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Synthesis of crosslinked nanostructured saccharidic vinyl copolymers and their functionalization

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Abstract—Saccharidic monomers and a macromonomer were synthesized and copolymerized in the presence of divinylbenzene (DVB) as crosslinker in conditions of separation of phases to give hydrophilic nanostructured sugar-based vinyl copolymers. Appropriate model molecules such as *N*-benzyl-D-gluconamide for the saccharidic copolymers and 4-(4-chlorobutoxy)benzaldehyde and (*E*)-4-(4-chloro-2-butenyloxy)benzaldehyde for electrophilic reagents prefiguring possible copper amine oxidase inhibitors allowed identification of conditions for useful monofunctionalizations mainly at the position 2 of the saccharidic units. The examined samples of the nanostructured copolymers from one of the monomers proved to be stable enough to tolerate the functionalization reactions without loss of morphology.

1. Introduction

Synthetic glycopolymers[1–4] find increasing applications in various biological and biomedical fields such as affinity separations, molecular recognition processes, tissue engineering and targeting of drugs.

In particular, glycopolymers possessing a polystyrene backbone with pendant mono- or oligosaccharidic units have been found to specifically interact with lectins[5,6] and influenza virus[7] and be useful as surface materials for hepatocyte cultures.[8–12] Polystyrene nanospheres having saccharidic branches have been prepared and used in protein[13–15] or cell[16] interaction studies or in drug delivery.[17] The amphiphilic character of polystyrene-based polymers with saccharidic branches forces them to assume partially ordered structures in water where the saccharidic moieties extend towards the aqueous medium[18–20] thus making these polymers good candidates for the generation of nanostructured materials as it is known in the self assembly of amphiphilic diblock copolymers.[21]

Copper amine oxidases (CAOs)[22] are ubiquitous enzymes that catalyze the oxidative deamination of biogenic primary amines, a reaction that is at the base of important cellular processes such as cell proliferation and crosslinking of elastin and collagen. The contribution of our laboratory in this field has concerned the synthesis of the first reversible inhibitors with 2,6-dialkoxybenzylamine structure[23] selective for benzylamine oxidase, the synthesis of inhibitors with 4-aminomethylpyridine structure highly active and selective, totally reversible for the same enzyme,[24,25] the synthesis of various types of soluble copolymers and resins containing functions with substrate or inhibitor activity towards benzylamine oxidase[26,27] and the synthesis of several very active substrate-like, reversible, nonselective inhibitors useful for correlating various CAOs from animals, plants and prokaryotes.[28]

Reasonably appropriate nanostructured glycopolymers incorporating in the saccharidic portion selected inhibitor residues, either selective or generally active towards CAOs, could constitute useful tools for localized studies of angiogenesis and cell proliferation. With this final target, we report in this work the synthesis of new nanostructured sugar-based vinyl copolymers crosslinked with divinylbenzene (DVB), stable enough to tolerate reactions of superficial functionalizations exploiting the formation of ether bridges with the saccharidic hydroxyl groups.

To take advantage of spectroscopic methodologies and to incorporate functions which are immediately detectable, the study of functionalization is performed with model molecules for both the saccharidic moiety of the nanoparticles and the electrophilic reagents prefiguring possible CAO inhibitors.

2. Results and discussion

Among the various methods known for synthesizing glycopolymers,[1–4] the free radical polymerization of sugar-based

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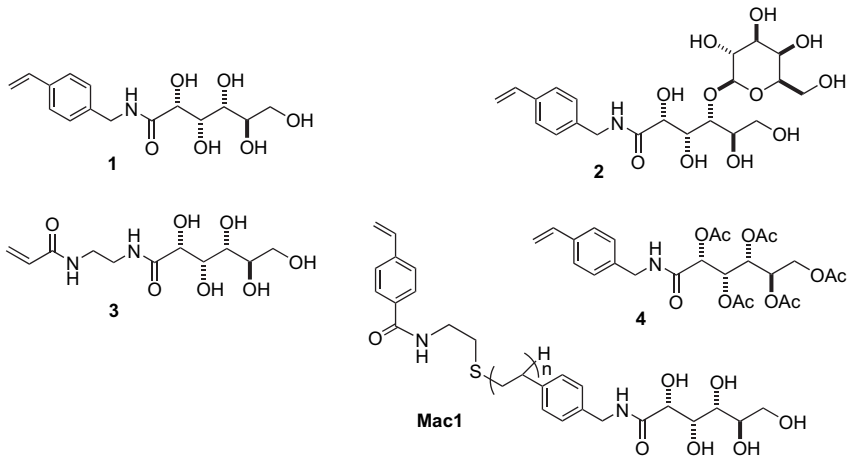


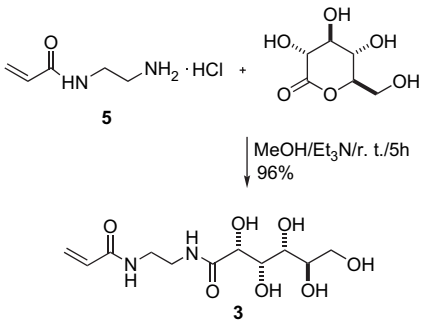
Chart 1.

vinyl monomers attracted our attention for the wide range of polymerization conditions and tolerance to many functionalities. In addition, the free radical polymerization has proved really useful in the production of polymeric nanospheres through the copolymerization of a sugar-based styrene macromonomer with styrene in aqueous systems,[15] and the radical precipitation polymerization has been successfully applied to the preparation of monodisperse crosslinked core–shell microspheres having a styrenic core and a functional shell.[29]

After that, we considered two known styrenic monomers such as **1**[30] and **2**[5] and two new compounds such as acrylamidic monomer **3** and styrenic macromonomer **Mac1** (Chart 1) to explore the possibility of obtaining stable nanospheric glycopolymers through radical precipitation polymerization. The structures of monomers **1**–**3** are characterized by different hydrophilic and hydrophobic portions whose balance can be crucial in the formation of polymeric nanospheres. The structurally more complex **Mac1**, not significantly changing the hydrophilic–hydrophobic balance with respect to **1**, is characterized by quite a different branched shape.

