Bioorganic & Medicinal Chemistry 23 (2015) 1276–1283

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of dansyl-labeled probe of thiophene analogue of annonaceous acetogenins for visualization of cell distribution and growth inhibitory activity toward human cancer cell lines

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ARTICLE INFO

Article history:
Received 4 December 2014
Revised 20 January 2015
Accepted 21 January 2015
Available online 29 January 2015

Keywords:
Annonaceous acetogenin
Antitumor agent
Convergent synthesis
Fluorescent-labeled analogue
Growth inhibitory activity

ABSTRACT

The convergent synthesis of the dansyl-labeled probe of the thiophene-3-carboxamide analogue of annonaceous acetogenins, which shows potent antitumor activity, was accomplished by two asymmetric alkynylations of the 2,5-diformyl THF equivalent with an alkyne having a thiophene moiety and another alkyne tagged with a dansyl group. The growth inhibitory profiles toward 39 human cancer cell lines revealed that the probe retained the biological function of its mother compound, and would be useful for studying cellular activity.

1. Introduction

The development of novel antitumor agents, particularly ones with hitherto unexplored modes of action, is strongly advocated because no effective treatment for all kinds of cancers exists and the number of cancer patients continues to rise with the accelerated aging of the world's population.

Our group has been engaged in the syntheses of the analogues of annonaceous acetogenins,[1] which are polyketides isolated from the *Annonaceae* plant growing in tropical and subtropical regions, for use as novel anticancer agents. In Figure 1, the structure of solamin, a mono-THF acetogenin, is shown as an example of acetogenins.[2] Annonaceous acetogenins are long-chain fatty acids (C32 or C34) whose terminal carboxylic acid combines with a 2-propanol unit at the C-2 position to form methyl-substituted α,β-unsaturated γ-lactones.

Most of the annonaceous acetogenins have one to three 2,5-disubstituted tetrahydrofuran (THF) systems with one or two flanking hydroxy groups at the center of a long hydrocarbon chain. It was revealed that acetogenins potently suppressed the growth of human cancer cell lines by inhibiting NADH ubiquinone oxidoreductase (complex I) in the mitochondrial electron transport system.[3] Many total syntheses of natural acetogenins[4] and their analogues[5] have been reported.

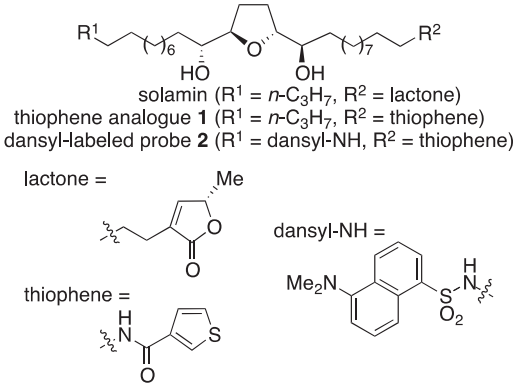

Figure 1. Structures of solamin and its analogues.

We have previously reported the synthesis and biological evaluation of the analogues of solamin,[6] a mono-THF acetogenin. Our analogues are characterized by the presence of various heterocycles instead of an α,β-unsaturated γ-lactone,[7] and most of them

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http://dx.doi.org/10.1016/j.bmc.2015.01.037
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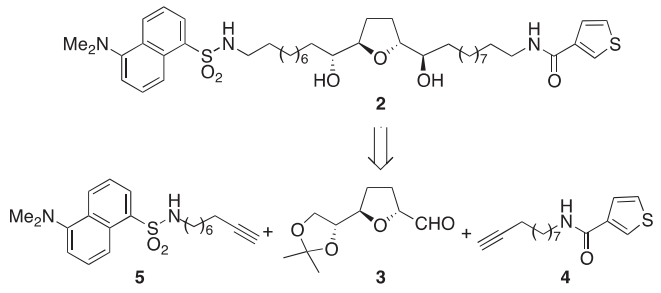
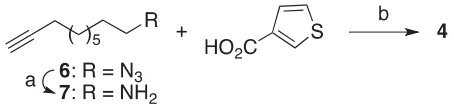


Scheme 1. Retrosynthetic analysis of dansyl-labeled probe **2** of thiophene-3-carboxamide analogue.



Scheme 2. Reagents and conditions: (a) PPh_3, H_2O, Et_2O, 0 °C to rt; (b) EDC, DMAP, THF, 0 °C to rt, 77% in two steps.

show stronger inhibitory activity toward human cancer cell lines than solamin. One of them, thiophene-3-carboxamide **1**, potently inhibited the growth of NCI-H23, a human lung cancer cell line, without critical toxicity in a mouse xenograft assay.[8] To elucidate the mode of action of lead compound **1**, we attempted to visualize its cell distribution.[9] Herein we describe the synthesis of dansyl-labeled analogue **2** and its growth inhibitory activity toward human cancer cell lines.

2. Results and discussion

2.1. Synthesis of dansyl-labeled probe of thiophene analogue of annonaceous acetogenins

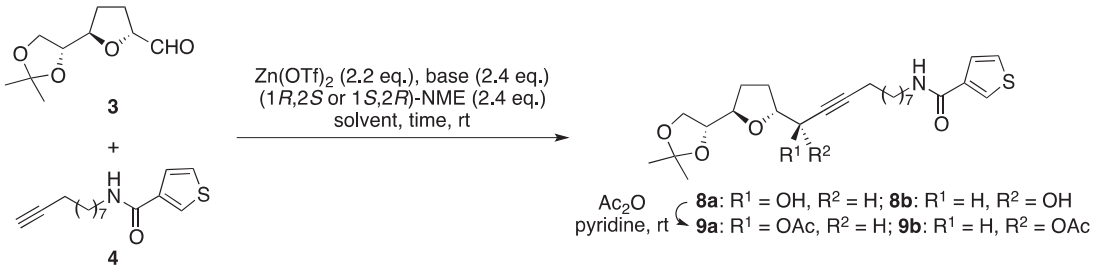
We designed fluorescent-labeled probe **2**, which has a dansyl group as the fluorescent group at the left end of the hydrocarbon chain, because an analogue of solamin, which has a dansyl group at the same position, gave good results in the elucidation of the mode of action of natural acetogenins.[9d–f] Scheme 1 outlines the synthesis of dansyl-labeled probe **2**. Probe **2** is divided into three fragments retrosynthetically: THF fragment **3**, thiophene fragment **4**, and dansyl fragment **5**. A convergent synthesis involving the sequential asymmetric alkynylations of 2,5-diformyl THF equivalent **3** with two alkynes **4** and **5** was planned. The direct alkynylation with **4** or **5** is a challenging approach because the amide proton may hinder the reaction.

