Synthesis of Arborescent Polypeptides for Controlled Drug Delivery Applications

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IPR Symposium May 1st, 2009

OUTLINE

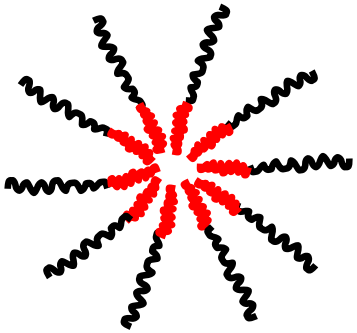
- Objectives
- Synthesis
- Results
- Future Work
- Acknowledgements

Project Objectives

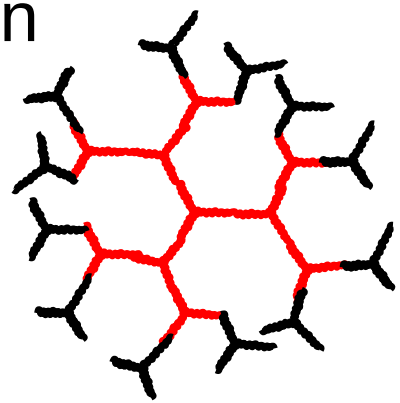
- Synthesize biocompatible branched polymers with narrow molecular weight distributions (MWD)
  - ➔Unimolecular micelles
- Demonstrate the feasibility of the synthetic scheme
  - ➔Grafting yield and coupling efficiency
- Demonstrate suitability for applications in microencapsulation (drug delivery)
  - ➔Solubilization and release of hydrophobic probe molecules

MICELLES

- Linear diblock copolymer micelles
  - Dynamic structure: Molecules in micelle interchange with free molecules
  - critical micelle concentrations (unstable at C < CMC)

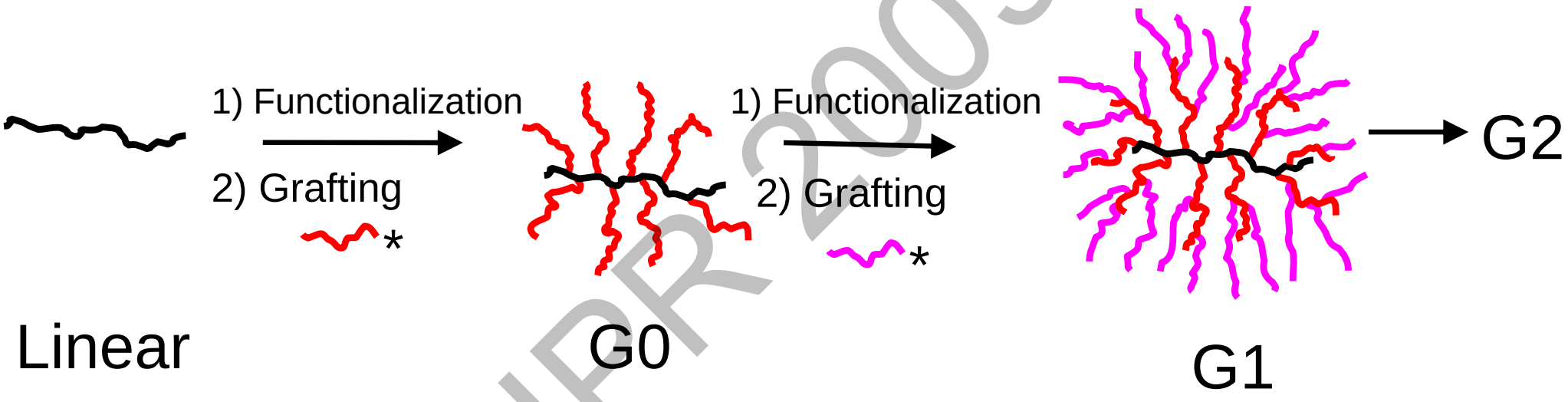


- Dendritic micelles
  - Narrow molecular weight distribution
  - low molecular weights



Arborescent Micelles

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



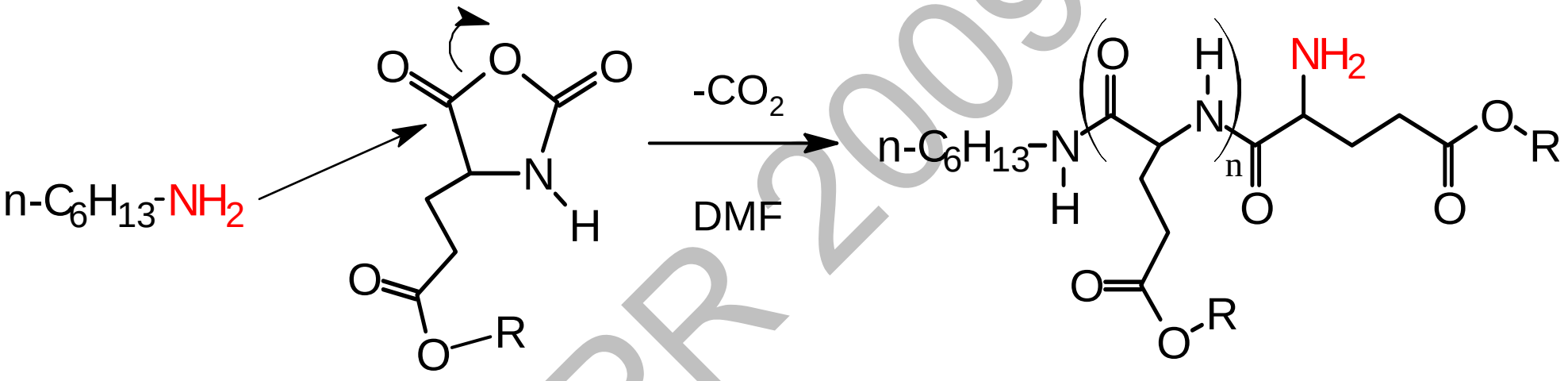
- ➔ Static structure (stable, no CMC)
- ➔ High molecular weights achieved in only a few grafting cycles
- ➔ Biocompatibility: Amino acid derivatives

SYNTHESIS

SYNTHETIC SCHEME

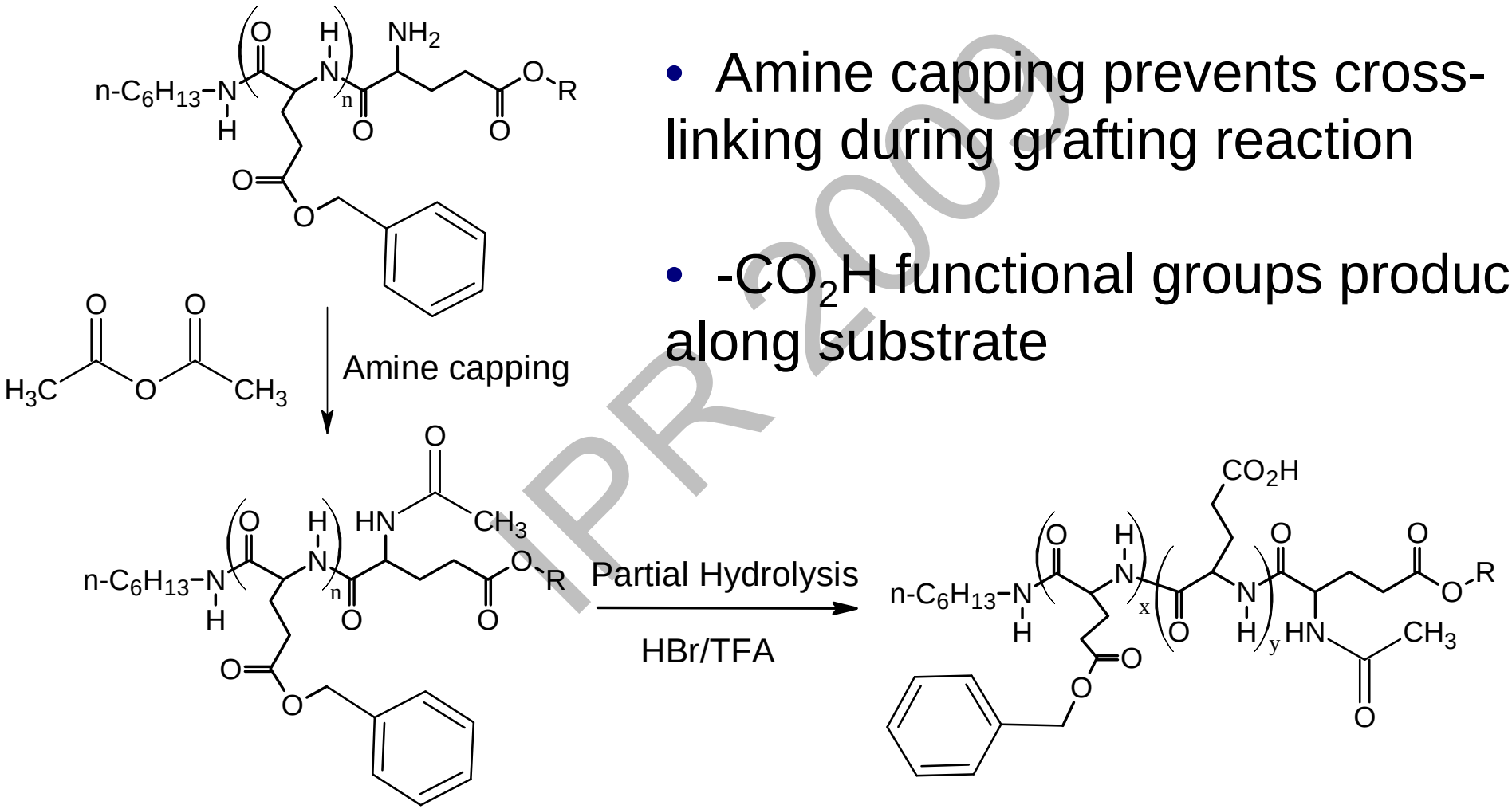
- Ring opening ‘living’ polymerization
  - γ-benzyl or γ-t-butyl L-glutamate monomers
- Partial deprotection of poly(γ-benzyl L-glutamate) (PBLG) substrate to generate randomly distributed grafting sites
- Grafting
  - Comb-branched polymer (Generation zero, G0)
  - Higher generations G1, G2 …..etc.
- Shell modification
  - Attach water-soluble components for micelle formation

