Glycoconj J (2016) 33:853–876
DOI 10.1007/s10719-016-9721-z

REVIEW

Synthetic glycoconjugates inhibitors of tumor-related galectin-3: an update

Vanessa Leiria Campo[1] · **Marcelo Fiori Marchiori**[1] · **Lílian Cataldi Rodrigues**[1] · **Marcelo Dias-Baruffi**[1]

Received: 4 April 2016 / Revised: 28 July 2016 / Accepted: 2 August 2016 / Published online: 15 August 2016

Abstract Galectin-3 is associated with the development and malignancy of several types of tumor, mediating important tumor-related functions, such as tumorigenesis, neoplastic transformation, tumor cell survival, angiogenesis, tumor metastasis and regulation of apoptosis. Therefore, synthetic galectin-3 inhibitors are of utmost importance for development of new antitumor therapeutic strategies. In this review we present an updated selection of synthetic glycoconjugates inhibitors of tumor-related galectin-3, properly addressed as monosaccharide- and disaccharide-based inhibitors, and multivalent-based inhibitors, disclosuring relevant methods for their synthesis along with their inhibitory activities towards galectin-3. In general, Cu(I)-assisted 1,3-dipolar azide-alkyne cycloaddition (CuAAC) reactions were predominantly applied for the synthesis of the described inhibitors, which had their inhibitory activities against galectin-3 evaluated by fluorescence polarization, surface plasmon resonance (SPR), hemagglutination, ELISA and cell imaging assays. Overall, the presented synthetic glycoconjugates represent frontline galectin-3 inhibitors, finding important biomedical applications in cancer.

Keywords Galectin-3 · Tumor · Glycoconjugates · Monosaccharides · Disaccharides · Multivalent-based inhibitors

✉ Vanessa Leiria Campo
vlcampo@fcfrp.usp.br

[1] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Av. Café S/N, CEP, Ribeirão Preto, SP 14040-903, Brazil

Introduction

Carbohydrate-protein interactions have been in evidence since they are involved in many biochemical and biological processes, such as downstream cell signaling, cell-cell and matrix- extracellular interactions, cell growth regulation, apoptosis and cancer metastasis [1–3]. During tumor development the alteration in cell survival and growth, as well as cell migration and antitumor immunity can be correlated with a diversity of glycosylation mechanisms on cell surface, leading to wide complex structures to be decoded by glycan-binding proteins (lectins) [3–6]. Thus, for deep understanding of what factors take place during the initial and late stages of neoplastic diseases it is crucial to consider the identification and expression of carbohydrate-binding proteins, such as galectins, as important targets for development of antitumor drugs [7–9]. Members of galectins (around 15), which have been isolated and sequenced in a wide variety of tissues from different species [9], are defined based on their affinity for β-galactosides-containing saccharides and amino acid sequence similarity in their CRDs (Carbohydrate Recognition Domain). Galectin-3, for instance, is the only chimeric galectin found in vertebrates that has a C-terminal CRD, which binds to β-galactosides, and the N-terminal domain that contains a serine phosphorylation site, which is critical for galectin-3 cell signaling, multivalency and cross-linking activity [8, 9].

Galectin-3 has been largely implicated in tumor development, being involved in cell proliferation, apoptosis, cell adhesion, invasion, angiogenesis and metastasis [8, 11, 12]. Galectin-3 has differential expression and location dependent on the type of tumor, being highly expressed in colon, head and neck, gastric, endometrial, thyroid, liver, lung, bladder and breast cancer. Moreover, decreased expression of galectin-3 has been shown in colorectal, prostate, kidney and pituitary cancer, although increased levels in the cytoplasm

were observed in colorectal, tongue, and prostate cancer, both correlated with disease progression and metastasis [8, 12, 13]. Cytoplasmic galectin-3 presents anti-apoptotic function related with several but not completely understood mechanisms, such as galectin-3 phosphorylation promoting its nuclear exportation into the cytoplasm and possible interaction with the B-cell lymphoma 2 (Bcl-2) protein resulting in inhibition of cytochrome C release. Although the mechanism is not well known, some relevant papers have described that galectin-3 has an Asp-Trp-Gly-Arg (NWGR) motif in the C-terminal domain that shows similarity with Bcl-2 and therefore seems to participate in interaction between both galectin-3 and this apoptosis regulator. The consequence of this inhibitory effect is the impairment of apoptosis from this pathway [8, 12–14].

Indeed, galectin-3 anti-apoptosis role is assigned by the tumor suppressor p53, which suppresses the transcription of galectin-3, leading to p53 induced apoptosis. Thus, this galectin can be considered a target for apoptosis and downstream events via p53 [15]. In contrast, pro-apoptotic function has been associated with the nucleus and with extracellular activity in activated T cells and in B-cell lymphoma [8, 12–14] (Fig. 1).

In addition, galectin-3 may mediate cell transformations and growth by interacting with oncogenic Ras (KRas), promoting Ras signal transduction through subsequently phosphatidylinositol-3-kinase (PIK-3) activation, and also by controlling cell cycle process upon regulation of cyclins, such as downregulation of A, E; upregulation of the cell-cycle inhibitors p21 (WAF 1) and p27 (KIP1); and induction of D1 followed by β-catenin binding [8, 12]. Lastly, galectin-3 promotes angiogenesis and indirectly metastasis by inducing endothelial cells migration through increased pro-inflammatory cytokines production and, therefore, up-regulation of molecules, such as Integrins, E-selectin, Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1) [16] (Fig. 1). Thus, considering all these tumor-promoting effects of galectin-3, the development of synthetic galectin-3 inhibitors represents a frontline strategy in the search for new antitumor drugs and accurate cancer diagnosis.

CRDs of galectins contain approximately 135 amino acid (aa) residues, which directly lead to the specificity of galectins for saccharides and are composed by subsites (A-E) which, along their 135 aa, form a groove able to bind up to a tetrasaccharide [17–19].Galactose binds to the most conserved subsite C, whereas the second more important subsite D is occupied by another pyranoside represented by (1 → 4)Glc/GlcNAc or (1 → 3) GlcNAc/GalNAc linked to galactose (subsite C) [17, 19, 20]. Interestingly, the preferential interaction of these subsites of each galectin CRD with different carbohydrates illustrate the diversity in their binding specificity and biological activities [17, 21]. The CRD of galectin-3 is comprised of eight conservative amino acids (Arg144, His158, Asn160, Arg162, Asn174, Trp181, Glu184 and Arg186) responsible for the lectin binding to carbohydrates. In general, the main interactions of galectin-3 to the natural disaccharide ligands Lac/LacNAc are represented by hydrogen bonds between the OH groups of Gal (C-4 and C-6) and Glc/GlcNAc (C-3) with His158, Asn160, Arg162, Glu184 and Asn174, and by Van der Waals contacts of Gal and Glc/GlcNAc residues with Trp181 and Arg186 [22]. Therefore, chemical modifications of natural ligands, such as at C-3 of Gal and at C-1 of Glc/GlcNAc can maintain the cited interactions and increase additional contacts, such as with Arg144 (subsite B) [22–25]. In the search for effective galectin-3 inhibitors, the synthesis of carbohydrate based 1,2,3-triazole analogs by Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition (CuAAC) has been largely applied, considering the several advantages of the formed triazole ring, such as its property to act as a rigid link, its stability toward oxidation, reduction and hydrolysis, besides the fact that CuAAC reactions are, generally, easily executed, fast and highly selective [26, 27].

On these bases, this review encompasses an updated selection (2006–2016) of synthetic glycoconjugates inhibitors of tumor-related galectin-3, disclosuring relevant methods for their synthesis along with their inhibitory activities towards galectin-3. It's noteworthy to mention that important published reviews on galectin-3 inhibitors rely mostly on crystallographic and biological aspects [12, 28–33], lacking deeper description on their synthesis.

Synthetic glycoconjugates as galectin-3 inhibitors

For the sake of clearer disposal and better comprehension, the selected synthetic galectin-3 inhibitors were properly divided in monosaccharide- and disaccharide-based inhibitors, and multivalent-based inhibitors, as follows in the next sections.

Monosaccharide-and disaccharide-based inhibitors

Taking into account the low affinities of naturally occurring carbohydrate ligands for galectins, besides their low physiological stabilities due to acid sensitive glycosidic bonds, and their high polarity, synthesis of anomeric and O-3 triazole analogs of galactosides has been developed to obtain higher affinity inhibitors of galectin-3. Thus, Cu(I)-assisted 1,3-dipolar azide-alkyne cycloaddition (CuAAC) reactions between galactosyl alkynes **1** and **2** with azides **3** or **4**, using CuI/DIPEA as catalytic system in THF, provided the triazole-galactosyl derivatives **5**–**7** (Scheme 1) [34]. The best inhibitory activities in the qualitative hemagglutination inhibition assays were verified for compounds **1** and **5**, which showed to be 40 times more active than galactose at 1.25 mM concentration (Table 1) [34].

The distinct sugars galactose and mannose display common structural features in that the stereochemical relationship

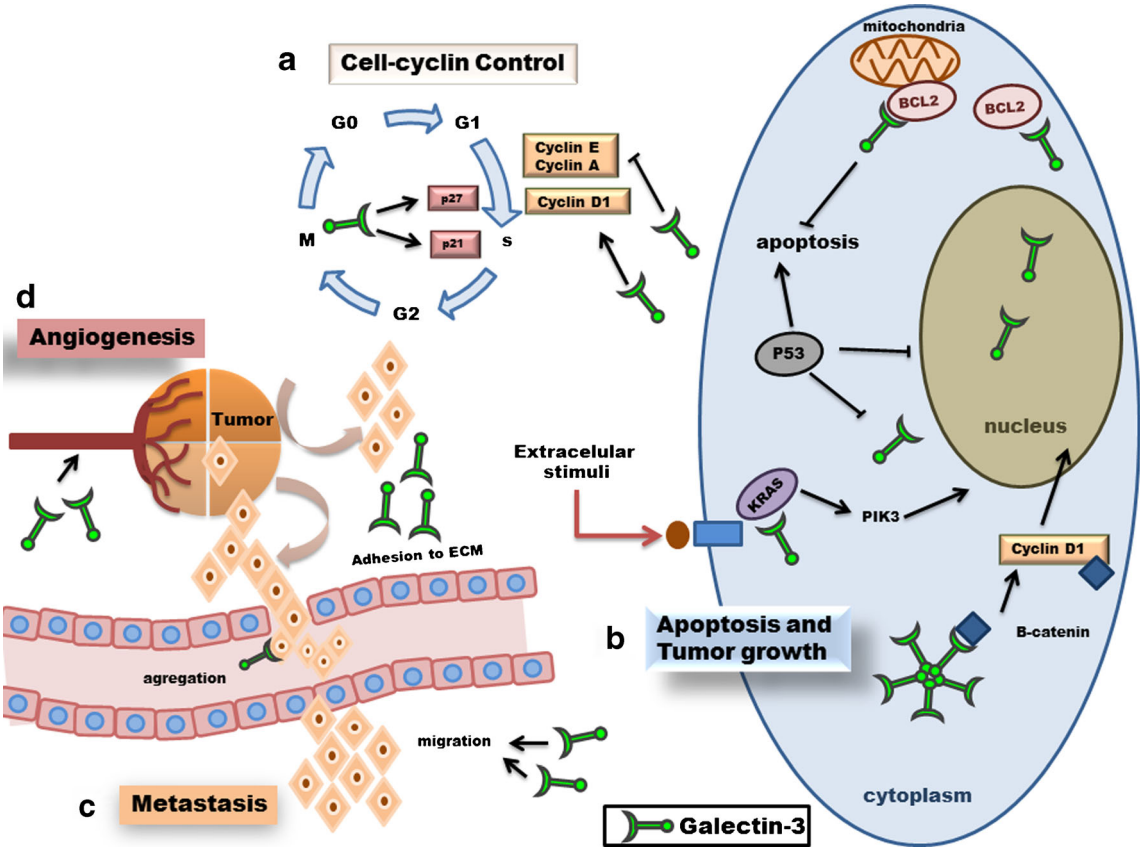
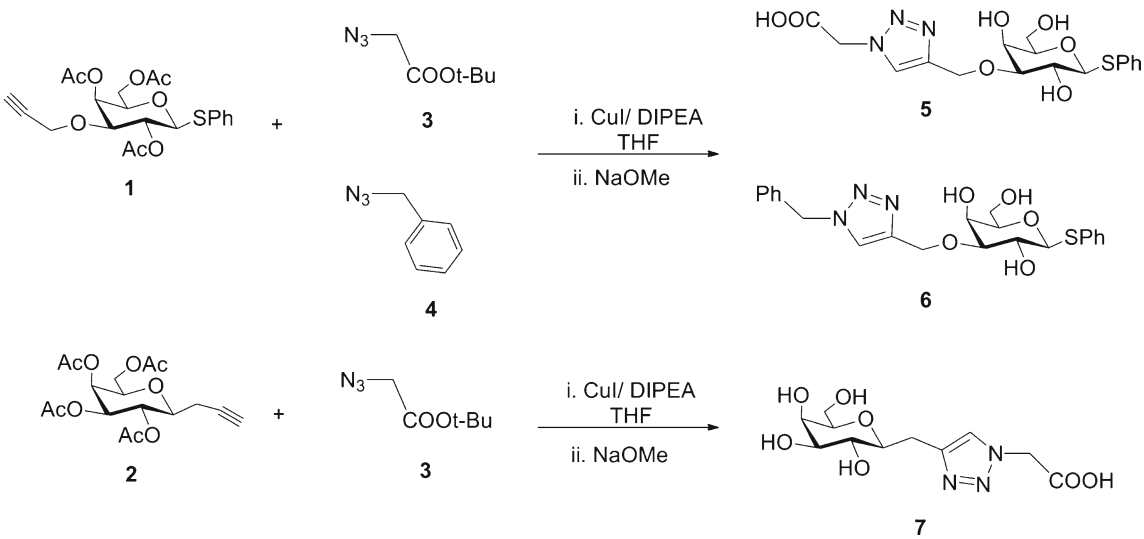


Fig. 1 Modulation of tumor development by Galectin-3 (Gal-3). Gal-3 participates in cell-cycle progression by promoting downregulation of A and E Cyclins **a**; upregulation of inhibitors p21 (WAF 1) and p27 (KIP1) **a**; and also induction of D1 after β-catenin binding **b**. An extracelullar stimulus favors Gal-3 tumor growth through oncogenic Ras (KRas) interaction with subsequently phosphatidylinositol-3-kinase (PIK-3) signal transduction **b**. Gal-3 antiapoptotic function is shown by Bcl2 binding that can stabilize the mitochondrial membrane potential inhibiting apoptosis **b**. Otherwise, Gal-3 transcription can be suppressed by apoptotic molecule p53 **b**. Gal-3 modulates angiogenesis **d** and indirectly metastasis **c** followed by up-regulation of Integrin and Adhesion Molecules expression and also, endothelial cells migration with increased pro-inflammatory cytokines production

of the galactose axial O-4 and equatorial O-3 resembles the axial O-2 and equatorial O-1 of β-mannose. Thus, this similarity suggested the possibility to synthesize β-mannoside-based galectin-3 inhibitors, by exploring the easier access to C-1 in mannose as compared to C-3 in galactose. In this regard, a series of 1*H*-(1,2,3)-triazol-1-yl β-mannosides was



Scheme 1 Synthesis of triazoles **5**–**7** by CuAAC reaction

Table 1 Summary of synthetic modifications and inhibitory activities against galectin-3 of described monosaccharide-, disaccharide- and multivalent-based inhibitors

	Inhibitors	Synthetic modification position	Inhibitory activity against Gal-3	Ref
Monosaccharide	5	Gal O3	40-fold potency than Gal at 1.25 mM (HI)	[34]
	39		Kd 4.56 μM (SPR)	[10]
	9	Man C1	Kd 1.4 mM (FP)	[35]
	14	Gal O1 and C3	Kd 11 μM (FP)	[19, 29]
	31	Tal O2	Kd 0.25 mM (FP)	[40]
	35	Tal O2 and C3	Kd 0.57 mM	[41, 42]
	53	Gal O2 and C3	Kd 87 μM (FP)	[45, 46]
Disaccharide	24	Gal_2S C3	Kd 0.029 μM (FP)	[38]
	70		Kd 0.046 μM (FP)	[44]
	74		Kd 0.052 μM (FP)	[44]
	79		Kd 0.9 μM (FP)	[51]
	85		Kd 0.013 μM (FP)	[43]
	28	LacNAc NH	Kd 10.6 μM (FAC)	[39]
	62	Lac O1	IC_{50} 0.31 mM (HI)	[47]
	68	LacNAc O1	4-fold potency than Lac at 0.4 mM (SP)	[48]
	95	Gal_2Se C1	IC_{50} 1.4 mM (SP)	[53]
Multivalent	102	Lac O1/ Gal C1	Kd 16 μM (FP)	[56]
	105	Lac O1	Kd 17 μM (FP)	[58]
	113		Kd 0.15 μM (SPR)	[10]
	109	Galfuranose C3	Kd 50 μM (FP)	[59]
	119	LacS/ Lac C1	IC_{50} 160 μM (HI)	[64]
	120	Lac C1	IC_{50} 0.07 μM (SP)	[35, 65]
	126	Sialoglycopeptide (GlcNAc C1)	EC_{50} 0.073 μM (SPR)	[67]
	142f	Neo-glycoprotein (LacDiNAc-LacNAc C1)	Kd 0.03 μM (ELISA)	[72]

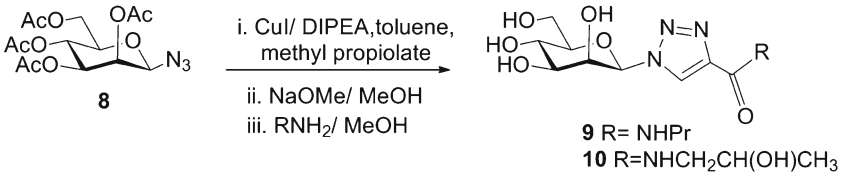
HI Hemagglutination inhibition assay, *SPR* Surface plasmon resonance, *FP* Fluorescence polarization assay, *FAC* Frontal affinity chromatography, *SP* Solid phase assay

synthesized by copper(I)-catalyzed 1,3-dipolar cycloaddition of azido **8** with methyl propiolate, followed by Zemplén transesterification (NaOMe/MeOH) and treatment with different amines, affording a panel of trizole amides (Scheme 2). The best affinities for galectin-3, as determined by fluorescence polarization assay, were verified for compounds **9** (Kd 1.4 mM) and **10** (Kd 1.5 mM) (Table 1), which, although active, did not represent optimal mimetics of 3-(triazol-1-yl)-galactosides for galectin-3 [35].