Thiophene fragment **4** was synthesized by reducing known azide **6**[7a] into primary amine **7** followed by condensation of commercially available 3-thiophenecarboxylic acid with EDC and DMAP in THF in good yield (Scheme 2).

We examined the applicability of Carreira's asymmetric alkynylation[10] to the reaction of α-tetrahydrofuranyl aldehyde **3**, which was prepared with our procedure,[11] with alkyne **4** (Table 1). Our first attempted reaction with Et_3N in the presence of (1*R*,2*S*)-*N*-methylephedrine (NME) in toluene gave propargyl alcohol **8a** in low yield with moderate diastereoselectivity (entry 1).[12] The diastereoselectivity was determined by analyzing the ^1H NMR spectra of acetate **9** after acetylation with Ac_2O in pyridine. The use of *i*-Pr_2NEt instead of Et_3N accelerated the reaction, but the decomposition of aldehyde **3** was observed (entry 2). It was revealed that this asymmetric alkynylation gave a higher yield when it was conducted in the presence of a higher solvent concentration.[11f] However, the reaction hardly proceeded when the solvent concentration exceeded 0.2 M because of the low solubility of alkyne **4** in toluene. Therefore, the solvent was changed to CH_2Cl_2 as **4** showed good solubility in it. As a result, the yield was improved to 65% with good diastereoselectivity (85:15) when the reaction was carried out in 0.4 M CH_2Cl_2 solution (entry 4). When the solvent concentration was increased further, the diastereoselectivity was slightly reduced (entry 5). Diastereomer **8b** was obtained in good yield with moderate diastereoselectivity by use of the antipode of NME (entry 6).

Separation of the two diastereomers (**8a**–**b** or **9a**–**b**) was difficult by silica gel chromatography. As mentioned above, acetylation of the mixture of **8a** and **8b** with Ac_2O in pyridine gave acetates **9a** and **9b** while retaining the diastereomeric ratio. Interestingly, we found that acetylation of the mixture of **8a** and **8b** (88:12) by using NaH as the base in THF at 0 °C afforded acetate **9a** with high diastereoselectivity (96:4), although the yield was low (Table 2, entry 1). By increasing the equivalent of Ac_2O and the reaction time, the

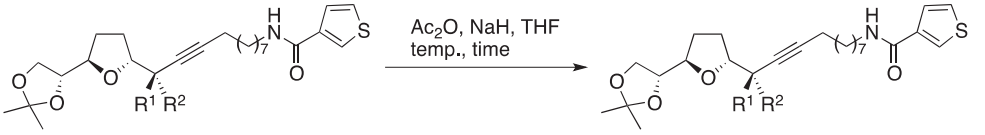
Table 1
Asymmetric alkynylation of α-tetrahydrofuranyl aldehyde **3** with alkyne bearing thiophene **4**



Entry	NME	Solvent[a] (concn, M)	Base	Time (h)	Yield[b] (%, **8a**:**8b**)
1	1*R*,2*S*	Toluene (0.2)	Et_3N	16	47 (80:20)
2	1*R*,2*S*	Toluene (0.2)	*i*-Pr_2NEt	4	49 (86:14)
3	1*R*,2*S*	CH_2Cl_2 (0.3)	*i*-Pr_2NEt	3	63 (88:12)
4	1*R*,2*S*	CH_2Cl_2 (0.4)	*i*-Pr_2NEt	2	65 (85:15)
5	1*R*,2*S*	CH_2Cl_2 (0.8)	*i*-Pr_2NEt	2.5	70 (79:21)
6	1*S*,2*R*	CH_2Cl_2 (0.4)	*i*-Pr_2NEt	3.5	85 (12:88)

[a] Concentration of aldehyde in reaction solvent.
[b] Isolated yield. Diastereomer ratio (dr) was determined by ^1H NMR measurement after acetylation of resulting secondary alcohol with Ac_2O and pyridine.

Table 2
Diastereoselective acetylation of secondary alcohol

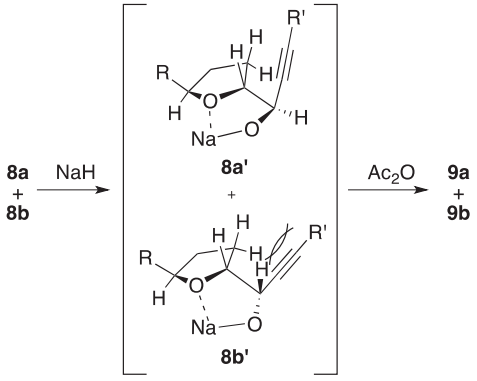


8a: R^1 = OH, R^2 = H; **8b**: R^1 = H, R^2 = OH

9a: R^1 = OAc, R^2 = H; **9b**: R^1 = H, R^2 = OAc

Entry	Substrate (dr)	Ac_2O (equiv)	Temp	Time (h)	Yield[a] (%)
1	**8a** (88:12)	3	0 °C	3	**9a** (22, dr = 96:4), **8a** (70)
2	**8a** (88:12)	6	0 °C	12	**9a** (44, dr = 95:5), **8a** (38)
3	**8a** (88:12)	15	0 °C	16	**9a** (65, dr = 96:4), **8a** (32)
4	**8a** (85:15)	15	0 °C to rt	5	**9a** (73, dr = 95:5)
5	**8a** (85:15)	15	0 °C to rt	10	**9a** (84, dr = 96:4)
6	**8b** (12:88)	15	0 °C to rt	10.5	**9b** (63, dr = 20:80)

[a] Isolated yield. Dr was determined by ^1H NMR.