SYNTHESIS OF SIDE CHAINS



'living' primary amine end group

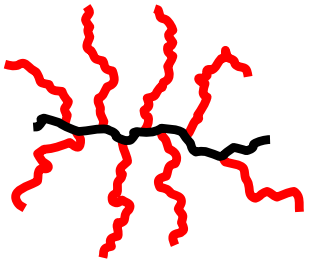
SYNTHESIS OF SUBSTRATE



- Amine capping prevents cross-linking during grafting reaction
- -CO_2H functional groups produced along substrate

GRAFTING

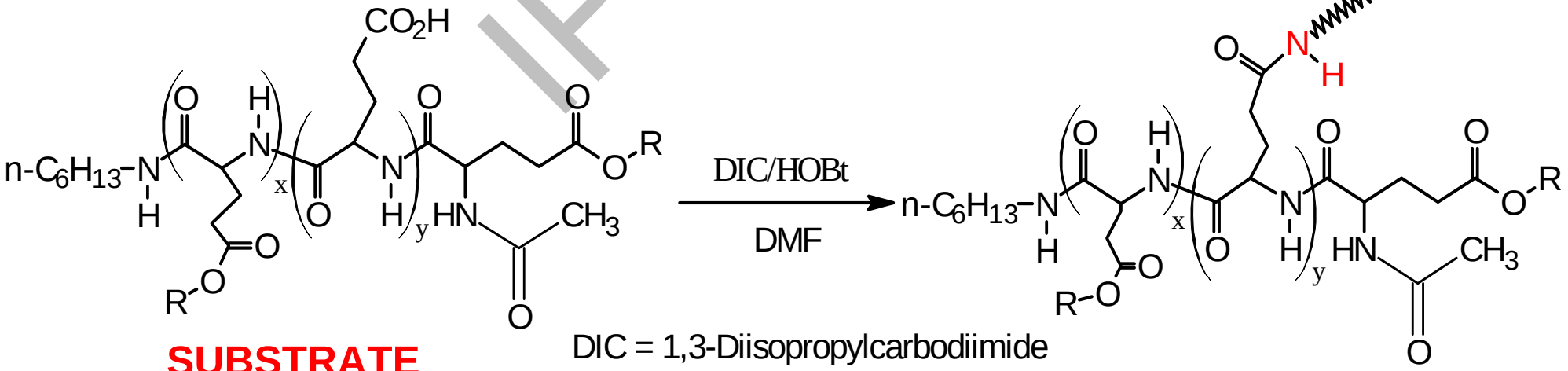
- Substrate coupling with side chains
  - Grafting sites activated by carbodiimide technique
    - ➔ DIC/HOBt
  - Stoichiometry varied to maximize grafting yield



G0 polymer

SIDE CHAINS

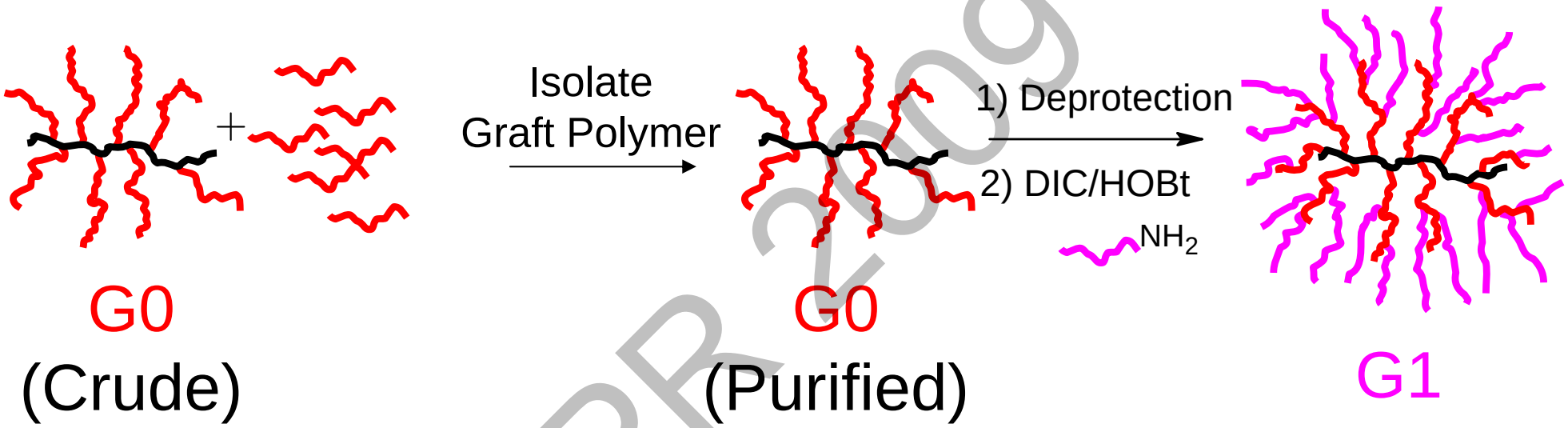
+



SUBSTRATE

DIC = 1,3-Diisopropylcarbodiimide
HOBt = 1-Hydroxybenzotriazole

GENERATIONS 1, 2,…



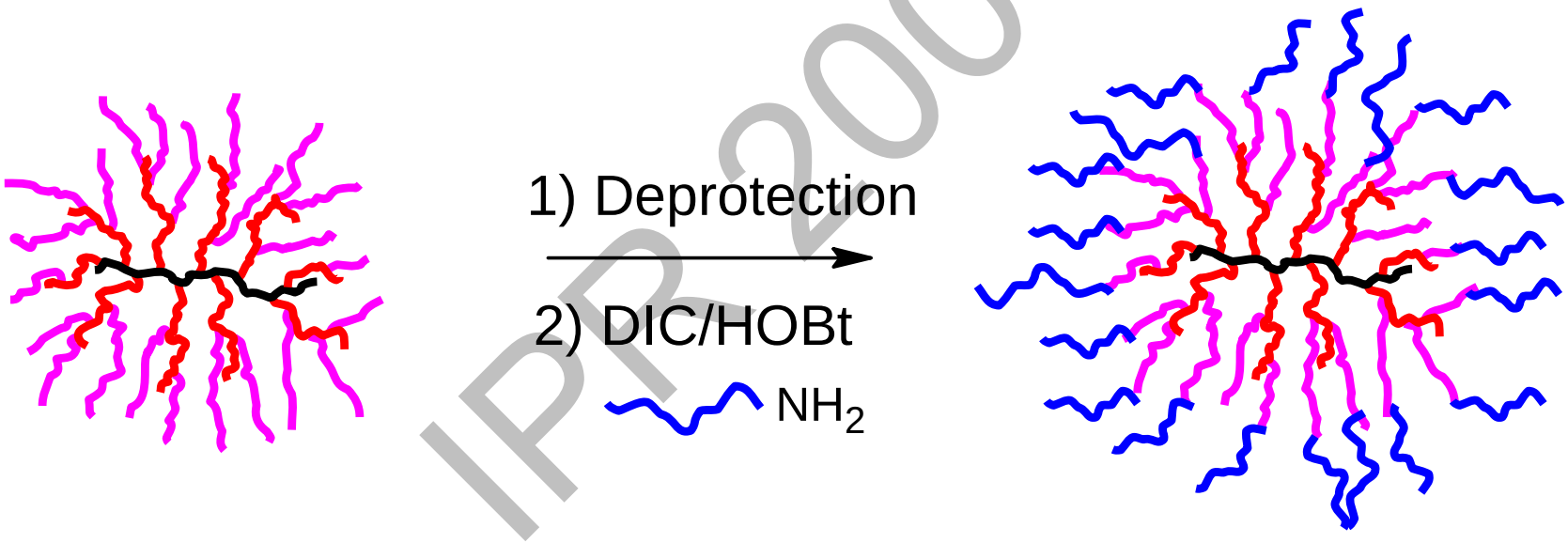
- Graft polymer isolated with preparative GPC column

  Fast separation of 60-80 mg crude product per injection

Analytical Column:	10 mm I.D.
Prep Column:	**22 mm** I.D.

