Article

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

Synthesis of Amino Acid-comprising Sialyltransferase Inhibitors and Their Antimetastatic Activities against Human Breast Cancer Cells

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(Received: Aug. 26, 2015; Accepted: Oct. 11, 2015; Published Online: Dec. 14, 2015; DOI: 10.1002/jccs.201500348)

The amino acid-containing lithocholic acids (LCA) represent a new class of human sialyltransferase (ST) inhibitors. In this study, we have reported their design, synthesis, and inhibitory activity against human STs. Among these derivatives, D-Glu-LCA **7**, L-Asp-L-Asp-LCA **13**, and L-Asp-L-Asp-Gly-Gly-LCA **22** with specific amino acid sequence were the most active ones with IC_{50} values of 2.3-5.6 and 4.2-6.2 μM toward α-2,3-ST and α-2,6-ST, respectively. The current study demonstrates that the new class of ST inhibitors inhibit cell migration in breast cancer cells by preventing closure of the wound rather than involving a direct antiproliferative effect.

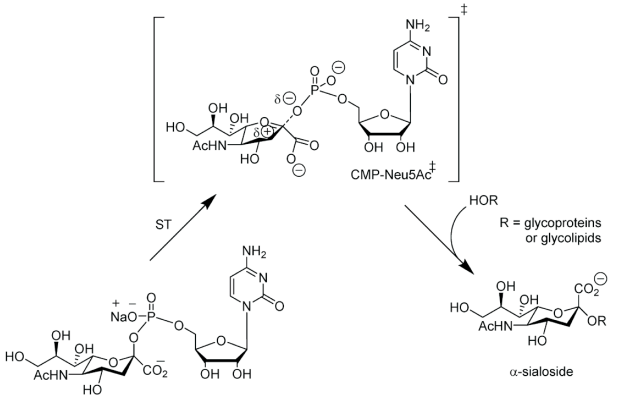
Keywords: Amino acid-containing lithocholic acids; Antiproliferative effect; Cell migration; Human sialyltransferase inhibitors; Wound healing.

INTRODUCTION

Cancer is the major diseases that awfully threaten the quality of human life and metastasis is the leading cause of cancer-related deaths.[1] Metastasis is the biologically complicated process including adhesion, degradation and migration of tumor cells from their initial site to the secondary organ.[2] Collapse at any of these steps could block the entire metastatic course and, as a result, could serve as a therapeutic strategy for the treatment of cancer metastasis.[3] Development of such therapeutic agents that inhibit tumor metastasis is highly attractive. However, study about the antimetastatic agents is very rare and limited.[4]

Hypersialylation at the non-reducing end of glycoproteins or glycolipids plays an important role in cellular interactions such as cell-cell adhesion, tumor cell metastasis and invasions.[5] Modifications of sialylation *in vivo* are mediated and regulated by various glycoprotein- and glycolipid-specific sialyltransferases (STs).[6] STs belong to a family of sialic acid-dependent glycosyltransferases and participate in the biosynthesis of sialylated glycoconjugates by catalyzing the transfer of the N-acetylneuraminic acid (NeuAc) portion of cytidine monophosphate-N-acetylneuraminic acid (CMP-Neu5Ac) with inversion of configuration to acceptor hydroxyl groups at or near the nonreducing terminus of oligosaccharide chains of glycoproteins and glycolipids via α2-3, α2-6, or α2-8 linkages (Scheme 1).[7] Recent studies have showed that hypersialylation of β1 integrins through ST6Gal-I promotes cancer progression by up-regulating cell motility *in vitro*,[8] then the deficiency of hypersialylation promotes carcinoma differentiation *in vivo*.[9] ST3Gal-I is acting as a tumor promoter in breast cancer and exerts its effect in tumor development in transgenic mice model.[10] ST6GalNAcV can mediate breast cancer metastasis to the brain.[11] Accordingly, there has been some development in using inhibition of ST functions as biological tools and perhaps therapeutics.[12] Therefore, the design of effective ST inhibitors would be highly desirable.

Scheme 1 Proposed mechanism of sialyltransferase-mediated sialylation



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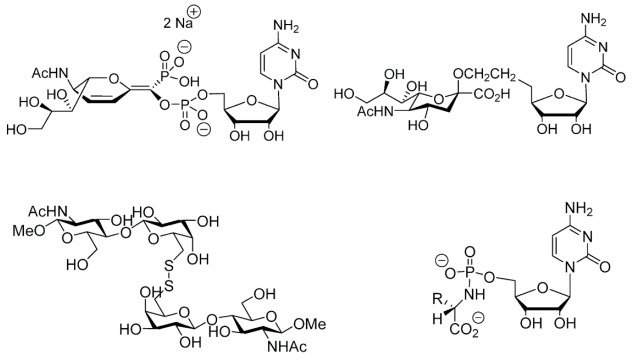
Supporting information for this article is available on the www under http://dx.doi.org/10.1002/jccs.201500348

Pioneering studies on ST inhibitors have provided essential information regarding the development of core skeleton based on mimetic of transition-state analogues, bisubstrate analogues, donor analogues, and acceptor analogues (Chart 1).[13] However, the lack of membrane permeability found in these inhibitors has hampered their biological and clinical applications. Instead, our studies have revealed the importance of ST inhibitors, lithocholic acid (LCA) derivatives,[14] with practical pharmaceutical applications. For example, we have shown that AL10, 7-nitro-2,1,3-benzoxadiazole analogue of L-Glu-LCA, significantly suppresses lung cancer metastasis *in vivo* without affecting liver and kidney functions of experimental animals.[15] Upon treatment of 4T1 cell animal model with ST inhibitor, L-Asp-LCA (**1**), results demonstrated that ST inhibitor-treated mice effectively delayed lung tumor metastasis/colonization.[16] Furthermore, inhibition of chemokine (C-C motif) receptor 7 sialylation appears to be the action for suppressing CCL19-stimulated proliferation, invasion and anti-anoikis.[17]

Chart 1 Examples of some of the sialyltransferase inhibitors



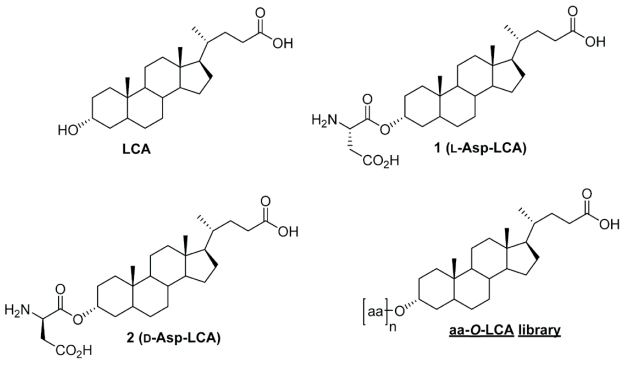
In this study, we developed and synthesized a series of amino acid conjugates of lithocholic acid (aa-*O*-LCA library; Chart 2), and studied their antimetastatic effects against human breast cancer cell lines, MCF-7 and MDA-MB231.