2.1. Monomers 3 and Mac1

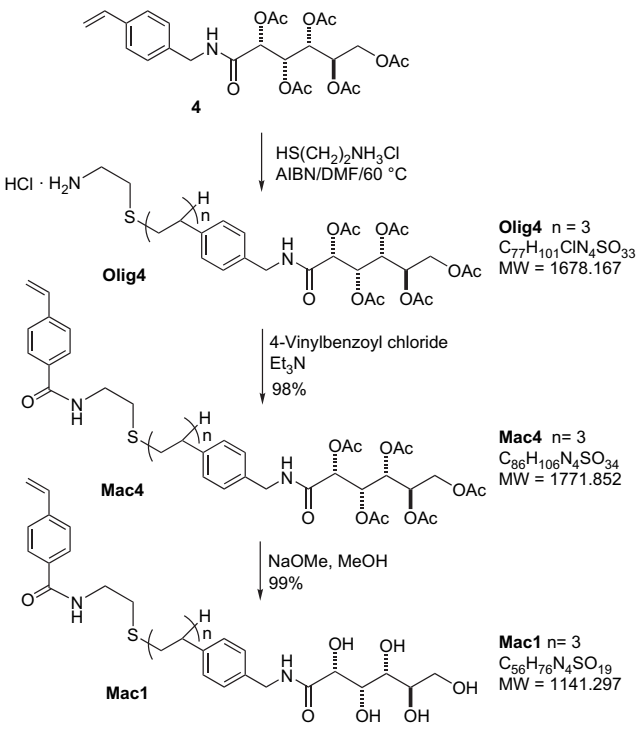
Monomer **3** was obtained in very good yield from *N*-acryloyl-1,2-diaminoethane hydrochloride (**5**)[31] and commercial D-glucono-1,5-lactone (Scheme 1).



Scheme 1.

Since preliminary experiments for the synthesis of macromonomer **Mac1** from **1** showed the need of protection of the hydroxyl groups, not considered in the preparation of different saccharidic macromonomers,[15] monomer **1** was converted into peracetylated **4** whose signals in the ^{13}C NMR spectrum of the saccharidic portion were assigned through COSY and HETCOR experiments.

Monomer **4** was then transformed into **Mac1** in three steps, which include oligomerization in the presence of 2-aminoethanethiol hydrochloride (AETHCl) as chain transfer agent,[32] reaction with 4-vinylbenzoyl chloride[33] and complete deacetylation (Scheme 2). Partial deacetylations modulating the hydrophilicity of the final macromonomer were not studied in the present work.



Scheme 2.

The analysis of the amino end group in two different samples of **Olig4**, performed by acidimetric titration, afforded the number average molecular weight (M_n) values 1497 and 1751, which fit the theoretical M_n=1678 calculated with a degree of oligomerization of three.

Macromonomer **Mac4** revealed the presence of the vinyl group by discolouring Br_2 in CCl_4 and aqueous $KMnO_4$ and the absence of the NH_2 group by a negative ninhydrin test. **Mac4** proved insoluble in hexane and water, partially soluble in Et_2O and soluble in $CHCl_3$, CH_2Cl_2, acetone, dioxane, THF, DMF, and DMSO.

The Zemplen deacetylation of **Mac4** was monitored by the disappearance of the strong absorptions at 1752 and 1220 cm^{-1} of the ester groups and the appearance of the very strong hydroxyl band at 3392 cm^{-1} in the FTIR spectra. Macromonomer **Mac1** showed solubilities drastically different from the precursor **Mac4** proving soluble in DMF, DMSO and water.

2.2. Polymerizations of monomers Mac1, 1, 2 and 3

Several preliminary copolymerization experiments performed with **Mac1**, **1**, **2** and **3** in the presence of styrene and AIBN at different feed molar compositions of the monomers and different solvent mixtures such as H_2O/EtOH, H_2O/2-propanol, H_2O/dioxane and H_2O/pyridine did not promote the separation of phases typical of precipitation polymerizations. The isolated polymeric products at the Scanning Electron Microscopy (SEM) observation resulted in amorphous irregular materials of little interest.

The separation of phases during polymerization was attained when each of the above monomers was polymerized in the presence of DVB as a crosslinker. With DVB in the range 3–12% by weight of the saccharidic monomer, **Mac1** afforded irregular nanostructured copolymers (**CM1**) (Fig. 1a), **1** gave spherically shaped copolymers (**C1**) with average diameter around 300 nm (Fig. 1b), **2** yielded copolymers (**C2**) made of coalesced nanospheres and **3** produced nanostructured copolymers (**C3**) only with high percent of DVB (32%) (Fig. 1c). The addition of PVP as dispersing agent in the copolymerization experiments from **1** reduced the assembling of nanospheres (Fig. 1b and d), changing their surface from smooth to slightly irregular, while it had no important effects on the copolymers from **2** and **3**. Table 1 collects some representative copolymerization data.

Chart 2 shows the structures of the copolymers prepared from **Mac1**/DVB (**CM1**), **1**/DVB (**C1**), **2**/DVB (**C2**) and **3**/DVB (**C3**).

