PROCESS FOR THE SYNTHESIS OF ARCHAEOL-BASED SULFATED CARBOHYDRATES

RELATED APPLICATION

The present application claims priority to U.S. provisional application No. 63/561,497 filed on March 5, 2024, the content of which is incorporated herein by reference in its entirety.

FIELD

The present description relates to a process for the synthesis of archaeol-based sulfated carbohydrates from Archaeol, and more particularly from the pure R diastereoisomer of Archaeol.

The present description refers to a plurality of documents, the contents of which are herein incorporated by reference in their entirety.

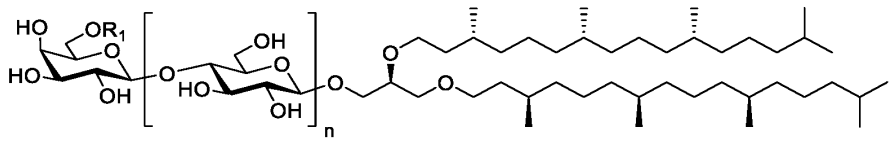
BACKGROUND

Adjuvants are vital components of modern vaccines, enhancing immune responses to disease-associated antigens and enabling protection against multiple pathogens. Archaeal lipid adjuvants formulated into liposome-like archaeosomes are a promising new class of adjuvants that have been shown to induce potent immune responses to a broad range of antigens, yet their complexity, heterogeneity, and relative high cost of manufacture as compared to other approved adjuvants, have represented significant obstacles to their widespread commercialization.

Traditional archaeosomal formulations consisted of liposomes formed from a mixture of total polar lipids (TPL) derived from various archaea species, and simpler semi-synthetic archaeosome formulations have since been developed, such as those based on sulfated S-lactosylarchaeols (SLAs). While these semi-synthetic archaeosome formulations have addressed some of the original drawbacks of TPL-based formulations, issues such as a reliance on microorganism fermentation, stereochemical heterogeneity, and high cost of manufacture per dose, remain significant obstacles to their widespread adoption in vaccines, as well as their accessibility on a global scale. Thus, a completely synthetic process for the manufacture of archaeosomal adjuvants of the type archaeal sulfated glycolipids, at reduced cost and with greater stereochemical homogeneity, would be highly desirable.

SUMMARY

In a first aspect, described herein is a process for synthesizing a sulfated glycoarchaeal of Formula (I)

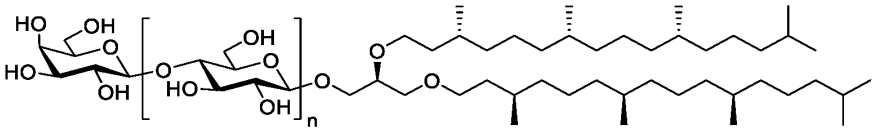


(I)

where R_1 is SO_3M with M being an alkali metal, and n is 0 or 1;

comprising:

reacting a compound of Formula (A)



(A)

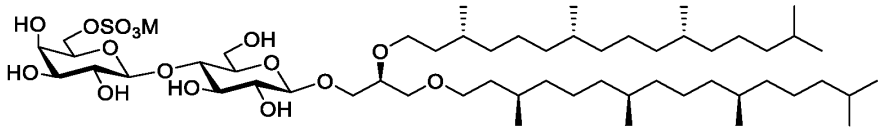
with a sulfur trioxide reagent to form an intermediate compound;

salification of the intermediate compound to form the compound of Formula (I).

In some embodiments, the sulfur trioxide reagent is a sulfur trioxide amine complex.

In some embodiments, the compound of Formula (A) can be prepared according to the process described herein.

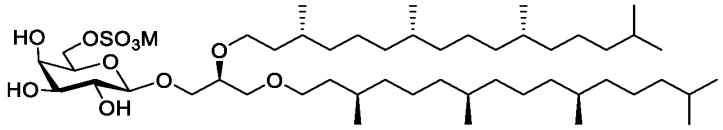
In some embodiments, n is equal to 1 and the sulfated glycoarchaeal has the following formula (II)



(II) .

In some embodiments, n is equal to 1 and the sulfur trioxide reagent is used in an amount providing about 1 molar equivalent of sulfur trioxide.

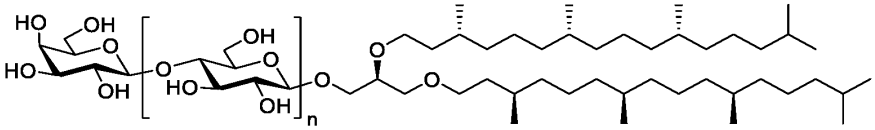
In some embodiments, n is 0 and the sulfated glycoarchaeal has the following formula (III)



(III) .

In some embodiments, n is equal to 0 and the sulfur trioxide reagent is used in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide.

In another aspect, described herein is a process for preparing a compound of Formula (A)

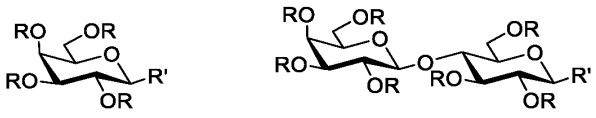


(A)

where n is 0 or 1;

comprising:

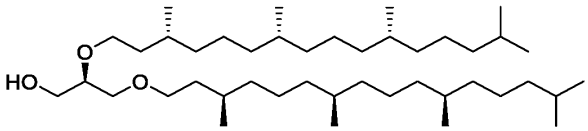
reacting a compound of Formula (B_0) when n is 0 or a compound of Formula (B_1) when n is 1



(B_0) (B_1)

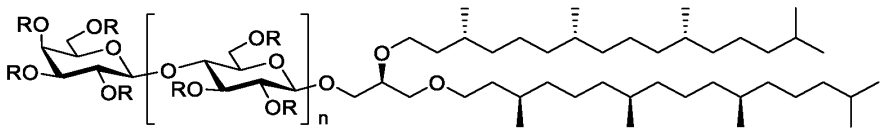
where R is a protecting group and R' is a leaving group

with a compound of Formula (C)



(C)

to form a compound of Formula (D)

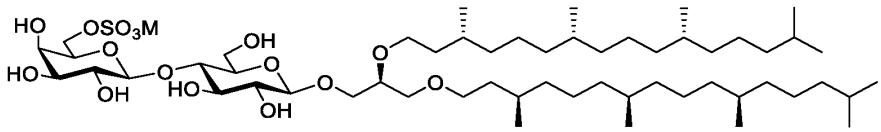


(D)

and

deprotecting the compound of Formula (D) to form the compound of Formula (A).

In yet another aspect, described herein is a process for synthesizing a sulfated glycoarchaeal of Formula (II)

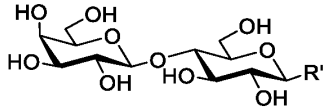


(II)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$,

comprising either:

i) silylating the -OH in position 6' of the lactopyranoside part of the compound of Formula (E)



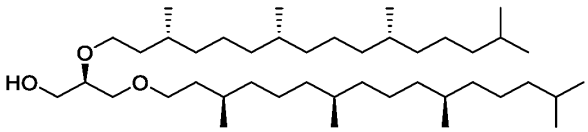
(E)

where R' is SEt, SPh or STol, SePh, S(O)Ph, or $S(O)_2Ph$;

to form a 6'-silylated compound (E1);

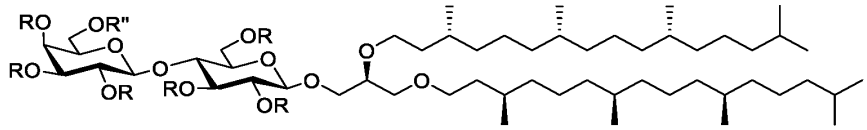
protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2);

reacting the 6'-silylated protected compound (E2) with a compound of Formula (C)



(C)

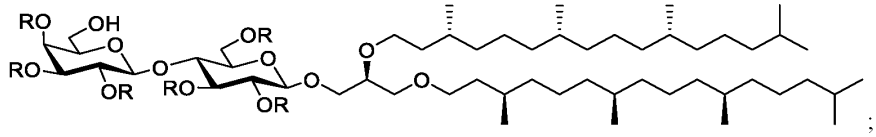
to form a compound of Formula (F)



(F)

where R is a protecting group and R" is a silyl group;

desilylating the compound of Formula (F) to form a compound of Formula (G)



;

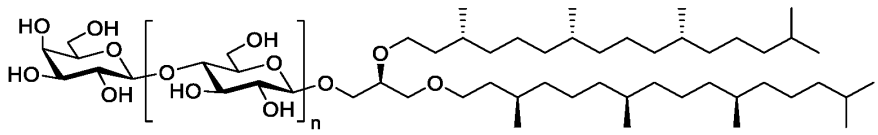
(G)

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with a sulfur trioxide reagent to form an intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

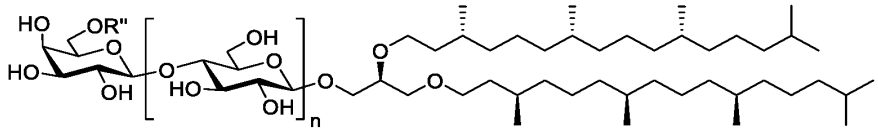
ii) silylating the -OH in position 6' of the compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A1)



(A1)

where n is 1 and R" is as defined above;

protecting the hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F);

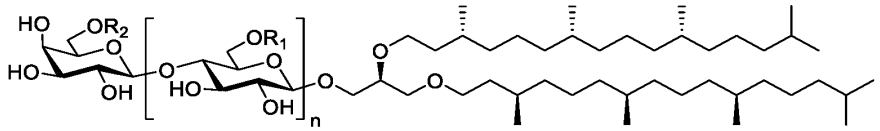
desilylating the compound of Formula (F) to form the compound of Formula (G);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with the sulfur trioxide reagent to form the intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

iii) silylating the compound of Formula (A) with a silylating agent to form a mixture of compounds of Formula (A2), (A3) and (A4)



(A2): n =1; R_1 = H and R_2 = R"

(A3): n = 1; R_1 = R" and R_2 = H

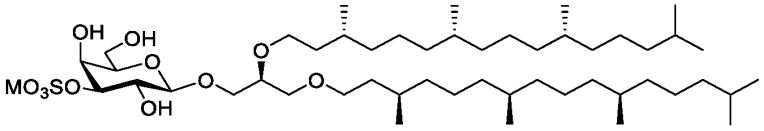
(A4): n = 1; R_1 = R" and R_2 = R";

separating compounds of Formula (A2), (A3) and (A4) to recover compound of Formula (A3);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (A3) with the sulfur trioxide reagent to form a 6'-sulfated 6-silylated compound (A5); and

desilylating the sulfated compound (A5) to form the compound of Formula (II).

In another aspect there is provided a process for synthesizing a sulfated glycoarchaeal of Formula (IV)

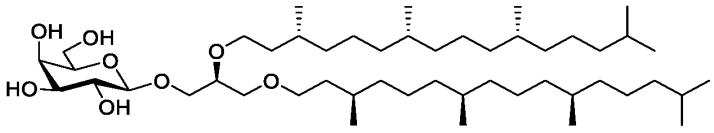


(IV)

where M is an alkali metal;

comprising:

reacting a compound of Formula (A0)



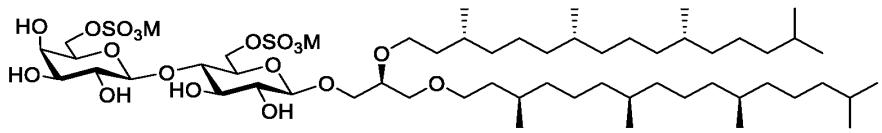
(A0)

with an organotin oxide or organotin dichloride compound to form a stannylene acetal on the galactopyranoside part of the compound (A0);

then reacting the stannylene acetal of the compound of Formula (A0) with a sulfur trioxide reagent to form an intermediate compound; and

salification of the intermediate compound to form the compound of Formula (IV).

In another aspect there is provided a process for synthesizing a disulfated glycoarchaeal of Formula (V)

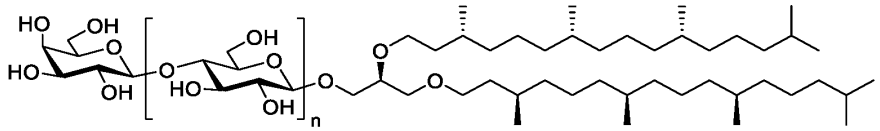


(V)

where M is an alkali metal;

the process comprising:

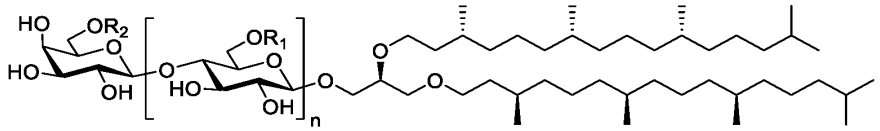
silylating the -OH in positions 6 and 6' of a compound of Formula (A)



(A)

where n is 1;

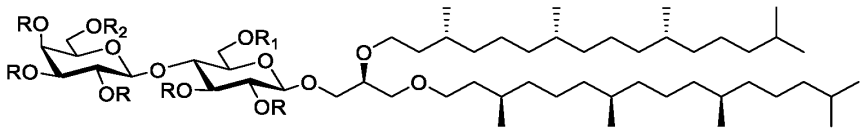
to form a 6- and 6'-silylated compound (A4)



(A4)

where n is 1 and R_1 and R_2 represent a silyl group;

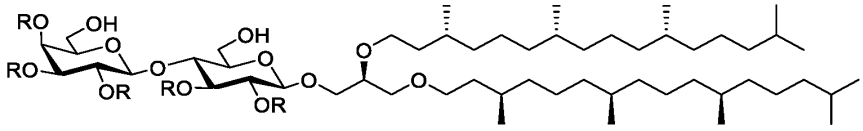
protecting the hydroxyl groups of the 6- and 6'-silylated compound (A4) to form the compound of Formula (F1)



(F1)

where R is a protecting group;

desilylating the compound of Formula (F1) to form the compound of Formula (G1);

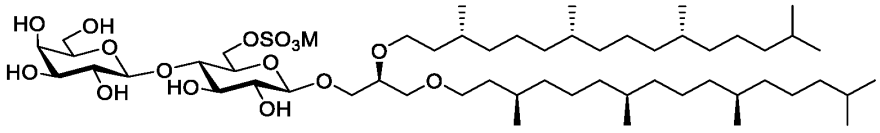


(G1)

sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) with a sulfur trioxide reagent to form an intermediate compound; and

deprotecting the intermediate compound to form the compound of Formula (V).

In another aspect there is provided a process for synthesizing a sulfated glycoarchaeal of Formula (VI)

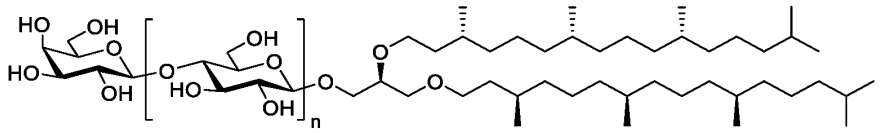


(VI)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$;

the process comprising:

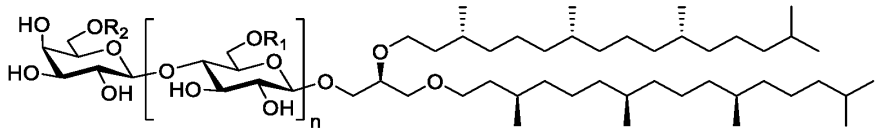
silylating the -OH in position 6' of a compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A2)



(A2)

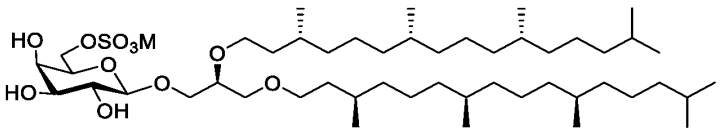
where n is 1, R_1 is H, and R_2 represents a silyl group;

sulfating the -OH in position 6 of the compound of Formula (A2) with a sulfur trioxide reagent to form a 6-sulfated 6'-silylated compound (A6);

desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$; and

optionally contacting the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$ with an alkali metal cation exchange resin to form the compound of Formula (VI) where M is alkali metal.

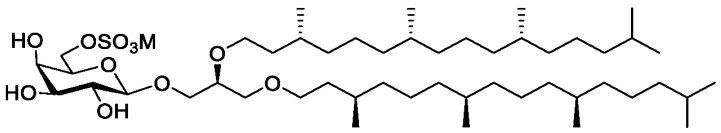
In another aspect there is provided a sulfated glycoarchaeal having the following formula (III)



(III)

wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$.

In yet another aspect there is provided a sulfated glycoarchaeal having the following formula (III)



(III)

wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$,

obtained by the process as described herein.

General Definitions

Headings, and other identifiers, e.g., (a), (b), (i), (ii), etc., are presented merely for ease of reading the specification and claims. The use of headings or other identifiers in the specification or claims does not

necessarily require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

The use of the word "**a**" or "**an**" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one" but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one".

The term "**about**" is used to indicate that a value includes the standard deviation of error for the device or method being employed in order to determine the value. In general, the terminology "about" is meant to designate a possible variation of up to 10%. Therefore, a variation of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% of a value is included in the term "about". Unless indicated otherwise, use of the term "about" before a range applies to both ends of the range.

As used in this specification and claim(s), the words "**comprising**" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The expression "**leaving group**" as used herein refers to a leaving group that provides improved reaction efficiency and/or specificity as compared to the corresponding functional group prior to replacement with the leaving group. In some embodiments, the leaving groups may be halogen (e.g., F, Cl, Br or I), OAc, SEt, SPh, STol, OTs, OMs, imidate such as trichloroacetimidate, SePh, S(O)Ph, or $S(O)_2Ph$.

The expression "**protecting group**" as used herein refers to a group that is introduced into a molecule by chemical modification of a functional group to generally obtain chemoselectivity in a subsequent chemical reaction. In some embodiments, the protecting groups may be Ac or Bz.

The expressions "**sulfated glycoarchaeal**", "**sulfated archaeal carbohydrates**", "**archaeal sulfated carbohydrates**" and "**archaeol-based sulfated carbohydrates**" are used herein interchangeably and refer to the compounds prepared by the described process using pure diastereoisomer R of Archaeol as also described herein.

The expressions "**providing X molar equivalent**" or "**to provide X molar equivalent**" of sulfur trioxide, mean that the amount of sulfur trioxide reagent to be used is calculated for providing to the reaction mixture X molar equivalent of sulfur trioxide SO_3 based on the molar content of the reactant which is sulfated. Indeed, in some embodiments, the sulfur trioxide reagent which can be a commercial product, is not pure and is characterized by its percentage of "active" SO_3. This should be considered in determining the amount of sulfur trioxide reagent to be used in the sulfation step.

Other objects, advantages and features of the present description will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents the dose response curve of the production of TNF-α by RAW264.7 cells to Compound **8** synthesized according to the present process.

Figure 2 represents a comparison of the production of TNF-α by RAW264.7 cells stimulated either by Compound **8** synthesized according to the present process or 6'-SLA (SLA-1) produced by a semi-synthetic process.

Figure 3 represents the results of the production of TNF-α from the dose-dependent stimulation of a murine macrophage cell line using 6'-SLA (Compound **8**) and variants thereof.

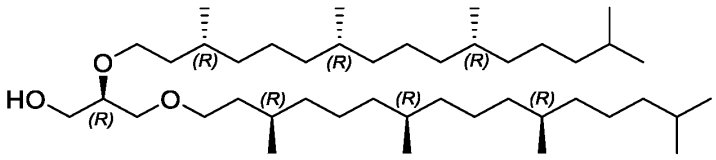
Figure 4 represents a comparison of the TF-specific antibody response in mice immunized with glycoprotein cross-reactive material 197 conjugated to the Thomsen-Friedenreich antigen (CRM197-TF), adjuvanted with either Compound **8** (SLA), SWE, Alum or PBS.

Figure 5 represents immunization test results on BALB/c mice immunized with CRM197-TF adjuvanted with SLA (Compound **8**) or PBS by intramuscular injection. (A) Total serum IgG2a and IgG1 concentration. (B) Splenocytes stimulation and production of INF-γ, TNF-α, IL-4, and IL-6.

Figure 6 represents immunization test results on BALB/c mice immunized with CRM197 adjuvanted with SLA (Compound **8**) or PBS by intramuscular injection. The graphs show the results of the proliferation of splenocytes labeled with CellTrace™ Violet that were re-stimulated with the indicated compounds. The dilution of CellTrace™ Violet in viable $CD19^{-}CD4^{-}CD8^{+}CD44+$, $CD19^{-}CD4^{+}CD8^{-}CD44+$ and $CD19^{+}CD4^{-}CD8^{-}CD44+$ cells was quantified by flow cytometry.

DETAILED DESCRIPTION

In some embodiments, the present description relates to a process for synthesizing sulfated archaeal carbohydrates from the "pure" diastereoisomer R of Archaeol, namely (2R)-3-(((3R,7R,11R)-3,7,11,15-tetramethylhexadecyl)oxy)-2-(((3R,7R,11R)-3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol, having the following structure:



Diastereoisomer R Archaeol

By "pure" diastereoisomer R of Archaeol, one means that the stereochemistry is R at all the stereochemical centers as identified in the above chemical structure. Another name of the diastereoisomer R of Archaeol is: (R)-2,3-bis(((3R, 7R, 11R)-3,7,11,15-tetramethylhexadecyl)-oxy)propan-1-ol.

In some embodiments, described herein is also a composition comprising a sulfated glycoarchaeal as described herein, and an antigen and/or an antigen-encoding polynucleotide. In some embodiments, the antigen may comprise a polypeptide antigen, glycoconjugate antigen (e.g., carbohydrate antigen covalently linked to a carrier), or an RNA molecule encoding a polypeptide antigen. In some embodiments, the antigen may be a carbohydrate antigen covalently linked to a carrier, wherein the carrier is, is from, or comprises: Tetanus Toxoid (TT), Diphtheria Toxoid (DT), cross-reacting material 197 (CRM197), Meningococcal Outer Membrane Protein Complex (OMPC), *H. Influenzae* Protein D (HiD), a virus-like particle (VLP), a

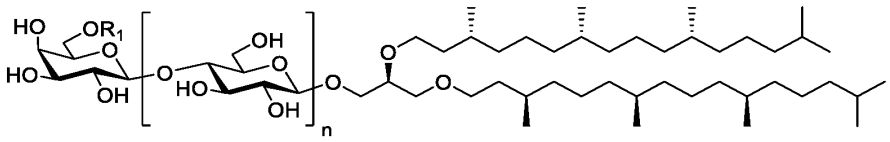
cytokine, albumin (such as bovine serum albumin or human serum albumin), keyhole limpet hemocyanin (KLH), or an immunogenic fragment thereof.

In some embodiments, there is provided the synthesis of sulfated archaeal carbohydrates (also referred to as "sulfated glycoarchaeals" herein) from the diastereoisomer R Archaeol described herein.

In some embodiments, there is thus provided the synthesis of sulfated archaeal carbohydrates comprising a diastereoisomer R Archaeol moiety as described herein.

Synthesis via "direct" sulfation

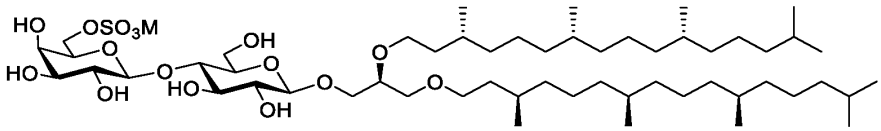
In some embodiments, there is thus provided a process for synthesizing a sulfated glycoarchaeal of Formula (I):



(I)

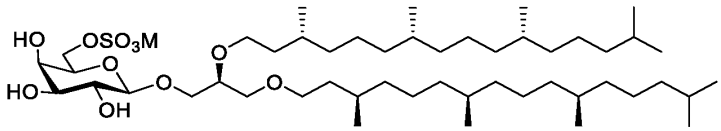
wherein R_1 is SO_3M with M being an alkali metal or $N(C_{1-4}alkyl)_4$, and n is 0 or 1.

In some embodiments, the sulfated glycoarchaeal of Formula (I) can be a disaccharide compound of Formula (II):



(II)

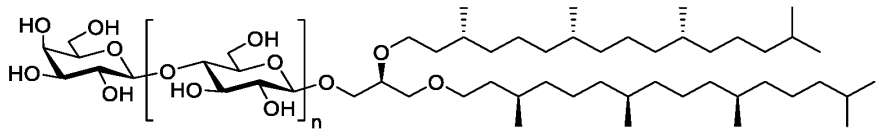
In some embodiments, the sulfated glycoarchaeal of Formula (I) can be a monosaccharide compound of Formula (III):



(III)

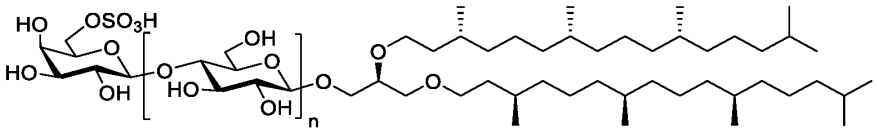
In some embodiments, the compounds of Formulae (I), (II), or (III) are such that M is Li, Na or K, preferably Na. In other embodiments, M can be an ammonium of formula $N(C_{1-4}alkyl)_4$, such as $N(Me)_4$, $N(Et)_4$, $N(Pr)_4$ or $N(Bu)_4$, preferably $N(Bu)_4$.

In some embodiments, a process of synthesizing the compound of Formula (I) can comprise reacting a compound of Formula (A)



(A)

where n is 0 or 1 with a sulfur trioxide reagent to form an intermediate compound and then performing a salification of the intermediate compound to form the compound of Formula (I). The intermediate compound can be a compound of formula (A_H)



(A_H)

which upon salification with an alkali metal reagent forms the corresponding alkali metal salt or ammonium salt of Formula (A). Hence, in some embodiments, the process for obtaining the sulfated glycoarchaeals of the present disclosure advantageously allows for direct sulfation of the hydroxylated glycoarchaeal without requiring a previous protection step of the hydroxyl groups on the sugar moiety.

In some embodiments, the sulfation of the compound of Formula (A) can be performed using a sulfur trioxide amine complex as the sulfur trioxide reagent. In some embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some preferred embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof. Preferably the sulfur trioxide reagent is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

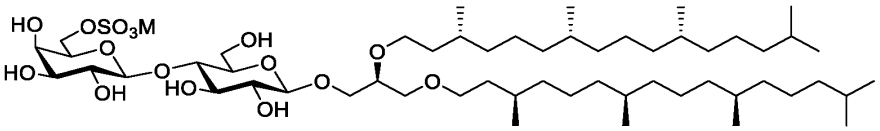
The reaction of the compound of Formula (A) with the sulfur trioxide reagent can generally be performed in solution, such as in solution in at least one organic solvent. In some embodiments, the organic solvent used for the sulfation with the sulfur trioxide reagent can be a polar or non-polar aprotic solvent. For instance, the sulfation solvent can be at least one organic solvent selected from CH_2Cl_2, dimethylformamide (DMF), pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene and tetrahydrofuran (THF). In some embodiments, the organic solvent used for the sulfation can be CH_2Cl_2, pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

The temperature at which the sulfation is performed can vary slightly but can generally be the room temperature (e.g., varying from about 15 °C to about 30°C, such as about 20 °C to 25 °C, or about 20 °C to 22 °C). In some embodiments, the reactants can be mixed in solution, at a lower temperature than room temperature and then the reaction mixture temperature is left to reach room temperature and the reaction is allowed to continue at room temperature until completion.

As noted above, the sulfation with the sulfur trioxide reagent forms an intermediate compound which can then be salified in the presence of an alkali metal agent to result in a more stable form consisting of the compound of Formula (I). In some embodiments, the salification can be performed with an alkali metal buffer, an alkali metal ion exchange resin, or using MeOM in MeOH or AcOM in AcOH, where M is the alkali metal. In preferred embodiments, the salification can be performed using an alkali metal ion exchange

resin, such as an alkali metal cation exchange resin, e.g., a sodium form cation exchange resin to result in the compound of Formula (I), such as the compound of Formula (II) or (III).

In some embodiments, the sulfated glycoarchaeal of Formula (I) is such that n is equal to 1 and the compound has the following formula (II)



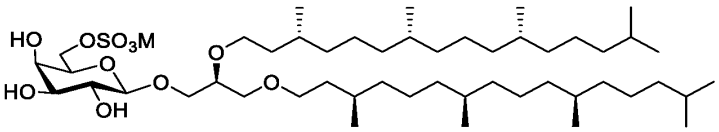
(II)

In some embodiments, the compound of Formula (II) can be prepared as mentioned above by reacting a sulfur trioxide reagent as defined herein with a glycoarchaeal, i.e., the compound of Formula (A) where n is equal to 1, in an amount providing about 1 molar equivalent of sulfur trioxide based to the molar content of glycoarchaeal, such as about 1.05 to about 1.1 equivalent for instance. By "providing X molar equivalent" of sulfur trioxide, one means that the amount of sulfur trioxide reagent to be used is calculated for providing to the reaction mixture X molar equivalent of sulfur trioxide SO_3 based on the molar content of the compound used in the sulfation reaction. Indeed, in some embodiments, the sulfur trioxide reagent which can be a commercial product, is not pure and is characterized by its percentage of "active" SO_3. This should be considered in determining the amount of sulfur trioxide reagent to be used in the sulfation step.

In some embodiments, the preparation of the compound of Formula (II) by reacting the compound of Formula (A) where n is equal to 1 with the sulfur trioxide reagent can be performed at room temperature for about 10 hours to about 72 hours. For instance, the reaction time can be from about 11 to about 72 hours, from about 12 to about 72 hours, from about 13 to about 72 hours, from about 14 to about 72 hours, from about 15 to about 72 hours, or from about 16 to about 72 hours. In some embodiments, the reaction time for preparing the compound of Formula (II) by reacting the compound of Formula (A) where n is equal to 1 with the sulfur trioxide reagent can be at least 15 hours, more preferably at least 16 hours.

In some embodiments, the preparation of the compound of Formula (II) by reacting the compound of Formula (A) where n is equal to 1 with the sulfur trioxide reagent can be performed by first mixing a solution of the compound of Formula (I) with the sulfur trioxide reagent at about 0 °C to then allow the reaction mixture to reach room temperature and further stirring the resulting mixture at room temperature, e.g., for about 10 hours to about 72 hours, more preferably for at least 15 hours, even more preferably for at least 16 hours, for instance for about 16 hours to about 72 hours.

In some embodiments, the sulfated glycoarchaeal of Formula (I) is such that n is equal to 0 and the compound has the following formula (III)



(III)

In some embodiments, the compound of Formula (III) can be prepared as mentioned above by reacting a sulfur trioxide reagent as defined herein with a compound of Formula (A) where n is 0, in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide (i.e., SO_3) based on the molar amount of the compound of Formula (A) used in the reaction. In some embodiments, the amount of sulfur trioxide reagent used is calculated to provide from about 3 to about 10 molar equivalents, or from about 4 to about 10 molar equivalents, or from about 5 to about 10 molar equivalents, or from about 6 to about 10 molar equivalents, or from about 7 to about 10 molar equivalents, or from about 8 to about 10 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (A) where n is equal to 0, used in the reaction. In some embodiments, the quantity of sulfur trioxide reagent to be used is calculated to provide at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (A) where n is equal to 0 used in the reaction. In some embodiments, the quantity of sulfur trioxide reagent to be used is calculated to provide from about 1.5 to about 7.5 molar equivalents, or from about 1.5 to about 5 molar equivalents, or from about 1.5 to about 2 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (A) where n is equal to 0 used in the reaction.

In some embodiments, the reaction between the sulfur trioxide reagent and the compound of Formula (A) where n is 0, can be performed at room temperature for at most about 4 hours, such as for about 1 to about 4 hours.

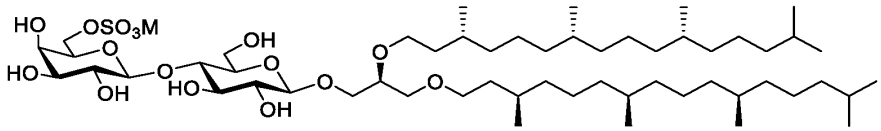
In some embodiments, the reaction between the sulfur trioxide reagent and the compound of Formula (A) where n is 0, can be performed using the sulfur trioxide reagent , in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide, preferably from about 1.5 to about 7.5 molar equivalents, more preferably from about 1.5 to about 5 molar equivalents, for instance from about 1.5 to about 2 molar equivalents, and for about 1 hour to about 4 hour.

In some embodiments, the reaction between the sulfur trioxide reagent and the compound of Formula (A) where n is 0, can be performed at room temperature for at most about 2 hours using at least 8 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (A) where n is 0, used in the reaction.

In some embodiments, once the reaction between the compound of Formula (A) and the sulfur trioxide reagent is completed, unreacted compound of Formula (A) can be recovered from the reaction mixture and can be advantageously reused in the process.

