Polymer-Supported Solution Synthesis of Oligosaccharides Using a Novel Versatile Linker for the Synthesis of D-Mannopentaose, a Structural Unit of D-Mannans of Pathogenic Yeasts

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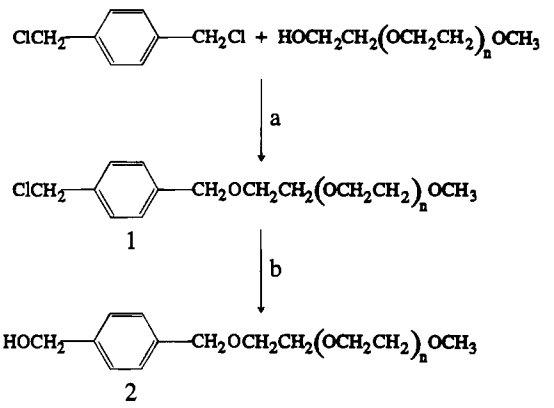
The emerging understanding of the critical role of oligosaccharides in biological processes and the promise of therapeutics based on oligosaccharides create an urgent need for an efficient synthetic methodology for oligosaccharides. The interest in polymer-supported strategies of oligosaccharide synthesis has gained momentum after the demonstration of the utility of solution phase polymer-supported strategy of oligosaccharide synthesis recently developed in our laboratory which utilizes as the supporting polymer poly(ethylene glycol) ω-monomethyl ether (MPEG).[1] Other ingenious variants of polymer-supported synthesis of oligosaccharides have since been described.[2]

The MPEG strategy requires the polymer-bound synthon to be soluble under reaction conditions of glycosylation and to be insoluble during the workup of reaction mixtures while all impurities are soluble. Thus, the major obstacle to any efficient synthesis of an oligomer, the difficult and time-consuming purification at each step, mainly by chromatography, is avoided. Originally MPEG was bound to a hydroxyl of the first monosaccharide through the succinoyl diester linker, successfully used in syntheses of oligopeptides and oligonucleotides[3] and in several laboratories for syntheses of oligosaccharides up to a decasaccharide.[4]

All glycosylations and protective group manipulations clearly should be compatible with the succinoyl ester linkages. Unfortunately, ester bonds have three main limitations: (1) they are base-labile, (2) they are prone to migrations in acidic environments, and (3) they cannot work at the anomeric center.[5] The succinoyl diester linker restricted the choice of reaction conditions and protective groups, and we searched for a more versatile linker. We have determined that α,α'-dioxyxylyl diether, $-CH_2C_6H_4CH_2O-$ (DOX), is a superior linker based on the following criteria: (a) it can be bound via an ether or an O-glycosidic linkage and it is stable under most reaction conditions including glycosylation; (b) it is removable from a finished oligosaccharide by hydrogenolysis,[6] either completely to give free OH, or MPEG can be removed selectively to leave the hydroxyl protected with $-CH_2C_6H_4CH_3$ (p-tolylmethyl, TM), whose properties resemble those of the benzyl protecting group; and (c) it is easily prepared from commercially available α,α'-dichloro-p-xylene.

Scheme 1[a]



***MPEG*DOXOH; n = about 110 or about 260**

[a] Reagents and conditions:(a) NaH, NaI, 30 equiv of xyleneCl_2, THF, 96 h; (b) 10% aqueous Na_2CO_3, 70 °C, 16 h.

The MPEG-DOX support−linker system is synthesized (see Scheme 1) from the monomethyl ether of MPEG and excess α,α'-dichloro-p-xylene by Williamson ether synthesis,[7] giving monochloro MPEG-DOX-Cl (**1**) or, after hydrolysis,[8] the alcohol MPEG-DOX-OH (**2**).

The alcohol MPEG-DOX-OH (**2**) can be glycosylated to link the support through the anomeric oxygen; such a connection would not survive during glycosylation reactions using the succinoyl linker. In an example of an iterative synthesis of D-mannopentaose, a structural moiety of cell surface D-mannans[9] of pathogenic yeasts (Scheme 2), MPEG-DOX-OH (**2**) is glycosylated using the trichloroacetimidate procedure[10] by 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**3**).[11] The mannosyl donor has an acyl group at O-2 that allows for stereocontrol via neighboring group participation and is readily removed by base treatment to yield an acceptor for the next glycosylation. Repetition of the glycosylation and hydrolysis steps gave the protected pentasaccharide, which after hydrogenolysis, acetylation, and purification gave, following deacetylation, pentamannopyranoside [Manp(α1−2)]$_4$Manp (**5**). As expected, no β-anomer formation was observed.

