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New hydroxyproline based methacrylic polybetaines: Synthesis, pH sensitivity and catalytic activity

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ABSTRACT

The synthesis of two new types of methacrylic polybetaines bearing *trans*-4-hydroxy-L-proline connected via its hydroxyl group is described, one with an aliphatic spacer of 6 carbon atoms and the other without any spacer. The pH sensitivity in aqueous media and the catalytic activity of the products in asymmetric aldol additions have been studied. The two polyzwitterions show an isoelectric point (IEP) close to 3. Swelling of networks prepared with the two monomers exhibit reversible pH sensitivity; the largest the pH distance from the IEP, the higher the net charge (positive or negative) and the higher the swelling. At basic pH and an ionic strength of 0.15, maximum swelling degrees of around 11 and 24 (g water/g polymer) have been found for the systems with and without spacer respectively. The polymers have been shown to be efficient catalysts for aldol reactions under homogeneous conditions in DMF but not in water.

1. Introduction

Amino acids are natural zwitterions; they have at least a cationizable *amino* and an anionizable *acid* function. Therefore, multifunctional amino acids may be used to obtain zwitterionic monomers and the corresponding biorelated polybetaines if at least one cationizable and one anionizable group are preserved (i.e. the *amino* and the *acid* group). To our knowledge the only relevant study that uses this strategy to obtain amino acid-based polybetaines is the work about *N*-acryloyl- and *N*-metacryloyl-L-histidines reported by Casolaro et al. [1–4]; the cationizable group of these polymers is the imidazole ring since the amine group is converted to an amide when introducing the polymerizable acrylic functionality.

Polybetaines are amphiphilic polymers that contain both anionic and cationic groups in the same monomeric unit and exhibit particular behaviour in water and biological interactions that make them very attractive macromolecules for specific bio-applications [5,6]. They are considered bio and haemocompatible, what is related to their highly hygroscopic nature [2,5,7]. Some of them – as for example the copolymers of the above mentioned *N*-methacryloyl-L-histidine with butyl methacrylate – have shown non-fouling performance [4,8,9]. Polybetaines are also pH sensitive and exhibit an isoelectric point (IEP), which is the pH at which no net charges are present and the macromolecules are electrically neutral. At this point the polymer is in its most compact conformation (in the case of crosslinked systems the networks show minimum swelling [3]). The larger the pH difference with respect to the IEP, the higher the net charge (positive or negative) and the more extended the conformation – or the degree of swelling in crosslinked materials. Polymers and networks sensitive to pH and other stimuli are promising materials in different biomedical and biotechnological areas [10].

We describe here the functionalization of *trans*-4-hydroxy-L-proline with a methacrylic moiety via its free hydroxyl group and the preparation of linear and crosslinked polybetaines bearing the proline moiety with free amino and carboxylic acid groups in the side chain (see Fig. 1). We have synthesized two molecules, one obtained by the direct esterification of *trans*-4-hydroxy-L-proline (polybetaine **A**) with methacrylic acid and a second one where a carbamate is used as an aliphatic spacer (polybetaine **B**). This flexible hydrophobic spacer will have an influence on the amphiphilic balance and the proline accessibility and therefore it may play a key role in the polymer performance.

L-Proline and 4-hydroxy-L-proline are the major constituents of collagen and they have also been used as building blocks in polymer chemistry to prepare macromolecules. Polymers containing the amino acid moiety in the main chain such as polyesters [11] or polyamides [12] have been reported as well as side chain type polymers such as several polyacrylics [13,14]. These polyacrylics

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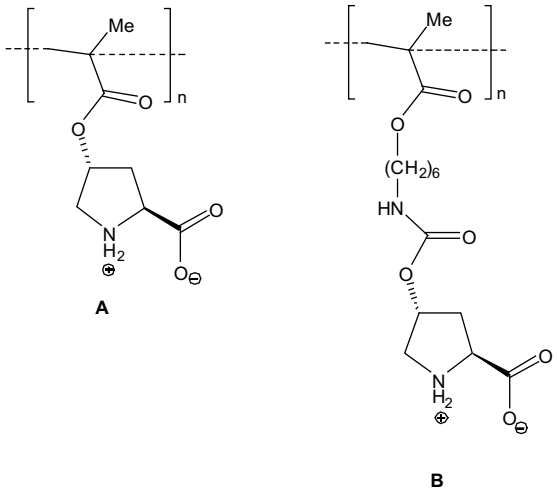


Fig. 1. Scheme of the zwitterionic monomeric units containing *trans*-4-hydroxy-L-proline described in this work. The ionized predominant forms near the IEP are shown.

such as *N*-acryloyl-L-prolines and *N*-acryloyl-L-hydroxyprolines are structurally related to the polymers described in this work but they are not polyzwitterionic since the amine group has been turned into an amide. These systems have shown anionizable character or thermosensitivity depending on the nature of the carboxylic moiety (COOH or COOMe respectively). Very recently, the O-acylation of hydroxyproline with an acryloyl function and the preparation of the corresponding polyacrylates has been reported [15]. The monomer and polymer described in this reference, which is devoted to the synthetic strategy to prepare supported catalyst, are the acrylate analogues of the methacrylate **A** described herein.

In addition to the above mentioned considerations, there is growing interest in the use of the amino acid proline [16–25] and its derivatives [26–33] in asymmetric organocatalysis [34–40]. In fact, proline is one of the first amino acid whose efficiency as an organocatalyst has been recognized [22]. Main advantages of using proline as a catalyst are that the reactions can be performed in a stereoselective manner, under mild conditions and without the need of any metal. In addition, both enantiomers of proline are available. From a practical point of view, the use of polymer-supported proline is of interest since it can facilitate the product purification and catalyst recovery [41–43]. Due to these reasons there is also much interest in the scientific community to attach proline, hydroxyproline or proline derivatives to different surfaces and polymers [43]. These methods usually link the proline derivative to a preformed polymer carrier, either a homogeneous one as PEG [44–46] or a heterogeneous one as PS-based solid supports [47–49].

In contrast, the design proposed here is a 'monomer-based' design with the advantage of the huge versatility of copolymerizations. This 'monomer-based' design allows the preparation of tailor-made macromolecules, i.e. incorporating comonomers as possible cocatalysts or comonomers for the control of the solubility in hydrophilic or hydrophobic solvents. We report here a preliminary evaluation of the catalytic activity of the two polybetaines **A** and **B** of Fig. 1 in asymmetric aldol additions.

2. Experimental

2.1. Materials

2,2′-Azobis-isobutyronitrile (AIBN, Merck) was recrystallized twice from ethanol. Other chemicals were purchased puriss p.A. from commercial suppliers or purified by standard techniques. Compound **1** was obtained as previously described [50].

Buffers from pH 1 to 10, ionic strength of 150 mM and 1 M, were prepared using the buffer calculator of the University of Liverpool [51]. Recipes for volumes of 1 L are indicated bellow. pH was always adjusted with HCl or NaOH. NaCl, which was the salt used to control the ionic strength, is indicated in brackets for the ionic strengths of 150 mM and 1 M respectively. pH 1: 5.805 g of maleic acid (8.433/58.179). pH 2: 5.805 g of maleic acid (7.099/56.817). pH 3: 4.9 g of phosphoric acid (6.163/55.912) .pH 4: 2.301 g of formic acid (6.714/56.44). pH 5: 3.002 g of acetic acid (6.725/56.45). pH 6: 4.307 g of piperazine (6.287/56.029). pH 7: 6 g of NaH_2PO_4 (2.18/51.667). pH 8: 6 g of NaH_2PO_4 (0.327/50.035). pH 9: 3.055 g of ethanol amine (6.857/56.576). pH 10: 3.055 g of ethanol amine (8.311/58.05).

