LIFE SCIENCE

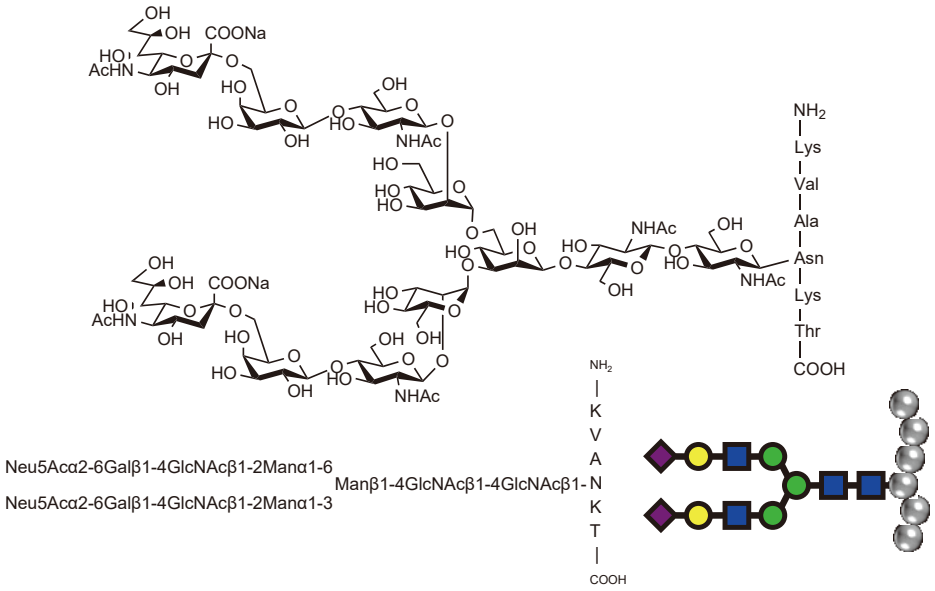
TCI

Sialylglycopeptide(SGP) and Disialyloctasaccharide

Structure-defined human-type *N*-glycans indispensable for glycoscience are available at affordable prices.

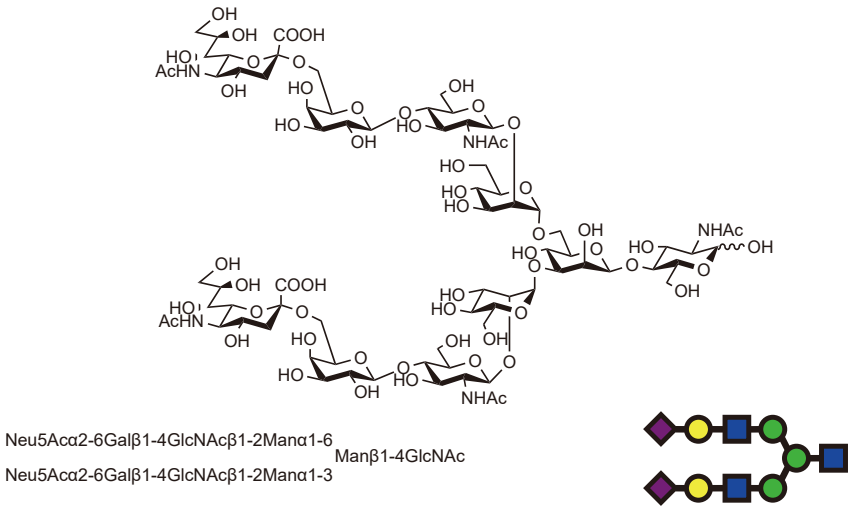
Sialylglycopeptide (SGP)

Sialylglycopeptide (= SGP) 10mg [S0523]



Disialyloctasaccharide

Disialyloctasaccharide 10mg [D4065]



Structure-defined Sialylglycopeptide (SGP) and Disialyloctasaccharide can be offered at high purity (HPLC >95%).

Please contact us about a request of several hundred grams-scale production.

