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Facing Glycoscience

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Abstract: This essay describes an approach facing some of the challenges of glycoscience, concentrating on the relation between structure and reactivity. This has resulted in new methods, precursors of carbohydrate-derived reactive intermediates, analysis of their reactivity, and application mostly in synthesis. It has also led to the design, synthesis, and use of enzyme inhibitors, and to novel carbohydrate mimics and nucleotide analogues.

Keywords: Carbohydrates · Diazirines · Glycosidase inhibitors · Nucleotides · Reactive intermediates

No account of our interests, short as it has to be, can start without mentioning the attraction of carbohydrates. Carbohydrates are *en vogue* – more than ever, and increasingly so. For this, there are many good reasons: the enormous diversity of their structure and functions, increasing information about their biological functions, the deepening of our understanding of the relation between structure and reactivity, the interest in enantiomerically pure, versatile building blocks for the synthesis of compounds with a 'pharmacophoric' structure and useful pharmacological properties, the interest in organic raw materials independent of oil, and the interest in 'green chemistry' – just to name a few of them. Thus, the molecular weight of carbohydrates ranges from a few hundred (glucose) to a few hundred thousand (celluloses with a DP of *ca.* 10–15000); *i.e.* it encompasses 'normal' organic chemistry and polymer chemistry likewise. If reactivity is the first property of interest, there are other properties such as the structural basis of sweetness, and for some polysaccharides at least the relation between structure and on the one hand, physicochemical properties (such as solubility, gelling, and friction) and, on the other hand, biological aspects (such as immunostimulation).

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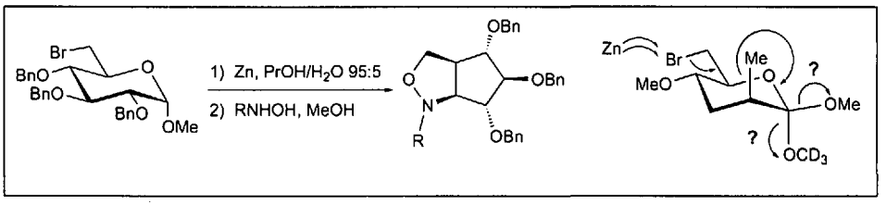
From a more narrowly chemical standpoint, monosaccharides are alcohols, aldehydes, and/or ketones; one finds acids, amines, amides, esters of organic and inorganic acids, acetals, sulphur and even (naturally occurring) arsenic derivatives, tetrahydropyrans and tetrahydrofurans, and saturated nitrogen heterocycles. Monosaccharides possess linear and branched-chain carbon skeletons (not to forget the closely related inositols, hydroxylated cyclohexanes). Moreover, pure carbohydrates combine with a large number of non-carbohydrate compounds to form both secondary metabolites (such as aromatic glycosides and carbohydrate-derived antibiotics) and primary metabolites (nucleotides, glycoproteins, glycolipids). Carbohydrates are produced in staggering amounts – cellulose is the most abundant natural product; and sucrose – enantiomerically pure and crystalline – is cheaper than most solvents, and freely available. I have not even mentioned the complex stereochemical aspects, questions of biosynthesis, the carbohydrate-transforming enzymes, and carbohydrate-directed receptors such as the lectins!

