Polymer 50 (2009) 2335–2340

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Synthesis and characterization of a methacrylic polyelectrolyte capable of reacting with primary amines at room temperature in water

Julien Barruet, Romain Molle, Julien Babinot, Jacques Penelle*

East Paris Institute of Chemistry and Materials Science, CNRS – Paris 12 University, 2-8 Rue Henri Dunant, 94320 Thiais, France

ARTICLE INFO

Article history:
Received 9 December 2008
Received in revised form
24 March 2009
Accepted 25 March 2009
Available online 7 April 2009

Keywords:
Blocked isocyanate
Water-soluble monomer
Reactive polymer

ABSTRACT

The synthesis of a water-soluble ionic methacrylate monomer containing a sodium 4-hydroxybenzenesulfonate reactive blocked isocyanate is described. The optimized synthetic procedure is fast and easy to use, yielding a monomer of high purity and in good yields. An investigation of the hydrolytic stability of this electrophilic reagent indicated that it could be stored under reasonable conditions. A polyelectrolyte exhibiting the pendant reactive functionalities as side groups was obtained by free-radical polymerization. The obtained polymer exhibited excellent reactivity toward a primary aliphatic amine in water at room temperature, supporting the possible use of this monomer in the bioconjugation area.

1. Introduction

Experimental procedures available for macromolecular bioconjugation, i.e. the covalent attachment of proteins and other biomolecules to synthetic polymers, are based on a limited array of highly efficient organic reactions [1–3]. Due to the practical importance of these procedures in modern biotechnology, many investigations over the years have concentrated on refining the experimental conditions of existing coupling reactions, identifying the most efficient experimental protocols, and screening for possible alternatives. Two very important classes of coupling reactions exploit the presence of nucleophilic residues on the periphery of most biomacromolecules. In particular, electrophilic moieties are often used to trap primary amines available on lysine residues, in a competitive reaction with less nucleophilic hydroxyl groups and/or ubiquitous water molecules.

A key issue associated with these otherwise highly efficient reactions is the poor compatibility of the needed reactive groups (acyl halides, nitro groups, activated C=C bonds, thiols, phenols…) with living/controlled free-radical polymerization techniques, making the synthesis of tailored architectures (block, star…) difficult or impossible. For instance, activated carbonyls such as acyl chlorides, thiols or nitro-containing groups are known to react with free radicals and to interfere with free-radical polymerizations.

In this contribution, an attempt is made to identify a novel functional group compatible with two central requirements: a high reactivity with primary amines in water at or near room temperature and a good compatibility with free-radical polymerizations. This preliminary report will focus on a negatively charged functional group of the blocked isocyanate family (see above), the 4-carbamoyl benzenesulfonate moiety whose structure is depicted in Fig. 1.

Blocked isocyanates derive from isocyanates, electrophilic agents that are highly reactive toward classical nucleophiles such as hydroxyl-, amine- or thiol- containing molecules (including water). In order to facilitate the storage of isocyanates in industrial applications such as textile or paper treatment [4,5], the reactivity of isocyanates is slightly toned down, using hydrogen labile nucleophiles such as phenols, lactams or oximes that can reversibly add on the C=N part of the isocyanate and regenerate it under appropriate thermal conditions [5]. Blocked isocyanates are also reactive with good nucleophiles, for example amino group, under certain temperature conditions (see Scheme 1). They are usually hydrophobic, although at least one example of a water-soluble blocked isocyanate has been described based on a reversible bisulfite addition [6,7].

In this contribution, the synthesis and polymerization of a water-soluble ionic methacrylate monomer containing a sodium 4-hydroxybenzenesulfonate reactive blocked isocyanate that could meet the above requirements are described and discussed.

* Corresponding author. Tel.: +33 1 49 78 12 75; fax: +33 1 49 78 12 08.
E-mail address: penelle@icmpe.cnrs.fr (J. Penelle).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.03.038

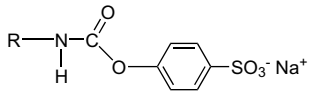


Fig. 1. Structure of the ionic blocked isocyanate investigated in this contribution.

2. Experimental part

2.1. Materials

n-Butylisocyanate (*n*-BuNCO) (Fluka), sodium 4-hydroxybenzenesulfonate dihydrate (NaHBS) (Aldrich), 2-isocyanatoethyl methacrylate (IEM) (Aldrich), *n*-hexylamine (Aldrich), *n*-butylamine (Aldrich), potassium persulfate (Prolabo), dimethylsulfoxide (DMSO) (Acros) were used without further purification. Water was purified with a Millipore Elix 3 before use.

