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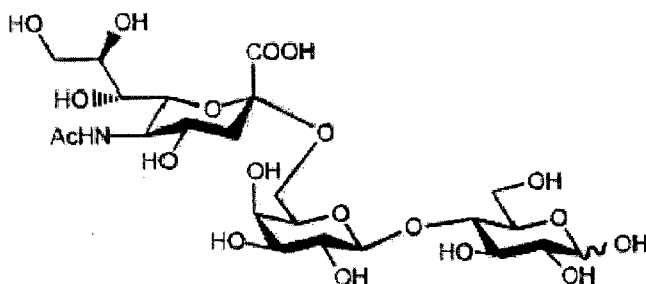
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[Continued on next page]

(54) Title: PRODUCTION OF 6'-O-SIALYLLACTOSE AND INTERMEDIATES



formula (I)

(57) Abstract: The present invention relates to a method for preparation of the trisaccharide 6'-O-sialyllactose (formula (I)) or salts thereof as well as intermediates in the synthesis and for the use of 6'-O-sialyllactose salts in pharmaceutical or nutritional compositions.

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PRODUCTION OF 6'-O-SIALYLLACTOSE AND INTERMEDIATES

FIELD OF THE INVENTION

The present invention provides novel oligosaccharides and derivatives thereof, and furthermore methods for the preparation of these oligosaccharides, especially in large scale.

5 BACKGROUND OF THE INVENTION

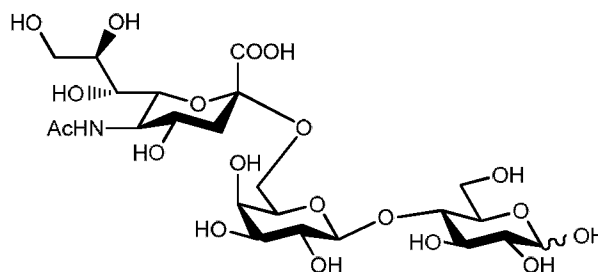
During the past decades the interest for preparation and commercialisation of human milk oligosaccharides has been increasing steadily. The importance of human milk oligosaccharides is directly linked to their unique biological activities such as antibacterial, antiviral, immune system and cognitive development enhancing activities [1].

- 10 Sialylated human milk oligosaccharides such as disialyllacto-*N*-tetraose, 3'-*O*-sialyl-3-*O*-fucosyllactose, 6'-*O*-sialyllactose, 3'-*O*-sialyllactose, 6'-*O*-sialylated-lacto-*N*-neotetraose, 3'-*O*-sialylated-lacto-*N*-tetraose etc. are major components of human milk.

- Among the above listed sialylated human milk oligosaccharides the sialic acid residue is always linked to the terminal 3'-*O*- and/or 6'-*O*- position(s) of D-galactose or to 6-*O*-
15 positions of non terminal sugar residues via α -glycosidic linkages.

- To date, access to large volumes of sialylated human milk oligosaccharides has not been possible by using isolation, biotechnology and synthetic methodologies. The chemical synthesis of sialylated human milk oligosaccharides is one of the most challenging fields of carbohydrate chemistry due to the nature of sialic acid donors themselves. In general,
20 stereoselective glycosylations are achieved via neighbouring group participations but the lack of a substituent at C-3 position of sialic acid prevents such an option. Thus, stereoselective sialylation has to be achieved via careful selection of a sialic acid donor – acceptor match, kinetic and solvent effects. Furthermore, the presence of the carboxylic moiety at the anomeric position of sialic acid also creates unfavoured steric and electronic effects for
25 stereoselective α -sialylations. The strong electron withdrawing effect of the carboxylic group initiates potential side reactions during the glycosylation via β -elimination.

Both the biological importance and the synthetic difficulties of sialylated oligosaccharides can be demonstrated via reviewing the background art of one of the simplest sialylated human milk oligosaccharides called 6'-*O*-sialyllactose (6'-SL, *O*-(*N*-acetyl- α -neuraminosyl)-(2 $\rightarrow$ 6)-*O*- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucose, Scheme 1).



Scheme 1. The structure of 6'-*O*-sialyllactose

6'-*O*-Sialyllactose is one of the sialylated human milk oligosaccharides found in high concentration in mother's milk.

Several biological roles of 6'-*O*-sialyllactose such as its prebiotic, antibacterial, antiviral, immune system and cognitive development enhancing etc. effects [1] have been demonstrated. These important features make 6'-SL an attractive target for large scale production and product development for the nutritional and therapeutic industries. 6'-*O*-Sialyllactose has been synthesised by chemical [2], enzymatic [3] and biotechnological [4] methodologies or it has been isolated from natural sources [5]. However, these methodologies have not been attractive for scale-up due to lack of efficient purification methodologies, use of expensive and toxic reagents and the involvements of many synthetic steps.

Several chemical synthetic methods have been developed towards 6'-*O*-sialyllactose [2a-c], Na, K, Mg and Ca salts thereof [2d, e] or intermediates thereof [2f-j]. In summary, these strategies gives 6'-*O*-sialylated lactose via stereoselective 6'-*O*-sialylation of either 4',6'-sugar diols or 6'-sugar alcohols using glycosylhalide, thioglycoside or diethylphosphite donor activations. The use of either very expensive or very toxic chemicals for the sialylation such as mercury cyanide, mercury bromide and silver carbonate is one of the reasons that make these methodologies less attractive for scale-up studies and production. Non efficient stereocontrol and/or yields likewise make(s) the strategies less suitable for large scale

technology developments. Additionally, severe purification difficulties characterize all the listed synthetic strategies.

In case of enzymatic production of 6'-*O*-sialyllactose glycosyltransferases and sialidases are the preferred enzymes used [3]. These complex enzymatic systems represent very expensive methodologies for scale up productions of 6'-*O*-sialyllactose. Similarly, sialidases could not be used successfully in large scale production methodologies due to their lack of regio- and stereoselectivity. Low yields and difficult purification protocols are likewise a hindrance for industrial scale technology developments.

The isolation of 6'-SL from human and other mammals' milk is rather difficult even in milligram quantities due to the presence of a large number of similar oligosaccharides. To date, only analytical HPLC methodologies have been developed for the isolation of 6'-SL from natural sources [5].

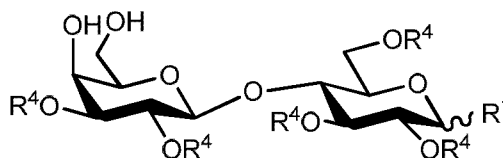
Some biotechnological methodologies are also described using genetically modified bacteria, yeast or other micro organisms [4]. Such methodologies have serious drawbacks in regulatory processes due to limiting commercialisation opportunities.

The present invention represents the first commercial approach suitable for industrial manufacture of 6'-*O*-sialyllactose and other sialylated bioactive oligosaccharides. The successful strategy is based upon the introduction of novel sialic acid donor-acceptor pairs, novel sialic acid donors, novel diol-type lactose acceptors, relevant crystalline intermediates, the use of cheap and non-toxic activators and robust purification methodologies.

SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for the preparation of 6'-*O*-sialyllactose or a salt thereof, comprising the steps of:

- a) reaction of an acceptor of general formula **3**

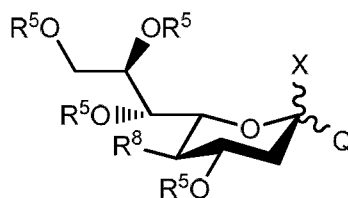


general formula 3

wherein R^1 is $-OR^2$, which R^2 is a group removable by catalytic hydrogenation, or R^1 is $-SR^3$, which R^3 is selected from optionally substituted alkyl, optionally substituted aryl and optionally substituted benzyl, or R^1 is $-NH-C(R'')=C(R')_2$,
 5 wherein each R' independently of each other is an electron withdrawing group selected from $-CN$, $-COOH$, $-COO$ -alkyl, $-CO$ -alkyl, $-CONH_2$, $-CONH$ -alkyl and $-CON(alkyl)_2$, or wherein the two R' -groups is linked together and represent $-CO-(CH_2)_{2-4}-CO-$ and thus form with the carbon atom to which they are attached a 5-7 membered cycloalkan-1,3-dione, in which dione any of the methylene groups is
 10 optionally substituted with 1 or 2 alkyl groups, and R'' is H or alkyl,

R^4 is selected from optionally substituted acyl and benzyl substituted by 1-3 methoxy group(s),

with a donor of general formula 4



general formula 4

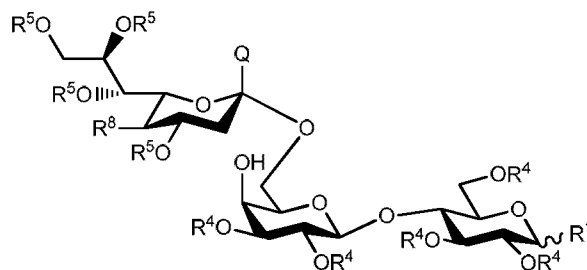
15 wherein R^5 is optionally substituted acyl,

R^8 is selected from $-NHAc$ and $-NAc_2$,

Q is COO -alkyl, which alkyl is optionally substituted, and

X is leaving group,

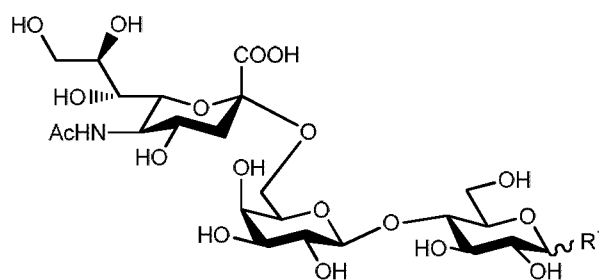
to yield a compound of general formula 2



general formula 2

wherein R^1 , R^4 , R^5 , R^8 and Q are as defined above,

b) deprotection of a compound of general formula **2** to give a compound of general formula **1** or salts thereof



general formula 1

wherein R^1 is as defined above, and

c) subsequently converting the compound of general formula **1** or salts thereof into 6'-*O*-sialyllactose or salts thereof.

The second aspect of the present invention provides 6'-*O*-sialyllactose Zn²⁺ salt.

10 The third aspect of the present invention relates to 6'-*O*-sialyllactose organic salts.

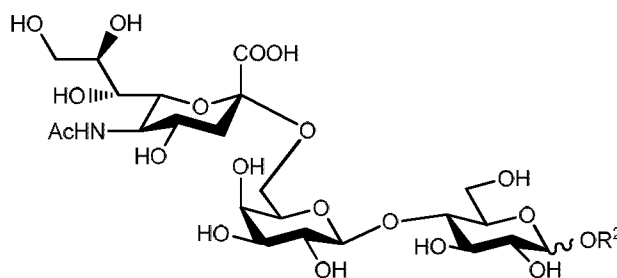
The fourth aspect of the present invention provides novel 6'-*O*-sialyllactose salts according to the second and thrid aspects for use as nutritional additive.

The fifth aspect of the present invention relates to novel 6'-*O*-sialyllactose salts according to the second and third aspects for use as pharmaceutical additive.

The sixth aspect of the present invention provides a nutritional composition comprising one or more 6'-*O*-sialyllactose salts according to the second and third aspects.

The seventh aspect of the present invention relates to a pharmaceutical composition comprising one or more 6'-*O*-sialyllactose salts according to the second and third aspects.

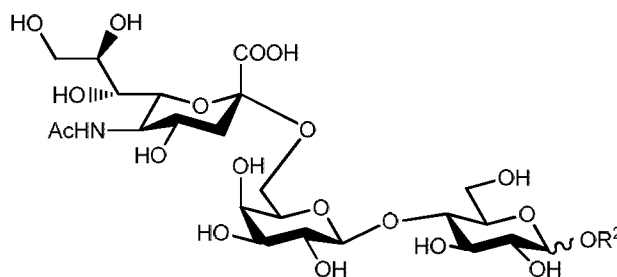
5 The eighth aspect of the present invention provides compounds of general formula **1A**



general formula 1A

wherein R² is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl and OR² is in β.

The ninth aspect of the present invention relates to compounds of general formula **1B**

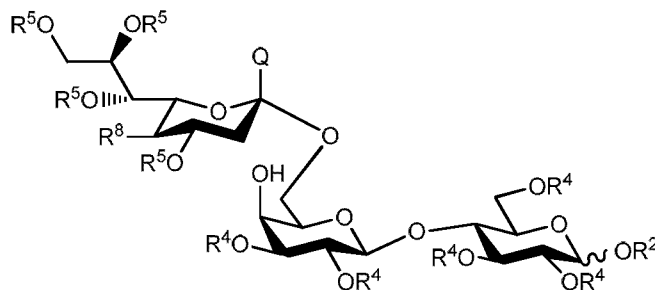


general formula **1B**

wherein R² is a group removable by catalytic hydrogenation,

in salt form selected from the Zn^{2+} , K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} and organic salts.

The tenth aspect of the present invention provides a compound of general formula **2A**

general formula **2A**

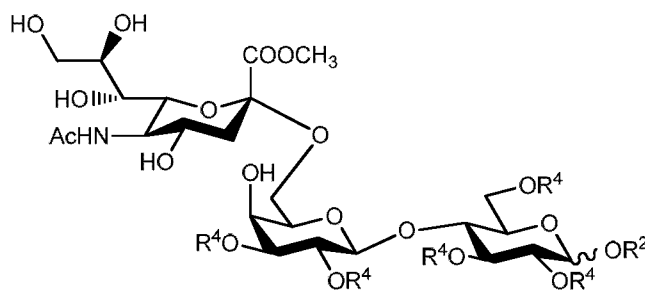
wherein R^2 is a group removable by catalytic hydrogenation,

R^4 and R^5 are independently from each other optionally substituted acyl,

R^8 is selected from $-NHAc$ and $-NAc_2$, and

5 Q is $-COO$ -alkyl, which alkyl is optionally substituted.

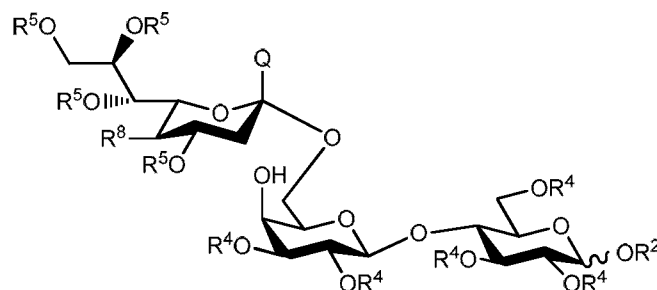
The eleventh aspect of the present invention relates to a compound of general formula **2C**

general formula **2C**

wherein $-OR^2$ is in β , R^2 is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and

10 R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably benzoyl.

The twelfth aspect of the present invention provides a compound of general formula **2D**

general formula **2D**

wherein R² is a group removable by catalytic hydrogenation,

R⁴ is selected from benzyl substituted by 1-3 methoxy group(s) and H,

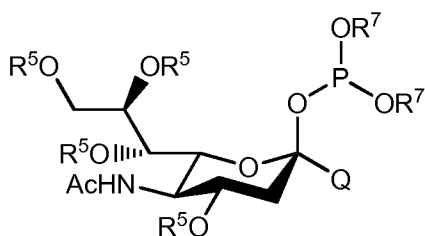
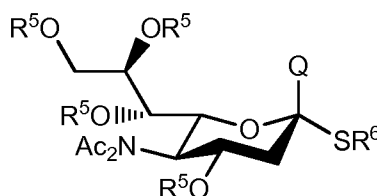
R⁵ is selected from optionally substituted acyl and H,

5 R⁸ is selected from -NHAc and NAc₂, and

Q is selected from -COO-alkyl, which alkyl is optionally substituted and -COOH in either protonated or deprotonated form,

provided that if Q is -COOH in either protonated or deprotonated form and R⁸ is -NHAc, then both R⁴ and R⁵ cannot be H simultaneously.

10 The thirteenth aspect of the present invention relates to a compound of general formula **4A** or **4B**

general formula **4A**general formula **4B**

wherein R⁵ is optionally substituted acyl, preferably acetyl,

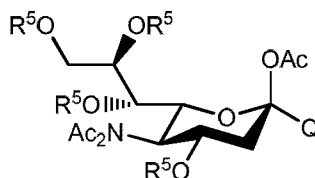
R⁶ is selected from C₂₋₆ alkyl, C₃₋₆ cycloalkyl and optionally substituted benzyl, preferably from ethyl, isopropyl, *t*-butyl, benzyl and cyclohexyl,

15

R^7 is substituted benzyl, preferably 4-chlorobenzyl or 4-bromobenzyl, and

Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

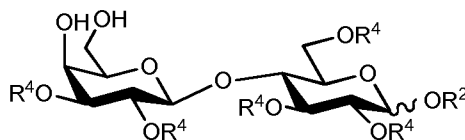
The fourteenth aspect of the present invention provides a compound of general formula 4C for use as sialyl donor



general formula 4C

wherein R^5 is optionally substituted acyl, preferably acetyl and Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

The fifteenth aspect of the present invention relates to a compound of general formula 3A



general formula 3A

wherein R^2 is a group removable by catalytic hydrogenation, and

R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and optionally substituted acyl provided that acetyl is excluded.

DETAILED DISCLOSURE OF THE INVENTION

Throughout the present description, the term “alkyl”, either alone or when attached to another atom or group, means a linear or branched hydrocarbon group with 1-6 carbon atoms, like methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *s*-butyl, *t*-butyl, etc.

“Cycloalkyl” means cyclic hydrocarbon residue with 3-6 carbon atoms like cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

In the present application the term “aryl” refers to homoaromatic groups like phenyl or naphthyl. Preferably, aryl means phenyl.

- 5 In the present description, the term “acyl” represent an R-C(=O)-, wherein R may be H, alkyl or aryl, like formyl, acetyl, propionyl, butyryl, pivaloyl, benzoyl, etc. The alkyl and aryl residues both may be substituted.

For the purpose of this specification with claims, the term “optionally substituted” means that the group in question may either carry a substituent or may be unsubstituted.

- 10 For the purpose of this specification with claims, the term “substituted” means that the group in question is substituted with a group which modifies the general chemical characteristics of the chain or ring. The substituents can be used to modify characteristics of the molecule as a whole, such as stability, solubility, and ability to form crystals. The person skilled in the art will be aware of other suitable substituents of a similar size and charge characteristics, which
15 could be used as alternatives in a given situation.

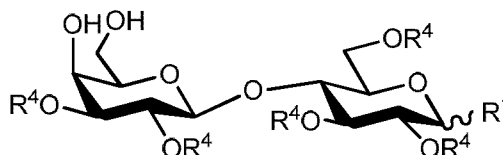
- More generally in connection with the terms “alkyl”, “cycloalkyl”, “aryl” and “acyl” the term “optionally substituted” is intended to mean that the group in question may be substituted one or several times, preferably 1-5 times, more preferably 1-3 times with group(s) selected from the group consisting of alkyl (only for cycloalkyl, aryl and aromatic acyl), hydroxy, alkoxy
20 (i.e. alkyl-oxy), carboxy, oxo (forming a keto or aldehyde functionality), alkoxycarbonyl, alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylamino, arylcarbonyl, amino, mono- and dialkylamino, carbamoyl, mono- and dialkyl-aminocarbonyl, alkylcarbonylamino, cyano, alkanoyloxy, nitro, alkylthio and halogen (F, Cl, Br, I).

- The “protecting group that is removable by catalytic hydrogenation” refers to groups whose
25 C-O bond is cleaved by addition of hydrogen in the presence of catalytic amounts of palladium, Raney nickel or another appropriate metal catalyst known for use in hydrogenolysis, resulting in the regeneration of the OH group. Such protecting groups are well known to the skilled man and are thoroughly discussed [7]. Suitable protecting groups

include benzyl, diphenylmethyl (benzhydryl), 1-naphthylmethyl, 2-naphthylmethyl or triphenylmethyl (trityl) groups, each of which may be optionally substituted by one or more groups selected from: alkyl, alkoxy, phenyl, amino, acylamino, alkylamino, dialkylamino, nitro, carboxyl, alkoxycarbonyl, carbamoyl, *N*-alkylcarbamoyl, *N,N*-dialkylcarbamoyl, azido, halogenalkyl or halogen. Preferably, such substitution, if present, is on the aromatic ring(s). Particularly preferred protecting groups are benzyl or 1-naphthylmethyl groups, both are optionally substituted with one or more groups selected from phenyl, alkyl or halogen. More preferably, the protecting group is selected from unsubstituted benzyl, unsubstituted 1-naphthylmethyl, 4-chlorobenzyl and 4-methylbenzyl. These particularly preferred and more preferable protecting groups have the advantage that the by-products of the hydrogenolysis are exclusively toluene, 1-methylnaphthalene, or substituted toluene or 1-methylnaphthalene derivatives. Such by-products can easily be removed even in multi ton scales from water soluble oligosaccharide products via evaporation and/or extraction processes.

First aspect of the present application relates to a method for the preparation of 6'-*O*-sialyllactose or a salt thereof, comprising the steps of:

a) reaction of an acceptor of general formula 3

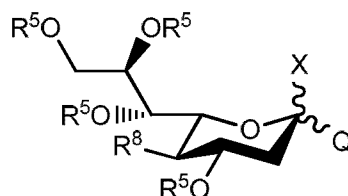


general formula 3

wherein R^1 is $-OR^2$, which R^2 is a group removable by catalytic hydrogenation, or R^1 is $-SR^3$, which R^3 is selected from optionally substituted alkyl, optionally substituted aryl and optionally substituted benzyl, or R^1 is $-NH-C(R'')=C(R')_2$, wherein each R' independently of each other is an electron withdrawing group selected from $-CN$, $-COOH$, $-COO$ -alkyl, $-CO$ -alkyl, $-CONH_2$, $-CONH$ -alkyl and $-CON(alkyl)_2$, or wherein the two R' -groups is linked together and represent $-CO-(CH_2)_{2-4}-CO-$ and thus form with the carbon atom to which they are attached a 5-7 membered cycloalkan-1,3-dione, in which dione any of the methylene groups is optionally substituted with 1 or 2 alkyl groups, and R'' is H or alkyl,

R^4 is selected from optionally substituted acyl and benzyl substituted by 1-3 methoxy group(s),

with a donor of general formula 4



general formula 4

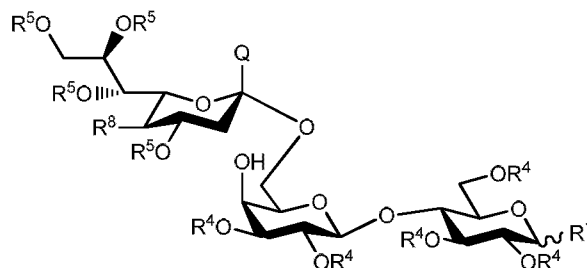
5 wherein R^5 is optionally substituted acyl,

R^8 is selected from $-NHAc$ and $-NAc_2$,

Q is COO -alkyl, which alkyl is optionally substituted, and

X is leaving group,

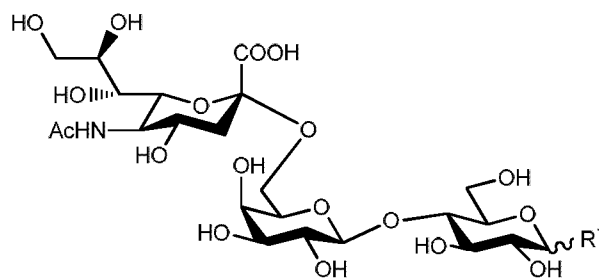
to yield a compound of general formula 2



general formula 2

10 wherein R^1 , R^4 , R^5 , R^8 and Q are as defined above,

b) deprotection of a compound of general formula 2 to give a compound of general formula 1 or salts thereof



general formula 1

wherein R^1 is as defined above, and

c) subsequently converting the compound of general formula 1 or salts thereof into 6'-*O*-sialyllactose or salts thereof.

5 With regard to step c) of the first aspect compounds of general formula 1 or salts thereof are treated to deprotect them anomerically thus 6'-*O*-sialyllactose or salts thereof can be obtained. Particularly, 6'-SL or salts thereof can be produced by subjecting a compound of general formula 1 or salts thereof

- 10 i) to catalytic reduction when R^1 is $-OR^2$, wherein R^2 is a group removable by catalytic hydrogenation,
- ii) to activation with a thiophilic reagent followed by hydrolysis when R^1 is $-SR^3$, wherein R^3 is selected from optionally substituted alkyl, optionally substituted aryl and optionally substituted benzyl,
- 15 iii) to treatment with an amine or halogen followed by hydrolysis at neutral or mild acidic pH when R^1 is $-NH-C(R'')=C(R')_2$, wherein each R' is an electron withdrawing group selected from $-CN$, $-COOH$, $-COO$ -alkyl, $-CO$ -alkyl, $-CONH_2$, $-CONH$ -alkyl and $-CON(alkyl)_2$, or wherein the two R' -groups is linked together and represent $-CO-(CH_2)_{2-4}-CO-$ and thus form with the carbon atom to which they are attached a 5-7 membered cycloalkan-1,3-dion, in which dion any of the methylene groups is
- 20 optionally substituted with 1-2 alkyl groups, and R'' is H or alkyl.

