Well-Defined Homopolypeptides, Copolypeptides and Hybrids of Poly(L-proline)

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Supporting Information
Attempts leading to impure LP-NCA

1. H-Pro-OH and triethylamine at various temperatures and solvents.

L-proline (5 g, 0.043 mol) was suspended in freshly distilled THF (200 ml) with magnetic stirring in a 500 ml two-necked round bottom flask and triphosgene (0.37 equiv., 4.68 g) was added in one portion, at 50 °C. After the complete dissolution (1h) of the powder, the solvent was distilled off in vacuo, until an oily product (N-carbamoyl chloride) was obtained. The oily residue was left to dryness in the high vacuum line for one hour in order for the excess phosgene to be removed and was finally dissolved in acetonitrile (or THF) (200 ml). Subsequently, freshly distilled triethylamine (1.1 equiv., 6.6 ml) was added dropwise at 0 °C, leading to the formation of the LP-NCA with instantaneous precipitation of TEA · HCl salt. After stirring for 6-24 h at -20 °C (or 0 or 25 °C) under Ar, the reaction mixture was filtered to allow complete precipitation of the salt. Crude LP-NCA was then dissolved in ethyl acetate, chilled and extracted with ice-cold water (50 mL) until neutral pH. The organic phase was then separated, dried over MgSO₄, filtered and the solvent was distilled off in the vacuum line. The oily contaminated LP-NCA was purified by repeated recrystallizations from THF/hexane. Finally, the product contained a significant amount of diketopiperazines.

2. H-Pro-OH and polystyrene resins bearing diethylamino groups (PS-DEAM).

L-proline (3 g, 0.026 mol) was suspended in freshly distilled THF (200 ml) with magnetic stirring in a two-necked round bottom flask of 500 ml and triphosgene (0.37 equiv., 2.83 g) was added in one portion, at 50 °C. After the complete dissolution (1h) of the powder, the solvent was distilled off in vacuo, until an oily product (N-carbamoyl chloride) was obtained. The oily residue was left to dry in the high vacuum line for one hour in order for the excess phosgene to be removed, and was redissolved in freshly distilled THF (200 mL). The solution was finally added onto the polymer-supported amine (3 equiv., 24.4 g), which had been swollen previously in dry THF in a specially designed apparatus, and
kept under high vacuum for 1 day. The N-carbamoyl chloride was left to react with the resin for 3 h at 25 °C under vacuum, leading to the formation of the LP-NCA. The reaction mixture was filtered to allow precipitation of the resin salt, and the filtrate was dried in high vacuum. Crude LP-NCA was then dissolved in ethyl acetate, chilled and extracted with ice-cold water (50 mL) until neutral pH. The organic phase was then separated, dried over MgSO₄, filtered and the solvent was distilled off in the vacuum line. The oily contaminated LP-NCA was purified by repeated recrystallizations with THF/hexane. The main side products were polymeric materials of proline.

Scheme S1: Diketopiperazine of L-proline

Figure F1. FTIR of the crude product after the synthesis of LP-NCA utilizing H-Pro-OH in ACN with Et₃N at –20 °C.
**Figure F2.** FTIR of the crude product after the synthesis of LP-NCA utilizing H-Pro-OH in THF and polystyrene resin bearing diethyamino groups at 25 °C.
**Figure F3:** Monitoring the purification of LP-NCA by $^1$H-NMR spectroscopy: (a) crude product; (b) after the recrystallization for removal of 1; (c) Boc-L-proline

**Figure F4:** $^{13}$C-NMR spectra (75 MHz, CDCl$_3$, 298 K) of purified LP-NCA.
**Figure F5**: Precursor ion scan of product ion $m/z$ 70, showing that the main precursor ions are $m/z$ 114 and $m/z$ 140 of LP-NCA.

**Scheme S2**: Highly pure needle-like LP-NCA.
## Table 1. Composition of Crude Products for the Synthesis of LP-NCA

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>base</th>
<th>Equivalents</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Pro-NCA (% w/w)</th>
<th>DKP (% w/w)</th>
<th>Cl side product b (%) w/w</th>
<th>Boc-Pro-OH Unreacted (%) w/w</th>
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</thead>
<tbody>
<tr>
<td>H-Pro-OH</td>
<td>Et$_3$N</td>
<td>1</td>
<td>ACN</td>
<td>0</td>
<td>35</td>
<td>45</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>H-Pro-OH</td>
<td>Et$_3$N</td>
<td>1</td>
<td>ACN</td>
<td>-20</td>
<td>42</td>
<td>55</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>H-Pro-OH</td>
<td>Et$_3$N</td>
<td>1</td>
<td>THF</td>
<td>0</td>
<td>45</td>
<td>20</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>H-Pro-OH</td>
<td>PS-DEAM$^a$</td>
<td>3</td>
<td>THF</td>
<td>25</td>
<td>75</td>
<td>5$^c$</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Boc-Pro-OH</td>
<td>Et$_3$N</td>
<td>1</td>
<td>THF</td>
<td>25</td>
<td>90</td>
<td>-</td>
<td>5</td>
<td>5</td>
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</tbody>
</table>