2.3. Functionalization of nanostructured copolymers C1

The study of the functionalization of the copolymeric nanoparticles, through deprotonation of hydroxyl groups of the saccharidic units with a strong base and aliphatic nucleophilic substitution with appropriate electrophilic reagents to form ether bridges, required the availability of easy spectroscopic analyses and functions, which are immediately detectable, so it was performed with the use of model molecules. *N*-Benzyl-D-gluconamide (**6**) was prepared as model for the saccharidic copolymers **C1**, and 4-(4-chlorobutoxy)benzaldehyde (**7**) and (*E*)-4-(4-chloro-2-butenyloxy)-benzaldehyde (**11**) were synthesized as models of possible CAO inhibitors. Compounds **7** and **11** contain a chlorine atom connected to a benzene ring through a saturated or unsaturated spacer and a benzaldehydic group well

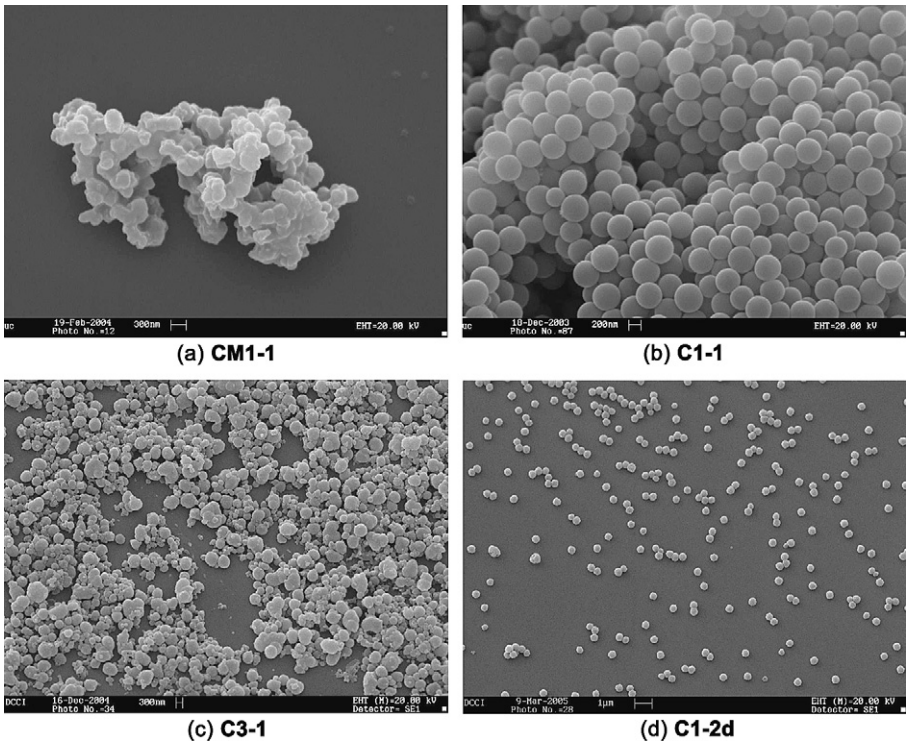


Figure 1. SEM images of copolymers from **Mac1** (a), **1** (b), **3** (c) and **1** with PVP (5%) (d).

Table 1. Typical copolymerization data of **Mac1**, **1**, **2** and **3** with DVB at 70 °C

Monomer, mg (mmol)	DVB, mg (%)	AIBN, mg (%)	PVP, mg (%)	Solvent, mL	Time, h	Polymer, mg (%)
Mac1, 350 (0.31)[a]	53 (12.0)	20 (5.1)	—	H_2O/EtOH 1:1.8, 70	24	**CM1-1**, 215 (55)
1, 450 (1.45)	18 (3.3)	9 (5.2)	—	MeOH, 95	42	**C1-1**, 110 (23)
1, 226 (0.73)	7 (3.2)	6 (2.5)	2.40 (5)	MeOH, 48	73	**C1-2d**, 89 (39)
2, 200 (0.42)	8 (3.2)	8 (4.0)	—	H_2O/EtOH 1:1.7, 44	96	**C2-1**, 72 (35)
2, 200 (0.42)	8 (3.2)	8 (4.0)	2.20 (5)	H_2O/EtOH 1:1.7, 44	96	**C2-1d**, 117 (56)
3, 200 (0.68)	90 (32.0)	4 (2.0)	—	MeOH, 45	24	**C3-1**, 117 (42)
3, 225 (0.77)	18 (6.4)	4 (3.1)	2.40 (5)	MeOH, 48	71	**C3-2d**, 14 (6)

DVB=divinylbenzene; AIBN=2,2′-azobis(2-methylpropionitrile); PVP=polyvinylpyrrolidone.
[a] Calculated from the nominal molecular weight of 1141 corresponding to a nominal loading of 0.876 mmol/g of the saccharidic units.

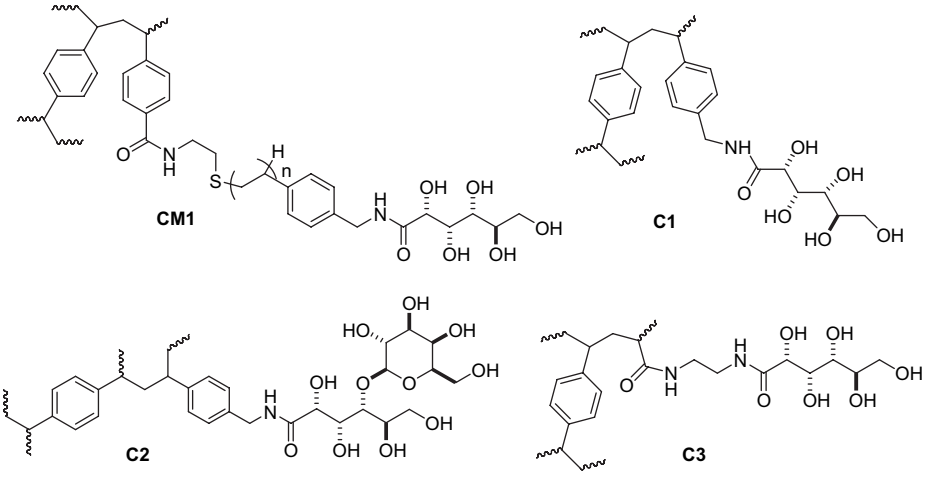
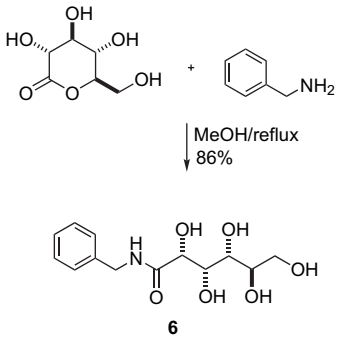


