

Controlled synthesis of sulfated alternating glycopolymers as glycosaminoglycan mimics and their functional properties

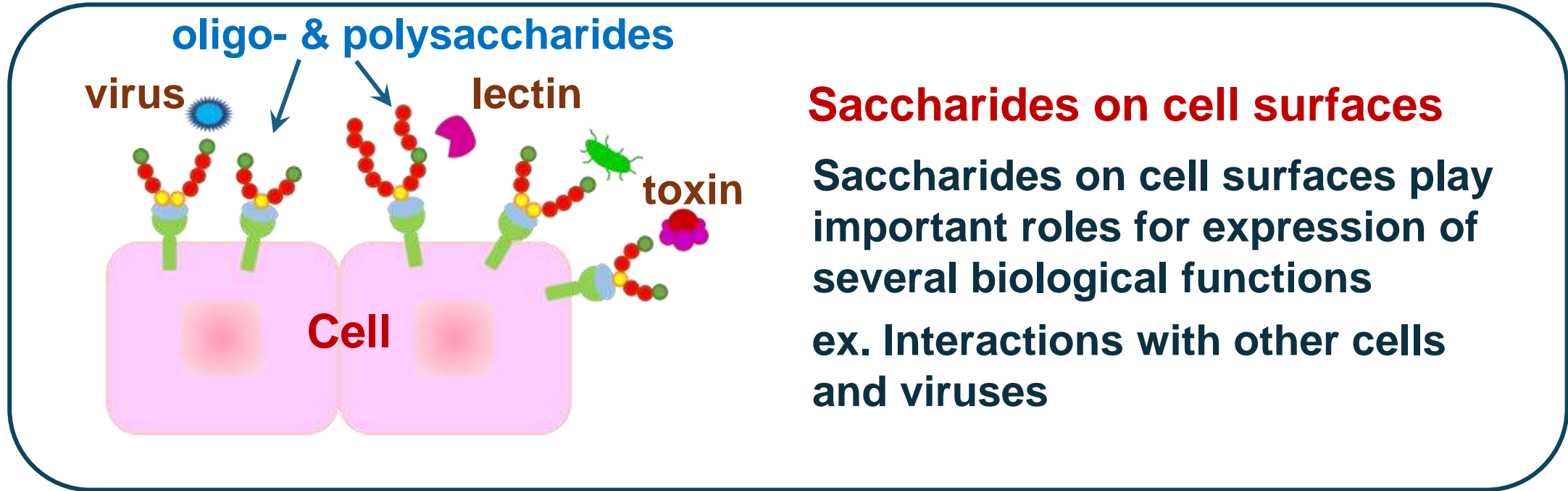


Faculty of Molecular Chemistry and Engineering,
Graduate School of Science and Technology,
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minoda@kit.ac.jp

Background: What are glycopolymers?



Saccharides on cell surfaces

Saccharides on cell surfaces play important roles for expression of several biological functions

ex. Interactions with other cells and viruses

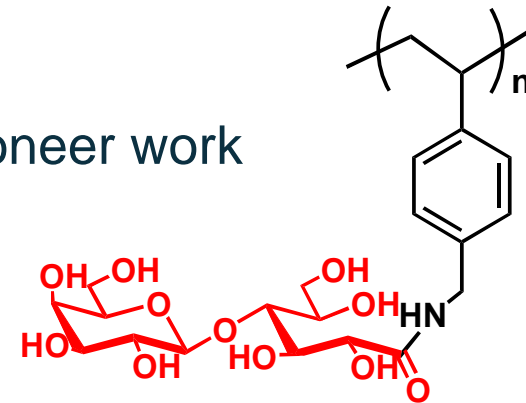
Mimics of glycoproteins and glycolipids



“Glycopolymers”

Synthetic polymers having pendant saccharide residues

Pioneer work



K. Kobayashi et al., *Polym J.* **1985**, 17, 567.

Saccharide-carrying styrene derivatives by conventional radical polymerization

Background: Structure control in glycopolymers

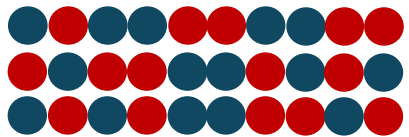
Glycopolymers so far synthesized by controlled radical polymerization



Polymer chain length (= MW)
Polymer chain length distribution (= MWD)



Incorporation of functions at the terminal



Composition ratio in random copolymers

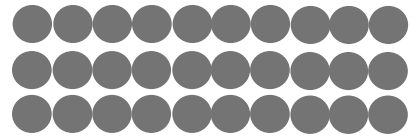


Block copolymer structure

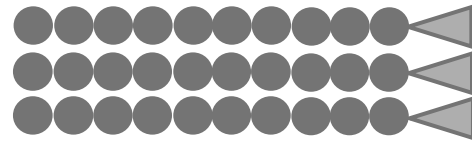


Background: Structure control in glycopolymers

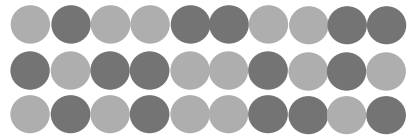
Glycopolymers **in this study** by **RAFT polymerization**



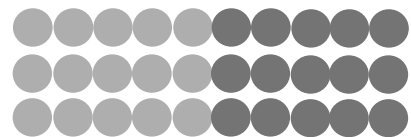
Polymer chain length (= MW)
Polymer chain length distribution (= MWD)



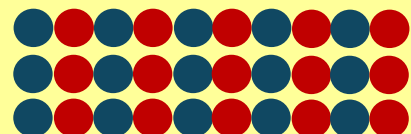
Incorporation of functions at the terminal



Composition ratio in random copolymers



Block copolymer architecture



Alternating copolymer architecture
(As sequence-controlled glycopolymers)



Background: Structure control in glycopolymers

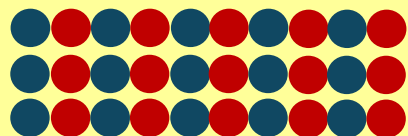
Glycopolymers in this study by **RAFT polymerization**

Polymer chain length (= MW)

RAFT polymerization

- Polymers with narrow MWD can be formed
- Metal-free process
- Tolerant to various functional groups
(preferred to saccharide-carrying monomers)

Block copolymer architecture



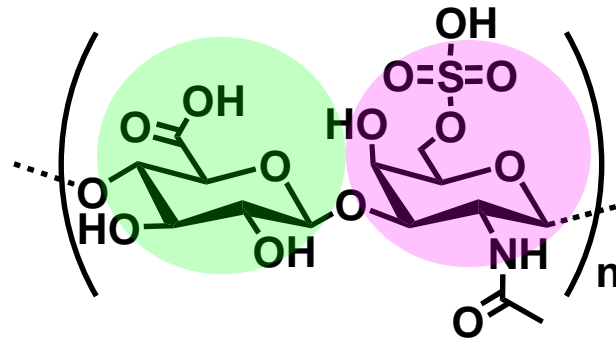
Alternating copolymer architecture
(As sequence-controlled glycopolymers)



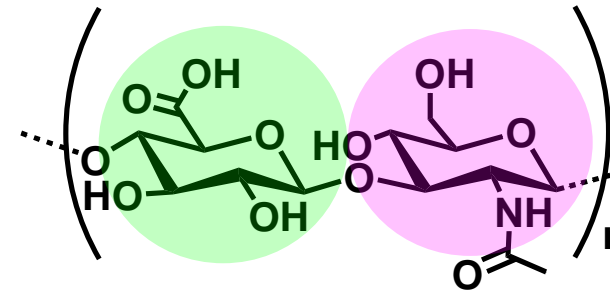
Motivation: Structure in “glycosaminoglycans”

“Glycosaminoglycans (GAGs)”

Naturally-occurring physiologically active polysaccharides



Chondroitin sulfate C (CS-C)



Hyaluronic acid (HA)

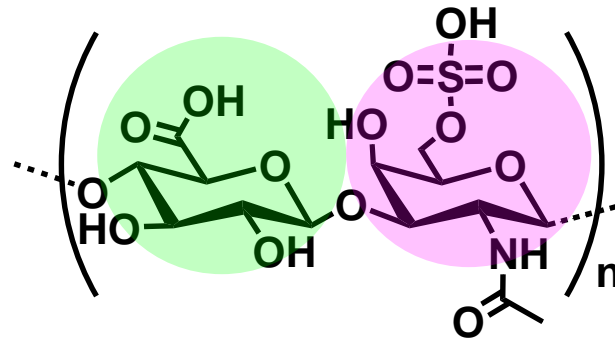
GAGs are long, linear, unbranched, and negatively-charged polysaccharides composed of repeating disaccharide units consisting of an amino sugar and an uronic acid. In addition, major GAGs are sulfated and distinguished each other by the disaccharides structures and various sulfation patterns.

Thus, GAGs are naturally occurring alternating copolymers.

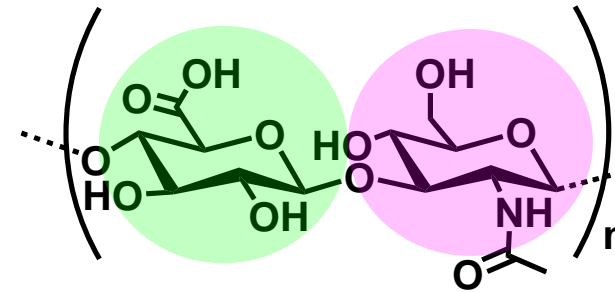
Motivation: Structure in “glycosaminoglycans”

“Glycosaminoglycans (GAGs)”

Naturally-occurring physiologically active polysaccharides

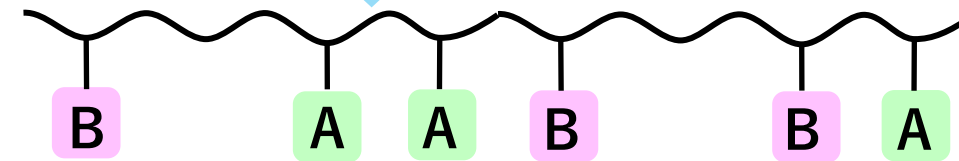
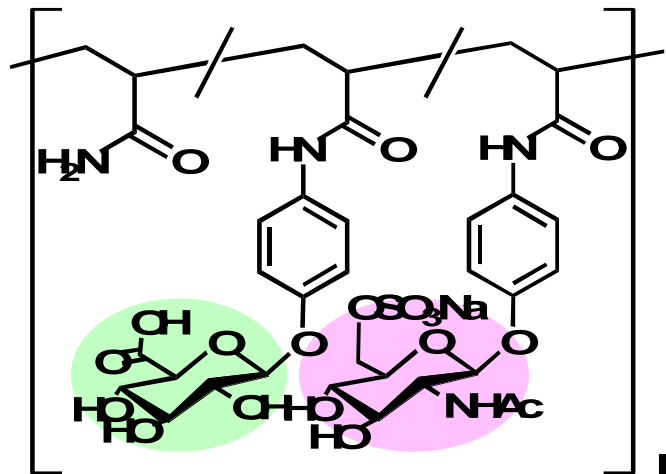


Chondroitin sulfate C (CS-C)



Hyaluronic acid (HA)

Several attempts for designing GAG mimics



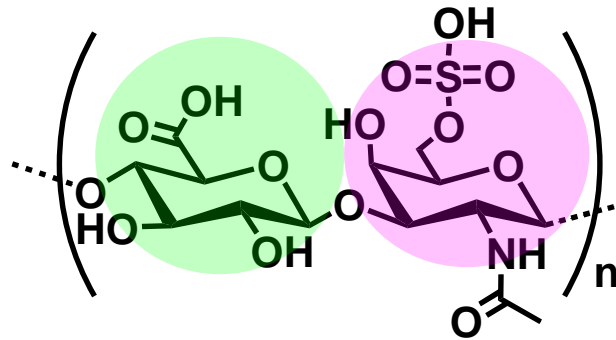
- GAG model **random** copolymers
- Inhibition of aggregation of Amyloid β which observed in Alzheimer disease
- **Sequence control are still beyond our reach**

Y. Miura et al., *Bull. Chem. Soc. Jpn.*, 2010, 83, 1004.

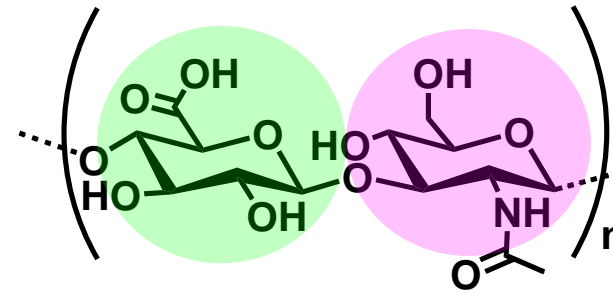
Aim: Synthesis of sulfated alternating glycopolymers