The affinity enhancement for the different subsites (A-D) of galectins by combining structural fragments to galactoside or lactoside derivatives may represent a significant approach to get effective galectin-3 inhibitors [36, 37]. In this perspective, two galactosyl oximes having C3-triazole fragments were prepared by CuAAC reactions between the 3-azido-Gal-indolcarbaldoxime **11** and the corresponding alkynes methyl propiolate **12** and phenyl acetylene **13**, in the presence of Cu wire in propanol (Scheme 3). The obtained compounds **14** and **15** showed promising affinities for galectin-3, with Kd values of 11 μM and 17 μM, respectively, in fluorescence polarization assays (Table 1). The improved affinity and selectivity of **14** and **15** for galectin-3 was further justified through their modeling into the binding sites of different galectins [19]. It was then observed that, apart from interacting with subsites B and C present in all galectins, these compounds were able to establish an extra strong interaction, through their indole aldoxime fragments, with the Arg144 residue present in a distinct cavity existent only in Gal-3 [19, 29].

Following the strategy of getting ligands able to interact with the different subsites (A-D) of galectins, a set of 3-triazol-galactosides, 3′-triazol-*N*-acetyllactosamine (LacNAc) and di-triazol-thiodigalactoside derivatives were synthesized, under Cu(I)-catalyzed reactions, as potential galectin-3 inhibitors [38]. Initially, a series of triazoles was obtained at the C3 position of galactose by click reaction (CuI/DIPEA in toluene, rt.-40 °C) of 3-azide-GalSMe **16** with different monosubstituted-aryl alkynes, as exemplified for triazole **17** (Scheme 4) Subsequently, considering that LacNAc is far superior to galactose as natural ligands for galectins, several LacNAc derivatives carrying 4-carbamoyl and 4-aryl triazoles at the galactose C3′ were obtained by click reactions of LacNAc C3′-azido **18** and corresponding alkynes, as

Scheme 2 Synthesis of β-mannoside-based triazoles **9** and **10**



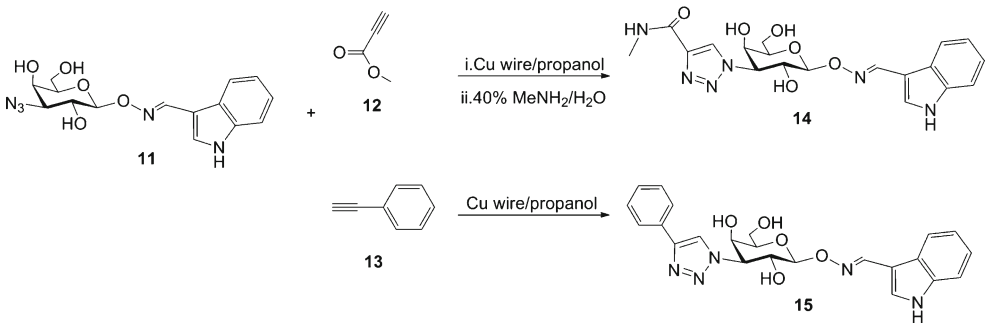
shown for compound **19** (Scheme 4). Lastly, a panel of thiodigalactoside triazole amides was obtained by a multi-step synthetic route, starting with the preparation of the thiodigalactoside triazole ester **20** by cycloaddition reaction between the galacto-azide **21** and methyl propiolate **12**. The formed triazole was then treated with HBr and to the resulting bromide **22** was added dried sodium sulfide, which led to its dimerisation. As the last step, reactions of **23** with a series of primary amines in methanol afforded the respective amides, as illustrated for the thiodigalactoside triazole butyl-amide **24** (Scheme 4). In general, the obtained compounds proved to be better inhibitors of galectin-3 if compared to other galectins subtypes (7, 8 N and 9 N), in competitive fluorescence polarization assays. The highest inhibitory activities toward galectin-3 were verified for compounds **19** (Kd 0.66 μM) and **24** (Kd 0.029 μM), related to LacNAc- and thiodigalactoside-triazoles series, respectively (Table 1) [38].

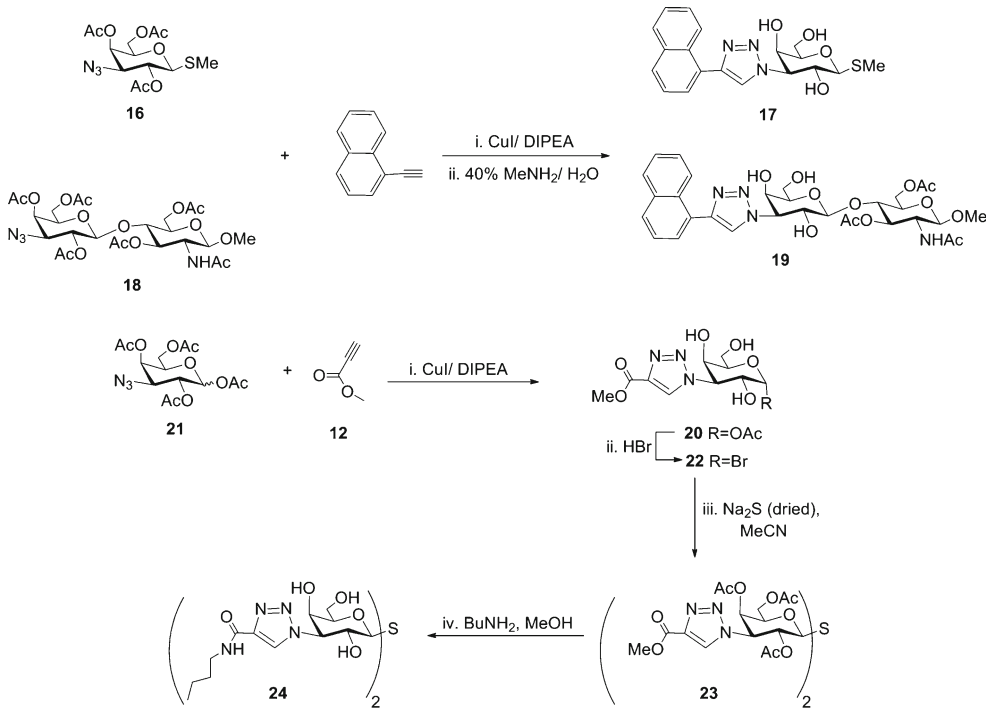
Considering the potential of β-D-Gal*p*-(1–3)-β-D-Glc*p*N (lacto-*N*-biose) disaccharide to interact with two essential galectin-3 subsites (C and D), a library of *N*-functionalized octyl-lacto-*N*-biose was synthesized aiming stronger binding to galectin-3 via interactions with its additional subsites [39]. The synthesis of this library was initiated by previous preparation of octyl-lacto-*N*-biose **25** by NIS/AgOTf-promoted glycosylation reaction between the suitably protected building blocks thioethyl galactoside **26** and octyl-*N*-Troc-glucosamine **27**, followed by *N*-Troc deprotection (Zn/AcOH) and *N*-acylation with a range of commercially available acetyl chlorides and carboxylic anhydrides in pyridine (Sheme 5) [39]. After removal of the benzylidene and benzyl groups ($Pd(OH)_2/C$), and *O*-deacetylation (NaOMe/MeOH), the obtained lacto-*N*-biose library was screened for galectin-3 binding by microscale frontal affinity chromatography coupled to mass spectrometry (FAC/MS), which is a technique that allows fast rank ligands and also gives access to their dissociation constants (Kd). It involves the preparation of a microscale tubing column by immobilizing a biotinylated galectin-3 onto streptavidin controlled-pore glass (CPG) beads, with subsequent elution of a library-containing solution. The affinities of compounds to galectin-3 are thus dictated by their order of elution, with the strongest-binding ligands eluting last. According to the screening performed by FAC/MS, best affinity to galectin-3 was verified for compound **28** (Kd 10.6 μM) (Table 1), which eluted much slower (retention time 11 min) than the sole lacto-*N*-biose (retention time 1.0 min and Kd 73.3 μM) [39] (Scheme 5).

The sugar talose has emerged as an attractive scaffold for the design of novel galectin-3 inhibitors since the inverted C2 configuration, relative to galactose, offers possibilities for inserting affinity-enhancing talose O2 substituents able to stablish interactions with polar amino acids, not previously accessed by galactose-based galectin-3 inhibitors. Moreover, considering that talosides appear not to be naturally present in mammalians, and hence no endogenous talose-processing enzymes are present, they may represent desired hydrolytically stable inhibitors [40]. Therefore, a series of methyl 3-*O*-(4-methylbenzoyl)-β-D-talopyranoside O2 derivatives was synthesized and tested as galectin-3 inhibitors. Starting from the known benzylidene-protected methyl β-D-talopyranoside **29**, subsequent synthetic steps involving acetylation, sulfation and PCl_3-mediated reactions, followed by cleavage of benzylidene using aqueous acetic acid, afforded the inhibitors **30**, **31** and **32** (Scheme 6). Evaluation of these talosides by fluorescence polarization assay showed corresponding dissociation constants of 0.55 mM, 0.25 mM and 0.60 mM for compounds **30**, **31** and **32** (Table 1), reflecting higher galectin-3 inhibition if compared to methyl β-D-galactopyranoside (Kd 4.4 mM) [40].

In continuous work to get β-D-talosides as galectins inhibitors, 3-amido-3-deoxy-talosides were synthesized via formation of idoside intermediates, followed by insertion of equatorial 3-NH_2 through a substitution or oxidation/reductive amination sequence, and subsequent conversion to aromatic

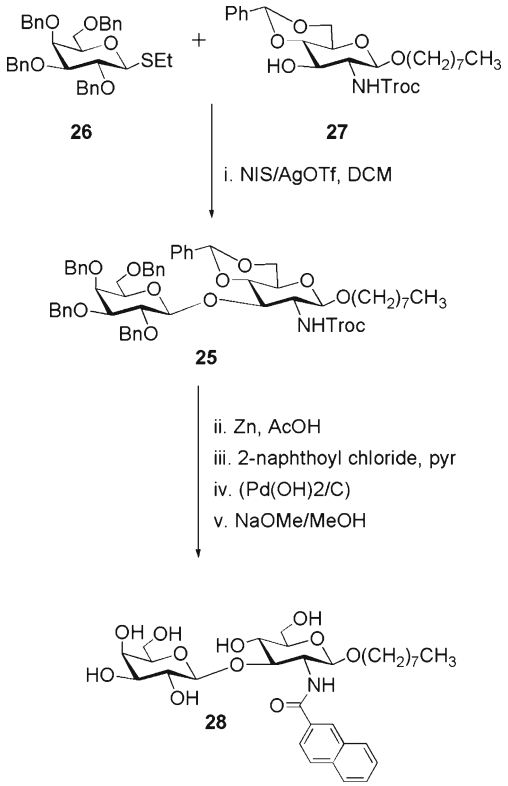
Scheme 3 Synthesis of galactosyl oximes **14** and **15** with subsite B-binding 1,2,3-triazoles at C3





Scheme 4 Synthesis of naphtyl-3-triazol-galactoside **17**, naphtyl-3′-triazol-*N*-acetyllactosamine **19** and butylamine-di-triazol-thiodigalactoside **24** by Cu(I)-catalyzed reactions

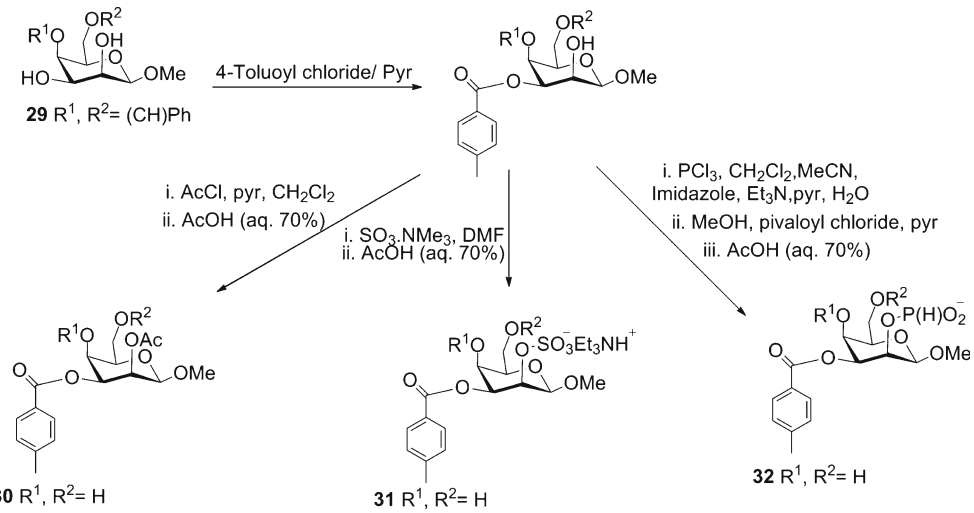
amides. Thus, a synthetic route starting from benzylidene-protected methyl β-D-galactopyranoside **33** afforded the 3-amino-3-deoxy-β-taloside **34**, which was then acylated to form selected aromatic amides, such as compounds **35** and **36** (Scheme 7). In fluorescence polarization assays, these compounds showed to be active against galectin-3, presenting corresponding Kd values of 0.57 mM and 0.72 mM (Table 1), which are higher than methyl β-galactoside (4.4 mM) [41, 42].



Scheme 5 Synthesis of NH derivatized octyl-lacto-*N*-biose **28**

The synthesis of anomeric and O3 modified analogs of natural monosaccharide (Gal) and disaccharides (Lac/LacNAc), especially those containing aromatic groups on the galactose C3 position, has been explored since they are able to establish favorable cation-π interactions with arginine residues, leading to galectin-3 inhibition [42–44]. Thus, aiming to favor these interactions and considering the potential of *O*-3 triazole-galactose analogs to interact with Gal-3 CRD, a series of 1,2,3-triazole amino acids-derived-3-*O*-galactosides, such as compounds **37–41**, derived from corresponding phenylalanine, tryptophan, lysine, aspartic acid and tyrosine amino acids, was synthesized. The synthesis of protected compounds **42–46** was carried out by click 1,3-dipolar cycloaddition reactions between azido-functionalized amino acids **47–51** and 3-*O*-propynyl-GalOMe **52**, in a microwave reactor utilizing the catalytic system $CuSO_4$/sodium ascorbate and DMF as solvent, followed by hydrogenolysis (10 % Pd-C/H_2) and deacetylation (NaOMe) reactions (Scheme 7). The obtained deprotected compounds **37–41** were then submitted to Surface Plasmon Resonance (SPR) assays for evaluation of their binding affinity to galectin-3. All tested 1,2,3-triazole amino acid-derived-3-*O*-galactosides **37–41** showed high binding affinity for galectin-3, with lower K_D values being verified for compounds **37** (7.96 μM) and **39** (4.56 μM), comprising derived-phenylalanine and lysine side chains amino acids, respectively (Table 1) [10] (Scheme 8).

