# Supramolecular Materials from Multifunctional Pyroglutamic Acid Derivatives

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**Introduction.** Pyroglutamic acid, the internally protected form of glutamic acid, is a naturally occurring $\alpha$-amino acid and is commonly found as an active component in biological systems. There has been recent interest in peptide-based drug design incorporating constrained amino acid residues for the purpose of strategic receptor interaction, and as a result, functional pyroglutamates have been investigated as bioactive drugs.[1] For example, pyroglutamic acid derivatives have been shown to act as nootropics, compounds that improve memory and learning.[2] When administered to mice, pyroglutamates countered the effect of memory impairing drugs and the effects of electroconvulsive shock in passive avoidance tests.[2] Pyroglutamate-based antagonists have also been found to inhibit the interaction of vascular cell adhesion molecule-1 (VCAM-1) and the integrin, very late antigen-4 (VLA-4).[3] Since the binding of VLA-4 to VCAM-1 plays a role in inflammation, pyroglutamate derivatives may be useful for treating rheumatoid arthritis and asthma.[3]

In organic synthesis, pyroglutamic acid has been frequently used as a starting material for the preparation of enantiomerically pure compounds, as chiral auxiliaries in asymmetric synthesis, and as a precursor for other amino acids.[4−6] For example, pyroglutamates may be converted to glutamic acid derivatives in solution or on solid phase supports by nucleophilic ring opening catalyzed by KCN.[7−9] In addition, $\gamma$-amino acids and conformationally constrained glutamic acid, alanine, lysine, and ornithine derivatives may also be prepared directly from pyroglutamic acid.[10−12]

Self-assembling supramolecular polymers based upon noncovalent binding has attracted considerable interest due to the potential applications in the areas of molecular devices and biological mimics.[13−16] One recent report in the literature indicates that pyroglutamic acid may be useful in forming molecular associations.[17] In that paper, the authors report that *N*-alkylammonium salts of pyroglutamic acid display smectic liquid crystalline character. In comparison, the authors note that, while analogous ammonium salts of polyacrylic and polymaleic acid show liquid crystalline character, the monomeric analogues do not. Since the *N*-alkylammonium pyroglutamates are not polymeric, the observation of liquid crystalline behavior probably results from the combination of van der Waals association of the long alkyl groups and hydrogen-bonding interactions of the pyroglutamate headgroups.

We have demonstrated recently that pyroglutamic acid derivatives may be easily prepared from the nucleophilic ring-opening reaction of pyroglutamic diketopiperazine (pyDKP) with amines.[18] As a result of this work, it was discovered that some *N*-alkyl pyroglutamides gel organic solvents through strong intermolecular associations.[19] By reacting pyDKP with diamines, multifunctional pyroglutamic acid derivatives have also been successfully synthesized. Presented here are observations that these compounds form supramolecular associations.

**Experimental Section. Materials.** Pyroglutamic diketopiperazine was prepared according to the published procedure.[18] 1,3-Diaminopropane and ethylenediamine were distilled from 4 Å molecular sieves under nitrogen. 2-Methyl-1,5-pentanediamine was distilled from sieves under vacuum. All other materials were purchased from commercial suppliers and used as received.

**Instrumentation.** $^{1}$H and $^{13}$C NMR analyses were performed on samples dissolved in DMSO-$d_6$ containing 0.1% TMS or $CDCl_3$ containing 0.1% TMS as internal reference using a Bruker AC-300 operating at 300.02 MHz for proton and 75.47 MHz for carbon. Differential scanning calorimetry (DSC) was performed using a TA Instruments DSC 2920 module (TA 2100 controller) at a heating rate of 10 °C/min in a nitrogen atmosphere. Thermogravimetric analysis (TGA) was conducted on a TA Instruments SDT 2960 module (TA 2100 controller) at a heating rate of 20 °C/min in a nitrogen environment. Optical micrographs were obtained using a Nikon Optiphot 2-pol. Melting points were obtained on a Mel-Temp hot block melting point apparatus.

