

AMINO ACIDS FOR THE SYNTHESIS
OF BRANCHED POLYMERS FOR
CONTROLLED DRUG DELIVERY
APPLICATIONS

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IPR Symposium May 15, 2007



OUTLINE

- Project Objectives
- Background
- Synthesis
- Results
- Future Work
- Acknowledgements



PROJECT OBJECTIVES

- Synthesize biocompatible dendritic (arborescent) polymers with a narrow molecular weight distribution (MWD)
- Suitable for microencapsulation
 - Intravenous drug delivery
- Demonstrate the feasibility of the synthetic scheme proposed



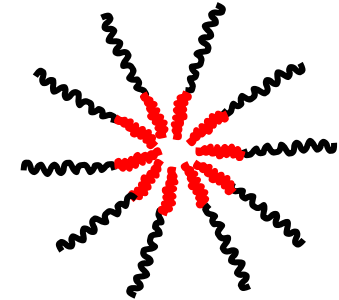
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BACKGROUND

MICELLES

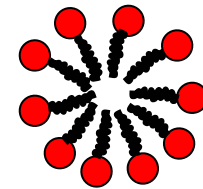
- Linear diblock copolymer micelles

(Mössmer et al. *Macromolecules* **2000**, 33, 4791)



- Surfactant micelles

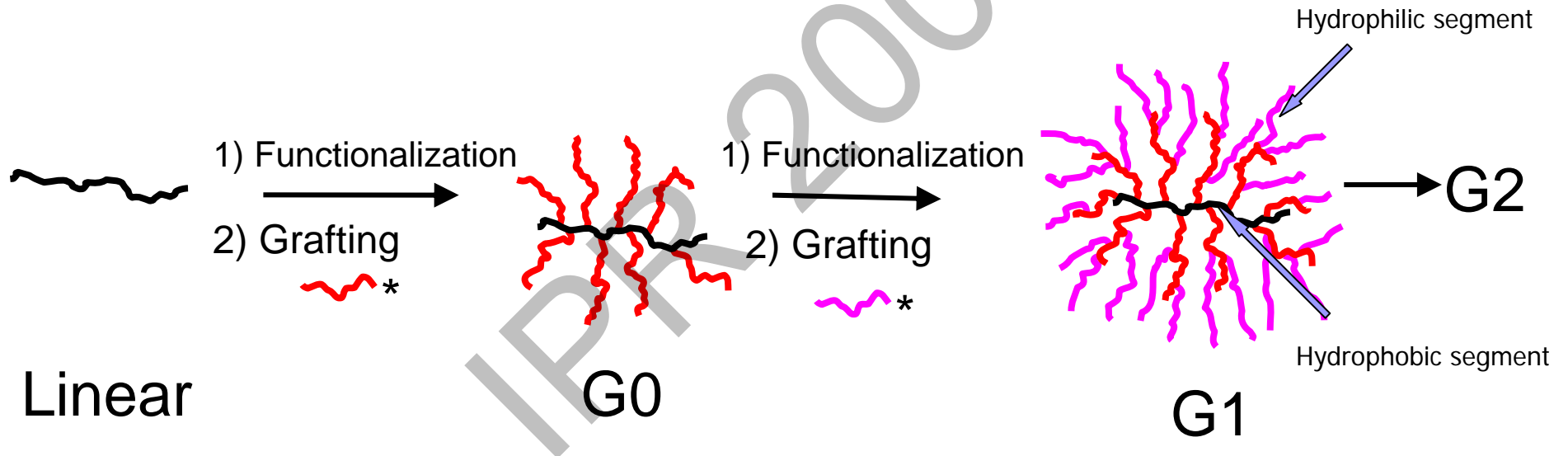
(Ohno et al. *Macromolecules* **2002**, 35, 8989)



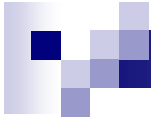
- Used for microencapsulation
- Both assemblies have critical micelle concentrations (unstable at $C < CMC$)
- Molecules in micelle interchange with free molecules (dynamic structure)

ARBORESCENT MICELLES

- Branched structure obtained from ***grafting onto*** scheme



- Static structure (stable, no CMC)
- Biocompatible? → Amino acids



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SYNTHESIS

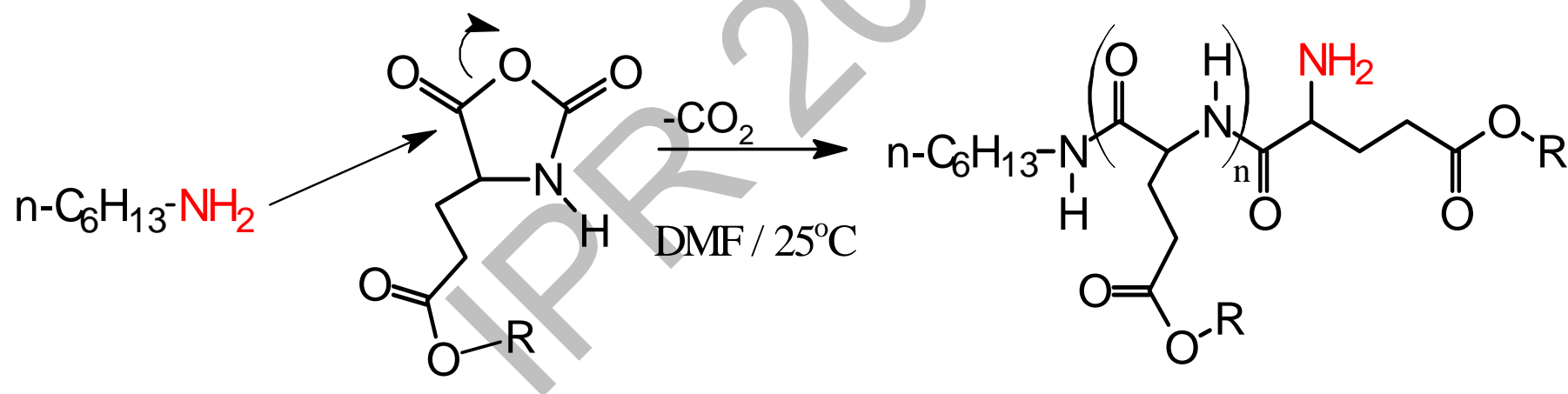


SYNTHETIC SCHEME

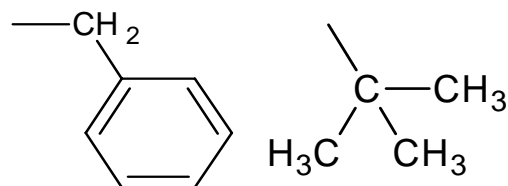
- Ring opening polymerization
 - Linear poly(γ -benzyl L-glutamate) (core side chains)
 - Linear poly(γ -tert-butyl L-glutamate) (shell side chains)
- Partial deprotection of benzyl ester
 - Substrate with randomly distributed grafting sites
- Grafting
 - Comb-branched polymer (Generation zero, G0)
 - Higher generations G1, G2etc.
- Characterization
 - Size Exclusion Chromatography
 - ^1H NMR Spectroscopy

RING OPENING POLYMERIZATION

- N-carboxyanhydride of amino acids
- Initiated by a primary amine (*n*-hexylamine)
- Susceptible to side reactions



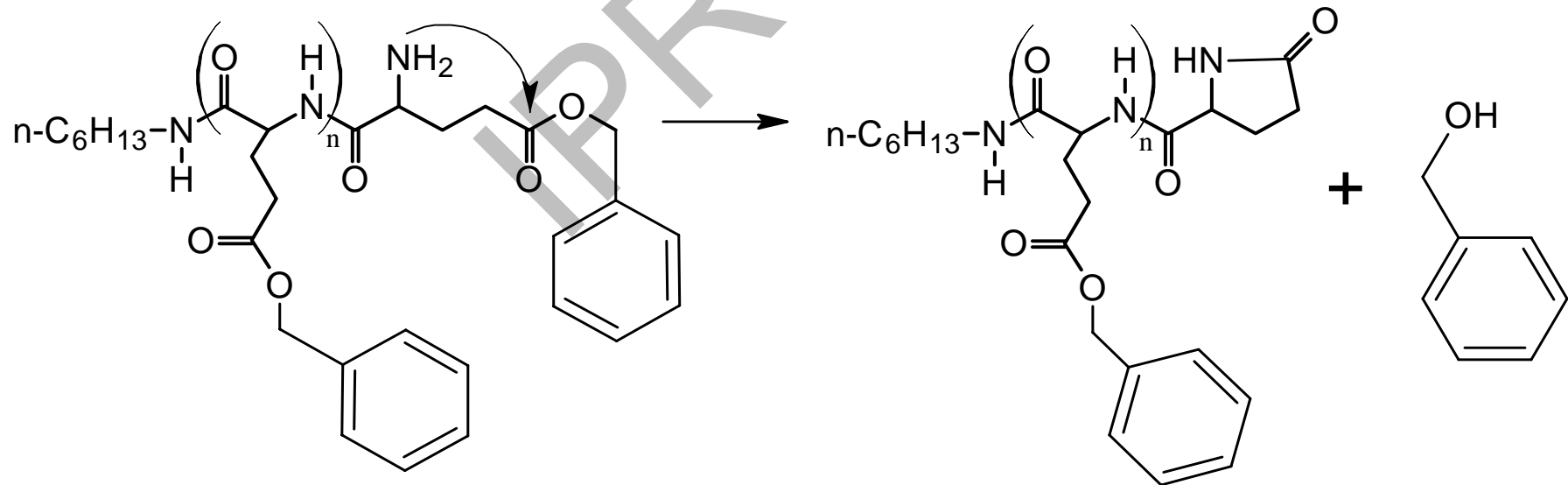
R = benzyl or *tert*-butyl



'living' primary amine end group

SIDE REACTIONS IN ROP

- Destroy 'living' primary amine
- Broaden MWD
- E.g. end group cyclization
 - Specific to poly(γ -benzyl L-glutamate)



SOLUTION TO SIDE REACTIONS

- Several methods in literature
 - To preserve 'living' character (primary amine) of chain
 - To maintain a narrow MWD
- E.g. lower reaction temperature
 - Propagation and side reactions have different activation energies (E_a)

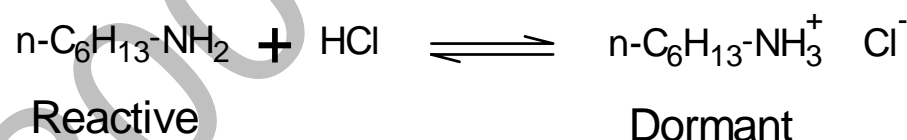
Arrhenius $k = Ae^{-E_a/RT}$

- Side reaction $E_a >$ Propagation E_a
- Lowering T decreases side reactions more rapidly