RESULTS AND DISCUSSION

Design and synthesis of amino acid conjugates of lithocholic acid

LCA, which potentially mimics a pentacyclic ring of Soyasaponin I,[18] showed a promising inhibitory property toward α-2,3-ST, indicating an acceptable pharmacophore.[14]

Chart 2 Structures of LCA, L-Asp-LCA (**1**), D-Asp-LCA (**2**) and a series of amino acid conjugates of LCA used in this study

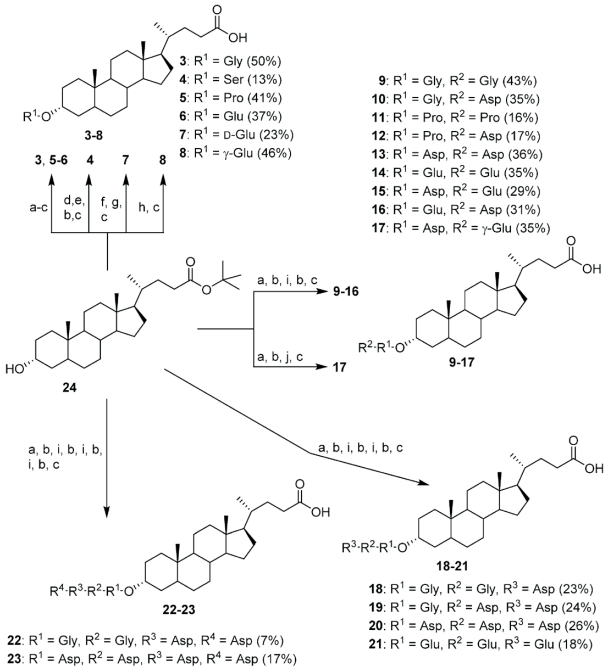


Interestingly, cell-permeable property appeared to be presented for LCA analogues, such as L-Asp-LCA (**1**) and D-Asp-LCA (**2**). Despite current efforts, however, other amino acid conjugates of LCA and their biological functions toward ST activity and cancer metastasis have not been explored. We reasoned that a hit library of this type should first inactivate ST function. Therefore, enzyme activity-based strategy and lead optimization approach were employed by using the results of prior round of screening to generate four amino acid sequence patterns of ST inhibitors sequentially, monopeptide-LCAs (R_1-LCAs, **3-8**), dipeptide-LCAs (R_2-R_1-LCAs, **9-17**), tripeptide-LCAs (R_3-R_2-R_1-LCAs, **18-21**), and tetrapeptide-LCAs (R_4-R_3-R_2-R_1-LCAs, **22-23**).

All peptides attached to LCA were synthesized on solution phase utilizing Fmoc- and Boc-amino acid building blocks. The coupling and activating reagents, dicyclohexylcarbodiimide (DCC), ethyl-(N',N'-dimethylamino) propylcarbodiimide hydrochloride (EDC) and N-hydroxybenzotriazole (HOBt), in dichloromethane (DCM) or N,N-dimethylformamide (DMF) were used, respectively. Scheme 2 illustrates the preparation of peptidyl LCA conjugates (**3-8**, **9-17**, **18-21** and **22-23**). The monopeptide-LCAs **3**, **5**, **6** and **8** were obtained by condensing the corresponding Fmoc- and Boc-amino acid, Fmoc-Gly-OH, Fmoc-Pro-OH, Fmoc-L-Glu(OBut)-OH and Boc-L-Glu-OBut, with **24** using DCC as coupling agent, followed by alkaline hydrolysis of the Fmoc and acidolytic cleavage of the remaining *t*Bu or Boc groups. To prevent the side reactions, hydroxyl group of Fmoc-Ser-OH was *tert*-butyldiphenylsilyl (TBDPS)-protected prior to coupling. Thus reaction of Fmoc-Ser(OTBDPS)-OH with **24** followed by treatment with tetrabutylammonium fluoride (n-Bu_4NF)

afforded the amino alcohol which could be subsequently converted to **4** using a similar deprotection procedure. To prepare the D-form Glu-LCA **7**, esterification of the secondary alcohol **24** using Boc-D-Glu(OAll)-OH was followed by liberation of the δ–carboxylic acid of D-Glu with $Pd(PPh_3)_4$ in the presence of morpholine and subsequent removal of *t*Bu and Boc groups.

Scheme 2 Preparation of peptidyl LCA analogues **3-23**. Reagents and conditions: a) Fmoc-amino acid-OH, DCC, DMAP, DCM; b) DBU, DCM; c) TFA, 2% H_2O, RT; d) Fmoc-Ser (OTBDPS)-OH, DCC, DMAP, DCM; e) *n*-Bu_4NF, HOAc, THF; f) Boc-D-Glu(OAll)-OH, DCC, DMAP, DCM; g) $Pd(PPh_3)_4$, morpholine, THF; h) Boc-Glu-OBu*t*, DCC, DMAP, DCM; i) Fmoc-amino acid-OH, EDC, HOBt, DMF; j) Boc-Glu-OBu*t*, EDC, HOBt, DMF

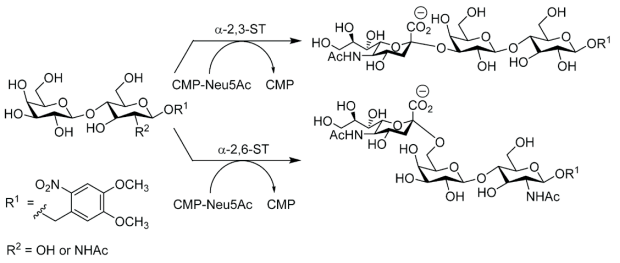


In addition, Scheme 2 illustrated the synthesis of dipeptide-LCAs **9-16** from monopeptide-LCAs. As a representative example, the assembly of dipeptide-LCA **10** was performed by using Fmoc solution-phase peptide synthesis strategy. Consecutively coupling of **24** with Fmoc-Gly-OH and Fmoc-Asp(OBut)-OH to give protected Gly-Asp-LCA. Dipeptide-LCA **10** was obtained in 35% yield after the similar deprotection procedures as described for monopeptide-LCA. Conjugate dipeptide-LCA **17** was obtained following the general peptide synthesis described in Scheme 2, in which **24** was first reacted with Fmoc-L-Asp(OBut)-OH using DCC/4-dimethylaminopyridine (DMAP) as coupling agent, followed by alkaline-mediated deprotection of Fmoc. The free amino group of protected L-Asp-LCA was reacted with Boc-Glu-OBut in the presence of EDC/HOBt followed by removal of the remaining Boc and *t*Bu groups to yield **17**. Similarly, the tripeptide-LCAs **18-21** and tetrapeptide-LCAs **22-23**, developed in this study, were accomplished by condensation of the corresponding Fmoc-amino acids with **24** within yields ranging from 7 to 26%. The peptide synthesis in this study could have low yields, *e.g.*, **4**, **22** or others, and might suffer from incorporation of lengthy protection/deprotection procedures as well as tedious HPLC separation of final products in some cases.

