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Generalized Anomeric Effect in *gem*-Diamines: Stereoselective Synthesis of α-*N*-Linked Disaccharide Mimics

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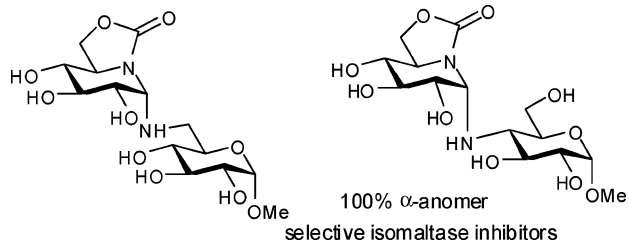
ABSTRACT



The orbital (negative hyperconjugation) contribution to the generalized anomeric effect is highly increased in bicyclic *gem*-diamines with a pseudoamide-type endocyclic nitrogen atom, which has been exploited for the stereoselective synthesis of configurationally stable α-*N*-linked azadisaccharide heteroanalogues of the natural disaccharides maltose and isomaltose as aglycon-sensitive inhibitors of isomaltase.

Since the structure elucidation of nephritogenoside (**1**), a nephritogenic glycopeptide isolated from the rat glomerular basement membrane,[1] the construction of the α-*N*-glycosidic bond has been a major challenge in carbohydrate chemistry. Although α-glycosylamines are direct intermediates toward this goal, anomerization of 1-aminoglycopyranosyl derivatives is so problematic that a number of alternative approaches have been developed which furnish glycosylamides[2] and other *N*-glycosyl derivatives[3] avoiding intermediate formation of the free amine. The lability of the aminoacetal functionality makes glycosylamines hydrolytically unstable and the preparation of α-configured diastereomers particularly problematic, due to the overwhelming thermodynamic preference for the β-configuration as determined by the reverse anomeric effect.[4] Replacing the *O*,*N*-acetal segment by an *S*,*N*-acetal (5-thiosugar glycosylamines) has been shown to weaken the reverse anomeric effect contribution to the anomeric equilibria, resulting in significant proportions of the axial isomers. Thus, 5-thioglucopyranosylamine (**2**)

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and 5-thiomannopyranosylamine (**3**) exist as 1:2 and 1:3 α:*β* mixtures in water solution, respectively.[5] Particularly interesting is the possibility of accessing α-*N*-linked disaccharide mimics,[6] a goal largely pursued in the design of inhibitors of the enzymes that process α-linked oligo or polysaccharides as drug candidates. Actually, the *S*,*N*-acetal analogue of maltose **4α** behaved as a low micromolar inhibitor of glucoamylase G2.[7] However, compound **4α** was in equilibrium with the corresponding *β*-anomer ***4β*** in aqueous solution, with a relative proportion 1:2.5 in favor of the latter (Figure 1).

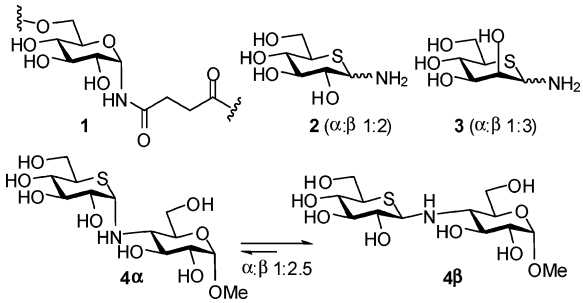

Figure 1. Partial structure of the α-N-glycopeptide nephritogenoside (**1**) and structures of 5-thiogluco(manno)pyranosylamines (**2** and **3**) and of the *N*-linked pseudodisaccharide **4**, with indication of the anomeric ratio.