MORE SOLUTIONS

■ Hydrochloride salt initiator

- Decreases reactivity of primary amine chain ends in reaction
- Longer reaction times

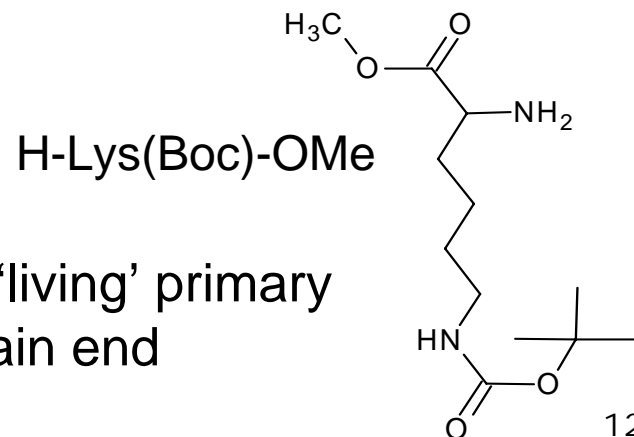


■ High vacuum techniques

- Removes water and other contaminants from reaction
- Azeotropic drying of monomer
- Time-consuming procedure

■ Difunctional initiator

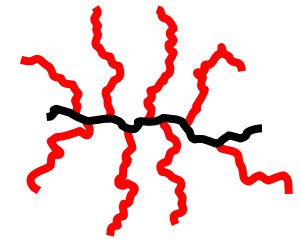
- Side reactions not prevented, but 'living' primary amine introduced as protected chain end



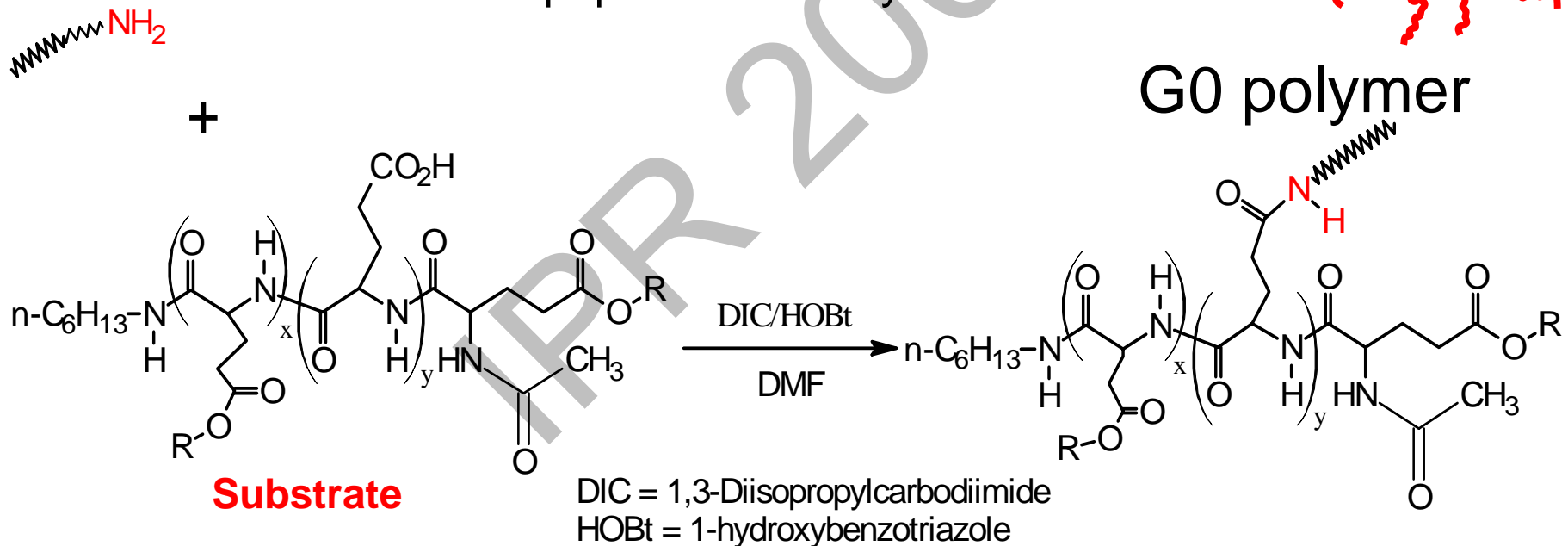
GRAFTING

- Substrate coupling with side chains

- Substrate activated with carbodiimide/HOBt
 - Standard peptide chemistry



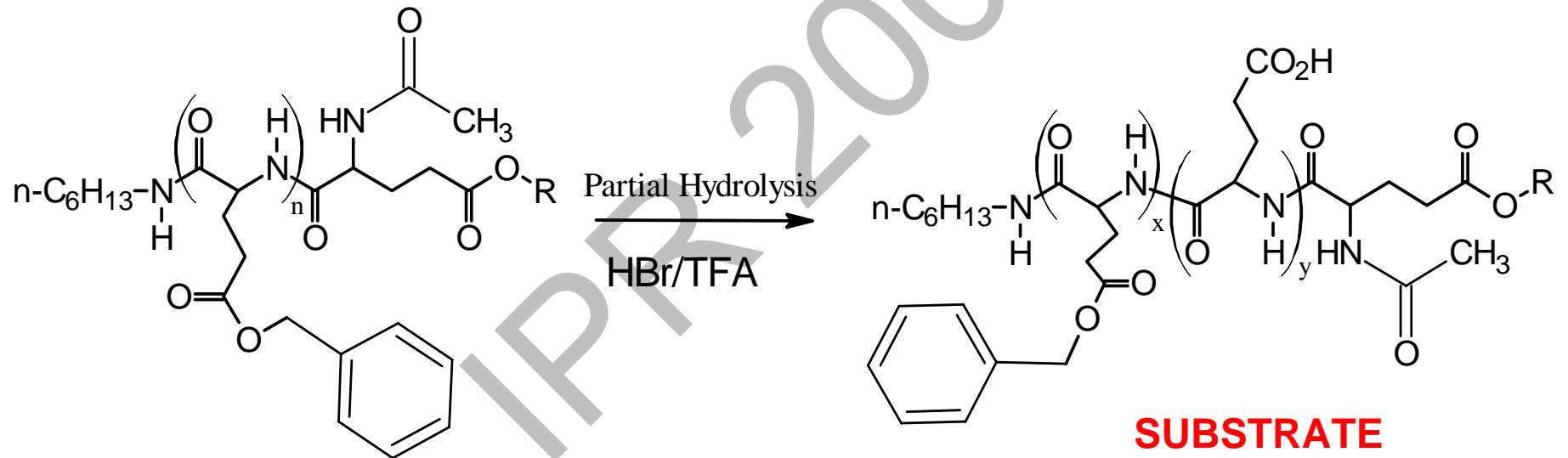
G0 polymer



- Stoichiometry varied to maximize grafting yield
- Process repeated for higher generations

DEPROTECTION OF Poly(L-glutamate esters)

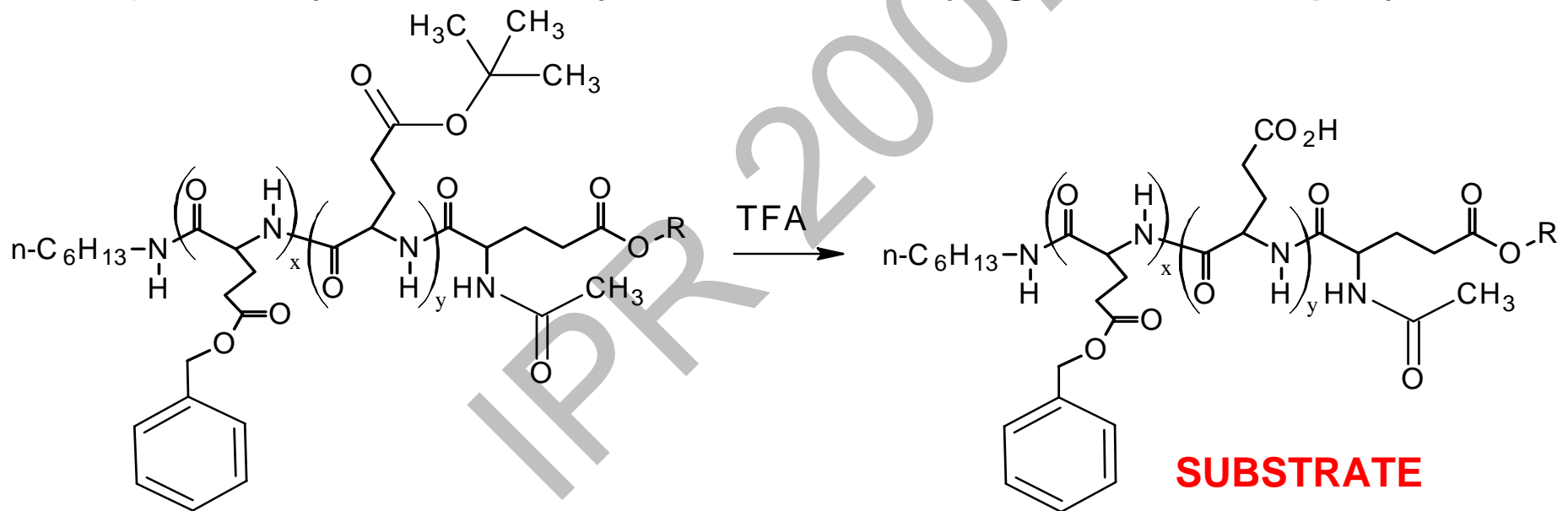
- 1) Acidolysis of poly(γ -benzyl L-glutamate) homopolymer



- Random acidolysis assumed
- Stoichiometry varied to obtain desired deprotection level

DEPROTECTION OF Poly(L-glutamate esters)

