Macromolecules

Article

pubs.acs.org/Macromolecules

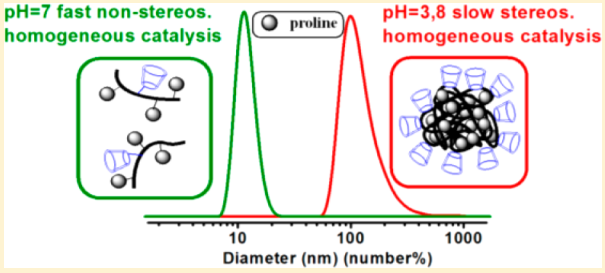
Water-Soluble Pendant Copolymers Bearing Proline and Permethylated β-Cyclodextrin: pH-Dependent Catalytic Nanoreactors

Elisa G. Doyagüez,[†] Juan Rodríguez-Hernández,[‡] Guillermo Corrales,[†] Alfonso Fernández-Mayoralas,[†,*] and Alberto Gallardo[‡,*]

[†]Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain

[‡]Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain

ABSTRACT: To achieve efficient proline-based catalysis in water, proline has been supported in the past to porous and hydrophobic solid resins leading to heterogeneous systems. These solid resins provide a hydrophobic environment to the active centers, mimicking what happens in natural enzymes. However, a more realistic mimetic approach would be to carry out the aldol reaction in a homogeneous way, maintaining the hydrophobic environment, using for example properly designed noncross-linked polymer carriers. In this work, we report the synthesis and aqueous catalytic evaluation of a linear copolymer bearing both pendant proline and permethylated β-cyclodextrin (β-CD) groups. It was designed on the basis that the presence of the hydrophobic cavity of the β-CD could bring aromatic substrates into close proximity to the surrounding catalytic proline residues through host−guest interactions. The compound is water-soluble and catalyzes aldol reactions in this medium without the need for any extra organic solvent. We employed a model reaction between cylohexanone and *p*-nitrobenzaldehyde, and we observed a decrease of the reaction rate when a competing aromatic compound, known to form a strong inclusion complex with β-CD, was added. The copolymeric catalyst showed a pH-dependent behavior. At pH 7, the copolymer is found in solution as extended single chains with negative charge, catalyzing the reaction in a fast and nonstereoselective mode. At the isoelectric point (pH 3.8) where the positive and negative charges of the zwitterionic proline are canceled by forming charge complexes, the copolymer forms multichain hydrophobic nanoaggregates most probably stabilized by the permethylated β-CD. Although the reaction inside these "nanoreactors" is slower, it exhibits high stereoselectivity. It is proposed that the observed stereoselectivity is caused by the exclusion of water from the core of these homogeneous entities.



INTRODUCTION

It is well-known that proline catalyzes aldol reactions.[1,2] Proline derivatives such as hydroxyproline, bearing an extra functional group for conjugation, have been used to support proline mainly on polymeric entities.[3,4] Some of the most representative polymeric supports are the solid resins. These catalysts are basically porous beads of highly cross-linked polymers (polystyrene-PS or acrylics),[5] which obviously catalyze in a heterogeneous mode. Noncross-linked polymer supports are also feasible, such as the well-known linear polymer polyethylenglycol (PEG), which is soluble in many different media. The use of soluble polymeric carriers such as PEG derivatives allows the reaction to be performed in homogeneous conditions.[6] Besides, different strategies for separation can be applied to these soluble supports profiting from their macromolecular nature.[7]

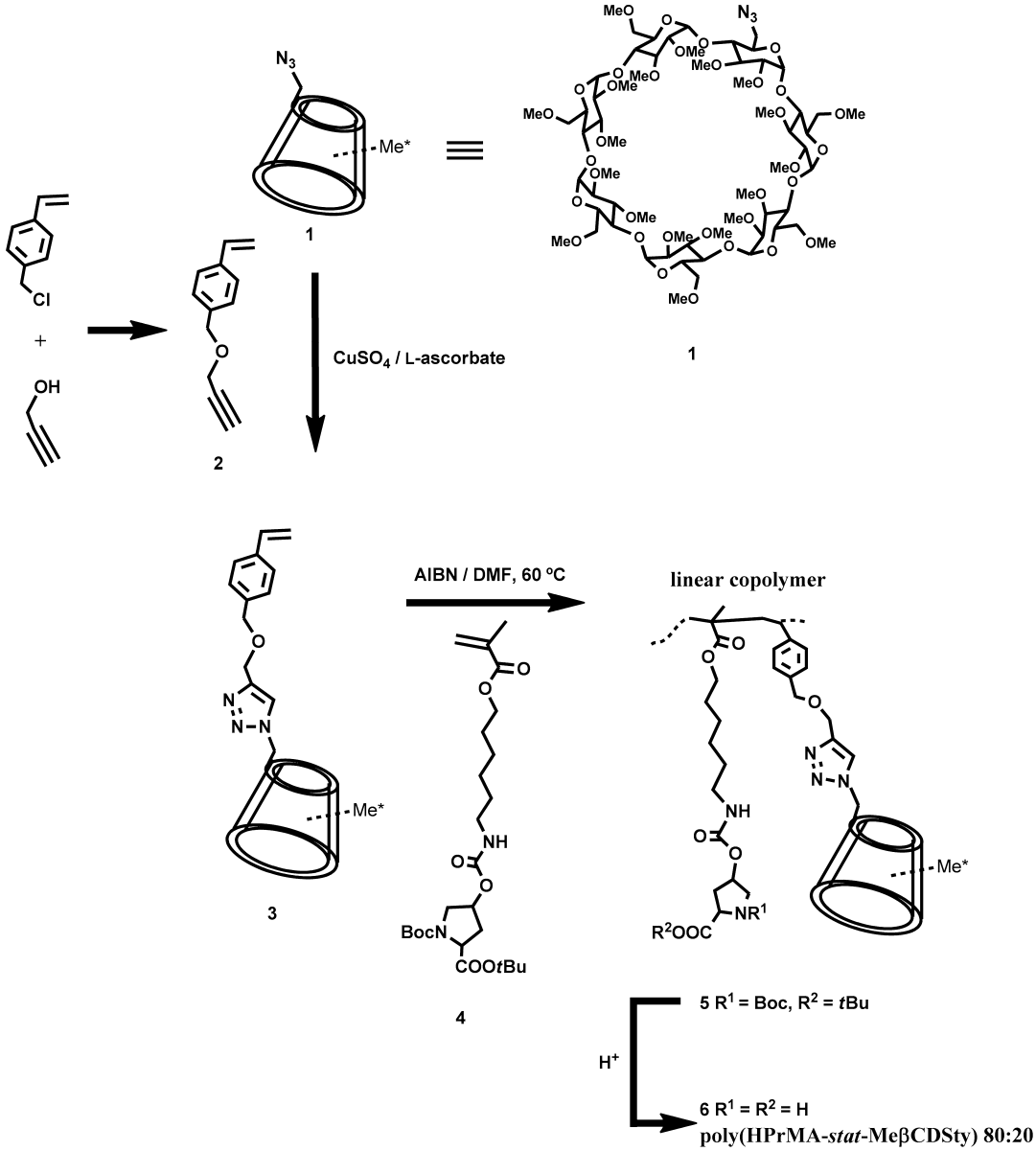
The use of polymer supports (cross-linked or un-cross-linked), not only facilitates the recovery and recycling of the catalyst[8] but it may also enlarge the application of proline-catalyzed reactions from nonpolar solvents to water. Particularly relevant is the use of water from a green chemistry perspective. Proline works properly in polar media but not in water where it exhibits poor efficiency despite its good solubility.[9,10] Recent works have shown that good efficiencies in water may be achieved through "hydrophobic activation", linking proline derivatives to hydrophobic moieties.[11] Thus, some of the hydrophobic porous solid resins exhibited excellent catalytic properties in water and lead to aldol products in high yields and stereoselectivity.[12−14] These resins actually provide hydrophobic environments next to the active proline mimicking at least to some extent natural enzymes that generally make use of a hydrophobic "pocket" at the active center.

