Direct Amidation of Poly(γ-glutamic acid) with Benzylamine In Dimethyl Sulfoxide

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Partially benzylamidated, amphipathic poly(γ-glutamic acid) (BzPGA) was synthesized from poly(γ-glutamic acid) (PGA) and benzylamine by direct amidation in dimethyl sulfoxide (DMSO). Benzylamine and PGA were heated in DMSO for 1 to 26 h at temperatures between 110 and 130 °C, producing derivatives of various degrees of benzylamidation as a function of the reaction time and temperature. Neither any carboxyl-activating agent nor catalyst is needed for the reaction to proceed. After purification by dialysis, the product was identified by ^{1}H and ^{13}C 1D and 2D NMR in DMSO-d_6. BzPGA prepared by the new direct amidation method was identical to that obtained with a conventional carbodiimide-mediated reaction in water. The one-pot amidation procedure described in the present article can probably be applied to the synthesis of amides from other amines and carboxylic acids.

Introduction

Polymers having polar side groups (e.g., hydroxyl, amino, or carboxyl) are easily modified by chemical reactions to yield cross-linked or derivatized products.[1−7] Polymeric carboxylic acids may be modified by esterification or amidation among others, the latter gives generally better chemical resistance against hydrolysis but is more difficult to perform, as, in contrast to esters, amides do not form readily from their precursors.[8] In protic solvents, especially in water, the acid−base equilibrium of amines and acids leads to ion formation, leaving very little unprotonated species, which are required for the amidation process. At temperatures high enough, crystalline amine salts of carboxylic acid do react by condensation; these reactions are mostly performed at 160−200 °C under inert gases or *in vacuo* to favor the release of water molecules.[9,10] These are rather harsh conditions which are not suitable for many organic molecules. To overcome this problem, most syntheses use activating agents which react with either the carboxylic or the amine groups, forming active intermediates and allowing for the amidation to take place at lower temperatures by circumventing ion formation.[11−13] Activation of the carboxyl group is generally made by halogenation, anhydratization, esterification (yielding respectively acyl halides, acid anhydrides, or esters), or with carbodiimides, although several other activators are in use.[13] Acyl halogenides are however difficult to prepare from polymers bearing other reactive functional groups beside carboxyls, or from those which are easily hydrolyzable (like some polyesters, polysaccharides, polyamides, or polypeptides, where partial or complete hydrolysis of the polymer backbone would occur). With the less reactive acid anhydrides or esters this can at least partially be overcome, but nevertheless the polymer needs first to be converted into the anhydride or ester form; thus, the procedure becomes lengthier and more laborious than typical one-pot reactions. The most popular activating agents in use today are the various carbodiimides (used often together with active esters) which can form *in situ* the activating complex even at low temperatures and are subsequently displaced by the amines acting as nucleophiles to yield amides with the concurrent formation of carbamides.[13] Besides these advantages, there are some drawbacks which make carbodiimides the less than ideal coupling agents. For example, the popular dicyclohexylcarbodiimide (DCC) is converted to dicyclohexylurea during the amidation.[13] While dicyclohexylurea generally precipitates from the reaction media, it is often significantly soluble in some of them and needs to be separated from the amide produced. For polymers, this mostly results in tedious precipitation/wash cycles.[14] Water-soluble carbodiimides (WSC) have the advantage of being soluble in aqueous media and can be eliminated by simple dialysis. However, they as well as the cheaper DCC may lead to racemization and acyl transfer as side reactions, and the *N*-acylurea subsequently formed cannot easily be split off the polymer.[15] The incidence of acyl transfer can be diminished by the use of low temperatures for coupling, or with the addition of certain nucleophiles like 4-hydroxybenzotriazole.[13] Newer condensing reagents with higher selectivity than carbodiimides were also investigated.[16] Other novel dehydrating agents include tetraalkoxysilanes, which are also effective in promoting amide bond formation.[17]

Certain authors have reported the use of microwave for the direct amidation of amines with carboxylic acids in organic media. This procedure is very fast, producing amides after only 5 min of irradiation under appropriate conditions.[18−20]

During the past few years, a very promising new way of amidation was published, which uses boron compounds in catalytic amounts to promote the condensation reaction.[21−23] Amines and carboxylic acids are generally dissolved in toluene or other high-boiling solvents containing small amounts of the catalyst (usually at concentrations less than 0.25 mol equiv) and heated under reflux for several hours. Water as a byproduct is eliminated from the reaction mixture by azeotropic distillation. Reaction yields are often very good, sometimes higher than 97%. For carboxylic polymers, the process variant using simple boric acid is particularly appealing, as this catalyst is very cheap and relatively nontoxic and can easily be separated from the product by dialysis in water.[24,25]

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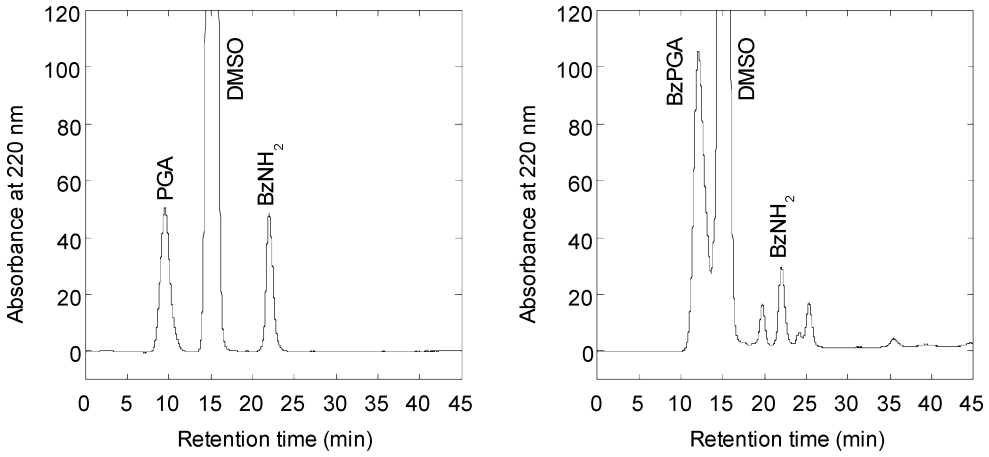

Figure 1. SEC chromatogram of the reaction mixture under nitrogen at initial time (left) and after 8 h at 130 ± 5 °C (right). Reaction conditions: 0.1 M PGA-H (as repeat unit) and 0.1 M $BzNH_2$ in DMSO. Chromatographic conditions as described in Experimental Section.