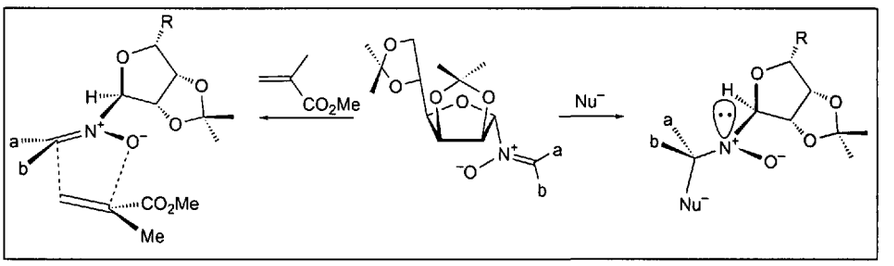
In face of this humbling wealth of potential and problems, we have been interested in a few aspects only, first in the influence of steric and stereoelectronic effects on reactions involving the anomeric centre. Since carbohydrates provide so many constitutional and configurational variants of tetrahydropyrans and -furans (in enantiomerically pure form), they allow to study the influence of the nature and orientation of substituents on the reactivity of the structurally varied anomeric centre. For this, we were interested in novel, or new and if possible useful transformations starting with the question of how to cleave the endocyclic C(5),O bond in glycosides [1]. This was achieved by a Zn-mediated fragmentation of 6-halo-6-deoxyglycosides under weakly acidic conditions, yielding useful hexenoses, and constituting a method that became popular (see *e.g.* [2]). We used it for the first preparative transformation of saccharides into cyclopentanes (Scheme 1), a methodology that confronted us with the question of the concerted or semi-concerted nature of the reductive fragmentation (such as the fate of the depicted ortholactone [3]) and the stereoselectivity of intramolecular nitrone-alkene cycloadditions. So, we studied the stereoelectronic effects of alkoxy substituents on the reactivity and selectivity of LUMO-controlled 1,3-dipolar cycloadditions of N-glycosyl nitrones (Scheme 2), and compared the cycloadditions to the addition of nucleophiles, making enantiomerically pure isoxazolidines, N-hydroxyamino phosphonates and aminophosphonic acids and nojirimycin from furan (see [4] and refs. cited there). Remarkably, in these reactions the initial interaction of the nitrone function with the alkoxy group lowering the ε(LUMO) is replaced by the lone-pair polar-bond interaction of the nitrogen lone pair with the coplanar (!) σ*(C,O) orbital, *i.e.* by a kinetic (= transition state stabilizing) anomeric effect. Selectivity is here essentially linked to the stabilisation of the relevant transition state, and not to

destabilisation of the transition state of competing reactions.

An interest in neuraminic acids and in the 'Umpolung' of the naturally electrophilic anomeric centre led us to make and study 1-deoxy-1-nitroaldoses. On the way to the nitro derivatives, we made glyconhydroximo lactones, halonitroso, and halonitro ethers, new carbohydrate derivatives (see [5][6] and refs. cited there; Scheme 3). The nitro ethers allowed for an 'Umpolung' and led to a synthesis of chain-elongated uloses, ulose-derived nucleosides, and C-glycosides without concomitant β-elimination [7][8]. The halonitroso ethers proved useful as reactive heterodienophiles in highly diastereoselective [4+2] cycloadditions [9], and the halonitro ethers allowed for $S_{RN}1$ reactions [10]. These derivatives allowed the generation of several reactive intermediates: (stabilised) carbanions, radicals, and oxycarbenium cations from nitro ethers, and radical anions from halonitro ethers. The nitro ethers (prepared by a shorter route [11]) indeed led to two complementary syntheses of neuraminic acids [12], later followed by a third one [13] allowing the preparation of a plethora of Neu5Ac analogues (see *e.g.* [14]).