2.2. Synthesis

2.2.1. *tert*-Butyl (2*S*,4R)-N-*Boc*-4-(methacryloyloxy)prolinate (**2**)

To a solution of compound **1** (540 mg, 1.88 mmol) in pyridine (2.17 mL) and CH_2Cl_2 (2.17 mL), was added methacryloyl chloride (348 μL, 3.76 mmol) under stirring at r.t. for 4.5 h. After this time, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc 1:1) giving a colourless oil that still contained traces of methacryloyl chloride. The oil was dissolved in dichloromethane (50 mL) and washed with an aqueous solution of NaOH 5% (75 mL × 3). The organic layer was dried and concentrated, giving a colourless oil (514 mg, 77%).

$[\alpha]_D^{25} - 39.8^\circ$ (*c* 1.63, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 5.99 (s, 1H, CH=C), 5.43 (s, 1H, CH=C), 5.1–5.0 (m, 1H, CH-4), 4.1–4.0 (m, 1H, CH-2), 3.7–3.4 (m, 2H, CH_2-5), 2.4–2.3 (m, 1H, CH-3_A), 2.2–2.0 (m, 1H, CH-3_B), 1.80 (s, 3H, CH_3), 1.4–1.2 (m, 18H, 6 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7 (COO*t*Bu), 166.7 (C=O acrylate), 153.9 (NCOO*t*Bu), 136.1 (CH=C), 126.3 (CH=C), 81.4 (COOCMe$_3$), 80.3, 80.2 (NCOOCMe$_3$), 73.1, 72.2 (C-4), 58.6 (C-2), 52.3, 52.0 (C-5), 36.7, 35.6 (C-3), 28.4–28.1 (CH_3 Boc), 18.2 (CH_3 acrylate). EM (ES): *m*/*z* 378.3 (M + 23); elemental analysis calcd (%) for $C_{18}H_{29}NO_6$: C 60.83, H 8.22, N 3.94; found: C 60.75, H 8.41, N 3.86.

2.2.2. 4-(Methacryloyloxy)pyrrolidine-2-carboxylic acid (**4**)

To a solution of **2** (350 mg, 0.99 mmol) in CH_2Cl_2 (27 mL), TFA (5.38 mL) was added. The reaction mixture was stirred at r.t. for 5 h. After this time, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc 1:1 → EtOAc/MeOH 1:1 → MeOH), to give a white solid (145 mg, 74%). $[\alpha]_D^{25} - 20.8^\circ$ (*c* 0.48, MeOH); m.p.: 102–105 °C; ^{1}H NMR (300 MHz, CD_3OD, 298 K): δ 6.17 (s, 1H, CH=C), 5.70 (s, 1H, CH=C), 5.5–5.4 (m, 1H, CH-4), 4.20 (dd, 1H, *J* = 12 Hz, 6 Hz, CH-2), 3.68 (dd, 1H, *J* = 12 Hz, 6 Hz, CH_2-5_A), 3.33 (d, 1H, *J* = 12 Hz, CH_2-5_B), 2.6–2.5 (m, 1H, CH-3_A), 2.4–2.2 (m, 1H, CH-3_B), 1.95 (s, 3H, CH_3); ^{13}C NMR (75 MHz, CD_3OD): δ 171.9 (COOH), 166.5 (C=O acrylate), 136.0 (CH=C), 126.1 (CH=C), 74.0 (C-4), 60.5 (C-2), 50.8 (C-5), 35.5 (C-3), 17.1 (CH_3 acrylate). EM (ES): *m*/*z* 200.0 (M + 1); elemental analysis calcd (%) for $C_9H_{13}NO_4$: C 54.26, H 6.58, N 7.03; found: C 54.33, H 6.41, N 6.89.

2.2.3. *tert*-Butyl (2*S*,4R)-N-*Boc*-4-(1H-imidazole-1-carboxyloyloxy)prolinate (**5**)

N,*N*′-Carbonyldiimidazole (2.24 g, 13.81 mmol) was added to a solution of **1** (3.31 g, 11.52 mmol) in dry dioxane (13 mL). The mixture was stirred at r.t. under Ar for 15 h. After this time, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/hexane 1:2, 1% of Et_3N) to give a white solid (4.04 g, 92%).

$[\alpha]_D^{20} - 54.0^\circ$ ($c = 0.47$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.20$ (s, 1H, NCHN), 7.40 (s, 1H, NCH=CHN), 7.12 (s, 1H, NCH=CHN), 5.55 (s, 1H, H-4), 4.5–4.2 (m, 1H, H-2), 4.0–3.4 (m, 2H, H-5), 2.7–2.5 (m, 1H, H-3_A), 2.4–2.2 (m, 1H, H-3_B), 1.6–1.1 (m, 18H, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.2$ (COO*t*Bu), 153.9

(NCOO*t*Bu), 153.7 (NCOO), 134.0 (N=CH–N), 130.2 (C=CH–N), 105.3 (C=CH–N), 81.9 (COOCMe$_3$), 80.8 (NCOOCMe$_3$), 69.5 (C-4), 58.2 (C-2), 51.7 (C-5), 36.5 (C-3), 28.3 (Me C-*t*Bu), 28.0 (Me N*t*Bu); MS (ES): *m*/*z* (%):382.3 [M + 1]; elemental analysis calcd (%) for $C_{18}H_{27}N_3O_6$: C 56.68, H 7.13, N 11.02; found: C 56.57, H 7.24, N 10.95.

2.2.4. *tert*-Butyl (2*S*,4R)-N-*Boc*-4-[(6-hydroxyhexyl)-carbamoyl]prolinate (**6**)

Compound **5** (1.6 g, 13.65 mmol) was dissolved in anhydrous THF (53 mL) and 6-amino-1-hexanol (4.04 g, 10.59 mmol) and Et$_3$N (17 mL) were added. The mixture was stirred at 50 °C under Ar for 1.5 h. After this time, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc/hexane 1:1) giving a colourless oil (3.65 g, 80%).

$[\alpha]_D^{25} - 48.70°$ ($c = 0.78$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.2–5.1$ (m, 1H, H-4), 4.9–4.8 (m, 1H, NH), 4.3–4.1 (m, 1H, H-2), 3.7–3.5 (m, 4H, CH_2-5, CH_2OH), 3.2–3.1 (m, 2H, CH_2NH), 2.4–2.3 (m, 1H, H-3$_A$), 2.2–2.0 (m, 1H, H-3$_B$), 1.6–1.4 (m, 4H, HOCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-CH$_2$NH), 1.5–1.4 (m, 18H, *t*Bu), 1.4–1.2 (m, 4H, HOCH$_2$CH$_2$-CH$_2$CH$_2$CH$_2$CH$_2$NH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.65$ (COO*t*Bu), 155.63 (NHCOO), 153.84 (NCOO*t*Bu), 81.29 (COOCMe$_3$), 80.21 (NCOOCMe$_3$), 72.94, 72.05 (C-4), 62.47 (CH_2OH), 58.47 (C-2), 52.12 (C-5), 40.74 (CH_2NH), 36.89 (C-3), 29.74 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 28.29 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 28.24 (Me C-*t*Bu), 27.92 (Me N*t*Bu), 26.30, 25.23 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH); MS (ES): *m*/*z* (%): 453.5 [M + 23]; elemental analysis calcd (%) for $C_{21}H_{38}N_2O_7$: C 58.58, H 8.90, N 6.51; found: C 58.39, H 9.15, N 6.73.

