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The First Combinatorial Library of Azasugar 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.

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] Through creation and screening of libraries of many compounds, the objective of this field is to save time and money 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[2] 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 a another sugar residue to create **3** resulted in a 60 fold increase in inhibition of glucoamylase.[3] This suggested that substitution at N-1 of **1** with the extra glucose residue increased affinity by mimicking the

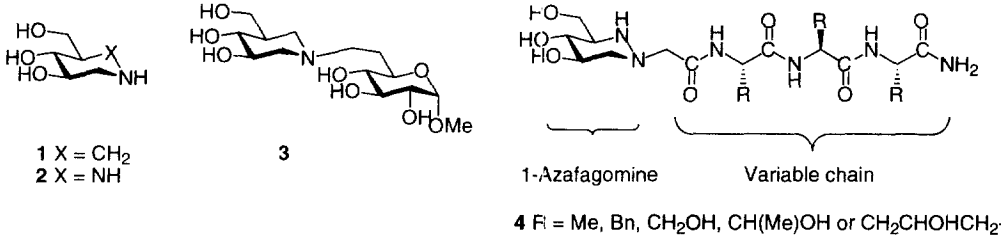

Fig 1. The proposed strategy for a 1-azasugar library.

aglycone (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.[4] Furthermore the L-stereoisomer is completely inactive.[5] Our target

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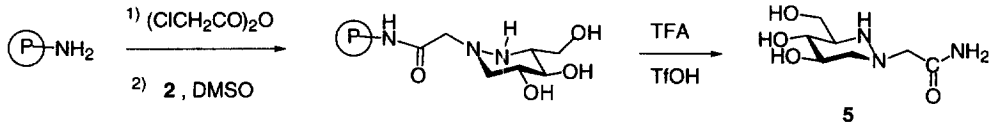
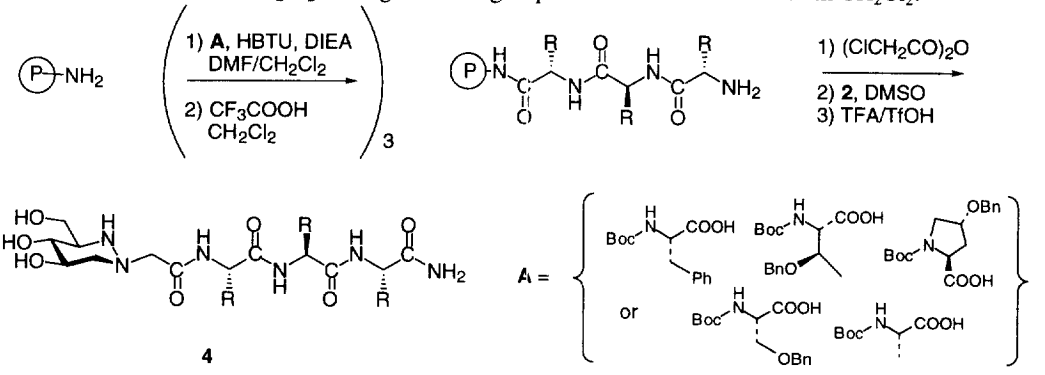
library is shown as **4** in Fig 1. It contains **2** linked through an acetic acid linker to a variable domain consisting of a tripeptide supposed to mimic the aglycone of the transition state.



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

Synthesis of **4** was planned to be carried out by solid phase synthesis using the split and mix method[1a,1b,1d] 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**. The chloroacetylation and nucleophilic substitution with **2** were studied in advance with the model experiment shown in Scheme 1. A 4-methyl benzhydrylamine resin (MBHA, P-NH_2) 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.[6] 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.

The synthesis was carried out as shown in Scheme 2. The tripeptide was synthesised in a 5 chamber reactor on a MBHA resin. Peptide couplings were carried out with the 5 amino acid derivatives shown using HBTU[7] and DIEA in DMF/CH_2Cl_2 as reagents. Boc groups were removed with TFA in CH_2Cl_2.



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

After three couplings the result was five sub-libraries of 25 peptides in each reactor, which were individually treated according to 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 %. 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.

Library	$\underline{K_i}$/μM	K_i/μM	Library	$\underline{K_i}$/μM	K_i/μM	Compound	$\underline{K_i}$/μM	K_i/μM
Aza-Ala-X-X	1225	25	Aza-Hyp-Ala-X	115	12	Aza-Hyp^2-Ala	212	106
Aza-Hyp-X-X	750	15	Aza-Hyp^2-X	80	8	Aza-Hyp^3 (**4a**)	40	20
Aza-Phe-X-X	>2500	>50	Aza-Hyp-Phe-X	360	36	Aza-Hyp^2-Phe	406	203
Aza-Ser-X-X	>2500	>50	Aza-Hyp-Ser-X	410	41	Aza-Hyp^2-Ser	308	154
Aza-Thr-X-X	>2500	>50	Aza-Hyp-Thr-X	270	27	Aza-Hyp^2-Thr	>400	>200

Table 1. Almond β-glucosidase inhibition constants for sublibraries. Aza means azafagomine (**2**), X is an unknown amino acid. $\underline{K_i}$ is the inhibition constant calculated from average molecular weight, and assuming average inhibition; K_i value assumes only one compound and stereoisomer in the library is an inhibitor.