Practical example: Oligosaccharide remodeling of *N*-glycan on antibodies with 2 types of oligosaccharide donors and Endo-M related enzymes

A. Structure-defined oligosaccharide donor

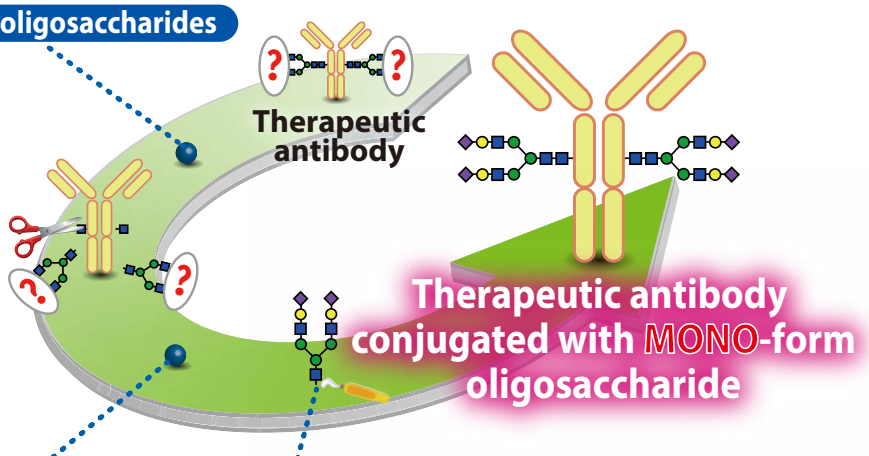
By chemoenzymatic glycoengineering with Endo-M and Glycosynthase, heterogenous *N*-linked oligosaccharides attached to an antibody are replaced by a fine-defined oligosaccharide with focused substrate specificity toward non-corefucosylated biantennary *N*-glycan.[Ref.7]
First, Endo-M hydrolyses a glycoside-bond of chitobiose included in *N*-glycan of IgG via an oxazoline intermediate, which subsequently exposes the innermost GlcNAc residue. Next, Glycosynthase bearing a point-mutation on Endo-M performs transglycosylation targeting the GlcNAc residue. The oligosaccharide donors, not only the activated Disialyloctasaccharide with oxazoline formation[Ref.8] but also the Sialylglycopeptide (SGP), are available to the oligosaccharide remodeling.[Ref.9]

(The reaction for oligosaccharide remodeling toward IgG with Endo-M and Glycosynthase is conducted under non-reducing conditions, whereas the analysis is performed under reducing conditions with denaturing.)

Endo-M (= *endo*-β-*N*-Acetylglucosaminidase) [A1651]

? : Multi-form oligosaccharides

Cleavage of multi-form oligosaccharides



Coupling of MONO-form oligosaccharide

Glycosynthase (= Endo-M-N175Q) [G0365]

Disialyloctasaccharide [D4065]

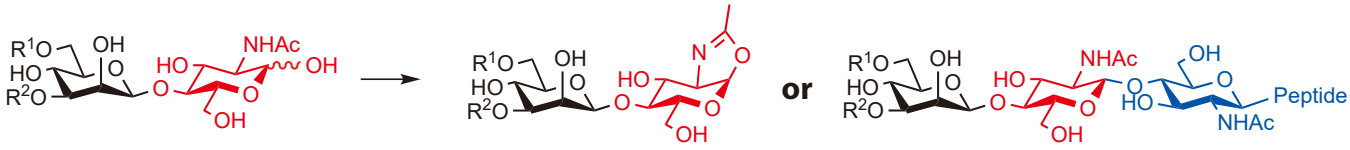
Activation is required to form the oxazoline intermediate.

or

Sialylglycopeptide (= SGP) [S0523]

This donor is available as is.

