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[Continued on next page]

(54) Title: SIALYLATED GLYCOPROTEINS

(57) Abstract: Glycoproteins having particular sialylation patterns, and methods of making and using such glycoproteins, are described.

SEQ ID NO:1

MTRLIyLALLAGLLASSRAGSSPLFAMEWSHPQFEKLEGGGSGGGSGGSWSHPQFEKHAFAHSR
KDHLIHNVHKEEHAHAHNKELGTAVFQGPMRM IRGPSFQVWNKDSSSKNLIPRLQKIWKNYLS
MHKYKVSYKGPGPGIKFSASALRCHLRDHVKPvASMVEVTDFPFNTSEMSGYLPKESIRTKAGPWG
RCAVVSSAGSLKSSQLGREIDDHDAVLRFNGAPTANFQQDVGTKTTIRLMNSQLVTTEKRFLKD
SLYNEGILIVWDPSVYHSDIPKWYQNPDYNFFNNYKTYRKLHPNQPFYILKPQMPWELWDILOE
ISPEEIQPNPPSSGMLGTIIMMTLCDQVDIYEFLPSKRKTDVCYYYQKFFDSACIMGAYHPLLY
EKNLVKHLNQGXDEDIYLLGKAILPGFRTIHCPG

FIG. 5A

SEQ ID NO:2

GSYYDSFKLQTKEFQVLKSLGKLAMGSDSQSVSSSSTQDPHRGRQTLGSLRGLAKAKPEASFQV
WNKDSSSKNLIPRLQKIWKNYLSMNKYKVSYKGPGPGIKFSAEALRCHLRDHVSVSWEVTDFP
FNTSEWEGYLPKESIRTKAGPWGRCAVVSSAGSLKSSQLGREIDDHDAVLRFNGAPTANFQQD¥
GTKTT IRLMNSQLVTTEKRFLKDSLYNEGILIVWDPSVYHSDI?KWYQN?DYNFFNNYKTYRKL
HPNQPFYILKPQMPWELWDILQEISPEEIQPNPPSSGMLGIIIMMTLCDQVDIYEFLPSKRKTD
VCYYYQKFFDEACTMGAYHPLLYEKNLVKHLNGGTDEDIYLLGKAILPGFRTIHC

FIG. 5B

SEQ ID NO:3

MIHTNLKKKFSYFILAFLLFALICVWKKGSYEALKLQAKEFQVTKSLEKLAIGSGSQST$ASIK
QDSKPGSQVLSHLRVTAKVKPQSPYQVWDKNSSSKNLNPRLQKILKNYLSMNKYKVSYKGPGPG
VKFSVEALRCHLRDRVNVSMIEATDFPFNTTEWEGYLPKENFRTKAGPWHRCAVVSSAGSLKSS
HLGKEIDSHDAVLRFNGAPVADFQQDVGMKTTIRLMNSQLITTEKQFLKDSLYNEGILIVWDPS
LYHADIPNWYKKPDYNFFETYKSYRKLYPSQPFYILRPQMPWELWDIIQEIAPDRIQPNPPSSG
MLGIIIMMTLCDQVDVYEFLPSKRKTDVCYYHQKFFDSACTMGAYHPLLFEKNMVKQLNEGTDE
DIYIFGKATLSGFRTIHC

FIG. 5C

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MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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29 January 2015

