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Synthetic Inhibitors of Galectins: Structures and Syntheses

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Abstract

The recent discovery of the critical involvement of galectins in cancer progression, and in inflammatory and immune responses, has raised this family of β-D-galactoside-binding proteins to the rank of high-priority drug targets by the scientific community. This report will highlight the relevance of glycochemistry toward the efficient development of synthetic galectins inhibitors with high affinity and selectivity, as small molecules or multivalent glycoconjugates.

A. Introduction

Lectins are carbohydrate-binding proteins that are grouped into several families (1, 2). Among them, galectins (15 mammalian members identified to date) share a consensus amino acid sequence and are recognized for their ability to bind β-galactoside residues (3, 4). Natural ligands of galectins are *N*-acetyl lactosamine, lactose and any glycoconjugates with a non-reducing galactoside terminus. The most striking features of galectins are their ability to regulate numerous biological processes (5), including an active involvement in a number of cellular events including neoplastic transformation, tumor cell survival processes, angiogenesis and tumor metastasis (6, 7). They are also known to regulate important cell phenomena that are critical for immune cell homeostasis (8–10). Their functions are directly related to the ability of galectins to cross-link glycoconjugates harboring multiple galactopyranoside residues (11). This phenomenon can be explained by the fact that galectins can assemble in three different and specific architectures, *i.e.*, proto-type, tandem repeat-type, and chimera-type (12). This diversity opens the door for the exploration of a myriad of structural combinations in the quest for the synthesis of potent and optimized inhibitors. Consequently, the scientific community recently directed efforts toward synthetic compounds having high affinities with galectins with the aim of deciphering their biological roles.

Synthetic strategies to prepare galectin inhibitors have already been nicely summarized in reviews by the groups of Nilsson (13, 14), Mayo (15), Pieters (16), and Kiss (17).To complement these reports, we wish to shed some light on the synthetic pathways specifically leading to sugar-based inhibitors, emphasizing a comprehensive analysis of their chemical structures that is required to properly identify potential clinical candidates. In that purpose and in the interest of clarity, different structural families including monosaccharides, disaccharides, glycoclusters and glycodendrimers will be described separately. It is noteworthy that combinatorial peptide (18) or glycopeptide (19) libraries will not be covered in this review.

B. Structural Features of Galectins

Galectins contain conserved carbohydrate recognition domain (CRD) of about 135 amino acids (20). For human galectin-3, eight amino acids (Arg144, His158, Asn160, Arg162, Arg186, Asn174, Trp181, and Glu184) interact with the carbohydrate. Considering the fact that the glycan binding site is described by five subsites A–E (21, 22), the galactose residue of lactose binds in subsite C, while subsite D accommodates the glucose moiety. Fig. 1 summarizes key features of the galectin–carbohydrate interactions based on the X-ray structure of galectin complexed to lactose (PDB 2NN8) (23) and allows the rationalization of the potential chemical functionalizations that can be realized toward the synthesis of optimized ligands. It is important to point out that if the 2-OH group on the glucose residue is replaced with an acetamide (LacNAc as ligand), similar interactions are possible with Arg186.

i) The endocyclic oxygen of the galactose residue directly hydrogen bonds with Arg162;
ii) 3′-OH group interacts indirectly with Arg144 *via* a water molecule;
iii) 4′-OH has hydrogen bonding interactions directly with His158, Asn160, Arg162 and indirectly with Arg144 by the

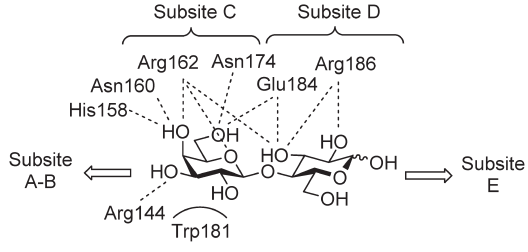


Fig. 1. Interactions between lactose and the CRD amino acids of galectin-3. Subsite C encloses the galactose residue and subsite D encloses the glucose residue. Dotted lines denote hydrogen bonding.

intermediary of a water molecule (not shown);

iv) The 6′-OH directly hydrogen bonds with Arg174 and Glu184;

v) A tryptophan residue is located underneath subsite C, allowing hydrophobic interactions with CH-3, CH-4 and CH-5 (proximity of the π orbitals of the aromatic residue) (24);

vi) 2-OH directly hydrogen bonds with Arg186;

vii) 3-OH group directly hydrogen bonds with Arg162 and Arg186;

viii) 6-OH group indirectly hydrogen bonds with Glu184 *via* a water molecule.

C. Synthetic Inhibitors of Galectins

Galectin inhibitors could be used as anti-inflammatory agents, as immunomodulators or in cancer therapy. For instance, knockout mice lacking galectin-1 or -3 suggest that inhibitors of these proteins could have therapeutic potential in inflammation or asthma intervention (25, 26). Intense investigations have also been devoted to cancer therapy with galectin-3 as the most studied lectin in this research field. This is exemplified with **TD139** (a ditriazolylthiodigalactoside, see section C-2-3), a lead compound resulting from two decades of research; this compound recently passed a Ib/IIa phase clinical trial in idiopathic pulmonary fibrosis patients (27, 28). Considering the major role of glycochemistry in this particular field, the main purpose of the present section will be centered on the description of the synthesis of some of the most relevant galectin inhibitors developed to date, with a logical classification of synthetic modifications in connection with targeted subsites.

C-1. Monosaccharides as Synthetic Galectin Inhibitors

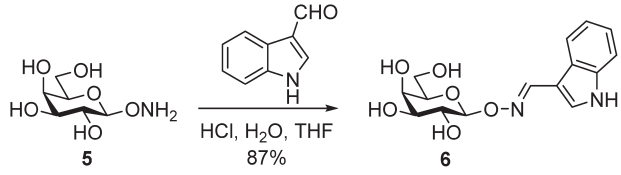
C-1-1. Anomeric Modification: Targeting Subsites D-E

In 2005, the group of Nilsson prepared a phenyl thio-β-D-galactopyranoside library as efficient inhibitors of galectin-7 (29). The general strategy was based on the use of 1,5-difluoro-2,4-dinitrobenzene that was successively substituted by various amine or thiol nucleophiles (Scheme 1). The sequence was thus initiated with the introduction of one galactoside residue **1** on difluorodinitrobenzene *via* a controlled nucleophilic aromatic substitution to provide thiogalactoside **2** in 77% yield. Subsequent functionalization was achieved with phenylmethanethiol providing intermediate **3**, which was finally transformed into dibenzamide **4** using tin(II) chloride, followed by de-*O*-acetylation using butylamine. The 28-member library was evaluated against galectins-1, -3, -8N and -9N. Using a competitive fluorescence-polarisation assay, the best inhibitor was compound **4** with a K_d value of 0.18 mM against galectin-7 and 2.2 mM against galectin-8N (30–32). Also, compound **4** proved to be active against galectin-3 (K_d=1.8 mM) and galectin-8N (K_d=0.14 mM).

The same year, a 50-member library of anomeric oxime ether derivatives was synthesized as a LacNAc-mimetic (33). Thus, the group of Nilsson treated galactopyranosylhydroxylamine **5** with various aldehydes providing the corresponding aldoximes in good yields (Scheme 2). The best inhibitor was [*E*]-*O*-(β-D-galactopyranosyl)-indole-3-carbaldoxime **6** (K_d=180 μM against galectin-3), which was 24 times better than methyl β-D-galactopyranoside (K_d=4400 μM) using the same fluorescence polarization assay. A follow-up study provided C3-triazole-substituted *O*-galactosyl aldoximes (34). Combination of subsite B-binding triazole fragment with a subsite D-binding indole-3-carbaldoxime fragment yielded selective galectin-3 inhibitors.

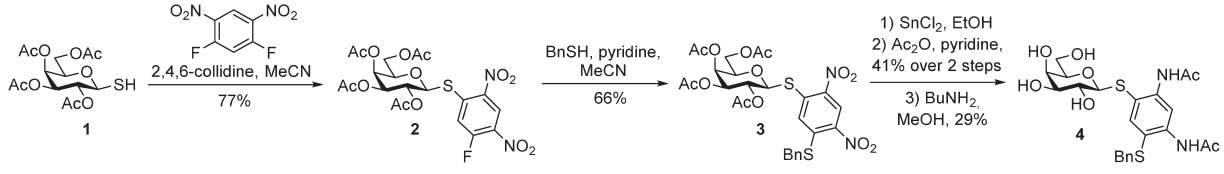
The group of Roy used a phase-transfer catalyzed nucleophilic displacement reaction to synthesize aryl *O*- and *S*-galactosides in a straightforward way (35). Under these conditions, the best monosaccharide inhibitor was obtained by the formation of the desired thioether linkage from **7** and 4-methoxythiophenol. Full deprotection of derivative **8** led to candidate **9**, which showed inhibitory properties of 2.5 mM against galectin-3 using a hemagglutination assay (Scheme 3). Interestingly, selectivity was achieved as this compound was inactive against galectin-1.

Galactoside-bearing triazoles and isoxazoles were also prepared by the group of Roy (36). Triazole **11** and isoxazole **13** were prepared using regioselective [1,3]-dipolar cycloadditions, from the corresponding *C*- and *O*-alkynes, **10** and **12**, respectively (Scheme 4). Compound **11** was active against galectins-1 and -3



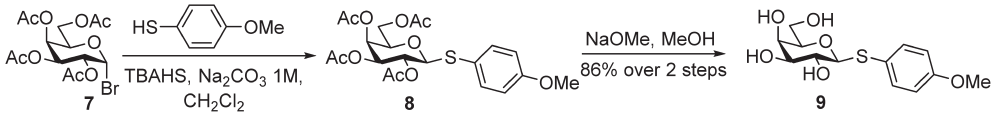
5 6

Scheme 2. Synthesis of galactopyranosyl-indole-3-carbaldoxime 6.

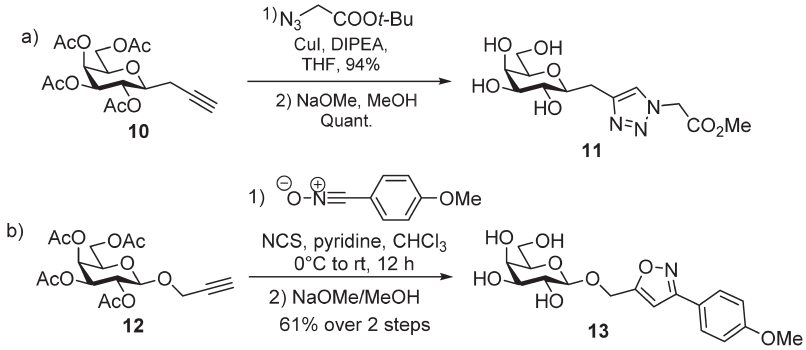


1 2 3 4

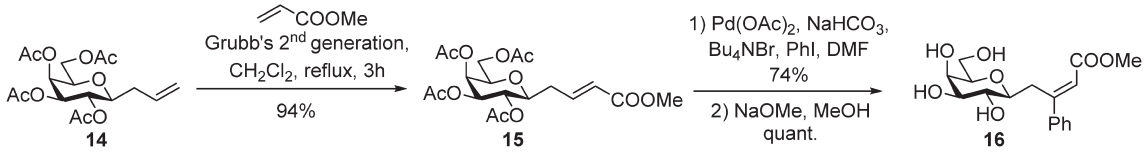
Scheme 1. Synthesis of phenyl thio-β-D-galactopyranoside 4 from 1,5-difluoro-2,4-dinitrobenzene.



