pK_a Modulation of Pyrrolidine-Based Catalytic Polymers Used for the preparation of Glycosyl Hydrazides at Physiological pH and Temperature

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ABSTRACT. Inspired by the ability of enzymes to use the surrounding hydrophobic and/or polarizable groups to modulate the pK_a of a given amino acid, we designed a series of soluble polymers able to decrease the basicity of pyrrolidine (from 11,2 to 8,6 pK_a units) which clearly increases its aminocatalytic activity at physiological pH in C=N bond formation reactions *via* ion iminium activation. Other parameters like charge density, hydrophobic/hydrophilic balance and aggregation state have been studied as important factors in the catalytic activity of the polymers for a given substrate. To demonstrate the utility of our approach, the optimal pyrrolidine-based catalytic polymer has been used for the formation of C-N bonds between hydrazides and free sugars as model system for the preparation of glycoconjugates.

Keywords: Enzyme-Like Organocatalysis, Iminium activation, pyrrolidine, pKa modulation, polymers, hydrazones

INTRODUCTION

The design of artificial catalysts imitating the astonishing behavior of naturally occurring enzymes is an interesting but challenging research topic. In this research area, the analysis of the conformations in the transition state and its stabilization are probably the most studied aspects.[1] The factors that affect the catalytic efficiency of an enzyme or an artificial one are difficult to quantify in a separate manner, as it is the result of a delicate combination of effects. An important feature of enzymes is the skillful modulation of the pK_a of a polarizable group of an amino acid by surrounding it with other hydrophobic and/or polarizable groups.[2,3] This variation of the pK_a allows for carrying out the catalytic reactions in a neutral pH range close to physiological conditions.[4,567]

Synthetic polymers may mimic to some extent the tertiary structure of enzymes since polymer chemistry offers tools to tailor the macromolecular architecture and the surrounding environment of a given organocatalyst. A straightforward way to modulate this issue is the use of a bottom-up approach in which designed monomers are prepared and polymerized, as it has been shown by us[8,9,10] and others.[11,12,13,14,15] This strategy provides high flexibility towards the synthesis of copolymers with specific features, by choosing the right co-monomers in the correct ratio. A proper design of a synthetic polymer chain may also control pK_a of polarizable groups and this strategy has been used to modulate the pK_a of carboxylic acids[16] and pyridines.[17]

We have recently demonstrated that pyrrolidine is a very efficient catalyst in the preparation of C=N bonds.[18,19,20,21] This metal and acid-free process takes place through iminium ion activation under very mild reaction conditions, allowing for the formation of a wide variety of imines,[18a]

nitrones[18b] as well as oximes or hydrazones[18c] in remarkable yields using an extremely simple procedure (Figure 1a). The presence of the secondary amine leads to very reactive iminium ion intermediates, what dramatically rises the rate of these reactions by increasing the electrophilicity of the carbonyl group and facilitating the removal of pyrrolidine in the intermediate hemiaminal without the need of using acidic media. Nevertheless, the extension of this methodology to biological applications has a difficult drawback as pyrrolidine (pK_a= 11,2) at physiological pH is nearly completely protonated and consequently inactive (Figure 1a).

Hydrazones are usually much more stable than other C=N functionalized compounds such as imines[22] and therefore it is a functional group frequently used in a variety of applications,[23,24,25,26,27,28,29,30,31,32,33,34,35,36] even in physiological media and in particular in bioconjugation linkages.[37,38,39,40] The formation of hydrazones can be readily accelerated under general acid catalysis as water elimination is the rate-determining step. Nevertheless, the reaction can be sluggish at low concentrations and physiological conditions. Based on previous reports by Jenks,[41,42] Dawson *et al.* generalized the use of an excess of aniline in acidic media to accelerate this type of linkages in biological systems.[43,44] The use of aniline avoids the water elimination step via imine formation, activation *via* protonation and transimination mechanism. Generally, a very high concentration of the cytotoxic aniline is needed to achieve the desired conversion. Therefore, strategies to reduce the use of the excess of toxic aniline have emerged.[45,46,47,48,49,50,51,52,53,54,55] The development of new approaches that allow for the formation of conjugates at physiological pH and temperature would be highly desirable.

Our working hypothesis has been that the backbone chain and the pending groups in the polymer could shift the pK_a to less basic values (Figure 1b). This could be achieved either by the proximity of other charged pyrrolidine moieties or by creating a hydrophobic microenvironment that makes

the secondary amine more resistant to protonation. Consequently, based on our previous experience,[8] we have designed a variety of polymer-pyrrolidine conjugates from a *bottom-up* approach, using three methacrylate monomers (**A-C**) bearing pyrrolidine linked to the main chain through three spacers (with different length and hydrophobic/hydrophilic balance). With each of these monomers, we prepared statistical copolymers with different load of hydrophobic styrene (S) as comonomer, in order to obtain a platform of soluble polymers where parameters like charge density, hydrophobic/hydrophilic balance or spacer nature have been varied. We have studied the catalytic performance, at physiological pH, of the polymers in a model reaction for hydrazone formation, analyzing the effect of pK_a, charge density, hydrophobic/hydrophilic balance and aggregation state of each polymer. Finally, we have paid special attention to the application of these polymers to form C-N bond between hydrazides and free sugars, as carbohydrates play an important role in numerous biological processes and therefore the construction of glycoconjugates from unprotected carbohydrates is an important issue.[56,57,58]

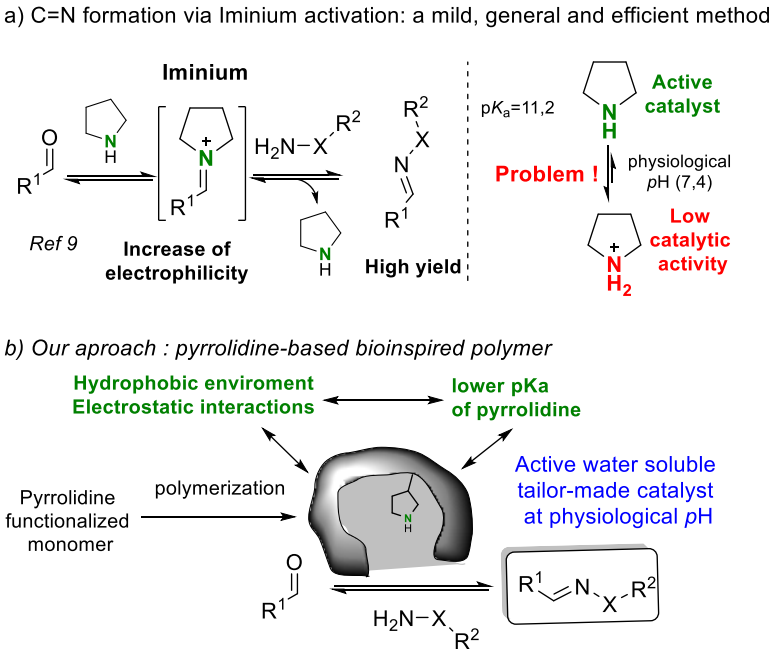


Figure 1. (a) Description of the addressed catalytic reaction (b) Present work hypothesis.

EXPERIMENTAL SECTION

General Remarks

(*R*)-1-Boc-3-hydroxypyrrolidine and (±)-1-Boc-3-hydroxymethylpyrrolidine were supplied by Fluorochem. Methacryloyl chloride (Aldrich) was freshly distilled. 2,2'-Azobis-isobutyronitrile was supplied by Glentham Life Sciences. Other chemicals were purchased puriss p.A. from commercial suppliers or purified by standard techniques. Thin-layer chromatography (TLC) was performed on aluminium sheets 60 F_{254} Merck silica gel and compounds were visualized by irradiation with UV light and/or by treatment with a solution of Ce_2MoO_4 in water, a solution of ninhydrin in *n*-BuOH/EtOH or H_2SO_4 (5%) in EtOH followed by heating. Flash chromatography was performed on silica gel (Merck 60: 0.040-0.063 mm). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a 300 MHz (Inova 300 or Bruker 300) and 400 MHz (Inova 400 or Mercury 400) spectrometers, using $CDCl_3$, DMSO-d_6 or D_2O as solvents at room temperature. Chemical shift (δ) are reported in parts per million relatives to tetramethylsilane (TMS) in ^{1}H and $CDCl_3$ (δ =77.0) in ^{13}C NMR. Coupling constants (*J* values) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbol: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). The HR MS analysis was carried out by using an Agilent 1200 Series LC system (equipped with a binary pump, an autosampler, and a column oven) coupled to a 6520 quadrupole-time of flight (QTOF) mass spectrometer. Acetonitrile:water (75:25, v:v) was used as mobile phase at 0.2 mL min-1. The ionization source was an ESI interface working in the positive-ion mode.

Gel permeation chromatography (GPC) analyses were carried out using a Perkin-Elmer apparatus with an isocratic pump serial 200 connected to a differential refractometric detector (serial 200a). Two Resipore columns (Varian) were conditioned at 70 ºC and used to elute the samples (1 mg/mL concentration) at 1 mL/min. HPLC-Grade *N,N'*-dimethyl formamide (DMF)

supplemented with 0.1% v/v LiBr was used as eluent. Calibration of SEC was carried out with monodisperse standard polystyrene samples in the range of 2.9×10^3 to 480×10^3 obtained from Polymer Laboratories.

The turbidity changes of the aqueous solutions of the polymers (2 mg/mL) as a function of pH was monitored measuring the absorbance at 500 nm in a UV-VIS Lambda 35 spectrophotometer (Perkin Elmer Instruments). The initial polymer solution was freshly prepared in an aqueous solution of 0.1 M of NaOH. A standard aqueous solution 1 M of HCl was delivered stepwise. pH was monitored with a Beckman 40 pH-Meter (Beckman Instruments, Fullerton, CA, USA).

The determination of pK_a by acid base titration was made using *Tiamo titration and more software.* To a solution of 0.06 M of each compound, 4.43 mmol of HCl were added, then, an acid base titration was carried out by adding NaOH 0.1M dropwise.

The polymer aggregation was investigated by dynamic light scattering (DLS) using a Malvern NanoZS. The polymer solutions measured were identical to those employed for the catalytic experiments that are summarized in each table throughout the manuscript. All the measurements were carried out at room temperature. The correlation curves and the hydrodynamic radius reported are the average result of 5 measurements comprising 10 runs of 10 seconds.

The phosphate buffered saline (PBS) was prepared under the conditions indicated by the manufacturer, one tablet (Aldrich) was dissolved in 200 mL of deionized water at 25 ºC.

