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Synthesis and Inhibition Study of Bicyclic Iminosugar-Based Alkaloids, Scaffolds, and Libraries towards Glucosidase

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Abstract: A small library of bicyclic iminosugar-based alkaloids and scaffolds possessing a polyhydroxylated pyrrolidine and a varied ring skeleton have been synthesized. Through rapid diversification of the scaffold via an amide coupling with random carboxylic acids, structurally diverse bicyclic iminosugar-based libraries were prepared with substituent diversity, core diversity, and configurational diversity. This discovery process allowed us to efficiently sieve out potent and specific glycosidase inhibitors, and a bicyclic, conformationally restricted iminosugar was demonstrated to be more potent than the monocyclic ones in this study. The most potent and selective inhibitor discovered was found to have a K_i value of 71 nM against α-glucosidase.

Keywords: alkaloids · combinatorial chemistry · glycosidase inhibitors · iminosugars · natural products

1. Introduction

The design and synthesis of new scaffolds and molecular libraries is often inspired by the skeletons of natural products or their mimics.[1] Such a research approach, referred to as "natural-product-based combinatorial synthesis", has attracted a great deal of attention in modern drug discovery.[2] Bicyclic iminosugar-based alkaloids such as polyhydroxylated pyrrolizidines, indolizidines, and other bicyclic structures have versatile inhibitory activities against various sugar-processing enzymes, which are involved in diseases such as diabetes, cancer, viral infections, and lysosomal storage disorders (Figure 1).[3] For example, swainsonine is a potent human GMII inhibitor and shows anticancer activity. Australine is a specific inhibitor of amyloglucosidase and shows antiviral and anti-HIV activities. In a rational design theory, the protonated nitrogen of the iminosugar may mimic the partial positive charge of the oxocarbenium transition-state analogues, to increase the interactions with enzyme residues.[3] A bicyclic molecule or skeleton might constitute a conformational constraint, to decrease internal molecular rotations and thus improve binding potency.[4]

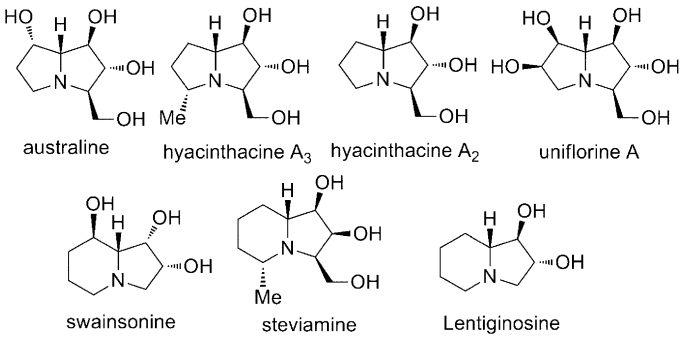

Figure 1. Examples of naturally occurring bicyclic iminosugars.

To date, polyhydroxylated pyrrolidines and piperidines, all classified as monocyclic iminosugars, have been extensively reported by us[5] and others.[6] In addition, we have successfully used these monocyclic iminosugar-based molecules as scaffolds to build diverse libraries.[5e] To the best of our knowledge, however, the use of bicyclic iminosugar-based scaffolds to prepare molecular libraries and increase molecular complexity in chemical space has yet to be explored,[7] although several bicyclic iminosugars such as pyrrolizidine and indolizidine alkaloids or their ana-

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logues have been isolated or synthesized.[3,8] Herein, we report a straightforward approach toward the synthesis of bicyclic iminosugar-based alkaloids and scaffolds, followed by the preparation of molecular libraries using microtiter-plate-based combinatorial chemistry, and an in situ enzyme-based inhibitory study. These natural-product-like libraries possess three classes of diversity: substituent diversity, core diversity (ring size), and configurational diversity. We also demonstrate how these points of diversity can be used to efficiently discover new inhibitors against glycan-processing enzymes such as α-glucosidase.

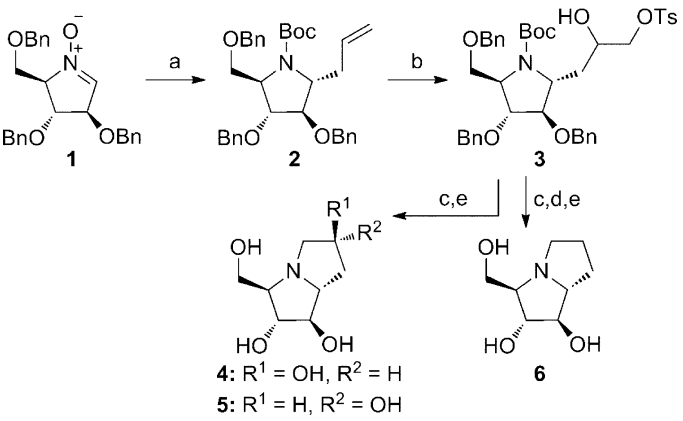
2. Results and Discussion

2.1. Synthetic Design towards Bicyclic Iminosugars

Our initial synthetic design is illustrated in Scheme 1. Of the eight possible enantiopure five-membered cyclic nitrones,[5a] the 2*R*,3*R*,4*R* configuration pattern was chosen as our A-ring skeleton as it has the same stereochemistry as naturally occurring (2*R*,5*R*)-di(hydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine (DMDP) and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (see Scheme 1). Both of these are well-known and potent glucosidase inhibitors, but unfortunately suffer from only poor to moderate inhibitory selectivity between α- and β-glucosidases.[9] To increase the diversity of our synthetic molecules, diastereoselective nucleophilic addition of cyclic nitrone **1**[5a] was applied instead of 1,3-dipolar cycloaddition[10] because the former is more flexible by using different nucleophiles for the B-ring formation (n or m ≥ 1) and further structural diversity (Scheme 1).