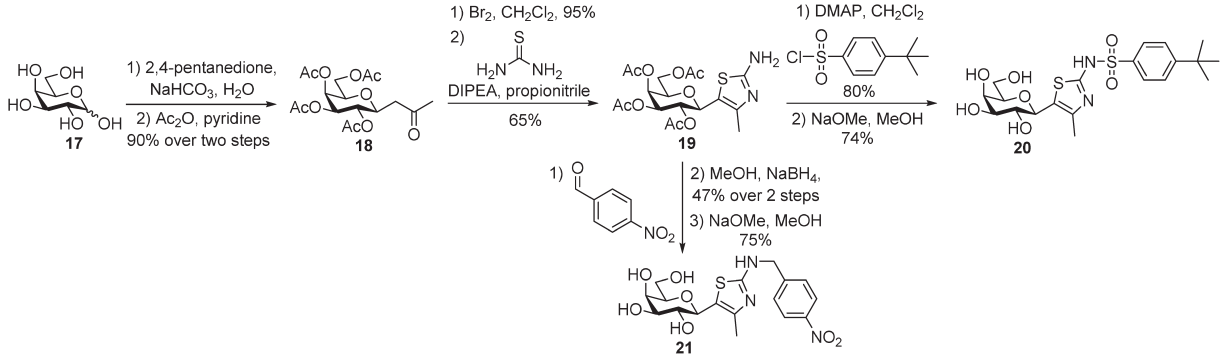
Scheme 3. Synthesis of *p*-methoxyphenyl *S*-galactopyranoside 9.



Scheme 4. Synthesis of a) triazole galactoside 11 and b) isoxazole galactoside 13.



Scheme 5. Synthesis of methyl cinnamate *C*-galactoside 16.

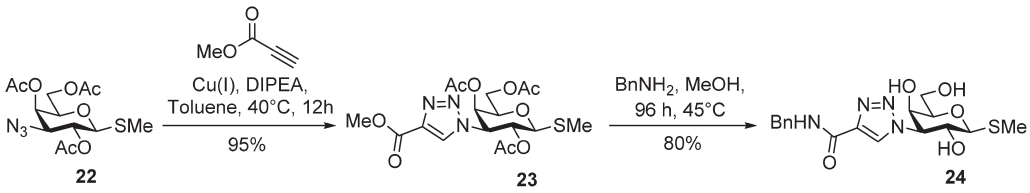


Scheme 6. Synthesis of aminothiazoles 20 and 21.

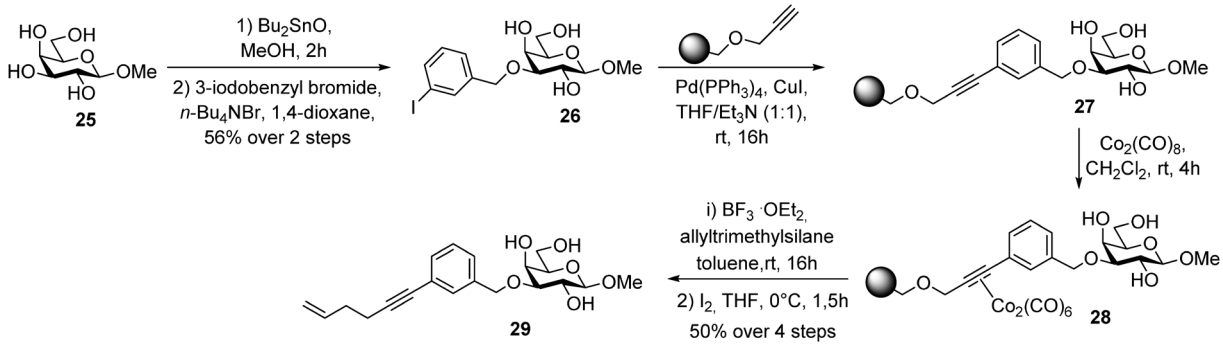
(IC_{50}=5 mM) while compound **13** selectively inhibited galectin-1 (IC_{50}=1.25 mM) as measured with a hemagglutination assay.

Targeting hydrolytically stable analogs, the group of Roy used *C*-allyl galactoside **14** as a precursor for chemical diversifications in a "drug-like" strategy (Scheme 5) (37). First, a Grubbs' cross-metathesis reaction using methyl acrylate afforded compound **15** (38). Then, a Heck palladium-catalyzed coupling reaction was efficiently performed using phenyl iodide, yielding the corresponding methyl cinnamate derivative under phase-transfer catalyzed conditions. Deprotection upon treatment with base afforded inhibitor **16**, which was active against galectin-1 (IC_{50}=0.313 mM), but inactive toward galectin-3 as evaluated using a hemagglutination inhibition assay.

An additional library of fifteen stable glycosides harbouring a *β*-*C*-aminothiazole scaffold (37) was also described (Scheme 6). Galactose derivative **17**, under Knoevenagel conditions, afforded *β*-*C*-glycosidic ketone **18** in 90% yield over two steps. Bromine installation was followed by treatment with thiourea allowing formation of aminothiazole **19** in 65% yield. This key intermediate reacted with 4-*tert*-butylbenzene-sulfonyl chloride to give the corresponding sulfonamide **20** (galectins-1 and -3: IC_{50}=5 mM). In parallel, compound **19** was treated with 4-nitrobenzaldehyde under $NaBH_4$-mediated reductive amination conditions, allowing formation of amine **21** after de-*O*-acetylation (galectins-1 and -3: IC_{50}=2.5 mM).



Scheme 7. Synthesis of 3-(1,2,3-triazol-1-yl)-1-thio-galactoside.

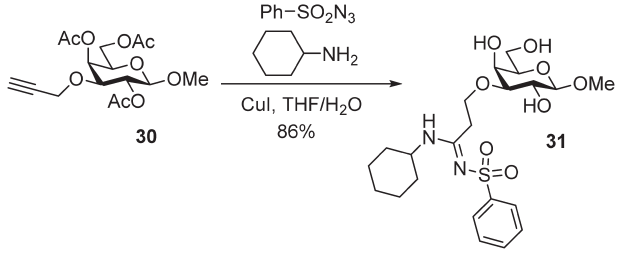


Scheme 8. Cobalt-mediated solid phase synthesis of 3-*O*-alkynylbenzyl galactosides.

C-1-2. Modifications on O-3/C-3 Position: Targeting Subsite A–B

Targeting subsite A and B has been accomplished by substitution at O-3/C-3-modified galactosides. Interestingly, the presence of an aromatic group was assumed to enhance interactions with Arg144 (Fig. 1) through stacking interactions together with desolvation effects. In 2005, the group of Nilsson reported a series of 3-(1,2,3-triazol-1-yl)-1-thio-galactosides as potent inhibitors of galectin-3 (39). The 3-azido-3-deoxy-galactose derivative **22** was used as a versatile starting material for cycloaddition with various alkynes (Scheme 7). Accordingly, triazole ester **23** was isolated in high yield and transformed into the corresponding amide with benzylamine. After amide synthesis and *O*-acetyl deprotection, compound **24** was determined as the best galectin-3 inhibitor (K_d=107 μM) out of a series of 12 compounds, as determined by a competitive fluorescence assay. Other 3-(1,2,3-triazol-1-yl)-1-thio-galactoside derivatives were synthesized using the same synthetic strategy and described a few years later (40). Interestingly, the derivative having a 1-naphthyl moiety on the 1,2,3-triazole core was determined as the best candidate with a K_d of 120 μM against galectin-3 using the same biological assay.

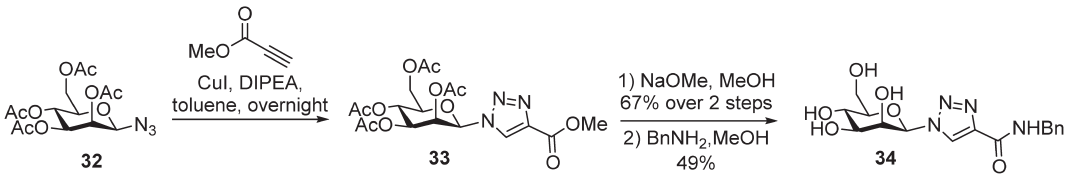
Novel 3- or 4-substituted benzyloxy ethers at C-3 of galactose recently emerged as galectin inhibitors (41). Alkyne-substituted benzyl ethers appeared attractive, as alkyne derivatives can be exploited in various reactions allowing straightforward functionalization. In this respect, methyl β-D-galactoside **25** was initially converted to the 3,4-*O*-stannylene acetal, then selectively benzylated with 3-iodobenzyl bromide to afford compound **26** in 56%



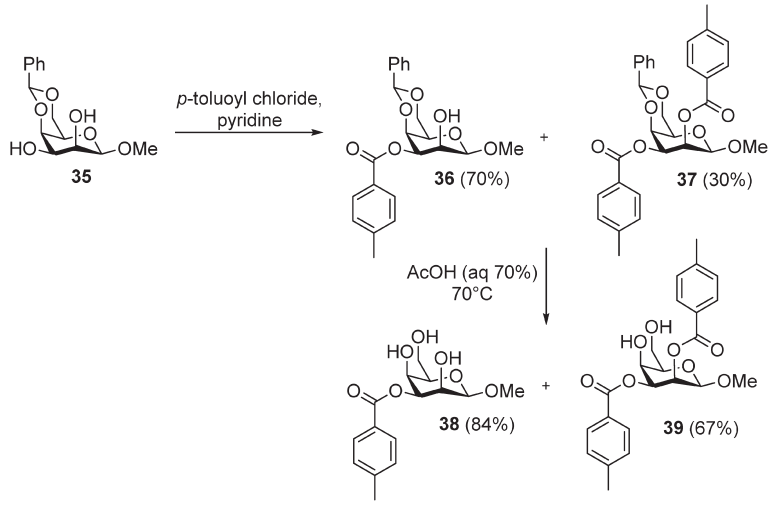
Scheme 9. Synthesis of amidine galactoside 31.

yield. The latter compound reacted with propargylated Merrifield resin using Sonogashira's coupling under basic conditions to generate intermediate **27**. Cobalt octacarbonyl was used to create the polymer-bound alkyne–cobalt complex **28**. A Nicholas reaction using allyltrimethylsilane led to desired product **29** after treatment with iodine. A library of 12 compounds was screened against galectins-1, -3, -7, -8N and -9N in a competitive fluorescence polarisation assay and inhibitor **29** was the most active and selective toward galectin-7 (K_d=0.39 mM).

The last example of modification of O3/C3 on a galactoside core has been recently described by Nilsson and coworkers (42). The authors used a three-component reaction between galactosyl alkyne **30**, various tosyl azides, and amines, to give *N*-sulfonyl amidine galactose derivatives (Scheme 9). This work resulted from a previous study based on the preparation and evaluation of coumaryl and iminocoumaryl derivatized glycosides (43). As an example of the method used, propargyl galactoside **30** was treated with phenylsulfonyl azide and cyclohexylamine in the presence of CuI to give the corresponding *N*-phenylsulfonylamidine **31** in an excellent



Scheme 10. Synthesis of 1*H*-(1,2,3-triazol-1-yl)-mannoside 34.



Scheme 11. Synthesis of taloside derivatives 38 and 39.

86% yield. This compound showed a K_d of 42 μM against galectin-9N measured in a competitive fluorescence polarization assay.

