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Synthesis and characterization of novel glycopolymers based on ethyl α-hydroxymethylacrylate

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Abstract

Novel methacrylate derivatives bearing β-D-glucopyranoside and β-D-galactopyranoside residues are synthesized. The construction of the corresponding glycosidic linkages are performed using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide as glycosyl donors, with ethyl α-hydroxymethylacrylate. New glycopolymers are obtained by homo and copolymerization with methyl methacrylate of the corresponding glycomonomers. The reactivity ratios of two protected copolymer systems are determined. Deprotected monomers and polymers are further obtained and characterized by treatment with sodium methoxide. The binding of deprotected polymer with galactose residues to specific receptor protein (RCA_{120}) is achieved.

Keywords: Carbohydrates; Glycomonomer; Glycopolymer; Copolymerization, Lectin

1. Introduction

Carbohydrates are involved in various biological functions in living systems. It is recognized today that they participate in a variety of mutual recognition events including immunological protection, virus infection, recognition in the nervous system, and so forth (Lowe, 1994). These recognition processes are thought to proceed by specific carbohydrate–protein interaction.

Along with the remarkable progress in glycoscience and glycotechnology, highly functionalized synthetic biomacromolecules such as glycopolymers (Okada, 2001; Varma, Kennedy, & Galgali, 2004), have emerged as important materials for the basic exploration of protein–saccharide interactions (Bovin & Gabius, 1995; Kawaguchi, Tagawa, Senda, Matsunaga, & Kitano, 1999; Lee & Lee, 1995; Ohno, Fukuda, & Kitano, 1998). In consequence, new synthetic complex carbohydrates and synthetic carbohydrate-based polymers, "glycomimics", are increasingly used as an important well-defined tool for investigating carbohydrate-based interactions (Simanek, McGarvey, Jablonoswki, & Wong, 1998). Therefore, carbohydrate-based monomers and polymers are of main interest with respect to very specialized applications in basic biochemical, pharmaceutical and biomedical research such as molecular recognition processes (Wassarman, 1987), drug delivery systems (Palomino, 1994; Sihorkar & Vyas, 2001), cell culture (Karamunt, Mayer, Wintermantel, & Akaike, 1999) and treatment of infectious diseases (Petronio et al., 1997).

In this context, recently, considerable attention has been paid to the design of biofunctional materials carrying saccharide moieties on synthetic polymers, mainly polystyrenes, polyacrylates and polymethacrylates. These can be synthesized by chemical methods from a sugar-containing monomers; by modification of a polymer backbone or by chemo-enzymatic method (Albertin, Kohlert, Stenzel, Foster, & Davis, 2004; Ladmiral, Melia, & Haddleton, 2004; Okada, 2001; Wolfenden & Cloninger, 2005). We will focus on the first polymer reaction method.

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The aim of this work is to obtain novel glycomonomers for the preparation of new carbohydrate-based polymers. In this paper, we describe the preparation of methacrylate derivatives with saccharide moiety, glucose and galactose, leading to a proper comparison of the influence of sugar type. These carbohydrate-monomers are suitable for the preparation of protected glycopolymers. The removal of protecting groups will be carried out either before or after the polymerization. Thus, the corresponding water soluble glycomonomers and glycopolymers were obtained. All the compounds are characterized by FTIR and NMR spectroscopy. In addition, the synthesis of novel statistical copolymers of these protected glycomonomers with methyl methacrylate are described.

2. Experimental

2.1. Materials

All chemicals were used without further purification. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Fluka, 95%), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (Sigma, 95%), Silver trifluoromethanesulfonate AgOTf (Aldrich, 99%), Sodium methoxide (Fluka, 95%), Cation-exchange resin Dowex 50WX2-200 (Aldrich). Dichloromethane (DCM, anhydrous 99.8%, Aldrich), chloroform (anhydrous 99%, Aldrich), and methanol (anhydrous 99.8%, Aldrich) were used as received. 2,2′-Azobisisobutyronitrile (AIBN, Fluka, 98%), was purified by recrystallization from methanol; Methyl methacrylate (MMA, Aldrich; 99%) was washed with a sodium hydroxide solution (5%) and water to remove the radical inhibitor, refluxed over calcium hydride, and distilled under reduced pressure before use; Ethyl α-hydroxymethylacrylate, (EHMA), was obtained by the treatment of an aqueous formaldehyde solution with triethyl phosphonate and potassium carbonate (Fernández-Monreal, Cuervo, & Madruga, 1992). Glycomonomers were synthesized according to the method described by others (Ambrosi et al., 2002) with minor changes. Concanavalin A, (ConA, Fluka) and Ricinus Communis Agglutinin (RCA_{120}, Atom) were used without further purification.