This "hydrophobic activation" applies also to soluble polymeric supports. Recently, Meijer et al. highlighted that in order to create a versatile synthetic catalyst the polymer should be compartmentalized to create a catalytic core.[15] In line with this study, we have reported recently that water-soluble linear copolymers of hydroxyproline methacrylate and styrene (that

Received: August 1, 2012
Revised: September 18, 2012
Published: September 25, 2012

   dx.doi.org/10.1021/ma301615a | *Macromolecules* 2012, 45, 7676−7683

Scheme 1. Synthesis of the Monomers and Copolymer (Me* Stands for Permethylated)



have a tendency to form alternating sequences) catalyzes the reaction in water with a high rate while the homopolymers without styrene are inactive.[16,17] The increased activity was explained by hydrophobic interactions between the phenyl ring of the styrene moiety and the substrates.[16] A similar hydrophobic substituent effect on proline catalysis in water, supported by quantum mechanical calculations, has recently been described by Schafmeistes et al.[18] These copolymers however did not exhibit stereoselectivity in pure homogeneous mode (soluble individual chains), although aggregates obtained by adding $MgCl_2$ indeed worked stereoselectively, probably by formation of hydrophobic regions where the reaction takes place with the exclusion of water.[16] This is in agreement with the role of water since water alters the highly organized transition states that are thought to be responsible for the stereoselectivity.[19−21] Unlike the coupling to preformed PS-resins or PEG conjugates, the above-mentioned water-soluble copolymers of hydroxyproline methacrylate and styrene were prepared using a "bottom-up" methodology, that is, acrylic proline-monomers were prepared in a first step and then copolymerized. The first examples of the preparation of supported prolines using this 'bottom-up' approach were described recently by us[17] and by Hansen et al.[22] This approach gives high flexibility to the synthesis since the properties of the chains may be easily modulated just by choosing the right comonomers in the right ratio. In this work, we have used this flexibility to design a new family of water-soluble polymer catalysts. Our previous studies indicated that the presence of hydrophobic pockets is needed for the reaction to proceed. Thus, in this work we have designed a new styrenic monomer bearing permethylated β-cyclodextrin (β-CD) to be copolymerized with a hydroxyproline methacrylate which has been previously reported.[17] It is well-known that cyclodextrins have the ability to form inclusion complexes with organic compounds in water, due to the hydrophobic character of their internal cavity.[23,24] There are a few examples describing organocatalysts that combine the catalytic properties of proline with the capabilities of cyclodextrin to form inclusion

dx.doi.org/10.1021/ma301615a | Macromolecules 2012, 45, 7676−7683

complexes.[25−31] The permethylated structure of the CD, instead of the nonpermethylated, has been chosen to tip the amphiphilic balance in favor of the hydrophobicity. In this work, the bottom-up synthesis and catalytic evaluation in water of linear copolymers incorporating a 20 mol % of cyclodextrin, are described.

EXPERIMENTAL SECTION

General Remarks. 2,2′-Azobis(isobutyronitrile) (AIBN, Merck) was recrystallized twice from ethanol. Other chemicals purchased from commercial suppliers were of analytical purity or purified by standard techniques. Thin-layer chromatography (TLC) was performed on aluminum sheets 60 F_{254} Merck silica gel and compounds were visualized by irradiation with UV light and/or by treatment with a solution of Ce_2MoO_4 in water, a solution of ninhydrin in *n*-BuOH/EtOH or H_2SO_4 (5%) in EtOH followed by heating. Flash chromatography was performed using thick walled columns, employing silica gel (Merck 60: 0.040−0.063 nm). NMR (^{1}H, ^{13}C NMR) spectra were recorded on a 300 MHz (Inova 300 or Bruker 300) and 400 MHz (Inova 400 or Mercury 400) spectrometers, using $CDCl_3$ or D_2O as solvents at room temperature. Chemical shift values are reported in parts per million (δ) relative to tetramethylsilane (TMS) in ^{1}H and $CDCl_3$ (δ =77.0) in ^{13}C NMR. Coupling constants (J values) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbol: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Diastereomeric and enantiomeric excess were calculated by NMR and HPLC Dionex P680 with DAD detector (lecture at 254 nm). Mass spectra were recorded on a HP series 1100 MSD spectrometer or in an Agilent 6250 Accurate Mass Q-TOF spectrometer.

Gel permeation chromatography (GPC) analyses were carried out using a Perkin-Elmer apparatus with an isocratic pump serial 200 connected to a differential refractometric detector (serial 200a). Two Resipore columns (Varian) were conditioned at 70 °C and used to elute the samples (1 mg/mL concentration) at 1 mL/min. HPLC-Grade N,N-dimethyl formamide (DMF) supplemented with 0.1% v/v LiBr was used as eluent. Calibration of SEC was carried out with monodisperse standard polystyrene samples in the range of 2.9 × 10^3 to 480 × 10^3 obtained from Polymer Laboratories.