Chart 2.

detectable through the very sensitive and simple chromatic Schiff's test.

N-Benzyl-D-gluconamide (**6**) was prepared from D-glucono-1,5-lactone by analogy to the synthesis of monomer **1** (Scheme 3).



Scheme 3.

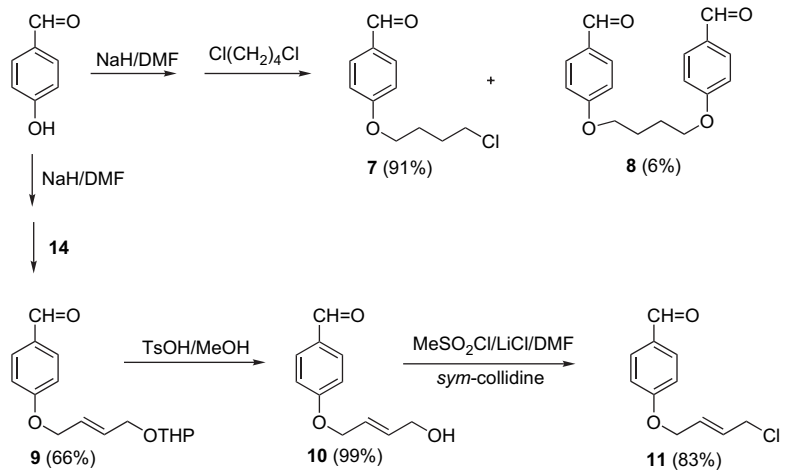
The structure of compound **6**, obtained in high yields as a stable solid, was confirmed by its spectroscopic properties. In particular the ^{13}C NMR spectrum, COSY, HETCOR and decoupling experiments allowed the assignment of C-2 (73.7 ppm), C-3 (70.1 ppm) and C-6 (63.3 ppm) of the saccharidic portion of the molecule leaving uncertain the assignment of C-4 and C-5 because the corresponding CH signals were too close in the proton spectrum. The ^1H NMR spectrum, though complex, is characterized by a doublet at 5.45 ppm of the OH at C-2 and by a narrow triplet of the amidic NH at 8.16 ppm as well-separated signals.

Compound **7** was obtained in high yields from 4-hydroxybenzaldehyde and a 6-fold molar excess of 1,4-dichlorobutane, and easily isolated from the product of double alkylation **8** by distillation at reduced pressure (Scheme 4).

Compound **11** was prepared by alkylation of 4-hydroxybenzaldehyde with (*E*)-4-(2-tetrahydropyranyloxy)-1-chloro-2-butene[34] (**14**), followed by deprotection and conversion of the allylic hydroxyl group into chloride using Meyers' method[35] (Scheme 4). A synthesis parallel to that of **7** was discarded owing to the low thermal stability of (*E*)-1,4-dichloro-2-butene prepared according to the literature.[36] Reagent **14** was prepared from (*E*)-2-butene-1,4-diol[37] (**12**) through its monoprotected tetrahydropyranyl derivative **13**, which was transformed into the allyl chloride as described for **11** (Scheme 4).

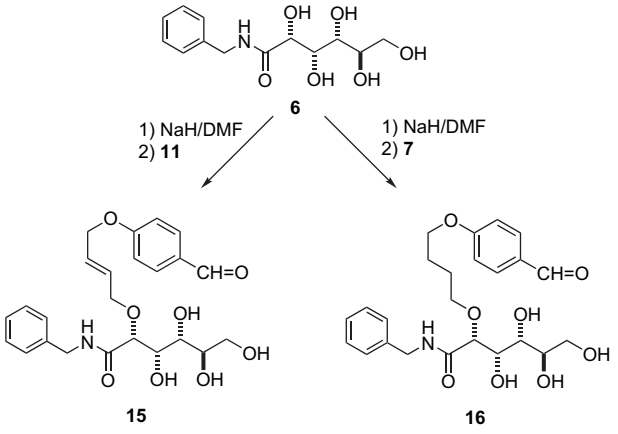
Benzaldehydic compounds **7** and **11** being sensitive to oxidation to the corresponding acids, especially **11**, were stored under nitrogen at −20 °C.

The functionalization of **6** through salification of the hydroxyl groups with NaH followed by aliphatic nucleophilic substitution with **11** or **7** (Scheme 5) was performed using 0.9 mol of NaH per mole of **6**, in order to limit the introduction of more than one aldehydic group per saccharidic unit. The reaction with the more reactive allyl chloride **11** slowly afforded at rt a mixture of three monoalkylated products with yields increasing with the reaction time. Their separation by PLC afforded the main compound **15** and a mixture of two minor products with unknown position of O-alkylation (Table 2, runs 1 and 2). The structure of **15** showing the



Scheme 4.