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/36413

A. CLASSIFICATION OF SUBIECT MATTER
IPC(8) - A61K 38/14 (2014.01)
CPC - A61K 39/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 38/14; C07K 1/00, 14/00, 17/00 (2014.01)
CPC: A61K 38/00, 38/1709, 39/00; C07K 9/005, 14/705; USPC: 514/20.9, 1.1, 1; 530/395, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C.B, DE-A, DE-T, DE-U, GB-A, FR-A); PubMed; Google Scholar, ScienceDirect; sialyltransferase, 'sialic acid,' 'ST6,' 'reaction condition,' donor, glycan, glycoprotein, ratio, 'predetermined proportion,' 'alpha-1,3,' 'alpha-1,6'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOZIASSE, D et al. Branch Specificity Of Bovine Colostrum CMP-Sialic Acid: Gal beta 1->4GlcNAc-R alpha 2->6-Sialyltransferase: Sialylation Of Bi-, Tri-, And Tetraantennary Oligosaccharides And Glycopeptides Of The N-Acetyllactosamine Type. J Biol Chem. 15 February 1987; Vol. 262, No. 5; pages 2025-2033; page 2026, Table V; page 2029, left column, sixth paragraph; page 2033, figure 7.	1-40, 45-51
Y	US 2004/0137106 A1 (SCHULTZ, PG et al.) July 15, 2004; paragraphs (0009], [0016], [0017], [0181]	1-40, 45-51
Y	US 8278072 B2 (MATTA, KL et al.j October 2, 2012; figure 2; column 2, lines 45-63	12, 26, 30, 36-40, 45/12, 48, 49
Y	US 2010/01 13294 A1 (VENKATARAMAN, G et al.) May 6, 2010; paragraphs [0151]-[0154]	7, 8, 17, 18
Y	LANCE, P et al. Isolation And Characterization Of A Partial cDNA For A Human Sialyltransferase. Biochem Biophys Res Commun. 16 October 1989; Vol. 164, No. 1; pages 225-232; abstract; page 229, figure 3.	10, 20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/36413

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

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

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

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

3. ☒ Claims Nos.: 41-44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

-""-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. ConsequenUy, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Groups I+: Claims 1-9, 10 (in-part), 11-19, 20 (in-part), 21-40, 45-51; Amino Acid residues 95-416 of SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

-"-Continued from Box No. III: Observations Where Unity of Invnetion Is Lacking:

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

Groups I+: Claims 1-40 and 45-51 are directed toward a method of producing a preparation of glycoproteins comprising Fc regions comprising branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm, the preparation comprising (i) a target level of branched glycans having a sialic acid on an alpha-1,3 arm and/or (ii) a target level of branched glycans having a sialic acid on an alpha-1,6 arm, the method comprising: providing a plurality of glycoproteins comprising Fc regions comprising branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm; and contacting the glycoproteins with an ST6 sialyltransferase in the presence of a reaction condition, thereby producing a glycoprotein preparation having (i) the target level of branched glycans having a sialic acid on the alpha-1,3 arm and/or (ii) a target level of branched glycans having a sialic acid on an alpha-1,6 ami; as well as associated methods of removing a sialic acid from a branched glycan, and methods of modulating sialylation of Fc region branched glycans.

The methods of producing a preparation of glycoproteins comprising contacting the glycoproteins with an ST6 sialyltransferase will be searched to the extent that the sialyltransferase has at least 90% identity to amino acid residues 95-416 of SEQ ID NO:1 (Homo sapiens amino acid sequence). It is believed that Claims 1-9, 10 (in part), 11-19, 20 (in-part), 21-40 and 45-51 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass amino acid residues 95-416 of SEQ ID NO: 1 (Homo sapiens amino acid sequence). Applicants must specify the claims that encompass any additional elected SEQ ID NOs. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: SEQ ID NO: 2 (Homo sapiens amino add sequence).

