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Foreword

Chemoenzymatic synthesis of carbohydrates

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This issue is devoted to the chemoenzymatic synthesis of carbohydrates, and the contributions to which can be grouped into three categories. The first deals with the synthesis of natural and unnatural monosaccharides and related substances, the second with the use of glycosidases, mainly in glycoside and oligosaccharide synthesis, and the third with the use of glycosyltransferases.

Since Whitesides' pioneering work 20 years ago, chemoenzymatic methods in carbohydrate chemistry have undergone extensive development and have proved to be very attractive for the synthesis of natural and unnatural monosaccharides as well as complex oligosaccharides involved in biological recognition processes. One of the best examples is the synthesis of the sialyl Lewis^x tetrasaccharide α-D-NeuAc-(2→3)-β-D-Gal-(1→4)-[α-L-Fuc-(1→3)]-D-GlcNAc achieved by Cytel Corporation on a kilogram scale using recombinant glycosyltransferases [1].

1. Synthesis of natural and unnatural monosaccharides and related substances

The aldolases represent the major class of enzymes investigated for use in monosaccharide synthesis. They can be classified into several groups according to the type of donor substrate required. The first group uses dihydroxyacetone phosphate (DHAP). The most representative enzyme of this group is fructose diphosphate aldolase (FDPA), which catalyses the reversible asymmetric aldol addition of DHAP and D-glyceraldehyde 3-phosphate to form fructose 1,6-diphosphate, creating a new C-3–C-4 bond with D-threo stereochemistry. Three other aldolases having different stereospecificities are also available for the asymmetric synthesis of carbohydrates [2]. The ability of these aldolases to accept a great variety of electrophilic substrates has been widely exploited in the synthesis of sugar analogs, such as sugars with nitrogen in the ring, that are potent glycosidase inhibitors [3]. On the other hand, aldolases are known to exhibit a strict specificity toward the nucleophilic donor. As a contribution to this issue, **Arth and Fessner** report that the phosphonomethyl substrate analog of DHAP can be used as a donor in aldolase-catalysed reactions to give sugar phosphonate analogs. However, the DHAP mimic was accepted by only three out of the six aldolases tested. The two first fructose diphosphate aldolases from rabbit muscle and *Staphylococcus carnosus* belong to class I, which bind the donor through Schiff base formation with an active-site lysine residue, whereas the third, the rhamnulose phosphate aldolase from *Escherichia coli*, belongs to class II, which activates the substrate by Zn^{2+}-dependent chelation.

The second group of aldolases uses pyruvate as the donor substrate. Aldolases of this class generate a new chiral center at C-4 leading to 3-deoxy-2-keto-ulosonic acids. The most important representative of this family is *N*-acetylneuraminic acid (NeuSAc). Following the published procedure of David et al. [4], whereby *N*-acetylmannosamine,

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obtained by alkaline epimerization of *N*-acetylglucosamine, reacts with pyruvate in the presence of immobilized sialyl aldolase, Glaxo–Wellcome is now producing this sialic acid on a ton scale. It is used as the starting material for the synthesis of a powerful inhibitor of the influenza A and B virus sialidase, 2,3-didehydro-2,4-dideoxy-4-guanidinyl-*N*-acetylneuraminic acid, which is currently under clinical trials [5]. Numerous sialic acid derivatives and analogs, including *N*-glycolylneuraminic acid and Kdn, the deaminated neuraminic acid discovered 10 years ago in trout eggs, have been prepared according to this procedure [6]. In an extension of this work, **Kong and von Itzstein** have synthesized 9-azido- and 9-bromo-Kdn from the corresponding 6-substituted mannose derivatives.

One of the most important enzymatic processes operated in the food industry is the conversion of D-glucose into 'high-fructose syrup' with immobilized glucose isomerase. Some years ago Stütz's group reported a novel synthetic application of this enzyme: the quantitative conversion of D-glucofuranose derivatives modified at position five into the corresponding D-fructopyranoses [7]. As an extension of this work, **Ebner and Stutz** describe in this issue the conversion of aldofuranoses such as D-and L-erythrose, and 5-azido- and 5-fluoro-deoxy-D-ribose, into the corresponding acyclic 2-ketoses. Interestingly the authors mention that the reverse reaction is not observed.

