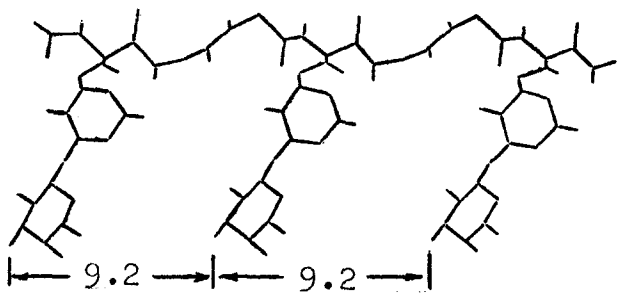

carbo-carbohydrate interactions, but also the one, in maximum degree blocking the centres of crystallization on the surface of the embrional stage of ice along the axis of this preferential growth by the compete formation of hydrogen bonds with them due to conformational accordance of terminal groups of Gal-residues and totally active structure AFGP with crystallographic latter of the ice I.

S9.8

Modeling Carbohydrate Conformations Using the Maximum Entropy Principle

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Modeling oligosaccharides is a difficult task, as they generally exhibit conformational flexibility. The modeling process requires experimentally determining a sufficiently large number of molecular constraints. Typical constraints resulting from NMR spectroscopic measurements are averaged internuclear distances (from cross relaxation rates) and torsional angles (from vicinal *J*-couplings). Since this procedure for oligosaccharides invariably results in underdetermined conformations, help is typically invoked from theoretical computational modeling. For example, some of our previous efforts to determine oligosaccharide conformations were based on a "distance mapping" procedure [1], including hydroxyl protons as "long-range conformational sensors" [2], or on a combined NMR – Monte Carlo analysis [3]. These procedures are all biased because a necessarily imperfect force field is used to describe the oligosaccharide. Here we report on an attempt to overcome this basic limitation by applying the maximum entropy principle. This procedure yields an explicit description of the probability distribution of an oligosaccharide in conformational space. The procedure uses experimental constraints in such a way that the distribution remains "maximally noncommittal" with respect to missing information. We applied this methodology to gentiobiose [Glcβ(1→6)Glc] to characterize the conformational flexibility around the C5-C6 bond. Taking advantage of a relatively large data set of experimentally determined constraints, our results describe a conformational flexibility around the C5-C6 bond of gentiobiose that differs significantly from the results of traditional rotameric distribution calculations based on analysis of proton-proton vicinal *J*-couplings by a Karplus-type equation.

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S9.9

Structural Characterisation of the Sulphated Saccharide Epitopes of Bovine Pro-Opiomelanocortin

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The *N*-terminal glycopeptide of pro-opiomelanocortin (POMC), designated as the 16 K fragment, is highly conserved throughout vertebrates and is therefore believed to have important biological functions. The 16 K fragment isolated from bovine pituitaries is *N*-glycosylated at Asn-65 and *O*-glycosylated at Thr-45. *O*-Glycosylation is thought to be involved in the tissue specific processing of the 16 K fragment in the anterior pituitary gland[1] but functions for the *N*-glycans have not been established although it is possible that they have a role in targeting and/or clearance. The possible biological significance of the glycosylation prompted us to initiate a complete structural characterisation of both the *N*- and *O*-glycan populations[2].

Fast Atom Bombardment-Mass Spectrometry (FAB-MS) analyses of the permethyl and peracetyl derivatives of the *N*-glycans released by PNGase F revealed the presence of both sulphated and neutral biantennary complex type structures. In conjunction with sugar and linkage analysis, the sulphated antennae was characterised as SO_4-4GalNAc1-4GlcNAc1-2Man, identical to the previously defined recognition signal which mediates the clearance of pituitary glycohormones carrying this moiety. In contrast to other pituitary glycohormones, this sulphated sequence is, however, located exclusively on the 3-arm of biantennary structures. In addition, a significant portion of the *N*-glycans also carries the Lewis x epitope together with a variety of other antennae including a rarely found fucosylated GalNAc-GlcNAc structure. These structure differences probably reflect the absence of a tripeptide motif in bovine POMC which fully conforms to the criteria previously defined for the recognition sequence for the specific *N*-acetylgalactosamine transferase[3]. FAB-MS analyses also identified the presence of sulphated *O*-glycans, not previously reported for any of the glycohormones, in addition to neutral glycans. The compositions of the major *O*-glycan structures were defined as $(SO_4)_1Hex_1HexNAc_3$ and $Hex_2HexNAc_2$.

Characterisation of the complete structures of both the neutral and sulphated *O*-glycans is being effected using a combination of enzymatic and GC-MS analyses.

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S9.10

Identification and Structural Analysis of the Tetrasaccharide NeuAcα2→6Galβ1→4GlcNAcβ1→3 Fucα1→*O* Linked to Ser-61 of Human Factor IX