“Glycosaminoglycans (GAGs)”

Naturally-occurring physiologically active polysaccharides

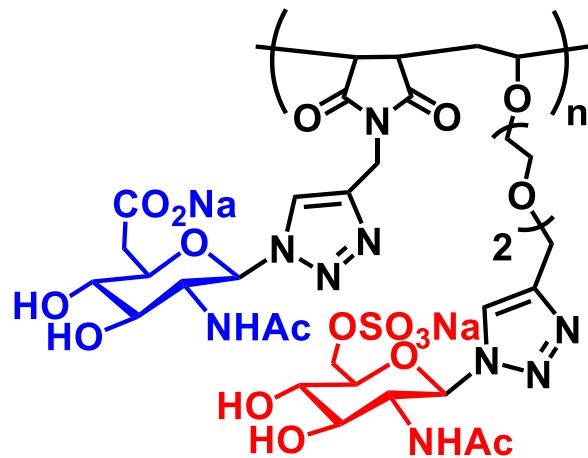


Chondroitin sulfate C (CS-C)

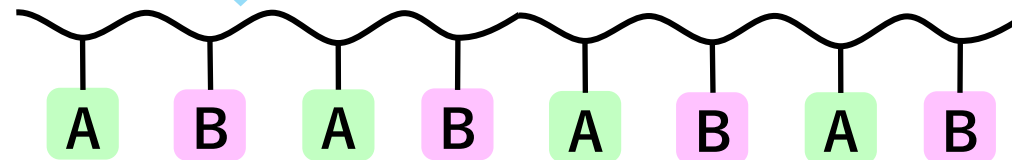


Hyaluronic acid (HA)z

This study aims to synthesize alternating glycopolymers as GAG mimics



CS-C mimic glycopolymer

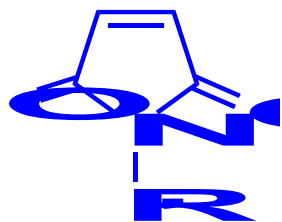


- **Sulfated alternating glycopolymers** in which two kinds of saccharide-substituted repeating units are alternatingly connected each other.

Alternating copolymers by RAFT copolymerization

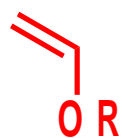
Copolymerization of electron-deficient and electron-rich vinyl monomers

Electron-Deficient
Monomers



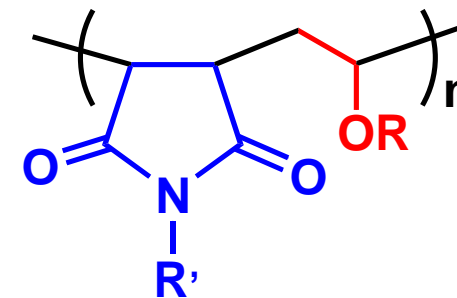
N-Substituted
Maleimide (MI)

Electron-Rich
Monomers



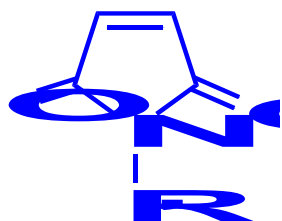
Vinyl Ether
Derivatives (VE)

RAFT
copolymerization

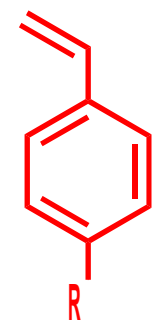


Poly(MI-*alt*-VE)

*Alternating copolymers
with controlled structure*

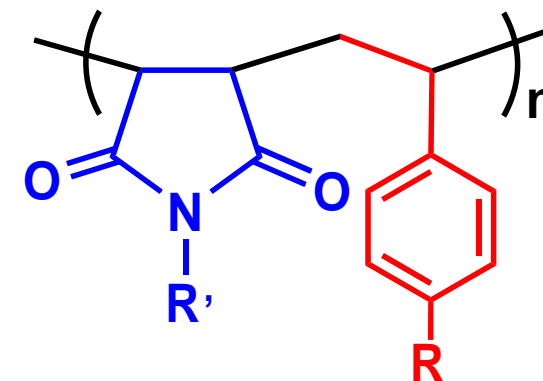


N-Substituted
Maleimide (MI)



Styrene
Derivatives (St)

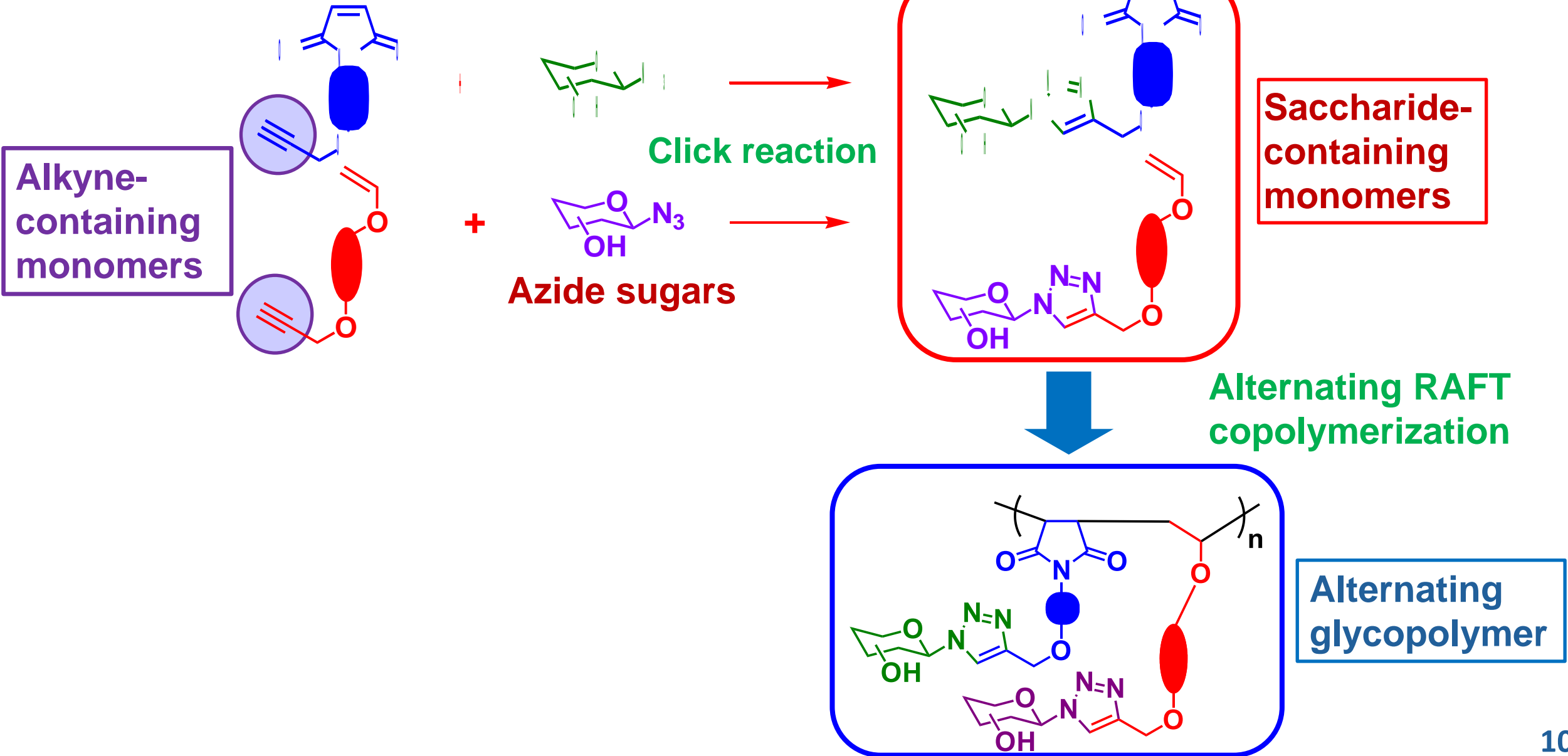
RAFT
copolymerization



Poly(MI-*alt*-St)

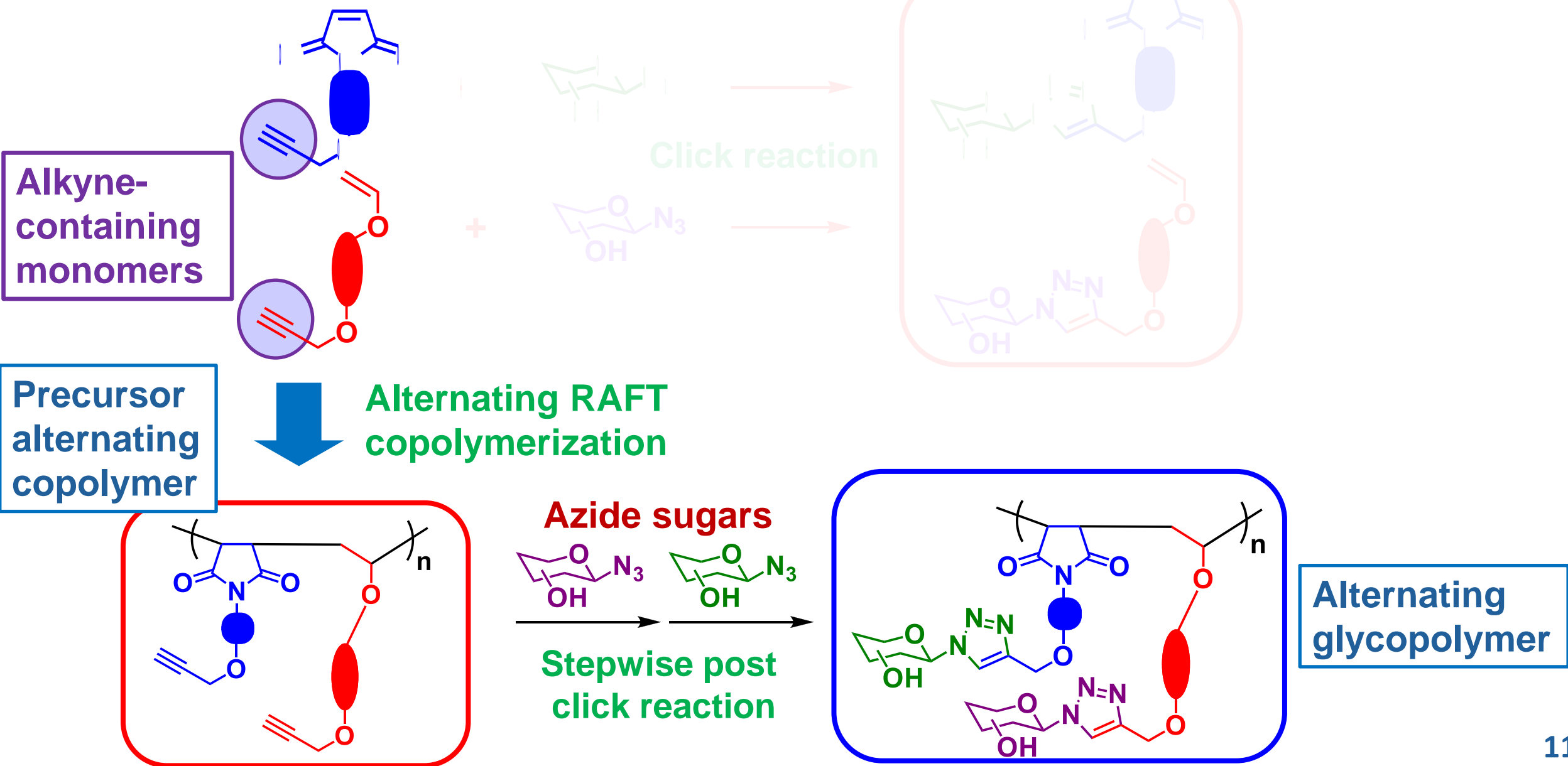
Synthetic strategy of alternating glycopolymers: Approach 1

Approach 1 (Direct synthesis)



