Bioorganic & Medicinal Chemistry 18 (2010) 8630–8641

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Convergent synthesis of fluorescence-labeled probes of *Annonaceous* acetogenins and visualization of their cell distribution

Naoto Kojima [a,*], Takekuni Morioka [a], Daisuke Urabe [a], Masahiro Yano [a], Yuki Suga [a], Naoyoshi Maezaki [b], Ayako Ohashi-Kobayashi [c], Yasuyuki Fujimoto [c], Masatomo Maeda [c], Takao Yamori [d], Takehiko Yoshimitsu [a], Tetsuaki Tanaka [a,*]

[a] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-Kita, Tondabayashi, Osaka 584-8540, Japan*
[c] *School of Pharmacy, Iwate Medical University, Yahaba, Shiwa-gun, Iwate 028-3694, Japan*
[d] *Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan*

ARTICLE INFO

Article history:
Received 17 August 2010
Revised 1 October 2010
Accepted 2 October 2010
Available online 16 October 2010

Keywords:
Annonaceous acetogenins
Antitumor agents
Fluorescence-labeled analogues
Convergent synthesis
Cell distribution

ABSTRACT

The convergent synthesis of fluorescence-labeled solamin, an antitumor *Annonaceous* acetogenin, was accomplished by two asymmetric alkynylations of 2,5-diformyl tetrahydrofuran with an alkyne tagged with fluorescent groups and another alkyne with an α,β-unsaturated γ-lactone. Assay for the growth inhibitory activity against human cancer cell lines revealed that the probe with the fluorescent groups at the end of the hydrocarbon chain may have the same mode of action as natural acetogenins. The merged fluorescence of dansyl-labeled solamin and MitoTracker Red suggests that *Annonaceous* acetogenins localize in the mitochondria.

1. Introduction

Over 400 natural polyketides called *Annonaceous* acetogenins have been isolated from *Annona* plants growing in tropical and subtropical regions.[1] Most acetogenins are white waxy derivatives of long-chain fatty acids (C32 or C34) whose terminal carboxylic acid is combined with a 2-propanol unit at the C-2 position to form a methyl-substituted α,β-unsaturated γ-lactone (Fig. 1). One interesting feature of their chemical structures is the single, adjacent, or nonadjacent 2,5-disubstituted tetrahydrofuran (THF) system with one or two flanking hydroxyl groups(s) at the center of a long hydrocarbon chain. Much attention has also been paid to their broad spectrum of biological activities, including immunosuppressive, antimalarial, insecticidal, and probably the most impressive, antitumor activity. Several strategies for the total synthesis of natural acetogenins[2] and their analogues[3] have been developed, motivated by their unique structures and attractive biological activities.

It is generally accepted that the mode of action of acetogenins is the inhibition of NADH-ubiquinone oxidoreductase (complex I) in the mitochondria, thereby suppressing ATP production particularly for cancer cells having high metabolic activity, and inducing apoptosis.[4–6] However, the structure–activity relationship for the inhibition of complex I is not completely related to its cytotoxicity. McLaughlin and co-workers suggested that as the mitochondrial assay is cell-free, it does not take into consideration such factors as membrane transport, intracellular transport, and metabolic inactivation.[7] Poupon and co-workers synthesized the first

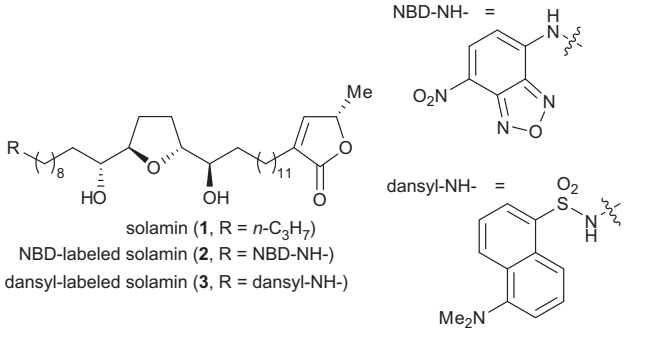


Figure 1. Structures of a *mono*-THF acetogenin, solamin, and its fluorescent derivatives.

* Corresponding authors. Tel.: +81 6 6879 8212; fax: +81 6 6879 8214 (N.K.); tel.: +81 6 6879 8210; fax: +81 6 6879 8214 (T.T.).
E-mail addresses: kojima@phs.osaka-u.ac.jp (N. Kojima), t-tanaka@phs.osaka-u.ac.jp (T. Tanaka).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.10.004

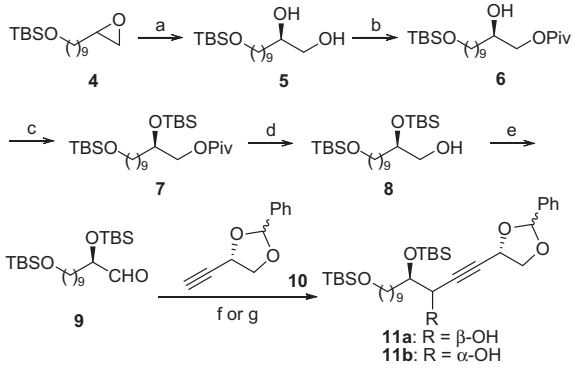
fluorescence-labeled acetogenin, dansyl-labeled squamocin, in which γ-lactone was replaced with a fluorescence label, and visualized its cell distribution.[8] Yao and co-workers reported that a fluorescence-labeled analogue, in which the THF ring moiety of natural acetogenin was mimicked by ethylene glycol ether unit replacements, showed different distributions in human normal cells and cancer cells.[9] Miyoshi and co-workers reported an ^{125}I-labeled acetogenin analogue in which the (trifluoromethyl)phenyldiazirine group served as a substitute for the γ-lactone moiety and a photoreactive group photo-cross-linked to the ND1 subunit of bovine heart mitochondrial complex I with high specificity.[10] These reports prompted us to synthesize a novel fluorescence-labeled acetogenin that retains all functionalities,[11] because in living cells, the hydrophilic THF moiety in natural acetogenins may be located at the surface of the mitochondrial inner membrane and the γ-lactone moiety may interact with the enzyme active site.[12] Miyoshi and co-workers revealed that the hydrocarbon chain on the left side of the THF moiety is not essential for the inhibitory activity against complex I.[13] We planned the synthesis of novel fluorescence-labeled acetogenins having a fluorescent group at the left end of the THF moiety. After starting our synthetic study, Sinha and co-workers reported the first synthesis of photoactive asimicin with all functionalities, which has a benzophenone group at the end of the hydrocarbon chain.[14] However, the photoactive probe was not evaluated.[15] Herein, we describe the full details of the first[16] and second-generation[17] syntheses of fluorescence-labeled acetogenins, the evaluation of their growth inhibitory activities against human cancer cell lines, and their cell distribution.

2. Results and discussion

2.1. First-generation synthesis of fluorescence-labeled solamin

We planned to synthesize fluorescence-labeled acetogenin with our developed stereoselective synthesis of the THF moiety[18,19] and selected solamin,[20] which has a comparatively simple structure yet all the functionalities, as the lead compound. The 7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl-amino (NBD-NH–) group was employed as a fluorescent tag due to its strong fluorescence with a long wavelength, which is advantageous for the observation of cell distribution.[21]

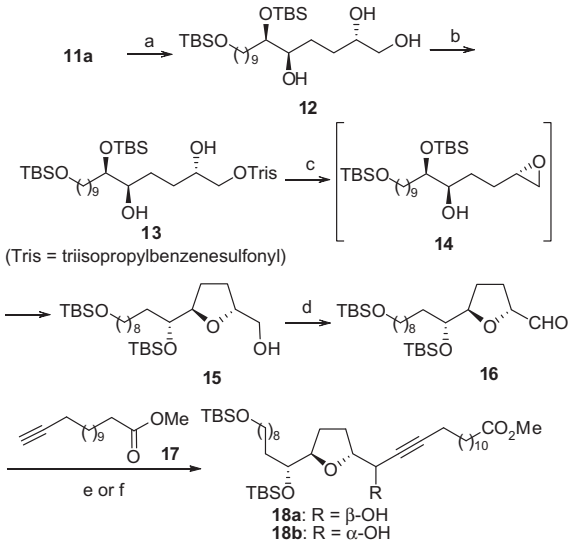
The synthesis was started from Jacobsen's kinetic optical resolution[22] of known racemic epoxide **4**[23] to give diol **5** with >98% ee (Scheme 1).[24,25] Diol **5** was converted into α-siloxyaldehyde **9** in a sequential reaction: (1) selective pivaloylation of the primary alcohol, (2) TBS protection of the secondary alcohol, (3) deprotection of the pivaloyl ester, and (4) oxidation with Dess–Martin periodinane. The asymmetric alkynylation of aldehyde **9** with chiral C4-unit **10**[18d] under Carreira's conditions[26] with (1*R*,2*S*)-*N*-methylephedrine (NME) as the chiral ligand afforded propargyl alcohol **11a** in high yield and selectivity. The stereochemistry was confirmed by comparison with the ^{1}H NMR spectral data of corresponding *anti*-adduct **11b** synthesized with the antipode of NME.[27]



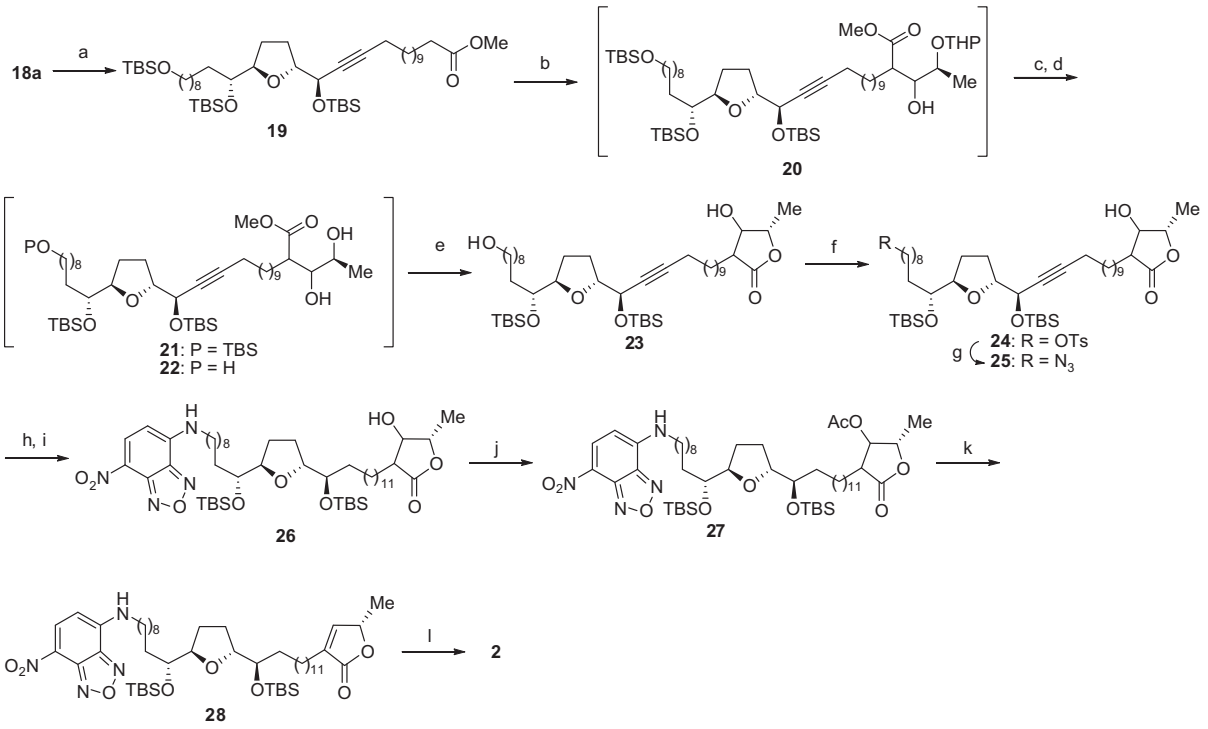
Scheme 1. Reagents and conditions: (a) (*S*,*S*)-(+)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt (III) acetate (0.5 mol%), Bu^{t}OMe, H_2O, 0 °C to rt, 48% (>98% ee); (b) PivCl, pyridine, CH_2Cl_2, 0 °C to rt, 95%; (c) TBSCl, imidazole, DMF, 0 °C to rt, 97%; (d) DIBAL-H, CH_2Cl_2, −78 °C, quantitative; (e) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C, 91%; (f) $Zn(OTf)_2$, Et_3N, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 91% (**11a**:**11b** = >97:3); (g) $Zn(OTf)_2$, Et_3N, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 85% (**11a**:**11b** = 3:>97).