Groups I+ share the technical features including a method of producing a preparation of glycoproteins comprising Fc regions comprising branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm, the preparation comprising (i) a target level of disialylated branched glycans having a sialic add on an alpha-1,3 arm and on an alpha-1,6 arm, (ii) a target level of monosialylated branched glycans having a sialic add on an alpha-1,3 arm and/or (iii) a target level of monosialylated branched glycans having a sialic add on an. alpha-1,6 arm the method comprising: providing a plurality of glycoproteins comprising Fc regions comprising branched glycans comprising alpha-1,3 ami and an alpha-1,6 arm; and contacting the glycoproteins with an ST6 sialyltransferase in the presence of a limited, intermediate or extended reaction condition, or a mixture thereof; thereby producing a sialylated preparation of glycoproteins; and formulating the preparation into a drug product if the preparation meets (i) the target level of disialylated branched glycans having a sialic add on an alpha-1,3 arm and on an alpha-1,6 arm; (ii) the target level of monosialylated branched glycans having a sialic add on an alpha-1,3 arm and/or (iii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,6 arm; a method of producing a preparation of glycoproteins comprising Fc regions comprising branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm, the preparation comprising (i) a target level of branched glycans having a sialic add on an alpha-1,6 arm and/or (ii) a target level of branched glycans having a sialic add on an alpha-1,3 arm, the method comprising: providing a plurality of glycoproteins comprising Fc regions comprising branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm; and contacting the glycoproteins with an ST6 sialyltransferase in the presence of an initial reaction condition suffident for the ST6 sialyltransferase substantially to add a sialic acid to an alpha-1,3 arm and/or to add a sialic add to an alpha-1,6 arm of a branched glycan in the presence of an extended reaction condition, thereby producing a glycoprotein preparation having (i) the target level of disialylated branched glycans having a sialic add on an alpha-1,3 arm and on an alpha-1,6 arm, (ii) the target level of monosialylated branched glycans having a sialic add on an alpha-1,3 arm and/or (iii) the target level of monosialylated branched glycans having a sialic add on an alpha-1,6 arm; a method of removing a sialic add from a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm, the method comprising: providing a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm and comprising a sialic add on the alpha-1,3 arm; contacting the branched glycan with an ST6 sialyltransferase in the presence of an initial reaction condition suffident for the ST6 sialyltransferase to add a sialic add to the alpha-1,6 arm to produce a disialylated branched glycan; and contacting the disialylated branched glycan with the ST6 sialyltransferase in the presence of an extended reaction condition, thereby removing the sialic add from the alpha-1,3 arm of the branched glycan; a method of modulating sialylation of Fc region branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm, the method comprising: providing a reaction solution comprising (i) Fc region branched glycans comprising an alpha-1,3 arm and an alpha-1,6 arm, (ii) a ST6 sialyltransferase, and (iii) a sialic add donor; and incubating the reaction solution under reaction conditions suffident for the ST6 sialyltransferase to catalyze transfer of a sialic add primarily to the alpha-1,3 arm only, primarily to the alpha-1,6 arm only, or to both the alpha-1,3 arm and the alpha-1,6 arm, wherein: a) incubating the reaction solution under reaction conditions suffident for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1,3 arm comprises controlling reaction kinetics such that: (i) the sialic acid addition rate for the alpha-1,3 arm (Ra1,3) exceeds the sialic acid addition rate for the alpha-1,6 arm (Ra1,6); or (ii) the sialic add removal rate for the alpha-1,6 arm (Rr1,6) exceeds Ra1,6; b) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1,6 arm comprises controlling reaction kinetics such that: (i) Ra1,6 exceeds Rr1,6; and (ii) the sialic acid removal rate for the alpha-1,3 arm (Rr1,3) eventually exceeds Ra1,3; or c) incubating the reaction solution under reaction conditions suffident for the sialyltransferase to catalyze transfer of the sialic add to both the alpha-1,3 and alpha-1,6 arms comprises controlling reaction kinetics such that: (i) Ra1,3 exceeds Rr1,3; and (ii) Ra1,6 exceeds Rr1,6; thereby modulating sialylation of a branched glycan.