2.2.5. *tert*-Butyl (2*S*,4R)-N-*Boc*-4-[6-(methacryloyloxy)-hexylcarbamoyl]prolinate (**7**)

To a solution of **6** (250 mg, 0.58 mmol) in pyridine (0.7 mL) and anhydrous dichloromethane (0.7 mL), methacrylic anhydride (173 μL, 1.16 mmol) was added. The mixture was stirred at r.t. under Ar for 2 h. After this time, the solvent was evaporated under reduced pressure and the residue was eluted through column chromatography (EtOAc/hexane 1:2) giving a colourless oil, containing traces of methacrylic anhydride. The oil was dissolved in dichloromethane (50 mL) and washed with an aqueous solution of NaOH 5% (75 mL × 3). The organic layer was dried and concentrated, giving a colourless oil (214 mg, 74%).

$[\alpha]_D^{25} - 45.70°$ ($c = 3.66$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.18$ (s, 1H, CH_2=C), 5.53 (s, 1H, CH_2=C), 5.2–5.1 (m, 1H, H-4), 4.8–4.7 (m, 1H, NH), 4.3–4.2 (m, 1H, H-2), 4.13 (t, 2H, $J = 1.6$ Hz, CH_2OCOC=CH_2), 3.7–3.5 (m, 2H, CH_2-5), 3.2–3.1 (m, 2H, CH_2NH), 2.5–2.3 (m, 1H, H-3$_A$), 2.2–2.1 (m, 1H, H-3$_B$), 1.93 (s, 3H, Me), 1.67 (q, 2H, $J = 1.7$ Hz, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 1.6–1.5 (m, 2H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 1.5–1.4 (m, 18H, *t*Bu), 1.4–1.3 (m, 4H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.68$ (COO*t*Bu), 167.49 (COC=CH_2), 155.65 (NHCOO), 154.18, 153.86 (NCOO*t*Bu), 136.44 (C=CH_2), 125.23 (C=CH_2), 81.29 (COOCMe$_3$), 80.20, 79.99 (NCOOCMe$_3$), 73.04, 72.14 (C-4), 64.5 (OCH_2), 58.46 (C-2), 52.53 (C-5), 40.85 (CH_2NH), 36.98, 35.79 (C-3), 29.78 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 28.49 (OCH$_2$CH$_2$CH$_2$CH$_2$-CH$_2$CH$_2$NH), 27.97 (Me C-*t*Bu), 27.89 (Me N*t*Bu), 26.33, 25.55 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 18.29 (Me acrylate); MS (ES): *m*/*z* (%):521.5 [M + 23]; elemental analysis calcd (%) for $C_{25}H_{42}N_2O_8$: C 60.22, H 8.49, N 5.62; found: C 59.88, H 8.42, N 5.72.

2.3. General polymerization and deprotection procedure

Polymers **3** and **8** were prepared by free radical polymerization in *N*,*N*-dimethyl formamide (DMF) at 60 °C for 24 h and using AIBN as initiator. Polymer **A′** was prepared under the same conditions but using distilled water with 0.1 M of NaCl as solvent. Reactions were carried out in the absence of oxygen by bubbling nitrogen for 40 min before sealing the system. The monomer and initiator concentrations were 1 and 0.015 mol/L respectively. The crosslinked systems (labelled as **3**$_{cross}$ and **8**$_{cross}$) were prepared using the same recipe as for **3** and **8** but adding ethylenglycol dimethacrylate (0.02 mol/L). After the polymerization procedure in DMF, both the linear and crosslinked systems dissolved or swollen in DMF were immersed in dichloromethane (DCM)/trifluoroacetic acid (TFA) 1:2 overnight at r.t. and with magnetic stirring to carry out the acid cleavage of protecting groups, obtaining the polymers **A** and **B** or their corresponding networks **A**$_{cross}$ and **B**$_{cross}$. The volume ratio DCM–TFA/DMF was 5:1. After this treatment, linear polymers used in catalysis were precipitated in ether and dried under vacuum overnight. Polymer **A′** was obtained by precipitation and washing in acetone followed by drying in vacuum. Linear polymers used in the turbidimetry studies were purified by sequential dialysis in ethanol and water (48 h each), and finally lyophilized. The crosslinked networks were sequentially washed with a large volume of ethanol, water and buffer at pH = 7.4 (48 h each), and used for the swelling experiments. After the completion of these experiments, the gels were equilibrated with distilled water and lyophilized to obtain the reference gravimetrical data (of dry networks).

2.3.1. Polymer **3**

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 5.2–5.0 (m, 1H, H-4), 4.4–4.2 (m, 1H, H-2), 3.8–3.4 (m, 2H, CH_2-5), 2.4–2.2 (m, 1H, CH-3$_A$), 2.2–2.0 (m, 1H, CH-3$_B$), 2.0–1.6 (m, 2H, (CH_2CMe)), 1.6–1.4 (m, 18H, 6 × CH_3), 1.2–0.8 (m, 3H, Me (methacryl)); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 178.5–177.0$ (OCOCMe), 171.5–171.4 (COO*t*Bu), 153.55, 153.33 (NCOO*t*Bu), 81.20 (COOCMe$_3$), 79.85 (NCOOCMe$_3$), 73.13, 72.92 (C-4), 58.36 (C-2, CH_2CMe), 54.5–51.5 ((CH_2CMe), 50.76 (C-5), 47.0–45.0 (CMeCOO), 36.45 (C-3), 30.73, 28.27 (Me C-*t*Bu), 27.93 (Me N*t*Bu), 25.41, 21.0–18.0 (Me acrylate); IR (KBr): $\nu = 2979$, 2934 (C–H), 1739 (C=O), 1706 (C=O), 1479, 1457 (*t*Bu), 1399 (*t*Bu), 1367, 1257, 1222 (COO), 1153 (OCC), 1063, 993, 939, 842, 770, 554 cm^{-1}; elemental analysis calcd (%) for $C_{18}H_{29}NO_6$: C 60.83, H 8.22, N 3.94; found: C 60.59, H 7.98, N 3.92.

2.3.2. Polymer **A** and **A′**

^{1}H NMR (300 MHz, D_2O, 298 K): δ 5.2–5.0 (m, 1H, H-4), 4.4–4.2 (m, 1H, H-2), 3.8–3.4 (m, 2H, CH_2-5), 2.4–2.2 (m, 2H, CH_2-3), 2.0–1.7 (m, 1H, CH_2CMe), 1.0–0.8 (m, 3H, Me (methacryl)); ^{13}C NMR (100 MHz, D_2O): $\delta = 178.5–177.0$ (OCOCMe), 173.52 (COOH), 75.16 (C-4), 60.48 (C-2), 54.0–51.5 (CH_2CMe), 50.44 (C-5), 47.0–45.0 (CMeCOO), 35.03 (C-3), 21.0–18.0 (Me acrylate); IR (KBr): $\nu = 3431$ (br, N–H, COOH), 2997 (C–H), 1736 (C=O), 1667 (C=O), 1177 (COO, OCC) cm^{-1}; elemental analysis calcd (%) for $C_{11}H_{14}F_3NO_6$: C 42.18, H 4.51, N 4.47; found (**A**): C 42.75, H 4.24, N 4.83.

