RAFT-derived antimicrobial polymethacrylates: Elucidating the impact of end-groups on activity and cytotoxicity

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Polymer synthesis and Characterization

Synthesis of Amine Polymers
Reversible addition–fragmentation chain transfer (RAFT) polymerization of 2-AEMA and MMA was performed in DMSO at 70 °C for 18 h using 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA1), 2-cyanopropan-2-yl dodecyl carbonitrithioate (CTA2) or 4-cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA3) as the RAFT agent and AIBN as the radical initiator.

PA1
2-AEMA (4.64 g, 28 mmol), MMA (1.20 g, 12 mmol), AIBN (98 mg 0.6 mmol), and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA1, 807 mg, 2 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze–evacuate–thaw cycles under high vacuum (10⁻⁴ Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (5.66 g, 85% yield).

PA2
2-AEMA (2.32 g, 14 mmol), MMA (601 mg, 6 mmol), AIBN (98 mg 0.6 mmol), and 4-cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA3, 263 mg, 1 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze–evacuate–thaw cycles under high vacuum (10⁻⁴ Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (2.80 g, 77% yield).

PA3
2-AEMA (2.32 g, 14 mmol), MMA (601 mg, 6 mmol), AIBN (98 mg 0.6 mmol), and 2-cyanopropan-2-yl dodecyl carbonitrithioate (CTA2, 263 mg, 1 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze–evacuate–thaw cycles under high vacuum (10⁻⁴ Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (3.14 g, 96% yield).

Representative ¹H NMR for Amine Polymers
Synthesis of Guanidine Polymers PG1-PG3

A post polymerization guanylation method was used to convert amine polymers PA1, PA2 and PA3 to the corresponding guanidine functionalized polymers PG1, PG2 and PG3.

**PG1**

To a solution of PA1 (4 g, 1 mmol) in anhydrous methanol (50 mL), was added 1H-pyrazole-1-carboxamidine hydrochloride (3.78 g, 26 mmol) and N,N-diisopropylethylamine base (6.34 g, 49 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol–acetone three times to obtain PG1 as a slight yellow powder in quantitative yield.

**PG2**

To a solution of PA2 (2 g, 0.5 mmol) in anhydrous methanol (25 mL), was added 1H-pyrazole-1-carboxamidine hydrochloride (1.76 g, 12 mmol) and N,N-diisopropylethylamine base (3.10 g, 24 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol–acetone three times to obtain PG2 as a slight yellow powder in quantitative yield.

**PG3**

To a solution of PA2 (2 g, 0.5 mmol) in anhydrous methanol (25 mL), was added 1H-pyrazole-1-carboxamidine hydrochloride (1.87 g, 13 mmol) and N,N-diisopropylethylamine base (3.30 g, 26 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol–acetone three times to obtain PG3 as a slight yellow powder in quantitative yield.

Representative 1H NMR for Guanidine Polymers
Radical Reduction Removal of RAFT End-Groups to give PA4 and PG4

A radical induced reduction method was used to convert PA1 and PG1 into the corresponding proton terminated PA4 and PG4. A representative procedure is given below.

**PA4**

To a solution of PA1 (600 mg, 0.15 mmol) in DMSO (5 mL) was added Vazo-88 (13 mg, 0.075 mmol, 0.5 eq) and EPHP (367 mg, 1.5 mmol, 10 eq) in a 50 mL Schlenk flask. The reaction underwent three high vacuum (10^{-3} Torr) freeze-evacuation-thaw cycles before being heated to 100°C for 16 h. The product was isolated as the hypophosphite salt via three precipitations from methanol-acetone followed by high vacuum to remove trace solvent. This gave PA4 as a white powder (421 mg, 76% yield). The complete removal of RAFT end-groups was confirmed using UV-Vis and ¹H NMR analysis (see below for relevant spectra).

**PG4**

To a solution of PG1 (600 mg, 0.15 mmol) in DMSO (5 mL) was added Vazo-88 (13 mg, 0.075 mmol, 0.5 eq) and EPHP (367 mg, 1.5 mmol, 10 eq) in a 50 mL Schlenk flask. The reaction underwent three high vacuum (10^{-3} Torr) freeze-evacuation-thaw cycles before being heated to 100°C for 16 h. The product was isolated as the hypophosphite salt via three precipitations from methanol-acetone followed by high vacuum to remove trace solvent. This gave PG4 as a white powder (388 mg, 70% yield). The complete removal of RAFT end-groups was confirmed using UV-Vis and ¹H NMR analysis.

**Confirmation of End Group Removal – Comparison of ¹H NMR Spectra**

![1H NMR Spectra Comparison](image)

**Confirmation of End Group Removal – Comparison of UV Spectra**

![UV Spectra Comparison](image)
DLS measurements

PA-series

Figure SI1: DLS measurements of PA1

Figure SI2: DLS measurements of PA2
Figure SI3: DLS measurements of PA3

Figure SI4: DLS measurements of PA4
**PG-series**

Figure SI5: DLS measurements of PG1

![DLS measurements of PG1](image1)

Figure SI6: DLS measurements of PG2

![DLS measurements of PG2](image2)
Figure SI7: DLS measurements of PG3

Figure SI8: DLS measurements of PG4
Antibacterial Results

Table SI1. Antimicrobial and haemolytic results

<table>
<thead>
<tr>
<th>Polymer</th>
<th>VISA</th>
<th>S. epidermidis</th>
<th>C. albicans</th>
<th>Haemolysis (%)a</th>
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<tbody>
<tr>
<td>PA1</td>
<td>32</td>
<td>32</td>
<td>32</td>
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<tr>
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<td>32</td>
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<td>PA4</td>
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<td>16</td>
<td>32</td>
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<tr>
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<td>32</td>
<td>64</td>
<td>13.4</td>
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MIC as measured in μg/mL according to CLSI standards; a Haemolysis was determined as the percentage of lysed cells at the MIC concentration of S. epi

Haemolysis Results

Figure SI9. Haemolysis results for PAI and PGI polymer series.
Haemagglutination Results

Table S12. Haemagglutination results obtained from PA and PG polymer series.

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<th>Polymer</th>
<th>Concentration (µg/mL)</th>
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Hemagglutination strength: ++++ strong, ++++moderate, ++ mild, + weak, 0 no hemagglutination, (L) hemolysis observed