The paper by **Huwig et al.** describes work directed towards synthesis of a rare sugar, L-tagatose, from galactitol, making use of the galactitol dehydrogenase from *Rhodobacter sphaeroides* D in the presence of NAD, which is regenerated in situ using lactate dehydrogenase.

2. Glycosidases

Many contributions in this issue deal with glycosidases. Apart from the papers referring to the use of these enzymes in glycoside or oligosaccharide synthesis, two contributions propose original applications. The first one is by **Kappes and Waldmann**. Waldmann has been very much interested in enzymatic protecting-group techniques as alternatives to classical chemical methods [8]. In a search for enzyme labile protecting groups for the *N*-terminus of peptides, he has developed a new carbohydrate-derived protecting group that is compatible with peptide bond synthesis. This is the tetrabenzylglucosyloxycarbonyl group that can be cleaved, after hydrogenolysis, by means of α- or β-glucosidase. Indeed glycosidases are able to hydrolyze the bond between the anomeric carbon and the oxygen of a glycosyloxycarbonyl group attached to peptides releasing, without any undesired side reactions, the peptide with a free *N*-terminus. It is noteworthy that the strategy for antibody-directed enzyme prodrug therapy (ADEPT) is based on the same principle [9].

The second original contribution (Vulfson et al.) is concerned with diastereoselectivity of glycosidases. Glycosidases are well known to exhibit diastereoselectivity in transglycosylation reactions involving racemic and meso alcohols as acceptors [10], but there have only been a few examples describing diastereoselectivity in hydrolysis reactions. These authors have shown that glycosidases are able to discriminate between diastereomeric pairs of 6-substituted (sulfoxides, methyl, epoxides) *p*-nitrophenyl-β-D-galactopyranosides.

As an alternative to the use of glycosyltransferases implicated in the biosynthesis of the sugar chains of glycoproteins and glycolipids, glycosidases that cleave glycosidic bonds in vivo have been used for in vitro glycoside and oligosaccharide synthesis. Two broad approaches have been exploited: the thermodynamically controlled approach, exploiting reverse hydrolysis activity, or the kinetically controlled approach, based on the transglycosylation reaction. This latter generally gives better results, with yields ranging from 20% to 80% or higher, and has been intensively studied. It relies on the trapping of the covalent glycosyl enzyme intermediate with exogenous nucleophiles, such as alcohols, glycosides, monosaccharides or higher oligosaccharides. Suitable glycosyl donors for this reaction include disaccharides, aryl glycosides and glycosyl fluorides. Interestingly, it has been found that in oligosaccharide synthesis the regioselectivity of transglycosylation can be controlled by the anomeric configuration of the glycoside acceptor [11]. Moreover regioselectivity also depends on the source of glycosidases. Thus the β-galactosidase from *Bacillus circulans* preferentially catalyses (1→4) glycosidic bond formation. This has been exploited in the large-scale synthesis of *N*-acetyllactosamine from lactose and GlcNAc [12]. Six contributions in this issue are concerned with transglycosylation reactions, expanding the range of useful applications of this type of reaction. Although it cannot yet be regarded as a general

method, it has proved to be very efficient with particular enzymes. In most cases, thioethyl or thiophenyl glycosides have been chosen as acceptors because of their stability towards the hydrolytic activity of glycosidases, and because they can be activated to be used as building blocks in chemical syntheses of oligosaccharides.

Singh et al. previously reported an efficient preparation of *N*-acetylchitooligosaccharides using the β-*N*-acetylhexosaminidase of *Aspergillus oryzae* [13]. Following this work, they have studied the transfer of *p*-nitrophenyl β-D-*N*-acetylgalactosaminide onto thioethyl glycosides of disaccharides such as maltose, cellobiose and gentiobiose in reactions catalysed by the same enzyme. The β-(1→4) linked trisaccharide was exclusively formed when maltose was the acceptor (29% yield), whereas in both of the other cases β-(1→3) and β-(1→4) trisaccharide mixtures were obtained.

