

The accordance with experiment is within  $\simeq 4\text{kJ/mol}$ , but these results are of particular significance because they correctly predicted the preference of  $\alpha\text{-D-Ido}$  for the inverted chair conformer.

The only other pyranose for which experimental measurement of inverted chair population via NMR could be feasible is altrose. Some NMR conformational studies regarding  $\alpha\text{-D-Alt}$  in macrocycles<sup>68,69</sup> in solution show indeed that  ${}^4\text{C}_1$ ,  ${}^1\text{C}_4$ , and  ${}^0\text{S}_2$  have roughly the same population. To the best of our knowledge, however, no results about altrose monosaccharide conformer populations have ever been published (NMR measurements on altrose are currently being carried on in our laboratory, and preliminary results<sup>70</sup> seems to be compatible with the theoretical predictions of Refs. 62 and 63). For the other pyranoses considered in this work, only lower bounds can in principle be determined from the sensitivity of experimental probes. In this sense, semi-empirical methods predict correctly the extremely high free energy of inverted chairs of glucose, galactose and mannose.

Some more recent experiments involving atomic force microscope (AFM) spectroscopy have allowed researchers to estimate the puckering free energy of conformers different from the chair ones. Marszalek, Lee, and coworkers<sup>44,71,72</sup>, for example, estimated the puckering free energy of glucose twisted boats employing AFM pulling on dextran, an  $\alpha\text{-(1}\rightarrow\text{6)}$  linked glucan. The conformational changes involved in the elongation of this polysaccharide are quite complex, involving ring deformation due to its intrinsic elasticity, rotations around the C5–C6 bond and chair to twisted boat transitions. In order to isolate these different contributions, the authors performed AFM pulling also on  $\beta\text{-(1}\rightarrow\text{4)}$ -D-Glc (cellulose) and on a  $\beta\text{-(1}\rightarrow\text{6)}$  linked glucan, namely, pustulan. These polysaccharides are both homopolymers of glucose like dextran but in the elongation process, due to the particular linkages, cellulose presents (apart from chain flexibility) only ring deformation, while pustulan presents both ring deformation and rotation around the C5–C6 bond, but no transition to the twisted boat conformer. By subtracting the free energy differences related to the various processes, the puckering free energy of the glucose twist boat was estimated to be about  $25\text{ kJ/mol}$ <sup>72</sup>. It is worth mentioning, that AFM spectroscopy was first employed to estimate puckering free energies of glucose boats conformers on carboxymethyl amylose (CMA) by Marszalek and coworkers<sup>73</sup>, and Li and coworkers<sup>74</sup>, independently. The two groups reported values of the boat free energy which were in agreement and in the range  $15 - 18\text{ kJ/mol}$ . These results differ considerably from the estimate of twisted boat free energy obtained from the analysis