-""-Continued on Next Supplemental Page-" *-

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However, these shared technical features are previously disclosed by US 2004/0138106 A1 to Schultz, et al. (hereinafter 'Schultz') in view of the publication entitled 'Branch Specificity Of Bovine Colostrum CPM-Sialic Acid: Gal-Beta1-4 GlcNAc-R a2-6-Sialyltransferase' by Joziasse, et al. (hereinafter 'Joziasse'). Schultz discloses a method of producing a preparation of glycoproteins (a method of producing a preparation of glycoproteins; abstract) comprising Fc regions (antibodies (comprising Fc regions); paragraph [0083]) comprising branched glycans (comprising biantennary or triantennary oligosaccharide structures (comprising branched glycans); paragraph [0009]), the preparation comprising (i) a target level (a desired amount (a target level); paragraph [0079]) of disialylated branched glycans (sialylated biantennary glycans (disialylated branched glycans); paragraphs [0009], [0040], [0057]), the method comprising: providing a plurality of glycoproteins (providing glycoproteins (a plurality of glycoproteins); paragraph [0007]) comprising Fc regions (antibodies (comprising Fc regions); paragraph [0083]) comprising branched glycans (comprising biantennary or triantennary oligosaccharide structures (comprising branched glycans); paragraph [0009]); and contacting the glycoproteins with a sialyltransferase (contacting the glycoproteins with a sialyltransferase); paragraph [0017]) in the presence of a limited, intermediate or extended reaction condition, or a mixture thereof (in the presence of divalent cations, phosphate ions, organic solvents, an activated nucleotide sugar donor, temperature, pH, and solubilizing detergents or solvents, and amount of enzyme or specific activity thereof (in the presence of a limited, intermediate or extended reaction condition); paragraphs [0061] [0062]); thereby producing a sialylated preparation of glycoproteins (thereby producing a sialylated preparation of glycoproteins; paragraphs [0016], [0017]); and formulating the preparation into a'drug product (producing a composition comprising the glycoprotein and a pharmaceutically acceptable excipient (formulating the preparation into a drug product); paragraph [0181]); a method of producing a preparation of glycoproteins (abstract) comprising Fc regions (antibodies (comprising Fc regions); paragraph [0083]) comprising branched glycans (comprising biantennary or triantennary oligosaccharide structures (comprising branched glycans); paragraph [0009]), the preparation comprising (i) a target level (a desired amount (a target level); paragraph [0079]) of branched glycans having a sialic acid (sialylated glycans (sialylated branched glycans); paragraphs [0009], [0040], [0057]) the method comprising: providing a plurality of glycoproteins (providing glycoproteins (a plurality of glycoproteins); paragraph [0007]) comprising Fc regions (antibodies (comprising Fc regions); paragraph [0083]) comprising branched glycans (comprising biantennary or triantennary oligosaccharide structures (comprising branched glycans); paragraph [0009]); and contacting the glycoproteins with an sialyltransferase (contacting the glycoproteins with a sialyltransferase; paragraph [0017]) in the presence of an initial reaction condition (in the presence of reaction conditions (in the presence of an initial reaction condition); paragraphs [0061], [0062]; initial reaction condition being the reaction conditions at the initiation of any reaction) sufficient for the sialyltransferase substantially to add a sialic acid to a branched glycan (sufficient for the sialyltransferase substantially to add a sialic acid to a branched glycan; paragraphs [0009], [0040]), thereby producing a glycoprotein preparation (thereby producing a glycoprotein preparation; paragraphs [0007], [0181]) having disialylated or monosialylated branched glycans (having sialylated monoantennary or biantennary glycans (having disialylated or monosialylated branched glycans); paragraphs [0009], [0040], [0057]); a method of modulating sialylation (a method of sialylating (a method of modulating sialylation); paragraphs [0007], [0009], [0040]) of Fc region (antibody (Fc regions); paragraph [0083])) branched glycans (biantennary or triantennary oligosaccharide structures (branched glycans); paragraph [0009]), the method comprising: providing a reaction solution (the method comprising: providing a reaction solution; paragraphs [0061], [0062]) comprising (i) Fc region branched glycans (comprising antibodies having antennary glycan structures including biantennary and triantennary glycans (comprising (i) Fc region branched glycans); paragraphs [0009], [0083]) (ii) a sialyltransferase (a sialyltransferase; paragraphs [0009], [0040]), and (iii) a sialic acid donor (an activated nucleotide sugar that acts as a sugar donor for the glycosyltransferase (a sialic add donor); paragraph [0062]);,and incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of a sialic acid (incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of a sialic acid; paragraphs [0009], [0040], [0062]); and wherein producing proteins having a desired pattern of glycosylation depends upon the cell line expressing the glycoprotein, which may then be modified directly with saccharides (producing proteins having a desired pattern of glycosylation depends upon the cell line expressing the glycoprotein, which may then be modified directly with saccharides; paragraph [0005]).

