

HEXOSE DERIVATIVES, PREPARATION AND USES THEREOF

This application claims the priority of U.S. Provisional Application No. 61/953,392, filed March 14, 2014, the contents of which are hereby incorporated by reference.

5 Throughout this application, various publications are referenced, including referenced in parenthesis. Full citations for publications referenced in parenthesis may be found listed in alphabetical order at the end of the specification immediately preceding the claims. The
10 disclosures of all referenced publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

15 Low molecular weight organic compounds termed compatible solutes have been identified in the cytoplasm of many halophilic or halotolerant organisms which counterbalance the osmotic pressure of the external medium and which promote correct protein folding, inhibit protein aggregation, and
20 prevent heat-induced denaturation (Faria 2008, Faria 2013). Compatible solutes are therefore industrially useful, for example, for stabilizing proteins in pharmaceutical and cosmetic formulations (Luley-Goedl 2011, Lentzen 2006).

Compatible solutes are usually amino acids, carbohydrates,
25 polyols, betaines and ectoines. Trehalose, glycerol, glycine-betaine and ectoine are typical compatible solutes of mesophiles. The discovery of extreme thermophilic and hyperthermophilic microorganisms led to the discovery of additional compatible solutes, such as mannosylglycerate (MG)
30 and dimyo-inositol-1,3'-phosphate (Faria 2008).

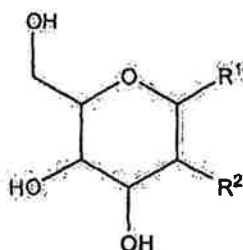
Compounds structurally related to MG, namely (2S)-2-(1-O- α -D-Mannopyranosyl)propionate (ML), 2-(1-O- α -Dmannopyranosyl)

acetate (MGlyc), 1-O-(2-glyceryl)- α -D-mannopyranoside (MGOH),
have been synthesized and tested for their ability to
5 stabilize model proteins against thermal stress. (Faria 2008).

New compounds for the stabilization of biological materials
are needed.

SUMMARY OF THE INVENTION

The invention provides a compound of formula I:



Formula I

5 or a salt thereof, wherein:

R^1 is $-OC(H)(X)(CH_2)_nC(=O)OH$;

R^2 is $-OH$, $-N_3$, or $-N(H)C(=O)CH_3$; or

R^1 and R^2 together with the carbon atoms to which they are attached form



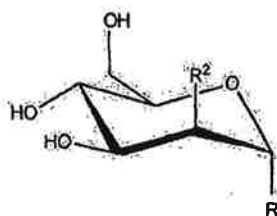
10

R^3 is $-H$, $-CH_3$, $-CH_2C(=O)OH$, or $-CH_2OH$;

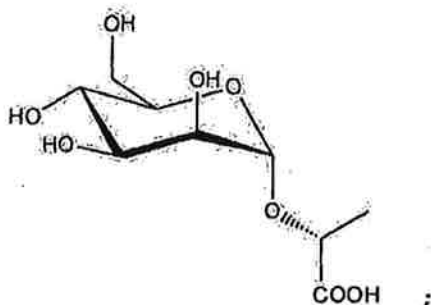
X is $-H$, $-CH_3$, $-CH_2OH$, or $CH_2C(=O)OH$; and

n is 0 or 1;

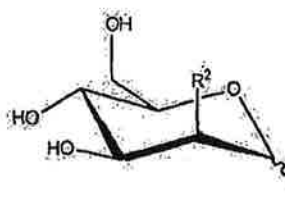
wherein when the compound is



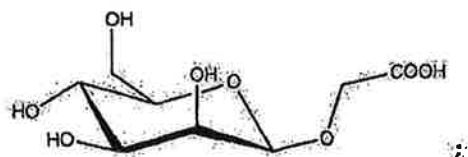
R^1 , and R^2 is OH, X is CH_3 , and n is 0, then the compound is



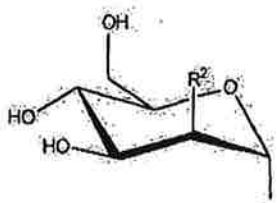
wherein when the compound is



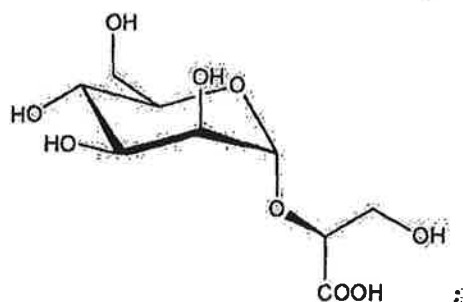
5 R^1 , and R^2 is OH, X is H, and n is 0, then the compound is



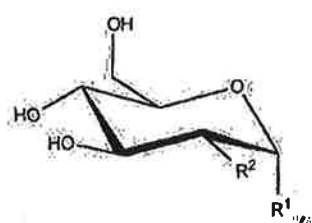
wherein when the compound is



10 R^1 , and R^2 is OH, X is CH_2OH , and n is 0, then the compound is

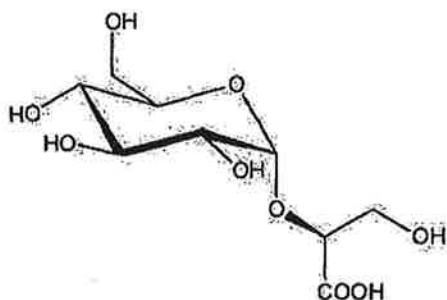


and wherein when the compound is



R¹ is OH, and R² is OH, X is CH₂OH, and n is 0,

then the compound is



5

The invention further provides a composition comprising at least one compound of Formula I, or a salt thereof, and a biological material.

The invention further provides a method of stabilizing a biological material, comprising adding at least one compound of Formula I, or a salt thereof, to a solution containing the biological material to form a stabilized solution.

The invention further provides a compound of Formula I or a salt thereof, for stabilizing a biological material.

The invention further provides a use of the compound of Formula I, or a salt thereof, for stabilizing a biological material.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Increment in the melting temperature (T_M) of malate dehydrogenase (MDH, grey bars), staphylococcal nuclease (SNase, black bars) and lysozyme (white bars) in the presence of 0.5 M of different solutes. The melting temperatures (T_M) in the absence of solutes were 50 °C for MDH, 52 °C for SNase and 71°C for lysozyme.

FIG. 2: Stabilising effect of different glucose derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

FIG. 3: Stabilising effect of different galactose derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

FIG. 4: Stabilising effect of different lactate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

FIG. 5: Stabilising effect of different malate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa

axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinates axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

- 5 **FIG. 6:** Stabilising effect dependence on concentration of AcOK alone and in conjugation with different hypersolutes (0.5M).

FIG. 7: Stabilising effect of different galactosyl glycerate derivatives against thermal denaturation of malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme. In the abscissa axis the increment in the melting temperature of MDH induced by 0.5 M of several compounds, and in the ordinate axis the increment in the melting temperature of SNase (solid symbols) and Lysozyme (open symbols) are plotted.

10

- 15 **FIG. 8:** Dependence of SNase melting temperature on the concentration of solutes.

FIG. 9: Dependence of MDH melting temperature on the concentration of solutes.

- FIG. 10:** Dependence of lysozyme melting temperature on the concentration of solutes.
- 20

FIG. 11: Increment in melting temperature of porcine insulin obtained for different solutes at 0.1 and 0.25 M. The left bar for each solute shows the result at 0.25 M and the right bar shows the result at 0.1 M.

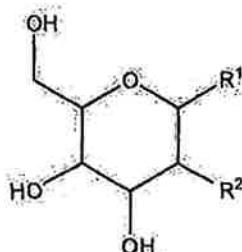
DETAILED DESCRIPTION OF THE INVENTION

The following abbreviations are used throughout this application.

	Ac	Acetate
5	BnBr	Benzyl bromide
	DIP	di- <i>myo</i> -inositol phosphate
	DGP	di-Glycerol phosphate
	DMAP	4-Dimethylaminopyridine
	DMF	Dimethylformamide
10	DSF	Differential Scanning Fluorimetry
	Et	Ethyl
	Et ₂ O	Diethyl ether
	EtOAc	Ethyl acetate
	GG	α -D-Glucosyl-D-glycerate
15	GGG	α -D-glucopyranosyl-(1 $\rightarrow$ 6)- α -D-glucopyranosyl-(1 $\rightarrow$ 2)-D-glycerate
	GL	α -D-Glucosyl-S-lactate
	Hex	Hexane
	MDH	Malate dehydrogenase
20	Me	Methyl
	MeOH	Methanol
	MG	α -D-Mannosyl-D-glycerate
	MGA	α -D-Mannosyl-D-glyceramide
25	MGG	α -D-Mannopyranosyl-(1 $\rightarrow$ 2)- α -D-glucopyranosyl-(1 $\rightarrow$ 2)-D-glycerate
	MGly	α -D-Mannosyl-glycolate
	MGGly	(2R)-2-(1-O- α -D-mannopyranosyl)-3-(1-O- α -D-glucopyranosyl)-glycerate

	ML	α -D-Mannosyl-S-lactate
	NaOMe	Sodium methoxide
	NIS	N-Iodosuccinimide
	NMR	Nuclear magnetic resonance
5	Ph	Phenyl
	TLC	Thin layer chromatography
	SNase	Staphylococcal nuclease
	TBAF	Tetra- <i>n</i> -butylammonium fluoride
	TBDPSCl	<i>tert</i> -Butylchlorodiphenylsilane
10	TBDMS	<i>tert</i> -Butyldimethylsilane
	TfOH	Trifluoromethanesulfonic acid
	THF	Tetrahydrofuran

The invention provides a compound of formula I:



15

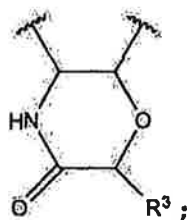
Formula I

or a salt thereof, wherein:

R^1 is $-OC(H)(X)(CH_2)_nC(=O)OH$;

R^2 is $-OH$, $-N_3$, or $-N(H)C(=O)CH_3$; or

R^1 and R^2 together with the carbon atoms to which they are
20 attached form

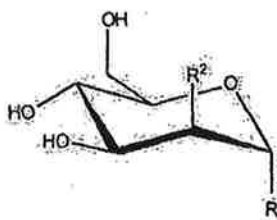


R^3 is $-H$, $-CH_3$, $-CH_2C(=O)OH$, or $-CH_2OH$;

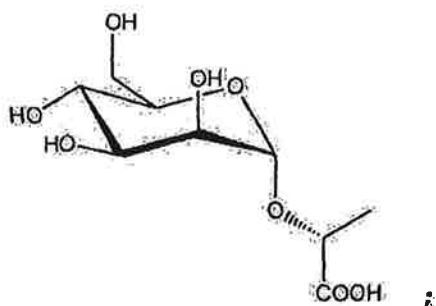
X is $-H$, $-CH_3$, $-CH_2OH$, or $CH_2C(=O)OH$; and

n is 0 or 1;

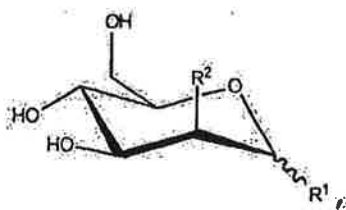
5 wherein when the compound is



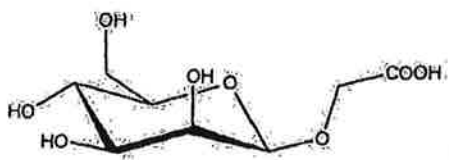
R^1 , and R^2 is OH , X is CH_3 , and n is 0, then the compound is



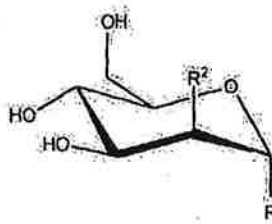
wherein when the compound is



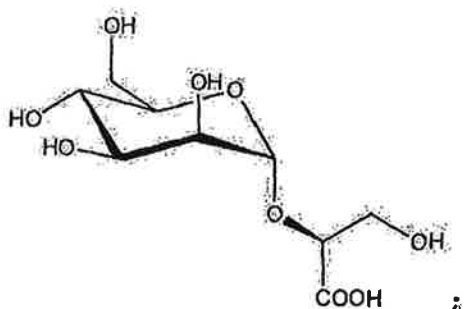
10 R^1 , and R^2 is OH , X is H , and n is 0, then the compound is



wherein when the compound is

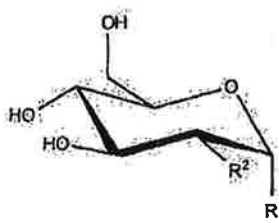


R^1 , and R^2 is OH, X is CH_2OH , and n is 0,
then the compound is

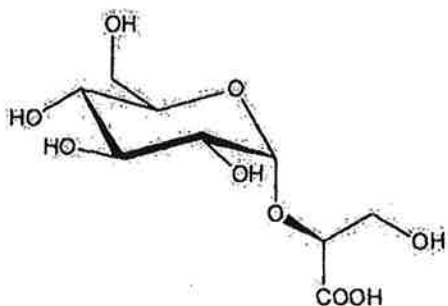


5

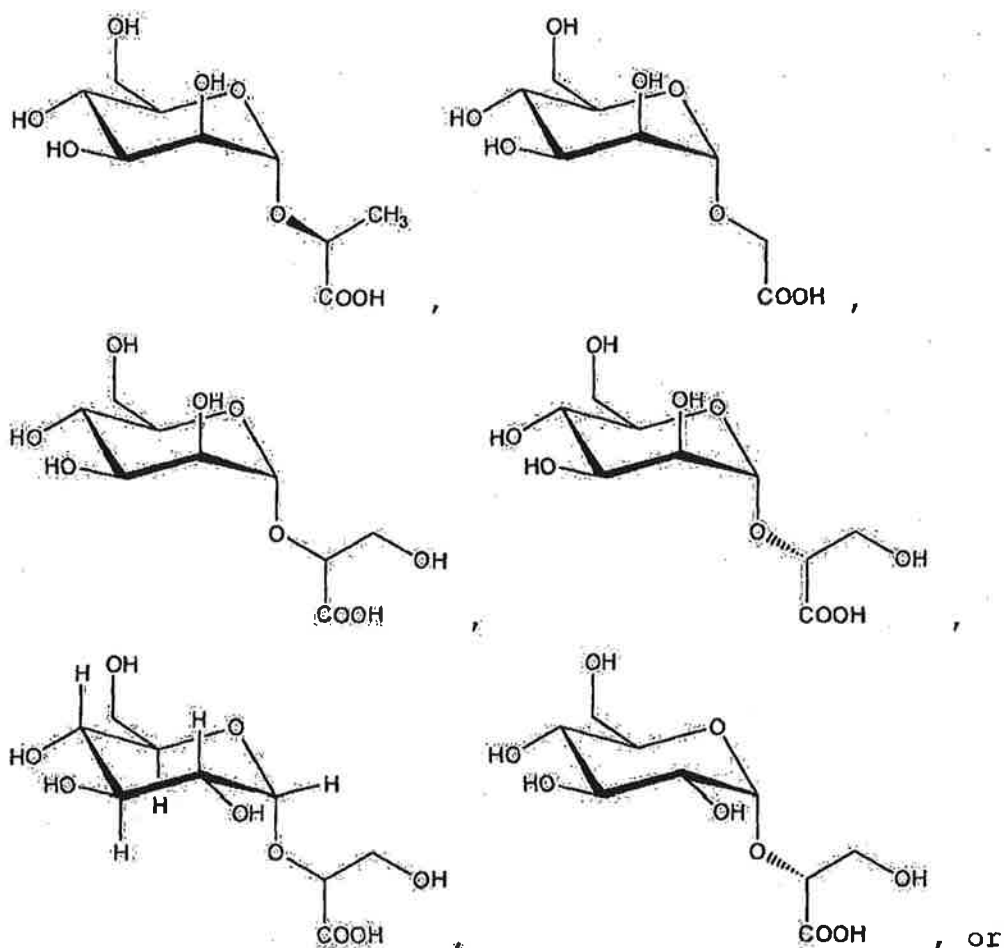
and wherein when the compound is



R^1 , and R^2 is OH, X is CH_2OH , and n is 0,
then the compound is



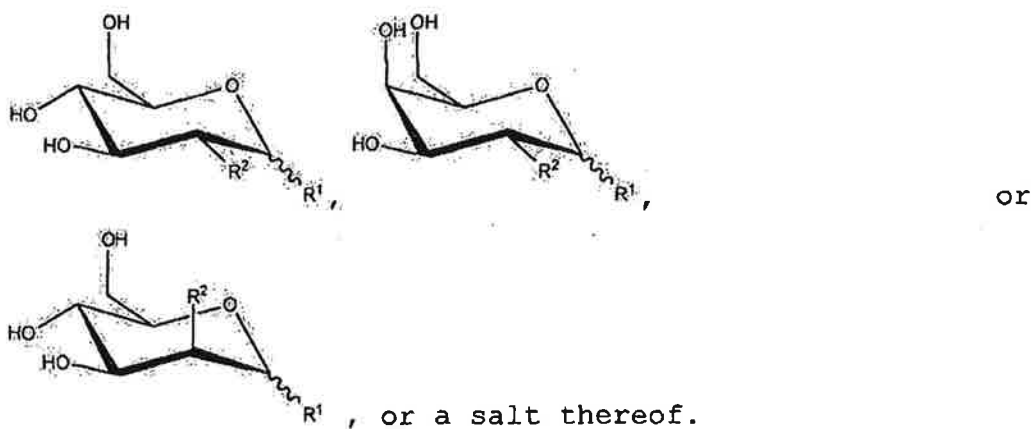
In an embodiment, the compound is not



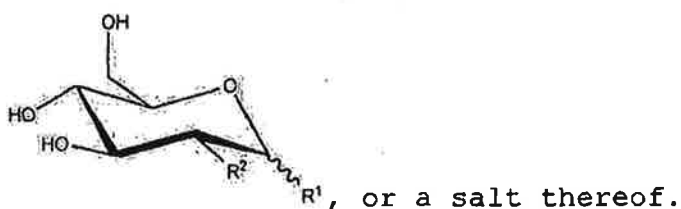
5 a salt thereof.

