Syntheses and Transformations of Glycohydrolase Substrates into Protein Conjugates Based on Michael Additions

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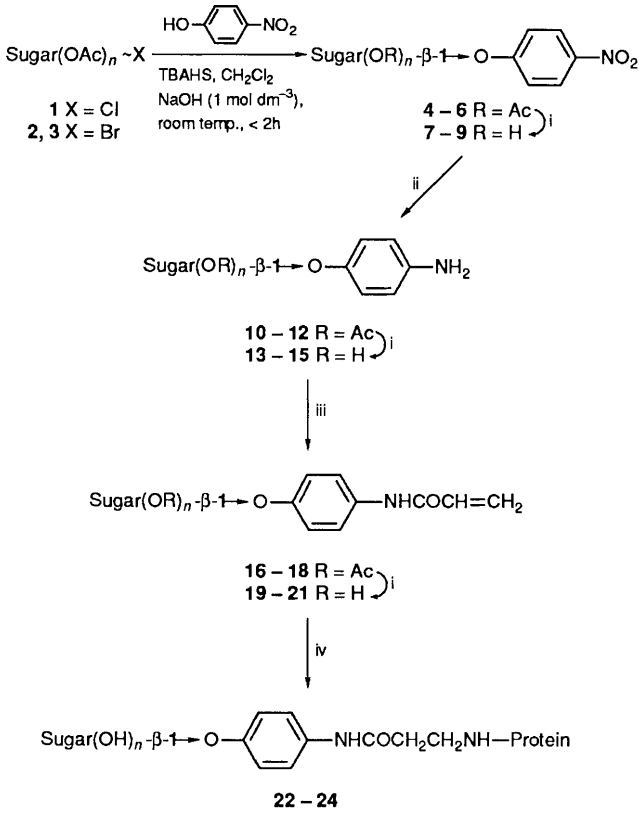
The glycosyl chloride **1** and bromides **2** and **3** were stereospecifically transformed into *p*-nitrophenyl glycosides by phase transfer catalysis; these glycohydrolase substrates were reduced and *N*-acryloylated to afford Michael acceptors which reacted with amine functions of proteins.

It is well established that artificial carbohydrate protein conjugates constitute useful antigens and immunogens from which immunodiagnostic reagents as well as animal and human vaccines can be derived.[1] They are also important tools for receptor studies, for the inhibition of adherence of bacteria and viruses, and for cell targeted drug delivery. In order to expand the methodologies[2] for the conjugation of functionalized carbohydrates to proteins and polymers, we present herein another application[3] of the Michael-type additions of protein-amine functionality (ε-lysine group) onto *p*-*N*-acryloylated phenyl glycosides. These model glycosides were easily derived from *p*-nitrophenyl glycosides obtained by phase transfer catalysis (PTC).[4,5] It is also of interest that some of the above *p*-nitrophenyl glycosides, which constitute useful glycohydrolase substrates, are commercially available and, therefore, the strategy described here represents a practical entry into this class of conjugates. This paper also describes for the first time the use of PTC conditions for the stereospecific synthesis of *p*-nitrophenyl glycosides in the series of 2-acetamido-2-deoxy-β-D-glucopyranoside **4**[5] and disaccharide **6**.[5,6]

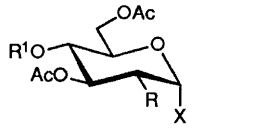
Among the previous conjugation methodologies, the diazonium[7] and the isothiocyanate[8] derivatives prepared from *p*-aminophenyl glycosides or derived aminoalditols still find widespread applications in spite of evident drawbacks. In continuation of our endeavours[5,6] to use PTC conditions for the introduction of a number of anomeric glycosyl substituents, we were interested to use *p*-*N*-acryloylphenyl glycosides as Michael acceptors. The general strategy for the preparation and transformation of these useful precursors is depicted in Scheme 1.

Starting from the glycosyl donors **1–3**, the known[4] peracetylated *p*-nitrophenyl glycosides **4–6**† were obtained in 73,

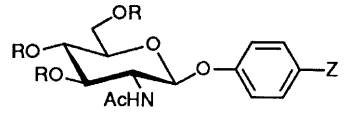
† All intermediates showed satisfactory spectral and analytical data. Yields refer to isolated crystalline materials.



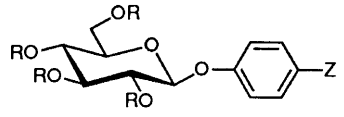
Scheme 1 *Reagents and conditions*: i, NaOMe, MeOH; ii, HCO_2NH_4, 10% Pd/C, MeOH, reflux, 5 min; iii, CH_2=CHCOCl, Et_3N, 0 °C; iv, protein (BSA), carbonate buffer (0.1 mol dm^{-3}), pH 9.0, 37 °C, 3 days dialysis



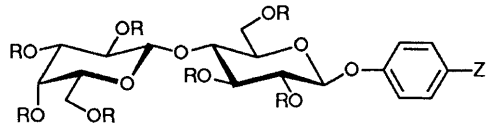
1 X = Cl, R = NHAc, R^1 = Ac
2 X = Br, R = OAc, R^1 = Ac
3 X = Br, R = OAc, R^1 = Gal(OAc)$_4$



