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A drug targeting motif for glycosidase inhibitors: an iminosugar–boronate shows unexpectedly selective β-galactosidase inhibition

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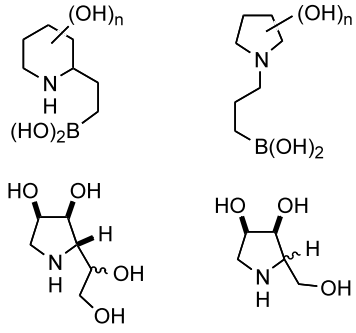
Abstract—Boronic acids were tethered to iminosugars in compounds such as **8** and **13** in order to increase their affinity for cell surfaces where glycoprotein processing enzymes are operative. Surprisingly, this modification diminished α-mannosidase inhibition while increasing β-galactosidase inhibitory activity (**8**: $K_i = 2.0\times10^{-4}$ M versus *E. coli* β-galactosidase). The presence of a boronate in **8** and **13** has a profound impact on the specificity of this inhibition.

The effect of glycosidase enzymes on the structure of cell-surface carbohydrates translates into important biological consequences for processes such as viral infection[1] and tumor metastasis.[2] This has attracted research to identify novel inhibitors of glycosidases as candidates for antitumor,[3,4] antiviral[5–8] and antidiabetic therapies.[9] There are a variety of known glycosidase inhibitors, many of which are naturally-occurring alkaloids that mimic the carbohydrate substrate.[10–13] In many cases, these iminosugars are believed to become protonated in the active site and thus serve as mimics of the electron-deficient transition state that must be involved in glycoside hydrolysis.[14] While some inhibitors are highly selective for a given glycosidase, the abundance of closely related enzymes within the body makes selective inhibition a challenge. Here, we report that attachment of a boronic acid to **1** converts the α-mannosidase inhibitor to a β-galactosidase inhibitor.

We are interested in targeting drugs to specific cell-surface carbohydrate structures as a means of increasing their selectivity and efficacy. Boronic acid derivatives of iminosugars would allow for reversible binding of glycoprotein carbohydrates based on the known affinity of boronates for *cis*-vicinal diols in mannose and galactose. This would target the inhibitor directly to the site of enzyme action for the glycoprotein trimming enzymes Mannosidase I and II (i.e. the boronate will mask the mannose substrate and present the inhibitor to the enzyme).

We are currently synthesizing both pyrrolidine (e.g. **1**–**4**) and piperidine based ring systems of the general structures shown in Fig. 1 to test this hypothesis. The first chemistry that we sought to develop was a means of installing boronic acids onto previously constructed iminosugar systems. The target compounds were envisaged to arise from *N*-allyl amines through hydroboration with excess borane followed by conversion to the boronic acid. Accordingly, the dimesylate **5** was synthesized by the procedure described by Fleet[15] and a



1: 5α-OH **3**: 4α-H
2: 5β-OH **4**: 4β-H

Figure 1. Generalized target structures and known glycosidase inhibitors.