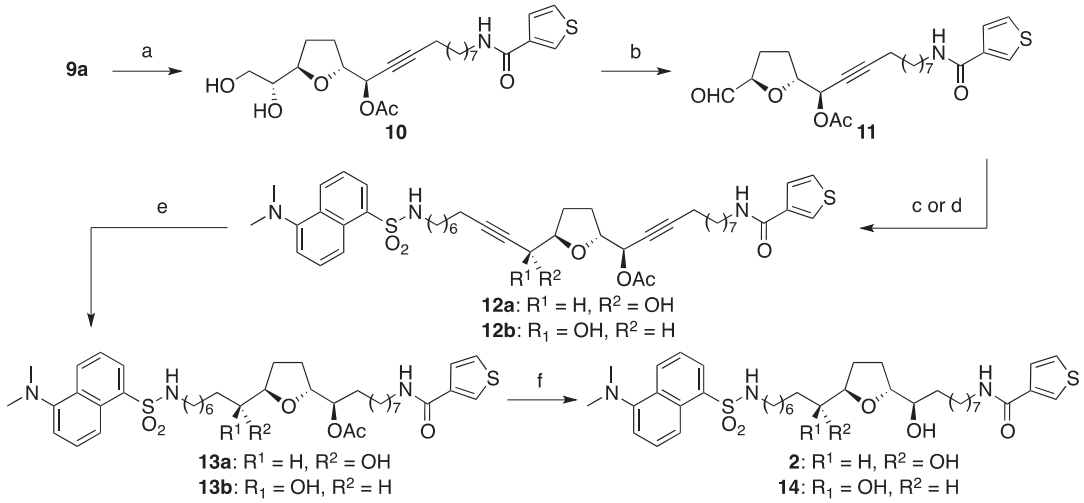


Scheme 3. Plausible mechanism of diastereoselective acetylation.

yield was improved to 65% while retaining the high diastereoselectivity (entry 3). Moreover, the yield reached 84% with high diastereoselectivity when the reaction was conducted at room temperature for 10 h (entry 5). On the other hand, the acetylation of the mixture containing **8b** as the major isomer under the same conditions afforded acetate **9b** with a lower diastereomeric ratio than the starting material (entry 6).

A plausible mechanism of the diastereoselective acetylation is shown in Scheme 3. In the reaction mixture, sodium alkoxide generated from **8a** may form five-membered ring **8a′** by chelation between the sodium atom and the oxygen atom in the THF ring. On the other hand, the sodium alkoxide from **8b** may not be able to form similar structure **8b′** because of steric repulsion between the alkyne moiety and the hydrogen atom in the THF ring. Therefore, it can be assumed that the reaction preferentially proceeds from more reactive intermediate **8a′** to give acetate **9a** with a high diastereomeric ratio and unreacted starting material **8** with a low diastereomeric ratio.

With acetate **9a** having an acceptable diastereomeric ratio in hand, the introduction of the fluorescent group was examined (Scheme 4). Hydrolysis of the dimethylacetal in **9a**, followed by oxidative cleavage of resulting diol **10** gave aldehyde **11** in good yield. The asymmetric alkynylation of aldehyde **11** with alkyne **5** bearing the dansyl group by using (1*R*,2*S*)-NME in CH_2Cl_2 smoothly proceeded to give alcohol **12a** in 80% yield with good diastereoselectivity (87:13). Diastereomer **12b** was also obtained by use of the antipode of NME in high yield with good diastereoselectivity (88:12). The mixture of two diastereomers **12a–b** was used in the next reaction because it was difficult to separate them. Acetate **13a** was synthesized by hydrogenation of the triple bond in **12a** with Wilkinson's catalyst in 77% yield. Finally, deacetylation with K_2CO_3 in MeOH gave dansyl-labeled probe **2** in an optically pure



Scheme 4. Reagents and conditions: (a) 60% aq AcOH, rt, 99%; (b) $NaIO_4$, THF/H_2O (3:1), rt, 92%; (c) $Zn(OTf)_2$, *i*-Pr_2NEt, (1*R*,2*S*)-*N*-methylephedrine, CH_2Cl_2, rt, 80% (**12a**:**12b** = 87:13); (d) $Zn(OTf)_2$, *i*-Pr_2NEt, (1*S*,2*R*)-*N*-methylephedrine, CH_2Cl_2, rt, 91% (**12a**:**12b** = 12:88); (e) H_2 (1 atm), $Rh(Ph_3P)_3Cl$, benzene/MeOH (1:1), 77% from **12a**, 85% from **12b**; (f) K_2CO_3, MeOH, rt, 62% from **13a**, 79% from **13b**.

form after purification by flash silica gel chromatography. Diastereomer **14** was also obtained from **12b** by employing the same procedure as that used for the synthesis of **2**. The stereochemistries around the THF moiety of **2** and **14** generated by the two asymmetric alkynylations were confirmed by comparison with the ^{13}C NMR data of Fujimoto's model compounds.[13] The chemical shifts around the THF moiety of **2** and **14** were in good agreement with those of *threo*/*trans*/*threo*- or *threo*/*trans*/*erythro*-type model compounds, respectively (For details, see Supplementary data).

2.2. Growth inhibitory activity of dansyl-labeled probe toward 39 human cancer cell lines

We used COMPARE analysis to confirm the anticancer effect of dansyl-labeled probe **2** and estimate the similarity between **2** and thiophene analogue **1**.[14] 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 have fingerprints, the growth inhibitory profiles across JFCR39, closely resembling each other. Dansyl-labeled probe **2** was tested for in vitro antiproliferative activity against 39 human cancer cell lines. Figure 2 shows the fingerprint of **2**. The activities of **1** are also shown for comparison. Although the potencies of **2** were weaker than that of **1**, the fingerprint of dansyl-labeled probe **2** was similar to that of mother compound **1**. The COMPARE analysis revealed that the fingerprint of dansyl-labeled probe **2** moderately correlated with that of **1** (**2** vs **1**: Pearson's $r = 0.517$, $P < 0.001$), suggesting that fluorescent probe **2** may have a similar mode of action to **1**.

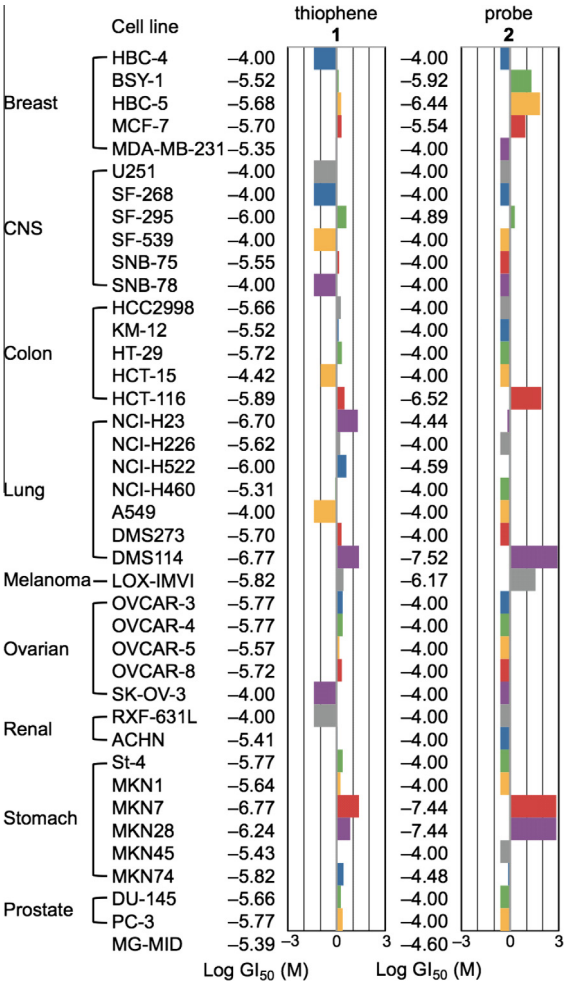


Figure 2. Growth inhibitory profiles (fingerprints) of thiophene analogue **1** and dansyl-labeled probe **2** across JFCR39. Cell growth inhibition was assessed by measuring the change in total cellular protein following 48 h of treatment with a given test compound, using the sulforhodamine B colorimetric 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 for all the cell lines tested. CNS = central nervous system.

