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METATHESIS OF OLIGOSACCHARIDES. RELATIVE STABILITIES OF ACTIVATED AND DEACTIVATED GLYCOSIDES OF POLYETHYLENE GLYCOL.

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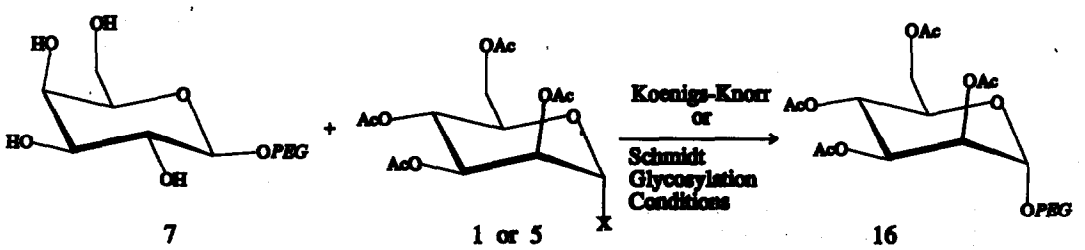
Abstract: *The preparation of glycosides and orthoesters of the monomethylether of polyethyleneglycol are described. The attempted glycosylation of these glycosides unexpectedly led to glycosyl exchange instead of the expected oligosaccharides. This observation suggests that PEG as aglycon acts both as a leaving group and a nucleophile. A hypothesis explaining metathesis of oligosaccharides under glycosylation conditions has been formulated.*

An efficient methodology for the preparation of oligosaccharides, and their incorporation into glycopeptides and glycolipids, is essential for the application of these compounds in biomedical sciences.[1] We have recently described a polymer-supported synthetic design combining the anomeric control of solution chemistry with the ease and speed of solid-state-supported work-up.[2] The strategy employs as the supporting polymer polyethyleneglycol monomethylether [$HOCH_2CH_2(OCH_2CH_2)_nOCH_3$, n=80-160; *PEG*, average MW 5,000][3,4] and is based on solubility of a carbohydrate-*PEG* synthon under reaction conditions, and its insolubility during work up. We have reported linking *PEG* using succinate linker to positions other than the anomeric carbon. Linked to an anomeric carbon, *PEG* could be considered a convenient protective group. We describe in this communication interesting observations suggesting that *PEG* as aglycon acts simultaneously both as a leaving group and a nucleophile under several glycosylation conditions. Since *PEG* glycosides can be easily isolated at any stage of the reaction by precipitation with an ether, and the ratio of exchanged to unexchanged *PEG* glycosides can be quantitatively measured by ^1H NMR *PEG* glycosides could be used for the evaluation of the relative reactivities of differently protected glycosylating agents.

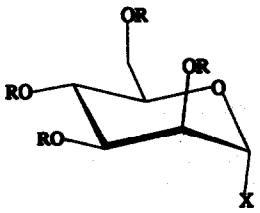


7 1 or 5 16

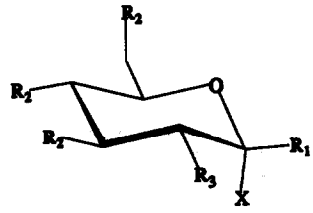
Scheme I.

Although the hydroxyl group on *PEG* is primary, its reactivity in glycosylation reactions is reduced, in comparison with the usual reactivity of carbohydrate primary hydroxy groups. For instance, reactions between 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (**1**) or 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**2**) and *PEG* using silver triflate and 2,6-di-*t*-butyl-4-methylpyridine as a base (> 1 eq.) in CH_2Cl_2 gives orthoesters, cf. **10**. Trichloroacetimidates **3**, **4** and **5**, on the other hand, yield *PEG trans* glycosides eg. **16**, since the acidic conditions used for the reaction are not favourable for orthoester formation. However,

