Review

Multivalent Pyrrolidine Iminosugars: Synthesis and Biological Relevance

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Abstract: Recently, the strategy of multivalency has been widely employed to design glycosidase inhibitors, as glycomimetic clusters often induce marked enzyme inhibition relative to monovalent analogs. Polyhydroxylated pyrrolidines, one of the most studied classes of iminosugars, are an attractive moiety due to their potent and specific inhibition of glycosidases and glycosyltransferases, which are associated with many crucial biological processes. The development of multivalent pyrrolidine derivatives as glycosidase inhibitors has resulted in several promising compounds that stand out. Herein, we comprehensively summarized the different synthetic approaches to the preparation of multivalent pyrrolidine clusters, from total synthesis of divalent iminosugars to complex architectures bearing twelve pyrrolidine motifs. Enzyme inhibitory properties and multivalent effects of these synthesized iminosugars were further discussed, especially for some less studied therapeutically relevant enzymes. We envision that this comprehensive review will help extend the applications of multivalent pyrrolidine iminosugars in future studies.

Keywords: iminosugar; pyrrolidine; multivalent effect; glucosidase inhibitors

1. Introduction

Iminosugars, containing an endocycling nitrogen atom that effectively mimics carbohydrates by facilitating the reversible and competitive inhibition of their processing enzymes, have generated much attention in recent years as targets in the treatment of a wide range of illnesses (e.g., diabetes, cancer, tuberculosis, and lysosomal storage disorders, etc.) [1–6]. Given the enormous range of biochemical events in which carbohydrate processing enzymes are implicated, iminosugars have enormous potential to be developed as inhibitors of glycosidases (glycoside hydrolases), glycosyltransferases (glycoside synthases), metalloproteinases, and nucleoside-processing enzymes [7–11]. It is worth noting that structural modifications to find potent inhibitors of the above enzymes among two of the most studied classes of iminosugars, polyhydroxylated pyrrolidines and piperidines, arouse great interest because of the wide range of their biological properties, such as glycosidase inhibition, shown over the past five decades [12–16].

The most famous representative iminosugars belonging to piperidine are derivatives of 1-deoxynojirimycin (DNJ, Figure 1). Since DNJ’s isolation from white mulberry root bark in 1976, hundreds of artificial iminosugars based on DNJ have been synthesized and their bioactivities evaluated [17–21]. Two N-alkylated DNJ derivatives are approved drugs, N-hydroxyethyl-1-deoxynojirimycin (Miglitol, Figure 1) to treat type II diabetes, and N-butyl-1-deoxynojirimycin (Miglustat, Figure 1) to treat lysosomal storage disorders (e.g., Gaucher disease). Similarly, five-membered iminocyclitols, also known as pyrrolidine iminosugars, exhibited excellent inhibition toward glycosidases. For example, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, Figure 1), the first pyrrolidine iminosugar extracted from the leaves of *Derris elliptica* in 1976 [22], proved to be a potent glycosidase inhibitor; subsequently, its analogs were also found to have significant effects.
on glycosidases [23–25]. 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB, Figure 1), isolated from the fruit of Angylocalyx boutiquanus, exhibited strong inhibition of glycogen phosphorylase, and is currently being explored for the treatment of type II diabetes [26,27]. 1,4-Dideoxy-1,4-imino-L-arabinitol (LAB, Figure 1), the enantiomer of DAB, displayed more potent specific glycosidase inhibition. A new α-glucosidase inhibitor based on LAB was reported by Kato et al. in 2012, which showed huge potential in reducing elevated plasma glucose after food intake when tested in vivo with a carbohydrate load at doses approximately ten times lower than the required dose of miglitol [27–30]. In addition, Radicamines A and B have attracted extensive interest because of their potent inhibition of α-glucosidases and potential pharmaceutical applications [31–33]. Overall, ample evidence has established that iminosugars have anti-diabetic effects [34–38]. Both classes of iminosugar derivatives would be promising drug candidates, and therefore the development of synthetic strategies and the evaluation of bioactivities are of decisive importance.

However, as previously introduced, only a few drugs are on the market. New strategies for developing iminosugar-based glycosidase inhibitors to understand vital biological processes or as clinical candidates are therefore major challenges in both academia and the pharmaceutical industry [39–41]. In the last decade, a multivalent glycosidase inhibition effect, which has been extensively used in developing lectin inhibitors to seek new therapeutic opportunities for carbohydrate-related diseases, was found and rapidly developed [42–49]. Johns and Johnson first reported the synthesis of divalent iminosugars and explored the contribution of the multivalent strategy to biological activity in 1998 [50]. Probably due to the reported multivalent compounds, which could not exhibit the expected inhibition ability to glycosidase enzymes, the design of glycosidase inhibitors has not been able to attract the interest of researchers [51,52]. The factors that hampered the application of multivalency to iminosugars may be as follows: Firstly, there is an intrinsic structural difference between glycosidases and lectins. The surface of lectins shows multiple carbohydrate-binding sites, while glycosides and other carbohydrate-processing enzymes are usually monomeric and therefore bind relatively weakly to multivalent substrates [53–55]. Secondly, the synthesis of multivalent iminosugars is challenging. Since the click reaction was immature before 2001, it was particularly hard to graft several monomers to a skeleton simultaneously [56,57]. In addition, the experimental results obtained were not encouraging [50–52]. It was not a rapidly emerging area with exciting potential until the discovery of a small but quantitative multivalent effect in α-mannosidase inhibition [58]. Based on the extensive literature involving lectins and glycoclusters, several potential interactions have been proposed to explain the multivalent effect. The “bind-and-recapture” process is a classical mode due to the increased concentration of active molecules concentration in proximity to the binding site (Figure 2a). The chelate effect can occur when the enzyme presents more than one active site (Figure 2b). In addition, stronger interactions will occur when some non-catalytic subsites interact with glycoclusters (Figure 2c). Moreover, cross-linking and aggregation

Figure 1. Examples of natural and synthetic iminosugars.
processes may prevail with glycoclusters if the enzyme possesses a multimetric nature (Figure 2d) [59,60].

![Figure 2. Proposed binding models accounting for the multivalent effect: (a) Bind and recapture; (b) Chelate process; (c) Subsite binding; (d) Cross-linking and aggregation.](image)

The construction of multivalent iminosugars follows conventional strategies, including modifications and protecting group chemistry of the iminosugars [50,61,62], coupling reactions using click chemistry [45,48,63], and recently developed supramolecular self-assembly based on \( \pi-\pi \) stacking or hydrophobic interactions [64–66]. Multimerization of the pyrrolidines using these strategies resulted in some interesting results. For example, multimeric pyrrolidine iminosugars were reported to be the first example of multivalent enhancers of human \( \alpha \)-galactosidase A (\( \alpha \)-Gal A), an enzyme involved in Fabry disease [49]. Multivalent dendrimers decorated with the DAB exhibited a relevant multivalent effect toward the lysosomal enzyme \( N \)-acetylglactosamine-6-sulfatase (GALNS), which is involved in a rare metabolic disorder [67]. Other multivalent effects of multivalent pyrrolidine iminosugars were obtained with carbohydrate-active enzymes such as Golgi \( \alpha \)-mannosidase II [68], \( \beta \)-N-acetylglucosaminidase [69], and \( \alpha \-L\)-fucosidase [70]. This unique class of compounds may provide new pharmaceutical opportunities to treat diseases involving carbohydrate-processing enzymes.

