Synthesis of Oligosaccharide-Polylysine Conjugates: A Well Characterized Sialyl Lewis[a] Polymer for ELISA

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Received March 3, 1997

Linear polymers containing carbohydrate residues which are covalently linked to the backbone represent an important class of macromolecules and have found broad application.[1] In the first place, such glycopolymers have been synthesized by radical copolymerization of glycosylated and nonglycosylated monomers.[2,3] This approach is convenient but sometimes lacks reproducibility and therefore predictability of the product composition due to differing polymerization properties of the monomers. Furthermore, very broad molecular mass distributions are obtained especially for acrylate polymerizations.[4] Fractionation is laborious and leads to loss of material. As an alternative, the derivatization of a preformed homopolymer which contains reactive groups is a less common but superior strategy (Figure 1): coupling with substoichiometric amounts of a carbohydrate component is followed by treatment with an excess of a capping agent to quench the remaining reactive functionalities of the intermediate. *To achieve a predictable product composition these conversions have to be quantitative and side reactions with respect to the polymer backbone have to be excluded.*

Thus, active esters of polyacrylic acid obtained by radical polymerization of 4-nitrophenyl acrylate[5] or *N*-oxysuccinimidyl acrylate[6] have been derivatized with residues containing a primary amino group. The products are not biodegradable due to the alkyl-backbone and, in some cases, contain variable amounts of carboxylic acid functions due to unwanted concomitant hydrolysis of active ester residues.[7] This restricts both the predictability of the composition and the applicability to charge-neutral preparations. Furthermore, the activated starting polymers are not readily available with narrow molecular weight distributions.

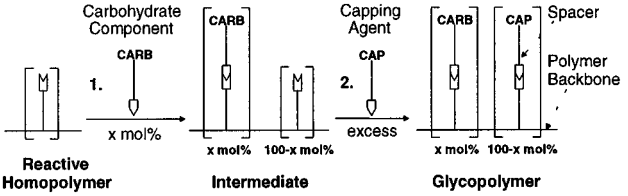

Figure 1. Synthesis of a tailored glycopolymer: 1. quantitative reaction of a reactive homopolymer with a small amount of a suitably functionalized carbohydrate and 2. capping of the remaining reactive residues.

Biodegradable glycopolymers were obtained from polyamino acids. Polyaspartimide (PAI) was functionalized with carbohydrates containing an amino substituent at the reducing end.[8] However, the carbohydrate incorporation can be incomplete and partial hydrolysis of PAI occurs. Polylysine has been functionalized directly. Michael addition to a carbohydrate containing an acrylamide residue gave a highly charged glycopolymer with more than 80 mol % of free amino functions.[9] Neither capping nor further functionalization of the amino groups was reported. The coupling of polylysine and carbohydrates containing carboxylic acid functions has been reported but the carbohydrate incorporation was incomplete.[10]

We became interested into glycopolymers in the course of our work on the selectin−carbohydrate interaction.[11] Control over this process is of high pharmaceutical interest as it mediates the leukocyte recruitment to sites of inflammation.[12] To set up competitive selectin binding assays which allow the determination of IC_{50} values of potential selectin antagonists we required well characterized glycopolymers containing a selectin ligand (sialyl Lewis[a]) and biotin as a handle for ELISA.

Here, we present a convenient access to complex, biodegradable glycopolymers. We describe the new, chloroacetylated poly-L-lysine **1** which is readily prepared from the commercially available poly-L-lysine hydrobromide (**2**) and its transformation into complex neoglycoconjugates by reaction with thiolated oligosaccharides and other thiols.[13] ^{1}H-NMR analysis of the products proves that the functionalizations are quantitative, and no side reactions occur.