Synthesis involving glycosilylation steps

According to the present disclosure, there is also provided alternative processes for the synthesis of the compound of Formula (II), i.e., the disaccharide version of the compound of Formula (I)



(II)

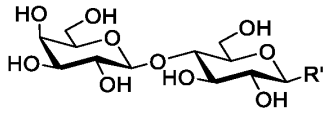
where M is an alkali metal or $N(C_{1-4}alkyl)_4$.

Such alternative processes will be detailed below.

In some embodiments, alternative processes for synthesizing a sulfated glycoarchaeal of Formula (II) can comprise the following methods **i)**, **ii)** or **iii)**.

In some embodiments, **method i)** comprises:

a step of silylating the -OH in position 6' of the lactopyranoside part of a compound of Formula (E)

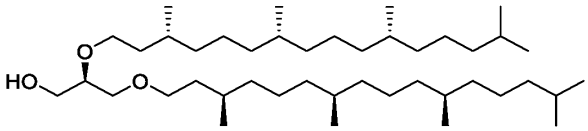


(E)

where R' is a leaving group as defined herein, such as OAc, SEt, SPh, STol, OTs, OMs, imidate such as trichloroacetimidate, SePh, S(O)Ph, or $S(O)_2Ph$, to form a 6'-silylated compound (E1);

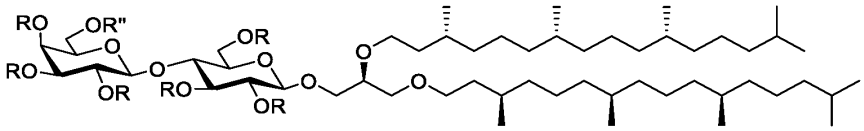
a step of protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2);

a step of reacting the 6'-silylated protected compound (E2) with a compound of Formula (C)



(C)

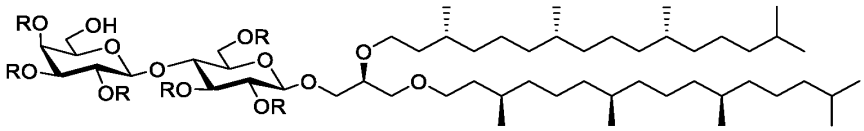
to form a compound of Formula (F)



(F)

where R is a protecting group and R" is a silyl group;

a step of desilylating the compound of Formula (F) to form a compound of Formula (G)



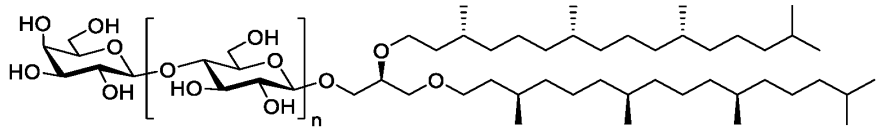
(G) ;

a step of sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with a sulfur trioxide reagent as defined above to form an intermediate compound; and

a step of deprotecting the intermediate compound to form the compound of Formula (I).

In some embodiments, **method ii)** comprises:

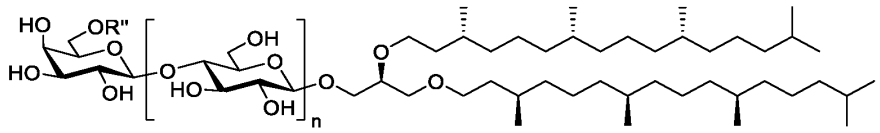
a step of silylating the -OH in position 6' of the compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A1)



(A1)

where n is 1 and R" is as defined above;

a step of protecting the hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F);

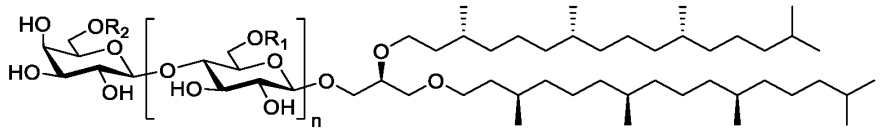
a step of desilylating the compound of Formula (F) to form the compound of Formula (G);

a step of sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with the sulfur trioxide reagent to form the intermediate compound;

a step of deprotecting the intermediate compound to form the compound of Formula (II).

In some embodiments, **method iii)** comprises:

a step of silylating the compound of Formula (A) with a silylating agent to form a mixture of compounds of Formula (A2), (A3) and (A4)



(A2): n =1; R_1 = H and R_2 = R"

(A3): n = 1; R_1 = R" and R_2 = H

(A4): n = 1; R_1 = R" and R_2 = R";

a step of separating compounds of Formula (A2), (A3) and (A4) to recover compound of Formula (A3);

a step of sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (A3) with the sulfur trioxide reagent to form a 6'-sulfated 6-silylated compound (A5); and

a step of desilylating the sulfated compound (A5) to form the compound of Formula (II).

In some embodiments, the leaving group R' in the compound of Formula (E) can be SEt, SPh or STol, SePh, S(O)Ph, or $S(O)_2Ph$. In some embodiments, the leaving group R' can particularly be SEt, SPh or STol.

In some embodiments, the silylating step in method i) can comprise first reacting the compound of Formula (E) with an organotin oxide or organotin dichloride compound to form a stannylene acetal of the

lactopyranoside part of the compound of Formula (E) and then reacting the stannylene acetal of the compound of Formula (E) with a silylating agent.

In some embodiments, the silylating step in method ii) can comprise first reacting the compound of Formula (A) with an organotin oxide or organotin dichloride compound to form a stannylene acetal of the lactopyranoside part of the compound of Formula (A) and then reacting the stannylene acetal of the compound of Formula (A) with a silylating agent.

In some embodiments, the organotin oxide or organotin dichloride compound used in the silylating step of method i) and/or ii) can comprise a di(C_1-C_4alkyl)tin oxide or dichloride, preferably dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride, most preferably dibutyltin oxide.

In some embodiments, the silylating agent used in any of methods i), ii) and ii) to form the group R" can comprise tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

In some embodiments, the silylating step in method i) can comprise reacting the compound of Formula (E) with a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

In some embodiments, the silylating step in method ii) can comprise reacting the compound of Formula (E) with a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

In some embodiments, the step of protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2) in method i) can comprise reacting the 6'-silylated compound (E1) with a compound RX, where X is halogen (e.g., F, Cl, Br or I, preferably Cl or Br) and R is Ac or Bz.

In some embodiments, the step of protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2) in method i) can comprise reacting the 6'-silylated compound (E1) with a compound RX where X is halogen (e.g., F, Cl, Br or I, preferably Cl or Br) and R is Bz.

In some embodiments, the step of protecting the hydroxyl groups of the 6'-silylated compound (A1) to form a 6'-silylated protected compound (F) in method ii) can comprise reacting the 6'-silylated compound (A1) with a compound RX, where X is halogen (e.g., F, Cl, Br or I, preferably Cl or Br) and R is Ac or Bz.

In some embodiments, the step of protecting the hydroxyl groups of the 6'-silylated compound (A1) to form a 6'-silylated protected compound (F) in method i) can comprise reacting the 6'-silylated compound (A1) with a compound RX where X is halogen (e.g., F, Cl, Br or I, preferably Cl or Br) and R is Bz.

In some embodiments, the step of reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) in method i) can be performed in the presence of a Lewis acid. In some embodiments, the step of reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) can be performed in the presence of N-iodosuccinimide (NIS) in combination with Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$),

Scandium(III) triflate ($Sc(OTf)_3$), or Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$); or any mixture thereof.

In some embodiments, the step of desilylating the compound of Formula (F) to form a compound of Formula (G) in method i) can be performed according to the method known in the art, such as using Tetra-n-butylammonium fluoride (TBAF) under acidic pH. In an alternative embodiment, the step of desilylating the compound of Formula (F) to form a compound of Formula (G) can comprise reacting the compound of Formula (F) with $BF_3.Et_2O$ or with Triethylamine trihydrofluoride (TEA.3HF).

In some embodiments, the step of desilylating the compound of Formula (F) to form a compound of Formula (G) in method i) can be performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

In some embodiments, the step of desilylating the sulfated compound of Formula (A5) to form a compound of Formula (II) in method iii) can be performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

The sulfation step whereby the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) is sulfated in methods i) or ii), can be performed using a sulfur trioxide reagent as defined previously, i.e., a sulfur trioxide amine complex. For instance, the sulfur trioxide reagent can be $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide reagent is preferably $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, more preferably $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

The sulfation step whereby the -OH in position 6' of the lactopyranoside part of the compound of Formula (A3) is sulfated in methods iii), can be performed using a sulfur trioxide reagent as defined previously, i.e., a sulfur trioxide amine complex. For instance, the sulfur trioxide reagent can be $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide reagent is preferably $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, more preferably $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

In some embodiments, the organic solvent that can be used to perform the sulfation step in methods i), ii) and ii) can be a polar or non-polar aprotic solvent. In some embodiments, the organic solvent used to perform the sulfation step in methods i), ii) and ii) can be CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, and/or tetrahydrofuran (THF). In some embodiments, the organic solvent comprises CH_2Cl_2, pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

In some embodiments, the sulfation step in methods i), ii) and ii) can be performed at room temperature. In some embodiments, the sulfation step in methods i), ii) and ii) can be performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours.

In some embodiments, the sulfation step in methods i), ii) or iii) can be performed using the sulfur trioxide reagent in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide

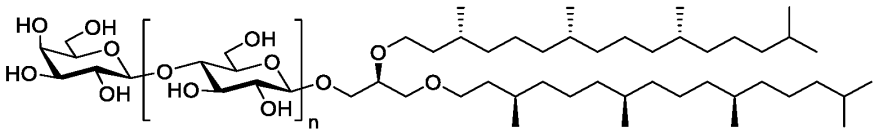
(i.e., SO_3) based on the molar amount of the compound of Formula (G) or Formula (A3). In some embodiments, the amount of sulfur trioxide reagent used is calculated to provide from about 1.5 to about 10 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (G) or Formula (A3). In some embodiments, the amount of sulfur trioxide reagent used is calculated to provide from about 2 to about 10 molar equivalents, or from about 3 to about 10 molar equivalents, or from about 4 to about 10 molar equivalents, or from about 5 to about 10 molar equivalents, or from about 6 to about 10 molar equivalents, or from about 7 to about 10 molar equivalents, or from about 8 to about 10 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (G) or Formula (A3). In some embodiments, the quantity of sulfur trioxide reagent to be used is calculated to provide at least 1.5, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8 molar equivalents sulfur trioxide based on the molar amount of the compound of Formula (G) or Formula (A3). In some embodiments, the sulfur trioxide reagent can be used in an amount to provide about 1.5 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (G). In some embodiments, the sulfur trioxide reagent can be used in an amount to provide about 8 molar equivalents of sulfur trioxide based on the molar amount of the compound of Formula (G).

Finally, the last step of deprotecting the intermediate compound obtained after the sulfation step in methods i) and ii) can be performed with a basic solution, such as a solution comprising at least one base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, more preferably the base is sodium methoxide.

Synthesis of intermediate unsulfated glycoarchaeal

According to the present disclosure, there is also provided a process of synthesizing a glycoarchaeal compound of Formula (A), which can comprise a disaccharide moiety (n = 1) or a monosaccharide moiety (n = 0), and which can be used in the above-described process for synthesizing the sulfated glycoarchaeal compound of Formula (I).

In some embodiments, there is thus provided a process for preparing a compound of Formula (A)

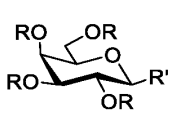


(A)

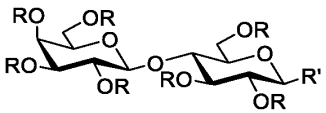
where n is 0 or 1;

which comprises:

a step of reacting a compound of Formula (B_0) when n is 0 or a compound of Formula (B_1) when n is 1



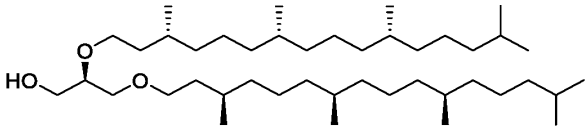
(B_0)



(B_1)

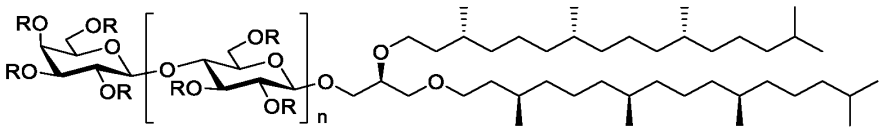
where R is a protecting group and R' is a leaving group

with a compound of Formula (C)



(C)

to form a compound of Formula (D)



(D)

and

a step of deprotecting the compound of Formula (D) to form the compound of Formula (A).

In some embodiments, the protecting group R in the compounds of Formulae (B_0), (B_1) and (D) can be Ac or Bz.

In some embodiments, the leaving group R' in the compounds of Formulae (B_0) and (B_1) can independently represent halogen (e.g., Cl or Br), OAc, SEt, SPh, STol, OTs, OMs, imidate such as trichloroacetimidate, SePh, S(O)Ph, or $S(O)_2Ph$, preferably OAc, SEt, SPh, or STol, more preferably OAc, SEt, SPh, or STol, even more preferably OAc.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in the presence of a Lewis acid reagent.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be further performed in the presence of molecular sieve.

In some embodiments, the leaving group R' can be OAc and the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), Trimethylsilyl trifluoromethanesulfonate (TMSOTf), Trifluoromethanesulfonic acid (TFOH), silver trifluoromethanesulfonate (AgOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$), Silver trifluoroacetate (CF_3CO_2Ag), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, optionally in combination with a metal-containing zeolite.

In some embodiments, the leaving group R' can be OAc and the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in the

presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof.

In some embodiments, the leaving group R' can be OAc and the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, in combination with a metal-containing zeolite.

In some embodiments, the synthesis of the compound of Formula (A) where n is 1 can be performed by reacting the compound of Formula (B_1) where the leaving group R' is OAc with the compound of Formula (C), in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), or in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite.

In some embodiments, the synthesis of the compound of Formula (A) where n is 1 can be performed by reacting the compound of Formula (B_1) where the leaving group R' is OAc with the compound of Formula (C), in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite.

In some embodiments, the synthesis of the compound of Formula (A) where n is 1 can be performed by reacting the compound of Formula (B_1) where the leaving group R' is OAc with the compound of Formula (C) in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite, and further in combination with a molecular sieve.

In some embodiments, the synthesis of the compound of Formula (A) where n is 1 can be performed by reacting the compound of Formula (B_1) where the leaving group R' is Br, with the compound of Formula (C) in the presence of a metal-containing zeolite in combination with a molecular sieve.

In some embodiments, the synthesis of the compound of Formula (A) where n is 0 can be performed by reacting the compound of Formula (B_0) where the leaving group R' is OAc, with the compound of Formula (C) in the presence of Tin(IV) chloride ($SnCl_4$) and $BF_3.OEt_2$.

In some embodiments, the synthesis of the compound of Formula (A) where n is 0 can be performed by reacting the compound of Formula (B_0) where the leaving group R' is OAc, with the compound of Formula (C) in the presence of Tin(IV) chloride ($SnCl_4$) and a metal-containing zeolite.

In some embodiments, the synthesis of the compound of Formula (A) where n is 0 can be performed by reacting the compound of Formula (B_0) where the leaving group R' is OAc, with the compound of Formula (C) in the presence of Tin(IV) chloride ($SnCl_4$) and a metal-containing zeolite, in further combination with a molecular sieve.

In some embodiments, the synthesis of the compound of Formula (A) where n is 0 can be performed by reacting the compound of Formula (B_0) where the leaving group R' is Br, with the compound of Formula (C) in the presence of a metal-containing zeolite in combination with a molecular sieve.

In some embodiments, the metal in the metal-containing zeolite can comprise Ag, Al, Cd, Co, Cu, Fe, Ga, In, Mo, Pd, Pt, Sn, Sb, V, Zr or any mixture thereof.

In some embodiments, the metal-containing zeolite can be a zeolite selected from the group consisting of Ag-zeolite, Sn-Beta, Zr-Beta, Al-Beta(OH), Al-Beta(F), Pt@MCM-22, K-PtSn/MFI, 0.3Pt/0.5Sn-Si-

Beta, Pt/Sn 2.0-Beta, 0.5CoSi-Beta, V-Beta, H-[Fe]ZSM-5, Fe-BEA, Ga-Beta, Ga-Beta-200, Mo/HZSM-5, and any mixture thereof.

In some embodiments, the metal-containing zeolite can be a silver-containing zeolite. Examples of molecular sieves that can be used include molecular sieves of type 3Å, type 4Å, type 5Å, type 13X. Any mixture thereof can also be used. In some embodiments, the molecular sieves are activated before use.

In some embodiments, the synthesis of the compound of Formula (A) can be performed by reacting the compound of Formula (B_0) or the compound of Formula (B_1) where the leaving group R' is SEt, SPh or STol, with the compound of Formula (C), in the presence of N-iodosuccinimide (NIS) in combination with triflic acid, triflate (TfOH), BF_3-OEt_2, AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or in the presence of trifluoromethanesulfonic anhydride (Tf_2O) with 2,6-di-terbutyl-4-methylpyridine (DTBMP); or any mixture thereof.

In some embodiments, the synthesis of the compound of Formula (A) can be performed by reacting the compound of Formula (B_0) or the compound of Formula (B_1) where the leaving group R' is SEt, SPh or STol, with the compound of Formula (C), in the presence of N-iodosuccinimide (NIS) in combination with BF_3-OEt_2 or $Yb(OTf)_3$.

In some embodiments, the synthesis of the compound of Formula (A) can be performed by reacting the compound of Formula (B_0) or the compound of Formula (B_1) where the leaving group R' is trichloroacetimidate, with the compound of Formula (C), in the presence of triflic acid, triflate (TfOH), AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or any mixture thereof.

In some embodiments, the synthesis of the compound of Formula (A) can be performed by reacting the compound of Formula (B_0) or the compound of Formula (B_1) where the leaving group R' is trichloroacetimidate, with the compound of Formula (C), in the presence of TMSOTf.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in at least one organic solvent.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in at least one organic solvent selected from CH_2Cl_2, toluene, DMF, DMSO, Dimethyl acetamide (DMAC), THF, Dioxane, 1,3 Dioxane, acetonitrile, 2,5-dimethyl tetrahydrofuran (DMTHF), Gamma-vaterolactone (GVL), Dihydrolevoglucoserone (Cyrene),

methyl levulinate (ML), Ethyl levulinate (EL), Ethyl levulinate propyleneglycol ketal (ELPK), Dimethyl glutarate (DMG), Dimethylpropylene urea (DMPU), Poly(propyleneglycol) (PPG), Glycofurol (THFP), 1-Ethyl-3-methylimidazolium acetate ([emim][OAc]), or any mixture thereof.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed in at least one organic solvent selected from is CH_2Cl_2, toluene, or a mixture thereof.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed at room temperature.

In some embodiments, the step of reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) can be performed under heating.

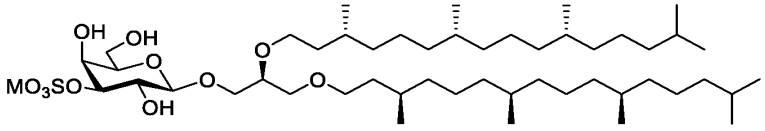
In some embodiments, the step of reacting the compound of Formula (B_0) with the compound of Formula (C) can be performed for about 1 hour to about 3 hours. In some embodiments, the step of reacting the compound of Formula (B_0) with the compound of Formula (C) can be performed for at most about 2 hours. In some embodiments, the step of reacting the compound of Formula (B_0) with the compound of Formula (C) can be performed for about 1 hour to about 2 hours.

In some embodiments, the step of reacting the compound of Formula (B_1) with the compound of Formula (C) can be performed for about 1 hour to about 72 hours. For instance, the reaction time between the compound of Formula (B_1) and the compound of Formula (C) can be from about 11 to about 72 hours, from about 12 to about 72 hours, from about 13 to about 72 hours, from about 14 to about 72 hours, from about 15 to about 72 hours, or from about 16 to about 72 hours. In some embodiments, the reaction time between the compound of Formula (B_1) and the compound of Formula (C) can be at least 15 hours, for instance at least about 16 hours, or from about 16 hours to about 72 hours. The reaction time can be adjusted depending on whether the reaction is performed under heating or simply at room temperature. Monitoring the reaction advancement can allow adjusting the reaction time properly.

Finally, the last step of the preparation of the compound of Formula (A), involving deprotecting the compound of Formula (D), can be performed by reacting the compound of Formula (D) with a basic solution. In some embodiments, deprotecting the compound of Formula (D) can be performed by reacting the compound of Formula (D) with a basic solution comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, preferably the base is sodium methoxide.

<u>Synthesis of other sulfated glycoarchaeal derivatives</u>

According to the present disclosure, there is also provided a process of synthesizing a sulfated glycoarchaeal of Formula (IV)

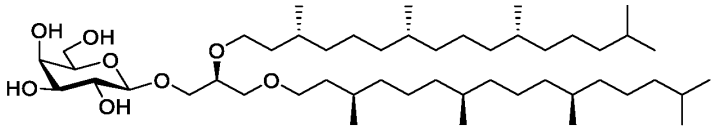


(IV)

where M is an alkali metal;

which comprises:

reacting a compound of Formula (A0)



(A0)

with an organotin oxide or organotin dichloride compound to form a stannylene acetal on the galactopyranoside part of the compound (A0);

then reacting the stannylene acetal of the compound of Formula (A0) with a sulfur trioxide reagent to form an intermediate compound; and

salification of the intermediate compound to form the compound of Formula (IV).

In some embodiments, the preparation of the sulfated glycoarchaeal of Formula (IV) can comprise: forming a mixture of the compound of Formula (A0) and the organotin oxide or organotin dichloride compound in a first organic solvent and then heating the mixture; concentrating the mixture to form a concentrated mixture; redissolving the concentrated mixture in a second organic solvent to form a redissolved mixture; reacting the redissolved mixture with the sulfur trioxide reagent to form the intermediate compound; and then salification of the intermediate compound to form the compound of Formula (I).

In some embodiments, the first organic solvent and the second organic solvent involved in the preparation of the sulfated glycoarchaeal of Formula (IV) are independently selected from CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, tetrahydrofuran (THF), and any combination thereof. In some embodiments, the first organic solvent and the second organic solvent are independently selected from CH_2Cl_2, pyridine, toluene and any combination thereof. In some embodiments, the first organic solvent and the second organic solvent are independently selected from pyridine, toluene and a combination thereof.

In some embodiments, the preparation of the sulfated glycoarchaeal of Formula (IV) can comprise forming the mixture of the compound of Formula (A0) and the organotin oxide or organotin dichloride compound in the first organic solvent and then heating the mixture at reflux. In some embodiments, after heating, the mixture is concentrated and then the concentrated mixture is redissolved in the second organic solvent as defined above, to form a redissolved mixture. The redissolved mixture can then be reacted with the sulfur trioxide reagent. In some embodiment, the reaction with the sulfur trioxide reagent can be performed at room temperature for about 1 hour to about 72 hours, preferably for about 10 hours to 72 hours, more preferably for at least 15 hours, for instance from about 15 hours to about 72 hours.

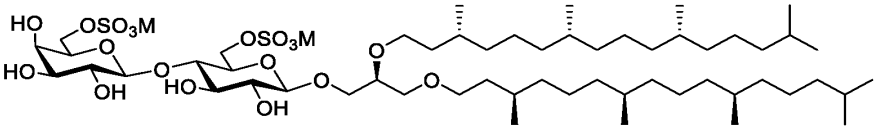
In some embodiments, the preparation of the sulfated glycoarchaeal of Formula (IV), the sulfur trioxide reagent can be used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide. In some embodiments, the sulfur trioxide reagent can be used in an amount providing from about

1 to about 7.5 molar equivalents of sulfur trioxide, or from about 1 to about 5 molar equivalents of sulfur trioxide, or from about 1.05 to about 2 molar equivalents of sulfur trioxide.

In some embodiments, the organotin oxide or organotin dichloride compound reacted with the compound of Formula (A0), in the first step of the preparation of the sulfated glycoarchaeal of Formula (IV), can comprise a di(C_1-C_4alkyl)tin oxide or dichloride. In some embodiments, the organotin oxide or organotin dichloride compound can be dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride. In some embodiments, the organotin oxide or organotin dichloride compound is dibutyltin oxide.

In some embodiments, the sulfur trioxide reagent used in the preparation of the sulfated glycoarchaeal of Formula (IV), is a sulfur trioxide amine complex. In some embodiments, the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof. In preferred embodiments, the sulfur trioxide reagent can be $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

According to the present disclosure, there is also provided a process for synthesizing a disulfated glycoarchaeal of Formula (V)

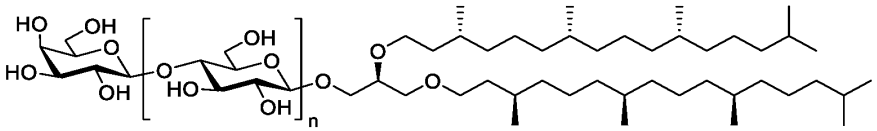


(V)

where M is an alkali metal;

which comprises:

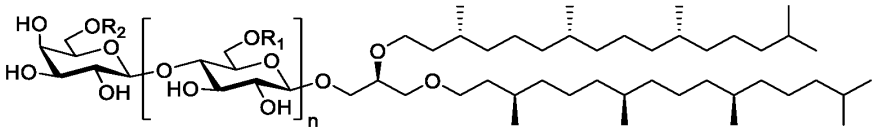
silylating the -OH in positions 6 and 6' of a compound of Formula (A)



(A)

where n is 1;

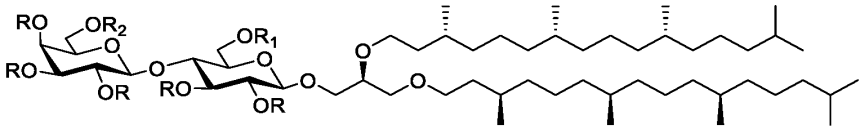
to form a 6- and 6'-silylated compound (A4)



(A4)

where n is 1 and R_1 and R_2 represent a silyl group;

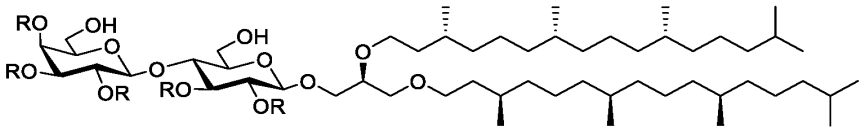
protecting the hydroxyl groups of the 6- and 6'-silylated compound (A4) to form the compound of Formula (F1)



(F1)

where R is a protecting group;

desilylating the compound of Formula (F1) to form the compound of Formula (G1);



(G1)

sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) with a sulfur trioxide reagent to form an intermediate compound; and

deprotecting the intermediate compound to form the compound of Formula (V).

In some embodiments, the step of silylating the -OH in positions 6 and 6' of the compound of Formula (A) to form the 6- and 6'-silylated compound (A4) can comprise reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

In some embodiments, the step of protecting the hydroxyl groups of the 6 and 6'-silylated compound (A4) to form the compound of Formula (F1) can comprise reacting the compound (A4) with a compound RX, where X is halogen and R is Ac or Bz. In some embodiments, the step of protecting the hydroxyl groups of the 6 and 6'-silylated compound (A4) can comprise reacting the compound (A4) with a compound RX where X is halogen (e.g., Br or Cl) and R is Bz.

In some embodiments, the step of desilylating the compound of Formula (F1) to form the compound of Formula (G1) can be performed with tetra-n-butylammonium fluoride (TBAF), or with triethylamine trihydrofluoride (TEA.3HF).

In some embodiments, the step of desilylating the compound of Formula (F1) to form the compound of Formula (G1) can be performed with tetra-n-butylammonium fluoride (TBAF) in the presence of $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF).

In some embodiments, the step of desilylating the compound of Formula (F1) to form the compound of Formula (G1) can be performed with tetra-n-butylammonium fluoride (TBAF).

In some embodiments, the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) can be performed with a sulfur trioxide amine complex as the sulfur trioxide reagent.

In some embodiments, the sulfur trioxide amine complex used in the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) can be $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof. In other

embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

In some embodiments, the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) is performed with the sulfur trioxide amine complex being used in an amount providing from about 2 to about 10 molar equivalents of sulfur trioxide. In some embodiments, the sulfur trioxide amine complex is used in an amount to provide from about 5 to about 10 molar equivalents. In some embodiments, the sulfur trioxide amine complex is used in an amount to provide from about 7 to about 10 molar equivalents. In some embodiments, the sulfur trioxide amine complex is used in an amount to provide about 8 molar equivalents.

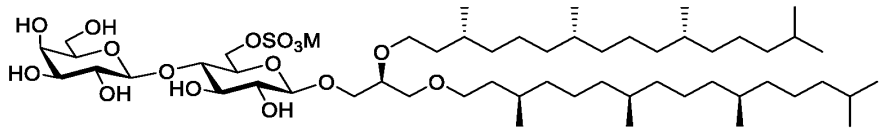
In some embodiments, the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) is performed in solution in an organic solvent. In some embodiments, the organic solvent is a polar or non-polar aprotic solvent. In some embodiments, the organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, tetrahydrofuran (THF), or any combination thereof. In some embodiments, the organic solvent comprises pyridine, toluene or a combination thereof.

In some embodiments, the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) is performed at room temperature.

In some embodiments, the step of sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) is performed at room temperature for at least 1 hour, such as for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

In some embodiments, the intermediate compound formed by sulfation of the -OH in positions 6 and 6' of the compound of Formula (G1) is deprotected, to form the compound of Formula (V), by reacting the intermediate compound with a basic solution. In some embodiments, the deprotection can be performed in the presence of a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate. In some embodiments, the base is sodium methoxide.

According to the present disclosure, there is also provided a process for synthesizing a sulfated glycoarchaeal of Formula (VI)

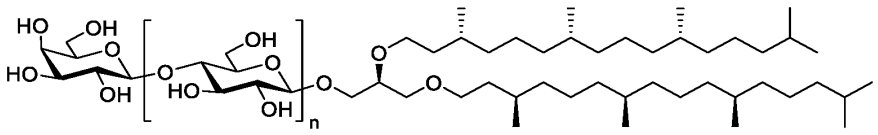


(VI)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$;

which comprises:

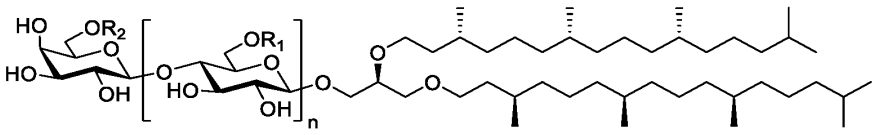
silylating the -OH in position 6' of a compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A2)



(A2)

where n is 1, R_1 is H, and R_2 represents a silyl group;

sulfating the -OH in position 6 of the compound of Formula (A2) with a sulfur trioxide reagent to form a 6-sulfated 6'-silylated compound (A6);

desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$; and

optionally contacting the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$ with an alkali metal cation exchange resin to form the compound of Formula (VI) where M is alkali metal.

In some embodiments, silylating the -OH in position 6' of a compound of Formula (A) to form the 6'-silylated compound (A2) can comprise reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride. In some embodiments, the silylating agent can include triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed with a sulfur trioxide reagent which is a sulfur trioxide amine complex. In some embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide amine complex can be $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof. In some embodiments, the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed with the sulfur trioxide reagent used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide. In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed with the sulfur trioxide reagent used in an amount from about 1 to about 5 molar equivalents. In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed using the sulfur trioxide reagent in an amount from about 1 to about 3 molar equivalents. In some embodiments, the sulfur trioxide reagent is used in an amount of about 2 molar equivalents.

In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed in solution in an organic solvent. In some

embodiments, the organic solvent is a polar or non-polar aprotic solvent. In some embodiments, the organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, tetrahydrofuran (THF) or a combination thereof. In some embodiments, the organic solvent comprises pyridine, toluene or a combination thereof.

In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed at room temperature.

In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed at room temperature for at least 1 hour. In some embodiments, sulfating the -OH in position 6 of the compound of Formula (A2) to form the 6-sulfated 6'-silylated compound (A6) is performed at room temperature for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

In some embodiments, the step of desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) can be performed with tetra-n-butylammonium fluoride (TBAF), or with triethylamine trihydrofluoride (TEA.3HF).

In some embodiments, the step of desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) can be performed with tetra-n-butylammonium fluoride (TBAF) in the presence of with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF).

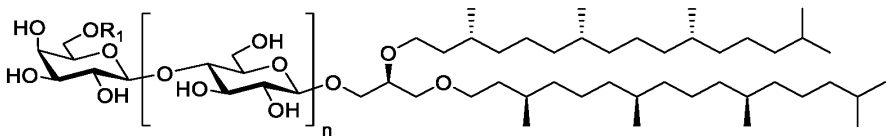
In some embodiments, the step of desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) can be performed with tetra-n-butylammonium fluoride (TBAF).

Several reaction conditions and parameters to perform the synthesis processes have been described herein. Variations thereof are contemplated within the scope of protection which should not be limited to such specific conditions and parameters.

ITEMS

Described herein are one or more of the following items.

