Synthesis of neoglycoconjugate dendrimers

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A series of polydentate dendritic neoglycoconjugates which contain 4, 8, 16, and 32 B-disaccharide ligands were designed as probes to assess the influence of inter-ligand distances on binding to *anti*-B-disaccharide immunoglobulins.

Interactions of natural proteins and glycoconjugates which contain clustered oligosaccharide ligands play an important role in cell recognition processes.[1] The affinity of carbohydrate ligands to proteins depends on spatial organisation of clusters and particularly on the distance between oligosaccharide ligands.[2] These characteristics depend on the properties of the carrier molecule used for the preparation of neoglycoconjugates.

To date, mainly linear polymers are used as carriers of carbohydrate ligands.[1] However, linear polymer-matrix-based conjugates as probes have some disadvantages which are related to the uncertainty and unpredictability of the attachment of ligands within the carrier chain. These problems can be solved by substitution of linear polymeric matrices by dendritic ones (dendrimers), which are highly ordered compounds with a hyper-branched structure with branching sites on each monomeric unit.[3] Dendrimers from symmetrical monomeric units are structures of special interest, because they can form polymeric molecules which have a spherical shape and a dense surface.[4] Properties of dendrimers can be tuned by changing structure, geometry, size of monomeric units and initiator core. First examples of the preparation of dendritic neoglycoconjugates were published earlier.[5–11]

In this paper we describe the synthesis of dendritic neoglycoconjugates which contain 4, 8, 16, and 32 B-disaccharide [B_{di}; -D-Gal(1→3) -D-Gal] ligands. Such glycodendrimers were designed as probes to assess the influence of inter-ligand distances on binding to *anti*-B_{di} immunoglobulins which cause graft rejection in pig to human xenotransplantation.[12] For the preparation of conjugates we used polyaminoamide (PAMAM) dendrimers as carriers of carbohydrate ligands. PAMAM carriers were selected due to their availability, high solubility in organic and aqueous phases and low toxicity.[13]

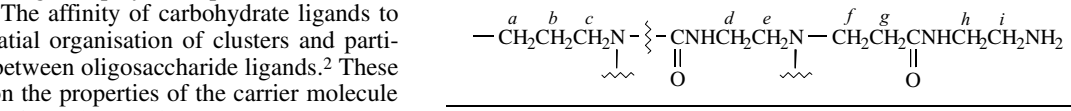
Synthesis of PAMAM dendrimers **2b–5b** was performed according to Tomalia[14] with the use of hexamethylene-diamine as the initial core (Scheme 1). Elongation and branching of dendritic chains was achieved by a sequence of stepwise reiterative reactions which included alkylation with methyl acrylate (5 equiv. CH_2=CHCOOMe, MeOH, room temperature, 18 h) and amidation by an excess of ethylenediamine (5 equiv. $NH_2CH_2CH_2NH_2$, MeOH, room temperature, 48 h). Purification of aminoesters **2a–5a** (Scheme 2) was performed by column chromatography on Kieselgel 60 (Merck) in ethanol, and aminoamides **2b–5b** were purified by chromatography on TSK HW-40F gel in a 0.5% aqueous NH_3 solution. All compounds were obtained as amorphous colourless solids.

Structural assessment of PAMAM matrices was performed using ^{1}H and ^{13}C NMR including 2D ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy and APT experiments. NMR spectra were recorded on a DRX-500 Bruker instrument; characteristic NMR data are presented in Tables 1 and 2. The completeness of elongation of side chains during iterative elongation steps was confirmed by integration of the signals of groups *a* + *b*, *d* + *h* and *g* (Table 1) in the spectra of polyamines **2b–5b**.

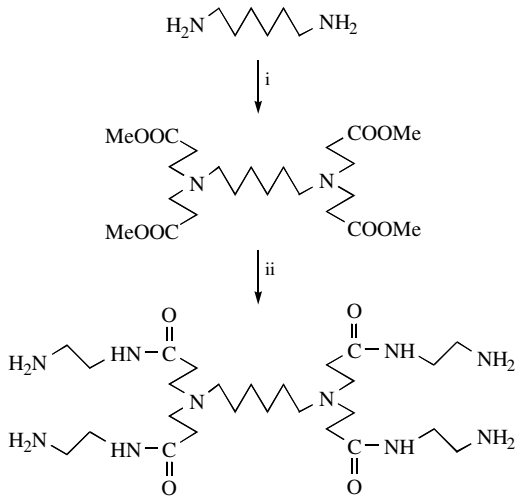
Signals in the NMR spectra of PAMAM derivatives depended on the pH values of solutions. Major changes in the ^{1}H NMR spectra of PAMAM matrices were observed for the signals of fragments with amino groups due to their ability to form ions (Table 1). On the contrary, major changes of chemical shifts in ^{13}C NMR spectra of the same compounds were observed for CH_2 groups connected to carboxyamide fragments.