In one embodiment R^1 represents $-OR^2$, wherein R^2 is a group removable by catalytic hydrogenation, that is optionally substituted benzyl, diphenylmethyl (benzhydryl), 1-

naphthylmethyl, 2-naphthylmethyl or triphenylmethyl (trityl) groups, whose removal typically takes place in a protic solvent or in a mixture of protic solvents. A protic solvent may be selected from a group consisting of water, acetic acid or C₁-C₆ alcohol. Mixture of one or more protic solvents with one or more proper aprotic organic solvents miscible partially or fully with the protic solvent(s) (such as THF, dioxane, ethyl acetate, acetone, etc.) may also be applied. Water, one or more C₁-C₆ alcohols or a mixture of water and one or more C₁-C₆ alcohols are preferably used as solvent system. Solutions containing the carbohydrate derivatives in any concentration or suspensions of the carbohydrate derivatives with the solvent(s) used are also applicable. The reaction mixture is stirred at 10-100 °C temperature range, preferably between 20-60 °C in hydrogen atmosphere of 1-50 bar in the presence of a catalyst such as palladium, Raney nickel or any other appropriate metal catalyst, preferably palladium on charcoal or palladium black, until reaching the completion of the reaction. Catalyst metal concentrations generally range from 0.1 % to 10 % based on the weight of carbohydrate. Preferably, the catalyst concentrations range from 0.15 % to 5 %, more preferably 0.25 % to 2.25 %. Transfer hydrogenation may also be performed, when the hydrogen is generated *in situ* from cyclohexene, cyclohexadiene, formic acid or ammonium formate. Addition of organic or inorganic bases/acids and/or basic and/or acidic ion exchange resins can also be used to improve the kinetics of the hydrogenolysis. The use of basic substances is especially preferred when halogen substituents are present on the substituted benzyl moieties of the precursors. Preferred organic bases are including but not limited to triethylamine, diisopropyl ethylamine, ammonia, ammonium carbamate, diethylamine, etc. Preferred organic/inorganic acids are including but not limited to formic acid, acetic acid, propionic acid, chloroacetic acid, dichloroacetic acid, trifluoroacetic acid, HCl, HBr, etc. The conditions proposed above allow simple, convenient and delicate removal of the solvent(s) giving rise to pure 6'-SL. 6'-SL and salts thereof can be isolated from the reaction mixture using conventional work-up procedures in crystalline, amorphous solid, syrupy form or concentrated aqueous solution.

In another embodiment of producing 6'-O-sialyllactose or salts R¹ is -SR³ and R³ is optionally substituted alkyl, optionally substituted aryl or optionally substituted benzyl in general formula 1. Thioglycosides typically act as donor in glycosidation reactions, thus conducting a glycosidation reaction in the presence of water results in the formation of the corresponding reducing sugar. In a typical glycosylation a thioglycoside according to general

formula **1** is dissolved in water or dipolar aprotic solvents containing water followed by addition of a thiophilic activator such as mercury(II) salts, Br₂, I₂, NBS, NIS, triflic acid or triflate salts, or mixtures thereof. The activated intermediate reacts easily with water being present in the reaction milieu and 6'-*O*-sialyllactose or salts thereof are produced.

- 5 In another embodiment 6'-*O*-sialyllactose and salts thereof can be formed by removal of acyclic vinylogous amine group of compounds of general formula **1**, wherein R¹ is -NH-C(R'')=C(R')₂, and R' is electron withdrawing groups selected from -CN, -COOH, -COO-alkyl, -CO-alkyl, -CONH₂, -CONH-alkyl, -CONH-benzyl, -CON(alkyl)₂ and -CON(benzyl)₂, or two R'-groups linked together represent -CO-(CH₂)₂₋₄-CO- and thus form
10 with the adjacent carbon atom an 5-7 membered cycloalkan-1,3-dione and any of the methylene groups may be substituted with 1 or 2 alkyl groups, and R'' represents H or alkyl. The enamine structure can be split by treatment with amino compounds or halogen. Solvents used for the reaction are including but not limited to methanol, ethanol, water, acetic acid, ethyl acetate, etc., and mixtures thereof can be also applied. Amino compounds used for
15 transamination are typically aqueous or anhydrous primary amines, like ethylamine, propylamine, butylamine etc.; hydrazines, like hydrazine hydrate, hydrazine acetate etc.; hydroxylamine derivatives; aqueous ammonia solution or ammonia gas in anhydrous conditions. The acyclic vinylogous amine can also be cleaved by using halogen such as chlorine gas or bromine. Both types of reaction yield amine functionality at the anomeric
20 position whose hydrolysis under neutral or slightly acidic pH (pH ≈ 4-7) readily provides 6'-*O*-sialyllactose.

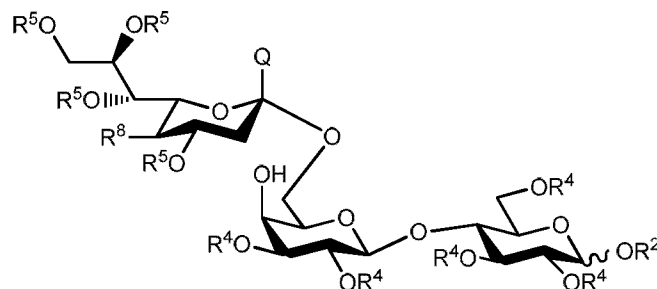
If 6'-*O*-sialyllactose is obtained in the abovementioned reactions, it is converted to a salt thereof, and if desired, a salt of 6'-*O*-sialyllactose is converted to another salt of 6'-*O*-sialyllactose.

- 25 In a preferred embodiment R¹ in a compound of general formula **1** is -OR², which R² is a group removable by catalytic hydrogenation, preferably selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl.

- With regard to step b) of the first aspect of the present application in compounds according to general formula **2** all of the functional groups are protected except for 4'-OH, which
30 protective groups are frequently used in carbohydrate chemistry. The protecting groups can

be removed either successively giving rise to partially protected trisaccharides (*vide infra*) and at last compounds of general formula 1 or salts thereof or in one step leading to directly compounds of general formula 1 or salts thereof. All combination of deprotection steps as used herein can be performed in any order, thus selecting the proper conditions needed to remove them as well as conducting deprotection steps in succession fall under the skilled person's competence.

According to one embodiment in step b) of the first aspect compounds of general formula 1 or salts thereof can be obtained from a group of compounds of general formula 2 characterized by general formula 2A



general formula 2A

wherein R^2 is a group removable by catalytic hydrogenation,

R^4 and R^5 are independently from each other optionally substituted acyl,

R^8 is selected from $-NHAc$ or $-NAc_2$ and

Q is $-COO$ -alkyl, which alkyl is optionally substituted,

comprising the following steps:

- a) base catalyzed transesterification reaction followed by a basic hydrolysis, optional acidification and optional salt formation, or
- b) basic hydrolysis, optional acidification and optional salt formation.

The term "base catalyzed transesterification deprotection" means a reaction, where the acyl protective groups from hydroxyls are removed in an alcohol solvent such as methanol,

ethanol, propanol, *t*-butanol, etc. in the presence of an alcoholate like NaOMe, NaOEt, KO^tBu, etc. at 20-100 °C temperatures. The alcohol and the alcoholate should be matched. The use of co-solvent as toluene or xylene might be beneficial in order to control particle size of the product and to avoid gel formations. Under this condition only *O*-acyls can be readily
5 deprotected and when R⁸ is -NAC₂ one of the acetyl is also removed. The Q group may be transesterified. In a preferred embodiment catalytic amount of NaOMe is used in methanol (Zemplén de-*O*-acylation). The term “basic hydrolysis” generally means base catalyzed hydrolysis in water, alcohol or water-organic solvent mixtures, in homogeneous or heterogeneous reaction conditions at temperatures varying from 0–100 °C. The base of
10 choice is generally a strong base, e.g. LiOH, NaOH, KOH, Ba(OH)₂, K₂CO₃, basic ion exchange resins, tetraalkylammonium hydroxides, etc. The bases can be used in the form of an aqueous solution as well. This condition affects *O*-acyls, when Q is ester it is also hydrolyzed and when R⁸ is -NAC₂ one of the acetyl is also removed. In a preferred embodiment the base is NaOH and the solvent is methanol.

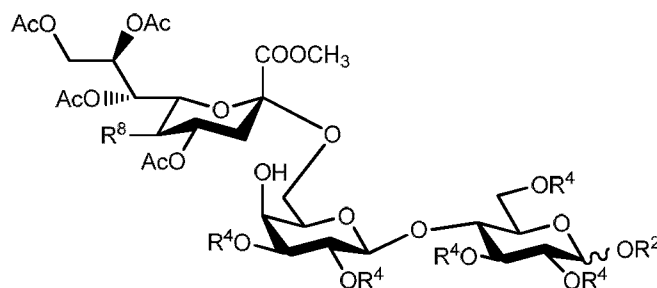
15 “Optional acidification” intends to mean that an acidification step can be performed when the acid form of compounds of general formula **1** is needed after base catalyzed transesterification deprotection or basic hydrolysis.

“Optional salt formation” means that a compound of general formula **1** in acidic form, if needed, is converted into its salt different to that formed after base catalyzed
20 transesterification deprotection or basic hydrolysis. A salt of a compound of general formula **1** means an associated ion pair consists of the negatively charged acid residue of compound of general formula **1** and a cation in any stoichiometric proportion. Cations, as used in the present context, can be atoms or molecules with positive charge. The cation may be inorganic as well as organic cation. Preferred inorganic cations are ammonium ion, alkali metal, alkali
25 earth metal and transition metal ions, more preferably Na⁺, K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Fe²⁺, Zn²⁺, Mn²⁺ and Cu²⁺, most preferably K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Fe²⁺ and Zn²⁺. Basic organic compounds in positively charged form may be relevant organic cations. Such preferred positively charged organic molecules are diethyl amine, triethyl amine, diisopropyl ethyl amine, ethanolamine, diethanolamine, triethanolamine, imidazol, piperidine, piperazine,
30 morpholin, benzyl amine, ethylene diamine, meglumin, pyrrolidine, choline, tris-(hydroxymethyl)-methyl amine, N-(2-hydroxyethyl)-pyrrolidine, N-(2-hydroxyethyl)-piperidine, N-(2-hydroxyethyl)-piperazine, N-(2-hydroxyethyl)-morpholine, L-arginine, L-

lysine, oligopeptides having L-arginine or L-lysine unit or oligopeptides having free amino group on *N*-terminal, etc., all in protonated form. An inorganic and organic salt of compound of general formula **1** can be obtained from a compound of general formula **1** in acidic form. The pH of the solution of the free acid in alcohol or alcohol/water is adjusted to 8,5-11 with a base; if the base is an inorganic one, it can be selected from alkali metal, alkali earth metal and transition metal hydroxides, carbonates and bicarbonates. The mixture is then diluted with alcohol and concentrated in vacuo. The slurry obtained is then filtered and washed with alcohol.

In a preferred realization compounds of general formula **2A**, wherein R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably pivaloyl and benzoyl, R^5 is acetyl, Q is $-\text{COOCH}_3$ and $-\text{OR}^2$ is in β are deprotected.

According to another embodiment in step b) of the first aspect compounds of general formula **2B** belonging to compounds of general formula **2**



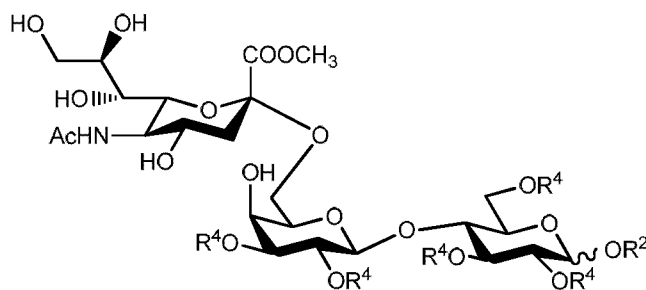
general formula **2B**

wherein R^2 is a group removable by catalytic hydrogenation,

R^4 is optionally substituted acyl provided that acetyl is excluded, and

R^8 is selected from $-\text{NHAc}$ and $-\text{NAC}_2$,

are subjected to selective acidic deprotection to give compounds of general formula **2C**

general formula **2C**

wherein R^2 and R^4 are as defined above,

followed by

a) base catalyzed transesterification reaction followed by basic hydrolysis,
optional acidification and optional salt formation, or

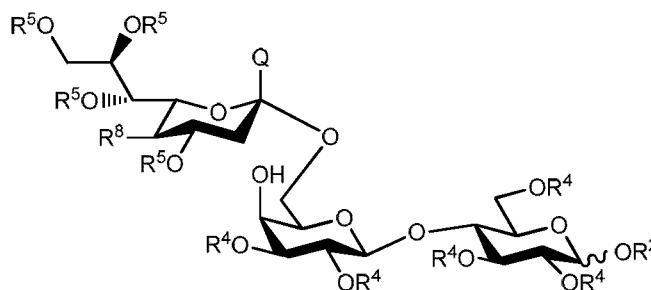
b) basic hydrolysis, optional acidification and optional salt formation

to give a compound of general formula **1** or salts thereof.

Compounds of general formula **2C** can be obtained in a delicate way from compounds of compounds **2B**. The present inventors recognized that the acetyl groups in the sialic acid residue (R^5 is acetyl) can be selectively removed when the lactose portion is protected by acyls different from acetyl (R^4 is optionally substituted acyl provided that acetyl is excluded) due to the higher reactivity of acetyls towards acidic transesterification than other acyls. The deprotection step can be carried out in a C_{1-6} alcohol or mixture of C_{1-6} alcohols, preferably methanol and ethanol in the presence of an acid, generally a protic acid selected from but not limited to acetic acid, trifluoroacetic acid, HCl, formic acid, sulphuric acid, perchloric acid, oxalic acid, *p*-toluenesulfonic acid, benzenesulfonic acid, cation exchange resins, etc., preferably strong inorganic acid which may be present in from catalytic amount to excess. The hydrolysis may be conducted at temperatures between 0 and 20 °C, preferably at 5-10 °C until TLC shows complete or nearly complete reaction which takes from about 2 hours to 3 days depending on temperature, concentration and pH.

In a preferred embodiment $-OR^2$ is in β , R^2 is benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably benzoyl and the acid catalyst of the selective acidic deprotection is sulphuric acid.

According to another realization in step b) of the first aspect a group of compounds of general formula **2** characterized by general formula **2D**



general formula **2D**

wherein R^2 is a group removable by catalytic hydrogenation,

5 R^4 is benzyl substituted by 1-3 methoxy group(s),

R^5 is optionally substituted acyl,

R^8 is selected from $-NHAc$ or $-NAc_2$ and

Q is $-COO$ -alkyl, which alkyl is optionally substituted

is deprotected comprising the steps of:

10 a) base catalyzed transesterification reaction resulting in another compounds of general formula **2D**, wherein R^2 is as defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is $-NHAc$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, followed by

15 aa) dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, each of R^4 and R^5 are H, R^8 is $-NHAc$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, followed by basic hydrolysis, optional acidification and optional salt formation, or

20 ab) basic hydrolysis, optional acidification and optional salt formation resulting in another compound of general formula **2D**, wherein R^2 is as

defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is -NHAc and Q is -COOH in either protonated or deprotonated form, followed dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation

5 to give a compound of general formula **1** or salts thereof,

or

10 b) dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, R^4 is H, R^5 is optionally substituted acyl, R^8 is selected from -NHAc and -NAC₂ and Q is -COO-alkyl, which alkyl is optionally substituted, followed by

ba) base catalyzed transesterification reaction followed by basic hydrolysis, optional acidification and optional salt formation, or

bb) basic hydrolysis, optional acidification and optional salt formation

to give a compound of general formula **1** or salts thereof,

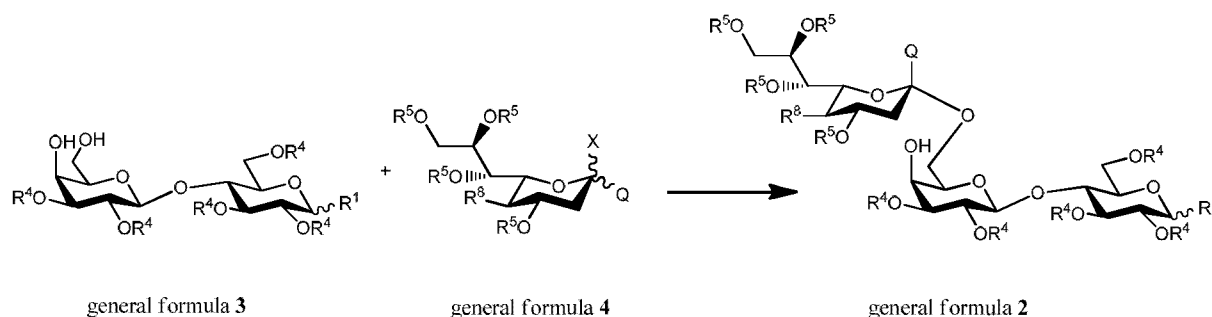
15 or

20 c) basic hydrolysis, optional acidification and optional salt formation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is -NHAc and Q is -COOH in either protonated or deprotonated form, followed by dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation to give a compound of general formula **1** or salts thereof.

25 The term “-COOH in either protonated or deprotonated form” in certain compounds of general formula **2D** intends to mean that the carboxylic group in those compounds of general formula **2D** is in either acidic (protonated) form thus signifying they are acids or the carboxylic group in those compounds of general formula **2D** is in carboxylate (-COO⁻) form signifying they are salts, in which salts the positively charged counter-ion may be an inorganic or organic cation. These inorganic and organic cations are specified above.

The protected 6'-*O*-sialyllactose may contain 1, 2 or 3 methoxy substituted benzyl groups on the lactose portion, preferably *p*-methoxybenzyl ($R^4 = \text{PMB}$). It is well-known that methoxy substituted benzyl ethers are more readily cleaved oxydatively than unsubstituted benzyl ethers, thus a selective deprotection may be possible if the aglycon or Q contains benzyl group. Typical oxidative agents are dichlorodicyanoquinone (DDQ) and cerium(IV) ammonium nitrate (CAN), the reaction runs in organic solvent in the presence of water, preferably in dichloromethane or acetonitrile, resulting in smooth removal of methoxy-benzyl ether protecting group.

With regard to step a) of the first aspect of the present invention a compound of general formula 3 (R^1 is $-\text{OR}^2$, which R^2 is a group removable by catalytic hydrogenation, or R^1 is $-\text{SR}^3$, which R^3 is optionally substituted alkyl, optionally substituted aryl or optionally substituted benzyl, or R^1 is $-\text{NH}-\text{C}(\text{R}'')=\text{C}(\text{R}')_2$, wherein each R' independently of each other is an electron withdrawing group selected from $-\text{CN}$, $-\text{COOH}$, $-\text{COO-alkyl}$, $-\text{CO-alkyl}$, $-\text{CONH}_2$, $-\text{CONH-alkyl}$ and $-\text{CON(alkyl)}_2$, or wherein the two R' -groups is linked together and represent $-\text{CO}-(\text{CH}_2)_{2-4}-\text{CO}-$ and thus form with the carbon atom to which they are attached a 5-7 membered cycloalkan-1,3-dione, in which dione any of the methylene groups is optionally substituted with 1 or 2 alkyl groups, R'' is H or alkyl, $\text{R}^4 =$ optionally substituted acyl or benzyl substituted by 1, 2 or 3 methoxy) is reacted with a compound according to general formula 4 ($\text{R}^5 =$ optionally substituted acyl, $\text{R}^8 = -\text{NHAc}$ or $-\text{NAC}_2$, Q is $-\text{COO-alkyl}$, which alkyl is optionally substituted, X is a leaving group) to obtain compounds of general formula 2 (R^4 is optionally substituted acyl or benzyl substituted by 1-3 methoxy group(s), R^5 is optionally substituted acyl, R^8 is $-\text{NHAc}$ or NAC_2 and Q is $-\text{COO-alkyl}$, which alkyl is optionally substituted) (Scheme 2.).



Scheme 2.

The coupling of the lactose acceptor of general formula 3 with the sialyl donor of general formula 4 can be carried out in an aprotic solvent or in a mixture of aprotic solvents in the presence of an activator (promoter or catalyst) so as to lead to the desired glycosylated product. The new interglycosidic linkage is formed by the nucleophilic displacement of the leaving group X of donor according to general formula 4 with the 6'-OH group of acceptor according to general formula 3. Other functional groups in both participating reactants have to be masked with protecting groups. Particular care has to be taken with regard to the stereoselectivity. The stereochemical outcome may be affected by different factors like the presence or absence of a participating group at the ring carbon adjacent to the anomeric centre of the donor, the nature of the leaving group X, solvent effect, nature of the protective groups on both the donor and acceptor, nature of the promoters or catalysts, temperature, pressure, steric interactions between the donor and acceptor, and like. In case of sialic acid derivatives an array of anomeric activation for glycosylation is developed and available to a skilled person engaged in sialic acid chemistry. These methodologies are expansively discussed by reviews [6]. For the sake of examples some general considerations are briefly mentioned below depending on the X-group.

The glycosyl halides (X means F, Cl, Br, I) are frequently used in glycosylation reaction because of their easy accessibility and satisfactory reactivity. Typically, anomeric halides follow the reactivity order $F < Cl < Br < I$ for nucleophilic displacement. The glycosylation reactions are generally promoted by heavy metal ion, mainly mercury or silver, and Lewis acids.

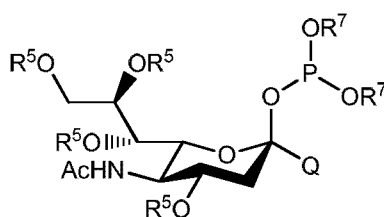
Glycosyl trichloroacetimidates ($X = -OC(=NH)CCl_3$) can be easily prepared by the addition of the free anomeric OH to trichloroacetonitrile under inorganic or organic base catalysis. In a typical glycosidation reaction catalytic amount of Lewis acid, such as trimethylsilyl triflate or BF_3 -etherate, promotes the coupling.

Thioglycosides (X denotes alkylthio- or phenylthio-group) can be activated by thiofilic promoters such as mercury(II) salts, Br_2 , I_2 , NBS, NIS, triflic acid, triflate salts, BF_3 -etherate, trimethylsilyl triflate, dimethyl-methylthio sulphonium triflate, phenylselenenyl triflate, iodonium dicollidine perchlorate, tetrabutylammonium iodide or mixtures thereof, in condensation reactions, preferably by Br_2 , NBS or NIS.

Glycosyl phosphite donors (X is $-\text{O}-\text{P}(\text{O}[\text{alkyl or benzyl}])_2$), can be promoted with NBS, NIS, TMSOTf, TfOH, Ti_2O , ZnCl_2 , $\text{BF}_3\cdot\text{OEt}_2$, LiClO_4 , DTBPI, Bu_4NI , AgClO_4 , LiClO_4 , $\text{Sn}(\text{OTf})_2$ or mixtures thereof, preferably TMSOTf.

5 Glycosyl acetates (X represents $-\text{OAc}$) in glycosylation reaction are first subjected to electrophilic activation providing a reactive intermediate, then treated with the nucleophilic OH-acceptor. Typical activators of choice are Bronsted acids (such as TsOH, HClO_4 , sulfamic acid), Lewis acids (such as ZnCl_2 , SnCl_4 , triflate salts, BF_3 -etherate, trityl perchlorate, AlCl_3 , triflic anhydride) and their mixtures.

10 According to a preferred method a group of compounds of general formula 4, which is characterized by general formula 4A



general formula 4A

15 wherein R^5 is optionally substituted acyl, Q is $-\text{COO}$ -alkyl, which alkyl is optionally substituted and R^7 is substituted benzyl, are employed in the coupling reactions. The reaction runs in aprotic solvent, preferably in dichloromethane, THF, toluene, acetonitrile or in mixtures thereof, more preferably in dichloromethane/acetonitrile mixture, at temperatures between $-78 - 0^\circ\text{C}$, in the presence of promoter like NBS, NIS, TMSOTf, TfOH, Ti_2O , ZnCl_2 , $\text{BF}_3\cdot\text{OEt}_2$, LiClO_4 , DTBPI, Bu_4NI , AgClO_4 , LiClO_4 , $\text{Sn}(\text{OTf})_2$ or mixtures thereof, preferably TMSOTf.

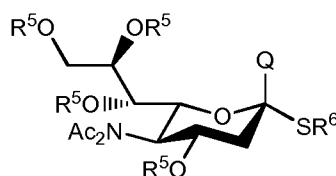
20 In a preferred embodiment the donor and acceptor are dissolved in the solvent mixture of THF/DCM in ratios 1:1 to 1:5 and temperature is kept between -40°C to -10°C . A promoter of choice including but limited to TMSOTf, TfOH, $\text{BF}_3\cdot\text{Et}_2\text{O}$, DTPI, tetrabutylammonium iodide, etc. is added and the reaction is stirred until it reaches completion.

In another preferred embodiment the donor and acceptor are dissolved in the solvent mixture of CH₃CN/DCM in ratios 1:1.7 to 1:5 and temperature is kept between -40 °C to -30 °C. A promoter of choice is added including but limited to TMSOTf, TfOH, BF₃·Et₂O, DTPI, tetrabutylammonium iodide, etc. and the reaction is stirred till it reaches completion.

- 5 In another preferred embodiment the donor and acceptor are dissolved in the solvent mixture of Tol/CH₃CN/DCM preferably in a ratio 1:1:1 and temperature is kept between -40 °C to -20 °C. A promoter of choice is added including but limited to TMSOTf, TfOH, BF₃·Et₂O, DTPI, tetrabutylammonium iodide, etc. and the reaction is stirred till it reaches completion.

10 In a preferred donor of general formula **4A** R⁵ is acetyl, R⁷ is 4-chlorobenzyl or 4-bromobenzyl, and Q is -COOMe.

With regard to the production of compounds of general formula **2** starting from acceptor of general formula **3** and donor of general formula **4**, another preferred realization comprises the use of another group of compounds of general formula **4**, which is characterized under general formula **4B**

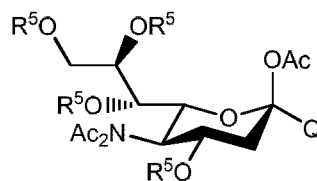


general formula **4B**

15 wherein R⁵ is optionally substituted acyl, Q is -COO-alkyl, which alkyl is optionally substituted and R⁶ is selected from C₂₋₆ alkyl, C₃₋₆ cycloalkyl and optionally substituted benzyl. The glycosylation is carried out in aprotic solvent(s) like chloroform, dichloromethane, toluene, dioxane, THF, acetonitrile or mixture thereof, preferably
 20 chloroform or dichloromethane, under the activation of NIS, NBS, Br₂, triflic acid, silver triflate, BF₃-etherate or mixture thereof.

