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Datasheet for the decision of 4 December 2020

Case Number: T 0989/16 - 3.3.08

Application Number: 07825657.5

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Language of the proceedings: EN

Title of invention:
LABELING AND DETECTION OF POST TRANSLATIONALLY MODIFIED PROTEINS

Patent Proprietor:
Life Technologies Corporation

Opponents:
Merck Patent GmbH
Graf von Stosch, Andreas

Headword:
Post translationally modified glycoproteins/LIFE TECHNOLOGIES CORPORATION

Relevant legal provisions:
EPC Art. 83, 123(2), 54, 56

Keyword:
Sufficiency of disclosure - main request (no)
Auxiliary request 1 - requirements of the EPC met - (yes)

Decisions cited:

Catchword:

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Case Number: T 0989/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 4 December 2020

Appellant: (Patent Proprietor)	Life Technologies Corporation 5823 Newton Drive Carlsbad, CA 92008 (US)
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Representative:	Graf von Stosch, Andreas Graf von Stosch Patentanwaltsgesellschaft mbH Prinzregentenstraße 22 80538 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 7 January 2016 concerning maintenance of the European Patent No. 1991273 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	D. Rogers

Summary of Facts and Submissions

I. European patent No. 1 991 273, based on European patent application No. 07825657.5, was opposed on the grounds of Articles 100(a), (b) and (c) EPC. An opposition division considered that the main request before it (patent as granted) contravened both Article 83 EPC and Article 56 EPC and took the view that the auxiliary request and the description adapted thereto complied with the requirements of the EPC.

II. The patent proprietor (appellant I), and opponents 1 and 2 (appellants II and III respectively) lodged an appeal against the decision of the opposition division. Appellant II submitted new documents E28 to E30 in support of its case.

III. The patent proprietor (appellant I) replied to appellant's II and III statements of grounds of appeal.

The parties were summoned to oral proceedings.

In response to the board's communication pursuant to Article 17(1) RPBA 2020, appellant I submitted new auxiliary requests 1, 1a, 2 and 2a.

Appellants II and III announced that they would not attend the oral proceedings.

IV. Oral proceedings took place on 4 December 2020, in the absence of appellants II and III.

V. Claims 1, 9 and 10 of the main request (claims as granted) read as follows:

"1. A method for enzymatically labeling a glycoprotein with an azide moiety, wherein the method comprises:
a) contacting the glycoprotein with UDP-GalNAz in the presence of a β-1,4-galactosyltransferase Y289L mutant enzyme to form an azido modified glycoprotein.

9. An azido modified glycoprotein obtainable by the method of any of claims 1-8.

10. A kit comprising: UDP-GalNAz; a β-1,4-galactosyltransferase Y289L mutant enzyme; an azide reactive reporter molecule, carrier molecule or solid support."

VI. Claims 1 and 9 of auxiliary request 1 read:

"1. A method for enzymatically labeling a glycoprotein **comprising a terminal GlcNAc group** with an azide moiety, wherein the method comprises:
a) contacting the glycoprotein with UDP-GalNAz in the presence of a β-1,4-galactosyltransferase Y289L mutant enzyme to form an azido modified glycoprotein.

9. An azido modified glycoprotein obtainable by the method of Claim 1."

VII. The following documents are cited in this decision:

D1 H.C. Hang and C.R. Bertozzi "The chemistry and biology of mucin-type O-linked glycosylation." Bioorganic Medicinal Chemistry 2005 Sep 1; 13(17), pages 5021-34;

D2 H.C. Hang *et al*. "A metabolic labeling approach toward proteomic analysis of mucin-type O-linked

glycosylation", PNAS, Dec 2003, 100 (25), pages 14846-14851;

D3 R. Chatterjee *et al.*, "Mutation of the ptsG gene results in increased production of succinate in fermentation of glucose by Escherichia coli" Appl. Environ. Microbiol. 2001; 67(1), p.148-154;

D4 US2005/0130235 (published 16 June 2005);

D16 A. Varki *et al.*, Essentials of Glycobiology, (1999), Cold Spring Harbor, Chapter 38, pages 581-598;

E1 Experimental Evidence submitted by opponent 2/ Appellant III;

E4 E. Saxon *et al.* "Investigating cellular metabolism of synthetic azidosugars with the Staudinger ligation." J Am Chem Soc. 2002 Dec 18; 124(50), pages 14893-902.

VIII. The submissions made by **appellant I**, insofar as relevant to the present decision, may be summarized as follows:

Main request
Sufficiency of disclosure (Article 100 (b) EPC)

The skilled person would have interpreted claim 1 when reading the patent application, based on its technical knowledge with respect to the enzyme specificity, as excluding methods relating to glycoproteins that could not be labelled by an enzyme-based reaction with UDP-GalNAz. These glycoproteins, including antibodies and α-crystallin, lacked an enzyme accessible terminal

GlcNAc, especially an O-GlcNAc and/or at least one terminal N-acetylglucosamine residue (see paragraphs [0108], [0112], [0280], Figures 6A-B, Figure 11B, Figure 17, Examples 18, 19 and 26). Document D16 - a general reference on glycobiology - taught that treatment of glycoproteins with endoglycosidases, e.g. endoglycosidase H, resulted in a mobility change on one or more of the bands on the SDS PAGE gel, indicating the presence of N-glycans (see page 584, Figure 38.1). Endo-H enzyme was known to specifically cleave the chitobiose core of N-linked glycans, exposing an N-linked terminal GlcNAc group. The "Endo-H (endo-β-N-acetylglucosaminidase H) enzyme could be used to generate a truncated chain which terminates with one N-acetylglucosamine residue (Figure 11B)", which inferred that the "terminal GlcNAc group" was either O- or N-linked to the remainder of the glycoprotein (see paragraph [0112]). Example 26 established that the Endo H_f deglycosylated goat antibody had a terminal N-acetylglucosamine residue, which was then labelled with a β-1,4-galactosyltransferase Y289L mutant and UDP-GalNAz (see paragraph [0280], Figure 17 A, lane 3, and paragraph [0112]).

The term "azide moiety" specified that a glycoprotein was contacted with a UDP-GalNAz in the presence of the claimed mutant enzyme to form an azido modified glycoprotein. The azide moiety was accordingly clearly defined.

Moreover, independent claim 9 had to be interpreted as a product formed in accordance with the method of claim 1 based on the same original starting glycoproteins, whereas independent claim 10 was defined by its components: UDP-GalNAz and β-1,4-galactosy1transferase Y289L mutant enzyme, which, for consistency reasons,

had to be interpreted as in claim 1.

