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Synthesis and Deconvolution of the First Combinatorial Library of Glycosidase Inhibitors

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Abstract—A combinatorial library of 125 compounds with a structure consisting of 1-azafagomine linked at N-1 via an acetic acid linker to a variable tripeptide was synthesised. The library was synthesised by Merrifield split and mix synthesis of the peptide, followed by capping with chloroacetate, regioselective nucleophilic substitution with 1-azafagomine and cleavage from the polymeric support. The library was screened for inhibition of β-glucosidase, α-glucosidase and glycogen phosphorylase and found to display β-glucosidase inhibition. Deconvolution of the library revealed that some inhibition was caused by all library members but the strongest inhibitor was clearly a compound having three hydroxyproline residues in the peptide fragment. This compound was a weaker but more selective inhibitor than 1-azafagomine itself.

Introduction

Combinatorial chemistry is an interesting new chemical/analytical technique that has emerged, which potentially facilitates the search for compounds with interesting chemical or biological properties.[1–10] Through creation and screening of libraries of many compounds the objective of this field is to save time and cost compared with conventional chemical synthesis and investigation of individual compounds. The challenge at the moment is, however, to find ways in which combinatorial chemistry can be applied effectively to specific problems.

Specific inhibitors of glycosidases and related enzymes have recently been the subject of much interest[11–21] either as potential drugs against various diseases and disorders, as glycobiochemical tools or as agents that provide information about the chemistry of enzymatic glycoside cleavage. In particular, it is the various forms of azasugars that have been found to be the most potent and specific inhibitors so far. These compounds are relatively laborious to synthesise, and the inhibitor discovery process might be facilitated considerably if combinatorial chemistry could be applied to these compounds. In this paper we report the first synthesis of a combinatorial library of glycosidase inhibitors and the subsequent deconvolution and identification of individual inhibitors from the library.

Previous work from our laboratory has shown that modification of the inhibitor isofagomine (**1**) at *N*-1 with another sugar residue to create **3** resulted in a 60-fold increase in inhibition of glucoamylase.[22] This suggested that substitution at *N*-1 of **1** with the extra glucose residue increased affinity by mimicking the leaving group (the 2nd saccharide residue) in the transition state. We were therefore interested in investigating the effect of *N*-1 substitution more broadly and chose this as a target for a combinatorial approach. We replaced **1** with the analogue 1-azafagomine (**2**) since this compound, albeit racemic, is also a potent glycosidase inhibitor and is readily prepared in gram quantities.[23] Our target library is shown as **4** in Figure 1. It contains **2** linked through an acetic acid linker to a variable domain consisting of a tripeptide supposed to mimic the leaving group of the transition state. The resemblance between an acyclic tripeptide and a rigid saccharide unit or chain may seem remote, but peptides, due to hydrogen bonding, often have rather rigid structures and, provided hydroxy groups were included in the peptide resemblance, might be attained.

Key words: Azasugar; nucleophilic substitution; solid-phase; hydrazine; peptide.
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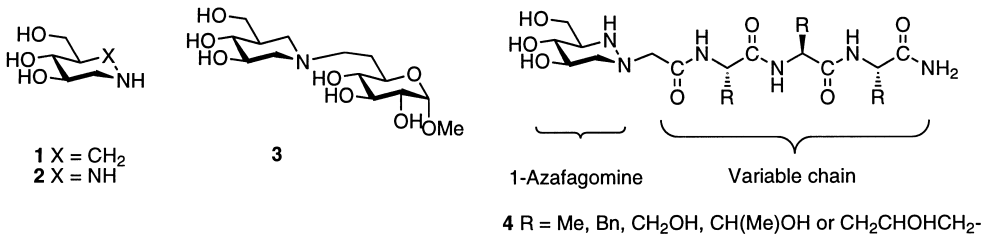


Figure 1. The proposed strategy for a 1-azasugar library.

