Finally, the ultrafast heating and cooling rates (in excess of 10^{14} K s^{-1}) of finite aggregates that may be achieved by high-velocity collisions of clusters with liquids exceed the record laboratory rates reported to date (*17, 18*). Consequently, the methods suggested here may open new experimental avenues for ultrafast heat processing and for the preparation and exploration of nanoglass materials aggregates.

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19 We acknowledge assistance by C. L. Cleveland, in particular his help in generating Fig. 3. We thank J. R. Stevenson for bringing the work in (*18*) to our attention and J. P. Rose and R. S. Berry for sending a preprint of their work (*16*) before publication. Supported by U.S. Department of Energy (DOE) grant FG05-86ER-45234. Calculations performed at the National Energy Research Supercomputer Center, Livermore, CA, and at the Florida State Supercomputer Center through computer time grants from the DOE.

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A Strategy for the Solid-Phase Synthesis of Oligosaccharides

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Repeating glycosidic linkages of oligosaccharides can be synthesized by solid-phase methods. Glycals were attached to a polystyrene copolymer with a silyl ether bond and were activated to function as glycosyl donors with 3,3-dimethyldioxirane. Glycosidation was performed by reactions with a solution-based acceptor (itself a glycal). Excess acceptor and promoter were removed by rinsing after each coupling, and the desired oligosaccharides were then easily obtained from the polymer by the addition of tetra-*n*-butylammonium fluoride. By this method, glycosidations are stereospecific and interior deletions are avoided.

Of the three major classes of biooligomers, the synthesis of polysaccharides has proven to be the most difficult. In the assembly of polypeptides and poly(2-desoxynucleotides), selection is not a problem as there is no stereochemistry in the repeating bond constructions (amide and phosphate, respectively). In contrast, each glycosidic bond to be fashioned in a growing oligosaccharide constitutes a new locus of stereogenicity and possible complexity (Fig. 1).

Furthermore, the problem of differential protection of potentially competing functions is rather formidable in the case of oligosaccharides. For instance, the extension of an oligosaccharide at either its "reducing" or "nonreducing" end by a single glucose entity requires the identification of one of five hydroxyls of the glucose to function as the glycosyl donor or acceptor, respectively (Fig. 2). In peptide bond formation, on the other hand, one need ordinarily be concerned with a single amino group (acyl acceptor) and a single carboxyl function (acyl donor) or, less commonly, two such moieties per unit (compare with lysine and aspartic acid) (*1*). In the synthesis of oligomers of 2-desoxynucleotides, one of two grossly different hydroxyl groups must be distinguished, per molecule, for each elongation (*2*).

Synthesis of structurally defined oligopeptides and oligonucleotides has benefited greatly from the feasibility of conducting such processes on various polymer supports. The anchoring of one component to an insoluble support allows, in principle, the use of a large excess of the coupling partner (as well as promoter reagents) while keeping the final purification problem manageable. Thus, limiting yields are improved by mass action. In some cases, such syntheses can be engineered for nearly automated execution. In contrast, the overwhelming majority of carbohydrate constructions are still conduct-

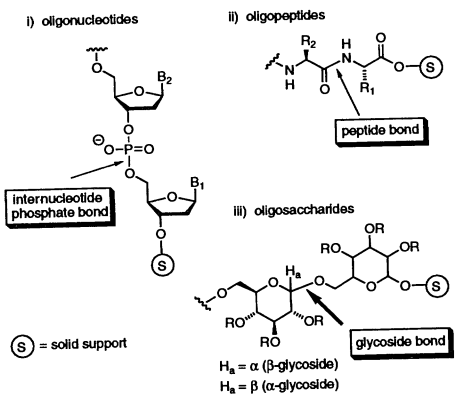

Fig. 1. Solid-phase synthesis of biopolymers.

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ed in solution (3). Although methodology has reached the stage where complex oligosaccharides and other glycoconjugate arrays can indeed be assembled in this way, the work is labor-intensive.

We have begun to study possible approaches to solid-phase oligosaccharide synthesis. The long-term hope is that success in this regard could be translated to much simplified (possibly automated) protocols that would be carried out in a wider range of laboratory settings than is currently the case. The criteria that guided this exploratory study are (i) ease of attachment of the starting material to the polymer support; (ii) significant relief from the rigors of protecting group manipulations; (iii) stereospecificity of the coupling step; (iv) ease of removal of the oligomer from the solid support; and (v) purification of the final product by avoidance of deletions in the elongation of the oligomer.