Several reviews on the topic of multivalent iminosugars have been published; however, large parts deal with the synthesis and biological properties of multivalent piperidine iminosugars rather than pyrrolidine [12,14,71,72]. Moreover, the latter research field was scarcely reviewed in the decade of its rapid development (2012–2022) [73]. The present review illustrates the detailed synthesis and multivalent effects of all multivalent pyrrolidine iminosugars that have been assayed against various glycosidases and provide an overview of the main achievements made to date.

2. Syntheses of Multivalent Pyrrolidine Iminosugars
2.1. Synthesis of Di- and Trivalent Iminosugars

The first synthesis of tethered di- and trivalent pyrrolidine iminosugars to interfere with carbohydrate processing enzymes was reported by Robina and co-workers in 2013 [70]. At that time, the advantages of the multivalent effect for glycosidase inhibition over the corresponding monomer were gradually realized (e.g., for \( \alpha \) - and \( \beta \)-glucosidases [74], for \( \beta \)-galactosidases [75]). Taking advantage of their experience in designing glycosidase inhibitors [76,77], the authors investigated the multivalent approach by comparing the \( \alpha \)-L-fucosidase inhibitory activities of multi- and mono-pyrrolidine iminosugars. For this purpose, four di- and trivalent pyrrolidine derivatives (1–4) were synthesized based on \( L\)-configured 1,4-imino-cyclitols 5 and 6, which displayed good inhibitory activity towards \( \alpha \)-L-fucosidase (Figure 3). The benzylamino pyrrolidine 5, designed as a monovalent reference for dimer 1 and trimers 2 and 3, was synthesized for the first time, while the furyl-substituted pyrrolidine 6, previously reported by the same research group [78], was selected for comparison with trimer 4.
The authors employed a classical amide coupling reaction to synthesize the desired iminosugars, starting from commercially available benzylamine and the O- and N-protected carboxylic acid 7 [70], using PyBOP as the coupling agent and DIPEA as base gave protected intermediates. Then, the excess benzylamine was easily separated by column chromatography. A similar method was used to remove excess amines for the preparations of 1–4. Finally, isopropylidene deprotection with HCl and subsequent catalytic hydrogenation with H2/Pd/C gave the corresponding target product 5 in 84% yield. Di- and trivalent iminosugars were obtained with commercially available m-xylyленедiamine 9 and triamine 10 as the scaffolds for bi- and trivalent glycomimetics, respectively. Moreover, the long-tethered triamine 11 reported by the same research group [79] was chosen as C-3 symmetric template to yield the long spacer trivalent iminosugar 3. Amide coupling reactions between scaffolds and compound 7 under the same condition above gave the corresponding target products 1–3 in moderate-to-good yields. Similarly, trimer 4 was obtained by the coupling reaction between a previously synthesized pyrrolidine-furan carboxylic acid 12 [78] and template 10, followed by standard reductive hydrogenation in 46% yield (Scheme 1).

To further understand the complicated multivalent effect on α-fucosidase inhibition, Behr, Robina, and co-workers reported a library of divalent pyrrolidine iminosugars 13–17 using polyamine and triazole benzene as spacers to evaluate the contributions of the length and rigidity of the bridge, the number of nitrogen atoms present, and the moieties close to the pyrrolidine to the biological activity of divalent inhibitors [80]. Since there is no report of the monovalent references 18–20, their synthesis routes were also introduced. The inhibitory effect of chemically diverse spacers in dimers on α-fucosidase was systematically investigated, and a potent and specific α-fucosidase inhibitor (compound 17, Ki = 3.7 nM) was thus discovered (Figure 4).

The target monovalent inhibitors 18 and 19 were synthesized from the known allylpyrrolidines 21 and 24 [81]. Starting from the (2R)-configured 21, after dihydroxylation, oxidative cleavage with NaO4 gave a stable intermediate 22. Pyrrolidinyl ethanol 18a was obtained in 37% yield by reducing 22 with sodium borohydride, followed by deprotection with hydrogen and acidification in three steps. The congener 18b was obtained by reacting 23 with benzylamine followed by deprotection with H2/Pd–C (10%), in 43% yield. However, the stereoisomers 19a and 19b of compounds 18a and 18b could not be obtained by the same synthetic route through (2S)-configured 24, mainly due to the key intermediate...
25 after reaction with 24. The authors reported that the aldehyde 25 would go through an epimerization process, which spontaneously opened the pyrrolidine ring to form the conjugated aldehyde 22, impeding the synthetic purpose [81,82]. Alternatively, protection of the amino group by switching from Bn to Boc solved this problem and gave the clean and stable (2S)-configured 26. Target iminosugars 19a and 19b were afforded by reduction and reductive amination under the same conditions as introduced above (Scheme 2).

Figure 4. Structures of divalent iminosugars 13–17 and monovalent references 18–20.
The synthetic routes for dimers 13 and 16 were similar to those of their corresponding monomers. An excess of compound 26 (2-fold excess) reacted with hexamethylene-diamine or spermine to produce the corresponding di-imines, which were then reduced via sodium borohydride. Silica gel chromatography was employed to separate the excess 26 and yield the corresponding protected dimers 13 and 16, which were further deprotected to give the target products. It is worth noting that under this method, homologue 16 with a spermine bridge contained some impurities. Hence, a further sequence of Boc protection/purification/deprotection (MeOH: HClaq) was required to obtain pure 16 in 29% yield (Scheme 2).

