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New cytotoxic annonaceous acetogenin mimetics having a nitrogen-heterocyclic terminal and their application to cell imaging

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ABSTRACT

A terminal unsaturated lactone or its equivalent is commonly believed to be essential for the cytotoxicity of natural annonaceous acetogenins and their artificial mimetics. In this work, we discovered a series of new cytotoxic ethylene glycol ether-containing mimetics, in which a variety of simple aliphatic nitrogen-heterocycles were introduced to replace the lactone terminal of AA005 (**1**), a representative bioactive polyether mimic identified from our previous research, for the first time. Among these, mimic **4** bearing a terminal piperazine was found to be the most potent compound against the proliferation of three cancer cells. Based on our new findings, a fluorescent probe **7** was also developed and successfully applied to the imaging of cancer cells. This work provides a new strategy for developing simpler cytotoxic mimetics of natural annonaceous acetogenins and molecular tools for biological imaging.

1. Introduction

Annonaceous acetogenins are a large family of linear fatty acid-derived natural products.[1,2] Many members of this family display a wide spectrum of biological activities, among which the most remarkable is the antitumor activity.[1,3] Our group has been devoted to simplify and mimic this class of natural products and further investigate their antitumor activities for several years. Several notable analogues without natural THF functionalities, such as AA005[4] and AA019,[5] were developed, and they were found to exhibit more potent or comparable activities as those of natural products. AA005 usually presents an IC_{50} of nM range against the proliferation of various cancer cells, such as 0.276 μM (MCF-7 cells), 0.305 μM (A549 cells), and 0.041 μM (Bel-7404 cells).[6,7] More importantly, some of them show selective actions predominately on cancerous cells over normal cells. Our previous works also found that the representative mimetic AA005 could inhibit NADH:ubiquinone oxidoreductase (complex I) of the mitochondrial electron transport system, and therefore induced cancer cell death through autophage processes.[8]

In order to study the structure–activity relationship of representative bioactive mimic AA005 (Fig. 1, **1**), several different series of AA005 analogues have been designed, synthesized, and evaluated, including inserting additional hydroxyl(s) to the middle hydrocarbon chain,[5,9] and displacing the terminal unsaturated γ-lactone with equivalent lactam.[6] Furthermore, our previous efforts proved that removal of the terminal unsaturated lactone or its equivalent (lactam) would result in significant loss of the cytotoxicity both in natural acetogenins and the artificial mimetics.[9] Very recently, we also synthesized a number of AA005 analogues with embedment of a certain nitrogen-heterocycle in the middle hydrocarbon region (remaining the terminal lactone) to improve water solubility of these lipophilic compounds, and they were also found to show low micromolar inhibitory activities against the proliferation of several cancer cell lines.[10] Such elaboration is commonly applied in development of small-molecule drugs to either increase water solubility or provide a new modification site (on nitrogen atom) without introducing any new chiral center.

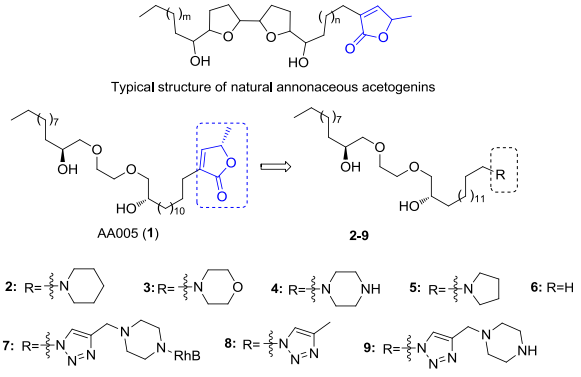

Fig. 1. Design of new acetogenin mimics having a nitrogen-heterocyclic terminal.

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In parallel to our previous works, several aromatic nitrogen-heterocycles were introduced by other groups to the natural acetogenin skeleton individually.[11] In addition, Hocquemiller and coworkers once applied alicyclic nitrogen-heterocycles to replace the terminal lactone of natural acetogenin squamocin in an α-acetonylamide form,[12] though the definite function of the heterocycles is unclear yet. As an expansion of our previous findings, we further explored the biological effects through replacement of the biologically 'essential' lactone terminal with a nitrogen-heterocyclic functionality (**2**–**9**) using AA005 (Fig. 1, **1**) as an examination platform. In this study, a molecule without any heterocycles (**6**) was designed as the negative control to verify the roles of the newly introduced terminal heterocycle(s) accordingly, and another *N*-bounded florescent derivative (**7**) was designed as a potential molecular tool for cell imaging. Herein, we wish to report our discovery of a new series of cytotoxic acetogenin mimetics based on the structure of AA005, in which the terminal lactone functionality was replaced with simple nitrogen-heterocycle(s) for the first time.

2. Results and discussion

Because they show structural difference only at their right terminals, most of the newly designed mimetics could be synthesized by a diverse approach from the common mesylate **10** (Fig. 2). Compounds **2**–**5** could be synthesized via the corresponding nucleophilic substitutions,[13] and **7**–**9** could be provided via proper Click chemistry.[10,14] Compound **6** without a terminal heterocycle also could be prepared from **10** by reduction with $NaBH_4$.[15] The common intermediate, mesylate **10**, could be prepared by reduction of ester **11** and mesylation. Further analysis suggested that ester **11** could be assembled through epoxide-opening, hydroxyl elimination, and hydrogenation[16] from the known alkyne **12**[17] and epoxide **13**.[18]

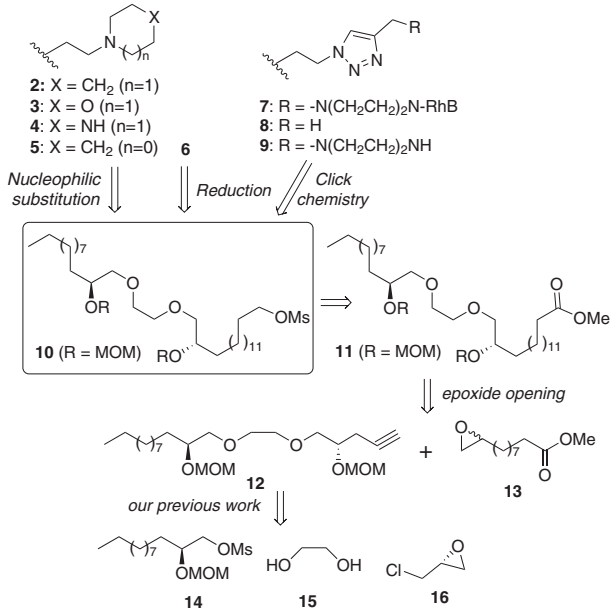
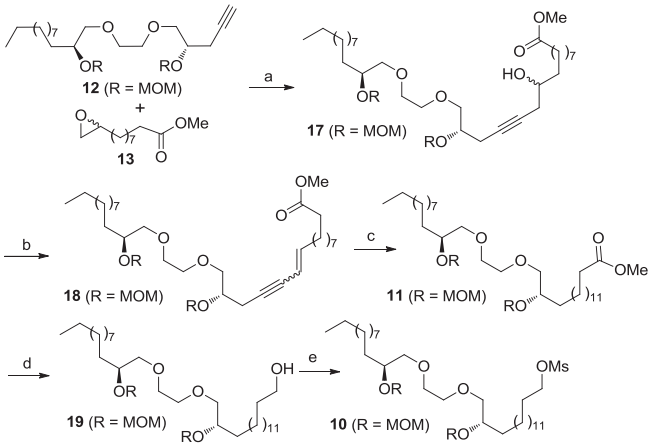


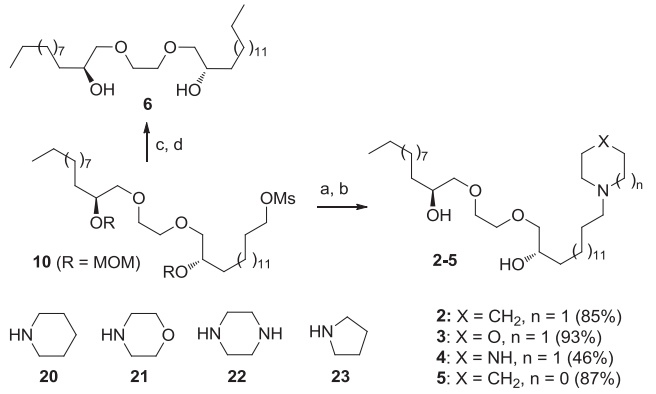
Fig. 2. Retrosynthetic analysis of the newly designed compounds.