Hydrogenation of *syn*-adduct **11a** using 10% Pd–C afforded saturated triol **12**, and subsequent selective sulfonylation of the primary alcohol with 2,4,6-triisopropylbenzenesulfonyl chloride (TrisCl) furnished sulfonate **13** (Scheme 2). The formation of a 2,5-disubstituted THF ring in a domino reaction via epoxide **14** was achieved by treatment of **13** with K_2CO_3 in MeOH. Oxidation of the primary alcohol of **15** was carried out with Dess–Martin periodinane to give α-tetrahydrofuranyl aldehyde **16**. The introduction of a linker with a methyl ester at the end was achieved by asymmetric alkynylation of aldehyde **16** with methyl 13-tetradecynoate **17** in 93% yield with high diastereoselectivity (>97:3 dr).[28,29]

After protection of the secondary hydroxyl group of **18a** as a TBS ether, the aldol reaction of tris-TBS ether **19** with THP-protected (*S*)-lactaldehyde[30] gave an inseparable mixture of aldol product **20** (mixture of diastereomers) and the unreacted aldehyde (Scheme 3). The selective deprotection of the THP group in **20** by treatment with $MgBr_2$ proceeded successfully, but the purification of diol **21** was difficult because partial γ-lactonization occurred during silica gel column chromatography. Therefore, we attempted to promote γ-lactonization with CSA. However, although the deprotection of the terminal TBS group proceeded, the γ-lactonization proceeded very slowly to give a mixture containing triol **22**. Fortunately, the γ-lactonization was completed by quenching the reaction with $NaHCO_3$, giving desired γ-lactone **23** in 41% yield in three steps from **19**. Construction of the α,β-unsaturated-γ-lactone moiety was conducted at the late stage of the synthesis because the methyl group on the γ-lactone easily epimerized.[31] Azidation of the terminal alcohol in **23** via tosylation gave azide **25** in good yield. After the reduction of the azide to the primary amine, the treatment with NBDCl in the presence of Et_3N in MeOH afforded NBD-labeled



Scheme 2. Reagents and conditions: (a) H_2 (3 atm), 10% Pd–C, EtOAc, rt, 94%; (b) TrisCl, pyridine, CH_2Cl_2, 0 °C to rt, 78%; (c) K_2CO_3, MeOH, 0 °C to rt, 67%; (d) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C to rt, 70%; (e) $Zn(OTf)_2$, Et_3N, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 93% (**18a**:**18b** = >97:3); (f) $Zn(OTf)_2$, Et_3N, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 68% (**18a**:**18b** = 3:>97).



Scheme 3. Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C to rt, 96%; (b) THP-protected-(*S*)-lactaldehyde, LDA, THF, −78 °C; (c) $MgBr_2$, Et_2O, rt; (d) CSA, MeOH–CH_2Cl_2 (1:1), 0 °C; (e) $NaHCO_3$, 41% from **19**; (f) TsCl, pyridine, CH_2Cl_2, 0 °C to rt, 85%; (g) NaN_3, DMSO, rt, 83%; (h) H_2 (3 atm), 10% Pd–C, MeOH, rt; (i) NBDCl, Et_3N, MeOH, 0 °C to rt, 95% in two steps; (j) Ac_2O, pyridine, rt, 77%; (k) DBU, THF, rt, 93%; (l) 48% aq HF, MeCN–THF (5:3), rt, 91%.

derivative **26** in 97% yield in two steps. The synthesis of fluorescent solamin **2** was completed via acetylation of the secondary alcohol, DBU-promoted β-elimination, and deprotection with HF in MeCN–THF.

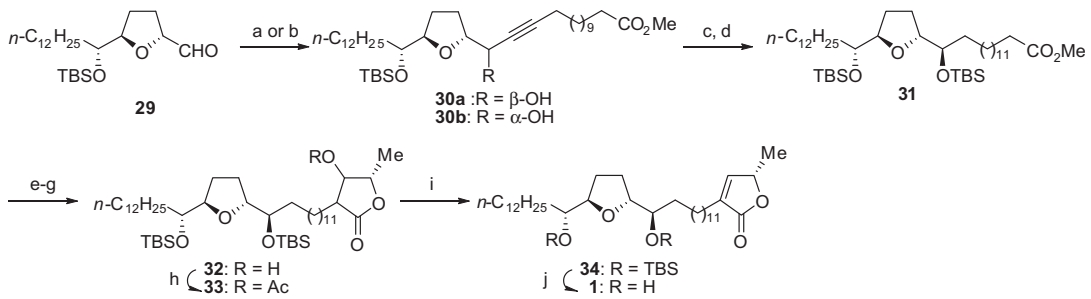
To confirm that the bioactivity of fluorescence-labeled solamin **2** is similar to that of natural solamin **1**, we would need a sample of **1**. The total synthesis of solamin **1** was easily accomplished with our strategy from our previously synthesized aldehyde **29**[18d] over nine steps (Scheme 4).[32]

With fluorescence-labeled solamin **2** and synthetic solamin **1** in hand, we attempted to visualize cell distribution with **2**. Unfortunately, we could not observe in detail the cell distribution of **2** because the quenching of the fluorescence of **2** was very fast. We had to prepare a new analogue with a different fluorescent tag from the NBD group, but the present synthetic route was long and the total yield was low because the synthetic route was linear. These problems prompted us to develop a novel approach to the fluorescence-labeled acetogenins.

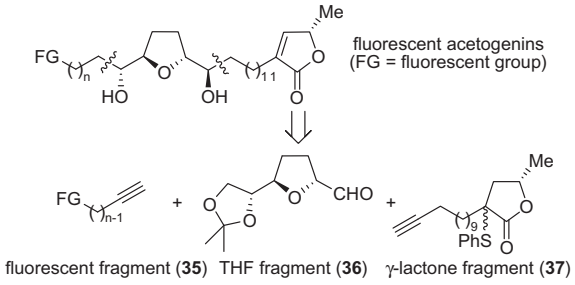
2.2. Second-generation synthesis of fluorescence-labeled solamin

Scheme 5 outlines the second-generation synthesis of fluorescence-labeled acetogenins. Fluorescence-labeled acetogenins are divided into three fragments retrosynthetically: fluorescent fragment **35**, THF fragment **36**, and γ-lactone fragment **37**. This synthesis should provide ready access to diverse fluorescent derivatives that have other fluorescent groups or long hydrocarbon chains as linker.

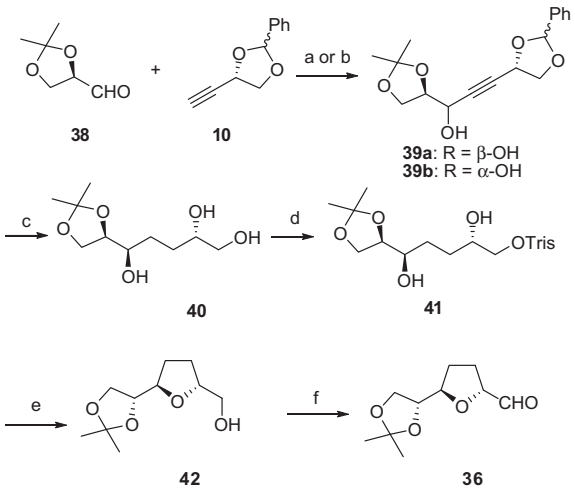
The preparation of THF fragment **36** started from the asymmetric alkynylation of 2,3-*O*-isopropylidene-D-glyceraldehyde **38**[33] with alkyne **10** (Scheme 6). Asymmetric alkynylation of **38** with **10** proceeded with high stereoselectivity to give propargyl alcohol **39a**.[34,35] Hydrogenation of the triple bond accompanied by deprotection of the benzylidene acetal with 10% Pd–C afforded saturated triol **40** in 84% yield. After selective sulfonylation of the primary alcohol with TrisCl, treatment of resulting sulfonate **41** with



Scheme 4. Reagents and conditions: (a) **17**, $Zn(OTf)_2$, Et_3N, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 85% (**30a**:**30b** = >97:3); (b) **17**, $Zn(OTf)_2$, Et_3N, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 91% (**30a**:**30b** = 3:>97); (c) H_2 (1 atm), 10% Pd–C, EtOAc, rt; (d) TBSCl, imidazole, DMF, 0 °C to rt, 69% from **30a**; (e) THP-protected-(*S*)-lactaldehyde, LDA, THF, −78 °C; (f) $MgBr_2$, Et_2O, rt; (g) $NaHCO_3$, 49% from **31**; (h) Ac_2O, pyridine, rt, 90%; (i) DBU, CH_2Cl_2, rt, quantitative; (j) 48% aq HF, MeCN–THF (5:3), rt, 91%.



Scheme 5. Retrosynthesis of second-generation approach of fluorescent acetogenins



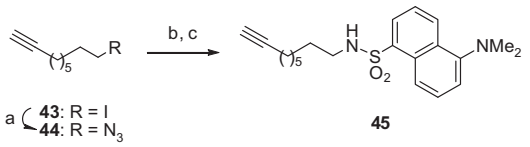
Scheme 6. Reagents and conditions: (a) $Zn(OTf)_2$, Et_3N, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 93% (**39a**:**39b** = >97:3); (b) $Zn(OTf)_2$, Et_3N, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 96% (**39a**:**39b** = 3:>97); (c) H_2 (3 atm), 10% Pd–C, EtOAc, rt, 84%; (d) TrisCl, pyridine, CH_2Cl_2, 0 °C to rt, 79%; (e) K_2CO_3, MeOH, 0 °C to rt, 98%; (f) SO_3·pyridine, DMSO, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 89%.

K_2CO_3 in MeOH gave *threo*/*trans*-THF alcohol **42** in good yield. DMSO oxidation of **42** with SO_3·pyridine complex gave α-tetrahydrofuranyl aldehyde **36** in high yield.[36]

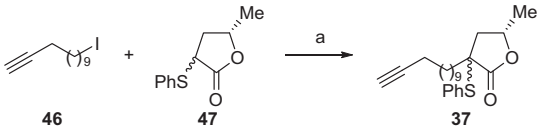
Fluorescent fragment **45** was synthesized by the coupling reaction of dansyl chloride with 8-nonyn-1-amine **44**, which was prepared by the azidation of 9-iodo-1-nonyne **43** followed by reduction under Staudinger conditions (Scheme 7).

γ-Lactone fragment **37** was easily prepared by α-alkylation of known α-sulfenyl-γ-lactone **47**[37] with 12-iodo-1-dodecyne **46**[38] under basic conditions in an almost quantitative yield (Scheme 8).

With all the fragments in hand, we examined the assembly of THF fragment **36** and γ-lactone fragment **37** because we thought it best that the expensive fluorescent fragment **45** be introduced at the late stage of the synthesis, and this synthesis should provide ready access to diverse fluorescent derivatives that have other fluorescent groups (Scheme 9). Asymmetric alkynylation of α-tetrahydrofuranyl aldehyde **36** with alkyne **37** proceeded with high diastereoselectivity (95:5 dr).[39,40] Protection of the secondary



Scheme 7. Reagents and conditions: (a) NaN_3, DMSO, rt, 98%; (b) PPh_3, Et_2O–H_2O (17:1), 0 °C; (c) dansyl chloride, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 96% in two steps.



Scheme 8. Reagents and conditions: (a) LDA, THF–HMPA (2:1), −78 °C to rt, 97%.