In principle, two alternatives for the solid-phase–supported chemical "growing" of an oligosaccharide present themselves (4). In one motif, the first carbohydrate is anchored to the support at its "reducing" end (case 1) (Fig. 3A). Thus, in each elongation, the glycosyl acceptor is linked to the solid phase and coupling occurs with a solution-based donor. As the next cycle is contemplated, a unique acceptor hydroxyl must be exposed in the solid phase. This requires the donor used in the previous glycosidation to be furnished with a uniquely deprotectable blocking group at the site of elongation. Of course, coexistence of this free hydroxyl in the solution-based donor, which already bears the intact glycosyl donating function, is unlikely under the conditions of glycosidation. The need to eventually expose the unique hydroxyl group on the polymer support must be met by recourse to multistep functional group adjustments in the synthesis of the glycosyl donor. The attractiveness of the method is correspondingly compromised.

Alternatively, the oligomer undergoing elongation is mounted to the support at its "nonreducing" end (case 2) (Fig. 3B). In anticipation of each iteration, a glycosyl donor function must be installed uniquely at

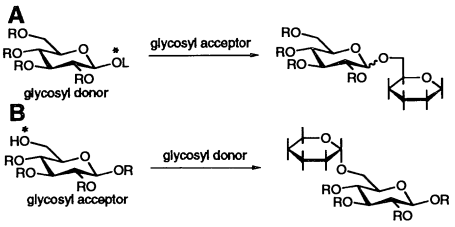

Fig. 2. Hydroxyl group differentiation requirements for glycosidation. (**A**) OL, activating group fashioned from a unique anomeric hydroxyl group. (**B**) Asterisk indicates the unique hydroxyl group.

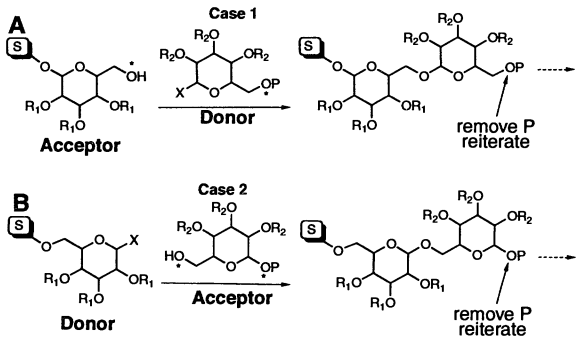

Fig. 3. Glycosyl (**A**) acceptor (case 1) and (**B**) donor (case 2) bound to the solid support. S, solid support; P, unique protective group; X, activating group; *, uniquely differentiated hydroxyl group. In case 1, reiteration requires the removal of P; in case 2, it requires the removal of P and the activation of the anomeric carbon.

the anomeric carbon of the support-bound saccharide. Coupling occurs with a solution-based acceptor. The "reducing" end of this acceptor is functionalized so that a new donor capability can be installed, after coupling, at its anomeric carbon. As with case 1, a serious question of compatibility under the conditions of glycosidation would arise if one attempted to enter an acceptor with its anticipated anomeric donor function already in place.

Such advances in polymer-based oligosaccharide synthesis were achieved with case 1 (5). The problem in case 2 of fashioning the anomeric donor function for each iteration on the support-bound saccharide has apparently been intimidating (6). Moreover, both plans need differentiated building blocks, which are difficult to obtain.

Previous studies in our laboratory have indicated some attractive possibilities for the use of glycals (1) (Fig. 4) in the solution synthesis of carbohydrate ensembles (7, 8). One important advantage offered by glycals is the ease of differential protection. It is much more straightforward to distinguish the three hydroxyls of a pyranosidal glycal than those of a pyranose (9). In addition, a range of protocols now exists for the initiation of the glycosyl donating capacity of a glycal (10, 11), and glycals can function as glycosyl acceptors (see structure 3) (7). We developed the reiterative strategy summarized in Fig. 4. The chemical activation device E^+ conscripts the intended donor glycal 1 to glycosylate the designated acceptor 3. Thus 1, when suitably primed, becomes 2, which glycosylates 3 to produce 4. Reiteration leads to 5. The priming phase (1 → 2) may consist of in situ generation of an active glycosylating species (11). Alternatively, 2 may be an isolable entity that is administered to the acceptor 3 under circumstances of stoichiometric definition (12). In the work reported here, E^+ corresponds to an epoxidizing agent (3,3-dimethyldioxirane) (13). Thus, donor 2 is a 1,2-anhydrosugar (E = O).