Using also the Malvern NanoZS zeta potential of B1, B2 and B3 was evaluated through the measurement of the electrophoretic mobility of the aggregates. The electrophoretic mobility of the aggregates was converted into zeta potential using an extension of the Henry Equation applying the approximation of Smoluchowski. The measure of the zeta potential is expressed in mV. The polymers were dissolved in PBS at 5mM concentrations and the measurements were carried out at

room temperature. The results provided are the average values obtained for 10 measurements of 24 runs of 5 seconds.

Synthesis of the monomers

***tert*-butyl 3-[(methacryloyloxy)methyl]pyrrolidine-1-carboxylate, A-Boc.** To a solution of 1-Boc-3-hydroxymethylpyrrolidine (6.0 g, 30 mmol) and 4.2 mL NEt_3 in methylene chloride (60 mL), freshly distilled methacryloyl chloride (2.9 mL, 30 mmol) was added under stirring at 0-5 ºC. After this time, the mixture was heated at 35 ºC for two hours. Then, it was extracted twice with water, the solvent dried using $MgSO_4$ and evaporated under reduced pressure and the residue was purified by column chromatography (hexane-ethyl acetate 10:1) giving a colorless oil), (6.6 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.03 (s, 1H, HCH=C), 5.50 (s, 1H, HCH=C), 4.12-3.98 (m, 2H, O-CH_2), 3.49-3.03 (m, 4H, CH_2-N-CH_2), 2.49 (m, 1H, CH_2-CH), 1.96-1.60 (m, 2H, CH_2-CH_2-N), 1.87 (s, 3H, CH_2=C-CH_3), 1.39 (s, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2 (COO), 154.4 (NCOO), 136.1 (C=CH_2), 125.7 (C=CH_2), 79.2 (C(CH_3)$_3$), 65.7 (O-CH_2), 48.5 (CH-CH_2-N), 45.1 (CH_2-CH_2-N), 37.6 (CH_2-CH), 28.5 (C(CH_3)$_3$), 28.1 (CH_2-CH_2-N), 18.2 (CH_2=CH-CH_3). HRMS (ESI) *m/z* calcd. for $C_{14}H_{23}NO_4$ [M^++Na]: 292.15193; found: 292.152.

***tert*-Butyl (*R*)-3-(methacryloyloxy)pyrrolidine-1-carboxylate, B-Boc**. To a solution of (*R*)-1-Boc-3-hydroxypyrrolidine (8.0 g, 42 mmol) and 6.5 ml NEt_3 in methylene chloride (120 mL), methacryloyl chloride (5.8 g, 55 mmol) was added under stirring at 0-5 ºC. After this time, the mixture was heated at 35 ºC for two hours. Then, it was extracted twice with water, the solvent dried using $MgSO_4$ and evaporated under reduced pressure and the residue was purified by column chromatography (hexane/ethyl acetate 20:1) giving colorless oil), (5.0 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.03 (s, 1H, HCH=C), 5.51 (s, 1H, HCH=C), 5.26 (m, 1H, O-CH), 3.55-3.37 (m,

4H, $\underline{CH_2}$-N-$\underline{CH_2}$) , 2.01 (m, 2H, $\underline{CH_2}$-CH_2-N), 1.86 (s, 3H, CH_2=C-$\underline{CH_3}$), 1.39 (s, 9H, $\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8 ($\underline{C}$OO), 154.4 (N$\underline{C}$OO), 136.1 ($\underline{C}$=CH_2), 125.9 (C=$\underline{CH_2}$), 79.5 ($\underline{C}(CH_3)_3$), 73.6 (O-$\underline{CH}$), 51.8 (CH-$\underline{CH_2}$-N), 43.7 (CH_2-$\underline{CH_2}$-N), 31.2 ($\underline{CH_2}$-CH_2-N), 28.4 (C($\underline{CH_3}$)$_3$), 18.1 (CH_2=CH-$\underline{CH_3}$). HRMS (ESI) *m/z* calcd. for $C_{13}H_{21}NO_4$ [M^++Na]: 278.13628; found: 278.13626.

***tert*-butyl (*R*)-3-[((3-methacrylamidopropyl)carbamoyl)oxy]pyrrolidine-1-carboxylate, C-Boc.** To a solution of triphosgene (0.53 g, 1.8 mmol) in methylene chloride (50 mL) (*R*)-1-Boc-3-hydroxymethylpyrrolidine (1.0 g, 5.3 mmol) and NEt_3 (0.75 mL), were added. Then, a solution of 3-aminopropyl methacrylamide hydrochloride (955 mg, 5.3 mmol) and 1.5 mL NEt_3 in 50 ml methylene chloride was added and the mixture was heated at 35 ºC for two hours. After extraction with water and drying with $MgSO_4$ the solvent of the organic phase was evaporated under reduced pressure and the residue was purified by column chromatography (methylene chloride/methanol 14:1) giving a colorless oil), (1.2 g, 68 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (s, 1H, CO$\underline{NH}$), 5.66 (s, 1H, H$\underline{CH}$=C), 5.60 (s, 1H, $\underline{NH}$COO), 5.27 (s, 1H, $\underline{HC}$H=C), 5.13 (m, 1H, O-$\underline{CH}$), 3.46-3.14 (m, 8H, $\underline{CH_2}$-N-$\underline{CH_2}$, $\underline{CH_2}$-CH_2-$\underline{CH_2}$) , 1.97-1.60 (m, 4H, CH-$\underline{CH_2}$-CH_2, CH_2-$\underline{CH_2}$-CH_2), 1.90 (s, 3H, CH_2=C-$\underline{CH_3}$), 1.39 (s, 9H, $\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9 ($\underline{C}$ONH), 156.6 (NH$\underline{C}$OO), 154.5 (N$\underline{C}$OO), 139.8 ($\underline{C}$=CH_2), 119.7 (C=$\underline{CH_2}$), 79.2 ($\underline{C}(CH_3)_3$), 73.7 (O-$\underline{CH}$), 54.1 (CH-$\underline{CH_2}$-N), 43.7 (CH-CH_2-$\underline{CH_2}$-N), 37.4 (CONH-$\underline{CH_2}$-), 36.0 ($\underline{CH_2}$-NHCOO) 31.3 (CH-$\underline{CH_2}$-CH_2-N), 29.9 (CH_2-$\underline{C}H_2$-CH_2), 28.5 (C($\underline{CH_3}$)$_3$), 18.6 (CH_2=CH-$\underline{CH_3}$). HRMS (ESI) *m/z* calcd. for $C_{17}H_{29}N_3O_5$ [M^++Na]: 378.19994; found: 378.19965.

General polymerization procedure. Protected copolymers were prepared by free radical polymerization of **A-Boc**, **B-Boc** and **C-Boc** and styrene (in the case of the copolymers) in *N*,*N*-dimethyl formamide (DMF) at 60 ºC for 24 hours using AIBN as initiator. The total concentration

of comonomers was 1 mol/L and the initiator concentration was 0.015 mol/L. Reactions were carried out in the absence of oxygen by gently bubbling nitrogen for 20-30 min before sealing the system. After 24 h, the reaction mixture was poured into water, and the resulting precipitate was dried under vacuum overnight.

General deprotection procedure. The protected polymers were dissolved in 2:1 dichloromethane/trifluoroacetic acid (2 mL per 100 mg of polymer) and the mixture was stirred for 24 h. After this time, the reaction mixture was concentrated and a mixture of DMF and water was added to solubilise them. The solutions were dialyzed in distilled water for 3 days. Polymers were recovered by freezing and lyophilisation.

Copolymer composition. Compositions of the copolymers were calculated from the spectra of the deprotected polymer (see SI) by using the area of the aromatic signals at 8-6 ppm (A_{ar}, 5 protons of S) and the area of the rest of the signals (A_{rest}) by using this equation:

$$fS = \frac{{A_{Ar}}/{5}}{{A_{Ar}}/{5} + \left(A_{rest} - {3A_{Ar}}/{5}\right)/{y}} \qquad [1]$$

where *y* is the number of protons of the proline-containing units, which is 14, 12 and 18 for the **A**, **B** and **C**, respectively.

HLB (Griffin). The hydrophilic-lipophilic balance of the compounds was determined by calculating values for the different regions of the molecule, as described by Griffin[59]:

$$HLB = 20\frac{M_h}{M}$$

where M_h is the molecular mass of the hydrophilic portion of the molecule, and M is the molecular mass of the whole molecule. The amine was considered to be protonated (>NH.H_2O).

Synthesis of compounds 2a, 2b and 3b.

***tert*-butyl 3-((benzoyloxy)methyl)pyrrolidine-1-carboxylate.** Benzoyl chloride (385µL, 3.32 mmol) was added dropwise at room temperature to a solution of 1-Boc-3-hydroxymethylpyrrolidine (317 mg, 1.5 mmol) and 4-(dimethylamino)pyridine (19.0 mg, 0.15 mmol) in dry pyridine (5 mL). The mixture was stirred for four hours and dissolved in CH_2Cl_2, then, it was washed with water and brine. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (cyclohexane/ethyl acetate, 4:1) (250 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.7 Hz, 2H, ArH), 7.60 (t, *J* = 7.6 Hz 1H, ArH), 7.45 (t, *J* = 7.5 Hz 2H, ArH), 4.38-4.22 (m, 2H, CH_2OC=O), 3.64-3.08 (m, 4H, $(CH_2)_2$-N), 2.67 (m, 1H, $CHCH_2O$), 2.13-1.73 (m, 2H, CH_2CH_2N) 1.47 (s, 9H, 3 x CH_3). ^{13}C NMR (75 MHZ, $CDCl_3$): δ 166.4 (C), 154.5(C), 133.08 (CH), 130.0 (CH), 129.6 (2 x CH), 128.4 (2 x CH), 79.3 (C), 67.9 (CH_2), 66.0 (CH_2), 48.6 (CH_2), 45.1 (CH), 28.5 (3 x CH_3), 27.7(CH_2). MS (ESI+): m/z 305 [M^++H] (0.10), m/z 105 [M-$C_5H_{10}NO$] (100). HRMS (ESI+): calcd. for $C_{17}H_{23}NO_4$ [M^++H]: 205.1195; found: 205.1094.

