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(54) Title: USE OF SPECIFIC GLYCOSIDE PHOSPHORYLASES FOR THE IMPLEMENTATION OF PHOSPHOROLYSIS OR REVERSE PHOSPHOROLYSIS REACTIONS

(57) Abstract: The present invention relates to the use of specific glycoside phosphorylases for the implementation of phosphoro lysis or reverse phosphoro lysis reactions involving a mannosyl donor compound or a mannosyl acceptor compound.

USE OF SPECIFIC GLYCOSIDE PHOSPHORYLASES FOR THE IMPLEMENTATION OF PHOSPHOROLYSIS OR REVERSE PHOSPHOROLYSIS REACTIONS

The present invention relates to the use of specific glycoside phosphorylases for the implementation of phosphorolysis or reverse phosphorolysis reactions involving a mannosyl donor compound or a mannosyl acceptor compound.

The present invention also relates to the use of specific glycoside phosphorylases in a process of degradation, by phosphorolysis, of mannosyl donor compounds that were not known to be degradable by phosphorolysis.

α-D-mannopyranose-1-phosphate is the donor substrate used in reverse phosphorolysis processes catalysed by mannosyl phosphorylases. However, until now, no glycoside phosphorylase was known to be able to catalyse the preparation of said α-D-mannopyranose-1-phosphate from inexpensive mannosyl donor compounds, like mannan.

The present invention also relates to the use of specific glycoside phosphorylases in a process of grafting by reverse phosphorolysis a mannosyl residue on a mannosyl acceptor compound.

Mannosylated compounds present nutritional or medical interest. In particular, beta-linked mannooligosaccharides (MOS) have prebiotic properties that can probably be modulated by varying the mannosyl acceptor molecule to render the oligosaccharide more or less fermentable in the host (human or animal) gut. Moreover, MOS are described as effective in lowering total body fat, because fat excretion has been shown to be increased with MOS consumption by humans or animals (Salinardi, T.C. et al. 2010, J. Nutr. 140, 1943–1948).

In addition, the oligosaccharide Man-GlcNAc is a component of the N-glycans that cover the gastrointestinal tract, to constitute a physical and chemical barrier between the intestinal contents and the underlying epithelia. N-glycans are a source of endogenous substrates for the GI microbial community that uses them as carbon source in addition to plant polysaccharides. Alterations in the structure and/or quantity of N-glycans alter their barrier function and could play roles in initiating and maintaining mucosal inflammation in inflammatory bowel diseases (IBD), and in driving cancer development in the intestine. Restoring the mucosal barrier in chronic inflammation is likely to diminish the risk of cancer development and should be part of the therapeutic goal, particularly in extensive colitis (Sheng, Y.H. et al. 2012, J. Gastroenterol. Hepatol. 27, 28–38).

Glycoside phosphorylases (GPs) are carbohydrate active enzymes which play a crucial role in the metabolism of all living organisms. They catalyse the breakdown of an osidic linkage from oligosaccharides or polysaccharides as donor substrates and the concomitant phosphate glycosylation, to generate a glycosyl phosphate product and a sugar chain of reduced chain length. These enzymes are also able to perform reverse-phosphorolysis, also called synthetic reaction, to form a glycosidic bond between the glycosyl unit coming from the glycosyl-phosphate, which plays the role of sugar donor, and a carbohydrate acceptor.

The GPs ability to form glycosidic linkages in reverse-phosphorolysis is of prime interest for the in vitro synthesis of glycosides, which can be used in medicine, nutrition and cosmetics applications fields.

Other known enzymatic beta-mannoside synthesis processes are based on the use of :

- mannosyl-transferases (glycoside transferases (GT) belonging to GT2, GT4, GT33 families); however, GTs use sugar nucleotides as substrates, which are expensive to produce;

- mannosidases or mannanases (glycoside-hydrolases (GH) belonging to GH2 and GH5 families) that are used for transmannosylation; these enzymes use oligosaccharides or polysaccharides as mannosyl donors, and monosaccharides, oligosaccharides or hydroxylated compounds as acceptors.

In the CAZy database (www.cazy.org), GPs are classified both in glycosyl transferase (GT) and glycoside hydrolase (GH) families, depending on the fold and catalytic mechanism similarities their share with typical GTs and GHs, respectively.

The natural structural and functional diversity of GPs appears to be highly restricted, since (i) they are found in only seven of the 226 GH and GT families listed in the CAZy database (March 2013); (ii) approximately only 15 EC entries are currently assigned to GPs, (iii) their specificity towards glycosyl phosphates is limited to α- and β-D-glucopyranose-1-phosphate which are the most prevalent substrates, α-D-galactopyranose-1-phosphate, *N*-acetyl-α-D-glucosamine-1-phosphate, and α-D-mannopyranose-1-phosphate.

α-D-Mannopyranose-1-phosphate specificity has been described for just four enzymes in the recently created GH130 family, which comprises a total of 533 entries, from archaea, bacteria, and eukaryotes :

- the *B. fragilis* NCTC 9343 mannosylglucose phosphorylase (BfMP), which converts β-D-mannopyranosyl-1,4-D-glucopyranose and phosphate into α-D-mannopyranose-1-phosphate and D-glucose (Senoura, T. et al. (2011), Biochemical and Biophysical Research Communications 408, 701–706). BfMP exhibits a very narrow specificity towards β-D-Man*p*-1,4-D-Glc, meaning that β-D-Man*p*-1,4-D-Glc is the only tolerated mannosyl donor during

BfMP mediated phosphorolyis and that D-glucose is the only tolerated mannosyl acceptor during BfMP mediated reverse phosphorolyis;

- RaMP1 and RaMP2 from the ruminal bacterium *Ruminococcus albus* NE1. These enzymes have also been proposed to participate in mannan catabolism in the bovine rumen, and are assisted by an endo-mannanase and an epimerase, via RaMP2 and RaMP1-catalysed phosphorolysis of β-1,4 mannooligosaccharides and 4-*O*-β-D-mannopyranosyl-D-glucopyranose, respectively (Kawahara R. *et al.* (2012), J Biol Chem 287: 42389–42399. doi:10.1074/jbc.M112.390336);

- Bt1033, a β-D-mannosyl-*N*-acetyl-1,4-D-glucosamine phosphorylase produced by the human gut inhabitant *B. thetaiotamicron* VPI-5482 (Nihira *et al.* (2013), J Biol Chem 288: 27366–27374. doi:10.1074/jbc.M113.469080). This enzyme is not able to phosphorolyse β-D-Man*p*-1,4-D-Glc and β-1,4 mannooligosaccharides (polymerisation degree ≥2), contrary to RaMP2 and UhgbMP.

One of the aims of the present invention is to provide specific glycoside phosphorylases able to degrade, by phosphorolysis, mannosyl donor compounds that were not known to be degradable by phosphorolysis, for example mannan.

Another aim of the present invention is to provide enzymatically synthesized α-D-mannopyranose-1-phosphate from beta-linked mannooligosaccharides and beta-linked mannopolysaccharides, in particular mannan.

Another aim of the present invention is to provide enzymatically synthesized oligosaccharides and glycoconjugates containing beta-linked mannosyl residues from α-D-mannopyranose-1-phosphate.

Still another aim of the invention is to provide inflammatory bowel diseases treatment by inhibiting glycoside-phosphorylases.

The present invention relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, for the implementation of a phosphorolysis or reverse phosphorolysis reaction involving α-D-mannopyranose-1-phosphate,

with the proviso that said glycoside-phosphorylase is not RaMP2.

The present invention also relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, for the implementation of

a phosphorolysis or reverse phosphorolysis reaction involving α-D-mannopyranose-1-phosphate,

with the proviso that said glycoside-phosphorylase is not RaMP2 or Bt1033.

The present invention also relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, to degrade by phosphorolysis a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2.

The present invention also relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, to degrade by phosphorolysis a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2 or Bt1033.

The glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1 is refered to UhgbMP (Unknown human gut bacterium Mannoside Phosphorylase, GenBank

accession number ADD61463.1), encoded by the gene situated between the nucleotides 2620 and 3603 of the nucleotidic sequence with GenBank accession number GU942931.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 80%, in particular of at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, more particularly of at least 90%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 50% and less than 64%, in particular an identity percentage comprised from 50% to 63%, more particularly an identity percentage of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62% or 63%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 64% and less than 80%, in particular an identity percentage comprised from 65% to 79%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 64% and less than 90%, in particular an identity percentage comprised from 65% to 89%, more particularly an identity percentage of 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 50% and less than 66%, in particular an identity percentage comprised from 50% to 65%, more particularly an identity percentage of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64% or 65%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 66% and less than 80%, in particular an identity percentage comprised from 67% to 79%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 66% and

less than 90%, in particular an identity percentage comprised from 67% to 89%, with the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, said glycoside-phosphorylase is characterized, in relation with phosphorolysis, generating α-D-mannopyranose-1-phosphate and a $(Man)_{m-1}$-donor compound from a mannosyl donor of formula $(Man)_m$-donor and inorganic phosphate, by the quantification of said α-D-mannopyranose-1-phosphate, said $(Man)_{m-1}$-donor compound or said inorganic phosphate.

In an advantageous embodiment, said glycoside-phosphorylase is characterized by the quantification, in particular by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), of α-D-mannopyranose-1-phosphate, after having contacted said glycoside-phosphorylase with a mannosyl donor and inorganic phosphate.

The quantification of α-D-mannopyranose-1-phosphate is for example performed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), according to the procedure described by Orvisky, E. et al. (2003), Analytical Biochemistry 317, 12–18.

In an advantageous embodiment, said glycoside-phosphorylase is characterized by the quantification, in particular by high-performance liquid chromatography (HPLC), of said $(Man)_{m-1}$-donor compounds, after having contacted said glycoside-phosphorylase with said $(Man)_m$-donor compounds.

The quantification of the (Man)m-1-donor compounds is for example performed by high-performance liquid chromatography (HPLC), according to the procedure described by Kawahara, R. et al. (2012), J. Biol. Chem. 287, 42389–42399.

In an advantageous embodiment, said glycoside-phosphorylase is characterized by the quantification, in particular by a colorimetric method, of inorganic phosphate, after having contacted said glycoside-phosphorylase with α-D-mannopyranose-1-phosphate and an mannosyl acceptor, in particular D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, or (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

The quantification of inorganic phosphate is for example performed by the colorimetric method described by Gawronski, J.D. et al. (2004), Analytical Biochemistry 327, 114–118, or by Chao, C. et al. (2011), Enzyme and Microbial Technology 49, 59–65.

In an advantageous embodiment, the apparent catalytic efficiency $kcat_{app}/ Km_{app}$ of said glycoside-phosphorylase is above about 0.02 $s^{-1}mM^{-1}$, in particular above about 0.9 $s^{-1}mM^{-1}$, for phosphorolysis in presence of inorganic phosphate and a mannosyl donor selected

from the list constituted by β-D-mannopyranosyl-1,4-D-mannose, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose and β-1,4-D-Mannan.

Apparent phosphorolysis catalytic efficiency is for example determined according to a procedure described by Kawahara, R. et al. (2012), J. Biol. Chem. 287, 42389–42399, with 0.01 mg/ml of purified enzyme by quantifying α-D-mannopyranose-1-phosphate release rate from 5 to 10 mM inorganic phosphate at pH 7.0 and between 1 and 20 mM of β-D-mannopyranosyl-1,4-D-mannose, between 1 and 10 mM of β-D-mannopyranosyl-1,4-D-glucose, between 0.05 and 0.5 mM of β-D-mannopyranosyl-1,4-N,N'-diacetyl chitobiose or between 0.4 and 4 mM of β-1,4-D-Mannan.

The apparent kinetic parameters, $kcat_{app}$ and Km_{app}, are, regarding phosphorolysis, for example determined by fitting the initial rates of α-D-mannopyranose-1-phosphate release, to the Michaelis-Menten equation (*ibid.*). Non-linear regression is for example performed with SigmaPlot Enzyme Kinetics module, version 1.3 (Systat Software, Inc., San Jose California USA).

α-D-Mannopyranose-1-phosphate concentration is for example quantified by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate are in particular separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and an isocratic step of 300 mM sodium acetate in 150 mM NaOH is applied at a 1 mL.min-1 flow rate. Detection is performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

By “identity percentage” is meant the percentage of identical amino acids between two aligned sequences, one of them being the amino acid sequence SEQ ID NO: 1, the other one being the amino acid sequence of interest.

In particular, the "identity percentage" between two polypeptide sequences is determined by comparing both optimally aligned sequences through a comparison window.

The portion of the amino-acid sequence in the comparison window may thus include additions or deletions (for example "gaps") as compared to the reference sequence (which does not include these additions or these deletions) so as to obtain an optimal alignment between both sequences.

The identity percentage is calculated by determining the number of positions at which an identical amino-acid residue, can be noted for both compared sequences, then by dividing the number of positions at which identity can be observed between both amino-acid residues,

by the total number of positions in the comparison window, then by multiplying the result by hundred to obtain the percentage of amino acid identity between the two sequences.

The comparison of the sequence optimal alignment may be effected by a computer using known algorithms.

Most preferably, the sequence identity percentage is determined using the BLASTP software (version 2.2.29), the parameters being set as follows: (1) Max target sequences=500 ; (2) short queries (Automatically adjust parameters for short input sequences)= `—yes ; (3) Expect threshold=10; (4) Word size=3; (5) Max matches in a query range=0; (6) Matrix=BLOSUM62 ; (7) Gap Costs= `Existence: 11 Extension: 1; (8) Compositional adjustments—conditional compositional score matrix adjustment"; (9) Filter (Low complexity regions) — no; (10) Mask (Mask for lookup table only) — no ; (11) Mask (Mask lower case letters) — no.

By “RaMP2” is meant the glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 3 (GenBank accession number ADU20661.1 or YP_004103295.1).

By “Bt1033” is meant the glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 7 (GenBank accession number WP_011107586.1).

By “mannosyl donor” is meant a donor of mannosyl, in other words a compound comprising a mannoside residue bonded to the rest of the compound (i.e. the “donor” part of the compound), the mannoside residue of which being able to be transferred to another compound, in particular inorganic phosphate, by phosphorolysis.

By “phosphorolysis” is meant the breakdown of the glycosidic bond joining said mannosyl to the rest of the mannosyl donor, and the concomitant phosphate mannosylation, to generate α-D-mannopyranose-1-phosphate, said reactions being catalysed by a glycoside-phosphorylase.

Interestingly, the inventors have found that UhgbMP and enzymes having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with UhgbMP are able to phosphorolyse β-D-mannopyranosyl-1,4-D-glucopyranose, β-1,4-linked D-manno-oligosaccharides and mannan (β-D-Man*p*-1,4-(D-Man*p*)$_n$, with n = 1 to 15). Regarding mannan, said phosphorolysis is characterized by a notable parallel increase in specific activity with the degree of polymerisation (DP).

By “specific activity” is meant the activity of an enzyme per milligram of total protein (expressed in $\mu mol.min^{-1}.mg^{-1}$).

By “enzyme activity” is meant the moles of substrate converted by said enzyme per unit of time.

