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Synthesis of *O*-galactosyl aldoximes as potent LacNAc-mimetic galectin-3 inhibitors

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Abstract—A panel of anomeric oxime ether derivatives of β-galactose were synthesized via the reaction of *O*-β-D-galactopyranosylhydroxylamine with aldehydes. The oxime ethers were evaluated as inhibitors against galectin-3 in a competitive fluorescence polarization assay. The best inhibitor, [*E*]-*O*-(β-D-galactopyranosyl)-indole-3-carbaldoxime (E-**52**), had a K_d value of 182 μM, which is 24 times better than methyl β-D-galactopyranoside (K_d = 4400 μM) and in the same range as methyl lactoside (K_d = 222 μM).

Galectin-3[1] is a member of a family of so far 14 mammalian cytosolic proteins (12 known in humans) defined in 1994[2] as follows: "Membership in the galectin family requires fulfilment of two criteria: affinity for β-galactosides and significant sequence similarity in the carbohydrate-binding site…". Galectin-3 is an intra- and extra-cellular lectin with a correlation of expression and functional implication in inflammation[3-4] and the aggressiveness and metastatic potential of cancer[5]. Galectin-3 appears to mediate most of its functions via interaction of its carbohydrate recognition domain (CRD) with glycoproteins (extracellular and at cell surface), and also non-glycosylated molecules (intracellular). The CRD of galectin-3 is a 130 aa beta-sandwich with a groove on one side that fits about a tetrasaccharide and can be described as four subsites—A, B, C and D[1]. Subsite C is built from most of the conserved amino acids characteristic for galectins and binds galactose with many interactions. In natural saccharides, the galactose residue glycosylates another pyranoside that in turn occupies subsite D. The residue in subsite D is typically (1→4)-linked Glc or GlcNAc or (1→3)-linked GlcNAc or GalNAc .

Natural saccharides have been proposed as inhibitors of galectins. However, they are difficult to synthesize, sensitive to hydrolysis and they are typically to polar to be used as drugs. One approach to circumvent these disadvantages of glycosides is to prepare inhibitors of galectins in which the saccharide in site D is replaced by simpler and less polar structures. This is reasonable as site D interacts only with a small part of the bound saccharide and therefore might accommodate other structures. Within this context, we herein report the synthesis of anomeric aldoxime derivatives of β-galactose via the reaction of *O*-β-D-galactopyranosyl hydroxylamine[6] (**1**) with aldehydes. Non-anomeric carbohydrate aldoxime ethers were recently reported as inhibitors of mucin-type *O*-glycosylation[7]. The anomeric oxime ethers are expected to possess improved stability against enzymatic hydrolysis and are relatively stable at physiological pH. In addition, more hydrophobic aldoximes may have improved cell membrane permeability. Hence, a panel of 51 oxime ethers **2**-**52** were prepared in good to excellent yields by reacting **1**[6,8] with different aldehydes of varying size, polarity and geometry[9] (Scheme 1). The lower yields obtained for some of the aldoximes are mainly due to lower purities of starting aldehydes, which resulted in uncharacterized by-products