Since poly(γ-glutamic acid) (PGA) is insoluble in toluene, we have used dimethyl sulfoxide (DMSO) during our initial experiments on boric acid-catalyzed amidation with benzylamine ($BzNH_2$) to yield partially benzylamidated PGA derivatives (BzPGA). We discovered that amidation in this solvent took place equally well either in the absence or in the presence of boric acid. The complete lack of acyl activating agent makes the process even more simple; a one-pot reaction is easily performed by heating PGA and benzylamine in a suitable vessel. The resulting derivative can be purified by dialysis, or precipitation−wash cycles, or obtained in crude form by simply evaporating DMSO *in vacuo*. The presence of air does not seem to interfere significantly with the amidation process; however, it increases the amount of chromatographically detectable byproducts.

Experimental Section

Biosynthesis of PGA. PGA was produced by bacterial fermentation, using *Bacillus licheniformis* ATCC 9945A grown aerobically on medium "E" at 37 °C.[5] At the end of the fermentation, cells, debris, and spores were harvested by centrifugation, and the clear supernatant mixed with an equal volume of acetone. Precipitated raw PGA was recovered, washed further with pure acetone, and then frozen in a freezer until the subsequent purification procedure.

Purification of PGA and Conversion into the Acidic Form. Raw PGA was redissolved in distilled water and then heated above 70 °C for 2 h in order to eliminate traces of acetone as well as to inactivate proteolytic enzymes possibly present from cell lysates. When this solution was cooled, 1 g/L of salicylic acid was added as a chelator for multivalent metal ions and preservative against fungal growth (salicylic acid is washed away during diafiltration and does not remain in the purified PGA solution). After the dissolution of salicylic acid, the PGA solution was filtered on polyvinylidene difluoride membranes of decreasing porosity, the smallest being 0.2 μm. Then pH was adjusted to 2.5 with dropwise addition of hydrochloric acid in order to bring PGA into its acidic form (PGA-H), as the sodium salt of PGA (PGA-Na) is only slightly soluble in DMSO. Upon lowering the pH, the solution became much less viscous, indicating that protonation of the carboxylic groups took place. The acidified solution was subsequently diafiltered with a VIVAFLO 200 or 500 unit (Sartorius Vivascience GmbH, Germany) equipped with a 100 000 MWCO polyethersulfone membrane until the solution's volume was reduced to one-fifth of the original. Diluted hydrochloric acid was added to the filtrate (final pH = 2.5) and the diafiltration cycle repeated three times. Additional extensive distilled water wash was applied until the effluent permeate was free from HCl (checked by $AgNO_3$ solution). The purified PGA was further concentrated by diafiltration, frozen, and lyophilized.

Direct Amidation Reaction. PGA-H was dissolved in DMSO by stirring and gentle heating to 50 °C at a carboxyl group concentration of 0.2 M (the molar weight of a single PGA repeat unit is 129). Benzylamine (95 mol %) (versus the COOH groups) was added, and then the mixture was heated with continuous stirring in a three-necked bottle, equipped with a cotton plugged outlet, a nitrogen inlet, and a thermometer. The temperature was adjusted to 110 °C by means of a silicone oil bath, and the nitrogen bubbling rate was set to one bubble per 2−3 s. Alternatively, various molar ratios of benzylamine were added to 0.2 M carboxyl-equivalent PGA solution in DMSO and then heated either in stoppered glass vials under nitrogen or in open microcentrifuge tubes/HPLC glass vials for different amounts of time and at various temperatures in order to prepare partially amidated PGA. In each case the reaction was stopped by cooling the reaction mixture to room temperature.

Amidation in the Presence of Carbodiimide. PGA-H (0.25 mmol) was dissolved in 2.5 mL of water containing 0.25 mmol of benzylamine at room temperature with continuous stirring. To this solution was added 0.0875 mmol (equivalent to 0.35 mol % in respect to PGA free carboxyl groups) of solid 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (Aldrich, Sigma-Aldrich Kft, Budapest, Hungary), and then the mixture was shaken vigorously in order to dissolve the carbodiimide. The amidation reaction was allowed to proceed for 44 h at room temperature under constant stirring. Alternatively, to 2.5 mL of ice-cold aqueous solution containing 0.25 mmol of PGA-H and 0.25 mmol of $BzNH_2$ was added the mixture of 2.5 mL water and 0.2 mmol of the above-mentioned carbodiimide dropwise with continuous stirring, and the reaction was continued at room temperature for 20 h. Samples were then dialyzed and freeze-dried as described previously.

Size Exclusion Chromatography. Periodically, samples were drawn from the reaction mixtures, diluted with HPLC eluent, and then analyzed by size exclusion chromatography (SEC) on an HP 1090 liquid chromatograph (formerly Hewlett-Packard, now Agilent Technologies, Santa Clara, CA), equipped with a Waters Ultrahydrogel Linear (Waters Kft, Budapest, Hungary) column (300 × 7.8 mm). The eluent contained 5 mM NaH_2PO_4, 7.5 mM Na_2HPO_4, 140 mM NaCl, and 20% (V/V) acetonitrile in water. The flow rate was set to 0.7 mL/min and the column thermostated at 40 °C. The effluent was monitored with the built-in diode array detector of the HP 1090, allowing to record chromatograms simultaneously at several wavelengths.