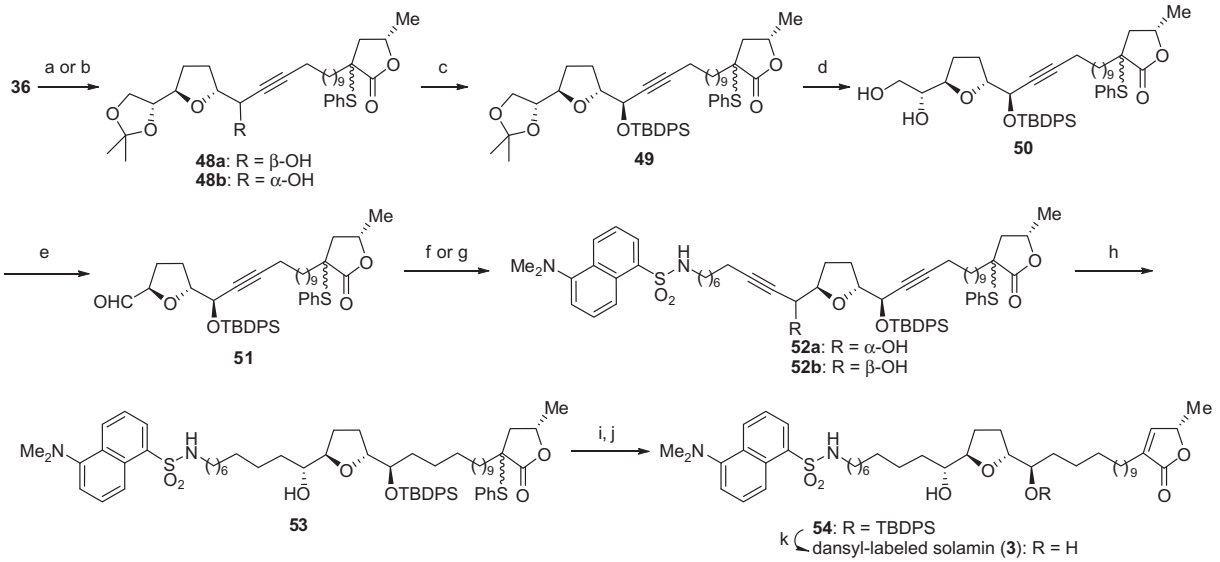
alcohol of **48a** as TBDPS ether, followed by hydrolysis of acetonide, gave diol **50** in high yield. Oxidative cleavage of the 1,2-diol moiety in **50** afforded α-tetrahydrofuranyl aldehyde **51** in 99% yield. The assembly of aldehyde **51** and fluorescent fragment **45** was performed by asymmetric alkynylation and high diastereoselectivity (91:9 dr) was achieved.[41] Unexpectedly, the reduction of diyne **52a** by catalytic hydrogenation over Pd–C, or the diimide reduction ($TsNHNH_2$, AcONa) was difficult to perform and gave a complex mixture of olefinic products that were obtained by partial reduction. Fortunately, hydrogenation with Wilkinson's catalyst under 3 atm pressure of hydrogen in benzene gave desired saturated alcohol **53** in 73% yield. Oxidation of the sulfide, followed by thermal elimination of the resulting sulfoxide, afforded α,β-unsaturated-γ-lactone **54**. The synthesis of dansyl-labeled solamin **3** was completed via deprotection of TBDPS ether with 48% HF in MeCN.[42]

2.3. Growth inhibitory activity of fluorescence-labeled solamins against human cancer cell lines

To examine the influence of the introduction of fluorescent groups at the end of the hydrocarbon chain on the biological activity, we used COMPARE analysis.[43,44] It is well established in a panel of 39 human cancer cell lines (JFCR39) that a pair of compounds potentially share the same mode of action when they indicate fingerprints closely resembling each other (r >0.75; r = Pearson correlation coefficient). Synthesized fluorescence-labeled solamins **2** and **3** and solamin **1** were tested for in vitro antiproliferative activity against JFCR39. Figure 2 shows the growth inhibitory profiles across the JFCR39 (fingerprints) of the three compounds. We noted that the either fingerprint of the fluorescent probes **2** or **3** was very similar to that of solamin **1** although **2** and **3** showed stronger growth inhibition against some cancer cell lines than solamin **1**. The COMPARE analysis revealed that the fingerprints of fluorescence-labeled solamins **2** and **3** significantly correlated with that of solamin **1** (**2** versus **1**: r = 0.864, p <0.01; **3** vs **1**: r = 0.857, p <0.01), suggesting that fluorescent probes **2** and **3** may have the same mode of action as natural solamin **1**.

2.4. Cell distribution of fluorescence-labeled solamin

To identify where in the cell solamin and its derivatives might localize, dansyl-labeled solamin **3** was introduced into CHO-K1 cells and localization was examined by confocal laser scanning microscopy. MitoTracker Red and ER-Tracker Red were used as organelle markers. To visualize the localization of the probes in lysosome, CHO-K1 cells in which GFP-tagged hTAPL (human transporter associated with antigen processing-like) was expressed stably were used. It is known that hTAPL localizes in lysosomal membranes.[45] The observation of cells treated with 10 μM of fluorescence-labeled solamin **3** for 5 h and stained with MitoTracker Red revealed the localization of dansyl-labeled solamin **3** in the mitochondria, as confirmed by the overlapping patterns of fluorescence (green and red). This result confirms the previous proposal that *Annonaceous* acetogenins would act as an inhibitor of complex I in living cells. Moreover, the fluorescence derived from ER-Tracker Red partially co-localized with the signal of **3**, indicating that part of dansyl-labeled solamin **3** localized in the endoplasmic



Scheme 9. Reagents and conditions: (a) **37**, $Zn(OTf)_2$, *i*-Pr_2NEt, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 74% (**48a**:**48b** = 95:5); (b) **37**, $Zn(OTf)_2$, *i*-Pr_2NEt, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 66% (**48a**:**48b** = 4:96); (c) TBDPSCl, imidazole, DMF, 0 °C to rt, quantitative; (d) Dowex 50 W, THF–MeOH (1:1), rt to 60 °C, 96%; (e) $NaIO_4$, THF–H_2O (4:1), rt, 99%; (f) **45**, $Zn(OTf)_2$, *i*-Pr_2NEt, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 66% (**52a**:**52b** = 91:9); (g) **45**, $Zn(OTf)_2$, *i*-Pr_2NEt, (1*S*,2*R*)-*N*-methylephedrine, toluene, rt, 81% (**52a**:**52b** = 4:96); (h) H_2 (3 atm), $(Ph_3P)_3RhCl$, benzene, rt, 73%; (i) *m*CPBA, CH_2Cl_2, 0 °C; (j) toluene, reflux, 68% in two steps; (k) 48% HF aq, MeCN, rt, 71%.

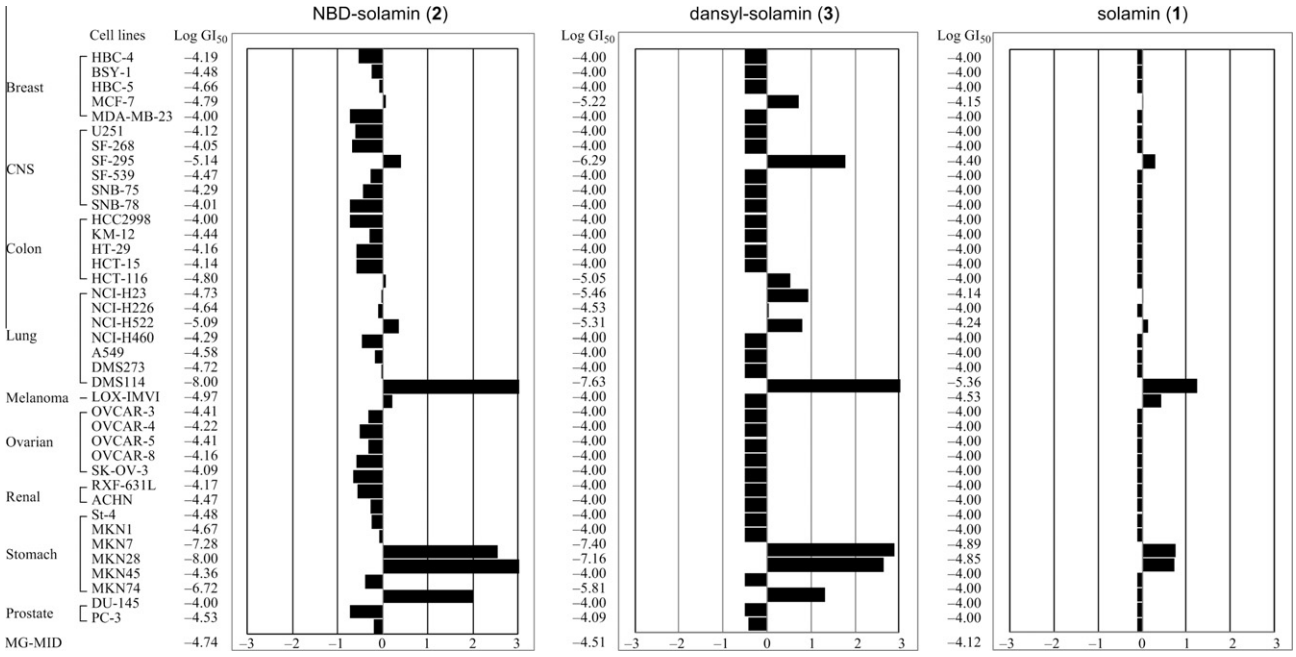


Figure 2. Growth inhibitory profiles (fingerprints) of fluorescence-labeled solamins **2** and **3** and solamin **1** across the JFCR39. Inhibition of cell growth was assessed by the change in total cellular protein following 48 h of treatment with a given test compound, and was measured using the sulforhodamine B colorimetric assay. Fingerprints were produced by computer processing of the 50% growth inhibition (GI_{50}) values. The logarithm of the GI_{50} value for each cell line is indicated. In the plot, columns to the right of zero indicate sensitivity of the cell line to the compound, and columns to the left of zero indicate resistance to the compound. The *x*-axis represents the logarithm of difference between the mean of GI_{50} values for the 39 cell lines and the GI_{50} value for each cell line. One scale represents one logarithm difference. MG-MID = mean of logarithm of GI_{50} values of all cell lines tested. CNS = central nervous system.

reticulum. However, localization in the lysosome was not observed. McLaughlin and co-workers reported that natural acetogenins showed selective inhibitory activity against cancer cells but not normal cells.[46] Wu, Yao and co-workers also described the selectivity of their analogues having the ethylene glycol moiety instead of bis-THF rings for cancer but not normal cells.[9] They suggested that a special mechanism is involved at the level of the cancer cell membrane. On the other hand, our fluorescence-labeled analogue **3** easily penetrated CHO-K1 cells, which are 'normal cells'. Interestingly, however, no toxicity was observed when cells treated with 10 μM of **3** were incubated for 24 h. This result suggests the existence of different mechanism from membrane recognitions that would account for the selective action of *Annonaceous* acetogenins, though the detail is unclear because no study of localization of our probes in cancer cells has yet been conducted.

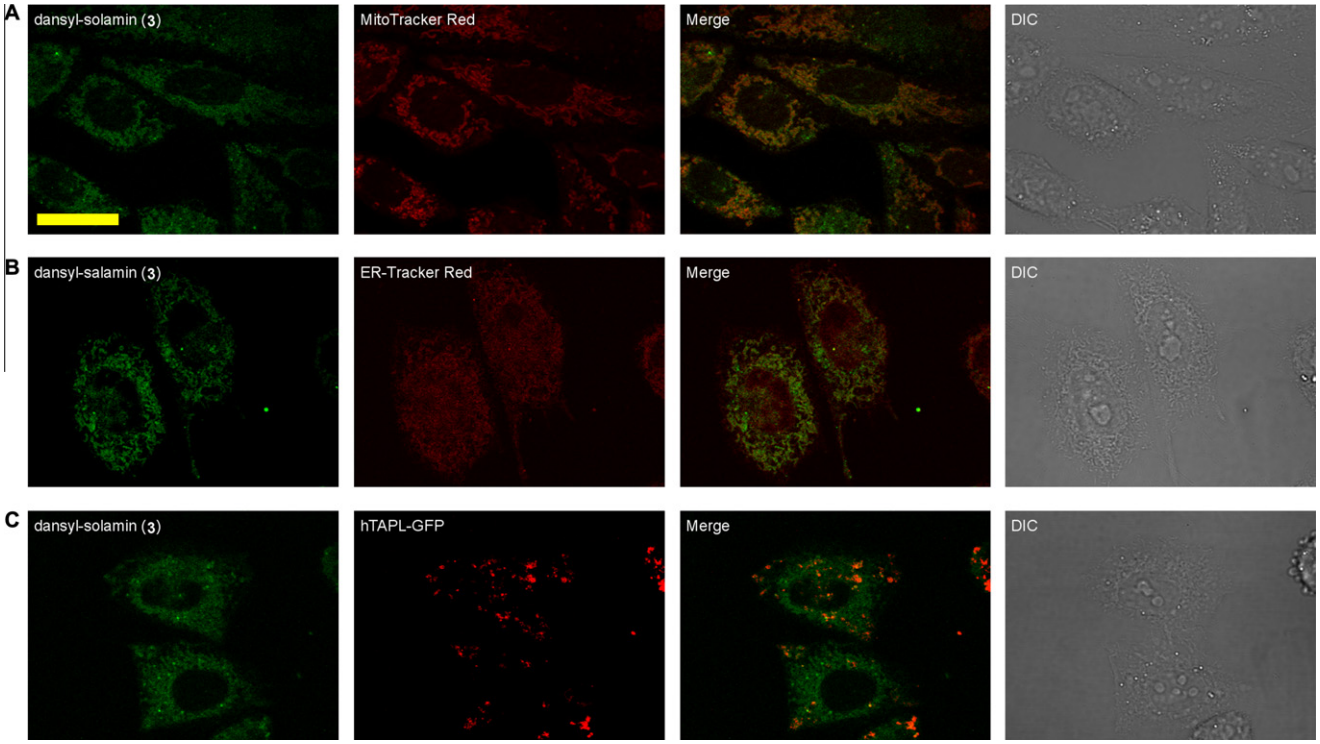


Figure 3. Visualization of dansyl-labeled solamin **3** by fluorescence microscopy. CHO-K1 cells were pretreated with 10 μM dansyl-labeled solamin **3** for 5 h before the cells were stained with organelle markers. (A) CHO-K1 cells labeled with dansyl-labeled solamin **3** were stained with MitoTracker Red and then observed under a confocal microscope. The signal of MitoTracker Red (second from left) merged (third from left) with that of **3** (left) in living cells. (B) CHO-K1 cells labeled with dansyl-labeled solamin **3** were stained with ER-Tracker Red and then observed under a confocal microscope. The signal of ER-Tracker Red (second from left) merged (third from left) with that of **3** (left) in living cells. (C) CHO-K1 cells expressing GFP-fusion proteins labeled with dansyl-labeled solamin **3** and then observed under a confocal microscope. The signal derived from hTAPL-GFP (second from left, red pseudo-color) merged (third from left) with that of **3** (left) in living cells. Bar indicates 20 μm. DIC = Differential interference contrast.