In a preferred donor of general formula **4B** R⁵ is acetyl, R⁶ is selected from ethyl, isopropyl, *t*-butyl, benzyl and cyclohexyl, and Q is -COOMe.

A further preferred embodiment relates to the use of compounds of general formula **4C** in the sialylation reaction

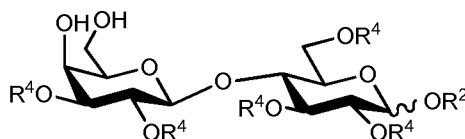


general formula **4C**

wherein R^5 is optionally substituted acyl, and Q is $-\text{COO-alkyl}$, which alkyl is optionally substituted. The glycosylation is carried out in aprotic solvent(s) like chloroform, dichloromethane, toluene, dioxane, THF, acetonitrile or mixture thereof, preferably chloroform or dichloromethane, under the activation of Bronsted or Lewis acid. A compound of general formula **4C** wherein R^5 is acetyl and Q is $-\text{COOMe}$ is the preferred donor of choice.

10 In a preferred donor of general formula **4C** R^5 is acetyl, and Q is $-\text{COOMe}$.

In another embodiment the synthesis of compounds according to general formula **2** comprises the use of compounds of the general formula **3A**



general formula **3A**

wherein R^2 is a group removable by catalytic hydrogenation, and

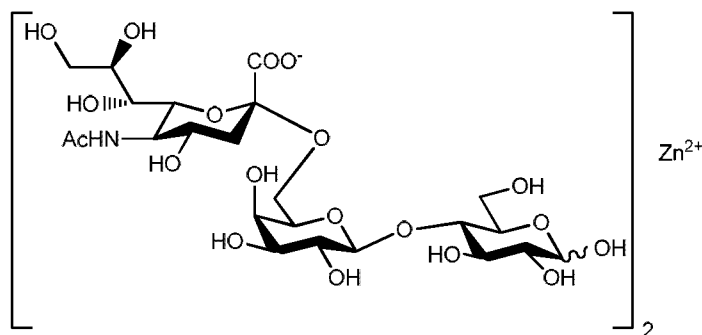
15 R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and optionally substituted acyl provided that acetyl is excluded.

In a preferred embodiment $-\text{OR}^2$ is in β , R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and R^4 is selected from isobutyryl, pivaloyl and benzoyl.

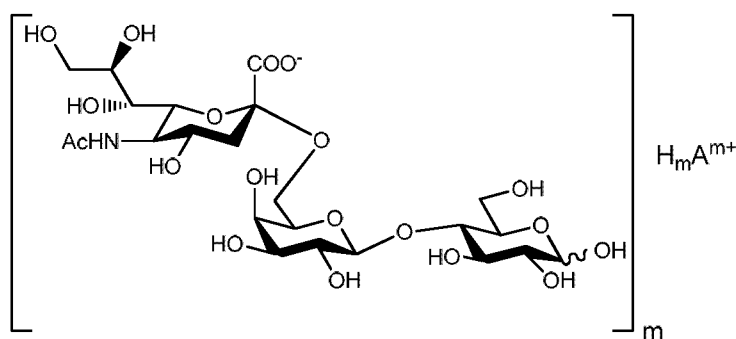
6'-*O*-Sialyllactose and several 6'-*O*-sialyllactose intermediates in acidic form have limited chemical stability in aqueous solution due to the high acidity of the sialic acid structural motif and to the low hydrolytic stability of the α -*O*-sialyl linkage. For example, 6'-*O*-sialyllactose decomposes in aqueous solution at room temperature into sialic acid and lactose by autocatalysis. The low acid stability of sialyllactose itself in aqueous solutions is the major obstacle of isolation, and of enzymatic or chemical technologies targeting the preparation of 6'-*O*-sialyllactose. Furthermore, 6'-*O*-sialyllactose is not crystalline preventing the development of efficient purification methodologies required for the preparation of high quality 6'-*O*-sialyllactose product.

- 10 The present invention comprises providing new salts of 6'-*O*-sialyllactose. The term "salt of 6'-*O*-sialyllactose" means an associated ion pair consists of the negatively charged acid residue of 6'-*O*-sialyllactose and a cation in any stoichiometric proportion. Cations, as used in the present context are atoms or molecules with positive charge. The cation may be inorganic as well as organic cation. Preferred inorganic cations are ammonium ion, alkali metal, alkali earth metal and transition metal ions, more preferably Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} , Zn^{2+} , Mn^{2+} and Cu^{2+} , most preferably K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} and Zn^{2+} . Basic organic compounds in positively charged form may be relevant organic cations. Such preferred positively charged counterparts are diethyl amine, triethyl amine, diisopropyl ethyl amine, ethanolamine, diethanolamine, triethanolamine, imidazol, piperidine, piperazine, morpholin, benzyl amine, ethylene diamine, meglumin, pyrrolidine, choline, tris-(hydroxymethyl)-methyl amine, N-(2-hydroxyethyl)-pyrrolidine, N-(2-hydroxyethyl)-piperidine, N-(2-hydroxyethyl)-piperazine, N-(2-hydroxyethyl)-morpholine, L-arginine, L-lysine, oligopeptides having L-arginine or L-lysine unit or oligopeptides having free amino group on *N*-terminal, etc., all in protonated form. Such salt formations can be used to modify characteristics of the complex molecule as a whole, such as stability, compatibility to excipients, solubility and ability to form crystals.

Thus it is provided the Zn^{2+} salt of 6'-*O*-sialyllactose as novel inorganic 6'-SL salt:



In addition the present application relates to providing 6'-SL organic salts of formula I



formula I

wherein A means any organic base and m is an integer number.

- 5 Compound A as organic base comprises preferably amine-type bases like diethyl amine, triethyl amine, diisopropyl ethyl amine, ethanolamine, diethanolamine, triethanolamine, imidazol, piperidine, piperazine, morpholine, benzyl amine, ethylene diamine, meglumin, pyrrolidine, choline, tris-(hydroxymethyl)-methyl amine, N-(2-hydroxyethyl)-pyrrolidine, N-(2-hydroxyethyl)-piperidine, N-(2-hydroxyethyl)-piperazine, N-(2-hydroxyethyl)-morpholine, L-arginine, L-lysine, oligopeptides having L-arginine or L-lysine unit or oligopeptides having free amino group on N-terminal, etc. These bases are in protonated form when associated with 6'-SL. The especially preferred organic amines are selected from diethyl amine, tris-(hydroxymethyl)-methyl amine, ethanolamine and choline.
- 10

Inorganic and organic salts of 6'-SL can be obtained from acidic 6'-SL. The pH of the solution of the free acid in alcohol or alcohol/water is adjusted to 8,5-11 with the selected base; if the base is an inorganic one it can be chosen from metal hydroxides, carbonates and

15

bicarbonates. The mixture is then diluted with alcohol and concentrated in vacuo. The slurry obtained is then filtered and washed with alcohol.

In an alternative way an inorganic or organic salt a compounds of general formula **1** wherein R^1 is $-OR^2$, which R^2 is a group removable by catalytic hydrogenation (see above) is subjected to catalytic hydrogenolysis to give rise to the corresponding inorganic or organic salt of 6'-SL.

It is strongly emphasised that the novel 6'-*O*-sialyllactose salts may be crystalline, they can be considered as sole chemical entities such as α - or β -anomers or even an anomeric mixture thereof. They might exist in anhydrous, hydrated as well as solvated forms. In addition, the novel 6'-SL salts can be isolated as amorphous solid, syrup or concentrated aqueous solution as well.

6'-*O*-Sialyllactose and sialylated human milk oligosaccharides are of great importance which is directly linked to their unique biological activities such as antibacterial, antiviral, immune system and cognitive development enhancing activities. Sialylated human milk oligosaccharides including 6'-SL, optionally in combination with other sialic acid and/or N-acetyllactosamine and/or fucose containing human milk oligosaccharides, are found to act as prebiotics in the human intestinal system helping to develop and maintain the intestinal flora. Furthermore they have also proved to be anti-inflammatory, and therefore these compounds are attractive components in the nutritional industry for the production of, for example, infant formulas, infant cereals, clinical infant nutritional products, toddler formulas, or as dietary supplements or health functional food for children, adults, elderly or lactating women, both as synthetically composed and naturally occurring compounds and salts thereof. Thus 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention are suitable for pharmaceutical and nutritional use.

In another aspect, the present invention provides pharmaceutical composition comprising 6'-SL Zn^{2+} salt and/or 6'-SL organic salts as active ingredient and one or more pharmaceutically acceptable carriers including but not limited to additives, adjuvants, excipients and diluents (water, gelatine, talc, sugars, starch, gum arabic, vegetable gums, vegetable oils, polyalkylene glycols, flavouring agents, preservatives, stabilizers, emulsifying agents, lubricants, colorants, fillers, wetting agents, etc.). Suitable carriers are described in the most recent

edition of Remington's Pharmaceutical Sciences, a standard reference text in the field. The dosage form for administration includes, for example, tablets, powders, granules, pills, suspensions, emulsions, infusions, capsules, syrups, injections, liquids, elixirs, extracts and tincture.

- 5 In a further embodiment 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention are used for the preparation of pharmaceutical compositions. Pharmaceutical compositions can be manufacture by means of any usual manner known in the art, e.g. described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field.
- 10 In a further embodiment it is provided nutritional formulations comprising one or more 6'-SL salts selected form 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention such as foods, drinks or feeds. The nutritional formulation may contain edible micronutrients, vitamins and minerals as well. The amounts of such ingredient may vary depending on whether the formulation is intended for use with normal, healthy infants,
- 15 children, adults or subjects having specialized needs (e.g. suffering from metabolic disorders). Micronutrients include for example edible oils, fats or fatty acids (such as coconut oil, soy-bean oil, monoglycerides, diglycerides, palm olein, sunflower oil, fish oil, linoleic acid, linolenic acid etc.), carbohydrates (such as glucose, fructose, sucrose, maltodextrin, starch, hydrolized cornstarch, etc.) and proteins from casein, soy-bean, whey or skim milk, or
- 20 hydrolysates of these proteins, but protein from other source (either intact or hydrolysed) may be used as well. Vitamins may be chosen from the group consisting of vitamin A, B1, B2, B5, B6, B12, C, D, E, H, K, folic acid, inositol and nicotinic acid. The nutritional formula may contain the following minerals and trace elements: Ca, P, K, Na, Cl, Mg, Mn, Fe, Cu, Zn, Se, Cr or I.
- 25 In a preferred embodiment the nutritional formulation is an infant formula. Infant formula means a foodstuff intended for particular nutritional use by infants during the first 4-6 months of life and satisfying by itself the nutritional requirements of infants. It may contain one or more probiotic *Bifidobacterium* species, prebiotics such as fructooligosaccharides and galactooligosaccharides, proteins from casein, soy-bean, whey or skim milk, carbohydrates
- 30 such as lactose, saccharose, maltodextrin, starch or mixtures thereof, lipids (e.g. palm olein, sunflower oil. safflower oil) and vitamins and minerals essential in a daily diet. The infant

formula may contain 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention in a total amount of 0.1-3.0 g/100 g formula.

In another preferred embodiment the nutritional formulation may be a food supplement including 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention. The food supplement may comprise one or more probiotics in an amount sufficient to achieve the desired effect in an individual, preferably in children and adults. The food supplement may also contain vitamins, minerals, trace elements and other micronutrients as well. The food supplement may be for example in the form of tablets, capsules, pastilles or a liquid. The supplement may contain conventional additives selected from but not limited to binders, coatings, emulsifiers, solubilising agents, encapsulating agents, film forming agents, adsorbents, carriers, fillers, dispersing agents, wetting agents, jellifying agents, gel forming agents, etc. The daily dose of 6'-SL Zn^{2+} salt and/or 6'-SL organic salts ranges from 0.1 to 3.0 g.

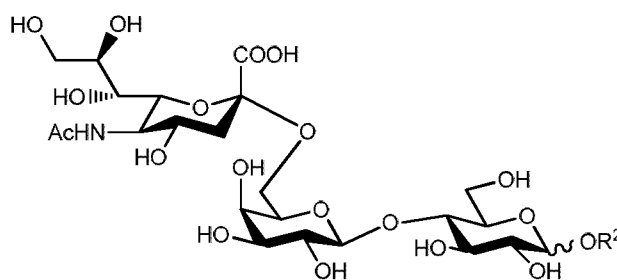
According to a more preferred embodiment the food supplement is digestive health functional food as the administration of 6'-SL Zn^{2+} salt and/or 6'-SL organic salts provides a beneficial effect on digestive health. Digestive health functional food is a processed food used with intention enhance and preserve digestive health by 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention as physiologically functional ingredient or component in forms of tablet, capsule, powder, etc. Different terms such as dietary supplement, nutraceutical, designed food, health product may also be used to refer to functional food.

In a further embodiment 6'-SL Zn^{2+} salt and/or 6'-SL organic salts according to the present invention are used for the preparation of nutritional formulation including foods, drinks and feeds, preferably infant formulas, food supplements and digestive health functional food. The nutritional formulation may be prepared in any usual manner.

Compounds of general formula **1**, **2**, **3** and **4** are believed to be valuable synthetic intermediates towards 6'-SL. The present inventors surprisingly recognized some of the compounds of general formula **1**, **2**, **3** and **4** can be obtained in crystalline form. Crystallization or recrystallization is one of the simplest and cheapest methods to isolate a product from a reaction mixture, separate it from contaminations and obtain pure substance.

Isolation or purification that uses crystallization makes the whole technological process robust and cost-effective, thus it is advantageous and attractive compared to other procedures. The present invention has a great commercial value in large scale production of 6'-SL providing high purity of intermediates, which cannot be achieved by any other known purification methods. Although some other intermediates have not shown the ability to crystallize, they can be prepared in clean, high-yielding and less by-product forming reactions where usual work-up (extraction, evaporation, precipitation, etc.) procedures have been sufficient to obtain high purity products which have been used without further purification in the next step.

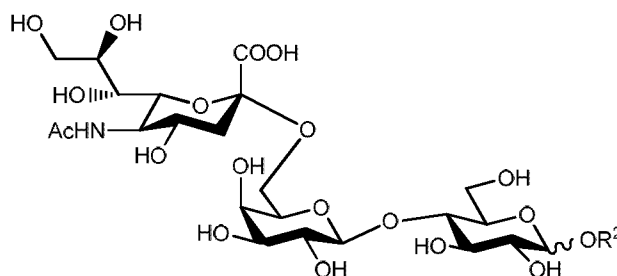
- 10 Thus it is provided as valuable 6'-SL intermediate a group of compounds of general formula 1 or a salt thereof, namely compounds of general formula 1A



general formula 1A

wherein R^2 is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl and OR^2 is in β ,

- 15 and compounds of general formula 1B



general formula 1B

wherein R^2 is a group removable by catalytic hydrogenation, in salt form selected from the Zn^{2+} , K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} and organic salts.

It is strongly emphasised that novel derivatives characterized by general formulae **1A** and **1B** can be considered as sole chemical entities such as either α or β anomers, preferably the β anomer, or even an anomeric mixture of α and β isomers. Novel 6'-SL intermediates of general formulae **1A** and **1B** can be characterized as crystalline solids, oils, syrups, precipitated amorphous material or spray dried products, preferably as crystalline solids. If crystalline, compounds of general formulae **1A** and **1B** might exist either in anhydrous or in hydrated crystalline forms by incorporating one or several molecules of water into their crystal structures. Similarly, novel compounds characterized by general formulae **1A** and **1B** might exist as crystalline substances incorporating ligands such as organic molecules and/or ions into their crystal structures.

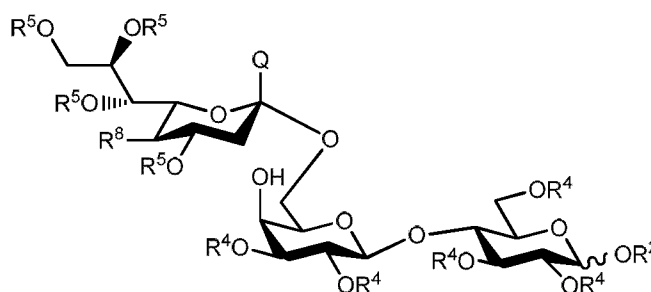
A compound of general formula **1B** as salt means an associated ion pair consists of the negatively charged acid residue of a compound of general formula **1B** and a cation in any stoichiometric proportion. The cation may be K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} , Zn^{2+} , Mn^{2+} and Cu^{2+} , most preferably K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} and Zn^{2+} . Basic organic compounds in positively charged form may be relevant organic cations as well. Such preferred positively charged organic molecules are diethyl amine, triethyl amine, diisopropyl ethyl amine, ethanolamine, diethanolamine, triethanolamine, imidazol, piperidine, piperazine, morpholin, benzyl amine, ethylene diamine, meglumin, pyrrolidine, choline, tris-(hydroxymethyl)-methyl amine, N-(2-hydroxyethyl)-pyrrolidine, N-(2-hydroxyethyl)-piperidine, N-(2-hydroxyethyl)-piperazine, N-(2-hydroxyethyl)-morpholine, L-arginine, L-lysine, oligopeptides having L-arginine or L-lysine unit or oligopeptides having free amino group on N-terminal, etc., all in protonated form.

A preferred embodiment relates to compounds of general formula **1B** wherein R^2 is benzyl, -OR² is in β and the salt is selected from the Zn^{2+} salt and organic salts, and the organic salt is preferably selected from ethanolammonium, diethyl ammonium, tris-(hydroxymethyl)-methyl ammonium and choline salt.

Novel compounds of general formulae **1A** and **1B** provided by the present invention can be used for the preparation of 6'-SL itself and other 6'-SL derivatives by using

chemical/enzymatic methodologies known in the Art. Novel compounds of general formulae **1A** and **1B** can also be used as advanced precursors/intermediates for the production/preparation of numerous human milk oligosaccharides. Novel compounds of general formulae **1A** and **1B** can also be considered as valuable intermediates for the synthesis of complex oligosaccharides/glycoconjugates suitable for therapeutic/nutritional use.

It is provided compounds of general formula **2A**



general formula **2A**

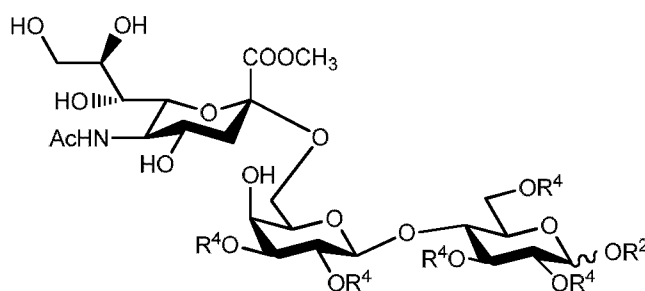
- 10 wherein R^2 is a group removable by catalytic hydrogenation,
 R^4 and R^5 are independently from each other optionally substituted acyl,
 R^8 is selected from $-NHAc$ and $-NAC_2$, and
 Q is $-COO$ -alkyl, which alkyl is optionally substituted.

15 It is strongly emphasised that novel derivatives characterized by general formula **2A** can be considered as sole chemical entities such as either α or β anomers, preferably the β anomer, or even an anomeric mixture of α and β isomers. Novel 6'-SL intermediates of general formula **2A** can be characterized as crystalline solids, oils, syrups, precipitated amorphous material or spray dried products, preferably as crystalline solids. If crystalline, compounds of general formula **2A** might exist either in anhydrous or in hydrated crystalline forms by
 20 incorporating one or several molecules of water into their crystal structures. Similarly, novel compounds characterized by general formula **2A** might exist as crystalline substances incorporating ligands such as organic molecules and/or ions into their crystal structures.

Compounds of general formula **2A** wherein $-OR^2$ is in β , R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably pivaloyl and benzoyl, R^5 is acetyl, Q is $-COOCH_3$ are especially preferred.

- 5 Novel compounds of general formula **2A** provided by the present invention can be used for the preparation of 6'-SL itself and other 6'-SL derivatives by using chemical/enzymatic methodologies known in the Art. Novel compounds of general formulae **2A** can also be used as advanced precursors/intermediates for the production/preparation of numerous human milk oligosaccharides. Novel compounds of general formulae **2A** can also be considered as
- 10 valuable intermediates for the synthesis of complex oligosaccharides/glycoconjugates suitable for therapeutic/nutritional use.

Further preferred compounds of general formula **2** are compounds of general formula **2C**



general formula **2C**

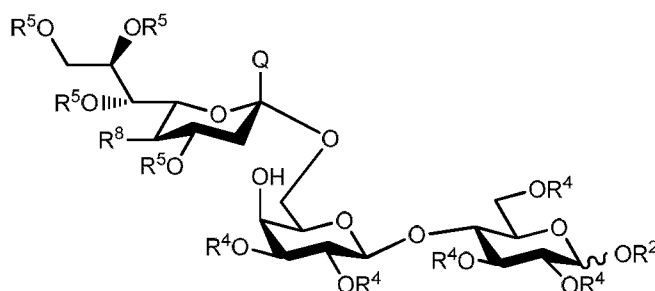
- 15 wherein $-OR^2$ is in β , R^2 is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and
- R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably benzoyl.

- It is strongly emphasised that novel derivatives characterized by general formula **2C** can be considered as sole chemical entities such as either α or β anomers, preferably the β anomer,
- 20 or even an anomeric mixture of α and β isomers. Novel 6'-SL intermediates of general formula **2C** can be characterized as crystalline solids, oils, syrups, precipitated amorphous material or spray dried products, preferably as crystalline solids. If crystalline, compounds of

general formula **2C** might exist either in anhydrous or in hydrated crystalline forms by incorporating one or several molecules of water into their crystal structures. Similarly, novel compounds characterized by general formula **2C** might exist as crystalline substances incorporating ligands such as organic molecules and/or ions into their crystal structures.

- 5 Novel compounds of general formula **2C** provided by the present invention can be used for the preparation of 6'-SL itself and other 6'-SL derivatives by using chemical/enzymatic methodologies known in the Art. Novel compounds of general formulae **2C** can also be used as advanced precursors/intermediates for the production/preparation of numerous human milk oligosaccharides. Novel compounds of general formulae **2C** can also be considered as
- 10 valuable intermediates for the synthesis of complex oligosaccharides/glycoconjugates suitable for therapeutic/nutritional use.

Another aspect of the present application relates to providing compounds of general formula **2D**



general formula **2D**

- 15 wherein R^2 is a group removable by catalytic hydrogenation,
- R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and H,
- R^5 is selected from optionally substituted acyl and H,
- R^8 is selected from -NHAc and NAc₂, and
- Q is selected from -COO-alkyl, which alkyl is optionally substituted and -COOH in
- 20 either protonated or deprotonated form,

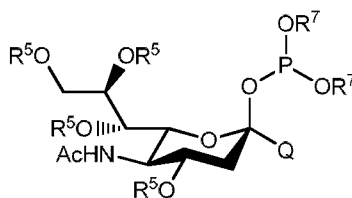
provided that if Q is -COOH in either protonated or deprotonated form and R⁸ is -NHAc, then both R⁴ and R⁵ cannot be H simultaneously.

It is strongly emphasised that novel derivatives characterized by general formula **2D** can be considered as sole chemical entities such as either α or β anomers, preferably the β anomer, or even an anomeric mixture of α and β isomers. Novel 6'-SL intermediates of general formula **2D** can be characterized as crystalline solids, oils, syrups, precipitated amorphous material or spray dried products, preferably as crystalline solids. If crystalline, compounds of general formula **2D** might exist either in anhydrous or in hydrated crystalline forms by incorporating one or several molecules of water into their crystal structures. Similarly, novel compounds characterized by general formula **2D** might exist as crystalline substances incorporating ligands such as organic molecules and/or ions into their crystal structures.

In a preferred embodiment -OR² is in β , R² is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R⁴ and R⁵ are H, R⁸ is -NHAc and Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

Novel compounds of general formula **2D** provided by the present invention can be used for the preparation of 6'-SL itself and other 6'-SL derivatives by using chemical/enzymatic methodologies known in the Art. Novel compounds of general formulae **2D** can also be used as advanced precursors/intermediates for the production/preparation of numerous human milk oligosaccharides. Novel compounds of general formulae **2D** can also be considered as valuable intermediates for the synthesis of complex oligosaccharides/glycoconjugates suitable for therapeutic/nutritional use.

It has to be emphasized that compounds of general formula **4A** are novel. Thus the present invention provides compounds of general formula **4A**



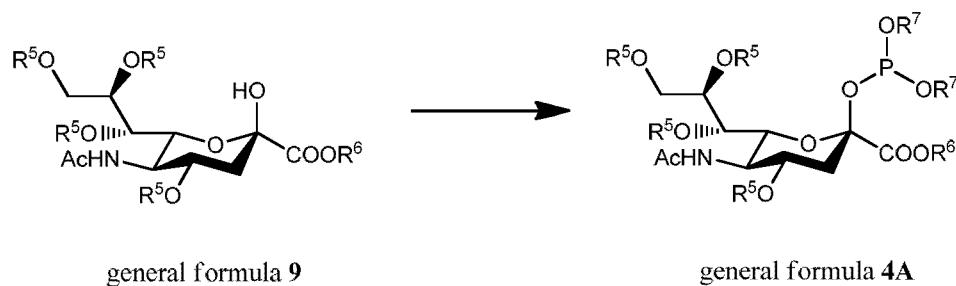
general formula **4A**

wherein R^5 is optionally substituted acyl, Q is -COO-alkyl, which alkyl is optionally substituted and R^7 is substituted benzyl. In preferred embodiments R^5 is acetyl, Q is -COOCH₃ and R^7 is substituted benzyl, preferably substituted by chloro or bromo in position 4.