Auxiliary request 1
Amendments (Article 123(2) EPC)

The features of amended claim 1 could be found in paragraph [0009] of the patent application, except that the enzyme is "a modified Gal T enzyme" rather than the claimed "β-1,4-galactosyltransferase Y289L mutant enzyme". The missing feature was disclosed in paragraphs [0111] and [0115] of the patent application, where it was stated that "... antibodies can be enzymatically labeled with UDP-GalNAz using GalT via reaction of the terminal O-GlcNAc molecules present on antibody carbohydrates" and "... if no O-GlcNAc sugars are present on an antibody, then the use of the Endo-H (endo-β-N-acetylglucosaminidase H) enzyme may be used to generate a truncated chain, which terminates with one N-acetylglucosamine residue (Figure 11B)". The enzymatic labeling of goat IgG antibody with UDP-GalNAz in the presence of a GalT1 Y289L mutant, which "can catalyze the transfer of galactose from uridine diphosphate-GalNAz (UDP-GalNAz) to terminal GlcNAc groups" was expressly disclosed in paragraph [0111] and Example 26 of the patent application (see Figure 17).

The methods could be used to obtain an azido modified glycoprotein (see paragraph [0009], lines 1 to 4), while paragraph [00231] referred to a kit containing "a GalT enzyme", a term which included specific GalT enzymes, such as β-1,4-galactosyltransferase (GalT) Y289L (see paragraph [0111] of the patent application).

Hence claims 1, 9 and 10 were based on the disclosure of the patent application.

Clarity (Article 84 EPC)

The expression "terminal GlcNAc group" designated a "terminal" location of a GlcNAc group covalently attached at a terminal glycan position of another glycan residue or of an amino acid residue of the glycoprotein, which could be O-linked or N-linked, but lacking any further residue attached to it. This expression reliably establishes what falls under the scope of claims 1 and 9. This interpretation was confirmed by paragraph [0112] of the patent.

Sufficiency of disclosure (Article 83 EPC)

The patent disclosed α-crystallin labelled at its terminal O-GlcNAc with an azide from UDP-GalNAz, using a modified b-GalT1 enzyme in accordance with the method of claim 1 (see example 18), and the generation of a terminal N-linked GlcNAc with Endo Hf, followed by azide labeling of the glycoprotein by contacting the glycoprotein with UDP-GalNAz in the presence of a GalT1 Y289L mutant (see Example 26). These examples used the Y289L mutant b-GalT1, since it was the only tangible modified enzyme of the patent (see paragraphs [0108] and [0280]), and two glycoproteins with different types of terminal GlcNAc (see paragraphs [0108], [0111] and [0112]).

Inventive step Article 56 EPC

Document D4 was the closest prior art. It concerned "methods for rapid and sensitive detection of post-translationally modified proteins ... that were at the limits of detection using traditional models" (D4, abstract). The aim of document D4 was achieved by using a glycosyl donor comprising an unnatural ketone

functionality. Even if GalT having a Y289L mutation could tolerate substrates with minor substitutions at the C-2 position, specifically 2-deoxy, 2-amino, and 2-N-acetyl substituents (D4, paragraph [0131]), these substituents differed significantly from the C-2 substituent both in size and by means of its polar azide group. Due to this difference, it was unknown whether GalT or a mutant thereof could use the UDP-GalNAz substrate with a reasonable expectation of success. Document D15 confirmed that the polarity of the substrate played an important role in the progress of the enzymatic reaction. Thus, the polar azide group of the UDP-GalNAz interfered with the network of polar interactions in the substrate binding pocket of GalT and thereby destroyed its ability to transfer the GalNAz group of UDP-GalNAz to an acceptor glycoprotein.

The subject-matter of claim 1 was for this reasons inventive over the content of document D4 alone.

Document E4 related to the metabolic incorporation of sugar analogues, including Ac_4ManNAz and Ac_4GlcNAz (see page 14897, Figures 3 and 5)

Thus, documents D4 and E4 failed to disclose the use of UDP-GalNAz, much less the use of UDP-GalNAz in conjunction with Y289L GalT to form an azido labelled glycoprotein.

Document D1 discussed mucin-type O-linked glycosylation initiated by the polypeptide *N*-acetyl-α-galactosaminyltransferases (ppGalNAcTs) (abstract, third sentence). ppGalNAcTs are not β-1,4-galactosyltransferases. Documents D2 and D3 described the transfer of GalNAz with a ppGalNAcT using

a UDP-GalNAz (see document D2, Figure 1; document D3, page 6, column 1, second paragraph, line 5).

Based on the enzyme specificity used in documents D1 to D3, the skilled person would not have considered combining any of them with document D4. But even if the skilled person considered such a combination possible, it was still unknown whether UDP-GalNAz represented a valid substrate for the mutant Y289L enzyme of document D4 or not. In any event, none of the R groups of the modified UDP-Gal compounds were attached to the glycosyl group via a nitrogen atom. The UDP-GalNAz of claim 1 was not covered by the Genus A derivatives of document D4.

Thus, applying the problem-solution approach, the skilled person would not have considered any of these citations in combination with D4.

This reasoning applied mutatis mutandis to product-by-process claim 9 and kit claim 10. All of the claims are therefore inventive.

IX. The submissions made by **appellant II**, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 100(b) EPC

Claim 1 extended to the labelling of glycoproteins without a specific terminal glycosyl residue, such as O-GlcNAc or N-GlcNAc. However, only the O-GlcNAc terminal residue and the GlcNAc terminal residue resulting from an Endo H or Endo H_f enzymatic glycosyl chain truncation on glycoprotein were a substrate for

the claimed method (see patent paragraphs [0108], [0112], [0280]). There was no support in the patent for other glycoproteins, known or yet to be identified, which could be used in the method of claim 1. Since the term "glycoprotein" had a recognized meaning in the prior art, it was inappropriate to apply a narrower interpretation than the definition given to this term in the prior art (see paragraphs [0085], [0108], Figures 6A-B, Figure 11b, examples 18, 19 and 26). Thus, the subject matter of claim 1 and its dependent claims was insufficiently disclosed.

The method of claim 2 was directed to a method of forming a glycoprotein conjugate. It defined the method of claim 1 as its first step, resulting in a glycoprotein with an azide functional group, which was then involved in a second conjugation step resulting in a product having consumed the azide functional group. Thus, the subject-matter of claim 9, directed at an azido modified glycoprotein inter alia obtainable specifically by a method of claim 2 was not enabled.

Thus, the set of claims failed to meet the requirements of Article 83 EPC.