Micelles: Shell Addition

- Last grafting cycle: Hydrophilic side chains



Water-soluble
Unimolecular micelle

CHARACTERIZATION

- Gel Permeation Chromatography (GPC)
  - Apparent and absolute molecular weights (M_n, M_w)
  - PDI for linear and graft polymers
  - Grafting yield
- NMR Spectroscopy
  - Number-average degree of polymerization (DP_n)
    - Absolute M_n
  - Deprotection level of substrate
  - Derivatization/^{19}F NMR to detect primary amine chain ends
- Static Light Scattering
  - Absolute M_w , PDI
  - Coupling efficiency
- UV and Fluorescence Spectroscopy
  - Monitoring of probe solubilization capacity and kinetics

Results

RING OPENING POLYMERIZATION

Preparation of Poly(γ-benzyl L-glutamate) Linear Side Chains

Sample #	% Yield	^{1}H NMR		GPC[a]	
		DP_n	M_n	$M_n^{apparent}$	M_w/M_n
51	89	18.1	4,300	6,500	1.10
55	87	21.0	4,900	8,600	1.10
56	89	19.2	4,500	7,000	1.13
57	94	16.7	4,000	4,600	1.12
58	86	18.9	4,500	5,900	1.14
59	94	16.3	3,900	6,000	1.14

[a] DRI detector

➔PDI = **1.10-1.14**, satisfactory

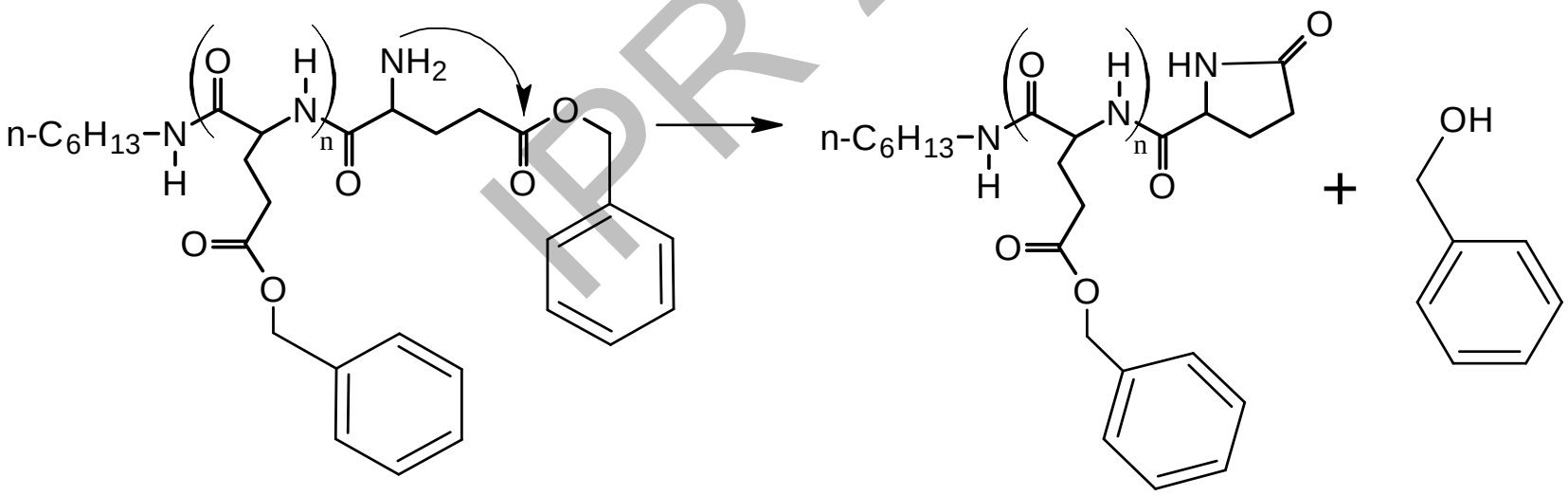
➔Chain functionality (-NH_2) level determines coupling efficiency

Side Reactions in ROP

- Destroy ‘living’ primary amine
- Broaden MWD

➔ Mostly end group cyclization

- Specific to poly(γ-benzyl L-glutamate)

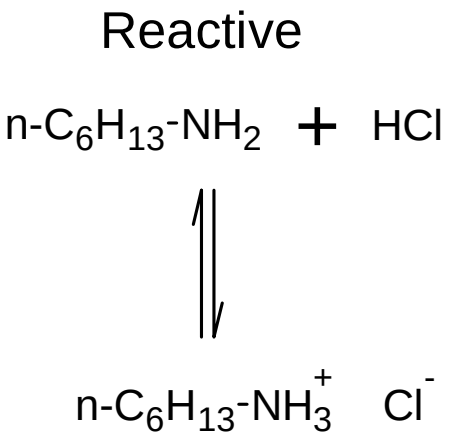


➔ Modify reaction conditions to reduce side reactions

Strategies To Avoid Side Reactions

- Hydrochloride salt initiator
  - ➔ Long reaction times (5-10 days)

    W Vayaboury et al. *Macromol. Rapid.Commun.* **2004**



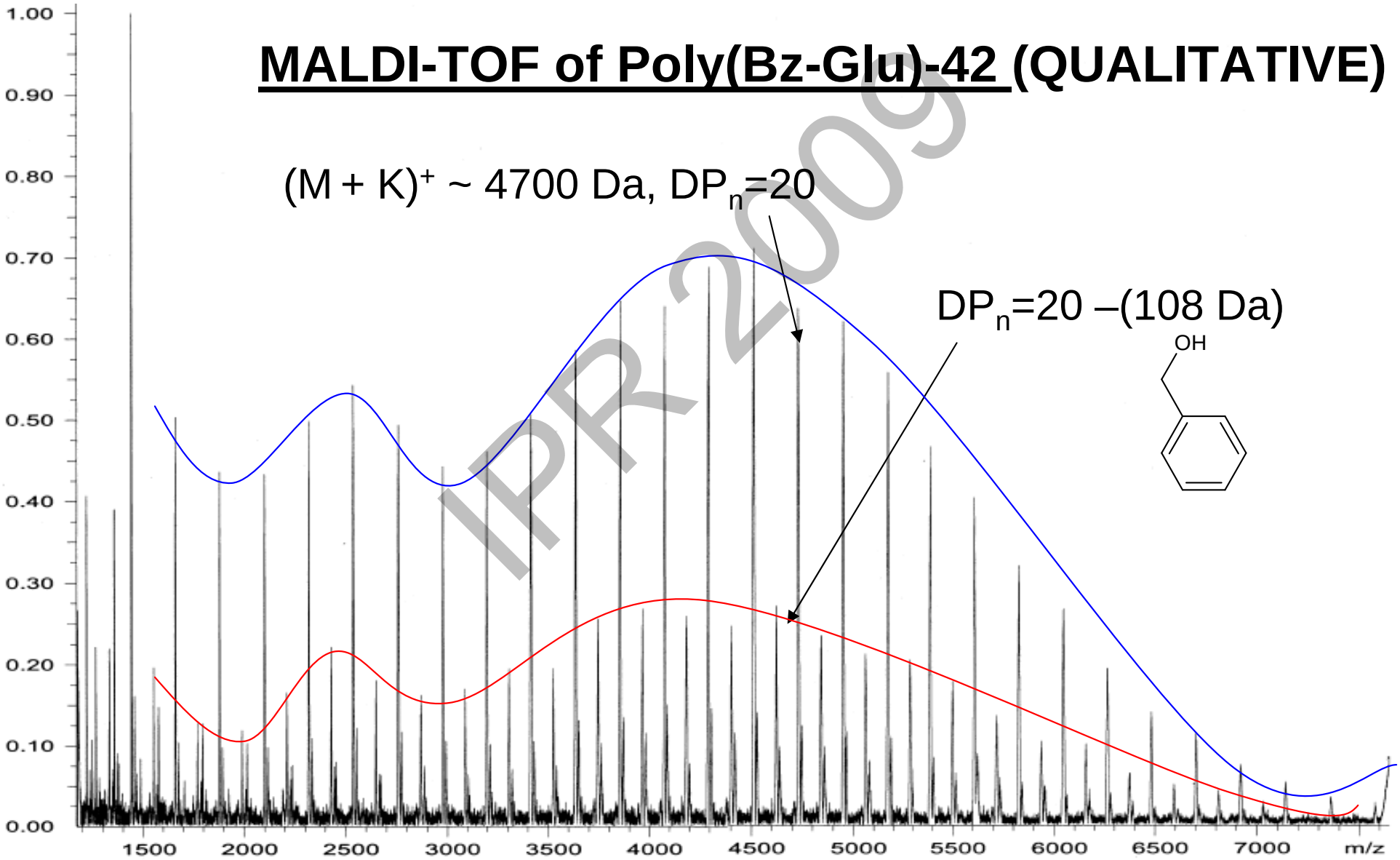
- High vacuum techniques
  - ➔ Time-consuming

    T Aliferis et al. *Biomacromolecules* **2004.**

- Lower polymerization temperature
  - ➔ Moderate reaction times (2-3 days), easy setup

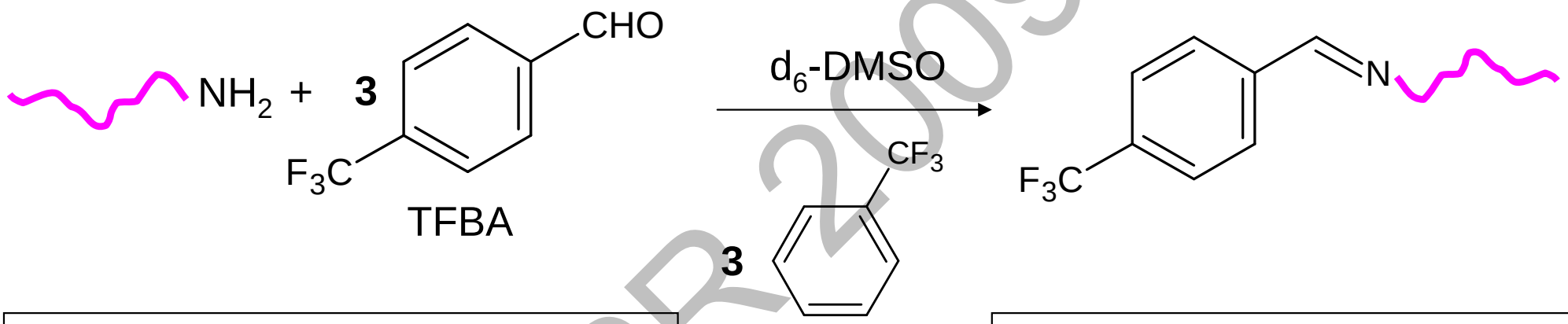
    I Dimitrov et al. *Chem. Commun.* **2003.**