Signals of the hexamethylenediamine core were pronounced in the spectra of tetra- and octaamines **2b** and **3b**. In the case of 16-dentate conjugate **3b**, we detected these signals only at pH 1, and they were invisible at all pH values in the spectra of 32-mer **4b**. Broadening and low intensity of some signals in the NMR spectra of dendrimers corresponded to published data.[15-17]

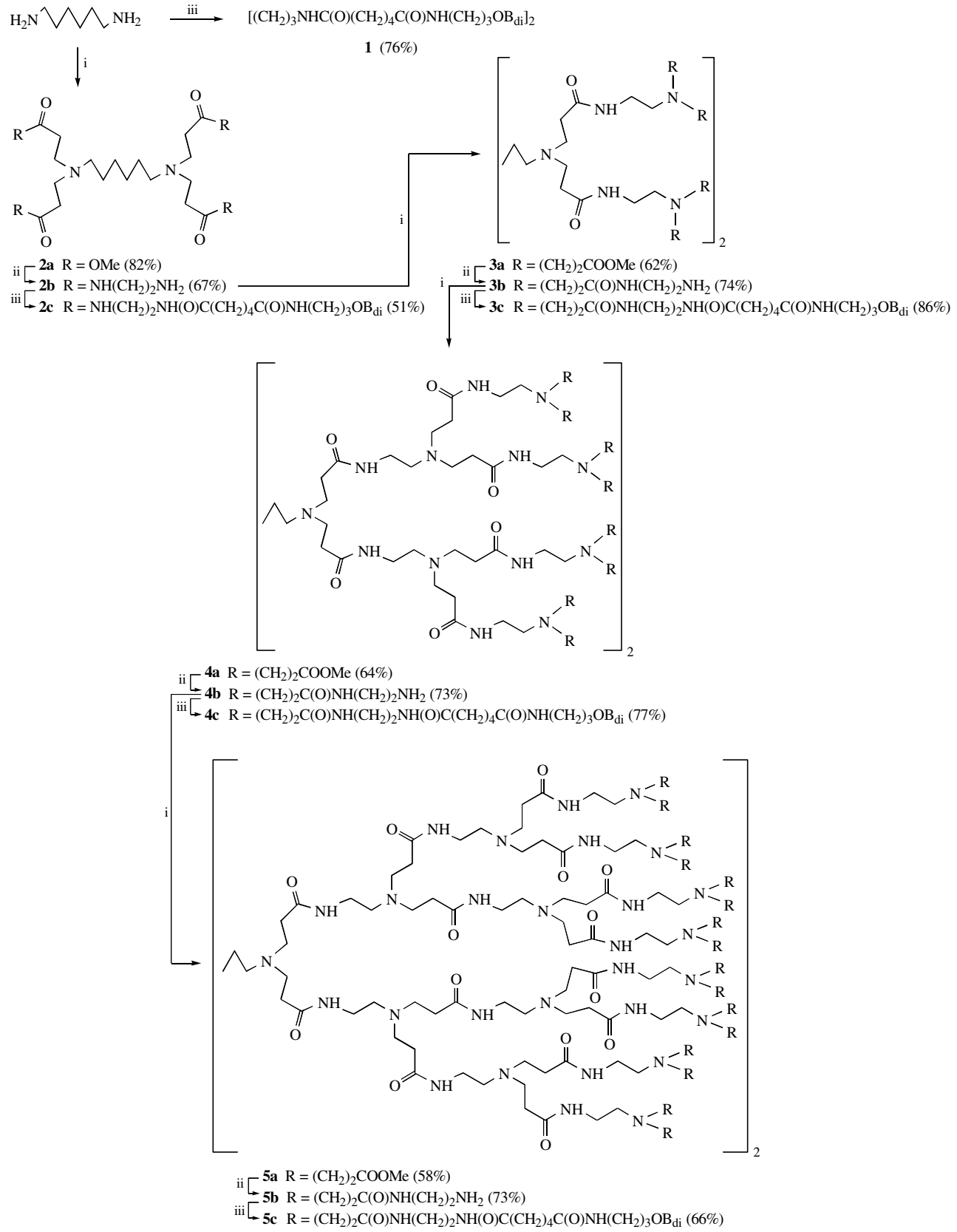
Table 1 ^{1}H and ^{13}C NMR shifts for groups in aminoamide matrices **2b–5b** (D_2O, δ/ppm).