DLS experiments were carried out using a Malvern Zetasizer (Zetasizer NS Malvern Instruments, Malvern, UK), working at a scattering angle of 173° relative to the source. This apparatus is equipped with a 4 mW He/Ne laser emitting at 633 nm, a measurement cell, an autocorrelator and a photomultiplier. The measurements were carried out in the fully automatic mode. Intensity auto correlation functions were analyzed by a General Purpose Algorithm (integrated in the Malvern Zetasizer software) in order to determine values of ζ potential (in mV) and ζ average diameter (in nm). The measurements were carried out using a 50 mM PBS solution. To study the influence of the polymer concentration on the aggregation we employed three different concentrations 25 mM (5.5 mg/mL), 100 mM (19.6 mg/mL), and 330 mM (73.3 mg/mL). The influence of the pH on the size was evidenced measuring the polymer samples in pH 7 and pH 3.8. Moreover, the isoelectric point was determined by ζ potential measurements varying the solution pH between 2.4 and 11.

The turbidity change of the aqueous solutions of the polymers (2 mg/mL) as a function of pH was monitored measuring the absorbance at 600 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 of NaCl and 0.1 M of NaOH. A standard aqueous solution 1 M of HCl was delivered stepwise. pH was monitored with a Beckman 40 pH-Meter (Beckman Instruments, Fullerton, CA).

Synthesis. The synthesis of the protected hydroxyproline methacrylate **4** and the permethylated 6^I-azido-6^I-deoxi-β-cyclodextrin (**1**)—used as precursor of the styrenic compound **3**—are described elsewhere.[16,28] The structure of the monomers can be found in Scheme 1.

Propargyl 4-Vinylbenzyl Ether (2). To a solution of propargyl alcohol (224 mg, 4 mmol) in THF (5 mL), sodium hydride (150 mg, 6.25 mmol) was added, and the mixture was stirred for 15 min in an ice bath. After this time, reaction was let to reach room temperature and 4-vinylbenzyl chloride (610 mg, 4 mmol) and tetrabutylammonium bromide (750 mg, 2.36 mmol) were added, and the reaction was stirred at room temperature for 5 h. Then, methanol (0.5 mL) was added to eliminate the excess of sodium hydride, and after, dichloromethane (40 mL). The mixture was washed with water (3 × 15 mL), and the organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (hexane:EtOAc, 6:1) to give 2 (420 mg, 59%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.5−7.2 (m, 4H, CH−Ar), 6.63 (dd, 1H, J = 10.9 Hz, 17.8 Hz, CH=CH_2), 5.78 (d, 1H, J = 15.7 Hz, CH=CH_2), 5.30 (d, 1H, J = 15.7 Hz, CH=CH_2), 4.65 (s, 2H, ArCH_2O), 4.20 (s, 2H, ArCH_2OCH_2), 2.51 (s, 1H, C≡CH). ^{13}C NMR (75 MHz, CD_3OD): δ 137.5 (C−Ar), 137.1 (C−Ar), 136.7 (CH=CH_2), 128.6 (CH−Ar), 126.5 (CH−Ar), 114.2 (CH=CH_2), 79.8 (C≡CH), 74.9 (C≡CH), 71.5 (ArCH_2O), 57.2 (ArCH_2OCH_2). HRMS (ESI) m/z (%): calcd for: m/z $C_{12}H_{12}ONa$ $[M + Na]^+$, 195.0786; found, 195.0781. Anal. Calcd (%) for $C_{12}H_{12}O$: C, 83.69; H, 7.02. Found: C, 83.22; H, 6.73.

Monomer MeβCDSty (Compound 3). To a solution of permethylated 6^I-azido-6^I-deoxi-β-cyclodextrin (1.970 mg, 0.673 mmol) in DMF:H_2O (1:1, 42 mL) were added propargyl 4-vinylbenzyl ether (2.191 mg, 1.108 mmol), $CuSO_4$·$5H_2O$ (185 mg), and sodium L-ascorbate (156 mg, 0.873 mmol) successively. The mixture was stirred at 80 °C for 35 min in a microwave. After this time, the mixture was diluted with water, and it was extracted with CH_2Cl_2 (4 × 10 mL). The organic layer was dried (Na_2SO_4) and solvent was evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc:MeOH, 20:1) to give 3 (0.87 g, 80%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.63 (s, 1H, NCH=C), 7.35 (d, 2H, J = 8.19 Hz, CH−Ar), 7.27 (d, 2H, J = 8.19 Hz, CH−Ar), 6.67 (dd, 1H, J = 10.9 Hz, 17.6 Hz, CH=CH_2), 5.71 (dd, 1H, J = 0.98 Hz, 17.6 Hz, CH=$CH_{2\ trans}$), 5.26 (d, 1H, J = 3.51 Hz, CH−anom), 5.22 (dd, 1H, J = 0.78 Hz, 10.9 Hz, CH=$CH_{2\ cis}$), 5.15−5.05 (m, 6H, CH−anom), 4.63 (s, 2H, ArCH_2OCH_2), 4.55 (s, 2H, ArCH_2O), 3.9−3.7 (m, 10H, CH and CH_2 CD), 3.6−3.5 (m, 32H, CH and CH_2 CD, OMe), 3.5−3.4 (m, 34H, CH and CH_2 CD, OMe), 3.4−3.2 (m, 14H, CH and CH_2 CD, OMe), 3.2−3.1 (m, 12H, CH and CH_2 CD). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.5 (C-triazol), 137.2 (C−Ar), 137.1 (C−Ar), 136.3 (CH=CH_2), 128.0 (CH−Ar), 126.2 (CH−Ar), 124.8 (CH−triazol), 113.9 (CH=CH_2), 99.1−98.7 (CH−anom), 82.0−81.0 (CH CD), 80.2−77.2 (CH CD), 72.2 (ArCH_2O), 71.3−70.2 (CH_2−CD), 63.6 (CH_2 triazol), 61.6−

dx.doi.org/10.1021/ma301615a | *Macromolecules* 2012, 45, 7676−7683

61.2 (CH CD), 59.1−58.3 (CH CD). HRMS (ESI) *m/z* (%): calcd for $C_{74}H_{121}N_3O_{35}Na$ $[M + Na]^+$, 1634.7678; found, 1634.7691.

General Polymerization Procedure. Protected copolymer poly(*p*HPrMA-*stat*-Me*β*CDSty) 80:20 (compound **5**) was prepared by free radical polymerization in *N*,*N*-dimethyl formamide (DMF) at 60 °C for 24 h using AIBN as initiator. The total concentration of comonomers was 0.5 mol/L and the initiator concentration was 0.025 mol/L. Example of recipe: 50.4 mg, 62.3 mg and 1.3 mg of **3**, **4**, and AIBN, respectively, were dissolved in 0.5 mL of DMF. Reactions were carried out in the absence of oxygen by gently bubbling nitrogen for 20−30 min before sealing the system. After 24 h, the reaction mixture was poured into water, and the resulting precipitate was dried under vacuum overnight. Poly(*p*HPrMA$_{4n}$-*stat*-Me*β*CDSty$_n$) was obtained as a white solid (82%).

