

# **Comprehensive and Efficient Synthesis of Heparan Sulfate Oligosaccharides**

**2023.10.07 Literature Seminar  
M2 Hiromu Kakizawa**

# Contents

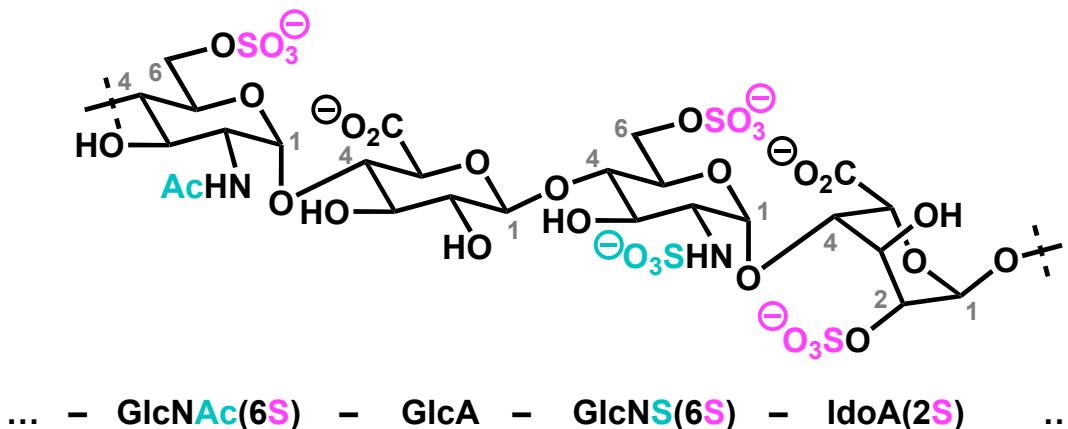
1. Introduction
2. Automated solid-phase preparation of a 16-membered heparan sulfate disaccharide library (*Org. Chem. Front.* 2022)
3. Generation of a library of 64 heparan sulfate tetrasaccharides utilizing fluorous chemistry (main paper, *Nat. Chem.* 2023)

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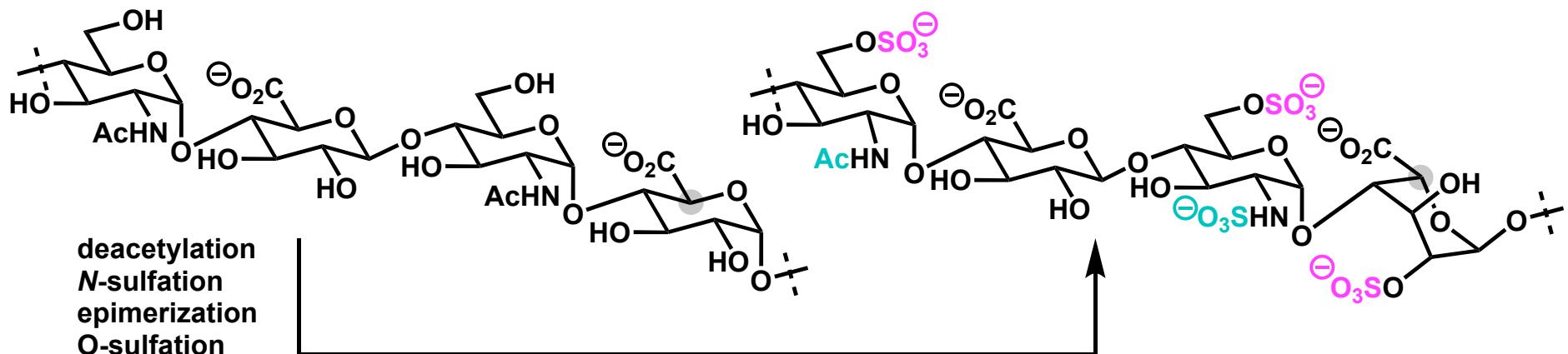
# Heparan Sulfate (HS)

## structural characteristics



- linear polysaccharides existing in various animals
- typically composed of D-glucosamine (GlcN), D-glucuronic acid (GlcA) and L-iduronic acid (IdoA)
- partially acetylated at NH<sub>2</sub> and sulfated at NH<sub>2</sub> and OHs

## biosynthesis of HS

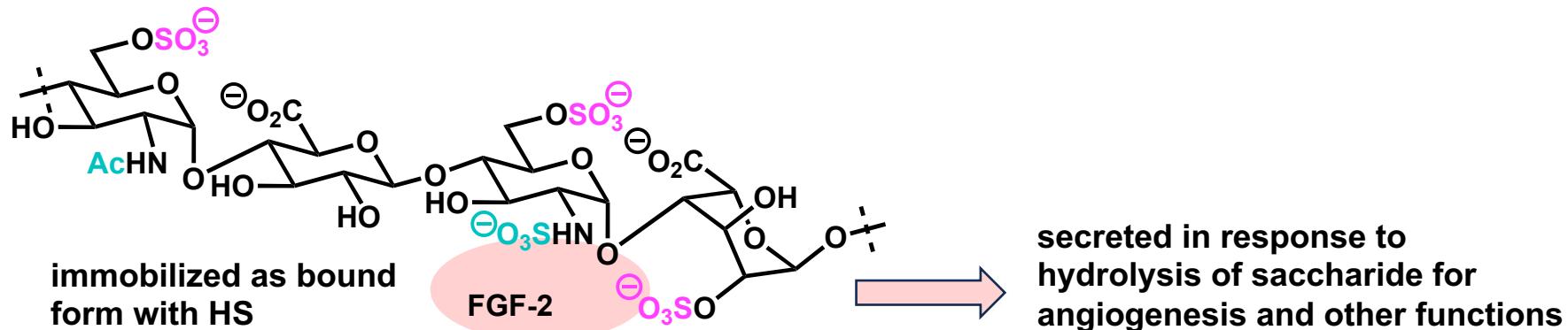


Various saccharide backbone and sulfation patterns are generated.

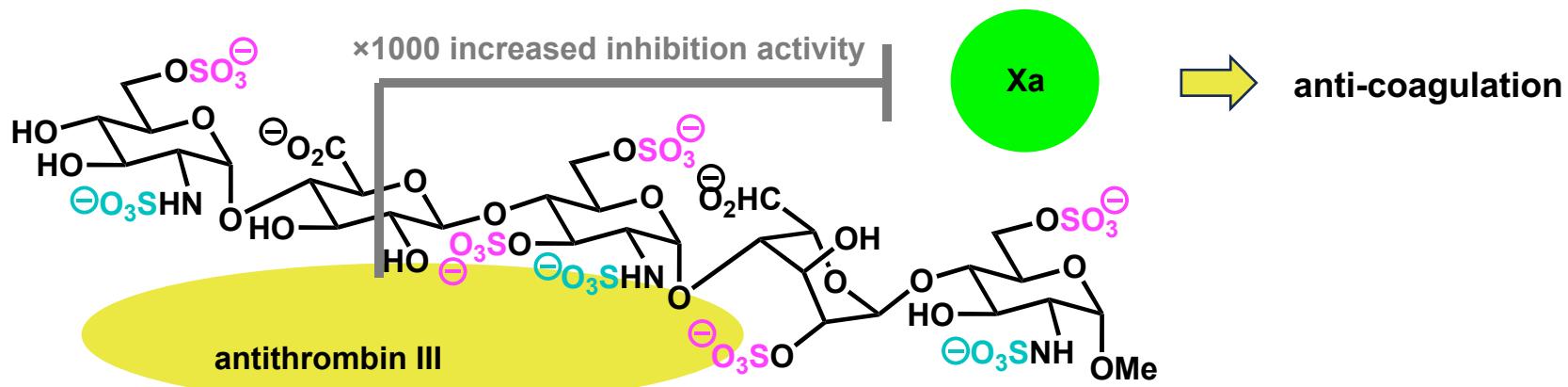
# Various Sulfation Pattern of HS

HSs interact with various proteins depending on their sulfation patterns, mainly by their negative charge.<sup>1)</sup>

## fibroblast growth factor 2 (FGF-2)



## antithrombin III ... applied for approved pentasaccharide drug fondaparinux (shown below)

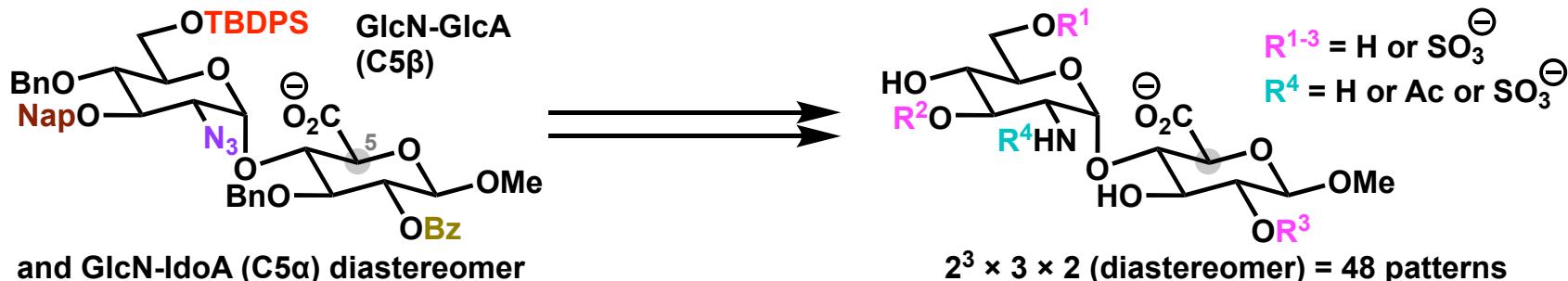


Elucidation of the relationships between sulfation pattern and protein binding is important for more use of HSs as bioactive molecules.

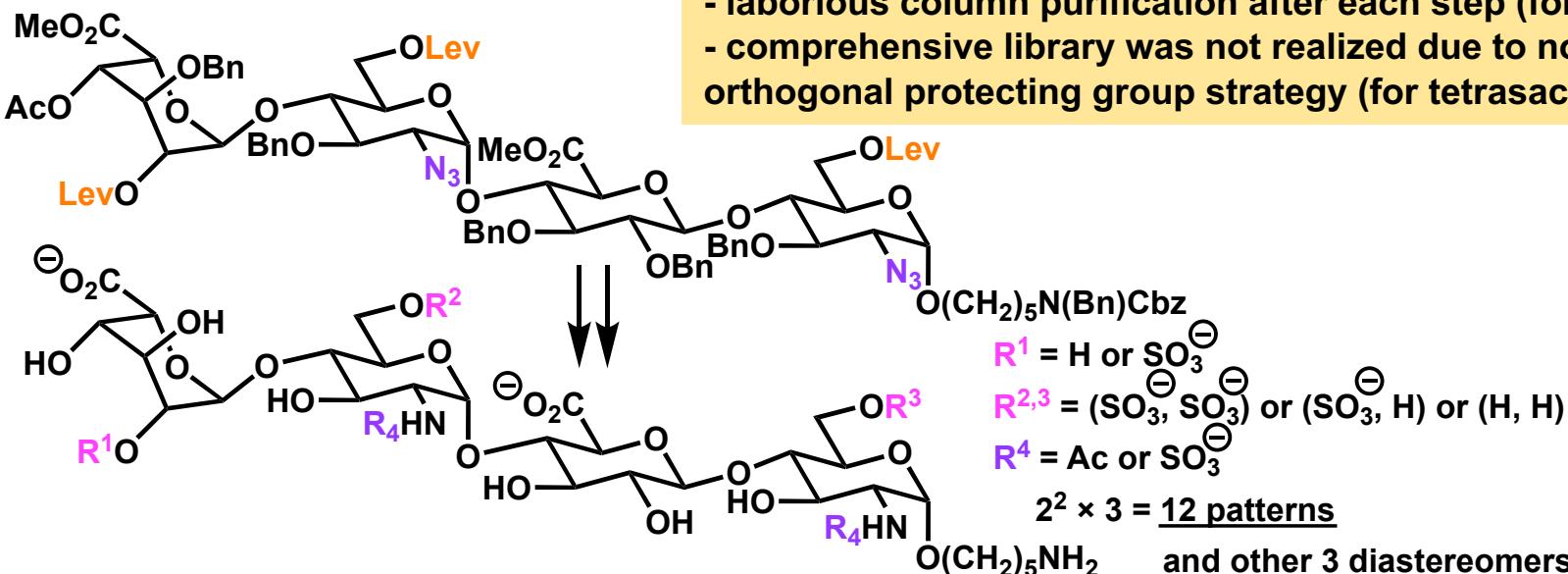
# Previous Synthetic Approach

**Efficient and comprehensive chemical synthesis** is crucial for evaluation of activity of extremely diversely sulfated HSs, which cannot be fully accessed by isolation from nature/enzymatic synthesis.