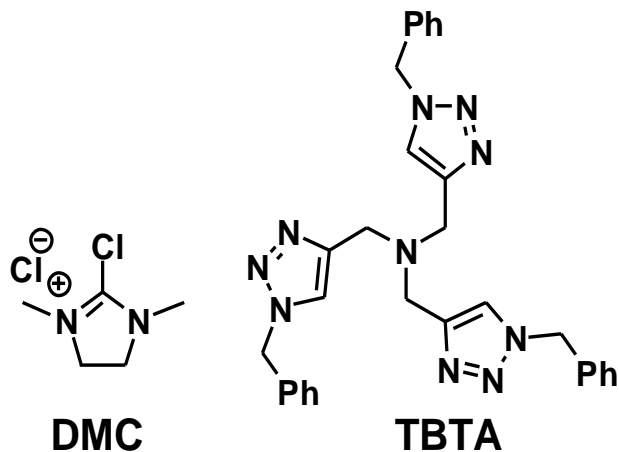
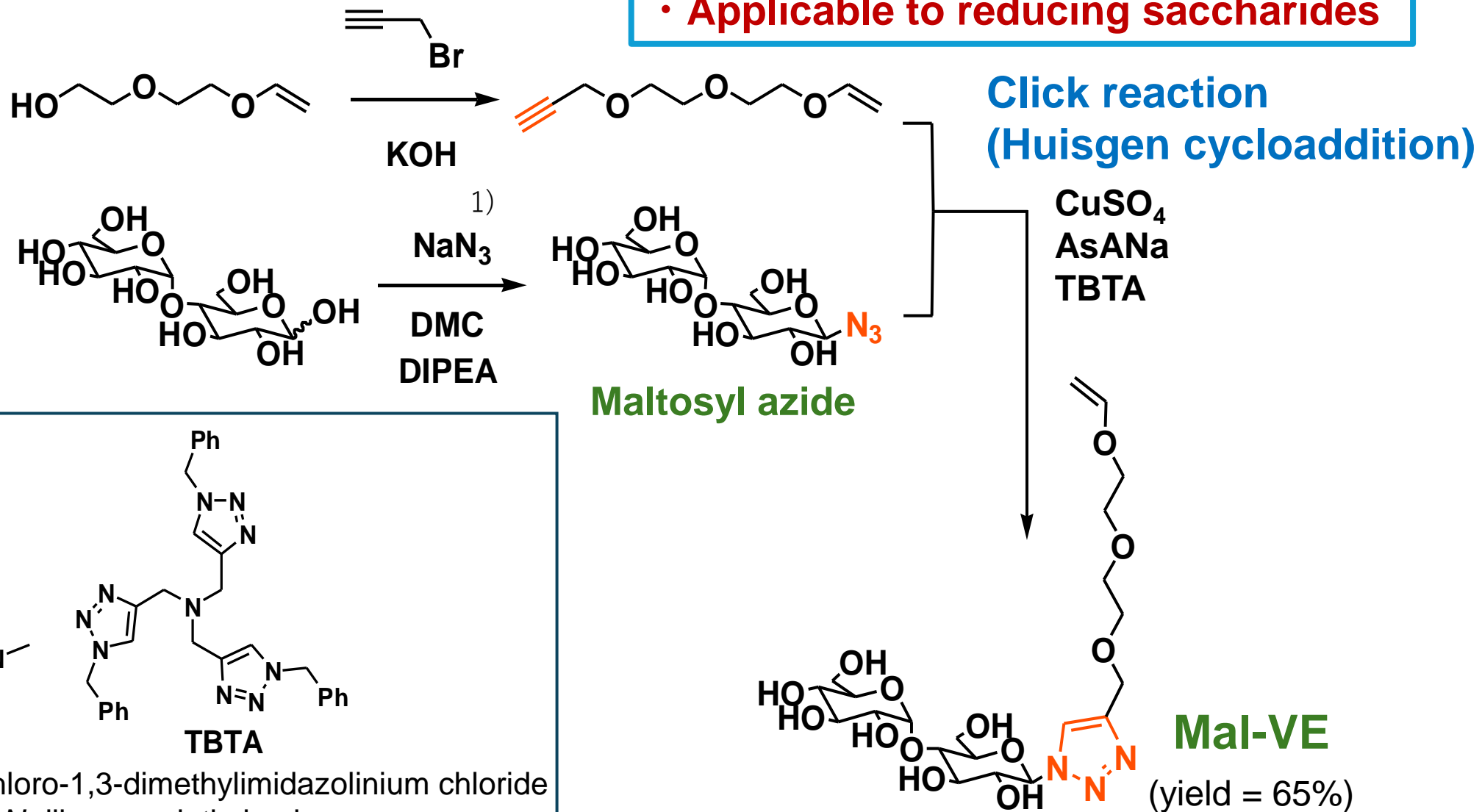
Synthetic strategy of alternating glycopolymers: Approach 2

Approach 2 (Stepwise synthesis)



Synthesis of maltose-containing VE (Mal-VE)

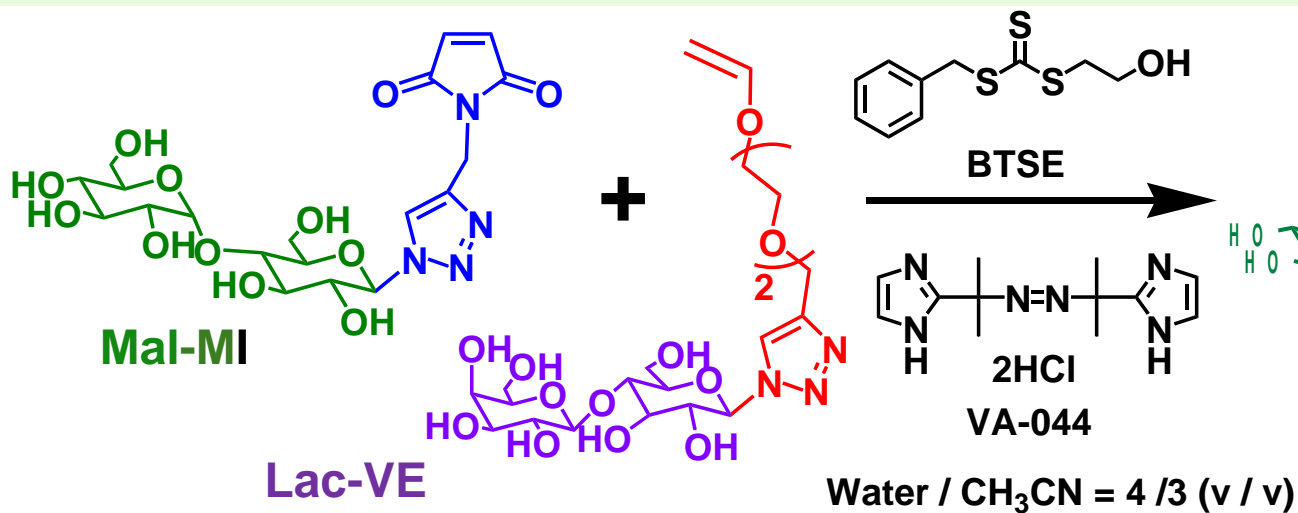
- Protecting group-free process
- Applicable to reducing saccharides



DMC; 2-chloro-1,3-dimethylimidazolium chloride
DIPEA; *N,N*-diisopropylethylamine
AsANA; Sodium ascorbate

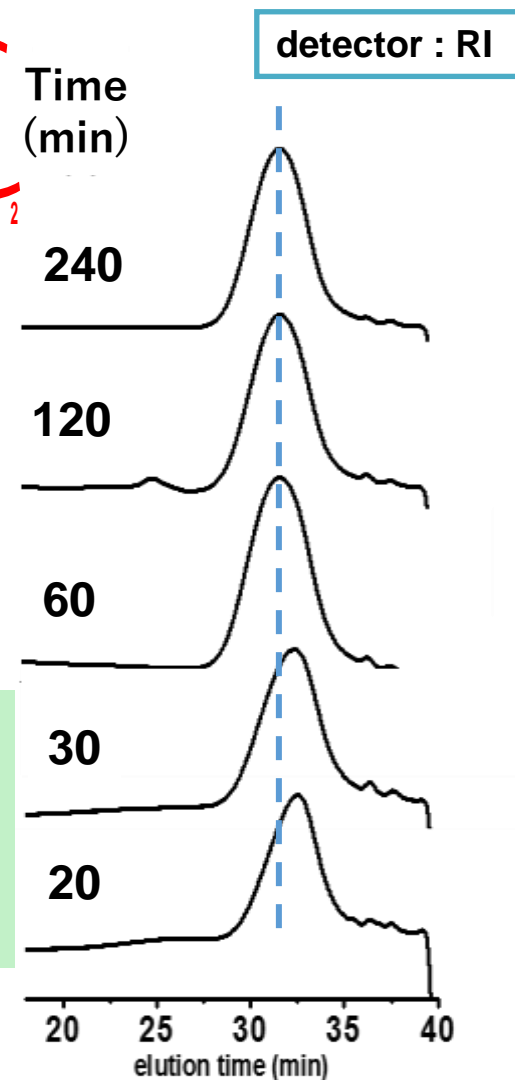
¹⁾ S. Shoda *et al.*, *Chem. Commun.*, **2009**, 3378.

Alternating RAFT copolymerization of Mal-MI and Lac-VE



$x \text{ (Lac-VE)} : y \text{ (Mal-MI)}$
 $= 36 : 64$

An excess use of
 Lac-VE for copolymer
 → **Alternating structure**
 $(x : y \approx 50 : 50)$



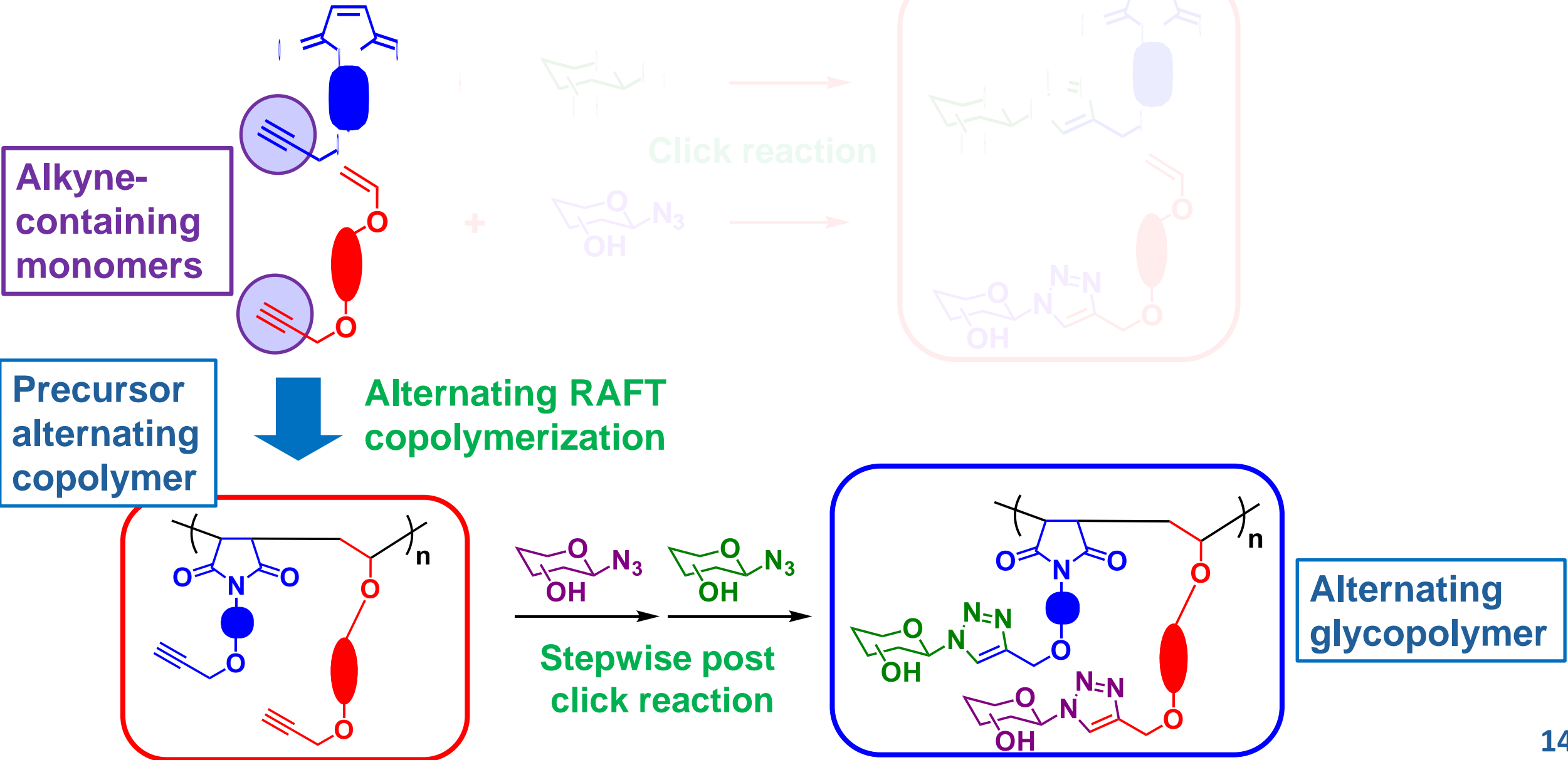
eluent: 0.20 M NaNO₃ aq.