Scheme 6 Synthesis of O2 and O3-derivatized methyl β-D-talopyranosides **30–32**

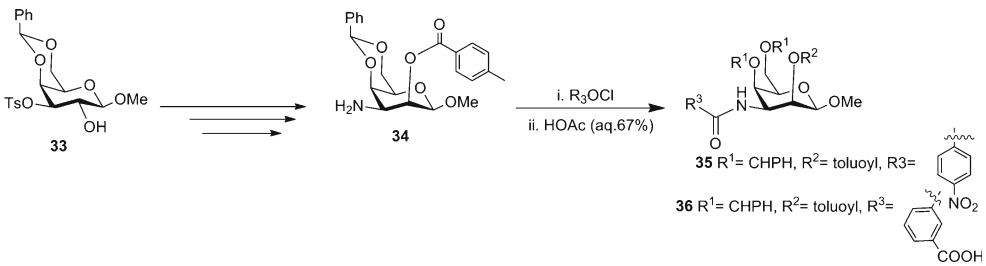


Apart from effective cation-π interactions with arginine residues, computer simulations have shown that additional anionic O2 substituents of inhibitors may provide favorable polar interactions around the arginine guanidinium hydrogens [45]. In this context, anionic O2 derivatives of methyl 3-deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside, such as compounds **53**, **54** and **55** containing sulfate or phosphate, have been synthesized as inhibitors against galectin-3. Starting from the known galactoside **56**, the synthesis of **53**–**55** involved the previous preparation of the key intermediate **57** (Scheme 9a), suitable for further derivatization of O2 position. Thus, sulfation of **57** using sulfur trioxide was straightforward to obtain **58**, whereas treatment of **57** with phosphorus trichloride or pivaloyl chloride/benzyl alcohol led to the phosphonate-derived **59** and benzylphosphate **60**, respectively (Scheme 9a). Finally, debenzylidenation of **58**–**60** afforded the final deprotected inhibitors **53**–**55**, which showed corresponding Kd values of 87 μM, 150 μM and 120 μM against galectin-3, as measured by fluorescence polarization assay (Table 1) [45, 46].

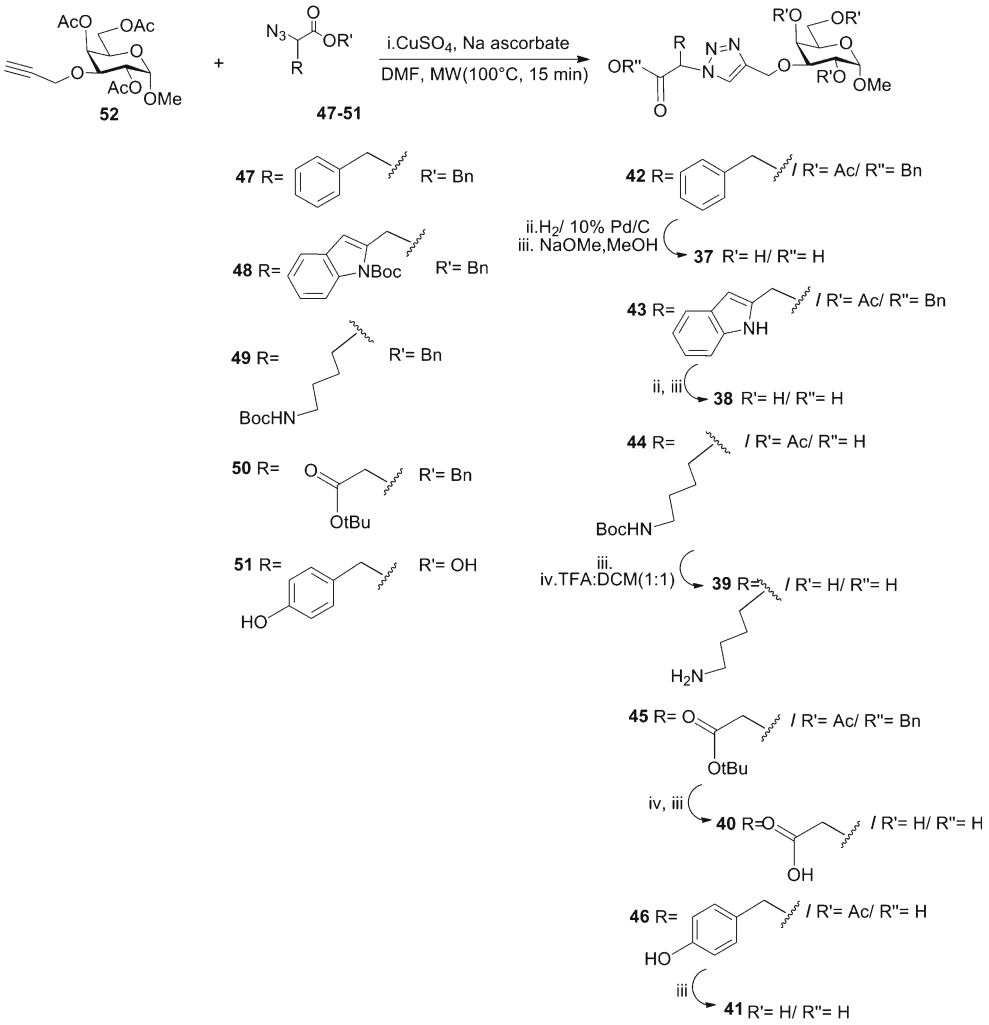
In a different approach aiming to compare the effects of different aglycon moieties on galectin-3 binding, a library of aryl thiolactosides was prepared by phase transfer catalysis reaction (PTC) [47]. Starting from acetobromolactose **61**, the phase transfer catalyzed nucleophilic displacement of bromide by aryl thiols (TBAHS), followed by oxidation of the formed sulfides (*m*CPBA) (Scheme 9b), afforded a library of anomeric sulfones, which were submitted to hemagglutination assays for evaluation of galectin-3 inhibition. Among the tested S-lactosides, best activities were verified for those containing aromatic aglycons, such as compound **62** that showed to be 3 times more active than lactose, with an inhibitory property of 0.31 mM (Table 1) [47]. It's valuable to mention that the same compound showed an increased inhibitory activity against galectin-1 (40 μM), reflecting its distinct binding modes towards galectins-3 and −1, as further evaluated by electron density calculations [47].

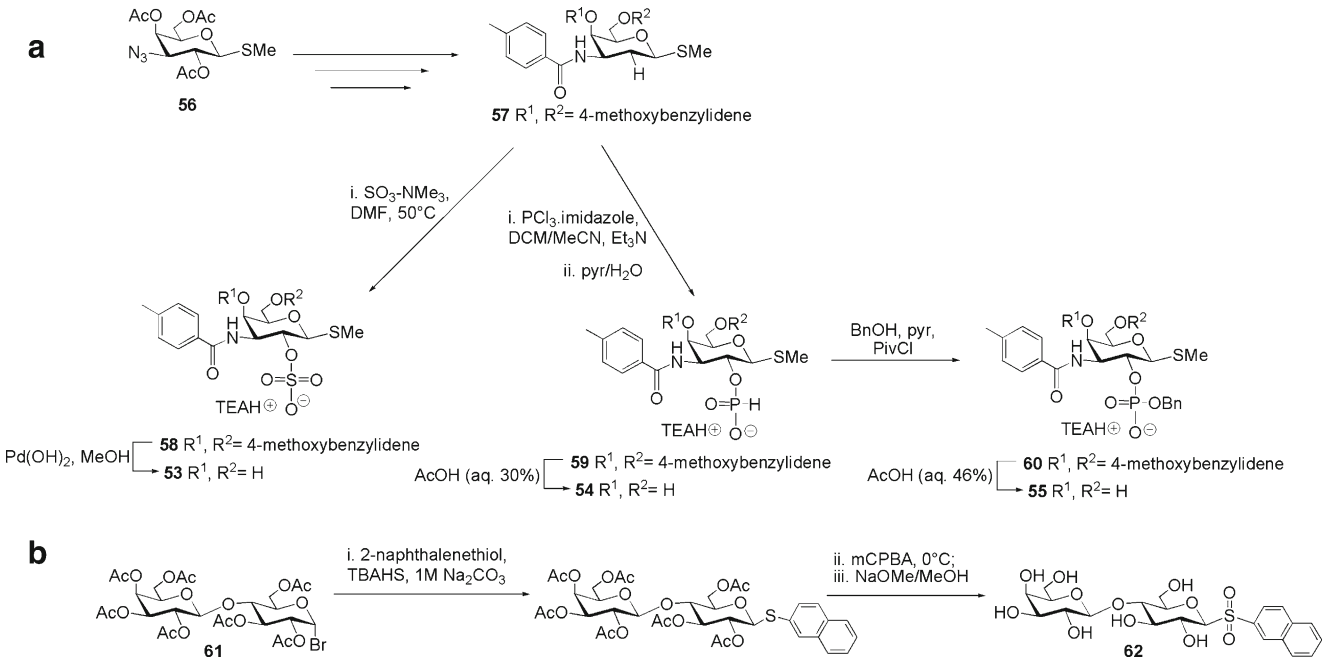
The effect of aglyconic modifications on galectin-3 inhibition was also investigated by means of the preparation of a library of triazolyl lactoside/ *N*-acetyllactosamine derivatives [48]. Firstly, the CuAAC reactions ($CuSO_4$, ascorbate, THF/H_2O) between the lactosyl azide **63** and disctinct alkynyl alcohols, propargyl aryl ethers and naphthyl propargyl ether afforded a panel of seven lactosides, subsequently *O*-deacetylated using NaOMe/ MeOH (Scheme 10). Regarding LacNAc derivatives, their synthesis started with the preparation of the propargyl β-D-LacNAc disaccharide **64** by a highly regioselective glycosylation between the prop-2-ynyl-6-*O*-protected GlcNAc **65** and galactosyl trichloroacetimidate **66** ($BF_3{\cdot}Et_2O$, DCM), followed by CuAAC reactions with a family of triazoles, furnishing a series of eight triazolyl LacNAc derivatives, after removal of the TBDPS protecting group (TBAF) and de-*O*-acetylation (NaOMe/MeOH) (Scheme 10) [48]. Both Lac/LacNAc libraries were then submitted to solid

Scheme 7 Synthesis of 3-amido-D-talopyranosides **35** and **36** as galectin-3 inhibitors

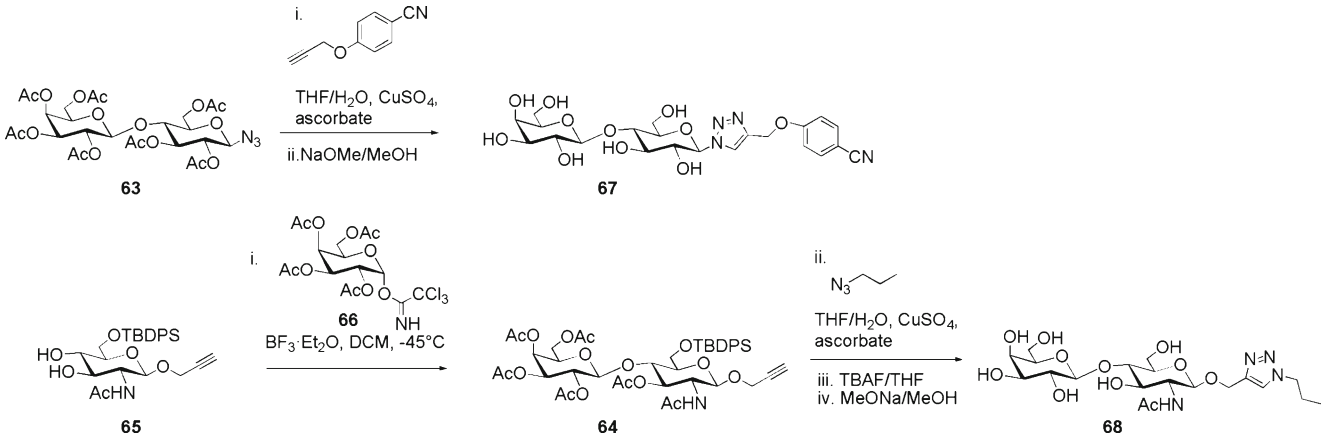


Scheme 8 Synthesis of 1,2,3-triazole-linked glycoconjugates **37–41** by click chemistry



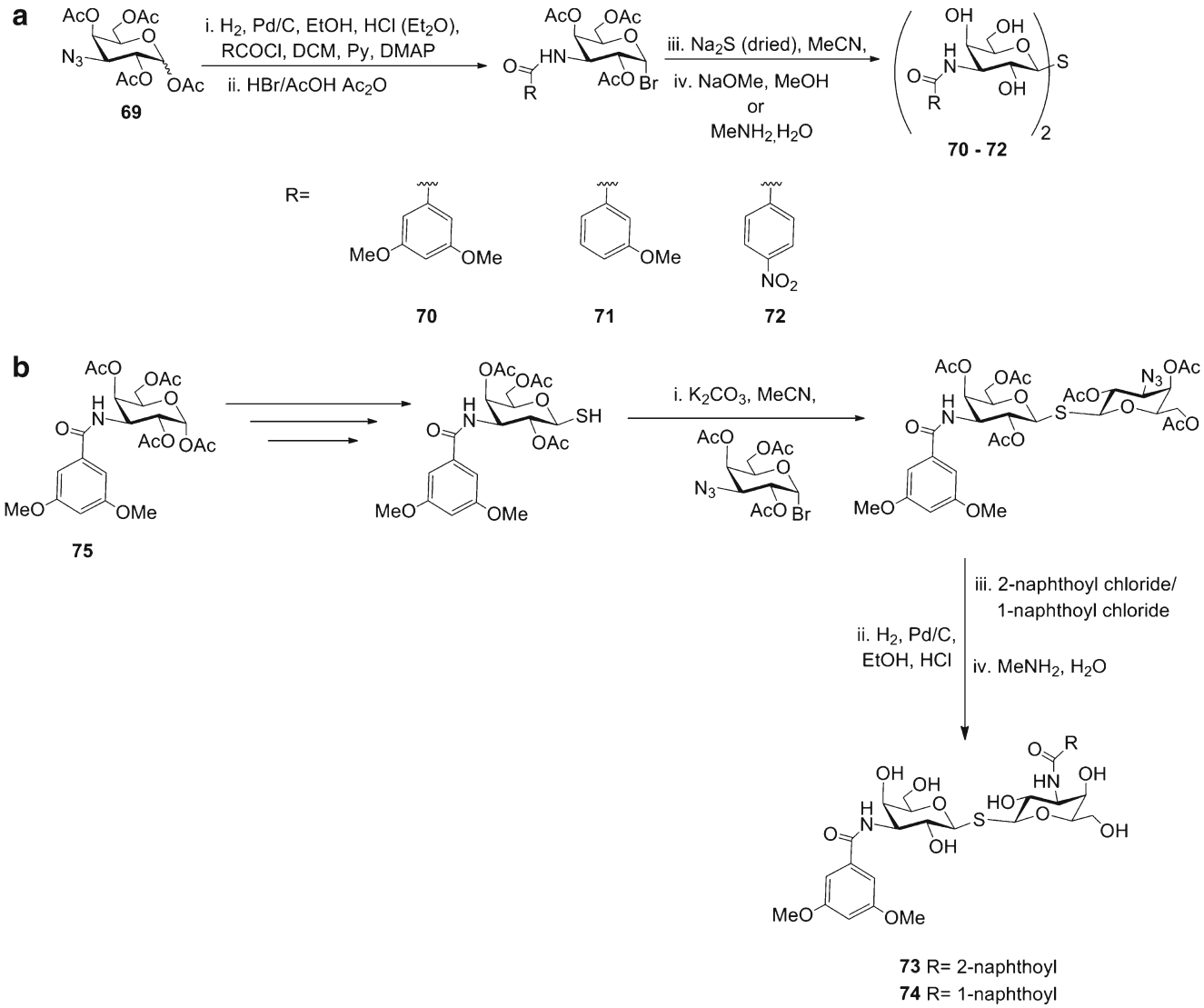


Scheme 9 **a** Synthesis of anionic O2 derivatives of methyl 3-deoxy-3-(4-methylbenzamido)-1-thio-β-D-galactopyranoside **53–55**. **b** Synthesis of the aromatic S-lactoside **62**



Scheme 10 Synthesis of corresponding triazolyl lactoside/ *N*-acetyllactosamine derivatives **67** and **68**

phase assays, based on the adsorption of the glycoprotein asialofetuin on microtiter plate wells, which is a known galectin-3 ligand, and on the use of labeled galectin-3. The tested compounds were then expected to interfere with the glycoprotein-galectin-3 interaction, being verified three- to fourfold potency in relation to Lac for compounds **67** and



Scheme 11 **a** Synthesis of symmetrical thiodigalactosides **70–72**. **b** Synthesis of unsymmetrical thiodigalactosides **73** and **74**