3. Conclusions

The convergent synthesis of the dansyl-labeled probe of the thiophene-3-carboxamide analogue of annonaceous acetogenins, which showed potent antitumor activity, was accomplished by two asymmetric alkynylations of the 2,5-diformyl THF equivalent with two alkynes, one having a thiophene moiety and the other, a dansyl group. The diastereomers formed by the asymmetric alkynylation could be separated by the diastereoselective acetylation of the secondary alcohol with Ac_2O and NaH. The growth inhibitory profiles of the probe for 39 human cancer cell lines were similar to those of the thiophene-3-carboxamide analogue, suggesting that the probe possessed the biological function of its mother compound. Visualization of cell distribution with the probe is under way.

4. Experimental

Melting points are uncorrected. Optical rotations were measured by using a JASCO DIP-360 digital polarimeter or a JASCO P-1020 digital polarimeter. ^1H NMR spectra were recorded in the specified solvent with a JEOL JNM-GX-500 spectrometer (500 MHz) or a JEOL JNM-EX-270 spectrometer (270 MHz). ^{13}C NMR spectra were recorded in the specified solvent with a JEOL JNM-AL300 spectrometer (75 MHz), a JEOL JNM-ECS-400 spectrometer (100 MHz), or a JEOL JNM-GX-500 spectrometer (125 MHz). Chemical shifts are reported in ppm relative to the internal solvent signal [$CDCl_3$: 7.26 ppm (^1H NMR), 77.0 ppm (^{13}C NMR); DMSO-d_6: 39.5 ppm (^{13}C NMR)] or tetramethylsilane [0 ppm] as the internal standard. The following abbreviations are used: broad singlet = br s, 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 Shimadzu FTIR-8400S, and only noteworthy absorptions (in cm^{-1}) are listed. Mass spectra were obtained with JEOL JMS-600H and JEOL JMS-700 mass spectrometers. 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 (40–63 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, if necessary. All organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure with a rotary evaporator.

4.1. *N*-(Undec-9-yn-1-yl)thiophene-3-carboxamide (4)

PPh_3 (17.1 g, 65.2 mmol) was added to a solution of **6** (11.6 g, 64.7 mmol) in Et_2O (62 mL) with stirring at 0 °C. After stirring for 10 min at the same temperature, the whole was stirred for 17 h at rt. Water (2.34 mL, 130 mmol) was added to the reaction mixture and the whole was stirred for 7 h at the same temperature.

4 N HCl aq was added to the mixture, the mixture was washed with Et_2O. After the aqueous layer was basified using 4 N NaOH aq, the mixture was extracted with Et_2O prior to drying and solvent evaporation. To a solution of the residue in THF (310 mL) were added *N*,*N*′-dimethyl-4-aminopyridine (7.57 g, 62.0 mmol), 3-thiophenecarboxylic acid (9.31 g, 72.6 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (13.2 g, 68.9 mmol) at 0 °C. After stirring for 15 h at rt, water was added and the mixture was extracted with Et_2O prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with CH_2Cl_2/EtOAc (5:1) as eluent yielded **4** (13.1 g, 77% in two steps) as a colorless powder. Mp 85.6–87.3 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29–1.41 (m, 8H), 1.50 (qn, 2H, *J* = 7.3 Hz), 1.57 (qn, 2H, *J* = 7.3 Hz), 1.93 (t, 1H, *J* = 2.7 Hz), 2.16 (td, 2H, *J* = 6.9, 2.7 Hz), 3.39 (q, 2H, *J* = 6.9 Hz), 6.20 (br s, 1H), 7.31 (dd, 1H, *J* = 5.0, 2.8 Hz), 7.38 (dd, 1H, *J* = 5.0, 1.4 Hz), 7.85 (dd, 1H, *J* = 2.8, 1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.3, 26.8, 28.3, 28.6, 28.9, 29.1, 29.6, 39.8, 68.1, 84.7, 126.0, 126.3, 127.9, 137.7, 163.1; IR (NaCl) cm^{-1}: 3304, 1630; MS (FAB) *m*/*z*: 264 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{15}H_{22}NOS$: 264.1422. Found: 264.1414 [*M*+H]$^+$.

4.2. *N*-((*R*)-11-{(2*R*,5*R*)-5-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-tetrahydrofuran-2-yl}-11-hydroxyundec-9-ynyl)thiophene-3-carboxamide (8a, Table 1, entry 3)

A flask was charged with $Zn(OTf)_2$ (2.17 g, 5.97 mmol). Vacuum (10 mmHg) was applied and the flask was heated to 120 °C for 3 h. After the flask was cooled to rt, the vacuum was released. Then, (1*R*,2*S*)-*N*-methylephedrine (1.17 g, 6.52 mmol), CH_2Cl_2 (9.1 mL), and *i*-Pr_2NEt (1.11 mL, 6.52 mmol) were added to the flask with stirring at rt. After 3 h, **4** (858 mg, 3.26 mmol) was added to the mixture at the same temperature. After stirring for 30 min, **3** (544 mg, 2.72 mmol) in CH_2Cl_2 (3.0 mL) was added to the reaction mixture and the whole mixture was stirred for 3 h at rt. The reaction was quenched with saturated NH_4Cl 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 column chromatography over silica gel with *n*-hexane/EtOAc (2:3) as eluent yielded **8a** (790 mg, 63%, dr = 88:12) as a colorless oil. $[\alpha]_D^{25}$ +10.2 (*c* 1.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.24–1.40 (m, 8H), 1.38 (s, 3H), 1.43 (s, 3H), 1.49 (qn, 2H, *J* = 7.3 Hz), 1.60 (qn, 2H, *J* = 7.3 Hz), 1.69 (dq, 1H, *J* = 12.2, 7.9 Hz), 1.79–1.88 (m, 1H), 1.97–2.07 (m, 1H), 2.09–2.16 (m, 1H), 2.19 (td, 2H, *J* = 7.0, 2.1 Hz), 3.40 (q, 2H, *J* = 6.7 Hz), 3.71 (t, 1H, *J* = 7.6 Hz), 3.99–4.05 (m, 2H), 4.07–4.13 (m, 2H), 4.23 (dt, 1H, *J* = 7.3, 2.1 Hz), 6.00 (br s, 1H), 7.33 (dd, 1H, *J* = 5.1, 3.1 Hz), 7.37 (dd, 1H, *J* = 5.1, 1.2 Hz), 7.85 (dd, 1H, *J* = 3.1, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (major isomer) δ: 18.6, 25.5, 26.4, 26.8, 27.90, 27.94, 28.3, 28.6, 28.8, 29.0, 29.6, 39.8, 65.6, 65.8, 77.9, 78.3, 80.0, 83.1, 86.5, 109.7, 126.0, 126.3, 127.9, 137.8, 163.1; IR (NaCl) cm^{-1}: 3335, 3327, 1632; MS (FAB) *m*/*z*: 464 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{25}H_{38}NO_5S$: 464.2471. Found: 464.2465 [*M*+H]$^+$.

