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# Synthesis of a Benzylamidine Derived from D-Mannose. A Potent Mannosidase Inhibitor

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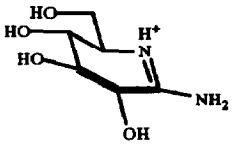
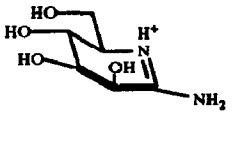
**Abstract**: The synthesis of a substituted mannopyranose-based amidine is described and its potential as glycosidase inhibitor evaluated. This new aminosugar derivative acts as a potent glycosidase inhibitor by virtue of its charge and shape similarities to the mannopyranosyl cation. The benzyl group of this pseudodisaccharide may also contribute to enzyme transition-state interactions.

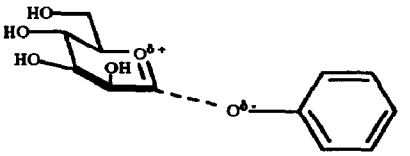
There is an increasing interest in the isolation and synthesis of glycosidase inhibitors due to their potential as chemotherapeutic agents[1,2]. Furthermore, they may constitute useful tools to unravel the catalytic mechanism of the corresponding enzymes[3,4]. The design of effective enzyme inhibitors generally relies on the mechanism of the enzyme catalyzed reaction. The enzymatic glycosidase mechanism is thought to involve a transient oxocarbonium with a flattened chair conformation stabilized by an active site catalytic residue with a complementary charge, identified as a carboxylate in most glycosidases[4]. Recently, amidine derivatives of sugars, **1** and **2** respectively, whose structure, shape and charge closely resemble the transient glycosyl cation have been proved to be potent and broad spectrum inhibitors of glycosidases[5,6]. However, less consideration has been given to mimick the aglycon part of the glycoside which plays an important role in the interaction of the inhibitor with the glycosidase[7]. Here, we report the synthesis of a benzylamidine **4** derived from D-mannose which contains features (the phenyl ring in this case) capable of mimicking the rate-determining transition state **3** for a mannosidase catalyzed hydrolysis. A phenyl aglycone part was chosen because phenylglycosides are often accepted as substrates by glycosidases. The incorporation of a methylene group between the phenyl and the amidine is supposed to mimick the stretching of the glycosidic bond that occurs along the glycosidic bond cleavage process[8]. This atom insertion could match the longer interatomic distance in the transition state[9].



