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Structural and Synthetic Investigations of Tanikolide Dimer, a SIRT2 Selective Inhibitor, and Tanikolide *seco*-Acid from the Madagascar Marine Cyanobacterium *Lyngbya majuscula*

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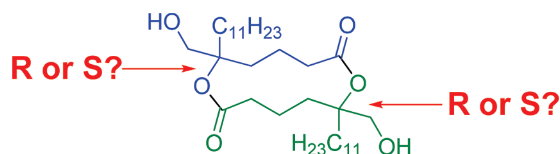

SIRT2 Inhibition IC$_{50}$ = 176 nM to 2.4 μM

Tanikolide *seco*-acid **2** and tanikolide dimer **3**, the latter a novel and selective SIRT2 inhibitor, were isolated from the Madagascar marine cyanobacterium *Lyngbya majuscula*. The structure of **2**, isolated as the pure *R* enantiomer, was elucidated by X-ray experiment in conjunction with NMR and optical rotation data, whereas the depside molecular structure of **3** was initially thought to be a *meso* compound as established by NMR, MS, and chiral HPLC analyses. Subsequent total synthesis of the three tanikolide dimer stereoisomers **4**, **5**, and *ent*-**5**, followed by chiral GC−MS comparisons with the natural product, showed it to be exclusively the *R*,*R*-isomer **5**. Tanikolide dimer **3** (= **5**) inhibited SIRT2 with an IC$_{50}$ = 176 nM in one assay format and 2.4 μM in another. Stereochemical determination of symmetrical dimers such as compound **3** pose intriguing and subtle questions in structure elucidation and, as shown in the current work, are perhaps best answered in conjunction with total synthesis.

Introduction

Tanikolide (**1**) is a biologically active δ-lactone that was originally isolated from the lipid extract of a collection of *Lyngbya majuscula* from Madagascar.[1] Tanikolide possesses a single chiral center, a quaternary carbon with hydroxyl and hydroxymethyl groups, as well as two multicarbon chains and exhibits potent antifungal activity against *Candida albicans* (13 mm diameter zone inhibition using 100 μg/disk). In addition, it shows strong toxicity in the brine shrimp *Artemia salina* and schistosomiasis-carrying snail *Biomphalaria glabrata* (LC$_{50}$ = 3.6 and 9.0 μg/mL, respectively) biological assays. Owing to its juxtaposition of functional groups, tanikolide (**1**) has been the target of numerous enantioselective syntheses.[2] As part of our ongoing search for structurally and pharmacologically interesting substances

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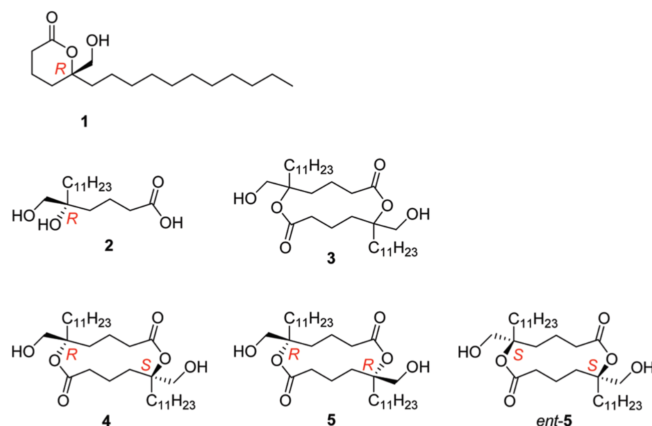
from cyanobacteria,[3-5] a detailed exploration of another Malagasy *L. majuscula* collection was undertaken. Using a human sirtuin type 2 (SIRT2) bioassay-guided approach, we report here the isolation and structural elucidation of both tanikolide *seco*-acid **2** and tanikolide dimer **3**.



SIRT2 is a NAD^+-dependent cytoplasmic protein that is co-localized with HDAC6 on microtubules. SIRT2 has been shown to deacetylate α-tubulin and to control mitotic exit from the cell cycle.[6] Human SIRT2 proteins are closely related in structure to the NAD-dependent deacetylases of other species.[7] The natural substrate for SIRT2 is believed to be p53.[8] Binding of p53 to DNA is activated by its acetylation in the C-terminal domain;[9] thus, deacetylation of p53 by SIRT2 could be important in the regulation of cellular responses to DNA-damaging agents.[10] Because SIRT2 functions to silence apoptotic responses mediated by p53,[11] inhibitors of these proteins are of interest as potential anticancer drugs. SIRT2 inhibitors have also emerged as agents with potential utility in neuroprotection.[12] Tanikolide dimer **3** was found to be a potent inhibitor of SIRT2 (IC_{50} = 176 nM in one assay format; 2.4 μM in another) as well as active in a sodium channel blocking assay (54% inhibition at 5.2 μM). Conversely, the *seco*-acid **2** exhibited only moderate cytotoxicity against the H-460 cancer cell line and was inactive in both the SIRT2 and sodium channel blocking assays.

Determination of the stereochemistry of symmetrical dimeric compounds can be technically challenging because such substances often possess subtle issues of chirality. For example, our initial chiral analysis of tanikolide dimer **3** suggested it to be a *meso* compound (e.g., **4**);[13] however, total chemical synthesis of the three stereoisomers of **3** and comparison by chiral GC–MS showed it to be the *R*,*R*-stereoisomer **5** and of high enantiomeric purity. Here, we report the isolation of this new dilactone as a potent SIRT2 inhibitor, elucidation of its planar structure, and determination of its stereostructure through enantio-specific synthesis and careful comparisons by chiral gas chromatography–mass spectrometry.

Results and Discussion

L. majuscula was collected from near Tanikely Island, Madagascar, extracted with CH_2Cl_2/MeOH (2:1), and fractionated by Si VLC. The resulting fractions were tested for SIRT2 inhibitory activity and revealed that the 40% EtOAc/hexanes eluting fraction was strongly active (IC_{50} = 2.5 μg/mL). This material was subjected to further fractionation by RP solid-phase extraction (SPE), and two fractions (D3 and D4, see the Experimental Section) were found to be active (81% and 75% inhibition at 10 μg/mL, respectively). Analytical reversed-phase HPLC purification of D3 and D4 led to the isolation of tanikolide dimer **3**, which initially showed an IC_{50} = 176 nM to SIRT2. Compound **2** was isolated as a crystalline substance from a VLC fraction eluting with 25% MeOH in EtOAc and was inactive in the SIRT2 assay.

HR FABMS analyses of compound **2** gave an $[M+Na]^+$ ion at m/z 325.2366 for a formula of $C_{17}H_{34}O_4Na$ (one less DBE than tanikolide). ^{13}C NMR revealed the presence of a carbonyl group, which on the basis of its chemical shift was likely that of a carboxylic acid (δ 176.8). The C-5 quaternary carbon shift (δ 74.4) was present in **2**, indicating that hydroxyl, hydroxymethyl, and two alkyl groups were attached at this position. Indeed, by the data presented above and COSY, HSQC, and HMBC, four distinct sections of the molecule were formulated: a hydroxy group, a hydroxymethyl group, a butanoic acid chain, and an undecanyl chain, all of which were connected to the C-5 quaternary carbon (Table 1). Because X-ray quality crystals of **2** were deposited from a fraction eluting with 25% MeOH in EtOAc, these structural features were confirmed via a diffraction study (see the Supporting Information).

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TABLE 1. ^{1}H and ^{13}C NMR Assignments for Tanikolide *seco*-Acid 2[a]

position	δ_C	δ_H
1	176.8	
2	34.6	2.29 (t, J = 6.7 Hz)
3	19.1	1.67 (m)
4	35.5	1.49 (m)
5	74.4	
6	36.2	1.49 (m)
7	22.7	1.29 (br s)
8	29.9	1.29 (br s)
9	29.6	1.29 (br s)
10	29.6	1.29 (br s)
11	29.6	1.29 (br s)
12	29.6	1.29 (br s)
13	29.6	1.29 (br s)
14	32.3	1.29 (br s)
15	23.1	1.29 (m)
16	13.4	0.91 (t, J = 6.9 Hz)
17	67.1	3.59 (d, J = 14.1 Hz) 3.68 (d, J = 14.1 Hz)

[a] 400 MHz ^{1}H and 100 MHz for ^{13}C NMR; CD_3OD as solvent.