Shifting the anomeric equilibrium in C−X−C−Y segments, where X represents an element with lone pairs, toward the gauche (α) orientation can be achieved by enhancing the generalized anomeric effect (GAE).[8] We conceived that the negative hyperconjugation contribution to the GAE would be particularly favorable when X is an sp^2-hybridized nitrogen atom, due to a very efficient overlapping of the *π*-type orbital hosting the lone pair in this case and the *σ** antibonding orbital of the contiguous C−Y bond. As a proof of principle, configurationally stable sp^2-iminosugar glycomimetics[9,10] with a hemiaminal portion anchored in the α-configuration, such as the bicyclic α-D-glucopyranose and α-D-mannopyranose analogues **5** and **6**, were prepared and shown to behave as anomer-selective glycosidase inhibitors.[11] We hypothesized that a similar strategy might be applied to stabilize the elusive α-diastereomer in sp^2-iminosugar glycosylamine analogues by replacing the *N*,*O*-acetal group by a *gem*-diamine functionality (**7**, Figure 2). This concept has

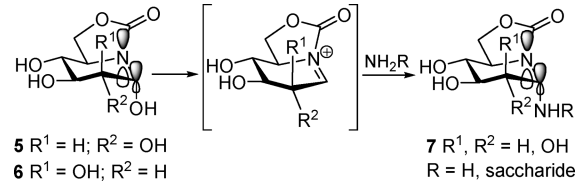

Figure 2. Structures of sp^2-iminosugars with hemiaminal (**5**, **6**) and *gem*-diamine-type pseudoanomeric centers (**7**). The orbitals involved in the negative hyperconjugation contribution to the GAE and the presumably structure of the azacarbonium ion intermediate are shown.

now been translated into the synthesis of the first mono- and disaccharide analogues bearing a configurationally and conformationally stable α-*N*-glycosidic linkage.

The *gem*-diamine structural motif is found in several biologically active natural and synthetic 2-acylaminopyrrolidine and -piperidine derivatives, including sugar-like compounds.[12] However, the incorporation of the 2-acylamino group in these molecules in a diastereoselective manner continues to be a major problem. Moreover, the resulting *gem*-diamines suffer from instability under physiological conditions.[13] In our prototype **7**, we expected that the preformed endocyclic carbamate functionality would direct

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(9) By analogy with the accepted name "iminosugar", we are using here the term "sp^2-iminosugar" 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. For recent monographs on iminosugars as glycosidase inhibitors, see: (a) *Iminosugars: From Synthesis to Therapeutic Applications*; Compain, P., Martin, O. R., Eds.; Wiley-VCH; Weinheim, 2007. (b) *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999.

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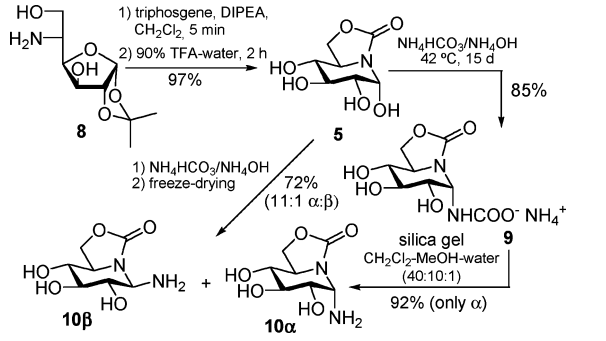
the stereochemical outcome of the reaction to the axial derivative by virtue of the incipient GAE in an azacarbonium ion intermediate (Figure 2). Because the ring sp^2-nitrogen should activate the pseudoanomeric C-1 carbon toward glycosylation-type reactions in a manner not unlike that of a pyranosyl anomeric carbon, formation of the target *gem*-diamine functionality by reaction of **5** and **6** with amine nucleophiles seemed feasible.

Treatment of crude **5**, accessible in only two steps from 5-amino-5-deoxy-1,2-*O*-isopropylidende-α-D-glucofuranose **8**,[11] with ammonium bicarbonate and ammonium hydroxide at 42 °C resulted in slow formation of the corresponding ammonium glycosylcarbamate[14] **9**. The transformation was complete in 15 days (TLC), and the unstable carbamate salt could be isolated by evaporation of the solvents and fast purification on a silica gel filter. ^{1}H NMR (500 MHz) analysis evidenced the presence of a single anomer having the α-configuration in D_2O solution. Attempts to further purify **9** by SiO_2 column chromatography resulted in elimination of carbon dioxide and ammonia to give the corresponding α-glucosylamine analogue **10α** as the sole product. In a separate experiment, the reaction mixture containing the carbamate salt was concentrated, lyophilized twice from water, and directly chromatographed to afford a mixture of the *gem*-diamine diastereomers **10α** and **10*β*** (72% yield) in 11:1 relative proportion (H-1 integration). Further SiO_2 column chromatography afforded the α-anomer in pure form (Scheme 1). An analogous transformation of the

Scheme 1. Synthesis of Configurationally Stable α-D-Glucopyranosylamine Mimics with *gem*-Diamine Structure



bicyclic D-mannopyranose mimic **6**, obtained from the *vic*-amino alcohol precursor **11** by carbonylation and further furanose → piperidine rearrangement,[11] afforded a mixture of the *gem*-diamines **12α** and **12*β*** in 17:1 relative proportion (H-6b integration), from which the α-anomer was isolated in pure form (Scheme 2).

