Electronic Supplementary Material (ESI) for Chemical Society Reviews

β1,2-Mannans Conformationally Interesting Molecules

β1,2-Mannan a Protective Epitope

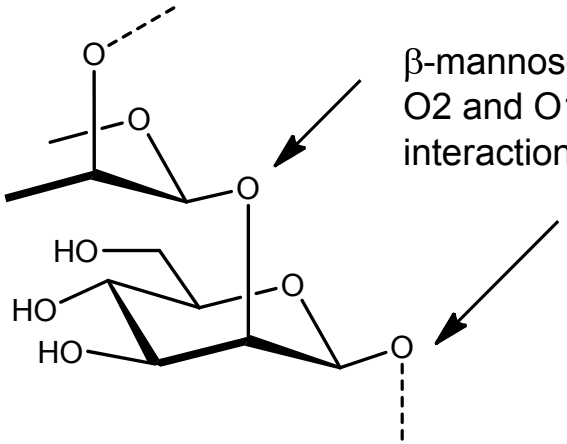
Predicted to have unique conformational properties

Polysaccharide Conformation. Part V1. Computer Model-Building for Linear and Branched Pyranoglycans. Correlations with Biological Function. Preliminary Assessment of Inter-Residue Forces in Aqueous Solution. Further Interpretation of Optical Rotation in Terms of Chain Conformation

D. A. Rees' and W. E. Scott, Chemistry Department,
The University of Edinburgh

***J. Chem. Soc. B*, 1971, 469-479.**

β1,2 linked glucans and mannans are crumpled and rigid



β-mannose residues at O2 and O1 will have steric interactions

Properties that could influence biological activity?

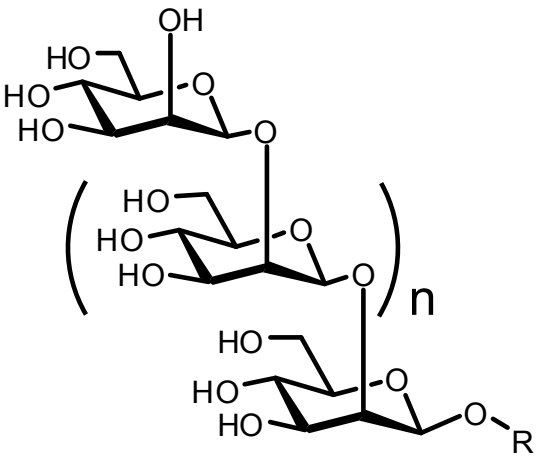
Electronic Supplementary Material (ESI) for Chemical Society Reviews

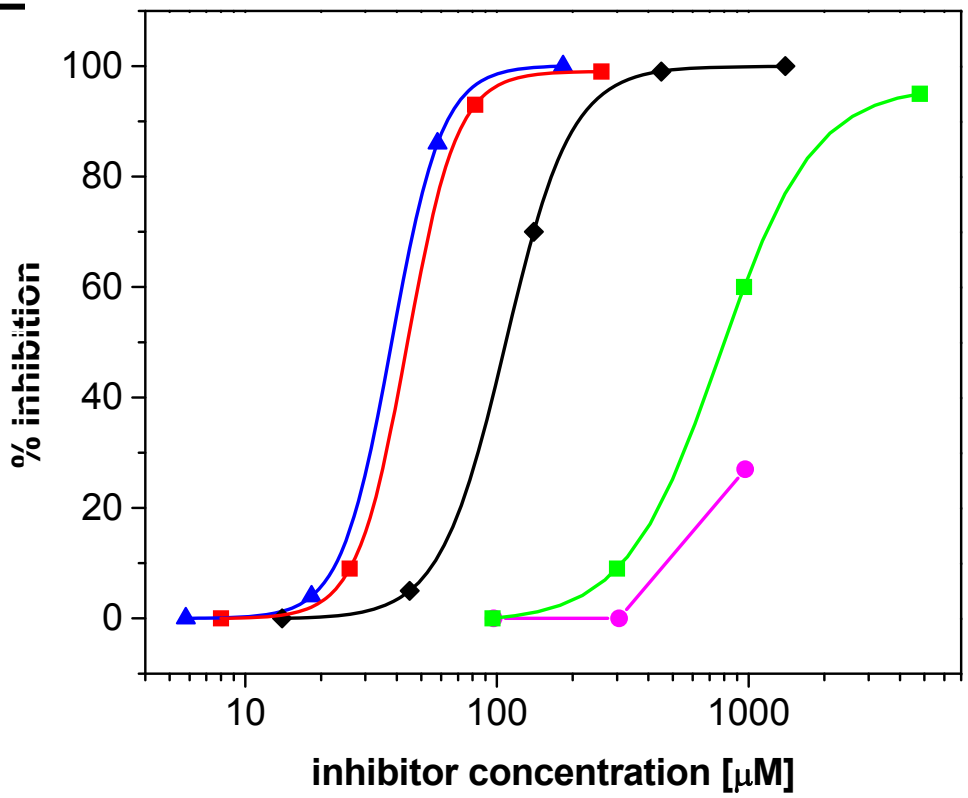
Oligosaccharide inhibition of mAb C3.1 binding to cell wall β-mannan

