Versatile Functionalization of Polylysine: Synthesis, Characterization, and Use of Neoglycoconjugates

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Received December 3, 1998

Abstract: Glycopolymers are useful macromolecules with a non-carbohydrate backbone for presenting saccharides in a multivalent form. Here, a new methodology is described which allows easy access to water-soluble, biodegradable glycopolymers with both predeterminable composition and molecular weight distribution. Thus, chloroacetylation of commercially available polylysine hydrobromide **3** gave the reactive homopolymer **4**, whose chloroacetamide functions allowed subsequent coupling with thiol-containing components. Water-soluble homopolymers such as **8** and **13** were available by treatment with an excess of hydrophilic thiols. Heteroglycopolymers were obtained via quantitative incorporation of substoichiometric amounts of carbohydrates with a mercapto functionality linked to the reducing end; the remaining chloroacetamide groups were capped with an excess of thioglycerol. A variety of glycopolymers with up to four different components was prepared. The composition and purity of the products were reliably analyzed by ^{1}H NMR. Generally, the quantitative incorporation of substoichiometric components was verified. The polymer backbone was not altered under the applied reaction conditions, as indicated by very similar polydispersities and degrees of polymerization of starting polylysine **3** and functionalized homo- and heteropolymers **8**, **13**, and **14**. Glycopolymer **25**, containing sialyl Lewis^a and biotin as a functional group for enzyme-linked immuno sorbent assay, was used for developing cell-free selectin ligand binding assays. The inhibition of E-selectin by glycopolymers **16**, containing sialyl Lewis^x (sLe^x), was evaluated in a cell adhesion assay under flow conditions using activated human umbilical vein endothelial cells and polymorphonuclear neutrophils. The sLe^x polymers **16** showed no significant inhibition, whereas conjugates with additional charged groups (carboxylates **18**, sulfonates **21**) in addition to sLe^x gave 30−35% reduction of the number of interacting cells at the same concentration of 100 μM sLe^x.

Introduction

The assembly of oligosaccharides using only a few monosaccharide building blocks gives nature access to an enormous structural variety. The recognition of such oligosaccharides by proteins (lectins) represents the basis of many biologically important events.[1] Individual protein−carbohydrate interactions are generally weak ($K_D = 10^{-3}-10^{-4}$ M^{-1}).[2] To overcome this, many processes mediated by oligosaccharide−lectin interactions involve multivalent binding.[2] Hence, tailored macromolecules that present oligosaccharides in a multivalent form are of high interest due to a variety of possible applications, e.g., as receptor blockers, for cell targeting, and for generation of monoclonal antibodies.[3] Such neoglycoconjugates can also be used for ELISA as coating reagents and multivalent ligands.[3]

A variety of neoglycoconjugates has been prepared. Neoglycoproteins derived from well-defined natural carrier proteins such as BSA and HSA were obtained by linking oligosaccharides to the ω-amino functions of lysine residues.[4] Liposomes formed by carbohydrate−lipid conjugates represent an alternative approach toward the multivalent presentation of complex oligosaccharides.[5] High-density presentation of carbohydrates was realized with dendrimers which were prepared either by carbohydrate functionalization of preformed core structures[6] or by linking of carbohydrate-containing building blocks in a convergent manner.[7]

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10.1021/ja984183p CCC: $18.00
Published on Web 06/11/1999

Glycopolymers are another important class of neoglycoconjugates. Homopolymers were prepared by ring-opening metathesis polymerization of carbohydrate-containing monomers[8a−c] and polymerization of carbohydrate-containing acrylamides.[8d] Heteroglycopolymers have been obtained by copolymerization of alkenyl-[9] or acryloyl-containing carbohydrates[10] and acrylamide (Figure 1). The ratios of applied and incorporated quantities of the monomers can vary significantly because carbohydrate-containing monomers and acrylamide often behave differently in the polymerization process. In these cases, product compositions can be both difficult to predetermine and difficult to reproduce. The control of the molecular weight distribution of the copolymers is tricky, and usually very broad molecular weight ranges are obtained.[11] Fractionation of the crude product to obtain a more uniform composition is laborious and leads to loss of valuable material.

The modification of a preformed, reactive homopolymer is an elegant approach toward neoglycoconjugates with predictable composition since the above-mentioned drawbacks of copolymerization can be avoided (Figure 1). Ideally, a well-characterized homopolymer is reacted first with a substoichiometric quantity of the carbohydrate-containing component and then, after complete consumption of the saccharide, with an excess of a capping agent to "quench" the remaining reactive functionalities. *To achieve a predeterminable product composition, these conversions ought to be quantitative, and side reactions altering the polymer backbone have to be excluded*. With these prerequisites, such an approach is also applicable for very complex preparations containing more than two different components.

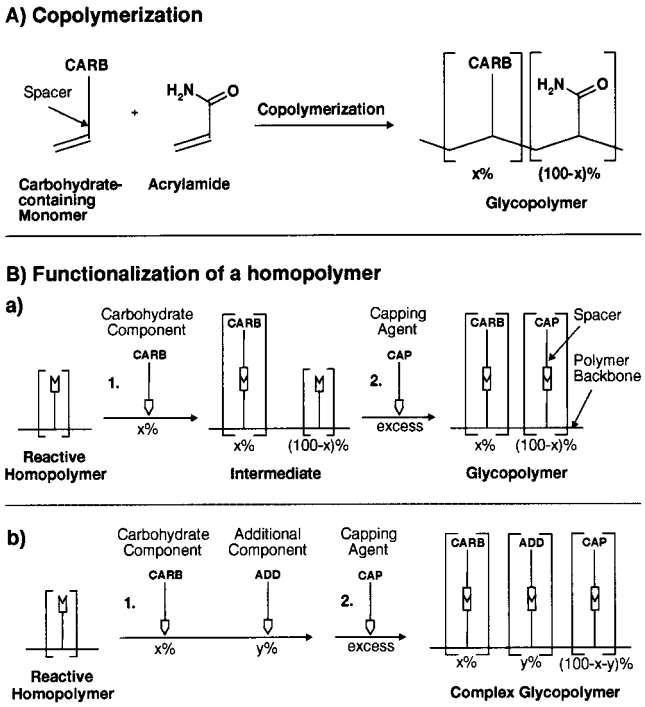

Figure 1. Strategies for the synthesis of glycopolymers. (A) Copolymerization of a carbohydrate-containing olefin and acrylamide. (B) Derivatization of a preformed homopolymer: (a**)** (two components) (1) quantitative reaction with a substoichiometric amount of a suitably functionalized carbohydrate component and (2) capping of the remaining reactive residues; (b**)** (three components) (1) quantitative reaction with substoichiometric amounts of a carbohydrate component and an additional component and (2) capping of the remaining reactive residues.

Thus, active esters of poly(acrylic acid), such as poly[4-nitrophenyl acrylate][12] and poly[*N*-oxysuccinimidyl acrylate],[13] have been functionalized with residues containing a primary amino group. Unfortunately, the reactive homopolymers, prepared by radical acrylate polymerization of the corresponding monomers, are not readily available with narrow molecular weight distribution. In some cases, partial hydrolysis of the active ester groups cannot be avoided, leading to unforseen and variable amounts of carboxylic acid functions in the product. As an example, the model homopolymer **1** derived from poly-4-nitrophenyl acrylate **2** by treatment with excess amounts of ethanolamine at room temperature contained approximately 8% of carboxylic acid functions (Scheme 1).[14] More complex glycopolymers of this type, which are prepared by applying elevated reaction temperatures, may contain an even higher carboxylate fraction. Furthermore, depending on the application, the nondegradable backbone of such polyacrylates might be an undesired feature.

As an alternative, polyamino acids have been derivatized giving biodegradable glycopolymers. Polyaspartimide (PAI) was functionalized with carbohydrates containing an amino substituent at the reducing end.[15] However, complete consumption of the saccharide was not always achieved, even when extended

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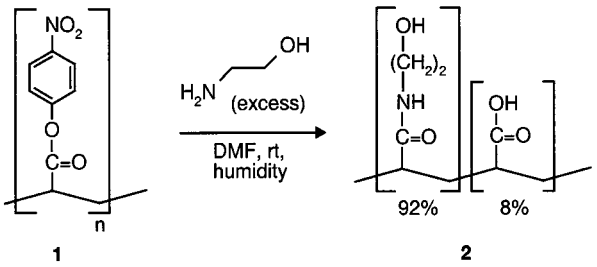
Scheme 1



1 **2**

reaction times and elevated temperatures were applied. Moreover, partial hydrolysis to unwanted carboxylic acid functions in the polymer cannot be avoided completely. Polylysine has been functionalized directly. Michael addition to a carbohydrate containing an acrylamide residue gave a highly charged glycopolymer with more than 80% of free amino functions.[16] Neither capping nor further functionalization of the amino groups was reported. Coupling of polylysine and carbohydrates with carboxylic acid functions has also been reported, but the carbohydrate incorporation was incomplete.[17]

Our interest in such glycopolymers arose from our involvement in studying the selectin oligosaccharide recognition.[18] E- and P-selectins are expressed on endothelial cells upon stimulation and mediate the recruitment of leukocytes to sites of injury or infection, recognizing sialyl Lewis^x (sLe^x)-related carbohydrate epitopes on the leukocyte surface.[19] Control over this process is of pharmaceutical interest because chronic expression occurs in certain inflammatory conditions, such as psoriasis and rheumatoid arthritis. Acute disorders such as reperfusion injury or asthma caused by excessive leukocyte recruitment represent another important target.[19] Several recent reports suggest that multivalency plays an important role in the inflammatory response.[20] To probe this, we required a reliable access to multivalent sLe^x ligands. We were also interested in well-characterized glycopolymers containing sLe^x or sLe^a and biotin as a functional group for competitive selectin binding assays, allowing the determination of IC_{50} values of potential selectin antagonists.