"*p*" denotes the pyranose form of a monosaccaride. For example, by "Man*p*" is meant mannopyranose.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl donor compound is β-1,4-D-Mannan.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase is chosen among glycoside-phosphorylases identified by the following GenBank Accession numbers: ADD61463.1, AEY67872.1, AFN75330.1, ADO59098.1, CCI71609.1, ADD61810.1, CBL14186.1, AFK85719.1, ADA67643.1, AAD36300.1, ACB09929.1, ABQ47551.1, ACM23522.1, ABV33993.1, AGB28392.1, AFL78596.1, EDS13361.1, ABX42090.1, AFG35891.1, ACJ75237.1, ADL43426.1, AEM72946.1, ACM61623.1, ABR30874.1, AFK07300.1, AFK07371.1, AFL98126.1, AAO76140.1, ACB75595.1, ZP_08158270.1, ZP_03299783.1, ZP_02422496.1, ZP_02205887.1, ZP_02090881.1, ZP_02071200.1, ZP_02067106.1, ZP_01958898, YP_210978.1 and YP_001297942.1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase is chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413. The proteins having the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413 are also identified by the GenBank Accession numbers presented in following table A:

Protein name	Accession number	Idendity to UhgbMP	Sequence coverage	E-value	SEQ ID NO
Uhgb_MP	ADD61463.1	100%	100%	0.0	1
MULTISPECIES: glycosidase [Bacteroides]	WP_005657882.1 (EDS13361.1)	99%	100%	0.0	46
MULTISPECIES: glycosidase [Bacteroides]	WP_005776041.1	92%	97%	0.0	49
glycosidase [Bacteroides fragilis]	WP_005809670.1	92%	97%	0.0	51
MULTISPECIES: glycosidase [Bacteroides]	WP_005786056.1 (YP_210978.1)	91%	97%	0.0	50
glycosidase [Bacteroides sp. 2_1_56FAA]	WP_009291829.1	91%	97%	0.0	109

hypothetical protein M101_1274 [Bacteroides fragilis str. 1007-1-F #8]	EXY14005.1	91%	97%	0.0	307
hypothetical protein M118_1182 [Bacteroides fragilis str. 3783N1-2]	EXY47258.1	91%	97%	0.0	308
hypothetical protein M072_1228 [Bacteroides fragilis str. DS-208]	EXZ06325.1	91%	97%	0.0	309
hypothetical protein M069_1383 [Bacteroides fragilis str. B1 (UDC16-1)]	EXZ84244.1	91%	97%	0.0	310
hypothetical protein M087_1235 [Bacteroides fragilis str. S23 R14]	EYA01159.1	91%	97%	0.0	311
hypothetical protein M132_1192 [Bacteroides fragilis str. S24L15]	EYA72129.1	91%	97%	0.0	312
pF04041 domain protein [Tannerella sp. CAG:118]	WP_021930111.1	88%	97%	0.0	235
glycosidase [Bacteroides dorei]	WP_007841086.1	87%	97%	0.0	81
glycosidase [Porphyromonas sp. oral taxon 279]	WP_009433021.1	87%	97%	0.0	114
hypothetical protein [Porphyromonas sp. oral taxon 278]	WP_021666340.1	87%	97%	0.0	227
hypothetical protein [Porphyromonas catoniae]	WP_005468873.1	86%	97%	0.0	43
MULTISPECIES: glycosidase [Bacteroides]	WP_005840987.1 (YP_001297942.1)	86%	97%	0.0	53
hypothetical protein [Bacteroides massiliensis]	WP_005941607.1	86%	99%	0.0	58
MULTISPECIES: glycosidase [Bacteroides]	WP_007832896.1 (ZP_03299783.1)	86%	97%	0.0	79
glycosidase [Bacteroides sp. 9_1_42FAA]	WP_008672798.1	86%	97%	0.0	92
glycosidase [Bacteroides sp. CAG:98]	WP_022506919.1	86%	99%	0.0	282
hypothetical protein M097_3456 [Bacteroides vulgatus str. 3775 SL(B) 10 (iv)]	KDS27775.1	86%	97%	0.0	354
glycosidase [Parabacteroides gordonii]	WP_028728909.1	85%	97%	0.0	401
MULTISPECIES: glycosidase [Bacteroidales]	WP_005857736.1	84%	97%	0.0	55

glycosidase [Dysgonomonas gadei]	WP_006797616.1	84%	97%	0.0	62
glycosidase [Bacteroides sp. 2_1_33B]	WP_008772434.1	84%	97%	0.0	95
MULTISPECIES: hypothetical protein [Bacteroidales]	WP_016275348.1	84%	97%	0.0	188
glycosidase [Porphyromonas macacae]	WP_025003950.1	84%	97%	0.0	326
hypothetical protein M095_3316 [Parabacteroides distasonis str. 3999B T(B) 4]	KDS64210.1	84%	97%	0.0	357
glycosidase [Prevotella stercorea]	WP_007897166.1	83%	79%	5,00E-167	82
glycosidase [Bacteroides propionicifaciens]	WP_018108189.1	83%	97%	0.0	198
glycosidase [Porphyromonas macacae]	WP_018361032.1	83%	97%	0.0	201
putative uncharacterized protein [Parabacteroides sp. CAG:2]	WP_022192625.1	83%	97%	0.0	255
glycoside hydrolase [Bacteroides reticulotermitis JCM 10512]	GAE84647.1	83%	97%	0.0	305
hypothetical protein M091_4429 [Parabacteroides distasonis str. 3776 D15 i]	KDS39128.1	83%	97%	0.0	355
hypothetical protein HMPREF1002_01378 [Porphyromonas sp. 31_2]	KEJ87210.1	83%	97%	0.0	411
glycosidase [Prevotella buccalis]	WP_004350808.1	82%	97%	0.0	31
glycosidase [Prevotella oralis]	WP_004368520.1	82%	97%	0.0	34
MULTISPECIES: glycosidase [Parabacteroides]	WP_007655989.1	82%	97%	0.0	75
glycosidase [Prevotella timonensis]	WP_008122173.1	82%	97%	0.0	84
PF04041 domain protein [Prevotella pleuritidis]	WP_021584220.1	82%	97%	0.0	223
PF04041 domain protein [Prevotella baroniae]	WP_021590755.1	82%	97%	0.0	224
putative uncharacterized protein [Prevotella sp. CAG:520]	WP_022158713.1	82%	97%	0.0	252
putative uncharacterized protein [Prevotella stercorea CAG:629]	WP_022429863.1	82%	97%	0.0	273
PF04041 domain protein [Prevotella sp. BV3P1]	WP_023057032.1	82%	97%	0.0	290

hypothetical protein [Prevotella oralis]	WP_023984840.1	82%	97%	0.0	292
glycosidase [Prevotella enoeca]	WP_025065587.1	82%	97%	0.0	327
glycosidase [Prevotella timonensis]	WP_025072541.1	82%	97%	0.0	329
glycosidase [Prevotella baroniae]	WP_027444955.1	82%	97%	0.0	383
glycosidase [Bacteroidales bacterium ph8]	WP_019129862.1	81%	97%	0.0	210
putative uncharacterized protein [Prevotella sp. CAG:474]	WP_022311711.1	81%	97%	0.0	265
putative uncharacterized protein [Bacteroides sp. CAG:770]	WP_022284896.1	80%	97%	0.0	262
predicted glycosylase [Alistipes sp. CAG:29]	WP_022332491.1	80%	93%	0.0	267
glycosidase [Alistipes onderdonkii]	WP_026318229.1	80%	97%	0.0	351
glycosidase [Prevotella dentalis]	WP_005844805.1 (AGB28392.1)	79%	97%	0.0	54
glycosidase [Alistipes sp. HGB5]	WP_009597303.1	79%	97%	0.0	117
MULTISPECIES: glycosidase [Alistipes]	WP_009597427.1 (AFL78596.1)	79%	97%	0.0	118
Predicted glycosylase [Alistipes shahii]	WP_015547179.1	79%	97%	0.0	179
predicted glycosylase [Alistipes sp. CAG:53]	WP_022061059.1	79%	97%	0.0	243
glycosidase [Hallella seregens]	WP_027952368.1	79%	97%	0.0	387
putative uncharacterized protein [Bacteroides sp. CAG:927]	WP_016564452.1	78%	97%	0.0	193
putative uncharacterized protein [Bacteroides sp. CAG:545]	WP_022015682.1	78%	97%	0.0	239
putative uncharacterized protein [Bacteroides sp. CAG:709]	WP_022147864.1	78%	97%	0.0	250
glycosidase [Alistipes onderdonkii]	WP_026318218.1	78%	100%	0.0	350
glycosidase [Porphyromonas levii]	WP_018358458.1	77%	97%	0.0	200

putative uncharacterized protein [Porphyromonas sp. CAG:1061]	WP_021903730.1	77%	97%	0.0	233
putative uncharacterized protein [Alistipes sp. CAG:268]	WP_022307455.1	77%	97%	0.0	264
hypothetical protein [Prevotella sp. oral taxon 473]	WP_009437337.1	76%	97%	0.0	116
glycosidase [Porphyromonas somerae]	WP_018028730.1	76%	97%	0.0	197
MULTISPECIES: hypothetical protein [Paenibacillus]	WP_021256311.1	76%	97%	0.0	222
glycosidase [Porphyromonas bennonis]	WP_025081440.1	76%	97%	0.0	331
hypothetical protein [uncultured bacterium contig00010(2014)]	AIA99576.1	76%	97%	0.0	332
glycosidase [Paenibacillus taiwanensis]	WP_028547193.1	76%	97%	0.0	398
glycosidase [Paenibacillus alvei]	WP_005543591.1	75%	97%	0.0	44
glycosidase [Paenibacillus dendritiformis]	WP_006678432.1	74%	97%	5,00E-178	60
glycosidase [Paenibacillus sp. OSY-SE]	WP_019420855.1	74%	97%	6,00E-178	211
glycosidase [Paenibacillus assamensis]	WP_028594496.1	74%	97%	7,00E-179	400
glycosidase [Herpetosiphon aurantiacus]	WP_012188447.1	73%	97%	2,00E-172	135
glycosidase [Caldanaerobius polysaccharolyticus]	WP_026486530.1	72%	96%	1,00E-169	360
glycosidase [Clostridium phytofermentans]	WP_012199744.1 (ABX42090.1)	71%	97%	9,00E-170	136
glycosidase [Mahella australiensis]	WP_013781285.1	71%	96%	7,00E-165	160
glycosidase [Caldanaerobius polysaccharolyticus]	WP_026485574.1	71%	96%	3,00E-169	359
MULTISPECIES: glycosidase [Roseburia]	WP_006856800.1	70%	95%	9,00E-157	64
glycosidase [Caldicellulosiruptor saccharolyticus]	WP_011915905.1	70%	95%	2,00E-158	130
glycosidase [Caldicellulosiruptor bescii]	WP_015908872.1 (ACM61623.1)	70%	96%	2,00E-160	183

hypothetical protein [Treponema lecithinolyticum]	WP_021686473.1	70%	97%	2,00E-165	230
glycosidase [Treponema phagedenis]	WP_002698257.1	69%	97%	3,00E-160	17
glycosidase [Caldicellulosiruptor obsidiansis]	WP_013291420.1 (ADL43426.1)	69%	96%	2,00E-159	144
glycosidase [Caldicellulosiruptor hydrothermalis]	WP_013402243.1	69%	96%	6,00E-159	150
glycosidase [Caldicellulosiruptor kronotskyensis]	WP_013429364.1	69%	96%	8,00E-159	151
glycosidase [Caldicellulosiruptor kristjanssonii]	WP_013433376.1	69%	96%	8,00E-160	152
MULTISPECIES: glycosidase [Caldicellulosiruptor]	WP_014041916.1 (AEM72946.1)	69%	96%	2,00E-159	162
glycosidase [Fervidobacterium pennivorans]	WP_014452319.1 (AFG35891.1)	69%	96%	3,00E-157	166
putative uncharacterized protein [Roseburia sp. CAG:100]	WP_022518503.1	69%	96%	1,00E-158	285
hypothetical protein [Catonella morbi]	WP_023355711.1	69%	97%	8,00E-164	291
glycosidase-like protein [Paenibacillus sp. FSL R7-277]	ETT59152.1	69%	95%	1,00E-158	302
glycosidase [Treponema phagedenis]	WP_024751802.1	69%	97%	4,00E-160	319
glycosidase [Thermotogae bacterium JGI 0000106-O11]	WP_029684040.1	69%	96%	1,00E-157	410
MULTISPECIES: glycosidase [Lachnospiraceae]	WP_003021946.1	68%	96%	3,00E-159	21
putative glycosylase [[Clostridium termitidis]	WP_004630151.1	68%	96%	2,00E-158	40
glycosidase [Prevotella sp. oral taxon 306]	WP_009434539.1	68%	99%	4,00E-160	115
glycosidase [Mariprofundus ferrooxydans]	WP_009851056.1	68%	96%	2,00E-155	122
glycosidase [Dictyoglomus thermophilum]	WP_012547681.1	68%	97%	1,00E-157	140
glycosidase [Dictyoglomus turgidum]	WP_012584146.1	68%	97%	2,00E-157	142

glycosidase [Mesotoga prima]	WP_014731198.1 (AFK07300.1 ; AFK07371.1)	68%	96%	4,00E-153	169
glycosidase [Paenibacillus sp. HW567]	WP_019913476.1	68%	96%	8,00E-161	216
hypothetical protein [Prevotella sp. F0091]	WP_021671911.1	68%	99%	2,00E-160	228
putative uncharacterized protein [Blautia sp. CAG:37]	WP_022463168.1	68%	97%	9,00E-161	278
glycosidase-like protein [Paenibacillus sp. FSL R7-269]	ETT40362.1	68%	95%	5,00E-158	300
glycosidase [Mariprofundus ferrooxydans]	WP_026195333.1	68%	96%	3,00E-156	349
glycosidase [Butyrivibrio sp. LC3010]	WP_026511627.1	68%	97%	2,00E-160	363
glycosidase [Thermosipho africanus]	WP_004100329.1 (ACJ75237.1)	67%	96%	6,00E-153	26
glycosidase [Prevotella melaninogenica]	WP_004359255.1	67%	99%	1,00E-159	33
glycosidase [Prevotella veroralis]	WP_004384352.1	67%	99%	2,00E-158	38
Glycosidase related protein [Mesotoga sp. VNs100]	WP_006489307.1	67%	96%	9,00E-153	59
glycosidase [Bacteroides finegoldii]	WP_007760098.1	67%	98%	1,00E-159	77
glycosidase [Imtechella halotolerans]	WP_008237718.1	67%	93%	3,00E-148	87
glycosidase [Prevotella histicola]	WP_008822147.1	67%	99%	2,00E-160	96
glycosidase [Prevotella sp. C561]	WP_009012765.1	67%	99%	1,00E-159	98
glycosidase [Paenibacillus sp. oral taxon 786]	WP_009223904.1	67%	96%	2,00E-155	104
MULTISPECIES: glycosidase [Bacteroides]	WP_010537903.1	67%	98%	3,00E-157	128
glycosidase [Zunongwangia profunda]	WP_013073563.1	67%	97%	8,00E-161	143
glycosidase [Prevotella veroralis]	WP_018910261.1	67%	99%	8,00E-159	208
hypothetical protein [Bacteroides pyogenes]	WP_021646149.1	67%	97%	5,00E-156	225

