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Efficient Biocatalytic Cleavage and Recovery of Organic Substrates Supported on Soluble Polymers

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Abstract: The applicability of novel solution-phase supports in combination with enzymes for biocatalytic transformations is reported. *Ex novo* designed styrene-based copolymers, bearing a phenylacetic residue in variable loadings and linked as a pendant group to the macromolecular backbone, through a spacer of variable length, have been synthesized and characterized. These derivatives are compatible and can be used as soluble supports in combination with immobilized penicillin G acylase (PGA – EC 3.5.1.11) for the biocatalytic cleavage of the covalently anchored organic substrate in quantitative yields, in water or water/dimethylformamide solvent mixtures, with recovery of the immobilized enzyme with negligible losses in activity.

Keywords: enzyme catalysis; immobilization; polymers; synthetic methods

Introduction

The introduction of solid-phase synthesis, pioneered by Merrifield and co-workers in the 1960s,[1] has been inspired by the necessity, in the organic synthesis of complex biopolymers, to introduce easy work-up procedures, in a repetitive growth scheme, and to render simpler the use of excess organic reagents. Since then, solid-phase synthesis has experienced a tremendous growth in terms of availability of supports, linkers and methods of analysis, particularly in combination with its use in combinatorial chemistry; innovative approaches such as "mix-and-split" for the creation of complex libraries of several thousands or more organic compounds have been conceived with the use of cross-linked beads, since the positive lead identified on the bead can then be mechanically separated, and the chemical structure of the "positive" determined by deconvolution or encoding methodologies.[2]

Cross-linked, insoluble polymer supports, however, do present disadvantages: generally speaking, the characteristic which is their main advantage, their insolubility, is also the origin of many operational drawbacks. Soluble polymers, on the contrary, can provide the primary advantage of heterogeneous systems, i.e., facile product/reagent separation by precipitation in a poor solvent or non-solvent, while overcoming the main limitations of insoluble supports, since they allow homogeneous reaction conditions.[3] Polyethylene glycols (PEG: soluble in water and most organic solvents; insoluble in diethyl ether),[4] or polystyrene derivatives (soluble in non-polar and chlorinated solvents; insoluble in methanol) have been extensively used for this purpose.[5] As in PEG supports the organic reagent/catalyst is introduced by functionalization of one or both terminal OH groups, in polystyrene matrices the organic reagent/catalyst can be introduced in variable loadings, since the active functionalities are pendants from the main polymeric backbone, through covalent incorporation into a styrenic derivative and subsequent copolymerization, or post-modification of a preformed functional polymer. The use of polystyrene derivatives may be advantageous since: a) the loading of the substrate of interest can be tuned in order to maintain the precipitability characteristics of the polymeric matrices thus obtained in the non-solvent (MeOH); b) the copolymers can be easily obtained through conventional free-radical techniques, which are highly functional group tolerant; c) controlled or "living" free-radical polymerization techniques, already developed for a variety of styrenic derivatives, could give access to a higher degree of control of poyldispersity, of the degree of

polymerization and of polymer topology (whether random or block) for the matrices thus obtained.[6]

Enzymatic methods have opened up advantageous alternatives to classical chemical techniques since enzyme-catalyzed transformations often proceed under very mild conditions (pH 5–8, 25–37 °C) and very high chemo-, regio- and stereoselectivity, and they have found applications in large-scale industrial syntheses.[7] However, in many cases the substrates are very hydrophobic and therefore soluble only in organic solvents, where most enzymes are inactive. Stability limitations of the biocatalyst when drastic conditions are required may be overcome by the use of immobilization-stabilization techniques.[8] The combination of immobilized enzymes for the biocatalytic transformations of substrates anchored on insoluble, crosslinked supports is therefore a strategy unlikely to succeed, as a consequence of the biopolymer and the synthetic polymer being *both* heterogeneous in the reaction mixture. For example, Waldmann et al. have demonstrated that soluble, PEG-based polymers could be used, in combination with the immobilized PGA acting as an activator for a safety-catch linker, to achieve reasonable yields of released products. The combination of immobilized enzyme-insoluble, crosslinked polymer was found to be detrimental for reaction yields.[9]

In this paper, we report the synthesis of novel modified styrene-based polymeric platforms supporting a PGA-cleavable model residue, and the optimization of linker and reaction conditions for the biocatalytic high-yielding cleavage of the residue.

Results and Discussion

Monomer and Polymer Synthesis

The design of our soluble polymeric platforms to be used in combination with natural enzymes, and in particular PGA, is reported in Figure 1. A phenylacetic residue, which is known to be cleaved by immobilized or free PGA,[8a] is covalently linked by means of a suitable spacer, to a styrenic derivative, and its loading within the copolymer composition is to be optimized to preserve the precipitability characteristics of the "parent" polystyrene in suitable solvents, e.g., MeOH. The linker group plays a crucial role in our design, ensuring a proper distance of the active residue from the bulky polymer backbone, in order to favour the interaction with the enzyme active site and the subsequent cleavage of the residue.

