JOC*Note*

Pseudoamide-Type Pyrrolidine and Pyrrolizidine Glycomimetics and Their Inhibitory Activities against Glycosidases

M. Isabel García-Moreno,† David Rodríguez-Lucena,† Carmen Ortiz Mellet,*,† and José M. García Fernández*,‡

Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41071 Sevilla, Spain, and Instituto de Investigaciones Químicas, CSIC, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain

jogarcia@iiq.csic.es; mellet@us.es

Received January 13, 2004

Abstract: Coupling reaction of (2*R*,3*R*,4*R*,5*R*)-2,5-hydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) with isothiocyanates afforded the corresponding thiourea adducts, which were transformed into isourea-type bicyclic oxapyrrolizidine glycomimetics by mercury(II) oxide-assisted intramolecular sulfur displacement. Cyclic carbamate and thiocarbamate analogues were also prepared by direct carbonylation or thiocarbonylation of DMDP. Evaluation of the glycosidase inhibitory properties demonstrated that remarkable specificities in enzyme inhibition can be achieved upon modifications on the pseudoaglyconic side chain and on the nature of the sp^2-hybridized endocyclic ring nitrogen.

Glycosyl hydrolases represent an important class of biocatalysts involved in the metabolism of carbohydrates and in the assembly of specific oligosaccharide structures which, in turn, play an essential role in biological communication, including fertilization, infection, the inflammatory response, cell adhesion, and cancer metastasis. Consequently, specific inhibitors of such enzymes show high promise as probes for structure/function studies of enzymatic catalysis and as chemotherapeutic drugs for the treatment of viral infections, cancer, and metabolic disorders such as diabetes.[1]

In the past decade, the natural and synthetic polyhydroxyalkaloids termed generically iminosugars ("azasugars"),[2] nitrogen in the ring carbohydrate mimics, have emerged as the most important class of reversible glycosidase inhibitors.[3] Among them, the five-membered iminocyclitols of the homoazasugar[4] (aza-*C*-glycoside) family, of which (2*R*,3*R*,4*R*,5*R*)-2,5-hydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, **1**) is one of the most prominent representatives,[5] have gained special importance. Homoazasugars usually retain the same type of biological activity of the parent azasugar while exhibiting a higher stability as compared with the corresponding aminoacetal counterparts (aza-*O*-glycosides). Moreover, the pyrrolidine ring of these compounds adopts conformations that resemble well the twisted half-chair conformation of the incipient oxocarbenium cation postulated as the transition state of enzymatic glycoside hydrolysis. However, the strong inhibitory activity of pyrrolidine glycomimetics is frequently accompanied by low enzyme specificity. Thus, compound **1** inhibits simultaneously several α and β-glycosidases, which represents a serious limitation for the above-mentioned applications. A higher enzyme specificity has been reported for the related pyrrolizidine azasugar australine (**2**),[6] probably due to the spatial requirements imposed by the rigid bicyclic structure. Nevertheless, the bridgehead location of the nitrogen atom in pyrrolizidines prevents incorporation of pseudoaglyconic *N*-substituents, a strategy that has been exploited in monocyclic azasugars to identify more potent and specific glycosidase inhibitors suitable for clinical trials.[1,7]

Recently, we found that a subtle change in the structure of azasugars, by replacing the sp^3 ring nitrogen atom with a pseudoamide-type nitrogen (urea, thiourea, carbamate, thiocarbamate, isourea) with substantial sp^2 character, led to a new group of glycosidase inhibitors ("sp^2-azasugars")[8] with high anomer selectivity.[9] Interestingly, this electronic feature is also present in the natural

* To whom correspondence should be addressed. (C.O.M.) Phone: +34 954557150. Fax: +34 954624960. (J.M.G.F.) Phone: +34 954489559. Fax: 954460564.

† Universidad de Sevilla.

‡ Instituto de Investigaciones Químicas.

(1) For recent leading reviews, see: (a) N. Asano, N. *Glycobiology* **2003**, *13*, 93R. (b) Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Top. Med. Chem*. **2003**, *3*, 513. (c) Butters, T. M.; Dwek, R. A.; Platt, F. M. *Curr. Top. Med. Chem*. **2003**, *3*, 561. (d) Vasella, A.; Davies, G. J.; Böhm, M. *Curr. Opin. Chem. Biol*. **2002**, *6*, 619. (e) Heightman, T. D.; Vasella, A. *Angew. Chem., Int. Ed*. **1999**, *38*, 750. (f) Sears, P.; Wong, C.-H. *Chem. Commun*. **1998**, 1161. (g) Dwek, R. A. *Chem. Rev*. **1996**, *96*, 683.