- 5 Compounds of general formula **4A** can be considered as crystalline materials in β -anomeric form. They are stable, can be stored for longer period of time without significant decomposition, can be easily activated in glycosylation reactions and shows excellent α -selectivity. As their unsubstituted benzyl phosphite counterparts are known not to be solid, compounds of general formula **4A** according to the present application has obviously
10 advantageous applicability in sialylation reactions.

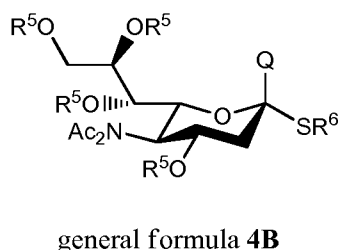
Accordingly, the present invention provides the use of compounds of general formula **4A** in the synthesis of sialooligosaccharides, preferably sialylated human milk oligosaccharides. Such sialylated HMOs are e.g. 6'-sialyllactose, 3'-sialyllactose, 3'-sialyl-3-fucosyllactose, disialyllacto-*N*-tetraose, disialyl monofucosyllacto-*N*-hexaose, sialyllacto-*N*-tetraose a,
15 sialyllacto-*N*-tetraose b, sialyllacto-*N*-tetraose c, sialyllacto-*N*-fucopentaose II, monofucosyl monosialyllacto-*N*-hexaose I, monosialyllacto-*N*-neohexaose I, monosialyl monofucosyllacto-*N*-neohexaose.

The present invention relates to also the production of compounds of general formula **4A** (Scheme 3.). The readily available sialic acid derivatives according to general formula **9** are
20 phosphitylated either by directly with $(R^7O)_2PY$, in which Y is halogen or dialkylamino group, in the presence of tertiary amine, preferably Et₃N, in aprotic solvent, preferably dichloromethane, or in two consecutive steps: first adding PCl₃ then R⁷OH (R^7 is substituted benzyl, preferably halo- or methylbenzyl). In both steps a tertiary amine base is needed to neutralize the hydrochloric acid formed: in the first step imidazole, in the second step Et₃N
25 are the preferred base. The solvent used is selected from aprotic solvents, preferably dichloromethane is the solvent of choice.



Scheme 3.

It has to be emphasized that compounds of general formula **4B** are novel. Thus the present invention provides compounds of general formula **4B**



- 5 wherein R^5 is optionally substituted acyl, preferably acetyl,
- R^6 is selected from C_{2-6} alkyl, C_{3-6} cycloalkyl and optionally substituted benzyl, preferably from ethyl, isopropyl, *t*-butyl, benzyl and cyclohexyl,
- Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

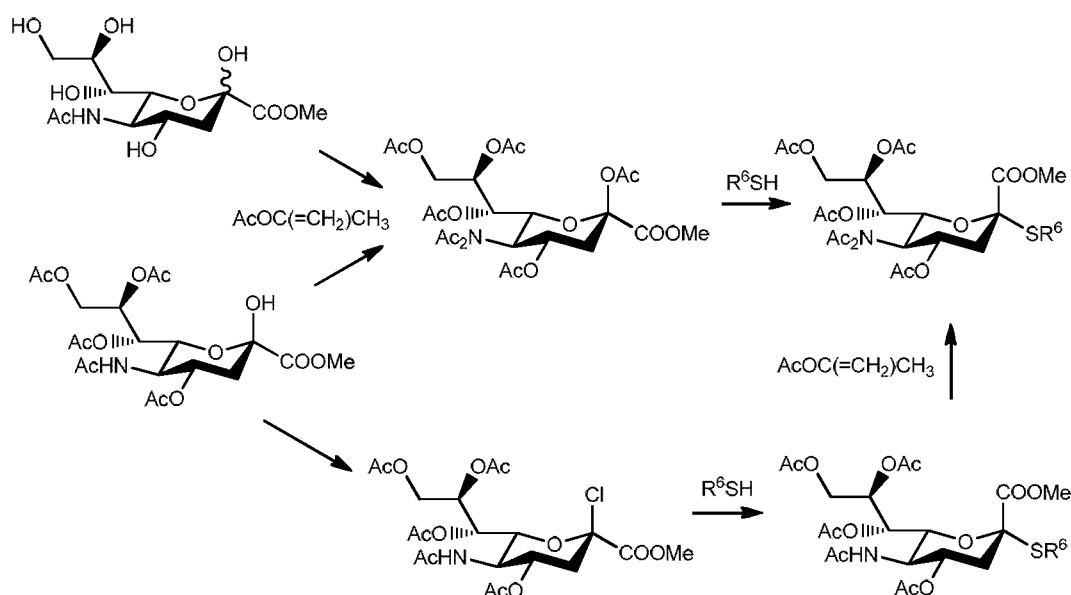
10 Compounds of general formula **4B** can be considered as crystalline materials in α -anomeric form. They are stable, can be stored for longer period of time without significant decomposition, can be easily activated in glycosylation reactions and shows excellent α -selectivity. As their analogues such as methyl, phenyl or 4-methylphenyl thioglycoside are known not to be solid, compounds of general formula **4B** according to the present application has obviously advantageous applicability in sialylation reactions.

15 Accordingly, the present invention provides the use of compounds of general formula **4B** in the synthesis of sialooligosaccharides, preferably sialylated human milk oligosaccharides. Such sialylated HMOs are e.g. 6'-sialyllactose, 3'-sialyllactose, 3'-sialyl-3-fucosyllactose, disialyllacto-*N*-tetraose, disialyl monofucosyllacto-*N*-hexaose, sialyllacto-*N*-tetraose a,

sialyllacto-*N*-tetraose b, sialyllacto-*N*-tetraose c, sialyllacto-*N*-fucopentaose II, monofucosyl monosialyllacto-*N*-hexaose I, monosialyllacto-*N*-neohexaose I, monosialyl monofucosyllacto-*N*-neohexaose.

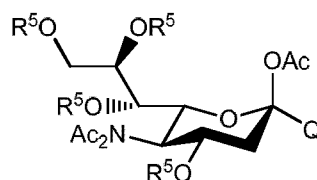
The present invention relates to also the production of compounds of general formula **4B**.

- 5 Sialic acid ester or tetra-*O*-acetyl sialic acid ester can be acetylated with isopropenyl acetate in the presence of acid, preferably tosic acid, at elevated temperature. The resulting 2,4,7,8,9-penta-*O*-acetyl-5-(*N*-acetylacetamido)-3,5-dideoxy-2-thio-D-glycero-D-galacto-non-2-ulopyranosonate is then reacted with R^6SH (R^6 is C_{2-6} alkyl, C_{3-6} cycloalkyl or optionally substituted benzyl, preferably ethyl, isopropyl, *t*-butyl, benzyl or cyclohexyl) to form
- 10 compounds of general formula **4B** under Lewis acid (e.g. boron trifluoride etherate, TfOH, TMSOTf, etc.) activation in an aprotic solvent or in mixture of aprotic solvents like dichloromethane, dichloroethane, chloroform, THF, dioxane, acetonitrile, DMF, etc. Activated alkylthio derivatives like trimethylsilyl- SR^6 can be also applied. According to a
- 15 different approach tetraacetyl sialyl chloride can be coupled with R^6SH in the presence of base and the resulting thioglycoside can be subsequently N-acetylated with isopropenyl acetate (Scheme 4.).



Scheme 4.

It has to be emphasized that the use compounds of general formula **4C** in sialylation reactions has not been mentioned in the art. Thus the present invention provides compounds of general formula **4C** for use as sialyl donor



general formula **4C**

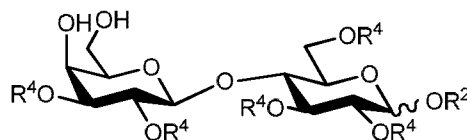
- 5 wherein R^5 is optionally substituted acyl, preferably acetyl and Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

Compounds of general formula **4C** can be considered as crystalline materials in β -anomeric form. They are stable, can be stored for longer period of time without significant decomposition, can be easily activated in glycosylation reactions and shows excellent α -
 10 selectivity.

Accordingly, in a more preferred embodiment the present invention provides the use of compounds of general formula **4C** in the synthesis of sialooligosaccharides, preferably sialylated human milk oligosaccharides. Such sialylated HMOs are e.g. 6'-sialyllactose, 3'-sialyllactose, 3'-sialyl-3-fucosyllactose, disialyllacto-*N*-tetraose, disialyl monofucosyllacto-
 15 *N*-hexaose, sialyllacto-*N*-tetraose a, sialyllacto-*N*-tetraose b, sialyllacto-*N*-tetraose c, sialyllacto-*N*-fucopentaose II, monofucosyl monosialyllacto-*N*-hexaose I, monosialyllacto-*N*-neohexaose I, monosialyl monofucosyllacto-*N*-neohexaose.

It is emphasised that compounds of general formula **3A** are novel, crystalline, they can be considered as sole chemical entities such as α - or β -anomers or even an anomeric mixture
 20 thereof, preferably in β -anomer. They might exist in anhydrous, hydrated as well as solvated forms.

Thus the present invention provides compounds of general formula **3A**

general formula **3A**

wherein R^2 is a group removable by catalytic hydrogenation, and

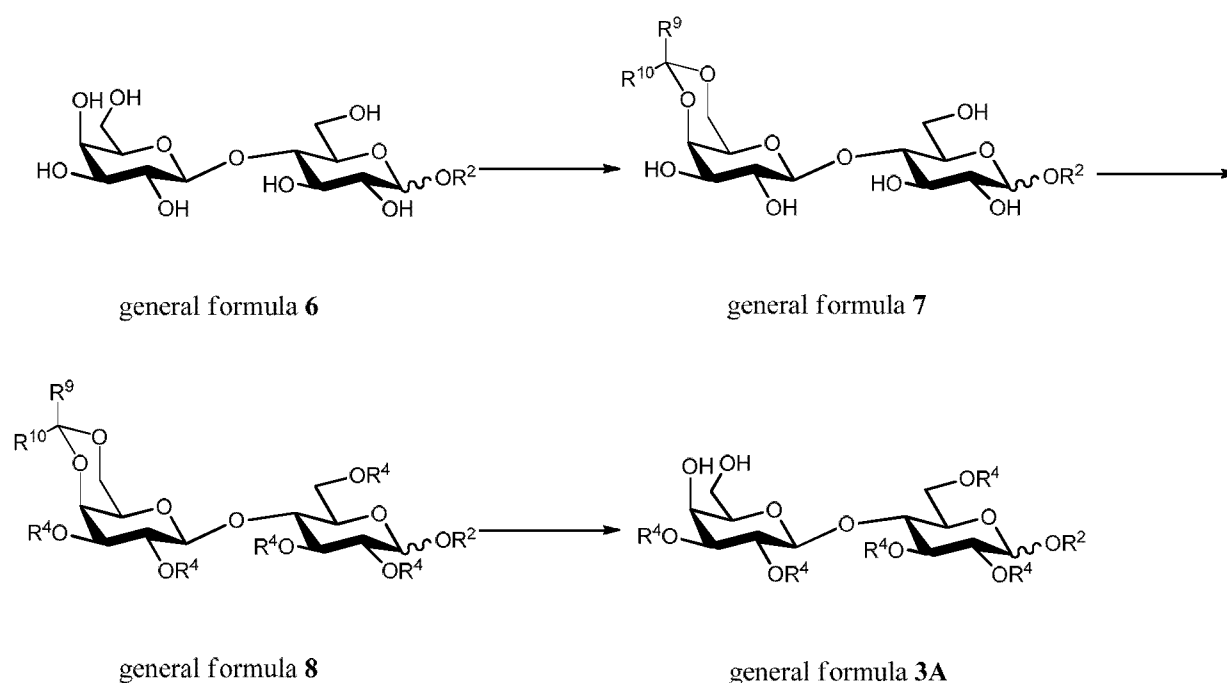
R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and optionally substituted acyl provided that acetyl is excluded.

- 5 In a more preferred embodiment $-OR^2$ is in β , R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and R^4 is selected from isobutyryl, pivaloyl and benzoyl.

The present inventors realized that acetyl groups are inconvenient protective groups when compounds of general formula **3A** act as glycosyl acceptor in sialylation reactions. Under the conditions of coupling acetyl migration always occurred to give complex mixture containing
 10 substances with similar physical characteristics which compounds can be separated only by lengthy and/or sophisticated and/or laborious techniques, e.g. chromatography. Choosing bulkier acyl protective group that don't tend or tend less to migrate results in an acceptor whose coupling product is formed almost exclusively in glycosidation, making the work-up procedure and isolation process of the desired compounds simpler, quick, powerful and cost-
 15 effective, e.g. by crystallization, which is one of the paramount concerns in large scale preparation or industrial process.

Another aspect of the present invention is to prepare glycosyl acceptor derivatives characterized by general formula **3A** above (Scheme 5.). The starting *O*-lactoside can be easily formed by Fischer glycosidation (lactose treated with the corresponding alcohol in the presence of acid catalysts). The so obtained *O*-lactosides of general formula **6** are then
 20 protected selectively at the 4',6'-position with R^9 -CHO (R^9 = phenyl optionally substituted with 1-2 alkoxy or nitro), benzophenone or di-*O*-acetals thereof in known manner giving rise to compounds of general formula **7** followed by acylation or methoxy-benzylation to obtain compounds of general formula **8**. The acylation can be performed with acyl halides,
 25 anhydrides, active esters etc. in the presence of base in known manner. A typical benzyl ether formation runs by adding the appropriate benzyl halogenide (chloride, bromide or iodide) and

NaH or powdered KOH or NaOH in an inert organic aprotic solvent like THF, dioxane, DMF, etc., even the reagent benzyl halogenide can be used as solvent. Phase-transfer catalysis conditions can be also applied in benzylation, the organic phase solvent is selected from the group of aromatic hydrocarbons (toluene, benzene, xylols) and basic aqueous solution, preferably 50 % NaOH or KOH solution is used as aqueous phase. Quaternary ammonium or phosphonium salts, or crown ethers can be added as catalyst, preferably TBAI. Removal of the acetal or ketal type protecting group from compounds of general formula 8 by acidic hydrolysis results in compounds of general formula 3A.



Scheme 5.

In a preferred embodiment of preparing compounds of general formula 3A β -benzylactoside is used as starting material ($R^1 = \text{OBn}$ in general formula 6). In a further preferred realization benzaldehyde-dimethylacetal is the reagent for protecting 4',6'-positions. To protect the free hydroxyls in compounds of general formula 7 pivaloyl and optionally substituted benzoyl groups are favoured. In case of removing benzylidene acetal from compounds of general formula 8 diluted strong inorganic acids, organic acids (e.g. alkanolic acids, sulfonic acids) and H^+ form ion exchange resin are given preference as acid catalyst, preferably p-

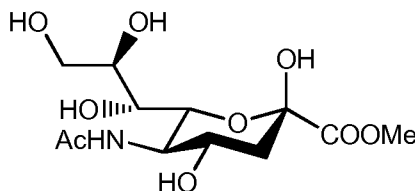
toluenesulfonic acid, and among the solvents alcohols, aromatic hydrocarbons, chlorinated alkanes or mixtures thereof are favourable, preferably DCM, methanol or mixtures thereof.

According to a further aspect the compounds of general formula **3A** defined and claimed above can be used for preparing 6'-SL. Compounds of general formula **3A** as acceptor reacts readily with any suitable sialyl donor giving rise to protected 6'-SL intermediates according to general formula **2** above, which can then be transformed into 6'-SL and salts thereof by means of deprotective manipulations as described above.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not to be limiting thereof.

EXAMPLES

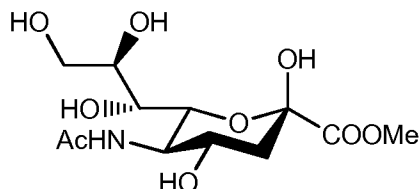
Example 1.



A mixture of anhydrous sialic acid (100 g, 323 mmol) and dried Amberlite IR-120 (H^+) ion exchange resin (100 g) in MeOH (1500 mL) was stirred for 15 hours at RT. The ion exchange resin was filtered off and washed with MeOH (2x100 mL). The washes were combined with the filtrate and concentrated to 300 mL. The concentrated residue crystallized upon seeding at RT. The crystals were collected by filtration giving 73.8 g (71 %) sialic acid methyl ester. The mother liquor was concentrated (10.5 g) and recrystallized from MeOH (30 mL) to yield 7.4 g (7 %) sialic acid methyl ester. Total yield 81.2 g (78 %).

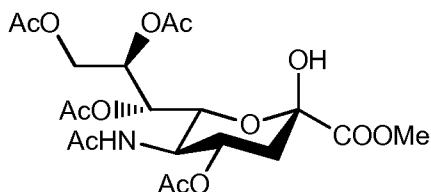
^1H NMR (D_2O) δ in ppm: 1.89 (dd, 1H, $J=13.0Hz$, $J=11.4Hz$); 2.03 (s, 3H); 2.28(dd, 1H, $J=13.0Hz$, $J=4.7Hz$); 3.52 (dd, 1H, $J=8.9Hz$, $J=3.0Hz$); 3.59 (dd, 1H, $J=11.6Hz$, $J=6.1Hz$); 3.71 (ddd, $J=8.9Hz$, $J=2.5Hz$, $J=11.6Hz$); 3.82 (dd, 1H, $J=11.6Hz$, $J=2.5Hz$); 3.82 (s, 3H); 3.90 (dd, 1H, $J=10.1Hz$, $J=10.0Hz$); 3.98-4.10 (m, 2H, H-6, H-4).

Example 2.



To a suspension of anhydrous sialic acid (100g, 323 mmol) in MeOH (1200 ml) [0.03 % water content] 8 % HCl in MeOH (50 ml) was added and the reaction mixture was stirred for 6 hours at RT. The reaction mixture was neutralized with triethylamine (15 ml) and the clear solution was concentrated to 270 mL. The concentrated residue crystallized upon seeding at RT for 2 hours. The solid was collected by filtration yielding 104.9 g (100 %).

Example 3.



A suspension of sialic acid methyl ester (50 g, 155 mmol) and acetic anhydride (73 ml, 775 mmol) in DCM (175 mL) was stirred at RT and 70 % perchloric acid (1 mL) was then added dropwise within 30 minutes. During the addition the temperature of the mixture increased until reflux. The reaction mixture was stirred at reflux for 2.5 h, and after this time MeOH (7.5 ml, 185 mmol) was added dropwise and the reaction mixture was stirred for a further hour at RT. The reaction mixture was diluted with DCM (175 mL) and washed with water (3x50 mL). The combined water phases were extracted with DCM (2x100 mL). The combined organic phases were washed with saturated NaHCO₃ (2x100 mL) and evaporated. The residue (59.6 g) was dissolved in *i*BuOAc at 50 °C and the mixture was cooled down to RT and let overnight complete the crystallization. The solid was collected by filtration yielding 39.2 g (52 %) of tetraacetyl sialic acid methyl ester.

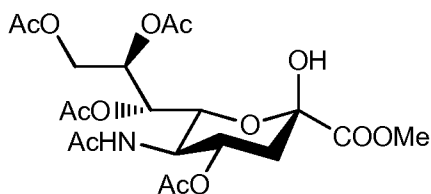
¹H NMR (C₆D₆) δ in ppm: 1.60, 1.63, 1.70, 1.85, 1.92 (5s, 15H); 2.19 (dd, 1H, J=12.8Hz, J=5.7Hz); 2.25 (ddd, 1H, J=12.8Hz, J=10.8Hz); 3.28 (s, 3H); 4.23 (dd, 1H, J=12.4Hz, J=7.6Hz); 4.26 (dd, 1H); 4.54 (ddd, 1H, J=10.8Hz); 4.78 (d, 1H, J=10.2Hz); 5.02 (dd, 1H,

$J=12.4\text{Hz}$, $J=2.0\text{Hz}$); 5.26 (ddd, 1H, $J=10.8\text{Hz}$, $J=5.7\text{Hz}$, $J=10.5\text{Hz}$); 5.61 (ddd, 1H, $J=2.0\text{Hz}$, $J=7.6\text{Hz}$); 5.64 (dd, 1H, $J=2.3\text{Hz}$, $J=4.2\text{Hz}$).

^1H (CDCl_3) δ in ppm: 1.91, 2.02, 2.03, 2.11, 2.15 (5s, 15H); 2.19 (dd, 1H, $J=12.8\text{Hz}$, $J=11.4\text{Hz}$); 3.26 (ddd, 1H, $J=12.8\text{Hz}$, $J=5.4\text{Hz}$); 3.86 (s, 3H); 4.03 (dd, 1H, $J=12.4\text{Hz}$, $J=7.5\text{Hz}$); 4.13-4.21 (m, 2H); 4.51 (dd, 1H, $J=12.4\text{Hz}$, $J=2.4\text{Hz}$); 5.22 (ddd, 1H, $J=11.4\text{Hz}$, $J=5.4\text{Hz}$, $J=9.5\text{Hz}$); 5.25 (ddd, 1H, $J=2.4\text{Hz}$, $J=7.5\text{Hz}$, $J=5.6\text{Hz}$); 5.36 (dd, 1H, $J=1.5\text{Hz}$, $J=5.6\text{Hz}$); 5.71 (m, 1H).

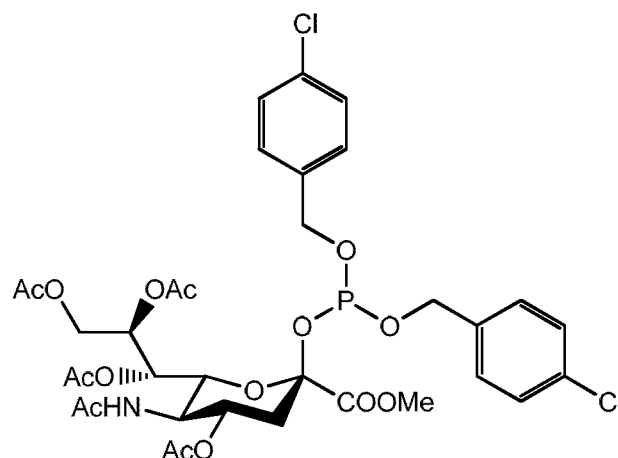
^{13}C NMR: 20.65, 20.75, 20.93, 22.93, 36.11, 49.04, 53.18, 62.50, 68.3, 69.12, 71.32, 72.07, 94.84, 168.93, 170.12, 170.30, 170.72, 171.04, 171.43

10 Example 4.



To a mixture of sialic acid methyl ester (60.8 g, 188 mmol) and acetic anhydride (89 ml, 940 mmol) in DCM (220 mL), perchloric acid 70 % (1.22 mL) was added dropwise within 30 minutes. During the addition the temperature of the mixture increased until reflux. The reaction mixture is stirred at reflux for 2.5 h. Subsequently, MeOH (9.2 mL, 225 mmol) was added dropwise and the reaction mixture was stirred for one additional hour at RT. The clear solution was added dropwise to a suspension of Na_2CO_3 (60.8 g; 573 mmol) in DCM and the mixture was stirred at room temperature for 2 hours. The remaining solid was removed by filtration and was washed with DCM (2x50 mL). The combined DCM phase was concentrated to 150 mL, $i\text{-BuOAc}$ (150 mL) was added and the remaining DCM was removed and let crystallize overnight. The crystals were collected by filtration yielding 65 g (71%) of tetraacetyl sialic acid methyl ester.

Example 5.

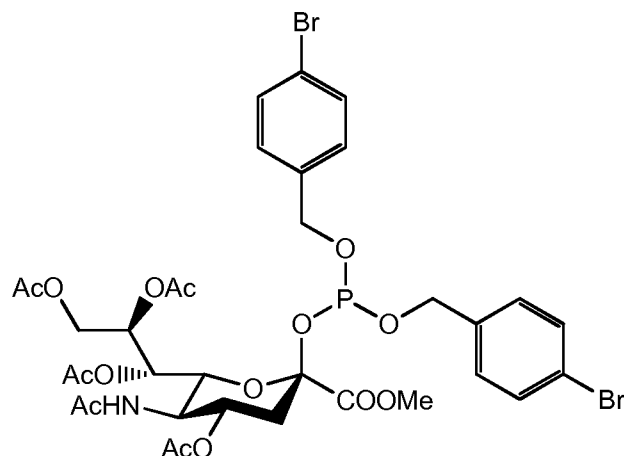


A suspension of imidazole (1.88 kg) in DCM (20 L) was cooled down to 5 °C, PCl₃ (690 ml) was added, followed by the addition of Et₃N (4.16 L). The heavy slurry was stirred for 60 min. at 0 °C and the tetraacetate (2.59 Kg) was added in DCM solution (3 liter/kg). The temperature was set to 25 °C and the reaction mixture stirred for 3h. After this time *p*-Cl-benzyl alcohol (2.64 Kg) was added as solid and the reaction mixture stirred for another 2 h. The reaction mixture was transferred to the 110 L extraction vessel, was neutralized with 1N HCl (26 L) and the phases were separated. The organic phase was concentrated to an oil and kept in the fridge until next day. The residue was dissolved in 15 L of *t*-BuOMe and washed with water (3 x 7 L); the organic phase was the evaporated. The residue was dissolved in *i*-Pr₂O (5 L) and the turbid solution turned into a solid after. The slurry was filtered off, the solid was washed with *i*-Pr₂O (2x1.5L) and dried in the vacuum oven. (2.65 Kg with 95% purity).