O-alkylation at C-2 was determined by comparing the ^{13}C NMR spectra of **6** and **15** and by observing in DEPT-135 experiments the jump of the C-2 signal of the saccharidic unit from 73.7 to 81.9 ppm typical for the conversion of a hydroxyl group into an alkoxyl one and in the ^{1}H NMR spectrum of **15** the disappearance of the doublet at 5.45 ppm of the OH at C-2. The 1:1 ratio of the integrals of the NH signal at 8.40 ppm and of the aldehydic proton at 9.88 ppm confirmed the monoalkylation of the saccharidic unit.



Scheme 5.

The two minor products, useful in any case for the functionalization of **6**, were assigned as monoalkylated products by observing that the sum of the integrals of the NH signals at 8.25 and 8.56 ppm was in a 1:1 ratio with that of the aldehydic protons at 9.90 and 9.91 ppm. Prolonged reaction times (Table 2, run 3), increase of the reaction temperature (Table 2, run 4) and excess of the chloride reagent (Table 2, run 5) gave, besides **15** and the mixture of the two monoalkylated products, a mixture of two doubly *O*-alkylated compounds recognized by observing in the ^{1}H NMR spectrum that the sum of the integrals of the NH signals at 8.36 and 8.62 ppm was in a 1:2 ratio with that of the aldehydic protons at 9.86 and 9.87 ppm.

The functionalization of **6** with the alkyl chloride **7** reproduced reaction pattern and ^{13}C and ^{1}H NMR analysis similar to those of the allyl chloride **11**, recording lower yields for the less reactive **7**. At rt for 96 h (Table 2, run 6) a mixture of three monoalkylated products containing the main compound **16** and two other minor products of O-alkylation were obtained. The PLC allowed the isolation of **16** corresponding to the O-alkylation at the C-2 of the saccharidic unit from the other two products with unknown position of O-alkylation. An excess of chloride reagent (Table 2, run 7) afforded, besides **16** and the two monoalkylated products, a mixture of three doubly *O*-alkylated compounds recognized by observing in the ^{1}H NMR spectrum that the sum of the integrals of the NH signals at 8.14, 8.31 and 8.54 ppm was in a 1:2 ratio with that of the aldehydic protons at 9.85, 9.86 and 9.87 ppm.

The conditions for the production of only monoalkylated saccharidic units found with the model **6** can avoid the formation of spurious systems and give degrees of functionalization largely satisfactory for biological interaction studies when transferred to the nanostructured copolymers.

Table 2. Reaction data of the alkylation of **6** with **7** or **11**

Run	**6**, mg (mmol)	Chloride	Molar ratio, **6**/NaH/chloride	*T*, °C	Time, h	Main product yield (%)	A yield (%)	B yield (%)
1	112.6 (0.395)	**11**	1:0.9:1	24	24	**15** (36.1)	(3.4)	—
2	114.4 (0.401)	**11**	1:0.9:1	24	72	**15** (45.9)	(4.5)	—
3	104.3 (0.365)	**11**	1:0.9:1	24	240	**15** (52.8)	(4.2)	(11.7)
4	109.2 (0.383)	**11**	1:0.9:1	60	4	**15** (51.9)	(3.6)	—
5	110.8 (0.388)	**11**	1:0.9:3	24	24	**15** (56.1)	(4.0)	(8.7)
6	114.1 (0.400)	**7**	1:0.9:1	24	96	**16** (16.7)	(6.2)	—
7	108.2 (0.379)	**7**	1:0.9:3	24	48	**16** (30.3)	(5.9)	(7.4)

A=mixture of two monoalkylated products; B=mixture of double alkylation products.

Functionalization experiments of copolymer **C1-1** with allyl chloride **11** performed under monofunctionalization conditions at rt and different reaction times, submitted to Schiff's test after removal of any trace of low molecular weight aldehydic compound, allowed to evidence directly on the copolymers the presence of aldehydic groups by formation of colours from pale to deep fuchsia in agreement with different degrees of functionalization.

3. Conclusion

With the final target of synthesizing glycopolymers functionalized with molecules active as inhibitors of CAOs for localized studies of angiogenesis and cell proliferation, we prepared monomers **1**–**3** and macromonomer **Mac1**, and from them we obtained through precipitation copolymerizations new nanostructured sugar-based hydrophilic vinyl copolymers the best of which resulted in spheres with average diameter around 300 nm (Fig. 1b), stable enough to tolerate functionalization reactions without loss of morphology.

This interesting property, probably exploitable also in fields different from topical CAO inhibition, was elucidated with the use of model molecules in controlled functionalizations by salification of some saccharidic hydroxyl groups with a strong base and formation of ether bridges with selected electrophilic reagents.

Compound **6** was prepared as model for the synthesized saccharidic copolymers, and **7** and **11** as chlorine containing electrophilic reagents prefiguring CAO inhibitors,[23,24,28] even though with benzaldehydic groups well detectable through Schiff's test. With such model molecules we individuated useful conditions for the monofunctionalization of the saccharidic units suitable for avoiding the formation of spurious systems. The reaction afforded a main product *O*-alkylated at the C-2 of the saccharidic unit as determined by NMR spectroscopic analysis and two minor products with unknown position of O-alkylation, useful in any case as active monosubstituted derivatives.

The occurrence of functionalizations performed with samples of nanostructured copolymers was confirmed by positive Schiff's tests showing colour intensities increasing with the degree of functionalization.

4. Experimental

4.1. Materials

N-(4-Vinylbenzyl)-D-gluconamide (**1**),[30] *N*-(4-vinylbenzyl)-[*O*-β-D-galactopyranosyl-(1→4)]-D-gluconamide (**2**),[5] *N*-acryloyl-1,2-diaminoethane hydrochloride (**5**),[31] 4-vinylbenzoyl chloride,[33] (*E*)-2-butene-1,4-diol (**12**)[37] and 4-vinylbenzoic acid[38] were prepared according to the literature.