Item 1. A process for synthesizing a sulfated glycoarchaeal of Formula (I)

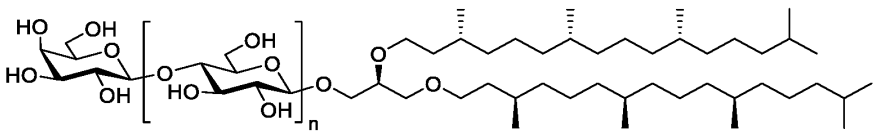


(I)

where R_1 is SO_3M with M being an alkali metal, and n is 0 or 1;

comprising:

reacting a compound of Formula (A)



(A)

with a sulfur trioxide reagent to form an intermediate compound;

salification of the intermediate compound to form the compound of Formula (I).

Item 2. The process of claim 1, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex.

Item 3. The process of claim 2, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof.

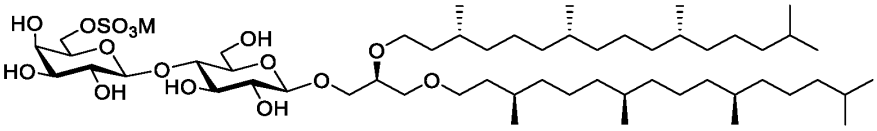
Item 4. The process of claim 2, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, preferably $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

Item 5. The process of any one of claims 1 to 4, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene or tetrahydrofuran (THF), even more preferably the organic solvent comprises CH_2Cl_2, pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

Item 6. The process of any one of claims 1 to 5, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

Item 7. The process of any one of claims 1 to 6, wherein the salification is performed with an alkali metal buffer, an alkali metal ion exchange resin, or using MeOM in MeOH or AcOM in AcOH, where M is the alkali metal, preferably the salification is performed with the alkali metal ion exchange resin.

Item 8. The process of any one of claims 1 to 7, wherein n is equal to 1 and the sulfated glycoarchaeal has the following formula (II)



(II) .

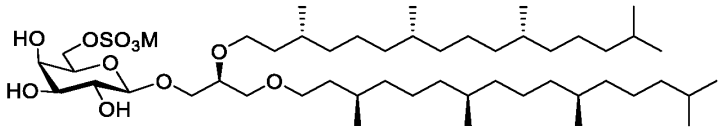
Item 9. The process of any one of claims 1 to 8, wherein n is equal to 1 and the sulfur trioxide reagent is used in an amount providing about 1 molar equivalent of sulfur trioxide, such as about 1.05 to about 1.1 molar equivalent of sulfur trioxide.

Item 10. The process of any one of claims 1 to 9, wherein n is equal to 1 and the reaction with the sulfur trioxide reagent is performed at room temperature for about 10 hours to about 72 hours, preferably for at least 15 hours, more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

Item 11. The process of any one of claims 1 to 10, wherein n is equal to 1 and the reaction with the sulfur trioxide reagent comprises mixing a solution of the compound of Formula (I) with the sulfur trioxide reagent at about 0 °C and then stirring the resulting mixture at room temperature,

preferably for about 10 hours to about 72 hours, more preferably for at least 15 hours, even more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

Item 12. The process of any one of claims 1 to 7, wherein n is 0 and the sulfated glycoarchaeal has the following formula (III)



(III) .

Item 13. The process of any one of claims 1 to 7 and 12, wherein n is 0 and the sulfur trioxide reagent is used in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide, preferably from about 1.5 to about 7.5 molar equivalents, more preferably from about 1.5 to about 5 molar equivalents, for instance from about 1.5 to about 2 molar equivalents.

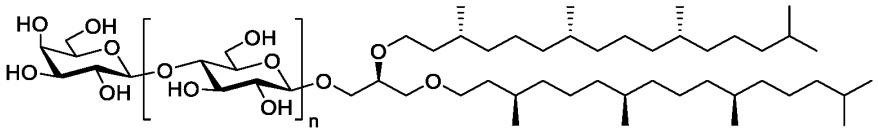
Item 14. The process of any one of claims 1 to 7, 12 and 13, wherein n is 0 and the reaction with the sulfur trioxide reagent is performed at room temperature for at most about 4 hours, such as about 1 to about 4 hours.

Item 15. The process of any one of claims 1 to 14, wherein M is Li, Na or K, preferably Na.

Item 16. The process of any one of claims 1 to 15, further comprising recovering unreacted compound of Formula (A) after the reaction with the sulfur trioxide reagent.

Item 17. The process of claim 16, wherein the recovered unreacted compound of Formula (A) is reused in the process.

Item 18. A process for preparing a compound of Formula (A)

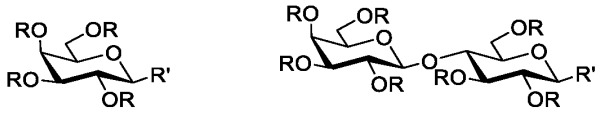


(A)

where n is 0 or 1;

comprising:

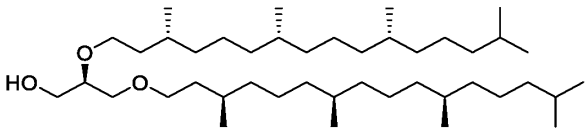
reacting a compound of Formula (B_0) when n is 0 or a compound of Formula (B_1) when n is 1



(B_0) (B_1)

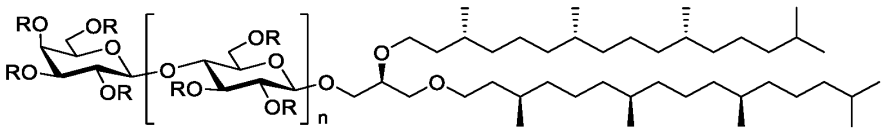
where R is a protecting group and R' is a leaving group

with a compound of Formula (C)



(C)

to form a compound of Formula (D)



(D)

and

deprotecting the compound of Formula (D) to form the compound of Formula (A).

Item 19. The process of claim 18, wherein the protecting group R is Ac or Bz.

Item 20. The process of claim 18 or 19, wherein the leaving group is halogen (e.g., Cl or Br), OAc, SEt, SPh, STol, OTs, OMs, imidate such as trichloroacetimidate , SePh, S(O)Ph, or $S(O)_2Ph$, preferably OAc, Br, trichloroacetimidate, SEt, SPh, or STol, more preferably OAc, Set, SPh, or STol, even more preferably OAc.

Item 21. The process of any one of claims 18 to 20, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of a Lewis acid reagent.

Item 22. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), Trimethylsilyl trifluoromethanesulfonate (TMSOTf), Trifluoromethanesulfonic acid (TFOH), silver trifluoromethanesulfonate (AgOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$), Silver trifluoroacetate (CF_3CO_2Ag), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, optionally in combination with a metal-containing zeolite.

Item 23. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, optionally in combination with a metal-containing zeolite.

Item 24. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate ($BF_3.OEt_2$), or in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite.

Item 25. The process of any one of claims 18 to 21, wherein the leaving group is OAc, n is 1, and reacting the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite, optionally further in combination with a molecular sieve.

Item 26. The process of any one of claims 18 to 24, wherein the leaving group is OAc, n is 0, and reacting the compound of Formula (B_0) with the compound of Formula (C) is performed in the presence of Tin(IV) chloride ($SnCl_4$) and a metal-containing zeolite, optionally in combination with a molecular sieve.

Item 27. The process of any one of claims 18 to 21, wherein the leaving group is Br and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of a metal-containing zeolite in combination with a molecular sieve.

Item 28. The process of any one of claims 22 to 27, wherein the metal in the metal-containing zeolite comprises Ag, Al, Cd, Co, Cu, Fe, Ga, In, Mo, Pd, Pt, Sn, Sb, V, Zr or any mixture thereof.

Item 29. The process of any one of claims 22 to 28, wherein the metal-containing zeolite is a zeolite selected from the group consisting of Ag-zeolite, Sn-Beta, Zr-Beta, Al-Beta(OH), Al-Beta(F), Pt@MCM-22, K-PtSn/MFI, 0.3Pt/0.5Sn-Si-Beta, Pt/Sn 2.0-Beta, 0.5CoSi-Beta, V-Beta, H-[Fe]ZSM-5, Fe-BEA, Ga-Beta, Ga-Beta-200, Mo/HZSM-5, and any mixture thereof.

Item 30. The process of any one of claims 22 to 29, wherein the metal-containing zeolite is a silver-containing zeolite.

Item 31. The process of any one of claims 18 to 21, wherein the leaving group is SEt, SPh or STol and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of N-iodosuccinimide (NIS) in combination with triflic acid, triflate (TfOH), BF_3-OEt_2, AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or in the presence of trifluoromethanesulfonic anhydride (Tf_2O) with 2,6-di-terbutyl-4-methylpyridine (DTBMP); or any mixture thereof; preferably reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is

performed in the presence of N-iodosuccinimide (NIS) in combination with BF_3-OEt_2 or $Yb(OTf)_3$.

Item 32. The process of any one of claims 18 to 21, wherein the leaving group is trichloroacetimidate and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of triflic acid, triflate (TfOH), AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or any mixture thereof; preferably reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of TMSOTf.

Item 33. The process of any one of claims 18 to 32, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in at least one organic solvent, preferably the at least one organic solvent is CH_2Cl_2, toluene, DMF, DMSO, Dimethyl acetamide (DMAC), THF, Dioxane, 1,3 Dioxane, acetonitrile, 2,5-dimethyl tetrahydrofuran (DMTHF), Gamma-vaterolactone (GVL), Dihydrolevoglucoserone (Cyrene), methyl levulinate (ML), Ethyl levulinate (EL), Ethyl levulinate propyleneglycol ketal (ELPK), Dimethyl glutarate (DMG), Dimethylpropylene urea (DMPU), Poly(propyleneglycol) (PPG), Glycofurol (THFP), 1-Ethyl-3-methylimidazolium acetate ([emim][OAc]), or any mixture thereof, most preferably, the at least one organic solvent is CH_2Cl_2, toluene, or a mixture thereof.

Item 34. The process of any one of claims 18 to 33, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed at room temperature and optionally further under heating.

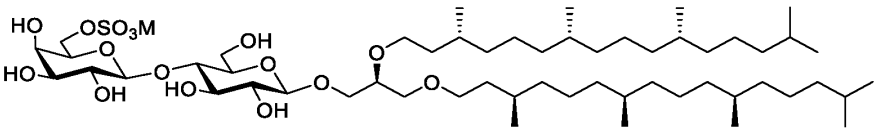
Item 35. The process of any one of claims 18 to 34, wherein reacting the compound of Formula (B_0) with the compound of Formula (C) is performed for about 1 hour to about 3 hours, preferably for at most 2 hours, more preferably for about 1 hour to about 2 hours.

Item 36. The process of any one of claims 18 to 34, wherein reacting the compound of Formula (B_1) with the compound of Formula (C) is performed for about 1 hour to about 72 hours, preferably for at least 15 hours, more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

Item 37. The process of any one of claims 18 to 36, wherein deprotecting the compound of Formula (D) to form the compound of Formula (A) is performed by reacting the compound of Formula (D) with a basic solution, preferably comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, preferably the base is sodium methoxide.

Item 38. The process of any one of claims 1 to 17, wherein the compound of Formula (A) is prepared according to the process of any one of claims 18 to 37.

Item 39. A process for synthesizing a sulfated glycoarchaeal of Formula (II)

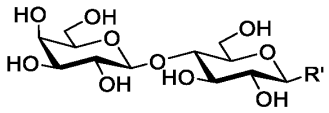


(II)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$,

comprising either:

i) silylating the -OH in position 6' of the lactopyranoside part of the compound of Formula (E)



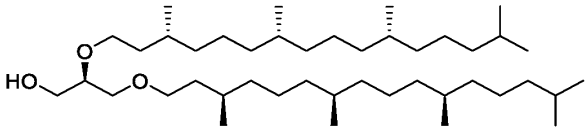
(E)

where R' is SEt, SPh or STol, SePh, S(O)Ph, or $S(O)_2Ph$;

to form a 6'-silylated compound (E1);

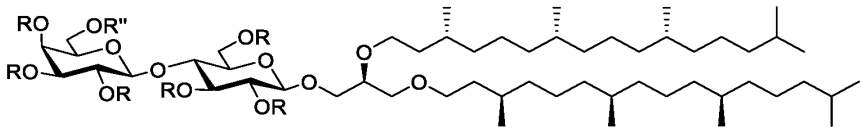
protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2);

reacting the 6'-silylated protected compound (E2) with a compound of Formula (C)



(C)

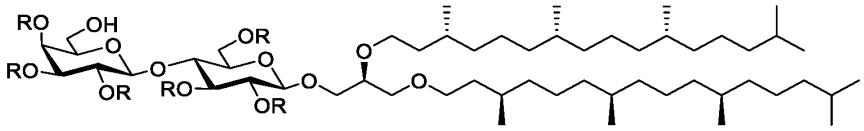
to form a compound of Formula (F)



(F)

where R is a protecting group and R" is a silyl group;

desilylating the compound of Formula (F) to form a compound of Formula (G)



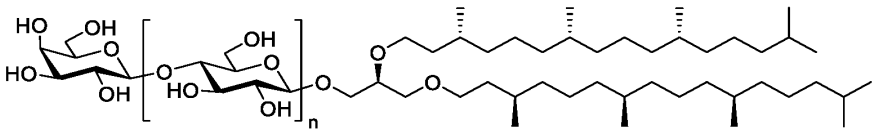
(G) ;

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with a sulfur trioxide reagent to form an intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

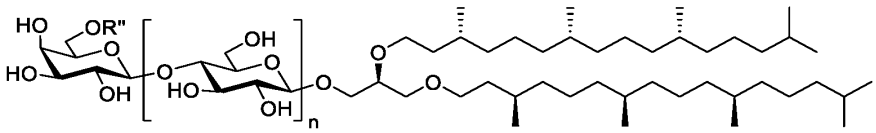
ii) silylating the -OH in position 6' of the compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A1)



(A1)

where n is 1 and R" is as defined above;

protecting the hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F);

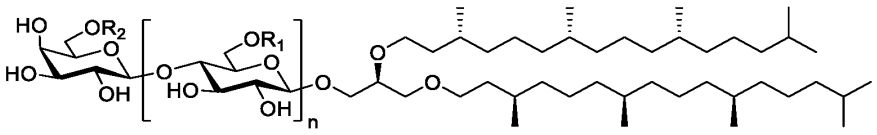
desilylating the compound of Formula (F) to form the compound of Formula (G);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with the sulfur trioxide reagent to form the intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

iii) silylating the compound of Formula (A) with a silylating agent to form a mixture of compounds of Formula (A2), (A3) and (A4)



(A2): n =1; R_1 = H and R_2 = R"

(A3): n = 1; R_1 = R" and R_2 = H

(A4): n = 1; R_1 = R" and R_2 = R";

separating compounds of Formula (A2), (A3) and (A4) to recover compound of Formula (A3);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (A3) with the sulfur trioxide reagent to form a 6'-sulfated 6-silylated compound (A5); and

desilylating the sulfated compound (A5) to form the compound of Formula (II).

Item 40. The process of claim 39, wherein R' is SEt, SPh or STol.

Item 41. The process of claim 39 or 40, wherein silylating in i) and/or ii) comprises first reacting the compound of Formula (E) or Formula (A) with an organotin oxide or organotin dichloride

compound to form a stannylene acetal of the lactopyranoside part of the compound of Formula (E) or Formula (A) and then reacting the stannylene acetal of the compound of Formula (E) or Formula (A) with a silylating agent.

Item 42. The process of claim 41, wherein the organotin oxide or organotin dichloride compound comprises a di(C_1-C_4alkyl)tin oxide or dichloride, preferably dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride, most preferably dibutyltin oxide.

Item 43. The process of any one of claims 39 or 42, wherein the silylating agent comprises a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

Item 44. The process of any one of claims 39 to 43, wherein protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2), and/or protecting the hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F) comprises reacting the 6'-silylated compound (E1) or (A1) with a compound RX, where X is halogen and R is Ac or Bz, preferably Bz.

Item 45. The process of any one of claims 39 to 44, wherein reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) is performed in the presence of a Lewis acid.

Item 46. The process of any one of claims 39 to 45, wherein reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) is performed in the presence of N-iodosuccinimide (NIS) in combination with Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), or Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$); or any mixture thereof.

Item 47. The process of any one of claims 39 to 46, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex.

Item 48. The process of claim 47, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof, preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

Item 49. The process of any one of claims 39 to 48, wherein the sulfur trioxide amine complex is used in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide, preferably from about 2 to about 10 molar equivalents, more preferably from about 5 to about 10 molar equivalents, for instance about 8 molar equivalents.

Item 50. The process of any one of claims 39 to 49, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises CH_2Cl_2,

pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

Item 51. The process of any one of claims 39 to 50, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

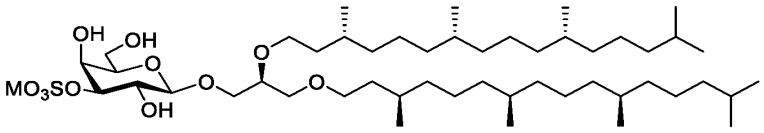
Item 52. The process of any one of claims 39 to 51, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours.

Item 53. The process of any one of claims 39 to 52, wherein deprotecting the intermediate compound comprises reacting the intermediate compound with a basic solution preferably comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, more preferably the base is sodium methoxide.

Item 54. The process of any one of claims 39 to 53, wherein desilylating the compound of Formula (F) to form the compound of Formula (G) and/or desilylating the sulfated compound of Formula (A5) to form the compound of Formula (I) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

Item 55. The process of any one of claims 39 to 54, wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$, preferably Li, Na, K or $N(Bu)_4$, more preferably Na.

Item 56. A process for synthesizing a sulfated glycoarchaeal of Formula (IV)

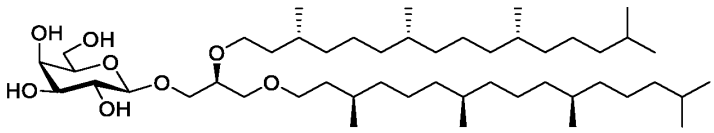


(IV)

where M is an alkali metal;

comprising:

reacting a compound of Formula (A0)



(A0)

with an organotin oxide or organotin dichloride compound to form a stannylene acetal on the galactopyranoside part of the compound (A0);

then reacting the stannylene acetal of the compound of Formula (A0) with a sulfur trioxide reagent to form an intermediate compound; and

salification of the intermediate compound to form the compound of Formula (IV).

Item 57. The process of claim 56, wherein the process comprises:

forming a mixture of the compound of Formula (A0) and the organotin oxide or organotin dichloride compound in a first organic solvent and then heating the mixture;

concentrating the mixture to form a concentrated mixture;

redissolving the concentrated mixture in a second organic solvent to form a redissolved mixture;

reacting the redissolved mixture with the sulfur trioxide reagent to form the intermediate compound; and

salification of the intermediate compound to form the compound of Formula (I).

Item 58. The process of claim 57, wherein the first organic solvent and the second organic solvent are independently selected from CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, tetrahydrofuran (THF), and any combination thereof; preferably the first organic solvent and the second organic solvent are independently selected from CH_2Cl_2, pyridine, toluene and any combination thereof; more preferably the first organic solvent and the second organic solvent are independently selected from pyridine, toluene and a combination thereof.

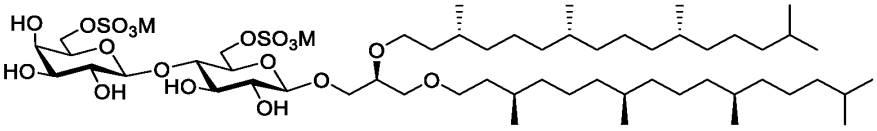
Item 59. The process of claim 57 or 58, wherein heating the mixture is performed at reflux and/or reacting the redissolved mixture with the sulfur trioxide reagent is performed at room temperature for about 1 hour to about 72 hours, preferably for about 10 hours to 72 hours, more preferably for at least 15 hours, for instance from about 15 hours to about 72 hours.

Item 60. The process of any one of claims 56 to 59, wherein the sulfur trioxide reagent is used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide, preferably from about 1 to about 7.5 molar equivalents, more preferably from about 1 to about 5 molar equivalents, for instance from about 1.05 to about 2 molar equivalents.

Item 61. The process of any one of claims 56 to 60, wherein the organotin oxide or organotin dichloride compound comprises a di(C_1-C_4alkyl)tin oxide or dichloride, preferably dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride, most preferably dibutyltin oxide.

Item 62. The process of any one of claims 56 to 61, wherein the sulfur trioxide reagent is as defined in any one of claims 2 to 4.

Item 63. A process for synthesizing a disulfated glycoarchaeal of Formula (V)

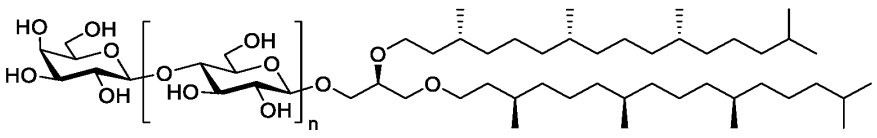


(V)

where M is an alkali metal;

the process comprising:

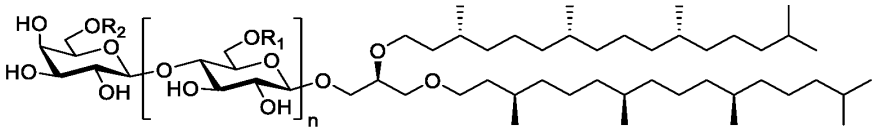
silylating the -OH in positions 6 and 6' of a compound of Formula (A)



(A)

where n is 1;

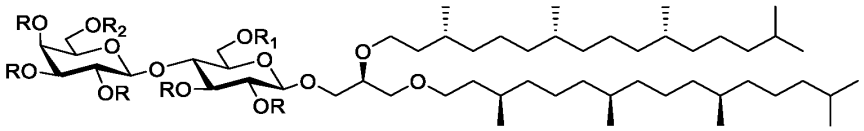
to form a 6- and 6'-silylated compound (A4)



(A4)

where n is 1 and R_1 and R_2 represent a silyl group;

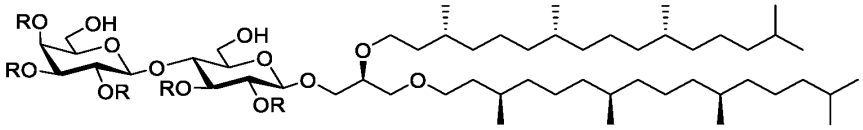
protecting the hydroxyl groups of the 6- and 6'-silylated compound (A4) to form the compound of Formula (F1)



(F1)

where R is a protecting group;

desilylating the compound of Formula (F1) to form the compound of Formula (G1);



(G1)

sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) with a sulfur trioxide reagent to form an intermediate compound; and

deprotecting the intermediate compound to form the compound of Formula (V).

Item 64. The process of claim 63, wherein silylating comprises reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

Item 65. The process of claim 63 or 64, wherein protecting the hydroxyl groups of the 6 and 6'-silylated compound (A4) comprises reacting the compound (A4) with a compound RX, where X is halogen and R is Ac or Bz, preferably Bz.

Item 66. The process of any one of claims 63 to 65, wherein desilylating the compound of Formula (F1) to form the compound of Formula (G1) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

Item 67. The process of any one of claims 63 to 66, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex; preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$,

Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof; more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof; even more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

Item 68. The process of claim 67, wherein the sulfur trioxide amine complex is used in an amount providing from about 2 to about 10 molar equivalents of sulfur trioxide, preferably from about 5 to about 10 molar equivalents, more preferably from about 7 to about 10 molar equivalents, for instance about 8 molar equivalents.

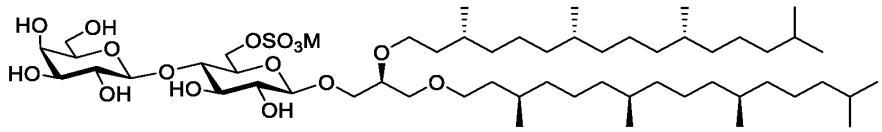
Item 69. The process of any one of claims 63 to 68, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises pyridine, toluene or a combination thereof.

Item 70. The process of any one of claims 63 to 69, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

Item 71. The process of any one of claims 63 to 70, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

Item 72. The process of any one of claims 63 to 71, wherein deprotecting the intermediate compound comprises reacting the intermediate compound with a basic solution preferably comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, more preferably the base is sodium methoxide.

Item 73. A process for synthesizing a sulfated glycoarchaeal of Formula (VI)

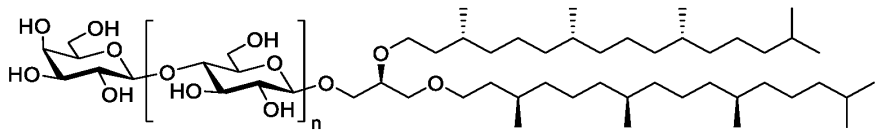


(VI)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$;

the process comprising:

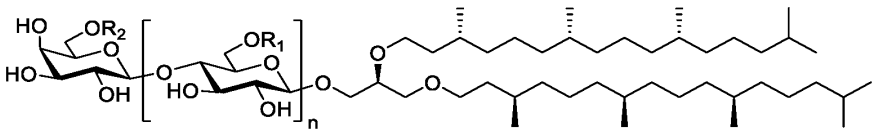
silylating the -OH in position 6' of a compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A2)



(A2)

where n is 1, R_1 is H, and R_2 represents a silyl group;

sulfating the -OH in position 6 of the compound of Formula (A2) with a sulfur trioxide reagent to form a 6-sulfated 6'-silylated compound (A6);

desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$; and

optionally contacting the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$ with an alkali metal cation exchange resin to form the compound of Formula (VI) where M is alkali metal.

Item 74. The process of claim 73, wherein silylating comprises reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

Item 75. The process of claim 73 or 74, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex; preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof; more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof; even more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

Item 76. The process of claim 75, wherein the sulfur trioxide amine complex is used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide, preferably from about 1 to about 5 molar equivalents, more preferably from about 1 to about 3 molar equivalents, for instance about 2 molar equivalents.

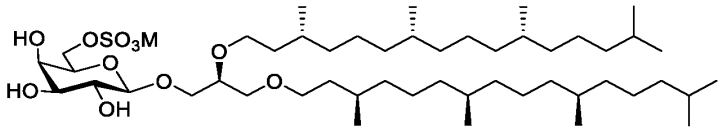
Item 77. The process of any one of claims 73 to 76, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises pyridine, toluene or a combination thereof.

Item 78. The process of any one of claims 73 to 77, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

Item 79. The process of any one of claims 73 to 78, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

Item 80. The process of any of claims 73 to 79, wherein desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

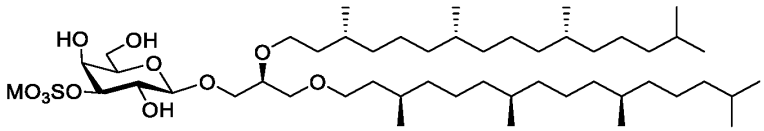
Item 81. A sulfated glycoarchaeal having the following formula (III)



(III)

wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$, wherein the sulfated glycoarchaeal is optionally obtained by the process of any one of claims 12 to 17 and 38.

Item 82. A sulfated glycoarchaeal having the following formula (IV)



(IV)

wherein M is Li, Na, or K, wherein the sulfated glycoarchaeal is optionally obtained by the process of any one of claims 56 to 62.

EXAMPLES

General Methods. Reactions were carried out under argon atmosphere using commercially available HPLC grade. Commercially available reagents (Sigma Aldrich, Toronto Research Chemicals, TCI America and Fisher Scientific, Canada) were used without further purification. Progress of reactions was monitored by thin-layer chromatography using silica gel 60 F_{254} coated plates (E. Merck). Flash chromatography was performed using ZEOprep™ silica gel 60 (40-63 μm) from Canadian Life Science or FlashPure™ system from BUCHI. Detection was carried out under UV light or by spraying with 20% ethanolic sulfuric acid or molybdate or $KMnO_4$ solution followed by heating. NMR spectra were recorded on Bruker ULTRASHIELD™ 300 MHz and Bruker Avance™III HD 400 and 600 MHz spectrometers. Proton and carbon chemical shifts (δ) are reported in ppm relative to the chemical shift of residual $CHCl_3$, which was set at 7.27 ppm (^1H) and 77.00 ppm (^{13}C). Coupling constants (*J*) are reported in Hertz (Hz), and the following abbreviations are used for peak multiplicities: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets with equal coupling constants (t_{ap}), triplet (t), multiplet (m). Analysis and assignments were made using COSY (COrrelated SpectroscopY) and HSQC (Heteronuclear Single Quantum Coherence) experiments. High-resolution mass spectra (HRMS) were measured with an LC-MS-TOF (Liquid Chromatography Mass Spectrometry Time Of Flight) instrument from Thermo Scientific in positive and/or negative electrospray mode. Either protonated ions $(M+H)^+$ or sodium adducts $(M+Na)^+$ were used for empirical formula confirmation. LC method: Samples were injected (2 μL) onto an PrePure C18 150x4.6 mm column with 5 μm particles (BUCHI) using a Dionex Ultimate 3000 system (Thermo Scientific) with water (A) and acetonitrile (B), both containing 0.1% acetic acid, at a flow rate of 800 μL/min at room temperature. The gradient started at 5% B, held for 0.5 min. It was increased to 15% B in 1 minute, then to 27% B in 14.5 minutes, and then to 95% B in 4 minutes. The gradient was held at 95% B for 2 minutes, and it was then decreased at 5% B in 1 minute. Finally, the gradient was held at 5% B for 1 minute. The total time was 24 minutes. MS method: MS spectra were collected on an TSQ Quantum Access Max (Thermo Scientific) equipped with a HESI ion source in positive ion mode set at 4,5 kV source voltage, 320°C source temperature. MS acquisition was from *m/z* 200-1000 in SCAN mode. The data was analyzed using Thermo XCalibur Qual Browser.

Analysis of archaeol: The archaeol sample was dissolved in methanol to obtain a concentration of 5 μg/μL. After addition of methanol, the sample was vortexed for 10 seconds, then sonicated for 5 minutes. This stock solution was then diluted 1:500 with methanol before being analyzed by mass spectrometry. A volume of 5 μL of the 1:500 dilution solution was injected on an HPLC (Transcend Ultimate 3000, Thermo Scientific) coupled to a Q-Exactive mass spectrometer (Thermo Scientific). The chiral column was a Hypersil Chiral-AT chiral column (3 μm, 250 mm X 4.6mm, Thermo Scientific). The mobile phase was methanol (100%). Chromatography was performed in isocratic mode at a flow rate of 400 μL/min, and the column was heated to 35°C. A post-column infusion set-up was prepared to infuse an aqueous solution of ammonium acetate (20 mM) to promote ionization by creating an ammonium adduct, at a flow rate of 10 μL/min with a Harvard infusion pump. The ammonium acetate solution was connected via a connecting

Tee between the column outlet and the mass spectrometer's electrospray probe. The total analysis time was 60 minutes. The mass spectrometer was programmed to perform a full scan from 100 to 1500 *m/z* in positive mode at a resolution of 70,000. The possible ion values sought for an archaeol molecule are as follows: $[M+H]^+$ at 653.6806 *m/z*, $[M+NH_4]^+$ at 670.7072 *m/z* and $[M+Na]^+$ at 675.6626 *m/z*.