2.2. Techniques

^{1}H and ^{13}C-NMR spectra were recorded with a Bruker DPX-300 and Bruker Avance AV-500 spectrometers. The solvent signals were used as chemical shift markers. The glycomonomers spectra were recorded at 25 °C using $CDCl_3$ or CD_3OD as deuterated solvents. Assignments of the observed signals to the hydrogen and carbon atoms were based on homonuclear decoupling experiments and homo- and heteronuclear correlations. The polymers spectra were acquired at 50 °C in dimethyl sulfoxide-d_6 or deuterated water. The infrared spectra were recorded with a Shimadzu 8300-FTIR spectrophotometer.

2.3. Preparation of glycomonomers

2.3.1. Ethyl α-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucosyloxymethyl)acrylate (AcGlEH)

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (7.69 mmol), EHMA (23.08 mmol) and 3 Å powdered molecular sieves (4 g) in dry dichloromethane were stirred at −40 °C under argon atmosphere and silver trifluoromethanesulfonate was added (9.23 mmol). The reaction mixture was stirred under the same conditions for 48 h. After this time, the mixture was gradually allowed to reach room temperature and was then filtered through Celite. The solution was concentrated and the residue was eluted from a column of silica gel (7:3 hexane–ethyl acetate), to give the glycomonomer as a white solid in yield of 48%.

2.3.2. Ethyl α-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactosyloxymethyl)acrylate (AcGaEH)

The mixture after the reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with EHMA in DCM at −40 °C in the presence of AgOTf for 66 h, was purified from a column of silica gel with 7:3 hexane-ethyl acetate. The glycomonomer was obtained as viscous oil (yield 63%).

2.3.3. Deacetylation of glycomonomers

General procedure: to a solution of *AcGlEH* or *AcGaEH* (1.09 mmol) in 3.5 ml of a dry mixed solvent ($CHCl_3$:CH_3OH, 1:2.5 v/v) under argon atmosphere, sodium methoxide in methanol (0.75 ml, 0.03 M) is added and the mixture is stirred at room temperature for 4 h. Then, 1.5 g of cation exchange resin (Dowex 50WX2-200) is added. After 30 min, the mixture is filtered, vacuum-evaporated and the remaining material is purified by column chromatography on silica gel (chloroform:methanol, 8:2). The deprotected glycomonomers, ethyl α-(β-D-glucosyloxymethyl)acrylate, *GlEH*, and ethyl α-(β-D-galactosyloxymethyl)acrylate, *GaEH*, were obtained as solids in 73% and 65% yield, respectively.

2.4. Preparation of glycopolymers

2.4.1. General polymerization procedure

Accurately weighed AIBN, *AcGlEH* or *AcGaEH* and MMA (initial concentration of monomers: 3.0 M and $[AIBN] = 3.0 \times 10^{-2}$ M) and a given amount of chlorobenzene were added to a glass ampoule and degassed down to 5×10^{-3} mmHg residual pressure using several freeze-pump-thaw cycles. The ampoules were sealed and immersed, for the required period of time, in an oil bath at 70 °C. After polymerization, the reaction mixture were cooled down in ice water and diluted with chloroform. The recovered polymers were precipitated into cold methanol under stirring, filtered and dried under vacuum. The conversions were determined gravimetrically.

2.4.2. Deacetylation of glycopolymers

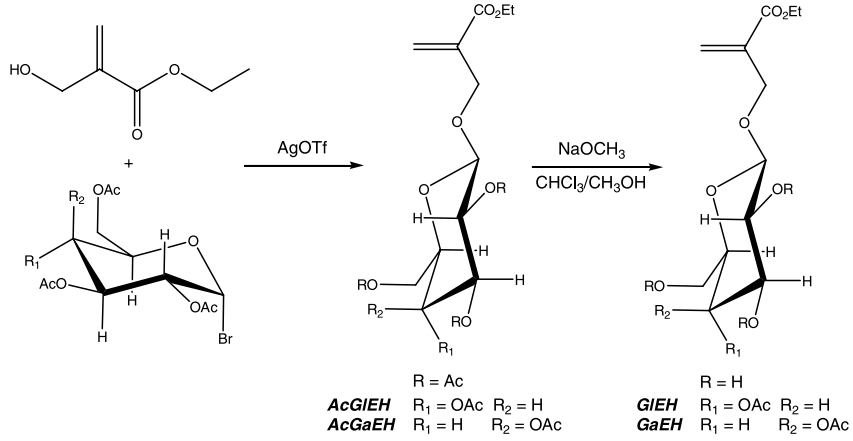
To a solution of protected homopolymer *PAcGlEH* or *PAcGaEH* (0.33 g) in dry mixed solvent ($CHCl_3$:CH_3OH,

