A (1→4)-"Trehazoloid" Glucosidase Inhibitor with Aglycon Selectivity

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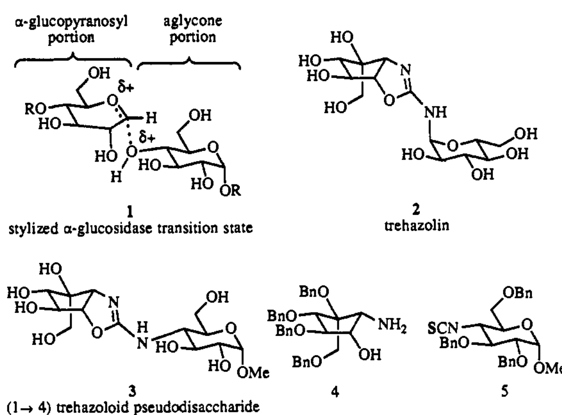
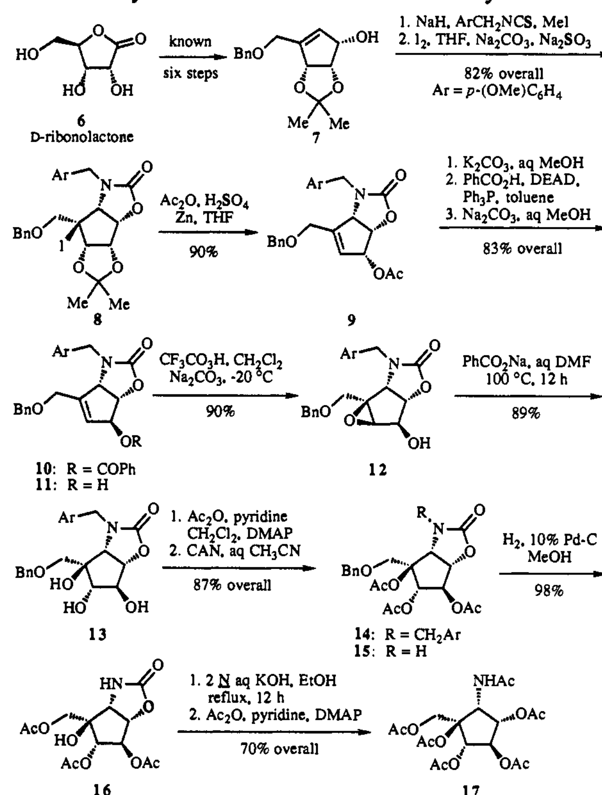
Mimics of the appropriate glycopyranosyl cation, or a transition state leading to it (see **1**), can function as glycosidase inhibitors by binding tightly in the enzyme active site.[1] The same active site also interacts with the *aglycon* portion of the natural substrate, as evidenced by the linkage specificity shown by many glycosidases.[2] This is particularly evident in glycoprotein biosynthesis and processing, where several glycosidases of a specific type may differ only with respect to their substrate aglycon and its mode of attachment.[3] Very recently, two crystal structures have appeared that show the binding of multisubunit inhibitors in the active sites of two completely different α-amylases. These show quite graphically that enzyme–aglycon interactions contribute to substrate binding in the active site, and also to distorting the shape of the bound substrate from its normal conformation.[4] Clearly, some of these interactions will also be present in the transition state for glycolysis. In principal, *effective and selective* inhibition of a particular glycosidase could be achieved by designing mimics of both portions of the natural substrate.[5] The structure of trehazolin (**2**), an α,α-trehalase inhibitor isolated from culture broth,[6] exemplifies this idea and also suggests that the analogous (1→4)-linked "trehazoloid" **3** might be an inhibitor of (1→4)-glucosidases. We report the synthesis of **3** and its effective competitive inhibition of yeast α-glucosidase and *Agrobacterium* β-glucosidase.



Scheme 1. Synthesis of the Trehazolin Aminocyclitol



Protected aminocyclitol **4** and sugar isothiocyanate **5** were targeted as logical component precursors to **3**; Scheme 1 shows the synthesis of the trehazolin aminocyclitol portion.[7,8] The allylic alcohol **7**, available from D-ribonolactone,[9] was converted to its carbonimidothioate derivative by condensation with *p*-methoxybenzyl isothiocyanate,[10] which in turn was cyclized with iodine[11] to afford upon quenching the iodo oxazolidinone **8**. This is a rare example of formal anti-Markovnikov iodo cyclization and may result from the kinetic preference for five-membered (rather than six-membered) and fused (rather than bridged) ring formation. One-pot treatment of **8** with acetic anhydride and sulfuric acid, followed by activated zinc, provided allylic acetate **9**, whose configuration was then inverted at C-2′ (trehazolin numbering) by the Mitsunobu procedure.[12] The resulting alcohol **11** was epoxidized to give **12** as the only stereoisomer detected, and then hydrolysis[13] of the epoxide at the less substituted position (C-1′) led to triol **13**. The cyclitol stereochemistry was proven by conversion of **13** to the aminocyclitol hexaacetate **17**, a known derivative first obtained by degradation of **2**.[6,7]