2.2. Preparation of Bicyclic Iminosugar-Based Alkaloids from Cyclic Nitrone 1

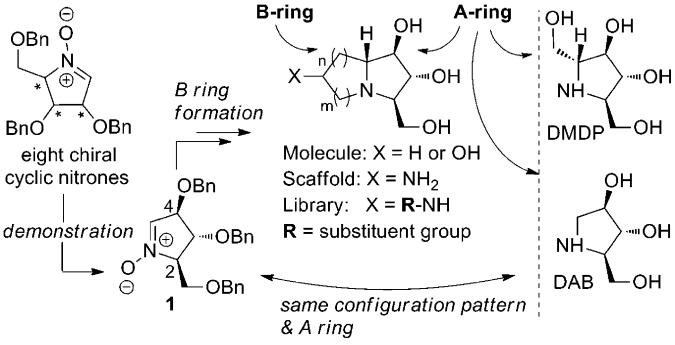
As shown in Scheme 2, intermediate **2** was obtained in 73% yield from cyclic nitrone **1** via a highly diastereoselective nucleophilic addition with allylmagnesium bromide (AllylMgBr),[11] reduction of the N–O bond, and *N*-Boc protection. Dihydroxylation of **2** with osmium tetroxide in the presence of *N*-morpholine oxide[12] followed by selective tosylation of the primary alcohol, *N*-Boc deprotection, intramolecular cyclization, and global deprotection gave two separable products, **4** and **5**, in a 1:1 ratio,[13] and their structures were confirmed by 2D NMR and NOE analysis. Likewise, **6** (hyacinthacine A_2,[14] 36% yield from **2** over six steps) was also prepared from **3** by using the $LiAlH_4$ reduction of the corresponding mesylated intermediate to remove the hydroxyl group on the B ring.



Scheme 2. Reagents and conditions: (a) (i) AllylMgBr, THF, 0°, 5 h, 76%; (ii) Zn, AcOH, 12 h, 96%; (iii) $(Boc)_2O$, NEt_3, DCM, 3 h, 100%. (b) (i) OsO_4, NMO, 1,4-dioxane/H_2O = 3:2, 60°, 12 h, 75% (two isomers, ratio = 1:1); (ii) TsCl, NEt_3, *n*-Bu_2SnO, 2 h, 88%. (c) TFA, DCM, 5 h, 96%. (d) (i) MsCl, NEt_3, DCM, 1 h, 92%; (ii) $LiAlH_4$, ether, 5 h, 62%. (e) $Pd(OH)_2/C$, H_2, 12 h, quant..

Under similar conditions, **7** was also prepared from **1** by using vinylmagnesium bromide instead of AllylMgBr as the nucleophile. After ozonolysis of **7**, the intermediate aldehyde **8** was reacted with AllylMgBr/CuCl, followed by variation of the alkyl chain length via an ozonolysis–reduction[15] or hydroboration–oxidation[16] sequence to give alcohols **10** and **14**, respectively (Scheme 3). Notably, the yield of **9** from **8** was improved from 30% to 71% when CuCl was added.[17] After mesylation of **10** followed by acid-mediated *N*-Boc deprotection and intramolecular cyclization, two epimers **11** (18%) and **12** (36%) were separated by silica gel column chromatography and structurally confirmed by 2D NMR and NOE analysis. Debenzylation of **12** under hydrogenation conditions [$Pd(OH)_2$/C, H_2] gave the natural product australine (**13**) in quantitative yield. Bicyclic iminosugars **15** and **16** were also prepared from intermediate **14**. This straightforward method starting from cyclic nitrone **1** allowed us to diversify the B-ring structure and systematically prepare various bicyclic iminosugars.

Likewise, intermediate **17**, prepared from **2**, could be converted to bicyclic iminosugars **18** and **19** through a series of transformations similar to those depicted in Scheme 3. To construct the B ring bearing a hydroxyl group at the desired position (Figure 2), either an ozonol-



Scheme 1. General design principles of bicyclic iminosugars in this study.

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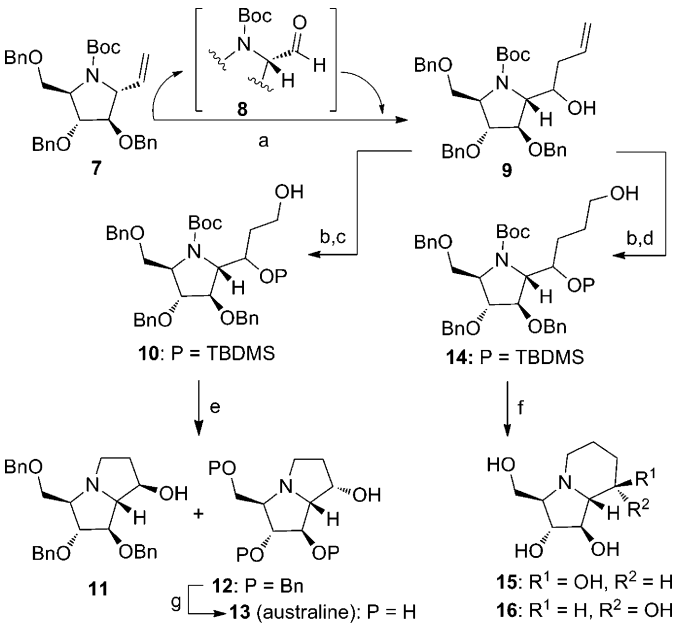

Scheme 3. Reagents and conditions: (a) O_3, DCM, then AllylMgBr, CuCl, THF, 0°, 5 h, 71%. (b) TBDMSOTf, NEt_3, DCM, 1 h, 95%. (c) O_3, DCM, then $NaBH_4$, MeOH, 3 h, 73%. (d) BH_3, THF, 1 h, then H_2O_2, $NaOH_{(aq)}$, 80%. (e) (i) MsCl, NEt_3, DCM; (ii) TFA, DCM; (iii) TBAF, THF, 18% (**11**) and 36% (**12**). (f) (i) MsCl, NEt_3, DCM, 0°; (ii) TFA, DCM, then K_2CO_3, MeCN; (iii) TBAF, THF, 40% (three steps); (iv) $Pd(OH)_2/C$, H_2, MeOH, 13% (**15**) and 26% (**16**). (g) $Pd(OH)_2/C$, H_2, 12 h, quant..

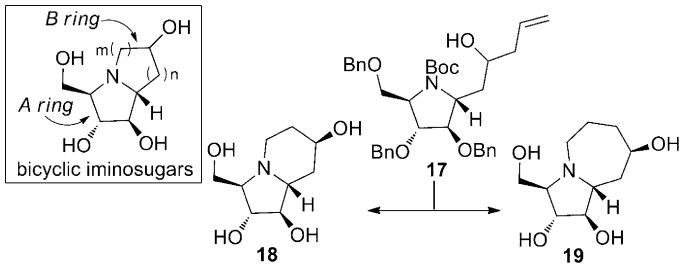

Figure 2. Chemical structures of bicyclic iminosugars **18** and **19**.

ysis–reduction–alkylation or a hydroboration–oxidation–alkylation sequence was utilized. Notably, **18** and **19** are new synthetic bicyclic alkaloids.