Wang: 48 HS disaccharides<sup>1)</sup>



Boons: 47 HS tetrasaccharides<sup>2)</sup>



1) Hu, Y.-P.; Zhong, Y.-Q.; Chen, Z.-G.; Chen, C.-Y.; Shi, Z.; Zulueta, M. M. L.; Ku, C.-C.; Lee, P.-Y.; Wang, C.-C.; Hung, S.-C. *J. Am. Chem. Soc.* **2012**, 134, 20722. 2) Zong, C.; Venot, A.; Li, X.; Lu, W.; Xiao, W.; Wilkes, J.-S. L. Salanga, C. L.; Handel, T. M.; Wang, L.; Wolfert, M. A.; Boons, G.-J. *J. Am. Chem. Soc.* **2017**, 139, 9534.

# Prof. Huang and Prof. Hsieh-Wilson



**Prof. Xuefei Huang**

- 1999 Ph. D. (chemistry) @Columbia University in the City of New York  
(under supervision of Prof. Koji Nakanishi)**
- 1999- postdoctorial fellow @The Scripps Research Institute  
(Prof. Chi-Huey Wong)**
- 2001- postdoctorial fellow @Columbia University  
(Prof. Koji Nakanishi)**
- 2002- assistant professor @University of Toledo**
- 2008- assistant professor @Michigan State University**
- 2012- professor @Michigan State University**

**research area: chemistry and biology of carbohydrates**



**Prof. Linda Hsieh-Wilson**

- 1996 Ph. D. (chemistry) @University of California Barkeley  
(under supervision of Prof. Peter Schultz)**
- 1996- postdoctorial fellow @The Rockefeller University  
(Prof. Paul Greengard)**
- 2000- assistant professor @California Institute of Technology**
- 2006- associate professor @California Institute of Technology**
- 2010- professor @California Institute of Technology**

**research area: chemistry and biology of carbohydrates**

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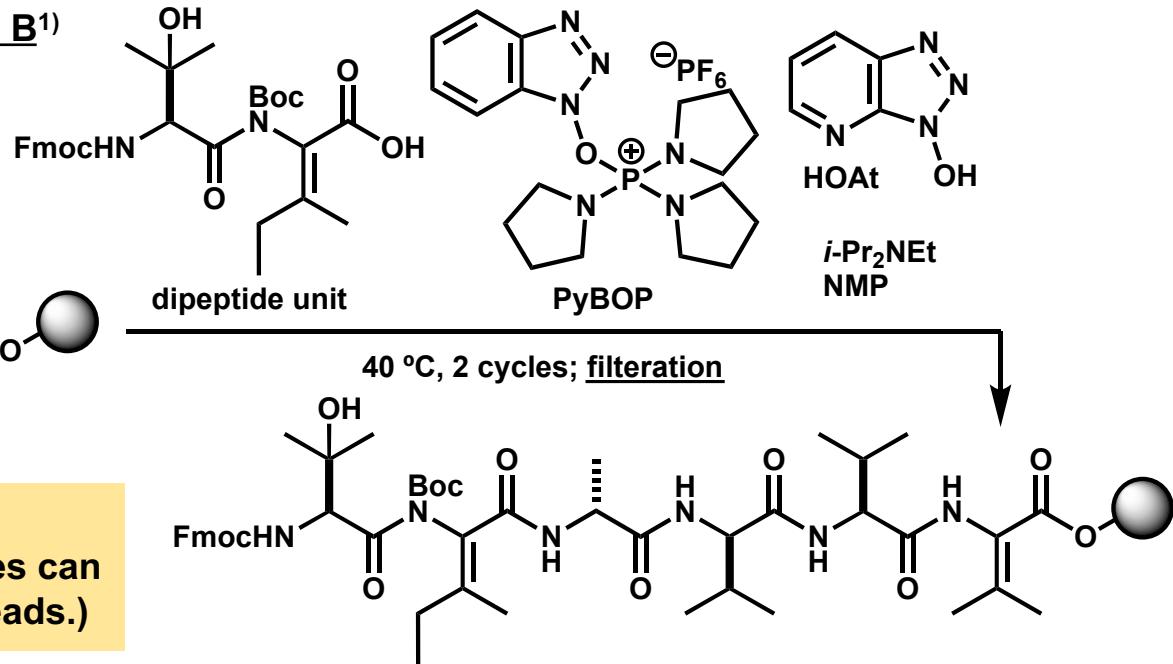
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# Solid-Phase Synthesis

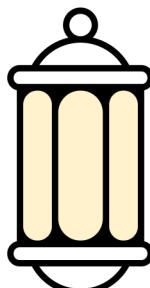
## solid-phase synthesis of yaku'amide B<sup>1)</sup>



: Wang-ChemMatrix resin  
(polymer bead, ~0.1 mm)



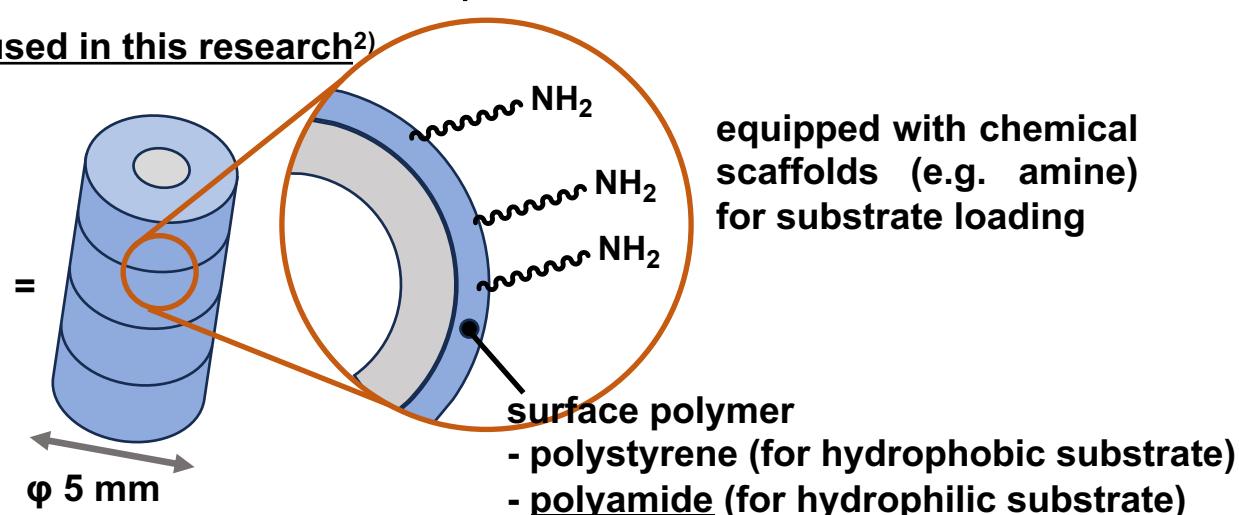
## solid support SynPhase Lantern used in this research<sup>2)</sup>



lantern



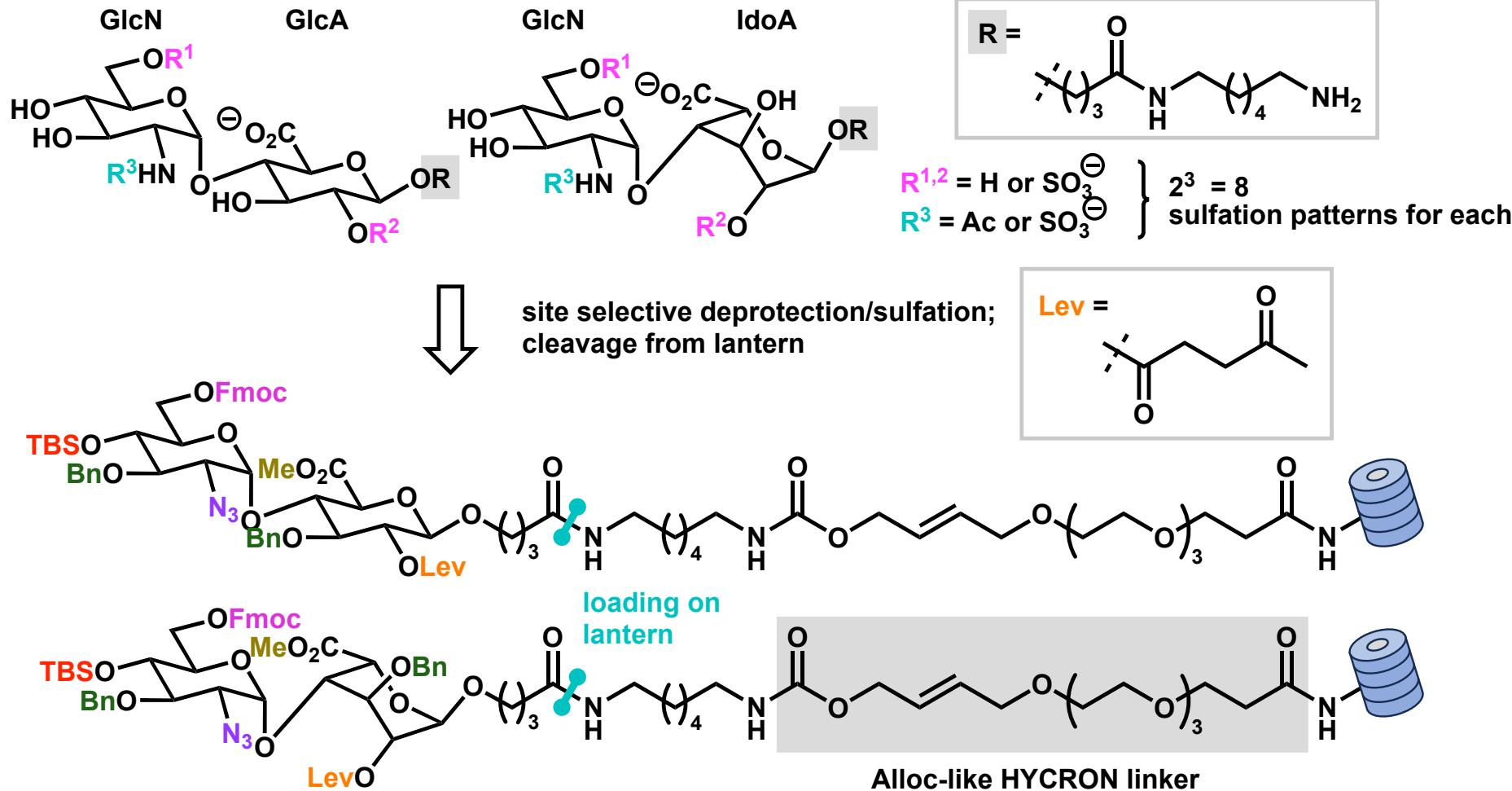
SynPhase Lantern



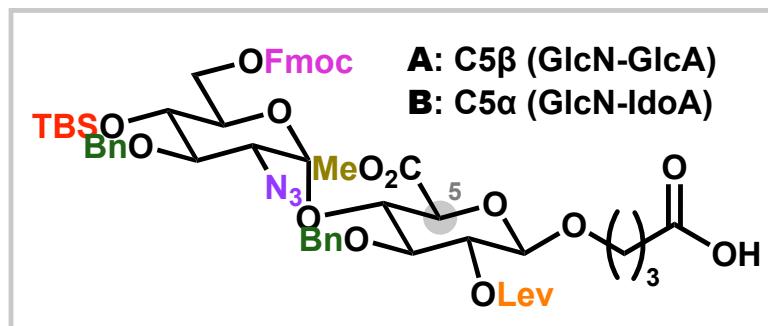
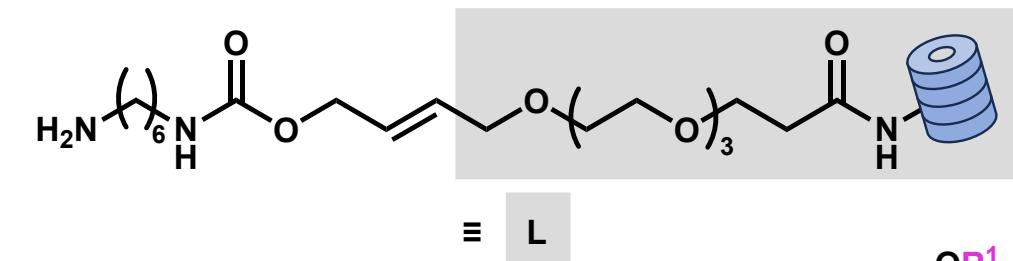
1) Itoh, H.; Miura, K.; Kamiya, K.; Yamashita, T.; Inoue, M. *Angew. Chem. Int. Ed.* **2020**, 59, 4564.