Structures of reducing terminals



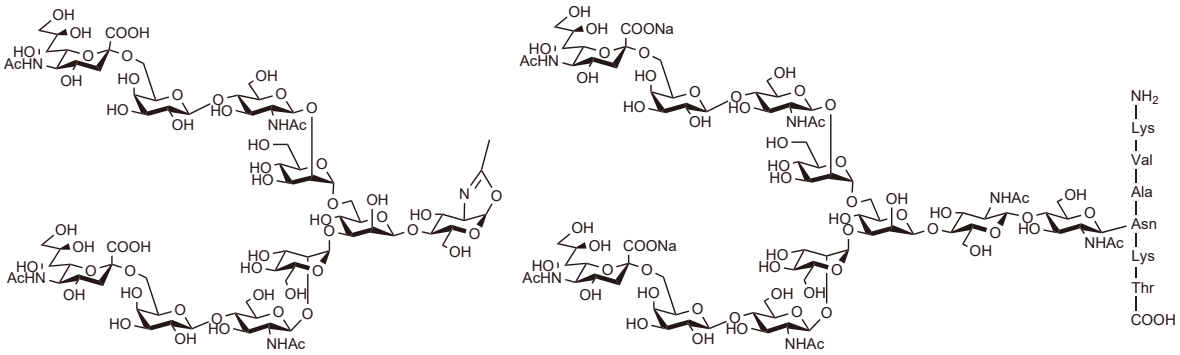
Disialyloctasaccharide → (Oxazolination) **Donor: Oxazoline-type**