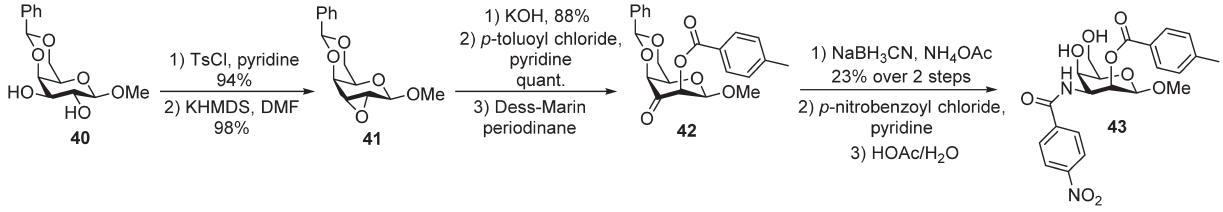
C-1-3. Mannosides: Targeting Subsite C

Nilsson used 1*H*-(1,2,3-triazol-1-yl) mannoside as a galactose mimetic that could interact with amino acids of the C-subsite region (44). The triazole moiety could adequately be accommodated in subsite B, while the mannose HO-2 hydrogen-bonding pattern is similar to galactose HO-4 (14). This strategy was also used for exploitation of other subsites using a taloside core (see next section). Starting from 2,3,4,6-tetra-*O*-acetyl-1-azido-1-deoxy-β-D-mannopyranose **32**, a copper(I)-catalyzed 1,3-dipolar cycloaddition with methyl propiolate afforded intermediate **33** (Scheme 10). The latter was subsequently deprotected in 67% yield and treated with benzylamine to afford compound **34** in 49% yield. A small library of amine derivatives was also prepared, but showed low affinity toward various galectins. Using a fluorescence polarization assay, a K_d of 540 μM against galectin-9N was observed for triazole **34**, which is similar to methyl β-LacNAc.

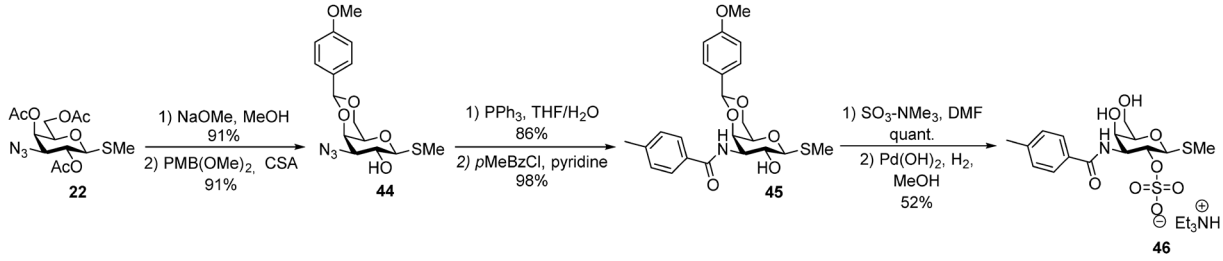
C-1-4. Talosides and O2-modifications: Targeting Other Subsites

The same logic was applied by Nilsson *et al.* to develop novel taloside derivatives as galectin inhibitors (45). Talosides were not only considered as galactose mimetics, but also allowed to target other perpendicular subsites to the A–E regions in quest for enhanced interactions. In this context, substitutions at O-2 and/or O-3 with aromatic esters resulted in promising candidates. Thus, starting from benzylidene acetal-protected methyl β-D-talopyranoside **35**, selective acylation on O-3 yielded intermediate **36**, along with bis-acylated taloside **37** (Scheme 11). Both compounds were evaluated in a competitive fluorescence polarization assay and were highly selective toward galectin-4 and -8. Toluoylation at O-3 resulted in a compound (**38**) with a K_d of 0.4 mM against galectin-8N. On the other hand, *bis*-toluoylation (**39**) provided a product active against galectin-4 (K_d=0.16 mM). This study thus suggested that selective inhibitors of galectins-4 or -8 could be achieved using a taloside scaffold.

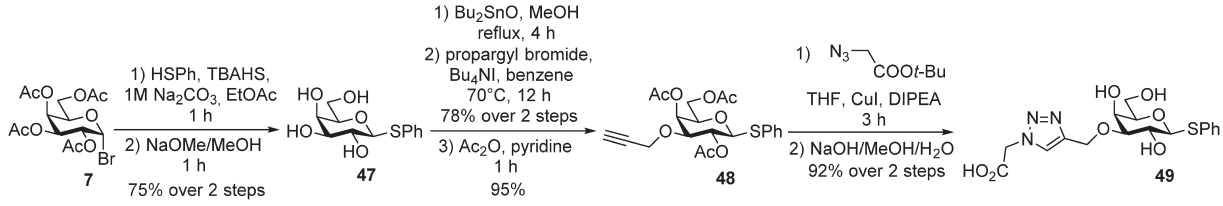
Taloside-based inhibitors have different specificity than galactoside inhibitors toward galectins. This was exemplified once more in complementary studies through the synthesis of 11 3-amido-3-deoxy-β-D-talopyranosides and their evaluation against a panel of galectins (46). The synthesis was initiated with benzylidene acetal-protected methyl β-D-galactopyranoside **40**, which was converted into the 2,3-anhydro guloside **41** following a selective 3-OH tosylation and a base-promoted oxirane formation (Scheme 12). Treatment with KOH afforded the desired *ido*-configured derivative. Then acylation with *p*-toluoyl chloride and Dess–Martin oxidation provided **42**. Finally, reductive amination gave the 3-amino-3-deoxy-β-D-taloside derivative, which was acylated with *p*-nitrobenzoyl chloride to yield **43** after benzylidene acetal de-



Scheme 12. Synthesis of 3-amino-3-deoxy-*β*-D-taloside derivative 43.



Scheme 13. Synthesis of galactose-derived arginine tweezers 46.



Scheme 14. Synthesis of 3′-triazole bearing carboxylic acid 49.

protection under acidic conditions. Compound **43** was a selective inhibitor of galectin-4C (K_d=94 μM) as compared to galectin-3 (K_d=570 μM). This study showed that this family of taloside derivatives had good chemical hydrolytic stability and could inhibit the cellular functions of galectin-4. Finally, elucidation of X-ray crystal structures of taloside analogs in complex with galectins-1 and -3 provided interesting information regarding the taloside/protein interactions (47).

The galactoside scaffold was also used to target other galectin subsites. Nilsson prepared galactose-derived arginine tweezers as galectin-3 inhibitors (48). Starting from the 3-azido thiogalactoside **22**, deacetylation was followed by benzylidenation to afford intermediate **44** (Scheme 13). Reduction of the azido group using polymer-supported triphenyl phosphine afforded the corresponding amine, which was *N*-acylated to yield compound **45**. Sulfation using sulfur trioxide allowed derivatization of the O-2 position and subsequent global deprotection gave galectin-3 tweezer **46**. A small library (9 compounds) was synthesized and tested *in vitro* against galectin-3 using a fluorescence polarization assay. With a K_d value of 87 μM, candidate **46** illustrated that substitution at the O-2 position of galactosides could offer a new affinity-enhancing approach. This was also demonstrated by generation of derivatives with various 2-*O*-substituants on the same scaffold (49). In fact, the methyl and benzyl 2-*O*-phosphate derivatives were interesting candidates as galectin-7 inhibitors (50). The X-ray crystal structure of galectin-7 in complex with 2-*O*-benzylphosphate galactoside showed that all the interactions involving the galactose moiety are conserved.

C-1-5. Anomeric and 3-modification: Targeting Subsites A-B and D-E

The group of Roy targeted subsites A-B and D-E with the use of a galactoside scaffold (36). Aryl *S*-galactosides proved to be interesting galectin inhibitors (35), and installation of a substituent at O-3 provided second generation inhibitors. Galactosyl bromide **7** was subjected to a phase-transfer catalyzed nucleophilic displacement with thiophenol to give **47** after de-*O*-acetylation (Scheme 14). Selective *O*-alkylation efficiently occurred after dibutylstannylene acetal treatment with dibutyltin oxide and then exposure to propargyl bromide. After acetyl protection, **48** was treated with *tert*-butyl azidoacetate under click chemistry conditions followed by methyl ester hydrolysis and de-*O*-acetylation to provide triazole derivative **49** in 92% yield over two steps. Activity was observed for **49** against both galectins-1 and -3 (inhibitory properties of 1.25 and 5 mM, respectively).

C-2. Disaccharides as Synthetic Galectin Inhibitors

C-2-1. Anomeric Modification: Targeting Subsites D-E

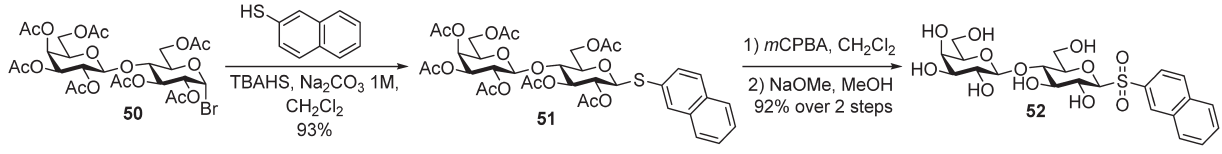
Disaccharides, having a non-reducing galactose residue are better galectin inhibitors than monosaccharides. This can be explained by the fact that the reducing sugar can be accommodated in subsite D in the CRD and create a hydrogen bonding network (see Fig. 1). The most popular scaffolds for the synthesis of high affinity galectin inhibitors are either lactosides or thiodigalactosides. In fact, even simple disaccharides have been shown to have interesting biological activities, as exemplified by allyl lactoside, which inhibits tumor-associated homotypic cell aggregation and apoptosis (51). This section will present efforts related to the preparation of disaccharide-based galectin inhibitors.

In 2006, the group of Roy reported a phase-transfer catalyzed reaction that efficiently yielded *O*- and *S*-aryl lactosides (Scheme 15) (35). Thus, lactosyl bromide precursor **50** was treated with *β*-thionaphthyl under basic conditions and afforded intermediate **51**. *m*-CPBA-mediated oxidation coupled to deprotection reactions generated the corresponding sulfone **52**, that was active against galectins-1 and -3 (inhibitory potency of 0.04 mM and 0.313 mM, respectively). In this study, *in silico* investigations showed that the electronic effects of the lactoside aglycons correlated with the electrostatic potential at O-3. Recently, the same group extended this study using 3′-*O*-sulfated derivatives upon derivatization of *O*- and *S*-aryl lactosides (52). Labeled tissue lectins were used as tools in histochemistry, enabling mapping the profiles of accessible binding sites in sections.

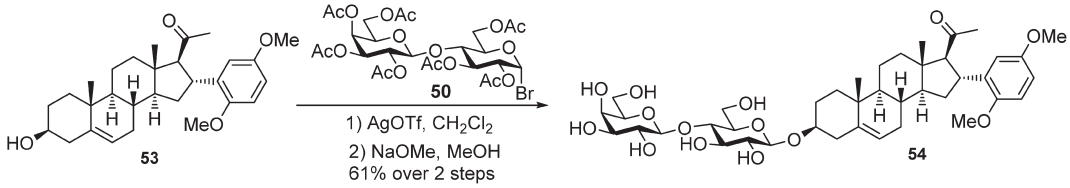
The last example of a disaccharide targeting only subsite D-E has been reported by the group of Kiss (53). Lactosylated steroid **54** (generated from glycosyl donor **50** and acceptor **53** in the presence of AgOTf activator), increased the survival of conventional mice grafted subcutaneously with P388 lymphoma and showed no cytotoxic effects on six different human cancer cell lines (Scheme 16). Moreover, *in vivo* studies also indicated that compound **54** increased the antitumor effectiveness of cisplatin in some lymphoma models.