2) For details, also see MIMOTOPE's homepage; <http://www.mimotopes.com/knowledgeBaseListing.asp?cid=26,34,57>

# Synthetic Target And Strategy



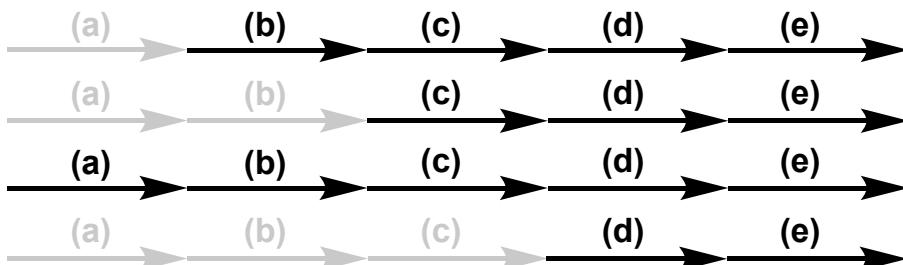
# Introduction on Lantern/O-Sulfation



**A or B, HATU  
HOAt, *i*-Pr<sub>2</sub>NEt**      **piperidine/DMF**

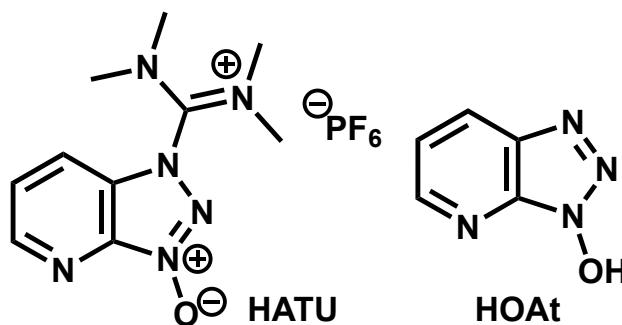
heated by microwave from **A**: 94%, from **B**: 86%\*  
60 °C for **A**, 50 °C for **B** (from UV of cleaved **Fmoc**)

\*calculated from the amount of **A** or **B** used for loading

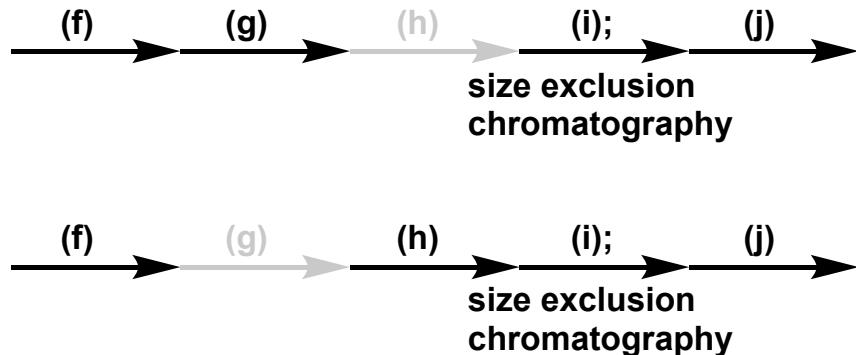
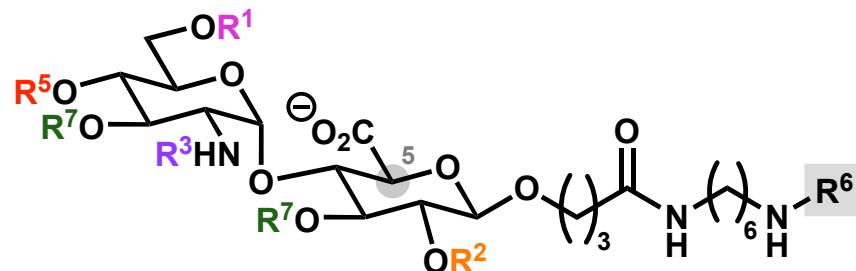


- |   |   |
|---|---|
| (a) Ac <sub>2</sub> O/pyridine, 60 °C                                       | ... Ac protection at <b>R<sup>1</sup></b>                       |
| (b) N <sub>2</sub> H <sub>4</sub> •H <sub>2</sub> O<br>pyridine/AcOH, 40 °C | ... Lev removal   |
| (c) SO <sub>3</sub> •Et <sub>3</sub> N, DMF/Et <sub>3</sub> N, 60 °C        | ... O-sulfation ( <b>R<sup>1</sup></b> , <b>R<sup>2</sup></b> ) |
| (d) Me <sub>3</sub> P, THF/H <sub>2</sub> O, 45 °C                          | ... N <sub>3</sub> reduction                                    |
| (e) LiOH, THF/H <sub>2</sub> O, 30 °C                                       | ... ester hydrolysis ( <b>Me</b> , <b>Lev</b> )                 |

<b>R<sup>1</sup></b>	<b>R<sup>2</sup></b>	<b>R<sup>3</sup></b>	<b>R<sup>4</sup></b>
SO <sub>3</sub> <sup>−</sup>	SO <sub>3</sub> <sup>−</sup>		
SO <sub>3</sub> <sup>−</sup>	H		
H	SO <sub>3</sub> <sup>−</sup>		
H	H	NH <sub>2</sub>	CO <sub>2</sub> <sup>−</sup>

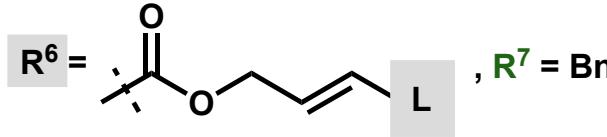


# N-Sulfation/Completion of Library Synthesis



- |   |                                       |
|---|---------------------------------------|
| (f) $n\text{-Bu}_4\text{NF}$ , THF, 35 °C   | ... TBS removal                       |
| (g) $\text{Ac}_2\text{O}$ , MeOH/ $\text{Et}_3\text{N}$ , 50 °C   | ... $N$ -acetylation ( $\text{R}^3$ ) |
| (h) $\text{SO}_3\text{-pyridine}$ , NaOH (pH = 8.5)<br>MeOH/ $\text{Et}_3\text{N}/\text{H}_2\text{O}$ , 60 °C       | ... $N$ -sulfation ( $\text{R}^3$ )   |
| (i) $\text{Pd}(\text{PPh}_3)_4$ , $\text{Me}_2\text{NH}\text{-BH}_3$<br>$\text{CH}_2\text{Cl}_2/\text{DMF}$ , 35 °C | ... cleavage from L                   |
| (j) Pd/C, $\text{H}_2$ , $t\text{-BuOH}/\text{H}_2\text{O}$   | ... $\text{Bn}$ removal               |

$\text{R}^1, \text{R}^2 = \text{H or SO}_3$ ,  $\text{R}^3 = \text{H}$ ,  $\text{R}^5 = \text{TBS}$



$\text{R}^1$	$\text{R}^2$	$\text{R}^3$	$\text{R}^5, \text{R}^6, \text{R}^7$	yield*
$\text{SO}_3^-$	$\text{SO}_3^-$			60%/48%
$\text{SO}_3^-$	H		Ac	33%/38%
H	$\text{SO}_3^-$			39%/49%
H	H			58%/39%
$\text{SO}_3^-$	$\text{SO}_3^-$			42%/40%
$\text{SO}_3^-$	H			41%/42%
H	$\text{SO}_3^-$		$\text{SO}_3^-$	37%/24%
H	H			57%/39%

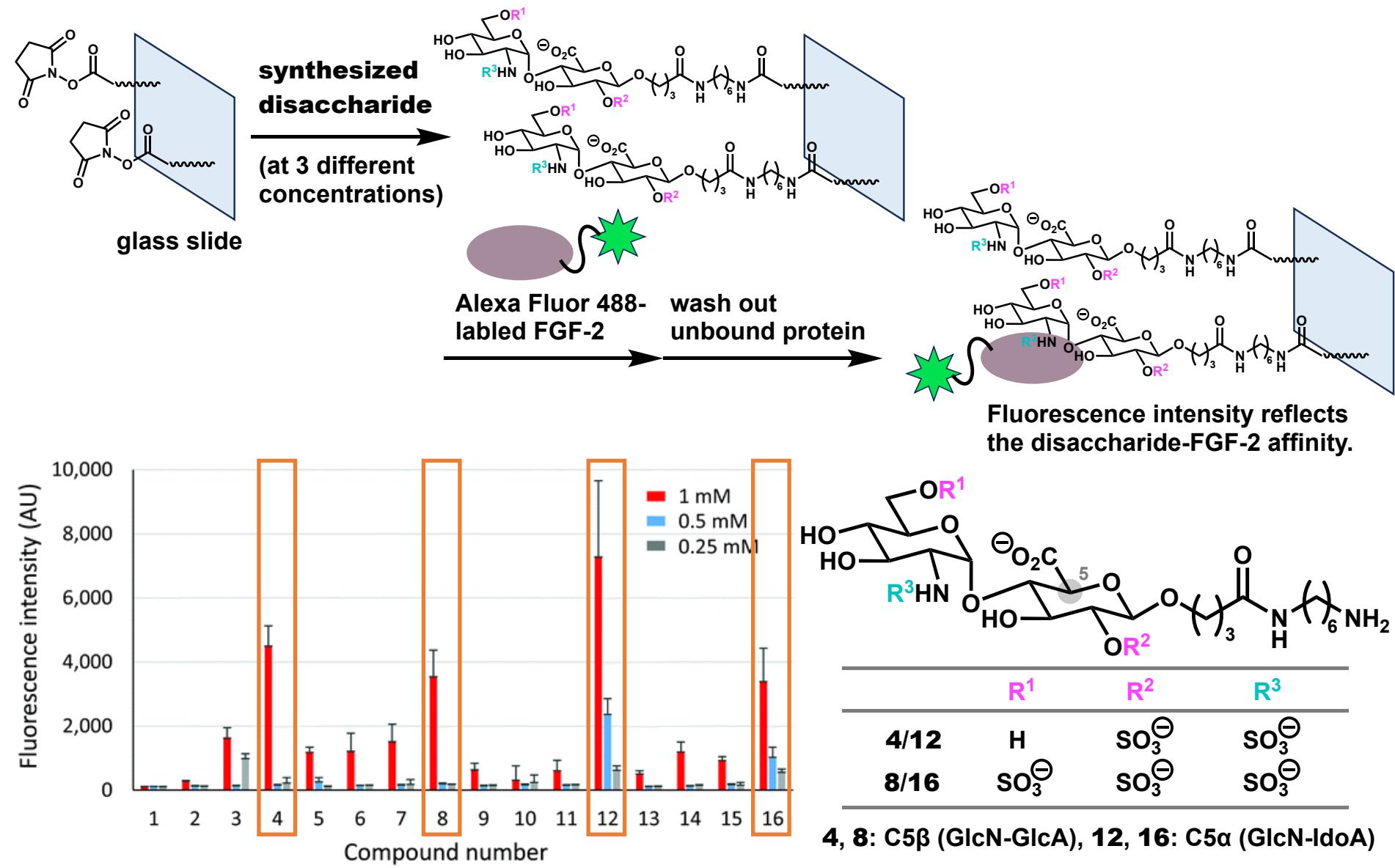
\*calculated based on the resin-loaded disaccharides shown as C5 $\beta$  (GlcN-GlcA)/C5 $\alpha$  (GlcN-IdoA)

Automation of synthesis was also realized.  
(a)-(i): 90 hours



Disaccharides with all the possible sulfation patterns were synthesized in 1-3 mg scale.