Starting from alkyne **12** and epoxide **13**, a five-step procedure was applied to synthesize the crucial mesylate **10** with the alkyne epoxide-opening protocol[16] (Scheme 1). Deprotonation of alkyne **12** with *n*-BuLi, activation with $BF_3{\cdot}Et_2O$, and followed by reaction with epoxide **13** afforded alcohol **17** as a diastereomeric mixture in 93% yield. Elimination of the newly born hydroxyl group was accomplished by conversion of the mixture into the corresponding mesylate and followed by treatment with DBU, affording eneyne **18** as an *E*/*Z*-olefin mixture. Eneyne **18** was then entirely reduced with $TsNHNH_2$ to give ester **11** in 94% yield. Reduction of ester **11** with $LiAlH_4$ followed by treatment with MsCl and Et_3N provided the common mesylate **10** in an excellent yield.



Scheme 1. Reagents and conditions: (a) *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 93%; (b) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt; (ii) DBU, THF, 0 °C to rt, 85% for two steps; (c) $TsNHNH_2$, NaOAc, DME/H_2O, reflux, 94%; (d) $LiAlH_4$, Et_2O, 0 °C to rt, 76%; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 96%.

With mesylate **10** in hand, synthesis of the first batch of AA005 analogues including the mimetics **2**–**5** equipped with a piperazine, morpholine, piperidine, or pyrrolidine moiety, was then conducted through parallel reactions with corresponding nitrogen-heterocycles (Scheme 2). Using **2** as a representative, treatment of mesylate **10** with piperidine in THF at reflux provided an amine intermediate,[13] of which the two MOM groups were then deprotected in 20% HCl solution in MeOH, giving **2** in 85% yield finally. Other compounds **3**–**5** were prepared correspondingly, in parallel. In addition, the reference compound **6** without any heterocycles was also prepared from mesylate **10** via reduction with $NaBH_4$,[15] and deprotection of the MOM protecting groups with 20% HCl solution in MeOH.



Scheme 2. Reagents and conditions: (a) **20**–**23** (in parallel), THF, rt to reflux for 4 h; (b) 20% HCl/MeOH, rt, 6 h, 46–93% for two steps; (c) $NaBH_4$, DMF, 0–70 °C; (d) 20% HCl/MeOH, rt, 91% for two steps.

For the availability of mimetics **2**–**6**, their in vitro cytotoxicities against cancer cells were evaluated using an MTT assay (MTT=3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Doxorubicin was used as a positive control, and cancer cell lines Bel-7404, A547, and MCF-7 were chosen as the cell models for the assessment (Table 1). To our delight, all the compounds bearing a terminal heterocycle (mimetics **2**–**5**) showed low micromolar inhibitory activities against the proliferation of all the tested cancer cell lines, while the compound without a terminal heterocycle (mimetic **6**, designed as a negative control) did not exhibit significant inhibitory activity (IC_{50}>40 μM). In addition, the small

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Table 1
In vitro cytotoxicities of analogues **2**–**6** against cancer cells[a,b]

Compounds	IC_{50} (μM)		
	A549	MCF-7	Bel-7404
2	2.91±0.38	3.84±0.11	3.23±0.27
3	11.66±1.78	13.01±0.67	25.76±1.09
4	2.70±0.30	2.88±0.05	2.05±0.05
5	2.81±0.27	2.96±0.16	2.44±0.14
6	>40	>40	>40
Doxorubicin[c]	1.32±0.11	0.19±0.02	0.68±0.08

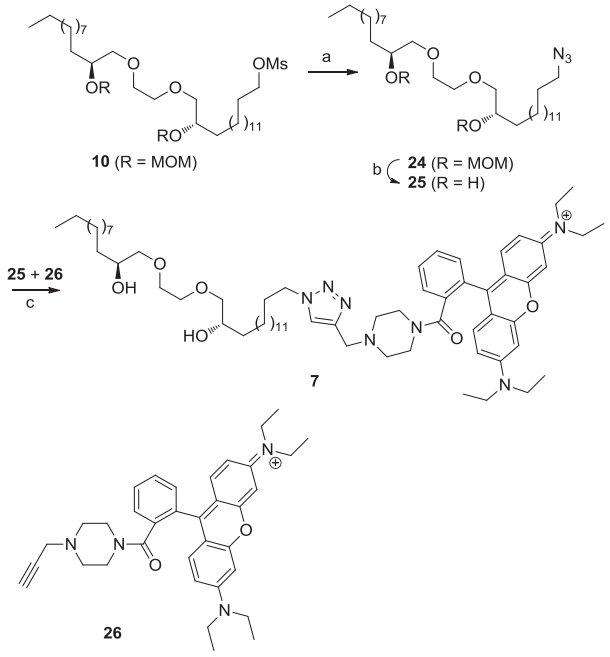
[a] Inhibition of cell growth by the listed compounds was determined using an MTT assay, and the inhibition time was 48 h, each data represent the mean+SD of three independent experiments (performed in triplicate).
[b] Bel-7404 (human hepatocellular carcinoma cells); A549 (human lung adenocarcinoma cells); MCF-7 (human breast adenocarcinoma cells).
[c] Doxorubicin was used as a positive control.

heterocycles **20**–**23** (see Scheme 2 for their structures) had no cytotoxicity (IC_{50}>40 μM, see Supplementary data). These results clearly indicated that the newly introduced terminal nitrogen-heterocycles are important structural factors for the bioactivity of new mimetics, and combination of the linear hydrocarbon chain containing an ethylene glycol ether moiety with the heterocycles was indispensable and very effective for the inhibitory activities.

Among these new mimetics, compounds **2**, **4**, and **5** all showed low micromolar inhibitory activities with an IC_{50} range from 2 to 4 μM approximately. Mimetic **3** (having a terminal morpholine) also exhibited inhibitory activity, but it was relatively weaker than other three compounds. The inhibitory difference between **2** and **3** suggests that the oxygen atom of the terminal morpholine moiety of **3** might be inappropriate for better bioactivity. Furthermore, little difference of inhibitory activity was observed between **2** (with a terminal piperidine) and **5** (with a terminal pyrrolidine). It is deduced that small change of the ring size of terminal heterocycle doesn't greatly affect the activity. According to IC_{50} values, **4** is the most potent compound among these mimetics. Compared with **2** (with a piperidine), an additional nitrogen atom was introduced into the terminal ring of mimetic **4** (with a piperazine). It demonstrates that introduction of the second nitrogen atom into the terminal heterocycle is helpful. It was also observed that the growth of all the tested cancer cells was inhibited without huge difference, suggesting that these new mimetics might have the potential of being inhibitors of a wide range of cancer cells.

Using mimetic **4** as a representative, the corresponding experiments of dose- and time-dependent inhibition were also conducted. To some extent, this compound could inhibit the proliferation of three cancer cell lines in a dose- and time-dependent fashion. The difference of inhibition rate was more obvious at lower doses, though the inhibition rate reached a maximum at higher doses (Fig. 3).