4.3. *N*-((*S*)-11-{(2*R*,5*R*)-5-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]tetrahydrofuran-2-yl}-11-hydroxyundec-9-ynyl)thiophene-3-carboxamide (8b, Table 1, entry 6)

The procedure was the same as that used for preparation of **8a** by use of (1*S*,2*R*)-*N*-methylephedrine, giving **8b** (85%, dr = 88:12). Colorless oil; $[\alpha]_D^{24}$ +16.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.24–1.39 (m, 8H), 1.37 (s, 3H), 1.43 (s, 3H), 1.48 (qn, 2H, *J* = 7.3 Hz), 1.58 (qn, 2H, *J* = 7.3 Hz), 1.61–1.67 (m, 1H), 2.01–2.08 (m, 3H), 2.18 (td, 2H, *J* = 7.3, 2.4 Hz), 3.39 (td, 2H *J* = 7.3, 6.1 Hz), 3.67 (t, 1H, *J* = 7.3 Hz), 4.00 (t, 1H, *J* = 7.3 Hz), 4.06 (td, 1H, *J* = 7.3, 6.1 Hz), 4.11 (dt, 1H, *J* = 7.3, 6.1 Hz), 4.16–4.19 (m, 1H), 4.51–4.53 (m, 1H), 6.41 (br s, 1H), 7.31 (dd, 1H, *J* = 4.9, 3.7 Hz), 7.41 (dd, 1H, *J* = 4.9, 1.2 Hz), 7.89 (d, 1H, *J* = 2.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (major isomer) δ: 18.5, 25.4, 25.9, 26.4, 26.8, 28.0, 28.3, 28.5, 28.8, 29.0, 29.5, 39.7, 64.0, 65.8, 77.7, 78.6, 81.0, 82.3, 86.5, 109.6, 126.0, 126.1, 127.8, 137.7, 163.1; IR (NaCl) cm^{-1}: 3335, 1634; MS (FAB) *m*/*z*: 464 [*M*+H]$^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{25}H_{38}NO_5S$: 464.2471. Found: 464.2475 [*M*+H]$^+$.

4.4. (*R*)-1-{(2*R*,5*R*)-5-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-tetrahydrofuran-2-yl}-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (9a, Table 2, entry 5)

NaH (60% in oil, 108 mg, 2.70 mmol) was added to a solution of **8a** (417 mg, 0.900 mmol, dr = 85:15) in THF (18 mL) with stirring at 0 °C. After stirring for 45 min at the same temperature, Ac_2O (1.28 mL, 13.5 mmol) was added. The whole was stirred for 10 h at rt. Water was added to the mixture and the mixture was extracted with CH_2Cl_2 prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with *n*-hexane/EtOAc (1:1) as eluent yielded **9a** (383 mg, 84%, dr = 96:4) as a colorless oil. $[\alpha]_D^{23}$ −13.6 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.23–1.39 (m, 8H), 1.36 (s, 3H), 1.41 (s, 3H), 1.48 (qn, 2H, *J* = 7.3 Hz), 1.59 (qn, 2H, *J* = 7.3 Hz), 1.66–1.74 (m, 1H), 1.89–1.95 (m, 1H), 1.99–2.06 (m, 1H), 2.09 (s, 3H), 2.10–2.15 (m, 1H), 2.19 (td, 2H, *J* = 7.3, 2.4 Hz), 3.40 (q, 2H *J* = 6.8 Hz), 3.72 (dd, 1H, *J* = 8.5, 6.1 Hz), 3.98 (dd, 1H, *J* = 8.5, 6.1 Hz), 4.03–4.08 (m, 2H), 4.20 (td, 1H, *J* = 7.3, 6.1 Hz), 5.35 (dt, 1H, *J* = 7.3, 2.4 Hz), 6.14 (br s, 1H), 7.32 (dd, 1H, *J* = 4.9, 2.4 Hz), 7.38 (dd, 1H, *J* = 4.9, 1.2 Hz), 7.85 (dd, 1H, *J* = 2.4, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.6, 21.1, 25.5, 26.3, 26.8, 27.5, 28.1, 28.2, 28.6, 28.8, 29.0, 29.6, 39.8, 65.8, 66.4, 75.2, 78.2, 79.6, 80.2, 87.2, 109.6, 126.0, 126.3, 127.8, 137.8, 163.1, 170.0; IR (NaCl) cm^{-1}: 3341, 1742, 1634; MS (FAB) *m*/*z*: 506 [*M*+H]$^+$; HRMS (FAB): *m*/*z*: Calcd for $C_{27}H_{40}NO_6S$: 506.2576. Found: 506.2563 [*M*+H]$^+$.

