exhibited potent apoptosis inhibitory activity, compound **64** was used as a positive control. Among the compounds tested, C1–trityl ether analogue **52** and C22·C24–diol **53** were highly cytotoxic against HeLa cells, while C24–hydroxyl analogue **50** and C1·C24–diol **51** showed weak cytotoxicity (Figure 4b). In contrast, BKA analogue **54**, which possessed three carboxylic acid groups, significantly recovered cell viability from STS-induced cell death in a pattern similar to that of **64**. These results indicated that all three carboxylic acid groups of BKA played an important role in apoptosis inhibitory activity. From the observation that C22·C24–diol **53** enhanced the toxic effect of STS compared with that of C24–hydroxyl analogue **50**, but C1·C24–diol **51** showed a similar level of cytotoxicity to that of **50**, we speculated that the hydroxy group in place of a carboxylic acid at the C22 position was most crucial of the three groups for cell toxicity. Treatment of HeLa cells with analogues **54** and **64**, which possessed three carboxylic acid groups, did not affect cell viability (see the Supporting Information). These findings show that the methyl group at C6 and the C8–C9 alkene moiety may not be essential for bioactivity. Further SAR exploration of BKA is necessary to design and develop more highly potent apoptosis inhibitors.

Conclusion

The second-generation total synthesis of (−)-BKA has been achieved. Our total synthesis was based on a convergent strategy that involved sequential three-component coupling to deliver BKA in 17 steps in the longest linear sequence. Notably, the last stage of the synthesis was achieved in only four steps after preparation of the three segments (A, B, and C): Kocienski–Julia coupling, Suzuki–Miyaura coupling, 1-Me-AZADO oxidation, and acidic deprotection; meanwhile 19 steps were needed after the preparation of each segment in our first-generation synthesis (32 steps in the longest linear sequence). It also enabled us to synthesize a series of BKA analogues for preliminary SAR studies and led to the development of potent apoptosis inhibitor **54**, which was more easily synthesized than BKA itself. The total number of steps for the construction of **54** was 10 steps fewer than that of BKA because of the structural simplification of segment B. Our present work provides important insights into the SAR of BKA[39] and a useful biological tool for mechanistic investigations into apoptosis. Further studies are currently underway to assess the biological potential and develop molecular probes to investigate cellular behavior.

Experimental Section

General

^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ by using JEOL JNM-EX-270 (^{1}H NMR at 270 MHz), JNM-AL-400 (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz), and JNM-ECA-600 spectrometers (^{1}H NMR at 600 MHz, ^{13}C NMR at 150 MHz) with standard references (^{1}H NMR at 0.00 ppm (TMS), ^{13}C NMR at 77.0 ppm ($CDCl_3$)). Chemical sifts are reported in ppm. Multiplicities are given by using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; sept, septet; m, multiplet; br, broad. IR spectra were recorded on Shimadzu FTIR-8300 and IRPrestige-21 spectrometers. Mass spectra and high-resolution mass spectra were obtained on JMS-K9, JEOL JMS-700, and Shimadzu LCMS-2010EV mass spectrometers. Elemental analyses were performed with a YANACO 026 CHN analyzer. Melting points were measured with a Yanaco MP-500D apparatus and Büchi 535 melting point apparatus and are uncorrected. TLC was performed on precoated plates (0.25 mm, silica gel Merck $60F_{245}$). Column chromatography was performed on silica gel (Kanto Chemical Co., Inc). Preparative HPLC was performed on a system utilizing a HITACHI L-6250 Intelligent Pump with a gradient solvent system of hexane and ethyl acetate and UV detector L-7400 at $\lambda = 254$ nm, and also a system utilizing a JASCO PU-2087 Intelligent Pump with Dynamic Mixer MX-2080–32 and UV detector UV-2075 and RI detector RI-2031. All reactions were performed under in air, unless otherwise noted. Dry CH_2Cl_2, Et_2O, and THF were purchased from Kanto Chemical Co., Inc., and other solvents were distilled. Unless otherwise noted, reagents were obtained from chemical sources and without further purification.

Syntheses

(2*R*)-1-(*tert*-Butyldimethylsiloxy)-6-(4-methoxybenzyloxy)-hex-4-yn-2-ol (28): *n*BuLi (94.9 mL, 150 mmol, 1.6 M in hexane) was added to a solution of alkyne **27** (26.6 g, 150 mmol) in THF (150 mL), cooled to −78 °C under argon, and stirred at −78 °C for 1 h. BF_3·OEt_2 (21.5 mL, 150 mmol) was added at −78 °C. The mixture was stirred for 10 min and a solution of oxirane **26** (18.8 g, 100 mmol) in THF (100 mL) was added. After being stirred at −78 °C for 1 h, the mixture was quenched with a saturated aqueous solution of $NaHCO_3$; extracted with AcOEt; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 15:85) to afford **28** as a colorless oil (28.0 g, 76%). $[\alpha]_D^{27} = -5.8$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.09$ (s, 6H), 0. 90 (s, 9H), 2.45–2.52 (m, 2H), 3.62 (dd, $J = 5.9$, 10.0 Hz, 1H), 3.72 (dd, $J = 4.1$, 10.0 Hz, 3H), 3.77–3.84 (m, 1H), 3.81 (s, 3H), 4.12 (t, $J = 2.2$ Hz, 2H), 6.85 (d, $J = 8.8$ Hz, 2H), 7.27 ppm (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.3$ (q), 18.3 (s), 23.5 (t), 25.8 (q), 55.2 (q), 57.2 (t), 65.6 (t), 70.2 (d), 71.0 (t), 78.1 (s), 82.7 (s), 113.7 (d), 129.4 (s), 129.5 (d), 159.2 ppm (s); IR (neat): $\tilde{\nu} = 3448$, 2283, 1612, 1513, 1250, 1073 cm^{-1}; MS (ESI): *m/z*: 387 [$M^+ + Na$]; HRMS (ESI): *m/z* calcd for $C_{20}H_{32}O_4NaSi$ [$M^+ + Na$]: 387.1968; found: 387.1963.