Auxiliary request 1
Amendments (Article 123(2) EPC)

Claim 1 was amended to a method of enzymatically labeling a "glycoprotein comprising a terminal GlcNAc group". The amendment contravened the requirements of Article 123(2) EPC as there was no basis in the patent application for using glycoproteins with a terminal GlcNAc other than O-GlcNAc groups. The sole explicit disclosure of the term "terminal GlcNAc", though embedded in an otherwise erroneous technical statement,

was found in the patent application in paragraph [0111]. First, even if a β-1,4-galactosyltransferase (GalT) enzyme could be used for this purpose, there was no direct and unambiguous disclosure to use a mutant enzyme, let alone a GalT Y289L mutant enzyme. Second, even if the use of a Y289L mutant of GalT on substrates (plural) was mentioned, it was nowhere specifically combined with a UDP-GalNAz substrate. Third, Figure 6 showed a geometric placeholder to represent the entire molecule except for the azide group of UDP-GalNAz. Fourth, Figure 11A depicted an erroneous side chain: -(C=O)-CH2-N3 instead of: -N-(C=O)-CH2-N3 for UDP-GalNAz, but there was no reason to consider Figure 11 rather than the corresponding written disclosure to be erroneous. Thus, both Figures could not serve as an adequate basis for amended claim 1 either.
Finally, paragraph [0115] of the patent application referred to antibodies, or otherwise to a "reaction of the terminal OGlcNAc molecules present on antibody carbohydrates". "If no OGlcNAc sugars are present on an antibody, the use of the Endo-H (...) enzyme will be used to generate a truncated chain which terminates with one O-linked N-acetylglucosamine residue (Figure 11B)" (see Example 26). Thus, paragraph [0115] only provided a basis for glycoproteins comprising O-GlcNAc groups but not for glycoproteins with other GlcNAc groups (see also paragraphs [0114], [0003] and [0004]). The same conclusion was applicable to independent claim 9.

Clarity (Article 84 EPC)

The patent provided no specific definition for the term "terminal GlcNAc". In consequence, the GlcNAc group was either located somewhere in a terminal part of a complex glycan motif on a glycoprotein or it

represented the terminus itself, which corresponded to its ordinary meaning. In some examples of the patent it defined the terminus of a truncated chain, illustrated as terminal O-GlcNAc, but this was not its sole logical interpretation (see paragraph [0112] and Figure 11, Figures 6A-B in paragraph [0108]). Thus, there was no reason to limit the scope of claim 1 to glycoproteins comprising specific positions on glycan allowing a transfer of the substituted monosaccharide UDP-GalNAz onto that glycoprotein by galactosyltransferases only, let alone by a Y289L mutated form. The term "terminal GlcNAc" introduced into claim 1 contravened Article 84 EPC.

The same conclusion applied to the dependent claims and to claim 9.

Article 83 EPC

Claim 1 referred to a terminal GlcNAc, encompassing O-GlcNAc and other terminal GlcNAc, such as N-GlcNAc, as well as simple or complex branched glyco-chains, wherein GlcNAc might be only one of several end groups. The patent provided no clear and unambiguous teaching regarding the nature of terminal GlcNAc groups that terminal N-GlcNAc and branched terminal GlcNAc were also suitable substrates for the claimed method. The labelling of a goat antibody, known to be O- and N-glycosylated, which was deglycosylated using Endo H_f enzyme was shown to occur on terminal O-GlcNAc residues (see Figure11, example 26). This example could not prove that the labelling of a terminal N-GlcNAc comprising glycoprotein also took place. Thus, the subject matter of claim 1 was not enabled across the entire scope of the claim 1. The same conclusion

applied mutatis mutandis to claims referring to it and dependent thereon.

Inventive step Article 56 EPC

Document D4 was the closest prior art. It described a method for the labeling of post-translationally modified proteins (e.g. paragraphs [0004], [0008]) with a chemical handle (see paragraph [0042]), wherein the method comprises contacting the post-translationally modified protein with a UDP-sugar derivative (see paragraph [0038]) in the presence of mutant Y289L β-1,4-galactosyltransferase (see paragraph [0097]) to form a modified glycoprotein (e.g. Figure 9, paragraphs [0183]-[0187]). The post-translationally modified protein was a glycoprotein (see paragraph [0093]), "having a pendant moiety", such as a glycosyl group or GlcNAc (paragraphs [0010]-[0013], [0027]-[0029]; claim 19). O-GlcNAc-glycosylated proteins were preferred embodiments and were used with Y289L mutant GalT and GalNAc sugar substrates, such as labelling agent 1, falling under the formula of genus A or A' (e.g. paragraphs [0094], [0125] to [0131], Figure 1B, [0038]). The R substituent was selected from the group consisting of straight chain or branched C_1-C_{12} carbon chain bearing a carbonyl group, azide group, straight chain or branched C_1-C_2 carbon chain bearing an azide group, straight chain or branched C_1-C_{12} carbon chain bearing an alkyne, and straight chain or branched C_1-C_{12} carbon chain bearing an alkene (see paragraph [0039]). The chemical handle attached to the UDP-sugar derivative was also described as an azide moiety (see paragraphs [0020], [0105]-[0107]) defined in paragraph [0104]. It explicitly disclosed the use of UDP-galactose derivatives, wherein the substituent R carrying the chemical handle appended at the C-2

position of the sugar moiety was an azide group or a linear or branched alkyl substituent bearing an azide group (see paragraph [0136]) inter alia in conjunction with a mutant GalT (see paragraph [0137]). It was immaterial that "the main focus appears to lie on ketone substituents".

The sole difference between the content of document D4 and the patent was the use of UDP-GalNAz. The sugar derivative of document D4 carried a chemical handle appended at the C-2 position of the galactose moiety whose purpose was to allow further modifications e.g. via Staudinger reaction (see paragraph [0110], second sentence). The use of GalNAz compared to the substrates disclosed in document D4 was not disclosed to have any advantage.

Thus, starting from document D4, the problem to be solved was to provide an alternative azido sugar substrate.
Document D4 disclosed that the engineered enzyme and the corresponding substrate "capitalize on the substrate tolerance of GalT, which allows for chemoselective installation of a non-natural functionality, such as a ketone chemical handle, to O-GlcNAc pendant moiety on modified proteins (Figure 1B)" (see paragraph [0130]) extending to "... unnatural substrates containing minor substitutions at the C-2 position, including 2-deoxy, 2-amino and 2N-acetyl substituents". The Y289L mutation enlarged the binding pocket of GalT and enhanced the catalytic activity toward GalNAc substrates without compromising specificity. Thus the skilled person was motivated to consider alternative substitutions at the C-2 position and was aware of the favourable properties of UDP-GalNAc sugar substrates. The chemical handle could be

an azido group capable of reacting in a Staudinger reaction, which was referring explicitly to document E4 (see paragraph [0107]) and thus incorporating its teaching (see decision T 153/85). The use of a substituent with a C_1-C_{12} carbon chain bearing an azide group was not expected to encounter steric hindrance (see document D4, paragraph [0136]).

Document E4 described azido modified N-acetyl analogues ManNAz and GlcNAz with an azido handle capable of reacting in a Staudinger reaction (see abstract).

Documents D4 and E4 prompted the skilled person to employ GalNAz instead of Gal-X-NAz and did not prevent him from using UPD-GalNAz in combination with the mutant Y289L GalT, since its binding pocket was enlarged, which enhanced its catalytic activity toward GalNAc substrates (see paragraphs [0130], [0131]; document D10).