3:1 v/v), sodium methoxide in methanol (3.3 mL, 1 M) is added and the mixture is stirred at room temperature for 1 h. Then, 7 mL of water and 3 g of cation exchange resin (Dowex 50WX2-200) is added. After 30 min, the mixture is filtered, and the filtrate was concentrated under reduced pressure until all organic solvents and about half of the water is removed. The solution is poured into a large excess of acetone. The deprotected glycopolymers, poly[ethyl α-(β-D-glucosyloxymethyl)acrylate], (*PGlEH*), and poly[ethyl α-(β-D-galactosyloxymethyl)acrylate], (*PGaEH*), were recovered as solids and dried in vacuum at room temperature (55% and 48% yield, respectively).

3. Results and discussion

3.1. Synthesis of the glycomonomers

As shown in Scheme 1, methacrylate derivatives of glucose and galactose, (*AcGlEH*), and (*AcGaEH*), were synthesized by coupling the glycosyl donors 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, respectively, with the glycosyl acceptor ethyl α-hydroxymethylacrylate (EHMA), using silver trifluoromethanesulfonate as catalyst. Although not commercially available, the hydrophilic



Scheme 1. Glycomonomer syntheses.

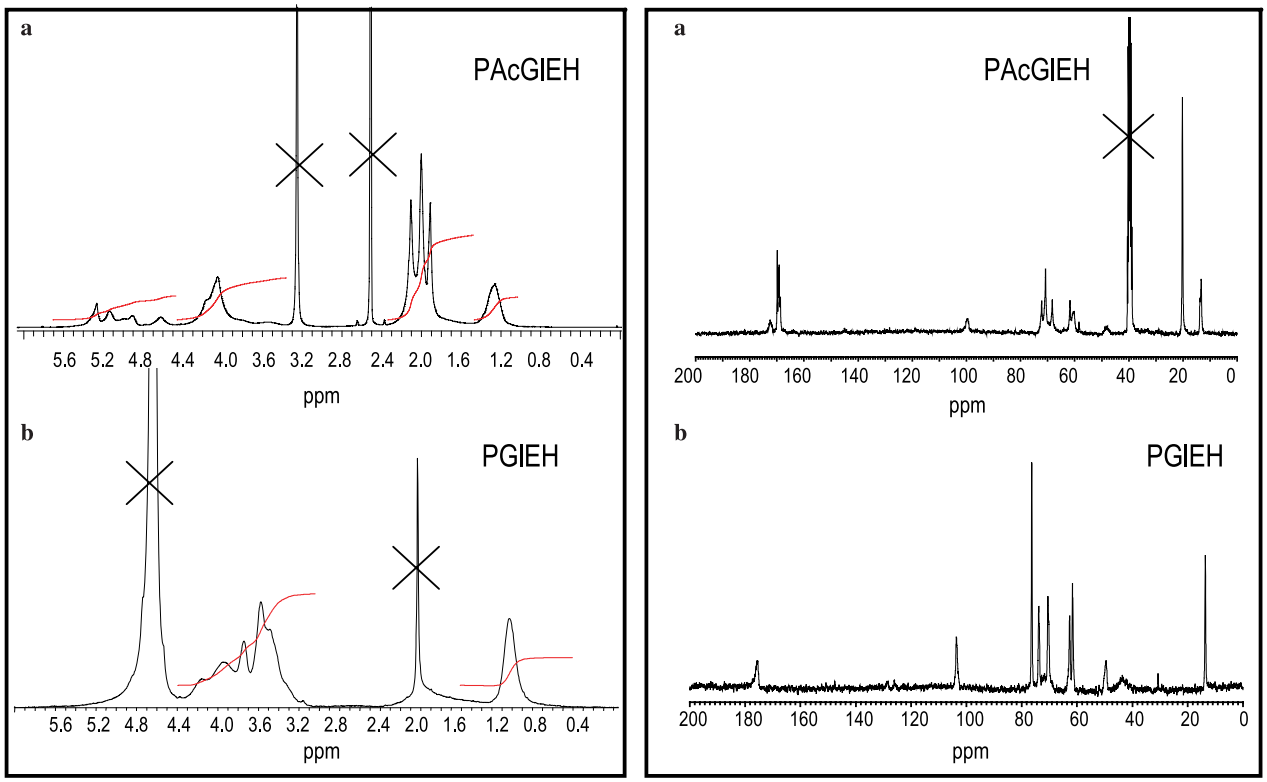


Fig. 1. ^{1}H and ^{13}C-NMR spectra of (a) *PAcGlEH* and (b) *PGlEH*.

monomer was prepared by a modified one-pot literature procedure (Fernández-Monreal et al., 1992). In addition to unreacted EHMA, ethyl α-acetoxymethylacrylate was also presented in each crude product. These were separable from the desired products by column chromatography on silica gel (7:3 hexane-ethyl acetate). In this manner, the protected glycomonomers were obtained and identified by ^{1}H-NMR spectroscopy as the β-anomers. Similar reactions have been reported previously (Ambrosi et al., 2002).