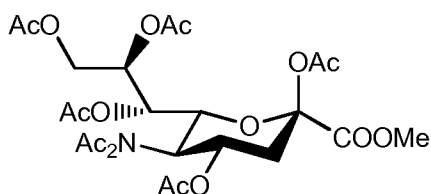
¹H NMR δ (CDCl₃) in ppm: 1.9-2.15 (m, 16H); 2.39 (dd, 1H, J=4.9Hz, J=13.00Hz); 3.75 (s, 3H); 3.85 (dd, 1H, J=10.6Hz, J=2.00Hz); 4.05 (m, 1H); 4.15 (dd, J=7.4, J=12.4); 4.6 (m, 2H); 4.8-5 (m, 4H); 5.15 (m, 2H); 5.22 (m, 1H).

Example 6.

Analogously prepared:



Example 7.



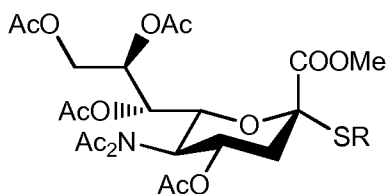
Method A: To a suspension of the N-acetyl neuraminic acid methyl ester tetra-*O*-acetate (100 g, 0.2 mol) in isopropenyl acetate (300 mL) TsOH was added and the reaction mixture was stirred for 16 h at 75 °C (oil bath). The reaction became a clear solution after couple of minutes. When TLC (toluene/acetone 3/2) showed completion the mixture was cooled, diluted with 500 mL of EtOAc and washed as follows: 1x NaHCO₃ (500 mL), 1x H₂O (500 mL) and 1x brine (300 mL). The residue was concentrated in vacuo, redissolved in EtOAc (200 mL) and heptane (280 mL) was added at 70 °C. The mixture was cooled to 0 °C and stirred for 2 h. The solid was collected by filtration to yield 102.12 g (87 %).

Method B: To a suspension of sialic acid methyl ester (100 g) in isopropenyl acetate (500 mL) TsOH was added and the reaction mixture stirred for 16 h at 75 °C (oil bath). The reaction became a clear solution after couple of hours. When TLC (toluene/acetone 3/2) showed completion the mixture was cooled, diluted with 500 mL of EtOAc and washed as follows: 1x NaHCO₃ (500 mL), 1x H₂O (500 mL) and 1x brine (300 mL). The residue was concentrated in vacuo, redissolved in EtOAc (200 mL) and heptane (280 mL) was added at 70 °C. The mixture was cooled to 0 °C and stirred for 2 h. The solid was collected by filtration to yield 101.3 g (85 %).

^1H NMR (CDCl_3) δ (ppm): 1.95-2.23 (m, 16H); 2.31 (s, 3H); 2.39 (s, 3H); 2.66 (dd, 1H, $J=13.5\text{Hz}$, $J=6.3\text{Hz}$); 3.77 (s, 3H); 4.15 (m, 2H); 4.35 (dd, 1H, $J=2.4\text{Hz}$, $J=12.4\text{Hz}$); 5.15 (m, 3H); 5.8 (m, 1H).

^{13}C NMR (CDCl_3) δ (ppm): 20.97, 21.13, 26.13, 28.24, 53.36, 54.66, 57.26, 62.03, 66.56,
5 67.72, 69.93, 70.57, 97.37, 166.80, 168.78, 169.80, 169.98, 170.49, 173.82

Example 8.



To a 0 °C cold solution of N,N-diacetyl neuraminic acid methyl ester penta-*O*-acetate (10 g, 17.4 mmol) and RSH (2.2 eq) in DCM (50 mL) $\text{BF}_3 \cdot \text{OEt}_2$ (2.79 mL, 1.3 eq) was added and
10 the reaction mixture was stirred at this temperature for 3 h (overnight in the case of *t*BuSH). Aqueous NaHCO_3 was added to neutralize (25 mL) the acid and the phases were separated, the organic phase was then washed with water, and the solvent was evaporated in *vacuo*. The residue was crystallized as follows.

R=*i*-Pr (crystallized from *i*-Pr₂O) yield 75%, mp: 110-111 °C

15 ^1H NMR (CDCl_3) δ (ppm): 1.25 (d, 3H, $J=6.7\text{Hz}$); 1.35 (d, 3H, $J=7\text{Hz}$); 1.9-2.2 (m, 13H); 2.3 (s, 3H); 2.4 (s, 3H); 2.65 (dd, 1H, $J=5.1\text{Hz}$, $J=13.7\text{Hz}$); 3.25 (m, 1H); 3.8 (s, 3H); 4.2 (m, 2H); 4.75 (dd, 1H, $J=2.3\text{Hz}$, $J=12.4\text{Hz}$); 5.1 (m, 1H); 5.25 (dd, 1H, $J=2.5\text{Hz}$, $J=3\text{Hz}$); 5.45 (dd, 1H, $J=9.9\text{Hz}$, $J=2.25\text{Hz}$); 5.8 (m, 1H).

^{13}C NMR (CDCl_3) δ (ppm): 20.88, 20.90, 21.04, 21.11, 24.83, 25.01, 26.13, 28.11, 34.29,
20 39.18, 52.98, 57.56, 62.59, 67.09, 69.16, 69.68, 72.57, 85.43, 169.18, 169.63, 170.35, 170.56, 170.58, 173.75, 174.49.

R=Bn (crystallized from MeOH) yield 79 %

^1H NMR (CDCl_3) δ (ppm): 1.9-2.2 (m, 13H); 2.3 (s, 3H); 2.4 (s, 3H); 2.7 (dd, 1H, $J=5.1\text{Hz}$, $J=13.9\text{Hz}$); 3.5 (s, 3H); 3.8 (s, 2H); 4.2 (m, 2H); 4.7 (dd, 1H, $J=2.4\text{Hz}$, $J=12.4\text{Hz}$); 5.2 (m, 1H); 5.3 (dd, 1H, $J=2\text{ Hz}$, $J=4.2\text{Hz}$); 5.55 (dd, 1H, $J=9.9\text{Hz}$, $J=2\text{ Hz}$); 5.8 (m, 1H); 7.3 (m, 5H).

5 ^{13}C NMR (CDCl_3) δ (ppm): 20.91, 20.94, 21.09, 21.11, 26.16, 28.18, 32.99, 38.63, 52.80, 57.59, 62.36, 67.16, 68.65, 69.53, 71.82, 85.17, 127.41, 128.71, 129.009, 136.45, 168.43, 169.65, 170.39, 170.50, 170.66, 173.71, 174.55.

R=*t*-Bu (crystallized from *i*-Pr₂O/Heptane) yield 80 %, mp: 95-98 °C

10 ^1H NMR (CDCl_3) δ (ppm): 1.4 (s, 9H); 1.9-2.2 (m, 13H); 2.3 (s, 3H); 2.4 (s, 3H); 2.7 (dd, 1H, $J=4.8\text{Hz}$, $J=13.5\text{Hz}$); 3.8 (s, 3H); 4.2 (m, 2H); 4.7 (dd, 1H, $J=1.5\text{Hz}$, $J=12.2\text{Hz}$); 5.1 (m, 1H); 5.3 (dd, 1H, $J=2.8\text{ Hz}$, $J=3.2\text{Hz}$); 5.55 (dd, 1H, $J=10\text{Hz}$, $J=2.7\text{ Hz}$); 5.8 (m, 1H); 7.3 (m, 5H).

^{13}C NMR (CDCl_3) δ (ppm): 20.87, 20.88, 21.02, 21.09, 26.07, 28.09, 31.46, 41.58, 47.81, 52.90, 57.77, 62.76, 66.99, 69.35, 69.50, 72.62, 86.45, 169.55, 169.81, 170.38, 170.61, 170.72, 174.02, 174.56.

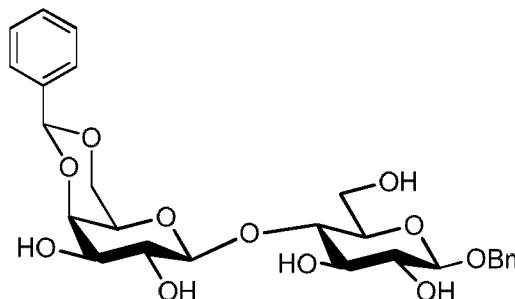
15 R=Et (crystallized from *i*-Pr₂O) yield 78 %, mp: 137-138 °C

R=cyclohexyl (crystallized form MeOH) yield 78 %, mp: 146-147 °C

20 ^1H NMR (CDCl_3) δ (ppm): 1.2-1.8 (m, 12H); 1.9-2.2 (m, 13H); 2.3 (s, 3H); 2.4 (s, 3H); 2.65 (dd, 1H, $J=5.2\text{Hz}$, $J=13.8\text{Hz}$); 2.8 (m, 1H); 3.8 (s, 3H); 4.2 (m, 2H); 4.8 (dd, 1H, $J=2.1\text{Hz}$, $J=12.4\text{Hz}$); 5.05 (m, 1H); 5.25 (t, 1H, $J=2.3\text{Hz}$); 5.45 (dd, 1H, $J=9.9\text{Hz}$, $J=2.3\text{Hz}$); 5.8 (m, 1H).

^{13}C NMR (CDCl_3) δ (ppm): 20.97, 20.99, 21.11, 21.15, 25.51, 26.23, 26.50, 28.16, 35.23, 35.29, 39.09, 42.05, 45.88, 53.01, 57.43, 63.05, 67.18, 69.53, 70.12, 73.33, 85.20, 169.32, 169.72, 170.45, 170.62, 170.68, 173.73, 174.51.

Example 9.

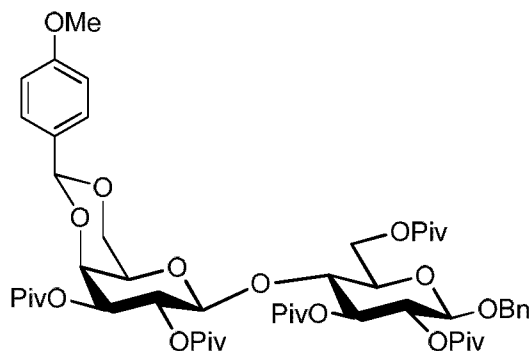


To a suspension of β -benzylactoside (700 g, 1.6 mol) in DMF (5 L) was added benzaldehyde dimethylacetal (389.5 mL, 2.6 mmol, 1.6 eq.) and p-TsOH $\cdot$ H₂O (31.5 g, 0.17 mmol, 0.1 eq.). The reaction mixture was then heated to 40-44 °C for approximately 22 h, after which a white suspension was obtained. It was cooled to ice bath temperature, i-Pr₂O (4 L) was added and the resulting suspension was further stirred for 1½ h at this temperature. This mixture was filtrated and the white solid obtained was washed/suspended with i-Pr₂O (2 × 1 L). After drying 702 g were obtained of the wanted product as a white free solid. The resulting mother liquor was allowed to stand at room temperature for approximately 3 days, during which time more white solid appeared. This was filtrated and washed/suspended with i-Pr₂O (2 × 150 mL). Obtained further 25 g of product. Combined amount: 727 g (86 %).

¹H NMR δ (CD₃OD) in ppm: 3.3-3.45 (m, 2H); 3.5-3.7 (m, 5H); 3.95 (m, 2H); 4.15 (m, 3H); 4.40 (d, 1H, J=7.8Hz); 4.5 (d, 1H, J=7.5Hz); 4.65 (d, 1H, J=11.8Hz); 4.9 (d, 1H, J=11.8Hz); 5.55 (s, 1H); 7.25 (m, 5H).

¹³C NMR: 61.75, 68.33, 70.17, 71.78, 71.86, 73.52, 74.89, 76.33, 77.35, 80.02; 102.3, 103.22, 104.87; 128.8-139.03

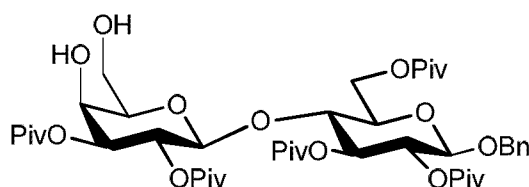
Example 10.



The starting pentaol (650 g, 1.16 mol), pyridine (3.25 l) and DMAP (7.1 g, 58 mmol) were loaded to the reactor and the PivCl (1.3 l, 10.6 mol) was added in one hour, the mixture was warmed up to 95 °C and stirred for 15 hours. The reaction mixture was cooled to 60 °C, 4.63 l MeOH was added, and the mixture was stirred in room temperature for 1,5 hours. The solid was filtrated, washed two times with 1.5 l MeOH, dried on open air for one night and in vacuum for three days.

^1H NMR δ (CD_3OD) in ppm: 1.05-1.2 (4s, 36H); 1.25 (s, 9H); 3.4 (m, 1H); 3.55 (m, 1H); 3.8 (s, 3H); 3.95 (t, 1H, $J=9.5\text{Hz}$); 4.05 (dd, 1H, $J=12.5\text{Hz}$, $J=1.7\text{Hz}$); 4.2 (dd, 1H, $J=11.8\text{Hz}$, $J=5.5\text{Hz}$); 4.35 (dd, 1H, $J=12.5\text{Hz}$, $J=0.9\text{Hz}$); 4.38 (dd, 1H, $J=0.3\text{Hz}$, $J=3.5\text{Hz}$); 4.55 (m, 4H); 4.8 (m, 2H); 4.95 (dd, 1H); 5.2 (m, 1H); 5.3 (m, 1H), 5.45 (s, 1H); 6.85 (m, 2H); 7.2-7.4 (m, 7H).

Example 11.

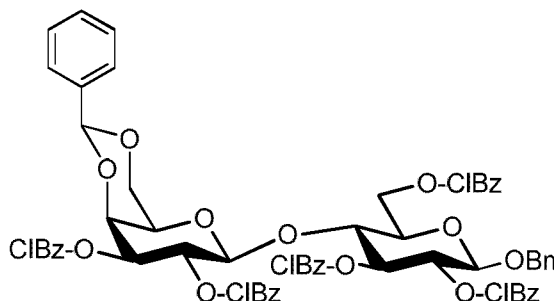


To a mixture of the starting material (example 10, 32.5 g, 33.5 mmol), DCM (65 ml) MeOH (16 ml) and water (6.5 ml), $p\text{TsOH}\cdot\text{H}_2\text{O}$ (2.6 g, 13.7 mmol) was added and then heated to reflux temperature. After two hours TLC (Toluene:Acetone 5:1) indicates the reaction is finished. The reaction mixture was cooled down to RT, diluted with DCM (65 ml) and washed with saturated NaHCO_3 (65 ml) and water (65 ml). The organic phase was dried over MgSO_4 and concentrated to get 33 g colourless oil. The crude product was dissolved in EtOAc (227 ml) at reflux temperature, petroleum ether (40-70 °C, 70 ml) was added and the

mixture was cooled to 0°C. The solid was filtrated, washed with petroleum ether (2x30 ml) and dried to get 22.6 g (79.2 %) acceptor.

¹H NMR δ (CD₃OD) in ppm: 1.05-1.2 (4s, 36H); 1.25 (s, 9H); 3.4 (m, 2H); 3.8 (m, 1H); 3.95 (m, 2H); 4.1 (m, 1H); 4.2 (dd, 1H, J=11.8Hz, J=5.5Hz); 4.55 (m, 4H); 4.8 (d, 1H, J=12.5Hz);
5 4.9 (dd, 1H); 5.0 (dd, 1H); 5.2 (m, 2H); 7.2-7.4 (m, 5H).

Example 12.

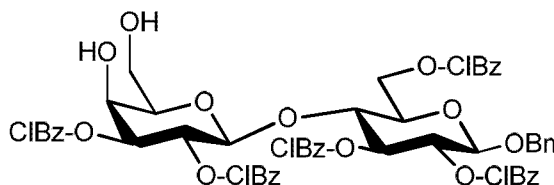


A solution of the pentaol (example 9, 5.6 g, 0.01 mol) in pyridine (20 mL) and DCM (50 mL) was cooled with ice bath and the p-chlorobenzoyl chloride (14 mL) was added slowly. The
10 reaction mixture stirred for 24 h at RT, and the solvent was evaporated in *vacuo*. The residue was dissolved in DCM (70 mL) and washed with 1N HCl (40 mL), H₂O (40 mL), NaHCO₃ (40 mL) and brine (30 mL), The organic phase was dried over Na₂CO₃ and the solvent was evaporated in *vacuo*. The solid was dissolved in DCM (60 mL) and 360 mL MeOH were added. The white solid was filtrated and washed with MeOH and dried to yield 10.6 g (81
15 %).

¹H NMR (CDCl₃) δ (ppm): 3.05 (m, 1H); 3.75 (m, 3H); 4.13 (dd, 1H, J=9.1Hz, J=9.5Hz); 4.31 (m, 1H); 4.42 (dd, 1H, J=4.1Hz, J=12.0Hz); 4.57 (m, 2H); 4.69 (d, 1H, J=7.7Hz); 4.75 (d, 1H, J=7.9Hz); 4.81 (d, 1H; 12.5Hz); 5.15 (dd, 1H, J=3.5Hz, J=10.4Hz); 5.30 (s, 1H); 5.38 (dd, 1H, J=7.9Hz, J=9.6Hz); 5.75 (m, 2H). 7.05-8.05 (m, 30H).

20 ¹³C NMR: 54.66, 62.92, 66.69, 68.13, 69.86, 70.76, 72.49, 72.73, 72.94, 73.13, 74.1, 76.42, 98.94, 100.81, 101.33, 126-140 (42C), 169.27, 164.46, 164.78, 165.13, 165.34.

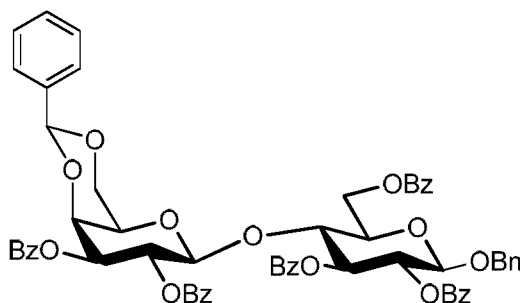
Example 13.



A solution of benzylidene acetal (example 12, 2.0 g, 1.65 mmol) in DCM/MeOH (4/1, 10 mL) and TsOH (126mg) was stirred at 40 °C for 24h. The reaction mixture was cooled to RT and diluted with DCM, washed with NaHCO₃, water and brine. The organic phase was dried over Na₂SO₄ and the solvent was removed in *vacuo*. The white solid was recrystallized from toluene to yield 1.28 g (70 %).

¹H NMR (CDCl₃): 2.95 (m, 1H); 3.40 (m, 3H); 3.85 (m, 1H); 4.15 (m, 2H); 4.40 (dd, 1H, J=5Hz, J=11.9Hz); 4.5-4.8 (m, 5H); 5.15 (dd, 1H; J=3.1Hz, 10.4Hz); 5.40 (dd, 1H, J=7.7Hz, J=9.4Hz); 5.65 (m, 2H), 7.0-8.0 (m, 25H).

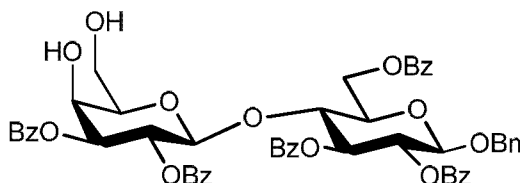
Example 14.



To an ice bath cooled solution of benzylidene-β-benzylactoside (example 9, 6.2 g, 11.9 mmol) in pyridine (40 mL), BzCl (13.8 mL) was added dropwise. The reaction mixture stirred 30 min at this temperature, and overnight at RT. The reaction was quenched with MeOH and the solvent was evaporated in *vacuo*. The residue was dissolved in DCM and washed with water, 1N HCl, water, NaHCO₃, and brine. The organic phase was dried over Na₂SO₄ and the solvent was removed in *vacuo*. The solid obtained was recrystallized from EtOAc/Hexane to yield 9.1g (73 %) of a white pure solid. M.p.: 162-164 °C.

^1H NMR (CDCl_3) δ (ppm): 2.95 (m, 1H); 3.58 (m, 1H); 3.78 (m, 2H); 4.25 (m, 2H); 4.40 (dd, 1H, $J=4.3\text{Hz}$, $J=12.1\text{Hz}$); 4.55 (d, 1H, $J=12.5\text{Hz}$); 4.65 (m, 1H); 4.70 (d, 1H, $J=7.7\text{Hz}$); 4.78 (d, 1H, $J=12.5\text{Hz}$); 4.85 (d, 1H, $J=7.9\text{Hz}$); 5.15 (dd, 1H, $J=3.4\text{Hz}$, $J=10.4\text{Hz}$); 5.30 (s, 1H); 5.40 (dd, 1H, $J=7.8\text{Hz}$, $J=9.2\text{Hz}$); 5.75 (m, 2H). 7.05-8.05 (m, 35H).

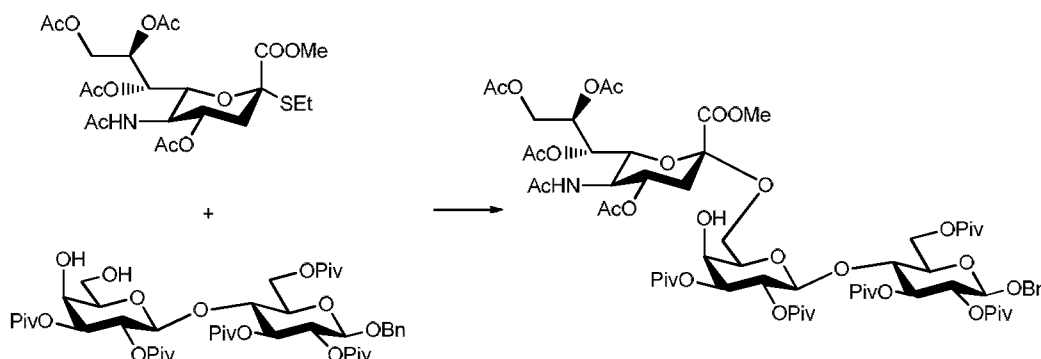
5 Example 15.



To a solution of the pentabenzoyl derivative (example 14, 22.0 g, 21 mmol) in DCM (140 mL) MeOH (20 mL) mixture TsOH monohydrate (1.6g, 0.4eq) was added, and the reaction mixture stirred for 2 days at 40 °C. After this time a saturated solution of NaHCO_3 was added and the mixture stirred for 15 min. The phases were separated and the organic one was washed with water and brine; after drying over Na_2SO_4 . The solvent was evaporated in vacuo and the solid obtained was suspended in EtOAc (200 mL) and the slurry stirred overnight, after filtration and drying 14.5 g (74 %) were obtained. Mp.: 220-222,5 °C.

^1H NMR (CDCl_3): 2.95 (m, 1H); 3.35 (m, 3H); 3.80 (m, 1H); 4.20 (m, 2H); 4.40 (dd, 1H, $J=5\text{Hz}$, $J=11.9\text{Hz}$); 4.5-4.9 (m, 5H); 5.07 (dd, 1H; $J=3.1\text{Hz}$, $J=10.4\text{Hz}$); 5.45 (dd, 1H, $J=7.7\text{Hz}$, $J=9.4\text{Hz}$); 5.70 (m, 2H), 7.0-8.1 (m, 30H).

Example 16.

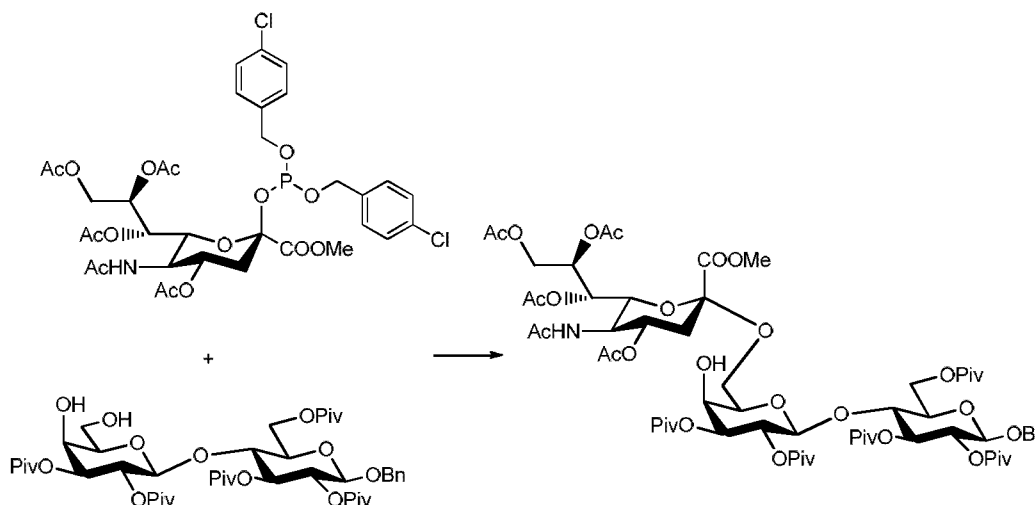


To a -40 °C cooled solution of the mixture donor-acceptor (100 g of the acceptor and 107 g of the donor) in CH₃CN/DCM 1/1 (1200 mL), NIS (78 g) was added and the mixture was stirred for 15 min at this temperature. After this time TfOH (5.1 mL) was added and the reaction was stirred between -40 and -50 °C for 2 h. Et₃N was then added (35 mL) and the solution was diluted with EtOAc (750 mL) and Na₂S₂O₃ (750 mL) was added and the solution was stirred until light yellow colour. The mixture was washed with Na₂S₂O₃ (750 mL) and brine (500 mL). The organic phase was dried over MgSO₄ and evaporated in vacuo. The crude was crystallized in 3L of TBME to give 80 g in two crops.