2.3.3. Polymer **8**

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 5.6–5.4 (m, 1H, NH), 5.2–5.1 (m, 1H, H-4), 4.3–4.1 (m, 1H, H-2), 4.0–3.8 (m, 2H, CH_2OCO), 3.7–3.4 (m, 2H, CH_2-5), 3.2–3.0 (m, 2H, CH_2NH), 2.5–2.3 (m, 1H, CH-3$_A$), 2.2–2.0 (m, 1H, CH-3$_B$), 1.7–1.6 (m, 4H, OCOCH$_2$CH$_2$, CH$_2$CMe), 1.6–1.5 (m. 2H, CH_2CH$_2$NH), 1.5–1.4 (m, 18H, 6 × CH_3), 1.4–1.3 (m, 4H, OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 1.2–0.8 (m, 3H, Me (methacryl)); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 178.5–176.5$ (OCOCMe), 171.94 (COO*t*Bu), 156.06 (NHCOO), 154.40, 154.06 (NCOO*t*Bu), 81.55 (COOCMe$_3$), 80.41, 80.22 (NCOOCMe$_3$), 73.08, 72.16 (C-4), 66.0–65.0 (OCH_2), 58.71 (C-2), 55.5–53.5 (CH_2CMe), 52.36 (C-5), 46.0–45.0 (CMeCOO), 41.16 (CH_2NH), 37.12, 36.71 (C-3), 35.98 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 30.05 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 28.55, 28.24 (Me C-*t*Bu), 28.15 (Me N*t*Bu), 26.68 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH), 21.0–16.0 (Me acrylate); IR: $\nu = 3504$ (br, N–H), 2974, 2932, 2869 (C–H), 1956, 1727 (C=O), 1678 (C=O), 1454 (*t*Bu), 1368 (*t*Bu), 1350, 1253 (COO), 1125

(OCC), 995, 950, 864, 771, 748, 659 cm^{-1}; elemental analysis calcd (%) for $C_{25}H_{42}N_2O_8$: C 60.22, H 8.49, N 5.62; found: C 59.84, H 8.70, N 5.39.

2.3.4. Polymer **B**

^1H NMR (300 MHz, D_2O, 298 K): δ 5.3–5.1 (m, 1H, H-4), 4.2–4.1 (m, 1H, H-2), 4.0–3.8 (m, 2H, $\underline{CH_2}OCO$), 3.6–3.4 (m, 2H, CH_2-5), 3.1–2.9 (m, 2H, CH_2NH), 2.5–2.3 (m, 1H, CH-3_A), 2.3–2.1 (m, 1H, CH-3_B), 1.7–1.1 (m, 10H, $OCOCH_2\underline{CH_2}CH_2CH_2\underline{CH_2}CH_2NH$, $OCH_2CH_2\underline{CH_2CH_2}$-$CH_2CH_2NH$, $\underline{CH_2}CMe$), 1.0–0.6 (m, 3H, Me (methacryl)); ^{13}C NMR (100 MHz, D_2O): δ = 180.0–177.0 (COO acrylate), 172.07 ($\underline{C}OOH$), 156.63 ($N\underline{C}OO$), 73.54 (C-4), 68.0–65.0 ($O\underline{CH_2}$), 59.13 (C-2), 55.5–53.5 ($\underline{CH_2}CMe$), 51.31 (C-5), 46.5–44.0 ($\underline{C}MeCOO$), 40.62 (CH_2NH), 35.21 (C-3), 34.97 ($OCH_2CH_2CH_2CH_2\underline{CH_2}CH_2NH$), 34.61 ($OCH_2\underline{CH_2}$-$CH_2CH_2CH_2CH_2NH$), 31.27, 29.20 ($\underline{OCH_2CH_2CH_2CH_2CH_2CH_2NH}$), 22.0–15.0 (Me acrylate); IR (KBr): ν = 3435 (br, N–H, COOH), 2929, 2851 (C–H), 1724 (C=O), 1631 (C=O), 1059 cm^{-1}; elemental analysis calcd (%) for $C_{18}H_{27}F_3N_2O_8$: C 47.37, H 5.96, N 6.14; found: C 46.98, H 5.82, N 6.31.

2.4. General procedure for the catalytic asymmetric aldol reaction of p-nitrobenzaldehyde (**9**) and ketone **10**

To a suspension/solution of ketone **10** (16.9 mg, 0.13 mmol) and the catalyst (30% mol) in the corresponding solvent (0.21 mL), aldehyde **9** was added (10 mg, 0.065 mmol). The mixture was stirred at r.t. for the time indicated in Table 1.Then, water was added (2 mL), and the mixture was extracted with ethyl acetate (3 × 2 mL). The combined organic layers were concentrated under vacuum. Yields and diastereoselectivities are reported in Table 1. Compound **11**: Spectroscopy data are consistent with those described in the literature [52]. Column chromatography on silica gel (hexane/AcOEt 3:1). ^1H NMR (300 MHz, $CDCl_3$, 298 K) δ major 8.21 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 8.1 Hz, 2H), 5.01 (d, J = 7.5 Hz, 1H), 4.5–4.1 (m, 3H), 3.8–3.7 (m, 1H), 1.39 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ major 210.6 (C), 146.5 (C), 138.3 (CH), 127.9 (CH), 123.2 (CH), 101.4 (C), 75.8 (CH), 71.7 (CH), 66.6 (CH_2), 23.4 (CH_3), 23.3 (CH_3); MS (EI): m/z 585.3 [$2M^+ + 23$]; elemental analysis calcd (%) for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98; found: C, 55.33; H, 5.22; N, 5.18; retention time (HPLC, Daicel Chiralpak OD-H, hexane/i-PrOH = 90:10, flow 1 mL/min, λ = 254 nm): t_R = 11.17 (*anti*, major), t_R = 12.79 (*anti*, minor), t_R = 16.62 (*syn*).

2.5. Equilibrium swelling measurements

The influence of pH and ionic strength on the swelling behaviour of the hydrogels was studied by comparing the swelling degree of gel samples at various pH values for two ionic strengths, 0.15 and 1. The gels obtained as described above (dry weight of 115 and 170 mg for polymers $\mathbf{A}_{cross}$ and $\mathbf{B}_{cross}$, respectively), were sequentially immersed in the buffers starting from pH 1 until pH 10, and placed in a thermostatized shaker at 37 °C. For each pH, the gels were allowed to swell until equilibrium for 24 h. The gravimetric measurements were obtained after removing carefully the aqueous solution. The swelling degree of the gels (g of water/g of polymer) was calculated according to the formula: $SD = (m - m_0)/m_0$, where m and m_0 are the masses of swollen and dried gels respectively.

2.6. Swelling/deswelling

For this study gels pre-equilibrated at pH 3 (ionic strength 0.15) were placed in a shaker at 37 °C and sequentially transferred to buffers at pH 7 and 3 (ionic strength 0.15). At appropriate times the gravimetrical data were obtained as described in the previous section.

