Qualifying Exam Presentation

Proposal I:
A Nonviral Gene Transfer Agent Based on N-(carboxymethyl)-*trans*-4-hydroxyl-L-proline

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Overview

I. Background
   A. Types of vectors
      1. Viral: retroviruses, adenoviruses, etc.
      2. Nonviral: liposomes, peptides, polymers, etc.
   B. Gene Delivery Process
   C. Gene Delivery Requirements
II. N-(carboxymethyl)-*trans*-4-hydroxyl-L-proline based polymer
   A. Reasoning
   B. How it meets requirements
III. Synthesis
   A. Monomer synthesis
   B. Polymer synthesis
IV. Characterization and evaluation
   A. NMR
   B. Toxicity
   C. Transfection efficiency

Types of Vectors

- Viral: retroviruses, adenoviruses, etc.
  - Must be de-evolved to be made safe.
  - Very expensive and sometimes dangerous.
  - Limits on size of DNA.

- Nonviral: liposomes, peptides, polymers, etc.
  - Toxicity.
  - Lack of targeting.
  - Ease of engineering.

Gene Delivery Process

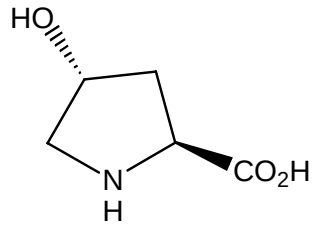
- DNA protection outside and inside cells.
- Bypass or escape from endocytotic pathways.
- Efficient release of DNA .
- DNA delivery to most of the target cells.
- At best:
  - Efficient nuclear targeting.
  - High, persistent and adjustable therapeutic levels.

Gene Delivery Requirements

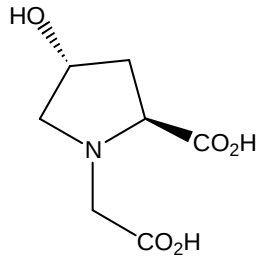
- Be minimally toxic.
- Efficiently transfect DNA.
  - Balance toxicity vs. transfection efficiency.
- Biodegradable ester linkages.
- Tertiary amine groups in interior.
- Primary amine groups on exterior.

N-(carboxymethyl)-*trans*-4-hydroxy-L-proline

- Reason for choice
  - 4-hydroxy-L-proline main component in collagen, which is nearly everywhere in the body of mammals.



