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SYNTHESIS AND CHARACTERIZATION OF POLYASPARTIC ACID-CARBOHYDRATE CONJUGATES: MULTIVALENT SIALYL LEWIS^x

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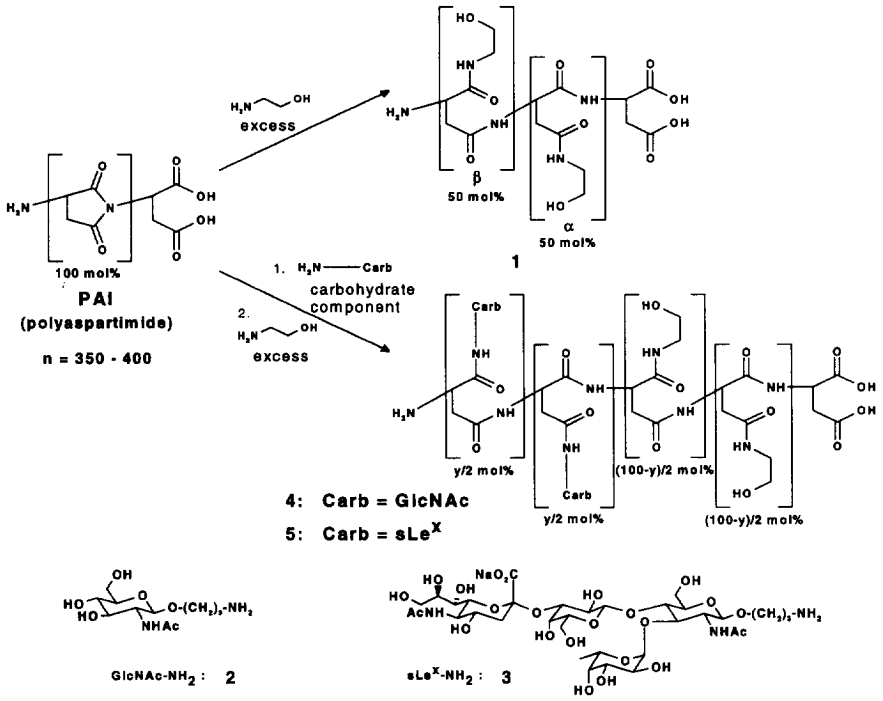
Abstract: A protocol for the functionalization of polyaspartimide (**PAI**) with carbohydrates containing an amino funtion at the reducing end to give biodegradable neoglycoconjugates such as **4** is presented. Scope and limitation of the strategy with respect to the preparation of multivalent sialyl Lewis^x **5** are discussed. © 1997 Elsevier Science Ltd.

Various biological cell-cell recognition events ranging from fertilization, growth, and development to inflammation and tumor metastasis are controlled by the recognition of oligosaccharides and proteins.[1] Therefore, polymers that present complex carbohydrates in a multivalent manner, so called neoglycoconjugates, have recently attracted attention and found broad application in the investigation of carbohydrate/protein interactions.[2]



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Although the copolymerization of carbohydrate-containing monomers and acrylamide[2] gives access to neoglycoconjugates, the resulting products often have a broad molecular weight distribution[3] and an unpredictable composition. Therefore, the functionalization of preformed, activated homopolymers represents a superior strategy. This was demonstrated with several active esters of polyacrylic acid, which have been transformed into neoglycoconjugates.[4]

Polyaspartimide (**PAI**)[5] is a well known activated polymer which reacts readily with primary amines such as ethanolamine under ring opening to give the linear polymer **1** with a biodegradable backbone consisting of a and b linked aspartic acid residues in a ratio of approximately 1:1.[6] Thus, coupling with substoichiometric amounts of **2** or **3** followed by treatment with an excess of ethanolamine to quench the remaining succinimide residues should give access to neoglycoconjugates such as multivalent GlcNAc **4** and multivalent sialyl Lewis^x (sLe^x) **5**. To achieve a predictable product composition these conversions have to be quantitative.