exporter of the RND superfamily [Bacteroides pyogenes JCM 10003]	GAE22054.1	67%	97%	1,00E-156	295
predicted glycoside hydrolase [Bacteroides pyogenes JCM 6292]	GAE14030.1	67%	97%	3,00E-156	296
PF04041 domain protein [Prevotella sp. ICM33]	ETT01200.1	67%	99%	1,00E-159	298
glycosidase [Bacteroides faecichinchillae]	WP_025074213.1	67%	98%	3,00E-157	330
glycosidase [Barnesiella viscericola]	WP_025278016.1	67%	99%	4,00E-162	333
glycosidase [Prevotella scopos]	WP_025839877.1	67%	99%	3,00E-160	345
glycosidase [Butyrivibrio sp. MB2005]	WP_026525522.1	67%	98%	1,00E-157	367
glycosidase [Butyrivibrio sp. AE3006]	WP_026669023.1	67%	97%	3,00E-157	369
glycosidase [[Clostridium cellobioparum]	WP_027628420.1	67%	96%	2,00E-158	385
glycosidase [Paenibacillus sp. J14]	WP_028537518.1	67%	96%	1,00E-155	395
glycosidase [Epulopiscium sp. 'N.t. morphotype B']	WP_029487932.1	67%	98%	1,00E-166	405
hypothetical protein [Bacteroides sp. HPS0048]	WP_002561891.1	66%	99%	7,00E-159	13
glycosidase [Capnocytophaga ochracea]	WP_002674213.1	66%	99%	1,00E-155	15
glycosidase [Capnocytophaga sputigena]	WP_002679718.1	66%	99%	1,00E-153	16
MULTISPECIES: glycosidase [Bacteroides]	WP_004306232.1	66%	98%	1,00E-156	28
MULTISPECIES: glycosidase [Bacteroides]	WP_004318517.1	66%	98%	3,00E-156	29
glycosidase [Prevotella bivia]	WP_004336686.1	66%	98%	2,00E-157	30
glycosidase [Prevotella denticola]	WP_004353840.1	66%	99%	8,00E-157	32
glycosidase [Prevotella oris]	WP_004371886.1	66%	98%	2,00E-158	35
glycosidase [Prevotella oris]	WP_004377652.1	66%	98%	6,00E-159	36
MULTISPECIES: glycosidase [Bacteroides]	WP_005681191.1	66%	98%	3,00E-156	48
glycosidase [Bacteroides coprosuis]	WP_006743673.1	66%	97%	3,00E-156	61

glycosidase [Bacteroides cellulosilyticus]	WP_007212742.1	66%	99%	4,00E-159	68
glycosidase [Prevotella multiformis]	WP_007367403.1	66%	99%	1,00E-156	70
glycosidase [Bacteroides nordii]	WP_007484755.1	66%	99%	1,00E-158	72
MULTISPECIES: glycosidase [Bacteroides]	WP_007559989.1	66%	99%	3,00E-157	73
glycosidase [Prevotella amnii]	WP_008448842.1	66%	98%	5,00E-159	89
glycosidase [Bacteroides sp. 1_1_14]	WP_008761672.1	66%	98%	1,00E-155	93
MULTISPECIES: glycosidase [Bacteroides]	WP_008765821.1	66%	98%	2,00E-156	94
glycosidase [Barnesiella intestinihominis]	WP_008860872.1	66%	99%	2,00E-162	97
glycosidase [Bacteroides sp. D22]	WP_009039638.1	66%	98%	9,00E-157	100
glycosidase [Prevotella sp. oral taxon 299]	WP_009227855.1	66%	98%	4,00E-159	106
glycosidase [Prevotella sp. oral taxon 317]	WP_009230884.1	66%	98%	5,00E-158	107
hypothetical protein [Capnocytophaga sp. oral taxon 324]	WP_009409904.1	66%	99%	7,00E-154	111
MULTISPECIES: glycosidase [Capnocytophaga]	WP_009417204.1	66%	99%	1,00E-154	113
hypothetical protein [Capnocytophaga sp. oral taxon 326]	WP_009750727.1	66%	99%	7,00E-155	119
glycosidase [Thermosipho africanus]	WP_012580125.1	66%	96%	2,00E-147	141
glycosidase [Bacteroides helcogenes]	WP_013547083.1	66%	99%	9,00E-158	155
glycosidase [Capnocytophaga canimorsus]	WP_013998379.1	66%	93%	1,00E-146	161
glycosidase [Ornithobacterium rhinotracheale]	WP_014791648.1 (AFL98126.1)	66%	99%	3,00E-156	171
glycosidase [Capnocytophaga ochracea]	WP_015781954.1	66%	99%	3,00E-155	182
hypothetical protein [Paenibacillus barengoltzii]	WP_016313761.1	66%	96%	2,00E-153	192
hypothetical protein [candidate division EM 19 bacterium SCGC AAA471-D06]	WP_018195826.1	66%	98%	1,00E-156	199

glycosidase [Prevotella nanceiensis]	WP_018362024.1	66%	98%	4,00E-159	202
glycosidase [Prevotella loescheii]	WP_018966875.1	66%	98%	1,00E-155	209
MULTISPECIES: hypothetical protein [Fervidibacteria]	WP_020250119.1	66%	98%	1,00E-157	219
uncharacterized protein BN772_03615 [Bacteroides sp. CAG:754]	CDA85485.1	66%	98%	8,00E-157	221
glycosidase related protein [Bacteroides sp. CAG:702]	WP_022039580.1	66%	99%	8,00E-157	240
putative uncharacterized protein [Firmicutes bacterium CAG:137]	WP_022181905.1	66%	97%	2,00E-154	253
uncharacterized protein [Bacteroides cellulosilyticus CAG:158]	WP_022210298.1	66%	99%	9,00E-159	256
putative uncharacterized protein [Bacteroides sp. CAG:875]	WP_022354087.1	66%	99%	3,00E-157	270
glycosidase [Draconibacterium orientale]	AHW59446.1	66%	99%	4,00E-158	314
glycosidase [Prevotella shahii]	WP_025815819.1	66%	98%	9,00E-157	342
glycosidase [Bacteroides nordii]	WP_025866909.1	66%	99%	6,00E-159	346
glycosidase [Capnocytophaga cynodegmi]	WP_026193925.1	66%	98%	6,00E-157	348
hypothetical protein M082_5855 [Bacteroides fragilis str. 3725 D9 ii]	KDS13138.1	66%	98%	5,00E-157	353
hypothetical protein M094_0359 [Bacteroides uniformis str. 3978 T3 ii]	KDS51703.1	66%	97%	8,00E-156	356
glycosidase [Bacteroides sp. 14(A)]	WP_026367858.1	66%	99%	6,00E-159	358
glycosidase [Butyrivibrio sp. WCD3002]	WP_026494966.1	66%	98%	9,00E-158	361
glycosidase [Bacteroides thetaiotaomicron] - BT1033	WP_011107586.1	66%	98%	8,00E-157	7
glycosidase [Bacteroides ovatus]	WP_004300473.1 (ZP_02067106.1)	65%	99%	9,00E-157	27
glycosidase [Prevotella oulorum]	WP_004381049.1	65%	96%	4,00E-153	37
glycosidase [Bacteroides caccae]	WP_005675734.1 (ZP_01958898.1)	65%	99%	3,00E-156	47

MULTISPECIES: glycosidase [Bacteroides]	WP_005828866.1 (ZP_02071200.1)	65%	99%	1,00E-158	52
MULTISPECIES: glycosidase [Bacteroides]	WP_005924061.1	65%	99%	6,00E-158	57
glycosidase [Prevotella salivae]	WP_007135680.1	65%	97%	1,00E-153	67
MULTISPECIES: glycosidase [Bacteroides]	WP_007666450.1	65%	99%	2,00E-156	76
glycosidase [Chryseobacterium sp. CF314]	WP_007840056.1	65%	98%	2,00E-157	80
glycosidase [Flavobacterium sp. F52]	WP_008467432.1	65%	97%	5,00E-158	90
glycosidase [Prevotella maculosa]	WP_008565315.1	65%	98%	3,00E-155	91
glycosidase [Bacteroides sp. D20]	WP_009037046.1	65%	99%	1,00E-158	99
glycosidase [Prevotella sp. oral taxon 472]	WP_009236589.1	65%	98%	9,00E-155	108
hypothetical protein [Capnocytophaga sp. oral taxon 332]	WP_009416958.1	65%	99%	2,00E-154	112
glycosidase [Marinilabilia salmonicolor]	WP_010665304.1	65%	99%	1,00E-151	129
glycosidase [Flavobacterium johnsoniae]	WP_012024105.1	65%	97%	7,00E-159	133
glycosidase [Maribacter sp. HTCC2170]	WP_013304978.1	65%	97%	1,00E-150	146
glycosidase [Spirochaeta thermophila]	WP_013312904.1	65%	97%	2,00E-151	148
glycosidase [Bacteroides salanitronis]	WP_013616878.1	65%	99%	2,00E-154	156
glycosidase [Spirochaeta thermophila]	WP_014623767.1	65%	97%	5,00E-152	168
MULTISPECIES: Predicted glycosylase [Ruminococcus]	WP_015559136.1	65%	97%	4,00E-155	180
hypothetical protein [Lachnospiraceae bacterium A4]	WP_016281590.1	65%	96%	3,00E-150	189
glycosidase [Paenibacillus sanguinis]	WP_018751671.1	65%	96%	8,00E-151	204
glycosidase [Prevotella maculosa]	WP_019966835.1	65%	98%	1,00E-154	217
PF04041 domain protein [Prevotella salivae]	WP_021824962.1	65%	97%	9,00E-155	231

uncharacterized protein [Bacteroides sp. CAG:20]	WP_021892132.1	65%	99%	3,00E-161	232
uncharacterized protein [Bacteroides sp. CAG:530]	WP_022130312.1	65%	99%	2,00E-156	248
uncharacterized protein [Bacteroides intestinalis CAG:315]	WP_022394484.1	65%	99%	1,00E-156	272
glycosidase [Zhouia amylolytica AD3]	ETN94324.1	65%	96%	7,00E-157	297
glycosidase [Prevotella oulorum]	WP_025069999.1	65%	96%	5,00E-152	328
glycosidase [Bacteroides rodentium]	WP_025833278.1	65%	99%	5,00E-158	343
glycosidase [Gelidibacter mesophilus]	WP_027125932.1	65%	96%	2,00E-155	376
glycosidase [Leeuwenhoekiella sp. Hel_I_48]	WP_028376565.1	65%	98%	1,00E-156	389
glycosidase [Prevotella sp. HJM029]	WP_028900669.1	65%	96%	1,00E-152	402
glycosidase [Ruminococcus albus]	WP_002847919.1 (ZP_08158270.1)	64%	97%	2,00E-152	19
glycosidase [Chryseobacterium gleum]	WP_002982641.1	64%	98%	9,00E-156	20
MULTISPECIES: glycosidase [Bacteroides]	WP_007566231.1	64%	99%	4,00E-155	74
glycosidase [Flavobacterium sp. CF136]	WP_007810298.1	64%	97%	9,00E-155	78
glycosidase [Bacteroides coprophilus]	WP_008144770.1	64%	99%	1,00E-154	85
MULTISPECIES: glycosidase [Elizabethkingia]	WP_009088587.1	64%	99%	2,00E-153	101
hypothetical protein [Bacteroides oleiciplenus]	WP_009129096.1	64%	99%	1,00E-153	102
MULTISPECIES: glycosidase [Porphyromonadaceae]	WP_009319210.1	64%	97%	2,00E-151	110
glycosidase [Leeuwenhoekiella blandensis]	WP_009778951.1	64%	98%	1,00E-154	120
glycosidase [Mesoflavibacter zeaxanthinifaciens]	WP_010518669.1	64%	98%	2,00E-156	126
glycosidase [Thermosipho melanesiensis]	WP_012057234.1 (ABR30874.1)	64%	96%	9,00E-148	134

hypothetical protein [Elizabethkingia meningoseptica]	WP_016198959.1	64%	99%	4,00E-152	186
glycosidase [Paenibacillus terrigena]	WP_018756021.1	64%	96%	2,00E-152	206
glycosidase [Paenibacillus fonticola]	WP_019637318.1	64%	96%	1,00E-147	212
glycosidase [Flavobacterium sp. SCGC AAA160-P02]	WP_020079936.1	64%	97%	1,00E-153	218
putative uncharacterized protein [Ruminococcus sp. CAG:579]	WP_022128289.1	64%	97%	4,00E-152	247
uncharacterized protein [Bacteroides sp. CAG:443]	WP_022232854.1	64%	99%	4,00E-156	258
uncharacterized protein [Bacteroides coprophilus CAG:333]	WP_022277494.1	64%	99%	3,00E-155	261
uncharacterized protein [Parabacteroides sp. CAG:409]	WP_022455924.1	64%	97%	3,00E-152	276
Glycosidase related protein [Elizabethkingia anophelis]	CDN80270.1	64%	99%	4,00E-153	313
glycosidase [Aquimarina sp. 22II-S11-z7]	EZH73463.1	64%	98%	3,00E-154	315
glycosidase [Elizabethkingia anophelis]	WP_024568043.1	64%	99%	3,00E-152	317
glycosidase [Ruminococcus albus]	WP_024858077.1	64%	97%	2,00E-152	322
glycosidase [Flavobacterium sp. JGI 0001001-D01]	WP_025571695.1	64%	97%	1,00E-155	335
glycosidase [Aquimarina megaterium]	WP_025663106.1	64%	98%	4,00E-154	336
glycosidase [Bacteroides stercorirosoris]	WP_025834961.1	64%	99%	2,00E-154	344
glycosidase [Flavobacterium daejeonense]	WP_026714400.1	64%	97%	9,00E-155	370
glycosidase [Flavobacterium denitrificans]	WP_026727657.1	64%	97%	4,00E-156	371
glycosidase [Gaetbulibacter saemankumensis]	WP_027136596.1	64%	97%	6,00E-154	377
glycosidase [Cytophaga fermentans]	WP_027473751.1	64%	99%	5,00E-151	384
glycosidase [Flavobacterium sp. KJJ]	WP_029273078.1	64%	97%	4,00E-157	404
glycosidase [[Eubacterium siraeum]	WP_005356317.1 (ZP_02422496.1)	63%	99%	2,00E-153	42

