Pergamon

Tetrahedron Letters 40 (1999) 7235–7238

TETRAHEDRON LETTERS

Convergent Synthesis of Fluorescein-labelled Lysine-based Cluster Glycosides

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Received 11 June 1999; accepted 11 August 1999

Abstract

The synthesis of fluorescein-labelled lysinyl trees, containing 2, 4 or 8 manno- or galactoside residues, is reported. These lysine-based cluster glycosides have been readily assembled by coupling amino-functionalized, *N*-chloroacetylated-L-lysinyl trees with fluorescein-isothiocyanate (FITC) and performing a thioether chemoselective ligation with fully deprotected glycoside derivatives. The reaction order is governed by the size of the lysinyl trees; the labelling/thioetherification steps can be performed in an one pot procedure, thus allowing an easy access to glycodendrimers designed to study the dendritic cells'mannose receptor.

Keywords: dendrimers, carbohydrate, mannose-receptor, fluorescein-labelling

The synthesis of carbohydrate-containing dendrimers has emerged as a major field in organic chemistry.[1] These constructions satisfy to the multivalency criterion, known as the cluster effect,[2] which characterizes most of the interactions of natural carbohydrate-ligands with their receptors.[3] As their structures are well-defined, it is also possible to envisage their use in drug composition or to modify their size and shape (*i.e.* by incorporating a spacer) in order to optimize their interaction[4] with or their presentation[5] to their receptors. Numerous glycodendrimers have been designed to inhibit the adherence of viral particles[6] or to get new insights into immunogenic diseases;[7] however none has been created to accomplish cell-specific drug delivery.

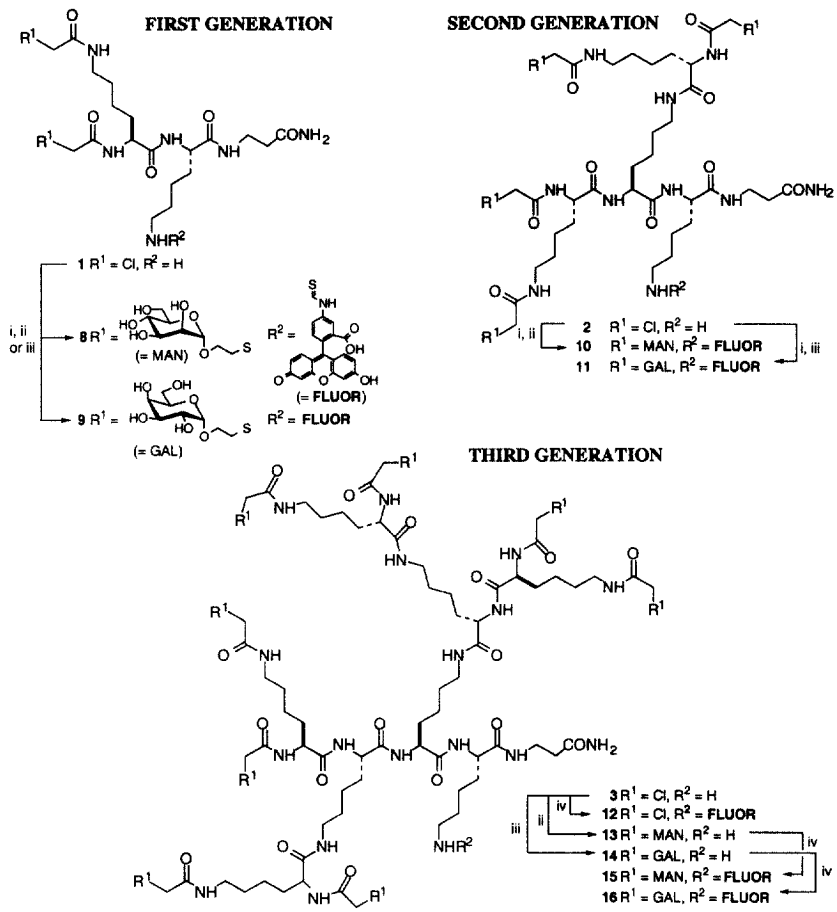
As part of a synthetic vaccine programme, we have envisaged the enhancement of the T-helper immune response by the targeting of major histocompatibility class II-restricted antigens to the dendritic cells' mannose receptor. It has been shown previously that mannose receptor-mediated uptake of antigens considerably improve their presentation by the dendritic cells as compared with endocytosis.[8] However, it is necessary to prepare mannosylated antigens to favour the former pathway. For example, lysine-based cluster mannosides have proved to be high affinity ligands for the mannose receptor.[8,9] However, these ligands have been synthesised using a recurrent strategy. To obtain greater flexibility, we sought to develop a convergent approach relying on (a) the synthesis of dendrimers, bearing two types of chemocompatible functional groups and (b) the sequential ligation of these dendrimers with fully deprotected glycoside derivatives and compounds of interest.