4.5. (*S*)-1-{(2*R*,5*R*)-5-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]-tetrahydrofuran-2-yl}-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (9b, Table 1)

Ac_2O (0.500 mL, 5.28 mmol) was added to a solution of **8b** (13.9 mg, 30.0 μmol, dr = 88:12) in pyridine (0.5 mL) with stirring at rt. The whole was stirred for 9.5 h at the same temperature. The solvent was evaporated under the reduced pressure. Purification by flash column chromatography over silica gel with *n*-hexane/EtOAc (1:1) as eluent yielded **9b** (15.6 mg, quant., dr = 88:12) as a colorless oil. $[\alpha]_D^{25}$ +34.8 (*c* 0.72, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) (major isomer): δ: 1.26–1.38 (m, 8H), 1.36 (s, 3H), 1.42 (s, 3H), 1.48 (qn, 2H, *J* = 7.3 Hz), 1.60 (qn, 2H, *J* = 7.3 Hz), 1.66–1.72 (m, 1H), 1.99–2.15 (m, 3H), 2.09 (s, 3H), 2.19 (td, 2H, *J* = 7.0, 2.1 Hz), 3.38–3.44 (m, 2H), 3.74 (dd, 1H, *J* = 7.9, 7.3 Hz), 4.00 (dd, 1H, *J* = 8.6, 6.7 Hz), 4.08 (q, 1H, *J* = 6.7 Hz), 4.13 (q, 1H, *J* = 6.1 Hz), 4.27 (td, 1H, *J* = 6.7, 3.0 Hz), 5.42 (dd, 1H, *J* = 4.3, 2.1 Hz), 6.08 (br s, 1H), 7.33 (dd, 1H, *J* = 4.9, 3.1 Hz), 7.38 (dd, 1H, *J* = 4.9, 1.2 Hz), 7.85 (dd, 1H, *J* = 3.1, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (major isomer) δ: 18.7, 21.1, 25.4, 26.4, 26.8, 27.1, 27.8, 28.2, 28.6, 28.8, 29.1, 29.6, 39.8, 65.8, 66.6, 74.9, 78.4, 80.6, 80.7, 87.3, 109.6, 126.0, 126.4, 127.8, 137.8, 163.1, 169.9; IR (NaCl) cm^{-1}: 3526, 1746, 1640; MS (FAB) *m*/*z*: 506 [*M*+H]$^+$; HRMS (FAB): *m*/*z*: Calcd for $C_{27}H_{40}NO_6S$: 506.2576. Found: 506.2573 [*M*+H]$^+$.

4.6. (*R*)-1-{(2*R*,5*R*)-5-[(*R*)-1,2-Dihydroxyethyl]tetrahydrofuran-2-yl}-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (10)

Compound **9a** (339 mg, 0.670 mmol) was dissolved in AcOH/H_2O (3:2, 3.0 mL), and the mixture was stirred for 10.5 h at rt. The solvent was evaporated under the reduced pressure.

Purification by flash column chromatography over silica gel with EtOAc as eluent yielded **10** (307 mg, 99%) as colorless solids. $[\alpha]_D^{24}$ −25.2 (*c* 1.40, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.24–1.40 (m, 8H), 1.49 (qn, 2H, *J* = 7.3 Hz), 1.59 (qn, 2H, *J* = 7.3 Hz), 1.84–1.94 (m, 2H,), 2.02 (dt, 1H, *J* = 11.0, 7.3 Hz), 2.10 (s, 3H), 2.14 (td, 1H, *J* = 7.3, 3.7 Hz), 2.19 (td, 2H, *J* = 7.3, 2.4 Hz), 2.65 (br s, 1H), 2.72 (br s, 1H), 3.41 (td, 2H, *J* = 7.3, 6.1 Hz), 3.54–3.59 (m, 1H), 3.64–3.70 (m, 2H), 4.05–4.09 (m, 1H), 4.17 (q, 1H, *J* = 7.3 Hz), 5.35 (dd, 1H, *J* = 7.3, 2.4 Hz), 6.20 (br s, 1H), 7.33 (dd, 1H, *J* = 4.9, 2.4 Hz), 7.39 (d, 1H, *J* = 4.9 Hz), 7.87 (d, 1H, *J* = 2.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.6, 21.0, 26.8, 27.6, 28.16, 28.22, 28.6, 28.8, 29.1, 29.6, 39.8, 64.6, 66.3, 72.7, 74.9, 80.2, 80.7, 87.5, 126.0, 126.4, 127.9, 137.7, 163.2, 170.1; IR (NaCl) cm^{-1}: 3322, 1740, 1634; MS (FAB) *m*/*z*: 466 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{24}H_{36}NO_6S$: 466.2263. Found: 466.2267 $[M+H]^+$.

4.7. (*R*)-1-[(2*R*,5*R*)-5-Formyltetrahydrofuran-2-yl]-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (11)

$NaIO_4$ (202 mg, 0.943 mmol) was added to a solution of **10** (275 mg, 0.590 mmol) in THF/H_2O (3:1, 5.9 mL) at rt. After stirred for 1.5 h, water was added to the reaction mixture. The mixture was extracted with CH_2Cl_2 prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with *n*-hexane/EtOAc (1:2) as eluent yielded **11** (235 mg, 92%) as a colorless oil. The aldehyde was unstable and therefore used immediately in the next reaction.

4.8. (*R*)-1-((2*R*,5*R*)-5-{(*R*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydec-2-ynyl}tetrahydrofuran-2-yl)-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (12a)

The procedure was the same as that used for preparation of **8a**, giving **12a** (80%, dr = 87:13). Pale green amorphous; $[\alpha]_D^{25}$ −11.8 (*c* 1.36, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.06–1.15 (m, 4H), 1.16–1.23 (m, 2H), 1.26–1.39 (m, 12H), 1.47 (qn, 2H, *J* = 7.3 Hz), 1.58 (qn, 2H, *J* = 7.3 Hz), 1.81–1.89 (m, 1H), 1.90–1.98 (m, 1H), 2.01–2.15 (m, 2H), 2.08 (s, 3H), 2.10–2.14 (m, 3H), 2.19 (td, 2H, *J* = 7.3, 2.4 Hz), 2.88 (q, 2H, *J* = 6.5 Hz), 2.88 (s, 6H), 3.38 (td, 2H, *J* = 7.3, 6.1 Hz), 4.06–4.24 (m, 3H), 5.10 (br s, 1H), 5.35 (d, 1H, *J* = 7.3 Hz), 6.43 (br s, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 7.30 (m, 1H), 7.42 (d, 1H, *J* = 4.9 Hz), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.55 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.89 (d, 1H, *J* = 2.4 Hz), 8.23 (d, 1H, *J* = 7.3 Hz), 8.31 (d, 1H, *J* = 8.5 Hz), 8.54 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (major isomer) δ: 18.5, 18.6, 21.0, 26.1, 26.7, 27.5, 28.0, 28.08, 28.11, 28.2, 28.3, 28.5, 28.8, 29.0, 29.3, 29.5, 39.7, 43.1, 45.3 (2C), 65.4, 66.3, 75.1, 78.0, 80.1, 82.9, 86.3, 87.3, 115.1, 118.8, 123.1, 126.1 (2C), 127.9, 128.2, 129.4, 129.6, 129.8, 130.2, 134.9, 137.6, 151.9, 163.1, 169.9; IR (NaCl) cm^{-1}: 3325, 1744, 1640, 1316, 1142; MS (FAB) *m*/*z*: 806 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{44}H_{60}N_3O_7S_2$: 806.3873. Found: 806.3856 $[M+H]^+$.