Since GalNAz can be produced from GalNAc with an azide moiety attached at the N-acetyl group using a method known in art and the advantages of azide groups were known, the choice of an azide chemical handle cannot justify an inventive step (see documents D1 to D3, especially D3, col.1, second paragraph).

Since the complexity of the technical problem to be solved was low and the labeling procedure completed in less than 24 hours, there was no technical prejudice which would have jeopardized the skilled person's expectation of success of a 'try and see' approach.

The subject matter of claim 1 was accordingly not inventive in the light of document D4 in combination with documents D1 to D3 or E4, explicitly referred to

in document D4 in paragraph [0107]. For the same reasons dependent claims 2-8 lacked inventive step as well.

The submissions made by **appellant III**, insofar as relevant to the present decision, may be summarized as follows:

Main request
Sufficiency of disclosure (Article 100(b) EPC)

The "modified b-GalT1 enzyme" used in example 18 could not only be reduced to the "β-1,4-galactosyltransferase Y289L mutant enzyme". The term encompassed a large number of enzymes characterized by their modifications. There was no reason to assume that the Y289L mutant, mentioned also elsewhere in the description and in the context of another embodiment, was the enzyme used in Example 18. Example 26 related to an experiment where deglycosylated goat IgG antibodies, whose glycosyl chains were first trimmed by Endo H_f to terminal sugar moieties, were then enzymatically labelled with a GalNAz moiety by a β-1,4 galactosyltransferase Y289L mutant enzyme to form an azido modified glycoprotein. Since the method of claim 1 defined neither the use of a deglycosylated glycoprotein nor the trimming step of a glycoprotein, a skilled person, applying the method of claim 1 to an antibody, would not know how to label the glycoprotein with a GalNAz moiety, i.e whether a prior deglycosylation or the introduction of a terminal GlcNAc was necessary or not. Example 26 was not sufficient to enable the skilled person to carry out the method of claim 1 over its entire scope without undue burden.

Examples 18 to 22 referred to a glycoprotein labelled on a terminal O-GlcNAc group, but provided no support on how the remaining embodiments falling also under claim 1, e.g. glycoproteins having a high-mannose comprising two innermost GlcNAc moieties (see document E1, RNAse B) or a terminal sugar moiety other than a terminal O-GlcNAc group, could be amenable to the claimed method without undue burden. The legend of Figure 11 related to an antibody and required the presence of terminal O-GlcNAc molecules on the antibody for its subsequent enzymatic labelling with UDP-GalNAz (see patent paragraph [0026]). Absent a disclosure how a non-terminal GlcNAc group was labelled and of the experimental conditions for achieving this labelling, the skilled person was not in a position to reproduce the claimed method over the entire breadth of the claim.

The Examples of the patent related to reactions where O-GlcNAc, but not "GlcNAc" or "N-GlcNAc" groups, were modified (see Figure 17; Figure 11 A or B). Except for glycoproteins comprising a O-GlcNAc, the skilled person had to identify first which sugar moieties allowed azido groups to be transferred and which experimental conditions were needed for glycoproteins to be modified. Document El supported this view. Hence, the skilled person could not carry out the invention of claim 1 over its entire scope pursuant to Article 83 EPC.

Claim 2 referred to "the method of claim 1 that is a method of forming glycoprotein conjugate", wherein the azido modified glycoprotein was contacted with a reporter molecule, carrier molecule or solid support comprising an azide reactive moiety to form a conjugate.

The reaction between an azido modified glycoprotein and an azide reactive moiety resulted in consumption of the azido group.

Claim 9 referred to an azido modified glycoprotein obtainable inter alia by the method of claim 2. As the method of claim 2 consumed the azido reactive group on the glycoprotein, claim 9 could not be implemented without carrying out an additional azido modification step.

Thus, the subject matter of claim 1 was not enabled across its entire scope, contrary to the requirements of Article 83 EPC. The same conclusion applied to claims 2-10.

Auxiliary request 1
Amendments (Article 123(2) EPC)

Paragraph [0004] of the background art of the patent application could not serve as a basis for the claimed invention.

The patent application stated erroneously that "β-1,4-galactosyltransferase (GalT) is an enzyme that can catalyze the transfer of galactose [sic!] from uridine diphosphate-GalNAz (UDP-GalNAz) to terminal GlcNAc groups" (see paragraph [0111]). This is technically impossible. Thus, even if a β-1,4-galactosyltransferase (GalT) enzyme could be used for this purpose, there was no direct and unambiguous disclosure to use a mutant enzyme, let alone a GalT Y289L mutant enzyme. Even if the use of a Y289L mutant of GalT on substrates (plural) was mentioned, it was not specifically combined with a UDP-GalNAz substrate. Neither example

18 and Figure 6 nor example 26 disclosed a method combining the specific features of claim 1.
In the method for enzymatically labeling a glycoprotein "the appropriate enzyme" is in one aspect a "Gal T enzyme" and in another aspect "a modified enzyme" (see paragraph [0009]). There was no direct and unambiguous basis for a modified enzyme being a mutant enzyme, let alone a Y289L mutant GalT enzyme. There was no basis in claim 1 of the patent application for a generic method for enzymatically labeling a glycoprotein with an azide moiety comprising step a) which is lacking its step b).

The subject-matter of claim 9 did not have a basis in the patent application, as no generic disclosure could be identified in the patent application for an "azido-modified glycoprotein" obtainable by the method according to claim 1. This was exacerbated by the fact that the method of claim 1 used the term "comprising", allowing the product of step a) to be modified by any additional process step, resulting in any potential azido modified glycoprotein.

The kit of claim 10 comprised added matter as it comprised a β-1,4-galactosyltransferase Y289L mutant enzyme, whereas the kit of claim 40 of the patent application comprised "a GalT enzyme" (see claim 40; paragraph [00231]).

Hence the subject-matter of claims 1, 9 and 10 violated the provisions of Article 123(2) EPC.

Clarity (Article 84 EPC)

Decision G 3/14 stipulated that amended claims had to comply with the requirements of Article 84 EPC, if the

introduced amendments resulted in a lack of clarity of the claims.
Claim 1 was amended to "[a glycoprotein] comprising a terminal GlcNAc group" only. However, the term "terminal" was highly ambiguous. It might either refer to the N-terminus or the C-terminus of the polypeptide chain of the glycoprotein or relate to the specific sugar moiety at the terminal position in a (branched or straight) chain of any polymer associated with the glycoprotein, e.g. a carbohydrate chain. It was equally unclear, which "terminus" of the glycoprotein the "terminal GlcNAc group" had to occupy, i.e. on a glycoside or a protein moiety and whether one single GlcNAc monomer attached to a side chain of the glycoprotein qualified as "terminal GlcNAc group" or not. It was furthermore unclear whether the "terminal GlcNAc group" of a glycoprotein had to be present at the outset of the method or whether it could be introduced by process steps not explicitly mentioned in claim 1. A skilled person would not interpret a terminal single monomeric GlcNAc group, having a relatively small size, buried in, or exposed on a larger glycoprotein at the terminus of a chain of units or on an amino acid residue on a glycoprotein as a "terminal GlcNAc group". Since the term "terminal" was assigned multiple definitions, it was unclear whether a single monomeric unit on a protein qualified as "terminal" (see Example 26 of the patent). Thus, claim 1 and all the claims dependent thereon contravened the requirements of Article 84 EPC.

