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Total synthesis of 27-hydroxy-bullatacin and its C-15 epimer, and studies on their inhibitory effect on bovine heart mitochondrial complex I functions

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Abstract

The total synthesis of 27-hydroxybullatacin and its C-15 epimer has been achieved using rhenium(VII) oxides-mediated and $Co(modp)_2$-catalyzed oxidative cyclization (OC), diastereoselective alkynylation, Brown's enantioselective allylboration, and Grubbs' cross metathesis as the key reactions. The inhibitory effect of these compounds on the complex I function, as determined by using bovine submitochondrial particles, was in low nanomolar range.

1. Introduction

27-Hydroxybullatacin, **1a**,[1] is an adjacent asimicin-type bis-THF acetogenin, isolated from the leaves extract of *Annona glabra* L., family Annonaceae.[2,3] The absolute structure of **1a** was determined on the basis of detailed NMR spectroscopy studies of the parent molecule and its Mosher's esters. Evident from its nomenclature, 27-hydroxy-bullatacin shares a framework identical to bullatacin, **2**,[4] possessing the *threo-trans-threo-trans-erythro* bis-THF system, though the structure of the former remains to be confirmed. Both bullatacin and 27-hydroxybullatacin are extremely potent anticancer compounds with reported activities of approximately 10^5 times higher than adriamycin against several cancer cell lines, including human prostate adenocarcinoma (PC-3) and pancreatic carcinoma (PACA-2). 27-Hydroxybullatacin is also very potent against the human kidney carcinoma (A-498) cell line. Therefore, annonaceous acetogenins and an asimicin-type bis-THF acetogenin (such as **1a** and **2**) in particular, can be considered potential drug candidates for the treatment of cancers. Our interest in such a compound is further augmented because of our ongoing program on the development of 'smart cytotoxins' for selective cancer therapy. Presumably, a smart acetogenin would target cancer cells preferentially over normal cells. Thus, to obtain the target molecule for the biological studies and to validate the proposed structure, we embarked on the total synthesis of **1a**.

Previously, we prepared a number of adjacent bis-THF acetogenins, including asimicin, bullatacin, squamotacin, trilobacin, trilobin, and uvaricin using a combination of modular, convergent and/or bidirectional approaches.[5,6] While most methods were highly stereoselective, and the target compounds and selected asimicin-type non-natural bis-THF acetogenins were obtained with very high diastereoselectivities, they often required many synthetic steps. Therefore, we sought to develop highly versatile methods that might not only rapidly afford the naturally occurring asimicin-type natural bis-THF acetogenins, but also their analogs. Here, we describe the initial findings of our study toward the stated goal, including a successful synthesis of 27-hydroxybullatacin **1a** and its C-15 epimer, **1b**, and validation of the proposed structure for the natural product. The key reactions used in the synthesis

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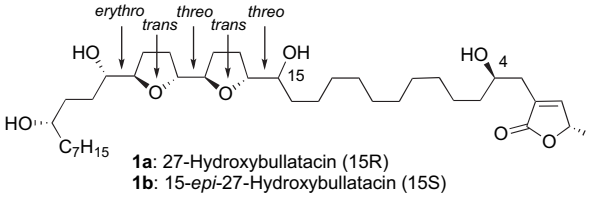
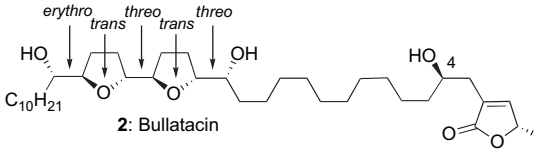
of **1a** and **1b** included the rhenium(VII) oxides-mediated trans oxidative cyclization (OC),[7] the Co(modp)$_2$-catalyzed Mukaiyama's trans-OC,[8] Ti-alkyne mediated diastereoselective alkynylation,[6b] the Brown's allylboration,[9] and Grubbs' cross metathesis[10] reactions. We also provide a report on the effect of **1a** and **1b** on the bovine mitochondrial enzyme functions as compared to **2**.



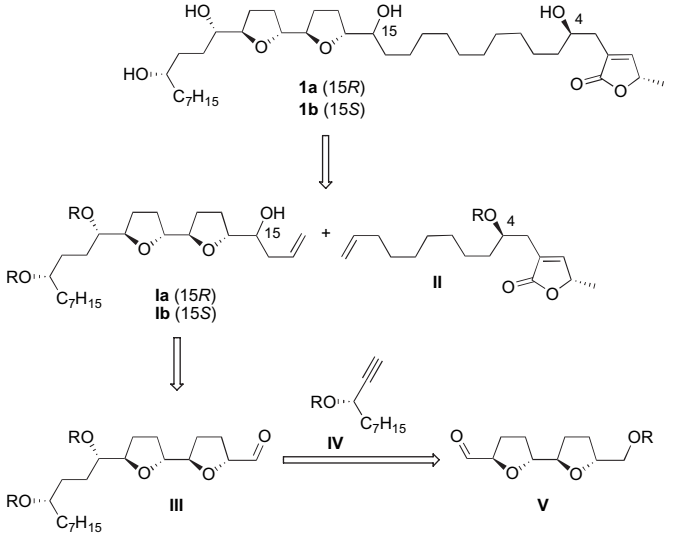
1a: 27-Hydroxybullatacin (15R)
1b: 15-*epi*-27-Hydroxybullatacin (15S)



2: Bullatacin

2. Results and discussion

Scheme 1 describes our retrosynthesis of 27-hydroxybullatacin, **1a**, involving a cross metathesis of alkene **I** with **II** to produce an alkene precursor of compound **1**. Whereas, compound **II** could be prepared analogous to one described earlier for its homologs,[11,12] compound **I** could be prepared by asymmetric allylation of aldehyde **III**. The latter product should be prepared by an asymmetric alkynylation of the previously reported bifunctional aldehyde **V**.[6b,13] Alternatively, we could develop a modified process to prepare aldehyde **V** starting with dieneyne, **3**. In a similar manner, compound **1b** could be prepared from **III** via **Ib**. The salient features of the current approach include the versatility and a comparatively less number of steps to produce the bis-THF fragments, **I**s', and quick assembly of the **I**'s to the butenolide fragment **II** by the Grubbs' cross metathesis reaction. Thus, four