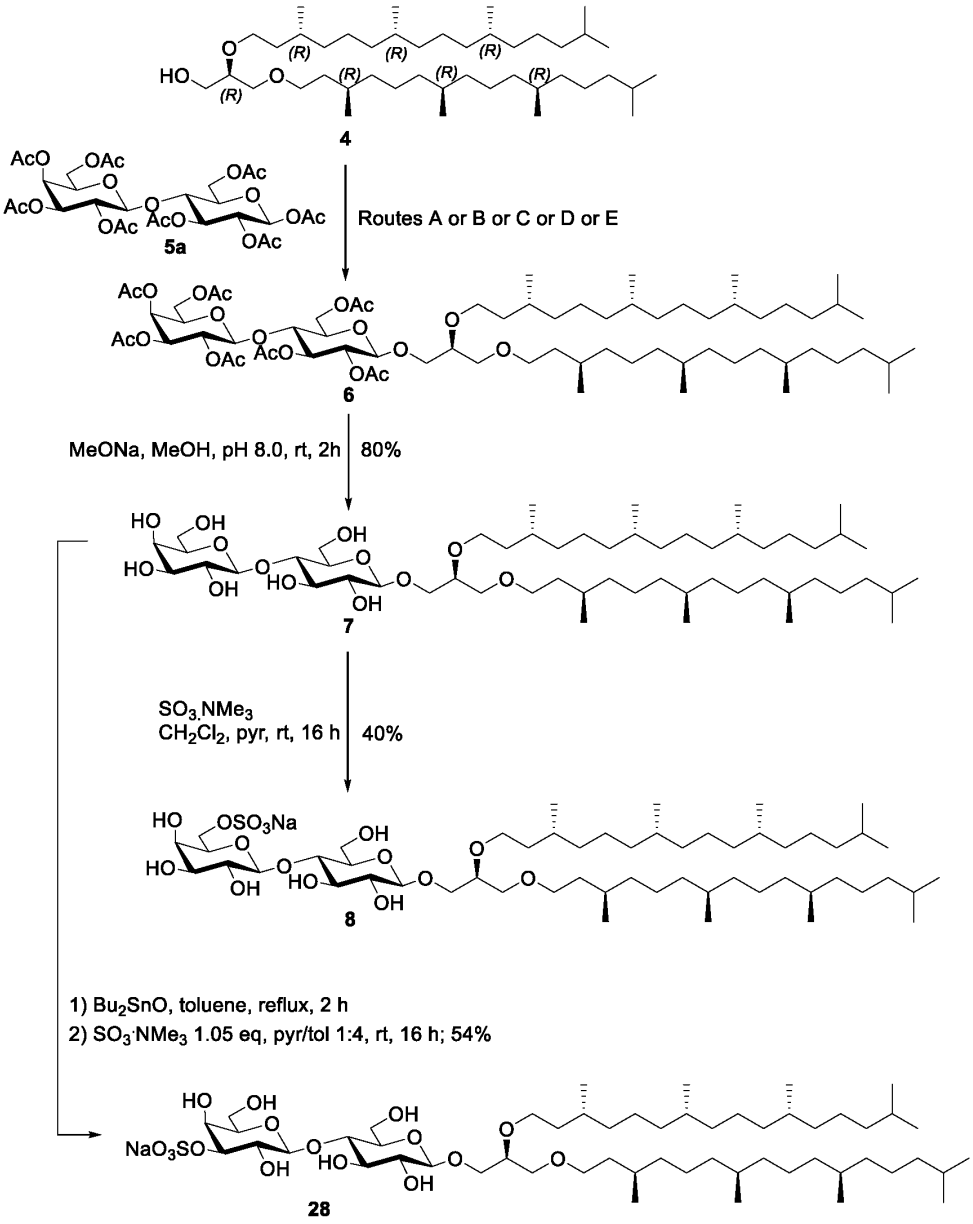
Analysis of SLA: The SLA sample was dissolved in methanol to obtain a concentration of 60 µg/µL. After addition of methanol, the sample was vortexed for 10 seconds, then sonicated for 5 minutes. This stock solution was then diluted 1:500 with methanol before being analyzed by mass spectrometry. A volume of 10 µL was injected on an HPLC (Transcend Ultimate 3000, Thermo Scientific) coupled to a Q-Exactive mass spectrometer (Thermo Scientific). Two columns with different stationary phases were used to characterize the sample. The first column was a Hypersil Gold C8 column (3 µm, 50 mm X 3 mm, Thermo Scientific). The second column was a PLRP-S (polystyrene/divinylbenzene, 3 µm, 50 mm X 4.6 mm, Agilent Technologies). The mobile phases were as follows. Phase A: Water containing ammonium formate (10 mM) and formic acid (0.1% v/v). Phase B: Methanol containing formic acid (0.1% v/v). Chromatography was performed at a flow rate of 500 µL/min. The gradient started at 55% B, increased to 97% B in 400 seconds, then to 98% B in 500 seconds. The total time analysis time was 15 minutes. A first acquisition on the mass spectrometer was done by first performing a full scan from 150 *m/z* to 2000 *m/z* in negative mode, then a second acquisition was done from 150 *m/z* to 2000 *m/z* in positive mode with the resolution set to 70,000 for both polarity modes. The ion value sought in negative mode for the SLA molecule is $[M-H]^-$ at 1055.7280 *m/z*. In positive mode the possible ions sought are: $[M+NH_4]^+$ at 1074.7702 *m/z* and $[M+Na]^+$ at 1079.7256 *m/z*.

Preparation of liposomes: SLA was hydrated in sterile 1X PBS at concentration of 40 mg/mL and vortexed for 60 seconds. The suspension was then sonicated for one minute, and vortexed for one minute again. The vortex and sonification process were repeated 3-5 cycles until most large aggregates were broken up. The lipid was then hydrated for one hour at 40 °C in a heating block, while being periodically mixed by vortex (one minute every 10 minutes). After one hour, the lipids were then subjected to 5 freeze/thaw/vortex cycles to further homogenize the liposomes and complete the hydration process. Lipids were first frozen in liquid nitrogen, then thawed in a water bath (37 °C). After each thawing, the mixture was vortexed for 30 seconds. Extruding the liposomes was carried out under aseptic conditions. The liposomes were passed through the polycarbonate membrane (100 nm pore size) for a total of 11 passes. The liposomes were filtered under 200 nm and transferred to a sterile vial for DLS, ELISA and bioassay analysis.

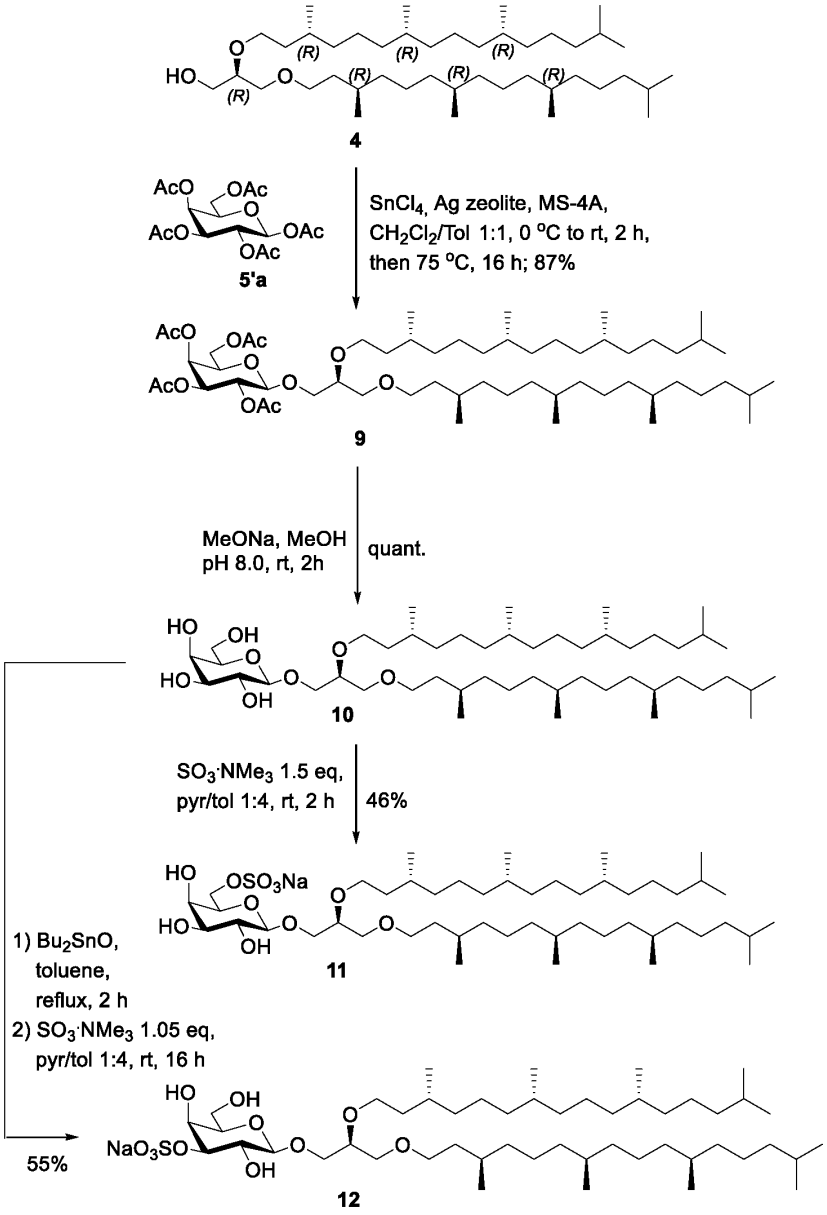
ELISA bioassays analysis: RAW264.7 cells, a murine macrophage-like cell line, were maintained in DMEM growth media supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin. RAW264.7 cells were grown to 70-80% confluency in a T75 flask and detached using enzyme-free dissociation buffer and a cell scrapper. Once resuspended in growth media, 200,000 cells were aliquoted per well in a 96-well plate. Immediately after seeding, serial dilutions of various forms of SLA were added to the 96-well plate. 800 ng/mL of CL307 and unstimulated cells were used as positive and negative controls, respectively (data not shown). RAW264.7 cells were stimulated for 24 hours, and

supernatant was subsequently collected. TNF-α concentration in supernatant was quantified by enzyme-linked immunosorbent assay (ELISA) using the Invitrogen™ TNF alpha Mouse Uncoated ELISA Kit (Fisher Scientific) as per the manufacturer's protocol. Each dot represents the mean of 3 technical replicates. Error bars, SD. Asymmetrical (five-parameter) logistic dose-response curves and EC50 values were derived using GraphPad Prism 10.3.0.

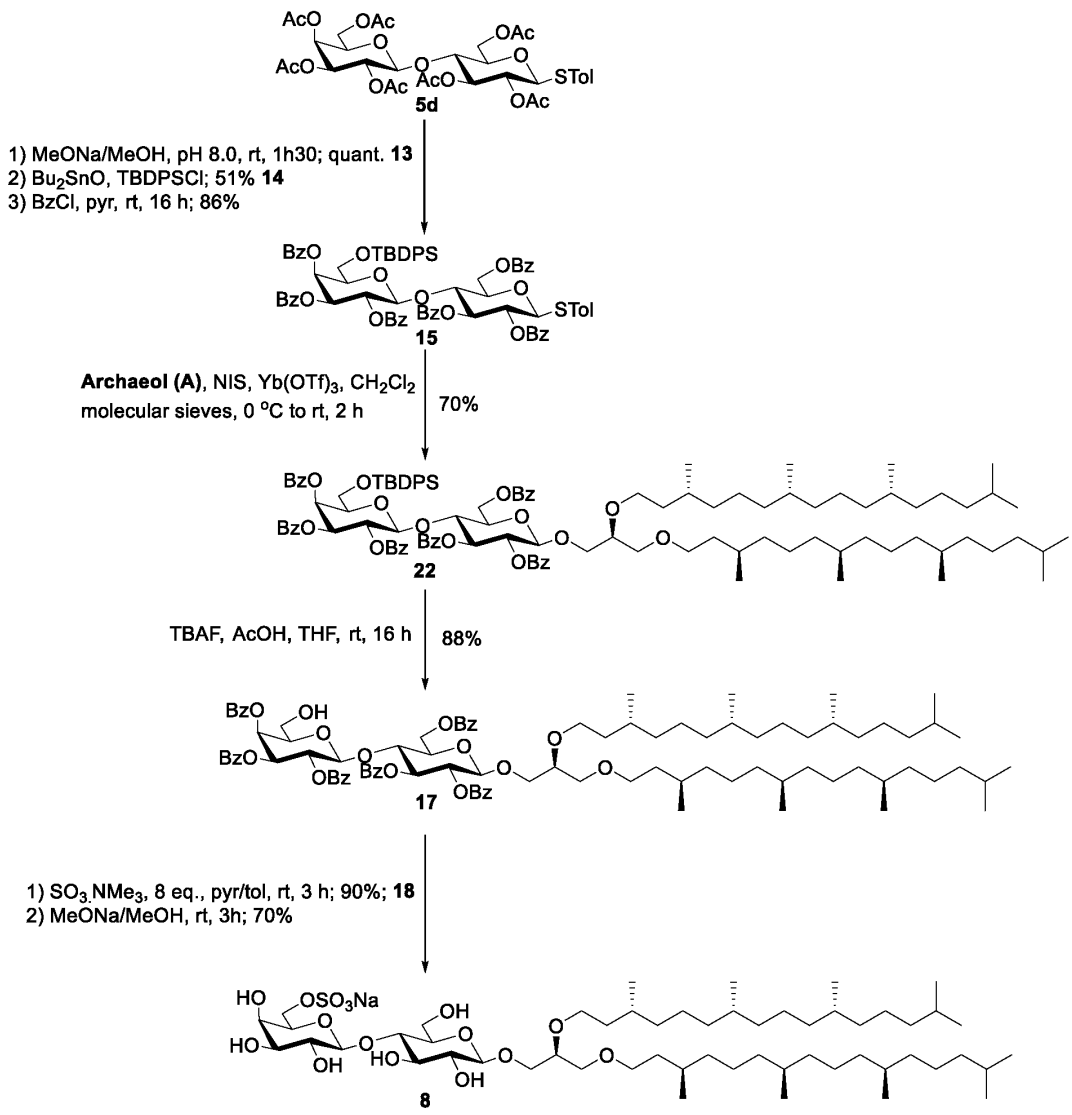
Synthesis schemes to prepare archaeol-based sulfated carbohydrates



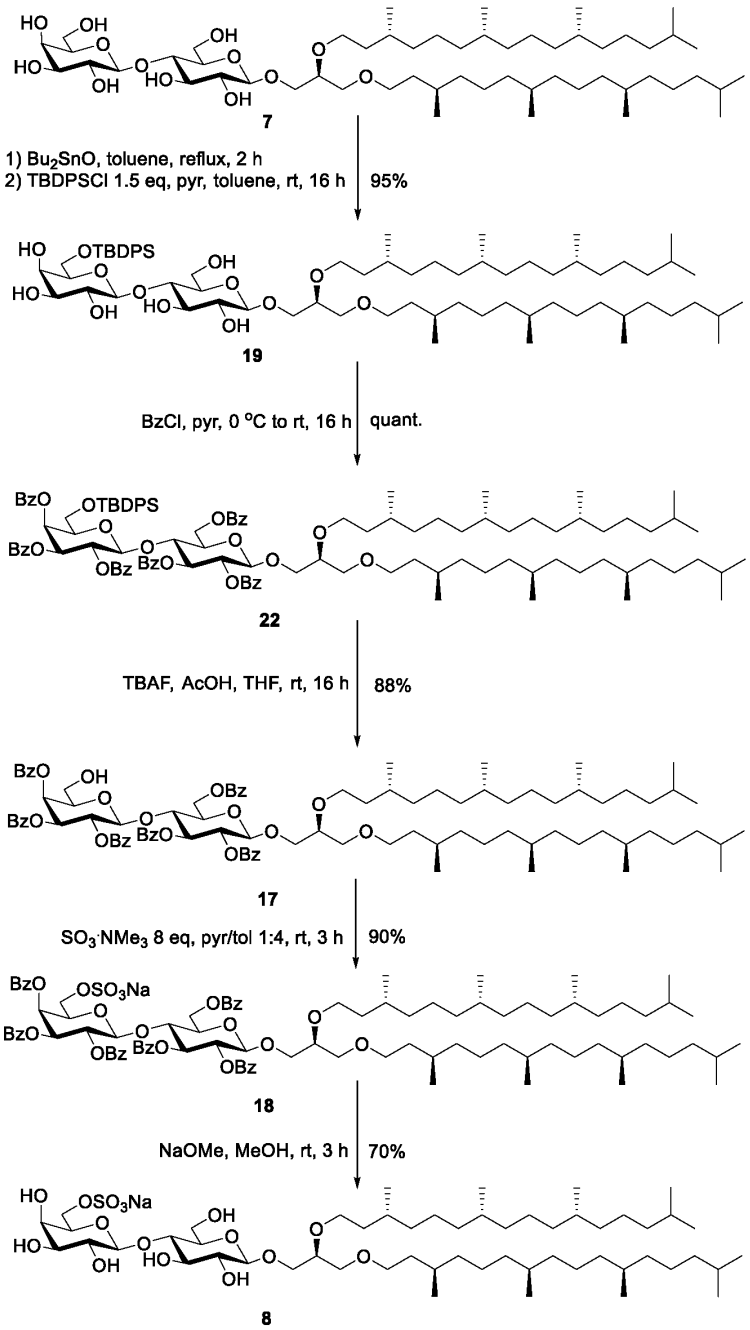
Scheme 1: Synthesis of 6'- and 3'-SLA by direct sulfation of archaeal carbohydrate



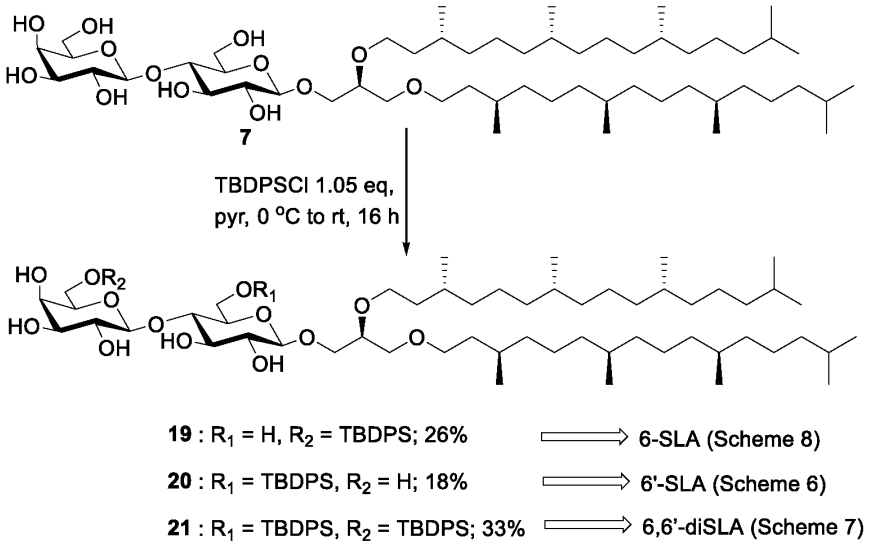
Scheme 2: Synthesis of 6- and 3-sulfated monosaccharide by direct sulfation of archaeal carbohydrate



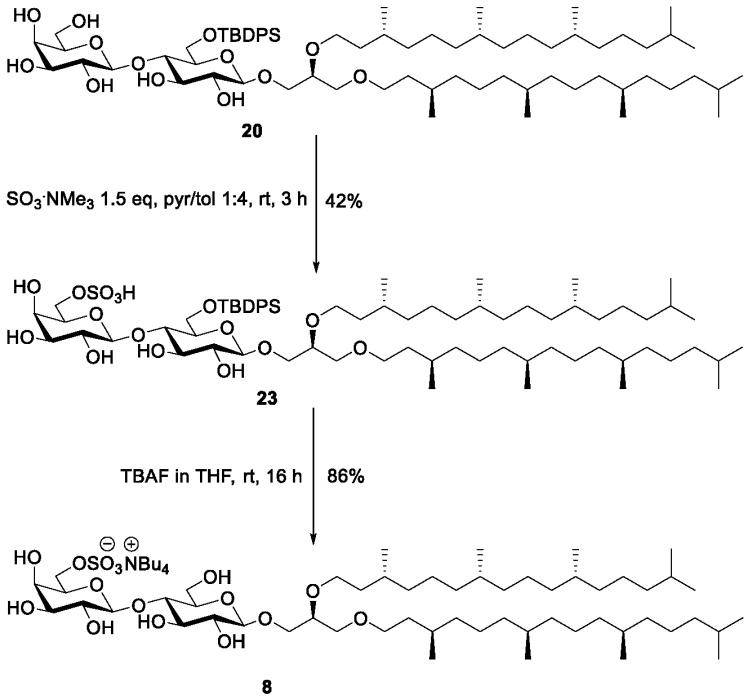
Scheme 3: Synthesis of 6'-SLA by selective sulfation involving orthogonal protection method I



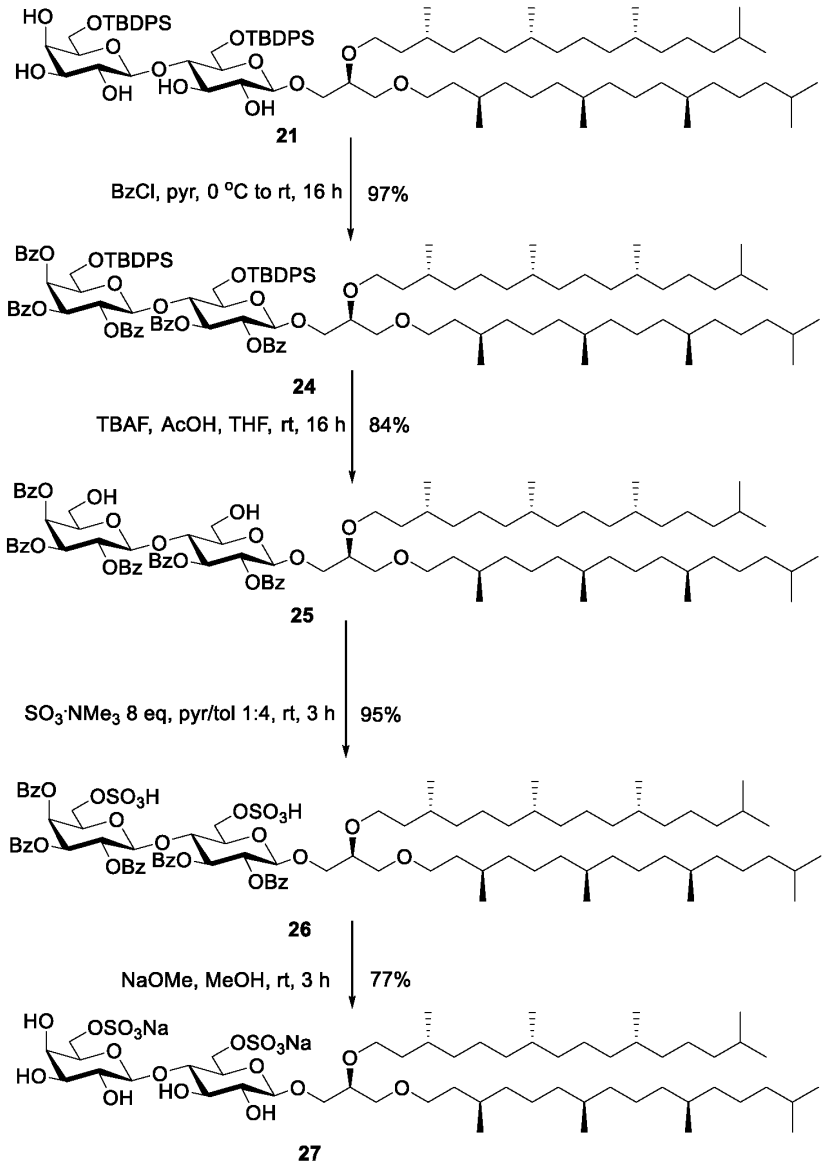
Scheme 4: Synthesis of 6'-SLA by selective sulfation involving orthogonal protection method II



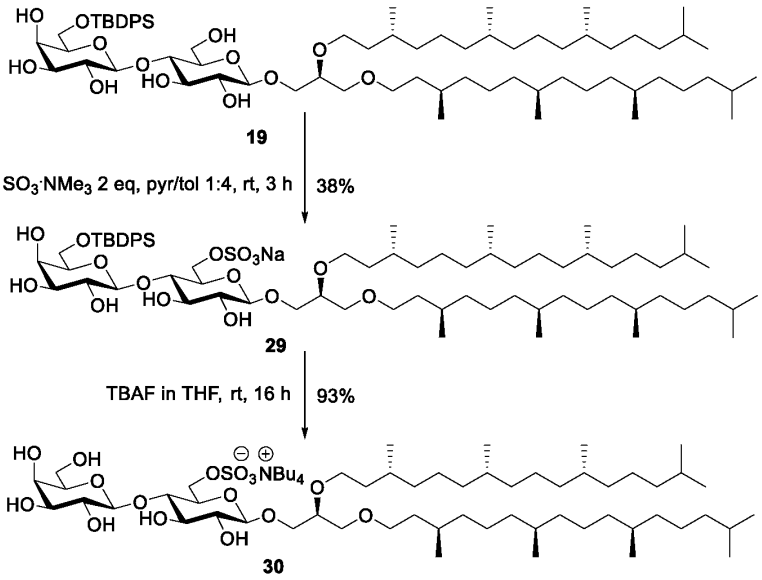
Scheme 5: Synthesis of 6-SLA, 6'-SLA and 6,6'-diSLA involving orthogonal protection



Scheme 6: Synthesis of 6'-SLA by selective sulfation with orthogonal protection selective



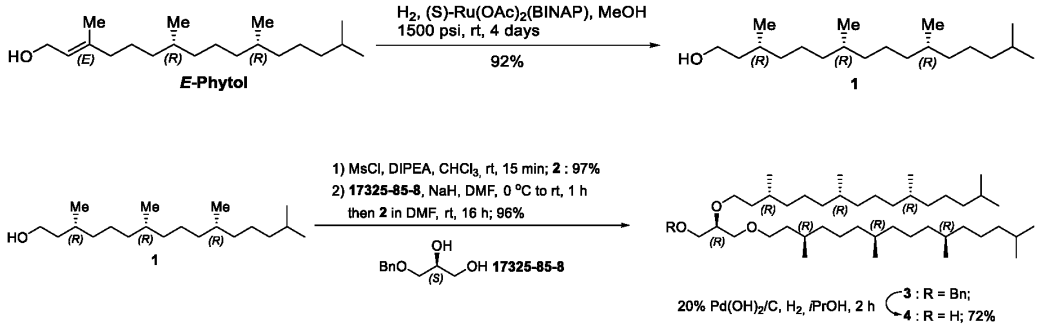
Scheme 7: Synthesis of 6,6'-diSLA by selective bis-sulfation with orthogonal protection selective



Scheme 8: Synthesis of 6-SLA by selective sulfation with orthogonal protection

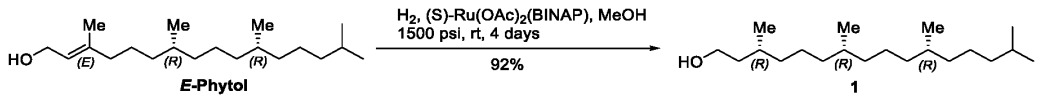
EXAMPLE 1: PREPARATION OF THE PURE DIASTEREOISOMER R ARCHAEOL - (R)-2,3-BIS(((3R, 7R, 11R)-3,7,11,15-TETRAMETHYLHEXADECYL)-OXY)PROPAN-1-OL (4)

General synthesis scheme



STEP 1: Synthesis of phytanol from pure commercial *E*-phytol - (3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecan-1-ol (1)

According to literature process: *J. Biol. Chem.* 2021, *296*, 100691



To an oven-dried 20 mL vial equipped with magnetic stirrer bar was added trans-phytol (1 g, 3.4 mmol) in 3 mL of dry methanol under nitrogen atmosphere. The resulting mixture was degassed by the freeze-pump-thaw technique after which (*S*)-BINAP-ruthenium(II) dicarboxylate (**II**) (6 mg, 0.07 mmol, 0.2 mol%) was added. The vial containing the reaction mixture was placed inside a 0.5 L Parr bomb and subjected to 75 bar or 1500 psi of H_2 pressure for 4 days. The resulting brown solution was concentrated *in vacuo* and

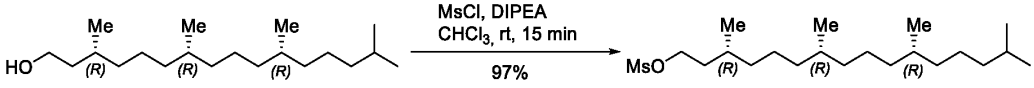
passed over a small silica column eluted with pentane. Evaporation of the solvent yielded diastereoisomerically pure (*R,R,R*)-phytanol **1** as a yellow oil (96% yield, 0.97 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76-3.61 (m, 2H), 1.99 (s, 1H), 1.73-1.45 (m, 3H), 1.45-0.96 (m, 21H), 0.95-0.76 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 61.2, 40.1, 39.5, 37.7, 37.6, 37.5, 37.5, 37.4, 32.9, 32.9, 29.7, 28.1, 24.9, 24.6, 24.5, 22.8, 22.7, 19.9, 19.9, 19.8. $[\alpha]_D$ = +1.90 (c = 0.1 in $CHCl_3$). HRMS (ESI+) calcd. for $C_{20}H_{46}NO$ $[M+NH_4]^+$: 316.3574, found 316.2253.

According to literature process: *J. Org. Chem.* 1997, *62*, 1924-1933

A mixture of *E*-phytol (12.9 g, 43.5 mmol) and [(*S*)-(-)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl]chloro(*p*-cymene)ruthenium chloride (97 mg) in degassed methanol (40 mL) was placed in a stainless steel autoclave under argon atmosphere. High purity hydrogen gas (99.999% purity) was introduced into the reaction apparatus (90 kgf/cm^2; 1500 psi). The mixture was stirred at room temperature for 4 days. The mixture was concentrated *in vacuo*, and the residue was chromatographed over silica gel with hexane-EtOAc (10:1) to give phytanol (**1**) (11.9 g, 92%) as an oil. $[\alpha]_D$ = +2.29° (*c* 1.02, $CHCl_3$) (lit.[2a] $[\alpha]_D$ = +2.4°). ^{1}H NMR (300 MHz) δ 0.84-0.91 (d, *J* = 6.6, 15H) 1.00-1.67 (m, 25H), 3.62-3.75 (m, 2H). ^{13}C NMR (75 MHz) δ 19.66, 19.74, 19.76, 22.62, 22.72, 24.35, 24.44, 24.78, 27.96, 29.49, 32.78, 37.26, 37.29, 37.36, 37.42, 37.47, 39.34, 39.93, 61.24. IR (neat): 737, 760, 1009, 1057, 1365, 1377, 1464, 2870, 2925, 2954, 3330 cm^{-1}.

STEP 2: (3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecyl methanesulfonate (2)

Reference: *J. Biol. Chem.* 2021, *296*, 100691

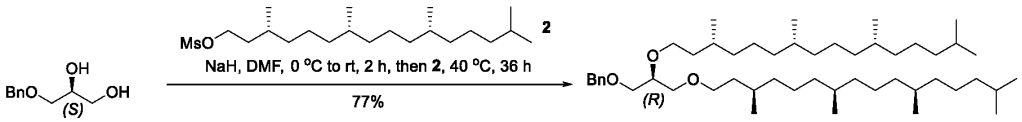


1 (Phytanol) **2**

To a stirring solution of phytanol **1** (955 mg, 3.20 mmol) and MsCl (372 µL, 4.80 mmol, 1.5 equiv.) in $CHCl_3$ (12 mL) at room temperature, was added N, N-diisopropylethylamine (DIPEA) (1.677 mL, 9.60 mmol, 3.0 equiv.). After 15 minutes, the mixture was quenched by the addition of 1) $NaHCO_3$ (sat) (50 mL) and then 2) brin (50 mL). The aqueous layer was extracted with dichloromethane (1x50 mL and 2x20mL). All the organic layers were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pression without heating (25 °C), the crude oil (1.54 g) was further purified by column chromatography (by gradient 100% hexanes to hexanes/EtOAc 95:5); (silica gel in Hexanes: 80 mL; column 30x150 cm; hexanes 100% 80 mL; hexanes/EtOAc 95:5 160 mL; start for collection); to give the desired mesylate **3** as a yellow light oil (97%, 1.168 g, 3.10 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.34-4.13 (m, 2H), 2.99 (s, 3H), 1.84-1.70 (m, 1H), 1.70-1.44 (m, 3H), 1.44-0.97 (m, 20H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.88-0.80 (m, 12H). HRMS (ESI+) calcd. for $C_{21}H_{48}NO_3S$ $[M+NH_4]^+$: 394.3349, found 394.3350.

STEP 3: 1-*O*-Benzyl-2,3-bis-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecan-1-yl]-*sn*-glycerol (3)

Reference: *J. Org. Chem.* 1997, *62*, 1924-1933

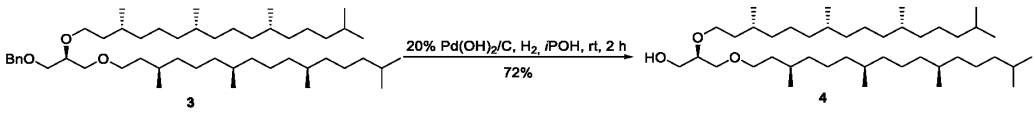


17325-85-8 3

To a suspension of NaH (60% in mineral oil, 705.6 mg, 17.64 mmol, 6.0 equiv.) in dry DMF (10.0 mL) was added at 0 °C under nitrogen a solution of 1-*O*-benzyl-*sn*-glycerol (CAS No. 17325-85-8) (535.7 mg, 2.94 mmol, 1.0 equiv.) in dry DMF (15.0 mL). After stirring at room temperature for 2 hours, a solution of mesylate **2** (3.54 g, 9.41 mmol, 3.2 equiv.) in dry DMF (25.0 mL) was added. The mixture was stirred at 40 °C for 36 h then quenched by cold brine (15.0 mL) and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel by gradient using 100% Hexanes to Hexanes/EtOAc (99:1) to afford **3** (1.68 g, 2.26 mmol, 77%) as colorless oil. $[\alpha]_D$ = +2.85° (*c* 0.64, $CHCl_3$). ^{1}H NMR (300 MHz) δ 0.83-0.88 (10 x d, 30H), 1.00-1.68 (m, 48H), 3.41-3.67 (m, 9H), 4.56 (s, 2H), 7.26-7.34 (m, 5H).