# Evaluation of Binding Affinity to FGF-2



Importance of 2-O- and N-sulfation on the FGF-2 interaction was shown by the **comprehensive** assay.

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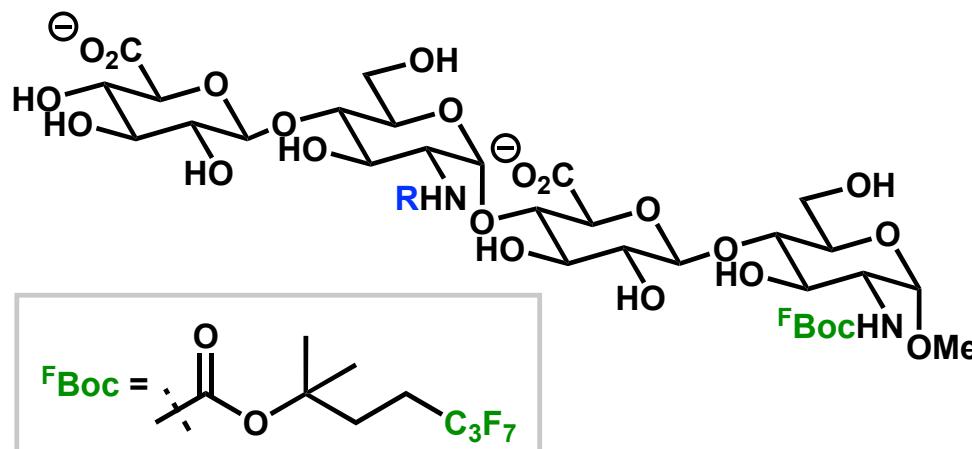
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# Fluorine Tag Method

fluorous chemistry: use of strong affinity between fluorinated compounds

Established in 1990's and used for isolation of compounds by liquid (aqueous) – liquid (organic) – liquid (fluorous) three phase separation<sup>1)</sup>.

application of “fluorous solid-phase extraction”<sup>2)</sup>

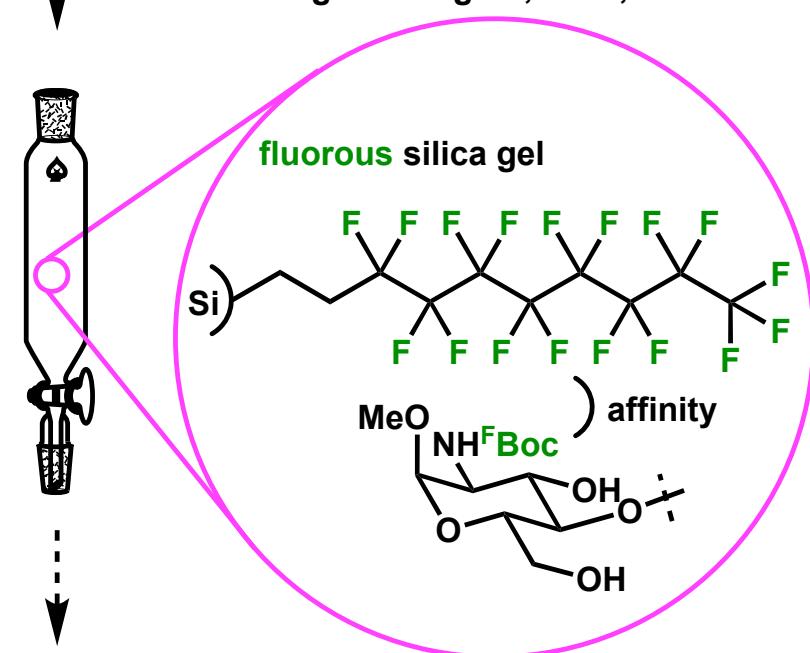


- $\text{R} = \text{COCF}_3$   
 $\text{R} = \text{SO}_3^-$
1.  $\text{Et}_3\text{N}$ ,  $\text{MeOH}/\text{H}_2\text{O}$   
 2.  $\text{SO}_3^-\text{Me}_3\text{N}$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{H}_2\text{O}$ ;  
solid-phase extraction

Fluorine tag method can...

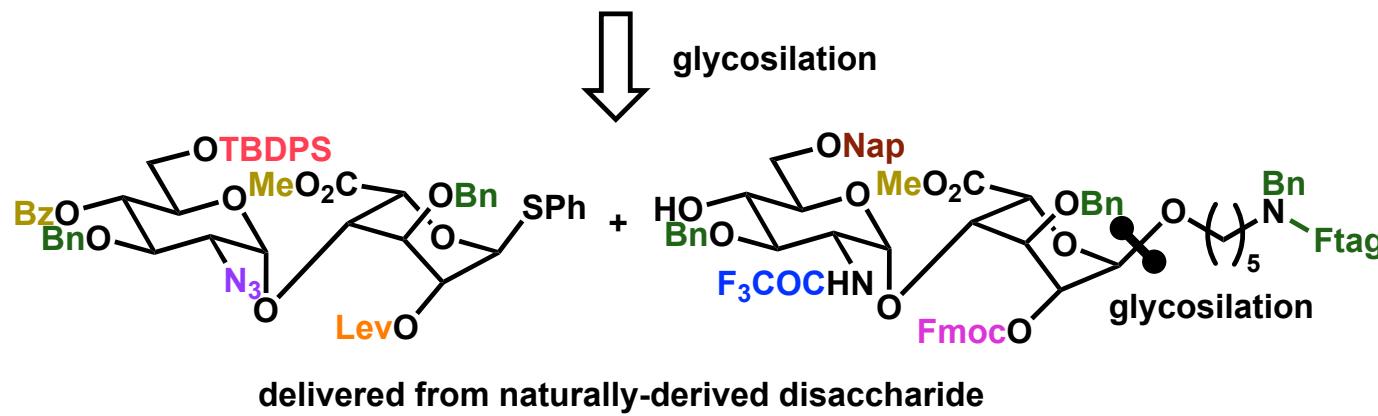
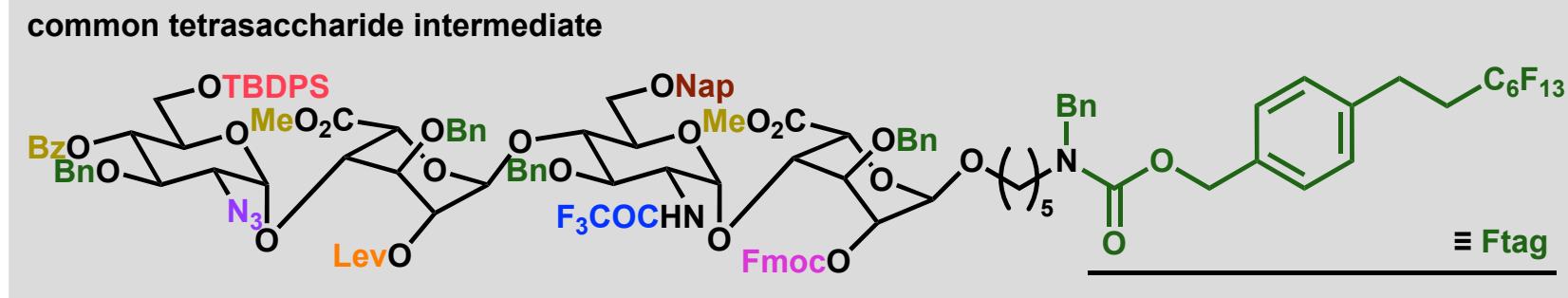
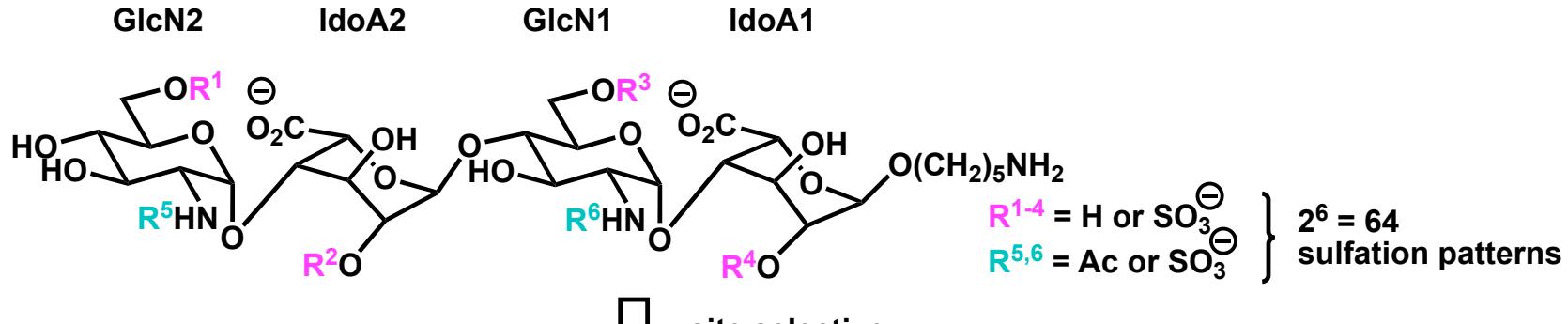
- simplify the **purification** of charged compounds
- take advantage of **liquid-phase reactions**  
 (easy monitoring by TLC, heterogeneous reactions)

- ↓
2. “fluorophilic” solvent (e.g.  $\text{MeOH}$ , acetone): for eluting **fluorine**-tagged product
1.  $\text{H}_2\text{O}$  (very low affinity with fluorine): for washing out reagent, salts, waste