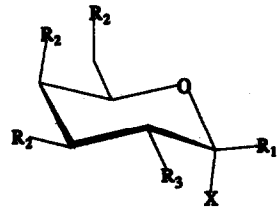
2,3,4,6-tetra-O-acetyl-α-D-galactosyl bromide (**6**) gives the ß-glycoside **14** even with AgTf and a base.[5a] *PEG*-sugar glycosides or *PEG*-sugar orthoesters were deacetylated using 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in methanol. Both Koenigs-Knorr[5b,c] and Schmidt[5d] glycosylation of these fully deacetylated *PEG*-orthoesters and *PEG*-glycosides was expected to take place regiospecifically on the primary hydroxyl. Surprisingly, mostly products of glycosyl exchange on *PEG*, rather than the expected disaccharides, were obtained (Scheme I).[6] This occurred under a variety of conditions.[7-9] For example, *PEG*yl-ß-D-galactopyranoside (**7**) gave upon treatment with 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (**1**)[7] exchanged peracetylated *trans* mannoside of *PEG* **16**, see scheme I. Moreover, **7** yielded *trans* glycosides of *PEG* with **2**,[7] **3**,[8] and **8**;[9] *PEG*yl α-D-mannopyranoside (**12**) yielded peracetylated *trans* glycosides of *PEG* with **3**[8] and **9**.[7] Similarly, orthoesters derived from glucose or mannose gave *trans* glycosides of *PEG* with peracetylated monosaccharides on treatment with **1**,[7] **2**,[7] **3**[8] and **6**,[7] for structures see scheme II. In all these exchanges no starting material was detectable. How is this happening?



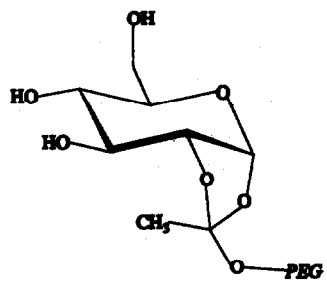
1, R = Ac, X= Br
5, R = Ac, X= OC(=NH)CCl$_3$
12, R = H, X= O*PEG*
16, R = Ac, X= O*PEG*



2, R$_1$= H, R$_2$ = OAc, R$_3$ = OAc, X= Br
4, R$_1$ = H, R$_2$ = OAc, R$_3$ = OAc, X= OC(=NH)CCl$_3$
9, R$_1$ = Br, R$_2$ = OAc, R$_3$= NPhth, X= H



6, R$_1$= H, R$_2$ = OAc, R$_3$ = OAc, X= Br
3, R$_1$ = H, R$_2$ = OAc, R$_3$ = OAc, X= OC(=NH)CCl$_3$
7, R$_1$ = O*PEG*, R$_2$ = OH, R$_3$ = OH, X= H
8, R$_1$= H, R$_2$ = OAc, R$_3$ = OAc, X= 
13, R$_1$ = OC(=NH)CCl$_3$, R$_2$ = OAc, R$_3$= NPhth, X= H
14, R$_1$= O*PEG*, R$_2$ = OAc, R$_3$ = OAc, X= H
15, R$_1$= O*PEG*, R$_2$ = OAc, R$_3$ = NPhth, X= H



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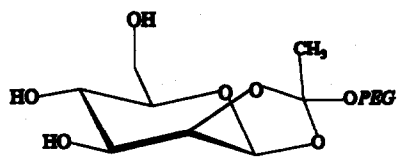