Time (min)	M_n^a	M_w/M_n^a	Conversion (%) ^{b)}	
			LacVE	MalMI
20	4700	1.40	3	22
30	5000	1.48	18	34
60	5800	1.52	30	49
120	5900	1.50	61	74
240	6000	1.47	67	81

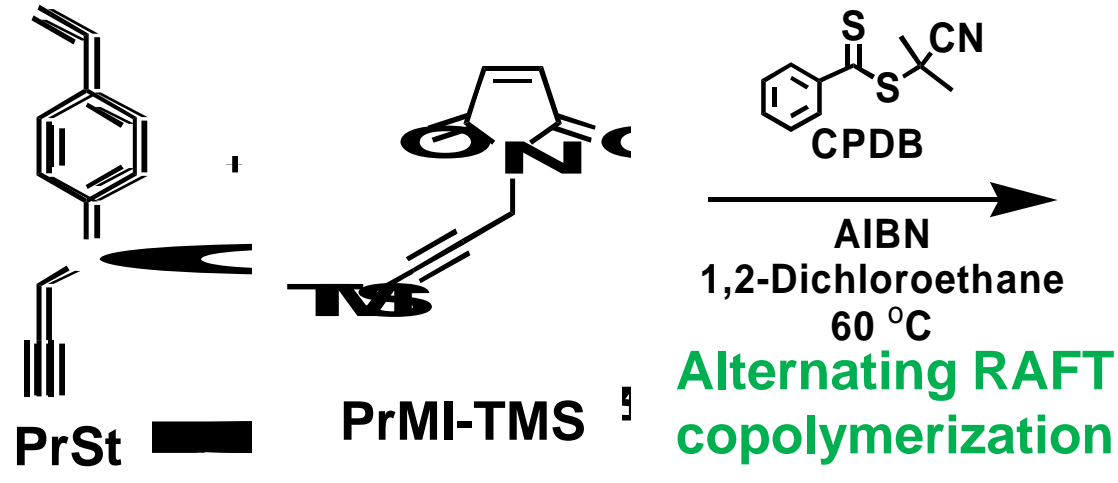
a) Estimated by PEG-calibrated GPC b) Determined by ¹H NMR.

Synthetic strategy of alternating glycopolymers: Approach 2

Approach 2 (Stepwise synthesis)



Precursor alternating copolymers having two-types of alkynes



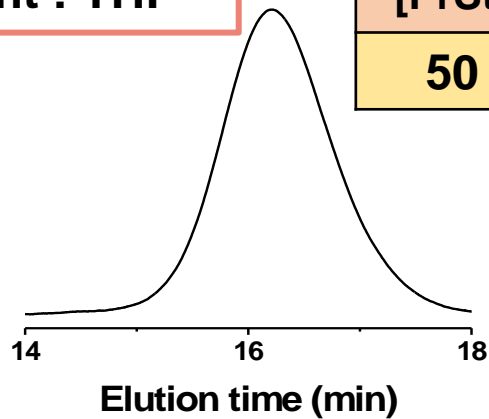
$[PrSt]_0 : [PrMI-TMS]_0 : [AIBN]_0 : [CPDB]_0 = 25 : 25 : 0.1 : 1$
 $[PrSt]_0 + [PrMI-TMS]_0 = 10 \text{ wt\%}$

Poly(PrSt-*alt*-PrMI-TMS)

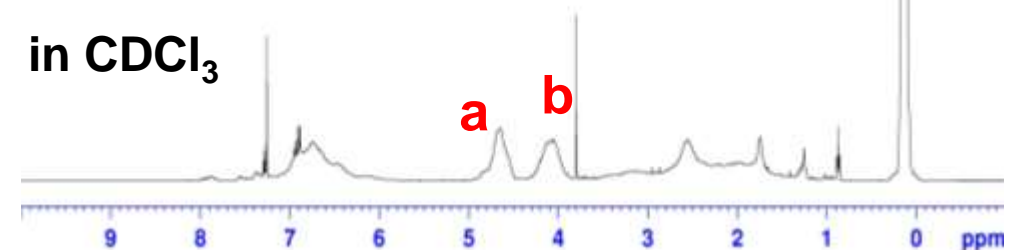
Detector : RI
 Eluent : THF

Composition (%) ^{a)}		M_n ^{b)}	M_w/M_n ^{b)}
[PrSt]	[PrMI-TMS]		
50	50	7000	1.19

a) Determined by 1H NMR.
 b) Estimated by PSt-calibrated GPC

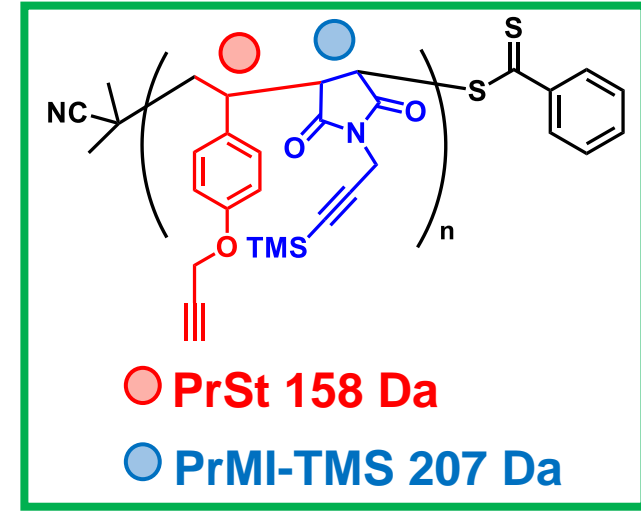
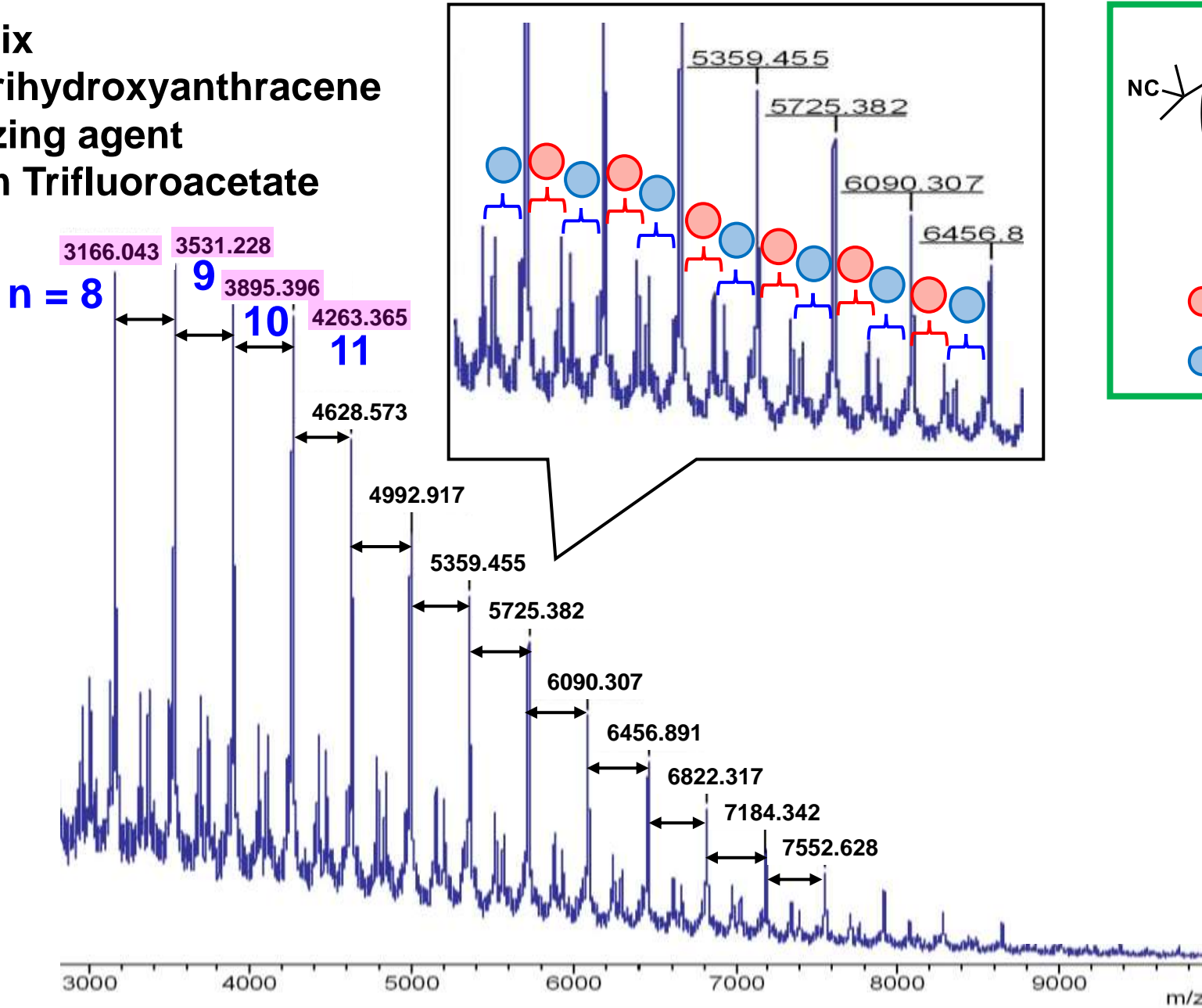


in $CDCl_3$



MALDI-TOF MS of the alternating polymer having two-types of alkynes

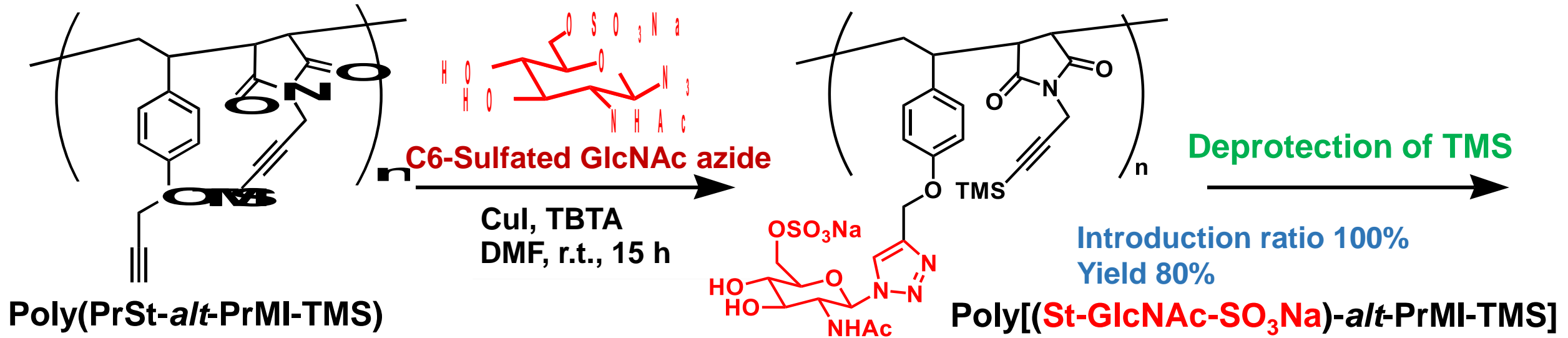
- ◆ Matrix
1,8,9-Trihydroxyanthracene
- ◆ Ionizing agent
Sodium Trifluoroacetate