C-2-2. 3′-modification: Targeting Subsites A-B

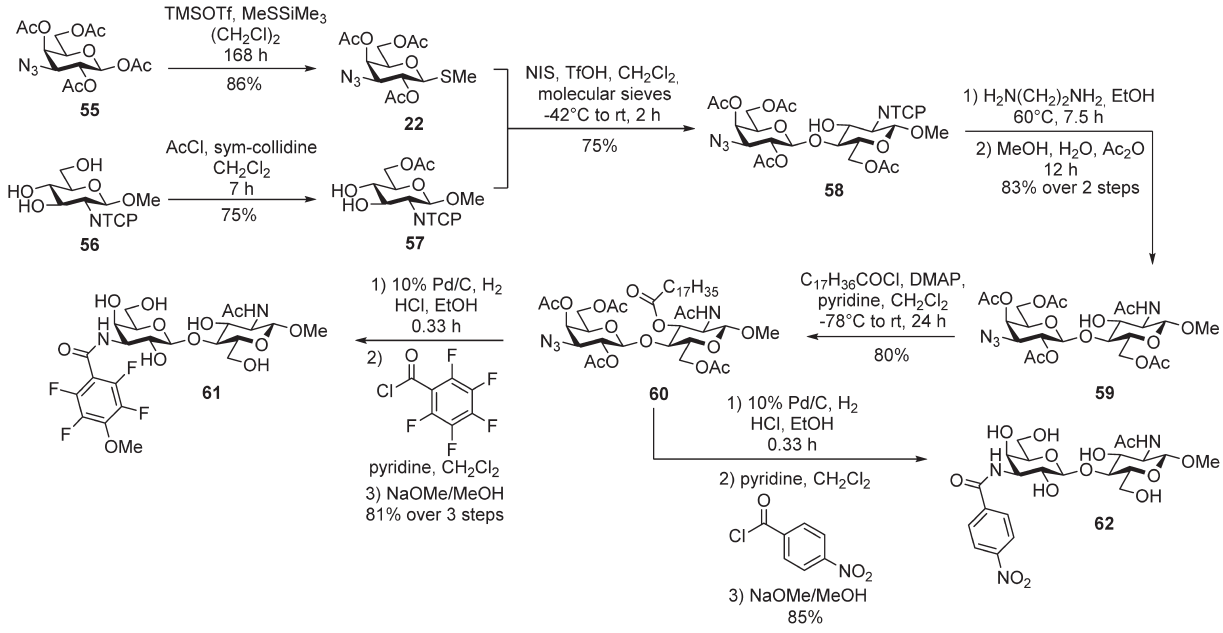
In 2002, the group of Nilsson prepared 12 compounds based on *N*-acylations or *N*-sulfonylations of 3′-amino-*N*-acetyllactosamine (54). Derivatization of the HO-3′ group was proposed to create extended interactions taking advantage of an extended groove in this region of the protein. Consequently, the installation of hydrophobic groups at the C-3′ position of the galactose moiety was considered. The chemistry was initiated with the transformation of 3-azido-galactoside **55** into thiogalactoside **22** with trimethylsilyl trifluoromethanesulfonate and (methylthio)trimethylsilane in 86% yield (Scheme 17). Glucosyl acceptor **57** was prepared by a regioselective acetylation of the corresponding *N*- tetrachlorophthalimido (TCP) glucosamine derivative **56** in 75% yield. Glycosylation between acceptor **57** and donor **22** was further accomplished using *N*-iodosuccinimide/TfOH in 75% yield. The *N*-TCP protecting group was easily converted into the corresponding *N*-acetate **59** in 83% and a stearic ester was installed on HO-3 in 80% yield. The latter was tagged with a hydrophobic chain (**60**) to allow rapid purification using reversed solid-phase extraction. Finally, reduction of the azido group was followed by *N*-derivatization and global deprotection. Compound **61** was the best inhibitor against galectin-3 with an IC_{50} of 4.4 μM. In a follow-up study, Nilsson's group, in collaboration with the groups of Rini and Leffler, confirmed that the 4-methoxy-2,3,5,6-tetrafluorobenzamido moiety stacked against the side chain Arg144 of galectin-3 (55). This cation-π interaction was shown by high-resolution X-ray crystal structures. To investigate the influence of the aromatic moiety at the 3′-position, a second generation library of 56 benzamido derivatives was prepared. Compound **62** was one of the best inhibitors (K_d=0.95 μM for galectin-3). This work clearly reinforced the



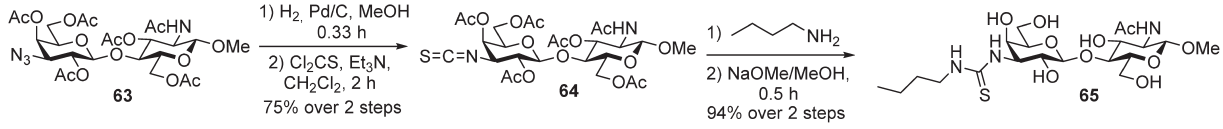
Scheme 15. Synthesis of *O*- and *S*-aryl lactoside 52 using phase-transfer reaction.



Scheme 16. Synthesis of a lactosylated steroid 54.



Scheme 17. Synthesis of 3′-amino of *N*-acetyllactosamine 61 and 62.



Scheme 18. Synthesis of 3′-thioureido *N*-acetyllactosamine 65.

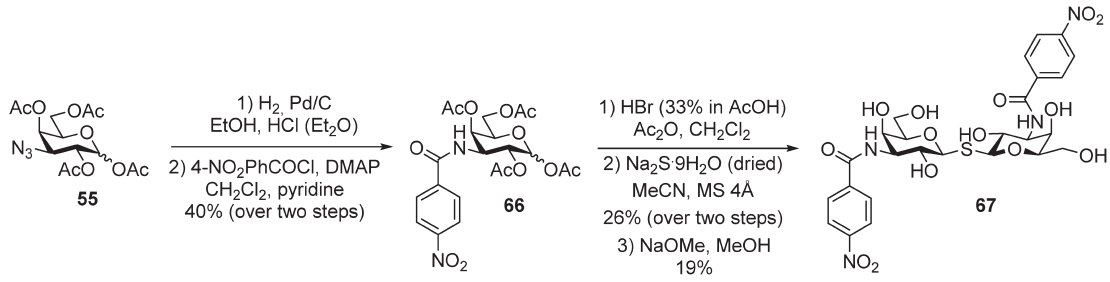
importance of cation-π interactions in drug design.

Based on their previous success with benzamido derivatives (30–32, 54), Nilsson *et al.* persisted in their quest for the optimal functional group to install at the C-3 position of the galactose moiety. Thus, a library of 3′-thioureido *N*-acetyllactosamine derivatives was proposed to improve affinity for the galectins (56). Azide **63** was first reduced over palladium on charcoal and the resulting amine was reacted with thiophosgene under basic conditions to give the corresponding isothiocyanate in 75% over two steps (Scheme 18). A collection of 13 thiourea derivatives was prepared by treatment of **64** with various amines followed by de-*O*-acetylation. Among then, compound **65** was the best inhibitor against galectins-1 and -3 (K_d=23 μM). When the thioureido substituent was 3-pyridylmethyl, a K_d of 23 μM was obtained against galectin-7. Other interesting biological results were obtained for the inhibition of galectin-9N, demonstrating that galactosides or lactosides bearing thioureido groups at C-3 could offer efficient inhibitors of galectins-7 or -9N.

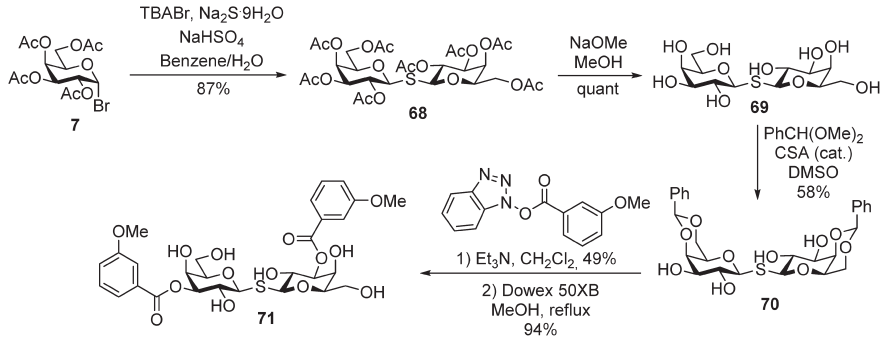
C-2-3. Targeting Subsites A-B and D-E

C_2-symmetrical thiodigalactoside bis-benzamido derivatives emerged in 2005 as a new class of galectin inhibitors, taking advantage of a double arginine–arene interaction (57). Their rational design was based on the fact that thiodigalactose was capable of binding to galectins as efficiently as LacNAc and its derivatization with aromatic amides at C-3 resulted in interaction with arginine. Another particular feature of thiodigalactose is it's resistance to glycosidase especially *in vivo*. This is critical, since Rivipansel, a pan-selectin inhibitor developed by GlycoMimetics is currently in phase III clinical trial (58). The latter compound still has difficulty in pharmacokinetics performance. As such, elongation of the serum half-life of such glycomimetic drugs must be improved in the future.

Thus, a symmetrical thiogalactoside was synthesized from 3-azido-3-deoxy-D-galactoside derivative **55** (Scheme 19). Reduction of the azido group into the corresponding amine was followed by acylation with *p*-nitrobenzoyl chloride and resulted in the formation of intermediate **66**. Finally, thiodigalactoside **67** was isolated in low yield following a three-step sequence. Compound **67** had a K_d value of 33 nM against galectin-3 based on a fluorescence-polarization assay. Docking experiments suggested favorable interactions between the aromatic amides and arginine. This pioneering work, based on the thiodigalactoside scaffold, was the first report of galectin inhibitors that generated candidates with nanomolar activity. Complementary studies based on a similar ap-



Scheme 19. Synthesis of C_2-symmetrical thiodigalactoside bis-benzamido 67.



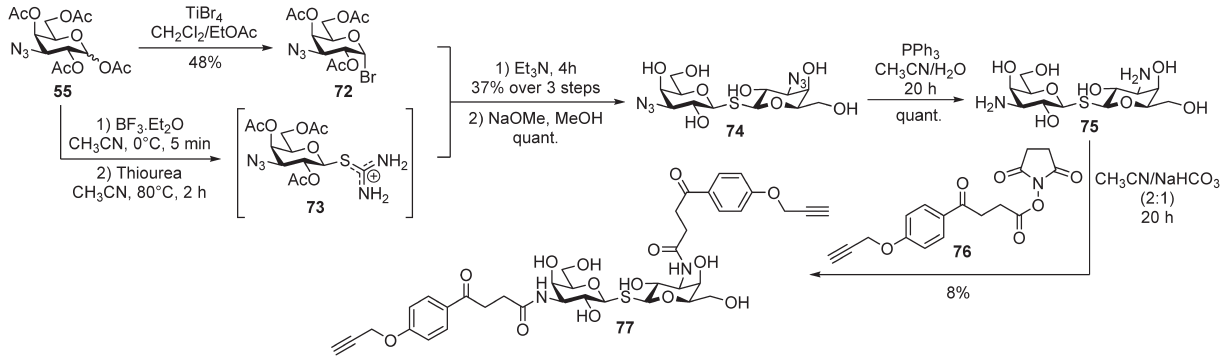
Scheme 20. Synthesis of thiodigalactoside aromatic 3,3′-diester 71.

proach were conducted for an evaluation against galectins-1, -3, -7, -8N and -9N terminal domains (59). The affinity was determined using competitive fluorescence-polarization and thermodynamic analysis by isothermal microcalorimetry. In addition, computational studies were used to provide structural requirements for the arginine–arene interactions. This work clearly demonstrated the relevance of the design since C_2-symmetrical thiodigalactoside *bis*-(3,5-dimethoxyphenyl)benzamido derivative had a K_d value as low as 46 nM against galectin-3.