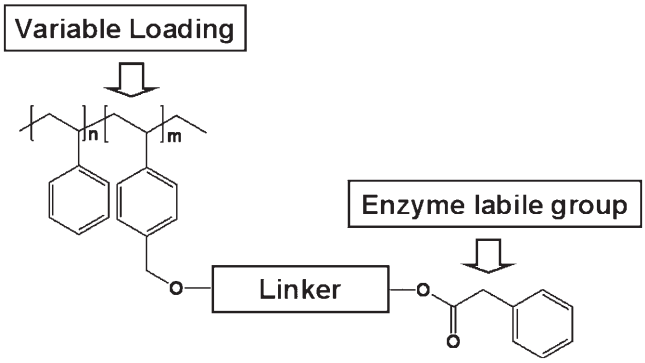
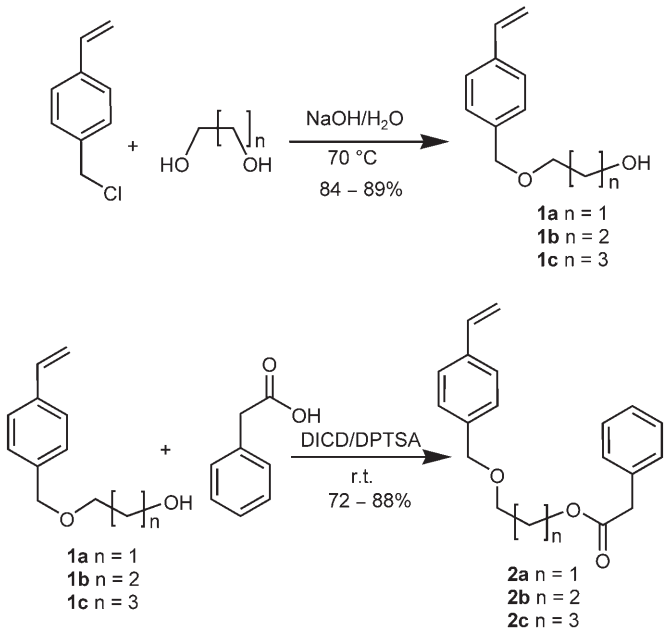


Figure 1. Design scheme for the linear polymers described in this paper.

In order to closely monitor the results of the polymerization processes and to be confident about the percentage of comonomer effectively incorporated in the polymeric mixtures, we chose to synthesize novel styrenic monomers, containing a phenylacetic ester residue linked, through a variable linker (two, three or four carbon atom aliphatic chains), and then copolymerize them with styrene, rather than functionalize a suitable modified styrenic copolymer with phenylacetic acid. The synthesis of the monomers is reported in Scheme 1. Compounds **1a–c** were obtained

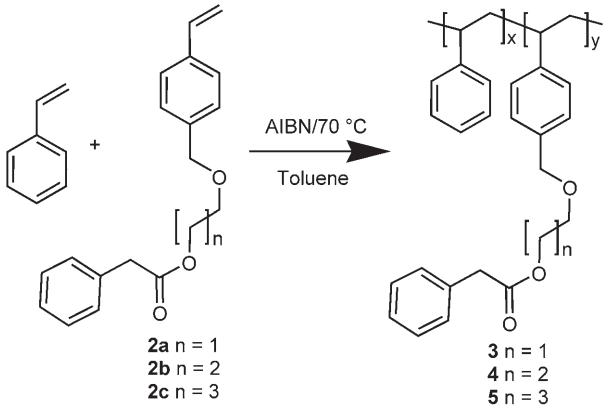


Scheme 1.

by reaction of the commercially-available 4-chloromethylstyrene with a large excess of the appropriate diol, in the presence of stoichiometric amounts of NaOH and H_2O, adapting a literature procedure.[10] The reaction was conducted at 70 °C in order to avoid thermal polymerization of the styrenic derivative, and the large excess of diol could be removed by simple partition in CH_2Cl_2/H_2O, the diol being much more soluble in the aqueous phase. After purification by column chromatography, compounds **1a–c** were sub-

jected to a coupling reaction with phenylacetic acid in standard conditions (DICD/DPTSA),[11] to obtain compounds **2a–c** in good yields.

The purified monomers were subjected to thermal free-radical copolymerization (Scheme 2) with styrene, using AIBN as the initiator, with different feed ratios, as reported in Table 1. The resulting polymers showed different characteristics in terms of precipitability in MeOH: at high functionalized monomer loadings (entries 1 and 5), independent of the aliphatic linker length, it was impossible to purify and obtain the polymer as a powder by a simple precipitation in the non-solvent (MeOH), but centrifugation of the suspension was needed. These samples were therefore not characterized further. When the loadings were reduced (entries 2, 3 and 6–9), the reaction mixture of the polymerization process could be directly purified by precipitation in MeOH; the samples were separated by filtration as gummy solids in good yields, and further treated by dissolving them in Et_2O; the solvent was removed under vacuum in order to azeotropically remove traces of solvents and reagents from the polymeric powders obtained. By drastically reducing the comonomer loading (entries 4 and 10), the precipitability characteristics of the resulting polymers were further improved, and the polymers could be easily obtained as white powders by filtration without any further manipulation. Given a certain comonomer loading, the role of the aliphatic spacer length appeared to influence only marginally the physicochemical characteristics of the resulting polymers and, therefore, their precipitability characteristics.



Scheme 2.