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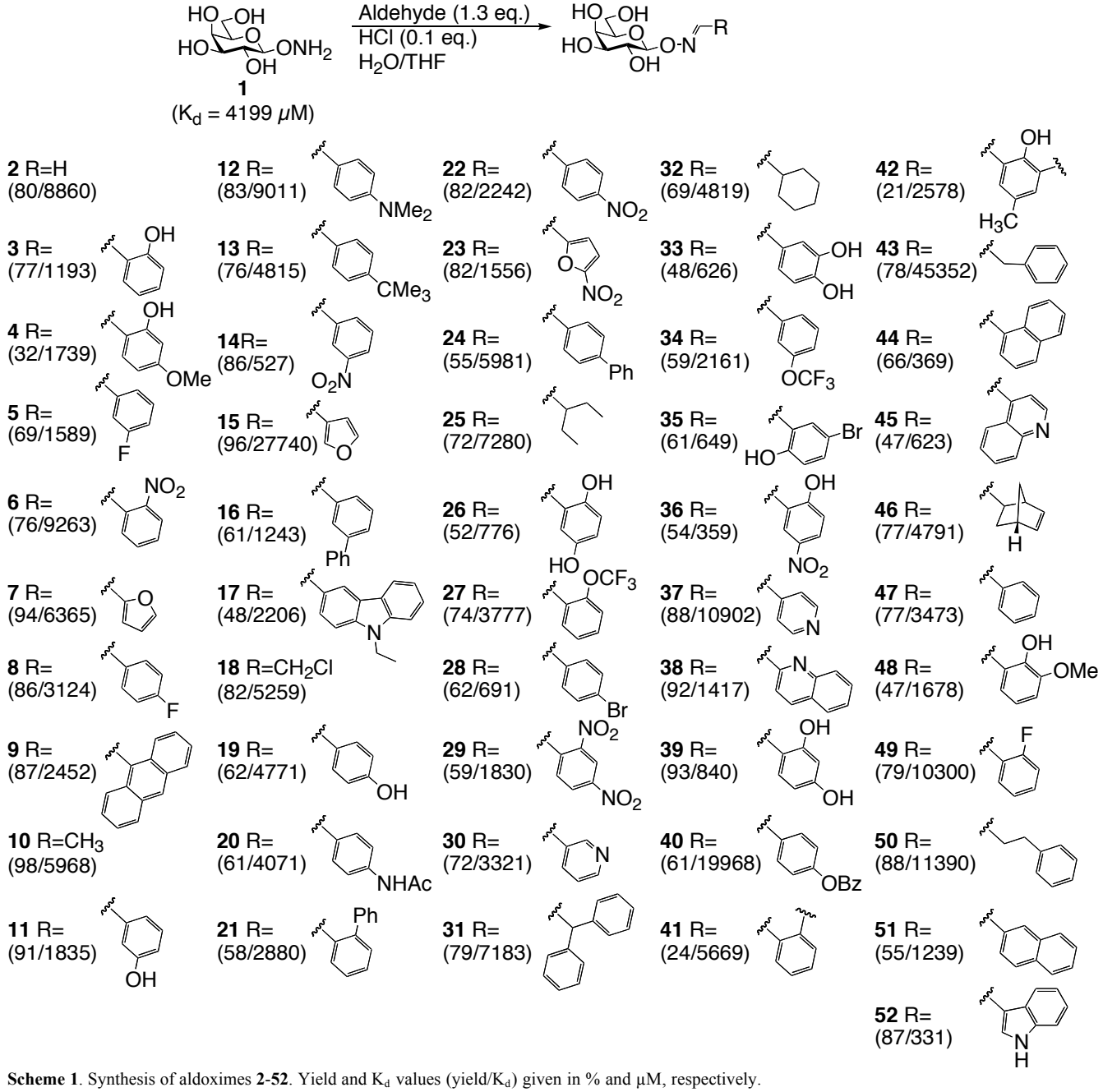
and in some cases incomplete reactions.

Dissociation constants for oxime ethers **2-52** (Scheme 1) with galectin-3 were determined by competitive fluorescence polarization[10] and compared to the known reference compounds D-galactose (K_d=4400 μM), lactose (K_d=222 μM) and N-acetyllactosamine (K_d=67 μM[11]) as methyl β-glycosides. The first important overall observation was that the structure of the aldehyde component greatly influenced the affinity of the inhibitors for galectin-3, as several inhibitors were superior to methyl β-D-galactoside and some were equal or even worse. The aminoxy compound **1** (K_d= 4199 μM) and oxime ether **2** (K_d= 8860 μM) had no improved binding as compared to the methyl galactoside. It can thus be concluded that the aminoxy moiety has no or only a minor influence on the K_d values. Aliphatic moieties (**2**, **10**, **18**, **25**, **31**, **32**, **43**, **46**, **50**) had no effect or resulted in even weaker binding as compared to **1**. Furthermore, only a minor improvement of binding, as compared to **1**, was seen with an unsubstituted phenyl **47** (K_d= 3473 μM). However, a hydroxyl group at ortho (**3**, K_d= 1193 μM) or meta (**11**, K_d= 1835 μM) position of phenyl oximes appeared important, while a hydroxyl at para position (**19**) resulted in slightly weaker binding. Dihydroxylation (**26**, **33**, and **39**) further improved the affinity for galectin-3, although the relatively strong inhibition by the 2,4- and 3,4-di-hydroxylated **39**



Scheme 1. Synthesis of aldoximes **2-52**. Yield and K_d values (yield/K_d) given in % and μM, respectively.