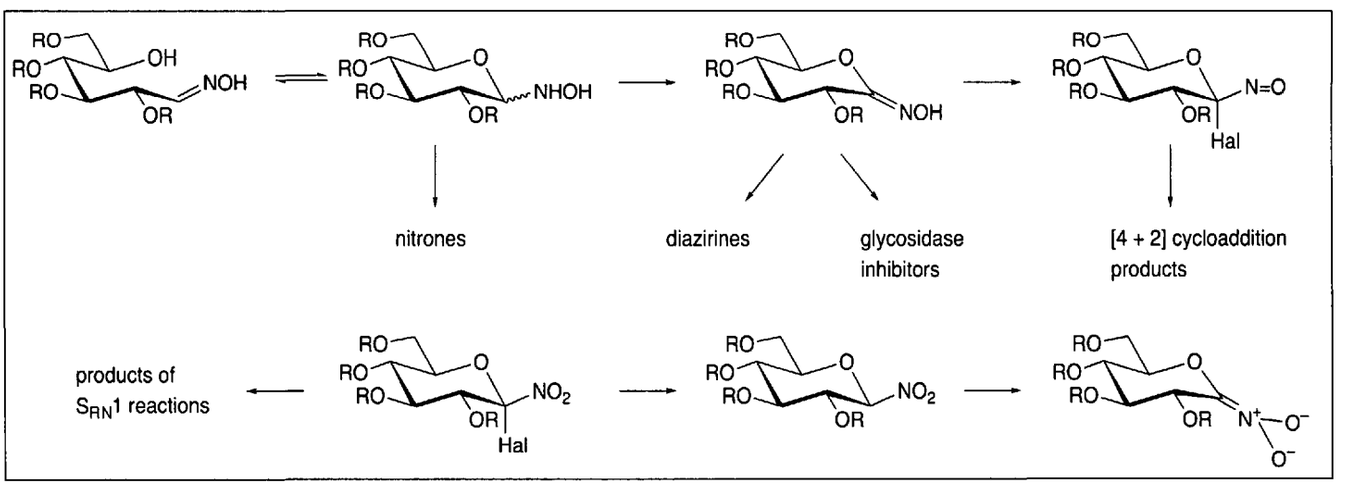
Scheme 1.



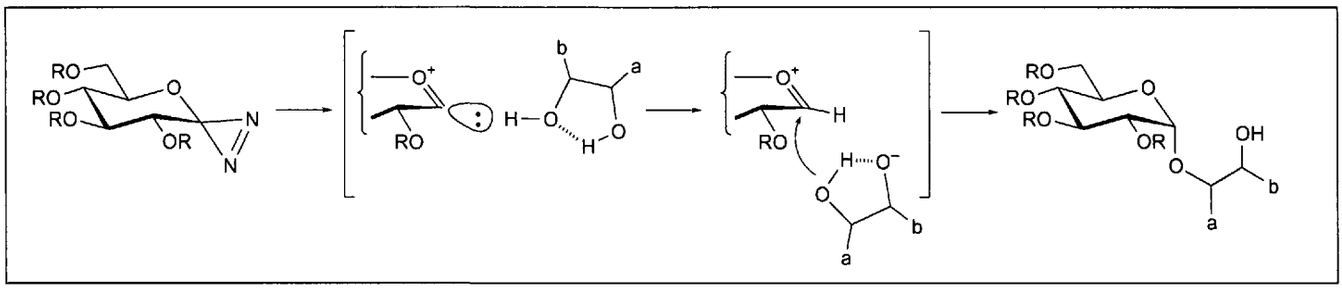
Scheme 2.

Glycosylidene carbenes were not known. We looked for suitable precursors, and diazirines looked promising. Alkoxydiazirines were known to be poorly stable, but we found a way to make them from hydroximolactones *via* alkoxydiaziridines (see [5][15][16] and refs. cited there). These diazirines are relatively stable, and proved useful and efficient precursors of glycosylidene carbenes. One of the attractive aspects of these alkoxy carbenes is their application to glycoside synthesis: mild thermolysis or photolysis of diazirines generates alkoxycarbenes, and these react with alcohols to lead by proton transfer to initially non-solvated ion pairs (generating both an activated glycosyl donor and acceptor) and hence to a reagent-free, regioselective glycoside synthesis (see [17][18] and refs. cited there; Scheme 4). The high reactivity and insensitivity to hindrance allowed, *inter alia* a high yielding glycosidation of the tertiary hydroxy group in ginkgolides [18]. In addition, C-, N-, S-, and Sn–glycosides are readily available [17][18], illustrating that these azi-compounds open a rapid access to new types of glycosyl derivatives, illustrated by the synthesis of the first fullerene C-glycoside [19] and of the first glycosyl boron derivatives [20] (Scheme 5). The course of the reaction, however, proved complex, depending on stereoelectronic control, hydrogen bonding (kinetic acidity of individual hydroxy groups), and steric factors. The dependence on hydrogen bonding is so strong that these carbenes can be used as a reactivity-based test for hydrogen bonding; it gives results – *e.g.* in the analysis of hydrogen bonds from hydroxy groups to F–C substituents – that agree with NMR analysis [21].

