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Novel *O*-glycosyl amino acid mimetics as building blocks for *O*-glycopeptides act as inhibitors of galactosidases

Lars Kröger, Dirk Henkensmeier, Andreas Schäfer and Joachim Thiem*

Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, Hamburg, Germany

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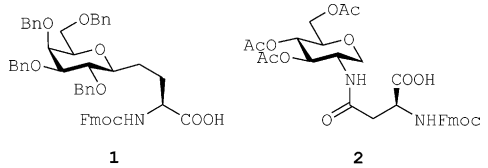
Abstract—As potential lead structures for a new class of glycosidase inhibitors the novel *O*-glycosyl amino acid mimetics 3′-*O*-[2,6-anhydro-D-*glycero*-L-gluco-heptitol-1-yl]-L-serine **3** and-L-threonine **4** were synthesized, employing regio- and stereoselective aziridine ring opening methodology. They proved to be stable in the presence of glycosidases and showed competitive inhibition of α-galactosidase from *Aspergillus niger*.

The important role of carbohydrates in many biological processes is evident, and tailored derivatives are discussed as promising leads for efficient pharmaceuticals. However, the lability of the glycosidic bond towards chemical and enzymatic degradation in vivo results in low bioavailability of carbohydrate derivatives and prevents their oral application.[1] The synthesis of glyco mimetics, representing the corresponding natural structures and offering higher metabolic stability, has already received much attention, and further effort on their synthesis are expected to be undertaken in the future.[2] A common approach is the application of *C*-glycosides, which are no longer susceptible to cleavage by glycosidases.[3–6]

Due to their therapeutic potential, the synthesis of glycopeptides and their mimetics represents a dynamically developing field of research.[7–9] Significant improvements in activity, bioavailability, stability and solubility of peptides were achieved by their glycosylation.[10,11] The glycosidic linkage is susceptible to enzymatic hydrolysis, but furthermore β-eliminations occur under mild basic conditions in the case of *O*-glycosides of serine and threonine.[12] Hence, glycopeptide mimetics can be considered promising leads to novel pharmaceuticals.

Bednarski et al. synthesized the β-Gal-*O*-serine mimetic **1**, stable against enzymatic hydrolysis, and incorporated it into a model peptide, inert against in vivo glycosidic cleavage.[13] In another approach connected with *N*-glycoproteins, we synthesized a *N*-glucoasparagine mimetic (**2**), in which the asparagine has been shifted from the anomeric center to position 2 of the carbohydrate.[14] In both these molecules the glycosidic acetal has changed into a stable ether bond (Fig. 1).

A novel approach for the stereoselective synthesis of β-alkoxy-α-amino acids was introduced by Nakajima and Okawa via ring opening of chiral activated aziridine-2-carboxylates with alcohols in the presence of Lewis acids.[15,16] A regioselective attack by nucleophiles such as alcohols, amines, carboxylic acids, thiols and indols occurs only at position 3.[17] Due to the moderate nucleophilicity of alcohols, only aziridine ring openings employing simple alcohols like methanol or ethanol are reported to date using the alcohol as solvent as well. Hence, we developed an optimized reaction protocol, enabling us to utilize the secondary hydroxyl group of a rather sterically demanding carbohydrate derivative as nucleophile.[18]



1 2

Figure 1. *C*-glycosidic β-Gal-*O*-Ser **1** and *N*-glucoasparagine **2** mimetic.

* Corresponding author. Tel.: +49-40-42838-4241; fax: +49-40-42838-4325; e-mail: thiem@chemie.uni-hamburg.de

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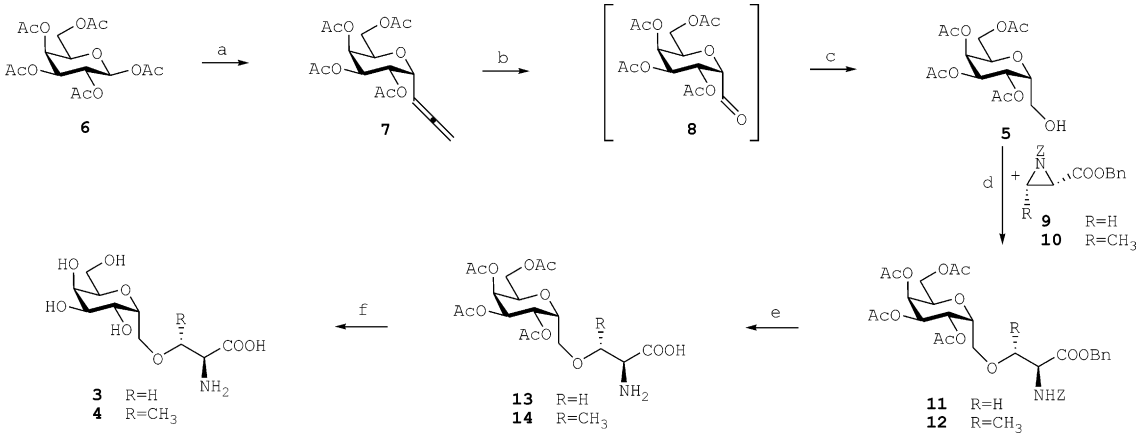