Inhibition of α-2,3-ST and α-2,6-ST by amino acid conjugates of lithocholic acid

The inhibition assays of α-2,3-ST and α-2,6-ST were performed following the general procedure of Schmidt *et al.*,[19] in which CMP-Neu5Ac was used as a substrate and the modified disaccharide[20] with a 4,5-dimethoxy-2-nitrobenzyl group was employed as an UV-labelled acceptor (Scheme 3). The resulting IC_{50} values of **1-23** are summarized in Table 1. The presence of the specific amino acid residue demonstrated to be essential for inhibitory potency. Among the monopeptide-LCAs, L-Asp-LCA **1**, D-Asp-LCA **2**, L-Glu-LCA **6** and D-Glu-LCA **7** exhibited a 1.2–8-fold potency increase over **3-5** and **8**, suggesting that these four amino acids are important for promoting affinity toward α-2,3-ST. Similarly, D-Glu-LCA **7** (IC_{50} = 6.2 μM) exhibited a 2.5-fold potency increase over L-Asp-LCA **1**, indicating that the presence of a D-Glu substituent in LCA leads to enhanced efficacy against α-2,6-ST.

Scheme 3 α-2,3-ST and α-2,6-ST mediated sialylations of UV-labelled acceptors



Of the 9 dipeptide-LCAs **9-17** designed for the

Table 1. Inhibition of α-2,3-ST and α-2,6-ST by selected peptidyl LCA analogues **1**–**23**

Compound [a]	α-2,3-ST (IC_{50}, μM)[a]	α-2,6-ST (IC_{50}, μM)[a]
1	12.2 ± 2.1[b]	15.8 ± 0.2[e]
2	6.8 ± 1.0[b]	-
3	14.3 ± 1.5	-
4	19.9 ± 2.0	-
5	42.1 ± 3.9	-
6	7.0 ± 0.9	13.2 ± 2.1
7	5.4 ± 0.7	6.2 ± 1.3
8	15.8 ± 1.5	-
9	~100	-
10	16.8 ± 2.6	-
11	> 100 (16)[c]	-
12	15.5 ± 1.8	-
13	5.6 ± 0.7	4.2 ± 1.2
14	> 15 (8)[d]	-
15	21.1 ± 1.9	-
16	22.0 ± 1.7	-
17	34.0 ± 3.8	-
18	19.9 ± 3.7	-
19	20.4 ± 2.7	-
20	31.6 ± 4.7	46.5 ± 5.3
21	> 15 (2)[d]	-
22	2.3 ± 0.3	6.1 ± 1.2
23	34.7 ± 3.8	-

[a] Inhibitor concentration at which half-maximal enzyme activity is obtained (IC_{50}, mean ± S.E.M., n = 3). [b] Value is from Ref. 14. [c] The percent (%) inhibition, in parentheses, at 100 μM is expressed as the percent (%) inhibition of enzyme activity. [d] The percent (%) inhibition, in parentheses, at 15 μM is expressed as the percent (%) inhibition of enzyme activity. [e] Value is from Ref. 16.

second round of screening, compound **13** showed the best inhibition constant with IC_{50} values of 5.6 and 4.2 μM against α-2,3-ST and α-2,6-ST, respectively. Dipeptide-LCAs containing two glycines (compound **9**) or two prolines (compound **11**), which are relatively hydrophobic, were shown to have low ability to suppress α-2,3-ST activity (at least 18-fold less potent than **13**), suggesting that the hydrophobic amino acid reduce ST activity. Surprisingly, replacement of the L-Asp-L-Asp with L-Glu-L-Glu, as in **14**, reduced activity dramatically when compared to that of **13**. Similarly, compounds **15-17**, where dipeptide is constructed by using different combination of two single amino acids (L-Asp, L-Glu and γ-Glu), displayed an approximately 4–6-fold lower inhibitory potency than **13**.

These results uncover the importance of specific amino acid sequence for improvement of inhibitory potency and provide us a clue to tackle the difficulty in the next design of tripeptide-LCAs **18-21** and tetrapeptide-LCAs **22-23**. Incorporation of Gly or L-Asp at position R^1 in tripeptide-LCAs **19** (IC_{50} = 20.4 μM) and **20** (IC_{50} = 31.6 μM), respectively, exhibited lower inhibitory potencies against α-2,3-ST when compared to that of corresponding dipeptide-LCA **13**. The relative IC_{50} value (46.5 μM) of 20 show similar trend against α-2,6-ST, an observation that indicates that the extra incorporation of Gly or L-Asp at position R^1 is not useful for suppressing ST activity. Unfavourable results were also observed for compounds **18** (L-Asp-Gly-Gly-LCA) and **21** (L-Glu-L-Glu-L-Glu-LCA).

However, tetrapeptide-LCA **22**, in which extra Gly is at position R^1 of corresponding tripeptide-LCA **19**, inhibited α-2,3-ST at the low micromolar level (IC_{50} = 2.3 μM). Compound **22** was at least 9-fold more potent than **19**, 2-fold more potent than **13**, 5-fold more potent than **1**, and 15-fold more potent than **23**. The inhibitory activity of compound **22** was also evaluated against α-2,6-ST with an IC_{50} value of 6.1 μM (Table 1), representing 2.6-7.6-fold improvement over L-Asp-LCA **1** and tripeptide L-Asp-L-Asp-L-Asp-LCA **20** and similar activity compared to monopeptide D-Glu-LCA **7** and dipeptide L-Asp-L-Asp-LCA **13**. Collectively, the results show that the presence of specific amino acid sequence in LCA such as D-Glu-LCA **7**, L-Asp-L-Asp-LCA **13**, and L-Asp-L-Asp-Gly-Gly-LCA **22**, leads to significantly enhanced efficacy against α-2,3-ST and α-2,6-ST.

***In Vitro* cell viability effects of amino acid conjugates of lithocholic acid**

The antiproliferative effect[21] of compounds **1**–**23** was tested on two breast cancer cell lines, MCF-7 (weakly metastatic human breast cancer cell) and MDA-MB-231 (strongly metastatic human breast cancer cell). **1**–**23** did not cause promising inhibitory effect on cell growth (> 90% survival) in both cell lines at the tested concentrations (20 and 50 μM). These results indicate that compounds **1-23** exhibit the inhibitory efficacy of ST by blocking the catalytic process of ST-mediated sialylation rather than involving a direct antiproliferative effect. The low antiproliferative effect of compounds **1-23** may also benefit the study of inhibition of migration of breast cancer cells in next section.