The attachment of MPEG-DOX to a carbohydrate hydroxyl other than the anomeric one is achieved by reaction of such a hydroxyl (e.g., **6**, Scheme 3) with MPEG-DOX-Cl (**1**) under

(1) (a) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5095. (b) Krepinsky, J. J.; Douglas, S. P.; Whitfield, D. M. *U.S. Patent* 5,278,303, 1994.

(2) For solid phase polymer-supported synthesis of oligosaccharides, for instance, for chemical approach, see: Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307. Yan, L.; Taylor, C. M.; Goodnow, R., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953. For enzymic approach, see: Schuster, M.; Wang, P.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1135 and references therein.

(3) (a) Bayer, E.; Mutter, M. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 2, p 286. (b) Bonora, G. M.; Scremin, C. L.; Colonna, F. P.; Garbesi, A. *Nucleic Acids Res.* **1990**, *18*, 3155. (c) Bonora, G. M.; Biancotto, G.; Maffini, M.; Scremin, C. L. *Nucleic Acids Res.* **1993**, *21*, 1213.

(4) (a) Leung, O. T.; Whitfield, D. M.; Douglas, S. P.; Pang, H. Y. S.; Krepinsky, J. J. *New J. Chem.* **1994**, *18*, 349. (b) Kandil, A. A.; Chan, N.; Chong, P.; Klein, M. *Synlett* **1992**, 555. (c) Verduyn, R.; van der Klein, P. A. M.; Douwes, M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 464. (d) Dreef-Tromp, C. M.; Willems, H. A. M.; Basten, J. E. M.; Westerduin, P.; van Boeckel, C. A. A. *Abstr., XVIIth Int. Carbohydr. Symp.* **1994**, 511.

(5) (a) Meah, M. Y.; Krepinsky, J. J. Unpublished observation. (b) Whitfield, D. M.; Douglas, S. P.; Krepinsky, J. J. *Tetrahedron Lett.* **1992**, *33*, 6795.

(6) For the mechanism of the hydrogenolysis of derivatives of benzyl alcohol see Kieboom, A. P. G.; de Kreuk, J. F.; van Bekkum, H. *J. Catal.* **1971**, *20*, 58. Further details of our hydrogenolysis studies will be published in a separate communication.

(7) Feuer, H.; Hooz, I. in *The Chemistry of the Ether Linkage*; Patai, S., Ed.; Interscience: New York, 1967; pp 446−450, 460−468.

(8) The reactions can be performed in most solvents except ethers; products are isolated from the reaction mixtures by precipitation after addition of an ether, generally *tert*-butyl methyl ether (MTBE). The latter of sufficient quality and degree of dryness for this purpose is available inexpensively from ARCO (Atlantic Richfield, Inc.) in bulk quantities.

(9) E.g.: Kobayashi, H.; Takaku, M.; Nishidate, Y.; Takahashi, S.-I.; Takikawa, M.; Shibata, N.; Suzuki, S. *Carbohydr. Res.* **1992**, *231*, 105. For a classical solution synthesis, see: Ogawa, T.; Yamamoto, H. *Carbohydr. Res.* **1982**, *104*, 271,

(10) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212.

(11) (a) Yamazaki, F.; Sato, S.; Nukada, T.; Ito, T.; Ogawa, T. *Carbohydr. Res.* **1990**, *201*, 31. (b) Paulsen, H.; Helpap, B. *Carbohydr. Res.* **1991**, *216*, 289.

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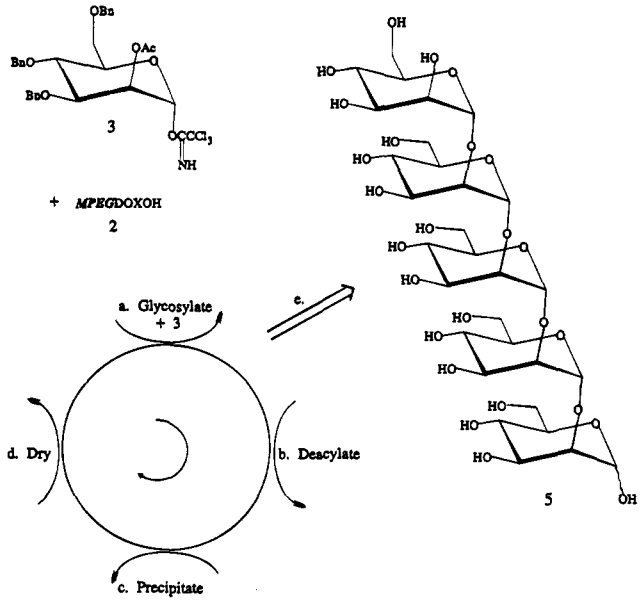
Scheme 2[a]



[a] Reagents and conditions: (a) imidate, TESOTf, 4 Å molecular sieves, CH_2Cl_2, 0−5 °C, 4 h; (b) DBU, CH_3OH, 16 h; (c) MTBE and then EtOH reprecipitation; (d) high vacuum; (e) Raney nickel W2, EtOH, reflux 16 h.