As mentioned above, the free piperazine NH-functionality existing in one terminal of mimic **4** also provided us a potential opportunity to explore other biological applications. For instance, proper modification of **4** might enable us to develop a molecular tool for cell imaging. To trace the behavior of live cells, florescent probe **7**, on the basis of **4**, was thus designed employing the widely applied Click chemistry,[10] and rhodamine B was employed as the fluorophore. Synthesis of fluorescent probe **7** was illustrated in Scheme 3. Firstly, treatment of mesylate **10** with NaN_3 in DMF gave azide **24**, of which the two MOM groups were then removed with 20% HCl in MeOH, affording azido-diol **25**. Under mild Click conditions, successful coupling of azide **25** and alkyne **26**[19,20] provided **7** in 65% yield.



Scheme 3. Reagents and conditions: (a) NaN_3, DMF, 70 °C, 81%; (b) 20% HCl/MeOH, rt, 90%; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/H_2O: 1:1, 65%.

Because an additional triazole was introduced to the structure of probe **7** via the above Click approach (Scheme 3), evaluation of its effects on the biological activities might be necessary. We further synthesized two fragment molecules **8** and **9** (Scheme 4) for comparison in the biological experiments. One of them was only equipped with a triazole ring at the right end (**8**), and the other was devised with both the triazole ring and the piperazine ring at the end (**9**). These two compounds were synthesized in parallel with

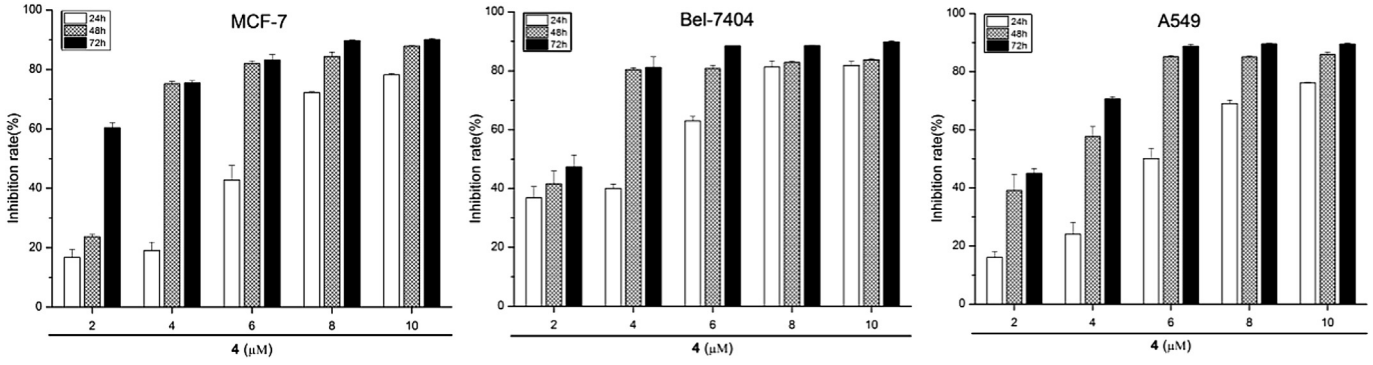


Fig. 3. Cancer cell lines MCF-7, Bel-7404, and A549 were treated with mimetic **4** at indicated concentrations and time points. Each column represents the mean+SD of three independent experiments (performed in triplicate).

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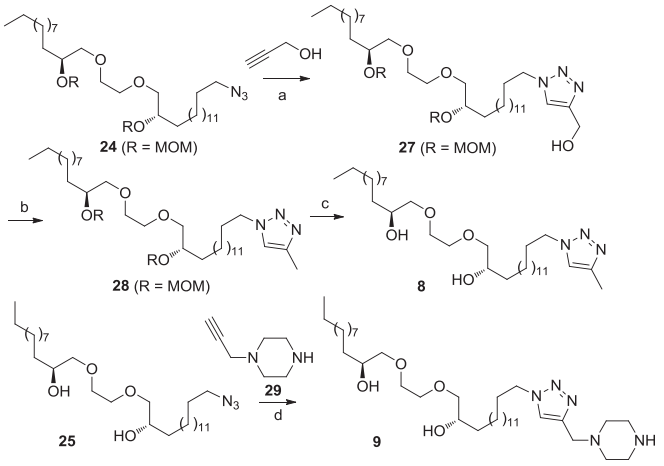

Scheme 4. Reagents and conditions: (a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/H_2O: 1:1, 86%; (b) (i) Et_3N, MsCl, DCM, 0 °C, overnight; (ii) $NaBH_4$, DMF, 0–70 °C, 58% for two steps; (c) 20% HCl/MeOH, rt, 85%; (d) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/H_2O: 1:1, 47%.

our previously used Click conditions. Compound **8** was elaborated from azide **24** in four steps, including Click reaction with propargyl alcohol, mesylation, $NaBH_4$ reduction,[15] and final deprotection of the two MOM groups. Preparation of compound **9** could be achieved by a direct Click coupling of azido-diol **25** with acetylene **29**.[21]

The cytotoxicity of compounds **7**–**9** was then evaluated and the results were summarized in Table 2. It is found that introduction of a triazole functionality into the molecules affects little on the cytotoxicity. By comparison with piperazine mimetic **4**, both **7** and **9** exhibited similar inhibitory activities against the three cancer cell lines with only slight decreases, while **8** having a triazole terminal showed significant decrease in its cytotoxicity. Based on such observation, we thought the fluorescent probe **7** proved itself to be qualified as a molecular tool for the future cell imaging.

Table 2
Cytotoxicity of newly synthesized compounds **7**–**9**[a]

Compounds	IC_{50} (μM)		
	A549	MCF-7	Bel-7404
7	1.61±0.06	2.01±0.01	1.16±0.11
8	8.42±1.03	>25	21.46±2.88
9	4.73±0.30	8.27±0.33	5.60±0.25
Doxorubicin[b]	1.32±0.11	0.19±0.02	0.68±0.08

[a] Inhibition of cell growth by the listed compounds in cells was determined using an MTT assay, and the inhibition time was 48 h, each data represent the mean+SD of three independent experiments (performed in triplicate).
[b] Doxorubicin was used as a positive control.

With rhodamine B-labeled probe **7**, the corresponding experiments of cell imaging were conducted. All cancer cells A549, MCF-7, and Bel-7404 were visualized by a confocal fluorescent microscopy after treatment with 100 nM of compound **7**. We obviously identified the probe in these cancer cells after 12 h of drug treatment. Furthermore, by co-staining with MitoTracker® Green FM, a specific mitochondrial probe, we found that the florescent probe **7** localized at almost exclusively the same area inside cells by the overlapping patterns of the green and red fluorescences (Fig. 4). This reveals that probe **7** also localize in the mitochondrial as our previous conclusions for AA005,[17,8a] indicating that the cell death induced by the new mimetics might be due to deficiency of ATP as the same mechanism by AA005.[8]

3. Conclusion

A new series of cytotoxic mimetics of natural annonaceous acetogenin have been designed and synthesized by replacing the original lactone terminal of AA005 with a simple nitrogen-heterocycle. Biological evaluation indicated that most of these compounds exhibited low micromolar cytotoxicity against the proliferation of all three tested cancer cell lines, and mimetic **4** containing a terminal piperazine was found to exhibit the most potent anti-proliferation activity. A fluorescent probe **7** was also developed through further modification of the newly introduced nitrogen atom of mimetic **4**, and applied to cell imaging successfully. Direct introduction of simple aliphatic nitrogen-heterocycles into one terminal of AA005 thus provides a new workable strategy to acquire unnatural anticancer agents derived from natural annonaceous acetogenins, and it is also helpful for developing molecular tools in various biomedical researches.