Purification of the Reaction Products. The reaction mixtures were dialyzed in conventional cellulose dialysis bags (MWCO = 10 000, Sigma-Aldrich Kft, Budapest, Hungary) twice against 1% acetic acid for at least 5 h, in order to wash out the ionically bound unreacted

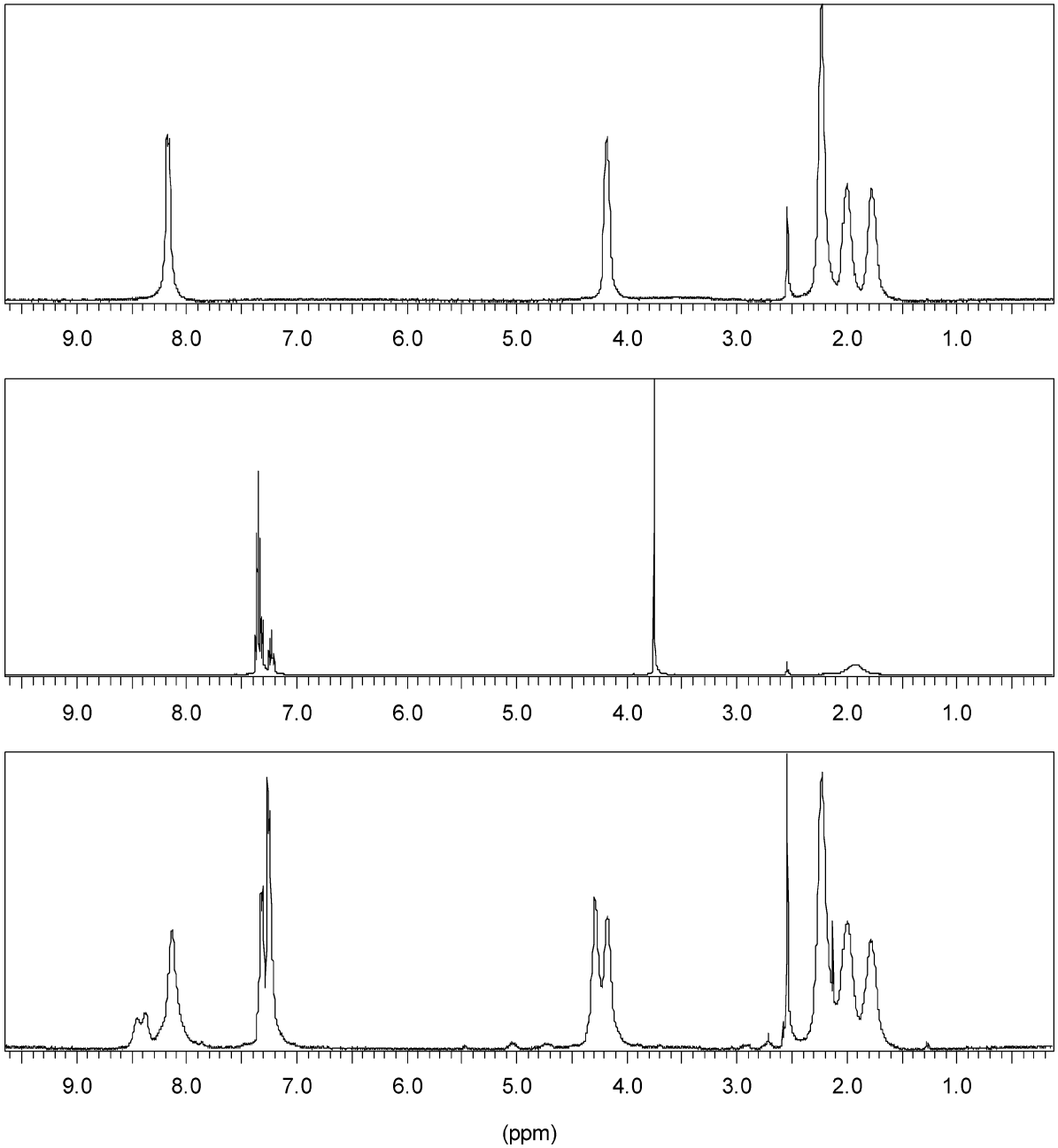

Figure 2. ^{1}H NMR spectra of PGA (upper graph), benzylamine (middle graph), and BzPGA with 30 mol % of incorporation (lower graph). Peaks from left to right: aromatic amide (8.39 ppm), aliphatic amide (8.11 ppm), aromatic ring (7.22 and 7.28 ppm), benzyl CH_2 (3.75 ppm for $BzNH_2$, shifted to 4.28 ppm for BzPGA), α CH (4.14 ppm), γ CH_2 (2.19 ppm), β and β' CH_2 (1.96 and 1.74 ppm). Solvent peak is at 2.5 ppm.

benzylamine, and then three times against distilled water. Sometimes a prewash consisting of a water−1% $NaHCO_3$ solution−water cycle preceded the acetic acid dialysis. Purification was considered to be finished when the wash solution had a pH of at least 5 and no free $BzNH_2$ was detectable by SEC. Finally, the washed benzylamidated PGA was frozen and then lyophilized.

NMR Measurements. Between 10 and 50 mg of PGA-H, benzylamine, and BzPGA were dissolved in 600 μL of hexadeuterated DMSO (DMSO-d_6) and analyzed with Bruker DRX 360 MHz and DRX 500 MHz NMR instruments. Routine ^{1}H NMR and ^{13}C NMR measuring programs were used to record spectra in 1D J-modulated (^{13}C) and 2D (COSY and HETCOR) mode.

Chemicals. Analytical grade DMSO (Spektrum-3D Kft, Debrecen, Hungary) was purified from possible thiolic contaminants by keeping it over powdered metallic silver overnight and subsequent vacuum distillation. Benzylamine (Aldrich, Sigma-Aldrich Kft, Budapest, Hungary) was also purified by distillation under reduced pressure. Other chemicals, unless noted otherwise, were of analytical grade.

Results

Synthesis of BzPGA. The mixture of PGA-H and benzylamine, when heated in DMSO, undergoes a slow conversion to BzPGA (Figure 1). Comparative ^{1}H NMR analysis of PGA-H, $BzNH_2$, and BzPGA confirms that the amidation reaction takes place (Figure 2).

The retention time of BzPGA by size exclusion chromatography (SEC) is larger than that of PGA, and both conversion and retention time increase with the reaction time (Figure 3).

