



(51) International Patent Classification:

C07J 9/00 (2006.01) C07J 15/00 (2006.01)
A61P 35/00 (2006.01) C12N 9/10 (2006.01)
C07J 11/00 (2006.01)

(21) International Application Number:

PCT/US2016/049084

(22) International Filing Date:

26 August 2016 (26.08.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/210,975 27 August 2015 (27.08.2015) US

(71) Applicants: **ACADEMIA SINICA** [CN/CN]; No. 128, Academia Road, Section 2, Nankang, Taipei, 115 (TW).
LIANG, Chi-Ming [US/US]; 6627 River Road, Bethesda, Maryland 20817 (US).

(72) Inventors: **LI, Wen-Shan**; 9F., No.100, Ln. 280, Sec. 6, Minguo E. Rd., Neihu Dist., Taipei City, 114 (TW).
HUNG, Wen-Chun; 13F. -2, No. 5, Aly. 3, Ln. 199, Jianguo 1st Rd., Lingya Dist., Kaohsiung City, 802 (TW).
SHEN, Chia-Ning; No.183, Liudu 1st St., Qidu Dist., Keelung City, 206 (TW).

(74) Agent: **MUNCY, GEISSLER, OLDS & LOWE, P.C.**;
4000 Legato Rd., Suite 310, Fairfax, Virginia 22033 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

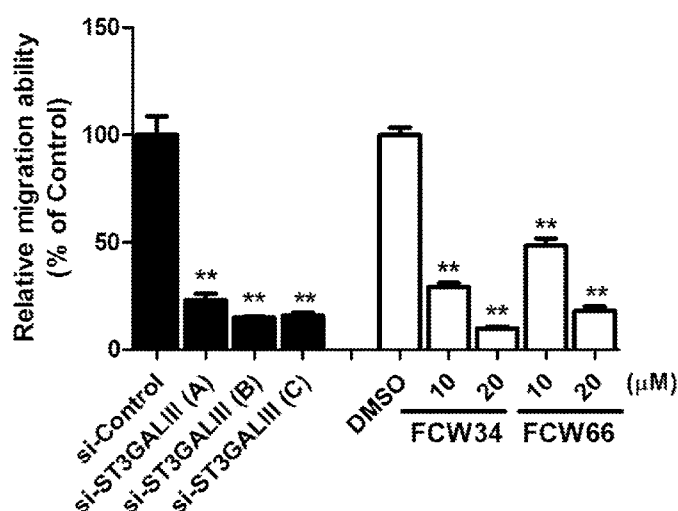
Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: SIALYLTRANSFERASE INHIBITORS AND USES THEREOF

FIG 1



(57) Abstract: Disclosed herein are novel sialyltransferase inhibitors, and compositions and methods for treating diseases and/or conditions associated with the activation of sialyltransferase, such as a cancer, an immune disease or an inflammatory disease.



-
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))* — *with sequence listing part of description (Rule 5.2(a))*

SIALYLTRANSFERASE INHIBITORS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/210,975, filed August 27, 2015, which is incorporated herein by reference in its entirety.

BACKGROUNDS OF THE INVENTION

[0002] 1. FIELD OF THE INVENTION

[0003] The present disclosure relates in general, to the field of sialyltransferase inhibitors. Compounds that inhibit sialyltransferase are provided. Also provided are methods for inhibiting sialyltransferase, and methods for treating diseases and/or conditions that involve sialylation.

[0004] 2. DESCRIPTION OF RELATED ART

[0005] Sialic acid containing epitopes are known to involve in various biological processes, such as adhesion and inflammation. There is also a correlation between sialyl content of glycoconjugates and the malignancy of tumor cells. Recently, sialylation and lymphocyte activation and immune function have also been reported.

[0006] Sialylglycoside are synthesized by sialyltransferase, which catalyzes the transfer of a sialic acid from a donor substrate, i.e., cytidine monophosphate (CMP)-sialic acid, to the subterminal sugars of glycoproteins or glycolipids via α 2,6-bond to N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), an α 2,3-bond to galactose (Gal) or through an α 2,8-bond to another sialic acid, forming polysialic acid.

[0007] Due to the widely impact of sialyltransferase on various biological process (e.g., inflammation and cancer progression), it is highly desirable to develop efficient inhibitors of sialyltransferase. In the past, most efforts in developing sialyltransferase inhibitors focused on CMP-based donor and

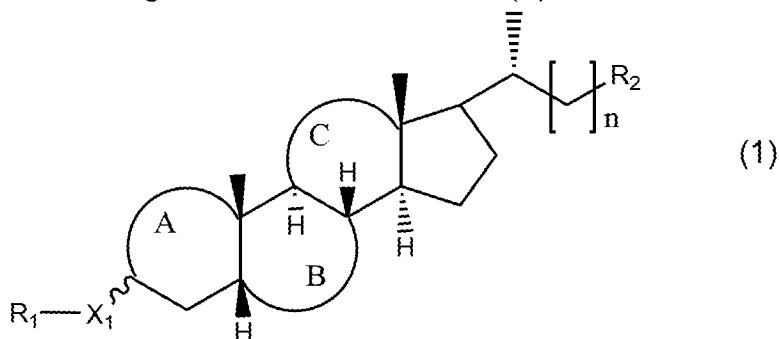
acceptor substrate analogues, however, few of them are cell permeable, and this lack of membrane permeability hampered their biological and clinical applications.

[0008] In view of the foregoing, there exists in this art a need of novel sialyltransferase inhibitors, which not only can suppress the activity of sialyltransferase, but also are membrane permeable; thus may be used as lead compounds for the development of medicaments for treating diseases and/or conditions that involve sialylation.

SUMMARY

[0009] The present disclosure is based, at least in part, unexpected discovery that certain lithocholic acid derivatives are effective in suppressing the activity of sialyltransferase, accordingly, these lithocholic acid derivatives are potential candidates that may act as lead compounds for the development of medicaments suitable for treating a disease and/or a condition associated with the activation of sialyltransferase, such as a cancer, an immune disease or an inflammatory disease.

[0010] Accordingly, it is the first aspect of the present disclosure to provide a compound having the structure of formula (1)



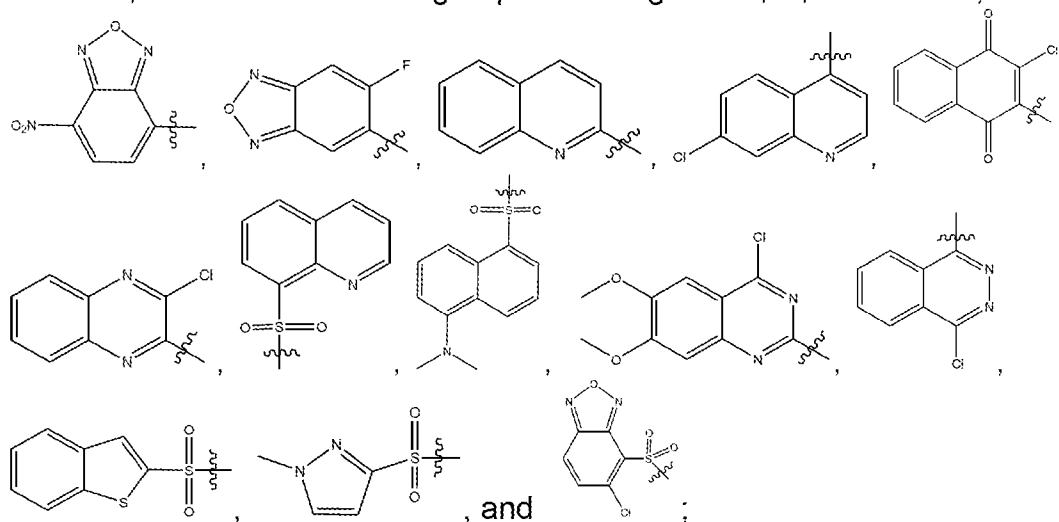
and pharmaceutically acceptable salts and solvates thereof, wherein,

n is an integral from 1 to 4;

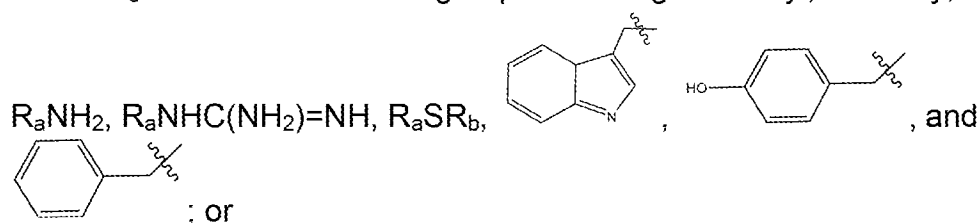
A, B, and C are independently a 6- or 7-membered saturated or unsaturated carbon cyclic ring, a homolactone or a homolactam, in which the saturated or unsaturated carbon cyclic ring, the homolactone or the homolactam is optionally substituted with one or more fluoro or hydroxy;

X_1 is O or N; or X_1 is fluoro, provided that R_1 is null;

R_1 is selected from the group consisting of null, H,



R_3 is selected from the group consisting of methyl, methoxy, $R_a\text{COOH}$,



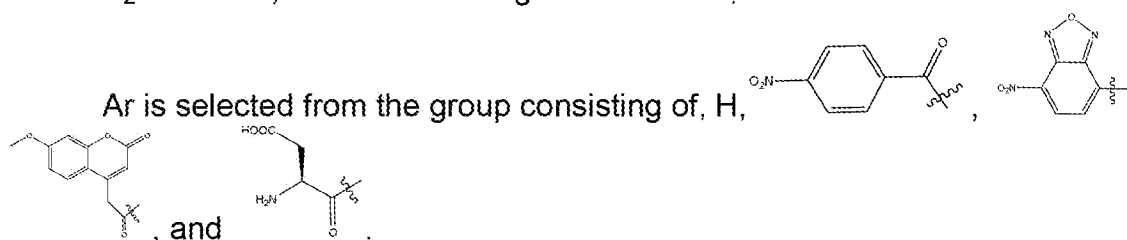
10 R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring;

R_a and R_b are independently H or C_{1-6} alkyl;

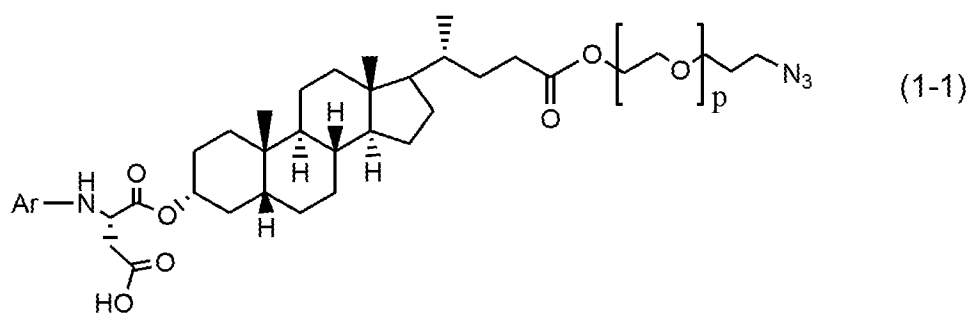
R_2 is COOH , $\text{CH}(\text{COOH})\text{COOH}$, or $\text{COX}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{N}_3$,

X_2 is O or N, and m is an integral from 1 to 4; and

15 Ar is selected from the group consisting of, H,



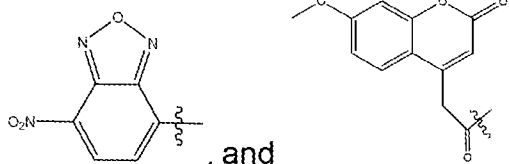
[0011] In some embodiments, particular compounds are of formula (1-1)



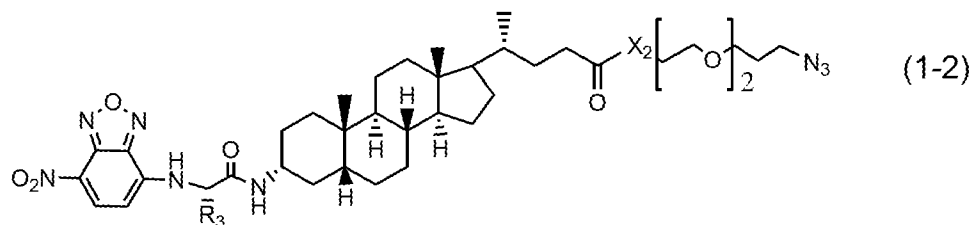
wherein,

p is an integral from 1 to 4; and

Ar is selected from the group consisting of,

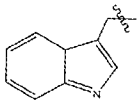
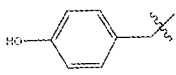
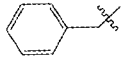


[0012] In other embodiments, particular compounds are of formula (1-2),



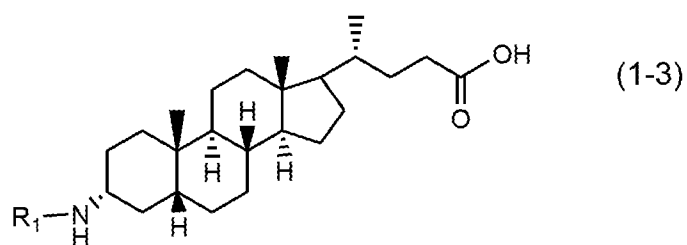
wherein, X_2 is O or N;

R_3 is selected from the group consisting of CH_3 , R_aCOOH , R_aNH_2 ,

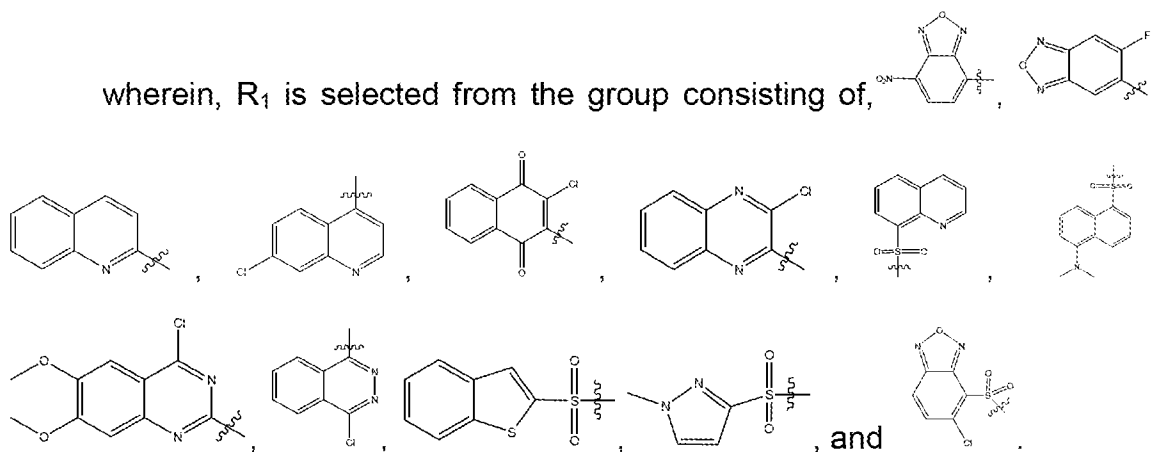
10 $R_aNHC(NH_2)=NH$, R_aSR_b , , , and ; or R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring; and

R_a and R_b are independently H or C_{1-6} alkyl.

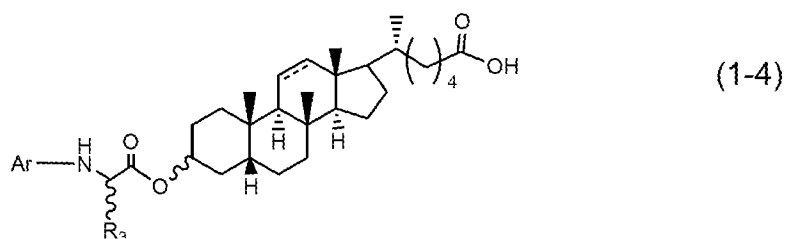
[0013] In some embodiments, particular compounds are of formula (1-3),



wherein, R_1 is selected from the group consisting of,

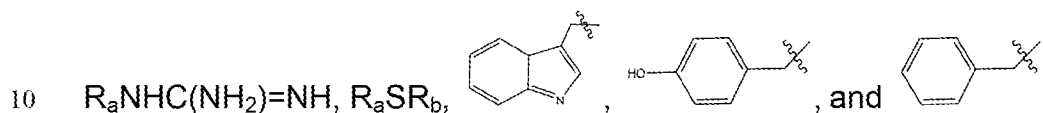


[0014] In further embodiments, particular compounds are of formula (1-4),

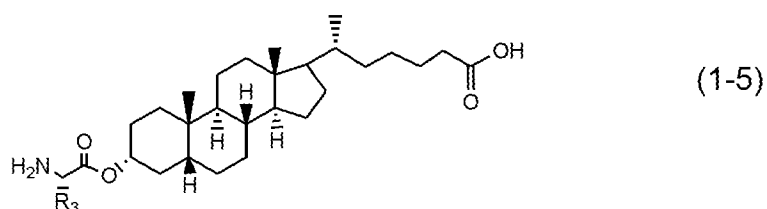


wherein, ----- is a single or a double bond;

R_3 is selected from the group consisting of, CH_2OH , R_aCOOH , R_aNH_2 ,

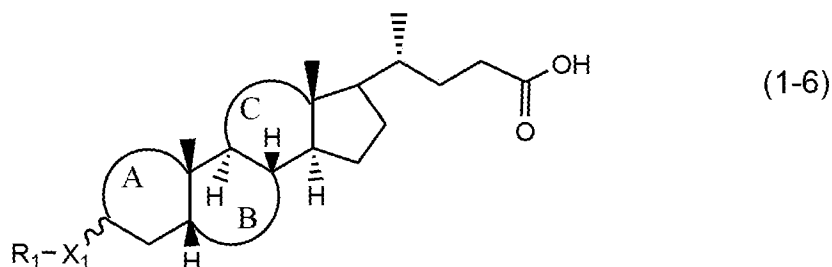


[0015] In some embodiments, particular compounds are of formula (1-5),



wherein, R_3 is CH_2COOH , or $\text{CH}_2\text{CH}_2\text{COOH}$.

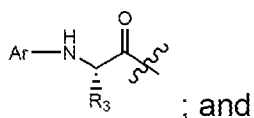
[0016] In other embodiments, particular compounds are of formula (1-6),



5

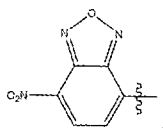
wherein, A, B, and C are independently a 6-membered saturated carbon cyclic ring substituted with at least one fluoro;

X_1 is H, O or fluoro, and in the case when X_1 is fluoro, then R_1 is null, H, or

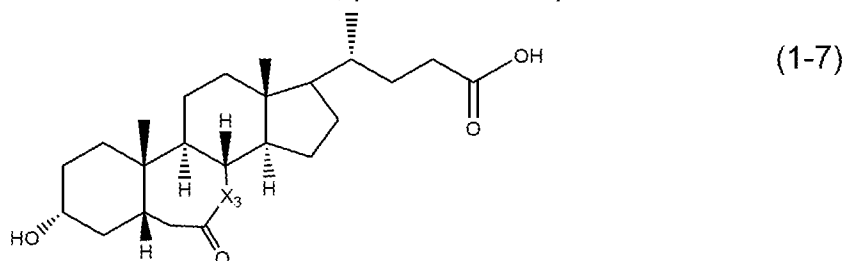


10

R_3 is CH_2COOH or

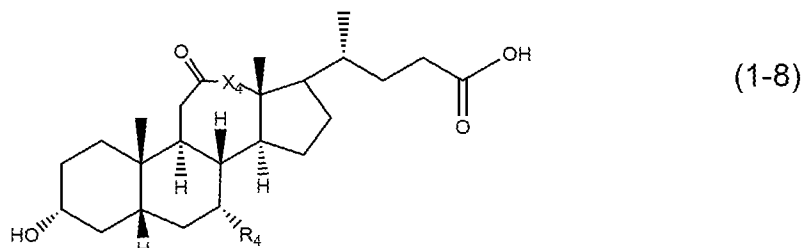


[0017] In still further embodiments, particular compounds are of formula (1-7),



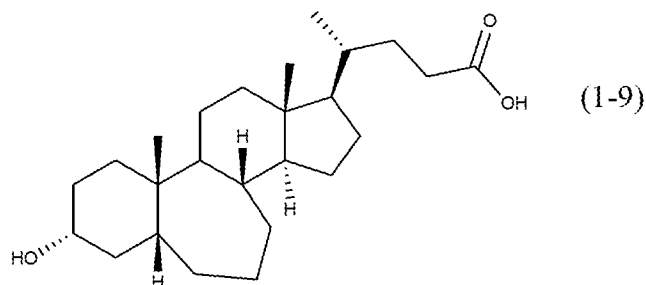
wherein, X_3 is O or N.

[0018] In some embodiments, particular compounds are of formula (1-8)



5 Wherein, X₄ is O or N; and R₄ is H or OH.

[0019] In still further embodiments, particular compound is of formula (1-9),



10 [0020] Accordingly, it is the second aspect of the present disclosure to provide a composition that suppresses the activity of sialyltransferase. The composition is therefore useful for treating a disease and/or a condition associated with the activation of sialyltransferase. The composition comprises an effective amount of the compound of formula (I), a salt or a solvate thereof; and a pharmaceutically acceptable excipient.

15 [0021] The compound of formula (I) is present in the composition about 0.1% to 99% by weight, based on the total amount of the composition. In certain embodiments, the compound of formula (I) is present in the composition at least about 1% by weight, based on the total amount of the composition. In other
20 embodiments, the compound of formula (I) is present in the composition at least about 5% by weight, based on the total amount of the composition. In further embodiments, the compound of formula (I) is present in the composition at least about 10% by weight, based on the total amount of the composition. In still further embodiments, the compound of formula (I) is present in the composition at
25 least about 25% by weight, based on the total amount of the composition.

[0022] According to some preferred embodiments, the composition further comprises a chemotherapeutic agent, an anti-inflammatory agent, or an immunosuppressive agent.

[0023] Accordingly, it is the third aspect of the present disclosure to provide a method of treating a disease and/or a condition associated with the activation of sialyltransferase. The method includes the step of, administering to the subject the composition of the present disclosure, so as to alleviate or ameliorate the symptoms related to the disease and/or condition.

[0024] According to some embodiments, the disease and/or condition that is associated with the activation of sialyltransferase may be a cancer, an immune disease or an inflammatory disease.

[0025] The details of one or more embodiments of the invention are set forth in the accompanying description below. Other features and advantages of the invention will be apparent from the detail descriptions, and from claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The present description will be better understood from the following detailed description read in light of the accompanying drawings, where:

[0027] **FIG 1** are bar graphs illustrating the effects of FCW34 and FCW66 on migrating MDA-MB-231 cells in accordance with one embodiment of the present disclosure;

[0028] **FIG 2A** is a line graph illustrating the quantified results of FCW34 suppressing the migration of MDA-MB-231 cells injected intraperitoneally in the test animal in accordance with one embodiment of the present disclosure;

[0029] **FIG 2B** is a line graph illustrating the effect of FCW34 on the tumor volume in the test animals of **FIG 2A**;

[0030] **FIG 3** is a bar graph illustrating the effects of FCW34 on sialyltransferase detected by SNA lectin staining in PANC-1 cells grown in glucose or fructose medium in accordance with one embodiment of the present disclosure;

[0031] **FIG 4** is a bar graph illustrating the effects of FCW34 in suppressing the migrating PANC-1 cells grown in glucose or fructose medium in accordance with one embodiment of the present disclosure;

[0032] **FIG 5** illustrates the effect of FCW34 on test animals injected with PANC-1 cells grown in glucose or fructose-containing medium in accordance with one embodiment of the present disclosure, in which (A) are representative bioluminescence IVIS images of the test animals, and (B) are histological staining of liver section taken from tumor lesions resulted from PANC-1 cells grown in glucose or fructose-containing medium in accordance with one embodiment of the present disclosure;

[0033] **FIG 6** illustrates the cytotoxicity of FCW551 on PANC-1 and LN cells in accordance with one embodiment of the present disclosure;

[0034] **FIG7** are bar graphs illustrating the effects of FCW551 on (A) the invasion of PANC-1 and LN cells; and (B) the migration of PANC-1 and LN cells in accordance with one embodiment of the present disclosure;

[0035] **FIG 8A** is a line graph illustrating the effect of FCW393 on the body weight of the test animals implanted with primary melanoma cells in accordance with one embodiment of the present disclosure;

[0036] **FIG 8B** is a line graph illustrating the effect of FCW393 on the the tumor volume in the test animals of **FIG 8A**;

[0037] **FIG 9** are bar graphs illustrating the effect of FCW393 on the weights of (A) lymph noew, (B) thymus, and (C) spleen of the the test animals of **FIG 8A**;

[0038] **FIG 10** are bar graphs illustrating the effect of FCW393 on the counts or levels of (A) white blood cells (WBC), (B) red blood cells (RBC), (C) platelets, (D) hemoglobin (HGB), (E) hematocrit (HCT), (F) mean corpuscular volume (MCV), (G) mean corpuscular hemoglobin (MCH), and (H) mean corpuscular hemoglobin concentration (MCHC) of the the test animals of **FIG 8A**;

[0039] **FIG 11** are bar graphs illustrating the effect of FCW393 on the levels of (A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT), (C)

albumin, (D) triglyceride, (E) blood urea nitrogen (BUN), (F) creatinine, and (G) uric acid of the the test animals of **FIG 8A**;

[0040] **FIG 12** are bar graphs illustrating the effect of FCW393 in suppressing the growth of metastatic melanoma based on quantified results of bioluminescence IVIS images of test animals bearing metastatic melanoma taken at (A) day 7, and (B) day 14 in accordance with one embodiment of the present disclosure, panel (C) is a bar graph depicting the effect of FCW393 on luciferase activity;

[0041] **FIG 13** are bar graphs illustrating the effect of FCW393 on the weights of (A) lymph node, (B) thymus, and (C) spleen of the the test animals of **FIG 12**;

[0042] **FIG 14** are bar graphs illustrating the effect of FCW393 on the counts or levels of (A) white blood cells (WBC), (B) red blood cells (RBC), (C) platelets, (D) hemoglobin (HGB), (E) hematocrit (HCT), (F) mean corpuscular volume (MCV), (G) mean corpuscular hemoglobin (MCH), and (H) mean corpuscular hemoglobin concentration (MCHC) of the the test animals of **FIG 12**; and

[0043] **FIG 15** are bar graphs illustrating the effect of FCW393 on the levels of (A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT), (C) albumin, (D) triglyceride, (E) blood urea nitrogen (BUN), (F) creatinine, and (G) uric acid of the the test animals of **FIG 12**.

DESCRIPTION

[0044] The detailed description provided below in connection with the appended drawings is intended as a description of the present examples and is not intended to represent the only forms in which the present example may be constructed or utilized. The description sets forth the functions of the example and the sequence of steps for constructing and operating the example. However, the same or equivalent functions and sequences may be accomplished by different examples.

[0045] 1. DEFINITIONS

[0046] For convenience, certain terms employed in the context of the present disclosure are collected here. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of the ordinary skill in the art to which this invention belongs.

5 [0047] Unless otherwise indicated, the term "alkyl" means a straight chain, branched and/or cyclic ("cycloalkyl") hydrocarbon having from 1 to 20 (e.g., 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 1) carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, 2-isopropyl-3-methyl butyl, pentyl, pentan-2-yl, hexyl, isohexyl, heptyl, 10 heptan-2-yl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl and dodecyl. Cycloalkyl moieties may be monocyclic or multicyclic, and examples include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. Unless otherwise specified, each instance of an alkyl group is independently optionally substituted, *i.e.*, unsubstituted (an "unsubstituted alkyl") or substituted (a 15 "substituted alkyl") with one or more substituents. In certain embodiments, the alkyl group is unsubstituted C₁₋₁₀ alkyl; preferably, unsubstituted C₁₋₆ alkyl. In one preferred example, the alkyl group is n-hexyl.

[0048] Unless otherwise indicated, the term "substituted," when used to describe a chemical structure or moiety, refers to a derivative of that structure or 20 moiety wherein one or more of its hydrogen atoms is substituted with one or more of: alkoxy, alkyl, aryl, halo, haloalkyl, or hydroxyl. Unless otherwise indicated, one or more adjectives immediately preceding a series of nouns is to be construed as applying to each of the nouns. For example, the phrase "optionally substituted alky, aryl, or heteroaryl" has the same meaning as "optionally 25 substituted alky, optionally substituted aryl, or optionally substituted heteroaryl."

[0049] It should also be noted that if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or the portion of the structure is to be interpreted as encompassing all stereoisomers of it. Similarly, names of compounds having one or more chiral

centers that do not specify the stereochemistry of those centers encompass pure stereoisomers and mixtures thereof. Moreover, any atom shown in a drawing with unsatisfied valences is assumed to be attached to enough hydrogen atoms to satisfy the valences. In addition, chemical bonds depicted with one solid line
5 parallel to one dashed line encompass both single and double (e.g., aromatic) bonds, if valences permit.

[0050] The term "subject" or "patient" refers to an animal including the human species that is treatable with the method of the present invention. The term "subject" or "patient" intended to refer to both the male and female gender unless
10 one gender is specifically indicated. Accordingly, the term "subject" or "patient" comprises any mammal which may benefit from the treatment method of the present disclosure.

[0051] The term "administered", "administering" or "administration" are used interchangeably herein to refer a mode of delivery, including, without limitation,
15 intravenously, intramuscularly, intraperitoneally, intraarterially, subcutaneously, or transdermally administering an agent (e.g., a compound or a composition) of the present invention.

[0052] The term "an effective amount" as used herein refers to an amount effective, at dosages, and for periods of time necessary, to achieve the desired
20 result with respect to the treatment of a disease. For example, in the treatment of an infection, an agent (i.e., a compound or a composition) which decrease, prevents, delays or suppresses or arrests any symptoms of the infection would be effective. An effective amount of an agent is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the
25 onset of the disease or condition is delayed, hindered or prevented, or the disease or condition symptoms are ameliorated. The effective amount may be divided into one, two or more doses in a suitable form to be administered at one, two or more times throughout a designated time period.

[0053] The term "treatment" as used herein are intended to mean obtaining a desired pharmacological and/or physiologic effect, e.g., inhibiting the growth of gram-positive bacteria. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein includes preventative (e.g., prophylactic), curative or palliative treatment of a disease in a mammal, particularly human; and includes: (1) preventative (e.g., prophylactic), curative or palliative treatment of a disease or condition from occurring in an individual who may be pre-disposed to the disease but has not yet been diagnosed as having it; (2) inhibiting a disease (e.g., by arresting its development); or (3) relieving a disease (e.g., reducing symptoms associated with the disease). According to specific embodiments of the present disclosure, an effective amount of a the present compound is administered to a subject suffering from a disease and/or disorder associated with the activation of sialyltransferase, as compared with that of the un-treated subject, and thereby alleviate or ameliorate one or more symptoms associated with the disease, the severity of one or more symptoms associated with the disease and/or the progression of the disease. In preferred embodiments, an effective amount of the compound of the present disclosure is administered to a subject suffering from a disease associated with the activation of sialyltransferase, so as to alleviate or ameliorate one or more symptoms associated with the disease, and thereby achieving the purpose of treating the disease.

[0054] The singular forms "a", "and", and "the" are used herein to include plural referents unless the context clearly dictates otherwise.

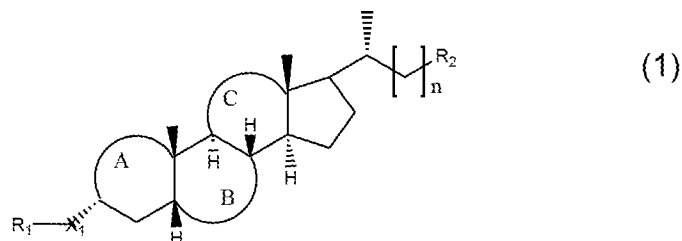
[0055] 2. The Present Sialyltransferase Inhibitors

[0056] The present disclosure is based, at least in part, unexpected discovery that some lithocholic acid derivatives, particularly the compound of formula (I), is capable of preventing the sialyltransferas from being activated, and thus the lithocholic acid derivatives of the present disclosure may be used as a lead

compound for the development of a medicament for treating diseases and/or conditions associated with the activation of sialyltransferas, such as cancer, immune disease or inflammatory diseases.

[0057] The lithocholic acid derivatives of the present disclosure are those of

5 formula (I),



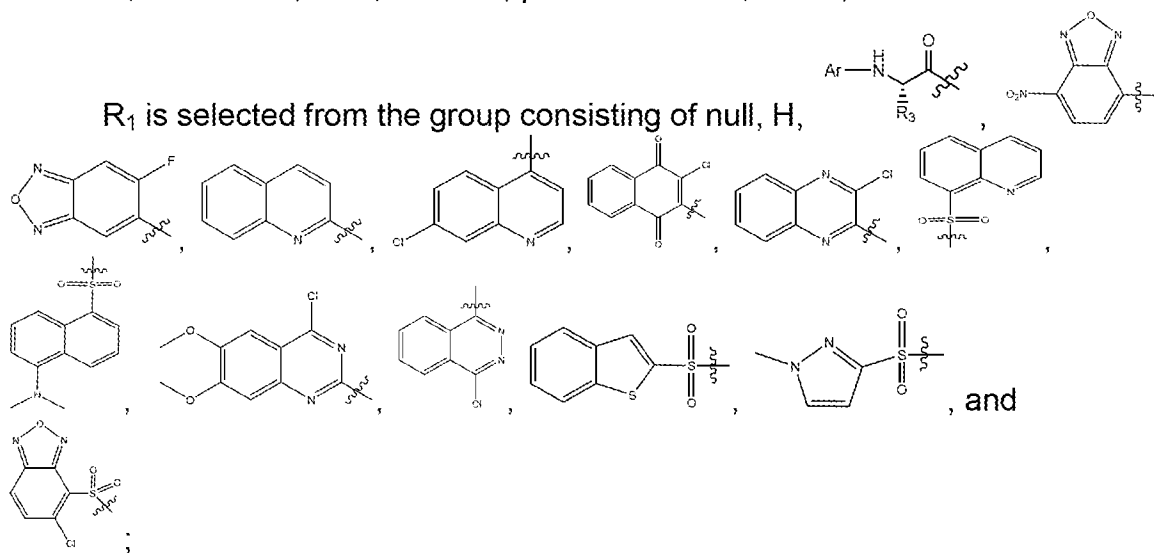
and pharmaceutically acceptable salts and solvates thereof, wherein,

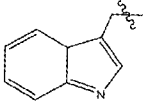
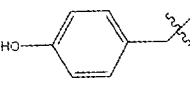
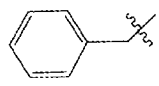
n is an integral from 1 to 4:

A, B, and C are independently a 6- or 7-membered saturated or unsaturated carbon cyclic ring, a homolactone or a homolactam, in which the carbon cyclic ring, the homolactone or the homolactam is optionally substituted with one or more fluoro or hydroxy;

X₁ is O or NH; or X₁ is fluoro, provided that R₁ is null;

R_1 is selected from the group consisting of null, H,



R_3 is selected from the group consisting of CH_3 , CH_2OH , R_aCOOH , R_aNH_2 ,
 $R_aNHC(NH_2)=NH$, R_aSR_b , , , and ; or

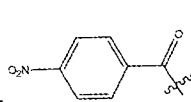
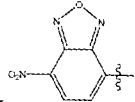
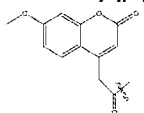
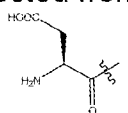
R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring;

5 R_a and R_b are independently H or C_{1-6} alkyl;

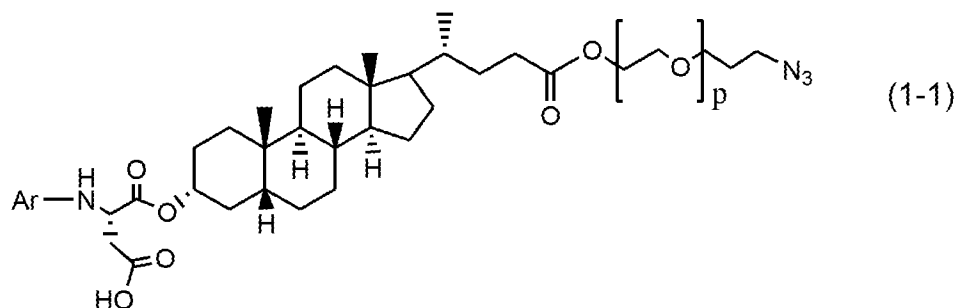
R_2 is $COOH$, $CH(COOH)COOH$, or $COX_2(CH_2CH_2O)_mCH_2CH_2N_3$,

X_2 is O or NH,

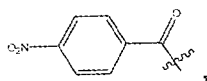
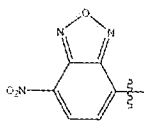
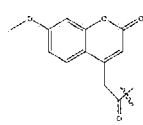
m is an integral from 1 to 4; and

10 Ar is selected from the group consisting of, H, , , , and .

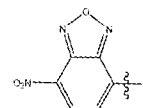
[0058] In some embodiments, particular compounds are of formula (1-1),



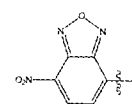
wherein p is an integral from 1 to 4; and Ar is selected from the group consisting of,

15 , , and .

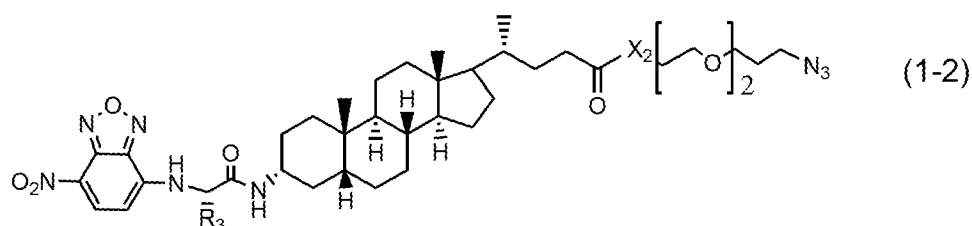
[0059] In one embodiment, in formula (1-1), p is 2, and Ar is



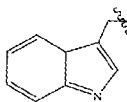
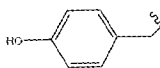
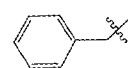
[0060] In another embodiment, in formula (1-1), p is 3, and Ar is



[0061] In some embodiments, particular compounds are of formula (1-2),

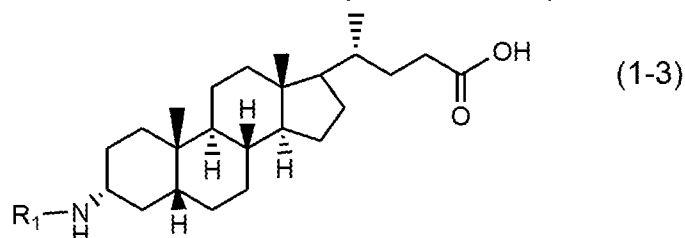


wherein, X_2 is O or N; R_3 is selected from the group consisting of CH_3 , R_aCOOH ,

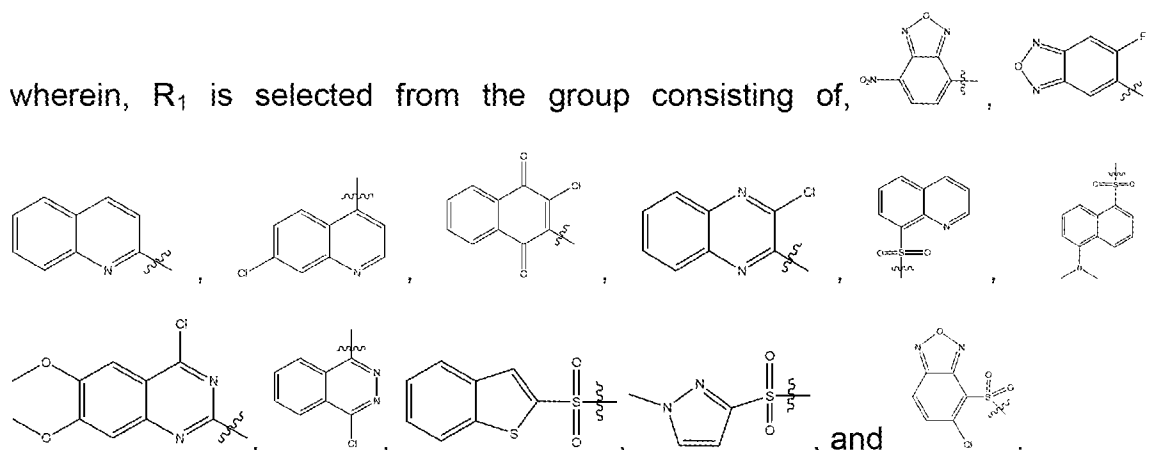
5 R_aNH_2 , $R_aNHC(NH_2)=NH$, R_aSR_b , , , and ; or R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring; and R_a and R_b are independently H or C_{1-6} alkyl.

[0062] According to one preferred embodiment, in formula (1-2), X_2 is N, R_3 is $-CH_2COOH$.

10 [0063] In some embodiments, particular compounds are of formula (1-3),

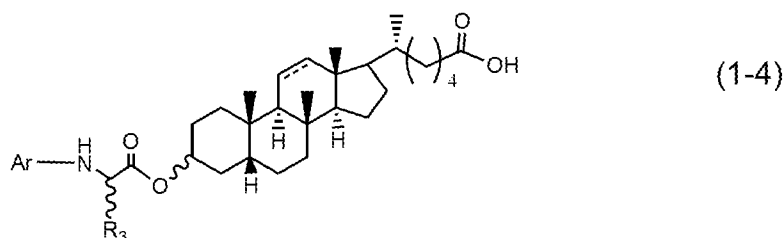


wherein, R_1 is selected from the group consisting of,

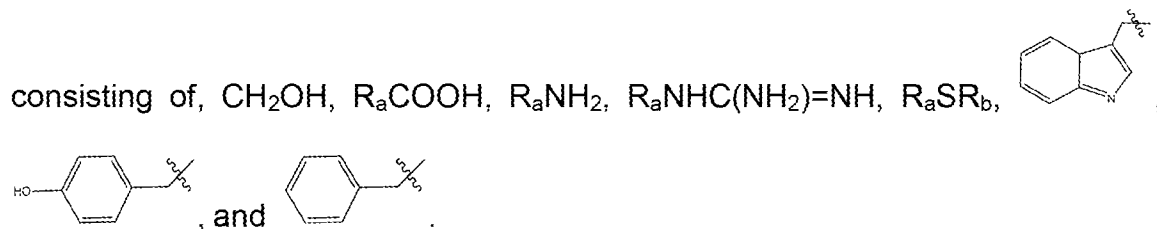


[0064] In further embodiments, particular compounds are of formula (1-4),

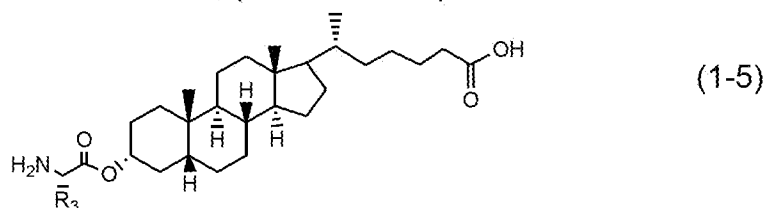
5



wherein, ----- is a single or a double bond; and R_3 is selected from the group

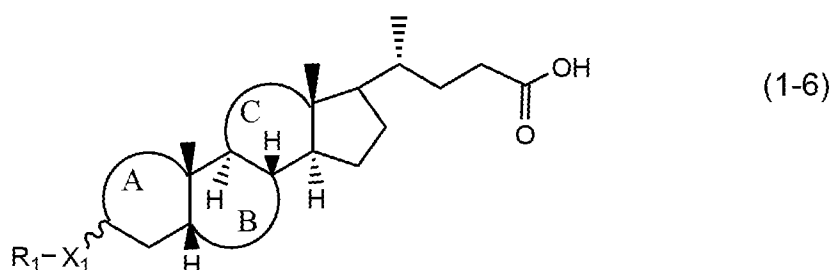


10 [0065] In some embodiments, particular compounds are of formula (1-5),

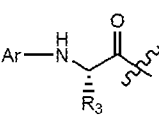


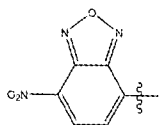
wherein, R_3 is CH_2COOH , or $\text{CH}_2\text{CH}_2\text{COOH}$.

[0066] In other embodiments, particular compounds are of formula (1-6),

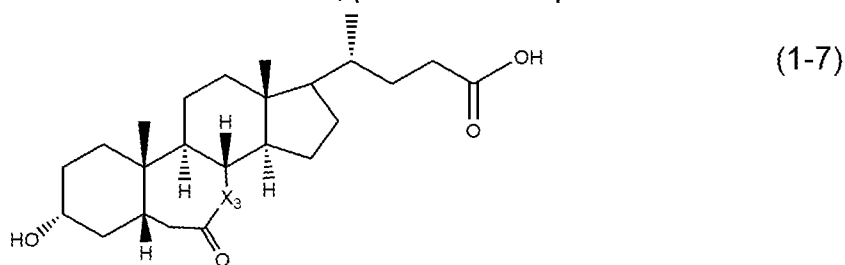


wherein, A, B, and C are independently a 6-membered saturated carbon cyclic ring substituted with at least one fluoro; X_1 is H, O or fluoro, and in the case when

5 X_1 is fluoro, then R_1 is null, H, or ; and R_3 is CH_2COOH or

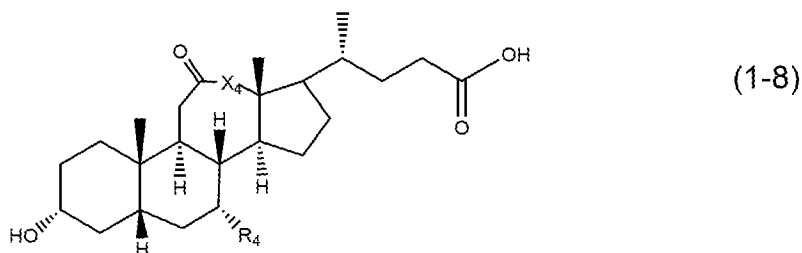


[0067] In still further embodiments, particular compounds are of formula (1-7),



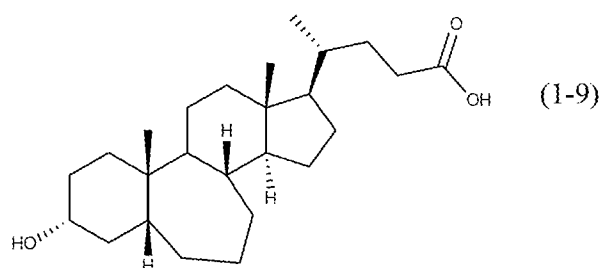
wherein, X_3 is O or N.

10 [0068] In some embodiments, particular compounds are of formula (1-8)



15 Wherein, X_4 is O or N; and R_4 is H or OH.

[0069] In still further embodiments, particular compound is of formula (1-9),

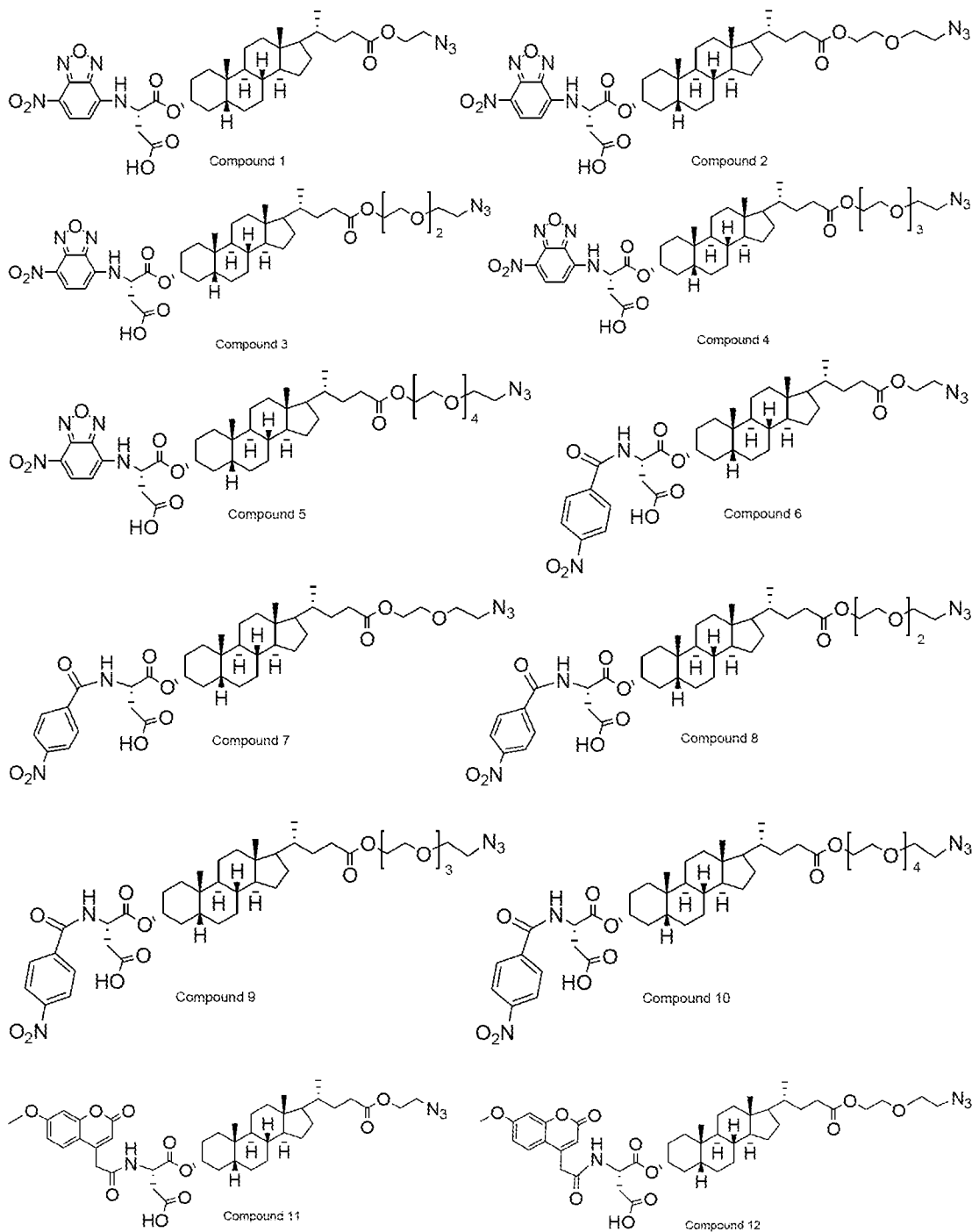


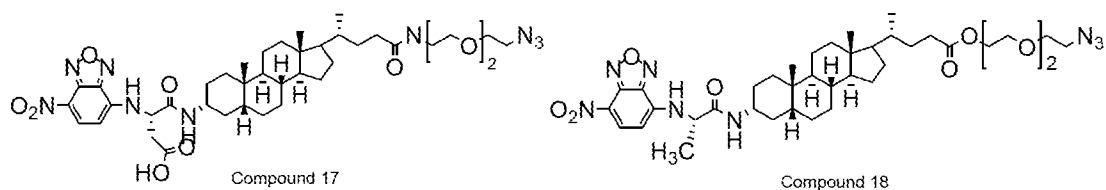
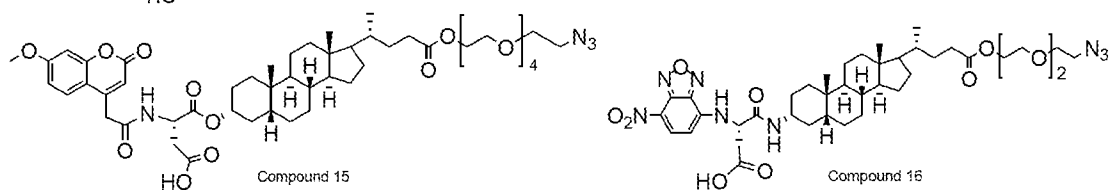
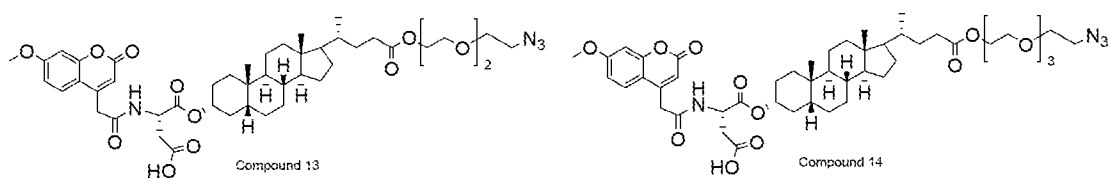
[0070] Compounds of the invention may contain one or more stereocenters, and can exist as racemic mixtures of enantiomers or mixtures of diastereomers. This invention therefore encompasses stereomerically pure forms of such compounds, such as the enantiomers, the diastereomers, as well as a racemic mixture thereof, and/or a combination thereof. The enantiomers of the compound of formula (I-1) to (1-9) may be prepared by chiral synthesis or enantioselective synthesis, in which a specific chiral compound is used as the starting material for the synthesis of a desired stereocompound. Alternatively, the stereoisomer of formula (I-1) to (1-9) may be obtained by routine isolating techniques, which include, and are not limited to, crystallization, chromatography, and the use of resolving agents. For example, each enantiomers may be isolated from the racemic mixture by use of HPLC. Alternatively, one enantiomer is separated from the other enantiomer by allowing its racemic mixture to react with a resolving agent, which allows one enantiomer to become solvable in the resolving agent while the other enantiomer remains precipitated. The present invention also encompasses the structural isoforms of the compound of formula (I-1) to (1-9), such as those in *cis*- and/or *trans*-conformations, either with or without the presence of double bond(s) in its structure. Accordingly, this invention further encompasses configurational isomers of compounds disclosed herein, either in admixture or in pure or substantially pure form, such as *cis* (Z) and *trans* (E) isomers.

[0071] Preferred compounds of the present invention are sialyltransferase inhibitors. Certain compounds of the present disclosure selectively inhibit

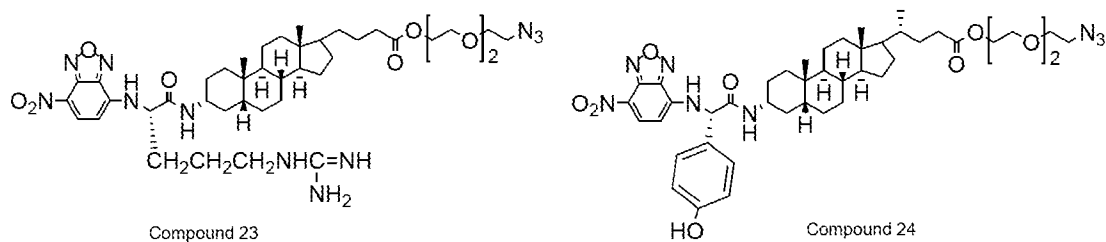
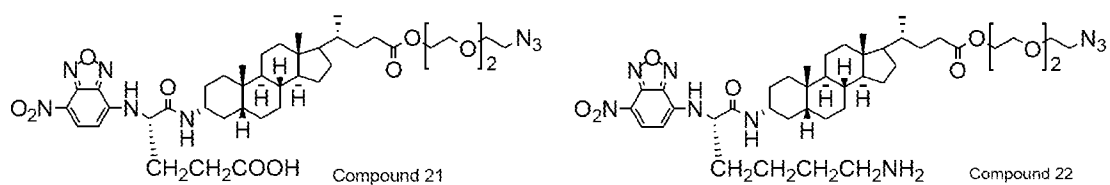
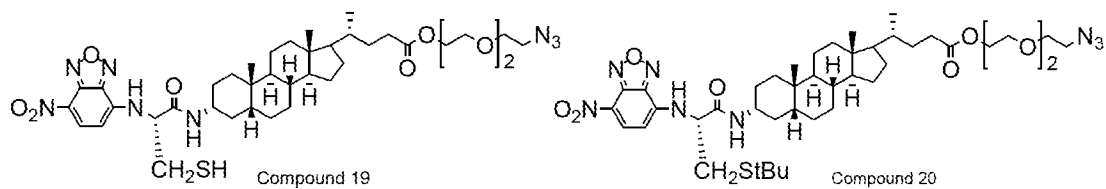
α 2,3(N)-sialyltransferase ST3GALIII and α 2,6(N)-sialyltransferases ST6GALI, but not α 2,3(O)-sialyltransferase ST3GALI.

[0072] Shown below are exemplary compounds, compounds 1-94, of this invention:

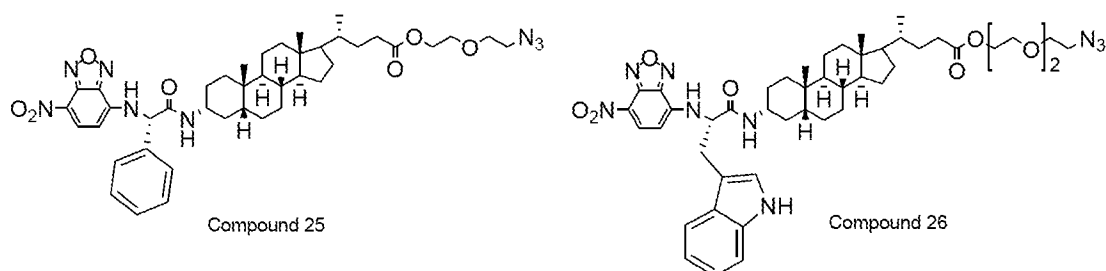


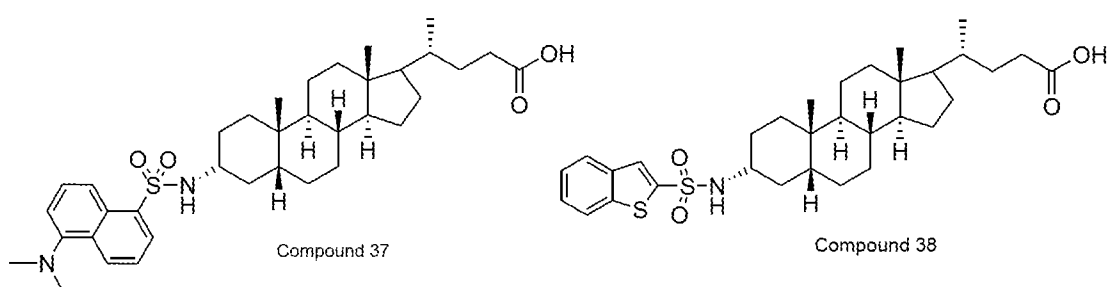
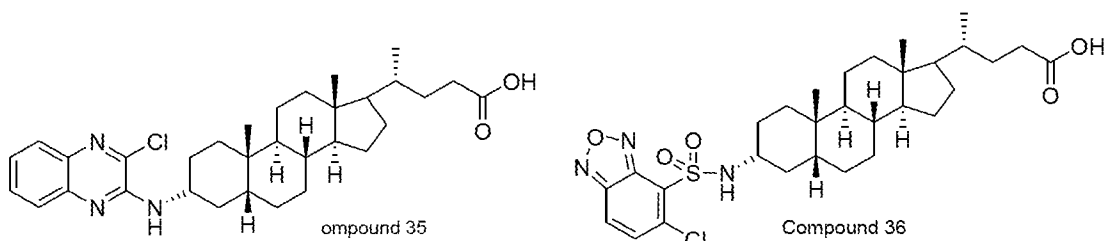
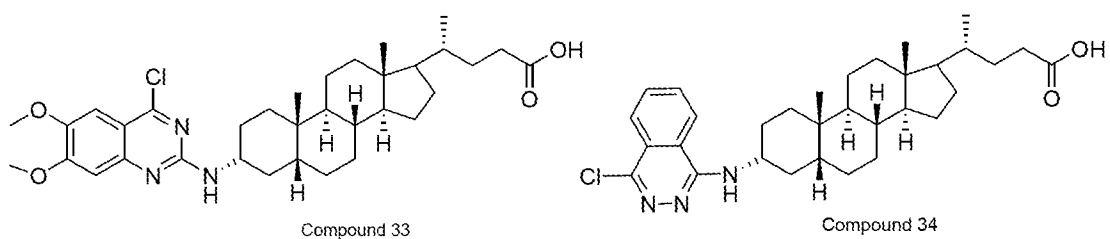
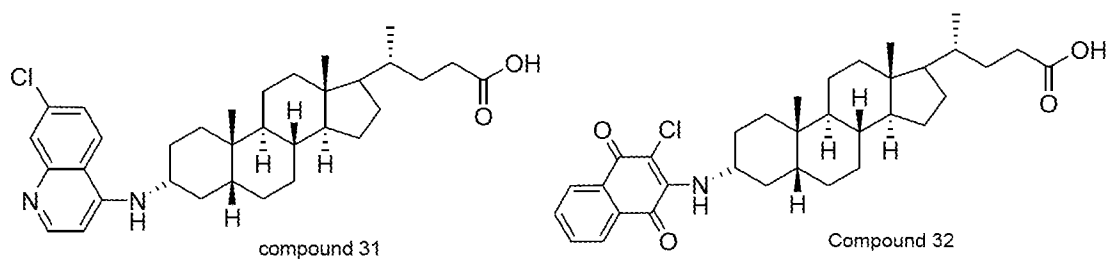
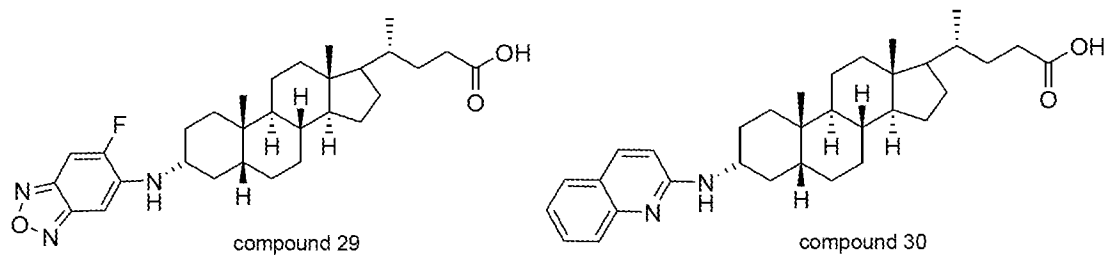
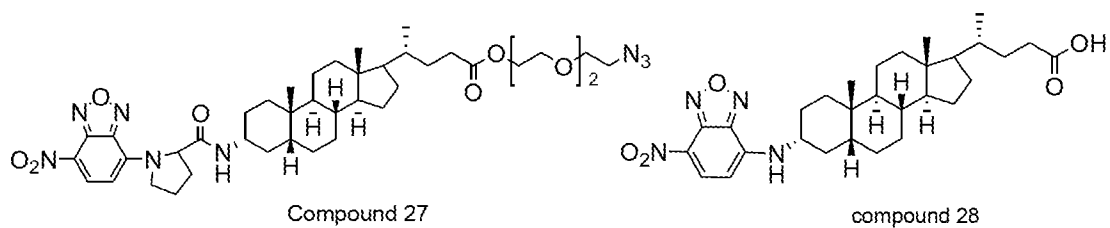


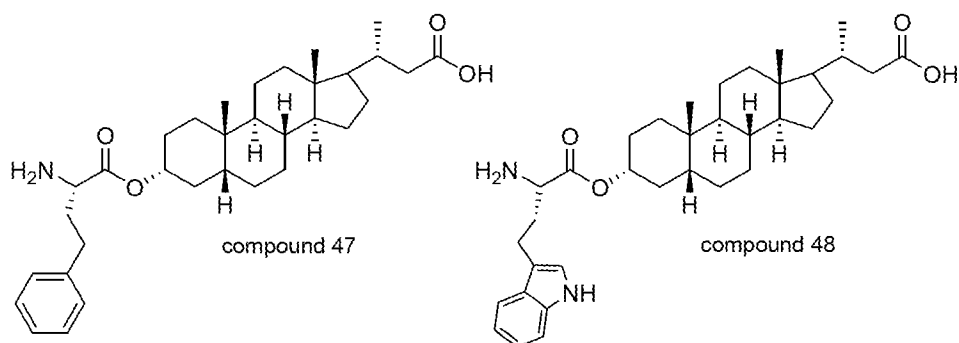
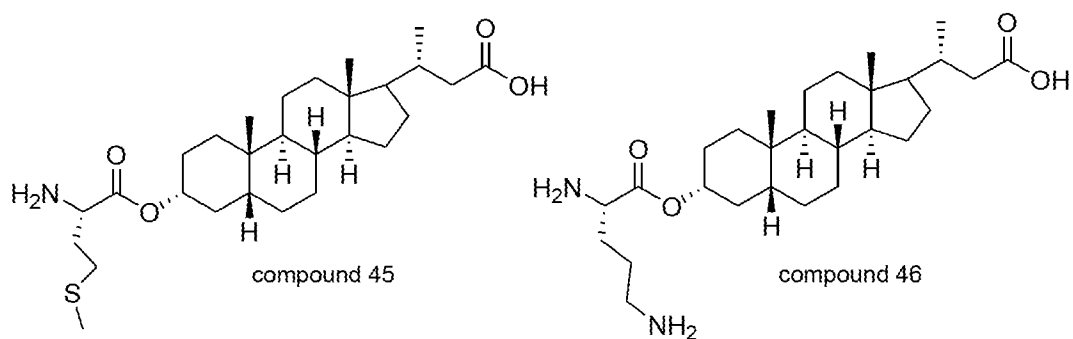
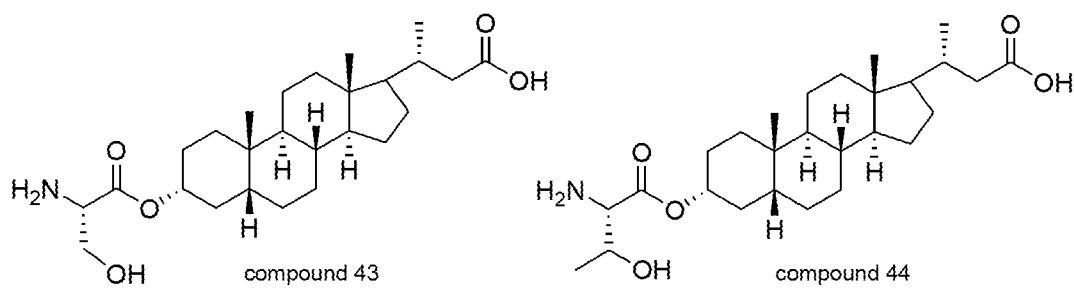
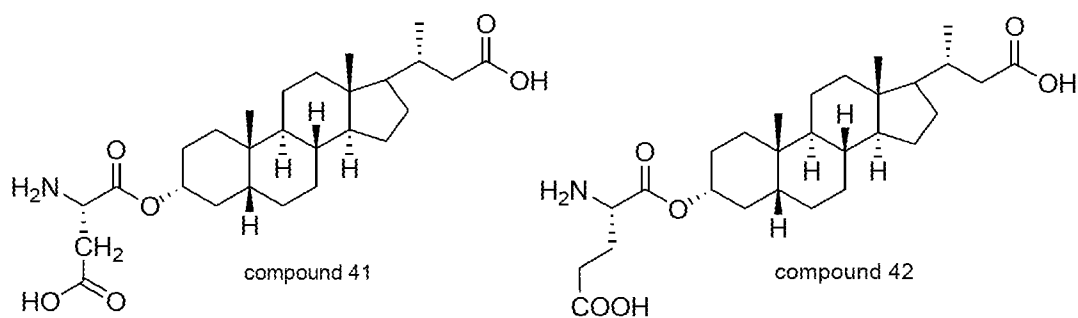
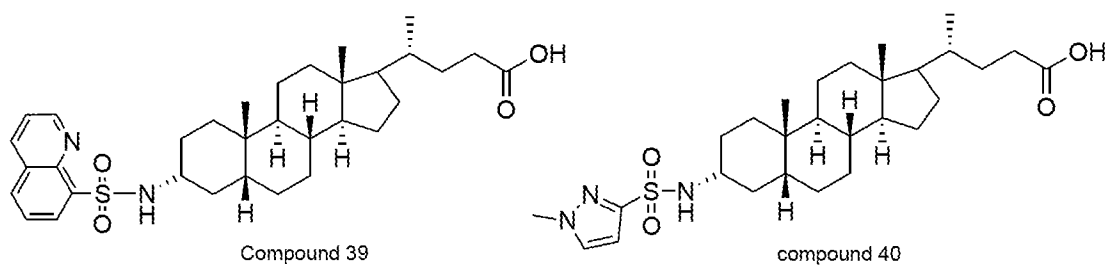
5

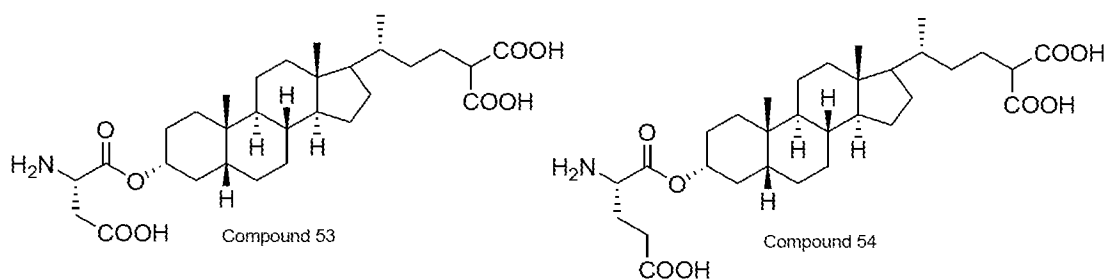
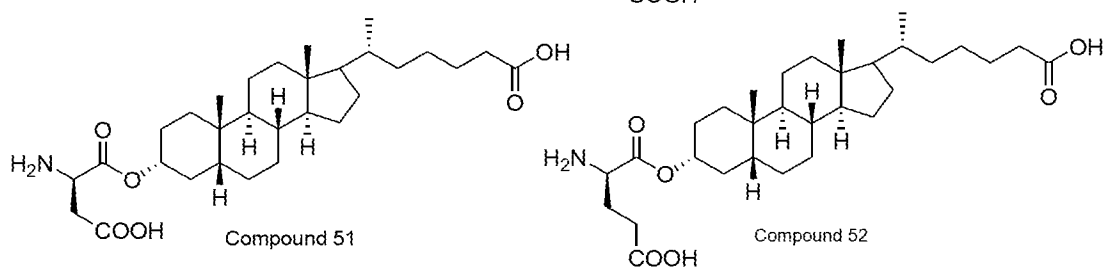
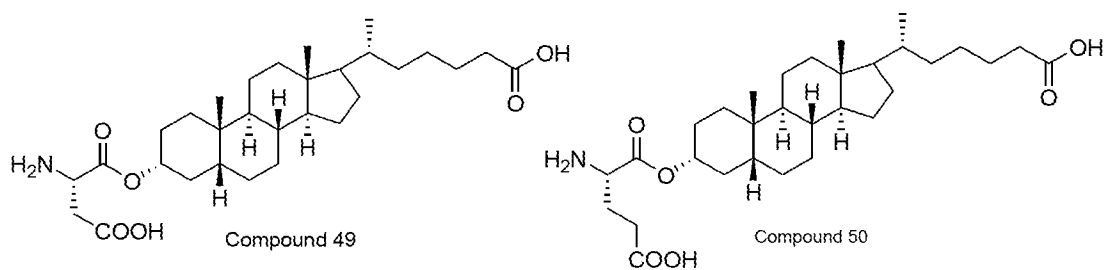


10

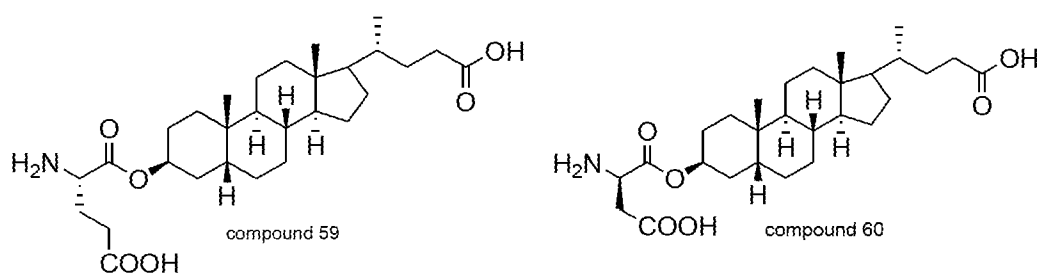
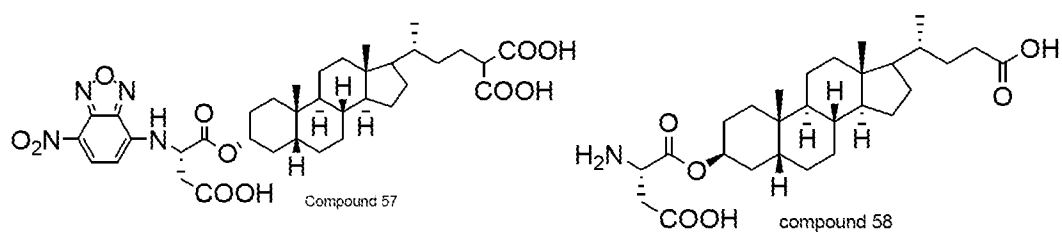
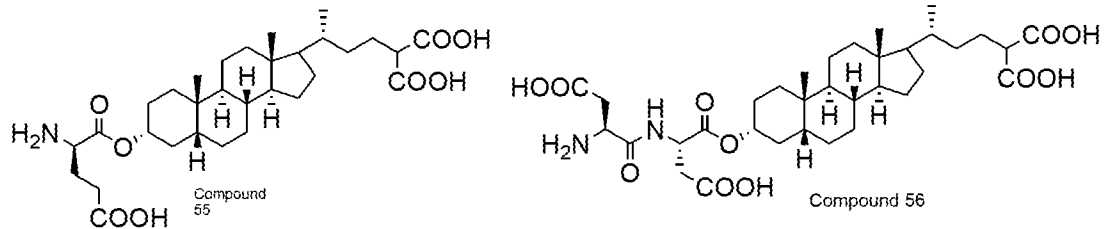




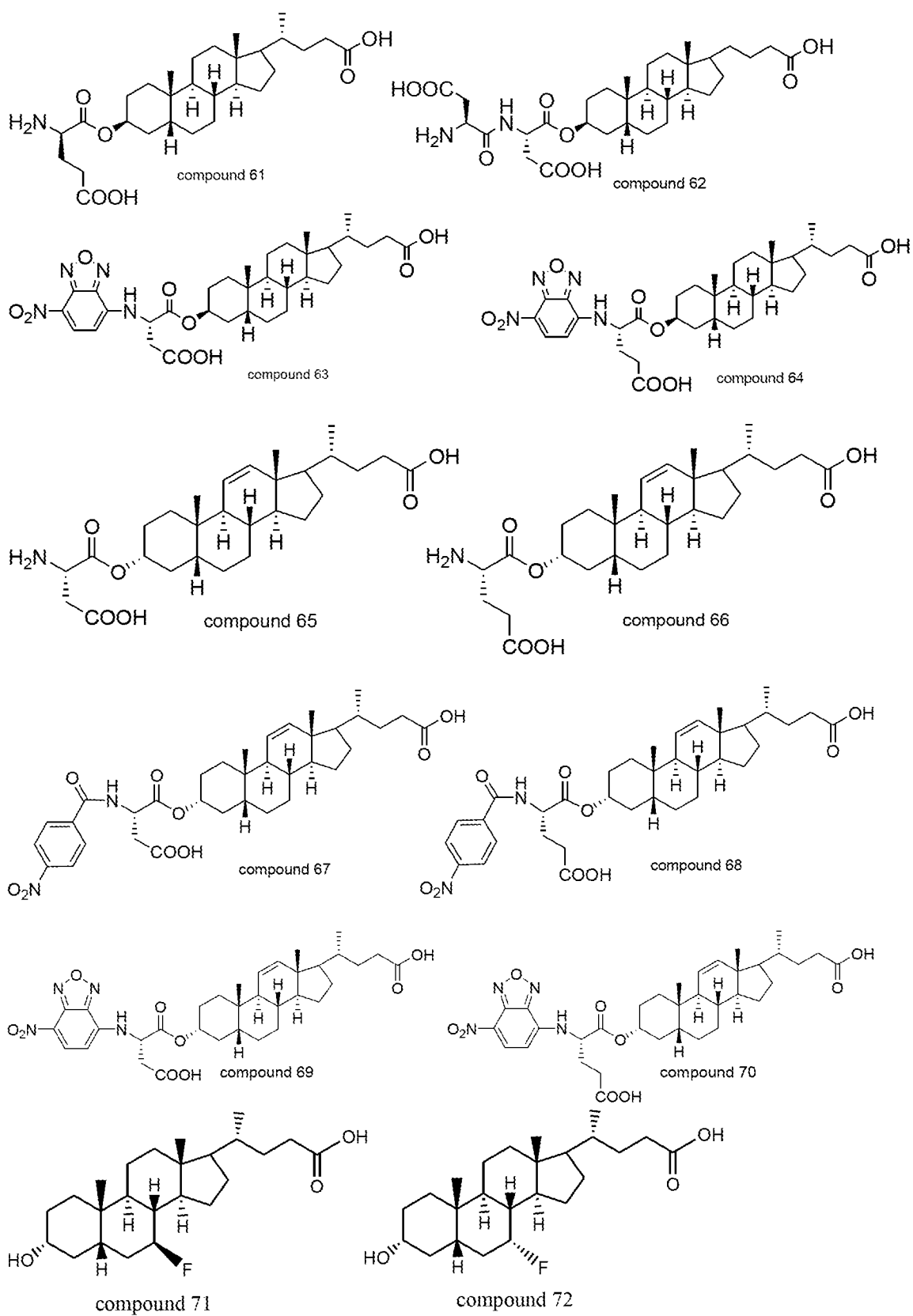


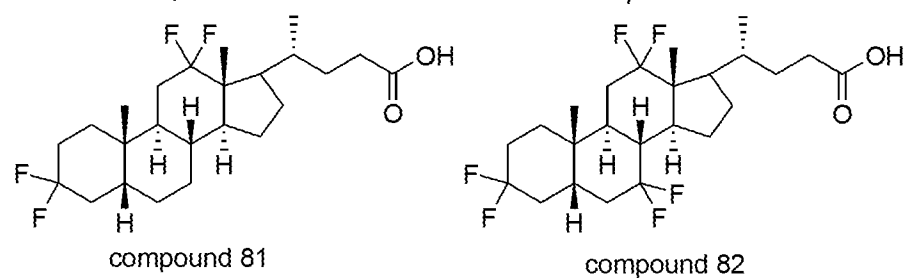
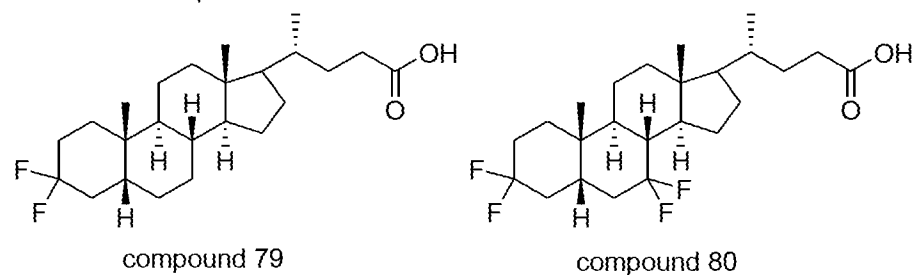
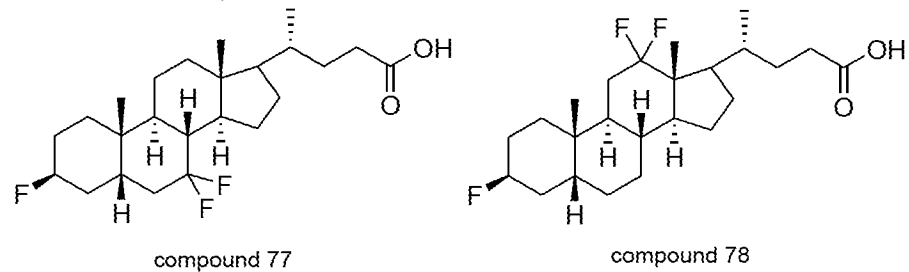
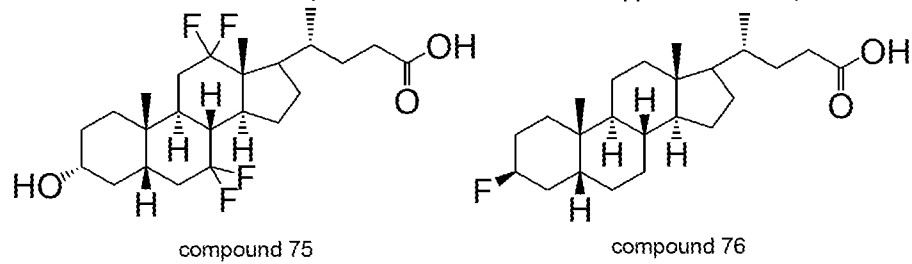
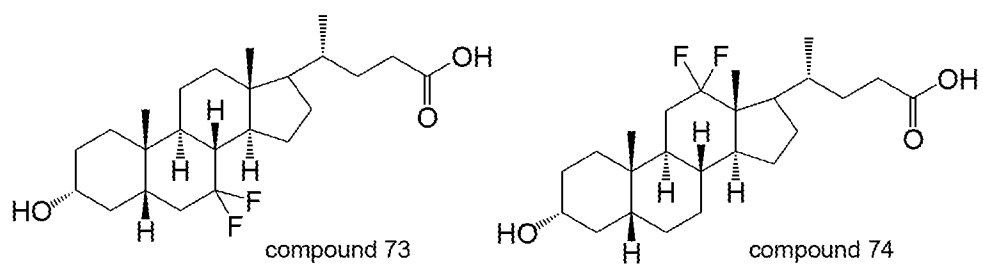


5

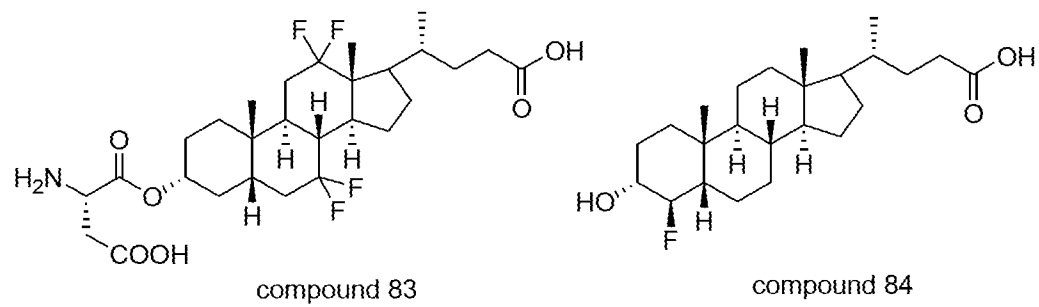


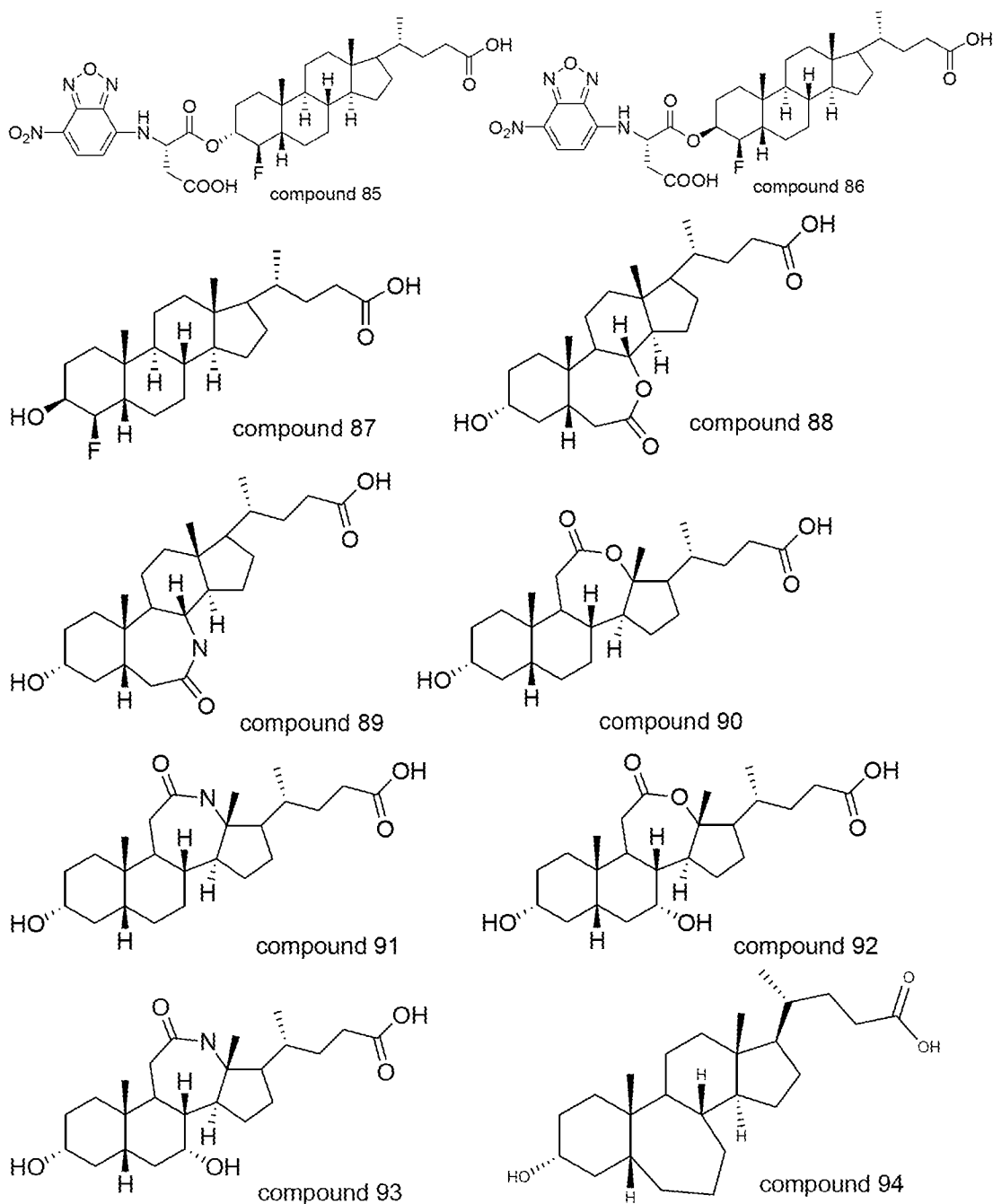
10





5





5

[0073] Each compounds of formula (I-1) to (1-9) is a derivative of lithocholic acid, and may be synthesized in accordance with methods set forth in the working examples of the present disclosure.