**1,3-Bis(pyroglutamido)propane (BPP).** To a 100 mL round-bottom flask were added pyroglutamic diketopiperazine (1.16 g, 0.0052 mol) and methanol (40 mL). The suspension was cooled to 0 °C in an ice bath, and 1,3-diaminopropane (0.389 g, 0.0052) was added. The reaction mixture was allowed to warm to room temperature and then stirred for 12 h. The resulting white precipitate was removed by vacuum filtration. The solvent was removed in vacuo to give the product as a white solid; yield 1.429 g (80%). $^{1}$H NMR (DMSO-$d_6$ with TMS): $\delta$ 8.23 (s, 2H), 7.85 (s, 2H), 4.02−3.97 (m, 2H), 3.07−3.03 (m, 4H), 2.29−2.00 (m, 6H), 1.90−1.80 (m, 2H), 1.60−1.50 (m, 2H). $^{13}$C NMR (DMSO-$d_6$ with TMS): $\delta$ 177.4, 172.3, 55.8, 36.2, 29.3, 28.8, 25.3.

Similarly prepared by pyDKP ring-opening reaction were the following:

**1,2-Bis(pyroglutamido)ethane (BPE).** $^{1}$H NMR (DMSO-$d_6$ with TMS): $\delta$ 8.23 (s, 2H), 7.82 (s, 2H), 4.01−3.97 (m, 2H), 3.15−3.13 (m, 4H), 2.29−2.05 (m 6H), 1.96−1.84 (m, 2H). $^{13}$C NMR (DMSO-$d_6$ with TMS): $\delta$ 177.3, 172.5, 55.9, 38.3, 29.3, 25.1.

**1,5-Bis(pyroglutamido)-2-methylpentane (BPMP).** $^{1}$H NMR (DMSO-$d_6$ with TMS): $\delta$ 8.23 (m, 2H), 7.89 (s, 2H), 4.10−4.02 (m, 2H), 3.04−2.92 (m, 4H), 2.30−2.05 (m, 6H), 1.91−1.82 (m, 2H), 1.58 (m, 1H), 1.43−1.29 (m, 4H). $^{13}$C NMR (DMSO-$d_6$ with TMS): $\delta$ 177.4, 172.4, 172.2, 55.8, 44.4, 39.0, 32.5, 31.1, 29.4, 25.4, 17.6.

**1,6-Bis(pyroglutamido)hexane (BPH).** $^{1}$H NMR (DMSO-$d_6$ with TMS): $\delta$ 8.33 (t, 2H), 7.90 (s, 2H), 4.05−4.01 (m, 2H), 3.00 (q, 4H), 2.26−2.01 (m, 6H), 1.87−

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**Scheme 1**

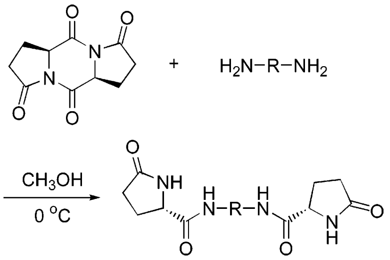

**Table 1. Reaction Products from pyDKP Reacted with Diamines**

| entry | diamine | R | yield (%) |
|---|---|---|---|
| 1 | 1,2-ethanediamine | $(CH_2)_2$ | 85 |
| 2 | 1,3-propanediamine | $(CH_2)_3$ | 86 |
| 3 | 2-methyl-1,5-pentanediamine | $CH_2CH(CH_3)(CH_2)_3$ | 67 |
| 4 | 1,6-hexanediamine | $(CH_2)_6$ | 92 |
| 5 | diethylenetriamine | $(CH_2)_2NH(CH_2)_2$ | 96 |

1.76 (m, 2H), 1.37 (s, 4H), 1.22 (s, 4H). $^{13}$C NMR (DMSO-$d_6$ with TMS): $\delta$ 177.5, 172.2, 55.8, 38.5, 29.4, 29.0, 26.1, 25.3.