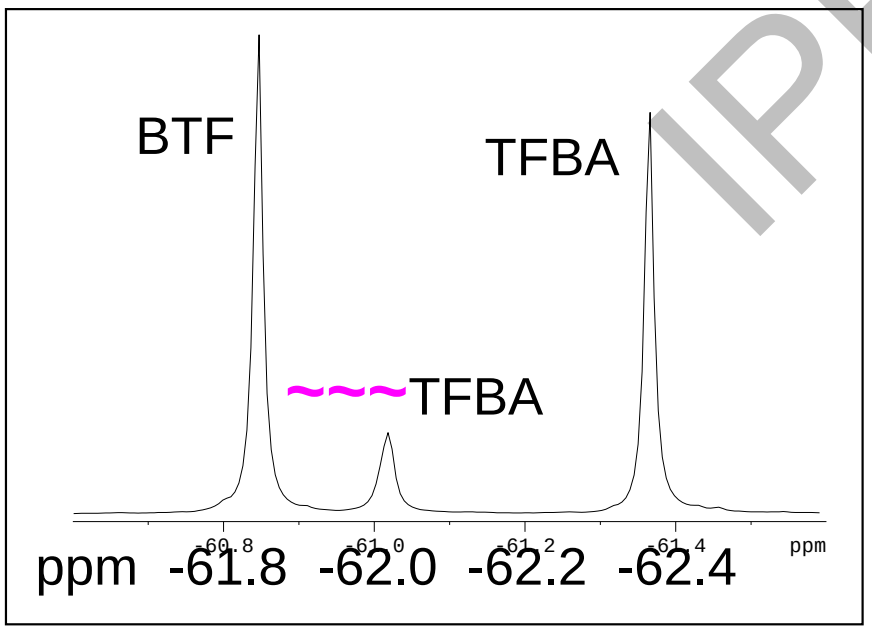
Detection of Side Reactions in ROP



DETECTION OF SIDE REACTIONS IN ROP

- Using **^{19}F NMR** spectroscopy to quantify $-NH_2$





	NH_2	TFBA	BTF
Before	1	3	3
After	1	2	3
Actual	1	2.52	3.20

TFBA *f* NH_2 = 2/2.25 = 79%

BTF *f* NH_2 = 3/3.58 = 84%

J Shengxiang, et al. *Macromolecules*, **2005**, 38, 4679.

DETECTION OF SIDE REACTIONS IN ROP

	% NH2 groups	
Sample #	**TFBA**	**BTF**
Poly(Bz-Glu)-20	20	25
Poly(Bz-Glu)-30	58	66
Poly(Bz-Glu)-50	15	20
Poly(Bz-Glu)-58	74	79
Poly(Bz-Glu)-64	79	84
Poly(Bz-Glu)-65	40	43.7
Poly(Bz-Glu)-71	41	46
PEG1900-NH2	72	79

- ~5% Discrepancy between TFBA and BTF methods, but sample grading is mostly consistent
- **QUANTITATIVE** method

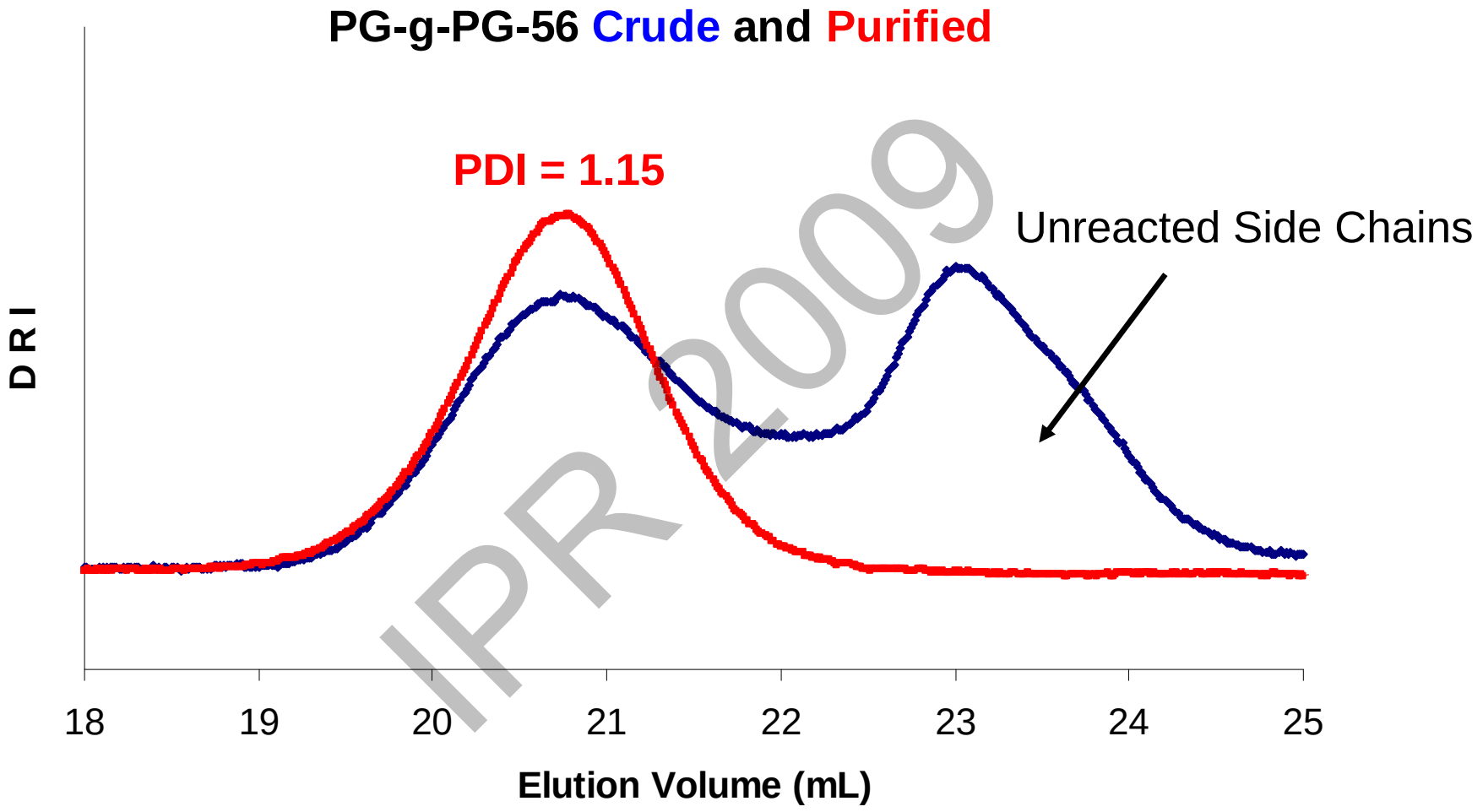
DEPROTECTION OF SUBSTRATES

Deprotection of Poly(γ-benzyl L-glutamate) Substrates

Sample	Target Deprotection	Mole Ratio of HBr to Benzyl ester Units	% Deprotection	
			^{1}H NMR	Titration
34	30	0.25:1	31	30
B	30	30:70	33	34
C	30	30:70	31	30
G0-52	30	0.3:1	32	--
G0-53	30	0.3:1	32	--
G1-2	20	0.2:1	16	--
G1-4	30	0.3:1	16	--
G2-3	30	0.3:2	26	--

- Good agreement between ^{1}H NMR analysis and titration
- Titration difficult for arborescent substrates

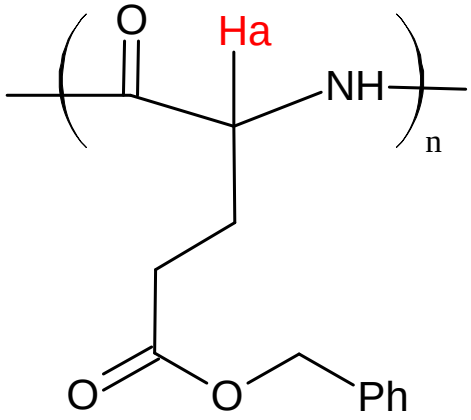
GRAFTING REACTION: GPC ANALYSIS

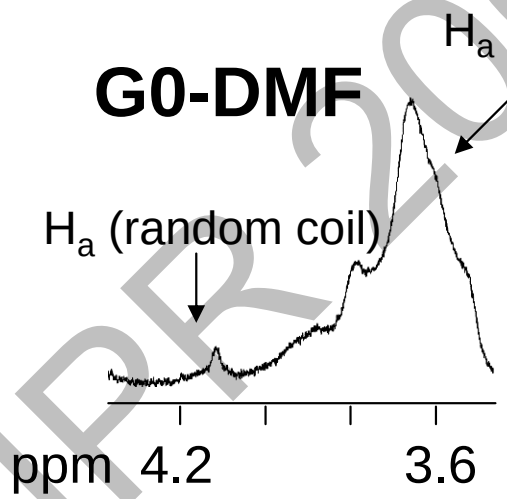


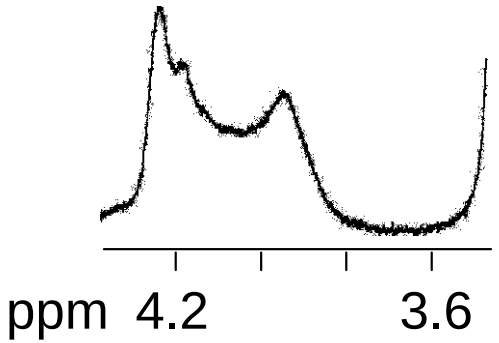
- Purification achieved after only one injection
- Narrow MWD maintained after purification

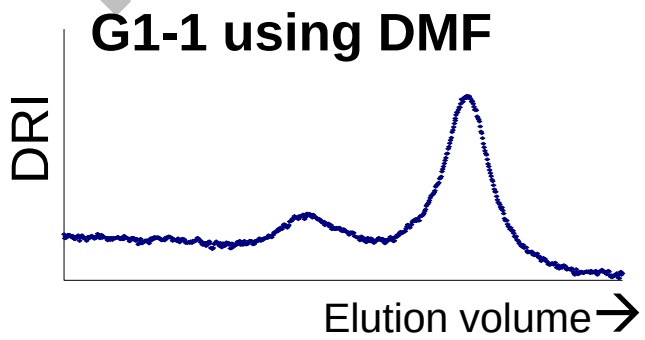
HINDERED GRAFTING REACTION

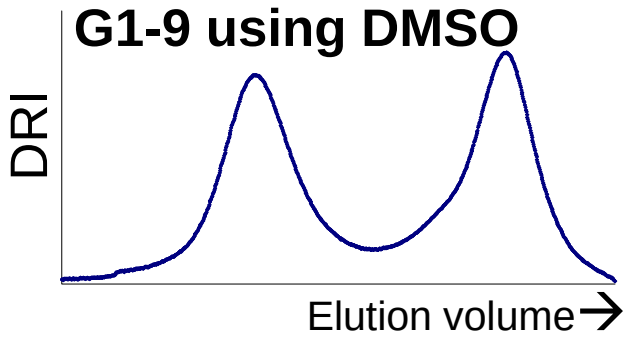
- No reaction for grafting on G0 in DMF
  - Possible α-helix conformation of G0 substrate and side chains
  - Run ^{1}H NMR to confirm (500 MHz)











K Maeda, et al. *Chem Eur. J.,* **2004**, *10*, 4000.

