Specific crosslinking of cell adhesive molecules by heterobifunctional glycopeptide synthesised on the basis of chemoenzymatic strategy

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Heterobifunctional glycopeptide 1 composed of Neu5Acα-(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAc (sialyl Lewis^x) and Lys-Gly-Arg-Gly-Asp-Ser (KGRGDS) having specific activity to bind concurrently with two different types of cell adhesive molecules such as selectins and integrins was synthesised on the basis of a combined chemical and enzymatic strategy.

It has been well documented that many kinds of cell adhesive molecules (CAMs) participate in a variety of biological phenomena such as cell–cell interactions and cell–extracellular matrix (ECM) interactions.[1–6] It should also be noted that collaboration of multiple and complex bindings between different types of cell surface receptors and their ligands greatly contribute to the construction of stable and specific cellular society.

In the present study, we designed a heterobifunctional glycopeptide **1** as a novel and potential glycoligand, because this artificial glycopeptide composed of Neu5Acα(2→3)Galβ≙(1→4)[Fucα(1→3)]GlcNAc (sialyl Lewis^x) and Lys-Gly-Arg-Gly-Asp-Ser (KGRGDS) was supposed to interact with two different types of families of cell adhesive proteins such as selectins and integrins concurrently. This type of synthetic heterobifunctional glycopeptide is strongly expected to become a novel class of antiflammatory and anticancer 'glycodrugs'.[7–9] Kunz *et al*. have reported a significant '*cis*' type interaction of the synthetic RGDS-sialyl Lewis^x with P-selectin as an IC_{50} value of 26 μM. In addition to this type of enhanced affinity of glycoligand with protein, we found an interesting '*trans*' type interaction of a novel glycoligand **1** involving a flexible peptide linker with two different protein receptors. Fig. 1 summarises some plausible mechanisms of '*cis*' and '*trans*' type interactions of glycoligand **1** with cell adhesive proteins.

Scheme 1 indicates a retrosynthetic route for compound **1**. Firstly, a key intermediate **2** was prepared by chemical synthesis from a simple *n*-pentenyl glycoside of *N*-acetyllactosamine **5**[10] and tetrapeptide derivative **4** as starting materials (Scheme 2). The terminal double bond of compound **5** was converted into a carboxy group by treating with potassium permanganate in 87% yield and the active ester of **6** was employed for the coupling reaction with the ε-amino group of Z-lysine to afford **7** in 71% yield. To achieve satisfactory flexibility in the spacer-arm between sialyl Lewis^x and RGDS and versatility in further chemical manipulation, H-Gly-OBn was combined with **7** to give *N*-acetyllactosamine-dipeptide derivative **8** in 83% yield. Consequently, deprotection of the benzyl ester and *O*-acetyl groups of **8** under alkaline conditions was allowed to proceed smoothly and gave compound **3** in 87% yield without racemization. Coupling reaction of the glycopeptide **3** with an unprotected disaccharide moiety with an amino-terminus generated from RGDS derivative **4** was carried out by employing diphenylphosphoryl azide[11] as a selective promoter of the carboxy group of the glycopeptide. A hexapeptide derivative **9** was obtained in 77% yield with no side reaction at the free hydroxy groups of the carbohydrate moiety. Removal of all