¹H NMR δ (CD₃OD) in ppm: 1.05-1.3 (m, 45H); 1.75-2.25 (m, 16H); 2.6 (dd, 1H, J=4.5Hz, J=12.7Hz); 3.5-3.75 (m, 8H); 3.9-4.10 (m, 5H), 4.3 (m, 2H); 4.5 (m, 4H); 4.75-5 (m, 4H); 5.2 (m, 3H); 5.3 (m, 2H); 7.25 (m, 5H).

¹³C NMR: 21.02; 21.1; 21.32; 23.09; 23.45; 27.33; 27.38; 27.43; 27.51; 38.90; 39.08; 53.24; 62.34; 62.68; 62.70; 62.72; 66.44; 67.46; 68.87; 69.09; 69.4; 70.56; 71.78; 71.83; 73.00; 73.13; 73.43; 73.54; 73.88; 73.89; 99.26; 99.38; 99.92; 128.17; 128.57; 136.84; 168.09; 170.38; 170.41; 171.00; 171.22; 178.00.

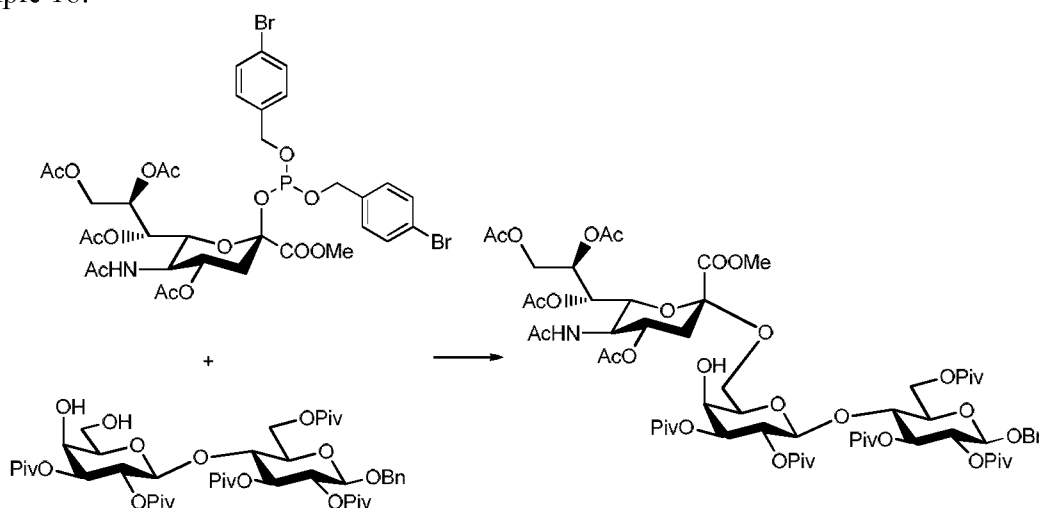
Example 17.



To a -40 °C cooled solution of the mixture donor-acceptor (acceptor: 5.3 g, donor: 8.5 g) in CH₃CN/DCM 1/1 (65 mL) TMSOTf (0.186 mL, 0.16 eq.) in 2 mL of MeCN was added. The reaction mixture was stirred for 3 h between -35- -40 °C and after this time Et₃N was added to neutralize the acid. The solution was diluted with 30 mL EtOAc and the organic phase was

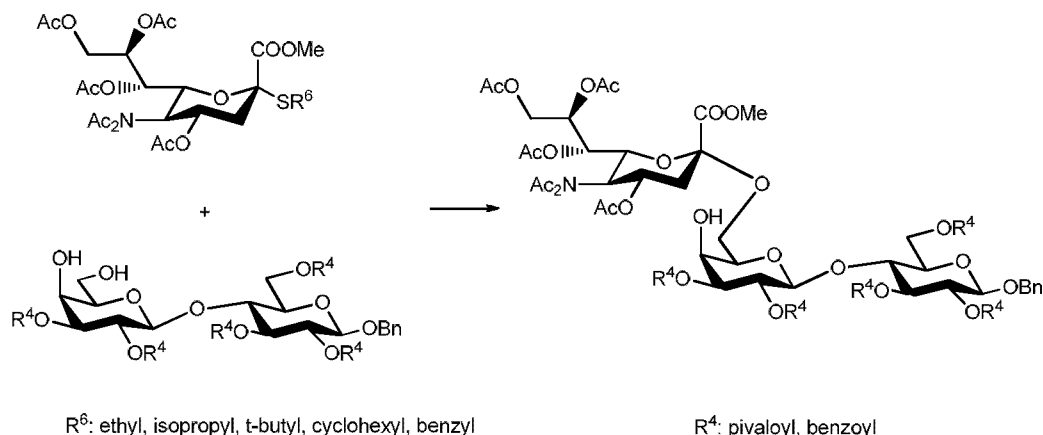
washed with 30 mL of water and 40 mL of brine, the organic phase was dried over Na_2SO_4 and the solvent was evaporated in vacuo. The residue was dissolved in 20 mL $i\text{-BuOAc}$ at 70 °C and 70 mL $i\text{-Pr}_2\text{O}$ were added. The solution crystallized overnight at 4 °C. The crystals were collected by filtration and washed with $i\text{-Pr}_2\text{O}$ to yield 4.3 g of pure product (51 %).

5 Example 18.



To a -40 °C cooled solution of the mixture donor-acceptor (acceptor: 5.3 g, donor: 9.42 g) in $\text{CH}_3\text{CN}/\text{DCM}$ 1/1 (65 mL) TMSOTf (0.186 mL) in 2 mL of MeCN was added. The reaction mixture was stirred for 3 h between -35- -40 °C and after this time Et_3N was added to neutralize the acid. The solution was diluted with 30 mL EtOAc and the organic phase was washed with 30 mL of water and 40 mL of brine, the organic phase was dried over Na_2SO_4 and the solvent was evaporated in vacuo. The residue was dissolved in 20 mL $i\text{-BuOAc}$ at 70 °C and 70 mL $i\text{-Pr}_2\text{O}$ were added. The solution crystallized overnight at 4 °C. The crystals were collected by filtration and washed with $i\text{-Pr}_2\text{O}$ to yield 4.3 g of pure product (51 %).

Example 19.



General procedure: To a -20 °C cooled solution of donor (1.4 eq.) and acceptor (10.5 mmol) in DCM/THF mixture (75 mL + 15 mL) NBS (1.6 eq.) was added followed by TfOH (0.42 eq.). The reaction mixture was stirred for 30 min at this temperature. An aq. solution of 7.5 % $\text{Na}_2\text{S}_2\text{O}_3$ in saturated aq. NaHCO_3 (45 mL) was added to quench, and the biphasic mixture was stirred for 20 min, the phases were separated and the organic one was washed once with water. The product was taken into the acidic deprotection step without further purification.

R^4 : benzoyl

^1H NMR (CDCl_3) δ (ppm): 1.85 (s, 3H); 1.9 (s, 3H); 2-2.2 (m, 13H); 2.48 (dd, 1H; $J=4.8\text{Hz}$, $J=12.9\text{Hz}$); 3.07 (m, 1H); 3.2 (m, 1H); 3.35 (m, 1H); 3.65 (m, 1H); 3.8 (m, 4H); 4.1 (m, 1H); 4.2-4.4 (m, 3H); 4.4-4.6 (m, 3H); 4.65-4.9 (m, 4H); 5.05 (m, 1H); 5.15 (M, 1H); 5.25 (m, 1H); 5.7 (m, 12H); 7-7.6 (m, 20H); 7.8-8.1 (m, 10H).

^{13}C NMR (CDCl_3) δ (ppm): 20.7, 20.8, 20.9, 21.0, 25.8, 27.4, 38.4, 52.7, 56.4, 62.2, 65.1 (2C), 67.0, 68.1, 68.8, 70.1, 70.2, 72.0, 72.3, 72.6, 73.1, 73.3, 74.3, 97.8, 98.5, 101.5, 127.6, 129.6, 132.8-133.4, 136.5, 164.9, 165.2 (2C), 165.6, 166.6, 169.4, 170.3, 170.8, 171.4, 173.4, 174.6.

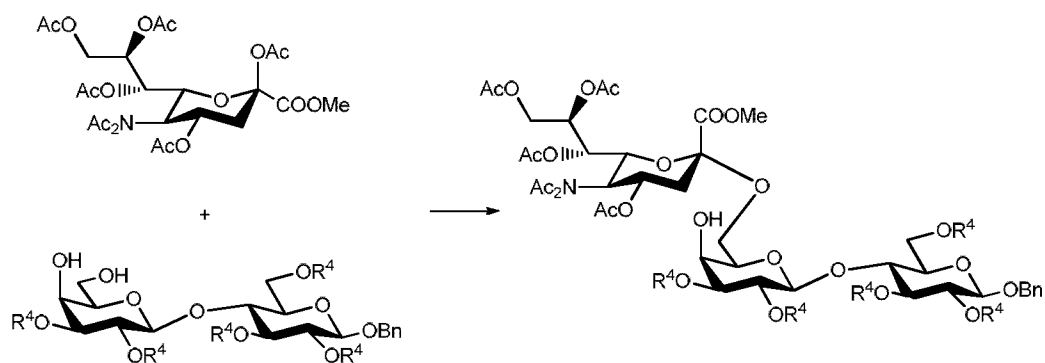
R^4 : pivaloyl

^1H NMR (CDCl_3) δ (ppm): 1.05-1.3 (m, 15H); 1.75 (dd, 1H, $J=10.9\text{Hz}$, $J=12.9\text{Hz}$); 1.95, 2.0, 2.05, 2.15, 2.25 (5s, 18H); 2.64 (dd, 1H, $J=5.5\text{Hz}$, $J=12.9\text{Hz}$); 3.18 (m, 1H); 3.35 (m, 1H); 3.53

(m, 1H); 3.75 (m, 1H); 3.85 (s, 3H); 4.15-4.25 (m, 4H); 4.35-4.45 (m, 2H); 4.45-4.65 (m, 5H); 4.8 (m, 1H); 4.95 (dd, 1H, J=7.9Hz, J=9.7Hz); 5.05 (m, 1H); 5.1-5.3 (m, 5H); 5.7 (m, 1H); 7.5 (m, 5H).

^{13}C NMR (CDCl_3) δ (ppm): 20.99, 21.14, 21.19, 21.23, 27.29, 27.40, 27.42, 38.76, 38.89, 38.90, 39.06, 39.12, 53.04, 54.67, 57.00, 60.39, 62.25, 65.66, 66.97, 68.86, 69.59, 69.82, 70.45, 71.69, 72.00, 72.61, 72.77, 73.40, 73.65, 98.30, 99.25, 100.25, 128.11, 128.16, 128.53, 136.82, 166.92, 169.71, 170.56, 170.80, 171.41, 173.72, 174.71, 176.42, 177.00, 177.12, 179.91.

Example 20.



R^4 : pivaloyl, benzoyl, isobutyryl, 4-methylbenzoyl

To a 4 °C cooled solution of donor (10g, 17.4 mmol) and acceptor (1.4 eq) in DCM (100 mL) $\text{BF}_3 \cdot \text{OEt}_2$ (1.6 mL, 0.75 eq) was added and the reaction stirred at this temperature for 16 h. The reaction was quenched with NaHCO_3 and the biphasic mixture was stirred for 10 min. The organic phase was washed with water and evaporated in *vacuo*. The residue was taken without further purification into the acidic deprotection step.

R^4 : 4-methylbenzoyl

^1H NMR (CDCl_3) δ (ppm): 1.85 (s, 3H); 1.9 (s, 3H); 2-2.2 (m, 13H); 2.25-2.44 (m, 15H); 2.48 (dd, 1H; J=4.8Hz, J=12.9Hz); 3.07 (m, 1H); 3.2 (m, 1H); 3.35 (m, 1H); 3.65 (m, 1H); 3.8 (m, 4H); 4.1 (m, 1H); 4.2-4.4 (m, 3H); 4.4-4.6 (m, 3H); 4.65-4.9 (m, 4H); 5.05 (m, 1H); 5.15 (M, 1H); 5.25 (m, 1H); 5.7 (m, 12H); 6.9 (m, 2H); 7-7.25 (m, 13H); 7.7-7.9 (m, 10H).

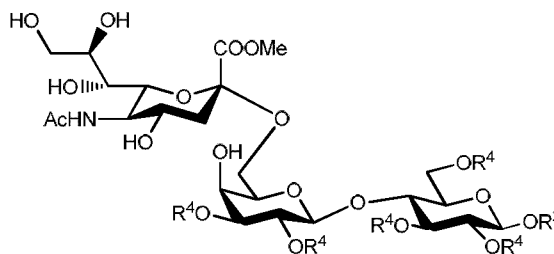
^{13}C NMR (CDCl_3) δ (ppm): 20.7, 20.8, 20.9, 21.0, 21.14, 21.27, 21.62, 21.66, 25.8, 27.4, 38.4, 52.7, 56.4, 62.2, 65.1 (2C), 67.0, 68.1, 68.8, 70.1, 70.2, 72.0, 72.3, 72.6, 73.1, 73.3, 74.3, 97.8, 98.5, 101.5, 127.6, 129.6, 132.8-133.4, 136.5, 164.9, 165.2 (2C), 165.6, 166.6, 169.4, 170.3, 170.8, 171.4, 173.4, 174.6.

5 R^4 : isobutyryl

^1H NMR (CDCl_3) δ (ppm): 1.0 (m, 30H), 1.6 (dd, 1H, $J=1.8\text{Hz}$, $J=2.05\text{Hz}$); 1.80, 1.87, 1.90, 1.97 (4s, 12H); 2.12 (2s, 6H); 2.2-2.6 (m, 6H); 3.15 (m, 1H); 3.25 (m, 1H); 3.4 (m, 1H); 3.55-3.8 (m, 6H); 3.95-4.3 (m, 4H); 4.3-4.55 (m, 5H); 4.67 (d, 1H, $J=12\text{Hz}$); 4.75-5.2 (m, 7H); 7.1 (m, 5H).

10 ^{13}C NMR (CDCl_3) δ (ppm): 18.59, 18.80, 18.86, 18.94, 19.07, 19.12, 19.28, 19.29, 19.32, 20.84, 20.98, 20.99, 21.11, 25.95, 27.98, 33.96, 34.03, 34.07, 50.28, 52.29, 54.67, 56.79, 60.16, 61.93, 62.21, 65.51, 68.73, 69.23, 69.63, 70.51, 71.49, 72.33, 72.39, 72.82, 73.17, 73.23, 75.74, 98.19, 99.10, 101.12, 127.92, 128.04, 128.47, 136.79, 166.78, 169.64, 170.42, 170.75, 171.49, 173.66, 174.83, 175.32, 175.54, 175.64, 175.70, 176.49.

15 Example 21.



R^2 : benzyl, 4-methylbenzyl, 4-chlorobenzyl, 1-naphthylmethyl; R^4 : pivaloyl, benzoyl, 4-methylbenzoyl

The syrup obtained in examples 19 or 20 was diluted with MeOH, cooled to 5 °C and sulfuric acid was added dropwise. The reaction mixture was stirred for 48 h at this temperature and neutralized with Et_3N . MeOH was evaporated and the residue was dissolved in EtOAc, washed with water once and with 5/1 water brine. The organic phase was evaporated in vacuo and the residue was crystallized (yield: 50-60 % for two steps).

R^2 : benzyl, R^4 : benzoyl

¹H (CD₃OD) δ (ppm): 1.5 (dd, 1H, J=11.7Hz, J=12.6Hz); 2.0 (s, 3H); 2.45 (dd, 1H, J=4.6Hz, J=12.8Hz); 3.2 (m, 1H); 3.4-3.7 (m, 7H); 3.7-3.85 (m, 5H); 3.95 (m, 1H); 4.1 (d, 1H, J=3.5Hz); 4.25 (dd, 1H, J=9.2Hz, J=9.5Hz); 4.45-4.65 (m, 3H); 4.75 (d, 1H, J=12.2Hz); 5.15 (dd, 1H, J=3.3Hz, J=10.4Hz); 5.35 (dd, 1H, J=8Hz, J=9.7Hz); 5.64 (dd, 1H, J=7.9Hz, J=10.3Hz); 5.69 (dd, 1H, J=9.2Hz, J=9.4Hz); 7.05-7.65 (m, 20H); 7.8-8.1 (m, 10H).

¹³C (CD₃OD) δ (ppm): 21.62, 39.03, 52.37, 52.44, 60.04, 63.14, 65.25, 67.44, 68.41, 70.50, 70.87, 72.58, 73.24, 73.39, 73.66, 73.97, 74.79, 77.15, 127.73, 128.16, 128.27, 128.37, 128.63, 129.08, 129.41, 129.46, 129.49, 129.54, 129.59, 129.72, 129.81, 129.89, 133.18, 137.18, 165.63, 165.66, 165.95, 166.01, 166.22, 169.35, 173.99.

10 R²: benzyl, R⁴: pivaloyl

¹H NMR (CDCl₃) δ (ppm): 1.0-1.3 (m, 45H); 1.9 (t, J=12.2Hz); 2.05 (s, 3H); 2.72 (m, 2H); 3.15 (m, 1H); 3.45 (m, 1H); 3.55 (m, 3H); 3.7-3.9 (m, 10H); 4.02 (m, 1H); 4.22 (dd, 1H, J=5.5Hz, J=11.5Hz); 4.4-4.6 (m, 4H); 4.72-4.85 (m, 3H); 4.95 (dd, 1H, J=7.9Hz, J=9.6Hz); 5.13 (dd, 1H, J=7.9Hz, J=10.3Hz); 5.18 (t, 1H, 9.4Hz); 6.65 (d, 1H, J=6.5Hz); 7.2-7.35 (m, 5H).

¹³C (CDCl₃) δ (ppm): 23.22, 27.23, 27.29, 27.37, 27.37, 27.44, 27.52, 27.59, 31.20, 38.94, 39.07, 39.12, 53.18, 53.65, 62.32, 63.68, 66.12, 67.77, 68.72, 69.41, 69.95, 70.92, 71.70, 71.79, 73.44, 73.70, 74.03, 99.13, 99.64, 99.83, 128.74, 129.32, 133.90, 135.38, 169.61, 174.25, 176.64, 177.19, 177.57, 177.87, 178.21.

20 R²: benzyl, R⁴: 4-methylbenzoyl

¹H (CD₃OD) δ (ppm): 1.5 (dd, 1H, J=11.7Hz, J=12.6Hz); 2.0 (s, 3H); 2.25 (s, 3H); 2.31-2.43 (m, 12H); 2.48 (dd, 1H, J=4.6Hz, J=12.8Hz); 3.2 (m, 1H); 3.4-3.7 (m, 7H); 3.7-3.85 (m, 5H); 3.90 (m, 1H); 4.1 (d, 1H, J=3.5Hz); 4.24 (dd, 1H, J=9.2Hz, J=9.5Hz); 4.44-4.64 (m, 3H); 4.73 (d, 1H, J=12.2Hz); 5.12 (dd, 1H, J=3.2Hz, J=10.4Hz); 5.32 (dd, 1H, J=8Hz, J=9.6Hz); 5.57-5.70 (m, 2H); 6.87 (m, 2H); 7.05-7.3 (m, 13H); 7.65-7.95 (m, 10H).

¹³C (CD₃OD) δ (ppm): 21.05, 21.14, 21.27, 21.62, 21.66, 39.03, 52.37, 52.44, 60.04, 63.14, 65.25, 67.44, 68.41, 70.50, 70.87, 72.58, 73.24, 73.39, 73.66, 73.97, 74.79, 77.15, 127.73,

128.16, 128.27, 128.37, 128.63, 129.08, 129.41, 129.46, 129.49, 129.54, 129.59, 129.72, 129.81, 129.89, 133.18, 137.18, 165.63, 165.66, 165.95, 166.01, 166.22, 169.35, 173.99.

R²: 4-chlorobenzyl, R⁴: pivaloyl

¹H NMR (CDCl₃) δ (ppm): 1.0-1.3 (m, 45H); 1.9 (t, J=12Hz); 2.05 (s, 3H); 2.64 (d, 1H, J=5.4); 2.75 (dd, 1H, J=3.7Hz, J=12.5Hz); 2.9 (m, 1H); 3.3 (m, 1H); 3.45 (m, 1H); 3.55 (m, 2H); 3.65-3.95 (m, 10H); 4.02 (m, 1H); 4.21 (dd, 1H, J=5.3Hz, J=11.7Hz); 4.4-4.6 (m, 4H); 4.67-4.85 (m, 2H); 4.95 (dd, 1H, J=8.3Hz, J=9.4Hz); 5.15 (m, 2H); 6.48 (d, 1H, J=7.5Hz); 7.18 (m, 2H); 7.28 (m, 2H).

¹³C (CDCl₃) δ (ppm): 23.22, 27.28, 27.30, 27.37, 27.43, 27.52, 27.58, 31.19, 38.93, 39.07, 39.12, 53.18, 53.65, 62.32, 63.66, 66.15, 67.81, 68.77, 69.50, 70.02, 70.99, 71.67, 71.81, 73.45, 73.59, 73.98, 99.09, 99.69, 100.01, 128.53, 129.11, 133.75, 135.19, 169.54, 174.21, 176.59, 177.08, 177.42, 177.57, 178.17.

R²: 1-naphthylmethyl, R⁴: pivaloyl

¹H NMR (CDCl₃) δ (ppm): 1.0-1.4 (m, 45H); 1.85 (t, J=12.2Hz); 2.05 (s, 3H); 2.69 (dd, 1H, J=3.7Hz, J=12.5Hz); 2.8 (m, 1H); 3.45 (m, 1H); 3.55 (m, 3H); 3.7-3.95 (m, 10H); 4.05 (m, 1H); 4.22 (dd, 1H, J=5.6Hz, J=11.8Hz); 4.44 (d, 1H, J=7.8Hz); 4.54 (m, 1H); 4.61 (d, 1H, J=7.9Hz); 4.78 (dd, 1H, J=3.2Hz, J=10.4Hz); 4.95 (m, 2H); 5.12 (m, 2H); 5.3 (d, 1H, J=11.7Hz); 7.35-7.5 (m, 4H); 7.8 (m, 2H); 8.0 (m, 1H).

¹³C (CDCl₃) δ (ppm): 22.96, 27.12, 27.27, 27.32, 27.37, 27.47, 31.15, 38.75, 38.83, 38.87, 39.00, 39.12, 53.62, 54.67, 55.07, 61.43, 62.27, 63.77, 65.94, 66.81, 68.61, 68.99, 69.41, 70.94, 71.65, 71.91, 73.32, 73.52, 74.31, 99.25, 99.36, 99.93, 123.92, 125.31, 126.04, 126.62, 127.06, 128.72, 129.16, 131.80, 132.26, 132.26, 133.77, 169.77, 174.51, 176.41, 176.91, 177.38, 177.76, 178.07.

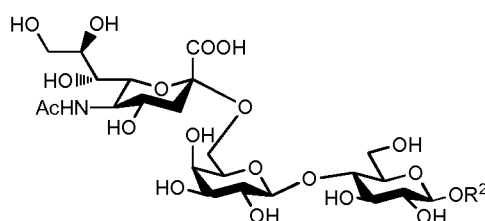
R²: 4-methylbenzyl, R⁴: pivaloyl

¹H NMR (CDCl₃) δ (ppm): 1.0-1.3 (m, 45H); 1.9 (t, J=12.3Hz); 2.05 (s, 3H); 2.25 (s, 3H); 2.65 (m, 1H); 2.83 (m, 1H); 3.35 (m, 1H); 3.45 (m, 1H); 3.55 (m, 3H); 3.6-3.85 (m, 12H);

3.95 (m, 1H); 4.15 (m, 1H); 4.32-4.5 (m, 3H); 4.63-4.8 (m, 2H); 4.85 (dd, 1H, $J=8\text{Hz}$, $J=9.4\text{Hz}$); 5.08-5.15 (m, 2H); 7.05 (m, 5H).

^{13}C (CDCl_3) δ (ppm): 21.10, 22.93, 27.02, 27.03, 27.09, 27.17, 38.64, 38.78, 38.84, 53.36, 54.89, 61.33, 62.32, 63.34, 65.87, 67.38, 68.38, 68.60, 69.16, 70.18, 70.65, 71.44, 71.69, 73.18, 73.33, 73.77, 98.71, 98.88, 99.63, 128.12, 128.99, 133.23, 137.75, 169.33, 173.98, 176.32, 176.98, 177.28, 177.56, 177.95.

Example 22.



Method A: To a solution of a protected or partially protected trisaccharide according to any of the examples 16-21 in 5 volumes of MeOH, 1M aqueous solution of NaOH was added slowly. The reaction mixture stirred overnight at RT and Amberlite IR 120 H^+ was added to neutralize the base. The mixture was filtered and the solvent was evaporated in vacuo. The material was then dissolved in MeOH and EtOH, and TBME was added slowly. The precipitate was filtered off and washed twice with TBME. The white solid obtained was dissolved in 1M NaOH and the reaction mixture was stirred for 6 h. IR-120 H^+ was used to neutralize the base and the solvent was evaporated in vacuo. After coevaporation with EtOH (twice) the product was dissolved in MeOH and i-PrOH was added slowly to give a solid in free acid form.

Method B: To a solution of a protected or partially protected trisaccharide according to any of the examples 16-21 in MeOH NaOMe was added and the reaction mixture was stirred for 5 h at 45 °C. The cooled solution was washed with heptane and acetone was added. The precipitate was filtered off and dissolved in 1M NaOH and the reaction mixture was stirred for 6 h. IR-120 H^+ was used to neutralize the base and the solvent was evaporated in vacuo. After coevaporation with EtOH (twice) the product was dissolved in MeOH and i-PrOH was added slowly to give a solid in free acid form.