Scheme 1. Synthesis of 3′-*O*-[2,6-anhydro-D-*glycero*-L-gluco-heptitol-1-yl]-L-serine **3** and -L-threonine **4**: (a) C_3H_3–$Si(CH_3)_3$, BF_3·Et_2O, MeCN, 48%; (b) O_3, DCM; (c) $NaBH_4$, THF, 62%; (d) BF_3·Et_2O, $CHCl_3$, 52% (R = H), 55% (R = CH_3); (e) H_2, Pd/C, MeOH, 89% (R = H), 95% (R = CH_3); (f) (1) NaOMe, MeOH; (2) Amberlite IR-120 (H^+), 59% (R = H), 92% (R = CH_3).

To further evaluate the potential of this method for the synthesis of *O*-glycosyl amino acid mimetics, we prepared the serine and threonine derivatives **3** and **4**, which can not be hydrolyzed. They have a *C*-glycosidic linkage and a stable ether-bridge between the carbohydrate and the amino acid part, resulting in a linkage elongated by one methylene group (Scheme 1).

The new *C*-glycosidic building block **5** was synthesized by reacting 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**6**) with propargyl trimethyl silane in the presence of a Lewis acid. The expected[19] α-linkage of the *C*-glycosidic allene **7** was confirmed by ^{1}H NMR, showing a $^3J_{1,2}$-coupling of 4.1 Hz. Cleavage of the allene with ozone gave the labile aldehyde **8**, which was reduced without isolation to the *C*-glycosidic methylene galactitol **5** employing sodium borohydride.[20]

Compound **5** was subjected to the Lewis acid catalyzed ring opening of the activated aziridines **9** and **10**, which gave the glycosyl-amino acid building blocks **11** an **12** in satisfactory yields of 52 and 55%, respectively.[21] Hydrogenolytic deprotection furnished the intermediates **13** and **14**, which were used, after Fmoc-protection, as versatile building blocks for glycopeptide solid phase synthesis of MUC1 mimetics. These results will be published in due course. Zemplén deacetylation of **13** and **14** gave the target compounds **3**[22] and **4**.[23]

To test the in vivo stability of the carbohydrate amino acid mimetics **3** and **4**, they were incubated with α-galactosidase from *Aspergillus niger*[24] and showed no hydrolysis. As these mimetics have structures closely related to the natural substrates of α-galactosidases, they should interact with the active center without being hydrolyzed by the enzyme. Thus, they might function as competitive glycosidase inhibitors. This hypothesis was tested by inhibition experiments of the α-galactosidase catalyzed cleavage of *para*-nitrophenyl α-D-galactopyranoside (Fig. 2). Comparison with the well known inhibitor 1-deoxynojirimycin (K_i = 18 μmol)[25] showed considerably lower results, but nevertheless a noticeable competitive inhibition was observed for **3** (K_i = 0.4 mmol) and **4** (K_i = 0.3 mmol). No optimization was attempted for these structures, which may be considered as promising leads for a new class of glycosidase inhibitors.

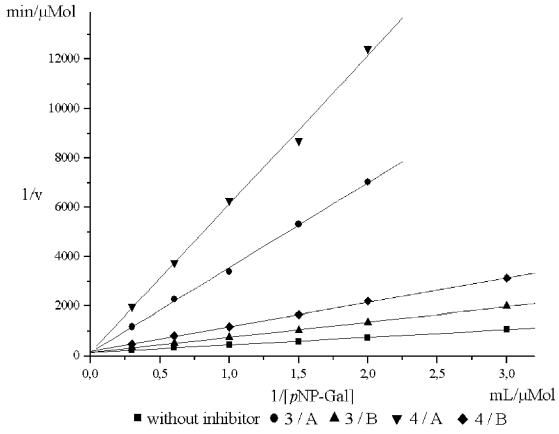

Figure 2. Lineweaver–Burk plot. Hydrolysis of *p*NP-α-Gal by α-galactosidase from *A. niger* with different concentrations of substrates **3** and **4** (A: substrate concentration 50 μmol; B: substrate concentration 10 μmol). The common point of intersection indicates a competitive inhibition.