The molecular weight characterization, using GPC with a calibration curve based on low polydispersity polystyrene standards, showed in all cases the presence of monomodal Gaussian distibutions with comparable degrees of polymerization and polydispersity indices above 1.5, as expected for this kind of thermally initiated free-radical polymerization. Given that all polymerizations were run with the same amount of free radical initiator, total monomer concentration and solvent, the molecular weight distributions, in terms of M_n and M_w values, turned out to be similar, as expected.

The polymers were also characterized by ^{1}H NMR spectroscopy, as shown in the example reported in Figure 2. Considering the expected disappearance of the vinyl proton resonances in the spectra of the copolymer when compared with the corresponding functionalized monomer, and the broadening of all the remaining signals, we were able to confirm a close correspondence between feed and observed ratios of monomers within the polymeric structure simply by integrating the broad, but well separated benzylic CH_2 proton resonances in the polymer (which can be attributed only to the functionalized comonomer) and comparing this value with the total aromatic signals of the polymer structure (see Supporting Information,

Table 1. Characterization of polymers synthesized in this work.

Entry	Polymer	Monomer [%][a]	Yield [%]	Precipitation[b]	M_n[c]	M_w[c]	PDI[c]
1	**3A**	**2a** [40]	-	-	-	-	-
2	**3B**	**2a** [23]	70	+	13590	23770	1.7
3	**3C**	**2a** [20]	81	+	9080	17630	1.9
4	**3D**	**2a** [7]	59	++	11080	18950	1.7
5	**4A**	**2b** [40]	-	-	-	-	-
6	**4B**	**2b** [23]	40	+	14880	25530	1.7
7	**4C**	**2b** [20]	67	+	8605	15800	1.8
8	**5A**	**2c** [22]	78	+	13590	24220	1.8
9	**5B**	**2c** [20]	76	+	9190	17420	1.9
10	**5C**	**2c** [15]	73	++	13080	21740	1.7

[a] Percentage of functionalized comonomer *vs.* total monomer concentration in the feed for copolymerizations.
[b] Precipitability characteristics in MeOH as the non-solvent; - = poor precipitation: ultracentrifugation needed to recover the polymer powder. + = good precipitation, the poylmer is filtered as a gummy solid and treated with solvent (see experimentals and text). ++ = excellent precipitation: the polymer is recovered as a white solid after filtration.
[c] As determined by GPC relative to polystyrene standards; PDI = polydispersity index.

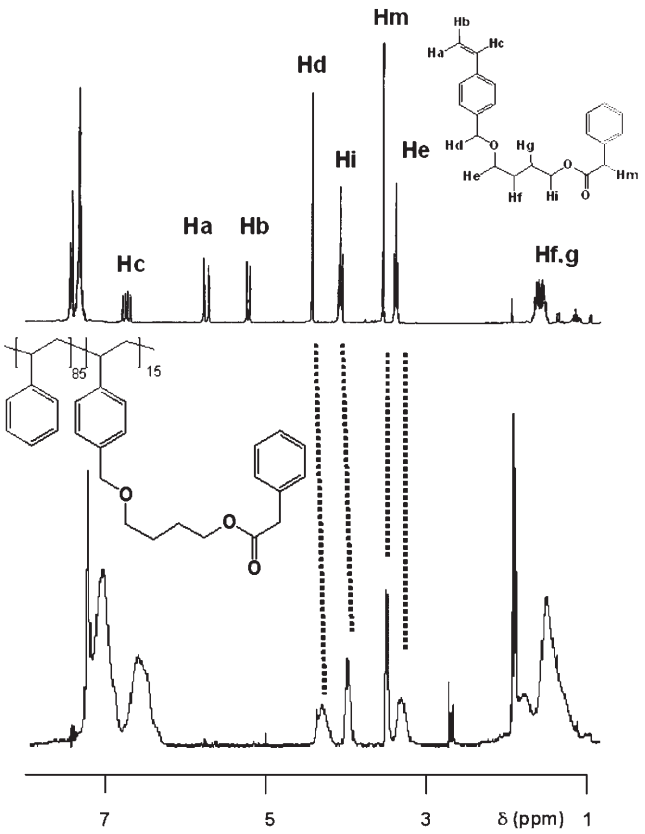


Figure 2. Comparison between the ^{1}H NMR (200 MHz, $CDCl_3$) spectra of monomer **2c** and polymer **5C.**

Table S1). These data confirmed in all cases very similar reactivity ratios of the functionalized monomers and styrene during the polymerization process.