(2) Although the term "azasugar" is widely used in the literature to refer to glycomimetics where the endocyclic oxygen atom has been replaced by nitrogen, the term is not strictly correct according to the IUPAC−IUMB nomenclature recommendations for carbohydrates, the accepted term being "iminosugar". "Azahexose" would actually imply that a carbon atom has been exchanged for a nitrogen atom. See: McNaught, A. D. *Pure Appl. Chem*. **1996**, *68*, 1919.

(3) For recent reviews on iminosugar glycosidase inhibitors, see: (a) Asano, N. *Curr. Top. Med. Chem*. **2003**, *3*, 471. (b) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev*. **2002**, *102*, 515. (c) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry* **2001**, *56*, 265. (d) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645. (e) Elbein, A. D.; Molyneux, R. J. Alkaloid Glycosidase Inhibitors. In *Comprehensive Natural Products Chemistry*; Barton, D., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier: Oxford, 1999; Vol. 3, p 129. (f) Simmonds, M. S. J.; Kite, G. C.; Porter, E. A. Taxonomic Distribution of Iminosugars in Plants and Their Biological Activities. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A., Ed.; Wiley-VCH: Weinheim, Germany, 1999; p 8. (g) Ossor, A.; Elbein, A. D. Glycoprotein Processing Inhibitors. In *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, Germany, 2000; Part II, Vol. 3, p 513.

(4) (a) Martin, O. R. Toward Azaglycosyl Compounds and Homoazaglycosides. In *Carbohydrate Mimics. Concepts and Methods*; Chapleur, Y., Ed.; Wiley-VCH: Weinheim, Germany, 1998; p 259. (b) Wong, C.-H-; Provencher, L.; Porco, J. A., Jr.; Jung, S.-H.; Wang, Y.-F.; Chen, L.; Wang, R.; Steensma, D. H. *J. Org. Chem*. **1995**, *60*, 1492.

(5) Evans, S. V.; Fellows, L. E.; Shing, T. K. M.; Fleet, G. W. J. *Phytochemistry* **1985**, *24*, 1953.

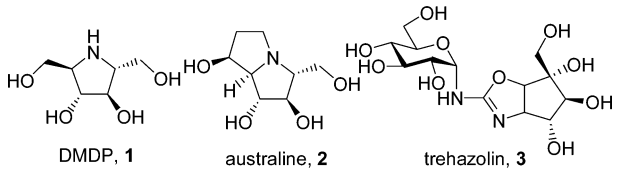
(6) (a) Molyneux, R. J.; Benson, M.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J. Nat. Prod*. **1988**, *51*, 1198−1206. (b) Kato, A.; Kano, E.; Adachi, I.; Molyneux, R. J.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Wormald, M. R.; Kizu, H.; Ikeda, K.; Asano, N. *Tetrahedron: Asymmetry* **2003**, *14*, 325.

(7) van den Broek, L. A. G. M. Azasugars: Chemistry and Their Biological Activity as Potential Anti-HIV Drugs. In *Carbohydrates in Drug Design*; Witczak, Z. J., Nieforth, K. A., Eds.; Marcel Dekker: New York, 1997; p 471.

(8) By analogy with the trivial name "azasugar", we are using here the term "sp^2-azasugar" to refer to glycomimetics where the endocyclic oxygen atom has been replaced by a nitrogen atom with substantial sp^2 character, typically a pseudoamide-type nitrogen.

10.1021/jo0499221 CCC: $27.50
Published on Web 04/21/2004

trehalase inhibitor trehazolin (**3**),[10] where the occurrence of the cyclic isourea function and the aglyconic glucopyranosyl substituent results in remarkable enzyme specificity. We hypothesized that by incorporating this functional group onto a preformed pyrrolidine template, new bicyclic pseudoamide-type inhibitors endowed with the advantages of trehalozoids and pyrrolizidines could be generated.