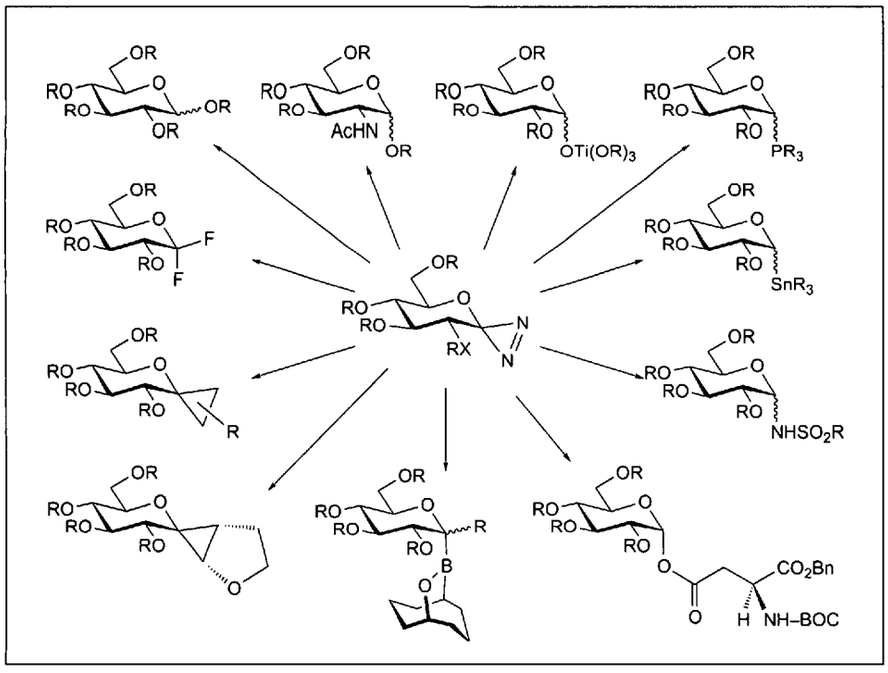
The essential role of hydrogen bonds directed our attention to their influence on the structure and physicochemical properties of celluloses. Also in view of the potential of acetylene chemistry, we decided to demonstrate the effects of intrachain interresidue hydrogen bonds in cellulose. These hydrogen bonds are considered to 'preorganise' cellulose molecules, favouring their association, and to be essential for the solubility of celluloses (or lack of it) and their material properties. The polymorphs of celluloses differ by hydrogen bonding, conformation, and packing properties (see [22][23] and refs. cited there). In a first approach to interrupting the crucial intrachain interresidue



Scheme 3.



Scheme 4.



Scheme 5.

H-bonds, we replaced the glycosidic oxygen by a buta-1,3-diyn-1,4-diyl group. The synthesis of these oligomers by directed cross-coupling of alkynes required efficient syntheses of monomers, the invention of orthogonal protecting groups for dialkynes, the optimisation and mechanistic study of transition-metal catalysed cross coupling of alkynes and haloalkynes [24][25]. We made up to a 16-mer and a 32-mer of these cellulose analogues [26] (Fig.1). Their solubility in DMSO is in keeping with the postulated hydrogen bonds. In this context, we had to analyse intra- and intermolecular hydrogen bonding in DMSO. This led to a set of useful rules and to the revision of prior interpretations [27].