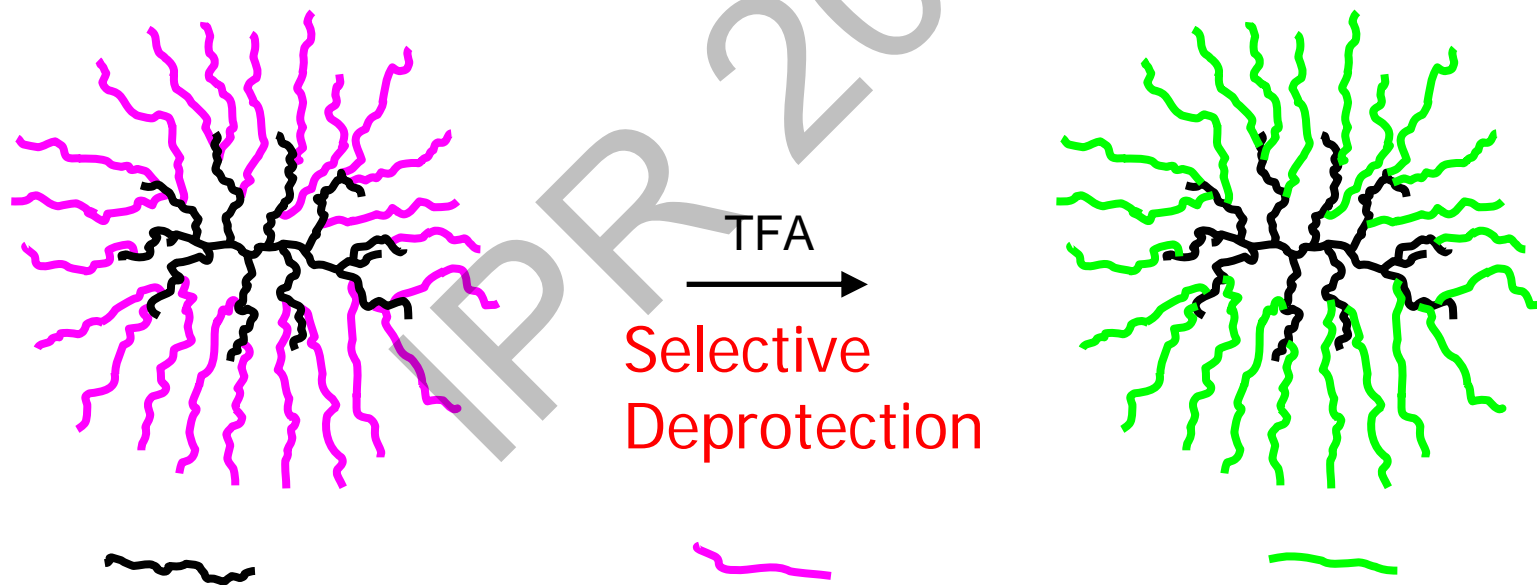
- 2) Acidolysis of benzyl- and *tert*-butyl-glutamate copolymer



- Random copolymerization assumed
- Stoichiometry varied for desired *tert*-butyl ester content

SHELL ADDITION

- Last cycle: poly(γ -tert-butyl L-glutamate) chains
- Selective cleavage of *tert*-butyl groups yields hydrophilic shell



Poly(benzyl L-glutamate) Poly(*tert*-butyl L-glutamate) Poly(L-glutamic acid)

- Result: Water-soluble dendritic micelles



Characterization

- Size Exclusion Chromatography (SEC)
 - Apparent molecular weights (M_n , M_w)
 - PDI for linear and graft polymers
 - Grafting yield
- ^1H NMR Spectroscopy
 - Number-average degree of polymerization (DP_n)
 - Absolute M_n for side chains
 - Deprotection level of substrate



RESULTS

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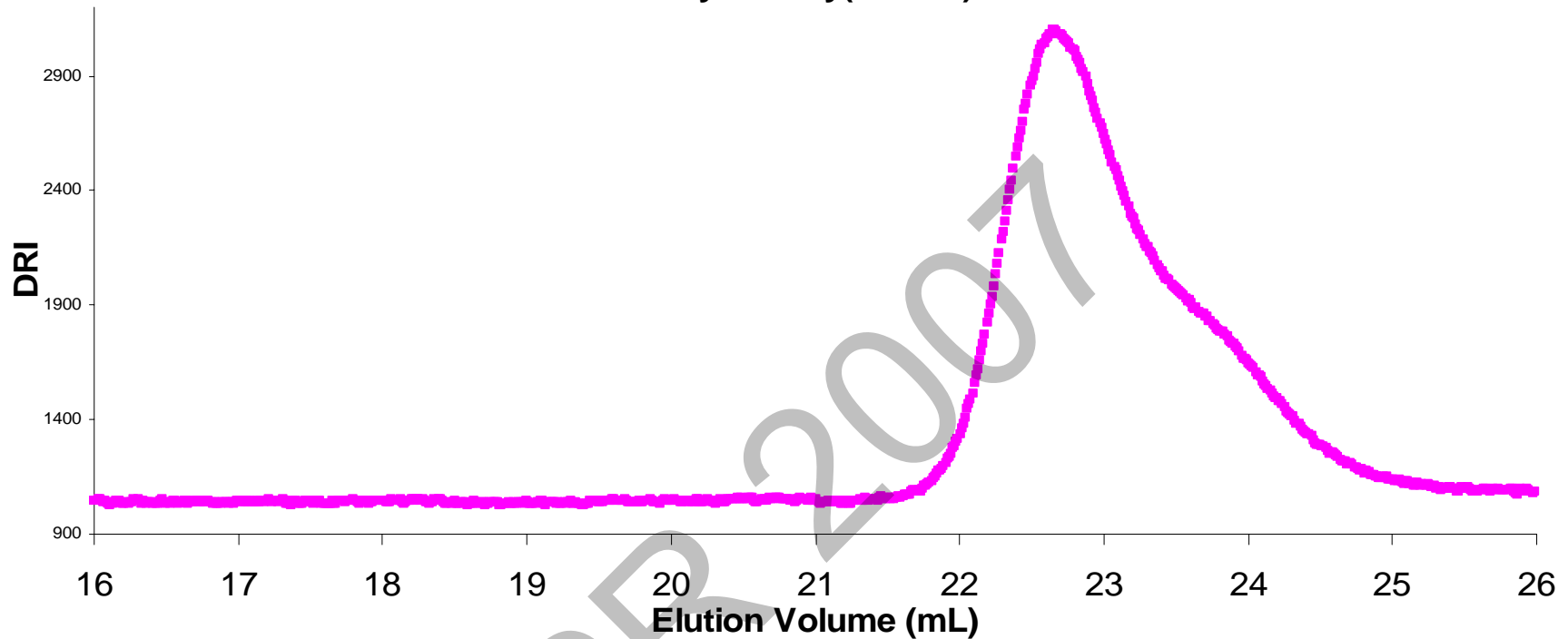
RING OPENING POLYMERIZATION

Sample Name*	Reaction Time	M_n^{app} (SEC)	PDI	Target DP	1H NMR DP_n	M_n^{HNMR}
Poly(Bz-Glu)-25^a	5 d	4,000	1.27	20	23	5,400
Poly(Bz-Glu)-26^b	24 h	4,300	1.28	20	20	4,700
Poly(Bz-Glu)-30^c	5 d	7,200	1.21	50	54	12,200
Poly(Bz-Glu)-35^a	4 d	4,000	1.14	20	20	4,700
Poly(Bz-Glu)-36^{ac}	10 d	5,200	1.24	40	35	8,000
Poly(Bz-Glu)-37	2 h	3,600	1.21	10	15	3,600

^a Polymerization temperature = 0°C; ^b H-Lys(Boc)-OMe initiator; ^c High vacuum technique; *number in name refers to run number

- PDI values ranging from 1.14-1.28, satisfactory
- Chain functionality (-NH₂) level will determine which method is best

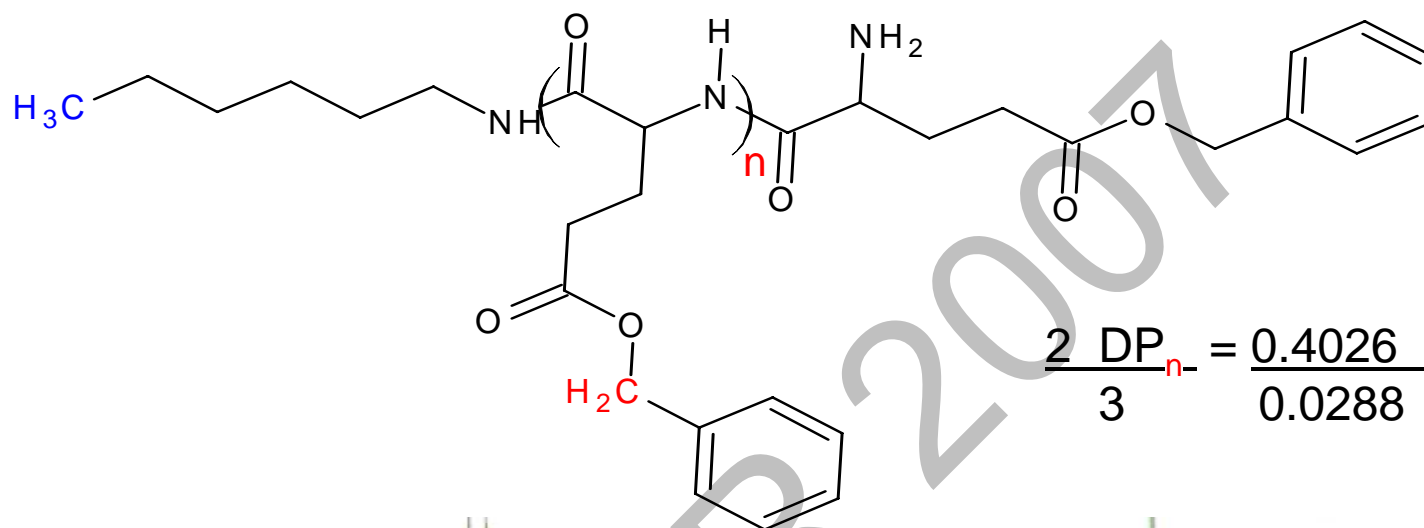
SEC Analysis Poly(Bz-Glu)-36



Solvent N,N-dimethylacetamide (1g/L LiCl) at ~80°C, flow rate 1 mL/min
(DRI = differential refractometer index detector signal intensity)