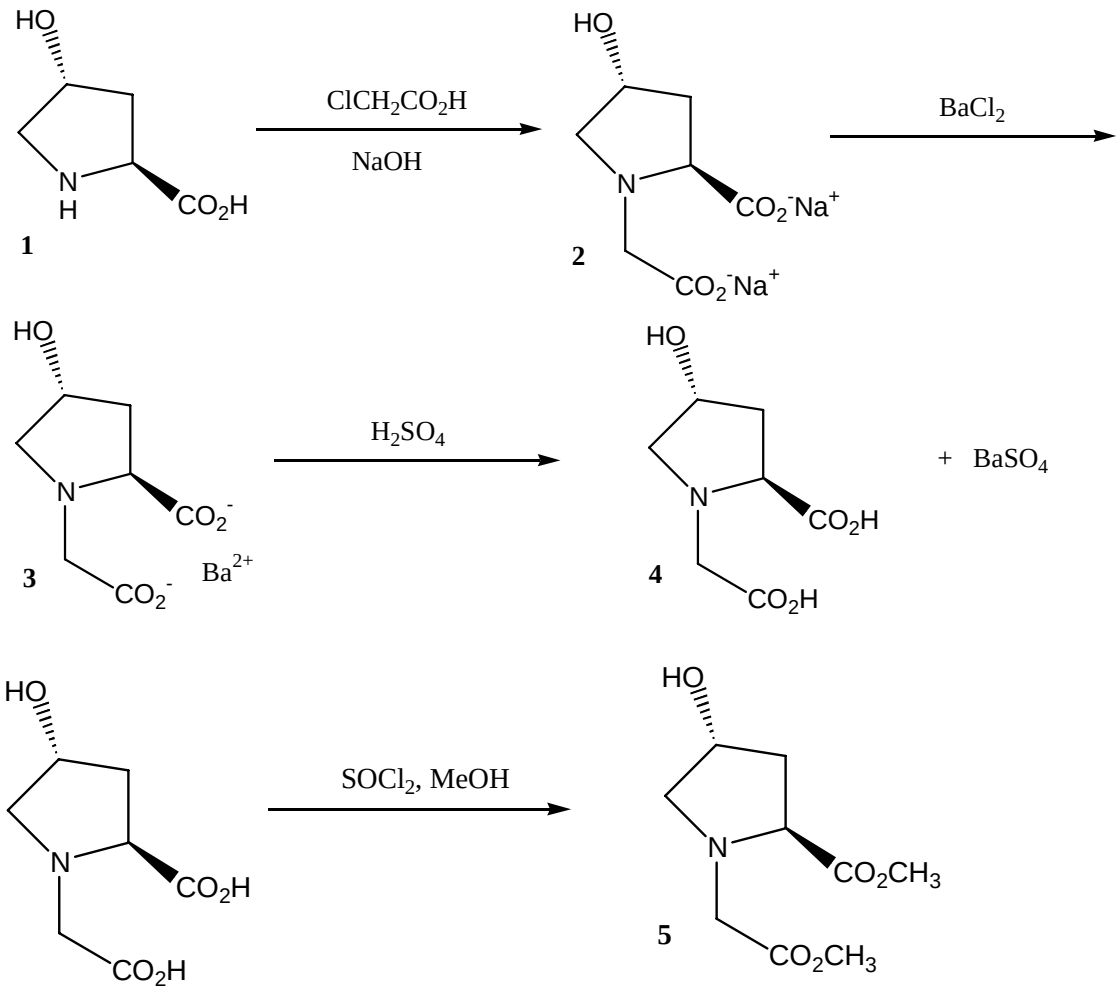
4-hydroxy-L-proline



N-(carboxymethyl)-*trans*-4-hydroxy-L-proline

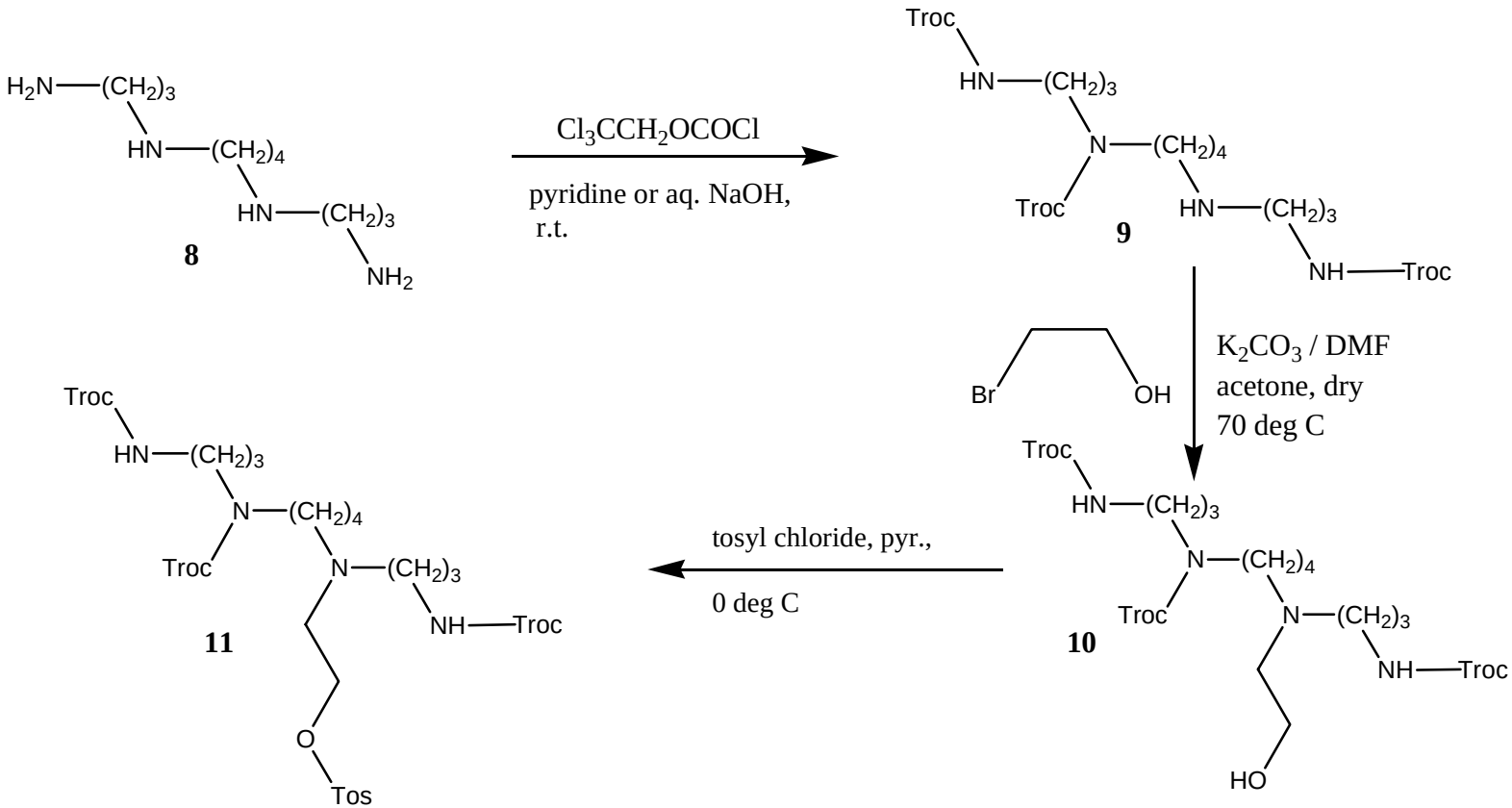
- How it will meet requirements
  - Biodegradable ester linkages.
  - Internal tertiary amine to act as buffer.