Group	pH 1		pH 10	
	^{1}H	^{13}C	^{1}H	^{13}C
a + *b*	1.25-1 .35 1.60-1 .70	23.8 26.3	1.35-1 .45 1.55-1 .65	26.4 27.6
c	2.65-2 .85	52.6	2.45-2 .60	53.3-5 4.0
d	3.53-3 .57	34.5–35.5	3.30-3 .43	49.8-5 0.2
e	3.23-3 .32	52.0–53.0	2.60-2 .80	33.1-3 3.7
f	3.40-3 .52	49.8–52.0	2.65-3 .00	41.5-4 2.0
g	2.65-2 .85	29.4–29.9	2.45-2 .60	51.5-5 3.0
h	3.03-3 .10	39.3–40.2	3.30-3 .43	38.0-3 9.5
i	3.40-3 .52	37.2–38.3	2.65-3 .00	40.2-4 0.8



Scheme 1 (i) Branching and (ii) elongation of dendritic chain. *Reagents and conditions*: i, 5 equiv. CH_2=CHCOOMe, MeOH, room temperature, 18 h; ii, 5 equiv. $(CH_2NH_2)_2$, MeOH, room temperature, 48 h.

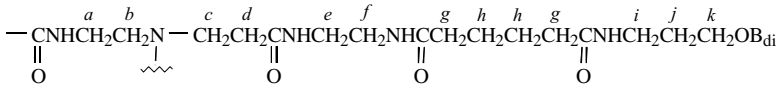


Scheme 2 Synthesis of dendritic PAMAM and neoglycoconjugates. *Reagents and conditions*: i, 5 equiv. CH_2=CHCOOMe, MeOH, room temperature, 18 h; ii, 5 equiv. $(CH_2NH_2)_2$, MeOH, room temperature, 48 h; iii, **7**, DMF, room temperature, 18 h.

Spacer-containing B_{di}-derivative **7** was used as a carbohydrate ligand for preparation of dendritic neoglycoconjugate targets. This compound was obtained in 75% yield by selective N-acylation of 3-aminopropyl glycoside **6**[18] with a 5 mol excess of bis(*p*-nitrophenyl) adipate (Scheme 3). The structure of compound **7** was confirmed by the data of ^{1}H and ^{13}C NMR spectra, which contained the complete series of expected signals.

Conjugation of hexamethylenediamine and aminoamide matrices **2b**–**5b** with spacer-containing B_{di}-derivative **7** (reaction iii in Scheme 2) was performed in DMF (room temperature, 18 h), resulting in bidentate conjugate **1** and glycodendrimers **2c**–**5c** in 51-86 % yields. These amorphous colourless compounds were purified by column chromatography on the gel TSK HW-55F by elution with a 0.5% aqueous NH_3 solution.

Table 2 ^{1}H and ^{13}C NMR shifts for matrices, spacer groups and B-disaccharide[a] ligands in glycoconjugates **2c–5c** (D_2O, δ/ppm).



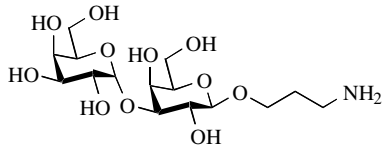
Group	pH 3		pH 5.5		pH 10	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
a	3.12–3.20	37.20	3.18–3.35	37.20	3.35–3.47	36.32
b	3.58–3.63	48.64	2.53–2.80	50.90	2.61–2.67	50.80
c	3.41–3.47	51.60	2.72–2.98	49.74	2.68–2.76	49.99
d	2.68–2.75	n/d	2.32–2.52	33.0–34.0	2.39–2.47	33.27
e + *f*	3.12–3.20	39.68, 39.08	3.18–3.35	39.62, 39.37	3.30–3.47	39.44, 39.28
g	2.10–2.14	36.16	2.15–2.27	36.32	2.22–2.30	36.19
h	1.42–1.47	25.62, 25.56	1.48–1.62	25.72, 25.68	1.55–1.65	25.59
i	3.12–3.20	37.20	3.18–3.35	37.20	3.35–3.47	37.16
j	1.76	29.21	1.81	29.34	1.70–1.80	29.21
k	3.81	69.01	3.92	69.10	3.95	68.45

[a]NMR signals of the B-disaccharide ligands are narrow lines, pH independent, and remain equal for all generations of glycodendrimers. ^{1}H NMR (D_2O) δ: 5.19 (d, H1 , ^2J 4 Hz), 3.91 (d, H2), 4.0 (dd, H3), 4.06 (br. d, H4), 4.23 (br. t, H5), 3.78 (m, H6,6′), 4.48 (d, H1 , ^2J 8 Hz), 3.87 (d, H2), 3.80 (m, H3), 4.20 (br. d, H4), 3.68 (m, H5), 3.78 (m, H6,6′), 3.36 (m, 2H, CH_2NH), 2.77 (m, 2H, NHCOCH_2), 1.89 (m, 2H, OCH$_2$CH_2), 1.55–1.65 (m, 4H, CH$_2$CH_2CH_2CH$_2$). ^{13}C NMR (D_2O) δ: 171.8 (CO), 171.1 (CO), 103.1 (C1), 69.6 (C2), 78.8 (C3), 64.3 (C4), 74.58 (C5), 60.3 (C6), 96.2 (C1), 66.5 (C2), 68.8 (C3), 68.4 (C4), 70.8 (C5), 60.2 (C6), 35.8 (CH$_2$N), 35.0 (*C*H$_2$COO), 33.2 (NHCO*C*H$_2$), 29.4 (CH$_2$), 24.6 and 23.8 (CH$_2$*C*H$_2$*C*H$_2$CH$_2$).