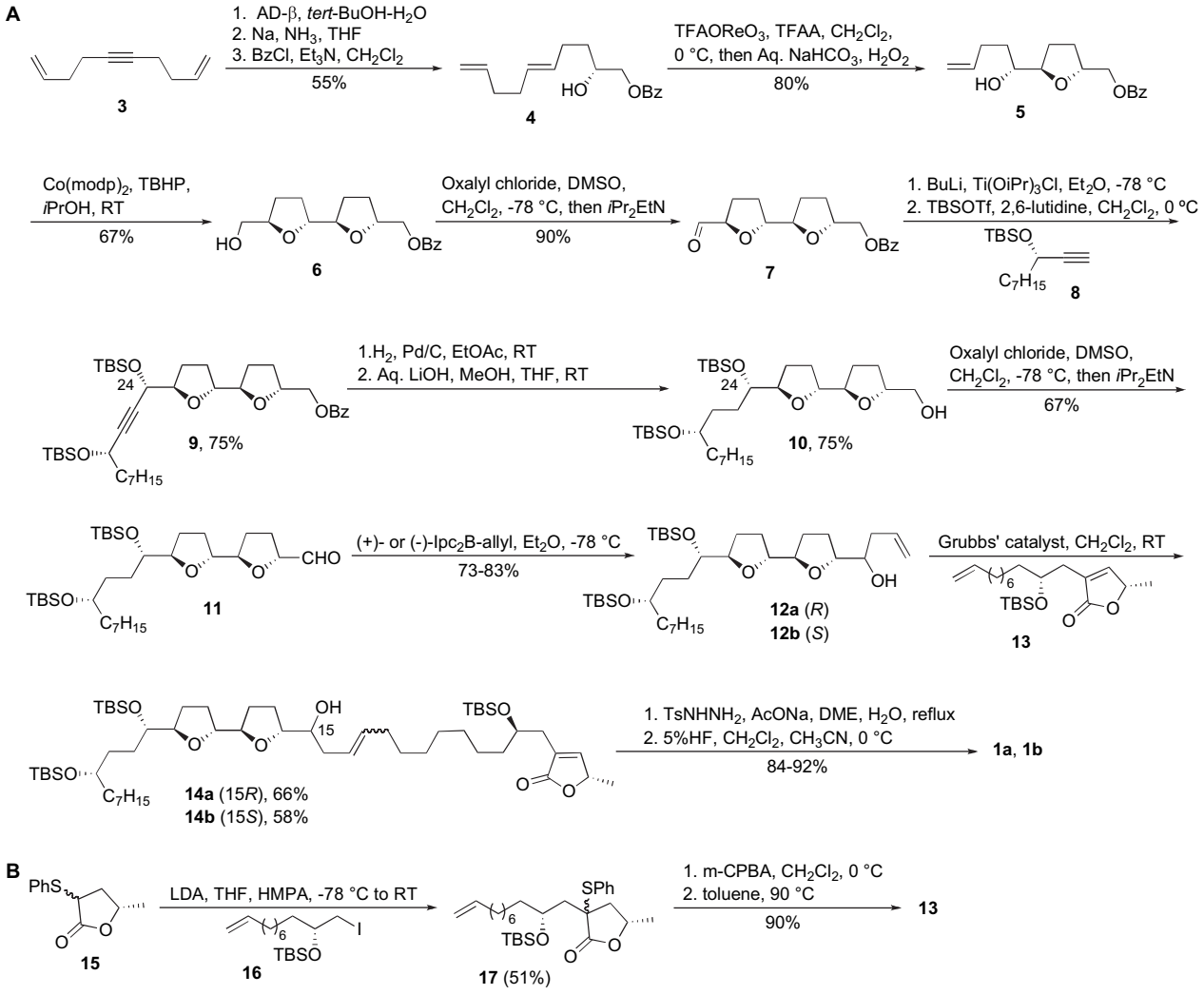


Scheme 1. Retrosynthesis of 27-hydroxybullatacin, **1a**, and its 15-epimer, **1b**.

stereoisomeric bis-THF acetogenins can be prepared by an asymmetric alkylation (alkynylation or allylation) of a single bis-THF aldehyde **V**. In other words, only 16 stereoisomeric **V**'s will be required to prepare 64 stereoisomeric asimicins or their alkene precursors, **I**'s. In contrast, our previous methods required a minimum of 36 bis-THF lactones to produce 64 stereoisomeric bis-THF alkene precursors analogous to **I**'s.[12] Furthermore, the cross metathesis approach eliminates any need of additional reactions on alkenes, such as the Wittig salt formation or an iodoalkene for the Pd coupling reaction.[5]

The synthesis of **1a** and **1b** started with the readily available deca-1,9-dien-5-yne, **3**. The diene **3** was mono-dihydroxylated with AD-β giving the corresponding monodiol in 67% yield and 86% ee. The alkyne function in the diol was reduced to *trans*-alkene using Na/NH_3, and the primary alcohol was selectively protected as benzoate ester giving compound **4**. Compound **4** was oxidatively cyclized using TFAOReO_3 affording the *trans*-monoTHF compound **5**. The latter product was then oxidatively cyclized using Co(modp)$_2$-catalyzed Mukaiyama's OC reaction affording the bifunctional intermediate **6**. An attempted second oxidative cyclization of **5** using TFAOReO_3 afforded a bis-THF compound that was different from **6**, and later identified as the corresponding cis analog. For the stereoselective addition of the alkyl and butenyl side chains on the two sides of **6**, we decided to use diastereoselective and enantioselective processes, respectively. Thus, compound **6** was oxidized to aldehyde **7** using a Swern oxidation, and reacted with the readily available alkyne **8**[14] using BuLi and ClTi(O-^{i}Pr)$_3$[6b] affording the alkynylated product with 9:1 diastereoselectivity, in favor of the desired *erythro* configuration. The major product was separated out from the undesired diastereomer, and the hydroxy function in the former was protected as a TBS ether giving compound **9**. The latter product was hydrogenated using 10% Pd–C catalyst under hydrogen atmosphere (balloon) and the benzoate group was removed by basic hydrolysis affording alcohol **10**. The latter was oxidized and the resulting aldehyde **11** was allylated using (+)-Ipc$_2$*B*-allyl yielding alcohols **12a** and **12b** in 83% combined yield and 4:1 diastereoselectivity. Using (−)-Ipc$_2$*B*-allyl, aldehyde **11** was converted to **12a** and **12b** in 73% combined yield and 1:10 diastereoselectivity. Pure products **12a** and **12b** were obtained by flash chromatography.

Separately, alkene **13** was synthesized from butyrolactone **15** and iodide **16**, as shown in Scheme 2B, using the process described earlier for similar compounds.[11] Iodide **16** was prepared from 1,10-undecadiene in four steps, including a Sharpless asymmetric dihydroxylation of the diene with AD-β to afford (*R*)-1,2-dihydroxyundec-10-ene, monotosylation at the primary alcohol, TBS protection of the secondary alcohol, and substitution of the tosylate function with an iodide. Alkylation of **15** with iodide **16** was achieved using LDA–HMPA, and the reaction product **17** was oxidized to its sulfoxide and then heated in toluene giving **13**. The latter compound was then cross-metathesized with compounds **12a** and **12b** in the presence of Grubbs' catalyst ($RuCl_2$[imid-H_2-Mes_2][CHPh]PCy_3), and the resulting products **14a** and



Scheme 2. Stereoselective synthesis of 27-hydroxybullatacin, **1a**, and 15-*epi*-27-hydroxybullatacin, **1b**.

14b were hydrogenated and deprotected to yield **1a** and **1b**.[15] The ^{1}H and ^{13}C NMR spectral data of synthetic compound **1a** was identical to the reported data for the natural 27-hydroxybullatacin.