The extent of the amidation depends on the temperature, time, and the ratio of the reactants. Upon a reaction time of 26 h at 110 °C with slow nitrogen purge, about 30 mol % benzylamine is incorporated into the product. At higher temperatures the reaction proceeds faster: about 50 mol % incorporation is attained at 130 °C after 8 h in closed vessel under nitrogen (Figure 3, left axis). However, increasing the temperature also

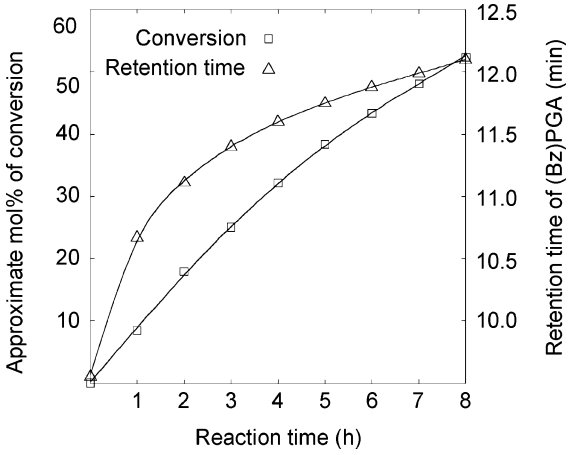

Figure 3. Time dependence of the conversion (left axis, squares) and retention time of the PGA or BzPGA peak by SEC (right axis, triangles) as a function of the reaction time. Reaction conditions: 0.1 M PGA-H (as repeat unit) and 0.1 M benzylamine in DMSO, closed vessel at 130 ± 5 °C under nitrogen. Conversion values calculated from the benzylamine concentration decrease, as measured by SEC (possible byproduct formation not taken into account).

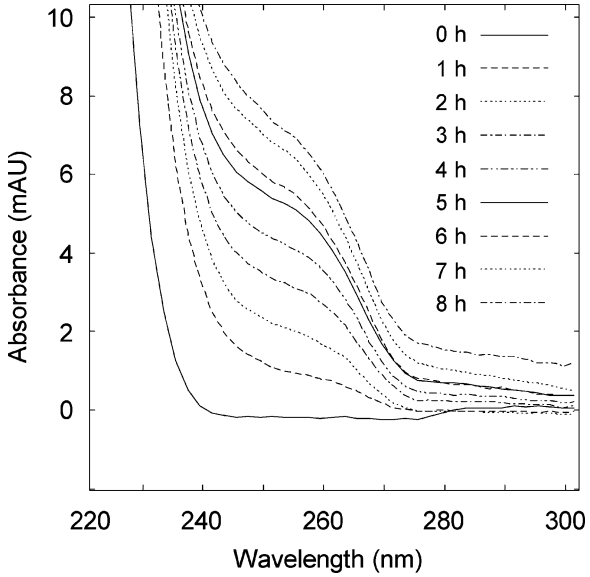

Figure 4. UV absorbance of the (Bz)PGA peak in function of the reaction time. Reaction conditions: 0.1 M PGA-H (as repeat unit) and 0.1 M benzylamine in DMSO, closed vessel at 130 ± 5 °C under nitrogen. Detection on-line by diode-array detector.

possibly increases the incidence of side reactions; it is therefore best not to exceed 130−140 °C since some decarboxylation of the polymer could occur. Incorporation percentages calculated from the benzylamine concentration decrease agree fairly well with data obtained from the ^{1}H NMR integrals: for the last data point in Figure 3, stoichiometric calculation gives 52 mol % and NMR analysis yields between 42 and 48 mol % benzylamide content. The difference is probably caused by the fact that stoichiometric calculation does not account for possible byproduct formation. A similar result is found for the experiment conducted at 110 °C for 26 h, where both $BzNH_2$ concentrations decrease and ^{1}H NMR integrals indicate around a 30 mol % conversion. Here the agreement between the two values is better, the incidence of side reactions being possibly related to the temperature.

During the course of the reaction, the initial water-clear color of the solution changes gradually to pale yellow, but this can be minimized with the use of high purity grade DMSO or by vacuum distilling the solvent from powdered metallic silver before use. We have observed that darkening of the reaction mixture is more pronounced when PGA-H is in excess; therefore, a (near) 1:1 molar ratio of PGA-H (as repeat unit) to benzylamine was used throughout the experiments. This setup has the additional benefit to facilitate the reaction rate order determination.

UV spectra of the chromatographic peaks reveal that while PGA has an UV cutoff of about $\lambda = 240$ nm above which its light transmittance is virtually complete, BzPGA presents a small "shoulder" at approximately $\lambda = 260$ nm, specific to the aryl ring incorporated into the product (Figure 4). The intensity of this shoulder correlates with the extent of amidation, but owing to its rather small amplitude, it is not a very sensitive marker of the amidation degree.

The reaction is not particularly sensitive to the presence of oxygen. When comparing the reaction under air and nitrogen, the only difference we have found is the higher area of some byproduct peaks when the nitrogen purge was omitted.

Condensation reactions often require the elimination of the small molar weight reaction products from the reaction medium in order to have a sufficiently high reaction rate, which is usually accomplished by evacuation, distillation, or inert gas bubbling. DMSO has a high boiling point (189 °C at atmospheric pressure[26]), permitting the use of temperatures exceeding 100 °C. Under these conditions, water evaporates from the reaction mixture in an open vessel. To minimize any possible deleterious effect of atmospheric oxygen onto the reaction or the medium, inert gas bubbling is often preferred to this method. It was however surprising to observe that the amidation proceeded very well even in a closed system, where water could not escape. We attribute this observation to the hygroscopic character of DMSO, water molecules being probably highly solvated and their hydrolytic activity greatly reduced, but also to the equilibrium constant of the amidation reaction being sufficiently large to accommodate a higher water content of the reaction medium in contrast to ester formation reactions.[9]

NMR Analysis. ^{1}H and ^{13}C NMR spectra assignments of γ-PGA were already published in the literature.[5] The symbols used for citing the different protons and carbons are shown in Figure 5, while chemical shift values are tabulated in Table 1.

The upper graphs in Figures 6 and **7** show the ^{1}H and ^{13}C carbon NMR spectra of partially amidated BzPGA (reaction in DMSO at 110 °C for 26 h under nitrogen). This sample was determined as being around 30 mol % of amidation by SEC. The benzylamidation of PGA changes the chemical shifts of the PGA skeleton only within the experimental error, and new aromatic and amide peaks appear in both the ^{1}H and ^{13}C NMR spectra of BzPGA (see Table 1). The assignment of the peaks was made by usual 2D ^{1}H−^{1}H and ^{1}H−^{13}C correlation techniques.