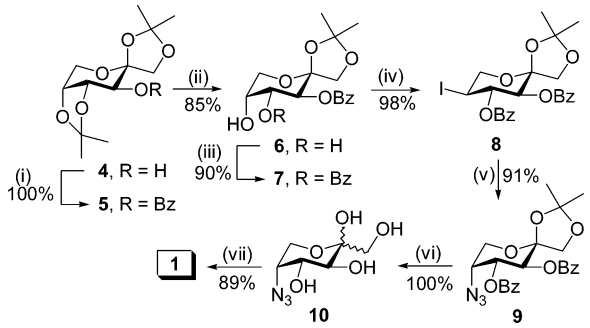
DMDP, **1** australine, **2** trehazolin, **3**

Since a rational design and synthesis to afford desired inhibition is often extremely difficult, due to the limited information regarding the structure of the active site, we have centered on synthetic methodologies that would be, eventually, amenable to combinatorial library strategies.[11] In a first approach, we decided to focus on creating a small, but diverse, group of compounds to test as potential glycosidase inhibitors, thus minimizing the risk that none of a series of compounds is inhibitory. DMDP has been selected as a suitable core to prove the potential of this concept to generate enzyme specificity, due to their broad and strong glycosidase inhibitory activity. Moreover, the C_2-symmetric structure of **1** makes it attractive in ring-closing schemes involving one of the hydroxymethyl substituents characteristic of the homoazasugar skeleton. Its synthesis was accomplished on a multigram scale from D-fructose 1,2:4,5-di-*O*-acetonide (**4**) by a modification of the method reported by Tatibouët et al.[12] involving benzoylation of the OH-3 to give **5**, selective removal of the 4,5-*O*-isopropylidene group, and regioselective benzoylation of the equatorial OH-4 to give **7**. Replacement of OH-5 was accomplished using a double-inversion strategy involving azide addition to 5-deoxy-5-iodo-L-sorbopyranose derivative **8**.[13] Deprotection of the hydroxy groups and final catalytic hydrogenation afforded **1** (Scheme 1).[14]

Previous syntheses of bicyclic isourea glycomimetics having a bridgehead nitrogen, in the aza-*O*-glycoside series, relied on the intramolecular nucleophilic addition of the endocyclic N-atom of 2-amino-2-oxazoline heterocycles, generated from hydroxycarbodiimide precursors, to suitably located aldehyde groups.[15] This approach is, however, unsuitable for accessing the corresponding aza-*C*-glycoside homologues. Instead, a two-step transformation was devised for assembling the bicyclo[3.3.0]octane framework from **1** involving (i) nucleophilic addition of the pyrrolidine nitrogen to an isothiocyanate electrophile and (ii) mercury(II) oxide-assisted sulfur displacement by one of the primary hydroxyl groups in the thiourea adduct. Both reactions proceed with total chemoselectivity, avoiding the use of protecting groups in the DMDP core. The first step provides, directly, sp^2-azasugars in the pyrrolidine series, while the second step affords trehazoloid−pyrrolizidine hybrid structures. Thus, reaction of **1** with phenyl isothiocyanate and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[16] gave the corresponding thiocarbamoyl pyrrolidines **11** and **12**, respectively, the pseudodisaccharide derivative **12** being further deacetylated to the fully unprotected compound **13**. The subsequent ring-closing reaction led to the corresponding phenylimino and glucopyranosylimino fused oxazolidine−pyrrolidine derivatives **14** and **15** (Scheme 2).

The DMDP-derived thioureas **11**−**13** exhibited a degenerate chemical exchange process in their NMR spectra due to slow rotation about the endocyclic nitrogen−thiocarbonyl bond in the NMR time scale.[17] This process becomes slow enough to break the symmetry of the pyrrolidine ring and to allow structure confirmation at temperatures below ambient (5−10 °C). Such an effect is absent in the case of the bicyclic compounds.

SCHEME 1[a]



[a] Reagents and conditions: (i) BzCl, pyridine, 0 °C to rt, 4 h; (ii) 50% aq AcOH, 50 °C, 3 h; (iii) 1.1 equiv of BzCl, pyridine, CH_2Cl_2, −65 °C, 1.5 h; (iv) PPh_3, I_2, imidazole, toluene, 70 °C, 16 h; (v) NaN_3, HMPT, 80 °C; (vi) (a) NaOMe, MeOH, (b) 9:1 TFA−water; (vii) 10% Pd/C, 4 bar, 24 h.