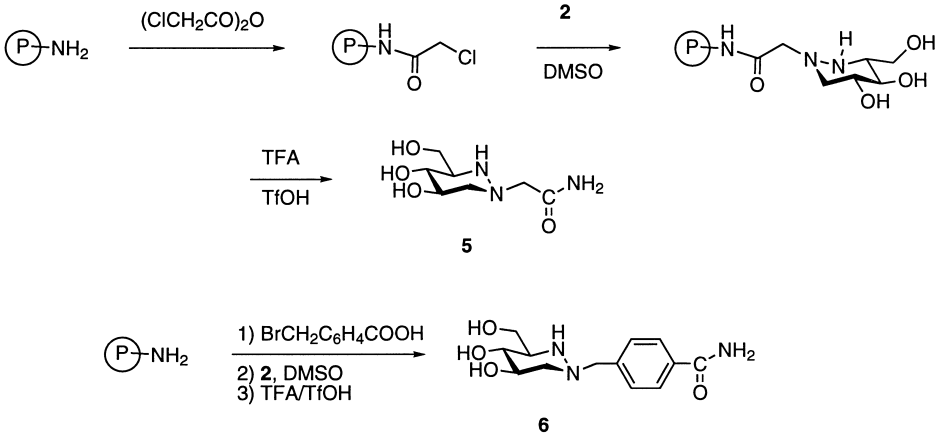
Results and Discussion

Synthesis of **4** was planned to be carried out by solid phase synthesis using the split and mix method[1,2,4] starting from the peptide C-terminus. This would result in formation of a combinatorial library of tripeptides, which could then be linked to a chloroacetate at the N-terminus. Finally, substitution of the chloride with (±)-**2** and cleavage from the resin should give **4**. While the peptide synthesis could be carried out using standard procedures, conditions for the chloroacetylation and nucleophilic substitution with **2** had to be studied in advance. Therefore the model experiment shown in Scheme 1 was carried out. A 4-methyl benzhydrylamine resin (MBHA, P-NH2) was exhaustively reacted with 4 equiv of chloroacetic anhydride in DMF/collidine, and then treated with 3 equiv of (±)-**2** in DMSO in the presence of 6 equiv diisopropylethylamine (DIEA). After extensive washing to ensure removal of excess **2**, cleavage from the resin was effected with trifluoroacetic acid (TFA)/trifluoromethanesulphonic acid (TfOH). A single product **5** was obtained in 48% yield after ion-exchange chromatography on an acidic resin, which ensured removal of possible neutral byproducts. The high selectivity for alkylation at *N*-1 may seem surprising, however it has previously been found that the selectivity between *N*-1 and *N*-2 in acetylation of **2** is 4:1.[24] Furthermore the reaction of **2** with 2,4-dinitrofluorobenzene (Sangers reagent) gave only *N*-1 arylation. Note that the solid phase alkylation of **2** ensures that di- and polyalkylation is avoided, which might otherwise occur (the reaction between MeI and **2** gives many products). This model experiment showed that the above synthesis plan to **4** was feasible and could be expected to give only one set of regioisomers.

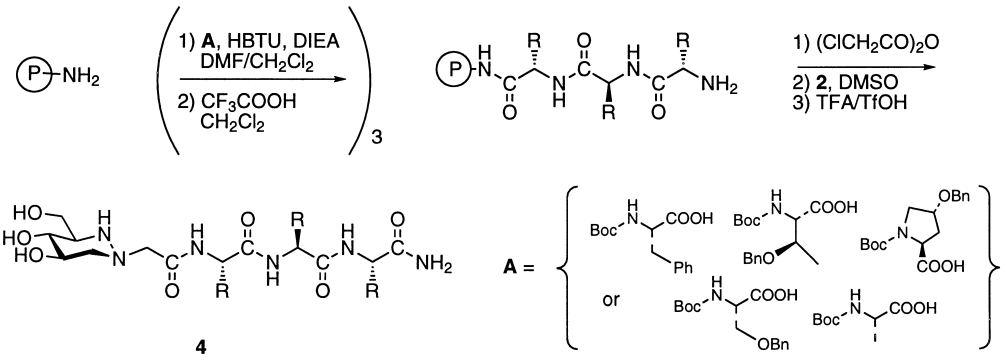
Another linker than glycine was also investigated (Scheme 1). Substituting chloroacetic anhydride in the coupling step in the above synthesis with 4-bromomethylbenzoic acid, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[25] and DIEA led to a 23% yield of **6**.

The synthesis of **5** paved the way for synthesis of the library **4** since it had been showed that the individual steps were successful. As constituents in the tripeptide fragment of **4** we chose five readily available amino acids: serine, threonine and 4-hydroxyproline were chosen because the presence of OH-groups were expected to resemble a carbohydrate moiety; phenylalanine and alanine were chosen as examples of amino acids with a large and small non-polar group. The amino acids were used as their *N*-Boc derivatives and with their hydroxyl groups benzylated. The benzyl groups would conveniently cleave under the strongly acidic conditions used for cleavage from the solid support. Using five amino acids should result in a final library with 125 compounds discounting the two stereoforms of **2**.

