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Amadori and Heyns Rearrangement Products as Possible Galectin-3 Ligands

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Introduction

The Amadori and Heyns rearrangements are two well-known reactions in carbohydrate chemistry occurring at the first stage of Maillard reaction. The Amadori rearrangement is a reaction between reducing sugars as *a*-hydroxy aldehydes and suitable amines leading to *a*-amino ketones. The introduction of an amino group at position C-1 of aldoses with concomitant isomerization leads to 1-amino-1-deoxyketoses (Figure 1A). The Heyns rearrangement employs ketoses as *a*-hydroxy ketones as starting materials and proceeds *via* a glycosylamine to the corresponding 2-amino-2-deoxyaldoses (Figure 1B). After the cascade of reactions, the final step of Maillard reaction called also the non-enzymatic browning produces advanced glycation end products (AGE). Maillard reaction occurs during the food processing as well as in the human body as a process of aging and especially during disease such as diabetes when the AGE products accumulate causing many problems.

Receptor for advanced glycation end products (RAGE) and its ligands have been considered as important pathogenic triggers for the progression of number of diseases. Preventive and therapeutic strategies focusing on RAGE is of great importance. Galectin-3 has been increasingly recognized as an important modulator of several biological functions, and galectin-3 participates in the pathogenesis of diabetic complications via its receptor function for advanced glycation end-products (AGEs) and advanced lipoxidation end-products (ALEs). We want to investigate the possibility of galectin-3 to react with early stage products of Maillard reaction before AGE formation.

Results and Discussion

In this study, the early products of the Maillard reactions 1-amino-1-deoxyketoses (Amadori compounds) (Figure 1A, **1**-**5**) [1] and 2-amino-2-deoxyaldoses (Heyns compounds) (Figure 1B, **6**-**8**) [2] were prepared from the endogenous opioid pentapeptide leucine-enkephalin (YGGFL, **4**), leucine-enkephalin methyl ester (**3**), structurally related tripeptide (YGG, **2**, **6**), amino acid (Y, **1**), and biologically important tetrapeptide LSKL (**5**, **7**, **8**). The strong evidence indicates that galectin-3 participates in the pathogenesis of diabetic complications *via* its receptor function for advanced glycation end-products (AGEs). Thus, the interactions of the early-stage Maillard reaction model compounds (**1**-**8**) with galectin-3 were investigated by using AlphaScreen competitive binding assay (Figure 2). The affinity level of galectin-3 for Amadori and Heyns compounds depends on sugar moiety, as well on peptide, or amino acid in model compounds.

AlphaScreen utilizes bead-based chemistry to study biological interactions in a high throughput manner in a multi-well format. Binding of molecules captured on the beads leads to a broad energy transfer from one bead to the other, ultimately producing an amplified luminescent/fluorescent signal. We used a histidine (nickel chelate) detection kit from PerkinElmer in this study. His-tagged galectin-3 was bound to nickel chelate acceptor beads (capacity = 600 nM), whereas streptavidin-coated donor beads (capacity = 30 nM) were used to attach to biotin asialofetuin (ASF) [3]. The multivalent glycoprotein ASF, which possesses nine *N*-acetyllactosamine (LacNAc) binding sites, was chosen as the galectin-3 binding partner since the binding constant of galectins for the first LacNAc epitope was reported to be in the nanomolar range with a gradient of decreasing binding constants of the remaining epitopes.

The best results were obtained for Amadori compound **4** and Heyns compound **6** (Figure 3) which suggest the importance of amino acids with aromatic side chains, Tyr and/or Phe, for binding to galectin-3. Interestingly, Amadori compound **3**, bearing the protected *C*-terminal carboxyl group,