4 R = Ac, Z = NO_2
7 R = H, Z = NO_2
10 R = Ac, Z = NH_2
13 R = H, Z = NH_2
16 R = Ac, Z = NHCOCH=CH_2
19 R = H, Z = NHCOCH=CH_2
22 R = H, Z = $NHCOCH_2CH_2NH$—BSA



5 R = Ac, Z = NO_2
8 R = H, Z = NO_2
11 R = Ac, Z = NH_2
14 R = H, Z = NH_2
17 R = Ac, Z = NHCOCH=CH_2
20 R = H, Z = NHCOCH=CH_2
23 R = H, Z = $NHCOCH_2CH_2NH$—BSA



6 R = Ac, Z = NO_2
9 R = H, Z = NO_2
12 R = Ac, Z = NH_2
15 R = H, Z = NH_2
18 R = Ac, Z = NHCOCH=CH_2
21 R = H, Z = NHCOCH=CH_2
24 R = H, Z = $NHCOCH_2CH_2NH$—BSA

48 and 57% yields, respectively under PTC conditions with tetrabutylammonium hydrogen sulphate (TBAHS) and 1 mol dm^{-3} sodium hydroxide (25 °C, <2 h).[5,6] The method was entirely stereospecific since only β-D-glycosides were detected and obtained from the α-glycosyl chloride **1** and bromides **2** and **3** and thus occurred with complete inversion. The NMR data of **4** {m.p. 237.4–238.4 °C, $[\alpha]_D^{23}$ −9.6° (Me_2SO)} displayed the anomeric proton signal at δ 5.56 with a characteristic 1,2-*trans*-β-D- relationship ($J_{1,2}$ 8.4 Hz) an anomeric carbon chemical shift at δ 97.1. The anomeric proton signals of **5** {m.p. 178.8–179.8 °C, $[\alpha]_D^{23}$ −40.5° ($CHCl_3$)} and **6** {m.p. 133.3–135.4 °C, $[\alpha]_D^{23}$ −21.1° ($CHCl_3$)} were obscured by the other signals. However, the chemical shifts of their anomeric carbon appeared at δ 97.9 and 97.6 (glucose residues), respectively, indicative of their β-D-configuration.[9] The anomeric proton signals of the reduced *p*-aminophenyl glycosides **10–12** were well defined and confirmed the above conclusions. The peracetylated glycosides **4–6** were deprotected under Zemplén conditions (NaOMe, MeOH) to give **7–9** in quantitative yields. Both the protected **4–6** or the unprotected **7–9** *p*-nitrophenyl glycosides were reduced to the *p*-aminophenyl glycosides **10–12** and **13–15** under catalytic transfer hydrogenation with ammonium formate and 10% Pd on charcoal[10] in excellent yields (87–98%). The protected glycosides **10–12** were also transformed into the free forms **13–15** under the above conditions. Finally, *N*-acryloylation of **10–12** and **13–15** with acryloyl chloride and triethylamine at 0 °C in methylene chloride (**10–12**) or methanol (**13–15**) afforded the desired *p*-*N*-acryloylphenyl glycosides **16–18** and **19–21** in 95 ± 2% yields. In the case of the unprotected series **13–15**, the excess of by-products had to be eliminated by treatment with ion-exchange resins. However, the scheme using the fully protected glycosides offered practical advantages since extraction of the products in organic solvent was effective and straightforward. As above, **16–18** were converted to **19–21** under Zemplén conditions (quantitative yields).

The use of *N*-acryloylated precursors has distinct advantages over previous methodologies since, as originally proposed by us,[11] these conjugated precursors offer two synthetic possibilities. First, they can be efficiently polymerized with acryloyl-type monomers and second, amine groups of proteins or of functionalized polymers can be used as nucleophiles to give glycoconjugates by Michael addition.[3] Thus, covalent attachment of **19–21** onto bovine serum albumin (BSA) was tested under various sets of reactant molar ratios (2 or 4 : 1, sugar : amine), buffers (phosphate, borate, carbonate) and pH (8.0 and 9.0). As previously observed,[3] the Michael reaction did not furnish appreciable conjugation in phosphate buffers at pH 8.0 or 9.0 (3 days, 37 °C), However, the addition

proceeded smoothly in both borate and carbonate buffers pH 8.0 and 9.0, the carbonate buffer being more efficient than the borate buffer. A pH of 9.0 was also more efficient in both buffers. There was a very small amount of carbohydrate conjugated in borate buffer pH 8.0. The glycoconjugates were isolated after dialysis and freeze-drying. The levels of incorporation were measured by comparing the absorbances at 250 and 280 nm using HPLC purified conjugates and *p*-*N*-acetamidophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside as a standard.[5] The HPLC separations were performed on a TSK G4000 SWXL column using NaCl (0.07 mol dm^{-3}) and phosphate buffer (0.02 mol dm^{-3}) pH 6.7 as the mobile phase. The sugar content of the conjugates varied from 6 to 10 (borate pH 9.0) to 30 to 60 (carbonate pH 9.0). The antigenicities of the conjugates were demonstrated with lectins by double radial immunodiffusion. Polymer conjugates have been prepared and will be presented in due course.

In conclusion, *p*-nitrophenyl glycosides, obtainable commercially or by phase transfer catalysed glycosidations, were transformed into *N*-acryloyl Michael acceptors which reacted with the amine of the proteins in aqueous buffers to afford antigenic carbohydrate protein conjugates.

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