2.2. Characterization

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II Ultrashield Plus 400 NMR spectrometer. All analyses in organic deuterated solvents were calibrated with TMS and all analyses in deuterated water were calibrated with DSS. Quantitative NMR experiments were performed under usual conditions (flip angle = 30°, recycle delay = 2 s, temperature = 296 K). Solid-state ^{13}C NMR experiments were performed at room temperature on a Bruker DSX 300 NMR spectrometer operating at a ^{13}C Larmor frequency of 75.46 MHz, equipped with a 4 mm magic angle spinning (MAS) double-resonance probe. The MAS spinning frequency was set to 8 kHz. The high-resolution ^{13}C NMR spectra were obtained using ^{1}H → ^{13}C cross-polarization (CP) experiments, with a ^{1}H $\pi/2$ pulse length of 3.8 μs, a proton (carbon) radio-frequency field of 66 kHz during the CP step and a contact time of 1 ms. The ^{13}C NMR signal was detected under high-power proton decoupling (DD, decoupling strength of 93 kHz). The recycle delay was set to 3 s. The ^{13}C chemical shifts were calibrated relative to TMS by taking the ^{13}C chemical shift of the carbonyl peak of glycine (α-form) as an external reference standard (176 ppm). FT-IR spectra were recorded on a Bruker Tensor 27 DTGS spectrometer in ATR mode. Melting point measurements were observed using a Labequip Electrothermal 9100. Elemental analyses were carried out by the CNRS Service Central d'Analyse.

2.3. Synthesis

2.3.1. Synthesis of sodium 4-(n-butylcarbamoyl)benzenesulfonate (*1*)

A solution of NaHBS·2H$_2$O (6.97 g, 0.030 mol) in 27 mL of deionized water was stirred at room temperature. *n*-BuNCO (3 mL, 0.027 mol) was added rapidly to the solution, which was stirred at room temperature. The originally colorless solution turned purple instantaneously. After 10 min, the solution turned slightly yellow and opaque. Finally, after 1 h, the solution was yellow and clear. The solution was precipitated in a large volume of acetone. After filtration and drying under vacuum at 50 °C overnight, 4.54 g of a slightly brown powder were obtained. A quantitative ^{1}H NMR indicated the presence of **1** (70 mol%) contaminated by residual NaHBS (30 mol%). No further efforts were devoted to purify the product. Chemical yield of **1** = 44% (as determined gravimetrically and corrected by NMR). ^{1}H NMR (D$_2$O) δ (ppm) = 0.90 (t, 3H), 1.36 (sext, 2H), 1.52 (quint, 2H), 3.19 (t, 2H), 7.25 (d, 2H), 7.85 (d, 2H). ^{13}C NMR (D$_2$O) δ (ppm) = 15.7 (CH_3), 21.8 (CH_3–$\underline{CH_2}$–CH_2), 33.5 (CH_2–$\underline{CH_2}$–CH_2), 43.2 (CH_2–$\underline{CH_2}$–NH), 124.9 (O–C–$\underline{CH}$–CH), 129.9 (CH–$\underline{CH}$–C–SO_3^- K^+), 142.5 (CH–$\underline{C}$–SO_3^- K^+), 155.4 (NH–C(=O)–O), 159.3 (O–$\underline{C}$–CH). FT-IR (cm^{-1}) = 3250 (–NH–), 1720 (–NH–C(=O)O–).

2.3.2. Synthesis of sodium 4-((2-(methacryloyloxy)ethyl)-carbamoyl)benzenesulfonate (*2*)

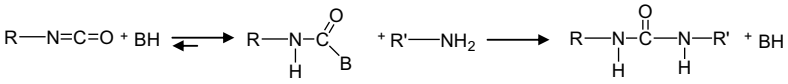
A solution of NaHBS (6.57 g, 0.028 mol) in 28 mL of deionized water was stirred at room temperature. IEM (5 mL, 0.035 mol) was added rapidly to the solution, which was stirred at room temperature. After 1 h, the solution was precipitated in a large volume of acetone. After filtration and drying under vacuum at 50 °C overnight, 7.18 g of white powder were obtained. Yield = 73%. ^{1}H NMR (D$_2$O) δ (ppm) = 1.92 (s, 3H), 3.53 (t, 2H), 4.29 (t, 2H), 5.72 (s, 1H), 6.14 (s, 1H), 7.22–7.24 (d, 2H), 7.83–7.86 (d, 2H). ^{13}C NMR (D$_2$O) δ (ppm) = 19.9 (CH_2=C($\underline{CH_3}$)–), 42.2 (–O–CH_2–$\underline{CH_2}$–NH–), 66.4 (–O–$\underline{CH_2}$–CH_2–NH–), 124.8 (–O–C_{arom}–$\underline{CH_{arom}}$–CH_{arom}–C_{arom}–SO_3^-), 129.7 ($\underline{CH_2}$=C(CH_3)–), 129.8 (–O–C_{arom}–CH_{arom}–$\underline{CH_{arom}}$–C_{arom}–SO_3^-), 138.3 (CH_2=$\underline{C}$(CH_3)–), 142.6 (–O–C_{arom}–CH_{arom}–$\underline{CH_{arom}}$–$\underline{C_{arom}}$–SO_3^-), 155.1 (–NH–$\underline{C}$(=O)–O–), 159.1 (–O–$\underline{C_{arom}}$–CH_{arom}–CH_{arom}–C_{arom}–SO_3^-), 172.1 (=C(CH_3)–$\underline{C}$(=O)O–). FT-IR (cm^{-1}) = 3250 (–NH–), 1720 (–NH–C(=O)O–), 1690 (–C(=O)O), 1650 (–C=C–). Elemental analysis (Calc) = C 44.45, H 4.02, N 3.99, Na 6.54, O 31.88, S 9.13; (Found) = C 44.11, H 4.05, N 3.59, S 8.49.