10

[0074] 2. Uses of Lithocholic Acid Derivatives

[0075] Also within the scope of the present disclosure is a method for treating a subject suffering from a disease and/or a condition associated with the activation

of sialyltransferase. The method includes steps of, administering an effective amount of the compound of formula (I), particularly the compounds of formula (1-1) to (1-9), to the subject, so as to alleviate or ameliorate one or more symptoms related to the disease and/or condition associated with the activation of sialyltransferase.

[0076] The disease and/or condition known to associate with the activation of sialyltransferase includes, but is not limited to, a cancer, an immune or inflammatory disease.

[0077] Cancer is a class of diseases in which a group of cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth and sometimes tumor metastasis. Examples of cancers include, but are not limited to, carcinoma and sarcoma such as breast cancer, cervical cancer, ovary cancer, endometrial cancer, melanoma, uveal melanoma, brain tumor, lung cancer, liver cancer, lymphoma, neuroepithelioma, kidney cancer, bladder cancer, pancreatic cancer, prostate cancer, stomach cancer, colon cancer, uterus cancer, hematopoietic tumors of lymphoid lineage, myeloid leukemia, thyroid cancer, thyroid follicular cancer, myelodysplastic syndrome (MDS), tumor of mesenchymal origin, teratocarcinoma, neuroblastoma, glioma, glioblastoma, keratoacanthomas, analplastic large cell lymphoma, esophageal squamous cell carcinoma, follicular dendritic cell carcinoma, intestinal cancer, muscle invasive cancer, seminal vesicle tumor, epidermal carcinoma, spleen cancer, head and neck cancer, stomach cancer, bone cancer, cancer of retina, biliary cancer, small bowel cancer, salivary gland cancer, uterine sarcoma, cancer of testicles, cancer of connective tissue, prostatic hypertrophy, myelodysplasia, Waldenstrom's macroglobulinemia, nasopharyngeal, neuroendocrine cancer, mesothelioma, angiosarcoma, Kaposi's sarcoma, oesophagogastric, fallopian tube cancer, peritoneal cancer, papillary serous mullerian cancer, malignant ascites, gastrointestinal stromal tumor (GIST), Li-Fraumeni syndrome or Von Hippel-Lindau syndrome (VHL). The

hematopoietic tumors of lymphoid lineage may be any of leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, B-cell lymphoma, Burkitt's lymphoma, multiple myeloma, Hodgkin's lymphoma, or Non-Hodgkin's lymphoma; whereas tumor of mesenchymal origin is fibrosarcomas or rhabdomyosarcomas.

5 [0078] The cancer may be metastatic or drug-resistant. According to one embodiment, the cancer is metastatic breast cancer. According to another embodiment, the cancer is metastatic pancreatic cancer.

[0079] An inflammatory disease is characterized by a local or systemic, acute or chronic inflammation. Examples include retinopathy (e.g., diabetic retinopathy and proliferative retinopathy), inflammatory dermatoses (e.g., dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, necrotizing vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, eosinophilic myositis, polymyositis, dermatomyositis, and eosinophilic fasciitis), inflammatory bowel diseases (e.g., Crohn's disease and ulcerative colitis), hypersensitivity lung diseases (e.g., hypersensitivity pneumonitis, eosinophilic pneumonia, delayed-type hypersensitivity, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, and ILD associated with rheumatoid arthritis), macular edema, asthma, and allergic rhinitis.

[0080] An immune disease is characterized by a hyper- or hypo-reaction of the immune system. Examples include, but are not limited to, autoimmune diseases (e.g., rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, myasthenia gravis, Type I diabetes mellitus, glomerulonephritis, autoimmune thyroiditis, ankylosing spondylitis, systemic sclerosis, and multiple sclerosis), acute and chronic inflammatory diseases (e.g., systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies, graft rejection, including allograft rejection, and graft-versus-host disease), Sjogren's syndrome, and human immunodeficiency virus infection.

[0081] One embodiment encompasses a method of treating a subject having or suffering from a cancer, which comprises administering to the subject an effective

amount of a compound of the present disclosure, particularly, a compound of formula (1-1) to (1-9), so as to alleviate or ameliorate one or more symptoms related to the cancer.

[0082] Another embodiment encompasses a method of treating an immune or inflammatory disease in a subject, which comprises administering to the subject an effective amount of a compound of the present disclosure, particularly, a compound of formula (1-1) to (1-9), so as to alleviate or ameliorate one or more symptoms related to the immune or inflammatory disease.

[0083] The amount, route of administration and dosing schedule of a compound will depend upon factors such as the specific indication to be treated, prevented, or managed, and the age, sex and condition of the patient. The roles played by such factors are well known in the art, and may be accommodated by routine experimentation. In general, the compound of formula (1-1) to (1-9) is administered to the subject in need thereof in an amount of about 1-100 mg/Kg body weight, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 mg/Kg body weight; preferably about 20-80 mg/Kg body weight, such as 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, and 80 mg/Kg body weight; more preferably about 40-60 mg/Kg body weight, such as 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, and 60 mg/Kg body weight. The amount may be administered in a single dosage or in multiple dosages in a day, such as in 2, 3, or 4 dosages per day. The actual amount of the compound of formula (1-1) to (1-9) will depend on the specific symptoms of the subject, and the physical conditions

of the subject such as age, sex, medical history and etc.; and may be readily determined by the attending physician in accordance with his/her experience.

[0084] In some embodiments, the method further includes administering another chemotherapeutic agent, an anti-inflammatory agent or an immunosuppressive agent, before, concurrently with, or after the administration of the compound of formula (I-1) to (1-9).

[0085] Examples of the chemotherapeutic agent include, but are not limited to, Bleomycin, Buserelin, Busulfan, Capecitabine, carboplatin, Carmustine, chlorambucil, cisplatin, Cladribine, Clodronate, cyclophosphamide, cyproterone, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, diethylstilbestrol, Docetaxel, Doxorubicin, Epirubicin, Estramustine, etoposide, Exemestane, Filgrastim, Fludarabine, Fludrocortisone, fluorouracil, Fluoxymesterone, Flutamide, Gemcitabine, Goserelin, hydroxyurea, Idarubicin, Ifosfamide, Imatinib, Interferon Alfa, Mnotecan, Letrozole, Leucovorin, Leuprolide, Levamisole, Lomustine, Mechlorethamine, Medroxyprogesterone, Megestrol, Melphalan, mercaptopurine, Mesna, methotrexate, mitomycin, Mitotane, Mitoxantrone, Nilutamide, Octreotide, Oxaliplatin, Paclitaxel, Pamidronate, Pentostatin, Plicamycin, Porfimer, procarbazine, Raltitrexed, Rituximab, streptozocin, Tamoxifen, Temozolomide, Teniposide, testosterone, thioguanine, Thiotepa, Topotecan, Trastuzumab, Tretinoin, Vinblastine, Vincristine, Vindesine, Vinorelbine and the like.

[0086] Examples of the anti-inflammatory agent include, but are not limited to, anthranilic acids, aspirin (5-acetylsalicylic acid), aclofenac, aloxiprin, aproxen, aspirin, acetaminophen, amtolmetin, azapropazone, benorilate, benoxaprofen, benzylamine hydrochloride, bromfenac, bufexamac, butibufen, carprofen, celecoxib, choline salicylate, clonixin, desoxysulindac, diflunisal, dipyrrone, droxicam, etodolac, etofenamate, etoricoxib, fenbufen, felbinac, fentiazac, fenoprofen, flufenamic acid, floctafenine, indomethacin, indoprofen, isoxicam, ketoralac, lomoxicam, loxoprofen, licofelone, fepradinol, mefenamic acid,

magnesium salicylate, meclofenamic acid, meloxicam, morniflumate, niflumic acid, nimesulide, oxaprozen, phenylbutazone, piroxicam, piketoprofen, priazolac, pirprofen, propyphenazone, proquazone, rofecoxib, salalate, salicylamide, salicylic acid, sodium salicylate, sodium thiosalicylate, sulindac, suprofen, 5 tolmetin, tenoxicam, tramadol, tenidap, tiaprofenic acid, trolamine salicylate, zomepirac, and the like.

[0087] Examples of the immunosuppressive agent include, but are not limited to, azathioprine, anthracyclines, bleomycin, cyclosporin, cyclophosphamide, cyclosporine A, fluorouracil, mercaptopurine, mitomycin C, methotrexate, 10 Tacrolimus, Sirolimus and the like.

[0088] 4. Pharmaceutical Composition

[0089] The present disclosure also encompasses a pharmaceutical composition for treating a disease and/or a condition associated with the activation of sialyltransferase. The pharmaceutical composition comprises a 15 therapeutically or prophylactically effective amount of the compound of formula (I); and a pharmaceutically acceptable excipient.

[0090] The compound of formula (I) of this invention is present at a level of about 0.1% to 99% by weight, based on the total weight of the pharmaceutical composition. In some embodiments, the compound of formula (I) of this 20 invention is present at a level of at least 1% by weight, based on the total weight of the pharmaceutical composition. In certain embodiments, the compound of formula (I) of this invention is present at a level of at least 5% by weight, based on the total weight of the pharmaceutical composition. In still other embodiments, the compound of formula (I) of this invention is present at a level of at least 10% 25 by weight, based on the total weight of the pharmaceutical composition. In still yet other embodiments, the compound of formula (I) of this invention is present at a level of at least 25% by weight, based on the total weight of the pharmaceutical composition.

[0091] The pharmaceutical composition is prepared in accordance with acceptable pharmaceutical procedures, such as described in Remington's Pharmaceutical Sciences, 17th edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa (1985). Pharmaceutically acceptable excipients are those
5 that are compatible with other ingredients in the formulation and biologically acceptable.

[0092] According to some optional embodiments, the pharmaceutical composition further includes, a chemotherapeutic agent, an anti-inflammatory agent or an immunosuppressive agent.

[0093] Examples of the chemotherapeutic agent include, but are not limited to, Bleomycin, Buserelin, Busulfan, Capecitabine, carboplatin, Carmustine, chlorambucil, cisplatin, Cladribine, Clodronate, cyclophosphamide, cyproterone, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, diethylstilbestrol, Docetaxel, Doxorubicin, Epirubicin, Estramustine, etoposide, Exemestane,
15 Filgrastim, Fludarabine, Fludrocortisone, fluorouracil, Fluoxymesterone, Flutamide, Gemcitabine, Goserelin, hydroxyurea, Idarubicin, Ifosfamide, Imatinib, Interferon Alfa, Mnotecan, Letrozole, Leucovorin, Leuprolide, Levamisole, Lomustine, Mechlorethamine, Medroxyprogesterone, Megestrol, Melphalan, mercaptopurine, Mesna, methotrexate, mitomycin, Mitotane, Mitoxantrone,
20 Nilutamide, Octreotide, Oxaliplatin, Paclitaxel, Pamidronate, Pentostatin, Plicamycin, Porfimer, procarbazine, Raltitrexed, Rituximab, streptozocin, Tamoxifen, Temozolomide, Teniposide, testosterone, thioguanine, Thiotepa, Topotecan, Trastuzumab, Tretinoin, Vinblastine, Vincristine, Vindesine, Vinorelbine and the like.

[0094] Examples of the anti-inflammatory agent include, but are not limited to, anthranilic acids, aspirin (5-acetylsalicylic acid), aclofenac, aloxiprin, aproxen, aspirin, acemetacin, amtolmetin, azapropazone, benorilate, benoxaprofen, benzydamine hydrochloride, benzydamine hydrochloride, bromfenal, bufexamac, butibufen, carprofen, celecoxib, choline salicylate, clonixin, desoxysulindac,
25

diflunisal, dipyrone, droxicam, etodolac, etofenamate, etoricoxib, fenbufen, felbinac, fentiazac, fenoprofen, flufenamic acid, floctafenine, indomethacin, indoprofen, isoxicam, ketoralac, lomoxicam, loxoprofen, licofelone, fepradinol, mefenamic acid, magnesium salicylate, meclofenamic acid meclofenamic acid, meloxicam, morniflumate, niflumic acid, nimesulide, oxaprozen, phenylbutazone, piroxicam, piketoprofen, priazolac, pirprofen, propyphenazone, proquazone, rofecoxib, salalate, salicylamide, salicylic acid, sodium salicylate, sodium thiosalicylate, sulindac, suprofen, tolmetin, tenoxicam, tramadol, tenidap, tiaprofenic acid, trolamine salicylate, zomepirac, and the like.

[0095] Examples of the immunosuppressive agent include, but are not limited to, azathioprine, anthracyclines, bleomycin, ciclosporin, cyclophosphamide, cyclosporine A, fluorouracil, mercaptopurine, mitomycin C, methotrexate, Tacrolimus, Sirolimus and the like.

[0096] The compound of formula (I) of the present invention may be formulated into a single dosage suitable for oral, transmembrane (such as intranasal, sublingual, intravaginal, buccal, and/or endorectal), and/or parenteral administration (e.g., subcutaneous, intravenous, intramuscular, intraperitoneal or bolus injection). Examples of the dosage include, but are not limited to, tablets, caplets, capsules (e.g., soft elastic gelatin capsules), cachets, troches, lozenges, dispersions, suppositories, ointments, cataplasms (or poultices), creams, plasters, solutions, patches, aerosols, or gels. The compound of formula (I) of the present invention may be formulated into a liquid pharmaceutical compositions, which are sterile solutions, suspensions (e.g., water solvable or insolvable liquid suspension, oil-in-water emulsion or water-in-oil emulsion) or elixirs that can be administered by, for example, oral ingestion, or intravenous, intramuscular, subcutaneous or intraperitoneal injection.

[0097] The compound of the present invention is formulated in accordance with the intended routes for its administration. For example, if the compound of the present invention is intended to be administered by oral ingestion, an enteric

coating may be applied on the formulation so as to prevent the compound of the present invention from being degraded in the acidic environment or until it reaches the intestines of the subject. The formulation may further include additional components that help deliver the compound of the present invention to its intended target site. In some examples, the compound of the present invention is enclosed in a liposome to prevent it from enzymatic degradation, and to help transporting the compound of the present disclosure through the circulation system of the subject, and/or across cell membrane to its intended cellular target site.

[0098] Further, the least soluble compound of the present invention may be formulated with additional agents, such as a solvating agent, an emulsifying agent and/or a surfactant, into a liquid formulation. Examples of the additional agent include, but are not limited to, cyclodextrin, and non-aqueous solvents, which include but are not limited to, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyl glycol, 1,3-butyl glycol, dimethyl formamide, dimethyl sulfoxide, biocompatible oils (e.g., cottonseed oil, peanut oil, corn oil, wheat germ oil, castor oil, olive oil, sesame oil, glycerol, tetrahydrogen furan, polyethyl glycol, fatty acid esters of sorbitan, and a combination thereof).

[0099] The amount of the compound of the present disclosure in the formulation varies with the route of administration. For example, formulations for acute treatment will contain larger amounts of one or more of the active compounds, as compared to formulations that are for chronic treatment. Similarly, parental formulations will comprise less amounts of one or more of the active compounds, as compared to formulations that are for oral ingestion. Also within the scope of the present disclosure are formulations suitable for other administration routes.

[00100] 4.1 Formulation for Oral Ingestion

[00101] The compound of present disclosure may be formulation into compositions suitable for oral ingestion. Examples of such formulations include,

but are not limited to, chewable tablets, tablets, capsules, and syrups, which may be prepared in accordance with procedures described in Remington's Pharmaceutical Sciences (18th ed., Mack Publishing, Easton, PA (1990)). The oral formulation is prepared by mixing a pre-determined amount of the active compound and one or more pharmaceutically acceptable excipients in accordance with procedures well known in the related art.

[00102] Tablets and capsules are two most common forms of oral formulation, which may be either liquid or solid composition form. In general, the tablets and capsules are manufactured by mixing the active components with liquid or milled solid excipients, then press into pre-determined forms. The solid formulation may further include disintegrants, which increase solubility; and lubricants.

[00103] **4.2 Formulation for Parental Administration**

[00104] Parental formulations are those suitable for subcutaneous, intravenous (which includes bolus injection), intramuscular, and intraperitoneal injection. To this purpose, sterile injectable or suspension are required so as to prevent the recipients from microorganism infections. Suitable diluents or solvent for manufacturing sterile injectable solution or suspension include, but are not limited to, 1,3-butanediol, mannitol, water, Ringer's solution, and isotonic sodium chloride solution. Fatty acids, such as oleic acid and its glyceride derivatives are also useful for preparing injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil. These oil solutions or suspensions may also contain alcohol diluent or carboxymethyl cellulose or similar dispersing agents. Other commonly used surfactants such as Tweens or Spans or other similar emulsifying agents or bioavailability enhancers that are commonly used in manufacturing pharmaceutically acceptable dosage forms can also be used for the purpose of formulation.

[00105] **4.3 Transmembrane Formulation**

[00106] Transmembrane formulations are those suitable for topical and transmucosal uses, which include but are not limited to, ophthalmic solutions,

sprays, aerosols, creams, lotions, ointments, gels, solutions, suspensions, skin patches and the like. The patches include reservoir type and matrix type skin patches, and may adhere onto the skin for a certain period of time to allow the active component to be adsorbed into the subject's body.

5 [00107] For topical administration, a wide variety of dermatologically acceptable inert excipients well known to the art may be employed. Typical inert excipients may be, for example, water, ethyl alcohol, polyvinyl pyrrolidone, propylene glycol, mineral oil, stearyl alcohol and gel-producing substances. All of the above dosages forms and excipients are well known to the pharmaceutical art. The
10 choice of the dosage form is not critical to the efficacy of the composition described herein.

[00108] For transmucosal administration, the pharmaceutical compositions of this invention may also be formulated in a variety of dosage forms for mucosal application, such as buccal and/or sublingual drug dosage units for drug delivery
15 through oral mucosal membranes. A wide variety of biodegradable polymeric excipients may be used that are pharmaceutically acceptable, provide both a suitable degree of adhesion and the desired drug release profile, and are compatible with the active agents to be administered and any other components that may be present in the buccal and/or sublingual drug dosage units.

20 Generally, the polymeric excipient comprises hydrophilic polymers that adhere to the wet surface of the oral mucosa. Examples of polymeric excipients include, but are not limited to, acrylic acid polymers and copolymers; hydrolyzed polyvinylalcohol; polyethylene oxides; polyacrylates; vinyl polymers and copolymers; polyvinylpyrrolidone; dextran; guar gum; pectins; starches; and
25 cellulosic polymers.

[00109] The present invention will now be described more specifically with reference to the following embodiments, which are provided for the purpose of demonstration rather than limitation.

[00110] **EXAMPLES**

[00111] **Materials and Methods**

[00112] *Cell Lines and Culture*

[00113] Cell lines used in the present disclosure include metastatic human breast cancer cell line MDA-MB-231; human pancreatic epithelial-like cell line PANC-1; and lymphatic-tropic cell line LN. Each cell lines were cultured and maintained in Dulbecco's modified Eagle media (DMEM) supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine in 5% CO₂ at 37°C, unless indicated otherwise.

[00114] *Established LN Cell Line*

[00115] The LN cell line was established by orthotopically injected 5 x10⁵ PANC-1 cells to the pancreas of SCID mice. After one month, metastasis was found in lymph nodes, cancerous cells were then isolated from lymph nodes. The genomic DNA of the isolated cancer cells from lymph nodes were extracted and sent for genotyping analysis, which confirmed that LN cells were indeed derived from PANC-1 cells (data not shown). Cell surface expression analysis conducted by real-time PCR and western blot also confirmed that α-2,3 sialic acid and α-2,6 sialic acid were significantly overexpressed in LN cells (data not shown).

[00116] *Experimental animals*

[00117] Athymic nude mice (BALB/cAnN.Cg-Foxn1nu/CrlNarl, 4-5 weeks old) were supplied from the Laboratory Animal Core Facility (BioLASCO Taiwan Co., Ltd) and given a standard laboratory diet and distilled H₂O *ad libitum* and kept on a 12 h light/dark cycle at 22 ± 2 °C. All experimental protocols were approved by the Institutional Animal Care and Utilization Committee (IACUC), Academia Sinica, Taiwan, R.O.C.

[00118] *Sialyltransferases Activity Assay*

[00119] The enzymes analyzed in the present study included rat ST3Gal(I), rat ST3Gal(III), and human ST6Gal(I). Rat ST3Gal(I) and rat ST3Gal(III), were

purchased from Calbiochem®, while human ST6Gal(I) was purchased from RnDSystems.com.

[00120] Sialyltransferase inhibition assay (α 2,6-N-ST) was performed as follows: Sialyltransferase activity was determined by measuring the level of the end product of sialylated saccharides with reverse-phase HPLC. The assay mixtures contained the following components: 200mM MES buffer; 100mM NaCl; 0.5mM EDTA; 0.01% Triton X-100; 1.4mU ST3GAL I; 2.5mM T-antigen; 1mM CMP-Neu5Ac and different concentration of Sialyltransferase inhibitors to a total volume of 50 μ l. The assay mixture was incubated at 37 °C for 15 mins and then quenched by heating up to 101°C for 10 mins. The sialylated product was resolved by reversed-phase HPLC. Kinetic data were obtained by fitting the initial rate data to the nonlinear regression equation.

[00121] Sialyltransferase inhibition assay (α 2,6-N-ST) was performed as follows: Sialyltransferase activity was determined by measuring the level of the end product of sialylated saccharides with reverse-phase HPLC. The assay mixtures contained the following components: 200mM MES buffer; 100mM NaCl; 0.5mM EDTA; 0.01% Triton X-100; 1.4mU ST6GAL I; 25 μ M Gal β 1-4GlcNAc; 1mM CMP-Neu5Ac and different concentration of Sialyltransferase inhibitors to a total volume of 50 μ l. The assay mixture was incubated at 37 °C for 15 mins and then quenched by heating up to 101°C for 10 mins. The sialylated product is resolved with reversed-phase HPLC. Kinetic data were obtained by fitting the initial rate data to the nonlinear regression equation.

[00122] Lith-O-Asp and **AL-10**, which were reported to have low inhibitory effects toward sialyltransferase (ST) were employed here as a comparative control.

[00123] *Knockdown the expression of ST3GAL(III)*

[00124] Human breast cancer cells were seeded onto the 6-well plates to 40-60% confluent. The siRNAs against Human ST3GALIII (Invitrogen, as

indicated in the following Table) were dissolved with DEPC water to form 20 μ M solutions.

Name of the Primer	Sequence	SEQ ID NO:
ST3GALIII	5'-GGACGCACAAUAUCCAGCGAGAGAA-3'	1
(HSS143938)	5'-UUCUCUCGCGUGGAUAUUGUGCGUCC-3'	2
ST3GALIII	5'-GGAAGCUGGUGAAAGCUCGCGUCAU-3'	3
(HSS143939)	5'-AUGACGCGAGCUUUCACCAGCUUCC-3'	4
ST3GALIII	5'-GGGACUCUUGGUAAUUGUGCGCAAU-3'	5
(HSS143940)	5'-AUUGCGCACAAAUACCAAGAGUCCC-3'	6

Lipofectamine RNAiMAX transfection reagent was diluted in Opti-MEM medium and siRNA was also diluted in Opti-MEM medium and mix with Lipofectamine RNAiMAX reagent in a 1:1 ratio and then added to cells. After 72h, cells were harvested for further analysis.

[00125] Transwell Migration Assay

[00126] Migration assays were done using a transwell, in which a polycarbonate filter pre-coated with Matrigel (8- μ m pores, Millicell; Millipore, Billerica, Mass) was inserted therein. Cells were serum-starved for 24 h and then seeded (5×10^5 cells per upper chamber) and treated with or without the present compound(s) (concentrations as indicated), and the assays were run for 6 h. Cells that had migrated to the underside of the inserted filter were stained with hematoxylin solution. The cells in the upper side of the inserted membrane were rubbed with a cotton swab. The migrated cells on the underside were pictured in a 200 $\times$ magnification field and quantified.

[00127] MTT Assay

[00128] MTT assay is a colorimetric assay that measures the activity of enzymes (i.e., reductase) that reduce (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide (MTT), a yellow tetrazole, to purple formazan, in living cells. This reduction only takes place

when cells are alive; hence MTT assay is generally used to assess the viability and proliferation of cells. Briefly, cells were challenged with 10 or 20 μM of the tested compound for 48 or 72 hours. Then, MTT dye (500 $\mu\text{g/ml}$) was added and the reaction was allowed to proceed for 4 hours before being terminated by the addition of 500 μl of isopropanol. The absorbance of the solution at 570 nm was measured by spectrophotometer.

[00129] *Inhibition of tumor genesis of MDA-MB-231 cells in BALB/c mice*

[00130] BALB/c mice (4-5 weeks old) were scutaneously injected with 1×10^6 MDA-MB-231 cells to the mammary fat pad on day 0. Tumors were allowed to grow in the mice until the size of the tumor reached 100 mm^3 , and then the animals were randomly assigned to 3 groups ($n = 6-8$ per group): tumor (vehicle) control, 10 mg/kg the present compound (e.g., **FCW34** or **FCW66**) treatment group. The present compound was intraperitoneally (*i.p.*) injected to test animals every three days. The growth and metastasis of the tumors were observed by IVIS imaging system, and the metastasized tumor tissues were collected on day 60, and measured for the volume (V) using calipers and calculated by the formula $V = (L \times W^2)/2$, where L is the length and W is the width of tumor.

[00131] *Inhibition of pancreatic tumor growth in SCID mice*

[00132] SCID mice (6 weeks old) were orthotopically injected with 1×10^5 human pancreatic cells PANC-1 grown in glucose-containing or fructose-containing medium with or without the presence of the present compound (e.g., **FCW34**, $5 \mu\text{M}$) on day 0. The progression of the tumor was followed by bioluminescence IVIS image analysis, and the test animals were then sacrificed by cervical dislocation after 14 weeks, and respective tumors and the liver sections were collected, and analyzed by histochemical staining with SNA lectin to measure 2,6-sialylation levels utilizing FACS analysis.

[00133] *Inhibition of melanoma tumor growth in C57BL mice*

[00134] Six C57BL mice (3-6 weeks old) were sbctaneously or intravenously injected with 5×10^5 primary melanoma cells (i.e., B16-F10/Luc cells) on day 0

and allowed the tumor to grow for 7 days to create primary or metastatic melanoma model. Then, the test compound (e.g., **FCW393**, 10 mg/Kg, i.p.) was administered by intraperitoneal injection at the interval of 3 times per week, until day 26 in each animal model. Tumor sizes were measured using calipers at designated days, and tumor volume was calculated using the formula: volume = width² × length × 0.52. The average body weight (BW) of the animals was measured daily, and the concentration of the test compound(s) (i.e., **FCW393**) in i.p. injection was adjusted proportionally to the BW respectively at the indicated dosage and time. After treatment, mice were sacrificed and their white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were respectively determined; their weights of lymph nodes, thymus, and spleen were measured; as well as the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, triglyceride, blood urea nitrogen (BUN), creatinine, and uric acid were measured.

[00135] *Statistical analysis*

[00136] All data are presented as mean ± standard deviation unless otherwise indicated. For multiple comparisons, analysis of variance (ANOVA) with Bonferroni adjustment was performed. A probability value of $P < 0.05$ was considered to represent statistical significance.

[00137] **EXAMPLE 1 Synthesis of The Compound of Formula (1)**

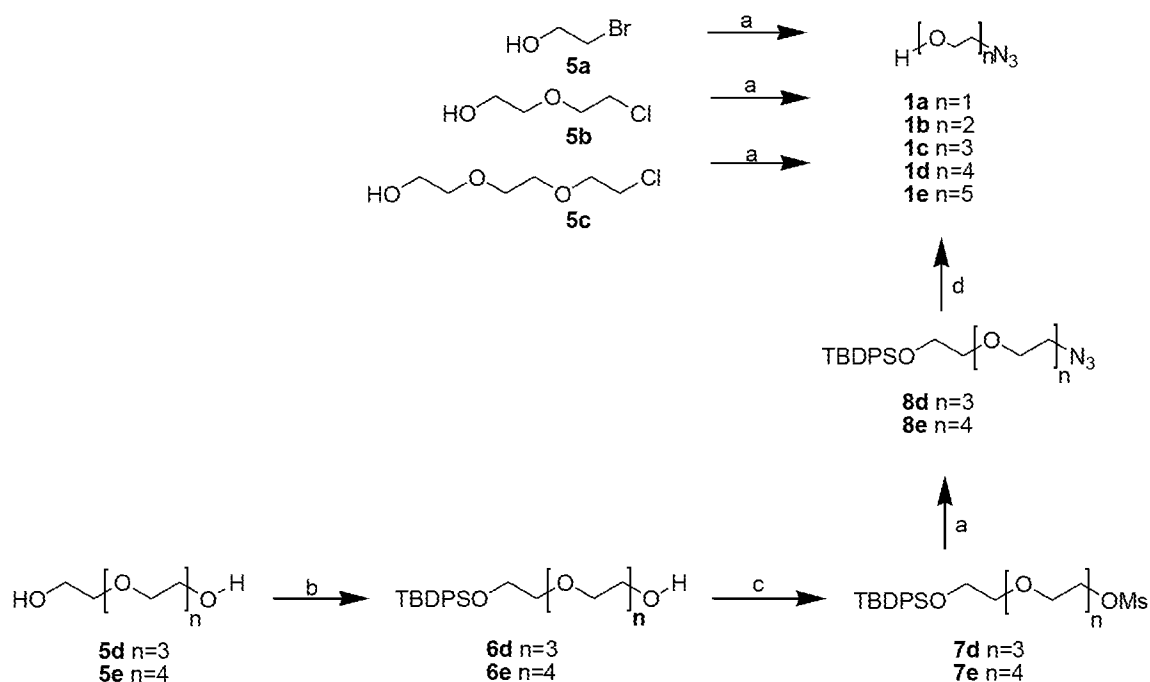
[00138] The compound of formula (I) were synthesized in accordance with schemes set forth in the following examples, the structures of the thus produced compounds were respectively confirmed by NMR spectra.

[00139] **1.1 Synthesis of Compounds 1 to 15**

[00140] In general, compounds **1** to **15** (i.e., compounds of formula (1-1)) were prepared by adding N3 linker to the carboxy group of lithocholic acid, in which compound **1-1-9** was reacted with a N₃ linker (i.e., compounds **1a** to **1e**) in

according to steps set forth in **scheme 2**. The N₃ linkers were synthesized in according to steps set forth in **scheme 1**.

[00141] **Scheme 1:**



Reagents and conditions: (a) NaN₃, EtOH, reflux, 16 h (**1a** 90 %, **1b** 85 %, **1c** 90 %, **8d** 96 %, **8e** 92 %), (b) TBDPSCI, imidazole, DMF, 0 °C to rt, 12 h (**6d** 45 %, **6e** 33 %), (c) MsCl, *N*-methyl morpholine, THF, 0 °C to rt, 3h (**7d** 95 %, **7e** 92 %), (d) TBAF (1 M in THF), THF, 0 °C to rt, 12 h (**1d** 90 %, **1e** 85 %).

[00142] *Synthesis of Compounds 1a to 1e*

[00143] Compounds **1a** to **1e** were respectively synthesized in accordance with the general procedures I or II, as described below.

[00144] General Procedure I. NaN₃ (24.0 mmol) was added to a stirred solution of halogen substituted ethanol (8.0 mmol) in 10 mL EtOH. The reaction was heated to reflux for 16 h. The reaction mixture was treated with water. After extraction with ethyl acetate (EA) (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, and

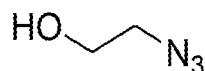
concentration under reduced pressure yielded the crude product. The residue was purified by flash column chromatography to produce the product as oil.

[00145] General Procedure II. Imidazole (2.57 mmol) was added to a stirred 0 °C solution of diol compound (5.15 mmol) in 5 mL dimethylformamide (DMF).

5 The reaction was stirred at 0 °C for 1 h, then added *tert*-butyl(chloro)diphenylsilane (TBDPSCI) (2.57 mmol) dropwisely. The reaction was stirred at room temperature for 12 h. The reaction mixture was quenched with H₂O. After extraction with EA (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, and
10 concentration under reduced pressure yielded the crude product. The residue was purified by flash column chromatography to afford the TBDPS protected alcohol as oil.

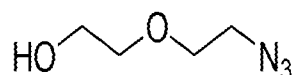
[00146] To a stirred 0 °C solution of TBDPS protected alcohol (1.68 mmol) in 10 mL dry tetrahydrofuran (THF), *N*-methyl morpholine (2.51 mmol) was added
15 dropwisely. The reaction was stirred at 0 °C for 30min, then methanesulfonyl chloride (3.35 mmol) was added dropwisely. The reaction was stirred at room temperature for 3 h. The reaction mixture was quenched with H₂O. After extracting with EA (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, and concentration under
20 reduced pressure yielded the crude product. The residue was purified by flash column chromatography to afford the TBDPS protected alcohol with mesylate OMs leaving group compound as oil.

[00147] *Synthesis of Compound 1a*



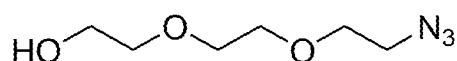
25 [00148] As described in general procedure I. Yield 90 % (oil), ¹H NMR (400 MHz, CDCl₃): δ 3.75(t, *J* = 4.8 Hz, 2H), 3.41 (t, *J* = 4.8 Hz, 2H), 2.02 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 61.4, 53.5.

[00149] *Synthesis of Compound 1b*



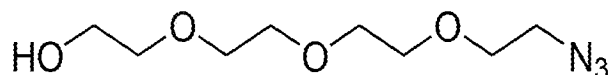
[00150] As described in general procedure I. Yield 85 % (oil), ¹H NMR (400 MHz, CDCl₃): δ 3.63-3.62 (m, 2H), 3.58 (t, *J* = 4.8 Hz, 2H), , 3.49 (t, *J* = 4.8 Hz, 2H), 3.31 (t, *J* = 4.8 Hz, 2H), 2.86 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 72.2, 69.6, 61.3, 50.4. HRMS calcd for C₄H₁₀O₂N₃ (M + H)⁺, 132.0773; found, 132.0774.

[00151] *Synthesis of Compound 1c*



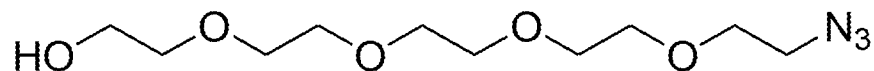
[00152] As described in general procedure I. Yield 90 % (oil), ¹H NMR (400 MHz, CDCl₃): δ 3.71-3.66 (m, 4H), 3.63-3.61 (m, 4H), 3.59-3.54 (m, 3H), 3.34 (t, *J* = 4.8 Hz, 1H), 2.66-2.64 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 72.5, 71.2, 70.5, 70.2, 61.4, 50.6. HRMS calcd for C₆H₁₄O₃N₃ (M + H)⁺, 176.1035; found, 176.1034.

[00153] *Synthesis of Compound 1d*



[00154] As described in general procedure II. Yield 40 % over four steps (oil), ¹H NMR (400 MHz, CDCl₃): δ 3.65 (t, *J* = 4.8 Hz, 2H), 3.61-3.59 (m, 10H), 3.54-3.52 (m, 2H), 3.32 (t, *J* = 4.8 Hz, 2H), 2.77 (brs, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 72.4, 70.6, 70.5, 70.4, 70.2, 69.9, 61.6, 50.5. HRMS calcd for C₈H₁₈O₄N₃ (M + H)⁺, 220.1297; found, 220.1300.

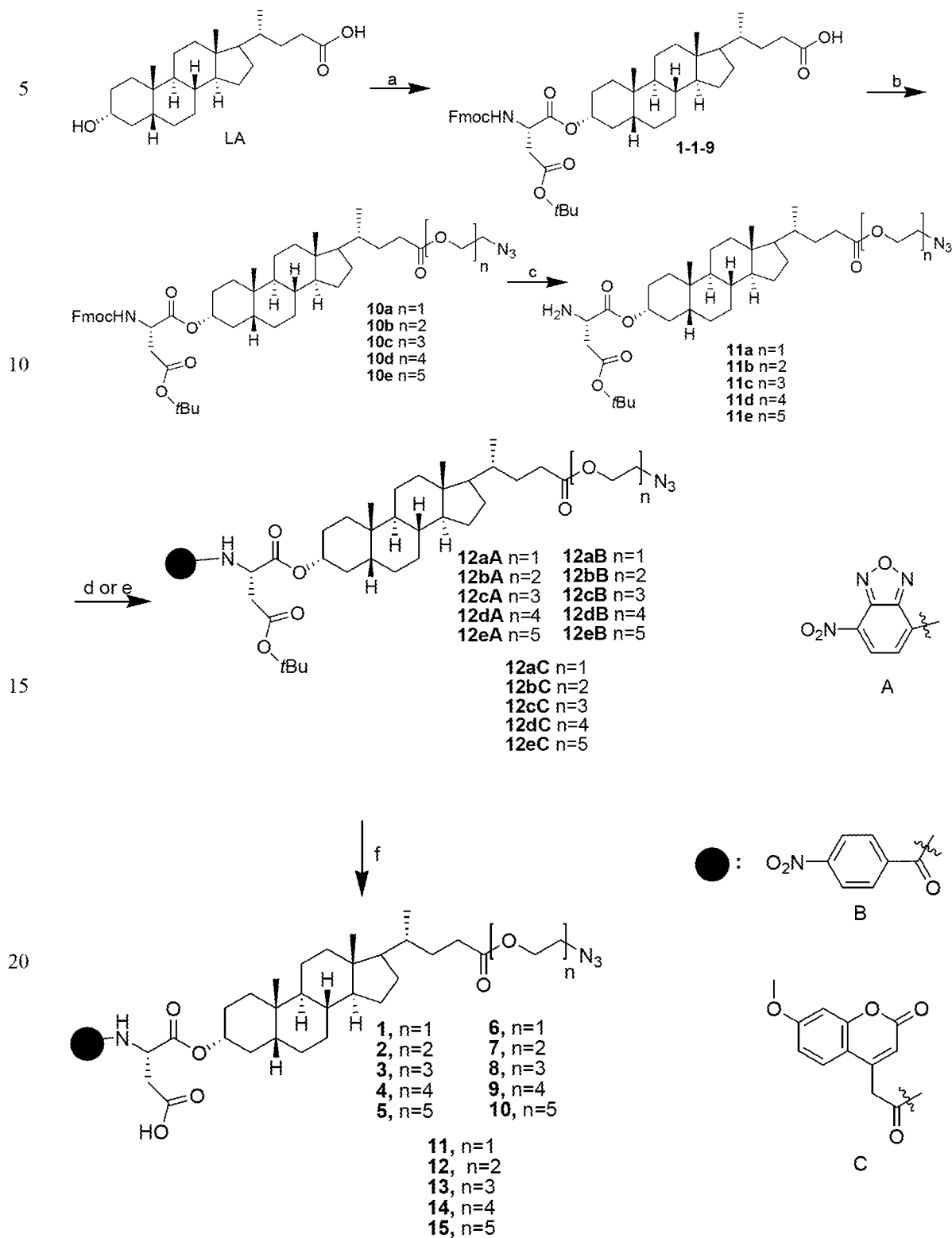
[00155] *Synthesis of Compound 1e*



[00156] As described in general procedure II. Yield 24% over four steps (oil), ¹H NMR (400 MHz, CDCl₃): δ 3.72 (t, *J* = 4.4 Hz, 2H), 3.68-3.66 (m, 14H), 3.61-3.59 (m, 2H), 3.40 (t, *J* = 5.2 Hz 2H), 2.42 (s, brs, 1H). ¹³C NMR (100 MHz,

CDCl_3 : δ 72.6, 70.6, 70.5, 70.1, 69.9, 61.6, 50.6. HRMS calcd for $\text{C}_{10}\text{H}_{22}\text{O}_5\text{N}_3$ ($\text{M} + \text{H}$), 264.1559; found, 264.1555.

[00157] **Scheme 2:**



Reagents and conditions: (a) Fmoc-L-Asp(O-*t*Bu)-OH, DCC, DMAP, CH₂Cl₂, rt, 8 h (50 %); (b) **1a** or **1b** or **1c** or **1d** or **1e**, DCC, DMAP, CH₂Cl₂, rt, 3 h (**10a** 88 %, **10b** 86 %, **10c** 80 %, **10d** 71 %, **10e** 75 %); (c) DBU, CH₂Cl₂, rt, 2h (**11a** 93 %, **11b** 92 %, **11c** 85 %, **11d** 81 %, **11e** 80 %); (d) NBD-Cl, NaHCO₃, THF-EtOH, rt, 2 days (**12aA** 48 %, **12bA** 35 %, **12cA** 40 %, **12dA** 38 %, **12eA** 40 %); (e) *p*-nitrobenzoic acid or 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetic acid, HBTU, DIPEA, CH₂Cl₂, rt, 3h (**12aB** 98 %, **12bB** 95 %, **12cB** 85 %, **12dB** 90 %, **12eB** 88 %, **12aC** 95 %, **12bC** 90 %, **12cC** 88 %, **12dC** 93 %, **12eC** 85 %); (f) TFA, H₂O, CH₂Cl₂, rt, 2h (**1** 85 %, **2** 82 %, **3(FCW34)** 91 %, **4(FCW66)** 88 %, **5** 85 %, **6** 80 %, **7** 82 %, **8** 90 %, **9** 82 %, **10** 84 %, **11** 90 %, **12** 82 %, **13** 85 %, **14** 80 %, **15** 82 %).

[00158] *Synthesis of Compound 1-1-9*

[00159] To a stirred solution of lithocholic acid (LA) (13.3 mmol) and Fmoc-L-Asp(O-*t*Bu)-OH (15.9 mmol) in 150 mL CH₂Cl₂, N,N'-dicyclohexylcarbodiimide (DCC) (15.9 mmol) and 4-dimethylaminopyridine (DMAP) (4.0 mmol) were added. The reaction was stirred at room temperature for 8 h. After reaction, removed solid by filtration, and the filtrate was extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, reduced pressure concentration resulted in the crude product. The residue was purified by flash column chromatography to afford the target product as powder.

[00160] *Synthesis of Compounds 10a to 10e*

[00161] In a flask, mixed compound **1-1-9** (1.30mmol) and a N₃ linker (i.e., any compounds **1a** to **1e**) (1.69 mmol) in 30 mL CH₂Cl₂, then DCC (1.69 mmol) and DMAP (0.39 mmol) were added to the mixed solution. The reaction was stirred at room temperature for 3 h. After reaction, removed solid by filtration, and the filtrate was extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, and concentration under reduced pressure yielded the crude products compounds.

The residue was purified by flash column chromatography to afford the target products, compounds **10a** to **10e**, as powder.

[00162] *Synthesis of Compounds 11a to 11e*

[00163] To a stirred solution of the Fmoc-protected compounds **10a** to **10d** (0.84 mmol) in 10 mL CH₂Cl₂, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1.00 mmol) was added and the mixture was stirred at room temperature for 2 h. Reaction mass was concentrated and product was purified by flash column chromatography to yield the desired compounds **11a** to **11e**.

[00164] *Synthesis of Compounds 12aA to 12eA*

[00165] To a stirred solution of any free amine compounds **11a** to **11e** (0.67 mmol) and nitrobenzoxadiazole chloride (NBD-Cl) (0.81 mmol) in 10 mL THF and 10 mL EtOH, NaHCO₃ (1.35 mmol) was added. The reaction was stirred in the dark at room temperature for 2 days. The reaction mixture was treated with H₂O. After extraction with EA (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, and concentration under reduced pressure yielded the crude product. The residue was purified by flash column chromatography to produce the target product, compounds **12aA** to **12eA**.

[00166] *Synthesis of Compounds 12aB to 12eB*

[00167] To a stirred solution of any free amine compounds **11a** to **11e** (0.31 mmol) and nitrobenzoic acid (NB) (0.37 mmol) in 10 mL CH₂Cl₂, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) (0.46 mmol) and N,N-diisopropylethylamine (DIPEA) (0.92 mmol) were added dropwisely. The reaction was stirred at room temperature for 3 h. The reaction mixture was treated with H₂O. After extraction with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration, concentration under reduced pressure yielded the crude product. The residue was purified by flash column chromatography to produce the target products, i.e., compounds **12aB** to **12eB**.

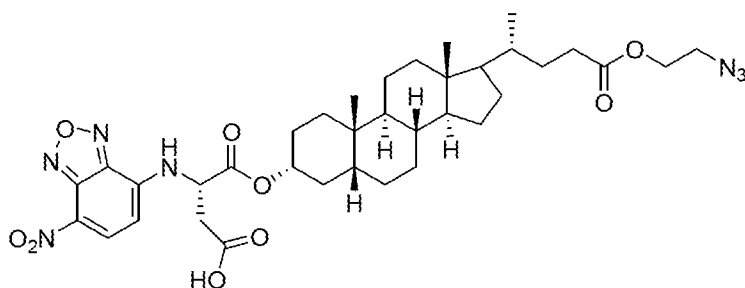
[00168] *Synthesis of Compounds 12aC to 12eC*

[00169] Synthetic procedures for compounds 12aC to 12eC were similar to those of compounds 12aB to 12eB except the reactant nitrobenzoic acid (NB) with replaced by 7-methoxycoumarin-4-acetic acid.

[00170] *Synthesis of Compounds 1-15*

[00171] Compounds **1-5**, **6-10**, and **11-15** were prepared by removing the protective tBu group from the compounds **12aA** to **12eA**, **12aB** to **12eB**, **12aC** to **12eC**, respectively. In general, the tBu protected compound (0.1 mmol) was dissolved in 5 mL trifluoroacetic acid (TFA) with ddH₂O (100 µL) and the reaction mixture was stirred at room temperature for 2 h. The TFA solution was then removed on rotary evaporator. The crude product was purified by reverse phase HPLC (RP-HPLC) (solvent A: water + 0.1% TFA; solvent B: acetonitrile + 0.05% TFA). The final product was obtained as oil or solid upon lyophilization.

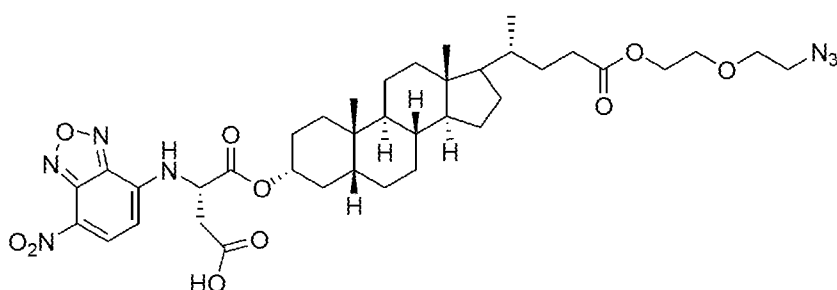
[00172] (3S)-4-(((3R,5R,8R,9S,10S,13R,14S)-17-((R)-5-(2-azidoethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (compound **1**)



[00173] Yield 17 % over five steps (powder), mp 78-79 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.46 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.26 (d, *J* = 8.4 Hz, 1H), 4.87-4.80 (m, 2H), 4.23 (t, *J* = 4.8 Hz, 2H), 3.46 (t, *J* = 4.8 Hz, 2H), 3.21-3.06 (m, 2H), 2.42-2.34 (m, 1H), 2.29-2.21 (m, 1H), 1.95-1.89 (m, 1H), 1.86-1.75 (m, 5H), 1.67-1.64 (m, 1H), 1.55-1.52 (m, 2H), 1.50-1.29 (m, 8H), 1.28-1.20 (m, 3H), 1.16-0.97 (m, 6H), 0.91-0.88 (sd, *J* = 6.4 Hz, 6H), 0.62 (s, 3H). ¹³C NMR (100

MHz, CDCl₃): δ 174.2, 173.3, 168.6, 144.3, 143.8, 142.3, 135.7, 125.5, 100.0, 77.8, 62.9, 56.4, 55.9, 52.6, 49.8, 42.7, 41.9, 40.5, 40.1, 35.7, 35.3, 34.8, 34.6, 32.0, 31.1, 30.9, 28.1, 26.9, 26.4, 26.3, 24.2, 23.3, 20.8, 18.2, 12.0. HRMS calcd for C₃₆H₄₈N₇O₉ (M — H)⁺, 722.3514; found, 722.3510.

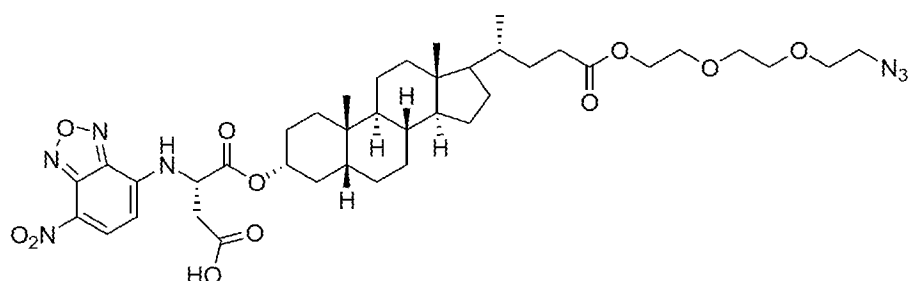
[00174] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-(2-azidoethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (compound 2)



[00175] Yield 11 % over five steps (powder), mp 75-76 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.45 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 6.27 (d, *J* = 8.4 Hz, 1H), 4.86-4.81 (m, 2H), 4.22 (t, *J* = 4.8 Hz, 2H), 3.70-3.65 (m, 4H), 3.37 (t, *J* = 4.8 Hz, 2H), 3.20-3.05 (m, 2H), 2.40-2.33 (m, 1H), 2.27-2.19 (m, 1H), 1.94-1.91 (m, 1H), 1.86-1.73 (m, 5H), 1.67-1.64 (m, 1H), 1.54-1.52 (m, 2H), 1.44-1.29 (m, 8H), 1.27-1.19 (m, 3H), 1.15-0.97 (m, 6H), 0.90-0.86 (sd, *J* = 6.4 Hz, 6H), 0.61 (s, 3H).

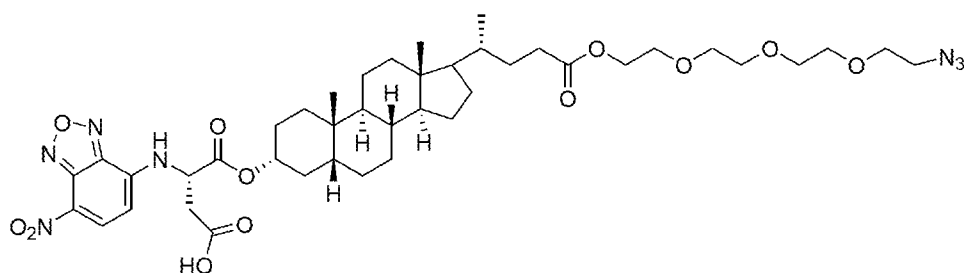
¹³C NMR (100 MHz, CDCl₃): δ 174.5, 173.5, 168.7, 144.3, 143.8, 142.3, 135.8, 125.3, 100.0, 77.7, 70.0, 69.2, 63.3, 56.4, 56.0, 52.6, 50.6, 42.7, 41.9, 40.5, 40.0, 35.7, 35.3, 34.8, 34.5, 32.0, 31.1, 30.9, 28.1, 26.9, 26.4, 26.3, 24.1, 23.2, 20.8, 18.2, 12.0. HRMS calcd for C₃₈H₅₂N₇O₁₀ (M — H)⁺, 766.3776; found, 766.3767.

[00176] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-(2-azidoethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (compound 3) – FCW34



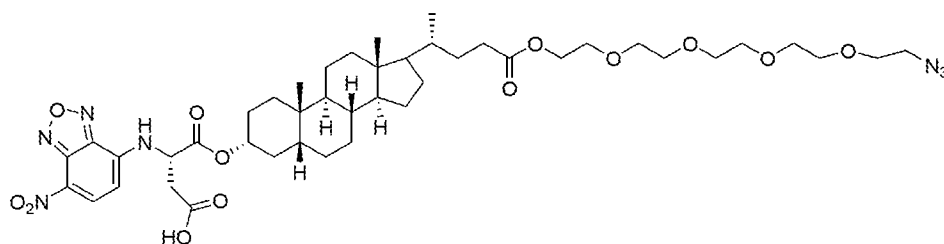
- 5 [00177] Yield 12 % over five steps (powder), mp 71-73 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.46 (d, *J* = 8.8 Hz, 1H), 6.98 (d, *J* = 6.0 Hz, 1H), 6.27 (d, *J* = 8.8 Hz, 1H), 4.87-4.81 (m, 2H), 4.21 (t, *J* = 5.2 Hz, 2H), 3.71-3.66 (m, 8H), 3.38 (t, *J* = 5.2 Hz, 2H), 3.19-3.05 (m, 2H), 2.40-2.32 (m, 1H), 2.26-2.18 (m, 1H), 1.95-1.92 (m, 1H), 1.86-1.72 (m, 5H), 1.67-1.63 (m, 1H), 1.55-1.52 (m, 2H), 1.45-1.29 (m, 8H),
 10 1.27-1.19 (m, 3H), 1.15-0.97 (m, 6H), 0.91-0.87 (sd, *J* = 6.4 Hz, 6H), 0.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.5, 172.9, 168.6, 144.3, 143.8, 142.3, 135.7, 125.4, 100.0, 77.7, 70.7, 70.6, 70.1, 69.3, 63.4, 56.4, 55.9, 52.6, 50.7, 42.7, 41.9, 40.5, 40.1, 35.7, 35.3, 34.8, 34.6, 32.0, 31.9, 31.1, 30.9, 28.2, 26.9, 26.5, 26.4, 26.3, 24.2, 23.3, 20.8, 18.3, 12.0. HRMS calcd for C₄₀H₅₇N₇O₁₁Na (M + Na)⁺,
 15 834.4014; found, 834.4014.

[00178] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-13-oxo-3,6,9,12-tetraoxaheptadecan-16-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (compound 4)-FCW66



[00179] Yield 10% over five steps (oil), ¹H NMR (500 MHz, CDCl₃): δ 8.44 (d, *J* = 8.5 Hz, 1H), 7.16 (brs, 1H), 6.26 (d, *J* = 8.0 Hz, 1H), 4.81 (brs, 2H), 4.19 (t, *J* = 4.5 Hz, 2H), 3.68-3.64 (m, 12H) 3.70 (t, *J* = 5.0 Hz, 2H), 3.14-3.07 (m, 2H), 2.37-2.31 (m, 1H), 2.24-2.18 (m, 1H), 1.92-1.90 (m, 1H), 1.85-1.72 (m, 5H), 1.64 (brs, 1H),
 5 1.53-1.52 (m, 2H), 1.43-1.27 (m, 8H), 1.25-1.19 (m, 3H), 1.13-0.96 (m, 6H), 0.89-0.85 (sd, *J* = 6.0 Hz, 6H), 0.60 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 174.4, 172.8, 168.8, 144.3, 143.8, 142.6, 135.8, 125.2, 100.1, 77.6, 70.7, 70.6, 70.5, 70.0, 69.2, 63.4, 56.4, 55.9, 52.9, 50.7, 42.7, 41.9, 40.5, 40.1, 35.7, 35.3, 34.8, 34.6, 32.0, 31.1, 30.9, 28.1, 26.9, 26.4, 26.3, 24.1, 23.3, 20.8, 18.3, 12.0. HRMS
 10 calcd for C₄₂H₆₀N₇O₁₂ (M — H) —, 854.4300; found, 854.4293.

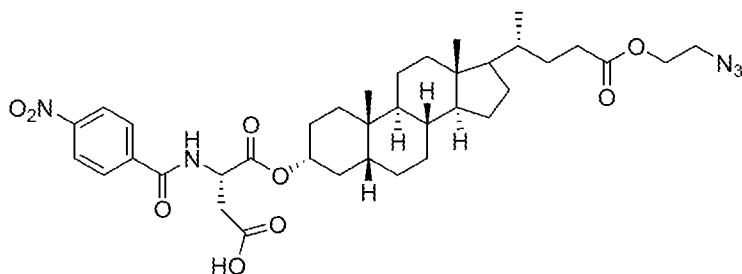
[00180] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-16-oxo-3,6,9,12,15-pentaoxaicosan-19-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (compound 5)



[00181] Yield 10% over five steps (oil), ¹H NMR (500 MHz, CDCl₃): δ 8.44 (d, *J* = 8.5 Hz, 1H), 6.26 (d, *J* = 7.5 Hz, 1H), 4.81 (brs, 2H), 4.20 (t, *J* = 4.5 Hz, 2H), 3.68-3.63 (m, 16H) 3.37 (t, *J* = 4.5 Hz, 2H), 3.14-3.06 (m, 2H), 2.37-2.31 (m, 1H), 2.24-2.18 (m, 1H), 1.92-1.90 (m, 1H), 1.85-1.75 (m, 5H), 1.65 (brs, 1H), 1.52-1.51 (m, 2H), 1.40-1.31 (m, 8H), 1.22-1.19 (m, 3H), 1.13-0.96 (m, 6H), 0.89-0.86 (sd, *J* = 6.0 Hz, 6H), 0.60 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 174.4, 168.8, 144.3, 143.8, 142.6, 135.8, 125.1, 100.1, 77.5, 70.6, 70.5, 70.0, 69.2, 63.4, 56.5, 55.9, 53.0, 50.7, 42.7, 41.9, 40.5, 40.1, 35.7, 35.3, 34.8, 34.6, 32.0, 31.0, 30.9, 28.1,

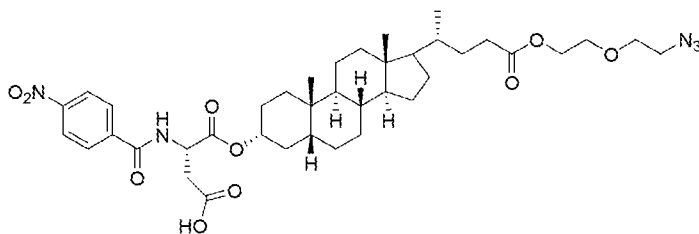
26.9, 26.4, 26.3, 24.1, 23.2, 20.8, 18.3, 12.0. HRMS calcd for $C_{44}H_{64}N_7O_{13}$ (M + H)⁺, 898.4562; found, 898.4556.

[00182] (3S)-4-(((3R,5R,8R,9S,10S,13R,14S)-17-((R)-5-(2-azidoethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-3-(4-nitrobenzamido)-4-oxobutanoic acid (compound 6)



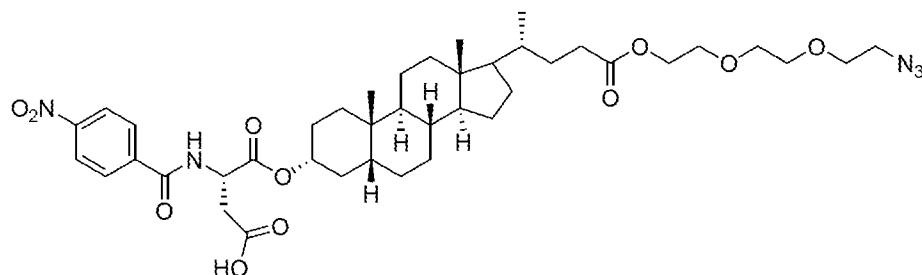
[00183] Yield 32 % over five steps (powder), mp 61-62 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.29 (d, *J* = 8.4 Hz, 2H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 7.6 Hz, 1H), 5.00-4.97 (m, 1H), 4.86-4.80 (m, 1H), 4.24 (t, *J* = 5.2 Hz, 2H), 3.47 (t, *J* = 5.2 Hz, 2H), 3.17 (dd, *J* = 17.4 Hz, 4.0 Hz, 1H), 3.06 (dd, *J* = 17.2 Hz, 4.0 Hz, 1H), 2.43-2.36 (m, 1H), 2.31-2.23 (m, 1H), 1.98-1.95 (m, 1H), 1.91-1.80 (m, 5H), 1.68-1.66 (m, 1H), 1.57-1.54 (m, 2H), 1.43-1.37 (m, 8H), 1.30-1.23 (m, 3H), 1.19-0.98 (m, 6H), 0.93-0.91 (sd, *J* = 7.6 Hz, 6H), 0.65 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.0, 169.8, 165.2, 149.9, 139.1, 128.4, 123.8, 62.8, 56.4, 55.9, 49.8, 49.3, 42.7, 41.9, 40.4, 40.0, 35.7, 35.3, 34.8, 34.5, 32.0, 31.0, 30.8, 28.1, 26.9, 26.4, 26.2, 24.1, 23.2, 20.8, 18.2, 12.0. HRMS calcd for $C_{37}H_{51}N_5O_9Na$ (M + Na)⁺, 732.3584; found, 732.3593.

[00184] (3S)-4-(((3R,5R,8R,9S,10S,13R,14S)-17-((R)-5-(2-(2-azidoethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)-3-(4-nitrobenzamido)-4-oxobutanoic acid (compound 7)



[00185] Yield 30% over five steps (powder), mp 57-58 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.26 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 6.8 Hz, 1H), 4.97-4.95 (m, 1H), 4.83-4.77 (m, 1H), 4.21 (t, *J* = 4.6 Hz, 2H), 3.70-3.64 (m, 4H), 3.37 (t, *J* = 5.2 Hz, 2H), 3.16-3.02 (m, 2H), 2.40-2.33 (m, 1H), 2.27-2.19 (m, 1H), 1.95-1.92 (m, 1H), 1.89-1.74 (m, 5H), 1.65-1.63 (m, 1H), 1.55-1.52 (m, 2H), 1.40-1.27 (m, 8H), 1.24-1.20 (m, 3H), 1.16-1.00 (m, 6H), 0.90-0.87 (sd, *J* = 6.4 Hz, 6H), 0.62 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.4, 169.8, 165.3, 149.9, 139.1, 128.4, 123.8, 70.0, 69.2, 63.2, 56.4, 56.0, 50.6, 49.3, 42.7, 41.9, 40.4, 40.1, 35.7, 35.3, 34.9, 34.6, 32.0, 31.1, 30.9, 28.1, 26.9, 26.4, 26.3, 24.1, 23.3, 20.8, 18.3, 12.0. HRMS calcd for C₃₉H₅₅N₅O₁₀Na (M + Na)⁺, 776.3847; found, 776.3840.

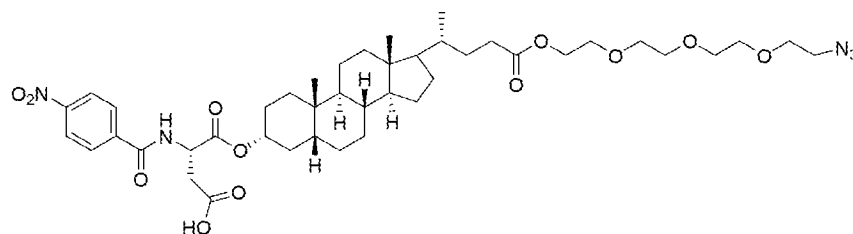
[00186] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(4-nitrobenzamido)-4-oxobutanoic acid (compound 8)



[00187] Yield 24 % over five steps (powder), mp 52-53 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.27 (d, *J* = 8.8 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 7.2 Hz, 1H), 4.98-4.95 (m, 1H), 4.83-4.77 (m, 1H), 4.20 (t, *J* = 4.8 Hz, 2H), 3.70-3.65 (m, 8H), 3.37 (t, *J* = 4.8 Hz, 2H), 3.16-3.00 (m, 2H), 2.39-2.32 (m, 1H), 2.26-2.18 (m, 1H), 1.95-1.92 (m, 1H), 1.89-1.73 (m, 5H), 1.66-1.64 (m, 1H), 1.55-1.52 (m, 2H), 1.40-1.26 (m, 8H), 1.23-1.16 (m, 3H), 1.14-0.96 (m, 6H), 0.90-0.87 (sd, *J* = 6.4 Hz, 6H), 0.62 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.4, 169.8, 165.2, 149.8, 139.1, 128.4, 123.8, 70.7, 70.6, 70.1, 69.3, 63.4, 56.4, 56.0, 50.7, 49.3, 42.7, 41.9,

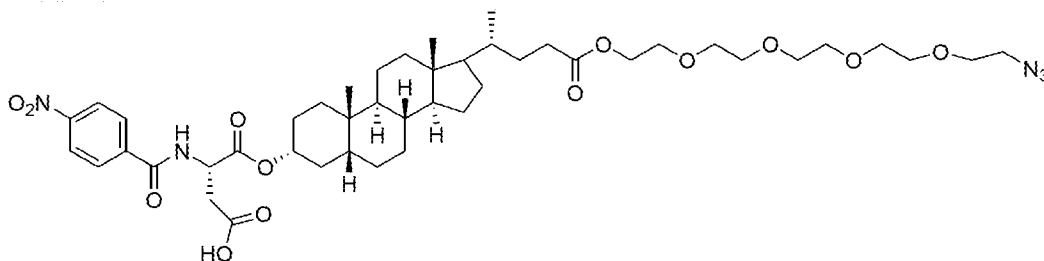
40.4, 40.1, 35.7, 35.3, 34.9, 34.6, 32.0, 31.1, 30.9, 28.1, 27.0, 26.4, 26.3, 24.1, 23.3, 20.8, 18.3, 12.0. HRMS calcd for $C_{41}H_{59}N_5O_{11}Na$ ($M + Na$)⁺, 820.4109; found, 820.4103.

[00188] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-13-oxo-3,6,9,12-*t*etraoxaheptadecan-16-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(4-nitrobenzamido)-4-oxobutanoic acid (compound **9**)



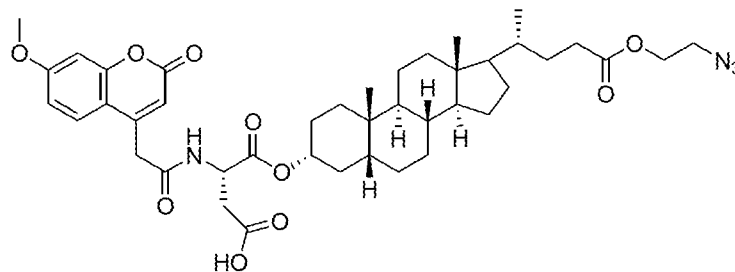
[00189] Yield 21 % over five steps (oil), ¹H NMR (400 MHz, CDCl₃): δ 8.27 (d, *J* = 8.8 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 1H), 4.7-4.96 (m, 1H), 4.80-4.79 (m, 1H), 3.72-3.58 (m, 14H), 3.38 (t, *J* = 4.8 Hz, 2H), 3.18-2.98 (m, 2H), 2.36-2.33 (m, 1H), 2.27-2.20 (m, 1H), 1.95-1.92 (m, 1H), 1.89-1.78 (m, 5H), 1.67-1.66 (m, 1H), 1.55-1.52 (m, 2H), 1.40-1.34 (m, 8H), 1.23-1.17 (m, 3H), 1.15-0.97 (m, 6H), 0.91-0.87 (sd, *J* = 4.0 Hz, 6H), 0.62 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 175.3, 174.4, 169.9, 165.3, 149.8, 139.1, 128.5, 123.8, 72.4, 70.6, 70.5, 70.2, 70.0, 69.2, 63.4, 61.6, 56.4, 55.9, 50.6, 49.3, 42.7, 41.9, 40.4, 40.1, 36.1, 35.7, 35.4, 34.9, 34.6, 32.0, 30.9, 30.7, 28.2, 27.0, 26.3, 24.1, 23.3, 20.9, 18.3, 12.0. HRMS calcd for $C_{43}H_{63}N_5O_{12}Na$ ($M + Na$)⁺, 864.4371; found, 864.4362.

[00190] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-16-oxo-3,6,9,12,15-pentaoxaicosan-19-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(4-nitrobenzamido)-4-oxobutanoic acid (compound **10**)



[00191] Yield 22 % over five steps (oil), ¹H NMR (400 MHz, CDCl₃): δ 8.25 (d, *J* = 8.4 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 6.4 Hz, 1H), 4.95 (brs, 1H), 4.78 (brs, 1H), 4.18 (t, *J* = 4.0 Hz, 2H), 3.63-3.62 (m, 16H), 3.35 (t, *J* = 4.4 Hz, 2H), 3.14-2.97 (m, 2H), 2.37-2.30 (m, 1H), 2.24-2.16 (m, 1H), 1.93-1.90 (m, 1H),
 5 1.84-1.76 (m, 5H), 1.64-1.62 (m, 1H), 1.53-1.51 (m, 2H), 1.39-1.33 (m, 8H), 1.25-1.19 (m, 3H), 1.15-0.94 (m, 6H), 0.89-0.86 (sd, *J* = 6.4 Hz, 6H), 0.60 (s, 3H).
¹³C NMR (100 MHz, CDCl₃): δ 174.4, 169.9, 165.2, 149.8, 139.1, 128.5, 123.8, 70.6, 70.5, 70.4, 69.9, 69.1, 63.4, 56.4, 55.9, 50.6, 49.4, 42.7, 41.9, 40.4, 40.0, 35.7, 35.3, 34.8, 34.5, 32.0, 31.1, 30.8, 28.1, 26.9, 26.4, 26.2, 24.1, 23.2, 20.8,
 10 18.2, 12.0. HRMS calcd for C₄₅H₆₇N₅O₁₃Na (M + Na)⁺, 908.4633; found, 908.4630.