(2*R*)-*tert*-Butyl-[2-methoxy-6-(4-methoxybenzyloxy)hex-4-ynyloxy]-dimethylsilane (29): Sodium hydride (1.9 g, 47.5 mmol) and methyl iodide (7.9 mL, 127 mmol) were added to a solution of alcohol **28** (11.6 g, 31.7 mmol) in THF (300 mL), cooled to 0 °C under argon, and

then stirred at RT for 2 h. The mixture was quenched with H_2O; extracted with AcOEt; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:6 to 1:4) to afford **29** as a pale yellow oil (11.2 g, quant). $[\alpha]_D^{21} = -2.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 0.07$ (s, 6H), 0. 90 (s, 9H), 2.42–2.60 (m, 2H), 3.34–3.45 (m, 1H), 3.46 (s, 3H), 3.71 (d, $J = 5.3$ Hz, 2H), 3.81 (s, 3H), 4.13 (t, $J = 2.1$ Hz, 2H), 4.52 (s, 2H), 6.88 (d, $J = 6.7$ Hz, 2H), 7.28 ppm (d, $J = 6.7$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.4$ (q), 18.3 (s), 20.9 (t), 25.8 (q), 25.9 (q), 55.2 (q), 57.3 (q), 57.8 (t), 63.8 (t), 70.9 (d), 77.4 (s), 80.2 (t), 83.4 (s), 113.7 (d), 129.7 (d), 159.3 ppm (s); IR (neat): $\tilde{\nu} = 1514$ cm^{-1}; MS (EI): *m/z* (%): 377 [$M^+ - H$], 121 (100); HRMS (EI): *m/z* calcd for $C_{21}H_{33}O_4Si$ [$M^+ - H$]: 377.2146; found: 377.2141.

(2*R*)-2-Methoxy-6-(4-methoxybenzyloxy)hex-4-en-1-ol (30): Quinoline (0.11 mL, 0.93 mmol) and Lindlar catalyst (382 mg) were added to a solution of alkyne **29** (3.8 g, 10.1 mmol) in hexane (50 mL) at RT and then stirred under a H_2 balloon for 30 min. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo to give a crude product, which was used without further purification. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 0.05$ (s, 6H), 0.89 (s, 9H), 2.20–2.35 (m, 2H), 3.23 (quin, $J = 5.8$ Hz, 1H), 3.40 (s, 3H), 3.55 (dd, $J = 5.8$, 10.4 Hz, 1H), 3.60 (dd, $J = 5.8$, 10.4 Hz, 1H), 3.80 (s, 3H), 4.07 (d, $J = 6.8$ Hz, 2H), 4.43 (s, 2H), 5.62–5.72 (m, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 7.27 ppm (d, $J = 8.7$ Hz, 2H).

The crude alkene was dissolved in THF (100 mL) and treated with 3 M HCl (16.8 mL, 50.5 mmol) at RT for 1 h. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$; extracted with AcOEt; and then the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:1) to afford **30** as a colorless oil (2.5 g, 93%, 2 steps). $[\alpha]_D^{21} = -24.8$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.22$ (t, $J = 6.2$ Hz, 1H), 2.25–2.42 (m, 2H), 3.26–3.35 (m, 1H), 3.40 (s, 3H), 3.41–3.68 (m, 2H), 3.81 (s, 3H), 4.04 (d, $J = 6.2$ Hz, 2H), 4.35 (s, 2H), 5.56–5.78 (m, 2H), 6.88 (d, $J = 8.6$ Hz, 2H), 7.27 ppm (d, $J = 8.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 28.4$ (t), 55.2 (q), 57.1 (q), 63.1 (t), 65.2 (t), 72.1 (d), 80.9 (t), 113.8 (d), 128.4 (d), 128.9 (d), 129.5 (d), 130.1 (s), 159.2 ppm (s); IR (neat): $\tilde{\nu} = 3445$, 1612, 1514, 1248 cm^{-1}; MS (EI): *m/z* (%): 266 [M^+], 121 (100); HRMS (EI): *m/z* calcd for $C_{15}H_{22}O_4$ [M^+]: 266.1518; found: 266.1518.