The synthesis was carried out as shown in Scheme 2. The tripeptide was first synthesised using the split and mix method[1,2,4] in a 5 chamber reactor on a MBHA resin. Peptide couplings were carried out with the amino acid derivatives described above using HBTU[25] and DIEA in DMF/CH_2Cl_2 as reagents. Boc groups were removed with TFA in CH_2Cl_2. After three couplings the



Scheme 1. Solid-phase alkylation of 1-azafagomine (**2**).



Scheme 2. Synthesis of the library **4**.

result was five sub-libraries of 25 peptides in each reactor, which were individually treated with the three steps from the synthesis of **5** above: reaction with chloroacetic anhydride, substitution with (±)-**2** in DMSO and cleavage from resin with TFA/TfOH. After purification of the libraries by ion-exchange chromatography on a strongly acidic resin to remove non-basic impurities the yields were 30–44% (Table 1). The ion-exchange column also conveniently removes TfOH, which would otherwise be difficult to remove from the polar product. The yields were calculated using a mean molecular weight of the compounds in the library.

The five sublibraries of **4**, each having a known N-terminal amino acid (of the peptide), were tested for inhibition of three enzymes that (±)-**2** was known to inhibit: almond β-glucosidase, yeast α-glucosidase and glycogen phosphorylase (Table 1). K_i values were calculated based on concentration of individual inhibitors in the library. Some inhibition of the three enzymes was observed in all the sublibraries. However, the sublibrary having hydroxyproline at the N-terminus expressed the most potent inhibition of β-glucosidase and glycogen phosphorylase and was chosen for further study. Thus synthesis of five new sublibraries of **4** was carried out which contained the hydroxyproline at the N-terminus and with a known amino acid in central position. Testing of these new sublibraries (each with five compounds) showed the strongest inhibition to be present in the library where the central amino acid was hydroxyproline. Finally, the five individual compounds present in that sublibrary were synthesised (Table 1). Of those the compound (**4a**), which had hydroxyproline residues at all positions in the peptide, was the strongest inhibitor having a K_i against almond β-glucosidase of 40 μM. **4a** was also the strongest inhibitor of glycogen phosphorylase but a very weak inhibitor of α-glucosidase. Characterisation of **4a** using ^{1}H NMR and FABMS confirmed both identity and purity; furthermore it confirmed that the combinatorial synthesis outlined in Scheme 2 worked, and that the sublibraries previously prepared had the expected identity.

Table 1. Enzyme inhibition constants for compounds or sublibraries (for glycogen phosphorylase the percent enzyme inhibition is shown at the given concentration of inhibitor (shown in parentheses)

Compound	Yield (%)	Enzyme, K_i/μM		
		β-Glucosidase	α-Glucosidase	Glycogen phosphorylase
4 (Ala-X-X)	30	49	87	20% (12 μM)
4 (Hyp-X-X)	34	30	67	40% (14 μM)
4 (Phe-X-X)	36	> 100	60	17% (15 μM)
4 (Ser-X-X)	44	> 100	63	17% (18 μM)
4 (Thr-X-X)	32	> 100	85	10% (14 μM)
4 (Hyp-Ala-X)	31	23	> 100	55% (93 μM)
4 (Hyp-Hyp-X)	31	16	> 100	60% (94 μM)
4 (Hyp-Phe-X)	32	72	> 100	35% (96 μM)
4 (Hyp-Ser-X)	36	82	79	25% (108 μM)
4 (Hyp-Thr-X)	48	54	83	40% (143 μM)
4 (Hyp-Hyp-Ala)	30	212	> 500	20% (400 μM)
4 (Hyp[22] = **4a**)	50	40	> 500(1325)	50% (200 μM)
4 (Hyp-Hyp-Phe)	31	406	> 500	10% (400 μM)
4 (Hyp-Hyp-Ser)	39	308	> 500	25% (400 μM)
4 (Hyp-Hyp-Thr)	39	> 400	> 500	15% (400 μM)
4b	20	501	—	30% (286 μM)
5	48	> 1000	> 1000	35% (714 μM)
6	23	76	500	65% (347 μM)
7	33	> 1000	—	45% (500 μM)
8	31	347	—	25% (186 μM)
9	46	70	—	50% (100 μM)
2 (1-azafagomine)[22]	—	0.65	3.9	50% (13.5 μM)

Thus, the result showed that the most potent β-glucosidase inhibitor of the 125 compounds in the original library was **4a**. Interestingly **4a** is also the compound in the library expected to have the closest resemblance with an oligosaccharide, because the rigid cyclic prolines should be expected to mimic the pyranoside rings better than acyclic amino acids.