Using a series of glycosyl donors and acceptors, **Prade et al.** have carefully investigated the transglycosylation activity of the *Agrobacterium* sp. β-glucosidase, originally involved in the exo-glucosidic cleavage of cello-oligomers. The enzyme was found to catalyse totally regioselective transfer of galactose from *p*-nitrophenyl β-D-galactopyranoside in one case only, when thiobenzyl β-D-mannopyranoside was the acceptor substrate, to give the β-(1→4) disaccharide in 24% yield. On the other hand, starting from β-D-mannopyranosyl fluoride as donor and thiophenyl β-D-gluco- or xylo-pyranoside as acceptors, only β-(1→3)-linked disaccharides were obtained but in 8% yield.

Montero et al. have tested a battery of six galactosidases in transgalactosylation reactions using D- or L-xylose as acceptors and *o*-nitrophenyl β-D-galactopyranoside as donor. With bovine testicular galactosidase, regioselectivity of 85% in favour of the β-D-Gal-(1→3)-D-Xyl was obtained (39% yield), whereas with the *E. coli* galactosidase, a regioselectivity of 81% in favour of β-D-Gal-(1→4)-D-Xyl (43% yield) was observed.

Koizumi et al. report transgalactosylation experiments on a cyclic homogeneous oligosaccharide composed of eight α-(1→6)-linked glucose units. Once again, the *B. circulans* β-galactosidase produced only one transgalactosylated product identified as the branched cycloisomaltooligosaccharide monosubstituted by a β-galactose residue at O-2.

The thermodynamically controlled approach allowed **Ajisaka et al.** to prepare α-D-Man(1→2)-D-Man and β-D-GlcNAc-(1→2)-D-Man, making use of the *A. niger* α-mannosidase and the *B. circulans* β-*N*-acetylglucosaminidase, respectively. Despite very low yields, this enzymatic route provided the authors with enough material to use these oligosaccharide blocks as starting compounds for the chemical synthesis of branched tetra- and hexasaccharides.

In a different context, the results presented by **Yamamoto et al.** are especially promising. These authors have exploited the transglycosylation activity of *Mucor hiemalis* endo-D-*N*-acetyl-β-glucosaminidase (endo M), the role of which in vivo is to split *N*,*N*′-diacetylchitobiosyl linkages in sugar chains of *N*-glycoproteins. Endo M acts on high-mannose and hybrid-type, as well as on complex-type asparagine-linked oligosaccharides. Yamamoto et al. report the transfer of the diantennary sialyl oligosaccharide of the glycopeptide isolated after pronase digestion of human transferrin, to chemically synthesized *N*-acetylglucosaminyl Peptide T. Peptide T, which can block infection of human T cells by human immunodeficiency virus, was thus glycosylated with a sialo diantennary complex-type oligosaccharide. Interestingly, the glycosylated peptide T showed a much higher resistance towards protease digestion than the non-glycosylated native peptide. With another related enzyme of specificity restricted to the high-mannose type, endo A, the synthesis of a glycopeptide analog with a C-glycosidic linkage has been recently reported [14]. This chemoenzymatic approach might have great potential in the future in the synthesis of useful neoglycopeptides and neoglycoproteins.

3. Glycosyltransferases

Unverzagt was able to prepare the sialyl diantennary *N*-glycan linked to asparagine that Yamamoto et al. obtained from transferrin hydrolysis. Four out of the eleven sugar units (two galactoses and two sialic acids) were transferred enzymatically at the end of the synthesis onto the chemically prepared heptasaccharide asparagine derivative. These four enzymatic steps were conducted as a one-pot reaction in 86% overall yield. Indeed glycosyltransferases are the catalysts of choice for elongation at the non-reducing end of the carbohydrate chains of glycoconjugates. A nice illustration is described in the contribution by Roy et al. In order to address the problem of the role of

multivalency in selectin-sialyl Lewis^x interactions, these authors have synthesized *N*-acetylglucosamine-containing dendrimers, which were subsequently elongated with glycosyltransferases (galactosyl, sialyl, fucosyl) affording di-, tetra- and octa-valent sialyl Lewis^x ligands scaffolded on dendrimers.