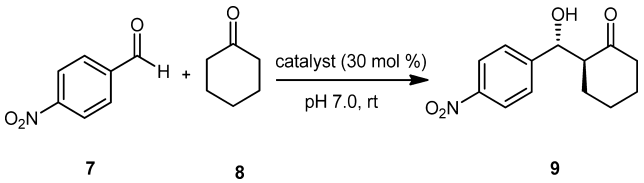
^{1}H NMR (300 MHz, $CDCl_3$, 298 K): *δ* 7.7−7.6 (wide singlet, *n* × 1H, triazol), 7.2−7.0 (wide singlet, Ar), 7.0−6.8 (wide singlet, Ar), 5.5−5.3 (m, 4*n* × 1H, H-4pro), 5.2−5.0 (m, *n* × 7H, CH-anom), 4.7−4.4 (m), 4.2−4.1 (m) 4.0−2.8 (m, *n* × 96H, 20 × OCH_3, CH-2^{I-VII}, CH-3^{I-VII}, CH-4^{I-VII}, CH_2-6^{I-VII} (CD), CH_2CHAr; 4*n* × 6H, $OCH_2CH_2CH_2CH_2$-CH_2CH_2NH, CH_2-5pro), 2.4−2.2 (m, 4*n* × 1H, CH_2-3$_A$pro), 2.2−2.0 (m, 4*n* × 1H, CH_2-3$_B$pro), 2.0−0.5 (m, *n* × 2H, CH_2CHAr; 4*n* × 31H, CH_2CMe, $OCH_2CH_2CH_2CH_2CH_2$-CH_2NH, CH_3 ^{t}Bu, CH_3 methacryl). GPC data: M_n = 33000 g/mol. PI (polydispersity index) = 2.8.

General Deprotection Procedure. Poly(*p*HPrMA-*stat*-Me*β*CDSty) (compound **5**) was dissolved in 1:2 dichloromethane/trifluoroacetic acid (2 mL per 100 mg of polymer) and the mixture was stirred for 24 h. After this time, the reaction mixture was concentrated and diethyl ether was added. The precipitate was decanted, washed with diethyl ether (3×), and dried under vacuum. The solution was dialyzed in distilled water for 1 week. Polymer was recovered by freezing and lyophilization. Poly(HPrMA$_{4n}$-*stat*-Me*β*CDSty$_n$) (compound **6**) was obtained as a white solid (100%).

^{1}H NMR (300 MHz, D_2O, 298 K): *δ* 8.1−7.8 (wide singlet, *n* × 1H, triazol) 7.4−6.9 (wide singlet, *n* × 4H, Ar), 5.2−5.0 (m, *n* × 7H, CH-anom), 4.2−3.9 (m), 3.8−2.7 (m), 2.5−2.2 (m, 4*n* × 1H, CH_2-3$_A$pro), 2.2−2.0 (m, 4*n* × 1H, CH_2-3$_B$pro), 2.0−0.5 (m, n x 2H, CH_2CHAr, 4*n* × 13H, CH_2CMe, $OCH_2CH_2CH_2CH_2CH_2NH$, CH_3 methacryl).

General Procedure for Asymmetric Aldol Reaction. To a solution of polymer (30 mol %, i.e. amount of proline on the polymer relative to *p*-nitrobenzaldehyde reactant) in phosphate buffer (50 mM, pH 7 or 3.8) (it could require sonication for complete solubilization), *p*-nitrobenzaldehyde (**7**) and cyclohexanone (**8**, 2 equiv) were added. Depending on the desired concentration, the quantities of the substrates were as follows: (1) 0.01 mmol of aldehyde (25 mM) and 0.02 mmol of ketone in 0.4 mL of buffer; (2) 0.01 mmol of aldehyde (100 mM) and 0.02 mmol of ketone in 0.1 mL of buffer; (3) 0.033 mmol of aldehyde (330 mM) and 0.165 mmol of ketone in 0.1 mL of buffer. The mixture was stirred at room temperature for the time indicated in Tables 1 and 2. After the specified time elapsed, water was added (1 mL) and the mixture was extracted with dichloromethane (3 × 1 mL). The organic phase was concentrated under reduced pressure. Conversions and stereoselectivities are summarized in Tables 1 and 2. In the experiments carried out with an inhibitor, sodium 2-naphthalenesulfonate (2 equiv., 0.02 mmol) was also added (Table 2).

Table 1. Aldol Reaction between 7 and 8 Using Copolymer Poly(HPrMA-*stat*-Me*β*CDSty) 80:20 as Catalyst (30 mol %) at pH 7.0



7 8 9

entry	aldehyde 7 (mmol/L)	time (h)	yield (%)[a]	anti:syn[a]	ee (%)[b]
1	25	4.5	43	1.5:1	2
		7	55	1.1:1	2
2[c]	25	4.5	19	1.5:1	4
		7	21	1.8:1	8
3	100	4.5	70	1.4:1	6
		7	91	1.4:1	18
4	330	20	56	>20:1	96

[a]Determined by NMR and HPLC. [b]Determined by HPLC. They are referred to the major isomer. [c]Same conditions as entry 1 except that sodium 2-naphthalenesulfonate (2 mol equiv with respect to the aldehyde) was added.

Table 2. Aldol Reaction between 7 (100 mM) and 8 (200 mM) Using Copolymer Poly(HPrMA-*stat*-Me*β*CDSty) 80:20 as Catalyst (30 mol %) in PBS (50 mM) at pHs 3.8 and 7.0

entry	pH	time (h)	yield (%)[a]	anti:syn[a]	ee (%)[b]
1	7.0	4.5	67	1.4:1	6
		7	91	1.4:1	18
2	3.8	24	26	>20:1	>99
		48	41	5:1	>99
3[c]	7.0	4.5	15	1.9:1	18
		7	22	1.3:1	14
4[c]	3.8	24	6	>20:1	>99
		48	9	>20:1	>99

[a]Determined by NMR and HPLC. [b]Determined by HPLC. They are referred to the major isomer. [c]Reaction was carried out in the presence of sodium 2-naphthalenesulfonate (2 mol equiv with respect to the aldehyde).