The synthesis of the divergent iminosugars 14 and 15 was started from the known compound 28 [83], which was reacted with benzylamine and sodium triacetoxylborohydrate, followed by acidification and deprotection to afford compound 15a in 26% yield. Dimer 15b was obtained by debenzylation of 15a under the H2/Pd/C system in 65% yield. Reacting 28 (2-fold excess) with hexamethylenediamine by similar methods (MgSO4 then NaBH4 or amine then NaBH(OAc)3) both gave dimer 29 in a low yield (25%) with an undesired trivalent product 31 (27%). Gratifyingly, the yield of 29 could be increased to 49% by reacting 28 with ethylenediamine in the presence of sodium borohydride and 2,2,2-trifluoroethanol. For exploration, the amino-protected dimer 29 and trimer 30 were both deprotected under hydrochloric acid to generate the target products 14 and 31, which were likewise tested towards α-fucosidase. Dimer 17 was synthesized due to the good inhibitory activities of (pyrrolidin-2-y)triazones shown by the researchers previously [83]. Thus, after the reduction of 28 to 32, target dimer 17 was generated through a two-step reaction by treating excess 32 with 1,3-bis(azidomethyl)benzene under the catalysts of Cul and DIPEA, followed by acidification. Column chromatography was carried out to remove unreacted 32 and yield 17 in 44% yield (Scheme 3). Increasing results began to highlight the advantages of multivalent effects. However, some contradictory experimental results were still reported. Elucidating the specific binding mechanisms of multi-ligands with enzymes is urgent and challenging. Behr and co-workers reported three stereoisomeric pyrrolidine dimers in 2016 to explore the divergent effect on fucosidase inhibition [84]. The divergent iminosugars (33, ent-33, and meso-33) were constructed based on a known fucosidase
inhibitory activities of (pyrrolidin-2-yl)triazoles shown by the researchers previously [83]. The monovalent iminosugars 35, ent-35, and 36 were also synthesized as referenced (Figure 5).

![Chemical structures and reactions](attachment:chemical_diagram.png)

Scheme 3. Synthesis of divalent iminosugars 14, 15, and 17.

Figure 5. Structures of divalent inhibitors and the corresponding monomers by Behr.

Known compound 37 and its enantiomer ent-37 were used as starting materials for synthesizing homodimer 33 and its enantiomer ent-33, respectively [86]. Hemiacetal ent-37 was converted to 38 by amination with benzylamine. The intermediate 38 was then subjected to a highly stereoselective ethynylmagnesium bromide-mediated nucleophilic addition to aminoalcohol ent-39 in 70% yield for two steps [87]. Then, azide ent-40 was obtained in 68% yield from ent-39 upon intramolecular nucleophilic reaction in the presence of MsCl, which was employed to activate the secondary hydroxyl to invert the configuration at C(OH). Homodimerization was carried out simply via the oxidation coupling of pyrrolidine ent-40 using Pd(PPh3)2Cl2 and CuI as catalysts in the presence of i-PrNH2 to
generate the diyne ent-41 in 84% yield. Finally, the target homodimer ent-33 was prepared in 38% yield by alkyne reduction, hydrogenolysis of the benzyl groups, and acidolysis of ent-41. Monomer ent-35 was readily prepared by the same reduction/acidolysis sequence from ent-40. The same synthetic route was applied to the known 37 to prepare compounds 33 and 35 (Scheme 4).

![Scheme 4. Synthesis of divalent iminosugars 33, ent-33 and monovalent references 35, ent-35.](image)

The meso analog meso-33 cannot be obtained by coupling ent-40 with its enantiomer directly, due to the formation of hard-to-remove mixture ent-41/41. In order to avoid reaction monitoring and isolation problems, ent-40 and known N-allyl protected enantiomer 42 [88] were employed to obtain meso-33 through the same way used to generate 41 described above. An excess of enantiomer 42 was necessary to decrease the production of compounds ent-41 and 43. As expected, the target hetero-diyne 44 was obtained and isolated in high yield (61%). Cleavage of the N-allyl group from 44 in the presence of NDMBA and Pd(PPh3)4, followed by hydrogenolysis of the benzyl groups and final acidolysis, afforded the target dimer meso-33. Monomer 36, an analog of 33 whose second pyrrolidine moiety was replaced by a phenyl group, was prepared from the known diyne 46 [33] using classic hydrogenation (H2, Pd/C, MeOH) in 93% yield (Scheme 5).

Two years later, Moreno Vargas and co-workers pioneered a valuable methodology for rapid, efficient screening of the divalent inhibitors to α-fucosidases and β-galactosidase, as well as studying the multivalent approach in the inhibition of glycosidases [89]. The Cu(I)-catalyzed alkyn-e-azide cycloaddition (CuAAC) reaction, a fantastic chemical reaction based on Huisgen 1,3-dipolar cycloaddition chemistry [90,91] and then developed by Meldal [56] and Sharpless [57], was employed to generate three libraries of divalent iminosugars (47a-l, 48a-l, and 49a-l) between alkyln y pyrrolidines 47-49 and the set of diazides a-i. Due to the high efficiency of the CuAAC reaction, the obtained crude products could be directly screened for enzyme inhibitors without purification. It is worth noting that the discovery of the CuAAC reaction extensively promoted the development of the multivalent approach (Figure 6).
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Known compounds D-lyxose and D-glucose, were employed as starting materials. Due to the high efficiency of the CuAAC reaction, the obtained crude diazides were prepared according to the design of the spacer. (Pyrrolidin-2-yl)triazole and (pyrrolidin-2-yl)furans were previously shown to exhibit significant glycosidase inhibition to α-fucosidases and β-galactosidases by the same group.

The Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction, a fantastic chemical reaction based on Huisgen 1,3-dipolar cycloaddition chemistry and then developed by Meldal and Sharpless, was employed to generate three libraries of divalent iminosugars.

Based on the previous report, generation of libraries of dimeric iminosugars (47a–l, 48a–l, and 49a–l) for in situ screening.

Alkynyl pyrrolidines 47–49 were selected as the skeletons because their analogs (pyrrolidin-2-yl)triazole and (pyrrolidin-2-yl)furans were previously shown to exhibit significant glycosidase inhibition to α-fucosidases and β-galactosidases by the same group. The initial step was to prepare the different tethered alkynyl pyrrolidine derivatives 47–49.
Known compounds 32 [84] and 53 [93], previously prepared by the same group from D-lyxose and D-glucose, were employed as starting materials. Alkynyl pyrrolidine 47 was readily prepared from 32 via Boc-deprotection in TFA in 87% yield. As expected, the CuAAC coupling reaction was carried out between alkynyl pyrrolidine 32 and azide 50 [94] to yield quantitative triazole 51. Then, propargylation of triazole 51 with NaH followed by acidic deprotection of derivative 52 quantitatively provided the desired alkynyl derivative 48. Classic amide coupling conditions (PyBOP/propargylamine) were employed to form the epimers 54a and 54b from compound 53, further separated by chromatography. Based on the previous report, cis-configured epimer 54b was chosen for deprotection to yield the target pyrrolidine-furan hybrid 49, since trans-configured epimer 54a exhibited weak α-fucosidase inhibition. Finally, the diazides a–l, another part for CuAAC coupling, were prepared according to the design of the spacer. (Pyrrolidin-2-yl)triazole libraries were generated by parallel CuAAC couplings between alkynyl functionalized pyrrolidines 47–49 and diazides a–l under the catalysts of CuI or CuSO₄. Due to the high efficiency of CuAAC coupling, granting almost quantitative yields with no side reactions, the desired products were all processed and directly screened for enzyme inhibition testing (Scheme 6).