The *seco*-acid **2** was found to be optically active, and only a single enantiomer was observed in the X-ray study. Further, when a sample of **2** was analyzed by chiral-phase HPLC, only a single peak was observed. Because the *R*-enantiomer of compound **2** has been chemically synthesized and its rotation measured {[α]$_D$ −0.8 (*c* 1.0, $CHCl_3$}, we conclude from the negative rotation of natural tanikolide *seco*-acid {[α]$^{25}_D$ −10 (*c* 0.87, $CHCl_3$)} that it is also of *R*-configuration at C-5, the same as in tanikolide (**1**) itself.[14]

The parent molecular ion for compound **3** was deduced by various modes of ionization, including FABMS in various matrices, ESIMS, and EIMS. LR FABMS displayed three peaks at *m/z* 591.4 [M + Na]$^+$, *m/z* 569.3 [M + H]$^+$, and *m/z* 551.2 [M − OH]$^+$. HR FABMS of the *m/z* 569.47804 [M + H]$^+$ peak showed a molecular formula of $C_{34}H_{65}O_6$, whereas HR EIMS of the *m/z* 551.4691 [M − OH]$^+$ peak showed a molecular formula of $C_{34}H_{63}O_5$. However, the ^{13}C NMR and DEPT spectra for **3** indicated the presence of only 17 carbon and 31 carbon-bound hydrogen atoms (Table 2). These data combined with the MS information indicated that only half of the signals were appearing in the NMR spectra and suggested that **3** was a symmetrical dimer. The three degrees of unsaturation implied by the molecular formula were accounted for by the presence of two carbonyls (δ 172.1) and one ring.

^{13}C NMR showed the presence of an ester carbonyl (δ 172.1; IR 1716 cm^{-1}), a methylene carbon attached to oxygen (δ 67.9), a quaternary carbon attached to oxygen (δ 86.9), a methyl group (δ 14.5), and 13 high-field methylene carbons. The ^{1}H NMR of **3** showed a pair of mutually coupled doublets (δ 3.68 and 3.59) for an isolated methylene attached to oxygen and a two-proton multiplet (δ 2.51) assigned to a methylene adjacent to a carbonyl group. A series of multiplets in the range δ 1.29−1.95 accounted for five methylene groups, and a broad singlet (δ 1.27) contained an additional 14 degenerate protons. A terminal methyl group was assigned to a three-proton triplet at δ 0.86.

(14) We cannot explain the discrepancy in the magnitude in the optical rotation of our crystalline natural sample of **2** from that of synthetic material, except to note that the rotation of the natural material was run on an older, less sensitive, and possibly inaccurate polarimeter (Perkin-Elmer 141).

TABLE 2. ^{1}H and ^{13}C NMR Assignments for Tanikolide Dimer 3[a]

position	δ_C	δ_H		HMBC
1/1′	172.1			
2/2′	30.2	2.51 (m)	3	1, 3, 4
3/3′	17.1	1.92 (m)	2, 4	1, 5
4/4′	27.0	1.78 (m) 1.95 (m)	3	3, 5
5/5′	86.9			
6/6′	37.1	1.66 (m) 1.75 (m)	7	4, 5, 17
7/7′	23.9	1.29 (m) 1.32 (m)	6	
8/8′	30.4	1.27 (br s)		
9/9′	30.2	1.27 (br s)		
10/10′	30	1.27 (br s)		
11/11′	30	1.27 (br s)		
12/12′	29.9	1.27 (br s)		
13/13′	29.7	1.27 (br s)		
14/14′	32.3	1.27 (br s)	15	
15/15′	23.1	1.30 (m)	14, 16	
16/16′	14.1	0.86 (t, J = 7.2 Hz)	15	15, 14
17/17′	67.9	3.59 (d, J = 11.3 Hz) 3.68 (d, J = 11.3 Hz)		4, 5, 6

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR; $CDCl_3$ as solvent.

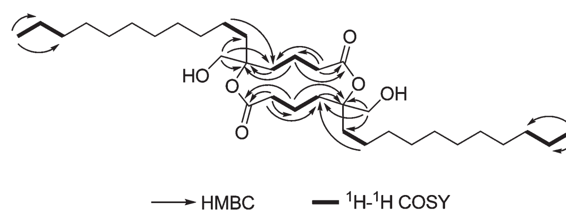
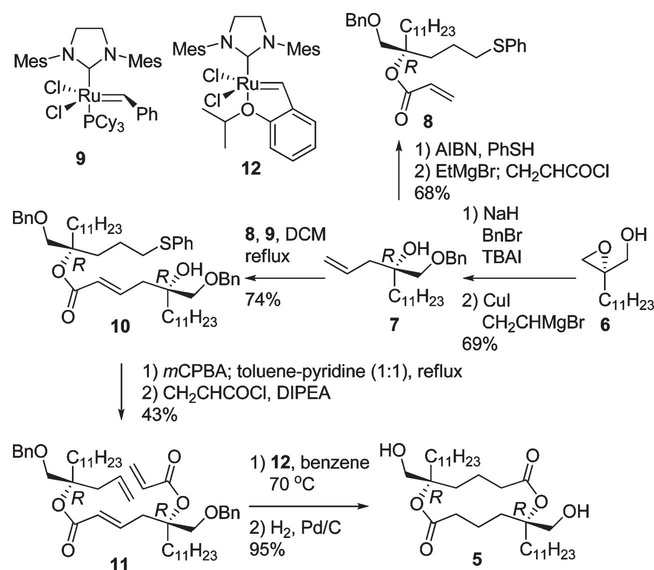


FIGURE 1. Selected HMBC and ^{1}H−^{1}H COSY correlations for tanikolide dimer **3**.

Analyses of ^{1}H−^{1}H COSY and HMBC experiments of **3** led to the deduction that it was closely related to tanikolide (**1**) (Figure 1).[1] For example, the oxymethylene protons at δ 3.68/3.59 (H_2-17) showed HMBC correlations with carbons at δ 86.9 (C-5), 27.0 (C-4), and 37.1 (C-6). Additionally, a pair of methylene protons at δ 2.51 (H_2-2) showed HMBC correlations with carbons at δ 172.1 (C-1), 17.1 (C-3), and 27.0 (C-4). ^{1}H−^{1}H COSY delineated a connected spin system for the methylenes at C-2, C-3, and C-4. A methyl group signal at δ 0.86 (H_3-16) showed HMBC correlations with two methylene carbons at δ23.1 (C-15) and 32.3 (C-14), a sequence confirmed by ^{1}H−^{1}H COSY analysis and, hence, was at the terminus of a long lipid tail. Based on the close concordance in chemical shifts and coupling patterns between **3** and **1**, and the MS analysis described above, compound **3** was deduced to be a symmetrical dimer of tanikolide (**1**).

Because tanikolide (**1**) and its *seco*-acid **2** had both been isolated as optically active metabolites and their lone stereocenters determined to be *R* (Mosher ester for the former and comparison with optical rotation for the latter), it was surprising that dimer **3** initially gave no rotation at the sodium D-line or any other wavelength examined [however, remeasurement of the optical rotation of natural tanikolide dimer on a more sensitive instrument subsequently gave an [α]$^{25}_D$ +1.0 (*c* 1.2, $CHCl_3$)]. This perplexing initial result was further examined by acid hydrolysis of **3** and analysis by chiral HPLC, first with diode array detection (DAD) and then with ESI LCMS. In both cases, a 1:1 ratio of two peaks

SCHEME 1. Synthesis of (*R*,*R*)-Tanikolide Dimer 5



was obtained which appeared to be due to the *seco* acid, suggesting that **3** was a *meso* compound deriving from one *R*-monomer and one *S*-monomer. To clarify this surprising result, we turned to an integrated total chemical synthesis approach of the three stereochemical possibilities for tanikolide dimer along with chiral GC–MS analysis. Remarkably, this approach gave quite different and unexpected results as described below.