Inhibition of migration of metastatic breast cancer cells by ST inhibitors

Increased cell migration is one of the important factors associated with tumor metastasis, the most universal cause of death in cancer patients. Therefore, the ability of ST inhibitors, representative compounds **2**, **5**, **6**, **13**, **20** and

22, to inhibit breast cancer cell migration was investigated *in vitro* using a qualitative wound healing assay.[22] For the control experiments, a sterile pipet tip was used to scratch the confluent MCF-7 cell monolayers and generated a clear wound strip at 0 h. The wound was almost closed at 24 h (Figure 1a). Addition of 20 μM of ST inhibitors inhibited MCF-7 cell migration and avoided closure of the wound at 24 h after scratching (Figure 1b). A greatly slower rate of wound closure was observed in the presence of compounds **5**, **6**, and, while compounds **13** and **22** were considered more effective. This result is in line with the previous observations, the similar trend of ST inhibition (Table 1). In contrast, compounds **2**, **5**, **6**, **13**, **20**, and **22** completely inhibited the migration of strongly metastatic human breast cancer cell, MDA-MB-231 (Figure 2), indicating that most of the LCA derivatives are selective for MDA-MB-231 cells rather than MCF-7 cells. Collectively, these findings suggest that migration of MDA-MB-231 rather than MCF-7 is relatively sensitive to these compounds. The cellular studies further demonstrate that potent ST inhibitor reduces α-2,3-ST/α-2,6-ST activities, prevents closure of the wound, and sequentially inhibits breast cancer cell migration.

In summary, in an effort to explore cell-permeable and low cytotoxic ST inhibitors for alteration of sialic acids on cancer cell surfaces, we disclose herein the rapid discovery and preparation of new amino acid conjugates of lithocholic acid based on the systematically rational design. Development of amino acid sequence-dependent ST inhibitors was achieved and some of these inhibitors, **7**, **13** and **22**, were found to have significant effect in not only suppression of α-2,3-ST and α-2,6-ST activities but also inhibition of breast cancer cell migration. Given the effective results that amino acid conjugate of LCA-based molecules inhibit cancer cell migration, application of using these cell-permeable ST inhibitors against tumor metastasis *in vivo* is of great promise. Meanwhile, further optimization of this class of potent ST inhibitors should pave the way for the development of new type of antimetastatic agents that are of clinical value.

EXPERIMENTAL

Materials: Amino acids were purchased from Advanced

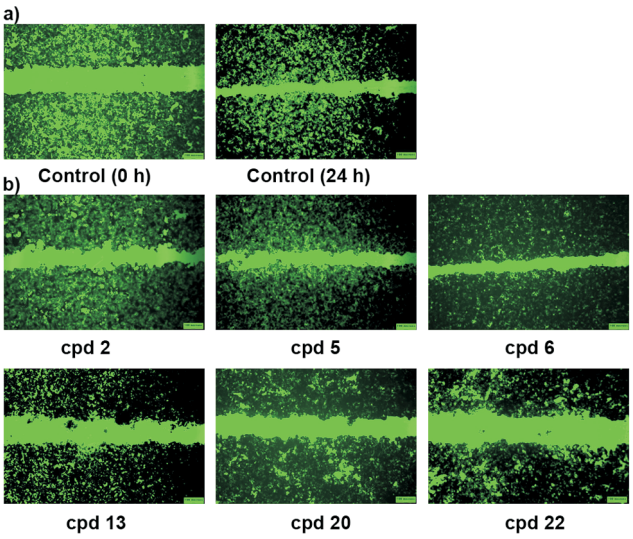


Fig. 1. Effect of ST inhibitors on wound healing of MCF-7 cells. Confluent cells were scraped with a pipette tip to produce a ~1 mm-wide wound area and the cells were treated with medium containing vehicle at 0 h and 24 h a), and ST inhibitors, compounds **2**, **5**, **6**, **13**, **20**, and **22**, were added to the medium at a final concentration of 20 μM, respectively. Individual imagines of the wound healing process were photographed after incubation of cells at 37 °C for 24 h b).

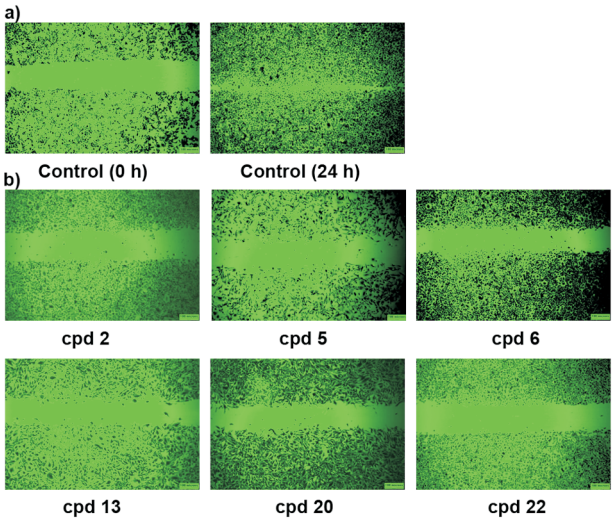


Fig. 2. Effect of ST inhibitors on wound healing of MDA-MB-231 cells. Confluent cells were scraped with a pipette tip to produce a ~1 mm-wide wound area and the cells were treated with medium containing vehicle at 0 h and 24 h a), and ST inhibitors, compounds **2**, **5**, **6**, **13**, **20**, and **22**, were added to the medium at a final concentration of 20 μM, respectively. Individual imagines of the wound healing process were photographed after incubation of cells at 37 °C for 24 h b).

Chemtech. Chemicals and buffers were purchased from Sigma, Aldrich, or Acros Organics. CMP-Neu5Ac was synthesized starting with sialic acid as described by Kajihara and co-workers.[23] Synthesis of lactose acceptor was prepared from lactose via the procedures similar to those of Halcomb.[20] Preparation of LacNAc-based acceptor was accomplished by modifying the procedure described in previous report[14] and the results will be published elsewhere. Rat liver α-2,3-ST and α-2,6-ST were obtained from CalBiochem at a concentration of 560 mU/mL and 0.76 mg/mL, stored at –80 °C and consumed within one week. ^{1}H and ^{13}C NMR spectra were recorded with Bruker AMX400 or 500 MHz instruments. Proton chemical shifts (δ) are reported in parts per million (ppm) relative to the methine singlet at 7.24 ppm for the residual $CHCl_3$ in the deuteriochloroform, or the methyl pentet at 3.30 ppm for the residual CHD_2OD in the methanol-d_4. Carbon chemical shifts are reported in parts per million relative to the internal ^{13}C signals in $CDCl_3$ (77.0 ppm) and CD_3OD-d_4 (49.0 ppm). Mass spectra were obtained with a FAB JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan) and ESI Finnigan LCQ mass spectrometer (Thermo Finnigan, San Jose, CA, United States) in negative mode. The final compounds were purified by reversed phase HPLC (Waters 1525 Binary Pump System with a Waters 2487 Dual λ Absorbance Detector), using Vydac 201SP510 C18 (1 cm × 25 cm) column to purity greater than 95% as judged by analytical HPLC. The purity of compounds was determined by reversed phase analytical HPLC (Waters 2695 System with a 996 PDA detector), using Vydac 201SP54 C18 column (250 × 4.6 mm, 5 μm) and Sephadex LH-20 (170 × 10 mm) column. The separation procedure was performed using H_2O/0.1% trifluoroacetic acid (TFA) and CH_3CN/0.05% TFA as eluents.

