

new applications for starch although many of these opportunities remain to be explored. Areas of interest are packaging, textiles, controlled release, cosmetics, pharmaceuticals and flocculation.

### Carbohydrate Chemistry Focused on Agrochemical Application

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In the last few years we have been devoted to the synthesis of biomolecules potentially interesting as agrochemicals. We now describe the preparation of the sugar moiety of Amipurimycin, of Miharamycin and of its analogues. These two molecules are antibiotics known to inhibit *Pyricularia oryzae*, responsible for the rice blast disease.

Pseudo-C-nucleosides are another group of compounds which have been investigated by us, in order to obtain new structures possessing bioactivity. In this work we also describe the synthesis and bioactivity of some thiazoles, tetrazoles, triazoles and pyrazoles, obtained by chain elongation of some sugar precursors.

The  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone unit is known from the literature to confer a great diversity of biological effects. We present the synthesis of fungitoxic sugar molecules, containing this unit in their structure, via Reformatsky-type reaction of the appropriate carbonyl compounds with ethyl  $\alpha$ -bromo-methyl acrylate and zinc. The relationship between structure, conformation, configuration of the molecules and the bioactivity detected will be evaluated.

### Carbohydrate Liquid Crystals

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Emil Fischer reported the *double melting points* of some long chain n-alkyl pyranosides, but failed to connect the observation with liquid crystal formation. It was not until nearly forty years later that these compounds were shown to form liquid crystals. This observation seemed to be overlooked, since some thirty years later none of the text books or reviews of the subject mentioned carbohydrates. The revival of interest came in 1984 when 17 examples were reported. Now a recent Liquid Crystal Data Base lists over 2000 Carbohydrates.

Liquid crystals fall into two classes; thermotropic and lyotropic. The thermotropic may be calamatic, discotic or chiral. The lyotropic phases, formed on contact with water, may be laminar, cubic or hexagonal.

Carbohydrates which illustrate these phases will be described. The potential commercial uses will be discussed.

### Carbohydrate Polymers as Wound Management Aids

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Polymeric materials composed of carbohydrate units, i.e. polysaccharides, are a diverse group of biological macromolecules that are showing increasing application in areas of wound management. A variety of neutral polysaccharides, e.g. dextran and starch, basic polysaccharides, e.g. chitin and chitosan, acidic polysaccharides, e.g. alginic acid and hyaluronic acid, and glycosaminoglycans, e.g. dermatan sulphate and heparin, and their respective derivatives, have been the focus of much interest with respect to biomedical and particularly woundcare applications over recent years, however there has been no directive that any one chemical structure is more efficacious than any other. To be suitable as a wound management aid a dressing material should exhibit a number of properties including abilities to: maintain high humidity at the wound-dressing interface provide protection against secondary infection, be able to remove excess wound exudate and toxic components, and maintain its strength when sterile and wet.

More recent investigations have examined some of the more unusual polysaccharides isolated from plant, bacterial and animal sources which possess potentially useful biological properties that may make them suitable for woundcare applications, e.g. arabinoxylan and  $\beta$ -D-(1  $\rightarrow$  3)-glucan derivatives. The precise structures of such unusual polysaccharide based materials are often unknown and detailed compositional and structural characterisation is required in order to satisfy regulatory authorities. The carbohydrate structure of a novel polysaccharide based material, namely Sterigel<sup>®</sup> (Seton Healthcare), used as a wound management aid has been determined. Enzymic hydrolysis and methylation analysis have shown the carbohydrate structure to be a highly substituted  $\beta$ -D-(1  $\rightarrow$  4)-xylan. This polysaccharide backbone is substituted with  $\alpha$ -L-arabinofuranoside residues and  $\alpha$ -D-glucopyranosyluronic acid residues. The total amino acid content of the Sterigel, as determined after acid hydrolysis, is 4.4% w/w with the amino acid hydroxyproline accounting for 0.22%. The cinnamic acid derivative ferulic acid has been identified in both alkaline (0.40%) and enzymic (0.25%) hydrolysates of the polysaccharide.

### Carbohydrate Substituted Porphyrins. Synthesis, Characterization and Lipoprotein Binding Properties

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The development of new photosensitisers for photodynamic therapy of tumours (PDT) is one of the most important fields of porphyrin chemistry today. PDT is a treatment that is based on the selectivity of porphyrinic compounds to tumour tissue and on the production of singlet oxygen by irradiation of the sensitiser with visible light. Thus formation of singlet oxygen in tumour cells causes cell death and tumour necrosis. Besides long wavelength absorption solubility and water and high selectivity to tumour cells are requirements a new photosensitiser has to fulfill. Therefore a number of different carbohydrate substituted porphyrins were synthesised. Although the mechanism of sensitiser uptake is not yet clarified, there is evidence that amphiphilic porphyrins associate to LDL and are introduced into the tumour cell via receptor mediated endocytosis.

We have synthesised some amphiphilic carbohydrate substituted porphyrins with different carbohydrate moieties. The compounds were synthesised by reaction of glycosyl imidates with the Nickel complex of 5-(4-hydroxymethylphenyl)-2,8,13,17-tetraethyl-3,7,12,18-tetramethylporphine in good yield and characterized by NMR and MS-spectroscopy. Investigation of the binding constant to different plasma proteins (LDL, HDL, VLDL) revealed, that the carbohydrate subunit is of great importance for the binding properties. Furthermore, time-resolved fluorescence spectroscopic measurements confirm that only a small amount of the porphyrinic sensitizer is associated with the apoprotein unit and most of the sensitizer is incorporated into the lipid compartment. These results are of great importance for the development of new sensitizers with enhanced tumour cell selectivity. Binding of sensitizers to the apoprotein unit may alter the interaction of LDL with cancer cells and has to be avoided.