(5*R*)-1-(7,7-Dibromo-5-methoxyhepta-2,6-dienyloxymethyl)-4-methoxybenzene (32): DMSO (75.7 mL, 80.7 mmol) was added to a solution of oxalyl chloride (5.6 mL, 64.6 mmol) in CH_2Cl_2 (140 mL), cooled to $-78\,^\circ$C under argon, and stirred at $-78\,^\circ$C for 10 min. A solution of alcohol **30** (7.2 g, 26.9 mmol) in CH_2Cl_2 (10 mL) was then added. After 30 min, triethylamine (28.2 mL, 202 mmol) was added and the resulting mixture was stirred at $-78\,^\circ$C for 30 min, then stirred at RT for 30 min. H_2O was added and the mixture was extracted with CH_2Cl_2; the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give crude product **31**, which was used without further purification. The crude aldehyde (3.6 g) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. Carbon tetrabromide (13.4 g, 40.4 mmol) and triphenylphosphine (21.2 g, 80.7 mmol) were successively added and the reaction mixture was stirred at RT for 25 min. The resulting mixture was diluted with hexane, filtered through a pad of Celite, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:9 to 1:7) to afford **32** as a pale yellow oil (4.3 g, 77%, 2 steps). $[\alpha]_D^{22} = +18.3$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.24$–2.42 (m, 2H), 3.32 (s, 3H), 3.81 (s, 3H), 3.93 (dt, $J = 6.5$, 8.4 Hz, 1H), 4.05 (d, $J = 6.2$ Hz, 2H), 4.44 (s, 2H), 5.55–5.79 (m, 2H), 6.33 (d, $J = 8.4$ Hz, 1H), 6.88 (d, $J = 7.0$ Hz, 2H), 7.27 ppm (d, $J = 7.0$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 32.4$ (t), 55.3 (q), 57.0 (q), 65.4 (t), 72.0 (d), 80.8 (t), 91.8 (s), 113.8 (d), 127.2 (d), 129.3 (d), 129.4 (d), 130.4 (s), 139.0 (d), 159.2 ppm (s); IR (neat): $\tilde{\nu} = 1514$, 1248 cm^{-1}; MS (EI): *m/z*: 420 [$M^+ + 2$]; HRMS (EI): *m/z* calcd for $C_{16}H_{20}{}^{79}Br^{81}BrO_3$ [$M^+ + 2$]: 419.9760; found: 419.9765.

(4*R*)-Ethyl 4-methoxy-8-(4-methoxybenzyloxy)oct-6-en-2-ynoate (33): *n*BuLi (86.4 mL, 225 mmol, 2.5 M in hexane) was added to a solution of alkene **32** (45 g, 107 mmol) in THF (1 L), cooled to $-78\,^\circ$C under argon, and stirred at $-78\,^\circ$C for 2 h. Ethyl chloroformate (20.5 mL, 214 mmol) was then added. After being stirred at $-78\,^\circ$C for 1.5 h, the reaction mixture was quenched with H_2O; extracted with AcOEt; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:8 to 1:6) to afford **33** as a pale yellow oil (32.3 g, 91%). $[\alpha]_D^{25} = +30.1$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.31$ (t, $J = 6.6$ Hz, 3H), 2.53 (dd, $J = 6.6$, 7.2 Hz, 2H), 3.42 (s, 3H), 3.81 (s, 3H), 4.04–4.09 (m, 3H), 4.23 (q, $J = 6.6$ Hz, 3H), 4.44 (s, 2H), 5.62–5.80 (m, 2H), 6.88 (d, $J = 7.8$ Hz, 2H), 7.26 ppm (d, $J = 7.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 14.0$ (q), 33.2 (t), 55.3 (q), 57.1 (q), 62.1 (t), 65.4 (t), 70.4 (d), 71.9 (t), 77.9 (s), 85.1 (s), 113.7 (d), 113.8 (d), 126.6 (d), 129.4 (d), 130.0 (d), 130.3 (s), 153.2 (s), 159.2 ppm (s); IR (neat): $\tilde{\nu} = 1713$ cm^{-1}; MS (EI): *m/z*: 332 [M^+]; HRMS (EI): *m/z* calcd for $C_{19}H_{24}O_5$ [M^+]: 332.1624; found: 332.1627.