$^a$ Polystyrene resin with diethylamine groups, $^b$ NCC for H-Pro-OH or carbonyl chlorocarbonate for Boc-Pro-OH, $^c$ Polymeric material

## Table 2. Molecular Characteristics of polymers containing poly(L-proline)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ first block $x 10^3$ (g/mol)$^a$</th>
<th>I of first block</th>
<th>$M_n$ total $x 10^3$ (g/mol)</th>
<th>Composition of PLP (% w/w)$^c$</th>
<th>I of final polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLP #1</td>
<td>4.6</td>
<td>1.26</td>
<td></td>
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<tr>
<td>PLP #2</td>
<td>9.3</td>
<td>1.23</td>
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<tr>
<td>PLP #3</td>
<td>13.0</td>
<td>1.24</td>
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<tr>
<td>PEO-$b$-PLP #1</td>
<td>9.2$^b$</td>
<td>1.13</td>
<td>36.8$^a$</td>
<td>75</td>
<td>1.10</td>
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<tr>
<td>PEO-$b$-PLP #2</td>
<td>17.3$^b$</td>
<td>1.13</td>
<td>34.6$^a$</td>
<td>50</td>
<td>1.10</td>
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<tr>
<td>PLP-$b$-PEO-$b$-PLP #1</td>
<td>8.5$^b$</td>
<td>1.12</td>
<td>19.3$^a$</td>
<td>56</td>
<td>1.10</td>
</tr>
<tr>
<td>PLP-$b$-PEO-$b$-PLP #2</td>
<td>8.5$^b$</td>
<td>1.12</td>
<td>14.9$^a$</td>
<td>43</td>
<td>1.09</td>
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<tr>
<td>PBLG-$b$-PLP #1</td>
<td>20.0$^d$</td>
<td>1.02</td>
<td>33.0$^e$</td>
<td>39$^f$</td>
<td>-</td>
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<tr>
<td>PBLG-$b$-PLP #2</td>
<td>16.0$^d$</td>
<td>1.08</td>
<td>45.0$^e$</td>
<td>64$^f$</td>
<td>-</td>
</tr>
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<td>PBocLL-$b$-PLP</td>
<td>99.1$^d$</td>
<td>1.18</td>
<td>239.1$^g$</td>
<td>58$^c$</td>
<td>1.10</td>
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$^a$ By SEC-TALLS in (0.1 M NaNO$_3$) water/ACN 80:20 at 35 °C. $^b$ $M_n$ of initial PEO-NH$_2$ and H$_2$N-PEO-NH$_2$. $^c$ Composition, according to $^1$H-NMR in CF$_3$COOD. $^d$ By SEC in (0.1 M LiBr) DMF at 60 °C. $^e$ The molecular weights of the PLP block are from stoichiometry, and added to the one of the PBLG obtained by TALLS. $^f$ Composition according to TGA. $^g$ The molecular weight of PBocLL-$b$-PLP is the sum of the $M_n$ of PBocLL block obtained by SEC-TALLS in DMF/LiBr (99.1 x 10$^3$ g/mol), and the $M_n$ of PLP block (140 x 10$^3$ g/mol) from the difference between the PLL precursor (68.0 x 10$^3$ g/mol) and the total molecular weight of PLL-$b$-PLP (208.0 x 10$^3$ g/mol) in (5% NaH$_2$PO$_4$) water/ACN 97:3 at 35 °C.
Figure F6: SEC chromatogram of PLP #3 in 0.1 M NaNO$_3$ solution of water/acetonitrile 80/20 at a flow rate of 0.8 mL/min at 35 °C.

Figure F7: $^1$H-NMR spectra (300 MHz, CF$_3$COOD, 298 K) of PLP II.
Figure F8: $^{13}$C-NMR spectra (75 MHz, CF$_3$COOD, 298 K) of PLP II.

Figure F9: CD spectra of left-handed PLP II in water at various temperatures.
Figure F10: FTIR spectra of PLP after precipitation (mainly form I) and PLP II.

Figure F11: $^1$H-NMR spectra (300 MHz, CF$_3$COOD, 298 K) of PLP-$b$-PEO-$b$-PLP #2.
Figure F12: $^1$H-NMR spectra (300 MHz, CF$_3$COOD, 298 K) of PEO-b-PLP #1.

Figure F13: $^1$H-NMR spectra (300 MHz, CF$_3$COOD, 298 K) of PLL-b-PLP.