Assignment of the peaks is very important because ^{1}H NMR is usually quantitative; therefore, the degree of amidation can be determined from the integrals. Three possibilities of calculations showed that the nominal (30 mol %) amidation is satisfactorily achieved. i. If we take the number of $\gamma+\beta$ methyl protons of one PGA repeat unit as 4 and the number of protons incorporated with one phenyl group as 5 then in case of complete (100 mol %) amidation a 4/5 ratio of integrals would be found (the integral of the backbone methylene protons divided by the integral of the phenyl protons should give 4/5). In our case this ratio was 4/1.57 which corresponds to (1.75/5) × 100 = 35 mol % of amidation. ii. The total integral of the amide peaks is 1.27; therefore, the increase of intensity from the new amide

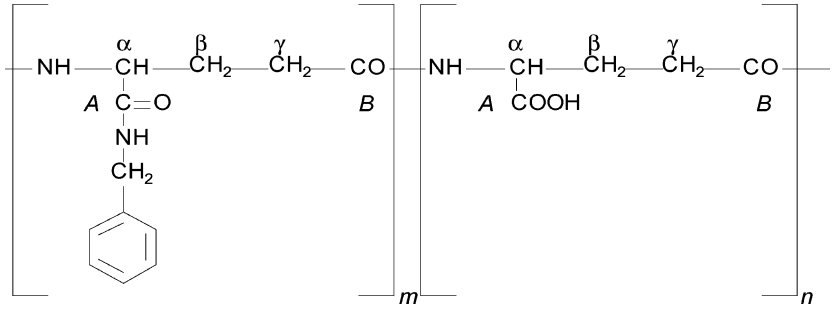

Figure 5. Scheme of partially benzylamidated PGA and the symbols used for citing protons and carbons.

Table 1. ^{1}H and ^{13}C NMR Shifts of PGA and BzPGA in DMSO-d_6[a]

	^{1}H NMR chemical shift (ppm)		^{13}C NMR chemical shift (ppm)	
group name/symbol	PGA	BzPGA	PGA	BzPGA
α (CH-PGA)	4.14	4.14	51.6	51.8
β (CH_2-PGA)	1.73	1.74	27.1	27.2
β (CH_2-PGA)	1.95	1.96	27.1	27.2
γ (CH_2-PGA)	2.19	2.19	31.6	31.7
benzyl (CH_2)	-	4.28		40.2
NH (PGA)	8.13	8.11	-	-
NH (BzPGA)		8.39	-	-
benzyl ring (two multiplets)		7.22, 7.28		126.7, 127.0, 127.2, 128.3
benzyl (quaternary C)				139.4, 139.6
CO	-	-	171.6 (A), 173.5 (B)	171.6 (A), 173.7 (B)

[a] The ^{1}H and ^{13}C peaks of DMSO were used for calibration as 2.50 and 39.5 ppm, respectively.

peak is approximately 27%. iii. Finally, if we integrate together the CH of the PGA skeleton and the benzyl-CH_2 overlapped signals and subtract 1 unit from the result (as CH from the backbone should give 1 unit) a ratio of 0.59/2 (i.e., 29.5% of amidation) is found. In light of these calculations, we tend to believe that 30 ± 5% is the real degree of amidation of this particular sample of BzPGA.

^{13}C NMR also shows the successful amidation. Peaks were assigned by means of COSY and HETCOR through proton−proton couplings and proton−carbon couplings, respectively. The ^{13}C spectrum is not as simple as the ^{1}H NMR spectrum; therefore, it holds some extra features to be explained. In Figure 7 one can find the newly appeared peaks of benzyl CH_2 carbons and aromatic carbon peaks of the benzyl group. However, the skeleton CH carbon peak number doubled, indicating a difference in chemical shift of the benzylated product compared to PGA. This is not surprising because this carbon is very close to the binding site of the benzyl group.

Interestingly only two CO peaks appear in the ^{13}C NMR spectrum of BzPGA, similarly to PGA. One of them belongs to the COO group (site A) while the other belongs to the CONH group (either at site A or B). The explanation of the difference in intensity, although ^{13}C NMR is only approximately quantitative, is that 30% of the COO groups became CONH groups (as measured from the ^{1}H NMR integrals). Since no more peaks are in the **C**O shift range, we suppose that the chemical environment of the newly formed **C**ONH (at site A) and of the original **C**ONH (site B) differs slightly and so does their chemical shift, but this shift is too small to be resolvable. That is, the peak at 173.5 ppm consists of two overlapped peaks.

The number of benzyl CH group peaks should be three, but four peaks are detected which all belong to benzyl CH protons according to the HETCOR experiment (data not shown). It indicates that in the secondary structure of BzPGA the benzyl group may have two chemical environments. This observation is a subject of further studies. 2 D ^{1}H $-^1$H NOESY experiment with 100 ms short mixing time shows cross-peaks between the benzyl CH_2 protons and the PGA skeleton β and γ protons. There is also a cross-peak between the original CONH protons and the benzyl CH_2 protons. These observations demonstrate that the benzylamide group is built onto the PGA chain. Moreover a pronounced cross-peak between the NH protons of the CO(B)NH group originally present and the benzyl CH_2 can help us to draw the secondary structure of the molecule. This study is under progress by means of NOE build-up experiments.

Similar results were found for other direct amidation experiments; therefore, NMR studies show that the synthetic procedure described in this article looks like a usable general technique to modify PGA polymers.