Deprotection of *tert*-butyl 3-((benzoyloxy)methyl)pyrrolidine-1-carboxylate, preparation of 2a. Trifluoroacetic acid (1.5 mL) was added dropwise at room temperature to a solution of *tert*-butyl 3-((benzoyloxy)methyl)pyrrolidine-1-carboxylate (250 mg, 0.81 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for three hours and neutralized with $NaHCO_3$, then, it was extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated under reduced pressure (200 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) : δ=8.06 (d, *J* = 7.8, 2H, Ar-H), 7.63 (t, *J* = 7.6, 1H, Ar-H), 7.50 (t, *J* = 7.4, 2H, Ar-H), 4.47-4.33 (m, 2H, CH_2-OCO), 3.54-3.09 (m, 4H, CH_2NHCH_2), 2.94-2.78 (m, 1H, $CHCH_2O$), 2.31-2.18 (m, 1H, CH_2CH_2NH), 1.95-1.81 (m, 1H, CH_2CH_2NH). ^{13}C NMR (75MHz, $CDCl_3$): δ 165.2 (C), 132.4 (CH), 130.0 (C), 128.6 (2 x CH), 127.5 (2 x CH), 66.7 (CH_2), 46.5 (CH_2), 44.1

(CH_2), 36.5 (CH), 26.6 (CH_2). MS (ESI+): m/z 205 [M^++H] (0.10), m/z 105 [M-$C_5H_{10}NO$] (100). HRMS (ESI+): calcd. for $C_{12}H_{15}NO_2$ [M^++H]: 205.1195; found: 205.1094.

***tert*-Butyl (*R*)-3-(benzoyloxy)pyrrolidine-1-carboxylate.** Benzoyl chloride (385µL, 3.32 mmol) was added dropwise at room temperature to a solution of (*R*)-1-*N*-Boc-3-hydroxy-pyrrolidine (290 mg, 1.5 mmol) and 4-(dimethylamino)pyridine (18.9 mg, 0.15 mmol) in dry pyridine (5 mL). The mixture was stirred for four hours and dissolved in CH_2Cl_2, then, it was washed with water and brine. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (cyclohexane/ethyl acetate, 4:1) (280 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, *J* = 7.7 2H, ArH), 7.56 (t, *J* = 7.4, 1H ArH), 7.43 (t, *J* = 7,6, 2H, ArH), 5.52 (m, 1H, CH), 3.65 – 3.49 (m, 4H, CH_2), 2.16 (m, 2H, CH_2), 1.46 (s, 9H, 3 x CH_3). The data agree with those described in the literature.[60]

Deprotection of *tert*-Butyl (*R*)-3-(benzoyloxy)pyrrolidine-1-carboxylate, preparation of 2b. Trifluoroacetic acid (1.5 mL) was added dropwise at room temperature to a solution of *tert*-butyl(*R*)-3-(benzoyloxy)pyrrolidine-1-carboxylate (280 mg, 0.96 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for three hours and neutralized with $NaHCO_3$, then, it was extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated under reduced pressure (200 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.2, 2H, ArH), 7.64 (t, *J* = 7.4, 1H, ArH), 7.49 (t, *J* = 7.6, 2H, ArH), 5.76 (m, 1H, CH), 3.62-6.54 (m, 4H, CH_2), 2.46 (m, 2H, CH_2). The data agree with those described in the literature.[61]

Preparation of 3b. This compound was prepared according to the method described in the literature.[62]

Synthesis of hydrazines.

**3,3`-dithiodipropanoylhydrazide.** In a round bottom flask, 3,3`dithiodipropanoic acid (5,0 g), methanol (50 mL) and sulfuric acid (2 drops) were added. The mixture was left under reflux for 2.5 hours under inert atmosphere. The mixture was cooled to room temperature and methanol was removed at reduced pressure. The product was extracted with ethyl acetate (20 mL) and water (10 mL). The organic layers were collected and dried with $MgSO_4$ and evaporated under reduced pressure. The oily product, dimethyl 3,3'-dithiopropionate, was used without purification for the next step. The product was added to 17 mL of ethanol and a solution of hydrazine monohydrate (11 mL) in ethanol (12.1 mL). The mixture was stirred for 2 hours at 50 ºC. Then, drops of hexane were added to precipitate the product (60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.05 (s, 2H, $NHNH_2$), δ = 4.20 (s, 4H, NH_2NH), δ = 2.88 (t, J = 7.2 Hz, 4H, CH_2CH_2S). δ = 2.40 (t, J = 7.2 Hz, 4H, CH_2SS). The data agree with those described in the literature.[63] ^{1}H NMR (300 MHz, D_2O): δ = 3.01-2.97 (t, J =6.8 Hz, 4H, CH_2CH_2S);2.69-2.64 (t, J = 6.8 Hz, 4H, CH_2CH_2S).

3-methylthiopropanoylhydrazide. To a solution of hydrazine monohydrate (11 mL) in EtOH (10 mL), a solution of methyl 3-(methylthio)propanoate (5 mL, 35 mmol) in anhydrous EtOH (15 mL) was added slowly. The mixture was stirred for three hours at 50 ºC. The product was concentrated under reduced pressure (98%). ^{1}H NMR (300 MHz, D_2O): δ = 2.80-2.76 (t, J = 6.9 Hz, 2H, CH_2CH_2S,), 2.55-2.50 (t, J = 6.8 Hz, 2H, CH_2CH_2S, 2H), 2.11 (s, 3H, CH_3S). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4 (C), 33.4 (CH_2), 29.1 (CH_2), 14.21 (CH_3). MS (ESI+): m/z 134 [M^++H] (76), m/z 60 [M-CH_3N_2O] (100). HRMS (ESI+): calcd. for $C_4H_{10}N_2OS$ [M^++H]: 134.0500; found: 134.0514.

Study of the catalytic activity in C=N bond formation. Reactions with *p*-chlorobenzaldehyde.

(*E*)-*N'*-(4-Chlorobenzylidene)benzohydrazide. In a round bottom flask, *p*-chlorobenzaldehyde (28.1 mg; 0.2 mmol) was dissolved in the buffer (40 mL), then, the benzohydrazide (27.5 mg; 0.2 mmol) and 10 mol% of the catalyst (polymeric, pyrrolidine or aniline) were added. The mixture was stirred for two hours at room temperature. It was extracted with diethyl ether (3 x 10 ml), the organic phases were dried with $MgSO_4$ and evaporated under reduced pressure. The resultant solid was dissolved in DMSO-d_6 and the rate of conversation was determined by NMR. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.96 (s, 1H, NH), 8.46 (s, 1H, CH=N), 7.92 (d, J = 7.4 Hz, 2H), 7.76 (d, J = 8.1 Hz, 2H), 7.60 –7.54 (m, 5H). The data agree with those described in the literature.[18c]

Study of the catalytic activity in C=N bond formation. Reaction with sugars, synthesis of glycosyl hydrazides.

1-(*β*-D-glucopyranosyl)-2-(benzoyl)-hydrazine. Into a 0.2 mL PCR tube, 20 μL of a 1M solution of glucose, 2 equivalents of benzohydrazide and 0.1 equivalents of catalyst (polymeric, pyrrolidine or aniline; see Figure 3) were added and, finally 100 mM phosphate buffer solution was added up to a final volume of 200 μL. The reaction was heated at 50 ºC for 2 or 20 h. The reaction was frozen and lyophilized. The resultant solid was dissolved in 0.7 mL of D_2O and the rate of conversion was determined by ^{1}H NMR (300 or 400 MHz). ^{1}H NMR (400 MHz, D_2O) δ 7.78 (d, J = 7.3 Hz, 2H), 7.66 (t, J = 6.9 Hz, 1H), 7.56 (t, J = 7.4 Hz, 2H), 4.25 (d, J = 8.9 Hz, 1H, H-1 β), 3.93 (d, J = 12.1 Hz, 1H, H-*α*), 3.75 (dd, J = 12.1, 5.6 Hz, 1H, H6b), 3.59 (t, J = 8.9 Hz, 1H), 3.51–3.42 (m, 3H). (M^++H) 299.12431; (M^++Na) 321.10608; ($2M^+$+Na) 619.22573. The data agree with those described in the literature.[64]

Reaction of glucose with different hydrazides. Into a 0.2 mL PCR tube, 20 μL of a 1M solution of glucose, 2 equivalents of benzohydrazide (3-methylthiopropanoylhydrazide or 3,3'-

ditiodipropanoilhidrazida) and 0.2 equivalents of polymeric catalyst **B_2** were added and, finally 100 mM phosphate buffer solution was added up to a final volume of 200 μL. The reaction was heated at 50 ºC or 37ºC during 20 h, whereupon it was frozen and lyophilized. The resultant solid was dissolved in 0.7 mL of D_2O and the conversion was determined by NMR.

Reaction of 3-methylthiopropanoylhydrazide with different sugars. Into a 0.2 mL PCR tube, 20 μL of a 1M solution of the saccharide, 2 equivalents of 3-methylthiopropanoylhydrazide and 0.2 equivalents of the polymeric catalyst B_2 were added and, finally 100 mM phosphate buffer solution was added up to a final volume of 200 μL. The reaction was carried out in duplicate, the first one was heated to 50 ºC for 20 h, the second one was heated to 37ºC during 20 h and the catalyst was preheated to 50 ºC previously. The reaction solutions were frozen and lyophilized. The resultant solid was dissolved in 0.7 mL of D_2O and the conversion was determined by NMR.

To prepare and purify the resultant hydrazones, the reaction was scaled up 4-fold and performed under the reaction conditions indicated above. The total solid was dissolved in 1.0 mL of a 1:1 mixture of methanol/ethyl acetate and the solution was applied in a 20x20 cm Silica 60 Aluminum TLC plate. The product was separated using a mixture 75:25:5 of isopropanol/water/ammonia (28%) as eluent. The silica was scrape out and was washed with 50 mL of a mixture 1:1 methanol/ethyl acetate. The solvent was evaporated at low pressure and, the final solid was characterized using NMR and MS-ESI. To recover the polymer, the glycoconjugates were purified using size exclusion chromatography, Sephadex G-15 as a stationary phase and mQ degassed water as a mobile phase. The process was followed by TLC, eluting the catalyst in the first fraction and the product in the following fractions.

1-(*β*-D-Glucopyranosyl)-2-(3-metylthiopropanoyl)-hydrazine. 10.8 mg (0.036 mmol, 60.8%). ^{1}H NMR (400 MHz, D_2O) δ 4.14 (d, J = 9.0 Hz, 1H, H-1 β), 3.93 (dd, J = 12.2, 1.8 Hz,

1H, H-6a), 3.74 (dd, *J* = 12.1, 5.8 Hz, 1H, H-6b), 3.55 (t, *J* = 9.0 Hz, 1H, H-3), 3.48 – 3.43 (m, 1H, H-5), 3.39 (m, 2H, H-4, H-2), 2.83 (t, *J* = 6.8 Hz, 2H, CH_2), 2.59 (t, *J* = 6.8 Hz, 2H, CH_2), 2.16 (s, 3H, S-CH_3).(M^++H) 297.110181.(M^++Na) 319.09289.($2M^+$+Na) 615.19996.