2.7. Instrumentation

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 followed by heating. Flash chromatography was performed using thick walled columns, employing silica gel (Merck 60: 0.040–0.063 nm). NMR (^1H, ^{13}C) spectra were recorded on a 300 MHz (Varian Unity 300 or Bruker 300) or 400 MHz (Varian Unity) spectrometer, using different deuterated solvents at room temperature. Chemical shift values are reported in parts per million (δ) relative to tetramethylsilane (TMS) in ^1H 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 symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). For the analysis of the tacticity of the polymers, a standard decoupled sequence with a relaxation delay of 10 s was used. The tactic distributions were determined by comparing the relative intensities of the carbons involved. One additional ^{13}C NMR experiment was performed with polymer **A** to check the quantitative character of the data, using inverse gated decoupling sequence with a relaxation delay of 5 s (decoupling was gated on only during acquisition to suppress any nuclear Overhauser enhancement and obtain quantitative or semi-quantitative data). The results were identical with those obtained via ^{13}C NMR with the standard methods. Therefore, we assumed that no differential spine lattice relaxation times are present for different stereoisomeric sequences (as mm, mr, and rr triads). Within this limit relative peak areas are proportional to the number of carbon atoms involved. Assuming Bernoullian statistic, which is common in polymethacrylates obtained by radical polymerization, we have used the quaternary chain carbon (around 45 ppm) and the α-CH_3 signals (between 15 and 22 ppm) to analyse the tacticity according to the classical tactical assignments of these two atoms in polymethacrylates (in order of increasing field, isotactic mm, heterotactic mr + rm and syndiotactic rr). The isotactic parameter σ was determined according to the following equation:

$$\sigma = 1 - \sqrt{f_{rr}}$$

where f_{rr} is the molar fraction of syndiotactic triads

$$f_{rr} = \frac{A_{rr}}{A_{total}}$$

being A_{rr} the integration of the syndiotactic signal and A_{total} the integration of the complete pattern of the selected carbon atom. Average data of signals from both carbon atoms were used.

Diastereomeric and enantiomeric excess was calculated by HPLC Dionex P680 with a DAD detector (lecture at 254 nm). The analytical column was Daicel Chiralpak OD-H. Optical rotations were recorded on a Perkin Elmer 241 Polarimeter (λ = 589 nm, 1 dm cell) and mass spectra on a hp series 1100 MSD spectrometer. IR spectra were recorded on a Perkin Elmer Spectrum One (240–4000 cm^{-1}) spectrometer and Elemental Analyses on a Heraeus CHN–O Rapid analyzer.

Gel permeation chromatography (GPC) analyses were carried out using Resipore (250 × 4.6 mm, 3 μm nominal particle size) Polymer Laboratories columns. DMF with 0.1% LiBr was used as a solvent. Measurements were performed at 70 °C at a flow rate of 0.3 mL/min using a RI detector. Molecular weights of polymers were referenced to PEG standards.

The turbidity change of the aqueous solutions of the polymers (2 mg/mL) as a function of pH was monitored measuring the absorbance at 400 nm in a UV–vis Lambda 35 spectrophotometer (Perkin Elmer Instruments). The initial polymer solution was freshly prepared in an aqueous solution of 0.15 M (or 1 M) of NaCl and 0.1 M

of HCl. A standard aqueous solution 0.1 M of NaOH was delivered stepwise. pH was monitored with a Beckman 40 pH-Meter (Beckman Instruments, Fullerton, CA, USA).

3. Results and discussion

3.1. Synthesis

The synthesis of polymers **A** and **B** was carried out in a few steps from *tert*-butyl (2*S*,4*R*)-*N*-Boc-4-hydroxyprolinate (**1**) as described in the scheme of Fig. 2. Thus, **1** was treated with methacryloyl chloride to give monomer **2**. Polymerization of **2** was performed in DMF in the presence of AIBN as radical initiator to form the linear polymethacrylate derivative **3**. Acid hydrolysis of protecting groups led to polymer **A**. Alternatively, polymer **A′** was obtained by polymerization of unprotected proline methacrylate **4**, previously prepared by treatment of **2** with trifluoroacetic acid and subsequent purification by silica gel chromatography. The product **A′** exhibited ^{1}H NMR spectral data identical to those of previously obtained polymer **A**. This fact confirms the complete deprotection and structural integrity of polymer **A** since the alternative route to obtain the identical product **A′** uses pure monomer **4** (see ^{1}H NMR spectrum in Supporting information) and a polymerization procedure where no possible structural modification may take place.

For the synthesis of polymer **B**, hydroxyprolinate derivative **1** was reacted with *N*,*N*′-carbonildiimidazole to give the imidazolylcarbonyl derivative **5**. Coupling of **5** with 6-amino-1-hexanol gave carbamate **6**, which was acylated by treatment with methacrylic anhydride to give monomer **7**. Similar polymerization and deprotection steps as described above for **7** furnished polymer **B**.

^{1}H NMR spectra of the polymers **A** and **B** were consistent with the proposed structures (Fig. 3). They exhibit the typical broad peaks of the polymeric species, with the peaks due to the resonances from the proline ring and, in the case of polymer **B**, from the spacer moiety. There were no sharp signals around 1.4 ppm assignable to the *tert*-butyl groups, indicating that removal of the protecting groups was readily achieved (see ^{1}H NMR spectra of precursors **3** and **8** in Supporting information). The polymers were characterized by GPC in the protected forms (the deprotected ones may cause big problems of interactions with the columns). Molecular weights were referenced to PEG standards. Number average molecular weights of polymers **3** and **8** were 71000D and 83000D, respectively with polydispersities of 2.6 and 3.1, which are typical values in radical polymerizations.

3.2. pH Sensitivity

Polybetaines and hydrophobic polyzwitterions are normally insoluble in aqueous media at the IEP, because the coulombic interactions between opposite charges are maximal at this point. Above and below it, the net charge is negative or positive respectively since the stoichiometry is lost allowing linear macromolecular chains to expand, solvate and eventually dissolve. The solubility changes were