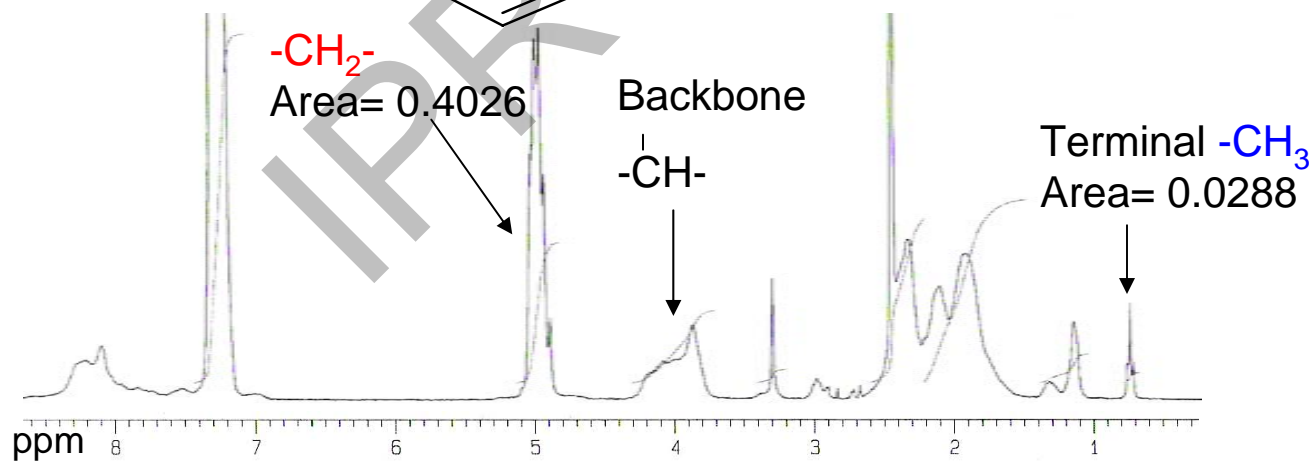
- Tail on low molecular weight side of peak
 - Possible side reactions, slow initiation, or SEC column adsorption
- $PDI = M_w/M_n = 1.24$

¹H NMR ANALYSIS



$$\frac{2}{3} DP_n = \frac{0.4026}{0.0288} \rightarrow DP_n = 21.0$$

$$\rightarrow M_n \sim 5,000$$



¹H NMR Spectrum of Poly(Bz-Glu)-33

DEPROTECTION OF Poly(L-glutamate esters)

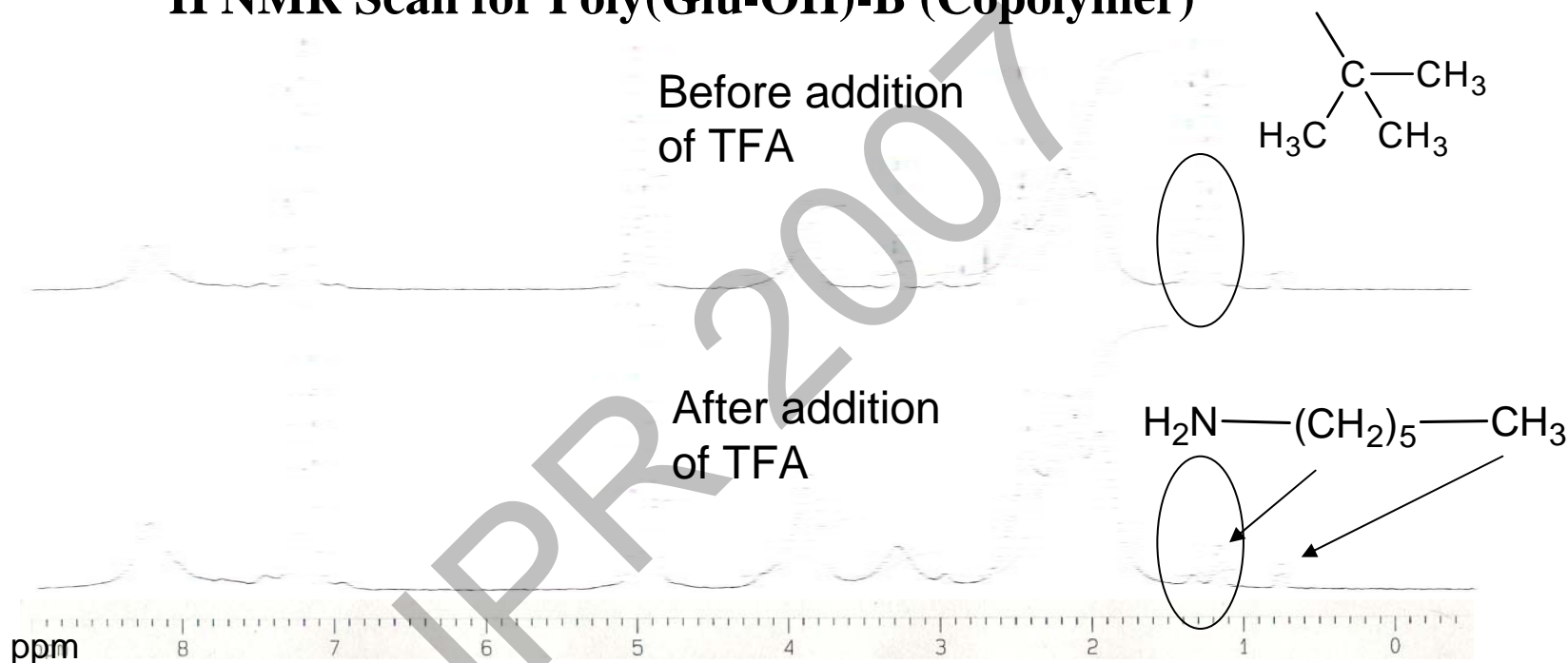
Sample Name	Reaction Size (g)	Mole Ratio of HBr or tert-butyl to Benzyl Units	¹ H NMR % Cleavage	Titration % Cleavage
Poly(Glu-OH)-18-2	0.25	0.35:1	49	46
Poly(Glu-OH)-18-3	0.25	0.2:1	27	25
Poly(Glu-OH)-19	1.0	0.25:1	34	31
Poly(Glu-OH)-34	1.5	0.25:1	31	30
Poly(Glu-OH)-B	0.5	0.30:0.70	33	34
Poly(Glu-OH)-C	1.0	0.30:0.70	31	30

Sample number or letter refers to homopolymer or copolymer used for reaction. Reaction time 3 h.

- Good correlation between ¹H NMR analysis and –CO₂H titration results
- Target acidolysis level of ~30% by both methods

SELECTIVE DEPROTECTION

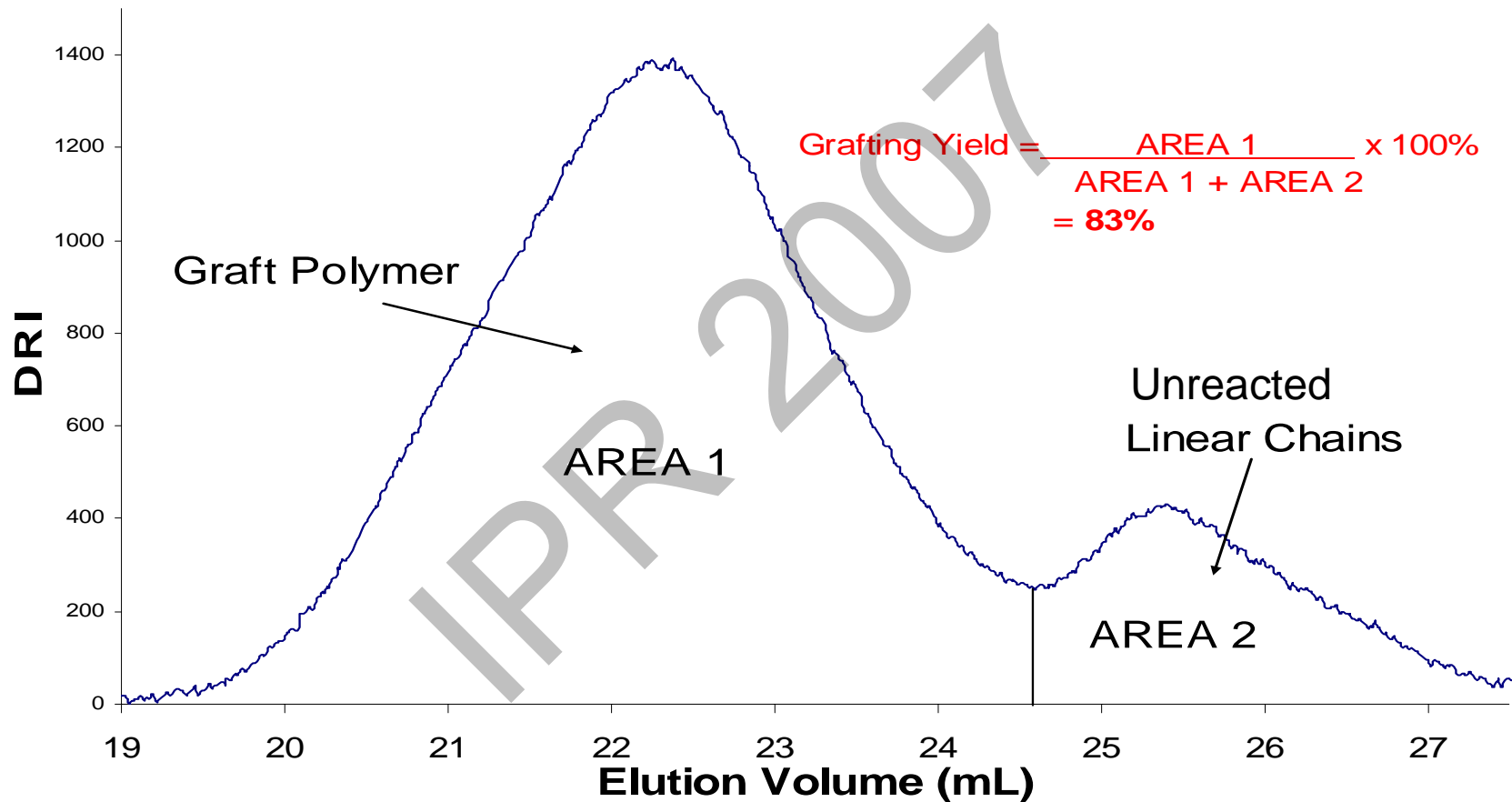
^1H NMR Scan for Poly(Glu-OH)-B (Copolymer)



- Essentially 100% of *tert*-butyl ester groups cleaved
- Remaining peaks from hexylamine initiator hydrocarbon chain ($-\text{CH}_2-$)
- No benzyl ester groups cleaved

GRAFTING REACTION

SEC Analysis PG-graft-PG-22



- Baseline resolution not achieved
 - Larger MW difference needed between graft and linear polymers