In an embodiment, the compound of formula I is not a naturally occurring compound.

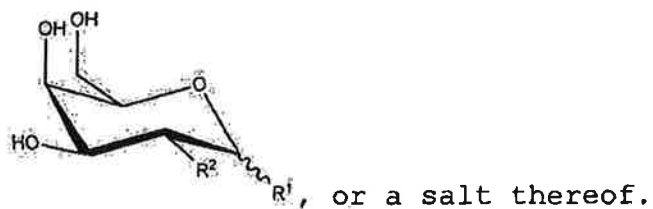
In an embodiment, the compound is



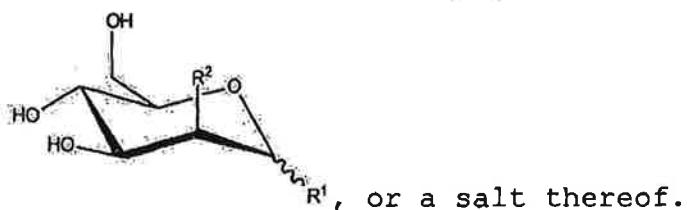
In an embodiment, the compound is



5 In an embodiment, the compound is



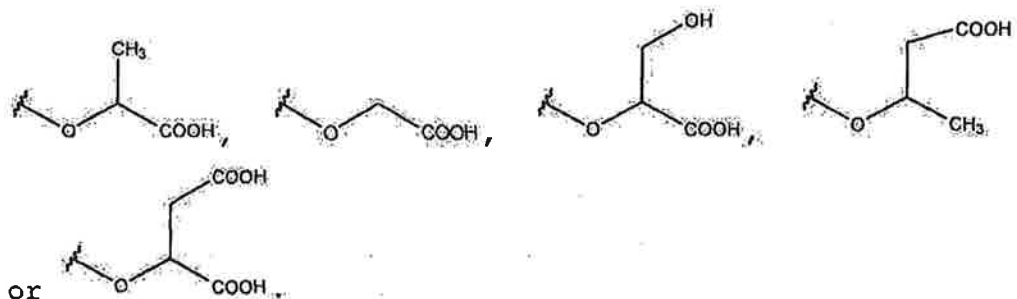
In an embodiment, the compound is



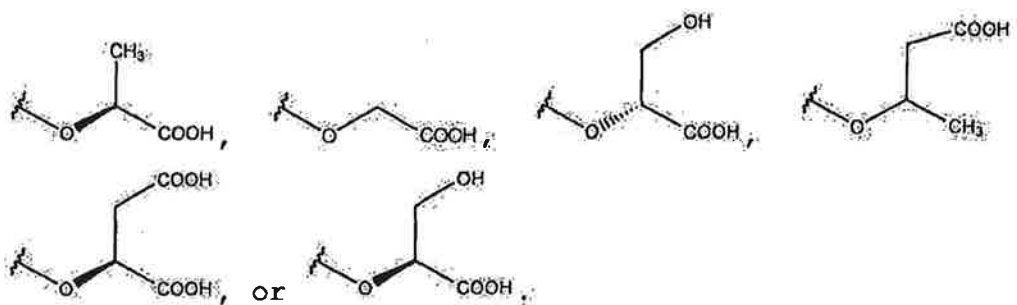
In an embodiment, the α/β anomer ratio of the compound or a salt thereof is 1:1 to 99:1. In an embodiment, the α/β anomer ratio of the compound or a salt thereof is 1:1 to 10:1. In an embodiment, the α/β anomer ratio of the compound or a salt thereof is 1:1 to 5:1. In another embodiment, α/β anomer

ratio of the compound or a salt thereof is 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1. In an embodiment, the α/β anomer ratio of the compound or a salt thereof is greater than 10:1. In an embodiment, α/β anomer ratio of the compound or salt thereof is greater than 99:1. In an embodiment, the compound or salt thereof is the α anomer.

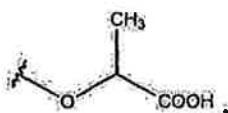
In an embodiment, R^1 is



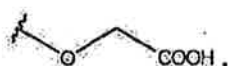
10 In an embodiment, R^1 is



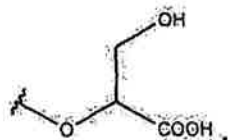
In an embodiment, R^1 is



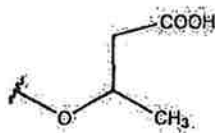
In an embodiment, R^1 is



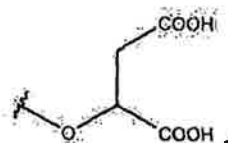
15 In an embodiment, R^1 is



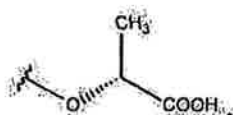
In an embodiment, R^1 is



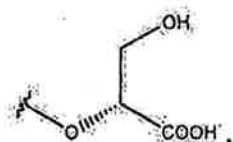
In an embodiment, R^1 is



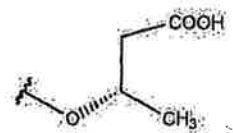
In an embodiment, R^1 is



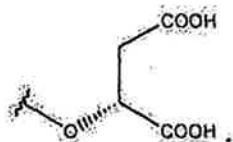
In an embodiment, R^1 is



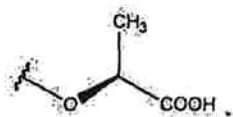
5 In an embodiment, R^1 is



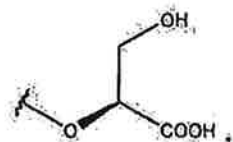
In an embodiment, R^1 is



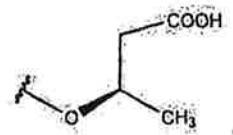
In an embodiment, R^1 is

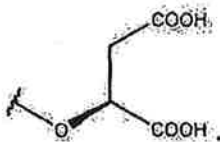


In an embodiment, R^1 is



In an embodiment, R^1 is





In an embodiment, R¹ is

In an embodiment, when an asymmetric center is present in R¹, the compound is a mixture of the two enantiomers.

In an embodiment, when an asymmetric center is present in R¹,
5 the compound is the *S* enantiomer.

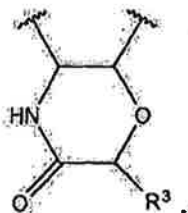
In an embodiment, when an asymmetric center is present in R¹, the compound is the *R* enantiomer.

In an embodiment, R² is -OH.

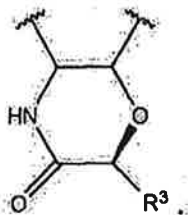
In an embodiment, R² is N₃.

10 In an embodiment, R² is -N(H)C(=O)CH₃.

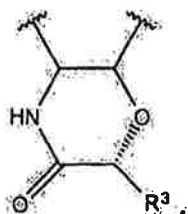
In an embodiment, R¹ and R² together with the carbon atoms to which they are attached form



In an embodiment, R¹ and R² together with the carbon atoms to
15 which they are attached form



In an embodiment, R¹ and R² together with the carbon atoms to which they are attached form



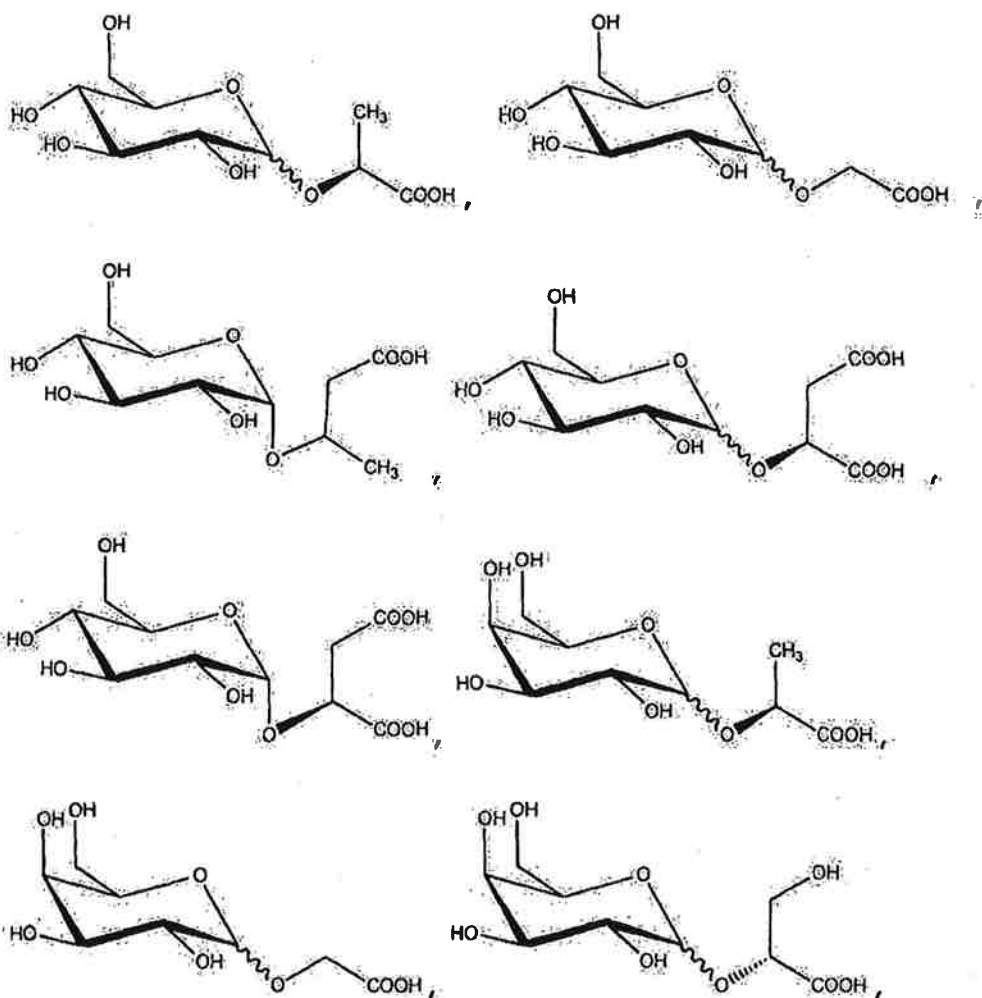
In an embodiment, R^3 is $-H$.

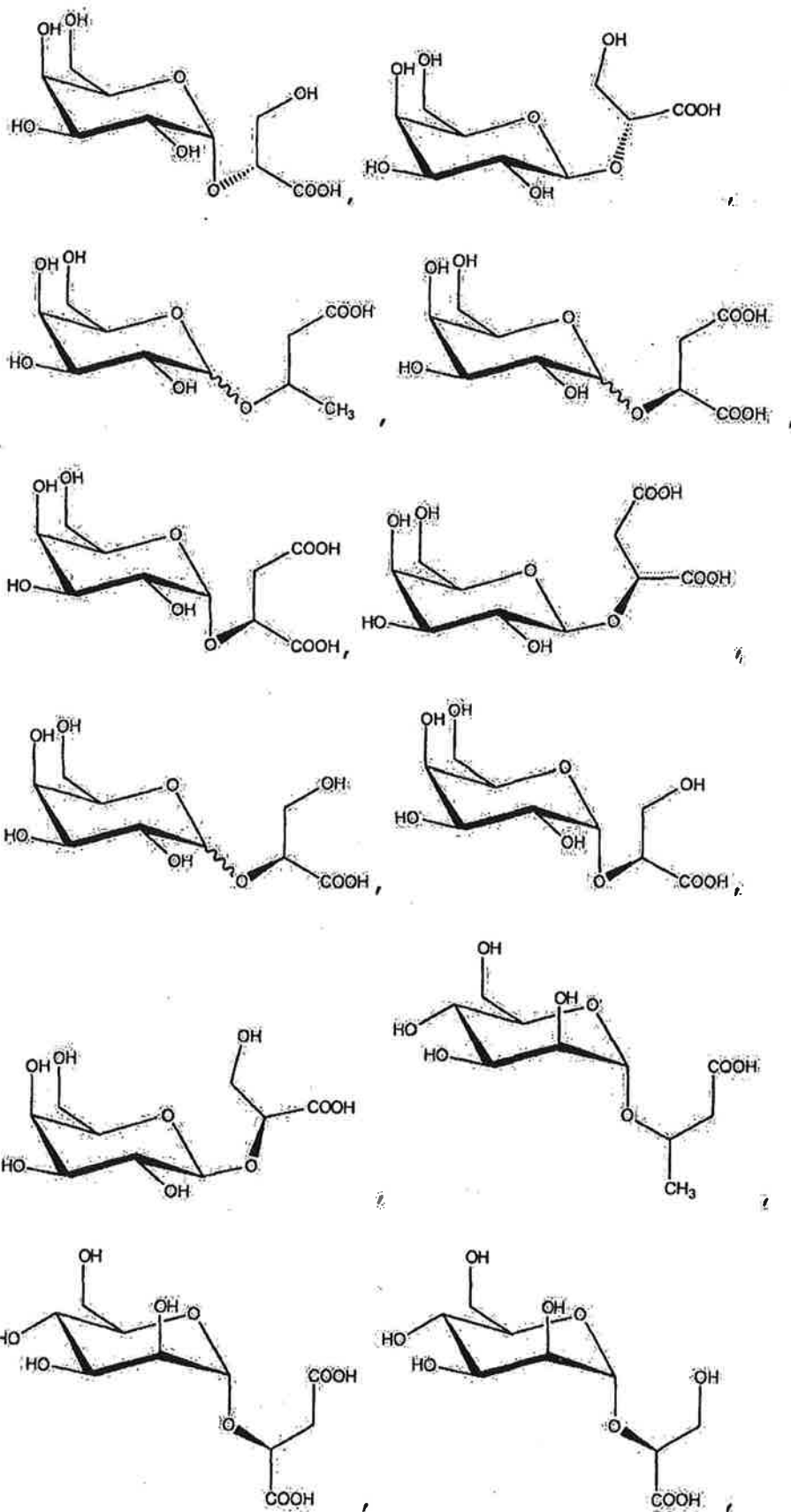
In an embodiment, R^3 is $-CH_3$.

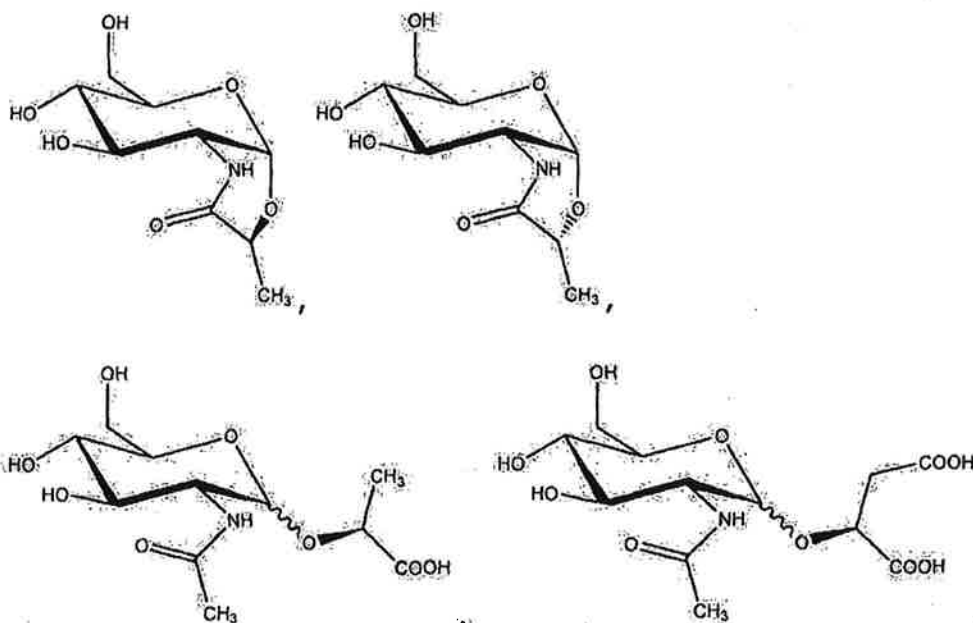
In an embodiment, R^3 is $-CH_2C(=O)OH$.

5 In an embodiment, R^3 is $-CH_2OH$.

In an embodiment, the compound is







or a salt thereof.

In an embodiment, the compound is any one compound of the
5 previous embodiment or a salt thereof.

In an embodiment, the compound is in the form of a salt.

In an embodiment, the compound is in the form of a
pharmaceutically acceptable salt.

In an embodiment, the compound is in the form of a potassium
10 salt.

In an embodiment, the compound is in the form of a sodium
salt.

The invention also provides a composition comprising at least
one compound of the invention, or a salt thereof, and a
15 biological material.

In an embodiment, the composition is a liquid. In an
embodiment, the composition is a solid. In an embodiment, the

composition is lyophilized. In an embodiment, the composition is freeze-dried.

In an embodiment, the composition is a liquid and the at least one compound of the invention is present in the composition at
5 a concentration of 0.01 to 1 M. In an embodiment, the at least one compound of the invention is present in a concentration of 0.1 to 0.5 M. In an embodiment, the at least one compound of the invention is present in a concentration of 0.01 to 1 M. In an embodiment, the at least one compound of the invention is
10 present in the composition in a concentration of 0.1 M, 0.2 M, 0.25 M, 0.3 M, 0.4 M, or 0.5 M.