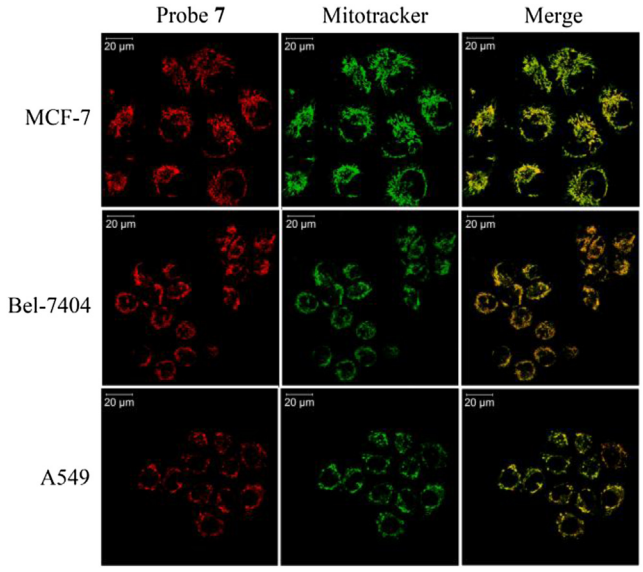

Fig. 4. The intracellular localization of probe **7**. The cells were incubated with **7** at 100 nM for 12 h, and analyzed by confocal microscopy using a MitoTracker® Green FM (invitrogen) to counter-stain mitochondria.

4. Experimental section

4.1. General methods

Optical rotations were measured at 25 °C. Infrared spectra (IR) were recorded on a Fourier transform infrared spectrometer (FTIR). ^1H NMR spectra were recorded at 300 MHz or 500 MHz, and are reported in ppm (δ) downfield relative to $CDCl_3$ as internal standard, and ^{13}C NMR spectra were recorded at 75 MHz or 100 MHz, and assigned in ppm (δ). HRMS spectra were recorded on Agilent G6500 TOF-MS. All melting points were uncorrected. Flash column chromatography was performed on 300–400 mesh silica gel.

4.2. Synthesis of compounds

*4.2.1. (5S,12S)-Methyl-5-decyl-17-hydroxy-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacos-14-yn-26-oate (**17**).* To a stirred solution of **12** (2.63 g, 6.3 mmol) in 24 mL anhydrous THF was added 2.4 M *n*-BuLi in hexane (2.92 mL, 7.0 mmol) dropwise at −78 °C under N_2 atmosphere. After the reaction mixture was stirred for 30 min at −78 °C, $BF_3 \cdot Et_2O$ (0.81 mL, 6.3 mmol) was added and the mixture was stirred for an additional 20 min. Epoxide **13** (0.83 g, 3.87 mmol) in 24 mL anhydrous THF was then slowly added to the reaction mixture. After being stirred at −78 °C for 2 h, the reaction was quenched by saturated aq NH_4Cl solution, and extracted with ethyl acetate for three times. The combined

organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **17** (2.27 g, 93%) as a colorless oil.[16] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.76 (d, J=6.8 Hz, 1H), 4.73 (s, 2H), 4.65 (d, J=6.8 Hz, 1H), 3.88–3.80 (m, 1H), 3.72–3.65 (m, 5H), 3.63–3.59 (m, 6H), 3.49 (d, J=5.1 Hz, 2H), 3.39 (s, 3H), 3.38 (s, 3H), 2.57–2.44 (m, 2H), 2.34–2.42 (m, 1H), 2.33–2.18 (m, 4H), 1.61 (t, J=7.2 Hz, 2H), 1.48 (dd, J=12.8, 6.8 Hz, 4H), 1.29–1.25 (m, 26H), 0.87 (t, J=6.7 Hz, 3H). IR (film, ν_{max}): 3484, 2927, 2855, 1741, 1464, 1359, 1212, 1150, 1106, 1040, 919, 724 cm^{-1}. MS (ESI, m/z): 653 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{35}H_{66}O_9Na$ 653.4599; found 653.4610.

*4.2.2. (5S,12S)-Methyl-5-decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacos-16-en-14-yn-26-oate (**18**).* To a solution of compound **17** (2.27 g, 3.60 mmol) and DMAP (22 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (300 mL) were added Et_3N (1.06 mL, 7.56 mmol) and MsCl (0.34 mL, 4.32 mmol) at 0 °C under N_2 atmosphere. The reaction mixture was stirred overnight at room temperature and quenched with water. The mixture was extracted by CH_2Cl_2 for three times. The combined organic phases were washed with 1 N aq HCl, water, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was used directly without purification. A solution of the above residue in anhydrous THF (35 mL) was treated with DBU (0.60 mL, 4.02 mmol) at 0 °C, and then stirred at room temperature for 24 h. The mixture was directly distilled in vacuum, and the residue was purified by flash chromatography to give **18** (1.87 g, 85% for two steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09–5.78 (m, 1H), 5.45–5.39 (m, 1H), 4.77–4.73 (m, 3H), 4.65 (d, J=6.8 Hz, 1H), 3.87 (dt, J=10.8, 5.3 Hz, 1H), 3.75–3.65 (m, 5H), 3.64–3.59 (m, 5H), 3.50 (d, J=5.1 Hz, 2H), 3.39 (s, 3H), 3.38 (s, 3H), 2.66–2.56 (m, 2H), 2.32–2.23 (m, 3H), 2.04 (t, J=6.8 Hz, 1H), 1.50 (t, J=6.6 Hz, 2H), 1.37–1.25 (m, 28H), 0.87 (t, J=6.7 Hz, 3H). IR (film, ν_{max}): 2926, 2855, 1741, 1464, 1359, 1150, 1106, 1040, 957, 919, 724 cm^{-1}. MS (ESI, m/z): 635 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{35}H_{64}O_8Na$ 635.4493; found 635.4506.

*4.2.3. (5S,12S)-Methyl-5-decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacosan-26-oate (**11**).* A mixture of **18** (1.24 g, 2.02 mmol) and *p*-toluenesulfono-hydrazide (45.26 g, 0.24 mol) in 1,2-dimethoxyethane (200 mL) was heated to reflux. An aqueous solution containing AcONa (23.63 g, 0.29 mol, 200 mL) was then dropped to the above mixture in 5 h, and then refluxed for additional 7 h. The mixture was cooled down to room temperature and extracted with diethyl ether for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **11** (1.17 g, 94%) as a colorless oil.[9] $[\alpha]_D^{25}$ −9.67 (*c* 1.08, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.76 (d, J=6.8 Hz, 2H), 4.65 (d, J=6.8 Hz, 2H), 3.72–3.64 (m, 5H), 3.64–3.57 (m, 4H), 3.49 (d, J=5.1 Hz, 4H), 3.38 (s, 6H), 2.30 (t, J=7.5 Hz, 2H), 1.50 (t, J=6.6 Hz, 4H), 1.25 (brs, 38H), 0.87 (t, J=6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.51, 96.22, 76.43, 74.32, 70.98, 55.63, 51.60, 34.31, 32.23, 32.11, 29.95, 29.82, 29.65, 29.54, 29.46, 29.36, 25.67, 25.16, 22.88, 14.30. IR (film, ν_{max}): 2925, 2854, 1742, 1465, 1361, 1105, 1040, 919, 723 cm^{-1}. MS (ESI, m/z): 641 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{35}H_{70}O_8Na$ 641.4963; found 641.4975.