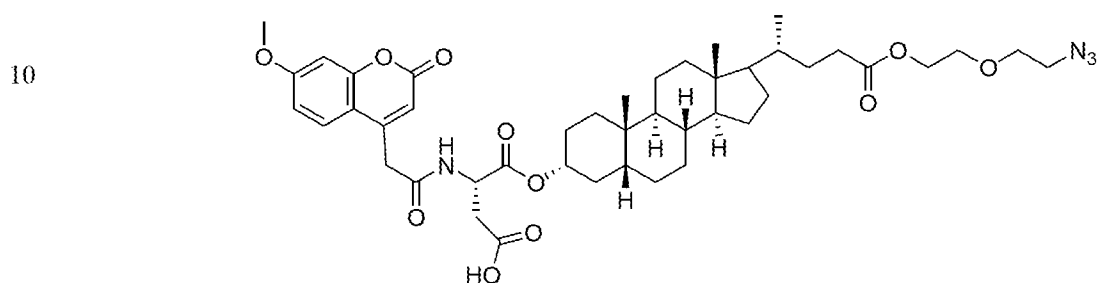
[00192] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-azidoethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)acetamido)-4-oxobutanoic acid
 15 (compound **11**)



[00193] Yield 35 % over five steps (powder), mp 151-152 °C, ¹H NMR (400 MHz, CDCl₃): δ 7.58 (d, *J* = 8.8 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 6.83 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 6.28 (s, 1H), 4.78-4.70 (m, 2H), 4.21 (t, *J* = 5.0 Hz, 2H), 3.82 (s, 3H), 3.74 (s, 2H), 3.45 (t, *J* = 5.0 Hz, 2H), 3.03-2.87 (m, 2H),
 20 2.41-2.33 (m, 1H), 2.28-2.20 (m, 1H), 1.93-1.91 (m, 1H), 1.84-1.74 (m, 5H), 1.61-1.53 (m, 2H), 1.49-1.46 (m, 1H), 1.39-1.27 (m, 8H), 1.23-1.14 (m, 4H), 1.10-0.93 (m, 5H), 0.89-0.88 (sd, *J* = 4.4 Hz, 6H), 0.61 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.3, 174.0, 169.8, 167.9, 163.0, 161.5, 155.4, 149.9, 126.0,

112.9, 112.8, 112.5, 101.0, 62.8, 56.3, 55.9, 55.8, 49.8, 49.2, 42.7, 41.9, 40.4, 40.0, 39.7, 35.8, 35.3, 34.9, 34.5, 32.0, 31.1, 30.9, 28.1, 27.0, 26.4, 26.3, 24.1, 23.3, 20.8, 18.2, 12.0. HRMS calcd for $C_{42}H_{56}N_4O_{10}Na$ ($M + Na$)⁺, 799.3894; found, 799.3890.

5 [00194] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-(2-azidoethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)acetamido)-4-oxobutanoic acid (compound **12**)

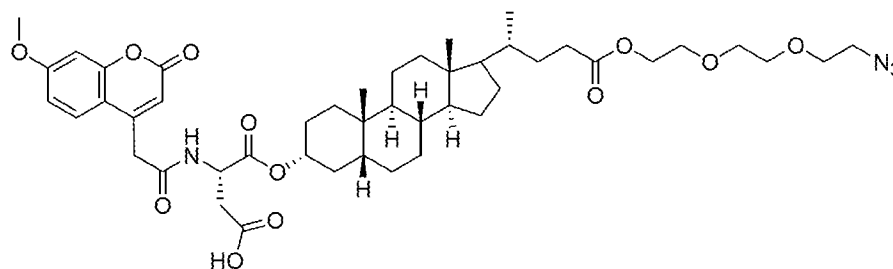


[00195] Yield 29 % over five steps (powder), mp 142-143 °C, ¹H NMR (400 MHz, CDCl₃): δ 7.58 (d, *J* = 9.2 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 6.83 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 6.28 (s, 1H), 4.77-4.70 (m, 2H), 4.21 (t, *J* = 4.8 Hz, 2H), 3.82 (s, 3H), 3.74 (s, 2H), 3.69-3.64 (m, 4H), 3.36 (t, *J* = 4.8 Hz, 2H), 3.02-2.87 (m, 2H), 2.40-2.32 (m, 1H), 2.26-2.18 (m, 1H), 1.93-1.91 (m, 1H), 1.83-1.74 (m, 5H), 1.61-1.46 (m, 3H), 1.39-1.26 (m, 8H), 1.23-1.10 (m, 4H), 1.08-0.94 (m, 5H), 0.88-0.86 (sd, *J* = 6.0 Hz, 6H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.3, 169.8, 167.9, 162.9, 161.5, 155.4, 149.9, 126.0, 112.9, 112.7, 112.5, 101.0, 70.0, 69.2, 63.2, 56.3, 56.0, 55.8, 50.6, 49.1, 42.7, 41.9, 40.4, 40.0, 39.7, 35.7, 35.3, 34.8, 34.5, 32.0, 31.1, 30.9, 28.1, 26.9, 26.4, 26.2, 24.1, 23.3, 20.8, 18.2, 12.0. HRMS calcd for $C_{44}H_{60}N_4O_{11}Na$ ($M + Na$)⁺, 843.4156; found, 843.4147.

25

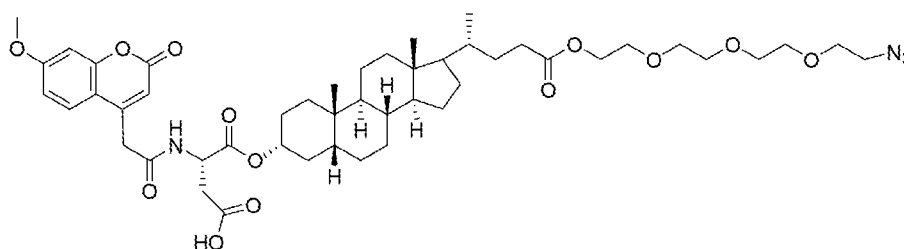
[00196] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-5-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]

phenanthren-3-yl)oxy)-3-(2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetamido)-4-oxobutanoic acid (compound 13)



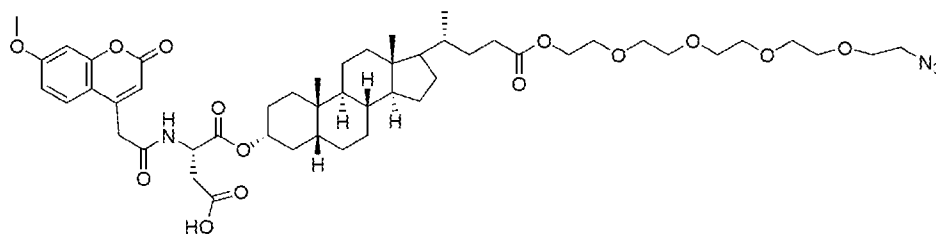
[00197] Yield 25 % over five steps (powder), mp 134-135 °C, ¹H NMR (400 MHz, CDCl₃): δ 7.57 (d, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 6.82 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 6.27 (s, 1H), 4.77-4.69 (m, 2H), 4.20 (t, *J* = 4.8 Hz, 2H), 3.81 (s, 3H), 3.74 (s, 2H), 3.70-3.64 (m, 8H), 3.36 (t, *J* = 5.2 Hz, 2H), 3.02-2.85 (m, 2H), 2.38-2.31 (m, 1H), 2.25-2.17 (m, 1H), 1.92-1.90 (m, 1H), 1.82-1.73 (m, 5H), 1.57-1.44 (m, 3H), 1.38-1.25 (m, 8H), 1.22-1.13 (m, 4H), 1.08-0.93 (m, 5H), 0.87-0.86 (sd, *J* = 5.2 Hz, 6H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.4, 174.3, 169.8, 168.0, 162.9, 161.5, 155.3, 149.9, 126.0, 113.0, 112.7, 112.5, 100.9, 70.6, 70.5, 70.0, 69.2, 63.3, 56.3, 56.0, 55.7, 50.6, 49.1, 42.7, 41.9, 40.3, 40.0, 39.6, 35.7, 35.3, 34.8, 34.5, 32.0, 31.2, 30.9, 28.1, 26.9, 26.3, 26.2, 24.1, 23.2, 20.8, 18.2, 12.0. HRMS calcd for C₄₆H₆₄N₄O₁₂Na (M + Na)⁺, 887.4418; found, 887.4417.

[00198] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-13-oxo-3,6,9,12-tetraoxaheptadecan-16-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl)oxy)-3-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)acetamido)-4-oxobutanoic acid (compound 14)



[00199] Yield 21 % over five steps (powder), mp 126-127 °C, ¹H NMR (500 MHz, CDCl₃): δ 7.57 (d, *J* = 9.0 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 6.83 (dd, *J* = 9.0 Hz, 2.0 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 6.27 (s, 1H), 4.77-4.69 (m, 2H), 4.19 (t, *J* = 4.5 Hz, 2H), 3.82 (s, 3H), 3.74 (s, 2H), 3.68-3.64 (m, 12H), 3.36 (t, *J* = 4.5 Hz, 2H),
 5 3.02-2.85 (m, 2H), 2.37-2.31 (m, 1H), 2.24-2.17 (m, 1H), 1.92-1.90 (m, 1H), 1.82-1.74 (m, 5H), 1.60-1.45 (m, 3H), 1.37-1.26 (m, 8H), 1.25-1.10 (m, 4H), 1.07-0.93 (m, 5H), 0.87-0.86 (sd, *J* = 6.5 Hz, 6H), 0.60 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 174.3, 174.0, 169.8, 167.9, 163.0, 161.4, 155.4, 149.8, 126.0, 113.0, 112.7, 112.5, 101.0, 70.7, 70.6, 70.5, 70.0, 69.2, 63.4, 56.4, 56.0, 55.8,
 10 50.7, 49.2, 42.7, 41.9, 40.4, 40.0, 39.7, 35.8, 35.3, 34.9, 34.6, 32.0, 31.2, 30.9, 28.1, 27.0, 26.4, 26.3, 24.1, 23.3, 20.8, 18.3, 12.0. HRMS calcd for C₄₈H₆₈N₄O₁₃Na (*M* + Na)⁺, 931.4681; found, 931.4681.

[00200] (3*S*)-4-(((3*R*,5*R*,8*R*,9*S*,10*S*,13*R*,14*S*)-17-((*R*)-1-azido-16-oxo-3,6,9,12,15-pentaoxaicosan-19-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene-3-yl)oxy)-3-(2-(7-methoxy-2-oxo-2*H*-chromen-4-yl)acetamido)-4-oxobutanoic acid (compound **15**)



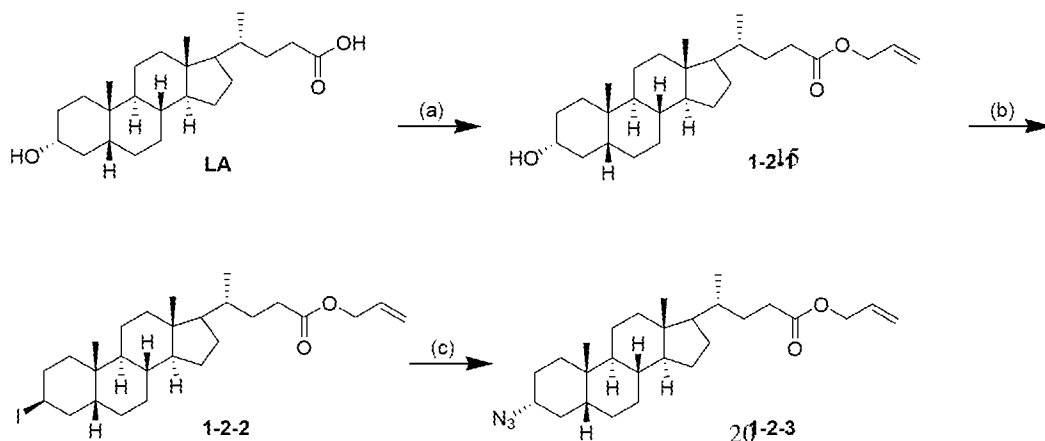
[00201] Yield 21 % over five steps (powder), mp 113-114 °C, ¹H NMR (500 MHz, CDCl₃): δ 7.57 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 7.0 Hz, 1H), 6.82 (dd, *J* = 9.0 Hz, 2.0 Hz, 1H), 6.73 (d, *J* = 2.0 Hz, 1H), 6.27 (s, 1H), 4.75-4.71 (m, 2H), 4.18 (t, *J* = 5.0 Hz, 2H), 3.81 (s, 3H), 3.73 (s, 2H), 3.67-3.62 (m, 16H), 3.35 (t, *J* = 5.0 Hz, 2H),
 25 3.01-2.85 (m, 2H), 2.37-2.31 (m, 1H), 2.23-2.17 (m, 1H), 1.92-1.90 (m, 1H), 1.79-1.73 (m, 5H), 1.59-1.44 (m, 3H), 1.35-1.26 (m, 8H), 1.25-1.10 (m, 4H), 1.07-0.92 (m, 5H), 0.87-0.85 (sd, *J* = 6.5 Hz, 6H), 0.59 (s, 3H). ¹³C NMR (125

MHz, CDCl₃): δ 174.2, 169.8, 167.9, 162.9, 161.3, 155.4, 149.7, 126.0, 113.1, 112.7, 112.5, 101.0, 76.6, 70.7, 70.6, 70.6, 70.5, 70.0, 69.2, 63.4, 56.3, 56.0, 55.7, 50.7, 49.2, 42.7, 41.9, 40.4, 40.0, 39.7, 35.8, 35.3, 34.9, 34.5, 32.0, 31.2, 30.9, 28.1, 26.9, 26.4, 26.3, 24.1, 23.2, 20.8, 18.3, 12.0. HRMS calcd for C₅₀H₇₂N₄O₁₄Na (M + Na)⁺, 975.4943; found, 975.4945.

[00202] **1.2 Synthesis of Compounds 16 to 27**

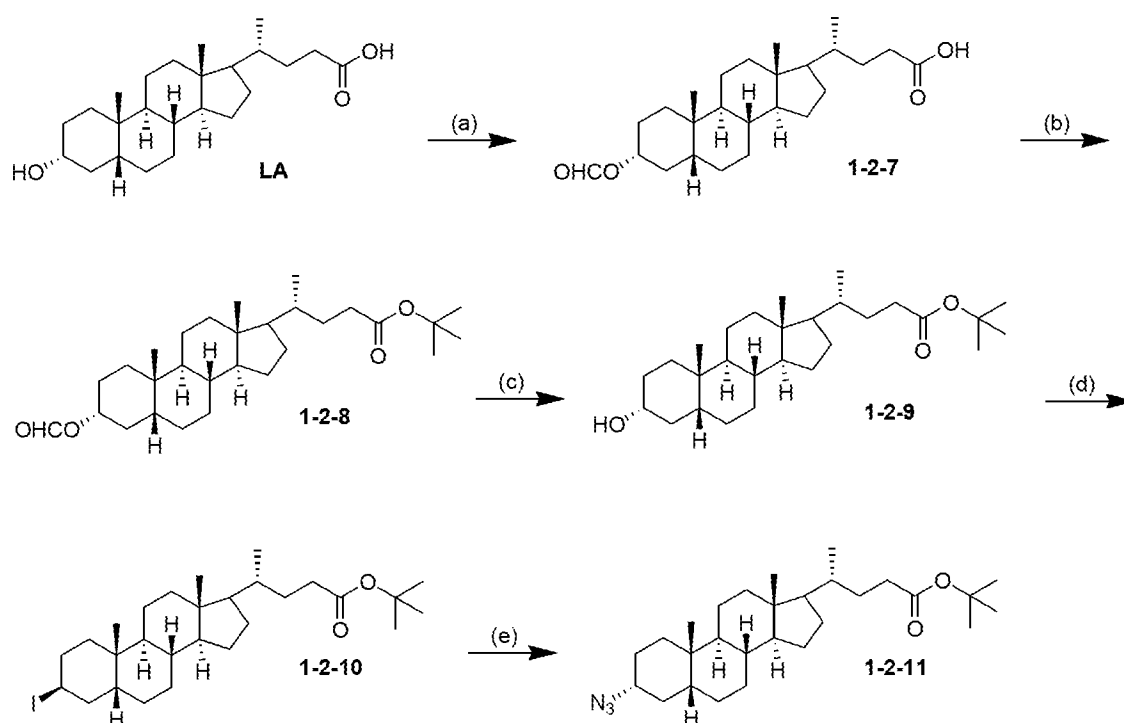
[00203] The compounds 16 to 27 (i.e., compounds of formula (1-2)) were synthesized in accordance with procedures set forth in **Schemes 3 to 8**, in which **Schemes 3 to 6** were for the synthesis of compounds with modification at α -3-NH₂; whereas **Schemes 7 and 8** were for the synthesis of compounds having amino acid side chain modification at α -3-NH₂ of lithocholic acid (LA).

[00204] **Scheme 3:**



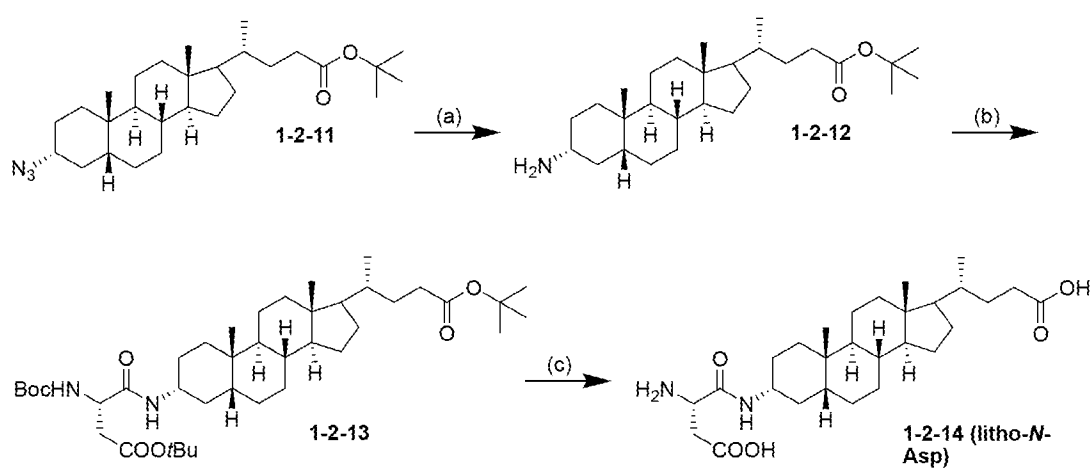
Reagents and conditions: (a) allyl bromide, K₂CO₃, DMF, 100 °C, 12 h, 99 %, (b) I₂, PPh₃, imidazole, CH₂Cl₂, 0 °C to rt, 3 h, 74 %, (c) NaN₃, DMF, 120 °C, 12 h, 99 %.

[00205] **Scheme 4:**



Reagents and conditions: (a) 90 % formic acid, 120 °C, 12 h, 99 %, (b) (Boc)₂O, DMAP, *t*-BuOH, CH₂Cl₂, rt, 1h, 60 %, (c) K₂CO₃, EtOH-CH₂Cl₂, rt, 4h, 99 %, (d) I₂, PPh₃, imidazole, CH₂Cl₂, 0 °C to rt, 3 h, 82 %, (e) NaN₃, DMF, 120 °C, 12 h, 99 %.

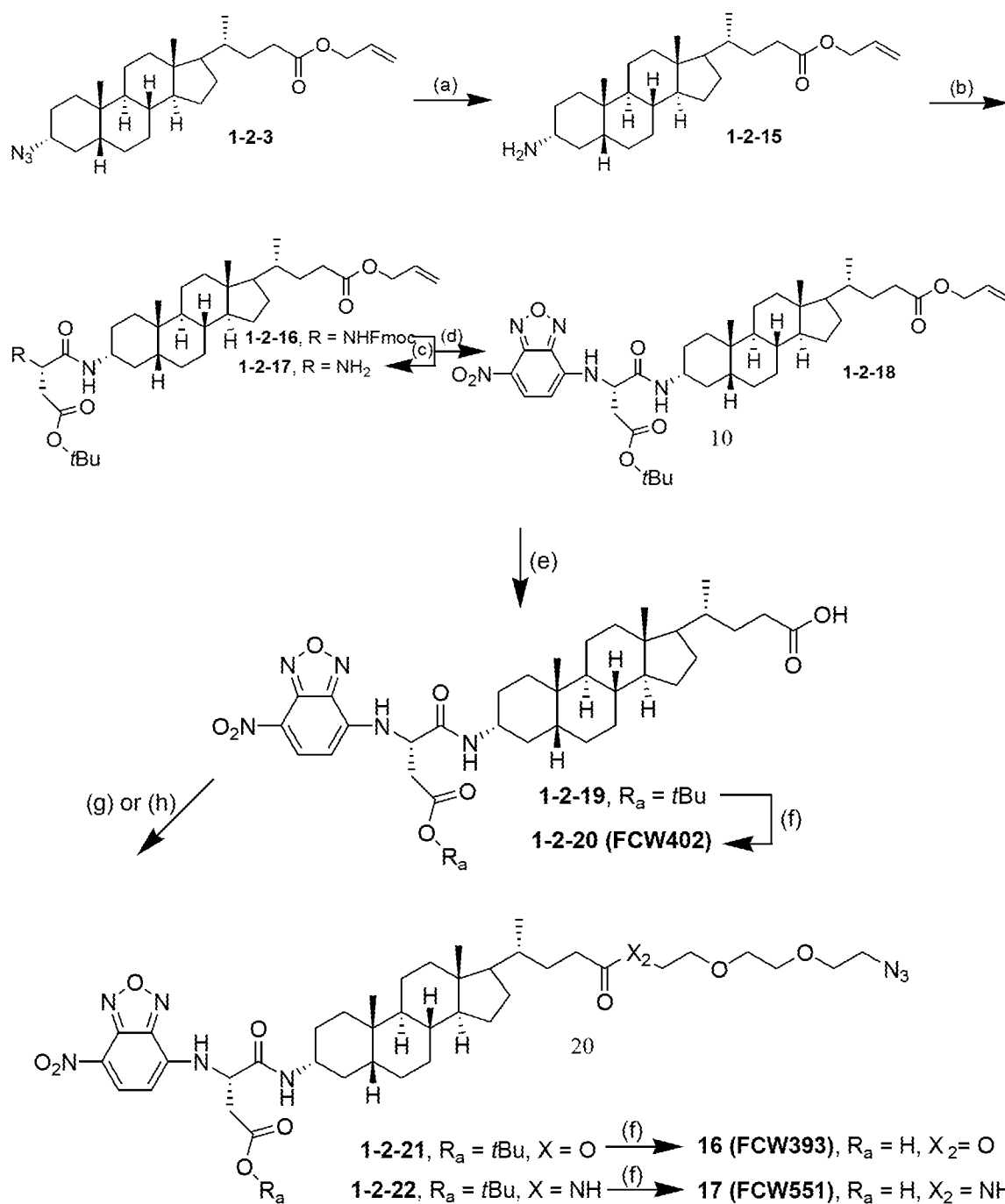
[00206] **Scheme 5:**



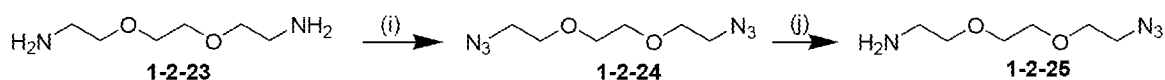
Reagents and conditions: (a) PPh_3 , $\text{THF-H}_2\text{O}$, rt, 12 h, 72 %, (b) Boc-L-Asp(O-*t*Bu), HBTU, DIPEA, CH_2Cl_2 , rt, 2 h, 95 %, (c) TFA, H_2O , CH_2Cl_2 , rt, 3 h, 90 %.

[00207] **Scheme 6:**

5



15



Reagents and conditions: (a) PPh_3 , THF- H_2O , rt, 12 h, 72 %, (b) Fmoc-L-Asp(O-*t*Bu), HBTU, DIPEA, CH_2Cl_2 , rt, 3 h, 93 %, (c) DBU, CH_2Cl_2 , rt, 0.5 h, 92 %, (d) NBD-Cl, NaHCO_3 , THF, EtOH, rt, 2 days, 42 %, (e) $\text{Pd}(\text{PPh}_3)_4$, PhSiH_3 , CH_2Cl_2 , rt, 2 h, 95 %, (f) TFA, H_2O , CH_2Cl_2 , rt, 3 h, **1-2-20**, 90 %; **16**, 88 %; **17**, 85 %, (g) **1C**, DCC, DMAP, CH_2Cl_2 , rt, 6 h, 72 %, (h) **1-2-26**, HBTU, DIPEA, CH_2Cl_2 , rt, 2 h, 95 %, (i) TfN_3 in CH_2Cl_2 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, K_2CO_3 , MeOH, H_2O , 0 °C to rt, 16 h, 92 %, (j) PPh_3 , Et₂O-THF-1M $\text{HCl}_{(\text{aq})}$, rt, 12 h, 88 %.

[00208] *General Procedures for Allyl Protection*

[00209] K_2CO_3 (45.53 mmol) and allyl bromide (16.70 mmol) were added dropwisely to a stirred solution of LA (15.18 mmol) in 20 mL DMF. The reaction was stirred at 100 °C for 12 h. After reaction remove solid by filtration, and filtrate was concentrated then extracted with CH_2Cl_2 (3X), the combined organic layers were washed with H_2O (2X) and brine (2X) and dried over MgSO_4 prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford the target product as powder.

[00210] *General Procedures for Iodination*

[00211] I_2 (60.71 mmol) and imidazole (124.46 mmol) were added to a stirred 0 °C solution of PPh_3 (54.64 mmol) in 125 mL CH_2Cl_2 . The reaction was stirred at 0 °C for 30min, then added compound **1-2-1** (15.18 mmol) in 125 ml CH_2Cl_2 dropwisely. The reaction was stirred at room temperature for 3 h. The reaction mixture was quenched with $\text{Na}_2\text{S}_2\text{O}_3$ (aq). After extraction with CH_2Cl_2 (3X), the combined organic layers were washed with H_2O (2X) and brine (2X) and dried over MgSO_4 prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford the compound **1-2-2**.

[00212] *General Procedures for Azide Formation*

[00213] NaN₃ (33.73 mmol) was added to a stirred solution of compound **1-2-2** (11.24 mmol) in 30 mL DMF. The reaction was stirred at 120 °C for 12 h. After reaction remove solid by filtration, and filtrate was concentrated then extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-3** as oil.

[00214] *General Procedures for CHO Protection*

[00215] In a flask, mixed LA (13.28 mmol) in 30 mL 90 % formic acid, then the reaction was stirred at 120 °C for 12 h. Collected solid by filtration, then solid was washed with water. The solid was dried by reduced pressure to afford the compound **1-2-7**.

[00216] *General Procedures for t-Bu Protection*

[00217] (Boc)₂O (13.62 mmol) and DMAP (2.04 mmol) were added to a stirred solution of compound **1-2-7** (6.81 mmol) in 30 mL CH₂Cl₂ and 30 mL *t*-butanol. The reaction was stirred at room temp for 1h, then reaction mass was concentrated and the product was purified by flash column chromatography to give compound **1-2-8**.

[00218] *Synthesis of Compound 1-2-9*

[00219] K₂CO₃ (34.56 mmol) was added to a stirred solution of compound **1-2-8** (4.32 mmol) in 20 mL CH₂Cl₂ and 40 mL EtOH. The reaction was stirred at room temp for 4 h. After reaction, removed solid by filtration, and the filtrate was concentrated, then extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-9** as oil.

[00220] *General Procedures for De-protecting CHO Group*

[00221] To a stirred solution of compound **1-2-8** (4.32 mmol) in 20 mL CH₂Cl₂ and 40 mL EtOH was added K₂CO₃ (34.56 mmol). The reaction was stirred at rt for 4 h. After reaction remove solid by filtration, and filtrate was concentrated then extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-9** as oil.

[00222] *General Procedures for Reducing Azide to Amine*

[00223] To a stirred solution of compound **1-2-3** (40.09 mmol) in 60 mL H₂O and 180 mL THF was added PPh₃ (80.18 mmol). The reaction was stirred at rt for 12 h then reaction mass was concentrated and crude product was extracted with CH₂Cl₂ (3X), the combined organic layers were washed with H₂O (2X) and brine (2X) and dried over MgSO₄ prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-15** as oil.

[00224] *General Procedures for De-protecting Allyl Group*

[00225] To a stirred solution of compound **1-2-18** (4.60 mmol) in 60 mL CH₂Cl₂ was added Pd(Ph₃)₄ (0.46 mmol) and phenyl silane (9.20 mmol) dropwise. The reaction was stirred at rt for 2h, then reaction mass was concentrated and product was purified by flash column chromatography to get compound **1-2-19**.

[00226] *General procedures for De-protecting Boc Group*

[00227] The Boc protected compound (0.1 mmol) was dissolved in 5 mL TFA with ddH₂O (100 µL) and the reaction mixture was stirred at room temperature for 3 h, then reaction mass was concentrated and product was purified by flash column chromatography to get target compound.

[00228] *General Procedures for Converting Amine to Azide*

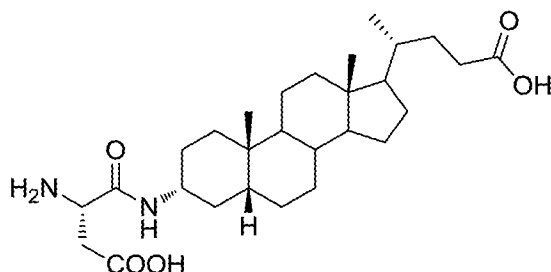
[00229] TfN₃ was prepared fresh prior to the reaction as follows. Six molar equivalents per substrate of NaN₃ (222.67 mmol) were dissolved in a minimum volume of water (40 mL) and cooled to 0 °C. An equal volume of CH₂Cl₂ (40 mL)

was added, followed by slow addition of Tf_2O (111.34 mmol) to the vigorously stirred solution. The reaction was stoppered and stirring was continued at 0 °C for 2 h. Saturated NaHCO_3 was added carefully while stirring continued and subsequently the mixture was transferred to a separatory funnel after CO_2 evolution had ceased. The aqueous phase was washed twice with CH_2Cl_2 and combined organic layers were washed with saturated NaHCO_3 . The TfN_3 in CH_2Cl_2 solution was used without further purification. Diamine compound **1-2-23** (37.11 mmol), K_2CO_3 (44.53 mmol) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.37 mmol) were dissolved in water (30 mL). The freshly prepared TfN_3 solution was added at once under vigorous stirring. Methanol (100 mL) was added slowly, then the reaction was stirred at rt for 16 h then reaction mass was concentrated in vacuo. The aqueous solution was acidified with 1N HCl to pH2 and was extracted with ethyl acetate (EA) (3X). The combined organic layers were dried over MgSO_4 prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-25** as oil.

[00230] *General Procedures for Reducing Azide to Amine*

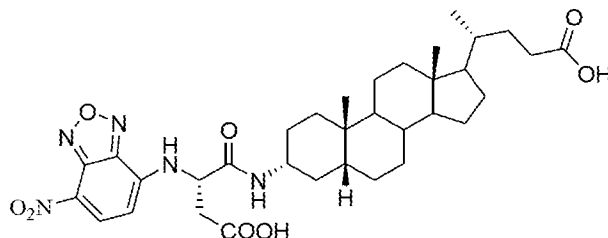
[00231] To a stirred solution of compound **1-2-25** (20.97 mmol) in 33 mL Et_2O , 6.6 mL THF and 26.4 mL 1N $\text{HCl}_{(\text{aq})}$ was added PPh_3 (20.97 mmol) in 33 mL Et_2O dropwise. The reaction was stirred at rt for 12 h then reaction mass was concentrated and crude product was extracted with CH_2Cl_2 (3X), the combined organic layers were washed with H_2O (2X) and brine (2X) and dried over MgSO_4 prior to filtration and concentration under reduced pressure to yield the crude product. The residue was purified by flash column chromatography to afford compound **1-2-26** as oil.

[00232] (4R)-4-((3R,5R,10S,13R)-3-((S)-2-amino-3-carboxypropanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid.
1-2-14 (litho-N-Asp)



[00233] mp 158-160 °C, ¹H NMR (400 MHz, MeOD): δ 4.07-4.01 (m, 1H), 3.72-3.65 (m, 1H), 2.82-2.68 (m, 2H), 2.35-2.29 (m, 1H), 2.24-2.16 (m, 1H), 2.04-2.01 (m, 1H), 1.95-1.78 (m, 5H), 1.62-1.59 (m, 2H), 1.48-1.42 (m, 6H), 1.34-1.22 (m, 6H), 1.18-1.04 (m, 6H), 0.98 (s, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.70 (s, 3H). ¹³C NMR (100 MHz, MeOD): δ 178.3, 174.5, 168.7, 58.1, 57.7, 51.9, 51.4, 44.0, 42.0, 41.6, 37.4, 37.3, 37.1, 36.8, 35.8, 34.2, 32.4, 32.1, 29.3, 28.3, 27.7, 25.4, 24.1, 22.1, 18.9, 12.6. HRMS calcd for C₂₈H₄₇N₂O₅ (M + H)⁺, 491.3485; found, 491.3480.

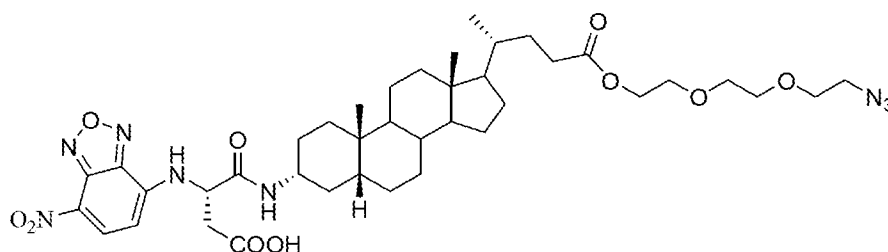
[00234] (4R)-4-((3R,5R,10S,13R)-3-((S)-3-carboxy-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-20 (FCW402)**



[00235] mp 134-136 °C, ¹H NMR (400 MHz, MeOD): δ 8.53 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 6.42 (d, *J* = 8.8 Hz, 1H), 3.76-3.65 (m, 1H), 3.08-2.96 (m, 2H), 2.35-2.27 (m, 1H), 2.22-2.14 (m, 1H), 2.00-1.94 (m, 1H), 1.93-1.73 (m, 5H), 1.61-1.53 (m, 2H), 1.43-1.35 (m, 7H), 1.33-1.22 (m, 5H), 1.15-0.99 (m, 6H), 0.95 (s, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.67 (s, 3H). ¹³C NMR (100 MHz, MeOD): δ 178.2, 170.8, 146.1, 145.8, 145.5, 125.0, 102.2, 58.1, 57.7, 56.1, 51.5, 44.1, 44.0, 41.9, 41.6, 37.3, 37.2, 36.8, 35.8, 34.1, 32.5, 32.2, 29.3, 28.3, 27.7, 25.3, 24.1.

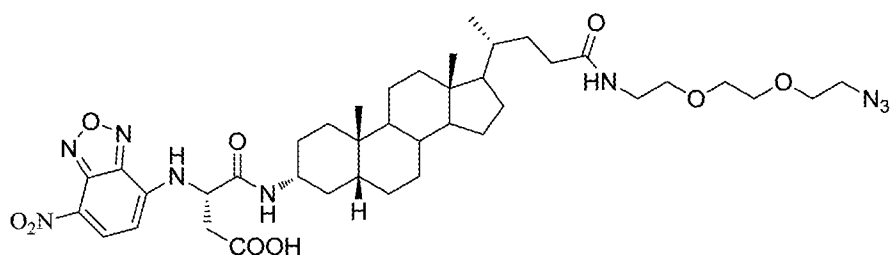
22.0, 18.9, 12.6. HRMS calcd for $C_{34}H_{46}N_5O_8$ ($M - H$), 652.3346; found, 652.3339.

[00236] (3S)-4-(((3R,5R,10S,13R)-17-((R)-5-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)amino)-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid. Compound **16** (FCW393)



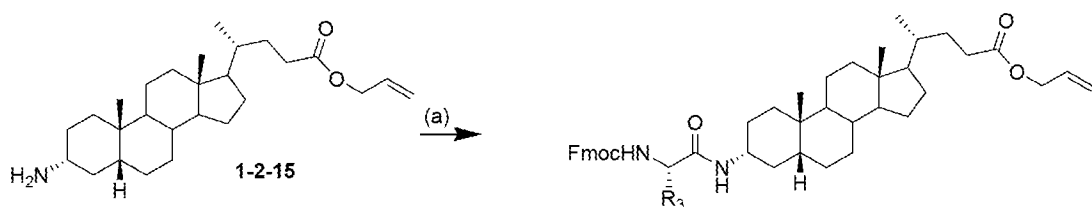
[00237] mp 80-81 °C, ^1H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.77 (s, 1H), 7.01 (s, 1H), 6.35 (s, 1H), 4.87-4.76 (m, 1H), 4.21 (s, 2H), 3.80-3.66 (m, 9H), 3.38 (s, 2H), 3.14-3.06 (m, 2H), 2.35-2.30 (m, 1H), 2.23-2.16 (m, 1H), 1.79-1.72 (m, 7H), 1.46-1.09 (m, 13H), 1.02-0.98 (m, 3H), 0.86 (s, 3H), 0.79 (d, $J = 4.0$ Hz, 3H), 0.74-0.67 (m, 3H), 0.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 173.2, 168.2, 144.3, 143.7, 142.8, 136.4, 124.5, 100.8, 70.6, 70.5, 70.0, 69.2, 63.5, 56.5, 56.1, 54.1, 50.7, 50.4, 42.6, 42.3, 40.4, 40.1, 35.7, 35.6, 35.3, 34.4, 32.9, 31.2, 30.9, 28.1, 27.3, 26.9, 26.2, 24.0, 23.4, 20.7, 18.2, 11.9. HRMS calcd for $C_{40}H_{58}N_8O_{10}Na$ ($M + Na$), 833.4174; found, 833.4181.

[00238] (3S)-4-(((3R,5R,10S,13R)-17-((R)-5-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)amino)-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid. Compound **17** (FCW551)



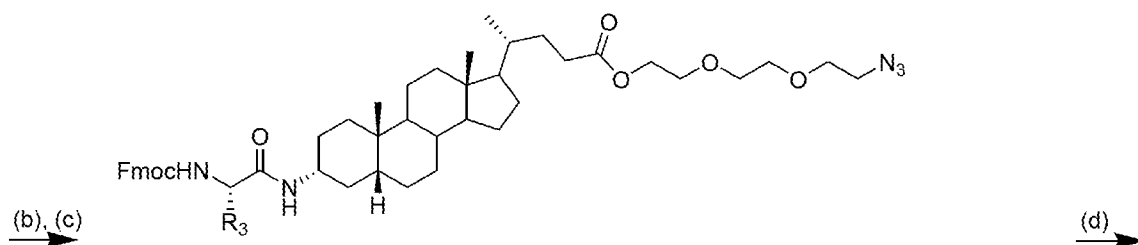
[00239] mp 90-91 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.33 (d, *J* = 8.8 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 6.51 (t, *J* = 4.8 Hz, 1H), 6.35 (d, *J* = 8.8 Hz, 1H), 4.85-4.76 (m, 1H), 3.85-3.76 (m, 1H), 3.67-3.61 (m, 6H), 3.54 (t, *J* = 5.2 Hz, 2H), 3.44-3.42 (m, 2H), 3.38 (t, *J* = 5.2 Hz, 2H), 3.10 (d, *J* = 5.2 Hz, 2H), 2.28-2.21 (m, 1H), 2.06-1.96 (m, 1H), 1.84-1.55 (m, 6H), 1.49-1.42 (m, 2H), 1.39-1.30 (m, 2H), 1.24-1.12 (m, 8H), 1.04-0.86 (m, 5H), 0.82 (s, 3H), 0.71 (d, *J* = 5.6 Hz, 3H), 0.67-0.51 (m, 3H), 0.46 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 175.4, 173.0, 167.9, 144.3, 143.5, 142.9, 136.6, 124.0, 100.5, 70.4, 70.1, 70.0, 69.6, 56.5, 56.2, 54.3, 50.6, 50.2, 42.4, 42.2, 40.2, 40.1, 39.5, 36.2, 35.7, 35.6, 35.4, 34.3, 33.6, 32.6, 31.8, 28.1, 27.0, 26.8, 26.1, 23.9, 23.3, 20.6, 18.1, 11.8. HRMS calcd for C₄₀H₅₈N₉O₉ (*M* - H)⁻, 808.4358; found, 808.4358.

[00240] **Scheme 7:**



1-2-16

- a, R₃ = CH₃
- b, R₃ = CH₂CH₂COO*t*Bu
- c, R₃ = CH₂St*t*Bu
- d, R₃ = CH₂STrt
- e, R₃ = CH₂Ph
- f, R₃ = CH₂-Ph-4-*Ot*Bu
- g, R₃ = 3-CH₂-indolylNBoc
- h, R₃ = CH₂CH₂CH₂CH₂NHBoc
- i, R₃ = CH₂CH₂CH₂NHC(=NH)NHPbf

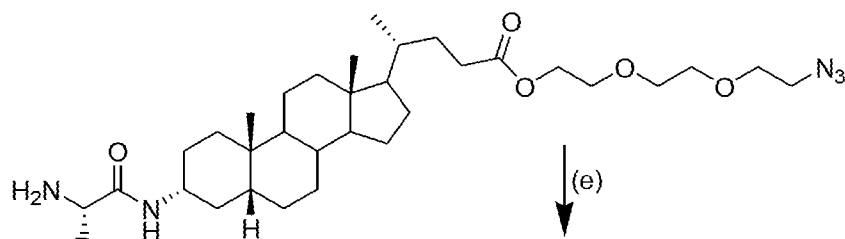
**1-2-17**

- a, R₃ = CH₃
 b, R₃ = CH₂CH₂COOtBu
 c, R₃ = CH₂StBu
 d, R₃ = CH₂STrt
 e, R₃ = CH₂Ph
 f, R₃ = CH₂-Ph-4-OTBu
 g, R₃ = 3-CH₂-indolylNBoc
 h, R₃ = CH₂CH₂CH₂CH₂NHBoc
 i, R₃ = CH₂CH₂CH₂NHC(=NH)NHPbf

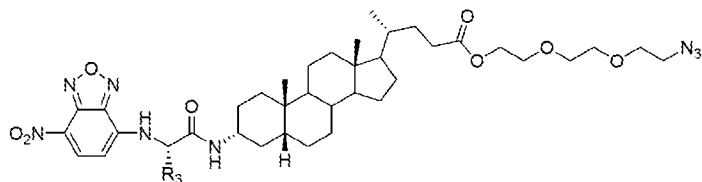
5

1-2-18

- a, R₃ = CH₃
 b, R₃ = CH₂CH₂COOtBu
 c, R₃ = CH₂StBu 10
 d, R₃ = CH₂STrt
 e, R₃ = CH₂Ph
 f, R₃ = CH₂-Ph-4-OTBu
 g, R₃ = 3-CH₂-indolylNBoc
 h, R₃ = CH₂CH₂CH₂CH₂NHBoc
 i, R₃ = CH₂CH₂CH₂NHC(=NH)NHPbf



15

**1-2-19**

- a, R₃ = CH₃
 b, R₃ = CH₂CH₂COOtBu $\xrightarrow{(f)}$
 c, R₃ = CH₂StBu $\xrightarrow{(g)}$
 d, R₃ = CH₂STrt $\xrightarrow{(f)}$
 e, R₃ = CH₂Ph $\xrightarrow{(f)}$
 f, R₃ = CH₂-Ph-4-OTBu $\xrightarrow{(f)}$
 g, R₃ = 3-CH₂-indolylNBoc $\xrightarrow{(f)}$
 h, R₃ = CH₂CH₂CH₂CH₂NHBoc $\xrightarrow{(f)}$
 i, R₃ = CH₂CH₂CH₂NHC(=NH)NHPbf $\xrightarrow{(g)}$

1-2-20

- i, R₃ = CH₂CH₂CH₂NHC(=NH)NH₂

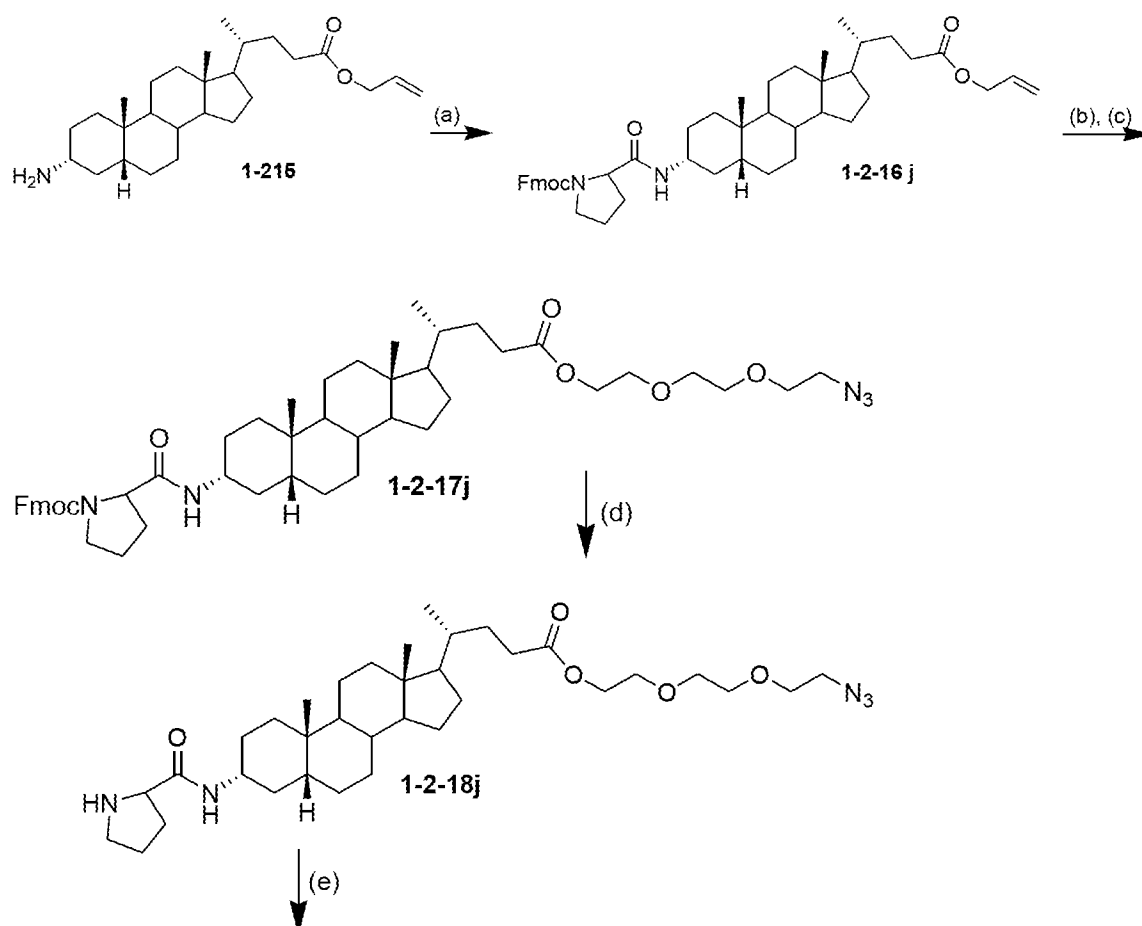
1-2-20

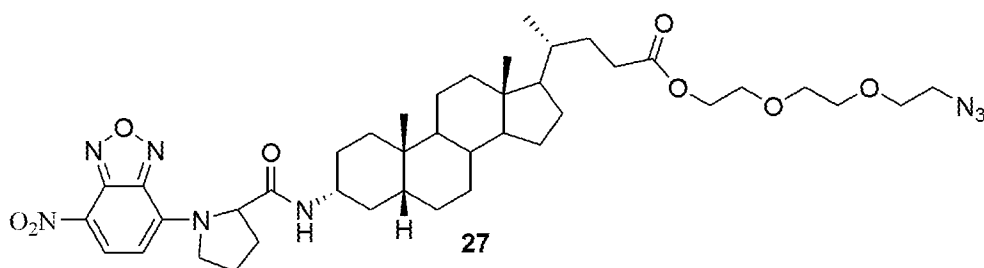
- b, R₃ = CH₂CH₂COOH
 d, R₃ = CH₂SH
 f, R₃ = CH₂-Ph-4-OH
 g, R₃ = 3-CH₂-indolylNH
 h, R₃ = CH₂CH₂CH₂CH₂NH₂

20

Reagents and conditions: (a) Different amino acids, HBTU, DIPEA, CH₂Cl₂, rt, 3 h, **1-2-16**: **a**, 99 %, **b**, 95 %, **c**, 90 %, **d**, 75 %, **e**, 96 %, **f**, 98 %, **g**, 82 %, **h**, 91 %, **i**, 62 %, (b) Pd(Ph₃)₄, PhSiH₃, CH₂Cl₂, rt, 2 h, (c) **1C**, DCC, DMAP, CH₂Cl₂, rt, 12 h, **1-2-17**: **a**, 74 %, **b**, 65 %, **c**, 73 %, **d**, 72 %, **e**, 60 %, **f**, 68 %, **g**, 70 %, **h**, 60 %, **i**, 55 % (two steps), (d) DBU, CH₂Cl₂, rt, 1 h, **1-2-18**: **a**, 98 %, **b**, 88 %, **c**, 90 %, **d**, 92 %, **e**, 85 %, **f**, 88 %, **g**, 91 %, **h**, 85 %, **i**, 90 % (e) NBD-Cl, NaHCO₃, THF, EtOH, rt, 2 days, **1-2-19**: **a**, 33 %, **b**, 35 %, **c**, 42 %, **d**, 38 %, **e**, 35 %, **f**, 38 %, **g**, 40 %, **h**, 31 %, **i**, 30 %, (f) TFA, H₂O, CH₂Cl₂, rt, 3 h, **1-2-21**: **b**, 92 %, **f**, 95 %, **g**, 85 %, **h**, 80 %, (g) TFA, TIPS, H₂O, CH₂Cl₂, rt, 4 h, **1-2-21**: **d**, 88 %, **41i**, 85 %.

[00241] **Scheme 8:**





5

Reagents and conditions: (a) Fmoc-Pro-OH, HBTU, DIPEA, CH₂Cl₂, rt, 3 h, **1-2-16j**, 98 %, (b) Pd(Ph₃)₄, PhSiH₃, CH₂Cl₂, rt, 2 h, (c) **1C**, DCC, DMAP, CH₂Cl₂, rt, 12 h, **1-2-17 j**, 78 % (two steps), (d) DBU, CH₂Cl₂, rt, 1 h, **1-2-18j**, 98 %, (e) NBD-Cl, NaHCO₃, THF, EtOH, rt, 2 h, **27**, 88 %.

10 [00242] *General Procedures for De-protecting Trt Group*

[00243] The compound **1-2-19d** (i.e., the Trt protected compound) (0.1 mmol) was dissolved in 10 mL TFA and 10 mL CH₂Cl₂ was added ddH₂O (500 μL) and TIPS (0.2 mmol) and the reaction mixture was stirred at room temperature for 4 h, then reaction mass was concentrated and product was purified by flash column chromatography to get target compound **1-2-20d** (or compound **19**).

15

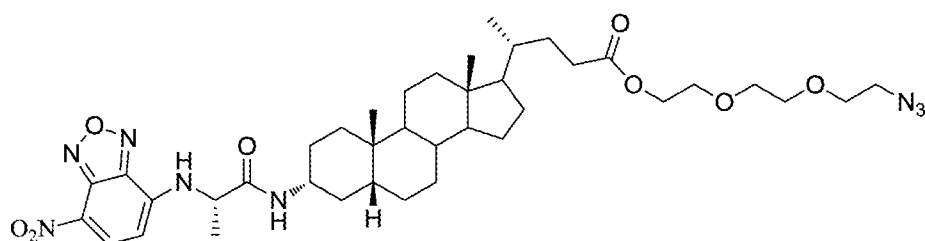
[00244] *General procedurs for de-protecting Pbf Group*

[00245] The compound **1-2-19i** (i.e., the Pbf protected compound) (0.05 mmol) was dissolved in 5 mL TFA and 5 mL CH₂Cl₂ was added ddH₂O (250 μL) and TIPS (0.1 mmol) and the reaction mixture was stirred at room temperature for 4 h, then reaction mass was concentrated and product was purified by flash column chromatography to get target compound **1-2-20i** (or compound **23**).

20

[00246] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-10,13-dimethyl-3-((S)-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-19a** (Compound **18**)

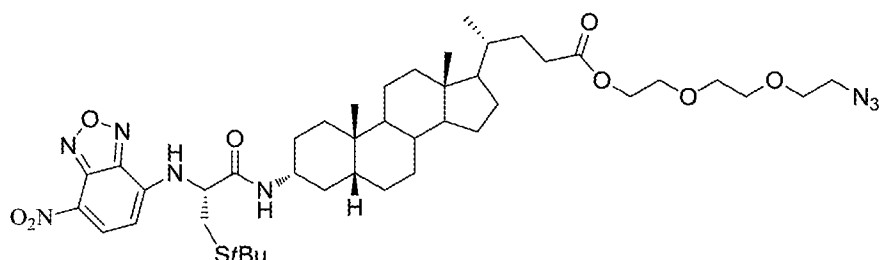
25



[00247] mp 69-70 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.44 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 6.4 Hz, 1H), 6.15 (d, *J* = 8.4 Hz, 1H), 5.95 (d, *J* = 8.0 Hz, 1H), 4.30 (brs, 1H), 4.21 (t, *J* = 4.8 Hz, 2H), 3.86-3.77 (m, 1H), 3.70-3.65 (m, 8H), 3.37 (t, *J* = 4.8 Hz, 2H), 2.38-2.31 (m, 1H), 2.25-2.17 (m, 1H), 1.92-1.89 (m, 1H), 1.86-1.71 (m, 6H), 1.64 (d, *J* = 6.8 Hz, 3H), 1.50-1.45 (m, 2H), 1.42-1.25 (m, 6H), 1.23-1.12 (m, 6H), 1.07-0.94 (m, 5H), 0.90 (s, 3H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.4, 169.4, 144.3, 143.9, 142.3, 135.9, 125.0, 100.1, 70.7, 70.6, 70.1, 69.3, 63.4, 56.7, 56.1, 53.7, 50.7, 50.1, 42.7, 42.3, 40.6, 40.2, 35.7, 35.6, 35.3, 34.5, 33.4, 31.1, 30.9, 28.2, 27.8, 26.9, 26.3, 24.1, 23.5, 20.8, 19.1, 18.3, 12.0. HRMS calcd for C₃₉H₅₈N₈O₈Na (M + Na)⁺, 789.4275; found, 789.4280.

[00248] 2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-19c**

(Compound **20**)

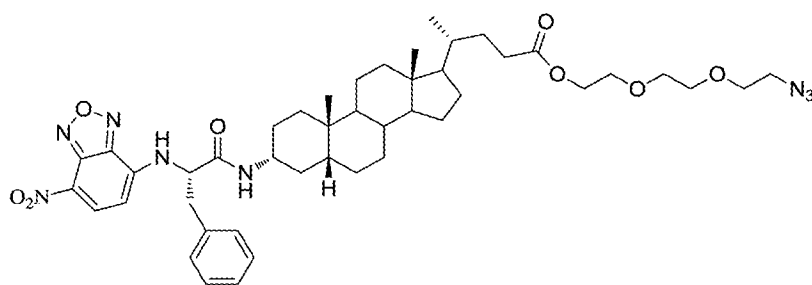


[00249] mp 64-65 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.43 (d, *J* = 8.4 Hz, 1H), 7.13 (brs, 1H), 6.28 (d, *J* = 8.0 Hz, 1H), 6.17 (d, *J* = 8.4 Hz, 1H), 4.30-4.27 (m, 1H), 4.19 (t, *J* = 4.8 Hz, 2H), 3.84-3.78 (m, 1H), 3.69-3.64 (m, 8H), 3.36 (t, *J* = 4.8 Hz, 2H), 3.12 (dd, *J* = 6.4, 12.8 Hz, 1H), 3.04 (dd, *J* = 6.4, 12.8 Hz, 1H), 2.36-2.29 (m, 1H),

2.24-2.14 (m, 1H), 1.90-1.87 (m, 1H), 1.84-1.73 (m, 5H), 1.68-1.59 (m, 2H), 1.50-1.39 (m, 3H), 1.34 (s, 9H), 1.30-1.13 (m, 9H), 0.89 (s, 5H), 0.84 (d, $J = 6.4$ Hz, 3H), 0.58 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 174.2, 167.3, 144.3, 143.7, 142.2, 135.8, 125.4, 100.4, 70.7, 70.6, 70.0, 69.2, 63.4, 57.2, 56.5, 56.0, 50.7, 50.3, 43.7, 42.7, 42.3, 40.5, 40.1, 35.7, 35.2, 34.5, 33.3, 31.1, 30.9, 30.8, 28.1, 27.7, 26.9, 26.3, 24.1, 23.4, 20.7, 18.2, 12.0. HRMS calcd for $\text{C}_{42}\text{H}_{66}\text{N}_8\text{O}_8\text{SNa}$ ($\text{M} + \text{Na}$) $^+$, 877.4617; found, 877.4654.

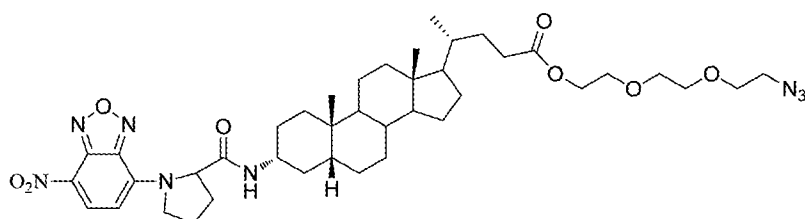
[00250] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-10,13-dimethyl-3-((S)-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-3-phenylpropanamido)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-19e**

(Compound **25**)



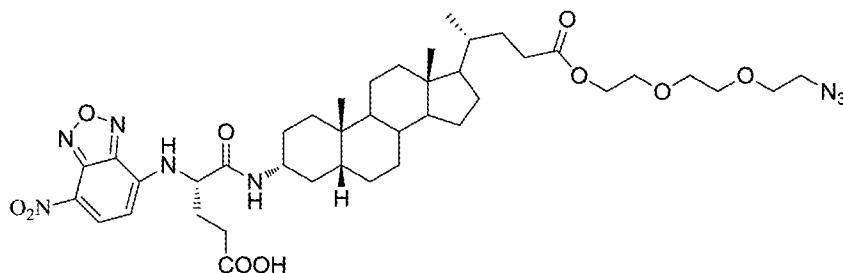
[00251] mp 65-66 °C, ^1H NMR (400 MHz, CDCl_3): δ 8.40 (d, $J = 8.4$ Hz, 1H), 7.36-7.25 (m, 5H), 6.91 (d, $J = 6.8$ Hz, 1H), 6.14 (d, $J = 8.4$ Hz, 1H), 5.55 (d, $J = 8.0$ Hz, 1H), 4.49-4.46 (m, 1H), 4.20 (t, $J = 4.8$ Hz, 2H), 3.72-3.65 (m, 9H), 3.37 (t, $J = 4.8$ Hz, 2H), 3.30 (dd, $J = 6.0, 13.6$ Hz, 1H), 3.18 (dd, $J = 6.0, 13.6$ Hz, 1H), 2.38-2.31 (m, 1H), 2.25-2.15 (m, 1H), 1.92-1.89 (m, 1H), 1.83-1.72 (m, 6H), 1.50-1.47 (m, 1H), 1.44-1.41 (m, 3H), 1.40-1.36 (m, 1H), 1.33-1.21 (m, 5H), 1.19-1.15 (m, 3H), 1.03-0.91 (m, 6H), 0.87 (d, $J = 6.0$ Hz, 3H), 0.85 (s, 3H), 0.59 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 174.3, 167.8, 144.2, 143.9, 142.4, 135.8, 135.2, 129.3, 129.2, 127.8, 100.3, 70.7, 70.6, 70.1, 69.3, 63.4, 59.3, 56.6, 56.0, 50.7, 50.0, 42.7, 42.2, 40.5, 40.2, 39.2, 35.7, 35.3, 34.5, 33.3, 31.1, 30.9, 28.2, 27.4, 26.9, 26.3, 24.1, 23.4, 20.7, 18.2, 12.0. HRMS calcd for $\text{C}_{45}\text{H}_{62}\text{N}_8\text{O}_8\text{Na}$ ($\text{M} + \text{Na}$) $^+$, 865.4588; found, 865.4579.

[00252] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-10,13-dimethyl-3-(1-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)pyrrolidine-2-carboxamido)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-19j** (Compound **27**)



[00253] mp 62-64 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.37 (d, *J* = 9.2 Hz, 1H), 6.08 (d, *J* = 9.2 Hz, 1H), 6.02 (s, 1H), 4.20 (t, *J* = 4.8 Hz, 2H), 3.86-3.65 (m, 10H), 3.37 (t, *J* = 4.8 Hz, 2H), 2.52-2.14 (m, 8H), 1.90-1.87 (m, 1H), 1.81-1.68 (m, 4H), 1.64-1.61 (m, 1H), 1.58-1.54 (m, 1H), 1.48-1.46 (m, 1H), 1.37-1.10 (m, 13H), 1.04-0.90 (m, 5H), 0.86 (d, *J* = 4.0 Hz, 3H), 0.84 (s, 3H), 0.58 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 174.3, 169.6, 144.8, 144.4, 143.5, 135.4, 123.0, 102.3, 70.7, 70.6, 70.1, 69.3, 65.7, 63.4, 56.6, 56.1, 50.7, 50.0, 42.7, 42.3, 40.6, 40.2, 35.7, 35.3, 34.5, 33.2, 31.7, 31.2, 31.0, 28.2, 27.6, 26.8, 26.3, 24.1, 23.4, 20.8, 18.2, 12.0. HRMS calcd for C₄₁H₆₀N₈O₈Na (M + Na)⁺, 815.4426; found, 815.4429.

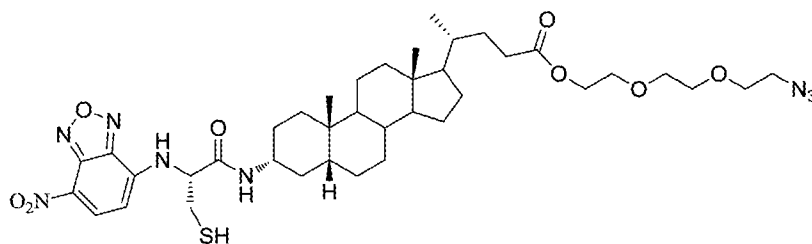
[00254] (4S)-5-(((3R,5R,10S,13R)-17-((R)-5-(2-(2-azidoethoxy)ethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)amino)-4-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-5-oxopentanoic acid. **1-2-20b** (Compound **21**)



[00255] mp 77-78 °C, ¹H NMR (500 MHz, CDCl₃): δ 8.38 (d, *J* = 8.0 Hz, 1H), 8.05 (brs, 1H), 6.98 (brs, 1H), 6.27 (d, *J* = 8.0 Hz, 1H), 4.48-4.46 (m, 1H), 4.22 (t, *J* =

4.5 Hz, 2H), 3.87-3.81 (m, 1H), 3.71-3.66 (m, 8H), 3.38 (t, $J = 4.5$ Hz, 2H), 2.68-2.63 (m, 2H), 2.36-2.32 (m, 3H), 2.22-2.20 (m, 1H), 1.84-1.52 (m, 7H), 1.45-1.15 (m, 14H), 1.01-0.90 (m, 4H), 0.86 (s, 3H), 0.81 (d, $J = 5.5$ Hz, 3H), 0.70-0.69 (m, 1H), 0.54 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3): δ 176.9, 174.7, 169.1, 144.4, 143.7, 143.3, 136.6, 124.3, 100.4, 70.7, 70.6, 70.1, 69.2, 63.5, 58.0, 56.6, 56.1, 50.7, 50.2, 42.6, 42.3, 40.5, 40.1, 35.7, 35.6, 35.3, 34.4, 33.0, 31.2, 31.0, 30.1, 28.2, 27.5, 27.3, 26.9, 26.2, 24.1, 23.4, 20.7, 18.2, 11.9. HRMS calcd for $\text{C}_{41}\text{H}_{60}\text{N}_8\text{O}_{10}\text{Na}$ ($\text{M} + \text{Na}$) $^+$, 847.4324; found, 847.4342.

[00256] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-3-((R)-3-mercapto-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-20d**
(Compound **19**)

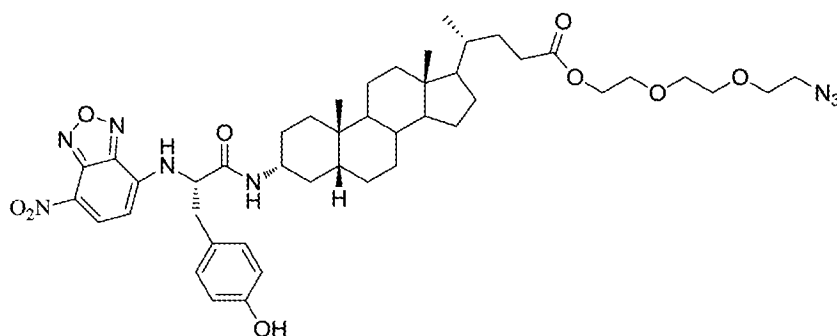


[00257] mp 68-69 °C, ^1H NMR (500 MHz, CDCl_3): δ 8.43 (d, $J = 8.0$ Hz, 1H), 7.20 (d, $J = 4.5$ Hz, 1H), 6.40 (d, $J = 7.0$ Hz, 1H), 6.24 (d, $J = 8.0$ Hz, 1H), 4.50-4.41 (m, 1H), 4.20 (t, $J = 4.0$ Hz, 2H), 3.83-3.65 (m, 10H), 3.37 (t, $J = 4.0$ Hz, 2H), 3.22-3.19 (m, 1H), 3.05-2.98 (m, 1H), 2.35-2.31 (m, 1H), 2.23-2.20 (m, 1H), 1.88-1.86 (m, 1H), 1.78-1.62 (m, 6H), 1.49-1.43 (m, 3H), 1.32-1.18 (m, 10H), 1.04-0.95 (m, 6H), 0.88 (s, 3H), 0.83 (d, $J = 6.0$ Hz, 3H), 0.57 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3): δ 174.6, 166.9, 144.3, 143.9, 142.4, 136.0, 125.2, 100.9, 70.7, 70.6, 70.2, 69.3, 63.6, 59.3, 56.7, 56.0, 50.7, 50.4, 42.7, 42.4, 40.6, 40.2, 35.7, 35.4, 34.6, 33.4, 31.1, 30.9, 28.2, 27.9, 27.3, 26.9, 26.3, 24.2, 23.5, 20.8, 18.3, 12.1. HRMS calcd for $\text{C}_{39}\text{H}_{58}\text{N}_8\text{O}_8\text{SNa}$ ($\text{M} + \text{Na}$) $^+$, 821.3996; found, 821.3990.

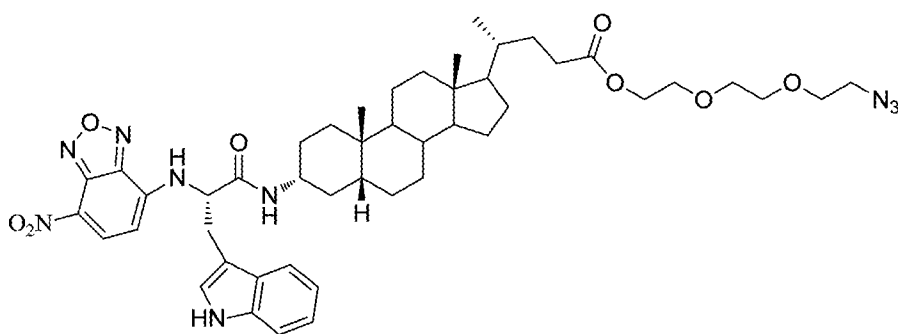
[00258] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-3-((S)-3-(4-hydroxyphenyl)-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)-10,1

3-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate.

1-2-20f (Compound 24)

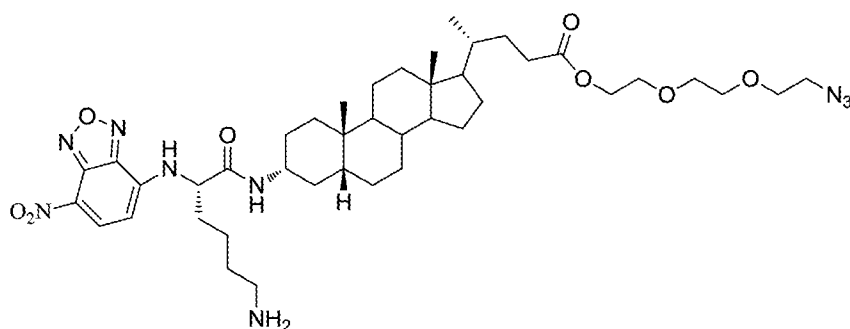


- 5 [00259] mp 72-73 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.34 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 4.4 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 8.4 Hz, 2H), 6.43 (d, *J* = 4.4 Hz, 1H), 6.15 (d, *J* = 8.4 Hz, 1H), 4.56-4.41 (m, 1H), 4.17 (t, *J* = 5.2 Hz, 2H), 3.84-3.73 (m, 1H), 3.70-3.63 (m, 8H), 3.36 (t, *J* = 5.2 Hz, 2H), 3.18 (d, *J* = 5.2 Hz, 2H), 2.36-2.28 (m, 1H), 2.24-2.16 (m, 1H), 1.86-1.80 (m, 1H), 1.73-1.58 (m, 5H),
- 10 1.55-1.48 (m, 1H), 1.42-1.34 (m, 4H), 1.25-1.20 (m, 5H), 1.15-1.10 (m, 5H), 0.99-0.87 (m, 3H), 0.84-0.81 (m, 9H), 0.55 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 174.7, 168.7, 155.8, 144.2, 143.8, 143.1, 136.4, 130.4, 126.5, 124.1, 116.0, 100.4, 70.6, 70.5, 70.0, 69.2, 63.5, 59.5, 56.5, 56.0, 50.6, 50.1, 42.6, 42.2, 40.4, 40.1, 38.4, 35.6, 35.3, 34.4, 33.1, 31.1, 30.9, 28.2, 27.3, 26.9, 26.2, 24.0, 23.4, 20.7,
- 15 18.2, 11.9. HRMS calcd for C₄₅H₆₂N₈O₉Na (M + Na)⁺, 881.4532; found, 881.4567. [00260] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-3-((S)-3-(1H-indol-3-yl)-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)propanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-20g (Compound 26)**



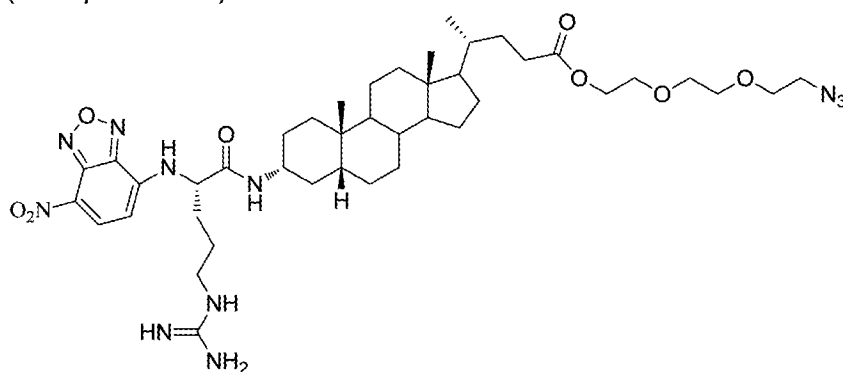
[00261] mp 86-87 °C, ¹H NMR (500 MHz, CDCl₃): δ 8.63 (s, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 2.0 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.13 (d, *J* = 8.5 Hz, 2H), 4.63-4.50 (m, 1H), 4.19 (t, *J* = 5.0 Hz, 2H), 3.74-3.59 (m, 9H), 3.50 (dd, *J* = 6.0, 14.5 Hz, 1H), 3.43 (dd, *J* = 6.0, 14.5 Hz, 1H), 3.36 (t, *J* = 5.0 Hz, 2H), 2.32-2.27 (m, 1H), 2.21-2.14 (m, 1H), 1.84-1.81 (m, 1H), 1.76-1.60 (m, 4H), 1.43-1.29 (m, 5H), 1.25-1.02 (m, 10H), 0.95-0.86 (m, 4H), 0.83 (s, 3H), 0.80 (d, *J* = 6.5 Hz, 3H), 0.76-0.70 (m, 2H), 0.53 (s, 3H). ¹³C NMR (125 MHz, CDCl₃): δ 174.5, 169.2, 144.1, 143.6, 142.8, 136.3, 136.2, 127.0, 124.4, 123.6, 122.6, 120.0, 118.3, 111.6, 108.9, 100.5, 70.5, 70.4, 70.0, 69.2, 63.4, 58.4, 56.5, 55.9, 50.6, 50.2, 42.7, 42.5, 42.1, 40.3, 40.0, 35.5, 35.2, 34.3, 32.7, 31.1, 30.8, 29.0, 28.1, 27.1, 26.8, 26.1, 24.0, 23.3, 20.6, 18.1, 11.9. HRMS calcd for C₄₇H₆₃N₉O₈Na (M + Na)⁺, 904.4692; found, 904.4696.

[00262] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-3-((S)-6-amino-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-20h**
(Compound **22**)



[00263] mp 84-85, ^1H NMR (400 MHz, CDCl_3): δ 8.26 (d, $J = 6.0$ Hz, 1H), 8.12 (d, $J = 2.4$ Hz, 1H), 7.91 (brs, 2H), 7.27 (brs, 1H), 6.29-6.22 (m, 1H), 4.21 (t, $J = 4.8$ Hz, 2H), 3.71-3.65 (m, 9H), 3.37 (t, $J = 4.8$ Hz, 2H), 3.03-2.99 (m, 1H), 2.70-2.65 (m, 3H), 2.35-2.33 (m, 1H), 2.25-2.18 (m, 1H), 2.04-2.02 (m, 2H), 1.84-1.54 (m, 10H), 1.42-1.16 (m, 13H), 0.94-0.83 (m, 12H), 0.57 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 174.5, 169.7, 144.2, 143.8, 137.1, 123.1, 115.2, 100.3, 70.7, 70.6, 70.1, 69.2, 63.5, 58.2, 56.4, 56.2, 50.7, 50.4, 42.6, 42.5, 40.4, 40.0, 39.5, 35.7, 35.4, 34.5, 32.9, 31.9, 31.4, 31.0, 28.2, 27.3, 27.0, 26.7, 26.3, 24.1, 23.5, 22.3, 20.8, 18.2, 12.0. HRMS calcd for $\text{C}_{42}\text{H}_{66}\text{N}_9\text{O}_8$ ($\text{M} + \text{H}$) $^+$, 824.5028; found, 824.5016.