1-(*α*-D-Mannopyranosyl)-2-(3-metylthiopropanoyl)-hydrazine. 21.7 mg (0.073 mmol, 91.6%). ^{1}H NMR (400 MHz, D_2O) δ 4.29 (s, 1H, H-1*α*), 4.09 (d, *J* = 2.6 Hz, 1H, H-2), 3.98 (dd, *J* = 12.0, 2.1 Hz, 1H, H-6a), 3.86 – 3.79 (m, 1H, H-5), 3.74 (dd, *J* = 12.1, 7.0 Hz, 1H, H-6b), 3.66 (dd, *J* = 9.6, 3.3 Hz, 1H, H-3), 3.57 (t, *J* = 9.6 Hz, 1H, H-4), 2.83 (t, *J* = 6.9 Hz, 2H, CH_2), 2.57 (t, *J* = 6.8 Hz, 2H, CH_2), 2.15 (s, 3H, S-CH_3).(M^++H) 297.11289.(M^++Na) 319.09437.($2M^+$+Na) 615.20106.

1-(*β*-D-Galactopyranosyl)-2-(3-metylthiopropanoyl)-hydrazine. 15.8 mg (0.06 mmol, 66.9%). ^{1}H NMR (400 MHz, D_2O) δ 4.06 (d, *J* = 8.9 Hz, 1H, H-1 *β*), 3.93 (d, *J* = 2.8 Hz, 1H, H-4), 3.83 – 3.69(m, 4H, H-5, H-3, H-6a, H-6b), 3.57 (t, *J* = 9.3 Hz, 1H, H-2), 2.81 (t, *J* = 6.9 Hz, 2H, -CH_2), 2.58 (t, *J* = 6.9 Hz, 2H, -CH_2), 2.14 (s, 3H, S-CH_3).(M^++H) 297.11196.(M^++Na) 319.09364.($2M^+$+Na) 615.2006.

1-(2-Acetamido-2-deoxy-*β*-D-glucopyranosyl)-2-(3-metylthiopropanoyl)-hydrazine. 16.8 mg (0.05 mmol, 83%). ^{1}H NMR (400 MHz, D_2O) δ 4.26 (d, *J* = 9.6 Hz, 1H, H-*β*), 3.96-3.75 (m, 3H, H-6a, H-6b, H-3) 3.63 – 3.56 (m, 1H, H-5), 3.49 – 3.43 (m, 2H, H-4, H-2), 2.81 (t, *J* = 6.9 Hz, 2H), 2.55 (t, *J* = 7.0 Hz, 2H), 2.16 (s, 3H), 2.07 (s, 3H, -$COCH_3$).(M^++H) 338.14082.(M^++Na) 360.11984.($2M^+$+Na) 697.25422.

1-(2-Acetamido-2-deoxy-*α*-D-mannopyranosyl)-2-(3-metylthiopropanoyl)-hydrazine. 21.9 mg (0.065 mmol, 81.2%). ^{1}H NMR (400 MHz, D_2O) δ 4.60 (d, *J* = 4.1 Hz, 1H, H-1 *α*), 4.40 (s, 1H, H-2), 3.96 (3, *J* = 12.1, 1.8 Hz, 1H, H-6a), 3.90 – 3.78 (m, 2H, H-3, H-6b), 3.72 – 3.61(m, 1H, H-5), 3.54 (t, *J* = 9.8 Hz, 1H, H-4, 2.82 (t, *J* = 6.8 Hz, 2H, -CH_2), 2.56 (t, *J* = 6.8 Hz, 2H, -CH_2),

2.15 (s, 3H, S-CH_3), 2.12 (s, 3H, CO-CH_3).(M^++H) 338.13287.(M^++Na) 360.12008.($2M^+$+Na) 697.25273.

1-(2-Acetamido-2-deoxy-*β*-D-galactopyranosyl)-2-(3-metylthiopropanoyl)-hidrazine. 18.2 mg (0.054 mmol, 67.6%). ^{1}H NMR (400 MHz, D_2O) δ 4.20 (d, *J* = 9.6 Hz, 1H, H-1 *β*), 4.04- 39.5 (m,3H), 3.84 – 3.66 (m, 3H, 2.81 (t, *J* = 7.1 Hz, 2H, -CH_2), 2.56 (t, *J* = 7.0 Hz, 2H, -CH_2), 2.15 (s, 3H, S-CH_3), 2.07 (s, 3H, CO-CH_3).(M^++H) 338.13924.(M^++Na) 360.12087.($2M^+$+Na) 697.25626.

1-(*β*-D-xylopyranosyl)-2-(3-metylthiopropanoyl)-hidrazine. ^{1}H NMR (400 MHz, D_2O) δ 4.08 (d, *J* = 8.9 Hz, 1H, H-*β*), 3.97 (dd, *J* = 11.4, 5.4 Hz, 1H, H-5eq), 3.61 (m, 1H), 3.49 (t, *J* = 9.1 Hz, 1H), 3.36 (t, *J* = 9.1 Hz, 1H), 3.31 (t, *J* = 11.1 Hz, 1H, H-5ax), 2.83 (t, *J* = 6.9 Hz, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 2.16 (s, 3H).(M^++H) 267.10236.(M^++Na) 289.08254.($2M^+$+Na) 555.17725.

1-(*α*-D-Arabinopyranosyl)-2-(3-metylthiopropanoyl)-hidrazine. ^{1}H NMR (400 MHz, D_2O) δ 4.03 (d, *J* = 8.7 Hz, 1H), 3.98 (s, 1H), 3.93 (dd, *J* = 13.0, 2.9 Hz, 1H), 3.73 (dd, *J* = 9.4, 3.4 Hz, 1H), 3.69 – 3.60 (m, 2H), 2.83 (t, *J* = 6.9 Hz, 2H), 2.60 (t, *J* = 6.9 Hz, 2H), 2.16 (s, 3H).(M^++H) 267.10126.(M^++Na) 289.08328.($2M^+$+Na) 555.17991.

1-(*α*-L-Arabinopyranosyl)-2-(3-metylthiopropanoyl)-hidrazine. ^{1}H NMR (400 MHz, D_2O) δ 4.03 (d, *J* = 8.7 Hz, 1H, H-1*α*), 3.98 (s, 1H), 3.93 (dd, *J* = 13.0, 2.9 Hz, 1H), 3.73 (dd, *J* = 9.4, 3.4 Hz, 1H), 3.69 – 3.60 (m, 2H), 2.83 (t, *J* = 6.9 Hz, 2H), 2.60 (t, *J* = 6.9 Hz, 2H), 2.15 (s, 3H).(M^++H) 267.10013 .(M^++Na) 289.08221.($2M^+$+Na) 555.16974.

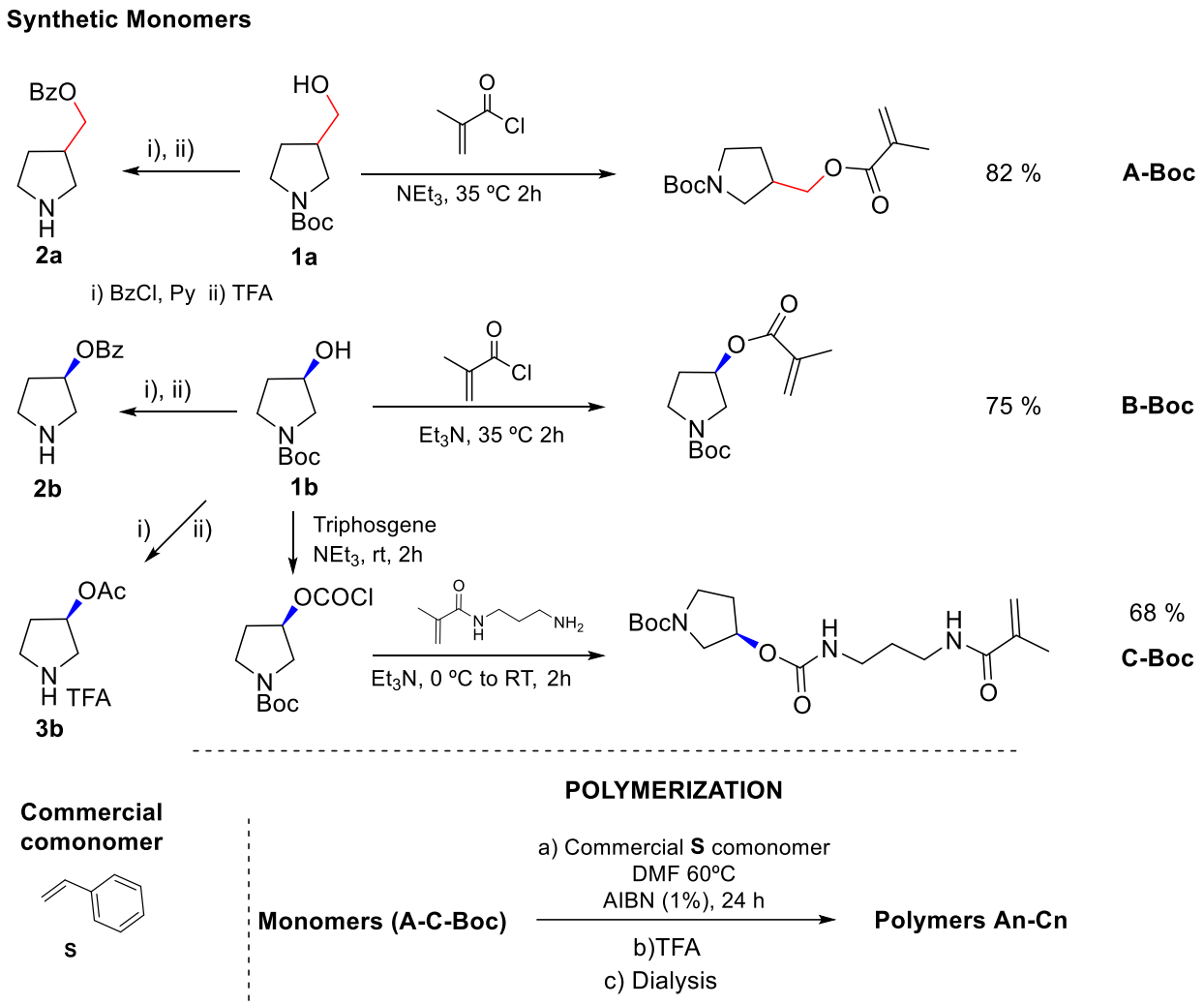
1-(*β*-D-Lactopyranosyl)-2-(3-metylthiopropanoyl)-hidrazine. ^{1}H NMR (400 MHz, D_2O) δ 4.47 (d, *J* = 7.8 Hz, 1H, H-1'), 4.17 (d, *J* = 9.0 Hz, 1H, H-1), 4.03 – 3.95 (m, 2H), 3.87 – 3.54 (m, 10H), 2.83 (t, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 6.8 Hz, 2H), 2.16 (s, 3H). (M^++H) 459.16494.(M^++Na) 481.14727.($2M^+$+Na) 939.31074.