*4.2.4. (5S,12S)-5-Decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacosan-26-ol (**19**).* To a stirred suspension of $LiAlH_4$ (0.20 g, 5.02 mmol) in anhydrous THF (40 mL) was added compound **11** (1.40 g, 2.25 mmol) in THF (20 mL) dropwise under N_2 atmosphere at 0 °C. The reaction mixture was stirred at room temperature overnight, and the mixture was poured to a suspension containing Et_2O, H_2O, and Na_2SO_4 at 0 °C. After being stirred for 1 h, the mixture was filtered, and the filtrate was directly concentrated. The residue was purified by flash chromatography to give **19** (1.01 g, 76%) as a colorless oil. $[\alpha]_D^{25}$ −8.31 (*c* 1.47, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.75 (d, J=6.8 Hz, 2H), 4.65 (d, J=6.8 Hz, 2H), 3.70 (dd, J=11.4, 5.5 Hz, 2H), 3.64 (d, J=6.6 Hz, 2H), 3.61–3.57 (m, 4H), 3.49 (d, J=5.1 Hz, 4H), 3.37 (s, 6H), 1.58–1.47 (m, 6H), 1.25 (br s, 38H), 0.87 (t, J=6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 96.01, 76.26, 74.14, 70.81, 62.83, 55.45, 32.86, 32.05, 31.97, 29.79, 29.68, 29.54, 29.40, 25.87, 25.51, 22.74, 14.17. IR (film, ν_{max}): 3499, 2925, 2854, 1464, 1359, 1261, 1214, 1144, 1104, 1040, 919, 802, 723 cm^{-1}. MS (ESI, m/z): 613 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{34}H_{70}O_7Na$ 613.5014; found 613.5031.

*4.2.5. (5S,12S)-5-Decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacosan-26-yl methanesulfonate (**10**).* To a solution of **19** (1.02 g, 1.72 mmol) in anhydrous CH_2Cl_2 (25 mL) were added Et_3N (0.3 mL, 2.064 mmol) and MsCl (0.17 mL, 2.06 mmol) under N_2 atmosphere at 0 °C. The reaction mixture was stirred overnight at room temperature and quenched by water. The mixture was extracted by CH_2Cl_2 for three times. The combined organic phases were washed with 1 N aq HCl, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **10** (1.1 g, 96%) as a colorless oil. $[\alpha]_D^{25}$ −7.14 (*c* 0.67, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, J=6.8 Hz, 2H), 4.63 (d, J=6.8 Hz, 2H), 4.20 (t, J=6.6 Hz, 2H), 3.72–3.63 (m, 2H), 3.63–3.53 (m, 4H), 3.48 (d, J=5.0 Hz, 4H), 3.36 (s, 6H), 2.98 (s, 3H), 1.77–1.66 (m, 2H), 1.48 (t, J=6.6 Hz, 4H), 1.24 (brs, 38H), 0.86 (t, J=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 96.18, 76.37, 74.27, 70.94, 70.38, 55.62, 37.52, 32.19, 32.09, 29.93, 29.80, 29.71, 29.60, 29.52, 29.30, 29.21, 25.65, 25.60, 22.86, 14.30. IR (film, ν_{max}): 2925, 2854, 1465, 1358, 1213, 1176, 1145, 1105, 1040, 951, 919, 824, 722, 528 cm^{-1}. MS (ESI, m/z): 691 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{35}H_{72}O_9SNa$ 691.4789; found 691.4803.

*4.2.6. Synthesis of **2**, **3**, **4**, **5**. General procedure (using **4** as an example).* To a solution of **10** (89 mg, 0.13 mmol) in anhydrous THF (5 mL) was added piperazine (57.3 mg, 0.67 mmol) under N_2 atmosphere. The reaction mixture was heated to reflux for 4 h. After water (15 mL) was added, the mixture was extracted by CH_2Cl_2 for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was re-dissolved in methanol (4 mL), and concd HCl (1 mL) was added. The mixture was stirred overnight at room temperature. The pH value of the mixture was adjusted to 11 using 1 M aq NaOH solution. The mixture was then extracted with CH_2Cl_2 for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel to give **4** (35 mg, 46% for two steps) as a white solid.[13]

The procedure for the synthesis of **2**, **3**, and **5** was analogous.

*4.2.6.1. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-(piperazin-1-yl)hexadecan-2-ol (**4**).* Mp 61–63 °C. $[\alpha]_D^{25}$ +11.00 (*c* 0.40, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80–3.75 (m, 2H), 3.72–3.60 (m, 4H), 3.53 (dd, J=9.8, 2.4 Hz, 2H), 3.31 (t, J=9.0 Hz, 2H), 2.92 (t, J=4.2 Hz, 4H), 2.43 (s, 4H), 2.31 (t, J=7.6 Hz, 2H), 1.47–1.25 (m, 44H), 0.87 (t, J=6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.11, 70.64, 70.37, 59.62, 54.54, 46.06, 33.23, 32.09, 29.87, 29.79, 29.76, 29.52, 27.78, 26.78, 25.75, 22.86, 14.30. IR (KBr, ν_{max}): 3490, 3440, 2919, 2850, 1468, 1400, 1375, 1325, 1143, 1116, 959, 909, 885, 860, 831, 724, 597 cm^{-1}. MS (ESI, m/z): 593 [M+Na]$^+$. HRMS (ESI, m/z): [M+Na]$^+$ calcd for $C_{34}H_{70}N_2O_4Na$ 593.5228; found 593.5236.

*4.2.6.2. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-(piperidin-1-yl)hexadecan-2-ol (**2**).* White solid, yield: 85%. Mp 55–57 °C.

$[\alpha]_D^{25}$ +12.21 (*c* 0.34, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.84–3.73 (m, 2H), 3.69–3.60 (m, 4H), 3.52 (dd, *J*=9.6, 2.1 Hz, 2H), 3.30 (t, *J*=9.0 Hz, 2H), 2.56 (s, 4H), 2.44 (t, *J*=7.9, 2H), 1.79–1.64 (m, 4H), 1.62–1.20 (m, 46H), 0.86 (t, *J*=6.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.09, 70.65, 70.42, 59.25, 54.36, 33.18, 32.09, 29.86, 29.80, 29.76, 29.62, 29.52, 27.70, 26.06, 25.74, 25.11, 23.99, 22.87, 14.31. IR (KBr, ν_{max}): 3493, 3436, 2920, 2851, 2803, 2765, 1467, 1327, 1143, 1115, 1095, 959, 725 cm^{-1}. MS (ESI, *m*/*z*): 592 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{35}H_{71}NO_4Na$ 592.5275; found 592.5287.

*4.2.6.3. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-morpholinohexadecan-2-ol (**3**).* White solid, yield: 93%. Mp 58–62 °C. $[\alpha]_D^{25}$ +12.25 (*c* 0.41, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.81–3.69 (m, 6H), 3.69–3.57 (m, 4H), 3.53 (dd, *J*=9.8, 2.5 Hz, 2H), 3.31 (t, *J*=9.0 Hz, 2H), 2.44 (s, 4H), 2.32 (t, *J*=7.8 Hz, 2H), 1.48–1.22 (m, 44H), 0.87 (t, *J*=6.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.11, 70.68, 70.46, 67.13, 59.44, 53.95, 33.19, 32.11, 29.81, 29.78, 29.54, 27.72, 26.68, 25.76, 22.89, 14.33. IR (KBr, ν_{max}): 3489, 3420, 2955, 2917, 2849, 2805, 2760, 1466, 1401, 1362, 1252, 1118, 1087, 1059, 1014, 917, 889, 868, 723 cm^{-1}. MS (ESI, *m*/*z*): 572 $[M+H]^+$, 594 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{34}H_{69}NO_5Na$ 594.5068; found 594.5081.