R²: benzyl

¹H (CD₃OD) δ (ppm): 1.63 (t, 1H, J=11.9Hz); 2.00 (s, 3H); 2.78 (dd, 1H, J=4.5Hz, J=12.2Hz); 3.28-3.49 (m, 4H); 3.50-3.79 (m, 9H); 3.80-3.97 (m, 5H); 4.02 (dd, 1H, J=7.5Hz, J=10Hz); 4.35 (m, 1H); 4.42 (d, 1H, J=7.8Hz); 4.66 (d, 1H, J=11.7Hz); 7.22-7.37 (m, 3H);
5 7.38-7.46 (m, 2H).

¹³C (CD₃OD) δ (ppm): 21.55, 41.26, 52.70, 60.81, 63.21, 63.42, 68.56, 69.01, 69.30, 70.66, 71.15, 72.12, 73.08, 73.55, 73.78, 74.47, 75.28, 75.32, 80.03, 100.29, 101.89, 103.36, 127.36, 127.87, 128.01, 128.12, 137.72, 173.36, 173.88.

R²: 4-chlorobenzyl

10 ¹H (CD₃OD) δ (ppm): 1.62 (t, 1H, J=12Hz); 2.00 (s, 3H); 2.77 (dd, 1H, J=4.7Hz, J=12.1Hz); 3.28-3.49 (m, 4H); 3.50-3.79 (m, 9H); 3.80-3.97 (m, 5H); 4.02 (dd, 1H, J=7.5Hz, J=10Hz); 4.35 (m, 1H); 4.42 (d, 1H, J=7.8Hz); 4.66 (d, 1H, J=11.7Hz); 7.24 (m, 2H); 7.32 (m, 2H).

¹³C (CD₃OD) δ (ppm): 21.52, 41.23, 52.72, 60.79, 63.23, 63.42, 68.61, 69.03, 69.31, 70.56, 71.14, 72.13, 73.11, 73.56, 73.79, 74.48, 75.23, 75.31, 80.09, 100.17, 101.91, 103.21, 127.36,
15 127.89, 133.01, 136.76, 173.29, 173.78.

R²: 1-naphthylmethyl

¹H (CD₃OD) δ (ppm): 1.62 (t, 1H, J=11.8Hz); 2.00 (s, 3H); 2.78 (dd, 1H, J=4.5Hz, J=12.2Hz); 3.28-3.49 (m, 4H); 3.50-3.79 (m, 9H); 3.80-3.97 (m, 5H); 4.02 (dd, 1H, J=7.6Hz, J=10.1Hz); 4.35 (m, 1H); 4.42 (d, 1H, J=7.8Hz); 4.68 (d, 1H, J=11.8Hz); 7.32-7.54 (m, 4H);
20 7.7 (m, 2H); 7.95 (m, 1H).

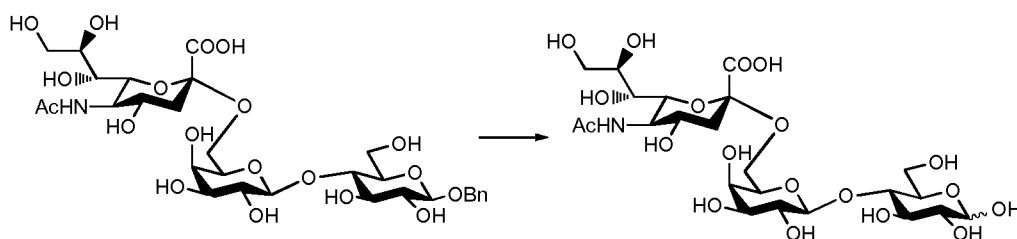
¹³C (CD₃OD) δ (ppm): 21.54, 41.28, 52.72, 60.81, 63.20, 63.44, 68.53, 69.06, 69.38, 70.64, 71.12, 72.14, 73.05, 73.54, 73.65, 74.45, 75.24, 75.33, 80.02, 100.25, 101.87, 103.21, 122.91, 124.82, 125.25, 125.93, 127.06, 128.72, 129.16, 132.80, 133.12, 135.97, 136.88, 173.36, 173.88.

25 R²: 4-methylbenzyl

^1H (CD_3OD) δ (ppm): 1.63 (t, 1H, $J=11.9\text{Hz}$); 2.00 (s, 3H); 2.32 (s, 3H); 2.78 (dd, 1H, $J=4.5\text{Hz}$, $J=12.2\text{Hz}$); 3.28-3.49 (m, 4H); 3.50-3.79 (m, 9H); 3.80-3.97 (m, 5H); 4.02 (dd, 1H, $J=7.5\text{Hz}$, $J=10\text{Hz}$); 4.35 (m, 1H); 4.42 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.7\text{Hz}$); 7.21 (m, 4H).

5 ^{13}C (CD_3OD) δ (ppm): 20.02, 21.55, 41.26, 52.70, 60.81, 63.21, 63.42, 68.56, 69.01, 69.30, 70.66, 71.15, 72.12, 73.08, 73.55, 73.78, 74.47, 75.28, 75.32, 80.03, 99.98, 100.89, 102.36, 127.36, 127.87, 128.01, 128.12, 133.26, 137.72, 173.36, 173.88

Example 23.



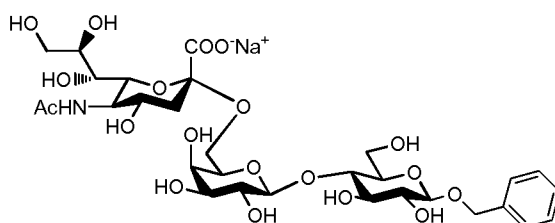
10 To a solution of 40 g of free acid in a mixture of methanol and water (250 mL + 300 mL) 4 g of Pd/C (10%) were added. The reaction mixture was stirred 2 d at RT under H_2 pressure (balloon). The mixture was then filtered through a pad of Celite and the solvent was evaporated in vacuo. The residue was dissolved in 80 mL of H_2O and dropped to 1200 mL of EtOH. The slurry was filtrated, the solid was washed with EtOH, acetone and a mixture of
15 1/1 acetone/ Et_2O . The solid was dried to give 35 g of 6'-O-sialyllactose.

^1H (D_2O) (anomeric mixture of glucose 0.6/0.4 β/α): 1.75 (dd, 1H, $J=12.0\text{Hz}$, $J=11.9\text{Hz}$); 2.05 (s, 3H); 2.7 (dd, 1H, $J=12.0\text{Hz}$, $J=4.6\text{Hz}$); 3.31 (dd, 0.6H, $J=7.8\text{Hz}$, $J=8.9\text{Hz}$); 3.5-3.75 (m, 11.4H), 3.76-4.05 (m, 8.9H); 4.43 (d, 1H, $J=7.8\text{Hz}$); 4.67 (d, 0.6H, $J=7.8\text{Hz}$), 5.23 (d, 0.4H, $J=3.8\text{Hz}$).

20 ^{13}C : 19.51, 24.78, 42.82, 54.51, 60.15, 62.83, 62.99, 65.36, 66.3, 71.1, 71.24, 72.68, 73.51, 73.78, 74.35, 74.53, 75.09, 75.24, 76.42, 76.45, 77.35, 77.39, 82.35, 82.46, 94.54, 98.37, 103.01, 105.92, 105.95, 176.21, 177.64.

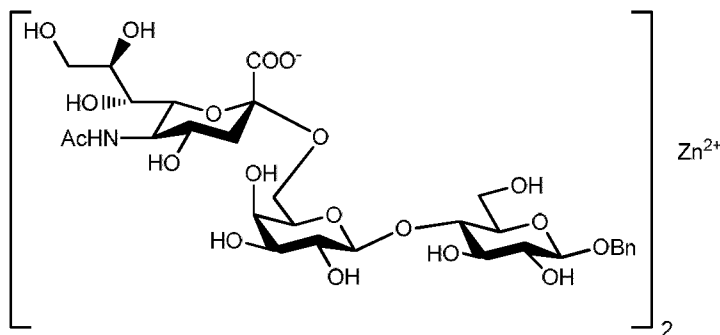
Example 24: salt formation

Inorganic and organic salts of 6'-SL or 6'-SL glycosides were obtained from acidic 6'-SL or 6'-SL glycosides. The pH of the acid in alcohol or alcohol/water was adjusted to 8,5-11 with an organic base selected from ethanolamine, diethylamine, tris-(hydroxymethyl)-methylamine base and choline hydroxide or with an inorganic base selected from metal hydroxide, carbonate and bicarbonate. The mixture was then diluted with alcohol and concentrated in vacuo. The slurry obtained was then filtered and washed with alcohol.



¹H (CD₃OD) δ (ppm): 1.63 (t, 1H, J=11.9Hz); 2.02 (s, 3H); 2.82 (m, 1H); 3.33-3.45 (m, 4H); 3.46-3.61 (m, 5H); 3.62-3.97 (m, 12H); 4.02 (dd, 1H, J=7.5Hz, J=10Hz); 4.37 (m, 2H); 4.66 (d, 1H, J=11.9Hz); 7.31 (m, 3H); 7.41 (m, 2H).

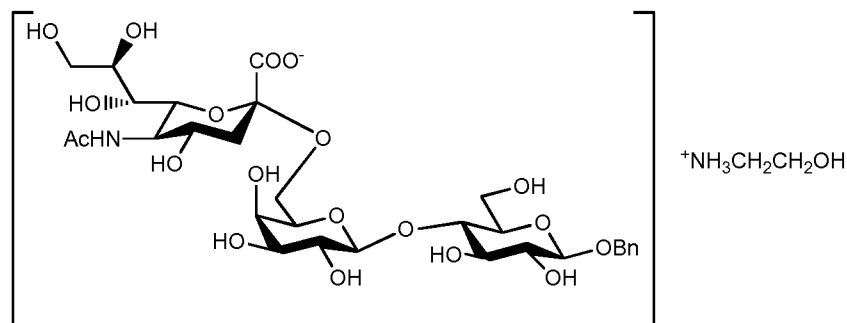
¹³C (CD₃OD) δ (ppm): 21.10, 40.60, 52.04, 60.22, 62.78, 67.90, 68.51, 68.69, 70.11, 70.61, 70.64, 71.44, 72.44, 72.83, 73.17, 73.92, 74.50, 74.70, 79.82, 99.76, 101.23, 103.52, 127.00, 127.44, 127.56, 137.21, 172.68, 173.28.



¹H (CD₃OD) δ (ppm): 1.71 (t, 1H, J=11.1Hz); 2.00 (s, 3H); 2.78 (dd, 1H, J=4.5Hz, J=12.2Hz); 3.28-3.49 (m, 4H); 3.50-3.79 (m, 9H); 3.80-3.97 (m, 5H); 4.02 (dd, 1H, J=7.5Hz,

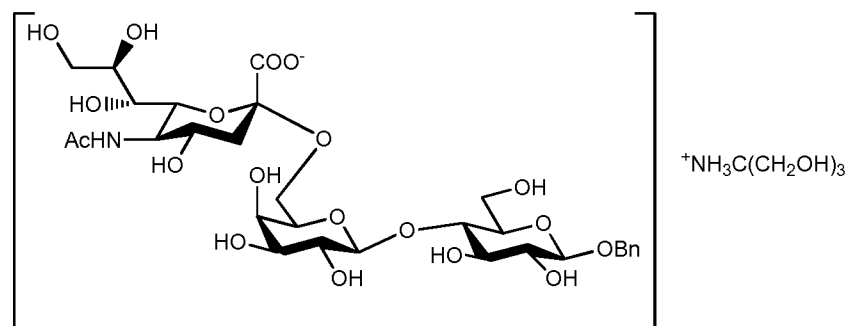
$J=10\text{Hz}$); 4.35 (m, 1H); 4.42 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.7\text{Hz}$); 7.22-7.37 (m, 3H); 7.38-7.46 (m, 2H).

^{13}C (CD_3OD) δ (ppm): 21.77, 41.09, 52.78, 60.87, 61.32, 63.06, 63.64, 63.21, 68.26, 69.11, 70.67, 71.22, 71.36, 72.01, 73.00, 73.37, 73.58, 73.66, 74.39, 75.19, 75.32, 75.87, 79.40,
5 80.35, 99.92, 101.86, 101.94, 103.86, 104.00, 127.55, 127.99, 128.13, 137.83, 174.03, 174.08



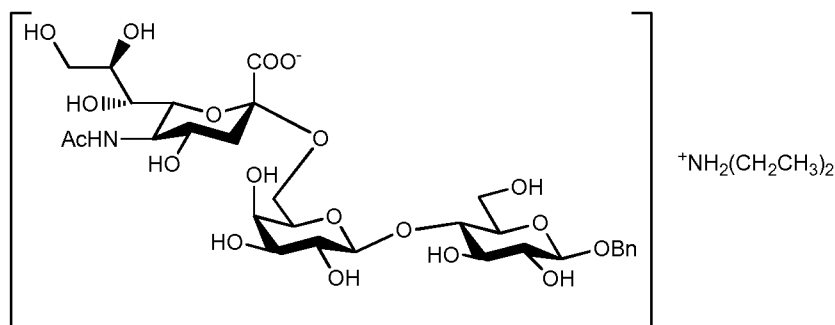
^1H (CD_3OD) δ (ppm): 1.63 (dd, 1H, $J=11.7\text{Hz}$, $J=12.2\text{Hz}$); 2.00 (s, 3H); 2.78 (dd, 1H, $J=4.5\text{Hz}$, $J=12.2\text{Hz}$); 2.92 (m, 4H); 3.33-3.47 (m, 2H); 3.46-3.57 (m, 5H); 3.58-3.78 (m, 9H); 3.78-3.90 (m, 5H); 3.93 (dd, 1H, $J=2.5$, $J=11.7\text{Hz}$); 4.02 (dd, 1H, $J=7.5\text{Hz}$, $J=10\text{Hz}$); 4.35 (m, 1H); 4.42 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.7\text{Hz}$); 7.22-7.37 (m, 3H); 7.38-7.46 (m, 2H).
10

^{13}C (CD_3OD) δ (ppm): 21.55, 41.26, 44.83, 52.70, 58.85, 60.81, 63.21, 63.42, 68.56, 69.01, 69.30, 70.66, 71.15, 72.12, 73.08, 73.55, 73.78, 74.47, 75.28, 75.32, 80.03, 99.98, 100.89, 102.36, 127.36, 127.87, 128.01, 128.12, 137.72, 173.36, 173.88



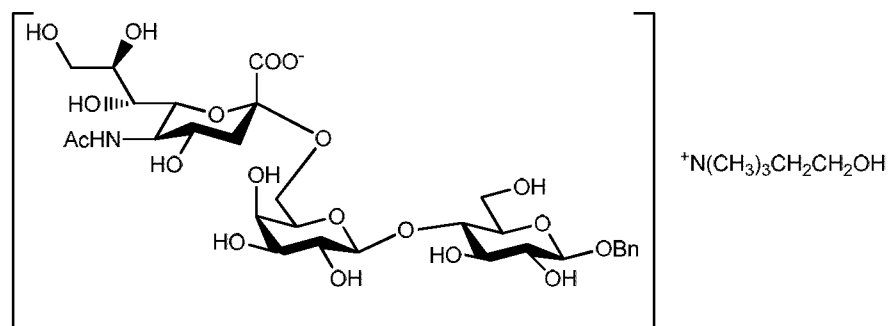
15 ^1H (CD_3OD) δ (ppm): 1.63 (dd, 1H, $J=11.9\text{Hz}$, $J=12.1\text{Hz}$); 2.00 (s, 3H); 2.78 (dd, 1H, $J=4.5\text{Hz}$, $J=12.2\text{Hz}$); 3.33-3.48 (m, 2H); 3.48-3.79 (m, 19H); 3.93 (dd, 1H, $J=2.5$, $J=11.8\text{Hz}$); 4.02 (dd, 1H, $J=7.5\text{Hz}$, $J=10\text{Hz}$); 4.35 (m, 1H); 4.42 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.7\text{Hz}$); 7.22-7.37 (m, 3H); 7.38-7.46 (m, 2H).

^{13}C (CD_3OD) δ (ppm): 21.60, 41.22, 52.71, 59.62, 60.89, 61.38, 63.23, 63.39, 68.64, 68.97, 69.25, 70.64, 71.26, 73.68, 75.24, 75.27, 80.16, 100.39, 101.87, 103.94, 127.52, 127.97, 128.00, 128.11, 137.85, 173.40, 173.90



5 ^1H (CD_3OD) δ (ppm): 1.28 (t, 6H, $J=11.8\text{Hz}$); 1.65 (dd, 1H, $J=11.9\text{Hz}$, $J=12.1\text{Hz}$); 2.00 (s, 3H); 2.79 (dd, 1H, $J=4.5\text{Hz}$, $J=12.2\text{Hz}$); 3.00 (q, 4H); 3.33-3.57 (m, 7H); 3.57-3.77 (m, 5H); 3.78-3.89 (m, 4H); 3.93 (dd, 1H, $J=11.9\text{Hz}$, $J=2.4\text{Hz}$); 4.00 (dd, 1H, $J=7.1\text{Hz}$, $J=9.7\text{Hz}$); 4.35 (m, 1H); 4.42 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.8\text{Hz}$); 7.22-7.37 (m, 3H); 7.38-7.46 (m, 2H).

10 ^{13}C (CD_3OD) δ (ppm): 10.64, 21.57, 41.29, 42.29, 52.74, 60.89, 63.23, 63.43, 68.63, 68.95, 69.30, 70.62, 71.25, 72.04, 73.06, 73.41, 73.73, 74.47, 75.26, 75.28, 80.15, 100.41, 101.90, 103.95, 127.51, 127.97, 127.99, 128.10, 137.88, 173.35, 173.85



15 ^1H (CD_3OD) δ (ppm): 1.63 (dd, 1H, $J=11.9\text{Hz}$, $J=12.1\text{Hz}$); 1.98 (s, 3H); 2.80 (dd, 1H, $J=4.5\text{Hz}$, $J=12.2\text{Hz}$); 3.19 (s, 9H); 3.33-3.56 (m, 8H); 3.56-3.77 (m, 7H); 3.78-3.94 (m, 5H); 3.95-4.04 (m, 4H); 4.34 (m, 1H); 4.39 (d, 1H, $J=7.8\text{Hz}$); 4.66 (d, 1H, $J=11.8\text{Hz}$); 7.22-7.36 (m, 3H); 7.40 (m, 2H).

^{13}C (CD_3OD) δ (ppm): 21.56, 40.95, 41.34, 52.75, 53.48, 53.53, 53.59, 55.92, 60.96, 63.15, 63.50, 63.84, 67.85, 68.65, 68.90, 69.31, 69.47, 70.61, 71.28, 72.05, 73.05, 73.41, 73.72, 74.45, 75.29, 76.89, 80.17, 100.42, 101.92, 103.97, 109.98, 127.52, 127.97, 128.11, 137.90, 138.29, 173.26, 173.85

5 6'-SL Na^+ salt

^1H (D_2O) δ (ppm): 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 3.10 (m, 1H); 3.27-3.55 (m, 10H); 3.56-3.82 (m, 9H); 4.23 (d, 1H, $J=7.8\text{Hz}$); 4.48 (d, 0.7H, $J=8\text{Hz}$); 5.03 (d, 0.3H, $J=3.7\text{Hz}$).

10 ^{13}C (D_2O) δ (ppm): 22.17, 40.19, 51.87, 52.10, 60.38, 62.71, 63.68, 68.46, 68.61, 70.87, 71.90, 72.44, 72.60, 73.78, 74.04, 74.68, 74.76, 79.80, 79.86, 96.16, 100.36, 103.33, 173.62, 174.99.

6'-SL Zn^{2+} salt

15 ^1H (D_2O) δ (ppm): 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 3.10 (m, 1H); 3.27-3.55 (m, 10H); 3.56-3.82 (m, 9H); 4.23 (d, 1H, $J=7.8\text{Hz}$); 4.48 (d, 0.7H, $J=8\text{Hz}$); 5.03 (d, 0.3H, $J=3.7\text{Hz}$).

^{13}C (D_2O) δ (ppm): 22.16, 40.16, 51.87, 60.15, 60.31, 62.69, 63.67, 68.45, 68.60, 70.02, 70.86, 71.12, 71.72, 71.88, 72.43, 72.59, 73.77, 74.71, 74.73, 79.69, 91.90, 95.72, 100.34, 103.31, 173.63, 174.99.

6'-SL ethanolammonium salt

20 ^1H (D_2O) δ (ppm): 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 2.92 (m, 2H); 3.10 (m, 1H); 3.27-3.55 (m, 10H); 3.56-3.82 (m, 11H); 4.23 (d, 1H, $J=7.8\text{Hz}$); 4.48 (d, 0.7H, $J=8\text{Hz}$); 5.03 (d, 0.3H, $J=3.7\text{Hz}$).

25 ^{13}C (D_2O) δ (ppm): 22.16, 40.19, 41.37, 51.87, 52.09, 57.93, 60.32, 62.70, 63.67, 68.46, 68.62, 70.03, 70.87, 71.13, 71.91, 72.43, 72.60, 73.80, 74.71, 74.74, 79.69, 79.80, 95.73, 100.35, 103.32, 173.61, 174.98.

6'-SL tris-(hydroxymethyl)-methyl ammonium salt

^1H (D_2O) δ (ppm): 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 3.10 (m, 1H); 3.56-3.82 (m, 9H); 4.24 (d, 1H, $J=7.8\text{Hz}$); 4.47 (d, 0.7H, $J=8\text{Hz}$); 5.02 (d, 0.3H, $J=3.7\text{Hz}$).

5 ^{13}C (D_2O) δ (ppm): 22.15, 40.19, 51.87, 59.90, 60.83, 62.70, 63.67, 68.46, 68.61, 70.03, 70.87, 71.13, 71.72, 71.90, 72.43, 72.60, 73.79, 74.71, 74.74, 79.70, 79.81, 95.73, 100.35, 103.32, 173.60, 174.98.

6'-SL diethyl ammonium salt

10 ^1H (D_2O) δ (ppm): 1.05 (t, 6H, $J=7.3\text{Hz}$); 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 2.84 (q, 4H, $J=7.3\text{Hz}$); 3.10 (m, 1H); 3.27-3.55 (m, 10H); 3.56-3.82 (m, 9H); 4.23 (d, 1H, $J=7.8\text{Hz}$); 4.48 (d, 0.7H, $J=8\text{Hz}$); 5.03 (d, 0.3H, $J=3.7\text{Hz}$).

^{13}C (D_2O) δ (ppm): 10.67, 22.13, 40.18, 42.35, 51.85, 52.09, 60.15, 60.31, 62.68, 63.67, 68.45, 68.58, 70.01, 70.84, 71.71, 71.88, 72.41, 72.58, 73.78, 74.72, 79.69, 79.80, 91.89, 95.72, 100.33, 103.31, 173.59, 174.95.

6'-SL choline salt

15 ^1H (D_2O) δ (ppm): 1.54 (t, 1H, $J=12.1\text{Hz}$); 1.82 (s, 3H); 2.52 (dd, 1H, $J=4.8\text{Hz}$, $J=12.3\text{Hz}$); 2.99, (s, 9H); 3.10 (m, 1H); 3.27-3.55 (m, 12H); 3.56-3.82 (m, 11H); 4.23 (d, 1H, $J=7.8\text{Hz}$); 4.48 (d, 0.7H, $J=8\text{Hz}$); 5.03 (d, 0.3H, $J=3.7\text{Hz}$).

20 ^{13}C (D_2O) δ (ppm): 22.14, 40.17, 51.85, 52.10, 53.87, 53.92, 53.97, 55.67, 60.31, 62.69, 63.67, 67.42, 67.46, 67.50, 68.45, 68.58, 70.85, 71.11, 71.88, 72.41, 72.58, 73.77, 74.72, 79.68, 79.80, 91.89, 95.72, 100.33, 103.31, 173.59, 174.95.

Example 25.

6'-SL K^+ salt

1g of 6'-SL was dissolved in 3 mL of water and passed through a cationic ion-exchange column (Amberlite IRC50 K^+ form). The fractions (2-7) were collected and lyophilized.

Example 26.

6'-SL Ca^{2+} salt

1g of 6'-SL was dissolved in 3 mL of water and passed through a cationic ion exchange column (Amberlite IRC50 Ca^{2+} form). The fractions (2-7) were collected and lyophilized.