2.3. Primary Enzyme Inhibition Study of Synthetic Alkaloids

With these structurally related molecules in hand, an examination of their capacity to inhibit glucosidases could commence, in order to determine which qualified as scaffolds for further library preparation (Table 1). Monocyclic alkaloids DMDP and DAB were used as reference compounds for comparison purposes, and as expected both were found to be potent but not selective inhibitors against glucosidases. As shown in Table 1, the size of the B ring was important for inhibition activity, and iminosugars **4–6** (bearing a five-membered B-ring system) had

Table 1. Enzyme inhibition study.

Compound	Inhibitory activity[a]	
	α-Glucosidase[b]	β-Glucosidase[c]
4	40%	10%
5	18%	–[d]
6 (hyacinthacine A_2)	28%	85%
13 (australine)	15%	–
15	–	<5%
16	–	50%
18	–	10%
19	–	–
DMDP	>95% (IC_{50} = 0.6 μM)	>95%
DAB	>95% (IC_{50} = 6.3 μM)	80%

[a] Inhibition % at concentration of 500 μM. [b] From *Bacillus stearothermophilus lyoph*. [c] From almonds. [d] "–" refers to no inhibition.

better activities compared to the other alkaloids **15**, **16**, **18** and **19** containing a six- or seven-membered B ring. Notably, alkaloids **4–6** and **13** were moderate α-glucosidase inhibitors, implying that the hydroxyl group on the B ring does not dramatically affect activity toward α-glucosidase. In contrast, **6** was a more potent inhibitor of β-glucosidase than the others, suggesting that a hydrophobic B ring in bicyclic iminosugars might increase interactions with enzyme residues and improve inhibitory potency.

Next, we planned to study the effect of substituent diversity on the B ring to investigate whether the inhibitory activity or selectivity against enzymes could be improved or not.

2.4. Preparation of Bicyclic Scaffolds and Library Synthesis

Based on the inhibition results in Table 1, scaffolds **23** and **25** bearing a primary amino group were proposed, inspired by the pyrrolizidine-type iminosugars **4–6**. As shown in Scheme 4, alcohols **20** and **21** were prepared from intermediate **3** via *N*-Boc deprotection and spontaneous intramolecular cyclization. After mesylation, azide replacement and hydrogenolysis of alcohol **20**, the desired scaffold **23** was obtained in 68% yield. Likewise, the other scaffold **25** was obtained from **21**. The scaffolds **23** and **25** were coupled with a randomly selected 48-membered acid library **26** (Table 2) via a parallel combinatorial approach.[18] Reactions were monitored by TLC or LC-MS and conversion was found to be almost complete after 48 h. All products were directly evaluated in the enzyme-based activity assay without further purification.[19]

Screening of library **27** at a concentration of 10 μM resulted in no hits. However, library **28** at the same concentration yielded seven hits (>70% inhibition against α-glucosidase). No potent hit for β-glucosidase was found from library **28**. Interestingly, the activity profiles of libraries **27** and **28** toward α-glucosidase revealed general trends in the inhibition potency. For example: (1) the stereocenter on the B ring and the structure of the substitu-

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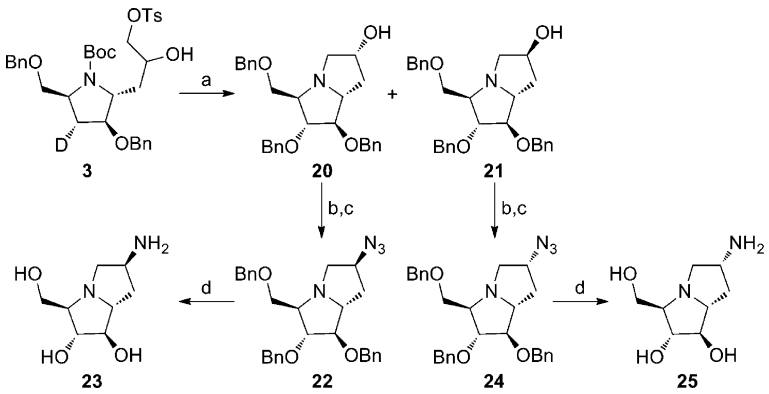
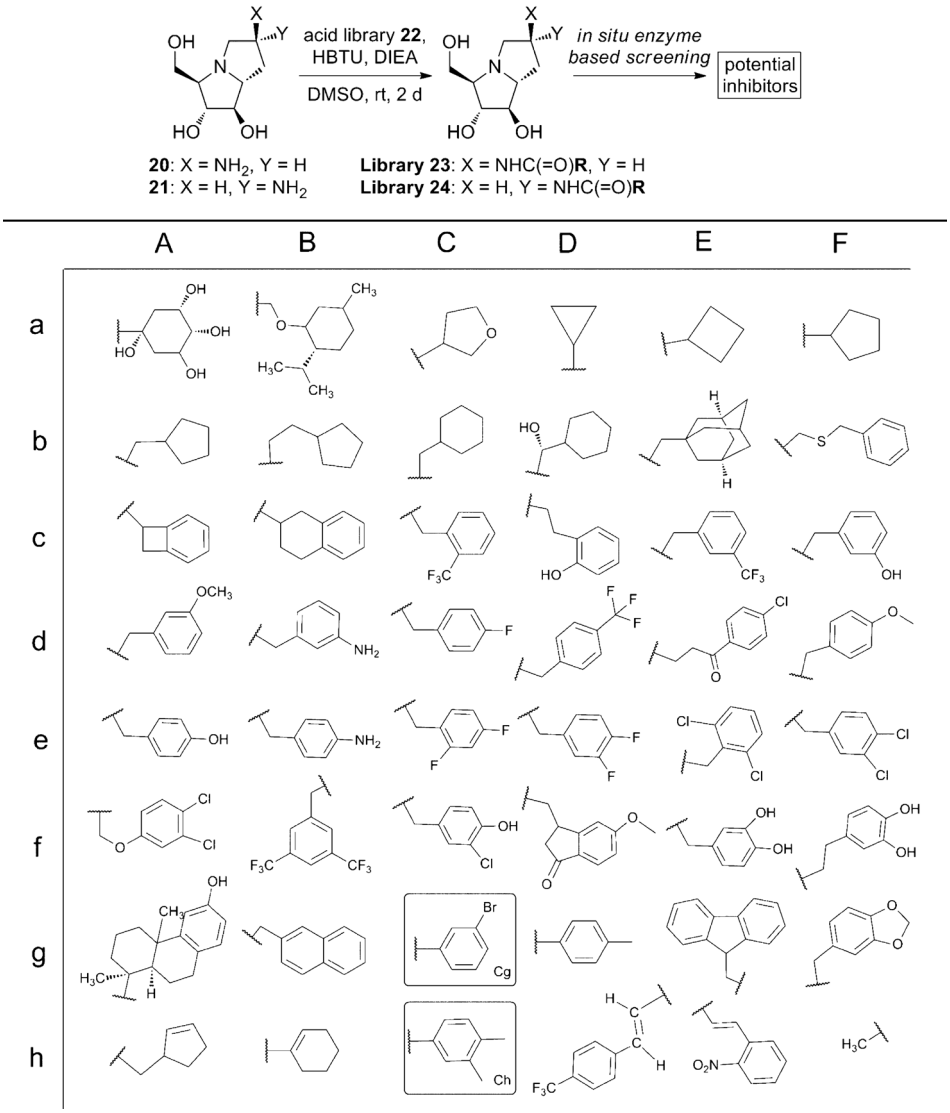