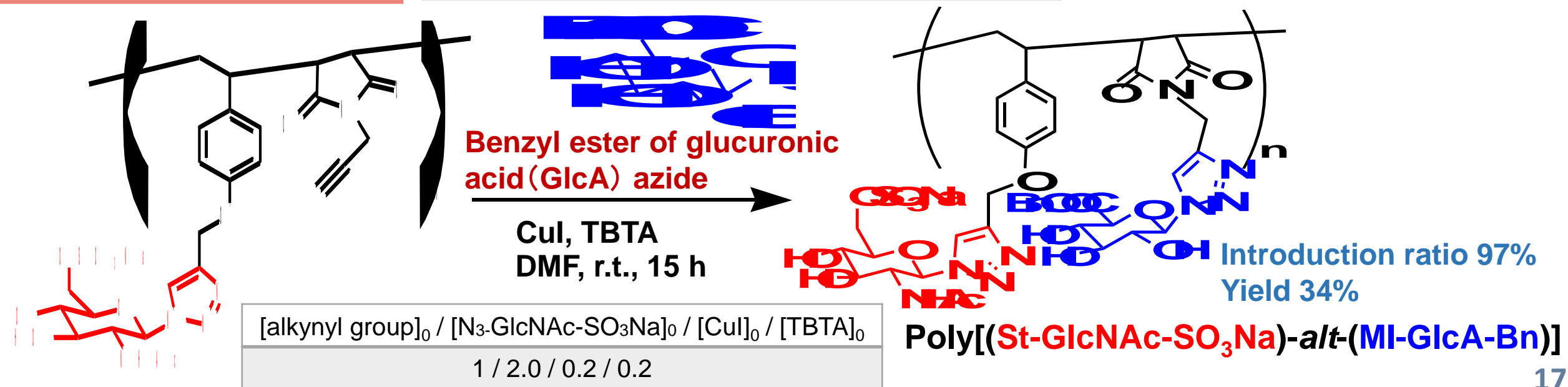
Theoretical molecular weight
 $= (158 + 207) \times n$
 $+ \text{CPDB (221)} + 23 (\text{Na}^+)$

n	Theoretical molecular weight (g/mol)
8	3168
9	3533
10	3899
11	4264

Sequential post click reactions using different carbohydrate-azides

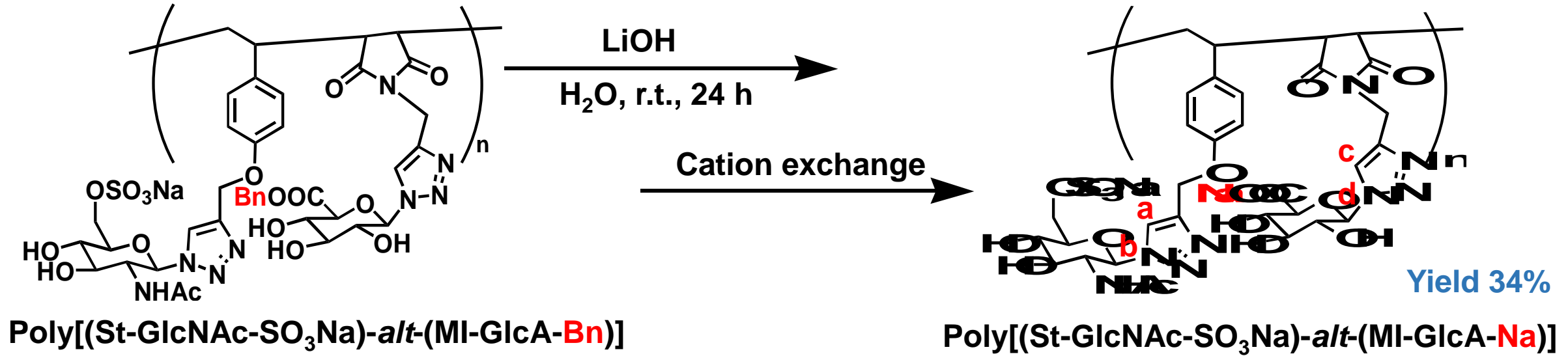


[alkynyl group] ₀ / [N ₃ -GlcNAc-SO ₃ Na] ₀ / [CuI] ₀ / [TBTA] ₀
1 / 1.5 / 0.15 / 0.15

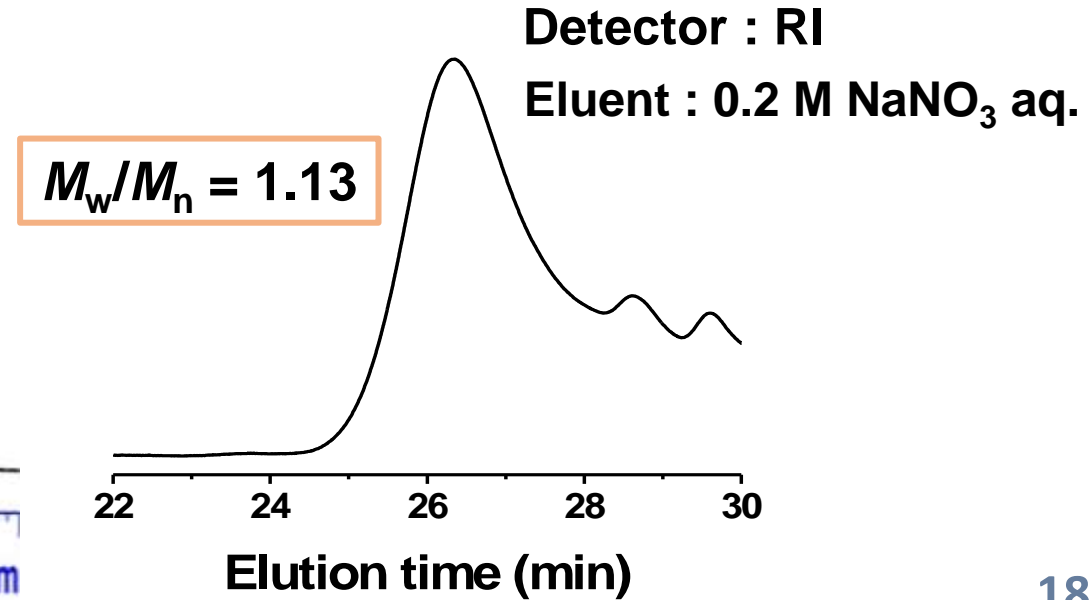
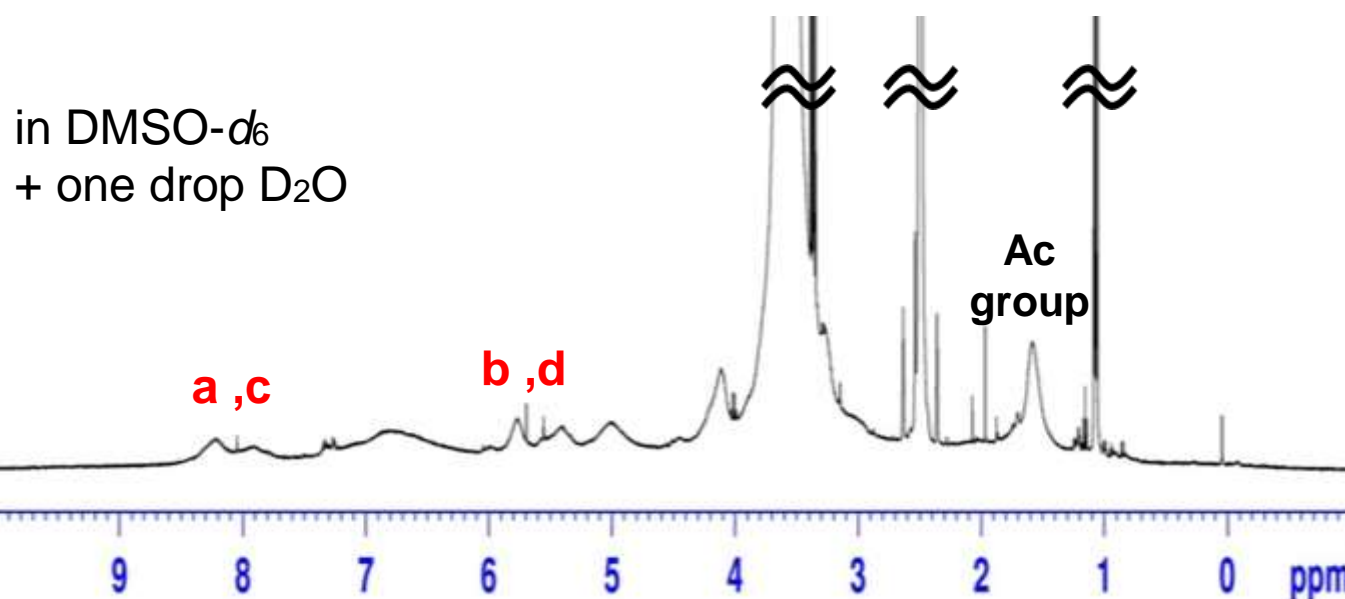


[alkynyl group] ₀ / [N ₃ -GlcNAc-SO ₃ Na] ₀ / [CuI] ₀ / [TBTA] ₀
1 / 2.0 / 0.2 / 0.2

Deprotection of benzyl ester to the target CS-C mimic glycopolymer



CS-C mimicking alternating glycopolymer



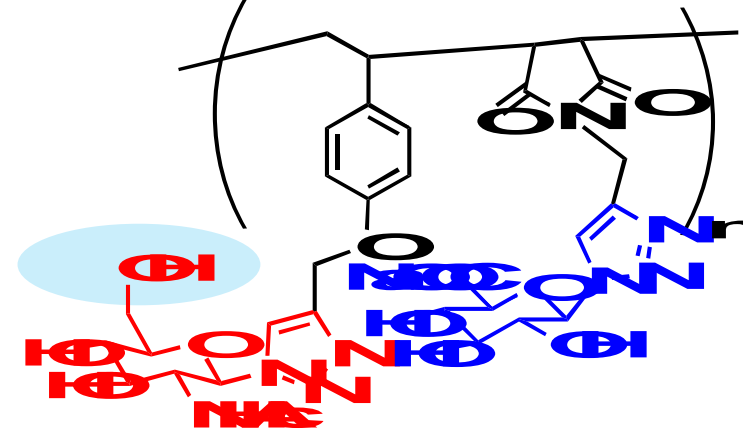
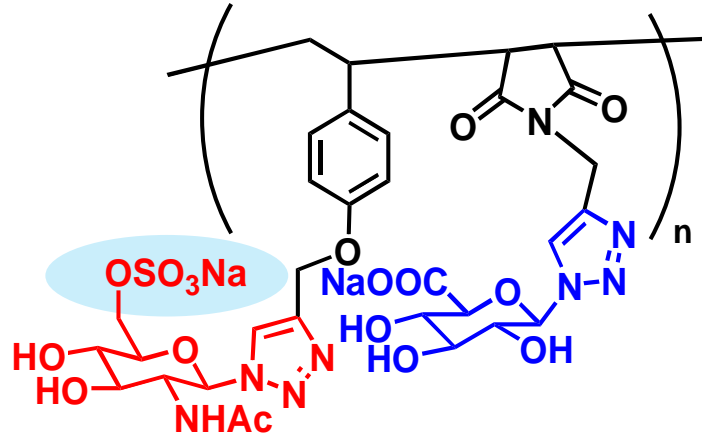
In vitro Cytotoxicity

Conditions

Cell: NIH3T3 cell (Mouse Embryonic Fibroblast); Cell density: 1.5×10^3 cells/well
WST-8 (Cell Counting Kit, Donjindo, Japan) was used to assess the cell.

Polymer concentrations: 1.0, 0.50, 0.10, 0.050, 0.010 mg/mL

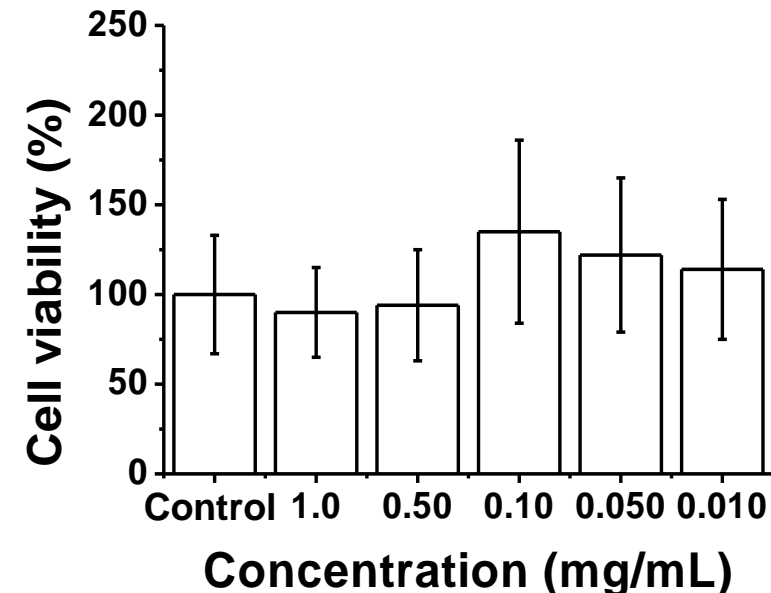
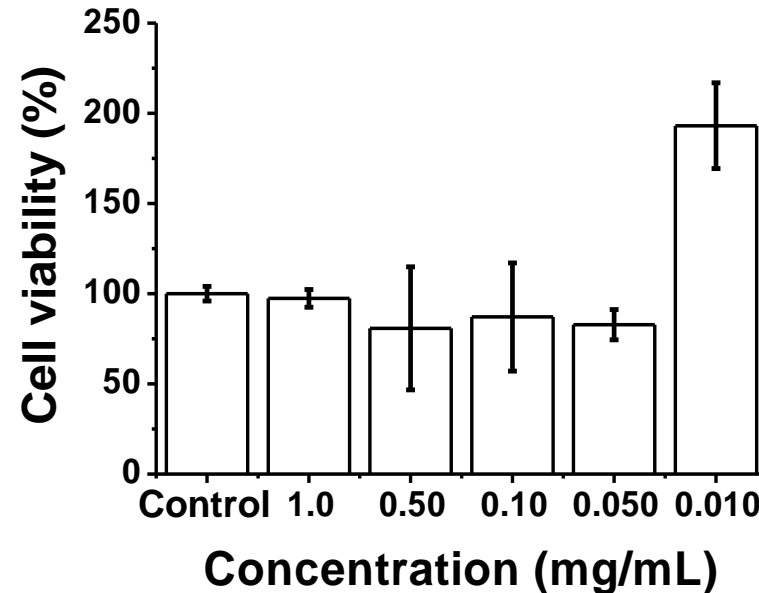
Samples



St-based Chondroitin sulfate C (CS-C) mimic

St-based Hyaluronic acid (HA) mimic

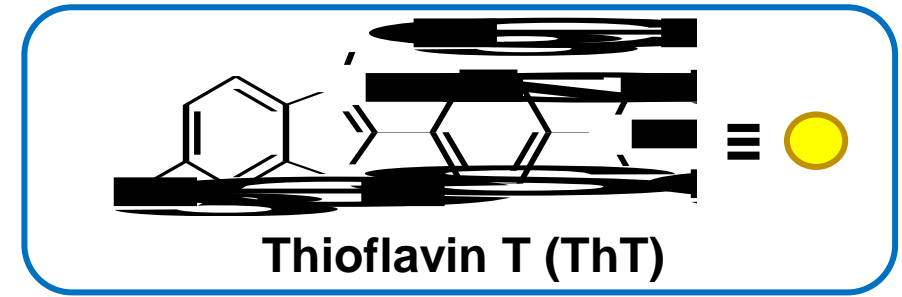
Both polymers may potentially cause no cytotoxicity for the cells (conc. < 1 mg mL⁻¹).