2.3.3. Polymerization of sodium 4-((2-(methacryloyloxy)ethyl)-carbamoyl)benzenesulfonate (*3*)

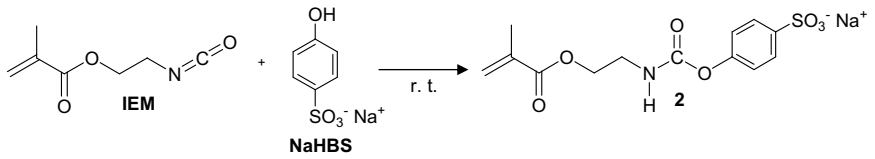
2 (1.0 g, 2.8 mmol) and potassium persulfate (10 mg, 0.036 mmol) were dissolved in 20 mL of deionized water. The solution was degassed for 30 min with an argon flow at room temperature. The solution was stirred and heated at 60 °C during 90 min. 20 mL of deionized water were added to decrease the viscosity of the solution. After precipitation in ethanol and drying under vacuum at 50 °C overnight, 0.420 g of a white solid were recovered. Solid state ^{13}C NMR (ppm) = 16.4 (–CH_2–C($\underline{CH_3}$)–), 39.5 (–$\underline{CH_2}$–C(CH_3)–), 45.0 (–CH_2–$\underline{C}$(CH_3)–), 57.5 (–O–CH_2–$\underline{CH_2}$–NH–), 63.4 (–O–$\underline{CH_2}$–CH_2–NH–), 122.2 (–O–C_{arom}–CH_{arom}–$\underline{CH_{arom}}$–C_{arom}–SO_3^-), 127.7 (–O–C_{arom}–$\underline{CH_{arom}}$–CH_{arom}–C_{arom}–SO_3^-), 141.2 (–O–C_{arom}–CH_{arom}–CH_{arom}–$\underline{C_{arom}}$–SO_3^-), 152.9 (–NH–$\underline{C}$(=O)–O–), 155.2 (–O–$\underline{C_{arom}}$–CH_{arom}–CH_{arom}–C_{arom}–SO_3^-), 177.5 (–$\underline{C}$(CH_3)–$\underline{C}$(=O)O–).

2.3.4. Synthesis of n-butyl-n-hexylurea (*4*)

A mixture of **1** (0.810 g, 2.74 mmol) and NaHBS (0.190 g, 0.82 mmol) was added to a solution of *n*-hexylamine (1.03 g, 0.01 mol) in 2.5 mL of deionized water. The solution was stirred overnight at room temperature. After adding 40 mL of hydrochloric acid (0.5 mol L^{-1}), the solution was stirred for 30 min. The crystalline product was filtered, washed with 100 mL of deionized water and dried at room temperature overnight. After drying, 0.47 g of a white powder were obtained. Yield = 87% of pure **4**. m.p. = 46 °C (after recrystallization in ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm) = 0.86–0.94 (m, 6H), 1.29–1.37 (m, 8H), 1.43–1.49 (quint, 4H), 3.12–3.18 (quint, 4H), 4.66 (s, 2H). ^{13}C NMR ($CDCl_3$) δ (ppm) = 13.8 ($\underline{CH_3}$–CH_2–CH_2–CH_2–CH_2–CH_2–NH–), 14.0 ($\underline{CH_3}$–



Scheme 1. Blocking of an isocyanate and subsequent reaction of the formed blocked isocyanate with an amine.



Scheme 2. Protection of IEM with NaHBS.