The known epoxy alcohol **6** served as the starting material for the synthesis of (5*R*,5′*R*)-tanikolide dimer **5**. The tertiary alcohol **7** was prepared from the benzyl ether derivative of **6** via reaction with vinylmagnesium bromide in the presence of cuprous iodide. Radical-mediated reaction of **7** with thiophenol and then reaction with acryloyl chloride produced acrylate **8**. Cross-metathesis reaction of **7** and **8** in the presence of the second-generation Grubbs catalyst[15] in hot dichloromethane led to the formation of the thioether **10**. Thermal elimination reaction of the sulfoxide derivative of **10** was carried out in hot toluene–pyridine, and the diene acrylate **11** was prepared from the elimination product. Ring-forming metathesis reaction of **11** in the presence of Hoveyda–Grubbs catalyst **12**[16] proceeded smoothly, and (5*R*,5′*R*)-tanikolide dimer **5** was obtained in high yield upon hydrogenation–hydrogenolysis (Scheme 1). The synthesis of (5*S*,5′*S*)-tanilkolide dimer *ent*-**5** was carried out in the same manner using the enantiomeric starting material.

The synthesis of (5*R*,5′*S*)-tanikolide dimer **4** also started with *ent*-**6**. Cross metathesis reaction between *ent*-**7** and **8** produced a new thioether **13**, which was converted into the diene acrylate **14** via oxidation–elimination and acrylate formation. Ring-forming metathesis reaction of **14** and hydrogenation–hydrogenolysis led to the formation of (5*R*,5′*S*)-tanikolide dimer **4** in high yield (Scheme 2).

When the NMR spectra of compounds **4** and **5** were compared, they looked very similar, and it was impractical to distinguish them on this basis (Supporting Information).

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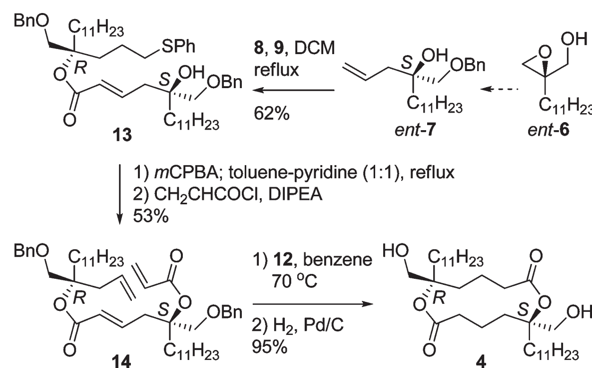
SCHEME 2. Synthesis of (*R*,*S*)-Tanikolide Dimer 4



Moreover, we were unable to develop a convincing HPLC-based method to conduct this analysis. Thus, we turned to a chiral GC–MS approach as described below.

Natural tanikolide dimer **3** and the synthetic stereoisomers (5*R*,5′*S*)-tanikolide dimer **4**, (5*R*,5′*R*)-tanikolide dimer **5**, and (5*S*,5′*S*)-tanikolide dimer *ent*-**5** were analyzed by GC–MS chromatography using a Cyclosil-B chiral column. When (5*R*,5′*S*)-tanikolide dimer **4** was injected, two peaks were observed at 35.99 and 36.37 min, each having a mass spectrum consistent with tanikolide (**1**) or its *seco*-acid **2** (e.g., each of these two compounds give the *seco*-acid following injection into the GC). The presence of two peaks in the chromatogram indicated that the dimer had undergone thermal hydrolysis, probably in the GC injection port. Consistent with this finding, when synthetic compounds **5** and *ent*-**5** were injected separately, they each gave a single peak, the former at 35.99 min and the latter at 36.37 min. When natural tanikolide dimer **3** was analyzed by this protocol, a single peak was observed at 35.99 min, thereby indicating it was composed of two identical monomers, each of *R* configuration.

We believe that the initial chiroptical and chiral HPLC analyses, which suggested that natural tanikolide dimer was a *meso* compound, were flawed for the following reasons. As determined with the synthetic materials, the optical rotations of both the *R*,*R* or *S*,*S* dimers are very weak such that an essentially zero measurement was made with the small amount of natural dimer material that we had originally isolated. This was exacerbated by the use of an older polarimeter in these initial measurements. More significantly, the conditions used for the acid hydrolysis (6 N HCl at 105 °C for 16 h) could very well have led to racemization of the seco acid; retrospectively, racemization of the tertiary alcohol under these vigorous conditions could have been predicted.

Pure compounds **2** and **3** (= **5**) were tested in the SIRT2 inhibitory assay as well as a mammalian cell sodium channel blocking assay.[17] Compound **3** (= **5**) was initially found to have an IC_{50} value of 176 nM, against SIRT2 whereas **2** was inactive at the highest concentration tested (50 μM). Comparing these data with those reported for sirtinol and 8,9-dihydroxybenzo[4,5]furo[3,2-*c*]chromen-6-one (IC_{50} values of 38 and 45 μM respectively)[18] indicates that **3** (= **5**) is

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TABLE 3. Inhibitory Properties of Synthetic Tanikolide Dimers to SIRT1 and SIRT2

compd	SIRT1 IC_{50} + SE (μM)	SIRT2 IC_{50} + SE (μM)
(*R*,*S*)-tanikolide dimer (**4**)	28.8 ± 4.3	2.4 ± 0.2
(*R*,*R*)-tanikolide dimer (**5**)	36.4 ± 8.1	3.3 ± 0.2
(*S*,*S*)-tanikolide dimer (*ent*-**5**)	34.5 ± 3.5	3.1 ± 0.3

200−300-fold more active than either of these agents. In the sodium channel blocking assay, compound **3** induced a 54% inhibition at 5.2 μM, while **2** was again inactive at the highest concentration tested (10 μM). However, cytotoxicity assays revealed that **2** was moderately toxic at 9.9 μM to the human lung H-460 cell line, while **3** did not have any activity at 10 μM. This observation is consistent with the limited cytotoxicity observed with other selective SIRT2 inhibitors[19] and suggests that tanikolide dimer may have more potential for use as a neuroprotectant.

Synthetic production of an additional supply of the natural (5*R*,5′*R*)-tanikolide dimer **5** as well as the stereoisomers (5*S*,5′*S*)-tanilkolide dimer *ent*-**5** and (5*R*,5′*S*)-tanikolide dimer **4** allowed for a further exploration of the SIRT1 and SIRT2 inhibitory properties of this compound class. Unfortunately, the original assay used for measuring these enzyme inhibitory properties was no longer available, but this problem was overcome using another assay system.[20,21] Surprisingly, all three stereoisomers (**4**, **5**, *ent*-**5**) showed similar potencies in their inhibition of SIRT1 (20−36 μM) or SIRT2 (2.4−3.3 μM) but were overall approximately 10-fold more potent to the SIRT2 isoform (Table 3). The decreased potency of natural tanikolide **3** (= **5**) in this second assay system relative to its initial assay may be due to small differences in the way the assays were run, the preparation of the enzymes, the substrates, duration of incubation, or a combination of factors. None of these three isomers showed any activity at 25 μM to a rat liver HDAC preparation composed of a mixture of HDACs,[20,21] and thus, the SIRT inhibitory properties of these tanikolide dimers is not a general and nonspecific property.

A study on novel inhibitors of SIRT2 reported that a phenol or hydroxyl group is important for inhibitory activity based on the structure of the putative SIRT2 active site.[22] These polar functional groups together with a hydrophobic moiety and hydrogen-bonding features are suggested to form an active SIRT2 pharmacophore. In addition, it is believed that SIRT2 inhibitors must sterically block the opening of a narrow channel near the putative active site, adjacent to Ile169 and Asp170, and continuing throughout the length of the enzyme.[22] Interestingly, the functional groups and overall dimeric structure of **3** meet these requirements; however, the results with the synthetic stereoisomers of tanikolide dimer (**4**, **5**, and *ent*-**5**) indicate that chirality is not important to this drug−protein interaction. That the *seco*-acid **2** was inactive despite its containing two hydroxyl groups suggests that either the free carboxyl functionality interferes with binding of the drug or that the entire dimeric structure is sterically required for effective inhibition.