2.1. Effect of 27-hydroxybullatacin and its C-15 epimer on the mitochondrial enzymes

Annonaceous acetogenins are among the most potent inhibitors of the mitochondrial enzymes, in particular complex I.[16] Though the exact mechanism of action still remains elusive, studies using the photoreactive acetogenins and competition assays with a variety of complex I inhibitors, including piericidin A and rotenone, have revealed that they share the common binding domain and that ND1 subunit constructs the inhibitor binding site.[17] Because both bullatacin and 27-hydroxybullatacin are known to possess comparable cytotoxicity against several human cancer cell lines, we decided to investigate their effect on the mitochondrial enzyme functions and compare. The mitochondrial activity of **1a**, **1b**, and **2** was determined by measuring the catalytic activity of the bovine heart submitochondrial particles (SMPs) in the presence and absence of these compounds, as described in the literature.[17] Briefly, compounds at various concentrations were added to 15 μg of SMPs/ml containing 0.25 M sucrose, 1 mM $MgCl_2$, and 50 mM phosphate buffer (pH 7.5) and mixed. After 5 min equilibration of SMPs with inhibitors, the reaction was started using 150 μM NADH, and progress of reaction was determined spectrometrically using a UV instrument at 340 nm. Under these conditions, all three compounds showed comparable activities in the low nanomolar range. Thus, the IC_{50} value for bullatacin was determined to be 3.3 nM, and those for 27-hydroxybullatacin and its C-15 epimer were recorded as 10 and 5 nM, respectively. Obviously, hydroxylation of bullatacin at C-27 caused three-fold reduction in its binding to the mitochondrial enzymes, and that was partially compensated when C-15 was epimerized in 15-*epi*-27-hydroxybullatacin.

In conclusion, the synthesis of 27-hydroxybullatacin and its C-15 epimer has been achieved using a bidirectional approach in 15 linear steps (approximately 5.6% yield) with compound **3** and 27 total number of steps from the commercially

available materials. The key steps used in the synthesis of the target compounds included rhenium(VII) oxides-mediated and the $Co(modp)_2$-catalyzed OC reactions to form the THF rings, Ti-alkyne mediated diastereoselective alkynylation, the asymmetric allylboration, and Grubbs' cross metathesis reactions. Using bovine submitochondrial particles, both 27-hydroxybullatacin and its 15-epimer were shown to discern low nanomolar inhibitory effect, comparable to bullatacin, on complex I functions.

3. Experimental section

3.1. General procedures

All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Tetrahydrofuran (THF) was distilled over potassium, diethyl ether over sodium and benzophenone, toluene over lithium aluminum hydride, and dichloromethane and triethyl amine over calcium hydride. Other solvents and reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light (λ_{max}=254) for visualization and an acidic mixture of *p*-anisaldehyde, phosphomolybdic acid, ceric ammonium molybdate or basic aqueous potassium permanganate ($KMnO_4$) and heat as the developing agents. E. Merck silica gel (60, particle size 0.043–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker DRX-500 or Varian Inova-400 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations (or combinations thereof) were used to explain the multiplicities: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. Low resolution mass spectra were recorded on Micromat-LCT. High-resolution mass spectra (HRMS) were recorded on Agilent LC/MSD TOF time-of-flight mass spectrometer by electrospray ionization time-of-flight reflectron experiments.

3.2. (R,E)-2-Hydroxydeca-5,9-dienyl benzoate **4**

Compound **3** (20 g, 150 mmol) was added to the well-stirred solution of potassium carbonate (61.5 g, 450 mmol), potassium ferricyanide (147 g, 45 mmol), DHQD(PHAL) (1.17 g, 1.5 mmol), and K_2OsO_4 (111 mg, 0.3 mmol) in 500 ml H_2O and 1000 ml *tert*-butanol at 0 °C, the reaction was stirred at 0 °C overnight, and then quenched with sodium sulfite (*Caution! Sodium sulfite should be added to reaction mixture in portions and slowly to avoid any spill due to effervescence*). The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified over silica gel (using CH_2Cl_2–CH_3OH) to yield the corresponding monodihydroxylated product, (9*R*)-dec-1-en-5-yne-9,10-diol (17 g, 67% yield, 86% ee). ^{1}H NMR: δ 5.83 (1H, m), 5.03 (2H, m), 3.84 (1H, m), 3.63 (1H, m), 3.45 (1H, m), 2.89 (2H, m), 2.85 (1H, m), 2.56 (1H, m), 2.27 (4H, m), 1.60 (2H, m).

Freshly cut pieces of Na metal (900 mg) were added in portions to liquid NH_3 (30 ml) in a round bottom flask at low temperature (−78 °C). After 10 min, a solution of the above-described diol (1.1 g, 6.5 mmol) in dry THF (10 ml) was added, and the mixture was stirred at this temperature for 4 h. The reaction mixture was quenched using solid NH_4Cl (2 g) and the cooling bath was removed. After ammonia has evaporated, the mixture was diluted with water and extracted using EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified over silica gel (using CH_2Cl_2–CH_3OH) to yield the corresponding *trans* olefin product, (9*R*)-dec-1,5-diene-9,10-diol (1.1 g, 100% yield). ^{1}H NMR: δ 5.83 (1H, m), 5.47 (2H, m), 4.98 (2H, m), 3.73 (1H, m), 3.64 (1H, m), 3.44 (1H, m), 2.21 (8H, m), 1.50 (2H, m); ^{13}C NMR: δ 138.32, 130.48, 129.84, 114.62, 71.80, 66.74, 33.68, 32.81, 31.89, 28.63.

Benzoyl chloride (0.37 ml, 3.1 mmol) was added dropwise to a solution of the above-described diol product (450 mg, 2.6 mmol) and Et_3N (0.56 ml, 4 mmol) in CH_2Cl_2 (20 ml). The solution was stirred overnight at 0 °C, then diluted with CH_2Cl_2, washed sequentially with saturated $NaHCO_3$ solution and brine, and evaporated. The residue was purified by flash chromatography (using hexane–EtOAc) to afford the mono protected compound **4** (600 mg, 82%). ^{1}H NMR: δ 8.06 (2H, d, *J*=8.0 Hz), 7.56 (1H, t, *J*=7.0 Hz), 7.44 (2H, t, *J*=7 Hz), 5.81 (1H, m), 5.47 (2H, m), 4.97 (2H, m), 4.37 (1H, m), 4.23 (1H, m), 3.99 (1H, m), 2.19 (6H, m), 1.62 (2H, m); ^{13}C NMR: δ 166.70, 138.31, 133.12, 133.12, 130.61, 129.82, 129.61, 129.59, 128.37, 114.58, 69.49, 69.04, 33.64, 33.09, 31.87, 28.45.