(4*R*)-Ethyl 4-methoxy-8-(4-methoxybenzyloxy)-3-methylocta-2,6-dienoate (34): MeLi (15.4 mL, 16.0 mmol, 1.0 M in Et_2O) was added to a suspension of copper(I) iodide (15.6 g, 8.0 mmol) in THF (70 mL), cooled to 0 °C under argon, and then cooled to $-78\,^\circ$C. A solution of alkynoate **33** (1.8 g, 5.3 mmol) in THF (30 mL) was added. After being stirred at $-78\,^\circ$C for 40 min, the mixture was quenched with H_2O; extracted with AcOEt; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:8 to 1:7) to afford **34** as a pale yellow oil (1.8 g, 94%). $[\alpha]_D^{24} = +75.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.27$ (t, $J = 7.2$ Hz, 3H), 1.83 (s, 3H), 2.27 (ddd, $J = 6.0$, 6.0, 14.8 Hz, 1H), 2.41 (ddd, $J = 4.8$, 8.0, 14.8 Hz, 1H), 3.23 (s, 3H), 3.80 (s, 3H), 4.06 (d, $J = 4.4$ Hz, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 4.31 (s, 2H), 5.11 (dd, $J = 6.0$, 8.0 Hz, 1H), 5.60–5.72 (m, 2H), 5.82 (s, 1H), 6.87 (d, $J = 8.4$ Hz, 2H), 7.27 ppm (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 14.2$ (q), 18.2 (q), 32.2 (t), 55.2 (q), 57.0 (q), 59.8 (t), 65.5 (t), 71.7 (t), 77.8 (d), 113.7 (d), 119.3 (d), 128.3 (d), 128.6 (d), 129.4 (d), 130.5 (s), 159.0 (s), 159.1 (s), 165.7 ppm (s); IR (neat): $\tilde{\nu} = 1713$, 1248 cm^{-1}; MS (FAB): *m/z*: 349 [$M^+ + H$]; HRMS (FAB): *m/z* calcd for $C_{20}H_{29}O_5$ [$M^+ + H$]: 349.2015; found: 349.2019.

(4*R*)-4-Methoxy-8-(4-methoxybenzyloxy)-3-methylocta-2,6-dien-1-ol (35): DIBAL (24.1 mL, 23.3 mmol, 0.98 M in hexane) was added to a solution of ester **34** (1.6 g, 4.7 mmol) in THF (65 mL), cooled to $-78\,^\circ$C under argon, and then stirred at $-78\,^\circ$C for 1.5 h. The reaction mixture was quenched with H_2O, stirred at RT for 1 h, diluted with AcOEt, filtered through a pad of Celite, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:1) to afford **35** as a colorless oil (1.4 g, 96%). $[\alpha]_D^{25} = +23.1$ (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.66$ (s, 3H), 2.24 (ddd, $J = 7.2$, 7.2, 14.0 Hz, 1H), 2.42 (ddd, $J = 6.8$, 6.8, 14.0 Hz, 1H), 3.20 (s, 3H), 3.81 (s, 3H), 3.99 (dd, $J = 6.8$, 7.2 Hz, 1H), 4.01 (d, $J = 6.8$ Hz, 2H), 4.01–4.18 (m, 2H), 4.44 (s, 2H), 5.50–5.74 (m, 3H), 6.88 (d, $J = 8.8$ Hz, 2H), 7.28 ppm (d, $J =$

8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.4 (q), 31.7 (t), 55.2 (q), 56.0 (q), 57.9 (t), 65.3 (t), 71.9 (t), 78.4 (d), 113.7 (d), 128.3 (d), 128.86 (d), 128.89 (d), 129.4 (d), 130.1 (s), 137.3 (s), 159.1 ppm (s); IR (neat): $\tilde{\nu}$ = 3418 cm^{-1}; MS (EI): *m/z*: 307 [M^++H]; HRMS (EI): *m/z* calcd for $C_{18}H_{27}O_4$ [M^++H]: 307.1909; found: 307.1905.

(6*R*)-Methoxymethyl 6-methoxy-10-(4-methoxybenzyloxy)-2,5-dimethyldeca-2,4,8-trienoate (39): MnO_2 (438 mg, 4.6 mmol) was added to a solution of alcohol **35** (70 mg, 0.23 mmol) in benzene (5 mL) and stirred at 50 °C for 50 min. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo to give crude product **40**, which was used without further purification. *t*BuLi (0.31 mL, 0.53 mmol, 1.7 M in pentane) was added to a solution of ethyl dibromopropionate[40] (34 mg, 0.13 mmol) in THF (0.5 mL), cooled to −78 °C under argon, and stirred at −78 °C for 15 min and then stirred at 0 °C for 30 min. A solution of crude aldehyde **40** (20 mg, 0.066 mmol) in THF (1 mL) was added and the resulting mixture was stirred at RT for 75 min and then MOMCl (25 μL, 0.33 mmol) was added at 0 °C. After being stirred at RT for 2 h, the reaction mixture was quenched with a saturated solution of NH_4Cl; extracted with Et_2O; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:8 to 1:7) to afford **39** as a pale yellow oil (20 mg, 75%). $[\alpha]_D^{24}$ = +52.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.83 (s, 3H), 1.97 (s, 3H), 2.25 (ddd, *J* = 7.2, 8.0, 14.4 Hz, 1H), 2.48 (ddd, *J* = 7.2, 7.2, 14.4 Hz, 1H), 3.21 (s, 3H), 3.48 (s, 3H), 3.80 (s, 3H), 4.04 (d, *J* = 6.8 Hz, 2H), 4.31 (dd, *J* = 7.2, 7.2 Hz, 1H), 4.43 (s, 2H), 5.32 (s, 2H), 5.48–5.74 (m, 2H), 6.37 (d, *J* = 12.4 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.58 ppm (d, *J* = 12.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 18.7 (q), 32.0 (t), 55.2 (q), 56.4 (q), 57.5 (q), 65.4 (t), 71.8 (t), 78.3 (d), 90.5 (t), 113.7 (d), 124.9 (d), 126.2 (s), 128.2 (d), 128.6 (d), 129.4 (d), 130.3 (s), 132.8 (d), 146.0 (s), 159.1 (s), 167.9 ppm (s); IR (neat): $\tilde{\nu}$ = 1711 cm^{-1}; MS (EI): *m/z*: 404 [M^+]; HRMS (EI): *m/z* calcd for $C_{23}H_{32}O_6$ [M^+]: 404.2199; found: 404.2191.

(6*R*)-Methoxymethyl 10-hydroxy-6-methoxy-2,5-dimethyldeca-2,4,8-trienoate (43): DDQ (14 mg, 0.060 mmol) was added to a solution of ether **39** (10 mg, 0.025 mmol) in CH_2Cl_2/H_2O (10:1, 0.5 mL) and stirred at RT for 51 h. The reaction mixture was treated with a saturated solution of $NaHCO_3$; extracted with CH_2Cl_2; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:2 to 1:1) to afford **43** as a pale yellow oil (6.0 mg, 84%). $[\alpha]_D^{21}$ = −17.9 (*c* = 0.84, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.87 (s, 3H), 1.98 (s, 3H), 2.20 (ddd, *J* = 6.4, 6.4, 14.0 Hz, 1H), 2.58 (ddd, *J* = 8.0, 8.0, 14.0 Hz, 1H), 3.22 (s, 3H), 3.50 (s, 3H), 4.09 (dd, *J* = 6.8, 12.8 Hz, 1H), 4.21 (dd, *J* = 7.6, 12.8 Hz, 1H), 4.32 (dd, *J* = 6.4, 8.0 Hz, 1H), 5.33 (d, *J* = 9.6 Hz, 1H), 5.36 (d, *J* = 9.6 Hz, 1H), 5.53–5.85 (m, 2H), 6.38 (d, *J* = 12.0 Hz, 1H), 7.59 ppm (d, *J* = 12.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1 (q), 18.8 (q), 32.0 (t), 56.5 (q), 57.5 (q), 57.8 (t), 78.1 (d), 90.6 (t), 124.5 (d), 126.2 (s), 128.1 (d), 131.4 (d), 132.8 (d), 146.2 (s), 168.0 ppm (s); IR (neat): $\tilde{\nu}$ = 3435, 1709 cm^{-1}; MS (EI): *m/z*: 284 [M^+]; HRMS (EI): *m/z* calcd for $C_{15}H_{24}O_5$ [M^+]: 284.1624; found: 284.1625.

(6*R*,17*S*)-Methoxymethyl 6-methoxy-2,5,17-trimethyl-19-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)nonadeca-2,4,8,10,14,18-hexaenoate (47): MnO_2 (280 mg, 2.9 mmol) was added to a solution of alcohol **43** (21 mg, 0.073 mmol) in benzene (2 mL) and stirred at RT for 1 h. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo to give crude product **44**, which was used without further purification. KHMDS (0.29 mL, 0.15 mmol, 0.5 M in toluene) was added dropwise to a solution of segment B (25, 40 mg, 0.085 mmol) in THF (1 mL), cooled to −78 °C under argon, and stirred at −78 °C for 30 min. A solution of **44** (21 mg, 0.073 mmol) in THF (1 mL) was added dropwise and the resulting solution was stirred at −78 °C for 3.5 h. The reaction mixture was quenched with H_2O; extracted with Et_2O; and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:9 to 1:6) to afford **47** as a colorless oil (35 mg, 92%). $[\alpha]_D^{22}$ = +73.4 (*c* = 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (d, 3H, *J* = 6.8 Hz), 1.27 (s, 12H), 1.85 (s, 3H), 1.94 (ddd, *J* = 7.2, 7.2, 13.6 Hz, 1H), 1.97 (s, 3H), 2.0–2.16 (m, 5H), 2.23 (ddd, *J* = 6.8, 6.8, 13.6 Hz, 1H), 2.38 (ddd, *J* = 7.2, 7.6, 14.4 Hz, 1H), 2.60 (ddd, *J* = 6.8, 7.2, 14.4 Hz, 1H), 3.22 (s, 3H), 3.48 (s, 3H), 4.34 (dd, *J* = 6.8, 7.6 Hz, 1H), 5.20 (ddd, *J* = 7.2, 7.2, 11.2 Hz, 1H), 5.33 (s, 2H), 5.35–5.42 (m, 3H), 5.68 (dt, *J* = 6.8, 15.2 Hz, 1H), 6.00 (dd, *J* = 11.2, 11.2 Hz, 1H), 6.27 (dd, *J* = 11.2, 15.2 Hz, 1H), 6.38 (d, *J* = 12.4 Hz, 1H), 6.56 (dd, *J* = 6.8, 18.0 Hz, 1H), 7.59 ppm (d, *J* = 12.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 12.2 (q), 18.6 (q), 18.7 (q), 24.7 (q), 32.0 (t), 32.4 (t), 33.0 (t), 39.1 (t), 39.4 (d), 56.4 (q), 57.5 (q), 78.4 (d), 83.0 (s), 90.5 (t), 124.1 (d), 124.9 (d), 125.0 (d), 125.5 (d), 126.1 (s), 128.7 (d), 130.6 (d), 131.1 (d), 133.0 (d), 135.1 (d), 146.2 (s), 159.4 (d), 168.0 ppm (s); IR (neat): $\tilde{\nu}$ = 1713, 1635, 1362 cm^{-1}; MS (FAB): *m/z*: 551 [M^++Na]; HRMS (FAB): *m/z* calcd for $C_{31}H_{49}BNaO_6$ [M^++Na]: 551.3520; found: 551.3516.

(6*R*,17*S*)-Bis(methoxymethyl) 20-(2-hydroxyethyl)-6-methoxy-2,5,17-trimethyldocosa-2,4,8,10,14,18,20-heptaenedioate (48): A solution of segment A (**11**, 73 mg, 0.26 mmol) in MeOH (2 mL) was added to a solution of $[PdCl_2(PPh_3)_2]$ (18 mg, 0.026 mmol) in MeOH (1 mL) at RT and stirred for 10 min. NEt_3 (0.22 mL, 1.5 mmol) and a solution of **47** (135 mg, 0.26 mmol) in MeOH (2 mL) were successively added and the resulting mixture was stirred at RT for 8 h. The mixture was evaporated to give a crude product, which was purified by column chromatography on silica gel (AcOEt/hexane 1:3 to 1:1) and HPLC (Kanto Migthysil Si 60 250–10 (5 μm), AcOEt/hexane 3:2, 5.0 mL min^{-1}) to afford **48** as a pale yellow oil (124 mg, 86%). $[\alpha]_D^{24}$ = +65.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.0 Hz, 3H), 1.52 (br s, 1H), 1.86 (s, 3H), 1.97 (s, 3H), 2.05 (ddd, *J* = 7.2, 7.2, 13.8 Hz, 1H), 2.08–2.15 (m, 5H), 2.34–2.44 (m, 2H), 2.58 (ddd, *J* = 7.2, 7.8, 13.8 Hz, 1H), 2.58–2.68 (m, 2H), 3.22 (s, 3H), 3.47 (s, 3H), 3.48 (s, 3H), 3.77 (dt, *J* = 6.0, 6.6 Hz, 2H), 4.34 (t, *J* = 7.2 Hz, 1H), 5.20 (dt, *J* = 7.2, 11.4 Hz, 1H), 5.27 (s, 2H), 5.33 (s, 2H), 5.32–5.43 (m, 2H), 5.67 (dt, *J* = 6.6, 15.0 Hz, 1H), 5.70 (s, 1H), 5.99 (dd, *J* = 10.8, 11.4 Hz, 1H), 6.11 (dd, *J* = 7.8, 15.6 Hz, 1H), 6.27 (dd, *J* = 10.8, 15.0 Hz, 1H), 6.38 (d, *J* = 12.0 Hz, 1H), 7.51 (d, *J* = 15.6 Hz, 1H), 7.59 ppm (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 12.1 (q), 18.7 (q), 19.4 (q), 32.0 (t), 32.2 (t), 32.8 (t), 37.5 (d), 37.6 (t), 39.8 (t), 56.3 (q), 57.5 (q), 61.8 (t), 78.4 (d), 89.8 (t), 90.5 (t), 116.0 (d), 124.2 (d), 124.8 (d), 124.9 (d), 125.5 (d), 126.0 (s), 128.2 (d), 130.6 (d), 131.5 (d), 132.9 (d), 135.0 (d), 145.0 (d), 146.2 (s), 153.2 (s), 165.4 (s), 168.0 ppm (s); IR (neat): $\tilde{\nu}$ = 3503, 1713 cm^{-1}.

BKA: Acetate buffer (0.75 mL, pH 4), 1-Me-AZADO (0.3 mg, 1.8 μmol), and $PhI(OAc)_2$ (15 mg, 0.046 mmol) were added to a solution of alcohol **48** (5 mg, 8.9 μmol) in CH_2Cl_2 (0.75 mL), cooled to 0 °C under argon, and stirred at RT for 3 h. The reaction mixture was quenched with a saturated solution of $Na_2S_2O_3$ at 0 °C and stirred for 30 min. The resulting mixture was extracted with Et_2O, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel ($CHCl_3$/hexane 1:1) to afford **49** as a pale yellow oil (5.1 mg, 99%). The monocarboxylic acid (**49**; 2.3 mg, 3.6 μmol) was dissolved in THF (0.5 mL) and treated with 6 M HCl (0.5 mL) at 0 °C. The reaction