STEP 4: 2,3-Di-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadecanyl]-*sn*-glycerol (4)

Reference: *J. Org. Chem.* 1997, *62*, 1924-1933

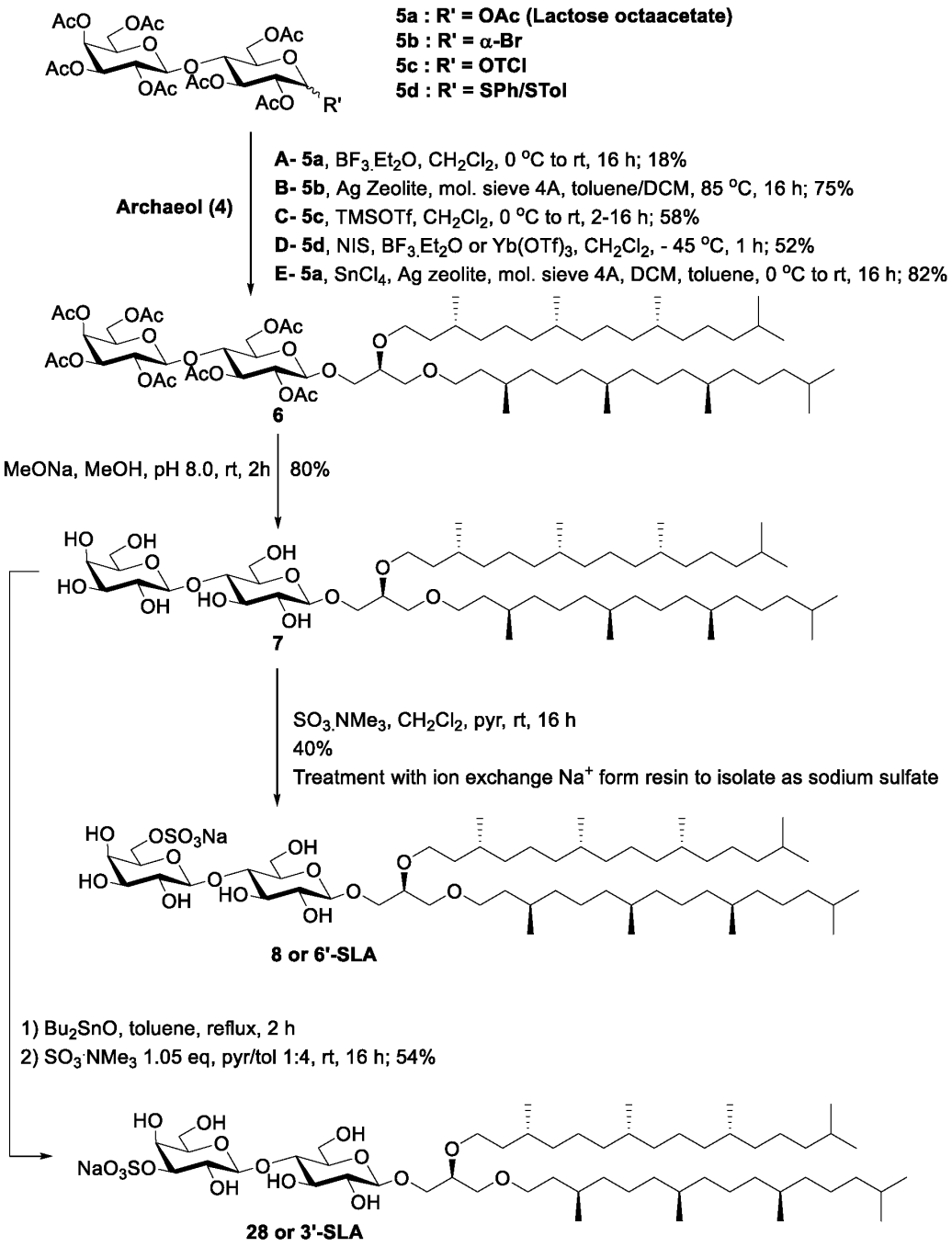


3 4

A mixture of **3** (979 mg, 1.32 mmol) and 20% $Pd(OH)_2$/C (50% water, 925 mg, 6.59 mmol, 5.0 equiv.) in isopropanol (44 mL) was stirred at room temperature under bubbled hydrogen atmosphere until total consumption of starting material **3**, checked by TLC (Hexanes/EtOAc 98:2): 2 hours. The catalyst was filtered through a pad mixture of Celite and silica gel (1:1 v/v) and washed with EtOAc. The filtrate and washings were combined and concentrated to dryness. The residue was purified by flash chromatography over silica gel with hexane-EtOAc (100:1) to give pure diastereoisomer archaeol **4** (620 mg, 0.95 mmol, 92%) as colorless oil. $[\alpha]_D$ = +8.66° (*c* 1.07, $CHCl_3$). ^{1}H NMR (300 MHz) δ 0.83-0.89 (m, 30H) 1.00-1.69 (m, 48H), 2.21 (t, *J* = 6.0, 1H), 3.44-3.76 (m, 9H). HRMS (ESI+) calcd. for $C_{43}H_{88}O_3Na$ $[M+Na]^+$: 675.6626, found 675.6601. Purity analysis of Archaeol **4** is performed by chiral LC-MS according to reference: J. Med. Chem. 2022, 65, 8332-8344.

EXAMPLE 2: SYNTHESIS OF 6'- AND 3'-SLA BY DIRECT SULFATION OF ARCHAEAL CARBOHYDRATE

According to general Scheme 1, further detailed below.



As noted from the above scheme, the preparation of the protected glycosylated Archaeal compound **6** can be performed according to various glycosylation conditions, which will be detailed below. The synthesis of compounds **5b-5d** is provided first.

STEP 1: SYNTHESIS OF LACTOSIDE DONORS 5b, 5c, AND 5d

Reference: *Carbohydr. Res.* **2010**, *345*, 214-229

Lactose octaacetate (5a) is commercially available (e.g., Sigma-Aldrich).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (5b)

Reference: ***Carbohydrate Chemistry: Proven Synthetic Methods*, 2014, *2*, Chap. 33, pp. 269-274**

A 33% solution of HBr in AcOH (2.28 mL, 13.0 mmol, 13.0 equiv.) was added in one portion to a solution of lactose octaacetate **5a** (679 mg, 1.0 mmol, 1.0 equiv.) in dry CH_2Cl_2 (3.0 mL, 0.33 M). The resulting mixture was stirred at room temperature for 1 hour. Upon completion of the reaction (TLC 3:2 EtOAc-Hexanes), the mixture was poured slowly into a cold, saturated aqueous solution of $NaHCO_3$ (50 mL), the flask was washed with CH_2Cl_2 (3 × 10 mL) and the contents were added to the mixture. The two phases were stirred until effervescence ceased (a large amount of CO_2 is produced during this operation, and slow addition of the reaction mixture is important when working on a large scale). The aqueous layer was decanted, and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 50 mL) and brine (50 mL). Drying over Na_2SO_4, filtration, and concentration under reduced pressure gave the bromide intermediate **5b** as a white solid. Compound **5b** could be used for the next step without further purification or be recrystallized from DCM-Et2O-petroleum ether. *Rf* = 0.27 hexanes/EtOAc (1:1); mp 138.5-139.0°C. ^{1}H NMR ($CDCl_3$) δ 6.52 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1I), 5.55 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3I), 5.35 (dd, 1H, $J_{3,4} = J_{4,5}$ 3.4 Hz, H-4II), 5.12 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.3 Hz, H-2II), 4.96 (dd, 1H, H-3II), 4.76 (dd, 1H, H-2I), 4.51 (d, 2H, H-1II), 4.51 (m, 1H, H-6aI), 4.18 (m, 3H, H-6aII, H-6bI and H-5II), 4.08 (dd, 1H, $J_{6a,6b}$ 11.2 Hz, $J_{5,6b}$ 7.3 Hz, H-6bII), 3.87 (m, 2H, H-5I and H-4I), 2.16, 2.13, 2.09, 2.06, 2.06, 2.05 and 1.96 ppm (7s, 21H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.3, 170.1, 170.1, 170.0, 170.0, 169.2, 168.9 (CO), 100.8 (C-1II), 86.4 (C-1I), 75.0 (C-4I), 72.9 (C-5I), 71.0 (C-3I), 70.8 (C-5II), 70.8 (C-3II), 69.6 (C-2I), 69.0 (C-2II), 66.6 (C-4II), 61.0 (C-6II), 60.8 (C-6I), 20.8, 20.8, 20.7, 20.6, 20.6, 20.6 and 20.5 ppm ($COCH_3$). ESI+-HRMS: $[M + NH_4]^+$ calculated for $C_{26}H_{39}NO_{17}Br$, 716.1396; found, 716.1383.

2,2,2-Trichloroacetimidate 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (5c)

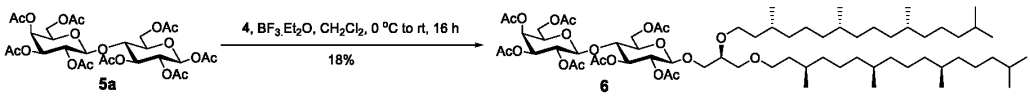
To a solution of β-lactose octoacetate **5a** (1.017 g, 1.5 mmol, 1 equiv.) in THF (6 mL) was added $BnNH_2$ (250 µL, 2.3 mmol, 1.5 equiv.). The mixture was stirred at room temperature for 16 h. Then, the crude was concentrated under reduced pressure, solubilized in EtOAc, and washed successively with 1M aqueous solution of HCl, water, a saturated aqueous solution of $NaHCO_3$, water and brin. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (Hexanes/Ethyl acetate from 1/1 to 3/7) gave the intermediate hemiacetal compound (950 mg, 1.5 mmol, quant.) as a brownish foaming oil. Without any characterization: To a solution of the hemiacetal (521 mg, 0.82 mmol, 1 equiv.) in dichloromethane (DCM) (4 mL) were successively added CCl_3CN (1.1 mL, 10.7 mmol, 13 equiv.), and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (16 µL, 0.1 mmol, 0.13 equiv.). The mixture was stirred at room temperature for 20 h and partially concentrated under reduced pressure. Flash column chromatography (Hexanes/Ethyl acetate from 7/3 to 1/1) gave the desired compound **5c** (587 mg, 0.75 mmol, 92%) as a colorless foaming oil. This imidate compound was not characterized and was used immediately in the following glycosylation step.

Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (5d)

To a mixture of lactose octaacetate **5a** (15.1 g, 22.25 mmol, 1.0 equiv.) and thiophenol (4.55 mL, 44.50 mmol, 2.0 equiv.) in dichloromethane (50 mL) was added dropwise boron trifluoride etherate (14.1 mL, 111.25 mmol, 5.0 equiv.) at 0 °C under nitrogen atmosphere. The mixture was stirred at under nitrogen at 0 °C for 2 h, the mixture was then washed with a saturated $NaHCO_3$ solution and brine, dried over sodium sulfate, and concentrated under reduced pressure. Crude residue was purified by flash chromatography on silica gel (gradient from Hexane 100% to Hexane/EtOAc 6:4) to afford the thiophenyl lactoside **5d** as white solid. (15.1 g, 20.7 mmol, 93% yield). R_f = 0.32 (Hexane/EtOAc 1:1).

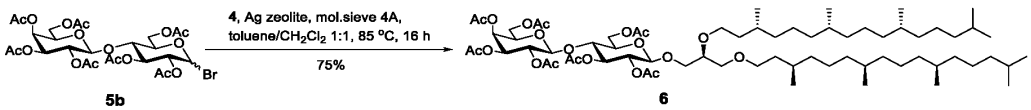
STEP 2: GLYCOSYLATION – Synthesis of Archaeal 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (6)

ROUTE 2A



To a solution of lactose octaacetate **5a** (166 mg, 0.25 mmol, 1.0 equiv.) and archaeol **4** (320 mg, 0.50 mmol, 2.0 equiv.) in dry dichloromethane (6 mL) under nitrogen atmosphere at 0 °C, was added dropwise a solution of BF_3-OEt_2 in dry dichloromethane (57 µL, 0.47 mmol, 1.9 equiv., 500 µL). The mixture was stirred at 0 °C to room temperature overnight (16 h). After TLC showed the incomplete reaction and the formation of byproduct ortho-ester, the mixture was diluted with dichloromethane (20 mL) and washed with a saturated $NaHCO_3$ solution (20 mL), water (10 mL), brin (10 mL) and extraction with dichloromethane twice (2 x 20 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel hexanes/EtOAc/DCM 6.5:2.5:1. The unreacted archaeol can be recovered by flash chromatography on silica gel first with hexanes/EtOAc 95:5 and isolated as colorless oil (235 mg, 0.36 mmol, 73% yield). The undesired byproduct lactose archaeal orthoester (on position 2) was isolated as colorless oil (18 mg, 0.014 mmol, 5.7% yield). The desired lactose archaeal compound **6** was isolated as colorless oil (58 mg, 0.046 mmol, 18% yield, 69% recover yield). R_f = 0.18 (Hexanes/EtOAc/DCM 6.5:2.5:1). HRMS (ESI+) calcd. for $C_{69}H_{122}O_{20}Na$ $[M+Na]^+$: 1293.8422, found 1293.8362.

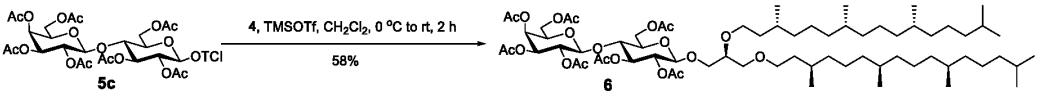
ROUTE 2B



To the solution of archaeol **4** (1.3 g, 2 mmol, 1.0 equiv.) in dry toluene (40 mL) was added activated molecular sieves 4Å powder (10 g) and silver-exchanged zeolite (30 g: 1.5 g/mmol of donor) at room temperature. The mixture was stirred under nitrogen at room temperature for 30 min. A solution of bromide **5b** (14 g, 20 mmol, 10.0 eq) in dry DCM (40 mL) was then added slowly into the mixture. The reaction

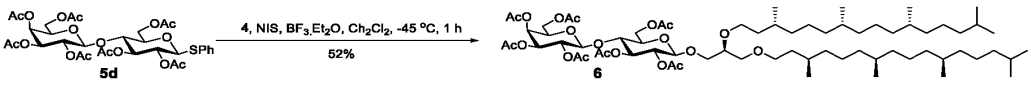
was then heated up to 85 °C and kept stirring overnight (16 h). After cooling down to room temperature, the mixture was filtered over a pad of Celite® and washed with EtOAc. The filtrate solution was concentrated under reduced pressure. The crude residue was then purified by flash chromatography on silica gel (gradient from hexanes 100% to hexanes/EtOAc/CH_2Cl_2 6.5:2.5:1) to afford the desired glycoside **6** as colorless oil (1.9 g, 1.49 mmol, 75% yield). R_f = 0.18 (Hexanes/EtOAc/ CH_2Cl_2 6.5:2.5:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.27 (t, *J* = 7.4 Hz, 1H), 5.12 (t, *J* = 9.3 Hz, 1H), 5.07-4.99 (m, 1H), 4.88 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.84 (dd, *J* = 9.5, 8.0 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.40 (dd, *J* = 8.9, 4.8 Hz, 2H), 4.13-3.96 (m, 3H), 3.84-3.77 (m, 2H), 3.72 (t, *J* = 9.5 Hz, 1H), 3.55-3.42 (m, 5H), 3.42-3.27 (m, 4H), 2.21-1.79 (m, 21H), 1.62-1.34 (m, 6H), 1.34-0.95 (m, 42H), 0.82-0.75 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.35, 170.16, 170.07, 169.76, 169.50, 169.07, 101.12, 100.83, 77.86, 76.34, 72.92, 72.57, 71.72, 71.01, 70.68, 70.54, 70.44, 70.12, 69.18, 69.11, 66.60, 62.05, 60.79, 39.38, 37.60, 37.55, 37.51, 37.47, 37.42, 37.40, 37.30, 37.14, 36.62, 32.81, 29.95, 29.85, 27.98, 24.80, 24.48, 24.37, 22.73, 22.64, 20.87, 20.83, 20.71, 20.65, 20.64, 20.52, 19.76, 19.71. HRMS (ESI+) calcd. for $C_{69}H_{122}O_{20}Na$ $[M+Na]^+$: 1293.8422, found 1293.8409.

ROUTE 2C



To a solution of archaeol **4** (336 mg, 0.51 mmol, 1 equiv.) and imidate **5c** (OTCl = trichloroacetimidate) (432 mg, 0.55 mmol, 1.1 equiv.) in dry DCM (6 mL) was added 4 Å molecular sieves (913 mg, 1.5 mg/mmol). The mixture was stirred at room temperature for 1.5 h. Then, the solution was cooled to 0 °C, and TMSOTf (10 µL, 0.06 mmol, 0.11 equiv.) was added. The mixture was allowed to warm to room temperature for 2 h before neutralization with 500 µL of Et_3N. The crude was concentrated under reduced pressure. Flash column chromatography (Hexanes/Ethyl acetate from 9/1 to 7/3) gave lactose archaeal compound **6** (329 mg, 0.26 mmol, 54%) as a mixture of the desired glycolipid and the corresponding orthoester, as a white amorphous solid oil. Both compounds were characterized by NMR. To a solution of the orthoester (236 mg, 0.19 mmol, 1 equiv.) in dry DCM (5 mL). The mixture was cooled to 0 °C before addition of TMSOTf (4.2 µL, 24 µmol, 0.13 equiv.), and the solution was stirred for 1.5 h before addition of 200 µL of Et_3N. The crude was concentrated under reduced pressure. Flash column chromatography (Hexanes/Ethyl acetate from 9/1 to 7/3) gave the desired lactose archaeal compound **6** (136 mg, 0.11 mmol, 58%) as a colorless oil. R_f = 0.18 (Hexanes/EtOAc/DCM 6.5:2.5:1).

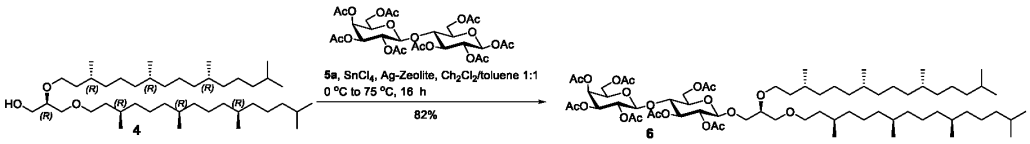
ROUTE 2D



Under literature conditions (*Carbohydr. Res.* 2010, *345*, 214-229), archaeol **4** (51 mg, 0.078 mmol, 1.0 equiv.) and thiophenyl lactoside donor **5d** (79 mg, 0.109 mmol, 1.4 equiv.) were dissolved in dry dichloromethane (1.5 mL) and cooled in an ice bath under an argon atmosphere. To this solution was added

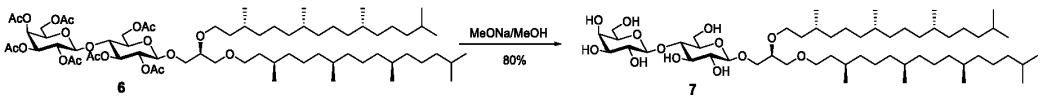
NIS (44 mg, 0.195 mmol, 2.5 equiv.) followed by a 0.25 M solution of BF_3-OEt_2 trifluoroethanol [156 µL, 0.5 equiv.; made from a solution of trifluoroethanol (360 µL) in dichloromethane (10.0 mL) at -45 °C (dry ice acetonitrile bath) to which was added BF_3-OEt_2 (314 µL) followed by treatment under vacuum, about 5 torr, for 20 min]. Stirring was continued for 1 h. The reaction mixture was then diluted with dichloromethane, and the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution followed by 10% aqueous $Na_2S_2O_3$. After drying with anhydrous Na_2SO_4, the organic layer was filtered and concentrated. The residue was purified by silica gel chromatography eluting with hexanes/EtOAc/DCM 6.5:2.5:1 to yield **6** (52 mg, 52%).

ROUTE 2E



To a mixture of archaeol **4** (78 mg, 0.12 mmol, 1.0 equiv.) and lactose **5a** (814 mg, 1.20 mmol, 10.0 equiv.) in dry dichloromethane (10 mL) and dry toluene (10 mL) was added activated molecular sieves 4Å powder (900 mg) and silver-exchanged zeolite (1.8 g: 1.5 g/mmol of donor) (preactivated at 300 °C) at room temperature. The mixture was stirred under nitrogen at room temperature for 30 min. A solution of 1M tin(IV) chloride in dichloromethane (1.44 mL, 1.44 mmol, 12.0 equiv.) was added into the reaction. The reaction was then stirred at room temperature or can heated up to 75 °C and kept stirring overnight (16 h). After cooling down to room temperature, the mixture was then neutralized by adding triethylamine until pH 7.0. The mixture was then filtered over a pad of Celite® and washed with EtOAc. The filtrate solution was concentrated under reduced pressure. The crude residue was then purified by flash chromatography on silica gel (gradient from hexanes 100% to hexanes/EtOAc 94:6) to recover unreacted archaeol reagent **4** and the purification system was changed to (Hexanes/EtOAc/DCM 6.5:2.5:1) to afford the desired glycoside **6** as colorless oil (125 mg, 0.098 mmol, 82%).

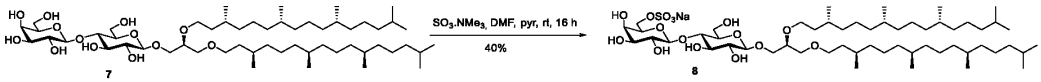
STEP 3: DEACETYLATION BY TRANS-ESTERIFICATION – Synthesis of Archaeal (β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (7)



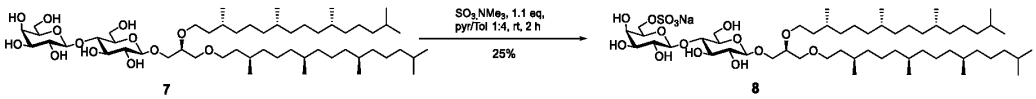
A solution of compound **6** (123 mg, 0.096 mmol, 1.0 equiv.) in 1M sodium methoxide in methanol (2.0 mL, pH 8-9) was stirred at room temperature until consumption of starting material. After 1.5 hours, the solution was neutralized by the addition of ion-exchange resin (Amberlite™ IR 120, H^+), filtered, washed with a mixture of MeOH and dichloromethane, and the solvent was removed under reduced pressure. The residue was chromatographed over silica gel with gradient dichloromethane 100% to DCM/MeOH (85:15) to afford the deacetylated lactose archaeal compound **7** as white foam after lyophilisation (75 mg, 0.077 mmol, 80%). R_f = 0.31 DCM/MeOH (85:15). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.27 (d, $J_{1',2'}$=7.8 Hz, 1H, $H_{1'}$), 4.23 (d, $J_{1,2}$=7.8 Hz, 1H, H_1), 3.88-3.83 (m, 1H, CH*H*-arch-a), 3.81-3.71 (m, 4H, H6ab, $H_{4'}$,

$H_{6'a}$), 3.64 (dd, J=11.7 Hz, 4.5 Hz, 1H, $H_{6'b}$), 3.57 (m, 4H, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c), 3.49 (m, 5H, H_3, H_4, $H_{2'}$, $H_{5'}$, CH*H*-arch-d), 3.44-3.38 (m, 4H, CH*H*-arch-d, CH_2-arch-e, $H_{3'}$), 3.31 (m, 1H, H_5), 3.23 (dd, 1H, H_2), 1.60-1.39 (m, 6H), 1.39-0.93 (m, 42H), 0.83-0.74 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.76 ($C_{1'}$), 103.31 (C_1), 79.93 (C_4), 77.80 (*C*H-arch-b), 75.61 ($C_{5'}$), 74.95 (C_5), 74.76 (C_3), 73.47 ($C_{3'}$), 73.19 (C_2), 71.16 ($C_{2'}$), 70.25 (*C*H_2-arch-d), 69.97 (*C*H_2-arch-e), 69.04 (*C*H_2-arch-a), 68.90 ($C_{4'}$), 68.62 (*C*H_2-arch-c), 61.31 ($C_{6'}$), 61.07 (C_6), 39.27, 37.38, 37.31, 37.27, 37.15, 36.83, 36.50, 32.66, 29.78, 29.69, 27.85, 24.66, 24.32, 24.27, 22.33, 22.24, 19.45, 19.42, 19.40, 19.38. HRMS (ESI+) calcd. for $C_{55}H_{108}O_{13}Na$ $[M+Na]^+$: 999.7682, found 999.7684.

STEP 4A: SULFATION – Synthesis of Archaeal *O*-(6'-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (8) (6'-SLA)



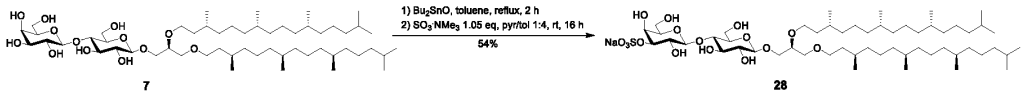
A solution of compound **7** (94 mg, 0.097 mmol, 1.0 equiv.) in dry pyridine (1.0 mL) was added into the solution of the sulfur trioxide/trimethylamine complex (40% active SO_3, 33.5 mg, 0.096 mmol, 1.0 equiv.) in dry DMF (2.0 mL) at 0 °C. The mixture was stirred at room temperature for 16 hours then concentrated. The resulting residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 10% MeOH. The unreacted compound **7** was recovered after the flash chromatography on silica gel (57 mg, 0.058 mmol, 58%) and the purified compound **8** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 6 mL) and the filtrate was concentrated under reduced pressure to afford the desired sodium salt compound **8** as white solid (41 mg, 38.4 µmol, 40%).



A solution of compound **7** (488.8 mg, 0.5 mmol, 1.0 equiv.) in dry pyridine (3.0 mL) and dry toluene (12.0 mL) was added sulfur trioxide trimethylamine complex (76.5 mg, 0.55 mmol, 1.1 equiv.) at room temperature. The mixture was stirred under nitrogen for 2 hours then quenched by MeOH (3.0 mL). The mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7.5% MeOH. The unreacted compound **7** was recovered after the flash chromatography on silica gel (151 mg, 0.154 mmol, 31%) and the purified compound **8** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 6 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **8** as white solid (133 mg, 0.123 mmol, 25%). R_f = 0.33; (CH_2Cl_2/MeOH 8:2). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.28-4.25 (m, 1H, $H_{6'a}$), 4.25-4.22 (m, 2H, $H_{1'}$, H_1), 4.05 (dd, J = 10.9, 3.1 Hz, 1H, $H_{6'b}$), 3.87 (dd, J = 10.3, 3.5 Hz, 1H, CH*H*-arch-a), 3.82-3.73 (m, 4H, $H_{5'}$, H_{6ab}, $H_{4'}$), 3.60-3.53 (m, 4H, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c), 3.53-3.39

(m, 8H, $H_{2'}$, $H_{3'}$, H_3, H_4, CH_2-arch-d, CH_2-arch-e), 3.35 (dt, J = 9.3, 3.5 Hz, 1H, H_5), 3.25-3.21 (m, 1H, H_2), 1.60-0.69 (m, 78H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 104.29 ($C_{1'}$), 102.79 (C_1), 82.47 (C_4), 77.76 (*C*H-arch-b), 74.68 (C_5), 74.26 (C_3), 73.61 ($C_{5'}$), 73.36 ($C_{3'}$), 72.88 (C_2), 70.95 ($C_{2'}$), 70.03 (*C*H$_2$-arch-d), 69.96 (*C*H$_2$-arch-e), 68.96 (*C*H$_2$-arch-a), 68.55 (*C*H$_2$-arch-c), 68.39 ($C_{4'}$), 66.94 ($C_{6'}$), 61.38 (C_6), 39.27, 37.38, 37.33, 37.27, 37.25, 37.16, 36.79, 36.50, 32.67, 29.80, 27.86, 24.67, 24.34, 24.27, 22.39, 22.30, 19.47, 19.41. HRMS (ESI-) calcd. for $C_{55}H_{107}O_{16}S$ [M-H]$^-$: 1055.7285, found 1055.7275. HRMS (ESI+) calcd. for $C_{55}H_{108}O_{16}SNa$ [M+Na]$^+$: 1079.7250, found 1079.7210.

STEP 4B: SULFATION – Synthesis of Archaeal *O*-(3'-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (28) (3'-SLA)

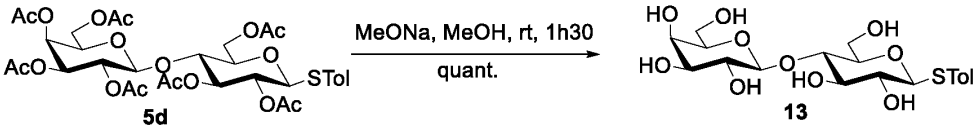


A mixture of compound **7** (98 mg, 0.1 mmol, 1.0 equiv.) and dibutyltin oxide (29.9 mg, 0.12 mmol, 1.2 equiv.) in toluene (5.0 mL) was heated to reflux until all reagents dissolve (2-4 hours). The solution was then concentrated under vacuum and dissolved in dry pyridine (1.0 mL) and dry toluene (4.0 mL) before addition of sulfur trioxide trimethylamine complex (14.6 mg, 0.105 mmol, 1.05 equiv.) at room temperature. The mixture was stirred under nitrogen overnight then quenched by MeOH (1.0 mL). The mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 10% MeOH. The purified compound **28** was treated with ion exchange Dowex Marathon sodium form (swell and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **28** as white solid (57.5 mg, 53.3 μmol, 54%). R_f = 0.3 (CH_2Cl_2/MeOH 8:2). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.40 (d, J = 7.8 Hz, 1H, $H_{1'}$), 4.23 (d, J = 7.8 Hz, 1H, H_1), 4.18 (dd, J = 9.8, 3.1 Hz, 1H, $H_{3'}$), 4.15 (d, J = 3.1 Hz, 1H, $H_{4'}$), 3.88-3.80 (m, 2H, CH*H*-arch-a, H_{6a}), 3.80-3.72 (m, 2H, H_{6b}, $H_{6'a}$), 3.71-3.64 (m, 2H, $H_{2'}$, $H_{6'b}$), 3.60-3.45 (m, 8H, CH*H*-arch-a, C*H*-arch-b, C*H*$_2$-arch-c, $H_{5'}$, H_4, H_3, CH*H*-arch-d), 3.42 (m, 3H, CH*H*-arch-d, C*H*$_2$-arch-e), 3.32 (dt, J = 9.7, 3.3 Hz, 1H, H_5), 3.26-3.22 (m, 1H, H_2), 1.57-1.50 (m, 2H), 1.48-1.41 (m, 4H), 1.34-0.95 (m, 42H), 0.83-0.75 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.32 ($C_{1'}$), 102.97 (C_1), 79.88 ($C_{3'}$), 79.80 (C_4), 77.45 (*C*H-arch-b), 74.90 ($C_{5'}$), 74.56 (C_5), 74.25 (C_3), 72.88 (C_2), 69.87, 69.78, 69.00 ($C_{2'}$), 68.82, 68.36, 67.15 ($C_{4'}$), 61.18 ($C_{6'}$), 60.63 (C_6), 39.00, 37.13, 37.06, 37.02, 36.90, 36.54, 36.23, 32.41, 29.54, 29.46, 27.59, 24.41, 24.08, 24.00, 22.16, 22.07, 19.24, 19.20, 19.18.

EXAMPLE 3: ALTERNATIVE SYNTHESIS OF 6'-SLA BY SELECTIVE SULFATION INVOLVING ORTHOGONAL PROTECTION – METHOD I

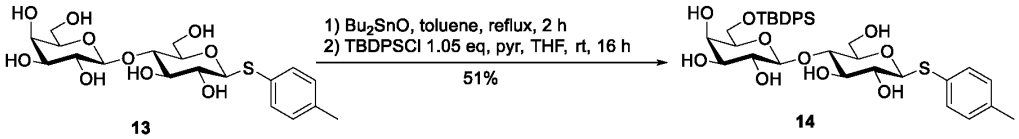
According to general Scheme 3, further detailed below.

STEP 1



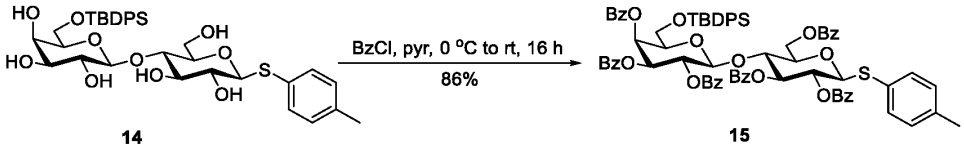
To a solution of **5d** prepared as previously mentioned (415 mg, 0.56 mmol, 1 equiv.) in MeOH (7.0 mL) was added MeONa 1.0 M in MeOH (56 µL, 0.05 mmol, 0.1 equiv.). The mixture was stirred at room temperature for 1.5 h. After 1.5 hours, the solution was neutralized by the addition of ion-exchange resin (Amberlite™ IR 120, H^+), filtered, washed with MeOH and the solvent was removed under reduced pressure. Lyophilization gave the desired compound **13** (250 mg, 0.56 mmol, quant.) as a white solid. R_f= 0.35; (CH_2Cl_2/MeOH 8:2). ^{1}H NMR (600 MHz, D_2O) δ 7.45-7.35 (m, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 4.65 (d, *J* = 9.9 Hz, 1H), 4.35 (d, *J* = 7.7 Hz, 1H), 3.92-3.79 (m, 2H), 3.75-3.40 (m, 9H), 3.28 (dd, *J* = 12.2, 6.6 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (150 MHz, D_2O) δ 139.0, 132.4, 129.9, 127.8, 102.8, 87.4, 78.7, 77.9, 75.8, 75.3, 72.4, 71.4, 70.9, 68.5, 61.0, 60.1, 20.1.