glycosidase [Halanaerobium praevalens]	WP_014554200.1	63%	99%	2,00E-153	167
Predicted glycosylase [[Eubacterium siraeum]	WP_015517885.1	63%	99%	8,00E-153	176
glycosidase [Bacteroides barnesiae]	WP_018711719.1	63%	99%	2,00E-151	203
uncharacterized protein [Eubacterium sp. CAG:786]	WP_021910734.1	63%	96%	2,00E-146	234
uncharacterized protein [Eubacterium sp. CAG:115]	WP_021955434.1	63%	96%	2,00E-146	237
glycosidase related protein [Ruminococcus sp. CAG:57]	WP_022288676.1	63%	99%	8,00E-152	263
uncharacterized protein [Bacteroides sp. CAG:714]	WP_022338980.1	63%	99%	1,00E-151	268
glycosidase [Aquimarina macrocephali]	WP_024771505.1	63%	98%	1,00E-152	320
glycosidase [Bacteroides paurosaccharolyticus]	WP_024993894.1	63%	98%	1,00E-147	325
glycosidase [Anaerophaga thermohalophila]	WP_026055203.1	63%	97%	1,00E-147	347
glycosidase [Chryseobacterium sp. UNC8MFCol]	WP_027371934.1	63%	98%	1,00E-151	382
glycosidase [Ruminococcus sp. FC2018]	WP_028506306.1	63%	99%	3,00E-152	390
glycosidase [Ruminococcus sp. NK3A76]	WP_028510924.1	63%	99%	3,00E-152	391
glycosidase [Spirochaeta cellobiosiphila]	WP_028974065.1	63%	97%	3,00E-144	403
glycosidase [Thermophagus xiamenensis]	WP_010528491.1	62%	99%	1,00E-148	127
uncharacterized protein [Eubacterium siraeum CAG:80]	WP_022271458.1	62%	99%	3,00E-152	260
predicted glycosylase [Ruminococcus sp. CAG:353]	WP_022473971.1	62%	97%	4,00E-148	279
Chain A - cristal structure of TM1225 [Thermotoga maritima MSB8]	AAD36300.1	61%	96%	7,00E-128	8
MULTISPECIES: glycosylase [Thermotoga]	WP_004080062.1 (ADA67643.1)	61%	97%	2,00E-130	25
glycosylase [Thermotoga sp. RQ2]	WP_012311206.1 (ACB09929.1)	61%	97%	5,00E-130	138

glycosylase [Thermotoga neapolitana]	WP_015919816.1 (ACM23522.1)	61%	97%	2,00E-130	184
glycosylase [Thermotoga sp. EMP]	WP_008195435.1	60%	97%	1,00E-129	86
glycosylase [Thermotoga petrophila]	WP_011943967.1 (ABQ47551.1)	60%	97%	6,00E-127	131
glycosylase [Thermotoga sp. A7A]	WP_029683035.1	60%	97%	2,00E-129	409
glycosylase [Opitutaceae bacterium TAV1]	WP_007359676.1	59%	96%	2,00E-125	69
glycosidase [Clostridium phytofermentans]	WP_029502036.1	59%	96%	1,00E-138	406
hypothetical protein [Vibrio cholerae]	WP_002028992.1	58%	93%	4,00E-124	12
glycosylase [Opitutus terrae]	WP_012375132.1 (ACB75595.1)	58%	96%	1,00E-124	139
putative uncharacterized protein [Ruminococcus sp. CAG:177]	WP_022188802.1	58%	97%	6,00E-127	254
putative uncharacterized protein [Clostridium sp. CAG:413]	WP_022240579.1	58%	97%	2,00E-127	259
MULTISPECIES: glycosylase [Vibrio]	WP_000111640.1	57%	99%	2,00E-130	10
glycosylase [Vibrio cholerae]	WP_001908866.1	57%	99%	1,00E-130	11
glycosylase [Marinomonas sp. MED121]	WP_009836219.1	57%	97%	7,00E-132	121
MULTISPECIES: glycosylase [Thermotoga]	WP_012003469.1 (ABV33993.1)	57%	96%	2,00E-121	132
glycosidase [[[Clostridium] stercorarium]	WP_015358820.1	57%	96%	3,00E-121	174
glycosidase related protein [Roseburia sp. CAG:100]	WP_022517033.1	57%	96%	5,00E-124	284
glycosylase [Ruminococcus flavefaciens]	WP_009984354.1	56%	95%	5,00E-120	123
glycosylase [Melioribacter roseus]	WP_014856762.1 (AFN75330.1)	56%	96%	1,00E-121	172

glycosylase [Amphibacillus xylanus]	WP_015010952.1	56%	96%	6,00E-125	173
glycosylase [Ruminococcus flavefaciens]	WP_019680777.1	56%	96%	7,00E-120	213
putative uncharacterized protein [Ruminococcus sp. CAG:563]	WP_022143092.1	56%	96%	8,00E-121	249
glycosylase [Ruminococcus flavefaciens 007c]	EWM52544.1	56%	96%	9,00E-120	306
glycosylase [Paenibacillus fonticola]	WP_026342093.1	56%	96%	8,00E-123	352
glycosylase [Aliagarivorans taiwanensis]	WP_026958751.1	56%	99%	7,00E-133	375
glycosylase [[Clostridium cellobioparum]	WP_027631321.1	56%	96%	4,00E-124	386
glycosylase [Ruminococcus flavefaciens]	WP_028520880.1	56%	95%	2,00E-118	394
hypothetical protein [Eubacterium plexicaudatum]	WP_004053156.1	55%	97%	3,00E-119	24
putative glycosylase [[Clostridium termitidis]	WP_004628984.1	55%	96%	3,00E-123	39
glycosylase [Faecalibacterium prausnitzii]	WP_005922713.1 (ZP_02090881.1)	55%	96%	2,00E-118	56
glycosylase [Verrucomicrobiae bacterium DG1235]	WP_008099469.1	55%	97%	3,00E-124	83
putative glycosylase [Clostridium sp. Maddingley MBC34-26]	WP_008421760.1	55%	96%	6,00E-118	88
Predicted glycosylase [Faecalibacterium prausnitzii]	WP_015537563.1	55%	96%	5,00E-118	178
putative uncharacterized protein [Ruminococcus sp. CAG:488]	WP_022080138.1	55%	96%	9,00E-119	244
putative uncharacterized protein [Blautia sp. CAG:37]	WP_022461809.1	55%	97%	3,00E-117	277
glycosidase related protein [Roseburia sp. CAG:182]	WP_022516286.1	55%	97%	4,00E-120	283
glycosylase [Ruminococcus flavefaciens]	WP_024861808.1	55%	95%	7,00E-118	323
glycosylase [Paenibacillus graminis]	WP_025708303.1	55%	96%	2,00E-119	340
MULTISPECIES: glycosylase [Butyrivibrio]	WP_026512558.1	55%	96%	2,00E-118	364
glycosylase [Ruminococcus flavefaciens]	WP_028516981.1	55%	96%	1,00E-119	393

MULTISPECIES: glycosylase [Coprococcus]	WP_004851781.1 (ZP_02205887.1)	54%	97%	5,00E-118	41
MULTISPECIES: conserved hypothetical protein [Firmicutes]	WP_005586402.1	54%	96%	4,00E-116	45
glycosylase [Oribacterium sp. oral taxon 078]	WP_009215050.1	54%	99%	1,00E-119	103
glycosylase [Clostridium phytofermentans]	WP_012200281.1	54%	95%	1,00E-117	137
glycosylase [Roseburia hominis]	WP_014080407.1	54%	97%	4,00E-119	163
putative glycosylase [Clostridium saccharoperbutylacetonicum]	WP_015395330.1	54%	96%	2,00E-115	175
glycosylase [[Clostridium cellulolyticum]	WP_015926354.1	54%	99%	2,00E-125	185
glycosidase [Clostridium sartagoforme]	WP_016208716.1	54%	96%	3,00E-117	187
glycosylase [Paenibacillus sp. PAMC 26794]	WP_017691679.1	54%	96%	7,00E-120	195
glycosidase related protein [Ruminococcus sp. CAG:55]	WP_020435906.1	54%	97%	2,00E-119	220
putative uncharacterized protein [Roseburia sp. CAG:18]	WP_022046103.1	54%	97%	8,00E-120	241
predicted glycosylase [Coprococcus sp. CAG:131]	WP_022216164.1	54%	97%	3,00E-118	257
uncharacterized protein [Faecalibacterium sp. CAG:74]	WP_022445741.1	54%	97%	3,00E-118	274
putative uncharacterized protein [Ruminococcus sp. CAG:624]	WP_022495503.1	54%	95%	1,00E-116	280
glycosidase related protein [Firmicutes bacterium CAG:95]	WP_022500747.1	54%	96%	4,00E-117	281
glycosylase [Butyrivibrio fibrisolvens]	WP_022755522.1	54%	97%	2,00E-116	286
glycosylase [Butyrivibrio fibrisolvens]	WP_022758436.1	54%	97%	3,00E-116	287
glycosylase [Butyrivibrio sp. XPD2006]	WP_022764543.1	54%	96%	2,00E-118	288
glycosidase like protein [Paenibacillus sp. FSL R5-192]	ETT35389.1	54%	96%	6,00E-119	299
glycosylase [Cellulomonas sp. KRMCY2]	WP_024285163.1	54%	96%	1,00E-111	316
MULTISPECIES: glycosylase [Paenibacillus]	WP_024629800.1	54%	96%	9,00E-121	318

glycosylase [Butyrivibrio sp. FCS014]	WP_024867154.1	54%	96%	3,00E-116	324
glycosylase [Butyrivibrio sp. VCB2001]	WP_026521011.1	54%	97%	4,00E-117	365
glycosylase [Butyrivibrio sp. VCB2001]	WP_026521090.1	54%	96%	8,00E-117	366
glycosylase [Butyrivibrio sp. VCD2006]	WP_026529031.1	54%	96%	1,00E-118	368
glycosylase [Clostridium beijerinckii]	WP_026885845.1	54%	96%	3,00E-117	373
glycosylase [Butyrivibrio fibrisolvens]	WP_027205091.1	54%	97%	2,00E-116	378
glycosylase [Pseudobutyrivibrio sp. MD2005]	WP_028235021.1	54%	97%	1,00E-117	388
glycosylase [Ruminococcus flavefaciens]	WP_028516231.1	54%	95%	2,00E-116	392
glycosylase [Roseburia intestinalis]	WP_006855602.1	53%	97%	2,00E-117	63
glycosylase [Marvinbryantia formatexigens]	WP_006861995.1	53%	97%	4,00E-113	66
glycosylase [Paenibacillus sp. oral taxon 786]	WP_009224131.1	53%	96%	2,00E-116	105
glycosylase [Clostridium cellulovorans]	WP_010073266.1	53%	96%	3,00E-116	124
glycosylase [Paenibacillus polymyxa]	WP_013373633.1 (ADO59098.1 ; CCI71609.1)	53%	96%	1,00E-117	149
glycosylase [Ethanoligenens harbinense]	WP_013484828.1	53%	97%	2,00E-116	153
glycosylase [Bacillus cellulosilyticus]	WP_013487973.1	53%	98%	3,00E-120	154
glycosylase [Cellulosilyticum lentocellum]	WP_013658367.1	53%	97%	1,00E-114	158
glycosylase [Clostridium sp. BNL1100]	WP_014315229.1 (AEY67872.1)	53%	99%	3,00E-120	165
MULTISPECIES: Predicted glycosylase [Roseburia]	WP_015520962.1	53%	97%	9,00E-118	177
Predicted glycosylase [Roseburia intestinalis]	WP_015561278.1	53%	97%	3,00E-117	181
hypothetical protein [Lachnospiraceae bacterium A4]	WP_016281854.1	53%	97%	2,00E-114	190

MULTISPECIES: glycosylase [Paenibacillus]	WP_016822703.1	53%	96%	5,00E-117	194
glycosylase [Paenibacillus sp. A9]	WP_017813118.1	53%	96%	1,00E-118	196
glycosylase [Paenibacillus sanguinis]	WP_018752750.1	53%	96%	3,00E-116	205
glycosylase [Paenibacillus massiliensis]	WP_018883934.1	53%	97%	1,00E-119	207
glycosylase [Paenibacillus polymyxa]	WP_019688927.1	53%	96%	1,00E-116	214
glycosylase [Paenibacillus sp. HW567]	WP_019912399.1	53%	96%	1,00E-116	215
putative uncharacterized protein [Clostridium sp. CAG:632]	WP_021940922.1	53%	97%	2,00E-114	236
uncharacterized protein [Ruminococcus sp. CAG:254]	WP_022050273.1	53%	98%	5,00E-115	242
glycosidase related protein [Clostridium sp. CAG:510]	WP_022123092.1	53%	97%	8,00E-117	246
glycosidase related protein [Firmicutes bacterium CAG:65]	WP_022154802.1	53%	97%	6,00E-118	251
predicted glycosylase [Clostridium sp. CAG:91]	WP_022313618.1	53%	97%	1,00E-117	266
glycosidase related protein [Firmicutes bacterium CAG:534]	WP_022352419.1	53%	97%	2,00E-114	269
glycosidase related protein [Firmicutes bacterium CAG:882]	WP_022367889.1	53%	97%	3,00E-116	271
uncharacterized protein [Faecalibacterium sp. CAG:74]	WP_022446695.1	53%	96%	1,00E-115	275
glycosylase [Butyrivibrio sp. AE2015]	WP_022776252.1	53%	97%	2,00E-116	289
glycosylase [Paenibacillus polymyxa]	WP_023990882.1	53%	96%	3,00E-117	293
glycosylase [Paenibacillus sp. FSL R7-269]	ETT46633.1	53%	96%	5,00E-115	301
glycosylase [Paenibacillus sp. FSL H8-237]	ETT68908.1	53%	96%	4,00E-117	303
glycosylase [Paenibacillus sp. FSL R7-277]	ETT77608.1	53%	96%	3,00E-115	304
glycosylase [Paenibacillus polymyxa]	WP_025366120.1	53%	96%	3,00E-117	334
glycosylase [Paenibacillus massiliensis]	WP_025681756.1	53%	96%	3,00E-118	338
glycosylase [Paenibacillus polymyxa]	WP_025721963.1	53%	96%	1,00E-116	341