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Abbreviations list

CMP-Neu5Ac cytidine 5'-monophospho-*N*-acetylneuraminic acid

DCM Dichloromethane

DMF *N,N'*-Dimethylformamide

5 DTPI 2,6-di-*tert*-butylpyridinium iodide

GlcNAc *N*-acetyl-glucosamine

HMOs Human Milk Oligosaccharides

6'-SL 6'-*O*-sialyllactose, Neu5Ac(α 2-6)Gal(β 1-4)Glc

NBS *N*-Bromosuccinimide

10 NeuAc Neuraminic acid

NIS *N*-Iodosuccinimide

RT Room temperature

THF tetrahydrofuran

TfOH Triflic acid

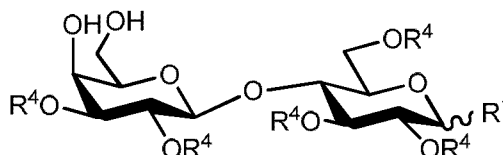
15 TMSOTf trimethylsilyl triflate

Tol toluene

CLAIMS

1. A method for the preparation of 6'-*O*-sialyllactose or a salt thereof, comprising the steps of:

5 a) reaction of an acceptor of general formula 3

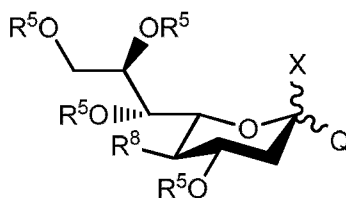


general formula 3

wherein R^1 is $-OR^2$, which R^2 is a group removable by catalytic hydrogenation, or R^1 is $-SR^3$, which R^3 is selected from optionally substituted alkyl, optionally substituted aryl and optionally substituted benzyl, or R^1 is $-NH-C(R'')=C(R')_2$,
 10 wherein each R' independently of each other is an electron withdrawing group selected from $-CN$, $-COOH$, $-COO$ -alkyl, $-CO$ -alkyl, $-CONH_2$, $-CONH$ -alkyl and $-CON(alkyl)_2$, or wherein the two R' -groups is linked together and represent $-CO-(CH_2)_{2-4}-CO-$ and thus form with the carbon atom to which they are attached a 5-7
 15 membered cycloalkan-1,3-dion, in which dion any of the methylene groups is optionally substituted with 1 or 2 alkyl groups, and R'' is H or alkyl,

R^4 is selected from optionally substituted acyl and benzyl substituted by 1-3 methoxy group(s),

with a donor of general formula 4



general formula 4

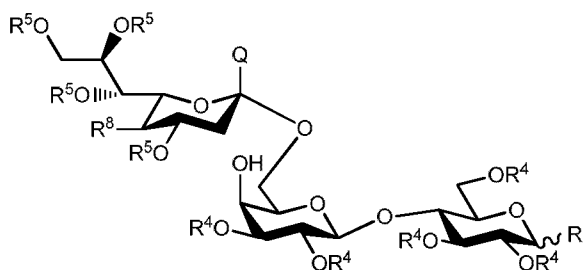
wherein R^5 is optionally substituted acyl,

R^8 is selected from $-NHAc$ and $-NAc_2$,

Q is COO -alkyl, which alkyl is optionally substituted, and

X is leaving group,

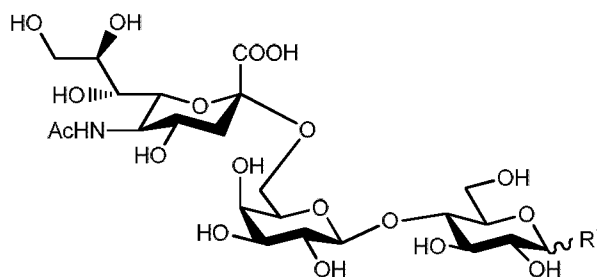
5 to yield a compound of general formula 2



general formula 2

wherein R^1 , R^4 , R^5 , R^8 and Q are as defined above,

b) deprotection of a compound of general formula 2 to give a compound of general formula 1 or salts thereof



general formula 1

wherein R^1 is as defined above, and

c) subsequently converting the compound of general formula 1 or salts thereof into 6'-*O*-sialyllactose or salts thereof.

2. The method according to claim 1, wherein a compound of general formula 1 or salt thereof

15 is subjected to

a) catalytic reduction when R^1 is $-OR^2$, wherein R^2 is a group removable by catalytic hydrogenation, or

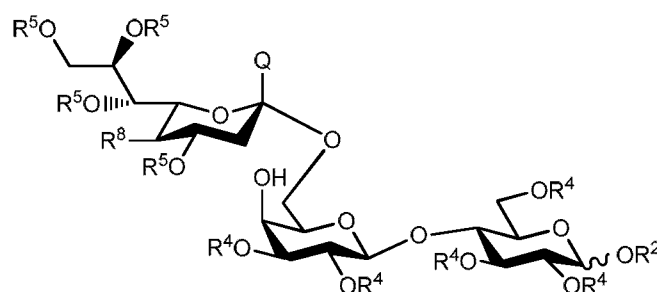
b) activation with a thiophilic reagent followed by hydrolysis when R^1 is $-SR^3$, wherein R^3 is selected from optionally substituted alkyl, optionally substituted aryl and optionally substituted benzyl, or

c) treatment with an amine or halogen followed by hydrolysis at neutral or mild acidic pH when R^1 is $-NH-C(R'')=C(R')_2$, wherein each R' is an electron withdrawing group selected from $-CN$, $-COOH$, $-COO$ -alkyl, $-CO$ -alkyl, $-CONH_2$, $-CONH$ -alkyl and $-CON(alkyl)_2$, or wherein the two R' -groups is linked together and represent $-CO-(CH_2)_{2-4}-CO-$ and thus form with the carbon atom to which they are attached a 5-7 membered cycloalkan-1,3-dione, in which dione any of the methylene groups is optionally substituted with 1-2 alkyl groups, and R'' is H or alkyl,

and when 6'-*O*-sialyllactose is obtained, it is optionally converted to a salt thereof, which salt of 6'-*O*-sialyllactose is optionally converted to another salt of 6'-*O*-sialyllactose.

3. The method according to claim 1 or 2, wherein R^1 is $-OR^2$, which R^2 is a group removable by catalytic hydrogenation, preferably selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl.

4. The method according to any of the claims 1-3, wherein a compound of general formula **2A** belonging to compounds of general formula **2** mentioned in claim 1



general formula **2A**

wherein R² is a group removable by catalytic hydrogenation,

R^4 and R^5 are independently from each other optionally substituted acyl,

R^8 is selected from $-\text{NHAc}$ or $-\text{NAc}_2$, and

Q is $-\text{COO-alkyl}$, which alkyl is optionally substituted,

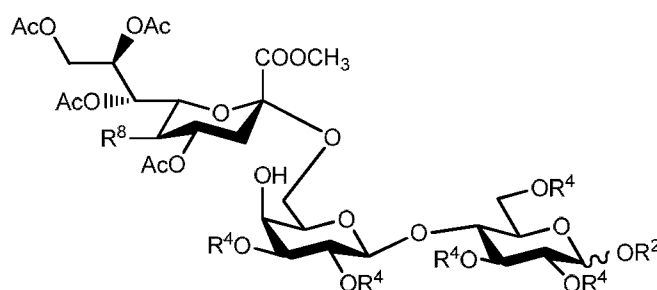
is deprotected comprising the steps of:

- 5 a) base catalyzed transesterification reaction followed by a basic hydrolysis, optional acidification and optional salt formation, or
- b) basic hydrolysis, optional acidification and optional salt formation

to give a compound of general formula **1** or salts thereof.

5. The method according to claim 4, wherein R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably pivaloyl and benzoyl, R^5 is acetyl, Q is $-\text{COOCH}_3$ and $-\text{OR}^2$ is in β .

6. The method according to any of the claims 1-3, wherein a compound of general formula **2B** belonging to compounds of general formula **2** mentioned in claim 1



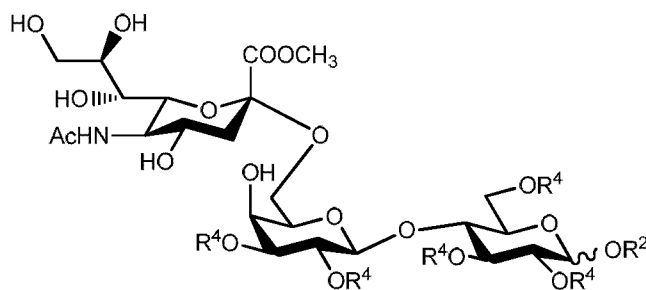
general formula **2B**

wherein R^2 is a group removable by catalytic hydrogenation,

R^4 is optionally substituted acyl provided that acetyl is excluded, and

R^8 is selected from $-\text{NHAc}$ and $-\text{NAc}_2$,

is subjected to selective acidic deprotection to give a compound of general formula **2C**

general formula **2C**

wherein R^2 and R^4 are as defined above,

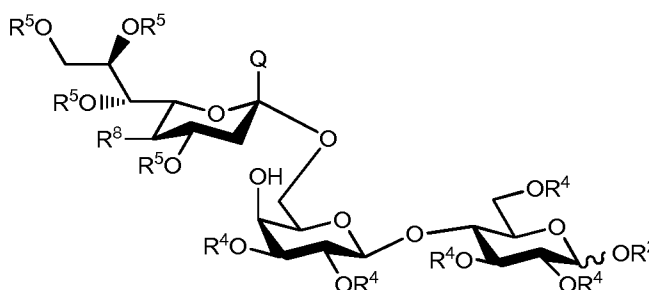
followed by

- a) base catalyzed transesterification reaction followed by basic hydrolysis,
optional acidification and optional salt formation, or
- b) basic hydrolysis, optional acidification and optional salt formation

to give a compound of general formula **1** or salts thereof.

7. The method according to claim 6, wherein the selective acidic deprotection is carried out in the presence of sulphuric acid on a compound of general formula **2B**, wherein $-OR^2$ is in β , R^2 is benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably benzoyl.

8. The method according to any of the claims 1-3, wherein a compound of general formula **2D** belonging to compounds of general formula **2** mentioned in claim 1

general formula **2D**

wherein R^2 is a group removable by catalytic hydrogenation,

R^4 is benzyl substituted by 1-3 methoxy group(s),

R^5 is optionally substituted acyl,

R^8 is selected from $-NHAc$ or $-NAc_2$ and

Q is $-COO$ -alkyl, which alkyl is optionally substituted

5 is deprotected comprising the steps of:

a) base catalyzed transesterification reaction resulting in another compound of general formula **2D**, wherein R^2 is as defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is $-NHAc$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, followed by

10 aa) dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, each of R^4 and R^5 are H, R^8 is $-NHAc$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, followed by basic hydrolysis, optional acidification and optional salt formation, or

15 ab) basic hydrolysis, optional acidification and optional salt formation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is $-NHAc$ and Q is $-COOH$ in either protonated or deprotonated form, followed dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate
20 (CAN) mediated oxidation

to give a compound of general formula **1** or salts thereof,

or

b) dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation resulting in another compound of general formula **2D**, wherein
25 R^2 is above, R^4 is H, R^5 is optionally substituted acyl, R^8 is selected from $-NHAc$ and $-NAc_2$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, followed by

ba) base catalyzed transesterification reaction followed by basic hydrolysis, optional acidification and optional salt formation, or

bb) basic hydrolysis, optional acidification and optional salt formation

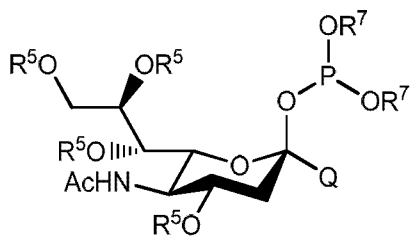
to give a compound of general formula **1** or salts thereof,

5 or

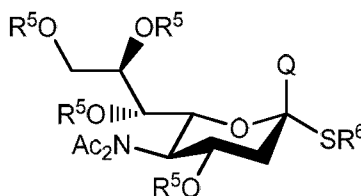
c) basic hydrolysis, optional acidification and optional salt formation resulting in another compound of general formula **2D**, wherein R^2 is as defined above, R^4 is benzyl substituted by 1-3 methoxy group(s), R^5 is H, R^8 is -NHAc and Q is -COOH in either protonated or deprotonated form, followed by dichlorodicyanoquinone (DDQ) or cerium(IV) ammonium nitrate (CAN) mediated oxidation to give a compound of general formula **1** or salts thereof.

10

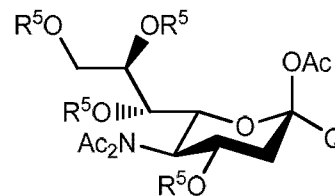
9. The method according to any of claims the 1-8, in said method the glycosylation reaction comprises the use of a donor of general formulae **4A**, **4B** or **4C**



general formula **4A**



general formula **4B**



general formula **4C**

15

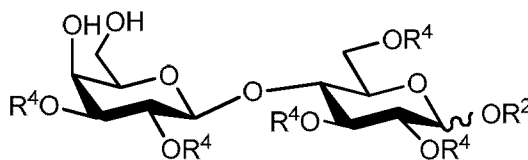
wherein R^5 is optionally substituted acyl,

R^6 is selected from C_{2-6} alkyl, C_{3-6} cycloalkyl and optionally substituted benzyl,

R^7 is substituted benzyl and

Q is -COO-alkyl, which alkyl is optionally substituted,

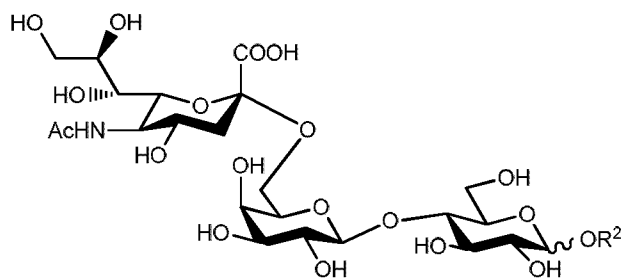
20 and of an acceptor of general formula **3A**

general formula **3A**

wherein R^2 is a group removable by catalytic hydrogenation, and

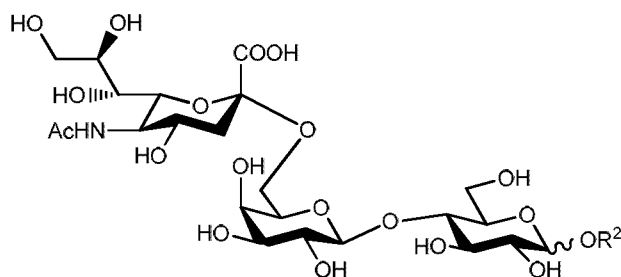
R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and optionally substituted acyl provided that acetyl is excluded.

- 5 10. 6'-*O*-Sialyllactose Zn^{2+} salt.
11. A 6'-*O*-sialyllactose organic salt, preferably 6'-*O*-sialyllactose diethyl ammonium salt, 6'-*O*-sialyllactose ethanolammonium salt, tris-(hydroxymethyl)-methyl ammonium salt or 6'-*O*-sialyllactose choline salt.
12. A 6'-*O*-sialyllactose salt according to any of the claims 10 or 11 for use as nutritional
10 additive.
13. A 6'-*O*-sialyllactose salt according to any of the claims 10 or 11 for use as pharmaceutical agent.
14. A nutritional composition comprising one or more 6'-*O*-sialyllactose salts according to any of the claims 10 or 11.
- 15 15. A pharmaceutical composition comprising one or more 6'-*O*-sialyllactose salts according to any of the claims 10 or 11.
16. A group of compounds of general formula **1** mentioned in claim 1, characterized by general formula **1A**

general formula **1A**

wherein R^2 is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl and OR^2 is in β .

17. A group of compounds of general formula **1** mentioned in claim 1, characterized by
5 general formula **1B**

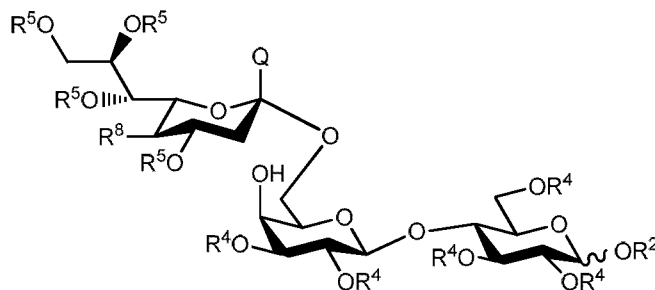
general formula **1B**

wherein R^2 is a group removable by catalytic hydrogenation,

in salt form selected from the Zn^{2+} , K^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Fe^{2+} , Mn^{2+} , Cu^{2+} and organic salts.

18. The compound according to claim 17, wherein R^2 is benzyl, $-OR^2$ is in β and the salt is
10 selected from the Zn^{2+} salt and organic salts, and the organic salt is preferably selected from ethanolammonium, diethyl ammonium, tris-(hydroxymethyl)-methyl ammonium and choline salt.

19. A compound of general formula **2A**



general formula **2A**

wherein R^2 is a group removable by catalytic hydrogenation,

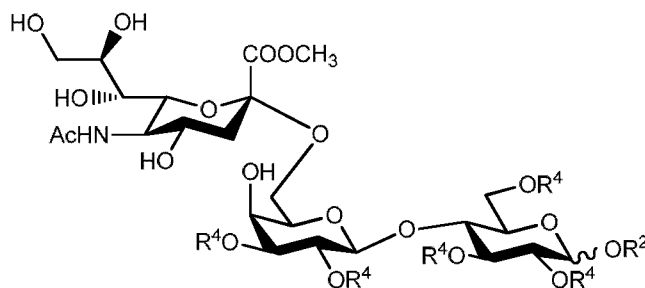
R^4 and R^5 are independently from each other optionally substituted acyl,

5 R^8 is selected from $-NHAc$ and $-NAc_2$, and

Q is $-COO-$ alkyl, which alkyl is optionally substituted.

20. The compound according to claim 19, wherein $-OR^2$ is in β , R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably pivaloyl and benzoyl, R^5 is acetyl, Q is –
10 $COOCH_3$.

21. A compound of general formula **2C**

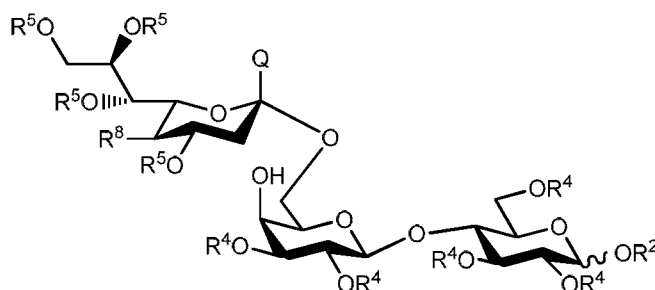


general formula **2C**

wherein $-OR^2$ is in β , R^2 is a group removable by catalytic hydrogenation, preferably benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and

R^4 is selected from isobutyryl, pivaloyl and optionally substituted benzoyl, preferably benzoyl.

22. A compound of general formula **2D**



general formula **2D**

5 wherein R^2 is a group removable by catalytic hydrogenation,

R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and H,

R^5 is selected from optionally substituted acyl and H,

R^8 is selected from $-NHAc$ and NAC_2 , and

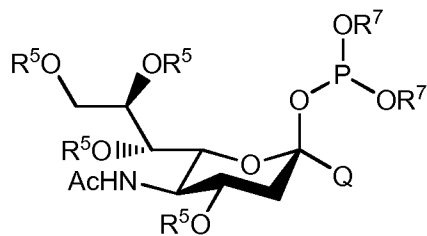
10 Q is selected from $-COO$ -alkyl, which alkyl is optionally substituted and $-COOH$ in either protonated or deprotonated form,

provided that if Q is $-COOH$ in either protonated or deprotonated form and R^8 is $-NHAc$, then both R^4 and R^5 cannot be H simultaneously.

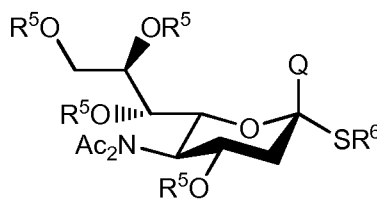
23. The compound according to claim 22, wherein $-OR^2$ is in β , R^2 is selected from benzyl, 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, R^4 and R^5 are H, R^8 is $-NHAc$ and Q is $-COO$ -alkyl, which alkyl is optionally substituted, preferably $-COOMe$.

15

24. A compound of general formula **4A** or **4B**



general formula **4A**



general formula **4B**

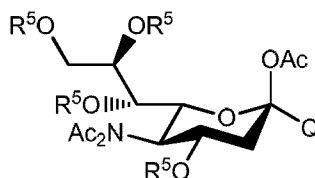
wherein R^5 is optionally substituted acyl, preferably acetyl,

R^6 is selected from C_{2-6} alkyl, C_{3-6} cycloalkyl and optionally substituted benzyl, preferably from ethyl, isopropyl, *t*-butyl, benzyl and cyclohexyl,

R^7 is substituted benzyl, preferably 4-chlorobenzyl or 4-bromobenzyl, and

Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

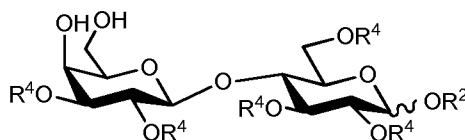
25. A compound of general formula **4C** for use as sialyl donor



general formula **4C**

wherein R^5 is optionally substituted acyl, preferably acetyl and Q is -COO-alkyl, which alkyl is optionally substituted, preferably -COOMe.

26. A compound of general formula **3A**



general formula **3A**

wherein R^2 is a group removable by catalytic hydrogenation, and

R^4 is selected from benzyl substituted by 1-3 methoxy group(s) and optionally substituted acyl provided that acetyl is excluded.

27. The compound according to claim 26, wherein $-OR^2$ is in β , R^2 is selected from benzyl,
5 4-methylbenzyl, 4-chlorobenzyl and 1-naphthylmethyl, and R^4 is selected from isobutyryl, pivaloyl and benzoyl.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK2011/050052

A. CLASSIFICATION OF SUBJECT MATTER

IPC: C07H 1/00 (2006.01), C07H 3/06 (2006.01), C07H 13/04 (2006.01), C07H 15/18 (2006.01), A61K 31/702 (2006.01), A23L 1/09 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07H, A61K, A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
DK, NO, SE, FI: Classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY, HCAPLUS, WPI, EPODOC, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RENCURSI, A. et al.: "Human milk oligosaccharides: an enzymatic protection step simplifies the synthesis of 3'- and 6'-O-sialyllactose and their analogues", Carbohydr. Res., Vol. 337, pages 473-483 See whole document	1-9
P,X	WO 2010/116317 A1 (INALCO S.P.A.) 2010.10.14 See whole document	1-9
A	Database REGISTRY, RN 35890-39-2, STN entry date 16 November 1984	
A	Database REGISTRY, RN 157574-76-0, STN entry date 09 September 1994	
A	Database REGISTRY, RN 70472-20-7, STN entry date 16 November 1984	
A	MATSUOKA, K. et al.: "Synthesis of a useful lauryl thioglycoside of sialic acid and its preparation", Tetrahedron Lett., 2004, Vol. 45, No. 51, pages 9383-9386	

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09/05/2011

Date of mailing of the international search report

10/05/2011

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2011/050052

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

See extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK2011/050052

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PAZYNINA, G. et al.: "Simple stereoselective synthesis of alpha-2,6 sialooligosaccharides", Tetrahedron Lett., 2002, Vol. 43, No. 45, pages 8011-8013 See compounds 5 and 7, table 1	
A	THOMAS, R.L. et al.: "Silver zeolite - effective catalyst for the regio-stereoselective formation of the Neu5Ac-alpha-2-6 glycosyl linkage - synthesis of several sialosaccharides", Tetrahedron Lett., 1990, Vol. 31, No. 20, pages 2825-2828 See table 1	
A	POZSGAY, V. et al.: "A novel approach to N-acetyl-neuraminic acid-containing oligosaccharides. Synthesis of a glycosyl donor derivative of alpha-N-acetyl-D-neuraminy-(2-6)-D-galactose	
A	OGURA, H. et al.: "Synthesis of 9-O-acetyl- and 4-O-acetyl-sialic acids", Carbohydr. Res., 1987, Vol. 167, pages 77-86	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/DK2011/050052

Patent documents cited in the search report	Publication date	Patent family member(s)	Publication date
WO2010116317 A1	20101014	ITFI20090071 A1	20101007

Continuation of box No. III

The international Searching Authority found that the application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. The result is a lack of unity between:

Invention 1 (claims 1-9, 16-27):

A method for the preparation of 6'-O-sialyllactose or a salt thereof and intermediates in the synthetic route

Invention 2 (claim 10 and claims 12-15 (in part)):

6'-O-Sialyllactose Zn²⁺ salt and the use thereof in nutritional or pharmaceutical compositions

Invention 3 (claim 11 and claims 12-15 (in part)):

A 6'-O-sialyllactose organic salt and the use thereof in nutritional or pharmaceutical compositions

The special technical feature linking inventions 1-3 is 6'-O-sialyllactose which is a known compound as also explained by the applicant (see also REGISTRY, RN 35890-39-2). Inventions 2-3 are linked by being salts of 6'-O-sialyllactose. However, salts of 6'-O-sialyllactose are also known (see REGISTRY, RN 157574-76-0 and RN 70472-20-7). Accordingly, there is no common patentable technical feature and a lack of unity arise.

Invention 1 relates to both a method of preparation of 6'-O-sialyllactose and to various intermediates in the synthesis. The technical interrelation between the method of preparation and the intermediates is dependent on the patentability of the method of preparation of 6'-O-sialyllactose.

Rencurosi et al. (D1) describes a synthesis of 6'-O-sialyllactose by reaction of sialyl phosphite donor 9 (falling within the scope of present general formula 4) and benzyl lactoside 6 using TMSOTf as promoter (see scheme 2). The reaction differs from present claim 1 in that the glycosyl acceptor of D1 contains a 3,4-O-isopropylidene and a free 6-OH, whereas the acceptor of present claim 1 contains a 3-O-acyl or 3-O-benzyl substituted by 1-3 methoxy groups, free 4-OH and 6-OH groups. The resulting protected 6'-sialyllactose was further deprotected to obtain benzyl 6'-O-sialyllactose which was subjected to hydrogenolysis (Pd/C, H₂) to afford the desired product. That is, the present claims 1-9 differ from D1 only in the acceptor having both a free 4-OH and 6-OH.

However, it is known for a person skilled in the art that reaction of a sialyl donor and a glycosyl acceptor having both free 4-OH and 6-OH results in generation of the corresponding 6'-sialyl glycoside (see e.g. Pazynina et al. and Thomas et al.). Therefore, the method of preparation of 6'-O-sialyllactose according to the present application does not constitute a special technical feature that makes a contribution over the prior art. Hence, the method of preparation (claims 1-9) and the intermediates are not so linked as to form a single inventive concept. As there is no interrelation between the various intermediates, the lack of unity results in a division of invention 1 above into the following 5 inventions:

Invention 1a (claims 1-9): A method for preparation of 6'-O-sialyllactose

Invention 1b (claims 16-18): A compound of general formula 1

Invention 1c (claims 19-23): A compound of general formula 2

Invention 1d (claims 24-25): A compound of general formula 4

Invention 1e (claims 26-27): A compound of general formula 3