3.3. ((2R,5R)-5-((R)-1-Hydroxypent-4-enyl)tetrahydrofuran-2-yl)methyl benzoate **5**

$TFAOReO_3$ was prepared in situ as described in literature.[18] In a general process, Re_2O_7 (484 mg, 1 mmol) was transferred into a flask, and THF (10 ml) and TFAA (170 µl, 1.2 mmol) were added sequentially. The mixture was stirred at room temperature for 1 h. The flask was cooled using an ice cold bath and solvents were removed in vacuo. The residue was washed twice using cold pentane and used directly for the OC reaction. Cold CH_2Cl_2 (10 ml) was added to the above prepared $TFAOReO_3$ and the mixture was cooled to 0 °C. A cold solution of TFAA (170 µl, 1.2 mmol) and compound **4** (140 mg, 0.5 mmol) in CH_2Cl_2 (5 ml) was added dropwise sequentially. After the reaction was stirred at 0 °C to room temperature for 4 h, the reaction mixture was worked up using a saturated solution of $NaHCO_3$ (5 ml) and 30% H_2O_2 (0.5 ml), then extraction with EtOAc. The organic layer was washed with saturated $NaHSO_3$ solution followed by brine, dried over Na_2SO_4, and solvents were removed. The resulting residue was purified over silica gel using hexanes–EtOAc as the eluting solvents affording the corresponding *trans*-mono OC product, **5**. ^{1}H NMR: δ 7.98 (2H, d, *J*=7.0 Hz), 7.54 (1H, t,

J=7.5 Hz), 7.41 (2H, t, J=7.0 Hz), 5.78 (1H, m), 5.04 (1H, dd, J=17.0, 1.0 Hz), 4.98 (1H, d, J=10.0 Hz), 4.56 (1H, m), 4.35 (2H, m), 4.26 (1H, m), 4.10 (1H, m), 3.04 (3H, s), 2.28–2.06 (4H, m), 1.78 (1H, m), 1.66 (3H, m); ^{13}C NMR δ 166.08, 136.81, 133.01, 129.75, 129.37, 128.29, 115.63, 85.38, 80.19, 76.71, 66.36, 38.71, 30.41, 28.90, 28.59, 28.33.

3.4. ((2R,2′R,5R,5′R)-5′-(Hydroxymethyl)octahydro-2,2′-bifuran-5-yl)methyl benzoate **6**

TBHP (0.1 ml, 0.5–0.6 mmol, 5–6 M in decane) was added dropwise to a well-stirred solution of **5** (150 mg, 0.5 mmol) and Co(modp)$_2$ (41 mg, 0.076 mmol) in 10 ml isopropanol and 0.05 ml water under 1 atm oxygen. The solution was warmed up to 50 °C and stirred for 24 h. Solvents were evaporated and the residue was purified by flash chromatography to give **6** (72 mg, 46%) and recovered starting material (36 mg). $[\alpha]_D^{20}$ −8.3 (*c* 2.25, $CHCl_3$); ^{1}H NMR: δ 8.04 (2H, d, J=7.5 Hz), 7.55 (1H, t, J=7.5 Hz), 7.42 (2H, t, J=7.5 Hz), 4.40 (1H, m), 4.36 (2H, m), 4.16 (1H, m), 4.01 (1H, dd, J=14.0, 6.5 Hz), 3.94 (1H, dd, J=14.0, 7.0 Hz), 3.71 (1H, dd, J=11.5, 2.5 Hz), 3.51 (1H, dd, J=11.5, 3.5 Hz), 2.13 (1H, m), 2.07–1.95 (3H, m), 1.82–1.66 (4H, m); ^{13}C NMR: δ 166.51, 132.94, 130.06, 129.65, 128.29, 82.37, 82.14, 80.03, 77.25, 66.78, 64.53, 28.77, 28.53, 28.41, 27.38. MS (ESI): *m/z*: 307 $[M+H]^+$, 329 $[M+Na]^+$.

3.5. ((2R,2′R,5R,5′R)-5′-Formyloctahydro-2,2′-bifuran-5-yl)methyl benzoate **7**

DMSO (0.4 ml, 5 mmol) in 2 ml dichloromethane was added to a pre-cooled solution of oxalyl chloride (2 ml, 4 mmol, 2 M in dichloromethane) in 10 ml dichloromethane at −78 °C. After 20 min, the bis-THF **6** (600 mg, 2 mmol) in 2 ml dichloromethane was added dropwise, and the reaction was stirred for 30 min at this temperature. Diisopropylethylamine (1.74 ml, 10 mmol) was added into the above solution, the reaction was warmed to room temperature slowly, quenched by saturated ammonium chloride solution, and extracted with dichloromethane. The organic layers were combined and washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography to yield aldehyde **7** (500 mg, 85%). ^{1}H NMR: δ 9.69 (1H, d, J=1.5 Hz), 8.03 (2H, d, J=7.5 Hz), 7.54 (1H, t, J=7.5 Hz), 7.42 (2H, t, J=7.5 Hz), 4.34 (3H, m), 4.04 (2H, m), 2.23–1.92 (5H, m), 1.82–1.70 (3H, m); ^{13}C NMR: δ 202.67, 166.43, 132.92, 129.59, 128.32, 128.27, 83.28, 83.17, 81.67, 77.32, 66.70, 28.47, 28.38, 27.90, 27.29.

3.6. ((2R,2′R,5R,5′R)-5′-((1S,4S)-1,4-di(tert-Butyldimethylsilyloxy)undec-2-ynyloctahydro-2,2′-bifuran-5-yl)methyl benzoate **9**

BuLi (2.5 M in hexanes, 1.1 ml, 2.75 mmol) was added dropwise to a solution of **8** (730 mg, 2.72 mmol) in 40 ml diethyl ether at 0 °C under argon atmosphere. After 30 min, the reaction mixture was cooled to −78 °C and ClTi(O-^{i}Pr)$_3$ (1 M in toluene, 2.75 ml, 2.75 mmol) was added. The solution was further stirred for 30 min, and aldehyde **6** (300 mg, 1.0 mmol) in 5 ml diethyl ether was added through a canula. Then the reaction was warmed to room temperature slowly. After 16 h the mixture was quenched with 10% tartaric acid solution and stirred for 0.5 h, and extracted with ethyl acetate. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue (10:1 dr in favor of the *erythro* isomer) was purified by flash chromatography on silica gel to afford the pure alkynylated product of **7** (480 mg, 86%) in the form of a colorless oil. ^{1}H NMR: δ 8.05 (2H, d, J=8.0 Hz), 7.56 (1H, t, J=7.5 Hz), 7.44 (2H, t, J=8.0 Hz), 4.57 (1H, s), 4.38 (4H, m), 4.18 (1H, m), 4.06–3.97 (2H, m), 2.35 (1H, d, J=4.5 Hz), 2.13 (1H, m), 2.04 (2H, m), 1.82–1.59 (6H, m), 1.39 (2H, m), 1.27 (9H, m), 0.89 (9H, s), 0.88 (3H, t, J=7.0 Hz), 0.11 (3H, s), 0.092 (3H, s); ^{13}C NMR: δ 165.21, 133.62, 132.95, 129.66, 128.31, 83.32, 82.35, 81.94, 81.26, 66.78, 64.06, 62.92, 38.58, 31.79, 29.19, 28.66, 28.54, 28.40, 26.01, 25.78, 25.19, 22.63, 18.21, 14.08, −4.50, −5.03. MS (ESI): *m/z*: 573 $[M+H]^+$, 595 $[M+Na]^+$.

