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Chemoselectively Template-Assembled Glycoconjugates as Mimics for Multivalent Presentation of Carbohydrates

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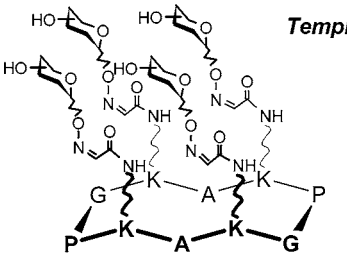
ABSTRACT



Template Assembled Glycopeptides

9	RAFT(βGlc)$_4$
10	RAFT(βGal)$_4$
11	RAFT(βGalNAc)$_4$
17	RAFT(αGlc)$_4$
18	RAFT(αGal)$_4$
19	RAFT(αGalNAc)$_4$

We report the highly efficient preparation of well-defined tetravalent glycoconjugates as potential tools for cell targeting and cell-surface mimics. Our strategy is based on chemoselective assembly of aminooxy carbohydrates to a cyclic decapeptide template presenting aldehydes, namely, a "regioselectively addressable functionalized template" (RAFT). Preliminary recognition assays of tetramannosyl glycoconjugate (20) with specific lectin (Concanavalin A) using the fluorescence anisotropy method are also reported.

Carbohydrates have become a subject of increasing interest during the past few years. They are widely expressed on cell surfaces where they play a crucial role in various biological recognition phenomena such as fertilization, cell adhesion, tissue formation, antigen/antibody interactions, cancer metastasis, and infection of viruses or bacteria.[1] A large number of these processes involve interactions between sugars and oligomeric biological receptors.[2] Particularly, some carbohydrate-binding proteins called lectins[3] contain two or more specific sugar-combining sites and comprise a large family of recognition molecules of the immune system. Despite the weak affinity between lectins and monosaccharides (K_d in the millimolar range), sugar/protein interactions prove to be highly efficient and specific due to multivalent events commonly known as the "glycoside cluster effect".[4]

Access to well-defined scaffolds presenting carbohydrates-based recognition elements to investigate these biological processes is essential.[5] In recent years, major advances in synthetic methodology have been accomplished for preparation of various kinds of glycosylated polymers,[6] cyclodextrin or calixarene-based glycoclusters,[7] glycodendrimers,[8] and glycocyclopeptides[9] or glycopeptides[10] as useful biorecog-

(1) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720.
(2) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754−2794.
(3) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674.
(4) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327. (b) Lundquist, J. J.; Toone, E. J. *Chem Rev.* **2002**, *102*, 555−578.
(5) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922−14933.
(6) Nishimura, S.-I.; Lee, Y. C. Synthetic Glycopolymers: New Tools for Glycobiology. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitriu, S., Ed.; Marcel Dekker: New York, 1998; pp 523−537.
(7) Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem.* **2001**, *12*, 655−672.
(8) (a) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692−702. (b) Zanini, D.; Roy, R. *Bioconjugate Chem.* **1997**, *8*, 187−192.
(9) Sprengard, U.; Schudok, M.; Kretzschmar, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 321−324.
(10) Veprek, P.; Jezek, J. *J. Peptide Sci.* **1999**, *5*, 5−23.

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nizable materials for therapeutic or medicinal applications (e.g., diagnosis and a synthetic anticancer vaccine). In this context, we have developed here a convenient method for chemical synthesis of topological peptide scaffolds presenting multiple carbohydrate recognition elements **9**−**12** and **17**−**20** as new potential tools for cell targeting and cell-surface mimics.

Previously, lysine-containing cyclodecapeptides called "regioselectively addressable functionalized templates" (RAFTs)[11] were described as stable scaffolds for de novo design of proteins or as peptidomimics.[12] Thanks to the lysine side-chain addressable sites, this topological template may provide an interesting framework to manipulate multivalent presentation of carbohydrates.[13] In addition, we selected the oxime bond for chemical attachments of carbohydrates as this chemoselective method has proved to be effective for glycoconjugate access[14] compared to the traditional method.[15] Following this, we report in this paper the facile synthesis of well-defined template-assembled glycoconjugates **9**−**12** and **17**−**20** by chemoselective assembly of various carbohydrates containing an aminooxy group at their anomeric position[16] to RAFT molecule **4** containing glyoxyl-aldehydes on its four lysine side-chains (Figure 1).