Such an approach toward multivalent sLe^x has been disclosed as a patent.[7] **PAI** was first treated with a substoichiometric amount of ethanolamine followed by the reaction with 7 mol% of a "sLex-amine" (hexylamine spacer at the reducing end) and NEt_3. Finally, an excess of ethanolamine was added. The reaction was carried out in a DMSO/water mixture at 35°C. The product was isolated by precipitation in 64% yield and contained 5 mol% of sLe^x and 95 mol% of ethanolamine according to ^{1}H-NMR. When we applied these conditions to our model carbohydrate **2** (propylamine spacer at the reducing end), we observed low sugar incorporation and, due to partial imide hydrolysis, more than 20 mol% of undesired carboxylic acid residues. Variable amounts of charged groups can restrict the applicability of neoglycoconjugates.[8]

In this communication we describe a modified protocol for the synthesis of polyaspartic acid-carbohydrate conjugates **4** and discuss the scope and limitation of this strategy with respect to the preparation of multivalent sialyl Lewis^x **5**.

Table 1:

Comp.	Carbohydr. (applied)	Conditions [a]	incorp. R_x [b]
4a	**2** (*10* mol %)	DMF/NEt_3/20°C/5d	*6* mol% [c]
4b	**2** (*5* mol %)	DMF/NEt_3/50°C/5d	*5* mol% [c]
4c	**2** (*10* mol %)	DMF/NEt_3/50°C/5d	*9* mol% [c]
4d	**2** (*20* mol %)	DMF/NEt_3/50°C/1d	*15* mol% [c]
4e	**2** (*20* mol %)	DMF/NEt_3/50°C/3d	*18* mol% [c]
4f	**2** (*20* mol %)	DMF/NEt_3/50°C/5d	*19* mol% [c]
4g	**2** (*20* mol %)	DMF/NEt_3/50°C/14d	*19* mol% [d]
4h	**2** (*20* mol %)	DMF/NEt_3/100°C/1d	*16* mol% [d]
4i	**2** (*20* mol %)	DMF/NEt_3/100°C/7d	*16* mol% [d]
5a	**3** (*5* mol %)	DMF/NEt_3/50°C/7d	*3* mol% [d]
5b	**3** (*20* mol %)	DMF/NEt_3/50°C/5d	*9* mol% [c]
5c	**3** (*20* mol %)	DMF/NEt_3/50°C/21d	*8* mol% [d]

[a] 3 eq of ethanolamine were added and the reaction continued for 1 day
[b] determined by ^{1}H-NMR
[c] hydrolysis of **PAI** leading to carboxylic acid functions was neglectable (<5 mol%);
[d] partial hydrolysis of **PAI** was detectable (10-30 mol%).

Treatment of **PAI**[6] (M_w of approximately 40.000; *table 2*) with 3 eq of ethanolamine in DMF furnished the water soluble homopolymer **1** which was isolated by precipitation in ethanol/ether (1:1) and purified by ultrafiltration. Next, **PAI** was reacted with 10 mol% of **2** in the presence of NEt_3 at r.t. for 5 days followed by treatment with an excess of ethanolamine at r.t. for 1 day. Precipitation and ultrafiltration gave compound **4a** which contained, according to ^{1}H-NMR, 6 mol% of monomers linked to carbohydrates and 94 mol% of

monomer units linked to ethanolamine (*table 1*). Longer reaction times and additives such as DMAP, DBU and LiCl did not increase the sugar incorporation. A reaction temperature of 50°C and a reaction time of 5 days turned out to be optimal and resulted in an almost quantitative sugar incorporation (**4b**, **4c**, **4f** with 5, 9 and 19 mol% sugar content; *table 1*). The yields were generally >90%. Shorter reaction times gave lower sugar incorporation (**4d**, **4e**) and more extended reaction times led to partial hydrolysis (**4g**). Obviously, in spite of the use of dried DMF traces of water could not be excluded over these long reaction times. Elevated temperatures did not improve the incorporation of the carbohydrate (**4h**, **4l**).