Cell Culture: Human breast carcinoma cell line MCF-7 and human adenocarcinoma cells MDA-MB-231 (BCRC-60436; BCRC-60425, Bioresource Collection and Research Center, Food Industry Research and Development Institute; Hsinchu, Taiwan) were grown in Dulbecco's modified Eagle's medium (DMEM) or L-15 medium with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO_2 in air.

Determination of Inhibitory Activity (IC_{50}) of ST Inhibitor: Each reaction solution containing 200 mM MES buffer, 100 mM sodium chloride, 0.5 mM disodium-EDTA, 0.01 % Triton X-100, 20 μM modified disaccharide, 1.4 mU α-2,3-ST (or α-2,6-ST) and inhibitor (in dimethyl sulfoxide) was adjusted to a total volume of 55 μL. The solution was incubated at 37 °C for 10 min, then 1 mM CMP-Neu5Ac (final concentration, 3 μL) was added to initiate the reaction. The solution was incubated at 37 °C until a detectable amount of product was produced. To terminate the enzyme reaction, the solution mixture was quenched by heat at 100 °C for 5 min. The activity was followed by measuring the quantity of sialylated disaccharide product by RP-HPLC (Waters 2695 System with a 996 PDA detector; Supelco Discovery® HS C18, 5 μm, 4.6 mm × 25 cm). Assays were performed in triplicate.

***In Vitro* Quantification of Cell Proliferation:** The cells were seeded with $3 \sim 5 \times 10^4$ cells/well in 200 μl DMEM or L-15 containing 10% FBS and incubated at 37 °C in 5% CO_2 for 24 h. The cells were exposed to each of the ST inhibitors, at the required concentration (a final concentration at 20 μM or ranging from 0.2 to 100 μM) and allowed to incubate for 48 h. The MTT solution (100 μl, 2 mg/ml) was added to each well for 3 h. Then, 100 μl DMSO/well was added and optical absorbance was detected at 540 nm by a Molecular Devices $Spectra_{max}$ Plus 384 microplate reader. Survival ratio is expressed in a percent of a value with respect to untreated cells. The IC_{50} values were determined as the ST inhibitor concentrations that inhibit cell growth by 50% compared with growth of vehicle-treated cells. All measurements were determined from replicates of 5 wells, and each experiment was repeated two times.

Wound Healing Migration Assay: Breast cancer cells (1×10^6 cells/mL) in DMEM or L-15 containing 10% FBS were seeded into 6-well culture plates for 24 h. After they grew to confluence, wounds were created with sterile pipette tips to obtain a clean ~1-mm-wide wound area followed by addition of ST inhibitors (20 μM) in the culture medium at 5% CO_2. The migration and cell movement was examined and photographed (Zeiss Axiovert 40CFL microscope) after 24 h.

General Procedure for Esterification of the 3-Hydroxy Lithocholic Acid (24) and Amino Acid: To a solution of the protected lithocholic acid 24 (0.35 mmol), amino acid (0.45 mmol) and 4-(dimethylamino)pyridine (0.10 mmol) in dichloromethane (8 cm^3) was added a solution of dicyclohexylcarbodiimide in dichloromethane (2 cm^3). The reaction was stirred at room temperature for 30 min and the solvent was removed by rotary evaporation. The resulting residue was purified by column chromatography on silica gel eluting with ethyl acetate and hexane to afford the pure product.

General Procedure for Peptide Bond Formation: To a solution of peptidyl lithocholic acid (0.17 mmol) and amino acid (0.18 mmol) in dimethylformamide (DMF) (3 cm^3) was added 1-hydroxybenzotriazole (HOBT) (0.18 mmol) and ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC) (0.18 mmol). The reaction was stirred at room temperature for 1 hour and the solvent was removed by vacuum system. The mixture was dissolved in dichloromethane (30 cm^3) and washed with water (30 cm^3) twice. The extracts were evaporated and dried to afford the

crude product as the sticky oil, which was purified by column chromatography on silica gel eluting with ethyl acetate and hexane to afford the pure product.

General Procedure for Deprotection of Allyl Group: To a solution of the Allyl-protected steroidal compound (0.36 mmol) in tetrahydrofuran (5 cm^3) was added tetrakis(triphenylphosphine)palladium(0) (0.03 mmol) and morpholine (1.5 cm^3) dropwise. The reaction was stirred at room temperature for 2 hours. Solvent was removed by rotary evaporation. The mixture was dissolved in dichloromethane (30 cm^3) and washed with water (30 cm^3) twice. The extracts were dried and evaporated to afford the crude product as the sticky oil, which was purified by flash column eluting with ethyl acetate and hexane to give the pure product.

General Procedure for Deprotection of Fmoc Group: To a solution of the Fmoc-protected steroidal compound (0.15 mmol) in dichloromethane (5 cm^3) was added neat 7,11-diazabicyclo[5.4.0]undec-11-ene (0.15 mmol) dropwise. The reaction was stirred at room temperature for 30 min. Solvent was removed by rotary evaporation to yield the crude product as oil, which was purified by flash column eluting with ethyl acetate and hexane to give the pure product.

General Procedure for Deprotection of Boc and *t*Bu Groups: A solution of protected compound (0.15 mmol) in TFA (2 cm^3) was treated at room temperature with 2% water (0.04 cm^3). After 30 min, solvent was removed by rotary evaporation. The resulting mixture was neutralized with saturated sodium bicarbonate and purified by reverse phase HPLC to afford the pure product.

Monopeptide-LCA **3**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.90 (m, 1H), 3.79 (s, 2H), 2.31 (m, 1H), 2.21 (m, 1H), 2.04 (m, 1H), 1.96-1.78 (m, 6H), 1.62-1.42 (m, 9H), 1.34-1.07 (m, 10H), 0.98 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.29, 168.15, 78.30, 58.03, 57.65, 44.06, 43.44, 42.00, 41.63, 41.35, 37.32, 36.85, 36.07, 35.83, 33.33, 32.46, 32.15, 29.35, 28.26, 27.66, 25.38, 23.88, 22.08, 18.89, 12.61; HRMS (ESI) calculated for $C_{26}H_{44}NO_4$ $(M + H)^+$, 434.3270; found, 434.3260.