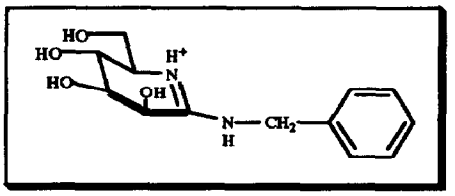
**1**



**2**

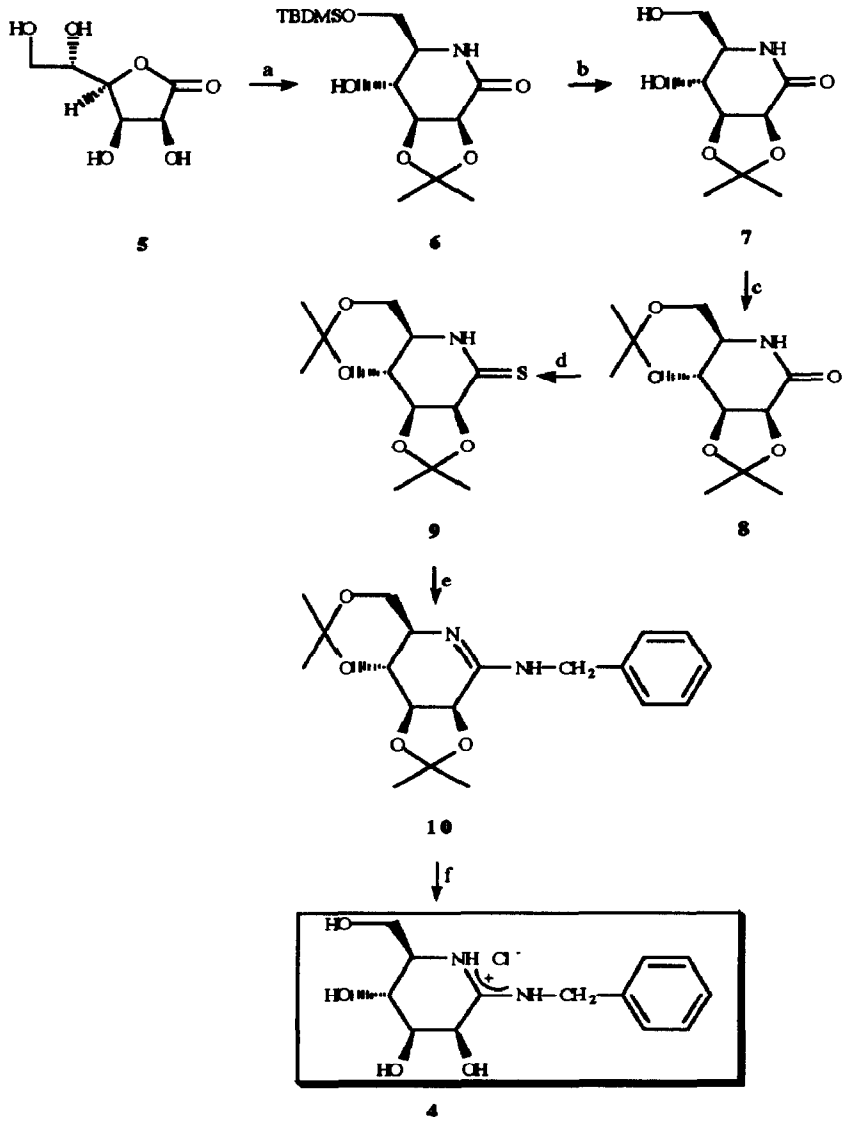


**3** Rate determining transition state



**4**

The synthesis has been achieved in 11 steps starting from commercially available L-gulonic acid lactone **5** which was transformed in six steps into a partially protected D-mannono-δ-lactam **6**[10]. Selective deprotection of the TBDMS group using aqueous acetic acid yielded the 2,3-O-isopropylidene-D-mannono-δ-lactam **7** which




**Scheme**: a) Ref.10; b) THF/$H_2O$/AcOH (1/1/3), 12h, 96%; c) 2,2 dimethoxypropane, APTS, dry acetone, 48 h, 70%; d) Lawesson's reagent (0.6 eq.), dry pyridine (3 eq.), dry benzene, reflux, 30 min., 77%; e) dry benzylamine (1.1 eq.), dry $CH_2Cl_2$, 48h, 68%; f) HCl/MeOH, 53%.

was then fully protected using an acetonide protective group to afford the 2,3:4,6-di-O-isopropylidene-D-mannono-δ-lactam **8**. Subsequent thionation of the lactam using Lawesson's reagent[11] under basic conditions yielded the fully protected 2,3:4,6-di-O-isopropylidene-D-mannono-δ-thionolactam **9** which was purified by flash column chromatography (ethyl acetate/petroleum ether/pyridine 5:15:2, 77%). The next step involved the reaction of this thiolactam with benzylamine in anhydrous dichloromethane under nitrogen for 48 h to afford the protected amidine **10**[12]. The last step consisted in the deprotection of the amidine to yield the target molecule **4** as an amidinium salt[13]. Amidine **4** is fully protonated[14] and stable for days at room temperature in neutral aqueous solution.