Since the carbohydrate moieties are highly hydrophilic and hence impart water solubility, of main interest in biological applications, methacrylate derivatives (*AcGlEH*) and (*AcGaEH*) were deprotected using a catalytic quantity of sodium methoxide in methanol from a modified literature procedure (Ambrosi et al., 2002). Despite exhaustive deprotecting conditions, the products resulting from the cleavage of the ester bond of the EHMA moiety were not observed. The purification of ethyl α-(β-D-glucosyloxymethyl)acrylate, (*GlEH*), and ethyl α-(β-D-galactosyloxymethyl)acrylate, (*GaEH*), afforded the products as solids in high purity.

Table 1
The glycomonomers molar fraction in the feed, f_{AcGlEH}, f_{AcGaEH}, the average molar fraction compositions of copolymers, F_{AcGlEH}, F_{AcGaEH}, and the final conversion

f_{AcGlEH}	Conv (%)	F_{AcGlEH}	f_{AcGaEH}	conv (%)	F_{AcGaEH}
0.1	2.5	0.07_9	0.1	2.4	0.06_7
0.2	2.4	0.13_9	0.2	2.6	0.11_7
0.3	2.4	0.20_1	0.3	2.9	0.15_5
0.4	2.6	0.23_1	0.4	2.7	0.20_8
0.5	2.5	0.30_9	0.5	2.4	0.27_4
0.6	2.8	0.36_2	0.6	3.0	0.31_1
0.7	2.6	0.41_8	0.7	2.8	0.36_4
0.8	2.9	0.42_6	0.8	3.0	0.43_2
0.9	2.7	0.55_3	0.9	2.9	0.49_5

The FTIR spectrum shows the complete O-deacetylation. The carbonyl absorption peak of acetyl group at 1750 cm^{-1} disappears, while the absorption peak ascribed to the ester bond of the EHMA monomer (1710 cm^{-1}) remains unchanged, and a characteristic broad absorption band corresponding to hydroxyl groups appears about 3400 cm^{-1}. Besides differences characteristic of acetyl removal in the ^{1}H and ^{13}C-NMR spectra (not shown) are observed. Thus, the proton peak at 2.0 ppm and the carbon peaks observed at about 20 and 170 ppm in the glycomonomers *AcGlEH*, and *AcGaEH*, give a clear evidence of the presence of acetyl groups. These signals disappear in the ^{1}H and ^{13}C-NMR spectra of *GlEH* and *GaEH*.

These deprotected glycomonomers can be polymerized in aqueous media, which points up a way to obtain polymers with applications in biomedical and biochemical fields. It will be object of further investigations.