Chain extension with glycosyltransferases was also achieved using carbohydrate substrates linked to water-soluble glycopolymers by **Yamada et al.** Of special interest was the study of the effect of the length of the spacer arm on the efficiency of enzymic glycosylation. Quantitative galactosylation could be only achieved with a long linker. Moreover one type of polymer containing an L-phenylalanine residue in the spacer-arm moiety could be successfully cleaved with α-chymotrypsin, releasing the oligosaccharide under very mild conditions.

Another example of enzyme-assisted synthesis of oligosaccharide chains is the contribution by **Hokke and van den Eijnden**. These authors have modified diantennary glycopeptides obtained by pronase digestion of desialylated bovine fibrinogen, by the sequential action of β-galactosidase, β-(1→4)-galactosaminyltransferase, α-(2→6)-sialyltransferase and α-(1→3)-fucosyltransferase. In this way, they have prepared a variety of *N*-glycans containing the β-D-GalNAc 4)-D-GlcNAc sequence, which specifically occurs on glycodelin A, a glycoprotein isolated from amniotic fluid, with interesting immunosuppressive and contraceptive properties. It is planned to test these *N*-glycans as inhibitors in sperm–egg binding assays.

The glycosyltransferases involved in these reactions, enzymes of the so-called Leloir pathway, require sugar—nucleotides as glycosyl donors. Regeneration in situ is the elegant way of using these cofactors [1], but this necessitates the use of additional enzymes, which is not always advantageous, especially for *N*-acetylneuraminic acid that is activated as the nucleoside monophosphate and not as the diphosphate. Accordingly, efficient chemical and enzymic syntheses of the eight sugar nucleotides, UDP-Glc, UDP-Gal, UDP-GalNAc, UDP-GalNAc, UDP-GlcNAc, UDP-GlcUA, GDP-Man, GDP-Fuc, CMP-NeuAc, have been developed [15]. Two papers in this issue are concerned with this topic. **Bülter et al.** report a novel synthesis of UDP-GalNAc as a new application of the plant glycosyltransferase sucrose synthetase. This; enzyme catalyses the synthesis of sucrose from UDP-Glc and fructose, but, owing to the reversible nature of the reaction it has been used also for the preparation of UDP-Glc (or other activated sugars) from sucrose and UDP (or other nucleoside diphosphates) [16]. The sucrose synthetase used, together with six other enzymes, all commercial, allowed the authors to achieve from UMP, phospho-enol pyruvate, sucrose and α-D-galactosamine-l-phosphate, an efficient preparation of UDP-GalNH_2, which was subsequently chemically acetylated. The contribution by **Fey et al.** deals with the enzymatic production of GDP-Man, starting from chemically prepared α-D-mannose 1- phosphate, GTP, and using recombinant GDP-Man pyrophosphorylase from *Salmonella enterica* expressed in *E. coli*, in addition to inorganic pyrophosphatase. The productivity of the process could be greatly improved using engineering techniques.

Glycosyltransferases are known to be very specific both for the sugar-nucleotide glycosyl donor and the acceptor sugar, which in most cases is a disaccharide. For example, up to seven fucosyltransferases, all acting on the disaccharide sequences β-D-Gal-β-(1→3/4)-D-GlcNAc, but with different specificities, are known [17]. A common feature of the family of enzymes transferring fucose onto the GlcNAc residue is that they do not tolerate substitution on position-6 of the galactose residue, indicating that the corresponding hydroxyl group is the key polar group essential for attachment of the substrate in the active site [18]. These data were obtained from substrate-mapping studies. Palcic et al. such a study using calf thymus α-(1→3)-galactosyltransferase, which typically transfers galactose to the non-reducing end of *N*-acetyllactosaminyl disaccharide. Ten new analogs of the trisaccharide α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc-OR, potential inhibitors of the anti-α-Gal antibody and toxin A of *Clostridium difficile*, could be prepared.

Stringent enzyme specificity can be exploited in particular syntheses, as illustrated in the contribution by **Räbinä et al.** Using human milk α-(1→3/4) fucosyltransferase together with β(1→4) galactosyltransferase and human placental α-(2→3) sialyltransferase, these workers have synthesized two dimers of *N*-acetyllactosamine sialylated at the non-reducing end and fucosylated either at the distal or the proximal end of the oligosaccharide, and the trimer sialylated at the non-reducing end and difucosylated at the other terminus. This work was based on the specificity of the fucosyltransferase

that does not accept as substrate a free GlcNAc residue or a GlcNAc linked to a bisubstituted galactose.