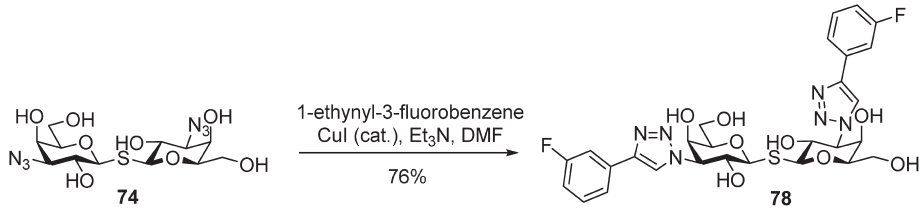
Further derivatization of the thiodigalactoside scaffold allowed generation of aromatic 3,3′-diesters (60). Five thiodigalactoside ester derivatives were prepared and evaluated against various galectins. The thiodigalactoside intermediate **68** was readily accessible from galactosyl bromide **7** (Scheme 20), and was then deprotected under basic conditions to afford compound **69**. The 4- and 6-positions were regioselectively protected as 4,6-benzylidene acetal under acidic conditions. The resulting compound **70** further reacted with an activated ester to generate **71** after acetyl deprotection. The latter inhibitor had a K_d value of 0.69 μM against galectin-3 using a fluorescence polarization assay. Interestingly, compound **71** also displayed potent anti-migratory properties against experimental lung and prostate cancer cells.

As illustrated above, thiodigalactoside represents an interesting scaffold for the preparation of high affinity galectin inhibitors. Following in the footsteps of these encouraging results, the group of Blanchard proposed to replace galactose by its C-2 epimer (talose) and thus generated thioditaloside (61). The synthesis of this new compound was reported along with crystal structures of it bound in the galectin-3 CRD. Fluorescence polarisation assays revealed that thiodigalactoside (K_d=50 μM) has better affinity with galectin-3 than thioditaloside (K_d=4.3 mM). This finding can be explained by the fact that the inner talose residue binds efficiently to galectin-3 (with the desired orientation of the axial O-2 into the bonding groove), but its distal talose residue is less capable of binding to the protein. In contrast to thiodigalactoside, the O-2′ of thioditaloside is directed away from the protein surface, within the bulk solvent (no interactions with Arg162 and Glu184).

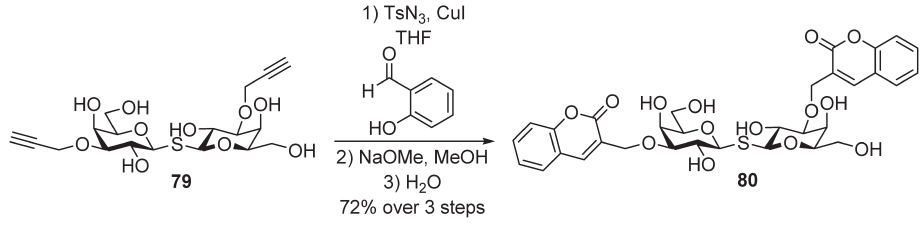
Taking advantage of the high affinity of C_2-symmetrical thiodigalactosides for galectins, photolabelled probes were developed based on this skeleton. Thus, the group of Pieters synthesized benzophenone or acetophenone thiodigalactosides for covalent attachment to proteins (62). In the process, a new synthetic route to symmetrical 3-azido-3-deoxy-thiodigalactoside was developed (Scheme 21). Azido-galactoside **55** was transformed into compound **73** using $BF_3{\cdot}OEt_2$ and thiourea. The latter reacted with galactosyl bromide **72** under basic conditions affording C_2-symmetrical thiodigalactoside in 37% yield. After an acetyl deprotection, which proceeded well to reach intermediate **74**, a double Staudinger reaction yielded diamine **75**. Subsequent coupling using activated ester **76** provided photolabelled probe **77** with a K_d value of 0.9 μM against galectin-3, as determined using a fluorescence polarization assay. The good results obtained for bis-acetophenone



Scheme 21. Synthesis of thiodigalactoside-based photolabel probe 77.



Scheme 22. Synthesis of ditriazolylthiodigalactoside 78.



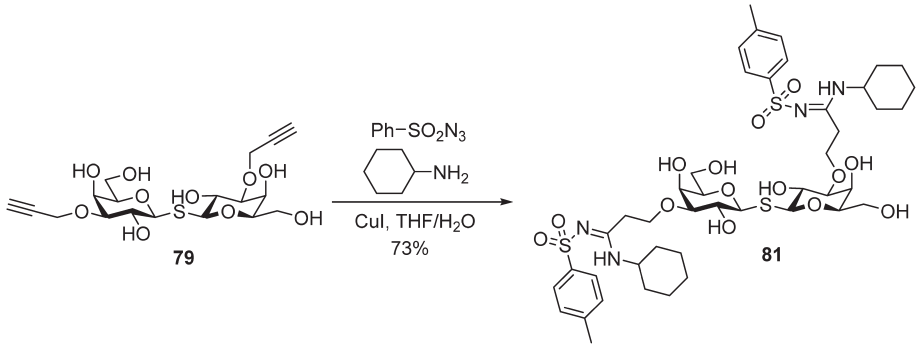
Scheme 23. Synthesis of 3,3′-substituted coumarylthiodigalactoside 80.

derivative **77** confirmed the efficiency of the labeling probe due to its high affinity to galectin-3, along with good flexibility allowing a more optimal stacking of an aryl moiety onto Arg144 and Arg186.

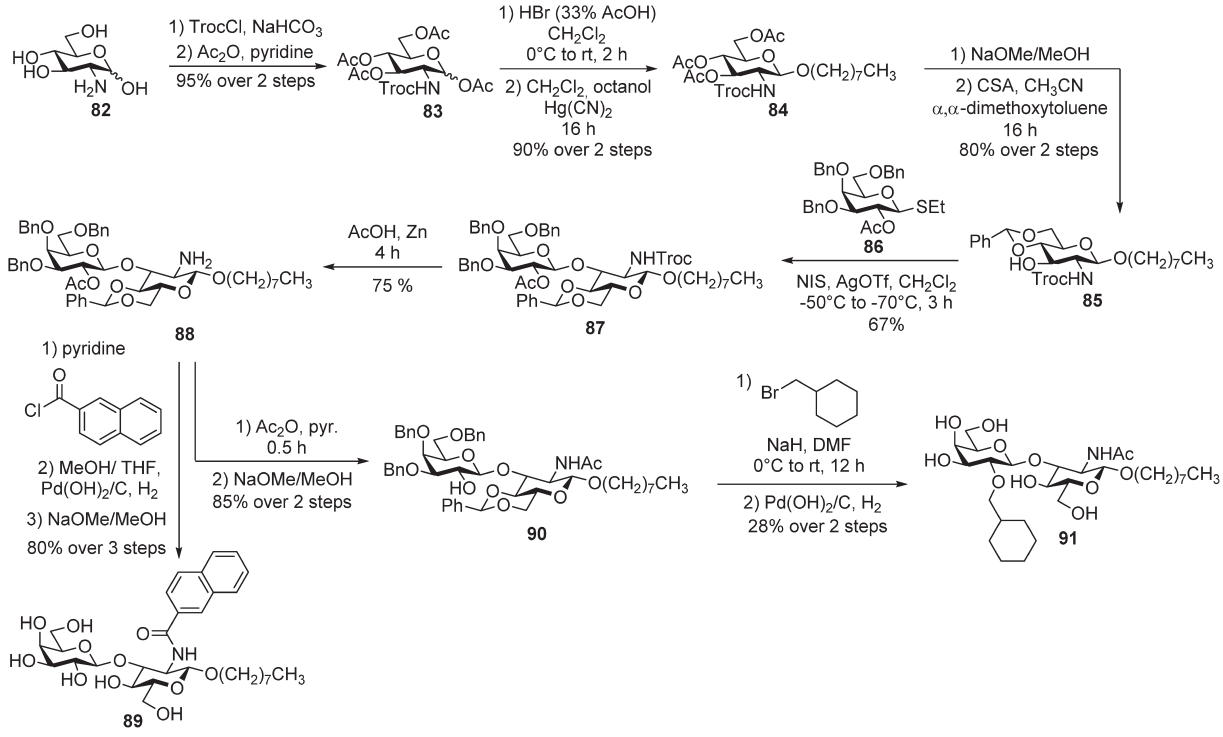
The exploitation of the high affinity of thiodigalactoside was once more demonstrated by the group of Nilsson (63). Novel 3,3′-*bis*-(4-aryltriazol-1-yl) thiodigalactosides appeared to reduce bleomycin-induced lung fibrosis and modulate intracellular glycan recognition. In this regard, a small library of nine compounds was prepared using copper-catalyzed cycloadditions between diazide **74** and various alkynes (Scheme 22). Compound **78** was isolated in 76% yield and showed a low K_d (14 nM) to galectin-3 as determined using a competitive fluorescence anisotropy assay. This excellent affinity was generated by the aryltriazole moieties forming stacking interactions with π system of aromatic amino acids in the CRD. Of interest, intracellular availability and activity were observed with compound **78** as it blocked amitriptyline-induced vesicle damage in breast carcinoma cells. In fact, this study originated from earlier work in 2010 (40) and 2013 (64), where similar compounds were prepared using the same approach. Finally, this work reported a promising therapeutic lead that disrupts cellular signal-sustaining galectin-3 lattices (27). Compound **78** passed clinical trial Ib/IIa phase in idiopathic pulmonary fibrosis patients.

Recently, the development of a coumarylthiodigalactoside series, described as a logical extension to coumaryl glycosides previously described (43), demonstrated the involvement of galectin-3 in a pulmonary fibrosis model (65). Inhibitor **80** was isolated in one step from propargyl **79** after acetates removal (Scheme 23). Thiodigalactoside **80** was active against galectin-3 (K_d=91 nM) using a fluorescence polarization assay. Also, the satisfactory results highlighted the efficiency of compound **80** in a bleomycin-induced mouse model of lung fibrosis, and suggested that galectin-3–glycan interactions limit the progression of lung fibrosis in this model.

A final example of galectin inhibitors targeting subsites A-B and D-E has been recently reported (42). Using a similar strategy as that described in Scheme 9, thiodigalactoside **81** was isolated in 73% yield from dipropargylated precursor **79** (Scheme 24). This compound was a good galectin-3 inhibitor with a K_d value in low μM range.



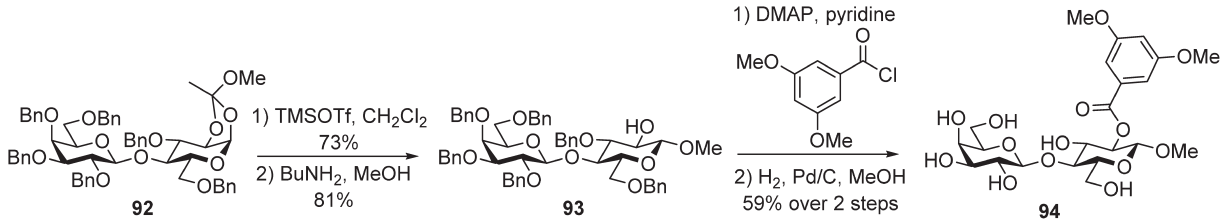
Scheme 24. Synthesis of amidine thiodigalactoside 81.