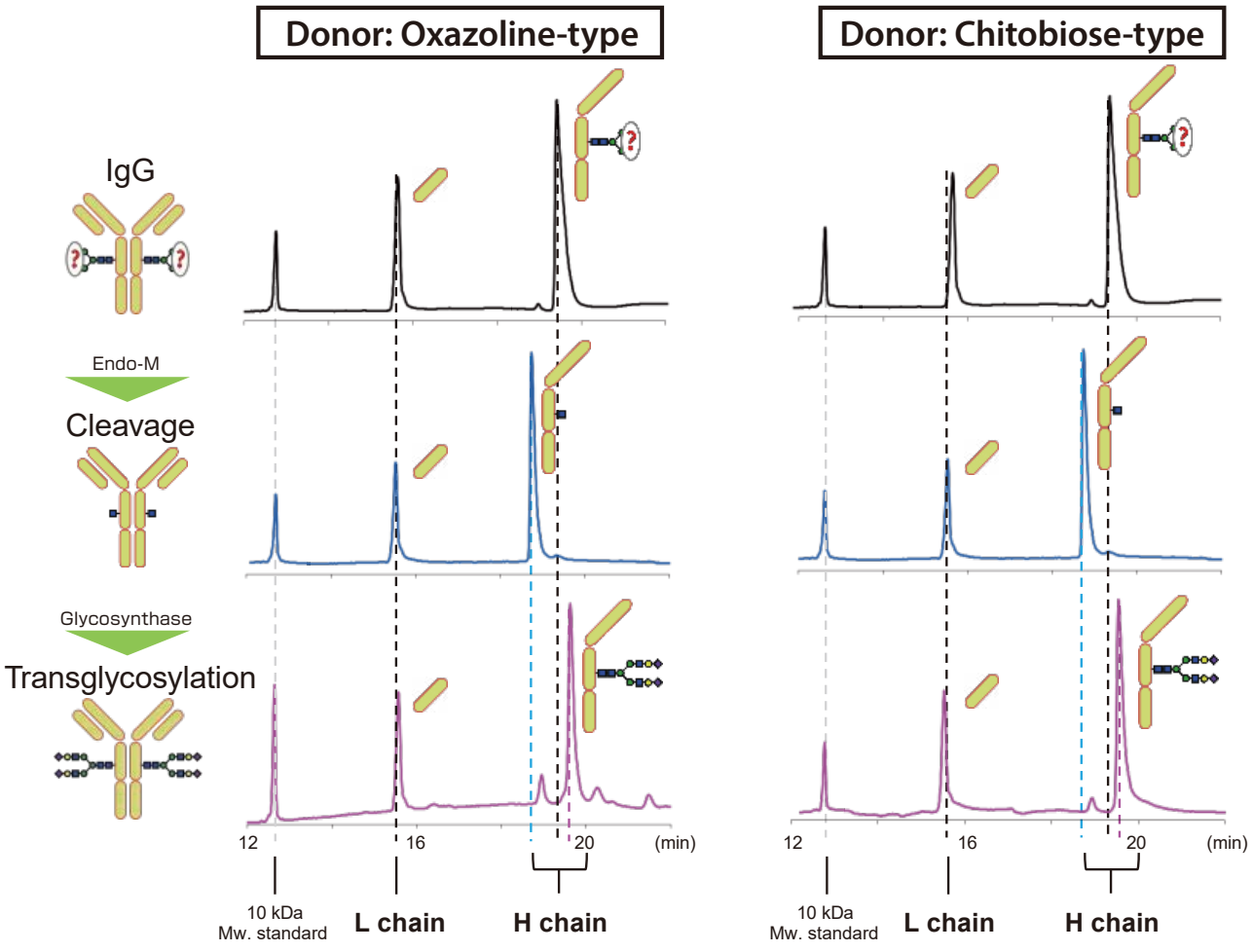
or

Sialylglycopeptide **Donor: Chitobiose-type**

B. Verification of oligosaccharide replacement with a non-fucosylated *N*-glycan attached to a heavy chain of antibody via capillary electrophoresis

Following the hydrolysis reaction for an *N*-glycan attached antibody (IgG) by Endo-M, the size reduction of the heavy chain can be verified via capillary electrophoresis (Blue line).
Next, the coupling between two types of sialylated oligosaccharide donors and a GlcNAc residue remaining on the antibody is conducted by Glycosynthase. Using an activated oxazoline donor derived from an oxazolinated Disialyloctasaccharide, would result in high reactivity. However, non-specific additional incorporation of an oxazoline-activated Disialyloctasaccharide onto any amino acid residue can be observed (Red line left).
Alternatively, the non-specific incorporation of oligosaccharides is not found with SGP donors (Red line right).
Finally, after the coupling of a uniform oligosaccharide to the GlcNAc-exposed antibody, a peak shift in the heavy chain is observed whereas it is not for the light chain.

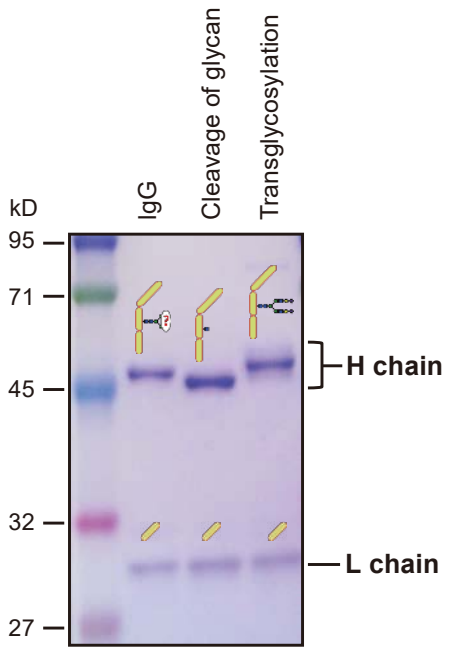




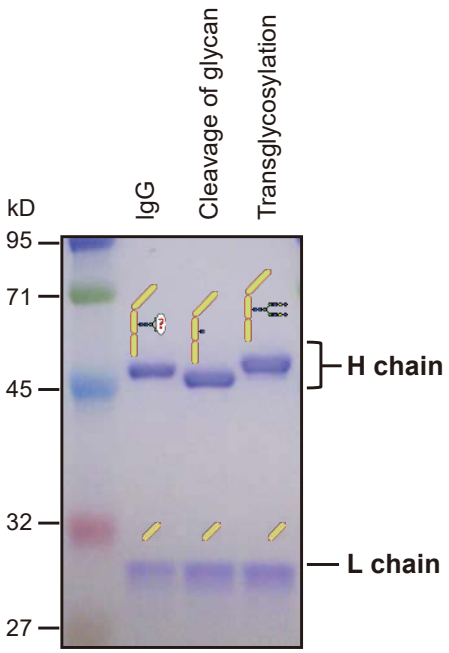
Capillary electrophoresis (under reducing condition)
[BECKMAN COULTER PA 800]
Detection : UV 214 nm

C. Verification of the heavy chain size by SDS-PAGE

Size reduction of the heavy chain is observed when an *N*-glycan attached to the heavy chain is hydrolyzed by Endo-M treatment. With regards to transglycosylation by Glycosynthase, two types of sialylated donors resulted in a larger molecular size compared with original IgG from capillary electrophoresis. A peak shift of the heavy chain is observed but not the light chain.



