

# Investigation of the Binding Affinity of a Broad Array of L-Fucosides with Six Fucose-Specific Lectins of Bacterial and Fungal Origin

Son Thai Le<sup>1,§</sup>, Lenka Malinovska<sup>2,3,§</sup>, Michaela Vašková<sup>4</sup>, Erika Mező<sup>1</sup>, Viktor Kelemen<sup>1</sup>, Anikó Borbás<sup>1</sup>, Petr Hodek<sup>4</sup>, Michaela Wimmerová<sup>2,3,5\*</sup> and Magdolna Csávas<sup>1\*</sup>

<sup>1</sup> Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032, Debrecen, Hungary; [le.thai.son@pharm.unideb.hu](mailto:le.thai.son@pharm.unideb.hu) (S. T. L.), [mezo.erika@science.unideb.hu](mailto:mezo.erika@science.unideb.hu) (E.M.), [kelemen.viktor@pharm.unideb.hu](mailto:kelemen.viktor@pharm.unideb.hu) (V.K.), [borbas.aniko@pharm.unideb.hu](mailto:borbas.aniko@pharm.unideb.hu) (A.B.), [csavas.magdolna@science.unideb.hu](mailto:csavas.magdolna@science.unideb.hu) (M.Cs.)

<sup>2</sup> Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; [malinovska@mail.muni.cz](mailto:malinovska@mail.muni.cz) (L.M.), [michaela.wimmerova@ceitec.muni.cz](mailto:michaela.wimmerova@ceitec.muni.cz) (M.W.)

<sup>3</sup> National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

<sup>4</sup> Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic, [michael.vaskova@gmail.com](mailto:michael.vaskova@gmail.com) (M.V.), [petr.hodek@natur.cuni.cz](mailto:petr.hodek@natur.cuni.cz) (P.H.)

<sup>5</sup> Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

\*Correspondence: [michaw@chemi.muni.cz](mailto:michaw@chemi.muni.cz) (M.W.); [csavas.magdolna@science.unideb.hu](mailto:csavas.magdolna@science.unideb.hu) (M.Cs.)

§These two authors contributed equally.

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**Abstract:** Series of multivalent  $\alpha$ -L-fucoside containing glycoclusters and variously decorated L-fucosides were synthesized to find potential inhibitors of fucose-specific lectins and study the structure-binding affinity relationships. Tri- and tetravalent fucoclusters were built up using copper-mediated azide-alkyne click chemistry. Series of fucoside monomers and dimers were synthesized using various methods, namely glycosylation, azide-alkyne click reaction, photoinduced thiol-en addition and sulfation. The interactions of compounds with six fucosyllectins of bacterial or fungal origin were tested using hemagglutination inhibition assay. As a result, a tetravalent,  $\alpha$ -L-fucose presenting glycocluster showed to be orders of magnitude better ligand than a simple monosaccharide for tested lectins in most cases, which can nominate it as a universal ligand for studied lectins. This compound was also able to inhibit adhesion of *Pseudomonas aeruginosa* cells to human epithelial bronchial cells. A trivalent fucocluster with protected amine functional group seems also to be a promising candidate to design glycoconjugates and chimeras.

**Keywords:** L-fucosides, multivalency, lectins, glycoclusters, hemagglutination, cystic fibrosis.

Inhibition of hemagglutination - AFL				
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 1	0.391 mM	0.195 mM	97.66 μM	48.83 μM
Compound 2	0.391 mM	0.195 mM	97.66 μM	48.83 μM
Compound 3	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Compound 4	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AFL)	
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 5	0.781 mM	0.391 mM	0.195 mM	97.66 μM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AFL)	

\* Standard. Standard experiment was done anew for every used batch of lectin or red blood cells.

\*\* Controls were done anew for every used batch of lectin or red blood cells.

S1. Influence of L-fucose, compounds 1, 2, 3, 4 and 5 on hemagglutination caused by lectin AFL.

Inhibition of hemagglutination - RSL				
L-fucose*	6.25 mM	3.125 mM	1.5625 mM	0.781 mM
Compound 1	12.2 μM	6.1 μM	3 μM	1.5 μM
Compound 2	12.2 μM	6.1 μM	3 μM	1.5 μM
Compound 3	12.2 μM	6.1 μM	3 μM	1.5 μM
Compound 4	12.2 μM	6.1 μM	3 μM	1.5 μM
Compound 14	97.66 μM	48.83 μM	22.41 μM	12.2 μM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin RSL)	
L-fucose*	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Compound 5	24.41 μM	12.2 μM	6.1 μM	3 μM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin RSL)	

\* Standard. Standard experiment was done anew for every used batch of protein or red blood cells.

\*\* Controls were done anew for every used batch of protein or red blood cells.

S2. Influence of L-fucose, compounds 1, 2, 3, 4, 14 and 5 on hemagglutination caused by lectin RSL.

Inhibition of hemagglutination - AAL				
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 1	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Compound 2	0.391 mM	0.195 mM	97.66 μM	48.83 μM
Compound 3	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Compound 4	1.5625 mM	0.781 mM	0.391 mM	0.195 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AAL)	
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 5	0.781 mM	0.391 mM	0.195 mM	97.66 μM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AAL)	

\* Standard. Standard experiment was done anew for every used batch of protein or red blood cells.

\*\* Controls were done anew for every used batch of protein or red blood cells.

S3. Influence of L-fucose, compounds 1, 2, 3, 4 and 5 on hemagglutination caused by lectin AAL.

Inhibition of hemagglutination - AOL				
L-fucose*	25 mM	12.5 mM	6.25 mM	3.125 mM
Compound 1	0.391 mM	0.195 mM	97.66 μM	48.83 μM
Compound 2	97.66 μM	48.83 μM	24.41 μM	12.2 μM
Compound 3	3.125 mM	1.5625 mM	0.781 mM	0.391 mM
Compound 4	3.125 mM	1.5625 mM	0.781 mM	0.391 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AOL)	
L-fucose*	25 mM	12.5 mM	6.25 mM	3.125 mM
Compound 5	0.195 mM	97.66 μM	48.83 μM	24.41 μM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin AOL)	

\* Standard. Standard experiment was done anew for every used batch of protein or red blood cells.

\*\* Controls were done anew for every used batch of protein or red blood cells.

S4. Influence of L-fucose, compounds 1, 2, 3, 4 and 5 on hemagglutination caused by lectin AOL.

Inhibition of hemagglutination – BC2L-C				
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 1	6.25 mM	3.125 mM	1.5625 mM	0.781 mM
Compound 2	3.125 mM	1.5625 mM	0.781 mM	0.391 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin BC2L-C)	
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 3	6.25 mM	3.125 mM	1.5625 mM	0.781 mM
Compound 4	3.125 mM	1.5625 mM	0.781 mM	0.391 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin BC2L-C)	
L-fucose*	50 mM	25 mM	12.5 mM	6.25 mM
Compound 5	3.125 mM	1.5625 mM	0.781 mM	0.391 mM
Controls**	Positive control (experiment without any inhibitor)		Negative control (experiment without lectin BC2L-C)	

\* Standard. Standard experiment was done anew for every used batch of protein or red blood cells.

\*\* Controls were done anew for every used batch of protein or red blood cells.

S5. Influence of L-fucose, compounds 1, 2, 3, 4 and 5 on hemagglutination caused by lectin BC2L-C.