glycosylase [Butyrivibrio sp. MC2013]	WP_026507173.1	53%	97%	9,00E-121	362
glycosylase [Clostridium akagii]	WP_026882155.1	53%	97%	2,00E-121	372
glycosylase [Butyrivibrio fibrisolvens]	WP_027207065.1	53%	97%	4,00E-116	379
glycosylase [Butyrivibrio fibrisolvens]	WP_027216979.1	53%	97%	8,00E-116	380
glycosylase [Butyrivibrio fibrisolvens]	WP_027218703.1	53%	97%	1,00E-114	381
glycosylase [Paenibacillus sp. J14]	WP_028539687.1	53%	96%	4,00E-116	396
glycosylase [Paenibacillus sp. UNCCL52]	WP_028540809.1	53%	96%	2,00E-117	397
glycosylase [Paenibacillus panacisoli]	WP_028589902.1	53%	97%	3,00E-118	399
glycosylase [Clostridium phytofermentans]	WP_029504721.1	53%	97%	3,00E-117	407
glycosylase [Treponema bryantii]	WP_022930997.1	53%	96%	4,00E-112	413
hypothetical protein [uncultured Chloroflexi bacterium]	CAI78545.1	52%	96%	2,00E-114	9
hypothetical protein [Clostridium butyricum]	WP_002579182.1	52%	96%	4,00E-112	14
glycosylase [Clostridium butyricum]	WP_003410166.1	52%	96%	4,00E-111	22
putative glycosidase [Clostridium butyricum]	WP_003424363.1	52%	96%	3,00E-112	23
glycosylase [Paenibacillus sp. Aloe-11]	WP_007432940.1	52%	97%	3,00E-115	71
glycosylase [Paenibacillus peoriae]	WP_010347083.1	52%	97%	8,00E-117	125
glycosylase [Paenibacillus polymyxa]	WP_013312515.1	52%	97%	3,00E-116	147
glycosylase [Paenibacillus terrae]	WP_014278667.1	52%	97%	6,00E-117	164
hypothetical protein [Lachnospiraceae bacterium M18-1]	WP_016298584.1	52%	97%	3,00E-111	191
hypothetical protein [Ruminococcus callidus]	WP_021681646.1	52%	96%	2,00E-114	229
predicted glycosylase [Ruminococcus sp. CAG:17]	WP_021977609.1	52%	97%	1,00E-114	238
hypothetical protein Q607_CBUC00205G0058 [Clostridium butyricum DORA_1]	ETI88253.1	52%	96%	5,00E-111	294

glycosylase [Clostridium sp. 12(A)]	WP_024836976.1	52%	97%	3,00E-108	321
glycosylase [Paenibacillus polymyxa]	WP_025676719.1	52%	96%	1,00E-116	337
glycosylase [Paenibacillus sp. 1-49]	WP_025683918.1	52%	97%	1,00E-115	339
glycosylase [[Clostridium aerotolerans]	WP_026890542.1	52%	97%	2,00E-109	374
glycosylase [Paenibacillus sp. WLY78]	WP_029518687.1	52%	97%	6,00E-117	408
glycosylase [Paenibacillus polymyxa]	KEO78836.1	52%	97%	3,00E-116	412
glycosylase [Treponema saccharophilum]	WP_002702282.1	51%	97%	2,00E-104	18
glycosylase [Roseburia intestinalis]	WP_006858634.1 (ADD61810.1 : CBL14186.1)	51%	97%	2,00E-109	65
glycosylase [Thermoanaerobacterium thermosaccharolyticum]	WP_013296705.1	51%	97%	2,00E-109	145
glycosylase [Cellulosilyticum lentocellum]	WP_013658330.1	51%	97%	2,00E-107	157
glycosylase [Thermoanaerobacterium saccharolyticum]	WP_014757630.1 (AFK85719.1)	51%	97%	1,00E-109	170
hypothetical protein [Clostridiales bacterium oral taxon 876]	WP_021653978.1	51%	96%	1,00E-111	226
putative uncharacterized protein [Roseburia intestinalis CAG:13]	WP_022113208.1	51%	97%	3,00E-108	245
glycosylase [Treponema brennaborense]	WP_013759040.1	50%	97%	4,00E-107	159

Table A

Table A further presents the identity to UhgbMP (%), the sequence coverage (%) and the E-value of said proteins.

Most preferably, the sequence identity percentage is determined using the BLASTP software (version 2.2.29), the parameters being set as follows: (1) Max target sequences=500 ; (2) short queries (Automatically adjust parameters for short input sequences)= `—yes ; (3) Expect threshold=10; (4) Word size=3; (5) Max matches in a query range=0; (6)

Matrix=BLOSUM62 ; (7) Gap Costs= `Existence: 11 Extension: 1; (8) Compositional adjustments—conditional compositional score matrix adjustment"; (9) Filter (Low complexity regions) — no; (10) Mask (Mask for lookup table only) — no ; (11) Mask (Mask lower case letters) — no;

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 80%, in particular of at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, more particularly of at least 90%, with the amino acid sequence SEQ ID NO: 1, and has an E-value comprised from 0 to 5,00E-167, in particular an E-value of 0.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 50% and less than 64%, in particular an identity percentage comprised from 50% to 63%, more particularly an identity percentage of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62% or 63%, with the amino acid sequence SEQ ID NO: 1, and has an E-value comprised from 1,00E-153 to 1,00E-106, in particular from 1,00E-153 to 1,00E-120 or from 1,00E-120 to 1,00E-106.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 64% and less than 80%, in particular an identity percentage comprised from 65% to 79%, with the amino acid sequence SEQ ID NO: 1, and has an E-value comprised from 0 to 1,00E-147, in particular from 0 to 1,00E-155, or from 1,00E-155 to 1,00E-147, the E-value being more particularly of 0.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of at least 50% and less than 66%, in particular an identity percentage comprised from 50% to 65%, more particularly an identity percentage of 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64% or 65%, with the amino acid sequence SEQ ID NO: 1, and has an E-value comprised from 1,00E-161 to 1,00E-106, in particular from 1,00E-161 to 1,00E-120 or from 1,00E-120 to 1,00E-106.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein glycoside-phosphorylase has an identity percentage of more than 66% and less than 80%, in particular an identity percentage comprised from 67% to 79%, with the amino acid sequence SEQ ID NO: 1, and has an E-value comprised from 0 to 1,00E-147, in

particular from 0 to 1,00E-155, or from 1,00E-155 to 1,00E-147, the E-value being more particularly of 0.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1 and wherein said mannosyl donor compound is β-1,4-D-Mannan.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase is recombinantly expressed.

For example, the gene encoding for the glycoside-phosphorylase, in particular UhgbMP, can be amplified by any means known in the art, for instance by PCR using an appropriate set of primers.

The amplified gene or PCR product can then be ligated into any appropriate expression vector, such as for example the expression vector pDEST17, which can be used to transform any host organism known in the art such as, for example, *E. coli*.

The enzyme produced by the host organism can then be extracted and purified by any method known in the art such as, for example, using a TALON resin (immobilized metal affinity chromatography (IMAC) resin, Clontech) loaded with cobalt to purify His-tagged UhgbMP.

In this regard, the invention further relates to a glycoside-phosphorylase, in particular UhgbMP, which contains at least one deletion, substitution or addition, or any combination thereof, which does not diminish the phosphorylase and/or reverse phosphorylase activity of said glycoside-phosphorylase by at most 5%, 10%, 20%, 30%, 40%, 50%, 60 %, 70%, 80% or 90%. In one embodiment, the glycoside-phosphorylase of the present invention, in particular UhgbMP, contains at least one deletion, substitution or addition, or any combination thereof, which does not diminish the phosphorylase and/or reverse phosphorylase activity of said glycoside-phosphorylase by at most 90%. In other words, the present invention also relates to a glycoside-phosphorylase, in particular UhgbMP, which contains at least one deletion, substitution or addition, or any combination thereof, and which retains 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10% of the phosphorylase and/or reverse phosphorylase activity of the wild type glycoside-phosphorylase. The phosphorylase and/or reverse phosphorylase activity can be measured by any method known to a skilled person, in particular by the determination of the apparent catalytic efficiency. A

non-limiting example of an addition that does not influence the enzyme's activity is an N- or C-terminal addition of a His-tag, or of another purification tag.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein a mannosyl residue is grafted on a mannosyl acceptor containing a hydroxyl group, in the presence of said α-D-mannopyranose-1-phosphate, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose.

In another aspect, the present invention relates to a process of degradation of a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose,

comprising a step of contacting said mannosyl donor with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, and inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2.

The present invention also relates to a process of degradation of a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose,

comprising a step of contacting said mannosyl donor with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, and inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2 or Bt1033.

In an advantageous embodiment, said glycoside-phosphorylase is characterized, in relation with phosphorolysis, generating α-D-mannopyranose-1-phosphate and a $(Man)_{m-1}$-donor compound from a mannosyl donor of formula $(Man)_m$-donor and inorganic phosphate, by the quantification of said α-D-mannopyranose-1-phosphate, said $(Man)_{m-1}$-donor compound or said inorganic phosphate.

In an advantageous embodiment, the apparent catalytic efficiency $kcat_{app}/ Km_{app}$ of said glycoside-phosphorylase is above about 0.02 $s^{-1}mM^{-1}$, in particular above about 0.9 $s^{-1}mM^{-1}$, for phosphorolysis in presence of inorganic phosphate and a mannosyl donor selected from the list constituted by β-D-mannopyranosyl-1,4-D-mannose, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose and β-1,4-D-Mannan.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said step of phosphorolysis is followed by a further step of contacting said compound of formula $(Man)_{m-1}$-donor with said glycoside-phosphorylase and inorganic phosphate, in a aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-2}$-donor, said further step being repeated p times, p being an integer comprised from 0 to (m-2), to obtain α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{(m-p)-2}$-donor.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said mannosyl donor compound is β-1,4-D-Mannan.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said glycoside-phosphorylase is chosen among glycoside-phosphorylases identified by the following GenBank Accession numbers: ADD61463.1, AEY67872.1, AFN75330.1, ADO59098.1, CCI71609.1, ADD61810.1, CBL14186.1, AFK85719.1, ADA67643.1, AAD36300.1, ACB09929.1, ABQ47551.1, ACM23522.1, ABV33993.1, AGB28392.1, AFL78596.1, EDS13361.1, ABX42090.1, AFG35891.1, ACJ75237.1, ADL43426.1, AEM72946.1, ACM61623.1, ABR30874.1, AFK07300.1, AFK07371.1, AFL98126.1, AAO76140.1, ACB75595.1, ZP_08158270.1,

ZP_03299783.1, ZP_02422496.1, ZP_02205887.1, ZP_02090881.1, ZP_02071200.1, ZP_02067106.1, ZP_01958898, YP_210978.1 and YP_001297942.1.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said glycoside-phosphorylase is chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1 and wherein said mannosyl donor compound is β-1,4-D-Mannan.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said glycoside-phosphorylase is recombinantly expressed.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, comprising:

- a step of contacting a compound of formula

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor;

- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to

obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said mannosyl acceptor is selected from the group constituted by carbohydrates, glycoproteins, glycopeptides, water and hydroxylated compounds, in particular polyols.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said mannosyl acceptor is selected from the list constituted by D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said mannose is grafted on said mannosyl acceptor via a β-linkage.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said phosphorolysis and said reverse phosphorolysis are performed in one-pot.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, wherein said reverse phosphorolysis is performed subsequently to said phosphorolysis.

In an advantageous embodiment, said phosphorolysis and said reverse phosphorolysis are performed independently.

In particular, said reverse phosphorolysis can be performed with commercial α-D-mannopyranose-1-phosphate.

In an advantageous embodiment, the present invention relates to the process of degradation as defined above, comprising:

- a step of contacting a compound of formula

(Man)$_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl

chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor;

- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate,
- a further step of contacting said mannosylated acceptor of formula Man-Acceptor with α-D-mannopyranose-1-phosphate and said glycoside-phosphorylase, said further step being repeated n times, n being an integer comprised from 0 to 9, to obtain a (n+2) times mannosyated acceptor of the following structure: $(Man)_n$-Man-Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

In another aspect, the present invention also relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, to graft a mannosyl residue on a mannosyl acceptor containing a hydroxyl group, in the presence of α-D-mannopyranose-1-phosphate, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose,
with the proviso that said glycoside-phosphorylase is not RaMP2.

The present invention also relates to the use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, to graft a mannosyl residue on a mannosyl acceptor containing a hydroxyl group, in the presence of α-D-mannopyranose-1-phosphate, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose,
with the proviso that said glycoside-phosphorylase is not RaMP2 or Bt1033.

By "mannosyl acceptor" is meant an acceptor of mannosyl, in other words a compound on which a mannoside residue is able to be grafted, by reverse phosphorolysis.

By "reverse phosphorolysis" is meant the formation of a glycosidic bond between the mannosyl unit of the α-D-mannopyranose-1-phosphate and the mannosyl acceptor, said formation being catalyzed by a glycoside-phosphorylase.

In an advantageous embodiment, said glycoside-phosphorylase is characterized, in relation with reverse phosphorolysis, generating a mannosylated acceptor and inorganic phosphate from α-D-mannopyranose-1-phosphate and a mannosyl acceptor, by the quantification of said α-D-mannopyranose-1-phosphate or said inorganic phosphate.

In an advantageous embodiment, the apparent catalytic efficiency $kcat_{app}$/ Km_{app} of said glycoside-phosphorylase is above about 0.05 $s^{-1}mM^{-1}$, in particular above about 0.4 $s^{-1}mM^{-1}$, for reverse phosphorolysis in presence of α-D-mannopyranose-1-phosphate and a mannosyl acceptor, in particular selected from D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is not L-arabinose, D-cellobiose, D-fucose, L-fucose, L-rhamnose, Xylitol, D-lyxose, L-xylose, D-mannitol, D-altrose, D-xylose or D-allose.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase is chosen among glycoside-phosphorylases identified by the following GenBank Accession numbers: ADD61463.1, AEY67872.1, AFN75330.1, ADO59098.1, CCI71609.1, ADD61810.1, CBL14186.1, AFK85719.1, ADA67643.1, AAD36300.1, ACB09929.1, ABQ47551.1, ACM23522.1, ABV33993.1, AGB28392.1, AFL78596.1, EDS13361.1, ABX42090.1, AFG35891.1, ACJ75237.1, ADL43426.1, AEM72946.1, ACM61623.1, ABR30874.1, AFK07300.1, AFK07371.1, AFL98126.1, AAO76140.1, ACB75595.1, ZP_08158270.1, ZP_03299783.1, ZP_02422496.1, ZP_02205887.1, ZP_02090881.1, ZP_02071200.1, ZP_02067106.1, ZP_01958898, YP_210978.1 and YP_001297942.1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase is chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is selected from the group constituted by carbohydrates, glycoproteins, glycopeptides, water and hydroxylated compounds, in particular polyols.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is a carbohydrate.

By "carbohydrates" is meant saccharides, in particular monosaccharides, oligosaccharides, and polysaccharides.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is selected from the list constituted by D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is selected from the list constituted by glycoproteins and glycopeptides.

By "glycoprotein" is meant a protein that contains saccharide chains, in particular oligosaccharide and/or monosaccharide chains, covalently attached to the side-chains of amino acids constituting said protein.

By "glycopeptide" is meant a peptide that contains saccharide chains, in particular oligosaccharide and/or monosaccharide chains, covalently attached to the side-chains of amino acids constituting said peptide.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl acceptor is water.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said mannosyl residue is grafted on said mannosyl acceptor via a β-linkage.

Considering α-D-mannopyranose-1-phosphate and said β-linkage between mannosyl residue and said acceptor, there is inversion of configuration at C-1 of the mannosyl residue of the α-D-mannopyranose-1-phosphate donor.

In an advantageous embodiment, the present invention relates to the use as defined above, wherein said α-D-mannopyranose-1-phosphate is obtained from degradation by phosphorolysis of a mannosyl donor compound of formula (Man)$_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15, said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with said glycoside-phosphorylase.

In another aspect, the present invention relates to the process of grafting a mannosyl residue on a mannosyl acceptor containing a hydroxyl group, comprising a step of contacting a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, with α-D-mannopyranose-1-phosphate and said mannosyl acceptor, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose and inorganic phosphate,
with the proviso that said glycoside-phosphorylase is not RaMP2.