The advantages of this method are considerable. The donor is formed in one easy step from the glycal. The epoxidation is nearly quantitative and is highly stereoselective

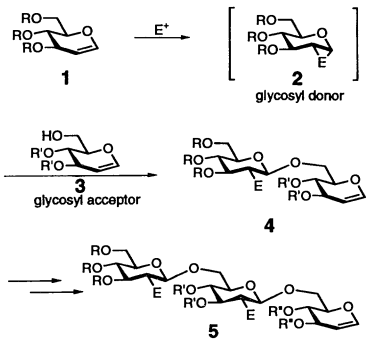

Fig. 4. General strategy for solution-phase oligosaccharide synthesis with glycals.

(14). Opening of the epoxide by a glycosyl acceptor gives a glycoside corresponding to inversion at the anomeric center (15). Finally, each glycosidation gives rise to a unique, free hydroxyl center at C-2 of the previous donor, which is of potentially great value for the synthesis of branched sugars.

In our method for solid-support oligosaccharide synthesis (Fig. 5), the oligomer is built with a polymer-bound donor (that is, case 2). The solid support is the known structure 6 (16, 17), which can be prepared from the inexpensive, commercially available copolymer of polystyrene cross-linked with 1% divinylbenzene. The first glycal 7 (derived from D-galactal) is attached by simple silylation (18). That 7 had indeed been attached was shown in several ways, including its isolation upon detachment [with tetra-*n*-butylammonium fluoride (TBAF) buffered with acetic acid]. Priming was readily accomplished by exposure of the insoluble 8 to the action of 3,3-dimethyldioxirane (9) (19), as shown, to give the 1,2-anhydrosugar 10 (13).

Coupling was achieved by exposure of insoluble 10 to a solution of 7 [in tetrahydrofuran (THF)] in the presence of zinc chloride. That a disaccharide was produced in linked form (see 11) was indeed demonstrated by the retrieval of 12 upon treatment of 11 with TBAF. Reiteration of the scheme led to 13 (from which 14 could have been retrieved by desilylation). We introduced diversity in the growing chain by conducting the next iteration with the D-glucal–derived