Enzymatic Hydrolysis

For a qualitative evaluation of reactivity rates, towards both monomers and polymers, our first approach was to compare the effect of not-immobilized PGA on the substrates in aqueous solvents. The cleavage of the phenylacetic ester was monitored using an automatic titrator, continuously adding an NaOH solution to keep the pH values of the enzymatic reactions constant and therefore monitoring the amount of free carboxylic acid cleaved from the polymer support. Although both the monomers and the polymers showed negligible solubilities in the aqueous medium and were in the form of a suspension, the biocatalytic activity was clearly detected. Initial results are shown in Table 2; it is evident how the monomer **2a–c** all react at high rates (entries 1–3); there is a descending trend in terms of reactivity from **2a** to **2c**, which has a parallelism in the already reported descending trend for the PGA hydrolysis of mandelic acid methyl, ethyl and *n*-butyl ester.[12] Polymers **3C**, **4C**, **5B** bearing a comonomer loading of about 20%, represented for us an ideal trade-off between precipitability characteristics (see Table 1) and loading, and they were therefore preliminary screened. As shown in Table 2, when compared to the data for monomers **2** in the same conditions, a huge decrease in reaction rates was detected using the polymers (entries 3–6), presumably as a consequence of two concurrent factors: the increased hydrophobicity of the polymers, which slows the catalytic effect of the hydrophilic enzyme, and the reduced accessibility of the phenylacetic residues to the enzyme active site because of the presence of the bulky, random-coil polymer chain. The same reactivity trend noticed for monomers **2a–c** could be found by comparing polymers **3–5** (entries 4–6 in Table 2): going from a one- to a three-carbon atom aliphatic spacer, the enzyme activity decreases. In order to improve reaction conditions and rates, we scaled up the synthesis of polymer **3C** (obtained on a 5-g scale with a yield comparable to that reported in Table 1).

Table 2. Biocatalyzed hydrolysis of phenylacetic acid residue bound to monomers **2** and polymers **3–5** in aqueous solutions (37 °C).[a]

Entry	Compound	Monomer [%][b]	Reaction rate[c]
1	**2a**	[100]	1.0
2	**2b**	[100]	0.84
3	**2c**	[100]	0.30
5[d]	**3C**	**2a** [20]	3.0×10^{-6}
6[d]	**4C**	**2b** [20]	1.0×10^{-6}
7[d]	**5B**	**2c** [20]	5.1×10^{-7}

[a] Substrate concentration: monomers *ca.* 3 mg mL^{-1}; polymers 3-5 mg mL^{-1}. Free PGA solution in phosphate buffer (10 mM), pH 8.
[b] Percentage of functionalized comonomer vs. total monomer concentration in the feed for copolymerizations.
[c] In mmol/(min×U_{PGA}).
[d] Polymer sample subjected to prehydration in the buffer for 24 h.

The solubility of **3C** in a series of polar solvents and solvent mixtures with H_2O in variable proportions was evaluated; amongst the solvents tested (DMF, MeCN, 1,2-dimethoxyethane, and DMSO; see Supporting Informatioon, Table S2), DMF proved to be the best solubilizing solvent for polymer **3C**, with good solubility values obtained even for solvent mixtures containing up to 80% H_2O. We focused on enzyme immobilization in order to explore the biocatalytic reactivity and stability in different solvent mixtures. The hydrolysis rates, calculated after the first 20 min of the hydrolysis, and normalized according to the enzyme units used, are reported in Table 3. Both derivatives of PGA on Eupergit and Agarose in an aqueous buffer solvent (entries 1 and 5) gave higher reaction rates when compared with the results ob-

Table 3. Biocatalyzed hydrolysis of phenylacetic acid residue bound to polymer **3C** in different conditions (37 °C).

Entry	Solvent[a]	Enzyme[b]	Reaction rate ($\times 10^6$)[c]
1	H_2O	B	91
2	H_2O/DMF, 80/20	B	155
3	H_2O/Me_2CO, 80/20	B	21[d]
4	H_2O/MeCN, 80/20	B	21[d]
5	H_2O	C	24
6	H_2O/DMF, 80/20	C	71
7	H_2O/Me_2CO, 80/20	C	19[d]
8	H_2O/MeCN, 80/20	C	14[d]

[a] Substrate concentration: 4 mg mL^{-1} in all cases. H_2O refers to phosphate buffer (10 mM), pH 8.
[b] Penicillin G acylase, conditions; B: PGA immobilized on Eupergit. C: PGA immobilized on Agarose.
[c] In mmol/(min×U_{PGA}). Calculated on values of NaOH addition after 1200 s.
[d] Enzyme denaturation occurs and the reaction stops after a few minutes.

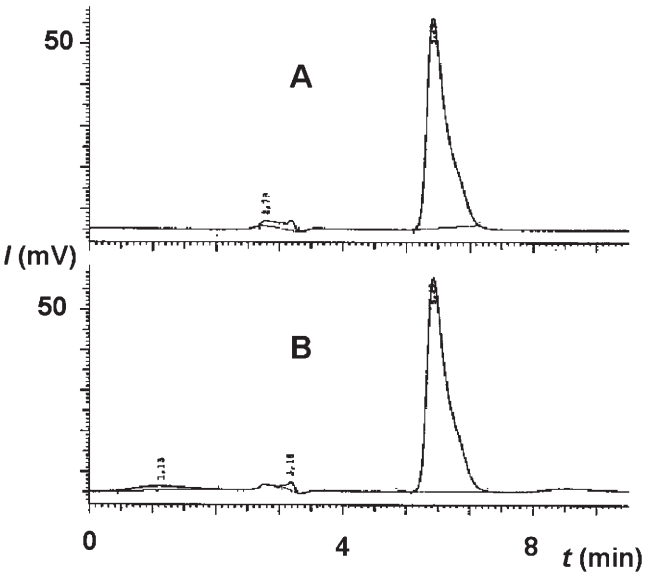


Figure 3. Reverse phase HPLC chromatograms of: **A**) phenylacetic acid in the hydrolysis reaction mixture at the end of the reaction; **B**) a 0.6 mM standard solution of phenylacetic acid used for the calibration curve.

