Conformation Analysis

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The ^2S_O Skew-Boat Conformation in L-Iduronic Acid**

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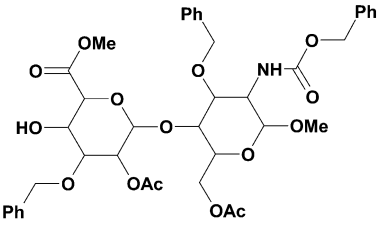
L-Iduronic acid (L-IdoA), an essential component of the glycosaminoglycans heparin, heparan sulfate, and dermatan sulfate, is biosynthesized in the polymeric form through a single epimerization at the C5-position of D-glucuronic acid. This locally controlled epimerization confers unique properties to L-IdoA-containing biomolecules, and the presence of L-IdoA in glycosaminoglycans explains their unique ability to control the activity of proteins such as growth factors, chemokines, blood coagulation enzymes, etc.[1] This unique ability of the highly flexible L-IdoA ring is related to its ability to adopt several conformations of comparable energies.[2] The iduronate ring conformation, initially thought to be restricted to an equilibrium between the $^1C_4$ and $^4C_1$ chair conformations, was further complicated when it was discovered that the $^2S_0$ skew-boat geometry could play a critical role in the control of blood coagulation.[3,4] Over the past decades, extensive solution NMR experiments[5] as well as theoretical computations[6] have been carried out, mostly using heparin fragments comprising up to 6 pyranose rings, to gain insight into the geometries of L-IdoA. Several macromolecular X-ray diffraction studies have also been performed, and a skew-boat conformation has been suggested based on studies of L-IdoA-containing polysaccharides complexed with their protein receptors.[7] Even though all these ingenious studies have given a convincing picture of L-IdoA with the ^2S_O geometry, an atomic-resolution model ($d \approx 1$ Å) is still required, especially as such a highly flexible ring may present a quasicontinuous distribution of conformations.[8] This model is presented herein through the study of the crystal structure of an L-idopyranosyluronic methyl ester, in the synthetic disaccharide **1** (Scheme 1).[9]



Scheme 1. Disaccharide **1**.

The crystallization of **1** was challenging and yielded only needlelike, multiply twinned microcrystals that were analyzed by X-ray powder diffraction by using various instruments (see the Supporting Information), including three synchrotron beam lines. These high-resolution data revealed the existence of a monoclinic (**II**) and an orthorhombic (**I**) polymorph with a common, surprisingly short, lattice parameter of approximately 5 Å, but all attempts at structure determination by means of ab initio generated molecular models were unsuccessful. Further progress would not have been possible without the scrutinization of numerous batches of crystals through a binocular microscope in polarized light. Finally, one tiny crystal was isolated and its structure elucidated, as the monoclinic form (**II**), by diffraction. However, a crystal of form **I** that was big enough for X-ray diffraction could not be found, and the structure determination had to be achieved by the powder method. By simply recalling the recent work on D-ribose,[10] one can easily imagine the computer resources required for determining the structure of a 56 atom molecule with 21 torsions in such poorly scattering material. Our knowledge of the molecular structure of **II**[11] enabled an essential reduction in the computing time needed for the successful determination of the stucture of form **I** by direct-space methods.[13]

The structure of **1** in form **II** is disordered (Figure 1), and the disordered region includes a benzyl group, the acetate moieties and a part of the glucopyranose unit; the L-iduronate ring in this structure is, however, well ordered. In agreement with the short cell parameter the molecule is rather flat; all substituents of D-glucosamine (conformation $^4C_1$) and L-IdoA lie in equatorial positions, except for the *O*-methyl at the reducing end. The dihedral angle between the mean planes of the pyranose rings is 29.4(4)°. The angles ϕ and ψ, defined as, $\measuredangle$(O5-C1-O1-C4P) and $\measuredangle$(C1-O1-C4P-C5P1), respectively, where P indicates primed atom-names on the glucopyranose, are −64.4(6)° and −114.2(8)° in form **II**; these angles are markedly different from those generally found in (one to four) equatorially linked pyranose rings (ca. −93° and −140°).[14] The iduronic carbons labelled C1, C3, C4, and C5 define a plane (r.m.s. = 0.0495 Å), and the two remaining ring atoms lie off this plane, (O5 by 0.707(7) Å and C2 by