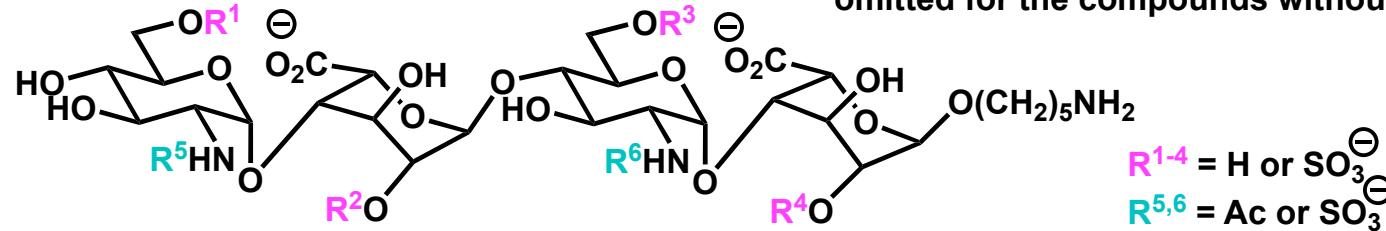
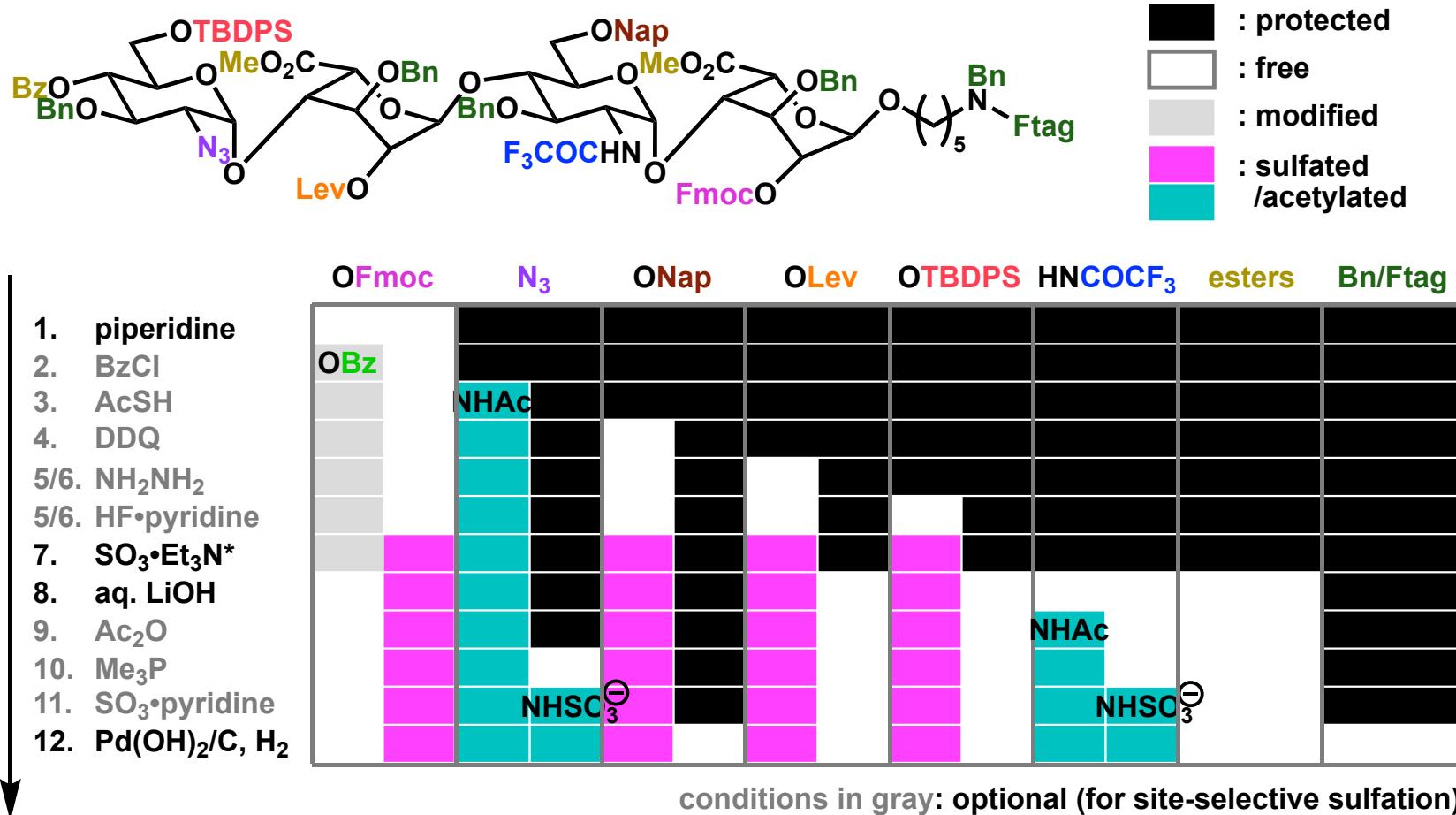


1) Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.-Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science*. **1997**, 275, 823. 2) Cai, C.; Dickinson, D. M.; Li, L.; Masuko, S.; Suflita, M.; Schultz, V.; Nelson, S. D.; Bhaskar, U.; Liu, J.; Linhardt, R. *J. Org. Lett.* **2014**, 16, 2240.

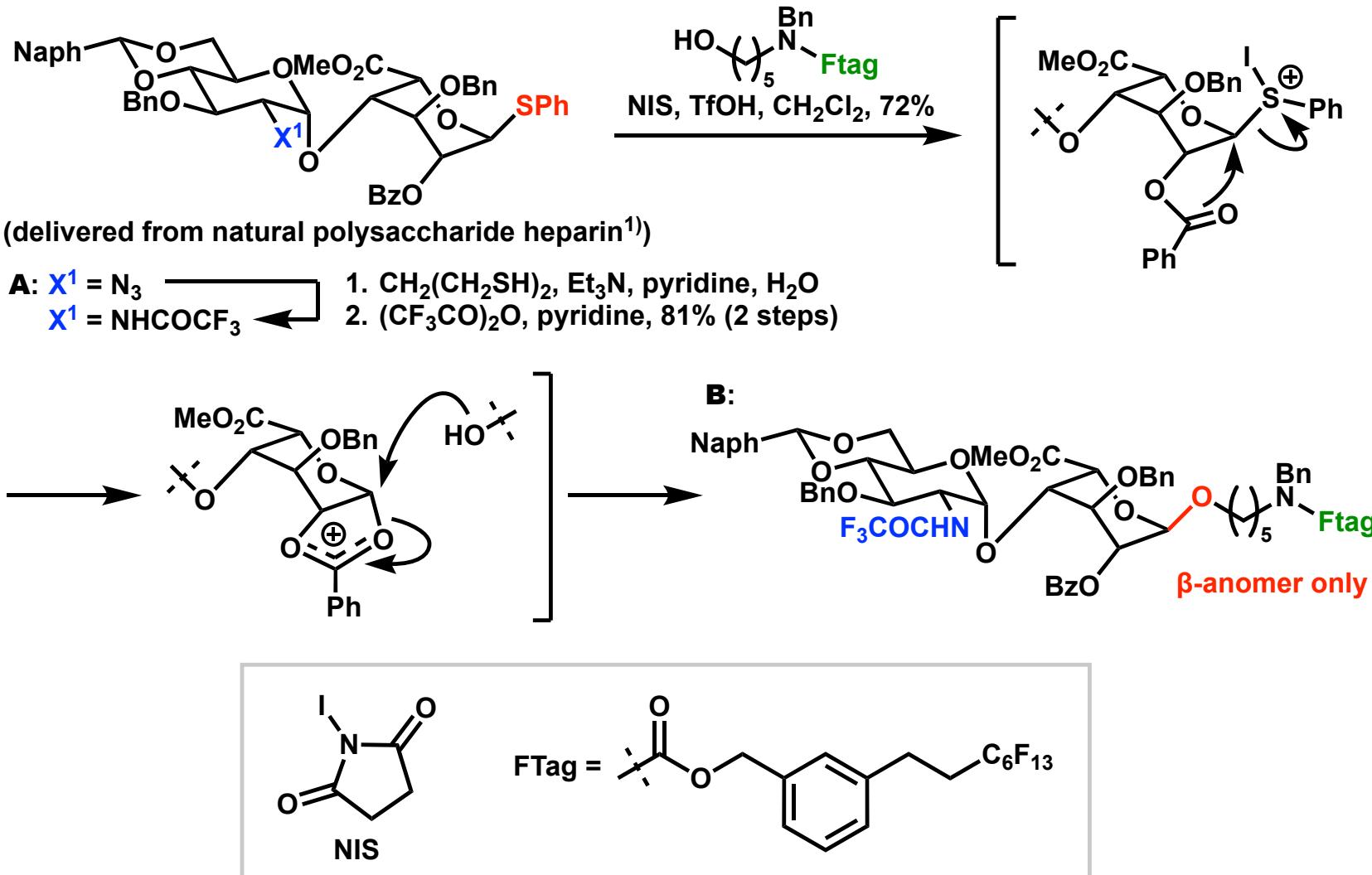
# Synthetic Target and Strategy



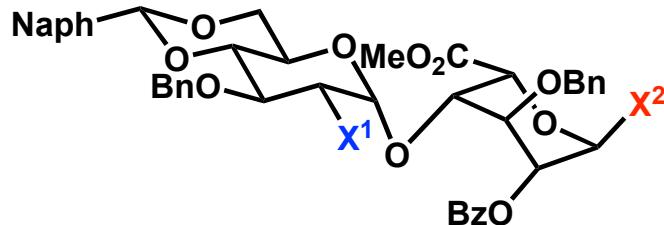
# Overview of Site-Selective Sulfation



# Introduction of Fluorous Tag

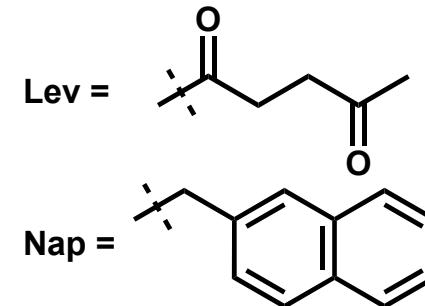
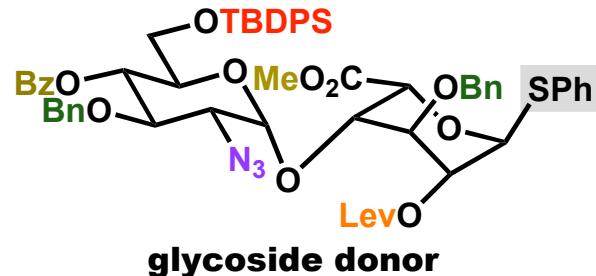


# Glycosylation toward Common Intermediate

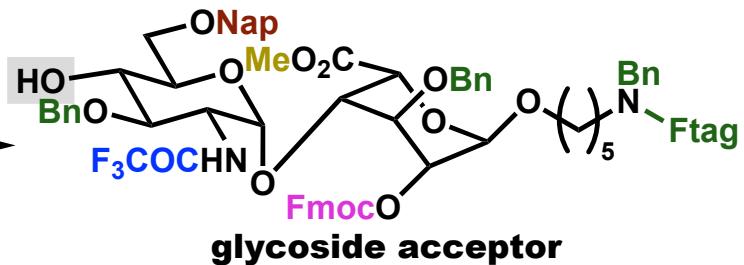


A:  $X^1 = N_3$ ,  $X^2 = SPh$   
 B:  $X^1 = NHCOCF_3$ ,  $X^2 = O(CH_2)_5N(Bn)Ftag$

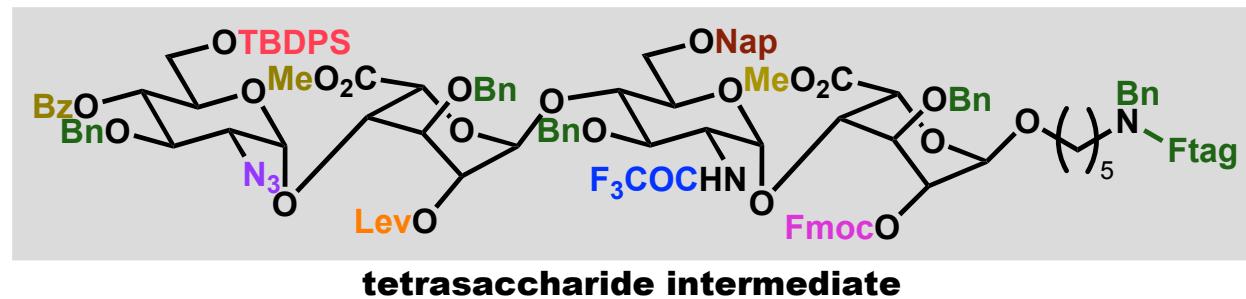
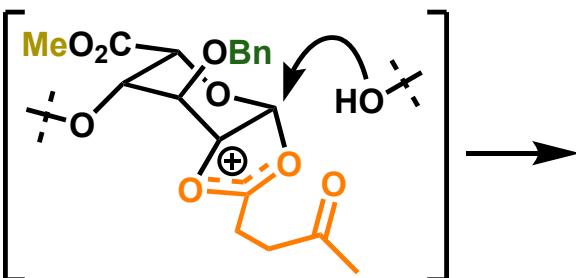
- A
1. NaOMe, MeOH
  2. LevOH, EtN=C=N(CH<sub>2</sub>)<sub>3</sub>NMe<sub>2</sub>, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 91%
  3. TsOH, MeOH
  4. TBDPSCl, imidazole, CH<sub>2</sub>Cl<sub>2</sub>
  5. BzCl, pyridine, 79% (3 steps)