tained for not-immobilized PGA (Table 2 entry 5). By using a mixed system H_2O/DMF (80/20), in which the polymeric substrate is soluble, a further 2–3-fold rate acceleration was observed (entries 2 and 6 *vs.* 1 and 5) with good correlation obtained by using a first order kinetic data treatment up to *ca.* 30% conversion (i.e., a biocatalyzed A to B transformation). In the case of solvent mixtures containing acetone or acetonitrile, and using either of the two immobilization techniques, low conversions and rates were observed as the reactions seemed to stop within a few minutes after the addition of the immobilized enzyme. These results are in agreement with a previously reported study concerning the stability of PGA in water/organic solvent systems.[13]

When selected runs reported in Table 3 (entries 1 and 6) were brought to the end point of the titration, the crude product in the solution was subjected to analysis by HPLC and proved to be pure phenylacetic acid (see Figure 3). Calculations of the yield of product obtained (on the basis of the residue incorporated in the copolymer used) indicated, by comparison with a calibration curve, quantitative release of products. The immobilized enzyme, previously filtered, could be washed with CH_2Cl_2, with the enzyme retaining 92.4% (in the case of Agarose immobilization) and 94.9% (in the case of Eupergit) of the original activity. Washings with acetone instead removed the polymer substrate and also dramatically reduced the enzyme activity to 10% (in the case of Eupergit) and 40% (in the case of Agarose) of the original one. The incompatibility of the immobilized enzyme in the presence of acetone was, however, already evident from the runs reported in Table 3.

Conclusions

We have presented the design and the realization of novel styrene-based polymeric supports bearing a pendant enzyme-cleavable residue from the main polymer backbone. The use of such polymers in biocatalysis is, to our knowledge, unprecedented. A linker of variable length has been used: both in the case of the monomers and, perhaps surprisingly, in the case of the polymers, there is a descending trend in reactivity as the phenylacetic residue is moved further away from the styrenic fragment. PGA immobilization on either Eupergit or Agarose brought about an increase in reaction rates with respect to the free enzyme. The biocatalyzed release of the organic compound anchored to the polymer support occurred quantitatively and in pure form. The identification of reaction conditions allowing "traditional" first order reaction kinetics, typical of commonly used biocatalyzed hydrolysis of small, monomeric substrates in solution, with immobilized enzymes is certainly promising for a rapid transfer of well-established protocols in biocatalysis from solution to soluble polymer-supported organic synthesis. We are currently addressing further improvements in the polymer design, in order for them to be used in a recyclable, repetitive scheme, particularly in cases where covalently anchored racemic substrates can be enantioselectively cleaved with biocatalytic techniques.

Experimental Section

General Remarks

Monomer and polymer synthesis: All commercially available compounds were purchased from Aldrich and used as received. THF (CaH_2) and CH_2Cl_2 (CaH_2) were dried and distilled before use. *N,N′*-4-Dimethylaminopyridinium *p*-toluenesulfonate (DPTSA)[11] was prepared according to literature procedures. Flash chromatography was carried out using silica gel (Merck 60, 0.040–0.063 mm). ^1H and ^{13}C NMR spectra were recorded from solutions in $CDCl_3$ or CD_3COCD_3 on Bruker 200 or AMX300 spectrometers with the solvent residual proton signal or tetramethylsilane (TMS) as a standard. Infrared spectra were recorded on a FT-IR PE Paragon 1000 spectrophotometer using potassium bromide with a diffuse reflectance accessory. Size-exclusion chromatography was carried out on a Waters system equipped with a DRI detector. Low polydispersity polystyrene standards (Fluka) were used for the calibration curve and the mobile phase was tetrahydrofuran (1 mL/minute, 40 °C). A bank of two columns (Styragel 4E and 5E) was used. Elemental analyses were done on a Carlo Erba 1106 elemental analyzer.

Biocatalysis: Penicillin G acylase crude extract from *E. coli* ATCC 11105 (EC 3.5.1.11) was kindly donated by Recordati (Milan, Italy); Eupergit C was kindly donated from Rohmpharma Rohm GmbH (Darmstadt, Germany); agarose (Sepharose CL-6B) was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Enzymatic activities were calculated on hydrolysis of penicillin G potassium salt (pH 8 and 37 °C) as previously reported.[12] HPLC analyses were run on a Kontron Instrument AG equipped with UV detector using a LiChroCART 250–4 RP-18 select-B 5 μm column, and the following conditions: flow 1 mL min^{-1}; eluents: phosphate buffer 10 mM 70 %-CH_3CN 30 %, pH 5. During the enzymatic hydrolysis reactions, the pH of the solutions was kept constant using an automatic titrator 718 Stat Titrino from Metrohm (Herisau, Switzerland). Immobilization of the free enzyme on Eupergit C and on Sepharose CL-6B was carried out as previously described.[12]

General Procedure for the Preparation of Diols 1a–c

The appropriate diol (0.9 mol) was added to a mixture of 4-vinylbenzyl chloride (0.033 mol), NaOH (0.038 mol) and H_2O (0.038 mol). The suspension was stirred with a magnetic stirrer at 70 °C in a thermostated oil bath for 24 h. After cooling to room temperature, H_2O (100 mL) was added and the solution was extracted with Et_2O (3 × 100 mL). The organic solution was dried (Na_2SO_4), the suspension filtered and the organic solvent removed under vacuum. The crude product was purified by column chromatography (SiO_2; C_6H_{12}/AcOEt).