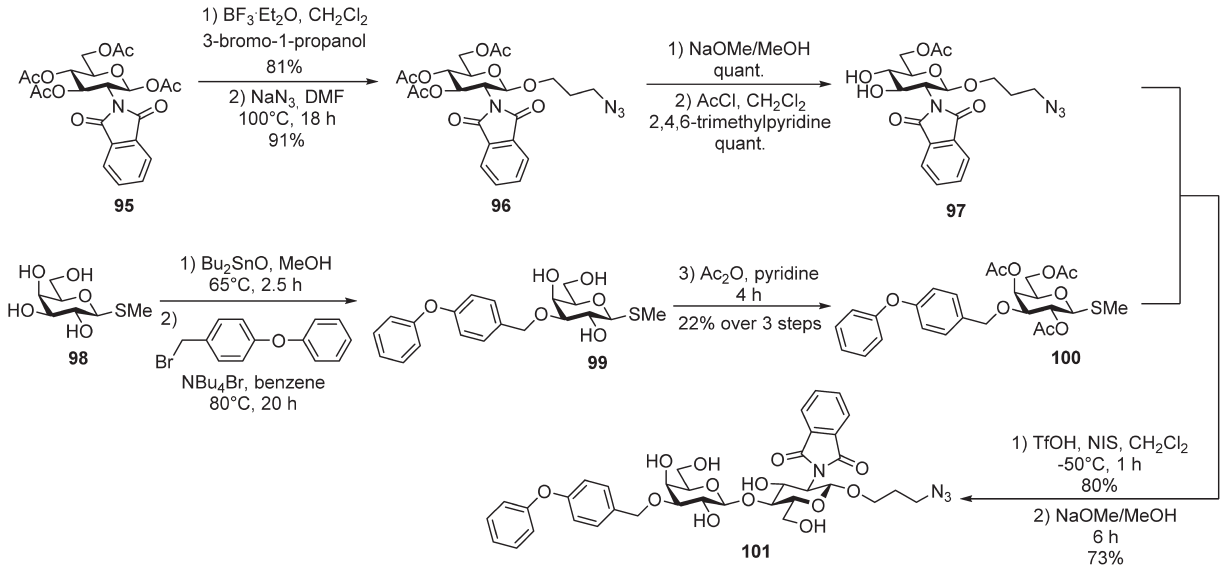
Scheme 25. Synthesis of lacto-*N*-biose inhibitors 89 and 91.

C-2-4. Targeting Other Subsites

Fort *et al*. discovered that synthetic *β*-D-Gal*p*-(1→3)-*β*-D-Glc*p*N (lacto-*N*-biose) derivatives were effective galectin-3 inhibitors (66). The screening of two libraries was performed using a microscale frontal affinity chromatography coupled to mass spectroscopy. The synthetic pathway and the best two inhibitors are shown in Scheme 25. Thus, glucosamine **82** was converted into intermediate **83** by reaction with (2,2,2-trichloroethoxy)carbonyl chloride followed by acetylation of the remaining free hydroxyl groups. Installation of the *O*-octyl aglycone was performed *via* the glycosyl bromide followed by mercuric cyanide-promoted glycosylation. Then, compound **84** was treated under Zemplèn conditions and protected as a 4,6-*O*-benzylidene acetal **85** in 80% yield over 2 steps. The latter underwent a glycosylation with donor **86** using NIS/AgOTf affording disaccharide **87**. The *N*-Troc group was then removed with zinc under acidic conditions and the free amine intermediate was used to generate two libraries. First, *N*-acylations of the amine were accomplished using nine acetyl chlorides and the resulting products were then de-*O*-benzylated and de-*O*-acetylated. Among these compounds, naphthyl derivative **89** was the best inhibitor (K_d=10.6 μM against galectin-3). The second strategy was initiated with *N*-acetylation followed by the application of classical Zemplèn conditions to recover selectively the desired OH-2′ group in intermediate **90** in 85% yield. Subsequent alkylation efficiently proceeded on this site in the presence of eight different alkyl bromides and the final preparation of the expected targets was realized under hydrogenolysis conditions. Cyclohexyl-bearing compound **91** was determined as the best inhibitor of this



Scheme 26. Synthesis of aromatic lactose 2-*O*-ester 94.



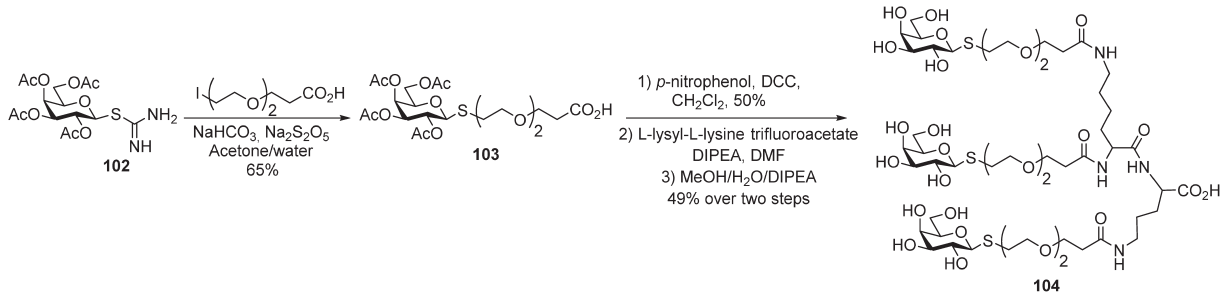
Scheme 27. Synthesis of 4-(4-phenoxybenzyl)ether linked lactosamine 101.

series (K_d=73.3 μM against galectin-3). This work highlighted the relevance of lacto-*N*-bioses as efficient scaffolds toward functionalized galectin inhibitors. In addition, the use of microscale frontal affinity chromatography coupled to mass spectroscopy as shown to be a useful tool for a rapid ranking of the different inhibitors.

In their ongoing studies to find the ideal group targeting arginine–arene interactions in the galectin CRD, the group of Nilsson prepared novel aromatic lactose 2-*O*-esters (67). A library of eight compounds was built, based on the derivatization of the 2-*O* position of a lactoside residue. As shown in Scheme 26, the chemistry was straightforward and initiated by a TMSOTf-mediated rearrangement of compound **92**, followed by deacylation of the 2-*O*-acetyl protecting group. Then, acylation of the corresponding free hydroxyl group furnished compound **94** after benzyl ether deprotections. Its binding properties to galectins-1, -3, -7, -8N, and -9N were evaluated by using a fluorescence-polarisation assay and revealed a potent affinity for galectin-3 with a K_d value of 2.2 μM.

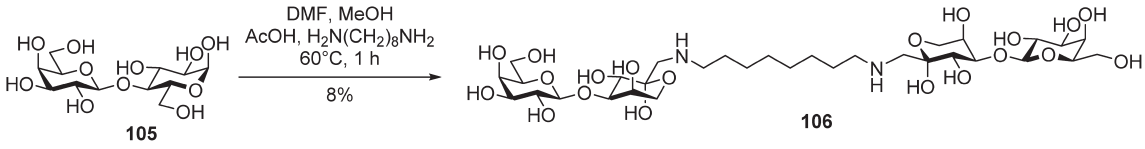
Another example of disaccharide targeting other subsites has been accomplished by the group of Pieters (64). Novel lactosamine derivatives possessing a 4-(4-phenoxybenzyl) ether linked at their C-3′ position were prepared. Thus, protected glucosamine **95** was glycosylated with 3-bromopropanol using $BF_3{\cdot}OEt_2$ followed by bromide displacement with sodium azide (Scheme 27). Acceptor **97** was generated after deprotection and selective installation of the *O*-6 acetate. On the other hand, thiomethylgalactoside donor **100** was easily generated using a three-step protocol. Galactoside **98** was treated with dibutyltin oxide to form the stannylidene acetal, which was subjected to 4-phenoxybenzyl bromide and acetic anhydride. Finally, lactosamine **101** was easily prepared under glycosylation conditions involving acceptor **97** and donor **100**, followed by removal of the acetate protecting groups. Interestingly, compound **101** was more selective for galectin-3 (K_d=1.2 μM) than galectin-1 (K_d=280 μM) as measured by a fluorescence polarization assay. This selectivity can be explained by a larger number of favorable aryl–arginine interactions for galectin-3 and possible steric impediments for galectin-1.

Finally, recently, the group of Grandjean designed galectin inhibitors from a lactosamine core, functionalized at key C2 and C3′ positions by aromatic substituents (68). The high affinity of those new inhibitors was demonstrated in cellular assays for an application to skin tissue repair. Moreover, the biophysical and structural characterization of lactosamine derivatives with human galectin-3



102 103 104

Scheme 28. Synthesis of trivalent galactoside 104.



105 106

Scheme 29. Synthesis of lactulosamine dimer 106.

were also accomplished (69). X-ray crystallography revealed cation-π interactions between lactosamine aryl substitutions and arginine residues from the CRD. This research was the foundation for the development of a sensitive microarray for the ranking of galectin inhibitors (70). Thus, glycan microarray based on evanescent-field fluorescence detection was used to screen novel lactosamine functionalized at C2 and C3′ positions by aromatic substituents.

C-3. Synthesis of Multivalent Inhibitors

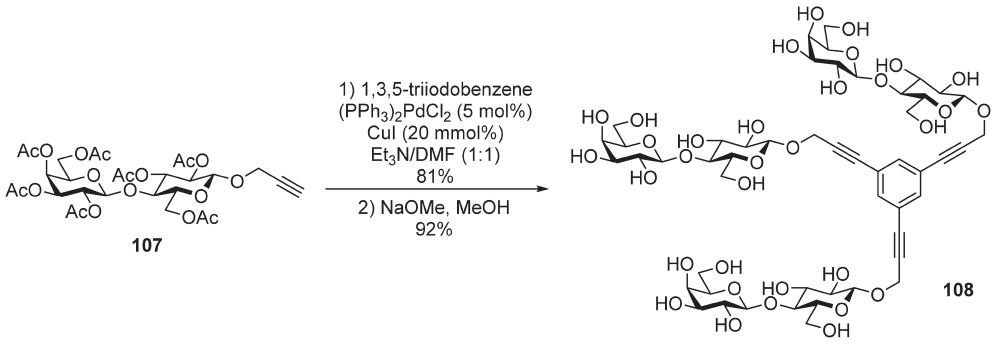
Multivalent carbohydrate-protein interactions mediate a large number of important physiological processes (71). Hence, the synthesis of multivalent glycoconjugates could be used to study, rationalize, and understand carbohydrate–galectin interactions (72). The proto type and tandem repeat type galectins are both divalent cross-linkers, as opposed to galectin-3, which is better known for its ability to act as efficient multivalent cross-linkers (73, 74). Over the years, various types of multivalent platforms were exploited for galectin inhibition, *i.e.*, dimers, small clusters, glycodendrimers and even self-assembled multivalent pseudopolyrotaxanes (75). This section will describe efforts on the preparation of multivalent glycoconjugates as galectin inhibitors. These studies often led to very potent candidates benefiting from a synergic and cooperative effect known as the "glycoside or dendritic effect." In fact, it is well established that multivalency could offer numerous benefits in terms of affinity and receptor selectivity *versus* weaker monovalent interaction (71).