Williamson conditions. Removal of phthalimido and silyl groups is compatible with the presence of MPEG-DOX. In the example illustrated in Scheme 3, this procedure, followed by other manipulations (steps a−c), gives MPEG-DOX-bound disaccharide **8**.

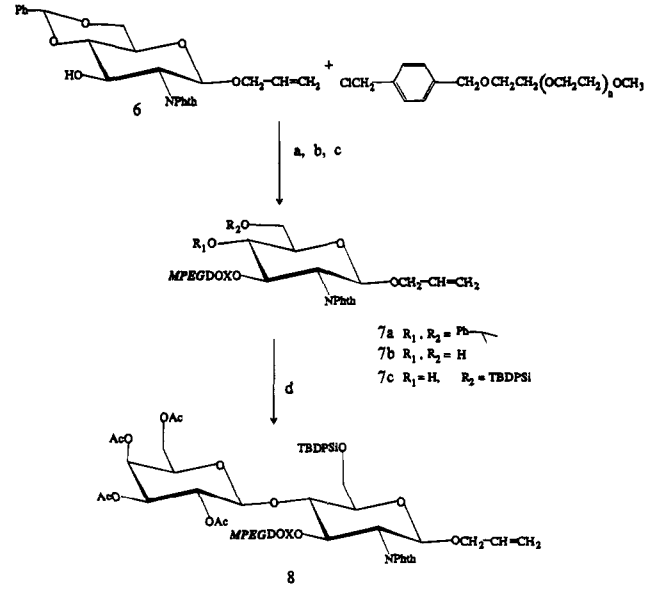
MPEG-DOX in **8** can be transformed into the TM group by hydrogenation under mild conditions.[12] MPEG-DOX is completely removed by more vigorous hydrogenation,[13] and the peracetate is formed after acetylation. MPEG-DOX support can be transformed into TM also when linked to the anomeric hydroxyl as shown in Scheme 4, e.g., perbenzoylated Galβ(1−4)Glcβ1-DOX-PEG (**9**) → perbenzoylated Galβ(1−4)Galβ1-O-TM (**10**). This selective hydrogenolysis gave reproducible results with 2-O-benzoylated, 2-O-isobutyrylated, 2-deoxy-2-*N*-phthalimido, and 2-O-glycosylated hexopyranoses. The MPEG-DOX group in 2-O-acetylated derivatives of hexopyranoses was removed completely under the same hydrogenolytic conditions.[6]

In summary, we have developed a versatile polymer-supported solution synthesis of protected oligosaccharides that can be manipulated further or deprotected to oligosaccharides required for biological testing. Finally, the MPEG-DOX-saccharides as such, because of their solubility in both polar and apolar media, promise interesting biomedical applications.

(12) E.g., using 5% Pd black in EtOH at room temperature and H_2 at 1 atm pressure.

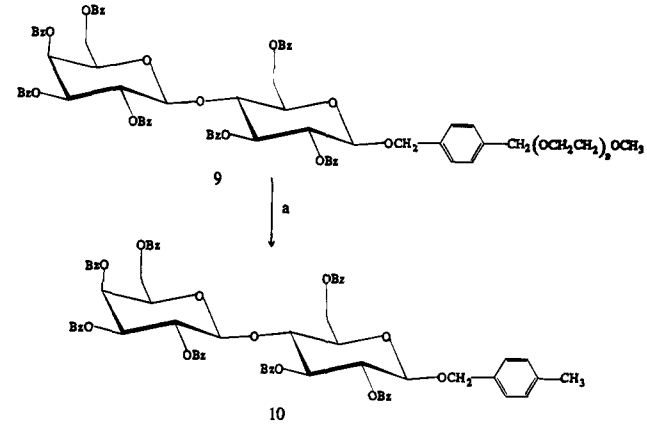
(13) Conditions: 10% Pd/C, 50% aqueous AcOH, 50 °C, H_2 at 3 atm pressure.

Scheme 3[a]



[a] Reagents and conditions: (a) NaH, NaI, THF, 50 °C 48 h; (b) 60% aqueous HOAc, 100 °C, 40 min; (c) TBDPSiCl, imidazole, CH_2Cl_2, 16 h; (d) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate, triflic anhydride, 4 Å molecular sieves, CH_2Cl_2, 0−5 °C, 2 h.

Scheme 4[a]



[a] Reagents and conditions: (a) H_2, Pd black, EtOH, 1 atm, 48 h.

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Supplementary Material Available: Experimental details, including procedures for the preparation of compounds **1**−**10** (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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