*4.2.6.4. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-(pyrrolidin-1-yl)hexadecan-2-ol (**5**).* White solid, yield: 87%. Mp 54–57 °C. $[\alpha]_D^{25}$ +11.65 (*c* 0.41, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.83–3.72 (m, 2H), 3.72–3.60 (m, 4H), 3.53 (dd, *J*=9.8, 2.7 Hz, 2H), 3.32 (t, *J*=9.0 Hz, 2H), 2.68 (s, 4H), 2.55 (t, *J*=7.9 Hz, 2H), 1.86 (dt, *J*=6.6, 3.5 Hz, 4H), 1.65–1.52 (m, 2H), 1.45–1.22 (m, 42H), 0.87 (t, *J*=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.09, 70.68, 70.43, 56.64, 54.23, 33.20, 32.10, 29.88, 29.81, 29.77, 29.63, 29.53, 28.27, 27.68, 25.75, 23.57, 22.88, 14.32. IR (KBr, ν_{max}): 3512, 3435, 2919, 2850, 2794, 1467, 1329, 1143, 1118, 1092, 959, 884, 725 cm^{-1}. MS (ESI, *m*/*z*): 556 $[M+H]^+$, 578 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{34}H_{69}NO_4Na$ 578.5119; found 578.5129.

*4.2.7. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)hexadecan-2-ol (**6**).* To a solution of **10** (20 mg, 0.03 mmol) in DMF (2 mL) was added $NaBH_4$ (2.3 mg, 0.06 mmol) at 0 °C. The mixture was warmed to room temperature, and gradually heated to 70 °C. After 7 h, the reaction was quenched by water. The whole mixture was extracted with Et_2O for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was re-dissolved in methanol (1.6 mL), and treated with concd HCl (0.4 mL). The reaction mixture was stirred overnight at room temperature. After water (20 mL) was added, the mixture was extracted with CH_2Cl_2 for several times. The organic phases were washed with saturated aq $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **6** (13.2 mg, 91% for two steps) as a white solid. Mp 62–65 °C. $[\alpha]_D^{25}$ +21.25 (*c* 0.16, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.85–3.74 (m, 2H), 3.74–3.58 (m, 4H), 3.54 (dd, *J*=9.8, 2.8 Hz, 2H), 3.31 (dd, *J*=9.7, 8.3 Hz, 2H), 1.44–1.24 (m, 44H), 0.88 (t, *J*=6.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.10, 70.72, 70.49, 33.20, 32.14, 29.91, 29.84, 29.58, 25.77, 22.91, 14.35. IR (KBr, ν_{max}): 3437, 2957, 2919, 2850, 1716, 1467, 1329, 1158, 1142, 1090, 958, 887, 724, 613 cm^{-1}. MS (ESI, *m*/*z*): 509 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{30}H_{62}O_4Na$ 509.4540; found 509.4552.

*4.2.8. (5S,12S)-5-(14-Azidotetradecyl)-12-decyl-2,4,7,10,13,15-hexaoxahexadecane (**24**).* To a solution of **10** (1.26 g, 1.89 mmol) in freshly distilled DMF (20 mL) was added NaN_3 (0.25 g, 3.78 mmol) in one portion. The mixture was heated to 70 °C for 8 h. After being cooled down to room temperature, the reaction was quenched by water and extracted by Et_2O for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **24** (0.94 g, 81%) as a colorless oil. $[\alpha]_D^{25}$ −7.50 (*c* 0.48, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 4.76 (d, *J*=6.8 Hz, 2H), 4.65 (d, *J*=6.8 Hz, 2H), 3.73–3.65 (m, 2H), 3.65–3.56 (m, 4H), 3.49 (d, *J*=5.1 Hz, 4H), 3.38 (s, 6H), 3.25 (t, *J*=7.0 Hz, 2H), 1.63–1.56 (m, 2H), 1.55–1.47 (m, 4H), 1.25 (brs, 38H), 0.87 (t, *J*=6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 96.09, 76.30, 74.21, 70.88, 55.51, 51.57, 32.12, 32.03, 29.86, 29.73, 29.66, 29.60, 29.45, 29.27, 28.95, 26.83, 25.57, 22.79, 14.22. IR (film, ν_{max}): 2926, 2855, 2096, 1464, 1355, 1258, 1214, 1145, 1106, 1041, 919, 722 cm^{-1}. MS (ESI, *m*/*z*): 638 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{34}H_{69}N_3O_6Na$ 638.5079; found 638.5093.

*4.2.9. (S)-16-Azido-1-(2-(((S)-2-hydroxydodecyl)oxy)ethoxy)hexadecan-2-ol (**25**).* To a solution of **24** (0.30 g, 0.49 mmol) in methanol (7 mL) was added concd HCl (1.6 mL). The reaction mixture was stirred overnight at room temperature. After water (20 mL) was added, the mixture was extracted with Et_2O for several times. The combined organic phases were washed with saturated aq $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **25** (0.23 g, 90%) as a white solid. Mp 49–51 °C. $[\alpha]_D^{25}$ +5.91 (*c* 0.88, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 3.84–3.74 (m, 2H), 3.73–3.58 (m, 4H), 3.53 (dd, *J*=9.8, 2.8 Hz, 2H), 3.31 (dd, *J*=9.8, 8.3 Hz, 2H), 3.25 (t, *J*=7.0 Hz, 2H), 2.79 (s, 2H), 1.65–1.53 (m, 2H), 1.45–1.21 (m, 42H), 0.87 (t, *J*=6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 76.11, 70.55, 70.28, 51.58, 33.13, 32.03, 29.82, 29.75, 29.60, 29.46, 29.27, 28.95, 26.83, 25.71, 22.80, 14.23. IR (KBr, ν_{max}): 3439, 2917, 2850, 2131, 1743, 1665, 1466, 1377, 1316, 1262, 1159, 1143, 1097, 1021, 957, 884, 864, 802, 725, 612 cm^{-1}. MS (ESI, *m*/*z*): 550 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{30}H_{61}N_3O_4Na$ 550.4554; found 550.4566.

*4.2.10. Synthesis of fluorescent probe **7**.* A mixture of alkyne **26** (66.5 mg, 0.011 mmol), azide **25** (50 mg, 0.095 mmol), $CuSO_4 \cdot 5H_2O$ (8.3 mg, 0.033 mmol), sodium ascorbate (15.1 mg, 0.076 mmol), water (4 mL), and THF (4 mL) was stirred at room temperature for 5 h under N_2 atmosphere. The mixture was extracted with CH_2Cl_2 for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography of the residue on silica gel (DCM/CH_3OH, 20:1) yielded **7** (68.5 mg, 65%) as a purple solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.73–7.56 (m, 3H), 7.56–7.47 (m, 1H), 7.34–7.27 (m, 1H), 7.23 (d, *J*=9.6 Hz, 2H), 6.96 (d, *J*=9.3 Hz, 2H), 6.73 (d, *J*=2.2 Hz, 2H), 4.31 (t, *J*=7.3 Hz, 2H), 3.85–3.73 (m, 2H), 3.73–3.50 (m, 16H), 3.47–3.26 (m, 6H), 2.36 (s, 4H), 1.91–1.82 (m, 2H), 1.48–1.38 (m, 6H), 1.21–1.35 (m, 48H), 0.87 (t, *J*=6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.51, 157.95, 156.51, 155.87, 143.74, 135.85, 132.43, 130.83, 130.29, 129.93, 129.04, 127.92, 123.48, 114.45, 114.00, 96.32, 76.03, 70.63, 70.53, 65.76, 53.29, 52.90, 52.83, 50.54, 47.78, 46.25, 41.87, 33.19, 32.11, 30.77, 30.48, 29.90, 29.88, 29.81, 29.79, 29.76, 29.72, 29.61, 29.53, 29.22, 26.71, 25.74, 22.88, 19.39, 14.32, 13.92, 12.79. IR (KBr, ν_{max}): 3441, 2924, 2853, 1735, 1632, 1597, 1528, 1489, 1467, 1416, 1348, 1277, 1262, 1184, 1133, 1082, 1048, 1017, 843, 684, 662, 557 cm^{-1}. MS (ESI, *m*/*z*): 1076 $[M]^+$. HRMS (ESI, *m*/*z*): $[M]^+$ calcd for $C_{65}H_{102}N_7O_6$ 1076.7886; found 1076.7906.