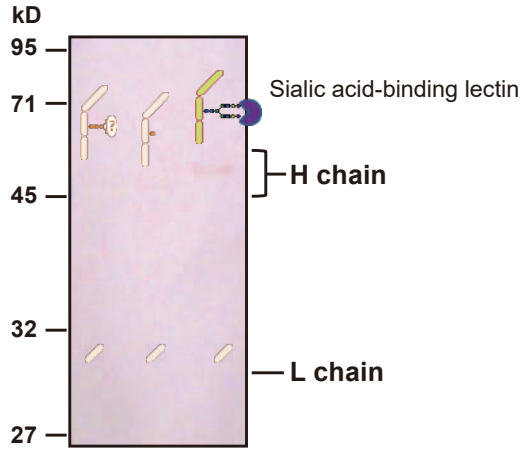
Detection : CBB staining



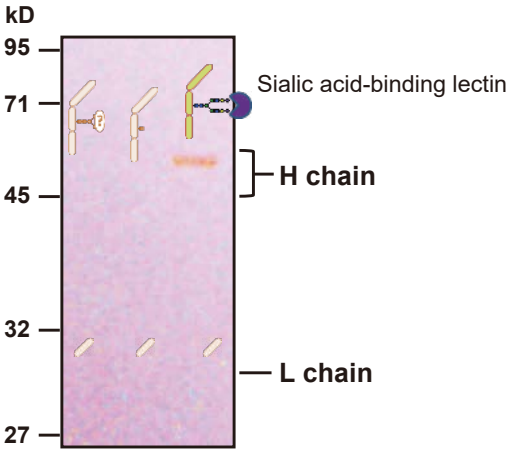
Detection : CBB staining

D. Detecting incorporation of sialylated oligosaccharides into Endo-M-treated antibody by lectin-blotting

Incorporation of the sialylated oligosaccharide is validated by lectin-blotting with a sialic acid-binding lectin (SSA: *Sambucus sieboldiana* agglutinin). Only the heavy chain of the transglycosylated antibody appears to exhibit susceptibility to SSA.



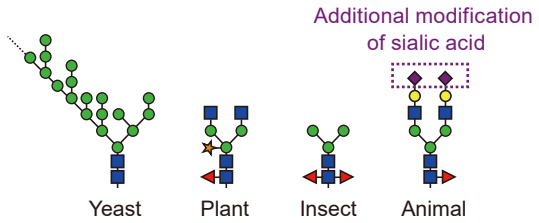
Detection : SSA-biotin + StAv-HRP
(DAB HRP substrate)

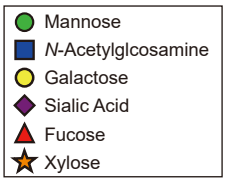


Detection : SSA-biotin + StAv-HRP
(DAB HRP substrate)

Biosynthesis and *N*-glycan

Oligosaccharide attachment during post-translational modification in protein biosynthesis is a ubiquitous biological process. While *N*-linked oligosaccharides are found in various eukaryotes, their oligosaccharide structures are of great diversity and heterogeneity between different organisms.[Ref.1] Unfortunately, this heterogeneity is difficult to replicate and heterogeneity for (for example) biotechnology-based medical remains a challenging problem to resolve.





