ELSEVIER

Carbohydrate Research 272 (1995) C11–C13

CARBOHYDRATE RESEARCH

Preliminary communication

N-Amidino-3,4,5-trihydroxypiperidine, a new efficient competitive β-glucosidase inhibitor

Jochen Lehmann *, Beatrice Rob

Institut für Organische Chemie und Biochemie, Universität Freiburg, Albertstr. 21, 79104 Freiburg, Germany

Received 17 March 1995; accepted 13 April 1995

Keywords: Glycosidases; Competitive inhibition; *N*-Amidinopiperidine

The guanidinium ion as an integral part of hydroxylated six-membered ring systems has been used to lend conformational rigidity, as well as a stable positive charge, to so-called transition state analogues resembling glycosyl cations [1]. In all cases described so far, the expected very low inhibition constants of < 10 μM were not reached. This disappointing result was, besides the missing "2-hydroxy group", in part attributed to the delocalised positive charge extending into an area which normally remains uncharged and to a shortening of the ring along the 1,4-axis [2]. These apparently unfavourable structural elements can be overcome by placing the guanidine "outside" the ring.

Guanidino monosaccharides have been prepared and their potential for binding to proteins discussed [3]. Extremely high inhibition of influenza sialidase [4] or proteases [5] was observed with guanidino derivatives.

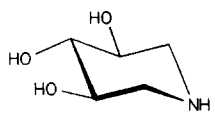
Led by the arguments explaining the weak inhibitory capacity of cyclic guanidinium systems and knowing that lactones, lactams, piperidines, and especially amidines can be very strong inhibitors [6], we prepared and studied a guanidine where one nitrogen is part of the ring skeleton.

Synthesis started from the known 1,5-dideoxy-1,5-iminoxylitol (**1**) [7], prepared from 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose [8] by catalytic reductive amination in hydrochloric acid using Pt/H_2. Subsequent treatment of compound **1** with formamidinesulfonic acid [9] in dry DMF in the presence of NEt_3 afforded *meso-xylo*-3,4,5-trihydroxypiperidine-1-carboxamidine (*meso-N*-amidino-*xylo*-3,4,5-trihydroxypiperidine) (**2**). Purification by ion-exchange chromatography (Dowex 50W-X8;2 M

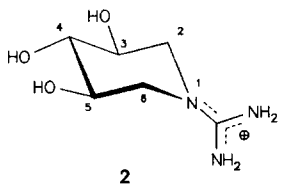
* Corresponding author.

Elsevier Science Ltd.
SSDI 0008-6215(95)00194-8

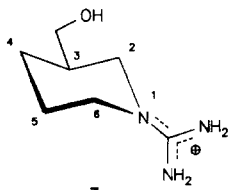
HCl) and crystallisation from MeOH yielded pure **2** as the hydrochloride (50%); mp 212°C (dec); R_f 0.20 (2:2:1 EtOAc–MeOH–25% NH_3, saturated with NH_4OAc); ^1H NMR (D_2O): δ 3.86 (ddd, 2 H, $J_{2e,2a} = J_{6e,6a} = 13.5$, $J_{2e,3} = J_{6e,5} = 4.5$, $J_{2e,4} = J_{6e,4} = 2.25$ Hz, H-2*e*, H-6*e*) 3.62 (ddd, 2 H, $J_{3,2a} = J_{5,6a} = 10.2$, $J_{3,4} = J_{5,4} = 8.7$ Hz, H-3, H-5), 3.44 (t, 1 H, H-4), 3.04 (dd, 2 H, H-2*a*, H-6*a*). Anal. Calcd for $C_6H_{14}ClN_3O_3$: C, 34.05; H, 6.67; N, 19.86. Found: C, 33.89; H, 6.77; N, 19.73.



1



2



3

Kinetic measurements with **2** on almond β-D-glucosidase using *p*-nitrophenyl β-D-glucopyranoside as substrate (K_M 227 μM) in Na–K–phosphate buffer (100 mM, pH 6.80, 30°C) indicated strong competitive inhibition with K_i 2.5 μM. The inhibition of yeast α-D-glucosidase was of the mixed type, uncompetitive inhibition was observed with jack bean α-D-mannosidase (both in the mmolar range). 1,5-Dideoxy-1,5-iminoxylitol (**1**) itself was shown by Ganem et al. [7] competitively to inhibit almond β-D-glucosidase with K_i of 430 μM. This clearly indicates the superiority of the guanidinium group by a factor well over 100. Since (*R*)-3-(hydroxymethyl)piperidine-1-carboxamidine [1] (**3**) does not show any inhibitory effect towards yeast α-D-glucosidase and jack bean α-D-mannosidase and only mixed competitive inhibition towards emulsin β-D-glucosidase in the mmolar range, the importance of hydroxy groups, especially in positions 4 and 5, for a "correct" binding adjustment of the ligand and the proper alignment of the positive charge is underlined.

Acknowledgments

We thank Dr K. Hilpert (Hoffmann-La Roche, Basel) for a gift of (*R*)-3-(hydroxymethyl)piperidine-1-carboxamidine and the Deutsche Forschungsgemeinschaft for financial support.

References

[1] J. Lehmann and B. Rob, *Liebigs Ann. Chem.*, (1994) 805–809; J. Lehmann and B. Rob, *Tetrahedron Asym.*, 5 (1994) 2255–2260; C.H. Fotsch and C.-H. Wong, *Tetrahedron Lett.*, 35 (1994) 3481–3484.

[2] J. Lehmann, B. Rob, and H.-A. Wagenknecht, *Carbohydr. Res.*, submitted for publication.

[1] A gift from Dr. K. Hilpert (Hoffmann-La Roche, Basel).

[3] H.P. Wessel, *J. Carbohydr. Chem.*, 12 (1993) 1173–1186; J. Yoshimura, T. Sekiya, and Y. Ogura, *Bull. Chem. Soc. Jpn.*, 47 (1974) 1219–1223.
[4] M. von Itzstein, W.-Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. Phan, M.L. Smythe, H.F. White, S.W. Oliver, P.M. Colman, J.N. Varghese, M. Ryan, J.M. Woods, R.C. Bethell, V.J. Hotham, J.M. Cameron, and C.R. Penn, *Nature (London)*, 363 (1993) 418–423.
[5] K. Hilpert, J. Ackerman, D.W. Banner, A. Gast, K. Gubernator, P. Hadváry, L. Labler, K. Müller, G. Schmid, T.B. Tschopp, and H. van de Waterbeemd, *J. Med. Chem.*, 37 (1994) 3889–3901.
[6] G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 319–384; T.M. Jespersen, W. Dong, M.R. Sierks, T. Skydstrup, I. Lundt, and M. Bols, *Angew. Chem. Int. Ed. Engl.*, 33 (1994) 1778–1779; M. Ichikawa, Y. Igarashi, and Y. Ichikawa, *Tetrahedron Lett.*, 36 (1995) 1767–1770.
[7] R.C. Bernotas, G. Papandreou, J. Urbach, and B. Ganem, *Tetrahedron Lett.*, 31 (1990) 3393–3396.
[8] W.A. Szarek and J.K.N. Jones, *Can. J. Chem.*, 43 (1965) 2345–2356.
[9] K. Kim, Y.-T. Lin, and H.S. Mosher, *Tetrahedron Lett.*, 29 (1988) 3183–3186.