![Synthesis of alkynyl pyrrolidines 47–49.](image)

Crude screening indicated that dimer 47i was the best inhibitor of α-fucosidases from the bovine kidney (kᵢ = 0.15 nM), and that dimer 49e was the best inhibitor of β-galactosidase from the bovine liver (kᵢ = 5.8 μM). Hence, compounds 47i and 49e were scaled up for detailed and complete analysis. To evaluate the multivalent effect on enzyme inhibition, monovalent references 56–59 were prepared. The synthesis routes adopted the same reaction conditions with compound 51 and CuAAC cycloaddition, and therefore will not be repeated here (Scheme 7).

The generation and in situ bio-screening of compound libraries mediated by efficient chemical reactions such as click reactions has proven to be an economical, rapid and efficient screening method for enzyme inhibitors, mainly in the context that the spatial structure of most enzymes is still unknown. More recently, Moreno and co-workers continued their work by screening a library of divalent pyrrolidine iminosugars to find inhibitors of human hexosaminidase [69]. A nanomolar and remarkably selective inhibitor of human nucleocytoplasmic β-N-acetylglucosaminidase was thus discovered.
Catalytic hydrogenation of 63 by H$_2$/Pd/C gave the amine 64 in 76% yield (Scheme 8).

The authors selected pyrrolidine derivative 60 as the skeleton of the divalent iminosugar libraries. Compound 60 was proved to be an outstanding inhibitor of β-N-acetylhexosaminidase in 2001 by Wong’s group [95], which is consistent with the purpose of this research. Amino and azido functional groups were introduced based on 60 through molecular modification to carry out the subsequent click reaction. Azide 63 was obtained via reduction of cyanide in the known compound 61 [96], followed by acylation and reductive amination with 6-azidohexanal using NaBH$_3$CN as catalyst in 49% yield (Scheme 8) [97]. Catalytic hydrogenation of 63 by H$_2$/Pd/C gave the amine 64 in 76% yield (Scheme 8).
A sub-library I was generated via CuAAC reaction between 63 (2.4–2.5 equiv.) and dialkynes a–e (1.0 equiv.) in the presence of CuSO$_4$ 5H$_2$O (0.14 equiv.), sodium ascorbate (0.44 equiv.), and t-BuOH/H$_2$O with high yield (Scheme 9). In parallel, (thio)urea-bond forming reactions between compound 64 (2.4–2.5 equiv.) and diisothiocyanates A–E (1.0 equiv.) were carried out in solvent DMSO to give a sub-library II (Scheme 10). Finally, the crude divalent iminosugars 63A–e and 64A–E were assayed as β-N-acetylglucosaminidase inhibitors, and thus compounds 63A and 64D, with the highest inhibitory potency, were screened and studied in detail. Similar to the previous protocol [89], the inhibition potency of divalent iminosugars was compared with corresponding monomers to evaluate the multivalent effect. Compound 65, the reference of 63A, was synthesized by CuAAC cycloaddition between 63 and methyl propargyl ether in 61% yield. Similarly, compound 66, as control of 64D, was generated through (thio)urea-bond forming reactions between 64 and phenyl isothiocyanate in 62% yield (Scheme 11).

As described above, several examples of multivalent pyrrolidine iminosugars were successfully prepared and used for biological activity exploration through the efficient CuAAC reaction. However, this reaction also brings some problems. For example, the catalyst copper ion required has a high chance of complexing with multiple nitrogen atoms in the triazole produced by the reaction, increasing the risk of metal ion contamination [98]. Moreover, the CuAAC reaction is usually carried out in the last step between the monovalent skeleton and scaffold, which limits the choice of monomer part of the
final iminosugars. Therefore, developing new strategies without metal catalysts for the preparation of bio-related iminosugars is highly desirable [61].

![Diagram of the synthesis of divalent iminosugars](image)

Scheme 11. Synthesis of monovalent references 65–66.

Cardona and co-workers reported an alternative way to synthesize multivalent pyrroldidine iminosugars without metals in 2019 [62]. The synthesis relies on iminosugar pyrrolidine DAB and three selective and high-yielding steps (1,3-dipole cycloaddition with nitrone 67, N–O bond cleavage of the adduct, and selective N- and/or O-allylation), and allows the preparation of different topologies in the DAB clusters. Nitrone 67, obtained from the commercially available tribenzylated D-arabinose [99], and allyl benzyl ether 68 [100] were employed to prepare exo-anti isoxazolidine 69 in 85% yield through 1,3-dipole cycloaddition (1,3 DC). The 1,3 DC process is a crucial step since a high degree of stereoselectivity in the reaction must be guaranteed to reduce isomer formation. Previous research showed that high exo-anti selectivity is ascribed to the trans-trans configured nitrone 67, whose C-3 and C-5 substituents on the same face are opposite to C-4. Thus, the exo mode was preferred to avoid repulsive steric interactions with a substituent at C-4 [99,101,102]. Then, the cleavage of the N–O bond of 69 in the presence of 10 equiv. of Zn afforded 70 quantitatively. Note that compound 70 is a key intermediate since the selective N- and/or O-allylation would generate new dipolarophiles 71–73, which would introduce a second or third DAB moiety by 1,3 DC with nitrone 67. MW-assisted selective N-allylation of 70 was carried out using electrophile allyl bromide to afford intermediate 71 in 86% yield. Protecting the amine of 70 using benzyl bromide, followed by selective O-allylation in the presence of allyl bromide and K₂CO₃, gave intermediate 72 in 71% yield. The N,O-bis allylated pyrrolidine 73 was obtained by treating 70 with a high excess of allyl bromide (6 equiv.) and NaH (8 equiv.) in 81% yield (Scheme 12).

The synthesis of multivalent DAB iminosugars was carried out as initially designed. 1,3 DC reaction between 71 and nitrone 67, followed by catalytic hydrogenation with Pd/C in MeOH/HCl, gave the bis-pyrrolidinium hydrochloride 75 in quantitative yield, which was submitted to the ion exchange resin Dowex 50WX8–200, followed by treatment with the strongly basic Ambersep 900-OH resin to afford the divalent DAB-based iminosugar 76 in 40% yield (Scheme 13). A similar approach was applied to the preparation of dimer 79 and trimer 82 from corresponding intermediates O-allylated 72 and N,O-allylated 73.
(Scheme 14). After three steps—1,3 DC, catalytic hydrogenation, and ion exchange resin—DAB-based iminosugars 79 and 82 were generated in 95% and 60% yield.

Scheme 12. Synthesis of crucial intermediates 71–73.

Scheme 13. Synthesis of DAB-based iminosugar 76.