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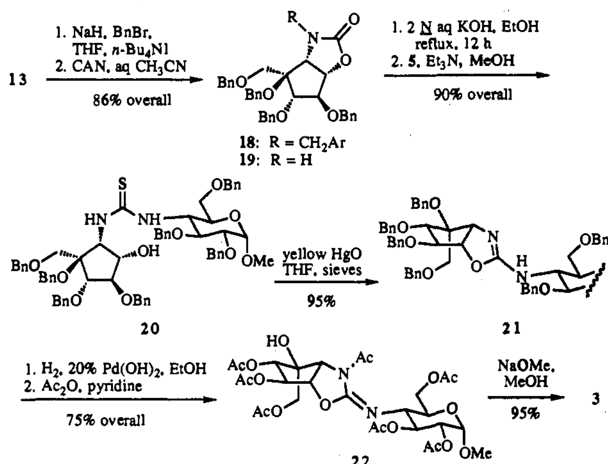
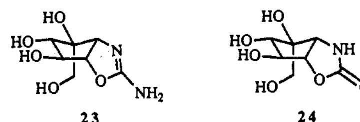
Scheme 2. Synthesis of the (1→4)-"Trehazoloid"



Conversion of **13** into a suitable coupling partner (Scheme 2) was achieved by O-benzylation, followed by oxidative removal of *N*-*p*-methoxybenzyl. Hydrolysis of the resulting oxazolidinone **19** gave amino alcohol **4**, which without isolation was coupled with sugar isothiocyanate **5**[14] to provide the thiourea **20**. Cyclization[8] of **20** to the isourea **21** was carried out with freshly prepared and dried yellow mercuric oxide, and the resulting oxazoline **21** was deprotected by hydrogenolysis. The pseudodisaccharide was isolated and characterized as its heptaacetate derivative **22** (which contains about 15% of its *exo* *N*-acetylisourea isomer[6a]) and then reconverted to **3** by treatment with sodium methoxide. The synthesis of **3** proceeds in about 16 steps (~26% overall yield) from **7**.

Trehazoloid **3** was found to be a highly effective competitive inhibitor of yeast α-glucosidase, with $K_i = 9.3\ \mu M$.[15] In contrast, **2** itself has previously been shown to bind poorly to yeast α-glucosidase and other α-glucosidases.[6,16] The pseudo-*gluco*-pyranosylamine portion of **2**, trehalamine (**23**),[16,17] bound about an order of magnitude more weakly ($K_i \approx 70\ \mu M$) than the pseudodisaccharide **3**, and the corresponding oxazolidinone[18] **24** scarcely bound ($K_i \approx 6$ mM). The enzyme, in keeping with its substrate specificity,[19] is therefore recognizing and deriving significant affinity from the presence and mode of linkage of the glucopyranose moiety of **3**.



Although its aglycon portion was designed to bind to an α-glucosidase, **3** nevertheless showed good competitive binding ($K_i = 48\ \mu M$) to *Agrobacterium* β-glucosidase. In comparison, **23** bound almost as well ($K_i \approx 90\ \mu M$), and **24** bound very weakly at $K_i \approx 700\ \mu M$. Trehazolin **2** exhibits poor inhibition of the kinetically similar[20] sweet almond β-glucosidase.[16] Thus the pseudo-*gluco*-pyranosylamine substructure of **3** (corresponding to **23**) accounts for much of the binding to *Agrobacterium* β-glucosidase, an enzyme with strong exoglucosidase action on cello-oligosaccharides, but the presence of the methyl α-glucopyranoside (rather than β) functionality in the aglycon portion may compromise the binding of **3** to some extent. For both the α- and β-glucosidase, the wrong mode of attachment of the aglycon portion (viz., **2**) is clearly detrimental, as is replacement of the interpyranosidic isourea nitrogen (viz., **24**).

Trehazoloid **3** is an example of a designed, linkage-spanning, glycosidase inhibitor with effective selectivity attributable to the aglycon portion, suggesting that enzyme inhibition may be generally tunable by this kind of structural modification. Work is underway to investigate the effect of other modes of linkage on glycosidase inhibitory action and selectivity.

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Supplementary Material Available: Experimental procedures and spectral data for the preparation of **3** and **17** from **7** (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(15) Kinetic studies on **3** with yeast α-glucosidase (type 3, Sigma Chemical Company, dialyzed prior to use) were performed at 37 °C by using *p*-nitrophenyl α-glucoside as substrate ($K_m = 0.18$ mM) in pH 6.8 buffer containing 50 mM sodium phosphate and 0.1% bovine serum albumin. Six substrate concentrations from 0.045 to 0.72 mM and five inhibitor concentrations ranging from 0 to 29 μM were employed. Kinetic studies with *Agrobacterium* β-glucosidase were performed at 37 °C by using *p*-nitrophenyl β-glucoside as substrate ($K_m = 0.08$ mM) in pH 7.0 buffer containing 50 mM sodium phosphate and 0.1% bovine serum albumin. Seven substrate concentrations from 0.02 to 0.06 mM and four inhibitor concentrations from 0 to 122 μM were employed. No time-dependent inactivation of either enzyme when incubated with inhibitor was observed over a 4-h period, indicating that inhibition is strictly reversible.

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(17) Trehalamine **23** was synthesized by KOH hydrolysis of **19**, followed by condensation with benzyl isothiocyanate as for **20** (85% overall), cyclization as for **21** (95%), and then hydrogenolysis as for **3** (90%). For previous synthesis, see refs 7a and 7c.

(18) The oxazolidinone **24** was prepared by catalytic hydrogenolysis of **19** over 10% Pd–C in ethanol solution (90% yield).

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