3. Conclusion

We have accomplished the convergent synthesis of fluorescence-labeled solamin, an antitumor *Annonaceous* acetogenin, by two asymmetric alkynylations of 2,5-diformyl tetrahydrofuran with two kinds of alkynes as the key steps. Examination of their growth inhibitory activity against cancer cells revealed that the introduction of fluorescent groups at the end of the hydrocarbon chain of acetogenins did not interfere with the mode of action of the probes. It was observed that dansyl-labeled solamin **3** localized in the mitochondria. Our convergent synthesis is a useful tool for the elucidation of the mechanism of action of *Annonaceous* acetogenins. New results of biological studies will be reported in due course.

4. Experimental section

4.1. Chemistry

Melting points are uncorrected. Optical rotations were measured by using a JASCO DIP-360 digital polarimeter or a JASCO P-1020 digital polarimeter. ^{1}H NMR spectra were recorded in $CDCl_3$ solution with a JEOL JNM-GX-500 spectrometer (500 MHz). ^{13}C NMR spectra were recorded in $CDCl_3$ solution with a JEOL JNM-AL300 spectrometer (75 MHz) or a JEOL JNM-GX-500 spectrometer (125 MHz). All signals are expressed as δ values in ppm downfield from the internal standard tetramethylsilane. The following abbreviations are used: broad = br, singlet = s, doublet = d, triplet = t, quartet = q, quintet = qn, sextet = sext, septet = sep and multiplet = m. IR absorption spectra (FT = diffuse reflectance spectroscopy) were recorded with KBr powder by using a Horiba FT-210 IR spectrophotometer, or as neat films on NaCl plates by using a Shimazu FTIR-8400S and only noteworthy absorptions (in cm^{-1}) are listed. Mass spectra were obtained with a JEOL JMS-600H and JEOL JMS-700 mass spectrometer. Column chromatography was carried out by using Kanto Chemical Silica Gel 60 N (spherical, neutral, 63–210 μm) and flash column chromatography was carried out by using Merck Silica Gel 60 (0.040–0.063 μm). All air- or moisture-sensitive reactions were carried out in flame-dried glassware under an atmosphere of Ar or N_2. All solvents were dried and distilled according to standard procedures. All organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure with rotary evaporator.

4.1.1. (1*R*)-1-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-[(2*RS*,4*S*)-2-phenyl-1,3-dioxolan-4-yl]-2-propyn-1-ol (39a)

A flask was charged with $Zn(OTf)_2$ (7.52 g, 20.7 mmol). Vacuum (7 mmHg) was applied and the flask was heated to 120 °C for 10 h. After the flask was cooled to rt, the vacuum was released. (1*R*,2*S*)-*N*-Methylephedrine (3.71 g, 20.7 mmol), toluene (10 mL), and Et_3N (3.13 mL, 22.4 mmol) were added to the flask with stirring at rt. After 2.5 h, a solution of **10** (3.27 g, 18.8 mmol) in toluene (5.7 mL) was added to the mixture at the same temperature. After 15 min, **38** (4.05 g, 31.1 mmol) in toluene (5 mL) was added to the reaction mixture and the whole mixture was stirred for 6 h at rt. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (2:1→1:1→2:3) as eluent yielded **39a** (5.32 g, 93%, **39a**:**39b** = >97:3) as a colorless oil. $[\alpha]_D^{26}$ +54.5 (*c* 3.19, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.38 (s, 1.5H), 1.39 (s, 1.5H), 1.46 (s, 1.5H), 1.48 (s, 1.5H), 2.45 (d, 0.5H *J* = 4.9 Hz), 2.50 (d, 0.5H, *J* = 4.9 Hz), 3.86 (dd, 0.5H, *J* = 7.9, 4.9 Hz), 3.93 (dd, 0.5H, *J* = 7.9, 4.9 Hz), 4.00 (dd, 0.5H, *J* = 7.9, 6.7 Hz), 4.06 (dd, 0.5H, *J* = 7.9,

6.7 Hz), 4.10–4.15 (m, 0.5H), 4.18 (dd, 0.5H, *J* = 6.7, 4.9 Hz), 4.19 (dd, 0.5H, *J* = 7.9, 6.7 Hz), 4.20–4.25 (m, 0.5H), 4.22 (dd, 0.5H, *J* = 6.7, 4.9 Hz), 4.34 (ddd, 0.5H, *J* = 6.7, 4.9, 1.8 Hz), 4.38 (dd, *J* = 7.9, 6.7 Hz, 0.5H), 4.39 (ddd, 0.5H, *J* = 6.7, 4.9, 1.8 Hz), 4.90 (ddd, 0.5H, *J* = 6.7, 4.9, 1.8 Hz), 4.94 (ddd, 0.5H, *J* = 6.7, 4.9, 1.8 Hz), 5.88 (s, 0.5H), 5.96 (s, 0.5H), 7.38–7.55 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.0 (0.5C), 25.1 (0.5C), 26.6, 63.8, 65.5 (0.5C), 65.89 (0.5C), 65.91 (0.5C), 65.93 (0.5C), 70.6 (0.5C), 70.9 (0.5C), 78.27 (0.5C), 78.30 (0.5C), 83.06 (0.5C), 83.12 (0.5C), 83.3 (0.5C), 83.9 (0.5C), 103.6 (0.5C), 104.8 (0.5C), 110.28 (0.5C), 110.32 (0.5C), 126.4, 126.7, 128.19, 128.24, 129.36 (0.5C), 129.41 (0.5C), 136.3 (0.5C, Ar), 136.8 (0.5C, Ar); IR (KBr) cm^{-1}: 3428, 2989; MS (FAB) *m*/*z*: 305 [*M*+H]$^+$; HRMS (FAB) calcd for $C_{17}H_{21}O_5$: 305.1389; found: 305.1391 [*M*+H]$^+$.

4.1.2. (1*S*)-1-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-[(2*RS*,4*S*)-2-phenyl-1,3-dioxolan-4-yl]-2-propyn-1-ol (39b)

The procedure was same as that used for preparation of **39a**. Colorless oil; $[\alpha]_D^{25}$ +59.7 (*c* 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.37 (s, 1.5H), 1.39 (s, 1.5H), 1.46 (s, 1.5H), 1.48 (s, 1.5H), 2.32–2.46 (br, 1H), 4.00 (t, 0.5H, *J* = 8.5 Hz), 4.01 (t, 0.5H, *J* = 8.5 Hz), 4.02–4.09 (m, 1H), 4.09 (m, 1H), 4.19 (dd, 0.5H, *J* = 7.3, 6.1 Hz), 4.22 (td, 0.5H, *J* = 6.1, 3.7 Hz), 4.27 (td, 0.5H, *J* = 6.1, 3.7 Hz), 4.37 (dd, 0.5H, *J* = 8.5, 7.3 Hz), 4.48–4.52 (m, 0.5H), 4.54 (ddd, 0.5H, *J* = 4.9, 3.7, 1.2 Hz), 4.91 (ddd, 0.5H, *J* = 7.3, 4.9, 2.4 Hz), 4.95 (ddd, 0.5H, *J* = 8.5, 7.3, 1.2 Hz), 5.87 (s, 0.5H), 5.97 (s, 0.5H), 7.35–7.55 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 25.1, 26.4, 62.6 (0.5C), 62.7 (0.5C), 65.2 (0.5C), 65.3 (0.5C), 65.7 (0.5C), 66.1 (0.5C), 70.8 (0.5C), 71.1 (0.5C), 77.6, 83.3 (0.5C), 83.4 (0.5C), 83.79 (0.5C), 83.81 (0.5C), 103.8 (0.5C), 105.0 (0.5C), 110.16 (0.5C), 110.21 (0.5C), 126.6, 126.8, 128.3, 128.4, 129.47 (0.5C), 129.53 (0.5C), 136.4 (0.5C), 137.0 (0.5C); IR (neat) cm^{-1}: 3439, 2988; MS (FAB) *m*/*z*: 305 [*M*+H]$^+$; HRMS (FAB) calcd for $C_{17}H_{21}O_5$: 305.1389; found: 305.1366 [*M*+H]$^+$.

4.1.3. (2*S*,5*R*)-5-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-1,2,5-pentanetriol (40)

A solution of **39a** (1.60 g, 5.26 mmol) in EtOAc (50 mL) was hydrogenated on 10% Pd–C (80 mg) with stirring at rt for 31 h under 3 atm pressure of hydrogen. The Pd–C was filtered off and the filtrate was concentrated under reduced pressure. Purification by column chromatography over silica gel with $CHCl_3$/MeOH (10:1) as eluent yielded **40** (974 mg, 84%) as a colorless solid. $[\alpha]_D^{28}$ +7.1 (*c* 3.97, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.36 (s, 3H), 1.44 (s, 3H), 1.45–1.79 (m, 4H), 3.25 (br s, 1H), 3.44 (dd, 1H, *J* = 11.5, 6.7 Hz), 3.50–3.65 (m, 2H), 3.72 (m, 2H), 3.80–4.30 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.9, 26.2, 28.8, 28.9, 65.5, 65.9, 71.6, 72.0, 78.6, 109.0; IR (KBr) cm^{-1}: 3384, 2938; MS (FAB) *m*/*z*: 221 [*M*+H]$^+$; HRMS (FAB) calcd for $C_{10}H_{21}O_5$: 221.1389; found: 221.1387 [*M*+H]$^+$.

4.1.4. (2*S*,5*R*)-5-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-2,5-dihydroxypentyl 2,4,6-triisopropylbenzene sulfonate (41)

2,4,6-Triisopropylbenzenesulfonyl chloride (206 mg, 0.681 mmol) was added to a solution of **40** (50.0 mg, 0.227 mmol) in pyridine (0.9 mL) and CH_2Cl_2 (1.4 mL) with stirring at 0 °C. After stirred for 15 min at the same temperature, the mixture was stirred at rt for 38 h. Water was added to the reaction mixture and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine prior to drying. Concentration followed by azeotropic removal of pyridine with toluene was repeated three times. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (3:1) as eluent yielded **41** (87.3 mg, 79%) as a colorless solid. Mp 108.3–109.4 °C (*n*-hexane–EtOAc); $[\alpha]_D^{28}$ +6.4 (*c* 1.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.26 (d, 18H, *J* = 6.1 Hz), 1.36 (s, 3H), 1.43 (s, 3H), 1.46–1.80 (m, 4H), 2.48 (br s, 1H), 2.75 (br s, 1H), 2.92 (sept, 1H, *J* = 6.1 Hz), 3.47–3.56 (m, 1H), 3.72 (dd, 1H, *J* = 8.5, 6.1 Hz), 3.89–4.06 (m, 4H), 4.02 (dd, 1H, *J* = 8.5, 7.3 Hz), 4.13 (sep, 2H, *J* = 6.1 Hz), 7.19 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.5 (2C), 24.66 (2C), 24.68 (2C), 25.2, 26.6, 29.1, 29.2, 29.6 (2C), 34.2, 66.0, 69.3, 72.3, 72.6, 78.9, 109.5, 123.8 (2C), 128.9, 150.8 (2C), 153.9; IR (KBr) cm^{-1}; 3473, 2958, 1601, 1566; MS (FAB) *m*/*z*: 487 [*M*+H]$^+$; HRMS (FAB) calcd for $C_{25}H_{43}O_7S$: 487.2729; found: 487.2722 [*M*+H]$^+$.

4.1.5. (2*R*,5*R*)-2-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-5-hydroxymethyltetrahydrofuran (42)

K_2CO_3 (9.66 g, 69.9 mmol) was added to a solution of **41** (3.40 g, 6.99 mmol) in MeOH (300 mL) with stirring at 0 °C. After 30 min at the same temperature, the mixture was stirred at rt for 39 h. Water was added to the reaction mixture and MeOH was removed under the reduced pressure. The mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (1:1→1:2) as eluent yielded **42** (1.39 g, 98%) as a colorless oil. $[\alpha]_D^{26}$ +2.6 (*c* 0.94, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.38 (s, 3H), 1.44 (s, 3H), 1.60–2.05 (m, 4H), 2.20 (br s, 1H), 3.52 (dd, 1H, *J* = 11.5, 4.8 Hz), 3.66 (t, 1H, *J* = 6.7 Hz), 3.72 (dd, 1H, *J* = 11.5, 3.0 Hz), 3.92–4.03 (m, 2H), 4.03–4.13 (m, 1H), 4.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.5, 26.5, 27.3, 28.3, 64.4, 65.9, 78.8, 80.2 (2C), 109.8; IR (KBr) cm^{-1} 3477, 2983; MS (FAB) *m*/*z*: 203 [*M*+H]$^+$; HRMS (FAB) calcd for $C_{10}H_{19}O_4$: 203.1283; found: 203.1285 [*M*+H]$^+$.