2.2. Synthesis of Multivalent Iminosugars

In 2016, Cardona and co-workers explored the inhibition of sulfatases using the first two examples of pyrrolidine clusters [67]. Nonavalent pyrrolidine iminosugars 83 and 84 were obtained from DAB and 1,4-dideoxy-1,4-imino-D-ribitol (86), prepared by the deprotection of the starting nitrones 67 [103] and 85 [104]. Upon selective N-alkylation with 1-azido-6-bromohexane [105] in the presence of K₂CO₃ under MW irradiation, DAB gave the deprotected azide 87 in 92% yield. Reacting 87 with the nonadiyne scaffold 89 [106] using the standard CuAAC cycloaddition condition (CuSO₄ (30 mol%), sodium ascorbate (60 mol%), THF/H₂O) afforded a mixture of unreacted 87 and desired product 83,
which was further purified by column chromatography and size exclusion chromatography Sephadex LH-20, obtaining pure 83 in 81% yield. Similar synthetic routes were applied for the synthesis of target iminosugar 84. Starting from a different configuration of bioactive 1,4-dideoxy-1,4-imino-D-ribitol (86), selective N-alkylation afforded 88, and CuAAC coupling gave ribose configured 84 in 71% yield (Scheme 15).

Scheme 14. Synthesis of DAB-based iminosugars 79 and 82.

Scheme 15. Synthesis of nonavalent iminosugars 83 and 84.

The monovalent references 90 and 91 corresponding to compounds 83 and 84 were synthesized to explore the multivalent effect further (Scheme 16). The reference 90 was synthesized from known D-arabinose derived nitrone 67. Two-step reduction by NaBH₄ followed by Zn in AcOH gave amine 92 in 98% yield, which was treated with 1-azido-6-
bromohexane in basic conditions under microwave irradiation to afford azide 93 in 88% yield. The CuAAC coupling between azide 93 and 3-buty-1-ol was carried out under MW irradiation to give 94 (93%), which was further deprotected using H₂ and Pd/C in acidic MeOH to give the final product 90 in 74% yield. Compound 91, generated through one-step synthesis, was obtained by cycloaddition of azide 88 to 3-buty-1-ol in the presence of CuSO₄ and sodium ascorbate under MW irradiation in 89% yield (Scheme 16).

![Scheme 16. Synthesis of monovalent references 90 and 91.](image)

Results showed that the nonavalent pyrrolidine iminosugar 83 exhibited impressive inhibition of N-acetylgalactosamine-6-sulfatase (GALNS). Considering that the DAB motif in 83 is a widely available glycosidase inhibitor, and the fact that GALNS and α-mannosidases both have dimer properties, the same research group continued to explore the interaction of α-mannosidases with different multivalent architectures based on iminosugar DAB in 2017 [107].

Similar to the method for synthesizing compound 83, the CuAAC cycloaddition of intermediate azide 87 and multivalent alkyne scaffolds was exploited to generate new tetra- and trivalent pyrrolidine iminosugars 96 and 98 (Scheme 17). Microwave-assisted CuAAC of azide 87 (3.5 or 4.5 equiv.) with trivalent scaffold tris[(propargyloxy)methyl]amino-methane 97 and tetravalent scaffold 95 (1.0 equiv.) in the presence of CuSO₄ (0.3 equiv.) and sodium ascorbate (0.6 equiv.) in THF/H₂O a, followed by purification through flash column chromatography and size-exclusion chromatography Sephadex LH-20 (H₂O) to separate the excess azide 87, gave the pyrrolidine iminosugar clusters 98 (48%) and 96 (76%) in good yields. In addition, inhibitory performance against a panel of glycosidases was evaluated among the nona-, tetra-, and trivalent iminosugars 83, 96, and 98, as well as monovalent references DAB and its derivative 90.

Two years later, Moreno Vargas and co-workers prepared four multivalent pyrrolidine iminosugars for GH1 β-glucosidases A and B (BglA and BglB) to continue their study on binding modes and key determinants responsible for the inhibitory effect displayed by pyrrolidine-based clusters [108]. Therefore, as before, the CuAAC click reaction between pyrrolidine-azide derivatives (99 and 101) and two different tri- or hexavalent alkynyl spacers (97 and 102) was exploited to give the target clusters (103–106). It was found that spacers containing aromatic moieties in multivalent inhibitors showed excellent inhibition against octameric BglA (µM range) compared to the similar monomeric BglB. Moreover, a modest multivalent effect was detected for the hexavalent inhibitor 106.

Starting from the azidomethyl pyrrolidine 107 reported by the same group, the protected azide 99 was obtained in good yield by reacting with the aromatic alkyne 108 [109] through CuAAC and then treating with NaN₃ in 93% yield. Then, classic conditions
(HCl/THF) were used to get rid of the protecting group to give the unprotected derivative 100 quantitatively. The same reactions performed in 107 and alkyne 109 [95] via a sequence of CuAAC coupling, nucleophilic displacement with NaN₃, and acidic deprotection afforded 101 in high yield. The synthesis of azides 100 and 101 was not only to obtain the final multivalent clusters but also as monomers for bioactivity control. However, due to the poor solubility of azide 100, protected azide 99 was selected in the subsequent synthesis and then deprotected again with hydrochloric acid (Scheme 18).

Finally, microwave-assisted CuAAC cycloaddition of azide 99 or 101 with scaffolds 97 [110] and 102 [108] in the presence of CuSO₄, sodium ascorbate in THF/H₂O (2:1), followed by checking reactions through ¹H NMR spectra of the crude mixtures, gave the pyrrolidine-based iminosugar clusters 103–106 in good yields (57–93%, Scheme 19). It is worth noting that the resulting crude, mixed with excess azide 99 or 101 and desired products, were purified by stirring with Quadrasil® MP followed by chromatography column (silica gel or Sephadex LH-20).

Moreno Vargas and co-workers have long worked on the design and synthesis of mono- and multivalent iminosugars to evaluate their inhibition activities toward various disease-related enzymes. Because two enzymes, β-glucocerebrosidase (GCase) and α-galactosidase (α-Gal A), are involved in Fabry and Gaucher diseases, respectively, and combined with the experimental results that pyrrolidine-3,4-diol skeleton-based iminosugars exhibit bioactivity to human lysosomal GCase reported by his group [111,112], exploring the multivalent effect on these two enzymes became their target. The author reported four
sets of multivalent pyrrolidine iminosugars with different valency, configuration, and spacers to perform a systematic analysis of the inhibition of the lysosomal glycosidases in 2020 [49].

Scheme 19. Synthesis of multivalent iminosugars 103–106.

The six azidoalkyl pyrrolidines shown in Figure 7 were selected as monovalent references as well as anchoring moieties for CuAAC coupling reactions. Compounds 100, 101, 110, and 111 were all known compounds reported by the same group [108,111], while 112 and 113 were newly synthesized. The synthetic routes of 112 and 113 were the same as the preparation for their epimers 101 and 100 described in Scheme 18 [108]. The known scaffolds shown in Figure 8 were selected to synthesize the tri-, tetra-, hexa- and nonavalent iminosugars via CuAAC coupling with azido derivatives.

Figure 7. Azidoalkyl pyrrolidines used as anchoring moieties.