and **33** is difficult to rationalize as they carry one hydroxy group para to the aldoxime group. However, as natural

ligands position a sugar moiety to form hydrogen bonds in subsite D, it is reasonable to assume that the affinity-enhancing effects of the hydroxylated aldoximes (**3**, **11**, **26**, **33**, and **39**) are due to hydrogen bonding to subsite D. A nitro group in meta position (**14**, K_d= 527 μM) was important, while a nitro group in ortho (**6**, K_d= 9263 μM) or para (**22**, K_d= 2242 μM) position did not result in strong inhibition. Thus, the position of the nitro group was critical for improved binding to galectin-3. An additive effect from hydroxyl and nitro groups was also observed; compare **3** and **14** with **36** (K_d=359 μM). A bromo substituent conferred low K_d values; compare **35** (K_d= 649 μM) with **3** and compound **28** (K_d= 691 μM) with **47**. Three of the best inhibitors contained bicyclic aromatic moieties, such as a naphthalene **44** (K_d=369 μM), quinoline **45** (K_d=623 μM) or indole **52** (K_d=331 μM). It is also clear that the carbohydrate should be attached at carbon 1 on naphthalene; compare **44** and **51**, or carbon 4 on quinoline **45**, i.e. the position next to the second aromatic ring, in order to confer low K_d values. Synthesis of oxime ethers normally yields predominantly the E-isomer and most compounds were obtained as pure E-isomers in this work. However, twelve oxime ethers (**7**,**14**, **15**, **18**, **22**, **23**, **25**, **32**, **36**, **44**, **46**, **52**) were obtained as mixtures (E/Z 10/1 to 3/1). Assignment of E/Z-configurations were based on chemical shifts of oxime protons[12-14]. A successful attempt to separate the isomers of the indole-3-carbaldoxime **52** (E/Z 3/1) was made using C-18 RP-HPLC and pure [E]- and [Z]-indole-3-carbaldoxime were isolated. Unfortunately, attempts to separate the E/Z-isomers of **14**, **44** and **45** were unsuccessful. The E- and Z-isomers of **52** displayed different K_d values with the E-isomer (K_d=182 μM) being better than the Z-isomer (K_d=549 μM), which suggests that a close contact between the indole ring and galectin-3 binding site confers selectivity towards the E-isomer. In order to exclude the possibility of E/Z-isomerization during biological evaluations, compound E-**52** was dissolved in deuterated bioassay buffer (PBS, pH 7.2) and analysed with ^{1}H NMR for 6 days. No E/Z-isomerization could be observed and as inhibitors were dissolved in buffer and evaluated as galectin-3 inhibitors within the same day, we conclude that E/Z-isomerizations do not interfere with evaluation of the pure isomers of **52**.

In conclusion, through a panel of structurally simple galactosyl oxime ethers, the glucose moiety of lactose has been replaced by less complex organic structures that provide improved affinity (K_d= 182 μM for E-**52**) for galectin-3 as compared to methyl lactoside (K_d= 222 μM). Furthermore, the indole derivative E-**52** show 24 times affinity enhancement for galectin-3 as compared to methyl β-D-galactoside. The use of oxime ethers results in less hydrogen bonding and less polar molecules, possibly with improved stability against enzymatic hydrolysis, and thus constitutes an advancement towards novel galectin inhibitors with improved pharmacological properties as compared to natural ligands. Finally, combination of the present strategy with the previously published targeting of subsite B[11,15] might give much stronger binding inhibitors with improved pharmacological properties.

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Ar-H), 7.49 (d, 1H, *J*=7.6 Hz, Ar-H), 7.61 (s, 1H, Ar-H), 7.98 (bd, 1H, *J*=6.9 Hz, Ar-H), 8.51 (s, 1H, NCH). For Z isomer δ 3.74-3.83 (m, 4H, H-3, H-5, H-6, H-6'), 3.90, (dd, 1H, *J*=8.2, 1.7 Hz, H-2), 3.99 (dd, 1H, *J*=3.3 Hz, H-4), 5.11 (d, 1H, *J*=8.2 Hz, H-1), 7.24-7.34 (m, 2H, Ar-H), 7.55 (bd, 1H, *J*=7.3 Hz, Ar-H), 7.84 (bd, 1H, *J*=7.2 Hz, Ar-H), 7.98 (s, 1H, Ar-H), 8.30 (s, 1H, NCH). The O-(β-D-galactopyranosyl)-carbaldoximes **2**-**52** were >99% pure as determined by ^{1}H NMR except for **36** (90% pure), **15** (85% pure), **9** (80% pure), **17** (75% pure), and **30** (95% pure).

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