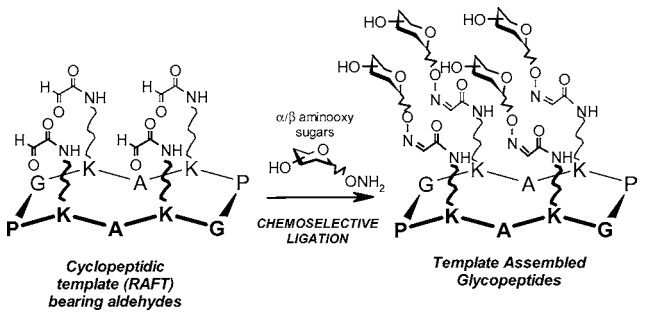
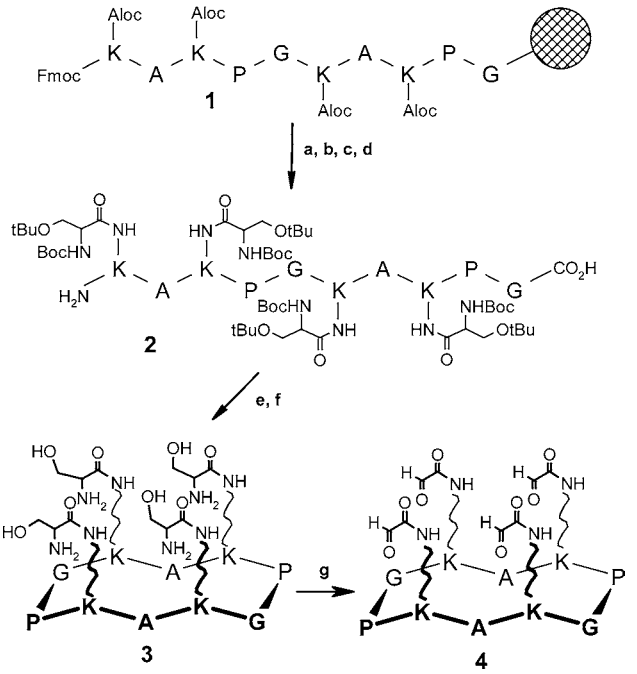


Figure 1. Synthesis of template-assembled glycopeptides.

The cyclopeptide template **4** containing aldehydes is prepared by combined solid-phase and solution strategy. The synthetic route followed is depicted in Scheme 1. Therein, linear peptide **2** is assembled from highly acid-labile *o*-chlorotrityl chloride resin following standard Fmoc/tBu solid-phase chemistry starting with a glycine residue to prevent epimerization during the cyclization reaction. Four lysine residues bearing Aloc protecting groups at the amino side chain are incorporated into the chain of peptide **1** during the elongation on the support. At the end of the peptide chain elongation, removal of Aloc is achieved using the typical procedure[17] with a catalytic amount of $Pd(Ph_3P)_4$ and a large excess of $PhSiH_3$ in CH_2Cl_2 (2 × 30 min cycle) under nitrogen gas.

Scheme 1. Synthesis of a RAFT Presenting Aldehyde **4**[a]



[a] Reaction conditons: (a) $PhSiH_3$, $Pd(Ph_3P)_4$, CH_2Cl_2 (90%); (b) BocSer(OtBu), PyBOP, DIEA, DMF (60%); (c) piperidine 20%/DMF (95%); (d) 1/1/8 ACOH/TFE/CH_2Cl_2; (e) PyBOP, DIEA, CH_2Cl_2 (90%); (f) TFA (90%); (g) $NaIO_4$, AcONa buffer pH 4.0 (83%).

Coupling of BocSer(OtBu) using standard conditions followed by N-terminal Fmoc deprotection with piperidine (20% v/v in DMF) and subsequent cleavage from resin in very mild acidic conditions (1/1/8 AcOH/TFE/DCM) afforded linear peptide **2**.[18] The cyclization of peptide **2** is readily performed "head-to-tail" by treatment with 1.1 equiv of PyBOP and 5 equiv of DIEA in CH_2Cl_2 at high dilution (0.5 mM). After 30 min at room temperature, the corresponding cyclodecapeptide is obtained as a white powder with 90% yield after evaporation and precipitation from diethyl ether. Further Boc and tBu removal from the serine residue with TFA gives pure peptide **3** containing four unprotected serines as masked glyoxo-aldehyde functions.[19] Finally, the oxidative cleavage with sodium periodate of amino-alcohol moiety from serine provides the desired

(11) Dumy, P.; Eggelston, I. M.; Cervigni, S.; Sila, U.; Sun, X.; Mutter, M. *Tetrahedron Lett.* **1995**, *36*, 1255−1258.