In this paper, we present a novel, reliable, and reproducible access to glycopolymers. Commercially available polylysine **3** is converted into its chloroacetamide **4**.[21] This new, DMF-soluble and reactive homopolymer can easily be transformed into water-soluble homopolymers by treatment with hydrophilic thiols. Glycopolymers with predeterminable carbohydrate content are available by reaction with substoichiometric amounts of saccharides containing a mercapto substituent, followed by treatment with an excess of thioglycerol to cap the remaining chloroacetamide groups.[22] The scope of the method is explored by preparing sLe^x- and sLe^a-glycopolymers with up to four different components. The appropriate characterization of the products is described in detail. Furthermore, the use of such conjugates as multivalent receptor blockers and multivalent ligands for assay development is discussed.

Results and Discussion

Acylation of Polylysine Hydrobromide. Polylysine of different well-characterized, narrow molecular weight fractions is commercially available in the L, D/L, and D forms. This polymer is nonimmunogenic and biodegradable and has already been explored as a drug carrier.[23] The polylysine hydrobromides **3** used in this work were purchased from Sigma and had a molecular weight M_w of approximately 50 000 (Table 2). We intended to transform poly-L-lysine hydrobromide (**L-3**)[24] into the per-*N*-chloroacetyl derivative **L-4** in order to subsequently modify this reactive homopolymer by thiol substitution (Scheme 2). A suspension of **L-3** in a 3:1 mixture of DMF and 2,6-lutidine was treated with chloroacetic anhydride. Formation of a clear solution indicated the consumption of **L-3**. The product **L-4** was isolated in 98% yield by precipitation. The compound is insoluble in water but readily dissolves in DMF and DMSO.

(14) Acryl polymer **2** was obtained from Synthesome, Gesellschaft fuer medizinische Biochemie mbH, Heimdall Str. 4, D-81739 Muenchen, Germany. ^{1}H NMR spectroscopy (500 MHz, D_2O, 60 °C) showed that the integral per proton of the ethanolamine side chain has approximately 90% of the value per proton of the polymer backbone, indicating 90% incorporation of ethanolamine. Accordingly, compound **2** contains 10% of carboxylic acid functions. Additional evidence for the presence of unwanted carboxylic acid functions in compound **2** was obtained as follows: Compound **2** was dissolved in 0.01 M HCl to transform carboxylates into the free carboxylic acid functions, followed by repeated ultrafiltration until the eluted solution became neutral. An ecxess of *N*,*N*-dimethylaminopyridine (DMAP, 5.0 equiv) was added to form the corresponding carboxylic acid salt. The solution was resubjected to ultrafiltration until the elute became neutral. Following lyophilization, the compound was analyzed by ^{1}H NMR (400 MHz, D_2O). A singlet at 3.10 ppm (6 H of DMAP) and two doublets at 6.80 and 7.95 ppm (2 H of DMAP each) indicated 8% DMAP with respect to the polymer backbone. Accordingly, **2** contains at least 8% of carboxylic acid functions. Within the experimental error,the sum of the integrals per proton of the ethanolamine side chain and of DMAP equaled the integral per proton of the polymer backbone. Similarly, commercially available glycopolymers are prepared by converting polyacrylate **2** first with substoichiometric amounts of carbohydrates (DMF, NEt_3, 40 °C, 24 h), followed by treatment with ethanolamine (see ref 12a). We have evidence that such compounds can contain even more carboxylic acid residues (>25%).

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Table 1. Heteroglycopolymers Containing *N*-Acetylglucosamine (R_{11}) or sLe^x(R_{17})

compd	11 or 17 (appl.) (%)	conditions[a]	R_i (incorp.) (%)[b]
L-14a	R_{11}-SH (50)	DMF/NEt_3, 2 equiv/16 h	(27)
L-14b	R_{11}-SH (50)	DMF/NEt_3, 10 equiv/16 h	(38)
L-14c	R_{11}-SH (50)	DMF/DBU, 1 equiv/1 h	(50)
L-14d	R_{11}-SH (20)	DMF/DBU, 1 equiv/1 h	(20)
D/L-14	R_{11}-SH (50)	DMF/DBU, 1 equiv/1 h	(50)
L-16a	R_{17}-SH (5)	DMF/H_2O/DBU, 1 equiv/1 h	(5)
L-16b	R_{17}-SH (10)	DMF/H_2O/DBU, 1 equiv/1 h	(10)
L-16c	R_{17}-SH (20)	DMF/H_2O/DBU, 1 equiv/1 h	(20)
L-16d	R_{17}-SH (30)	DMF/H_2O/DBU, 1 equiv/1 h	(30)
D/L-16a	R_{17}-SH (10)	DMF/H_2O/DBU, 1 equiv/1 h	(10)
D/L-16b	R_{17}-SH (30)	DMF/H_2O/DBU, 1 equiv/1 h	(30)

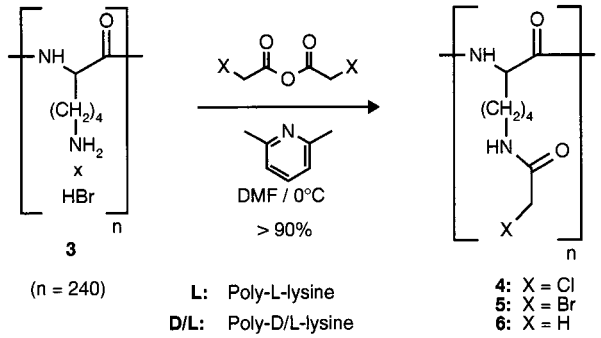
[a] In all cases, 3−5 equiv of thioglycerol was subsequently added, and the mixture was stirred for 16 h. [b] Product composition analyzed by 1H NMR (error, ±2%).

Table 2. Molecular Weight Distribution

compd	mol wt per unit	M_w (av)	polydispersity, M_w/M_n	degree of polymerization (av)
D/L-3	209	50 000[a]	1.2[a]	240
L-3	209	50 000[a]	1.2[a]	240
L-8	276	58 000[b]	1.4[b]	210
L-14	412	86 000[b]	1.6[b]	210
L-13	548	107 000[b]	1.3[b]	200

[a] In water; determined by coupling of size exclusion chromatography and low angle laser light scattering (SEC-LALLS), done on the succinimidyl derivative; performed by Sigma Chemical Co., 3050 Spruce St., St. Louis, MO 63103. [b] In DMF; determined by coupling of size exclusion chromatography and multiangle laser light scattering (SEC-MALLS); performed by Polymer Standards Services, Postfach 3368, D-55023 Mainz, Germany.

Scheme 2



It can be stored over months at room temperature without decomposition.[25] Treatment of **L-3** with bromoacetic anhydride or acetic anhydride under similar conditions furnished bromoacetamide **L-5** and acetamide **L-6**. Starting from **D/L-3** chloroacetamide, **D/L-4** was available. It is important to note that the use of 2,6-lutidine for chloro- or bromoacetylation is essential, as with pyridine or triethylamine decomposition was observed.

The chloroacetamides **4** were characterized by ^{1}H NMR in DMSO-d_6. The spectra show a singlet at 4.02 ppm for the chloroacetamide function (Figure 2). The integral of this signal corresponds to two hydrogens, indicating complete transformation. Signals of carbon-linked hydrogens of the polyamide backbone or close to it (H-2, H-3) are very broad, whereas distant H atoms of the side chain give rather sharp peaks (H-6, CH_2-Cl). While chemical shift and shape of the signals of H-6 and CH_2-Cl are identical for **L-4** and **D/L-4**, differences are observed for H-2 and H-3. Different conformational preferences of the backbone of **L-4** and **D/L-4** in DMSO could be an explanation.