Comparison with Carbodiimide-Mediated Amidation. Amidation reactions are nowadays mostly performed at or below room temperature in the presence of a carbodiimide, and most authors consider this coupling method as a standard way to form amides. We therefore compared BzPGA obtained by the novel direct amidation procedure with that from a more "conventional" water-soluble carbodiimide (WSC)-mediated reaction. By reacting 0.25 mmol of PGA-H and 0.25 mmol of benzylamine with 0.00875 mmol of [(dimethylamino)propyl]-3-ethylcarbodiimide methiodide for 44 h at room temperature in order to achieve the maximal possible conversion, a white fluffy product was obtained after dialysis and freeze-drying which was similar to the BzPGA samples obtained earlier. It must be noted though that while WSC was present at 35 mol % and benzylamine at 100 mol % versus the free PGA carboxyl groups, the yield of this reaction was significantly lower than expected (only about 2−3 mol % of conversion instead of the theoretical maximum of 35 mol %, as determined by ^{1}H NMR integrals). Therefore, another WSC-mediated amidation was performed, the quantity of WSC versus PGA and $BzNH_2$ was increased to 80 mol % but the reaction time shortened to 20 h. This time the incorporation of $BzNH_2$, as measured from the ^{1}H integrals, was found to be around 6 mol %. This gives an amidation degree similar to that with only 1 h of direct amidation in DMSO at 130 °C, demonstrating the time-effectiveness of the latter reaction. The extent of $BzNH_2$ incorporation was nevertheless enough to permit the comparison with BzPGA synthesized by the direct amidation method.

SEC chromatograms show that both the direct and the indirect amidation process yield products having increased retention times compared to PGA-H (11.2 min for the second WSC reaction; for direct amidation, see Figure 3, right axis). The UV absorption spectra are also very similar with a small shoulder

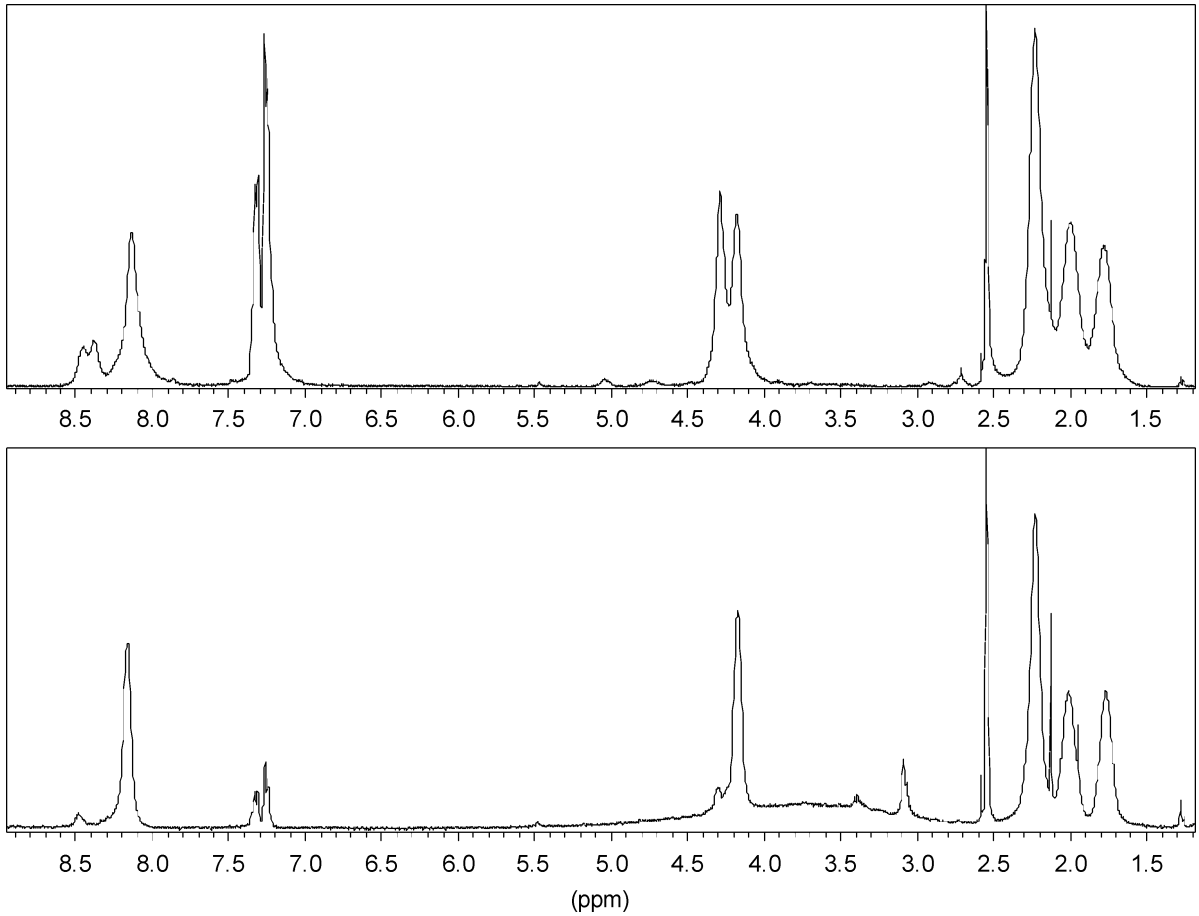

Figure 6. ^{1}H NMR spectra of BzPGA in DMSO-d_6. Upper graph: prepared by direct amidation (30 mol % conversion). Lower graph: prepared by WSC-mediated amidation (6 mol % conversion). The narrow peak at 2.1 ppm comes from acetic acid used during the dialysis; the DMSO solvent peak is at 2.5 ppm. For the exact reaction conditions, see the text.

around $\lambda = 260$ nm specific to the benzyl group (not shown), further indicating that the end-products are probably the same. This is also confirmed by comparing the ^{1}H and ^{13}C NMR spectra of the products obtained by the two procedures (Figures 6 and **7**). Our results confirm that the reaction of benzylamine and PGA-H in DMSO leads indeed to the amidation of the latter compound, and this compound is chemically identical to that obtained by WSC-mediated amide formation.

Overall Reaction Order. When PGA is reacted with benzylamine in equimolar amounts (PGA carboxyls versus $BzNH_2$), the logarithm of the concentration of unreacted benzylamine as a function of the reaction time gives a straight line with a good approximation (Figure 8). The overall reaction is therefore of first order, indicating the presence of a slower, rate-limiting step during the amidation process (which may well be the folding/unfolding of the PGA chain or the slow diffusion of benzylamine through the solvated polymer coil).

