Custom-designed Glycopolymer† Syntheses by Terpolymerizations‡

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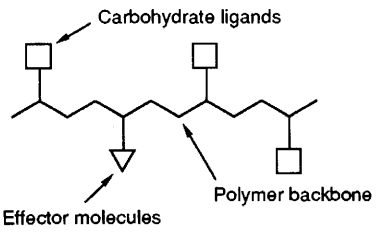
Carbohydrate derivatives having lectin binding properties and bearing *N*-acryloyl functionality have been copolymerized under three-component terpolymerization conditions with acrylamide and *N*-acryloylated effector molecules to provide water-soluble glycopolymers with custom designed physico-chemical properties.

Synthetic glycoconjugates are a family of compounds which include (neo)glycoproteins, (neo)glycolipids and glycopolymers. The latter derivatives have gained increased interest[1–3] since their introduction by Horejší *et al.*[4] due to the advantageous physico-chemical and structural properties of such glycopolymers. Moreover, recent observations of the 'cluster effect' of carbohydrate ligands for their specific receptors[5] can be fully addressed by virtue of the polymers' intrinsic properties. In order to further expand[6] the usefulness of glycopolymers in bio- and immuno-chemical assays, we have explored the possibility of using three-component copolymerization (terpolymerization) in the custom design syntheses of glycopolymers having defined physico-chemical properties.

The general structure of these new glycopolymers is depicted in Scheme 1. The strategy is very simple and relies on readily available *N*-acryloylated precursors.[1] The carbohydrate derivatives serve as ligands for lectins and antibodies. The acrylamide was used to confer the polymers with water-soluble properties while the probes or effector molecules were added to provide their specific properties. Biotin was chosen as an obvious candidate since many avidin or streptavidin-labelled conjugates are commercially available.[7] The *N*-acryloylated stearylamine device was incorporated in order to provide improved lipophilic properties which make these polymers suitable coating antigens in enzyme linked immunosorbent assays (ELISA or ELLA). The *N*-acryloylated tyramine moiety was produced because it provides glycopolymers on which radioactive iodine labelling can be readily accomplished under usual conditions. It permits mimicking tyrosine residues of proteins.



Scheme 1

The syntheses of the *N*-acryloylated carbohydrate precursors **1–3** have been described previously.[1,8] The effector molecules were prepared in a similar manner. Thus, *N*-acryloylation of biotinamidocaproylhydrazide **4** was accomplished with acryloyl chloride dissolved in dioxane added dropwise to ice-cold methanol containing anionic resin ($^-$OH). Filtration of the resin and evaporation of the solvents provided crude **5**. Crystallization from methanol and silica gel chromatography of the mother liquor afforded pure **5** in 78% combined yield {two crops; m.p. 185.7–187.1 °C, $[\alpha]_D^{23}$ +35.4 (dimethyl sulfoxide)}.§ *N*-Acryloylation of stearylamine **6** with acryloyl chloride in methylene chloride containing triethylamine at 0 °C provided pure **7** after crystallization from ethanol–water (96% yield; m.p. 74.5–75.1 °C). *N*-Acryloyation of tyramine **8** in methanol containing triethylamine at 0 °C as above provided **9** in 69% yield as an amorphous solid after silica gel chromatography (chemical ionization MS for $C_{11}H_{13}NO_2$: *m/z* 192, M + 1, 100%).

The required terpolymers **10–15** were obtained by copolymerization of the carbohydrate moieties **1–3** (10 equiv.), acrylamide (or methacrylamide only in two cases, **13**, **13a**) (100 equiv.) and the suitable effector molecules **5**, **7**, **9** (1

† This term has been recently defined by analogy to glycoconjugates, glycolipids and glycoproteins (see ref. 3).

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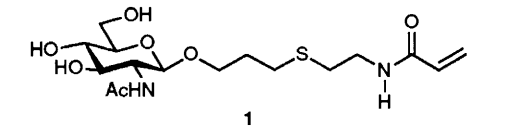
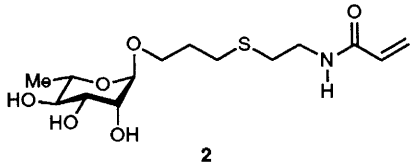
§ All intermediates showed satisfactory spectral and analytical data.