The present invention relates to the process of grafting a mannosyl residue on a mannosyl acceptor containing a hydroxyl group, comprising a step of contacting a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, with α-D-mannopyranose-1-phosphate and said mannosyl acceptor, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose and inorganic phosphate,
with the proviso that said glycoside-phosphorylase is not RaMP2 or Bt1033.

In an advantageous embodiment, said glycoside-phosphorylase is characterized, in relation with reverse phosphorolysis, generating a mannosylated acceptor and inorganic phosphate from α-D-mannopyranose-1-phosphate and a mannosyl acceptor, by the quantification of said α-D-mannopyranose-1-phosphate or said inorganic phosphate.

In an advantageous embodiment, the apparent catalytic efficiency $kcat_{app}/ Km_{app}$ of said glycoside-phosphorylase is above about 0.05 $s^{-1}mM^{-1}$, in particular above about 0.4 $s^{-1}mM^{-1}$, for reverse phosphorolysis in presence of α-D-mannopyranose-1-phosphate and a mannosyl acceptor, in particular selected from D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said glycoside-phosphorylase is chosen among glycoside-phosphorylases identified by the following GenBank Accession numbers: ADD61463.1, AEY67872.1, AFN75330.1, ADO59098.1, CCI71609.1, ADD61810.1, CBL14186.1, AFK85719.1, ADA67643.1, AAD36300.1, ACB09929.1, ABQ47551.1, ACM23522.1, ABV33993.1, AGB28392.1, AFL78596.1, EDS13361.1, ABX42090.1, AFG35891.1, ACJ75237.1, ADL43426.1, AEM72946.1, ACM61623.1, ABR30874.1, AFK07300.1, AFK07371.1, AFL98126.1, AAO76140.1, ACB75595.1, ZP_08158270.1, ZP_03299783.1,

ZP_02422496.1, ZP_02205887.1, ZP_02090881.1, ZP_02071200.1, ZP_02067106.1, ZP_01958898, YP_210978.1 and YP_001297942.1.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said glycoside-phosphorylase is chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said glycoside-phosphorylase has the amino acid sequence SEQ ID NO: 1.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl acceptor is selected from the group constituted by carbohydrates, glycoproteins, glycopeptides, water and hydroxylated compounds, in particular polyols.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl acceptor is a carbohydrate.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl acceptor is selected from the list constituted by D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl acceptor is selected from the list constituted by glycoproteins and glycopeptides.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl acceptor is water.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl residue is grafted on said mannosyl acceptor via a β-linkage.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, comprising:

- a step of contacting a compound of formula

  (Man)$_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor;

- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

Said embodiment concerns the grafting of a mannosyl residue from α-D-mannopyranose-1-phosphate to a mannosyl acceptor in presence of a glycoside-phosphorylase, said α-D-mannopyranose-1-phosphate being obtained by degradation of a mannosyl donor by said glycoside-phosphorylase.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said phosphorolysis and said reverse phosphorolysis are performed in one-pot.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said reverse phosphorolysis is performed subsequently to said phosphorolysis.

In an advantageous embodiment, said phosphorolysis and said reverse phosphorolysis are performed independently.

In particular, said reverse phosphorolysis can be performed with commercial α-D-mannopyranose-1-phosphate.

In an advantageous embodiment, the present invention also relates to the process of grafting as defined above, comprising:

- a step of contacting a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, with α-D-mannopyranose-1-phosphate and said mannosyl acceptor, in an aqueous medium, to obtain by reverse

phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose and inorganic phosphate,

- a further step of contacting said mannosylated acceptor of formula Man-Acceptor with α-D-mannopyranose-1-phosphate and said glycoside-phosphorylase, said further step being repeated n times, n being an integer comprised from 0 to 9, to obtain a (n+2) times mannosyated acceptor of the following structure: $(Man)_n$-Man-Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

Said embodiment concerns the grafting of one mannosylated residue on a mannosyl acceptor, said grafting being repeated (n+1) times to obtain a compound of the following formula: $(Man)_{n+2}$-Acceptor.

In other words, mannosyl residues are polymerized, through the creation of beta-1,4 linkages, yielding to the synthesis of manno-oligo and manno-polysaccharides grafted on an acceptor.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, comprising:

- a step of contacting a compound of formula

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor;

- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate;

- a further step of contacting said mannosylated acceptor of formula Man-Acceptor with α-D-mannopyranose-1-phosphate and said glycoside-phosphorylase, said further step being repeated n times, n being an integer comprised from 0 to 9, to obtain a (n+2) times mannosyated acceptor of the following structure: $(Man)_n$-Man-Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said mannosyl donor compound is β-1,4- D-Mannan.

In an advantageous embodiment, the present invention relates to the process of grafting as defined above, wherein said glycoside-phosphorylase is recombinantly expressed.

In another aspect, the present invention relates to a pharmaceutical composition comprising as active substance an inhibitor of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, said inhibitor being in particular D-altrose, D-xylose or D-allose, in association with a pharmaceutically acceptable vehicle.

In an advantageous embodiment, the present invention relates to the pharmaceutical composition as defined above, administrable by oral route at a dose comprised from about 0.1 mg/kg to about 1000 mg/kg of body weight.

In an advantageous embodiment, the present invention relates to the pharmaceutical composition as defined above, administrable by rectal route at a dose comprised from about 0.1 mg/kg to about 1000 mg/kg of body weight.

In an advantageous embodiment, the present invention relates to the pharmaceutical composition as defined above, under a form liable to be administrable by oral route, under the form of a unit dose comprised from about 5 mg to about 10,000 mg, in particular from about 10 mg to about 2,000 mg, in particular from about 50 to about 1,000 mg.

In an advantageous embodiment, the present invention relates to the pharmaceutical composition as defined above, under a form liable to be administrable by rectal route, under the form of a unit dose comprised from about 5 mg to about 10,000 mg, in particular from about 10 mg to about 2,000 mg, in particular from about 50 to about 1,000 mg.

In another aspect, the present invention relates to a composition comprising an inhibitor of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, for treating inflammatory bowel diseases.

In an advantageous embodiment, the present invention relates to the composition as defined above, wherein said inflammatory bowel diseases belong to the group consisting of Crohn's disease, ulcerative colitis and colon cancer.

DESCRIPTION OF THE DRAWINGS

Figure 1A presents the ^{1}H NMR spectra of the products synthesized by reverse phosphorolysis by UhgbMP from 1) 10 mM α-D-mannopyranose-1-phosphate and 10 mM D-mannose, 2) 10 mM α-D-mannopyranose-1-phosphate and 10 mM D-glucose, 3) 10 mM α-D-mannopyranose-1-phosphate and 10 mM *N*,*N*'-diacetyl chitobiose.

Figure 1B presents the ^{13}C (B) NMR spectra of the products synthesized by reverse phosphorolysis by UhgbMP from 1) 10 mM α-D-mannopyranose-1-phosphate and 10 mM D-mannose, 2) 10 mM α-D-mannopyranose-1-phosphate and 10 mM D-glucose.

Figure 2 presents the dependency of UhgbMP specific activity (phosphorolysis) with the polymerisation degree (DP) of the phosphorolysed β-1,4-D-mannoligosaccharides.

EXAMPLES

Example 1 : Recombinant UhgbMP production and purification

First, the UhgbMP (SEQ ID NO: 1) encoding gene (SEQ ID NO: 2) was PCR amplified from the E. coli metagenomic clone (Genbank accession number GU942931) using primers forward 5'AGTATGAGTAGCAAAGTTATTATTCCTTGG 3' (SEQ ID NO: 5) and reverse 5' TCAGATGATGCTTGTACGTTTGGTAAATTC 3' (SEQ ID NO: 6), by using the Expand Long Template PCR kit (Roche).

To allow heterologous UhgbMP production in E. coli with His(6) tag at the N-terminal extremity, the PCR product was purified and subsequently cloned into the pCR8/GW/TOPO entry vector (Invitrogen), and then into the pDEST17 destination vector (Invitrogen), according to the manufacturer's recommendations. E.coli BL21-AI cells (Invitrogen) harboring the UhgbMP-encoding plasmid were cultured at 20°C for 24 hours in ZYM-5052 autoinduction medium supplemented with 100 µg/mL ampicillin, inoculated at OD600nm 0.1. Cells were harvested and resuspended in 20mM Tris HCl, pH 7.0, 300 mM NaCl, and lysed by sonication. Soluble lysate was applied to a TALON resin loaded with cobalt (GE Healthcare) equilibrated in 20mM Tris HCl, pH 7.0, 300 mM NaCl. After column washing with 8 volumes of the same buffer supplemented with 10mM imidazole, the protein was eluted in 20mM Tris HCl, pH 7.0, 300 mM NaCl, 150 mM imidazole. Finally, the protein

sample was desalted on a PD-10 column (GE Healthcare) and eluted in 20 mM Tris-HCl pH7.0, Tween 80 0.1% (vol/vol). Protein concentrations were determined by spectrometry using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). In these conditions, 84 % of UhgbMP remained soluble after 8 days at 4°C.

Example 2 : Synthesis of manno-oligosaccharides from α-D-mannopyranose-1-phosphate and carbohydrate acceptors

Reaction was performed with 0.1 mg/ml purified UhgbMP during 24h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM of the α-D-mannopyranose-1-phosphate (reference M1755, Sigma), and 10 mM of carbohydrate acceptors.

When the acceptor is *N*,*N*'-diacetyl chitobiose (Dextra, reference C8002), the reaction can be performed with a concentration of said *N*,*N*'-diacetyl chitobiose comprised from 0.1 to 10 mM, in particular from 0.5 to 1 mM, more particularly with a concentration of 1mM.

The apparent kinetic parameters for reverse phosphorolysis were determined by fitting the initial rates of α-D-mannopyranose-1-phosphate release and consumption, respectively, to the Michaelis-Menten equation. Non-linear regression was performed with SigmaPlot Enzyme Kinetics module, version 1.3 (Systat Software, Inc., San Jose California USA).

Here the reaction conditions were different than those used for synthesis of mannooligosaccharides : Reaction was performed with 0.01 mg/ml purified UhgbMP at 37 °C in Tris HCl 20mM, pH 7.0. Substrate concentrations were as follows: 10 mM α-D-mannopyranose-1-phosphate and 5 to 40 mM of D-glucose, D-galactose, D-fructose, or *N*-acetyl-D-glucosamine, or, 5 or 10 mM α-D-mannopyranose-1-phosphate and 5 to 40 mM of D-mannose, or 5 mM α-D-mannopyranose-1-phosphate and 0.1 to 1 mM of *N*,*N*'-diacetyl chitobiose.

Carbohydrate analysis was performed by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate were separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and an isocratic step of 300 mM sodium acetate in 150 mM NaOH is applied at a 1 $mL.min^{-1}$ flow rate. Detection is performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

NMR Spectroscopy: Freeze-dried reaction media were exchanged twice with 99.9 atom% D_2O and lyophilized. Deuterium oxide was used as the solvent and sodium 2,2,3,3-

tetradeuterio-3-trimethylsilylpropanoate was selected as the internal standard. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 500 MHz spectrometer using a 5 mm z-gradient TBI probe at 298 K, an acquisition frequency of 500.13 MHz and a spectral width of 8012.82 Hz. Spectra were acquired and processed using TopSpin 3.0 software. The various signals were assigned by comparison with signals obtained from α-D-mannopyranose-1-phosphate, β-D-mannopyranosyl-1,4-D-mannose (Megazyme, Irleland, reference O-MBI), β-D-mannopyranosyl-1,4-D-glucose (Carbosynth, United Kingdom, reference OM04754), or β-D-mannopyranosyl-1,4-N,N'-diacetyl chitobiose (Dextra, United Kingdom, reference MC0320), used as standards.

Results

First, even without any carbohydrate acceptor, UhgbMP produces mannose and, further, mannooligosaccharides of DP ranging from 1 to 12, indicating that water itself plays the role of first acceptor at the beginning of the reaction. The β-1,4 regio-specific synthesis of manno-oligosaccharides was characterized by ^{1}H and ^{13}C NMR (Figure 1). Various carbohydrates were tested as acceptors (Table 1). D-Glc*p*NAc and β-D-Glc*p*NAc-1,4-D-Glc*p*NAc were the best recognized acceptors, given that the UhgbMP Km value for these compounds are 6 and 48 fold lower than for D-mannose, respectively. Starting from α-D-mannopyranose-1-phosphate and D-Glc*p*NAc or β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, UhgbMP generates series of mannooligosaccharides containing D-Glc*p*NAc or β-D-Glc*p*NAc-1,4-D-Glc*p*NAc at their reducing end, with a DP of up to 4. D-glucose, D-mannose, D-galactose and D-fructose are also recognized as acceptors.

Reverse-phosphorolysis			
Glycoside acceptors	**Km $_{app}$** (mM)	**kcat $_{app}$** (s^{-1})	**kcat $_{app}$/ Km $_{app}$** ($s^{-1}mM^{-1}$)
D-glucose	29.79±6.21	12.99±1.78	0.44
D-mannose (5 mM α-D-mannopyranose-1-phosphate)	11.21±1.64	7.23±0.39	0.64
D-mannose (10 mM α-D-mannopyranose-1-phosphate)	15.34±0.28	11.16±0.08	0.73
D-galactose	16.33±3.00	13.11±1.03	0.80
D-fructose	2.86±0.93	12.13±0.77	4.24
N-acetyl-D-glucosamine	3.78±0.55	13.51±0.45	3.57
N,N'-diacetyl chitobiose	0.28±0.03	9.31±0.35	33.25

Table 1

UhgbMP is the most effective phosphorylase for production of N-glycan core oligosaccharides, such as β-D-Man*p*-1,4-D-GlcNAc and β-D-Man*p*-1,4-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, whose commercial price today exceeds $ 10,000 per mg. In fact, the UhgbMP apparent catalytic efficiency for reverse phosphorolysis using *N*-acetyl-D-glucosamine and β-D-Glc*p*NAC-1,4-D-GlcNAc as acceptors is 24 and 262 times higher than that of RaMP2.

Example 3 : Phosphorolysis of oligosaccharides or polysaccharides by UhgbMP

Reaction was performed with 0.1 mg/ml purified UhgbMP during 24h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM inorganic phosphate (prepared from a stock solution of 100 mM phosphate obtained from a mix of 100 mM di-sodium hydrogen phosphate dodecahydrate (ref 28028.298 / VWR Prolabo) and of 100 mM sodium dihydrogen phosphate dihydrate (ref 28015.294 / VWR Prolabo) solutions to reach a pH of 7.0) and 10 mM carbohydrate donor, excepted for β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose (Dextra, United Kingdom, reference MC0320) which was used at 2 mM.

The apparent kinetic parameters for phosphorolysis were determined by fitting the initial rates of α-D-mannose-1-phosphate release and consumption, respectively, to the Michaelis-Menten equation. Non-linear regression was performed with SigmaPlot Enzyme Kinetics module, version 1.3 (Systat Software, Inc., San Jose California USA).