Considering that there are no small model compounds of celluloses I (native form), we embarked on attaching up to cellooctaose moieties corresponding to the two independent chains of the elementary cell of crystalline cellulose I in the appropriate distance to a template (1,8-disubstituted naphthalene in Fig. 1 [22]). Remarkably, this gave rise to a mimic of the thermodynamically preferred cellulose II. Presumably, the template did not sufficiently account for the phase shift between the two cellulose chains. We are pursuing this work, with a new template (1,8-disubstituted anthraquinone in Fig. 1 [23]), again directed to a mimic of cellulose I. We wonder if this type of crystal engineering will be successful.

During the last ten years, we became particularly interested in mechanistic aspects of glycosidases. The hydroximolactones we had prepared as intermediates of nitroaldoses are analogues of lactones, long-known competitive and selective inhibitors of β-glycosidases. We took advantage of the additional hydroxy group of hydroximolactones to introduce groups mimicking the aglycone moiety, and also hoped that the hydroximolactones may be more stable in aqueous solution than the lactones. We were lucky in finding strong (1 to 0.04 micromolar) inhibitors of glycosidases and hexosaminidases; even glycogen phosphorylase b was inhibited [28][29]. Later, we realised that the contention that glyconolactones are transition state analogues was not experimentally founded, just a hypothesis derived from structurally correlating these lactones to oxycarbenium cations, the putative reactive intermediates of the enzymatic glycoside hydrolysis [30]. While the lactones can, in principle, interact both with the catalytic acid (an undissociated carboxyl group) and the catalytic nucleophile (a carboxylate anion) of the catalytic machinery, the oxycarbenium cation can only interact with the carboxylate anion. Indeed, ammonium cations, such as nojirimycin, are well-known strong (micromolar) but not very selective inhibitors of many glycosidases. Are lactone-type inhibitors and/or piperidinium-type inhibitors transition state analogues? Are the lactones protonated by the enzyme? Is protonation important for their inhibitory activity? Is protonation really taking place from a direction perpendicular to the plane of the pyranoside ring, as inferred from early crystal structure analyses of lysozyme? Inhibitors proved a valuable tool to answer these questions, used in combination with kinetic studies and protein crystallography. Inhibition of glycosidases is here not so much a goal in itself as a means to better understand the mechanism of action of glycosidases. Obviously, a deeper understanding of the enzymatic mechanism will, in its turn, lead to the design of stronger inhibitors, useful for applications.

We first prepared a fused tetrazole as a stable analogue of gluconolactone [31] (Scheme 6). It is a micromolar, competitive and selective inhibitor of β-glucosidases, like gluconolactone, and not too dissimilar from hydroximolactams, prepared and studied by Bruce Ganem [32], and by us [33]. Steve Withers [34] determined that the tetrazole is a partial (*ca.* 55%) transition state analogue. Supposing that protonation of the inhibitor is important for its activity, we made pyrrole analogues of this tetrazole, but they were poor inhibitors. These observations, the