[00264] (4R)-2-(2-(2-azidoethoxy)ethoxy)ethyl-4-((3R,5R,10S,13R)-3-((S)-5-guanidino-2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)pentanamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate. **1-2-19j**
(Compound **23**)



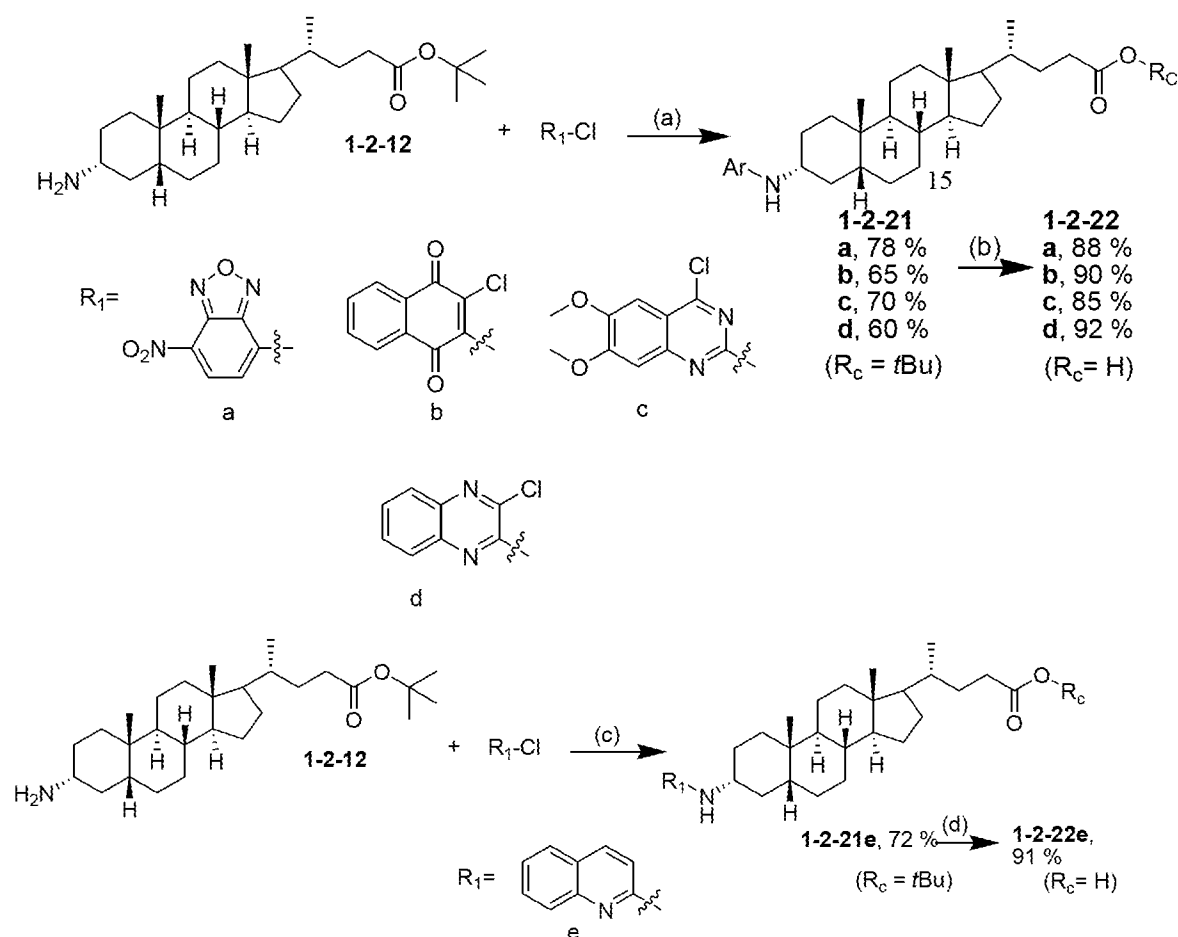
[00265] mp 78-79 $^\circ\text{C}$, ^1H NMR (500 MHz, $\text{d}_6\text{-DMSO}$): δ 9.26 (s, 1H), 8.58 (d, $J = 6.5$ Hz, 1H), 8.22 (s, 1H), 7.70 (s, 1H), 7.38-6.95 (m, 3H), 6.27 (d, $J = 6.5$ Hz, 1H), 4.30-4.20 (m, 1H), 4.10 (t, $J = 6.0$ Hz, 2H), 3.71-3.64 (m, 2H), 3.60-3.54 (m, 8H),

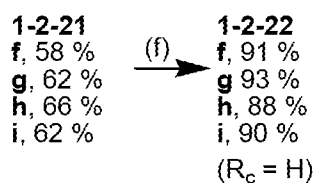
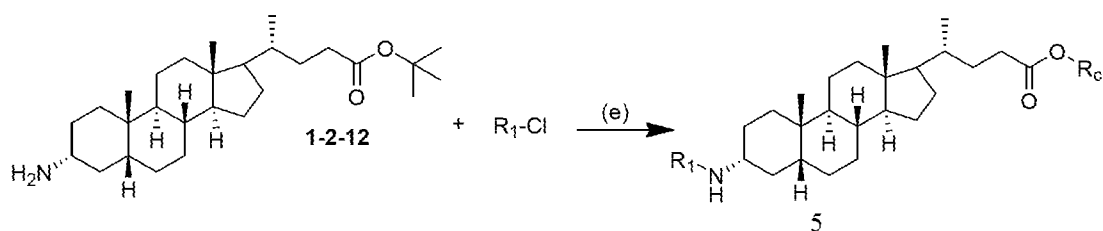
3.11 (t, $J = 6.0$ Hz, 2H), 2.35-2.29 (m, 1H), 2.23-2.17 (m, 1H), 1.92-1.90 (m, 3H), 1.80-1.78 (m, 2H), 1.72-1.68 (m, 3H), 1.58-1.52 (m, 3H), 1.43-1.42 (m, 1H), 1.39-1.27 (m, 7H), 1.21-1.18 (m, 6H), 0.88 (s, 3H), 0.85 (d, $J = 5.5$ Hz, 3H), 0.59 (s, 3H). ^{13}C NMR (125 MHz, d_6 -DMSO): δ 173.4, 168.6, 158.5, 156.7, 144.5, 144.1, 137.9, 121.9, 99.9, 70.6, 69.8, 69.7, 69.4, 68.4, 63.1, 57.4, 56.2, 55.7, 50.0, 49.0, 43.6, 42.3, 41.9, 40.4, 35.6, 35.4, 34.8, 34.3, 32.8, 30.6, 28.7, 27.8, 27.2, 26.7, 26.2, 25.4, 23.9, 23.3, 20.4, 18.1, 11.9. HRMS calcd for $\text{C}_{42}\text{H}_{64}\text{N}_{11}\text{O}_8$ ($M - \text{H}$) $^-$, 850.4939; found, 850.4932.

[00266] 1.3 Synthesis of Compounds 28 to 40

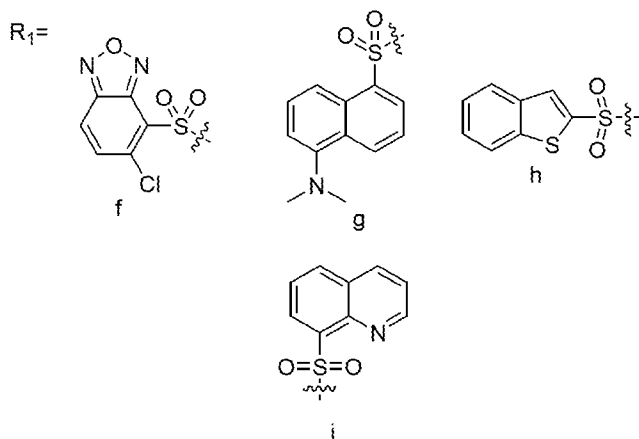
[00267] The compounds 28 to 40 (i.e., compounds of formula (1-3)) were synthesized in accordance with procedures set forth in **Scheme 9**.

[00268] Scheme 9:





10



15

Reagents and conditions: (a) Different R₁-Cl (a-d), NEt₃, 1-BuOH, 120 °C, 12 h, (b) TFA, H₂O, CH₂Cl₂, rt, 3 h, (c) R₁-Cl (e), Pd₂(dba)₃, P(*t*Bu)₃HBF₄, Cs₂CO₃, 1,4-dioxane, 120 °C, 4h, (d) TFA, H₂O, CH₂Cl₂, rt, 3 h, (e) Different R₁-Cl (f-i), DIPEA, CH₂Cl₂, rt, 6 h, (f) TFA, H₂O, CH₂Cl₂, rt, 3 h.

[00269] *General Procedures for SN-R₁ Reaction*

[00270] The compound **1-2-12** (1.02 mmol), R₁-Cl (1.02 mmol) and NEt₃ (2.04 mmol) were dissolved in 10 mL 1-butanol and the reaction mixture was stirred at 120 °C for 12 h. The reaction mass was concentrated and product was purified by flash column chromatography to get target compound.

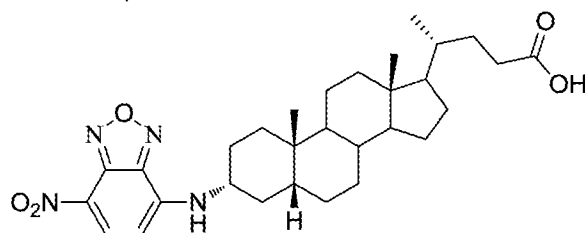
[00271] *General Procedures for Amination*

[00272] To a stirred solution of **1-2-12** (1.02 mmol) and R₁-Cl (1.02 mmol) in 10 mL 1,4-dioxane was added Pd₂(dba)₃ (0.10 mmol), P(*t*Bu)₃HBF₄ (0.20 mmol) and Cs₂CO₃ (2.04 mmol). The reaction mixture was stirred at 120 °C for 4 h. The reaction mass was concentrated and product was purified by flash column chromatography to get target compound.

[00273] *General Procedure for Sulforamide Formation*

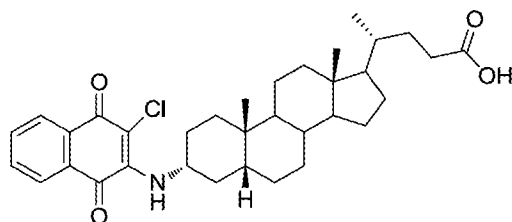
[00274] To a stirred solution of **1-2-12** (1.11 mmol) and R₁-Cl (1.33 mmol) in 10 mL CH₂Cl₂ was added DIPEA (1.67 mmol) dropwise. The reaction mixture was stirred at rt for 6 h. The reaction mass was concentrated and product was purified by flash column chromatography to get target compound.

[00275] (4R)-4-((3R,5R,10S,13R)-10,13-dimethyl-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22a** (compound **28**)



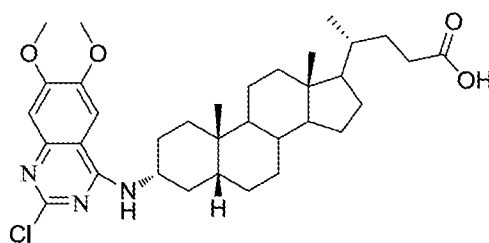
[00276] mp 137-139 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.45 (d, *J* = 8.8 Hz, 1H), 6.22 (d, *J* = 8.0 Hz, 1H), 6.16 (d, *J* = 8.8 Hz, 1H), 3.72-3.66 (m, 1H), 2.42-2.34 (m, 1H), 2.28-2.20 (m, 1H), 2.02-1.73 (m, 7H), 1.62-1.52 (m, 2H), 1.50-1.36 (m, 5H), 1.35-1.22 (m, 5H), 1.21-1.01 (m, 7H), 0.99 (s, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.64 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 179.1, 144.4, 144.0, 142.9, 136.6, 123.5, 98.6, 56.5, 56.0, 54.1, 42.7, 42.5, 40.7, 40.1, 35.8, 35.6, 35.3, 34.8, 33.0, 30.8, 30.7, 28.1, 27.2, 27.0, 26.3, 24.1, 23.5, 20.9, 18.2, 12.0. HRMS calcd for C₃₀H₄₁N₄O₅ (M- H)⁻, 537.3077; found, 537.3085.

[00277] (4R)-4-((3R,5R,10S,13R)-3-((3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22b** (compound **32**)



[00278] mp 120-122 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.12 (d, *J* = 7.2 Hz, 1H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.59 (t, *J* = 7.2 Hz, 1H), 6.01 (brs, 1H), 4.45-4.35 (m, 1H), 2.42-2.34 (m, 1H), 2.28-2.20 (m, 1H), 2.02-1.94 (m, 1H),
 5 1.89-1.72 (m, 6H), 1.69-1.61 (m, 1H), 1.59-1.47 (m, 2H), 1.43-1.23 (m, 10H), 1.18-1.02 (m, 6H), 0.94 (s, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 180.6, 179.5, 176.8, 143.5, 134.9, 132.9, 132.3, 129.7, 126.8, 56.5, 56.0, 53.8, 42.7, 42.3, 40.6, 40.1, 35.8, 35.5, 35.4, 35.3, 34.6, 30.9, 30.8, 29.6, 28.1, 27.1, 26.4, 24.2, 23.4, 20.8, 18.2, 12.0. HRMS calcd for C₃₄H₄₃NO₄Cl (M-H)⁻, 564.2881; found, 564.2872.

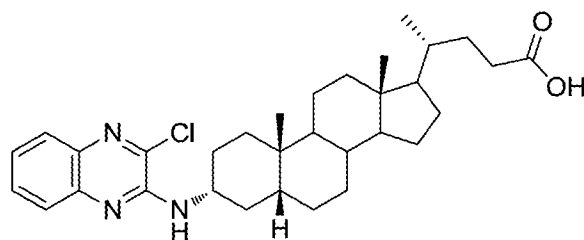
[00279] (4R)-4-((3R,5R,10S,13R)-3-((2-chloro-6,7-dimethoxyquinazolin-4-yl)amino)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22c** (compound **33**)



[00280] mp 178-180 °C, ¹H NMR (400 MHz, d₆-DMSO): δ 8.00 (d, *J* = 3.6 Hz, 1H), 7.63 (s, 1H), 7.04 (s, 1H), 4.21-4.10 (m, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 2.27-2.20 (m, 1H), 2.14-2.06 (m, 1H), 1.99-1.73 (m, 6H), 1.66-1.64 (m, 2H), 1.50-1.48 (m, 5H), 1.41-1.35 (m, 4H), 1.26-1.15 (m, 4H), 1.12-1.04 (m, 5H), 0.94
 20 (s, 3H), 0.87 (d, *J* = 6.0 Hz, 3H), 0.62 (s, 3H). ¹³C NMR (100 MHz, d₆-DMSO): δ 174.9, 159.1, 155.1, 154.4, 148.4, 147.1, 106.8, 106.4, 102.4, 56.2, 56.1, 55.8, 55.7, 50.4, 42.3, 42.0, 35.7, 35.3, 34.9, 34.3, 33.1, 32.5, 30.8, 30.6, 27.7, 26.7,

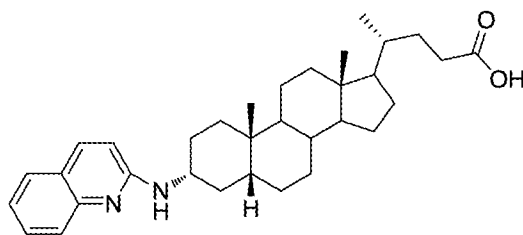
26.2, 23.8, 23.2, 20.4, 18.1, 11.8. HRMS calcd for $C_{34}H_{48}N_3O_4Cl$ ($M + H$)+, 598.3406; found, 598.3396.

[00281] (4R)-4-((3R,5R,10S,13R)-3-((3-chloroquinoxalin-2-yl)amino)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22d**
 5 (compound **34**)



[00282] mp 118-120 °C, ^1H NMR (400 MHz, $CDCl_3$): δ 7.79-7.73 (m, 2H), 7.59 (t, $J = 8.4$ Hz, 1H), 7.40 (t, $J = 8.4$ Hz, 1H), 5.69-5.67 (m, 1H), 4.15-4.09 (m, 1H), 2.43-2.35 (m, 1H), 2.29-2.21 (m, 1H), 1.98-1.95 (m, 1H), 1.92-1.73 (m, 6H),
 10 1.58-1.55 (m, 2H), 1.40-1.36 (m, 6H), 1.35-1.00 (m, 11H), 0.97 (s, 3H), 0.91 (d, $J = 6.4$ Hz, 3H), 0.64 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.3, 146.5, 139.0, 138.0, 135.7, 130.9, 128.0, 125.5, 124.4, 56.6, 56.0, 51.8, 42.8, 42.3, 40.7, 40.2, 35.9, 35.6, 35.3, 34.7, 33.4, 30.8, 28.2, 27.6, 27.1, 26.4, 24.2, 23.5, 20.9, 18.3, 12.1. HRMS calcd for $C_{32}H_{45}N_3O_2Cl$ ($M + H$)+, 538.3194; found, 538.3205.

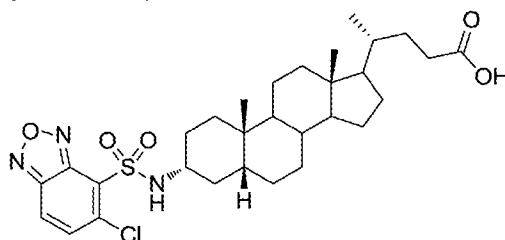
[00283] (4R)-4-((3R,5R,10S,13R)-10,13-dimethyl-3-(quinolin-2-ylamino)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22e** (compound **30**)
 15



[00284] mp 140-142 °C, ^1H NMR (400 MHz, $CDCl_3$): δ 10.43 (d, $J = 5.2$ Hz, 1H), 8.05 (d, $J = 9.2$ Hz, 1H), 7.82 (d, $J = 9.2$ Hz, 1H), 7.67-7.62 (m, 2H), 7.35 (t, $J = 8.0$ Hz, 1H), 6.85 (d, $J = 9.6$ Hz, 1H), 3.46-3.44 (m, 1H), 2.42-2.34 (m, 1H), 2.31-2.19 (m, 1H), 2.03-1.92 (m, 3H), 1.89-1.76 (m, 4H), 1.70-1.52 (m, 3H), 1.48-1.21 (m,
 20

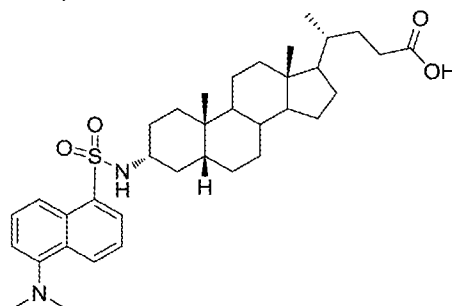
11H), 1.18-1.00 (m, 5H), 0.96 (s, 3H), 0.90 (d, $J = 6.4$ Hz, 3H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 179.3, 152.8, 143.2, 137.7, 133.0, 128.0, 124.8, 120.5, 118.1, 109.5, 55.9, 55.8, 54.0, 42.8, 42.7, 40.4, 39.8, 35.9, 35.8, 35.3, 34.8, 33.2, 31.0, 30.8, 28.1, 27.3, 27.2, 26.3, 24.1, 23.6, 20.9, 18.2, 12.0. HRMS calcd for $\text{C}_{33}\text{H}_{47}\text{N}_2\text{O}_2$ ($\text{M} + \text{H}$) $^+$, 503.3638; found, 503.3638.

[00285] (4R)-4-((3R,5R,10S,13R)-3-(5-chlorobenzo[c][1,2,5]oxadiazole-4-sulfonamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22f** (compound **36**)



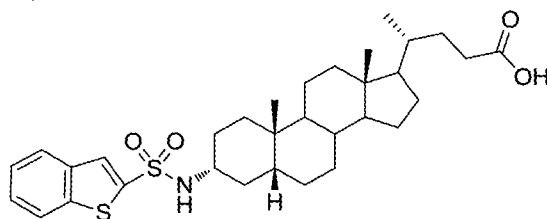
[00286] mp 128-130 $^{\circ}\text{C}$, ^1H NMR (400 MHz, CDCl_3): δ 7.96 (d, $J = 9.2$ Hz, 1H), 7.47 (d, $J = 9.2$ Hz, 1H), 5.49 (d, $J = 7.6$ Hz, 1H), 3.39-3.29 (m, 1H), 2.41-2.33 (m, 1H), 2.27-2.19 (m, 1H), 1.92-1.90 (m, 1H), 1.87-1.66 (m, 5H), 1.58-1.50 (m, 2H), 1.42-1.08 (m, 15H), 1.06-0.95 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.86 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 179.3, 148.0, 146.5, 138.3, 135.7, 128.2, 120.6, 56.4, 55.9, 54.9, 45.7, 42.7, 42.4, 40.4, 40.0, 35.7, 35.6, 35.3, 34.4, 34.3, 30.8, 30.7, 28.7, 28.1, 26.8, 26.2, 24.1, 23.3, 20.7, 18.2, 12.0. HRMS calcd for $\text{C}_{30}\text{H}_{42}\text{N}_3\text{O}_5\text{ClNa}$ ($\text{M} + \text{Na}$) $^+$, 614.2456; found, 614.2422.

[00287] (4R)-4-((3R,5R,10S,13R)-3-(5-(dimethylamino)naphthalene-1-sulfonamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid. **1-2-22g** (compound **37**)



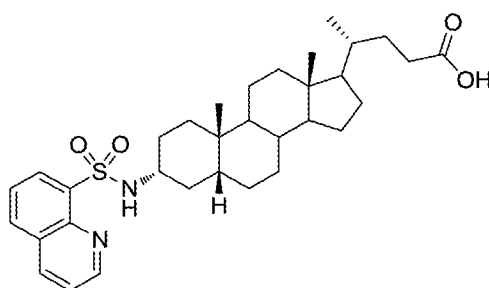
[00288] mp 136-138 °C, ¹H NMR (400 MHz, CDCl₃): δ 8.55 (d, *J* = 8.8 Hz, 1H), 8.47 (d, *J* = 8.8 Hz, 1H), 8.29 (dd, *J* = 1.2, 7.6 Hz, 1H), 7.62-7.56 (m, 2H), 7.38 (d, *J* = 7.6 Hz, 1H), 6.75 (s, 1H), 4.97 (d, *J* = 6.4 Hz, 1H), 3.09 (s, 6H), 2.39-2.31 (m, 1H), 2.25-2.17 (m, 1H), 1.92-1.88 (m, 1H), 1.86-1.56 (m, 5H), 1.55-1.48 (m, 1H), 1.43-1.16 (m, 12H), 1.14-0.95 (m, 7H), 0.86 (d, *J* = 6.0 Hz, 3H), 0.80 (s, 3H), 0.57 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 179.5, 147.0, 136.6, 129.6, 128.7, 128.5, 127.7, 124.7, 122.2, 116.3, 56.3, 55.9, 54.2, 45.9, 42.6, 42.5, 40.3, 40.0, 35.8, 35.6, 35.3, 34.6, 34.3, 30.9, 30.7, 28.8, 28.1, 26.8, 26.2, 24.1, 23.3, 20.7, 18.2, 11.9. HRMS calcd for C₃₆H₅₃N₂O₄S (M + H)⁺, 609.3726; found, 609.3718.

[00289] (4R)-4-((3R,5R,10S,13R)-3-(benzo[*b*]thiophene-2-sulfonamido)-10,13-dimethylhexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)pentanoic acid. **1-2-22h** (compound **38**)



[00290] mp 132-134 °C, ¹H NMR (400 MHz, CDCl₃): δ 7.86-7.82 (m, 3H), 7.48-7.41 (m, 2H), 4.88 (d, *J* = 7.6 Hz, 1H), 3.30-3.21 (m, 1H), 2.41-2.34 (m, 1H), 2.27-2.19 (m, 1H), 1.92-1.89 (m, 1H), 1.86-1.68 (m, 5H), 1.65-1.60 (m, 1H), 1.53-1.46 (m, 2H), 1.43-1.21 (m, 10H), 1.17-1.12 (m, 2H), 1.09-0.91 (m, 5H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.85 (s, 3H), 0.60 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 179.9, 142.5, 141.6, 137.6, 128.8, 127.0, 125.5, 125.3, 122.6, 56.3, 55.9, 54.5, 42.6, 42.5, 40.4, 40.0, 35.8, 35.7, 35.2, 34.5, 34.3, 30.9, 30.7, 28.8, 28.1, 26.8, 26.2, 24.1, 23.3, 20.7, 18.2, 11.9. HRMS calcd for C₃₂H₄₅NO₄S₂Na (M + Na)⁺, 594.2682; found, 594.2684.

[00291] (4R)-4-((3R,5R,10S,13R)-10,13-dimethyl-3-(quinoline-8-sulfonamido)hexadecahydro-1H-cyclopenta[*a*]phenanthren-17-yl)pentanoic acid. **1-2-22i** (compound **39**)

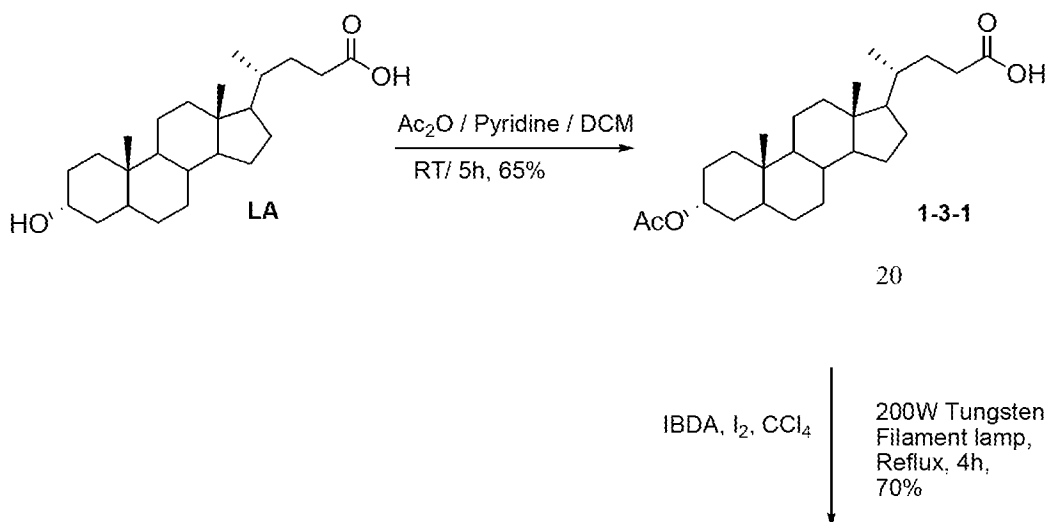


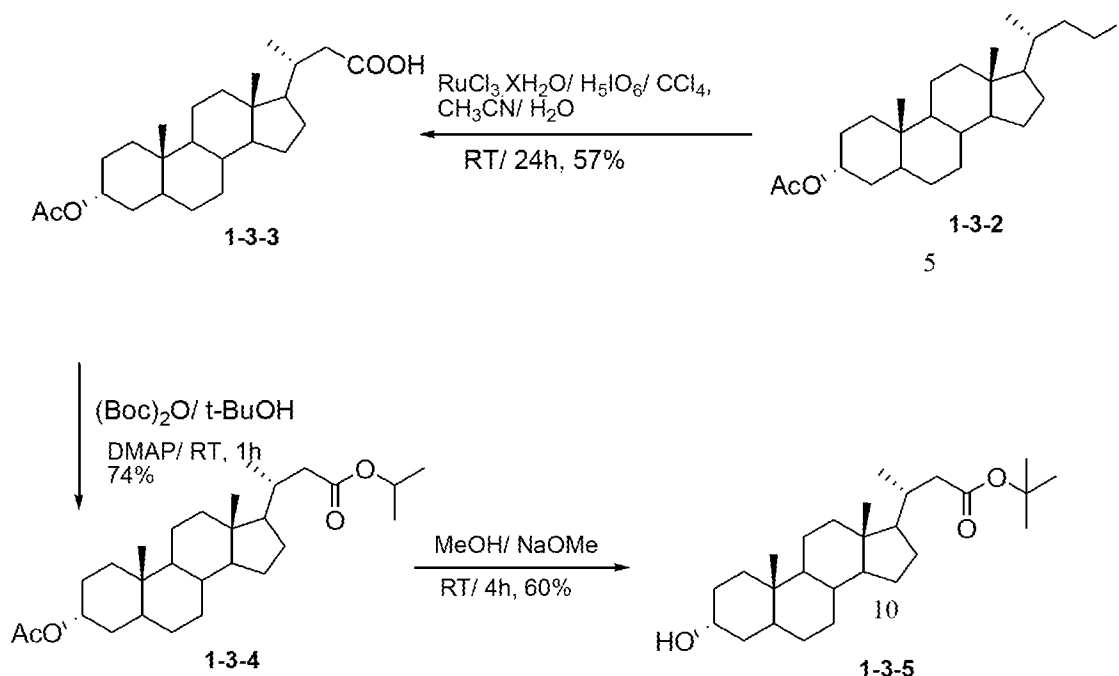
[00292] mp 138-140 °C, ¹H NMR (400 MHz, CDCl₃): δ 9.02 (dd, *J* = 1.6, 4.4 Hz, 1H), 8.41 (dd, *J* = 1.2, 7.6 Hz, 1H), 8.25 (dd, *J* = 1.6, 8.4 Hz, 1H), 8.02 (dd, *J* = 1.2, 8.4 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.54 (dd, *J* = 4.4, 8.0 Hz, 1H), 6.21 (brs, 1H),
 5 3.11-3.06 (m, 1H), 2.41-2.33 (m, 1H), 2.27-2.19 (m, 1H), 1.89-1.87 (m, 1H), 1.85-1.74 (m, 2H), 1.73-1.49 (m, 4H), 1.47-1.14 (m, 12H), 1.10-0.95 (m, 7H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.78 (s, 3H), 0.58 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 178.9, 150.9, 143.3, 137.5, 137.0, 132.9, 130.5, 128.8, 125.7, 122.1, 56.4, 55.9, 54.6, 42.7, 42.4, 40.4, 40.1, 35.7, 35.3, 34.5, 34.4, 30.8, 28.6, 28.1, 26.9, 26.2,
 10 24.1, 23.4, 20.7, 18.2, 12.0. HRMS calcd for C₃₃H₄₇N₂O₄S₂ (M + H)⁺, 567.3251; found, 567.3267.

[00293] **1.4 Synthesis of Compounds 41 to 48**

[00294] The compounds 41 to 48 (i.e., compounds of formula (1-3)) were synthesized in accordance with procedures set forth in **Schemes 10 to 19**.

15 [00295] **Scheme 10:**





[00296] *Synthesis of Compound 1-3-1*

[00297] To a solution of lithocholic acid (LA) (8 mmole) in dichloromethane (20ml) was added pyridine (23.90 mmole) followed by acetic anhydride (31.86 mmole) and resulting reaction mixture was stirred at room temperature for 24 hrs.

15 Reaction mass was concentrated, the residue was dissolved in dichloromethane (DCM) (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 70:30) to give the target product 1-3-1 (65%).

[00298] Compound 1-3-1: ^1H NMR (CDCl_3 , 400MHz): δ = 4.72 (m, 1H), 2.39-2.23 (m, 2H), 2.00 (s, 3H), 1.95 (d, J = 11.8Hz, 1H), 1.85-1.75 (m, 5H), 1.72-1.69 (m, 1H), 1.65-1.55 (m, 2H), 1.43-1.31 (m, 8H), 1.23-1.20 (m, 2H), 1.08-0.99 (m, 6H), 0.92-0.88 (m, 6H), 0.63 (s, 3H); ^{13}C NMR (100MHz): δ =
 20 180.03, 170.66, 74.36, 56.43, 55.98, 55.92, 42.69, 41.83, 40.35, 40.08, 35.73,

35.24, 34.97, 34.52, 32.19, 30.91, 30.70, 28.10, 26.95, 26.57, 26.25, 24.11, 23.26, 21.41, 20.77, 18.18, 11.98.

[00299] *Synthesis of Compound 1-3-2*

[00300] A solution of 3 α -acetoxy-5 β -cholan-24-oic acid 1-3-1 (2.38 mmole) in CCl₄ (10ml) containing IBDA (1.60 mmole) and iodine (1.45 mmole) was irradiated with 200-W tungsten-filament lamp for 45 min at reflux temperature. Another portion of iodosobenzene diacetate (IBDA) (1.50 mmole) and iodine (1.45 mmole) was then added and the irradiated at this temperature continue for 2 hrs. Reaction mixture was washed with sodium thiosulfate (2 x 30 ml) and water (2 x 30 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 90:10) to give the target products **1-3-2** (70%).

[00301] Compound 1-3-2: ¹H NMR (CDCl₃, 400MHz): δ = 4.73-4.67 (m, 1H) 3.28-3.26 (m, 1H), 3.08-3.06 (m, 1H), 2.00 (s, 3H), 1.85-1.95 (m, 1H), 1.82-1.77 (m, 4H), 1.75-1.67 (m, 1H), 1.53-1.50 (m, 4H), 1.40-1.35 (m, 7H), 1.24-1.20 (m, 3H), 1.14-1.10 (m, 2H), 1.04-1.00 (m, 4H), 0.90-0.88 (m, 6H), 0.64 (s, 3H); ¹³C NMR (100MHz): δ = 170.56, 74.33, 56.44, 55.85, 42.79, 41.83, 40.36, 40.30, 40.09, 37.15, 36.41, 35.73, 34.98, 34.53, 32.20, 28.14, 26.98, 26.58, 26.26, 24.09, 23.28, 21.42, 20.77, 17.79, 12.00, 5.09.

[00302] *Synthesis of Compound 1-3-3*

[00303] To a solution of 1-3-2 (6.00 mmole) in CCl₄ (15 ml), acetonitrile (15 ml) and water (26 ml) was added H₅IO₆ (6.38 mmole) and stirred for 15 minute. RuCl₃.H₂O (0.30mmole) was added and resulting reaction mixture stirred at room temperature for 24 hrs. Reaction mass was cooled to 0°C to it Et₂O was added with vigorous stirring and continue stirring for 10 min. The mixture was extracted with Et₂O (2 x 30 ml), organic layer washed with water (2 x 30 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 70:30) to give the target products **1-3-3** (57%)

[00304] Compound 1-3-3: ^1H NMR (CDCl_3 , 400MHz): δ = 4.72-4.66 (m, 1H), 2.46 (d, J = 12.2Hz, 1H), 2.01 (s, 3H), 1.95-1.90 (m, 2H), 1.88-1.77 (m, 4H), 1.75-1.65 (m, 1H), 1.56-1.50 (m, 2H), 1.43-1.35 (m, 7H), 1.27-1.16 (m, 5H), 1.10-1.04 (m, 4H), 0.99-0.94 (m, 3H), 0.84 (s, 3H) 0.64 (s, 3H); ^{13}C NMR (100MHz): δ = 179.12, 170.66, 74.38, 56.47, 55.97, 42.77, 41.82, 41.26, 40.34, 39.97, 35.74, 34.97, 34.53, 33.56, 32.19, 29.64, 28.25, 26.94, 26.57, 26.25, 24.10, 23.26, 21.41, 20.75, 19.45.

[00305] *Synthesis of Compound 1-3-4*

[00306] To a solution of 1-3-3 (6.67 mmole) in tert. butanol (30ml) was added Boc-anhydride (13.34 mmole) followed by DMAP (2 mmole) and resulting reaction mixture was stirred at room temperature for 1 h. Reaction mass was concentrated, the residue was dissolved in DCM (40mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products 1-3-4 (74%).

[00307] Compound 1-3-4: ^1H NMR (CDCl_3 , 400MHz): δ = 4.70-4.68 (m, 1H), 2.31 (d, J = 10.7Hz, 1H), 2.00 (s, 3H), 1.96-1.93 (m, 1H), 1.86-1.76 (m, 7H), 1.65-1.57 (m, 2H), 1.42 (s, 9H), 1.40-1.34 (m, 4H), 1.26-1.20 (m, 5H), 1.21-1.07 (m, 2H), 1.05-1.00 (m, 4H), 0.99 (d, J = 5.9Hz, 3H), 0.90 (s, 3H) 0.65 (s, 3H); ^{13}C NMR (100MHz): δ = 173.07, 170.63, 79.91, 74.39, 56.52, 56.17, 42.82, 41.88, 40.39, 40.06, 35.78, 35.02, 34.57, 33.78, 32.24, 29.68, 28.21, 28.14, 27.00, 26.62, 26.29, 24.14, 21.45, 20.80, 19.37, 12.04.

[00308] *Synthesis of Compound 1-3-5*

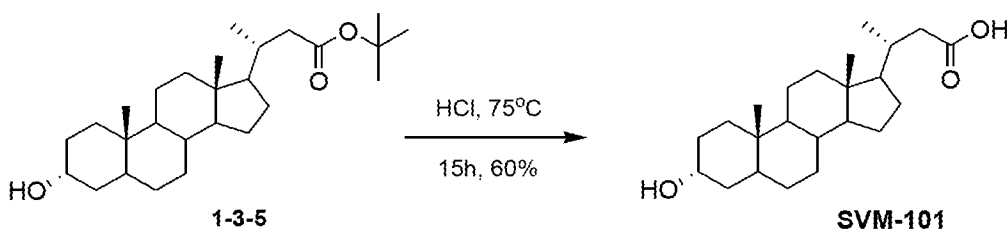
[00309] Compound 1-3-4 (4.88 mmole) was dissolved in methanol (40ml) to it sodium methoxide (14.65 mmole) was added and the resulting reaction mixture was stirred at room temperature for 12 h. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried

over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-3-5** (60%)

[00310] Compound **1-3-5**: ^1H NMR (CDCl_3 , 400MHz): δ = 3.62-3.57 (m, 1H), 2.31 (d, J = 10.7Hz, 1H), 1.92 -1.86 (m, 1H), 1.84-1.74 (m, 7H), 1.65-1.57 (m, 2H), 1.42 (s, 9H), 1.40-1.34 (m, 4H), 1.26-1.20 (m, 5H), 1.21-1.07 (m, 2H), 1.05-1.00 (m, 4H), 0.99 (d, J = 5.9Hz, 3H), 0.90 (s, 3H) 0.65 (s, 3H); ^{13}C NMR (100MHz): δ = 173.07, 79.90, 71.83, 56.51, 56.12, 42.80, 42.76, 42.06, 40.39, 40.07, 36.42, 35.81, 35.31, 34.54, 33.79, 30.52, 29.67, 28.21, 28.13, 27.15, 26.36, 24.14, 23.33, 20.77, 19.35, 12.02.

[00311] *Synthesis of SVM-101*

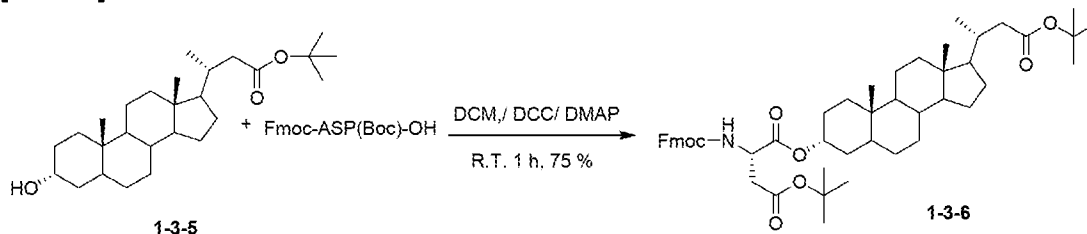
[00312] **Scheme 11:**

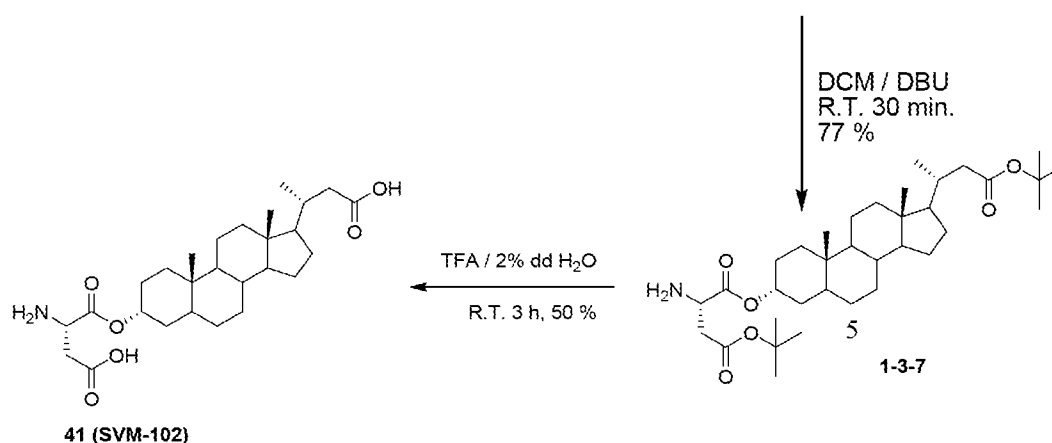


[00313] Compound **1-3-5** (0.477 mmole) was dissolved in HCl (5ml), and the reaction mixture was stirred at 75°C for 12 h. Reaction mass was neutralized with aq. NaOH solution and extracted with ethyl acetate. Organic layer washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 70:30) to give the target products **SVM101** (60%).

[00314] *Synthesis of Compounds 41 to 48*

[00315] **Scheme 12:**





[00316] General Procedures for

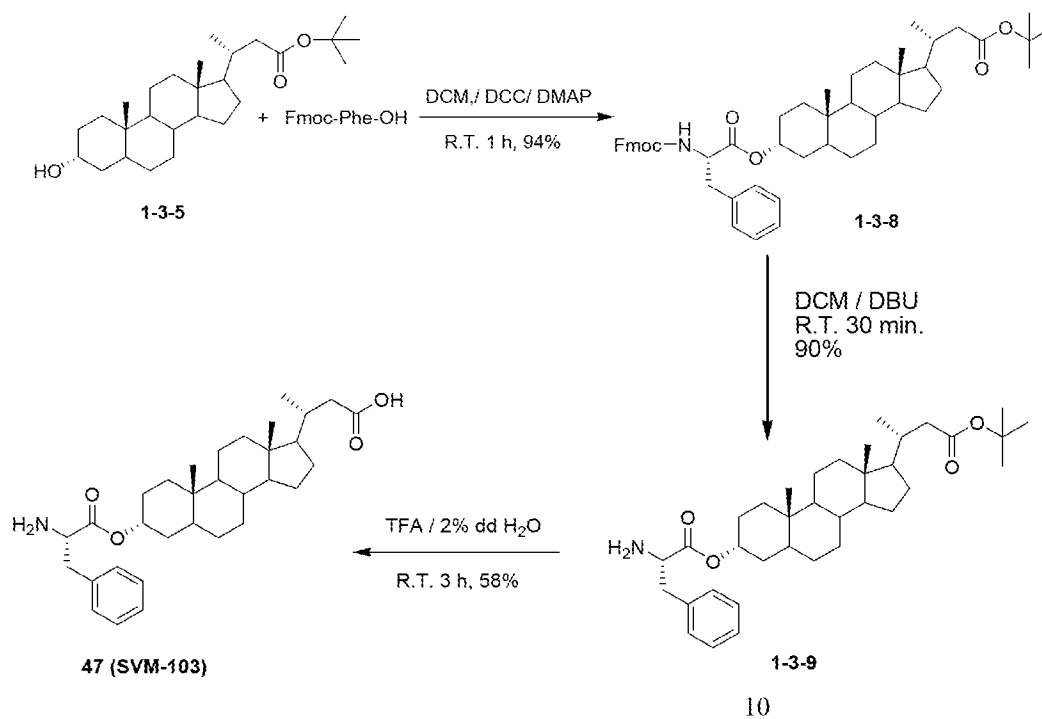
Synthesizing Compounds 1-3-6, 1-3-8, 1-3-10, 1-3-12, and 1-3-14

[00317] To a solution of corresponding Fmoc protected amino acid (0.286 mmole) in dichloromethane (5ml) was added compound **1-3-5** (0.238 mmole), and stirred for 10 min. A solution of DCC (0.382 mmole) in DCM (5ml) followed by DMAP (0.34 mmole) was added to above stirred solution and the resulting reaction mixture was stirred at room temperature for 1-2 hrs. Reaction mass was filtered and the filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-3-6, 1-3-8, 1-3-10, 1-3-12, and 1-3-14** (75-90%).

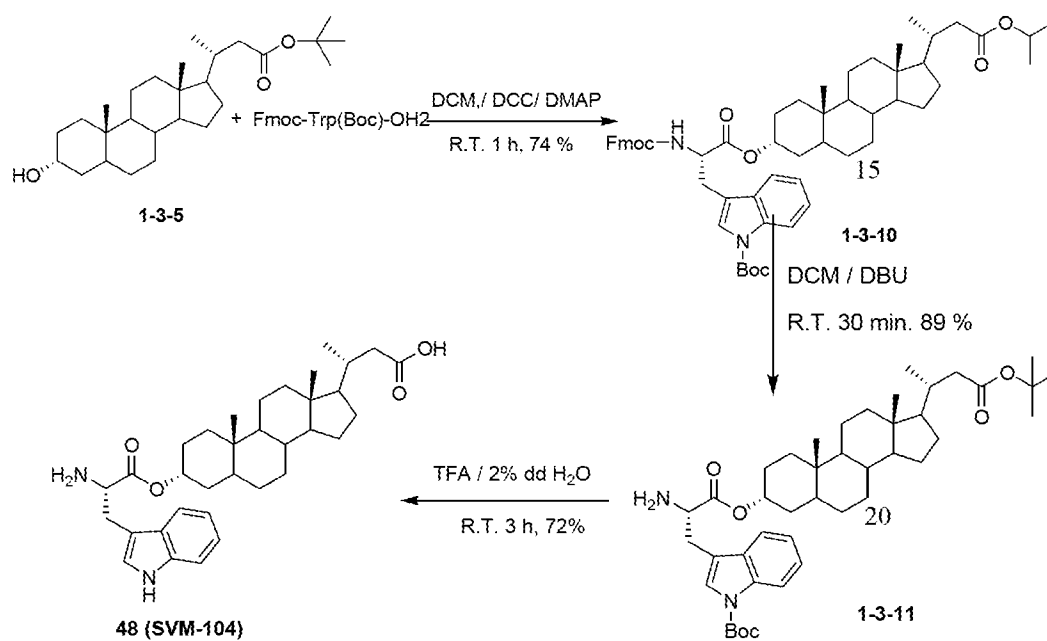
[00318] General Procedures for Synthesizing Compounds 1-3-7, 1-3-9, 1-3-11, 1-3-13 and 1-3-15

[00319] To a solution of compound **1-3-6, 1-3-8, 1-3-10, 1-3-13 and 1-3-15** (0.218 mmole) in dichloromethane (10ml) was added DBU (0.218 mmole) and stirred at room temperature for 1h. Reaction mass was concentrated and product purified by flash column chromatography to get desired compound **1-3-7, 1-3-9, 1-3-11, 1-3-13, and 1-3-15** (80-91%).

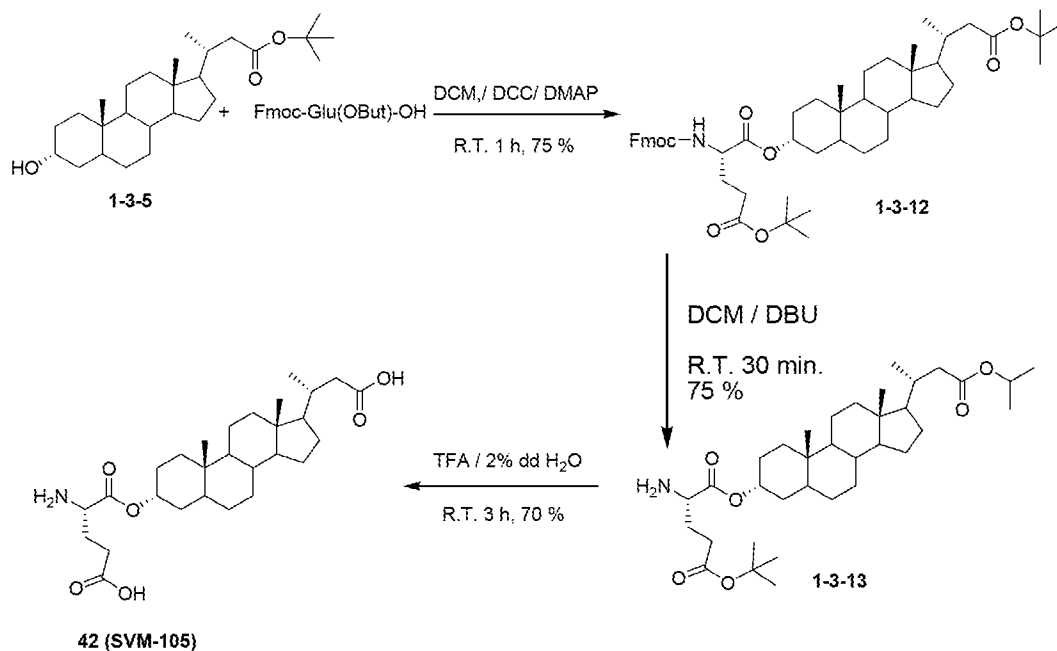
[00320] Scheme 13:



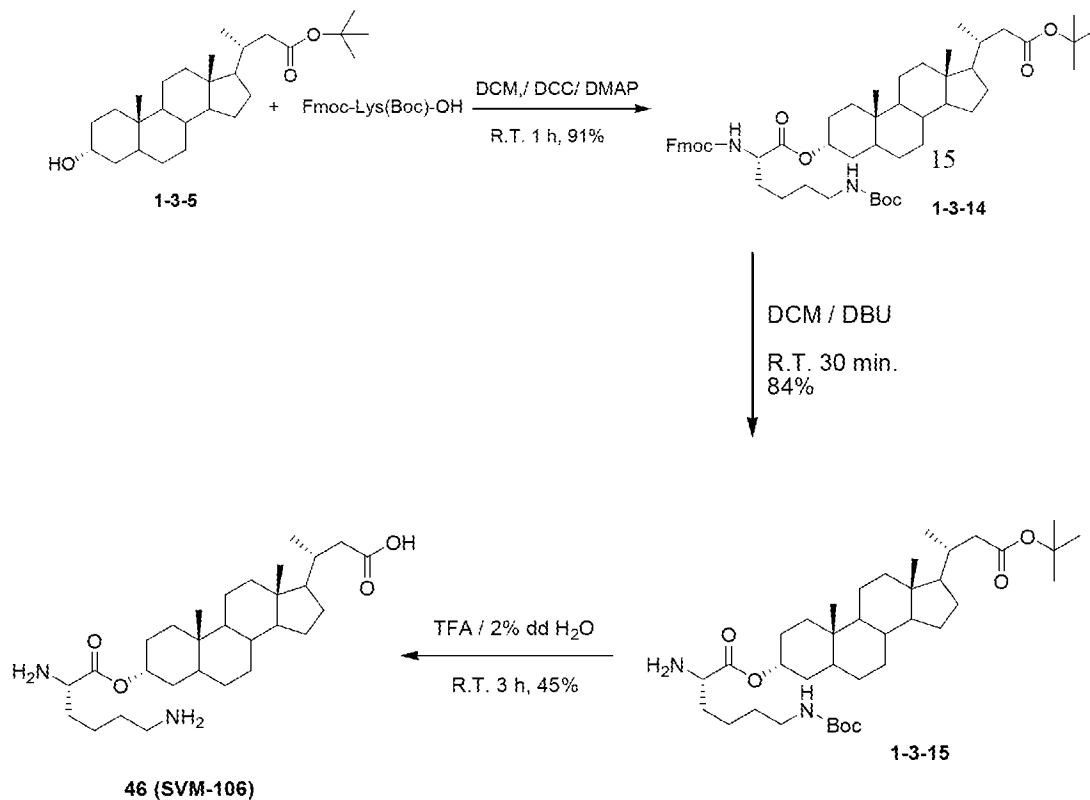
[00321] Scheme 14:



[00322] Scheme 15:

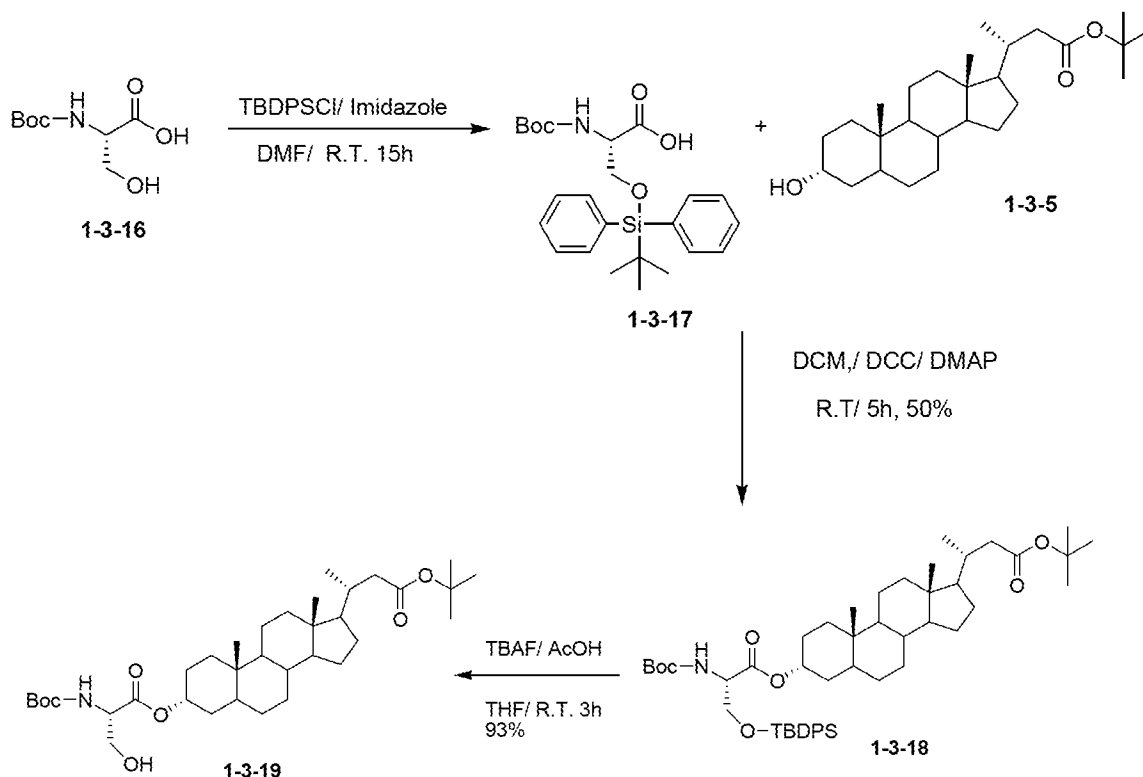


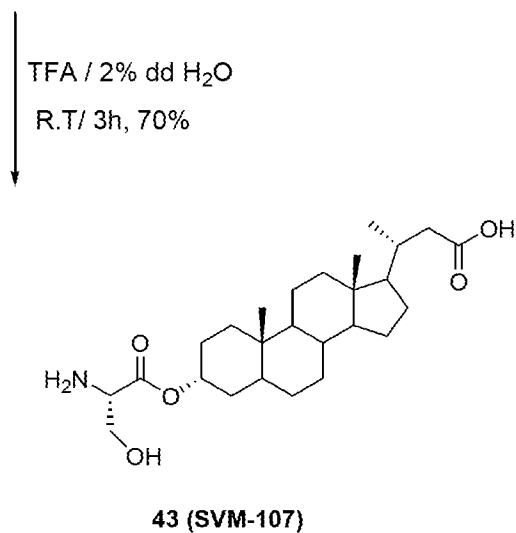
[00323] Scheme 16:



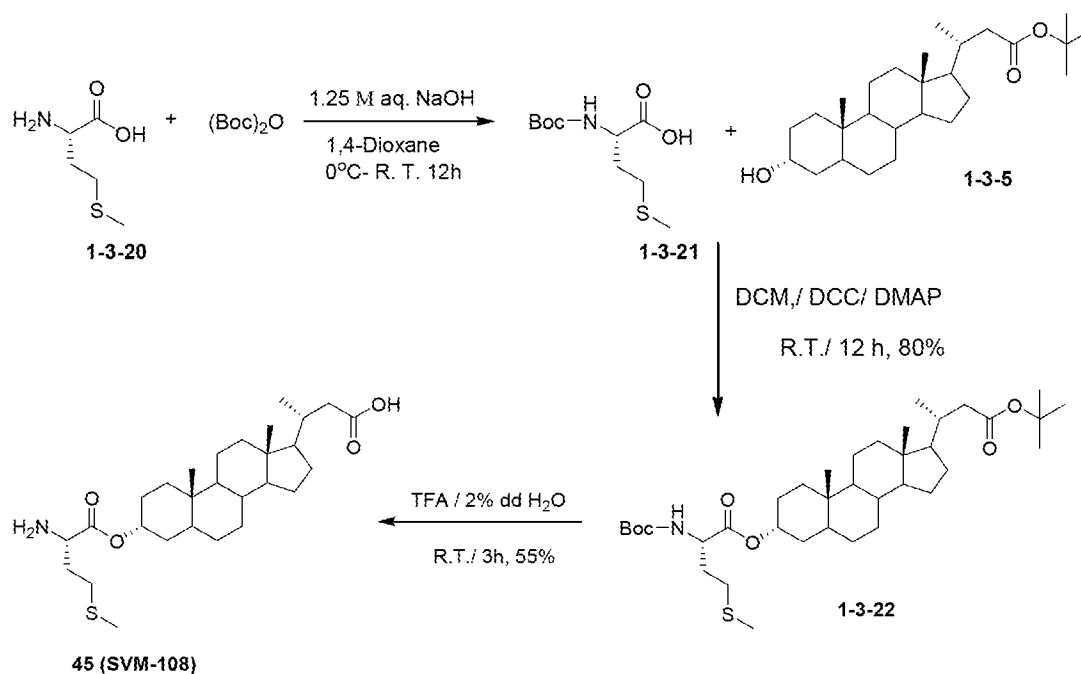
[00324] **Synthesis of compounds 1-3-17 and 1-3-22**

[00325] To a solution of compound **1-3-16** or **1-3-23** (2.43 mmole) in DMF (10ml) was added imidazole (6.33 mmole) and stirred for 10 min. TBDPS-Cl (6.091 mmole) was added slowly and resulting reaction mixture was stirred at room temperature for 16 hrs. Reaction mass was concentrated, the residue was dissolved in ethyl acetate (20mL) and washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. Obtained slurry was dissolved in methanol: water (70: 30) (10 ml) to it K_2CO_3 (6.090 mmole) was added and stirred at room temperature for 2hrs. Methanol: water were removed, residue was dissolved in DCM (20 ml) and acidify with 10% aq. citric acid up to PH 2, extracted with DCM. Organic layer was dried over sodium sulfate, filtered, and evaporated, final residue was purified by column chromatography (methanol/DCM, 80:20) to give the target products **1-3-17** or **1-3-22** (60%).

[00326] **Scheme 17:**



10 [00327] **Scheme 18:**



20

[00328] *Synthesis of Compound 1-3-21*

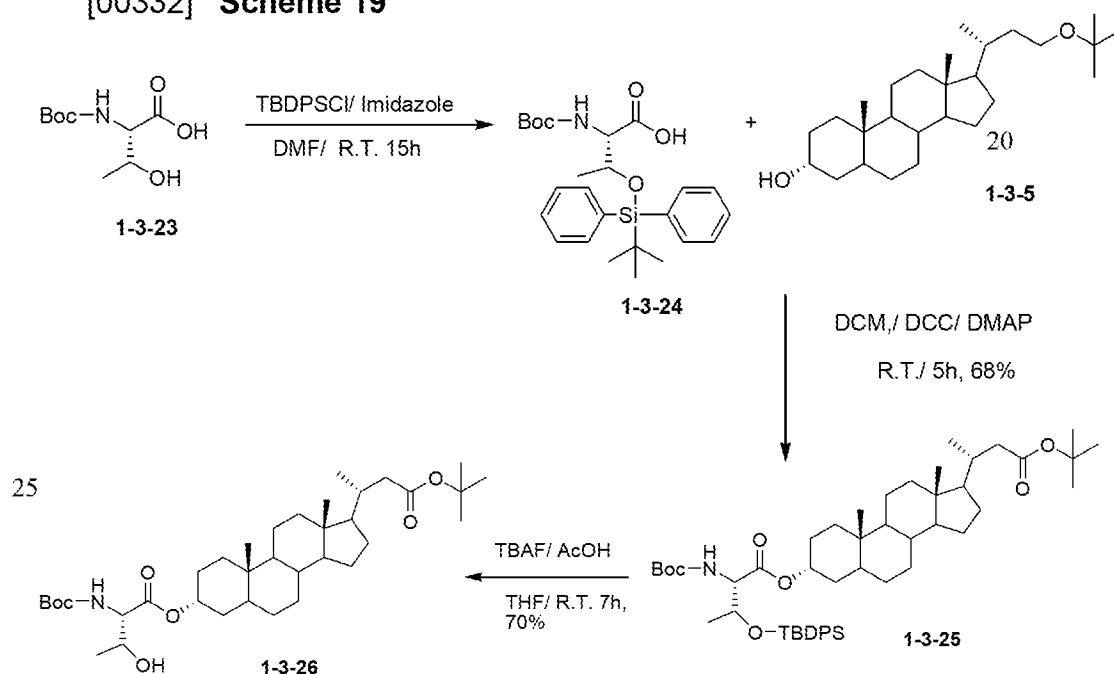
[00329] To a solution of compound 1-3-20 (3.35 mmole) in dioxane (10 ml) was added a solution of 1.25 M aq. NaOH (5 ml) at 0°C and stirred for 10 min. A solution of Boc-anhydride (3.35 mmole, in dioxane 5 ml) slowly in 30 minutes at

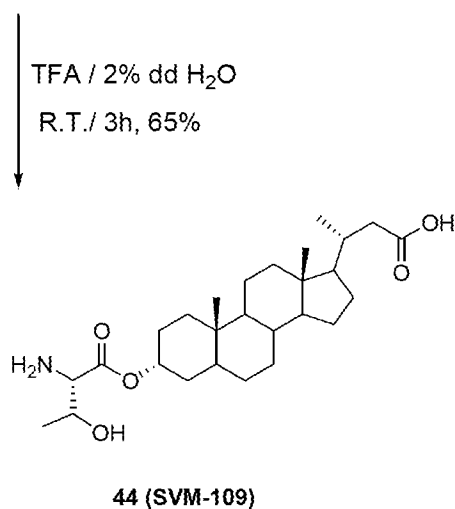
same temperature and resulting reaction mixture was stirred at room temperature for 12h. Reaction mass was concentrated, obtained slurry dissolved in water (50ml) and washed with hexane (3 X 20 ml). Water layer acidify with 10% aq. HCl up to PH 2 and extracted with DCM. Organic layer was dried over sodium sulfate, filtered, and evaporated, final residue was purified by column chromatography (methanol/DCM, 80:20) to give the target products **1-3-21** (70%).

[00330] *General Procedures for the Synthesis of Compounds 1-3-18, 1-3-22, and 1-3-25*

[00331] To a solution of corresponding Boc-protected amino acid (0.286 mmole) in dichloromethane (5ml) was compound **1-3-5** (0.238 mmole) stirred for 10 min. A solution of DCC (0.382 mmole) in DCM (5ml) followed by DMAP (0.34 mmole) was added to above stirred solution and resulting reaction mixture was stirred at room temperature for 1-2 hrs. Reaction mass was filtered and filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-3-18, 1-3-22, and 1-3-25** (50-80%).

[00332] **Scheme 19**





[00333] *Treatment of Final Products*

[00334] Compounds **1-3-7**, **1-3-9**, **1-3-11**, **1-3-13**, **1-3-15**, **1-3-18**, **1-3-22**, and **1-3-25** (0.107 mmole) was dissolved in TFA (5 ml) at 0°C water (50 µL) was added and the reaction mixture was stirred at room temperature for 3h. The TFA solution was then removed on rotary evaporator and the residue was neutralized with saturated solution of aq. NaHCO₃, dialyzed using membrane and purified by

perverse phase HPLC to get desired compounds as white solid.

[00335] Compound **SVM-101**: MP = 137-138°C, ¹H NMR (DMSO-D₆, 400MHz): δ = 4.47-4.45 (m, 1H), 2.30 (dd, *J* = 2.5Hz, *J* = 14.2Hz, 2H), 1.92-1.90 (m, 1H), 1.80-1.74 (m, 4H), 1.65-1.60 (m, 2H), 1.58-1.51 (m, 2H), 1.35-1.30 (m, 6H), 1.21-1.15 (m, 7H), 1.04-1.02 (m, 3H), 0.92 (d, *J* = 6.2Hz, 3H), 0.86 (s, 3H), 0.63 (s, 3H); ¹³C NMR (100MHz): δ = 174.57, 70.28, 56.48, 55.88, 42.71, 41.93, 41.59, 36.68, 35.80, 35.55, 34.61, 33.44, 30.75, 28.15, 27.28, 26.53, 24.19, 23.65, 20.78, 19.77, 12.24, HPLC conditions: retention time = 10.10 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Vydac C18.

[00336] Compound **41 (SVM-102)**: MP = 158-159°C, ¹H NMR (DMSO-D₆, 500MHz): δ = 4.73 (m, 1H), 4.25 (m, 1H), 2.88 (dd, *J* = 3.9Hz, *J* = 16.7Hz, 2H), 2.30-2.27 (m, 1H), 1.84-1.75 (m, 6H), 1.61-1.49 (m, 4H), 1.44-1.35 (m, 5H), 1.21-1.08 (m, 10H), 0.92-0.90 (m, 6H), 0.63 (s, 3H); ¹³C NMR (125MHz): δ = 174.53, 171.17, 168.24, 76.39, 56.36, 55.90, 49.06, 42.69, 41.59, 41.45, 35.70,

34.71, 34.62, 34.53, 33.40, 31.92, 28.12, 26.94, 26.30, 24.12, 23.29, 20.78, 19.76, 12.20. HRMS m/z calcd for $C_{27}H_{44}NO_6$, 478.3169; found 478.3175 ($M + H$)⁺, HPLC conditions: retention time = 10.70 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Vydac C18

5 [00337] Compound **47 (SVM-103)**: MP = 204-205°C, ¹H NMR (CD₃OD, 400MHz): δ = 7.39-7.34 (m, 3H), 7.28-7.26(m, 2H), 4.80-4.76 (m, 1H), 4.26 (t, J = 7.1Hz, 1H), 3.20 (d, J = 7.1Hz, 2H), 2.41-2.40 (m, 1H), 2.05-2.02 (m, 1H), 1.98-1.83 (m, 6H), 1.62-1.52 (m, 3H), 1.47-1.21 (m, 11H), 1.12-1.07 (m, 4H), 1.06 (d, J = 8Hz, 3H), 1.03 (s, 3H), 0.73 (s, 3H); ¹³C NMR (125MHz): δ = 175.94, 168.07, 133.97, 129.08, 128.62, 127.45, 76.91, 56.49, 56.06, 42.51, 41.73, 40.97, 40.30, 39.93, 36.22, 35.69, 34.39, 34.17, 33.44, 31.61, 27.79, 26.59, 26.00, 25.81, 23.73, 22.18, 20.42, 18.51, 10.97; HRMS m/z calcd for $C_{32}H_{48}NO_4$, 510.3590; found 510.3600 ($M + H$)⁺, HPLC conditions: retention time = 19.75 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil
15 300-5C4.

[00338] Compound **48 (SVM-104)**: MP = 144-145°C, ¹H NMR (CD₃OD, 500MHz): δ = 7.57 (d, J = 10Hz, 1H), 7.40 (d, J = 10Hz, 1H), 7.19 (s, 1H), 7.14 (t, J = 5Hz, 1H), 7.08 (t, J = 7.5Hz, 1H), 4.72-4.67 (m, 1H), 4.26 (t, J = 5Hz, 1H), 3.39-3.37 (m, 2H), 2.43-2.41 (m, 1H), 2.03-2.01 (m, 1H), 1.95-1.85 (m, 4H),
20 1.79-1.67 (m, 2H), 1.63-1.59 (m, 1H), 1.45-1.36 (m, 6H), 1.34-1.29 (m, 3H), 1.26-1.19 (m, 4H), 1.15-1.10 (m, 2H), 1.06-0.99 (m, 5H), 0.92 (s, 3H), 0.72 (s, 3H); ¹³C NMR (125MHz): δ = 177.58, 170.09, 138.42, 128.54, 122.57, 123.12, 120.42, 119.24, 112.79, 107.96, 78.51, 57.96, 57.66, 55.01, 44.09, 43.37, 42.60, 41.82, 41.46, 37.27, 36.02, 35.74, 35.06, 32.12, 29.40, 28.17, 27.96, 27.57, 27.17,
25 25.32, 22.01, 20.13, 12.57; HRMS m/z calcd for $C_{34}H_{49}N_2O_4$, 549.3692; found 549.3699 ($M + H$)⁺, HPLC conditions: retention time = 20.45 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00339] Compound **42 (SVM-105)**: MP = 149-150°C, ¹H NMR (CD₃OD/DMSO-D₆, 500MHz): 4.75-4.71 (m, 1H), 3.96 (t, *J* = 7Hz, 1H), 2.43-2.36 (m, 2H), 2.29-2.27 (m, 1H), 2.06-2.01 (m, 2H), 1.91-1.89 (m, 1H), 1.85-1.74 (m, 6H), 1.52-1.49 (m, 2H), 1.42-1.33 (m, 6H), 1.18-1.04 (m, 5H), 1.01-0.94 (m, 4H), 0.89 (d, *J* = 6Hz, 1H), 0.86 (s, 3H), 0.60 (s, 3H); ¹³C NMR (125MHz): δ = 175.54, 173.81, 168.49, 77.06, 56.41, 56.01, 51.97, 42.56, 41.84, 41.07, 40.36, 39.91, 35.75, 34.53, 34.31, 33.48, 31.79, 28.95, 27.89, 26.73, 26.10, 25.36, 23.83, 22.44, 20.54, 18.75, 11.20; HRMS *m/z* calcd for C₂₈H₄₆NO₆, 492.3325; found 492.3320 (M + H)⁺, HPLC conditions: retention time = 8.45 min.

Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00340] Compound **46 (SVM-106)**: MP = 219-220°C, ¹H NMR (CD₃OD, 500MHz): δ = 4.02 (t, *J* = 6.4Hz, 1H), 2.96 (t, *J* = 7.9Hz, 2H), 2.43 (d, *J* = 11.4Hz, 1H), 2.01-1.88 (m, 9H), 1.75-1.68 (m, 3H), 1.62-1.45 (m, 10H), 1.34-1.28 (m, 3H), 1.21-1.11 (m, 6H), 1.01 (d, *J* = 6Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H); ¹³C NMR (125MHz): δ = 177.57, 170.02, 78.60, 58.06, 57.67, 54.00, 44.11, 43.47, 42.60, 42.01, 41.52, 40.40, 37.34, 36.07, 35.85, 35.04, 33.34, 31.23, 29.39, 28.24, 28.17, 27.71, 27.64, 25.33, 23.85, 23.24, 22.07, 20.12, 12.59; HRMS *m/z* calcd for C₂₉H₅₁N₂O₄, 491.3849; found 491.3840 (M + H)⁺, HPLC conditions: retention time

= 19.80 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00341] Compound **43 (SVM-107)**: MP = 165-166°C, ¹H NMR (CD₃OD, 500MHz): δ = 4.07 (t, *J* = 3.6Hz, 1H), 3.98 (dd, *J* = 3.5Hz, *J* = 11Hz, 2H), 2.42 (d, *J* = 11.3Hz, 1H), 2.04-1.85 (m, 7H), 1.77-1.73 (m, 1H), 1.63-1.60 (m, 2H), 1.54-1.43 (m, 6H), 1.33-1.12 (m, 9H), 1.01(d, *J* = 6.1Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H); ¹³C NMR (125MHz): δ = 177.57, 168.55, 78.59, 60.89, 58.04, 57.64, 56.35, 44.10, 43.46, 42.60, 41.97, 41.50, 37.33, 36.07, 35.83, 35.05, 33.31, 29.39, 28.25, 27.64, 27.57, 23.85, 22.06, 20.11, 12.59; HRMS *m/z* calcd for C₂₆H₄₄NO₅, 450.3219; found 450.3227 (M + H)⁺, HPLC conditions: retention time = 19.70 min. Solvents:

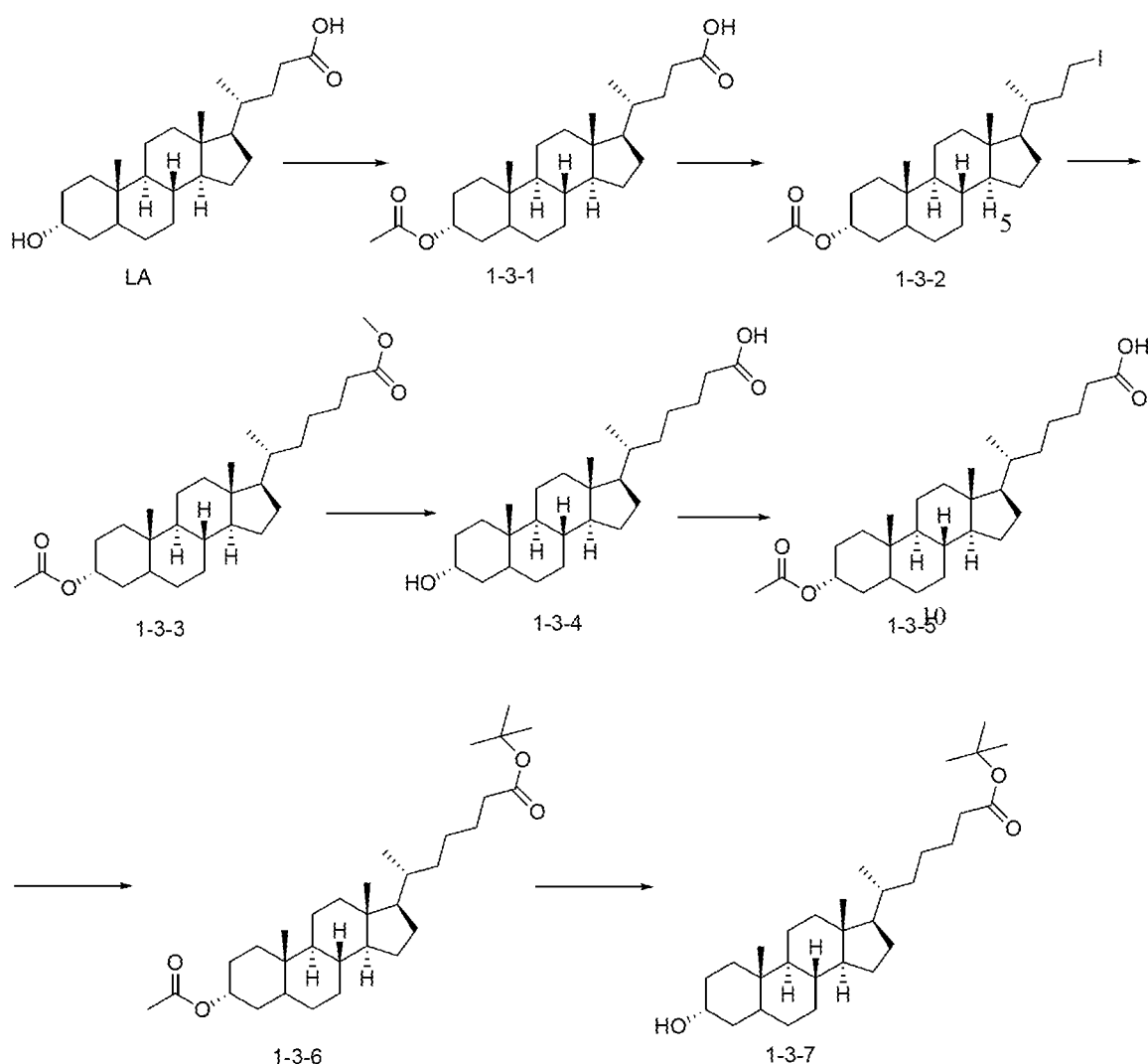
A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00342] Compound **45 (SVM-108)**: MP = 129-130°C, ^1H NMR (CD_3OD , 500MHz): δ = 4.167 (t, J = 6.4Hz, 1H), 2.66-2.63 (m, 2H), 2.43 (d, J = 11.3Hz, 1H), 2.23-2.14 (m, 2H), 2.13 (s, 3H), 2.04-1.86 (m, 7H), 1.76-1.74 (m, 1H), 1.63-1.61 (m, 2H), 1.54-1.48 (m, 6H), 1.35-1.28 (m, 3H), 1.24-1.09 (m, 6H), 1.01 (d, J = 6.1Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (125MHz): δ = 177.58, 170.00, 78.69, 58.01, 57.62, 53.10, 44.10, 43.45, 42.60, 41.98, 41.49, 37.33, 36.06, 35.84, 35.05, 33.32, 31.05, 30.26, 29.40, 28.24, 27.68, 27.63, 25.33, 23.85, 22.07, 20.11, 15.17, 12.59; HRMS m/z calcd for $\text{C}_{28}\text{H}_{48}\text{NO}_4\text{S}$, 494.3304; found 494.3295 ($\text{M} + \text{H}$) $^+$, HPLC conditions: retention time = 19.25 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00343] Compound **44 (SVM-109)**: MP = 181-182°C, ^1H NMR (CD_3OD , 500MHz): δ = 4.26-4.22 (m, 1H), 3.84 (d, J = 4.6Hz, 1H), 2.42 (d, J = 11.2Hz, 1H), 2.04-1.88 (m, 8H), 1.77-1.75 (m, 1H), 1.63-1.60 (m, 2H), 1.51-1.45 (m, 6H), 1.33 (d, J = 6.5Hz, 3H), 1.32-1.30 (m, 3H), 1.26-1.08 (m, 6H), 1.01 (d, J = 6.1Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (125MHz): δ = 177.56, 168.84, 78.54, 66.60, 60.15, 58.03, 57.63, 44.10, 43.45, 42.58, 41.98, 41.50, 37.33, 36.07, 35.84, 35.05, 33.32, 29.40, 28.24, 27.63, 27.56, 25.33, 23.86, 22.06, 20.82, 20.11, 12.59; HRMS m/z calcd for $\text{C}_{27}\text{H}_{46}\text{NO}_5$, 464.3376; found 464.3372 ($\text{M} + \text{H}$) $^+$, HPLC conditions: retention time = 18.25 min. Solvents: A) 100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00344] 1.5 Synthesis of Compounds 49 to 52

[00345] Compounds 49 to 52 were synthesized in accordance with procedures set forth in **Schemes 20 to 23**.

[00346] **Scheme 20:**[00347] *Synthesis of Compound 1-3-1*

[00348] To a solution of lithocholic acid **LA** (2.655 mmole, 1 eq) in dichloromethane was added pyridine (7.966 mmole, 3 eq) followed by acetic anhydride (10.62 mmole, 4 eq) and resulting reaction mixture stirred at room temperature for 4 h. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column

chromatography (hexane/ethyl acetate, 70:30) to give the target product **1-3-1** (65%).

[00349] *Synthesis of Compound 1-3-2*

[00350] A solution of **1-3-1** (1.72 mmole, 1 eq) in CCl₄ (10ml) containing IBDA
5 (5.17 mmole, 0.65 eq) and iodine (1.72 mmole, 1 eq) was irradiated with 200-W tungsten-filament lamp for 45 min at reflux temperature. Another portion of IBDA (0.65 eq) and iodine (0.5 eq) was then added and the irradiated at this temperature continue for 2 hrs. Reaction mixture was washed with sodium thiosulfate (2 x 30 ml) and water (2 x 30 ml). The organic layer was dried over
10 sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 90:10) to give the target product **1-3-2** (70%).

[00351] *Synthesis of Compound 1-3-3*

[00352] A solution of **1-3-2** (1.20 mmole, 1 eq) in toluene (10ml) containing methyl
15 acrylate (2.4 mmole, 1.2 eq), tris(trimethylsilyl)silane (TTMSS) (2.4 mmole, 1.2 eq) as a free radical mediator and AIBN (5mol%) heated at 70 °C under nitrogen atmosphere. for 2hr. Reaction mass was concentrated. The residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final
20 residue was purified by column chromatography (hexane/ethyl acetate, 95:5) to give the target product **1-3-3** (64%).

[00353] *Synthesis of Compound 1-3-4*

[00354] Compound **1-3-3** (0.77 mmole, 1eq) was dissolved in aqueous methanol (10ml) to it 1N sodium hydroxide (2.31 mmole, 3eq) was added and the resulting
25 reaction mixture was reflux for 5h. Reaction mass was concentrated, the residue was dissolved in water (30mL) and washed with ether (2 x 30 ml). Aqueous layer was acidify with 6%HCl, and washed with ether (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness to give the target product **1-3-4** (91%).