The surprizing result of lower activity as inhibitor size increase beyond a trisaccharide

Antibody Site Filled by Di- or Trisaccharide

Inhibitor	IC_{50} mM
Disaccharide	44
Trisaccharide	38
Tetrasaccharide	108
Pentasaccharide	770
Hexasaccharide	>1000

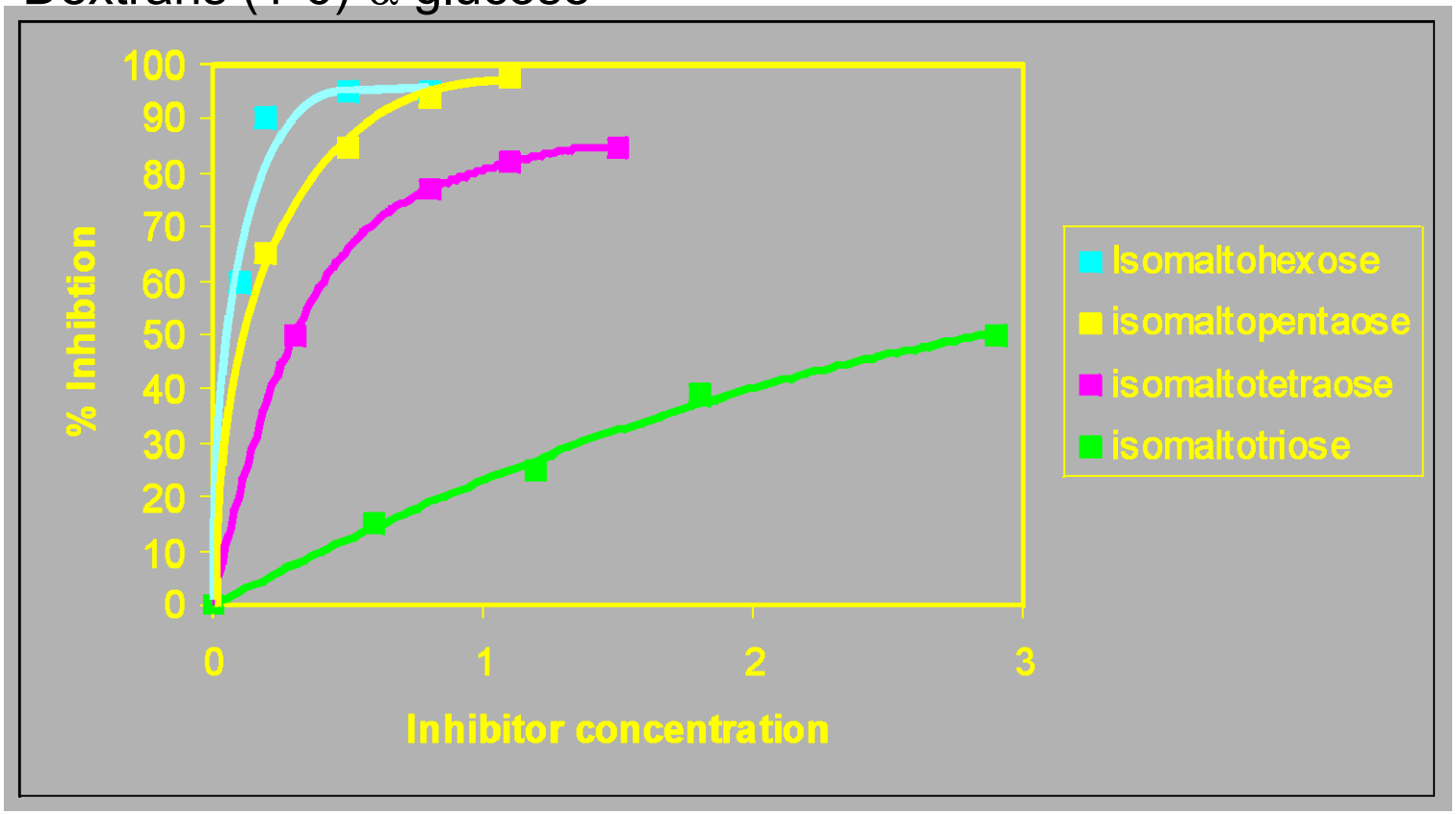




Nitz *et al*., J. *Biol. Chem.* **277,** 3440, 2002.