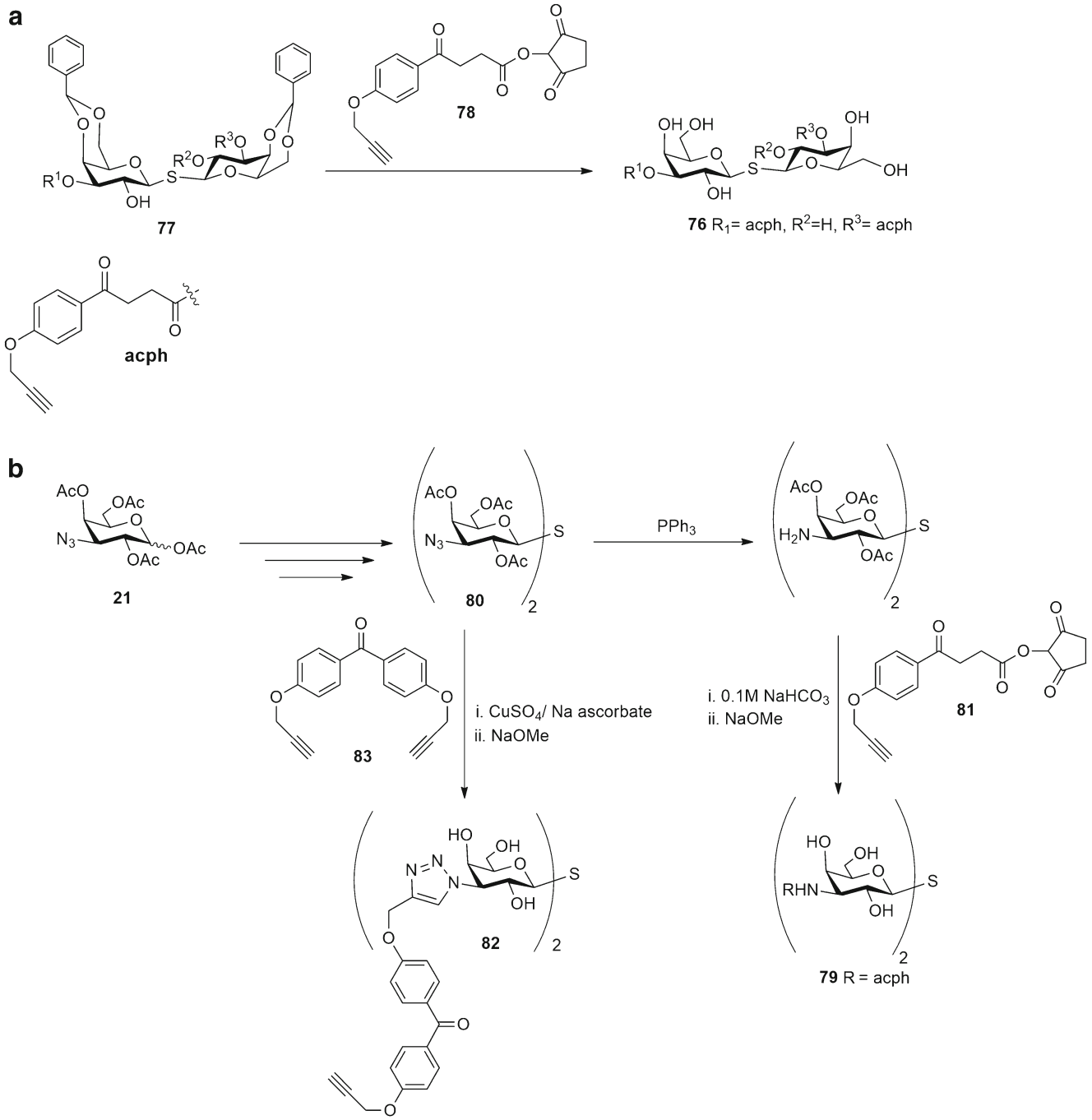
68, at galectin-3 concentration of 0.4–0.5 mM (Table 1). Further NMR spectroscopy analysis with ^{15}N-labeled galectins was also performed to assess the impact of structural differences among the tested compounds on selective binding to galectin-3 and the closely related galectin-1 [48].

Thiodigalactoside has also been shown to bind to galectin-3 with a similar affinity to lactose or LacNAc. In fact, X-ray crystallography and molecular modeling showed that the two galactose residues of thiodigalactoside (TDG) were bound identically in the β-galactoside binding site, whereas the second galactose of thiodigalactoside was bound in the same place as the GlcNAc of LacNAc, with identical hydrogen bonding networks between the disaccharides and the protein [24, 49]. Therefore, derivatization of thiodigalactoside at C-3 of galactose, especially with aromatic amides, may lead to effective cation-π interactions with arginine residues of galectin-3, such as Arg144 and Arg186 [44]. In this regard, thiodigalactosides bearing two identical amides at the two 3-positions (*i.e.* C_2-symmetrical compounds) were synthesized from galacto azide **69**, in a synthetic route involving a sequential reduction to amine, acylation with respective aromatic acyl chlorides to give the amides, bromination (HBr), treatment with dried sodium sulfide and final deprotection (Scheme 11a). Among the obtained diamides, **70** (Kd 0.046 μM), **71** (Kd 0.050 μM) and **72** (Kd 0.049 μM) (Scheme 11a) presented higher inhibitory activities against galectin-3 in fluorescence polarization assays. For the sake of comparison, unsymmetrical thiodigalactosides, such as **73** and **74**, having two different amides at C-3 and C-2 positions of galactose residues were synthesized from dimethoxybenzamide-substituted galactose **75**, in a sequential 5 steps route (Scheme 11b). Compounds **73** (Kd 0.069 μM) and **74** (Kd 0.052 μM) showed binding affinities to galectin-3 comparable to the symmetrical diamides **70–72** in fluorescence polarization assays (Table 1) [44].

Considering the limited understanding of galectin-3 roles both in and outside the cell, the development of molecular probes able to detect and identify galectin-3 in the organism is of great relevance [50]. Thus, a series of galectin-3 probes bearing acetophenone or benzophenone moieties as photolabels, and based on ester-, amide- or triazole-derived thiodigalactosides, was synthesized [51]. Firstly, the acetophenone-based probe **76** was obtained by coupling of suitably benzylidene-protected thiodigalactoside **77** with 4′-*O*-succinimide-ester-4-(prop-2-ynyloxy)acetophenone **78** in basic media, followed by deprotection reaction (AcOH/H_2O) (Scheme 12a). Subsequent synthesis of amide-linked probe **79** was carried out by Staudinger reduction of the azido-thiodigalactoside **80** using PPh_3, followed by coupling to the photolabel **81** (0.1 M $NaHCO_3$) and Zemplen deprotection (Scheme 12b). Regarding the triazole-linked probe **82**, it was obtained by click chemistry reaction between the azido-thiodigalactoside **80** and the photolabel-linked dialkyne **83**, using $CuSO_4$/ sodium ascorbate as catalytic system, followed by removal of the acetyl groups (Scheme 12b). According to fluorescence polarization assays, higher binding affinities to galectin-3 were verified for compounds **76** (Kd 2 μM) and **79** (Kd 0.9 μM) (Table 1), which also showed the most pronounced labeling under fluorescence SDS-PAGE gel-based assays [51].

Aiming to increase arginine-arene interactions and to improve galectin-3 selectivity, inhibitors for galectin-3 containing bulky aromatic groups were synthesized from thiodigalactoside and lactosamine by derivatization of the galactose C3. The synthesis of thiodigalactoside-based inhibitors, such as **84** and **85**, was carried by CuAAC reactions between the common intermediate azido-thiodigalactoside **80** and the aromatic alkynes phenylacetylene **86** and 3-hydroxyphenylacetylene **87**, using $CuSO_4$/sodium ascorbate under microwave irradiation, followed by deacetylation reactions (Scheme 13a). In relation to LacNAc derivatives with an ether-linked aromatic moiety, compound **88**, bearing the 4-(4-phenoxybenzyl) group, was obtained by glycosylation reaction between derivatized galactoside **89** and glucosamine **90**, followed by careful deacetylation without removal of the phthaloyl protecting group (Scheme 13b). For the synthesis of 4-(4-phenoxyphenyl)triazole-linked lactosamine **91**, the intermediate **92** was firstly synthesized by glycosylation reaction (TfOH, NIS) between the 3-azido functionalized methylthio donor **93** and the bromide acceptor **94**. Subsequently, the obtained **92** was submitted to click reaction ($CuSO_4$, sodium ascorbate) with 1-ethynyl-4-phenoxybenzene, followed by azide introduction (NaN_3) and careful deacetylation, affording compound **91** (Scheme 13c) [43]. The binding affinities of compounds **84**, **85**, **88** and **91** to galectin-3 were evaluated by fluorescence polarization assays, being verified low Kd values of 0.044 μM, 0.013 μM, 1.2 μM and 2.2 μM (Table 1), respectively, reflecting the positive influence of their larger aromatic groups to establish favourable aryl-arginine interactions with galectin-3 [43].

In order to investigate the binding properties of selenoglycosides toward galectin-3 [52], symmetrical selenodigalactoside (SeDG) **95** and diselenodigalactoside (DSeDG) **96** were prepared from β-galactopyranosyl isoselenuronium bromide **97**, in three-steps synthetic routes (Scheme 14) [53]. These two selenides were submitted to solid phase binding assays, based on their capacity to block binding of labeled galectin to surface-imobilized neoglycoprotein (Lac-BSA), and to fluorescent cell surface assays. SeDG and DSeDG presented corresponding IC_{50} values of 1.4 mM and 3.8 mM (Table 1), comparable to TDG (IC_{50} 1.1 mM), under fixed concentrations of 15 μg/mL for galectin-3 and 0.25 μg/well of lactosylated BSA. In fluorescent cell assays, involving evaluation of stained colon adenocarcinoma cells



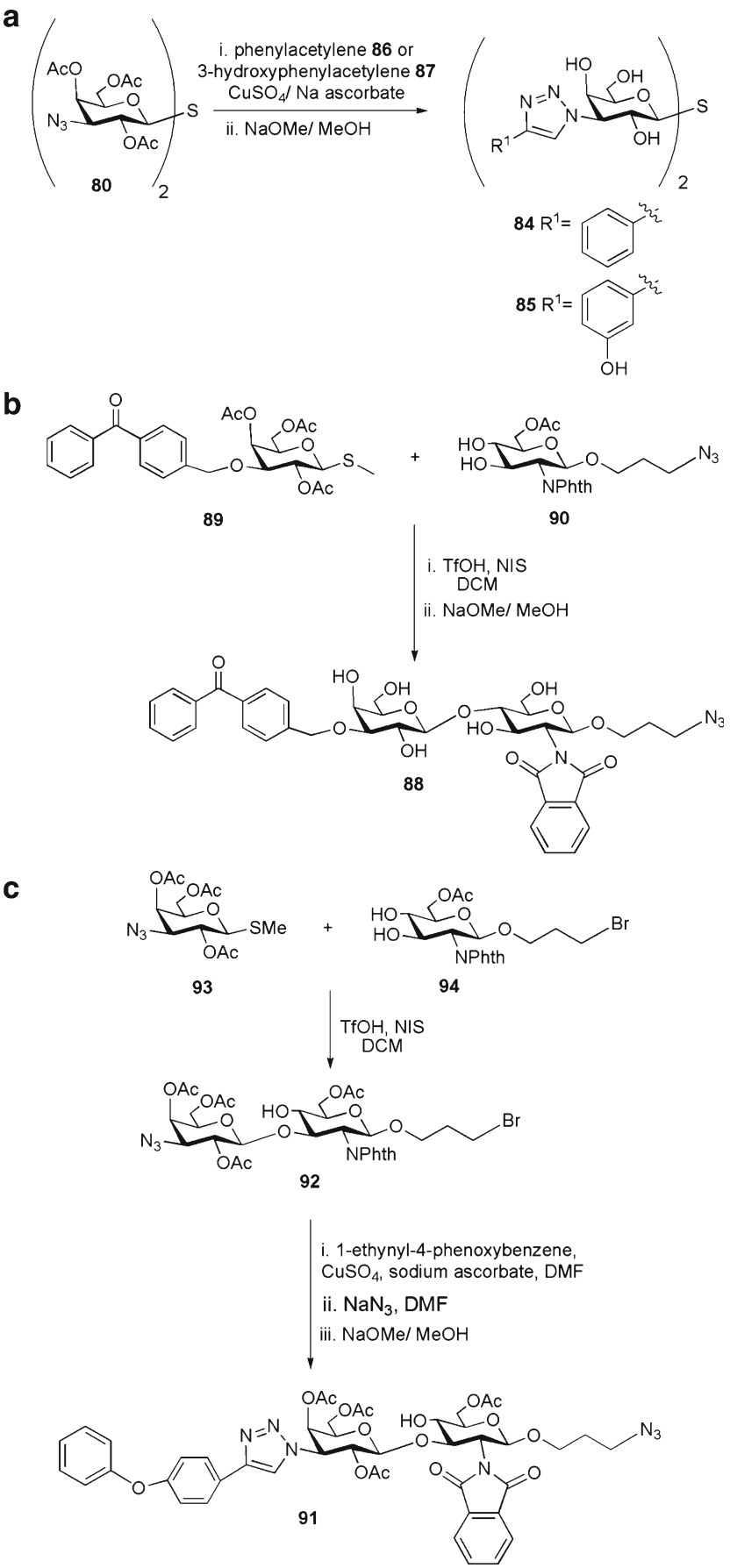
Scheme 12 Synthesis of acetophenone-based probe **76 a**, amide-linked probe **79** and triazole-linked probe **82 b**

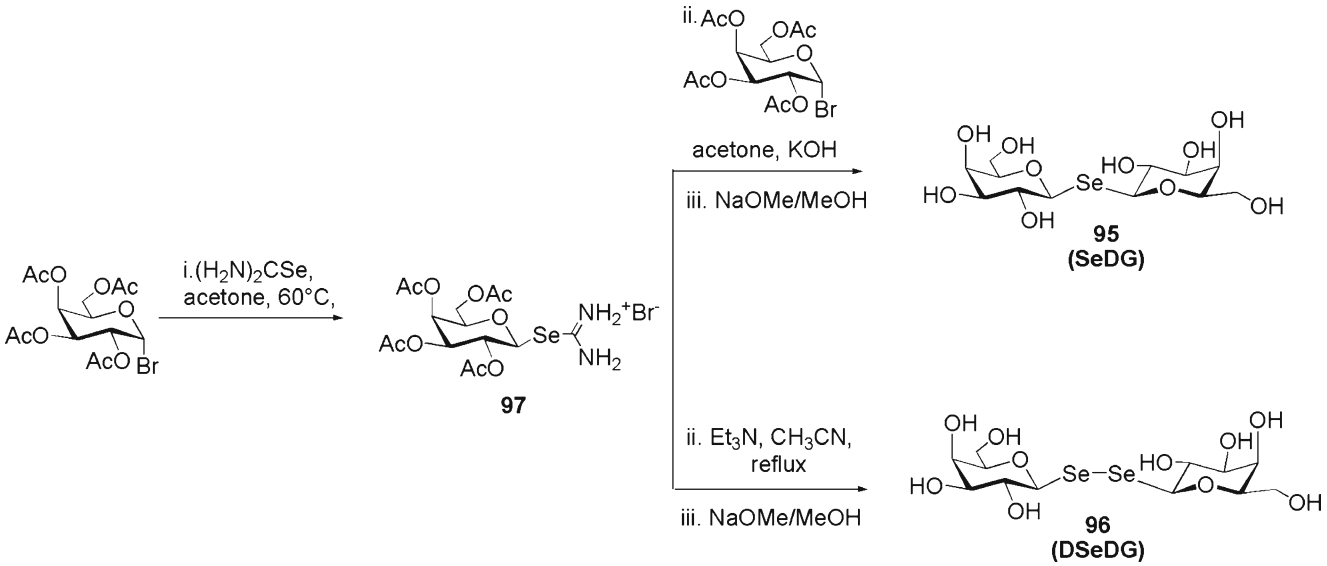
(SW480) in the presence of SeDG/ DSeDG or Lac (1 mM) and labeled galectin-3 (5 μg/mL), both selenides presented better galectin-3 inhibition than lactose, displaying fluorescence binding of 96 % [52]. In addition to inhibitory properties towards galectin-3, selenoglycosides opens up the way for development of new bioanalytical approaches directed to human lectins, such as sensor activity involving ^{77}Se -bearing carbohydrate ligands [52].