The reason the K_i value for some sublibraries was lower than the K_i value for **4a** was because the K_is were calculated based on the concentration of a single inhibitor in the library. Therefore the total inhibition will be greater than that of the strongest inhibitor present if the other library members also act as inhibitors.

The results in Table 1 also showed that some degree of inhibition is shown by all the compounds in the library. To investigate the span in inhibition potency we also synthesised a weak inhibitor of β-glucosidase. To do that we used the inhibition data for the sublibraries to determine which amino acid was the least favorable for each of the three positions. From the N-terminus these were threonine, serine and phenylalanine. The corresponding compound (**4b**) was synthesised and found to have a K_i of 501 μM. Thus the difference in inhibition by **4a** and **4b** was approximately 12-fold.

These results were compared with K_i values for (±)-**2**[23] and derivatives **5** and **6** (Table 1). Compound **4a** was a weaker β-glucosidase inhibitor than **2**, but was, unlike **2**, an extremely weak inhibitor of α-glucosidase. The selectivity for β-glucosidase inhibition is therefore higher for **4a** than **2**. This might be interpreted as an indication that the peptide moiety in general was unfavorable for binding. However, compound **5** was found to be an extremely weak inhibitor of both these enzymes which suggest that the *N*-substitution is unfavorable. Compound **4a** is at least 25 times more potent against β-glucosidase than **5**, and thus the triproline residue must contribute to binding. Therefore the loss in inhibition by **4a** compared to **2** must be attributed to the substitution. Aromatic derivative **6** was a stronger inhibitor than **5** having a K_i against β-glucosidase of 70 μM. To further investigate the effect of substitution on inhibition the derivatives **7** and **8** were synthesised (Fig. 2) using the general method outlined in Scheme 2. Surprisingly **7**, which only contained a single hydroxyproline, was a very poor inhibitor. Together with the data above this suggests that the 2nd and 3rd amino acid residues of **4a** contribute significantly to binding. The stronger inhibition of **6** compared to **5** suggested that the toluic amide linker might be better than the acetamide linker, and compound **8** was synthesised with the purpose of investigating this. It was however less potent than **4a**.

There are a number of recent examples in the literature that multivalent glycomimetics, such as clustered glycosides or glycodendrimers, show increased binding to carbohydrate binding proteins.[26,27] We felt it was interesting to investigate the effect of multivalency on glycosidase inhibition. In a recent report[19] bivalent azasugars were investigated so we decided to use the present technique to synthesise the tetravalent azasugar **9** (Scheme 3). This we did by using a diamino acid, ornithine, in the peptide synthesis. Thus a branched tripeptide was synthesised on the MBHA resin by coupling two times with Boc-protected ornithine using *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)/DIEA in DMF/DCM and using TFA for amino deprotection. The resulting tetraamine was reacted with chloroacetic anhydride followed by substitution with (±)-**2** in DMSO. Cleavage from the resin was as usual carried out with TfOH/TFA with