Compounds **10α**, **12α** and **10*β***, **12*β*** existed in water solution in the $^4C_1$ conformation, with the anomeric amino group in axial and equatorial dispositions, respectively, as the anomeric substituents in natural α- and *β*-glucosides.

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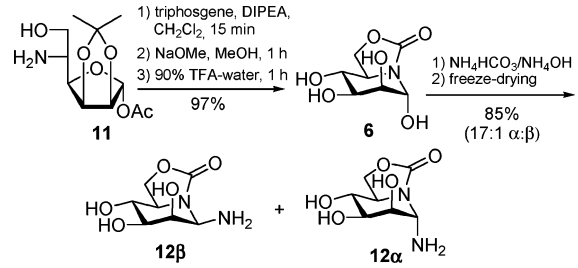
Scheme 2. Synthesis of Configurationally Stable α-D-Mannopyranosylamine Mimics with *gem*-Diamine Structure



Interestingly, pure **10α** and **12α** remained conformationally and configurationally stable after 48 h in water solution under slightly basic, neutral, or acidic conditions (pH 8−4), which is drastically different from the situation encountered in glycosylamines or glycosylamine mimics such as **2** and **3**. Reversion to the starting amino acetals **5** and **6** was only observed after heating a solution of **10α** or **12α**, respectively, in water at 60 °C for several hours. The *β*-anomers **10*β*** and **12*β*** were comparatively less stable under acidic conditions and underwent hydrolysis in the presence of silica gel during column chromatography using aqueous eluents (40:10:1 CH_2Cl_2−MeOH−H_2O), which facilitated purification of the α-anomers. Altogether, these results strongly support the prevalence of the anomeric versus the reverse anomeric effect in *gem*-diamine sp^2-iminosugar systems.

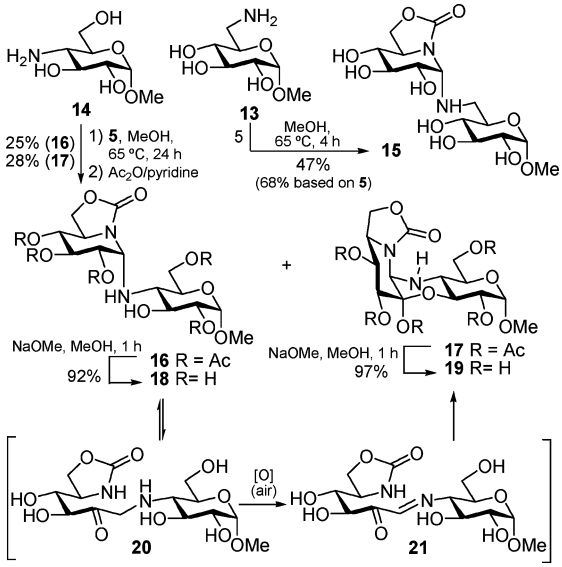
Compounds **10α** and **12α** can, in principle, mimic the developing gluco- and mannopyranosyl cations in the reaction coordinate of enzymatic hydrolysis of the corresponding α-glycosides by matching not only the hydroxylation pattern but also the α-anomeric configuration and the (partial) positive charge at the anomeric region. Notwithstanding, they exhibited a rather broad glycosidase inhibition profile and a poor anomeric selectivity.[15] Previous results have shown that the presence of an anomeric pseudoaglyconic hydroxyl group anchored in the axial position in glucomimetic structures does not warrant an increased selectivity toward α-glucosidases either.[11] In the absence of an aglycon portion, the glycomimetic can adapt to the active site of different glycosidases.[16] The existence of additional nonglyconic interactions can result even in totally reverse anomeric selectivity. In

