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(54) Title: GLYCOCONJUGATION OF POLYPEPTIDES USING OLIGOSACCHARYLTRANSFERASES

(57) Abstract: The current invention provides polypeptides and polypeptide conjugates that include an exogenous N-linked glycosylation sequence. The N-linked glycosylation sequence is preferably a substrate for an oligosaccharyltransferase (e.g., bacterial PglB), which can catalyze the transfer of a glycosyl moiety from a lipid-bound glycosyl donor molecule (e.g., a lipid-pyrophosphate-linked glycosyl moiety) to an asparagine (N) residue of the glycosylation sequence. In one example, the asparagine residue is part of an exogenous N-linked glycosylation sequence of the invention. The invention further provides methods of making the polypeptide conjugates that include contacting a polypeptide having an N-linked glycosylation sequence of the invention and a lipid-pyrophosphate-linked glycosyl moiety (or phospholipid-linked glycosyl moiety) in the presence of an oligosaccharyltransferase under conditions sufficient for the enzyme to transfer the glycosyl moiety to an asparagine residue of the N-linked glycosylation sequence. Exemplary glycosyl moieties that can be conjugated to the glycosylation sequence include GlcNAc, GlcNH, bacillosamine, 6-hydroxybacillosamine, GalNAc, GalNH, GlcNAc-GlcNAc, GlcNAc-GlcNH, GlcNAc-Gal, GlcNAc-GlcNAc-Gal-Sia, GlcNAc-Gal-Sia, GlcNAc-GlcNAc-Man, and GlcNAc-GlcNAc-Man(Man)<sub>2</sub>. The transferred glycosyl moiety is optionally modified with a modifying group, such as a polymer (e.g., PEG). In one example, the modified glycosyl moiety is a GlcNAc or a sialic acid moiety.



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**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US 09/30503

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(8) - C07K 1/107 (2009.01)

USPC - 530/402, 345

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)  
530/402, 345

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
530/402, 345

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
google scholar, PubWest: defrees, Ser, Asn, nh2, thr, arg, conjugate, glycan, recombinant, library, expression, vector, asn-x-ser/thr, asn-x-thr/ser, asparagine-X-serine/threonine, proline, 530/402, 345, @pd<20080108, n-linked, glycosyl\$, encoding, motif, parent, peptide, sequence, position, amino adj3 acid,

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -----	US 7,138,371 B2 (DeFrees et al.) 21 November 2006 (21.11.2006) Claims 1, 4, 13, 15; col 1, ln 57-61; col 8, ln 48-50; col 53, ln 21-30; col 70, ln 1-8; col 145, ln 5-11; col 163, ln 23-25; col 181, ln 8-10, 19-21, 25-32, 33-38, 42-45, 50-52; col 182, ln 8-10, 31-32, 38-39; col 183, ln 9-12, 16-22; col 186, ln 53-58; col 191, ln 60-65; col 193, ln 22-28; col 206, ln 26-28; col 227, ln 17-18; Figures 27A-G	1-10, 12-15, 34-36, 38-43, 45 -----
Y		11, 37, 44
Y	Feldman, M.R. et al. 'Engineering N-Linked Protein Glycosylation with Diverse O Antigen Lipopolysaccharide Structures in Escherichia Coli'. PNAS; 22 February 2005 (22.02.2005); Vol. 102, No. 8; pg. 3016-3021; Abstract, Materials and Methods, pg. 3018, col 1, para 2	11, 44
Y	US 2003/0195338 A1 (Chung et al.) 16 October 2003 (16.10.2003) Abstract, Para [0098]	37

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 09/30503

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

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

- 1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
- 2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
- 3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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.

Group I: claims 1-15 and 34-45, directed to a polypeptide, or library of polypeptides comprising an exogenous sequence, limited to SEQ ID NO: 1, wherein the peptide is covalently bonded to a polymer moiety through a linker molecule attached to the peptide at a site within the exogenous sequence.

Group II: claims 16-33 and 46-77, directed to a polymer molecule having a specified structure, which may optionally be covalently bonded to a peptide through a linker attached to the peptide at an N-linked glycosylation site.

- Please see extra sheet for continuation -

- 1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
- 4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-15 and 34-45, limited to 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.

INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of Box III: Lack of Unity of Invention

The inventions listed as Groups I - II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a peptide or library of peptides comprising an exogenous sequence, limited to SEQ ID NO: 1, wherein the peptide is covalently bonded to a polymer moiety through a linker molecule attached to the peptide at a site within the exogenous sequence. The special technical feature of the Group II claims is a polymer molecule having a specified structure, which may optionally be covalently bonded to a peptide through a linker attached to the peptide at an N-linked glycosylation site.

The common technical element shared by the above groups is that they are related to peptides bonded to polymers via a linker attached to the peptide at an N-linked glycosylation site. This common technical element does not represent an improvement over the prior art of US 2007/0254834 A1 to Defrees et al. (see SEQ ID NO: 1, abstract, para [0194]). Therefore, the inventions of Group I and Group II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

It should be noted that the applicant is entitled to a search of SEQ ID NO: 1 without additional payment. Should the applicant elect for SEQ ID NO: 2 to be searched, payment must be made for the search of the additional invention.