Solubility of BzPGA in Water. In contrast to PGA-H which is slightly but definitely soluble in water, the benzylamidated derivatives precipitate from the solution as white clumps during dialysis, when DMSO is gradually exchanged with dilute acetic acid. When BzPGA is first converted into the much more soluble Na^+ form by dialysis with 1% (W/V) $NaHCO_3$ solution, subsequent acetic acid dialysis yields a more stable, uniformly hazy solution, which is additionally purer, because impurities, which would be entrapped into the precipitating undissociated BzPGA if only acetic acid dialysis were used, are effectively washed out during the preceding alkaline wash.

Water solubility of BzPGA mainly in the H^+ form (namely at pH lower than 3−4) increases with decreasing benzylamidation degree. On the other hand, solubility of even the scarcely water-soluble PGA derivatives increases significantly when the pH is brought higher than about 5. It can be assumed that the remaining free, nonbenzylamidated carboxylic groups dissociate at higher pH and the outer layer of the molecule gains a more hydrophilic character. The benzylamide groups are probably expelled from the bulk solvent phase by the hydrogen bond network present between water molecules and are "folded" inside the molecule into a relatively water-poor environment; thus, the molecule acquires an amphiphilic character.[7] A similar effect (though on a multimolecular scale) is observed with micelle-forming compounds like soaps; hence, the BzPGA derivatives can be termed "unimolecular micelles" or "polysoaps".[14,27]

Discussion

In contrast to the popular carbodiimide-based coupling reactions, direct amidation has been known for more than 100 years. It is mainly used nowadays for the direct synthesis of polyamides by letting salts of dicarboxylic acids and diamines react in the melt at temperatures exceeding 160−200 °C and generally at low pressure. To our knowledge, this process has never been used for amidating a high molecular weight

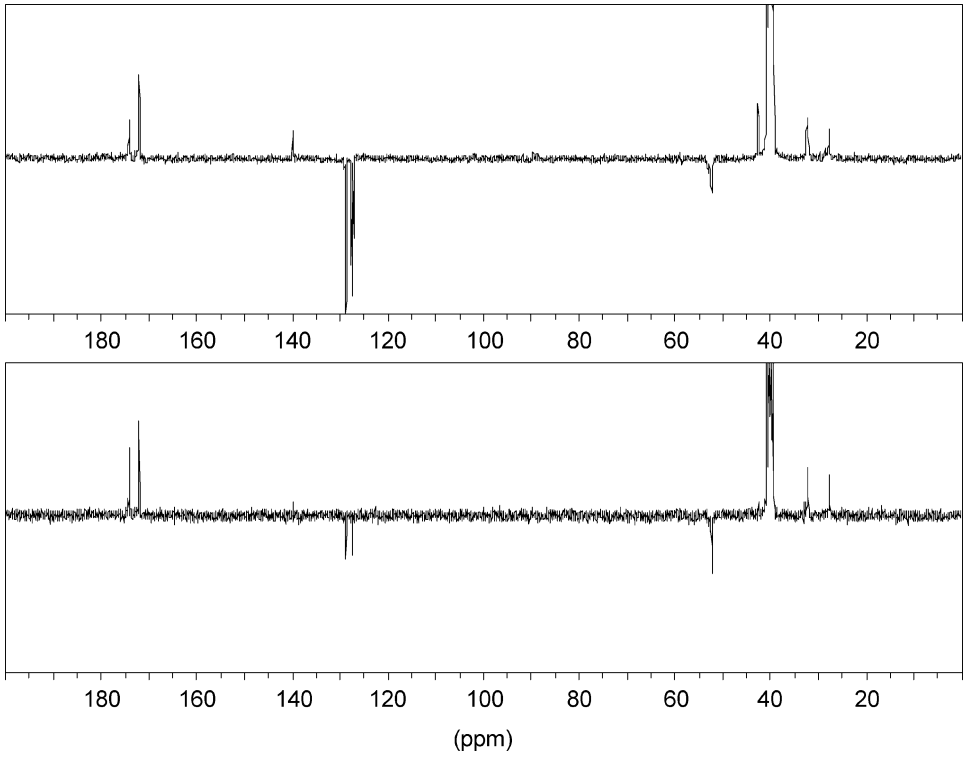

Figure 7. J-modulated ^{13}C NMR spectra of BzPGA in DMSO-d_6. Upper graph: prepared by direct amidation (30 mol % conversion). Lower graph: prepared by WSC-mediated amidation (6 mol % conversion). For the exact reaction conditions, see the text.

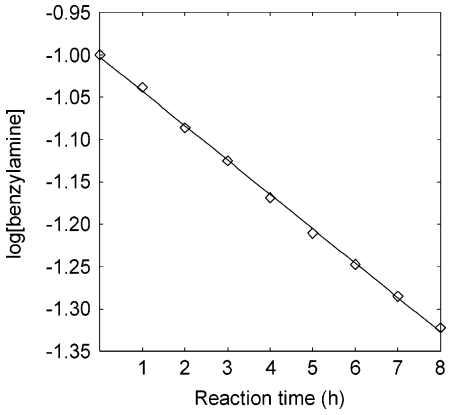

Figure 8. Logarithm of the remaining benzylamine concentration as a function of reaction time at 130 ± 5 °C. Reaction conditions: 0.1 M PGA-H and 0.1 M $BzNH_2$ in DMSO in a stoppered and stirred vial under nitrogen. Chromatographic conditions as described in Experimental Section.

polypeptide in DMSO. In protic solvents, carboxylic acids and amines form ions, as the acidic dissociation constant of the carboxylic group is much higher than that of the protonated amine. For example, in water the pK_A of acetic acid is about 4.8 at room temperature (we did not find unequivocal literature data concerning PGA), while the pK_{BH} of benzylammonium ($BzNH_3^+$) is 9.3. If our reactants were dissolved in water, the very large difference in pK_a values would cause the $BzNH_2$ + PGA-H ↔ $BzNH_3^+$ + PGA^- equilibrium to be shifted completely toward the right, almost no free benzylamine and protonated PGA being present, although both of them are required for the amidation to proceed.