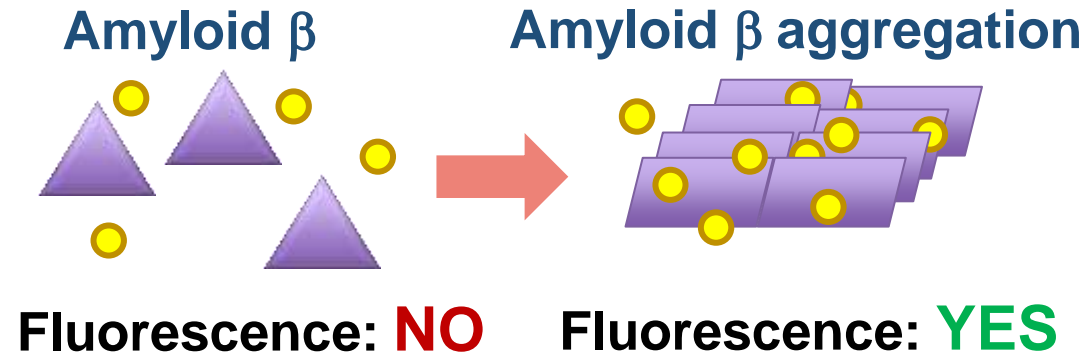
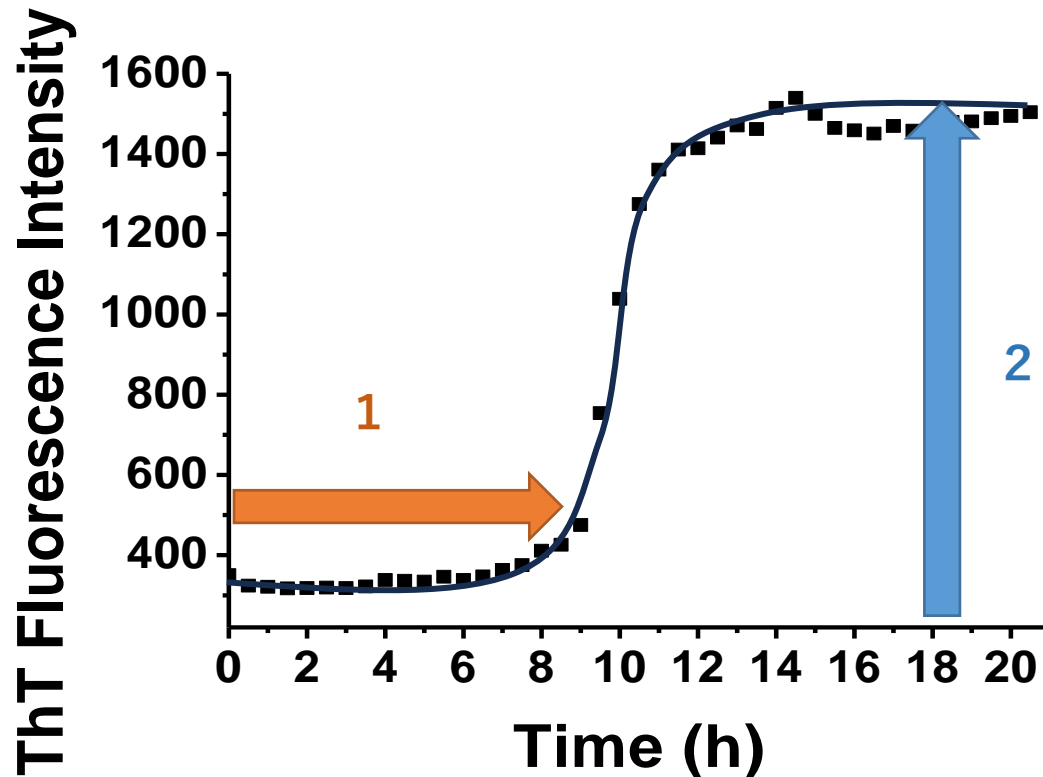
Inhibitory activity of Amyloid β aggregation by ThT fluorescence assay

Method: Thioflavin (ThT) fluorescence

Polymer concs: 1.0, 0.10, 0.010, 0.0010 mg/mL
(St-based CS-C mimic glycopolymer)
1.0, 0.10 mg/mL
(St-based HA mimic glycopolymer)



ThT fluorescence intensity change by A β aggregation



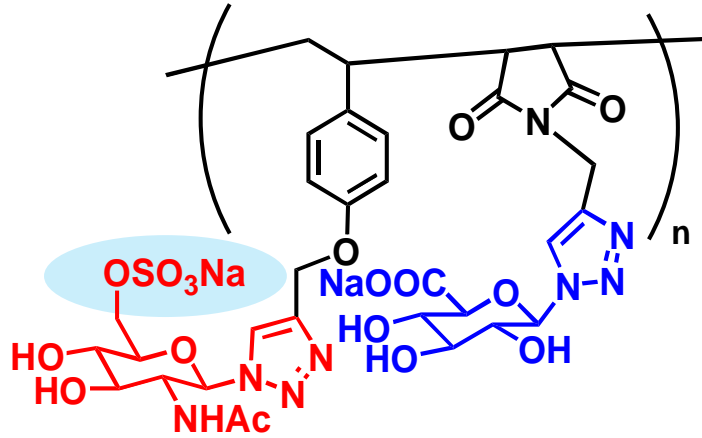
1. The lag time

The longer the lag time,
the more the aggregation is delayed.

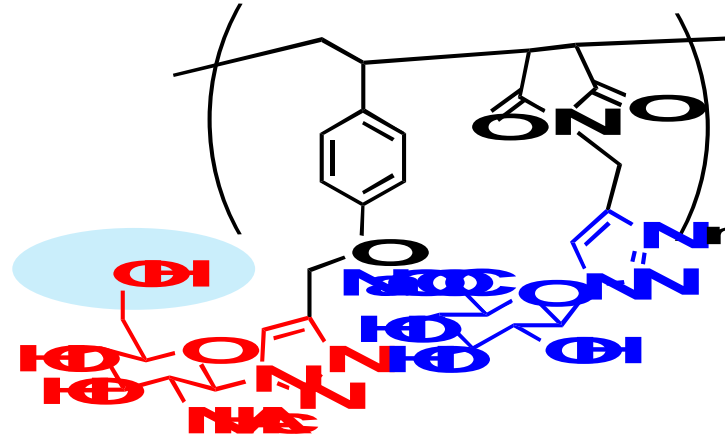
2. Fluorescence intensity at saturation

The lower the fluorescence intensity,
the greater the inhibitory activity.

Inhibitory activity of Amyloid β aggregation by ThT fluorescence assay



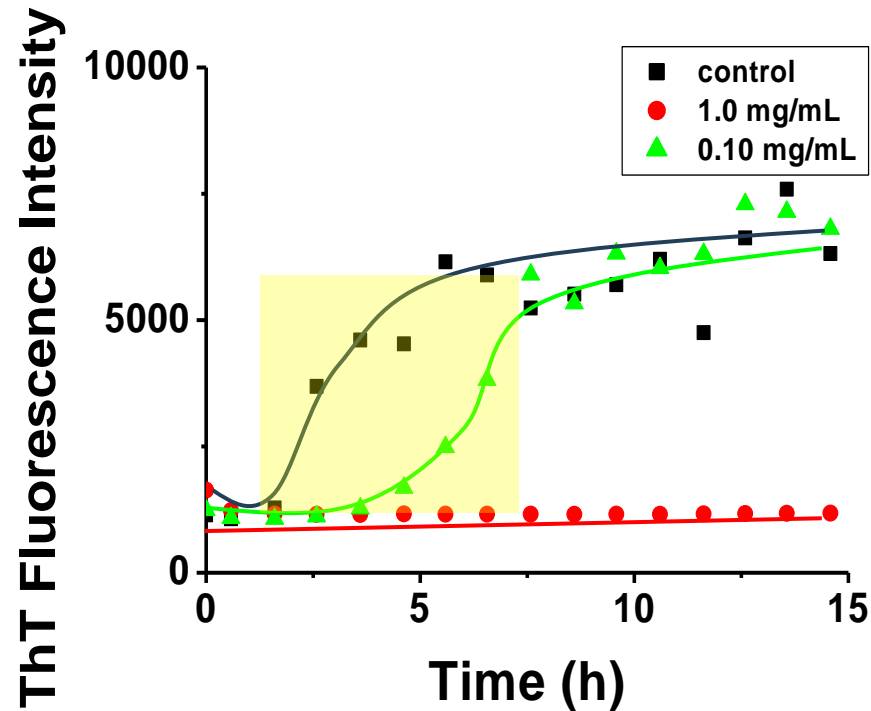
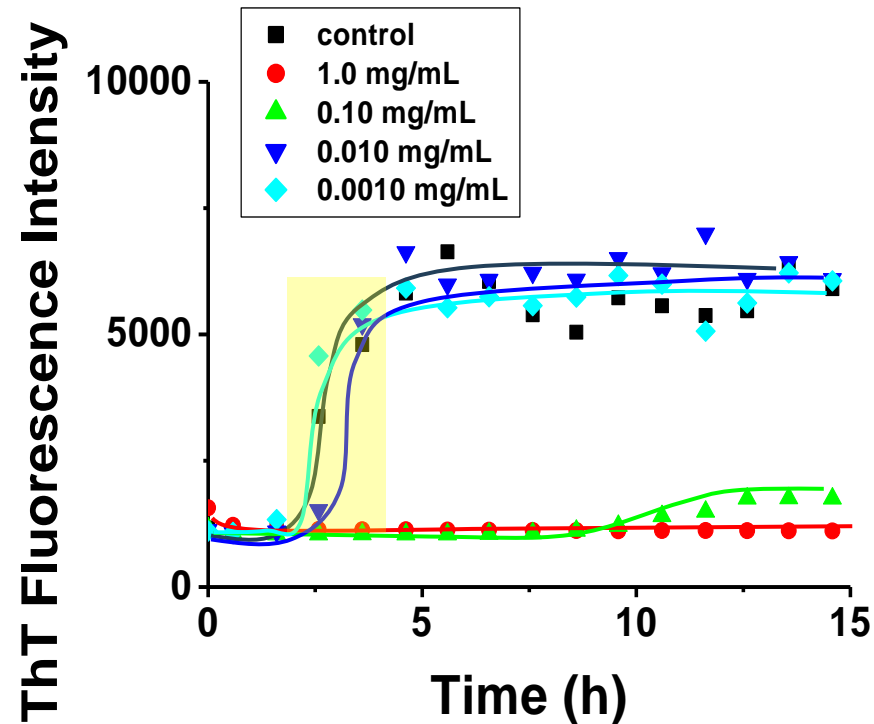
St-based CS-C mimic



St-based HA mimic

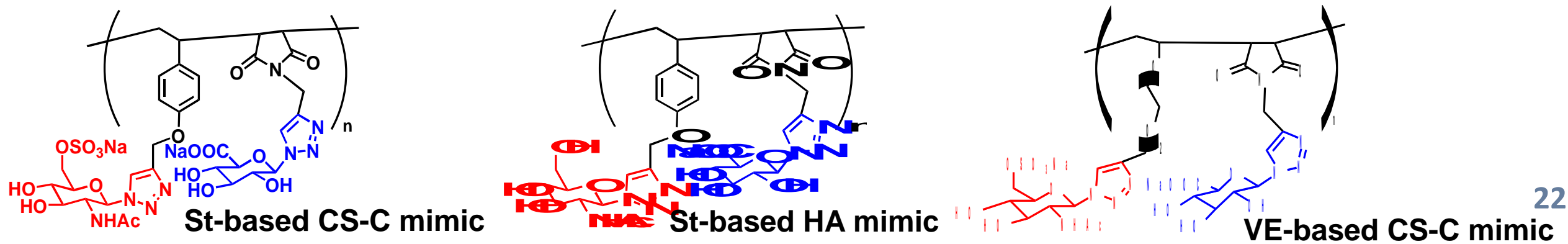
CS-C mimic alternating glycopolymer exhibited inhibitory activity of Amyloid β aggregation at concentrations of 0.1 mg/mL or higher.