Here the reaction conditions were different than those used for synthesis of mannooligosaccharides : Reaction was performed with 0.01 mg/ml purified UhgbMP at 37 °C in Tris HCl 20mM, pH 7.0. Substrate concentrations were as follows: 10 mM inorganic phosphate and 1 to 10 mM of β-D-mannopyranosyl-1,4-D-glucose, 10 mM inorganic phosphate and 0.4 to 4 mM of β-1,4-D-mannan, 5 or 10 mM inorganic phosphate and 1 to 20 mM of β-D-mannopyranosyl-1,4-D-mannose, 5 mM inorganic phosphate and 0.05 to 0.5 mM of β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose.

Carbohydrate analysis was performed by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate are separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and an isocratic step of 300 mM sodium acetate in 150 mM NaOH is applied at a 1 $mL.min^{-1}$ flow rate. Detection is performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

Results

UhgbMP exhibits a wide specificity towards carbohydrate donors (Table 2). UhgbMP is able to phosphorolyse β-D-mannopyranosyl-1,4-D-glucopyranose, β-1,4-linked D-manno-oligosaccharides and mannan (β-D-Man*p*-1,4-(D-Man*p*)$_n$, with n = 1 to 15), characterized by a notable parallel increase in specific activity with the degree of polymerisation (DP) (Figure 2). UhgbMP is thus to date the only characterized GH130 enzyme that is able to breakdown mannan, a constituent of hemicellulose in grains and nuts. Interestingly, the best recognized UhgbMP carbohydrate donor that we tested for UhgbMP is β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose (β-D-Man*p*-1,4- β-D-Glc*p*NAc-1,4-D-Glc*p*NAc), a signature motif of human N-glycans. The Km for this compound is respectively 10- and 57-fold lower than that obtained for β-1,4-D-mannobiose and β-D-Man*p*-1,4-D-Glc*p*. However, neither cellobiose (β-D-Glc*p*-1,4-D-Glc*p*) nor *N*,*N*'-diacetyl chitobiose (β-D-Glc*p*NAc-1,4-D-Glc*p*NAc) could be phosphorolysed, indicating that the UhgbMP subsite -1 is highly specific for mannosyl residues.

Phosphorolysis			
Glycoside donors	**Km $_{app}$** (mM)	**kcat $_{app}$** (s^{-1})	**kcat $_{app}$/ Km $_{app}$** ($s^{-1}mM^{-1}$)
β-1,4-D-mannopyranosyl-D-mannose (5 mM inorganic phosphate)	1.15±0.11	1.06±0.02	0.92
β -1,4-D-mannopyranosyl-D-mannose (10 mM inorganic phosphate)	1.06±0.04	1.10±0.01	1.04
β -1,4-D-mannopyranosyl-D-glucose	47.67±5.76	60.54±6,23	1.27
β -1,4-D-Mannan	2.17±0.39	3.07±0.26	1.41
β -1,4-D-mannopyranosyl-N,N'-diacetyl chitobiose	0.018±0.003	0.072±0.002	4.0

Table 2

Finally, no trace of oligosaccharide-phosphate was visible, at any reaction time, on HPAEC-PAD chromatograms of products obtained during phosphorolysis reactions, irrespective of the donor substrate. UhgbMP is thus an exo-acting enzyme, able to breakdown only the first beta-mannosidic linkage at the non-reducing end of donor oligosaccharides.

Example 4 : Quantification of UhgbMP inhibition by carbohydrates or hydroxylated compounds

The percentage of UhgbMP inhibition by carbohydrates or other hydroxylated compounds was measured with 0.1 mg/ml purified enzyme by quantifying α-D-mannopyranose-1-

phosphate consumption rate from 10 mM α-D-mannopyranose-1-phosphate as glycosyl donor, with and without 10 mM of L-rhamnose, D-altrose, D-allose, D-fucose, L-fucose, D-mannitol, D-lyxose, xylitol, L-xylose, D-xylose, L-arabinose, or D-cellobiose. It was checked by HPAEC-PAD that less than 10 % of α-D-mannopyranose-1-phosphate was consumed during the phase of activity measurement.

The presence of these compounds decreased enzyme-specific activity, in some cases quite markedly (Table 3). But concentrations of these compounds did not decrease during reaction, and no significant additional product was produced compared with reaction in the presence of_α-D-mannopyranose 1-phosphate as sole substrate. These carbohydrates thus act as UhgbMP inhibitors.

Glycoside donor	Inhibitor	% of inhibition
α-D-mannose-1-phosphate	/	0
	L-arabinose	2 ± 0.4
	D-cellobiose	37 ± 1
	D-fucose	12 ± 2
	L-fucose	11 ± 4
	L-rhamnose	31 ± 2
	Xylitol	29.4 ± 0.4
	D-lyxose	19 ± 2
	L-xylose	58 ± 1
	D-mannitol	37 ± 4
	D-altrose	84 ± 3
	D-xylose	86.2 ± 0.2
	D-allose	94 ± 3

Table 3

Example 5 : Test of various sugar-phosphates as glycosyl donors for reverse phosphorolysis

Reactions were performed with 0.1 mg/ml purified UhgbMP during 24h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM of the α-D-fructose-1- and -6-phosphate, D-ribose-1-phosphate, α-D-galactosamine-1-phosphate, α-D-glucosamine-1-phosphate, D-mannose-6-phosphate, α-D-glucopyranosyl-1 or -6-phosphate as putative glycoside donors.

Carbohydrate analysis was performed by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate are separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and a isocratic step of 300 mM sodium acetate in 150 mM NaOH is applied at a 1 $mL.min^{-1}$ flow rate. Detection is performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

All of said sugar-phophates (α-D-fructose-1- and -6-phosphate, D-ribose-1-phosphate, α-D-galactosamine-1-phosphate, α-D-glucosamine-1-phosphate, D-mannose-6-phosphate, α-D-glucopyranosyl-1 or -6-phosphate) remained untouched by UhgbMP.

Example 6 : Protocol to assay mannosylation of GlcNAc-GlcNAc-protein by UhgbMP - catalysed reverse-phosphorolysis

Mature human intestinal Muc2 (SEQ ID NO: 4) glycoprotein is purified from mucus, as described by Allen, A. et al. (1998), The International Journal of Biochemistry & Cell Biology 30, 797–801.

Muc2 glycoprotein containing GlcNAc-GlcNAc N-glycosylation motifs are obtained by hydrolysis of the mature Muc2 glycoprotein with mannosidases classified in the families GH92 and GH2 of glycoside-hydrolases (Tailford, L.E. et al. (2007), J. Biol. Chem. 282, 11291–11299 ; Cantarel, B.L. et al. (2009), Nucl. Acids Res. 37, D233–D238 ; Zhu, Y. et al. (2010), Nature Chemical Biology 6, 125–132).

UhgbMP reverse-phosphorolysis from α-D-mannopyranose-1-phosphate and the human intestinal Muc2 glycoprotein containing GlcNAc-GlcNAc N-glycosylation motifs is determined after incubation of 0.1 mg/ml purified enzyme during 24h at 37°C in Tris HCl 20mM, pH 7.0 with 10 mM α-D-mannopyranose-1-phosphate (reference M1755, Sigma) and between 1 and 100 µM of the Muc2 protein obtained as described above, by quantifying α-D-mannopyranose-1-phosphate consumption rate by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

Example 7 : Protocol to assay phosphorolysis of a Man-GlcNAc-GlcNAc-protein by UhgbMP

Mature human intestinal Muc2 glycoprotein is purified from mucus, as described by Allen, A. et al. (1998), The International Journal of Biochemistry & Cell Biology 30, 797–801.

Muc2 glycoprotein containing Man-GlcNAc-GlcNAc N-glycosylation motifs are obtained by hydrolysis of the mature Muc2 glycoprotein with mannosidases classified in the families GH92 of glycoside-hydrolases (Cantarel, B.L. et al. (2009), Nucl. Acids Res. 37, D233–D238 ; Zhu, Y. et al. (2010), Nature Chemical Biology 6, 125–132).

UhgbMP catalysed phosphorolysis of the human intestinal Muc2 glycoprotein containing Man-GlcNAc-GlcNAc N-glycosylation motifs is determined after incubation of 0.1 mg/ml purified enzyme during 24h at 37 °C in Tris HCl 20mM, pH 7.0 with 10 mM inorganic phosphate (prepared from a stock solution of 100 mM phosphate obtained from a mix of 100 mM di-sodium hydrogen phosphate dodecahydrate (ref 28028.298 / VWR Prolabo) and of 100 mM sodium dihydrogen phosphate dihydrate (ref 28015.294 / VWR Prolabo) solutions to reach a pH of 7.0) and between 1 and 100 µM of the Muc2 protein obtained as described above, by quantifying α-D-mannopyranose-1-phosphate release rate by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

Example 8 : Protocol for the one-pot synthesis of mannooligosaccharides containing a GlcNAc (or GlcNAC-GlcNAc) at their reducing end, from mannan, inorganic phosphate, and *N*-acetyl-D-glucosamine (or *N*,*N*'-diacetyl chitobiose) as substrates

Reaction is performed with 0.1 mg/ml purified UhgbMP during 10h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM inorganic phosphate (prepared from a stock solution of 100 mM phosphate obtained from a mix of 100 mM di-sodium hydrogen phosphate dodecahydrate (ref 28028.298 / VWR Prolabo) and of 100 mM sodium dihydrogen phosphate dihydrate (ref 28015.294 / VWR Prolabo) solutions to reach a pH of 7.0) and 10 mM mannan (Megazyme, Ireland, reference P-MANCB), 10 mM *N*-acetyl-D-glucosamine (Sigma, reference A8625), or 1 mM *N*,*N*'-diacetyl chitobiose (Dextra, reference C8002).

Carbohydrate analysis is performed by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate are separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and a isocratic step of 300 mM sodium acetate in 150 mM NaOH is applied at a 1 $mL.min^{-1}$ flow rate. Detection is

performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

Example 9 : Protocol for the two-step synthesis of mannooligosaccharides containing a GlcNAc (or GlcNAC-GlcNAc) at their reducing end, from mannan, inorganic phosphate, and *N*-acetyl-D-glucosamine (or *N*,*N*'-diacetyl chitobiose) as substrates

9a - Production of α-D-mannopyranose-1-phosphate from mannan and inorganic phosphate

Reaction is performed with 0.1 mg/ml purified UhgbMP during 10h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM inorganic phosphate (prepared from a stock solution of 100 mM phosphate obtained from a mix of 100 mM di-sodium hydrogen phosphate dodecahydrate (ref 28028.298 / VWR Prolabo) and of 100 mM sodium dihydrogen phosphate dihydrate (ref 28015.294 / VWR Prolabo) solutions to reach a pH of 7.0) and 10 mM mannan (Megazyme, Ireland, reference P-MANCB).

9b - Purification of α-D-mannopyranose-1-phosphate

α-D-mannopyranose-1-phosphate is purified from the reaction medium obtained at step 9b by preparative high-performance liquid chromatography (HPLC) or low-pressure liquid chromatography (LPLC) by using first a C18 column to eliminate oligosaccharides of polymerisation degree > 3, and secondly a H+ or K+ column to separate α-D-mannopyranose-1-phosphate from oligosaccharides of polymerisation degree ≤ 3.

9c - Production of mannooligosaccharides containing a GlcNAc (or GlcNAC-GlcNAc) at their reducing end, from α-D-mannopyranose-1-phosphate and *N*-acetyl-D-glucosamine (or *N*,*N*'-diacetyl chitobiose) as substrates

Reaction is performed with 0.1 mg/ml purified UhgbMP during 10h at 37 °C in Tris HCl 20mM, pH 7.0, with 10 mM of the α-D-mannopyranose-1-phosphate obtained in step 9b, and 10 mM *N*-acetyl-D-glucosamine (Sigma, reference A8625), or 1 mM *N*,*N*'-diacetyl chitobiose (Dextra, reference C8002).

Carbohydrate analysis is performed by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Carbohydrates and α-D-mannopyranose-1-phosphate are separated on a 4 x 250 mm Dionex Carbopac PA100 column. A gradient of sodium acetate (from 0 to 150 mM in 15 min) and an isocratic step of

300 mM sodium acetate in 150 mM NaOH is applied at a 1 $mL.min^{-1}$ flow rate. Detection is performed using a Dionex ED40 module with a gold working electrode and a Ag/AgCl pH reference.

Example 10 : *In vivo* study of effects of Uhgb_MP inhibitors on Inflammatory Bowel Deseases

Twenty to fifty patients with an inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis) are recruited. None of the included patients have overtly clinical symptoms during the time of the study and none are on medication for IBD problems. Thus, no nonsteroidal anti-inflammatory drugs, antibiotics, 5-aminosalicylic acid, corticosteroids, or other anti-inflammatory agents are used during the time of the study. After providing written informed consent, patients are randomly allocated to begin with Uhgb_MP inhibitor supplementation or with a placebo using a doubleblind crossover design. Five grams of Uhgb_MP inhibitor (D-altrose, D-xylose or D-allose) are dissolved in 200 ml of a commercially available milk-based beverage (Nutridrink®, Nutricia Nederland B.V.). The placebo consisted of the beverage Without enzyme inhibitor. The use of this standard beverage facilitates the double-blinded placebo-controlled character of the study. Patients are instructed to drink one bottle with 200 ml of the beverage in the morning at breakfast and a second in the evening at supper. After three weeks of consuming two bottles per day, patients return to the hospital to fill in a questionnaire and to undergo endoscopy. Patients brought fresh stools, produced in the morning immediately before they come to the hospital. Between the inhibitor and the placebo period there is a washout period of four weeks. The purpose of this washout period is to overcome effects of the first study period possibly intervening with effects of the final period. All investigators, including the endoscopist, are blinded to the randomization. The endoscopist and the pathologist do not participate in obtaining clinical data and are blinded to results until data analysis was finished. During endoscopy, findings were scored according to the Crohn's disease endoscopic index of severity (CDEIS) (Mary, J. Y. & Modigliani, R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). *Gut* 30, 983-989 (1989)) and to the ulcerative colitis endocscopic index of severity (UCEIS) (Travis, S. P. *et al.* Developing an ulcerative colitis endocscopic index of severity (UCEIS): results of pilot phase. *Gut* 40 (Suppl. 1), A-201 (2008)) criteria. At least three biopsy specimens are randomly taken from the mucosa in areas without apparent signs of ulceration or mucous exudate. Biopsy

specimens are encoded, fixed in formalin, and embedded in paraffin. Histologic sections are stained with hematoxylin and eosin. The average scores for all endoscopic and all histologic criteria of the CDEIS and UCEIS are called "total endoscopic score" and "total histologic score," respectively. Within two hours after defecation, stools are processed. pH is determined. Bile acids and short chain fatty acids in stools are quantified by gas chromatography(van Faassen A, Hazen MJ, van den Brandt PA, van den Bogaard AE, Hermus RJ, Janknegt RA. Bile acids and pH values in total feces and in fecal water from habitually omnivorous and vegetarian subjects. Am J Clin Nutr 1993;58:917–22. ; van den Bogaard AE, Hazen MJ, van Boven CP. Quantitative gas chromatographic analysis of volatile fatty acids in spent culture media and body fluids. J Clin Microbiol 1986;23:523–30).. Levels of significance are set at P _ 0.05. Differences between the enzyme inhibitor and the placebo period are statistically analyzed using Wilcoxon's signed-rank test (C. F. M.Welters, E.Heineman, F. B. J.M.Thunnissen, A. E. J.M. Van den Bogaard, P. B. Soeters, and C. G. M. I. Baeten, "Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis,c *Diseases of the Colon and Rectum*, vol. 45, no. 5, pp. 621 Rect 2002).