Sample Name	DP _n ^{SIDE}	DP _n ^{SUB}	% Graft Sites	Graft Yield
PG-g-PG-18	58	50	33	68
PG-g-PG-19	50	50	33	43
PG-g-PG-21	33	55	31	56
PG-g-PG-22	23	55	31	83
PG-g-PG-23	23	50	33	70
PG-g-PG-26	54	62	33	59
PG-g-PG-28	21	62	33	69

Molar ratios → NH₂ : CO₂H : DIC : HOBt : TEA
 1 : 2 : 10 : 10 : 20

- Grafting yield ranged from 43-83%
 - 83% Grafting yield obtained when using side chains obtained at 0°C and copolymer substrate



FUTURE WORK

- Determine 'living' chain content of samples by Non-aqueous Capillary Electrophoresis (NACE)
- Confirm random acidolysis and copolymerization by ^{15}N NMR spectroscopy
- Systematic variations in side chain DP_n , coupling site density to optimize grafting yield
- Synthesize G1, G2, ...etc. polymers
- Last grafting cycle with *tert*-butyl ester

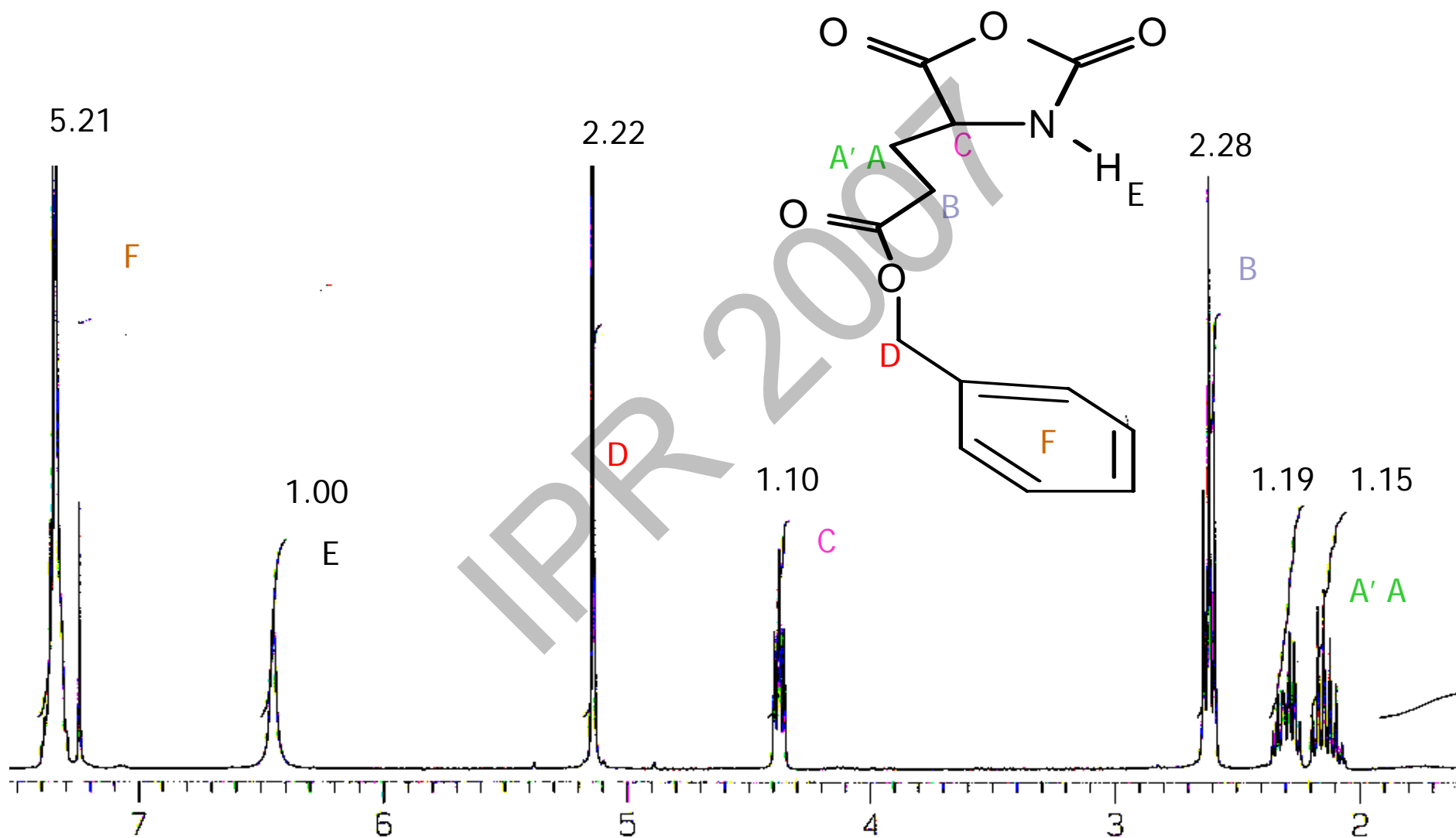


ACKNOWLEDGEMENTS

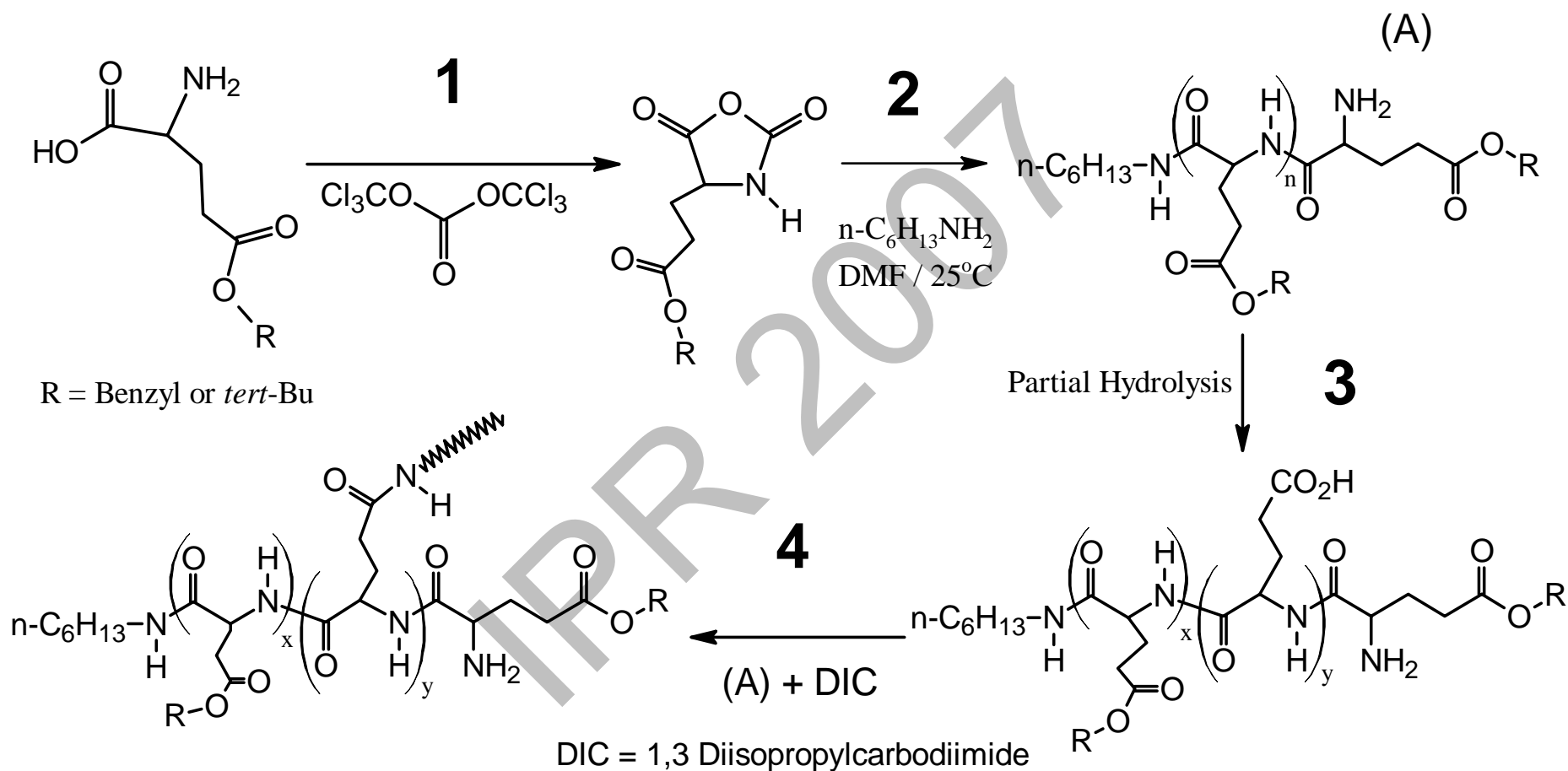
- Dr. Duhamel
- Members of the Gauthier and Duhamel Groups