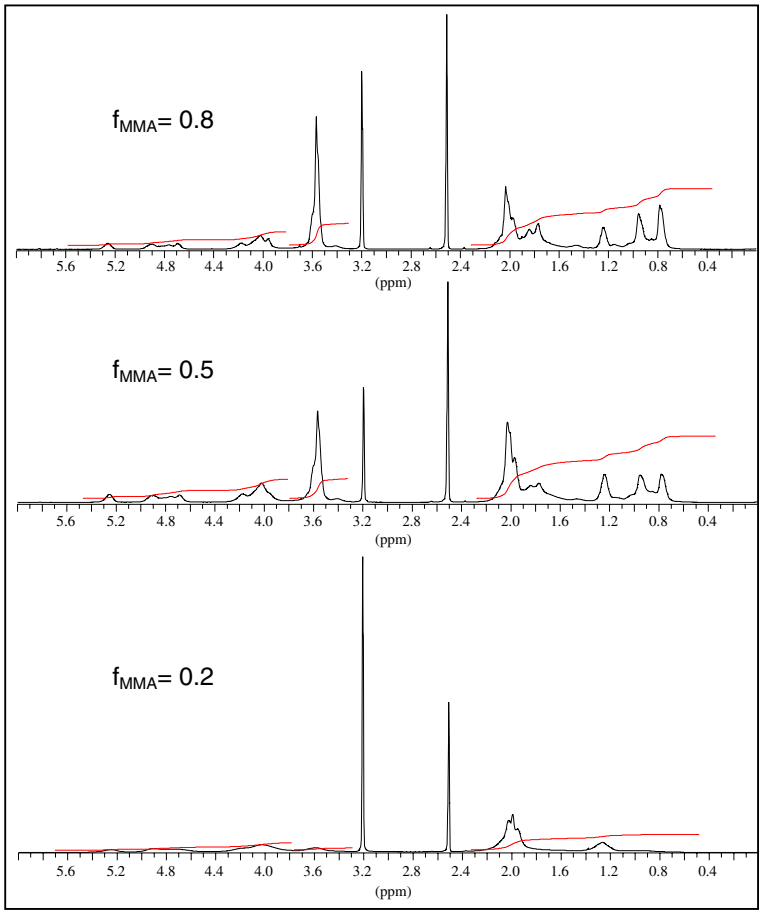


Fig. 2. ^{1}H-NMR spectra of glucose-carrying copolymers with $f_{MMA} = 0.2$, 0.5 and 0.8 obtained in chlorobenzene solution at 70 °C.

3.2. Polymerization reactions

The homopolymerization of the protected glycomonomers leads to poly[ethyl α-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucosyloxymethyl)acrylate], *PAcGlEH*, and poly[ethyl α-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactosyloxymethyl)acrylate], *PAcGaEH*. Thus, the polymerizations were conducted with AIBN by heating at 70 °C, using chlorobenzene as solvent.

Deprotection of the polymers according to the methods used for previous syntheses of poly(glyco-acrylates and -methacrylates) (Liang, Li, Chen, & Li, 1999) results in a complete deacetylation. A comparison between the FTIR spectrum before and after O-deacetylation indicates that the carbonyl absorption peak due to acetyl groups (1750 cm^{-1}) disappears whereas a broad adsorption band at 3400 cm^{-1} corresponding to hydroxyl groups is observed. The absorption peak owing to the ester bond remains at 1710 cm^{-1}.

Fig. 1 shows the proton and carbon NMR spectra of the synthesized containing glucose polymers. The signals due to methyl protons of the protecting acetyl groups vanishes in the ^{1}H-NMR spectra of (*PGlEH*) and (*PGaEH*) (range δ 1.8–2.3 ppm). No traces of residual protecting groups are also presented in the ^{13}C-NMR spectra (peaks in the range δ 20–21 ppm and δ 165–170 ppm). Therefore, under the present conditions, the O-protecting acetyl groups are removed quantitatively, while the ester bond connecting the main chain remains unaffected.