In an embodiment, the composition is a solid which was prepared by drying a liquid composition of the invention.

In an embodiment, the composition comprises at least one other
15 compatible solute in addition to the at least one compound of the invention. The at least one other compatible solute can be, for example, at least one other compatible solute known in the art. In compositions having at least one other compatible solute and/or more than one compound of the invention, the
20 amount of the other compatible solute and/or the amount of each compound of the invention necessary to stabilize the biological material may be less than the amount of each agent necessary to stabilize the biological material alone.

In an embodiment, the composition further comprises one or
25 more salts in addition to the at least one compound of the invention. In an embodiment, the one or more additional salts are pharmaceutically acceptable salts. In an embodiment, the one or more salts comprises potassium acetate.

In an embodiment, the composition is a pharmaceutical
30 composition, a cosmetic, or a food product.

In an embodiment, the biological material is a nucleic acid, a polypeptide, a whole cell, a virus, a virus like particle, a cell membrane, a cell component, a liposome, a tissue, or a mixture of any of the foregoing. In an embodiment, the
5 biological material comprises one, two, three, or more species of biological material.

In an embodiment, biological material is one or more species of nucleic acids. In an embodiment, the nucleic acid is RNA, DNA, or a mixture of RNA and DNA. In an embodiment, the RNA is
10 single stranded RNA. In an embodiment, the RNA is double stranded. In an embodiment, the RNA is mRNA. In an embodiment, the RNA is an antisense oligonucleotide. In an embodiment, the DNA is double stranded. In an embodiment, the DNA is single stranded.

15 In an embodiment, the biological material is one or more species of whole cells.

In an embodiment, the biological material is a polypeptide.

In an embodiment, the polypeptide is an enzyme, an antibody, a plasma protein, or a hormone.

20 In an embodiment, the polypeptide is insulin, malate dehydrogenase, staphylococcal nuclease or lysozyme. In an embodiment, the polypeptide is insulin.

In an embodiment, the polypeptide is a recombinant polypeptide. In an embodiment, the polypeptide is isolated
25 from a yeast or mammalian cell culture.

In an embodiment, polypeptide is not a recombinant polypeptide. In an embodiment, the polypeptide is isolated from a plant, an animal, a fungus, or a bacteria. In an embodiment, the polypeptide is an animal or human serum
30 polypeptide.

In an embodiment, the composition further comprises a buffer.

The invention also provides a method of stabilizing a biological material, comprising adding at least one compound of the invention, or a salt thereof, to a solution containing
5 the biological material to form a stabilized solution.

In an embodiment, the method further comprises a step of drying the stabilized solution.

In an embodiment, the drying is spray-drying or lyophilization.

10 In an embodiment, the at least one compound of the invention or a salt thereof is selected based upon the properties of the biological material to be stabilized. For example, if the biological material is a protein, a compound of the invention, or a combination of compounds of the invention may be selected
15 based upon the hydrophobicity and/or hydrophilicity of the surface of the protein.

The invention also provides a compound of the invention or a salt thereof for stabilizing a biological material.

The invention also provides a use of the compound of the
20 invention or a salt thereof for stabilizing a biological material.

In an embodiment, stabilizing is protecting a biological material from denaturation. In an embodiment, stabilizing is increasing the melting temperature of a biological material.

25 In an embodiment, stabilizing is protecting a biological material from dessication. In an embodiment, stabilizing is protecting a biological material from aggregation. In an embodiment, stabilizing is protecting a biological material from heat. In an embodiment, stabilizing is protecting a
30 biological material from freezing. In an embodiment,

stabilizing is protecting a biological material during drying.
In an embodiment, stabilizing is increasing the shelf-life of
a biological material.

The invention also provides a diagnostic kit comprising a
5 compound of the invention or a salt thereof.

In an embodiment, the diagnostic kit is a microarray, a
biosensor, or an enzymatic preparation. In an embodiment, the
microarray, biosensor, or enzymatic preparation comprises an
immobilized biological material. The compatible solutes of the
10 invention can be used in methods known in the art which make
us of compatible solutes to improve the performance of
techniques using immobilized biological materials. See, for
example, PCT International Application Publication No.
WO/2007/097653.

15 The invention also provides a cosmetic or other dermatological
composition comprising a compound of the invention, or a salt
thereof, and an excipient suitable for topical administration
to humans or animals.

In an embodiment, the cosmetic or dermatological composition
20 comprises one or more biological materials.

The invention also provides compounds, compositions, methods,
and uses as described above, wherein the compound is any
compound listed in Table 6 or 7, or a salt thereof. For
example, this invention provides a composition for stabilizing
25 a biological material comprising one or more of the compounds
listed in Tables 6 and 7 and the biological material. As
another example, this invention provides a method of
stabilizing a biological material, comprising adding at least
one compound listed in Tables 6 and 7, or a salt thereof, to a
30 solution containing the biological material to form a
stabilized solution.

The specific embodiments and examples described herein are illustrative, and many variations can be introduced on these embodiments and examples without departing from the spirit of the disclosure or from the scope of the appended claims.

5 Elements and/or features of different illustrative embodiments and/or examples may be combined with each other and/or substituted for each other within the scope of this disclosure and appended claims.

Each of the embodiments described herein as being applicable
10 to or including a compound of the invention is equally applicable to a salt of the compound.

For the foregoing embodiments, each embodiment disclosed herein is contemplated as being applicable to each of the other disclosed embodiments.

15 By any range disclosed herein, it is meant that all tenth and integer unit amounts within the range are specifically disclosed as part of the invention. Thus, for example, 0.1 M to 0.5 M means that 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M are embodiments within the scope of the invention.

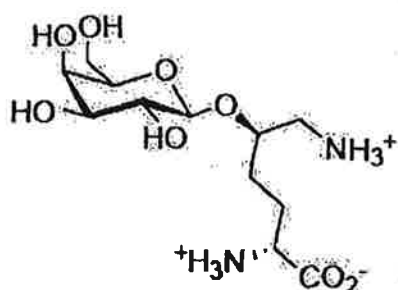
20 This invention will be better understood by reference to the Examples which follow, which are set forth to aid in an understanding of the subject matter but are not intended to, and should not be construed to, limit in any way the claims which follow thereafter.

25 *Example 1: Synthesis of novel compatible solutes*

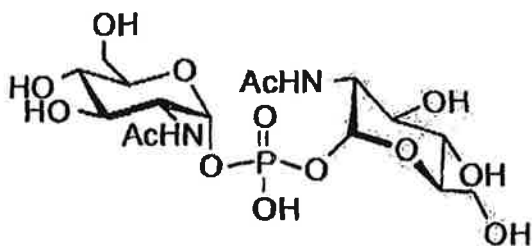
A chemical library based on sugar derivatives was prepared in order to identify new organic compounds with increased protein stabilization properties. The diversity of the analogue structures was introduced by using different hexoses, such as
30 glucose, galactose, mannose and glucosamine, and by using

different glycosyl acceptors during the glycosylation reaction.

Galactose and glucosamine analogues were synthesized in addition to mannosides and glucosides in order to assess the contribution of the sugar structure for the stabilization effect. To our knowledge, only one galactose containing compatible solute has been isolated from hyperthermophiles, the β -galactopyranosyl-5-hydroxylysine (GalHI) from *Thermococcus litoralis*:



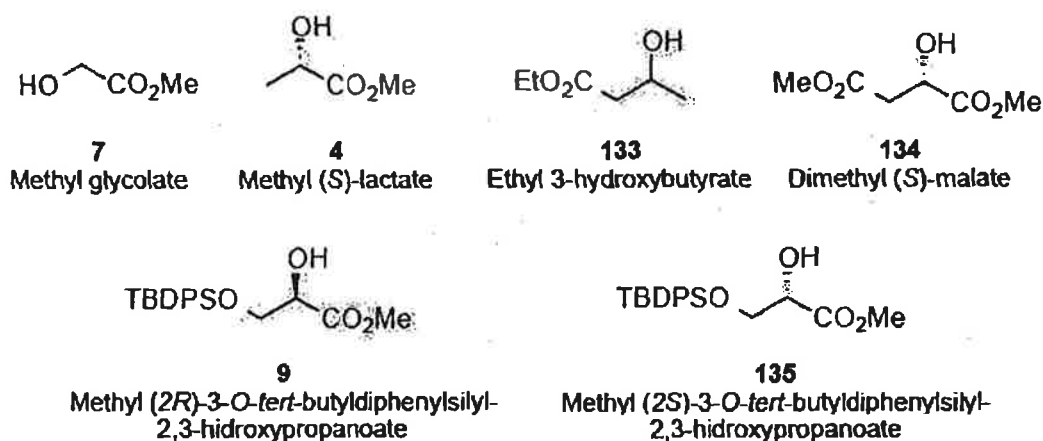
Several amino acids, like glutamate, proline, and glutamine, can function as compatible solutes in many mesophilic organisms and both α - and β -amino acids are used for osmoadaptation (Costa 1998). In order to determine if an amino group or the extra charge would enhance the stabilisation effect glucosamine derivatives were synthesized. To our knowledge, only one glucosamine containing compatible solute has been isolated from hyperthermophiles, di-N-acetylglucosamine phosphate (DAGAP) from *Rubrobacter xylanophilus*:



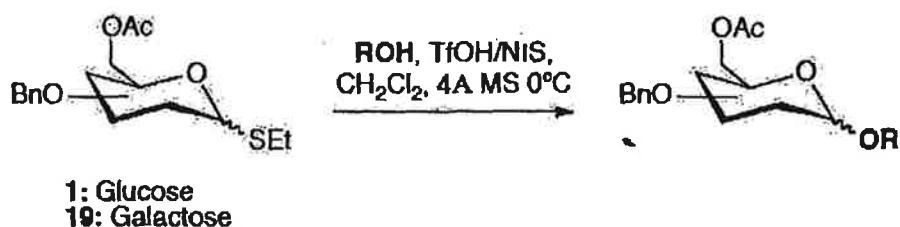
(Empadinhas 2007).

DAGAP is structurally similar to the phosphodiester compatible solutes found in hyperthermophiles, like DIP or DGP, however, the role as a compatible solute has been refuted due to the concentrations that are too low to contribute to the cell's osmotic balance.

All of the glycosyl acceptors chosen for this study are charged and structurally related to glycerate with point modifications, such as more or less carbon atoms, loss of a hydroxyl group, an additional carboxylic group and the configuration at the asymmetric center, when present:



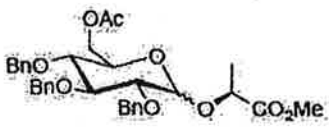
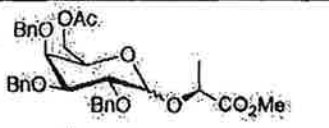
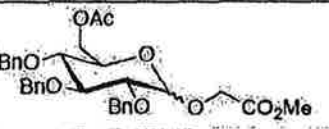
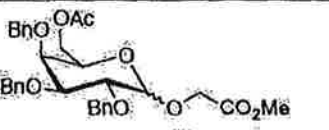
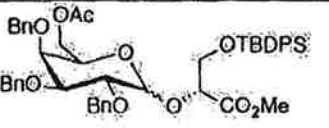
For the synthesis of the glucose and galactose derivatives, thioglycoside donors **1** and **19** were synthesized. The results obtained for the glycosylation reaction of donors **1** and **19** with the glycosyl acceptors above using NIS/TfOH system (Lourenco 2009) in dichloromethane are described in Table 1. All acceptors were commercially available with exception of the methyl glycerate derivatives **9** and **135**, which were synthesised according to the experimental procedures reported for D-serine (Lourenco 2009; Lok 1976).

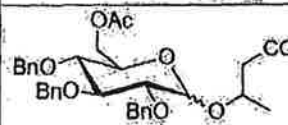
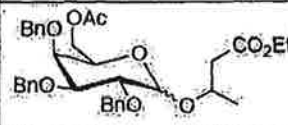
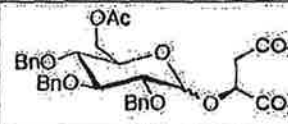
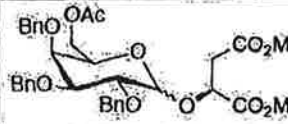
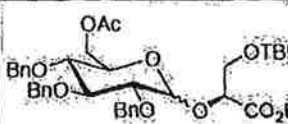
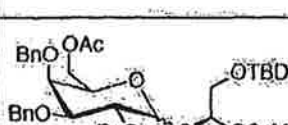


Scheme 1: Glycosylation reaction using thioglycoside donors 1 and 19

Reactions gave a mixture of anomers for most of the glycosyl acceptors. This provided the opportunity to test α and β anomers separately to determine the importance of the stereochemistry of the anomeric position for the stabilization effect. This was the case for the D/L-glycerate and malate galactosyl derivatives.

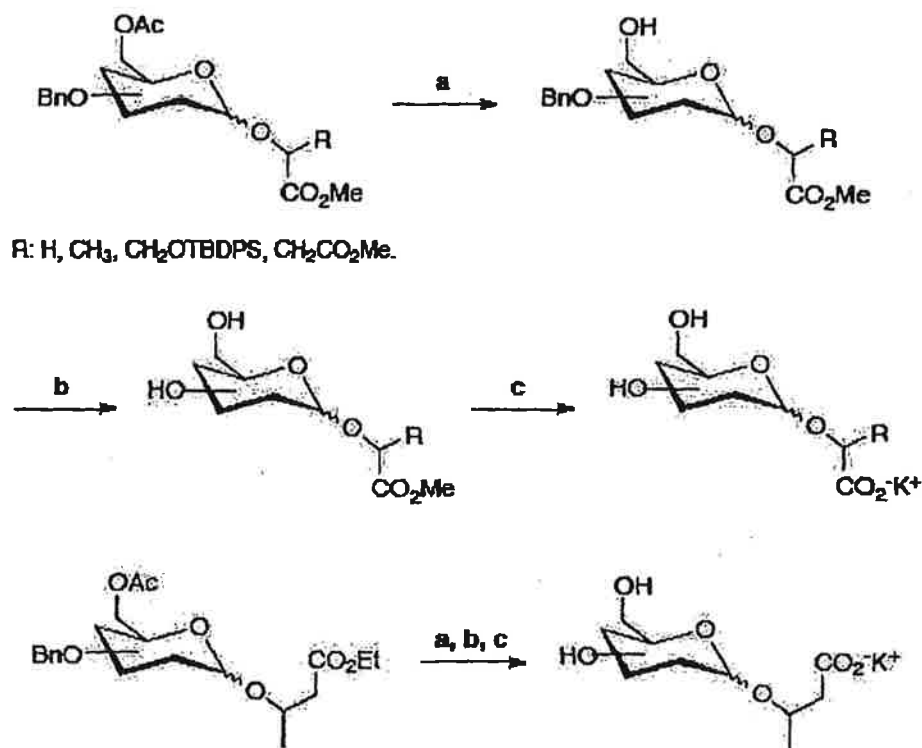
Table 1: Results obtained for the glycosylation reaction with the thioglycoside donors 1 and 19.

ROH	Donor	Product	Yield	α/β
4	1	 10	91	4:1
	19	 32	87	3:1
7	1	 13	93	4:1
	19	 34	95	3:1
9	19	 35	88	2:1

ROH	Donor	Product	Yield	α/β
133	1	 136	84	9:1 ^a
	19	 137	95	3:1 ^a
134	1	 138	94	7:1
	19	 139	87	5:1
135	1	 140	98	>10:1
	19	 141	88	2.6:1

^aCalculated after deprotection of the acetate group.

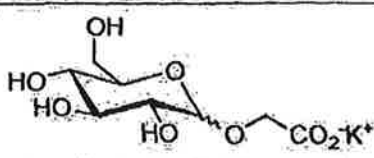
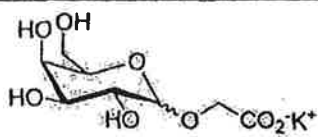
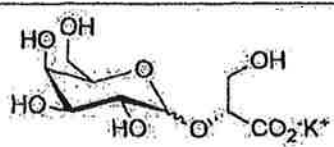
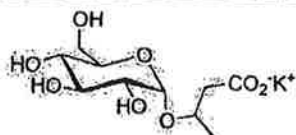
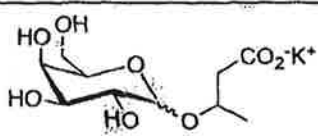
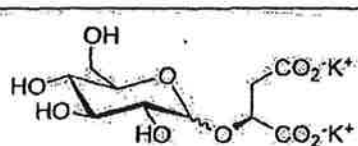
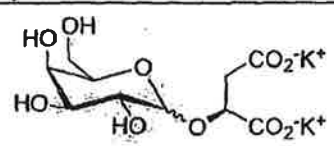
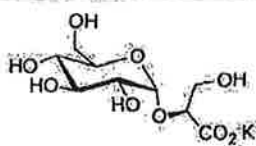
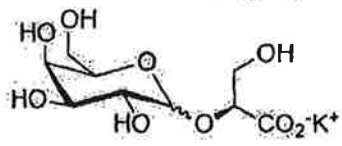
After the successive removal of the protective groups using common organic reactions, such as methanolysis of the acetates, fluorolysis of the silyl ether in the case of the glycerate analogues (compounds **35**, **140** and **141**) and hydrolysis of the ester group (Scheme 2), the desired products were obtained (Table 2).



Scheme 2: General deprotection scheme for the glucose and galactose analogues.

Table 2: Final products and overall yields^a for glucose and galactose derivatives.