RESULTS AND DISCUSION

Preparation of the polymers.

As nitrogen protected pyrrolidine subunit for the preparation of the corresponding polymers we used *N*-Boc-3-hydroxymethylpyrrolidine (**1a**) and (*R*)-(-)-*N*-Boc-3-pyrrolidinol (**1b**), providing the first one a system with a longer spacer. We have chosen to functionalize position 3 because preliminary tests have shown a higher reactivity of the pyrrolidine unit and an easier preparation of the monomers than those substituted in position 2. In a general way, the hydroxyl groups were functionalized with different methacrylate-based moieties, rendering structures **A-Boc**, **B-Boc** and **C-Boc** (Scheme 1). Then, from these three monomers, three polymeric series were prepared using styrene (S) as comonomer. The *tert*-butoxycarbonyl (Boc) protecting group was removed using TFA and the corresponding trifluoroacetate salt eliminated by dialysis using membranes with a molecular weight limit of 3.5 kDa in distilled water.[65,66] The ^{1}H NMR spectra of the deprotected polymers can be found in the Supplementary Information (SI).

In order to compare the effect of the polymer environment on both pK_a and catalytic activity of the pyrrolidine moiety, compounds **2a**, **2b** and **3b**, carrying substituents at the pyrrolidine unit that can be related to the surrounding structures along the polymer chains were also prepared by simple acylation and deprotection (Scheme 1, left).



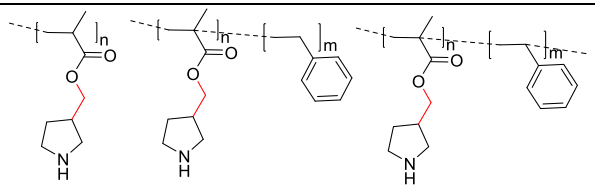
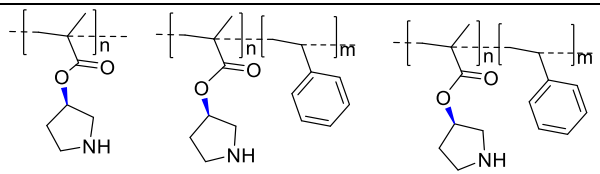
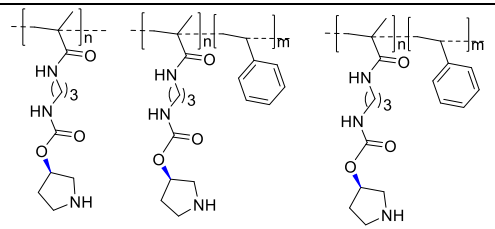
Scheme 1. General procedure for the preparation of pyrrolidine containing polymers.

Following the general procedure shown above, three series of polymers with different hydrophilic/hydrophobic balance represented in Table 1 were prepared (A_n, B_n, C_n, being n=1, 2 or 3). These differ from each other in two aspects, the composition of the copolymer and the nature of the spacer between the pyrrolidine ring and the polymer backbone. Catalysts $\mathbf{C_n}$ have longer spacers, polymers $\mathbf{A_n}$ and $\mathbf{B_n}$ have shorter spacers. The nomenclature $_1$, $_2$, $_3$ pretends to give an idea of the molar amount of comonomer **S** introduced in each case. Polymers designated as $_1$ indicate homopolymerization of the monomers as depicted in Table 1 ($\mathbf{A_1}$, $\mathbf{B_1}$, $\mathbf{C_1}$). Polymers $\mathbf{A_2}$, $\mathbf{B_2}$ and

C₂ are those that contain two units of pyrrolidine per each of styrene. **A₃**, **B₃** and **C₃** contain one unit of pyrrolidine monomer per each of styrene.

The copolymer compositions of the series **A** and **B,** which have been determined as indicated in the Experimental Section, are close to the nominal feed values, which indicate that both units have properly been incorporated into the chains. In the case of series **C**, and specially for copolymer $\mathbf{C}_3$, the copolymer is slight enriched in styrene. However, the composition determination of **C₃** has a larger error than the rest of the spectra because the peaks are very broad (see SI), probably because of the formation of aggregates. The molecular weights of the polymers were characterized by GPC in the protected form, using polystyrene standards as a reference. The number average molecular weights and dispersity indexes are quoted in Table 1.

Table 1. List of polymers prepared and properties.

	 n/m=2/1 n/m=1/1			 n/m = 2/1 n/m = 1/1			 n/m = 2/1 n/m = 1/1		
	A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Fs	-	0.33	0.50	-	0.33	0.50	-	0.33	0.50
fs	-	0.32	0.46	-	0.34	0.44	-	0.39	0.62
$\overline{Mn}$	122.000	44.000	35.000	87.000	49.000	36.000	23.000	15.000	18.000
$\overline{Mw}/\overline{Mn}$	2.5	2.3	1.6	2.5	2.6	1.7	1.3	1.1	1.2
Titration	-	9.2	8.8	8.6	8.5	8.7	9.0		
Turbidimetry	-	9.5 (8.7)	9.4 (8.1)	-	8.7 (7.9)	8.6 (7.6)	-	9.5 (8.5)	9.3 (8.2)

Compositional characteristics and number molecular weight ($\overline{Mn}$) and dispersity index ($\overline{Mw}/\overline{Mn}$). F_S and f_S are respectively the feed and molar fractions of styrene in the copolymer. Series 1: homopolymers, series 2: pyrrolidine: Ph=2/1, series 3: pyrrolidine:Ph 1/1. pK_a or Cloud Point (c.p.) of the conjugated acids of the different pyrrolidine derivatives as determined by acid-base titration and turbidimetry, respectively. In the case of turbidimetry, the pH value at which the solution becomes transparent upon decreasing pH is indicated between parentheses.

Titration and/or turbidimetry of the polymers (see Table 1 and figures S1-13 in SI) have shown that the pK_as of the amine in these polymeric series are in the interval 8.5-9.5 (pK_a of conjugated acid), which is a significant shift compared to pristine pyrrolidine (pK_a of 11.2). Copolymers with styrene, which are insoluble at basic pH, have been characterized by turbidimetry and a cloud point has been determined when increasing the pH. This cloud point is not the true pK_a but is strongly related to it, since it is the pH where the balance between protonated and neutral units makes polymer chains to precipitate.

The above mentioned pK_a shift can partially be assigned to the influence of the aliphatic oxycarbonyl derivatization at 3 position of the pyrrolidone ring, since the structural model (*R*)-pyrrolidin-3-yl acetate (**3b**) exhibited a pK_a shift to 9.7, as quoted in Figure 2a. An additional pK_a shift is achieved by the incorporation of the amine as side chain to a polymeric backbone (polymethacrylate or copolymers with styrene). This pK_a shift is especially relevant for series **B**, reaching values around 8.5 (see Figure 2b).

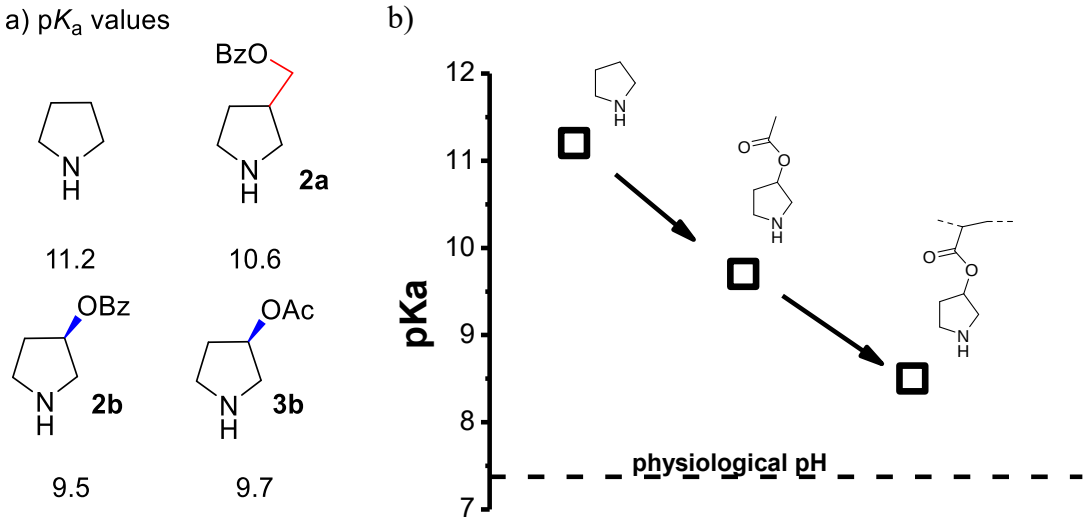
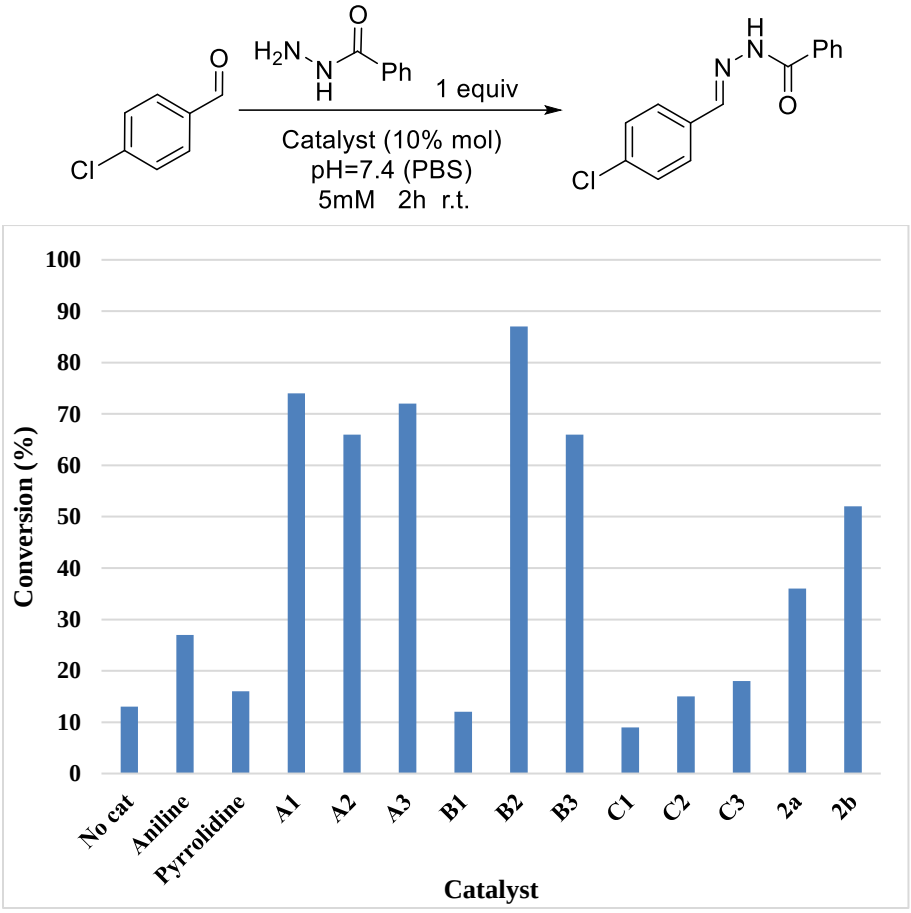


