

## Exercise 3

- Process for labelling of immunoglobulins, comprising a galactosyltransferase catalysed labelling of the glycans of the immunoglobulins with a C-6 modified UDP-galactose derivative.
- Labelled immunoglobulins prepared by this method.
- Process for the enzymatic preparation of UDP-6-biotinyl- $\alpha$ -D-galactose and UDP-6-biotinyl-N-acetyl- $\alpha$ -D-galactosamine starting from UDP-Glc or UDP-GlcNac, comprising combining a UDP-Glc(Nac) 4-epimerase (EC 5.1.3.2) with a galactose oxidase (EC 1.1.3.9) in a one-pot process.

### Analyze of the claims

1. Immunoglobulins+C-6 modified UDP-galactose derivative  
+ galactosyltransferase ->labelled Immunoglobulins

2. labelled Immunoglobulins prepared by claim 1.

3. UDP-Glc + 4-epimerase -> UDP-Gal

Known

+ Gal-oxidase + biotin

-> UDP-6-biotin-Gal

## Identify linking concept/feature

1. Immunoglobulins + C-6 modified UDP-galactose derivative  
+ galactosyltransferase -> labelled Immunoglobulins

2. labelled Immunoglobulins prepared by claim 1.

3. UDP-Glc + 4-epimerase -> UDP-Gal

+ Gal-oxidase + biotin

-> UDP-6-biotin-Gal

## Quick search

UDP-Gal + Gal-oxidase + biotin -> UDP-6-biotin-Gal

Strategy (NCBI PubMed and Mesh)

1. "Gal-oxidase"[Mesh]

Results 506

2. Biotin\*

Results 29243

3. #1 and #2

Results 5

PubMed History - Microsoft Internet Explorer

Address: http://www.ncbi.nlm.nih.gov/sites/entrez

NCBI PubMed A service of the U.S. National Library of Medicine and the National Institutes of Health

Search [PubMed] for [biotin\*] AND (#16) [Go] Clear Advanced Search (beta) Save Search

Limits Preview/Index History Clipboard Details

- Search History will be lost after eight hours of inactivity.
- Search numbers may not be continuous; all searches are represented.
- To save search indefinitely, click query # and select Save in My NCBI.
- To combine searches use #search, e.g., #2 AND #3 or click query # for more options.

Search	Most Recent Queries	Time	Result
#25 Search biotin*		08:47:22	29243
#24 Search (EC 1.1.3.9) AND (#22)		08:43:13	1
#23 Search EC 1.1.3.9		08:42:48	30
#22 Search UDP-galactose		08:42:00	2144
#21 Search UDPglucose UDPgalactose EC 5.1.3.2.		08:38:48	12
#20 Search UDPglucose UDPgalactose 4-Epimerase		08:38:11	375
#19 Search UDPglucose + Epimerase/chemical synthesis[Mesh]		08:35:26	1
#18 Search "Galactose Oxidase"[Mesh]		08:29:56	506
#17 Search ("Galactose Oxidase/chemical synthesis"[Mesh] OR "Galactose Oxidase/chemical synthesis"[Mesh]) AND "Galactose Oxidase"[Mesh]		08:28:07	8
#1 Search biotin* epimerase oxidase		08:14:47	1

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Display Summary Show [20] Sort By [ ] Send to [ ]

All: 5 [Review: 1]

Items 1 - 5 of 5 One page.

#1: Fortecave M, Ollagnier-de-Choudens S, Mulliez E. **Biological radical sulfur insertion reactions.** Chem Rev. 2003 Jun;103(6):2149-66. Review. No abstract available. PMID: 12797827 [PubMed - indexed for MEDLINE]

#2: Buzo T, Spornauer I, Namdjou DJ, Gutierrez Gallego R, Clausen H, Elling L. **Chemoenzymatic synthesis of biotinylated nucleotide sugars as substrates for glycosyltransferases.** Chembiochem. 2001 Dec 3;2(12):684-94. PMID: 11948877 [PubMed - indexed for MEDLINE]

#3: Diezel MR, Mau MM. **Biotin-conjugated reagents as site-specific probes of membrane protein structure: application to the study of the human erythrocyte hexose transporter.** Anal Biochem. 1990 Nov 1;190(2):297-303. PMID: 2127160 [PubMed - indexed for MEDLINE]

#4: Yabe H, Watanabe T, Kimura M, Niskane FK. **A trial of alloreactive T-cell depletion using biotinylated galactose oxidase for the prevention of acute graft-versus-host diseases.** Tokai J Exp Clin Med. 1988 Dec;13(4-5):227-38. PMID: 3256939 [PubMed - indexed for MEDLINE]

#5: Zehnb R, Orr GA. **Use of avidin-iminobiotin complexes for purifying plasma membrane proteins.** Methods Enzymol. 1986;122:87-94. No abstract available. PMID: 3702705 [PubMed - indexed for MEDLINE]

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## Analyze results

**CHEMBIOCHEM**

2001 Dec 3;2(12):884-94.

### **Chemoenzymatic Synthesis of Biotinylated Nucleotide Sugars as Substrates for Glycosyltransferases**

Thomas Bülter,<sup>[a]</sup> Thomas Schumacher,<sup>[a]</sup> Darius-Jean Namdjou,<sup>[a]</sup>  
Ricardo Gutiérrez Gallego,<sup>[b]</sup> Henrik Clausen,<sup>[c]</sup> and Lothar Elling<sup>\*[a]</sup>

The linking feature:

C-6 modified UDP-galactose derivative /or UDP-6-biotin-Gal

Lack of Unity

1. Immunoglobulins + C-6 modified UDP-galactose derivative  
+ galactosyltransferase → labelled Immunoglobulins

2. labelled Immunoglobulins prepared by claim 1.

3. UDP-Glc + 4-epimerase → UDP-Gal

+ Gal-oxidase + biotin

→ UDP-6-biotin-Gal

## Complete the search

the name of the inventor(Elling) in the closest prior art

non-Patent database  
PubMed, EBI,Google.....

Patent database  
EPO(Esp@cenet), USPTO.....

**CHEMBIOCHEM**

2001 Dec 3;2(12):884-94.

### **Chemoenzymatic Synthesis of Biotinylated Nucleotide Sugars as Substrates for Glycosyltransferases**

Thomas Bülter,<sup>[a]</sup> Thomas Schumacher,<sup>[a]</sup> Darius-Jean Namdjou,<sup>[a]</sup>  
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substrate. The presented method may be further exploited for the detection of agalactosyl glycans of glycoproteins related to diseases such as rheumatoid arthritis,<sup>[42]</sup> immunoglobulin A (IgA) nephropathy,<sup>[62]</sup> and breast cancer. A diagnostic marker for patients with rheumatoid arthritis are agalactosyl complex type N-glycans of immunoglobulin G (IgG).<sup>[42, 63]</sup> Patients with IgA

1. Immunoglobulins+C-6 modified UDP-galactose derivative  
+ galactosyltransferase ->labelled Immunoglobulins
2. labelled Immunoglobulins prepared by claim 1.

Claim 1 Lack of Novelty

Claim 2 Lack of Novelty

Thank you for your patience

Jungtae Kim  
Yi Suo  
Nathamon Choedsook  
Maria, Chia-Ying Wu