References and notes

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20. **Compound 5**: colorless oil; $[\alpha]_D^{20} = +31.3$ (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.00$, 2.01, 2.03, 2.05 (4×s, 12H, 4×C$\underline{H}_3$), 3.60 (dd, 1H, H-1, $^2J_{1,1'} = 11.7$ Hz, $^3J_{1,2} = 7.6$ Hz), 3.77 (dd, 1H, H-1′), 4.03 (dd, 1H, H-7, $^3J_{6,7} = 4.1$ Hz, $^2J_{7,7'} = 11.7$ Hz), 4.19–4.24 (m, 2H, H-2, H-6), 4.35 (dd, 1H, H-7′, $^3J_{6,7'} = 8.1$ Hz), 5.20 (dd, 1H, H-3, $^3J_{2,3} = 4.1$ Hz, $^3J_{3,4} = 8.1$ Hz), 5.24 (dd, 1H, H-4, $^3J_{4,5} = 3.1$ Hz), 5.38 (dd, 1H, H-5, $^3J_{5,6} = 3.6$ Hz) ppm. Anal. calcd for $C_{15}H_{22}O_{10}$: C, 49.42, H, 6.12. Found: C, 49.10, H, 6.25.
21. **General procedure for the aziridine ring opening**: alcohol component **5** (1 equiv) (2*S*)-benzyl-1-benzyloxycarbonyl-aziridin-2-carboxylate **9** or (2*S*,3*S*)-benzyl-1-benzyloxy-carbonyl-3-methylaziridin-2-carboxylate **10** (0.66 equiv), respectively, were dissolved in dry chloroform. The solution was degassed and kept under high vacuum for 30 min. After flushing the flask with argon, the resulting syrup was redissolved in the minimal amount of dry chloroform and treated with a catalytic amount of 10% $BF_3{\cdot}Et_2O$ in chloroform. The reaction was left at room temperature for 16 h, treated with a catalytic amount of $BF_3{\cdot}Et_2O$ again and left for further 16 h. After diluting with chloroform, saturated sodium hydrogencarbonate was added and the organic phase dried over magnesium sulfate. Evaporation of the solvent followed by column chromatography gave the desired products.
22. **Compound 3**: colorless solid; mp 134 °C; $[\alpha]_D^{20} = +24.3$ (*c* 0.5, H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta = 3.54$ (dd, 1H, H-7, $^3J_{6,7} = 4.4$ Hz, $^2J_{7,7'} = 11.8$ Hz), 3.58 (dd, 1H, H-7′, $^3J_{6,7'} = 7.7$ Hz), 3.58–3.64 (m, 2H, H-1, H-4), 3.74–3.83 (m, 6H, H-3, H-5, H-6, H-Ser-α, CH_2-Ser-β), 3.87 (dd, 1H, H-1′, $^2J_{1,1'} = 10.2$ Hz, $^3J_{1',2} = 6.2$ Hz), 4.10 (ddd, 1H, H-2, $^3J_{2,3} = 3.5$ Hz) ppm.
23. **Compound 4**: colorless solid; mp 148 °C; $[\alpha]_D^{20} = +21.4$ (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 1.18$ (d, 1H, CH_3-Thr, $^3J_{\beta\text{-Thr},CH3\text{-Thr}} = 6.5$ Hz), 3.52 (d, 1H, H-Thr-α, $^3J_{\alpha\text{-Thr},\ \beta\text{-Thr}} = 4.6$ Hz), 3.54–3.70 (m, 5H, C$\underline{H}_2$-1, H-4, C$\underline{H}_2$-7), 3.74 (ddd, 1H, H-6, $^3J_{5,6} = 1.8$ Hz, $^3J_{6,7} = 4.6$ Hz, $^3J_{6,7'} = 6.5$ Hz), 3.82 (dd, 1H, H-5, $^3J_{4,5} = 3.4$ Hz), 3.86 (dd, 1H, H-3, $^3J_{2,3} = 6.2$ Hz, $^3J_{3,4} = 9.7$ Hz), 3.93 (dd, 1H, H-Thr-β), 4.06 (ddd, 1H, H-2, $^3J_{1,2} = 3.9$ Hz, $^3J_{1',2} = 8.9$ Hz) ppm.
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