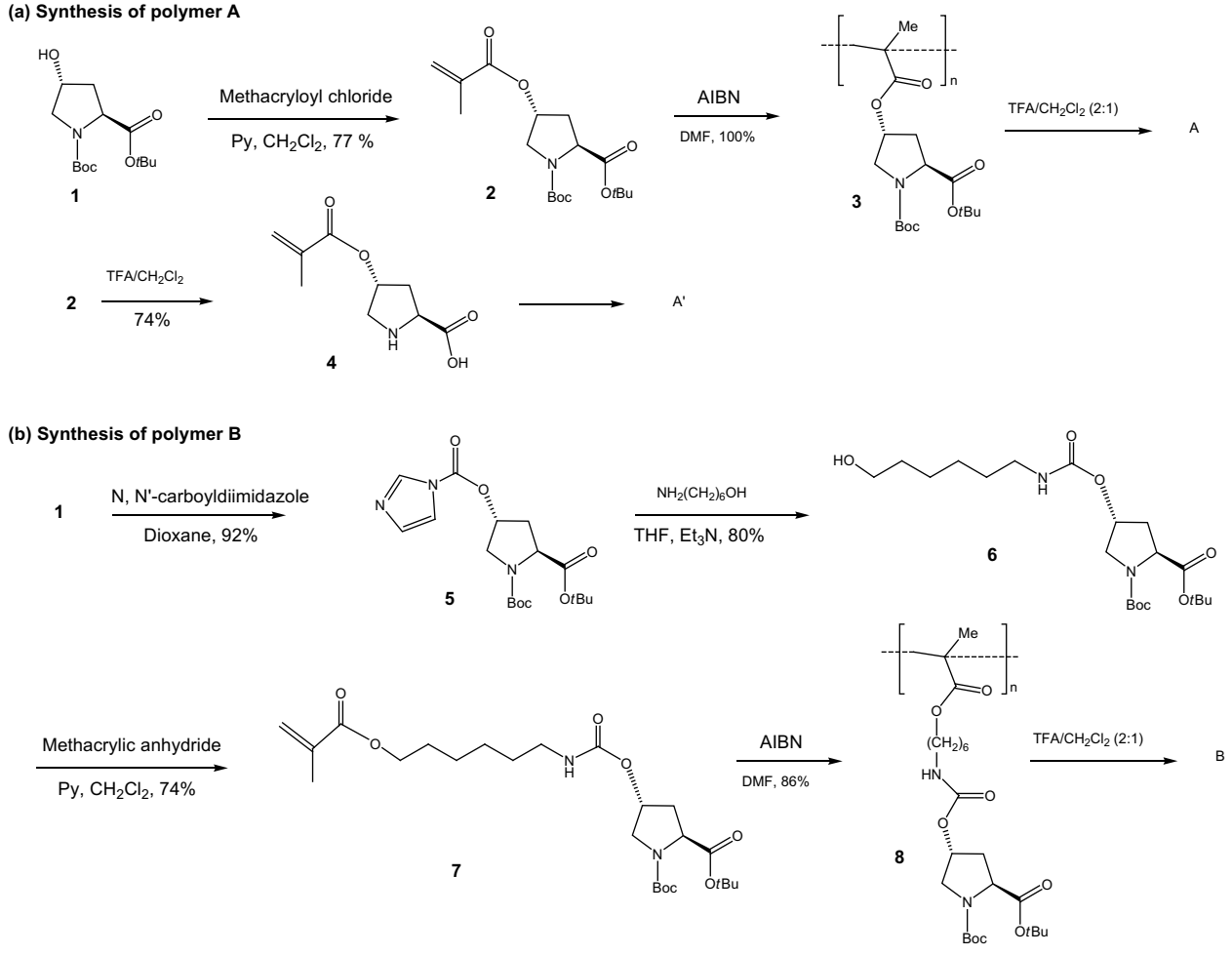


Fig. 2. Synthesis of polymers **A**, **A′** and **B**.

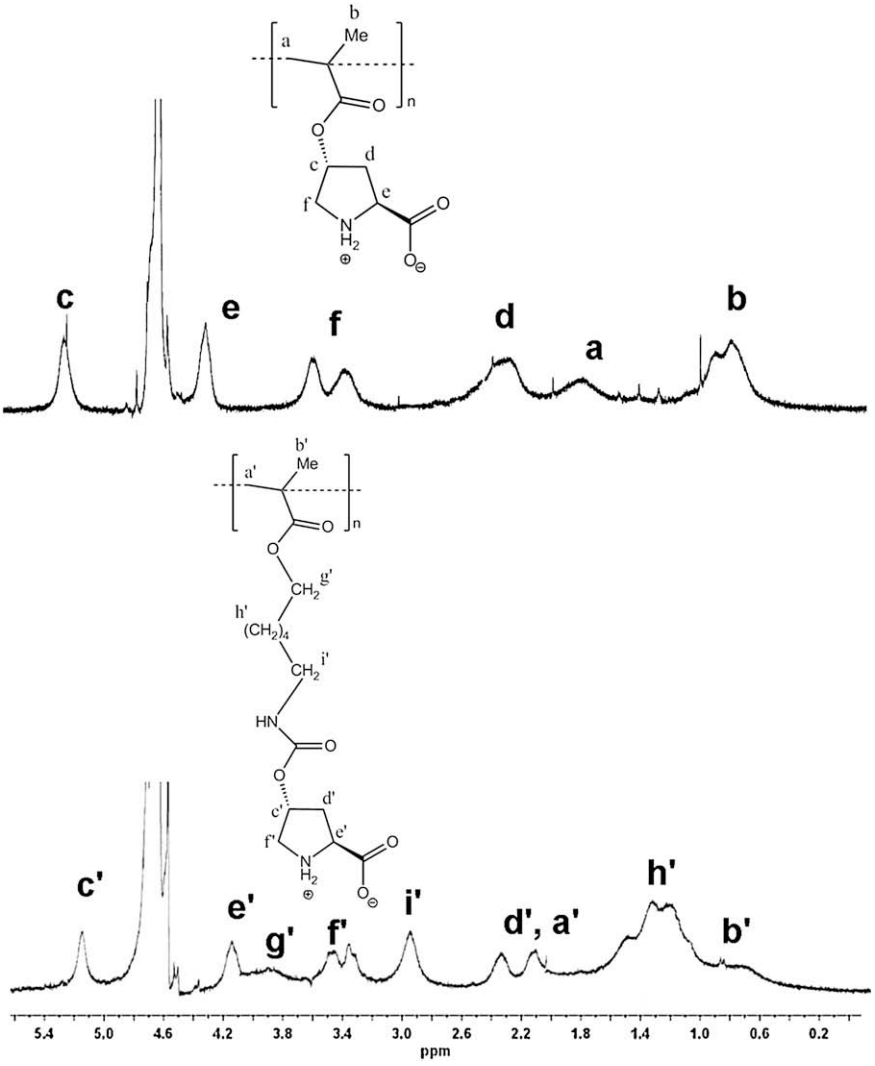


Fig. 3. ^{1}H NMR spectra of the polybetaines **A** and **B** (up and down respectively).

monitored by turbidity measurements (see Fig. 4) under different ionic strengths, 0.15 and 1.

If we take as IEP the average pH between cloud points (the two limiting pH values at which the solution changes from transparent and homogeneous to non-transparent and heterogeneous), the IEPs are very similar, approximately 3 and 3.5 for the **A** and **B** polymers respectively at ionic strength = 0.15. This low IEP (IEP of the amino acid proline is 6.3) may be related to the differences in accessibility for ionizable groups, the *weak* amino base and the *weak* carboxylic acid. A structurally homologous polybetaine derived from aminocrotonate has shown also a very low IEP, around 2, low value that was tentatively attributed to the different accessibility of carboxylic and secondary amine groups upon ionization [53]. This aminocrotonate polymer also bears *weak* carboxylic acid and *weak* secondary amine groups (polybetaines with both weak acid and weak base are very rare in literature).

With regards to this point, it is very interesting to point out the bimodal character of the swelling dependence of the crosslinked system of $\mathbf{A}_{cross}$ when pH is increased from IEP to 10 at ionic strength 0.15 (Fig. 5). There is a clear jump in the graph indicating the stronger swelling capacity of polymer $\mathbf{A}_{cross}$ (being $\mathbf{A}_{cross}$ the polymer with the proline moiety closer to the backbone compared to $\mathbf{B}_{cross}$). It seems as if polymer $\mathbf{A}_{cross}$ would consist of two types of amine, of which one is more easily ionizable than the other. We may tentatively relate this to the tactic arrangements of the macromolecular chains, which are known to be responsible for intermolecular arrangements and gelation effects in different macromolecular

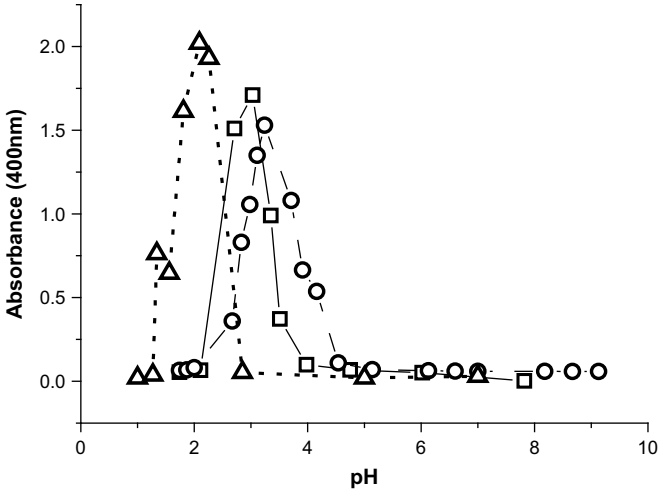


Fig. 4. Absorbance at 400 nm vs the pH for the polymers **A** (□) and **B** (○) at ionic strength = 0.15 and for polymer **A** (△) at ionic strength = 1.