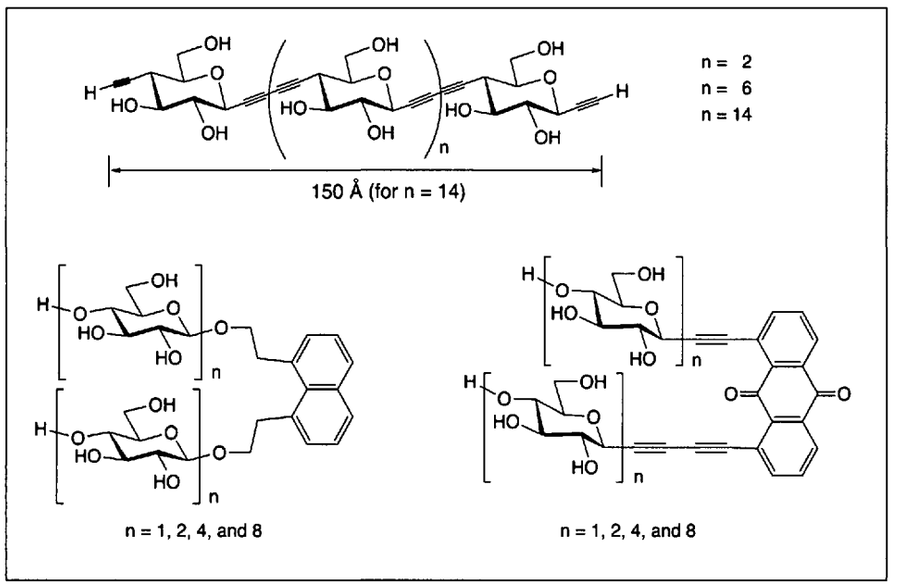


Fig. 1.

strong inhibition by related imidazoles, and the weak inhibition by a triazole that lacks a heteroatom at the position corresponding to the gluconolactone carbonyl oxygen (*i.e.* the 'glycosidic oxygen'), suggested a protonation trajectory in the plane of the imidazole ring, and not perpendicular to it [30]. Inspection of the available crystal structures of glycosidases showed no exception to lateral protonation, and led to the discovery that there are two classes of glycosidases, differing by their protonation trajectory, *syn*, or *anti* to the endocyclic C–O bond. The interaction with the catalytic acid and the catalytic base of a tetrazole contributes to a similar extent to the inhibition, as shown by calculations, validated by structural and inhibition data with glycogen phosphorylase b, in collaboration with the groups of Louise Johnson (Oxford) and Nikos Oikonomakis (Athens) [35]. With the help of inhibitors we also probed the known important interaction of C(2)OH of glucosides with the catalytic nucleophile [36]. More recently, we became interested in the conformational changes preceding or accompanying the enzymatic cleavage of β-glycosides, evidenced by three crystal structure analyses of *endo*-glycosidases [37]. Such a conformational change is required by the principles of stereoelectronic control, and we consider it important to demonstrate its operation during the cleavage of β-glycosides by different families of glycosidases using conformationally biased inhibitors (*e.g.* the isoquinuclidine in Scheme 6 [38]). We are also synthesising potential inhibitors that should be selective for *syn*- or *anti*-protonating glycosidases, and have embarked on the inhibition of some transferases [39] and other carbohydrate-transforming key enzymes.

More recently, we have yielded to the lure of nucleotides – they are far too important to be left aside. The ambitious project proposes to make new types of nucleotide analogues, where the nucleobases are included in the backbone, and not distinct from it, as schematically expressed in Fig. 2. According to model studies and calculations, several representatives of this novel class of analogues could form double helices. We have so far learned about important factors governing the conformation of these compounds [40–42]; we have started with the synthesis of second-generation analogues. In parallel to this project, we are pursuing work on new preparative methods and on the total synthesis of a complex nucleotide. Pursuing several projects at the same time has the advantage of offering ample opportunity for coworkers to learn about many aspects of organic chemistry, and particularly of glycoscience – the wonderful world of carbohydrates, catching, as in a mirror, so many of the treasures of structural and reactivity-related aspects of chemistry, and some of its equally thrilling neighbouring fields.