All the reagents including Schiff's fuchsin-sulfite reagent and solvents were purchased from Aldrich. The solvents were dried and distilled according to standard procedures. Petroleum ether refers to the fraction with boiling point 40–60 °C. Styrene (STY) and divinylbenzene (DVB, containing 80% of DVB isomers) were distilled at reduced pressure under N_2 and stored at −20 °C. 2,2′-Azobis-(2-methylproprionitrile) (AIBN) was crystallized from MeOH.

4.2. Methods

Melting points, determined on a Leica Galen III hot stage apparatus, and boiling points are uncorrected. FTIR spectra were recorded as films or KBr pellets on a Perkin Elmer System 2000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance DPX 300 spectrometer at 300 and 75.5 MHz, respectively, using TMS as internal standard and assigned through DEPT-135, COSY, HETCOR and decoupling experiments. GC–MS analyses were obtained with an Ion Trap Varian Saturn 2000 instrument EI or CI mode (filament current 10 μA) equipped with a DB-5MS (J&W) 30 m, i.d. 0.32 mm, film 1 μm capillary column.

HPLC analyses were performed at rt, constant flow rate (1 mL/min) and UV detection (254 nm) using a Merck LiChrocart 125-4 HPLC cartridge, with a mixture acetonitrile/water=60:40 as eluent.

Flash chromatographic separations were performed on Merck Silica gel (0.040–0.063 mm). TLCs were obtained on Merck F_{254} silica gel aluminium sheets. The saccharidic compounds were evidenced by spraying the TLC with a 0.3% solution of *o*-aminodiphenyl in H_2SO_4/EtOH (5:95) followed by heating. Preparative layer chromatographies (PLCs) were performed on Merck F_{254} 60 silica gel plates (20×20 cm, 0.5 mm).

Scanning Electron Microscopy (SEM) images were obtained with a Leo Stereoscan 440 instrument (LEO Electron Microscopy Ltd) at Dipartimento di Chimica e Chimica Industriale, Università di Genova.

Microanalyses of solid compounds **3**, **4** and **6** were obtained from Laboratorio di Microanalisi, Facoltà di Farmacia, Università di Pisa.

4.3. *N*-(2-Acrylamidoethyl)-D-gluconamide (3)

A mixture of *N*-acryloyl-1,2-diaminoethane hydrochloride (**5**)[31] (1.10 g, 8.17 mmol), commercial D-glucono-1,5-lactone (0.764 g, 4.29 mmol), Et_3N (1.2 mL, 8.61 mmol), dry MeOH (13 mL) and hydroquinone (few crystals) was magnetically stirred under N_2 at rt for 5 h up to the disappearance of D-glucono-1,5-lactone as evidenced by TLC (MeOH/EtOAc=50:50 as eluent). After removal of the volatiles at reduced pressure, the residue was taken with 2-propanol (60 mL), filtered and dried to afford **3** (1.20 g, yield 96%) as a white solid, which was crystallized from methanol. Mp 155–156 °C, purity 96% by HPLC. IR (KBr, ν, cm^{-1}) 3511, 3350 and 3320 (OH), 1664 and 1554 (amide), 1628 (C=C), 990 and 918 (vinyl). δ_H (300 MHz, DMSO-d_6) 3.21 (m, 4H), 3.44–3.65 (m, 4H), 3.92 (m, 1H), 4.00 (m, 1H), 4.36 (s, br O*H*), 4.45 (d, br O*H*, *J*=6 Hz), 4.53 (s, br O*H*), 4.57 (s, br O*H*), 5.40 (s, br O*H*), 5.59 (dd, 1H, *Jcis*=9.8 Hz, *Jgem*=2.5 Hz, CH_2=CH), 6.08 (dd, 1H, *Jtrans*=17.1 Hz, *Jgem*=2.5 Hz, CH_2=CH), 6.20 (dd, 1H, *Jtrans*=17.1 Hz, *Jcis*=9.8 Hz, C*H*=CH$_2$), 7.82 (s, br N*H*),

8.12 (s, br NH); δ_C (75.5 MHz, DMSO-d_6) 38.5, 38.7, 63.6, 70.5, 71.8, 72.5, 73.8, 125.5, 132.0, 165.2 (C=O), 173.2 (C=O). Anal. Calcd for $C_{11}H_{20}N_2O_7$: C, 45.20; H, 6.90; N, 9.58. Found: C, 45.31; H, 6.91; N, 9.55.

4.4. *N*-(4-Vinylbenzyl)-2,3,4,5,6-penta-*O*-acetyl-D-gluconamide (4)