[00355] *Synthesis of Compound 1-3-5*

[00356] To a solution of bishomolithocholic acid **1-3-4** (0.69 mmole, 1 eq) in dichloromethane (DCM) was added pyridine (2.07 mmole, 3 eq) followed by acetic anhydride (2.76 mmole, 4 eq) and resulting reaction mixture stirred at room temperature for 4 h. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 70:30) to give the target product **1-3-5** (65%).

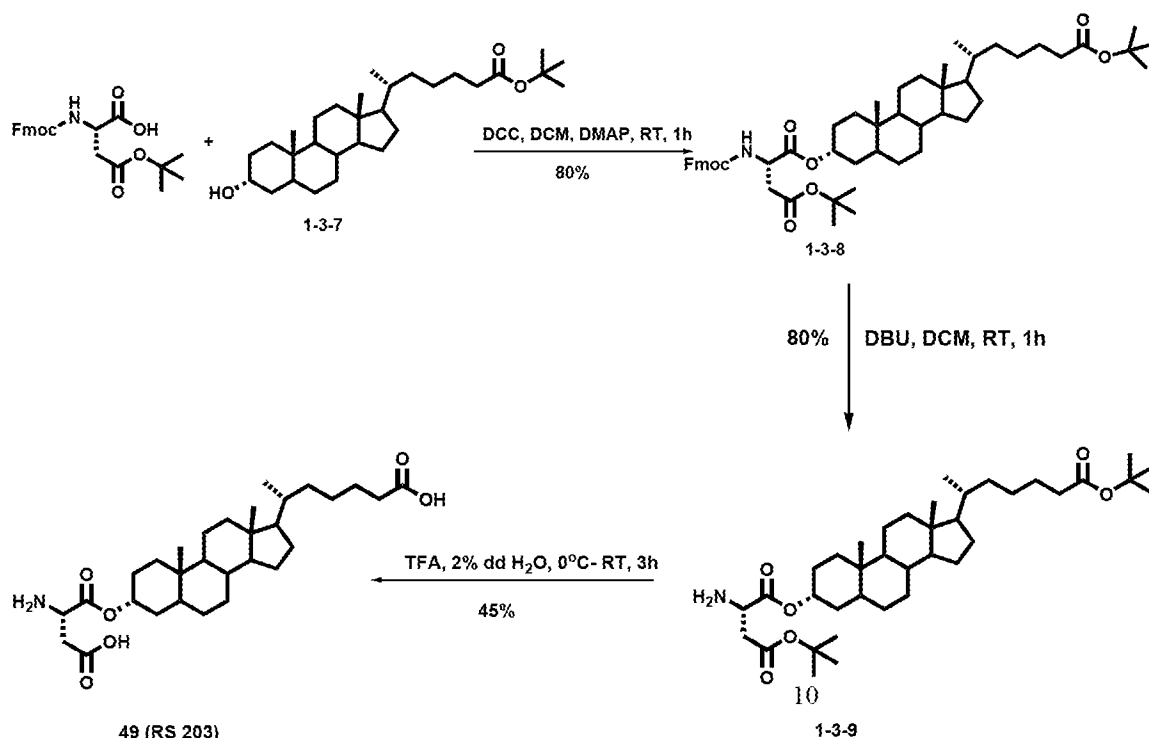
[00357] *Synthesis of Compound 1-3-6*

[00358] To a solution of **1-3-5** (0.45 mmole, 1eq) in tert. butanol (10 ml) was added Boc-anhydride (0.9 mmole, 2eq) followed by DMAP (0.14 mmole, 0.3eq) and resulting reaction mixture was stirred at room temperature for 1 h. Reaction mass was concentrated, the residue was dissolved in DCM (40mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product **1-3-6** (74%).

[00359] *Synthesis of Compound 1-3-7*

[00360] Compound **1-3-6** (0.33 mmole, 1eq) was dissolved in methanol (10ml) to it sodium methoxide (1.98 mmole, 6eq) was added and the resulting reaction mixture was stirred at room temperature for 5 h. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-3-7** (60%).

[00361] *Synthesis of Compound 49 (RS203)*

[00362] **Scheme 21**

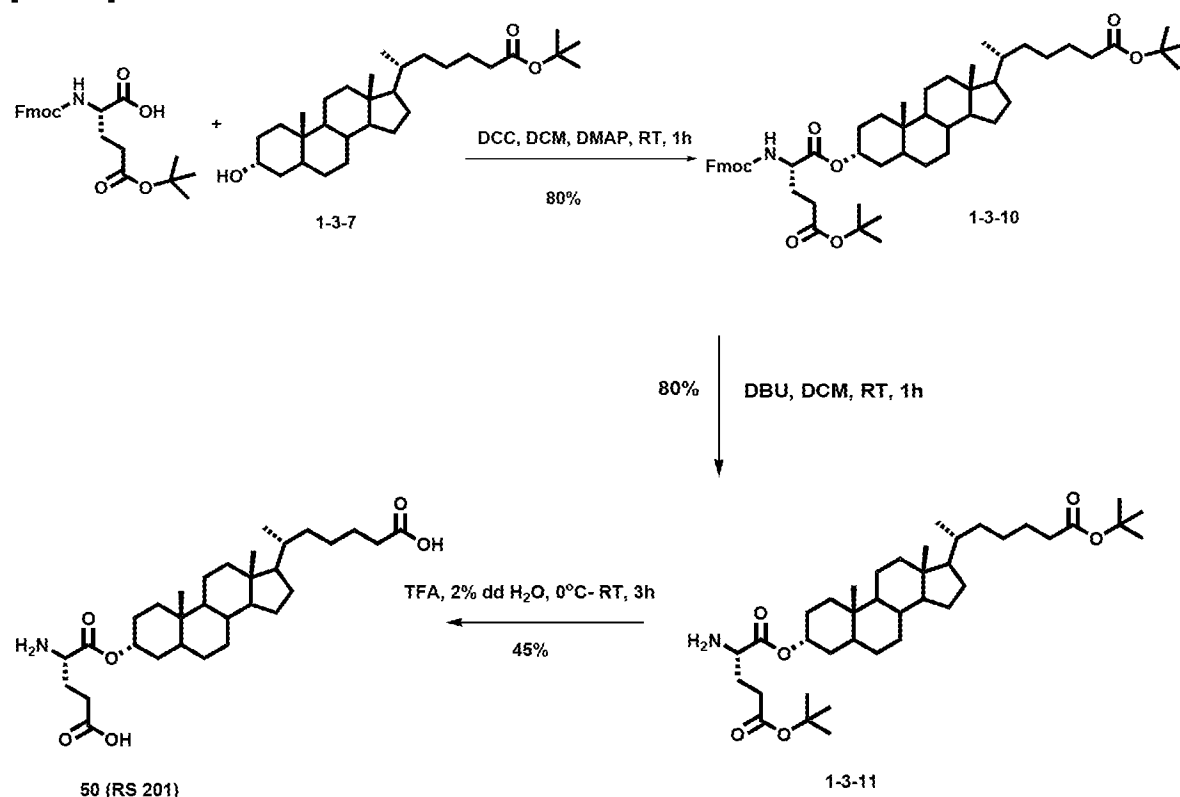
[00363] The solution of corresponding Fmoc protected amino acid (0.24 mmole, 1.2eq) in dichloromethane (5ml) was added to compound 1-3-7 (0.2 mmole, 1eq), stirred for 10 min. A solution of N,N'-dicyclohexylcarbodiimide (DCC) (0.32 mmole, 1.6eq) in DCM (5ml) followed by DMAP (0.06mmole, 0.3eq) was added to above stirred solution and resulting reaction mixture was stirred at room temperature for 1-2 hrs. Reaction mass was filtered and filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product **1-3-8** (75-90%).

[00364] To a solution of compound **1-3-8** (0.16 mmole, 1eq) in dichloromethane (10ml) was added DBU (0.16 mmole, 1eq) and stirred at room temperature for 1h. Reaction mass was concentrated and product purified by flash column chromatography to get desired compound **1-3-9** (80-91%).

[00365] Compound 1-3-9 (0.13mmole, 1eq) was dissolved in TFA (5 ml) at 0°C, water (50 µL) was added and the reaction mixture was stirred at room temperature for 3h. The TFA solution was then removed on rotary evaporator and the residue was neutralized with saturated solution of aq. NaHCO₃, dialyzed using membrane and purified by reverse phase HPLC to produce the desired compound 49 (**RS203**) as white solid.

[00366] *Synthesis of RS201*

[00367] **Scheme 22**



[00368] The solution of corresponding Fmoc protected amino acid (0.24 mmole, 1.2eq) in dichloromethane (5ml) was added to compound 1-3-7 (0.2 mmole, 1eq), stirred for 10 min. A solution of DCC (0.32 mmole, 1.6eq) in DCM (5ml) followed by DMAP (0.06 mmole, 0.3eq) was added to above stirred solution and resulting reaction mixture was stirred at room temperature for 1-2 hrs. Reaction mass was filtered and filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium

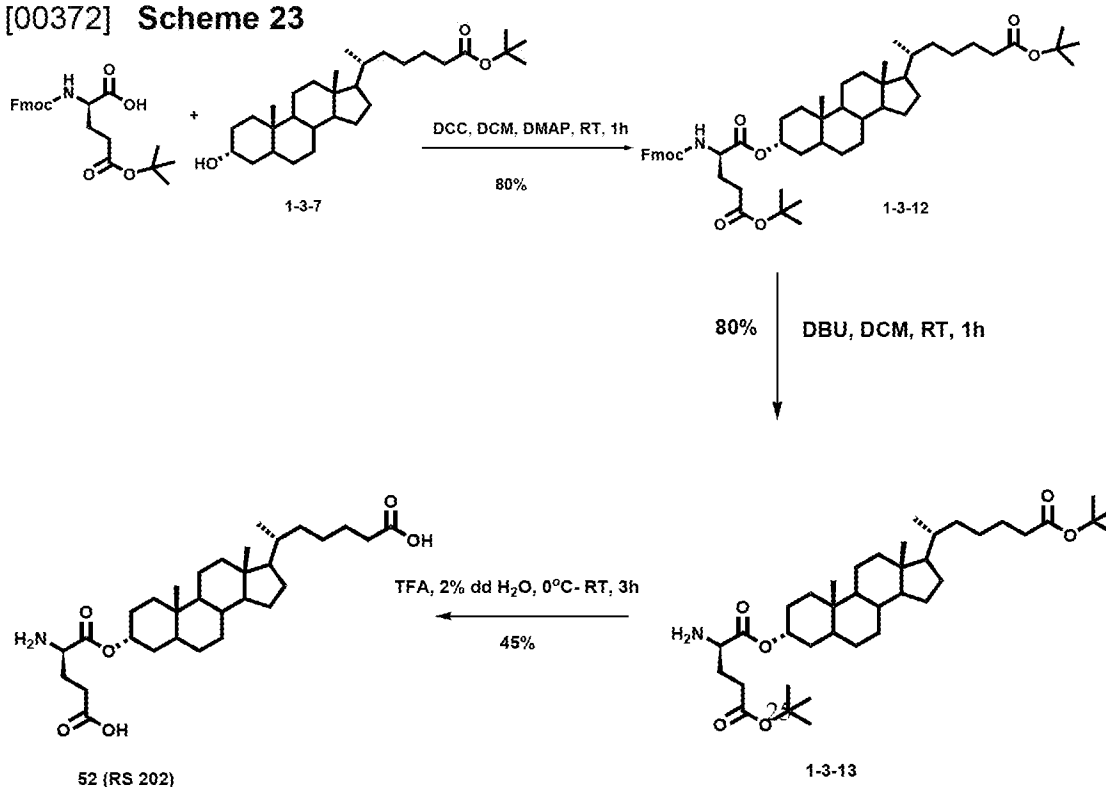
sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product 1-3-10 (75-90%).

[00369] To a solution of compound **1-3-10** (0.16 mmole, 1eq) in dichloromethane (10ml) was added DBU (0.16 mmole, 1eq) and stirred at room temperature for 1h. Reaction mass was concentrated and product purified by flash column chromatography to get desired compound **1-3-11** (80-91%).

[00370] Compound **1-3-11** (0.12 mmole, 1eq) was dissolved in TFA (5 ml) at 0°C water (50 μ L) was added and the reaction mixture was stirred at room temperature for 3h. The TFA solution was then removed on rotary evaporator and the residue was neutralized with saturated solution of aq. NaHCO₃, dialyzed using membrane and purified by reverse phase HPLC to get desired compounds 50 (RS201) as white solid.

[00371] *Synthesis of compound 52 (RS202)*

[00372] **Scheme 23**



[00373] The solution of corresponding Fmoc protected amino acid (0.24 mmole, 1.2eq) in dichloromethane (5ml) was added to compound **1-3-7** (0.2 mmole, 1eq), stirred for 10 min. A solution of DCC (0.32 mmole, 1.6eq) in DCM (5ml) followed by DMAP (0.06mmole, 0.3eq) was added to above stirred solution and resulting reaction mixture was stirred at room temperature for 1-2 hrs. Reaction mass was filtered and filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product **1-3-12** (75-90%).

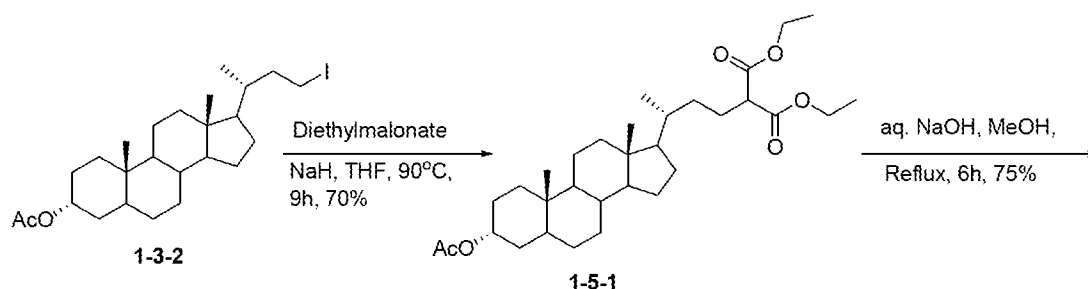
[00374] To a solution of compound **1-3-12** (0.16 mmole, 1eq) in dichloromethane (10ml) was added DBU (0.16 mmole, 1eq) and stirred at room temperature for 1h. Reaction mass was concentrated and product purified by flash column chromatography to get desired compound **1-3-13** (80-91%).

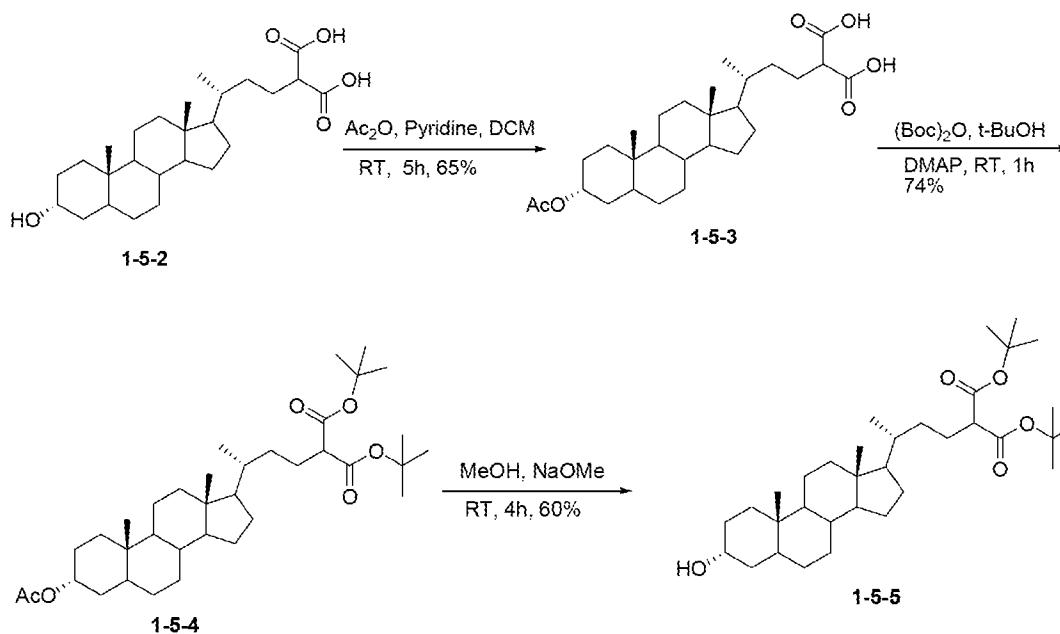
[00375] Compound **1-3-13** (0.12 mmole, 1eq) was dissolved in TFA (5 ml) at 0°C water (50 μ L) was added and the reaction mixture was stirred at room temperature for 3h. The TFA solution was then removed on rotary evaporator and the residue was neutralized with saturated solution of aq. NaHCO₃, dialyzed using membrane and purified by reverse phase HPLC to get desired compounds **52 (RS202)** as white solid.

[00376] 1.6 Synthesis of Compounds 51 to 57

[00377] Compounds **51** to **57** (or compounds of formula (1-5)) were synthesized in accordance with procedures set forth in **Schemes 24** to **28**.

[00378] Scheme 24:





10 [00379] *Synthesis of compound 1-5-1*

[00380] To a suspension of NaH (15 mmole) in dry THF (30ml) was added diethylmalonate (17.5 mmole) and heated at 40°C. To it a solution of compound 1-3-2 (5 mmole) in THF was added slowly and resulting reaction mixture stirred at 90°C for 6 hrs. Reaction mass was diluted with ethyl acetate and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product 1-5-1 (70%).

[00381] *Synthesis of compound 1-5-2*

[00382] Compound 1-5-1 (1 g) dissolved in methanol (10ml), to it 40% NaOH(1mL) was added and resulting reaction mixture reflux for 6 hrs under nitrogen. Reactions mass concentrated on rota, suspension acidify with 5% HCl solution, obtained solid filtered and washed with water. The white solid dried using high vacuum for several hours provides product 1-5-2 (75%). For biological study, compound purify by reverse phase HPLC.

[00383] *Synthesis of compound 1-5-3*

[00384] To a solution of compound **1-5-2** (8 mmole) in dichloromethane (20ml) was added pyridine (23.90 mmole) followed by acetic anhydride (31.86 mmole) and resulting reaction mixture stirred at room temperature for 24 hrs. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 70:30) to give the target product **1-5-3** (65%).

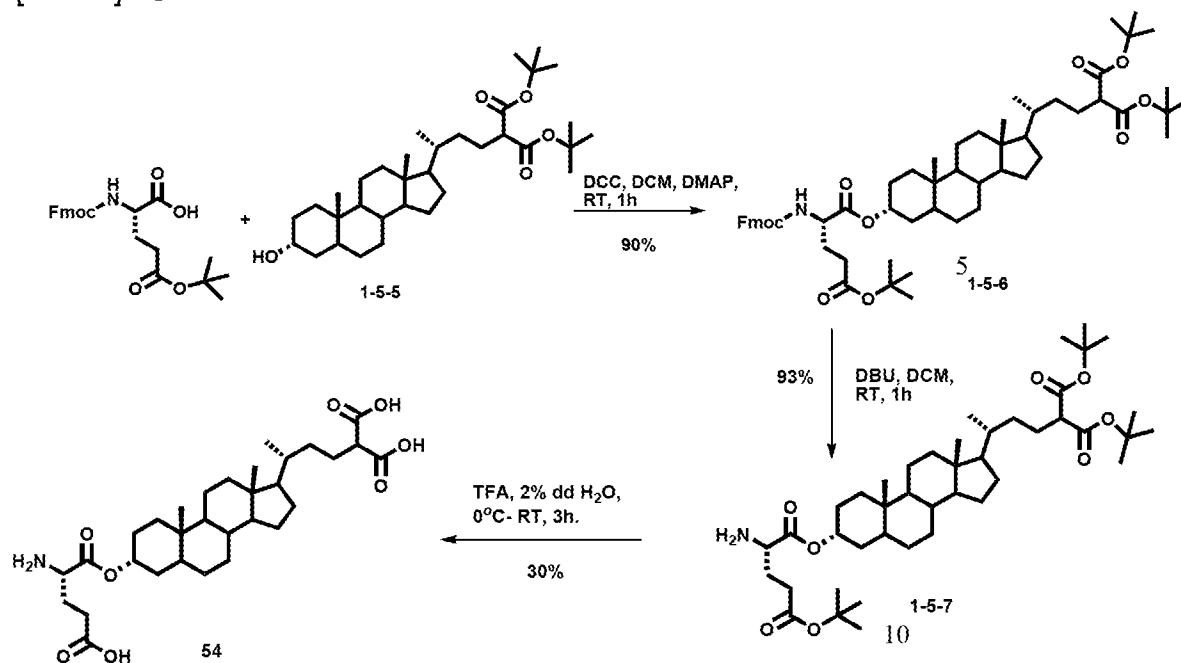
[00385] *Synthesis of compound 1-5-4*

[00386] To a solution of **1-5-3** (6.67 mmole) in tert. butanol (30ml) was added Boc-anhydride (13.34 mmole) followed by DMAP (2 mmole) and resulting reaction mixture was stirred at room temperature for 1 h. Reaction mass was concentrated, the residue was dissolved in DCM (40mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target product **1-5-4** (74%).

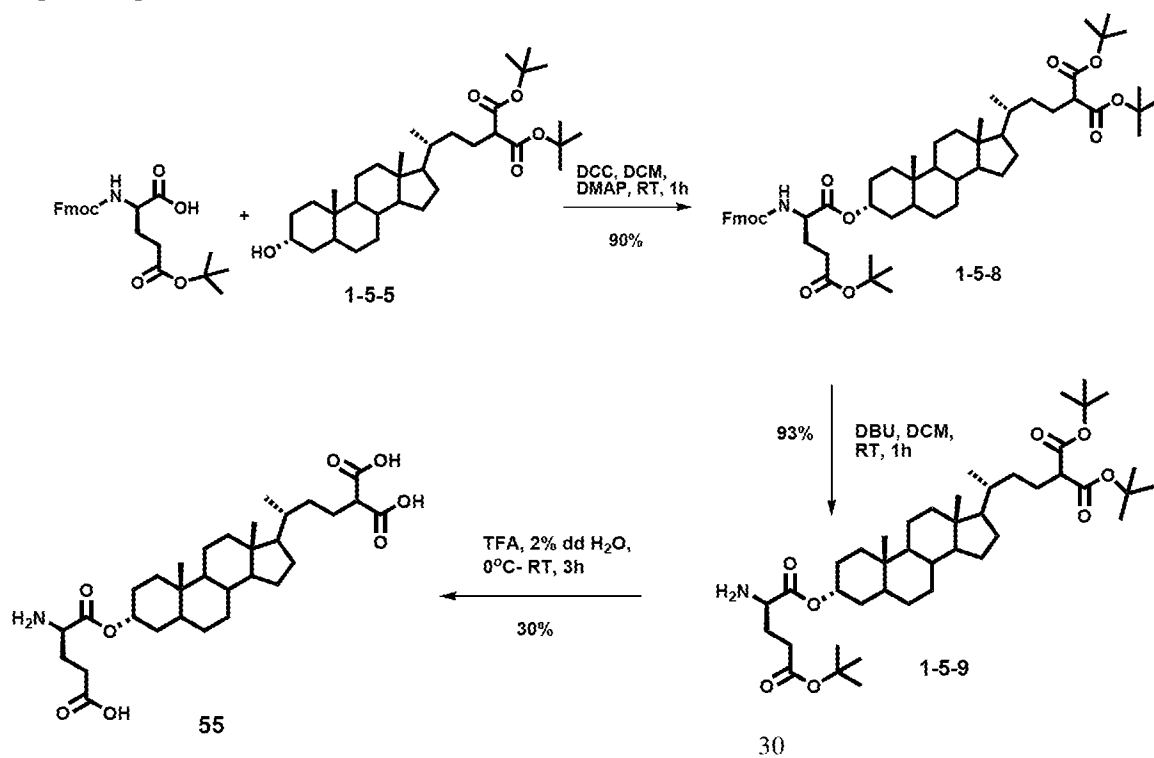
[00387] *Synthesis of compound 1-5-5*

[00388] Compound **1-5-4** (4.88 mmole) was dissolved in methanol (40ml) to it sodium methoxide (14.65 mmole) was added and the resulting reaction mixture was stirred at room temperature for 12 h. Reaction mass was concentrated, the residue was dissolved in DCM (30mL) and washed with water (2 x 30 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-5-5** (60%).

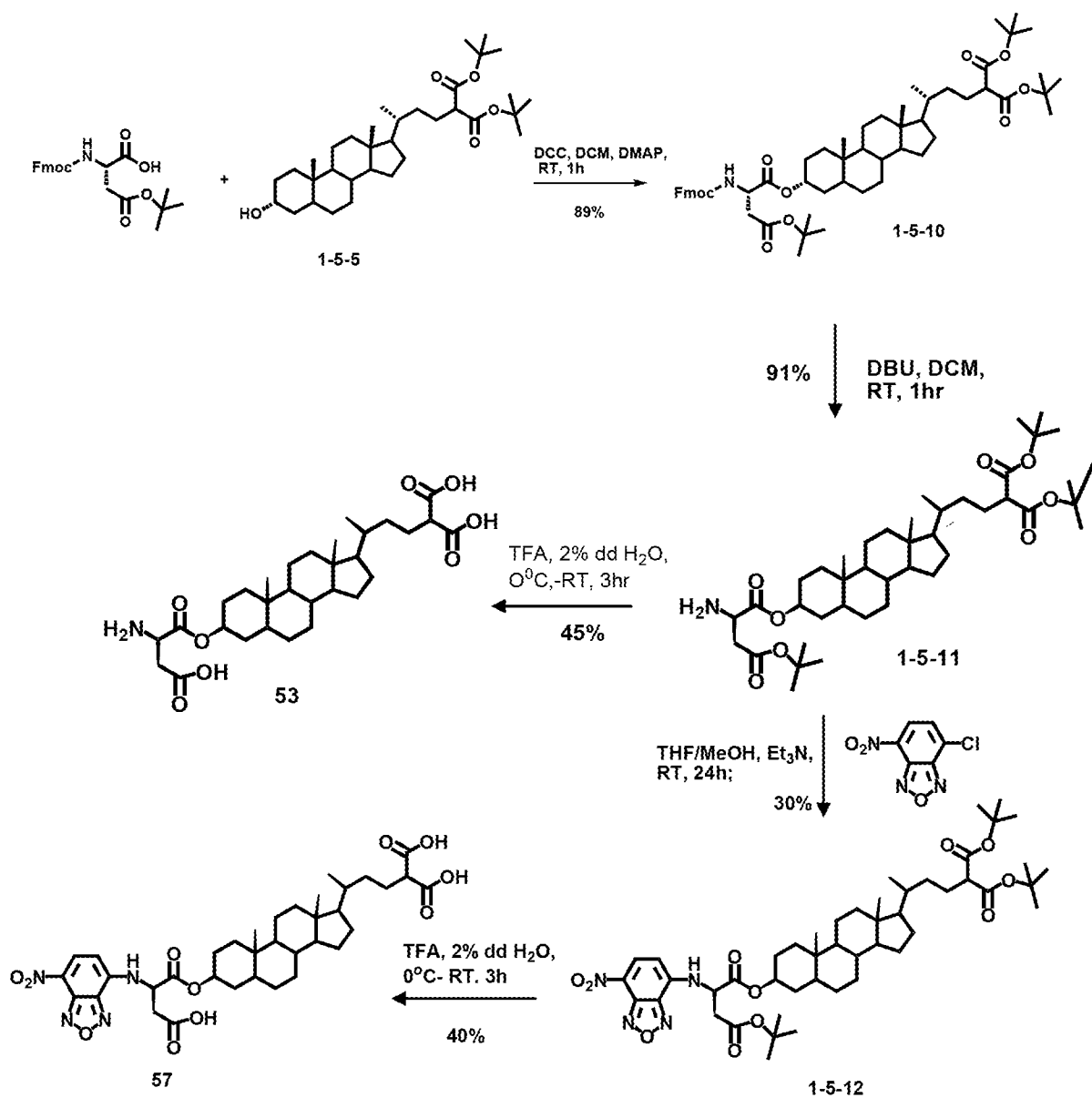
[00389] Scheme 25:

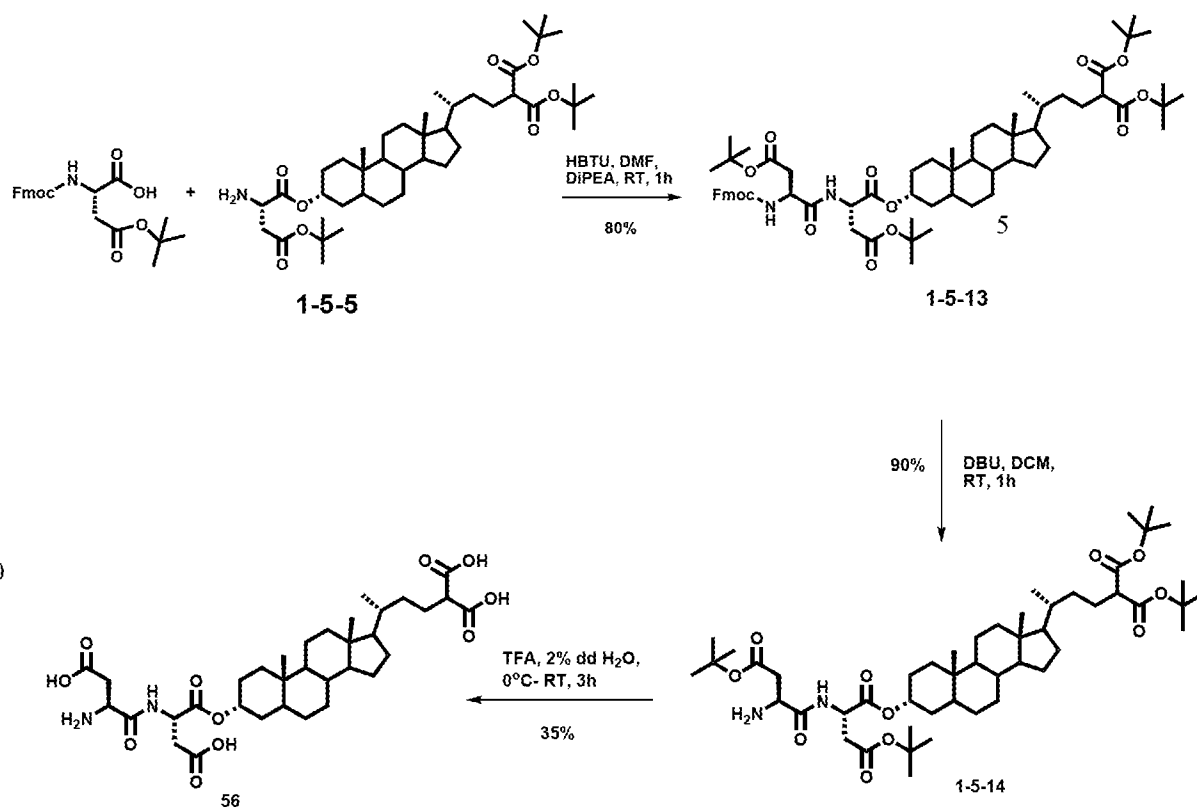


[00390] Scheme 26



[00391] Scheme 27



[00392] **Scheme 28**

[00393] *General Procedures for Synthesizing Compounds 1-5-6, 1-5-8, 1-5-10 and 1-5-13*

[00394] To a solution of corresponding Fmoc protected amino acid (0.510 mmole) in dichloromethane (10ml) was added compound 1-5-5, 1-5-11 (0.346 mmole) stirred for 10 min. A solution of DCC (0.553 mmole) in DCM (5ml) followed by DMAP (0.102 mmole) was added to above stirred solution and resulting reaction mixture was stirred at room temperature for 1h. Reaction mass was filtered and filtrate was washed with water (2 x 20 ml) followed by saturated sodium chloride solution (2 x 20 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The final residue was purified by

column chromatography (hexane/ethyl acetate, 80:20) to give the target products **1-5-6**, **1-5-8**, **1-5-10** and **1-5-13** (80-90%).

[00395] *General Procedures for Synthesizing Compounds 1-5-7, 1-5-9, 1-5-11, and 1-5-14*

5 [00396] To a solution of compound **1-5-6**, **1-5-8**, **1-5-10** or **1-5-13** (0.288 mmole) in dichloromethane (10ml) was added DBU (0.288 mmole) and stirred at room temperature for 1h. Reaction mass was concentrated and product purified by flash column chromatography to get desired compound **1-5-7**, **1-5-9**, **1-5-11**, and **1-5-14** (90-93%).

10 [00397] *General Procedures for Synthesizing Compound 1-5-12*

[00398] Compound **1-5-11** (0.250 mmole) was dissolved in a mixture of THF:MeOH (1:1) (5ml), then NaHCO₃ (0.375 mmole) was added, followed by NBD-Cl (0.275 mmole), and stirred at room temperature for 24h (in dark). Reaction mass was concentrated and product purified by column chromatography
15 to get desired compound **1-5-12** (30%).

[00399] *General Procedures for Synthesizing Compounds 53, 54, 55, 56, and 57*

[00400] Compound **1-5-7**, **1-5-9**, **1-5-11**, **1-5-12** or **1-5-14** (0.107 mmole) was dissolved in TFA (5 ml) at 0°C, water (50 µL) was added and the reaction mixture was stirred at 0°C to room temperature for 3h. The TFA solution was then
20 removed on rotary evaporator and the residue was neutralized with saturated solution of aq. NaHCO₃, dialyzed using membrane and purified by perverse phase HPLC to produce desired compounds as white solid.

[00401] **Compound 1-5-1**: ¹H NMR (CDCl₃, 300MHz): δ = 4.71-4.67 (m, 1H), 4.22-4.13 (m, 4H), 3.25 (t, J = 7.1Hz, 1H), 1.95 (s, 3H), 1.92-1.86 (m, 2H),
25 1.82-1.72 (m, 4H), 1.68-1.53 (m, 2H), 1.49-1.36 (m, 8H), 1.28-1.19 (m, 10H), 1.14-1.04 (m, 6H), 0.92-0.88 (m, 6H), 0.63 (s, 3H).

[00402] **Compound 1-5-5**: ¹H NMR (CDCl₃, 400MHz): δ = 3.62-3.56 (m, 1H), 3.04 (t, J = 7.6Hz, 1H), 1.94-1.92 (m, 1H), 1.82-1.78 (m, 4H), 1.75-1.68 (m, 7H), 1.43 (s, 18H), 1.37-1.35 (m, 7H), 1.23-1.21 (m, 5H), 1.10-1.01 (m, 6H), 0.92-0.88

(m, 6H), 0.63 (s, 3H); ^{13}C NMR (100MHz): δ = 169.41, 169.30, 81.43, 72.21, 56.80, 56.20, 54.73, 43.01, 40.78, 40.46, 36.82, 36.19, 35.79, 35.68, 34.90, 33.60, 30.91, 27.53, 26.74, 25.56, 24.52, 23.68, 21.14, 18.87, 12.32.

[00403] **Compound 54:** ^1H NMR (MeOD, 500MHz): δ = 4.88-4.86 (m, 1H), 3.19 (m, 1H), 2.56-2.51 (m, 2H), 2.19-2.12 (m, 2H), 2.04-1.88 (m, 6H), 1.76-1.74 (m, 2H), 1.62-1.47 (m, 11H), 1.30-1.20 (m, 4H), 1.19-1.08 (m, 7H), 0.98-0.96 (m, 6H), 0.70 (s, 3H); ^{13}C NMR (100MHz): δ = 175.68, 173.89, 173.84, 169.89, 78.72, 57.99, 57.46, 53.58, 44.01, 43.47, 41.98, 41.60, 37.33, 36.94, 36.07, 35.84, 34.68, 33.31, 30.55, 29.31, 28.27, 27.66, 27.62, 26.90, 26.86, 25.36, 23.86, 22.09, 19.92, 12.52, HRMS m/z calcd for $\text{C}_{31}\text{H}_{50}\text{NO}_8$, 564.3528; found 564.3536 ($\text{M} + \text{H}$) $^+$, HPLC conditions: retention time = 16.51 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00404] **Compound 55:** ^1H NMR (MeOD, 500MHz): δ = 4.87-4.85 (m, 1H), 4.08-4.05 (m, 1H), 2.56-2.51 (m, 1H), 2.19-2.12 (m, 2H), 2.04-1.88 (m, 6H), 1.77-1.75 (m, 2H), 1.61-1.59 (m, 2H), 1.54-1.45 (m, 8H), 1.42-1.31 (m, 3H), 1.29-1.08 (m, 7H), 0.98-0.96 (m, 6H), 0.70 (s, 3H); ^{13}C NMR (125MHz): δ = 175.64, 173.75, 173.72, 169.89, 78.73, 57.99, 57.46, 53.57, 44.02, 43.49, 41.99, 41.61, 37.34, 36.95, 36.08, 35.84, 34.71, 33.28, 30.55, 29.32, 28.24, 27.65, 26.92, 25.37, 23.87, 22.10, 19.18, 12.59, HRMS m/z calcd for $\text{C}_{31}\text{H}_{50}\text{NO}_8$, 564.3544; found 564.3536 ($\text{M} + \text{H}$) $^+$, HPLC conditions: retention time = 16.47 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00405] **Compound 53:** ^1H NMR (MeOD, 500MHz): δ = 4.91-4.83 (m, 1H), 4.27-4.05 (t, J = 4Hz, 1H), 3.22-3.19 (m, 1H), 3.04-2.93 (m, 2H), 2.03-2.01 (m, 1H), 1.97-1.90 (m, 5H), 1.75-1.71 (m, 2H), 1.61-1.59 (m, 2H), 1.51-1.42 (m, 8H), 1.29-1.22 (m, 3H), 1.20-1.11 (m, 7H), 0.98-0.96 (m, 6H), 0.70 (s, 3H); ^{13}C NMR (125MHz): δ = 173.72, 173.66, 173.00, 169.12, 78.79, 58.00, 57.49, 50.90, 44.02, 43.47, 41.98, 41.61, 37.33, 36.93, 36.05, 35.84, 35.11, 34.71, 33.21, 29.31, 28.27, 27.51, 26.81, 25.36, 23.86, 22.09, 19.18, 12.60, HRMS m/z calcd for $\text{C}_{30}\text{H}_{47}\text{NO}_8$,

550.3376; found 550.3380 ($M + H$)⁺, HPLC conditions: retention time = 18.72 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

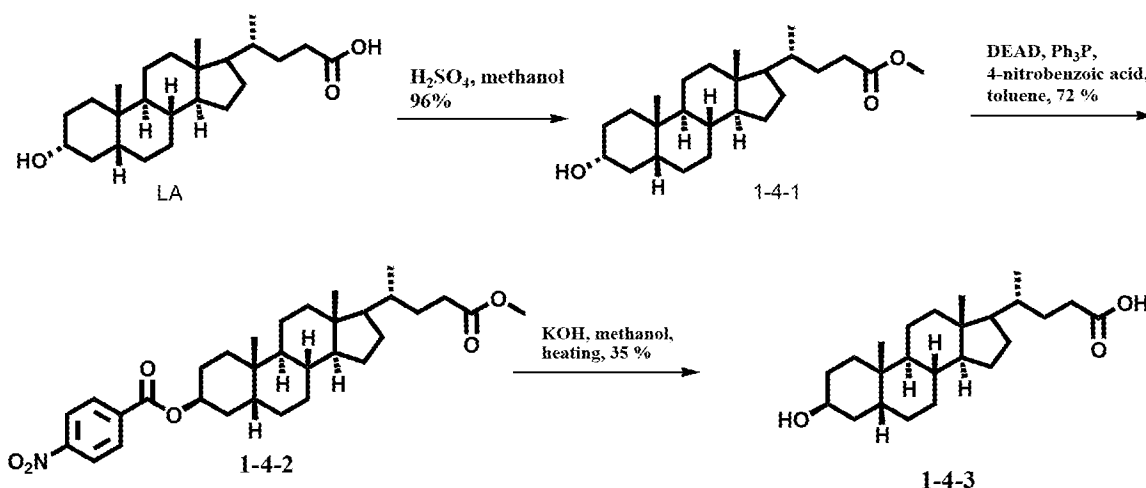
[00406] **Compound 57:** ¹H NMR (MeOD, 500MHz): δ = 8.53 (d, J = 8.5Hz, 1H), 6.53 (d, J = 9Hz, 1H), 4.82-4.76 (m, 1H), 3.23 (t, J = 7Hz, 1H), 3.15-3.04 (m, 2H), 3.04-2.93 (m, 1H), 1.94-1.77 (m, 6H), 1.74-1.68 (m, 2H), 1.56-1.35 (m, 10H), 1.24-1.19 (m, 3H), 1.11-0.96 (m, 6H), 0.93-0.92 (m, 6H), 0.63 (s, 3H); ¹³C NMR (125MHz): δ = 173.65, 173.45, 173.38, 171.02, 146.00, 145.54, 137.98, 124.95, 78.06, 57.85, 57.36, 53.58, 43.93, 43.45, 41.88, 41.53, 37.25, 36.93, 36.83, 36.11, 35.79, 34.79, 33.32, 29.28, 27.59, 26.84, 25.32, 23.88, 22.05, 19.18, 12.56, HRMS m/z calcd for C₃₆H₄₈N₄O₁₁, 713.3395; found 713.3398 ($M + H$)⁺, HPLC conditions: retention time = 18.75 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00407] **Compound 56:** ¹H NMR (MeOD, 500MHz): δ = 4.79-4.72 (m, 2H), 4.24-4.22 (m, 1H), 3.20 (t, J = 7.5Hz, 1H), 3.02-2.98 (m, 1H), 2.90-2.77 (m, 3H), 2.03-2.00 (m, 1H), 1.95-1.85 (m, 5H), 1.75-1.68 (m, 2H), 1.60-1.55 (m, 2H), 1.46-1.41 (m, 8H), 1.31-1.21 (m, 4H), 1.20-1.09 (m, 6H), 0.97-0.96 (m, 6H), 0.69 (s, 3H); ¹³C NMR (125MHz): δ = 174.04, 173.99, 173.79, 173.44, 171.42, 169.62, 77.59, 57.90, 57.43, 51.30, 50.30, 44.01, 43.51, 41.97, 41.60, 37.36, 36.95, 36.79, 36.70, 36.15, 35.85, 34.67, 33.39, 29.32, 28.30, 27.65, 26.93, 25.37, 23.91, 22.10, 19.18, 12.58, HRMS m/z calcd for C₃₄H₅₃N₂O₁₁, 665.3655; found 665.3649 ($M + H$)⁺, HPLC conditions: retention time = 18.75 min. Solvents: A)100% Water + 0.1% TFA, B)100% ACN + 0.05% TFA, Column: Kromasil 300-5C4.

[00408] **1.7 Synthesis of Compounds 58 to 70**

[00409] Compounds **58** to **64** (or compounds of formula (1-4)) were synthesized in accordance with procedures set forth in **Schemes 29** to **33**, whereas compounds 65 to 70 were synthesized in accordance with procedures set forth in **Schemes 34** to **40**.

[00410] **Scheme 29**



[00411] Synthesis of compound **1-4-1**

[00412] Placed 5.0 g of lithocholic acid (13.28 mmol) in the bottom of a 100mL round ended flask, added methanol and sulfuric acid (200 μL , 8M), allowed the mixture to react at room temperature for 3 hrs until the mixture turned into a clear solution. Reduced the volume of the clear solution by concentration, then added water to the concentrated solution to precipitate the product, washed with deionized water to give white solid compound **1-4-1** (5.00 g, 96%).

[00413] **Compound 1-4-1:** ^1H NMR (400 MHz, CDCl_3): δ 3.63 (s, 3H), 3.58 (m, 1H), 2.32 (m, 1H), 2.18 (m, 1H), 1.92 (m, 1H), 1.84 (m, 2H), 1.78 (t, $J = 3.1$ Hz, 1H), 1.75 (t, $J = 3.0$ Hz, 1H), 1.70 (t, $J = 8.4$ Hz, 1H), 1.63 (m, 1H), 1.50 (s, 4H), 1.38 (m, 5H), 1.3 (t, $J = 6.9$ Hz, 2H), 1.25 (m, 2H), 1.20 (d, $J = 11.0$ Hz, 2H), 1.13 (m, 1H), 1.08 (s, 1H), 1.03 (m, 2H), 0.96 (m, 1H), 0.90 (s, 4H), 0.86 (s, 2H), 0.61 (s, 3H).

[00414] Synthesis of compound **1-4-2**

[00415] In a 100 mL round ended flask, placed compound **1-4-1** (2.0 g, 5.12 mmol), Ph_3P (2.69 g, 10.24 mmol), and 4-nitrobenzoic acid (1.03 g, 6.13 mmol), 30 mL toluene was then added, and kept the mixture in an iced bath until the temperature of the reacting mixture reached 0°C , then diethyl azodicarboxylate was added, which turned the color of the mixture into orange, allowed the reaction to proceed for 24 hrs until its temperature returned to room temperature.

[00416] Concentrated the product to remove any un-reacted toluene, then dissolved the concentrated product in methylene dichloride, extracted it twice with brine. The organic layers were combined, dried with Na₂SO₄, then subjected to column chromatography, a white, non-crystallined solid or compound **1-4-2** was
5 yield (2.0 g, 72%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.40.

[00417] **Compound 1-4-2:** ¹H NMR (400 MHz, CDCl₃): δ 8.26 (d, *J* = 8.8 Hz, 2H), 8.17 (d, *J* = 8.8 Hz, 2H), 5.34 (s, 1H), 3.63 (s, 3H), 2.33 (m, 1H), 2.19 (m, 1H), 2.07 (m, 1H), 1.95 (t, *J* = 6.2 Hz, 1H), 1.84 (m, 2H), 1.72 (m, 3H), 1.59 (m, 4H), 1.38 (t, *J* = 6.5 Hz, 4H), 1.33 (d, *J* = 3.1 Hz, 1H), 1.29 (m, 2H), 1.23 (t, *J* = 5.9
10 Hz, 2H), 1.17 (m, 2H), 1.11 (d, *J* = 9.5 Hz, 1H), 1.04 (m, 3H), 0.99 (s, 3H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.62 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 174.9, 164.1, 150.6, 136.7, 130.7, 123.6, 13.0, 56.7, 56.1, 42.9, 10.3, 10.1, 37.9, 35.8, 35.4, 35.1, 31.2, 31.2, 31.1, 30.8, 28.3, 26.6, 26.3, 25.3, 24.3, 24.2, 21.3, 18.4, 12.2.

[00418] Synthesis of compound **1-4-3**

15 [00419] Mixed compound **1-4-2** (2.0 mg, 3.71 mmol), methanol (40 mL), KOH (200 μL, 40%) in a flask, stirred and heated the mixture to 60°C, and allowed the mixture to react for 3 hrs.

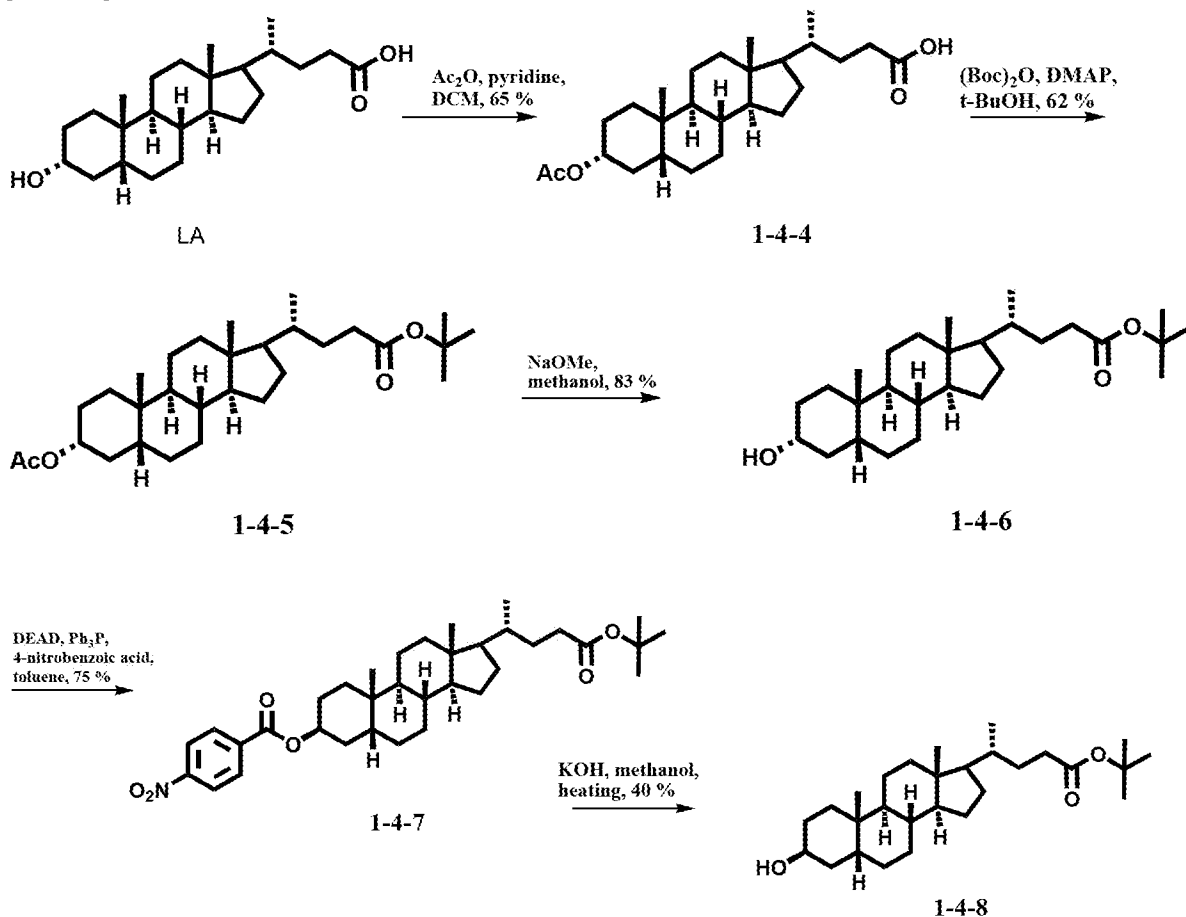
[00420] Concentrated the product to remove any un-reacted methanol, then dissolved the concentrated product in methylene dichloride, extracted it twice with
20 sodium bicarbonate. Collected the organic layer, extracted with brine, combined the collected organic layers, dried with Na₂SO₄, then subjected it to column chromatography (ethyl acetate/hexane = 1:9), a white, non-crystallined solid or compound **1-4-3** was obtained (500 mg, 35%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.20. m.p. = 194-196°C.

25 [00421] **Compound 1-4-3:** ¹H NMR (400 MHz, CDCl₃): δ 4.09 (m, 1H), 2.36 (m, 1H), 2.23 (m, 1H), 1.95 (m, 2H), 1.81 (m, 3H), 1.68 (t, *J* = 4.8 Hz, 1H), 1.55 (m, 2H), 1.45 (s, 2H), 1.36 (m, 6H), 1.25 (m, 6H), 1.14 (m, 2H), 1.03 (m, 4H), 0.93 (s, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.63 (s, 3H); ¹³C NMR (100 MHz, CDCl₃):

δ 179.1, 67.4, 56.8, 56.2, 43.0, 40.4, 39.9, 36.8, 35.8, 35.5, 35.3, 33.7, 31.0, 30.1, 29.9, 28.4, 28.0, 26.8, 26.4, 24.4, 24.1, 21.3, 18.4, 12.3

[00422] HRMS (ESI) calculated for $C_{24}H_{39}O_3$ (M-H)⁻ 375.2899; found 375.2900.

[00423] **Scheme 30**



10 [00424] **Synthesis of compound 1-4-6**

[00425] Dissolved lithocholic acid (4.00 mg, 10.6 mmol) in methylene dichloride (100 mL) in a 250 mL round ended flask, added pyridine (3.43 mL, 42.5 mmol), waited until all pyridine was dissolved, then added acetic anhydride (3.01 mL, 31.9 mmol), let the mixture to react at room temperature for 4 hrs.

15 [00426] Concentrated the product to remove any residual pyridine and acetic anhydride. Dissolved the concentrated product in methylene dichloride, extracted twice with 6% HCl, collected and combined the organic layers, extracted twice with sodium bicarbonate, dried with Na₂SO₄, then subjected it to column

chromatography (ethyl acetate/hexane = 1:9 to 3:7), vacuum dried to produce a white, non-crystalline solid or compound **1-4-4** (2.9 mg, 65%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.45.

[00427] Synthesis of compound **1-4-5**

5 [00428] Mixed and dissolved compound **1-4-4** (4.0 g, 9.55 mmol) in t-butanol (200 mL) in a round ended flask, added (Boc)₂O (4.09 mL, 19.11 mmol) and DMAP (350 mg, 2.87 mmol), and allowed the mixture to react at room temp for 3 hrs.

[00429] Concentrated the product to remove any residual t-butanol. Dissolved
10 the concentrated product in methylene dichloride, extracted twice with water, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (ethyl acetate/hexane = 1:9), vacuum dried to produce a white, non-crystalline solid or compound **1-4-5** (2800 mg, 62%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.80.

15 [00430] Synthesis of compound **1-4-6**

[00431] Mixed and dissolved compound **1-4-5** (5.0 g, 10.53 mmol) in methanol (200 mL) in a round ended flask, added NaOCH₃ (5.69g, 105.3 mmol), and allowed the mixture to react at room temp for 2 hrs.

[00432] Concentrated the product to remove any residual methanol.
20 Dissolved the concentrated product in methylene dichloride, extracted twice with water, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (ethyl acetate/hexane = 2:8), vacuum dried to produce a white, non-crystalline solid or compound **1-4-6** (3.8 g, 83%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.55.

25 [00433] **Compound 1-4-6:** ¹H NMR (400 MHz, CDCl₃): δ 3.59(m, 1H), 2.23 (m, 1H), 2.10 (m, 1H), 1.93 (m, 1H), 1.82 (m, 3H), 1.72 (m, 2H), 1.64 (s, 1H), 1.52 (s, 5H), 1.41 (s, 8H), 1.36 (t, J = 4.3 Hz, 6H), 1.24 (m, 5H), 1.09 (m, 6H), 0.95 (s, 2H), 0.88 (d, J = 7.6 Hz, 2H), 0.62 (s, 3H)

[00434] Synthesis of compound **1-4-7**

[00435] Mixed and dissolved compound **1-4-6** (2.0 g, 4.62 mmol), Ph₃P (1.82 g, 6.93 mmol), and 4-nitrobenzoic acid (910 mg, 5.55 mmol) in a 250 mL round ended flask, 100 mL dry toluene was then added, and kept the mixture in an iced bath until the temperature of the reacting mixture reached 0°C, then diethyl azodicarboxylate was added, which turned the color of the mixture into orange, allowed the reaction to proceed for 24 hrs until its temperature returned to room temperature.

[00436] Concentrated the product to remove any un-reacted toluene, then dissolved the concentrated product in methylene dichloride, extracted it twice with brine. Collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (Ethyl acetate /Hexane = 1 : 9), a white, non-crystallined solid of compound **1-4-7** was obtained (2.0 g, 75%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.40.

[00437] Synthesis of compound **1-4-8**

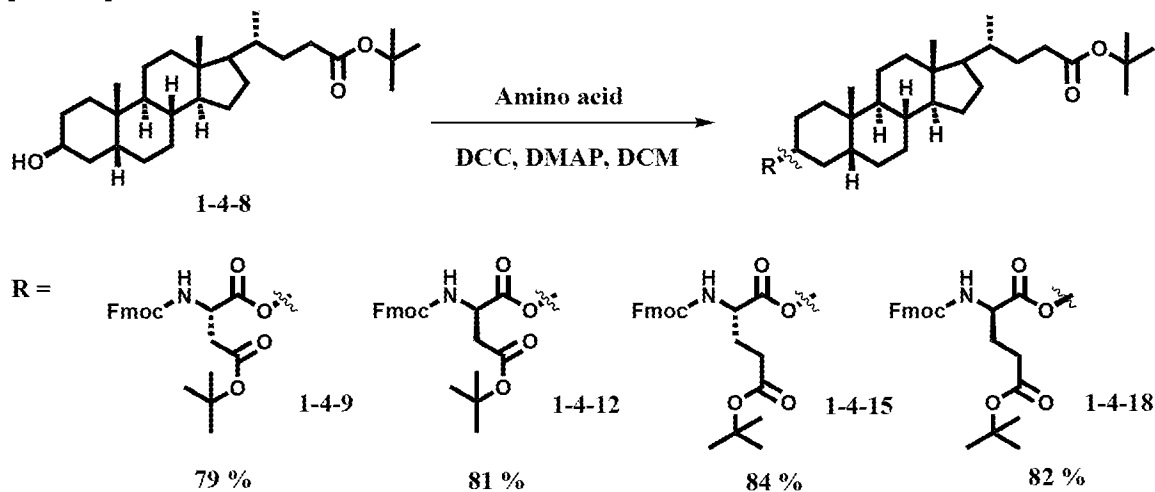
[00438] Mixed compound **1-4-7** (2.0 mg, 3.43 mmol), methanol (40 mL), KOH (200μL, 40%) in a flask, stirred and heated the mixture to 60°C, and allowed the mixture to react for 3 hrs.

[00439] Concentrated the product to remove any un-reacted methanol, then dissolved the concentrated product in methylene dichloride, extracted it twice with sodium bicarbonate. Collected the organic layer, extracted with brine, combined the collected organic layers, dried with Na₂SO₄, then subjected it to column chromatography (ethyl acetate/hexane = 1:9), a white, non-crystallined solid or compound **1-4-8** was obtained (600 mg, 40%).

[00440] **Compound 1-4-8**: ¹H NMR (400 MHz, CDCl₃): δ 4.07(s, 1H), 2.15 (m, 1H), 1.94 (m, 2H), 1.75 (m, 4H), 1.52 (m, 3H), 1.40 (s, 9H), 1.34 (m, 6H), 1.23 (m, 6H), 1.10 (m, 4H), 1.00 (m, 3H), 0.92 (s, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.61 (s, 3H)

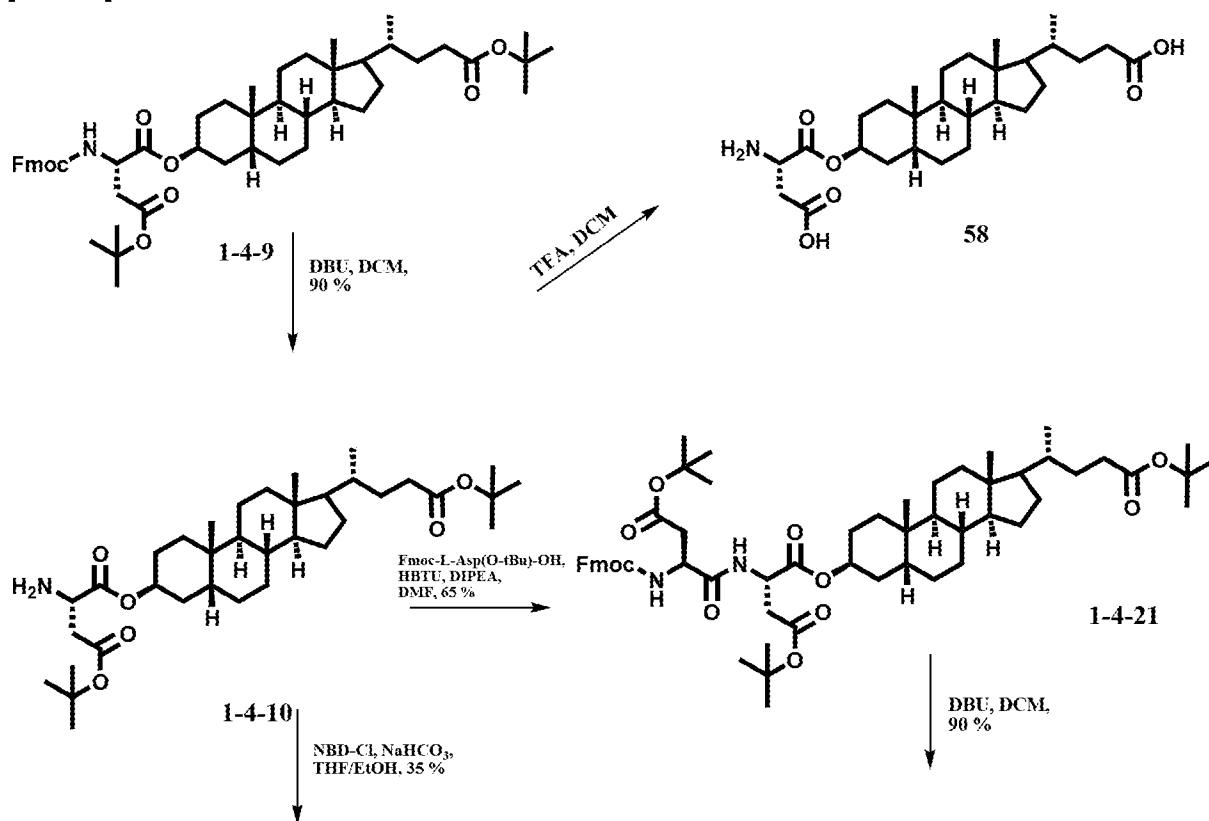
[00441] ¹³C NMR (100 MHz, CDCl₃): δ 173.7, 79.8, 66.9, 56.6, 56.1, 42.7, 40.3, 39.8, 36.5, 35.6, 35.3, 35.1, 33.5, 32.5, 31.1, 29.9, 28.2, 27.8, 26.7, 26.3, 24.2, 23.9, 21.1, 19.3, 12.1.

[00442] Scheme 31

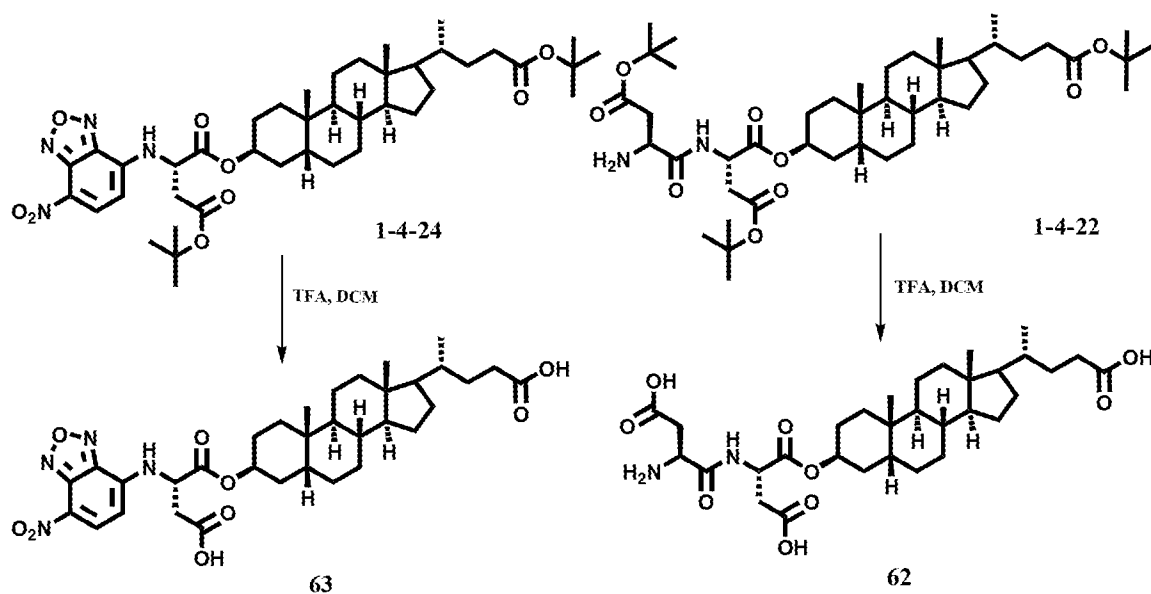


5

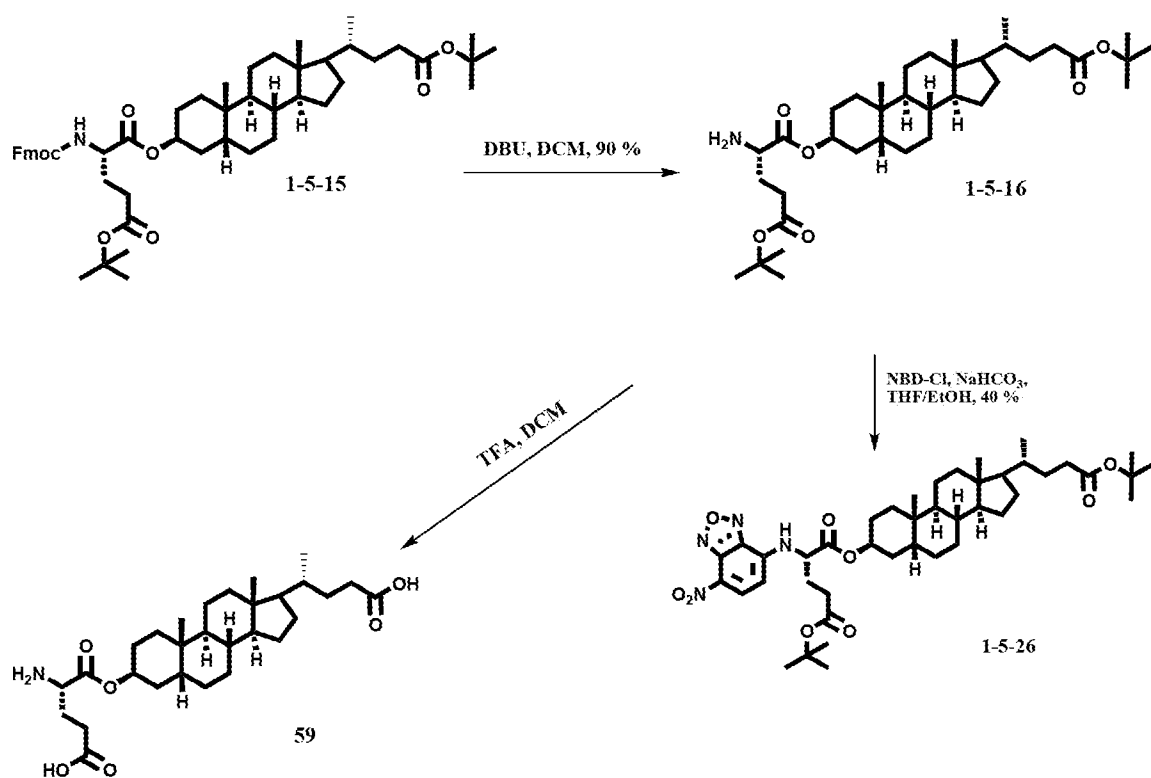
[00443] Scheme 32

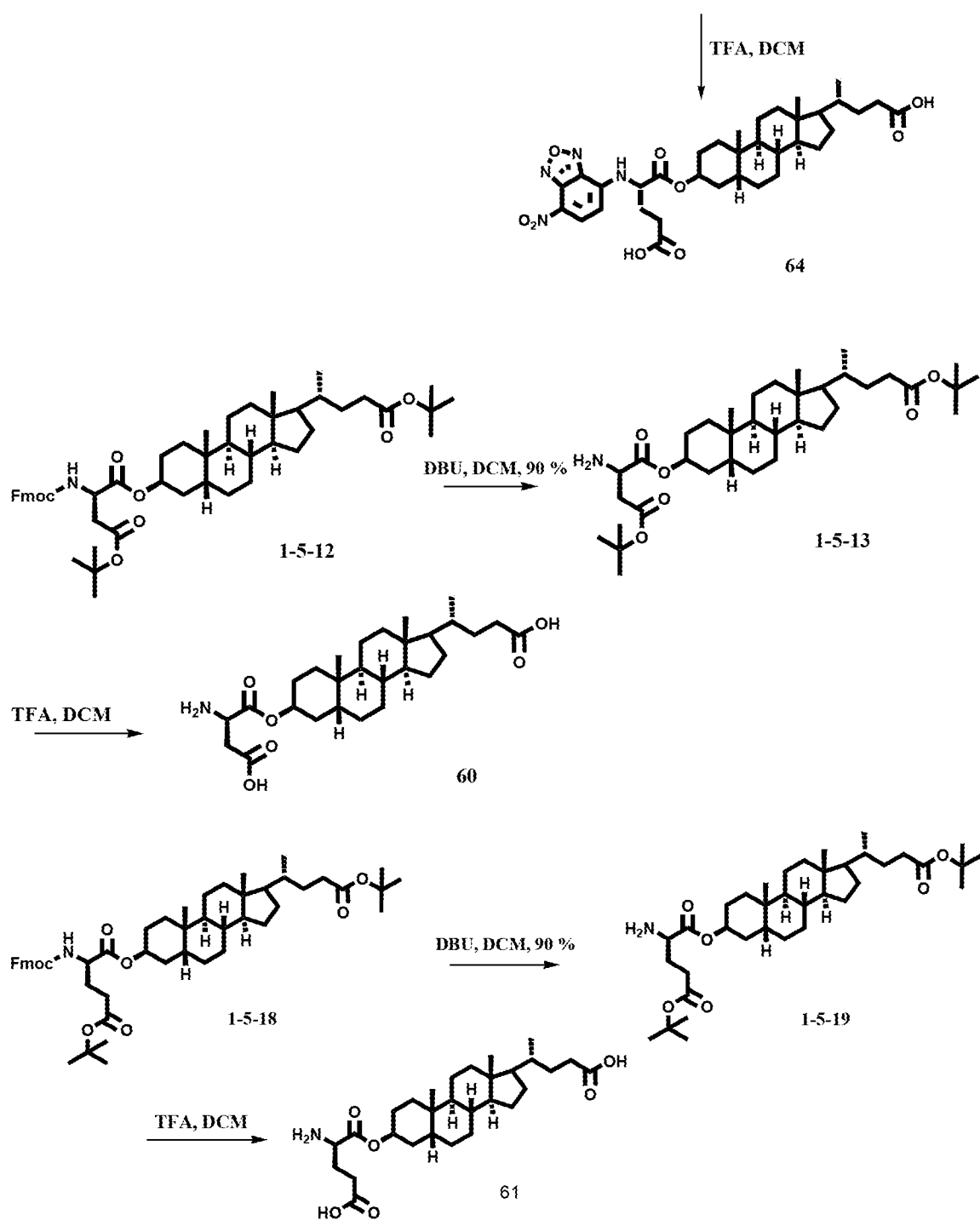


10



[00444] Scheme 33





[00445] Synthesis of compound 1-4-9

10 [00446] In a round ended flask, mixed and dissolved compound 1-4-8 (500 mg, 1.155 mmol) in methylene dichloride (25 mL), then added Fmoc-L-Asp-tert-butyl

(620 mg, 1.5 mmol), DCC (240 mg, 2.671 mmol), and DMAP (45 mg, 0.347 mmol), allowed the mixture to react at room temp for 1 hr.

[00447] Filtered the solution, discarded the precipitate, and extracted the filtrate with distilled water twice, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (ethyl acetate/hexane = 1:9), a white, non-crystallined solid or compound **1-4-9** was obtained (760 mg, 79%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

[00448] **Compound 1-4-9**: ¹H NMR (400 MHz, CDCl₃): δ 7.74(d, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 6.4 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 5.84 (d, *J* = 8.5 Hz, 1H), 5.14 (s, 1H), 4.55 (m, 1H), 4.34 (m, 2H), 4.22 (m, 1H), 2.86 (m, 1H), 2.21 (m, 1H), 2.10 (m, 1H), 1.94 (m, 2H), 1.82 (m, 2H), 1.71 (s, 1H), 1.58 (m, 5H), 1.43 (s, 9H), 1.41 (s, 9H), 1.34 (d, *J* = 5.7 Hz, 3H), 1.27 (s, 4H), 1.23 (s, 3H), 1.20 (s, 2H), 1.14 (m, 2H), 1.02 (m, 3H), 0.96 (s, 1H), 0.92 (s, 3H), 0.89 (s, 2H), 0.84 (s, 1H), 0.62 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 173.8, 170.3, 170.2, 156.2, 144.1, 144.1, 143.9, 141.4, 141.4, 127.8, 127.8, 127.2, 127.2, 125.3, 125.3, 120.1, 120.1, 81.9, 80.0, 72.8, 67.4, 56.7, 56.2, 50.9, 47.3, 42.9, 40.3, 40.1, 37.8, 37.6, 35.8, 35.4, 35.0, 32.7, 31.2, 31.0, 30.5, 28.3, 28.3, 28.3, 28.2, 28.2, 28.2, 26.6, 26.3, 25.1, 24.3, 24.1, 21.2, 18.4, 12.1. HRMS (ESI) calculated for C₅₁H₇₁NO₈Na (M+Na)⁺ 848.5077; found 848.5079

[00449] Synthesis of compound **58**

[00450] In a 100 mL round ended flask, mixed and dissolved compound **1-4-9** (200 mg, 0.242 mmol) in methylene dichloride (20 mL), then added DBU (40μL, 0.242 mmol), allowed the mixture to react at room temp for 0.5 hr.

[00451] Concentrated, and purified the product by flash column chromatography (eluted the column with hexane to hexane/EA = 3:7), a white, non-crystallined solid of compound **1-4-10** was obtained (130 mg, 90%). TLC (Ethyl acetate /Hexane = 4 : 6) : R_f = 0.45.

[00452] In a 50 mL round ended flask, mixed and dissolved compound **1-4-10** (100 mg, 0.165 mmol) in TFA (3 mL), then added deionized water (60μL), allowed

the mixture to react at room temp for 3 hrs. Concentrated the product to remove residual TFA, and a crude compound **58** was obtained (60 mg, 70%), which was further purified by HPLC to give a white, non-crystallized solid of compound 58.

[00453] M.P. = 143-145°C

5 [00454] **Compound 58**: ^1H NMR (400 MHz, MeOH- d_4): δ 5.21 (s, 1H), 4.30 (t, J = 5.2 Hz, 1H), 2.99 (m, 2H), 2.26 (m, 2H), 2.05 (m, 2H), 1.92 (m, 2H), 1.80 (m, 1H), 1.64 (m, 5H), 1.45 (m, 6H), 1.29 (m, 4H), 1.18 (m, 3H), 1.08 (m, 3H), 0.99 (s, 3H), 0.95 (d, J = 6.4 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (100 MHz, MeOH- d_4): δ 174.2, 172.8, 169.0, 75.8, 57.9, 57.6, 50.8, 44.0, 41.5, 41.2, 38.8, 37.1, 36.8, 36.0, 35.0, 32.4, 32.1, 31.9, 31.4, 29.3, 27.6, 27.9, 25.9, 25.3, 24.3, 22.3, 18.9, 12.6. HRMS (ESI) calculated for $\text{C}_{28}\text{H}_{46}\text{NO}_6$ ($\text{M}+\text{H}$) $^+$ 492.3325; found 492.3323

[00455] Synthesis of compound **1-4-12**

[00456] In a 100 mL round ended flask, mixed and dissolved compound **1-4-8** (500 mg, 1.155 mmol) in methylene dichloride (25 mL), then added
15 Fmoc-D-Asp-tert-butyl (620 mg, 1.5 mmol), DCC (240 mg, 2.671 mmol) and DMAP (45 mg, 0.347 mmol), allowed the mixture to react at room temperature for 1 hr.

[00457] Filtered the solution, discarded the precipitate, and extracted the filtrate with distilled water twice, collected and combined the organic layers, dried with
20 Na_2SO_4 , then subjected it to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (1:9)), a white, non-crystallined solid or compound **1-4-12** was obtained (780 mg, 82%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

[00458] **Compound 1-4-12**: ^1H NMR (400 MHz, CDCl_3): δ 7.73 (d, J = 7.5 Hz, 2H), 7.58 (t, J = 6.0 Hz, 2H), 7.38 (t, 2H), 7.29 (m, 2H), 5.85 (d, J = 8.5 Hz, 1H), 5.15 (s, 1H), 4.56 (t, J = 4.2 Hz, 1H), 4.38 (m, 2H), 4.22 (d, J = 7.2 Hz, 1H), 2.85 (m, 2H), 2.15 (m, 2H), 1.84 (m, 6H), 1.57 (m, 4H), 1.44 (s, 9H), 1.41 (s, 9H), 1.36 (m, 3H), 1.24 (m, 6H), 1.12 (m, 3H), 1.01 (m, 4H), 0.93 (s, 3H), 0.88 (d, J = 6.4 Hz, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 173.8, 170.3, 170.2, 156.2,

144.1, 143.9, 143.9, 141.4, 141.4, 127.8, 127.8, 127.2, 127.2, 125.4, 125.4, 125.3, 125.3, 120.1, 120.1, 81.9, 80.0, 72.8, 67.4, 56.2, 56.2, 50.9, 47.3, 42.9, 40.0, 37.9, 37.6, 35.8, 35.4, 34.0, 32.7, 31.2, 30.6, 28.3, 28.3, 28.3, 28.2, 28.2, 28.2, 26.5, 26.2, 24.9, 24.3, 24.1, 21.2, 18.4, 12.2. HRMS (ACPI) calculated for
5 $C_{51}H_{71}NO_8Na$ ($M+Na$)⁺ 848.5077; found 848.5081

[00459] Synthesis of compound **60**

[00460] In a 100 mL round ended flask, mixed and dissolved compound **1-4-12** (200 mg, 0.242 mmol) in methylene dichloride (20 mL), then slowly added DBU (40 μ L, 0.242 mmol), allowed the mixture to react at room temperature for 0.5 hr.

10 Concentrated, and purified the product by flash column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (3:7)), vacuume dried to produce a white, non-crystallined solid of compound **1-4-13** (130 mg, 90%). TLC (Ethyl acetate /Hexane = 4 : 6) : R_f = 0.45.