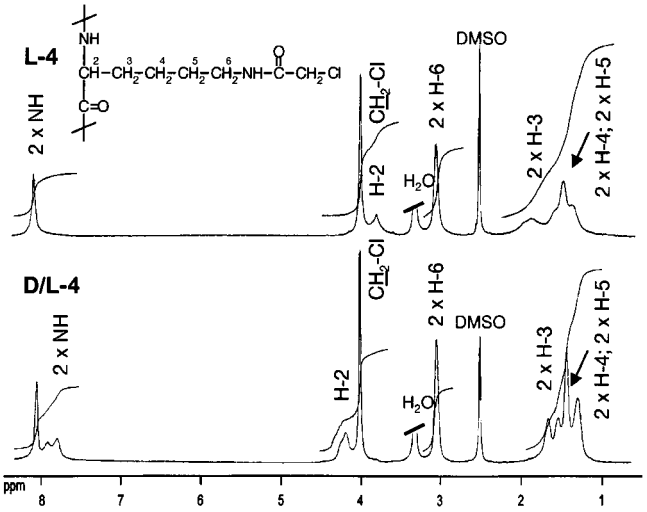


Figure 2. ^{1}H-NMR spectra (500 MHz, DMSO-d_6, room temperature) of the ω-chloroacetamides **L-4** and **D/L-4**.

Water-Soluble Homopolymers from Polylysine Derivatives 4. The reaction of thiols with halogenoacetamides usually proceeds with very good yields and has been used to link carbohydrates to dendrimer core structures, leading to carbohydrate clusters with up to 16 sugar residues.[6] To prepare water-soluble homopolymers, we treated **L-4** with excess amounts of hydrophilic mercaptans. The reaction of **L-4** with mercaptoethanol in DMF in the presence of NEt_3 led to an insoluble material which was not further characterized. We reasoned that one hydroxyl group per lysine residue is insufficient to compensate for the lipophilicity of the side chain. In fact, treatment of **L-4** with 3 equiv of the diol thioglycerol (**7**, Figure 3) and NEt_3 gave the perfectly water-soluble polymer **L-8** in quantitative yield after precipitation and ultrafiltration in water (Scheme 3).[26] Analogously, **D/L-8** was prepared from **D/L-4**. The compounds were analyzed by ^{1}H NMR in water (Figure 4), and the peaks were assigned by two-dimensional ^{1}H/^{1}H correlation (COSY) and ^{1}H/^{13}C correlation spectroscopy (HSQC). Most signals are baseline separated and become sharper and better resolved with increasing distance to the polyamide backbone. Most importantly, no signal of remaining chloroacetamide functions was observed, indicating the complete transformation of **4**. For **L-8** and **D/L-8**, the integral for one hydrogen of the lysine part (H-2−H-7, *lys*) matches the integral for one hydrogen of the glycerol part (H-8−H-10, *gly*), in line with quantitative side-chain functionalization. Similar to polychloroacetamides **L-4** and **D/L-4**, the chemical shifts of H-2 (**L-8**, 4.00 ppm; **D/L-8**, 4.20 ppm) and H-3 (**L-8**, 1.65−2.10 ppm; **D/L-8**, 1.50−1.80 ppm) differ substantially. The other signals are very similar with respect to both chemical shift and shape. The NMR data of **L-8** and **D/L-8** could again indicate different backbone conformations of these stereoisomers. Since

(25) High-molecular-weight poly-L-lysine hydrobromide ($M_w \approx 180\,000$; degree of polymerization ≈ 860; polydispersity $M_w/M_n = 1.1$) was also transformed into the corresponding polychloroacetamide in high yield. The ^{1}H NMR spectrum of the product was very similar to the spectrum of **L-4**. Treatment with thioglycerol in the presence of NEt_3 gave a water-soluble material in almost quantitative yield which showed, compared to **L-8**, slightly broadened ^{1}H NMR signals. The reaction of low-molecular-weight poly-L-lysine hydrobromide ($M_w \approx 8000$; degree of polymerization ≈ 40; polydispersity $M_w/M_n = 1.2$) with chloroacetic acid anhydride in DMF/2,6-lutidine gave an impure material. A clean product was obtained when the hydrobromide was transformed into the DMF-soluble tosylate prior to the acylation (for the transformation of polylysine hydrobromide into its tosylate, see ref 23a).

(26) **L-8** and all other polymers described are also soluble in DMF and DMSO.

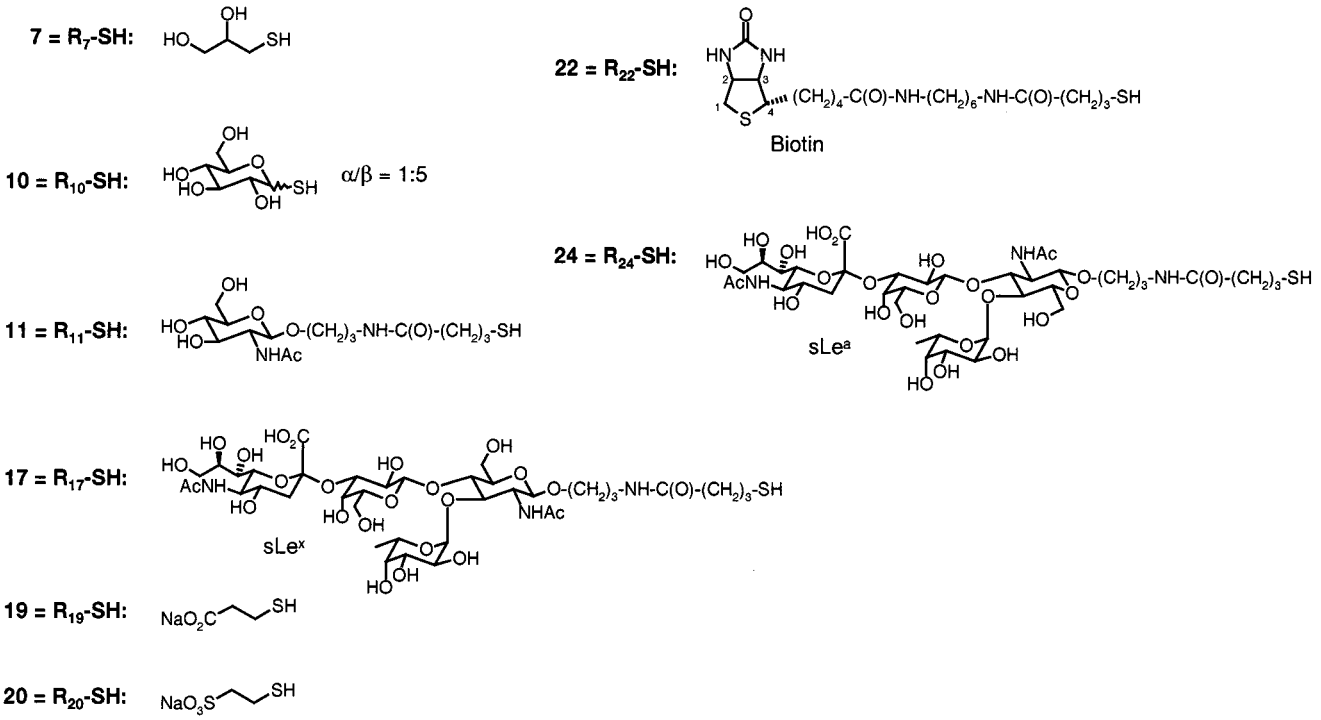
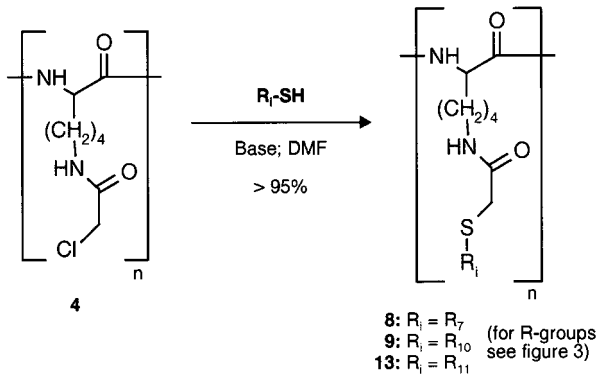


Figure 3. Components incorporated in polylysine conjugates.

Scheme 3



racemic thioglycerol was used for the preparations, the additional stereocenter, however, complicates the interpretation.

The circular dichroism (CD) spectrum of compound **L-8** was recorded in water and showed two negative bands at 208 and 222 nm, which can be taken as strong evidence for the presence of a significant amount of α-helix (see Supporting Information). This is an interesting finding since poly-L-lysine is unordered in water at pH 7.3 but adopts a *β*-pleated sheet conformation at pH 11.5 when it is uncharged.[27] An α-helical structure is observed during the pH-induced transition from random coil to *β*-sheet.[28]

As a first example of a polylysine-based homoglycopolymer compound, **L-9** was prepared by reaction of chloroacetamide **L-4** with 2 equiv of 1-thioglucose **10** (Figure 3) in the presence of NEt_3 (Scheme 3). The 5:1 *β*/α-ratio of **10** was reflected in the product, which contained 83% monomers linked to *β*-thioglucose and 17% monomers linked to α-thioglucose, as judged by integration of the signals of the anomeric hydrogen atoms. According to NMR, all chloroacetamide groups had been substituted.