At a concentration of 0.1 mg/mL, CS-C mimic clearly showed higher inhibitory activity of Amyloid β aggregation compared to HA mimic.

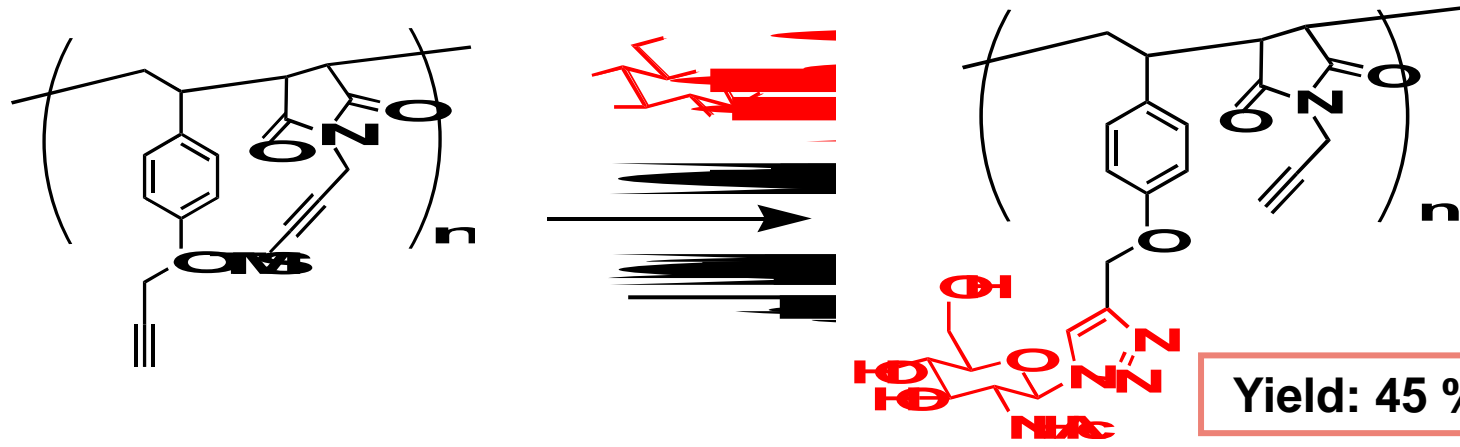


Conclusions

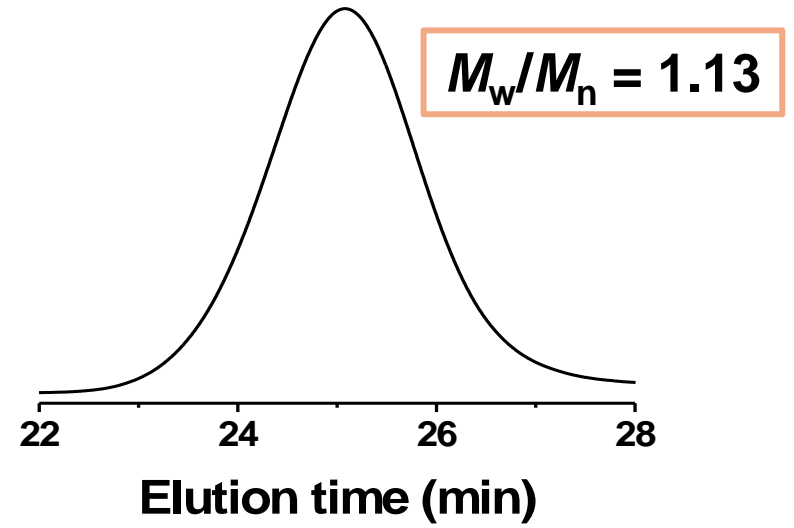
- Two strategies for the controlled synthesis of alternating glycopolymers were developed.
- RAFT alternating copolymerization of vinyl ether or styrene derivatives and maleimides lead to the successful synthesis of alternating glycopolymers.
- RAFT alternating copolymerization of a couple of alkyne-containing vinyl monomers afforded the precursor alternating copolymers with definite structure. Then, sequential post click reactions employing corresponding carbohydrate azides lead to the successful synthesis of vinyl ether- or styrene-based Chondroitin C (CS-C)- and Hyaluronic acid (HA)-mimicking alternating glycopolymers, respectively.
- The obtained periodic glycopolymers caused no cytotoxicity for the cells.
- CS-C mimicking alternating glycopolymer exhibited amyloid aggregation inhibitory activity at concentrations of 0.1 mg/mL or higher. At a concentration of 0.1 mg/mL, CS-C mimic clearly showed higher amyloid aggregation inhibitory activity compared to HA mimic counterpart.



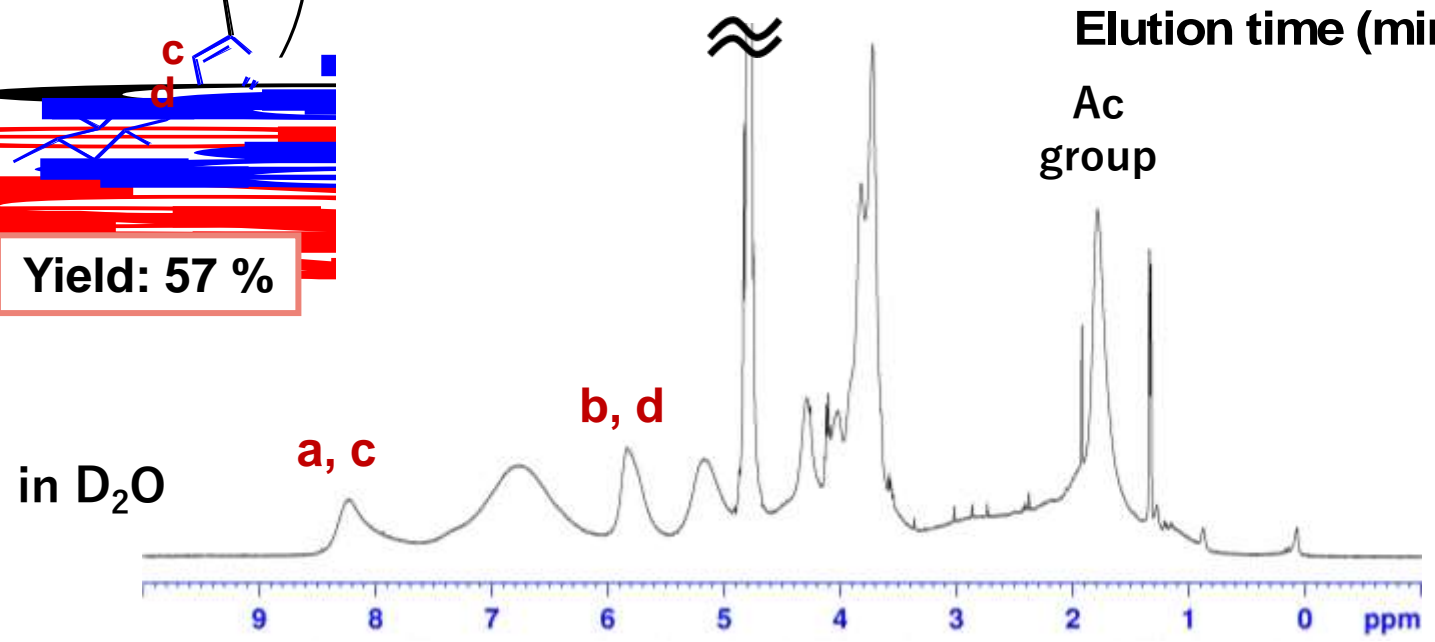
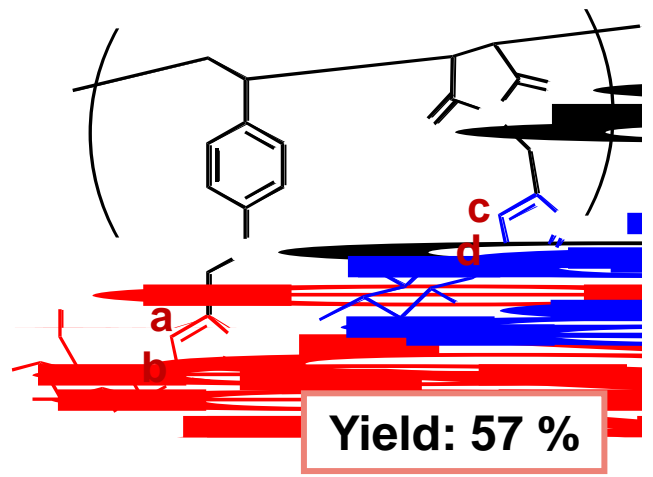
Synthesis of St-based HA mimicking glycopolymer



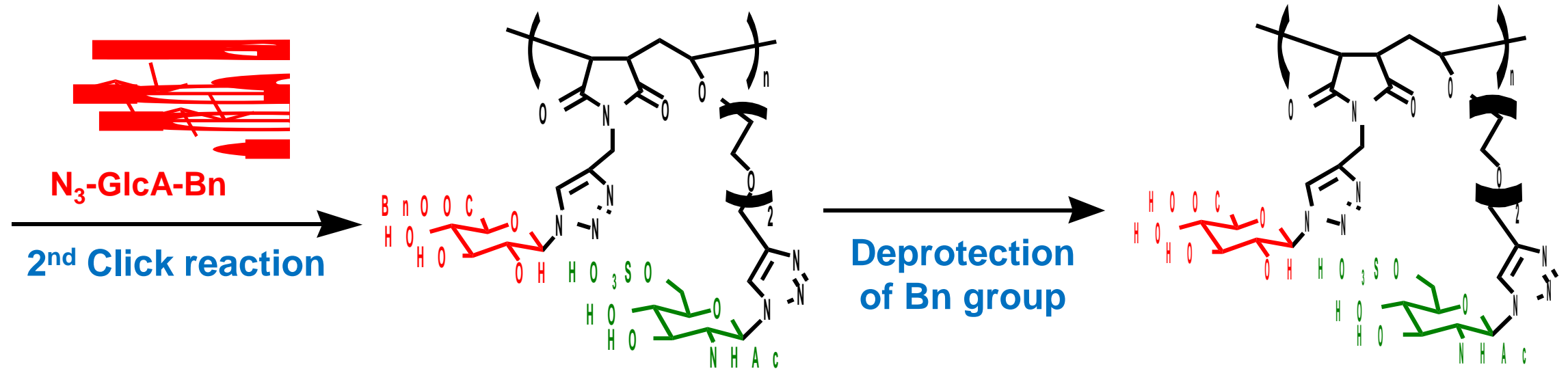
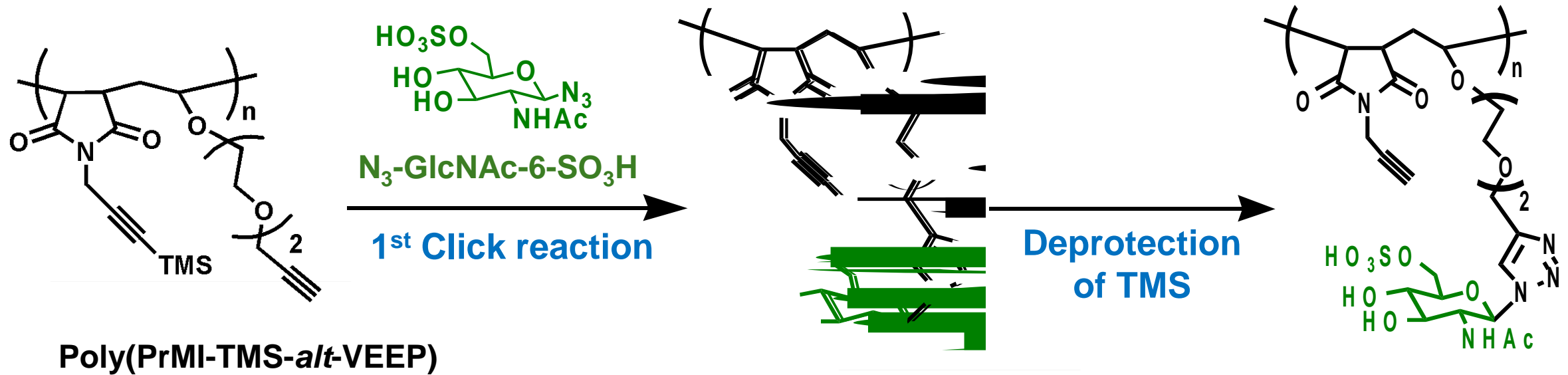
Detector : RI
Eluent : 0.2 M NaNO₃ aq.