Table 2:

Comp.	M_w [a)]	M_w [b)]	M_n [b)]	M_w/M_n [b)]
PAI	40.000 (DMF)	-----	-----	-----
1	40.000 (DMF)	64.000 (DMF)	55.000 (DMF)	1.2
4c	50.000 (water)	88.000 (DMF)	66.000 (DMF)	1.3

[a)] Low Angle Laser Light Scattering (LALLS); [b)] coupling of Gel Permeation Chromatography and Multy Angle Laser Light Scattering (GPC/MALLS)[9)]

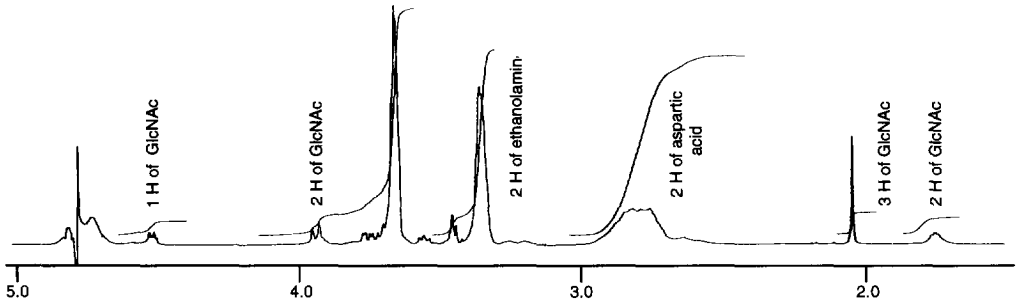

Figure 1: ^{1}H NMR (500 MHz, D_2O, rt) of compound **4c** containing 9 mol% of monomer units linked to GlcNAc and 91 mol% of monomer units linked to ethanolamine

The molecular weight M_w of **1** and **4c** was determined to be 64.000 and 88.000, respectively, which is in good agreement to the starting **PAI** demonstrating that the polymer backbone was not fragmented under the conditions applied. Polydispersities (M_w/M_n) of 1.2 and 1.3 indicate narrow molecular weight distributions (*table 2*). The ^{1}H NMR of compound **4c** is shown in figure 1. The integration of various well separated signals allows the quantitative analysis of the product composition.

When we applied the optimized conditions to the synthesis of multivalent sLe^x by reacting **PAI** with 5 mol% of "sLe^x-amine" **3** at 50°C for 7 days, complete consumption of the tetrasaccharide could not be achieved. Following the treatment with ethanolamine and ultrafiltration compound **5a** containing 3 mol% of sLe^x was isolated. Reaction of **PAI** with 20 mol% of **3** for 3 weeks furnished **5c** containing 8 mol% of sLe^x. Both polymers **5a** and **5c** contained up to 15 mol% of carboxylic acid residues as indicated by ^{1}H-NMR. The best results were obtained when **PAI** was treated with 20 mol% of **3** at 50°C for 5 days. Multivalent sLe^x **5b** with 9 mol% sugar content was obtained in 90% yield. Only trace amounts of carboxylic acid functions (<5%) could be detected.[9)]

In conclusion, we have demonstrated that **PAI** can be transformed under appropriate conditions into neoglycoconjugates by treatment with amino-functionalized saccharides. The model compound **2** led to products with predictable composition whereas "sLe^x-amine" **3** could only partially be incorporated.

Incomplete incorporation of primary amines into **PAI** has been observed earlier even in the presence of additives. [10] The reaction is not fully understood and the rate is not a simple function of the basicity.[11]

Experimental Procedure for the synthesis of 4c: To a solution of 1.00 g (10.31 mmol) of **PAI** in 20 ml of abs. DMF was added 0.29 g (1.03 mmol) of **2** and 0.16 g (1.55 mmol) distilled NEt_3 at 50°C and under argon. After stirring at 50°C for 5 d, the solution was cooled to r. t. and 1.89 g (31 mmol) of ethanolamine was added. After 24 h the clear, colorless solution was added dropwise to 200 ml of ether/ethanol (1:1). The precipitate which was formed was filtered off and washed with ethanol and ether. The crude product was dissolved in water, the solution adjusted to pH 11 with sodium hydroxide solution, and further purified by means of ultrafiltration (Amicon YM 3 membrane) (five times from 100 down to 20 ml, with the volume being made up with distilled water on each occasion). Following lyophilization polymer **4c** (1.69 g, 92%) was isolated as a colorless powder.

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