When **L-4** was treated with 1.5 equiv of thiolated *N*-acetylglucosamine **11** (Figure 3) in the presence of NEt_3 for 48 h, glycopolymer **L-12** was isolated (Scheme 4). ^{1}H NMR revealed that **L-12** contained 70% of monomers linked to *N*-acetylglucosamine besides 30% of parent chloroacetamide functionalities. This result shows the stability of the chloroacetamide group under the applied reaction conditions. Incomplete substitution was still observed at elevated temperatures and when DMSO was used instead of DMF. Probably, NEt_3 is not strong enough to effect complete incorporation of the aliphatic mercaptan **11**. With the more acidic anomeric thiol **10**, on the other hand, the reaction goes to completion. Homoglycopolymer **L-13** was finally obtained by treating a DMF solution of **L-4** and 1.3 equiv of **11** with 1.5 equiv (with respect to **L-4**) of the stronger base 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) at room temperature (Scheme 3). The product was isolated in quantitative yield by precipitation and analyzed by ^{1}H NMR (Figure 4).[29] The spectrum of **L-13** demonstrates the purity of the compound, and integration of several well-separated signals shows the complete carbohydrate derivatization. It should be noted that the polymer **L-4** and the thiol **11** have to be dissolved prior to the addition of DBU. In some experiments, the inversion of the order resulted in the formation of insoluble precipitates. Dissolution of **4** can be a slow process and *should not be accelerated* by physical methods such as sonication or with the use of high-velocity stirrers.

Model Polymers with Two Components. The reaction of 50% of thiolated *N*-acetylglucosamine **11** with chloroacetamide **L-4** in the presence of triethylamine (2 equiv) for 2 h at room temperature gave incomplete consumption of **11**, as indicated by TLC. Following the addition of thioglycerol, the product **L-14a** was isolated and the sugar content determined to be 27% by ^{1}H NMR (Scheme 5, Table 1). When 10 equiv of NEt_3 were used and the reaction time was extended to 24 h, the carbohy-

(27) (a) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108, (b) Keiderling, T. A.; Yasui, S. C.; Sen, A. C.; Toniolo, C.; Bonora, G. M. *Pept.: Sruct. Funct., Proc. Am. Pept. Symp., 9th* **1985**, 167.

(28) Peggion, E.; Cosani, A., Terbojevich, M.; Romanin-Jacur, L. *J. Chem. Soc., Chem. Commun.* **1974**, 314.

(29) Quantitative carbohydrate incorporation was also achieved when the sodium thiolate of **11** was used, which was prepared from **11** by treatment with 1 equiv of sodium methanolate.

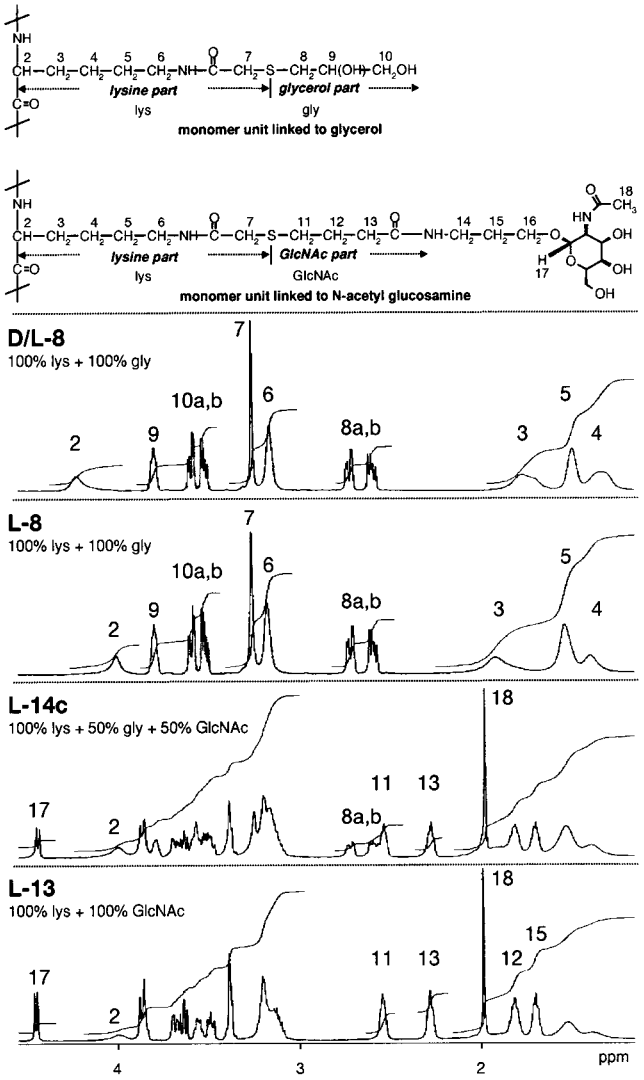
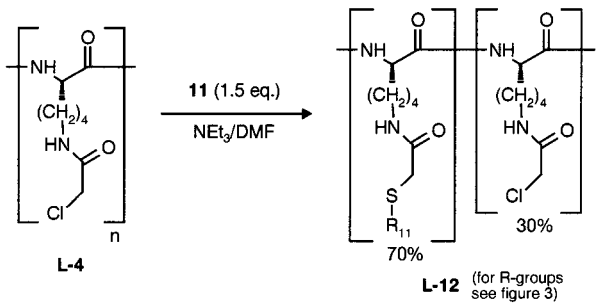


Figure 4. ^{1}H-NMR spectra (500 MHz, D_2O, room temperature) of homopolymers **D/L-8**, **L-8**, and **L-13** and heteroglycopolymer **L-14c**.

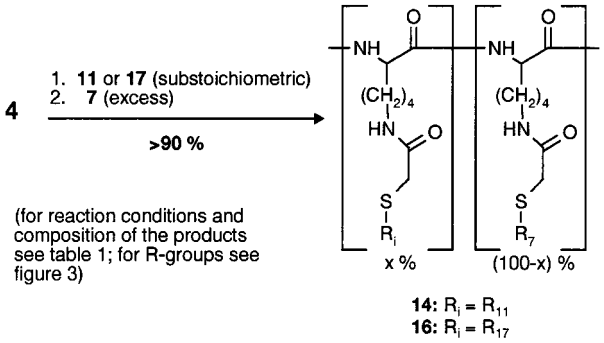
Scheme 4



drate content of the corresponding product **L-14b** increased to 38%, but complete incorporation could still not be achieved. In the presence of 1.0 equiv (with respect to **L-4**) of DBU, thiol **11** was consumed within 3 min, as indicated by TLC. After 1 h, excess amounts of thioglycerol (**7**) and NEt_3 were added, and the desired glycopolymer **L-14c** was isolated as described above (Scheme 5).

The NMR spectrum of heteropolymer **L-14c** looks exactly like that of a 1:1 mixture of the corresponding homopolymers **L-8** and **L-13**, allowing a straightforward peak assignment (Figure 4). Impurities were below the NMR detection level. Integration of several well-resolved signals allows the determination of the composition. Accordingly, compound **L-14c** consists of 50% of monomers linked to the carbohydrate and 50% of units containing thioglycerol. Generally, the NMR specra of multicomponent polymers can be easily interpreted by using the spectra of simple homopolymers or less complex heteropolymers with the corresponding components. Complete carbohydrate incorporation was also observed when **L-4** was reacted under the same conditions with 20% of thiolated *N*-acetylglucosamine **11**, leading to heteroglycopolymer **L-14d**. The analogous transformation of poly-D/L-lysine derivative **D/L-4** using 50% of **11** gave **D/L-14**, containing 50% of lysine units linked to the carbohydrate (Table 1). These data demonstrate the clean course of the derivatization and, hence, a high predictability of the resulting polymer composition.