Slow addition of an excess of chloroacetic anhydride to a suspension of poly-L-lysine hydrobromide (**2**) (M_w: 55 kD)[14] in DMF/2,6-lutidine (4:1) led to complete consumption of **2** within 1 h indicated by the formation of a clear solution. The addition of ethanol/ether (1:1) furnished **1** as a colorless precipitate which was isolated in 80−90% yield by filtration

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S0002-7863(97)00657-4 CCC: $14.00

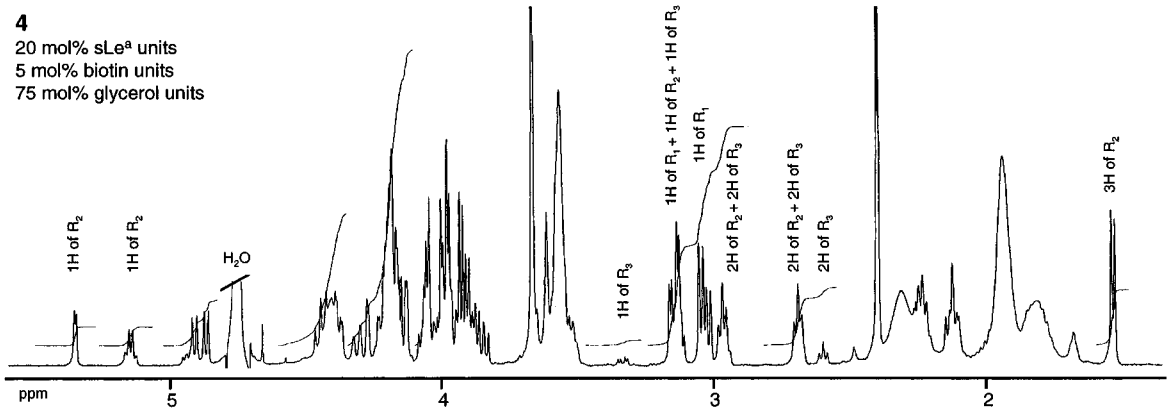
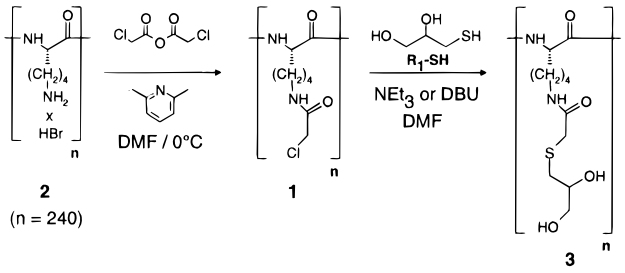


Figure 2. ^{1}H-NMR spectrum of compound **4** (600 MHz, D_2O, 60 °C): The assignments of the signals of R_1-R_3 are based on simpler model polymers. The integrals of the signals give the ratio of the incorporated residues.

Scheme 1



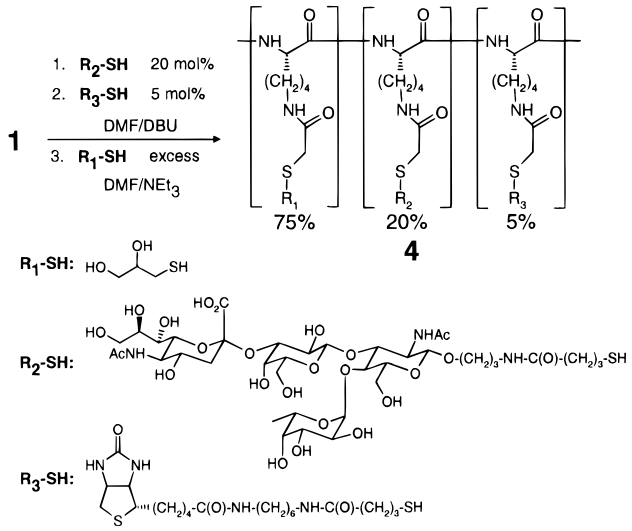
(Scheme 1). The stable, reactive polymer **1** is insoluble in water but readily dissolves in DMSO or DMF. ^{1}H-NMR analysis indicated quantitative chloroacetylation.

Treatment of **1** with an excess of thioglycerol (**R_1-SH**) in the presence of DBU or NEt_3 followed by precipitation and ultrafiltration gave the water soluble derivative **3** in quantitative yield (Scheme 1).[15] No remaining chloroacetamide groups could be detected by ^{1}H-NMR. For **3** a molecular weight of 60 kD[16] has been determined which is in good agreement with the molecular weight of the starting polylysine hydrobromide **2** indicating that the polylysine backbone was not altered.