DMSO is an aprotic solvent with a high dipole moment.[26] The polarization of the sulfur−oxygen bond makes oxygen bear a partial negative, and sulfur a positive charge. Owing to this charge repartition, DMSO solvates cations very well while anions are only poorly solvated.[28] As a consequence pK_A values of carboxylic acids become much larger in DMSO than in water (e.g., 12.6 and 4.8, respectively, for acetic acid), while the acidic strength of alkylammonium ions remains approximately the same (pK_{BH} of ethylammonium is 10.6 in water and 11.0 in DMSO) or is even increased (from 10.7 in water to 9 in DMSO for triethylammonium).[29] Therefore, this means that in DMSO, benzylammonium ion is probably a stronger acid than PGA; thus, formation of $BzNH_3^+$ and PGA^- is mostly suppressed, and there are enough undissociated precursors for the condensation reaction. Another benefit lies in the fact that DMSO has a boiling point of 189 °C at atmospheric pressure which enables the use of quite high temperatures to speed up the synthesis, while water evaporates from the medium without influencing the reverse reaction. It must be noted however, at least when the reactants are present in relatively small concentrations, e.g., around 0.1 mol/L, that the amidation process is practically not affected by water produced during the course of the reaction and the synthesis can easily be performed in a closed vessel. We assume this is made possible by the fairly high equilibrium constant of the amidation reaction which makes the process more tolerant toward water than, for example, esterification.[9] Finally, DMSO is a very good solvent for many polyamides.[30,31] This is a very useful benefit, for if the product was insoluble, its precipitation would slow down the incorporation of further benzylamido groups, and at the same time randomness of the substituents along the polymer chain would be compromised.

NMR data show that the expected amount of benzylamine builds into the polymer. However, there are some doubts which are to be the subject of a longer discussion. One of these doubts is whether amidation has really occurred or is benzylamine merely present as its PGA salt in the reaction product (the term "salt" is correct here since in contrast to what is happening in pure DMSO, dissociation of both PGA-H and $BzNH_2$ is complete in the aqueous dialysis solution). First, SEC chromatograms do not show the presence of free $BzNH_2$ in the purified products. This is very strong evidence which demon-

strates that our BzPGA samples are not simple benzylammonium salts of PGA but covalently linked amides. In our aqueous chromatographic eluent containing 160 mmol of Na^+ ions per liter, almost complete dissociation of any benzylammonium salt would instantly occur, which should lead to the complete separation of the PGA and $BzNH_2$ peaks during the chromatographic run. It was indeed observed that irrespective of their molar ratio, benzylamine and PGA are always well separated by SEC and can unequivocally be distinguished from each other as well as from DMSO. Second, the BzPGA peak's steady retention time increase as a function of the reaction time (and the concomitant decrease of the $BzNH_2$ peak area) shows that a chemical reaction happens with both PGA and $BzNH_2$ during heating (see Figure 3). We explain the increased retention time of BzPGA by the formation of a more compact, micelle-like structure. In aqueous solutions, the hydrophobic benzylamido groups are probably "folded" inside the molecule while the outer layer is composed of the free carboxyl groups. Such an effect is described for PGA modified by L-phenylalanine ethyl ester[7] to which BzPGA should behave very similarly. Also, the appearance of a new amide peak around 8 ppm on the ^1H NMR spectrograms indicate that the amidation indeed takes place. One further problem which should be addressed is the possibility of transamidation; that is, a peptide bond of the PGA skeleton might theoretically break by reacting with benzylamine, forming a chain fragment terminated by a benzylamide and another chain fragment with a newly liberated free NH_2 end-group. However, if this happened then probably very small molecules (e.g., oligomers) would also form and the polydispersity of BzPGA would increase during the course of the reaction, but there are no traces of such changes in the SEC chromatograms. Additionally, the overall appearance of the ^1H NMR spectrum of BzPGA indicates that no degradation happened. The peaks are broad; in other words, the transverse relaxation of BzPGA is faster (the rotation correlation time longer) than that of small molecules. The fact that the ^1H NMR peaks belonging to the pendent phenyl ring are somewhat narrower than those of the main PGA chain (Figures 2 and 6) is not surprising either, as their rotation around the methylene−phenyl bond is probably faster (their relaxation slower) than that of the bulky backbone. All together this indicates that transamidation does not play a significant role during direct amidation.

Conclusion

The reaction presented in this paper is very easy to set up. It does not require any carboxyl-activating agent or catalyst and is remarkably insensitive toward the presence of oxygen. While some oxidation or condensation products of low molecular weight might be formed in an open vessel, this can be minimized with the use of analytical grade DMSO; moreover, these products are easily washed out during dialysis. When these byproducts are nevertheless a concern, use of a stoppered vessel under inert gas or the use of an almost completely filled screw-capped bottle seems the easiest way to proceed. Such a system does not necessitate continuous inert gas sparging, and the formation of oxidative byproducts is greatly suppressed compared to an open vessel reactor. Moreover, a closed system setup facilitates the stoichiometric quantitation of the precursors and products.

In lieu of the time-consuming dialysis, a faster but less accurate cleaning procedure consists of diluting the reaction mixture with acetone or toluene in which DMSO, benzylamine, and most of the byproducts are soluble. BzPGA gradually precipitates from this solution, especially in the presence of small quantities of salts like $NaHCO_3$ (ionomer effect[32]). The relative amount of organic solvent needed depends primarily on the benzylamidation degree of the product.

Beside PGA and benzylamine, the direct amidation process in DMSO can probably be applied to the synthesis of other DMSO-soluble polymers as well, but care should be taken that in the presence of dehydrating agents, DMSO can effect oxidation on hydroxyl-containing compounds, yielding aldehydes or ketones with the concurrent formation of dimethyl sulfide.[33,34]

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Supporting Information Available. ^1H NMR spectrum of PGA in DMSO, ^{13}C NMR spectrum of PGA in DMSO, $^1H-^1H$ COSY spectrum of PGA, HETCOR of PGA, $^1H-^1H$ COSY of BzPGA, part of $^1H-^{13}C$ HETCOR of BzPGA indicating the assignment of benzyl CH_2, alkyl CH, and benzyl CH peaks, and 2D $^1H-^1H$ NOESY of BzPGA. Mixing time is 100 ms. This material is available free of charge via the Internet at http://pubs.acs.org.

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