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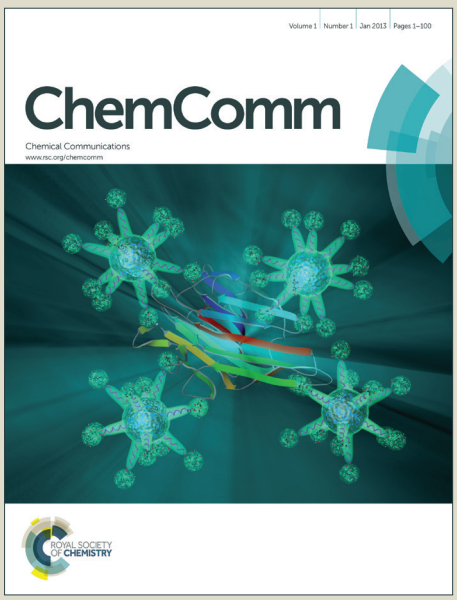

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ARTICLE TYPE

Iminosugar-based glycopolypeptides: glycosidase inhibition with bioinspired glycoprotein analogue micellar self-assemblies

Colin Bonduelle,[ab] Jin Huang,[c] Teresa Mena-Barragán,[d] Carmen Ortiz Mellet,[d] Camille Decroocq,[e] Emile Etamé,[e] Andreas Heise,[c] Philippe Compain*[ef], Sebastien Lecommandoux*[ab]

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Biomimetic nanoparticles prepared by self-assembly of iminosugar-based glycopolypeptides evidenced remarkable multivalency properties when inhibiting α-mannosidase activity. This approach paves the way to biologically active drug delivery systems having glycosidase inhibition potency.

Glycoproteins constitute an important class of biomacromolecules involved in fundamental biological processes that holds many promises for the development of therapeutics, diagnostics and vaccines.[1, 2] Chemical synthesis has been applied to reproduce the full structure of several natural glycoproteins via the use of, among other methodologies, solid-phase peptide synthesis and native chemical ligation.[3-5] Despite impressive progress in this field, this chemical approach is still limited because natural glycoproteins structures are highly complex. As an alternative approach, materials scientists have proposed bioinspired glycoprotein analogues obtained through smart synthetic polymer design.[6-9] Among them, glycopolypeptides are biomimetic glycopolymers with pendant carbohydrates on a polypeptide backbone. Their preparation involves recent progresses made with N-carboxyanhydride (NCA) controlled ring-opening polymerization (ROP) and efficient coupling reactions applicable in multiconjugation schemes (such as the so-called "click chemistry" ligation methods).[8-10]

An expedient way to prepare synthetic glycopolypeptides consists in the post-polymerization glycosylation by exploiting the copper(I)-catalyzed azide-alkyne cycloaddition coupling (CuAAC) between an existing polypeptide scaffold and a complementary sugar partner.[11-14] This approach has recently been the focus of several studies in which the polypeptide backbone was prepared from unnatural amino acid having "clickable" moieties at the side chain extremities.[13-16] By using sequential NCA ROP, easy modulation of the macromolecular composition and architecture can eventually be achieved to form amphiphilic structures incorporating carbohydrates.[17-19] For instance, glycosylation of poly(γ-benzyl-*L*-glutamate–*b*–*DL*-propargylglycine) **1** has been used to form promising drug delivery systems having targeting ability toward lectins showing that advanced polymer chemistry offer now opportunities to prepare biologically active nanomaterials.[18]

In this context, iminosugars that are carbohydrate mimics in which the endocyclic oxygen is replaced by a nitrogen atom, are historically known as very potent glycosidase inhibitors.[20-22] Very recently, the biological evaluation of iminosugar clusters based on a β-cyclodextrin or fullerene (C_{60}) core have led to an unprecedented strong multivalent effects in glycosidase inhibition with affinity enhancement close to 4 orders of magnitude over the corresponding monovalent ligand.[23,24] The potential of the multivalent effect for drug discoveries was subsequently explored with glycosidases of therapeutic interest and promising results were obtained in the field of Gaucher disease[25] and cystic fibrosis (CF).[26]