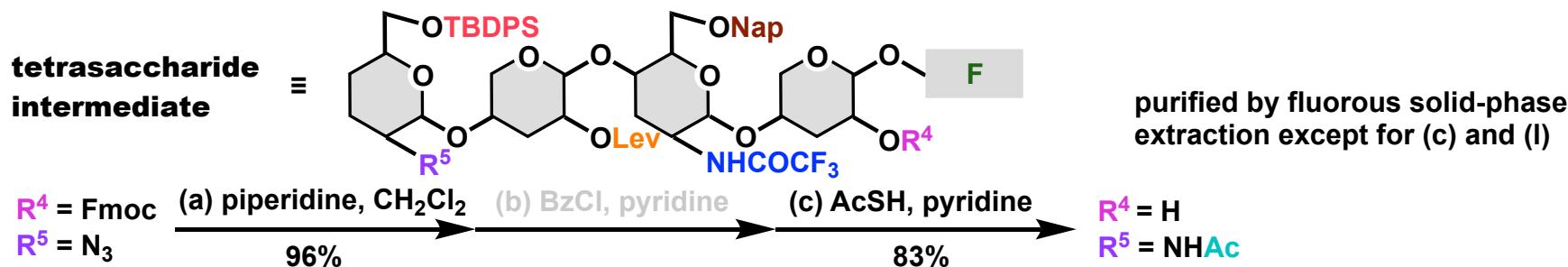
- B
1. NaOMe, MeOH
  2. FmocCl, pyridine, 81% (2 steps)
  3. CF<sub>3</sub>CO<sub>2</sub>H, (CF<sub>3</sub>CO)<sub>2</sub>O, Et<sub>3</sub>SiH, CH<sub>2</sub>Cl<sub>2</sub>, 80%



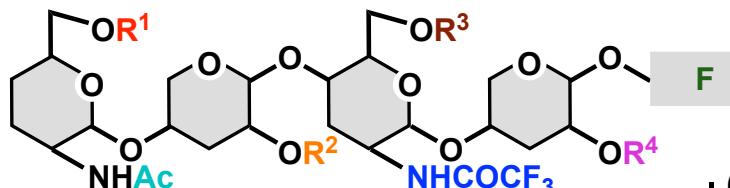
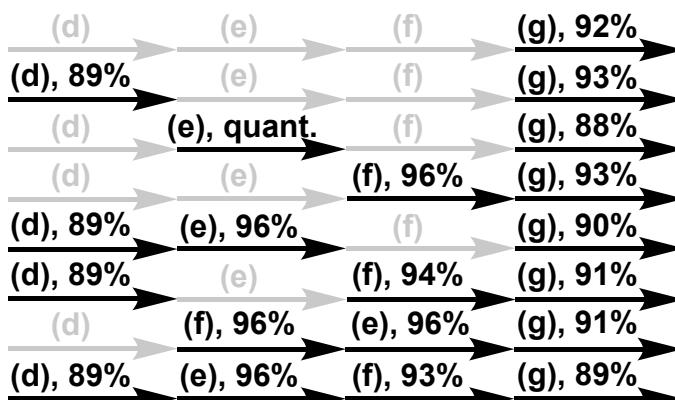
glycoside donor  
 NIS, AgOTf, MS4A, CH<sub>2</sub>Cl<sub>2</sub>, 80%



# Site-Selective Deprotection/Sulfation (1)



- (d) DDQ, 1,2-dichloroethane/MeOH/PBS
- (e) NH<sub>2</sub>NH<sub>2</sub>•AcOH, CH<sub>2</sub>Cl<sub>2</sub>/MeOH
- (f) HF•pyridine, pyridine
- (g) SO<sub>3</sub>•Et<sub>3</sub>N, DMF, 50 °C



R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
TBDPS	Lev	Nap	SO <sub>3</sub> Na
TBDPS	Lev	SO <sub>3</sub> Na	SO <sub>3</sub> Na
TBDPS	SO <sub>3</sub> Na	Nap	SO <sub>3</sub> Na
SO <sub>3</sub> Na	Lev	Nap	SO <sub>3</sub> Na
TBDPS	SO <sub>3</sub> Na	SO <sub>3</sub> Na	SO <sub>3</sub> Na
SO <sub>3</sub> Na	Lev	SO <sub>3</sub> Na	SO <sub>3</sub> Na
SO <sub>3</sub> Na	SO <sub>3</sub> Na	Nap	SO <sub>3</sub> Na
SO <sub>3</sub> Na	SO <sub>3</sub> Na	SO <sub>3</sub> Na	SO <sub>3</sub> Na

(h) LiOH  
H<sub>2</sub>O/MeOH/THF  
rt to 40 °C  
(TBDPS, Lev → H)

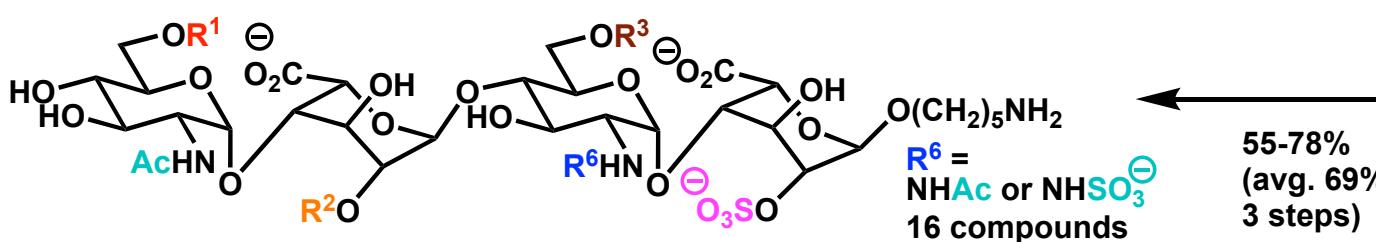
(i)\* Ac<sub>2</sub>O, Et<sub>3</sub>N  
MeOH

(j) Me<sub>3</sub>P, THF/H<sub>2</sub>O

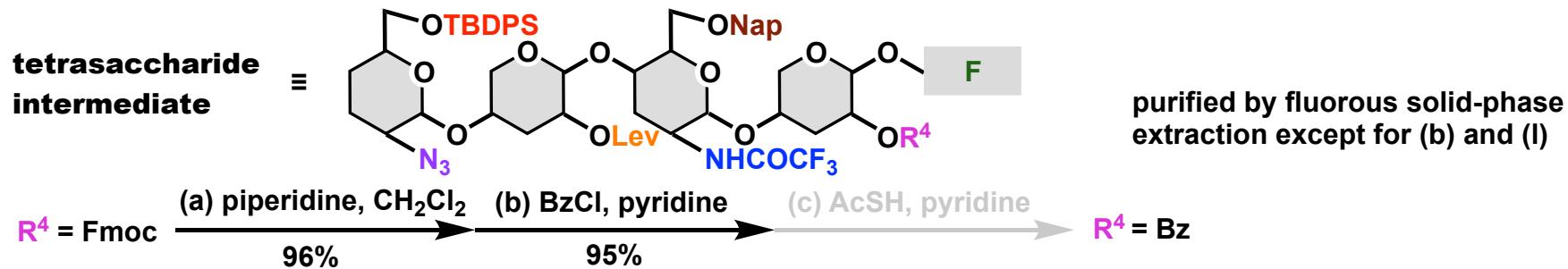
(k)\* SO<sub>3</sub>•pyridine  
Et<sub>3</sub>N, NaOH  
MeOH/CF<sub>3</sub>CH<sub>3</sub>/H<sub>2</sub>O

(l) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>  
t-BuOH/H<sub>2</sub>O  
(Nap → H)

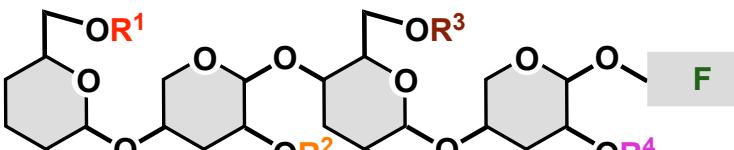
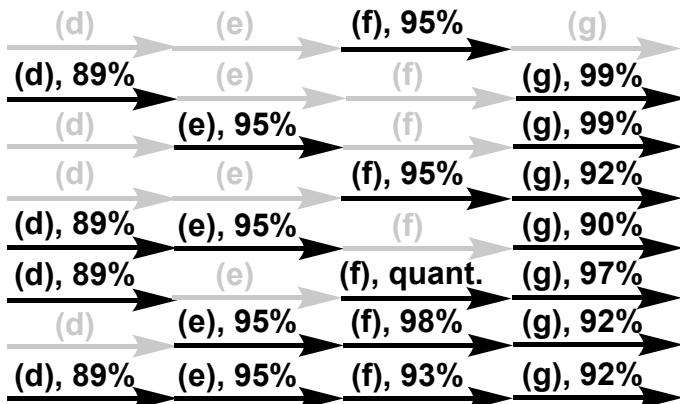
\*optional



# Site-Selective Deprotection/Sulfation (2)



- (d) DDQ, 1,2-dichloroethane/MeOH/PBS
- (e)  $\text{NH}_2\text{NH}_2 \cdot \text{AcOH}$ ,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$
- (f) HF-pyridine, pyridine
- (g)  $\text{SO}_3 \cdot \text{Et}_3\text{N}$ , DMF, 50 °C



	$R^1$	$R^2$	$R^3$	$R^4$
H	Lev	Nap	Bz	
TBDPS	Lev	$\text{SO}_3\text{Na}$	Bz	
TBDPS	$\text{SO}_3\text{Na}$	Nap	Bz	
$\text{SO}_3\text{Na}$	Lev	Nap	Bz	
TBDPS	$\text{SO}_3\text{Na}$	$\text{SO}_3\text{Na}$	Bz	
$\text{SO}_3\text{Na}$	Lev	$\text{SO}_3\text{Na}$	Bz	
$\text{SO}_3\text{Na}$	$\text{SO}_3\text{Na}$	Nap	Bz	
$\text{SO}_3\text{Na}$	$\text{SO}_3\text{Na}$	$\text{SO}_3\text{Na}$	Bz	

(h)  $\text{LiOH}$   
 $\text{H}_2\text{O}/\text{MeOH}/\text{THF}$   
rt to 40 °C  
(TBDPS, Lev → H)

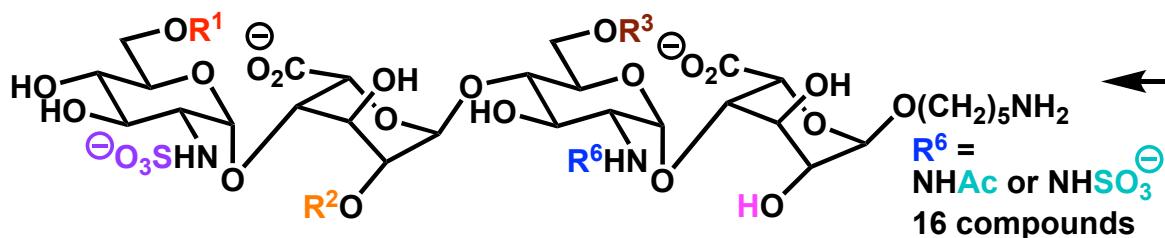
(i)\*  $\text{Ac}_2\text{O}, \text{Et}_3\text{N}$   
 $\text{MeOH}$

(j)  $\text{Me}_3\text{P}$ ,  $\text{THF}/\text{H}_2\text{O}$

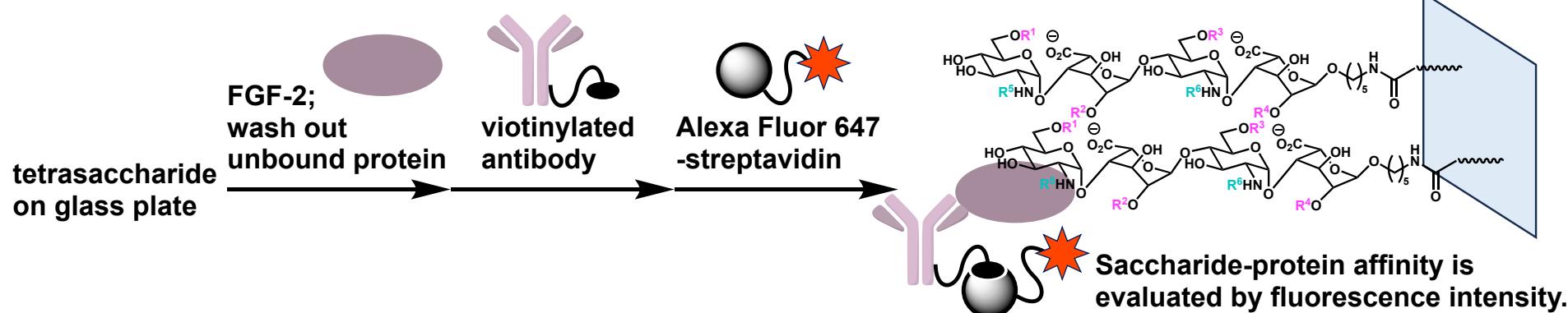
(k)  $\text{SO}_3 \cdot \text{pyridine}$   
 $\text{Et}_3\text{N}, \text{NaOH}$   
 $\text{MeOH}/\text{CF}_3\text{CH}_3/\text{H}_2\text{O}$