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^1H NMR Spectrum of Monomer



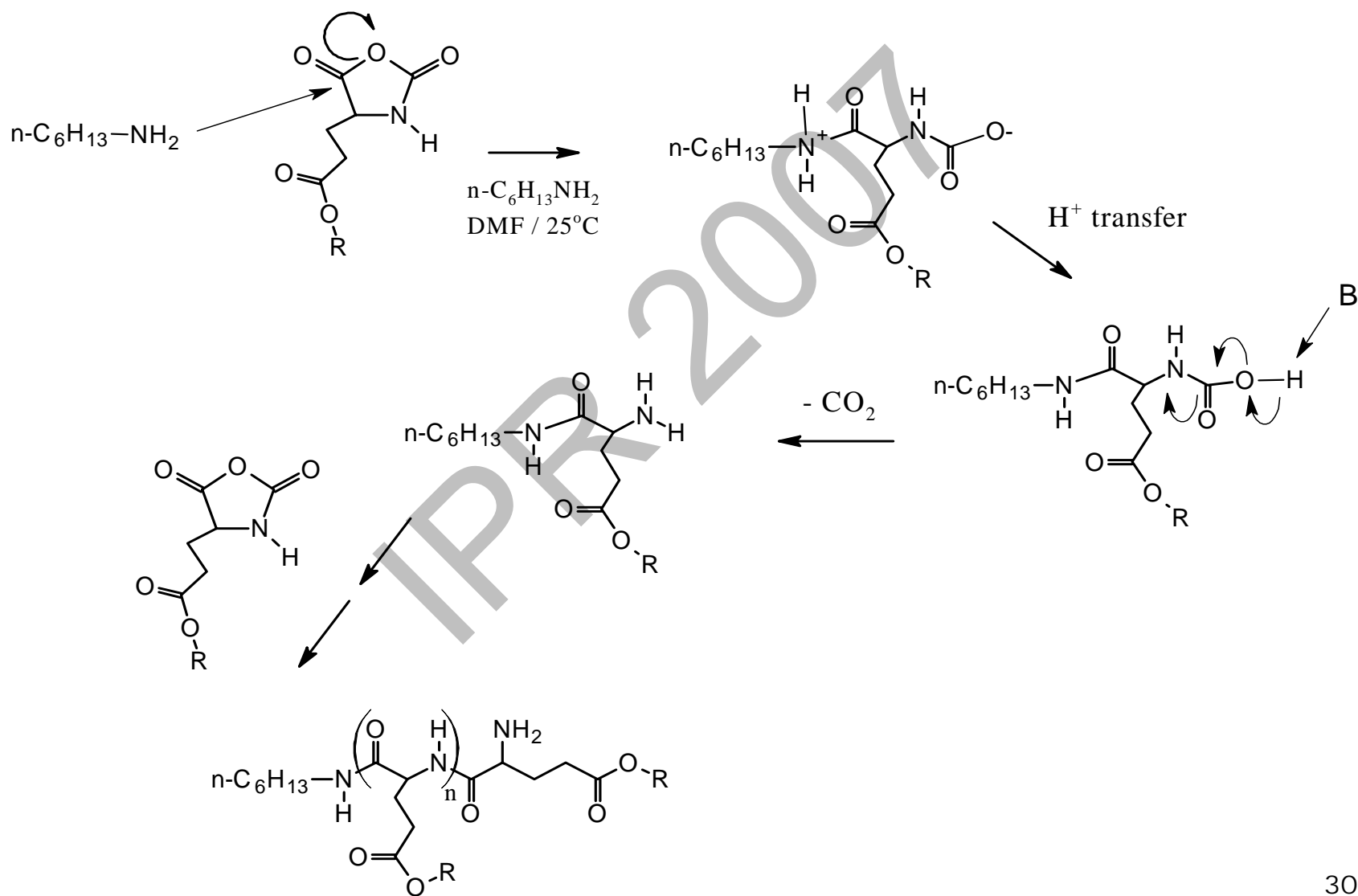
Overall Synthetic Scheme



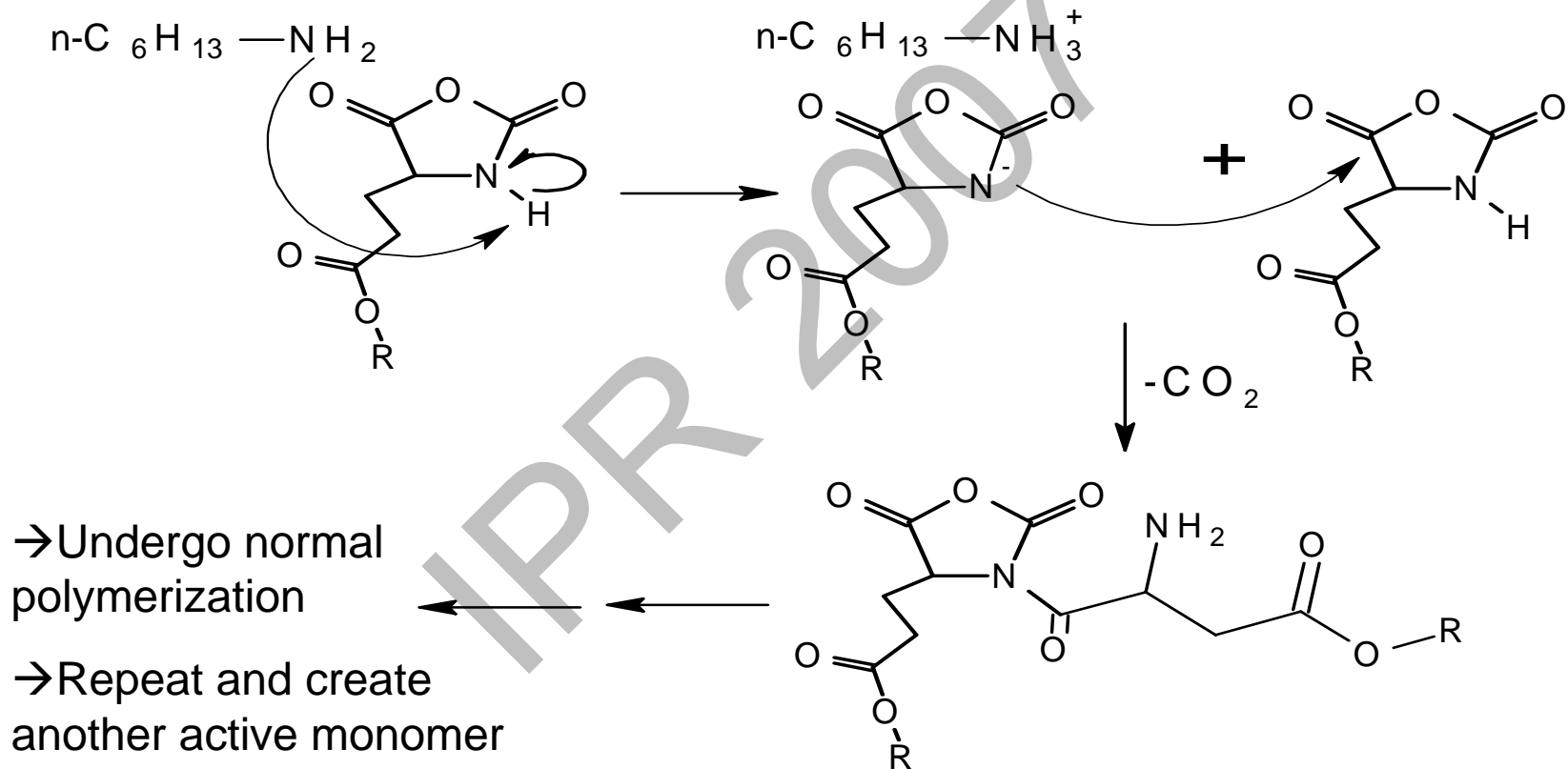
-Explain each step briefly so audience can get an idea of what is going on