(1) Click reaction
→
(2) Deprotection of Bn group



Synthesis of VE-based CS-C mimicking glycopolymer

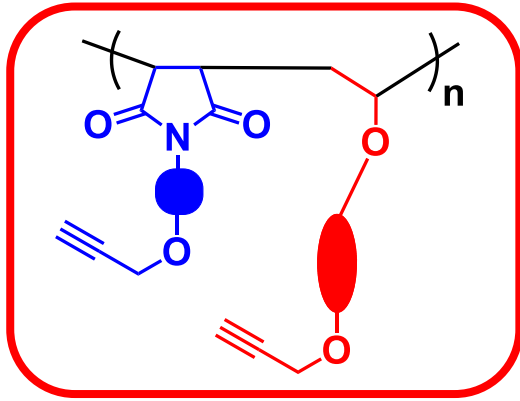


Poly[(MI-GlcA)-*alt*-(VE-GlcNAc-6-SO₃H)]

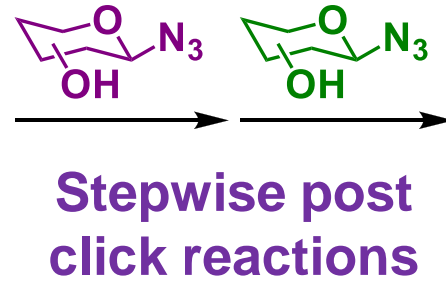
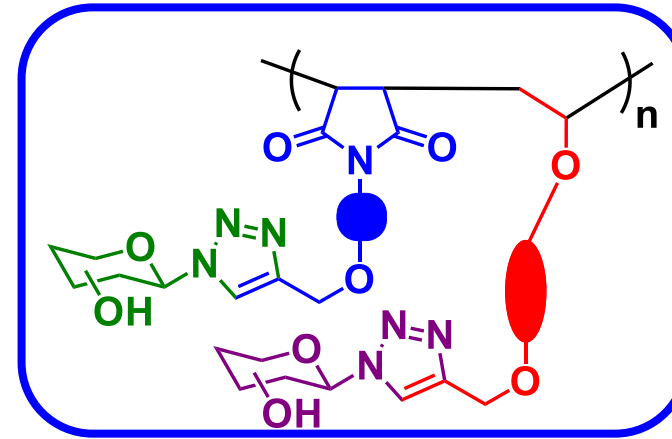
VE-based CS-C mimicking alternating glycopolymer

Synthesis of St- or VE-based alternating glycopolymers as GAG mimics

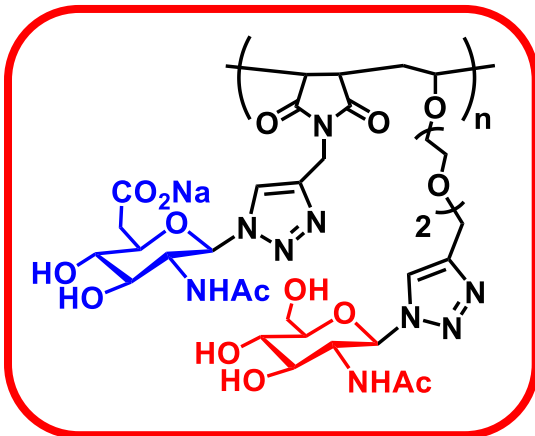
Precursor
alternating copolymer



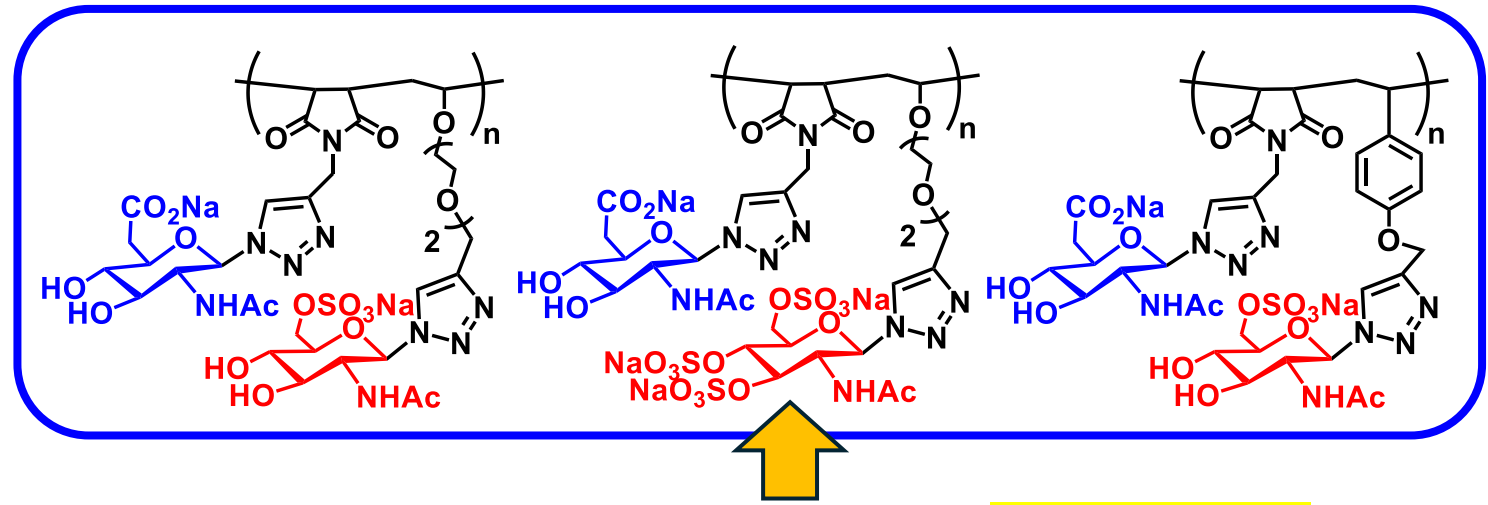
Alternating glycopolymer



HA mimic

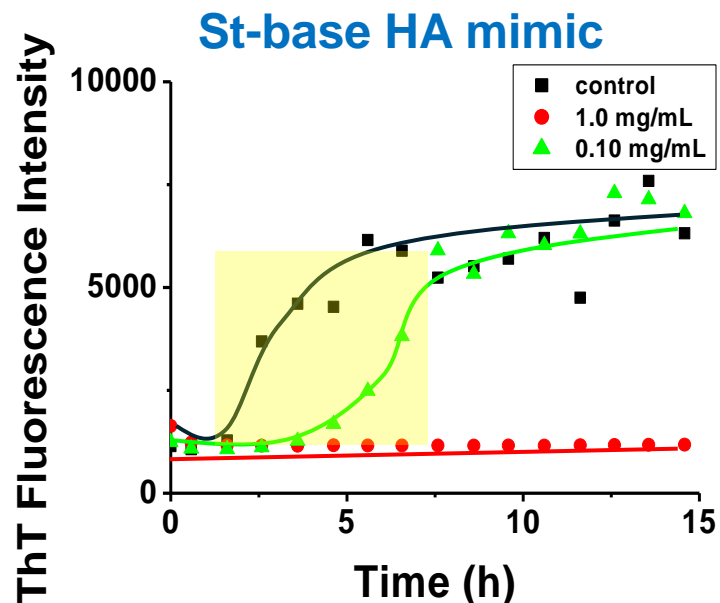
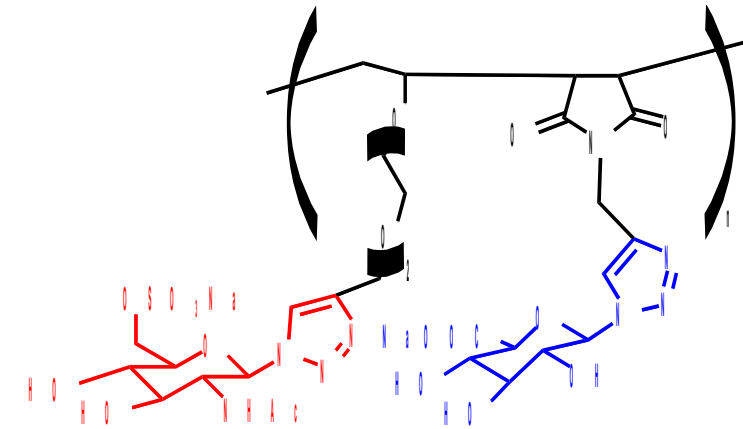
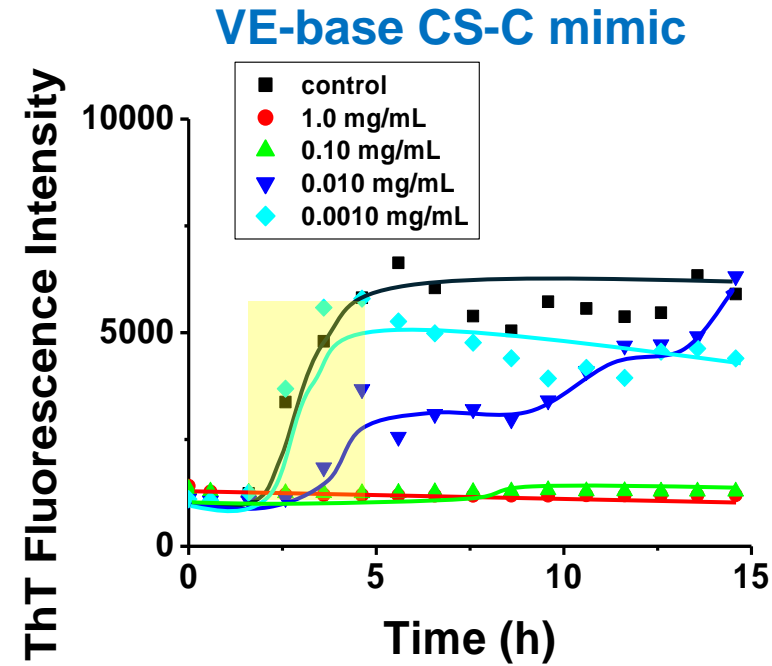
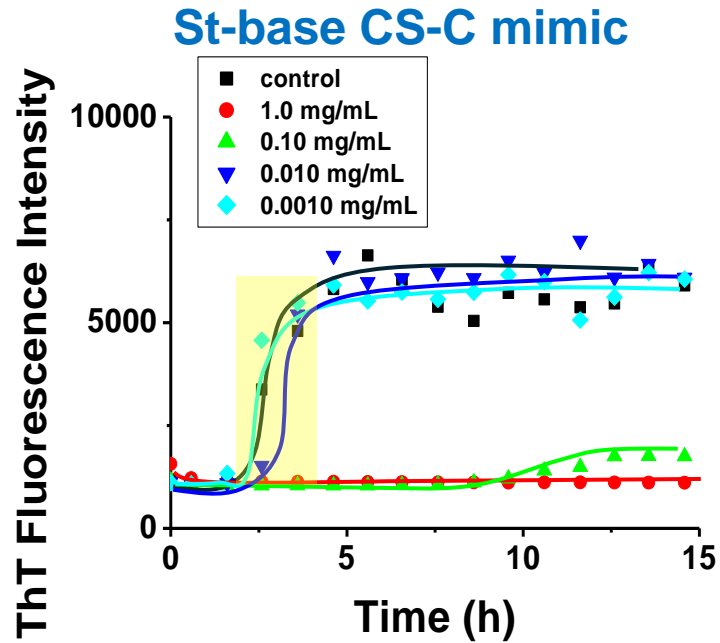


CS-C mimics



Poly[(MI-GlcA)-*alt*-(VE-GlcNAc-3,4,6-(SO₃Na)₃)]

Inhibition activity of Amyloid β aggregation by ThT fluorescence assay



VE-based CS-C mimic also showed amyloid β aggregation inhibitory activity, but a difference in concentration dependence was observed compared to St-based counterpart.