TBSOTf (230 μl, 1.0 mmol) was added to a solution of the above-described hydrogenated product (480 mg, 0.86 mmol) and 2,6-lutidine (140 μl, 1.2 mmol) in CH_2Cl_2 at 0 °C. The mixture was stirred at this temperature for 2 h and worked up using saturated $NaHCO_3$ solution and CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was purified over silica gel affording the corresponding TBS ether (605 mg, 100%). ^{1}H NMR: δ 8.05 (2H, d, J=7.5 Hz), 7.55 (1H, t, J=7.5 Hz), 7.43 (2H, t, J=7.5 Hz), 4.55 (1H, m), 4.34 (4H, m), 4.14 (1H, m), 3.98 (2H, m), 2.11 (2H, m), 1.98 (2H, m), 1.75 (2H, m), 1.61 (4H, m), 1.38 (2H, m), 1.25 (8H, m), 0.89 (9H, s), 0.87 (9H, s), 0.88 (3H, t, J=7.5 Hz), 0.12 (3H, s), 0.097 (6H, s), 0.080 (3H, s); ^{13}C NMR: δ 166.53, 132.88, 130.18, 129.67, 128.28, 82.83, 82.67, 81.97, 77.24, 66.93, 65.62, 62.94, 38.61, 31.78, 31.57, 29.22, 29.19, 28.28, 25.77, 25.59, 25.19, 22.62, 21.03, 18.21, 18.17, 14.08, −4.50, −4.71, −4.99, −5.06. MS (ESI): *m/z*: 687 $[M+H]^+$, 709 $[M+Na]^+$.

3.7. ((2R,2′R,5R,5′R)-5′-(1S,4S)-1,4-Di(tert-butyldimethylsilyloxy)undecyl)octahydro-2,2′-bifuran-5-yl)-methanol **10**

The TBS-protected compound **9** (605 mg, 0.86 mmol) and Pd–C (10% w/w, 60 mg) in EtOAc (30 ml) was stirred under H_2 atmosphere using balloon affording the desired hydrogenated product (600 mg, 99%). ^{1}H NMR: δ 8.04 (2H, dd, J=8.5, 1.5 Hz), 7.54 (1H, t, J=7.5 Hz), 7.42 (2H, t, J=8.0 Hz), 4.34 (3H, m), 3.99 (1H, m), 3.90 (2H, m), 3.77 (1H, m), 3.58 (1H, m), 2.11 (1H, m), 2.01 (1H, m), 1.92–1.67 (6H, m), 1.54 (1H, m), 1.45 (1H, m), 1.39–1.23 (16H, m) 0.89 (9H, s), 0.86 (9H, s), 0.87 (3H, t, J=6.5 Hz), 0.056 (3H, s), 0.047 (3H, s), 0.023 (3H, s), 0.015 (3H, s); ^{13}C NMR: δ 166.50,

132.86, 130.18, 129.05, 128.25, 82.30, 81.86, 81.57, 77.25, 73.85, 72.53, 66.96, 37.12, 32.77, 31.80, 30.45, 29.74, 29.27, 28.62, 28.54, 28.23, 25.97, 25.89, 25.84, 25.62, 25.29, 22.62, 18.13, 18.09, 14.07, −4.29, −4.43, −4.46, −4.48. MS (ESI): *m/z*: 691 $[M+H]^+$, 713 $[M+Na]^+$.

The above-described hydrogenated product (600 mg, 0.9 mmol) was hydrolyzed using 2 M aqueous LiOH (2.5 ml) in a mixture of THF–MeOH (1:1, 10 ml) at room temperature (overnight). The desired product **10** (520 mg, 99%) was obtained after usual work-up (using ether and water), and purification over silica gel. $[\alpha]_D^{20}$ 1.5 (*c* 2.95, $CHCl_3$); ^1H NMR: δ 4.10 (1H, m), 3.93–3.83 (3H, m), 3.79 (1H, m), 3.68 (1H, dd, *J*=11.5, 3.0 Hz), 3.58 (1H, m), 3.47 (1H, dd, *J*=11.5, 5.5 Hz), 1.97–1.82 (6H, m), 1.73–1.53 (5H, m), 1.46–1.23 (13H, m), 0.88 (9H, s), 0.87 (9H, s), 0.88 (3H, t, *J*=6.5 Hz), 0.052 (3H, s), 0.041 (3H, s), 0.023 (3H, s), 0.020 (3H, s); ^{13}C NMR: δ 82.31, 82.00, 81.86, 79.70, 73.84, 72.54, 64.67, 37.14, 32.82, 31.82, 30.49, 29.77, 29.29, 28.67, 27.43, 25.98, 25.92, 25.64, 25.33, 22.65, 18.15, 18.12, 14.10, −4.29, −4.41, −4.43, −4.46. MS (ESI): *m/z*: 587 $[M+H]^+$, 609 $[M+Na]^+$.

3.8. (2R,2′R,5R,5′R)-5′-((1S,4S)-1,4-Di(tert-butyldimethylsilyloxy)undecyl)octahydro-2,2′-bifuran-5-carbaldehyde ***11***

DMSO (340 ml, 4.4 mmol) in 2 ml dichloromethane was added to a pre-cooled solution of oxalyl chloride (1 ml, 2 mmol, 2 M in dichloromethane) in 10 ml dichloromethane at −78 °C. After 20 min, compound **10** (260 mg, 0.44 mmol) in 2 ml dichloromethane was added dropwise, and the reaction was stirred for further 30 min at this temperature. Diisopropylethylamine (1.74 ml, 10 mmol) was added into the above solution, the reaction was warmed to room temperature slowly, quenched by saturated ammonium chloride solution, extracted with dichloromethane. The organic layers were combined and washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography to yield aldehyde **11** (240 mg, 92%). ^1H NMR: δ 9.68 (1H, d, *J*=2.0 Hz), 4.04–3.86 (4H, m), 3.79–3.74 (1H, m), 3.59 (1H, m), 1.97–1.62 (10H, m), 1.60–1.26 (14H, m), 0.87 (18H, s), 0.88 (3H, t, *J*=6.5 Hz), 0.068 (3H, s), 0.054 (3H, s), 0.065 (6H, s); ^{13}C NMR: δ 203.10, 83.30, 83.07, 82.50, 82.34, 82.16, 73.81, 72.59, 72.54, 37.15, 32.82, 31.84, 30.50, 29.78, 29.31, 28.68, 27.96, 27.44, 26.00, 25.98, 25.94, 25.85, 25.75, 25.33, 22.66, 18.15, 18.13, 14.10, −4.21, −4.27, −4.27, −4.42.