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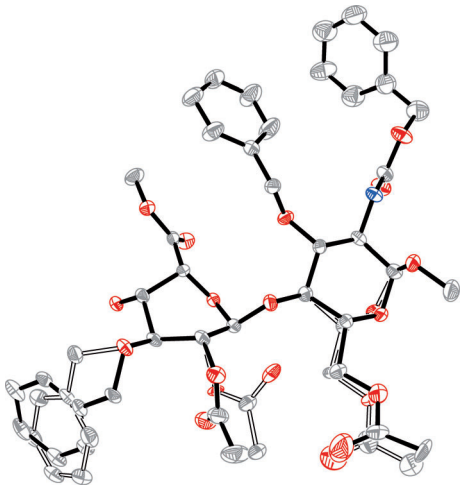

Figure 1. Disordered structure of **1** (**II**). Thermal ellipsoids are shown at 30 % probablilty and hydrogen atoms are omitted for clarity. C gray, O red, N blue.

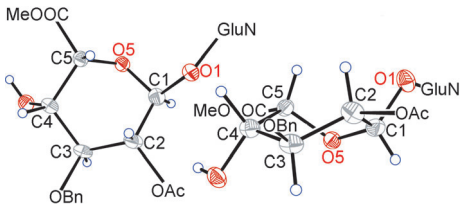

Figure 2. Top (left) and side (right) ORTEP drawings (thermal ellipsoids are shown at 20% probablilty) of the ^2S_O skew-boat conformation of L-IdoA in form **II**. Bn = benzyl, GluN = glucopyranose.

0.69(1) Å; Figure 2). The Rietveld refinement of form **I** (Figure 3,[15] and the Supporting Information) revealed the molecular structures in both modifications to be essentially similar. This similarity also extends to the packing, which consists of slightly waved layers perpendicular to [001] (Form **I**) and [010] (Form **II**; Figure 4). The Cremer–Pople parameters for the L-IdoA moiety in **II** are: $\phi,\theta,Q = 145.674, 94.714, 0.748°$, and those in **I** are: $\phi,\theta,Q = 156.945, 95.153, 0.719°$, values typical of a pure ^2S_O conformation.

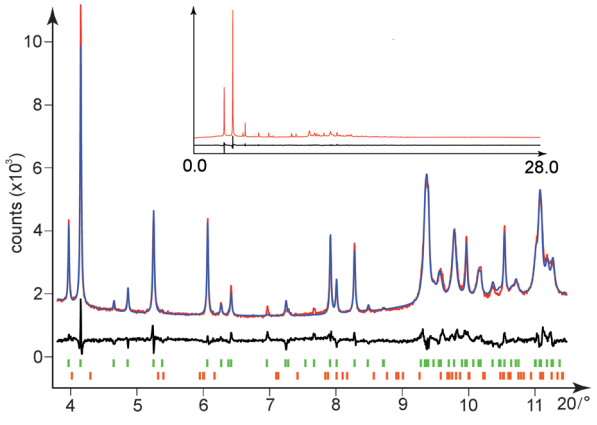

Figure 3. Details of the observed (red), calculated (blue), and difference (black) profiles from the Rietveld refinement of form **I**. Marks indicate reflections of form **I** (green) and **II** (red).

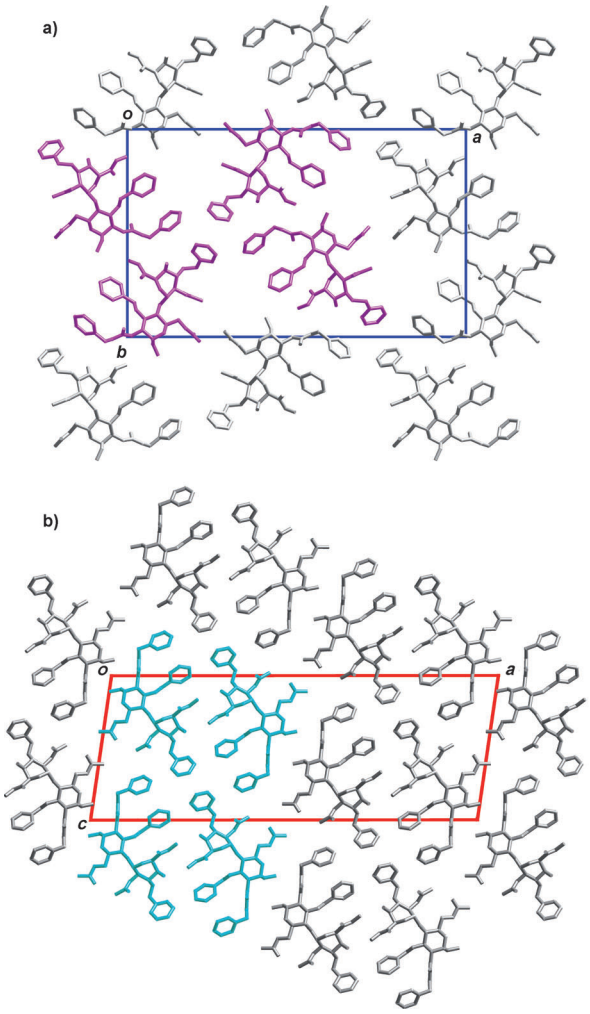

Figure 4. Layers of **1** contain C–H···π connected phenyl rings: four in form **I** (a), and six in form **II** (b). Adjacent layers are stabilized by N–H···O and C–H···O hydrogen bonds.