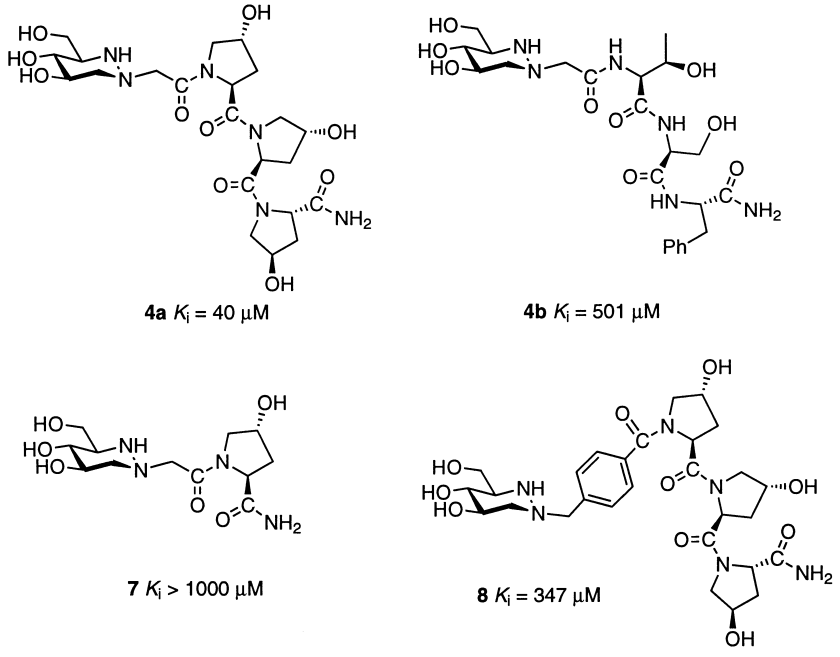
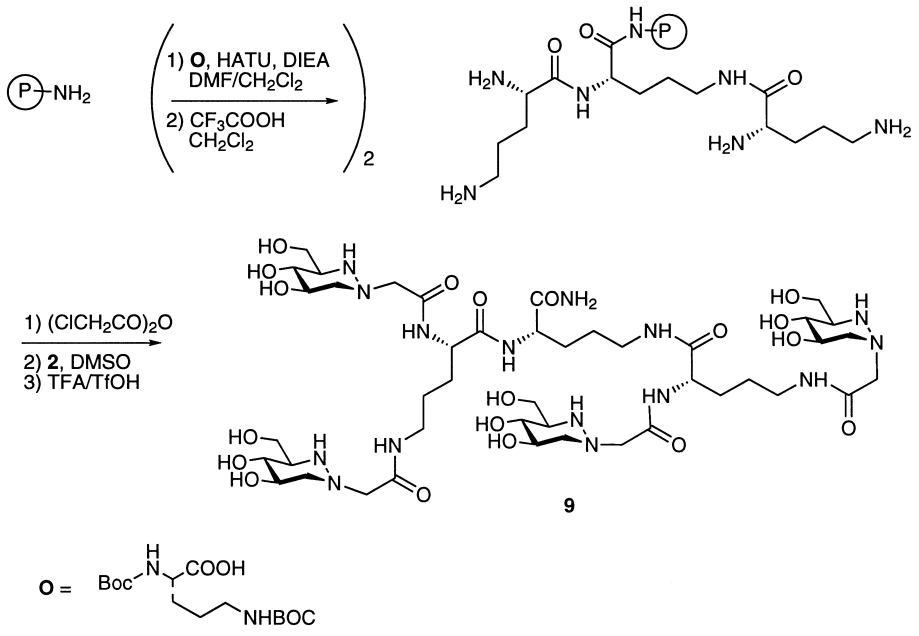


Figure 2. The most potent inhibitor in the library and analogues.



Scheme 3. Synthesis of dendrimer **9**.

purification by ion-exchange chromatography. The product **9** was isolated in 46% yield, and ^{1}H NMR and FABMS were consistent with the expected structure. K_i against β-glucosidase was 70 μM; thus **9** was more potent than most of the compounds in library **4**, but less potent than **4a**. Future studies will investigate ligand binding properties of **9** with carbohydrate binding proteins.

In this paper we have reported the synthesis of the first combinatorial library of azasugars using a flexible synthesis that can be applied to synthesis of much bigger libraries than those reported here. Thus 125 compounds were investigated for glycosidase inhibition in a manner that took only a fraction of the time conventional synthesis would have required. The results suggest that 1-*N* substitution of **2** with an acetic acid linker in general decreases inhibitory potency, but also suggests that a tri(hydroxyproline) actually contributes to increased binding, and that more selective inhibitors can be obtained.[28]

Experimental

General

^{13}C NMR and ^{1}H NMR spectra were recorded on a Varian Gemini 200 instrument using DHO (^{1}H NMR: δ 4.7 ppm) or acetone (^{1}H NMR: δ 2.05 ppm; ^{13}C NMR: δ 29.8 ppm) as reference. FAB mass spectra were obtained using a Kratos MS50RF mass spectrometer with EB geometry. The resolving power of the spectrometer was set to 1400–1600 (10% valley definition). The accelerating voltage was 8 kV, and the post accelerating detector potential was 9 kV. Ions were generated by fast atom bombardment (FAB) of the compounds dissolved in a glycerol matrix. The FAB gun employed Xenon gas, 99.99% and was operated at 9 kV. The instrument mass scale was externally calibrated using CsI, and the mass to charge ratio (m/z) of observed signals was corrected using the co-observed signals of known glycerol cluster ions, whereby an estimated mass accuracy of 100 ppm was achieved. The electrospray mass spectra (ESMS) were obtained using a Finnigan TSQ 700 triple quadropole instrument equipped with a nanospray source (Protana A/S, Odense, DK). Source conditions were: spray potential 800 V; heated (150°C) capillary: 20 V; tube lens: 70 V. Water solutions were approximately 1 mM in the analyte and 1 mM in glycerol. Again the mass to charge ratio of the observed M + 1 signals were corrected using the co-observed signals of known cluster ions.