STEP 2



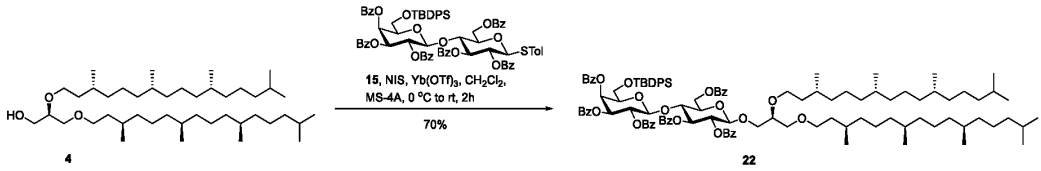
A mixture of compound **13** (40 mg, 89.2 µmol, 1.0 equiv.) and dibutyltin oxide (26.7 mg, 107 µmol, 1.2 equiv.) in toluene (5.0 mL) was heated to reflux until all reagents dissolve (2-4 hours). The solution was then concentrated under vacuum and dissolved in pyridine (1.0 mL) and THF (3.0 mL) before addition of TBDPSCl (24.3 µL, 93.7 µmol, 1.05 equiv.). After stirring at room temperature overnight, the mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel (gradient from CH_2Cl_2 100% to CH_2Cl_2/MeOH 92:8) to afford the desired compound **14** (31.3 mg, 45.6 µmol, 51%) as colorless oil. R_f= 0.43; (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.61-7.56 (m, 4H), 7.38-7.26 (m, 8H), 7.02 (d, *J* = 8.1 Hz, 2H), 4.40 (d, *J* = 9.8 Hz, 1H, H_1), 4.23 (d, *J* = 7.8 Hz, 1H, $H_{1'}$), 3.82-3.66 (m, 5H, $H_{4'}$, H_{6ab}, $H_{6'ab}$), 3.56-3.51 (m, 1H, $H_{5'}$), 3.43 (m, 3H, $H_{2'}$, H_3, H_4), 3.36 (dd, *J* = 9.7, 3.2 Hz, 1H, $H_{3'}$), 3.31 (ddd, *J* = 9.4, 3.9, 2.4 Hz, 1H, H_5), 3.13 (t, *J* = 9.1 Hz, 1H, H_2), 2.20 (s, 3H, S-Ph-C*H*$_3$), 0.94 (d, *J* = 12.5 Hz, 9H, *t*Bu); ^{13}C NMR (150 MHz, CD_3OD) δ 147.87, 138.15, 137.53, 135.34, 135.32, 133.11, 133.01, 132.51, 129.58, 129.55, 129.37, 129.17, 127.52, 127.46, 103.51 ($C_{1'}$), 87.99 (C_1), 79.04 (C_5), 78.56 (C_4), 76.30 (C_3), 75.41 ($C_{5'}$), 73.44 ($C_{3'}$), 72.03 (C_2), 71.06 ($C_{2'}$), 68.42 ($C_{4'}$), 62.38 (C_6), 60.53 ($C_{6'}$), 27.06, 25.96, 25.85, 19.75, 18.58, 12.59.

STEP 3



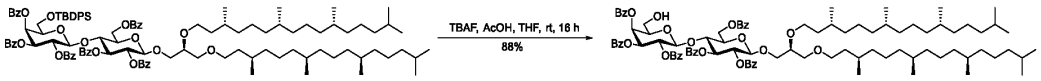
To a solution of 6'-*O*-silylated lactoside **14** (26 mg, 37.9 μmol, 1.0 equiv.) in dry pyridine (0.5 mL) was added benzoyl chloride (32 μL, 273 μmol, 7.2 equiv.) dropwise at 0 °C. The reaction was stirred under nitrogen at room temperature overnight. The solution was then diluted in CH_2Cl_2 and washed with ice water, saturated $KHSO_4$ solution, saturated $NaHCO_3$ solution and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Crude residue was purified by flash chromatography on silica gel (Hexane/EtOAc 8:2) to afford the desired lactoside **15** as colorless oil. (42.5 mg, 32.4 μmol, 86%). R_f = 0.4; (Hexane/EtOAc 8:2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07-6.59 (m, 44H), 5.81 (d, *J* = 5.2 Hz, 1H, $H_{4'}$), 5.59 (t, *J* = 9.4 Hz, 1H, H_3), 5.54 (dd, *J* = 10.3, 7.9 Hz, 1H, $H_{2'}$), 5.36 (dd, *J* = 10.3, 3.3 Hz, 1H, $H_{3'}$), 5.25 (t, *J* = 9.8 Hz, 1H, H_2), 4.71 (d, *J* = 10.0 Hz, 1H, H_1), 4.66 (d, *J* = 7.9 Hz, 1H, $H_{1'}$), 4.45 (qd, *J* = 11.9, 3.6 Hz, 2H, H_{6ab}), 3.93 (t, *J* = 9.5 Hz, 1H, H_4), 3.70 (ddd, *J* = 9.9, 4.9, 2.2 Hz, 1H, H_5), 3.62 (dd, *J* = 9.2, 5.7 Hz, 1H, $H_{5'}$), 3.08 (dd, *J* = 10.0, 5.2 Hz, 1H, $H_{6'a}$), 2.80 (t, *J* = 9.7 Hz, 1H, $H_{6'b}$), 2.17 (s, 3H, S-Ph-CH_3), 0.83 (d, *J* = 10.0 Hz, 9H, *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.21, 138.42, 136.57, 135.58, 135.43, 133.84, 133.54, 133.30, 133.23, 133.04, 132.77, 132.32, 130.16, 130.04, 129.94, 129.87, 129.84, 129.74, 129.55, 129.51, 129.45, 129.32, 128.99, 128.78, 128.46, 128.36, 128.19, 127.86, 127.68, 127.46, 123.99, 101.37 ($C_{1'}$), 85.93 (C_1), 77.14 (C_5), 76.17 (C_4), 74.07 (C_3), 73.41 ($C_{5'}$), 72.05 ($C_{3'}$), 70.24 (C_2), 70.20 ($C_{2'}$), 67.00 ($C_{4'}$), 62.66 (C_6), 59.41 ($C_{6'}$), 26.52, 21.18, 18.76.

STEP 4: Synthesis of 2,3-bis-*O*-[(3*R*,7*R*,11*R*)-3,7,11,15-tetramethylhexadexan-1-yl]-*sn*-glycer-1-yl 2,3,4-tri-*O*-Benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (22)



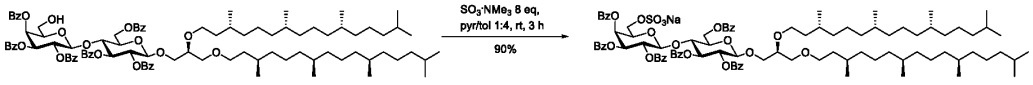
To a solution of **4** (52 mg, 80 μmol, 1.0 equiv.) and **15** (210 mg, 0.16 mmol, 2.0 equiv.) in dry DCM (3.0 mL) at 0 °C were successively added NIS (46 mg, 0.2 mmol, 2.5 equiv.), and $Yb(OTf)_3$ (14 mg, 24 μmol, 0.3 equiv.) under argon atmosphere. The mixture was then stirred at room temperature for 2 h. The crude was then quenched with Et_3N and concentrated under reduced pressure. Flash column chromatography (Hexanes/Ethyl acetate from 95:5 to 4:1) gave the desired compound **22** (103 mg, 56 μmol, 70%) as a colorless oil. R_f = 0.45; (Hexane/EtOAc 8:2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07-6.60 (m, 40H), 5.80 (d, *J* = 3.2 Hz, 1H, $H_{4'}$), 5.60 (t, *J* = 9.6 Hz, 1H, H_3), 5.55 (dd, *J* = 10.3, 7.9 Hz, 1H, $H_{2'}$), 5.33 (m, 2H, $H_{3'}$, H_2), 4.67 (d, *J* = 7.9 Hz, 1H, $H_{1'}$), 4.64 (d, *J* = 7.9 Hz, 1H, H_1), 4.47-4.37 (m, 2H, H_{6ab}), 4.06-3.99 (m, 1H, H_4), 3.79 (dd, *J* = 10.4, 3.2 Hz, 1H, CH*H*-arch-a), 3.67 (ddd, *J* = 9.9, 4.1, 2.4 Hz, 1H, H_5), 3.59 (dd, *J* = 9.4, 5.8 Hz, 1H, $H_{5'}$), 3.44-3.37 (m, 2H, CH*H*-arch-a, C*H*-arch-b), 3.34-3.22 (m, 2H, CH_2-arch-c), 3.22-3.13 (m, 4H, CH_2-arch-d, CH_2-arch-e), 3.10 (dd, *J* = 10.0, 5.2 Hz, 1H, $H_{6'a}$), 2.81 (t, *J* = 9.7 Hz, 1H, $H_{6'b}$), 1.47-1.36 (m, 4H), 1.32-0.90 (m, 44H), 0.83 (s, 9H, *t*Bu), 0.80-0.61 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.57, 135.42, 133.21, 133.03, 132.74, 132.31, 130.18, 130.05, 129.94, 129.83, 129.77, 129.49, 129.32, 129.00, 128.80, 128.50, 128.29, 128.18, 127.86, 127.45, 101.27 ($C_{1'}$), 101.25 (C_1), 77.64 (*C*H-arch-b),

76.15 (C_4), 73.39 ($C_{5'}$), 73.06 (C_5), 72.85 (C_3), 72.07 ($C_{3'}$), 71.66 (C_2), 70.58, 70.57, 70.19 ($C_{2'}$), 69.96, 69.11, 66.99 ($C_{4'}$), 62.51 (C_6), 59.42 ($C_{6'}$), 59.05, 57.28, 39.39, 37.47, 37.42, 37.31, 36.93, 36.51, 32.81, 32.65, 29.87, 29.65, 27.99, 26.52, 24.81, 24.49, 24.33, 24.29, 22.74, 22.64, 19.77, 19.63, 19.50, 18.77. HRMS (ESI+): $[M+Na]^+$ calculated for $C_{113}H_{150}NaO_{19}Si$, 1862.0438; found, 1862.0423.



22 17

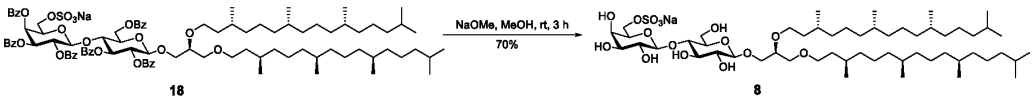
A mixture of compound **22** (83.6 mg, 45.4 μmol, 1.0 equiv.) in TBAF solution (1M in THF, 1.0 mL) was acidified to pH 6.5 with few drops of AcOH. After stirring overnight at room temperature, the mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel by gradient using 100% Hexanes to Hexanes/EtOAc (8:2) to afford **17** (63.8 mg, 39.8 μmol, 88%) as colorless oil. R_f= 0.26; (Hex/EtOAc 7:3). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92-7.01 (m, 30H), 5.69 (dd, *J* = 10.4, 7.8 Hz, 1H, $H_{2'}$), 5.63 (t, *J* = 9.4 Hz, 1H, H_3), 5.47 (d, *J* = 3.4 Hz, 1H, $H_{4'}$), 5.40 (dd, *J* = 9.8, 7.9 Hz, 1H, H_2), 5.25 (dd, *J* = 10.4, 3.4 Hz, 1H, $H_{3'}$), 4.75 (d, *J* = 7.8 Hz, 1H, $H_{1'}$), 4.69 (d, *J* = 7.9 Hz, 1H, H_1), 4.51 (dd, *J* = 11.9, 1.7 Hz, 1H, H_{6a}), 4.38 (dd, *J* = 12.0, 4.4 Hz, 1H, H_{6b}), 4.15 (t, *J* = 9.4 Hz, 1H, H_4), 3.81 (dd, *J* = 10.5, 3.4 Hz, 1H, CH*H*-arch-a), 3.73 (ddd, *J* = 9.8, 4.3, 1.9 Hz, 1H, H_5), 3.51 (t, *J* = 6.8 Hz, 1H, $H_{5'}$), 3.44 (dt, *J* = 12.2, 6.1 Hz, 1H, CH*H*-arch-a), 3.42-3.36 (m, 1H, C*H*-arch-b), 3.33-3.24 (m, 2H, CH_2-arch-c), 3.24-3.14 (m, 4H, CH_2-arch-d, CH_2-arch-e), 2.91-2.81 (m, 1H, $H_{6'a}$), 2.65 (dd, *J* = 11.4, 6.9 Hz, 1H, $H_{6'b}$), 1.53-1.38 (m, 4H), 1.31-1.12 (m, 44H), 0.77-0.62 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.44, 165.87, 165.38, 133.85, 133.39, 133.32, 133.14, 133.04, 130.15, 129.78, 129.75, 129.71, 129.57, 129.46, 129.33, 128.68, 128.64, 128.58, 128.53, 128.33, 128.31, 128.25, 101.27 (C_1), 101.00 ($C_{1'}$), 77.66 (*C*H-arch-b), 75.98 (C_4), 74.03 ($C_{5'}$), 73.46 (C_3), 72.90 (C_5), 71.86 ($C_{3'}$), 71.59 (C_2), 70.61, 70.56, 70.12 ($C_{2'}$), 69.98, 69.14, 68.38 ($C_{4'}$), 62.43 (C_6), 59.77 ($C_{6'}$), 39.38, 37.52, 37.47, 37.42, 37.38, 37.30, 36.94, 36.51, 32.81, 29.88, 29.66, 27.99, 24.81, 24.48, 24.33, 24.29, 22.74, 22.64, 19.76, 19.64, 19.51.



17 18

To a mixture of compound **17** (63 mg, 39.3 μmol, 1.0 equiv.) in dry pyridine (1.0 mL) and dry toluene (4.0 mL) was added sulfur trioxide trimethylamine complex (43.8 mg, 314.6 μmol, 8 equiv.) at room temperature. After stirring under nitrogen for 3 hours, the mixture was quenched by MeOH (1.0 mL) and then concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 5% MeOH. The purified compound **18** was treated with a Dowex Marathon sodium form cation-exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **18** as white waxy solid (60 mg, 35.2 μmol, 90%). R_f= 0.25; (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 8.03-7.10 (m, 30H), 5.72-5.67 (m, 1H, H_3), 5.66 (d, *J* =

3.4 Hz, 1H, $H_{4'}$), 5.51 (dd, J = 10.2, 7.9 Hz, 1H, $H_{2'}$), 5.37 (dd, J = 10.3, 3.4 Hz, 1H, $H_{3'}$), 5.33 (dd, J = 9.9, 8.0 Hz, 1H, H_2), 4.93 (d, J = 7.9 Hz, 1H, $H_{1'}$), 4.75 (d, J = 8.0 Hz, 1H, H_1), 4.56 (m, 1H, H_{6a}), 4.42 (dd, J = 12.0, 5.4 Hz, 1H, H_{6b}), 4.24 (t, J = 9.5 Hz, 1H, H_4), 4.07 (t, J = 6.9 Hz, 1H, $H_{5'}$), 3.81 (dd, J = 10.5, 3.5 Hz, 2H, H_5, CH*H*-arch-a), 3.65 (dd, J = 11.0, 5.9 Hz, 1H, $H_{6'a}$), 3.49-3.39 (m, 2H, CH*H*-arch-a, C*H*-arch-b), 3.35-3.04 (m, 3H, $H_{6'b}$, CH_2-arch-c), 3.22-3.12(m, 4H, CH_2-arch-d, CH_2-arch-e), 1.54-0.88 (m, 48H), 0.79-0.56 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 166.32, 166.02, 165.54, 165.49, 165.34, 165.22, 133.41, 133.36, 133.25, 133.17, 129.69, 129.62, 129.53, 129.42, 129.24, 129.22, 128.97, 128.74, 128.57, 128.40, 128.37, 128.31, 128.11, 101.15 (C_1), 100.62 ($C_{1'}$), 77.51(*C*H-arch-b), 75.75 (C_4), 73.14 (C_5), 72.91 (C_3), 72.08 ($C_{3'}$), 71.97 (C_2), 71.56 ($C_{5'}$), 70.35, 70.29 ($C_{2'}$), 70.19, 69.77, 68.87, 67.75 ($C_{4'}$), 63.98 ($C_{6'}$), 62.75 (C_6), 44.71, 39.25, 37.28, 37.22, 37.18, 37.13, 36.69, 36.32, 32.65, 29.67, 29.44, 27.83, 24.65, 24.29, 24.19, 24.16, 22.28, 22.19, 19.43, 19.39, 19.28, 19.19.

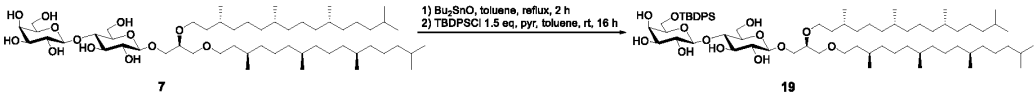


18 8

A solution of compound **18** (55 mg, 32.2 μmol, 1.0 equiv.) in 1M sodium methoxide in methanol (1.0 mL, pH 8-9) was stirred at room temperature until consumption of starting material. After 3 hours, the solution was ion-exchanged by resin (Amberlite™ IR 120, H^+), filtered, washed with MeOH/DCM 1:1, and the solvent was removed under reduced pressure. The crude residue was chromatographed over silica gel by gradient using 100% DCM to 15% MeOH and the purified compound **8** was treated with a Dowex Marathon sodium form cation-exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the debenzoylated lactose archaeal compound **8** as white foam after lyophilisation (24.5 mg, 22.7 μmol, 70%). R_f = 0.33; (CH_2Cl_2/MeOH 8:2). NMR ($CDCl_3$:CD_3OD 1:1) corresponds to our previous results.

EXAMPLE 4: ALTERNATIVE SYNTHESIS OF 6'-SLA BY SELECTIVE SULFATION INVOLVING ORTHOGONAL PROTECTION – METHOD II

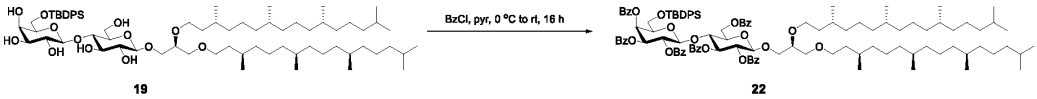
Synthesis of 6'-SLA referring to Scheme 4, further detailed below.



7 19

A mixture of compound **7** (195.5 mg, 0.2 mmol, 1.0 equiv.) and dibutyltin oxide (59.8 mg, 0.24 mmol, 1.2 equiv.) in toluene (10.0 mL) was heated to reflux until all reagents dissolve (2-4 hours). The solution was then concentrated under vacuum and dissolved in pyridine (2.5 mL) and toluene (10.0 mL) before addition of TBDPSCl (77.8 μL, 0.3 mmol, 1.5 equiv.). After stirring at room temperature overnight, the mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel (gradient from CH_2Cl_2 100% to CH_2Cl_2/MeOH 96:4) to afford the 6'-*O*-silylated lactoside **19** (230 mg,

0.189 mmol, 95%) as colorless oil. R_f= 0.2; (CH_2Cl_2/MeOH 95:5). NMR ($CDCl_3$:CD_3OD 1:1) corresponds to our previous results.



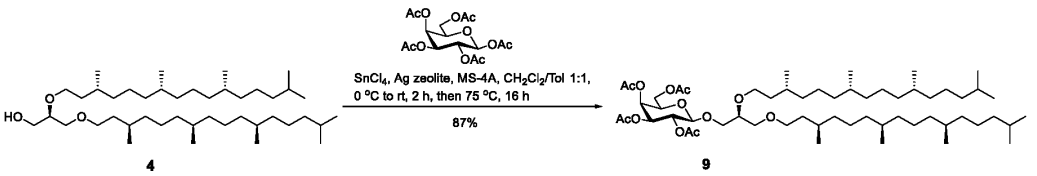
19 22

To a mixture of 6'-*O*-silylated lactoside **19** (60 mg, 49 μmol, 1.0 equiv.) in dry pyridine (1.5 mL) was added benzoyl chloride (41 μL, 353 μmol, 7.2 equiv.) dropwise at 0 °C. The reaction was stirred under nitrogen at room temperature overnight. The solution was then diluted in CH_2Cl_2 and washed with ice water, saturated $KHSO_4$ solution, saturated $NaHCO_3$ solution and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Crude residue was purified by flash chromatography on silica gel (Hexane/EtOAc 9:1) to afford the desired lactoside **22** as colorless oil. (90 mg, 49 μmol, quantitative).

The synthesis steps for obtaining Compound **8** from Compound **22**, are detailed above in Step 4 of Example 3.

EXAMPLE 5: SYNTHESIS OF 3- AND 6-SULFATED MONOSACCHARIDE BY DIRECT SULFATION OF ARCHAEAL CARBOHYDRATE
According to general Scheme 2 above, further detailed below.

STEP 1: Archaeal 2, 3, 4, 6-tetra-*O*-acetyl-β-D-galactopyranoside (9)

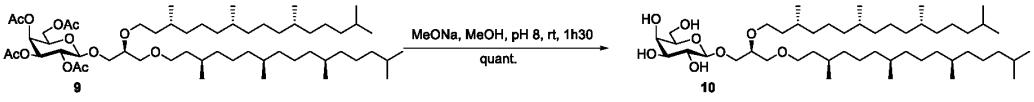


4 9

To a mixture of archaeol **4** (309 mg, 0.47 mmol, 1.0 equiv.) and galactose pentaacetate **5'a** (1.83 g, 4.7 mmol, 10 equiv.) in dry dichloromethane (40 mL) and dry toluene (40 mL) was added activated molecular sieves 4Å powder (5 g) and silver-exchanged zeolite (7.05 g: 1.5 g/mmol of donor) at room temperature. The mixture was stirred under nitrogen at room temperature for 30 min. A solution of 1M tin(IV) chloride in dichloromethane (1.04 mL, 5.64 mmol, 12.0 equiv.) was added into the reaction at 0 °C. The reaction was stirred at room temperature for 2 hours then heated up to 75 °C and kept stirring overnight (16 h). After cooling down to room temperature, the mixture was then neutralised by adding triethylamine until pH 7.0. The mixture was then filtered over a pad of Celite® and washed with EtOAc. The filtrate solution was concentrated under reduced pressure. The crude residue was then purified by flash chromatography on silica gel (gradient from hexanes 100% to Hexanes/EtOAc/DCM 7.5:1.5:1) to afford the desired archaeal glycoside **9** as colorless oil (400 mg, 0.407 mmol, 87%). R_f= 0.28 (Hexanes/EtOAc/DCM 7.5:1.5:1). ^1H NMR (600 MHz, $CDCl_3$) δ 5.32 (dd, $J_{4,3}$ = 3.4, $J_{4,5}$ = 0.8 Hz, 1H, H_4), 5.15 (dd, $J_{2,3}$ = 10.5, $J_{2,1}$ = 8.0 Hz, 1H, H_2), 4.94 (dd, $J_{3,2}$ = 10.5, $J_{3,4}$ = 3.4 Hz, 1H, H_3), 4.49 (d, $J_{1,2}$ = 8.0 Hz, 1H, H_1), 4.08 (qd, J = 11.2, 6.7 Hz, 2H, H_{6ab}), 3.87 (dt, J = 9.5, 6.8 Hz, 1H, C*H*-arch), 3.84-3.79 (m, 1H, H_5), 3.57-3.45 (m, 4H), 3.45-3.30 (m, 4H), 2.08 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.60-1.38 (m, 6H), 1.37-0.93 (m, 42H), 0.83-0.75 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.38, 170.29, 170.19, 169.27, 101.51 (C_1), 77.95 (*C*H-

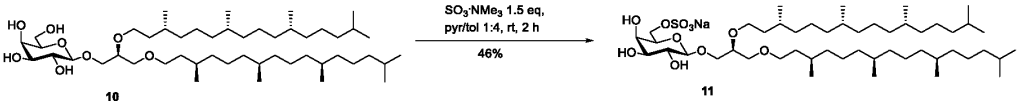
arch), 71.01 (C_3), 70.60 (C_5), 70.55, 70.52, 70.12, 69.22, 68.90 (C_2), 67.06 (C_4), 61.23 (C_6), 39.38, 37.56, 37.53, 37.47, 37.43, 37.40, 37.30, 37.18, 36.62, 32.81, 29.95, 29.87, 27.99, 24.81, 24.49, 24.38, 22.73, 22.64, 20.79, 20.69, 20.61, 19.76, 19.73.

STEP 2: Archaeal β-D-galactopyranoside (10)



A solution of compound **9** (105 mg, 0.106 mmol, 1.0 equiv.) in 1M sodium methoxide in methanol (1 mL, pH 8-9) was stirred at room temperature until consumption of starting material. After 2 hours, the solution was neutralized by addition of ion-exchange resin (Amberlite^{TM} IR 120, H^+), filtered, washed with a mixture of MeOH and dichloromethane, and the solvent was removed under reduced pressure. The residue was chromatographed over silica gel with gradient dichloromethane 100% to DCM/MeOH (95:5) to afford the deacetylated galactose archaeal compound **10** as white foam after lyophilisation (85 mg, 0.104 mmol, 98%). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.15 (d, $J_{1,2}$ = 7.6 Hz, 1H, H_1), 3.86 (dd, J = 10.3, 4.2 Hz, 1H, CH*H*-arch-a), 3.78 (d, $J_{4,3}$ = 2.9 Hz, 1H, H_4), 3.68 (qd, J = 11.4, 6.1 Hz, 2H, H_{6ab}), 3.61-3.52 (m, 4H, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c), 3.48 (m, 2H, CH*H*-arch-d, H_2), 3.44-3.37 (m, 5H, CH*H*-arch-d, CH_2-arch-e, H_3, H_5), 1.57-1.40 (m, 6H), 1.35-0.95 (m, 42H), 0.84-0.73 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.92 (C_1), 77.87 (*C*H-arch-b), 75.03 (C_3), 73.42 (C_5), 71.21 (C_2), 70.42, 69.88, 68.80, 68.72 (C_4), 68.54, 61.07 (C_6), 39.25, 37.36, 37.29, 37.25, 37.13, 36.83, 36.50, 32.67, 32.64, 29.75, 29.66, 27.83, 24.64, 24.30, 24.25, 22.26, 22.16, 19.39, 19.35, 19.31. HRMS (ESI+) calcd. for $C_{49}H_{98}O_8Na$ $[M+Na]^+$: 837.71539, found 837.7190.

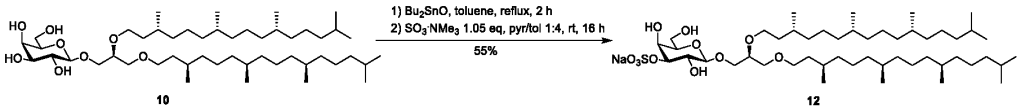
STEP 3: Archaeal 6-*O*-sulfo-β-D-galactopyranoside sodium salt (11)



Archaeal 6-*O*-sulfo-β-D-galactopyranoside sodium salt (11) The solution of compound **10** (84.6 mg, 0.103 mmol, 1.0 equiv.) in dry pyridine (0.3 mL) and dry toluene (1.2 mL) was added sulfur trioxide trimethylamine complex (21.7 mg, 0.156 mmol, 1.5 equiv.) at room temperature. The mixture was stirred under nitrogen for 2 hours then quenched by MeOH (0.3 mL). The mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7.5% MeOH. The purified compound **11** was treated with ion exchange Dowex Marathon sodium form (swell and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **11** as white solid (43 mg, 0.041 mmol, 46%). R_f = 0.33 (CH_2Cl_2/MeOH 8:2). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.15 (dd, J = 9.8, $J_{1,2}$ = 7.5 Hz, 2H, H_1, H_{6a}), 4.08 (dd, J = 10.5, 5.8 Hz, 1H, H_{6b}), 3.88 (d, J = 2.9 Hz, 1H, H_4), 3.85 (dd, J = 10.6, 4.4 Hz, 1H, CH*H*-arch-a), 3.66 (dd, J = 12.0, 5.0 Hz, 1H, H_5), 3.61-3.47

(m, 5H, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c, CH*H*-arch-d), 3.47-3.38 (m, 5H, H_2, H_3, CH*H*-arch-d, CH_2-arch-e), 1.50-1.41 (m, 6H), 1.35-1.09 (m, 30H), 1.08-1.04 (m, 4H), 1.02-0.96 (m, 8H), 0.84-0.75 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.56 (C_1), 77.37 (*C*H-arch-b), 72.67 (C_3), 72.36 (C_5), 70.86 (C_2), 70.17, 69.60, 68.61, 68.22, 67.62 (C_4), 64.99 (C_6), 38.94, 37.06, 36.99, 36.96, 36.83, 36.49, 36.20, 32.34, 29.47, 29.37, 27.52, 24.34, 24.01, 23.95, 21.99, 21.90, 19.08.

Archaeal 3-*O*-sulfo-β-D-galactopyranoside sodium salt (22)



A mixture of compound **10** (50 mg, 61.3 μmol, 1.0 equiv.) and dibutyltin oxide (18.3 mg, 73.6 μmol, 1.2 equiv.) in toluene (5.0 mL) was heated to reflux until all reagents were dissolved (2-4 hours). The solution was then concentrated under vacuum and dissolved in dry pyridine (1.0 mL) and dry toluene (4.0 mL) before addition of sulfur trioxide trimethylamine complex (9 mg, 64.4 μmol, 1.05 equiv.) at room temperature. The mixture was stirred under nitrogen overnight then quenched by MeOH (1.0 mL). The mixture was concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 10% MeOH. The purified compound **12** was treated with ion exchange Dowex Marathon sodium form (swell and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **12** as white solid (31 mg, 33.8 μmol, 55%). R_f = 0.28 (CH_2Cl_2/MeOH 85:15). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.24 (d, *J* = 7.8 Hz, 1H, H_1), 4.22 (d, *J* = 3.2 Hz, 1H, H_4), 4.16 (dd, *J* = 9.7, 3.2 Hz, 1H, H_3), 3.89 (dd, *J* = 9.9, 3.6 Hz, 1H, CH*H*-arch-a), 3.75-3.68 (m, 2H, H_{6ab}), 3.66 (dd, *J* = 9.7, 7.8 Hz, 1H, H_2), 3.61-3.51 (m, 4H, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c), 3.50-3.44 (m, 2H, CH*H*-arch-d, H_5), 3.41 (m, 3H, CH*H*-arch-d, CH_2-arch-e), 1.53-0.94 (m, 48H), 0.82-0.75 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.39 (C_1), 80.18 (C_3), 77.64 (*C*H-arch), 74.32 (C_5), 70.25, 70.12, 69.19 (C_2), 69.02, 68.63, 67.18 (C_4), 61.16 (C_6), 39.30, 37.46, 37.37, 37.33, 37.20, 36.81, 36.52, 32.71, 29.86, 29.79, 27.89, 24.71, 24.39, 24.30, 22.52, 22.43, 19.57, 19.53, 19.49. HRMS (ESI+) calcd. for $C_{49}H_{98}O_{11}SNa$ $[M+Na]^+$: 917.6722, found 917.6695.

EXAMPLE 6: SYNTHESIS OF 6-SLA, 6'-SLA AND 6,6'-DI-SLA BY SELECTIVE SULFATION BY ORTHOGONAL PROTECTION

Synthesis of 6-SLA, 6'-SLA and 6,6'-diSLA referring to Schemes 5 to 8, further detailed below.



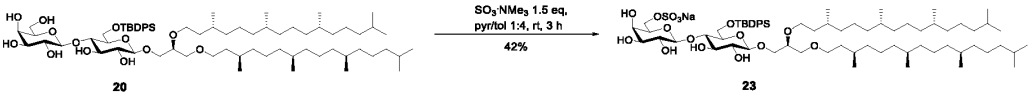
A solution of compound **7** (195.5 mg, 0.2 mmol, 1.0 equiv.) in dry pyridine (2.0 mL) was added dropwise TBDPSCl (55 μL, 0.21 mmol, 1.05 equiv.) at 0 °C. The solution was stirred under nitrogen at room

temperature overnight, then concentrated under vacuum. Crude residue was purified by flash chromatography on silica gel (gradient from CH_2Cl_2 100% to CH_2Cl_2/MeOH 96:4) to afford the disilylated lactoside **21** (94 mg, 0.065 mmol, 33%) and 6'-*O*-silylated lactoside **19** (63 mg, 0.052 mmol, 26%) and 6-*O*-silylated lactoside **20** (45 mg, 0.037 mmol, 18%) as colorless oil. F1: R_f = 0.64; F2: R_f = 0.48; (CH_2Cl_2/MeOH 9:1).