4.1.6. (2*R*,5*R*)-2-Formyl-5-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]tetrahydrofuran (36)

i-Pr_2NEt (2.00 mL, 11.8 mmol) and DMSO (0.601 mL, 7.85 mmol) were added to a solution of **42** (397 mg, 1.96 mmol) in CH_2Cl_2 (2 mL) with stirring at 0 °C. After 10 min at the same temperature, SO_3·py (625 mg, 3.93 mmol) was added to the mixture and the mixture was stirred for 30 min at the same temperature. After 1 h at rt, brine was added to the reaction mixture and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (2:1) as eluent yielded **36** (350 mg, 89%) as a colorless oil. The aldehyde was unstable and therefore used immediately in the next reaction.

4.1.7. 9-Azidonon-1-yne (44)

NaN_3 (1.28 g, 19.7 mmol) was added to a solution of **43** (2.46 g, 9.84 mmol) in DMSO (49 mL) with stirring at rt. After 3 h, water was added to the reaction mixture and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (10:1) as eluent yielded **44** (1.60 g, 98%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.30–1.64 (m, 10H), 1.95 (t, 1H, *J* = 2.4 Hz), 2.19 (td, 2H, *J* = 7.3, 2.4 Hz), 3.26 (t, 2H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.2, 26.4, 28.1, 28.35, 28.44, 28.6, 51.2, 68.1, 84.3; IR (neat) cm^{-1}: 3306, 2936, 2097; elemental Anal. Calcd for $C_9H_{15}N_3$: C, 65.42; H, 9.15; N, 25.43. Found: C, 65.15; H. 9.21; N, 25.11.

4.1.8. 5-(Dimetylamino)-*N*-(non-8-ynyl)naphthalene-1-sulfonamide (45)

PPh_3 (1.12 g, 4.28 mmol) was added to a solution of **44** (707 mg, 4.28 mmol) in Et_2O (2.7 mL) with stirring at 0 °C. After 2 h, water was added to the reaction mixture and the whole was stirred for

3 h at the same temperature. HCl (3 *N*) was added to the mixture, the mixture was washed with Et_2O. After the aqueous layer was basified using NaOH, the mixture was extracted with Et_2O, and the solvent was evaporated. To a solution of the residue in CH_2Cl_2 (11 mL) was added *i*-Pr_2NEt (0.853 mL, 5.02 mmol). After stirred for 10 min at the same temperature, 5-dimetylaminonaphthalenesulfonyl chloride (1.36 g, 5.02 mmol) was added to the reaction mixture and the whole was stirred for 3.5 h. After solvent evaporation, purification by column chromatography over silica gel with *n*-hexane/EtOAc (10:1) as eluent yielded **5** (1.53 g, 96% in two steps) as a pale green solid. Mp 58.1–59.1 °C (*n*-hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.06–1.44 (m, 10H), 1.92 (t, 1H, *J* = 2.4 Hz), 2.11 (td, 2H, *J* = 7.3, 2.4 Hz), 2.88 (q, 2H, *J* = 6.7 Hz), 2.89 (s, 6H), 4.56 (br t, 1H, *J* = 6.7 Hz), 7.19 (d, 1H, *J* = 7.3 Hz), 7.53 (t, 1H, *J* = 7.9 Hz), 7.57 (dd, 1H, *J* = 8.5, 7.9 Hz), 8.25 (d, 1H, *J* = 7.3 Hz), 8.28 (d, 1H, *J* = 8.5 Hz), 8.54 (d, 1H, *J* = 7.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.2, 26.1, 28.1, 28.29, 28.31, 29.32, 43.1, 45.4 (2C), 68.1, 84.5, 115.1, 118.7, 123.1, 128.3, 129.6 (2C), 129.8, 130.3, 134.7, 151.9; IR (neat) cm^{-1} : 3295, 2936, 2114, 1587; MS (FAB) *m*/*z*: 395 [*M*+Na]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{21}H_{28}N_2NaO_2S$: 395.1769; found: 395.1758 [*M*+Na]$^+$.

4.1.9. (3*RS*,5*S*)-5-methy-3-phenylsulfenyl-3-(dodec-11-ynyl)-tetrahydrofuran-2-one (37)

n-BuLi (1.58 M in *n*-hexane, 8.65 mL, 13.7 mmol) was added to a solution of *i*-Pr_2NH (1.92 mL, 13.7 mmol) in THF (5.6 mL) with stirring at −78 °C and the mixture was stirred for 30 min. A solution of **47** (3.03 g, 14.5 mmol) in THF (5.6 mL) was added to the mixture. After 1.5 h, a solution of **46** (2.66 g, 9.10 mmol) in THF/HMPA (1:2.7, 9.3 mL) was added to the mixture and the whole was stirred for 5 min at the same temperature. After stirred for 16 h at rt, the reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (10:1) as eluent yielded **37** (3.28 g, 97%) as a yellow oil. $[\alpha]_D^{25}$ −33.4 (*c* 1.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.18 (d, 3H, *J* = 6.1 Hz), 1.21–1.81 (m, 18H), 1.94 (t, 1H, *J* = 2.4 Hz), 1.96 (dd, 1H, *J* = 14.6, 7.3 Hz), 2.18 (td, 2H, *J* = 7.3, 2.4 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 4.9 Hz), 2.52 (dd, 0.85H, *J* = 14.6, 7.3 Hz), 4.49 (qt, 0.85H, *J* = 7.3, 6.1 Hz), 4.60 (qt, 0.15H, *J* = 13.4, 4.6 Hz), 7.31–7.44 (m, 3H), 7.49–7.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.2, 21.3, 24.4, 28.2, 28.5, 28.8, 29.1, 29.2 (2C), 29.3, 36.3, 39.9, 56.1, 68.0, 73.0, 84.5, 128.8 (2C), 129.5, 130.3, 136.6 (2C), 176.9; IR (neat) cm^{-1}: 3304, 2928, 2116, 1763; MS (FAB) *m*/*z*: 373 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{23}H_{33}O_2S$: 373.2201; found: 373.2202 [*M*+H]$^+$.

4.1.10. (3*RS*,5*S*)-3-{(13*R*)-13-Hydroxy-13-[(2*R*,5*R*)-5-[(1*R*)-2,2-dimethyl-1,3-dioxolan-1-yl]-5-hydroxy-methyltetrahydrofuran-2-yl]tridec-11-ynyl}-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-2-one (48a)

A flask was charged with $Zn(OTf)_2$ (447 mg, 1.23 mmol). Vacuum (8 mmHg) was applied and the flask was heated to 120 °C for 9 h. After the flask was cooled to rt, the vacuum was released. (1*R*,2*S*)-*N*-methylephedrine (240 mg, 1.34 mmol), toluene (0.8 mL), and *i*-Pr_2NEt (0.228 mL, 1.34 mmol) were added to the flask with stirring at rt. After 2.5 h, a solution of **37** (417 mg, 1.12 mmol) in toluene (0.8 mL) was added to the mixture at the same temperature. After 15 min, **36** (112 mg, 0.559 mmol) in toluene (0.9 mL) was added to the reaction mixture and the whole mixture was stirred for 5 h at rt. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (2:1) as eluent yielded **48a** (236 mg, 74%, **48a**:**48b** = 95:5) as a colorless oil. $[\alpha]_D^{26}$ −13.8 (*c* 1.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.18 (d, 3H, *J* = 7.3 Hz), 1.25–1.79 (m, 19H), 1.38 (s, 3H), 1.44 (s, 3H), 1.79–1.86 (m, 1H), 1.95–2.08 (m, 1H), 1.97 (dd, 1H, *J* = 13.4, 7.3 Hz), 2.10–2.16 (m, 1H), 2.20 (td, 2H, *J* = 7.3, 2.4 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 4.9 Hz), 2.52 (dd, 0.85H, *J* = 13.4, 7.3 Hz), 2.53 (br s, 1H), 3.70 (t, 1H, *J* = 7.3 Hz), 4.01 (t, 1H, *J* = 7.3 Hz), 4.00–4.05 (m, 1H), 4.07–4.14 (m, 2H), 4.21–4.25 (m, 1H), 4.49 (m, 0.85H), 4.61 (m, 0.15H), 7.33–7.43 (m, 3H), 7.51–7.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.7, 21.4, 24.6, 25.5, 26.4, 27.86, 27.88, 28.4, 28.7, 28.9, 29.2, 29.3 (2C), 29.5, 36.4, 40.0, 56.2, 65.5, 65.8, 73.2, 77.7, 78.3, 79.9, 83.1, 86.5, 109.7, 128.9 (2C), 129.6, 130.3, 136.7 (2C), 177.1; IR (neat) cm^{-1}: 3462, 2928, 2231, 1763, 1458, 1439; MS (FAB) *m*/*z*: 573 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{49}O_6S$: 573.3250; found: 573.3223 [*M*+H]$^+$.

4.1.11. (3*RS*,5*S*)-3-{(13*S*)-13-Hydroxy-13-[(2*R*,5*R*)-5-[(1*R*)-2,2-dimethyl-1,3-dioxolan-1-yl]-5-hydroxy-methyltetrahydrofuran-2-yl]tridec-11-ynyl}-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-2-one (48b)

The procedure was same as that used for preparation of **48a**. Colorless oil; $[\alpha]_D^{26}$ −7.2 (*c* 0.95, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.18 (d, 3H, *J* = 6.7 Hz), 1.25–1.82 (m, 19H), 1.38 (s, 3H), 1.44 (s, 3H), 1.97 (dd, 1H, *J* = 14.0, 6.7 Hz), 2.00–2.09 (m, 3H), 2.19 (td, 2H, *J* = 7.3, 1.8 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 5.5 Hz), 2.34 (d, 1H, *J* = 4.9 Hz), 2.52 (dd, 0.85H, *J* = 14.0, 6.7 Hz), 3.66 (dd, 1H, *J* = 7.3, 6.7 Hz), 4.01 (dd, 1H, *J* = 7.3, 6.7 Hz), 4.06 (q, 1H, *J* = 6.7 Hz), 4.08–4.14 (m, 1H), 4.19 (td, 1H, *J* = 7.3, 3.1 Hz), 4.49 (sext, 0.85H, *J* = 6.7 Hz), 4.52–4.56 (m, 1H), 4.57–4.64 (m, 0.15H), 7.32–7.44 (m, 3H), 7.50–7.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.6, 21.4, 24.5, 25.4, 25.8, 26.4, 28.0, 28.4, 28.7, 28.9, 29.2, 29.3 (2C), 29.4, 36.4, 40.0, 56.2, 63.9, 65.7, 73.1, 77.5, 78.6, 81.1, 82.3, 86.5, 109.6, 128.9 (2C), 129.6, 130.3, 136.7 (2C), 177.0; IR (neat) cm^{-1} : 3460, 2928, 1765; MS (FAB) *m*/*z*: 573 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{49}O_6S$: 573.3250; found: 573.3261 [*M*+H]$^+$.

4.1.12. (3*RS*,5*S*)-3-{(13*R*)-13-*tert*-Butyldiphenylthylsilyoxy-13-[(2*R*,5*S*)-5-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]tetrahydrofuran-2-yl]tridec-11-ynyl}-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-2-one (49)

Imidazole (164 mg, 2.41 mmol) was added to the solution of **48a** (627 mg, 1.10 mmol) in DMF (11 mL) at 0 °C. After stirred for 10 min, TBDPSCl (0.569 mL, 2.19 mmol) was added to the reaction mixture and the whole was stirred for 10 min at the same temperature. After stirred for 12 h at rt, water was added to the mixture. The mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (7:1) as eluent yielded **49** (888 mg, quant.) as a colorless oil. $[\alpha]_D^{25}$ −38.9 (*c* 1.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.06 (s, 9H), 1.18 (d, 3H, *J* = 6.1 Hz), 1.22–1.82 (m, 19H), 1.35 (s, 3H), 1.39 (s, 3H), 1.94–2.03 (m, 1H), 1.96 (dd, 1H, *J* = 14.0, 7.3 Hz), 2.05 (t, 2H, *J* = 7.3 Hz), 2.08–2.18 (m, 2H), 2.33 (dd, 0.15H, *J* = 13.4, 4.9 Hz), 2.51 (dd, 0.85H, *J* = 14.0, 7.3 Hz), 3.66 (dd, 1H, *J* = 7.3, 6.7 Hz), 3.96 (dd, 1H, *J* = 7.3, 6.7 Hz), 3.98–4.05 (m, 2H), 4.13 (q, 1H, *J* = 6.1 Hz), 4.44–4.52 (m, 1.85H), 4.57–4.64 (m, 0.15H), 7.30–7.43 (m, 9H), 7.51–7.57 (m, 2H), 7.65–7.76 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.6, 19.3, 21.4, 24.6, 25.5, 26.4, 26.8 (3C), 27.1, 27.9, 28.3, 28.7, 29.0, 29.3, 29.4 (2C), 29.5, 36.4, 40.0, 56.2, 65.8, 66.5, 73.2, 78.4, 78.9, 80.0, 82.0, 86.4, 109.5, 127.2 (2C), 127.4 (2C), 128.9 (2C), 129.4, 129.6 (2C), 130.4, 133.4, 133.7, 135.8 (2C), 135.9 (2C), 136.8 (2C), 177.1; IR (neat) cm^{-1} : 2930, 2238, 1767; MS (FAB) *m*/*z*: 833 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{49}H_{66}NaO_6S$-Si: 833.4247; found: 833.4240 [*M*+H]$^+$.