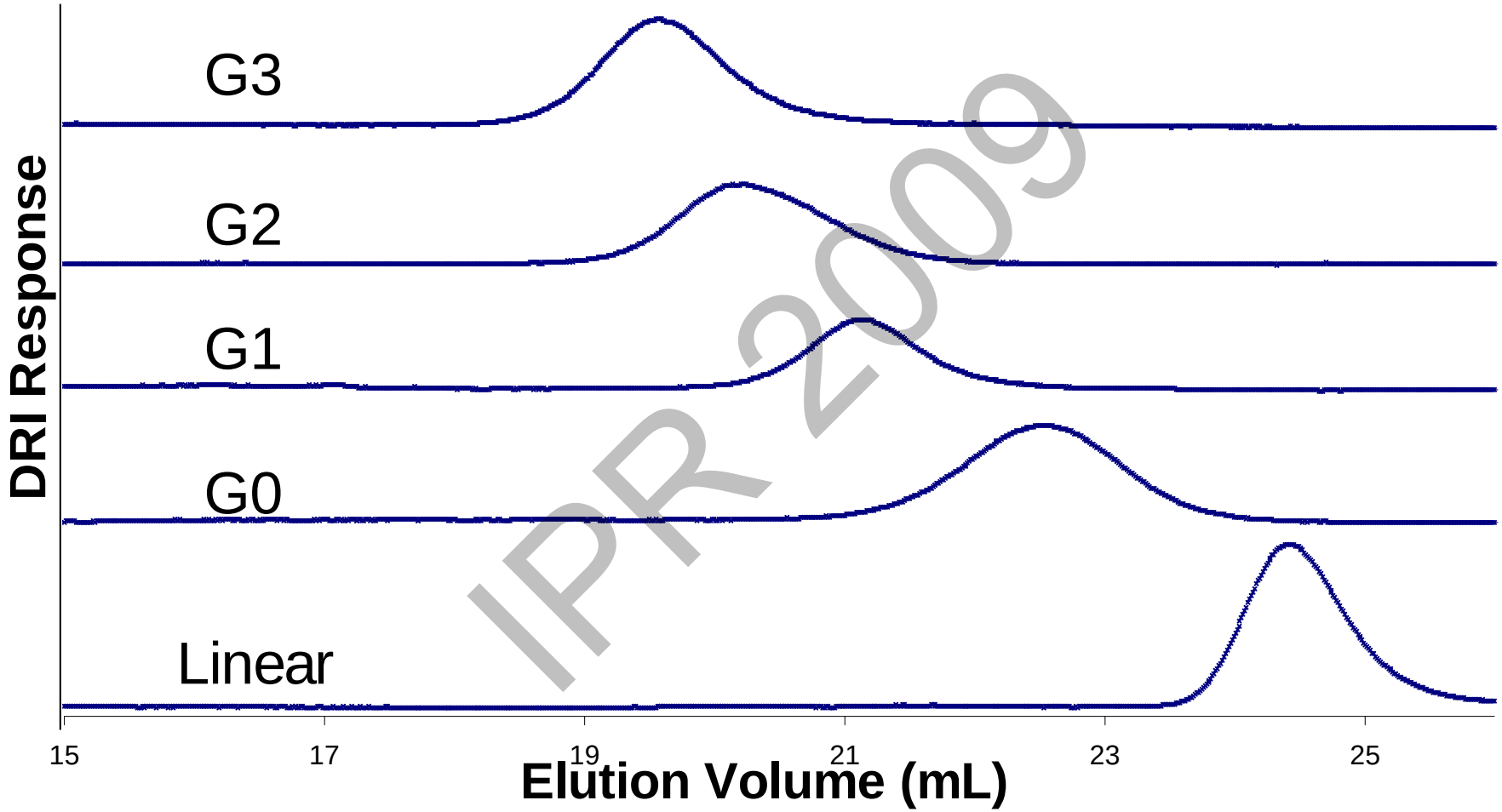
GENERATIONS 1, 2,…

Characteristics of Arborescent Poly(γ-benzyl L-glutamate) for Successive Generations

Sample #	M_n Side chain [a]	DRI	MALLS		Grafting Yield (%) [b]	Branching Functionality (%)[c]
		M_n^{app}	M_n	M_w/M_n		
G0	4,300	17,400	48,000	1.04	38	9
G1	4,000	39,300	133,000	1.06	63	21
G2	3,900	83,100	486,000	1.03	46	90
G3	3,900	134,000	1,060,000	1.03	32	147

[a] ^{1}H NMR, [b] DRI detector, [c] MALLS Detector

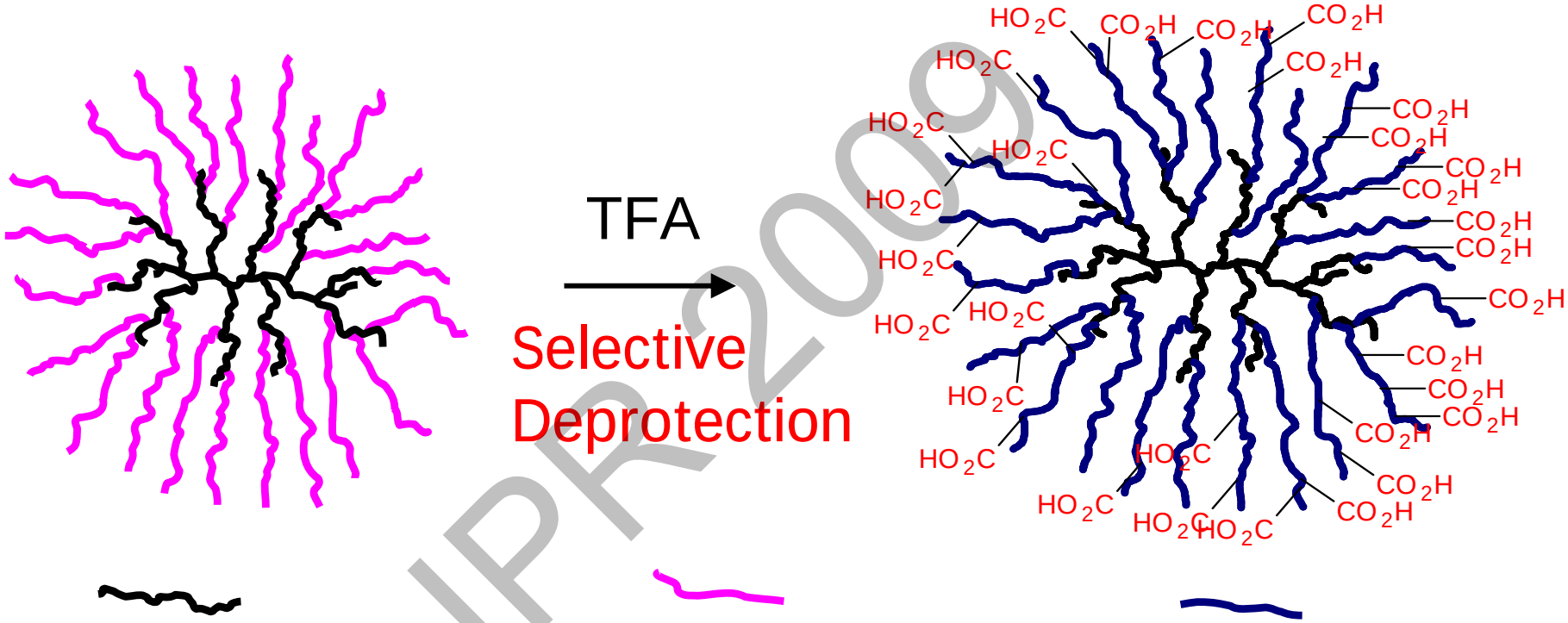
GENERATIONS 1, 2,…



- Narrow MWD maintained
- Graft polymers free of linear contaminant

MICELLES: SHELL ADDITION

- Last grafting cycle: Poly(γ-*tert*-butyl L-glutamate) side chains



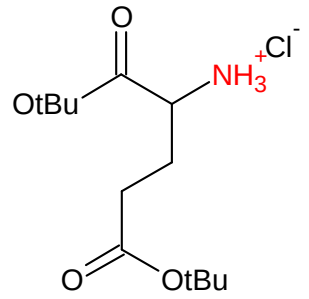
- Soluble in water and alkaline water solutions
  - → Gradual desolubilization by dialysis
- -CO_2H groups buried in core (→ less hydrophobic)