The vicinal coupling constants ($^3J_{1,2} = 6.5$, $^3J_{2,3} = 10.5$, $^3J_{3,4} = 3.0$, $^3J_{4,5} = 3.5$ Hz) were computed from the refined proton positions, at low temperature (**II** at 110 K), in the L-IdoA moiety in **1**.[18] It is revealing to see that experimental solution coupling constants[19] approach our solid-state ^3J constants ever more closely as a function of the increasing percentage of ^2S_O in various mixtures of $^1C_4$, $^4C_1$, and ^2S_O conformers.[19] This approach and the corresponding increase in the gap between the values of the $^3J_{2,3}$ and $^3J_{3,4}$ constants, which should be as large as possible for a high percentage of ^2S_O,[5] both corroborate the validity of our result (compare 7.54, 3.56 for 64 %; 8.3, 4.2 for 69 %; and 9.4, 4.4 for 78 %[19] with 10.5, 3.0 for our 100 % ^2S_O).

A unique property of **1** could be observed by comparing the L-IdoA geometry in the solid state with the geometry of other oligosaccharides, which contain iduronic acid with and without protecting groups; these geometries were studied by X-ray diffraction.[20] L-IdoA crystallizes in the $^1C_4$ or $^4C_1$ conformation in all di-, tetra-, and even pentasaccharides we have investigated. It therefore appears that bulky substituents do not necessarily imply a flat ^2S_O conformation for L-IdoA.

The planarity of the molecule shown in both crystal structures of **1** seems to be favored as a result of a significant number of C–H···O hydrogen bonds between the stacked L-IdoA, although we cannot exclude the possibility that the aromatic nature of some of the peripheral substituents could also play a part.

To further assess if the L-IdoA conformation found in both polymorphs of **1** is retained in solution, we studied the compound by NMR spectroscopy in fifteen solvents, including those used for crystallization. In each solvent all the coupling constants indicate a clear chair conformation for the L-IdoA moiety (mostly $^1C_4$; see the Supporting Information). Therefore, in contrast to other synthetic iduronic acids, which are designed to maintain a skew-boat conformation,[4,21] the geometry of the L-IdoA moiety in **1** appears unconstrained in solution.

To conclude, even though **1** must be considered unnatural, its L-IdoA moiety nevertheless adopts the three conformations $^1C_4$, $^4C_1$ (in solution), and ^2S_O (in the solid state) systematically observed for nonsubstituted (i.e. natural) L-IdoA moieties in heparin fragments. In view of the fact that this L-IdoA has a chair conformation in the liquid state, the fact that the ^2S_O geometry occurs in **1** is surprising and that it prevails in both polymorphs is even more so. The polymorphic structures of **1** therefore contain valuable information of interest toward several disciplines, such as fine-tuning of force fields, nucleation theory, structure prediction, and last but not least, our ^2S_O conformation might provide a new reference model for the structure of L-IdoA.

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[11] Crystal data for **1** form **II**: $C_{40}H_{47}NO_{15}$, *C*2, $M_r = 781.79$, $a = 47.1186(11)$, $b = 4.9602(3)$, $c = 17.309(3)$ Å, $\beta = 103.065(3)°$; $V = 3940.7(4)$ Å^3; $Z = 4$; $\rho_{calcd} = 1.318$ g cm^{-3}; $\mu(K\alpha) = 0.849$ mm^{-1}; $T = 110$ K; $2\theta_{max} = 127.5°$; 4434:3339 measured/independent reflections; $\lambda = 1.54178$ Å. Refinements:[12] R $[I > 2\sigma(I)] = 0.0683$, $R_{int} = 0.0489$; S(all data) = 1.091 for 3339 reflections; 711 parameters and 875 restraints;, largest peak/hole 0.33:–0.28 e Å^{-3}. A riding model was used for hydrogen atoms, except for those on the L-IdoA ring, which were refined isotropically. CCDC 755555, 835351, and 844630 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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