[00461] In a 50 mL round ended flask, mixed and dissolved compound **1-4-13**
15 (100 mg, 0.165 mmol) in TFA (3 mL), then added deionized water (60 μ L), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to remove any residual TFA, and a crude compound **60** was obtained (60 mg, 70%), which was further purified by HPLC to give a white, non-crystallized solid of compound **60**. Melting point = 184-186°C

20 [00462] **Compound 60**: ¹H NMR (400 MHz, MeOH- d_4): δ 5.21 (s, 1H), 4.31 (t, J = 4.9 Hz, 1H), 3.00 (m, 2H), 2.26 (m, 2H), 2.06 (m, 2H), 1.86 (m, 3H), 1.64 (m, 5H), 1.46 (m, 6H), 1.31 (m, 4H), 1.13 (m, 6H), 1.00 (s, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ¹³C NMR (100 MHz, MeOH- d_4): δ 178.2, 172.8, 169.0, 75.7, 57.9, 57.6, 50.8, 44.0, 41.5, 41.2, 38.8, 37.1, 36.8, 36.0, 35.0, 32.4, 32.1, 31.8, 31.6,
25 29.3, 27.6, 27.3, 25.7, 25.3, 24.3, 22.3, 18.9, 12.6; HRMS (ESI) calculated for $C_{28}H_{46}NO_6$ ($M+H$)⁺ 492.3325; found 492.3333

[00463] Synthesis of Compound **1-4-15**

[00464] In a 100 mL round ended flask, mixed and dissolved compound **1-4-8** (200 mg, 0.242 mmol) in methylene dichloride (25 mL), then added

Fmoc-L-Glu-tert-butyl (640 mg, 1.5 mmol), DCC (240 mg, 2.671 mmol) and DMAP (45 mg, 0.347 mmol), allowed the mixture to react at room temperature for 1 hr.

[00465] Filtered the solution, discarded the precipitate, and extracted the filtrate with distilled water twice, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (1:9)), a white, non-crystallined solid or compound **1-4-15** was obtained (800 mg, 84%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

[00466] **Compound 1-4-15:** ¹H NMR (400 MHz, CDCl₃): δ 7.72 (d, *J* = 7.3 Hz, 2H), 7.56 (m, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.27 (t, *J* = 7.46 Hz, 2H), 5.55 (d, *J* = 7.9 Hz, 1H), 5.10 (s, 1H), 4.35 (t, *J* = 7.0 Hz, 2H), 4.18 (t, *J* = 7.0 Hz, 1H), 2.23 (m, 4H), 1.94 (m, 3H), 1.83 (m, 3H), 1.70 (m, 2H), 1.56 (m, 5H), 1.42 (s, 9H), 1.41 (s, 9H), 1.35 (t, *J* = 4.4 Hz, 3H), 1.31 (d, *J* = 4.0 Hz, 1H), 1.24 (d, 5H), 1.10 (m, 4H), 1.00 (d, *J* = 8.3 Hz, 3H), 0.92 (s, 3H), 0.84 (d, *J* = 11.7 Hz, 3H), 0.61 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 173.7, 172.0, 171.4, 156.0, 143.9, 143.7, 143.7, 141.2, 141.2, 127.7, 127.7, 127.0, 127.0, 125.1, 125.1, 119.9, 119.9, 80.7, 76.7, 72.6, 67.0, 56.6, 56.5, 56.0, 53.7, 47.1, 42.7, 40.1, 39.9, 37.5, 36.5, 35.6, 35.2, 34.8, 33.5, 32.5, 31.0, 29.7, 28.1, 28.1, 28.1, 28.0, 28.0, 28.0, 26.4, 26.1, 24.9, 24.1, 23.8, 21.0, 18.3, 12.0; ; HRMS (ESI) calculated for C₅₂H₇₃NO₈Na (M+Na)⁺ 862.5234; found 862.5229

[00467] Synthesis of compound **59**

[00468] In a 100 mL round ended flask, mixed and dissolved compound **1-4-15** (200 mg, 0.238 mmol) in methylene dichloride (20 mL), then slowly added DBU (40 μL, 0.238 mmol), allowed the mixture to react at room temperature for 0.5 hr. Concentrated, and purified the product by flash column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (3:7)), vacuume dried to produce a white, non-crystallined solid of compound **1-4-16** (140 mg, 90%). TLC (Ethyl acetate /Hexane = 4 : 6) : R_f = 0.45.

[00469] In a 50 mL round ended flask, mixed and dissolved compound 1-4-16 (100 mg, 0.161 mmol) in TFA (3 mL), then added deionized water (60 μ L), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to remove any residual TFA, and a crude compound 59 was obtained (60 mg, 70%), which
5 was further purified by HPLC to give a white, non-crystallized solid of compound **59**. Melting point = 145-147°C

[00470] **Compound 59**: ^1H NMR (400 MHz, MeOH- d_4): δ 5.23(s, 1H), 4.09 (t, J = 6.6 Hz, 1H), 2.55 (m, 2H), 2.32 (m, 1H), 2.15 (m, 4H), 2.02 (m, 1H), 1.92 (m, 2H), 1.80 (d, J = 6.8 Hz, 1H), 1.65 (m, 4H), 1.54 (s, 1H), 1.48 (m, 6H), 1.32 (m, 4H),
10 1.20(m, 3H), 1.09 (m, 3H), 1.00 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, MeOH- d_4): δ 178.3, 175.4, 170.0, 75.7, 57.9, 57.6, 53.4, 44.0, 41.5, 41.2, 39.0, 37.1, 36.8, 36.1, 32.4, 32.1, 31.9, 31.6, 30.4, 29.3, 27.6, 27.3, 26.9, 25.9, 25.3, 24.3, 22.3, 18.8, 12.6; HRMS (ESI) calculated for $\text{C}_{29}\text{H}_{48}\text{NO}_6$ ($\text{M}+\text{H}$) $^+$ 506.3482; found 506.3481

15 [00471] Synthesis of Compound **1-4-18**

[00472] In a 100 mL round ended flask, mixed and dissolved compound **1-4-8** (500 mg, 1.155 mmol) in methylene dichloride (25 mL), then added Fmoc-D-Glu-tert-butyl (640 mg, 1.5 mmol), DCC (240 mg, 2.671 mmol) and DMAP (45 mg, 0.347 mmol), allowed the mixture to react at room temperature for
20 1 hr.

[00473] Filtered the solution, discarded the precipitate, and extracted the filtrate with distilled water twice, collected and combined the organic layers, dried with Na_2SO_4 , then subjected it to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (1:9)), a white, non-crystallized solid
25 or compound **1-4-18** was obtained (780 mg, 82%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

[00474] **Compound 1-4-18**: ^1H NMR (400 MHz, CDCl_3): δ 7.73 (d, J = 7.5 Hz, 2H), 7.55 (d, J = 7.2 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 5.57 (d, J = 8.2 Hz, 1H), 5.11 (s, 1H), 4.35 (m, 2H), 4.18 (t, J = 7.0 Hz, 1H), 2.33 (s, 2H),

2.21 (m, 2H), 2.09 (m, 2H), 1.94 (d, $J = 12.7$ Hz, 4H), 1.82 (m, 2H), 1.72 (m, 2H), 1.63 (d, $J = 12.8$ Hz, 2H), 1.54 (m, 3H), 1.43 (s, 9H), 1.41 (s, 9H), 1.34 (s, 3H), 1.27 (d, $J = 3.3$ Hz, 2H), 1.21 (s, 2H), 1.12 (d, $J = 11.8$ Hz, 3H), 1.06 (d, $J = 9.8$ Hz, 1H), 1.00 (d, $J = 7.8$ Hz, 3H), 0.92 (s, 3H), 0.87 (d, $J = 6.3$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 173.6, 172.0, 171.4, 156.0, 143.9, 143.7, 143.7, 141.2, 141.2, 127.7, 127.7, 127.0, 127.0, 125.1, 125.1, 119.9, 119.9, 80.7, 76.8, 72.5, 67.0, 56.6, 56.5, 56.0, 53.7, 47.1, 42.7, 40.1, 39.9, 37.3, 36.5, 35.6, 35.2, 34.8, 33.5, 32.5, 31.0, 29.7, 28.1, 28.1, 28.1, 28.0, 28.0, 28.0, 26.4, 26.1, 24.9, 24.1, 23.8, 21.1, 18.3, 12.0; HRMS (ESI) calculated for $\text{C}_{52}\text{H}_{73}\text{NO}_8\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 862.5234; found 862.5229.

[00475] Synthesis of compound **61**

[00476] In a 100 mL round ended flask, mixed and dissolved compound **1-4-18** (200 mg, 0.238 mmol) in methylene dichloride (20 mL), then slowly added DBU (40 μL , 0.238 mmol), allowed the mixture to react at room temperature for 0.5 hr.

Concentrated, and purified the product by flash column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (3:7)), vacuume dried to produce a white, non-crystallined solid of compound **1-4-19** (140 mg, 90%). TLC (Ethyl acetate /Hexane = 4 : 6) : $R_f = 0.45$.

[00477] In a 50 mL round ended flask, mixed and dissolved compound **1-4-19** (100 mg, 0.161 mmol) in TFA (3 mL), then added deionized water (60 μL), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to remove any residual TFA, and a crude compound **61** was obtained (60 mg, 70%), which was further purified by HPLC to give a white, non-crystallized solid of compound **61**. Melting point = 159-161 $^{\circ}\text{C}$

[00478] **Compound 61**: ^1H NMR (400 MHz, $\text{MeOH}-d_4$): δ 5.24 (s, 1H), 4.11 (t, $J = 6.8$ Hz, 1H), 2.57 (m, 2H), 2.32 (m, 1H), 2.15 (m, 4H), 1.90 (m, 4H), 1.64 (m, 5H), 1.47 (m, 6H), 1.31 (m, 4H), 1.13 (m, 6H), 1.00 (s, 3H), 0.94 (d, $J = 6.4$ Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (100 MHz, $\text{MeOH}-d_4$): δ 178.3, 175.5, 170.0, 75.7, 57.9, 57.6, 53.4, 44.0, 41.5, 41.2, 38.9, 37.1, 36.8, 36.1, 32.4, 32.1, 31.9, 31.6, 30.5,

29.3, 27.6, 27.3, 27.0, 25.9, 25.3, 24.3, 22.3, 18.8, 12.6; HRMS (ESI) calculated for $C_{29}H_{48}NO_6$ (M+H)⁺ 506.3482; found 506.3485.

[00479] Synthesis of compound **62**

[00480] In a 100 mL round ended flask, mixed and dissolved compound **1-4-13** (200 mg, 0.331 mmol) in methylene dichloride (25 mL), then added
5 Fmoc-D-Asp-tert-butyl (160 mg, 0.37 mmol), HBTU (165 mg, 0.431 mmol) and DiPEA (180 μ L, 0.993 mmol), allowed the mixture to react at room temperature for 2 hrs.

[00481] Concentrated to remove any residual DMF, dissolved the concentrated
10 product in methylene dichloride, then extracted the solution with NaHCO₃ twice, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (3:7)), a white, non-crystallined solid or compound **1-4-21** was obtained (220 mg, 65%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.45.

[00482] **Compound 1-4-21**: ¹H NMR (400 MHz, CDCl₃): δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.3 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.27 (t, *J* = 7.4 Hz, 2H), 5.98 (d, *J* = 8.3 Hz, 1H), 5.11 (s, 1H), 4.72 (m, 1H), 4.57 (d, *J* = 6.6 Hz, 1H), 4.36 (d, *J* = 7.2 Hz, 2H), 4.20 (t, *J* = 7.1 Hz, 1H), 2.90 (m, 2H), 2.74 (m, 1H), 2.63 (m, 1H), 2.21 (m, 1H), 2.09 (m, 1H), 1.92 (m, 2H), 1.81 (m, 2H), 1.70 (m, 1H), 1.55 (m, 4H), 1.43 (s, 9H), 1.41 (s, 9H), 1.38 (s, 9H), 1.36 (s, 2H), 1.32 (s, 3H), 1.23 (m, 5H), 1.11 (m, 3H), 0.99 (m, 4H), 0.91 (s, 3H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.60 (s, 3H).
20

[00483] In a 100 mL round ended flask, mixed and dissolved compound **1-4-21** (200 mg, 0.203 mmol) in methylene dichloride (20 mL), then slowly added DBU (30 μ L, 0.203 mmol), allowed the mixture to react at room temperature for 0.5 hr. Concentrated, and purified the product by flash column chromatography (eluted the column with methylene dichloride/methanol (9:1)), vacume dried to produce a
25 white, non-crystallined solid of compound **1-4-22** (145 mg, 90%). TLC (methylene dichloride/methanol = 8 : 2) : R_f = 0.70.

[00484] In a 50 mL round ended flask, mixed and dissolved compound **1-4-22** (100 mg, 0.129 mmol) in TFA (3 mL), then added deionized water (60 μ L), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to remove any residual TFA, and a crude compound **62** was obtained (60 mg, 70%), which
5 was further purified by HPLC to give a white, non-crystallized solid of compound **62**. Melting point = 155-157°C

[00485] **Compound 62:** ^1H NMR (400 MHz, MeOH- d_4): δ 5.11(s, 1H), 4.81 (t, J = 5.5 Hz, 1H), 4.26 (m, 1H), 2.87 (m, 3H), 2.33(m, 1H), 2.19 (m, 1H), 2.03 (m, 2H), 1.91 (m, 2H), 1.78 (m, 1H), 1.63 (m, 5H), 1.46 (m, 6H), 1.31 (m, 5H), 1.12 (m, 6H),
10 1.00 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, MeOH- d_4): δ 178.2, 173.7, 172.9, 171.2, 169.3, 74.3, 57.9, 57.5, 51.0, 50.7, 44.0, 41.6, 41.3, 38.9, 37.1, 36.8, 36.3, 36.0, 32.4, 32.1, 32.0, 31.7, 31.5, 29.3, 27.6, 27.4, 25.9, 25.3, 24.4, 22.3, 18.9, 12.6; HRMS (MALDI) calculated for $\text{C}_{32}\text{H}_{50}\text{N}_2\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 629.3414; found 629.3431.

15 [00486] Synthesis of compound **63**

[00487] In a 100 mL round ended flask, mixed and dissolved compound **1-4-10** (200 mg, 0.331 mmol) in THF/ethanol = 1:1 (20 mL), then added NBD-Cl (73 mg, 0.365 mmol), NaHCO_3 (42 mg, 0.497 mmol), allowed the mixture to react at room temperature for 24 hrs.

20 [00488] Concentrated the product, and then dissolved the concentrated product in methylene dichloride, extracted twice with water, collected and combined the organic layers, dried with Na_2SO_4 , then subjected it to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (1:9)), a white, non-crystallized solid or compound **1-4-24** was obtained (90 mg, 35%).

25 TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

[00489] In a 50 mL round ended flask, mixed and dissolved compound **1-4-24** (90 mg, 0.129 mmol) in TFA (3 mL), then added deionized water (60 μ L), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to remove any residual TFA, and a crude compound **63** was obtained (60 mg, 70%), which

was further purified by HPLC to give a white, non-crystallized solid of compound **63**. Melting point = 138-140°C

[00490] **Compound 63:**

[00491] ^1H NMR (400 MHz, Methanol- d_4): δ 8.57 (d, J = 8.7 Hz, 1H), 6.60 (d, J = 8.7 Hz, 1H), 5.15 (s, 2H), 3.12 (m, 2H), 2.27 (m, 4H), 2.00 (m, 2H), 1.79 (m, 2H), 1.59 (m, 3H), 1.46 (m, 4H), 1.33 (m, 8H), 1.17 (m, 2H), 1.04 (m, 3H), 0.93 (m, 3H), 0.70 (d, J = 5.5 Hz, 3H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, Methanol- d_4): δ 176.7, 172.2, 170.5, 169.2, 144.5, 136.3, 75.7, 73.1, 73.0, 68.8, 56.3, 56.0, 42.4, 41.9, 39.9, 39.6, 37.3, 35.5, 35.2, 34.3, 34.2, 30.8, 30.5, 30.2, 30.0, 27.7, 25.9, 25.7, 24.3, 23.7, 22.6, 22.3, 20.7, 17.3; HRMS (MALDI) calculated for $\text{C}_{34}\text{H}_{46}\text{N}_4\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 677.3162; found 677.3181.

[00492] Synthesis of compound **64**

[00493] In a 100 mL round ended flask, mixed and dissolved compound **1-4-16** (200 mg, 0.323 mmol) in THF/ethanol = 1:1 (20 mL), then added NBD-Cl (72 mg, 0.356 mmol), NaHCO_3 (41 mg, 0.485 mmol), allowed the mixture to react at room temperature for 24 hrs, at which time, the solution was yellow in color.

[00494] Concentrated the product, and then dissolved the concentrated product in methylene dichloride, extracted it twice with water, collected and combined the organic layers, dried with Na_2SO_4 , then subjected the product to column chromatography (eluted the column with a gradient of hexane to ethyl acetate/hexane (1:9)), a yellow, non-crystallized solid or compound **1-4-26** was obtained (100 mg, 40%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45.

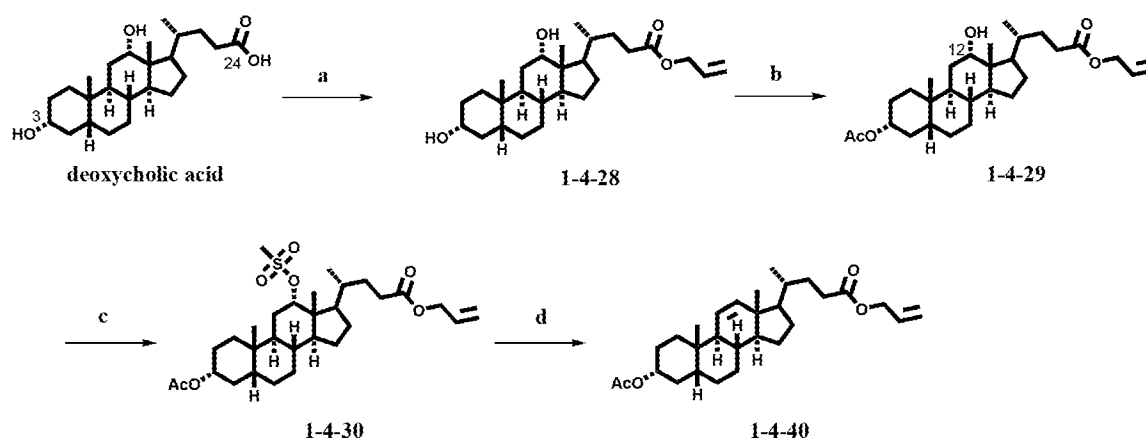
[00495] ^1H NMR (400 MHz, CDCl_3): δ 8.53 (d, J = 8.2 Hz, 1H), 6.32 (d, J = 8.2 Hz, 1H), 5.21 (m, 1H), 4.57 (m, 1H), 2.57 (s, 2H), 2.31 (m, 3H), 2.06 (m, 4H), 1.80 (m, 5H), 1.59 (m, 8H), 1.44 (t, J = 10.0 Hz, 18H), 1.28 (t, J = 26.0 Hz, 18H), 1.07 (m, 8H), 0.86 (m, 11H), 0.66 (s, 3H)

[00496] In a 50 mL round ended flask, mixed and dissolved compound **1-4-26** (100 mg, 0.1495 mmol) in TFA (3 mL), then added 2% deionized water (60 μL), allowed the mixture to react at room temp for 3 hrs. Concentrated the product to

remove any residual TFA, and a crude compound **64** was obtained (60 mg, 70%), which was further purified by HPLC to give a yellow, non-crystallized solid of compound **64**. Melting point = 195-197°C

[00497] **Scheme 34**

5

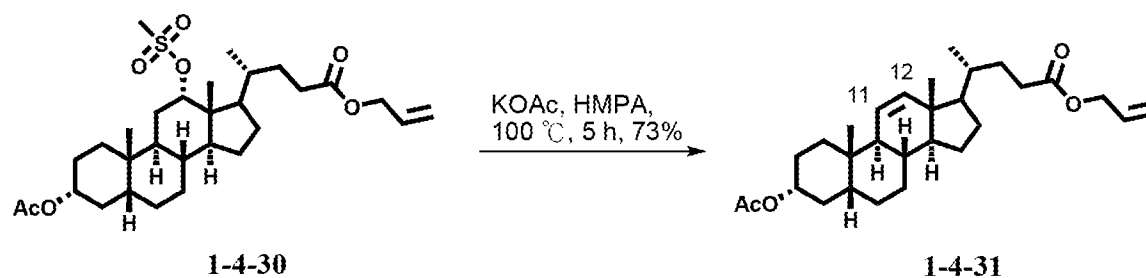


Reagent and conditions:

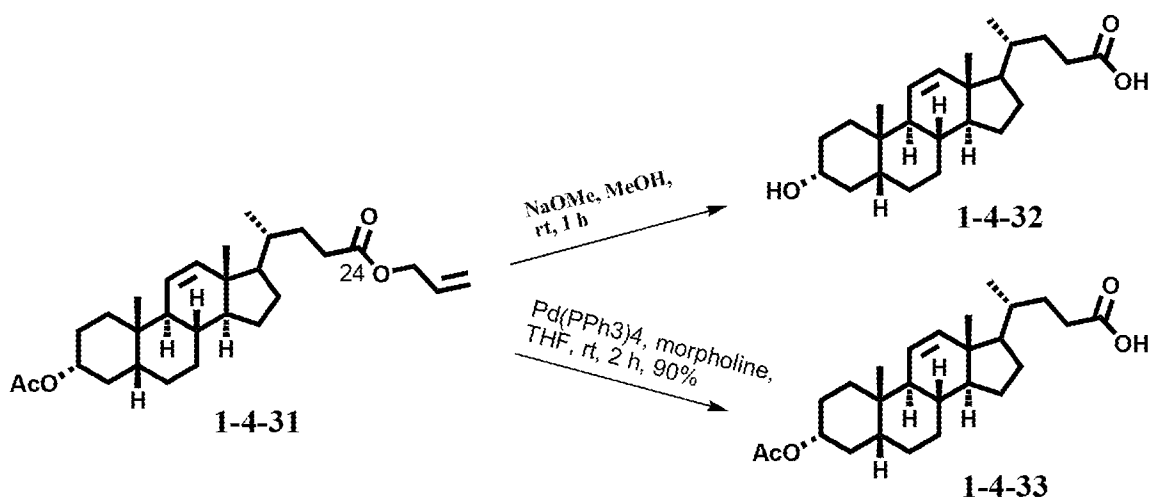
(a) allyl bromide, Cs_2CO_3 , DMF, rt, 6 h, 96%; (b) Ac_2O , pyridine, DCM, rt, 17 h, 65%; (c) MsCl , pyridine, 0 °C-rt, 5 h, 74%; and (d) KOAc , DMPU, 125 °C, 4 h, 80%.

10

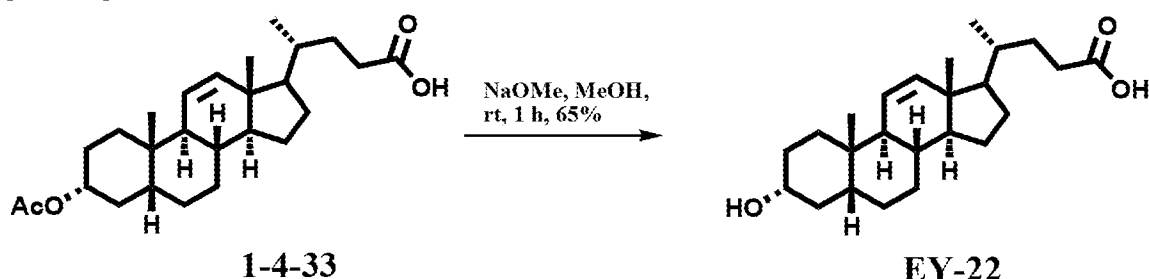
[00498] **Scheme 35**



15 [00499] **Scheme 36**



[00500] Scheme 37



5 [00501] Synthesis of compound 1-4-30

[00502] In a 100 mL double-necked flask, mixed and dissolved deoxycholic acid (2.00g, 5.09 mmol) in dry DMF (8 ml), then Cs₂CO₃ (1.66g, 5.09 mmol) and allyl bromide (3.1 mL, 35.87 mmol) were added, and allowed the mixture to react at room temperature for 6 hrs or until the color of the pH indicator, Bromocresol

10 Green, turned into blue, indicating the reaction had completed. Removed any of the residues by filtering, collected the filtrate and removed the residual DMF by vacume. Dissolved the product in ethyl acetate (EA), then extracted twice with double distilled water. Harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a

15 gradient of EA/hexane, from 1:6 to 1:1), a white, non-crystallined solid or compound 1-4-28 was obtained (2.11 g, 95%). TLC (Ethyl acetate /Hexane = 5 : 5) : R_f = 0.75.

[00503] In a 100 mL double-necked flask, mixed and dissolved compound 1-4-31 (2.00g, 4.63 mmol) in dry DCM (15 ml), then acetic anhydride (1.75 mL, 18.55 mmol) was added, folwed by the addition of pyridine (1.5 mL, 18.60 mmol) in dropwise manner, allowed the mixture to react at room temperature for 17 hrs.

5 Vaccume removed any residual pyridine and/or acetic anhydride, redissolved the product in DCM, extracted it twice with 6% HCl. Harvested and combined the organic layers, then extracted it twice with NaHCO₃, collected and combined the organic layers, dried with Na₂SO₄, then subjected it to flash column chromatography (eluted the column with a gradient of EA/hexane, from 1:5 to 3:7),
10 a sticky liquid of compound **1-4-29** was obtained (1.43g, 65%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.55.

[00504] In a 100 mL double-necked flask, mixed and dissolved compound 1-4-29 (1.50g, 3.16 mmol) in pyridine (10 ml), placed the entire system in an iced bath, then slowly added methanesulfonyl chloride (0.74 mL, 9.56 mmol), at which
15 time the temperature of the reacting mixture would rise from 0 °C to room temperature, allowed the mixture to react at room temperature for about 5 hrs. Once the reaction was completed, vacuumed removed any residual pyridine, redissolved the product in DCM, extracted it twice with 6% HCl. Harvested and combined the organic layers, then extracted it twice with NaHCO₃, collected and
20 combined the organic layers, dried with Na₂SO₄, then subjected it to flash column chromatography (eluted the column with a gradient of EA/hexane, from 1:5 to 3:7), a sticky liquid of compound **1-4-30** was obtained (1.43g, 65%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.55. Melting point = 95-97 °C

[00505] Compound **1-4-30**: ¹H NMR (400 MHz, CDCl₃) : δ 5.88 (m, 1H), 5.28 (dd, J = 17.2, 1.6 Hz, 1H), 5.20 (dd, J = 10.4, 1.6 Hz, 1H), 5.08 (s, 1H), 4.67 (m, 1H), 4.54 (d, J = 6.0 Hz, 2H), 3.03 (s, 3H), 2.40-2.15 (m, 2H), 1.99 (s, 3H), 1.97-1.60 (m, 9H), 1.59-1.39 (m, 8H), 1.35-0.99 (m, 7H), 0.96 (d, J = 6.4 Hz, 3H), 0.89 (s, 3H), 0.73 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) : δ 173.9, 170.8, 132.5, 118.4, 85.3, 74.3, 65.2, 48.9, 47.4, 46.2, 42.0, 39.9, 36.0, 35.4, 35.0, 34.4, 34.2, 32.6, 31.3,

31.0, 27.7, 27.3, 27.2, 26.6, 26.0, 23.7, 23.3, 21.7, 17.9, 12.6; HRMS-ESI : calculated for $C_{30}H_{48}O_7SNa$ ($M+Na$)⁺, 575.3018 ; found, 575.3022.

[00506] Synthesis of compound **1-4-31**

[00507] In a 100 mL double-necked flask, mixed and dissolved compound of
5 1-4-30 (2.00g, 3.62 mmol) in hexamethylphosphoramine (HMPA) (15 ml), then potassium acetate (5.33 g, 54.3 mmol) was added. The mixture was then heated to about 100°C, then allowed it to react for 5 hrs. Dissolved the product in EA, then extracted the solution twice with 6% HCl. Harvested and combined the organic layers, dried with $MgSO_4$, then subjected it to column chromatography
10 (eluted the column with a gradient of EA/hexane (1:25)), an oil compound **1-4-31** was obtained (1.2 g, 73%). TLC (Ethyl acetate /Hexane = 1 : 9) : R_f = 0.60.

[00508] Synthesis of **EY-22**

[00509] In a 100 mL double-necked flask, mixed and dissolved compound
1-4-31 (1.5 g, 3.32 mmol) in dry THF (15 ml), added $Pd(Ph_3)_4$ (0.12 g, 0.10 mmol),
15 followed by the addition of morpholine (5.74 mL, 66.40 mmol) in dropwise manner. Allowed the mixture to react at room temperature for 2 hrs or until the color of the mixture turned into golden yellow, indicating the reaction had completed. Vacuumed removed the residual DMF. Dissolved the product in DCM, then extracted the solution twice with 6% HCl. Harvested and combined the organic
20 layers, dried with Na_2SO_4 , then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:20 to 3:7), an oil compound **1-4-33** was obtained (1.2 g, 90%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.50.

[00510] In a 50 mL flask, mixed and dissolved compound **1-4-33** (0.2 g, 0.48 mmol) in methanol (8 mL), then NaOMe (0.13g, 2.40 mmol) was added, allowed
25 the mixture to react at room temperature for 1 hr. Vacuumed removed residual methanol once the reaction was completed, re-dissolved the product in DCM, then extracted the solution twice with double distilled water. Harvested and combined the organic layers, dried with Na_2SO_4 , then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:9 to 2:1),

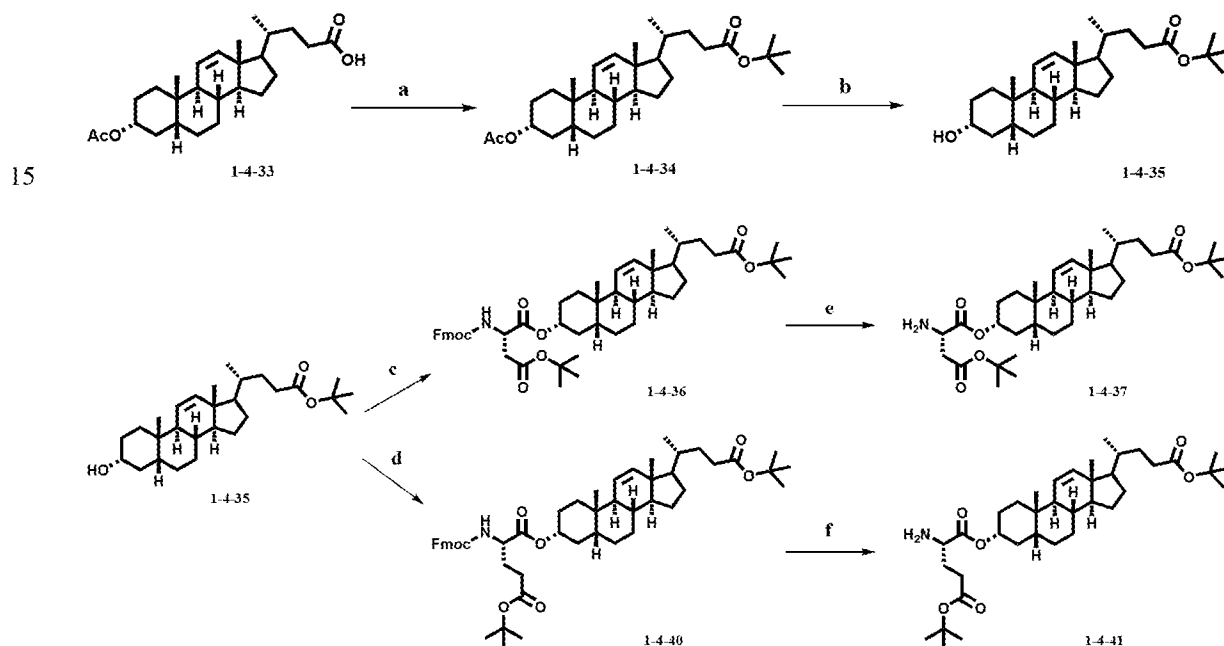
a white, non-crystalline solid or compound **EY-22** was obtained (117 mg, 65%).
TLC (Ethyl acetate /Hexane = 1 : 1) : R_f = 0.50.

[00511] Compound **EY-22** was further purified by HPLC, then freeze dried to produce a white solid of compound **EY-22**.

5 [00512] Melting point = 155-157°C

[00513] Compound **EY-22**: ^1H NMR (400 MHz, CDCl_3) : δ 6.07 (dd, J = 10.4, 2.8 Hz, 1H), 5.40 (d, J = 10.4 Hz, 1H), 3.62 (m, 1H), 2.4-2.18 (m, 3H), 1.96-1.62 (m, 7H), 1.60-1.38 (m, 6H), 1.37-1.10 (m, 8H), 0.99 (d, J = 6.4 Hz, 3H), 0.86 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) : δ 179.8, 138.9, 125.8, 72.0, 53.8, 52.1, 45.2, 43.4, 41.4, 37.2, 36.1, 35.4, 35.2, 34.6, 31.2, 30.9, 30.6, 28.6, 28.3, 25.7, 23.9, 23.2, 18.5, 16.9; HRMS-ESI : calculated for $\text{C}_{24}\text{H}_{37}\text{O}_3$ (M-H) $^-$, 373.2743 ;
10 found, 373.2750.

[00514] **Scheme 38**

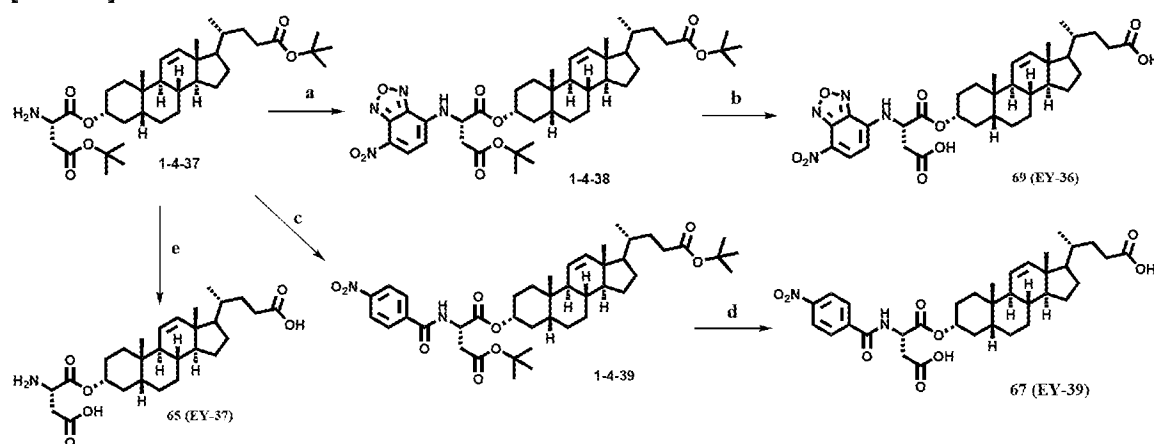


Reagents and conditions:

(a) $(\text{Boc})_2\text{O}$, DMAP, t -BuOH, rt, 1 h, 60%; (b) NaOMe, MeOH, rt, 1 h, 66%; (c)
20 Fmoc-L-Asp(OtBu)-OH, DCC, DMAP, DCM, rt, 18 h, 76%; (d)

Fmoc-L-Glu(OtBu)-OH, DCC, DMAP, DCM, rt, 18 h, 73%; (e) DBU, DCM, rt, 1 h, 91%; and (f) DBU, DCM, rt, 1 h, 75%.

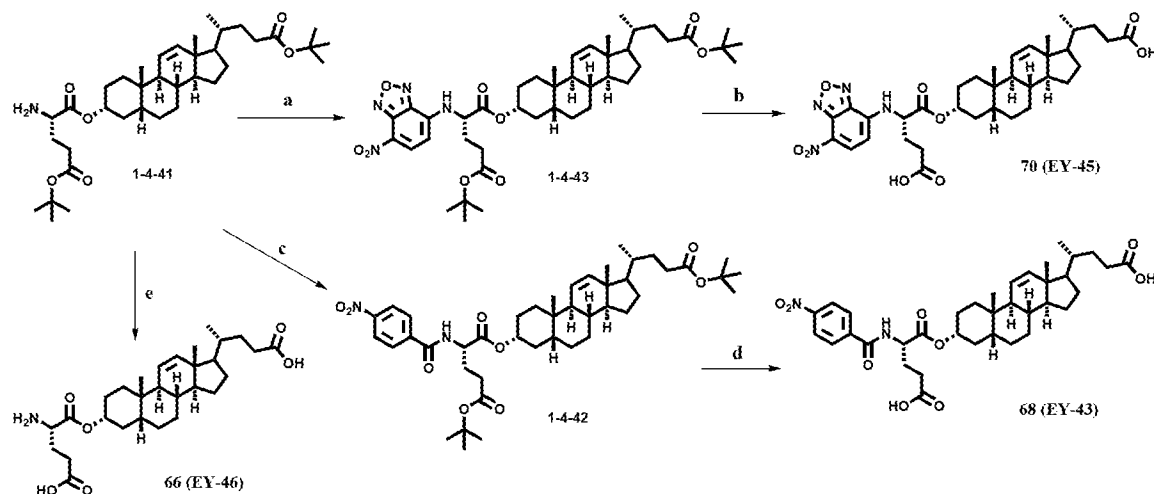
[00515] **Scheme 39**



Reagents and conditions:

(a) NBD-Cl, NaHCO₃, THF; EtOH (1:1), rt, 48 hr, 26%; (b) TFA, dd H₂O, DCM, rt, 4 hr, 90%; (c) 4-nitrobenzoic acid, HBTU, DiPEA, dry DMF, rt, 24 hr, 95%; (e) TFA, dd H₂O, DCM, rt, 4 hr, 85%; (f) TFA, dd H₂O, DCM, rt, 4 hr, 88%.

[00516] **Scheme 40**



Reagents and conditions:

(a) NBD-Cl, NaHCO₃, THF; EtOH (1:1), rt, 48 hr, 68%; (b) TFA, dd H₂O, DCM, rt, 4 hr, 91%; (c) 4-nitrobenzoic acid, HBTU, DiPEA, dry DMF, rt, 24 hr, 815%; (e) TFA, dd H₂O, DCM, rt, 4 hr, 93%; (f) TFA, dd H₂O, DCM, rt, 4 hr, 84%.

[00517] Synthesis of compound 1-4-36

5 [00518] In a 100 mL double-necked flask, mixed and dissolved compound 1-4-33 (1.5 g, 3.6 mmol) in t-BuOH (10 ml), added (Boc₂)O (1.54 mL, 7.2 mmol), followed by the addition of DMAP (0.13 g, 1.08 mmol). Allowed the mixture to react at room temperature for 1 hr. Vacuumed removed the residual t-BuOH once the reaction was completed. Dissolved the product in DCM, then extracted
10 the solution twice with double distilled water. Harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of hexane to EA/hexane (1:25)), a white solid compound **1-4-34** was obtained (1.0 g, 60%). TLC (Ethyl acetate /Hexane = 1 : 7) : R_f = 0.70.

15 [00519] In a 100 mL flask, mixed and dissolved compound **1-4-34** (0.2 g, 0.48 mmol) in methanol (10 mL), then NaOMe (0.57 g, 10.60 mmol) was added, allowed the mixture to react at room temperature for 1 hr. Vacuumed removed residual methanol once the reaction was completed, re-dissolved the product in DCM, then extracted the solution twice with double distilled water. Harvested
20 and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:25 to 1:10), a white solid of compound **1-4-35** was obtained (0.6 g, 66%). TLC (Ethyl acetate /Hexane = 1 : 9) : R_f = 0.30

[00520] In a 100 mL flask, mixed and dissolved Fmoc-L-Asp(OtBu)-OH (0.63 g, 1.53 mmol) in a mixture of 10 mL of DCM and 2% THF, then compound 1-4-35
25 (0.60g, 1.39 mmol) and DCC (0.57g, 2.78 mmol) were added, followed by the addition of DMAP (54 mg, 0.42 mmol), allowed the mixture to react at room temperature for 18 hrs.

[00521] Filtered and removed the residual, collected the filtrate, and extracted the filtrate twice with double distilled water. Harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:30 to 1:15), a white solid of compound **1-4-36** was obtained (0.87 g, 76%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.55

[00522] Synthesis of compound **69 (EY-36)**

[00523] In a 100 mL double-necked flask, mixed and dissolved compound **1-4-36** (0.65g, 0.793 mmol) in DCM (8 ml), then DBU (0.12 mL, 0.79 mmol), allowed the mixture to react at room temperature for 1 hr, at which time the solution appeared to have a light yellow color. Vacuum removed any residual solvent once the reaction was completed, then subjected it to flash column chromatography (eluted the column with a gradient of EA/hexane, from 1:0 to 1:1), a white solid of compound **1-4-37** was obtained (430 mg, 91%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.35.

[00524] In a 100 mL flask, mixed and dissolved compound **1-4-37** (0.43 g, 0.71 mmol) in a mixture of 8 mL THF/ethanol (1:1), then NaHCO₃ (119 mg, 1.42mmol) and NBD-Cl (156 mg, 0.78 mmol) were added, allowed the mixture to react at room temperature for 48 hrs, at which time the color of the reacting mixture would turn into golden yellow. Concentrated and subjected the concentrated product to flash column chromatography (eluted the column with a gradient of hexane to EA/hexane (1:10)), a yellow solid of compound **1-4-38** was obtained (140 mg, 26%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.70.

[00525] In a 50 mL flask, mixed and dissolved compound **1-4-38** (0.14 g, 0.18 mmol) in DCM (10 mL), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed residual TFA once the reaction was completed, a solid of compound **69 (EY-36)** was obtained (106 mg, 90%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.30.

[00526] Compound **69 (EY-36)** was further purified by HPLC, then freeze dried to produce a white solid of compound **69** or **EY-36**.

[00527] Melting point = 188-190°C

[00528] Compound **69 (EY-36)**: ^1H NMR (400 MHz, CD_3OD) : δ 8.52 (d, J = 8.8 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 6.07 (dd, J = 10.4, 2.8 Hz, 1H), 5.37 (d, J = 10.4 Hz, 1H), 4.80 (m, 2H), 3.08 (m, 2H), 2.40-2.15 (m, 3H), 2.0-1.67 (m, 7H), 1.67-1.40 (m, 5H), 1.39-1.24 (m, 6H), 1.20-1.06 (m, 3H), 1.00 (d, J = 6.4 Hz, 3H), 0.89 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) : δ 175.4, 172.4, 170.0, 145.6, 145.1, 145.0, 139.7, 137.5, 126.1, 124.9, 101.7, 76.9, 54.5, 54.4, 52.9, 45.8, 44.0, 41.8, 36.8, 36.4, 35.8, 35.4, 35.2, 33.5, 31.8, 31.3, 29.1, 28.7, 27.2, 26.2, 24.0, 23.7, 18.8, 17.1; HRMS-ESI : calculated for $\text{C}_{34}\text{H}_{44}\text{N}_4\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$, 675.3006 ; found, 675.3008.

[00529] Synthesis of compound **65 (EY-37)**

[00530] In a 50 mL flask, mixed and dissolved compound **1-4-37** (0.20g, 0.24 mmol) in DCM (5 ml), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed residual TFA once the reaction was completed, a solid of compound **65 (EY-37)** was obtained (103 mg, 88%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.25.

[00531] Compound **65 (EY-37)** was further purified by HPLC, then freeze dried to produce a white solid of compound **65** or **EY-37**.

[00532] Melting point = 164-166°C

[00533] Compound **65**: ^1H NMR (400 MHz, CD_3OD) : δ 6.14 (dd, J = 10.4, 2.8 Hz, 1H), 5.44 (d, J = 10.4 Hz, 1H), 4.85 (s, 1H), 4.28 (dd, J = 6.0, 4.4 Hz, 1H), 3.04 (dd, J = 18.0, 6.0 Hz, 1H), 2.96 (dd, J = 18.0, 4.4 Hz, 1H), 2.40-2.18 (m, 3H), 2.06-1.74 (m, 7H), 1.71-1.45 (m, 6H), 1.43-1.10 (m, 8H), 1.03 (d, J = 6.4 Hz, 3H), 0.93 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) : δ 178.2, 172.9, 169.1, 140.3, 126.2, 78.6, 55.2, 53.6, 50.8, 46.4, 44.6, 42.5, 37.4, 36.2, 35.9, 35.8, 35.0, 33.8, 32.3, 32.1, 29.6, 29.2, 27.5, 26.7, 24.2, 24.1, 19.0, 17.2; HRMS-ESI : calculated for $\text{C}_{28}\text{H}_{44}\text{NO}_6$ ($\text{M}+\text{H}$) $^+$, 490.3169 ; found, 490.3176

[00534] Synthesis of compound **67 (EY-39)**

[00535] In a 50 mL flask, mixed and dissolved 4-nitrobenzoic acid (57 mg, 0.34 mmol) in dry DMF (10 ml), then HBTU (129 mg, 0.34 mmol) and DIPEA (0.15 mL, 0.84 mmol) were added, followed by the addition of compound **1-4-37**, allowed the mixture to react at room temperature for 24 hrs. Vacuum removed residual TFA once the reaction was completed, re-dissolved the product in EA, then extracted the solution twice with ddH₂O and NaCl solution, harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:9 to 2:8), a white solid of compound **1-4-39** was obtained (0.2 g, 95%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.50

[00536] In a 50 mL flask, mixed and dissolved compound **1-4-39** (0.20g, 0.27 mmol) in DCM (5 ml), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed residual TFA once the reaction was completed, a solid of compound **67 (EY-39)** was obtained (150 mg, 85%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.35.

[00537] Compound **67 (EY-39)** was further purified by HPLC, then freeze dried to produce a white solid of compound **67** or **EY-39**.

[00538] Melting point = 127-129°C

[00539] Compound **67**: ¹H NMR (400 MHz, CDCl₃) : δ 8.29 (d, *J* = 8.4 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.26 (s, 1H), 6.08 (dd, *J* = 10.4, 2.4 Hz, 1H), 5.36 (d, *J* = 10.4 Hz, 1H), 4.96 (m, 1H), 4.80 (m, 1H), 3.19 (dd, *J* = 17.6, 4.0 Hz, 1H), 3.03 (dd, *J* = 17.6, 4.4 Hz, 1H), 2.50-2.15 (m, 3H), 2.0-1.65 (m, 7H), 1.64-1.40 (m, 6H), 1.39-1.05 (m, 8H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.88 (s, 3H), 0.72 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) : δ 180.6, 176.2, 170.0, 165.7, 150.1, 139.2, 139.1, 128.7, 128.7, 125.4, 124.1, 124.1, 77.0, 53.8, 52.1, 49.5, 45.2, 43.3, 41.1, 36.3, 36.1, 35.2, 34.8, 34.5, 32.8, 31.2, 30.8, 28.6, 28.0, 26.4, 25.6, 23.8, 23.2, 18.5, 16.8; HRMS-ESI : calculated for C₃₅H₄₆N₂O₉Na (M+Na)⁺, 661.3101 : found, 661.3093.

[00540] Synthesis of compound **1-4-40**

[00541] In a 100 mL flask, mixed and dissolved Fmoc-L-Glu(OtBu)-OH (1.0 g, 2.35 mmol) in a mixture of 10 mL of DCM and 2% THF, then compound 1-4-35 (0.93g, 1.97 mmol) and DCC (0.65g, 3.15 mmol) were added, followed by the addition of DMAP (72 mg, 0.59 mmol), allowed the mixture to react at room temperature for 18 hrs.

[00542] Filtered and removed the residual, collected the filtrate, and extracted the filtrate twice with double distilled water. Harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:40 to 1:15), a white solid of compound **1-4-40** was obtained (1.2 g, 73%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.65

[00543] Synthesis of compound **68 (EY-43)**

[00544] In a 50 mL flask, mixed and dissolved compound **1-4-40** (0.22g, 0.26 mmol) in DCM (8 ml), then slowly added DBU (39μL, 0.26 mmol), allowed the mixture to react at room temperature for 1 hr, at which time the color of the reacting solution appeared to be light yellow. Vacuumed removed any residual DCM once the reaction was completed, then subjected it to flash column chromatography (eluted the column with a gradient of EA/hexane, from 1:10 to 1:1), a white solid of compound **1-4-41** was obtained (120mg, 75%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.20

[00545] In a 50 mL flask, mixed and dissolved 4-nitrobenzoic acid (38 mg, 0.23 mmol) in DCM (8 ml), then HBTU (87 mg, 0.23 mmol) and DiPEA (0.1 mL, 0.57 mmol) were added, followed by the addition of compound **1-4-41**, allowed the mixture to react at room temperature for 24 hrs. Vacuumed removed residual TFA once the reaction was completed, extracted the product solution twice with ddH₂O and DCM, harvested and combined the organic layers, dried with Na₂SO₄, then subjected it to column chromatography (eluted the column with a gradient of EA/hexane, from 1:10 to 1:8), a white solid of compound **1-4-42** was obtained (118mg, 81%). TLC (Ethyl acetate /Hexane = 2 : 8) : R_f = 0.45

[00546] In a 50 mL flask, mixed and dissolved compound **1-4-42** (118 mg, 0.15 mmol) in DCM (5 ml), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed residual TFA once the reaction was completed, a solid of compound **68 (EY-43)** was obtained (91 mg, 93%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.40.

[00547] Compound **68 (EY-43)** was further purified by HPLC, then freeze dried to produce a white solid of compound **68** or **EY-43**.

[00548] Melting point = 110-112°C

[00549] Compound **68**: ^1H NMR (400 MHz, CDCl_3) : δ 8.28 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 7.2 Hz, 1H), 6.09 (dd, J = 10.4, 2.4 Hz, 1H), 5.38 (d, J = 10.4 Hz, 1H), 4.80 (m, 2H), 2.06-2.03 (m, 7H), 2.00-1.66 (m, 7H), 1.65-1.40 (m, 7H), 1.35-1.05 (m, 7H), 1.00 (d, J = 6.4 Hz, 3H), 0.89 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) : δ 180.3, 178.3, 171.5, 165.8, 150.0, 139.2, 139.2, 128.6, 128.6, 125.4, 124.0, 124.0, 76.7, 53.8, 52.9, 52.0, 45.2, 43.3, 41.1, 36.1, 35.2, 34.9, 34.5, 32.9, 31.1, 30.9, 30.4, 28.6, 28.0, 27.2, 26.7, 25.6, 23.7, 23.1, 18.5, 16.8; HRMS-ESI : calculated for $\text{C}_{36}\text{H}_{48}\text{N}_2\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$, 675.3258 ; found, 675.3255.

[00550] Synthesis of compound **70**

[00551] In a 100 mL flask, mixed and dissolved compound **1-4-37** (0.43 g, 0.71 mmol) in a mixture of 8 mL THF/ethanol (1:1), then NaHCO_3 (100 mg, 1.2 mmol) and NBD-Cl (140 mg, 0.70 mmol) were added, allowed the mixture to react at room temperature for 48 hrs, at which time the color of the reacting mixture would turn into golden yellow. Concentrated and subjected the concentrated product to flash column chromatography (eluted the column with a gradient of hexane to EA/hexane (1:10)), a yellow solid of compound **1-4-43** was obtained (310 mg, 68%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.75.

[00552] In a 50 mL flask, mixed and dissolved compound **1-4-43** (0.31 g, 0.40 mmol) in DCM (8 ml), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed

residual TFA once the reaction was completed, a solid of compound **70** (**EY-45**) was obtained (245 mg, 91%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.30.

[00553] Compound **70** (**EY-45**) was further purified by HPLC, then freeze dried to produce a white solid of compound **70** or **EY-45**.

5 [00554] Melting point = 214-216°C

[00555] **Compound 70**: ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) : δ 8.55 (d, J = 8.8 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 6.51 (d, J = 8.8 Hz, 1H), 6.11 (dd, J = 10.4, 2.8 Hz, 1H), 5.42 (d, J = 10.4 Hz, 1H), 4.83 (m, 2H), 2.64 (t, J = 7.2 Hz, 2H), 2.50-2.17 (m, 5H), 2.02-1.70 (m, 7H), 1.68-1.37 (m, 7H), 1.36-1.10 (m, 7H), 1.02 (d, J = 6.8 Hz, 3H), 0.91 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) : δ 175.1, 174.3, 170.9, 145.6, 145.2, 145.1, 139.7, 137.5, 126.1, 125.0, 101.4, 76.8, 57.2, 54.5, 53.0, 45.9, 44.0, 41.8, 36.8, 35.8, 35.4, 35.2, 33.6, 31.8, 31.3, 30.4, 29.1, 28.7, 27.4, 27.3, 26.2, 24.0, 23.7, 18.8, 17.1; HRMS-ESI : calculated for $\text{C}_{35}\text{H}_{45}\text{N}_4\text{O}_9$ (M-H) $^-$, 665.3187 ; found, 665.3190.

15 [00556] Synthesis of compound **66**

[00557] In a 50 mL flask, mixed and dissolved compound **1-4-41** (157 mg, 0.25 mmol) in DCM (5 ml), then TFA (2 mL) and 2% double distilled water were added, allowed the mixture to react at room temperature for 4 hrs. Vacuum removed residual TFA once the reaction was completed, a solid of compound **66** (**EY-46**) was obtained (108 mg, 84%). TLC (Ethyl acetate /Hexane = 3 : 7) : R_f = 0.30.

[00558] Compound **66** (**EY-46**) was further purified by HPLC, then freeze dried to produce a white solid of compound **66** or **EY-46**.

[00559] Melting point = 233-235°C

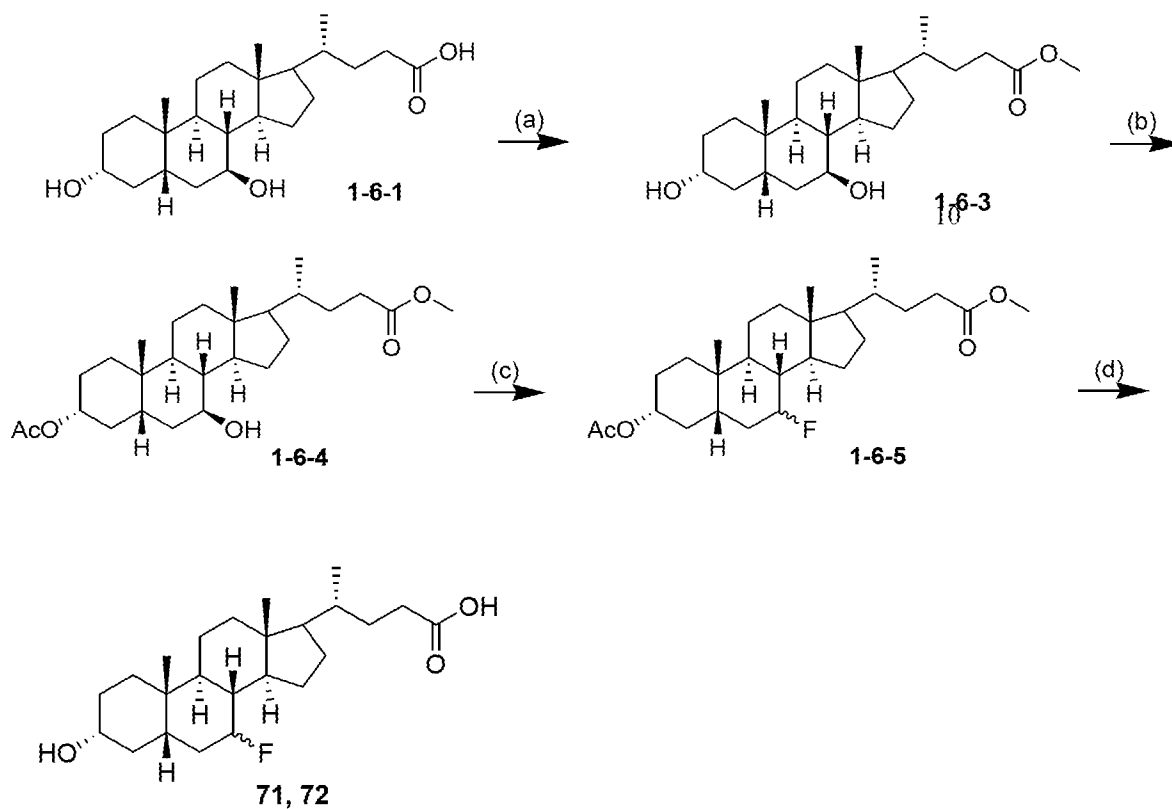
[00560] Compound **66**: ^1H NMR (400 MHz, CD_3OD) : δ 6.15 (dd, J = 10.4, 2.8 Hz, 1H), 5.45 (d, J = 10.4 Hz, 1H), 4.85 (s, 1H), 4.07 (t, J = 6.8 Hz, 1H), 2.54 (m, 2H), 2.40-2.08 (m, 5H), 2.07-1.73 (m, 7H), 1.72-1.43 (m, 7H), 1.40-1.12 (m, 7H), 1.03 (d, J = 6.4 Hz, 3H), 0.94 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) : δ 178.2, 176.0, 170.0, 140.3, 126.3, 78.5, 55.1, 53.6, 53.6, 46.4, 44.6, 42.5, 37.4, 36.3, 35.9, 35.9, 33.9, 32.3, 32.2, 30.9, 29.6, 29.2, 27.6, 27.0, 26.7, 24.2, 24.1,

19.0, 17.2; HRMS-ESI : calculated for $C_{29}H_{46}NO_6$ ($M+H$)⁺, 504.3325 ; found, 504.3316.

[00561] **1.8 Synthesis of Compounds 71 to 87**

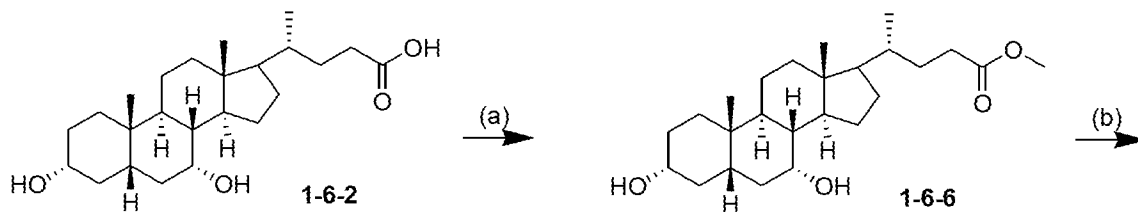
[00562] Compounds **71** to **87** (or compounds of formula (1-6)) were synthesized
5 in accordance with procedures set forth in **Schemes 41** to **49**.

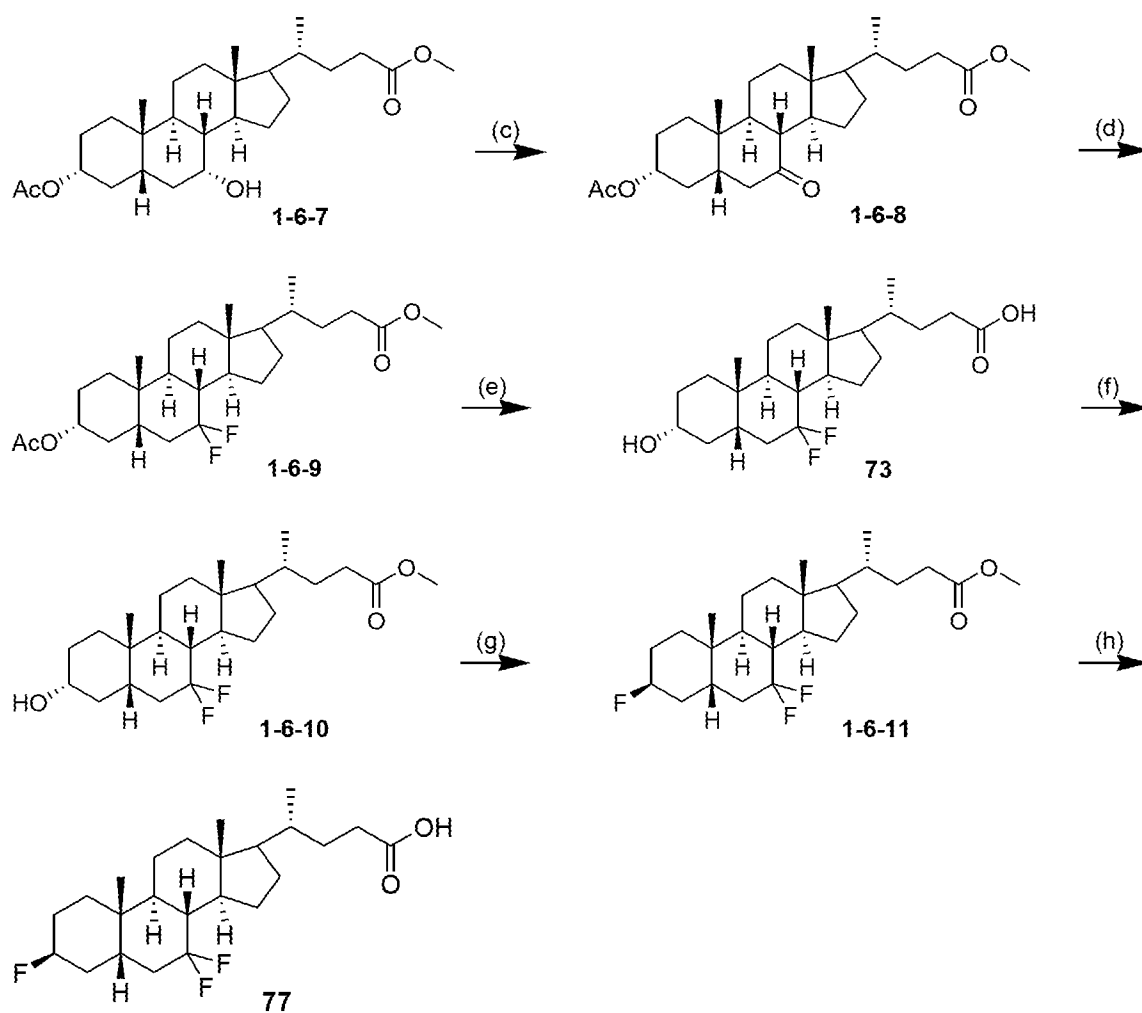
[00563] **Scheme 41:**



Reagents and conditions: (a) H_2SO_4 , MeOH, 97 %, (b) Ac_2O , pyridine, CH_2Cl_2 , 30
20 %, (c) DAST, CH_2Cl_2 , $-78\text{ }^\circ C$, 45 %, (d) KOH, MeOH, $80\text{ }^\circ C$, 86 %.

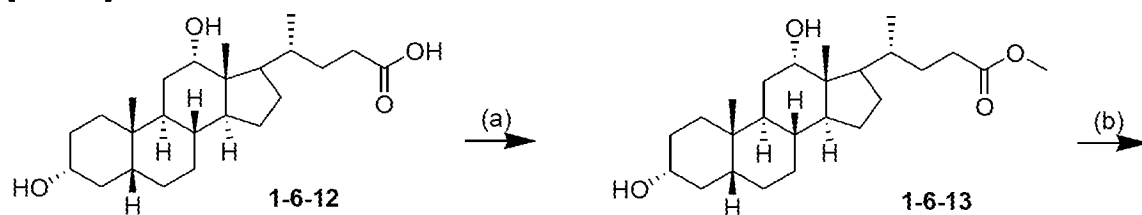
[00564] **Scheme 42:**

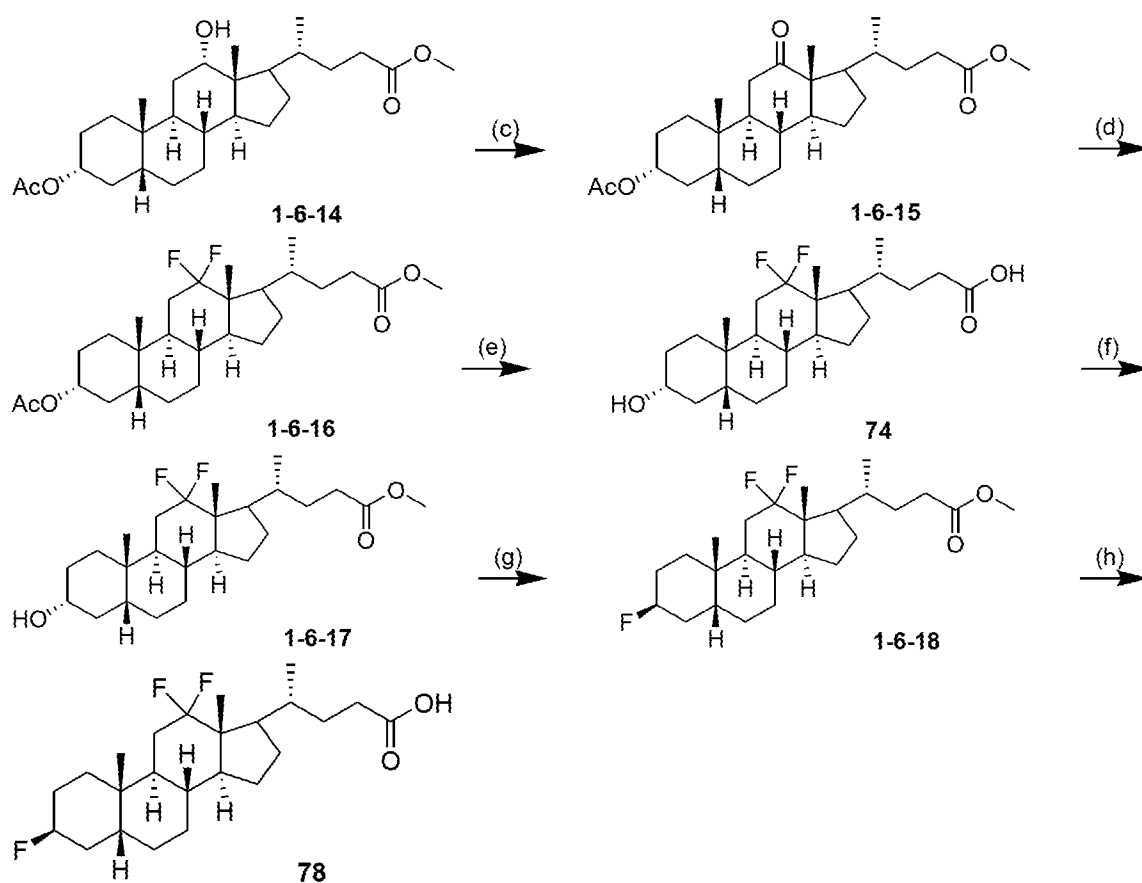




Reagents and conditions: (a) H_2SO_4 , MeOH, 96 %, (b) Ac_2O , pyridine, CH_2Cl_2 , 53
 10 %, (c) CrO_3 , AcOH, 83 %, (d) DAST, 80°C , 55 %, (e) KOH, MeOH, 80°C , 80 %, (f)
 H_2SO_4 , MeOH, 99 %, (g) DAST, CH_2Cl_2 , -78°C , 46 %, (h) KOH, MeOH, 80°C ,
 83 %.

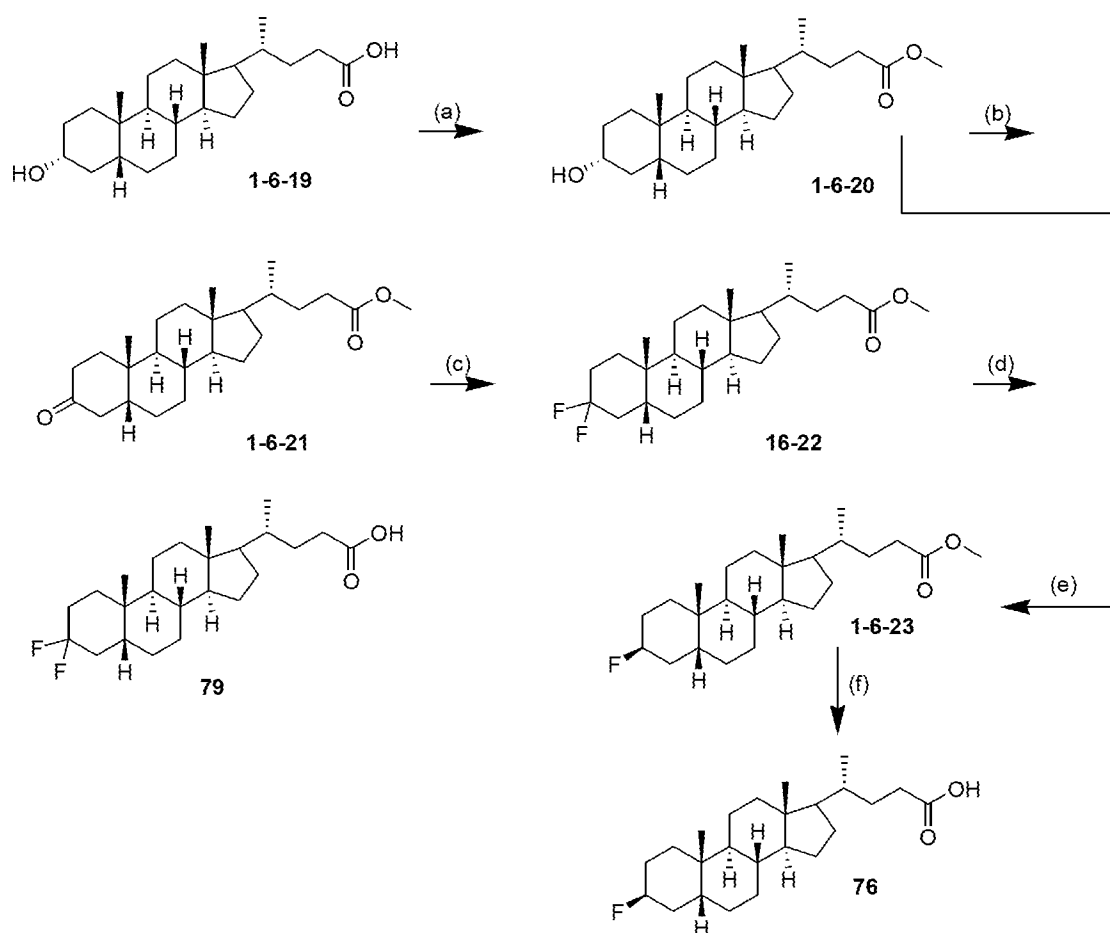
[00565] **Scheme 43:**





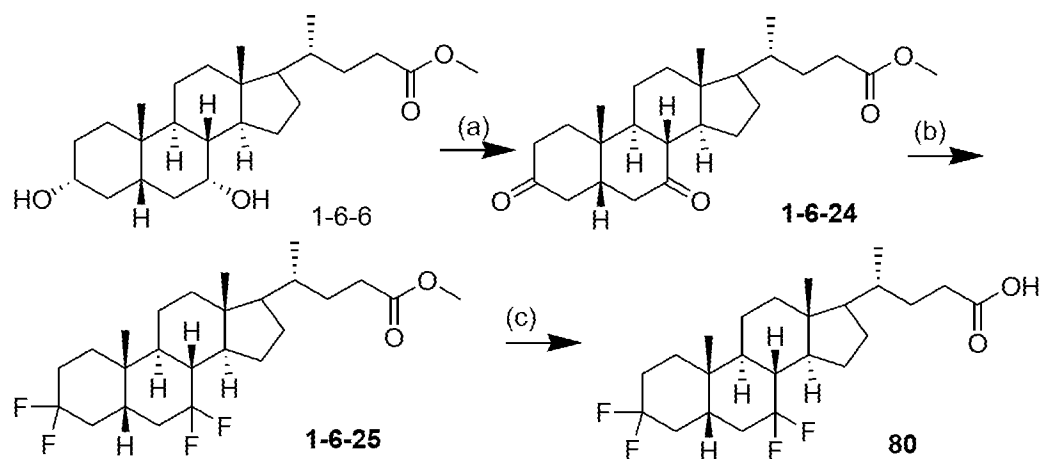
- 5 Reagents and conditions: (a) H_2SO_4 , MeOH, 87 %, (b) Ac_2O , pyridine, CH_2Cl_2 , 59 %, (c) CrO_3 , AcOH, 64 %, (d) DAST, 80 °C, 76 %, (e) KOH, MeOH, 80 °C, 88 %, (f) H_2SO_4 , MeOH, 99 %, (g) DAST, CH_2Cl_2 , -78 °C, 86 %, (h) KOH, MeOH, 80 °C, 80 %.

[00566] **Scheme 44:**



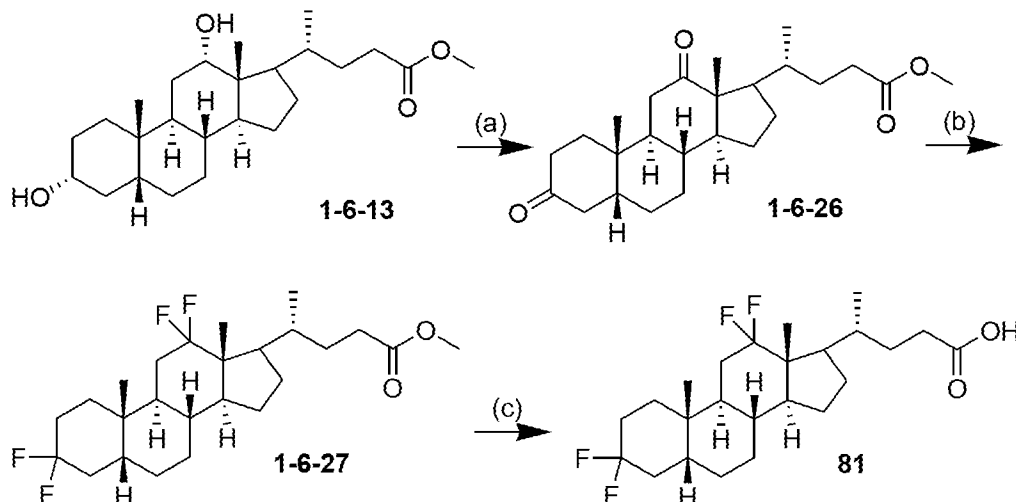
Reagents and conditions: (a) H_2SO_4 , MeOH, 99 %, (b) CrO_3 , AcOH, 40 %, (c) DAST, 80 °C, 76 %, (d) KOH, MeOH, 80 °C, 90 %, (e) DAST, CH_2Cl_2 , -78 °C, 37 %, (f) KOH, MeOH, 80 °C, 95 %

5 [00567] **Scheme 45:**



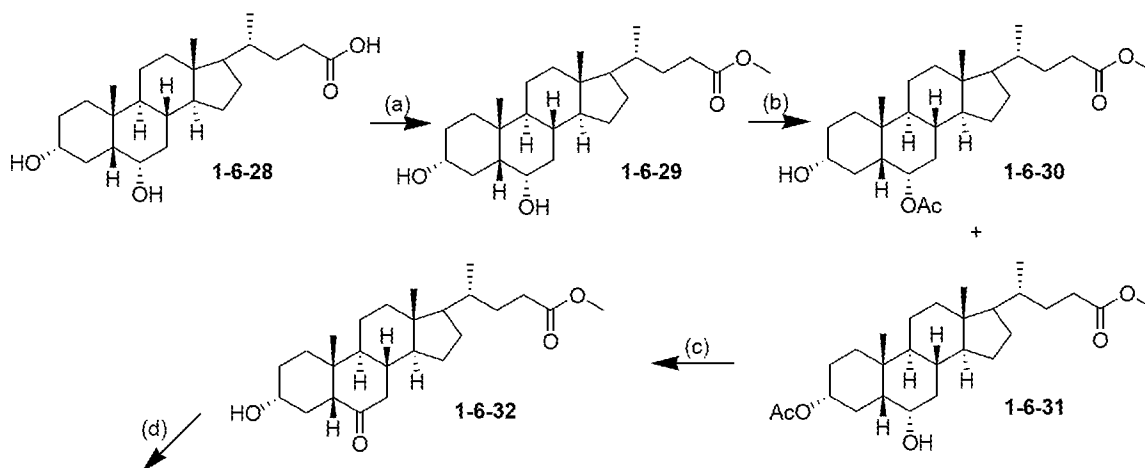
Reagents and conditions: (a) CrO_3 , AcOH, 58 %, (b) DAST, 80 °C, 34 %, (c) KOH, MeOH, 80 °C, 93 %.

[00568] **Scheme 46:**

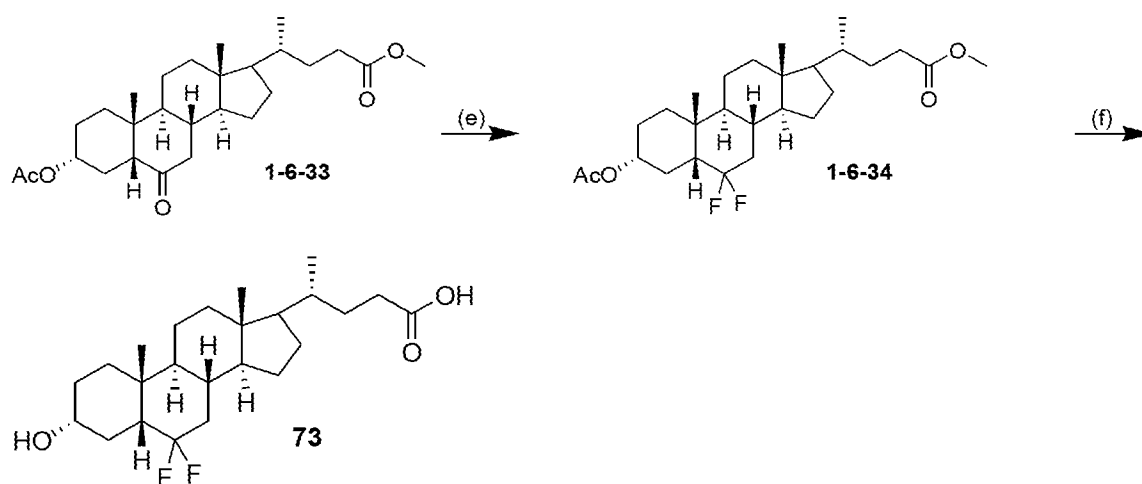


Reagents and conditions: (a) CrO_3 , AcOH, 63 %, (b) DAST, 80 °C, 70 %, (c) KOH, MeOH, 80 °C, 90 %.