Scheme 4. Reagents and conditions: (a) TFA, DCM, 96%; (b) MsCl, NEt_3, DCM, 92%; (c) NaN_3, DMF, 60°, 74%; (d) $Pd(OH)_2/C$, H_2, MeOH, quant..

Table 2. Library preparation and bioevaluation.



ent moiety both significantly influenced the potency; (2) inhibitors bearing phenyl moieties were more potent than those bearing alkyl, benzyl, or alicyclic groups; (3) inhibitors **28Cg** (with a bromo group at the *meta* position) and **28Ch** (with methyl groups at the *meta* and *para* positions) showed that hydrophobic substituents on the phenyl moiety might increase the inhibition potency. Notably, these preliminary results might serve as an initial starting point for inhibitor study. After re-synthesis and purification of the two selective inhibitors **28Cg** and **28Ch**, their inhibitory activities were re-examined and their IC_{50} values were determined to be 0.2 and 0.4 μM, respectively (Table 3). Surprisingly, both were more potent than DAB and one, the bicyclic iminosugar **28Cg**, was even more potent than monocyclic DMDP. This result shows how a weak inhibitor such as **4** can be dramatically improved to a potent inhibitor such as **28Cg** with simple chemical modifications.

The Lineweaver–Burk plot of α-glucosidase kinetics is shown in Figure 3. The kinetic results demonstrated that the inhibition pattern of **28Cg** was competitive with a K_i

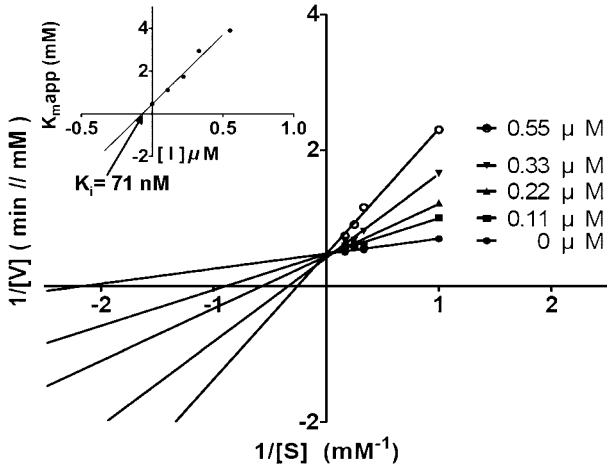


Figure 3. Lineweaver–Burk double-reciprocal plots of **28Cg**.

value of 71 nM. Presumably, this inhibitor is a transition-state mimic and the substituent moiety interacting with enzyme residues around the aglycan part significantly improves the binding potency and selectivity. For comparison purposes, monocyclic **29** (structurally similar to **28Cg**; Figure 4) was prepared and found to be a surprisingly

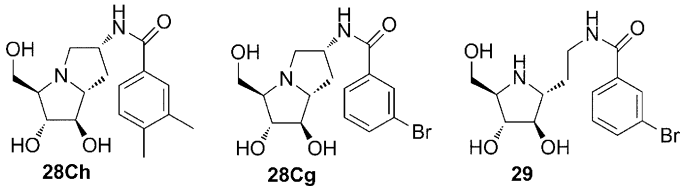


Figure 4. Chemical structures of **28Ch**, **28Cg** and **29**.

weak inhibitor against α-glucosidase (Table 3). The IC_{50} value of **29** was 0.1 mM, 500 times weaker than that of bicyclic **28Cg**.

Table 3. Inhibition activity against various glycosidases.

Enzyme	IC_{50} (μM)		
	28Cg	**28Ch**	**29**
α-Glucosidase[a]	0.2 (K_i = 71 nM)	0.4	100
β-Glucosidase[b]	NI[g]	NI	NI
α-Galactosidase[c]	NI	NI	NI
α-Mannosidase[d]	NI	NI	NI
Amylase[e]	(68 %)[h]	1.2	(33 %)[h]
Naringinase[f]	NI	NI	NI

[a] From *Bacillus stearothermophilus*. [b] From almonds. [c] From green coffee beans. [d] From *Canavalia ensiformis* (jack bean). [e] From *Aspergillus niger*. [f] From *Penicillium decumbens*. [g] NI refers to less than 50% inhibition at 200 μM. [h] Inhibition % at 50 μM.

Under the slightly acidic assay conditions (pH 6.8), the amine groups in **28Cg** and **29** are expected to be protonated (calculated pK_a of approximately 8–9).[20] These positively charged iminosugars might mimic the proposed transition states.[21] Notably, our results show that the bicyclic conformationally restricted scaffold contributes more significantly to inhibitory activity than charge distribution.

As shown in Table 3, **28Cg**, **28Ch**, and **29** were examined to study their inhibitory potency and selectivity towards various glycosidases. Interestingly, **28Cg** showed better potency and selectivity against α-glucosidase and weak potency against amylase (68 % inhibition at 50 μM). Furthermore, no significant inhibition was observed against other glycosidases such as β-glucosidase and α-galactosidase.