Human milk α-(1→3/4) fucosyltransferase, which is in fact a mixture of two enzymes [19], has been widely used in synthesis. The paper by **Lubineau et al.** reports a straightforward preparative synthesis of 3^{IV}, 6^{III} disulfated Lewis^x pentasaccharide, a good candidate ligand for human L-selectin. This synthesis was achieved in height steps from the lactosyl derivative precursor, using this enzyme together with β-galactosyltransferase. It is worth mentioning that the fucosyltransferase used was adsorbed on SP-Sephadex.

Glycosyltransferases may display some flexibility with respect to their substrate requirements, especially in vitro when the substrates are provided in high concentration. This point is exemplified by two contributions. **Stangier et al.** describe the synthesis of modified Lewis^a and Lewis^x trisaccharides, with L-galactose and 3-deoxy-L-galactose residues instead of L-fucose, based on the transfer of these sugars from the corresponding sugar nucleotides catalysed by human milk fucosyltransferase. **Kren et al.** have exploited the fact that UDP-Glc can serve as the donor substrate for bovine galactosyltransferase. They prepared glucosylated chitooligomers that were of interest in measurements of the affinity in binding to natural killer-cell protein NKR-PlA. Glycosylated alkaloids have been found to be substrates for glycosyltransferases [20]. In relation to the galactosylation of poorly water soluble molecules, the contribution by **Riva et al.** describing a systematic study of the effects of various organic solvents on activity and stability of β-(1→4)-galactosyltransferase is of particular interest.

Cloning and overexpression of glycosyltransferases has made these enzymes readily available in soluble form and quantity. This improved availability makes it possible to test various substrates, sometimes very different from the natural ones. In some cases, unexpectedly broad substrate tolerance was observed. For example, and surprisingly, fucosyltransferases III and VI have been found to recognize fucosyl donors in which the natural guanosine has been replaced by other nucleosides [21].

The contribution by **Watt et al.** describes the use of a recombinant β-mannosyltransferase expressed in *E. coli*, adsorbed on a nickel(II)-charged affinity column, for the synthesis of the core trisaccharide of *N*-linked glycoproteins, β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-D-GlcNAc.

Non-Leloir transferases, typically using glycosyl phosphates as glycosyl donors, also have synthetic applications. **Percy et al.** have evaluated different sugars, D-arabinose, D-altrose and L-fucose as acceptors in synthetic reactions of cellobiose phosphorylase from *Cellvibrio gilvus*. Thus the disaccharide β-D-Glc-(1→4)-D-Alt could be prepared from α-D-glucose-l-phosphate and D-altrose in 19% yield.

Among these transferases, dextransucrase has until now attracted the greatest attention in synthetic applications. This is a glucosyltransferase that catalyses the synthesis of dextran from sucrose, but which can also catalyse the transfer of glucosyl units to an auxiliary acceptor such as maltose, thereby generating oligosaccharides. Thus oligosaccharides terminating in α-(1→2) glucosyl residues at the non-reducing end and resistant to glucoamylase are now produced on an industrial scale by the action of *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase on maltose and sucrose [22]. In their article **Dols et al.** have further characterized these oligosaccharides using MALDI-TOF mass spectrometry and NMR spectroscopy.

Of great interest is the contribution by Driguez's group concerning cyclodextrin-α-(1→4)-glucosyltransferase (CGTase). CGTase catalyses not only the cyclization of starch and oligosaccharides to give mixtures of cyclic oligosaccharides (α, β, γ cyclodextrins) but also the cyclization of α-D-glucosyl fluoride [23]. **Bornaghi et al.** report the CGTase-catalysed conversion of 4-thio-α-maltosyl fluoride into cyclo α-(1→4^2)-4-thiomalto-tetraoside, -pentaoside and -hexaoside, in addition to linear thiomaltooligosaccharides, in which *S*- and *O*-glycosidic bonds alternate. Using the same strategy, but starting from 4-*C*-α-maltosyl fluoride, the synthesis of *C*-maltooligosaccharides (dimer, trimer and tetramer) could be also achieved. However, in this case no cyclic molecules were observed. To our knowledge, this is the first report of the enzymatic preparation of oligosaccharides containing *C*-glycosidic bonds using non-Leloir transferases.