**1,5-Bis(pyroglutamido)-3-iminopentane (BPIP).** To a 100 mL round-bottom flask were added pyroglutamic diketopiperazine (2.0 g, 0.009 mol), methanol (60 mL), and a magnetic stir bar. The flask was capped with a septum, purged with nitrogen gas, and cooled to −15 °C in an ice−acetone bath. Diethylenetriamine (0.98 mL, 0.009 mol) was added, and the reaction mixture was stirred for 3 h. Methanol was removed in vacuo, and the resulting solid was recrystallized from 1-propanol and 2-propanol (2:1, v:v) to give the product as a white solid; yield 2.8 g (96%), mp 150−160 °C. $^1$H NMR (DMSO-$d_6$ with TMS): $\delta$ 7.94 (t, 2H), 7.82 (s, 2H), 3.97 (m, 2H), 3.13, (q, 4H), 2.57 (m, 5H), 2.34−2.06 (m, 6H), 1.93−1.79 (m, 2H). $^{13}$C NMR (DMSO-$d_6$ with TMS): $\delta$ 177.49, 172.43, 55.89, 48.13, 38.81, 29.34, 25.36.

**Results and Discussion.** PyDKP was prepared from pyroglutamic acid using a modified published procedure[18] and then reacted with a variety of diamines to form bis(pyroglutamide)s in good yield (Scheme 1). The reactions were carried out in a variety of solvents including $CH_3OH$, $CH_2Cl_2$, $CHCl_3$, DMAc, and DMF. Since pyDKP was only partially soluble in most organic solvents, the reaction was initially heterogeneous but gradually became homogeneous as reaction took place. Thus, either low solubility of pyDKP allowed gradual reaction after pyDKP dissolution or reaction took place at the liquid−solid interface with the highly crystalline reagent. In either case, particle size reduction, use of catalysts, or use of phase transfer agents may accelerate the reaction and help control regioselectivity. Polar solvents and low reaction temperatures promoted regioselective six-membered ring-opening reaction while minimizing the formation of DKP byproducts resulting from five-membered ring-opening. The desired products were isolated by solvent evaporation but were also obtained by precipitation into acetone and filtration.

Since the ring-opening reaction of pyDKP is most efficient with primary amines,[18] a series of analogous difunctional compounds were prepared from diamines (Table 1). All of these primary diamines are commercially available and were chosen to be representative.

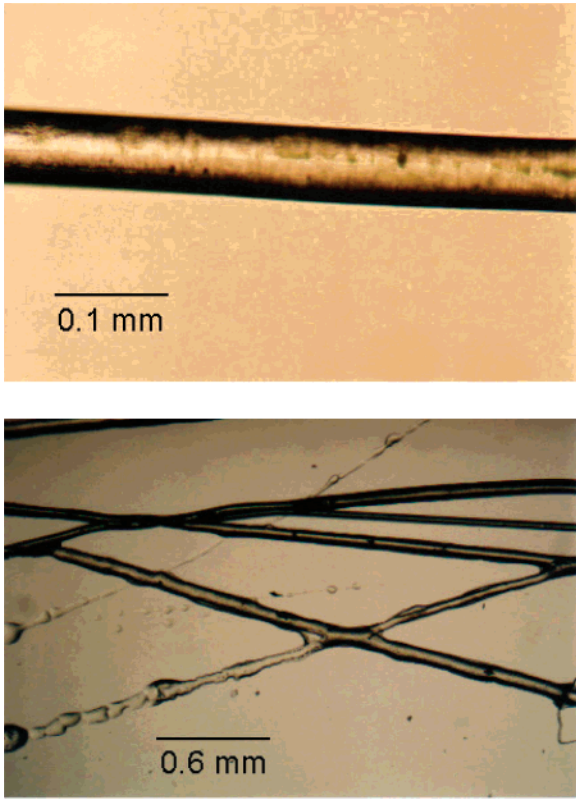

**Figure 1.** (a) Upper optical image, BPP fiber. (b) Lower image after 24 h in air.

By taking advantage of the inability of secondary amines to react with pyDKP, diethylenetriamine was used to prepare a reactive bis(pyroglutamide) (entry 5, Table 1). This bis(pyroglutamide) was used to make a tetrafunctional pyroglutamide (BDPIO) by reaction with 1,18-octadecanedicarbonyl dichloride, demonstrating potential for entire families of tetrapyroglutamides and multipyroglutamides from amine-terminated dendrimers and hyperbranched polymers.