General reaction conditions of the click reaction involved using CuSO₄ and sodium ascorbate as catalysts and in THF–H₂O under microwave irradiation at 80 °C. Similar to the methods for compounds 103–106, chromatography column (silica gel or Sephadex LH-20) was employed to separate the unreacted azidoalkyl pyrrolidines. Multimeric derivatives (114–133) were generated in high yields (55–99%). The monovalent references (134–139) were prepared via CuAAC reaction between monomers (110 and 111) and the corresponding alkynes in high yields (79–91%), which were submitted to evaluate inhibition
against human GCase and α-Gal A along with the corresponding multivalent iminosugars 114–133 (Figure 9, Scheme 20).

Figure 8. Alkynyl scaffolds for CuAAC reaction.

Figure 9. Tri-, tetra-, hexa- and nonavalent iminosugars 114–133.

Scheme 20. Synthesis of monovalent references 134–139.
More recently, Gaeta, Cardona, and co-workers constructed pyrrolidine-based multivalent clusters, employing the less researched scaffold resorcinarene [113], which exhibited conformationally mobile ability [114–116], to explore the role of both conformability and valency in the inhibition of therapeutically relevant enzyme Golgi α-mannosidase IIb (GMIIB) [68].

Resorcinarene 140–142 [113,116] are macrocycles consisting of four to six rings of resorcinol obtained by resorcinol/aldehyde acid-catalyzed condensation reaction (Scheme 21). Interestingly, each aromatic moiety of the macrocycle contains two hydroxyl functional groups, making it suitable for constructing multivalent iminosugars. Azides 87 and 143, belonging to the DAB-derived pyrrolidine family, were selected as skeletons (Scheme 21). Azide 87 [67] was reported by the same group previously, and new azido-ending ligand 143, which possessed a more hydrophilic linker, was newly synthesized to explore the role of the nature of linkers in bioactivity [68].

Scheme 21. Synthesis of multivalent iminosugars 147–150.

Different scaffolds C-methyl-resorcin [4] arene 140, resorcin [4] arenes 141, and resorcin [6] arenes 142 were allowed to react with propargyl bromide 2 equivalents per hydroxyl in acetone by treatment with excess K$_2$CO$_3$ to give alkyne-ending scaffolds 144–146 in high yields (58–98%). Then, target resorcinarene-based iminosugars 147–149 were obtained by reacting azide 87 with scaffolds 144–146 through the CuAAC click reaction in moderate yields (27–44%). The unsubstituted azides 87 and 143 were purified through chromatographic column (silica gel, gradient: from MeOH to ammonia solution 4 M in MeOH). Finally, the compound 150, featuring a more hydrophilic linker, was obtained by CuAAC reaction between azide 143 and scaffold 146 (Scheme 21). It is worth pointing out that, although the valency of 147 and 148 was the same, the scaffold resorcin [4] arene (140) was conformationally blocked in a cone conformation, thanks to the presence of CH$_3$CH bridges between aromatic rings [117]. As a result, iminosugar 147 was more flexible than 148.
3. Biological Activity of Multivalent Pyrrolidine Iminosugars

3.1. Inhibition of α-Fucosidases

α-Fucosidases (AFU) are lysosomal acid hydrolase enzymes that catalyze the hydrolysis of α-fucose units located on the cell surface oligosaccharides and participate in various biological processes, including immune response, signal transduction, and antigenic determination [118–120]. Changes in the activity of AFU in serum or tissue significantly correlate with the occurrence of tumors, such as hepatocellular carcinoma [121], colon adenocarcinoma [122,123], and gastric cancer [124]. Since the discovery of pyrrolidine 1,4-iminocyclitols as potent inhibitors of AFU [77,78], Robina and co-workers first explored the AFU-inhibitory activity of di- (1) and trivalent pyrrolidine iminosugars 2–4 in 2013 (Figure 3) [70]. Results showed that all the newly synthesized compounds displayed high AFU inhibition (IC\textsubscript{50}: 1.6–17 µM) and excellent selectivity. However, compared with the monomer references 5 and 6 (Figure 3), the effect of multivalency was not convincing, except for the trivalent iminosugar 2 (K\textsubscript{i} = 0.3 µM, Table 1), which showed seven-fold more potent inhibition activity than monovalent reference 5 (K\textsubscript{i} = 2.1 µM). Compounds 2 and 3 (K\textsubscript{i} = 0.4 µM, Table 1) displayed almost equivalent activities, indicating that the increase in the length of multivalent iminosugars was not clearly linked to the inhibitory properties of the enzyme. Divalent iminosugars with more diverse spacers were also reported subsequently [80]. Polyamino and triazole-benzyl bridged iminosugars (13–17, Figure 4) were constructed to develop potential inhibitors for AFU. Dimers 13, 14, and 16 showed stronger inhibition than their corresponding monomers, while compounds 14, 15, and 31 yielded the opposite results. Triazole-benzyl bridged iminosugar 17 showed excellent enzyme inhibition to AFU (IC\textsubscript{50} = 74 nM, K\textsubscript{i} = 3.7 nM, Table 1) while dimer 13 (IC\textsubscript{50} = 1.2 µM, Table 1) indicated the existence of multivalency compared with its control 19b (IC\textsubscript{50} = 13 µM, Figure 4), a 10.8-fold potency enhancement. The result that compound 17 exhibited excellent inhibition toward AFU was consistent with the fact that the presence of an additional aromatic or heteroaromatic binding component close to the five-membered iminosugars notably increases their inhibitory activity to AFU, which was shown by Robina [83], Behr [125], and Wong [126]. To further explore the ligand-enzyme binding modes, stereoisomeric pyrrolidine dimers (33, ent-33, and meso-33, Figure 5) with short and flexible space were synthesized [84]. Dimer 33 showed potential inhibition of AFU (IC\textsubscript{50} = 0.108 µM, K\textsubscript{i} = 23 nM, Table 1) when compared to its monovalent reference 35 (IC\textsubscript{50} = 2.0 µM, K\textsubscript{i} = 0.18 µM, Figure 5), which to some extent confirmed the existence of the multivalent effect. The divalent meso-33 also showed potential inhibition of AFU (IC\textsubscript{50} = 0.365 µM, K\textsubscript{i} = 0.051 µM), while compound ent-33 was significantly less potent (IC\textsubscript{50} = 84 µM, K\textsubscript{i} = 12 µM). Through detailed controlled trials and structural analysis, the authors suggested that the inhibition enhancement obtained with divalent compounds could be explained by additional interactions of the hydrophobic moiety with a lipophilic binding pocket other than the active site. This hypothesis was confirmed by the 3-D structure of the bacterial fucosidase BjFuc2970 complexed with the best divalent inhibitor 33. However, other mechanisms such as rebinding could not be completely ruled out. In 2018, Moreno Vargas and co-workers successfully screened a batch of AFU inhibitors through the CuAAC click reaction followed by in situ biological screening and identified one of the most effective enzyme inhibitors, 47i (IC\textsubscript{50} = 48 nM, K\textsubscript{i} = 15 nM, Figure 6, Table 1) [89]. The higher inhibition shown by dimer 47i compared to its analogue 48i could be argued to be due to non-specific interactions of the diphenylsulfone spacer in the loop regions near the GH29 family’s enzymatic active site. Due to controversy over the reference selection, a valid multivalent effect could not be given, but the discovery of compound 47i proved the rapidity and efficiency of the methodology, which should be highlighted in the screening of enzyme inhibitors. To some extent, these results indicated that the multivalent effect of iminosugars on α-fucosidases is probably due to the additional unspecific interactions with a noncatalytic subsite, which would be beneficial to medicinal chemists in the rational design of α-fucosidase inhibitors.
Table 1. Inhibition activities (Kᵢ or IC₅₀ [µM]) and relative inhibition potencies (rp and rp/n) of some selected pyrrolidine iminosugars described in the previous section of the present review.

