ARTICLE

OBC
www.rsc.org/obc

Synthesis and biological evaluation of mannose-6-phosphate-coated multivalent dendritic cluster glycosides

Oruganti Srinivas,[a] S. Radhika,[a] Narasimha Murthy Bandaru,[a] Siva Kumar Nadimpalli*[b] and Narayanaswamy Jayaraman*[a]

[a] *Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India*

[b] *Protein Biochemistry and Molecular Biology Laboratory, Department of Biochemistry, University of Hyderabad, Hyderabad 500 046. E-mail: jayaraman@orgchem.iisc.ernet.in; Fax: 91-80-2360-0529*

Received 6th May 2005, Accepted 23rd September 2005
First published as an Advance Article on the web 31st October 2005

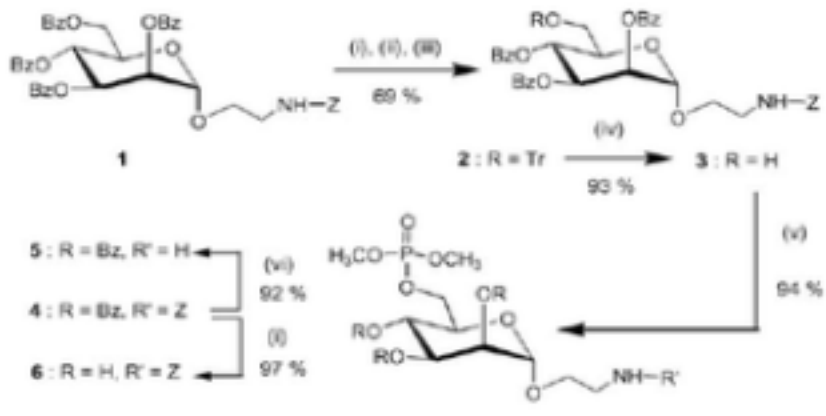
The synthesis of multivalent dendritic cluster glycosides of mannopyranosyl-6-phosphate is presented. Poly(amido amine)-based dendrimers of 0.5–3.5 generations, containing carboxylic acid peripheral functionalities, were utilized so as to install 4, 8, 16 and 32 mannopyranosyl-6-phosphate residues at the peripheries of the dendrimers. Amide bond formation between an amine-tethered mannopyranosyl-6-phosphate monomer unit and carboxylic acid-functionalized dendrimers was conducted to synthesize the dendritic cluster glycosides. The constitutions of the Man-6-P-containing dendrimers were assessed by ^{1}H, ^{13}C and ^{31}P NMR spectroscopies and the sugar content analysis by a resorcinol assay. Preliminary biological studies with few newly synthesized Man-6-P-containing dendrimers showed that these compounds could bind the purified goat liver mannose 6-phopshate receptor (MPR 300) protein.

Introduction

Phosphorylated and phosphosugar structures form major components of natural glycosylated biomacromolecules, such as proteophosphoglycans and lipophosphoglycan, present abundantly in pathogenic parasites and poly(glycosyl phosphates).[1] In some of these phosphoglycans, the phosphoryl components are composed of repeating units of Man-1-P and Gal-β-(1–4)-Man-α-1-P residues, as in the case of *Leishmania genus*.[2] These phosphorylated glycosides act as biological signals. Mannose-6-phosphate (Man-6-P), in particular, is known to be involved in the selective targeting of newly synthesized enzymes to lysosomes.[3] It is known that the presence of multiple Man-6-P residues on *N*-linked oligosaccharides lead to binding affinity enhancements to the cation-independent Man-6-P receptors in macrophages and such enhanced binding affinities have been attributed to the so-called 'glycoside cluster effect'.[4] In the light of the importance of the glycoside cluster effect in carbohydrate–protein interactions, a large number of synthetic cluster glycosides have been synthesized and their lectin recognition properties resulting from clustering the sugar ligands studied.[5] Cluster glycosides built up on dendritic scaffolds have occupied considerable interest in recent years.[6] The hyperbranched and unimolcular nature of dendrimers add as yet another newer scaffold for the presentation of clustered sugar ligands, and on the basis of dendritic design principles, several dendritic cluster glycosides have been synthesized and their properties studied.[7] Dendrimers constructed with phosphorylated building blocks might be considered as highly branched analogs of naturally-occurring phosphorylated glycosides and thus have a potential to incorporate them in studies directed towards understanding the functions of phosphorylated glycoconjugates. We report herein the synthesis of phosphorylated sugar-containing dendrimers, in which the phosphorylated sugar units are presented at the peripheries of the dendrimers. In the synthesis of phosphorylated sugar-coated dendrimers, Man-6-P sugar units were attached at the peripheries of a series of poly(amido amine) (PAMAM) dendrimers.

Results and discussion

The covalent modification of the peripheries of dendrimers was performed by forming amide bonds between amine-tethered sugar units and carboxylic acid-functionalized PAMAM dendrimers. Pre-formed PAMAM dendrimers[8] of 0.5, 1.5, 2.5 and 3.5 generations, possessing 4, 8, 16 and 32 carboxylic acid groups, respectively, were utilized to obtain the Man-6-P-containing dendrimers. Synthesis of amine-tethered Man-6-P derivative **5** was initiated from *N*-(benzyloxycarbonylamino)-ethyl-2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**1**). Derivative **1** was modified sequentially as: (i) deprotection of the *O*-benzoyl protecting groups; (ii) *O*-tritylation of the primary hydroxy group; and (iii) *O*-benzoylation of the remaining hydroxy groups to afford **2** (Scheme 1). De-*O*-tritylation of **2** and phosphorylation of **3**, with chlorodimethyl phosphate in pyridine, provided the Man-6-P derivative **4**. Hydrogenolysis of the benzyloxycarbonyl protecting group in **4** led to the isolation of the free amine-tethered Man-6-P derivative **5**. Alternatively, *O*-benzoyl group deprotection of **4** led to the isolation of the free hydroxy group-containing Man-6-P derivative **6**.



Scheme 1 *Reagents and conditions*: (i) 0.5 M NaOMe/MeOH; (ii) TrCl, Py, 70 °C, 3 h; (iii) BzCl, Py, 12 h; (iv) HCOOH–THF–H_2O (1 : 1 : 0.1), 50 °C, 2 h; (v) (O)P(OMe)$_2$Cl, Py, −40 °C → rt, 24 h; (vi) H_2, 10% Pd–C, MeOH–EtOAc (1 : 1), 12 h, rt.

Amine **5** was subjected to amide bond formation with pre-formed PAMAM dendrimers. Thus, the reaction of **5** with PAMAM dendrimers (0.5 generation), having four carboxylic acid groups, in the presence of di-isopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt), followed by deprotection of the *O*-benzoyl groups with NaOMe/MeOH afforded the tetravalent Man-6-P-functionalized first generation dendrimer **7**

DOI: 10.1039/b506348e