### Cellulases in the Textile Industry – An Overview

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Cellulases are well established in textile wet processing as agents for fibre and fabric surface modification. The most known applications are the ageing of fabric surfaces, like the stone washed look of Denim garments, and also the cleaning and renewing of fabric surfaces from microfibrils, fuzz and loss fibres. Apparently these opposite effects can be obtained with the same enzymes. However, cellulases are a multi-component enzyme system, with endoglucanases (EGs) that hydrolyze randomly cellulose chains, cellobiohydrolases (CBHs) that hydrolyze cellobiose from cellulose ends and cellobiases that hydrolyze cellobiose to glucose. The different effects can be obtained with different enzyme compositions, EG or EG rich preparations are best for ageing and defibrillation of fibre surfaces while complete cellulase systems are best for cleaning and dippilling effects. The finishing effects delivered by cellulases are always obtained in process (rotating drum washers and jets) where strong mechanical agitation into the fabrics are provided during the treatments. In this paper a overview is done about the actual knowledge of the processes and future directions of this research field.

### Construction of Recombinant BHK Cell Lines Expressing Wild-type and Mutants of Human $\alpha$ 1,3/4-fucosyltransferase

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Stable BHK-21 cell lines were constructed expressing i) the wild-type form of human  $\alpha$ 1,3/4-fucosyltransferase (FT3T2), ii) the secretory form of the enzyme where amino acids 46-361 (S2FT3TS) were coupled at their amino terminus to the signal sequence of interleukin-2, and iii) a membrane bound form (FT3NPT2) where the amino acid residues Cys-16, Gln-23, Cys-29, and Tyr-33 from the transmembrane domain of the enzyme were replaced by Leu residues.

Cell lines expressing similar amounts of total fucosyltransferase activity were used to localize the three constructs

by immunofluorescence microscopy studies. The S2FT3T2 was detected as small vesicles in the cells. The FT3T2 was found to be present within the Golgi and trans-Golgi-network. Most of the FN3NPT2 was detected on the plasma membrane of the recombinant cells. These results suggest that the amino acid residues Cys-16, Gln-23, Cys-29 and Tyr-33 residues of the transmembrane domain of the  $\alpha$ 1,3/4-fucosyltransferase specify location of the enzyme in the Golgi.

The S2FT3T2 was purified on GDP-Fractogel resin and its specificity towards oligosaccharides, N-glycans, glycolipids, glycopeptides and glycoproteins was studied. The soluble forms of  $\alpha$ 1,3/4-fucosyltransferase may be used for *in vitro* synthesis of the Lewis<sup>x</sup> determinant on carbohydrates and glycoproteins, whereas Lewis<sup>x</sup> and sialyl-Lewis<sup>x</sup> structures cannot be synthesized.

### Cyclodextrins, Supramolecular Devices for Drug Transport and Targeting

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Cyclomaltooligosaccharides (cyclodextrins, CDs) are almost ideal supramolecular devices for the bioavailability enhancement of bioactive compounds, in view of their almost starch like innocuity, and their ability to form inclusion complexes with a wide variety of poorly water soluble molecules, and their recent availability at low costs from well handled biotechnological processes. Problems with such carriers are however still encountered, related with their relatively low solubility in water which affects their solubilization properties, relatively high hemolytic character at least for the more common heptose entity which limit their parenteral use, and their absence of recognition sites *in vivo*.

Selective chemical modification, still almost restricted to the narrower primary hydroxyl side of the torus, have been designed in order to overcome these shortcomings. Substitution at C-6, as with branched mono- and per-(6-O and 6-S) linked glycosyl-CDs enhances drastically the solubility and solubilization properties. Interestingly, the thiourea functionality, which was initially introduced as spacer, enhances by itself the solubility as shown with the 6<sup>l</sup>-methylthioureido derivative which shows solubility improvements  $\times 43$  as compared to  $\beta$ -CD, probably due to the hydrogen bond interaction between thiourea NH protons and water molecules. Bioactive compounds, belonging to various therapeutic classes, have been considered as guests in order to define the optimal parameters for their transport in biological fluids. Using NMR spectroscopy as a main tool, it was shown that a balance between inclusion parameters and solubilization properties had to take into account, not only the size of the cavity, but also the possibility of interaction with the primary hydroxyl bearing side of the torus. In situations where the stabilization of the complex involves the formation of hydrogen bonds, the 6<sup>l</sup>-branched derivative exhibits larger binding constants as compared to the persubstituted analog. In addition, when the guest compound interacts from the primary hydroxyls side of the host, as it is the case with the potent anticoagulant 2-phenylindane-1,3-dione, the steric hindrance of the C-6 substituent reduces the affinity. Conversely, the solubilization properties are greatly improved when the host-guest interaction occurs from the secondary hydroxyls side, as with the analgesic carbamazepine or the antidepressant dothiepine. Stabilisation of the trilactonic active form of the