As early as 1999, multivalent lactosides were developed to probe multivalent ligand functions in galectins (76). Later, the group of Gabius presented simple divalent and trivalent galactosides and lactosides (77). The best inhibitor against galectin-1 is presented in Scheme 28 and followed a route previously described (78). Thus, galactoside **102** reacted with the bifunctional spacer under basic conditions affording PEGylated intermediate **103**. The carboxylic moiety was then activated as a *p*-nitrophenyl ester and smoothly reacted with L-lysyl-L-lysine. Deprotection under standard basic conditions afforded trivalent galactoside **104**. Out of eight compounds, trivalent **104** proved to be the superior inhibitor of galectin-1 (IC_{50} = 1.6 mM) as compared with the monovalent derivative (IC_{50} = 12 mM) as determined in a solid-phase assay with surface-immobilized lactosylated bovine serum albumin.

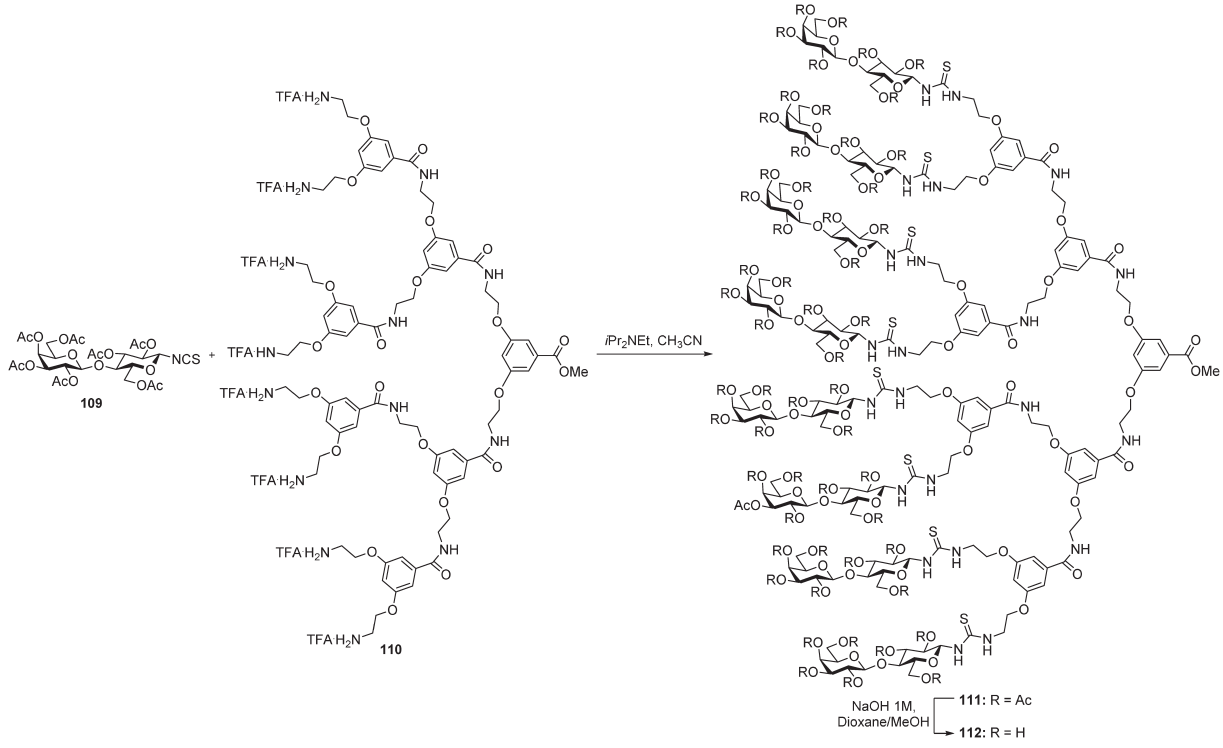
Simpler dimeric structures also demonstrated interesting features as exemplified by the work of Rabinovich *et al.* who proposed the synthesis of low molecular weight lactulosamine derivatives in only one step (Scheme 29) (79). Galectin binding was observed with IC_{50} value on the order of 20–40 mM for compound **106**. In addition, this dimer possessed a selective regulatory effect in different events linked to tumor progression. The authors also proposed that subtle differences in the carbohydrate structures may be potentially useful tool to block specifically different steps of tumor growth and metastasis.

The group of Roy demonstrated inhibition of lectin binding using synthetic multivalent glycoclusters (80). Hence, 2-propynyl lactoside **107** was subjected to Sonogashira conditions with 1,3,5-triiodobenzene. Trivalent lactoside **108** was isolated in high yield and was the only candidate to induce a strong cluster effect with an IC_{50} value of 31 μM against chimeric-type galectin-3 (*vs.* 700 μM for free lactose standard). In this study, haemmagglutination and cytofluorometry studies showed that cluster **108** was more active for this galectin than other inhibitors with higher lactoside valency, by efficiently blocking its binding to native cell surfaces.

The group of Pieters prepared a family of glycodendrimers (up to G(3), as shown in Scheme 31) based on the 3,5-di-(2-aminoethoxy)benzoic acid branching units (81). In this context,



Scheme 30. Synthesis of multimeric lactoside using Sonogashira palladium-catalyzed cross-coupling.

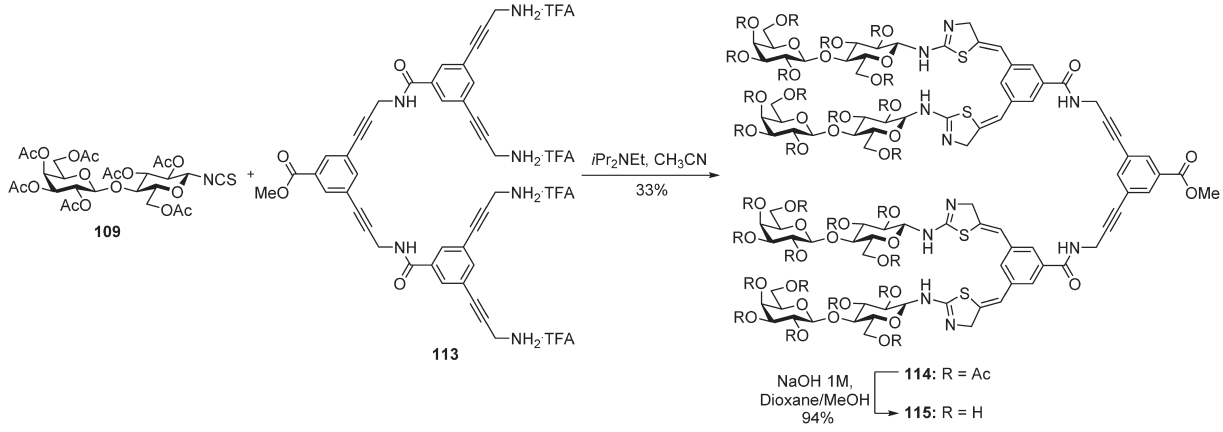


Scheme 31. Synthesis of multivalent lactoside 112.

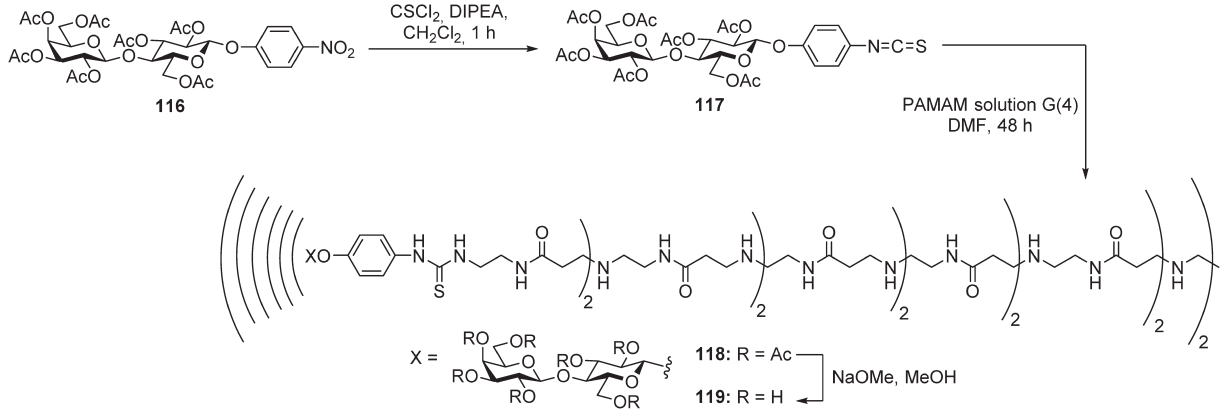
lactose isothiocyanate **109** directly reacted with scaffold **110** containing eight aminated termini to anchor *β*-lactoside derivatives through isothiocyanate carbohydrate linkages. After full deprotection under basic conditions, hydroxylated glycodendrimer **112** showed minimal inhibitory concentrations of 10.8 mM and 1.3 mM against galectins-1 and -3 respectively (as determined in a solid-phase assay with surface-immobilized (neo)glycoproteins). Of interest, this work clearly demonstrated that optimal inhibition does not necessarily correlate with high valency, but rather requires a distinct mode of tridimensional glycan presentation.

Multivalent lactosides were also synthesized by the group of Pieters using a more rigid propargylic amine spacer (82). Thus, lactose isothiocyanate **109** was reacted with aryl propargylic amine **113** to generate thiourea linkers (Scheme 32), conversion into 2-aminothiazoline moieties in the presence of *i*-Pr_2NEt and subsequent acid treatment facilitated their formation. Tetravalent lactoside **114** was subjected to standard Zemplèn deacetylation conditions to yield the desired inhibitor **115**, which showed multivalent effects against galectin-3, with an 4300-fold enhancement of activity relative to lactose standard (IC_{50} = 70 nM) in a solid phase assay. No significant multivalency effect was observed for galectin-5.

Poly(amidoamine) starburst glycodendrimers also offered the potential to serve as efficient scaffolds for high-affinity ligands. In this context, the group of Gabius presented five generations of *p*-isothiocyanate linkages with *p*-aminophenyl-*β*-D-lactoside as head groups (83). The synthesis followed previous work of Roy's group (84). The 4-nitrophenyl lactoside **116** was transformed into the corresponding 4-isothiocyanate **117**, which reacted with den-



Scheme 32. Synthesis of multivalent lactoside 115.



Scheme 33. Synthesis of the G4 poly(amidoamine) glycodendrimer 119.