3.9. (S)-1-((2R,2′R,5R,5′R)-5′-((1S,4S)-1,4-Di(tert-butyldimethylsilyloxy)undecyl)octahydro-2,2′-bifuran-5-yl)-but-3-en-1-ol ***12a*** *and (R)-1-((2R,2′R,5R,5′R)-5′-((1S,4S)-1,4-Di(tert-butyldimethylsilyloxy)undecyl)octahydro-2,2′-bifuran-5-yl)but-3-en-1-ol* ***12b***

To a solution of (−)-*B*-allyldiisopinocampheylborane (prepared from 0.5 ml allylmagnesium bromide, 0.5 mmol, 1 M solution in Et_2O, and (+)-*B*-methoxydiisopinocampheylborane (160 mg, 0.5 mmol) in 10 ml Et_2O at 0–25 °C, 1 h) aldehyde **11** (60 mg, 0.1 mmol in 5 ml Et_2O) was added at −78 °C. The reaction was stirred overnight, and quenched with MeOH and treated with Et_3N (0.5 ml) and 30% H_2O_2 (2 mmol, 0.15 ml). Standard work-up and purification of the resulting crude product by chromatography on silica gel gave the homoallylic alcohols **12b** and **12a** in a ratio of 10:1. Similarly aldehyde **10** reacted with (+)-*B*-allyldiisopinocampheylborane affording **12a** and **12b** in a ratio of 4:1. *Physical data of compound* ***12a***. $[\alpha]_D^{20}$ +7.0 (*c* 0.97, $CHCl_3$); ^1H NMR: δ 5.88 (1H, m), 5.08 (2H, m), 3.93–3.82 (4H, m), 3.78 (1H, m), 3.58 (1H, m), 3.49 (1H, m), 2.47 (1H, br), 2.24 (2H, m), 1.99–1.82 (6H, m), 1.71–1.62 (5H, m), 1.55–1.26 (13H, m), 0.88 (18H, s), 0.87 (3H, t, *J*=7.5 Hz), 0.063 (3H, s), 0.052 (3H, s), 0.022 (6H, s); ^{13}C NMR: δ 134.88, 116.99, 82.36, 81.97, 81.92, 81.69, 73.83, 73.25, 72.53, 38.32, 37.13, 32.82, 31.82, 30.42, 29.76, 29.29, 28.71, 28.33, 25.98, 25.91, 25.73, 25.73, 25.31, 22.64, 18.14, 18.12, 14.09, −4.28, −4.42, −4.44, −4.46. MS (ESI): *m/z*: 627 $[M+H]^+$, 649 $[M+Na]^+$. *Physical data of compound* ***12b***. $[\alpha]_D^{20}$ +7.7 (*c* 0.57, $CHCl_3$); ^1H NMR: δ 5.85 (1H, m), 5.11 (2H, m), 3.94–3.78 (6H, m), 3.58 (1H, m), 2.20 (2H, m), 1.99–1.81 (7H, m), 1.69–1.51 (5H, m), 1.48–1.21 (12, m), 0.88 (21H, s and m), 0.061 (3H, s), 0.052 (3H, s), 0.023 (3H, s), 0.018 (3H, s); ^{13}C NMR: δ 134.77, 117.24, 82.29 (2), 81.97, 81.84, 73.85, 72.53, 70.97, 32.83, 31.82, 30.48, 29.76, 29.66, 29.29, 28.63, 28.57, 25.98, 25.92, 25.59, 25.31, 25.18, 22.64, 18.14, 18.11, 14.09, −4.30, −4.42, −4.43, −4.46. MS (ESI): *m/z*: 627 $[M+H]^+$, 649 $[M+Na]^+$.

3.10. (S)-3-((R)-2-(tert-Butyldimethylsilyloxy)undec-10-en-yl)-5-methylfuran-2(5H)-one ***13***

3.10.1. Preparation of iodide ***16***

To a well-stirred solution of potassium carbonate (8.2 g, 60 mmol), potassium ferricyanide (19.6 g, 60 mmol) in 200 ml *tert*-butanol and 100 ml water was added (DHQD)-PHAL (156 mg, 0.2 mmol), K_2OsO_4 (14.8 mg, 0.02 mmol) and 1,10-undecadiene (3.8 g, 25 mmol) at 0 °C. After the solution was stirred for 24 h at the same temperature, it was quenched with sodium sulfite (*Caution! Sodium sulfite should be added to reaction mixture in portions and slowly to avoid any spill due to effervescence*), diluted with water and extracted with ethyl acetate. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography to give (10R)-undece-1-en-10,11-diol (2.2 g, 60% yield, 85% ee) in the form of a solid. ^1H NMR: δ 5.82 (1H, m), 5.06 (1H, dd, *J*=17.5, 2.0 Hz), 4.95 (1H, d, *J*=10.5 Hz), 3.70 (1H, s), 3.65 (1H, d, *J*=11.0 Hz), 3.43 (1H, t, *J*=9.5 Hz), 2.15 (1H, s), 2.03 (3H, m), 1.43–1.25 (12H, m); ^{13}C NMR: 139.14, 114.15, 72.30, 66.83, 33.76, 33.18, 29.56, 29.36, 29.02, 28.87, 25.51.

TsCl (2.37 g, 12 mmol) was added in portions to a solution of the above-described diol (1.86 g, 10 mmol) and triethyl amine (2.8 ml, 20 mmol), in dry dichloromethane at 0 °C. The mixture was stirred at room temperature for 24 h, and

then diluted with dichloromethane, washed using saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated, and the residue was purified by flash chromatography to give the corresponding monotosylate (2.7 g, 91% yield) of the primary alcohol function. ^{1}H NMR: δ 7.78 (2H, d, J=8.0 Hz), 7.33 (2H, d, J=8.0 Hz), 5.78 (1H, m), 4.96 (1H, J=17.5, 2.0 Hz), 4.90 (1H, d, J=10.5 Hz), 4.00 (1H, dd, J=10.0, 3.0 Hz), 3.86 (1H, dd, J=10.0, 7.0 Hz), 3.80 (1H, s), 2.43 (3H, s), 2.33 (1H, s), 2.02 (2H, m), 1.34 (4H, m), 1.23 (8H, m); ^{13}C NMR: δ 144.92, 138.99, 132.61, 129.84, 127.85, 114.08, 73.89, 69.30, 60.31, 33.65, 32.56, 29.27, 29.18, 28.88, 28.75, 25.09, 21.54.

