

# Novel, Lipophilic Derivatives of 2,5-Dideoxy-2,5-imino-D-mannitol (DMDP) Are Powerful $\beta$ -Glucosidase Inhibitors<sup>†</sup>

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**Abstract**—Novel derivatives of the D-glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol bearing lipophilic aliphatic or aromatic amides attached to C-1 have been found to inhibit  $\beta$ -glucosidase from *Agrobacterium* sp. in the nanomolar range. One of them, a coumarin derivative, ranks amongst the most active compounds in the class of reversible glycosidase inhibitors of the iminoalditol type. © 2001 Elsevier Science Ltd. All rights reserved.

The iminoalditol 2,5-dideoxy-2,5-imino-D-mannitol (**1**), a natural product,<sup>1</sup> is a potent reversible inhibitor of D-glucosidases and invertase rivaling the activity of 1-deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol, **2**), the paradigmatic D-glucosidase inhibitor in the class of compounds under consideration (Fig. 1).<sup>2</sup>

This fact was attributed to its  $C_2$ -axis of symmetry and the relatively flat five-membered ring which was assumed to be more closely mimicking the putative transition state of enzymatic glycoside hydrolysis (Scheme 1).

Recently, we have gained access to the 1-aminodeoxy derivative of compound **1** taking advantage of the Amadori rearrangement reaction of 5-azidodeoxy-D-glucofuranose (**3**) with benzyl amine to give **4** followed by catalytic hydrogenation providing intramolecular reductive amination and concomitant nitrogen deprotection in 75% yield from **3** to compound **5**.<sup>3,4</sup>

C-1 modified derivatives, designed for immobilisation studies,<sup>3</sup> were also synthesised and found to exhibit inhibitory properties comparable to or even better than the ones of parent compound **1**.<sup>4</sup>

To gain a better understanding for the structure–activity relationships applying in this family of compounds, we have now extended the range of derivatives by some

examples substituted with markedly lipophilic side chains. Synthetic approaches were based on the coupling of carboxylic chlorides directly to N-1 of the inhibitor **5**.<sup>5</sup> Alternatively, the carboxylic acid was employed in the presence of HBTU as the coupling reagent for the synthesis of compound **10** (80%).

In all cases reported, the primary amine reacted highly chemo- and regioselectively in the presence of the unprotected ring nitrogen of the iminoalditol giving practically exclusively the desired 1-N-acylated or -sulfonylated products **6–10**.<sup>6</sup>

Gratifyingly, strong inhibition in the nanomolar range was observed with all new compounds.<sup>7</sup>

The inhibitory power of coumarin derivative **10** was found to be two orders of magnitude better than the one of parent compound **1** ( $K_i$  200 nM) (Schemes 2 and 3).

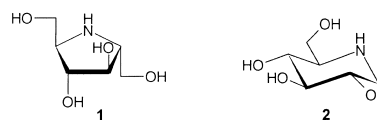
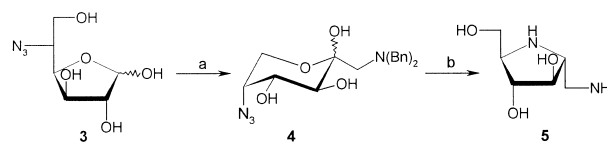


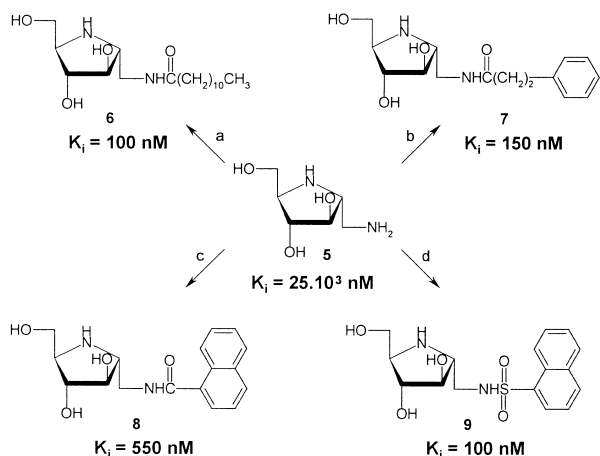
Figure 1.



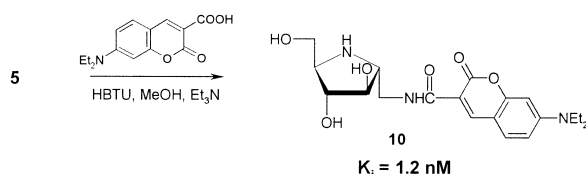
Scheme 1. (a) HN(Bn)<sub>2</sub>, HOAc, MeOH; (b) H<sub>2</sub>, Pd/C (10%), MeOH.

<sup>†</sup>Dedicated to Prof. Dr. Peter Köll on the happy occasion of his 60th birthday.

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**Scheme 2.** (a)  $[\text{CH}_3(\text{CH}_2)_{10}\text{CO}]_2\text{O}$ , MeOH, 67%; (b)  $\text{Ph}(\text{CH}_2)_2\text{COCl}$ , MeOH, 53%; (c)  $\text{C}_{10}\text{H}_7\text{COCl}$ , MeOH, 50%; (d)  $\text{C}_{10}\text{H}_7\text{SO}_2\text{Cl}$ , MeOH, 54%.