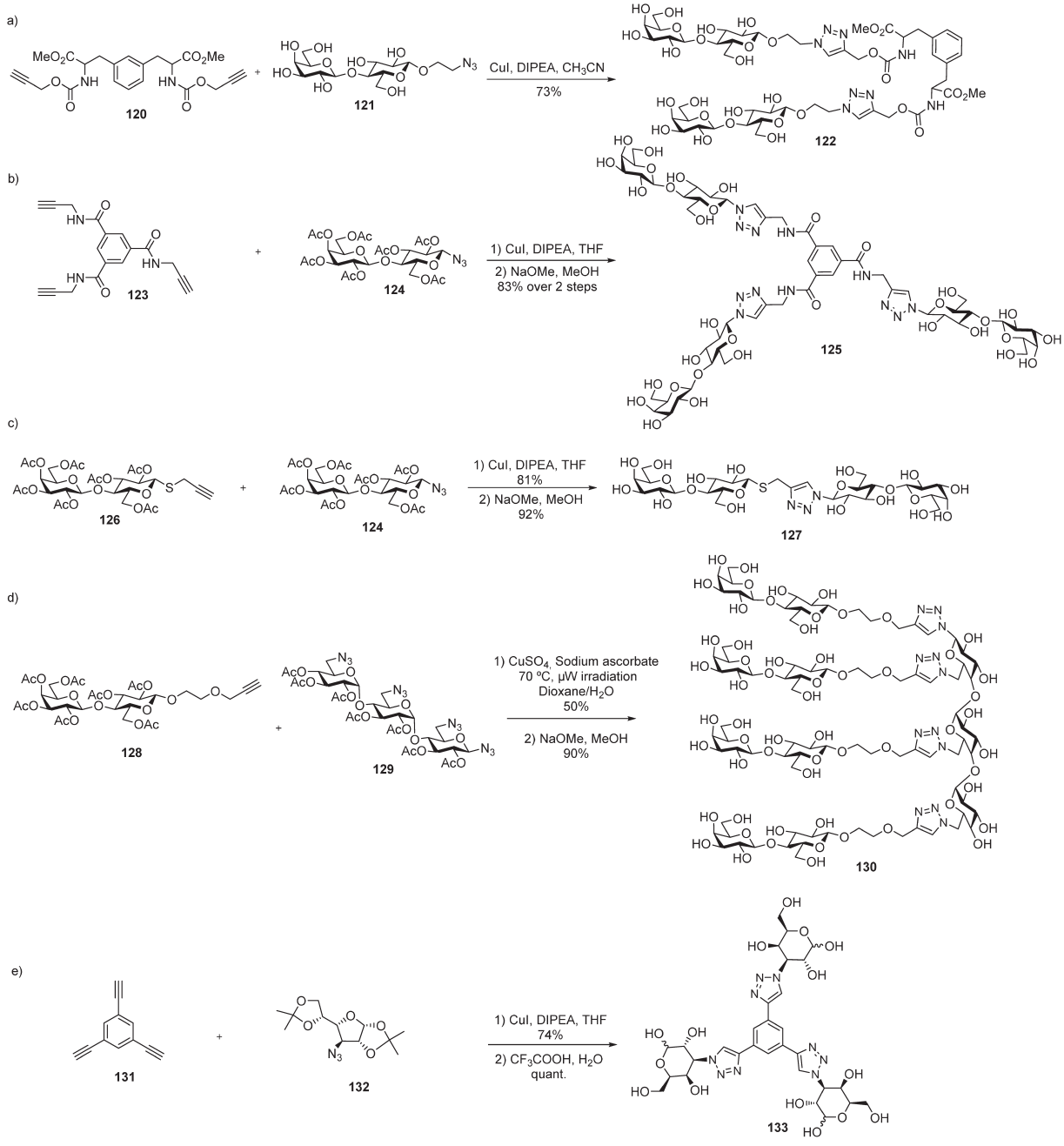
dritic poly(amidoamine) (Scheme 33). The G(4) poly(amidoamine) glycodendrimer **119** was the best inhibitor against galectin-1. The affinity constant for the interaction of surface-immobilized glycodendrimers with labeled sugar receptors resulted in a K_d of 163 nM against galectin-1 and a K_d of 767 nM against galectin-3.

As an alternative mode of conjugation, the copper(I)-catalyzed azide–alkyne 1,3-dipolar cycloaddition (CuAAc) has been extensively used in the glycosciences (85). This so-called click reaction is as a powerful tool for the preparation of multivalent glycosides to study galectin inhibition. The click reaction is ideal for preparing multivalent galactoside or lactoside clusters, because it is performed under mild conditions, and is compatible with a wide range of solvents and functionalities.

In this regard, the group of Nilsson prepared acetylenic natural and unnatural amino acids for functionalization with 2-azidoethyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (86). A library of nine compounds was made and dimer **122** was the best inhibitor against galectin-1, with a K_d of 3.2 μM (Scheme 34a). Moreover, compound **122** had a relative potency of 59 as compared to methyl β-lactoside as determined in a fluorescence polarization assay. This work demonstrated that galectin-1 undergoes cross-linked aggregation while compound **122** had the strongest cluster effect of the library.

The same year, the group of Roy prepared carbohydrate triazoles and isoxazoles as inhibitors of galectins-1 and -3 (36). C_3-symmetric tris-lactoside **125** was prepared from the cycloaddition of azide **124** and *N*-*N*′-*N*″-tripropargyl-1,3,5-carboxamidobenzene partner **123** (Scheme 34b). Trivalent lactoside had inhibitory properties of 20 μM against galectin-1. No multivalent effect was detected when this compound was evaluated against galectin-3 (inhibitory properties of 250 μM). Interestingly, trivalent lactoside **125** reduced galectin-1-mediated HIV-1 attachment to target cells (87) thus suggesting that galectin-1-specific inhibitors could potentially pave the way for the development of a new class of compounds to treat HIV-1 infection.

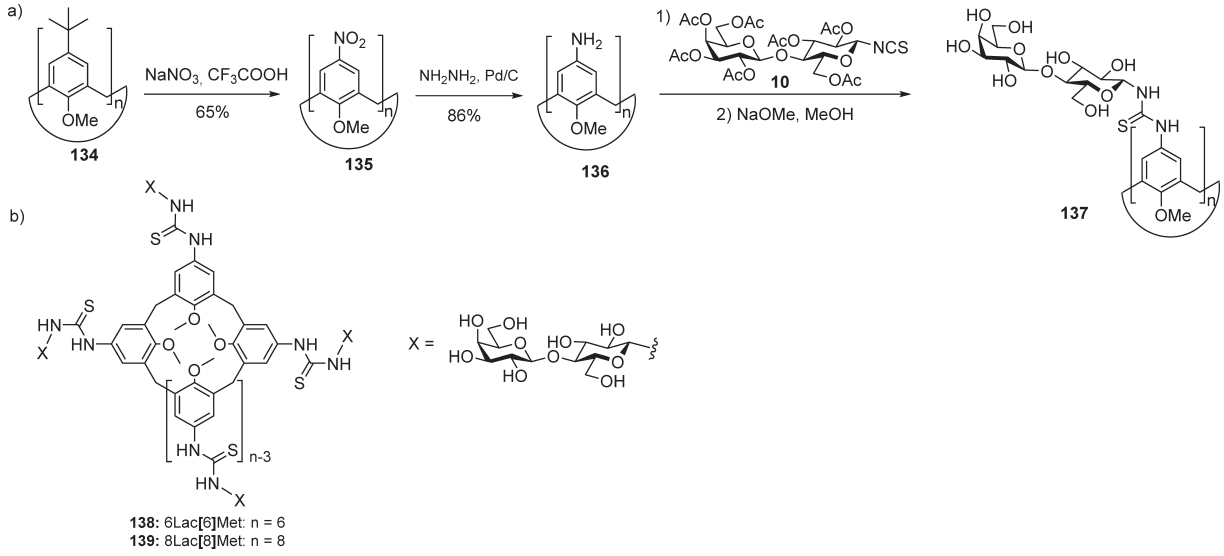
The group of Roy also described the preparation and inhibitory properties of simple homo- and hetero-lactoside and galactoside dimers (37). Out of the four triazole dimers prepared, compound **127** was active against galectins-1 and -3 (inhibitory properties of 0.3 mM and 0.16 mM, respectively) (Scheme 34c).



Scheme 34. Synthesis of multimeric lactoside "click clusters" 122, 125, 127, 130, and 133.

The group of Gouin used multimeric lactosides as tools to investigate the effect of linker length on specific interactions with galectins-1 and -3 (88). Tetravalent lactoside **130**, accessible using CuAAc reactions from alkyne **128** and azidosugar **129**, was evaluated in a fluorescent polarization assay and an enzyme-linked lectin assay (Scheme 34d). The K_d of **130** against galectin-3 was 16 μM with a relative potency of 4.6 compared to monovalent lactoside. This worked highlighted that multivalent derivatives bearing the longest spacers were more efficient for cross-linking lectins.

Finally, 1,2,3-triazole-linked galactohybrids were prepared by the group of Turks (Scheme 34e) (89). Using 1,3,5-triethynylbenzene **131** and azido sugar **132** as complementary partners for CuAAc reaction, trimer **133** was isolated in 74% yield over 2 steps. The multivalent 3-deoxy-3-triazolyl-galacto-conjugate had a K_d value as low as 50 μM against galectin-3 (100-fold better than galactose), as determined using a fluorescent anisotropy assay. This is an impressive result considering the absence of natural disac-



Scheme 35. a) General strategy to access various calix[*n*]arene-based glycoclusters; b) Structure of 6Lac[*6*]Met 138 and 8Lac[*8*]Met 139.

charide structural moieties and the simplicity of the synthetic approach.

The flexibility in varying ring size, molecular shape, conformational flexibility, symmetry, and valency makes calix[*n*]-arenes unique tools to study glycocluster–protein interactions. The group of Gabius and Ungaro synthesized calix[*n*]arene-based glycoclusters for their potential selectivity and avidity against galectins and the synthetic route is described in Scheme 35a (90). Thus, the *p-tert*-butyl calixarene precursor **134** was first converted into the corresponding nitro calixarene **135** using $NaNO_3$ and CF_3CO_2H. Reduction of the nitro groups using hydrazine hydrate in the presence of Pd/C led to the corresponding polyamine **136**, which reacted with *β*-galactosyl or *β*-lactosyl isothiocyanates. After deprotection of acetyl groups using Zemplèn conditons, the general procedure generated 14 calix[*n*]arene-based glycoclusters ("*calixsugars*") that were isolated and evaluated against *Viscum album agglutinin* and galectins-1, -3 and -4 using solid-phase inhibition assays. Inhibitory potencies showed that 6Lac[*6*]Met **138** was the best inhibitor of galectin-1 (IC_{50}=600 μM, concentration of 10 μg/mL) and galectin-4 (IC_{50}=5 μM, concentration of 5 μg/mL) (Scheme 35b). Also, 8Lac[*8*]Met **139** has better affinity for galectin-3 (IC_{50}=400 μM, concentration of 5 μg/mL). This work unequivocally demonstrated cluster effects for the tandem-repeat-type galectin-4 and the influence of conformational properties of glycoclusters on the inhibition of various galectins. A follow-up study presented glycoclusters of *N*-acetyllactosamine with aromatic substituents at 2-*N* or 3′-positions on calix[*4*/*6*]arenes (91). They were able to achieved good inhibition of galectin-3 (IC_{50}=0.4 μM, concentration of 3 μg/mL) and galectin-4 (IC_{50}=0.8 μM, concentration of 5 μg/mL).

D. Conclusion and Outlook

Considering the role of these galectins in cancer-related processes it is clear that the quest for optimized galectin ligands represents a major challenge that is necessary for public health. Thus, the long-standing synthetic efforts carried out by numerous research groups is an important endeavour. The present review emphasizes the synthetic strategies used to prepare galectin inhibitors that could have a highly promising future as drug targets. Ideally, high-avidity ligands combining specificity, selectivity, and synthetic accessibility with simple carbohydrate-based structures could rapidly emerge as efficient lead candidates. Because glycochemists already possess powerful synthetic and biological assets, the hope of seeing the development of optimized galectin inhibitors in clinical trials in the next decade is a likely reality. We are convinced that the clever combination of both intrinsic ligand fine-tuning and multivalency strategies could lead to potent candidates ready to enter clinical trials.

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From left to right: Denis Giguère, Danny Lainé, Vincent Denavit, Thomas Tremblay, and Jacob St-Gelais