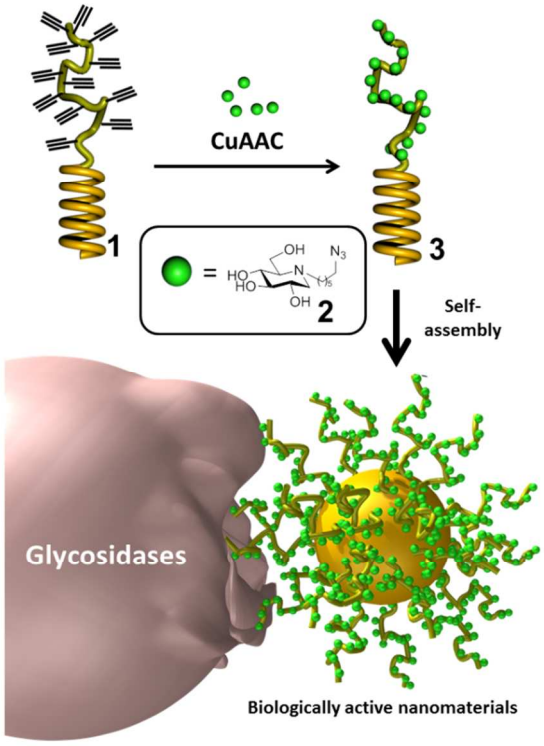
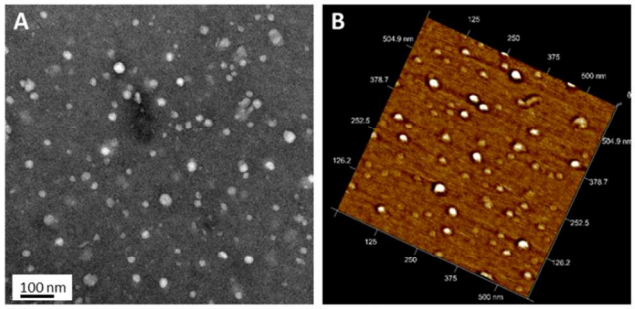


Figure 1 DNJ-based glycopolypeptides : synthesis, self-assembly and glycosidase inhibition

We present in this contribution the first synthesis and biological evaluation of glycopolypeptides-based clusters incorporating iminosugars inhibitors. Due to the biological relevance of this moiety[20] and for comparison purposes,[23,24] an *N*-alkyl analogue of 1-deoxynojirimycin (*N*-alkyl DNJ) was selected as the peripheral ligand. *N*-(6-azidohexyl)-DNJ **2**[23,24] has been

incorporated onto a poly(γ-benzyl-*L*-glutamate–*b*–*DL*-propargylglycine) **1** scaffold to design amphiphilic structures **3** having self-assembly properties in aqueous solutions (*see figure 1*). The glycosylated nanomaterials thus obtained have been fully characterized and their multivalent inhibition potency evaluated towards several models of sugar-binding enzymes.

According to previous reports,[18] the polypeptide backbone was prepared by sequential polymerization, *DL*-propargylglycine N-carboxyanhydride (PG-NCA) being macroinitiated by a poly(γ-benzyl-*L*-glutamate) block in dimethyl sulfoxide (DMSO) at room temperature at a 20:25 benzyl-*L*-glutamate NCA to PG-NCA ratio. Analysis of **1** by size exclusion chromatography (SEC) and ^{1}H-NMR confirmed an average molecular weight (Mn) of 5900 g mol^{-1}, a low polydispersity of 1.16 and good agreement of the copolymer composition with the monomer feed ratio (ESI). In a second step, CuAAC multiconjugation was carried out with **2** (figure 1) by using $(PPh_3)_3CuBr$ as catalyst. The success of the click reaction and the presence of the iminosugars in the block copolymer were monitored by ^{1}H NMR, SEC and FT-IR (ESI). ^{1}H-NMR signals belonging to the *N*-alkyl DNJ moiety were clearly identified after cycloaddition and relative integrations evidenced a nearly quantitative coupling of **2**. Analysis of **3** by SEC clearly indicated a decrease in retention time (Mn of 15000 g.mol^{-1}, polydispersity 1.4) and no residual N_3 stretching was detected by IR spectroscopy after purification.