Schultz does not disclose glycans comprising an alpha-1,3 arm and an alpha-1,6 arm; disialylated branched glycans having a sialic acid on an alpha-1 ,3 arm and on an alpha-1 ,6 arm; having a sialic acid on an alpha-1 ,3 arm and on an alpha-1 ,6 arm, (ii) a target level of monosialylated branched glycans having a sialic acid on an alpha-1,3 arm and/or (iii) a target level of monosialylated branched glycans having a sialic acid on an alpha-1 ,6 arm; contacting the glycoproteins with an ST6 sialyltransferase; each of a limited, intermediate and extended reaction condition; formulating the preparation into a drug product if the preparation meets (i) the target level of disialylated branched glycans having a sialic acid on an alpha-1,3 arm and oh an alpha-1,6 arm, (ii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,3 arm and/or (iii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1 ,6 arm; sufficient for the ST6 sialyltransferase substantially to add a sialic acid to an alpha-1 ,3 arm and/or to add a sialic acid to an alpha-1,6 arm of a branched glycan in the presence of an extended reaction condition; thereby producing a glycoprotein preparation having (i) the target level of disialylated branched glycans having a sialic acid on an alpha-1 ,3 arm and on an alpha-1 ,6 arm, (ii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,3 arm and/or (iii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,6 arm; a method of removing a sialic acid from a branched glycan of an Fc region, the branched glycan comprising an alpha-1 ,3 arm and an alpha-1 ,6 arm, the method comprising: providing a branched glycan of an Fc region, the branched glycan comprising an alpha-1 ,3 arm and an alpha-1 ,6 arm and comprising a sialic acid on the alpha-1 ,3 arm; contacting the branched glycan with an ST6 sialyltransferase in the presence of an initial reaction condition sufficient for the ST6 sialyltransferase to add a sialic acid to the alpha-1,6 arm to produce a disialylated branched glycan; and contacting the disialylated branched glycan with the ST6 sialyltransferase in the presence of an extended reaction condition, thereby removing the sialic acid from the alpha-1 ,3 arm of the branched glycan; incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of a sialic acid primarily to the alpha-1,3 arm only, primarily to the alpha-1,6 arm only, or to both the alpha-1,3 arm and the alpha-1,6 arm, wherein: a) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1 ,3 arm comprises controlling reaction kinetics such that: (i) the sialic acid addition rate for the alpha-1 ,3 arm (Ra1 ,3) exceeds the sialic acid addition rate for the alpha-1 ,6 arm (Ra1 ,6); or (ii) the sialic acid removal rate for the alpha-1,6 arm (Rr1,6) exceeds Ra1,6; b) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1 ,6 arm comprises controlling reaction kinetics such that: (i) Ra1 ,6 exceeds Rr1 ,6; and (ii) the sialic acid removal rate for the alpha-1 ,3 ami (Rr1 ,3) eventually exceeds Ra1 ,3; or c) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid to both the alpha-1,3 and alpha-1,6 arms comprises controlling reaction kinetics such that: (i) Ra1,3 exceeds Rr1,3; and (ii) Ra1,6 exceeds Rr1,6; thereby modulating sialylation of a branched glycan.