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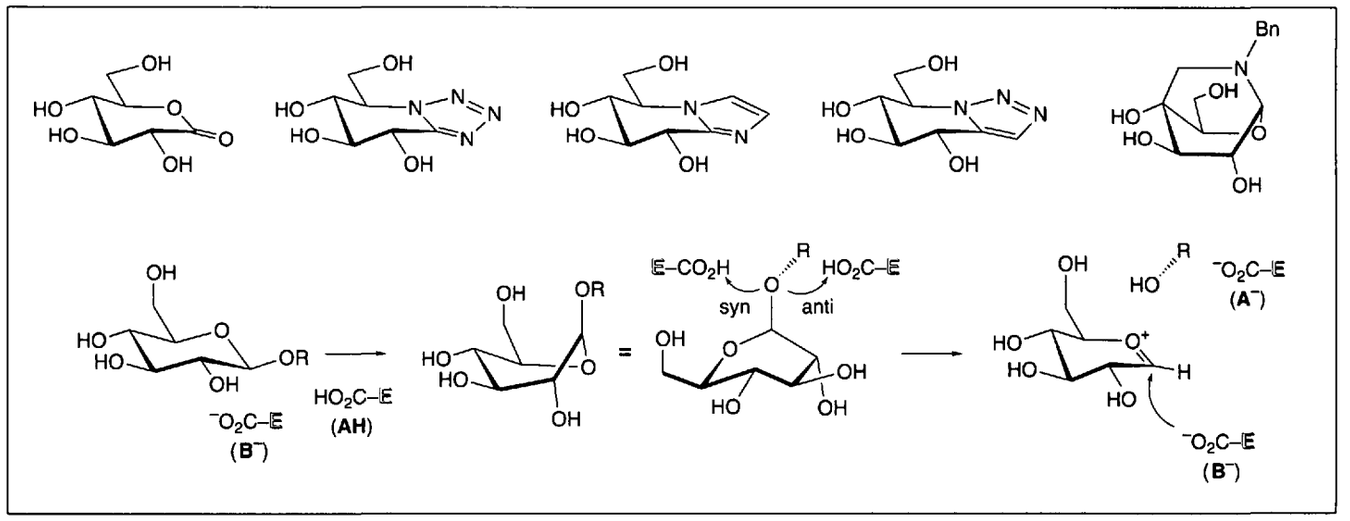

Scheme 6.

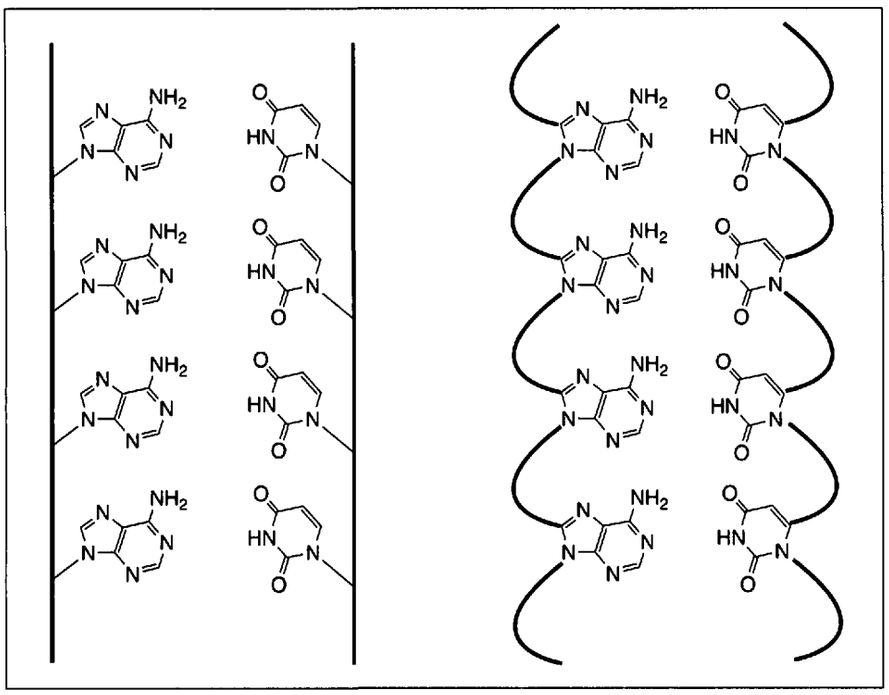

Fig. 2.

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