DNJ-based glycopolypeptide 3 content	[DNJ] for 1 mg/mL nanoparticle solution	Hydrodynamic diameter of the micelles	Polydispersity
0 %	0 mM	35 nm	0.24
10 %	0.18 mM	35 nm	0.22
33 %	0.56 mM	34 nm	0.24
50 %	0.86 mM	28 nm	0.22
66 %	1.12 mM	33 nm	0.25
100 %	1.70 mM	51 nm	0.25

Figure 2 Nanoassemblies incorporating DNJ-based glycopolypeptide **3**. A) TEM imaging; B) AFM imaging; C) Light-scattering analysis of the nanoparticles depending on the DNJ-based glycopolypeptide **3** content.

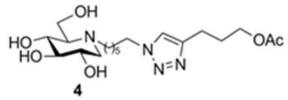
The DNJ-functionalized block copolymer **3** was subsequently self-assembled using a solvent displacement method that consisted in adding a copolymer solution in DMSO to deionized water at 50°C (ESI). The sample appeared perfectly limpid upon dialysis, which removed DMSO. TEM and AFM imaging (see figure 2) of the nanomaterials evidenced spherical micellar structures having sizes below 50 nm. Subsequently, a small library of micelles was prepared (figure 2C) by mixing DNJ-functionalized block copolymer **3** to glycopolypeptides functionalized with a natural monosaccharide, namely galactose (Figure S4, ESI). It is worth to note that this second glycopolypeptide does not inhibit glycosidases but recognizes specifically lectins proteins such as RCA120.[18] Following the same self-assembly procedure, different mixtures of copolymers were used to form micelles containing various densities of iminosugar **2** at the surface. After dialysis, the presence of spherical nanoparticles with hydrodynamic diameters below 50 nm, irrespective of the content of copolymer **3**, was observed by dynamic light scattering (DLS) analysis (see figure 2C).

The DNJ-based glycopolypeptides micellar assemblies (figure 2C) were evaluated for their inhibitory properties against a panel of commercial glycosidases including α- or β-*Glc*ase, α- or β-*Gal*ase and α- or β-*Man*ase (ESI). Higher inhibition potencies were observed for Jack bean α-*Man*ase, an enzyme class having an open active site and several accessible aglycon binding sites, which probably facilitates multipoint interactions.[23] α-*Man*ase emerges as a key model system for the study of the recently discovered multivalent effect in glycosidase inhibition. The best enhancements in binding affinity has been indeed obtained with this enzyme to date.[23,24] Analogy can be made also with Concanavalin A which has been used in numerous works as a model protein to study the interaction of glycoclusters with lectins. The corresponding K_i values are collected in Table I in comparison with data already reported for monomer.[23]

Table 1. Inhibitory activity of DNJ-based glycopolypeptides against α-Mannosidase (K_i, μM).[a]

DNJ-functionalized block copolymer **3** content	α-*Man*ase (Jack bean)	Relative Potency[b]	Relative potency/valency[c]
Monomer (4)[23]	516	-	-
0%	NI[a]	-	-
10%	7.0	74	30
33%	1.2	430	52
50%	0.60	860	69
66%	0.15	3440	206
100%	0.15	3440	138

[a] Due to the maximum DNJ-concentration in the nanoparticles solution was 7 μM, only K_i values in the low micromolar range were measurable. [b] Relative values with respect to K_i of monomer **4**.[c] A 100% DNJ-copolymer contains 25 DNJ units.