We also investigated the possibility of glycodendrimer preparation using an alternative procedure based on the application of activated esters of dendrimeric matrices which were terminated with activated carboxyl groups. This way was less effective because the hydrolysis of PAMAM aminoesters (*e.g.* of compound **2a**) and subsequent transesterification with $CF_3C(O)OSu$/Py or $CF_3C(O)ONp$/Py was accompanied by destruction processes and thus gave complex mixtures of products.

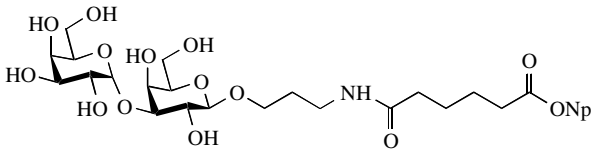
The structural assessment of dendritic neoglycoconjugates was performed by ^{1}H and ^{13}C NMR as in the cases of parent PAMAM matrices. NMR spectra contained expected series of signals for the matrix part and spacer-containing B-disaccharide fragments (Table 2). The completeness of conjugation of terminal amino groups in PAMAM matrices with carbohydrate ligands was confirmed by the integration of signals for groups *c* and *d* in the matrix part and groups *h*, *g* and *j* in the spacer-containing ligand fragments (see scheme in Table 2) in the ^{1}H NMR spectra of glycodendrimers **2c–5c**.

Chemical shifts of some signals in NMR spectra of glycoconjugates **2c–5c** and particularly of CH_2 groups in NHCH$_2$-CH$_2$N(CH$_2$CH$_2$CONH)$_2$ fragments (but not methylenes in spacer of B_{di}-ligands) were pH-dependent (Table 2). It is remarkable that the signals of carbon NCH$_2$*C*H$_2$CO were well resolved at pH 10, but were broadened at pH 5.5 and were not observed in the spectra at pH 3. ^{1}H and ^{13}C NMR signals of B_{di}-parts in the spectra of glycoconjugates were pH-independent and consistent with the data reported earlier[18] for parent amino-propyl glycoside **6**.



6 (B_{di}-OCH$_2$CH$_2$CH$_2$NH$_2$)

5 equiv. (CH$_2$CH$_2$COONp)$_2$
DMSO/DMF, 75%



7 (B_{di}-OCH$_2$CH$_2$CH$_2$NHCOCH$_2$CH$_2$CH$_2$CH$_2$COONp)

Scheme 3 Synthesis of activated spacer-containing B_{di}-derivative **7**.

Investigation of the ability of dendrimers to bind to human xenoantibodies (IgG) was performed as described in refs. 19 and 20. Binding of the antibodies to a xenoantigen applied to immunological plates was inhibited with glycodendrimers **1**,**2c–5c** and two reference substances: monomeric B_{di} and a conjugate of the B_{di}-ligand with polyacrylamide (B_{di}-PAA) with a known high activity.[18] The inhibitory activities of di-, tetra- and octameric glycoconjugates **1**,**2c** and **3c** were similar to those of the monomeric B_{di}-ligand (IC_{50} ~ 500 M); 16- and 32-dentate glycodendrimers had higher activities (IC_{50} ~ 60 M and ~ 40 M, respectively), but lower than that of B_{di}-PAA (IC_{50} ~ 9 M).

For strong binding to antibodies, inter-ligand distances of the glycoconjugates should be comparable to the distance between antigen binding sites of an IgG immunoglobulin that has a value of 80–120 Å.[21] In such a case, co-operative blockage of multiple binding sites can occur. The results of molecular-dynamics simulations (15 ps, *in vacuo*, HyperChem 4.5) showed that, in the case of 16-mer **4c** and 32-mer **5c**, the inter-ligand distance reached the desirable values. Smaller activities of **4c** and **5c** as compared to that of B_{di}-PAA may be due to the absence of an optimal topology in **4c** and **5c** for multiple and co-operative interaction with immunoglobulin. One can assume that an increased activity can be reached with larger dendrimers. To prove this assumption, we are currently performing a new synthesis of larger glycodendrimers, whose shape and size may elicit a higher inhibiting activity.

In conclusion, we report a convenient way for the preparation of glycodendrimers with specified inter-ligand distances, which can be used as a tool for probing the interaction of carbohydrate receptors with antibodies and clustered lectins.

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