-mention more details on synthesis later

ROP MECHANISM



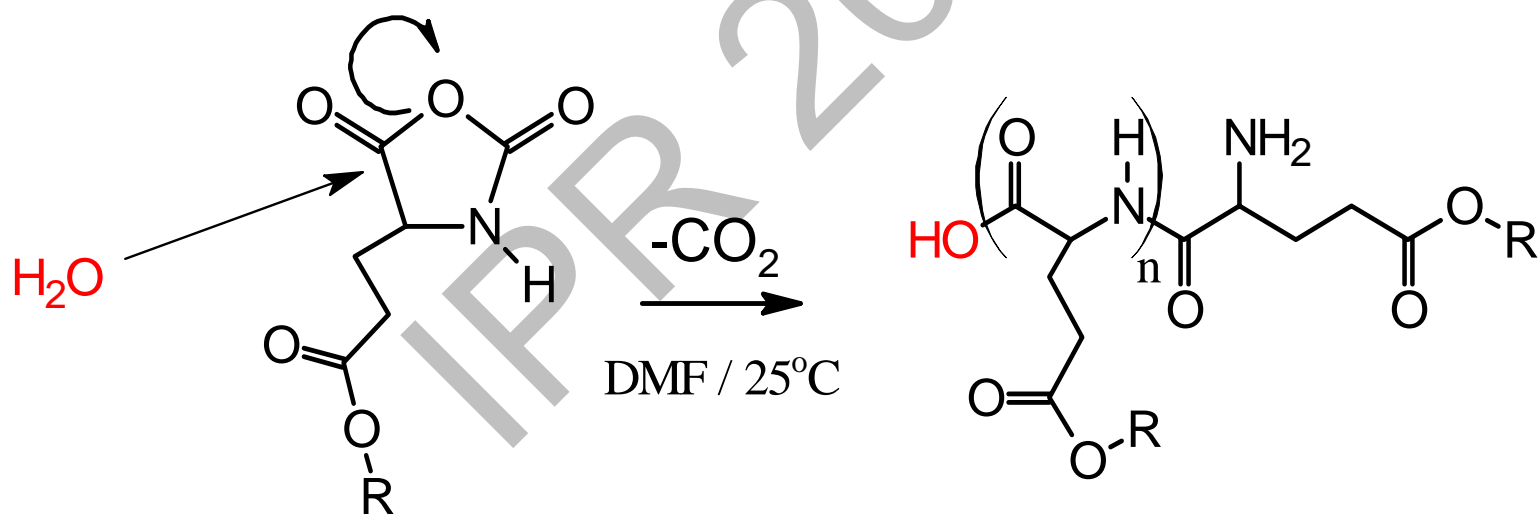
Activated Monomer Mechanism



- Result: No initiator unit in chain, ill-defined polypeptides

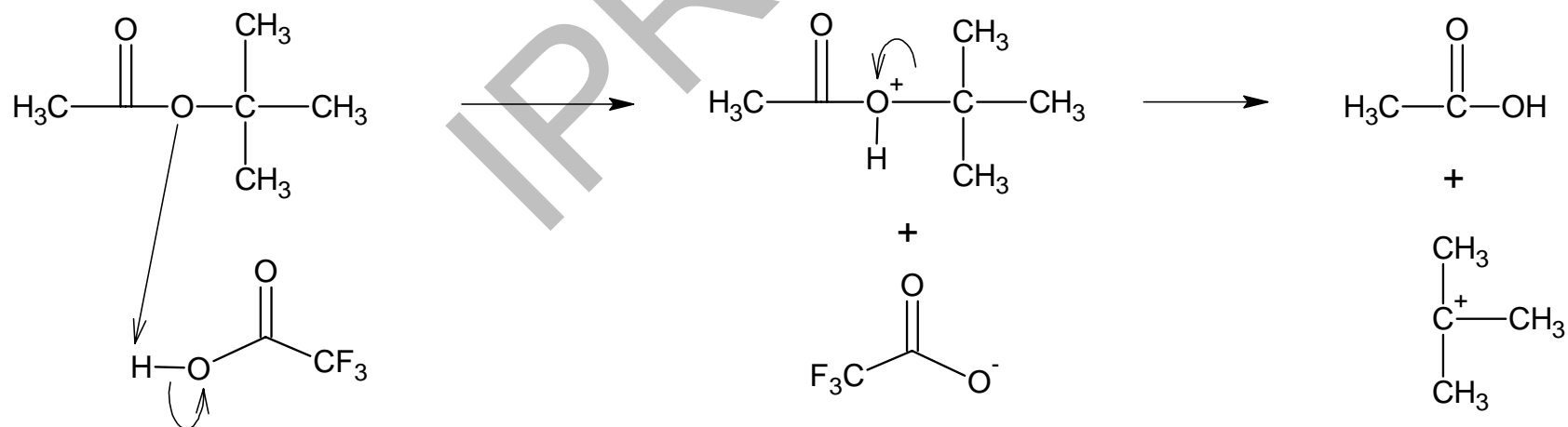
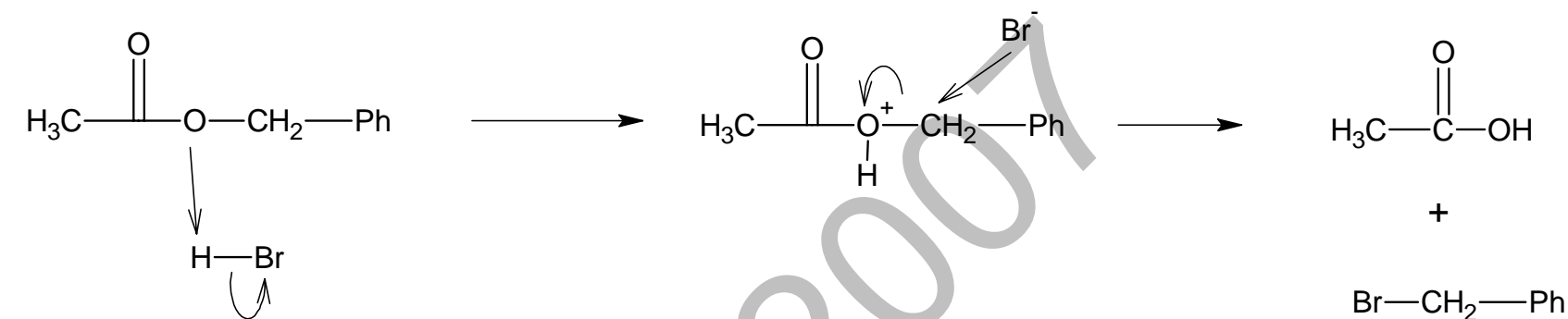
Water-Induced Polymerization

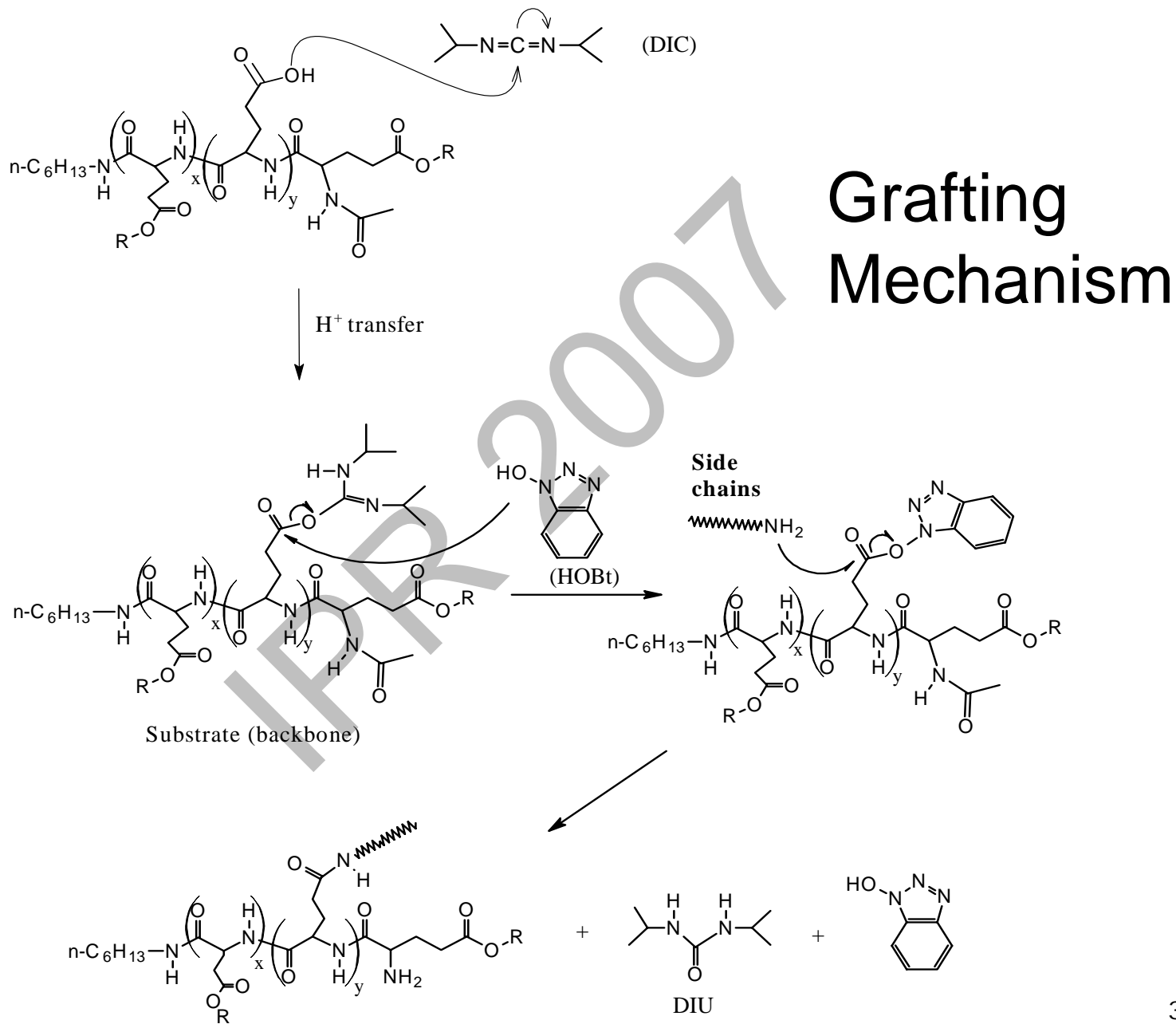
- Water not as nucleophilic as n-hexylamine
- Appears in solvent, glassware, atmosphere