(2*S*,1′*R*)-2-(Hydroxy(4-nitrophenyl)methyl)-cyclohexan-1-one (9). ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): *δ* (major isomer) 8.19 (d, *J* = 8.7 Hz, 2H, ArH), 7.49 (d, *J* = 8.7 Hz, 2H, ArH), 4.88 (d, *J* = 8.4 Hz, 1H, CHOH), 4.02 (wide singlet, 1H), 2.63−2.54 (m, 1H), 2.50−2.33 (m, 1H), 2.31−2.21 (m, 1H), 2.14−2.06 (m, 1H), 1.83−1.79 (m, 1H), 1.73−1.52 (m, 3H), 1.49−1.28 (m, 1H). All spectroscopic data were in agreement with reported values.[32,33] Retention time (HPLC, Daicel Chiralpak AD-H, hexane/*i*-PrOH = 80:20, flow rate 0.5 mL/min, *λ* = 254 nm): t_R = 23.79 (*syn*, minor), t_R = 25.40 (*syn*, major), t_R = 27.30 (*anti,* minor), 34.60 (*anti,* major).

RESULTS AND DISCUSSION

The structure of the two monomers as well as a schematic detail of a dyad along the statistical copolymer are shown in Scheme 1. The synthesis of the styrenic monomer Me*β*CDSty (**3**) was carried out by a copper catalyzed azide−alkyne Huisgen's cyclization between the permethylated *β*-cyclodextrin (Me*β*-CD) functionalized with azide **1** and the alkyne **2**, obtained by etherification of propargyl alcohol with 4-vinylbenzyl chloride.

dx.doi.org/10.1021/ma301615a | *Macromolecules* 2012, 45, 7676−7683

The synthesis of the protected hydroxyproline methacrylate *p*HPrMA (**4**, Scheme 1), to be used as comonomer, has been described previously.[17] This methacrylate contains an aliphatic and flexible spacer with six carbons to facilitate the interaction of the catalytic proline residue with the CD-cavity. The hydrophobic nature of the spacer together with the permethylated structure of the CD (instead of the hydroxylated CD) has been chosen to tip the amphiphilic balance in favor of the hydrophobicity. It has to be also noted that the Me*β*CD is linked to the macromolecular backbone through a spacer with two aromatic rings. Due to the large size of the cyclodextrin moiety, a monomer ratio HPrMA: Me*β*CDSty of 4:1 (molar) was chosen for this study to balance the proline load and the presence of the hydrophobic cavity. A facile standard radical copolymerization of the two units (see Experimental Section) results in the statistical copolymers indicated in the Scheme, which exhibit a tendency to form alternating sequences. This tendency guarantees that most of the CD cavities are surrounded by proline methacrylates, which is the catalytic residue, thus optimizing the possible synergistic effect of both structures. According to our previous analysis, the population of (Me*β*CDSty)−(Me*β*CDSty) dyads is negligible.[16] ^{1}H NMR spectra of the copolymer before and after deprotection are shown in Figure 1.

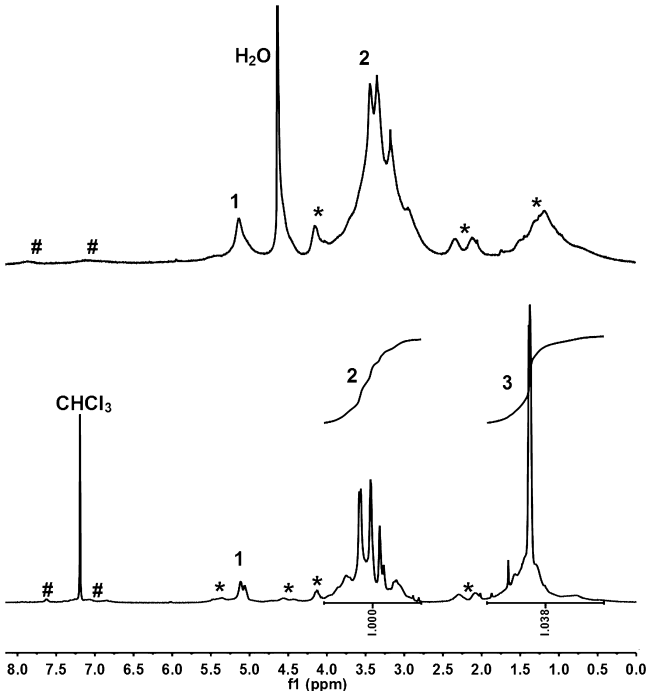


Figure 1. ^{1}H NMR spectrum of **5** (bottom) and **6** (up), protected and unprotected copolymeric forms respectively, see Scheme 1. 1 = anomeric protons of the CD (7H). 2 and 3 = groups of peaks used to determine the molar fractions as described in the text. ∗ and # = other peaks of the *p*HPrMA/HPrMA and Me*β*CDSty units respectively.

Due to the presence of *tert*-butyl groups and permethylated cyclodextrin, *p*HPrMA and Me*β*CDSty have a high amount of protons in two differential regions of the spectrum: 4.0−2.8 ppm (integral 2 of the figure has 96 and 6 protons of the Me*β*CDSty and *p*HPrMA units respectively) and 2.0−0.5 ppm (integral 3 of the figure corresponding to 2 and 31 protons of the Me*β*CDSty and *p*HPrMA units, respectively). This allows for the determination of the molar fraction of the copolymer with relatively high accuracy, according to:

$$A_2 = 96H_{\mathrm{Me}\beta\mathrm{CDSty}} + 6H_{p\mathrm{HPrMA}}$$

$$A_3 = 2H_{\mathrm{Me}\beta\mathrm{CDSty}} + 31H_{p\mathrm{HPrMA}} \qquad ([1])$$

Using this formula, a molar fraction of Me*β*CDSty, $f_{\mathrm{Me}\beta\mathrm{CDSty}}$, of 0.21 has been obtained, which is close to the molar fraction of the feed, $F_{\mathrm{Me}\beta\mathrm{CDSty}} = 0.20$, indicating that both components are incorporated into the copolymers.

The molecular weight of the copolymer was characterized by GPC in the protected form, using polystyrene standards as a reference. The number average molecular weight and polydispersity index were found to be 33000 g/mol and 2.8, which are expected values in conventional radical polymerizations.

The copolymer, which is soluble in water, was catalytically tested in an aqueous buffer at pH 7, in the aldol reaction between *p*-nitrobenzaldehyde **7** and cyclohexanone **8** (2 mol equiv with respect to the aldehyde) as a model reaction (scheme of Table 1). Table 1 shows the results obtained at this pH using different concentrations of the aldehyde.

The copolymer is active and the rate of the aldol reaction is reasonably good; in diluted conditions (25 mM of aldehyde, entry 1) it reaches a yield of 55% after 7 h. At higher concentrations of reactants and catalyst (100 mM of aldehyde, entry 2) the reaction is obviously faster with 91% conversion after 7 h. Both reactions are non stereoselective. Since the homopolymer poly(HPrMA) did not exhibit any significant activity,[17] these results must be attributed to the Me*β*CDSty and its binding capacity. A complementary experiment has evidenced the role of the CD cavity. The addition to the media of the aromatic sodium 2-naphthalenesulfonate (entry 2), which is known to form an inclusion complex with *β*-CD,[34,35] decreases the reaction rate. Therefore, the cavity of the *β*-CD plays a role in the catalytic function of the polymer in water, probably by binding the aromatic aldehyde substrate and bringing it close to the catalytic proline unit.