Figure 2. a) pK_a values of pyrrolidines **2a**, **2b** and **3b** determined by acid-base titration. b) Comparison of the pK_a of pyrrolidine, **3b** and $\mathbf{B_1}$.

Study of the catalytic activity using *p*-clorobenzaldehyde as model aldehyde.

It is important to point out that the reactions between aldehydes and hydrazides work smoothly at slightly acidic pH (around 5), nevertheless the big challenge is to perform them at physiological pH, so that they can be used as bioorthogonal reactions.[23] Therefore, the catalytic ability of all the polymers (**A-C**) at physiological pH and room temperature was analyzed using *p*-chlorobenzaldehyde and benzohydrazide as model aldehyde and hydrazide respectively at 5 mM (Figure 3). We also performed the reactions in the absence of catalyst, using aniline, pyrrolidine and substituted pyrrolidines **2a** and **2b**; all the experiments were performed using 10 % of catalysts. As expected, pyrrolidine offered a similar conversion relative to the uncatalyzed reaction (16 % and 13 % respectively), but aniline, which is not protonated at physiological pH, was slightly more efficient (27%). In general, the polymers gave higher conversions than pyrrolidine, which may be related to some extent to the decrease of the pK_a described above. It is especially relevant that the best results have been obtained with some systems of series **B**, which were the polymers with higher shift of pK_a.

Figure 3. Conversions obtained for the formation of the hydrazone from *p*-clorobenzaldehyde.



The conversion values have been depicted versus pKa-7.4 being 7.4 the pH of the reaction medium (see Figure 4a). For the small models (pyrrolidine, **2a**, **2b**) and for some of the polymers (**B**$_2$, **B**$_3$, **A**$_2$, **A**$_3$), there is a clear correlation between pK_a shift and catalytic activity, which is in agreement with our initial hypothesis.

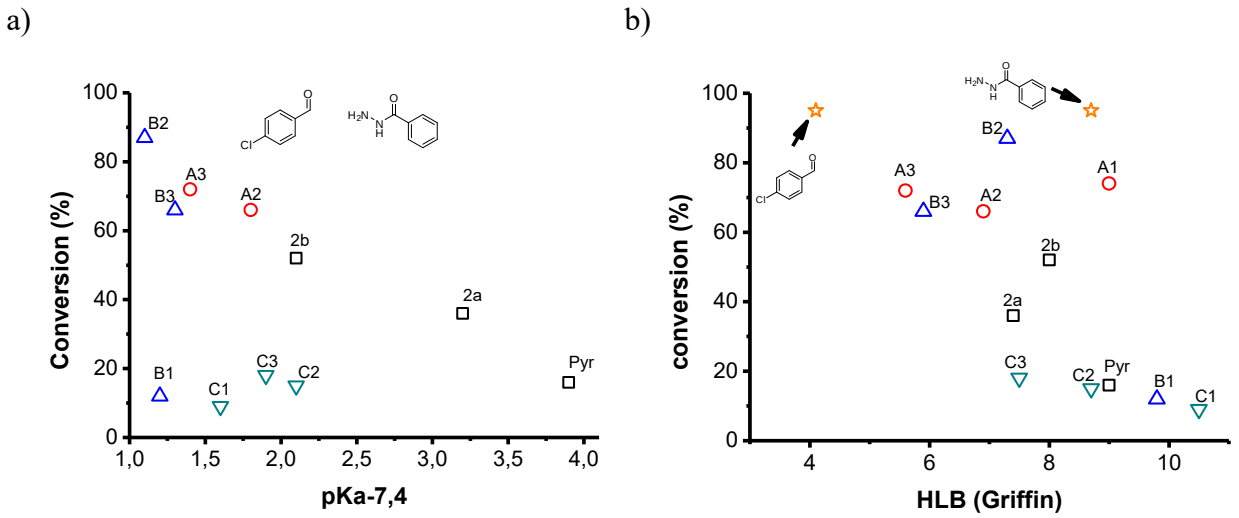


Figure 4. a) Conversions vs pK_a-7.4 for the reactions of Figure 3. b) Conversion of the reactions quoted in Figure 3 vs HLB (according to Griffin). The HLB of the substrates have been included (orange stars).

However, other systems with a clear pK_a shift (**B**$_1$, **C**$_1$, **C**$_2$, **C**$_3$) exhibited low conversion values and did not fit the mentioned correlation. It seems that the catalytic performance could also be related to other structural characteristics such as the copolymer composition and the spacer between the pyrrolidine ring and the polymer backbone. Thus, catalysts **C** with longer spacer gave lower conversions than the polymers with shorter linkers independently of the molar ratio of comonomers. Within the polymer series **B,** with shorter spacers, some differences between homo- and copolymers can be observed. While homopolymer **B**$_1$ gave a conversion similar to that of the uncatalyzed reaction, **B**$_2$ and **B**$_3$ with molar ratios of 2:1 and 1:1 of pyrrolidine methacrylate/styrene gave a substantial increase in conversion relative to the reaction without catalyst (87 % and 66 % respectively, Figure 3). These results pointed to a beneficial effect in polymers where the hydrophobic phenyl ring is situated close to the catalytic pyrrolidine. In the case of polymers **A**, very similar conversions were obtained in all cases (66-74 %, Figure 3).

In terms of hydrophilic/hydrophobic balance, it seems that the complete series **A**, as well as **B**$_2$ and **B**$_3$, present a beneficial balance for the catalytic reaction of the hydrophobic substrates *p*-chlorobenzaldehyde and benzohydrazide, unlike **B**$_1$ or series **C**, which may be too hydrophilic. This is in agreement with the catalytic performance exhibited by **2a** and **2b**, which have an amphiphilic structure and provide higher conversions than pyrrolidine but lower ones than the corresponding polymers. In this sense, it has to be noted that polymers **B**$_1$, **C**$_1$, **C**$_2$, **C**$_3$, in which no correlation between conversion and pK_a shift were found, as mentioned before (see Figure 4b), are actually the more hydrophilic. In order to gain some insight into this issue, the hydrophilic/lipophilic balance (HLB), as defined by Griffin,[59] has been determined for each structure as indicated in the Experimental Section, and has been related to the catalytic activity (see Figure 4b). A slight correlation may be observed for all the systems, and it may explain, to some extent, the low catalytic performance of some of the more hydrophilic systems (**B**$_1$, **C**$_1$, **C**$_2$) as compared to the more hydrophobic ones.

In addition to the previous structural discussion, which was centered on the monomeric structures, polymers in solution may form different entities according to their structure. In order to clarify this point, dynamic light scattering (DLS) and zeta potential (ZP) experiments were carried out for the **B** polymers, which were the best catalysts. ZP measurements of the copolymers B1, B2 and B3 were carried out at room temperature using PBS (neutral pH values) as solvent. The values measured varied gradually from +13.1mV for B1, +6.9mV for B2 and +4.9mV for B3. Therefore, a decrease in the magnitude of the ZP can be directly associated with the copolymer composition. B1, the homopolymer present the largest amount of pyrrolidine methacrylate and thus possess the largest zeta potential values. In B2 the amount of pyrrolidine decreased to 66% and finally in

copolymer B3 the ratio pyrrolidine/styrene is 50%, thus provoking a gradual decrease in the ZP values.

The first series of DLS experiments were focused on understanding the temperature effect on the aggregation. For this purpose, the polymers were heated at 50 ºC and then cooled to room temperature and compared to those that were directly dissolved in the aqueous media. According to the correlation curves the polymers exposed to temperature presented more homogenous aggregates. However, the relaxation times did not change significantly before and after heating indicating that the aggregation remains (see SI).

Also, the role of the composition was analyzed. In Figure 5, both the correlation curves as well as the resulting hydrodynamic size distribution measured for **B_1**, **B_2** and **B_3** are plotted. First of all, it is worth mentioning that all three polymers formed aggregates in aqueous media forming rather large structures as evidenced by the correlation curves that exhibited a sigmoidal distribution. More interestingly, the analysis of the hydrodynamic size distribution curves in number indicates the formation of different types of aggregates directly related to the polymer hydrophilicity. Therefore, **B_3** with a larger hydrophobic contribution forms aggregates with larger sizes (around 200 nm in average) than **B_2** (~50 nm), and finally **B_1** (~20 nm) forms smaller aggregates in comparison to **B_2** and **B_3**. The conversions observed previously could be explained not only in terms of hydrophobic micro-environment but also in terms of size of the aggregates. As it has been mentioned before, the presence of aromatic styrene is clearly beneficial for the reaction, which explains the low catalytic activity of styrene-free **B_1** in despite of its smaller size and higher accessibility (as compared to **B_2** and **B_3**). **B_3** possesses the largest number of hydrophobic units but it exhibits lower catalytic activity than **B_2**, which may be explained by the larger size of the aggregates that may decrease the accessibility to the active centers (as compared to **B_2**). **B_2**

presented a combination of small aggregate size and a hydrophobic effect high enough to result in an optimal catalytic activity.