CH_2–CH_2–CH_2–NH–), 20.1 (CH_3–$\underline{CH_2}$–CH_2–CH_2–NH–), 22.6 (CH_3–$\underline{CH_2}$–CH_2–CH_2–CH_2–CH_2–NH–), 26.6 (CH_3–CH_2–CH_2–$\underline{CH_2}$–CH_2–$\underline{CH_2}$–NH–), 30.3 (CH_3–CH_2–CH_2–CH_2–$\underline{CH_2}$–CH_2–NH–), 31.6 (CH_3–CH_2–$\underline{CH_2}$–CH_2–CH_2–CH_2–NH–), 32.4 ($\overline{CH_3}$–CH_2–$\underline{CH_2}$–CH_2–NH–), 40.3 ($\overline{CH_3}$–CH_2–CH_2–CH_2–CH_2–$\underline{CH_2}$–NH–), 40.6 ($\overline{CH_3}$–CH_2–CH_2–$\underline{CH_2}$–NH–), 158.6 (–NH–C(=O)–$\overline{NH}$–). FT-IR (cm^{-1}) = 1570 and $\overline{1615}$(–NH–C(=O)–NH–). Elemental analysis (Calc) = C 65.95, H 12.08, N 13.98, O 7.99; (Found) = C 65.77, H 11.78, N 13.82, O 8.17.

*2.3.5. Modification of **4** with n-butylamine (**5**)*

3 (0.4 g) was added to a solution of *n*-butylamine (0.241 g, 3.3 mmol) in 50 mL of deionized water. The solution was stirred at room temperature for 3 days. 66 mL of a hydrochloric acid solution (0.1 mol L^{-1}) were added. The solution was stirred during 30 min at room temperature. The solid was collected on a fritted funnel, washed with water, dried at 50 °C under vacuum overnight to obtain 0.205 g of a white solid. Solid state ^{13}C NMR (ppm) = 14.0 (–CH_2–CH_2–$\underline{CH_3}$); 20.3 (CH_3–C) et (–CH_2–$\underline{CH_2}$–CH_3); 32.6 (–NH–CH_2–$\underline{CH_2}$–CH_2–); 39.1 (–$\underline{CH_2}$–C(CH_3)–); 44.7 (–CH_2–$\underline{C}$(CH_3)–); 54.7 (–$\underline{CH_2}$–NH–C(=O)–NH–$\underline{CH_2}$–); 64.2 (–O–$\underline{CH_2}$–CH_2–); 159.6 (–NH–$\overline{C(=O)}$–NH–), 177.3 (–$\overline{C(=O)}$O).

3. Results and discussion

3.1. Monomer design and blocking of model compound n-butylisocyanate with NaBHS

Water-soluble blocked isocyanates are usually prepared using bisulfite anions (HO–SO_2^-) as blocking agents [6,7]. To obtain a vinyl monomer according to this approach, the most convenient line of attack involves the direct addition of bisulfite anions such as potassium bisulfite on commercially available 2-isocyanatoethyl methacrylate (IEM). Unfortunately, work in our group has indicated the formation of a hydrogel under these conditions, the *in situ* generation of free radicals initiating a concomitant polymerization [8]. In order to synthesize a water-soluble blocked IEM, the use of an almost phenylogous equivalent to the bisulfite anion HO–SO_2^-, sodium 4-hydroxybenzenesulfonate HO–Ar–SO_3^- (NaBHS), as an alternative blocking agent is proposed here. Phenols are known to be good blocking agents for many isocyanates, including IEM [5,9,10]. Water solubility should be obtained from the anionically charged sulfonate moiety.

In order to explore this strategy, a first experiment was attempted with *n*-butylisocyanate (*n*-BuNCO) as a model reagent. The synthesis was inspired by a patent procedure described by Nakao and coworkers [11]. To a solution of NaHBS in water was added *n*-BuNCO in slight excess. After a one-hour reaction and a precipitation in acetone, a product was filtered and dried overnight under vacuum at 50 °C. A product was obtained as a slightly brown powder whose ^1H NMR spectrum, in D_2O, showed the proton signals corresponding to the alkyl chain and four peaks in the aromatic area: two at 7.25 and 7.85 ppm corresponding to **1** and two others at 6.98 and 7.72 ppm corresponding to the aromatic protons of NaHBS. ^{13}C NMR analysis confirmed the structure of **1**, with the presence of a signal at 155.4 ppm corresponding to the carbon of the urethane functional group (–NH–C(=O)–O–); no isocyanate signal at 122.5 ppm was observed. The absence of an isocyanate was also observed on the FT-IR spectrum, with no signal at 2250 cm^{-1} that would typically correspond to an isocyanate.