| Enzyme                          | Compound | Valency (n) | Kᵢ a | IC₅₀ b  | Rp c | Rp/n d | Ref. |
|---------------------------------|----------|-------------|------|---------|------|--------|------|
| α-fucosidase e                  | 2        | 3           | 0.3  | 1.6     | 7.0  | 2.3    | [70] |
| "                               | 3        | 3           | 0.4  | 3.8     | 5.3  | 1.8    | [70] |
| "                               | 13       | 2           | -    | 1.2     | 10.8 | 5.4    | [80] |
| "                               | 17       | 2           | -    | 0.0037  | 0.074| 4.1    | [80] |
| "                               | 33       | 2           | 0.023| 0.108   | 7.8  | 3.9    | [84] |
| "                               | 47i      | 2           | -    | 4.8 × 10⁻³| 0.15 × 10⁻³| -    | [89] |
| α-mannosidase f                 | 83       | 9           | -    | 0.095   | 13,684| 1520  | [107]|
| "                               | 147      | 8           | -    | 5.3     | 245  | 31     | [68] |
| "                               | 148      | 8           | -    | 14.8    | 88   | 11     | [68] |
| "                               | 149      | 12          | -    | 1.2     | 1083 | 90     | [68] |
| "                               | 150      | 12          | -    | 10.5    | 124  | 10     | [68] |
| Golgi α-mannosidase IIb g       | 147      | 8           | -    | 3.7     | 47   | 6      | [68] |
| "                               | 148      | 8           | -    | 5.3     | 33   | 4.1    | [68] |
| "                               | 149      | 12          | -    | 0.7     | 250  | 21     | [68] |
| "                               | 150      | 12          | -    | 28.5    | 6    | 0.5    | [68] |
| N-acetylgalactosamine-6-sulfatase b| 83     | 9           | -    | 47      | 83   | 9.2    | [67] |
| iduronate-2-sulfatase i         | 84      | 9           | -    | 85      | 59   | 6.5    | [67] |
| β-N-acetylgalactosaminidase i   | 64D     | 2           | 168  | -       | 1.9  | 0.96   | [69] |
| β-N-acetylgalactosaminidase k   | 64D     | 2           | 0.0061| -       | 7.8  | 3.9    | [69] |
| α-galactosidase A l             | 133     | 9           | 0.2  | 1.2     | 378  | 42     | [40] |

a Glycosidase inhibition constant (Kᵢ, µM). The Kᵢ on glycosidases was calculated from the measured IC₅₀ value using the Cheng–Prusoff equation. b Half maximal inhibitory concentration (IC₅₀, µM). c Relative inhibitory potency: Kᵢ (monovalent reference)/Kᵢ (glycocluster) or IC₅₀ (monovalent reference)/IC₅₀ (glycocluster). d Inhibitory potency per iminosugar unit. e Bovine kidney. f Jack bean. g Drosophila melanogaster. h Human leukocytes. i Human recombinant enzyme (pichia pastoris). j Human recombinant enzyme (escherichia coli). k Human lysosome.

3.2. Inhibition of α-Mannosidases

α-Mannosidases are mainly involved in the biosynthesis and catabolism of N-glycans in cells. Such processes are, for instance, involved in the treatment of cancers and lysosomal diseases [127–129]. The first evidence of the multivalent effect on iminosugars was gained through the interaction between Jack bean α-mannosidase (JBMan) and a trivalent DNJ conjugate [58]. Due to the successful analysis of its crystal structure and the ease of purchase, JBMan has become the most investigated enzyme for multimeric inhibition studies [48]. Novel tri-, tetra-, and nonavalent pyrrolidine iminosugars (98, 96, and 83, Schemes 15 and 17) were constructed by Cardona and co-workers to investigate the binding modes to α-mannosidases [107]. A large multivalent effect was observed from the three iminosugars (rp/n >> 1). The DAB-based nonavalent iminosugar 83 (Scheme 15) was the best inhibitor of JBMan (IC₅₀ = 95 nM, Table 1), with a 13,684-fold (rp/n = 1520) stronger inhibitory potency than the corresponding the monovalent reference 90 (IC₅₀ = 1300 µM, Scheme 16). The trivalent compound 98 and the tetravalent 96 also showed good multivalent effects towards JBMan, with rp/n values of 46 and 10, respectively. Transmission electron microscope (TEM) analysis, nuclear magnetic resonance (NMR), and molecular dynamic studies were carried out to elucidate the binding mode of the multivalent iminosugars and α-mannosidases. NMR studies showed the existence of specific interactions of the multivalent ligands with JBMan, which presumably take place within the enzyme active site. TEM studies indicated that the binding mode would probably be intermolecular cross-linking, due to the formation of ligand–JBMan aggregates. It is worth noting that a remarkable selectivity of iminosugars (83, 96, and 98) for Golgi α-mannosidase IIb (GMMIIb) over lysosomal α-mannosidase II (LManII), two biologically relevant enzymes (GMMIIb: tumor growth and cell metastasis; LManII: disorder mannosidosis), was observed.
interesting selectivity appeared particularly relevant for selective application of multivalent compounds in anticancer therapy without the undesirable side effect of mannosidosis syndrome. Subsequently, scaffold resorcinarene was employed to explore the role of both the conformability and the valency of multivalent iminosugars to therapeutically relevant target GMIIb [68]. Similarly, both the 8-valent (147, 148, Scheme 21) and 12-valent iminosugars (149, 150, Scheme 21) exhibited greater selectivity to JBMan and GMIIb over LManII. Biological assay indicated that 12-valent 149 had stronger inhibition, for example, towards GMIIb (IC$_{50}$ = 0.7 μM, Table 1) than 8-valent 147 (IC$_{50}$ = 3.7 μM, Table 1) and 148 (IC$_{50}$ = 5.3 μM, Table 1), which further showed that the inhibitory activity of resorcinarene-based conjugates was related to their valency. The 12-valent iminosugar 150, possessing a more hydrophilic group, showed weaker inhibition (GMIIb, IC$_{50}$ = 28.5 μM, Table 1) than the same valent 149 (GMIIb, IC$_{50}$ = 0.7 μM, Table 1). This was ascribed to the unfavorable repulsions between oxygen atoms on the linker with electron-rich atoms of the amino acid residues of the GMIIb protein. In addition, the 12-valent 149 showed a remarkable multivalent effect towards JBMan (IC$_{50}$ = 1.2 μM) compared to its monovalent reference 90 (Scheme 16, IC$_{50}$ = 1300 μM, rp/n = 90). Computational studies suggest that the binding mode should be the rebinding process, since the resorcinarene ligands bind the dimer of the JBMan by coordination of one Zn ion at a time. From these results, we can know that the multivalent effect of pyrrolidine iminosugars on α-mannosidases has a great relationship with valency. Generally, higher valency iminosugars usually exhibit better inhibitory activities. The multivalent effect is also affected by the type of linker and the conformation of the scaffold. In addition, the proposed binding modes—cross-linking and aggregation, and bind and recapture (Figure 2)—are more likely involved in better responses of multivalent pyrrolidine iminosugars toward α-mannosidases. However, a binding mode that involves both the active site and non-catalytic subsites cannot be completely excluded.