1



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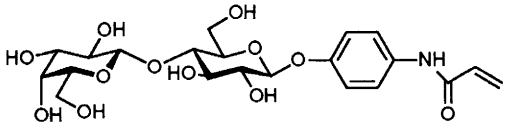

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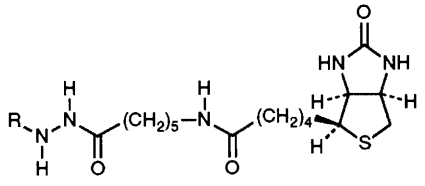
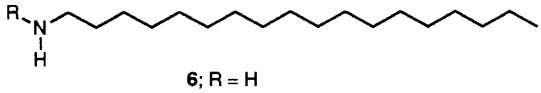
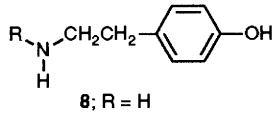


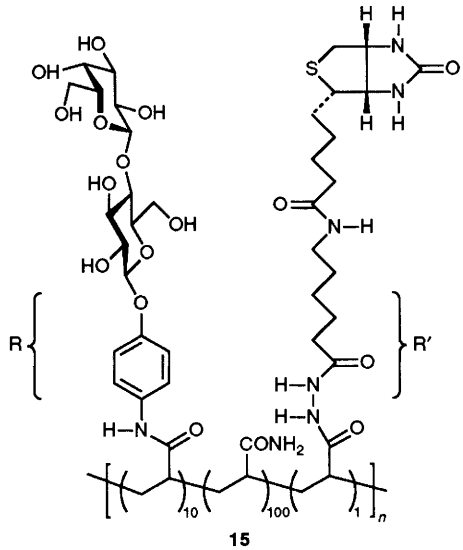
4; R = H
5; R = CO–CH=CH_2



6; R = H
7; R = CO–CH=CH_2



8; R = H
9; R = CO–CH=CH_2



15

10; R = 1, R′ = 5
11; R = 1, R′ = 7
12; R = 1, R′ = 9
13; R = 1, co-methacrylamide
13a; R = 1, co-acrylamide
14; R = 1, R′ = 2
15; R = 3, R′ = 5

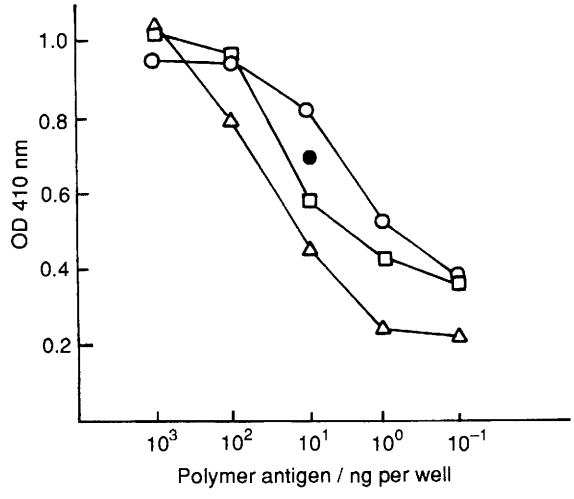


Fig. 1 Enzyme linked lectin assay (ELLA) of the terpolymers **11–13a** used as coating antigens with horseradish peroxidase-labelled wheat germ agglutinin (WGA) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as substrate; **11** (○), **12** (□), **13** (●, one point), **13a** (△)