The integrated areas of peaks assigned to CH_{arom} ($I_{7.25}$, 2 hydrogens) of **1** (7.25 ppm) and to CH_{arom} ($I_{6.98}$, 2 hydrogens) of NaHBS (6.98 ppm) were used to estimate the purity of **1** according to the following relationship:

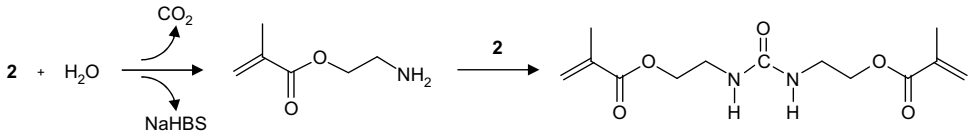
$$\text{Purity} = [I_{7.25}/(I_{7.25} + I_{6.98})] \times 100 \quad (1)$$

where $I_{6.98}$ and $I_{7.25}$ correspond to the intensity of peaks characteristic of NaHBS and **1** respectively. The purity of **1** was estimated to be about 77% according to this technique, indicating a chemical yield of 44% for the reaction. As an excess of isocyanate had been used, the residual presence of NaHBS clearly suggests that the synthesis under these conditions is not complete. This preliminary experiment indicates that the investigated synthetic route is simple, fast, and does not require the use of a catalyst as it is generally the case when isocyanates are protected in organic media. It is quite probable that better yields and purities can be obtained by optimizing the experimental conditions for this model reaction, but no specific efforts were devoted in that direction.

1 was stored in a small container without removing air. After ten months, a ^1H NMR spectrum showed that no significant further modification had taken place, indicating that the product can be stored in the solid state without any drastic precaution.

3.2. Monomer synthesis

The methacrylate monomer targeted in this study was first synthesized using an experimental procedure identical to the one used during the protection of *n*-BuNCO (see Scheme 2 and Section 3.1). Monomer **2** was obtained as a white powder. The ^1H NMR spectrum shows signals corresponding to all the expected protons, but, as previously observed for the model compound, four peaks are obtained in the aromatic area: two major ones at 7.25 and 7.85 ppm assigned to **2** and two minor ones at 6.98 and 7.72 ppm corresponding to NaHBS. The fraction of NaHBS contaminating **2**, according to ^1H NMR, amounts to 3.5 mol%. The two additional



Scheme 3. Hydrolytic degradation of monomer **2**.

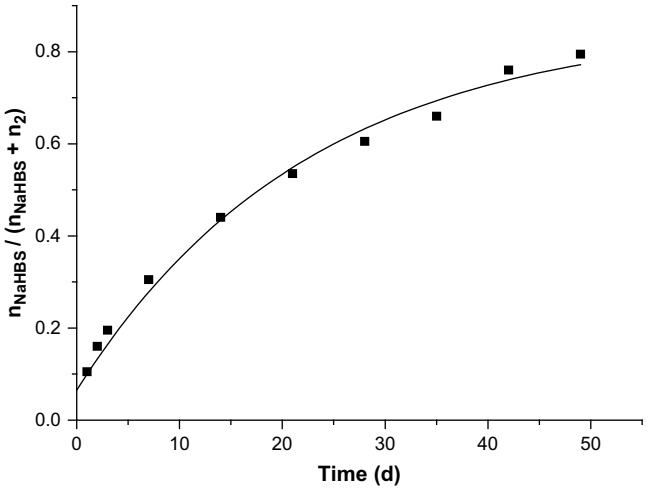


Fig. 2. Hydrolytic stability of the protected monomer **2** as kinetically monitored by NMR.

peaks observed at 5.74 and 6.12 ppm correspond to the protons of the vinyl group. The structure of **2** was further confirmed by ^{13}C NMR, with a peak at 155.4 ppm for the carbonyl carbon of the expected urethane, and by FT-IR with the absence of the typical band for the isocyanate. A band observed at 1650 cm^{-1} on the FT-IR confirmed the presence of the methacrylic functional group.

Surprisingly, the reaction led to a much smaller residual amount of NaHBS than in the model reaction and, consequently, to a higher purity for **2** (96% instead of 77%), despite the fact the reactions for both the model compound and the monomer had been carried out under the exact same conditions. The purity was confirmed by the good agreement between theoretical and experimental values in the elemental analysis (see Experimental part).

In order to distinguish between two explanations for the presence of traces of NaHBS (unreacted reagent or product degradation according to Scheme 3), a stability study was conducted by following by ^1H NMR the evolution of a sample of **2** (64 mg mL^{-1}) in D_2O. Fig. 2 presents the data extracted from the evolution spectra of **2**, using the following parameter:

$$f(t) = I_{6.98}/(I_{7.25} + I_{6.98}) = n_{NaHBS}/(n_{NaHBS} + n_2) \quad (2)$$

It appears that the kinetics with respect to **2** could not be described by a simple first or second order law. Half of the initial quantity of **2** was consumed after 15–17 days, but only 3% after 5 h, meaning that only a small quantity would be hydrolyzed under typical free-radical polymerization conditions. This observation is crucial to the success of the polymerization for two reasons: (a) it means that the presence of phenolic NaHBS would be minimal during the polymerization, limiting the extent of inhibition that could arise by hydrogen abstraction of the propagating chain to the phenol (although inhibition data from the literature on related phenols [12] suggest that this side-reaction might be of limited concern), and (b) it also limits the occurrence of cross-linking that would happen in the presence of the bismethacrylate urea that accompanies the hydrolytic degradation (see Scheme 3). This hydrolytic experiment also indicates that the amount of NaHBS contaminating **2** does not result significantly from a hydrolytic side-reaction. It must be noted that the intermediate amine resulting from the hydrolysis of **2** is also capable of undergoing aza Michael reactions as well as isomerization to *N*-2-hydroxyethyl methacrylamide although these reactions may not be kinetically competitive with urea-forming reactions [13].