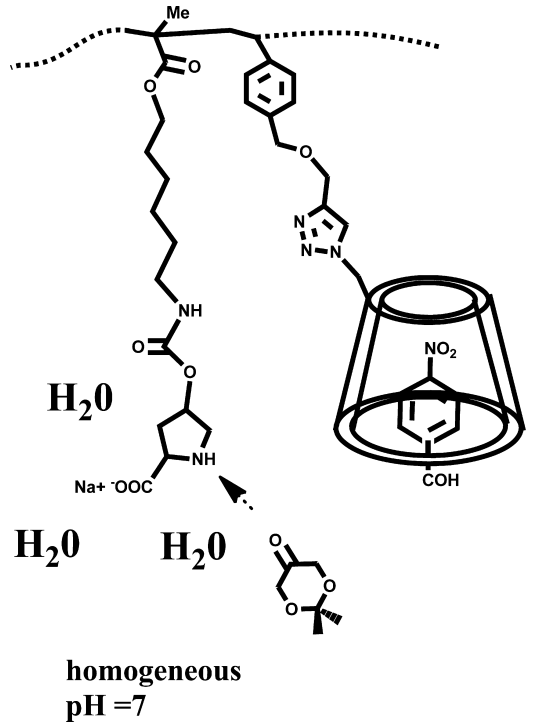
It is remarkable that under conditions where the catalytic polymer is not soluble (at 330 mM of aldehyde, entry 4) the reaction is much slower but stereoselective. This result can be rationalized as the reaction takes place in water-free hydrophobic domains of a thick paste; the exclusion of water in these regions favors the stereoselectivity of the reaction as discussed below. On the other hand, at 25 and 100 mM of aldehyde the copolymer is soluble and water may influence the process. The cartoon shown in Scheme 2 is proposed to explain the results of the soluble forms at pH 7. The data previously discussed indicates that the CD cavity has an active role, probably forming inclusion complexes with the aromatic aldehyde. According to our previous studies[16,17] the polymer has a net negative charge at this pH 7; therefore the anions along the chains and their solvation may cause H_2O to be located in the active center, probably influencing the transition states and preventing stereoselectivity.

The copolymer behavior in solution has been studied by dynamic light scattering (DLS) experiments. Figure 2 shows the correlation curves obtained for 25 mM and 100 mM at pH 7, which indicates the presence of soluble chains of around 10 nm in diameter.

These entities are probably individual extended chains since, as mentioned above, the copolymer at this pH 7 has a net negative charge,[17] and the chains may be seen as "hydrophobic"

dx.doi.org/10.1021/ma301615a | *Macromolecules* 2012, 45, 7676−7683

Scheme 2. Tentative Scheme Showing the Solvation of the Carboxylate Unit at pH 7 as Well as the Active Role of the Cyclodextrin Cavity



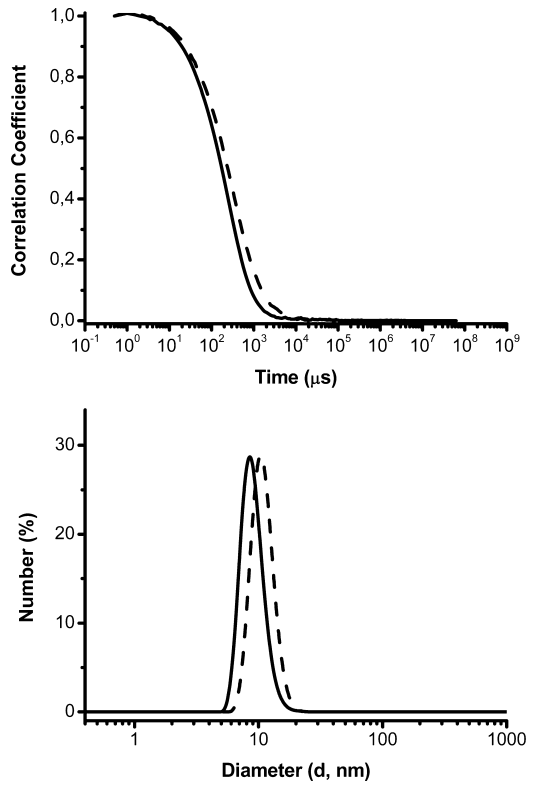


Figure 2. Correlation curves (up) and size distribution curves (bottom) for the copolymer solutions. (a) Solid line: 5.5 mg/mL copolymer (7.5 mM pendant proline) and (b) Dashed line: 22.2 mg/mL of copolymer (30 mM pendant proline). The measurements were carried out at pH 7 in a 50 mM PBS solution.

polyanions with columbic repulsions between carboxylate units and solvation of the ions with water molecules. The correlation curve obtained for the sample at 330 mM (not shown here) did not show a single decay but rather a multimodal decay, evidencing the presence of polydisperse and aggregated polymer chains. In this case, the reaction proceeds in a nonhomogeneous media, which precludes us from performing appropriate DLS analysis. Under these conditions, hydrophobic areas where water can be excluded could explain the data observed in Table 1. It has to be mentioned that this explanation is in agreement with other previous results[16] and with the performance of some solid resins.[12−14] Equally, the reaction rate decreases due both to the limited diffusion of the reactants and the accessibility to the catalytic centers. Similar observations were previously obtained in styrene-proline copolymers in which the addition of magnesium salts provokes the aggregation of the copolymer and enhances the enantioselectivity.[16]

It has been previously reported that the homopolymer poly-HPrMA is insoluble in aqueous media at the isoelectric point (IEP),[17] which is around 3.5. The proline units are zwitterions in a certain pH range where both the anionic and cationic charges coexist. The pH at which the two charges cancel each other is the IEP. Above and below the IEP, the net charge is negative or positive, respectively, since the stoichiometry is lost allowing linear macromolecular chains to expand, solvate, and eventually dissolve. Figure 3 (top) shows the results of a turbidimetry study of the homopolymer. Poly-HPrMA is soluble at pHs far enough from IEP (i.e., 7) and precipitates in the pH region between 4.5 and 2 approximately. The copolymer poly(HPrMA-*stat*-MeβCDSty) 80:20, however, is soluble in aqueous solutions at all pHs (see turbidimetry studies