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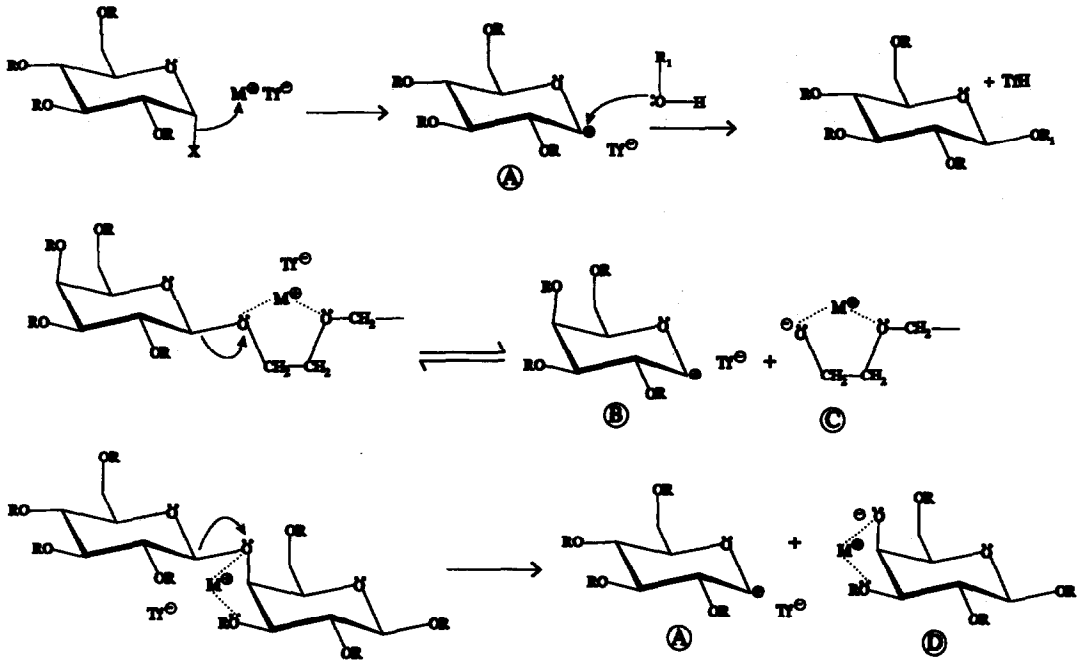
Scheme II.

A glycosylation reaction is generally defined as a nucleophilic substitution by a hydroxyl group at the anomeric carbon as portrayed in Scheme III, X being a leaving group such as halogen or trichloroacetimide. The promoter M$^+$ is an electron deficient reagent such as BF_3 or Ag^+ that generates an electrophilic species A. In the *PEG* glycosides M$^+$ can coordinate to the oxygens of *PEG* and the sugar, leading to the activation of the anomeric carbon. Subsequent dissociation leads to an electrophilic species B and nucleophilic *PEG* C. During glycosylation of *PEG* glycosides, the glycosylating agents become activated and are available for reaction with any nucleophiles present, including the *PEG* species C. The relative stabilities of A and B, and

their relative electrophilicities, as well as the relative stabilities of the final products A-C and B-C, will decide which *PEG* glycosides will be isolated.

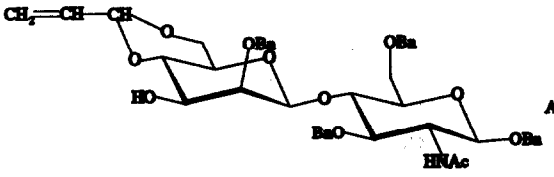


Scheme III.

Thus it is understandable that the unprotected monosaccharides in *PEG* glycosides are exchanged for the acetylated species since the hydroxy group containing monosaccharides are not expected to exhibit particularly high solubility under the conditions of glycosylation reactions. The insolubility removes this unprotected species from the competition with the acetylated donor saccharide. These principles are exemplified by glycosylation of *PEG*yl α-D-mannopyranoside (**12**) with a mixture of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl trichloroacetimidate (**3**) and 2-deoxy-2-phthalimido 3,4,6-triO-acetyl-ß-D-galactopyranosyl trichloroacetimidate (**13**) in CH_2Cl_2 promoted by $BF_3.Et_2O$ (1 eq. to *PEG*). The *PEG*-bound reaction products included *PEG*yl 2,3,4,6-tetra-O-acetyl-ß-D-galactopyranoside (**14**) [40%; δ(H-1) 4.57], *PEG*yl 2-deoxy-2-phthalimido-3,4,6-tri-O-acetyl-ß-D-galactopyranoside (**15**) [40%; δ(H-1) 5.37], and two disaccharides; mannopyranosides glycosylated with 2,3,4,6-tetra-O-acetyl-ß-D-galactopyranose and 2-deoxy-2-phthalimido-3,4,6-tri-O-acetyl-ß-D-galactopyranose most likely in O-6 [20%; δ(Man H1) 4.90 and 4.74]. No starting *PEG*yl α-D-mannopyranoside (**12**) [δ(H1) 4.83] could be detected. Glycosylated mannose might be soluble enough to compete with both donors for *PEG*. Next, to provide a further example of the scenario where all glycosyls were endowed with similar reactivity, we have examined a reaction of *PEG*yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**16**) with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl trichloroacetimidate (**3**) using $BF_3.Et_2O$ as a promoter.[8] A mixture of *PEG*yl 2,3,4,6-tetra-O-acetyl-ß-D-galactopyranoside (**14**) and *PEG*yl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**16**) (approximately 1:1) resulted. Similar observations were made with various combinations of *gluco*-, *galacto*-, and mannopyranosides.