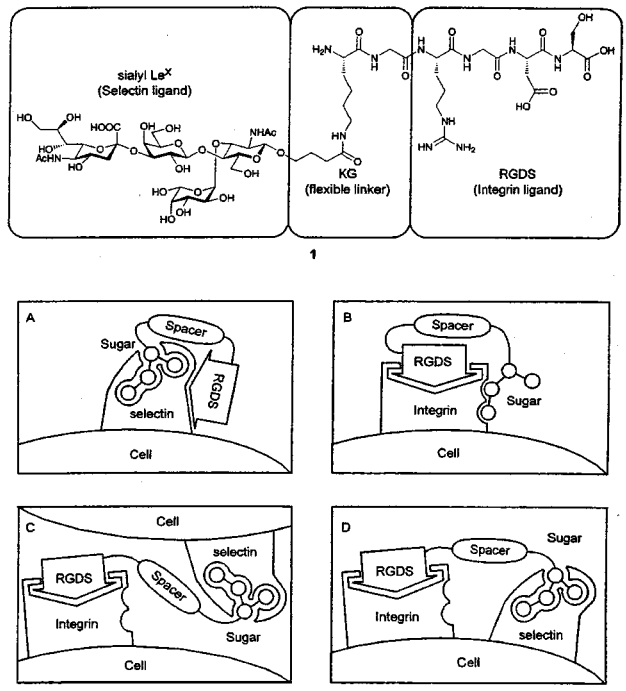
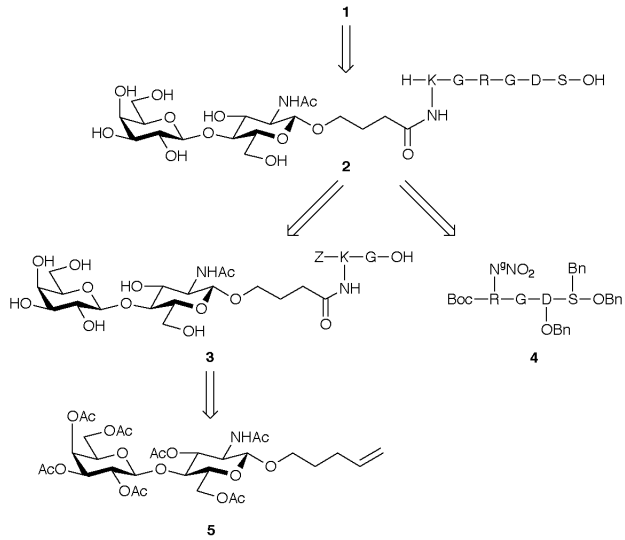
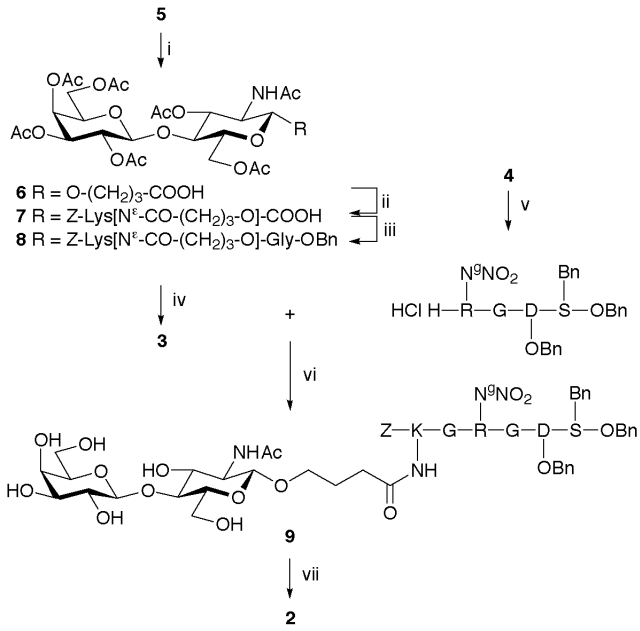


Fig. 1 Chemical structure of **1** and plausible mechanisms of binding of **1** with receptor molecules. (A) and (B) represent cooperative-type '*cis*' interactions, and (C) and (D) represent crosslinking-type '*trans*' interactions (crosslinking by glycopeptide), respectively.



Scheme 1 Retrosynthetic route to cell adhesive glycopeptide **1** (CAGP **1**).

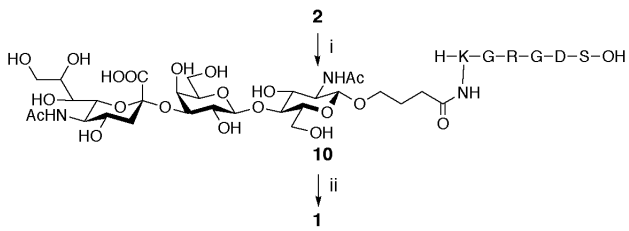


Scheme 2 *Reagents and conditions*: i, $KMnO_4$, AcOH aq., rt, 87%; ii, HONSu, DCC, DMF, 0 °C→rt, then Z-Lys, TEA, DMF–H_2O (9:1), rt, 71%; iii, DPPA, Gly-OBn, TEA, DMF, 0 °C→rt, 83%; iv, NaOMe, dry MeOH, rt, then 1 M NaOH aq., rt, ion exchange resin (Dowex 50W-X8), 87%; v, 2 M HCl–dioxane, 0 °C→rt; vi, DPPA, TEA, DMF, 0 °C→rt, 77% (over all yield from **4**); vii, Pd/C, H_2 gas, MeOH–H_2O, (1:1) rt, 99%.

protecting groups of the peptide moiety of **9** such as benzyl, nitro, and benzyloxycarbonyl groups was performed by standard hydrogenation in the presence of palladium on charcoal to give the intermediate **2** in 92% yield.

Subsequently, the glycopeptide **2** was employed as a substrate for the corresponding glycosyltransferases. It is of interest to examine the substrate specificity of the enzymatic glycosyltransfer reactions against non-natural glycopeptide **2**, since acceptability of synthetic glycoconjugates to the glycosyltransferases depends strongly on the nature of aglycons or supporting backbones.[12] In fact, a synthetic hexapeptide bearing an *N*-acetyllactosamine side chain through an appropriate hydrophobic linker (**2**) was proved to be an excellent substrate both for α(2"→3)sialyltransferase and for α(1→3)fucosyltransferase. The sialylation and fucosylation of compound **2** proceeded smoothly and glycopeptide **1** was isolated by preparative HPLC in 45% overall yield from **2** (Scheme 3).