[00569] **Scheme 47:**

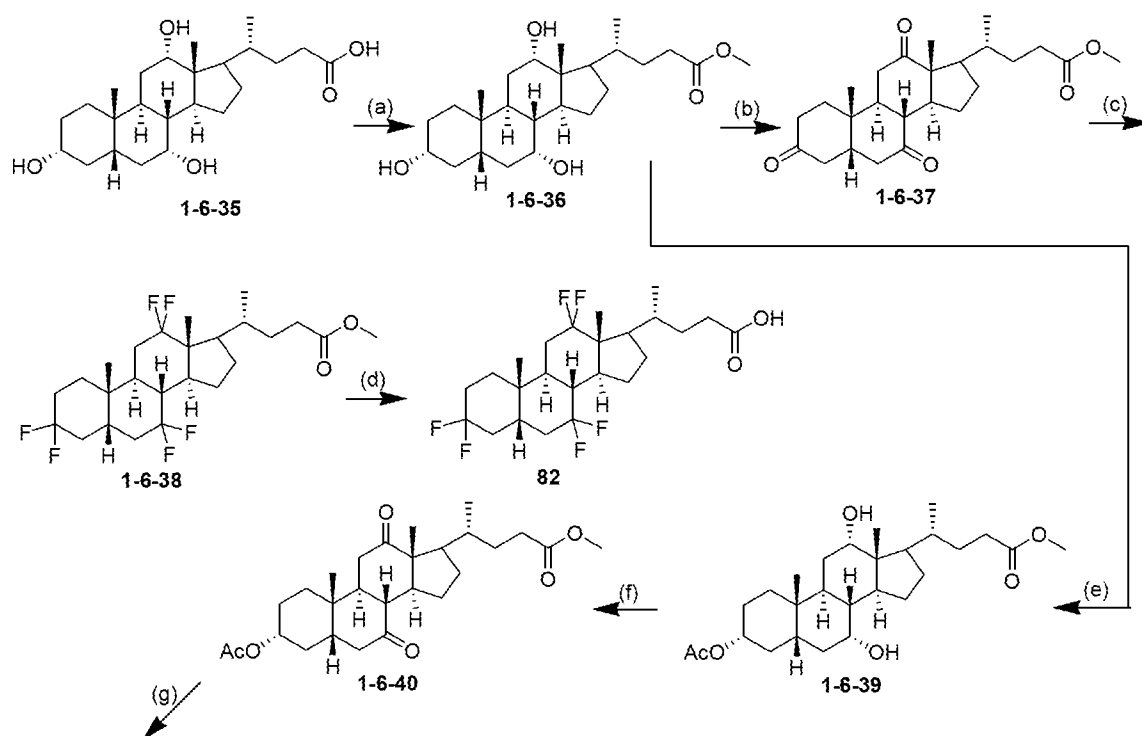


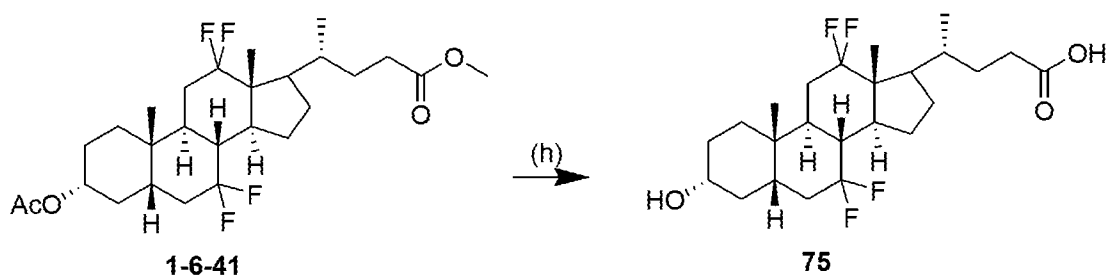
15



Reagents and conditions: (a) H_2SO_4 , MeOH, 98 %, (b) Ac_2O , pyridine, CH_2Cl_2 , 30
 5 %, (c) PCC, CH_2Cl_2 , 69 %, (d) Ac_2O , pyridine, CH_2Cl_2 , 53 %, (e) DAST, 80 °C, 53
 %, (f) KOH, MeOH, 80 °C, 89 %.

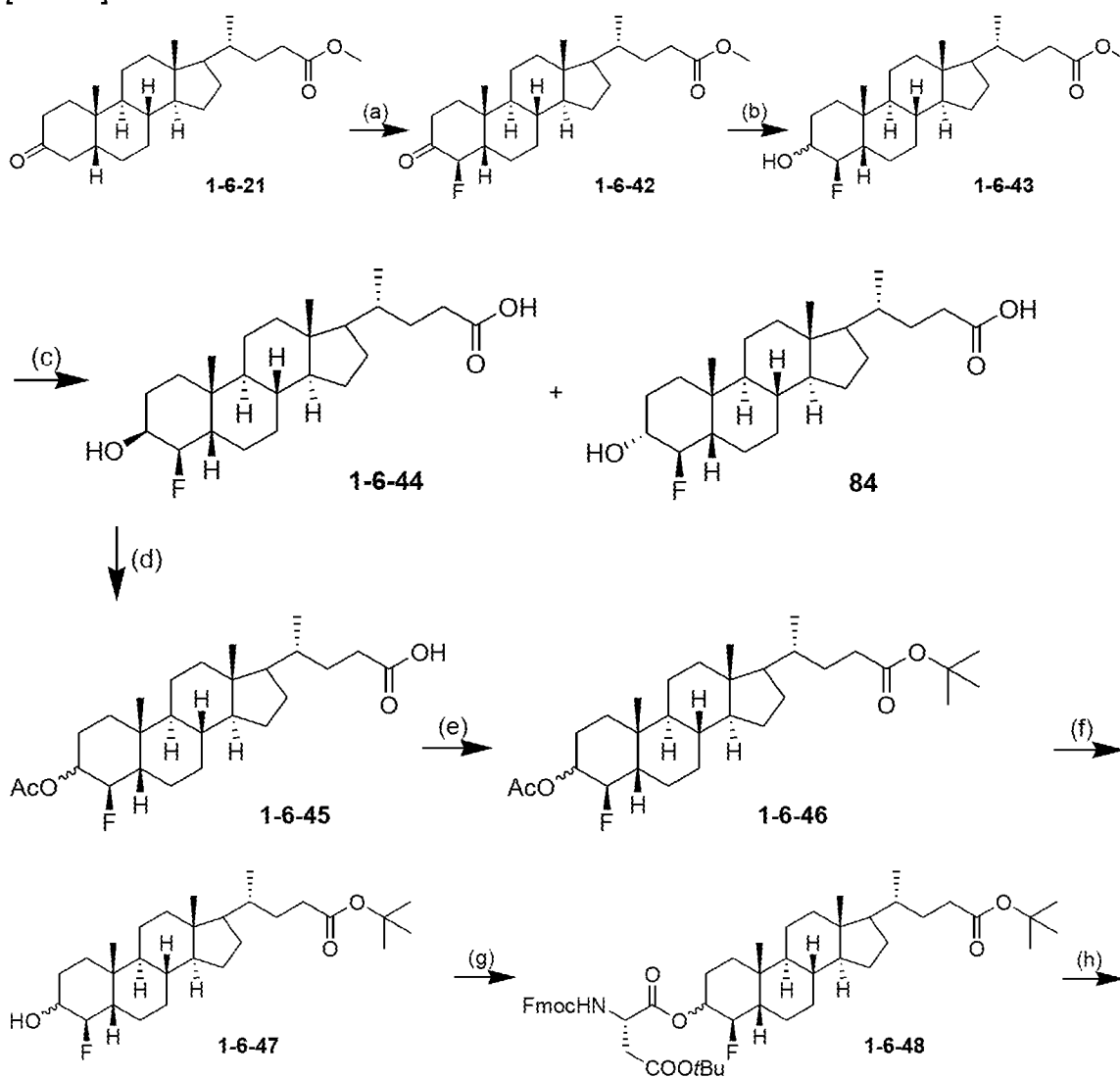
[00570] **Scheme 48:**

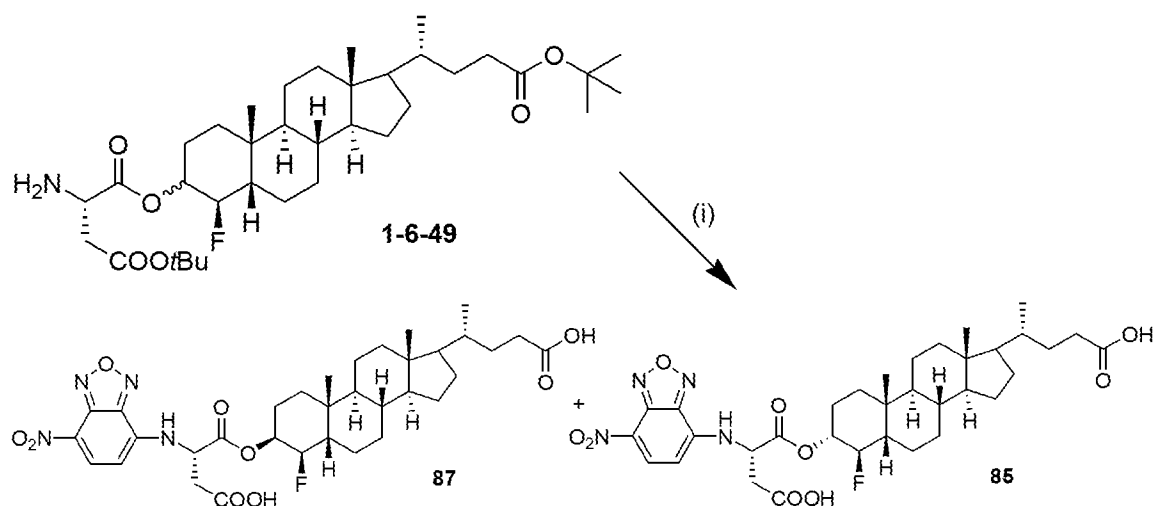




Reagents and conditions: (a) H_2SO_4 , MeOH, 99 %, (b) PDC, CH_2Cl_2 , 72 %, (c) DAST, 80 °C, 25 %, (d) KOH, MeOH, 80 °C, 72 %, (e) Ac_2O , pyridine, CH_2Cl_2 , 22 %, (f) PDC, CH_2Cl_2 , 77 %, (g) DAST, 80 °C, 56 %, (h) KOH, MeOH, 80 °C, 92 %.

5 [00571] **Scheme 49:**





- 5 Reagents and conditions: (a) selectfluor, 85 °C, 24 h, (b) $\text{LiAl}(\text{O}t\text{Bu})_3$, THF, rt, 1 h, (c) KOH, MeOH, H_2O , (d) Ac_2O , pyridine, CH_2Cl_2 , rt, 24 h, (e) $(\text{Boc})_2\text{O}$, DMAP, $t\text{-BuOH}$, rt, 4 h 80 %, (f) NaOMe, MeOH, 60 °C, 6 h, 92 %, (g) Fmoc-L-Asp(OtBu)-OH, DCC, DMAP, CH_2Cl_2 , rt, 6 h, 85 %, (h) DBU, CH_2Cl_2 , rt, 1 h, 87 %, (i) 1. NBD-Cl, NaHCO_3 , THF, EtOH, rt, 48 h, 2. TFA, H_2O , CH_2Cl_2 , rt, 2 h, 24 % (two steps).
- 10

[00572] Compound **71/72**: ^1H NMR (400 MHz, CDCl_3): δ 4.58, 4.40 (dm, dm, $2J_{\text{F}7\text{H}7\beta} = 48$ Hz, $2J_{\text{F}7\text{H}7\alpha} = 48$ Hz, 1H), 3.59-3.48 (m, m, isomer1+ isomer2, 1H), 2.41-2.33 (m, m, isomer 1+ isomer 2, 1H), 2.27-2.19 (m, m, isomer 1+ isomer 2, 1H), 1.99-1.79 (m, m, isomer 1+ isomer 2, 7H), 1.71-1.62 (m, m, isomer 1+ isomer 2, 4H), 1.53-1.41 (m, m, isomer 1+ isomer 2, 5H), 1.36-1.26 (m, m, isomer 1+ isomer 2, 5H), 1.19-1.02 (m, m, isomer 1+ isomer 2, 5H), 0.94, 0.87 (s, s, isomer 1 + isomer2, 3H), 0.91 (d, $J = 6.3$ Hz, isomer 1 + isomer 2, 3H), 0.65, 0.63 (s, s, isomer 1+ isomer 2, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 179.2, 93.8 [d, $1J(19\text{F}, 13\text{C}) = 171$ Hz], 91.2 [d, $1J(19\text{F}, 13\text{C}) = 171$ Hz], 71.9, 71.4, 55.7, 55.2, 50.1, 43.2, 42.7, 42.5, 42.4, 41.8, 41.6, 40.9, 39.9, 39.5, 39.2, 39.0, 38.7, 38.6, 37.2, 35.3, 35.2, 35.1, 34.6, 34.1, 33.8, 33.7, 33.6, 32.6, 32.4, 30.9, 30.8, 30.4, 30.2, 28.3, 28.1, 25.9, 23.5, 23.3, 22.5, 21.0, 20.6, 18.3, 12.0, 11.7 (two isomer); ^{19}F

15

20

NMR (376 MHz, CDCl_3) : δ -172.49 (s, 1F), -186.53 (s, 1F) (two isomer);
HRMS-ESI : calculated for $\text{C}_{24}\text{H}_{38}\text{FO}_3$ (M - H) —, 393.2805 ; found, 393.2797.

[00573] Compound **73**: ^1H NMR (400 MHz, CDCl_3) : δ 3.55-3.50 (m, 1H),
2.43-2.35 (m, 1H), 2.27-2.25 (m, 1H), 2.16-1.97 (m, 3H), 1.83-1.75 (m, 4H),
5 1.70-1.67 (m, 5H), 1.57-1.45 (m, 4H), 1.34-1.27 (m, 3H), 1.24-1.05 (m, 5H), 0.93
(s, 3H), 0.92 (d, J = 7.1 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) : δ
179.2, 124.6 [t, $1J$ (19F, ^{13}C) = 243.2 Hz], 71.3, 55.0, 48.6, 43.1, 41.8 [t, $2J$ (19F,
 ^{13}C) = 22.6 Hz], 41.2 [d, $3J$ (19F, ^{13}C) = 10.5 Hz], 39.4, 37.2, 37.0 [d, $3J$ (19F,
 ^{13}C) = 8.9 Hz], 36.2 [t, $2J$ (19F, ^{13}C) = 23.1 Hz], 35.3, 34.7, 34.3, 30.9, 30.8,
10 30.1, 28.3, 25.3, 22.6, 20.9, 18.3, 11.8; ^{19}F NMR (376 MHz, CDCl_3) : δ -84.39 (d,
 $2J$ (19F, 19F) = 236.9 Hz, 1F), -101.21 (d, $2J$ (19F, 19F) = 240.6 Hz, 1F);
HRMS-FAB : calculated for $\text{C}_{24}\text{H}_{38}\text{F}_2\text{O}_3\text{Na}$ (M + Na)⁺, 435.2686 ; found, 435.2687.

[00574] Compound **77**: ^1H NMR (400 MHz, CDCl_3) : δ 4.82 (dm, $2J_{\text{F}_{30}\text{H}_{38}}$ = 48.8
Hz, 1H), 2.42-2.34 (m, 1H), 2.28-2.20 (m, 1H), 2.25-1.92 (m, 3H), 1.92-1.76 (m,
15 6H), 1.72-1.57 (m, 3H), 1.56-1.44 (m, 5H), 1.44-1.32 (m, 5H), 1.27-1.10 (m, 4H),
0.98 (s, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) :
 δ 179.8, 124.9 [t, $1J$ (19F, ^{13}C) = 243.8 Hz], 89.3 [t, $1J$ (19F, ^{13}C) = 166.3 Hz],
55.0, 48.7, , 43.2, 41.8 [t, $2J$ (19F, ^{13}C) = 22.5 Hz], 39.5, 36.5 [d, $3J$ (19F, ^{13}C)
= 8.8 Hz], 36.3 [d, $3J$ (19F, ^{13}C) = 10.5 Hz], 35.6 [t, $2J$ (19F, ^{13}C) = 22.9 Hz],
20 35.2, 34.5, 32.5 [d, $3J$ (19F, ^{13}C) = 4.4 Hz], 32.3 [d, $3J$ (19F, ^{13}C) = 4.1 Hz], 30.8
[t, $2J$ (19F, ^{13}C) = 20.0 Hz], 29.6, 28.3, 25.7 [t, $2J$ (19F, ^{13}C) = 21.1 Hz], 25.3
[d, $3J$ (19F, ^{13}C) = 3.4 Hz], 23.0, 21.2, 18.3, 11.8; ^{19}F NMR (376 MHz, CDCl_3) :
 δ -83.99 (d, $2J$ (19F, 19F) = 240.6 Hz, 1F), -101.15 (d, $2J$ (19F, 19F) = 240.6 Hz,
1F), -184.9 (s, 1F); HRMS-FAB : calculated for $\text{C}_{24}\text{H}_{38}\text{F}_3\text{O}_2$ (M + H)⁺, 415.2820 ;
25 found, 415.2824.

[00575] Compound **74**: ^1H NMR (400 MHz, CDCl_3) : δ 3.65-3.57 (m, 1H),
2.44-2.40 (m, 2H), 2.32-2.24 (m, 1H), 1.99-1.70 (m, 5H), 1.67-1.53 (m, 6H),
1.53-1.36 (m, 5H), 1.36-1.18 (m, 5H), 1.18-1.02 (m, 3H), 0.98 (d, J = 6.0 Hz, 3H),
0.90 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) : δ 178.3, 126.5 [t, $1J$ (19F,

13C) = 249.0 Hz], 71.6, 52.5 [d, 3J(19F, 13C) = 7.1 Hz], 49.2 [t, 2J(19F, 13C) = 20.5 Hz], 47.8, 41.6, 37.2 [d, 3J(19F, 13C) = 9.4 Hz], 36.2, 35.1, 34.7, 34.1, 33.2, 31.3 [t, 2J(19F, 13C) = 25.3 Hz], 31.2, 30.1 [d, 3J(19F, 13C) = 9.4 Hz], 26.9, 26.3, 25.5, 23.1, 23.0, 18.9, 18.8, 10.5; 19F NMR (376 MHz, CDCl₃): δ -90.8
 5 (d, 2J(19F, 19F) = 229.4 Hz, 1F), -111.9 (d, 2J(19F, 19F) = 229.4 Hz, 1F); HRMS-ESI : calculated for C₂₄H₃₇F₂O₃ (M - H) —, 411.2711 : found, 411.2714.

[00576] Compound **78**: 1H NMR (400 MHz, CDCl₃): δ 4.86 (dm, 2J_{F_{3α}H_{3β}} = 48.8 Hz, 1H), 2.44-2.32 (m, 1H), 2.32-2.24 (m, 1H), 1.92-1.83 (m, 5H), 1.83-1.70 (m, 5H), 1.62-1.47 (m, 7H), 1.47-1.22 (m, 6H), 1.19-0.99 (m, 3H), 0.99-0.95 (m, 6H),
 10 0.80 (s, 3H); 13C NMR (125 MHz, CDCl₃): δ 179.3, 126.5 [t, 1J(19F, 13C) = 245.1 Hz], 89.8 [t, 1J(19F, 13C) = 165 Hz], 52.7, 49.3 [t, 2J(19F, 13C) = 21.3 Hz], 47.8, 36.6 [d, 3J(19F, 13C) = 9.3 Hz], 36.5, 34.4, 34.4 [d, 3J(19F, 13C) = 7.6 Hz], 33.2, 31.6 [t, 2J(19F, 13C) = 25.8 Hz], 31.5, 31.3, 30.2, 30.0, 26.4, 26.0, 25.8 [t, 2J(19F, 13C) = 21.3 Hz], 25.2, 23.3, 23.0, 18.9 [d, 3J(19F, 13C) = 10.9 Hz], 10.5; 19F NMR (376 MHz, CDCl₃): δ -90.93 (d, 2J(19F, 19F) = 229.4 Hz, 1F), -111.97 (d, 2J(19F, 19F) = 229.4 Hz, 1F), -182.70 (s, 1F); HRMS-FAB :
 15 calculated for C₂₄H₃₉F₃O₂Na (M + Na)⁺, 437.2643 : found, 437.2658.

[00577] Compound **79**: 1H NMR (400 MHz, CDCl₃): δ 2.42-2.34 (m, 1H), 2.28-2.20 (m, 1H), 2.15-1.94 (m, 2H), 1.85-1.45 (m, 5H), 1.66-1.56 (m, 3H),
 20 1.46-1.34 (m, 5H), 1.29-1.23 (m, 5H), 1.17-1.05 (m, 5H), 0.95 (s, 3H), 0.90 (d, J = 6.4 Hz, 3H), 0.64 (s, 3H); 13C NMR (100 MHz, CDCl₃): δ 180.1, 124.6 [t, 1J(19F, 13C) = 238.5 Hz], 56.4, 55.9, 42.8, 40.0, 39.9, 35.2, 35.3, 34.5, 34.5 [t, 2J(19F, 13C) = 18.5 Hz], 33.1, 33.0, 31.0, 30.7, 29.3 [t, 2J(19F, 13C) = 24.5 Hz], 28.1, 26.1, 26.0, 24.1, 22.8, 21.1, 18.2, 12.1; 19F NMR (376 MHz, CDCl₃): δ -88.24 (d, 2J(19F, 19F) = 233.1 Hz, 1F), -99.4 (d, 2J(19F, 19F) = 233.1 Hz, 1F); HRMS-ESI :
 25 calculated for C₂₄H₃₇F₂O₂ (M - H) —, 395.2762 : found, 395.2768.

[00578] Compound **76**: 1H NMR (400 MHz, CDCl₃): δ 4.85 (dm, 2J_{F_{3α}H_{3β}} = 49.2 Hz, 1H), 2.41-2.33 (m, 1H), 2.23-2.19 (m, 1H), 1.96-1.87 (m, 2H), 1.82-1.72 (m, 3H), 1.69-1.57 (m, 4H), 1.39-1.32 (m, 7H), 1.23-1.11 (m, 3H), 1.11-1.01 (m, 4H),

1.19-1.13 (m, 3H), 0.94 (s, 3H), 0.90 (d, $J = 6.4$ Hz, 3H), 0.63 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3): δ 179.6, 90.2 [d, $1J(19\text{F}, 13\text{C}) = 165.4$ Hz], 56.6, 56.0, 42.8, 40.2, 39.9, 36.9, 35.6, 35.3, 34.8, 31.7, 31.5, 30.9, 30.8, 30.2, 28.2, 26.3, 26.1, 24.2, 23.7, 21.1, 18.3, 12.1; ^{19}F NMR (376 MHz, CDCl_3): δ -182.1 (s, 1F);

5 HRMS-FAB: calculated for $\text{C}_{24}\text{H}_{40}\text{FO}_2$ ($M + H$), 379.3016; found, 379.3012.

[00579] Compound **78**: ^1H NMR (400 MHz, CDCl_3): δ 2.42-2.35 (m, 1H), 2.29-2.21 (m, 1H), 2.18-1.98 (m, 3H), 1.90-1.73 (m, 8H), 1.73-1.58 (m, 3H), 1.48-1.36 (m, 4H), 1.36-1.06 (m, 7H), 0.99 (s, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.66 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3): δ 179.3, 124.3 [t, $1J(19\text{F}, 13\text{C}) = 243.1$ Hz],

10 123.5 [t, $1J(19\text{F}, 13\text{C}) = 240$ Hz], 55.0, 48.5, 43.2, 41.7 [t, $2J(19\text{F}, 13\text{C}) = 22.4$ Hz], 39.3, 39.2 [t, $2J(19\text{F}, 13\text{C}) = 11.6$ Hz], 36.5 [d, $3J(19\text{F}, 13\text{C}) = 8.7$ Hz], 35.3 [t, $2J(19\text{F}, 13\text{C}) = 23.3$ Hz], 35.2, 34.3, 32.4 [d, $3J(19\text{F}, 13\text{C}) = 9.0$ Hz], 30.8, 30.7, 29.1 [t, $2J(19\text{F}, 13\text{C}) = 22.9$ Hz], 28.3, 25.3, 25.3, 22.1, 21.2, 18.3, 11.8; ^{19}F NMR (376 MHz, CDCl_3): δ -87.7 (d, $2J(19\text{F}, 19\text{F}) = 240.6$ Hz, 1F), -89.7 (d, $2J(19\text{F}, 19\text{F}) = 231.88$ Hz, 1F), -101.27 (d, $2J(19\text{F}, 19\text{F}) = 169.2$ Hz, 1F), -101.9 (d, $2J(19\text{F}, 19\text{F}) = 157.9$ Hz, 1F); HRMS-ESI: calculated for $\text{C}_{24}\text{H}_{35}\text{F}_4\text{O}_2$ ($M - H$) —, 431.2573; found, 431.2586.

[00580] Compound **81**: ^1H NMR (400 MHz, CDCl_3): δ 2.44-2.40 (m, 1H), 2.28-2.24 (m, 1H), 2.12-1.91 (m, 1H), 1.90-1.83 (m, 4H), 1.75-1.67 (m, 5H),
20 1.63-1.56 (m, 4H), 1.48-1.40 (m, 5H), 1.34-1.07 (m, 5H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.95 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3): δ 179.9, 126.2 [t, $1J(19\text{F}, 13\text{C}) = 246.1$ Hz], 124.1 [t, $1J(19\text{F}, 13\text{C}) = 239.4$ Hz], 52.5, 52.4, 49.2 [t, $2J(19\text{F}, 13\text{C}) = 20.4$ Hz], 47.8, 39.6 [d, $3J(19\text{F}, 13\text{C}) = 8.8$ Hz], 36.6 [d, $3J(19\text{F}, 13\text{C}) = 9.1$ Hz], 34.4 [t, $2J(19\text{F}, 13\text{C}) = 22.3$ Hz], 34.1, 33.2, 32.8 [d, $3J(19\text{F}, 13\text{C}) = 9.1$ Hz],
25 31.5 [t, $2J(19\text{F}, 13\text{C}) = 25.8$ Hz], 31.4, 30.2, 29.1 [t, $2J(19\text{F}, 13\text{C}) = 23.6$ Hz], 26.3, 25.8, 25.1, 23.0, 22.4, 18.9 [d, $3J(19\text{F}, 13\text{C}) = 10.6$ Hz], 10.5; ^{19}F NMR (376 MHz, CDCl_3): δ -88.8 (d, $2J(19\text{F}, 19\text{F}) = 233.1$ Hz, 1F), -91.0 (d, $2J(19\text{F}, 19\text{F}) = 233.1$ Hz, 1F), -99.7 (d, $2J(19\text{F}, 19\text{F}) = 233.1$ Hz, 1F), -111.9 (d,

2J (19F, 19F) = 233.1 Hz, 1F); HRMS-ESI : calculated for C₂₄H₃₅F₄O₂ (M - H) - ,431.2573 ; found, 431.2568.

[00581] Compound **73**: ¹H NMR (400 MHz, CDCl₃) : δ 3.65-3.57 (m, 1H), 2.41-2.34 (m, 1H), 2.28-2.20 (m, 1H), 1.98-1.91 (m, 2H), 1.90-1.74 (m, 5H),
 5 1.73-1.42 (m, 6H), 1.41-1.31 (m, 4H), 1.30-1.20 (m, 2H), 1.19-1.01 (m, 5H), 0.94 (d, J = 4.6 Hz, 3H), 0.91 (d, J = 6.37 Hz, 3H), 0.65 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) : δ 179.5, 125.0 [t, 1J (19F, 13C) = 244.5 Hz], 70.4, 55.8 [d, 3J (19F, 13C) = 11.7 Hz], 49.1 [t, 2J (19F, 13C) = 18.5 Hz], 42.8, 39.8, 39.6, 35.7 [d, 3J (19F, 13C) = 5.5 Hz], 35.2, 35.2 [t, 2J (19F, 13C) = 20.5 Hz], 33.0 [d, 3J (19F, 13C) = 9.5 Hz], 32.8, 31.0, 30.7, 29.8, 28.0, 27.9, 24.1 [d, 3J (19F, 13C) = 7.17 Hz], 24.0, 22.5, 20.4, 18.2, 11.9; ¹⁹F NMR (376 MHz, CDCl₃) : δ -87.5 (d, J = 210.6 Hz, 1F), -91.1 (d, J = 240.6 Hz, 1F).

[00582] Compound **82**: ¹H NMR (400 MHz, CDCl₃) : δ 2.40-2.33 (m, 1H), 2.32-2.25 (m, 1H), 2.18-1.90 (m, 5H), 1.89-1.86 (m, 5H), 1.83-1.74 (m, 4H),
 15 1.74-1.53 (m, 3H), 1.44-1.24 (m, 5H), 1.00 (s, 3H), 0.98 (dm, 3H), 0.84 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) : δ 180.1, 125.3 [t, 1J (19F, 13C) = 245.0 Hz], 123.7 [t, 1J (19F, 13C) = 243.8 Hz], 123.1 [t, 1J (19F, 13C) = 240 Hz], 49.4 [t, 2J (19F, 13C) = 20.5 Hz], 46.7, 45.2, 40.7 [t, 2J (19F, 13C) = 21.8 Hz], 39.0 [t, 2J (19F, 13C) = 10.2 Hz], 23.3 [t, 2J (19F, 13C) = 23.3 Hz], 33.9, 33.7 [d, 3J (19F, 13C) = 9.2 Hz], 33.0, 32.2 [d, 3J (19F, 13C) = 9.2 Hz], 31.5, 31.4 [t, 2J (19F, 13C) = 26.5 Hz], 30.0, 28.9 [t, 2J (19F, 13C) = 23.1 Hz], 26.0, 24.1, 24.0, 21.8, 19.1 [d, 3J (19F, 13C) = 10.2 Hz], 10.5; ¹⁹F NMR (376 MHz, CDCl₃) : δ -85.4 (d, J = 244.4 Hz, 1F), -90.2 (d, J = 244.4 Hz, 1F), -93.1 (d, J = 244.4 Hz, 1F), -101.4 (d, J = 244.4 Hz, 1F), -102.1 (d, J = 236.9 Hz, 1F), -111.74 (d, J = 236.9 Hz, 1F);
 25 HRMS-FAB : calculated for C₂₄H₃₄F₆O₂Na (M + Na)⁺, 491.2361 ; found, 491.2376.

[00583] Compound **75**: ¹H NMR (400 MHz, CDCl₃) : δ 3.58-3.48 (m, 1H), 2.46-2.37 (m, 1H), 2.34-3.24 (m, 1H), 2.21-1.96 (m, 3H), 1.92-1.83 (m, 4H), 1.79-1.61 (m, 7H), 1.61-1.43 (m, 3H), 1.38-1.09 (m, 5H), 0.98 (d, J = 6.0 Hz, 3H),

0.95 (s, 3H), 0.83 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 177.8, 125.5 [t, 1J(19F, 13C) = 248.7 Hz], 124.0 [t, 1J(19F, 13C) = 248.7 Hz], 71.1, 49.4 [t, 2J(19F, 13C) = 20.4 Hz], 46.7, 45.2, 40.9 [d, 3J(19F, 13C) = 16.7 Hz], 40.8 [t, 2J(19F, 13C) = 21.5 Hz], 37.1, 36.1 [t, 2J(19F, 13C) = 21.6 Hz], 34.5, 34.2 [t, 2J(19F, 13C) = 10.2 Hz], 33.9, 33.0, 31.2 [t, 2J(19F, 13C) = 22.5 Hz], 30.1 [d, 3J(19F, 13C) = 12.5 Hz], 30.0, 29.7, 26.0, 24.1, 22.3, 19.1 [d, 3J(19F, 13C) = 10.9 Hz], 10.5; ¹⁹F NMR (376 MHz, CDCl₃): δ -85.1 (d, J = 244.4 Hz, 1F), -93.0 (d, J = 244.4 Hz, 1F), -101.3 (d, J = 244.4 Hz, 1F), -111.8 (d, J = 244.4 Hz, 1F); HRMS-ESI: calculated for C₂₄H₃₅F₄O₃ (M - H) —, 447.2522; found, 447.2515.

[00584] Compound **86**: ¹H NMR (500 MHz, DMSO): δ 4.71 (ddd, J = 4.7 Hz, 1H), 3.97 (m, 1H), 2.26-2.05 (m, 2H), 1.89 (d, J = 10.0 Hz, 1H), 1.83-1.74 (m, 2H), 1.71-1.49 (m, 4H), 1.49-1.29 (m, 8H), 1.29-0.98 (m, 9H), 0.93 (s, 3H), 0.86 (d, 3H), 0.61 (s, 3H); ¹³C NMR (125 MHz, DMSO): δ 175.3, 91.7, 90.3, 66.2, 66.1, 56.2, 56.0, 42.6, 41.3, 41.2, 41.1, 37.4, 37.3, 35.5, 35.2, 31.2, 31.1, 29.1, 28.1, 26.2, 26.1, 25.9, 24.2, 23.7, 21.2, 20.3, 18.5, 12.3.

[00585] Compound **84**: ¹H NMR (500 MHz, DMSO): δ 4.99 (d, J = 4.8 Hz, 1H), 4.47 (td, J = 51.1 Hz, 1H), 3.47-3.30 (m, 1H), 2.27-2.05 (m, 2H), 1.94-1.87 (d, J = 12.0, 1H), 1.85-1.75 (m, 1H), 1.73-1.51 (m, 6H), 1.47-0.98 (m, 16H), 0.92 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.61 (s, 3H); ¹³C NMR (125 MHz, DMSO): δ 175.3, 95.5, 94.1, 73.0, 72.8, 56.2, 55.9, 47.0, 46.9, 42.6, 42.1, 37.5, 37.4, 35.4, 35.2, 34.3, 31.2, 31.1, 28.1, 28.0, 27.9, 26.0, 24.2, 23.3, 20.8, 18.5, 12.3.

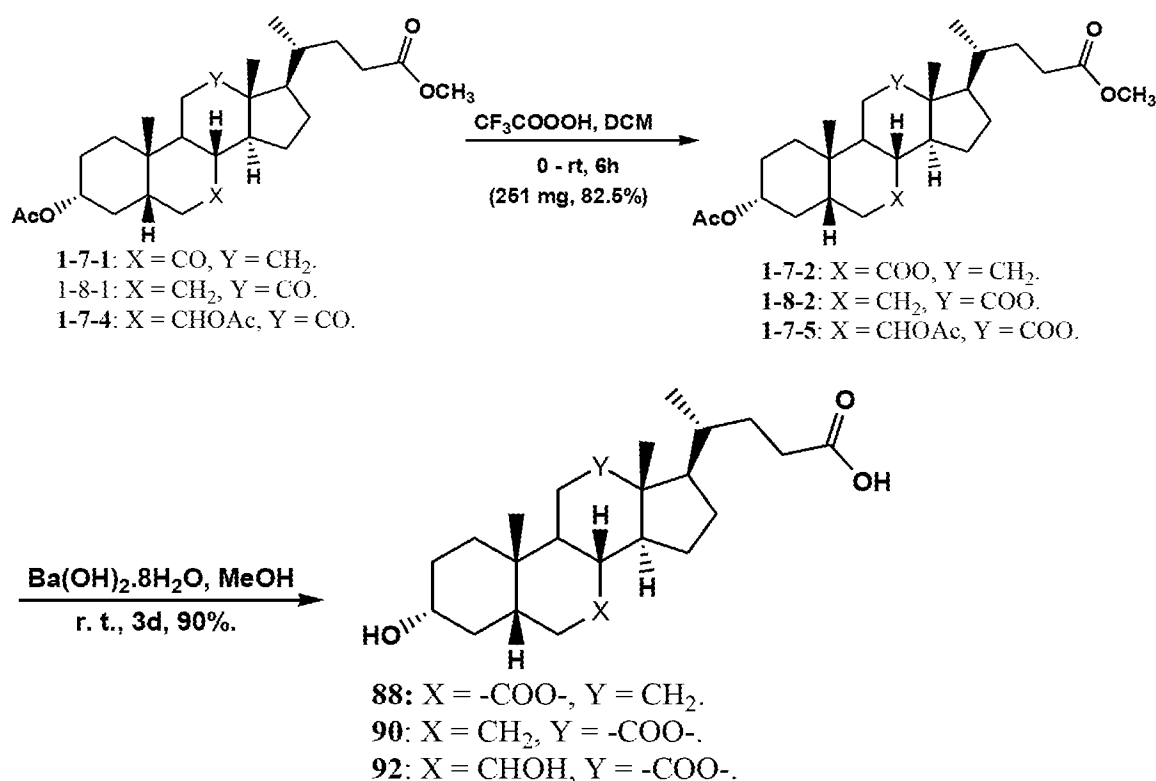
[00586] Compound **87**: ¹H NMR (500 MHz, DMSO): δ 8.59 (d, J = 8.5 Hz, 1H), 8.19 (d, J = 9.23 Hz, 1H), 6.70 (d, J = 8.8 Hz, 1H), 5.44-5.32 (br, 1H), 5.39-5.21 (br, 1H), 5.02-4.84 (ddd, J = 46.9 Hz, 1H), 3.24 (d, J = 2.3 Hz, 2H), 2.36-2.25 (m, 2H), 2.24-2.13 (m, 2H), 1.98-1.91 (d, J = 9.87 Hz, 1H), 1.85-1.70 (m, 3H), 1.68-1.33 (m, 10H), 1.32-1.04 (m, 9H), 0.91 (d, J = 6.7 Hz, 3H), 0.73 (s, 3H), 0.65 (s, 3H); ¹³C NMR (125 MHz, DMSO): δ 174.1, 171.0, 168.7, 144.6, 144.1, 136.3, 124.0, 101.3, 88.3, 86.9, 71.8, 71.7, 56.1, 56.0, 42.7, 42.6, 42.4, 41.2, 39.8, 36.8, 36.7, 35.3, 35.1, 35.0, 30.8, 30.2, 27.8, 25.2, 23.7, 23.0, 22.9, 22.4, 20.9, 19.7, 17.7, 11.4.

[00587] Compound **85**: ^1H NMR (500 MHz, DMSO) : δ 9.03 (d, J = 9.05 Hz, 1H), 8.58-8.45 (m, 1H), 7.11 (d, J = 8.8 Hz, 1H), 5.71-5.58 (m, 1H), 5.47-5.31 (m, 1H), 5.38-5.18 (td, J = 51.3Hz, 1H), 3.70 (d, J = 5.7Hz, 2H), 2.85-2.74 (m, 2H), 2.71-2.60 (m, 2H), 2.46-2.39 (m, 1H), 2.38-2.13 (m, 7H), 2.13-1.80 (m, 9H),
 5 1.86-1.52 (m, 11H), 1.48 (s, 3H), 1.39 (d, J = 6.4Hz, 3H), 1.14 (s, 3H); ^{13}C NMR (125 MHz, DMSO): δ 174.1, 170.8, 169.1, 144.6, 144.1, 136.4, 124.2, 100.9, 91.0, 89.6, 77.7, 77.5, 56.1, 56.0, 53.1, 47.2, 47.1, 42.3, 42.0, 39.8, 37.0, 35.3, 35.2, 33.4, 30.8, 30.2, 27.8, 25.5, 23.8, 22.2, 20.6, 20.3, 17.7, 11.4.

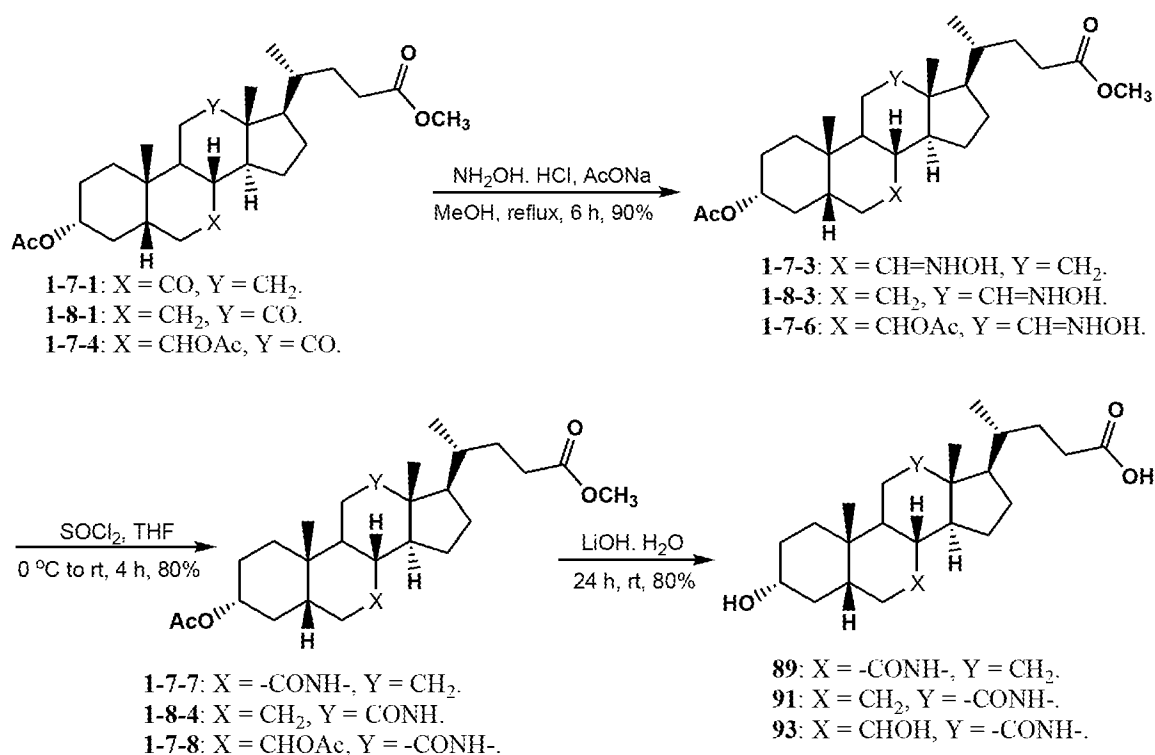
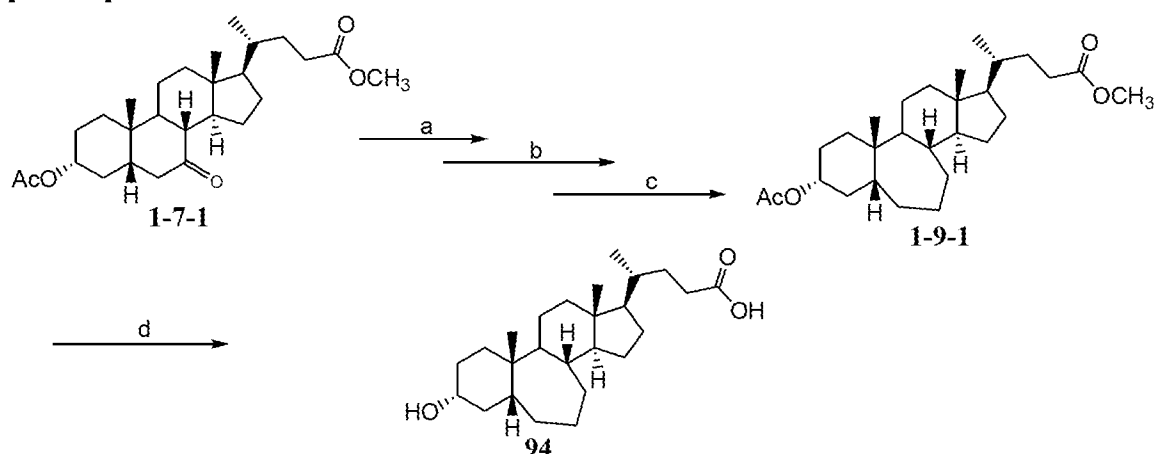
[00588] **1.9 Synthesis of Compounds 88 to 94**

10 [00589] Compounds 88 to 94 (or compounds of formula (1-7), (1-8) and (1-9)) were synthesized in accordance with procedures set forth in **Schemes 50 to 52**.

[00590] **Scheme 50:**



[00591] **Scheme 51:**

5 [00592] **Scheme 52:**

- (a) (1) 2.8 equiv. of TMSCHN₂, 4.2 equiv. of BF₃·Et₂O, CH₂Cl₂, -15 °C, 3 h,
 (2) 2N HCl, SiO₂, Et₂O, rt, 6 h.
 (b) TsNHNH₂, MeOH, rt, 24 h.
 (c) (1) catecholborane, CHCl₃, (2) NaOAc, reflux. 33% yield (3 steps)
 (d) 20% KOH, EtOH, reflux, 4 h. 70% yield

[00593] *Synthesis of Compounds 88, 90 and 92*

[00594] Homolactones (compds. **88**, **90** and **92**; scheme 37) were synthesized

10 by Bayer-Viliger oxidation of the known ketocholanes (compounds **1-7-1**, **1-8-1**,

and 1-7-4), using trifluoroperacetic acid. Baeyer-Villiger oxidation of ketocholanes was chosen for the ring expansion to incorporate an additional oxygen atom to give lactone. This reaction proceeds with complete retention of the configuration and regioselectivity, thus offering possibilities for a reliable synthetic design. Hence, Baeyer-Villiger oxidation of ketocholanes furnished the lactones 1-7-2, 1-8-2, 1-7-5 which was purified by rapid column chromatography. The lactones were saponified with methanolic barium hydroxide in analogy to a reported procedure (Peterson et al., Tetrahedron, 51, 9467-9486 (1995)) and subsequently acidified with concentrated hydrochloric acid, extracted with ethyl acetate, washed with brine, dried and evaporated under vacuum to afford the title compounds **88, 90, and 92**.

[00595] *General procedure for Baeyer-Villiger oxidation*

[00596] The Methyl ketocholanate (0.66 mmol) in dichloromethane (12 mL) was added at 0 °C to a solution of trifluoroperacetic acid in dichloromethane prepared by adding trifluoroacetic anhydride (4.5 mL) to 30% aqueous hydrogen peroxide (0.75 mL) in dichloromethane (12 mL) at 0 °C. The mixture was stirred at this temperature for 2 h then was warmed to room temperature and maintained for 4 h. The reaction mixture was diluted with dichloromethane (30 mL), and the resulting solution was washed with saturated sodium bicarbonate solution, brine solution, dried over anhydrous sodium sulfate, and concentrated. The residue was chromatographed (eluting with ethyl acetate/hexane; 2/1) to afford the lactone in 82%.

[00597] *General procedure for saponification of lactone*

[00598] A mixture of lactone (0.11 mmol) and barium hydroxide octahydrate (powdered 3.5 g) in MeOH (5 mL) was stirred at room temperature for 97 h. The heavy white suspension was diluted with brine (3 ml) and acidified with 1 M HCl to give a clear solution of pH = 3-4. This aqueous solution was extracted with EtOAc (2 x 50 + 2 x 20) and the combined organic extracts washed with brine (20 mL), dried, concentrated in vacuo, washed with chloroform to give 80-90% yield.

[00599] **Methyl 3 α -acetoxy-8-oxa-7-oxo-B-homo-5 β -cholanate (1-7-2).** R_f = 0.25 (hexane/EtOAc, 2:1). ^1H NMR (400 MHz, CDCl_3) 4.62-4.58 (m, H-3 ax.), 4.11 (dd, $J=9.2$ and 10.0 Hz, H-8 ax), 3.55 (s, CH_3O), 2.95 (br. d, $J=15.0$ Hz, H-6), 2.30-2.05 (m, 3H), 1.95-1.07 (m, 24H, steroidal CH and CH_2), 0.98 (s, CH_3 -19),
 5 0.81 (d, $J=6.8$ Hz, CH_3 -21), 0.56 (s, CH_3 -18). ^{13}C NMR (75 MHz, CDCl_3) 35.2 (C-1), 26.2 (C-2), 72.3 (C-3), 31.4 (C-4), 41.2 (C-5), 35.2 (C-6), 173.6 (C-7), 79.2 (C-8), 39.7 (C-9), 37.2 (C-10), 22.6 (C-11), 38.1 (C-12), 42.9 (C-13), 54.1 (C-14), 24.7 (C-15), 27.4 (C-16), 55.4 (C-17), 11.2 (C-18), 23.1 (C-19), 34.7 (C-20), 17.9 (C-21), 30.5 (C-22), 30.6 (C-23), 174.0 (C-24), 51.1 (CH_3 -O), 170.0 (AcO).

10 [00600] **Methyl 3 α -acetoxy-13-oxa-12-oxo-C-homo-5 β -cholanate (1-8-2).** R_f = 0.30 (hexane/EtOAc, 2:1). ^1H NMR (400 MHz, CDCl_3) 4.67-4.59 (m, H-3 ax.), 3.57 (s, CH_3O), 2.52 (br. d, $J=12.0$ Hz, H-11), 2.37-2.25 (m, 3H), 1.92 (s, 3H, CH_3CO), 1.84-1.02 (m, 24H, steroidal CH and CH_2), 0.95 (d, $J=6.5$ Hz, CH_3 -21), 0.8 (s, CH_3 -18). ^{13}C NMR (75 MHz, CDCl_3) 14.3, 17.0, 20.3, 20.5, 21.0, 22.1, 23.8,
 15 25.5, 25.8, 26.2, 26.3, 29.9, 30.5, 31.6, 33.9, 34.5, 35.2, 36.2, 37.3, 38.8, 40.8, 51.2, 54.9, 55.1, 73.4 (C-3), 87.2 (C-13), 170.5 (AcO), 174.2 (C-12), 175.2 (C-24).

[00601] **Methyl 3 α , 7 α -diacetoxy-13-oxa-12-oxo-B-homo-5 β -cholanate (1-7-5).** R_f = 0.30 (hexane/EtOAc, 2:1). ^1H NMR (400 MHz, CDCl_3) 4.60 (br. d, $J=2.0$ Hz, H-7) 4.46-4.39 (m, 1H, H-3 ax.), 3.49 (s, CH_3O), 2.52 (br. d, $J=13.2$ Hz, H-11), 2.37-2.25 (m, 4H), 2.10-0.99 (m, 30H, steroidal CH and CH_2 , 2 Ac), 0.88 (d, $J=6.6$ Hz, CH_3 -21), 0.76 (s, CH_3 -18). ^{13}C NMR (75 MHz, CDCl_3) 14.2, 17.0, 21.0, 21.7, 23.7, 24.7, 26.3, 29.9, 30.4, 30.6, 32.7, 33.8, 34.2, 34.7, 35.1, 35.6, 39.8, 40.6, 49.8, 51.1, 55.0, 71.0 (C-3), 73.1 (C-3), 86.2 (C-13), 169.7 (AcO), 170.1
 25 (C-12), 173.9 (C-24).

[00602] **3 α -Hydroxy-8-oxa-7-oxo-B-homo-5 β -cholanoic acid (88).** 90% yield, R_f = 0.23 (dichloromethane/MeOH, 5:1). Mp. 212-213 $^\circ\text{C}$ ^1H NMR (400 MHz, acetone- d_6) 4.40 (dd, $J=9.2$ and 10.0 Hz, H-8 ax), 3.59-3.54 (m, H-3 ax.), 3.20 (br. d, $J=14.8$ Hz, H-6), 2.35-2.19 (m, 3H), 1.99-1.26 (m, 22H, steroidal CH and CH_2),

1.12 (s, CH₃-19), 0.96 (d, J=6.4 Hz, CH₃-21), 0.72 (s, CH₃-18). ¹³C NMR (100 MHz, acetone-d₆) 36.3 (C-1), 28.4 (C-2), 70.9 (C-3), 35.8 (C-4), 41.1 (C-5), 31.7 (C-6), 173.9 (C-7), 79.5 (C-8), 39.4 (C-9), 37.0 (C-10), 23.2 (C-11), 38.4 (C-12), 42.6 (C-13), 55.7 (C-14), 25.7 (C-15), 28.4 (C-16), 56.7 (C-17), 11.9 (C-18), 23.9 (C-19), 36.3 (C-20), 18.6 (C-21), 31.2 (C-22), 31.3 (C-23), 175.0 (C-24). HRMS calcd for C₂₄H₃₇O₅ (M - H)⁻, 405.2641; found, 405.2635.

[00603] **3 α -Hydroxy-13-oxa-12-oxo-C-homo-5 β -cholanoic acid (90).** 80% yield, R_f = 0.21 (dichloromethane/MeOH, 5:1). Mp. 102-103 °C. ¹H NMR (400 MHz, MeOH-d₄) 3.56-3.50 (m, H-3 ax.), 2.37-2.17 (m, 4H), 2.00--1.20 (m, 20H, steroidal CH and CH₂), 1.05 (d, J=5.6 Hz, CH₃-21), 0.93 (s, CH₃-19), 0.82 (s, CH₃-18). ¹³C NMR (100 MHz, MeOH-d₄) 17.8, 20.3, 23.9, 25.8, 26.6, 28.1, 31.7, 31.9, 32.0, 35.7, 36.5, 37.2, 37.6, 43.2, 52.9, 54.4, 72.4 (C-3), 82.2 (C-13), 178.3 (C-12), 179.0 (C-24). HRMS calcd for C₂₄H₃₇O₅ (M + Na)⁺, 429.2617; found, 429.2613.

[00604] **3 α , 7 α -Dihydroxy-13-oxa-12-oxo-C-homo-5 β -cholanoic acid (92).** 85% yield, R_f = 0.2 (dichloromethane/MeOH, 5:1). Mp. 99-101 °C. ¹H NMR (400 MHz, MeOH-d₄) 3.70 (d, J=2.4 Hz, H-7), 3.44-3.39 (m, H-3 ax.), 2.7.-2.5 (m, 2H), 2.40--1.26 (m, 26H, steroidal CH and CH₂, CH₃-19), 1.05 (d, J=6.4 Hz, CH₃-21), 0.91 (s, CH₃-18). ¹³C NMR (100 MHz, MeOH-d₄) 14.7, 17.8, 22.6, 25.1, 26.0, 26.5, 31.3, 31.9, 32.3, 33.7, 35.5, 36.3, 37.3, 40.4, 42.5, 43.7, 51.7, 57.0, 68.7 (C-7), 72.5 (C-3), 88.9 (C-13), 178.1 (C-12 and C-24). HRMS calcd for C₂₄H₃₈O₆ (M - H)⁻, 421.2590; found, 421.2583.

[00605] *Synthesis of Compounds 89, 91, and 93*

[00606] Compounds **1-7-1**, **1-8-1**, and **1-7-4** (Scheme 38) were transformed into the corresponding oximes **1-7-3**, **1-8-3**, and **1-7-6** under standard reaction conditions (Iuliano et al., Tetrahedron: Asymmetry 2001, 12, 2811-2825). Compounds **1-8-3** and **1-7-6** underwent Beckmann rearrangement with SOCl₂/THF at 0 °C to corresponding lactams **1-7-7**, **1-8-4**, and **1-7-8**, then

converted to compounds **89**, **91** and **93** by reacting with LiOH at room temperature for 24 hrs.

[00607] *General procedure for oxime synthesis (1-7-3, 1-8-3, and 1-7-6)*

[00608] Sodium acetate (1.03 g, 13.42 mmol, 5.75 equiv.) and hydroxylamine hydrochloride (0.288 g, 4.13 mmol, 2 equiv) were added to a solution of ketone (1.0 g, 1 equiv) in methanol (20 mL). The reaction mixture was stirred at reflux for 6 h. The solvent was removed under vacuum and the residue dissolved in CH₂Cl₂. The organic solution was washed with water then dried over Na₂SO₄. The solvent was evaporated under reduced pressure and the crude product recrystallized from aqu. Ethanol to afford chemically pure oxime in 90% yield.

[00609] *General procedure for Beckmann rearrangement (1-7-7, 1-8-4, 1-7-8)*

[00610] Freshly distilled thionyl chloride (1.5 mL) in 4 mL dry THF was added dropwise to a solution of oxime (**1-7-3**, **1-8-3** or **1-7-6**, 355 mg, 0.77 mmol) in 12 mL dry THF cooled to 0 °C, while maintaining the temperature at 0 °C. After this addition, the reaction mixture was stirred at 0–10 °C for 1 h and then at room temperature for 4h under argon atmosphere. The mixture was then poured into ice–water, neutralized with a solution of NH₃, and extracted with dichloromethane. The organic layer was washed with water and saturated brine, dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure gave a solid residue, which was chromatographed over silica gel using hexane/EtOAc (1:2) as the eluent to give 347 mg (80%) as a white solid.

[00611] *General Procedures for the Hydrolysis of the ester: (89, 91, and 93)*

[00612] LiOH (91 mg, 2.2 mmol, 10 equiv.) in water (1 ml) was added to a solution of 1-7-7, 1-8-4, or 1-7-8 (100 mg, 0.21 mmol) in THF–methanol 1:2 (7 mL) and the mixture was stirred at room temperature for 24 h. Then acidified with 10% aqueous HCl. The solvents were removed at reduced pressure and ethyl acetate (20 mL) was added. The organic solution was washed with brine then dried (MgSO₄), evaporated. The solid residue was recrystallized from ethyl acetate to furnish the target compounds (**89**, **91** or **93**) 70 to 80%.

[00613] Methyl 3 α -acetoxy-7-oximino-5 β -cholan-24-oate (1-7-3) R_f = 0.43 (hexane/EtOAc, 5:1). ^1H NMR (400 MHz, CDCl_3): 7.35 (br. s, 1H, NOH), 4.75-4.65 (m, 1H, H-3 ax.), 3.65 (s, CH_3O), 3.08 (dd, $J=2.0$ and 12.8 Hz, H-6), 2.34-2.15 (m, 4H), 2.05-1.10 (m, 24H, steroidal CH and CH_2 , Ac), 1.07 (s, CH_3 -19), 0.91 (d, $J=6.4$ Hz, CH_3 -21), 0.66 (s, CH_3 -18). ^{13}C NMR (100 MHz, CDCl_3 , δ): 12.2, 18.5, 21.2, 21.5 (CH_3CO), 23.3, 25.4, 26.4, 27.5, 28.2, 31.1, 31.2, 32.8, 34.3, 35.4, 35.6, 39.2, 42.4, 42.9, 43.1, 44.6, 49.5, 51.6 (CH_3O), 55.0, 73.4 (C3), 161.0 (C=NOH), 170.7 ($\text{CH}_3\text{C}=\text{O}$); 174.8 (24 C=O).

[00614] **Methyl 3 α -acetoxy-8-aza-7-oxo-B-homo-5 β -cholanate (1-7-7).** R_f = 0.16 (hexane/EtOAc, 1:1). ^1H NMR (400 MHz, CDCl_3) 5.37 (br. s., 1H, NH), 4.79-4.72 (m, H-3 ax.), 3.65 (s, CH_3O), 3.31 (dd, $J=6.0$ and 9.6 Hz, H-8 ax), 2.92 (br. d, $J=14.4$ Hz, H-6), 2.34-2.13 (m, 3H), 1.97 (s, 3H, Ac), 1.97-1.08 (m, 24H, steroidal CH and CH_2), 1.06 (s, CH_3 -19), 0.91 (d, $J=6.4$ Hz, CH_3 -21), 0.66 (s, CH_3 -18). ^{13}C NMR (100 MHz, CDCl_3 , δ): 11.6, 18.3, 21.4, 21.9, 23.2, 25.2, 26.6, 27.7, 29.8, 30.9, 31.1, 31.5, 35.2, 36.3, 36.8, 38.5, 38.8, 41.0, 41.7, 42.7, 51.6, 51.9, 54.7, 55.7, 73.1 (C3), 170.5 ($\text{CH}_3\text{C}=\text{O}$); 174.5 (C7), 175.5 (24 C=O). HRMS calcd for $\text{C}_{27}\text{H}_{43}\text{NO}_5$ ($\text{M} + \text{Na}$) $^+$, 484.3039; found, 484.3036.

[00615] **Methyl 3 α -acetoxy-13-aza-12-oxo-C-homo-5 β -cholanate (1-8-4).** R_f = 0.16 (hexane/EtOAc, 1:1). ^1H NMR (400 MHz, CDCl_3) 6.00 (br. s., 1H, NH), 4.79-4.72 (m, H-3 ax.), 3.65 (s, CH_3O), 2.38-2.22 (m, 3H), 1.98-1.14 (m, 29H, steroidal CH and CH_2 , CH_3 , CH_3CO), 1.03 (d, $J=6.4$ Hz, CH_3 -21), 0.86 (s, CH_3 -18). ^{13}C NMR (1100 MHz, CDCl_3) 15.0, 18.0, 21.4, 22.7, 25.9, 26.6, 26.8, 26.83, 27.0, 29.8, 30.3, 30.9, 32.1, 34.8, 35.1, 35.7, 37.0, 37.0, 40.0, 41.4, 51.6, 56.5, 58.6, 58.6, 60.7, 74.0 (C-3), 170.7 (AcO), 174.2 (C-12), 176.6 (C-24). HRMS calcd for $\text{C}_{27}\text{H}_{43}\text{NO}_5$ ($\text{M} + \text{Na}$) $^+$, 484.3039; found, 484.3031.

[00616] **Methyl 3 α , 7 α -diacetoxy-13-aza-12-oxo-C-homo-5 β -cholanate (1-7-8).** R_f = 0.14 (hexane/EtOAc, 1:1). ^1H NMR (400 MHz, CDCl_3) 6.00 (br. s., 1H, NH), 4.89 (br. d, $J=2.8$ Hz, H-7) 4.60-4.53 (m, 1H, H-3 ax.), 3.65 (s, CH_3O), 2.45-2.19 (m, 4H), 2.05-1.10 (m, 36H, steroidal CH and CH_2 , CH_3 , 2 Ac), 0.87 (d,

J=6.4 Hz, CH₃-21), 0.83 (s, CH₃-18). ¹³C NMR (100 MHz, CDCl₃) 14.6, 18.0, 21.4, 21.6, 22.3, 25.8, 26.3, 26.8, 29.8, 30.3, 30.6, 30.9, 31.1, 32.9, 34.8, 35.3, 35.6, 35.8, 36.2, 40.4, 41.9, 51.7, 53.1, 56.7, 60.2, 71.9 (C-3), 73.8 (C-7), 170.2 (AcO), 170.2 (AcO), 170.7 (AcO), 174.1 (C-12), 176.2 (C-24). HRMS calcd for C₂₉H₄₅NO₇ (M + Na)⁺, 542.3094; found, 542.3099.

[00617] **3α-Hydroxy-8-aza-7-oxo-B-homo-5β-cholanoic acid (compound 89).**

79.5 % yield, R_f = 0.45 (dichloromethane/MeOH, 10:1). Mp. 285-286 °C ¹H NMR (400 MHz, MeOH-d₄) 3.61-3.58 (m, H-3 ax.), 3.44 (dd, J=10.0 and 10.4 Hz, H-8 ax), 3.10 (br. d, J=14.0 Hz, H-6), 2.36-2.17 (m, 2H), 2.03-1.18 (m, 21H, steroidal CH and CH₂), 1.10 (s, CH₃-19), 0.97 (d, J=6.4 Hz, CH₃-21), 0.73 (s, CH₃-18). ¹³C NMR (100 MHz, MeOH-d₄) 36.4 (C-1), 28.6 (C-2), 71.6 (C-3), 36.3 (C-4), 41.5 (C-5), 31.9 (C-6), 178.0 (C-7), 53.4 (C-8), 39.9 (C-9), 37.4 (C-10), 23.5 (C-11), 39.6 (C-12), 42.8 (C-13), 55.0 (C-14), 25.9 (C-15), 28.6 (C-16), 56.9 (C-17), 11.8 (C-18), 25.9 (C-19), 37.44 (C-20), 18.7 (C-21), 30.9 (C-22), 28.6 (C-23), 178.7 (C-24). HRMS calcd for C₂₄H₃₉NO₄ (M - H)⁻¹, 404.2801; found, 404.2805.

[00618] **3α-Hydroxy-13-aza-12-oxo-C-homo-5β-cholanoic acid (compound 91).** 70% yield, R_f = 0.42 (dichloromethane/MeOH,10:1). Mp. 154-156 °C ¹H NMR (400 MHz, MeOH-d₄) 3.59-3.50 (m, H-3 ax.), 2.56-2.24 (m, 4H), 2.04--1.20 (m, 25H, steroidal CH and CH₂, CH₃-19), 1.08 (d, J=6.4 Hz, CH₃-21), 0.91 (s, CH₃-18).

¹³C NMR (100 MHz, MeOH-d₄) 15.0, 17.8, 23.1, 25.8, 26.8, 27.7, 27.9, 31.4, 31.6, 36.1, 36.4, 36.7, 36.9, 37.4, 39.1, 41.2, 42.9, 56.8, 59.6, 62.0, 72.1 (C-3), 179.7 (C-12 and C-24). HRMS calcd for C₂₄H₃₉NO₄ (M - H)⁻¹, 404.2801; found, 404.2803.

[00619] **3α, 7α-Dihydroxy-13-aza-12-oxo-C-homo-5β-cholanoic acid**

(compound 93). 80% yield, R_f = 0.25 (dichloromethane/MeOH,10:1). Mp. 263-264 °C ¹H NMR (400 MHz, MeOH-d₄) 3.86 (d, J=2.8 Hz, H-7) 3.43-3.37 (m, H-3 ax.), 2.56 (t, J=12.8 Hz, H-11), 2.39-1.28 (m, 25H, steroidal CH and CH₂, CH₃-19), 1.08 (d, J=6.4 Hz, CH₃-21), 0.91 (s, CH₃-18). ¹³C NMR (100 MHz, MeOH-d₄) 14.7, 17.8, 22.6, 26.7, 27.2, 31.5, 31.8, 33.3, 35.9, 36.1, 36.7, 37.3,

40.4, 42.6, 44.5, 54.1, 57.0, 61.6 (C-13), 69.0 (C-7), 72.6 (C-3), 177.7 (C-12 and C-24). HRMS calcd for $C_{24}H_{39}NO_5$ (M+H)⁺, 422.2906; found, 422.2902.

[00620] *Synthesis of Compound 1-7-9*

[00621] A 2 M solution of TMSCHN₂ in hexane (0.65 mL, 1.31 mmol) was added dropwise at -20°C to a stirred solution of 1-7-1 (211 mg, 0.474 mmol) and BF₃·Et₂O (250 µL, 1.98 mmol) in dry CH₂Cl₂ (20 mL). The mixture was stirred at -15°C for 3 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried over Na₂SO₄, and evaporated, thus giving a pale yellow residue. The residue was dissolved into Et₂O (70 mL), to which silica gel (1.2 g) and 2N HCl (0.3 mL) was added. The heterogeneous solution was stirred at room temperature for 3 h, and Na₂SO₄ (500 mg) was added. After filtration through a glass-filter and evaporation, the residue was dissolved in methanol (10 mL) and reacted directly with *p*-toluenesulfonhydrazide (71 mg, 0.474 mmol, 1 equiv.) under nitrogen and at room temperature with stirring for 10 h. The resultant mixture was filtered and the solid washed with cold methanol. Evaporation under reduced pressure afforded tosylhydrazone. This was dissolved in chloroform (15 mL) under argon and was cooled to 0 °C. Catecholborane (4.5 mL, 1.0 M solution in THF, 4.43 mmol, 10 equiv.) was added dropwise. The resultant mixture was stirred for 10 h at 0 °C. Sodium acetate (1.2 g, 8.86 mmol) was added in portions. The mixture was allowed to warm to room temperature over 30 min and then heated under reflux for 8 h. The reaction mixture was cooled to room temperature and filtered. The solid material was washed with dichloromethane (10 mL), and the combined filtrates were evaporated under reduced pressure to dryness. The remaining residue was purified by chromatography on a silica gel column with 5% ethyl acetate in hexane to give compound **1-7-9** as a white solid (130 mg, 33%, 3 steps).

[00622] **Methyl 3 α -acetoxy-8-carba-B-homo-5 β -cholanoate (1-7-9).** R_f = 0.52 (hexane/EtOAc, 10:1). ¹H NMR (400 MHz, CDCl₃): 4.75-4.67 (m, 1H, H-3 ax.), 3.63 (s, CH₃O), 2.37-2.15 (m, 2H), 2.00 (s, 3H, Ac), 1.98-1.00 (m, 28H,

steroidal CH and CH₂), 0.89 (s, CH₃-19), 0.86 (d, J=6.4 Hz, CH₃-21), 0.62 (s, CH₃-18). ¹³C NMR (100 MHz, CDCl₃, δ): 11.8, 18.3, 21.3, 21.5, 23.6, 23.9, 26.1, 27.3, 27.6, 29.7, 30.0, 30.9, 31.0, 32.8, 33.6, 35.4, 37.1, 37.5, 38.7, 40.1, 42.3, 43.1, 45.9, 51.4 (CH₃O), 56.0, 56.4, 74.2 (C3), 170.6 (Ac), 174.7 (24 C=O).

5 [00623] *Synthesis of Compound 94*

[00624] Compound **1-7-9** (130 mg, 0.29 mmol) was dissolved in ethanol (10 mL) and refluxed with 20% aqueous KOH (10 mL) for 6 h. After cooling to room temperature, the solution was acidified with 6 N HCl until a precipitate was formed. The resulting suspension was then extracted with CH₂Cl₂ (20 mL × 3). The
10 organic extracts were combined, and the solvent was removed in vacuo. the product was recrystallized from ethyl acetate/hexane to yield compound 94 (80 mg, 70%) as a white solid

[00625] **3 α -Hydroxy-8-carba-B-homo-5 β -cholanoic acid (compound 94).** R_f = 0.62 (hexane/EtOAc, 1:2). Mp. 204-206 °C. ¹H NMR (400 MHz, CDCl₃):
15 3.59-3.54 (m, 1H, H-3 ax.), 2.35-2.10 (m, 2H), 2.05-1.11 (m, 28H, steroidal CH and CH₂), 0.87 (s, 6H, CH₃-19 and CH₃-21). ¹³C NMR (100 MHz, CDCl₃, δ): 12.3, 18.9, 22.5, 24.5, 24.7, 27.1, 28.6, 31.3, 31.9, 33.1, 34.2, 34.4, 37.0, 38.5, 38.56, 39.0, 39.7, 41.5, 43.4, 44.4, 47.6, 57.6, 57.7, 72.4 (C3), 181.1 (24 C=O).

[00626] **Example 2 Characterization of The Compounds of Example 1**

20 [00627] **2.1 Compounds 1-5 Selectively Suppress α 2,3(N)-sialyltransferase ST3GALIII or α 2,6(N)-sialyltransferases ST6GALI, but not α 2,3(O)-sialyltransferase ST3GALI**

[00628] Effects of the present compounds on the activity of sialyltransferase, including α 2,3(N)-sialyltransferase ST3GALIII, α 2,6(N)-sialyltransferases
25 ST6GALI, and α 2,3(O)-sialyltransferase ST3GALI, were determined in accordance with procedures described in the "Materials and Methods" section, and results are summarized in Table 1.

[00629] As evidenced from the data presented in Table 1, compounds **1-5, 6-10** and **11-15**, exhibited a comparative inhibitory activity against ST6GALI with IC₅₀

values in the range 3–7 μM (Table 1). Similarly, the results reveal that compounds **2aA–2eA** were also potent inhibitors of ST3GALIII with IC_{50} values in the range 0.77–1.74 μM . It is surprising that none of the compounds **1–5** displayed inhibitory activity toward ST3GALI, at concentrations as high as 500 μM , with modest inhibitory activity being observed at the highest concentration (1 mM). The inhibition constants of compounds **1–5** indicate up to 287- to 649-fold (or 575- to 1299-fold) selectivity in favor of for ST3GALIII versus ST3GALI. The three series LA analogues (i.e., compounds **1–5**; **6–10**, and **11–15**) also exhibited approximately 2 orders of magnitude improvement over ST6GALI versus ST3GALI. These results suggest that compounds **1–5**, **6–10** and **11–15**, possessing the respective EG or PEG linker units, are thus the first known inhibitors with potency and selectivity for the ST3GALIII and ST6GALI rather than ST3GALI to modify the $\alpha 2,3$ - and $\alpha 2,6$ -sialylated proteins with only *N*-linked glycans.

[00630] Table 1 Inhibitory potency and specificity of the present sialyltransferase inhibitors

Compound	$\alpha 2,3$ - <i>N</i> -link	$\alpha 2,6$ - <i>N</i> -link	$\alpha 2,3$ - <i>O</i> -link	selectivity ratio	
	IC_{50} (μM)			ST3GALI / ST3GALIII	ST3GALI / ST6GALI
	ST3GALIII	ST6GALI	ST3GALI		
1	0.80 ± 0.11	4.20 ± 0.26	0 % ^a	> 625 ^d	> 119
1	0.77 ± 0.02	4.33 ± 0.04	0 % ^a (74%) ^b	> 649 ^d (< 1299) ^e	> 116
3 (FCW34)	1.74 ± 0.09	3.59 ± 0.38	0 % ^a (60%) ^b	> 287 ^d (< 575) ^e	> 139
4 (FCW66)	1.01 ± 0.07	4.90 ± 0.08	0 % ^a (50%) ^b	> 495 ^d (≤ 990) ^e	> 102
5	0.97 ± 0.14	5.03 ± 0.20	0 % ^a	> 516 ^d	> 99
6	^c	7.21 ± 0.10	0 % ^a	^c	> 70
7	^c	6.72 ± 0.30	0 % ^a	^c	> 74
8	^c	5.35 ± 0.46	0 % ^a	^c	> 94
9	^c	5.70 ± 0.36	0 % ^a	^c	> 88
10	^c	7.45 ± 0.36	0 % ^a	^c	> 67
11	^c	5.59 ± 0.24	0 % ^a	^c	> 89
12	^c	5.14 ± 0.11	0 % ^a	^c	> 97
13	^c	4.61 ± 0.35	0 % ^a	^c	> 109
14	^c	4.95 ± 0.37	0 % ^a	^c	> 101
15	^c	5.69 ± 0.40	0 % ^a	^c	> 88
Lith-O-Asp	12	15.78 ± 0.13	38.95 ± 1.26	3	3

AL10	0.88 ± 0.07	1.50 ± 0.46	13.21 ± 0.59	15	9
------	-------------	-------------	--------------	----	---

^a Inhibition percentage was measured at 500 μM.

^b Inhibition percentage was measured at 1 mM.

^c Not determined.

^d The selectivity ratio was calculated based on the concentration, 500 μM, of ST inhibitor used toward α2,3-O-ST3GAL.

^e The selectivity ratio was calculated based on the concentration, 1 mM, of ST inhibitor used toward α2,3-O-ST3GAL.

[00631] 2.2 FCW34 and FCW66 Respectively Suppress The Migration of Breast Cancer Cells

[00632] In this example, effects of the present compounds, particularly, **FCW34** and **FCW66**, on cancer cell motilities were investigated by Transwell analysis.

[00633] Briefly, breast cancer cells were first treated with 2-30 μM **FCW34** or **FCW66** prior to transwell migration assay, and then allowed the cells to invade to the lower side of the chamber for 16 hours. The IC₅₀ of the compound **3 (FCW34)** and **4 (FCW66)** on migration were 10.64 ± 0.09 μM and 12.01 ± 0.13 μM, respectively (data not shown).

[00634] To find out the mediator of the inhibitory effects of the compounds **3** or **4** on the migration of breast cancer cells, the gene of ST3GAL(III) was knocked out in MDA-MD-231 cells in accordance with the procedures set forth in the "Materials and Methods" section. Quantified results are summarized in **FIG 1**.

[00635] As depicted in **FIG 1**, knock down the expression of ST3GAL(III) significantly inhibited the migration activity of MDA-MD-231 cells, such effect was seen when MDA-MD-231 cells were treated with **FCW34** or **FCW66** (20 μM). Taken together, the results suggest that **FCW34** or **FCW66** inhibited the migration of MDA-MD-231 cells via inhibiting sialyltransferase, particularly ST3GAL(III).

[00636] 2.3 FCW34 Suppresses the Genesis of Breast Cancer

[00637] The effects of the sialyltransferase inhibitor in tumor growth and metastasis were investigated in a nude mice model of human MDA-MB-231 breast cancer established in accordance with procedures set forth in the "Materials and Methods" section. The MDA-MB-231/Luc cells (5 × 10⁶ cells) were orthotopically injected into the mammary fat pad of nude mice. Then the mice were given **FCW34** and vehicle, respectively, on day 20, and the tumor

volume were evaluated by bioluminescence every 3 days using IVIS image analysis. Results are illustrated in **FIGs 2A** and **2B**.

[00638] As evidenced in **FIG 2A**, the level of bioluminescence continued to rise throughout the entire time course of the experiment, indicating continued growth of tumor in the control mice or the mice treated with vehicle; whereas in mice treated with **FCW34** (10mg/Kg/mouse/3 days), bioluminescence remained at a relatively low level, indicating the growth of the tumor was suppressed. This finding was confirmed afterwards, when the test animals were sacrificed on day 60, and the respective tumors were harvested and weighted. Results in **FIG 2B** confirmed that administering **FCW34** to mice bearing MDA-MB-231 breast cancer cells, may suppresses the growth of the cancer cells. Further, the biochemistry tests of blood and the body weight assessment indicated that **FCW34** had limited toxic effects on the treated mice (data not shown).

[00639] **2.4 FCW34 Suppresses the Invasion and/or Migration of Pancreatic Cancer Cells**

[00640] In this example, the ability of the **FCW34** in suppressing the migration and/or invasion of pancreatic cancer cells was investigated using *in vitro* transwell analysis and *in vivo* mice model bearing pancreatic cancer.

[00641] PANC-1 cells were first grown in glucose-containing or fructose-containing medium treated with or without the presence of the test compound (i.e., **FCW34** or **AL10**, which was used as a positive control), then the cells were stained with SNA lectin to measure 2,6-sialylation level by FACS analysis. It was found that PANC-1 cells exhibited a significantly higher expression of the 2,6-sialylation if they were grown in fructose-containing medium, and the level of 2,6-sialylation was greatly reduced by the treatment of either the compound **3** or **AL10** (**FIG 3**).