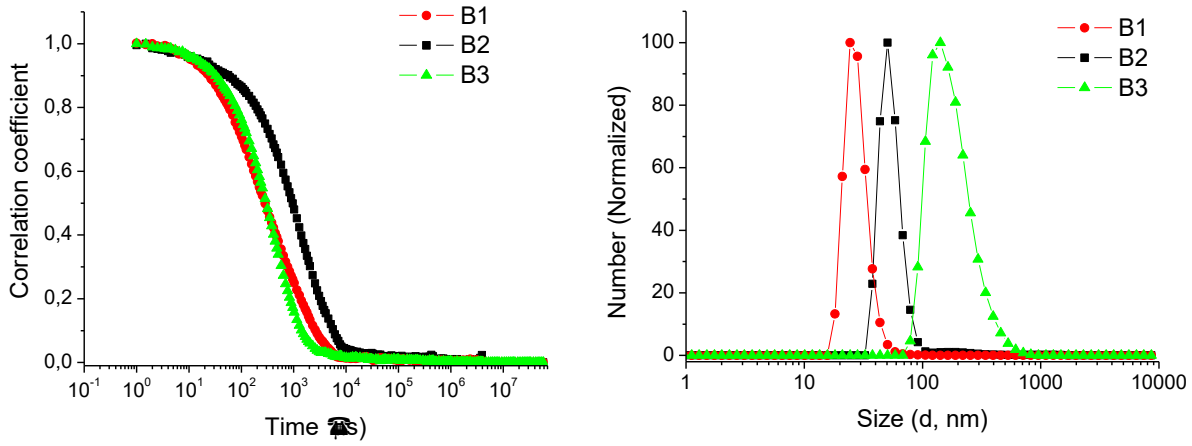


Figure 5. DLS autocorrelation function (left plot) and hydrodynamic size distribution in number (right plot) of the aggregates formed by **B1**, **B2** and **B3** in aqueous media at 0.5 mM which is the concentration of the active moiety for the reaction performed at 5 mM of substrate using a 10 mol% loading.

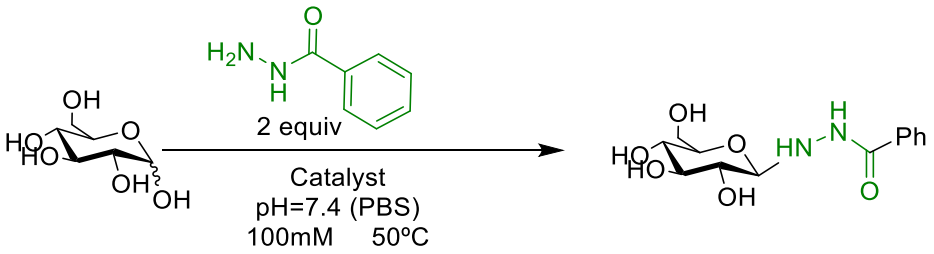
In any case, Figures 4 and 5 confirm that the relationship between catalytic activity and structure is not trivial. Several structural parameters, such as pK_a shift, spacer length, HLB or chain aggregation must be jointly considered.

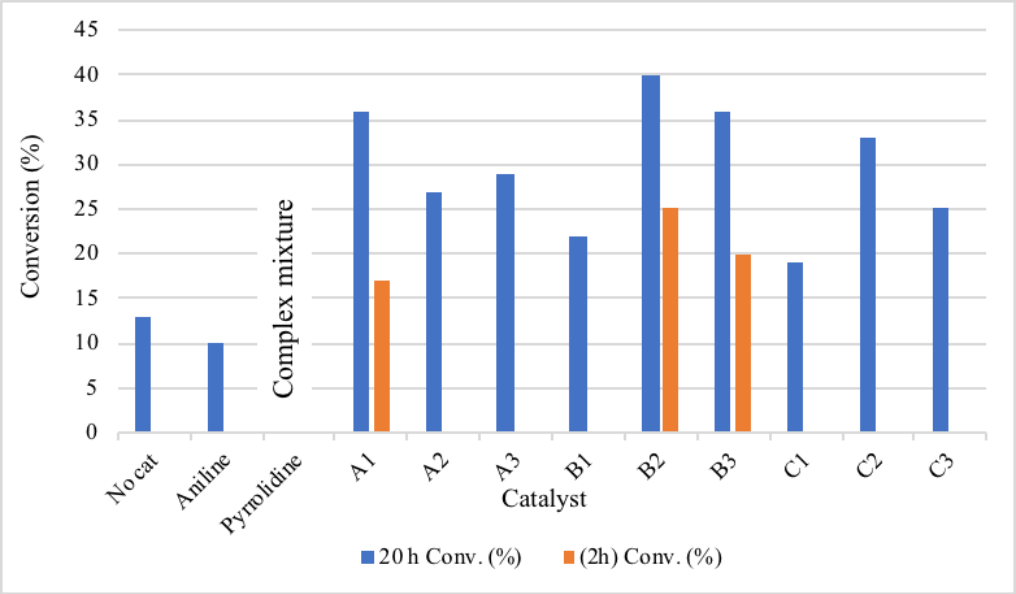
Study of the catalytic activity using glucose as model for free sugar.

Once studied the reaction for a non-polar aldehyde, we turned our attention towards glucose as model substrate for the formation of glycoconjugates. We started studying the reaction of D-glucose (100 mM) and benzylhydrazide as model transformation using the same catalytic loading than above (10 mol %) at physiological pH.[37b] This reaction is more difficult due to several reasons; the aldehyde moiety is masked in the form of a hemiacetal and the final product is less

stable than that formed using aromatic aldehydes.[67] Therefore, to determine the efficiency of the different catalysts we increased both, temperature (50 ºC) and reaction time (20 h) (Figure 6). In the absence of any catalyst, the reaction took place to a low extend; a similar result was obtained in the presence of 10% of aniline. The use of the same amount of pyrrolidine afforded a very complex reaction mixture. The polymers, on the other hand, provided much higher conversions in cleaner reaction mixtures. It is important to remark that the pyrrolidine moiety presents a chiral center and polymers **B** were prepared in enantiomerically pure form. As glucose is a chiral molecule, we wondered if $\mathbf{B_2}$ was able to exhibit some chiral recognition presenting higher catalytic activity for one of the enantiomers of glucose.[68,69] Nevertheless, when we performed the reaction with L-Glucose, after 20 h only a slightly lower conversion (33%) was obtained than that obtained at the same reaction time for D-glucose (40%). In addition, we also studied the catalytic activity of the best polymers $\mathbf{B_2}$, $\mathbf{B_3}$ and $\mathbf{A_1}$ at shorter reaction times; the reaction seems to work faster at the beginning as conversions after 2h are more than half of the conversion after 20h (in orange, Figure 6).

Figure 6. Conversions of the formation of hydrazones D-glucose using different polymers.





For this reaction, since the catalytic differences between the different polymers are smaller than those found for the previous model reaction, the representation of the conversion versus pK_a-7.4 or HLB indicates just a slight dependence (see Figures 7). Being glucose much more hydrophilic than *p*-chlorobenzaldehyde, it seems that in this case there are more polymers with a suitable hydrophilic/hydrophobic balance to interact with both substrates.

a) b)

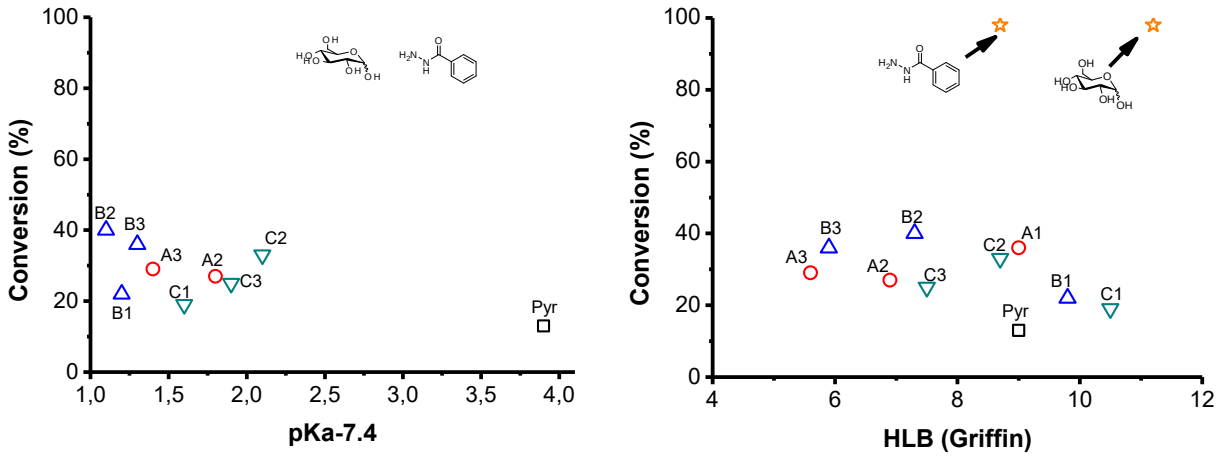


Figure 7. a) Conversions vs (pK_a-7.4) for the reactions of Figure 6. b) Conversion of the reactions quoted in Figure 6 vs HLB (according to Griffin). The HLB of the substrates have been included (orange stars).

Interestingly, DLS analysis of the **B** polymers indicated a strong influence of the concentration on the aggregation. Two series of **B** solutions prepared at 0.5 mM and 10 mM (which were the concentrations of active unit used in the catalytic reactions Figures 3 and 6 respectively) were measured and the aggregation of each polymer was compared. As evidenced in Figure 8 (a and b) the concentration plays a key role in the aggregation; the size of the aggregates clearly decreased by increasing the polymer concentration. This behavior could be explained in terms of ionic interactions between the polymer, which may be considered as a weak polyelectrolyte, and the dissolved ions (medium is PBS).[70,71] Associated to these interactions, there is an electrostatic screening and a reduced repulsion between charged units of a macromolecule, which ultimately favors aggregation. A 20-fold increase of the polymer concentration (as it happens in the two reactions studied), involves a decrease on the ionic ratio (dissolved ions/polymer charges) and a reduced screening and higher repulsion between polyelectrolytes, that is, less aggregation. Thus,

at higher polymer concentrations smaller aggregates are formed (eventually unimolecular micelles as observed for the cases of **B_2** and **B_1**, according to the sizes measured in DLS ~ 6-14 nm). Interestingly, the presence of glucose does not affect dissociation or the formation of aggregates. As evidenced by Figure 8d, in both cases the size distribution in number indicates the formation of unimolecular micelles.