The copolymerization reactions of the glycomonomers, *AcGlEH*, and *AcGaEH*, with MMA at 70 °C in chlorobenzene solutions were performed at low conversions. Total monomer concentration was 3.0 M and the glycomonomer molar fraction in the feed was varied from 0.1 to 0.9. The copolymers were obtained at low degree of conversion (below 3%, Table 1) to satisfy the differential copolymerization equation (Mayo & Lewis, 1944). The average molar fraction composition of copolymers was quantitatively determined from the corresponding ^{1}H-NMR spectra of copolymer samples prepared with different monomer feeds. The analysis was performed from the relative areas of the signals assigned to eleven protons of the glucose or galactose-carrying monomers (5.5–3.8 ppm) and the peaks correspond to fifteen glycomonomer and eight MMA protons (3.8–0.5 ppm). Fig. 2 shows ^{1}H-NMR spectra of AcGlEH-MMA copolymers with different compositions. The glycomonomer molar fraction in the feed, f_{AcGlEH} or f_{AcGaEH}, and the average molar fraction composition of copolymers, F_{AcGlEH} or F_{AcGaEH}, are collected in Table 1. The monomer reactivity ratios have been determined from the average composition of copolymers through the nonlinear least-squares analysis suggested by Tidwell and Mortimer (Tidwell & Mortimer, 1965). The obtained values for AcGlEH-MMA system are $r_{AcGlEH} = 0.049$ and $r_{MMA} = 1.372$ and $r_{AcGaEH} = 0.020$ and $r_{MMA} = 1.819$ for AcGaEH-MMA system. This indicates that MMA radical has higher tendency to react with galactose-carrying vinyl monomer than with glucose one. Fig. 3a shows the accuracy of the estimated data where the 95% joint confidence intervals are drawn. For these systems a fundamental statistical copolymer rich in methyl methacrylate units can be expected at low conversions. At high conversions most of the glycomonomer molecules will polymerize, giving rise to copolymer chains with relatively long blocks of glycomonomer units. The copolymer composition for the different molar fractions in the feed is shown in Fig. 3b. The theoretical curves were calculated with the obtained monomer reactivity ratios using the integrated copolymer composition equation. It can be seen that the experimental data are in agreement with the Mayo-Lewis terminal model in both systems. It is important to remark that the behavior of both copolymer systems is quite similar, as can be expected.

Preliminary qualitative studies of deprotected homopolymers demonstrate that glucose-carrying polymer does not show interaction with *ConA* in aqueous buffer solution

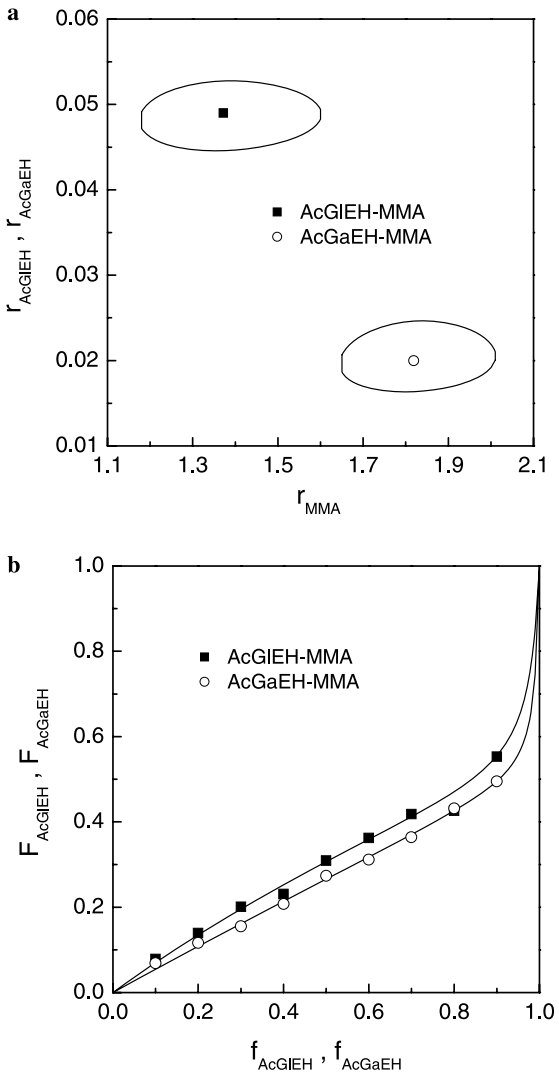


Fig. 3. Copolymerization of AcGlEH-MMA and AcGaEH-MMA systems in chlorobenzene solutions at 70 °C. (a) The 95% joint confidence interval of monomer reactivity ratios. (b) The experimental composition data and the calculated theoretical curves.

(pH 7.2), since *PGlEH* contains the β-anomer of glucose. While *PGaEH* is effectively recognized by the galactose-specific lectin, RCA_{120} at pH 7.8.

4. Conclusions

In conclusion, novel monomeric and polymeric methacrylate derivatives bearing β-D-glucopyranoside and β-D-galactopyranoside residues have been successfully synthesized, in an efficient stereocontrolled manner, and characterized. It has been shown that deprotection of monomers and polymers according to the method used results in a complete deacetylation. The copolymerization of these glycomonomers with methyl methacrylate has been successfully described by the terminal model. Therefore, we consider that these glucose and galactose-carrying vinyl monomers may be useful for the synthesis of a variety of glycopolymers architectures with potential biological and biomedical relevance.

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