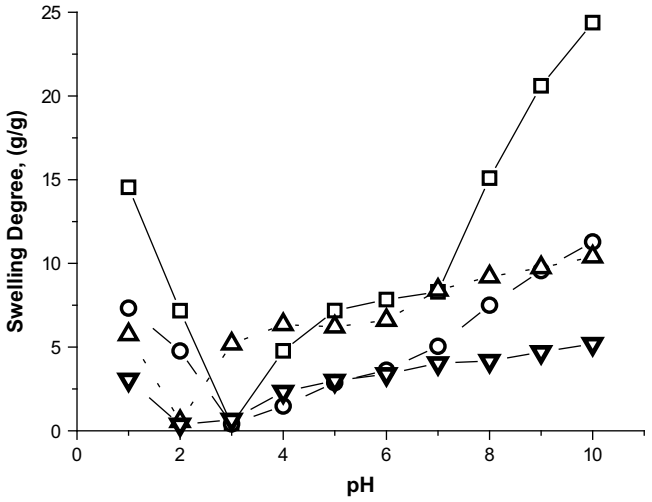


Fig. 5. Equilibrium swelling degrees (g water/g gel) as a function of pH. $\mathbf{A}_{cross}$ (□) and $\mathbf{B}_{cross}$ (○) gels at ionic strength = 0.15, and $\mathbf{A}_{cross}$ (△) and $\mathbf{B}_{cross}$ (▽) gels at ionic strength of 1. Theoretical crosslinking degree of 2% (molar).

networks [54,55]. Polymethacrylate chains are usually predominantly syndiotactic, with isotactic parameters between 0.2 and 0.3 [56], which means that a 20–30% of the diads are isotactic. Fig. 6 shows the expanded ^{13}C decoupled spectra of the α-CH_3 and quaternary resonances for both polymers. These signals exhibited complex patterns that have been analysed in terms of the content of different stereochemical sequences as indicated in the figure and following classical assignments of polymethacrylates [56,57]. As expected, polymers are predominantly syndiotactic with isotactic parameters calculated as indicated in experimental of $\sigma = 0.30$ and 0.23 for polymers **A** and **B** respectively. If a tacticity effect would influence the amine ionization, the higher neighbour interaction should show up between isotactic units. In a very rough approximation, the ratio of the swelling values of the two regions at pH values higher than IEP is 8/25 for polymer $\mathbf{A}_{cross}$ (see Fig. 5 ionic strength 0.15), close to the isotactic parameter.

The dependence of the swelling degree from pH shown in Fig. 5 is in very good agreement with the turbidity data displayed in Fig. 4. The minimum swelling at ionic strength of 0.15 has been found again at or near pH 3. Besides, polymer $\mathbf{B}_{cross}$ that bears the hydrophobic spacer swells approximately half than the more hydrophilic polymer $\mathbf{A}_{cross}$. This lower swelling together with the flexibility of the spacer explains the high dimensional stability of the $\mathbf{B}_{cross}$ gel, which may be submitted without breakage to many swelling–deswelling cycles of high dimensional changes. The $\mathbf{A}_{cross}$ gel however breaks when subjected to high dimensional variations.

In Figs. 4 and 5 also the influence of an increasing ionic strength is pointed out. When passing from an ionic strength 0.15 to 1, the IEP as well as the swelling degree change. IEP of polymer **A** has clearly shifted to lower values (around 2) according to both the turbidimetry data and the swelling minima. Also in the case of polymer **B** a slight decrease of the IEP seems to have taken place. It is well known that the ionic strength influences the p*K*s values of weak acids and amines of macromolecules such as proteins [58,59]. On the other hand, the influence of the ionic strength on the swelling variation with pH is in agreement with the classical theories of antipolyelectrolytical and polyelectrolytical behaviour near or far from the IEP respectively [5]. Near the IEP the systems exhibited polyzwitterion characteristics (antipolyelectrolytic) and far enough from IEP they showed polyelectrolytic behaviour. That is, between the IEP and pH 5 in Fig. 5, swelling of both gels has been stronger for the higher ionic strength, and above pH 5 the opposite behaviour has been found. Note that the 'jump' that what measured at ionic strength 0.15 is also observed at high ionic strength but less pronounced, which may be related to the higher shielding effect of the small electrolytes.

Pulsatile experiments in response to a change in pH were performed at two different pH values: at pH 3 because at this pH that is very close to IEP the networks show strong contraction and at pH 7 because at this value that is close to the physiological one a remarkable network expansion and swelling was observed (see Fig. 7). The gels were subjected to pH changes every few hours, not allowing the networks to reach the equilibrium state. They exhibited a reversible swelling behaviour, adsorbing system $\mathbf{B}_{cross}$ a much smaller amount of water, which is in agreement with the data of Fig. 5 and is again related to the hydrophobic nature of the spacer. It has to be noted that the swelling at pH 3 is quite similar for the two systems.

3.3. Evaluation of the catalytic activity

The proposed mechanism for proline-catalyzed aldol reactions between ketones and aldehydes is depicted in Fig. 8 [60,61]. A donor ketone reacts with proline to form an enamine intermediate. Next, the acceptor aldehyde reacts with the chiral enamine and C–C bond formation takes place resulting in an iminium intermediate which, after hydrolysis, gives the enantiomerically enriched aldol product and the catalytic cycle can be repeated.

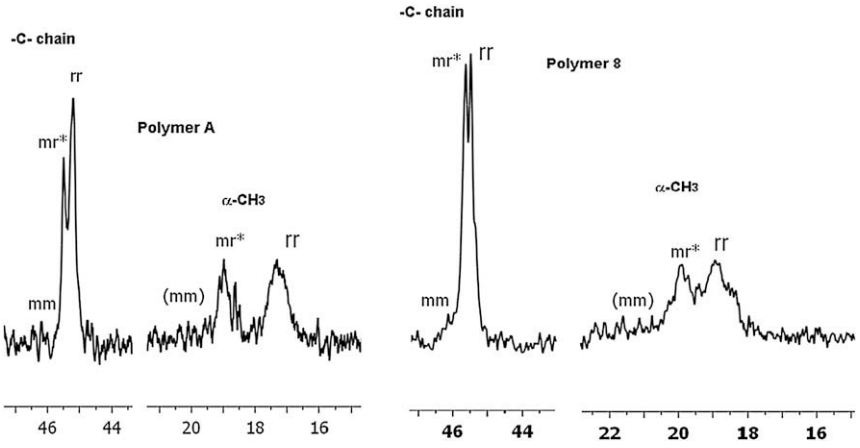


Fig. 6. ^{13}C NMR enhanced resonance signals of the α-CH_3 and quaternary carbons of polymers **A** and **8** in D_2O and $CDCl_3$ respectively.