**Scheme 3.**

Thus, compound **10** ranks amongst the most potent representatives<sup>2,8</sup> of this type of  $\beta$ -glucosidase inhibitor known thus far.

Clearly, additional investigations will be necessary to understand this dramatic increase in inhibitory potency by the extended aromatic system.

In conclusion, the compounds reported here represent a novel type of reversible iminoalditol based glycosidase inhibitor. In particular, compound **10** features excellent inhibitory properties suitable for exploitation in a wide range of applications in glycobiology and glycotechnology.

### Acknowledgements

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### References and Notes

1. Welter, A.; Jadot, J.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, 15, 747.

2. *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; VCH-Wiley: Weinheim, 1999, and references cited therein.

3. Wrodnigg, T. M.; Stütz, A. E.; Withers, S. G. *Tetrahedron Lett.* **1997**, 38, 5463.

4. Wrodnigg, T. M.; Gaderbauer, W.; Greimel, P.; Häusler, H.; Sprenger, F. K. (Fitz); Stütz, A. E.; Virgona, C.; Withers, S. G. *J. Carbohydr. Chem.* **2000**, 19, 975.

5. General procedure for compounds **6–9**: To a 1% methanolic solution of **5**, 1 equiv of the respective acyl or sulfonyl chloride was added and the mixture was kept at ambient temp (TLC:  $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$  concn 300:100:1). Concentration under reduced pressure and chromatography (silica gel Merck 60) gave the desired products.

Compound **10**: To a 1% methanolic solution of **5**, 1 equiv each of 7-dimethylaminocoumarin-3-carboxylic acid, HBTU [*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate], and  $\text{NEt}_3$  were added at 0°C. After 2 h at ambient temp, DMF was added until the precipitate had re-dissolved. Concentration under reduced pressure followed by chromatography yielded compound **10**.

6. Selected data: Compound **6**:  $[\alpha]_D^{20}$  19.9 (*c* 1.0, MeOH);  $^{13}\text{C}$  NMR (50.9 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  176.4, 78.4, 76.8, 64.0, 62.4, 60.3, 40.5, 36.5, 35.8, 31.9, 29.6, 29.5, 29.3, 29.2, 26.0, 25.7, 22.5, 13.3.

Compound **7**:  $[\alpha]_D^{20}$  27.8 (*c* 1.5, MeOH);  $^{13}\text{C}$  NMR (50.9 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.9, 77.4, 76.0, 64.4, 63.1, 59.4, 39.8, 37.5, 31.4.

Compound **8**:  $[\alpha]_D^{20}$  28.0 (*c* 2.0, MeOH);  $^{13}\text{C}$  NMR (50.9 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  171.9, 80.0, 78.1, 63.7, 61.8, 42.5.

Compound **9**:  $[\alpha]_D^{20}$  24.9 (*c* 2.7, MeOH);  $^{13}\text{C}$  NMR (50.9 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  79.5, 78.2, 63.4, 62.2, 61.6, 45.1.

Compound **10**:  $[\alpha]_D^{20}$  32.4 (*c* 0.7, MeOH);  $^{13}\text{C}$  NMR (50.9 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  164.7, 80.0, 78.0, 63.4, 61.8, 61.4, 44.8 (2C), 42.2, 11.5 (2C).

Signals of aromatic systems appear in the expected regions and are not listed explicitly.

7. *Agrobacterium* sp.  $\beta$ -glucosidase was purified and assayed as described (Prade, H.; Mackenzie, L. F.; Withers, S. G. *Carbohydr. Res.* **1998**, 305, 371). Kinetic studies were performed at 37°C in pH 7.0 sodium phosphate buffer (50 mM) containing 0.1% bovine serum albumin, using  $7.2 \times 10^{-5}$  mg/mL enzyme. Approximate values of  $K_i$  were determined using a fixed concentration of substrate, 4-nitrophenyl  $\beta$ -D-glucopyranoside (0.11 mM =  $1.5 \times K_m$ ) and inhibitor concentrations ranging from 0.2 to 5 times the  $K_i$  value ultimately determined. A horizontal line drawn through  $1/V_{\max}$  in a Dixon plot of this data ( $1/v$  vs  $[S]$ ) intersects the experimental line at an inhibitor concentration equal to  $K_i$ . Full  $K_i$  determinations were performed using the same range of inhibitor concentrations while also varying substrate (4-nitrophenyl glucoside) concentrations from approximately 0.015 to 0.6 mM. Data were analysed by direct fit to the Michaelis–Menten equation describing reaction in the presence of inhibitors using the program GraFit (Leatherbarrow, R. J. GraFit Version 3.0, Erithacus Software, Staines, UK, 1992).

8. For other, related types of highly potent  $\beta$ -glucosidase inhibitors see: Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem.* **1994**, 106, 1858; *Angew. Chem., Int. Ed. Engl.* **1994**, 33, 1778; Legler, G.; Finken, M.-T.; Felsch, S. *Carbohydr. Res.* **1996**, 292, 91; Panday, N.; Canac, Y.; Vasella, A. *Helv. Chim. Acta* **2000**, 83, 58.