(9) (a) García-Moreno, M. I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2001**, *66*, 7604. (b) Díaz Pérez, V. M.; García-Moreno, M. I.; Ortiz Mellet, C.; Fuentes, J.; García Fernández, J. M.; Díaz Arribas, J. C.; Cañada, F. J. *J. Org. Chem.* **2000**, *65*, 136. (c) Jiménez Blanco, J. L.; Díaz Pérez, V. M.; Ortiz Mellet, C.; Fuentes, J.; García Fernández, J. M.; Díaz Arribas, J. C.; Cañada, F. J. *Chem. Commun.* **1997**, 1969.

(10) For reviews on trehazolin and close analogues ("trehazoloids"), see: (a) Berecibar, A.; Grandjean C.; Siriwardena, A. *Chem. Rev.* **1999**, *99*, 779. (b) Kobayashi, Y. *Carbohydr. Res.* **1999**, *315*, 3.

(11) For recent reports on combinatorial synthetic strategies applied to the identification of selctive glycosidase inhibitors in the pyrrolidine azasugar series, see: (a) Gerber-Lemaire, S.; Popowycz, F.; Rodríguez-García, E.; Carmona Asenjo, A. T.; Robina, I.; Vogel, P. *ChemBioChem* **2002**, *3*, 466. (b) Saotome, C.; Wong, C.-H.; Kanie, O. *Chem. Biol.* **2001**, *8*, 1061−1070. (c) Takebayashi, M.; Hiranuma, S.; Kanie, Y.; Kajimoto, T.; Kanie, O.; Wong, C.-H. *J. Org. Chem.* **1999**, *64*, 5280.

(12) Tatibouët, A.; Lefoix, M.; Nadolny, J.; Martin, O. R.; Rollin, P.; Yang, J.; Holman, G. D. *Carbohydr. Res.* **2001**, *333*, 327.

(13) 5-Azido-5-deoxy-D-fructopyranose derivatives have also been obtained from L-sorbose, an alternative starting material for DMDP synthesis; see: (a) Murphy, D. *J. Chem. Soc. C* **1967**, 1732. (b) Kuzuhara, H.; Emoto, S. *Tetrahedron Lett.* **1973**, *14*, 5051.

(14) For alternative recent syntheses of DMDP, see: (a) Donohoe, T. J.; Headley, C. E.; Cousins, R. P. C.; Cowley, A. *Org. Lett.* **2003**, *5*, 999. (b) Dondoni, A.; Giovannini, P. P.; Perrone, D. *J. Org. Chem.* **2002**, *67*, 7003. (c) Izquierdo, I.; Plaza, M. T.; Franco, F. *Tetrahedron: Asymmetry* **2002**, *13*, 1503. (d) Le merrer, Y.; Poitout, L.; Depezay, J.-C.; Dosbaa, I.; Geoffroy, S.; Foglietti, M.-J. *Bioorg. Med. Chem.* **1997**, *5*, 519.

(15) (a) García-Moreno, M. I.; Díaz Pérez, P.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2003**, *68*, 8890. (b) García-Moreno, M. I.; Díaz Pérez, P.; Ortiz Mellet, C.; García Fernández, J. M. *Chem. Commun.* **2002**, 848.

(16) Camarasa, M. J.; Fernández-Resa, P.; García-López, M. T.; de las Heras, F. G.; Méndez-Castrillón, P. P.; San Félix, A. *Synthesis* **1984**, 509.

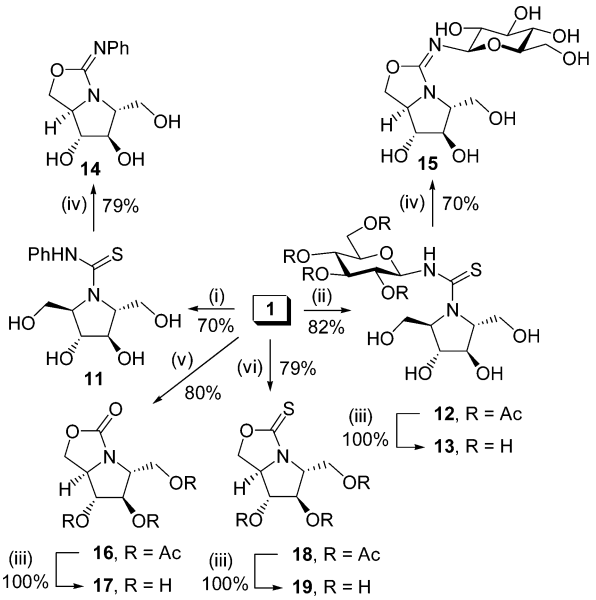
(17) For a discussion of the structural properties and reactivity of sugar thioureas, see: Ortiz Mellet, C.; García Fernández, J. M. *Adv. Carbohydr. Chem. Biochem.* **1999**, *55*, 35.