Sufficiency of disclosure (Article 83 EPC)

The objections raised against the main request under Article 83 EPC were applicable mutatis mutandis to this request.

Inventive step Article 56 EPC

Document D4 was the closest prior art. It related to methods for labeling and detecting glycosylated proteins comprising a GlcNAc group (see paragraph [0008]). The Y289L mutant enzyme was used to transfer galactose or its analogue to terminal GlcNAc groups (see paragraph [0097]) and taught that "the single Y289L mutation enlarged the binding pocket of GalT and enhanced the catalytic activity toward GalNAc substrates without compromising specificity" (see paragraph [0131]). It provided specific embodiments of substrates for Y289L mutant enzyme, such as UDP analogues according to "Genus A" or "Genus A'" (see paragraphs [0135] to [0138]), wherein the C2 substituent was - amongst others - a C2 carbon chain bearing an azide group. Thus, document D4 disclosed generically substrates including UDP-GalNAz and an UDP-Gal analogue with a ketone chemical handle (see formula 1, paragraph [0133]).

The only difference between the specific embodiment disclosed in document D4 and the subject-matter of claim 1 was the "azido modified N-acetyl group" at position C2 instead of the "ketone chemical handle". The choice of an azide handle (instead of a ketone handle), both representing well known chemical handles in sugar chemistry, was merely an arbitrary alternative, as there is no unexpected effect connected to that structural substitution.

The objective technical problem was therefore the provision of an alternative UDP-Gal analogue.

It was common technical knowledge that in addition to its galactosyltransferase activity, the mutant enzyme exhibited GalNAc transferase activity. Changes in enzymatic activity and substrate specificity of the Y289L mutant enzyme accepting other Gal C2 analogues was known (see document D4, paragraph [0131]): "Importantly, the single Y289L mutation enlarges the binding pocket of GalT and enhances the catalytic activity toward GalNAc substrates without compromising specificity."
The plural of the expression "GalNAc substrates" in the above sentence, provided a further hint for the skilled person that GalNAc itself as well as other structurally similar compounds could be used, all of which acting as substrates for that mutant enzyme.

There was a clear pointer in document D4 to use UDP-Gal analogues to replace UDP-Gal ketone and for the use of GalNAc transferases. The GalNAc transferases and the analogue UDP-GalNAz as substrate for GalNAc transferases were reported in documents D1, D2 and D3 (see D3, page 6, right column, first paragraph; Figure 2; D1, page 5026, right column, last sentence; D2, page 14850, left column, penultimate paragraph). The skilled person would therefore have selected the UDP-GalNAz as inevitable "chemical handle" candidate analogue substrate with a reasonable expectation of success (cf. decision T 249/88) and was aware of all the advantages of azide over ketone as a chemical handle.

The subject-matter of claim 1 did not involve an inventive step, as (i) its underlying object was not solved over the entire scope, (ii) the subject-matter claimed did not represent any advantageous effects and (iii) its subject-matter was most obviously suggested by the prior art.

In view of the above arguments, the azido modified glycoprotein obtainable by the method of claim 1 lacked an inventive step as well.
Finally, both the Y289L mutant enzyme and the use of UDP-GalNAz were known in the art, rendering their use in a kit together with an azide reactive reporter molecule, carrier molecule or solid support obvious.
Thus, claims 1, 9 and 10 lack an inventive step.

X. Appellant I (patent proprietor) requested, as its main request, that the decision under appeal be set aside and the patent be maintained as granted, or alternatively, that appellant II's and III's appeals be dismissed, or that the patent be maintained on the basis of any of auxiliary requests 1a, 2 and 2a, filed under cover of letters dated 4 March 2020 and 30 September 2016 respectively.

XI. Appellant II requested that the decision under appeal be set aside and the patent be revoked.

XII. Appellant III requested that the decision under appeal be set aside and the patent be revoked as well as that documents E23 to E27 be admitted into the appeal proceedings.

Reasons for the Decision

Procedural issues
Basis of the appeal proceedings

1. Concerning novelty, appellant II stated: "The novelty objections submitted in the opposition writ are upheld." Similarly, appellant III stated: "As far as dependent claims are concerned, it is referred to our submissions filed before the opposition division." Both

appellants considered these submissions to form part of their appeal case.

1.1 Appellants' understanding as to the requirements for submissions in appeal proceedings is not correct. Article 12(1) of the Rules of Procedure of the Boards of Appeal (RPBA 2020) stipulates that appeal proceedings shall be based on inter alia (a) the decision under appeal and minutes of any oral proceedings before the department having issued that decision, (b) the notice of appeal and statement of grounds of appeal, (c) any written reply of the other party or parties to be filed within four months of the notification of the grounds of appeal, and (d) any communication sent by the board and any answer thereto filed pursuant to directions of the board. The statement of grounds of appeal must therefore contain appellant's complete case (see Article 12(3) RPBA 2020).

1.2 The requirement under Article 12(3) RPBA 2020 is not fulfilled by a passing reference to the facts and evidence put forward in opposition proceedings. It is not for the board to identify issues which arose in opposition proceedings and may (or may not) still be a matter of dispute in appeal proceedings, but for the appellants to put forward in their statement of grounds of appeal their line(s) of argument and each of the facts and evidence on which they want to rely in appeal proceedings.

1.3 Appellant II provided no reason why the decision of the opposition division under Article 54 EPC was incorrect. Appellant III provided no reason why the lines of argument with regard to the dependent claims were still applicable. Since these objections were not adequately

substantiated, they are not admitted under Article 12(3) RPBA 2020.

Admission of documents E23 to E30 into the appeal proceedings (Article 12(4) RPBA 2007)

2. With its statement of grounds of appeal, appellant II submitted new documents E28 to E30. Document E28 was a copy of a communication of the Examining Division dated 18 November 2011 in relation to the present matter. Document E29 consisted of a re-submission of E24. Document E30 was supplementary evidence to establish that only a very small number of azide sugar substrates was actually available at the relevant date.

2.1 With its statement of grounds of appeal, appellant III resubmitted documents E23 to E27 and requested their admission into the appeal proceedings.

2.2 In the decision under appeal, it was concluded that documents E23 to E27 were not prima facie relevant and/or the reason for their late filing could not be established. They were therefore not admitted into the opposition proceedings.

