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Preliminary communication

A general synthesis of model glycoproteins: coupling of alkenyl glycosides to proteins, using reductive ozonolysis followed by reductive amination with sodium cyanoborohydride

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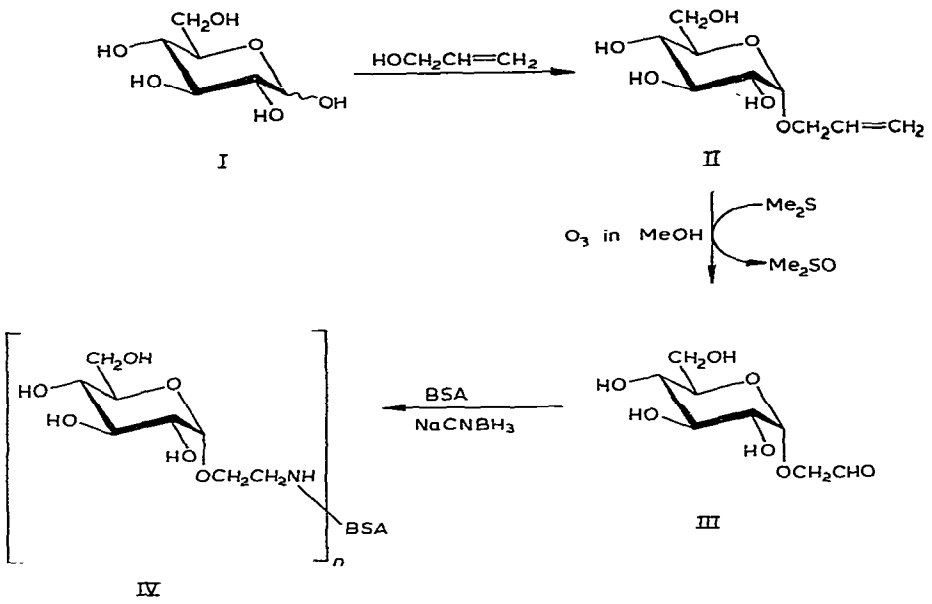
Concomitant with the growing interest[1] in the properties and biological functions of glycoconjugates, much attention[2–15] has been directed to their *in vitro* synthesis. In the glycoprotein area, sugars of known structure have been covalently attached to a protein aglycon. Most notable in this regard are the recent studies of Lemieux and co-workers[15], in which various oligosaccharide, blood-group determinants have been synthesized, coupled to bovine serum albumin (BSA), and the resultant synthetic antigens used to raise antibodies.

Each procedure described in the literature for the synthesis of model glycoproteins has some limitation, and the one presented here was designed with the following criteria in mind. The reaction should (*a*) be directly compatible with the reagents and protecting groups used in oligosaccharide synthesis, (*b*) not cause modification of the reducing (terminal) sugar residue, (*c*) have inbuilt variability of the spacer between the sugar and the protein in terms of length, hydrophobicity, and antigenic characteristics, (*d*) be convenient, and rapid at, or near, physiological pH in aqueous media, using stable reagents, (*e*) give high-yielding coupling to a specific amino acid *via* a hydrolytically stable, covalent linkage, and (*f*) not dramatically alter the surface charge of the protein.

The coupling procedure that we have been using for over a year to prepare sugar–protein conjugates is summarized in Scheme 1. In its simplest form, the sugar (I) is glycosylated with an alkenyl alcohol using standard methods, such as those developed for the synthesis of allyl glycosides[16,17]. The resultant glycoside (II) is treated in methanol solution, first with ozone, typically for <5 min* at −78°, and subsequently with dimethyl sulfide to complete the reductive ozonolysis[18] and afford aldehyde III† quantitatively. Compound III, together with BSA, is then dissolved in water, and subjected to reductive amination[20] with sodium cyanoborohydride at pH 9.0. After a chosen time, the BSA–sugar conjugate (IV) can be isolated by chromatography on a column of Sephadex G 25 followed by exhaustive dialysis, and a sample analyzed for sugar by, for example, the phenol–sulfuric acid method[21]. Attachment of a protected sugar, such as a peracetate, can

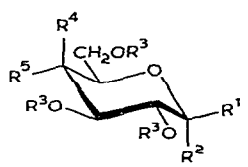
*Prolonged ozonolysis (1–10 h) in acetic anhydride at room temperature can lead to side reactions[19].
†The structure of such products has been confirmed chemically, and by p.m.r. spectroscopy at 400 MHz.



Scheme 1

be achieved in a similar way, but with 4:1 MeOH–CH_2Cl_2 as the solvent for the reductive ozonolysis, and H_2O–MeOH for the reductive amination. If a long spacer-arm is desired, glycosides of, for example, 10-undecenyl alcohol can be readily prepared.

In typical reactions with BSA, we found that use of a molar ratio of sugar:lysine of 5:1 resulted in the attachment of 20 mol of the α-D-glucoside **1** to each mol of BSA within 48 h (a 36% "yield"); for the α-D-galactoside **2**, a molar ratio of 2:1 gave a 25% yield (14 mol/mol) within the same time-period, and reaction for a further time resulted in little increase in yield. Using the fully *O*-acetylated allyl β-D-glucoside **3**, 38 lysyl amine groups per BSA unit were derivatized within 48 h; after 170 h, 45 sugar residues had been attached. In a preliminary experiment only, the *O*-acetylated 10-undecenyl glucoside **4** was coupled to the extent of 8 mol/mol of BSA within 48 h.



	1	2	3	4
R^1	H	H	$OCH_2CH{=}CH_2$	$O(CH_2)_9CH{=}CH_2$
R^2	$OCH_2CH{=}CH_2$	$OCH_2CH{=}CH_2$	H	H
R^3	H	H	Ac	Ac
R^4	H	OH	H	H
R^5	OH	H	OAc	OAc

One of the several advantages of this approach is that each of the individual reactions has previously been well documented, and, hence, their incorporation into this scheme does not necessitate the development of an extensive array of "control" experiments. For example, a detailed study by Schwartz and Gray[7] proved that reductive amina-

tion of BSA with disaccharides gives reaction at the 6-amino groups of lysine, and, hence, it is not necessary to check this point for every reaction. Reductive ozonolysis has an advantage over the periodate–ruthenium dioxide oxidation[22], which we have also tried[23], in that it can be applied to free glycosides.

We consider that this approach to the synthesis of model sugar–protein conjugates has substantial generality, and we are now including it in our spectroscopic studies[24,25] of specific, associative phenomena involving the carbohydrate moieties of glycoproteins.

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