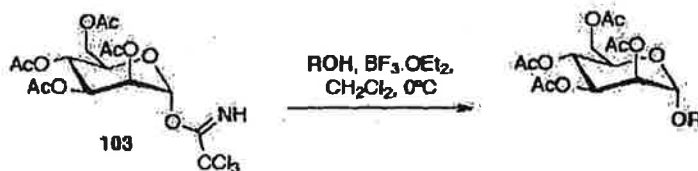
Compound number	Final Product	Yield (%) ^a
10	 142	78
32	 143	83

Compound number	Final Product	Yield (%) ^a
13	 144	73
34	 145	77
35	 146 α 147 β	61 21
136	 148	68
137	 149	86
138	 150	42
139	 151 α 152 β	57 16
140	 153	60
141	 154 α 155 β	43 14

^a Calculated from the glycosylation reaction.

Overall the products were successfully obtained in good yields and the α anomer was the major product. Although in some cases a mixture of α and β anomers was obtained (enriched in α), they were used for a preliminary screening where the most promising compounds would then be tested separately. The lowest yields were obtained for the derivatives of the dimethyl (*S*)-malate (**150**, **151** and **152**, Table 2) due to the use of base in the removal of the acetates and in the hydrolysis of the methyl ester, which promoted the hydrolysis of the anomeric position, by elimination of the malate moiety. This problem has been reported in the literature in the synthesis of bacillithiol (BSH) (Sharma 2011). To minimize the elimination of the malate moiety, careful ester hydrolysis left traces of the mono-ester in the final product.

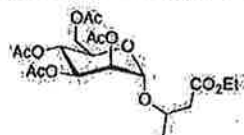
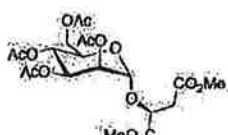
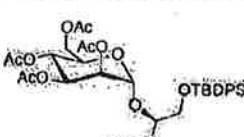
For the synthesis of the 1,2-trans mannosides, the C-2 acyl neighboring group participation strategy was applied using acetates as protecting groups, and trichloroacetamides as glycosyl donors, which are relatively fast to prepare and inexpensive. Glycosylation reaction between the mannose trichloroacetimidate donor **103** and the glycosyl acceptors, using BF_3OEt_2 as the promoter, afforded as expected exclusively the α -products. The results obtained for the glycosylation reaction with the mannose donor **103** are presented in Table 3.



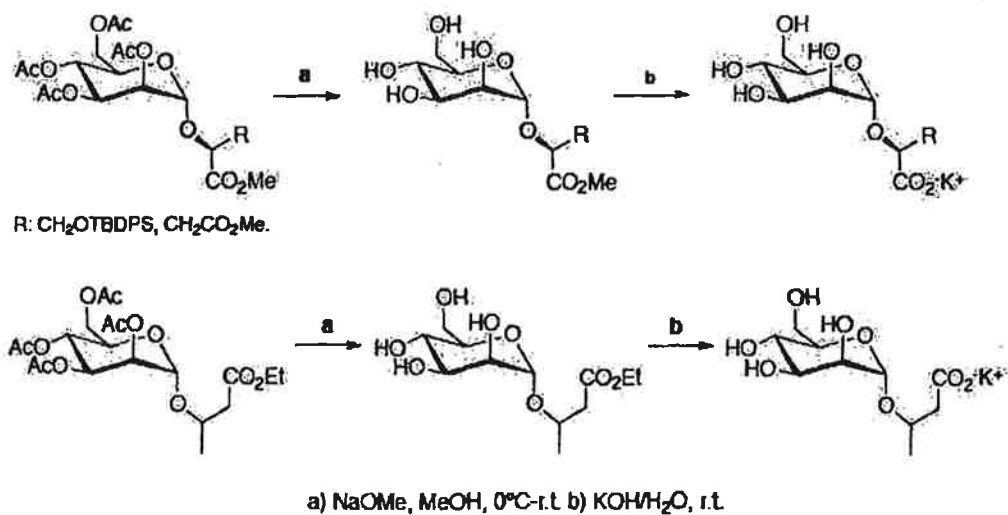
Scheme 3: Glycosylation reaction with the mannose trichloroacetimide donor **103**.

Table 3: Results obtained for the glycosylation reaction with the mannose trichloroacetimide donor **103**.

5

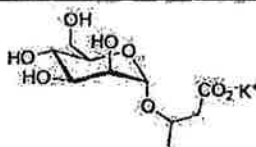
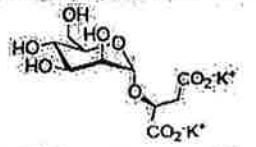
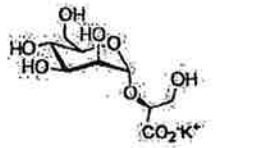
ROH	Product	Yield (%)
133		156 91
134		157 88
135		158 73

Successive removal of the protective groups using common organic reactions (Scheme 4), afforded the desired products (Table 4) in good overall yields.



Scheme 4: General deprotection scheme for the mannose analogues.

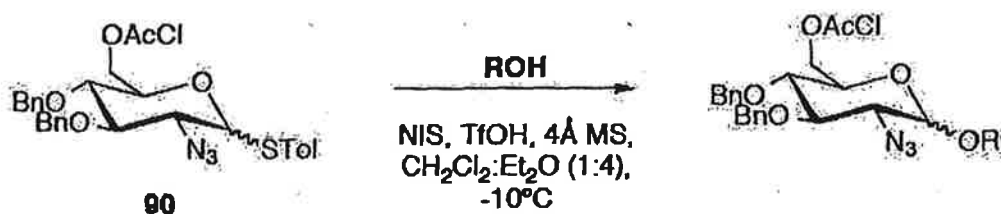
Table 4: Final products and overall yields^a for mannose products.

Compound Number	Final Product	Overall Yield (%) ^a
156		73
157		69
158		50

5 ^a Calculated from the glycosylation reaction

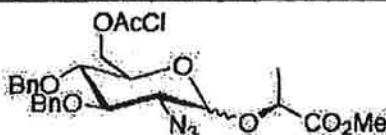
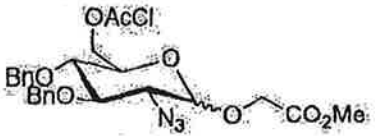
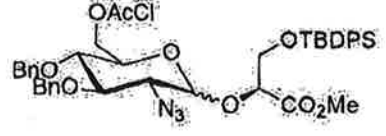
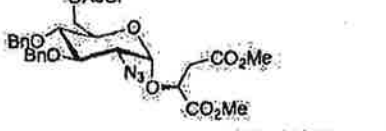
Hydrolysis of the acetate groups and of the methyl ester of mannosyl dimethyl (S)-malate derivative presented the same problem described above for the glucose and galactose derivatives.

10 The 1,2-*cis* glucosamine derivatives were synthesised from the 2-azido-2- deoxythiogluco-
 15 side donor **90**, and the glycosylation reaction with the glycosyl acceptors conducted in a mixture of CH₂Cl₂/Et₂O (4:1) at -10°C and using NIS/TfOH as promotor. The results are presented in Table 5.

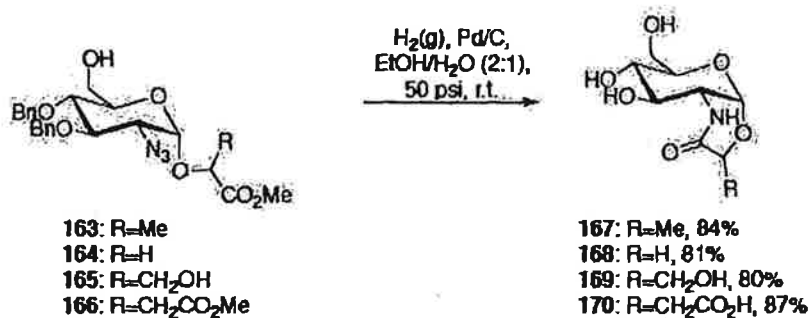


Scheme 5: Glycosylation reaction with the 2-azido-2-deoxythioglucoside donor **90**.

5 Table 5: Results obtained for the glycosylation reaction with the 2-azido-2-deoxythioglucoside donor **90**.

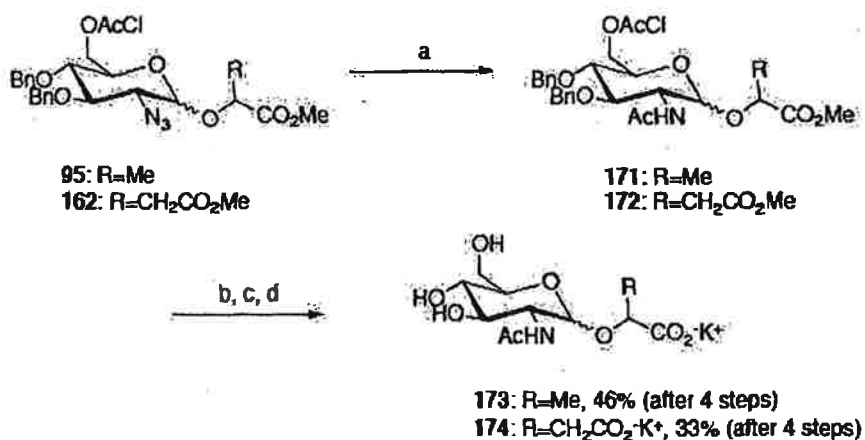
ROH	Product	Yield (%)	α/β	
4		95	86	8.7:1
7		96	76	12:1
9		97	84	>10:1
134		162	86	1:0

10 The α anomer was the major product for all of the acceptors used. After methanolysis of the acetate group and in the case of the glycerate derivative fluorolysis of the silyl ether, catalytic hydrogenation of the benzyl group with simultaneous reduction of the azide promoted the formation of an undesired cyclic amide (Scheme 6). This effect was only observed for the α anomers.



Scheme 6: Hydrogenation of the 2-azido-2-deoxyglucoside derivatives.

Since charge is important for the stabilisation effect, to overcome this problem previous reduction of the azide using a Staudinger reaction, followed by protection of the resulting amine group with acetate (Scheme 7) blocked the amine and avoided cyclisation. After removal of the protecting groups and hydrolysis of the methyl ester the final *N*-acetyl glucosamine derivatives were obtained.



a) (i) Ph₃P, THF, AcOH, 0°C-r.t. (ii) Ac₂O, 0°C. b) NaOMe, MeOH, 0°C. c) H₂ (g), Pd/C, AcOEt, 50 psi, r.t. d) KOH/H₂O, r.t.

Scheme 7: Synthesis of the *N*-acetyl glucosamine derivatives.

Experimental Details

Experiment 1. Synthesis of Ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio- α/β -D-glucopyranoside (1)

The synthesis of compound 1 was carried out according to
5 the procedure described in the literature (Lourenco 2009).

Experiment 2. General glycosylation procedure

A suspension of thioglycoside donor (0.15 mmol), acceptor
(0.15 mmol) and 4Å MS in the solvent/mixture of solvents
indicated (1 mL) was stirred for 1 h at room temperature then
10 cooled to 0°C. *N*-Iodosuccinimide (0.19 mmol) and TfOH (0.9
μL) were added at 0°C and when the reaction was complete
(TLC), 10% Na₂S₂O₃ aqueous solution (2 mL) and saturated
aqueous NaHCO₃ (1 mL) were added and the mixture was
15 extracted with CH₂Cl₂ (3x5 mL); the combined organic phases
were dried (MgSO₄), filtered and the solvent was removed
under vacuum. The crude product was purified by preparative
TLC (3:7, EtOAc/Hex). The α/β ratio of the isolated product
was measured by ¹H NMR (400 MHz, CDCl₃) spectra.

Experiment 3. Synthesis of Methyl (2S)-2-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-glucopyranosyl)propanoate (10)

The glycosylation reaction of donor 1 with acceptor 4 was
performed according to the procedure described in
experiment 2.

Experiment 4. Synthesis of Methyl 2-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-glucopyranosyl)acetate (13)

The glycosylation reaction of donor 1 with acceptor 7 was
performed according to the procedure described in
experiment 2.

Experiment 5. Synthesis of Ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio- α/β -D-galactopyranoside (19)

To a stirred solution of methyl α -D-galactopyranoside (2.0 g, 10.3 mmol) in DMF (20 mL) was added benzyl bromide (6.3 mL, 51.5 mmol). The mixture was cooled to 0°C and sodium hydride (1.48 g, 61.8 mmol) was added portion-wise. The reaction was kept over-night at room temperature (r.t.) and after complete conversion of the starting material MeOH was added at 0°C. The mixture was extracted with Et₂O and the combined organic phases dried, filtered and concentrate. Purification by flash column chromatography on silica gel (10:90, EtOAc/Hex) afforded the product as a viscous colourless foam (5.14 g, 90%).

Concentrated sulphuric acid (1.0 mL) was added dropwise to a stirred solution of the methyl tetra-O-benzylgalactopyranoside (5.72 g, 10.7 mmol) in acetic acid/acetic anhydride (1:1, 50 mL) at 0°C. After complete conversion of the starting material the reaction mixture was quenched with saturated NaHCO₃ solution and ice-cold distilled water until pH 7. The mixture was extracted with EtOAc (3x70 mL) and the combined organic dried, filtered and concentrated in vacuum. The residue was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) to give the diacetate (4.29 g, 75%, $\alpha:\beta=3.7:1$) as a viscous colourless foam.

Ethanethiol (1.56 mL, 20.7 mmol) was added to a stirred solution of diacetate (3.69 g, 6.9 mmol) in DCM (30 mL). The reaction mixture was cooled to 0°C and boron trifluoride diethyl etherate (1.31 mL, 10.35 mmol) added dropwise. After complete conversion of starting material the reaction mixture was diluted with CH₂Cl₂ (2x40 mL) and quenched with saturated NaHCO₃ solution until pH 7.

The aqueous phase was extracted with CH₂Cl₂ (2×40 mL) and the combined organic extracts dried, filtered and concentrated in vacuum. The residue was purified by flash column chromatography (20:80, EtOAc/Hex) to give the
5 thiogalactoside **19** (3.39 g, 91%, $\alpha/\beta=2.4:1$) as a viscous colourless foam.

Experiment 6. Synthesis of Methyl (2S)-2-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)propanoate (32)

The glycosylation reaction of donor **19** with acceptor **4** was
10 performed according to the procedure described in experiment 2.

Experiment 7. Synthesis of Methyl 2-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)acetate (34)

The glycosylation reaction of donor **19** with acceptor **7** was
15 performed according to the procedure described in experiment 2.

Experiment 8. Synthesis of Methyl 3-O-tert-butyldimethylsilyl-(2R)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)-2,3-dihydroxypropanoate (35)

20 The glycosylation reaction of donor **19** with acceptor **9** was performed according to the procedure described in experiment 2.

Experiment 9. Synthesis of Phenyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-1-thio- α/β -D-glucopyranoside (85)

25 To a solution of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy- α/β -D-glucopyranose (Goddard-Borger 2007) (6.42 g, 17.2 mmol) and thiophenol (3.55 mL, 34.4 mmol) in CH₂Cl₂ (60 mL) at 0°C was added and BF₃OEt₂ (9.81 mL, 77.4 mmol). The reaction mixture was stirred at r.t. for 48 hours, then

diluted with CH_2Cl_2 and washed with NaHCO_3 . The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried with MgSO_4 , filtered and concentrated under vacuum. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/hexane) to afford **85** (5.40 g, 74%, $\alpha/\beta=2.5:1$) as a colourless viscous foam, and to recover the initial tetraacetate (1.30 g, 20%).

Experiment 10. Synthesis of p-Tolyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-1-thio- α/β -D-glucopyranoside (86)

To a solution of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy- α/β -D-glucopyranose (Goddard-Borger 2007) (3.87 g, 10.4 mmol) and p-toluenethiol (2.57 g, 20.7 mmol) in CH_2Cl_2 (60 mL) at 0°C was added and BF_3OEt_2 (6.57 mL, 51.8 mmol). The reaction mixture was stirred at r.t. for 60 hours, then diluted with CH_2Cl_2 and washed with NaHCO_3 . The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried with MgSO_4 , filtered and concentrated under vacuum. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/hexane) to afford **85** (2.26 g, 50%, $\alpha/\beta=1.8:1$) as a colourless viscous foam, and to recover the initial tetraacetate (1.69 g, 44%).

Experiment 11. Synthesis of Phenyl 2-azido-6-O-tert-butylidiphenylsilyl-2-deoxy-1-thio- α/β -D-glucopyranoside (87)

A solution of NaOMe 1N (6.73 mL, 6.73 mmol) in MeOH was added to a stirred solution of **85** (4.75 g, 11.21 mmol) in MeOH (20 mL) at 0°C . After 3 hours the starting material had been consumed. The reaction mixture was diluted with MeOH and Dowex- H^+ resin was added until neutral pH. Filtration and evaporation of the solvents afforded the triol (3.23 g, 97%) as a viscous gum. To a solution of triol (2.46 g, 8.27 mmol) in pyridine (20

mL) at r.t. was added TBDPSCl (2.36 mL, 9.10 mmol), followed by a catalytic amount of DMAP. The mixture was stirred overnight, then quenched with H₂O (20 mL), extracted with CH₂Cl₂ (3x20 mL) and the combined organic phases were dried (MgSO₄) and concentrated. Purification by flash column chromatography (30:70 AcOEt/hexane) afforded the product **87** (4.12 g, 93%, $\alpha/\beta=1.8:1$) as a white solid.

10 **Experiment 12. Synthesis of p-Tolyl 2-azido-6-O-tert-butylidiphenylsilyl-2-deoxy-1-thio- α/β -D-glucopyranoside (88)**

The procedure of experiment 11 was applied to compound **86** affording compound **88** as a colourless viscous gum in 98% yield ($\alpha/\beta=1.8:1$) over the two steps.