Monomer Synthesis

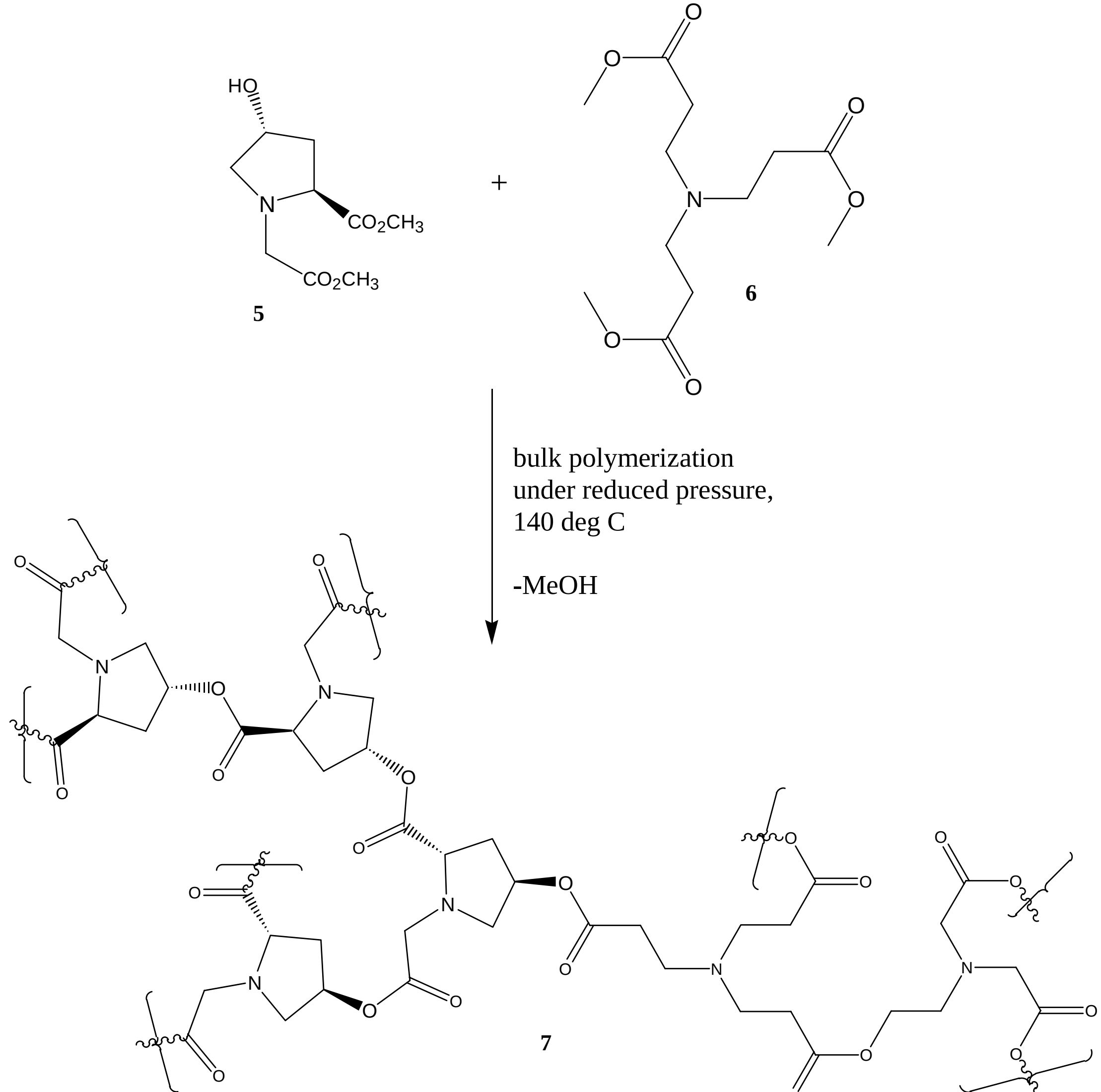


Chaouk, H.; Middleton, S.; Jackson, W. R.; Hearn, M. T. W.; *International Journal of Bio-Chromatography*; **1997**, 2 (3), 153.