4.9. (*R*)-1-((2*R*,5*R*)-5-{(*S*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydec-2-ynyl}tetrahydrofuran-2-yl)-11-(thiophene-3-carboxamido)undec-2-ynyl acetate (12b)

The procedure was the same as that used for preparation of **8a** by use of (1*S*,2*R*)-*N*-methylephedrine, giving **12b** (91%, dr = 88:12). Pale green amorphous; $[\alpha]_D^{25}$ −8.5 (*c* 0.79, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.06–1.40 (m, 18H), 1.48 (qn, 2H, *J* = 7.3 Hz), 1.57 (qn, 2H, *J* = 7.3 Hz), 1.89–1.96 (m, 1H), 2.01–2.20 (m, 7H), 2.08 (s, 3H), 2.72 (br s, 1H), 2.86–2.90 (m, 8H), 3.38 (td, 2H, *J* = 7.3, 6.1 Hz), 4.12-4.18 (m, 1H), 4.23–4.27 (m, 1H), 4.42–4.48 (m, 1H), 5.14 (br s, 1H), 5.33 (d, 1H, *J* = 7.3 Hz), 6.45 (br s, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 7.28–7.30 (m, 1H), 7.41 (d, 1H, *J* = 4.9 Hz), 7.50–7.56 (m, 2H), 7.89 (d, 1H, *J* = 2.4 Hz), 8.23 (d, 1H, *J* = 7.3 Hz), 8.32 (d, 1H, *J* = 8.5 Hz), 8.53 (d, 1H, *J* = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) (major isomer) δ: 18.47, 18.53, 21.0, 25.7, 26.1, 26.7, 28.1 (2C), 28.17, 28.21, 28.3, 28.5, 28.7, 29.0, 29.3, 29.5, 39.7, 43.1, 45.3 (2C), 64.1, 66.4, 75.1, 77.8, 80.8, 82.3, 86.4, 87.2, 115.1, 118.7, 123.1, 126.1 (2C), 127.9, 128.2, 129.4, 129.6, 129.8, 130.2, 134.9, 137.7, 151.9, 163.1, 169.9; IR (NaCl) cm^{-1}: 3525, 1746, 1641, 1317, 1142; MS (FAB) *m*/*z*: 806 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{44}H_{60}N_3O_7S_2$: 806.3873. Found: 806.3855 $[M+H]^+$.

4.10. (*R*)-1-((2*R*,5*R*)-5-{(*R*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydecyl}tetrahydrofuran-2-yl)-11-(thiophene-3-carboxamido)undecyl acetate (13a)

A solution of **12a** (20.4 mg, 0.0253 mmol) in benzene/MeOH (1:1, 0.5 mL) was hydrogenated on $Rh(PPh_3)_3Cl$ (9.4 mg, 0.0101 mmol) for 6 h with stirring at rt under 1 atm pressure of hydrogen. The solvent was evaporated under the reduced pressure. Purification by flash column chromatography over flash silica gel with *n*-hexane/EtOAc (3:2) as eluent yielded **13a** (15.9 mg, 77%, dr = 87:13) as a pale green oil. $[\alpha]_D^{24}$ +9.3 (*c* 0.88, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.06–1.40 (m, 30H), 1.41–1.49 (m, 1H), 1.52–1.74 (m, 6H), 1.92–2.02 (m, 2H), 2.08 (s, 3H), 2.89 (q, 2H, *J* = 6.1 Hz), 2.89 (s, 6H), 3.37 (q, 1H, *J* = 6.1 Hz), 3.40 (td, 2H, *J* = 7.3, 6.1 Hz), 3.80 (td, 1H, *J* = 7.3, 6.1 Hz), 3.98 (dt, 1H, *J* = 7.3, 6.1 Hz), 4.64 (br s, 1H), 4.87 (dt, 1H, *J* = 7.3, 6.1 Hz), 6.04 (br s, 1H), 7.19 (d, 1H, *J* = 7.3 Hz), 7.32 (dd, 1H, *J* = 4.9, 2.4 Hz), 7.37 (d, 1H, *J* = 4.9 Hz), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.56 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.84 (d, 1H, *J* = 2.4 Hz), 8.24 (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 (125 MHz, $CDCl_3$) (major isomer) δ: 21.1, 25.3, 25.5, 26.4, 26.9, 28.2, 28.4, 28.9, 29.2 (2C), 29.32, 29.35, 29.37, 29.38, 29.39, 29.5, 29.6, 29.7, 30.8, 33.5, 39.8, 43.3, 45.4 (2C), 73.8, 75.1, 79.6, 82.3, 115.2, 118.8, 123.2, 126.0, 126.4, 127.8, 128.3, 129.6, 129.7, 129.9, 130.3, 134.9, 137.8, 152.0, 163.1, 170.9; IR (NaCl) cm^{-1}: 3325, 1738, 1640, 1312, 1144; MS (FAB) *m*/*z*: 814 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{44}H_{68}N_3O_7S_2$: 814.4499. Found: 814.4493 $[M+H]^+$.

4.11. (*R*)-1-((2*R*,5*R*)-5-{(*S*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydecyl}tetrahydrofuran-2-yl)-11-(thiophene-3-carboxamido)undecyl acetate (13b)

The procedure was the same as that used for preparation of **13a**, giving **13b** (85%, dr = 88:12). Pale green oil; $[\alpha]_D^{24}$ +5.7 (*c* 0.84, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) (major isomer) δ: 1.06–1.48 (m, 30H), 1.52–1.68 (m, 6H), 1.77–1.90 (m, 2H), 1.95–2.05 (m, 1H), 2.09 (s, 3H), 2.89 (q, 2H, *J* = 6.1 Hz), 2.89 (s, 6H), 3.40 (td, 2H, *J* = 7.3, 6.1 Hz), 3.77 (td, 1H, *J* = 6.1, 3.7 Hz), 3.87 (ddd, 1H, *J* = 8.5, 6.1, 3.7 Hz), 4.02 (dt, 1H, *J* = 7.3, 6.1 Hz), 4.62–4.65 (m, 1H), 4.86 (dt, 1H, *J* = 7.3, 6.1 Hz), 6.03 (br s, 1H), 7.18 (d, 1H, *J* = 7.3 Hz), 7.32 (dd, 1H, *J* = 4.9, 2.4 Hz), 7.37 (d, 1H, *J* = 4.9 Hz), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.56 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.84 (d, 1H, *J* = 2.4 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 (125 MHz, $CDCl_3$) (major isomer) δ: 21.2, 24.8, 25.3, 25.9, 26.3, 26.9, 28.4, 28.86, 28.87, 29.2 (2C), 29.27, 29.34, 29.37, 29.39 (2C), 29.5, 29.7, 30.9, 32.6, 39.8, 43.3, 45.4 (2C), 71.6, 75.4, 80.2, 82.2, 115.2, 118.7, 123.2, 126.0, 126.4, 127.8, 128.3, 129.6, 129.7, 129.9, 130.3, 134.8, 137.8, 152.1, 163.1, 170.9; IR (NaCl) cm^{-1}: 3318, 1734, 1636, 1310, 1144; MS (FAB) *m*/*z*: 814 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{44}H_{68}N_3O_7S_2$: 814.4499. Found: 814.4487 $[M+H]^+$.