Scheme 5

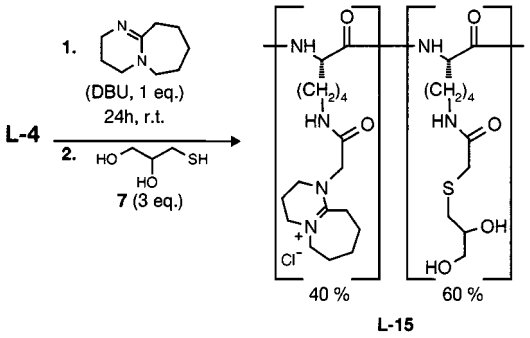


Determination of Molecular Weight and Polydispersity. Cleavage of the polylysine backbone under the applied reaction conditions (chloroacetylation, thiol addition) would alter both the degree of polymerization and the polydispersity (M_w/M_n) of the product. To verify the stability of the amide backbone of polylysine, the molecular weight distributions of polymers **L-3**, **L-8**, **L-13**, and **L-14c** were determined by coupling of size exclusion chromatography and light scattering (Table 2). The molecular weight M_w of the poly-L-lysine hydrobromide **L-3** was approximately 50 000, corresponding to an average degree of polymerization of 240. The derivatives **L-8**, **L-13**, and **L-14c** were prepared from **L-3** and had molecular weights M_w of 58 000, 86 000, and 107 000, respectively. Their degrees of polymerization of 210, 210, and 200 indicate that the polymer backbone was not altered significantly under the applied reaction conditions. Furthermore, very similar polydispersities of all four polymers show that the molecular weight distribution was not changed substantially.

It is likely that the distribution of different components in the polymers is statistical because, otherwise, an activation of still intact chloroacetamide funtionalities adjacent to lysine units which are already linked to **11** would have to be assumed. Nevertheles, the applied analytical methods prove neither the homogeneous distribution of the components nor the absence of carbohydrate clusters within the polymer chain. Clustering of either carbohydrate **11** or polylysine **4** in the medium (DMF) could also contribute to nonstatistical distributions.

Side Reaction: N-Alkylation of DBU. It is very important to note that extended treatment of the activated polylysine derivative **L-4** with DBU in the absence of a thiol leads to partial N-alkylation of the base (Scheme 6). When **L-4** was reacted with 1 equiv of DBU for 18 h followed by the addition of 3 equiv of thioglycerol and NEt_3, polymer **L-15** was isolated, containing 40% of lysine residues linked to DBU and 60% of thioglycerol side chains (DBU signals at 2.08, 2.60, and 4.31 ppm). When excess amounts of thiols were used, leading to homopolymers **L-13** and **L-8** (**L-8** was also prepared using DBU

Scheme 6



Scheme 7

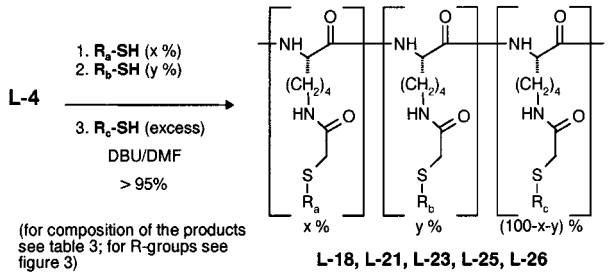


Table 3. Glycopolymers with Three Components

compd	R_a	(%)[a]	R_b	(%)[a]	R_c	(%)[b]
L-18	R_{17}	20	R_{19}	25	R_7	55
L-21	R_{17}	20	R_{20}	25	R_7	55
L-23	R_{17}	20	R_{22}	5	R_7	75
L-25	R_{24}	20	R_{22}	5	R_7	75
L-26	R_{17}	20	R_{22}	5	R_{20}	75

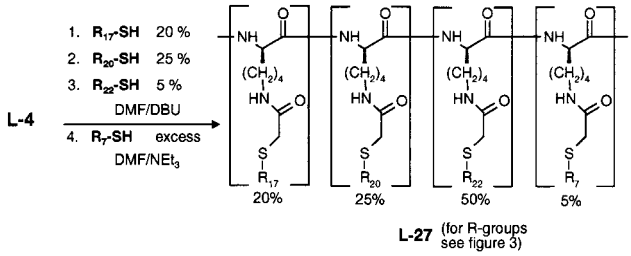
[a] Applied and incorporated amounts were identical. [b] 3−5 equiv of the capping reagent was used.

instead of NEt_3), N-alkylation was below the NMR detection level (<2%). Thiol addition to chloroacetamides in the presence of DBU (complete reaction within minutes) seems to be much faster than N-alkylation (incomplete reaction after 18 h). To obtain pure heteropolymers, the reaction time with substoichiometric quantities of thiols in the presence of DBU should be limited to <1 h. Then, thioglycerol has to be added for capping the remaining chloroacetamide functionalities.

Synthesis of sLe^x and sLe^a Conjugates. A series of sialyl Lewis^x glycopolymers **16** with different carbohydrate contents (Scheme 5, Table 1) was prepared by reacting chloroacetamide **4** with sLe^x thiol **17** (Figure 3) in the presence of DBU. It is advantageous to dissolve **4** in DMF, add the tetrasaccharide **17** as a solid, and then add water dropwise until the solution becomes clear. Addition of too much water has to be avoided because otherwise polymer **4** can precipitate. Glycopolymers **L-16** with 5, 10, 20, and 30% sLe^x content were obtained from **L-4**. Precipitation followed by ultrafiltration in water gave pure products in almost quantitative yields. Poly-D/L-chloroacetamide **D/L-4** was transformed into **D/L-16**, containing 10 and 30% of sLe^x. According to NMR, quantitative carbohydrate incorporation was achieved in all cases.

Glycopolymers containing three components are also accessible in a very predictable manner (Scheme 7, Table 3). Substoichiometric quantities of the oligosaccharide and an additional component were reacted with **L-4** in the presence of DBU, followed by the additon of an excess of thioglycerol. Thus, multivalent sialyl Lewis^x with additional carboxylate groups **L-18** was obtained using 20% of sLe^x thiol **17** and 25% of mercaptopropionic acid **19** (Figure 3) . Reaction of **L-4** with

Scheme 8



20% of sLe^x thiol **17** and 25% and mercaptoethanesulfonate **20** (25%) (Figure 3) gave glycopolymer **L-21**.

A series of biotinylated glycopolymers was prepared to serve as multivalent ligands for ELISA. Treatment of **L-4** with biotin thiol **22** (5%) (Figure 3) and sLe^x thiol **17** (20%), followed by the addition of thioglycerol, gave **L-23**. The use of sLe^a thiol **24** (20%) (Figure 3) instead of sLe^x thiol **17** gave **L-25**. Highly acidic multivalent sLe^x **L-26** was obtained by reacting **L-4** with biotin thiol **22** (5%) and sLe^x thiol **17** (20%), followed by the addition of an excess of mercaptoethanesulfonate **20**. The products were analyzed, and, within the accuracy of NMR spectroscopy, the applied substoichiometric amounts of the components were reflected by the product compositions, demonstrating again the quantitative course of the functionalization.

The scope of the method was explored by preparing the four-component derivative **L-27** (Scheme 8). DBU was added to a solution of chloroacetylated polylysine **L-4**, sLe^x thiol **17** (20%), sulfonate **20** (25%), and biotin thiol **22** (5%). When TLC control showed complete consumption of the thiols, the mixture was treated with an excess of thioglycerol and NEt_3. The highly complex conjugate **L-27** was analyzed by ^{1}H NMR at elevated temperatures (60 instead of 20 °C) to obtain sharper signals. The individual protons of the various components reflected by complex signals were deduced from the spectra of simpler conjugates and/or the spectra of the nonconjugated components (as shown for **L-14c**, Figure 4). The spectrum of **L-27** (Figure 5) and the indicated assignments therein demonstrate the reliability of the analysis of the composition. According to the integrals, **L-27** contains 50% of R_7, 20% of R_{17}, 25% of R_{20}, and 5% of R_{22}, corresponding to the fractions of thiols applied in the preparation. It is conceivable that compounds of even higher complexity are accessible.

Preparation of Mercaptans from Primary Amines. The mercaptans **11**, **17**, **22**, and **24** were prepared by reacting the corresponding primary amines **28**, **29**, **30**, and **31** with thiobutyrolactone (10 equiv) in refluxing, oxygen-free methanol in the presence of triethylamine (10 equiv) for 16 h (Scheme 9).[30] The mercaptans were purified by chromatography on silica gel, and the purity was checked by ^{1}H NMR. Only trace amounts of disulfides were detected (<5%). The products were isolated in yields ranging from 60 to 90%. In some cases, the aprotic solvent DMF is better suited for these transformations, since less side product formation with respect to thiobutyrolactone occurs.

When Traut's iminothiolane[31] was applied, we obtained unsatisfactory results. Instead of the desired amides, we observed the formation of amidines. This could lead to positively charged

(30) This method has been described earlier in a patent. (a) Thoma, G.; Duthaler, R. O.; Ernst, B.; Magnani, J. L.; Patton, J. T. PCT Int. Appl., WO 97/19105, 1997. A similar procedure has recently been published. (b) Blixt, O.; Norberg, T. *J. Carbohydr. Chem.* **1997**, *16*, 143.

(31) (a) Traut, R. R.; Bollen, A.; Sun, T.-T.; Hershey, J. W. B.; Sundberg, J.; Pierce, L. R. *Biochemistry* **1973**, *12*, 3266. Jue, R. J.; Lambert, J. M.; Pierce, L. R.; Traut, R. R. *Biochemistry* **1978**, *17*, 5399.