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Joziasse discloses an ST6 sialyltransferase (Gal-Beta1-4GlcNAc-R alpha2-6-sialyltransferase (an ST6 sialyltransferase); abstract), glycans an alpha-1,3 arm and an alpha-1,6 arm (glycans an alpha-1,3 arm and an alpha-1,6 arm; abstract; Table V); contacting glycoproteins with an ST6 sialyltransferase (contacting glycoproteins with an ST6 sialyltransferase; abstract); and wherein said sialyltransferase demonstrates an acceptor preference for a Man alpha1-3 branch over a Man alpha1-6 branch (wherein said sialyltransferase demonstrates an acceptor preference for a Man alpha1-3 branch over a Man alpha1-6 branch; abstract), including the production of mono and disailylated alpha 1-3 and alpha1-6 glycan branches (production of mono and disailylated alpha 1-3 and alpha1-6 glycan branches; Table V), wherein the monosialylated is preferentially on the alpha1-3 branch (wherein the monosialylated is preferentially on the alpha1-3 branch; Table V); and extended reaction conditions (reaction products were assessed after intervals, including a last reaction product (extended reaction conditions); Supplementary Material; page 2029, column 2, paragraph 2).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Schultz, for integrating the use of an ST6 sialyltransferase, including one capable of modifying alpha1-3 and alpha1-6 branches of a branched glycan, as previously disclosed by Joziasse, and to have implemented the use of common enzymatic reaction profiling to determine the reaction conditions sufficient to have produced a target level of disialylated branched glycans having a sialic acid on an alpha-1,3 arm and on an alpha-1,6 arm, (ii) a target level of monosialylated branched glycans having a sialic acid on an alpha-1,3 arm and/or (iii) a target level of monosialylated branched glycans having a sialic acid on an alpha-1,6 arm, thereby enabling, upon analysis of the reaction products formulating the preparation into a drug product if the preparation meets (i) the target level of disialylated branched glycans having a sialic acid on an alpha-1,3 arm and on an alpha-1,6 arm, (ii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,3 arm and/or (iii) the target level of monosialylated branched glycans having a sialic acid on an alpha-1,6 arm for providing a pharmaceutically useful glycoprotein, as previously disclosed by Schultz. Although Schultz does not disclose a method of removing a sialic acid from a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm, the method comprising: providing a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm and comprising a sialic acid on the alpha-1,3 arm; contacting the branched glycan with an ST6 sialyltransferase in the presence of an initial reaction condition sufficient for the ST6 sialyltransferase to add a sialic acid to the alpha-1,6 arm to produce a disialylated branched glycan; and contacting the disialylated branched glycan with the ST6 sialyltransferase in the presence of an extended reaction condition, thereby removing the sialic acid from the alpha-1,3 arm of the branched glycan; incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of a sialic acid primarily to the alpha-1,3 arm only, primarily to the alpha-1,6 arm only, or to both the alpha-1,3 arm and the alpha-1,6 arm, wherein: a) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1,3 arm comprises controlling reaction kinetics such that: (i) the sialic acid addition rate for the alpha-1,3 arm (Ra1,3) exceeds the sialic acid addition rate for the alpha-1,6 arm (Ra1,6); or (ii) the sialic acid removal rate for the alpha-1,6 arm (Rr1,6) exceeds Ra1,6; b) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic add primarily to the alpha-1,6 arm comprises controlling reaction kinetics, such that: (i) Ra1,6 exceeds Rr1,6; and (ii) the sialic acid removal rate for the alpha-1,3 arm (Rr1,3) eventually exceeds Ra1,3; or c) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid to both the alpha-1,3 and alpha-1,6 amis comprises controlling reaction kinetics such that (i) Ra1,3 exceeds Rr1,3; and (ii) Ra1,6 exceeds Rr1,6; thereby modulating sialylation of a branched glycan, the manipulation of reaction conditions to favor a reverse reaction (e.g. by failing to provide a nucleotide sugar donor) was known to enable an enzyme to catalyze a reverse reaction, wherein the removal of a sialic acid by a sialyltransferase would have been obvious to a person of ordinary skill in the art, as would the fact that the overall reaction rates of the addition or removal of sialic acid moieties from the alpha 1-3 and alpha 1-6 branches represent an overall balance of addition and removal rates, wherein changing reaction conditions, particularly concentrations of reactants and pH, would have enabled modulation of the specificties of the enzyme to favor producing a particular sialylation pattern, and thereby providing a method of removing a sialic acid from a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm, the method comprising: providing a branched glycan of an Fc region, the branched glycan comprising an alpha-1,3 arm and an alpha-1,6 arm and comprising a sialic acid on the alpha-1,3 arm; contacting the branched glycan with an ST6 sialyltransferase in the presence of an initial reaction condition sufficient for the ST6 sialyltransferase to add a sialic acid to the alpha-1,6 arm to produce a disialylated branched glycan; and contacting the disialylated branched glycan with the ST6 sialyltransferase in the presence of an extended reaction condition, thereby removing the sialic acid from the alpha-1,3 arm of the branched glycan; incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of a sialic acid primarily to the alpha-1,3 arm only, primarily to the alpha-1,6 arm only, or to both the alpha-1,3 arm and the alpha-1,6 arm, wherein: a) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1,3 arm comprises controlling reaction kinetics, such that: (i) the sialic acid addition rate for the alpha-1,3 arm (Ra1,3) exceeds the sialic acid addition rate for the alpha-1,6 arm (Ra1,6); or (ii) the sialic acid removal rate for the alpha-1,6 arm (Rr1,6) exceeds Ra1,6; b) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid primarily to the alpha-1,6 arm comprises controlling reaction kinetics such that: (i) Ra1,6 exceeds Rr1,6; and (ii) the sialic acid removal rate for the alpha-1,3 arm (Rr1,3) eventually exceeds Ra1,3; or c) incubating the reaction solution under reaction conditions sufficient for the sialyltransferase to catalyze transfer of the sialic acid to both the alpha-1,3 and alpha-1,6 arms comprises controlling reaction kinetics such that: (i) Ra1,3 exceeds Rr1,3; and (ii) Ra1,6 exceeds Rr1,6; thereby modulating sialylation of a branched glycan.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Schultz and Joziasse references, unity of invention is lacking.