*4.2.11. (1-((5S,12S)-5-Decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxahexacosan-26-yl)-1H-1,2,3-triazol-4-yl)methanol (**27**).* The same procedure was applied as that for synthesis of **7**, using azide **24** (60 mg, 0.097 mmol) and propargyl alcohol (13.8 μL, 0.29 mmol), yielding **27** (57 mg, 86%) as a brown oil. $[\alpha]_D^{25}$ −5.48 (*c* 0.58, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (s, 1H), 4.78 (s, 2H), 4.75 (d, *J*=6.8 Hz, 2H), 4.64 (d, *J*=6.8 Hz, 2H), 4.33 (t, *J*=7.2 Hz, 2H), 3.73–3.64 (m, 2H), 3.63–3.56 (m, 4H), 3.48 (d, *J*=5.0 Hz, 4H), 3.37 (s, 6H), 1.92–1.84 (m, 2H), 1.56–1.45 (m, 4H), 1.39–1.21 (m, 38H), 0.86 (t, *J*=6.5 Hz, 3H). ^{13}C

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NMR (75 MHz, $CDCl_3$) δ 147.93, 121.66, 96.12, 76.34, 74.22, 70.88, 56.45, 55.57, 50.51, 32.14, 32.04, 30.43, 29.88, 29.75, 29.64, 29.51, 29.47, 29.13, 26.62, 25.60, 22.81, 14.25. IR (film, ν_{max}): 3425, 3139, 2924, 2854, 1465, 1359, 1261, 1216, 1144, 1105, 1040, 918, 803, 722 cm^{-1}. MS (ESI, *m*/*z*): 672 $[M+H]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{37}H_{73}N_3O_7Na$ 694.5341; found 694.5351.

*4.2.12. 1-((5S,12S)-5-Decyl-12-(methoxymethoxy)-2,4,7,10-tetraoxa hexacosan-26-yl)-4-methyl-1H-1,2,3-triazole (**28**).* To a solution of **27** (250 mg, 0.37 mmol) in anhydrous CH_2Cl_2 (9 mL) were added Et_3N (0.13 mL, 0.93 mmol) and MsCl (72 μL, 0.93 mmol) at 0 °C under N_2 atmosphere. The reaction mixture was stirred overnight at 0 °C and quenched by water. The mixture was extracted by CH_2Cl_2 for three times. The combined organic phases were washed with 1 N aq HCl, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was re-dissolved in DMF (3 mL) and treated with $NaBH_4$ (57 mg, 0.08 mmol) at 0 °C. The reaction mixture was warmed to room temperature, and then heated at 70 °C for 5 h. The reaction was quenched by water, and extracted by Et_2O for several times. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel to give **28** (140 mg, 58% for two steps) as a colorless oil. $[\alpha]_D^{25}$ −5.36 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 (s, 1H), 4.75 (d, *J*=6.8 Hz, 2H), 4.64 (d, *J*=6.8 Hz, 2H), 4.28 (t, *J*=7.2 Hz, 2H), 3.68 (dt, *J*=11.1, 5.4 Hz, 2H), 3.63–3.57 (m, 4H), 3.48 (d, *J*=5.0 Hz, 4H), 3.37 (s, 6H), 2.34 (s, 3H), 1.89–1.80 (m, 2H), 1.49 (t, *J*=6.2 Hz, 4H), 1.24 (brs, 38H), 0.86 (t, *J*=6.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.07, 96.19, 76.39, 74.29, 70.95, 55.63, 50.34, 32.21, 32.10, 30.55, 29.94, 29.81, 29.71, 29.58, 29.53, 29.21, 26.69, 25.66, 22.87, 14.31, 11.04. IR (film, ν_{max}): 2925, 2855, 1557, 1464, 1359, 1215, 1144, 1105, 1041, 918, 722 cm^{-1}. MS (ESI, *m*/*z*): 656 $[M+H]^+$, 678 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{37}H_{73}N_3O_6Na$ 678.5392; found 678.5398.

*4.2.13. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-(4-methyl-1H-1,2,3-triazol-1-yl)hexadecan-2-ol (**8**).* To a solution of **28** (18.0 mg, 0.027 mmol) in methanol (1.6 mL) was added concd HCl (0.4 mL). The reaction mixture was stirred overnight at room temperature, and quenched with water (20 mL). The mixture was extracted with ethyl acetate for several times. The combined organic phases were washed with saturated aq $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography to give **8** (13.2 mg, 85%) as a white solid. Mp 79–82 °C. $[\alpha]_D^{25}$ +9.09 (*c* 0.53, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (s, 1H), 4.29 (t, *J*=7.2 Hz, 2H), 3.83–3.74 (m, 2H), 3.73–3.58 (m, 4H), 3.53 (dd, *J*=9.8, 2.7 Hz, 2H), 3.32 (t, *J*=9.0 Hz, 2H), 2.35 (s, 3H), 1.94–1.80 (m, 2H), 1.44–1.22 (m, 42H), 0.87 (t, *J*=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.10, 76.09, 70.70, 70.49, 50.37, 33.20, 32.12, 30.58, 29.91, 29.82, 29.72, 29.59, 29.56, 29.23, 26.71, 25.76, 22.90, 14.33, 11.06. IR (KBr, ν_{max}): 3464, 3141, 2920, 2848, 1558, 1463, 1438, 1405, 1373, 1261, 1213, 1143, 1098, 1081, 875, 831, 724, 571 cm^{-1}. MS (ESI, *m*/*z*): 590 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{65}N_3O_4Na$ 590.4867; found 590.4880.

*4.2.14. (S)-1-(2-(((S)-2-Hydroxydodecyl)oxy)ethoxy)-16-(4-(piperazin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)hexadecan-2-ol (**9**).* The same procedure was applied as that of synthesizing compound **7**, using azide **25** (100 mg, 0.19 mmol) and alkyne **29**[21] (70.6 mg, 0.57 mmol), yielding **9** (58 mg, 47%) as a white solid. Mp 81–83 °C. $[\alpha]_D^{25}$ +12.96 (*c* 0.22, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 1H), 4.32 (t, *J*=7.3 Hz, 2H), 3.83–3.73 (m, 2H), 3.72–3.58 (m, 6H), 3.52 (dd, *J*=9.8, 2.6 Hz, 2H), 3.31 (t, *J*=9.0 Hz, 2H), 2.89 (t, *J*=4.7 Hz, 4H), 2.49 (s, 4H), 1.88 (dt, *J*=12.9, 6.4 Hz, 2H), 1.49–1.21 (m, 42H), 0.87 (t, *J*=6.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.23, 122.55, 76.06, 70.57, 70.28, 54.12, 54.00, 50.43, 45.85, 33.20, 32.02, 30.40, 29.81, 29.73, 29.70, 29.58, 29.46, 29.08, 26.61, 25.70, 22.80, 14.25. IR (KBr, ν_{max}): 3468, 2919, 2849, 1466, 1371, 1320, 1273, 1143, 1114, 1098, 1082, 1051, 1006, 874, 831, 797, 721, 574 cm^{-1}. MS (ESI, *m*/*z*): 674 $[M+Na]^+$. HRMS (ESI, *m*/*z*): $[M+Na]^+$ calcd for $C_{37}H_{73}N_5O_4Na$ 674.5555; found 674.5569.

4.3. Biological experiments

4.3.1. Cell culture conditions. The human lung adenocarcinoma cells A549, human breast adenocarcinoma cells MCF-7 were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS; Gibco/BRL, Grand Island, NY), 100 U/mL penicillin and 100 μg/mL streptomycin. Human hepatocellular carcinoma cells Bel-7404 were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. All cell lines were cultivated in a humidified atmosphere of 5% CO_2 at 37 °C.