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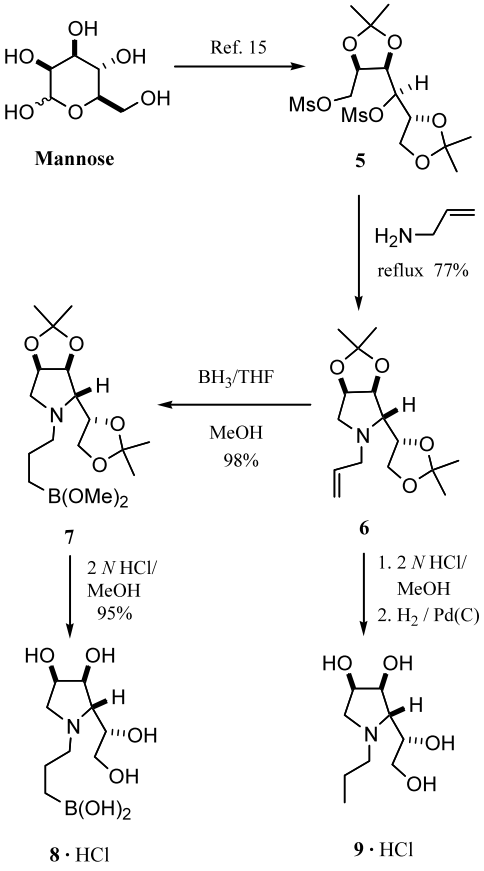
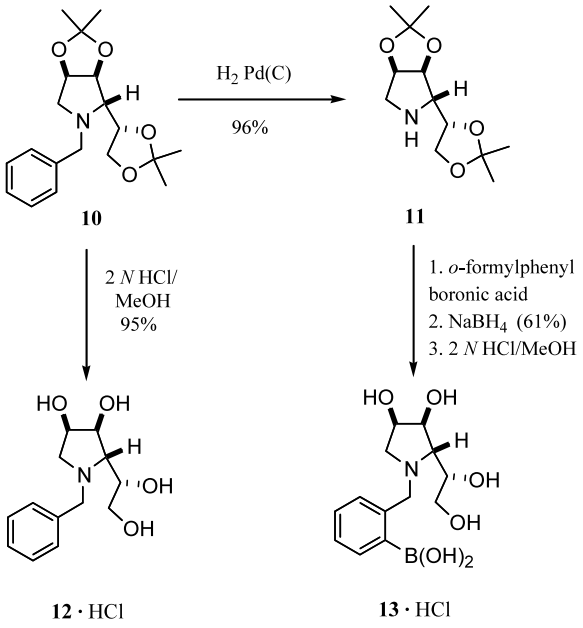
double displacement with allylamine provided **6** in excellent overall yield from mannose. This compound was converted to both the boronated target **8** and non-boronated compound **9** through two separate steps shown in Scheme 1. Use of a 20-fold excess of borane ensures the formation of a monoboronate ester upon methanol quench. The unreacted borane is converted to trimethylborate that can be removed in vacuo. Compound **6** was also converted to *N*-allyl-1,4-dideoxy-1,4-imino-D-talitol by reaction with methanolic HCl and the olefin was reduced through catalytic hydrogenation to yield **9** (94% for two steps).

The phenyl derivatives **12**[15] and **13** were synthesized according to Scheme 2. The known benzylamine **10**[15] was again synthesized from **5** by double displacement of the dimesylate. Acetonide deprotection of **10** furnished compound **12** while benzyl deprotection provided the secondary amine **11**. This allowed the phenylboronate to be installed through reductive amination to yield the final analog **13** (58% for three steps).[16] As with compounds **8** and **9**, the aromatic iminosugars **12** and **13** differ only in the substitution of a hydrogen for a boronic acid, thus offering control compounds to study the effect of boronate substitution on enzyme inhibition.

Each compound was isolated as its hydrochloride salt and was tested for inhibition of three common glycosidases (Table 1). The boronic acids **8** and **13** displayed



Scheme 1. Synthesis of inhibitors **8** and **9**.



Scheme 2. Synthesis of inhibitors **12** and **13**.

very little inhibition (ca. 5–10%) of α-mannosidase (Jack bean) and actually gave a slight increase in α-galactosidase (green coffee bean) activity at 1.0 mM concentration. Remarkably, both compounds were much more effective at inhibiting β-galactosidase (*E. coli*) than their non-boronated counterparts **9** and **12**. An electrophilic boron species alone, in the form of boric acid, is not sufficient to inhibit the enzyme.

Fleet has shown that the 1,4-iminotalitol compound **1** lacking *N*-alkyl substitution is a specific and competitive inhibitor of human liver α-mannosidase.[15] A closely related class of compounds based on the 5-OH epimer **2** shows alternating α-mannosidase or α-fucosidase inhibition depending on the nature of the *N*-alkyl group.[17] The structurally reduced lyxitol compound **3** is also epimeric to the talitol stereochemistry at the bridgehead. It is a potent inhibitor of α-galactosidase while inhibiting β-galactosidase (*A. niger*) only at threefold higher concentration.[18] Huber and Gaunt discovered that L-ribose inhibits β-galactosidase from *E. coli* more effectively than its enantiomer.[19] The talitol compounds here match the stereochemical disposition of L-ribofuranose and might bind in a similar manner such that it mimics distorted transitions of D-galactopyranose found on the reaction coordinate.