A mixture of **1** (3.00 g, 9.64 mmol), dry pyridine (7.5 mL) and acetic anhydride (5.19 g, 49.87 mmol) was magnetically stirred at rt for 25 h up to the disappearance of **1** as revealed by TLC (petroleum ether/EtOAc=50:50 as eluent), hydrolyzed with iced water (pH=6) and extracted with CH_2Cl_2 (30×3 mL). The organic extracts were dried over $MgSO_4$ and concentrated at reduced pressure to afford crude **4**, which was crystallized from absolute ethanol (4.55 g, yield 91%). Mp 132–133 °C, purity 98% by HPLC. IR (KBr, ν, cm^{-1}) 1761, 1752 and 1740 (ester), 1655 and 1567 (amide), 1239 and 1220 (C–O), 996 and 913 (vinyl). δ_H (300 MHz, $CDCl_3$) 1.94 (s, 3H, CH_3C=O), 2.05 (s, 3H, CH_3C=O), 2.08 (s, 3H, CH_3C=O), 2.09 (s, 3H, CH_3C=O), 2.19 (s, 3H, CH_3C=O), 4.13 (dd, 1H, *J*=5.4 Hz, *J*=12.2 Hz), 4.34 (m, 2H), 4.50 (dd, 1H, *J*=6.2 Hz, *J*=14.7 Hz), 5.05 (m, 1H), 5.25 (dd, 1H, *Jcis*=10.9 Hz, *Jgem*=0.8 Hz, CH_2=CH), 5.36 (d, 1H, *J*=5.1 Hz), 5.46 (m, 1H), 5.70 (m, 1H), 5.73 (dd, 1H, *Jtrans*=17.7 Hz, *Jgem*=0.8 Hz, CH_2=CH), 6.36 (t, br, NH, *J*=5.6 Hz), 6.69 (dd, 1H, *Jtrans*=17.7 Hz, *Jcis*=10.9 Hz, CH=CH_2), 7.29 (m, 4H); δ_C (75.5 MHz, $CDCl_3$) 20.3, 20.7, 20.8, 43.2 (CH_2N), 61.5 (C-6), 68.9 (C-5), 69.1 (C-3), 69.5 (C-4), 71.7 (C-2), 114.2, 126.6, 128.1, 136.3, 137.1, 137.2, 166.1, 169.2, 169.7, 169.9, 170.6. Anal. Calcd for $C_{25}H_{31}NO_{11}$: C, 57.58; H, 5.99; N, 2.69. Found: C, 57.68; H, 6.01; N, 2.68.

4.5. Oligomerizations of monomer 4

The peracetylated monomer **4**, AETHCl (20% by weight of the monomer) and AIBN (4% by weight of the monomer) were dissolved under N_2 in dry DMF in a vial with side arm, submitted to two freeze–pump–thaw cycles at −78 °C, siphoned into the polymerization flask and magnetically stirred at 60 °C for 72 h. The mixture was cooled, concentrated at reduced pressure and poured into a large excess of Et_2O (400 mL/g of oligomer). The oligomer was filtered and further purified through two dissolution/precipitation cycles in a mixture of THF (solvent)/Et_2O (non-solvent) up to the disappearance in the ether washing of the residual monomer **4** as checked by TLC using petroleum ether/acetone 1:1 as eluent (*Rf* **4**=0.49, *Rf* **Olig4**=0). The presence of NH_2 groups in the oligomers was confirmed by the pink colour that developed when **Olig4** was eluted on a TLC sheet with a 0.2% ninhydrin solution in 95% EtOH.

The loading of NH_2 groups in the oligomers was determined by dissolving the oligomer (60–90 mg) in THF (1–2 mL), treating the solution with a known excess of 0.1 N NaOH and titrating the unreacted NaOH with 0.1 N HCl.

The FTIR spectra of **Olig4** are characterized by strong absorption bands at 1752 cm^{-1} (ester), 1677 cm^{-1} (amide) and 1220, 1048 cm^{-1} (C–O).

4.6. Macromonomer Mac1

A solution of **Olig4** (0.65 mmol, as determined by titration of NH_2 group) in dry $CHCl_3$ (25 mL) and Et_3N (1.3 mmol) was cooled to 0 °C, treated under N_2 with 4-vinylbenzoyl chloride[33] (0.65 mmol), allowed to reach rt and magnetically stirred up to the disappearance of the purple colour developed on TLC after elution with 0.2% solution of ninhydrin in 95% EtOH. The mixture was then hydrolyzed with water (10 mL), extracted with $CHCl_3$ (3×20 mL) and dried over anhydrous $MgSO_4$. After removal of the solvent at reduced pressure, the solid **Mac4** was purified by repeated dissolution in $CHCl_3$ and precipitation in Et_2O and brought to constant weight at reduced pressure (yield 98%).

A solution of **Mac4** (1.07 g, 0.60 mmol calculated from a nominal molecular weight of 1772) in dry dioxane (39 mL) was treated with 1.135 M MeONa in MeOH at rt for 2 h up to the disappearance of the acetyl macromonomer as shown both by TLC (petroleum ether/acetone=1:1 as eluent) and by the lack in the FTIR spectrum of the ester band at 1755 cm^{-1}. The milky suspension was then treated with MeOH in large excess to favour coagulation and filtered. The solid hydroxyl macromonomer **Mac1** was dried and brought to constant weight at reduced pressure (0.6777 g, yield 99%).

4.7. Precipitation copolymerizations of Mac1, 1, 2 and 3 with DVB

Saccharidic monomer, AIBN (2–5% by weight of the monomer), and PVP (5% by weight of the monomer) when required, were dissolved under N_2 with the appropriate solvent (H_2O/EtOH=1:1.8 for **Mac1**, H_2O/EtOH=1:1.7 for **2** and MeOH for **1** and **3**) in a vial with side arm and siphoned in the polymerization flask equipped with silicone septum and a Teflon stirring bar. After 30 min of N_2 purging at rt, the solution was added with DVB (3–32% by weight of the monomer) through a microsyringe, and then it was magnetically stirred at 70 °C for 24–96 h during which a separation of phases was observed. The milky suspension was then centrifuged at 3500 rpm for 30 min and the solid was submitted to cycles of washing/sonication/centrifugation (15 min each) first with THF and then with Et_2O. The copolymer was then brought to constant weight at reduced pressure (yields 6–59%). Table 1 collects some typical data.