A 40 year old paradigm: The Size of an Antibody Site that Binds Polysaccharide Antigens

Dextrans (1-6)-α glucose



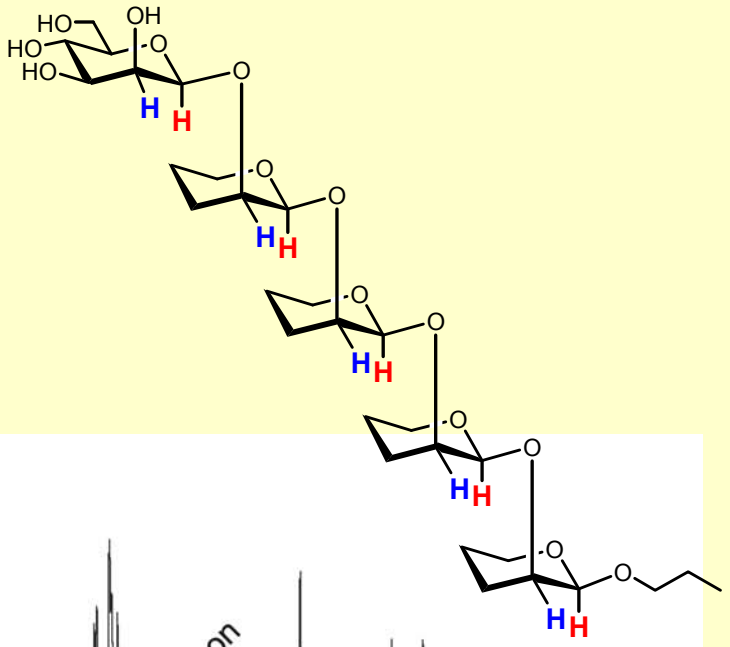
Kabat E.A and H.H. Mayer, **1961**, In *immunochemistry* Charles C. Thomas, Springfield

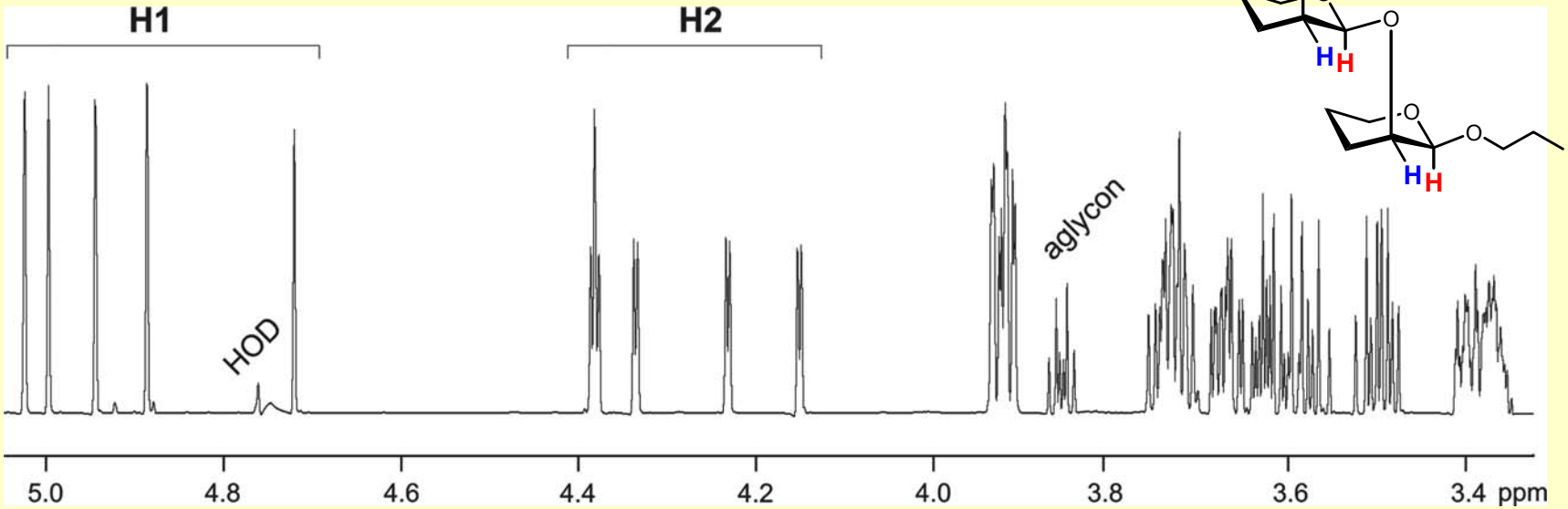
Electronic Supplementary Material (ESI) for Chemical Society Reviews