(12) (a) Mutter, M.; Dumy, P.; Garrouste, P.; Lehmann, C.; Mathieu, M.; Peggion, C.; Peluso, S.; Razaname, A.; Tuchscherer, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1482−1485. (b) Peluso, S.; Dumy, P.; Nkubana, C.; Yokokawa, Y.; Mutter, M. *J. Org. Chem.* **1999**, *64*, 7114−7120.

(13) (a) Dumy, P.; Eggleston, I. M.; Esposito, G.; Nicula, S.; Mutter, M. *Biopolymer* **1996**, *39*, 297−308. (b) Peluso, S.; Rückle, T.; Lehmann, C.; Mutter, M.; Peggion, C.; Crisma, M. *ChemBioChem* **2001**, *2*, 432−437.

(14) For a recent review, see: Hang, H.; Bertozzi, C. R. *Acc. Chem. Res*. **2001**, *34*, 727−736.

(15) For recent reviews, see: (a) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495−4537. (b) Seitz, O. *ChemBioChem* **2000**, *1*, 214−246. (c) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579−601.

(16) Renaudet, O.; Dumy, P. *Tetrahedron Lett.* **2001**, *42*, 7575−7578.

(17) Thieriet, N.; Alsina, J.; Girald, E.; Guibé, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275−7278.

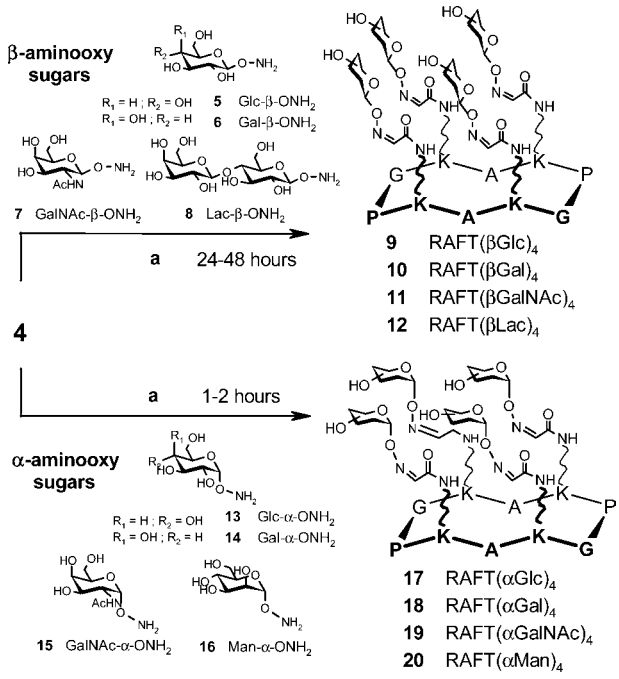
(18) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513−520.

(19) Geoghegan, K. F.; Stroh, J. G. *Bioconjugate Chem.* **1992**, *3*, 138−146.

glyoxyaldehyde RAFT(CHO)$_4$ **4** in 30 min with 83% yield after semipreparative HPLC purification.

The final chemoselective oxime bond formation is carried out between aldehyde compound **4** and various aminooxy-lated-carbohydrates **5**−**8** and **13**−**16** as described for the preparation of carbohydrate oligonucleotide conjugates (COCs).[20] The latter compounds were readily prepared through glycosylation with *N*-hydroxyphthalimide and subsequent deprotection by methylhydrazinolyse.[16] As a typical chemoselective conjugation procedure, the reaction occurs easily at room temperature with 4−8 equiv of sugars **5**−**8** or **13**−**16** and RAFT(CHO)$_4$ **4** in aqueous conditions, without any other chemical manipulation (Scheme 2).

Scheme 2. Chemoselective Incorporation of Aminooxy Carbohydrate Moieties into a RAFT Molecule[a]



[a] Reaction conditons: (a) 0.1 M AcONa buffer, pH 4.0 (70−80%).