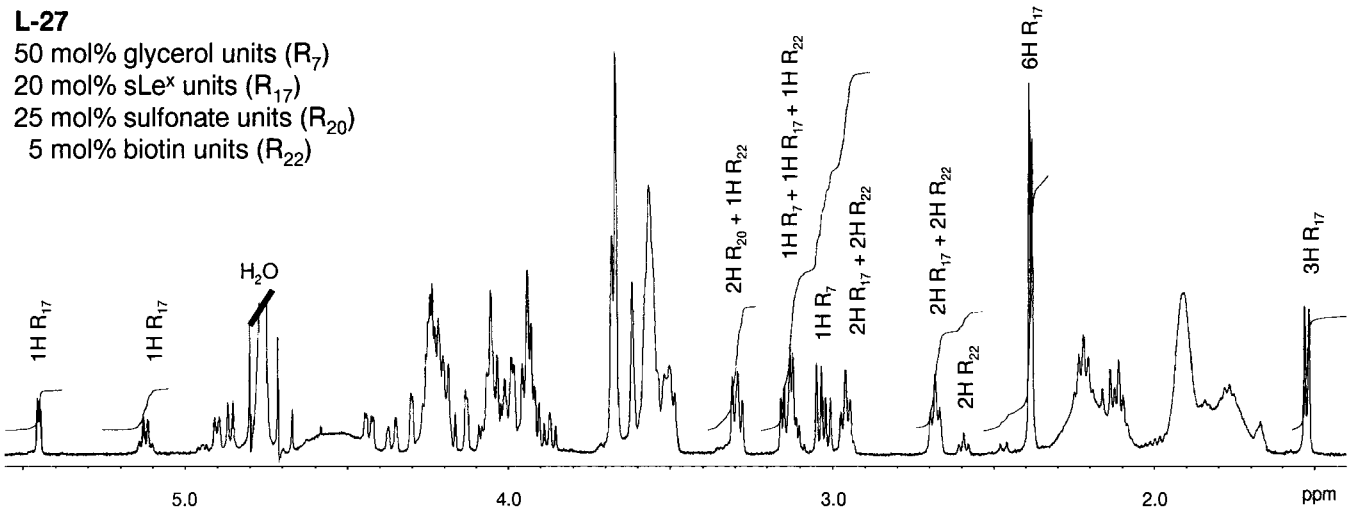
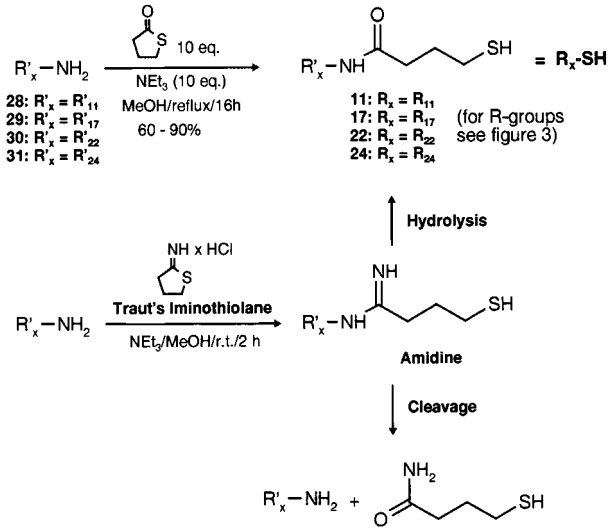


Figure 5. ^{1}H-NMR spectrum of **L-27** (500 MHz, D_2O, 60 °C).

Scheme 9



polymers. In addition, the amidine linkages are susceptible to both hydrolysis and cleavage, which could result in the formation of impure products and incomplete incorporation of the components (Scheme 9).

Investigation of sLe^x-Containing Polylysine Derivatives 16, L-18, and L-21 as Multivalent E-Selectin Inhibitors. We consider the determination of IC_{50} values of multivalent inhibitors in static, cell-free assays as inappropriate because, compared to cell surfaces, plastic wells coated with recombinant E-selectin exhibit completely different properties with respect to both selectin density and mobility. These factors are essential, especially for multivalent interactions. Therefore, a parallel plate flow chamber coated with human umbilical vein endothelial cells (HUVECs) was used to study the rolling of polymorphous neutrophils (PMNs) in contact with selectins under hydrodynamic flow (Figure 6). This in vitro assay mimics the nonequilibrium conditions in vivo. Digital image acquisition was used to determine the number of interacting cells in the presence and absence of the potential E-selectin inhibitors (for details, see the Experimental Section and ref 30a). Monovalent sLe^x gave no significant inhibition at 0.5 mM, but the more potent, monovalent sLe^x analogue CGP69669A[32] showed up to 45−75% reduction at 0.20 mM. Compounds **L-16** and **D/L-16**, which contain 5−30% of sLe^x, were inactive at 0.1 mM per sLe^x. The compounds **16** are functional in the static assay

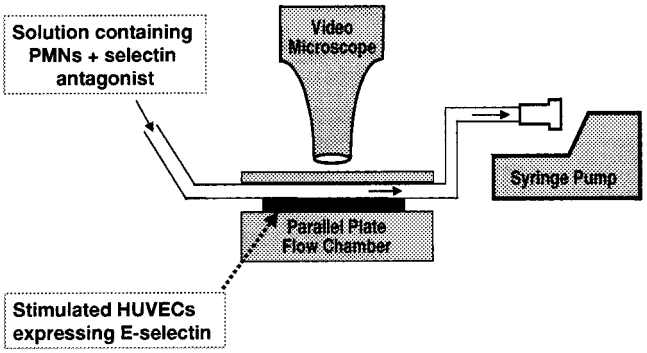
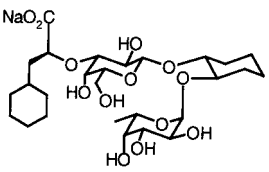


Figure 6. Cell-based E-selectin flow assay.

described below and inhibit E-selectin (IC_{50} = 0.5−1.0 mM sLe^x) similarly to monovalent sLe^x (IC_{50} = 1.0−2.0 mM). Interestingly, glycopolymer **L-18**, containing sLe^x (20%) and additional carboxylic acid functions (25%), gave 30% reduction of the number of interacting cells at 0.1 mM sLe^x. Multivalent sLe^x **L-21**, with 25% of sulfonate residues, also showed 35% reduction of the number of interacting cells. A control polymer with 100% of carboxylates but no sLe^x showed no effect. The observed activities of the charged polymers are too weak to indicate multivalent E-selectin−sLe^x recognition and rather highlight the important but not fully understood interactions of multiple acidic functions with selectins. Selectin inhibition by macromolecules which contain multiple acid functions such as acidic ion-exchange resins has been observed before.[33] P-selectin inhibition using liposomes with carbohydrates and additional carboxylic acid funtions has also been reported.[20b]

Use of Polymer L-24 as Multivalent Ligand for Competitive Selectin Ligand Binding Assays. Compound **L-24**, containing 75% of glycerol, 20% of sLe^a, and 5% of biotin,

(32) Sialyl Lewis^x mimic CGP69669A



(IC_{50} = 0.1 mM) is 10 times more active in the static E-selectin ligand binding assay than sLe^x. Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, *3*, 1571.

(33) Kretzschmar, G.; Toepfer, A.; Huels, C.; Krause, M. *Tetrahedron* **1997**, *53*, 2485.

was used as a polymeric ligand for a cell-free, competitive E-selectin ligand binding assay (for details, see Experimental Section and ref 22). Interestingly, attempts to use the corresponding biotinylated sLe^x polymer **L-23** failed. A similar assay has been described that uses a commercially available biotinylated sLe^a-containing polyacrylate derived from 4-nitrophenyl polyacrylate.[34] As shown above, such compounds can contain variable quantities of additional carboxylates (Scheme 1).[14] Since the binding properties of multivalent carbohydrate ligands with additional carboxylates is not fully understood, it can be problematic to use these polymers for selectin assays. Compound **L-24** is well characterized, can be prepared with reproducible composition and molecular weight distribution, and has no additional acid functions (besides the sialic acid and one end group). Our assay allows reliable evaluation of a broad variety of E-selectin antagonists. The data are predictive for the in vivo behavior of the tested compounds. The application for similar P- and L-selectin ligand binding assays was less successful.

Conclusion

The new homopolymer **4** with reactive chloroacetamide functions was prepared from commercially available polylysine hydrobromide. As functionalization with various thiols is quantitative and the polylysine backbone is not affected, this methodology gives easy access to complex, biodegradable glycopolymers with predictable composition and molecular weight distribution. Both product purity and composition can be readily analyzed by ^{1}H NMR. This new method is not limited to the synthesis of glycopolymers and could be broadly applied to the design and preparation of soluble polylysine conjugates for a variety of uses.