Future Work

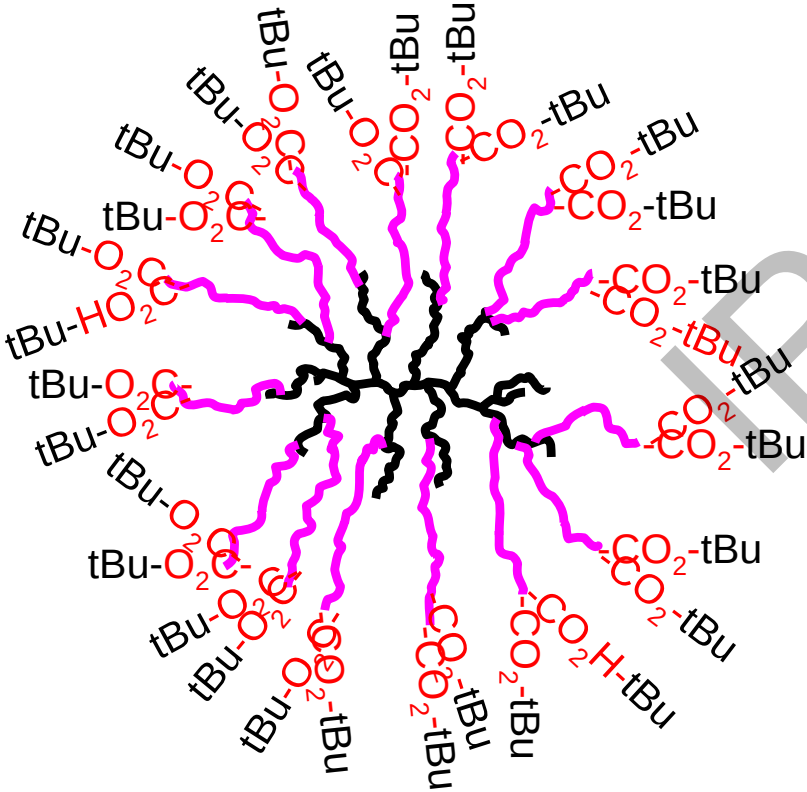
- Modification to generate water-soluble micelles
  - Addition of longer or more PEG or Poly(OtBu-Glu) segments
  - Addition of hydrophilic segments at chain ends
- Solubilization studies with hydrophobic probes
  - UV-monitored
- Study of release kinetics
  - Fluorescence- and UV-monitored

MICELLES: SHELL ADDITION 2

- Last grafting cycle: Side chains with H-Glu(OtBu)-OtBu initiator
- Remove protecting groups to functionalize ONLY chain ends (two –CO_2H per chain end)

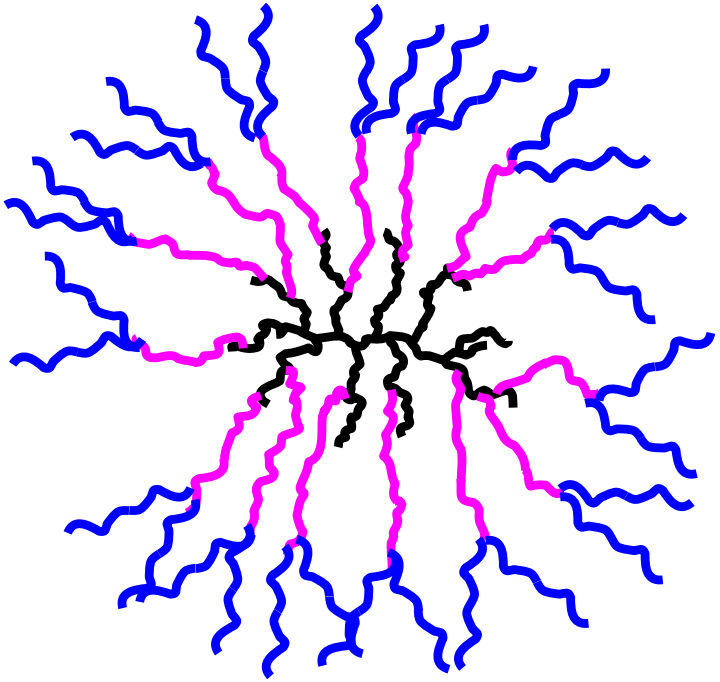


H-Glu(OtBu)-OtBu



1) TFA

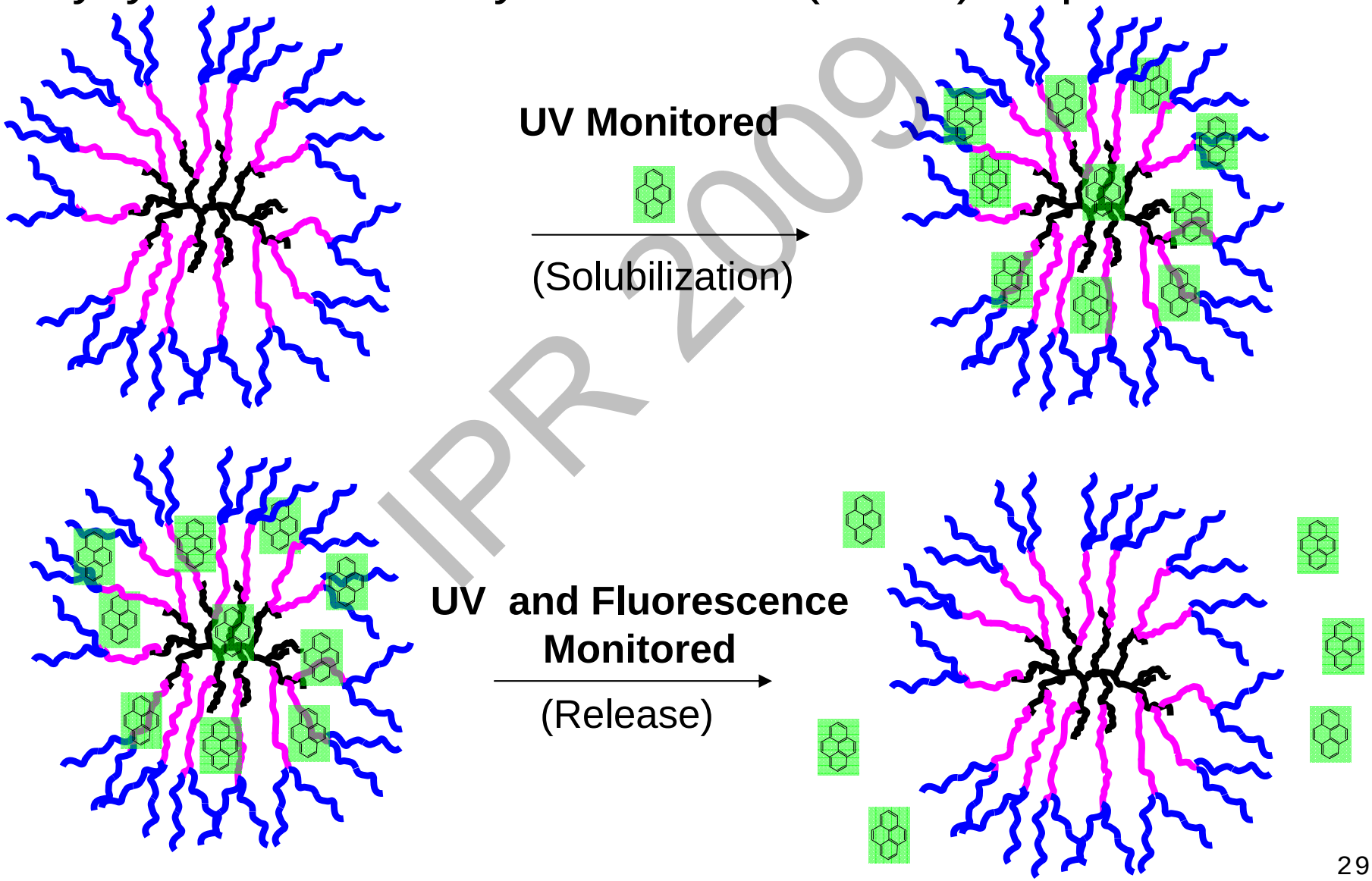
2) PEG-NH_2 or Poly(OtBu-Glu)



Unimolecular micelle

SOLUBILIZATION AND RELEASE STUDIES

- Polycyclic aromatic hydrocarbons (PAHs) as probes



ACKNOWLEDGEMENTS

- Dr. Gauthier
- Dr. Duhamel

- Members of the Gauthier and Duhamel Groups

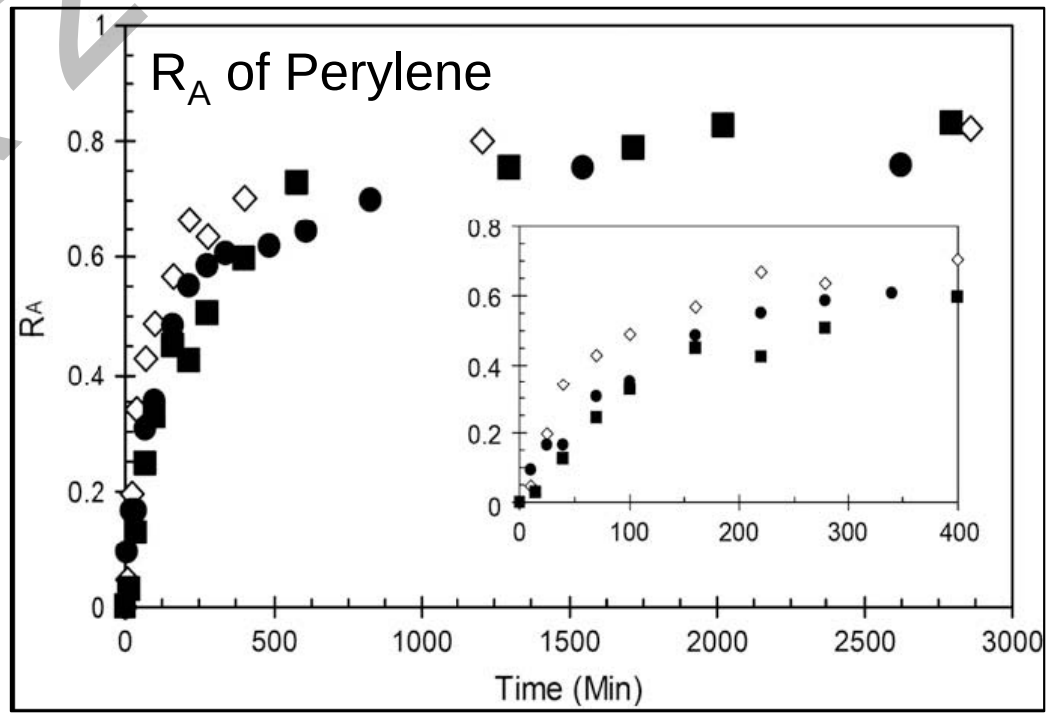
Release Kinetics

- Fluorescence Resonance Energy Transfer (FRET) experiments
  - Characterize release kinetics of hydrophobic probes from arborescent micelles

$$R_A = \frac{I_A(t) - I_A(0)}{I_A(\infty) - I_A(0)}$$

R_A = Normailized experimental fluorescence intensity ratio (time-dependant)