Based on these information, the influence of the reaction time (between 5 min and 3 h) and solvent type (H_2O or DMSO) on the yield and purity was investigated for a synthesis of **2** carried out under stoichiometric conditions. Results are presented in Table 1. Small variations in these data are complex to interpret as results are based on isolated products, and as such include the influence of work-up and separation efficiencies in addition to conversion rates and reactions selectivities. The best yield (78%) is observed after 1 h. The purity is initially weak at low conversion and increases after 15 min to remain relatively stable. The best conditions (reaction time, yield and purity) were estimated to correspond to a reaction time of 1 h (see entry 5).

Two experiments were carried out using dimethylsulfoxide (DMSO) as solvent (see entries 8 and 9). At the used NaHBS concentration, the initial solution in DMSO is perfectly clear, whereas in water the solution is slightly cloudy. The yield was higher when the synthesis was made in DMSO (compare entries 3 and 8 and entries 7 and 9), but the purity of the product is clearly weaker.

The influence of the stoichiometric ratio between NaHBS and IEM was also investigated. Results are presented in Table 2. When the quantity of NaHBS ranges between 0.75 and 1.00 equivalent, yields and purities are clearly not affected.

Based on the above experiments, optimized conditions for the synthesis of **2** are as follows: 1 h in water with 0.8 equivalent of NaHBS. This protocol leads to a monomer whose contamination by NaHBS (as measured by ^1H NMR) is so low that it most probably arises from some hydrolysis during sample preparation. It was used in all polymerization experiments described below.

3.3. Polymerization of monomer *2*

A solution prepared from the monomer and potassium persulfate as initiator (10 wt% with respect to the monomer) in deionized water was stirred at room temperature under argon for 30 min, then heated at 60 °C for 90 min. Polymer **3** was obtained as a white powder by precipitation in ethanol and filtration, and drying

Table 1
Protection of IEM using NaHBS: influence of time and solvent.[a]

Entry	Time (min)	Solvent	Isolated yield (%)	$n_{NaHBS}/(n_{NaHBS} + n_2)$[b]
1	5	H_2O	4	0.72
2	10	H_2O	55	0.26
3	15	H_2O	67	0.04
4	30	H_2O	76	0.04
5	60	H_2O	78	0.04
6	120	H_2O	77	0.03
7	180	H_2O	63	0.07
8	15	DMSO	80	0.11
9	180	DMSO	80	0.40

[a] $n_{IEM} = 7$ mmol, $n_{NaHBS} = 7$ mmol, $V_{Solvent} = 7$ mL.
[b] As defined in equation (2), integrations measured by ^1H NMR immediately after dissolution of the product in D_2O.

Table 2
Protection of IEM using NaHBS: influence of the stoichiometric ratio of reagents.[a]

Entry	NaHBS equivalent with respect to IEM	Yield with respect to NaHBS (%)	$n_{NaHBS}/(n_{NaHBS} + n_2)$[b]
1	1	78	0.04
2	0.95	73	0.03
3	0.8	73	0.02
4	0.75	73	0.02
5	0.5	28	0.03

[a] Time = 1 h, $n_{IEM} = 7$ mmol, $V_{H_2O} = 7$ mL.
[b] As described in equation (2), integrations measured by ^1H NMR immediately after dissolution of the product in D_2O.