To confirm structures proposed for the bis(pyroglutamide)s, BPP (R = $(CH_2)_3$) was also synthesized directly from pyroglutamic acid and 1,3-diaminopropane using DCC and 1-hydroxybenzotriazole. $^1$H and $^{13}$C NMR chemical shifts were identical when compared to the product from the ring-opening reaction. NMR chemical shifts for all products correlated closely with previously reported pyroglutamides, confirming that six-membered ring-opening of pyDKP is occurring in these reactions.[18] The $^{13}$C NMR spectrum of BPMP was more complex due to the unsymmetrical nature of the diamine component which resulted in two peaks at 172 ppm for the amide carbonyls.

Routine melting point analysis provided no visible melting when the sample was heated slowly from ambient temperature to well above 200 °C. However, insertion of a fresh sample at temperatures near 200 °C gave instantaneous melting. Upon melting a sample on a glass slide, it was further discovered that fibers up to three feet in length could be drawn from the melt (Figure 1a). It was found that the entire series of bis(pyroglutamide)s, as well as the tetrafunctional compound, formed melt-drawn fibers. All fibers, however, were found to be hygroscopic. Exposure of the fibers to atmospheric moisture for a period of 24 h or longer resulted in fiber "degradation" (Figure 1b). Water absorption would be expected to break up amide−amide

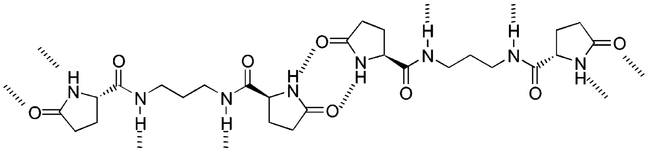

**Figure 2.** Proposed supramolecular association.

hydrogen bonding that is apparently the key factor in fiber formation. Fibers stored under nitrogen retained their integrity, confirming this hypothesis.

Intrigued by the polymeric behavior, BPP, BPE, and BPMP were analyzed using differential scanning calorimetry (DSC). Weak thermal transitions at 125, 126, and 110 °C, respectively, were observed. DSC traces showed no melting near 200 °C. Upon reexamining the melting point of BPP with a polarizing microscope equipped with a heating stage, no visible change at 125 °C was observed, and the sample remained a solid up to 200 °C.

Thermogravimetric analysis (TGA) showed that the compounds were stable up to 300 °C before decomposition. Samples collected by solvent precipitation and subsequent filtration exhibited a 6% weight loss at 100 °C followed by the onset of decomposition near 300 °C, indicating the presence of absorbed water. Therefore, all samples were dried at 200 °C before analysis to remove bound water.

The observations of thermal transitions resembling glass transition temperatures, solvent precipitation, high thermal stability, and the formation of melt-drawn fibers indicate that bis(pyroglutamide)s form hydrogen-bonded supramolecular associations. However, in contrast to the typical polymer properties of nylons, for example, these compounds are readily soluble in water, alcohol, and chlorinated solvents. They also displayed low solution viscosities (e.g., an intrinsic viscosity of 0.13 dL/g was found for BPP in DMAc at 35 °C). While this value is low for a polymer, it is high for a small molecule solute and provides additional evidence for an associated structure (Figure 2).

**Conclusions.** Multifunctional pyroglutamides have been successfully synthesized in good yields by ring-opening reaction of primary diamines with pyDKP at the six-membered ring. The products display good thermal stability and thermal transitions below the visible melting range. On the basis of polymer-like fiber formation, as well as good solubility but with significant solution viscosity of these nonpolymeric species, it is proposed that this class of multifunctional species forms hydrogen-bonded supramolecular associations.

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