4.1.13. (3*RS*,5*S*)-3-{(13*R*)-13-*tert*-Butyldiphenylsilyloxy-13-[(2*R*,5*R*)-5-[(1*R*)-1,2-dihydroxyethan-1-yl]tetrahydrofuran-2-yl]tridec-11-ynyl}-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-2-one (50)

Dowex 50 W (418 mg) was added to a solution of **49** (424 mg, 0.523 mmol) in THF/MeOH (1:1, 12 mL) at rt. After stirred for 10 h at 60 °C, the catalyst was filtered off and the filtrate was concentrated under reduced pressure. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (2:1) as eluent yielded **50** (386 mg, 96%) as a colorless solid. $[\alpha]_D^{26}$ −36.8 (*c* 1.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.07 (s, 9H), 1.17 (d, 3H, *J* = 6.1 Hz), 1.18–1.83 (m, 19H), 1.85–1.99 (m, 2H), 1.96 (dd, 1H, *J* = 13.4, 6.1 Hz), 2.01–2.13 (m, 1H), 2.05 (t, 2H, *J* = 7.3 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 6.1 Hz), 2.45 (br s, 1H), 2.51 (dd, 0.85H, *J* = 13.4, 7.3 Hz), 2.54 (br, 1H), 3.44–3.51 (m, 1H), 3.54–3.67 (m, 2H), 3.87 (dt, 1H, *J* = 8.5, 4.9 Hz), 4.11 (q, 1H, *J* = 6.1 Hz), 4.32 (d, 1H, *J* = 6.1 Hz), 4.44–4.51 (m, 0.85H), 4.56–4.63 (m, 0.15H), 7.32–7.43 (m, 9H), 7.51–7.56 (m, 2H), 7.71 (d, 2H, *J* = 7.3 Hz), 7.75 (d, 2H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.5, 19.3, 21.4, 24.6, 26.8 (3C), 27.9 (2C), 28.3, 28.7, 29.0, 29.3, 29.4 (2C), 29.5, 36.4, 40.0, 56.2, 64.6, 66.9, 73.0, 73.2, 78.7, 80.7, 82.6, 86.6, 127.2 (2C), 127.4 (2C), 128.9 (2C), 129.5, 129.57, 129.63, 130.3, 133.51, 133.54, 135.7 (2C), 136.0 (2C), 136.7 (2C), 177.1; IR (neat) cm^{-1} : 3458, 2930, 1767; MS (FAB) *m*/*z*: 771 $[M+H]^+$; HRMS (FAB) *m*/*z*: calcd for $C_{46}H_{63}O_6SSi$: 771.4115; found: 771.4107 $[M+H]^+$.

4.1.14. (3*RS*,5*S*)-3-{(13*R*)-13-*tert*-Butyldiphenylsilyloxy-13-[(2*R*,5*R*)-5-formyltetrahydrofuran-2-yl]tridec-11-ynyl}-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-2-one (51)

$NaIO_4$ (193 mg, 0.902 mmol) was added to a solution of **50** (580 mg, 0.752 mmol) in THF/H_2O (4:1, 10 mL) at rt. After stirred for 2 h, $NaIO_4$ (48.3 mg, 0.226 mmol) was added to the mixture and the whole was stirred for 2 h. Water was added to the reaction mixture and the mixture was extracted with $CHCl_3$. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by column chromatography over silica gel with *n*-hexane/EtOAc (3:1→2:1) as eluent yielded **51** (550 mg, 99%) as a colorless oil. The aldehyde was unstable and therefore used immediately in the next reaction.

4.1.15. 5-Dimethylamino-*N*-[(10*R*)-10-hydroxy-10-((2*R*,5*R*)-5-{(1*R*)-1-*tert*-butyldiphenylsilyloxy-13-[(3*RS*,5*S*)-2-oxo-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-3-yl]tridec-2-ynyl}tetrahyrofran-2-yl)dec-8-ynyl]naphthalene-1-sulfonamide (52a)

A flask was charged with $Zn(OTf)_2$ (125 mg, 0.343 mmol). Vacuum (8 mmHg) was applied and the flask was heated to 120 °C for 10 h. After the flask was cooled to rt, the vacuum was released. (1*R*,2*S*)-*N*-methylephedrine (67.1 mg, 0.375 mmol), toluene (0.2 mL), and *i*-Pr_2NEt (0.106 mL, 0.624 mmol) were added to the flask with stirring at rt. After 2.5 h, a solution of **45** (116 mg, 0.312 mmol) in toluene (0.3 mL) was added to the mixture at the same temperature. After 15 min, **51** (77.3 mg, 0.104 mmol) in toluene (0.7 mL) was added to the reaction mixture and the whole mixture was stirred for 4 h at rt. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with *n*-hexane/Et_2O (3:1) as eluent yielded **52a** (76.2 mg, 66%, **52a**:**52b** = 91:9) as a pale green amorphous solid. $[\alpha]_D^{24}$ −25.1 (*c* 1.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.04–1.64 (m, 28H), 1.07 (s, 9H), 1.16 (d, 3H, *J* = 6.1 Hz), 1.66–1.82 (m, 1H), 1.91–2.14 (m, 3H), 1.96 (dd, 1H, *J* = 14.6, 7.3 Hz), 2.09 (td, 2H, *J* = 7.3, 2.4 Hz), 2.10 (td, 2H, *J* = 7.3, 2.4 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 4.9 Hz), 2.42 (br, 1H), 2.51 (dd, 0.85H, *J* = 14.6, 7.3 Hz), 2.86 (q, 2H, *J* = 6.1 Hz), 2.87 (s, 6H), 3.94 (q, 1H, *J* = 6.1 Hz), 4.05–4.16 (m, 2H), 4.31–4.37 (m, 1H), 4.42–4.52 (m, 0.85H), 4.54–4.63 (m, 0.15H), 4.74–4.90 (m, 1H), 7.17 (d, 1H, *J* = 7.3 Hz), 7.30–7.40 (m, 9H), 7.48–7.58 (m, 4H), 7.69 (d, 2H, *J* = 7.3 Hz), 7.75 (d, 2H, *J* = 7.3 Hz), 8.24 (d, 1H, *J* = 7.3 Hz), 8.30 (d, 1H, *J* = 8.5 Hz), 8.53 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.5, 18.6, 19.3, 21.4, 24.6, 26.1, 26.8 (3C), 27.5, 27.9, 28.1, 28.2, 28.3, 28.7, 29.0, 29.26, 29.29, 29.35, 29.38, 29.5, 29.6, 36.4, 39.9, 43.1, 45.3 (2C), 56.2, 65.6, 66.8, 73.2, 77.8, 78.6, 82.5, 82.9, 86.1, 86.4, 115.0, 118.6, 123.1, 127.2 (2C), 127.4 (2C), 128.3, 128.9 (2C), 129.4, 129.5 (2C), 129.56, 129.62, 129.7, 130.2, 130.3, 133.5 (2C), 134.7, 135.7 (2C), 136.0 (2C), 136.7 (2C), 151.8, 177.1; IR (neat) cm^{-1} : 3526, 3310, 2930, 2239, 1761; MS (FAB) *m*/*z*: 1111 $[M+H]^+$; HRMS (FAB) *m*/*z*: calcd for $C_{66}H_{87}N_2O_7S_2Si$: 1111.5724; found: 1111.5709 $[M+H]^+$.

4.1.16. 5-Dimethylamino-*N*-[(10*S*)-10-hydroxy-10-((2*R*,5*R*)-5-{(1*R*)-1-*tert*-butyldiphenylsilyloxy-13-[(3*RS*,5*S*)-2-oxo-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-3-yl]tridec-2-ynyl}tetrahyrofran-2-yl)dec-8-ynyl]naphthalene-1-sulfonamide (52b)

The procedure was same as that used for preparation of **52a**. Pale green amorphous solid; $[\alpha]_D^{26}$ −21.2 (*c* 0.95, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00–1.82 (m, 28H), 1.07 (s, 9H), 1.16 (d, 3H, *J* = 6.1 Hz), 1.87–2.20 (m, 4H), 1.96 (dd, 1H, *J* = 14.6, 7.3 Hz), 2.06 (t, 2H, *J* = 7.3 Hz), 2.10 (t, 2H, *J* = 7.3 Hz), 2.33 (dd, 0.15H, *J* = 13.4, 6.1 Hz), 2.39 (br, 1H), 2.51 (dd, 0.85H, *J* = 13.4, 7.3 Hz), 2.87 (q, 2H, *J* = 6.1 Hz), 2.90 (s, 6H), 4.01 (td, 1H, *J* = 7.3, 2.4 Hz), 4.19 (m, 1H), 4.31–4.39 (m, 2H), 4.44–4.51 (m, 0.85H), 4.55–4.62 (m, 0.15H), 5.05 (br t, 1H, *J* = 6.1 Hz), 7.20 (d, 1H, *J* = 7.3 Hz), 7.29–7.43 (m, 9H), 7.47–7.59 (m, 4H), 7.70 (d, 2H, *J* = 7.3 Hz), 7.75 (d, 2H, *J* = 7.3 Hz), 8.24 (d, 1H, *J* = 7.3 Hz), 8.35 (d, 1H, *J* = 8.5 Hz), 8.57 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 18.46, 18.53, 19.2, 21.3, 24.5, 26.0 (2C), 26.8 (3C), 27.5, 28.1, 28.16, 28.23, 28.3, 28.7, 28.9, 29.2, 29.25, 29.3 (2C), 29.4, 36.3, 39.9, 43.0, 45.4 (2C), 56.2, 64.1, 66.7, 73.1, 77.7, 78.7, 82.1, 83.0, 86.1, 86.4, 115.2, 119.1, 123.2, 127.2 (2C), 127.3 (2C), 128.1, 128.9 (2C), 129.36, 129.41 (2C), 129.5 (2C), 129.6, 130.0, 130.3, 133.45, 133.53, 134.8, 135.7 (2C), 135.9 (2C), 136.7 (2C), 151.3, 177.1; IR (neat) cm^{-1}: 3509, 3298, 2930, 1761; MS (FAB) *m*/*z*: 1111 $[M+H]^+$; HRMS (FAB) *m*/*z*: calcd for $C_{66}H_{87}N_2O_7S_2Si$: 1111.5724; found: 1111.5723 $[M+H]^+$.