I_A = emission intensity at start (0), given time (t), and at equilibrium (∞)



GN Njikang et al. *Polymer*, **2008**, 49, 5474

RELEASE KINETICS

- Fluorescence quenching experiments
  - Using **Pyrene** and ionic quencher thallium nitrate (**$TlNO_3$**)

Stern-Volmer equation

$$\frac{I_0}{I} = 1 + k_q \tau_0 [Q]$$

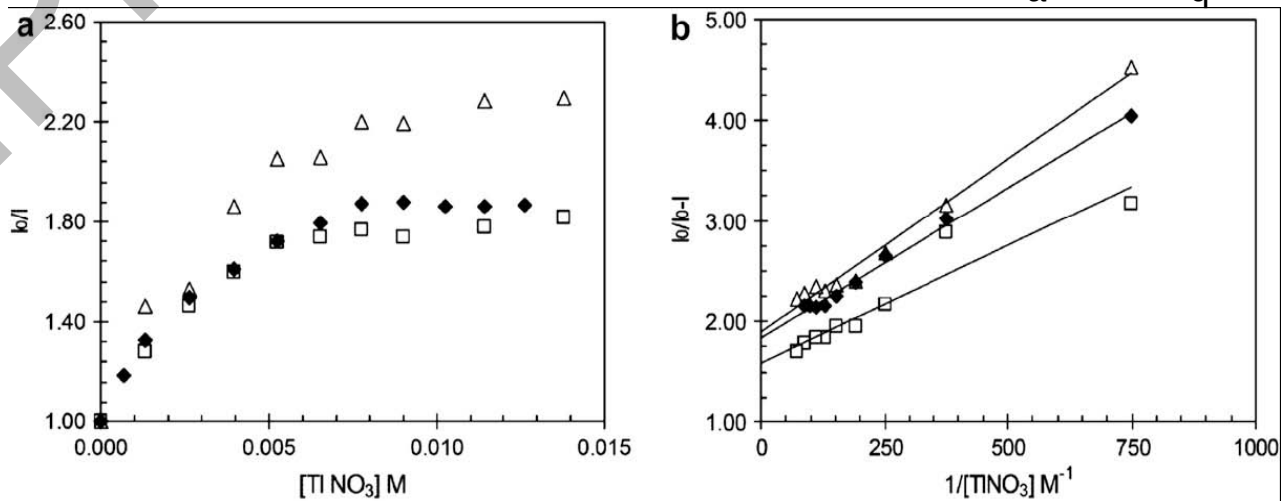
Modified Stern-Volmer equation

$$\frac{I_0}{I_0 - I} = \frac{1}{f_a} + \frac{1}{f_a k_q \tau_0 [Q]}$$

f_a = fraction of pyrene accessible to quencher