The effect of **4** on various glycosidases was next examined. Inhibition studies were performed under steady-state conditions with 5 inhibitor concentrations on different glycosidases at their optimum pH. Competitive inhibition has been observed for all the enzymes tested. Kinetic measurements on the jack bean α-mannosidase (p-nitrophenyl-α-D-mannopyranoside as substrate, pH 4.5, 30°C, $K_m$ = 2.5 mM) indicated a value of $K_i$ = 550 nM using non linear regression analyses[15]. Strong inhibition of **4** (Ki = 6 μM) was also observed on the β-mannosidase from *Achatina achatina* snail[16] (p-nitrophenyl-β-D-mannopyranoside as substrate, pH , 30°C, $K_m$ = 2.9 mM). Kinetic measurements on sweet almond β-glucosidase (p-nitrophenyl-β-D-glucopyranoside as substrate, pH 5.6, 30°C, $K_m$ = 2.5 mM) gave a value of $K_i$ = 25 μM. Finally kinetic measurements on *Aspergillus oryzae* β-galactosidase (p-nitrophenyl-β-D-galactopyranoside as substrate, pH 4.5, 30°C, $K_m$ = 1mM) indicated no inhibition at an inhibitor concentration of 0.4 mM. This new substituted mannoamidine is a more potent inhibitor of mannosidases than the corresponding piperidine analogue (deoxymannojirimycin)[17]. This may be attributed to its half-chair conformation which better mimicks the transition-state involved in glycosidase mechanism. Amidine **4** also showed a broad spectrum of inhibition against α and β mannosidases like the previous reported amidines. It has been suggested[18] that the lack of stereochemical discrimination observed for half-chair like inhibitors could be attributed to the overriding electrostatic interaction between the enzyme carboxylate groups and the positive charge of the flattened chair of the inhibitor. The incorporation of a hydrophobic group in the aglycon moiety of **4** did not significantly improve the binding of the inhibitor to jack bean α-mannosidase compared to the mannoamidine **2**[5]. However, a narrower specificity was observed with **4** compared to **1** and **2**. Almost no inhibition was observed on *Aspergillus oryzae* and *E. coli* β-galactosidases and the binding constant was reduced 50 fold for β-glucosidase. This suggests that the enzyme-aglycone interaction might contribute to the stereoselectivity of this binding.

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12. **10**: Rf = 0.75 (ethyl acetate/petroleum ether/pyridine = 5/15/2); $^1$H NMR (250 MHz, $CDCl_3$/TMS): δ 1.54 (3H, s, $CH_3$), 1.46 (3H, s, $CH_3$), 1.38 (6H, s, $CH_3$), 3.09 (1H, ddd, J = 5.5Hz, 9.9Hz and 10.6Hz), 3.56 (1H, dd, J = 6.6Hz and 9.9Hz), 3.79 (1H, dd, J = 11.7Hz and 10.6Hz), 4.22 (1H, dd, J = 5.5Hz and 11.7Hz), 4.44 (2H, s, $CH_2N$), 4.46 (1H, dd, J = 6.6Hz and 8.4Hz), 4.59 (1H, d, J = 8.4Hz), 7.22-7.44 (5H, m, $C_6H_5$). $^{13}$C NMR (250 MHz, $CDCl_3$): δ 19.26, 25.07, 26.92, 29.50, 45.04, 50.61, 65.82, 71.19, 74.42, 77.29, 99.21, 111.43, 127.44, 127.88, 128.65, 138.52, 159.73. MS (EI, 70 eV): *m/z* $M^+$= 347, (CI, $CH_4$): *m/z* $M+H^+$= 348.
13. **4**: Rf = 0.7 ($CH_3CN/H_2O$/AcOH = 20/4/1); $^1$H NMR (250 MHz, $D_2O$/acetone): δ 3.42 (1H, m), 3.67 (1H, dd, J = 6.1Hz and 11.8 Hz), 3.82 (1H, dd, J = 4.2Hz and 11.8Hz), 3.90 (1H, dd, J = 5.2Hz and 7.5Hz), 4.12 (1H, dd, J = 3.5Hz and 5.2Hz), 4.61 (2H, s, $CH_2N$), 4.76 (1H, d, J = 3.5Hz), 7.28-7.44 (5H, m, $C_6H_5$). δ $^{13}$C NMR (250 MHz, $D_2O$/acetone): δ 44.82, 58.04, 60.13, 65.64, 67.67, 71.61, 126.87, 127.97, 128.70, 133.59, 164.13. MS (EI, 30 eV): **4** derivatized with 4 TMS on hydroxyl groups *m/z* $M^+$= 554.
14. $^1$H NMR pH titration indicated that amidine **4** starts to decompose at pH = 9.
15. The non linear regression analyses have been performed using the "Enzyme Kinetics" program, Trinity Software.
16. We thank Pr. B. Colas for having kindly provided a sample of β-mannosidase from *Achatina achatina* snail.
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