References

[1] H.J.M. Gijsen, L. Qiao, W. Fitz and C.-H. Wong, *Chem. Rev.*, 96 (1996) 443–473.

[2] W.D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G.E. Schulz, J. Badia and J. Arguilar, *Angew. Chem., Int. Ed. Engl.*, 30 (1991) 555–558.

[3] K.K.-C. Liu, T. Kajimoto, L. Chen, Z. Zhong, Y. Ichikawa and C.-H. Wong, *J. Org. Chem.*, 56 (1991) 6280–6289.
[4] S. David, C. Augé and C. Gautheron, *Adv. Carbohydr. Chem. Biochem.*, 49 (1991) 176–237.
[5] I.D. Starkey, M. Mahmoudian, D. Noble, P.W. Smith, P.C. Cherry, P.D. Howes and S.L. Sollis, *Tetrahedron Lett.*, 36 (1995) 299–302.
[6] C. Augé and C. Gautheron-Le Narvor, in S. Hanessian (Ed.), *Preparative Carbohydrate Chemistry*, Marcel Dekker, New York, 1997, pp 469–484.
[7] A. Berger, A. Deraadt, G. Gradnig, M. Grasser, H. Low and A.E. Stütz, *Tetrahedron Lett.*, 33 (1992) 7125–7128.
[8] M. Schelhaas and H. Waldmann, *Angew. Chem.*, Int. Ed. Engl., 35 (1996) 2056–2083.
[9] D.B.A. de Bont, R.G.G. Leenders, H.J. Haisma, I. van der Meulen-Muileman and H.W. Scheeren, *Bioorg. Med. Chem.*, 5 (1997) 405–414.
[10] D.H.G. Crout, D.A. MacManus and P. Critchley, *J. Chem. Soc., Perkin Trans.*, I, (1991) 376–378.
[11] K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
[12] G.F. Herrmann, U. Kragl and C. Wandrey, *Angew. Chem., Int. Ed. Engl.*, 32 (1993) 1342–1343.
[13] S. Singh, J. Packwood, C. J. Samuel, P. Critchley and D.H.G. Crout, *Carbohydr. Res.*, 279 (1995) 293–305.
[14] L.-X. Wang, J.-Q. Fan and Y.C. Lee, *Tetrahedron Lett.*, 37 (1996) 1975–1978.
[15] J.E. Heidlas, K.W. Williams and G.M. Whitesides, *Acc. Chem. Res.*, 25 (1992) 307–314.
[16] A. Zervosen, A. Stein, H. Adrian and L. Elling, *Tetrahedron*, 52 (199) 2395–2404.
[17] S. Sueyoshi, S. Tsuboi, R. Sawada-Hirai, U.N. Dang, J.B. Lowe and M. Fukuda, *J. Biol. Chem.*, 269 (1994) 32342–32350.
[18] S. Gosselin and M.M. Palcic, *Biorg. Med. Chem.*, 4 (1996) 2023–2028.
[19] P.H. Johnson, A.S.R. Donald, J. Feeney and W.M. Watkins, *Glycoconjugate J.*, 9 (1992) 251–264.
[20] V. Kren, A. Fiserova, C. Auge, P. Sedmera, V. Havlicek and P. Sima, *Biorg. Med. Chem.*, 4 (1996) 869–876.
[21] G. Baisch, R. Ohrlein and A. Katopodis, *Biorg. Med. Chem. Lett.*, 6 (1996) 2953–2956.
[22] F. Paul, A. Lopez-Munguia, M. Remaud, V. Pelenc and P. Monsan, US Patent 5 141 858 (1992); *Chem. Abstr.*, 112 (1990) 215440.
[23] W. Treder, J. Thiem and M. Schlingmann, *Tetrahedron Lett.*, 27 (1986) 5605–5608.