It seems likely that the biosynthesis of tanikolide (**1**), its *seco*-acid **2**, and its dimer **3** proceeds via a PKS biosynthetic pathway. However, the occurrence of a branching carbon atom at C-5(5′) in these metabolites, a site on the polyketide backbone logically deriving from C-1 of acetate, suggests the involvement of an HMGCoA synthase-like enzyme as has recently been deduced in curacin A and jamaicamide A biosynthesis.[23,24] With clarification of the configuration of this stereocenter in tanikolide dimer **3** from combined synthetic/chiral GCMS analysis, it appears that the condensation of acetate with a β-carbonyl functionality occurs with the same chiral preference for both monomers present in the dimer.

Conclusions

In summary, the structure elucidation of tanikolide dimer **3** (= **5**) raised a number of intriguing configurational and biosynthetic questions for further study, and these were effectively answered by the combined approach of synthesis of the three candidate stereoisomers and chiral GC−MS analysis. It was intriguing that all three stereoisomers had equivalent activity in the SIRT2 assay, suggesting an achiral interaction between inhibitor and enzyme. Nevertheless, given tanikolide dimer's potent biological effects to SIRT2, expanding a deeper understanding of its structural subtleties and mechanism of formation is important to developing its lead compound and biotechnological potential.

Experimental Section

Collection, Extraction, and Isolation Procedures. *L. majuscula* was collected by hand using scuba in April 2000 near Tanikely Island, Nosy-Be Madagascar (voucher specimen available from WHG as collection no. MNT-26/Apr/00−02). The alga was stored at −20 °C in 70% EtOH until workup. A total of 944 g (dry wt) of the alga was extracted three times with CH_2Cl_2−MeOH (2:1) to yield 8.73 g of crude extract. A portion of this (1.5 g) was fractionated using vacuum−liquid chromatography (VLC) on Si gel by a stepwise gradient of hexanes−EtOAc and EtOAc−MeOH to give nine fractions (A through I). Fraction D (96.7 mg, 40% EtOAc in hexanes) showed inhibitory activity against SIRT2 and was subjected to further fractionation by solid-phase extraction (NP SPE) to yield five fractions (D1-D5). Fraction D3 and D4 gave 80% and 75% inhibition at 10 μg/mL, respectively, in the SIRT2 assay. Analytical reversed-phase HPLC (Phenomenex Sphereclone ODS, 250 × 10.0 mm, 5 μm, 2.5 mL/min, RI detection, MeOH/H_2O, 95:5) purification of D3 and D4 led to the isolation of 15 mg of **3** (t_R = 8 min) as a colorless oil. Fraction H from the initial Si gel chromatography (25% MeOH-EtOAc) directly deposited crystals of compound **2** in several milligram yield, and these were used in X-ray crystallographic and other experiments without any further purification.

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X-ray Crystallography of Tanikolide *seco*-Acid 2. The crystalline sample of **2** obtained from the fraction eluting with 25:75 MeOH/EtOAc was used without further preparation. Determination of the crystallographic parameters, data collection, and structure solution and refinement were done as described elsewhere,[25] with the following details.

A well-shaped crystal of dimensions 0.30 × 0.10 × 0.02 mm was selected and mounted on the tip of a thin glass fiber using a dab of Paratone.[25] Crystal quality evaluation and preliminary indexing were performed from four images of 10° rotation about ω, each separated from the others by 50°. Proving to be a satisfactory crystal, a full set of 160 frames of 5° rotation about ω was collected. The frames were integrated using the determined unit cell, using the program TwinSolve as included in Rigaku/MSC's software package CrystalClear to yield a redundant data set of 8951 reflections. Correction for the effects of absorption anisotropy was carried out by means of multiscans as programmed in TwinSolve.[26] Finally, a data set consisting of 2294 unique reflections in the range (0−6, −9−9, −24−24) was generated with an R(merge) of 0.1640. The reported unit cell was refined using all 2048 reflections with intensities greater than 10 times their esd's in the range $2.25° < \theta < 64.64°$.

The structure was solved using direct methods as programmed in SHELXS-90[26] and refined using the program SHELXL-97.[27] Although all hydrogen atoms could be clearly identified from the Fourier map, in order to preserve a favorable data-to-parameter ratio the hydrogen atoms were placed in geometrically idealized positions. The hydrogen atoms were given a displacement parameter equal to 1.5 times (methyl group) or 1.2 times (all other hydrogens) the equivalent isotropic displacement parameter of the atom to which it was attached. During the final cycle of least-squares refinement, all non-hydrogen atoms were refined with anisotropic displacement parameters. The refined value of the absolute structure parameter (Flack parameter)[28] of 0.5(7) indicates that no clear indication of the absolute structure of the molecule can be derived from the diffraction experiment alone. An ORTEP[29] of the final model for compound **2** is given in the Supporting Information, with displacement ellipsoids drawn at the 50% probability level.

Initial SIRT2 Inhibitory Assay. The assay was run in 96-well format in a final volume of 50 μL. All reaction components were prepared in buffer A. Blank wells contained no NAD. The reaction was run for 2 h at 37 °C and stopped with 50 μL of stop buffer. Development was allowed to proceed for 20 min, and then the plate was read with a fluorescence plate reader (excitation at 360 nm, emission at 460 nm). Final assay concentrations contained 50 μM fluor de Lys substrate (purchased from Biomol and used according to the manufacturer's instructions at http://www.biomol.com/Online_Catalog/Online_Catalog/Products/Product_Detail/38/?categoryId=226&productId=749&mid=75), 270 nM SIRT2 (12.5 μg/50 μL), 1 mM NAD, ± inhibitor, and adjusted to a 50 μL volume with buffer A. Buffer A was composed of 25 mM Tris−HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$. Stop buffer was composed of 2.7 mg/mL trypsin in buffer A.

Secondary SIRT1 and SIRT2 Inhibitory Assays. These secondary assays for inhibition to the sirtuins and HDACs followed the procedures outlined in Heltweg et al.[20] except that the SIRT1 and SIRT2 enzymes were overexpressed and their activities assayed as described in Uciechowska et al.[21]

Sodium Channel Blocking Assay. Chemicals were evaluated for their capacity to either activate or block sodium channels using the following modifications to the cell-based bioassay of Manger et al.[17] Twenty-four hours prior to chemical testing, mouse neuroblastoma (neuro-2a) cells were seeded in 96-well plates at 6.0×10^4 cells/well in a volume of 200 μL. Test chemicals dissolved in DMSO were serially diluted in medium without fetal bovine serum and added at 10 μL/well. DMSO was less than 0.5% final concentration. Plates to evaluate sodium channel activating activity received 20 μL/well of either a mixture of 3 mM ouabain and 0.3 mM veratridine (Sigma Chemical Co.) in 5 mM HCl or 5 mM HCl in addition to the test chemical. Plates were incubated for 18 h and results compared to similarly treated solvent controls with 10 μL of medium added in lieu of the test chemical. The sodium channel activator brevetoxin PbTx-3 (Calbiochem) was used as the positive control and added at 10 ng/well in 10 μL of medium. Sodium channel blocking activity was assessed in a similar manner except that ouabain and veratridine were 5.0 and 0.5 mM, respectively, and the sodium channel blocker saxitoxin (Calbiochem) was used as the positive control. Plates were incubated for approximately 22 h.

Cytotoxicity Assay. Cytotoxicity was measured in NCI-H460 lung tumor cells and neuro-2a cells using the method of Alley et al.[30] with cell viability being determined by MTT reduction.[17] Cells were seeded in 96-well plates at 6000 cells/well in 180 μL. Twenty-four hours later, the test chemical dissolved in DMSO and diluted into medium without fetal bovine serum was added at 20 μL/well DMSO was less than 0.5% final concentration. After 48 h, the medium was removed and cell viability determined.

Tanikolide *seco*-acid 2:. $[\alpha]^{25}_{D}$ −10 (c 0.87, $CHCl_3$); no UV absorbance observed; IR ν_{max} (neat) 3425, 3410, 2850, 2360, 1715, 1250, 1050, 939 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; LR FABMS (nba) obsd m/z (rel int) 325.3 (100) $[M + Na]^+$; HR FABMS obsd $[M + Na]^+$ m/z 325.23668 for $C_{17}H_{34}O_4Na$ (−1.2 mmu).