(l)  $\text{Pd}(\text{OH})_2/\text{C}, \text{H}_2$   
 $t\text{-BuOH}/\text{H}_2\text{O}$   
(Nap → H)

\*optional



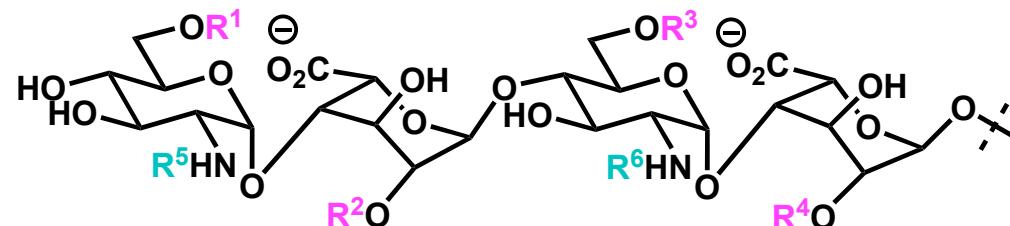
# Evaluation of Binding Affinity to FGF-2



FGF2	N-sulfation			
	NAc-NAc	NAc-NS	NS-NAc	NS-NS
6H-2H-6H-2H	1 0.1 ± 0.1	2 0.4 ± 0.3	3 0.5 ± 0.1	4 0.9 ± 0.1
6H-2H-6H-2S	5 0.1 ± 0.1	6 21 ± 1	7 0.6 ± 0.2	8 32 ± 2
6H-2H-6S-2H	9 0.2 ± 0.3	10 0.3 ± 0.1	11 0.7 ± 0.1	12 2 ± 1
6S-2H-6H-2H	13 0.0 ± 0.7	14 0.4 ± 0.1	15 18 ± 1	16 21 ± 1
6S-2H-6H-2H	17 0.0 ± 0.1	18 0.4 ± 0.1	19 0.4 ± 0.2	20 1 ± 1
6H-2H-6S-2S	21 0.1 ± 0.0	22 29 ± 2	23 2 ± 1	24 38 ± 2
6H-2S-6H-2S	25 0.2 ± 0.0	26 31 ± 2	27 30 ± 1	28 68 ± 3
6S-2H-6H-2S	29 0.0 ± 0.0	30 26 ± 1	31 1 ± 1	32 22 ± 3
6H-2S-6S-2H	33 0.2 ± 0.2	34 2 ± 1	35 38 ± 5	36 36 ± 1
6S-2H-6S-2H	37 0.0 ± 0.1	38 2 ± 4	39 2 ± 1	40 3 ± 1
6S-2S-6H-2H	41 0.1 ± 0.0	42 0.6 ± 0.2	43 26 ± 1	44 24 ± 1
6H-2S-6S-2S	45 0.9 ± 0.1	46 37 ± 1	47 57 ± 2	48 77 ± 2
6S-2H-6S-2S	49 1 ± 1	50 36 ± 1	51 3 ± 1	52 45 ± 1
6S-2S-6H-2S	53 0.6 ± 0.4	54 38 ± 2	55 36 ± 1	56 63 ± 4
6S-2S-6S-2H	57 3 ± 1	58 5 ± 3	59 68 ± 10	60 39 ± 2
6S-2S-6S-2S	61 2 ± 1	62 28 ± 3	63 70 ± 3	64 100 ± 4

0 100

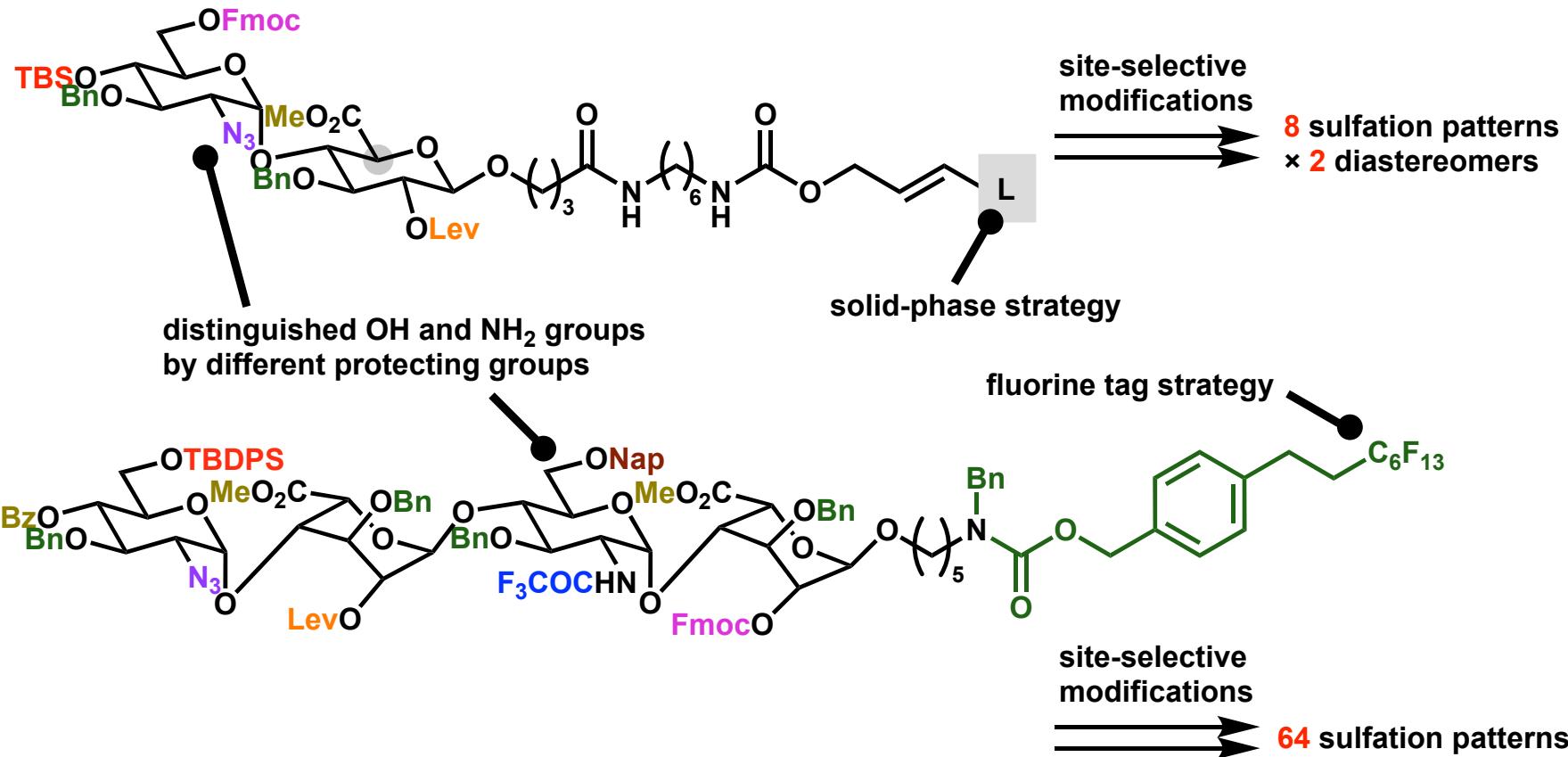
structures with the best 5 affinity and almost no affinity:



	R <sup>1</sup>	R <sup>5</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>6</sup>	R <sup>4</sup>	#S
64	SO <sub>3</sub> <sup>-</sup>	6					
48	H	SO <sub>3</sub> <sup>-</sup>	5				
63	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	Ac	SO <sub>3</sub> <sup>-</sup>	5
28	H	SO <sub>3</sub> <sup>-</sup>	H	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	5
59	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	Ac	H	4
40	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	H	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	H	4
58	SO <sub>3</sub> <sup>-</sup>	Ac	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	SO <sub>3</sub> <sup>-</sup>	H	4

Comprehensive investigations for the structural determinants for protein recognition of HSs was realized.

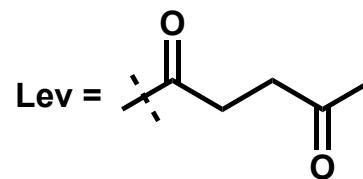
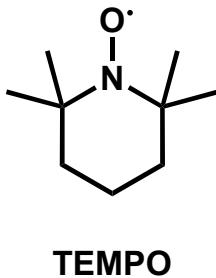
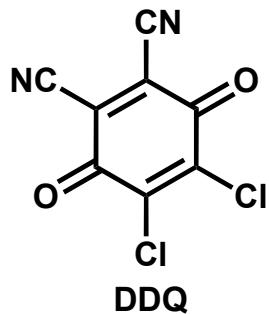
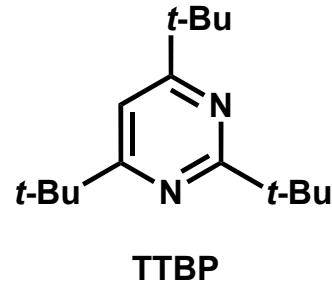
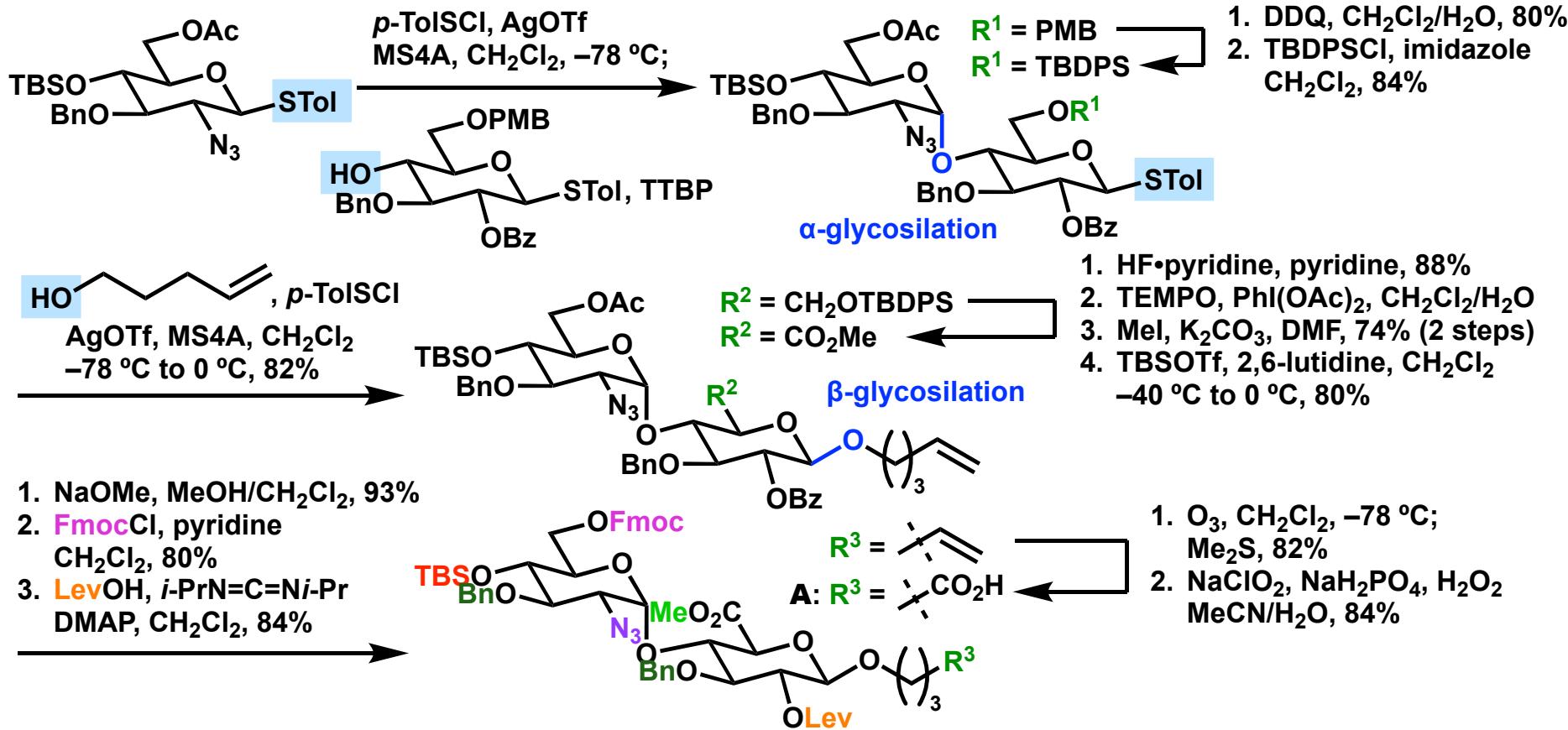
# Summary