In the present case, it is not clear exactly how the addition of a boronic acid imparts selective inhibitory activity in this series. It is possible that the boron is binding to an active site nucleophile in the aglycone cavity since boronates make up a powerful class of enzyme inhibitors, particularly versus serine proteases.[20–22] Inspection of the three-dimensional structure of the *E. coli* β-galactosidase reveals that there are residues outside of the catalytic carboxylates that might act as nucleophiles toward the boron center, most importantly Met-502.[23] The sulfur of this highly-conserved amino acid has been alkylated with an

Table 1. Glycosidase inhibition at 1.0 mM inhibitor concentration

Compds	*E. coli* β-galactosidase	Coffee bean α-galactosidase	Jack bean α-mannosidase
8	75–86	(10)	<5
9	13	NI	10
12	NI	<5	NI
13	45–55	(<5)	10
Boric acid	NI	NT	NT

Values are expressed as a percentage of activity lost (gained) in the presence of inhibitor. Results are from three independent determinations where values fall within 5% range unless a range is noted. UV assays were performed at the enzyme's optimal pH with requisite *p*-nitrophenylglycoside substrates (NI, no inhibition; NT, not tested).

anomeric diazomethane derivative of galactose,[24] and is believed to participate as a nucleophile during carbohydrate hydrolysis in the absence of Glu-461.[25] The electrophilic site of the *C*-galactosyl affinity label used by Sinnott[24] is roughly similar to the position of the boronate in **8** and **13**. Alternatively, the boronate might form a cyclic ester with the C-5/C-6 vicinal diol to create the active species.[26] Nonetheless, the specificity change that the boronic acid imparts on **1**, a selective mannosidase inhibitor ($K_i = 1.2\times10^{-4}$ M versus human liver lysosomal α-mannosidase), is dramatic (**8**: $K_i = 2.0\times10^{-4}$ M versus β-galactosidase) and should prove an effective way to enhance other, more potent β-galactosidase inhibitors.

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26. The ^{11}B NMR spectrum of **8** in D_2O is simple at pD 2.0, but becomes complex above pD 7.0 indicative of free amine-catalyzed esterification of the boronate. The hydrochloride salts **8**, **9**, **12** and **13** were collected by filtration and deemed of sufficient purity (>95% by ^{1}H NMR) for use in the initial enzyme assays. Compound **8** was purified by precipitation with HCl in Et_2O for determining its K_i value versus β-galactosidase. ***N*-(Propyl-3-**

boronate)-1,4-dideoxy-1,4-imino-D-talitol HCl (8): ^{1}H NMR (500 MHz, pD 2.0 D_2O): δ 0.82 (2H, t), 1.81 (2H, m), 3.36 (1H, m), 3.39 (1H, m), 3.49 (1H, m), 3.56 (1H, m), 3.70 (1H, m), 3.72 (1H, m), 3.80 (1H, m), 3.96 (1H, m), 4.25 (1H, m), 4.41 (1H, m); ^{13}C NMR (125 MHz, pD 2.0 D_2O): δ 11.4, 20.18, 50.34, 56.34, 63.34, 68.34, 70.00, 73.00, 73.34; ^{11}B NMR (160 MHz, pD 2.0 D_2O): δ 18.65 (boric acid); HRMS (ESI+) m/z 250.1447 [(M+H$^+$) calcd for $C_9H_{21}BNO_6$ 250.1462]. ***N*-Allyl-1,4-dideoxy-1,4-imino-D-talitol HCl**: ^{1}H NMR (300 MHz, pD 3.0 D_2O): δ 3.02 (1H, dd, J=3.3, 12.9 Hz), 3.20 (2H, m), 3.30 (1H, dd, J=4.8, 12.3 Hz), 3.39 (1H, AB d, J=3.3 and 11.7 Hz), 3.53 (2H, m), 3.73 (1H, dd, J=6.3, 12.9), 3.88 (1H, dd, J=4.1, 6.9 Hz), 3.99 (1H, app. q, J=3.9, 8.1 Hz), 5.18 (2H, m), 5.53 (1H, m); ^{13}C NMR (75 MHz, pD 3.0 D_2O): δ 55.37, 62.12, 62.73, 69.61, 69.74, 71.64, 72.90, 126.45, 126.89; HRMS (ESI+) m/z 204.1236 [(M+H$^+$) calcd for $C_9H_{18}NO_4$ 204.1258]. ***N*-Propyl-1,4-dideoxy-1,4-imino-D-talitol HCl (9)**: ^{1}H NMR (300 MHz, pD 3.0 D_2O): δ 0.53 (3H, t), 1.33 (2H, m), 2.98 (3H, m), 3.15 (1H, t, J=6.4 Hz), 3.34 (3H, m), 3.55 (1H, m), 3.84 (1H, dd, J=4.1 and 5.8 Hz), 3.99 (1H, app. q, J=4.1, 8.8 Hz). Like **9**, the allyl talitol derivative does not show significant inhibition of β-galactosidase at 1.0 mM concentration [<10%].