4.12. *N*-[(*R*)-11-((2*R*,5*R*)-5-{(*R*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydecyl}tetrahydrofuran-2-yl)-11-hydroxyundecyl]thiophene-3-carboxamide (2)

K_2CO_3 (5.4 mg, 0.0391 mmol) was added to a solution of **13a** (15.9 mg, 0.0195 mmol) in MeOH (0.4 mL) at rt. After stirred for 7 h, satd. NH_4Cl was added to the reaction mixture. The mixture was extracted with CH_2Cl_2 prior to drying and solvent evaporation. Purification by flash column chromatography over silica gel with *n*-hexane/EtOAc (2:3) as eluent yielded **2** (9.4 mg, 62%) as a pale green oil. $[\alpha]_D^{24}$ +3.8 (*c* 0.45, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.06–1.54 (m, 30H), 1.59 (qn, 2H, *J* = 7.3 Hz), 1.64–1.72 (m, 4H), 1.94–2.04 (m, 2H), 2.38 (br s, 2H), 2.85–2.91 (m, 2H), 2.89 (s, 6H), 3.37–3.43 (m, 4H), 3.77–3.83 (m, 2H), 4.63 (td, 1H, *J* = 6.1, 1.2 Hz), 6.01 (br s, 1H), 7.19 (d, 1H, *J* = 7.3 Hz), 7.32 (dd, 1H, *J* = 4.9, 3.7 Hz), 7.37 (d, 1H, *J* = 4.9 Hz), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.56 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.84 (d, 1H, *J* = 3.7 Hz), 8.24 (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 (125 MHz, $CDCl_3$) δ: 25.51, 25.54, 26.4, 26.9, 28.7 (2C), 28.9, 29.2, 29.25, 29.31, 29.43 (2C), 29.47, 29.51, 29.55, 29.62, 29.7, 33.45, 33.49, 39.8, 43.3, 45.4 (2C), 74.0 (2C), 82,6 (2C), 115.2, 118.7, 123.2, 126.0, 126.4, 127.8, 128.3, 129.6, 129.7, 129.9, 130.3, 134.8, 137.8, 152.1, 163.1; IR (NaCl) cm^{-1}: 3323, 1640, 1314, 1142; MS (FAB) *m*/*z*: 772 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{42}H_{66}N_3O_6S_2$: 772.4393. Found: 772.4417 $[M+H]^+$.

4.13. *N*-[(*R*)-11-((2*R*,5*R*)-5-{(*S*)-10-[5-(Dimethylamino)naphthalene-1-sulfonamido]-1-hydroxydecyl}tetrahydrofuran-2-yl)-11-hydroxyundecyl]thiophene-3-carboxamide (14)

The procedure was the same as that used for preparation of **2**. Pale green oil; $[\alpha]_D^{24}$ +4.9 (*c* 1.12, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.05–1.42 (m, 28H), 1.42–1.54 (m, 2H), 1.59 (qn, 2H, *J* = 7.3 Hz), 1.60–1.76 (m, 3H), 1.81–1.92 (m, 2H), 1.96–2.03 (m, 1H), 2.12 (br s, 1H), 2.40 (br s, 1H), 2.86–2.88 (m, 2H), 2.89 (s, 6H), 3.37–3.43 (m, 3H), 3.76–3.81 (m, 1H), 3.82 (dt, 1H, *J* = 7.3, 6.1 Hz), 3.86 (ddd, 1H, *J* = 8.5, 6.1, 3.7 Hz), 4.63–4.67 (m, 1H), 6.02 (br s, 1H), 7.19 (d, 1H, *J* = 7.3 Hz), 7.32 (dd, 1H, *J* = 4.9, 2.4 Hz), 7.37 (d, 1H, *J* = 4.9 Hz), 7.52 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.56 (dd, 1H, *J* = 8.5, 7.3 Hz), 7.84 (d, 1H, *J* = 2.4 Hz), 8.24 (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 (125 MHz, $CDCl_3$) δ: 25.4, 25.5, 25.9, 26.4, 26.9, 28.6, 28.9, 29.20, 29.24, 29.27, 29.28, 29.4 (2C), 29.46, 29.50, 29.6, 29.7, 32.5, 33.2, 39.8, 43.3, 45.4 (2C), 71.6, 74.3, 82.2, 83.2, 115.1, 118.7, 123.2, 126.0, 126.4, 127.8, 128.3, 129.6, 129.7, 129.9, 130.3, 134.8, 137.8, 152.0, 163.1; IR (NaCl) cm^{-1}: 3390, 3325, 1634, 1310, 1142; MS (FAB) *m*/*z*: 772 $[M+H]^+$; HRMS (FAB) *m*/*z*: Calcd for $C_{42}H_{66}N_3O_6S_2$: 772.4393. Found: 772.4399.

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

This experiment was carried out at the Cancer Chemotherapy 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, NCI-H226, 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 measuring changes in total cellular protein levels following 48 h treatment with a given test compound, 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.[14] COMPARE analysis was performed by calculating the Pearson correlation coefficient (*r*) between the GI_{50} mean graphs of 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 ($n = 39$). The Pearson correlation coefficients were used to determine the degree of similarity. The larger the coefficient is, the higher the similarity between *X* and *Y* is.[15]

Acknowledgments

In vitro antiproliferative activities of acetogenin analogues 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 (MEXT). This work was supported in part by Grants-in-Aid for Young Scientists (B) [Nos. 21790113, 23790130], a Grant-in-Aid for Scientific Research (C) [No. 25460159], a Grant-in-Aid for Scientific Research (B) [No. 22390021] from Japan Society for the Promotion of Sciences (JSPS), and MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2008-2012.

Supplementary data

Supplementary data (Synthesis and characterization data of **6** and determination of stereochemistry around THF moiety by comparison with Fujimoto's model compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.037.

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