3. Conclusions

We have successfully developed a straightforward and flexible synthetic route for the preparation of various bicyclic iminosugar-based alkaloids from a chiral five-membered cyclic nitrone. Preliminary bioevaluation of these bicyclic iminosugars led us to generate appropriate bicyclic scaffolds bearing an amino group. Through rapid diversification of the scaffolds via an amide coupling with random carboxylic acids, structurally diverse bicyclic iminosugar-based libraries were prepared with substituent diversity, core diversity, and configurational diversity. This discovery process allowed us to efficiently sieve out several potent and specific glycosidase inhibitors. We also demonstrated that the bicyclic conformationally restricted iminosugars are more potent than monocyclic ones in this study. Since all eight five-membered cyclic nitrones with three stereogenic centers have been prepared in our lab,[5] we strongly believe this powerful strategy can be applied to

develop more biologically interesting bicyclic iminosugar-based inhibitors against other glycan-processing enzymes.

4. Experimental Section

4.1. General Information

Samples were concentrated by rotary evaporation. Cyclic nitrone **1**, **2** and **7** were prepared as previously described.[5a] The substituent diversity via amide bond formation was performed as previously described.[18] Enzymes, including α-glucosidase from *Bacillus stearothermophilus lyoph* and β-glucosidase from almond, were purchased from commercial suppliers and the inhibitory assay was performed as previously described.[5b,19b]

4.2. Synthesis

Compound **3**

To a solution of **2** (940 mg, 1.7 mmol) in 1,4-dioxane/H_2O (3:2) were added OsO_4 (43 mg, 0.17 mmol) and NMO (220 mg, 1.9 mmol) at 60° for 12 h. The reaction was quenched with aqueous $Na_2S_2O_3$ and then the reaction mixture was stirred for 1 h at 0°. The mixture was extracted with brine, dried ($MgSO_4$) and concentrated to give the crude intermediate (740 mg, 1.3 mmol, 75%), which was mixed with TsCl (260 mg, 1.3 mmol), NEt_3 (180 μL, 1.3 mmol) and catalyst *n*-Bu_2SnO (32 mg, 0.13 mmol) in dry DCM at room temperature. After 2 h, the reaction was quenched with H_2O, dried ($MgSO_4$), concentrated, and purified by CC (eluent: 1:5 EtOAc/Hex, silica gel) to give **3** (0.83 g, 1.1 mmol, 88%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.80–1.95 (m, 2H), 2.41 (s, 3H), 3.40–3.46 (m, 1H), 3.71–3.73 (m, 2H), 3.81–3.85 (m, 2H), 3.87–3.90 (m, 1H), 3.95–4.01 (m, 2H), 4.11–4.19 (m, 1H), 4.32–4.62 (m, 6H), 7.14–7.34 (m, 17H), 7.74 (m, 2H). HRMS (ESI) calcd for $[C_{41}H_{49}NO_9S+H]^+$, 732.3201; found, 732.3253.

Compounds **4** and **5**[13]

To a solution of **3** (830 mg, 1.1 mmol) in DCM was added 50% TFA at room temperature. After 5 h, the reaction was quenched with sat. aqueous $NaHCO_3$ and extracted with DCM. The organic layer was washed with brine, dried ($MgSO_4$), concentrated, and then purified by CC (eluent: 3:1 EtOAc/Hex, silica gel) to give 96% of two separable cyclization adducts in a 1:1 ratio. To a solution of the adducts in MeOH were added 20% $Pd(OH)_2$/C (8 mg, 0.05 mmol) and one drop of conc. aqueous HCl under a hydrogen atmosphere at 1 atm and room temperature. After 12 h, the reaction solution was filtered through a Celite pad and the filtrate was concentrated. The residue was purified by CC (eluent: 1:9 NH_4OH/*n*-propanol, silica gel) to give **4** (50 mg, 0.28 mmol, 48% over two steps) and **5** (50 mg, 0.28 mmol, 48% over two steps). Compound **4**. ^{1}H NMR (600 MHz, D_2O, δ): 2.09–2.14 (m, 1H), 2.19–2.23 (m, 1H), 2.98 (br, 1H), 3.11 (dd, 1H, J=4.2, 11.8 Hz), 3.21 (d, 1H, J=11.8 Hz), 3.62 (d, 1H, J=6 Hz), 3.74–3.78 (m, 2H), 3.89 (dd, 1H, J=4.2, 11.8 Hz), 3.95–4.00 (m, 1H), 4.68 (s, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 37.6, 61.7, 65.9, 69.6, 70.3, 72.0, 77.3, 80.0. HRMS (ESI) calcd for $[C_8H_{15}NO_4+H]^+$, 190.1079; found, 190.1081. Compound **5**. ^{1}H NMR (600 MHz, D_2O, δ): 2.05–2.10 (m, 1H), 2.18–2.22 (m, 1H), 3.04–3.07 (m, 1H), 3.11 (dd, 1H, J=4.0, 12.0 Hz), 3.27 (d, 1H, J=12.0 Hz), 3.66–3.73 (m, 2H), 3.84 (dd, 1H, J=3.6, 12.0 Hz), 3.91–3.97 (m, 2H), 4.62 (br, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 37.4, 60.4, 62.0, 66.8, 70.7, 71.9, 77.0, 79.4. HRMS (ESI) calcd for $[C_8H_{15}NO_4+H]^+$, 190.1079; found, 190.1015.

Compound **6**[22]

To a solution of the cyclization adduct of **3** (120 mg, 0.26 mmol) in dry DCM were added MsCl (30 μL, 0.4 mmol) and NEt_3 (50 μL, 0.4 mmol) at room temperature. After 1 h, the reaction was quenched with water and extracted with DCM. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated, and then purified by CC (eluent: 1:1 EtOAc/Hex, silica gel) to give a white solid (0.13 g). A mixture of the white solid and $LiAlH_4$ (35 mg, 0.96 mmol) was stirred at room temperature for 5 h, and then the reaction was quenched with 1 N aqueous HCl and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to give the intermediate (66 mg, 0.15 mmol), which was directly used for the next step without further purification. A mixture of the intermediate (65 mg), $Pd(OH)_2$/C (3 mg, 0.02 mmol), and one drop of conc. aqueous HCl in MeOH was stirred under a hydrogen atmosphere at room temperature for 12 h. The reaction solution was filtered through a Celite pad and the filtrate was concentrated to give **6** (26 mg, 0.15 mmol, 57% over three steps). ^{1}H NMR (600 MHz, D_2O, δ): 2.05–2.21 (m, 4H), 3.26–3.34 (m, 3H), 3.42–3.46 (m, 1H), 3.82–3.85 (m, 1H), 3.90–3.97 (m, 3H). ^{13}C NMR (150 MHz, D_2O, δ): 23.8, 28.2, 55.1, 56.7, 69.1, 69.9, 73.8, 77.6. HRMS (ESI) calcd for $[C_8H_{15}NO_3+H]^+$, 174.1125; found, 174.1158.