Function of human-type *N*-glycan

Sialylated *N*-glycan is a typical human-type glycan that is suggested to be strongly correlated with various physiological phenomena:

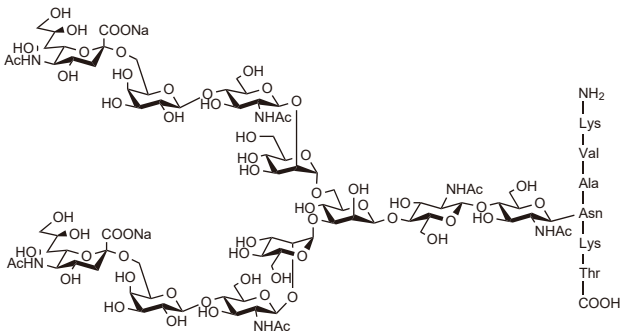
- Viral infection (by human influenzae, etc.) [Ref.2]
- Delayed blood glycoprotein half-life (owing to hindered clearance by the hepatic asialoglycoprotein receptor) [Ref.3]
- Anti-inflammatory activity with sialylated *N*-glycan of the IgG Fc region (via endogenous immune-receptors such as DC-SIGN, etc.)[Ref.4]
- Immunoregulation caused by endogenous lectins (such as Siglecs which preferentially bind to sialylated oligosaccharides) [Ref.5]
- Relevance to stability of biopharmaceuticals (e.g. erythropoietin: EPO) [Ref.6]

Sialylated *N*-glycan may contribute to various applications:

- Improvement of biophamaceutical function (via oligosaccharide remodeling)
- Virus scavenger (based on a matrix of conjugated oligosaccharides)
- Drug delivery systems (as an oligosaccharide-immobilized DDS)
- Oligosaccharide analysis of biological specimens and biophamaceuticals (internal or external standard)
- Stabilization of proteins (improved solubility)
- General Glycoscience (glycosidase, competitive inhibitors and etc.)

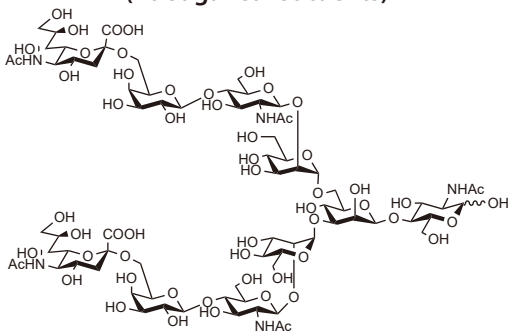
Sialylglycopeptide (SGP)

Glycopeptide containing sialic acids at non-reducing terminal (11 sugar constituents)



Disialyloctasaccharide

Oligosaccharide containing sialic acids at non-reducing terminal (10 sugar constituents)



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Related Products

[Reagent for Oxazolination]

2-Chloro-1,3-dimethylimidazolinium Chloride	5g / 25g	[C1408]

[Related Oligosaccharides]

Disialylnonasaccharide-β-*p*NP	1mg	[N0913]
Disialylnonasaccharide-β-Ethylazide	1mg	[D4217]
DNS-SGN	1mg	[D3690]
Neu5Acα(2-6) *N*-Glycan	1mg	[N1065]
Neu5Acα(2-6) *N*-Glycan 2AB	500pmol/vial	[N1073]
G2-peptide	5mg	[G0466]
G2 Glycan	1mg	[G0487]
G2 2AB	500pmol/vial	[G0493]

[Endo-M and Related-Reagents]

MANT-M3GN2-DNP (= MM3D)	1mg	[M3174]
Endo-M (= *endo*-β-*N*-Acetylglucosaminidase) from *Mucor hiemalis* expressed in *Candida boidinii*	100m units/vial	[A1651]
Glycosynthase (= Endo-M-N175Q) from *Mucor hiemalis* expressed in *Escherichia coli*	100m units/vial	[G0365]
Endo-M-W251N from *Mucor hiemalis* expressed in *Escherichia coli*	100m units/vial	[E1339]
Anti-Endo-M Polyclonal Antibody	0.2mg/vial	[A2958]
Anti-Endo-M Polyclonal Antibody Biotin Conjugate	0.2mg/vial	[A2959]

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