Multivalent-based inhibitors

Since lectin-carbohydrate interactions are generally weak, special efforts are often required to achieve tighter binding of carbohydrate-based ligands to lectins. One of the established strategies to improve such interactions is based on the concept of the "glycoside cluster effect", which is related to the number and geometry of carbohydrate residues and also depends on their steric bulk, density and relative

Scheme 13 Synthesis of aromatic thiodigalactosides **84** and **85 a**, and lactosamines **88** and **91 b** and **c**



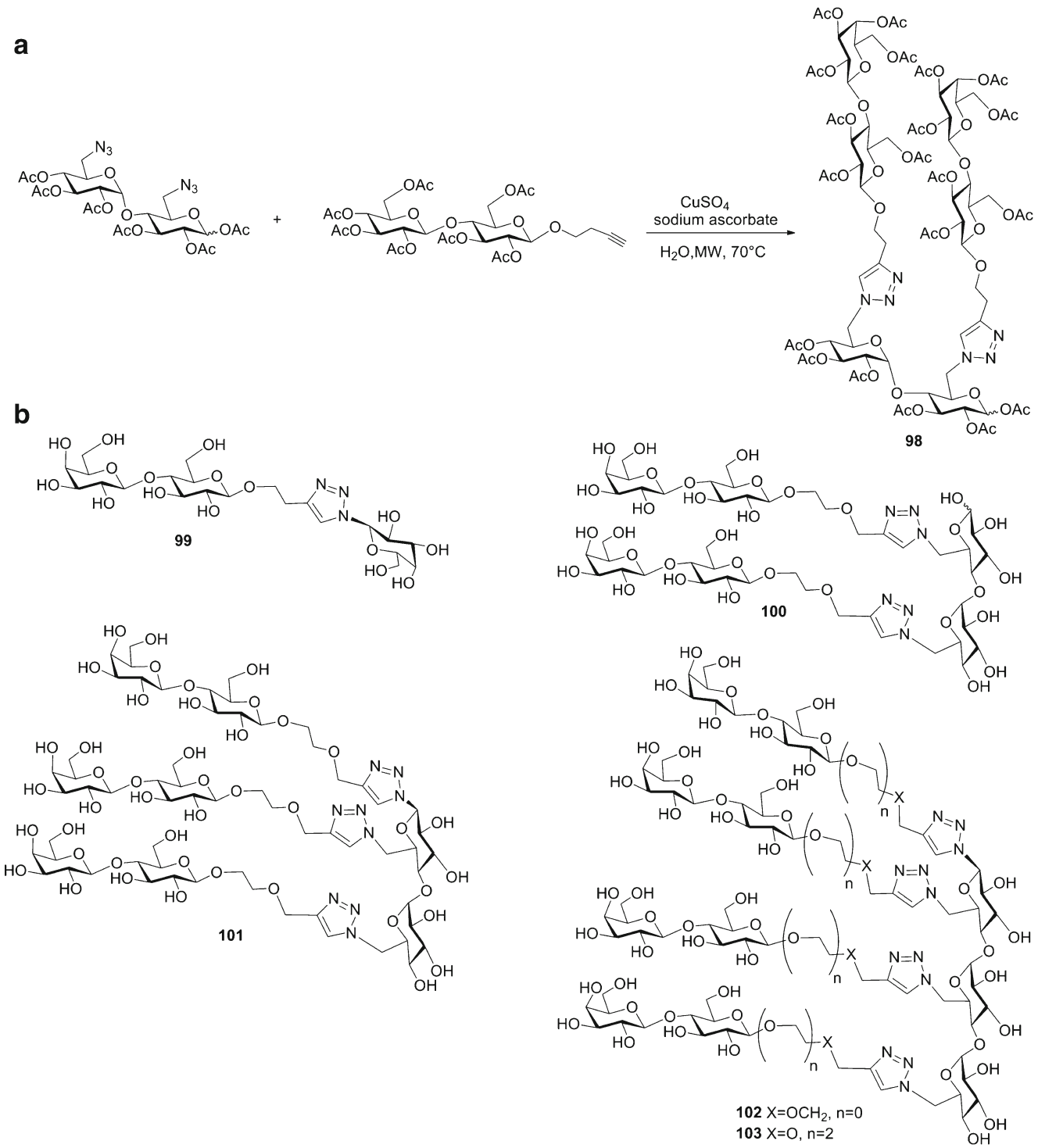


Scheme 14 Synthesis of symmetrical selenodigalactoside (SeDG) **95** and diselenodigalactoside (DSeDG) **96**

distance, as well as on their three-dimensional arrangement [54, 55]. According to this concept, the affinity enhancements observed for multimeric ligands over monomeric species is often greater than predicted from the sum of the constitutive interactions. Within this context, multimeric lactosides based on carbohydrate scaffolds with valences ranging from 1 to 4 and different linker lengths were synthesized by CuAAC reactions as inhibitors of galectin-3 (Scheme 15) [56]. To achieve this purpose, commercially available glucose, maltose and maltotriose were used as core structures to generate multivalency, by means of the preparation of their corresponding azido derivatives, in parallel to the synthesis of lactoside moieties tethered by alkyne-containing oligo(ethylenoglycol) (EG) linkers. Subsequently, click reactions between the obtained azido-sugar cores and alkyne lactosides, in the presence of $CuSO_4$/sodium ascorbate under microwave irradiation, led to a series of mono- and multivalent lactoside triazoles such as compounds **98**–**103** (Scheme 15) [56]. According to competitive fluorescent polarization assays, the highest affinity for galectin-3 was observed for the tetravalent derivative **102** (Kd 16 μM) (Table 1), which showed four times higher inhibitory potency than the corresponding derivative with one lactose residue.

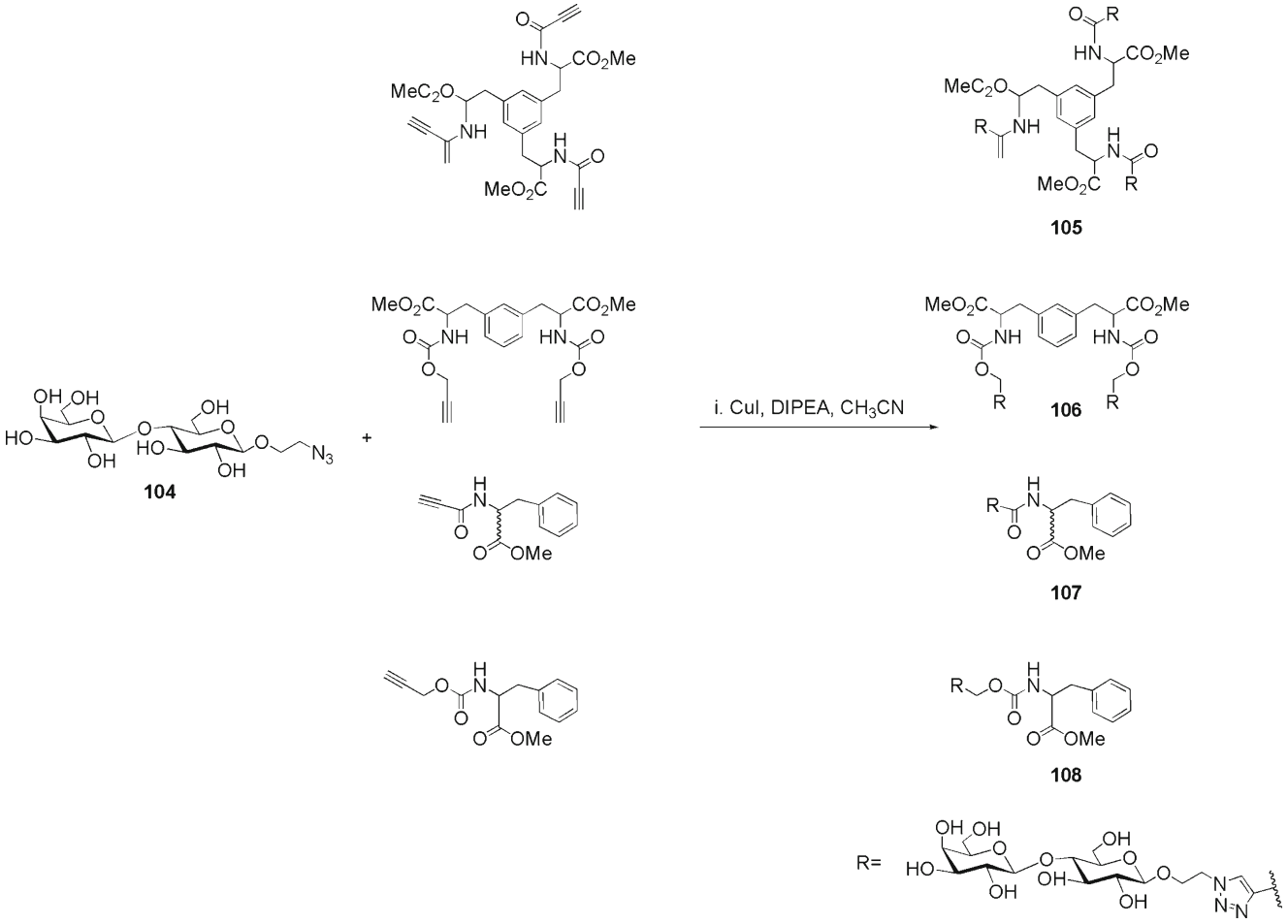
Following the purposes of evaluating the glycoside cluster effect between multivalent ligands and galectin-3, polyfunctional unnatural amino acids like phenyl-bis-alanine (PBA) and phenyl-trisalanine (PTA), bearing two orthogonal functionalities (amine and ester), were employed as scaffolds for the synthesis of a panel of mono-, di- and trivalent lactoside derivatives. Alkyne precursors derived from the PBA and PTA were thus firstly prepared by coupling reactions with propiolic acid and then reacted with 2-azidoethyl-lactose **104**, by means of 1,3-dipolar cycloaddition reaction (copper (I) iodide, DIPEA), generating a series of triazole-lactosides (Scheme 16) [57]. The binding affinities of the obtained compounds to galectin-3 were assessed by competitive fluorescent polarization assays, being the highest affinity verified for compounds **105** (Kd 17 μM) and **106** (Kd 27 μM) (Table 1), which were about 10 times more potent than methyl β-lactoside (Kd 220 μM). Further evaluation of the cluster effect of the trivalent-**105** and divalent-**106** lactoside towards galectin-3 showed cluster effect values around 4.0 and 1.5, when comparing **105** and **106** to the corresponding methyl β-lactoside or to the monomers **107** and **108**. Thus, these different values point out the importance to calculate the cluster effect with respect to a reference monomer, such as **107** or **108**, instead of lactose when analyzing multivalent galectin-3 inhibitors. It's interesting to mention that compound **106** presented higher inhibition toward the closely related galectin-1 (Kd 3.2 μM) and also much stronger cluster effects of 30 and 7.7 when compared to methyl β-lactoside and to monomer **108**, respectively. This stronger binding of **105** and **106** to galectin-1 may be due to additional interactions of the second lactose on another site on the same CRD, or, in a lesser extent, to their possible cluster effect towards this dimeric galectin [58].

In the search for novel galactose-1,2,3-triazole glycoconjugates, the trimeric triazole galactohybrid **109** was synthesized from the sugar 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **110**. Thus, CuAAC reaction between the azidogalactofuranose **110** and the 1,3,5-triethynylbenzene **111** gave the trivalent galacto-cluster **112**, which was then treated with an aqueous solution of trifluoroacetic acid for cleavage of isopropylidene protecting groups, affording the final galactohybrid **109** as a mixture of its α- and β-pyranose forms (Scheme 17). As determined by fluorescence anisotropy assay, compound **109** exhibited interesting galectin-3 binding with Kd 50 μM (Table 1) [59].



Scheme 15 **a** General procedure of Cu(I)-catalyzed reactions between azido-sugar cores and alkyne lactosides, exemplified for **98**. **b** Structures of mono- **99** and multivalent lactoside triazoles **100**–**103**

Envisioning the possibility to interact with two CRDs from distinct galectin-3 monomers, the divalent lactoside represented by 1,2,3-triazole di-lactose-derived glycoconjugate **113** was synthesized as a potential galectin-3 inhibitor. The synthesis of **113** was carried out by click chemistry reaction between the corresponding azide and alkyne precursors **114** and **115**, affording the per-acetylated product **116**, which was then deacetylated (NaOMe/ MeOH) to give the final divalent lactoside **113** (Scheme 18a) [10]. Compound **113** was submitted to surface plasmon resonance (SPR) assays, which increase the chance of multivalency effects due to immobilization of galectin-3 on the SPR surface, being verified strong

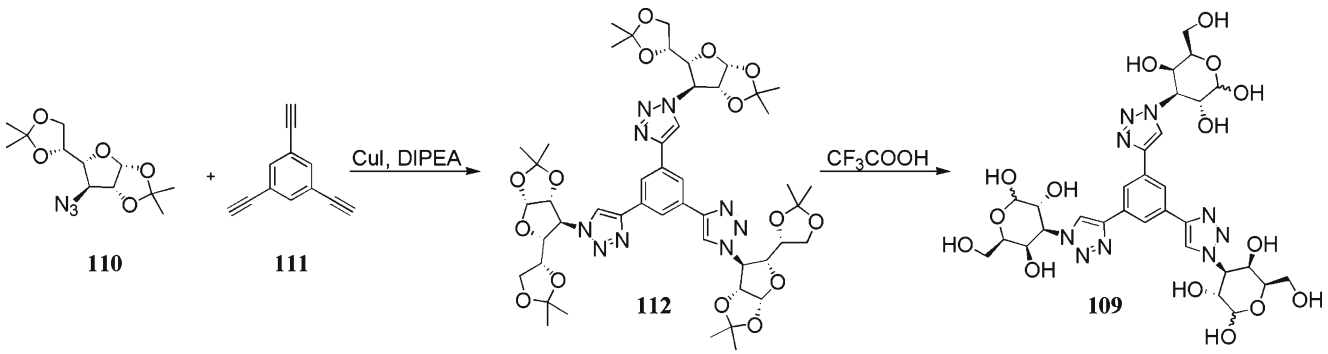


Scheme 16 Synthesis of PBA and PTA-derived lactosides **105–108** by CuAAC reactions

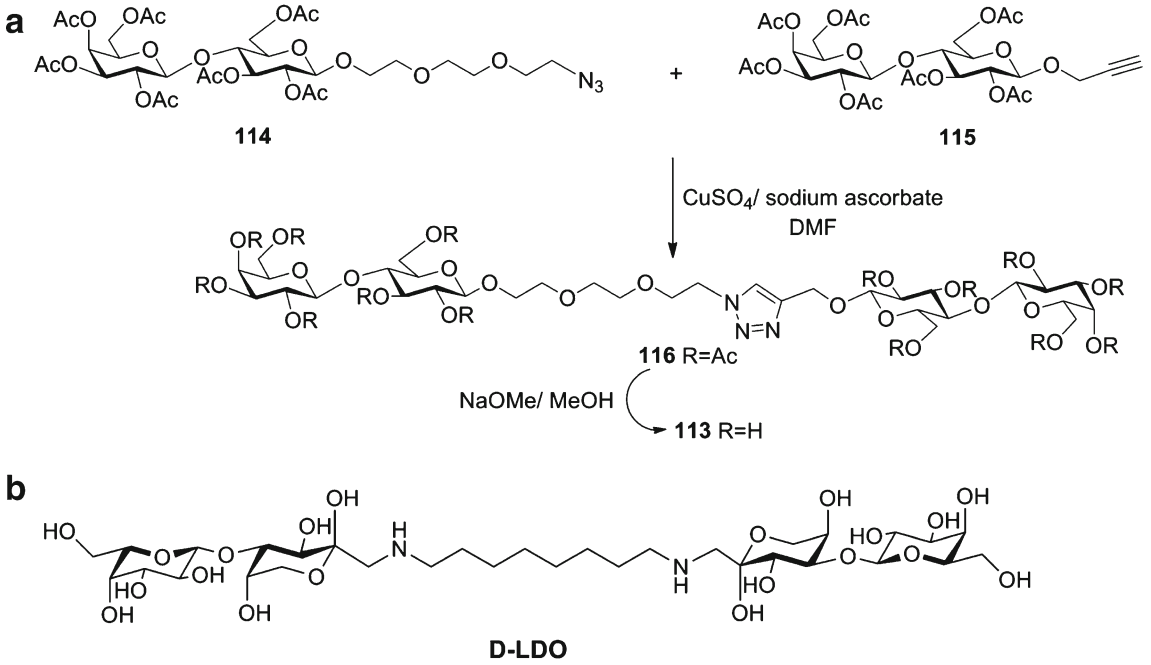
interaction with galectin-3 (Kd 0.15 μM) (Table 1). In fact, molecular dynamics simulations revealed the capacity of glycoconjugate **113** to bridge two independent galectin-3 CRDs, creating a non-covalent cross-link between two monomers, which may explain its submicromolar affinity towards galectin-3 [10].

The structure of glycoconjugate **113** resembles described lactulose amines [60, 61], such as *N*,*N*′-dilactulose-octamethylenediamine (D-LDO) (Scheme 18b), which satisfactorily inhibited the binding of galectin-3 to microwells coated with 90 K (a highly glycosylated protein previously shown to interact with galectin-3), with IC_{50} in the order of 20–40 mM [61].