### 3.3. Inhibition of Other Disease-Related Glycosidases

Besides the α-fucosidases and α-mannosidases introduced above, Cardona and co-workers explored the impact of multivalency on sulfatases involved in lysosomal storage disorders (LSD) for the first time [67]. A decrease in two lysosomal enzymes, N-acetylgalactosamine-6-sulfatase (GALNS) and iduronate-2-sulfatase (IDS) could cause diseases of mucopolysaccharidoses: Morquio A syndrome and Hunter disease, respectively [130–132]. Nonavalent DAB-based iminosugars 83 (GALNS: IC$_{50}$ = 47 μM, IDS: IC$_{50}$ = 140 μM, Scheme 15, Table 1) and 84 (GALNS: IC$_{50}$ = 85 μM, IDS: IC$_{50}$ = 31 μM, Scheme 15, Table 1) exhibited strong inhibition to both enzymes compared to the negligible monovalent references 90 (GALNS: IC$_{50}$ = 3900 μM, IDS: IC$_{50}$ = 3200 μM, Scheme 16) and 91 (GALNS: IC$_{50}$ = 5000 μM, IDS: IC$_{50}$ = 5500 μM, Scheme 16). The results demonstrated that a good multivalent effect was achieved with pyrrolidine-based clusters towards sulfatases. For example, 84 showed a remarkable multivalent effect toward IDS (rp/n = 19.7, Table 1). However, detailed kinetic studies and proposed binding modes were not given. On the basis of this result, DAB-based iminosugars 79 (Scheme 14) and 82 (Scheme 14) with different ligand topologies were synthesized for GALNS inhibition two years later by changing CuAAC coupling to a new strategy which avoided contamination with copper ions [62]. Dimer 79 and trimer 82 showed IC$_{50}$ to GALNS in the low micromolar range (0.3 and 0.2 μM, respectively), confirming that multimerization of DAB epitopes generates potent GALNS inhibitors. Comparing the inhibitory activities of 79 and 82 with 83 and 84, we can learn that the ligand topology strongly affected the affinity of the DAB-based multivalent iminosugars for GALNS. Divalent iminosugar 64D (Scheme 10) was discovered to be a potent inhibitor of human hexosaminidases, the potential pharmacological targets for drug development, via the screening of two libraries of divalent pyrrolidine iminosugars [69]. The results showed that compound 64D exhibited remarkable inhibition of human β-N-acetylgalcosaminidase (hOGA) in the nanomolar range (K$_{i}$ = 6.1 nM, Table 1) compared to the monovalent reference 66 (K$_{i}$ = 47.6 nM, Scheme 11). No significant multivalent effect was observed in the inhibition of any of the hexosaminidases by dimers.
However, compound 64D displayed excellent selectivity towards hOGA compared with human lysosomal β-N-acetylhexosaminidases (hHexB, \( K_i = 168 \mu M \), Table 1), with an approximately 27500-fold enzyme affinity enhancement. It was observed very clearly from the result that multivalency could also be a promising tool to modulate the inhibition selectivity of multivalent iminosugars. Similarly, the (2R)-nonavalent iminosugar 133 (Figure 9) was screened by Moreno-Vargas and co-workers for human α-galactosidase A (α-Gal A), which is involved in a common lysosomal storage disorder, Fabry disease [49]. Compound 133 displayed remarkably potent inhibition and multivalent effect (\( K_i = 0.2 \mu M \), \( rp/n = 42 \), Table 1) towards α-Gal A, being a 375-fold more potent inhibitor than the monovalent reference 139 (\( K_i = 75 \mu M \), Scheme 20). The author suggested that the multivalent effect was probably due to the involvement of interaction mechanisms such as statistical rebinding, additional binding with allosteric sites, and/or aggregative processes. More importantly, the activity enhancement effect of compound 133 towards α-Gal A in Fabry fibroblasts constitutes the first evidence of the potential of multivalent iminosugars to act as pharmacological chaperones in the treatment of this LSD.

4. Conclusions

The last decade has witnessed the rapid development of multivalent effects in glycosidase inhibition and drug discovery. In this review, we systematically summarized the process of fabricating multivalent iminosugars based on pyrrolidine in terms of design strategies, synthesis routes, and glycosidase inhibition investigations. Up to 12-valent pyrrolidine iminosugars were synthesized through classic click reactions, and thus several outstanding inhibitors were discovered. For example, nonavalent inhibitors based on DAB and one of its epimers demonstrated the existence of the multivalent effect in sulfatases for the first time [67]. Moreover, nonavalent iminosugars based on pyrrolidine-triazole moieties exhibited a remarkable multivalent effect on one important therapeutic enzyme, human α-galactosidase A, and constitute the first evidence of a multivalent enzyme activity enhancer for Fabry disease [49]. Despite advances in the design and investigation of multivalent iminosugars based on pyrrolidines, some problems and challenges remain.

The enzymes used for studying the multivalent approach are mostly limited to the more researched models, such as α-mannosidase and α-fucosidase, which means the importance of some therapeutically relevant glycosidases is overlooked. The complex and confusing enzyme–ligand binding mechanism is not a negligible issue when developing new relevant multivalent inhibitors. Elucidating the binding mode(s) would improve glycosidase inhibition efficiency and selectivity, two major problems currently existing. Despite the many challenges, we hope that, with the information presented in this review, researchers in this field will continue to explore multivalent effects based on pyrrolidine for developing new glycosidase inhibitors, as well as for candidates for advanced clinical trials or markets.

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