4.8. Synthesis of *N*-benzyl-D-gluconamide (6)

A mixture of D-glucono-1,5-lactone (1.51 g, 8.5 mmol) and MeOH (50 mL) was treated at reflux with a solution of benzylamine (1.00 g, 9.3 mmol) in MeOH (10 mL). The clear solution was refluxed for 3 h and cooled to rt to afford **6** as a pearly solid that was refluxed in benzene to eliminate traces of water, filtered and crystallized from MeOH (2.09 g, yield 86%). Mp 177–178 °C, purity 99% by HPLC. IR (KBr, ν, cm^{-1}) 3499 and 3223 (OH), 1649 and 1535 (amide), 1099, 1080 and 1038 (C–O). δ_H (300 MHz, DMSO-d_6) 3.31–3.43 (m, 1H), 3.50 (s, br 2H), 3.55–3.64 (m, 1H), 3.97 (m, 1H), 4.08 (dd, 1H, *J*=4.0 Hz, *J*=5.5 Hz), 4.33 (m, 3H), 4.46 (d, 1H, *J*=7.2 Hz), 4.51 (d, 1H, *J*=4.7 Hz), 4.56 (d, 1H, *J*=4.3 Hz), 5.45 (d, 1H, *J*=4.7 Hz), 7.18–7.34 (m, 5H), 8.16 (t, NH, *J*=6.2 Hz);

δ_C (75.5 MHz, DMSO-d_6) 41.7 (CH_2N), 63.3 (C-6), 70.1 (C-3), 71.4 (C-4 or C-5), 72.4 (C-4 or C-5), 73.7 (C-2), 126.5, 127.0, 128.0, 139.4, 172.5 (C=O). Anal. Calcd for $C_{13}H_{19}NO_6$: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.52; H, 6.73; N, 4.93.

4.9. Alkylation of 6 with 7 or 11

4.9.1. General procedure. NaH (60% suspension in white oil, 0.32 mmol, 0.9 equiv) was washed under N_2 with dry pentane (2×2 mL), dried, suspended in dry DMF (2 mL), treated with a solution of **6** (0.35 mmol) in dry DMF (2 mL) and magnetically stirred at rt until to get clear solution (30 min). The clear solution was treated with **7** or **11** (1 or 3 equiv) under stirring until no further modifications of the composition of the mixture was evidenced by TLC ($CHCl_3$/MeOH=4:1 as eluent). The mixture was then treated with MeOH (2 mL) and evaporated at 60 °C/300 mmHg to give a syrup that was added with acetone to precipitate unreacted **6**, which was filtered and washed with THF. Washings and filtrate were combined, concentrated and chromatographed by PLC using the same eluent as above. Reaction conditions and product yields are collected in Table 2.

Compound **15**. Purity 97% by HPLC. IR (in CH_2Cl_2 0.1 mm, ν, cm^{-1}) 3413 (OH), 2686 and 1692 (aldehyde), 1660 (amide). δ_H (300 MHz, DMSO-d_6) 3.38 (m, 2H+HOD), 3.57 (m, 2H), 3.94 (m, 2H), 4.08 (m, 2H), 4.33 (m, 4H), 4.55 (m, 2H), 4.67 (m, 2H), 5.98 (m, 2H), 7.30–7.13 (m, 7H), 7.88 (m, 2H), 8.40 (t, NH, J=6.0 Hz), 9.88 (s, 1H, CH=O); δ_C (75.5 MHz, DMSO-d_6) 41.8 (CH_2N), 63.5 (C-6), 67.8 (CH_2O chain), 69.9 (CH_2O chain), 70.3 (C-3), 71.0 (C-4 or C-5), 71.3 (C-4 or C-5), 81.9 (C-2), 115.0, 126.5, 126.8, 127.0, 128.1, 129.6, 130.4, 131.7, 139.4, 163.1, 170.7 (C=O), 191.2 (CH=O).

Compound **16**. Purity 98% by HPLC. IR (in CH_2Cl_2 0.1 mm, ν, cm^{-1}) 3413 (OH), 2686 and 1687 (aldehyde), 1658 (shoulder, amide). δ_H (300 MHz, DMSO-d_6) 1.77 (m, 4H), 3.40–3.53 (m, 6H+HOD), 3.89 (m, 2H), 4.09 (m, 2H), 4.33 (m, 2H), 4.56 (m, 2H), 7.11 (m, 2H), 7.28 (m, 5H), 7.86 (m, 2H), 8.37 (t, NH, J=6.0 Hz), 9.87 (s, 1H, CH=O); δ_C (75.5 MHz, DMSO-d_6) 25.7 (CH_2 chain), 26.2 (CH_2 chain), 42.3 (CH_2N), 64.0 (C-6), 68.3 (CH_2O chain), 70.4 (CH_2O chain), 70.7 (C-3), 71.5 (C-4 or C-5), 71.8 (C-4 or C-5), 82.9 (C-2), 115.3, 127.0, 127.4, 128.5, 129.9, 132.2, 139.9, 164.1, 171.4 (C=O), 191.7 (CH=O).

4.10. Functionalizations of copolymers C1-1 with 11

NaH (60% suspension in white oil) was washed under N_2 with dry pentane (1 mL), dried, suspended in dry DMF (2 mL), added with solid **C1-1** (30 mg, loading of the saccharidic unit 3.10 mmol/g) and magnetically stirred at rt for 30 min. The suspension was treated with **11** (molar ratio of **C1-1** saccharidic units/NaH/**10**=1:0.9:1), stirred at rt for 2–72 h and then treated with MeOH (2 mL) and THF (2 mL). The solid coploymer was separated from the liquid phase by centrifugation at 3500 rpm (15 min) and submitted to washing/centrifugation cycles in THF followed by Et_2O up to the removal of aldehydic compounds as evidenced by a negative Schiff's test in the last washings. The isolated copolymer, dried at reduced pressure up to constant weight, submitted to Schiff's test and centrifuged at 3500 rpm (15 min) showed fuchsia colour scaled from pale to deep with reaction time.

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Supplementary data

Supplementary data concerning the preparation of **Olig4**, synthesis and characterization of **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14**, ^1H and ^{13}C NMR spectra of **6**, **15** and **16** can be found in the online version. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.08.106.

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