Solid-phase synthesis of 4. General procedure

MBHA resin (5×100 mg, loading 1.05 mmol/g) was swollen in dichloromethane (DCM, 2 mL) for 1 h in five separate reactors. Each of the five protected amino acids (Boc-Ala-OH, Boc-Hyp-(OBn)-OH, Boc-Phe-OH, Boc-Ser-(OBn)-OH, Boc-Thr-(OBn)-OH, 0.32 mmol) was dissolved together with HBTU (110 mg, 0.29 mmol) in a mixture of DMF and DCM (1:1, 1 mL). To each of the five solutions were added DIEA (110 mL, 0.63 mmol) and the mixtures were allowed to react for 5 min, before adding them to the resin. The reactors were shaken for 1 h, and excess reagents were removed by suction before washing thoroughly with 50% DMF in DCM (5×), followed by DCM (3×). Removal of the Boc-groups was achieved by treatment with 50% TFA in DCM (2 mL, 2×10 min), followed by washing with 50% DMF in DCM (3×) and pyridine (3×). The resins were combined in a bigger reactor and shaken for 5 min to ensure complete mixing, and then split into the five reactors again. Another two coupling and deprotection cycles were done in a likewise manner. After the third deprotection

step the resins were kept in the separate reactors and each treated with chloroacetic anhydride (72 mg, 0.42 mmol) dissolved in a mixture of DMF and collidine (8:2, 1 mL) for 40 min. The resins were washed with pyridine (3×) then 50% DMF in DCM (5×) before adding a solution of 1-azafagomine[23] (**2**, 47 mg, 0.32 mmol) and DIEA (110 mL, 0.63 mmol) in DMSO (1 mL) to each of the reactors. The resins were shaken for 3 days, washed with DMF (5×), then 50% DMF in DCM (5×), and finally DCM (3×) and dried under high vacuum for at least 2 h. The finished peptides were cleaved from the resins using a mixture of TFA and TfOH (3:1, 0.60 mL, 2×, 1 h). The resins were washed with an additional amount of TFA (2×, 0.4 mL) and the combined acidic washings were diluted with water (10 mL) before applying them to an ion-exchange column (Amberlite IR-120, H^+, 10 mL). Excess acid from the cleavage and neutral compounds were washed off with water (50 mL) and the final products were liberated by treatment of the column with ammonium hydroxide solution (2.5%, 50 mL). Evaporation of the solvent gave **4** in an overall yield of 23–50% based on the original loading of the resin.

2-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-)acetyl-(4-hydroxy-)L-prolyl-(4-hydroxy-)L-prolyl-(4-hydroxy-)L-prolyl amide (4a). White amorphous solid. Yield: 27 mg (50%). ^{1}H NMR (D_2O) δ 4.4–4.9 (m, 6H, 3 CH-N, 3 CHOH), 3.1–3.9 (m, 13H, 3 CH_2-N, CH_2CO, 2 H-3′, H-4, H-5, H-6), 2.8 (m, 1H, H-3) 2.5 (t, 1H, H-6ax), 2.3 and 2.0 (2 m, 6H, 3 CH_2). MS(FAB), *m*/*z* calcd for $C_{22}H_{36}N_6O_{10}+H^+$: 545.26, obsd: 545.25.

2-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-)acetyl-L-threonyl-L-serinyl-L-phenylalanyl amide (4b). White amorphous solid. Yield: 15 mg (20%). MS(FAB), *m*/*z* calcd for $C_{23}H_{36}N_6O_9+H^+$: 541.26, obsd: 541.28.