Thus, each dendrimer will give rise to a wide range of potential ligands differing in their glycosides and, for example, their antigen components. To illustrate this strategy, we report

PII: S0040-4039(99)01535-X

here the synthesis of fluorescein-labelled lysinyl trees having 2, 4 or 8 mannopyranoside residues. The fluorescent probe will allow flow cytometric analysis of the interaction and the uptake of the constructions by human dendritic cells. In order to discriminate specific and non-specific interactions during biological evaluation, corresponding galactopyranoside-containing dendrimers have also been synthesised to provide *a priori* low affinity ligands of the mannose receptor.[10]

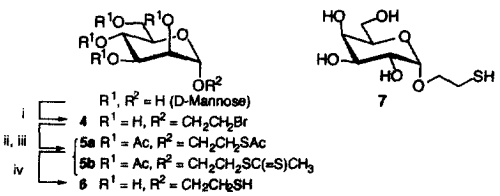
For our purpose, we prepared modified *N*-chloroacetylated-L-lysinyl trees **1**, **2**, and **3** on a 4-methylbenzydrylamine resin using the Boc/benzyl strategy[11] in 52, 42 and 20% overall yields, respectively (Scheme 1).[13] The ε-amino group of the first lysine was not incorporated into the dendrimeric scaffold to allow its linkage with FITC.



Scheme 1 *Reagents and conditions:* i, 1.1 eq FITC, 4 eq $iPrNEt_2$, DMF, rt, 2 h; ii, 1.5 eq/Cl **6**, K_2CO_3, DMF/H_2O 9:1 (pH 8-8.5), rt, 48 h; iii, 1.5 eq/Cl **7**, K_2CO_3, DMF/H_2O 9:1 (pH 8-8.5), rt, 48 h; iv, 2+1 eq FITC, DMF/NaOAc 0.1 M (pH 7.9) 1:1, rt, 120 h.

Mercapto-derivatives of mannose and galactose have been synthesised since a thioether ligation has been envisaged for their attachment to the trees (Scheme 2).

Thus, D-mannose was reacted with 2-bromoethanol in the presence of a catalytic amount of camphorsulfonic acid at 50°C for 2 hours to give **4**.[13] Crude intermediate **4** was further acetylated and finally treated with potassium thioacetate in dry THF to furnish **5a** and the corresponding dithioester **5b** in 48 and 8% yield, respectively. 2-Thioethyl α,D-mannopyranoside **6** was finally obtained following removal of the acetyl groups of compound **5a** using Zemplén's conditions. Compound **7** was obtained analogously from D-galactose in a 42% yield.



Scheme 2 *Reagents and conditions*: i, CSA cat., $HO(CH_2)_2Br$, 50°C, 2 h; ii, Ac_2O, AcOH, $HClO_4$ cat., 2 h; iii, 1.1 eq KSAc, THF, reflux, 1 h; MeONa, MeOH, rt, 45 min.