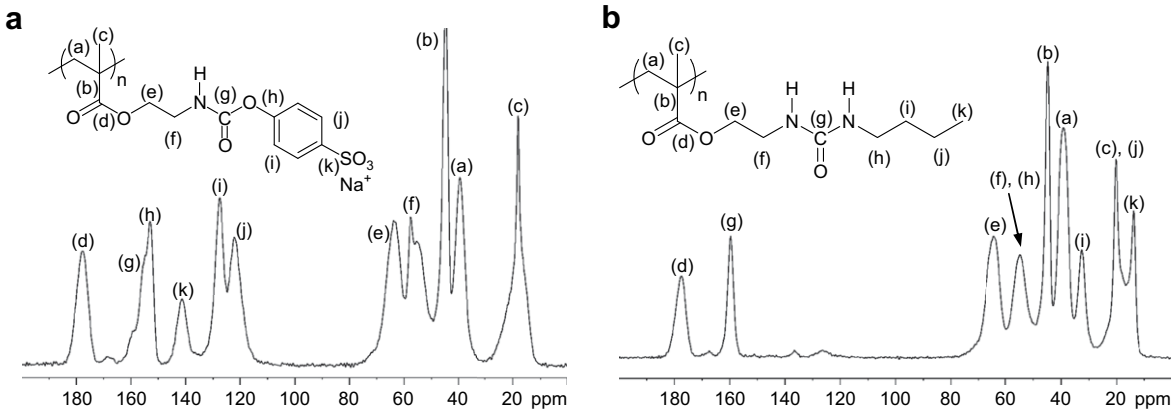
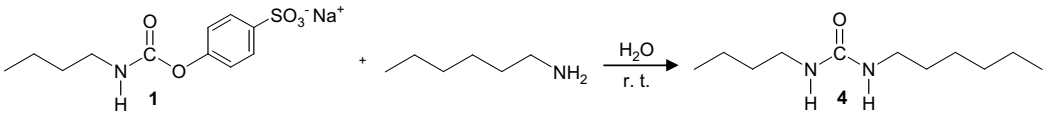


Fig. 3. Solid-state ^{13}C NMR spectrum of (a) a dry sample of polyelectrolyte **3** and (b) the polyelectrolyte after reaction with *n*-butylamine **5**.



Scheme 4. Reaction of the blocked *n*-butylisocyanate with *n*-hexylamine.

overnight under vacuum at 50 °C. A conversion of 42% was determined by gravimetry.

The obtained polyelectrolyte is soluble in water at low concentrations, but the high concentrations needed to obtain high quality NMR spectra could not be reached under normal conditions. It was found to be also insoluble in acetone, $CHCl_3$, DMSO, methanol and ethanol. As a result, the polymer was analyzed by solid-state ^{13}C NMR. A dry sample was ground in order to obtain a fine powder. Fig. 3a displays the obtained spectrum, which is fully compatible with the proposed structure. The blocked isocyanate functional group is observed, with a peak at 155 ppm corresponding to the carbon of the urethane functional group –NH–C(=O)–O– and peaks of the aromatic moiety at 122 and 128 ppm corresponding to the two substituted carbons and two peaks at 141 and 153 ppm corresponding to the two unsubstituted carbons.

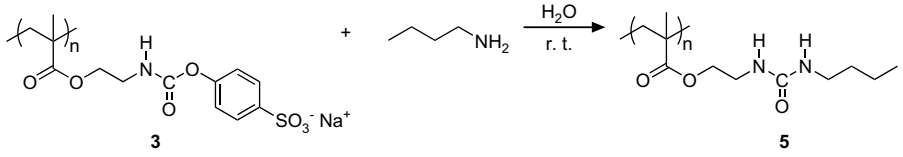
3.4. Reactivity of model compound *1* with n-hexylamine

In order to estimate the reactivity of the synthesized blocked isocyanate toward primary amines, **1** was added to a large excess (3 equivalents) of *n*-hexylamine in water at room temperature (see Scheme 4). A product precipitated during the synthesis (one night). After filtration and drying, a yield of 87% could be evaluated (under the assumption that proved to be correct (see below) that the obtained product is urea **4**). The ^1H NMR spectrum of **4** shows a good agreement to the expected structure, in particular the presence at 4.66 ppm of a signal corresponding to the two protons of the urea group. The ^{13}C NMR spectrum confirms the synthesis of **4** with a signal at 158.6 ppm corresponding to the carbon of the formed urea (–NH–C(=O)–NH–). Melting points before and after recrystallization in ethanol were constant at 46 °C. This preliminary experiment indicates that as expected, the addition of a primary amine on this type of blocked isocyanate in water is efficient.

3.5. Reactivity of polyelectrolyte *3* with n-butylamine

The reactivity of polymer **3** toward primary amines was investigated under conditions similar to those used for model **1**. The solid polymer was added to an excess of *n*-butylamine (3 equivalents) in deionized water in order to obtain a moderately diluted solution (8 g L^{-1}), which was stirred at room temperature (see Scheme 5). After 3 days, the solution was filtered and the resulting solid was dried. As it was not possible to solubilize the product in conventional solvents such as acetone, methanol, chloroform, dimethylsulfoxide or water, the polymer was characterized by solid-state ^{13}C NMR (see Fig. 3b).