Accordingly, the complex glycopolymer **4** was prepared (Scheme 2). At room temperature DBU was added to a solution of chloroacetylated polylysine **1**, sLe^a-thiol **R_2-SH** (20 mol %), and biotin-thiol **R_3-SH** (5 mol %). After complete consumption of the thiols (1 h) the mixture was treated with an excess of thioglycerol **R_1-SH** and NEt_3 to cap the remaining chloroacetamide groups. The ^{1}H-NMR spectrum of **4** proves the purity of the compound. In spite of its complexity several well separated signals of all three R groups could be assigned. According to the integrals **4** contains 75 mol % of R_1, 20 mol % of R_2, and 5 mol % of R_3 (Figure 2). These data clearly demonstrate that chloroacetylated polylysine **1** reacts quantitatively with the thiols used. The distribution of the residues is expected to be statistical.

Compound **4** was used as a polymeric ligand to set up a cell-free, competitive E-selectin ligand binding assay.[17] Recently, it was shown that acidic ion exchange resins bind to selectins.[18] Therefore, it is not advisable to use polymers with variable amounts of acid functions in selectin assays. As mentioned above, the amount of unwanted carboxylates cannot be controlled for several types of glycopolymers. Compound **4** has

Scheme 2



no additional acid functions (besides the sialic acid and one end group) and allowed a reliable and reproducible evaluation of a broad variety of E-selectin antagonists.[11,19]

In conclusion, we have transformed polylysine into the reactive chloroacetamide **1** which can be derivatized with different thiols in a quantitative reaction to give biodegradable neoglycoconjugates. The product composition can be reliably analyzed by ^{1}H-NMR. Our new methodology is not limited to the synthesis of carbohydrate containing compounds and should find broad application.

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(14) Poly-L-lysine (M_w 55 kD, determined by the coupling of size exclusion chromatography and low angel laser light scattering, SEC-LALLS) was purchased from SIGMA; Polydispersity: $M_w/M_n = 1.20$. Polylysine is also avalable with different molecular masses and in the D and D/L form.

(15) The reaction of **1** with mercapto ethanol gave a poorly water soluble product which was not further characterized.

(16) Determined by the coupling of size exclusion chromatography and multy angel laser light scattering (SEC-MALLS); Polydispersity: $M_w/M_n = 1.40$; done by Polymer Standards Service, Postfach 3368, D-55023 Mainz, Germany.

(17) Cell-free E-selectin ligand binding assay: Wells in a microtiter plate (plate 1, Falcon probind) are coated with E-selectin/hIg chimera by incubation of 100 mL of the purified chimeric protein at a concentration of 200 ng/well in 50mM Tris, 0.15 M NaCl, 2 mM $CaCl_2$, pH 7.4 (Tris-Ca^{2+}). After 2 h, 100 mL of a 1:1 mixture of 1% BSA in Tris-Ca^{2+} and Stabilcoat are added to each well and incubated at 22 °C to block nonspecific binding. During this incubation, inhibitory test compounds, diluted in Tris-Ca^{2+}, 1% BSA, are titrated by a 2-fold serial dilution in a second U-shaped bottom low-bind microtiter plate (plate 2, Costar, Inc.). An equal volume of a preformed complex of a biotinylated sialyl Lewis^a polymer **4** and horseradish peroxidase-labeled streptavidin (KPL, Gathersburg, MD) at 1 mg/mL in Tris-Ca^{2+}, 1% BSA is added to each well. After 2 h at 22 °C, plate 1 is washed with Tris-Ca^{2+} and 100 mL/well are transferred from plate 2 to plate 1. The binding reaction is allowed to proceed for 2 h at 22 °C while rocking. Plate 1 is then washed with Tris-Ca^{2+}, and 100 mL of TMB substrate reagent (KPL, Gaithersburg, MD) is added to each well. After 3 min, the colorimetric reaction is stopped by adding 100 mL/well of 1 M H_3PO_4 and the optical density is determined at 450 nm.

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(19) A similar set up has been described using a commercially available sLe^a containing polyacrylate derived from 4-nitrophenyl polyacrylate; see ref 4 (Synthesome, Gesellschaft fuer medizinische Biochemie mbH, Heimdall Str. 4, D-81739 Munic, Germany). Weitz-Schmidt, G.; Stockmaier, D.; Scheel, G.; Nifant'ev, N. E.; Tuzikov, A. B.; Bovin, N. V. *Anal. Biochem.* **1996**, *238*, 184.