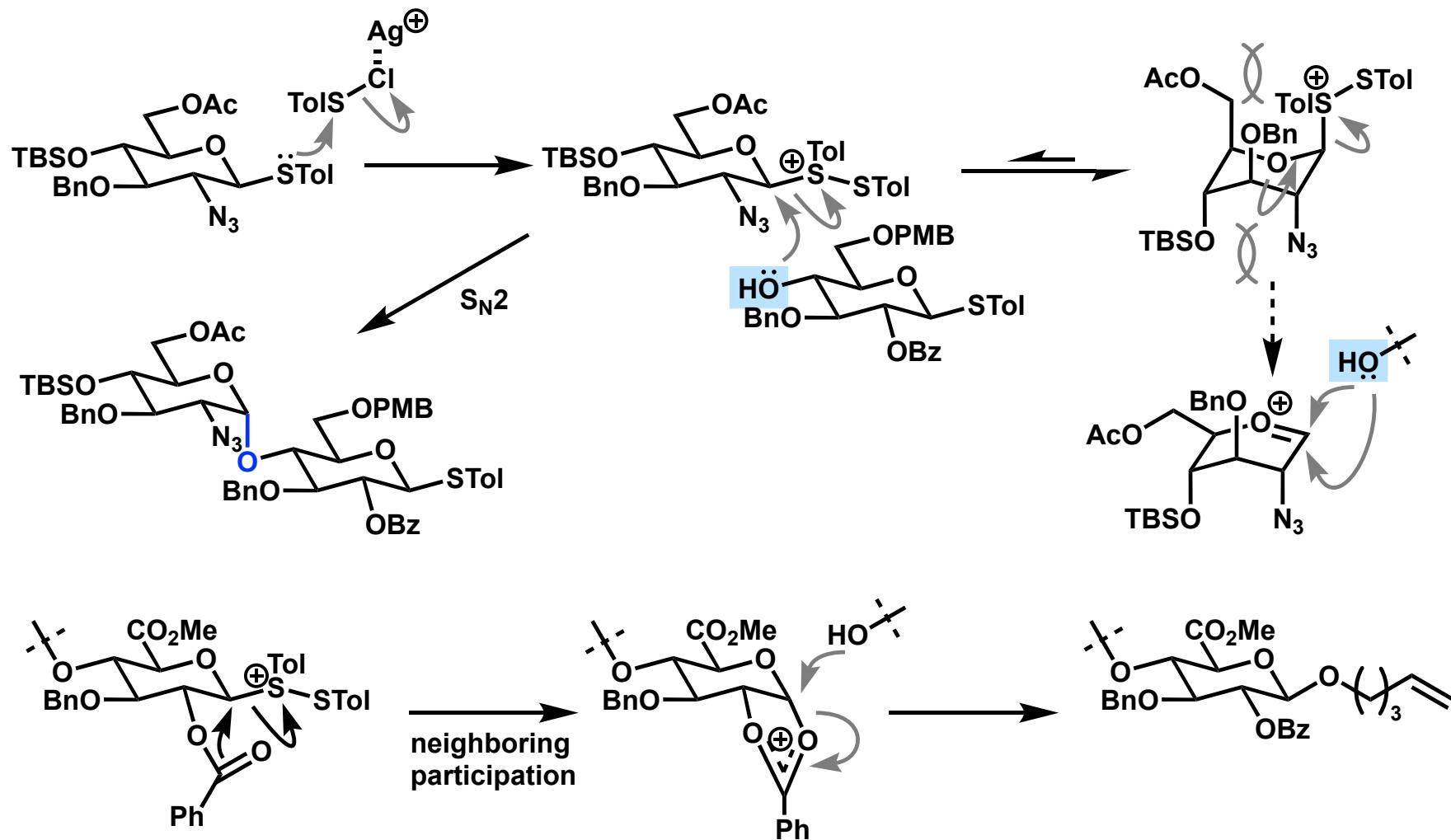
Diversely sulfated HS libraries were constructed **systematically and efficiently**, enabling **comprehensive** investigation into sulfation requirements for binding to various proteins.

# **Appendix**

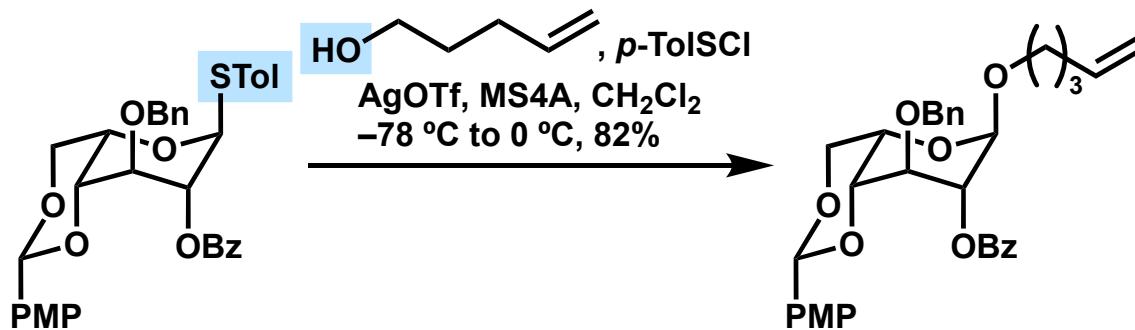
# Synthesis of GlcN-GlcA Building Block



# Stereoselectivity of Glycosylation

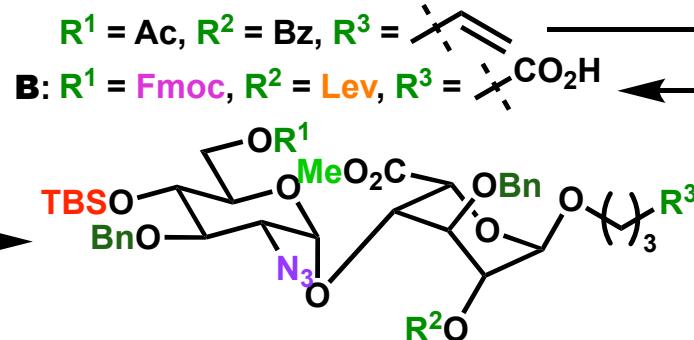
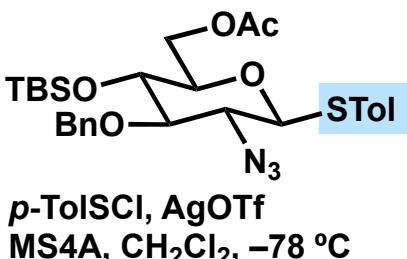
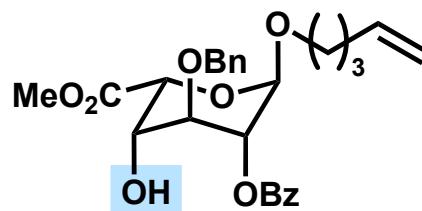


# Synthesis of GlcN-IdoA Building Block



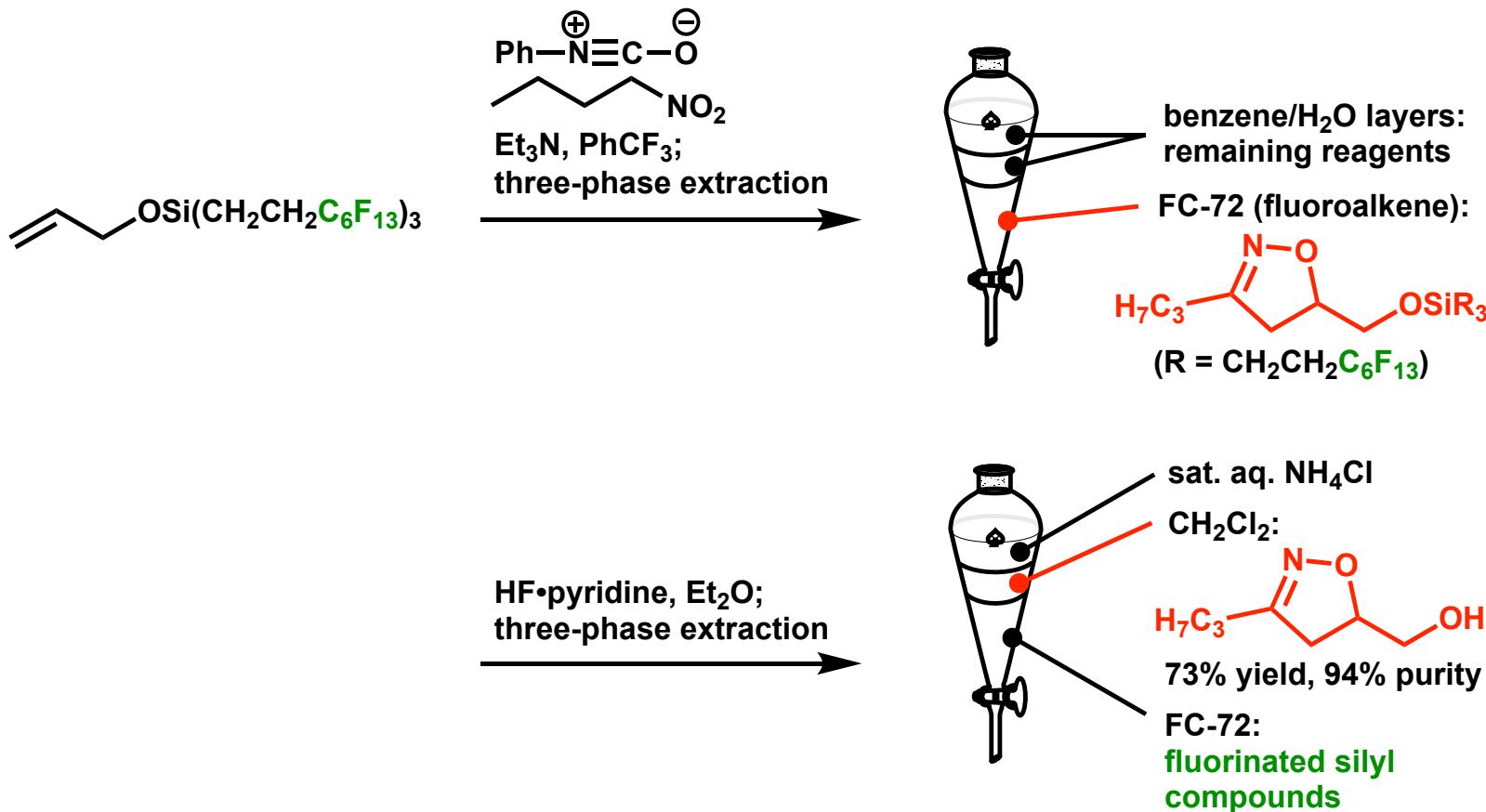
1. *p*-toluenesulfonic acid  
CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 90%

2. TEMPO, PhI(OAc)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O  
3. MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, 86% (2 steps)



1. NaOMe, MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 79%
2. FmocCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 71%
3. LevOH, *i*-PrN=C=N*i*-Pr  
DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 75%
4. O<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C; Me<sub>2</sub>S, 70%
5. NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>  
MeCN/H<sub>2</sub>O, 80%

# organic-aqueous-fluorous separation<sup>1)</sup>



Pure [3 + 2] cycloadduct was obtained without column chromatography.

1) Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.-Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science*. **1997**, 275, 823.

# Modifications of Naturally Derived Disaccharide<sup>1)</sup>