The progress of the reaction is monitored by reverse-phase HPLC (Figure 2). The appearance of each glycoconjugate is observed at 214 and 250 nm (absorbance of the peptide bond as well as the glyoxylic oxime linkage). A difference of reactivity is noticed between α and *β* sugars; conjugation with *β*-aminooxy sugars generally required up to 48 h to be completed (Figure 2, C). Several peaks are detected at an intermediate time (Figure 2, B), which may correspond to an expected mixture of the corresponding mono-, di-, tri- and tetraconjugates. No difference of reactivity is found between monosaccharide and disaccharide lactose. Conversely, with α-aminooxy sugars, formation of glycoconjugates is much faster because the chemoselective ligation is achieved after 2 h under the same experimental conditions (Figure 2, D). In any case, very clean crude reaction mixtures are observed, confirming the high efficiency of this reaction.

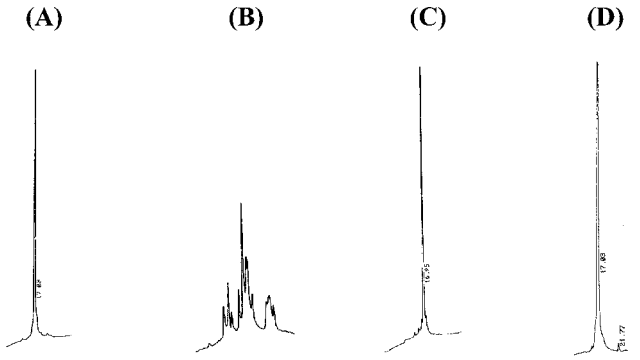
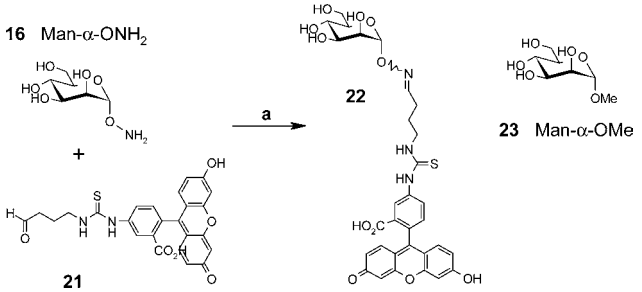


Figure 2. HPLC profiles (detection at 214 nm) of (**A**) the starting RAFT(CHO)$_4$ **4**, (**B**) a crude mixture of the ligation between RAFT-(CHO)$_4$ **4** and Gal-*β*-ONH$_2$ **6** after 2 h, (**C**) a crude mixture of the ligation between RAFT(CHO)$_4$ **4** and Gal-*β*-ONH$_2$ **6** after 24 h, and (**D**) a crude mixture of the ligation between RAFT(CHO)$_4$ **4** and Glc-α-ONH$_2$ **13** after 2 h.

Stable glycoconjugates **9**−**12** and **17**−**20** are finally isolated in almost 80% yield after semipreparative HPLC purification. These were fully characterized by ES-MS. Moreover, NMR studies of compound **20** confirm its structure and prove the unique and homogeneous cis configuration of the oxime linkages.[21]

To inspect the affinity of our multivalent ligands with specific carbohydrate-binding proteins, preliminary recognition assays were performed using a fluorescence anisotropy method, which is based on the detection of changes in the anisotropy of fluorescence (*r*) of a labeled carbohydrate ligand.[22] To this end, we have chosen to determine the binding affinity of tetramannosyl peptide **20** for well-known Concanavalin A (Con A), which is a tetrameric α-mannose-specific plant lectin.[23] As a model fluorescent ligand, fluoromannosyl **22** was prepared by chemoselective ligation between mannose α-aminooxy **16** and the fluorescein presenting aldehyde linker **21** (Scheme 3).[24]

Scheme 3. Synthesis of Fluorescent α-Mannosyl **22**



[a] Reaction conditons: (a) 0.1 M AcONa buffer, pH 4.0 (90%).

(20) (a) Forget, D.; Renaudet, O.; Defrancq, E.; Dumy, P. *Tetrahedron Lett.* **2001**, *42*, 7829−7832. (b) Forget, D.; Renaudet, O.; Boturyn, D.; Defrancq, E.; Dumy, P. *Tetrahedron Lett.* **2001**, *42*, 9171−9174.

After it was demonstrated that fluorescent compound **22** interacts specifically with carbohydrate binding sites of Con A,[25] competition experiments were performed using tetramannosyl glycopeptide **20** and commercially available methyl α-D-mannopyranoside **23**.