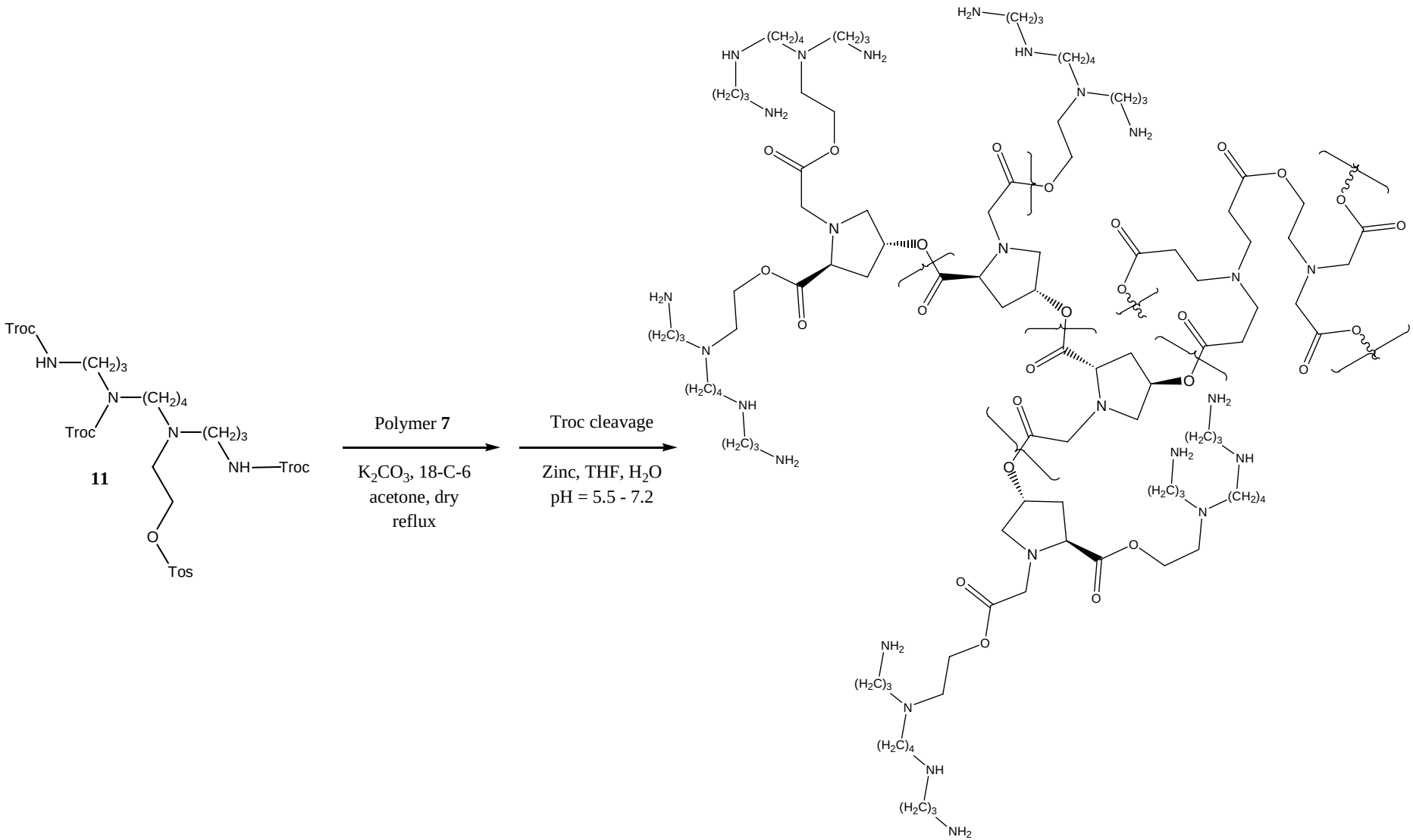
End Group Synthesis



Polymer Synthesis

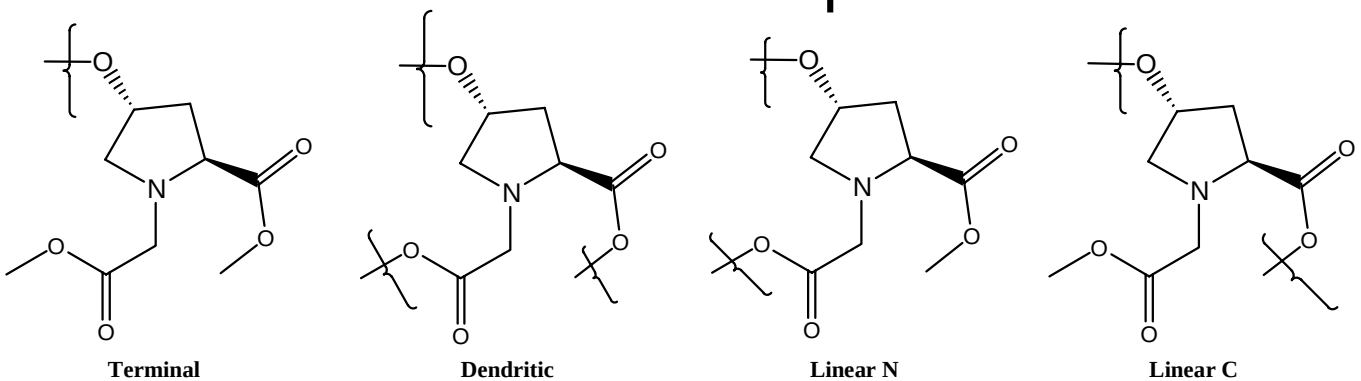


Polymer Synthesis



Characterization

- ^{1}H and ^{13}C NMR
  - Determination of DB (degree of branching) by ^{13}C NMR with aid of model compounds.



  - $DB = (N_d + N_t)/(N_d + N_t + N_{lC} + N_{lN})$ *
- SEC with polystyrene and PAMAM as standards

* Hawker, C. J.; Lee, R.; Frechet, J. M. J.; *J. Am. Chem. Soc.,* **1991**, *113*, 4583.

Evaluation

- Toxicity
  - Measured using an MTT assay.
  - Pale yellow color of MTT (3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) turns to blue when cleaved by living cells.
- Transfection efficiency
  - Measure decrease or increase in target protein levels.
  - Include reporter gene in transfected DNA, such as green fluorescent protein (GFP).

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