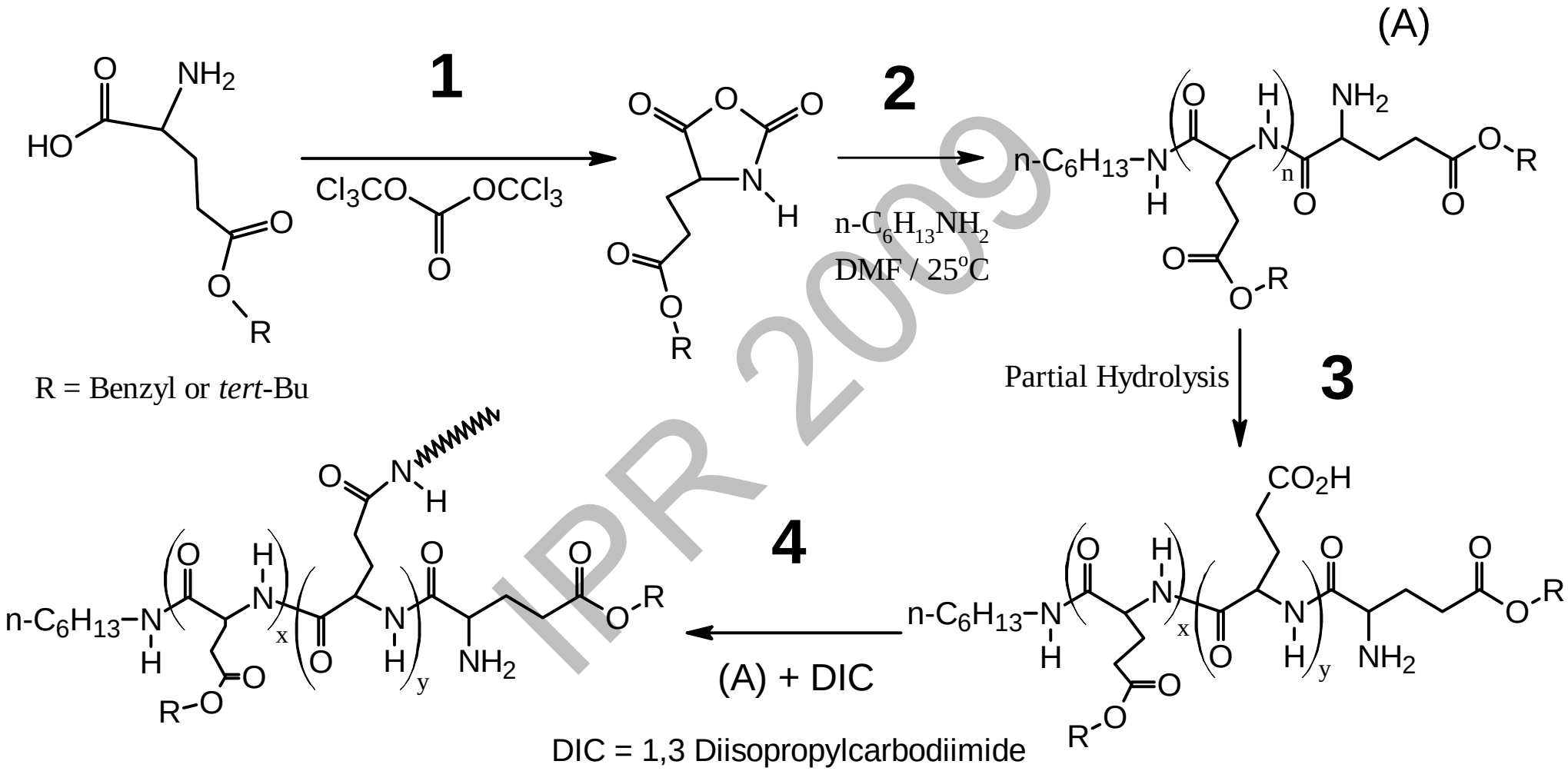
k_q = bimolecular quenching constant

estimate f_a and k_q



GN Njikang et al. *Polymer*, **2008**, 49, 5474

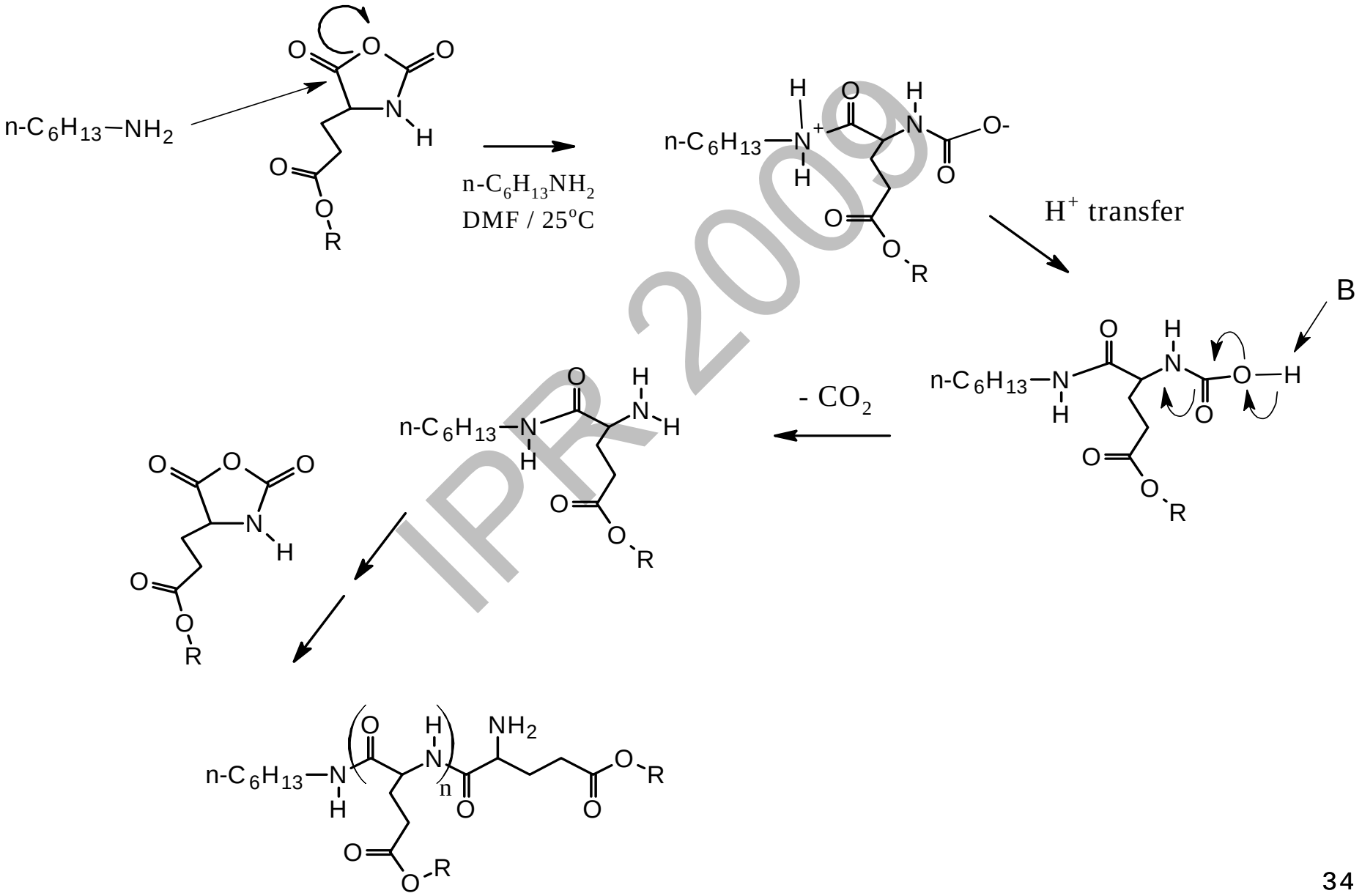
Overall Synthetic Scheme



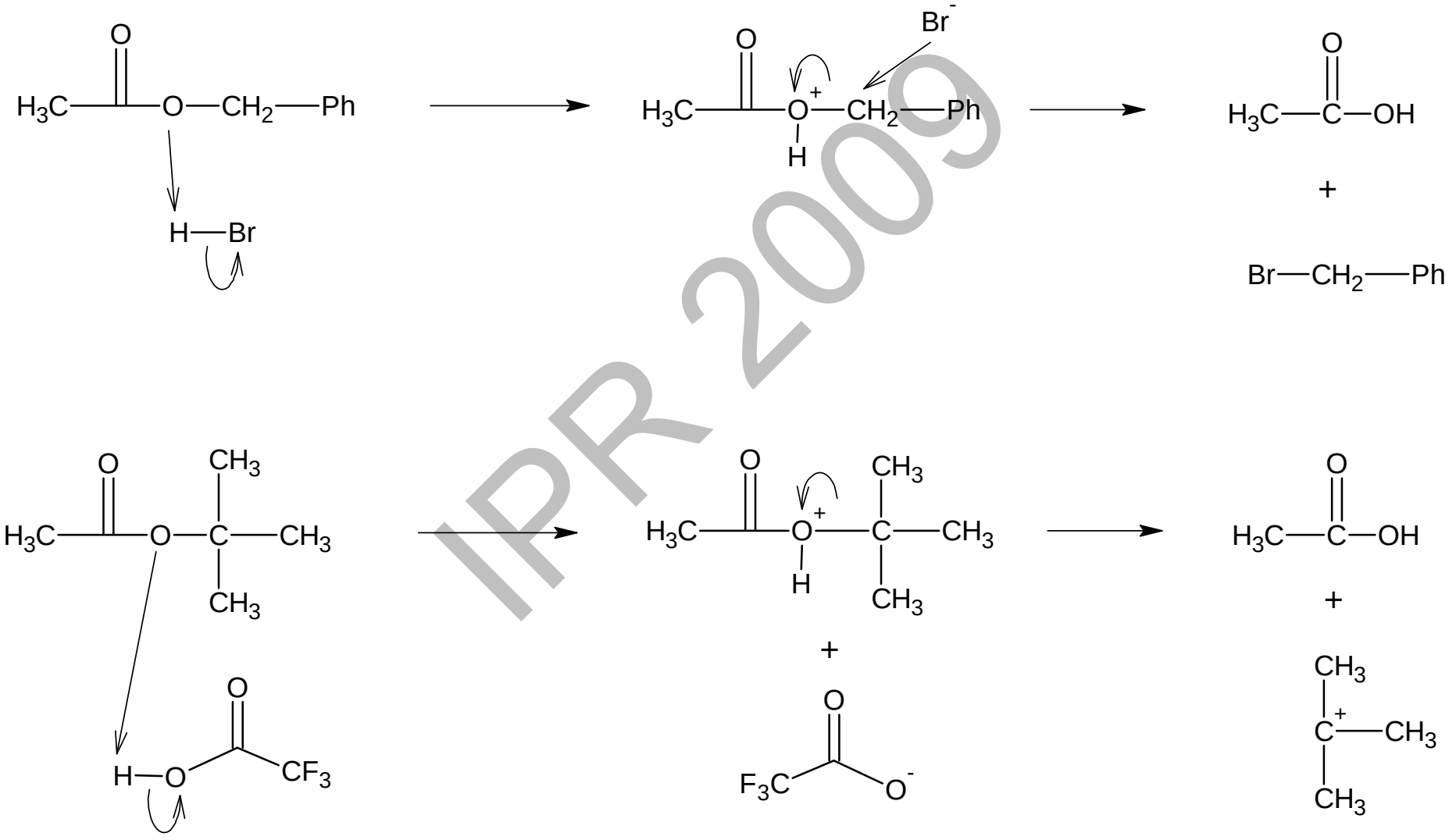
-Explain each step briefly so audience can get an idea of what is going on

-mention more details on synthesis later

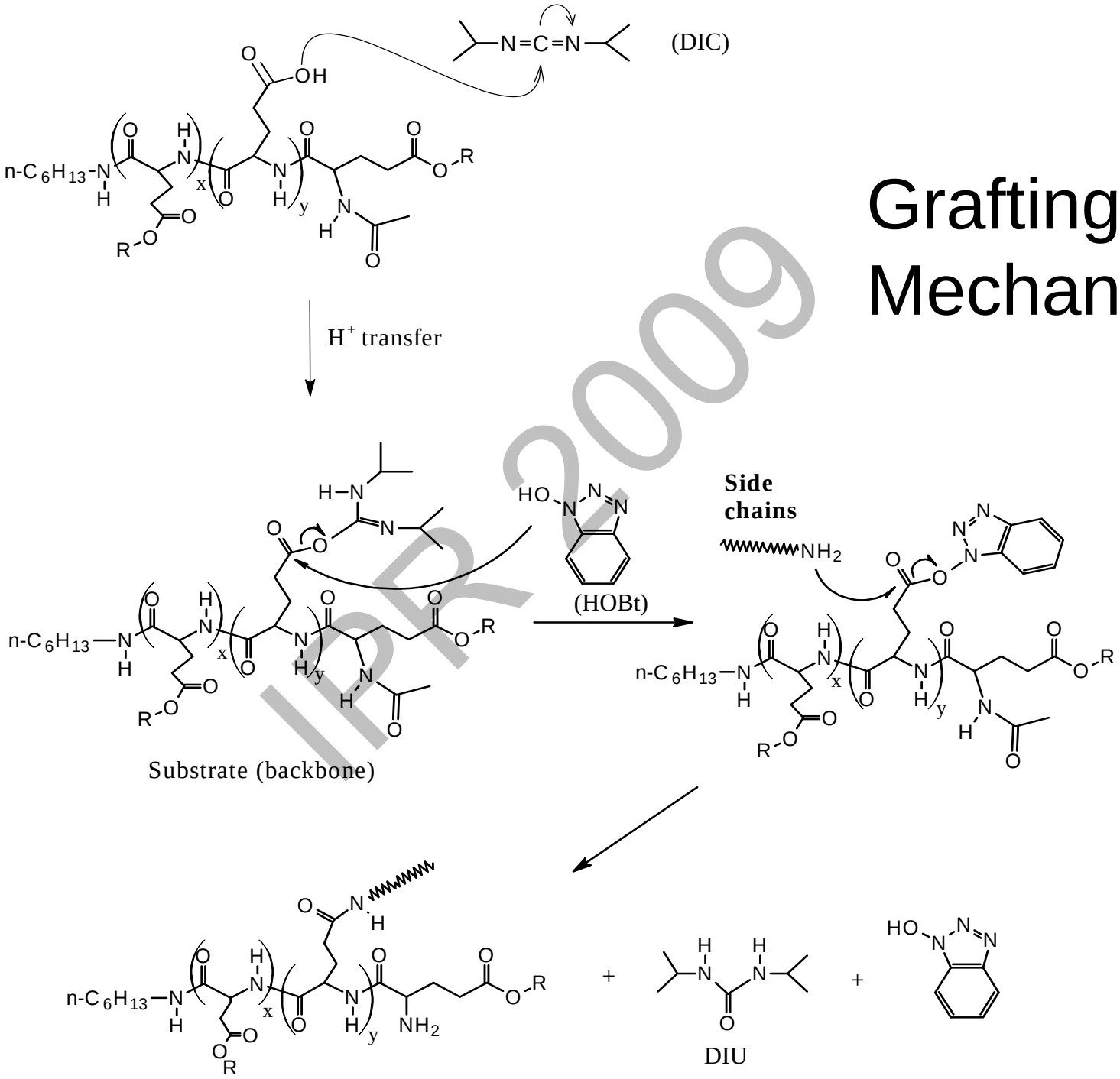
ROP MECHANISM



Deprotection Mechanism



Grafting Mechanism



Grafting Side Reaction