2.3 Since no reasons were submitted why the opposition division had exercised its discretion not to admit documents E23 to E27, document E29 being identical to E24, according to the wrong principles or in an unreasonable way, the board sees no reason to overrule the decision under appeal. Since no reasons were provided as to why documents E28 and E30 could not have been submitted earlier and why they were prima facie relevant to the present case, the board exercises its discretion not to admit these documents into the proceedings (Article 12(4) (RPBA 2007)). None of the

documents E23 to E30 were admitted into the appeal proceedings.

Main request
Article 100(b) EPC

3. Appellant I appeared to accept that the skilled person cannot readily and without undue burden achieve the stated goal of the claimed method across the entire breadth of claim 1 when interpreting it literally.

It argued however that the claim must be construed by a mind willing to understand not a mind desirous of misunderstanding (see decision T 396/99, ultimate paragraph of section 3.5). When considering claim 1, a skilled person ruled out interpretations which were illogical or which did not make technical sense and took into account the whole disclosure of the patent. The method of claim 1 was therefore implicitly limited to the enzymatic labelling of glycoproteins with an azide moiety.

4. The board follows the established case law that construing a claim with a mind willing to understand does not require that a technically broad term is interpreted more narrowly (see for example decision T 1408/04, reasons 1).

4.1 The method of claim 1 defines one step for enzymatically labelling a glycoprotein. This step comprises contacting two substrates with an enzyme. The use of UDP-GalNAz and a Y289L mutant β-1,4-galactosyltransferase enzyme, however, does not impose any structural or functional limitation on the nature of the glycoprotein to be labelled. The term glycoprotein used in claim 1 does not exclude

glycoproteins lacking a terminal GlcNAc group for the sole reason that they cannot be labeled.

4.2 It is undisputed that the subject-matter of claim 1 needs to be interpreted in the light of the patent's teaching and the skilled person's common general knowledge when considering sufficiency of disclosure. However, a limiting feature mentioned in the description only (see e.g. paragraphs [0104], [0111], [0112], [0269], [0280] of the patent) which is missing in the claims cannot be read into them and so inserted merely through interpretation. The only way is to amend the claims to incorporate it.

Since the method of claim 1, comprising the step of contacting the glycoprotein with UDP-GalNAz in the presence of a β-1,4-galactosyltransferase Y289L mutant enzyme to form an azido modified glycoprotein, does not comprise any unclear term requiring a further interpretation, there is no reason and no leeway to limit the clear wording of claim 1 to "a method for enzymatically labelling a glycoprotein comprising a terminal GlcNAc group".

4.3 Since the aim of the method cannot be achieved across the full scope of claim 1, the board sees no reason to deviate from the decision under appeal and concludes that the claimed subject matter is insufficiently disclosed.

Auxiliary request 1 (claims 1-10 of the decision under appeal)

Claim 1 of the auxiliary request 1 differs from claim 1 of the main request in that it is directed at a method for... labeling a glycoprotein comprising "a terminal GlcNAc group with" an azide moiety.

Article 123(2) EPC

5. Both, appellant II and III argued that the proposed amendment contravened Article 123(2) EPC as there was no basis in the patent application for using glycoproteins with a generic terminal GlcNAc group but only for O-GlcNAc groups.

5.1 Appellant I argued that the features of amended claim 1 could be found in paragraph [0009] of the patent application, except that the enzyme was "a modified Gal T enzyme" rather than the claimed "β-1,4-galactosyltransferase Y289L mutant enzyme". The missing feature of claim 1 was disclosed in paragraphs [0111] and [0115] of the patent application.

6. The board notes that it was undisputed that the subject-matter of claim 1 does not find an explicit basis in the patent application.

6.1 The decisive question is whether the subject matter of claim 1 is directly and unambiguously albeit implicitly disclosed, keeping in mind that an implicit disclosure means a disclosure which any person skilled in the art would objectively consider as necessarily implied in the explicit content. It means nothing more than the clear and unambiguous consequence of what is explicitly mentioned.

6.2 Paragraph [0111] of the patent application discloses that the proteins described in the patent can be modified in vitro using enzymatic post-translational modifications, which include but are not limited to, glycosylation, isoprenylation, lipoylation and phosphorylation. In certain embodiments these

modifications serve to modify proteins with azide moieties, alkyne moieties, or phosphine moieties. "Certain embodiments utilize β-1,4-galactosyltransferase (GalT), or a mutant thereof, to modify glycosylated proteins with azide moieties, alkyne moieties, or phosphine moieties. β-l,4-galactosyltransferase (GalT) is an enzyme that can catalyze the transfer of galactose from uridine diphosphate-GalNAz (UDP-GalNAz) to terminal GlcNAc groups. Thus, glycoproteins are enzymatically labeled in vitro with azide modified sugar moieties, alkyne modified sugar moieties, or phosphine modified sugar moieties."

6.2.1 The skilled person derives that the patent application implicitly discloses a method for labeling a glycoprotein at any of its glycosylation sites by contacting it with a mutant β-1,4-galactosyltransferase (GalT) and with UDP-GalNAz so as to result in an azido modified glycoprotein. Since the transfer of "galactose" from UDP-GalNAz to the terminal GlcNAc groups with β-1,4-galactosyltransferase (GalT) is technically impossible, the skilled person would have construed the "galactose" to be "GalNAz" as it was immediately evident that nothing else could be intended in this case.

6.2.2 The use of a mutant β-GalT enzyme to label the O-GlcNAc containing protein with a UDP-GalNAz is also disclosed. Since β-1,4-galactosyltransferase (GalT) Y289L is the only specific mutant identified throughout the patent application, the skilled person considers this mutant as the β-GalT mutant capable of labeling an O-GlcNAc containing protein using UDP-GalNAz (see paragraph [0111] and Figures6A-B). This viewpoint is confirmed by example 18, Figure 6 and example 26, which disclose

methods of enzymatically labelling either an α-crystallin at an O-GlcNac glycosylation site using UDP-GalNAz with a modified b-GalT1 enzyme or a goat IgG using a UDP-GalNAz with a GalT1 enzyme Y289L. Thus, paragraph [0111] discloses implicitly a method for labeling a glycoprotein at any of its glycosylation sites, wherein a mutant β-1,4-galactosyltransferase (GalT) Y289L contacted with UDP-GalNAz transfers GalNAz to the glycoprotein's terminal GlcNAc groups so as to result in an azido modified glycoprotein.

6.3 The patent application refers to glycoproteins comprising O-GlcNAc groups and that if no O-GlcNAc sugars are present on an antibody, Endo-H (endo-β-*N*-acetylglucosaminidase H) enzyme will generate a truncated chain terminating with one N-acetylglucosamine residue (see paragraph [0115]). Endo-H (endo-β-*N*-acetylglucosaminidase H) enzyme or Endo-H_f are known to cleave the chitobiose of the linked glycans so as to expose an N-linked terminal GlcNAc group (see example 26; document D16, page 584). Thus, the "terminal GlcNAc group" referred to in this paragraph consists of a terminal GlcNAc group attached by an O- or N-covalent linkage to the glycoprotein. For this reason, the board considers that there is an adequate basis in the patent application for the subject-matter of claim 1.