TABLE 1. Glycosidase Inhibitory Activities (K_i, μM) for Pseudoamide-Type Pyrrolidines 11 and 13 and Pyrrolizidines 14, 15, 17, and 19, in Comparison with Data for the Parent Azasugars 1 and 2

	compound							
enzyme	**1**[18]	**2**[18]	**11**	**13**	**14**	**15**	**17**	**19**
α-glucosidase (yeast)[a]	0.73	n.i.[b]	72	n.i.	>1000	n.i.	132	408
β-glucosidase (almonds)[c]	1.7	n.i.	9.2	334	465	n.i.	340	433
β-glucosidase/β-galactosidase (bovine liver)[c]	3.3	n.d.[d]	39	n.i.	153	n.i.	644	347
isomaltase (yeast)[a]	2.5	n.d.	28	n.i.	341	251	29	72
trehalase (pig kidney)[e]	355	n.d.	n.i.	n.i.	n.i.	20	n.i.	n.i.
invertase (yeast)[b]	2.9	28	>1000	n.i.	n.i.	n.i.	n.i.	n.i.
amyloglucosidase (*Aspergillus niger*)[f]	2.1	1.5	n.i.	n.i.	170	249	n.i.	n.i.

[a] pH 6.8. [b] n.i. = no inhibition detected. [c] pH 7.3. [d] n.d. = not determined. [e] pH 6.0. [f] pH 4.5.

SCHEME 2[a]



[a] Reagents and conditions: (i) PhNCS, pyridine, rt, 24 h; (ii) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate, pyridine, rt, 24 h; (iii) NaOMe, MeOH; (iv) HgO, MeCN, rt, 24 h; (v) (a) triphosgene, 10% aq $NaHCO_3$, toluene, 5 h, (b) Ac_2O, pyridine; (vi) (a) CS_2, DCC, DMF, −10 °C to rt, 16 h, (b) Ac_2O, pyridine.

To broaden the range of DMDP-derived sp^2-azasugar glycomimetics for structure/activity studies, the preparation of the carbonyl and thiocarbonyl pyrrolizidine derivatives **17** and **19** seemed attractive. Their preparation was accomplished in a straightforward manner by direct carbonylation and thiocarbonylation of **1** with triphosgene and carbon disulfide/dicyclohexylcarbodiimide, respectively. Purification was better effected on the corresponding triacetates **16** and **18**, which after conventional catalytic transesterification afforded the fully unprotected compounds in pure form (Scheme 2). All compounds here reported were found to be stable for months as solids or as aqueous solutions in a refrigerator.

The inhibitory activities of the new pseudoamide-type pyrrolidine and pyrrolizidine azasugar mimics **11**, **13** and **14**, **15**, **17**, **19** for α-glucosidase (yeast), β-glucosidase (almonds), β-glucosidase/β-galactosidase (bovine liver, cytosolic), isomaltase (yeast), trehalase (pig kidney), invertase (yeast), and amyloglucosidase (*Aspergillus niger*) are summarized in Table 1. None of the prepared compounds inhibited α-galactosidase (green coffee beans), α-mannosidase (Jack beans), and α-fucosidase (bovine kidney), which is in agreement with the configurational specificity of the parent azasugars **1** and **2**.