Monopeptide-LCA **4**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.04 (m, 1H), 3.98 (dd, J = 11.6, 4.7 Hz, 1H), 3.91 (dd, J = 11.6, 3.4 Hz, 1H), 3.69 (m, 1H), 2.30 (m, 1H), 2.20 (m, 1H), 2.04-1.74 (m, 7H), 1.63-1.42 (m, 9H), 1.36-1.04 (m, 11H), 0.98 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.42, 168.71, 78.55, 60.99, 58.05, 57.68, 56.36, 44.05, 43.45, 41.99, 41.63, 37.31, 36.86, 36.07, 35.84, 33.32, 32.49, 32.24, 29.36, 28.27, 27.68, 27.58, 25.38, 23.87, 22.08, 18.89, 12.60; HRMS (ESI) calculated for $C_{27}H_{46}NO_5$ $(M + H)^+$, 464.3376; found, 464.3380.

Monopeptide-LCA **5**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.87 (m, 1H), 4.38 (m, 1H), 3.35 (m, 2H), 2.33 (m, 1H), 2.20 (m, 1H), 2.11-1.71 (m, 11H), 1.63-1.07 (m, 19H), 0.98 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.29, 169.83, 78.81, 61.03, 58.02, 57.65, 47.31, 44.05, 43.44, 41.98, 41.62, 37.32, 36.85, 36.03, 35.83, 33.25, 32.45, 32.14, 29.63, 29.35, 28.25, 27.67, 27.57, 25.38, 24.65, 23.85, 22.08, 18.88, 12.60; HRMS (ESI) calculated for $C_{29}H_{48}NO_4$ $(M + H)^+$, 474.3583; found, 474.3583.

Monopeptide-LCA **6**. ^{1}H NMR (400 MHz, CD_3OD): δ 5.48 (d, J = 6.6 Hz, 1H), 4.07 (t, J = 6.7 Hz, 1H), 2.53 (m, 2H), 2.28-2.15 (m, 4H), 2.03-1.89 (m, 6H), 1.75-1.69 (m, 2H), 1.62-1.58 (m, 3H), 1.45-1.35 (m, 8H), 1.30-1.26 (m, 6H), 1.13-1.09 (m, 6H), 0.98 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.29, 174.55, 169.83, 77.21, 56.53, 56.19, 52.23, 42.60, 42.01, 40.52, 40.15, 35,88, 35.40, 34.63, 34.39, 31.86, 31.01, 30.73, 27.90, 26.82, 26.21, 23.93, 22.42, 20.64, 17.45, 11.16; HRMS (FAB) calculated for $C_{29}H_{48}NO_6$ $(M + H)^+$, 506.3482; found, 506.3484.

Monopeptide-LCA **7**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.07 (t, J = 6.7 Hz, 1H), 2.55 (m, 2H), 2.28-2.15 (m, 4H), 2.03-1.89 (m, 6H), 1.75-1.69 (m, 2H), 1.62-1.58 (m, 2H), 1.45-1.35 (m, 7H), 1.30-1.26 (m, 5H), 1.13-1.09 (m, 6H), 0.98 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.70, 175.55, 168.29, 77.11, 56.37, 56.03, 51.93, 42.44, 41.86, 40.37, 40.00, 35.72, 35.24, 34.46, 34.23, 31.67, 30.85, 30.54, 28.88, 27.75, 26.63, 26.03, 25.29, 23.77, 22.26, 20.48, 17.28, 10.99; HRMS(FAB) calculated for $C_{29}H_{48}NO_6$ $(M + H)^+$, 506.3482; found, 506.3489.

Monopeptide-LCA **8**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.76-4.70 (m, 1H), 4.00 (t, J = 6.6 Hz, 1H), 2.56-2.54 (m, 2H), 2.20-2.21 (m, 4H), 1.92-1.86 (m, 6H), 1.65-1.48 (m, 4H), 1.46-1.42 (m, 7H), 1.32-1.28 (m, 4H), 1.16-1.09 (m, 5H), 0.97 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.27, 173.48, 171.75, 76.50, 58.03, 57.62, 53.50, 44.06, 43.49, 42.00, 41.64, 37.33, 36.83, 36.21, 35.85, 33.50, 32.45, 32.14, 31.14, 29.35, 28.30, 27.78, 27.70, 26.90, 25.40, 23.97, 22.09, 18.92, 12.64; HRMS(FAB) calculated for $C_{29}H_{48}NO_6$ $(M + H)^+$, 506.3482; found, 506.3489.

Dipeptide-LCA **9**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.76 (m, 1H), 4.00 (s, 2H), 3.74 (s, 2H), 2.31 (m, 1H), 2.21(m, 1H), 2.04-1.57 (m, 9H), 1.50-1.05 (m, 18H), 0.96 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.29, 170.82, 167.94, 77.25, 58.03, 57.64, 44.06, 43.48, 42.39, 42.00, 41.64, 41.54, 37.33, 36.86, 36.16, 35.85, 33.45, 32.46, 32.14, 29.36, 28.30, 27.73, 27.69, 25.39, 23.91, 22.09, 18.89,

12.60; HRMS (ESI) calculated for $C_{28}H_{47}N_2O_5$ $(M + H)^+$, 491.3485; found, 491.3484.

Dipeptide-LCA **10**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.76 (m, 1H), 4.26 (q, J = 4.3 Hz, 1H), 3.98 (dd, J = 38.5, 17.6 Hz, 2H), 3.02 (dd, J = 17.9, 5.9 Hz, 1H), 2.85 (dd, J = 17.9, 8.7 Hz, 1H), 2.31 (m, 1H), 2.22 (m, 1H), 2.04-1.35 (m, 16H), 1.34-1.05 (m, 10H), 0.96 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.27, 172.99, 170.54, 170.02, 77.20, 57.96, 57.59, 51.12, 44.05, 43.45, 42.54, 41.97, 41.62, 37.32, 36.84, 36.31, 36.15, 35.83, 33.44, 32.45, 32.14, 28.29, 27.74, 27.66, 25.39, 23.93, 22.09, 18.91, 12.63; HRMS (ESI) calculated for $C_{30}H_{49}N_2O_7$ $(M + H)^+$, 549.3540; found, 549.3545.