4.1.17. 5-Dimethylamino-*N*-[(10*R*)-10-hydroxy-10-((2*R*,5*R*)-5-{(1*R*)-1-*tert*-butyldiphenylsilyloxy-13-[(3*RS*,5*S*)-2-oxo-5-methyl-3-(phenylsulfenyl)tetrahydrofuran-3-yl] tridecyl}tetrahyrofran-2-yl)decanyl]naphthalene-1-sulfonamide (53)

A solution of **52a** (74.0 mg, 0.0666 mmol) in benzene (1.5 mL) was hydrogenated on $Rh(PPh_3)_3Cl$ (43.0 mg, 0.0466 mmol) for 67.5 h with stirring at rt under 3 atm pressure of hydrogen. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. Purification by column chromatography over flash silica gel with *n*-hexane/Et_2O (3:1) as eluent yielded **53** (54.7 mg, 73%) as a pale green oil. $[\alpha]_D^{24}$ −7.3 (*c* 0.45, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.02 (s, 9H), 1.02–1.82 m, 43H), 1.18 (d, 3H, *J* = 6.1 Hz), 1.82–1.90 (m, 1H), 1.97 (dd, 1H, *J* = 13.4, 6.1 Hz), 2.33 (dd, 1H, *J* = 13.4, 6.1 Hz), 2.51 (dd, 1H, *J* = 13.4, 7.3 Hz), 2.87 (q, 2H, *J* = 6.1 Hz), 2.89 (s, 6H), 3.03–3.12 (m, 1H), 3.43 (dt, 1H, *J* = 7.3, 6.1 Hz), 3.45–3.54 (m, 1H), 3.92 (dt, 1H, *J* = 8.5, 7.3 Hz), 4.42–4.62 (m, 1H), 4.56 (t, 1H, *J* = 6.1 Hz), 7.19 (d, 1H, *J* = 7.3 Hz), 7.29–7.43 (m, 9H), 7.45–7.60 (m, 4H), 7.70 (d, 2H, *J* = 8.5 Hz), 7.74 (d, 2H, *J* = 7.3 Hz), 8.25 (d, 1H, *J* = 7.3 Hz), 8.28 (d, 1H, *J* = 8.5 Hz), 8.54 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) (Major isomer) δ: 19.6, 21.5, 24.7, 24.9, 25.5, 26.4, 27.1 (3C), 28.7, 28.9, 29.0, 29.3, 29.4 (2C), 29.46 (2C), 29.48 (2C), 29.6 (4C), 29.7, 33.2, 33.4, 36.5, 40.0, 43.3, 45.4 (2C), 56.3, 73.2, 74.0, 76.6,

82.2, 82.3, 115.1, 118.6, 123.2, 127.1 (2C), 127.3 (2C), 128.3, 129.0 (2C), 129.2, 129.3, 129.6, 129.65, 129.68, 129.8, 130.3, 130.4, 133.9, 134.6, 135.2, 135.7 (2C), 136.2 (2C), 136.8 (2C), 152.0, 177.2; IR (neat) cm^{-1} : 3547, 3296, 2926, 1765; MS (FAB) *m*/*z*: 1196 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{66}H_{95}N_2O_7S_2Si$: 1119.6350; found: 1119.6305 [*M*+H]$^+$.

4.1.18. 5-Dimethylamino-*N*-[(10*R*)-10-hydroxy-10-((2*R*,5*R*)-5-{(1*R*)-1-*tert*-butyldiphenylsilyloxy-13-[(5*S*)-2-oxo-5-methyl-2,5-dihydrofuran-3-yl)tridecyl]tetrahyrofran-2-yl}decanyl]-naphtalene-1-sulfonamide (54)

*m*CPBA (75% in water, 6.8 mg, 0.0294 mmol) was added to a solution of **53** (24.5 mg, 0.0219 mmol) in CH_2Cl_2 (1.0 mL) with stirred at 0 °C. After stirred for 15 min, the reaction was quenched with satd. $NaHCO_3$ and the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was dissolved in toluene (1.0 mL), and the mixture was stirred for 20 min under reflux. Purification by flash column chromatography over silica gel with *n*-hexane/EtOAc (3:1) as eluent yielded **54** (15.1 mg, 68% in two steps) as a pale green oil. $[\alpha]_D^{23}$ +13.6 (*c* 1.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.00–1.89 (m, 44H), 1.02 (s, 9H), 1.40 (d, 3H, *J* = 7.3 Hz), 2.26 (t, 2H, *J* = 7.3 Hz), 2.87 (q, 2H, *J* = 6.1 Hz), 2.89 (s, 6H), 3.05–3.13 (m, 1H), 3.43 (q, 1H, *J* = 7.3 Hz), 3.47–3.54 (m, 1H), 3.92 (q, 1H, *J* = 7.3 Hz), 4.64 (t, 1H, *J* = 6.1 Hz), 4.99 (q, 1H, *J* = 7.3 Hz), 6.98 (s, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 7.31–7.43 (m, 6H), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.56 (t, 1H, *J* = 8.5, 7.3 Hz), 7.70 (d, 2H, *J* = 7.3 Hz), 7.74 (d, 2H, *J* = 7.3 Hz), 8.25 (d, 1H, *J* = 7.3 Hz), 8.29 (d, 1H, *J* = 8.5 Hz), 8.54 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.2, 19.6, 24.9, 25.1, 25.5, 26.4, 27.1 (3C), 27.4, 28.7, 28.9, 29.0, 29.27, 29.30, 29.4, 29.47, 29.49, 29.52, 29.56 (2C), 29.58, 29.60, 29.67, 29.70, 33.2, 33.4, 43.3, 45.4 (2C), 74.0, 77.4, 76.6, 82.2, 82.3, 115.1, 118.6, 123.2, 127.1 (2C), 127.3 (2C), 128.3, 129.2, 129.3, 129.59, 129.65, 129.8, 130.3, 133.9, 134.3, 134.6, 135.2, 135.7 (2C), 136.2 (2C), 148.9, 152.0, 173.9; IR (neat) cm^{-1} : 3565, 3291, 2926, 1755; MS (FAB) *m*/*z*: 1009 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{60}H_{89}N_2O_7SSi$: 1009.6160; found: 1009.6153 [*M*+H]$^+$.

4.1.19. 5-Dimethylamino-*N*-[(10*R*)-10-hydroxy-10-((2*R*,5*R*)-5-{(1*R*)-1-hydroxy-13-[(5*S*)-2-oxo-5-methyl-2,5-dihydrofuran-3-yl)tridecyl]tetrahyrofran-2-yl}decanyl]naphthalene-1-sulfonamide (3)

Two drops of 48% aq HF were added to a solution of **54** (25.0 mg, 0.0248 mmol) in MeCN (1.0 mL) with stirring at rt. After stirred for 41 h, the reaction was quenched with satd. $NaHCO_3$, and the mixture was extracted with CH_2Cl_2, The combined organic layers were washed with brine prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with $CHCl_3$/EtOAc (4:1) as eluent yielded **3** (13.6 mg, 71%) as a pale green powder. Mp 58.9–60.2 °C; $[\alpha]_D^{25}$ +17.3 (*c* 0.60, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.09–1.58 (m, 38H), 1.40 (d, 3H, *J* = 7.3 Hz,), 1.60–1.78 (m, 2H), 1.94–2.05 (m, 2H), 2.26 (t, 2H, *J* = 7.3 Hz), 2.40 (br, 2H), 2.87 (q, 2H, *J* = 6.1 Hz), 2.89 (s, 6H), 3.35–3.44 (m, 2H), 3.75–3.84 (m, 2H), 4.62 (t, 1H, *J* = 6.1 Hz), 5.00 (q, 1H, *J* = 7.3 Hz), 6.99 (s, 1H), 7.19 (d, 1H, *J* = 7.3 Hz), 7.53 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.57 (dd, 1H, *J* = 8.5, 7.3 Hz), 8.25 (d, 1H, *J* = 7.3 Hz), 8.28 (d, 1H, *J* = 8.5 Hz), 8.53 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.2, 25.1, 25.5, 25.6, 26.3, 27.4, 28.7 (2C), 28.9, 29.1, 29.2, 29.27, 29.30, 29.46, 29.48, 29.53, 29.6 (4C), 29.7, 33.38, 33.43, 43.3, 45.4 (2C), 74.00, 74.02, 77.4, 82.58, 82.61, 115.1, 118.6, 123.2, 128.3, 129.6, 129.7, 129.8, 130.3, 134.3, 134.7, 148.9, 152.0, 174.0; IR (neat) cm^{-1} : 3503, 3310, 2926, 1751; MS (FAB) *m*/*z*: 771 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: calcd for $C_{44}H_{71}N_2O_7S$: 771.4982; found: 771.4985 [*M*+H]$^+$.

4.2. Biology

4.2.1. Determination of cell growth inhibition profiles (fingerprint) and COMPARE analysis

This experiment was carried out at the Cancer Chemo therapy Center, Japanese Foundation for Cancer Research. The screening panel consisted of the following 39 human cancer cell lines (JFCR39): breast cancer HBC-4, BSY-1, HBC-5, MCF-7, and MDA-MB-231; brain cancer U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; colon cancer HCC2998, KM-12, HT-29, HCT-15, and HCT-116; lung cancer NCI-H23, NCIH-226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; melanoma LOX-IMVI; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancer RXF-631L and ACHN; stomach cancer St-4, MKN1, MKN7, MKN28, MKN45, and MKN74; and prostate cancer DU-145 and PC-3. Inhibition of cell growth was assessed by the change in total cellular protein following 48 h of treatment with a given test compound, and was measured using the sulforhodamine B colorimetric assay. The molar concentration of a test compound required for 50% growth inhibition (GI_{50}) of cells was calculated as reported previously. A detailed method is described elsewhere.[43] A graphic representation (termed fingerprint) of the differential growth inhibition of each compound for the cells in the JFCR39 panel was plotted based on a calculation employing a set of GI_{50} values. We used COMPARE analysis to compare two compounds for the similarity of their mode of action based on their fingerprints. COMPARE analysis was performed by calculating the Pearson correlation coefficient (r) between the GI_{50} mean graphs of the compounds **X** and **Y** using the following formula: $r = ((x_i - x_m)(y_i - y_m))/((x_i - x_m)^2 (y_i - y_m)^2)^{1/2}$, where x_i and y_i are Log GI_{50} of the two compounds, respectively, for each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively (n = 39).[43,44] The Pearson correlation coefficients were used to determine the degree of similarity. The greater the coefficient is, the higher the similarity between **X** and **Y**.

4.2.2. Fluorescence imaging study

CHO-K1 cells were inoculated in 35 mm glass-bottomed dishes (Matsunami, Japan) and cultured for two days in Ham's F12 medium (Sigma, USA) with 7% fetal calf serum at 37 °C. To accomplish double staining with dansyl-labeled solamin **3** and MitoTracker Red, cells were first treated with 10 μM dansyl-labeled solamin **3** in the medium for 5 h. Then, the dishes were washed five times with fresh medium. Thereafter, the cells were further incubated for 30 min in the presence of 50 nM MitoTracker Red CMX-Ros (Molecular Probes, USA) in Ham's F12 medium or for 40 min in the presence of 1 μM ER-Tracker Red (Molecular Probes) in Hank's balanced salt solution. After washing with fresh medium, fluorescence images were obtained with a confocal microscope (FV1000-IX81; Olympus, Japan) with 405 nm (dansyl-labeled solamin **3**) and 559 nm (MitoTracker and ER-Tracker) excitation lines. For the simultaneous detection of dansyl-labeled solamin **3** and lysosomes, a CHO-K1 sub-line stably expressing GFP-tagged hTAPL[45] was cultured and stained with dansyl-labeled solamin **3** as above, and GFP fluorescence was observed with the 473 nm excitation line. For each experiment, samples were run in duplicate, and any fields were similarly stained. Representative images are displayed in Figure 3. No toxicity of compound **3** against CHO-K1 cells was confirmed because desquamated and suspended cells were not observed by microscope observation in dishes which were incubated for 24 h after the distribution study by a microscopy.

Acknowledgments

In vitro antiproliferative activities of fluorescence-labeled solamins and natural solamin against human cancer cell lines were examined by the Screening Committee of New Anticancer Agents

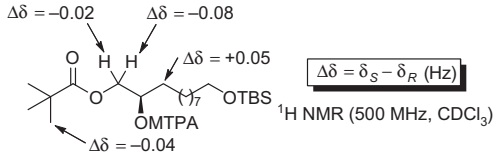
supported by a Grant-in-Aid for Scientific Research on Priority Area 'Cancer' from The Ministry of Education, Culture, Sports, Science and Technology, Japan. We acknowledge financial support in the form of a Grant-in-Aid for Young Scientists (B) [No. 21790113], a Grant-in-Aid for Scientific Research (B) [No. 22390021], and a Grant-in Aid for Scientific Research on Priority Area 'Creation of Biologically Functional Molecules' [No. 18032048] from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supplementary data

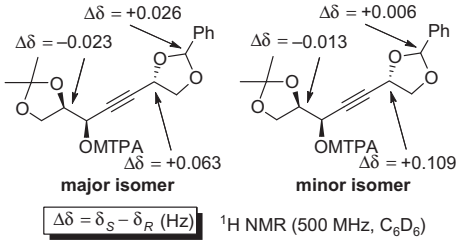
Supplementary data (experimental procedures and characterization data of novel compounds not listed in the Experimental Section) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.10.004.