CLAIMS

1. Use of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, to degrade by phosphorolysis a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2,

said mannosyl donor compound being in particular β-1,4-D-Mannan,

said glycoside-phosphorylase being in particular chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413,

said glycoside-phosphorylase having more particularly the amino acid sequence SEQ ID NO: 1,

said glycoside-phosphorylase being in particular recombinantly expressed.

2. Process of degradation of a mannosyl donor compound of formula:

$(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-

mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose,

said mannosyl donor compound being in particular β-1,4-D-Mannan,

comprising a step of contacting said mannosyl donor with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, and inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor,

with the proviso that said glycoside-phosphorylase is not RaMP2,

said glycoside-phosphorylase having more particularly the amino acid sequence SEQ ID NO: 1.

3. Process according to claim 2, wherein said step of phosphorolysis is followed by a further step of contacting said compound of formula $(Man)_{m-1}$-donor with said glycoside-phosphorylase and inorganic phosphate, in a aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-2}$-donor, said further step being repeated p times, p being an integer comprised from 0 to (m-2), to obtain α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{(m-p)-2}$-donor.

4. Process according to anyone of claims 2 to 3, wherein said glycoside-phosphorylase is chosen among proteins comprising or constituted by the amino acid sequence SEQ ID NO: 1, 7 and 8 to 413.

5. Process according to anyone of claims 2 to 4, wherein said glycoside-phosphorylase is recombinantly expressed.

6. Process according to anyone of claims 2 to 5, wherein said glycoside-phosphorylase is characterized, in relation with phosphorolysis, generating α-D-mannopyranose-1-phosphate and a $(Man)_{m-1}$-donor compound from a mannosyl donor of formula $(Man)_m$-donor and inorganic phosphate, by the quantification of said α-D-mannopyranose-1-phosphate, said $(Man)_{m-1}$-donor compound or said inorganic phosphate.

7. Process according to anyone of claims 2 to 6, wherein the apparent catalytic efficiency $kcat_{app}$/ Km_{app} of said glycoside-phosphorylase is above about 0.02 $s^{-1}mM^{-1}$, in particular above about 0.9 $s^{-1}mM^{-1}$, for phosphorolysis in presence of inorganic phosphate and a mannosyl donor selected from the list constituted by β-D-mannopyranosyl-1,4-D-mannose, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose and β-1,4-D-Mannan.

8. Use according to claim 1, wherein a mannosyl residue is grafted on a mannosyl acceptor containing a hydroxyl group, in the presence of said α-D-mannopyranose-1-phosphate, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose.

9. Process according to anyone of claims 2 to 7, comprising:

- a step of contacting a compound of formula

  $(Man)_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

  said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula $(Man)_{m-1}$-donor;
- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

10. Process according to claim 9, wherein said mannosyl acceptor is selected from the group constituted by carbohydrates, glycoproteins, glycopeptides, water and hydroxylated compounds, in particular polyols.

11. Process according to anyone of claims 9 to 10, wherein said mannosyl acceptor is selected from the list constituted by D-glucose, D-mannose, D-galactose, D-fructose, (β-D-Man*p*-1,4)$_i$-D-Glc*p*NAc, i being equal to 0 or 1, and (β-D-Man*p*-1,4)$_j$-β-D-Glc*p*NAc-1,4-D-Glc*p*NAc, j being equal to 0, 1 or 2.

12. Process according to anyone of claims 9 to 11 wherein:

- said phosphorolysis and said reverse phosphorolysis are performed in one-pot, or
- said reverse phosphorolysis is performed subsequently to said phosphorolysis.

13. Process according to anyone of claims 9 to 12, comprising:

- a step of contacting a compound of formula

(Man)$_m$-donor, wherein Man represents mannose and m is an integer comprised from 1 to 1000, in particular from 1 to 100, more particularly from 1 to 20, even more particularly from 1 to 15,

said mannosyl donor compound being selected from the list constituted by β-1,4-D-mannan, β-1,4-D-mannooligosaccharides, β-D-mannopyranosyl-1,4-D-glucose, β-D-mannopyranosyl-1,4-*N*-acetyl-D-glucosamine, β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, and glycoproteins constituted by a protein glycosylated by β-D-mannopyranosyl-1,4-*N*,*N*'-diacetyl chitobiose, with a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, in the presence of inorganic phosphate, in an aqueous medium, to obtain by phosphorolysis α-D-mannopyranose-1-phosphate and a compound of formula (Man)$_{m-1}$-donor;

- a step of contacting said glycoside-phosphorylase with said α-D-mannopyranose-1-phosphate and a mannosyl acceptor containing a hydroxyl group, in an aqueous medium, to obtain by reverse phosphorolysis a mannosylated acceptor of formula Man-Acceptor wherein Man represents mannose, and inorganic phosphate,
- a further step of contacting said mannosylated acceptor of formula Man-Acceptor with α-D-mannopyranose-1-phosphate and said glycoside-phosphorylase, said further step being

repeated n times, n being an integer comprised from 0 to 9, to obtain a (n+2) times mannosyated acceptor of the following structure: $(Man)_n$-Man-Man-Acceptor wherein Man represents mannose, and inorganic phosphate.

14. Pharmaceutical composition comprising as active substance an inhibitor of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, said inhibitor being in particular D-altrose, D-xylose or D-allose, in association with a pharmaceutically acceptable vehicle,

in particular administrable by oral or rectal route at a dose comprised from about 0.1 mg/kg to about 1000 mg/kg of body weight,

or in particular under a form liable to be administrable by oral or rectal route, under the form of a unit dose comprised from about 5 mg to about 10,000 mg, in particular from about 10 mg to about 2,000 mg, in particular from about 50 to about 1,000 mg.

15. Composition comprising an inhibitor of a glycoside-phosphorylase having the amino acid sequence SEQ ID NO: 1, or having an identity percentage of at least 50%, in particular at least 60, 70, 80 or 90%, with said amino acid sequence, said inhibitor being in particular D-altrose, D-xylose or D-allose, for treating inflammatory bowel diseases, in particular inflammatory bowel diseases belonging to the group consisting of Crohn's disease, ulcerative colitis and colon cancer.

Figure 1A

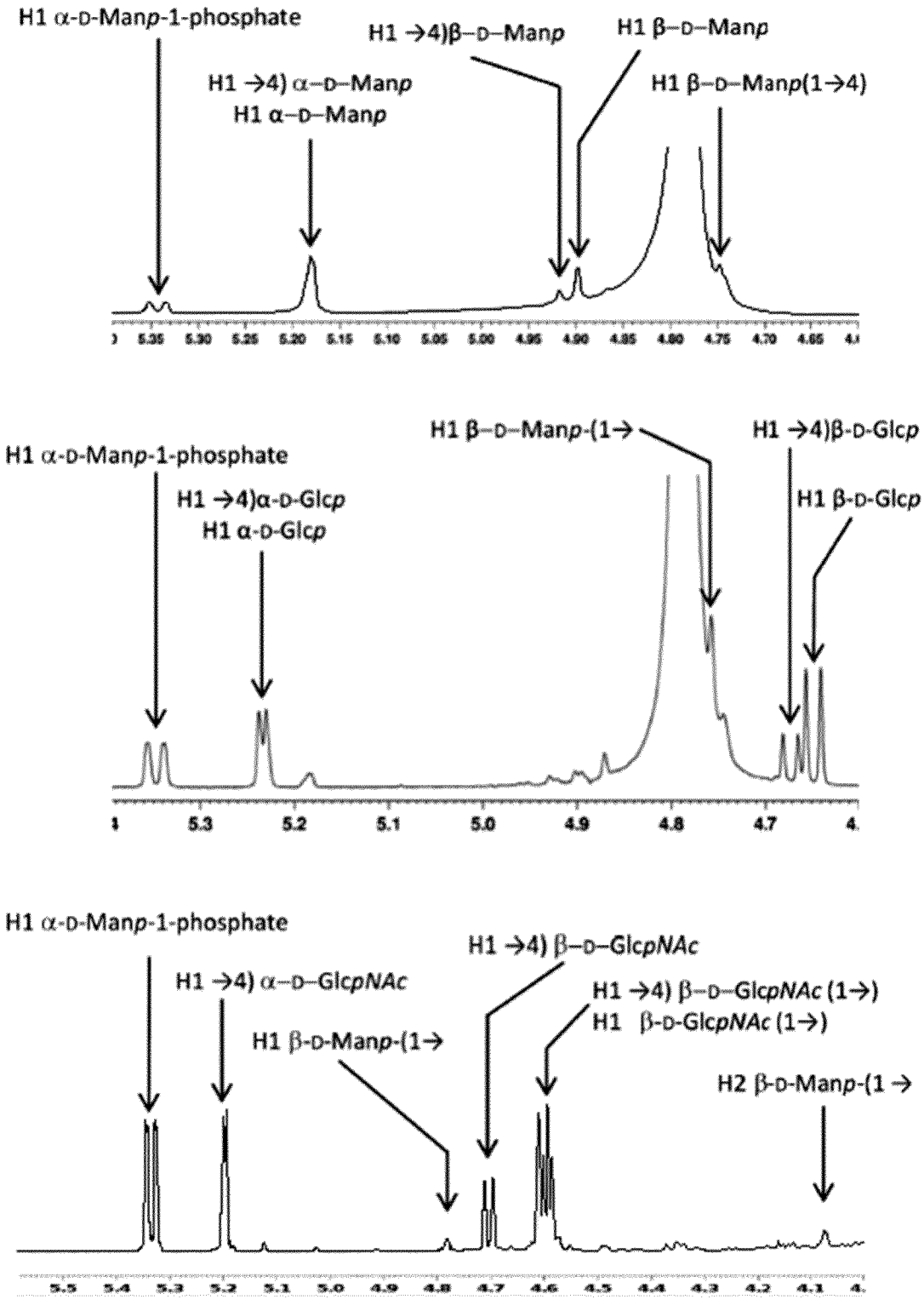
H1 α-D-Manp-1-phosphate
H1 →4)β–D–Manp
H1 β–D–Manp
H1 →4) α–D–Manp
H1 α–D–Manp
H1 β–D–Manp(1→4)
H1 α-D-Manp-1-phosphate
H1 β–D–Manp-(1→
H1 →4)β-D-Glcp
H1 →4)α-D-Glcp
H1 α-D-Glcp
H1 β-D-Glcp
H1 α-D-Manp-1-phosphate
H1 →4) β–D–GlcpNAc
H1 →4) α–D–GlcpNAc
H1 →4) β–D–GlcpNAc (1→)
H1 β-D-Manp-(1→
H1 β-D-GlcpNAc (1→)
H2 β-D-Manp-(1 →

Figure 1B

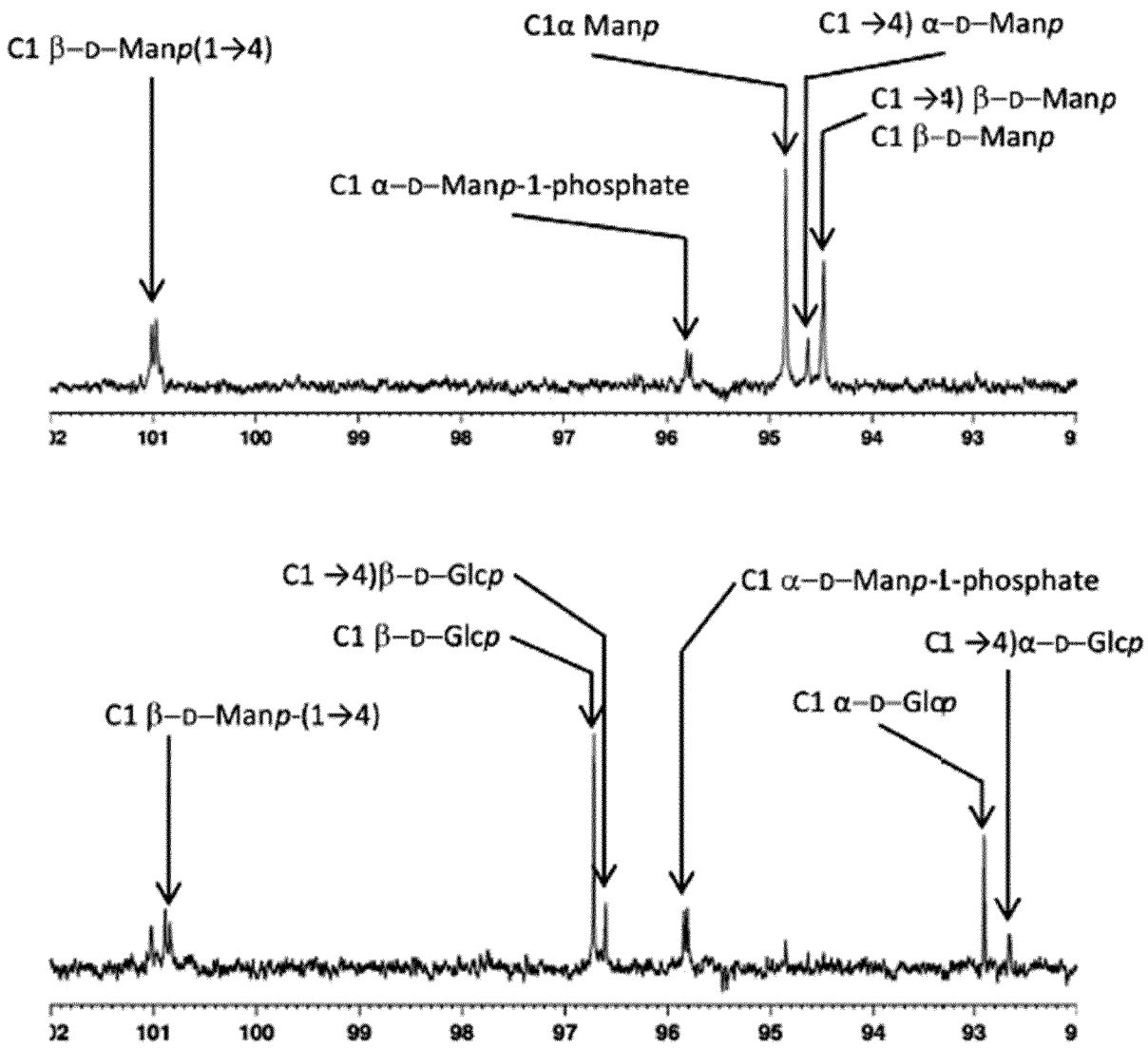

Figure 2