Experimental Section

General. All reactions were carried out under an atmosphere of dry argon. Chemical shifts of the ^{1}H NMR signals of water-soluble compounds are reported relative to the shift of the DHO peak (4.75 ppm). The signal assignments which are given in the Supporting Information are based on two-dimensional ^{1}H/^{1}H correlation (COSY) and ^{1}H/^{13}C correlation spectroscopy (HSQC). The SEC-MALLS measurements of **L-8**, **L-13**, and **L-14c** were done in DMF at 30 °C using PSS SDV 1000 Å, 10 μm, 8 × 300 mm, and PSS SDV 10^5 Å, 10 μm, 8 × 300 colums, a Wyatt DAWN-F multiangle laser light scattering detector, and a Shodex differential refractometer SE-61.

Syntheses of Mercaptans 11, 17, 22, and 24 from the Corresponding Primary Amines. General Procedure. Under rigorous exclusion of oxygen, a solution of $\mathbf{R_x'}$**-SH** (0.05 mmol), thiobutyrolactone (0.5 mmol), and NEt_3 (0.5 mmol) in 5 mL of degassed methanol was heated under reflux for 16 h. The solvent and volatile side products were removed in vacuo, and the residue was subjected to chromatography on silica gel.

11 from 28:[35] eluent, ethyl acetate/methanol 3:1; yield, 89%; MS/EI 403 $(M + Na)^+$.

17 from 29:[36] eluent, chloroform/methanol/water 55:45:10; yield, 74%; MS/EI 1002 $(M + Na)^+$.

22 from 30:[37] eluent, chloroform/methanol 10:1; yield, 78%; MS/EI 445 $(M + H)^+$.

24 from 31:[36] eluent: chloroform/methanol/water 55:45:10; yield, 72%; MS/EI 1002 $(M + Na)^+$.

Acylation of Polylysine Hydrobromide. General Procedure. Polylysine hydrobromide **3** (1.0 mmol) was suspended in a mixture of 4 mL of DMF and 1 mL of 2,6-lutidine. At 0 °C, a solution of acid anhydride (3.0 mmol) in 1 mL of DMF was added within 15 min, and the resulting clear solution was stirred for 16 h at 0 °C. The product was precipitated by dropwise addition of the solution to 40 mL of a stirred 1:1 mixture of ethanol and ether. The solid was filtered off, washed with ethanol/ether 1:1, and dried to yield the acylated polylysine derivative.

L-4: yield, 98%. **D/L-4**: yield, 94%. **L-5**: yield, 94%. **L-6**: yield, quantitative.

Functionalization Reactions of 4. General Procedure for the Purification of Water-Soluble Polymers. The reaction mixture was added dropwise to 30 mL of a 1:1 mixture of ethanol and ether. The formed precipitate was filtered off and washed with ethanol. The crude product was dissolved in water and further purified by means of ultrafiltration. Ultrafiltrations were performed using Amicon stirred cells 8010 (volume, 10 mL, diameter, 25 mm) and Amicon disk membranes YM3 (molecular weight cutoff, 3000). Ultrafiltrations were repeated five times from 10 down to 2 mL, with the volume being made up with distilled water on each occasion. When charged components were incorporated, the aqueous solution was adjusted to pH 11 by adding 2 M NaOH prior to the first run to obtain the sodium salts. Lyophilization afforded the product polymers as colorless powders.

L-8. A solution of DBU (29.8 mg, 0.196 mmol, 2.0 equiv) in 1 mL of DMF was added at room temperature to a solution of **L-4** (20.0 mg, 0.098 mmol) and **7** (31.8 mg, 0.294 mmol, 3.0 equiv) in 2 mL of DMF, and the mixture was stirred for 3 h (**L-8**, 26 mg, 96%).

D/L-8. As described for **L-8**; yield, 99%.

L-9. At room temperature, NEt_3 (20.2 mg, 0.200 mmol, 2 equiv) was added to a solution of **L-4** (10.0 mg, 0.050 mmol) and **10**[38] (5:1 α/β-mixture; 19.2 mg, 0.100 mmol, 2 equiv) in 2 mL of DMF, and the mixture was stirred for 16 h (**L-9**; 17.0 mg, 93%). According to ^{1}H NMR, the product contained 83% of monomer units linked to β-R_{10} and 17% of monomer units linked to α-R_{10}.

L-12. At room temperature, NEt_3 (30.3 mg, 0.300 mmol, 3 equiv) was added to a solution of **L-4** (20.5 mg, 0.100 mmol) and **11** (57 mg, 0.150 mmol, 1.5 equiv) in 1 mL of DMF, and the mixture was stirred for 48 h (**L-12**; 28 mg, 72%). According to ^{1}H NMR, the product contained 70% of monomer units linked to GlcNAc and 30% of chloroacetamide units.

L-13. A solution of DBU (37.0 mg, 0.244 mmol, 2.0 equiv) in 1 mL of DMF was added at room temperature to a solution of **L-4** (25.0 mg, 0.122 mmol) and **11** (60.4 mg, 0.159 mmol, 1.3 equiv) in 2 mL of DMF and stirred for 3 h (**L-13**; 65 mg, 97%).

L-14c. A solution of DBU (13.7 mg, 0.092 mmol, 0.75 equiv) in 1 mL of DMF was added at room temperature to a solution of **L-4** (25.0 mg, 0.122 mmol) and **11** (23.2 mg, 0.061 mmol, 0.5 equiv) in 2 mL of DMF and stirred for 1 h. Then, **7** (29.8 mg, 0.276 mmol, 3 equiv) and NEt_3 (27.9 mg, 0.276 mmol, 3 equiv) were added, and stirring was continued for 2 h (**L-14c**; 47.5 mg, 95%). According to ^{1}H NMR, the product contained 50% of monomer units linked to thioglycerol and 50% of monomer units linked to GlcNAc.

L-14d. As described for **L-14c**, but by using 20% of **11** instead of 50%; yield, 95%. According to ^{1}H NMR, the product contained 20% of monomer units linked to GlcNAc and 80% of monomer units linked to thioglycerol.

D/L-14. as described for **L-14c**; yield, 95%. According to ^{1}H NMR, the product contained 50% of monomer units linked to GlcNAc and 50% of monomer units linked to thioglycerol.

L-15. DBU (22.3 mg, 0.147 mmol, 1.0 equiv) was added at room temperature to a solution of **L-4** (30.0 mg, 0.147 mmol) in 6 mL of

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(38) Compound **10** (5:1 α/β-mixture) was obtained from the corresponding tetraacetate by saponification using sodium methanolate in methanol. The reaction mixture was neutralized with Dowex 50Wx8 ion-exchange resin.

DMF and stirred for 18 h. Then, **7** (47.7 mg, 0.441 mmol, 3.0 equiv) and NEt_3 (74.2 mg, 0.735 mmol, 5.0 equiv) were added, and stirring was continued for 24 h (**L-15**; 21 mg, 44%). According to ^{1}H NMR, the product contained 60% of monomer units linked to thioglycerol and 40% of monomer units linked to DBU.

L-16d. **4L** (7.6 mg, 0.037 mmol) was dissolved in 1.4 mL of degassed DMF. Next, **17** (12.0 mg, 0.0111 mmol, 0.3 equiv) followed by 0.1 mL of degassed water were added. To the clear solution was added at room temperature a solution of DBU (8.5 mg, 0.056 mmol, 1.5 equiv) in 0.4 mL of DMF. After the solution was stirred for 1 h, **7** (12.0 mg, 0.111 mmol, 3 equiv) and NEt_3 (11.2 mg, 0.111 mmol, 3 equiv) were added, and stirring was continued for 16 h (**L-16d**; 20.0 mg, quantitative). According to ^{1}H NMR, the product contained 70% of monomer units linked R_7 (thiglycerine) and 30% of monomer units linked to R_{17} (sLe^x).

Accordingly, **L-16a**, **L-16b**, and **L-16c** were prepared from **L-4**. Polymers **D/L-16a** and **D/L-16b** were prepared from **D/L-4** (for yields and carbohydrate contents, see Table 1).

L-18. A solution of DBU (8.5 mg, 0.056 mmol, 1.5 equiv) in 0.4 mL of DMF was added at room temperature to a solution of **L-4** (6.6 mg, 0.0324 mmol), **17** (7.0 mg, 0.0065 mmol, 0.2 equiv), and **19** (0.92 mg, 0.0081 mmol, 0.25 equiv) in 1.0 mL of DMF and 0.05 mL of water and stirred for 1 h. Then, **7** (10.5 mg, 0.097 mmol, 3 equiv) and NEt_3 (9.8 mg, 0.097 mmol, 3 equiv) were added, and stirring was continued for 16 h. (**L-18**; 14.5 mg, quantitative). According to ^{1}H NMR, the product contained 55% of monomer units linked to R_7, 20% of monomer units linked to R_{17}, and 25% of monomer units linked to R_{19}.