15 **Experiment 13. Synthesis of Phenyl 6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-1-thio- α/β -D-glucopyranoside (89)**

To a stirred solution of **87** (3.91 g, 7.30 mmol) and benzyl bromide (1.97 mL, 22.6 mmol) in DMF (15 mL) at 0°C was added portion-wise sodium hydride (0.45 g, 18.6 mmol). After 2 hours, MeOH was added at 0°C and the reaction mixture was quenched with a saturated aqueous solution and extracted with Et₂O. The combined organic phases were dried with MgSO₄, filtered and evaporated in vacuum. Purification by flash column chromatography (10:90 AcOEt/hexane) afforded the dibenzylated product (4.45 g, 85%) as a white solid, and the tribenzylated product **92** (0.31 g, 7%) as a viscous gum.

To a solution of the dibenzylated product (2.42 g, 3.38 mmol) in THF (10 mL) at r.t. was added TBAF (1.15 g, 4.39 mmol). The reaction mixture was stirred for 3 hours and then water was added. The mixture was extracted with AcOEt

(3x20 mL), dried (MgSO_4) and concentrated to furnish a yellow viscous residue. Purification by flash column chromatography (30:70, AcOEt/hexane) afforded the alcohol (1.43 g, 88%) as a viscous gum.

- 5 To a stirred solution of the alcohol (1.396 g, 2.92 mmol) in pyridine (5 mL) at 0°C was added acetic anhydride (0.55 mL, 5.85 mmol) and a catalytic amount of DMAP. After complete conversion of the starting material water was added. The mixture was extracted with EtOAc, dried
10 (MgSO_4) and concentrated to furnish a viscous residue. Filtration through celite with a mixture of EtOAc/hexane (10/90) afforded the product **89** as a viscous colourless gum (1.38 g, 91%, $\alpha:\beta=1.4:1$).

Experiment 14. Synthesis of Phenyl 2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy-1-thio- α/β -D-glucopyranoside (90)

- The procedure of experiment 13 was applied to compound **87** using chloroacetic anhydride and affording compound **90** as a colourless viscous gum in 66% ($\alpha/\beta=1.6:1$) yield over
20 the three steps. Characterisation data of compound **90** identical to the literature (Csiki 2010).

Experiment 15. Synthesis of p-Tolyl 2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy-1-thio- α/β -D-glucopyranoside (91)

- 25 The procedure of experiment 13 was applied to compound **88** using chloroacetic anhydride and affording compound **91** as a colourless viscous gum in 82% yield ($\alpha/\beta=1:1$) over the three steps.

Experiment 16. Synthesis of Methyl (2S)-2-(2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy- α/β -D-glucopyranosyl)propanoate (95)

The glycosylation reactions of donor 90 and 91 with acceptor 4 were performed according to the procedure described in experiment 2.

Experiment 17. Synthesis of Methyl 2-(2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy- α/β -D-glucopyranosyl)acetate (96)

The glycosylation reaction of donor 91 with acceptor 7 was performed according to the procedure described in experiment 2.

Experiment 18. Synthesis of Methyl (2R)-tert-butyldimethylsilyl-3-(2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy- α/β -D-glucopyranosyl)-2,3-dihydroxypropanoate (97)

The glycosylation reaction of donor 91 with acceptor 9 was performed according to the procedure described in experiment 2.

Experiment 19. Synthesis of 2,3,4,6-Tetra-O-acetyl- α -D-mannopyranosyl trichloroacetimidate (103)

The synthesis of compound 103 was carried out according to the procedure described in the literature (Hanessian 1997).

Experiment 20. Synthesis of Ethyl 3-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-glucopyranosyl)-3-hydroxybutyrate (136)

A suspension of thioglucoside donor 1 (0.815 g, 1.52 mmol), ethyl 3-hydroxybutyrate 133 (0.197 mL, 1.52 mmol) and 4Å MS in CH₂Cl₂ (6 mL) was stirred for 1 h at room temperature then cooled to 0°C. N-Iodosuccinimide (0.434 g, 1.93 mmol)

and TfOH (0.112 mL) were added at 0°C and when the reaction was complete, 10% Na₂S₂O₃ aqueous solution (6 mL) and saturated aqueous NaHCO₃ solution (3 mL) were added. The mixture was extracted with CH₂Cl₂ (3x6 mL), the combined organic phases were dried (MgSO₄), filtered and the solvent was removed under vacuum. The crude product was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) to afford product **136** as a viscous colourless foam (0.771 g, 84 %).

10 **Experiment 21. Synthesis of Ethyl 3-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)-3-hydroxybutyrate (137)**

The glycosylation reaction of thiogalactoside donor **19** (0.638 g, 1.19 mmol) and ethyl 3-hydroxybutyrate **133** (0.170 mL, 1.31 mmol) was performed according to the procedure described in experiment 20. The crude was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) affording the product **137** as a viscous colourless gum (0.685 g, 95 %).

20 **Experiment 22. Synthesis of Dimethyl (2S)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-glucopyranosyl)-2-hydroxysuccinate (138)**

The glycosylation reaction of thiogalactoside donor **1** (0.850 g, 1.58 mmol) and dimethyl (S)-malate **134** (0.208 mL, 1.58 mmol) was performed according to the procedure described in experiment 20. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **138** as a viscous colourless gum (0.949 g, 94 %, $\alpha/\beta=7:1$).

Experiment 23. Synthesis of Dimethyl (2S)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)-2-hydroxysuccinate (139)

The glycosylation reaction of thiogalactoside donor **19** (1.20 g, 2.23 mmol) and dimethyl (S)-malate **134** (0.294 mL, 2.23 mmol) was performed according to the procedure described in experiment 20. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **139** as a viscous colourless gum (1.235 g, 87 %, $\alpha/\beta=5:1$).

Experiment 24. Synthesis of Methyl 3-O-tert-butyltrimethylsilyl-(2S)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-glucopyranosyl)-2,3-dihydroxypropanoate (140)

The glycosylation reaction of thiogalactoside donor **1** (0.300 g, 0.56 mmol) and acceptor **135** (0.200 g, 0.56 mmol) was performed according to the procedure described in experiment 20. The crude was purified by flash column chromatography on silica gel (10:90, EtOAc/Hex) affording the product **140** as a viscous colourless gum (0.463 g, 98 %, $\alpha/\beta>10:1$).

Experiment 25. Synthesis of Methyl 3-O-tert-butyltrimethylsilyl-(2S)-2-O-(6-O-acetyl-2,3,4-tri-O-benzyl- α/β -D-galactopyranosyl)-2,3-dihydroxypropanoate (141)

The glycosylation reaction of thiogalactoside donor **1** (0.300 g, 0.56 mmol) and acceptor **135** (0.200 g, 0.56 mmol) was performed according to the procedure described in experiment 20. The crude was purified by flash column chromatography on silica gel (10:90, EtOAc/Hex) affording the product **140** as a viscous colourless gum (0.463 g, 98 %, $\alpha/\beta>10:1$).

Experiment 26. Synthesis of Potassium (2S)-2-(α -D-glucopyranosyl)propanoate (142)

A solution of NaOMe 1N (0.443 mL, 0.443 mmol) in MeOH was added to a stirred solution of **10** (0.427 g, 0.74 mmol) in MeOH (4 mL) at 0 °C. After 1 h the reaction mixture was neutralized with saturated aqueous NH_4Cl . The aqueous phase was extracted with EtOAc and the combined organic extracts were dried (MgSO_4), filtered and the solvent was removed. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afford the α (0.310 g, 78%) and β -alcohol (0.027 g, 7%) as viscous colourless gums.

A solution of the α -alcohol (0.300 g, 0.56 mmol) in EtOAc was hydrogenated at 50 psi in the presence of Pd/C 10% (0.25 equiv). After 5 hours, the reaction mixture was filtered and the solvent was evaporated to afford the ester as a very viscous colourless foam (0.149 g, quantitative). A solution of 2 M KOH (0.28 mL) was added to a stirred solution of the ester (0.149 g, 0.56 mmol) in H_2O (2 mL). After all of the starting material had been consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **142** as a viscous colorless foam (0.162 g, quantitative). $[\alpha]^{20}_{\text{D}} = +107.2$ ($c = 0.60$, H_2O). $^1\text{H NMR}$ (D_2O): δ 4.93 (d, $J = 3.9$ Hz, 1H, H-1), 3.96 (q, $J = 6.8$ Hz, 1H, CHCH_3), 3.75-3.68 (m, 5H), 3.44 (dd, $J = 9.9$ Hz, $J = 4.0$ Hz, 1H, H-2), 3.35 (t, $J = 9.3$ Hz, 1H, H-4), 1.28 (d, $J = 6.8$ Hz, 3H, CHCH_3) ppm. $^{13}\text{C NMR}$ (CDCl_3): δ 181.0 (CHCO_2^-), 97.3 (C-1), 75.5 (CHCH_3), 73.1 (C-3), 71.9 (C-5), 71.5 (C-2), 69.4 (C-4), 60.1 (C-6), 17.5 (CHCH_3) ppm.

Experiment 27. Synthesis of Potassium (2S)-2-(α/β -D-galactopyranosyl)propanoate (143)

The methanolysis of the acetate group of the galactoside **32** (1.720 g, 2.63 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (1.350 g, 96 %, $\alpha/\beta=3:1$). After catalytic hydrogenation of the benzyl ethers (1.323 g, 2.46 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 23, the compound **143** was obtained as a viscous colorless foam (0.715 g, quantitative, $\alpha/\beta=3:1$). **FTIR** (film) $\nu_{\max}$: 1635 (C=O), 3332 (O-H) cm^{-1} . **^1H NMR** (D_2O): δ 4.97 (d, $J = 3.9$ Hz, H-1 (α)), 4.58 (q, $J = 7.0$ Hz, CHCH_3 (β)), 4.37 (d, $J = 7.7$ Hz, H-1 (β)), 4.25 (q, $J = 6.9$ Hz, CHCH_3 (α)), 3.93-3.88 (m), 3.84-3.79 (m), 3.76-3.64 (m), 3.63-3.52 (m), 3.47 (dd, $J = 9.9, 7.7$ Hz,), 1.38 (d, $J = 7.0$ Hz, CHCH_3 (β)), 1.35 (d, $J = 6.8$ Hz, CHCH_3 (α)) ppm. **^{13}C NMR** (CDCl_3): δ 187.3 (CHCO_2^-), 101.8 (C-1 (β)), 98.9 (C-1 (α)), 75.2, 73.7, 73.1, 72.6, 71.4, 70.7, 69.21, 69.07, 68.5, 68.0, 60.84, 60.80, 52.68, 52.62, 17.1 (CHCH_3) ppm.

Experiment 28. Synthesis of Potassium 2-(α/β -D-glucopyranosyl)acetate (144)

The methanolysis of the acetate group of the glucoside **13** (0.940 g, 1.66 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.765 g, 88 %, $\alpha/\beta=11:1$). After catalytic hydrogenation of the benzyl ethers (0.715 g, 1.37 mmol) and hydrolysis of the methyl ester according to the

procedure described in experiment 26, the compound **144** was obtained as a viscous colorless foam (0.378 g, quantitative, $\alpha/\beta=10:1$). $^1\text{H NMR}$ (D_2O): δ 4.88 (d, $J = 3.8$ Hz, H-1 (α)), 4.41 (d, $J = 7.9$ Hz, H-1 (β)), 4.22 (d, $J = 15.6$ Hz, $\text{CHH}'\text{CO}_2^-$ (β)), 4.06 (d, $J = 15.5$ Hz, $\text{CHH}'\text{CO}_2^-$ (α)), 4.03 (d, $J = 15.8$ Hz, $\text{CHH}'\text{CO}_2^-$ (α)), 3.88 (d, $J = 15.5$ Hz, $\text{CHH}'\text{CO}_2^-$ (β)), 3.79-3.61 (m), 3.46 (dd, $J = 9.8, 3.8$ Hz), 3.34 (t, $J = 9.5$ Hz) ppm. $^{13}\text{C NMR}$ (CDCl_3): δ 177.4, 102.3 (C-1 (β)), 98.3 (C-1 (α)), 75.9 (β), 75.5 (β), 73.1, 71.9, 71.5, 69.5, 68.5 (CH_2CO_2^- (β)), 66.8 (CH_2CO_2^- (α)), 60.61 (C-6 (β)), 60.43 (C-6 (α)) ppm.

Experiment 29. Synthesis of Potassium 2-(α/β -D-galactopyranosyl)acetate (145)

The methanolysis of the acetate group of the galactoside **34** (1.079 g, 1.91 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.800 g, 81 %, $\alpha/\beta=2:1$). After catalytic hydrogenation of the benzyl ethers (0.787 g, 1.50 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **145** was obtained as a viscous colorless foam (0.416 g, quantitative, $\alpha/\beta=2:1$). $^1\text{H NMR}$ (D_2O): δ 4.91 (d, $J = 3.9$ Hz, H-1 (α)), 4.34 (d, $J = 7.7$ Hz, H-1 (β)), 4.24 (d, $J = 15.6$ Hz, $\text{CHH}'\text{CO}_2^-$ (β)), 4.06 (d, $J = 15.6$ Hz, $\text{CHH}'\text{CO}_2^-$ (α)), 4.03 (d, $J = 15.6$ Hz, $\text{CHH}'\text{CO}_2^-$ (β)), 3.92-3.84 (m), (d, $J = 15.6$ Hz, $\text{CHH}'\text{CO}_2^-$ (α)), 3.75-3.71 (m), 3.69-3.58 (m), 3.52 (dd, $J = 10.0$ Hz, $J = 7.6$ Hz, H-2 (β)) ppm. $^{13}\text{C NMR}$ (CDCl_3): δ 177.5, 102.9 (C-1 (β)), 98.3 (C-1 (α)), 75.2 (β), 72.6 (β), 71.1 (α), 70.8 (β), 69.5 (α), 69.2 (α), 68.6 (β), 68.5 (CH_2CO_2^- (β)), 68.4 (α), 66.8 (CH_2CO_2^- (α)), 61.15 (C-6 (α)), 60.95 (C-6 (β)) ppm.

Experiment 30. Synthesis of Potassium (2R)-2-O-(α/β -D-galactopyranosyl)-2,3-dihydroxypropanoate (146, 147)

The methanolysis of the acetate group of the galactoside **35** (1.078 g, 1.29 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (20:80, EtOAc/Hex) affording the α (0.671 g, 66 %) and the β -alcohol (0.286 g, 28 %) as viscous colourless gums.

TBAF (1M in THF; 0.83 mL, 0.83 mmol) was added to a solution of the α -galactoside (0.655 g, 0.83 mmol) in THF (4 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried (MgSO_4) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the α -diol as a viscous colourless gum (0.457 g, 92%). After catalytic hydrogenation of the benzyl ethers from the α -diol (0.140 g, 1.50 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **146** was obtained as a viscous colorless foam (0.416 g, quantitative). **Alpha product 146:** $[\alpha]^{20}_{\text{D}} = +127.6$ ($c = 0.62$, H_2O). $^1\text{H NMR}$ (D_2O): δ 4.97 (d, $J = 3.9$ Hz, 1H, H-1), 4.13 (dt, $J = 4.7$ Hz, $J = 2.3$ Hz, 1H, CHCH_2OH), 3.99 (t, $J = 6.2$ Hz, 1H), 3.93-3.87 (m, 2H), 3.81 (dd, $J = 12.1, 3.2$ Hz, 1H), 3.77-3.70 (m, 2H), 3.69-3.64 (m, 2H, H-6, H'-6) ppm. $^{13}\text{C NMR}$ (D_2O): δ 177.1 (CHCO_2^-), 97.6 (C-1), 79.2 (CHCH_2OH), 71.3, 69.6, 69.3, 68.5, 63.1 (CHCH_2OH), 61.2 (C-6) ppm.

The same strategy was applied for the deprotection of the β -galactoside (0.266 g, 0.34 mmol). After fluorolysis (0.140 g, 76%), catalytic hydrogenation of the benzyl

ethers from the β -diol (0.340 g, 0.615 mmol) and hydrolysis of the methyl ester, the compound **147** was obtained as a viscous colorless foam (0.188 g, quantitative). **Beta product 147**: $^1\text{H NMR}$ (D_2O): δ 4.42 (d, J = 7.5 Hz, 1H, H-1), 4.11 (dd, J = 6.5 Hz, J = 3.2 Hz, 1H, CHCH_2OH), 3.83-3.77 (m, 2H), 3.73-3.67 (m, 2H), 3.64-3.53 (m, 4H) ppm. $^{13}\text{C NMR}$ (D_2O): δ 177.9 (CHCO_2^-), 102.6 (C-1), 81.3 (CHCH_2OH), 75.1, 72.6, 70.9, 68.7, 62.6 (CHCH_2OH), 60.9 (C-6) ppm.

10 **Experiment 31. Synthesis of Potassium 3-O-(α -D-glucopyranosyl)-3-hydroxybutyrate (148)**

The methanolysis of the acetate group of the glucoside **136** (0.823 g, 1.36 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the α (0.594 g, 82 %) and the β -alcohol (0.066 g, 9 %) as viscous colourless gums.