After additions of inhibitor solution (3.5 mM for **20** and 13 mM for **23**)[26] to labeled compound **22** bound to Con A,[25] the decrease in *r* is measured. Inhibition profiles thus obtained are shown in Figure 3. It is worth mentioning that

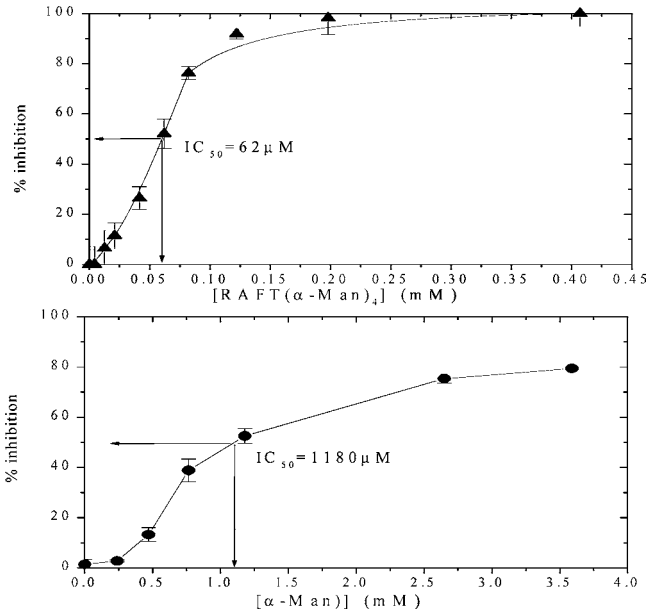


Figure 3. Fluorescence anisotropy competition experiments: inhibition of binding Con A/fluorescent α-mannosyl **22** versus the concentration of tetramannosyl glycopeptide **20** (top) and methyl α-D-mannopyranoside **23** (bottom) as an inhibitor.

compound **20**, presenting a mannose cluster, released the fluorescent mannose probe from Con A completely, thus indicating a complete inhibition of the binding process. In sharp contrast, entire displacement of the complex was not achieved with α-methyl mannose despite the use of a 10-fold more concentrated solution of this inhibitor. IC_{50} values can be thus estimated to a value of 1.2 mM for compound **23** and 20-fold less for compound **20** (62 μM). This binding enhancement toward Con A observed with our template-assembled mannose conjugate **20** compared to the monovalent mannose is consistent with a glycoside cluster effect resulting from multivalent presentation of the ligand by the template.

In conclusion, we have developed a convenient route for preparation of well-defined glycopeptides presenting multiple copies of carbohydrate recognition motifs.

Furthermore, the use of a topological template having defined faces[13] may offer advantages for the oriented presentation of carbohydrate recognition elements compared to dendrimer or linear peptide scaffolds.[7,9] Therefore, these compounds may represent useful tools for studying protein−carbohydrate interactions as well as for vectorization and cell-targeting systems. The chemoselective assembly of more complex glycoconjugates combining arrays of carbohydrates and peptides is currently being investigated in our laboratory and will be reported in due time.

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Supporting Information Available: Experimental procedure for chemoselective reactions and structural characterization data of glycopeptides **9**−**12** and **17**−**20** and of fluorescent mannosyl **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) ES-MS and NMR data obtained during structural studies are given in Supporting Information.

(22) (a) Weatherman, R. V.; Kiessling, L. L. *J. Org. Chem.* **1996**, *61*, 534−538. (b) Seethala, R. *Methods* **2000**, *22*, 61−70.

(23) Bittiger, H.; Schnebli, H. P. *Concanavalin A as a Tool*; Wiley & Sons: London, 1976.

(24) Trévisiol, E.; Defrancq, E.; Lhomme, J.; Laayoun, A.; Cros, P. *Eur. J. Org. Chem.* **2000**, 211−217.

(25) Our results are consistent with binding experiments described by Kiessling[19a] using a fluorescent mannose derivative ($\Delta G° \approx 5$ kcal/mol ± 0.5 for compound **22**).

(26) Experimental values of the anisotropy of fluorescence were measured at 23 °C with a Perkin-Elmer Fluorescence Data Manager LS50. Competition titration experiments were performed using 150 μM concanavalin A in 0.1 M HEPES buffer, pH 7.4, containing 0.9 M NaCl, $CaCl_2$, 1 mM $MnCl_2$, and 160 nM **22**. The unlabeled ligand concentration (3.5 mM for **20** and 13 mM for **23**) was then gradually increased until until further addition of ligand failed to significantly affect the anisotropy measurement.