With the aim to get glycolytically stable lactosides as effective multivalent galectin-3 inhibitors, transition metal-catalyzed cross-coupling reactions have been efficiently used to introduce carbon-carbon bonds [62, 63], generating dimeric thio-lactosides, such as **117**. Compound **117** was synthesized by Glaser Cu(I)-catalyzed homo-coupling reaction between two units of the lactoside alkyne **118**, followed by



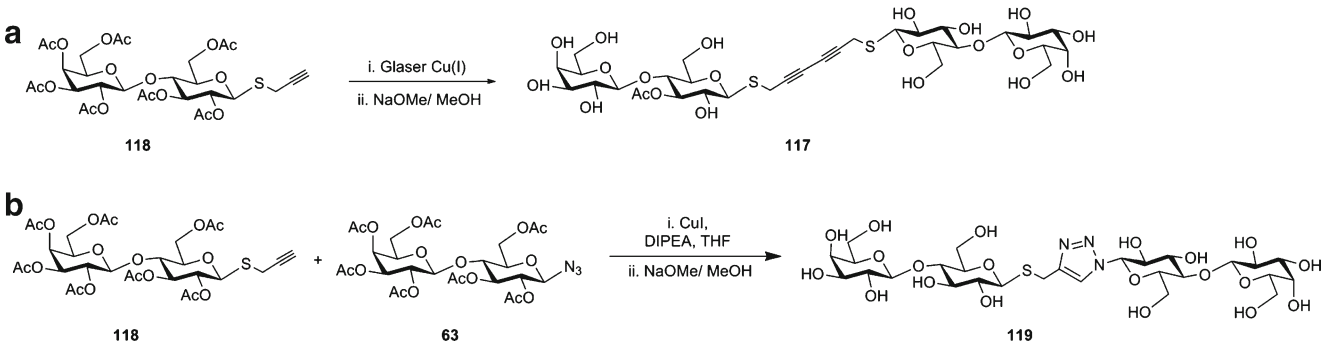
Scheme 17 Synthesis of the trimeric triazole galactohybrid **109** by CuAAC reaction



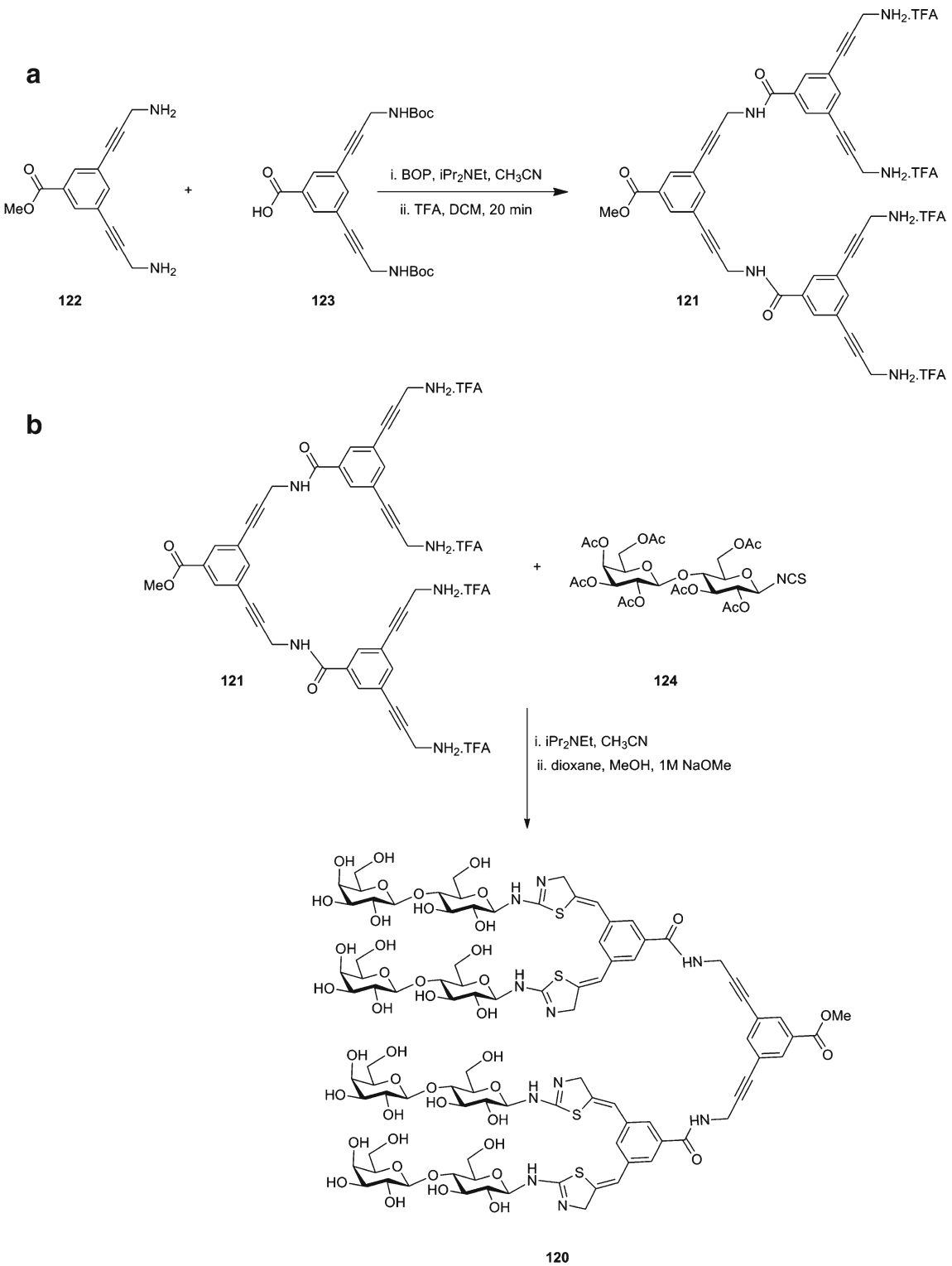
Scheme 18 **a** Synthesis of the 1,2,3-triazole lactose-derived glycoconjugate **113** by click chemistry. **b** Chemical structure of *N*,*N*′-dilactulose-octamethylenediamine (D-LDO)

deacetylation reaction (NaOMe/MeOH) (Scheme 19a). In addition, the Cu(I) catalyzed 1,3-dipolar cycloaddition reaction also proved to be effective to get the glycolytically stable triazole dimeric lactoside **119**, which was obtained after click reaction (CuI/ DIPEA) between the lactoside alkyne **118** and the lactoside azide **63**, and final deacetylation reaction (NaOMe/MeOH) (Scheme 19b). Compounds **117** and **119** were tested by hemagglutination assays at a 1 μM concentration of galectin-3, presenting inhibitory IC_{50} of 160 μM against galectin-3 (Table 1) [64].

Following the concept of multivalency for gaining improved affinity toward galectin-3, the rigid multivalent lactose ligand **120** was prepared by a synthetic procedure, which mechanism involves the initial formation of thiourea-linked sugar, with subsequent conversion to a rigid 2-aminothiazoline moiety. Therefore, the synthesis of **120** started with the previous preparation of the alkyne-branched tetraamino **121** by conjugation of the building blocks **122** and **123**, containing the corresponding free amino and carboxyl groups, in the presence of the coupling reactant BOP (Scheme 20a). Subsequently, compound **121** was reacted with lactose β-isothiocyanate **124** in acetonitrile/*i*Pr_2Net and then deacetylated (NaOMe/MeOH), yielding the tetravalent lactose derivative **120** (Scheme 20b). The inhibitory potency of compound **120** toward galectin-3 was determined with the use of a solid phase based assay, according to what the biotin-labeled galectin-3 was allowed to bind to the carbohydrate moieties of asialofetuin (ASF) coated onto the surface of microtiter plate wells (the matrix) in competition with the lactoside **120**. It was verified an IC_{50} of 0.07 μM for compound **120**, representing an enhancement, relative to lactose, of almost 4300-fold. However, under a direct binding assay with galectin-3 using Trp fluorescence (fluorescence titration), an apparent Kd value of 14 μM was observed for compound **120**, corresponding



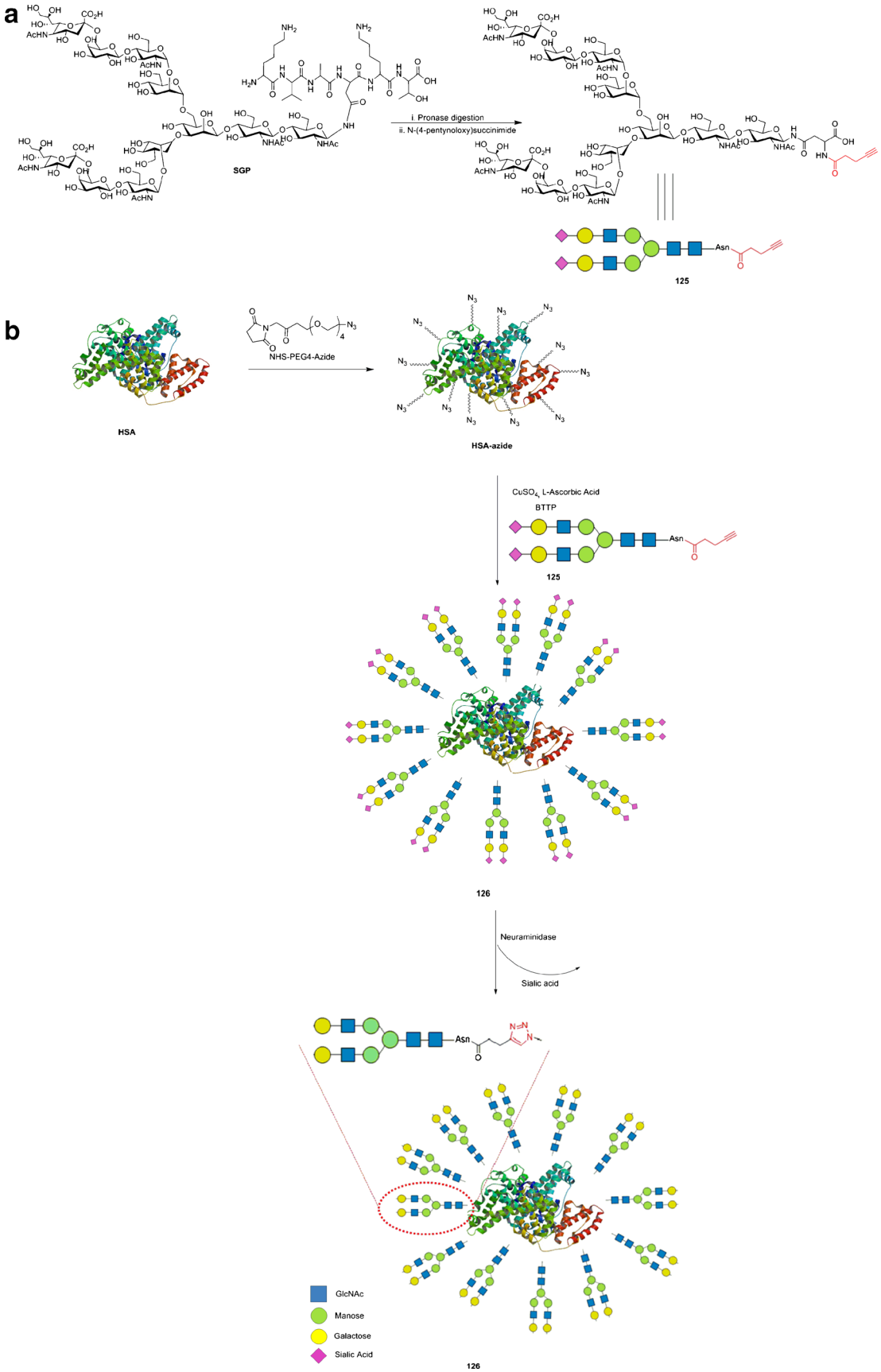
Scheme 19 Synthesis of multivalent dimeric lactosides **117** **a** and **119** **b**



Scheme 20 **a** Synthesis of the alkyne-branched tetraamino **121**. **b** Synthesis of the tetravalent lactose derivative **120**

to much lower potency (44-fold relative to lactose) if compared to the previous solid phase assay (Table 1) [65].

The synthesis of selective multivalent glycoprotein-based glyco-ligands as galectin-3 inhibitors may have a great impact on tumor-related mechanisms mediated by this lectin [66]. In this regard, human serum albumin (HSA) was used as the scaffold and an Asn-linked complex type *N*-glycan was used as the glycan building block. Sialoglycopeptide (SGP), isolated from chicken egg yolk, was thus used as starting glycan by means of previous treatment with pronase to remove the peptide portion,

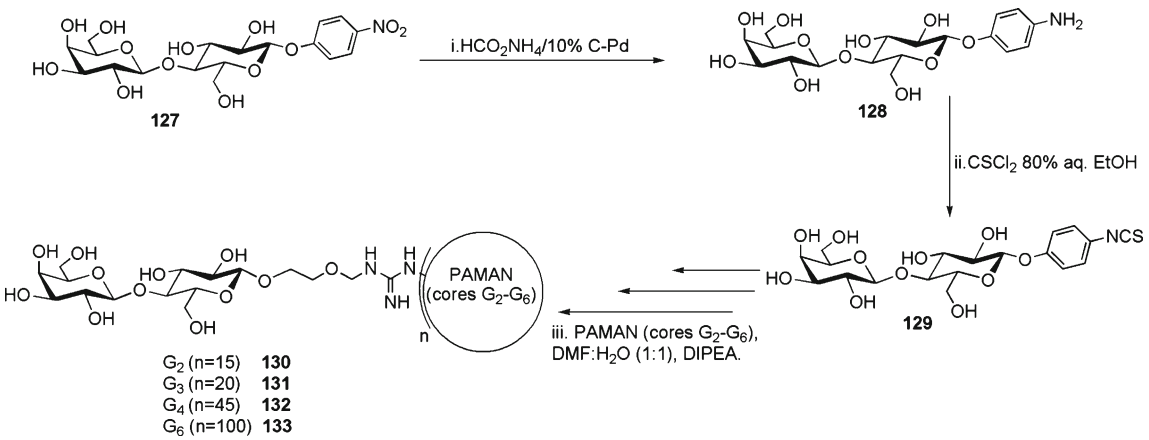


Scheme 21 **a** Synthesis of alkyne-labeled N-glycan **125**. **b** Synthesis of the HSA-based glycoprotein **126**

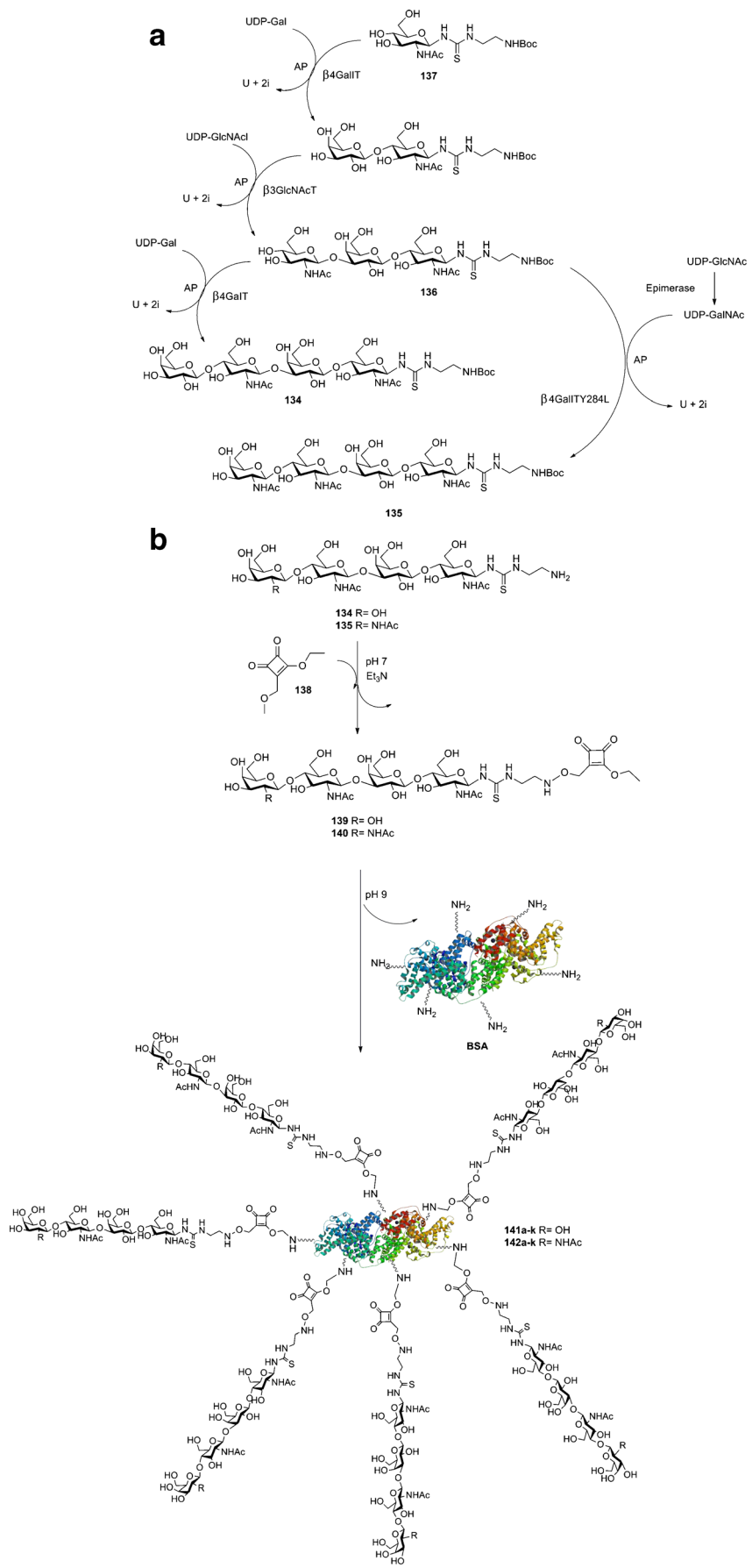
leaving the asparagine (Asn)-linked *N*-glycan (SCT-Asn), which was then labeled with an alkyne group by reaction with an activated ester, *N*-(4-pentynoyloxy)succinimide in an aqueous solution (Scheme 21a). In paralell, HSA, containing free lysine residues on its surface, was tagged with azide functionality by treatment with an excess of the activated azide derivative, NHS-PEG4-azide, in an aqueous $NaHCO_3$ solution resulting in the introduction of multiple azide groups in the protein (Scheme 21b). Subsequently, the SCT-Asn-alkyne **125** and HSA-azide were conjugated via Cu(I)-catalyzed alkyne-azide cycloaddition reaction, utilizing $CuSO_4$, L-ascorbic acid and the copper ligand BTTP, in phosphate buffer (pH .5), affording the HSA-based neoglycoprotein **126**, which was treated with neuraminidase for removal of sialic acids and exposure of galactose moieties (Scheme 21b). The HSA-Gal glycoprotein **126**, containing about 20 *N*-glycans, showed high affinity to galectin-3 in SPR assays, with EC_{50} 0.073 μM (Table 1), demonstrating its strong multivalent effect on galectin-3 inhibition. In addition, the inhibition of the attachment of galectin-3 to immobilized cancer cells by HSA-Gal glycoprotein **126** was assessed by ELISA assays, which showed that **126** was able to block the attachment of galectin-3 to both PC3 prostate and A549 alveolar cancer cells in a dose-dependent manner (IC_{50} for PC3 cells, 0.122 μM; IC50 for A549 cells, 0.338 μM), suggesting that this synthetic HSA based glyco-ligand may find applications for blocking the function of galectin-3 in distinct tumor processes such as cancer cell aggregation, adhesion and metastasis [67].