[00642] According to the results from the *in vitro* transwell analysis, PANC-1 cells grown in fructose-containing medium possessed higher invasion ability, and

treatment of **FCW34** significantly inhibited its invasion ability promoted by replacing the culturing medium from glucose to fructose (**FIG 4**).

[00643] According to results from mice bearing pancreatic tumor, mice injected with PANC-1 cells grown in fructose-containing medium developed more advanced tumor than those injected with PANC-1 cells grown in glucose-containing medium, and if the PANC-1 cells were pre-treated with **FCW34**, the progression of the tumor would be halted significantly (**FIG 5**). In addition, histological analysis of the liver sections taken from the test animals also indicated that PANC-1 cells grown in fructose-containing medium caused metastatic lesions in livers, which was suppressed by the treatment of **FCW34** (Table 2).

[00644] Table 2 Liver lesions in mice bearing pancreatic cancer

	Incidence	P value
Glucose medium	0/4	
Fructose medium	3/4	0.028
Treated with FCW34	0/3	N/A

[00645] 2.5 **FCW551 Possesses No Significant Cytotoxicity Toward PANC-1 and LN Cells**

[00646] The cytotoxicity of **FCW551** on PANC-1 and LN cells were determined by MTT assay in according to procedures described in the "Materials and Methods" section. Results are presented in **FIG 6**.

[00647] As evidenced in **FIG 6**, no significant cytotoxicity was found for PANC-1 and LN cells, respectively treated with various doses of **FCW551** (1, 5, 10, 25 and 50 μ M) for 24 and 48 hours.

[00648] 2.6 **FCW551 Inhibits the Invasion and/or Migration of PANC-1 and LN Cells**

[00649] In this example, the ability of the **FCW551** in suppressing the migration and/or invasion of pancreatic cancer cells was investigated. Briefly, PANC-1 and LN cells were respectively treated with 1 and 20 μ M **FCW551** for 24 and 48 hours before subjecting to the migration and invasion analysis. Results are illustrated in **FIG 7**.

[00650] As evidenced in **FIG 7**, LN cells possessed much higher capability in terms of migrating and invading, as compared to those of PANC-1 cells; and both the migration and invasion abilities were greatly suppressed by the treatment of **FCW551**.

[00651] **Example 3 Effects of FCW393 on melanoma**

[00652] In this example, effects of **FCW393** (or compound **16**) on animals implanted with primary melanoma cells were investigated, in which the body weight; the size of the tumor; the respective weights of lymph nodes, thymus, and spleen; the respective functions of liver, kidney, and vascular system; as well as the levels of inflammation and/or stress markers were determined. Results are illustrated in **FIGs 8-15**.

[00653] **3.1 Effects of FCW393 on primary melanoma-bearing mice**

[00654] As evidenced in **FIGs 8A** and **8B**, **FCW393** was able to suppress the growth of tumor by reducing the tumor volume to about 1/3 as compared with that of the control animal (**FIG 8A**), while at the same time, without significantly affecting the body weights of the test animals (**FIG 8B**). Similarly, **FCW393** had no effects on the weight of lymph node, thymus or spleen of the test animals either (**FIG 9**).

[00655] Further, except the counts of white blood cells (WBC), which exhibited a 2-fold increases after **FCW393** treatment (**FIG 10**, panel A), **FCW393** had no effects on the levels of red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (**FIG 10**, panels B to H).

[00656] **FCW393** also had no effect on the level of inflammation marker (*i.e.*, albumin), (**FIG 11**, panel C), nor did **FCW393** affect the vascular and kidney functions either (**FIG 11**, panels D, E and F). Regarding the liver function, while **FCW393** did not affect the level of alanine aminotransferase (ALT), yet it did
5 reduce the level of aspartate aminotransferase (AST) (**FIG 11**, panels A and B). In addition, the level of the stress marker (*i.e.*, uric acid) was slightly increased after **FCW393** treatment (**FIG 11**, panel G).

[00657] **3.2 Effects of FCW393 on metastatic melanoma-bearing mice**

[00658] In this example, effects of **FCW393** on mice bearing metastatic
10 melanoma were investigated, and results are provided in **FIGs 12 to 15**.

[00659] According to the quantified results of bioluminescence IVIS images of test animals bearing metastatic melanoma taken at days 7 and 14, respectively, it was evident that **FCW393** may suppress the growth of tumor (**FIG 12**, panels A and B). This observation was further confirmed by direct measurement of the
15 luciferase activity (**FIG 12**, panel C).

[00660] Similar to the findings in Example 3.1, **FCW393** did not affect the weight of lymph node, thymus, or spleen of the the test animals (**FIG 13**); nor did it possess any significant effects on the levels of WBC, RBC, platelets, HGB, HCT, MCV, MCH, and MCHC (**FIG 14**) of the test animals.

[00661] Further, **FCW393** did not affect the level of inflammation marker or stress marker, nor did it affect the function of liver, kidney or vascular system (**FIG 15**).

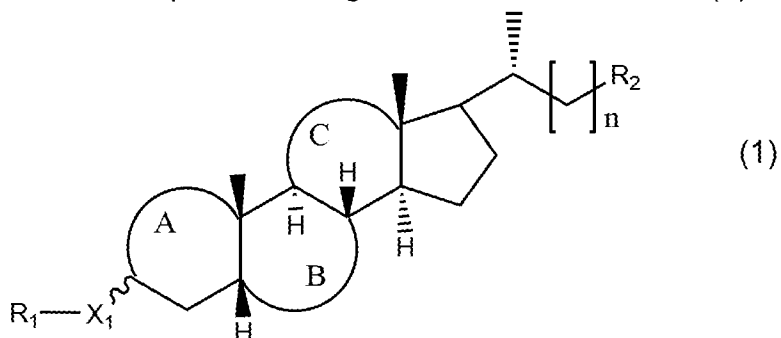
[00662] Taken together, the findings in the examples presented above suggest that the compounds of the present application are sialyltransferase (ST) inhibitors,
25 and some of them are selective toward $\alpha 2,3(N)$ -sialyltransferase ST3GALIII or $\alpha 2,6(N)$ -sialyltransferases ST6GALI, but not $\alpha 2,3(O)$ -sialyltransferase ST3GALI. In addition, the identified ST inhibitors are potent anticancer agents for the treatment of cancers, particularly the metastatic cancers.

[00663] It will be understood that the above description of embodiments is given by way of example only and that various modifications may be made by those with ordinary skill in the art. The above specification, examples and data provide a complete description of the structure and use of exemplary embodiments of the invention. Although various embodiments of the invention have been described above with a certain degree of particularity, or with reference to one or more individual embodiments, those with ordinary skill in the art could make numerous alterations to the disclosed embodiments without departing from the spirit or scope of this invention.

10

WHAT IS CLAIMED IS:

1. A compound having the structure of formula (1)

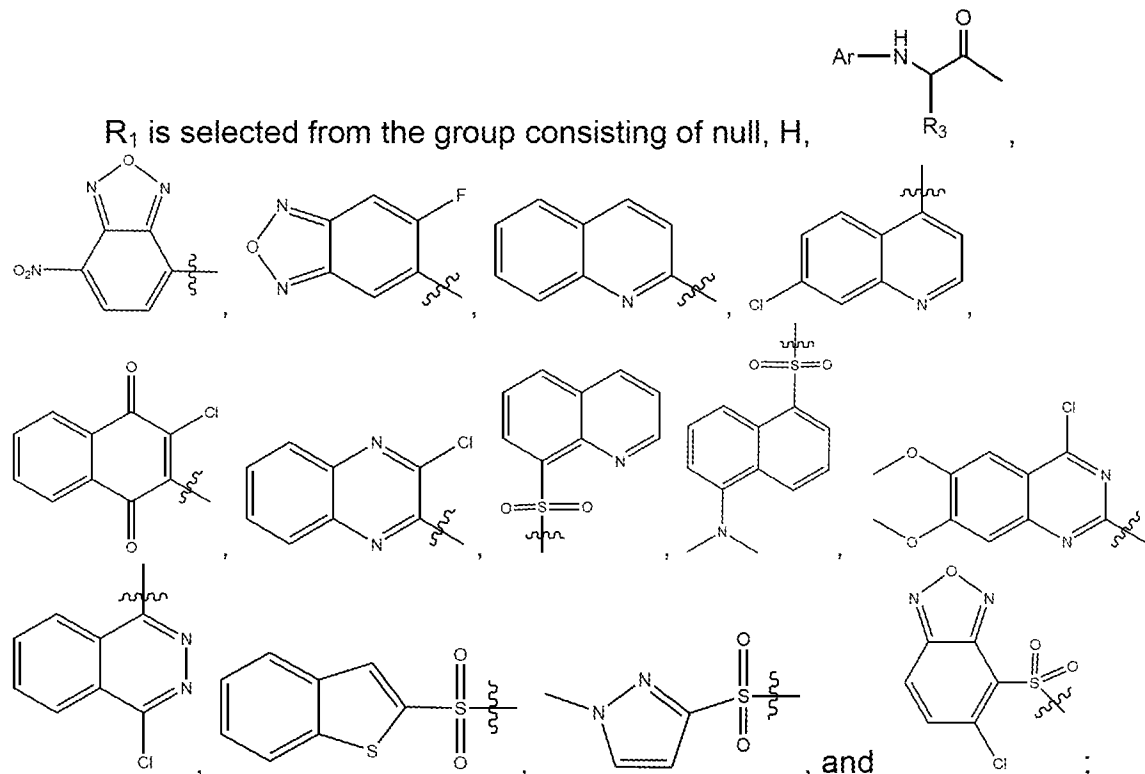


and pharmaceutically acceptable salts and solvates thereof, wherein,

- 5 n is an integral from 1 to 4;

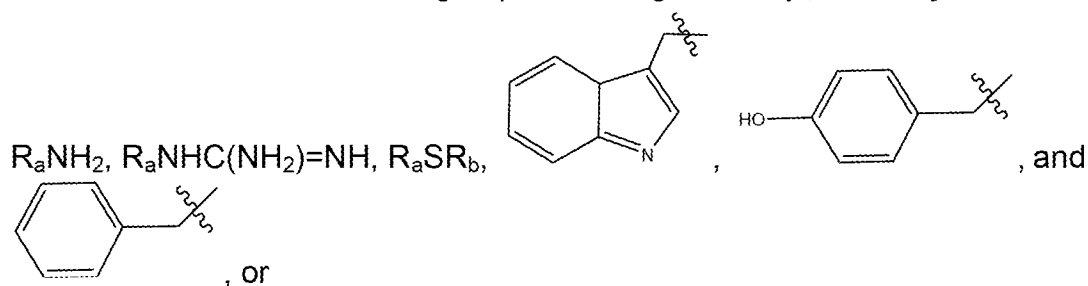
A, B, and C are independently a 6- or 7-membered saturated or unsaturated carbon cyclic ring, a homolactone or a homolactam, in which the saturated or unsaturated carbon cyclic ring, the homolactone or the homolactam is optionally substituted with one or more fluoro or hydroxy;

- 10 X₁ is O or N; or X₁ is fluoro, provided that R₁ is null;



15

R_3 is selected from the group consisting of methyl, methoxy, $R_a\text{COOH}$,



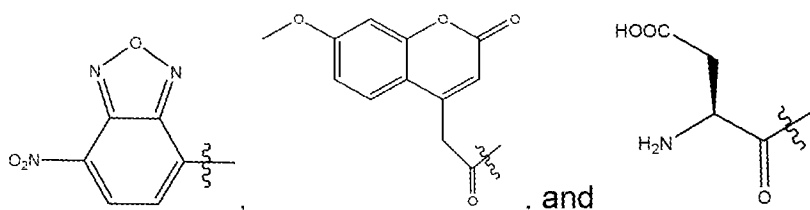
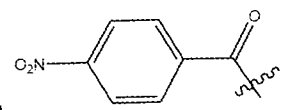
R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring;

R_a and R_b are independently H or C_{1-6} alkyl;

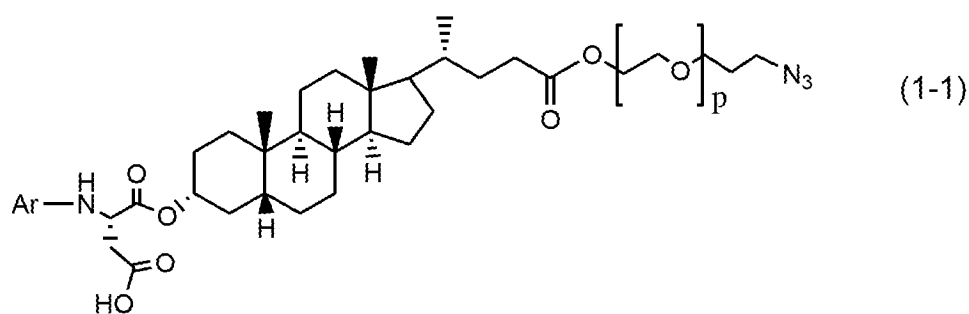
R_2 is COOH , $\text{CH}(\text{COOH})\text{COOH}$, or $\text{COX}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{N}_3$,

X_2 is O or N, and m is an integral from 1 to 4; and

Ar is selected from the group consisting of, H,



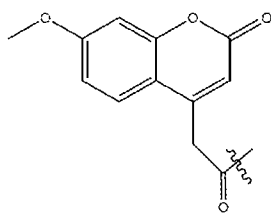
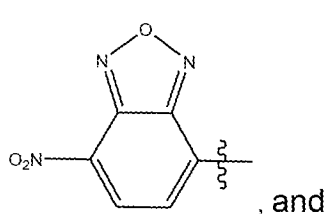
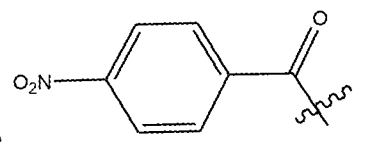
2. The compound of claim 1, wherein the compound has the structure of formula (1-1)



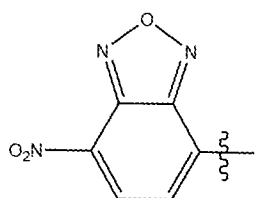
wherein,

p is an integral from 1 to 4; and

Ar is selected from the group consisting of,

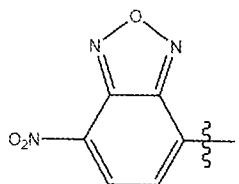


3. The compound of claim 2, wherein in formula (1-1), p is 2, and Ar is



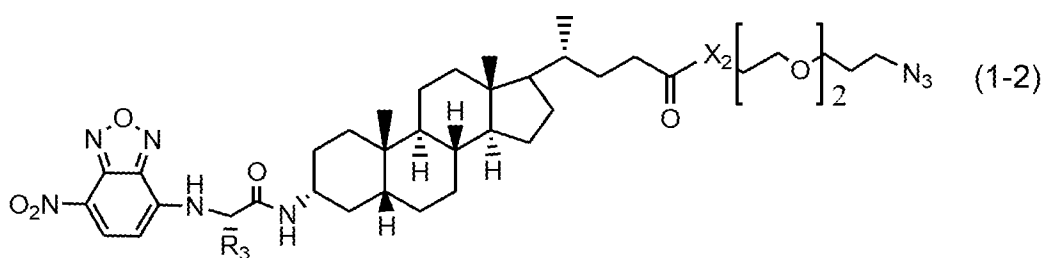
5

4. The compound of claim 2, wherein in formula (1-1), p is 3, and Ar is



10

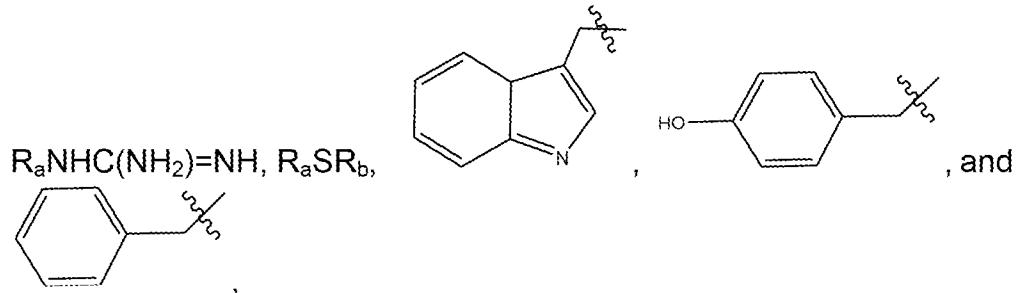
5. The compound of claim 1, wherein the compound has the structure of formula (1-2)



wherein,

X_2 is O or N; and

R_3 is selected from the group consisting of CH_3 , R_aCOOH , R_aNH_2 ,



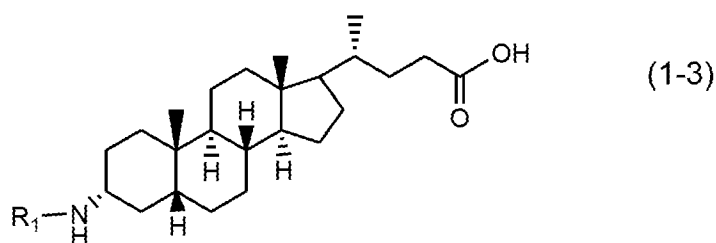
or R_3 and the neighboring carbon and nitrogen atoms are taken together to form a 5-membered heterocyclic ring;

R_a and R_b are independently H or C_{1-6} alkyl.

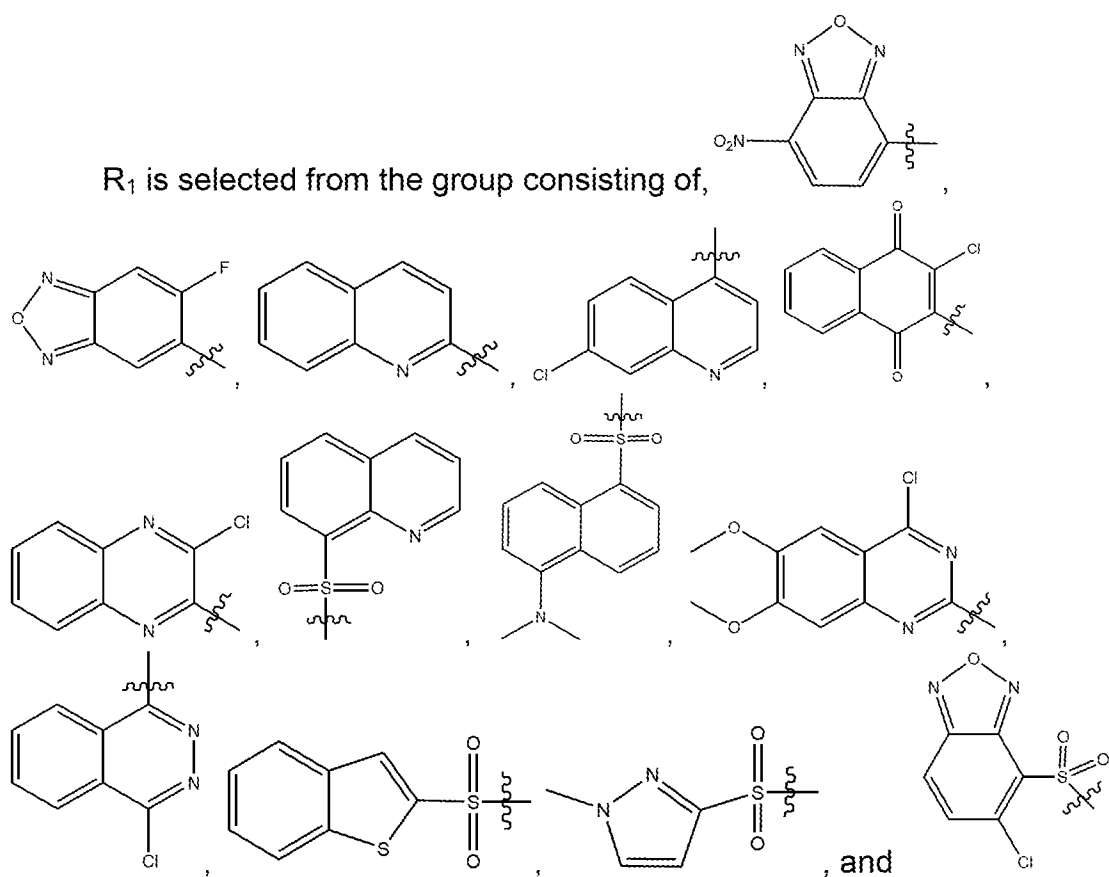
6. The compound of claim 5, wherein in formula (1-2), X_2 is O, R_3 is R_aCOOH , and R_a is H.

7. The compound of claim 5, wherein in formula (1-2), X_2 is N, R_3 is R_aCOOH , and R_a is H.

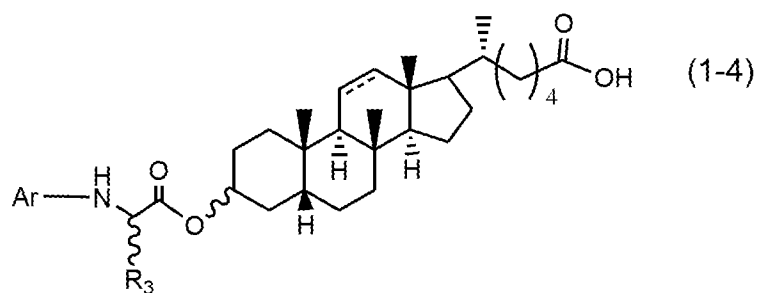
8. The compound of claim 1, wherein the compound has the structure of formula (1-3)



wherein,



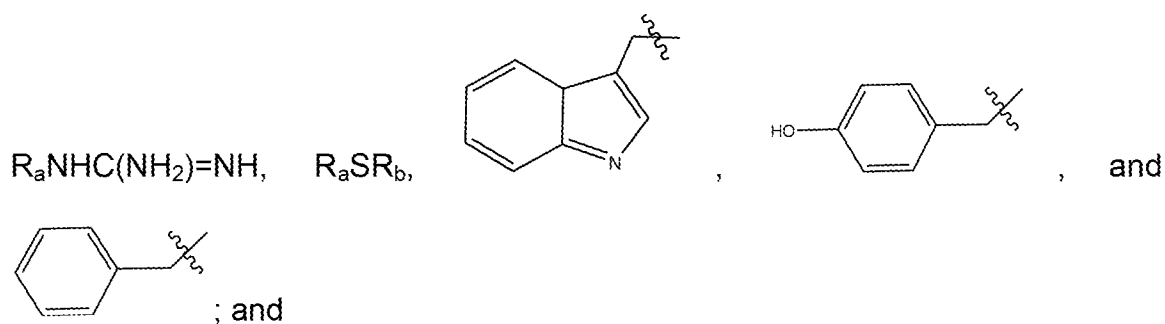
9. The compound of claim 1, wherein the compound has the structure of formula (1-4)

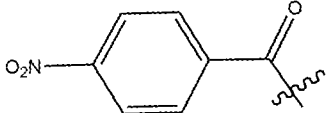


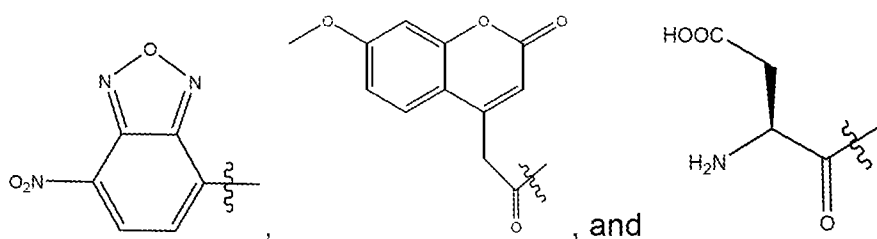
wherein,

----- is a single or a double bond;

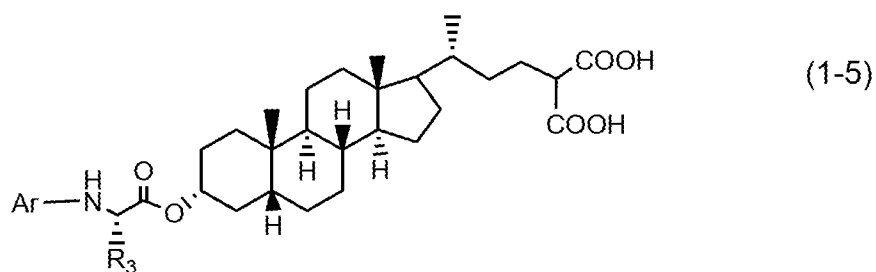
R_3 is selected from the group consisting of, CH_2OH , R_aCOOH , R_aNH_2 ,



Ar is selected from the group consisting of H , ,

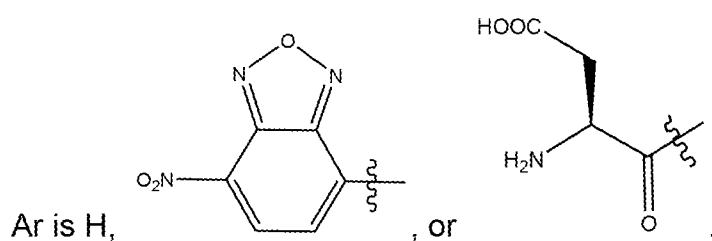


10. The compound of claim 2, wherein the compound has the structure of formula (1-5)

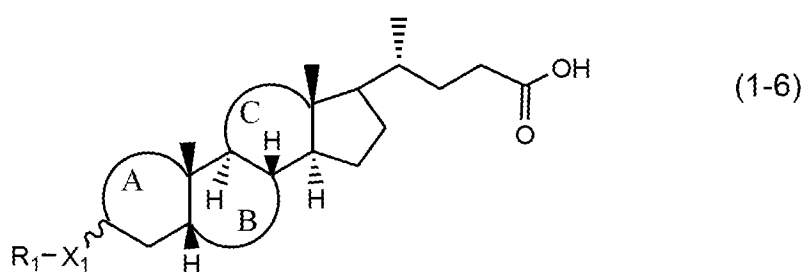


wherein,

R_3 is CH_2COOH , or CH_2CH_2COOH ; and



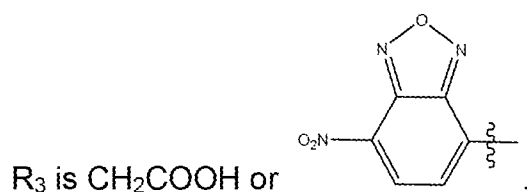
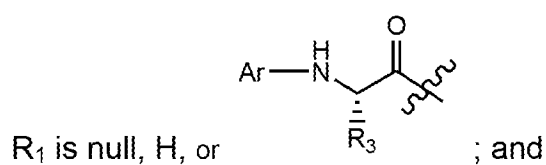
11. The compound of claim 1, wherein the compound has the structure of formula (1-6),



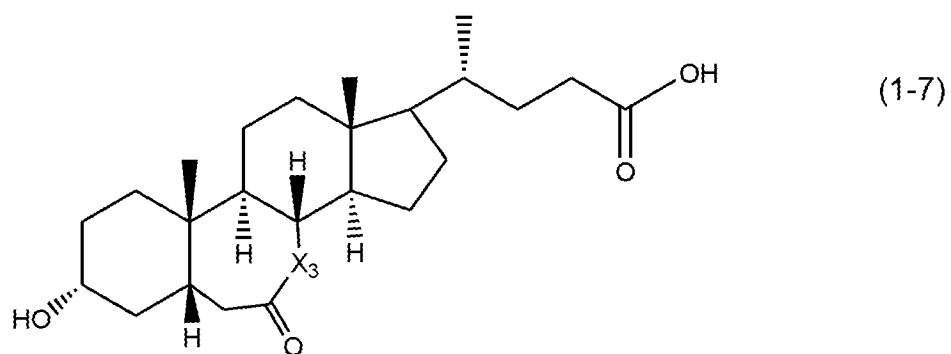
wherein,

A, B, and C are independently a 6-membered saturated carbon cyclic ring substituted with at least one fluoro;

X_1 is H, O or fluoro, and in the case when X_1 is fluoro, then R_1 is null;



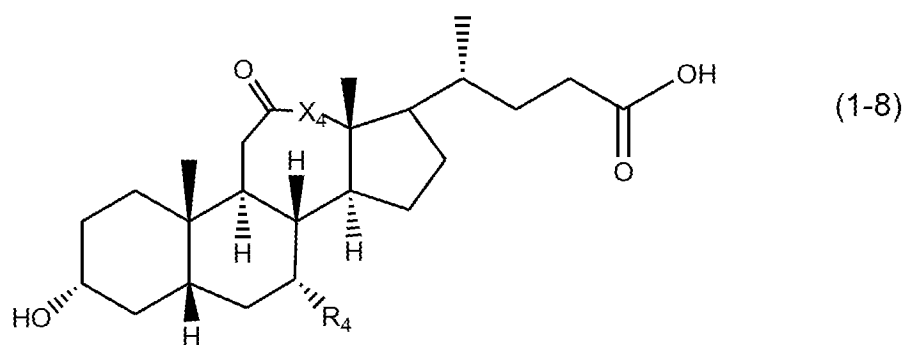
12. The compound of claim 1, wherein the compound has the structure of formula (1-7)



Wherein,

X_3 is O or N.

- 5 13. The compound of claim 1, wherein the compound has the structure of formula (1-8)

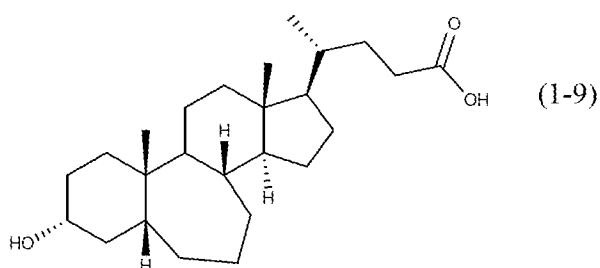


Wherein,

X_4 is O or N; and

10 R_4 is H or OH.

14. The compound of claim 1, wherein the compound has the structure of formula (1-9)



15. The compound of claim 1, wherein the compound of formula (1-1) to (1-3) is an inhibitor of α 2,3(N)-sialyltransferase ST3GALIII or α 2,6(N)-sialyltransferases ST6GALI, but not α 2,3(O)-sialyltransferase ST3GALI.

16. A pharmaceutical composition comprising an effective amount of a compound of claims 1 to 15, a salt or a solvate thereof; and a pharmaceutically acceptable excipient.

17. A method of treating a disease associated with the activation of sialyltransferase of a subject, comprising administering to the subject the pharmaceutical composition of claim 16, so as to alleviate or ameliorate the symptoms of the disease.

18. The method of claim 17, wherein the disease is a cancer, an inflammatory disease or an immune disease.

19. The method of claim 18, wherein the cancer is metastatic.

20. The method of claim 18, wherein the cancer is any of breast cancer, cervical cancer, ovary cancer, endometrial cancer, melanoma, uveal melanoma, brain tumor, lung cancer, liver cancer, lymphoma, neuroepithelioma, kidney cancer, bladder cancer, pancreatic cancer, prostate cancer, stomach cancer, colon cancer, uterus cancer, hematopoietic tumors of lymphoid lineage, myeloid

leukemia, thyroid cancer, thyroid follicular cancer, myelodysplastic syndrome (MDS), tumor of mesenchymal origin, teratocarcinoma, neuroblastoma, glioma, glioblastoma, keratoacanthomas, anaplastic large cell lymphoma, esophageal squamous cell carcinoma, follicular dendritic cell carcinoma, intestinal cancer, muscle invasive cancer, seminal vesicle tumor, epidermal carcinoma, spleen cancer, head and neck cancer, stomach cancer, bone cancer, cancer of retina, biliary cancer, small bowel cancer, salivary gland cancer, uterine sarcoma, cancer of testicles, cancer of connective tissue, myelodysplasia, Waldenstrom's macroglobulinemia, nasopharyngeal, neuroendocrine cancer, mesothelioma, angiosarcoma, Kaposi's sarcoma, oesophagogastric, fallopian tube cancer, peritoneal cancer, papillary serous mullerian cancer, malignant ascites, gastrointestinal stromal tumor (GIST), Li-Fraumeni syndrome or Von Hippel-Lindau syndrome (VHL).

21. The method of claim 20, wherein the hematopoietic tumors of lymphoid lineage is any of leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, B-cell lymphoma, Burkitt's lymphoma, multiple myeloma, Hodgkin's lymphoma, or Non-Hodgkin's lymphoma.

22. The method of claim 20, wherein the tumor of mesenchymal origin is fibrosarcomas or rhabdomyosarcomas.

23. The method of claim 18, wherein the immune disease is any of rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, myasthenia gravis, Type I diabetes mellitus, glomerulonephritis, autoimmune thyroiditis, ankylosing spondylitis, systemic sclerosis, multiple sclerosis, systemic anaphylaxis, drug allergies, insect sting allergies, graft rejection, Sjogren's syndrome, or human immunodeficiency virus infection.

24. The method of claim 18, wherein the inflammatory disease is any of diabetic retinopathy, proliferative retinopathy, dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, necrotizing vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, eosinophilic myositis, polymyositis, 5 dermatomyositis, eosinophilic fasciitis, Crohn's disease, ulcerative colitis, hypersensitivity pneumonitis, eosinophilic pneumonia, delayed-type hypersensitivity, interstitial lung disease (ILD), idiopathic pulmonary fibrosis, and ILD associated with rheumatoid arthritis, macular edema, asthma, or allergic rhinitis.

10

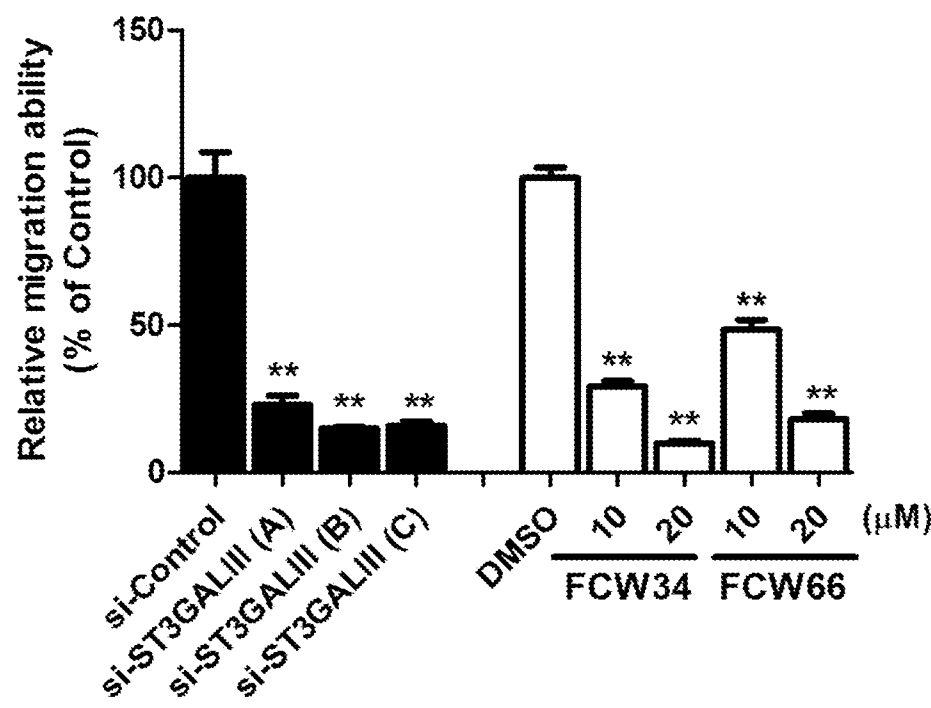
25. The method of claim 17, wherein the pharmaceutical composition comprises the compound of claim 3.

26. The method of claim 17, wherein the pharmaceutical composition 15 comprises the compound of claim 4.

27. The method of claim 17, wherein the pharmaceutical composition comprises the compound of claim 6.

20 28. The method of claim 17, wherein the pharmaceutical composition comprises the compound of claim 7.

FIG 1



2/13

FIG 2A

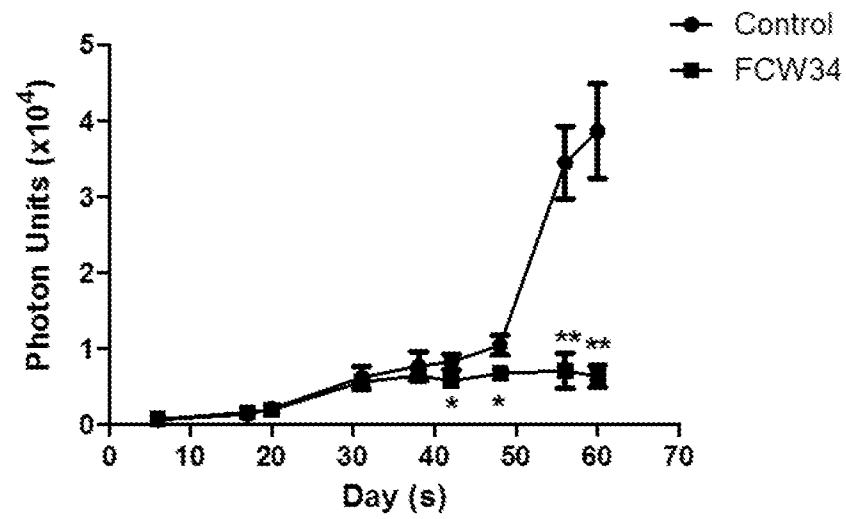


FIG 2B

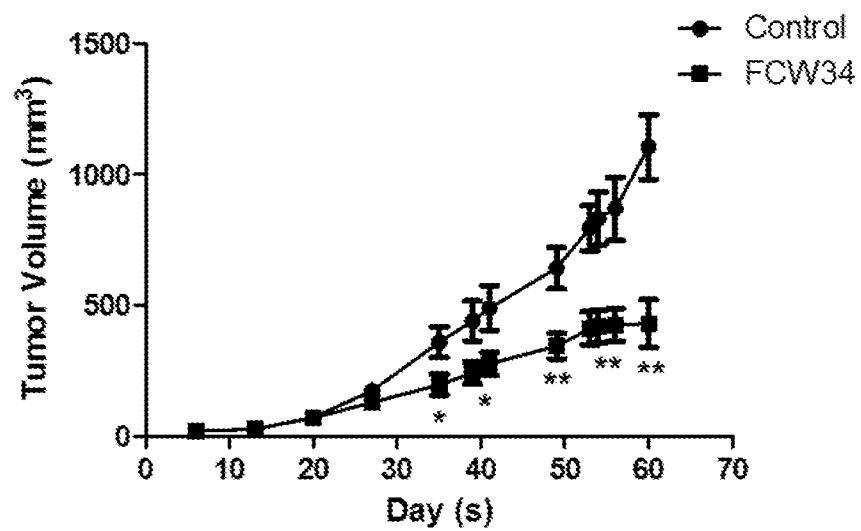


FIG 3

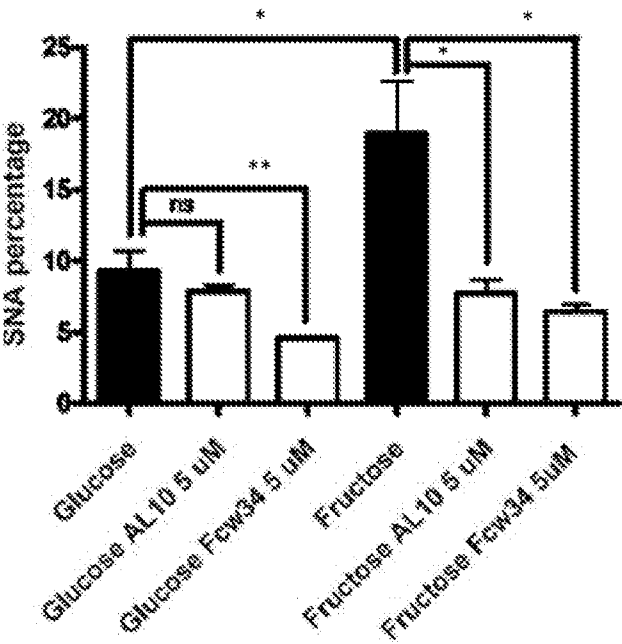


FIG 4

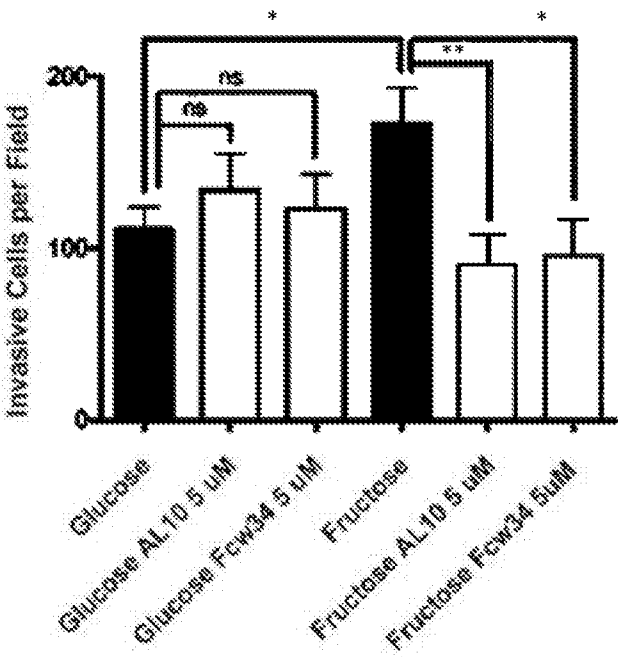


FIG 5

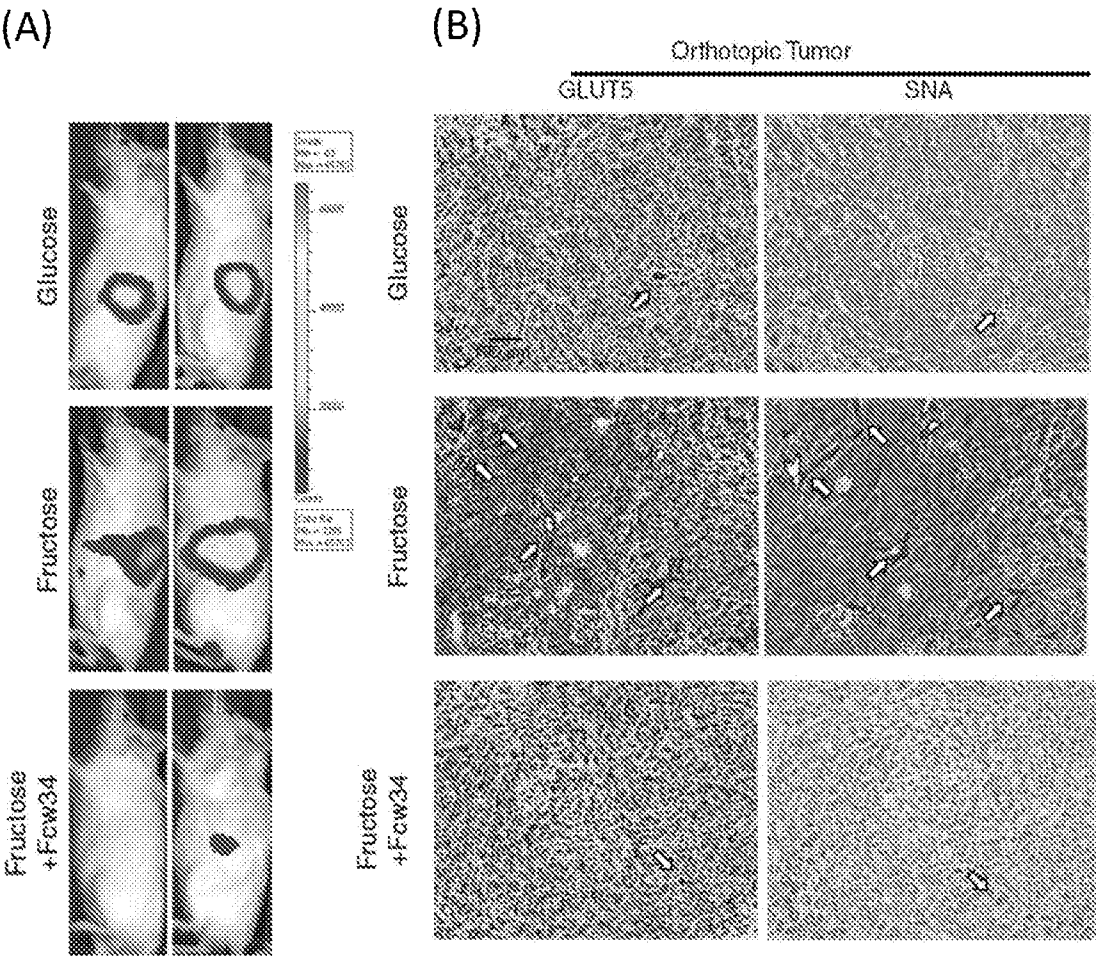


FIG 6

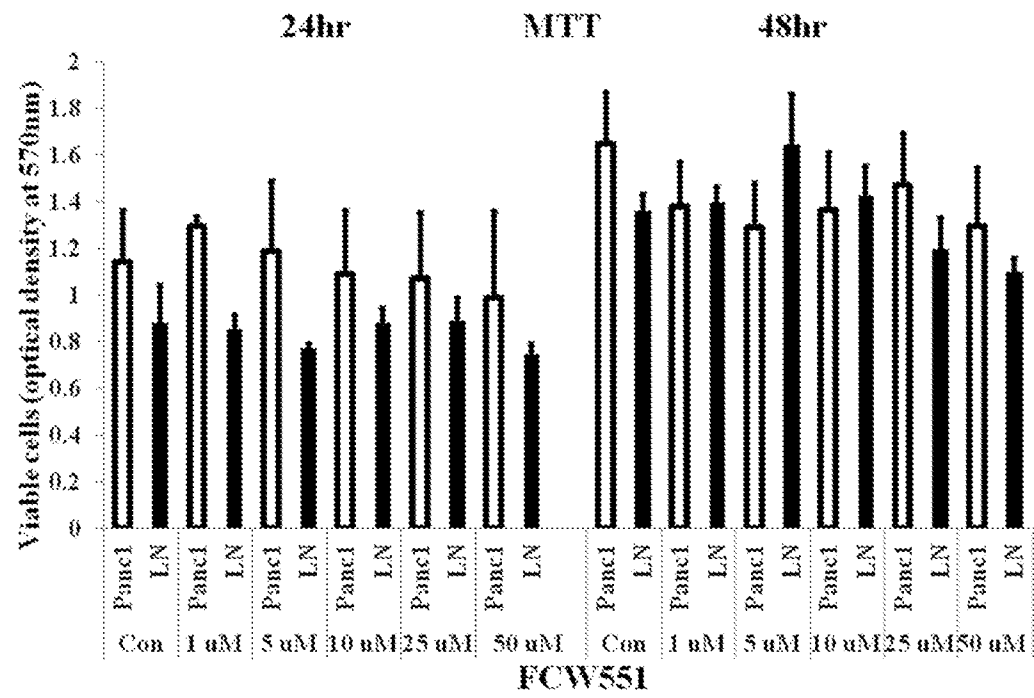
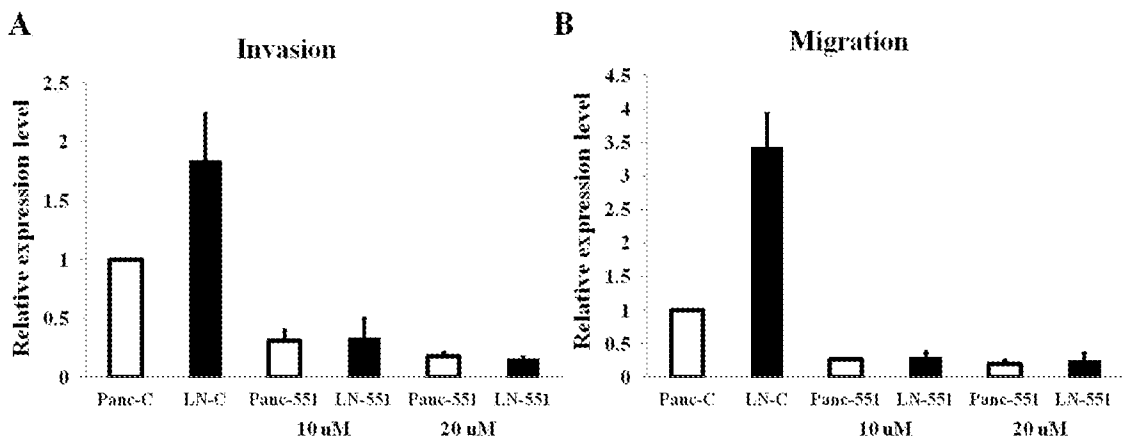


FIG 7



6/13

FIG 8A

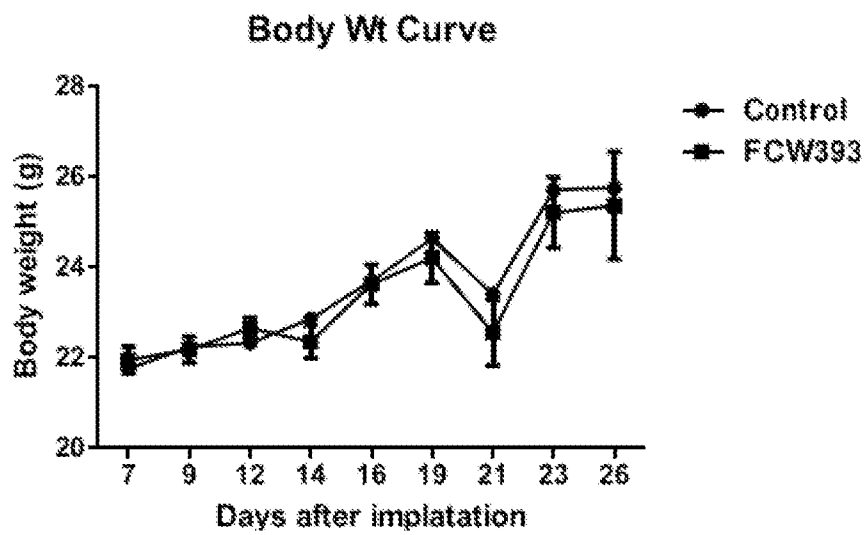
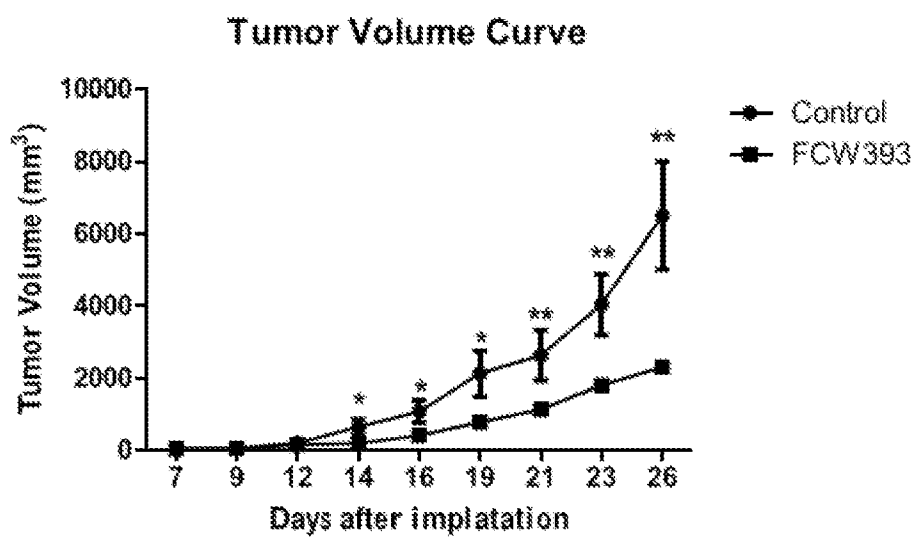
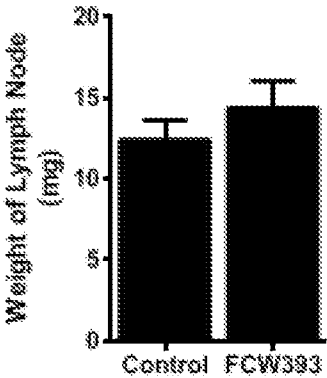


FIG 8B

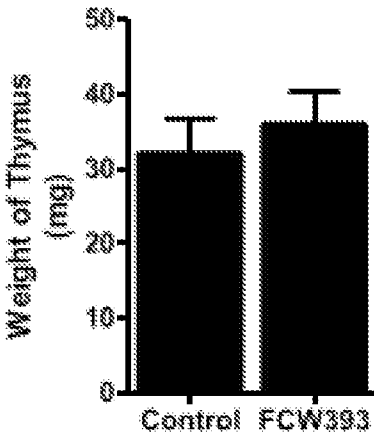


7/13

FIG 9
(A)



(B)



(C)

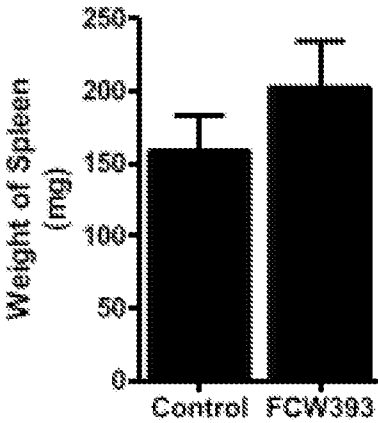


FIG 10

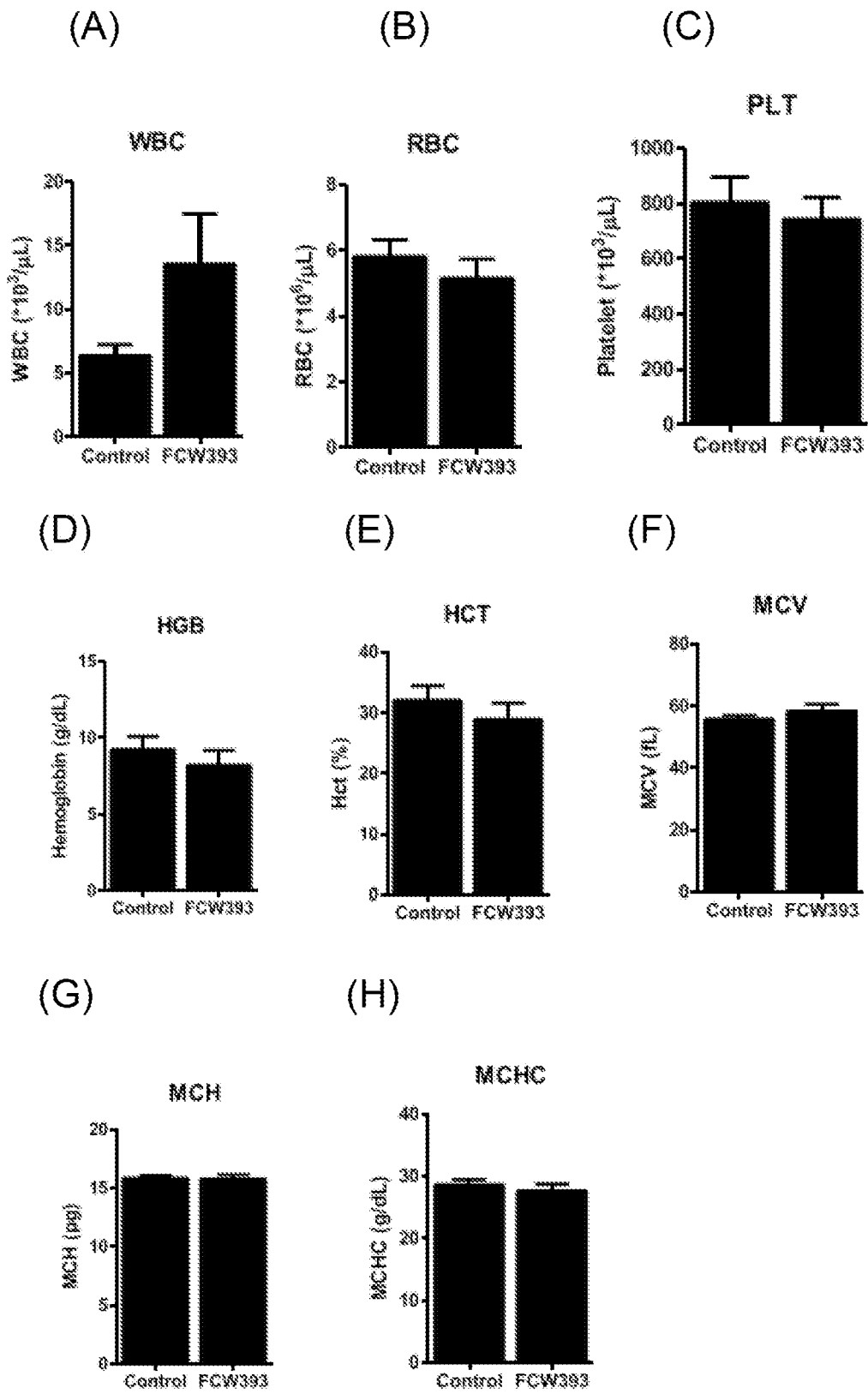


FIG 11

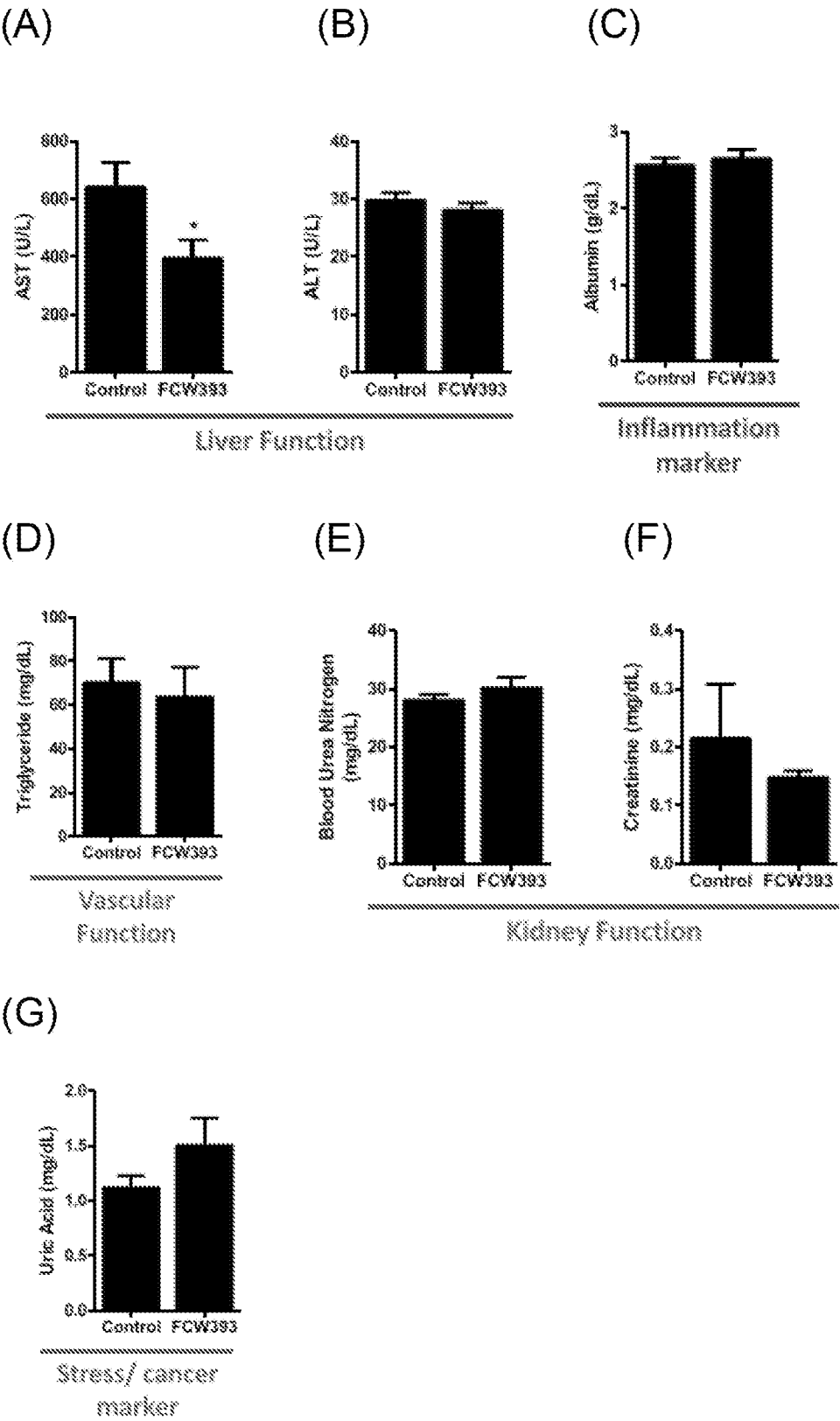
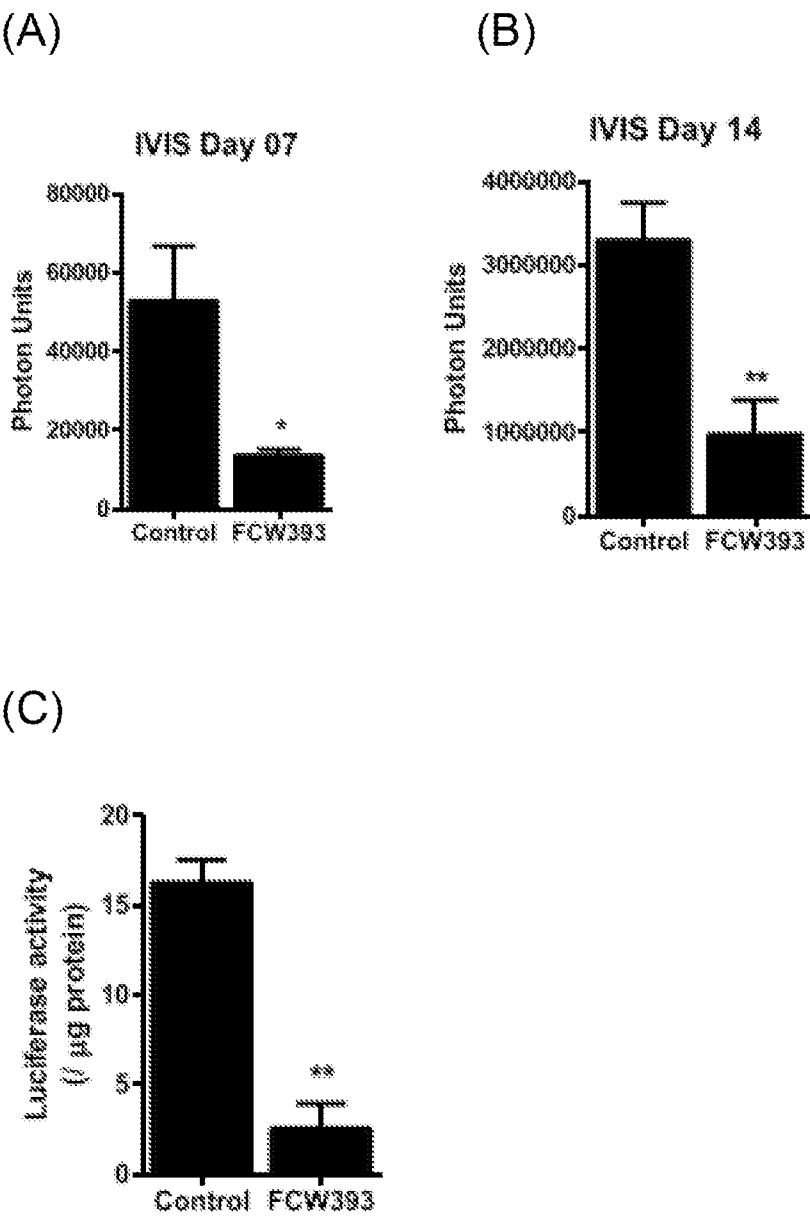


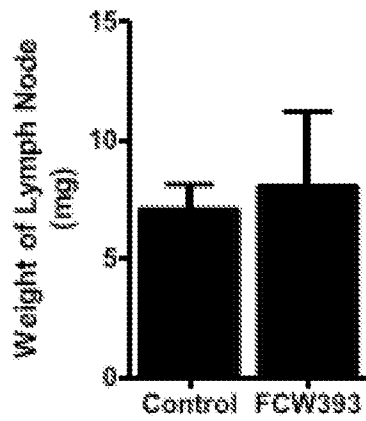
FIG 12



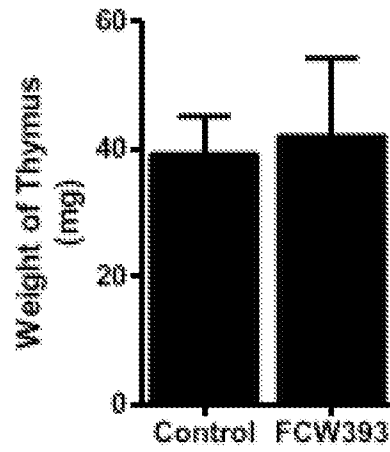
11/13

FIG 13

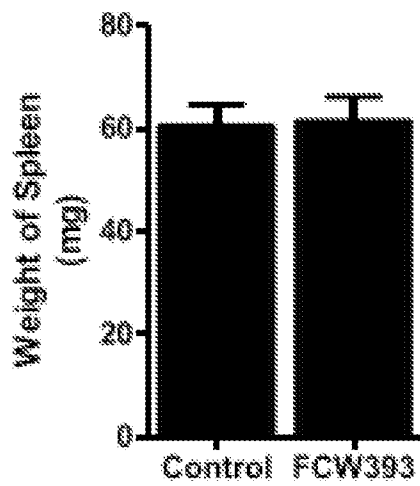
(A)



(B)



(C)



12/13

FIG 14

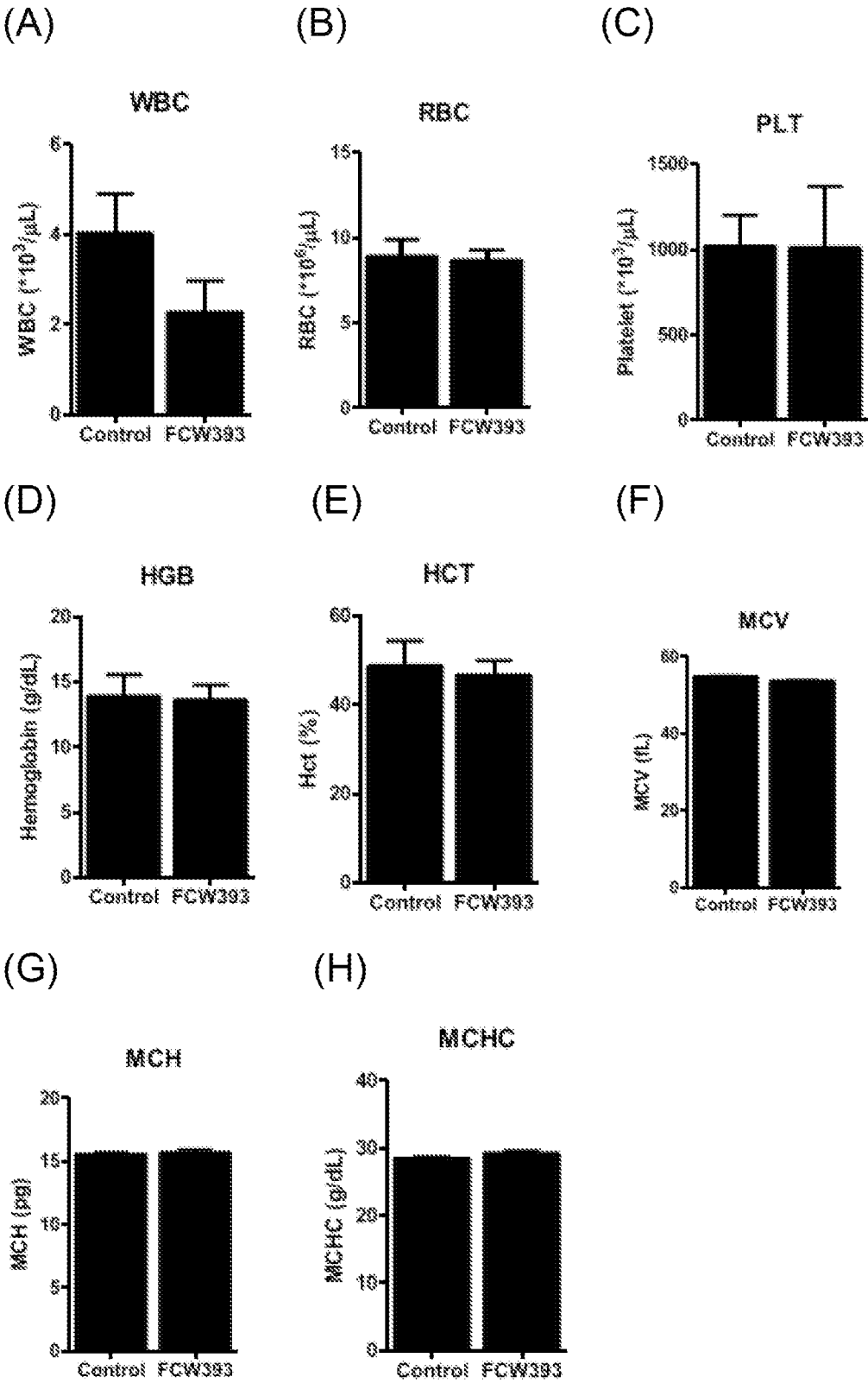
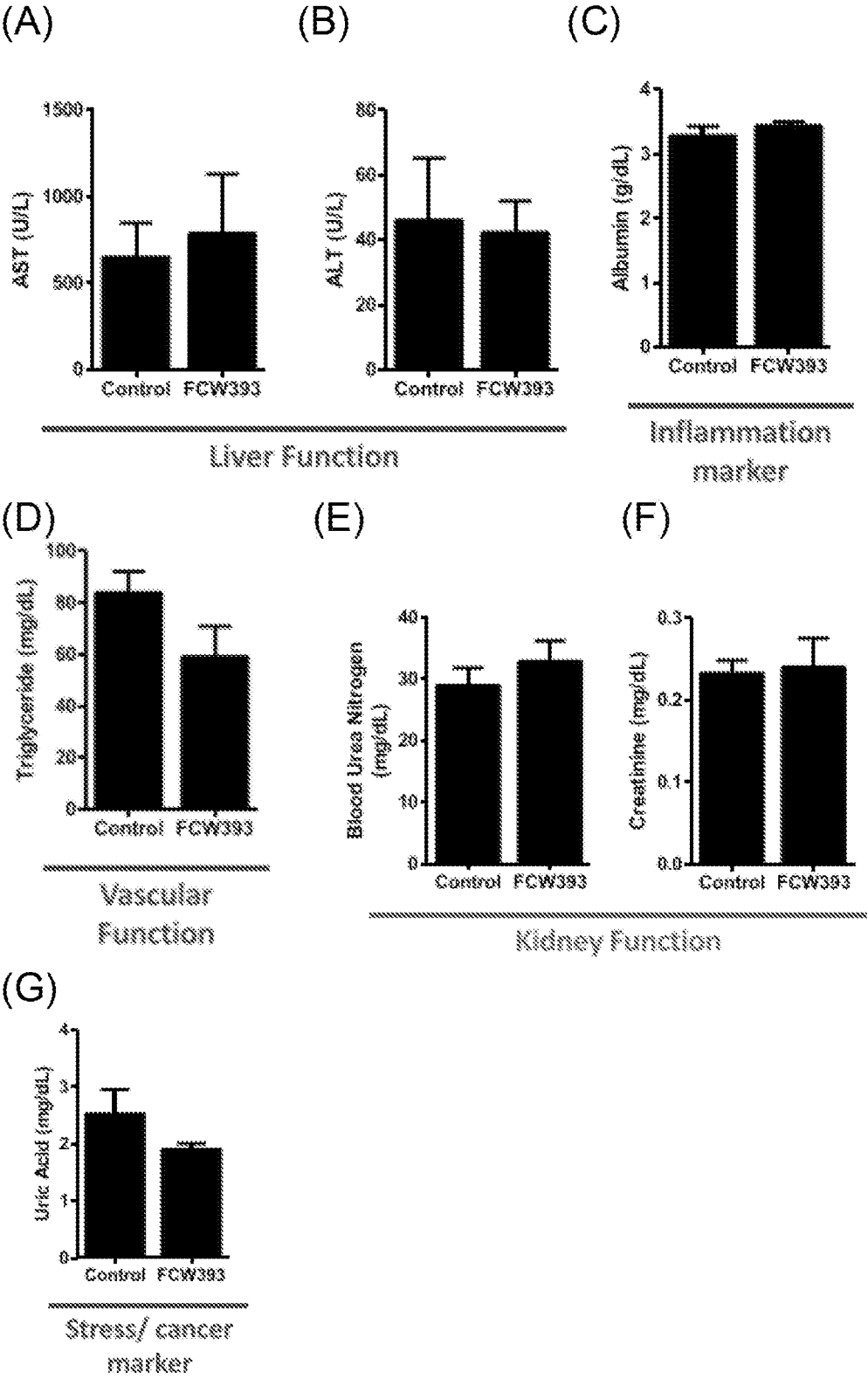


FIG 15



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/049084

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C07J 9/00; A61P 35/00; C07J 11/00; C07J 15/00; C12N 9/10 (2016.01) CPC - C07J 9/00; C07J 9/005; C07J 11/00; C07J 15/00; C12N 9/10 (2016.11) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC - A61P 35/00; C07J 9/00; C07J 11/00; C07J 15/00; C12N 9/10 CPC - C07J 9/00; C07J 9/005; C07J 11/00; C07J 15/00; C12N 9/10 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 514/169; 514/182; 552/540; IPC - A61P 35/00; C07J 9/00; C07J 11/00; C07J 15/00; C12N 9/10; CPC - C07J 9/00; C07J 9/005; C07J 11/00; C07J 15/00; C12N 9/10 (keyword delimited) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Orbit, Google Patents, Google, PubChem, SureChem, STN Search terms used: sialyltransferase inhibitor, sialylglycoside, cyclopentaphenanthrene, fluoro		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,439,899 A (CUSHMAN et al) 08 August 1995 (08.08.1995) entire document	1, 15-24
A	US 2011/0311651 A1 (DJABALLAH et al) 22 December 2011 (22.12.2011) entire document	1, 15-24
A	US 2013/0183250 A1 (FOAMIX LTD) 18 July 2013 (18.07.2013) entire document	1, 15-24
A	US 2009/0181854 A1 (THORSON et al) 16 July 2009 (16.07.2009) entire document	1, 15-24
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 06 December 2016		Date of mailing of the international search report 05 JAN 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300		Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/049084

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 25-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

Claims 1 and 15-24 have been analyzed subject to the restriction that the claims read on a compound having the structure of formula (1) and pharmaceutically acceptable salts and solvates thereof, wherein n is 1; A, B, and C are independently a 6-membered saturated carbon cyclic rings, in which the saturated carbon cyclic ring is unsubstituted; X1 is fluoro; R1 is null; R2 is COOH; pharmaceutical compositions thereof; and methods of treating a disease associated with the activation of sialyltransferase of a subject.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 15-24

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-24 are drawn to compounds having the structure of formula (1), and pharmaceutically acceptable salts and solvates thereof, pharmaceutical compositions thereof, and methods of treating a disease associated with the activation of sialyltransferase of a subject.

The first invention of Group I+ is restricted based on the proviso X1 is fluoro, provided that R1 is null; and is restricted to a compound having the structure of formula (1) and pharmaceutically acceptable salts and solvates thereof, wherein n is 1; A, B, and C are independently a 6-membered saturated carbon cyclic rings, in which the saturated carbon cyclic ring is unsubstituted; X1 is fluoro; R1 is null; R2 is COOH; pharmaceutical compositions thereof; and methods of treating a disease associated with the activation of sialyltransferase of a subject. It is believed that claims 1 and 15-24 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a compound having the structure of formula (1) and pharmaceutically acceptable salts and solvates thereof, wherein n is 4; A, B, and C are independently a 7-membered saturated carbon cyclic rings, in which the saturated carbon cyclic ring is unsubstituted; X1 is N; R1 is the second shown structure; R2 is CH(COOH)COOH; pharmaceutical compositions thereof; and methods of treating a disease associated with the activation of sialyltransferase of a subject. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables A, B, C, X1, R1, n, and R2.

The Groups I+ share the technical features of a compound having the core structure of formula (1), and pharmaceutically acceptable salts and solvates thereof; a pharmaceutical composition comprising an effective amount of a compound, a salt, or a solvate thereof; and a pharmaceutically acceptable excipient; and a method of treating a disease associated with the activation of sialyltransferase of a subject, comprising administering to the subject the pharmaceutical composition so as to alleviate or ameliorate the symptoms of the disease. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 5,439,899 A to Cushman et al. teach a compound having the core structure of formula (1) (Cols. 8 and 9, Compound X; ...see shown structure...); a pharmaceutical composition (Claims 8 and 9) comprising an effective amount of a compound, a salt, or a solvate thereof (Col. 4, Lns 5-22); and a pharmaceutically acceptable excipient (Col. 4, Lns 5-22); and a method of treating a disease associated with the activation of sialyltransferase of a subject (Col. 3, Ln. 57 through Col. 4, Ln. 4; Col. 1, Lns. 10-17; See Para. [0080] of the Applicant's Specification where the disease associated with the activation of sialyltransferase can be human immunodeficiency virus.), comprising administering to the subject the pharmaceutical composition (Col. 3, Ln. 57 through Col. 4, Ln. 22) so as to alleviate or ameliorate the symptoms of the disease (Col. 3, Ln. 57 through Col. 4, Ln. 4,...a method of preventing an animal, particularly, a human, from being infected with a human immunodeficiency virus, comprising administering a quantity of a compound as defined in Formulas I, II or III, sufficient to be prophylactically effective in preventing such an infection.)

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.