After catalytic hydrogenation of the benzyl ethers of the α -alcohol (0.516 g, 0.94 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **148** was obtained as a viscous colorless foam (0.285 g, quantitative). $^1\text{H NMR}$ (D_2O): δ 4.98 (d, J = 4.0 Hz, H-1), 4.97 (d, J = 4.2 Hz, H-1), 4.14-4.04 (m, CHCH_3), 3.80-3.59 (m), 3.45-3.39 (m, H-2), 3.34-3.28 (m, H-4), 2.47 (dd, J = 14.2 Hz, J = 6.9 Hz, $\text{CHCH}_2\text{CO}_2^-$), 2.40-2.22 (m, $\text{CHCH}_2\text{CO}_2^-$), 1.21 (d, J = 6.1 Hz, CHCH_3), 1.14 (d, J = 5.9 Hz, CHCH_3) ppm. $^{13}\text{C NMR}$ (D_2O): δ 180.19, 180.16, 97.6 (C-1), 94.9 (C-1), 73.4 (CHCH_3), 73.15 (CHCH_3), 73.06, 71.9, 71.51, 71.48, 71.32, 70.5, 69.62, 69.56, 60.6 (C-6), 60.3 (C-6), 45.6 ($\text{CHCH}_2\text{CO}_2^-$), 44.5 ($\text{CHCH}_2\text{CO}_2^-$), 20.6 (CHCH_3), 18.0 (CHCH_3) ppm.

Experiment 32. Synthesis of Potassium 3-O-(α/β -D-galactopyranosyl)-3-hydroxybutyrate (149)

The methanolysis of the acetate group of the galactoside **137** (0.709 g, 1.17 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol as a viscous colourless gum (0.584 g, 88 %, $\alpha/\beta=3:1$). After catalytic hydrogenation of the benzyl ethers (0.573 g, 1.01 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **145** was obtained as a viscous colorless foam (0.309 g, quantitative, $\alpha/\beta=3:1$). $^1\text{H NMR}$ (D_2O): δ 5.01 (d, $J = 3.9$ Hz, H-1 (α)), 4.99 (d, $J = 3.8$ Hz, H-1 (α)), 4.42 (d, $J = 8.4$ Hz, H-1 (β)), 4.40 (d, $J = 8.1$ Hz, H-1 (β)), 4.23-4.04 (m), 3.97-3.89 (m), 3.84 (t, $J = 3.9$ Hz), 3.78-3.54 (m), 3.40 (dd, $J = 9.6, 8.2$ Hz), 2.54-2.44 (m), 2.40-2.22 (m), 1.22-1.13 (m) ppm. $^{13}\text{C NMR}$ (D_2O): δ 180.3, 179.9, 101.9 (C-1 (β)), 101.1 (C-1 (β)), 97.9 (C-1 (α)), 95.1 (C-1 (α)), 75.14, 75.10, 74.99, 74.0, 73.4, 72.79, 72.65, 71.02, 71.01, 70.8, 70.52, 70.44, 69.58, 69.46, 69.30, 69.1, 68.72, 68.56, 68.46, 68.2, 68.72, 68.56, 68.46, 68.2, 61.23, 61.09, 60.9, 45.75 ($\text{CHCH}_2\text{CO}_2^-$), 45.57 ($\text{CHCH}_2\text{CO}_2^-$), 44.5 ($\text{CHCH}_2\text{CO}_2^-$), 20.6 (CHCH_3), 19.3 (CHCH_3), 18.0 (CHCH_3) ppm.

Experiment 33. Synthesis of Potassium (2S)-2-O-(α/β -D-glucopyranosyl)-2-hydroxysuccinate (150)

The methanolysis of the acetate group of the glucoside **138** (0.263 g, 0.41 mmol) was performed according to the procedure described in experiment 26. The crude was purified by preparative TLC (50:50, EtOAc/Hex) affording the desired alcohol (0.117 g, 48 %) as a viscous

colourless gum, and recovery of the initial **138** (0.068 g, 26 %) and the product of the hydrolysis at the anomeric position (0.046 g, 25 %). After catalytic hydrogenation of the benzyl ethers (0.565 g, 0.95 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **145** was obtained as a viscous colorless foam (0.290 g, 94 %). ¹H NMR (D₂O): δ 4.94 (d, *J* = 3.9 Hz, H-1 (α)), 4.39 (d, *J* = 7.9 Hz, H-1 (β)), 4.19 (dd, *J* = 10.4, *J* = 3.1 Hz, 1H, CO₂⁻CHCH₂CO₂⁻), 3.80-3.63 (m), 3.45-3.28 (m), 3.21-3.15 (m), 2.59 (dd, *J* = 15.1 Hz, *J* = 2.9 Hz, CHCH₂CO₂⁻ (β)), 2.52 (dd, *J* = 15.2 Hz, *J* = 3.2 Hz, CHCH₂CO₂⁻ (α)), 2.42 (dd, *J* = 15.2 Hz, *J* = 10.4 Hz, CHCH₂CO₂⁻ (α)) ppm. ¹³C NMR (D₂O): δ 179.5, 179.1, 135.3 (C-1 (β)), 99.7 (C-1 (α)), 95.9, 79.0, 75.92, 75.72, 74.1, 73.1, 72.2, 71.8, 71.4, 69.6, 69.2, 60.7, 60.0, 41.5 ppm.

Experiment 34. Synthesis of Potassium (2S)-2-O-(α/β-D-galactopyranosyl)-2-hydroxysuccinate 151, 152)

The methanolysis of the acetate group of the galactoside **139** (1.215 g, 1.91 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (50:50, EtOAc/Hex) affording the α (0.642 g, 57 %) and β-alcohol (0.176 g, 16%) as viscous colourless gums, and recovery of the starting material **139** (0.020 g, 16 %) and the product of the hydrolysis at the anomeric position (0.067 g, 8 %). After catalytic hydrogenation of the benzyl ethers from the α (0.640 g, 1.07 mmol) and β-alcohol (0.159 g, 0.27 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compounds **151** (0.401 g, quantitative) and **152** (0.100 g, quantitative) were obtained as viscous colorless foams. **Alpha product 151: FTIR** (film) *v*_{max}: 1634 (C=O), 3332 (O-H) cm⁻¹. ¹H NMR (D₂O): δ 4.96 (d, *J* = 4.0

Hz, 1H, H-1) 4.20 (dd, $J = 10.3, 3.1$ Hz, $\text{CO}_2^- \text{CHCH}_2\text{CO}_2^-$),
 4.05-4.02 (m), 3.94 (d, $J = 2.8$ Hz), 3.86 (dd, $J = 10.4$
 Hz, $J = 3.3$ Hz), 3.70-3.54 (m), 2.54 (dd, $J = 15.2$ Hz,
 3.2 Hz, 1H, $\text{CHCH}_2\text{CO}_2^-$), 2.43 2.42 (dd, $J = 15.2$ Hz, J
 5 = 10.3 Hz, 1H, $\text{CHCH}_2\text{CO}_2^-$) ppm. **Beta product 152: FTIR** (film)
 ν_{max} : 1736 (C=O), 3410 (O-H) cm^{-1} . **^1H NMR** (D_2O): δ 4.52 (dd,
 $J = 10.0$ Hz, $J = 3.1$ Hz, 1H, $\text{CO}_2^- \text{CHCH}_2\text{CO}_2^-$), 4.33 (d, $J =$
 7.5 Hz, 1H, H-1), 3.83-3.82 (m, 1H), 3.77-3.50 (m, 5H),
 2.62 (dd, $J = 15.2, 3.1$ Hz, 1H, $\text{CHCH}_2\text{CO}_2^-$), 2.44 (dd, $J =$
 10 15.2, 10.0 Hz, 1H, $\text{CHCH}_2\text{CO}_2^-$). **^{13}C NMR** (D_2O): δ 179.4
 (CO_2^-), 179.0 (CO_2^-), 102.0 (C-1), 77.6 ($\text{CHCH}_2\text{CO}_2^-$), 75.4,
 72.8, 70.9, 68.8, 61.3 (C-6), 41.9 ($\text{CHCH}_2\text{CO}_2^-$) ppm.

Experiment 35. Synthesis of Potassium (2S)-2-O-(α -D-glucopyranosyl)-2,3-dihydroxypropanoate (153)

15 The methanolysis of the acetate group of the glucoside
140 (0.450 g, 0.54 mmol) was performed according to the
 procedure described in experiment 26. The crude was
 purified by flash column chromatography on silica gel
 (20:80, EtOAc/Hex) affording the α (0.299 g, 70 %) and
 20 the β -alcohol (0.030 g, 7 %) as viscous colourless gums.

TBAF (1M in THF; 0.37 mL, 0.37 mmol) was added to a
 solution of the α -glucoside (0.290 g, 0.37 mmol) in THF (2
 mL) at r.t. The reaction mixture was stirred for 4 hours
 and then water was added. The mixture was extracted with
 25 EtOAc, dried (MgSO_4) and concentrated to give a yellow
 viscous residue. Purification by flash column
 chromatography on silica gel (80:20, EtOAc/hexane)
 afforded the α -diol as a viscous colourless gum (0.162 g,
 80%). After catalytic hydrogenation of the benzyl ethers
 30 from the α -diol (0.150 g, 0.27 mmol) and hydrolysis of the
 methyl ester according to the procedure described in
 experiment 26, the compound **153** was obtained as a viscous

colorless foam (0.077 g, quantitative). ¹H NMR (D₂O): δ 4.96 (d, J = 3.9 Hz, 1H, H-1), 3.96 (dd, J = 3.3 Hz, J = 6.5 Hz, 1H, CHCH₂OH), 3.79- 3.75 (m, 3H), 3.72-3.63 (m, 3H), 3.48 (dd, J = 3.9 Hz, J = 9.9 Hz, 1H, H-2), 3.37 (t, J = 9.6 Hz, 1H, H-4) ppm. ¹³C NMR (D₂O): δ 177.4 (CO₂⁻), 99.2 (C-1), 81.4 (CHCH₂OH), 72.9, 72.2, 71.7 (C-2), 69.3 (C-4), 62.6 (CHCH₂OH), 60.0 (C-6) ppm.

Experiment 36. Synthesis of Potassium (2S)-2-O-(α-D-galactopyranosyl)-2,3-dihydroxypropanoate (154)

10 The methanolysis of the acetate group of the α-galactoside **141** (0.640 g, 0.77 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the alcohol (0.572 g, 94 %) as a viscous colourless residue.

TBAF (1M in THF; 0.83 mL, 0.88 mmol) was added to a solution of the α-galactoside (0.695 g, 0.88 mmol) in THF (5 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried (MgSO₄) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the diol as a viscous colourless gum (0.343 g, 71%). After catalytic hydrogenation of the benzyl ethers from the diol (0.310 g, 0.56 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **154** was obtained as a viscous colorless foam (0.172 g, quantitative). ¹H NMR (D₂O): δ 5.01 (d, J = 3.9 Hz, 1H, H-1), 4.28 (t, J = 4.2 Hz, 1H, CHCH₂OH), 4.18-3.92 (m, 4H), 3.85-3.58 (m, 4H) ppm.

Experiment 37. Synthesis of Potassium (2S)-2-O-(β -D-galactopyranosyl)-2,3-dihydroxypropanoate (155)

The methanolysis of the acetate group of the β -galactoside **141** (0.275 g, 0.33 mmol) was performed according to the procedure described in experiment 26. The crude was purified by flash column chromatography on silica gel (40:60, EtOAc/Hex) affording the alcohol (0.243 g, 93 %) as a viscous colourless residue.

TBAF (1M in THF; 0.83 mL, 0.44 mmol) was added to a solution of the β -galactoside (0.350 g, 0.44 mmol) in THF (3 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried (MgSO_4) and concentrated to give a yellow viscous residue. Purification by flash column chromatography on silica gel (80:20, EtOAc/hexane) afforded the diol as a viscous colourless gum (0.151 g, 62 %). After catalytic hydrogenation of the benzyl ethers from the diol (0.140 g, 0.25 mmol) and hydrolysis of the methyl ester according to the procedure described in experiment 26, the compound **155** was obtained as a viscous colorless foam (0.078 g, quantitative). ^1H NMR (D_2O): δ 4.38 (d, J = 7.4 Hz, 1H, H-1), 4.28 (dd, J = 6.2 Hz, J = 2.9 Hz, 1H, CHCH_2OH), 3.84 (dt, J = 7.2, 3.8 Hz, 2H), 3.76-3.68 (m, 3H), 3.66-3.53 (m, 4H) ppm. ^{13}C NMR (D_2O): δ 177.1 (CO_2^-), 102.5 (C-1), 81.4 (CHCH_2OH), 75.3, 72.8, 71.0, 68.6, 63.2 (CHCH_2OH), 61.0 (C-6) ppm.

Experiment 38. Synthesis of Ethyl 3-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-3-hydroxybutyrate (156)

Ethyl 3-dihydroxybutyrate (0.348 mL, 2.68 mmol) was added to a solution of trichloroacetamidate **103** (1.100 g, 2.23 mmol) in dry CH_2Cl_2 (6 mL). The solution was cooled to 0°C

and BF_3OEt_2 (0.0.282 mL, 2.23 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of NaHCO_3 was added, followed by extractions with CH_2Cl_2 (3x15 mL). The combined organic phases were dried (MgSO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (40:60, EtOAc/hexane) to afford **156** (0.943 g, 91 %) as a viscous colourless residue.

Experiment 39. Synthesis of Dimethyl (2S)-2-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-2-hydroxysuccinate (157)

The glycosylation reaction of trichloroacetamidate donor **103** (1.540 g, 3.12 mmol) and ethyl dimethyl dimethyl (S)-malate **134** (0.494 mL, 3.75 mmol) was performed according to the procedure described in experiment 38. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **157** as a viscous colourless gum (1.359 g, 88 %).

Experiment 40. Synthesis of Methyl 3-O-tert-butyldimethylsilyl-(2S)-2-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-2,3-dihydroxypropanoate (158)

The glycosylation reaction of trichloroacetamidate donor **103** (1.30 g, 2.64 mmol) and acceptor **135** (0.946 g, 2.64 mmol) was performed according to the procedure described in experiment 38. The crude was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) affording the product **158** as a viscous colourless gum (1.33 g, 73 %).

Experiment 41. Synthesis of Potassium 3-O-(α -D-mannopyranosyl)-3-hydroxybutyrate (159)

A solution of NaOMe 1N (0.36 mL, 0.36 mmol) in MeOH was added to a stirred solution of **156** (0.276 g, 0.60 mmol)

in MeOH (3 mL) at 0 °C. After complete conversion of the starting material, previously activated Dowex-H⁺ resin was added until neutral pH. After filtration with MeOH and water, the solvent was removed in vacuum to yield
5 the deprotected mannoside as a viscous colourless gum (0.157 g, 90 %).

A solution of 2 M KOH (0.78 mL) was added to a stirred solution of the previously mannoside (0.431 g, 1.56 mmol) in H₂O (4 mL). After all of the starting material had been
10 consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **159** as a viscous colorless foam (0.446 g, quantitative). ¹H NMR (D₂O): δ 4.91 (d, *J* = 7.5 Hz), 4.18-4.09 (m, (CHCH₃)), 3.84-3.77 (m), 3.74-3.63 (m), 3.57 (t, *J* = 8.9 Hz), 2.43-2.21
15 (m, (CHCH₂CO₂⁻)), 1.20 (d, *J* = 6.1 Hz, CHCH₂CO₂⁻), 1.14 (d, *J* = 5.6 Hz, CHCH₂CO₂⁻) ppm. ¹³C NMR (CDCl₃): δ 179.9 (CH₂CO₂⁻), 179.8 (CH₂CO₂⁻), 99.7 (C-1), 96.5 (C-1), 73.4, 72.9, 72.5, 70.57, 70.52, 70.41, 70.29, 70.24, 66.84, 66.72, 61.0 (C-6), 60.7 (C-6), 45.5 (CHCH₂CO₂⁻), 44.8 (CHCH₂CO₂⁻),
20 20.8 (CHCH₃) ppm.

Experiment 42. Synthesis of Potassium (2S)-2-O-(α-D-mannopyranosyl)-2-hydroxysuccinate (160)

The methanolysis of the acetate groups of the mannoside **157** (1.343 g, 2.72 mmol) was performed according to the
25 procedure described in experiment 41. The crude was purified by column chromatography on silica gel (20:80, MeOH/CH₂Cl₂) affording the desired deprotected mannoside (0.685 g, 78 %) as a viscous colourless gum, and the product of the hydrolysis at the anomeric position, the
30 D-mannopyranoside (0.100 g, 20 %). After hydrolysis of the methyl ester according to the procedure described in experiment 38 compound **160** was obtained as a

viscous colorless foam (0.0.786 g, quantitative). ^1H NMR (D_2O): δ 4.85 (t, $J = 15.6$ Hz, 1H), 4.85 (s, 1H, H-1), 4.24 (dd, $J = 8.2$ Hz, $J = 4.7$ Hz, 1H), 3.90-3.58 (m, 5 H), 2.79-2.64 (m, 2H) ppm.

5 **Experiment 43. Synthesis of Potassium (2S)-2-O-(D-mannopyranosyl)-2,3-dihydroxypropanoate (161)**

TBAF (1M in THF; 0.38 mL, 0.38 mmol) was added to a solution of the **158** (0.220 g, 0.32 mmol) in THF (3 mL) at r.t. The reaction mixture was stirred for 4 hours and
10 then water was added. The mixture was extracted with EtOAc, dried (MgSO_4) and concentrated to give a yellow viscous residue. Purification by preparative TLC (60:40, EtOAc/hexane) afforded the alcohol as a viscous colourless gum (0.103 g, 72 %). The methanolysis of the
15 acetate groups of the alcohol (0.518 g, 1.15 mmol) was performed according to the procedure described in experiment 41. After complete conversion of the starting material, previously activated Dowex- H^+ resin was added until neutral pH. After filtration with MeOH and water,
20 the solvent was removed in vacuum to yield the deprotected mannoside as a viscous colourless gum (0.312 g, 96 %). Hydrolysis of the methyl ester according to the procedure described in experiment 41, the compound
161 was obtained as a viscous colorless foam (0.300 g, 25 quantitative). ^1H NMR (D_2O): δ 4.89 (d, $J = 1.4$ Hz, 1H, H-1), 4.02 (dd, $J = 7.1, 3.2$ Hz, 1H, CHCO_2^-), 3.99 (dd, $J = 3.4, 1.6$ Hz, 1H, H-), 3.89 (dd, $J = 9.5, 3.4$ Hz, 1H), 3.79 (dd, $J = 12.2, 3.1$ Hz, 1H, H-), 3.74-3.61 (m, 5H) ppm. ^{13}C NMR (CDCl_3): δ 100.8, 80.6 (C-1), 73.2, 70.5, 70.1,
30 66.5, 62.5, 60.5 ppm.