L-21. A solution of DBU (8.5 mg, 0.056 mmol, 1.5 equiv) in 0.4 mL of DMF was added at room temperature to a solution of **L-4** (6.6 mg, 0.0324 mmol), **17** (7.0 mg, 0.0065 mmol, 0.2 equiv), and **20** (1.33 mg, 0.0081 mmol, 0.25 equiv) in 1.0 mL of DMF and 0.05 mL of water and stirred for 1 h. Then, **4** (10.5 mg, 0.097 mmol, 3 equiv) and NEt_3 (9.8 mg, 0.097 mmol, 3 eqiv) were added, and stirring was continued for 16 h (**L-21**; 15.0 mg, quantitative). According to ^{1}H NMR, the product contained 55% of monomer units linked to R_7, 20% of monomer units linked to R_{17}, and 25% of monomer units linked to R_{20}.

L-23. DBU (7.5 mg, 0.050 mmol, 1 equiv) was added at room temperature to a solution of **L-4** (10.0 mg, 0.050 mmol), **17** (10.6 mg, 0.010 mmol, 0.2 equiv), and **22** (1.11 mg, 0.0025 mmol, 0.05 equiv) in 2.0 mL of DMF and stirred for 1 h. Then, **7** (27.0 mg, 0.25 mmol, 5 equiv) and NEt_3 (25.3 mg, 0.25 mmol, 5 equiv) were added, and stirring was continued for 16 h (**L-23**; 22.0 mg, 95%). According to ^{1}H NMR, the product contained 75% of monomer units linked to R_7, 20% of monomer units linked to R_{17}, and 5% of monomer units linked to R_{22}.

L-25. DBU (7.5 mg, 0.050 mmol, 1 equiv) was added at room temperature to a solution of **L-4** (10.0 mg, 0.050 mmol), **24** (10.6 mg, 0.010 mmol, 0.2 equiv), and **22** (1.11 mg, 0.0025 mmol, 0.05 equiv) in 2.0 mL of DMF and stirred for 1 h. Then, **7** (27.0 mg, 0.25 mmol, 5 equiv) and NEt_3 (25.3 mg, 0.25 mmol, 5 equiv) were added, and stirring was continued for 16 h (**L-25**; 22.0 mg, 95%). According to ^{1}H NMR, the product contained 75% of monomer units linked to R_7, 20% of monomer units linked to R_{24}, and 5% of monomer units linked to R_{22}.

L-26. DBU (18.5 mg, 0.122 mmol, 1 equiv) was added at room temperature to a solution of **L-4** (25.0 mg, 0.122 mmol), **17** (10.6 mg, 0.024 mmol, 0.2 equiv), and **22** (2.7 mg, 0.0061 mmol, 0.05 equiv) in 2.5 mL of DMF and stirred for 1 h. Then, **20** (60.0 mg, 0.366 mmol, 3 equiv), NEt_3 (61.6 mg, 0.61 mmol, 5 equiv), and 0.5 mL of degassed water were added, and stirring was continued for 16 h (**L-26**; 62.0 mg, quantitative). According to ^{1}H NMR, the product contained 20% of monomer units linked to R_{17}, 75% of monomer units linked to R_{20}, and 5% of monomer units linked to R_{22}.

L-27. To an oxygen-free solution of 25.0 mg (0.122 mmol) of **L-4** in 2.5 mL of dimethylformamide and 0.5 mL water were added 5.0 mg (0.0305 mmol, 0.25 equiv) of **20**, 26.4 mg (0.024 mmol, 0.2 equiv) of **17**, and 2.70 mg (0.0061 mmol, 0.05 equiv) of **22** at room temperature and under argon. Then, 27.8 mg (0.183 mmol) of DBU was added to the clear solution. After the solution was stirred at room temperature for 1 h, 39.5 mg (0.366 mmol) of thioglycerol **7** and 100 mL of distilled triethylamine were added, and stirring was continiued for 2 h (**L-27**; 60 mg, quantitative). According to ^{1}H NMR, the product contained 50% of monomer units linked to R_7, 20% of monomer units linked to R_{17}, 25% of monomer units linked to R_{20}, and 5% of monomer units linked to R_{22}.

Cell-Free E-Selectin Ligand Binding Assay. Wells in a microtiter plate (plate 1, Falcon probind) are coated with E-selectin/hIg chimera at a concentration of 200 ng/well. After coating, the wells are blocked for a minimum of 2 h. During this incubation, inhibitory test compounds 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 the biotinylated sialyl Lewis^a polymer **L-15** and horseradish peroxidase-labeled streptavidin (KPL, Gaithersburg, MD) is added to each well. After 2 h at 22 °C, plate 1 is washed with buffer, and 100 μL/well is 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 buffer, and 100 μL of TMB substrate reagent (KPL) is added to each well. After 3 min, the colorimetric reaction is stopped by adding 100 μL/well of 1 M H_3PO_4, and the optical density is determined at 450 nm.

Assay To Measure Cell Adhesion under Flow Conditions. A flow assay was employed using a parallel plate flow chamber (GlycoTech, Rockville, MD) to measure the activity of multivalent sLe^x polymers to inhibit the rolling of polymorphonuclear neutrophils (PMNs) on human umbilical vein endothelial cells (HUVECs).

HUVECs were isolated from umbilical cords using collagenase (Worthington Biochemicals, LS004196), washed, and expanded in T-175 flasks. After about 10 days, when the HUVECs reached confluence, they were passaged to 35-mm tissue culture dishes coated with fibronectin (FN) (Gibco 33016-023). The dishes were used in 3−5 days once a confluent monolayer was obtained.

PMNs were isolated from fresh blood the day of each experiment and used within 5 h of the isolation. The neutrophils were suspended in HBSS with Ca and Mg (Sigma H9269) and 12 mM Hepes (Biofluids, MD No. 305) at 10^6 cells/mL for use in the flow assay.

Prior to the flow assay, the confluent HUVEC monolayers were stimulated with TNF-α (30 U/mL, Genzyme) for 3 h to induce the expression of E-selectin on the cell surface. Test compounds were incubated with HUVECs and PMNs for 20 min prior to flow in the presence or absence of human serum albumin (HSA, 3 mg/mL, Sigma, A-6784). The cell suspension of PMNs (10^6 cells/mL) containing the test compound was perfused through the chamber at a shear rate corresponding to a wall shear stress of 0.9 dyn/cm^2. The wall shear stress (τ_w, dyn/cm^2) is given by $\tau_w = 6\mu Q/a^2 b$, where μ is the apparent viscosity of the media (for H_2O at 37 °C = 0.0076 P), a is the channel height (i.e., gasket thickness of 254 μm), b is the channel width (i.e., gasket width of 0.25 cm), and Q is the volumetric flow rate (mL/min). The cell suspension was allowed to flow through the chamber for 3 min before digital images were collected to quantify each experiment.

The digital image system consisted of a Silicon Graphics Indigo 2 workstation interfacing to Inovision's IC300 digital image system. A CCD camera (Dage-MTI CCD72) was mounted on a Zeiss inverted stage microscope (ICM 405), operated in the phase contrast mode using a 10× objective to provide the signal to the digital image system. The experiments were recorded on a video recorder (Sony model SVO-1610).

After 3 min of perfusing cells through the flow chamber, digital images were acquired at 7−10 different locations on each of three dishes for every experimental condition. The image acquisition program collected 10 images at each location to provide sufficient image data for subsequent image processing. Each of the 10 images was a result of a real-time minimization function of three frames to remove all moving cells in the bulk flow which are not in contact with HUVEC monolayer in the flow chamber. Once the 10 images were collected, image analysis was performed to generate composite images containing only rolling cells, only arrested cells, or images with the total number of interacting cells (i.e., both the rolling and arrested cells). The images with the rolling cells are created to show the rolling cells as vertical

streaks corresponding to the distance traveled by the cells during image acquisition.

The number of interacting cells was determined by a segmentation program based on pixel intensity and size. The digital image analysis system is able to count the number of objects that meet the pixel intensity and size criteria and report the number of interacting cells for each image. The number of arrested cells is usually minimal, so they were counted visually. Quantification of the rolling behavior was performed by analysis of images containing the rolling cells as vertical streaks. The measure of rolling was the rolling index defined as the total area (i.e., total pixel count) of all the vertical streaks in each image.

Acknowledgment. We thank Gabriele Baisch, Beatrice Wagner, Franz Schwarzenbach, and Bernhard Wyss for excellent technical assistance.

Supporting Information Available: CD spectum of **L-8**; NMR data and peak assignment for thiols **11**, **17**, **22**, **24** and for polymers **4**−**6**, **9**, **12**−**16**, **18**, **21**, **23**, **25**−**27** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA984183P