The effect of hybridisation of the functional carbohydrate and peptide on the '*cis*' type interaction between glycoligand **1** and each protein was investigated by surface plasmon resonance (SPR). As anticipated, compound **1** binds P- and E-selectins more strongly than native sialyl Lewis^x, and association constants of **1** with P- and E-selectins were assumed to be 6.6×10^7 M^{-1} and 4.5×10^5 M^{-1}, respectively. It was also found that **1** inhibited interaction of human integrin β_1 with its monoclonal antibody more effectively than RGDS (IC_{50} = 0.55 mM).



Scheme 3 *Reagents and conditions*: i, **2** (20 mg), CMP-Neu5Ac (25 mg), α-2,3-sialyltransferase (0.3 unit), calf intestine alkaline phosphatase (20 unit), BSA (10 mg), $MnCl_2 \cdot 4H_2O$, Triton CF-54, 50 mM sodium cacodylate buffer (2.0 mL, pH 7.4), 37 °C, 3 d, 80%; ii, **10** (15 mg), GDP-Fuc (10.3 mg), α-1,3-fucosyltranseferase V (10 munit), calf intestine alkaline phosphatase (1 unit), NaN_3, $MnCl_2 \cdot 4H_2O$, 100 mM HEPES buffer (1.0 mL, pH 7.5), 37 °C, 3 d, 56%.

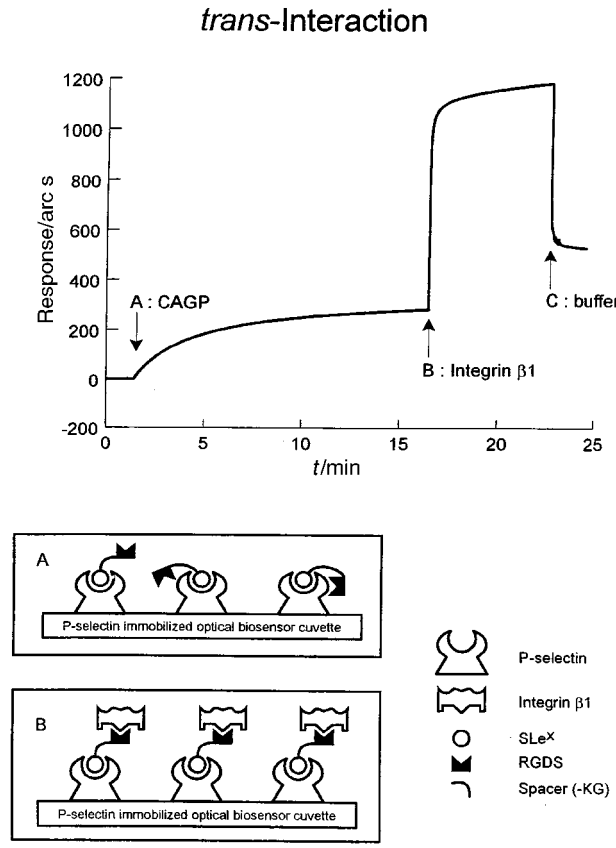


Fig. 2 Crosslinking of selectin and integrin by cell adhesive glycopeptide (CAGP) monitored by SPR method. The first arrow 'A' indicates the time point of the beginning of the injection of cell adhesive glycopeptide (20 μL, 1.3 mg mL^{-1}) to the P-selectin immobilised cuvette. The second arrow 'B' indicates the time point of the injection of integrin β1 (20 μL, 9.1 μg mL^{-1}) to this cuvette. The third arrow 'C' indicates the beginning of the buffer washout (200 μL of phosphate buffer solution). Integrin β1 was found to bind to CAGP-P-selectin complex as indicated by a significant increase in the response unit.

These successful results of cooperative '*cis*' interactions prompted us to examine the artificial crosslinking of selectin and integrin by the synthetic glycoligand. As indicated in Fig. 2, glycopeptide **1** exhibited the specific capacity to bind P-selectin and human integrin β_1 concurrently. We also have evidence that **1** could block the integrin-mediated adhesion of activated T cells to a collagen-coated plate at the same level as inhibition by a monoclonal antibody to integrin. Therefore, it is strongly suggested that synthetic glycopeptide **1** will become an effective practical tool, 'cell adhesive glycopeptide (CAGP)', to modulate immune responses through its inhibitory effect on lymphocyte-endothelial cell interaction.

Notes and references

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