Dipeptide-LCA **11**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.71 (m, 1H), 4.56 (dd, J = 8.6, 5.6 Hz, 1H), 4.48 (dd, J = 8.7, 5.2 Hz, 1H), 3.69 (m, 1H), 3.60 (m, 1H), 3.44 (m, 1H), 3.33 (m, 1H), 2.56 (m, 1H), 2.35-2.12 (m, 4H), 2.10-1.76 (m, 14H), 1.70-1.52 (m, 2H), 1.47-1.05 (m, 15H), 0.97 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.27, 172.72, 168.38, 77.06, 61.08, 60.45, 58.10, 57.67, 48.25, 47.81, 44.06, 43.43, 42.04, 41.68, 37.32, 36.86, 36.11, 35.85, 33.40, 32.45, 32.14, 30.18, 29.64, 29.36, 28.28, 27.70, 26.04, 25.38, 25.22, 23.91, 22.08, 18.89, 12.61; HRMS (ESI) calculated for $C_{34}H_{55}N_2O_5$ $(M + H)^+$, 571.4111; found, 571.4111.

Dipeptide-LCA **12**. ^{1}H NMR (500 MHz, CD_3OD): δ 4.72 (m, 1H), 4.50 (m, 2H), 3.36 (m, 2H), 3.02 (dd, J = 18.0, 3.1 Hz, 1H), 2.74 (dd, J = 18.0, 9.9 Hz, 1H), 2.32 (m, 2H), 2.20 (m, 1H), 2.08-1.80 (m, 8H), 1.62-1.04 (m, 20H), 0.97 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.32, 172.68, 172.64, 168.08, 77.08, 57.89, 57.58, 50.17, 48.35, 44.05, 43.45, 41.99, 41.60, 37.33, 36.87, 36.11, 35.84, 33.25, 32.46, 32.15, 30.18, 29.37, 28.28, 27.70, 27.63, 26.11, 25.38, 23.90, 22.09, 18.89, 12.60; HRMS (ESI) calculated for $C_{33}H_{53}N_2O_7$ $(M + H)^+$, 589.3853; found, 589.3850.

Dipeptide-LCA **13**. ^{1}H NMR (500 MHz, CD_3OD): δ 4.77 (m, 2H), 4.25 (dd, J = 9.2, 4.0 Hz, 1H), 3.04 (dd, J = 18.1, 4.0 Hz, 1H), 2.85 (m, 3H), 2.31 (m, 1H), 2.21 (m, 1H), 2.03-1.85 (m, 8H), 1.70-1.41 (m, 12H), 1.34-1.05 (m, 13H), 0.95 (s, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.31, 171.73, 170.96, 169.40, 167.49, 75.58, 55.90, 55.62, 49.00, 48.82, 42.05, 41.49, 39.96, 39.60, 35.34, 34.87, 34.64, 34.40, 34.13, 33.85, 31.39, 30.47, 30.15, 27.38, 26.29, 25.64, 23.39, 21.90, 20.09, 16.89, 10.60; HRMS (ESI) calculated for $C_{32}H_{51}N_2O_9$ $(M + H)^+$, 607.3589; found, 607.3595.

Dipeptide-LCA **14**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.79-4.76 (m, 1H), 4.50-4.47 (m, 1H), 3.96 (t, J = 6.4 Hz, 1H), 3.64 (s, 1H), 2.57-2.51 (m, 2H), 2.44-2.42 (m, 2H), 2.18-2.03 (m, 4H), 1.81-1.66 (m, 10H), 1.54-1.48 (m, 3H), 1.38-1.20 (m, 10H), 1.07-1.02 (m, 5H), 0.97 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.75, 174.62, 174.27, 170.71, 168.41, 75.86, 70.09, 56.30, 55.96, 52.14, 42.43, 41.89, 40.32, 39.98, 35.74, 35.26, 34.56, 34.25, 31.82, 30.86, 30.54, 29.51, 28.60, 26.68, 26.29, 26.15, 26.05, 23.78, 22.29, 20.47, 17.28, 10.99; HRMS (ESI) calculated for $C_{34}H_{55}N_2O_9$ $(M + H)^+$, 635.3908; found, 635.3907.

Dipeptide-LCA **15**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.75-4.72 (m, 2H), 3.90 (t, J = 6.4 Hz, 1H), 2.80-2.77 (m, 2H), 2.53-2.47 (m, 2H), 2.25-2.22 (m, 1H), 2.10-2.07 (m, 3H), 1.93-1.80 (m, 5H), 1.81-1.66 (m, 4H), 1.42-1.36 (m, 7H), 1.25-1.21 (m, 5H), 1.07-1.02 (m, 6H), 0.89 (s, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.62 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.71, 174.31, 172.13, 169.84, 168.18, 76.09, 56.29, 56.25, 52.13, 49.14, 42.43, 41.89, 40.32, 39.98, 35.74, 35.26, 34.56, 34.25, 31.82, 30.86, 30.54, 29.51, 28.60, 26.68, 26.29, 26.15, 26.05, 23.78, 22.29, 20.47, 17.28, 10.99; HRMS (FAB) calculated for $C_{33}H_{53}N_2O_9$ $(M + H)^+$, 621.3751; found, 621.3755.

Dipeptide-LCA **16**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.75-4.73 (m, 1H), 4.46-4.45 (m, 1H), 2.97-2.95 (m, 1H), 2.83-2.80 (m, 1H), 2.43 (t, J = 7.3 Hz, 2H), 2.40-2.38 (m, 1H), 2.23-2.20 (m, 2H), 2.01-1.89 (m, 7H), 1.75-1.67 (m, 3H), 1.48-1.41 (m, 8H), 1.31-1.24 (m, 6H), 1.16-1.09 (m, 6H), 0.97 (s, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.88, 174.79, 171.97, 170.75, 168.29, 75.79, 56.29, 55.96, 52.21, 49.73, 42.44, 41.87, 40.35, 39.97, 35.73, 35.26, 35.09, 34.53, 34.24, 31.82, 30.86, 30.54, 29.51, 28.60, 26.68, 26.29, 26.15, 26.05, 23.78, 22.29, 20.47, 17.31, 11.02; HRMS (FAB) calculated for $C_{33}H_{53}N_2O_9$ $(M + H)^+$, 621.3731; found, 621.3729.

Dipeptide-LCA **17**. ^{1}H NMR (400 MHz, CD_3OD): δ 5.48 (s, 1H), 4.10-4.08 (m, 1H), 3.97-3.95 (m, 1H), 2.80-2.77 (m, 2H), 2.53-2.47 (m, 2H), 2.25-2.22 (m, 1H), 2.10-2.07 (m, 3H), 1.93-1.80 (m, 5H), 1.81-1.66 (m, 4H), 1.42-1.36 (m, 7H), 1.25-1.21 (m, 5H), 1.07-1.02 (m, 6H), 0.95-0.90 (m, 6H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 177.18, 173.32, 172.78, 170.86, 170.76, 76.35, 56.81, 56.47, 49.69, 42.90, 42.37, 40.81, 40.46, 36.19, 35.71, 35.01, 34.72, 31.74, 31.34, 31.31, 31.00, 28.22, 27.16, 26.54, 26.49, 24.24, 22.78, 22.69, 20.94, 17.75, 13.42, 11.45; HRMS (FAB) calculated for $C_{33}H_{53}N_2O_9$ $(M + H)^+$, 621.3751; found, 621.3767.