21 : ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.67-7.65 (m, 4H), 7.60-7.54 (m, 4H), 7.35-7.25 (m, 12H), 4.42 (d, *J* = 7.7 Hz, 1H, $H_{1'}$), 4.20 (d, *J* = 7.8 Hz, 1H, H_1), 4.07 (dd, *J* = 11.5, 3.2 Hz, 1H, H_{6a}), 3.90 (d, *J* = 11.4 Hz, 1H, H_{6b}), 3.85 (m, 2H, $H_{4'}$, CH*H*-arch-a), 3.81-3.71 (m, 3H, $H_{6'a}$, H_4, $H_{6'b}$), 3.64-3.54 (m, 3H, C*H*-arch-b, CH*H*-arch-a, CH*H*-arch-c), 3.46 (m, 8H, $H_{2'}$, $H_{5'}$, H_3, CH*H*-arch-c, CH_2-arch-d, CH_2-arch-e), 3.36 (dd, *J* = 9.6, 3.2 Hz, 1H, $H_{3'}$), 3.32 (d, *J* = 9.7 Hz, 1H, H_5), 3.28 (dd, *J* = 9.2, 7.9 Hz, 1H, H_2), 1.57-1.49 (m, 2H), 1.49-1.40 (m, 4H), 1.34-1.08 (m, 30H), 1.07-0.97 (m, 12H), 0.96-0.95 (2s, 18H, 2*t*Bu), 0.83-0.70 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 136.13, 133.76, 133.67, 130.21, 128.28, 128.17, 103.89 ($C_{1'}$), 103.70 (C_1), 78.48 (*C*H-arch-b), 78.40 (C_4), 75.94 ($C_{5'}$), 75.88 (C_5), 75.12 (C_3), 74.40 ($C_{3'}$), 74.16 (C_2), 71.86 ($C_{2'}$), 71.35, 70.60, 69.38, 69.27, 68.76 ($C_{4'}$), 62.75 (C_6), 62.61 ($C_{6'}$), 39.95, 38.06, 38.00, 37.95, 37.84, 37.58, 37.21, 33.34, 30.45, 30.37, 28.53, 27.20, 27.17, 25.35, 25.01, 22.99, 22.90, 20.13, 20.09.

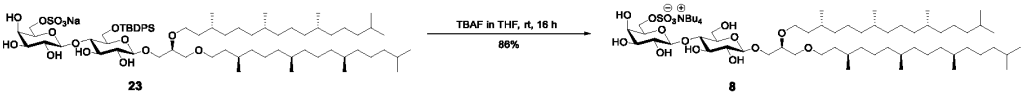
19 : ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.62-7.56 (m, 4H), 7.36-7.28 (m, 6H), 4.24 (d, *J* = 7.8 Hz, 1H, $H_{1'}$), 4.21 (d, *J* = 7.8 Hz, 1H, H_1), 3.86-3.73 (m, 6H, $H_{4'}$, $H_{6'ab}$, H_{6ab}, CH*H*-arch-a), 3.59-3.51 (m, 5H, $H_{5'}$, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c), 3.51-3.37 (m, 8H, $H_{2'}$, H_4, H_3, $H_{3'}$, CH_2-arch-d, CH_2-arch-e), 3.30 (dt, *J* = 9.3, 3.4 Hz, 1H, H_5), 3.22-3.18 (m, 1H, H_2), 1.52 (dt, *J* = 19.0, 6.1 Hz, 2H), 1.49-1.40 (m, 4H), 1.34-1.08 (m, 30H), 1.08-0.97 (m, 12H), 0.97 (s, 9H, *t*Bu), 0.82-0.74 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 135.44, 135.39, 132.99, 129.69, 129.67, 127.64, 127.59, 103.79 ($C_{1'}$), 103.27 (C_1), 79.72 (C_4), 77.81 (*C*H-arch-b), 75.27 ($C_{5'}$), 74.93 (C_5), 74.57 (C_3), 73.55 ($C_{3'}$), 73.29 (C_2), 71.10 ($C_{2'}$), 70.34, 69.85, 68.97, 68.55, 68.13 ($C_{4'}$), 62.03 ($C_{6'}$), 60.86 (C_6), 39.24, 37.33, 37.27, 37.23, 37.12, 36.81, 36.49, 32.63, 29.73, 29.63, 27.82, 26.38, 24.63, 24.28, 24.24, 22.22, 22.13, 19.36, 19.32, 19.28, 18.87.

20 : ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.70-7.63 (m, 4H), 7.36-7.26 (m, 6H), 4.42 (t, *J* = 6.9 Hz, 1H, $H_{1'}$), 4.21 (d, *J* = 7.8 Hz, 1H, H_1), 4.06 (dd, *J* = 11.6, 3.5 Hz, 1H, H_{6a}), 3.93 (dd, *J* = 11.4, 1.4 Hz, 1H, H_{6b}), 3.86 (dd, *J* = 10.6, 4.5 Hz, 1H, CH*H*-arch-a), 3.78-3.71 (m, 3H, H_4, $H_{4'}$,$H_{6'a}$), 3.64-3.55 (m, 4H, $H_{6'b}$, CH*H*-arch-a, C*H*-arch-b, CH*H*-arch-c), 3.53-3.46 (m, 4H, H_3, $H_{2'}$, CH*H*-arch-c, CH*H*-arch-d), 3.42 (m, 4H, $H_{5'}$, CH*H*-arch-d, CH_2-arch-e), 3.36 (dd, *J* = 9.6, 3.3 Hz, 1H, $H_{3'}$), 3.34-3.28 (m, 2H, H_5, H_2), 1.58-1.50 (m, 2H), 1.49-1.39 (m, 4H), 1.35-1.08 (m, 30H), 1.08-0.97 (m, 12H), 0.96 (s, 9H, *t*Bu), 0.83-0.73 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 135.75, 135.51, 133.69, 133.09, 129.53, 127.59, 127.49, 103.24 ($C_{1'}$), 103.02 (C_1), 78.06 (C_4), 77.77 (*C*H-arch-b), 75.64 ($C_{5'}$), 75.30 (C_5), 74.64 (C_3), 73.54 ($C_{3'}$), 73.27 (C_2), 71.23 ($C_{2'}$), 70.50, 70.02, 68.93 ($C_{4'}$), 68.72, 68.64, 62.16 (C_6), 61.46 ($C_{6'}$), 39.28, 37.41, 37.34, 37.29, 37.17, 36.88, 36.53, 32.71, 32.68, 29.81, 29.74, 27.86, 26.56, 24.68, 24.35, 24.28, 22.43, 22.34, 19.53, 19.51, 19.49, 19.47, 19.28.



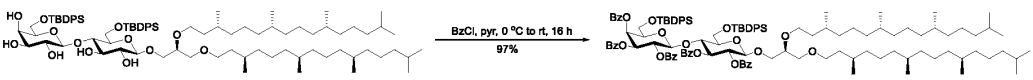
20 23

To a mixture of 6-*O*-silylated lactoside **20** (39 mg, 32 μmol, 1.0 equiv.) in dry pyridine (0.3 mL) and dry toluene (1.2 mL) was added sulfur trioxide trimethylamine complex (6.7 mg, 48 μmol, 1.5 equiv.) at room temperature. After stirring under nitrogen for 3 hours, the mixture was quenched by MeOH (0.3 mL) and then concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7.5% MeOH. The unreacted compound **20** was recovered after the flash chromatography on silica gel (13 mg, 10.7 μmol, 33%) and the purified compound **23** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **23** as white solid (17.5 mg, 13.3 μmol, 42%). R_f= 0.44; (CH_2Cl_2/MeOH 85:15). HRMS (ESI+): $[M+H]^+$ calculated for $C_{71}H_{126}NaO_{16}SSi$, 1317.8434; found, 1317.8435.



23 8

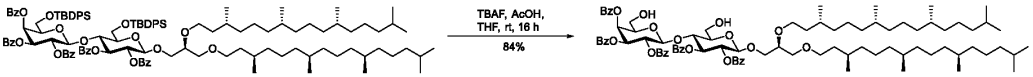
A mixture of compound **23** (15 mg, 11.3 μmol, 1.0 equiv.) in TBAF solution (1M in THF, 0.2 mL) was stirred at room temperature overnight. The solution was then concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7.5% MeOH. The purified compound **8** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4 to remove excess ammonium salts. The resin was filtered off (MeOH/DCM 1:1 v/v, 2 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired tetrabutyl ammonium salt compound **8** as white solid (12.6 mg, 9.7 μmol, 86%). R_f= 0.33; (CH_2Cl_2/MeOH 8:2). NMR ($CDCl_3$:CD_3OD 1:1) corresponds to our previous results.



21 24

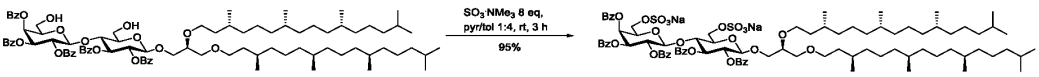
To a mixture of disilylated lactoside **21** (87 mg, 60 μmol, 1.0 equiv.) in dry pyridine (1.5 mL) was added benzoyl chloride (42 μL, 360 μmol, 7.2 equiv.) dropwise at 0 °C. The reaction was stirred under nitrogen at room temperature overnight. The solution was then diluted in CH_2Cl_2 and washed with ice water, saturated $KHSO_4$ solution, saturated $NaHCO_3$ solution and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Crude residue was purified by flash chromatography on silica gel (Hexane/EtOAc 85:15) to afford the desired lactoside **24** as colorless oil. (115 mg, 58.2 μmol, 97%). R_f= 0.27; (Hexane/EtOAc 85:15). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06-6.56

(m, 45H), 5.90 (d, J = 3.3 Hz, 1H, $H_{4'}$), 5.54 (t, J = 9.7 Hz, 1H, H_3), 5.49 (dd, J = 10.4, 7.9 Hz, 1H, $H_{2'}$), 5.43 (dd, J = 10.4, 3.4 Hz, 1H, $H_{3'}$), 5.29 (dd, J = 9.9, 8.0 Hz, 1H, H_2), 5.07 (d, J = 7.9 Hz, 1H, $H_{1'}$), 4.57 (d, J = 8.0 Hz, 1H, H_1), 4.36 (t, J = 9.6 Hz, 1H, H_4), 3.90-3.82 (m, 3H, H_{6a}, H_{6b}, CH*H*-arch-a), 3.82-3.76 (m, 1H, $H_{5'}$), 3.49-3.42 (m, 1H, C*H*-arch-b), 3.42-3.35 (m, 2H, $H_{6'a}$, CH*H*-arch-a), 3.35-3.18 (m, 7H, H_5, CH_2-arch-c, CH_2-arch-d, CH_2-arch-e), 2.98 (t, J = 9.7 Hz, 1H, $H_{6'b}$), 1.69-1.40 (m, 6H), 1.32-1.13 (m, 30H), 1.12 (s, 9H, *t*Bu), 1.08-0.91 (m, 14H), 0.86 (s, 9H, *t*Bu), 0.82-0.61 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 136.01, 135.49, 135.45, 135.44, 133.75, 133.62, 133.06, 133.02, 132.98, 132.62, 132.59, 132.33, 130.36, 130.21, 129.99, 129.86, 129.81, 129.58, 129.52, 129.47, 128.51, 128.33, 128.27, 128.21, 127.95, 127.83, 127.72, 127.54, 101.11 (C_1), 100.12 ($C_{1'}$), 77.65 (*C*H-arch-b), 75.17 (C_5), 73.38 (C_4), 73.30 ($C_{5'}$), 72.89 (C_3), 72.07 ($C_{3'}$), 72.00 (C_2), 70.88, 70.24 ($C_{2'}$), 70.18, 70.03, 69.25, 67.18 ($C_{4'}$), 61.01 (C_6), 59.67 ($C_{6'}$), 39.38, 37.57, 37.48, 37.43, 37.31, 36.99, 36.59, 32.82, 31.60, 29.92, 27.99, 27.03, 26.55, 24.81, 24.49, 24.30, 22.74, 22.64, 19.76, 19.68, 19.55, 19.52, 18.84, 14.13.



24 **25**

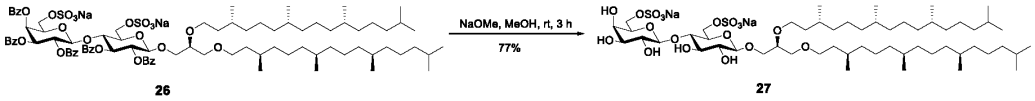
A mixture of compound **24** (237 mg, 0.12 mmol, 1.0 equiv.) in THF (3.0 mL) was acidified to pH 6.5 with few drops of AcOH before adding TBAF solution (1M in THF, 1.44 mL, 12.0 equiv.). After stirring overnight at room temperature, the mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel by gradient using 100% Hexanes to Hexanes/EtOAc (6:4) to afford compound **25** (150 mg, 0.1 mmol, 84%) as colorless oil. R_f = 0.18; (Hex/EtOAc 6:4). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.92-7.06 (m, 25H), 5.63-5.53 (m, 3H, $H_{4'}$, H_3, $H_{2'}$), 5.39 (dd, J = 10.3, 3.4 Hz, 1H, $H_{3'}$), 5.28 (dt, J = 19.8, 5.9 Hz, 1H, H_2), 4.95 (d, J = 7.9 Hz, 1H, $H_{1'}$), 4.65 (d, J = 8.0 Hz, 1H), 4.25 (m, 1H, H_4), 3.84 (dd, J = 10.5, 3.5 Hz, 1H, CH*H*-arch-a), 3.77 (t, J = 6.9 Hz, 1H, $H_{5'}$), 3.74-3.65 (m, 2H, H_{6ab}), 3.45 (dd, J = 10.6, 6.2 Hz, 1H, CH*H*-arch-a), 3.42-3.28 (m, 4H, C*H*-arch-b, H5, CH_2-arch-c), 3.26-3.16 (m, 4H, CH_2-arch-d, CH_2-arch-e), 3.02 (dd, J = 11.5, 6.2 Hz, 1H, $H_{6'a}$), 2.76 (dd, J = 11.5, 7.3 Hz, 1H, $H_{6'b}$), 1.50-0.96 (m, 48H), 0.79-0.64 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 166.09, 165.87, 165.69, 165.46, 165.18, 133.57, 133.44, 133.28, 129.80, 129.64, 129.59, 129.56, 129.20, 128.95, 128.86, 128.68, 128.52, 128.48, 128.35, 128.22, 128.06, 101.08 (C_1), 100.30 ($C_{1'}$), 77.60 (*C*H-arch-b), 75.34 (C_5), 74.16 (C_4), 73.67 ($C_{5'}$), 73.26 (C_3), 72.27 ($C_{3'}$), 71.88 (C_2), 70.41 ($C_{2'}$), 70.21, 70.09, 69.95, 69.09, 68.13 ($C_{4'}$), 59.49 (C_6), 59.25 ($C_{6'}$), 39.29, 37.35, 37.30, 37.26, 37.19, 36.76, 36.39, 32.70, 29.76, 29.58, 27.88, 24.70, 24.37, 24.25, 24.22, 22.48, 22.38, 19.57, 19.55, 19.43, 19.36, 13.50.



25 **26**

To a mixture of compound **25** (145 mg, 96.8 μmol, 1.0 equiv.) in dry pyridine (1.0 mL) and dry toluene (4.0 mL) was added sulfur trioxide trimethylamine complex (107.8 mg, 774.4 μmol, 8 equiv.) at room temperature. After stirring under nitrogen for 3 hours, the mixture was quenched by MeOH (1.0 mL) and

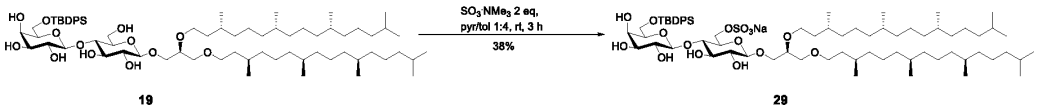
then concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 10% MeOH. The purified compound **26** was treated with a Dowex Marathon sodium form cation-exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **26** as white waxy solid (156 mg, 91.6 μmol, 95%). R_f = 0.22; 6(CH_2Cl_2/MeOH 9:1). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 8.05-7.18 (m, 25H), 5.67 (d, *J* = 3.5 Hz, 1H, $H_{4'}$), 5.58 (t, *J* = 9.7 Hz, 1H, H_3), 5.47 (dd, *J* = 10.2, 7.8 Hz, 1H, $H_{2'}$), 5.41 (dd, *J* = 10.2, 3.4 Hz, 1H, $H_{3'}$), 5.27-5.20 (m, 2H, H_2, $H_{1'}$), 4.65 (d, *J* = 8.0 Hz, 1H, H_1), 4.39 (d, *J* = 9.6 Hz, 1H, H_{6a}), 4.35-4.30 (m, 2H, $H_{5'}$, H_4), 4.14 (d, *J* = 9.6 Hz, 1H, H_{6b}), 3.82 (dd, *J* = 10.2, 2.9 Hz, 1H, CH*H*-arch-a), 3.69 (dd, *J* = 11.6, 6.6 Hz, 1H, $H_{6'a}$), 3.53-3.48 (m, 2H, H_5, $H_{6'b}$), 3.43-3.36 (m, 2H, CH*H*-arch-a, C*H*-arch-b), 3.30 (ddt, *J* = 10.7, 9.4, 4.6 Hz, 2H, CH_2-arch-c), 3.23-3.20 (m, 2H, CH_2-arch-d), 3.17 (ddd, *J* = 16.5, 8.6, 6.7 Hz, 2H, CH_2-arch-e), 1.42-0.91 (m, 48H), 0.79-0.62 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 166.03, 165.68, 165.33, 136.92, 136.92, 133.30, 133.17, 129.85, 129.76, 129.65, 129.59, 129.50, 129.45, 128.83, 128.55, 128.39, 128.36, 128.20, 128.15, 101.35 (C_1), 99.53 ($C_{1'}$), 77.59 (*C*H-arch), 73.28 (C_5), 73.11 (C_4), 72.90 (C_3), 72.50 ($C_{3'}$), 71.86 (C_2), 71.32 ($C_{5'}$), 70.39, 70.36, 70.09 ($C_{2'}$), 69.88, 69.05, 68.16 ($C_{4'}$), 65.09 ($C_{6'}$), 64.39 (C_6), 54.48, 44.92, 39.27, 37.34, 37.28, 37.24, 37.16, 36.76, 36.37, 32.67, 29.74, 29.53, 27.85, 24.67, 24.33, 24.20, 22.39, 22.30, 22.28, 19.50, 19.48, 19.38, 19.29.



26 **27**

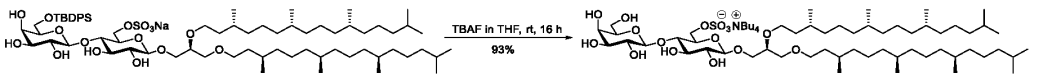
A solution of compound **26** (156 mg, 91.6 μmol, 1.0 equiv.) in 1M sodium methoxide in methanol (1.0 mL, pH 8-9) was stirred at room temperature until consumption of starting material. After 3 hours, the solution was ion-exchanged by resin (Amberlite™ IR 120, H^+), filtered, washed with MeOH/DCM 1:1, and the solvent was removed under reduced pressure. The crude residue was chromatographed over silica gel by gradient using 100% DCM to 30% MeOH and the purified compound **27** was treated with a Dowex Marathon sodium form cation-exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the debenzoylated lactose archaeal compound **27** as white foam after lyophilisation (82.7 mg, 70 μmol, 77%). R_f= 0.43; (CH_2Cl_2/MeOH 7:3). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.40-4.33 (m, 1H, $H_{1'}$), 4.28-4.25 (m, 1H, $H_{6'a}$), 4.25-4.20 (m, 3H, H_1, H_{6a}, H_{6b}), 4.04 (dd, *J* = 10.8, 3.2 Hz, 1H, $H_{6'b}$), 3.85 (dd, *J* = 10.7, 4.1 Hz, 1H, CH*H*-arch-a), 3.82 (dd, *J* = 9.4, 3.0 Hz, 1H, $H_{5'}$), 3.77 (s, 1H, $H_{4'}$), 3.61-3.46 (m, 10H, $H_{2'}$, $H_{3'}$, H_3, H_4, H_5, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c, CH*H*-arch-d), 3.43-3.37 (m, 3H, CH*H*-arch-d, CH_2-arch-e), 3.26-3.22 (m, 1H, H_2), 1.54 (dt, *J* = 12.8, 6.9 Hz, 2H), 1.45 (tq, *J* = 13.3, 6.6 Hz, 4H), 1.34-0.95 (m, 42H), 0.83-0.74 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.92 ($C_{1'}$), 102.73 (C_1), 80.95 (C_4), 77.74 (CH-arch), 74.33 (C_5), 73.49 ($C_{5'}$), 73.09 ($C_{3'}$),73.05 (C_3), 72.76 (C_2), 71.10 ($C_{2'}$), 70.28, 69.89, 68.97, 68.50 ($C_{4'}$), 68.43, 66.97 ($C_{6'}$), 66.11 (C_6), 39.25, 37.37, 37.27, 37.23, 37.13, 36.80,

36.51, 32.64, 29.77, 27.83, 27.81, 24.64, 24.30, 24.26, 22.26, 22.17, 19.38, 19.36, 19.31. HRMS (ESI+) calcd. for $C_{55}H_{108}O_{19}S_2Na$ $[M+Na]^+$+ [-H_2O]: 1141.6713, found 1141.6730.



19 29

To a mixture of 6'-*O*-silylated lactoside **19** (230 mg, 189 μmol, 1.0 equiv.) in dry pyridine (1.0 mL) and dry toluene (4.0 mL) was added sulfur trioxide trimethylamine complex (52.6 mg, 378 μmol, 2.0 equiv.) at room temperature. After stirring under nitrogen for 3 hours, the mixture was quenched by MeOH (1.0 mL) and then concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7% MeOH. The unreacted compound **19** was recovered after the flash chromatography on silica gel (88 mg, 72.3 μmol, 39%) and the purified compound **29** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4. The resin was filtered off (MeOH/DCM 1:1 v/v, 3 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired sodium salt compound **29** as white solid (93 mg, 70.6 μmol, 38%). R_f= 0.44; (CH_2Cl_2/MeOH 85:15). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 1:1) δ 7.59 (ddd, *J* = 13.4, 7.9, 1.4 Hz, 4H), 7.37-7.27 (m, 6H), 4.41-4.37 (m, 1H, $H_{1'}$), 4.27 (dd, *J* = 11.0, 3.7 Hz, 1H, H_{6a}), 4.22-4.19 (m, 2H, H_1, H_{6b}), 3.89 (s, 1H, $H_{4'}$), 3.81 (ddd, *J* = 15.7, 10.4, 5.7 Hz, 2H, $H_{6'a}$, CH*H*-arch-a), 3.73 (dd, *J* = 10.0, 6.0 Hz, 1H, $H_{6'b}$), 3.62 (dd, *J* = 12.9, 6.2 Hz, 1H, $H_{5'}$), 3.59-3.36 (m, 13H, $H_{2'}$, $H_{3'}$, H_3, H_4, H_5, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c, CH_2-arch-d, CH_2-arch-e), 3.21 (dd, *J* = 9.0, 8.0 Hz, 1H, H_2), 1.56-1.49 (m, 2H), 1.48-1.40 (m, 4H), 1.34-1.08 (m, 30H), 1.08-0.97 (m, 12H), 0.96 (s, 9H, *t*Bu), 0.84-0.73 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 135.45, 135.38, 132.94, 129.71, 129.68, 127.67, 127.61, 103.68 ($C_{1'}$), 103.24 (C_1), 79.77 (C_4), 77.72 (CH-arch), 75.10 ($C_{5'}$), 74.38 (C_5), 73.52 (C_3), 73.14 (C_2), 73.05 ($C_{3'}$), 71.54 ($C_{2'}$), 70.47, 69.88, 69.02, 68.55, 68.17 ($C_{4'}$), 65.95 (C_6), 61.85 ($C_{6'}$), 44.68, 39.25, 37.37, 37.27, 37.14, 36.82, 36.50, 32.67, 32.65, 29.77, 29.66, 27.83, 26.41, 24.65, 24.30, 24.26, 22.28, 22.19, 19.41, 19.38, 19.35, 19.32, 18.89.



29 30

A mixture of compound **29** (78 mg, 59.2 μmol, 1.0 equiv.) in TBAF solution (1M in THF, 1.0 mL) was stirred at room temperature overnight. The solution was then concentrated under vacuum and the crude residue was purified by flash chromatography on silica gel by gradient using 100% DCM to 7.5% MeOH. The purified compound **30** was treated with a Dowex Marathon sodium form cation exchange resin (swelled and prewashed with MeOH for 10 min) until pH 7.4 to remove excess ammonium salts. The resin was filtered off (MeOH/DCM 1:1 v/v, 2 mL) and the filtrate was centrifuged at 6000 rpm for 5 min. The clear supernatant obtained after centrifugation was concentrated under reduced pressure to afford the desired compound **30** as white solid (71.4 mg, 55 μmol, 93%). R_f= 0.33; (CH_2Cl_2/MeOH 8:2). ^{1}H NMR

(600 MHz, $CDCl_3$:CD_3OD 1:1) δ 4.46-4.40 (m, 1H, $H_{1'}$), 4.28 (dd, *J* = 11.0, 3.3 Hz, 1H, H_{6a}), 4.22 (m, 2H, H_{6b}, H_1), 3.84 (dd, *J* = 10.7, 4.3 Hz, 1H, CH*H*-arch-a), 3.78 (s, 1H, $H_{4'}$), 3.73 (dd, *J* = 11.7, 7.2 Hz, 1H, $H_{6'a}$), 3.65 (dd, *J* = 11.6, 4.6 Hz, 1H, $H_{6'b}$), 3.60-3.36 (m, 14H, $H_{2'}$, H_3, $H_{3'}$, H_4, H_5, $H_{5'}$, CH*H*-arch-a, C*H*-arch-b, CH_2-arch-c, CH_2-arch-d, CH_2-arch-e), 3.24-3.21 (m, 1H, H_2), 1.52 (dd, *J* = 13.2, 8.0 Hz, 2H), 1.48-1.41 (m, 4H), 1.32-1.10 (m, 30H), 1.08-0.97 (m, 12H), 0.83-0.74 (m, 30H); ^{13}C NMR (150 MHz, $CDCl_3$:CD_3OD 1:1) δ 103.46 ($C_{1'}$), 103.26 (C_1), 79.34 (C_4), 77.75 (*C*H-arch), 75.47 ($C_{5'}$), 74.57 (C_5), 73.52 (C_3), 73.21 (C_2), 73.16 ($C_{3'}$), 71.53 ($C_{2'}$), 70.47, 69.92, 69.05 ($C_{4'}$), 68.99, 68.55, 65.90 (C_6), 61.29 ($C_{6'}$), 58.46, 39.27, 37.40, 37.31, 37.29, 37.27, 37.15, 36.84, 36.52, 32.68, 32.66, 29.79, 29.69, 27.85, 27.82, 24.66, 24.33, 24.27, 23.62, 22.34, 22.25, 22.20, 19.53, 19.46, 19.43, 19.40, 19.38, 13.17.

EXAMPLE 7: FORMULATION OF LIPOSOMES OF COMPOUND 8

The 6'-SLA (Compound **8)** was hydrated in 1X sterile PBS to 40 mg/mL, then extruded by 11 passages through a 100 nm polycarbonate membrane using the Avanti mini extruder (Fisher Scientific Catalog no. NC9273238) to produce monodisperse and homogeneous liposomes. The number of extrusion passages can be repeated up to 21 times, as required. The size distribution of the prepared liposomes was then analyzed by dynamic light scattering (DLS), having an average diameter of 115 nm and a PI (polydispersity index) of 0.06.

EXAMPLE 8: BIOLOGICAL TESTING

Example 8A- Compound 8 (6'-SLA) stimulates the dose-dependent production of TNF-α by a murine macrophage cell line

Method: RAW264.7 cells were split at 70-80% confluency and seeded into 96-well flat bottom plates at a density of 200,000 cells per well. Cells were stimulated with varying concentrations of Compound **8** (10mg/mL) for 24 hours, after which the cell culture supernatant was collected. TNF-α was quantified in the cell culture supernatant by ELISA as per the manufacturer's protocol (Thermo Fisher catalog # 88-7324-88). Each dot represents the mean of 3 technical replicates. Error bars represent SD. EC50 was calculated using the asymmetrical (5-parameter) dose response curve function in Prism 10.0.2.

Results: The results are shown in Figure 1. Compound **8** (6'-SLA) stimulates the dose-dependent production of TNF-α by a murine macrophage cell line.

Example 8B- The fully synthetic 6'-SLA Compound 8 stimulates comparable amounts of TNF-α compared to the archaeol-based glycolipid produced by a semi-synthetic process

Methods: RAW264.7 cells were split at 70-80% confluency and seeded into 96-well flat bottom plates at a density of 200,000 cells per well. Cells were stimulated with varying concentrations of Compound **8**, or with SLA-1 obtained according to the semi-synthetic process by Régnier S. et al. (J. Med. Chem. 2022, 65, 8332−8344), for 24 hours, after which the cell culture supernatant was collected. TNF-α was quantified in the cell culture supernatant by ELISA as per the manufacturer's protocol (Thermo Fisher catalog # 88-7324-88). Each dot represents an average of six technical replicates from two independent experiments. Bar represents the mean.

Results: The results are shown in Figure 2. The graph shows that stimulation of RAW264.7 cells with the indicated concentration of either compound induces the production of TNF-α in a dose response manner with a similar effective concentration (EC50).

Example 8C- Fully synthetic 6'-SLA (Compound 8) and variants thereof can stimulate the dose-dependent production of TNF-α by a murine macrophage cell line

Methods: RAW264.7 cells were split at 70-80% confluency and seeded into 96-well flat bottom plates at a density of 200,000 cells per well. Cells were stimulated with the same range of concentrations of the various compounds from 11.56 µM to 1480.00 µM for 24 hours, after which the cell culture supernatant was collected. TNF-α was quantified in the cell culture supernatant by ELISA as per the manufacturer's protocol (Thermo Fisher catalog # 88-7324-88). Each dot represents the mean of 3 technical replicates. Error bars represent SD. The EC50 was calculated using the asymmetrical (5-parameter) dose response curve function in Prism 10.0.2.

Results: The results are shown in Figure 3 and Table 1. The results show that RAW264.7 cells respond similarly to Compound **8** whether extruded or not when compared to the other compounds. Nonetheless, Compounds **27** and **28** may have varying degrees of immunostimulatory capacity. Compound **8** (EC50 = 159.4 µM, TNF-α max. = 597.3 pg/mL), Compound **8** extruded (EC50= 175.5 µM, TNF-α max.= 510.6 pg/mL), Compound **27** (EC50 = 718.8 µM, TNF-α max. = 535.8 pg/mL), Compound **27** extruded (EC50 = na, TNF-α max. = na)

Compound #	EC_{50} (µM) not extruded	TNF-α max. (pg/mL) not extruded	DLS (nm)/ PI not extruded	EC_{50} (µM) extruded	TNF-α max. (pg/mL) extruded	DLS (nm)/ PI extruded
8	159.4	597.3	78 / 0.42	175.5	510.6	115 / 0.06
27	718.8	535.8	143 / 0.52	na	na	95 / 0.58
28	117.5	666.4	78-106 / 0.50	206.4	435.2	94 / 0.10
30	na	na	109 / 0.50	na	na	60/ 0.15

Example 8D- The adjuvant 6'-SLA induces a more robust antigen-specific antibody response compared to other adjuvants

Method: BALB/c mice were immunized with 25 µg of the glycoprotein cross-reactive material 197 conjugated to the Thomsen-Friedenreich antigen (CRM197-TF), adjuvanted with either; 1mg of 6'-SLA, 50 µL of Sepivac SWE™, 25 µL alum (aluminum hydroxide - Imject Alum™) or Phosphate-buffered saline (PBS) in a total volume of 100 µL by intramuscular injection. Mice were immunized at 2-week intervals for a total of 3 immunizations. Stars indicate immunization time points. Serum TF-specific IgG concentration was quantified by ELISA at the time points indicated. Error bars indicate SD.

Results: The results are shown in Figure 4. The results show that mice immunized with CRM197-TF adjuvanted with 6'-SLA developed a more robust anti-TF specific antibody response than with the other adjuvants.

Example 8E: SLA induces both a type 1 and 2 immune response

Method: BALB/c mice were immunized with 25 µg of CRM197-TF adjuvanted with 1 mg of SLA (Compound **8**) or PBS by intramuscular injection. Mice were immunized for a total of 4 immunizations. Spleens were harvested 7 days after the last immunization. (A) Total serum IgG2a and IgG1 concentration quantified by ELISA at the time points indicated. Each dot represents the mean of 15 biological replicates. Error bars represent SD. (B) 500,000 splenocytes were unstimulated and stimulated with the indicated concentration of CRM197-TF or 2 µg/mL of the mixture of soluble anti-CD3 and anti-CD28 as positive control, for 72 hours. Cytokine concentration was quantified by ELISA from cell culture supernatant. Each dot represents an individual mouse.