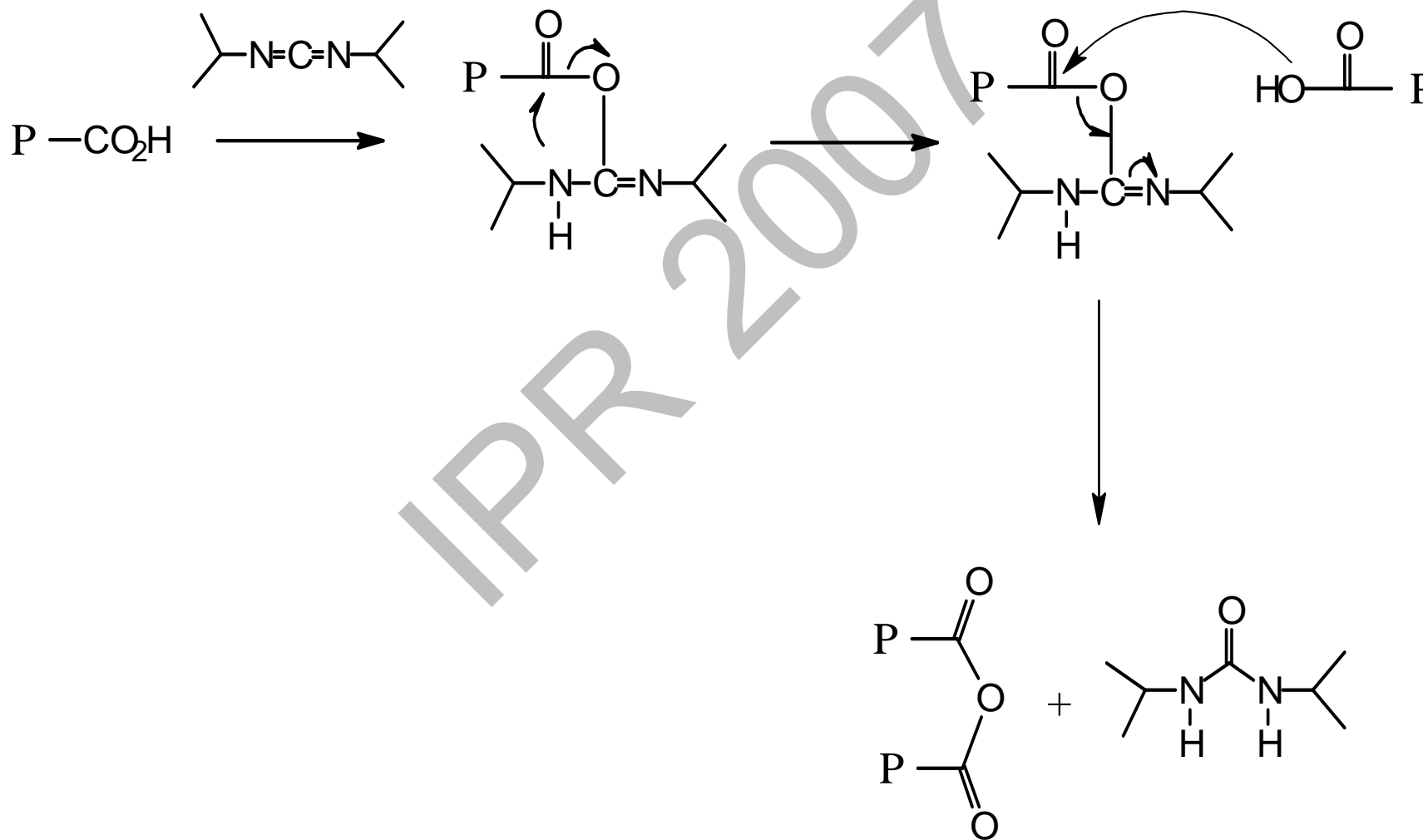
- No initiator unit in chain

Deprotection Mechanism





Grafting Side Reaction



RESULTS

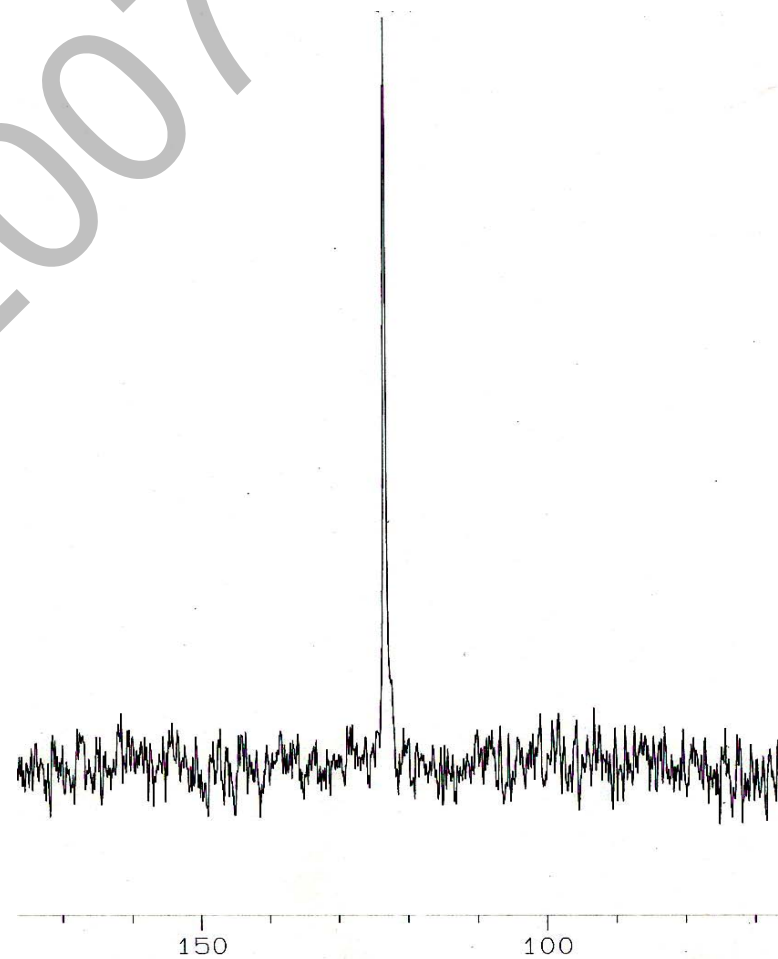
Table 1: Polymerization of Glu-NCA Under Different Reaction Conditions

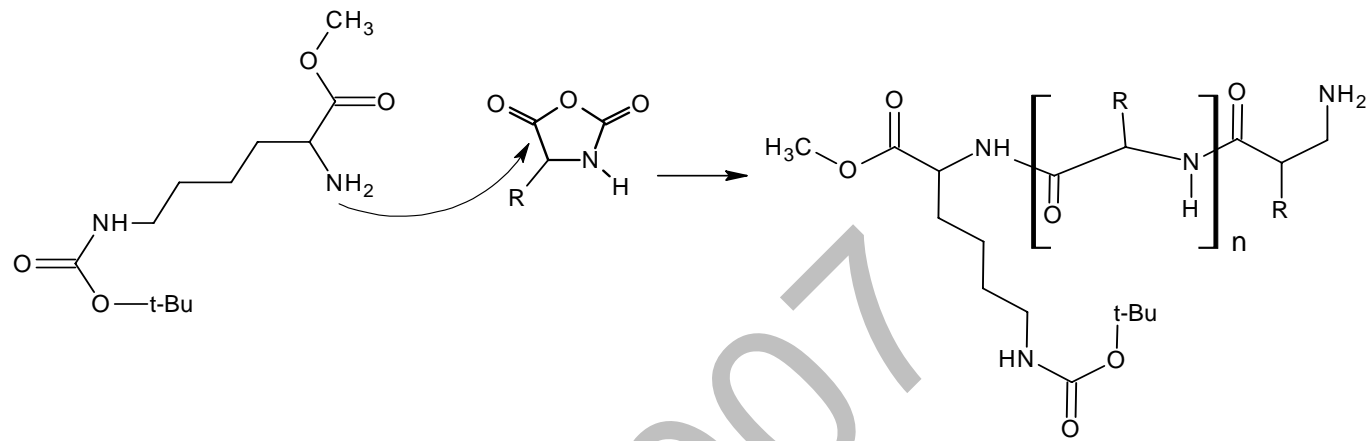
Sample Name*	Reaction Time	M_n^{app}	PDI	Target DP	1H NMR DP	$M_n^{H NMR}$
Poly(Bz-Glu)-18	2 h	11,500	1.18	20	25	5,800
Poly(Bz-Glu)-19	2 h	16,100	1.18	60	50	11,300
Poly(Bz-Glu)-20 ^a	3 d	7,960	1.11	20	31	7,200
Poly(Bz-Glu)-20 ^a	5 d	9,220	1.14	20	33	7,600
Poly(Bz-Glu)-21 ^a	13 d	9,530	1.13	20	14	3,500
Poly(Bz-Glu)-22	4 h	11,200	1.19	20	22	5,100
Poly(Bz-Glu)-23 ^b	24 h	11,300	1.15	20	22	5,100
Poly(Bz-Glu)-24 ^d	2 h	8,140	1.12	20	18	4,200
Poly(Bz-Glu)-25 ^b	5 d	11,500	1.14	20	23	5,400
Poly(Bz-Glu)-26 ^d	24 h	12,500	1.19	20	20	4,700
Poly(Bz-Glu)-27 ^d	24 h	11,700	1.20	20		
Poly(Bz-Glu)-29 ^c	6 d	10,500	1.16	20	21	4,900
Poly(Bz-Glu)-29 ^c	10 d	11,200	1.15	20	24	5,600
Poly(Bz-Glu)-30 ^e	4 d	19,400	1.12	50		
Poly(Bz-Glu)-30 ^e	5 d	21,200	1.17	50	54	12,200
Poly(Bz-Glu)-31 ^e	2 h	16,400	1.12	20	29	6,600
Poly(Bz-Glu)-32 ^e	2 h	15,400	1.15	20	28	6,500
Poly(Bz-Glu)-33 ^b	3 d	14,000	1.14	20	21	4,900

a hydrochloride salt initiator, b temperature decrease to 00C, c temperature decrease to -150C, d H-Lys(Boc)-OMe initiator e High vacuum technique.*number in sample name refers to run number

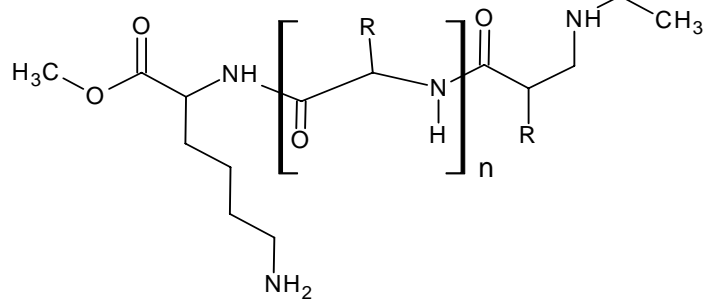
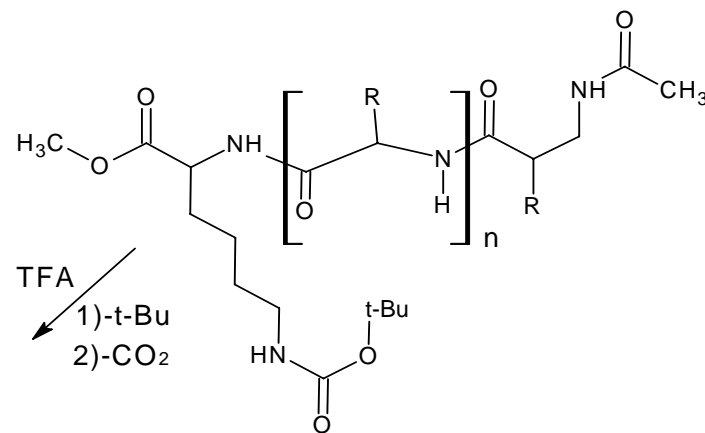
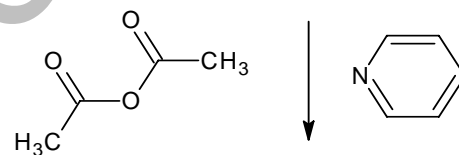
^{15}N NMR COPOLYMER

- One peak suggests similar environment
 - Similar reactivities for copolymerization
- Might need better resolution to confirm

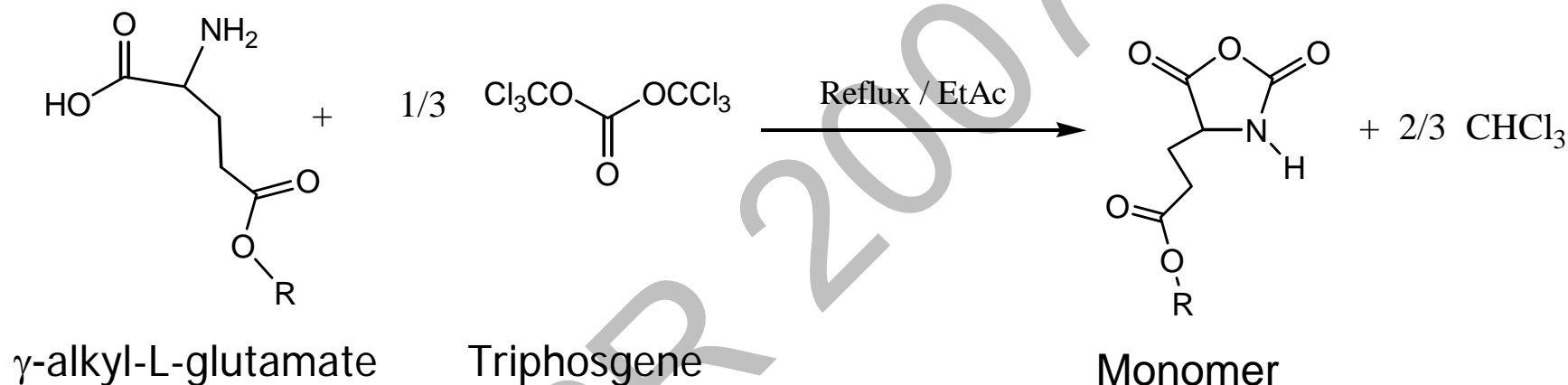




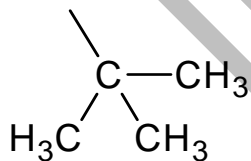
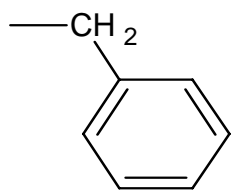
Lysine initiation/deprotection



N-CARBOXYANHYDRIDE (MONOMER) SYNTHESIS



R = Benzyl or *tert*-Butyl



- 92% product yield on 10 g reaction scale
- Product purity confirmation by ^1H NMR