Compounds **11** and **12**

The reactions were carried out as described above for **4** and **5** starting from **7** to give **11** and **12**. Compound **11**. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.69–1.74 (m, 1H), 1.95–2.03 (m, 1H), 2.83–2.87 (m, 1H), 2.90 (dt, 1H, J=5.8, 11.8 Hz), 3.19–3.23 (m, 1H), 3.27 (t, 1H, J=5.3 Hz), 3.48 (dd, 1H, J=5.8, 9.4 Hz), 3.54 (dd, 1H, J=4.6, 9.4 Hz), 3.81 (t, 1H, J=5.3 Hz), 4.00 (t, 1H, J=6.6 Hz), 4.11–4.16 (m, 1H), 4.49–4.65 (m, 6H), 7.22–7.33 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 34.1, 52.6, 68.1, 71.8, 72.0, 72.5, 73.3, 75.9, 76.7, 85.0, 86.6, 127.5, 127.6 (×2), 127.71 (×2), 127.78 (×2), 127.8, 127.9 (×2), 128.33 (×2), 128.38 (×2), 128.4, 138.1, 138.2, 138.3. HRMS (ESI) calcd for $[C_{29}H_{33}NO_4+H]^+$, 460.2482; found, 460.2480. Compound **12**. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.87 (dd, 1H, J=5.7, 12.9 Hz), 1.92–1.98 (m, 1H), 2.77 (dt, 1H, J=6.4, 10.2 Hz), 3.08 (td, 1H, J=5.7, 11.4 Hz), 3.20 (t, 1H, J=

8.2 Hz), 3.48 (dd, 1H, J=6.4, 9.7 Hz), 3.53–3.55 (m, 2H), 4.11 (br, 1H), 4.15 (t, 1H, J=4.3 Hz), 4.27 (t, 1H, J=4.3 Hz), 4.50–4.66 (m, 6H), 7.24–7.33 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 36.9, 52.7, 69.8, 71.6, 71.7, 72.0, 72.3, 73.2, 73.3, 81.5, 85.6, 127.4 (×2), 127.6 (×2), 127.72 (×2), 127.79 (×2), 127.8 (×2), 128.3 (×2), 128.43 (×2), 128.46, 137.8, 138.2, 138.5. HRMS (ESI) calcd for $[C_{29}H_{33}NO_4+H]^+$, 460.2482; found, 460.2447.

Australine (**13**)[23]

The reaction was carried out as described above for **4** starting from **12** to give **13** (69 mg, 0.37 mmol, 99%). ^{1}H NMR (600 MHz, D_2O, δ): 2.20–2.26 (m, 1H), 2.28–2.33 (m, 1H), 3.35–3.41 (m, 2H), 3.81 (m, 1H), 3.88–3.93 (m, 2H), 3.99 (dd, 1H, J=3.0, 13.2 Hz), 4.15 (dd, 1H, J=7.3, 10.5 Hz), 4.48 (t, 1H, J=7.3 Hz), 4.68 (dd, 1H, J=4.2, 7.3 Hz). ^{13}C NMR (150 MHz, D_2O, δ): 34.3, 52.2, 55.9, 68.1, 70.6, 71.5, 72.5, 75.4. HRMS (ESI) calcd for $[C_8H_{15}NO_4+H]^+$, 190.1079; found, 190.1020.

Compounds **15** and **16**[7a]

The reactions were carried out as described above for **4** and **5** starting from **14** (400 mg, 0.59 mmol) to give **15** (15 mg, 0.076 mmol, 13% over four steps) and **16** (30 mg, 0.15 mmol, 26% over four steps). Compound **15**. ^{1}H NMR (600 MHz, D_2O, δ): 1.73 (br, 1H), 1.85 (br, 1H), 1.99–2.06 (m, 2H), 3.27 (t, 1H, J=10.8 Hz), 3.48–3.58 (m, 3H), 3.99–4.05 (m, 2H), 4.22 (br, 2H), 4.31 (s, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 17.1, 27.7, 48.0, 61.1, 65.8, 68.3, 68.8, 73.3, 75.3. HRMS (ESI) calcd for $[C_9H_{17}NO_4+H]^+$, 204.1230; found, 204.1285. Compound **16**. ^{1}H NMR (600 MHz, D_2O, δ): 1.62–1.66 (m, 1H), 1.86–1.93 (m, 1H), 2.01–2.07 (m, 1H), 2.12–2.17 (m, 1H), 3.33 (td, 1H, J=3.1, 13.5 Hz), 3.42 (br, 1H), 3.48 (dt, 1H, J=4.1, 13.5 Hz), 3.63 (q, 1H, J=4.9 Hz), 3.98 (dd, 1H, J=4.9, 13.5 Hz), 4.04 (dd, 1H, J=3.9, 12.9 Hz), 4.08–4.12 (m, 1H), 4.23 (dd, 1H, J=3.2, 4.9 Hz), 4.40 (br, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 17.3, 28.6, 47.6, 57.5, 63.2, 69.7, 70.5, 75.8, 76.9. HRMS (ESI) calcd for $[C_9H_{17}NO_4+H]^+$, 204.1230; found, 204.1279.

Compound **18**

The reactions were carried out as described above for **13** starting from **17** (400 mg, 0.59 mmol) to give **18** (30 mg, 0.15 mmol, 25% over four steps). ^{1}H NMR (600 MHz, D_2O, δ): 1.92 (br, 1H), 2.12 (br, 3H), 3.45 (d, 1H, J=12.7 Hz), 3.63 (t, 1H, J=11.0 Hz), 3.69 (br, 1H), 3.87 (br, 1H), 3.99 (dd, 1H, J=4.6, 13.0 Hz), 4.07 (dd, 1H, J=3.3, 13.0 Hz), 4.13 (br, 1H), 4.17 (br, 1H), 4.21 (br, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 25.5, 28.2, 43.1, 57.3, 60.5, 62.6, 68.5, 76.7, 77.4. HRMS (ESI) calcd for $[C_9H_{17}NO_4+H]^+$, 204.1230; found, 204.1260.