800 MHz NMR Studies of a Pentasaccharide in D_2O solution show well dispersed resonances that facilitate assignment and NOE measurements pointing to a constrained solution conformation

Solution Conformation of β-1,2-Mannan

Pentasaccharide: 800 MHz

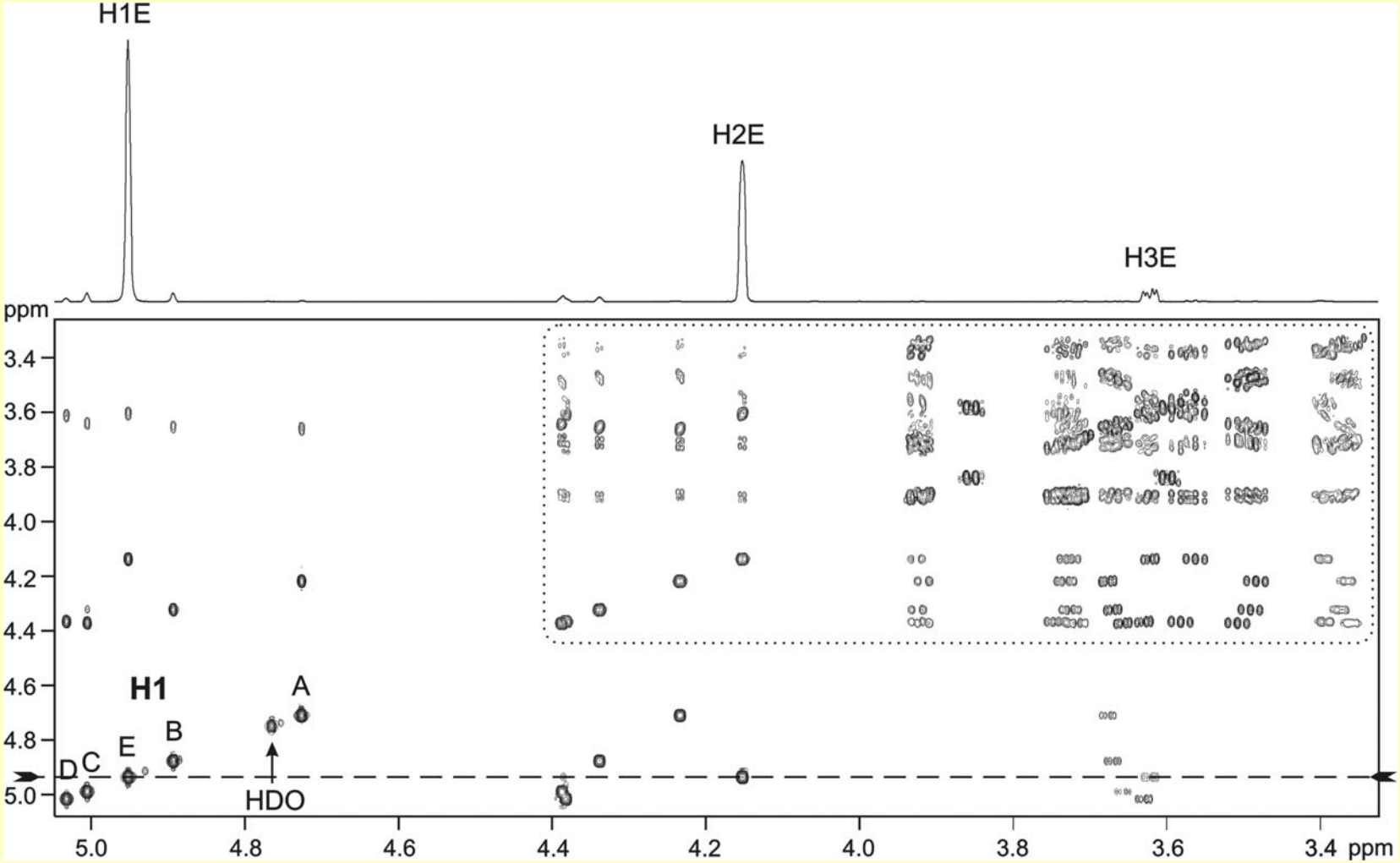




5 mM in D_2O, 30.0°C

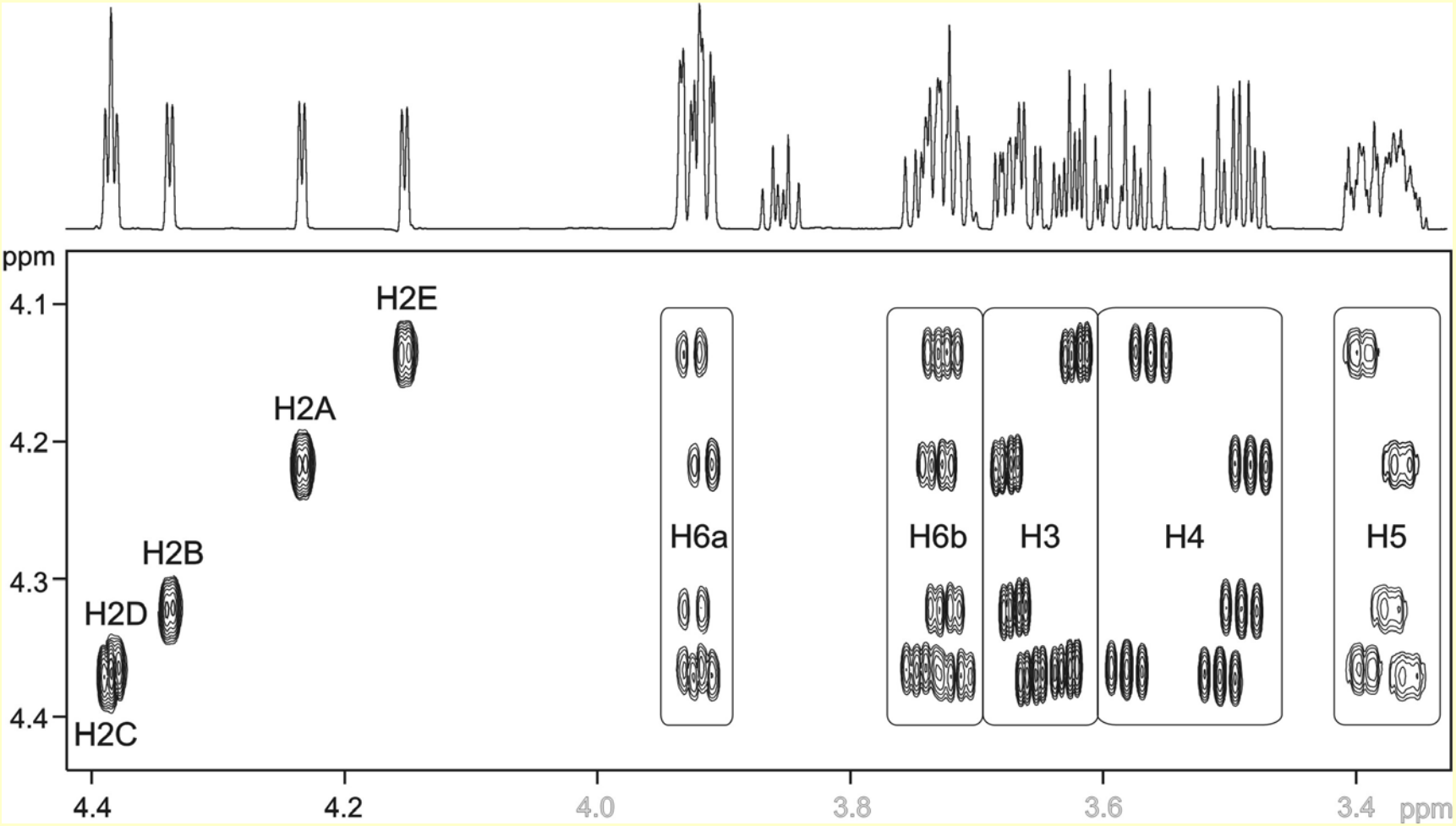
Nitz *et al*., *J. Biol. Chem.* **277,** 3440, 2002.