2-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-)acetyl amide (5). The general procedure for the preparation of **4** was used without the three initial peptide coupling steps. White amorphous solid. Yield: 10 mg (48%). ^{1}H NMR (D_2O) δ 3.6–3.8 (m, 3H, H-5, 2 H-3′), 3.34 (d, 2H, CH_2CO), 3.24 (t, 1H, J_{34} and $J_{45}=9$ Hz, H-4), 3.18 (dd, 1H, $J_{6ax6eq}=11$, $J_{56eq}=5$ Hz, H-6eq), 2.80 (ddd, 1H, $J_{33'a}=5$, $J_{33'b}=3$ Hz, H-3), 2.40 (t, 1H, $J_{56ax}=11$ Hz, H-6ax). MS(ES), *m*/*z* calcd for $C_7H_{15}N_3O_4+H^+$: 206.11, obsd: 206.13.

4-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-methyl)benzoyl amide (6). MBHA resin (100 mg, loading 1.05 mmol/g) was swollen in DCM and treated with a preactivated (5 min) solution of 4-bromomethylbenzoic acid (90 mg, 0.42 mmol), HBTU (150 mg, 0.40 mmol), and DIEA (110 mL, 0.63 mmol) in DMF:DCM (1:1, 1 mL) for 1 h. Alkylation, cleavage and purification was done as described for compound **4**. White amorphous solid. Yield: 6.7 mg (23%). ^{1}H NMR (D_2O) δ 7.8 (d, 2H, 2 ArH), 7.4 (d, 2H, 2 ArH), 3.5–4.0 (m, 5H, 2 H-3′, H-5, CH_2Ar), 3.2 (m, 2H, H-4, H-6eq), 2.8 (m, 1H, H-3), 2.2 (t, 1H, $J_{56ax}=11$ Hz, H-6ax). MS(ES), *m*/*z* calcd for $C_{13}H_{19}N_3O_4+H^+$: 282.14, obsd: 282.14.

2-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-)acetyl-(4-hydroxy-)L-prolyl amide (7). The general procedure for the preparation of **4** was used without the two initial peptide coupling steps. White amorphous solid. Yield: 11 mg (33%). ^{1}H NMR (D_2O) δ 4.4–4.7 (m, CH-N, CHOH), 3.1–3.8 (m, CH_2-N, CH_2CO, 2 H-3′, H-4, H-5, H-6), 2.8 (m, H-3) 2.5 (m, H-6ax), 2.3 and 2.0 (2 m, 3 CH_2). MS(FAB), *m*/*z* calcd for $C_{12}H_{22}N_4O_6+H^+$: 319.16, obsd: 319.20.

4-((3*RS*,4*RS*,5*RS*)-4,5-Dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-methyl)benzoyl-(4-hydroxy)-L-prolyl-(4-hydroxy-)L-prolyl-(4-hydroxy-)L-prolyl amide (8). The general procedure was followed except for the reaction with chloroacetic anhydride, which was substituted with a coupling step with 4-bromomethylbenzoic acid, HBTU and DIEA (as described in the preparation of **6** above). White amorphous solid. Yield: 17 mg (26%). MS(FAB), *m*/*z* calcd for $C_{28}H_{40}N_6O_{10}+H^+$: 621.29, obsd: 621.30.

***N*²,*N*⁵-Bis-(*N*²,*N*⁵-di-(2-((3*RS*,4*RS*,5*RS*)-4,5-dihydroxy-3-hydroxymethylhexahydropyridazin-1-yl-)acetyl)-L-ornitoyl)-L-ornitoyl amide (9).** HMBA, HCl resin (150 mg, loading 0.80 mmol/g) was swollen in DCM for 1 h and washed with a solution of DIEA in DCM (10%, 2 mL, 2×, 3 min). Boc-Orn(Boc)-OH (160 mg, 0.48 mmol) and HATU (173 mg, 0.46 mmol) were dissolved in a mixture of DMF in DCM (1:1, 2 mL) and DIEA (125 mL, 0.72 mmol) were added. The mixture was allowed to react for 5 min before adding it to the resin. After shaking for 1 h the resin was washed and deprotected like previously described. Another coupling cycle with Boc-Orn(Boc)-OH was done with double the amount of reagents just described. At this point the general procedure for the chloroacetylation and substitution was followed. White amorphous solid. Yield: 61 mg (46%). ^{1}H NMR (D_2O) δ 4.2 (m, 3H, 3 CH-N), 3.1–3.8 (m, 34H, 4 CH_2CO), 2.8 (m, 4H, 4 H-4), 2.4–2.6 (m, 4H, 4 H-6eq), 1.5–1.8 (m, 12H, 3 CH_2CH_2). MS(FAB), *m*/*z* calcd for $C_{43}H_{81}N_{15}O_{19}+H^+$: 1112.59, obsd: 1112.65. MS(ES), *m*/*z* calcd for $C_{43}H_{81}N_{15}O_{19}+H^+$: 1112.5911, obsd: 1112.5931.