Compound **19**

The reactions were carried out as described above for **15** starting from **17** (400 mg, 0.59 mmol) to give **19** (28 mg, 0.13 mmol, 27% over four steps). ^{1}H NMR (600 MHz, D_2O, δ): 1.75 (br, 1H), 1.93 (br, 3H), 2.24 (dd, 1H, J=4.5, 11.5 Hz), 2.38 (dd, 1H, J=8.1, 11.5 Hz), 3.27 (br, 1H), 3.49 (br, 1H), 3.75 (d, 1H, J=8.1 Hz), 3.97 (dd, 1H, J=3.2, 9.3 Hz), 4.03–4.07 (m, 4H), 4.32 (s, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 19.1, 33.2, 34.8, 52.2, 56.0, 62.4, 65.2, 70.6, 73.0, 78.3. HRMS (ESI) calcd for $[C_{10}H_{19}NO_4+H]^+$, 218.1387; found, 218.1335.

Compounds **20** and **21**

To a solution of **3** (0.83 g, 1.1 mmol) in DCM was added 50% TFA at room temperature. After 5 h, the reaction was quenched with aqueous $NaHCO_3$ and extracted with DCM. The organic layer was washed with brine, dried ($MgSO_4$), concentrated, and then purified by CC (eluent: 3:1 EtOAc/Hex, silica gel) to give **20** (0.25 g, 0.55 mmol, 48%) and **21** (0.25 g, 0.55 mmol, 48%). Compound **20**. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.88 (ddd, 1H, J=4.4, 10.3, 13.2 Hz), 2.16 (dd, 1H, J=7.1, 13.2 Hz), 3.02 (dd, 1H, J=4.4, 12.1 Hz), 3.23 (td, 1H, J=7.1, 12.1 Hz), 3.52 (d, 1H, J=12.1 Hz), 3.61 (dd, 1H, J=5.0, 9.6 Hz), 3.68 (dd, 1H, J=7.5, 9.6 Hz), 3.89 (t, 1H, J=4.4 Hz), 4.05–4.12 (m, 2H), 4.44–4.63 (m, 7H), 7.20–7.34 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 40.1, 64.1, 67.8, 70.0, 70.3, 72.0, 72.5, 73.1, 73.4, 85.2, 86.6, 127.6, 127.72, 127.79 (×2), 127.8, 127.93 (×2), 127.99 (×2), 128.0, 128.30, 128.38, 128.4, 128.50, 128.54, 137.2, 137.4, 137.8. HRMS (ESI) calcd for $[C_{29}H_{33}NO_4+H]^+$, 460.2482; found, 460.2459. Compound **21**. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.80 (ddd, 1H, J=4.2, 9.0, 13.1 Hz), 2.04 (dd, 1H, J=6.9, 13.1 Hz), 2.91 (dd, 1H, J=4.2, 11.5 Hz), 3.00 (td, 1H, J=6.9, 11.5 Hz), 3.13 (d, 1H, J=11.5 Hz), 3.51 (dd, 1H, J=3.1, 9.0 Hz), 3.57 (dd, 1H, J=4.9, 9.0 Hz), 3.68–3.71 (m, 1H), 3.83 (t, 1H, J=5.4 Hz), 4.06 (t, 1H, J=5.4 Hz), 4.43–4.45 (m, 1H), 4.46–4.48 (m, 6H), 7.24–7.33 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 40.6, 63.6, 66.1, 68.9, 71.9, 72.1, 72.5, 73.2, 74.0, 86.0, 88.7, 127.5 (×2), 127.6 (×2), 127.70 (×2), 127.75 (×2), 127.77 (×2), 128.34 (×2), 128.37 (×2), 128.4, 138.0, 138.3, 138.4. HRMS (ESI) calcd for $[C_{29}H_{33}NO_4+H]^+$, 460.2482; found, 460.2426.

Compound **22**

To a solution of **20** (0.12 g, 0.26 mmol) in dry DCM were added MsCl (0.03 mL, 0.4 mmol) and NEt_3 (0.05 mL, 0.4 mmol) at room temperature. After 1 h, the reaction was quenched with water and extracted with DCM. The organic layer was washed with brine, dried ($MgSO_4$), concentrated, and then purified by CC (eluent: 1:1 EtOAc/Hex, silica gel) to give the mesylate (0.13 g, 0.24 mmol, 92%). To a solution of the mesylate (0.06 g, 0.11 mmol) in dry DMF was added NaN_3 (0.008 g, 0.12 mmol) at 60°. The mixture was stirred at room temperature for 12 h. After quenching the reaction, the organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The residue was purified by CC (eluent: 1:1 EtOAc/Hex, silica gel) to give **22** (0.04 g, 0.08 mmol, 74%). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.88–1.92 (m, 1H), 2.03–2.07 (m, 1H), 3.02 (q, 1H, J=6.2 Hz), 3.07 (dd, 1H, J=5.2, 11.7 Hz), 3.16 (dd, 1H, J=3, 11.64 Hz), 3.53 (t, 1H, J=9.2 Hz), 3.59 (dd, 1H, J=5.1, 9.3 Hz), 3.66 (dd, 1H, J=7.4, 12.4 Hz), 4.05 (t, 1H, J=5.3 Hz), 4.16–4.19 (m, 1H), 4.51–4.69 (m, 6H), 7.27–7.38 (m, 15H).

Compound **23**

A suspension of the azide **22** (40 mg, 82 μmol), palladium hydroxide on carbon (20 wt % on activated carbon, 10 mg) and a catalytic amount of conc. HCl in methanol (2 mL) was vigorously stirred for 14 h under an atmosphere of hydrogen. The reaction mixture was then filtered and concentrated and the residue was purified by CC (eluent: 1:9 NH_4OH/IPA then 1:4, silica gel) to give **23** (15 mg, quant.). ^{1}H NMR (600 MHz, D_2O, δ): 2.29–2.34 (m, 1H), 2.49–2.53 (m, 1H), 3.16 (s, 1H), 3.35–3.38 (m, 1H), 3.56 (dd, 1H, J=6.2, 12.3 Hz), 3.77 (dd, 1H, J=6.2, 12.3 Hz), 3.83 (br, 1H), 3.88 (dd, 1H, J=3.3, 12.3 Hz), 3.92–4.03 (m, 2H), 4.16 (t, 1H, J=6.9 Hz). ^{13}C NMR (150 MHz, D_2O, δ): 32.8, 49.1, 56.4, 59.7, 66.6, 70.5, 75.4, 78.7. HRMS (ESI) calcd for $[C_8H_{16}N_2O_3+H]^+$, 189.1234; found, 189.1259.