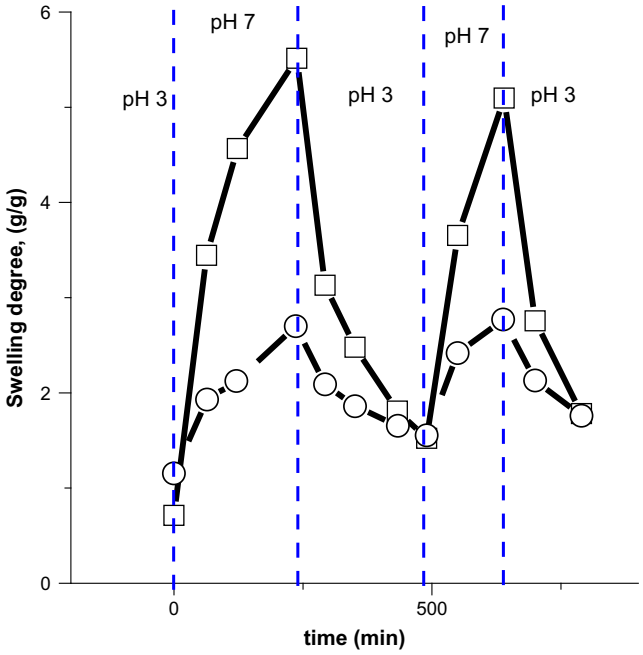
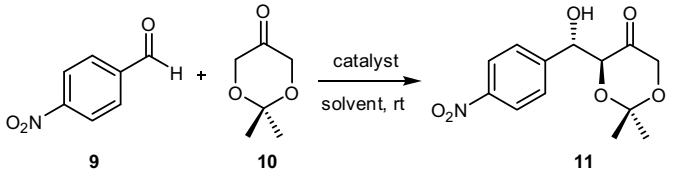


Fig. 7. Swelling–deswelling experiments carried out as described in Experimental part. $\mathbf{A}_{cross}$ (□) and $\mathbf{B}_{cross}$ (○) polymers. Ionic strength = 0.15.

We have evaluated the activity of proline-containing polymers **A** and **B** as catalysts of the aldol addition of *p*-nitrobenzaldehyde (**9**) with 2,2-dimethyl-1,3-dioxan-5-one (**10**) in water and in DMF (Table 1). The polymers were soluble in both solvents. In DMF the polymers were tested with the proline moiety in two ionized forms. In the acid form as obtained after the deprotection step with trifluoroacetic acid (entries 6 and 7), and in the form of the triethylammonium salt obtained after treatment with Et_3N (1 equiv) in water and lyophilization (entries 4 and 5). For comparative purposes the results using L-proline in DMF are also included (entry 1). While no reaction was observed with polymer **A** in water after 48 h, polymer **B**, bearing the hydrocarbon spacer group, gave aldol products although in low conversion and

Table 1
Aldol reaction between *p*-nitrobenzaldehyde **9** and dioxanone **10** in the presence of proline-containing polymers **A** and **B**.



Entry	Catalyst	Solvent	Time (h)	Conv.[a] (%)	d.r.[a] *anti*:*syn*	e.e.[a,b] (%)
1	L-Proline	DMF	48	88	2:1	63
2	A	H_2O (pH 7.0)	48	–	–	–
3	B	H_2O (pH 7.0)	48	11	1:1	46
4	A[c]	DMF	48	78	8:1	78
			64	90	7:1	88
5	B[c]	DMF	48	84	3:1	58
			64	99	3:1	48
6	A[d]	DMF	24[e]	26	8:1	80
7	B[d]	DMF	24[e]	25	5:1	78

[a] Determined by HPLC analysis.
[b] The e.e. values referred to the major diastereomer.
[c] The polymers were used after treatment with Et_3N.
[d] The proline moiety was in the acid form.
[e] No reaction progress was observed after 24 h.

stereoselectivity. This small but different behaviour between **A** and **B** is in agreement with previous observations indicating that a hydrophobic group linked to the proline moiety is beneficial for proline-catalyzed reactions to proceed in water [62,63].

Better results were obtained in DMF. Thus, polymer **A** gave aldol products in a yield comparable to that obtained with L-proline but with better diastereo- and enantioselectivity (d.r and e.e.) (entry 4 versus entry 1). Polymer **B** furnished excellent conversion but lower stereoselectivity. The ionic form of the proline moiety turned out to be important. The reactions in the presence of polymers obtained after the acid hydrolysis step and without treatment with Et_3N, stopped after 25–30% conversion (entries 6 and 7).

We also evaluated the catalytic properties of polymers **A** and **B** under heterogeneous conditions. Thus, the reaction was tried in

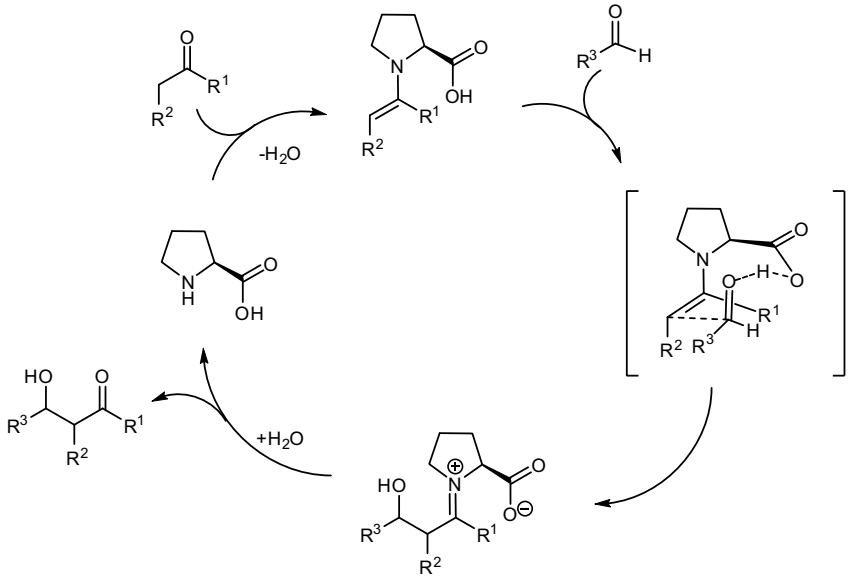


Fig. 8. The L-proline-mediated enamine catalytic cycle.

a non-polar solvent, CH_2Cl_2, in both the absence and presence of added water (2, 5, and 6 mol equiv with respect to the aldehyde **8**), as it has been reported that small amounts of water are sometimes beneficial in proline-catalyzed aldol additions [64]. However, only traces of aldol products were observed after 4 days (results not shown), probably indicating that the polymer collapsed blocking the access to the proline moiety. Similar results were obtained in hydrophobic toluene as solvent.

In conclusion, new L-hydroxyproline-based methacrylic polybetaines have been synthesized. In addition to the hydroxyproline-acrylate recently reported [15], to our knowledge, these are the only examples describing polybetaines where the native *amino* and carboxylic *acid* groups are preserved. The polybetaines have shown pH sensitivity with IEP near 3, with maximum swellings degrees of around 11 and 24 (g water/g polymer) at ionic strength of 0.15 for the polymers with and without aliphatic spacer respectively. The introduction of the spacer leads to approximately half the swelling compared to the polymer without spacer. The polybetaines have been shown to be efficient catalysts of aldol reaction under homogeneous conditions in DMF. However, no appreciable activity was found in water. Due to the unique characteristics offered by water as a solvent, the design and synthesis of new polymers functionalized with residues derived from proline that have been shown to catalyze reactions in water [65], will be the subject of our future investigations.

Acknowledgments

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2009.07.022.

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