Experiment 44. Synthesis of Dimethyl (2S)-2-O-(2-azido-3,4,di-O-benzyl-6-O-chloroacetyl-2-deoxy- α -D-glucopyranosyl)-2-hydroxysuccinate (162)

A suspension of thioglucoside donor **91** (0.750 g, 1.32 mmol), methyl (S)- malate **134** (0.197 mL, 1.52 mmol) and 4Å MS in $\text{CH}_2\text{Cl}_2:\text{Et}_2\text{O}$ (1:4, 20 mL) was stirred for 1 h at room temperature then cooled to 0°C. A solution of N-iodosuccinimide (0.0594 g, 2.64 mmol) and TfOH (0.027 mL) in $\text{CH}_2\text{Cl}_2:\text{Et}_2\text{O}$ (1:1, 20 mL) was added at 0°C. After complete conversion of the starting material, 10% $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution (20 mL) and saturated aqueous

NaHCO_3 solution (10 mL) were added. The mixture was extracted with CH_2Cl_2 (3x20 mL), the combined organic phases were dried (MgSO_4), filtered and the solvent was removed under vacuum. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afford product **162** as a viscous colourless foam (0.672 g, 84 %).

Experiment 45. Synthesis of Methyl (2S)-2-(2-azido-3,4,di-O-benzyl-2-deoxy- α -D-glucopyranosyl)propanoate (163)

A solution of NaOMe 1N (0.46 mL, 0.46 mmol) in MeOH was added to a stirred solution of **95** (0.470 g, 0.77 mmol) in MeOH (5 mL) at 0 °C. After 1 hour the reaction mixture was neutralized with saturated aqueous NH_4Cl . The aqueous phase was extracted with EtOAc and the combined organic extracts were dried (MgSO_4), filtered and the solvent was removed. The crude product was purified by flash column chromatography on silica gel (30:70, EtOAc/Hex) to afford **163** (0.355 g, 98%) as a viscous colourless gum.

Experiment 46. Synthesis of Methyl 2-(2-azido-3,4,di-O-benzyl-2-deoxy- α -D-glucopyranosyl)acetate (164)

The procedure of experiment 45 was applied to compound **96** (0.500 g, 0.94 mmol) affording compound **164** as a viscous
5 colourless gum (0.393 g, 92 %).

Experiment 47. Synthesis of Methyl (2R)-tert-butyltrimethylsilyl-3-(2-azido-3,4,di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-2,3-dihydroxypropanoate (165)

TBAF (1M in THF; 1.14 mL, 1.14 mmol) was added to a
10 solution of **97** (0.830 g, 1.03 mmol) in THF (7 mL) at r.t. The reaction mixture was stirred for 4 hours and then water was added. The mixture was extracted with EtOAc, dried (MgSO₄) and concentrated to give a yellow viscous residue. Purification by flash column
15 chromatography on silica gel (50:50, EtOAc/hexane) afforded the alcohol as a viscous colourless gum (0.401 g, 72%). The procedure of experiment 45 was applied to the alcohol (0.296 g, 0.55 mmol) affording **165** as a viscous colourless gum (0.261 g, 92 %).

20 **Experiment 48. Synthesis of Dimethyl (2S)-2-O-(2-azido-3,4,di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-2-hydroxysuccinate (166)**

The procedure of experiment 45 was applied to compound **162** (0.670 g, 1.10 mmol) affording compound **166** as a viscous
25 colourless gum (0.429 g, 73%).

Example 2: Protein stabilizing effects of the compatible solutes of Example 1 in three model proteins

The ability of the new synthetic analogues to stabilize three model proteins against thermal stress was assessed using
30 differential scanning fluorimetry (DSF). In this study, malate

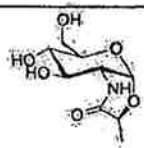
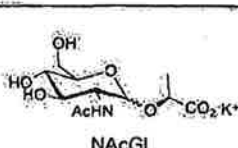
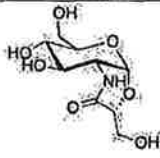
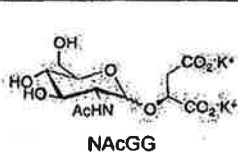
dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme were used as model proteins, and the stabilising effect of the synthetic compounds was compared with the effect of natural solutes, like MG and GG as well as potassium chloride, and other previously synthesised non-natural solutes, like MGlyc and ML.

The compounds tested are shown in Tables 6 and 7.

Table 6: Chemical structures of the natural and synthetic glucose, galactose and mannose derivatives tested in this study.

Glucose derivatives	Galactose derivatives	Mannose derivatives
GL	GaL	ML
GGlyc	GaGlyc	MGlyc
GG (D)	GaG (α, D) GaG (β, D)	MG (D)
GBut	GaBut	MBut
GMal	GaMal (α) GaMal (β)	MMal
GG (L)	GaG (α, L) GaG (β, L)	MG (L)

Table 7: Chemical structures of the synthetic glucosamine and N-acetyl glucosamine derivatives tested in this study.

Glucosamine derivatives	N-Acetyl Glucosamine derivatives
 GNHL	 NAcGL
 GNHG	 NAcGG

The DSF based stability assays were performed at each protein working pH and the melting temperatures (T_M) values determined in the absence (control experiments) and in the presence of solutes, and at different solute concentrations. The denaturation curves for each assay were analysed, and the melting temperatures determined by the calculation of the first derivative, which corresponds to the midpoint temperature of the protein-unfolding transition. In the absence of solutes, malate dehydrogenase (MDH), staphylococcal nuclease (SNase) and lysozyme have melting temperatures (T_M) of 50, 52 and 71°C respectively. The unfolding temperature shifts (ΔT_M) were calculated by comparing the T_M values obtained in the presence of solutes with the T_M values of the control experiments (absence of solutes). Positive ΔT_M values correspond to an increase in the T_M meaning that the protein is more stable and more energy (heat) is needed to unfold it. Negative ΔT_M values correspond to a decrease in the T_M meaning that the protein is less stable.

The increment in the melting temperature (ΔT_M) of the three enzymes induced by the presence of the synthetic and the natural solutes is depicted in Figure 1.

Analysis of different glucose derivatives (Figure 2) and galactose (Figure 3) showed the importance of the non-glycosidic group attached to the hexose.

Concerning the importance of the sugar structure different lactate (Figure 4) and malate derivatives (Figure 5) were analysed.

When plotting the increment of the melting temperature of MDH versus those of SNase and lysozyme (Figures 2-5), a general view of the results arises.

10 General conclusions for the tested proteins:

- charged compounds are better stabilisers;

- malate (the best) and lactate derivatives give higher stabilization;

15 - the non-sugar moiety has greater influence in the stabilisation effect than the hexose structure; and

- glucose and galactose derivatives are better stabilisers.

The stabilising effect of potassium acetate salt (AcOK) on lysozyme was studied in conjugation with the hypersolutes (Figure 6). The results showed that alone AcOK is not a good stabiliser, stabilising only at high salt concentrations. However, in conjugation with the hypersolutes it is able to enhance their stabilisation properties.

25 To determine the importance of the glycosidic linkage of the sugar for the stabilisation effect, different α and β anomers of D and L-galactosyl glycerates were studied (Figure 7). Results obtained for the three enzymes showed that L-glycerates were better stabilisers than the natural D-glycerate derivatives, and that the β -anomers were

better stabilisers than those with the β configuration.

In order to study the dependence of the increment of the melting temperature on the concentration of the solutes, the proteins were tested at different solute concentrations - 0.1, 0.25 and 0.5 M (Figure 8, Figure 9, and Figure 10). For the three proteins, results showed that independent of the degree of stabilisation, the stabilisation effect was directly proportional to the concentration of the solute. Although the results obtained seem to follow a general trend, when taking a closer look in the case of SNase (Figure 8) and lysozyme (Figure 10) α -galactosyl malate is clearly the best stabiliser. In the case of MDH the results show that glucosyl malate was the best stabiliser for this enzyme.

Materials

Mannosylglycerate (MG), glucosylglycerate (GG), glucosylglucosylglycerate (GGG), mannosyl glycolate (MGly) and mannosyl lactate (ML) were obtained by chemical synthesis as described in literature (Costa 1998). New synthetic compounds were obtained by chemical synthesis as described in Example 1. The desired compounds were purified by size exclusion chromatography on a Sephadex G-10 column eluted with water. The fractions containing the pure compounds were pooled, lyophilized. Purity and concentration of the compounds was assessed by ^1H NMR spectra obtained at 500 MHz spectrometer in D_2O . For quantification purposes, spectra were acquired with a repetition delay of 60 s with formate as concentration standard. Only samples with purity higher than 98% were used. Mitochondrial malate dehydrogenase from pig heart (MDH) was purchased from Roche, and hen egg white lysozyme was purchased from Sigma-Aldrich. These enzymes were used without further purification. Recombinant staphylococcal nuclease A (SNase)

was produced and purified from *Escherichia coli* cells as described by Faria 2008. Protein concentration was determined from UV absorbance at 280 nm, using $0.28 \text{ (mg/mL)}^{-1}\text{cm}^{-1}$ for the extinction coefficient of MDH, $2.58 \text{ (mg/mL)}^{-1}\text{cm}^{-1}$ for lysozyme and $0.93 \text{ (mg/mL)}^{-1}\text{cm}^{-1}$ for SNase.

DSF assay

The protein melting temperature (T_M) determination was performed by monitoring protein unfolding with the fluorophore SYPRO Orange dye (Molecular Probes), which although completely quenched in aqueous environment, emits fluorescence upon binding to protein hydrophobic patches. Such increase in fluorescence can be measured as a function of temperature using Differential Scanning Fluorimetry. In a typical assay with a total volume of 20 μL , a protein concentration from 0.14 to 0.21 mg/mL, and a dye concentration of 5 fold were used to guarantee the best signal to noise ratio. Protein stock solutions of SNase or MDH were prepared in phosphate buffer (20 mM of sodium phosphate, pH 7.6), and lysozyme was prepared in citrate buffer (40 mM sodium citrate, 110 mM NaCl, pH 6.0). These stock solutions were extensively dialyzed against the same buffer before the assays. Protein concentrations approximately 1.9 μM were used for MDH, 12.4 μM for SNase and 13 μM for lysozyme. Solute solutions were prepared in water with the respective concentrations. The assay was prepared by adding 2 μL of protein to 8 μL of dye buffer solution, and 10 μL of solute solution, all prepared in the protein purification buffer except for the solutes solutions. Fluorescence intensities versus temperature are used to calculate the protein melting temperature (T_M) by determining the first derivative ($d(R_{fu})/dT$) to extract the exact transition inflection point.

Example 3: Stabilizing effect of six compatible solutes of Example 1 on porcine insulin

The ability of galactosyl lactate **143**, galactosyl butyrate **149**, galactosyl glycerate **146**, glucosyl butyrate **148**, glucosyl glycolate **144**, and glucosyl malate **150** to stabilize porcine insulin was studied using the DSF assay described in Example 2.

For the assay, solutions comprising porcine insulin at a concentration of 129 μ M and the compatible solutes at concentrations of 0.1 and 0.25 M were made. The increase in melting temperature observed for each solute at 0.1 M and 0.25 M is shown in Figure 11.

All six solutes stabilized porcine insulin at both concentrations tested, with glucosyl glycolate and glucosyl malate providing the highest increases in melting temperature.

Discussion

The effectiveness of the new compounds in the protection of model proteins against heat-induced inactivation was assessed using DSF, and compared with the effect of natural solutes, like MG and GG as well as potassium chloride, and other previously synthesised non-natural solutes, like MGlyc and ML. DSF proved to be an excellent high-throughput method to obtain rapid information about the stabilising properties of the molecules.

Analysis of the results obtained showed that the stabilisation effect is not general, and strongly depends on specific protein-solute interactions. Although some solutes showed superior thermostabilisation properties, the degree of stabilisation is different for each protein.

The presence of charge is one the most important features for the stabilisation effect. Uncharged solutes, like the glucosamine cyclic derivatives, gave the lowest stabilisation, and malate derivatives, bearing a double charge, were the best stabilisers.

Concerning the use of different hexoses, glucose and galactose derivatives were better stabilisers than the respective mannose and N-acetylglucosamine derivatives. However, the results showed that the group attached to the sugar had more influence for the stabilisation effect than the nature of the sugar.

The results obtained with the α and β anomers of galactosyl glycerates showed that the α derivatives were better stabilisers.

It is expected that the new compounds described herein will stabilize additional proteins as well as other biological materials. Thus, the new compounds of the invention are useful for the stabilization of biological materials used in pharmaceuticals, e.g. biologics such as antibodies and hormones, cosmetics, food products, etc. The compounds of the invention can be used to protect biological materials against temperature stress, aggregation, and high salinity. For example, compounds of the invention can be used to stabilize biologics during processing, e.g. purification, formulation and/or drying, transportation, and storage.

REFERENCES

- Costa, M.S. et al., "An overview of the role and diversity of compatible solutes in Bacteria and Archaea," *Biotechnology of Extremophiles*, Antranikian, G., Ed., Springer Berlin Heidelberg: 1998; vol. 61, pp 117-153.
- Csiki, Z. and Fugedi, P., "Synthesis of glycosaminoglycan oligosaccharides. Part 4: Synthesis of aza-L-iduronic acid-containing analogs of heparan sulfate oligosaccharides as heparanase inhibitors." *Tetrahedron* 2010, 66, 7821-7837.
- 10 Empadinhas, N. et al., "Organic solutes in *Rubrobacter xylanophilus*: the first example of di-myo-inositol-phosphate in a thermophile." *Extremophiles* 2007, 11, 667-673.
- Faria, C. et al., "Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms," *Carbohydrate Research* 343 (2008) 3025-3033.
- 15 Faria, C. et al., "Inhibition of formation of α -synuclein inclusions by mannosylglycerate in a yeast model of Parkinson's disease," *Biochimica et Biophysica Acta* 1830 (2013) 4065-4072.
- 20 Goddard-Borger, E. D. et al., "An efficient, inexpensive, and shelf-stable diazotransfer reagent: Imidazole-1-sulfonyl azide hydrochloride," *Organic Letters* 2007, 9, 3797-3800.
- Hanessian, S., *Preparative Carbohydrate Chemistry*. Taylor & Francis: 1997.
- 25 Lentzen, G. and Schwarz, T., "Extremolytes: natural compounds from extremophiles for versatile applications," *Appl Microbiol Biotechnol* (2006) 72:623-634.

Lok, C. M et al., "Synthesis of Chiral Glycerides Starting from D-Serine and L-Serine." *Chemistry and Physics of Lipids* 1976, 16, 115-122.

5 Lourenco, E. C, "Synthesis of potassium (2R)-2-O-alpha-D-glucopyranosyl-(1 -> 6)-alpha-D-glucopyranosyl-2,3-dihydroxypropanoate a natural compatible solute." *Carbohydrate Research* 2009, 344, 2073-2078.

10 Luley-Goedl, C. and Nidetzky B, "Glycosides as compatible solutes: biosynthesis and applications," *Nat. Prod. Rep.*, (2011), 28, 875-896.

Pais, T. M. et al., "Structural determinants of protein stabilization by solutes - The importance of the hairpin loop in rubredoxins. *Febs Journal* 2005, 272, 999-1011.

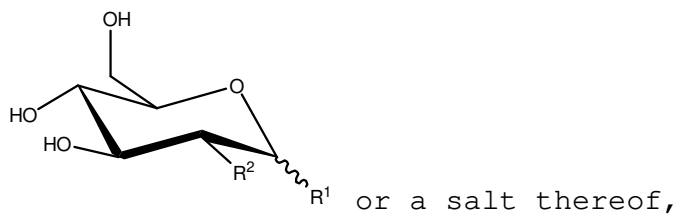
15 PCT International Application Publication No. WO/2007/097653, published August 30, 2007 (Pereira de Oliveira da Silva Santos et al.).

20 Sharma, S. V. et al., "Chemical and chemoenzymatic syntheses of Bacillithiol: A unique low-molecular-weight thiol amongst low G+C gram-positive bacteria," *Angewandte Chemie-International Edition* 2011, 50, 7101-7104.