Compound **24**

The title compound was synthesized from compound **21** as per the procedure for the preparation of compound **22**. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.80 (ddd, 1H, J=6.1, 12.7 Hz), 2.20 (ddd, 1H, J=6.6, 13.7 Hz), 2.89 (dd, 1H, J=5.1, 11.4 Hz), 3.19–3.23 (m, 2H), 3.45 (dd, 1H, J=6.0, 9.6 Hz), 3.49 (dt, 1H, J=6.4, 13.7 Hz), 3.55 (dd, 1H, J=3.9, 9.6 Hz), 3.98 (t, 1H, J=6.2 Hz), 4.02 (t, 1H, J=7.3 Hz), 4.07–4.11 (m, 1H), 4.49–4.70 (m, 6H), 7.24–7.35 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 36.8, 59.1, 62.4, 66.4, 68.2, 71.8, 72.2, 72.4, 73.3, 85.0, 88.5, 127.5, 127.6 (×2), 127.71 (×2), 127.75 (×2), 127.82 (×2), 127.84 (×2), 128.34, 128.38 (×2), 128.5, 138.0, 138.2, 138.3. HRMS (ESI) calcd for $[C_{29}H_{32}N_4O_3+H]^+$, 485.2547; found, 485.2520.

Compound **25**

The title compound was synthesized from compound **24** as per the procedure for the preparation of compound **23**. ^{1}H NMR (600 MHz, D_2O, δ): 1.72 (q, 1H, J=10.5 Hz), 2.47–2.52 (m, 1H), 2.61 (t, 1H, J=9.9 Hz), 2.89–2.92 (m, 1H), 3.29 (q, 1H, J=7.6 Hz), 3.5 (dd, 1H, J=6.6, 9.9 Hz), 3.59 (dd, 1H, J=7.0, 11.6 Hz), 3.64–3.69 (m, 1H), 3.75 (dd, 1H, J=3.7, 11.6 Hz), 3.86 (t, 1H, J=8.2 Hz), 3.98 (t, 1H, J=7.6 Hz). ^{13}C NMR (150 MHz, D_2O, δ): 35.9, 52.0, 59.4, 62.3, 65.4, 70.6, 78.1, 80.5. HRMS (ESI) calcd for $[C_8H_{16}N_2O_3+H]^+$, 189.1234; found, 189.1276.

Compound **28Cg**

To a solution of **25** (0.03 g, 0.16 mmol) in 1:1 DMF/H_2O were added HBTU (75 mg, 0.2 mmol), DIEA (60 μL, 0.34 mmol) and 3-bromobenzoic acid (34 mg, 0.17 mmol) at room temperature. The mixture was stirred for 12 h and the residue was purified by CC (eluent: 70:10 DCM/MeOH, silica gel) to give **28Cg** (35 mg, 0.094 mmol, 60 %) as an oil. ^{1}H NMR (600 MHz, D_2O, δ): 1.84–1.89 (m, 1H), 2.52–2.56 (m, 1H), 2.74 (t, 1H, J=9.6 Hz), 2.95 (br, 1H), 3.33–3.36 (m, 1H), 3.48–3.50 (m, 1H), 3.62 (dd, 1H, J=7.0, 11.7 Hz), 3.77 (dd, 1H, J=7.0, 11.7 Hz), 3.88 (t, 1H, J=8.0 Hz), 4.02 (t, 1H, J=8.0 Hz), 4.48–4.53 (m, 1H), 7.40 (t, 1H, J=7.9 Hz), 7.67 (d, 1H, J=7.6 Hz), 7.75 (d, 1H, J=7.9 Hz), 7.89 (s, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 35.1, 52.0, 58.5, 62.4, 65.3, 70.5, 78.1, 80.6, 121.9, 125.8, 130.1, 130.3, 134.8, 135.5, 169.7. HRMS (ESI) calcd for $[C_{15}H_{19}BrN_2O_4+H]^+$, 371.0601; found, 371.0649.

Compound **28Ch**

Following the procedure for the preparation of **28Cg**, **28Ch** was obtained as an oil. ^{1}H NMR (600 MHz, D_2O, δ): 2.18–2.20 (m, 1H), 2.23 (s, 3H), 2.24 (s, 3H), 2.75 (ddd, 1H, J=7.1, 13.6 Hz), 3.19 (t, 1H, J=10.3 Hz), 3.52 (br, 1H), 3.86 (dd, 1H, J=6.5, 12.6 Hz), 3.93–3.97 (m, 3H), 4.14 (t, 1H, J=7.1 Hz), 4.28 (t, 1H, J=6.5 Hz), 4.61–4.65 (m, 1H), 7.22 (d, 1H, J=7.9 Hz), 7.45 (d, 1H, J=7.9 Hz), 7.48 (s, 1H). ^{13}C NMR (150 MHz, D_2O, δ): 18.7, 18.8, 33.6, 50.6, 57.6, 58.3, 68.4, 72.2, 76.6, 78.9, 124.6, 128.0, 129.6, 130.1, 137.4, 142.1, 170.7. HRMS (ESI) calcd for $[C_{17}H_{24}N_2O_4+H]^+$, 321.1809; found, 321.1852.

4.3. General Procedure for the Inhibition Assay with Various Glycosidases

The initial velocities of hydrolysis at room temperature were measured spectrophotometrically at 405 nm at various concentrations of *p*-nitrophenyl-glycopyranoside (from 62.5 mM to 0 mM) using a multidetection reader (SpectraMax M5, Molecular Devices). The obtained data were fitted into the Michaelis–Menten equation by using GraphPad to determine the K_m and V_{max} values. The compounds that showed activities were selected and further tested at lower concentration to determine IC_{50}. The assays performed in 96-well microtiter plates contained sodium phosphate buffer [100 mM, pH 6.8, for α-glucosidase (E.C. 3.2.1.20) and β-glucosidase (E.C. 3.2.1.21)].

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