We then investigated both labelling and ligation with the glycosyl-vectors of the L-lysinyl cores (Scheme 1).[14] These reactions were performed with first generation L-lysine tree **1** in an one pot procedure. Compound **1** was first reacted with FITC in DMF in the presence of DIEA. After completion (monitored by RP-HPLC), glycosides **6** or **7**, dissolved in H_2O, were added to the crude mixture, and the pH adjusted to 8-8.5 (paper) by adding solid K_2CO_3. Constructs **8** and **9** were finally obtained in 39 and 25% yield, respectively, following RP-HPLC purification.

We applied the above procedure to lysinyl tree **2**. Construct **10** was obtained following RP-HPLC purification in 55% yield together with a more hydrophilic by-product whose structure corresponds to a cyclic *N*-arylisothiourea, containing three mannoside residues, in 24% yield. The cyclization happened upon labelling (as observed by RP-HPLC) by intramolecular nucleophilic substitution of one chlorine atom by the newly formed thiourea bond.[15] Glycodendrimer **11** was obtained similarly in 30% yield.

On attempting the labelling of the third generation L-lysinyl core **3**, only traces of the expected product were obtained even under forcing conditions (excess FITC, heating). We anticipated that the amino group would be more accessible if the reaction was carried out in a partially aqueous medium Therefore, the reaction was performed in a 1:1 mixture of DMF/aqueous NaOAc 0.1M. Although the reaction was very slow (5 days), we observed the formation of the fluorescein-labelled intermediate **12**, which further evolved into cyclized products (as determined by ES-MS). The use of more basic conditions did not improve the kinetics but resulted into the decomposition of the products. In view of these results, we decided to branch the glycosides first and then, to introduce the fluorescent probe. L-lysinyl core **3** was reacted with **6** or **7** in DMF/H_2O 9:1, at an apparent pH of 8-8.5, to give glycodendrimers **13** and **14** in 36% and 50% yield, respectively, following RP-HPLC purification. Compounds **15** and **16** were actually obtained after reaction with FITC in 29 and 35% yield, respectively (together with 7 and 4% of starting material **6** or **7**, respectively).

Thus, we were able to synthesise labelled cluster glycosides, containing up to 8 residues, by simply switching the reaction order.

These results demonstrate the consistency of a convergent strategy based on double chemical ligation for the preparation of glycodendrimers as drug vectors. By modifying the set of the reacting groups on the dendrimeric cores, it should be possible to extend this approach to the delivery of antigens (*i.e.* by transforming the ε-amino group of the first lysine into an hydrazino function on the solid phase).[16]

Acknowledments: This work was financially supported by the ANRS and by the Fondation pour la Recherche Médicale (Sidaction), (CG). We are grateful to G. Ricart and H. Drobecq for recording ES-MS data, J.-M. Wieruszeski and G. Montagne for the recording of NMR spectra, and S. Brooks for proofreading the manuscript.

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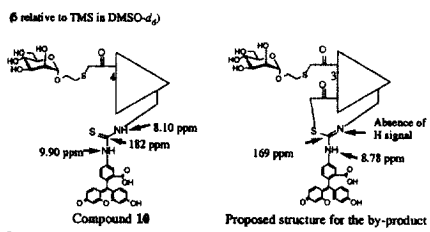
15 Only one among the eight possible cyclic by-products has been obtained, but the cyclization site has not been determined yet. The structure is suggested by the expected $(M-H)^-$ 1871 *m/z* value determined by negative ESI and by the comparison of the ^1H and ^{13}C NMR spectra of this compound and compound **10**. These data are compatible with those given in the literature, for example: B. G. Shearer, S. Lee, J. A. Oplinger, L. W. Frick, E. P. Garvey, and E. S. Furfine, *J. Med. Chem.*, **1997**, *40*, 1901-1905.



Compound **10** Proposed structure for the by-product

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