6.4 Since the subject matter of claim 1 is directly and unambiguously disclosed in the patent application, the same must be true for the product obtainable by performing said method (claim 9).

6.5 Appellant III considered that the kit of claim 10 comprising a β-1,4-galactosyltransferase Y289L mutant enzyme, contravened Article 123(2) EPC, as the kit of

claim 40 of the patent application referred to "a GalT enzyme" only.

6.6 Even if the patent application in paragraph [00231] only mentions that the present invention provides kits that include "a GalT enzyme", the board cannot ignore that this paragraph belongs to a section entitled "Kits of the invention". Since the invention is directed at a method for enzymatically labeling a glycoprotein with an azide moiety and a Y289L mutant GalT, which is the only β-1,4-galactosyltransferase (GalT) identified which is capable of transferring the GalNAz from the UDP-GalNAz to the glycoprotein's terminal GlcNAc groups, the skilled person would have directly and unambiguously derived that the kit of the invention comprises the Y289L mutant β-1,4-galactosyltransferase (GalT). Thus, claim 10 finds an adequate basis in the patent application.

7. The board concludes that the subject-matter of claims 1, 9 and 10 of auxiliary request 1 does not extend beyond the content of the application as filed. Hence, auxiliary request 1 meets the requirements of Article 123(2) EPC.

Article 84 EPC

8. Appellant III raised a clarity objection under Article 84 EPC against amended claims 1, 9 and 10. It considered the expression "terminal GlcNAc group" unclear.

8.1 This expression either refers to the N-terminus, or the C-terminus of the polypeptide chain of the glycoprotein, or relates to the specific sugar moiety at the terminal position in a (branched or straight)

chain of any polymer associated with the glycoprotein, e.g. a carbohydrate chain.

8.2 The board observes that the expression "terminal GlcNAc group" does not assign any particular position on the glycoprotein, consisting of a glycosyl and a protein part. The expression "a terminal GlcNAc group" has to be interpreted in the widest sense in accordance with what it means in the art: an end GlcNAc group attached to the glycoprotein at any terminal distal position, i.e. on any type of available polymer chain in said glycoprotein, be it the amino acid backbone, an amino acid side chain or a saccharide polymer. Hence, a single GlcNAc monomer covalently attached to a glycoprotein at an amino acid side-chain or at outer positions of a glycan chain clearly qualifies as "terminal GlcNAc group", irrespective of its relative size compared to the remaining glycan structure, the steric constraints, or whether it is exposed on a larger glycoprotein at the terminus of a chain of units or not. The mere fact that the "terminal GlcNAc group" covers different embodiments does not mean that the feature is ambiguous, and that a claim including it lacks clarity within the meaning of Article 84 EPC. A claim is only considered to lack clarity if the exact distinctions which delimit the scope of protection conferred by the claim cannot be established from it (see decision T 0006/01 of 2 December 2003, paragraph 14).

8.3 Hence, the board concludes that claims 1, 9 and 10 fulfil the requirements of Article 84 EPC.

Article 83 EPC

9. Appellant II submitted that claim 1 referred to a terminal GlcNAc, encompassing O-GlcNAc and other terminal GlcNAc, such as N-GlcNAc, as well as simple or complex branched glyco-chains, wherein GlcNAc might be only one of several end groups. The patent provided however no clear teaching that terminal N-GlcNAc and branched terminal GlcNAc were also suitable substrates for the claimed method. Example 26 could not prove the labelling of a terminal N-GlcNAc comprising glycoprotein. Thus, the subject matter of claim 1 was not enabled across the entire scope of the claim 1. The same conclusion applied mutatis mutandis to claims referring to it and dependent thereon.

9.1 Appellant III considered that example 18 of the patent disclosed the enzymatic labeling of α-crystallin O-GlcNAc using a "modified b-GalT1 enzyme" and UDP-GalNAz, without mentioning the GalT1 Y289L enzyme. The skilled person was left in the dark about which "modified b-GalT1 enzyme" to use. Example 26 described the enzymatic labeling of goat IgG antibodies using a GalT1 Y289L enzyme and UDP-GalNAz respectively, but involved an Endo H_f deglycosylation step before the mutant enzyme was capable of labeling the glycoprotein. As a result, there was not sufficient guidance to the skilled person as to when deglycosylation was required and how the glycoproteins had to be trimmed to generate a terminal GlcNAc glycoprotein, so that the invention could be implemented without undue burden.

9.2 The onus is on the appellants to prove a lack of sufficiency of disclosure. To this end, appellant III submitted experimental evidence E1. Considering that experiment A of document E1 explicitly states that RNAse B lacks a terminal GlcNAc moiety and that Endo H treatment of trastuzumab in experiment B did not remove

complex glycans, both experiments do not fall under the ambit of claim 1 and as such cannot support appellant's case (see last sentence of the first paragraph on page 2 of document E1).

9.3 The board concludes that since the examples of the patent provide sufficient guidance on how to perform the claimed invention and what to do if no terminal GlcNAc group is available, the findings of the decision under appeal with regard to Article 83 EPC were correct.

Article 54 EPC

10. Appellant III considered that the azido modified glycoprotein of claim 9 lacked novelty. Document D2 disclosed: "[G]lycoproteins expressed both recombinantly and at endogenous levels in mammalian cells were labelled with GalNAz, enabling their detection from complex cellular lysates with phosphine probes (Figure 1B)" (see document D2, page 4846, right column, penultimate paragraph). Since, the azido modified glycoprotein of claim 9 was defined as a "product-by-process", obtainable by the method of claim 1 which was itself characterized by an open-ended wording ("comprising"), it did not exclude the presence of additional method steps having an impact on the structure of the resulting "azido modified glycoprotein". Claim 1 encompassed methods where the removal of the "azido modification" introduced by step a) of claim 1 was followed by a different "azido modification" using another enzyme and/or another azido substrate, or alternatively the addition of sugar moieties so that the GalNAz group was located on an inner-chain member of the final "azido-modified glycoprotein". In consequence, any azido modified

glycoprotein, as disclosed in documents D1 to D3, anticipated the subject-matter of claim 9.

10.1 The board notes that documents D1 to D3 disclose GalNAz modified glycoproteins wherein **α-1** galactose is bound to mucin (see document D1, Figure 6, chair form of the sugar; D2, Figure 1B, D3, Figure 2), whereas the method of claim 1, comprising contacting a glycoprotein with UDP-GalNAz in the presence of a **β-1**,4 galactosyltransferase Y289L mutant enzyme, results in a GalNAz(**β**1-4)terminal Glc acceptor. The **β** glycosidic bond between the carbohydrates obtained by the method of claim 1 differs from the **α** glycosidic bond obtained when using a different enzyme as described in documents D1 to D3. In addition, none of these documents discloses a glycoprotein with a terminal GlcNAc group modified by a GalNAz monosaccharide.