Natural tanikolide dimer 3:. initial measurement $[\alpha]^{25}_{D}$ 0 - (c 1.0, $CHCl_3$, Perkin-Elmer 141); second measurement $[\alpha]^{25}_{D}$ +1.0 (c 1.2, $CHCl_3$, JASCO P-2000); UV ($CHCl_3$) λ_{max} 202 nm (log ε = 2); IR ν_{max} (neat) 3420, 3402, 2853, 2360, 1716, 1248, 1049, 940 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; LR FABMS (otg matrix) obsd m/z (rel int) 591.4 (5) $[M + Na]^+$, 569.3 (35) $[M + H]^+$, 267.1 (100); LR FABMS (nba matrix) obs m/z (rel int) 591.4 (7) $[M + Na]^+$, 569.3 (14) $[M + H]^+$, 551.2 (20) $[M - OH]^+$, 533.2 (10) $[M - 2OH - H]^+$, 267.1 (100); LR TOF MS ES+ obsd m/z (rel int) 607.4 (5) $[M + K]^+$, 591.5 (5) $[M + Na]^+$, m/z 569.5 (10) $[M + H]^+$, m/z 551.4 (60) $[M - OH]^+$, and m/z 533.4 (20) $[M - 2OH - H]^+$; HR FABMS obs $[M + H]^+$ m/z 569.47804 for $C_{34}H_{65}O_6$ (0.1 mmu); HR EI MS m/z 551.4691 $[M - OH]^+$ and 533.4567 $[M - 2OH - H]^+$ for $C_{34}H_{63}O_5$ (−0.5 mmu) and $C_{34}H_{61}O_4$ (2.8 mmu), respectively.

Initial Chiral HPLC Analysis of Tanikolide Dimer 3 and Its Acid Hydrolysate. Chiral LCMS analyses of **3** employed a Chirobiotic T (4.6 × 100 mm, 10 μm) column and yielded a single peak (t_R = 3.0 min) using a gradient elution beginning with 70:30 MeOH/H_2O and ending in pure MeOH. Chiral LCMS analyses on the same column of the hydrolysate of **2** (6 N HCl at 105 °C for 16 h) yielded two peaks (t_R = 4.73 and 5.22 min) using an isocratic elution (40:60 H_2O/EtOH).

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Chiral GC−MS Analysis of Natural Tanikolide Dimer 3, (5*R*,5′*S*)-Tanikolide Dimer 4, (5*R*,5′*R*)-Tanikolide Dimer 5, and (5*S*,5′*S*)-Tanikolide Dimer *ent*-5. Natural tanikolide dimer **3** and the synthetic standards **4**, **5**, and *ent*-**5** were analyzed by GC−MS using a Cyclohexil-B chiral column. All of the samples were analyzed using a ramp from 80 to 240 °C at 7 °C/min. Natural tanikolide dimer **3** gave a single peak at 35.99 min with the following mass spectrum (the dimer thermally hydrolyzes in the GC-injection inlet to its component "monomers"): EIMS *m*/*z* 253 (38), 225 (34), 129 (70), 97 (52), 57 (57), 55 (100). Synthetic (*R*,*S*)-tanikolide dimer **4** gave two peaks at 35.99 min [EIMS *m*/*z* 253 (52), 225 (25), 129 (68), 97 (42), 57 (69), 54 (100)] and 36.37 min [EIMS *m*/*z* 253 (47), 225 (42), 129 (59), 97 (38), 71 (48), 57 (62), 54 (100)], while synthetic (*R*,*R*)-tanikolide dimer **5** gave a single peak at 35.99 min [EIMS *m*/*z* 253 (63), 225 (32), 153 (12), 129 (73), 97 (59), 57 (68), 55 (100)], and synthetic (*S*,*S*)-tanikolide dimer *ent*-**5** gave a single peak at 36.37 min [EIMS *m*/*z* 253 (58), 225 (38), 129 (67), 83 (42), 71 (58), 57 (77), 55 (100)].

Formation of the Benzyl Ether of Epoxide 6. NaH (60% in oil, 87 mg, 2.18 mmol) was added to a solution of epoxy alcohol **6** in THF (10 mL) at 0 °C. After the mixture was stirred 30 min, benzyl bromide (0.17 mL, 1.42 mmol) and TBAI (80 mg, 0.22 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. After dilution with Et_2O (10 mL), the reaction mixture was cooled to 0 °C and treated with satd NH_4Cl solution (10 mL). The aqueous phase was extracted with Et_2O (10 mL × 2), and the combined organic extracts were washed with brine (15 mL × 2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 20:1) to give the benzyl ether of epoxide **6** (328 mg, 95%): R_f 0.51 (hexanes−EtOAc, 8:1); $[\alpha]^{25}_D$ +2.3 (*c* 0.55, $CHCl_3$); IR (neat) ν_{max} = 3034, 2925, 2856, 1459, 1371, 1208, 1103, 1022, 953, 903, 815 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.18−7.43 (m, 5 H), 4.58 and 4.53 (ABq, *J* = 12.0 Hz, 2 H), 3.60 and 3.46 (ABq, *J* = 11.0 Hz, 2 H), 2.70 and 2.64 (ABq, *J* = 4.8 Hz, 2 H), 1.72−1.88 (m, 1 H), 1.47−1.64 (m, 1 H), 1.14−1.43 (m, 18 H), 0.88 (t, *J* = 6.7 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 128.6, 127.9, 127.9, 73.5, 72.1, 58.9, 50.5, 32.2, 32.2, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 24.9, 22.9, 14.4; MS *m*/*z* (FAB, relative intensity) 319 ($M^+ + 1$, 13), 289 (2), 227 (11), 181 (17), 154 (22), 137 (24), 107 (39), 91 (100), 55 (30), 43 (32), 29 (9); HRMS (FAB) calcd for $C_{21}H_{35}O_2$ (M^++1) 319.2637, found 319.2641.

Homoallylic Alcohol 7. Vinylmagnesium bromide (1 M solution in THF, 1.5 mL, 1.5 mmol) was added slowly to a suspension of CuI (44 mg, 0.2 mmol) in THF (6 mL) at −20 °C. After being stirred for 30 min, a solution of the benzyl ether of epoxide **6** (320 mg, 1.0 mmol) in THF (3 mL) was added, and the reaction mixture was stirred at −20 °C for 2 h. After dilution with Et_2O (10 mL), the reaction mixture was warmed to 0 °C and treated with satd NH_4Cl solution (10 mL). The aqueous phase was extracted with Et_2O (15 mL × 2), and the combined organic extracts were washed with brine (15 mL×2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 20:1) to give homoallylic alcohol **7** (309 mg, 89%): R_f 0.40 (hexanes−EtOAc, 8:1); $[\alpha]^{25}_D$ +2.4 (*c* 2.00, $CHCl_3$); IR (neat) ν_{max} = 3457, 3071, 3031, 2925, 2857, 1817, 1639, 1459, 1371, 1208, 1101, 1002, 915, 800, 740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.23−7.41 (m, 5 H), 5.74−5.87 (m, 1 H), 5.08 (d, *J* = 12.5 Hz, 2 H), 4.54 (s, 2 H), 3.34 and 3.31 (ABq, *J* = 9.0 Hz, 2 H), 2.25−2.38 (m, 2 H), 2.22 (s, 1 H), 1.42−1.52 (m, 2 H), 1.18−1.36 (m, 18 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 134.1, 128.6, 127.9, 127.9, 118.4, 75.5, 73.4, 73.7, 41.6, 36.9, 32.2, 30.5, 29.9, 29.9, 29.9, 29.8, 29.6, 23.4, 22.9, 14.4.

Sulfide Derivative of 7. AIBN (7.5 mg, 0.05 mmol) was added to a solution of homoallylic alcohol **7** (81 mg, 0.23 mmol) in thiophenol (1.2 mL) at room temperature. The mixture was heated to 80 °C and stirred for 12 h. The mixture was cooled to room temperature. After dilution with Et_2O (3 mL), the solution was washed successively with 5% NaOH solution (5 mL) and brine (5 mL × 2), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 15:1) to give the sulfide derivative of **7** (104 mg, quant): R_f 0.30 (hexanes−EtOAc, 8:1); $[\alpha]^{25}_D$ −1.1 (*c* 1.50, $CHCl_3$); IR (neat) ν_{max} = 3469, 3063, 3030, 2925, 2857, 1950, 1872, 1582, 1459, 1370, 1299, 1204, 1099, 1021, 739, 696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.10−7.43 (m, 10 H), 4.52 (s, 2 H), 3.29 (s, 2 H), 2.80−3.00 (m, 2 H), 2.18 (s, 1 H), 1.57−1.74 (m, 4 H), 1.38−1.50 (m, 2 H), 1.13−1.35 (m, 18 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 136.9, 129.2, 129.1, 128.7, 128.0, 127.9, 126.0, 75.7, 74.0, 73.7, 36.8, 35.8, 34.4, 32.2, 30.5, 29.9, 29.9, 29.8, 29.6, 23.7, 23.5, 23.0, 14.4; MS *m*/*z* (FAB, relative intensity) 456 (M^+, 3), 439 (13), 347 (3), 331 (6), 239 (5), 225 (15), 123 (10), 91 (100), 55 (8), 43 (9), 29 (2); HRMS (FAB) calcd for $C_{29}H_{44}O_2S$ (M^+) 456.3062, found 456.3048.

Acrylate 8. Ethylmagnesium bromide (1 M solution in THF, 5.3 mL, 5.3 mmol) was added dropwise to a solution of the sulfide derivative of **7** (1.2 g, 2.65 mmol) in THF (27 mL) at room temperature. After being stirred for 20 min, acryloyl chloride (0.75 mL, 9.3 mmol) was added to this mixture at room temperature. After being stirred for 2 h, the reaction mixture was diluted with Et_2O (20 mL) and quenched with satd $NaHCO_3$ solution (20 mL). The aqueous phase was extracted with Et_2O (30 mL × 1), and the combined organic extracts were washed with brine (30 mL x 2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 10:1) to give acrylate **8** (1.1 g, 79%): R_f 0.27 (hexanes−EtOAc, 8:1); $[\alpha]^{25}_D$ −1.6 (*c* 0.25, $CHCl_3$); IR (neat) ν_{max} = 3030, 2925, 2857, 1945, 1722, 1628, 1583, 1459, 1405, 1285, 1198, 1105, 980, 810, 740, 476 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.08−7.39 (m, 10 H), 6.28 (d, *J* = 17.24 Hz, 1 H), 6.02 (dd, *J* = 17.2, 10.3 Hz, 1 H), 5.73 (d, *J* = 10.3 Hz, 1 H), 4.48 (s, 2 H), 3.66 (s, 2 H), 2.89 (t, *J* = 7.3 Hz, 2 H), 2.05 (m, 2 H), 1.78−1.94 (m, 2 H), 1.55−1.67 (m, 2 H), 1.11−1.37 (m, 18 H), 0.88 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 138.5, 136.8, 130.2, 129.9, 129.2, 129.1, 128.6, 127.8, 127.8, 126.0, 86.1, 73.5, 71.2, 34.0, 34.0, 33.3, 32.2, 30.1, 29.9, 29.9, 29.9, 29.8, 29.6, 23.3, 23.2, 22.9, 14.4; MS *m*/*z* (FAB, relative intensity) 510 (M^+, 4), 439 (83), 403 (6), 347 (9), 331 (29), 239 (16), 199 (4), 136 (76), 123 (55), 91 (100), 55 (87); HRMS (FAB) calcd for $C_{32}H_{46}O_3S$ (M^+) 510.3168, found 510.3150.

Ester 10. Grubbs' second-generation catalyst (8 mg, 0.0063 mmol) was added to a solution of homoallylic alcohol **7** (90 mg, 0.25 mmol) and acrylate **8** (195 mg, 0.37 mmol) in CH_2Cl_2 (4 mL) at room temperature. The reaction mixture was heated under reflux for 5 h, and the solvent was evaporated. The residue was purified by flash column chromatography (hexanes−EtOAc, 8:1) to give ester **10** (164 mg, 77%): R_f 0.5 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ −1.5 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} = 3471, 2925, 2854, 1719, 1652, 1457, 1381, 1270, 1175, 1101, 739, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.12−7.40 (m, 15 H), 6.81−6.92 (m, 1 H), 5.75 (d, *J* = 15.4 Hz, 1 H), 4.54 (s, 2 H), 4.51 (s, 2 H), 3.66 (s, 2 H), 3.32 (s, 2 H), 2.88 (t, *J* = 7.1 Hz, 2 H), 2.13−2.25 (m, 2 H), 1.80−1.94 (m, 4 H), 1.55−1.69 (m, 4 H), 1.48 (d, *J* = 4.7 Hz, 2 H), 1.10−1.40 (m, 34 H), 0.88 (t, *J* = 6.5 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 144.1, 138.5, 138.2, 136.9, 129.2, 129.1, 128.7, 128.6, 128.0, 127.9, 127.8, 127.8, 125.9, 125.4, 85.8, 75.4, 74.0, 73.7, 73.6, 71.4, 40.1, 37.2, 34.1, 34.1, 33.4, 32.2, 30.4, 30.2, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.6, 29.6, 23.4, 23.4, 23.3, 22.9, 14.4.

Ester 13. Grubbs' second-generation catalyst (10 mg, 0.0078 mmol) was added to a solution of homoallylic alcohol *ent*-**7** (110 mg, 0.31 mmol) and acrylate **8** (246 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) at room temperature. The reaction mixture was heated under reflux for 5 h, and the solvent was evaporated. The residue

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was purified by flash column chromatography (hexanes−EtOAc, 8:1) to give ester **13** (161 mg, 76%): R_f 0.5 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ +1.6 (*c* 0.60, $CHCl_3$); IR (neat) ν_{max} = 2925, 2853, 2349, 1713, 1650, 1456, 1272, 1100, 738, 696 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13−7.33 (m, 15 H), 6.82−6.87 (m, 1 H), 5.76 (d, *J* = 15.4 Hz, 1 H), 4.52 (s, 2 H), 4.48 (s, 2 H), 3.65 (s, 2 H), 3.30 (s, 2 H), 2.89 (t, *J* = 7.3 Hz, 2 H), 2.40 (d, *J* = 7.3 Hz, 2 H), 2.03−2.06 (m, 2 H), 1.80−1.91 (m, 2 H), 1.57−1.61 (m, 2 H), 1.45−1.47 (m, 2 H), 1.18−1.31 (m, 36 H), 0.88 (t, *J* = 7.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 144.1, 138.5, 138.2, 136.9, 129.2, 129.1, 128.7, 128.6, 128.0, 127.9, 127.8, 127.8, 125.9, 125.4, 85.7, 75.4, 74.0, 73.7, 73.5, 71.4, 40.1, 37.2, 34.1, 34.1, 33.4, 32.2, 30.4, 30.2, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.6, 29.6, 23.4, 23.4, 23.2, 22.9, 14.4.

Diene Product of 10. *m*-CPBA (50 mg, 0.21 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **10** (190 mg, 0.22 mmol) in CH_2Cl_2 (25 mL) at −78 °C. After 10 min, the reaction mixture was quenched with satd $NaHCO_3$ solution (20 mL) at −78 °C and then warmed to room temperature. The aqueous phase was extracted with CH_2Cl_2 (30 mL × 1), and the combined organic extracts were washed with brine (30 mL × 2), dried over $MgSO_4$, filtered, and concentrated. This crude sulfoxide was dissolved in freshly prepared toluene (13 mL) and pyridine (13 mL), and the solution was heated under reflux for 72 h. This mixture was concentrated, and the residue was purified by flash column chromatography (hexanes−EtOAc, 15:1) to give the diene product of **10** (131 mg, 83%): R_f 0.45 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ −4.1 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} = 3460, 3067, 3031, 2925, 2854, 2100, 1714, 1648, 1495, 1455, 1271, 1102, 735, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21−7.41 (m, 10 H), 6.80−6.92 (m, 1 H), 5.79 (d, *J* = 15.8 Hz, 1 H), 5.71 (dd, *J* = 17.1, 9.7 Hz, 1 H), 4.98−5.15 (m, 2 H), 4.51 (s, 4 H), 3.71 and 3.65 (ABq, *J* = 9.6 Hz, 2 H), 3.31 (s, 2 H), 2.73 and 2.65 (ABX, J_{AB} = 13.9, J_{AX} = 7.3, J_{BX} = 7.3 Hz, 2 H), 2.41 (d, *J* = 7.3 Hz, 2 H), 1.83−1.95 (m, 2 H), 1.42−1.53 (m, 2 H), 1.25 (m, 36 H), 0.88 (t, *J* = 6.8 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 144.0, 138.5, 138.2, 133.2, 128.7, 128.5, 128.0, 127.9, 127.8, 127.8, 125.5, 118.7, 85.4, 75.4, 74.0, 73.7, 73.5, 71.3, 40.1, 38.6, 37.2, 33.8, 32.2, 30.4, 30.2, 29.9, 29.9, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.6, 29.6, 23.4, 23.2, 22.9, 14.4; MS *m*/*z* (FAB, relative intensity) 718 (M^+, 2), 644 (2), 643 (1), 548 (3), 458 (5), 284 (3), 258 (8), 156 (2), 121 (3), 91 (100), 55 (87); HRMS (FAB) calcd for $C_{47}H_{74}O_5$ (M^+) 718.5536, found 718.5532.

Triene 11. Acryloyl chloride (0.1 mL, 2.85 mmol) was added to a solution of the diene product of **10** (205 mg, 0.285 mmol) in CH_2Cl_2 (2.8 mL) and DIPEA (2.8 mL) at 0 °C. Acryloyl chloride (0.1 mL) was added two more times every 2 h at 0 °C. After 6 h, the reaction mixture was quenched with satd NH_4Cl solution (5 mL). The aqueous phase was extracted with CH_2Cl_2 (5 mL × 1), and the combined organic extracts were washed with brine (5 mL × 2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 10:1) to give triene **11** (70 mg, 51%): R_f 0.5 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ −2.73 (*c* 0.25, $CHCl_3$); IR (neat) ν_{max} = 3460, 3067, 3031, 2925, 2854, 2100, 1714, 1648, 1495, 1455, 1271, 1102, 735 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21−7.37 (m, 10 H), 6.74−6.85 (m, 1 H), 6.31 and 6.28 (ABq, *J* = 1.5 Hz, 1 H), 6.02 (dd, *J* = 17.2, 10.3 Hz, 1 H), 5.81 (d, *J* = 15.4 Hz, 1 H), 5.75 and 5.72 (ABq, *J* = 1.4 Hz, 1 H), 5.65−5.72 (m, 1 H), 4.97−5.12 (m, 2 H), 4.51 (s, 4 H), 3.71 and 3.65 (ABq, *J* = 9.6 Hz, 4 H), 2.86 and 2.80 (ABX, J_{AB} = 14.2, J_{AX} = 7.5, J_{BX} = 7.7 Hz, 2 H), 2.72 and 2.64 (ABX, J_{AB} = 13.8, J_{AX} = 7.2, J_{BX} = 7.5 Hz, 2 H), 1.82−2.00 (m, 4 H), 1.10−1.37 (m, 36 H), 0.88 (t, *J* = 6.9 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 165.3, 142.9, 138.6, 138.3, 133.2, 130.5, 129.7, 128.6, 128.5, 127.9, 127.8, 127.8, 126.0, 118.7, 85.4, 85.3, 73.6, 73.5, 71.3, 71.2, 38.6, 37.2, 34.1, 33.8, 32.2, 30.6, 30.2, 30.0, 29.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 23.2, 23.2, 22.9, 14.4; MS *m*/*z* (FAB, relative intensity) 772 (M^+, 1), 699 (1), 665 (1), 593 (3), 503 (1), 355 (3), 329 (8), 328 (2), 265 (4), 235 (3), 91 (100); HRMS (FAB) calcd. for $C_{50}H_{76}O_6$ (M^+) 772.5642, found 772.5646.

Diene Product of 13. *m*-CPBA (50 mg, 0.21 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **13** (270 mg, 0.33 mmol) in CH_2Cl_2 (30 mL) at −78 °C. After 10 min, the reaction mixture was quenched with satd $NaHCO_3$ solution (30 mL) at −78 °C and warmed to room temperature. The aqueous phase was extracted with CH_2Cl_2 (50 mL × 1), and the combined organic extracts were washed with brine (50 mL × 2), dried over $MgSO_4$, filtered, and concentrated. The crude sulfoxide was dissolved in freshly prepared toluene (30 mL) and pyridine (30 mL), and then the mixture was heated under reflux for 7 d. This mixture was concentrated and flash column chromatography (hexanes−EtOAc, 15:1) gave the diene product of **13** (220 mg, 94%): R_f 0.45 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ +3.0 (*c* 0.35, $CHCl_3$); IR (neat) ν_{max} = 3469, 2925, 2854, 1715, 1650, 1457, 1270, 1102, 991, 917, 737, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26−7.35 (m, 10 H), 6.83−6.88 (m, 1 H), 5.79 (d, *J* = 16.2 Hz, 1 H), 5.71 (dd, *J* = 20.7, 9.9 Hz, 1 H), 5.04−5.09 (m, 2 H), 4.53 (s, 4 H), 3.71 and 3.65 (ABq, *J* = 9.7 Hz, 2 H), 3.31 (s, 2 H), 2.73 and 2.66 (ABX, J_{AB} = 16.8, J_{AX} = 7.4, J_{BX} = 7.5 Hz, 2 H), 2.41 (d, *J* = 7.7 Hz, 2 H), 1.88−1.90 (m, 2 H), 1.45−1.48 (m, 2 H), 1.25−1.31 (m, 36 H), 0.88 (t, *J* = 6.8 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 144.0, 138.6, 138.2, 133.2, 128.7, 128.6, 128.1, 127.9, 127.8, 127.8, 125.5, 118.7, 85.4, 75.5, 74.1, 73.8, 73.6, 71.3, 40.1, 38.7, 37.2, 33.8, 32.2, 30.4, 30.2, 29.9, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.6, 23.4, 23.2, 22.9, 14.4; MS *m*/*z* (FAB, relative intensity) 719 (M^+ + 1, 1), 611 (1), 519 (1), 463 (1), 373 (2), 329 (16), 265 (2), 221 (5), 181 (12), 131 (7), 91 (100); HRMS (FAB) calcd for $C_{47}H_{75}O_5$ (M^++1) 719.5615, found 719.5613.

Triene 14. Acryloyl chloride (0.1 mL, 2.85 mmol) was added to a solution of the diene product of **13** (40 mg, 0.057 mmol) and DIPEA (0.9 mL) in CH_2Cl_2 (0.9 mL) at 0 °C. Acryloyl chloride (0.04 mL) was added two more times every 2 h at 0 °C. After 6 h, the reaction mixture was quenched with satd NH_4Cl solution (2 mL). The aqueous phase was extracted with CH_2Cl_2 (5 mL × 1), and the combined organic extracts were washed with brine (3 mL × 2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (hexanes−EtOAc, 10:1) to give triene **14** (28 mg, 62%): R_f 0.5 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ +0.98 (*c* 1.00, $CHCl_3$); IR (neat) ν_{max} = 3066, 3032, 2925, 2854, 1722, 1653, 1457, 1402, 1273, 1197, 1114, 985, 917, 809, 738, 698, 609 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26−7.34 (m, 10 H), 6.77−6.83 (m, 1 H), 6.32 and 6.28 (ABq, *J* = 1.4 Hz, 1 H), 6.02 (dd, *J* = 17.2, 10.3 Hz, 1 H), 5.81 (d, *J* = 15.4 Hz, 1 H), 5.75 and 5.73 (ABq, *J* = 1.5 Hz, 1 H), 5.65−5.71 (m, 1 H), 5.03−5.08 (m, 2 H), 4.51 (s, 4 H), 3.70 (d, *J* = 10.0 Hz, 2 H), 3.63 (dd, *J* = 11.2, 2.5 Hz, 2 H), 2.86 and 2.80 (ABX, J_{AB} = 17.5, J_{AX} = 7.1, J_{BX} = 7.7 Hz, 2 H), 2.72 and 2.64 (ABX, J_{AB} = 16.8, J_{AX} = 7.3, J_{BX} = 7.5 Hz, 2 H), 1.86−1.95 (m, 4 H), 1.10−1.37 (m, 36 H), 0.88 (t, *J* = 6.9 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 165.3, 142.9, 138.6, 138.3, 133.2, 130.5, 129.7, 128.6, 128.6, 127.9, 127.9, 126.0, 118.7, 85.5, 85.3, 73.6, 73.6, 71.3, 71.2, 38.6, 37.2, 34.1, 33.8, 32.2, 30.2, 30.1, 29.9, 29.9, 29.8, 29.8, 29.6, 29.6, 23.2, 23.2, 23.0, 14.4; MS *m*/*z* (FAB, relative intensity) 773 (M^+ + 1, 1), 699 (1), 665 (1), 593 (1), 463 (1), 373 (1), 329 (5), 265 (4), 219 (2), 154 (10), 136 (11), 91 (100); HRMS (FAB) calcd for $C_{50}H_{77}O_6$ (M^+ + 1) 773.5720, found 773.5706.

Diene 12. Hoveyda−Grubbs' second-generation catalyst (8 mg, 0.00625 mmol) was added to a solution of triene **11** (48 mg, 0.062 mmol) in benzene (6 mL) at room temperature. The reaction mixture was warmed to 70 °C. After 2 h, the solvent was evaporated. The residue was purified by flash column chromatography (hexanes−EtOAc, 8:1) to give diene **12** (36 mg, 77%): R_f 0.5 (hexanes−EtOAc, 4:1); $[\alpha]^{25}_D$ −0.45 (*c* 1.15, $CHCl_3$); IR (neat) ν_{max} = 3031, 2924, 2854, 1723, 1458, 1383,

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1252, 1113, 1031, 957, 812, 739, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.36 (m, 10 H), 6.70–6.74 (m, 2 H), 5.97 (td, J = 7.2, 4.0 Hz, 2 H), 4.54 (d, J = 2.6 Hz, 4 H), 3.58 and 3.46 (ABq, J = 9.7 Hz, 4 H), 2.35–2.70 (m, 4 H), 1.68–1.81 (m, 4 H), 1.22–1.32 (m, 36 H), 0.88 (t, J = 9.9 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.6, 143.8, 138.1, 128.7, 128.0, 127.9, 120.8, 83.5, 73.8, 72.9, 37.2, 32.1, 30.1, 29.9, 29.8, 29.8, 29.7, 29.6, 23.4, 22.9, 14.3; MS m/z (FAB, relative intensity) 745 ($M^+ + 1$, 3), 655 (1), 598 (1), 508 (1), 463 (1), 395 (2), 373 (19), 307 (2), 289 (1), 281 (2), 263 (2), 181 (2), 154 (14), 137 (12), 91 (100); HRMS (FAB) calcd for $C_{48}H_{73}O_6$ ($M^+ + 1$) 745.5407, found 745.5422.

Diolide 5. Pd/C was added to a solution of diene **12** (46 mg, 0.062 mmol) in hexane (2 mL) under H_2. After 3 h, the mixture was filtered and concentrated to give diolide **5** (34 mg, quant): R_f 0.3 (hexanes–EtOAc, 1:1); $[\alpha]^{25}_D$ +2.9 (c 0.25, $CHCl_3$); IR (neat) ν_{max} = 3384, 2925, 2855, 1734, 1660, 1586, 1496, 1459, 1382, 1243, 1088, 1052, 755, 722, 700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 and 3.56 (ABX, J_{AB} = 14.3, J_{AX} = 6.8, J_{BX} = 6.8 Hz, 4 H), 2.47–2.51 (m, 4 H), 1.84–1.94 (m, 6 H), 1.71–1.77 (m, 4 H), 1.59–1.65 (m, 2 H), 1.12–1.42 (m, 36 H), 0.88 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 86.8, 67.8, 36.8, 32.2, 30.2, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 26.8, 23.7, 22.9, 16.9, 14.4; MS m/z (FAB, relative intensity) 591 (M^+ + Na, 7), 551 (18), 533 (10), 391 (5), 339 (3), 267 (85), 154 (100), 136 (78), 91 (56), 89 (51); HRMS (FAB) calcd for $C_{34}H_{64}O_6$ (M^+ + Na) 591.4601, found 591.4604.

Initial olefin metathesis product of triene **14**: Hoveyda–Grubbs' second-generation catalyst (6 mg, 0.0048 mmol) was added to a solution of triene **14** (37 mg, 0.048 mmol) in benzene (5 mL) at room temperature. The reaction mixture was warmed to 70 °C. After 2 h, the solvent was evaporated. The residue was purified by flash column chromatography (hexanes–EtOAc, 8:1) to give the diene product from metathesis of **14** (34 mg, 96%): R_f 0.5 (hexanes–EtOAc, 4:1); $[\alpha]_D$ +0.16 (c 2.00, $CHCl_3$); IR (neat) ν_{max} = 3031, 2925, 2854, 1723, 1458, 1383, 1252, 1113, 1031, 957, 812, 739, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.36 (m, 10 H), 6.70–6.74 (m, 2 H), 5.97 (td, J = 7.2, 4.0 Hz, 2 H), 4.54 (d, J = 2.6 Hz, 4 H), 3.57 and 3.46 (ABq, J = 9.7 Hz, 4 H), 2.35–2.70 (m, 4 H), 1.68–1.81 (m, 4 H), 1.22–1.32 (m, 36 H), 0.88 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.6, 143.8, 138.0, 128.6, 128.0, 127.9, 120.7, 83.4, 73.8, 72.8, 37.2, 32.1, 30.1, 29.8, 29.8, 29.8, 29.7, 29.6, 23.3, 22.9, 14.3; MS m/z (FAB, relative intensity) 745 ($M^+ + 1$, 2), 668 (1), 626 (1), 546 (1), 508 (1), 463 (1), 395 (6), 373 (13), 355 (2), 281 (2), 265 (7), 251 (5), 181 (3), 154 (5), 136 (5), 91 (100); HRMS (FAB) calcd for $C_{48}H_{73}O_6$ ($M^+ + 1$) 745.5407, found 745.5412.

Diolide 4. Pd/C was added to a solution of the initial olefin metathesis product of **14** (19 mg, 0.026 mmol) in hexane (2 mL) under H_2. After 3 h, the mixture was filtered and concentrated to give diolide **4** (14 mg, quant): R_f 0.3 (hexanes–EtOAc, 1:1); $[\alpha]^{25}_D$ +0.00 (c 0.90, $CHCl_3$); IR (neat) ν_{max} = 3444, 2923, 2850, 1716, 1467, 1385, 1252, 1173, 1103, 1051, 928, 723 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 and 3.55 (ABX, J_{AB} = 14.2, J_{AX} = 6.6, J_{BX} = 6.6 Hz, 4 H), 2.47–2.50 (m, 4 H), 1.87–1.92 (m, 6 H), 1.71–1.79 (m, 4 H), 1.59 - 1.65 (m, 2 H), 1.12–1.42 (m, 36 H), 0.88 (t, J = 7.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 86.7, 67.8, 36.9, 32.1, 30.2, 30.0, 29.8, 29.8, 29.8, 29.7, 29.6, 26.8, 23.7, 22.9, 16.9, 14.3; MS m/z (FAB, relative intensity) 569 ($M^+ + 1$, 25), 537 (1), 421 (2), 391 (2), 341 (1), 285 (48), 267 (100), 253 (12), 225 (8), 154 (51), 137 (64); HRMS (FAB) calcd for $C_{34}H_{65}O_6$ ($M^+ + 1$) 569.4781, found 569.4766.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and X-ray crystal structure data including ORTEP drawing and CIF for compound **2** and ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and mass spectrometric data for compound **3**; ^{1}H and ^{13}C NMR spectra for synthetic intermediates **6**–**14** and products **4** and **5**; chiral GC–MS analysis of compounds **3**–**5** and *ent*-**5**. Inhibition dose–response curves for compounds **4**, **5**, and *ent*-**5** to SIRT1 and SIRT2. This material is available free of charge via the Internet at http://pubs.acs.org.

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