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The data reflected a DNJ content-dependent increase in the inhibitory potency towards Jack bean α-*Man*ase that reached a maximum for a 66% proportion of the iminosugar (K_i 0.15 μM). No difference was indeed observed in DNJ-molar basis when nanoparticles incorporating 100% of DNJ-based glycopolypeptide **3** were used. Based on this interesting feature, hybrid systems with additional properties may be envisioned without losing inhibition potency by mixing DNJ-functionalized block copolymer **3** to polypeptides functionalized for example, with a fluorophore moiety, a targeting ligand or a polyethylene glycol (PEG) oligomer. The molar relative enhancement of the inhibition potency above 66% copolymer **3** content were the highest ever reported for a polymeric based nanoassemblies and further underline the possible use of such multivalency to modulate the biological activity of iminosugars when exposed at the surface of biomimetic nanomaterials. For all the iminosugar-based glyconanoparticles, one can hypothesize that it is difficult

for the DNJ moieties close to the core to participate to the inhibition because of the steric hindrance, meaning that all the relative potency values are possibly influenced by the thickness of the hydrophilic layer from which DNJ inhibitors are displayed. K_i modulation from micelles having different glycosylated poly(propargylglycine) molecular-weight is currently under study to accurately determine the balance between the multivalency and this steric hindrance. It is worthwhile to highlight here that three different approaches have been explored so far to control the surface chemistry of glycopolymeric nanoassemblies:[27] conjugation of glycan to preformed nanomaterials,[28] formation of nanomaterials from end-functionalized synthetic block copolymers[29] and formation of nanomaterials from biomolecule-containing hydrophilic blocks comprising polymers.[30] Only this last approach favours the formation of nanomaterials with highly functionalized outer surfaces.[27] Grafting density higher than 50% was required in our case to promote a strong enhancement of the inhibition potency (*see table 1*), a grafting density that is hardly achieved with approaches involving end-functionalized block copolymers.

Synthetic glycopolypeptides are innovative materials that are expected to bring important breakthrough in applications belonging to fields that merge materials science and biology.[8] We present in this paper the first preparation of synthetic glycopolypeptide incorporating glycomimetics By carefully designing the scaffold, amphiphilic copolymers have been obtained that presented self-assembly properties in aqueous solution. Resulted nanomaterials were presented at the surface glycomimetic groups and were able to increase by up to 3 orders of magnitude over the corresponding monovalent analogue the inhibition potency towards Jack bean α-mannosidase. This Jack bean α-mannosidase belongs to the same glycoside hydrolase family than the human lysosomal and Golgi α-mannosidases,[31] two enzymes involved in key steps of glycosphingolipid degradation and *N*-glycoprotein biosynthesis, respectively.[32, 33] It is then expected that our polymer-based nanodevices could be exploited in multimodal strategies aiming to modulate the activity of such human glycosidases of therapeutic interest.

Notes and references

[a] *Université de Bordeaux/IPB, ENSCBP, 16 avenue Pey Berland, 33607 Pessac Cedex, France. E-mail: lecommandoux@enscbp.fr*
[b] *CNRS, Laboratoire de Chimie des Polymères Organiques (UMR5629), Pessac, France*
[c] *School of Chemical Sciences, Dublin City University, Dublin 9, Ireland. E-mail: andreas.heise@dcu.ie*
[d] *Department of Organic Chemistry, Faculty of Chemistry, University of Seville, C/ Professor Garcia Gonzalez 1,41012-Seville, Spain. E-mail: mellet@us.es; Fax: +34 624960; Tel: +34 954559806.*
[e] *Laboratoire de Synthèse Organique et Molécules Bioactives, Université de Strasbourg et CNRS (UMR 7509), Ecole Européenne de Chimie, Polymères et Matériaux, 25 rue Becquerel, 67087 Strasbourg (France). E-mail: philippe.compain@unistra.fr; Fax: (+33) 3-6885-2754 ; Tel: (+33) 3-6885-27592.*
[f] *Institut Universitaire de France, 103 Bd Saint-Michel, 75005 Paris, (France).*

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