Results: The results are shown in Figure 5: The results show that immunization of mice with a vaccine adjuvanted with SLA induces a mixed immune response of the Th1 phenotype, characterized by the production of IgG2a antibodies and the cytokines INF-γ and TNF-α, and of the Th2 phenotype characterized by the production of IgG1 antibodies, and the cytokines IL-4 and IL-6.

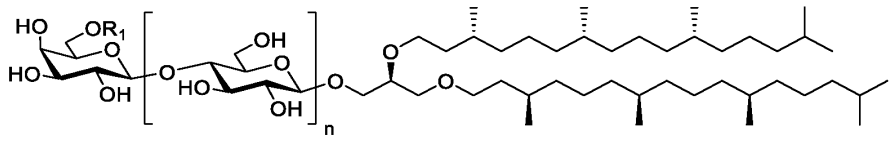
Example 8F: SLA induces a CRM197-specific cell proliferation

Method: BALB/c mice were immunized with 25 µg of CRM197 adjuvanted with 0.5 mg of SLA (Compound **8**) or PBS by intramuscular injection. Mice were immunized for a total of 3 immunizations 14 days apart and splenocytes were isolated 7 days after the last immunization. 500,000 splenocytes labeled with CellTrace™ Violet were stimulated for 72 hours with 50 µg/mL of CRM197. The dilution of CellTrace™ Violet in viable $CD19^{-}CD4^{-}CD8^{+}CD44+$, $CD19^{-}CD4^{+}CD8^{-}CD44+$ and $CD19^{+}CD4^{-}CD8^{-}CD44+$ cells was quantified by flow cytometry. Data were analyzed by one-way ANOVA. Dots in graphs represent individual mice. $***p < 0.001$, $**p < 0.01$, and $*p < 0.05$. Error bars represent SD; ns, not significant.

Results: The results are shown in Figure 6: The graphs represent the dilution of CellTrace™ Violet where each peak (P) quantifies the frequency of cells that have proliferated P(x) number of times. Splenocytes derived from SLA-adjuvanted mice proliferated significantly more upon re-stimulation with CRM197 compared to those derived from mice that received no adjuvant. This is representative of a more robust adaptive immune response and larger memory cell pool.

CLAIMS

1. A process for synthesizing a sulfated glycoarchaeal of Formula (I)

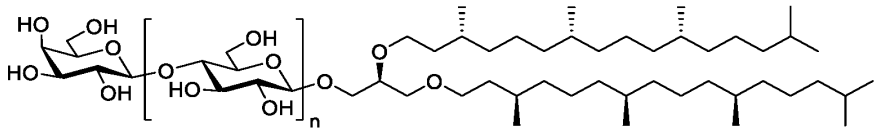


(I)

where R_1 is SO_3M with M being an alkali metal, and n is 0 or 1;

comprising:

reacting a compound of Formula (A)



(A)

with a sulfur trioxide reagent to form an intermediate compound;

salification of the intermediate compound to form the compound of Formula (I).

2. The process of claim 1, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex.

3. The process of claim 2, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof.

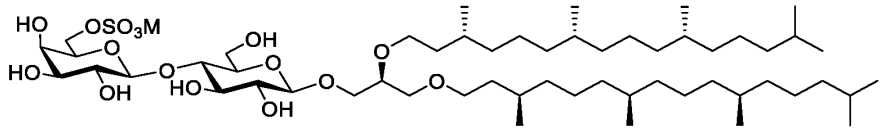
4. The process of claim 2, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, preferably $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

5. The process of any one of claims 1 to 4, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene or tetrahydrofuran (THF), even more preferably the organic solvent comprises CH_2Cl_2, pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

6. The process of any one of claims 1 to 5, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

7. The process of any one of claims 1 to 6, wherein the salification is performed with an alkali metal buffer, an alkali metal ion exchange resin, or using MeOM in MeOH or AcOM in AcOH, where M is the alkali metal, preferably the salification is performed with the alkali metal ion exchange resin.

8. The process of any one of claims 1 to 7, wherein n is equal to 1 and the sulfated glycoarchaeal has the following formula (II)



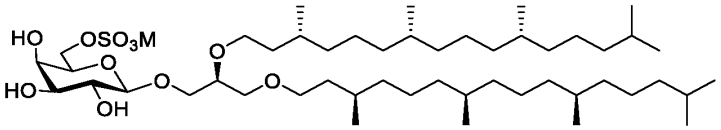
(II)

9. The process of any one of claims 1 to 8, wherein n is equal to 1 and the sulfur trioxide reagent is used in an amount providing about 1 molar equivalent of sulfur trioxide, such as about 1.05 to about 1.1 molar equivalent of sulfur trioxide.

10. The process of any one of claims 1 to 9, wherein n is equal to 1 and the reaction with the sulfur trioxide reagent is performed at room temperature for about 10 hours to about 72 hours, preferably for at least 15 hours, more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

11. The process of any one of claims 1 to 10, wherein n is equal to 1 and the reaction with the sulfur trioxide reagent comprises mixing a solution of the compound of Formula (I) with the sulfur trioxide reagent at about 0 °C and then stirring the resulting mixture at room temperature, preferably for about 10 hours to about 72 hours, more preferably for at least 15 hours, even more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

12. The process of any one of claims 1 to 7, wherein n is 0 and the sulfated glycoarchaeal has the following formula (III)



(III)

13. The process of any one of claims 1 to 7 and 12, wherein n is 0 and the sulfur trioxide reagent is used in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide, preferably from about 1.5 to about 7.5 molar equivalents, more preferably from about 1.5 to about 5 molar equivalents, for instance from about 1.5 to about 2 molar equivalents.

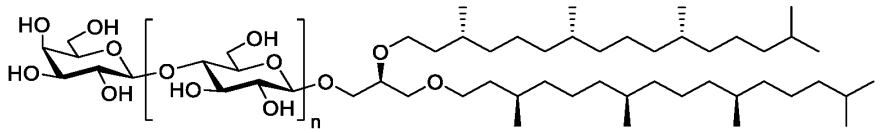
14. The process of any one of claims 1 to 7, 12 and 13, wherein n is 0 and the reaction with the sulfur trioxide reagent is performed at room temperature for at most about 4 hours, such as about 1 to about 4 hours.

15. The process of any one of claims 1 to 14, wherein M is Li, Na or K, preferably Na.

16. The process of any one of claims 1 to 15, further comprising recovering unreacted compound of Formula (A) after the reaction with the sulfur trioxide reagent.

17. The process of claim 16, wherein the recovered unreacted compound of Formula (A) is reused in the process.

18. A process for preparing a compound of Formula (A)

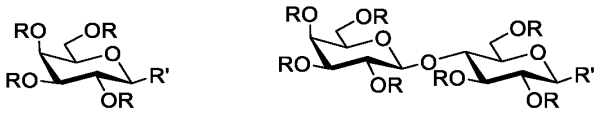


(A)

where n is 0 or 1;

comprising:

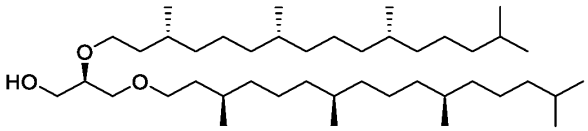
reacting a compound of Formula (B_0) when n is 0 or a compound of Formula (B_1) when n is 1



(B_0) (B_1)

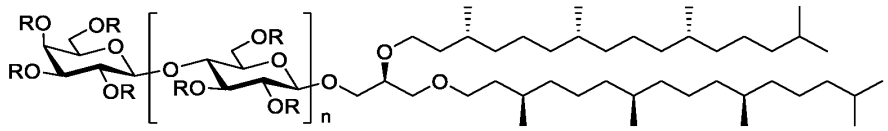
where R is a protecting group and R' is a leaving group

with a compound of Formula (C)



(C)

to form a compound of Formula (D)



(D)

and

deprotecting the compound of Formula (D) to form the compound of Formula (A).

19. The process of claim 18, wherein the protecting group R is Ac or Bz.

20. The process of claim 18 or 19, wherein the leaving group is halogen (e.g., Cl or Br), OAc, SEt, SPh, STol, OTs, OMs, imidate such as trichloroacetimidate , SePh, S(O)Ph, or $S(O)_2Ph$, preferably OAc, Br, trichloroacetimidate, SEt, SPh, or STol, more preferably OAc, Set, SPh, or STol, even more preferably OAc.

21. The process of any one of claims 18 to 20, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of a Lewis acid reagent.

22. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate (BF_3OEt_2), Trimethylsilyl trifluoromethanesulfonate (TMSOTf), Trifluoromethanesulfonic acid (TFOH), silver trifluoromethanesulfonate (AgOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$), Silver trifluoroacetate (CF_3CO_2Ag), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, optionally in combination with a metal-containing zeolite.

23. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate (BF_3OEt_2), Tin(IV) chloride ($SnCl_4$), Tin(IV) bromide ($SnBr_4$) or any mixture thereof, optionally in combination with a metal-containing zeolite.

24. The process of any one of claims 18 to 21, wherein the leaving group is OAc and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of boron trifluoride diethyl etherate (BF_3OEt_2), or in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite.

25. The process of any one of claims 18 to 21, wherein the leaving group is OAc, n is 1, and reacting the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of Tin(IV) chloride ($SnCl_4$) in combination with a metal-containing zeolite, optionally further in combination with a molecular sieve.

26. The process of any one of claims 18 to 24, wherein the leaving group is OAc, n is 0, and reacting the compound of Formula (B_0) with the compound of Formula (C) is performed in the presence of Tin(IV) chloride ($SnCl_4$) and a metal-containing zeolite, optionally in combination with a molecular sieve.

27. The process of any one of claims 18 to 21, wherein the leaving group is Br and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of a metal-containing zeolite in combination with a molecular sieve.

28. The process of any one of claims 22 to 27, wherein the metal in the metal-containing zeolite comprises Ag, Al, Cd, Co, Cu, Fe, Ga, In, Mo, Pd, Pt, Sn, Sb, V, Zr or any mixture thereof.

29. The process of any one of claims 22 to 28, wherein the metal-containing zeolite is a zeolite selected from the group consisting of Ag-zeolite, Sn-Beta, Zr-Beta, Al-Beta(OH), Al-Beta(F), Pt@MCM-22, K-PtSn/MFI, 0.3Pt/0.5Sn-Si-Beta, Pt/Sn 2.0-Beta, 0.5CoSi-Beta, V-Beta, H-[Fe]ZSM-5, Fe-BEA, Ga-Beta, Ga-Beta-200, Mo/HZSM-5, and any mixture thereof.

30. The process of any one of claims 22 to 29, wherein the metal-containing zeolite is a silver-containing zeolite.

31. The process of any one of claims 18 to 21, wherein the leaving group is SEt, SPh or STol and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of N-iodosuccinimide (NIS) in combination with triflic acid, triflate (TfOH), BF_3-OEt_2, AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or in the presence of trifluoromethanesulfonic anhydride (Tf_2O) with 2,6-di-terbutyl-4-methylpyridine (DTBMP); or any mixture thereof; preferably reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of N-iodosuccinimide (NIS) in combination with BF_3-OEt_2 or $Yb(OTf)_3$.

32. The process of any one of claims 18 to 21, wherein the leaving group is trichloroacetimidate and reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of triflic acid, triflate (TfOH), AgOTf, Trimethylsilyl trifluoromethanesulfonate (TMSOTf), copper(I) trifluoromethanesulfonate (CuOTf), Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$), Zinc trifluoromethanesulfonate ($Zn(OTf)_2$), Aluminium trifluoromethanesulfonate ($Al(OTf)_3$), Bismuth(III) trifluoromethanesulfonate ($Bi(OTf)_3$), Iron(III) trifluoromethanesulfonate ($Fe(OTf)_3$), or Manganese bis(trifluoromethanesulfonate) ($Mn(OTf)_2$); or any mixture thereof; preferably reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in the presence of TMSOTf.

33. The process of any one of claims 18 to 32, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed in at least one organic

solvent, preferably the at least one organic solvent is CH_2Cl_2, toluene, DMF, DMSO, Dimethyl acetamide (DMAC), THF, Dioxane, 1,3 Dioxane, acetonitrile, 2,5-dimethyl tetrahydrofuran (DMTHF), Gamma-vaterolactone (GVL), Dihydrolevoglucoserone (Cyrene), methyl levulinate (ML), Ethyl levulinate (EL), Ethyl levulinate propyleneglycol ketal (ELPK), Dimethyl glutarate (DMG), Dimethylpropylene urea (DMPU), Poly(propyleneglycol) (PPG), Glycofurol (THFP), 1-Ethyl-3-methylimidazolium acetate ([emim][OAc]), or any mixture thereof, most preferably, the at least one organic solvent is CH_2Cl_2, toluene, or a mixture thereof.

34. The process of any one of claims 18 to 33, wherein reacting the compound of Formula (B_0) or the compound of Formula (B_1) with the compound of Formula (C) is performed at room temperature and optionally further under heating.

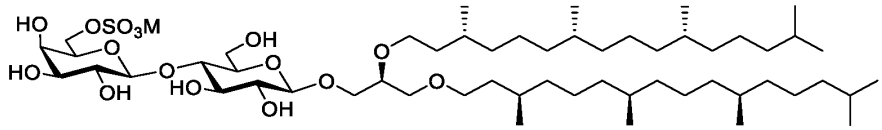
35. The process of any one of claims 18 to 34, wherein reacting the compound of Formula (B_0) with the compound of Formula (C) is performed for about 1 hour to about 3 hours, preferably for at most 2 hours, more preferably for about 1 hour to about 2 hours.

36. The process of any one of claims 18 to 34, wherein reacting the compound of Formula (B_1) with the compound of Formula (C) is performed for about 1 hour to about 72 hours, preferably for at least 15 hours, more preferably for at least 16 hours, for instance from about 16 hours to about 72 hours.

37. The process of any one of claims 18 to 36, wherein deprotecting the compound of Formula (D) to form the compound of Formula (A) is performed by reacting the compound of Formula (D) with a basic solution, preferably comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, preferably the base is sodium methoxide.

38. The process of any one of claims 1 to 17, wherein the compound of Formula (A) is prepared according to the process of any one of claims 18 to 37.

39. A process for synthesizing a sulfated glycoarchaeal of Formula (II)

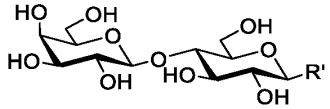


(II)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$,

comprising either:

i) silylating the -OH in position 6' of the lactopyranoside part of the compound of Formula (E)



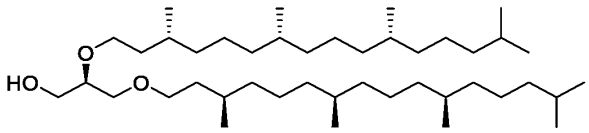
(E)

where R' is SEt, SPh or STol, SePh, S(O)Ph, or $S(O)_2Ph$;

to form a 6'-silylated compound (E1);

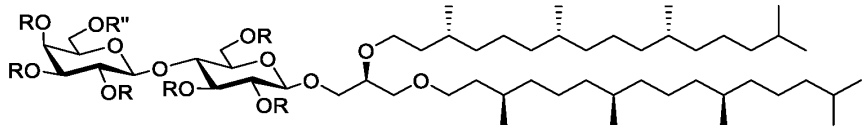
protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2);

reacting the 6'-silylated protected compound (E2) with a compound of Formula (C)



(C)

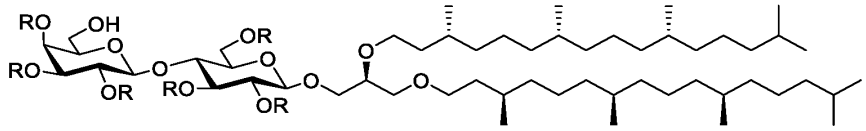
to form a compound of Formula (F)



(F)

where R is a protecting group and R" is a silyl group;

desilylating the compound of Formula (F) to form a compound of Formula (G)



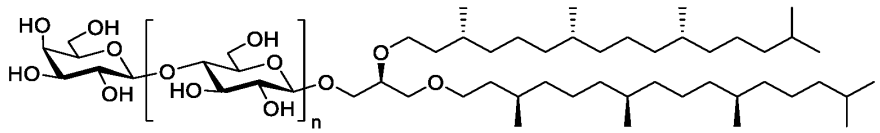
(G) ;

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with a sulfur trioxide reagent to form an intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

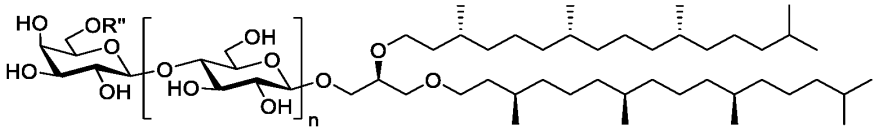
ii) silylating the -OH in position 6' of the compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A1)



(A1)

where n is 1 and R" is as defined above;

protecting the hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F);

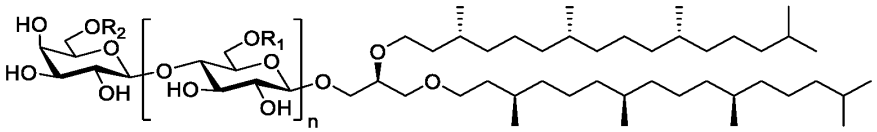
desilylating the compound of Formula (F) to form the compound of Formula (G);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (G) with the sulfur trioxide reagent to form the intermediate compound;

deprotecting the intermediate compound to form the compound of Formula (II);

or

iii) silylating the compound of Formula (A) with a silylating agent to form a mixture of compounds of Formula (A2), (A3) and (A4)



(A2): n =1; R_1 = H and R_2 = R"

(A3): n = 1; R_1 = R" and R_2 = H

(A4): n = 1; R_1 = R" and R_2 = R";

separating compounds of Formula (A2), (A3) and (A4) to recover compound of Formula (A3);

sulfating the -OH in position 6' of the lactopyranoside part of the compound of Formula (A3) with the sulfur trioxide reagent to form a 6'-sulfated 6-silylated compound (A5); and

desilylating the sulfated compound (A5) to form the compound of Formula (II).

40. The process of claim 39, wherein R' is SEt, SPh or STol.

41. The process of claim 39 or 40, wherein silylating in i) and/or ii) comprises first reacting the compound of Formula (E) or Formula (A) with an organotin oxide or organotin dichloride compound to form a stannylene acetal of the lactopyranoside part of the compound of Formula (E) or Formula (A) and then reacting the stannylene acetal of the compound of Formula (E) or Formula (A) with a silylating agent.

42. The process of claim 41, wherein the organotin oxide or organotin dichloride compound comprises a di(C_1-C_4alkyl)tin oxide or dichloride, preferably dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride, most preferably dibutyltin oxide.

43. The process of any one of claims 39 or 42, wherein the silylating agent comprises a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

44. The process of any one of claims 39 to 43, wherein protecting the hydroxyl groups of the 6'-silylated compound (E1) to form a 6'-silylated protected compound (E2), and/or protecting the

hydroxyl groups of the 6'-silylated compound (A1) to form the compound of Formula (F) comprises reacting the 6'-silylated compound (E1) or (A1) with a compound RX, where X is halogen and R is Ac or Bz, preferably Bz.

45. The process of any one of claims 39 to 44, wherein reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) is performed in the presence of a Lewis acid.

46. The process of any one of claims 39 to 45, wherein reacting the 6'-silylated protected compound (E2) with the compound of Formula (C) is performed in the presence of N-iodosuccinimide (NIS) in combination with Indium(III) trifluoromethanesulfonate ($In(OTf)_3$), Ytterbium(III) trifluoromethanesulfonate hydrate ($Yb(OTf)_3$), Scandium(III) triflate ($Sc(OTf)_3$), or Lanthanum(III) trifluoromethanesulfonate ($La(OTf)_3$); or any mixture thereof.

47. The process of any one of claims 39 to 46, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex.

48. The process of claim 47, wherein the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof, preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof, more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

49. The process of any one of claims 39 to 48, wherein the sulfur trioxide amine complex is used in an amount providing from about 1.5 to about 10 molar equivalents of sulfur trioxide, preferably from about 2 to about 10 molar equivalents, more preferably from about 5 to about 10 molar equivalents, for instance about 8 molar equivalents.

50. The process of any one of claims 39 to 49, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises CH_2Cl_2, pyridine, toluene or a combination thereof, such as a combination of CH_2Cl_2 and pyridine or a combination of CH_2Cl_2 and toluene.

51. The process of any one of claims 39 to 50, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

52. The process of any one of claims 39 to 51, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours.

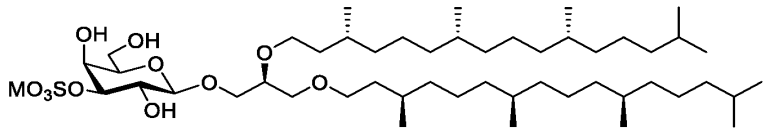
53. The process of any one of claims 39 to 52, wherein deprotecting the intermediate compound comprises reacting the intermediate compound with a basic solution preferably comprising a base

selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, more preferably the base is sodium methoxide.

54. The process of any one of claims 39 to 53, wherein desilylating the compound of Formula (F) to form the compound of Formula (G) and/or desilylating the sulfated compound of Formula (A5) to form the compound of Formula (I) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

55. The process of any one of claims 39 to 54, wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$, preferably Li, Na, K or $N(Bu)_4$, more preferably Na.

56. A process for synthesizing a sulfated glycoarchaeal of Formula (IV)

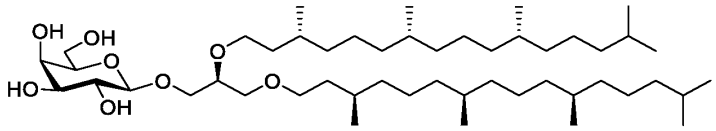


(IV)

where M is an alkali metal;

comprising:

reacting a compound of Formula (A0)



(A0)

with an organotin oxide or organotin dichloride compound to form a stannylene acetal on the galactopyranoside part of the compound (A0);

then reacting the stannylene acetal of the compound of Formula (A0) with a sulfur trioxide reagent to form an intermediate compound; and

salification of the intermediate compound to form the compound of Formula (IV).

57. The process of claim 56, wherein the process comprises:

forming a mixture of the compound of Formula (A0) and the organotin oxide or organotin dichloride compound in a first organic solvent and then heating the mixture;

concentrating the mixture to form a concentrated mixture;

redissolving the concentrated mixture in a second organic solvent to form a redissolved mixture;

reacting the redissolved mixture with the sulfur trioxide reagent to form the intermediate compound; and

salification of the intermediate compound to form the compound of Formula (I).

58. The process of claim 57, wherein the first organic solvent and the second organic solvent are independently selected from CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, tetrahydrofuran (THF), and any combination thereof; preferably the first organic solvent and the second organic solvent are independently selected from CH_2Cl_2, pyridine, toluene and any combination thereof; more preferably the first organic solvent and the second organic solvent are independently selected from pyridine, toluene and a combination thereof.

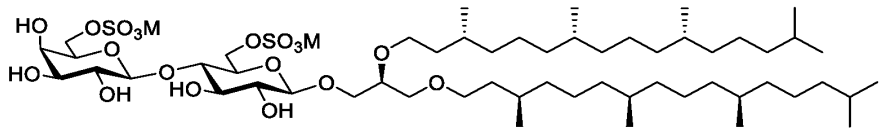
59. The process of claim 57 or 58, wherein heating the mixture is performed at reflux and/or reacting the redissolved mixture with the sulfur trioxide reagent is performed at room temperature for about 1 hour to about 72 hours, preferably for about 10 hours to 72 hours, more preferably for at least 15 hours, for instance from about 15 hours to about 72 hours.

60. The process of any one of claims 56 to 59, wherein the sulfur trioxide reagent is used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide, preferably from about 1 to about 7.5 molar equivalents, more preferably from about 1 to about 5 molar equivalents, for instance from about 1.05 to about 2 molar equivalents.

61. The process of any one of claims 56 to 60, wherein the organotin oxide or organotin dichloride compound comprises a di(C_1-C_4alkyl)tin oxide or dichloride, preferably dibutyltin oxide, dibutyltin dichloride or dimethyltin dichloride, most preferably dibutyltin oxide.

62. The process of any one of claims 56 to 61, wherein the sulfur trioxide reagent is as defined in any one of claims 2 to 4.

63. A process for synthesizing a disulfated glycoarchaeal of Formula (V)

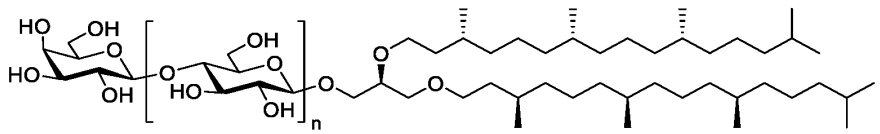


(V)

where M is an alkali metal;

the process comprising:

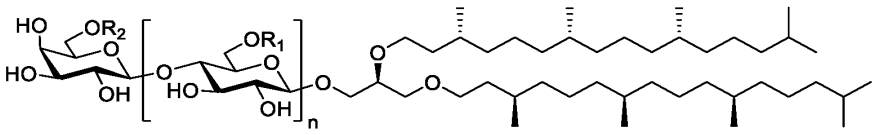
silylating the -OH in positions 6 and 6' of a compound of Formula (A)



(A)

where n is 1;

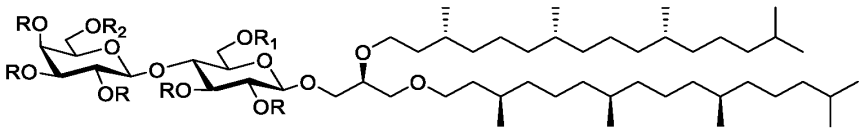
to form a 6- and 6'-silylated compound (A4)



(A4)

where n is 1 and R_1 and R_2 represent a silyl group;

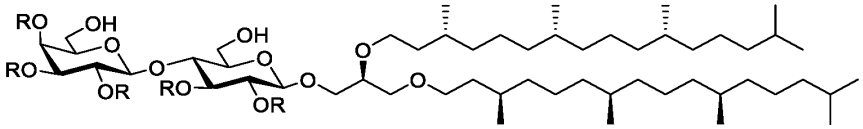
protecting the hydroxyl groups of the 6- and 6'-silylated compound (A4) to form the compound of Formula (F1)



(F1)

where R is a protecting group;

desilylating the compound of Formula (F1) to form the compound of Formula (G1);



(G1)

sulfating the -OH in positions 6 and 6' of the compound of Formula (G1) with a sulfur trioxide reagent to form an intermediate compound; and

deprotecting the intermediate compound to form the compound of Formula (V).

64. The process of claim 63, wherein silylating comprises reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

65. The process of claim 63 or 64, wherein protecting the hydroxyl groups of the 6 and 6'-silylated compound (A4) comprises reacting the compound (A4) with a compound RX, where X is halogen and R is Ac or Bz, preferably Bz.

66. The process of any one of claims 63 to 65, wherein desilylating the compound of Formula (F1) to form the compound of Formula (G1) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

67. The process of any one of claims 63 to 66, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex; preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$,

Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof; more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof; even more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

68. The process of claim 67, wherein the sulfur trioxide amine complex is used in an amount providing from about 2 to about 10 molar equivalents of sulfur trioxide, preferably from about 5 to about 10 molar equivalents, more preferably from about 7 to about 10 molar equivalents, for instance about 8 molar equivalents.

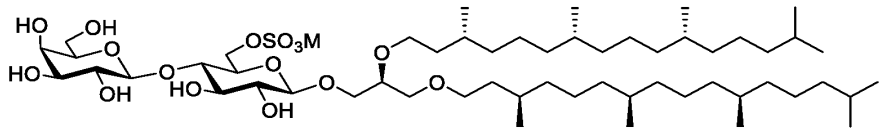
69. The process of any one of claims 63 to 68, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises pyridine, toluene or a combination thereof.

70. The process of any one of claims 63 to 69, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

71. The process of any one of claims 63 to 70, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

72. The process of any one of claims 63 to 71, wherein deprotecting the intermediate compound comprises reacting the intermediate compound with a basic solution preferably comprising a base selected from the group consisting of a methoxide, ethoxide and 2-methylpropan-2-olate, more preferably the base is sodium methoxide.

73. A process for synthesizing a sulfated glycoarchaeal of Formula (VI)

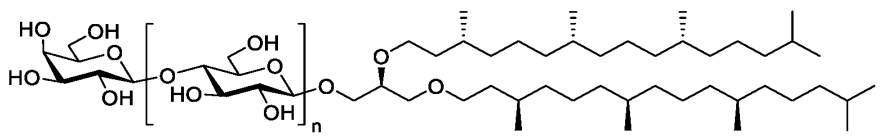


(VI)

where M is an alkali metal or $N(C_{1-4}alkyl)_4$;

the process comprising:

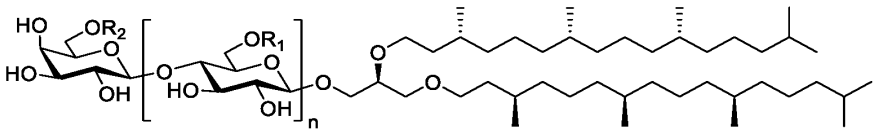
silylating the -OH in position 6' of a compound of Formula (A)



(A)

where n is 1;

to form a 6'-silylated compound (A2)



(A2)

where n is 1, R_1 is H, and R_2 represents a silyl group;

sulfating the -OH in position 6 of the compound of Formula (A2) with a sulfur trioxide reagent to form a 6-sulfated 6'-silylated compound (A6);

desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$; and

optionally contacting the compound of Formula (VI) where M is $N(C_{1-4}alkyl)_4$ with an alkali metal cation exchange resin to form the compound of Formula (VI) where M is alkali metal.

74. The process of claim 73, wherein silylating comprises reacting the compound of Formula (A) with a silylating agent comprising a tri(C_1-C_4alkyl)silyl chloride, preferably triisopropylsilyl chloride (TIPSCl), tert-Butyldimethylsilyl chloride (TBDMSCl) or tert-Butyldiphenylsilyl chloride (TBDPSCl).

75. The process of claim 73 or 74, wherein the sulfur trioxide reagent is a sulfur trioxide amine complex; preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Dimethylaniline.SO_3, Dimethylformamide.SO_3, Pyridine.SO_3, or any combination thereof; more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, $NEt_3.SO_3$, Pyridine.SO_3, or any combination thereof; even more preferably the sulfur trioxide amine complex is $NMe_3.SO_3$, Pyridine.SO_3 or a combination thereof.

76. The process of claim 75, wherein the sulfur trioxide amine complex is used in an amount providing from about 1 to about 10 molar equivalents of sulfur trioxide, preferably from about 1 to about 5 molar equivalents, more preferably from about 1 to about 3 molar equivalents, for instance about 2 molar equivalents.

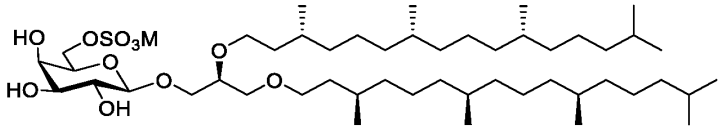
77. The process of any one of claims 73 to 76, wherein the reaction with the sulfur trioxide reagent is performed in solution in at least one organic solvent, preferably the at least one organic solvent is a polar or non-polar aprotic solvent, more preferably the at least one organic solvent is CH_2Cl_2, dimethylformamide, pyridine, chloroform, Dimethyl sulfoxide (DMSO), acetone, toluene, or tetrahydrofuran (THF), even more preferably the organic solvent comprises pyridine, toluene or a combination thereof.

78. The process of any one of claims 73 to 77, wherein the reaction with the sulfur trioxide reagent is performed at room temperature.

79. The process of any one of claims 73 to 78, wherein the reaction with the sulfur trioxide reagent is performed at room temperature for at least 1 hour, preferably for about 2 hours to about 72 hours, for instance about 1 hour to about 5 hours.

80. The process of any of claims 73 to 79, wherein desilylating the 6-sulfated 6'-silylated compound (A6) to form the compound of Formula (VI) is performed with tetra-n-butylammonium fluoride (TBAF), optionally with $BF_3.Et_2O$, or with triethylamine trihydrofluoride (TEA.3HF), preferably with tetra-n-butylammonium fluoride (TBAF).

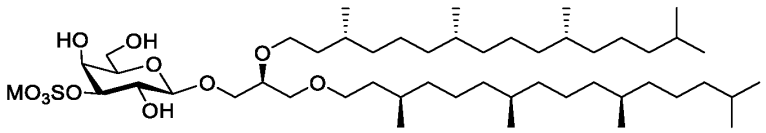
81. A sulfated glycoarchaeal having the following formula (III)



(III)

wherein M is Li, Na, K or is $N(C_{1-4}alkyl)_4$, wherein the sulfated glycoarchaeal is optionally obtained by the process of any one of claims 12 to 17 and 38.

82. A sulfated glycoarchaeal having the following formula (IV)



(IV)

wherein M is Li, Na, or K, wherein the sulfated glycoarchaeal is optionally obtained by the process of any one of claims 56 to 62.