The involvement of tumor-related galectin-3 is also known to be related to its capacity to bind to TF antigen on MUC1 mucin, a large transmembrane protein that is overexpressed and heavily glycosylated on cancer cells [68]. In fact, clustering formation in response to MUC1-galectin binding exposes adhesion molecules and increases homotypic aggregation with heterotypic adhesion to endothelium, which favors tumor escape in the circulation and eventually metastasis [69]. In this context, galectin-3 inhibitors represented by multivalent lactose-functionalized dendrimers may have a fundamental role on the elucidation of the mechanism by which cancer cellular aggregation occurs. Lactose-functionalized poly(amidoamine) (PAMAM) dendrimers were thus prepared by a synthetic route involving initial catalytic transfer hydrogenation (ammonium formiate/ 10 % Pd-C) of *p*-nitrophenyl-β-D-lactoside **127**, giving the *p*-aminophenyl derivative **128**, which was then treated with thiophosgene in 80 % aqueous ethanol for conversion to the isothiocyanate **129**. Subsequently, **129** was submitted to conjugation reaction with dendritic PAMAM, in a mixture of DMF:H_2O (1:1) containing DIPEA, to afford glycodendrimers **130**–**133**, displaying 15 to 100 lactose end groups attached to the dendrimer framework (Scheme 22) [70, 71]. The effects of lactose-functionalized dendrimers **130**–**133** on cellular aggregation in three different cancer cell lines (A549, DU-145, and HT-1080) and in the presence of galectin-3, were then evaluated by cells image analysis, Western blotting and fluorescence microscopy. According to the obtained results, the smaller dendrimer **130** inhibited galectin-3 induced aggregation for all three tested cancer cell lines, whereas the larger dendrimer **133** caused cancer cells to aggregate through a galectin-3 pathway. These distinct observed effects indicate that inhibition by **130** occurred in a competitive mode, diverting galectin-3 binding to TF antigen on MUC1, while dendrimer **133** provided too many sites for galectin-3, increasing cross-linking of the cells and thus enhancing aggregation [71]. Thus, aggregation can be favored or not depending on the number of lactose groups on the dendrimer [71].

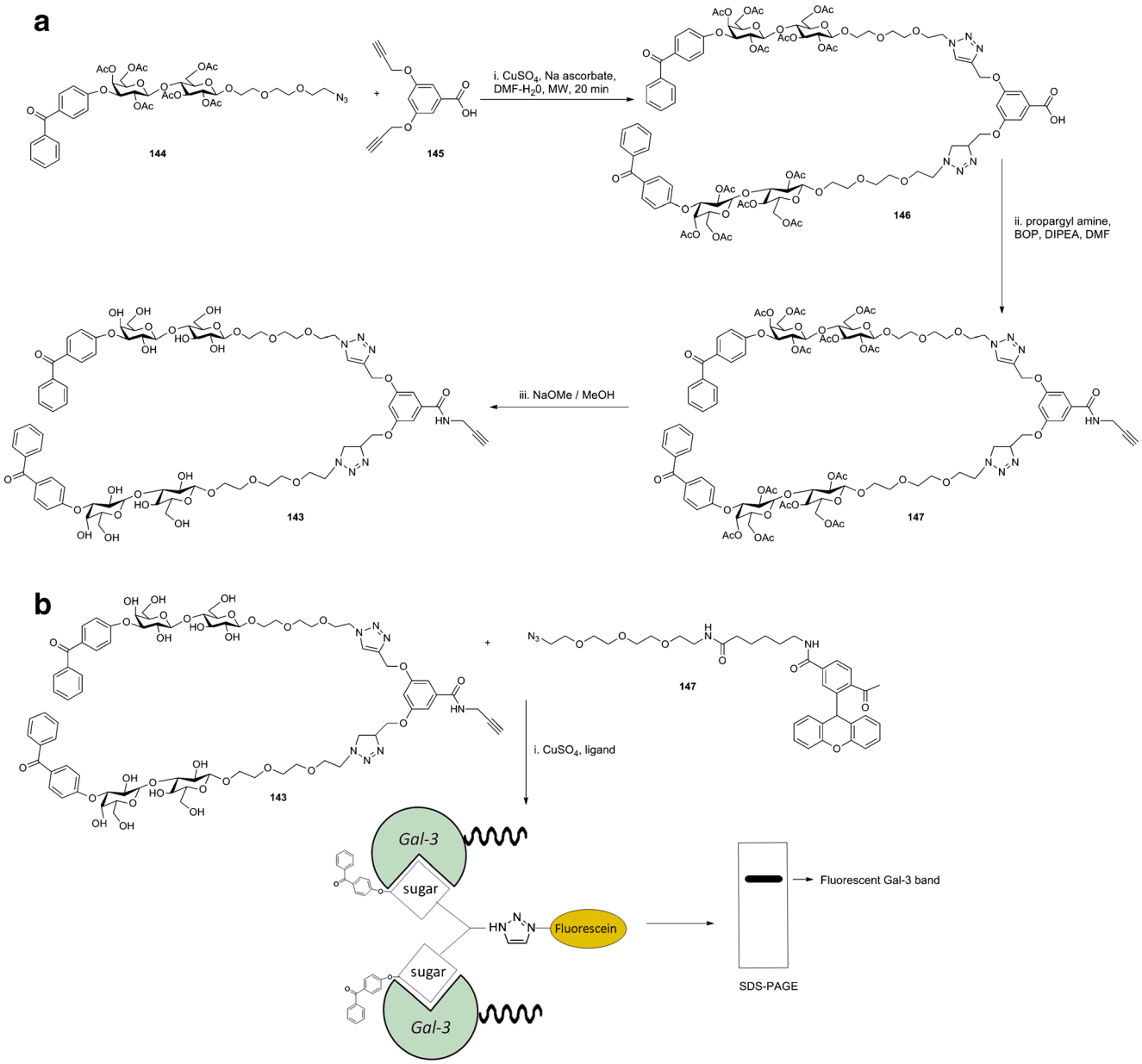
Considering the potential of multivalent glycan ligands to improve binding affinity towards galectin-3, designed neoglycoproteins constituted by two kinds of tetrasaccharides (LacNAc-LacNAc **134** or LacDiNAc-LacNAc **135**) and having bovine serum albumin (BSA) as scaffold for multivalent presentation were prepared by combination of chemoenzymatic synthesis and chemical conjugation to lysine residues of BSA [72]. Initially, the trisaccharide intermediate **136** was synthesized from the monosaccharide GlcNAc-linker-*t*Boc **137**, in a sequential chemo-enzymatic route involving



Scheme 22 Synthesis of lactose-functionalized dendrimers **130–133**



Scheme 23 **a** Chemo-enzymatic synthesis of tetrasaccharides **134** and **135** using activated nucleotide sugars as donor substrates and recombinant glycosyltransferases for complete conversions. **b** Two-step synthesis of neoglycoproteins **141a**–**k** and **142a**–**k**



Scheme 24 **a** Synthesis of the probe **143** using click chemistry strategy. **b** Schematic representation of galectin-3 detection by treatment of Gal-3-probe formed complex with the fluorescent reporter **147**

the glycosyltransferases β4GalT (galactosyltransferase) and β3GlcNAcT (*N*-acetylglucosaminyltransferase), and the corresponding UDP-Gal and UDP-GlcNAc activated nucleotide sugars as donor substrates (Scheme 23a). Once the intermediate **136** was obtained, it was reacted with the donors UDP-Gal and UDP-GalNAc, respectively, in the presence of the corresponding enzymes β4GalT and β4GalTY284L (*N*-acetylgalactosaminyltransferase), affording the tetrasaccharides LacNAc-LacNAc **134** and LacDiNAc-LacNAc **135**, respectively (Scheme 23a) [72–74]. In order to favor further coupling to BSA, tetrasaccharides **134** and **135** were previously reacted with squaric acid diethyl ester **138** (Et_3N/ EtOH) to form the corresponding squaric acid monoamide esters **139** and **140** (Scheme 23b). Subsequently, conjugation of **139** and **140** to lysine groups of BSA was carried out in borate buffer (pH .0), with an incubation period of six days, affording neo-glycoproteins **141a-k** and **142a-k** with variable numbers of LacNAc-LacNAc and LacDiNAc-LacNAc, reaching numbers for modified lysine residues between 2 and 29 per BSA molecule (Scheme 23b). The binding properties of neo-glycoproteins **141a-k** and **142a-k** as ligands for human galectin-3 were then evaluated by an ELISA-type assay, based on the incubation of different amounts of galectin-3 (2–12 μM) on immobilized neo-glycoproteins (5 pmol per well). According to the obtained results, lowered Kd values were verified for **142d-f** with about 9, 14 and 21

LacDiNAc-LacNAc glycans, respectively, reaching a Kd value for galectin-3 in the nanomolar range (30 nM for **142f**) (Table 1). Thus, these data emphasize high selectivity of LacDiNAc-LacNAc glycans toward galectin-3, finding important biomedical applications, especially in cancer related research [72].

Considering the correlation of galectin-3 expression with cancer aggressiveness, metastasis and apoptosis, it can be considered an emerging cancer marker [75]. Therefore, the development of chemical probes is of great relevance for detection of the cancer-linked galectin-3. In fact, they represent alternative tools to the often used antibody-based methods to detect active galectin-3 [76]. In this context, the synthesis of multivalent probe **143** was initiated by linking the lactoside azide **144** to the divalent alkyne **145** using click chemistry reaction ($CuSO_4$/ sodium ascorbate) (Scheme 24a). Subsequently, an alkyne group was introduced into the obtained triazolic compound **146** by means of a BOP coupling of propargyl amine to the scaffold carboxylic acid function, giving compound **147**, which was then deacetylated (NaOMe/ MeOH) to afford the probe **143**. For probe evaluation, distinct biological protein mixtures containing galectin-3 were sequentially treated with varying concentrations of the probe **143** and the fluorescein-N_3 construct **147** (reporter molecule) (Scheme 24b). Based on what was verified in SDS-PAGE, the obtained fluorescent gel images showed a strong and dominant band corresponding to galectin-3 in the tested protein mixtures, being the most favourable concentration of probe **143** determined to be around 5 μM, with a two-fold excess of the N_3 reporter **147**. In fact, this type of probe in combination with various reporter molecules may represent a valuable tool for deciphering galectin-3 roles, especially in cancer, besides aiding in its diagnosis [76].

Conclusions

In this review we presented relevant synthetic routes for obtaining monosaccharide-, disaccharide- and multivalent-based galectin-3 inhibitors, involving regioselective modifications of distinct carbohydrate scaffolds (galactose, mannose, talose, lactose or thiodigalactose) and glycoproteins. Cu(I)-assisted 1,3-dipolar azide-alkyne cycloaddition (CuAAC) reactions were predominantly applied to the synthesis of the described inhibitors, allowing the insertion of varying substituent groups at different sugar positions via triazole moiety. When comparing the inhibitory activities towards galectin-3, thiodigalactoside derivatives, especially those containing aromatic aglycons, showed to be the most effective galectin-3 inhibitors, highlighting their capacity to interact with extended galectin-3 subsites. In general, multivalent carbohydrate ligands strongly inhibited galectin-3 and it is also worth mentioning that lower Kd values were verified for multivalent inhibitors tested by solid phase assays such as ELISA and SPR, which can be explained by increased chances of multivalency effects when immobilizing galectin-3 on a solid surface.

Taken together, the synthetic glycoconjugates described in this review represent frontline galectin-3 inhibitors, finding important therapeutic and diagnostic applications in galectin-3 mediated tumor processes, and open up the way for the design and synthesis of novel galectin-3 inhibitors candidates.

Acknowledgments We acknowledge the financial support and fellowships from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - Brazil) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Boligan K.F., Mesa C., Fernandez L.E., von Gunten S.: Cancer intelligence acquired (CIA): tumor glycosylation and sialylation codes dismantling antitumor defense. Cell. Mol. Life Sci. **72**, 1231–1248 (2015)
2. Dall'Olio F., Malagolini N., Trinchera M., Chiricolo M.: Mechanisms of cancer-associated glycosylation changes. Front. Biosci. (Landmark Ed). **17**(670–699), (2012)
3. Stowell S.R., Ju T., Cummings R.D.: Protein glycosylation in cancer. Annu. Rev. Pathol. **10**, 473–510 (2015)
4. Nakahara S., Raz A.: Biological modulation by lectins and their ligands in tumor progression and metastasis. Anti Cancer Agents Med. Chem. **8**, 22–36 (2008)
5. Boscher C., Dennis J.W., Nabi I.R.: Glycosylation, galectins and cellular signaling. Curr. Opin. Cell Biol. **23**, 383–392 (2011)
6. Garner O.B., Baum L.G.: Galectin-glycan lattices regulate cell-surface glycoprotein organization and signalling. Biochem. Soc. Trans. **36**, 1472–1477 (2008)
7. Hockl P.F., Wolosiuk A., Sáez J.M., Bordoni A.V., Croci D.O., Terrones Y.T., Illia G.J., Rabinovich G.A.: Glyco-nano-oncology: Novel therapeutic opportunities by combining small and sweet. Pharmacol. (2016). doi:10.1016/j.phrs.2016.02.005
8. Ahmed H., AlSadek D.M.: Galectin-3 as a potential target to prevent cancer metastasis. Clin. Med. Insights Oncol. **25**, 113–121 (2015)
9. Arthur, C. M.; Baruffi, M. D.; Cummings, R. D.; Stowell, S. R.: Galectins: methods and protocols. Stowell, S. R.; Cummings, R. D. (eds.); human press, Vol. 1, Chapter 1, pp 1–35 (2015)
10. Marchiori M.F., Souto D.E., Bortot L.O., Pereira J.F., Kubota L.T., Cummings R.D., Baruffi M.D., Carvalho I., Campo V.L.: Synthetic 1,2,3-triazole-linked glycoconjugates bind with high affinity to human galectin-3. Bioorg. Med. Chem. **23**, 3414–3425 (2015)
11. van den Brüle F., Califice S., Castronovo V.: Expression of galectins in cancer: a critical review. Glycoconj. J. **19**, 537–542 (2004)
12. Liu F.T., Rabinovich G.A.: Galectins as modulators of tumour progression. Nat. Rev. Cancer. **5**, 29–41 (2005)