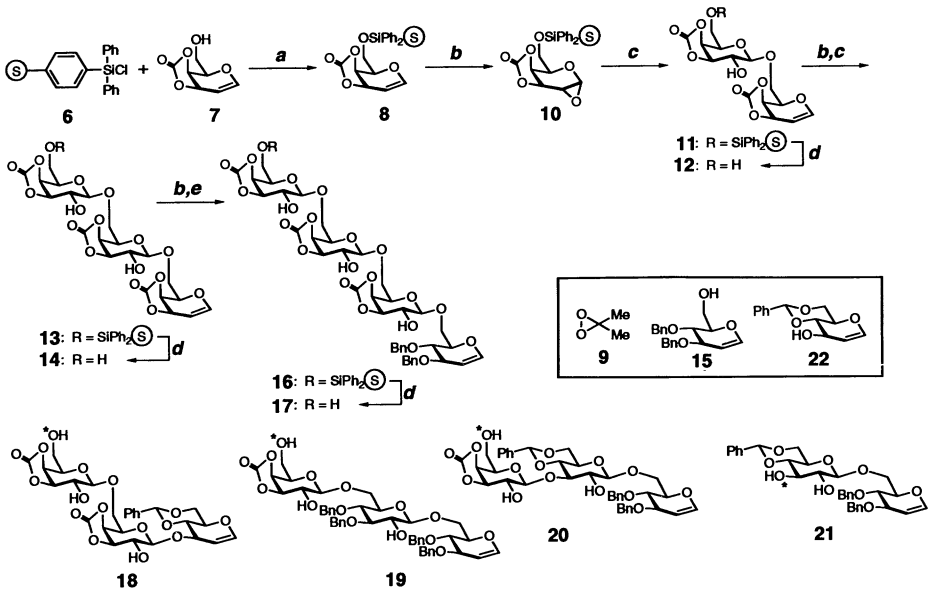

Fig. 5. Synthesis of oligosaccharides with polymer-bound glycals. Compounds **18** to **21** are alternate products. Reagents: (*a*) Hunig's base and CH_2Cl_2; (*b*) **9** and CH_2Cl_2; (*c*) **7**, $ZnCl_2$, and THF; (*d*) TBAF, acetic acid, and THF; (*e*) **15**, $ZnCl_2$, and THF. Asterisk, anchorage site of first glycal to the solid support; circled S, copolymer of styrene crosslinked with 1% divinylbenzene. A typical coupling cycle involves addition of a solution of **9** in acetone (~10 molar equivalents) to a stirred suspension of the polymer-bound glycal in CH_2Cl_2 (~1 ml per 50 mg of polymer) at 0°C, followed by 1 hour of stirring at 0°C. The solvents are filtered off, and the polymer-bound 1,2-anhydrosugar is dried in vacuo. The polymer is placed under a dry nitrogen atmosphere and treated with a 0.2 to 0.3 M solution of the acceptor (10 to 20 molar equivalents) in anhydrous THF, followed by 2 molar equivalents of $ZnCl_2$, added as a 1.1 M solution in THF. The mixture is stirred at 40°C for several hours, and the reagents are removed by filtration, followed by a thorough wash with THF.

acceptor **15**, which yielded **16**. Retrieval of **17** was accomplished (32% overall yield based on **8**) again through the action of TBAF (*20*).

It is remarkable that the method shown here produces little or no products from which saccharide blocks are deleted: the desired product, tetrasaccharide **17**, is purified from highly polar by-products with very straightforward chromatography on silica gel. One possible explanation is that each coupling failure is "corrected" by hydrolysis of the unreacted 1,2-anhydrosugar during the rinsing procedure. The resultant, highly polar hydrolysis product or products, or further degradation products, do not complicate purification of the final target. Assuming that all of the glycal is epoxidized in each cycle (*21*), the method precludes formation of oligomers with interior deletions.

Several other features of our scheme can be discerned. Secondary hydroxyl centers in glycals do function as glycosyl acceptors (see formation of **18**) (*22*). Furthermore, D-glucal–based glycosyl donors are also accommodated (see formation of **19**, **20**, and **21**). Finally, initial attachment of a D-glucal unit to a polymer, at the unit's secondary (C-3) hydroxyl, is tolerated (see product **21**) (*23*). The inclusion of systems **18** to **21**, without complication in our protocols, underscores the diversity that can be programmed.

At present, the average yield per coupling cycle, consisting of epoxidation and glycosidation, is ~70% (assuming quantitative retrieval upon desilylation). Clearly, more experimentation, including the screening of other polymers, other priming protocols, and other coupling conditions, are necessary before this chemistry can be considered optimal. Although we lay no claim to having solved the problem of oligosaccharide synthesis on a solid support, this method is already workable because of its capacity to "self police" its coupling failures, thereby rendering the final purification a straightforward matter.

We have demonstrated here only the case in which the oligomer is released in a form that contains undifferentiated hydroxyl groups. It is tempting to envision a scenario in which diverse capping of the C-2 hydroxyl, produced after each iteration, can be used to fully (and easily) distinguish every hydroxyl group of the oligomer. In that case, convergent solid-support synthesis of very large oligosaccharides (and other large glycoconjugate arrays) can be contemplated. The block structures that would be released from the solid phase could function as solution-phase acceptors toward supported glycal-triggered donors.

Note added in proof: Since submission of this report, we have achieved considerable progress in extending glycal chemistry to other glycoside patterns.

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21. We assessed the completeness of epoxidation by treating an aliquot of the polymer-bound 1,2-anhydrosugar with TBAF and checking for the presence of unreacted glycal by thin layer chromatography.
22. Trisaccharide **18** was obtained from **11** by epoxidation with **9**, followed by coupling with D-glucal–derived acceptor **22**, and finally retrieval by desilylation with TBAF
23. Compound **22** was reacted with **6** with use of the method of Chan and Huang (*16*).

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