References and notes

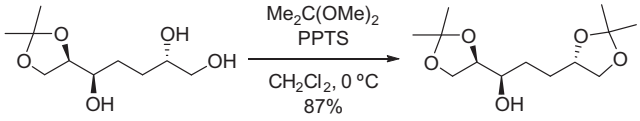
1. Recent review of *Annonaceous* acetogenins: (a) McLaughlin, J. L. *J. Nat. Prod.* **2008**, *71*, 1311; (b) Bermejo, A.; Figadère, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269.
2. Recent review of total synthesis of *Annonaceous* acetogenins: Li, N.; Shi, Z.; Tang, Y.; Chen, J.; Li, X. *Beilstein J. Org. Chem.* **2008**, *4*, 48.
3. Recent review of synthesis of acetogenin analogues: Kojima, N.; Tanaka, T. *Molecules* **2009**, *14*, 3621.
4. (a) Morré, R. D.; de Cabo, J.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343; (b) Wolvetang, E. J.; Johnson, K. L.; Krauer, K. S.; Ralph, J.; Linnane, A. W. *FEBS Lett.* **1994**, *339*, 40.
5. Recently, Nagle and Zhou reported that *Annonaceous* acetogenins inhibited hypoxia-inducible factor-1 (HIF-1) mediated hypoxic signaling pathways in tumor cells: Coothankandaswamy, V.; Liu, Y.; Mao, S.-C.; Morgan, J. B.; Mahdi, F.; Jekabsons, M. B.; Nagle, D. G.; Zhou, Y.-D. *J. Nat. Prod.* **2010**, *73*, 956.
6. Höglinger and co-workers have described that complex I inhibitor including acetogenins may contribute to the pathogenesis of neurodegenerative disorders: Höllerhage, M.; Matusch, A.; Champy, P.; Lombès, A.; Ruberg, M.; Oertel, W. H.; Höglinger, G. U. *Exp. Neurol.* **2009**, *220*, 133.
7. Oberlies, N. H.; Chang, C.; McLaughlin, J. L. *J. Med. Chem.* **1997**, *40*, 2102.
8. Derbré, S.; Roué, G.; Poupon, E.; Susin, S. A.; Hocquemiller, R. *ChemBioChem* **2005**, *6*, 979.
9. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Jiang, S.; Wu, J.-R.; Yao, Z.-J. *ChemBioChem* **2007**, *8*, 172.
10. (a) Sekiguchi, K.; Murai, M.; Miyoshi, H. *Biochim. Biophys. Acta* **2009**, *1787*, 1106; (b) Murai, M.; Ishihara, A.; Nishioka, T.; Yagi, T.; Miyoshi, H. *Biochemistry* **2007**, *46*, 6409.
11. After the publication of our first-generation synthesis of fluorescence-labeled acetogenins, Poupon and co-workers reported the semisynthesis of fluorescence-labeled squamocin having all functionalities, but their analogues have a bulky fluorescent group near the THF moiety: Derbré, S.; Gil, S.; Taverna, M.; Boursier, C.; Nicolas, V.; Demey-Thomas, E.; Vinh, J.; Susin, S. A.; Hocquemiller, R.; Poupon, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5741.
12. (a) Kuwabara, K.; Takada, M.; Iwata, J.; Tatsumoto, K.; Sakamoto, K.; Iwamura, H.; Miyoshi, H. *Eur. J. Biochem.* **2000**, *267*, 2538. and references cited therein; (b) Shimada, H.; Grutzner, J. B.; Kozlowski, J. F.; McLaughlin, J. L. *Biochemistry* **1998**, *37*, 854; (c) Shimada, H.; Kozlowski, J. F.; McLaughlin, J. L. *Pharmacol. Res.* **1998**, *37*, 357.
13. Motoyama, T.; Yabunaka, H.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2089.
14. Han, H.; Sinha, M. K.; D'Souza, L. J.; Keinan, E.; Sinha, S. C. *Chem. Eur. J.* **2004**, *10*, 2149.
15. During the preparation of this manuscript, Nakamaru-Ogiso and Sinha et al. reported that the ND2 subunit in the mitochondria is labeled by their asimicin analogue, which has a photoaffinity tag at the end of the left side of the THF moiety: Nakamaru-Ogiso, E.; Han, H.; Matsuno-Yagi, A.; Keinan, E.; Sinha, S. C.; Yagi, T.; Ohnishi, T. *FEBS Lett.* **2010**, *584*, 883.
16. Maezaki, N.; Urabe, D.; Yano, M.; Tominaga, H.; Morioka, T.; Kojima, N.; Tanaka, T. *Heterocycles* **2007**, *73*, 159.
17. Kojima, N.; Morioka, T.; Yano, M.; Suga, Y.; Maezaki, N.; Tanaka, T. *Heterocycles* **2009**, *79*, 387.
18. (a) Maezaki, N.; Kojima, N.; Tanaka, T. *Synlett* **2006**, 993; (b) Kojima, N. *Yakugaku Zasshi* **2004**, *124*, 673; (c) Kojima, N.; Maezaki, N.; Tominaga, H.; Yanai, M.; Urabe, D.; Tanaka, T. *Chem. Eur. J.* **2004**, *10*, 672; (d) Kojima, N.; Maezaki, N.; Tominaga, H.; Asai, M.; Yanai, M.; Tanaka, T. *Chem. Eur. J.* **2003**, *9*, 4980; (e) Maezaki, N.; Kojima, N.; Tominaga, H.; Yanai, M.; Tanaka, T. *Org. Lett.* **2003**, *5*, 1411; (f) Maezaki, N.; Kojima, N.; Asai, M.; Tominaga, H.; Tanaka, T. *Org. Lett.* **2002**, *4*, 2977.
19. (a) Kojima, N.; Hayashi, H.; Suzuki, S.; Tominaga, H.; Maezaki, N.; Tanaka, T.; Yamori, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6451; (b) Kojima, N.; Fushimi, T.; Maezaki, N.; Tanaka, T.; Yamori, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1637; (c) Tominaga, H.; Maezaki, N.; Yanai, M.; Kojima, N.; Urabe, D.; Ueki, R.; Tanaka, T. *Eur. J. Org. Chem.* **2006**, 1422; (d) Maezaki, N.; Tominaga, H.; Kojima, N.; Yanai, M.; Urabe, D.; Ueki, R.; Tanaka, T.; Yamori, T. *Chem. Eur. J.* **2005**, *11*, 6237; (e) Maezaki, N.; Tominaga, H.; Kojima, N.; Yanai, M.; Urabe, D.; Tanaka, T. *Chem. Commun.* **2004**, 406.
20. (a) Nakanishi, Y.; Chang, F.-R.; Liaw, C.-C.; Wu, Y.-C.; Bastow, K. F.; Lee, K.-H. *J. Med. Chem.* **2003**, *46*, 3185; (b) Sinha, S. C.; Keinan, E. *J. Am. Chem. Soc.* **1993**, *115*, 4891; (c) Myint, S. H.; Cortes, D.; Laurens, A.; Hocquemiller, R.; Leboeuf, M.; Cavé, A.; Cotte, J.; Quéro, A.-M. *Phytochemistry* **1991**, *30*, 3335.
21. Imai, K.; Watanabe, Y. *Anal. Chim. Acta* **1981**, *130*, 377.
22. Schaus, S. E.; Brånalt, J.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 4876.
23. Vyvyan, J. R.; Meyer, J. A.; Meyer, K. D. *J. Org. Chem.* **2003**, *68*, 9144.
24. The stereochemistry of diol **5** was confirmed by the modified Mosher method. The enantiomeric excess was determined by comparison of the ^{1}H NMR spectroscopic data of the (*R*)- and (*S*)-MTPA esters of **5**.



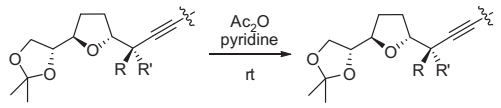
25. The diol was synthesized by Sinha using asymmetric epoxidation; see: Sinha, S. C.; Sinha-Bagchi, A.; Keinan, E. *J. Org. Chem.* **1993**, *58*, 7789.
26. (a) El-Sayed, E.; Anand, N. K.; Carreira, E. M. *Org. Lett.* **2001**, *3*, 3017; (b) Anand, N. K.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 9687; (c) Sasaki, H.; Boyall, D.; Carreira, E. M. *Helv. Chim. Acta* **2001**, *84*, 964; (d) Boyall, D.; López, F.; Sasaki, H.; Frantz, D.; Carreira, E. M. *Org. Lett.* **2000**, *2*, 4233; (e) Frantz, D. E.; Fässler, R. C.; Tomooka, S.; Carreira, E. M. *Acc. Chem. Res.* **2000**, *33*, 373; (f) Frantz, D. E.; Fässler, R.; Carreira, E. M. *J. Am. Chem. Soc.* **2000**, *122*, 1806.
27. In a comparison of the related compounds synthesized in our laboratory, the hydroxyl proton of the *anti*-adduct appears at a higher field than that of the *syn*-adduct. On the other hand, the signals of methine protons at both C2 and C3 positions showed a greater downfield shift in the *anti*-adduct than in the *syn*-adduct. For details, see Ref. 18d.
28. The stereochemistry of **18a** was determined by comparison with the ^{13}C NMR data of Fujimoto's model compounds after reduction of the triple bond and deprotection of the TBS group with HF. For details, see Supplementary data.
29. Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175.
30. (a) Yao, Z.-J.; Wu, Y.-L. *J. Org. Chem.* **1995**, *60*, 1170; (b) Yao, Z.-L.; Wu, Y.-L. *Tetrahedron Lett.* **1994**, *35*, 157.
31. Duret, P.; Figadère, B.; Hocquemiller, R.; Cavé, A. *Tetrahedron Lett.* **1997**, *38*, 8849.
32. For the total synthesis of solamin, see: (a) Makabe, H.; Kuwabara, A.; Hattori, Y.; Konno, H. *Heterocycles* **2009**, *78*, 2369; (b) Raghavan, S.; Subramanian, S. G.; Tony, K. A. *Tetrahedron Lett.* **2008**, *49*, 1601; (c) Prestat, G.; Baylon, C.; Heck, M.-P.; Grasa, G. A.; Nolan, S. P.; Mioskowski, C. *J. Org. Chem.* **2004**, *69*, 5770; (d) Kuriyama, W.; Ishigami, K.; Kitahara, T. *Heterocycles* **1999**, *50*, 981; (e) Makabe, H.; Tanaka, A.; Oritani, T. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1975; (f) Trost, B. M.; Shi, Z. *J. Am. Chem. Soc.* **1994**, *116*, 7459.
33. Jackson, D. Y. *Synth. Commun.* **1988**, *18*, 337.
34. The stereochemistry of propargyl alcohol **39a** was confirmed by the modified Mosher method. The enantiomeric excess was determined by comparison of the ^{1}H NMR spectroscopic data of **39a** and **39b**.



35. The stereochemistry at C4 position in compound **39a** was also determined by transformation to known bis-acetal **I** (Goto, A.; Fujiwara, K.; Kawai, A.; Kawai, H.; Suzuki, T. *Org. Lett.* **2007**, *9*, 5373.) via acetalization of **40**.



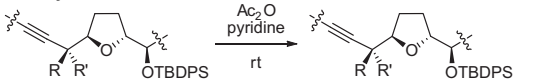
36. Oxidation of alcohol **42** with Dess–Martin periodinane gave aldehyde **36** in 49% yield.
37. White, J. D.; Somers, T. C.; Reddy, G. N. *J. Org. Chem.* **1992**, *57*, 4991.
38. Valicenti, A. J.; Pusch, F. J.; Holman, R. T. *Lipids* **1985**, *20*, 234.
39. The stereoselectivity was determined by comparison of the ^{1}H NMR spectra data of acetyl derivatives **IIa–b**.



48a: R = OH, R' = H
48b: R = H, R' = OH

IIa: R = OAc, R' = H, 92% (95:5 dr)
IIb: R = H, R' = OAc, quant. (96:4 dr)

40. The stereochemistry of **48a–b** was confirmed by comparison of their ^{1}H and ^{13}C NMR spectra with those of model compounds that we have synthesized previously; see Ref. 18d.
41. The stereoselectivity was determined by comparison of the ^{1}H NMR spectra data of acetyl derivatives **IIIa–b**.



52a: R = H, R' = OH
52b: R = OH, R' = H

IIIa: R = H, R' = OAc, quant. (91:9 dr)
IIIb: R = OAc, R' = H, quant. (96:4 dr)

42. The stereochemistry around the THF moiety of **3** was determined by comparison with the ^{13}C NMR data of Fujimoto's model compounds. For details, see Supplementary data.
43. (a) Dan, S.; Okamura, M.; Seki, M.; Yamazaki, K.; Sugita, H.; Okui, M.; Mukai, Y.; Nishimura, H.; Asaka, R.; Nomura, K.; Ishikawa, Y.; Yamori, T. *Cancer Res.* **2010**, *70*, 4982; (b) Yaguchi, S.; Fukui, Y.; Koshimizu, I.; Yoshimi, H.; Matsuno, T.; Gouda, H.; Hirono, S.; Yamazaki, K.; Yamori, T. *J. Natl. Cancer Inst.* **2006**, *98*, 545; (c) Yamori, T.; Matsunaga, A.; Saito, S.; Yamazaki, K.; Komi, A.; Ishizu, K.; Mita, I.; Edatsugi, H.; Matsuba, Y.; Takezawa, K.; Nakanishi, O.; Kohno, H.; Nakajima, Y.; Komatsu, H.; Andoh, T.; Tsuruo, T. *Cancer Res.* **1999**, *59*, 4042.
44. Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
45. Kamakura, A.; Fujimoto, Y.; Motohashi, Y.; Ohashi, K.; Ohashi-Kobayashi, A.; Maeda, M. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 847.
46. Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; McLaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55.