mixture was stirred at RT for 1 h and then diluted with H_2O. The mixture was extracted with Et_2O and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude product, which was purified by column chromatography on silica gel ($CHCl_3$/hexane 4:1) to afford BKA (1.6 mg, 92%) as a white amorphous solid. $[\alpha]_D^{23} = -51.3$ ($c = 1.5$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.07$ (d, $J = 7.2$ Hz, 3H), 1.88 (s, 3H), 1.88–1.98 (m, 1H), 1.94 (s, 3H), 2.02–2.20 (m, 5H), 2.27 (ddd, $J = 6.0$, 9.0, 13.8 Hz, 1H), 2.32–2.42 (m, 1H), 2.46 (ddd, $J = 5.4$, 7.8, 13.8 Hz, 1H), 3.21 (s, 3H), 3.33 (d, $J = 16.2$ Hz, 1H), 3.47 (d, $J = 16.2$ Hz, 1H), 4.33 (dd, $J = 5.4$, 9.0 Hz, 1H), 5.32–5.44 (m, 3H), 5.72 (s, 1H), 5.74 (dt, $J = 6.6$, 15.0 Hz, 1H), 6.01 (dd, $J = 7.8$, 15.6 Hz, 1H), 6.05 (dd, $J = 10.8$, 12.0 Hz, 1H), 6.30 (dd, $J = 10.8$, 15.0 Hz, 1H), 6.34 (d, $J = 12.6$ Hz, 1H), 7.44 (d, $J = 15.6$ Hz, 1H), 7.64 ppm (d, $J = 12.6$ Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 11.8$ (q), 18.6 (q), 20.5 (q), 32.2 (t), 32.6 (t), 33.2 (t), 38.8 (d), 40.0 (t), 40.7 (t), 56.8 (q), 80.0 (d), 118.2 (d), 124.1 (d), 124.3 (d), 124.7 (d), 125.2 (s), 125.3 (d), 128.1 (d), 131.4 (d), 131.5 (d), 134.6 (d), 135.9 (d), 145.5 (d), 148.5 (s), 148.9 (s), 170.4 (s), 174.7 (s), 176.8 ppm (s); IR (neat): $\tilde{\nu} = 2930$, 1697, 1684 cm^{-1}; MS (FAB): m/z: 509 [$M^+ + Na$]; HRMS (FAB): m/z calcd for $C_{28}H_{38}NaO_7$ [$M^+ + Na$]: 509.2515; found: 509.2509.