Compound 1a: Prepared from ethylene glycol (33.1 g, 0.53 mol), 4-vinylbenzyl chloride (3.05 g, 0.02 mol), NaOH (0.02 mol) and H_2O (0.02 mol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 7/3), **1a** was obtained as a yellow oil; yield: 2.96 g (84 %). IR: n = 3417, 3086, 3006, 2862, 1629 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ = 7.50–7.25 (4 H, m, Ar*H*), 6.75 (1 H, dd, -C*H*=CH_2), 5.75 (1 H, d, -CH=CH_2), 5.25 (1 H, d, -CH=CH_2), 4.5 (2 H, s, Ar-CH_2-O-), 3.6 (4 H, m, -O-CH_2-CH_2-O-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.7, 138.4, 137.7, 129.2, 127.5, 115.2, 74.2, 72.6, 63.1.

Compound 1b: Prepared from propane-1,3-diol (67.0 g, 0.88 mol), 4-vinylbenzyl chloride (5 g, 0.033 mol), NaOH (0.033 mol) and H_2O (0.033 mol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 6/4), **1b** was obtained as a yellow-orange oil; yield: 5.6 g (89 %). IR: ν = 3386, 2863, 1629, 1511, 1363, 1089 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ = 7.5 (4 H, dd, Ar*H*), 6.7 (1 H, dd, -C*H*=CH_2), 5.7 (1 H, d, -CH=CH_2), 5.3 (1 H, d, -CH=CH_2), 4.5 (2 H, s, Ar-CH_2-O-), 3.8 (2 H, t, -O-CH_2-CH_2-CH_2-OH), 3.7 (2 H, m, -O-CH_2-CH_2-CH_2-OH), 2.75 (1 H, s, OH), 1.85 (2 H, m, -O-CH_2-CH_2-CH_2-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.5, 136.9, 136.3, 127.7, 126.1, 113.7, 72.7, 68.8, 61.2, 32.0; anal. calcd. for $C_{12}H_{16}O_2$: C 74.9 %, H 8.4 %; found: C 74.8 %, H 8.8 %.

Compound 1c: Prepared from butane-1,4-diol (81.5 g, 0.90 mol), 4-vinylbenzyl chloride (5 g, 0.033 mol), NaOH (0.033 mol) and H_2O (0.033 mol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 8/2), **1c** was obtained as a dark yellow oil; yield: 5.8 g (86 %). IR: ν = 3404, 3086, 2939, 2863, 1711, 1629, 1362, 1100 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ = 7.4 (4 H, m, Ar*H*), 6.7 (1 H, dd, -C*H*=CH_2), 5.8 (1 H, d, -CH=CH_2), 5.25 (1 H, d, -CH=CH_2), 4.5 (2 H, s, Ar-CH_2-O-), 3.7–3.5 (4 H, m, -O-CH_2-CH_2-CH_2-CH_2-OH), 2.5 (1 H, s, O*H*), 1.7 (4 H, m, -O-CH_2-CH_2-CH_2-CH_2-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.5, 136.8, 136.3, 127.7, 126.1, 113.6, 72.6, 70.1, 62.4, 29.9, 24.5; anal. calcd. for $C_{13}H_{18}O_2$: C 75.7 %, H 8.8 %; found: C 75.1 %, H 9.3 %.

General Procedure for the Preparation of Esters 2a–c

A solution of alcohol **1** (12 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise over a few minutes to a solution of phenylacetic acid (9 mmol), DICD (11 mmmol) and DPTSA (5.8 mmol) in dry CH_2Cl_2 (40 mL). The solution was stirred for 24 h, then quenched with H_2O. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), the suspension filtered and the organic solvent removed under vacuum. The crude product was purified by column chromatography (SiO_2; C_6H_{12}/AcOEt).

Compound 2a: Prepared from alcohol **1a** (2.05 g, 12 mmol), DICD (1.38 g, 11 mmol), phenylacetic acid (1.23 g, 9 mmol), DPTSA (1.71 g, 5.8 mmol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 8/2), **2a** was obtained as a yellow oil;yield: 1.95 g (74 %); IR: ν = 2868, 1732, 1629, 1257 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.25 (9 H, m, Ar*H*), 6.75 (1 H, dd, -C*H*=CH_2), 5.75 (1 H, d, -CH=CH_2), 5.25 (1 H, d, -CH=CH_2), 4.5 (2 H, s, Ar-CH_2-O-CH_2-), 4.25 (2 H, t, -COOCH_2-), 3.65 (4 H, m, -O-CH_2-CH_2-O-and ArCH_2COO-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.3, 137.2–125.4 (9 signals), 113.7, 76.8, 67.06, 63.5, 41.05; anal. calcd. for $C_{19}H_{20}O_3$: C 77.0 %, H 6.8 %; found: C 77.5 %, H 7.2 %.