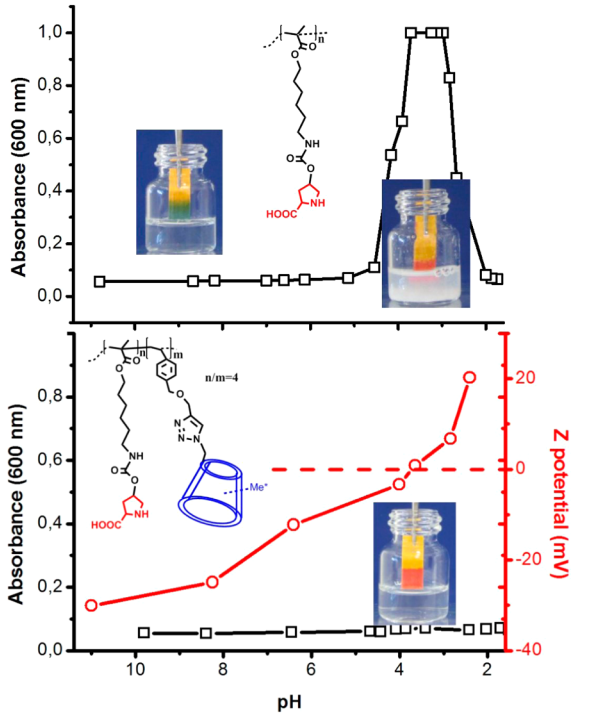


Figure 3. Absorbance at 600 nm vs the pH for the copolymer poly(HPrMA-*stat*-MeβCDSty) 80:20 (□, bottom) and the homopolymer poly(HPrMA) ((□, top) at ionic strength = 0.15. The graph includes the ζ potential (mV) values (○) as a function of the pH.

dx.doi.org/10.1021/ma301615a | *Macromolecules* 2012, 45, 7676−7683

in Figure 3 (bottom)). In spite of this solubility, the ionization of the proline groups (carboxylic and amine) as a function of the pH seems to be quite similar to the behavior of these groups in the homopolymer, as it is shown in Figure 3 using ζ potential measurements. These measurements reveals a charge cancellation, this is, an IEP, at pH = 3.8, very close to the IEP observed for the homopolymer. Thus, it seems that the permethylated β-cyclodextrin stabilizes the hydrophobic interpolymeric stoichiometric complexes near IEP avoiding precipitation. This result is in agreement with some recent studies reported in the literature on the stabilization properties of amphiphilic permethylated β-cyclodextrin functionalized with hydrophobic moieties.[36,37]

It is worth recalling that in the case of the homopolymer, the global hydrophobia causes the interpolymer complexes to precipitate. The presence of permethylated cyclodextrin, however, seems to stabilize these complexes, thus avoiding precipitation. The DLS analysis of the copolymeric entities at this IEP, as compared to the analysis at pH 7, can be found in Figure 4.

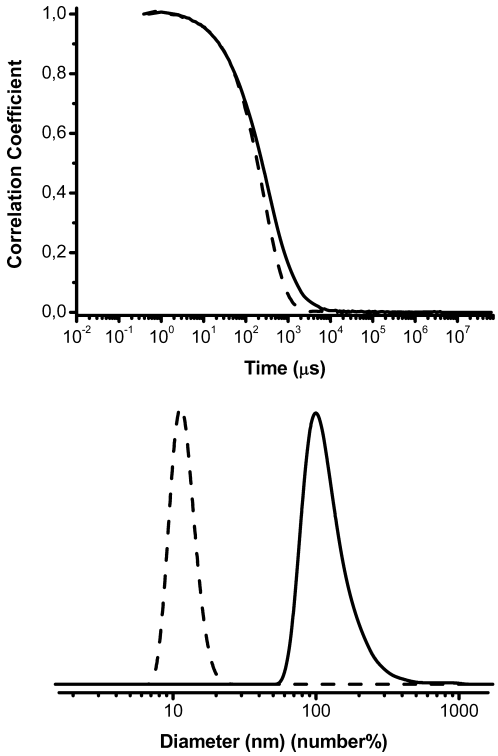
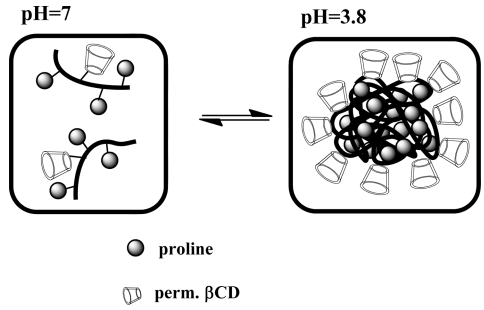


Figure 4. Correlation curves (up) and size distribution curves (bottom) for copolymer solutions containing 30 mM of pendant proline in the copolymer obtained at two different pH values: 3.8 (solid line) and 7 (dashed line).

Whereas at pH 7 the copolymers exist as individual entities with average diameters of 10−12 nm, at pH 3.8 (the IEP) objects with sizes 1 order of magnitude bigger than at pH 7 (around 100 nm) were observed. The solution is homogeneous (transparent) but DLS shows that there are no single chains anymore but homogeneous nanoaggregates. A model to explain this behavior has been proposed in Scheme 3. We suggest that the formation of hydrophobic domains upon charge cancellation at the IEP (as observed for the homopolymer) and a stabilization effect of the amphiphilic CD prevents the formation of large aggregates. A similar stabilization role of the permethylated cyclodextrin has been reported recently.[36,37]

Scheme 3. Schematic Cartoon Showing the Proposed Entities at pH 7 and 3.8, Individual Extended Chains and Multichain Nano-Aggregates, Respectively



The copolymer was evaluated at pH 3.8, and the obtained results were compared to those obtained at pH 7. As depicted in Table 2 their behavior is remarkably different. A concentration of 100 mM of aldehyde was used in this study.

At pH 3.8 the reaction rate decreases while the enantioselectivity is almost absolute (see entries 1 and 2 in Table 2). To explain this result, an 'exclusion of water' mechanism is proposed. Considering that the nonionizable structure of the copolymer backbone is globally hydrophobic, the interpolymer complexation and the formation of larger aggregates are compatible with an expulsion of water from the vicinities of the inner active centers, allowing the reaction to occur in a stereoselective way. The decrease of the reaction rate is in agreement with a poorer accessibility to these active sites. Moreover, the addition of sodium 2-naphthalenesulfonate decreases the reaction rate at both pHs (entries 3 and 4 in Table 2). This again supports the proposed active role of the CD cavity. Interestingly, the enantioselectivity of the reaction is not affected by the presence of the inhibitor.