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[1] A. G. van Veen, W. K. Mertens, *Recl. Trav. Chim. Pays-Bas* **1934**, *53*, 257–266.
[2] H. Wen-Juan, C. Xial-Ming, M. Hong-De, M. Zhao-He, *Biomed. Environ. Sci.* **1989**, *2*, 65–71.
[3] P. J. F. Henderson, H. A. Lardy, *J. Biol. Chem.* **1970**, *245*, 1319–1326.
[4] a) N. Zamzami, P. Marchetti, M. Castedo, T. Hirsch, S. A. Susin, B. Masse, G. Kroemer, *FEBS Lett.* **1996**, *384*, 53–57. For a review, see: b) D. R. Green, J. C. Reed, *Science* **1998**, *281*, 1309–1312.
[5] M. Muranyi, P.-A. Li, *Neurosci. Lett.* **2005**, *384*, 277–281.
[6] S.-P. Chen, H.-L. Yang, G. M. Her, H.-Y. Lin, M.-F. Jeng, J.-L. Wu, J.-R. Hong, *Virology* **2006**, *347*, 379–391.
[7] L. Malekova, V. Kominkova, M. Ferko, P. Stefanik, O. Krizanova, A. Ziegelhoffer, A. Szewczyk, K. Ondrias, *Biochim. Biophys. Acta Bioenerg.* **2007**, *1767*, 31–44.
[8] The cost of BKA is $875 for 1 mg from BIOMOL.
[9] a) G. W. M. Lumbach, H. C. Cox, W. Berends, *Tetrahedron* **1970**, *26*, 5993–5999; b) G. W. M. Lumbach, H. C. Cox, W. Berends, *Tetrahedron* **1971**, *27*, 1839–1858.
[10] J. de Bruijn, D. J. Frost, D. H. Nugteren, A. Gaudemer, G. W. M. Lijmbach, H. C. Cox, W. Berends, *Tetrahedron* **1973**, *29*, 1541–1547.
[11] J. Zylber, F. Gaudemer, A. Gaudemer, *Experientia* **1973**, *29*, 648–649.
[12] E. J. Corey, J. Tramontano, *J. Am. Chem. Soc.* **1984**, *106*, 462–463.
[13] M. Shindo, T. Sugioka, Y. Umaba, K. Shishido, *Tetrahedron Lett.* **2004**, *45*, 8863–8866.
[14] M. Kanematsu, M. Shindo, M. Yoshida, K. Shishido, *Synthesis* **2009**, 2893–2904.
[15] Y. Sato, Y. Aso, M. Shindo, *Tetrahedron Lett.* **2009**, *50*, 4164–4166.
[16] a) A. Français, A. Leyva, G. Etxebarria-Jardi, S. V. Ley, *Org. Lett.* **2010**, *12*, 340–343; b) A. Français, A. Leyva, G. Etxebarria-Jardi, J. Pena, S. V. Ley, *Chem. Eur. J.* **2011**, *17*, 329–343.
[17] B. S. Bal, W. E. Childers Jr., H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091–2096.
[18] For a review, see: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457–2483.
[19] For a review, see: P. R. Blakemore, *J. Chem. Soc. Perkin Trans. 1* **2002**, 2563–2585.
[20] D. A. Evans, M. D. Ennis, D. J. Mathre, *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
[21] K. Takai, N. Shinomiya, H. Kaihara, N. Yoshida, T. Moriwake, K. Utimoto, *Synlett* **1995**, 963–964.
[22] a) S. Ohira, *Synth. Commun.* **1989**, *19*, 561–564; b) G. J. Roth, B. Liepold, S. G. Müller, H. J. Bestmann, *Synthesis* **2004**, 59–62.
[23] E. J. Corey, P. L. Fuchs, *Tetrahedron Lett.* **1972**, *13*, 3769–3772.
[24] E. J. Corey, J. A. Katzenellenbogen, *J. Am. Chem. Soc.* **1969**, *91*, 1851–1852.
[25] a) M. Shindo, Y. Sato, T. Yoshikawa, R. Koretsune, K. Shishido, *J. Org. Chem.* **2004**, *69*, 3912–3916. For a review, see: b) M. Shindo, S. Mori, *Synlett* **2008**, 2231–2243; c) M. Shindo, S. Mori, *J. Synth. Org. Chem. Jpn.* **2008**, *66*, 28–38.
[26] a) M. Shindo, K. Matsumoto, K. Shishido, *Org. Synth.* **2007**, *84*, 11–21; b) M. Shindo, K. Matsumoto, *Top. Curr. Chem.* **2012**, *327*, 1–32.
[27] F. Glaus, K.-H. Altmann, *Angew. Chem. Int. Ed.* **2012**, *51*, 3405–3409; *Angew. Chem.* **2012**, *124*, 3461–3465.
[28] a) M. Zhao, J. Li, E. Mano, Z. Song, D. M. Tschaen, E. J. J. Grabowski, P. J. Reider, *J. Org. Chem.* **1999**, *64*, 2564–2566; b) S. Mannam, G. Sekar, *Tetrahedron Lett.* **2008**, *49*, 2457–2460; c) M. Zhao, J. Li, Z. Song, R. Desmond, D. M. Tschaen, E. J. J. Grabowski, P. J. Reider, *Tetrahedron Lett.* **1998**, *39*, 5323–5326.
[29] a) M. Shibuya, M. Tomizawa, I. Suzuki, Y. Iwabuchi, *J. Am. Chem. Soc.* **2006**, *128*, 8412–8413; b) T. Watanabe, T. Imaizumi, T. Chinen, Y. Nagumo, M. Shibuya, T. Usui, N. Kanoh, Y. Iwabuchi, *Org. Lett.* **2010**, *12*, 1040–1043.
[30] M. Shibuya, T. Sato, M. Tomizawa, Y. Iwabuchi, *Chem. Commun.* **2009**, 1739–1741.
[31] The specific optical rotation value of our $[\alpha]_D^{23} = -51.3$ ($c = 1.5$, $CHCl_3$) is identical to that of Ley's synthetic BKA $[\alpha]_D^{25} = -47.2$ ($c = 0.175$, $CHCl_3$), and not that of the natural product, see: D. H. Nugteren, W. Berends, *Recl. Trav. Chim. Pays-Bas* **2010**, *76*, 13–27.
[32] The purity of the synthesized BKA was confirmed by HPLC analysis, see the Supporting Information for more details.
[33] Y. Pei, A. K. Carroll, C. M. Anderson, W. H. Moos, S. S. Ghosh, *Synthesis* **2003**, *11*, 1717–1721.
[34] a) S. L. Budd, L. Tenneti, T. Lishnak, S. A. Lipton, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6161–6166; b) I. J. Furlong, C. L. Mediavilla, R. Ascaso, A. L. Rivas, M. K. L. Collins, *Cell Death Differ.* **1998**, *5*, 214–221; c) M. Tafani, D. A. Minchenko, A. Serroni, J. L. Farber, *Cancer Res.* **2001**, *61*, 2459–2466.
[35] a) J.-K. Koh, M. B. Wie, B. J. Gwag, S. L. Sensi, L. M. T. Canzoniero, J. Demaro, C. Csernansky, D. W. Choi, *Exp. Neurol.* **1995**, *135*, 153–159; b) A. J. Krohn, E. Preis, J. H. M. Prehn, *J. Neurosci.* **1998**, *18*, 8186–8197.
[36] K. Okuda, K. Hasui, M. Abe, K. Matsumoto, M. Shindo, *Chem. Res. Toxicol.* **2012**, *25*, 2253–2260.
[37] Recently, Yamada et al. reported thatthe synthesized BKA showed the antiapoptosis effect in HeLa cells treated with VP-16 as an apoptosis inducer. They also demonstrated that the antiapoptosis activity was significantly increased by using a MITO-porter, which is a mitochondrial

delivery system originally developed by them; see: Y. Yamada, K. Nakamura, R. Furukawa, E. Kawamura, T. Moriwaki, K. Matsumoto, K. Okuda, M. Shindo, H. Harashima, *J. Pharm. Sci.* **2013**, *102*, 1008 – 1015.

[38] BKA was slightly cytotoxic against HeLa cells, even at 200 μM of BKA; see also ref. [35].

[39] As far as we know, this is the first SAR study on BKA in terms of the apoptosis inhibitory activity.

[40] M. Shindo, Y. Sato, R. Koretsune, T. Yoshikawa, K. Matsumoto, K. Itoh, K. Shishido, *Chem. Pharm. Bull.* **2003**, *51*, 477 – 478.

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