Enzymatic assays. α- and β-glucosidase. 4-Nitrophenyl-α- and β-D-glucopyranoside, α-glucosidase (yeast, Sigma G 5003, 4.5 sigma units/mg) and β-glucosidase (sweet almonds, Sigma G 0395, 7 sigma units/mg) were purchased from Sigma Chemical Co. 4-Nitrophenyl-α- or β-D-glucopyranoside were used as substrate and α- or β-glucosidase as catalyst in a sodium phosphate buffer (0.05 M, pH 6.8) at 25°C. Formation of the product, 4-nitrophenol, was measured continually at 400 nm using a Milton Roy Genesys 5 spectrometer. In all kinetic runs less than 1% of the initial substrate was consumed assuring the constancy of the substrate concentration. K_i determinations were performed as follows: two thermostatted solutions of (1) 1 mL of 0.1 M buffer, 800 mL substrate in varied concentration (α-glucosidase: 0.05–2.5 μM; β-glucosidase: 1–40 μM) and 100 μL

water and (2) 100 μL enzyme (1 mg/mL) were mixed, and the reaction was immediately monitored. From four experiments with varied substrate concentration initial reaction rates were calculated from the slope of the 1st order plot of product absorption versus reaction time. K_M and V_{max} were calculated from a Hanes plot. Similarly, reactions with inhibitors or inhibitor libraries present were carried out by substituting the 100 μL water in solution (1) in the procedure above with 100 μL inhibitor solution in a concentration [I] relatively close to the expected K_i. For inhibitor libraries [I] was calculated by dividing the weight concentration of the total library with the mean molecular mass of the compounds present and dividing with the number of compounds. From four experiments K'_M was calculated from the Hanes plot and from K_M and $K_M'K_i$ was calculated.

Acknowledgements

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References

1. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, 1233.
2. Lowe, G. *J. Chem. Soc.* **1995**, *24*, 309.
3. Brady, P. A.; Sanders, J. K. M. *J. Chem. Soc.* **1997**, *26*, 327.
4. Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82.
5. Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84.
6. Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem. Int. Ed.* **1995**, *34*, 2720.
7. Ohlmeyer, M. H.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *J. Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922.
8. Cheng, S.; Comer, D. D.; Williams, J. P.; Myers, P. L.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 2567.
9. Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.
10. Byrgesen, E.; Nielsen, J.; Willert, M.; Bols, M. *Tetrahedron Lett* **1997**, *38*, 5697.
11. Sinott, M. L. *Chem. Rev.* **1990**, *90*, 1171.
12. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319.
13. Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199.
14. Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340.
15. Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1.
16. Kraehenbuehl, K.; Picasso, S.; Vogel, P. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 893.
17. Parr, I. B.; Horenstein, B. A. *J. Org. Chem.* **1997**, *62*, 7489.
18. Panday, N.; Granier, T.; Vasella, A. *Helv. Chim. Acta* **1998**, *81*, 475.
19. Johns, B. A.; Johnson, C. R. *Tetrahedron Lett.* **1998**, *39*, 749.
20. Katzmaier, U.; Schneider, C. *Tetrahedron Lett.* **1998**, *39*, 817.
21. Berges, D. A.; Ridges, M. D.; Dalley, N. K. *J. Org. Chem.* **1998**, *63*, 391.
22. Dong, W.; Jespersen, T. M.; Skrydstrup, T.; Bols, M.; Sierks, M. R. *Biochemistry* **1996**, *35*, 2788.
23. Bols, M.; Hazell, R.; Thomsen, I. *Chem. Eur. J.* **1997**, *3*, 940.
24. Thomsen, I.; Ernholt, B. V.; Bols, M. *Tetrahedron* **1997**, *53*, 9357.
25. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillesen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
26. Roy, R. *Top. Curr. Chem.* **1997**, *187*, 241.
27. Hansen, H. C.; Haataja, S.; Finne, J.; Magnusson, G. *J. Am. Chem. Soc.* **1996**, *119*, 6974.
28. A preliminar report of some of this work has been published: Lohse, A.; Jensen, K. B.; Bols, M. *Tetrahedron Lett.* **1999**, *40*, 3033.