The reported findings help to explain frequently observed decomposition of larger oligosaccharides used as reactants in glycosylation reactions. Such decompositions are usually ascribed to hydrolysis due to a failure

to maintain the reaction mixture rigorously anhydrous. The results reported in this communication suggest, however, that a part of an oligosaccharide may act to a certain extent as a "leaving group" analogously to *PEG*. The glycosidic bond involved could split as shown in Scheme III giving A and D, and it could be either recreated to give back the original or the other anomer (or both), or the "fragments" could undergo other reactions. The latter would lead to irreversible structural changes, in particular during the work-up of the reaction mixture. In order to simulate *PEG*, the oligosaccharide should have the appropriate dihedral angle of vicinal ethers -O-CH-CH-O-, for instance 60° diequatorial *trans* or axial-equatorial *cis* geometries for pyranosides. As an example from this laboratory, glycosylation of the disaccharide **17** with trisaccharide **18**-derived glycosylating agents[10] led to the extensive formation of not further identified monosaccharide and disaccharide derivatives (for general portrayal see Scheme III).



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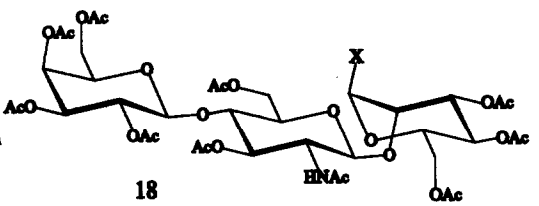

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References and Notes.

1. Carbohydrate Recognition in Cellular Function (Ed. G. Bock and S. Harnett), CIBA Foundation Symposium 145; Wiley, Chichester 1989.
2. S. P. Douglas, D. M. Whitfield, and J. J. Krepinsky, J. Amer. Chem. Soc. **113**, 5095 (1991).
3. a)Technical Bulletin: Polyglycols Hoechst. Polyethylene Glycols: Properties and Applications; Hoechst, Frankfurt/Main 1983. (b), J. Dale, Isr. J. Chem. **20**, 3 (1980).
4. Since *PEG* contains a single O-CH_3 group (δ=3.380 ppm), the reaction course is easily monitored by NMR spectroscopy using the signal of this methyl as an internal standard.
5. a) N. K. Kochetkov, A. J. Khorlin, and A. F. Bochkov, Tetrahedron **23**, 693 (1967); (b) H. Paulsen, Angew. Chem. Int. Ed. Engl. **21**, 155 (1982); (c) **29**, 823 (1990); (d), R. R. Schmidt, Angew. Chem. Int. Ed. Engl. **25**, 212 (1986).
6. The yields of precipitated *PEG* derivatives are generally in the 65-95% range. This variability reflects only the variations in the work-up conditions (precipitation and filtration). The precipitation ability of *PEG* derivatives is not noticeably affected by the properties of the "glyco" portion since the solubility properties are dictated by the overwhelming influence of the polymeric *PEG*.
7. Conditions: Silver triflate, 2,6-di-*t*-butyl-4-methylpyridine, in CH_2Cl_2, room temperature, using 1.2 eq. of a bromide, for 16 hours.
8. Conditions: $BF_3.Et_2O$ in CH_2Cl_2 at room temperature using 1.1 eq. of a trichloroacetimidate for 16 hours.
9. Conditions: CH_3I in CH_2Cl_2 at 50 °C for 72 hours in a sealed reaction vessel.
10. Conditions: 7 or 8.

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