Compound 2b: Prepared from alcohol **1b** (4.54 g, 23 mmol), DICD (2.66 g, 21 mmol), phenylacetic acid (2.88 g, 21 mmol), DPTSA (1.64 g, 5.6 mmol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 8/2), **2b** was obtained as a yellow oil; yield: 4.70 g (72 %). IR: ν = 3030, 2860, 1734, 1629, 1511, 1363, 1258, 1103 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.5 (9 H, m, Ar*H*), 6.75 (1 H, dd, -C*H*=CH_2), 5.75 (1 H, d, -CH=CH_2), 5.25 (1 H, d, -CH=CH_2), 4.5 (2 H, s, Ar-CH_2-O-CH_2-), 4.25 (2 H, t, -COOCH_2-),

3.6 (2H, s, ArCH_2COO-), 3.5 (2H, t, Ar-CH_2-O-CH_2-), 2.05 (2H, m, Ar-CH_2-O-CH_2-CH_2-CH_2-O-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 137.8, 136.9, 136.4, 129.5–126.1 (6 signals), 113.69, 72.6, 66.4, 61.9, 41.3, 28.9; anal. calcd. for $C_{20}H_{22}O_3$: C 77.4%, H 7.1%; found: C 76.8%, H 7.6%.

Compound 2c: Prepared from alcohol **1c** (0.21 g, 1.02 mmol), DICD (0.13 g, 2.4 mmol), phenylacetic acid (0.135 g, 0.99 mmol), DPTSA (0.155 g, 0.52 mmol). Purified by column chromatography (SiO_2; C_6H_{12}/AcOEt: 8/2), **2c** was obtained as a yellow oil; yield: 0.28 g (88%). IR: ν = 2952, 2854, 1733, 1628, 1254 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.25 (9H, m, Ar*H*), 6.75 (1H, dd, -C*H*=CH_2), 5.8 (1H, d, -CH=CH_2), 5.20 (1H, d, -CH=CH_2), 4.5 (2H, s, Ar-CH_2-O-CH_2-), 4.20 (2H, t, -COOCH_2-), 3.7 (2H, s, ArCH_2COO-), 3.5 (2H, t, Ar-CH_2-O-CH_2-), 2.6 (4H, m, Ar-CH_2-O-CH_2-CH_2-CH_2-CH_2-O-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 138.1, 136.9, 136.5, 134.1, 129.2–126.2 (5 signals), 113.7, 72.5, 69.6, 64.6, 41.4, 26.2, 25.5; anal. calcd. for $C_{21}H_{24}O_3$: C 77.7%, H 7.5%; found: C 77.6%, H 8.0%.

General Procedure for the Free-Radical Polymerization

The monomers and the initiator (AIBN, 2% mol *vs.* total monomers) were dissolved in toluene (0.5 M of total monomer concentration). The solution was deoxygenated by bubbling N_2 for 30 min, and then heated under magnetic stirring at 70 °C in a temperature-controlled oil bath for 48 h. The solvent was then removed under vacuum, the remaining solid was dissolved in the minimum amount of CH_2Cl_2, and the solution was added dropwise to MeOH (20 times its co-solvent volume). The purified, precipitated polymer sample was filtered and dried (work-up procedure **A**). In other cases (see main text), the precipitated polymeric mixture was treated with Et_2O, and the solvent removed under vacuum to remove azeotropically traces of solvents and volatile monomers (work-up procedure **B**).

Polymers 3

Polymer 3A: From styrene (44.6 mg, 0.43 mmol, 0.6 equivalents) and compound **2a** (87 mg, 0.427 mmol, 0.4 equivalents). The crude reaction mixture was treated according to work-up procedure **B**. IR: ν = 3057, 3022, 2917, 2844, 2360, 1945, 1736, 1601, 1493, 1453 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.6–6.9 (bs, ArH), 4.7–4.2 (bs, Ar-CH_2-O-CH_2- and -COOCH_2-), 3.9–3.4 (bs, -O-CH_2-CH_2-O-and ArCH_2COO-), 2.3–1.0 (-CH- and -CH_2- polymer chains); gel permeation chromatography: M_n = 13580; M_w = 23110; PDI = 1.7.

Polymer 3B: From styrene (0.6 g, 5.76 mmol, 0.77 equivalents) and compound **2a** (0.502 g, 1.7 mmol, 0.23 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **3B** as a white powder; yield: 0.78 g (70%). IR and ^{1}H NMR spectra were qualitatively identical to those of polymer **3A**. Gel permeation chromatography: M_n = 13590; M_w = 23770; PD = 1.7; anal. calcd. for $C_{10.53}H_{10.76}O_{0.69}$: C 85.2%, H 7.3%; found: C 85.5%, H 7.3%.

Polymer 3C: From styrene (0.7 g, 8.4 mmol, 0.8 equivalents) and compound **2a** (0.5 g, 1.7 mmol, 0.2 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **3C** as a white powder; yield: 0.97 g (81%). IR and ^{1}H NMR spectra were qualitatively identical to those of polymer **3A**. Gel permeation chromatography: M_n = 9080; M_w = 17630; PD = 1.9; anal. calcd. for $C_{10.2}H_{10.4}O_{0.6}$: C 85.8%, H 7.3%; found: C 86.1%, H 7.3%.