Iminosugars are thought to be good, but rather nonspecific, inhibitors of glucosidases because they mimic the incipient glycosyl cation involved in the mechanism of action of both α- and β-glycosidases, as a result of the heterocyclic nitrogen atom being protonated at physiological pH. In *N*-thiocarbonyl compounds such as **11** and **13**, the basicity is drastically decreased (by 14 p*K* units) as compared to the corresponding amine, while keeping a positive charge density resulting from delocalization of the lone electron pair into the thiocarbonyl group.[17] This scenario is probably closer to that encountered in the transition state of enzymatic glycoside hydrolysis at the endocyclic oxygen atom region,[19] which was expected to result in improved enzyme specificities. Actually, compound **11** is a rather good competitive inhibitor of β-glucosidase and isomaltase (K_i 9.2 and 28 μM, respectively) and only a moderate inhibitor of α-glucosidase and β-glucosidase/β-galactosidase, indicative of a reverse, and 20-fold higher, selectivity as compared to DMDP for these particular enzymes. In sharp contrast with **1**, no inhibition was detected for invertase and amyloglucosidase at mM concentration. The pseudoaglyconic substituent proved to have a decisive role in the biological activity. Thus, replacement of the aromatic group into a β-D-glucopyranosyl residue (**13**) virtually eliminated any interaction with the assayed glycosidases.

A further increase in enzyme specificity was observed for the bicyclic carbamate and thiocarbamate derivatives **17** and **19**, which can be considered as conformationally restricted *N*-(thio)carbonyl pyrrolidines. Thus, **17** and **19** retained the isomaltase inhibitory activity while they were 1 order of magnitude weaker inhibitors of β-glucosidase. The enzyme specificity was also strongly de-

(18) Ekhart, C. W.; Fechter, M. H.; Hadwiger, P.; Mlaker, E.; Stütz, A. E.; Tauss, A., Wrodnigg, T. M. Tables of Glycosidase Inhibitors with Nitrogen in the Sugar Ring and Their Inhibitory Activities. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A., Ed.; Wiley-VCH: Weinheim, Germany, 1999; p 253.

(19) Theoretical and experimental studies indicate that true transition state analogues must be essentially neutral or zwitterionic at physiological pH to keep specificity against the target enzyme. See: (a) Legler, G. Glycosidase Inhibition by Basic Sugar Analogues and the Transition State of Enzymatic Glycoside Hydrolysis. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A., Ed.; Wiley-VCH: Weinheim, 1999; p 31 (b) Jeong, J.-H.; Murray, B. W.; Takayama, S.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 4227. (c) Legler, G.; Finken, M.-T. *Carbohydr. Res.* **1996**, *292*, 103. (d) Blériot, Y.; Genre-Grandpierre, A.; Imberty, A.; Tellier, C. *J. Carbohydr. Chem.* **1996**, *15*, 985. (e) Deng, H.; Chan, A. W.-Y.; Bagdasianan, C. K.; Estupiñán, B.; Ganem, B.; Callender, R. H.; Scharamm, V. L. *Biochemistry* **1996**, *35*, 6037. (f) Ernert, P.; Vasella, A.; Weber, M.; Rupitz, K.; Withers, S. G. *Carbohydr. Res.* **1993**, *250*, 113.

pendent on the nature of the sp^2-hybridized bridgehead nitrogen. Thus, the more basic isourea-type pyrrolizidine analogues **14** and **15** were 10-fold weaker inhibitors of isomaltase. Interestingly, the glucopyranosyl derivative **15** turned to be a good inhibitor of porcine trehalase (K_i 20 μM), with virtually no inhibition of any other of the tested glycosidases.

In summary, the concept demonstrated herein of modifying the glycosidase inhibitory properties of pyrrolidine homoazasugars through the incorporation of the key ring nitrogen into linear or cyclic pseudoamide functionalities provides a new direction to the development of highly selective glycosidase inhibitors. The methodology allows the efficient introduction of molecular diversity by modifying the configuration of the homoazasugar core, the nature of the pseudoaglyconic substituent or the basicity of the pseudoamide group. The high efficiency of the proposed transformations, in combination with the abundance of commercially available isothiocyanate reagents, makes the approach particularly well suited for combinatorial library schemes. Work in that direction is currently underway in our laboratories.

Acknowledgment. We thank the Ministerio de Ciencia y Tecnología for financial support (Grant No. BMC2001-2366-CO3-03) and the Ministerio de Educación, Cultura y Deportes, for a doctoral fellowship (D.R.-L.).

Supporting Information Available: General experimental details, full purification and characterization data for the prepared compounds, and experimental procedure for determination of glycosidase inhibition constants (K_i). This material is available free of charge via the Internet at http://pubs.acs.org.

JO0499221