The five sublibraries of **4**, each having a known N-terminal amino acid (of the peptide), were tested[4,6] for inhibition of almond β-glucosidase (Table 1, Fig 2). The sublibrary having hydroxyproline at the N-terminus contained the most potent inhibition of β-glucosidase 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 5 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, Fig 2). 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 20 μM. Characterisation of **4a** using ^{1}H NMR and FAB-MS 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.

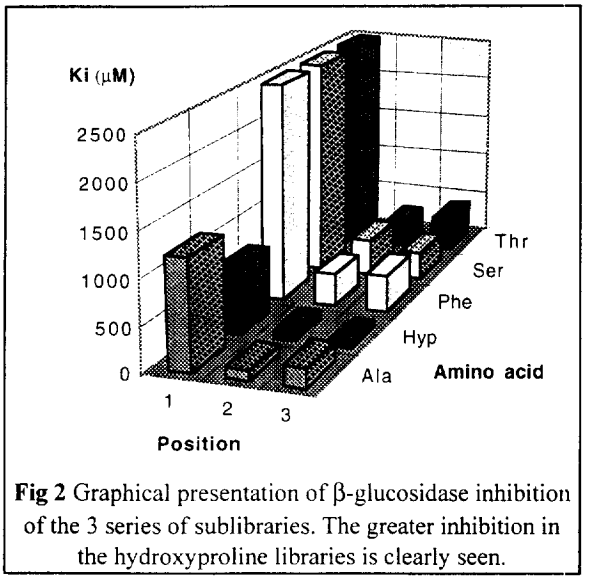


Fig 2 Graphical presentation of β-glucosidase inhibition of the 3 series of sublibraries. The greater inhibition in the hydroxyproline libraries is clearly seen.

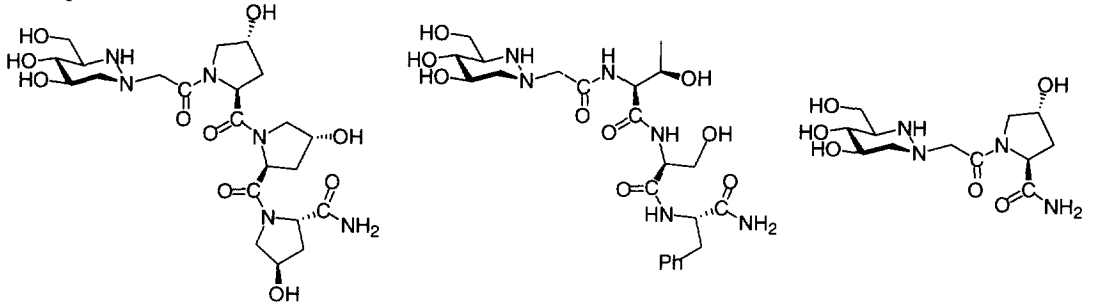
Thus the result showed that the most potent β-glucosidase inhibitor of the 125 compounds in the original library was **4a** (Fig 2). Interestingly **4a** is also the compound in the library expected to have the most rigid conformation, because of the cyclic amino acids.

The reason the K_i values for some sublibraries were lower than the K_i value for **4a** was because the K_i's 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 occured from other 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 (Fig 2). From the N-terminus these were threonine, serine and phenylalanine. The corresponding compound (**4b**, Fig 3) was synthesised and found to have a K_i of 251 μM. Thus the difference in inhibition by **4a** and **4b** was approximately 12 fold.

These results were compared with K_i values for (±)-**2**[4] and derivative **5** (Table 1). **4a** was a weaker β-glucosidase inhibitor than **2**. This might be interpreted as suggesting that the peptide moiety in general was

unfavorable, but compound **5** was found to be an extremely weak inhibitor (Ki >1000 μM), which suggests that it is the N-substitution that was 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 N-substitution.



4a K_i = 20 μM **4b** K_i = 251 μM **6** K_i > 1000 μM

Fig 3. The most potent inhibitor in the library and analogues.

To investigate further the effect of substitution on inhibition the derivative **6** was synthesised (Fig 3) using the general method outlined in Scheme 2. Surprisingly **6**, 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.

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 by mimicking an aglycone of the transition state, and that more selective inhibitors can be obtained.[8]

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