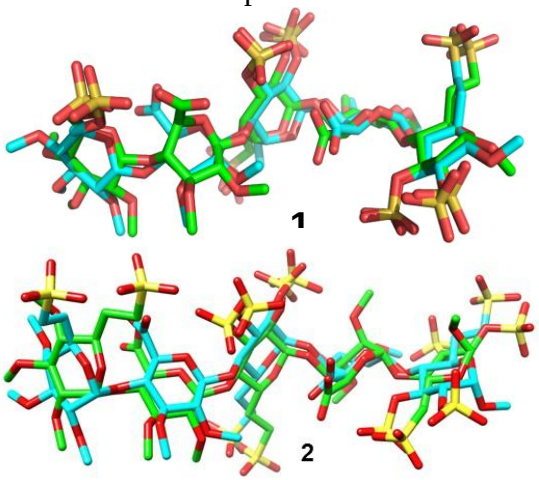
Principal Investigator: Anikó Borbás
Grant Title: Development of a novel class of heparinoid anticoagulants

Abstract

Objectives: I. Synthesis of novel anticoagulant pentasaccharides with sulfonatomethyl groups at the primary and secondary positions. Structure, dynamics and binding studies and in vitro anti-Xa inhibitory activity measurements of the new compounds.
II. Development of new methods for the synthesis of the L-idose/L-iduronic acid and replacement of the L-iduronate residue by potential structural substituents.
Methods used: Solution phase chemical synthesis was applied to achieve the synthetic goals. The interaction of the oligosaccharides with antithrombin was studied by 2D ^{1}H-^{1}H CLIP-COSY, TOCSY, ROESY, NOESY and ^{1}H-^{13}C HSQC-CLIP-COSY NMR methods, by Isothermal Titration Calorimetry (ITC) measurements and by Molecular Dynamics Simulations. The factor Xa inhibitory activity of the new oligosaccharide sulfonic acids was measured *in vitro* by Berichrom® Heparin chromogenic assay on a Siemens BCS-XP automated coagulometer, using pooled normal human plasma.
Results obtained: We prepared a series of heparinoid pentasaccharide sulfonic acids bearing up to three primary sulfonate ester moieties. These idraparinux-analogue pentasaccharides display excellent or modest anticoagulant activity depending on the position of the sulfonatomethyl groups. The prepared mono- and disulfonic acids bearing the sulfonatomethyl moiety at units **F** and **H** represent a new class of heparinoid anticoagulants.



Three-dimensional structures of the free and antithrombin-bound forms of a pentasaccharide disulfonic acid of high anticoagulant activity (**1**) and a pentasaccharide trisulfonic acid of low anticoagulant activity (**2**) were determined by NMR spectroscopy and molecular dynamics simulations. These studies revealed significant differences of their conformational preferences explaining the highly different biological activity observed.
C2 and C3 sulfonatomethyl glycosides prepared by Horner-Wadsworth-Emmons olefination were utilized in the synthesis of heparinoid pentasaccharides bearing secondary sulfonate goups.
Three novel idraparinux-analogue pentasaccharides containing a 6-deoxy-L-talopyranoside instead of the L-iduronic acid were prepared.
A short and highly efficient synthetic route to L-idose glycosyl donors was developed by C5 epimerization of orthogonally protected D-glucopyranosyl α-thioglycosides.