equiv.). The reactions were conducted in deoxygenated water and the radical initiated polymerization was performed with $(NH_4)_2S_2O_8$ and heat (15 min) as previously described.[1,3,6] The resulting viscous solutions were diluted with water and dialysed exhaustively against water. White spongy copolymers were obtained after lyophilization. In this manner, copolymers **10–12** were obtained in 62, 60 and 55% yields, respectively. However, when the reactions were conducted at room temperature (1.5 h) in water–dimethyl sulfoxide (DMSO) mixture (2:1) and in the presence of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) as catalyst, the polymers **10** and **15** were obtained in 97 and 93% yields, respectively. Polymer **15** was prepared in order to obtain an acrylamide : lactose : biotin ratio of 50 : 5 : 1 which was exactly the ratio of the reactants as previously observed for this type of *N*-acryloylated monomers.[1,3,6] The monomer ratio within the polymers was determined using ^{1}H NMR spectroscopy. For comparison purpose in ELISA assays, polymer **13** was prepared by copolymerization of **1** with methacrylamide as above without DMSO and TMEDA. Finally, the heterobifunctional terpolymer **14** was prepared by copolymerizing two different carbohydrate units **1** and **2** with acrylamide as above. These water-soluble terpolymers have broad molecular mass distributions centred at ~150 ku as measured by HPLC gel permeation chromatography using TSK G4000 SWXL columns (7.5 mm × 30 cm) in 0.1 mol dm^{-3} phosphate buffer and polyacrylamide as standards.[3]

All the polymers retained their specific lectin binding properties as determined by agar gel diffusion and ELLA with wheat germ agglutinin (WGA, for GlcNAc), *Ricinus communis* (castor bean lectin, for rhamnose) and peanut lectin (*Arachis hypogaea*, for lactose). Enzyme linked lectin assays (ELLA) in microtitre plates using GlcNAc-containing terpolymers **11–13** as coating antigens and horseradish peroxidase labelled WGA showed the effect of the effector molecules on the capacity of the copolymers to be adsorbed on the surface of the plastic well.[3] At 10 ng per well, the co-stearoyl terpolymer **11** was twice as efficient as the co-acrylamide alone (without effectors) taken as standard (Fig. 1). The biotin containing glyco(ter)polymers showed strong binding to both avidin and streptavidin. Similar terpolymers containing sialic acid residues were also synthesized and the results will be reported elsewhere.

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References

1 R. Roy and F. Tropper, *Glycoconjugate J.*, 1988, **5**, 203; R. Roy and C. A. Laferrière, *Carbohydr. Res.*, 1988, **88**, C1; R. Roy and F. D. Tropper, *J. Chem. Soc., Chem. Commun.*, 1988, 1058.
2 For other relevant examples, see: P. Kosma, P. Waldstätten, L. Daoud, G. Schulz and F.M. Unger, *Carbohydr. Res.*, 1989, **194**, 145; N. E. Byramova, L. V. Mochalova, J. M. Belyanchikov, M. N. Matrosovich and N. V. Bovin, *J. Carbohydr. Chem.*, 1991, **10**, 691; A Spaltenstein and G. M. Whitesides, *J. Am. Chem. Soc.*, 1991, **113**, 686; E. Kallin, H. Lönn, T. Norberg and M. Elofsson, *J. Carbohydr. Chem.*, 1989, **8**, 597; A. Y. Chernyak, G. V. M. Sharma, L. O. Kononov, P. R. Krishna, A. V. R. Rao and N. K. Kochetkov, *Glycoconjugate J.*, 1991, **8**, 82; H. Paulsen, A. Wulff and M. Brenken, *Liebigs Ann. Chem.*, 1991, 1127 and references cited therein.
3 R. Roy, F. D. Tropper and A. Romanowska, *Bioconjugate Chem.*, 1992, **3**, 256.
4 V. Horejší, P. Smolek and J. Kocourek, *Biochim. Biophys. Acta*, 1978, **538**, 293.
5 Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Lönngren, J. Arnap, M. Haraldsson and H. Lönn, *J. Biol. Chem.*, 1983, **258**, 199.
6 R. Roy and C. A. Laferrière, *J. Chem. Soc., Chem. Commun.*, 1990, 1709; R. Roy, F. O. Andersson, G. Harms, S. Kelm and R. Schauer, *Angew. Chem., Int. Ed. Engl.*, 1992, in the press; R. Roy, F. D. Tropper, A. Romanowska, R. K. Jain, C. F. Piskorz and K. L. Matta, *BioMed. Chem. Lett.*, 1992, **2**, 911.
7 M. Wilchek and E. A. Bayer, *Anal. Biochem.*, 1988, **171**, 1.
8 R. Roy, F. D. Tropper, T. Morrison and J. Boratynski, *J. Chem. Soc., Chem. Commun.*, 1991, 536.