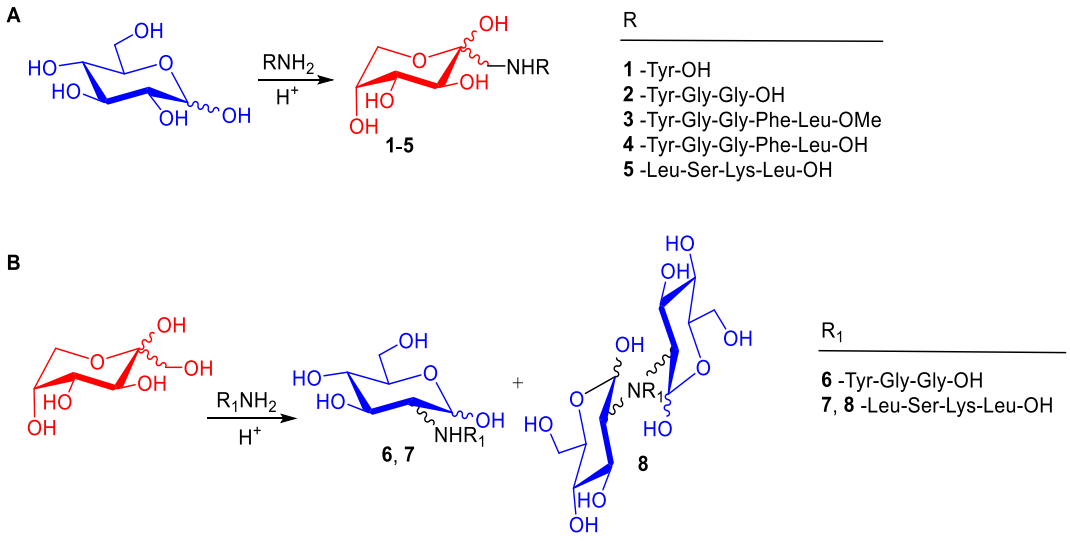

*Fig. 1. A) Amadori rearrangement reaction and Amadori products of Y (**1**), YGG (**2**), YGGFL-OMe (**3**), YGGFL (**4**) and LSKL (**5**); B) Heyns rearrangement reaction and Heyns products of YGG (**6**), and LSKL (**7**, **8**).*

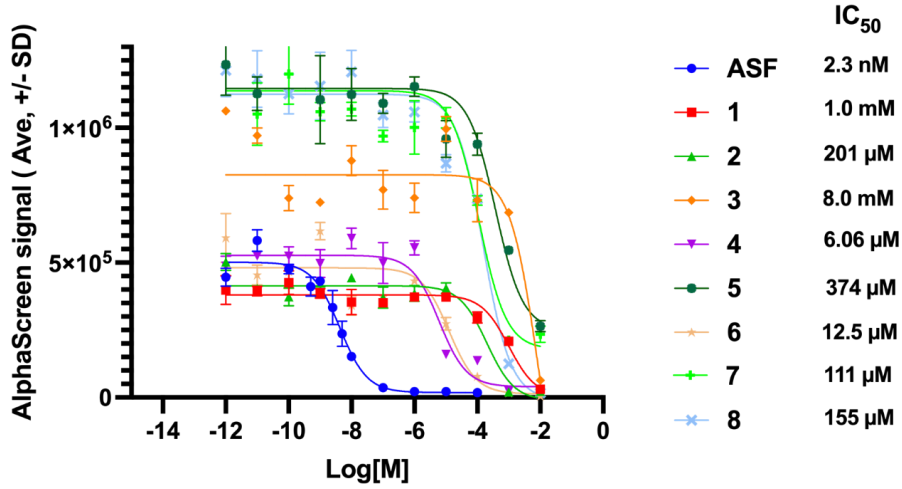

*Fig. 2. Inhibition of binding of biotinylated asialofetuin (ASF) (5 nM) to galectin-3 (200 nM) by **1**-**8** (final concentrations of 0–10 mM). The final concentration of the beads was 25 µg/mL. The assay buffer consisted of 25 mM Hepes (pH 7.4) containing 100 mM NaCl and 0.05% Tween 20. Curves, AlphaScreen signal counts (counts per second) vs log [inhibitor, M], were plotted as means of five replicate measurements.*

exhibited significant drop in affinity compared to **4**. This underscores the critical role carboxylate plays in improving binding affinity of **4**. Heyns compound **6**, that carries the same peptide sequence as Amadori compound **2**, but differs in sugar moiety, showed higher affinity for galectin-3. Neither Amadori or Hyens compounds of tetrapetide LSKL (**5**, **7**, **8**) showed notable affinity for galectin-3, possibly due to lack of aromatic residues.

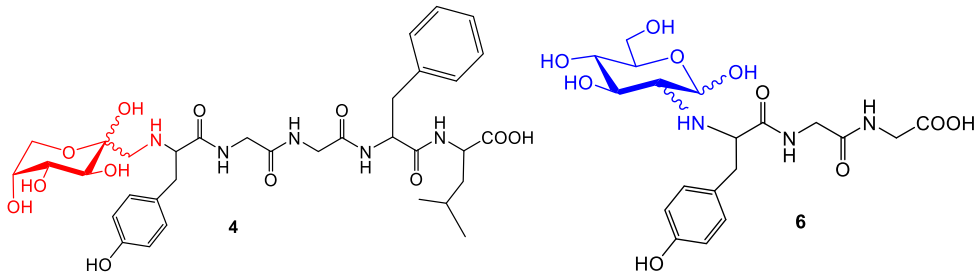

*Fig. 3. The most potent Amadori **4** and Heyns compound **6** for galectin-3 complexation.*

Acknowledgments

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