In conclusion, these studies provide new insights on the role of water and the effect of pH changes on the aldol reactions using supported proline. It has been confirmed that the reaction is efficient in water when there is "hydrophobic activation", in this study the hydrophobic activation is supplied by the component bearing CD. Besides, new evidence shows that a hydrophobic microenvironment around the active proline moiety is beneficial for stereoselectivity. As proline has two ionizable groups (carboxylic and amine), which are solvated in the ionized form, the exclusion of water can be achieved only by "blocking" that solvation, i.e., forming stoichiometric charge complexes at the IEP. This is in agreement with the previous studies on copolymers of hydroxyproline methacrylate and styrene at pH 7 using Mg^{2+} salts that strongly interact with the major carboxylate groups present at this pH.[16] The aggregates formed upon addition of Mg^{2+} excluded water and exhibited enantioselectivity as well.

This study has found that the permethylated β-CD plays a key role in stabilizing the hydrophobic complexes formed at the IEP, which ensures the catalysis process in a homogeneous manner.

AUTHOR INFORMATION

Corresponding Author

*(A.F.-M.) Fax: (+34)-91-564-48-53. E-mail: mayoralas@iqog.csic.es. (A.G.) Fax: (+34)-91-564-48-53. E-mail: gallardo@ictp.csic.es.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Ministry of Science and Innovation (MAT2010-20001, MAT2010-17016 and CTQ2010-15418), the Comunidad de Madrid (S2009/PPQ-1752), and the CSIC (201080E001 fellowship to E.G.D.).

■ REFERENCES

(1) Movassaghi, M.; Jacobsen, N., N *Science* **2002**, *298*, 1904−1905.
(2) List, B.; Lerner, R. A.; Barbas, C. F. *J. Am. Chem. Soc.* **2000**, *122*, 2395−2396.
(3) Benaglia, M.; Puglisi, A.; Cozzi, F. *Chem. Rev.* **2003**, *103*, 3401−3429.
(4) Lu, J.; Toy, P. H. *Chem. Rev.* **2009**, *109*, 815−838.
(5) Sherrington, D. C. *Chem. Commun.* **1998**, 2275−2286.
(6) Benaglia, M.; Cinquini, M.; Cozzi, F.; Puglisi, A. *Adv. Synth. Catal* **2002**, *344*, 533−542.
(7) Bergbreiter, D. E.; Tian, J.; Hongfa, C. *Chem. Rev.* **2009**, *109*, 530−582.
(8) Trindade, A. F.; Góis, P. M. P.; Afonso., C. A. M. *Chem. Rev.* **2009**, *109*, 418−514.
(9) Córdova, A.; Notz, W.; Barbas, C. F., III *Chem. Commun.* **2002**, 3024−3025.
(10) Chimni, S. S.; Mahajan, D. *Tetrahedron* **2005**, *61*, 5019−5025.
(11) Raj, M.; Singh, V. K. *Chem. Commun.* **2009**, 6687−6703.
(12) Kristensen, T. E.; Vestli, K.; Fredriksen, K. A.; Hansen, F. K.; Hansen, T. *Org. Lett.* **2009**, *11*, 2968−2971.
(13) Font, D.; Sayalero, S.; Bastero, A.; Jimeno, C.; Pericàs, M. A. *Org. Lett.* **2008**, *10*, 337−3340.
(14) Gruttadauria, M.; Giacalone, F.; Marculescu, A. M.; Meo, P. L.; Riela, S.; Noto, R. *Eur. J. Org. Chem.* **2007**, 4688−4698.
(15) Terashima, T.; Mes, T.; De Greef, T. F. A.; Gillissen, M. A. J.; Besenius, P.; Palmans, A. R. A.; Meijer, E. W. *J. Am. Chem. Soc.* **2011**, *133*, 4742−4745.
(16) Doyagüez, E. G.; Corrales, G.; Garrido, L.; Rodríguez-Hernández, J.; Gallardo, A.; Fernández-Mayoralas, A. *Macromolecules* **2011**, *44*, 6568−6276.
(17) Doyagüez, E. G.; Parra, F.; Corrales, G.; Fernández-Mayoralas, A.; Gallardo, A. *Polymer* **2009**, *50*, 4438−4446.
(18) Zhao, Q.; Lam, Y. H.; Kheirabadi, M.; Xu, C.; Houk, K. N.; Schafmeister, C. E. *J. Org. Chem.* **2012**, *77*, 4784−4792.
(19) Mase, N.; Nakai, Y.; Ohara, N.; Yoda, H.; Takabe, K.; Tanaka, F.; Barbas, C. F., III *J. Am. Chem. Soc.* **2006**, *128*, 734−735.
(20) Lindstrom, U. M. *Chem. Rev.* **2002**, *102*, 2751−2772.
(21) Kobayashi, S.; Manabe, K. *Acc. Chem. Res.* **2002**, *35*, 209−217.
(22) Kristensen, T. E.; Hansen, F. K.; Hansen, T. *Eur. J. Org. Chem.* **2009**, 387−395.
(23) Rekharsky, M.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.
(24) Connors, K. *Chem. Rev.* **1997**, *97*, 1325−1357.
(25) Watanabe, K. I.; Yamada, Y.; Goto, K. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1401−1406.
(26) Shen, Z.; Ma, J.; Liu, Y.; Jiao, C.; Li, M.; Zhang, Y. *Chirality* **2005**, *17*, 556−558.
(27) Liu, K.; Häussinger, D.; Woggon, W. D. *Synlett* **2007**, *14*, 2298−2300.
(28) Huang, J.; Zhang, X.; Armstrong, D. W. *Angew. Chem., Int. Ed.* **2007**, *46*, 9073−9077.
(29) Yuan, D. Q.; Lu, J.; Atsumi, M.; Yan, J. M.; Kai, M.; Fujita, K. *Org. Biomol. Chem.* **2007**, *5*, 2932−2939.
(30) Doyagüez, E. G.; Fernández-Mayoralas, A. *Tetrahedron* **2012**, *68*, 7345−7354.
(31) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997−2011.
(32) Font, D.; Sayalero, S.; Bastero, A.; Jimeno, C.; Pericàs, M. A. *Org. Lett.* **2008**, *10*, 337−340.
(33) Guillena, G.; Hita, M. C.; Nájera, C.; Viózquez, S. F. *J. Org. Chem.* **2008**, *73*, 5933−5943.
(34) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L. H.; Shen, B. J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475−481.
(35) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.
(36) Gervaise, C.; Bonnet, V.; Wattraint, O.; Aubry, F.; Sarazin, C.; Jaffrès, P. A.; Djedaïni-Pilard, F. *Biochimie* **2012**, *94*, 66−74.
(37) Bauer, M.; Fajolles, C.; Charitat, T.; Wacklin, H.; Daillant, J. *J. Phys. Chem. B* **2011**, *115*, 15263−15270.