10.2 Although it is undisputed that the method of claim 1 does not exclude further method/modification steps, a novelty objection against claim 9 cannot be based on presumptions but must be based on facts established beyond reasonable doubt. At present, there is no convincing evidence that a product as disclosed in documents D1 to D3 could be obtained by carrying out a method including the step of claim 1 in combination with yet undefined additional steps.

10.2.1 It follows that the products disclosed in documents D1 to D3 do not anticipate the subject-matter of claim 9 of auxiliary request 1.

Article 56 EPC

11. Appellant II's and III's objection under Article 56 EPC that the underlying technical problem is not solved

over the whole scope of claim 1, because a vast number of glycoproteins are allegedly inaccessible to azido modifications, is dealt with under Article 83 EPC as the goal of the method, the glycoprotein's labelling with an azide moiety, is a functional feature of claim 1 (see Decision G 1/03 OJ 2004, 413, point 2.5.2 of the Reasons).

12. It was common ground between the parties that document D4 represents the closest prior art for the method according to claim 1.

12.1 Document D4 discloses methods for labeling and detecting glycosylated proteins comprising a GlcNAc group (see paragraphs [0004], [0008]). The Y289L GalT mutant enzyme was suitable to transfer galactose or its analogues to terminal GlcNAc groups of a glycoprotein (see paragraph [0097]). "[T]he single Y289L mutation enlarges the binding pocket of GalT and enhances the catalytic activity toward GalNAc substrates without compromising specificity" (see paragraphs [0131], and [0183] to [0187], Figure9). An uridyl diphosphate analogue 1 with a ketone chemical handle at position C-2 of the galactose ring showed that the Y289L mutant tolerated unnatural substrates containing minor substitutions at the C-2 position (see paragraphs [0133], [0134], and Figure1B). Other UDP analogues like "Genus A" or "Genus A'" bearing an azide group on the substituent on position C-2 were also described as substrates for the Y289L mutant enzyme (see paragraphs [0135] to [0138]). Finally, it discloses UDP-analogues wherein the substituent R is selected from the group consisting of straight chain or branched C2-C4 carbon chain bearing a carbonyl group, azide group, straight chain or branched C2-C4 carbon chain bearing an azide group, straight chain or branched C2-C4 carbon chain

bearing an alkyne, and straight chain or branched C2-C4 carbon chain bearing an alkene, but does not disclose an UDP-analogue wherein R is an azidoacetyl galactosamine.

12.2 The difference between document D4 and the method of claim 1 is that the method claimed uses an UDP-analogue, where the R substituted galactosyl analogue consists of an *N*-azidoacetyl galactosamine, in combination with a β-1,4-galactosyltransferase Y289L mutant enzyme to form an azido modified glycoprotein.

12.3 The use of this alternative substrate is not associated with an unexpected technical effect.

12.4 Starting from document D4 and in the absence of any unexpected technical effect underlying said difference, the technical problem is defined as the provision of an alternative method for enzymatically labeling a glycoprotein comprising a terminal GlcNAc group

12.5 The solution to this problem is the method of claim 1.

12.6 It remains therefore to be assessed whether or not the skilled person starting from the closest prior art method and faced with the technical problem identified above would have arrived at the claimed method in an obvious manner.

12.7 The board cannot agree with Appellants II and III that the skilled person was motivated by paragraph [0131] in document D4 to use UDP-Gal analogues instead of UDP-Gal ketone and to consult documents D1 to D3 dealing with GalNAc transferases and their substrates.

12.7.1 First document D4 refers to a R substituted UDP-galactosyl substrate, wherein the substituent R is "selected from the group consisting of straight chain or branched C_1-C_{12} carbon chain bearing a carbonyl group, azide group, straight chain or branched C_1-C_{12} carbon chain bearing an azide group, straight chain or branched C_1-C_{12} carbon chain bearing an alkyne, and straight chain or branched C_1-C_{12} carbon chain bearing an alkene". However, there is no mention in this paragraph or elsewhere of an UDP galactosyl substrate having -NHAc as its substituent R, or of a *N*-acetylgalactosamine, let alone of the use of a N-azido-galactosamine substrate in combination with a mutant Y289L GalT enzyme. Thus, document D4 provides no motivation to turn to a glycosyl substrate.

12.8 Second, even if documents D1 to D3 disclosed the use of UDP-GalNAz as an analogue of UDP-GalNAc for GalNAc transferases, it was used with *N*-acetyl-α-galactosaminyltransferases (ppGalNAcTs), resulting in O-linked *N*-acetyl-α-galactosaminyl Tn-antigen (GalNAc-α-*O*-Thr/Ser-Tn antigen), which differs from the claimed Y289L mutant β-1,4-galactosyltransferase enzyme both by its substrate specificity and the resulting α-glycosidic bond.
The ppGalNAcTs utilize an UDP-GalNAc substrate to modify serine or threonine residues of proteins, in an α linkage, whereas the β-1,4-galactosyltransferase transfers an UDP-galactose analogue to acceptor sugars, such as *N*-Acetylglucosamine (GlcNAc), in a β(1→4) linkage. From this content, the skilled person derived that enzymes with another specificity, e.g. ppGalNAcTs, compared to the enzyme used in claim 1 were by no means interchangeable. The mere use of a glycosyl analogue only, as defined in claim 1, in combination with another ppGalNAcT enzyme results in an entirely

different product, lacking first a β-glycosidic bond and second said bond not occurring at a GlcNAc terminal group of the glycoprotein.

12.9 Likewise, since the azide-functionalized glycosylamine substrates disclosed in document E4, such as the tetraacetylated *N*-acetylmannosamine and the tetraacetylated *N*-acetyl glucosamine (Ac_4ManNAz and Ac_4GlcNAz), are different from the substrate GalNAz used in claim 1, document E4 does not motivate either the skilled person to use derivatives thereof which are neither explicitly disclosed nor suggested.

12.10 Since none of the prior art documents suggested a method of contacting UDP-GalNAz substrates with a specific Y289L mutant GalT transferase, the skilled person was never in a "try and see" situation which consisted in only verifying whether the potential solution conceived worked as suggested.

12.11 Thus, a skilled person starting with the disclosure of document D4, faced with the technical problem of providing an alternative method for enzymatically labeling a glycoprotein, would not have combined its teaching with that of any of documents D1 to D3 and E4. Even if it had, the skilled person would not have arrived at the subject-matter of claim 1 of auxiliary request 1.

12.12 Thus, the subject-matter of claim 1 and hence auxiliary request 1 involves an inventive step.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated