6-Guanidinopyranoses: Novel Carbohydrate-Based Peptidomimetics**

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Dedicated to Professor Hans Paulsen on the occasion of his 75th birthday

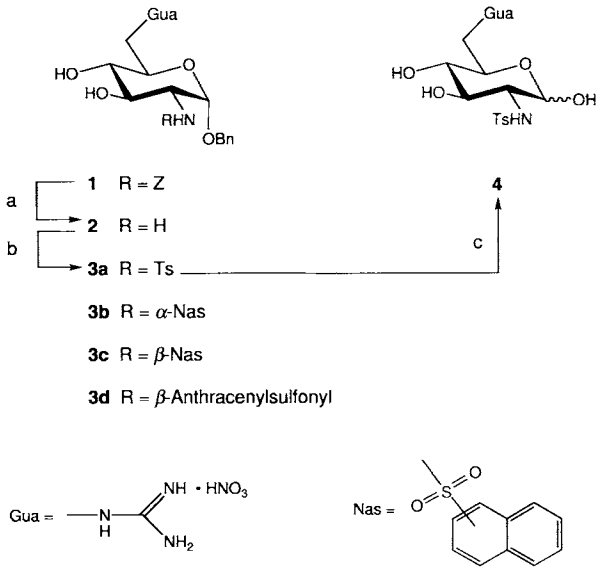
A classical approach to drug development is based on mimicking bioactive peptides that are enzyme substrates or receptor ligands. Non-peptidic structures are of particular interest. We report here on such peptidomimetics, which have been developed in a project directed at identifying thrombin inhibitors.

Thrombin is a key enzyme of the coagulation cascade. It cleaves fibrinogen to fibrin, activates the coagulation factors V and VIII, and is a main stimulator in the activation of platelets. Thrombin thus plays a central role in physiological hemostasis as well as pathological thrombosis.[1] Therefore, the direct inhibition of thrombin is an attractive paradigm for the development of a new antithrombotic agent; some thrombin inhibitors[2, 3] are already used in clinical trials.[4]

Thrombin is a serine protease with high specificity for arginine-containing peptide sequences.[5] As a result, known thrombin inhibitors contain arginine residues (for example Argatroban)[6] or guanidine (for example Ro46-6240)[7] or amidine derivatives (for example NAPAP)[8] as arginine mimetics. We have investigated 6-guanidinohexoses, which can be viewed as conformationally restricted arginine mimetics. Fitting of these structures into an enzyme model allowed the prediction that such compounds could be accommodated in the recognition pocket of thrombin.

The favored synthesis of 6-guanidinohexoses from the corresponding 6-amino derivatives with 3,5-dimethylpyrazolylformamidinium nitrate (DPFN) has been described.[9] We started from benzyl-2-benzyloxycarbonylamino-2,6-dideoxy-6-guanidinio-α-D-glucopyranoside nitrate (**1**),[9] which afforded the glucosamine derivative **2** on selective catalytic hydrogenation with palladium on charcoal (Scheme 1). The transformation of the free amino group to amides or sulfonamides was straightforward; reaction of the hydroxyl groups was prevented by the presence of water, and the pH was controlled so that a guanidinium salt was present. That is, protons served as a "protective group" for the guanidino functionality. Thus, reaction of **2** with aromatic sulfonyl chlorides led to the sulfonamides **3**.

While amide derivatives of **2** did not display any substantial biological activity, a thrombin inhibitor with micromolar activity was obtained with the tosyl derivative **3a**. The enlargement of the aromatic moiety to the α-naphthylsulfonyl derivative **3b** resulted in an inactive compound, whereas the analogous β-naphthylsulfonyl derivative **3c**[10] and the β-anthracenylsulfonyl derivative **3d** exhibited slightly increased thrombin inhibitory activity (Table 1). To judge the selectivity of thrombin inhibition, the related but less discriminating[11] serine protease trypsin was investigated (Table 1). Since, according to the enzyme model, an interaction of the anomeric center with the active site of thrombin seemed possible, the anomeric benzyl



Scheme 1. a) H_2, Pd/C, MeOH/H_2O 2/1, room temperature (RT), 4 h, quantitative; b) TsCl, THF/H_2O 2/3, NEt_3, 0 °C, 6 h, 36%; α-NasCl, H_2O/pyridine 5/3, NEt_3, RT, 5 h, 95%; β-NasCl, H_2O/pyridine 5/3, NEt_3, RT, 5 h, 79%; β-anthracenyl chloride, H_2O/pyridine 1/1, NEt_3, RT, 20 h, 78%; c) H_2, Pd/C, EtOH/H_2O 1/1, RT, 18 h, quantitative.

Table 1. Thrombin inhibition of some guanidino hexoses and their selectivities vs. trypsin [a].

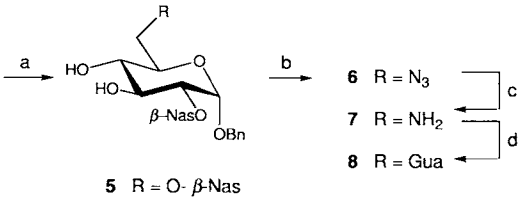
Compound	K_i [μM]	Selectivity
1	410	–
3a	4	19
3c	1.1	109
3d	0.9	>227
8	1.4	143

[a] Selectivity = K_i(trypsin) K_i(thrombin)$^{-1}$

group of **3a** was removed by hydrogenolysis. However, as the resulting free pyranose **4** showed no activity, we could conclude that the anomeric center does not interact with the serine of the catalytic triad of the serine proteases under investigation.

An analogous sulfonate was synthesized to study the influence of the sulfonamide nitrogen atom. Activation of benzyl-α-D-glucopyranoside with bis(tributyltin) oxide followed by acylation with β-naphthylsulfonyl chloride gave, as expected,[12] the bis(sulfonate) **5** as the main product. The guanidino derivative **8**[13] was prepared via azide **6** and amine **7** by the established synthetic strategy[9] (Scheme 2). This sulfonate had an activity comparable to that of the sulfonamide, but slightly higher selectivity (Table 1).

The position of **8** in the recognition pocket of thrombin was proven by an X-ray structure[14] of an inhibitor complex with



5 R = O-β-Nas

Scheme 2. a) Benzyl α-D-glucopyranoside [20, 21], $(Bu_3Sn)_2O$, toluene, reflux, 4 h; β-NasCl, 60 °C, 15 h, 34%; b) NaN_3, DMF, 55 °C, 16 h, 79%; c) PPh_3, THF/H_2O 20/1, RT, 24 h, 95%; d) DPFN, DMF, 80 °C, 24 h, 49%.

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[**] We thank Rudolf Minder and Roland Keller for experimental help.

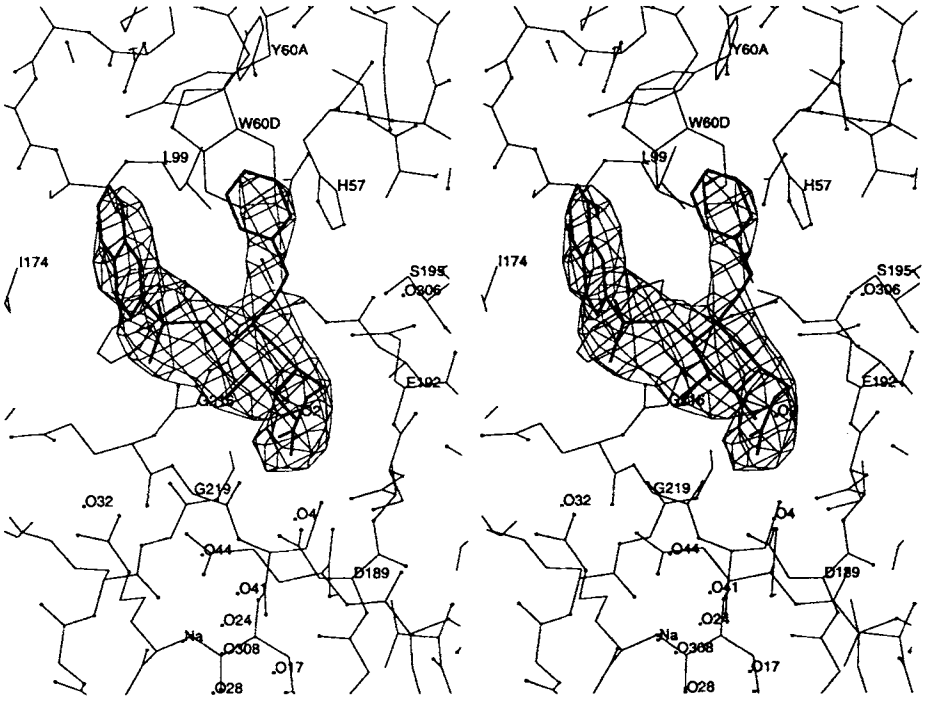

Figure 1. Steroview of the refined structure of the complex between **8** (electron density contour representation) and human thrombin (line representation). Water molecules (labeled "O") are the same as found for other inhibitors; only O 306 (right of **8**) in the oxyanion hole is new here. In contrast to most known complexes of thrombin inhibitors, there are no hydrogen bonds to glycine 216 (hidden by **8**). The sugar hydroxyl groups form only one hydrogen bond to the protein (between O3′ and -NH of glycine 219). The guanidinium group has very weak hydrogen bonds (3.4 and 3.9 Å) to the oxygen atoms of aspartic acid 89 at the bottom of the recognition pocket, but good hydrogen bonds (2.8 and 2.9 Å) to the C=O group of glycine 218 and the water molecule O2. Other possible conformations of the guanidinium group were examined, but were found to be less favorable. These nonoptimal hydrogen bonds of inhibitor **8** may explain its rather moderate activity.

thrombin. This guanidinohexose binds without direct contact to the active site serine ("inhibitor binding mode"[15]). The naphthyl residue binds in the D-pocket,[16] and the benzyl group in the P-pocket. The guanidinium moiety is found in the recognition pocket as expected (Figure 1). Thus, guanidinohexoses can indeed function as arginine mimetics. Analogous use of aminohexoses as conformationally restricted lysine mimetics might be envisaged. An advantage of this approach is that these readily accessible hexose derivatives may serve as templates,[17, 18] due to their high natural functionalization, for the introduction and variation of other functional groups.

Received: October 4, 1996 [Z 9612 IE]
German version: *Angew. Chem.* **1997**, *109*, 791–793

Keywords: carbohydrates · enzyme inhibitors · guanidino sugars · peptidomimetics

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[14] Crystals of the complex of human thrombin with **8** were prepared, and data measured to 3.3 Å resolution and processed as described [7, 16]. With initial atomic coordinates from the best available thrombin structure, the difference electron density clearly showed the inhibitor bound in the active site. The structure was refined with tight geometrical constraints to $R = 0.126$ with standard deviations of 0.011 Å for bond lengths, 1.88° for bond angles, and 1.96° for torsion angles relative to standard parameters [19].
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  0570-0833/97/3607-0752 $ 17.50+.50/0