To the above-described tosylate product (2.7 g, 7.88 mmol) and 2,6-lutidine (1.4 ml, 11.8 mmol) in 30 ml dichloromethane was added TBSOTf (2.2 ml, 9.46 mmol) at −78 °C. The reaction was stirred for 1 h at this temperature, quenched with water, and extracted with dichloromethane. The organic layer was dried and concentrated. The residue was purified by flash chromatography to give the corresponding TBS ether (the precursor of iodide **16**) (3.3 g, 92% yield). ^{1}H NMR: δ 7.78 (2H, d, J=8.0 Hz), 7.34 (2H, d, J=8 Hz), 5.80 (1H, m), 4.97 (1H, m), 3.85 (3H, m), 3.80 (1H, s), 2.45 (3H, s), 2.02 (2H, s), 1.53 (6H, m), 1.39 (4H, m), 1.26 (8H, m), 0.83 (9H, s), 0.02 (3H, s), 0.005 (3H, s); ^{13}C NMR: δ 144.87, 139.12, 132.60, 129.78, 127.96, 114.17, 73.19, 69.97, 34.05, 33.76, 29.52, 29.29, 28.99, 28.87, 25.74, 25.70, 24.73, 21.62, 18.02, −4.56, −4.81.

A mixture of the above-described TBS-protected tosylate compound (2.0 g, 4.4 mmol), sodium bicarbonate (440 mg, 5.0 mmol), and sodium iodide (7.7 g, 50 mmol) in acetone (40 ml) was refluxed for 24 h. Solvents were removed in vacuo and the residue was purified to give iodide **16** (1.7 g, 94% yield). ^{1}H MNR: δ 5.81 (1H, m), 4.92–5.02 (2H, m), 3.54 (1H, m), 3.18 (2H, d, J=5.0 Hz), 2.04 (2H, m), 1.60 (1H, m), 1.53 (1H, m), 1.38 (2H, m), 1.30 (8H, m), 0.91 (9H, s), 0.10 (3H, s), 0.07 (3H, s); ^{13}C NMR: δ 139.06, 114.15, 71.42, 36.87, 33.77, 29.46, 29.36, 29.01, 28.88, 25.83, 24.88, 18.05, 13.98, −4.36, −4.56.

3.10.2. Alkylation of lactone *15* to produce *17*

A solution of *N*,*N*-diisopropylamine (424 μl, 3.0 mmol) in 15 ml THF was treated with *n*-BuLi (1.25 ml, 3.125 mmol, 2.5 M in THF) at 0 °C for 15 min and then cooled to −78 °C. Lactone **15** (1.0 g, 2.4 mmol) in 5 ml THF was added dropwise via syringe and the mixture was stirred at 0 °C for 30 min. Iodide **16** (500 mg, 2.4 mmol) in 1.3 ml HMPA and 2 ml THF was added to the above solution and the mixture was stirred for 24 h. The solution was diluted with ethyl acetate and washed with saturated NH_4Cl and brine. The organic solution was dried, filtered, concentrated by rotary evaporation, and purified by flash column chromatography to afford compound **17** (620 mg, 51%). ^{1}H NMR: δ 7.57 (2H, m), 7.35 (3H, m), 5.80 (1H, m), 4.94 (2H, m), 4.52 (1H, m), 4.25 (1H, m), 3.02 (1H, m), 2.00 (3H, m), 1.85 (1H, m), 1.46–1.22 (17H, m), 0.90 (9H, s), 0.16 (3H, s), 0.12 (3H, s); ^{13}C NMR: δ 177.48, 139.09, 136.63, 130.39, 129.55, 128.92, 114.11, 73.25, 69.44, 60.31, 55.28, 41.18, 39.49, 37.94, 33.74, 29.67, 29.53, 28.95, 28.85, 25.96, 24.37, 21.29, 20.99, 17.98, 14.16, −3.85.

3.10.3. Conversion of *17* to *13*

Compound **17** (610 mg, 1.2 mmol) was dissolved in 10 ml dichloromethane and treated with *m*-CPBA (200 mg, 1.2 mmol) at 0 °C. After the reaction solution was stirred for 10 min, it was diluted with 20 ml dichloromethane and washed with saturated $NaHCO_3$. The organic layer was separated, dried, and concentrated. The residue was re-dissolved into 10 ml toluene, and then the solution was heated at 90 °C for 2 h, cooled down and concentrated and purified by flash chromatography to afford the desired compound **13** (410 mg, 90%). ^{1}H NMR: δ 7.11 (1H, s), 5.80 (1H, m), 4.96 (3H, m), 3.94 (1H, m), 2.40 (2H, d, J=5.0 Hz), 2.02 (2H, m), 1.41–1.22 (16H, m), 0.86 (9H, s), 0.038 (3H, s), 0.010 (3H, s); ^{13}C NMR: δ 173.94, 151.40, 139.11, 130.80, 114.07, 77.39, 70.13, 36.90, 33.73, 32.71, 29.59, 29.36, 28.98, 28.84, 25.82, 25.07, 18.92, 17.99, −4.50.

3.11. 4,24,27-Tri-(tert-butyldimethylsilyl)-12,13-dehydro-27-hydroxybullatacin *14a* and 4,24,27-tri-(tert-butyl-dimethylsilyl)-12,13-dehydro-15-epi-27-hydroxy-bullatacin *14b*

The homoallylic alcohol compound **12a** (13 mg, 0.02 mmol) and butenolide **13** (31 mg, 0.08 mmol) in 2 ml dichloromethane were added to the solution of the Grubbs' catalyst ($RuCl_2$[imid-H_2-Mes_2][CHPh]PCy_3, 4.0 mg, 0.004 mmol) in 2 ml dichloromethane at reflux over 12 h by syringe pump. The solution was concentrated by reduced pressure after further refluxing for an additional hour. Column chromatography on silica gel afforded compound **14a** (11 mg, 66%). ^{1}H NMR: δ 7.12 (1H, s), 5.48 (1H, qd, J_1=1.0 Hz, J_2=5.5 Hz), 4.99 (1H, m), 3.96–3.78 (6H, m), 3.59 (1H, m), 3.43 (1H, m), 2.42 (2H, m), 2.18 (2H, m), 2.04–1.88 (8H, m), 1.67–1.63 (4H, m), 1.56 (6H, m), 1.45–1.26 (20H, m), 1.41 (3H, d, J=7 Hz), 0.88 (27H, s), 0.87 (3H, t, J=7.5 Hz), 0.066 (3H, s), 0.054 (3H, s), 0.050 (3H, s), 0.023 (9H, s); ^{13}C NMR: δ 177.95, 151.43, 133.28, 125.88, 82.35, 80.01, 81.84, 81.68, 77.44, 73.83, 73.69, 72.54, 70.17, 37.14, 37.08, 36.95, 32.84, 32.74, 32.63, 31.82, 30.41, 29.77, 29.68, 29.45, 29.30, 29.16, 28.71, 28.32, 25.99, 25.92, 25.87, 25.70, 25.33, 25.14, 22.64, 18.97, 18.15, 18.12, 18.04, 14.09, −4.26, −4.41, −4.44. MS (ESI): *m*/*z*: 979 $[M+H]^+$, 1001 $[M+Na]^+$.