The spectrum displays all the expected signals for **5**. Peaks from (a) to (f) correspond to those unaffected by the polymer modification. In contrast, aromatic and urethane signals have disappeared. The addition of *n*-butylamine is confirmed by the presence of new peaks in the area of the alkyl carbons, corresponding to the alkyl chain of the *n*-butylamine, and by the presence at 160 ppm of a new peak, corresponding to the urea carbonyl group –NH–C(=O)–NH–. Fully quantitative analysis was not possible under the NMR conditions used during the experiment, but by considering the relative intensity of the signals, it appears that most of the urea functions are indeed of the *n*-butylurea type, confirming that the formation of the urea functions arises from the



Scheme 5. Reactivity of polymer **3** with n-butylamine.

addition of the amine and not from the hydrolysis of the polyelectrolyte.

During the previously described experiment, a low fraction of insoluble polymer is present throughout the reaction, and increases over time. This rising loss of solubility can be explained by the fact that progressive substitutions of the charged groups into neutral ureas occur during the reaction, lowering water solubility. This explanation was confirmed by the observation that precipitation occurs as well if the reaction is carried out on a fresh solution of polymer obtained by polymerization but without precipitation. The initially clear solution immediately became cloudy, and a white precipitate appeared rapidly. Solid-state ^{13}C NMR analysis of this product provides a spectrum identical to the one already described in Fig. 3b.

4. Conclusions

The synthesis of a water-soluble blocked isocyanate monomer has been achieved from 2-isocyanatoethyl methacrylate using sodium 4-hydroxybenzenesulfonate as a blocking agent. To the best of our knowledge, this monomer is the only water-soluble blocked isocyanate methacrylate monomer described in the literature. Optimization of the experimental procedure has led to an efficient synthesis and access to a highly pure product with a good yield. The monomer has been polymerized in water using free-radical polymerization conditions. The good reactivity of the blocked isocyanate located on the polymer toward an aliphatic primary amine in water at room temperature has been proved. The hydrolytic stability of the polymer in water at room temperature is limited but sufficient to maintain the efficient coupling with reactive nucleophiles such as amino compounds.

These encouraging results suggest that polymer **3** or derived (co)polymers could be used as a tool for the attachment of reactive biological molecules, a rather new area in bioconjugation [14–20].

Acknowledgments

The authors thank Cédric Lorthioir for the solid state NMR analyses and helpful discussions. J. Barruet benefited from a graduate fellowship from the French Ministry of Research and Higher Education. Funding by this ministry and CNRS is gratefully acknowledged.

This contribution is dedicated to Prof. I. de Aguirre whose pioneering work in the area of blocked isocyanate reactivity was a great source of inspiration.

References

[1] Wong SS. Chemistry of protein conjugation and cross-linking. Boca Raton, FL: CRC Press; 1993.
[2] Thordarson P, Le Droumaguet B, Velonia K. Appl Microbiol Biotechnol 2006;73(2):243–54.
[3] Gauthier MA, Klok HA. Chem Commun 2008;23:2591–611.
[4] Wicks DA, Wicks ZW. Prog Org Coat 2001;41(1):1–83.
[5] Wicks DA, Wicks ZW. Prog Org Coat 1999;36(3):148–72.
[6] Guise GB, Jackson MB, McLaren JA. Aust J Chem 1972;25(12):2583–95.
[7] Kirkpatrick A, Maclaren JA. Aust J Chem 1977;30(4):897–901.
[8] Barruet J, Gaillet C, Penelle J. Macromol Rapid Commun 2007;28(20):2007–11.
[9] Fravel HG, Reguiski TW, Thomas MR. Ind Eng Chem Prod Res Dev 1984; 23(4):586–90.
[10] Sadoun T, Clouet G, Brossas J. Makromol Chem 1987;188(6):1367–73.
[11] Nakao M, Nyudo T, Sato H. Japan Kokai Tokkyo Koho JP 55017357; 1980; Chem Abstr 1980;92:199244.
[12] Brandrup J, Immergut EH. Polymer handbook. New York: Wiley-Interscience; 1989.
[13] He L, Read ES, Armes SP, Adams DJ. Macromolecules 2007;40(13):4429–38.
[14] Sompuram SR, Kodela V, Ramanathan H, Wescott C, Radcliffe G, Bogen SA. Clin Chem 2002;48(3):410–20.
[15] Sompuram SR, Kodela V, Zhang KM, Ramanathan H, Radcliffe G, Falb P, et al. J Histochem Cytochem 2002;50(11):1425–33.
[16] Sompuram SR, McMahon D, Vani K, Ramanathan H, Bogen SA. J Histotechnol 2003;26(2):117–23.
[17] Sompuram SR, Vani K, Wei L, Ramanathan H, Olken S, Bogen SA. Anal Biochem 2004;326(1):55–68.
[18] Vigano M, Levi M, Chiari M, Damin F, Turri S. E-Polymers 2007:13.
[19] Vigano M, Levi M, Turri S, Chiari M, Damin F. Polymer 2007;48(14):4055–62.
[20] Vigano M, Suriano R, Levi M, Turri S, Chiari M, Damin F. Surf Sci 2007;601(5):1365–70.