Polymer 3D: From styrene (2.3 g, 22 mmol, 0.93 equivalents) and compound **2a** (0.5 g, 1.7 mmol, 0.07 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **3c** as a white powder; yield: 1.65 g (58%). IR and ^{1}H NMR spectra were qualitatively identical to those of polymer **3A**. Gel permeation chromatography: M_n = 11080; M_w = 18950; PD = 1.7; anal. calcd. for $C_{8.77}H_{8.84}O_{0.21}$: C 89.5%, H 7.5%; found: C 89.3%, H 7.6%.

Polymers 4

Polymer 4A: From styrene (44 mg, 0.42 mmol, 0.6 equivalents) and compound **2b** (87 mg, 0.28 mmol, 0.4 equivalents). The crude reaction mixture was treated according to work-up procedure **B**. IR: ν = 3060, 3027, 2918, 2860, 2360, 2336, 1944, 1734, 1654 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.6–6.9 (bs, ArH), 4.7–4.2 (bs, Ar-CH_2-O-CH_2- and -COOCH_2-), 3.8–3.5 (bs, -O-CH_2-CH_2-O-and ArCH_2COO), 2.0–1.8 (bs, Ar-CH_2-O-CH_2-CH_2-CH_2-O-), 1.7–1.0 (-CH- and -CH_2- polymer chains); gel permeation chromatography: M_n = 13770; M_w = 34770; PD = 2.5.

Polymer 4B: From styrene (0.67 g, 6.44 mmol, 0.77 equivalents) and compound **2b** (0.59 g, 1.9 mmol, 0.23 equivalents). The crude reaction mixture was treated according to work-up procedure **B**. IR and ^{1}H NMR spectra were qualitatively identical to those of polymer **4A**. Gel permeation chromatography: M_n = 14880; M_w = 25530; PD = 1.7; anal. calcd. for $C_{10.76}H_{11.22}O_{0.69}$: C 85.2%, H 7.4%; found: C 85.4%, H 7.6%.

Polymer 4C: From styrene (0.67 g, 6.44 mmol, 0.8 equivalents) and compound **2b** (0.5 g, 1.6 mmol, 0.2 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **4C** as a white powder; yield: 0.78 g (66%). IR and ^{1}H NMR spectra were qualitatively identical to those of polymer **4A**. Gel permeation chromatography: M_n = 8610; M_w = 15800; PD = 1.8; anal. calcd. for $C_{10.4}H_{10.8}O_{0.6}$: C 85.8%, H 7.4%; found: C 86.1%, H 7.6%.

Polymers 5

Polymer 5A: From styrene (0.78 g, 7.5 mmol, 0.78 equivalents) and compound **2c** (0.7 g, 2.1 mmol, 0.22 equivalents). The crude reaction mixture was treated according to work-up procedure **B**. IR: ν = 3081, 3025, 2923, 2851, 1734, 1601, 1493, 1452 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.4–6.2 (bs, ArH), 4.6–4.1 (bs, Ar-CH_2-O-CH_2- and -COOCH_2-), 3.7–3.3 (bs, -O-CH_2-CH_2-O-and ArCH_2COO), 2.0–0.9 (bs, Ar-CH_2-O-CH_2-CH_2-CH_2-O-, -CH- and -CH_2- polymer chains); gel permeation chromatography: M_n = 13590; M_w = 24220; PD = 1.8; anal. calcd. for $C_{10.86}H_{11.52}O_{0.66}$: C 85.4%, H 7.6%; found: C 85.6%, H 7.5%.

Polymer 5B: From styrene (0.64 g, 6.2 mmol, 0.8 equivalents) and compound **2c** (0.5 g, 1.5 mmol, 0.2 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **5B** as a white powder; yield: 0.86 g (76%). IR and ^{1}H NMR spectra were qualita-

tively identical to those of polymer **5A**. Gel permeation chromatography: $M_n = 9190$; $M_w = 17420$; PD = 1.9; anal. calcd. for $C_{10.6}H_{11.2}O_{0.6}$: C 85.6%, H 7.6%; found: C 86.3%, H 7.8%.

Polymer 5C: From styrene (1.31 g, 12.6 mmol, 0.85 equivalents) and compound **2c** (0.7 g, 2.1 mmol, 0.15 equivalents). The crude reaction mixture was treated according to work-up procedure **A** to obtain polymer **5C** as a white powder; yield: 1.46 g (73%). Gel permeation chromatography: $M_n = 13080$; $M_w = 21740$; PD = 1.7; anal. calcd. for $C_{9.95}H_{10.4}O_{0.45}$: C 87.0%, H 7.6%; found: C 87.4%, H 7.8%.

General Procedure for the Quantitative Determination of Solubility of Polymer 3C in Various Solvent Mixtures

The polymer was added to the solvent mixture (1.5 mL) indicated in Table S2 (see Supporting Information) until some undissolved product was visible after stirring and ultrasonication for 10 min. The solution was centrifuged for 2 min, after which 1.3 mL of it was filtered through a small glass wool plug. Exactly 1 mL of this saturated solution was transferred into a small flask, evaporated, dried under high vacuum and the residue weighed.

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