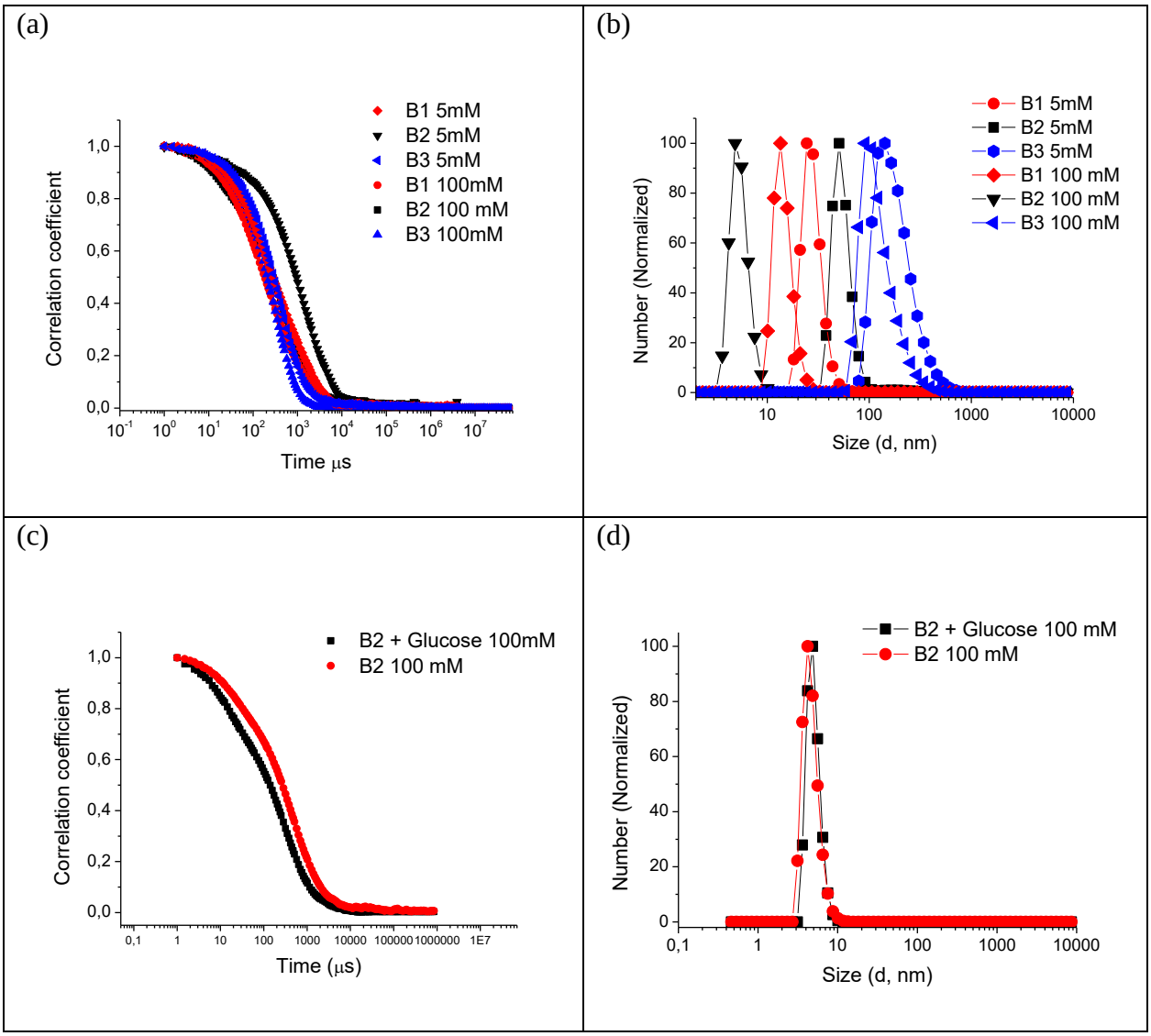


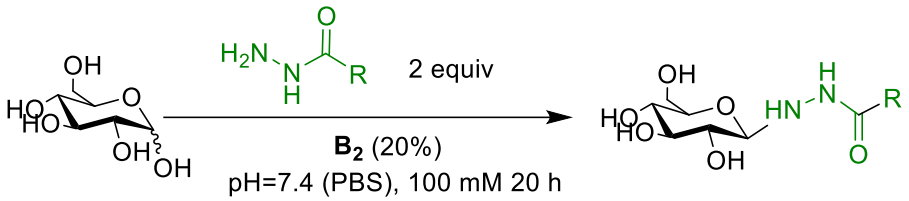
Figure 8. (a) and (b) DLS autocorrelation function (left plot) and hydrodynamic size distribution in number (right plot) of the aggregates formed by **B_1, B_2** and **B_3** in aqueous media at two different polymer concentrations 0.5 mM and 10 mM which correspond with the reactions performed at 5

mM and 100 mM of substrate respectively (c) and (d) DLS autocorrelation function (left plot) and hydrodynamic size distribution in number (right plot) of the aggregates formed by **B2** with and without glucose at 100 mM.

Moreover, we have performed ITC experiments on pyrrolidine-containing polymer **B2** and pyrrolidine using glucose as aldehyde (Supporting Information). The results highlighted differences between both reaction systems and pointed to a favorable interaction when pyrrolidine is linked to the polymer, which supports our hypothesis that a decrease of pyrrolidine basicity occurs by the presence of the backbone chain and the pending groups in the polymer.

According to results gathered in Figure 3, we decided to choose **B2** as the best catalyst and increase the catalytic loading to 20%, which allowed us to obtain the glycosylhydrazide in excellent yield after 20 h (Scheme 2). The reaction also worked with 3-methylthiopropanoylhydrazide as hydrazide, which proven to be more reactive than benzylhydrazide and the reaction was performed at 37ºC (Scheme 2).[72] Using 3-methylthiopropanoylhydrazide the reaction took place to some extend after 2h in the absence of catalysts, but no clear evolution was observed after this initial time but complex reaction mixtures were detected after 20 h.

Scheme 2. Conversions of the formation of different hydrazones of D-glucose using **B2**.

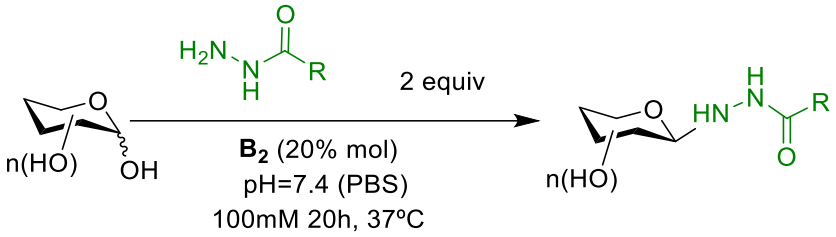


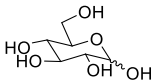
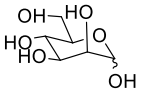
R=Ph	50ºC	98 %
R=-$(CH_2)_3$-SMe	37ºC	95 %

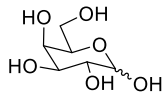
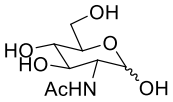
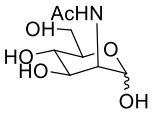
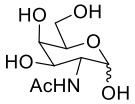
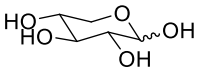
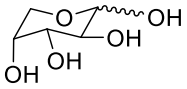
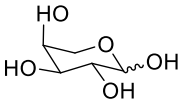
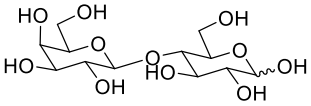
Study of the catalytic activity of B_2 using different sugars.

Providing that the experimental procedure is extremely simple, the polymers can be easily recovered by simple filtration through Sephadex and the products purified by flash chromatography; we decided to prove the generality of the method using 3-methylthiopropanoylhydrazide and different sugars. This hydrazide provides an excellent model for sulfur-containing bioconjugates and their use in the synthesis of glycol-gold nanoparticles[73] Moreover, its structure facilitates the monitoring of the reaction by ^{1}H NMR. Therefore, following this protocol, we could obtain the glycosyl hydrazides of a wide range of free sugars in moderate to good yields (Table 2). Only *N*-acetyl-D-glucosamine provided low yields (entry 4). Interestingly, *N*-acetyl-D-mannosamine and *N*-acetyl-D-galactosamine, which are more reactive than *N*-acetyl-D-glucosamine, evolved satisfactorily towards the corresponding glycosylhydrazides (entries 5 and 6).

Table 2. Reaction of 3-methylthiopropanoylhydrazide with different sugars.



Entry	Saccharide	Yield
1	 D-Glucose	81
2	 D-Mannose	89

3	 D-Galactose	79
4	 N-acetyl-D-glucosamine	12
5	 N-acetyl-D-mannosamine	95
6	 N-acetyl-D-galactosamine	74
7	 D-Xylose	84
8	 D-arabinose	95
9	 L-arabinose	98
10	 Lactose	48

CONCLUSIONS

Based on the assumption that the backbone chain and the pending groups of a polymer strategically designed could shift the pK_a of the pyrrolidine moiety and therefore increase its catalytic activity at physiological pH, we have prepared a series of pyrrolidine-anchored polymers by systematically

changing the number of polar groups, the hydrophobic environment and the nature of the spacer. As hypothesized, the obtained polymers exhibited several p*K*a units below that of free pyrrolidine and displayed better catalytic activity than pyrrolidine itself in the C=N bond formation at physiological pH via ion iminium activation. A methodic analysis of several parameters demonstrated that although the p*K*a of the polymer plays a crucial role in the catalysis, a good balance of charge density, hydrophobic/hydrophilic interactions and aggregation state are also decisive. The method has been applied to the preparation of a variety of glycosylhydrazides from unprotected carbohydrates. Based on the knowledge acquired, we are working on the design of new polymers that also modulate artificially the pKa of basic amines and allow for new applications.

ASSOCIATED CONTENT

Some more experimental details, graphics and NMRs of the compounds are included in the Supporting Information.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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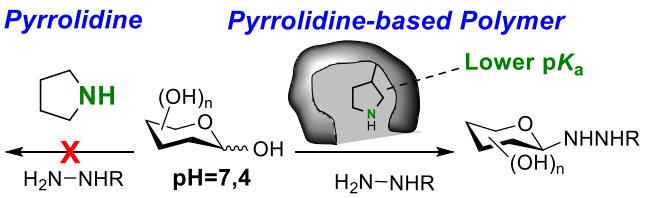

Catalysis: pK_a, a*ggregation state, hydrophilic/lipophilic balance*

pK_a Modulation of Pyrrolidine-Based Catalytic Polymers Used for the Preparation of Glycosyl Hydrazides at Physiological pH and Temperature