Similarly, compound **12b** was reacted with **13** affording **14b** in 58% yield. ^{1}H NMR: δ 7.12 (1H, s), 5.51 (1H, m), 5.41 (1H, m), 4.99 (1H, m), 3.97–3.81 (7H, m), 3.59 (1H, m), 2.42 (1H, d, J=5.5 Hz), 2.14 (2H, m), 2.02–1.80 (10H, m), 1.68–1.52 (6H, m), 1.41 (3H, d, J=6.5 Hz), 1.48–1.25 (24H, m), 0.88 (30H, s and m), 0.062 (3H, s), 0.051 (3H, s), 0.023 (6H, s), 0.020 (6H, s); ^{13}C NMR: δ 174.85, 151.43, 133.65, 125.70, 82.28, 81.95, 77.43, 73.85, 72.52, 71.42, 70.17, 37.12, 36.94, 36.19, 32.85, 32.73, 32.61, 31.82, 30.47, 29.76, 29.68, 29.42, 29.35, 29.29, 29.13, 29.09, 28.64, 28.56, 25.99, 25.92, 25.87, 25.53, 25.32, 25.28, 25.13, 22.65, 18.96,

18.15, 18.14, 14.09, −4.30, −4.41, −4.46. MS (ESI): *m/z*: 979 $[M+H]^+$, 1001 $[M+Na]^+$.

*3.12. 27-Hydroxybullatacin **1a** and 15-epi-hydroxybullatacin **1b***

A solution of NaOAc (100 mg, 1.2 mmol) in water (2 ml) was added to a stirred solution of metathesis product (**14a**, 11 mg, 0.011 mmol) and *p*-toluenesulfonyl-hydrazine (186 mg, 1 mmol) in dimethoxyethane (2 ml) at reflux over 5 h. The mixture was then cooled to room temperature, poured into water, and extracted with dichloromethane. The combined organic layers were dried and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexane−EtOAc 6:1) yielded the corresponding hydrogenated product (10 mg, 90%). ^{1}H NMR: δ 7.11 (1H, d, J=1.5 Hz), 5.01 (1H, m), 3.96−3.77 (6H, m), 3.58 (1H, m), 3.38 (1H, m), 2.42 (2H, m), 1.98−1.82 (5H, m), 1.71−1.60 (4H, m), 1.60−1.25 (35H, m), 1.41 (3H, d, J=6.5 Hz), 0.88 (27H, s), 0.87 (3H, t, J=7.5 Hz), 0.065 (3H, s), 0.054 (3H, s), 0.052 (3H, s), 0.023 (9H, s). MS (ESI): *m/z*: 981 $[M+H]^+$, 1003 $[M+Na]^+$.

The above-described hydrogenated product (10 mg, 0.01 mmol) was stirred with 2 ml 5% HF in MeCN and 2 ml THF at room temperature for 12 h, the reaction mixture was partitioned between dichloromethane and brine. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography on silica gel yielded **1a** (6.0 mg, 91%) as a colorless waxy solid. $[\alpha]_D^{20}$ +5.8 (*c* 1.04, $CHCl_3$); (lit.[1] +11.0); ^{1}H NMR: δ 7.18 (1H, d, J=1.0 Hz), 5.05 (1H, m), 3.93 (2H, m), 3.85 (4H, m), 3.65 (1H, m), 3.40 (1H, m), 2.52 (1H, m), 2.40 (1H, m), 2.34 (1H, m), 2.02−1.84 (6H, m), 1.74−1.39 (10H, m), 1.43 (3H, d, J=7.0 Hz), 1.36−1.16 (27H, m), 0.88 (3H, t, J=7.5 Hz); ^{13}C NMR: δ 174.60, 151.78, 131.23, 83.23, 82.81, 82.54, 82.22, 77.98, 74.10, 71.84, 71.53, 70.02, 37.49, 37.44, 34.04, 33.37, 31.94, 31.85, 29.71 (2), 29.67 (2), 29.52, 29.37, 29.30, 22.71, 22.67, 19.14, 14.11. HRMS (ESI-TOF, $M+H^+$) calcd for $C_{37}H_{66}O_8$ 639.483, found 639.4813.

Similarly, compound **14b** was hydrogenated affording the precursor of **1b**. ^{1}H NMR: δ 7.12 (1H, s), 5.00 (1H, m), 3.96−3.80 (7H, m), 3.59 (1H, m), 2.42 (2H, d, J=5.0 Hz), 2.13−1.84 (10H, m), 1.64−1.26 (36H, m), 1.41 (3H, d, J=6.5 Hz), 0.88 (30H, m), 0.050 (9H, s), 0.023 (9H, s). MS (ESI): *m/z*: 981 $[M+H]^+$, 1003 $[M+Na]^+$.

The hydrogenated product of **14b** was deprotected as above using HF affording **1b** in >90% yield. $[\alpha]_D^{20}$ +7.62 (*c* 0.84, $CHCl_3$); ^{1}H NMR: δ 7.18 (1H, d, J=1.5 Hz), 5.05 (1H, qd, J=13.5, 1.0 Hz), 3.95−3.83 (9H, m), 3.64 (1H, m), 2.54−2.51 (1H, m), 2.38 (1H, m), 1.98 (3H, m), 1.91−1.78 (6H, m), 1.73−1.52 (8H, m), 1.42 (3H, d, J=7.0 Hz), 1.50−1.23 (26H, m), 0.87 (3H, t, J=7.5 Hz); ^{13}C NMR: δ 174.58, 151.76, 131.21, 83.06, 82.98, 82.76, 77.96, 71.66, 71.52, 71.17, 69.99, 37.49, 37.42, 34.09, 33.36, 32.39, 31.84, 29.66, 29.58, 29.51, 29.48, 29.46, 29.26, 28.79, 28.76, 28.26, 26.01, 25.78, 25.57, 24.77, 24.45, 22.67, 19.13, 14.11. HRMS (ESI-TOF, $M+H^+$) calcd for $C_{37}H_{66}O_8$ 639.483, found 639.4813.

3.13. Determination of the effect of 27-hydroxybullatacin and its C-15 epimer on the mitochondrial enzymes' inhibitors

Bovine heart SMPs were provided by our collaborator, Takao Yagi, at The Scripps Research Institute, who prepared them using a sonication medium containing 0.25 M sucrose, 1 mM succinate, 1.5 mM ATP, 10 mM $MgCl_2$, 10 mM $MnCl_2$, and 10 mM Tris−HCl (pH 7.4) and stored in a buffer containing 0.25 M sucrose and 10 mM Tris−HCl (pH 7.4) at −84 °C. A solution of 27-hydroxy-bullatacin or its C-15 epimer at various concentrations between 1 and 20 nM were added to 15 μg of SMPs in 500 μl volume containing 0.25 M sucrose, 1 mM $MgCl_2$, and 50 mM phosphate buffer (pH 7.5), and mixed. After 5 min equilibration of SMP with inhibitors, the reaction was started using 150 μM NADH and progress of reaction was determined spectrometrically using a UV instrument at 340 nm. Minimum concentration of the compounds required to inhibit the oxidation of NADH by 50% was recorded as their IC_{50} value.

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