4.3.2. MTT cell growth inhibition assay.[7] A549 cells (9×10^3), MCF-7 cells (3×10^3), and Bel-7404 (7×10^3) cells were plated in flat-bottomed 96-well microplates per well. Background control wells lacking the cells but containing the same volume of media were included in each assay plate. Twelve hours after seeding, new medium containing increasing concentrations of tested compounds at 0.10–40 μM or vehicle control (DMSO) was added. Cells were further incubated for corresponding time (e.g., 24 h, 48 h or 72 h) and then treated with MTT (20 μL/well, 5 mg/mL) and incubated for another 4 h. Then the medium was removed carefully and 150 μL DMSO was added to each well, including controls and blanks. After the samples were shaken gently for 20 min, the absorbance in each well at 570 nm in a microtiter plate reader (Varioskan Flash) was measured with a reference wavelength at 690 nm. Experiments were independently repeated for three times (performed in triplicate), and growth inhibition rate was calculated as follows: growth inhibition rate (%)={1−[ΔOD(compounds)−ΔOD(blank)]/[ΔOD(controls)−ΔOD(blank)]}×100%. The growth inhibition (IC_{50}) for each compound was defined as the concentration of drug leading to a 50% reduction in A570 compared with controls.

4.3.3. Confocal microscopy analyses. Cells were grown on Φ 20 mm glass bottom cell culture dishes, and incubated at 37 °C for 24 h, florescent probe **7** sample in DMEM (or RPMI-1640) with 10% fetal bovine serum (FBS) at a concentration of 100 nM were added to replace the culture medium. After 12 h of incubation, the medium was removed and the cells were washed three times with PBS. Afterward, new medium containing 200 nM MitoTracker® Green FM (invitrogen) was added to co-stain mitochondria. After 20 min of incubation at 37 °C, the cells was again washed three times with PBS. Cell culture medium of 1 mL was added and the cells were examined by a Zeiss LSM 710 microscope equipped with a 63× oil-immersion objective. The fluorescence was examined under excitation at 543 nm for probe **7** and 488 nm for MitoTracker® Green FM. Image processing and analysis were done with ZEN 2008 software.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.05.047.

References and notes

1. Kojima, N.; Tanaka, T. *Molecules* **2009**, *14*, 3621–3661.
2. Bermejo, A.; Figadère, B.; Zafra-Polo, M. C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269–303.
3. (a) Liu, H.-X.; Shao, F.; Li, G.-Q.; Xun, G.-L.; Yao, Z.-J. *Chem.—Eur. J.* **2008**, *14*, 8632–8639; (b) Morré, D. J.; de Cabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1994**, *56*, 343–348.
4. Zeng, B.-B.; Wu, Y.; Yu, Q.; Wu, Y.-L.; Li, Y.; Chen, X.-G. *Angew. Chem. Int. Ed.* **2000**, *39*, 1934–1937.
5. Jiang, S.; Liu, Z.-H.; Sheng, G.; Zeng, B.-B.; Chen, X.-G.; Wu, Y.-L.; Yao, Z.-J. *J. Org. Chem.* **2002**, *67*, 3404–3408.
6. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Wu, J.-R.; Yao, Z.-J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3426–3430.
7. Xiao, Q.-C.; Liu, Y.-Q.; Qiu, Y.-T.; Zhou, G.-B.; Mao, C.; Li, Z.; Yao, Z.-J.; Jiang, S. *J. Med. Chem.* **2011**, *54*, 525–533.
8. (a) Liu, Y.-Q.; Cheng, X.; Guo, L.-X.; Mao, C.; Chen, Y.-J.; Liu, H.-X.; Xiao, Q.-C.; Jiang, S.; Yao, Z.-J.; Zhou, G.-B. *PLoS One* **2012**, *7*, e47049; (b) Huang, G.-R.; Jiang, S.; Wu, Y.-L.; Jin, Y.; Yao, Z.-J.; Wu, J.-R. *ChemBioChem* **2003**, *4*, 1216–1221.
9. Zeng, B.-B.; Wu, Y.-K.; Jiang, S.; Yu, Q.; Yao, Z.-J.; Liu, Z.-H.; Li, H.-Y.; Li, Y.; Chen, X.-G.; Wu, Y.-L. *Chem.-Eur. J.* **2003**, *9*, 282–290.
10. Mao, C.; Han, B.; Wang, L.-S.; Wang, S.-Z.; Yao, Z.-J. *Med. Chem. Commun.* **2011**, *2*, 918–922.
11. (a) Kojima, N.; Fushimi, T.; Maezaki, N.; Tanaka, T.; Yamori, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1637–1641; (b) Duval, R.; Lewin, G.; Hocquemiller, R. *Bioorg. Med. Chem.* **2003**, *11*, 3439–3446; (c) Duval, R.; Lewin, G.; Peris, E.; Chahboune, N.; Garofano, A.; Dröse, S.; Cortes, D.; Brandt, U.; Hocquemiller, R. *Biochemistry* **2006**, *45*, 2721–2728; (d) Kojima, N.; Fushimi, T.; Tatsukawa, T.; Yoshimitsu, T.; Tanaka, T.; Yamori, T.; Dan, S.; Iwasaki, H.; Yamashita, M. *Eur. J. Med. Chem.* **2013**, *63*, 833–839.
12. Duval, R. A.; Poupon, E.; Romero, V.; Peris, E.; Lewin, G.; Cortes, D.; Brandt, U.; Hocquemiller, R. *Tetrahedron* **2006**, *62*, 6248–6257.
13. Vijaykumar, B. V. D.; Mallesham, P.; Chandrasekhar, S. *Eur. J. Org. Chem.* **2012**, *5*, 988–994.
14. Ryu, M.-H.; Choi, J.-W.; Kim, H.-J.; Park, N.; Cho, B.-K. *Angew. Chem., Int. Ed.* **2011**, *50*, 5737–5740.
15. Lu, R.-J.; Tucker, J. A.; Zinevitch, T.; Kirichenko, O.; Konoplev, V.; Kuznetsova, S.; Sviridov, S.; Pickens, J.; Tandel, S.; Brahmachary, E.; Yang, Y.; Wang, J.; Freel, S.; Fisher, S.; Sullivan, A.; Zhou, J. Y.; Stanfield-Oakley, S.; Greenberg, M.; Bolognesi, D.; Bray, B.; Koszalka, B.; Jeffs, P.; Khasanov, A.; Ma, Y.-A.; Jeffries, C.; Liu, C. H.; Proskurina, T.; Zhu, T.; Chucholowski, A.; Li, R. S.; Sexton, C. *J. Med. Chem.* **2007**, *50*, 6535–6544.
16. Liu, H.-X.; Yao, Z.-J. *Tetrahedron Lett.* **2005**, *46*, 3525–3528.
17. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Jiang, S.; Wu, J.-R.; Yao, Z.-J. *ChemBioChem* **2007**, *8*, 172–177.
18. Reddy, T. V. K.; Devi, B. L. A. P.; Prasad, R. B. N.; Sujitha, P.; Kumar, C. G. *Eur. J. Med. Chem.* **2013**, *67*, 384–389.
19. Vangala, K.; Yanney, M.; Hsiao, C.-T.; Wu, W. W.; Shen, R.-F.; Zou, S.; Sygula, A.; Zhang, D.-M. *Anal. Chem.* **2010**, *82*, 10164–10171.
20. Nazemi, A.; Amos, R. C.; Bonduelle, C. V.; Gillies, E. R. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 2546–2559.
21. Zheng, H.-L.; Weiner, L. M.; Bar-Am, O.; Epsztejn, S.; Cabantchik, Z. I.; Warshawsky, A.; Youdim, M. B. H.; Fridkin, M. *Bioorg. Med. Chem.* **2005**, *13*, 773–783.

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