13. Califice S., Castronovo V., Bracke M., *et al*.: Dual activities of galectin-3 in human prostate cancer: tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3. Oncogene. **23**, 7527–7536 (2004)
14. Sun W., Li L., Yang Q., Shan W., Zhang Z., Huang Y.: G3-C12 peptide reverses galectin-3 from foe to friend for active targeting cancer treatment. Mol. Pharm. **12**, 4124–4136 (2015)
15. Cecchinelli B., Lavra L., Rinaldo C., Iacovelli S., Gurtner A., Gasbarri A., Ulivieri A., Del Prete F., Trovato M., Piaggio G., Bartolazzi A., Soddu S., Sciacchitano S.: Repression of the antiapoptotic molecule galectin-3 by homeodomain-interacting protein kinase 2-activated p53 is required for p53-induced apoptosis. Mol. Cell. Biol. **26**, 4746–4757 (2006)
16. Yang R.Y., Rabinovich G.A., Liu F.T.: Galectins: structure, function and therapeutic potential. Expert. Rev. Mol. Med. (2008). doi:10.1017/S1462399408000719
17. Leffler H., Carlsson S., Hedlund M., Qian Y., Poirier F.: Introduction to galectins. Glycoconj. J. **19**, 433–440 (2004)
18. Knibbs R.N., Agrwal N., Wang J.L., Goldstein I.J.: Carbohydrate-binding protein 35. II. Analysis of the interaction of the recombinant polypeptide with saccharides. J. Biol. Chem. **268**, 14940–14947 (1993)
19. Tejler J., Salameh B., Lefflerc H., Nilsson U.J.: Fragment-based development of triazole-substituted O-galactosyl aldoximes with fragment-induced affinity and selectivity for galectin-3. J. Org. Biomol. Chem. **7**, 3982–3990 (2009)
20. Bum-Erdene K., Gagarinov I.A., Collins P.M., Winger M., Pearson A.G., Wilson J.C., Leffler H., Nilsson U.J., Grice I.D., Blanchard H.: Investigation into the feasibility of thioditaloside as a novel scaffold for galectin-3-specific inhibitors. Chembiochem. **14**, 1331–1339 (2013)
21. Stowell S.R., Karmakar S., Stowell C.J., Baruffi M.D., McEver R.P., Cummings R.D.: Human galectin-1, −2, and −4 induce surface exposure of phosphatidylserine in activated human neutrophils but not in activated T cells. Blood. **109**, 219–227 (2007)
22. Rapoport E.M., Kurmyshkina O.V., Bovin N.V.: Mammalian galectins: structure, carbohydrate specificity, and functions. Biochem. Mosc. **73**, 393–405 (2008)
23. Giguere D., Andre S., Bonin M.A., Bellefleur M.A., Provençal A., Cloutier P., Pucci B., Roy R., Gabius H.J.: Inhibitory potential of chemical substitutions at bioinspired sites of β-D-galactopyranose on neoglycoprotein/cell surface binding of two classes of medically relevant lectins. J. Bioorg. Med. Chem. Lett. **19**, 3280–3287 (2011)
24. Cumpstey I., Sundin A., Leffler H., Nilsson U.J.: C2-symmetrical thiodigalactoside bis-benzamido derivatives as high-affinity inhibitors of galectin-3: efficient lectin inhibition through double arginine-arene interactions. J. Angew. Chem. Int. Ed. **44**, 5110–5112 (2005)
25. Cumpstey I., Salomonsson E., Sundin A., Leffler H., Nilsson U.J.: Double affinity amplification of galectin-ligand interactions through arginine-arene interactions: synthetic, thermodynamic, and computational studies with aromatic diamido thiodigalactosides. Chem. **14**, 4233–4245 (2008)
26. Aragão-Leoneti V., Campo V.L., Gomes A.S., Field R.A., Carvalho I.: Application of copper(I)-catalysed azide/alkyne cycloaddition (CuAAC) 'click chemistry' in carbohydrate drug and neoglycopolymer synthesis. Tetrahedron. **66**, 9475–9492 (2010)
27. Tiwari V.K., Mishra B.B., Mishra K.B., Mishra N., Singh A.S., Chen X.: Cu-catalyzed click reaction in carbohydrate chemistry. Chem. Rev. **116**, 3086–3240 (2016)
28. Blanchard H., Yu X., Collins P.M., Bum-Erdene K.: Galectin-3 inhibitors: a patent review (2008-present). Expert Opin. Ther. Pat. **24**, 1053–1065 (2008)
29. Téllez-Sanz R., García-Fuentes L., Vargas-Berenguel A.: Human galectin-3 selective and high affinity inhibitors. Present state and future perspectives. Curr. Med. Chem. **20**, 2979–2990 (2013)
30. Yang R.-Y., Rabinovich G.A., Liu F.-T.: Galectins: structure, function and therapeutic potential. Expert Rev. Mol. Med. **10**, e17 (2008)
31. Nakahara S., Raz A.: Biological Modulation by lectins and their ligands in tumor progression and metastasis. Anti Cancer Agents Med. Chem. **8**, 22–36 (2008)
32. Klyosov A.A., Traber P.G.: Galectins and disease implications for targeted therapeutics. ACS Symposium Series, American Chemical Society, Washington (2012)
33. Radosavljevic G.D., Pantic J., Jovanovic I., Lukic M.L., Arsenijevic N.: The two faces of galectin-3: roles in various pathological conditions. Ser. J. Exp. Clin. Res. **17**, 1–1 (2016)
34. Giguére D., Patnam R., Bellefleur M.-A., St-Pierre C., Sato S., Roy R.: Carbohydrate triazoles and isoxazoles as inhibitors of galectins-1 and −3. Chem. Commun. **14**, 2379–2381 (2006)
35. Tejler J., Skogman F., Lefflerc H., Nilsson U.J.: Synthesis of galactose-mimicking 1H-(1,2,3-triazol-1-yl)- mannosides as selective galectin-3 and 9 N inhibitors. Carbohydr. Res. **342**, 1869–1875 (2007)
36. Compagno D., Jaworski F.M., Gentilini L., Contrufo G., González Pérez I., Elola M.T., Pregi N., Rabinovich G.A., Laderach D.J.: Galectins: major signaling modulators inside and outside the cell. Curr. Mol. Med. **14**, 630–651 (2014)
37. Bum-Erdene K., Gagarinov I.A., Collins P.M., Winger M., Pearson A.G., Wilson J.C., Leffler H., Nilsson U.J., Grice I.D., Blanchard H.: Investigation into the feasibility of thioditaloside as a novel scaffold for galectin-3-specific inhibitors. ChemBiolChem. **14**, 1331–1342 (2013)
38. Salameh B.A., Cumpstey I., Sundin A., Leffler H., Nilsson U.J.: 1*H*-1,2,3-triazol-1-yl thiodigalactoside derivatives as high affinity galectin-3 inhibitors. Bioorg. Med. Chem. **18**, 5367–5378 (2010)
39. Fort S., Kim H.-S., Hindsgaul O.: Screening for galectin-3 inhibitors from synthetic lacto-N-biose libraries using microscale affinity chromatography coupled to mass spectrometry. J. Organomet. Chem. **71**, 7146–7154 (2006)
40. Öberg C., Blanchard H., Leffler H., Nilsson U.J.: Protein subtype-targeting through ligand epimerization: talose-selectivity of galectin-4 and galectin-8. Bioorg. Med. Chem. Lett. **18**, 3691–3694 (2008)
41. Öberg C., Noresson A.-L., Leffler H., Nilsson U.J.: Synthesis of 3-amido-3-deoxy-beta-D-talopyranosides: all-cis-substituted pyranosides as lectin inhibitors. Tetrahedron. **67**, 9164–9172 (2011)
42. Collins P.M., Oberg C.T., Leffler H., Nilsson U.J., Blanchard H.: Taloside inhibitors of galectin-1 and galectin-3. Chem. Biol. Drug Des. **79**, 339–346 (2012)
43. van Hattum H., Branderhorst H.M., Moret E.E., Nilsson U.J., Leffler H., Pieters R.J.: Tuning the preference of thiodigalactoside- and lactosamine-based ligands to galectin-3 over galectin-1. J. Med. Chem. **56**, 1350–1354 (2013)
44. Cumpstey I., Solomonsson E., Sundin A., Leffler H., Nilsson U.J.: Double affinity amplification of galectin–ligand interactions through arginine–arene interactions: synthetic, thermodynamic, and computational studies with aromatic diamido thiodigalactosides. Chem. Eur. J. **14**, 4233–4245 (2008)
45. Öberg C.T., Leffler H., Nilsson U.J.: Arginine binding motifs: design and synthesis of galactose-derived arginine tweezers as galectin-3 inhibitors. J. Med. Chem. **51**, 2297–2301 (2008)
46. Öberg C.T., Leffler H., Nilsson U.J.: Inhibition of galectins with small molecules. Chimia. **65**, 18–23 (2011)
47. Guigere D., Sato S., St-Pierre C., Sirois S., Roy R.: Aryl O- and S-galactosides and lactosides as specific inhibitors of human galectins-1 and −3: role of electrostatic potential at O-3. Bioorg. Med. Chem. Lett. **16**, 1668–1672 (2006)
48. Rauthu S.R., Shiao T.C., André S., Miller M.C., Madej E., Mayo K.H., Gabius H.-J., Roy R.: Defining the potential of aglycone modifications for affinity/selectivity enhancement against

medicallyrelevant lectins: synthesis, activity screening, and HSQCBased NMR analysis. Chembiochem. **16**, 126–139 (2015)
49. Bianchet M.A., Ahmed H., Vasta G.R., Amzel L.M.: Soluble β-galactosyl-binding lectin (galectin) from toad ovary: crystallographic studies of two protein-sugar complexes. Proteins: Struct., Funct., Bioinf. **40**, 378–388 (2002)
50. Pieters R.J.: Inhibition and Detection of Galectins. ChemBioChem. **7**, 721–728 (2006)
51. van Scherpen zeel M., Moret E.E., Ballell L., Liskamp R.M.J., Nilsson U.J., Leffler H., Pieters R.J.: Synthesis and Evaluation of New Thiodigalactoside-Based Chemical Probes to Label Galectin-3. ChemBioChem. **10**, 1724–1733 (2009)
52. André S., Kövér K.E., Gabius H.-J., Szilágyi L.: Thio- and selenoglycosides as ligands for biomedically relevant lectins: Valency–activity correlations for benzene-based dithiogalactoside clusters and first assessment for (di)selenodigalactosides. Bioorg. Med. Chem. Lett. **25**, 931–935 (2015)
53. Wagner G., Nuhn P.: Synthese von Selenoglykosiden mit Acetyl-glykosyl-isoselenuronium-bromiden. Arch. Pharm. **297**, 461–473 (1964)
54. Galante E., Geraci C., Sciuto S., Campo V.L., Carvalho I., Sesti-Costa R., Guedes P.M.M., Silva J.S., Hill L., Nepogodiev S.A., Field R.A.: Glycoclusters presenting lactose on calix[4]arene cores display trypanocidal activity. Tetrahedron. **67**, 5902–5912 (2011)
55. Campo, V. L., Carvalho, I.: Click chemistry applied to carbohydrate-based drug discovery click chemistry in Glycoscience. New developments and strategies. In: Witczak, Z. J., Bielski, R., (eds); Wiley; Vol. 1, Chapter 13, pp 325–358 (2013)
56. Gouin S.G., Fernández J.M.G., Vanquelef E., Dupradeau F.-Y., Salomonsson E., Leffler H., Ortega-Muñoz M., Nilsson U.J., Kovensky J.: Multimeric lactoside "click clusters" as tools to investigate the effect of linker length in specific interactions with peanut lectin, galectin-1, and −3. Chembiochem. **11**, 1430–1442 (2010)
57. Tejler J., Tullberg E., Frejd T., Leffler H., Nilsson U.J.: Synthesis of multivalent lactose derivatives by 1,3-dipolar cycloadditions: selective galectin-1 inhibition. Carbohydr. Res. **341**, 1353–1362 (2006)
58. Salomonsson E., Larumbe A., Tejler J., Tullberg E., Rydberg H., Sundin A., Khabut A., Frejd T., Lobsanov Y.D., Rini J.M., Nilsson U.J., Leffler H.: Monovalent interactions of galectin-1. Biochemistry. **49**, 9518–9532 (2010)
59. Mackeviča, J., Ostrovskis, P., Leffler, H., Nilsson, U. J., Rudovica, V., Viksna, A., Belyakov, S., Turks, M.: Synthesis of 1,2,3-triazole-linked galactohybrids and their inhibitory activities on galectins. ARKIVOC (iii) 90–112 (2014)
60. Glinsky G.V., Mossine V.V., Price J.E., Bielenberg D., Glinsky V.V., Ananthaswamy H.N., Feather M.S.: Inhibition of colony formation in agarose of metastatic human breast carcinoma and melanoma cells by synthetic glycoamine analogs. Clin. Exp. Metastasis. **14**, 253–267 (1996)
61. Rabinovich G.A., Cumashi A., Bianco G.A., Ciavardelli D., Iurisci I., D'Egidio M., Piccolo E., Tinari N., Nifantiev N., Iacobelli S.: Synthetic lactulose amines: novel class of anticancer agents that induce tumor-cell apoptosis and inhibit galectin-mediated homotypic cell aggregation and endothelial cell morphogenesis. Glycobiology. **16**, 210–220 (2006)
62. Dominique R., Liu B., Das S.K., Roy R.: Synthesis of `molecular Asterisks' via sequential cross-metathesis, Sonogashira and Cyclotrimerization Reactions. Synthesis. **6**, 862–868 (2000)
63. Roy R., Trono M.C., Giguère D.: Effects of linker rigidity and orientation of mannoside cluster for multivalent interactions with proteins. ACS Symp. Ser. **896**, 137–150 (2005)
64. Roy R., Trono M.C., Giguère D.: Synthesis of stable and selective inhibitors of human galectins-1 and −3. Bioorg. Med. Chem. **16**, 7811–7823 (2008)
65. Vrasidas I., André S., Valentini P., Böck C., Lensch M., Kaltner H., Liskamp R.M.J., Gabius H.-J., Pieters R.J.: Rigidified multivalent lactose molecules and their interactions with mammalian galectins: a route to selective inhibitors**.** Org. Biomol. Chem. **1**, 803–810 (2003)
66. Bian C.-F., Zhang Y., Sun H., Li D.-F., Wang D.-C.: Structural basis for distinct binding properties of the human galectins to Thomsen-Friedenreich antigen. PLoS One. **6**, (2011). doi:10.1371/journal.pone.0025007
67. Wang H., Huang W., Orwenyo J., Banerjee A., Vasta G.R., Wang L.-X.: Design and synthesis of glycoprotein-based multivalent glyco-ligands for influenza hemagglutinin and human galectin-3. Bioorg. Med. Chem. **21**, 2037–2044 (2013)
68. Campo V.L., Riul T.B., Carvalho I., Baruffi M.D.: Antibodies against mucin-based Glycopeptides affect *Trypanosoma cruzi* cell invasion and tumor cell viability. Chembiochem. **15**, 1495–1507 (2014)
69. Zhao Q., Barclay M., Hilkens J., Guo X., Barrow H., Rhodes J.M., Yu L.-G.: Interaction between circulating galectin-3 and cancer-associated MUC1 enhances tumour cell homotypic aggregation and prevents anoikis. Mol. Cancer. **9**, (2010). doi:10.1186/1476–4598–9-154
70. André S., Ortega P.J.C., Perez M.A., Roy R., Gabius H.-J.: Lactose-containing starburst dendrimers: influence of dendrimer generation and binding-site orientation of receptors (plant/animal lectins and immunoglobulins) on binding properties. Glycobiology. **9**, 1253–1261 (1999)
71. Michel A.K., Nangia-Makker P., Raz A., Cloninger M.J.: Lactose-functionalized dendrimers arbitrate the interaction of galectin-3/muc1 mediated cancer cellular aggregation. Chembiochem. **15**, 2106–2112 (2014)
72. Böcker S., Laaf D., Elling L.: Galectin binding to neo-glycoproteins: LacDiNAc conjugated BSA as ligand for human galectin-3. Biomolecules. **5**, 1671–1696 (2015)
73. Li J., Li W., Jinga J., Yua W.W.: Influencing factors on the synthesis of magnetically responsive lipases. J. Mol. Catal. B Enzym. **101**, 47–55 (2014)
74. Rech C., Rosencrantz R.R., Křenek K., Pelantová H., Bojarová P., Römer C.E., Hanisch F.-G., Křen V., Elling L.: Combinatorial one-pot synthesis of poly-*n*-acetyllactosamine oligosaccharides with leloir-glycosyltransferases. Adv. Synth. Catal. **353**, 2492–2500 (2011)
75. Takenaka Y., Fukumori T., Raz A.: Galectin-3 and metastasis. Glycoconj. J. **19**, 543–549 (2004)
76. Ballell L., van Scherpenzeel M., Buchalova K., Liskamp R.M.J., Pieters R.J.: A new chemical probe for the detection of the cancer-linked galectin-3. Org. Biomol. Chem. **4**, 4387–4394 (2006)