Tripeptide-LCA **18**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.74 (m, 1H), 4.00 (dd, J = 8.5, 4.6 Hz, 1H), 3.98 (m, 4H), 3.03 (dd, J = 17.9, 4.6 Hz, 1H), 2.87 (dd, J = 17.9, 8.4 Hz, 1H), 2.31 (m, 1H), 2.21(m, 1H), 2.04-1.53 (m, 10H), 1.50-1.05 (m, 18H), 0.96 (s, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.28, 172.99, 171.61, 170.90, 169.92, 77.10, 58.00, 57.61, 51.24, 44.06, 43.48, 43.31, 42.41, 41.98, 41.63, 37.34,

36.85, 36.18, 36.05, 35.85, 33.46, 32.46, 32.14, 29.36, 28.31, 27.74, 27.69, 25.39, 23.91, 22.09, 18.90, 12.61; HRMS (ESI) calculated for $C_{32}H_{52}N_3O_8$ $(M + H)^+$, 606.3754; found, 606.3745.

Tripeptide-LCA **19**. ^{1}H NMR (500 MHz, CD_3OD): δ 4.82 (dd, J = 8.1, 5.2 Hz, 1H), 4.74 (m, 1H), 4.20 (dd, J = 8.6, 4.5 Hz, 1H), 3.91 (dd, J = 23.4, 17.5 Hz, 2H), 3.06 (dd, J = 17.9, 4.4 Hz, 1H), 2.89 (m, 2H), 2.75 (dd, J = 17.0, 8.2 Hz, 1H), 2.31 (m, 1H), 2.21(m, 1H), 2.03-1.54 (m, 8H), 1.50-1.04 (m, 15H), 0.96 (s, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.30, 173.87, 173.26, 173.12, 170.71, 169.50, 77.06, 57.95, 57.58, 51.56, 51.21, 44.05, 43.47, 42.55, 41.96, 41.61, 37.34, 36.86, 36.78, 36.21, 36.17, 33.45, 32.46, 32.14, 29.37, 28.30, 27.74, 27.67, 25.39, 23.92, 22.09, 18.90, 12.61; HRMS (ESI) calculated for $C_{34}H_{54}N_3O_{10}$ $(M + H)^+$, 664.3809; found, 664.3799.

Tripeptide-LCA **20**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.80 (m, 1H), 4.72 (m, 2H), 4.20 (dd, J = 9.3, 4.3 Hz, 1H), 3.08-2.71 (m, 6H), 2.33 (m, 1H), 2.20 (m, 1H), 2.03-1.76 (m, 6H), 1.69-1.44 (m, 10H), 1.34-1.01 (m, 10H), 0.96 (m, 6H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.31, 174.11, 173.84, 173.36, 172.45, 171.67, 169.58, 77.46, 57.87, 57.58, 51.55, 51.21, 50.78, 44.04, 43.50, 41.91, 41.58, 37.34, 36.86, 36.82, 36.35, 36.16, 35.85, 33.34, 32.46, 32.15, 29.37, 28.29, 27.64, 25.39, 23.91, 22.09, 18.89, 12.60; HRMS (ESI) calculated for $C_{36}H_{56}N_3O_{12}$ $(M + H)^+$, 722.3864; found, 722.3856.

Tripeptide-LCA **21**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.84-4.79 (m, 1H), 4.45-4.42 (m, 2H), 3.95 (t, J = 6.4 Hz, 1H), 2.51-2.42 (m, 6H), 2.44-2.42 (m, 1H), 2.25-2.19 (m, 5H), 1.81-1.66 (m, 7H), 1.38-1.20 (m, 5H), 1.07-1.02 (m, 5H), 0.96 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.75, 175.05, 174.98, 171.86, 171.04, 168.38, 75.60, 56.28, 55.95, 52.77, 52.28, 52.06, 42.43, 41.89, 40.31, 39.99, 35.73, 35.26, 34.57, 34.25, 31.82, 30.86, 30.55, 29.70, 27.77, 26.68, 26.41, 26.14, 26.05, 26.00, 23.79, 22.30, 20.47, 17.28, 10.99; HRMS (FAB) calculated for $C_{39}H_{62}N_3O_{12}$ $(M + H)^+$, 764.4334; found, 764.4316.

Tertapeptide-LCA **22**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.72 (m, 1H), 4.46 (m, 1H), 4.31 (m, 1H), 3.91 (m, 4H), 2.87 (m, 4H), 2.31 (m, 1H), 2.21 (m, 1H), 2.03-1.52 (m, 10H), 1.44-1.04 (m, 17H), 0.94 (m, 6H), 0.69 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.33, 173.86, 172.88, 172.49, 170.95, 169.70, 169.48, 77.02, 57.97, 57.57, 53.56, 52.85, 44.05, 43.64, 43.48, 42.41, 41.95, 41.61, 40.19, 37.82, 37.35, 36.85, 36.18, 35.85, 33.44, 32.45, 32.14, 29.36, 28.31, 27.71, 25.40, 23.92, 22.08, 18.89, 12.60; HRMS (ESI) calculated for $C_{36}H_{53}N_4O_{10}$ $(M–H_2O–H)^-$, 701.3762; found, 701.3771.

Tertapeptide-LCA **23**. ^{1}H NMR (400 MHz, CD_3OD): δ 4.78-4.68 (m, 4H), 4.18 (dd, J = 8.4, 4.4 Hz, 1H), 3.03-2.74 (m, 8H), 2.31 (m, 1H), 2.21 (m, 1H), 2.03-1.78 (m, 6H), 1.65-1.42 (m, 10H), 1.34-1.00 (m, 10H), 0.96 (m, 6H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 178.35, 174.35, 174.29, 174.10, 173.76, 172.73, 172.35, 171.67,169.99,77.42, 57.85, 57.58, 52.11, 51.44, 50.91, 44.05, 43.53, 41.82, 41.56, 37.37, 37.00, 36.87, 36.68, 36.46, 36.20, 35.86, 33.30, 32.47, 32.15, 29.39, 28.32, 27.67, 27.59, 25.40, 23.91, 22.10, 18.90, 12.60; HRMS (ESI) calculated for $C_{40}H_{61}N_4O_{15}$ $(M + H)^+$, 837.4133; found, 837.4146.

ACKNOWLEDGEMENTS

We are grateful for financial support of this work provided by the Academia Sinica and the National Science Council (NSC 102-2325-B-001-025-). Instrumentation support was provided by the NMR and Mass Spectrometry facilities of the Institute of Chemistry at Academia Sinica, Taiwan.

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