(15) Inhibition constants (K_i) for **10α** and **12α** against commercial enzymes: *β*-glucosidase from almonds, 26 ± 2 and 49 ± 5 *μ*M; *β*-glucosidase/*β*-galactosidase from bovine liver, 4 ± 1 and 217 ± 15 *μ*M; naringinase (*β*-glucosidase/*β*-rhamnosidase) from *Penicillium decumbes*, 11 *μ*M and no inhibition; isomaltase from yeast, 8 and 184 *μ*M; amyloglucosidase from *Aspergillus niger*, 70 *μ*M and no inhibition; trehalase from pig kidney, 241 ± 15 and 462 ± 20 *μ*M; α-mannosidase from jack bean, 45 ± 3 and 7 ± 1 mM; *β*-mannosidase from *Helix pomatia*, 430 ± 20 and 25 ± 3 *μ*M, respectively. Neither **10α** nor **12α** inhibited *β*-galactosidase (*E. coli*) and α-galactosidase (coffee beans) at 1 mM concentration.

(16) In principle, the negative hyperconjugation contribution to the anomeric effect in nonprotonated **10α** and **12α** must be weaker as compared with the oxygen analogues **5** and **6**, while it would be expected to be stronger in the protonated state. The anomerization barriers at pH 7.3, used in the inhibition experiments toward *β*-glucosidases, are therefore expected to be lower, which might be at the origin of the observed low inhibition selectivity. Actually, traces of the *β*-anomers were observable after dissolving the α-configured free bases in D_2O (pH ∼8.5) by NMR, while this was not the case for the corresponding hydrochloride salts (pH ∼5).

principle, effective and selective inhibition of a particular glycosidase requires designing mimics of both portions, glyconic and aglyconic, of the natural substrate. To test this notion, the synthesis of the *gem*-diamine disaccharide mimics of isomaltose and maltose **15** and **18**, respectively, was next attempted (Scheme 3).

Scheme 3. Synthesis of α-*N*-Linked Disaccharide Mimics



We found that heating a 1:1.5 molar solution of **5** and methyl 6-amino-6-deoxy-α-D-glucopyranoside (**13**)[17] in methanol at 60 °C for 4 h afforded the target α-*N*-linked isomaltose mimic **15** in 47% yield (68% considering the recovered **5**) as a single diastereomer. A similar reaction using instead the 4-amino-4-deoxy derivative **14**[18] as the glycosidating partner led to a mixture of two compounds that could be separated, after acetylation, into the α(1→4)-*N*-linked pseudodisaccharide **16** (25%) and the tetracyclic *gem*-diamine **17** (28%). Further deacetylation provided the fully unprotected derivatives **18** and **19**.

Formation of the tetracyclic derivative **19** in the reaction of **5** and **14** is intriguing. It involves Amadori rearrangement of the glycosylamine-like derivative **16** to the open-chain 1-amino-1-deoxy-D-fructose derivative **20**. Spontaneous oxidation of the β-aminocarbonyl functionality to the transient iminoketone **21** seems then to occur. Subsequent double intramolecular attack of the carbamate nitrogen to the imine carbon and of the OH-3 hydroxyl at the glucose moiety to the carbonyl group takes place with total stereoselectivity, leading to the diastereomer having the heteroatom substituents at the central 1,4-oxazine ring, in a chair conformation, in axial disposition, fitting the anomeric effect.

Compounds **15** and **18** represent examples of designed, linkage-spanning glycosidase inhibitors. Actually, they behaved as highly selective, though modest inhibitors of isomaltase,[19] an enzyme involved in the hydrolysis of α(1→6) linkages in polysaccharides.[20] This is attributable to the interplay of a configurationally anchored "α" *gem*-diamine moiety, isosteric of the acetal functionality in the natural substrate, and the discriminating effect of the aglycon portion,[21] suggesting that enzyme inhibition may be generally tunable by this kind of structural modification. Exploitation of the generalized anomeric effect in sp^2-iminosugar systems as a tool to investigate the effect of other modes of linkage on glycosidase inhibitory action and selectivity is currently underway in our laboratories.

Acknowledgment. This work was supported by the Spanish MEC (contract nos. CTQ2006-15515-C02-01/BQU and CTQ2007-61180/PPQ) and the Junta de Andalucía (P08-FQM-03711).

Supporting Information Available: Experimental details and copies of the NMR spectra for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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