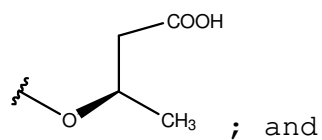
CLAIMS

1. A compound of formula:

(A)

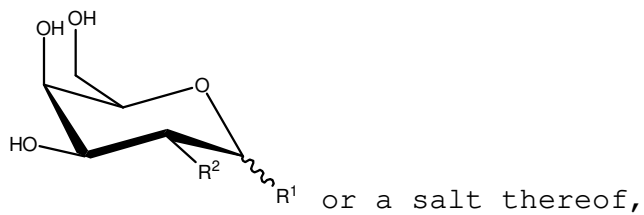


wherein R^1 is , or



R^2 is $-OH$; or

(B)

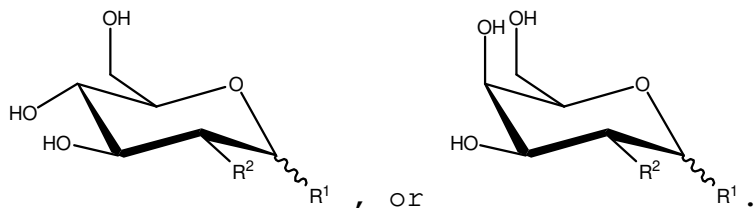


wherein R^1 is $-OC(H)(X)(CH_2)_nC(=O)OH$;

R^2 is $-OH$;

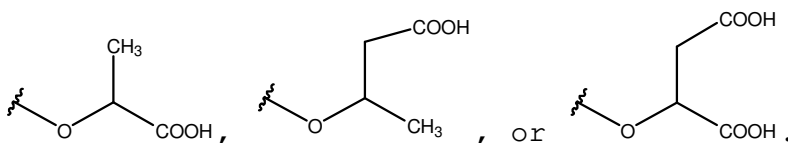
X is $-\text{CH}_3$ or $\text{CH}_2\text{C}(=\text{O})\text{OH}$; and
n is 0 or 1.

2. The compound of claim 1 or a salt thereof, wherein the compound is

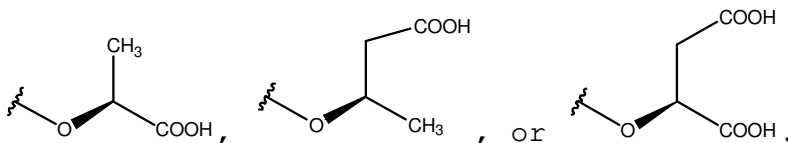


3. The compound of any one of claims 1-2 or a salt thereof, wherein the α/β anomer ratio of the compound is 1:1 to 99:1, or the α/β anomer ratio of the compound is greater than 99:1.

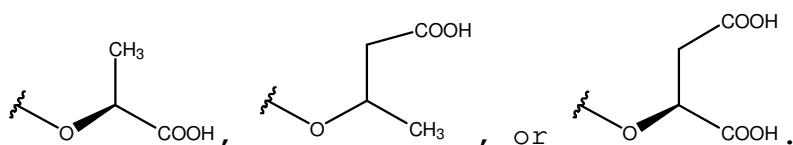
4. The compound of any one of claims 1-2 or a salt thereof, wherein in compound (B) R^1 is



5. The compound of claim 4 or a salt thereof, wherein R^1 is

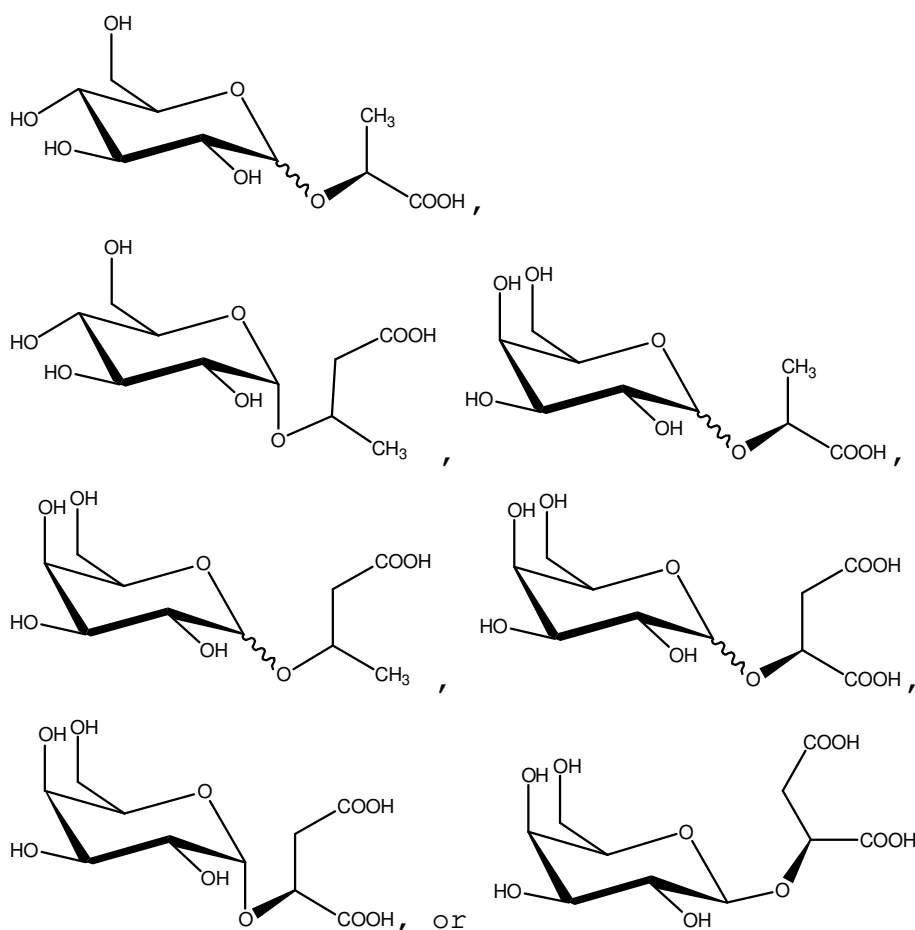


6. The compound of claim 4 or a salt thereof, wherein R^1 is



7. The compound any one of claims 1-2 or a salt thereof, wherein R^2 is -OH.

8. The compound of claim 1 or a salt thereof, wherein the compound is



9. A composition comprising at least one compound of any one of claims 1-2, or a salt thereof, and a biological material selected from the group consisting of a nucleic acid, a

polypeptide, a whole cell, a cell membrane, a cell component, a virus, a liposome, a tissue, and a mixture of any of the foregoing, the composition further comprising or not comprising a buffer.

10. The composition of claim 9, wherein the biological material is a polypeptide, an enzyme, an antibody, a plasma protein, or a hormone, insulin, malate dehydrogenase, staphylococcal nuclease, lysozyme, or a recombinant polypeptide.

11. A method of stabilizing a biological material, comprising adding at least one compound of any one of claims 1-2, or a salt thereof, to a solution containing the biological material selected from the group consisting of a nucleic acid, a polypeptide, a whole cell, a cell membrane, a cell component, a virus, a liposome, a tissue, and a mixture of any of the foregoing to form a stabilized solution, the method further comprising or not comprising a step of drying the stabilized solution.

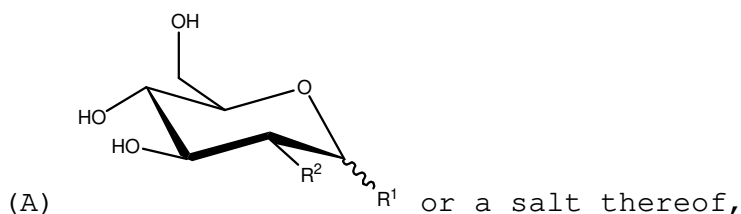
12. The method of claim 11, wherein the biological material is a polypeptide, an enzyme, an antibody, a plasma protein, or a hormone, insulin, malate dehydrogenase, staphylococcal nuclease, lysozyme, or a recombinant polypeptide.

13. The compound of any one of claims 1-2, or a salt thereof, for stabilizing a biological material selected from the group consisting of a nucleic acid, a polypeptide, a whole cell, a cell membrane, a cell component, a virus, a liposome, a tissue, and a mixture of any of the foregoing.

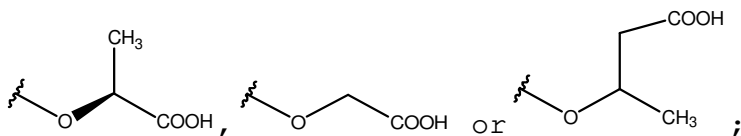
14. A diagnostic kit comprising a compound of any one of claims 1-2, or a salt thereof.

15. The diagnostic kit of claim 14, comprising a microarray, a biosensor, or an enzymatic preparation.

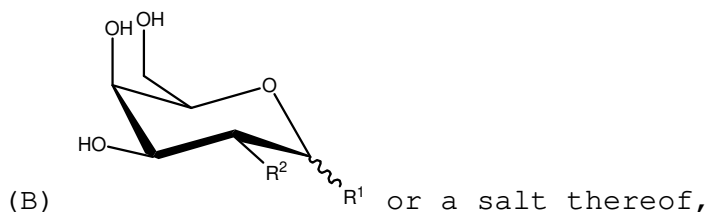
16. A method of stabilizing a biological material, comprising adding to a solution containing the biological material at least one compound of the formula (A) or (B):



wherein R¹ is



R² is -OH;



wherein R¹ is -OC(H)(X)(CH₂)_nC(=O)OH;

R² is -OH;

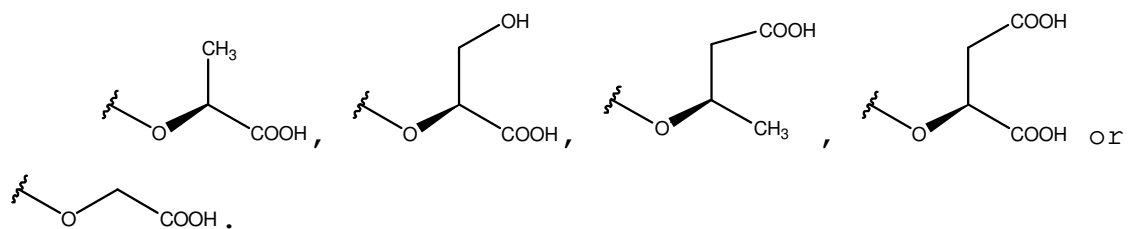
X is -CH₃, -CH₂OH, or CH₂C(=O)OH; and

n is 0 or 1,

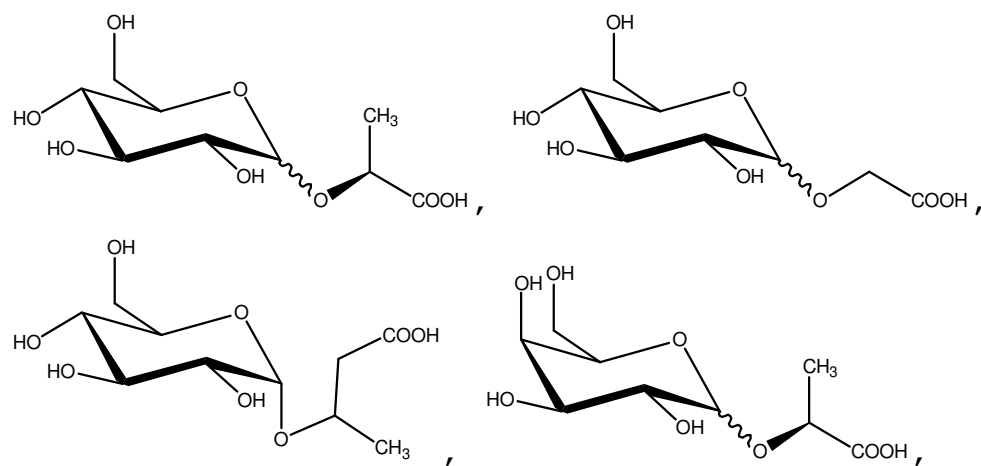
so as to thereby form a stabilized solution of the biological material.

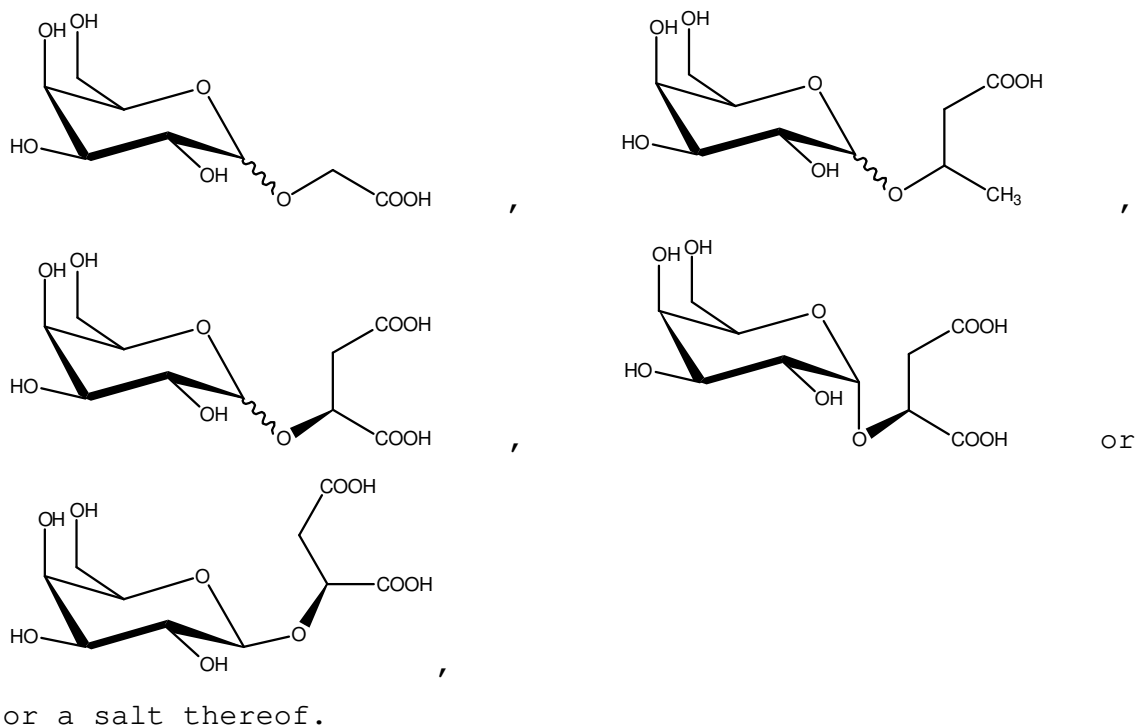
17. The method of claim 16 wherein the α/β anomer ratio of the compound is 1:1 to 99:1, or the α/β anomer ratio of the compound is greater than 99:1.

18. The method of claim 16 wherein in compound (B) R^1 is



19. The method of claim 16, wherein the compound is

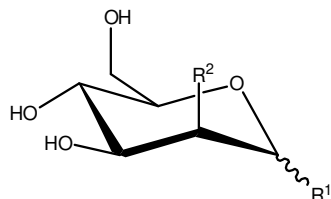




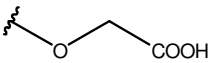
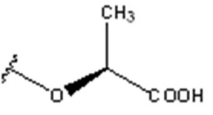
20. The method of claim 16, further comprising a step of drying the stabilized solution.

21. The method of claim 16, wherein the biological material is a polypeptide, an enzyme, an antibody, a plasma protein, a hormone, insulin, malate dehydrogenase, staphylococcal nuclease or lysozyme.

22. A method of stabilizing a biological material, comprising adding to a solution containing the biological material at least one compound of the following formula or a salt thereof,



wherein

R^1 is  or ; and

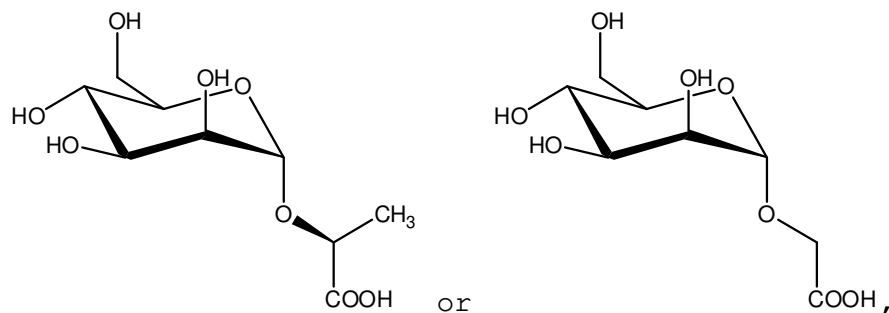
R_2 is OH,

so as to thereby form a stabilized solution of the biological material,

wherein the biological material is an antibody, a plasma protein, a hormone or insulin.

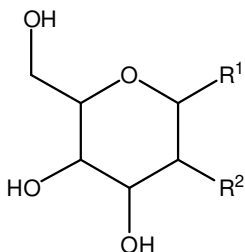
23. The method of claim 22, wherein the biological material is insulin.

24. The method of claim 22, wherein the compound is



or a salt thereof.

25. A method of stabilizing a biological material, comprising adding to a solution containing the biological material at least one compound of the formula I:

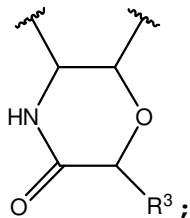


Formula I

or a salt thereof,

wherein:

R¹ and R² together with the carbon atoms to which they are attached form

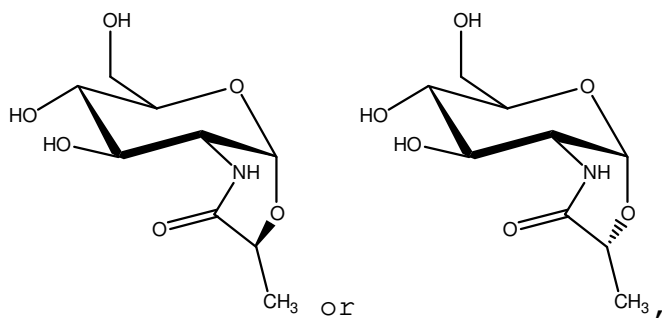


R³ is -H, -CH₃, -CH₂C(=O)OH, or -CH₂OH;

X is -H, -CH₃, -CH₂OH, or CH₂C(=O)OH; and

n is 0 or 1.

26. The method of claim 25, wherein the compound is



or a salt thereof.

27. The method of claim 25 or 26, wherein the biological material is a polypeptide, an enzyme, an antibody, a plasma protein, a hormone, insulin, malate dehydrogenase, staphylococcal nuclease or lysozyme.