Pentasaccharide: 800 MHz GTOCSY



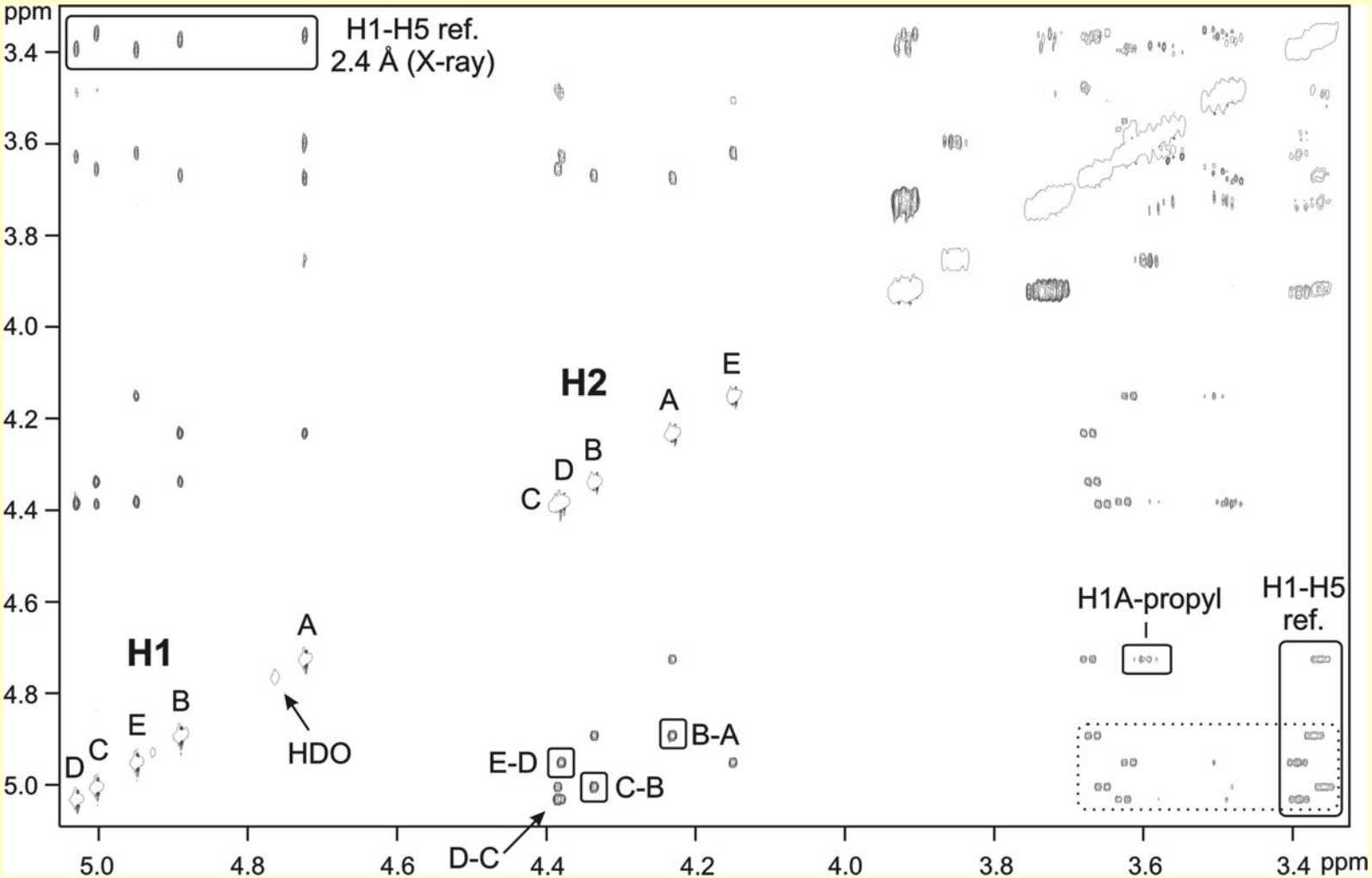
nt=1, spin lock: mix=130 ms, 6.6 kHz

Pentasaccharide: 800 MHz GTOCSY



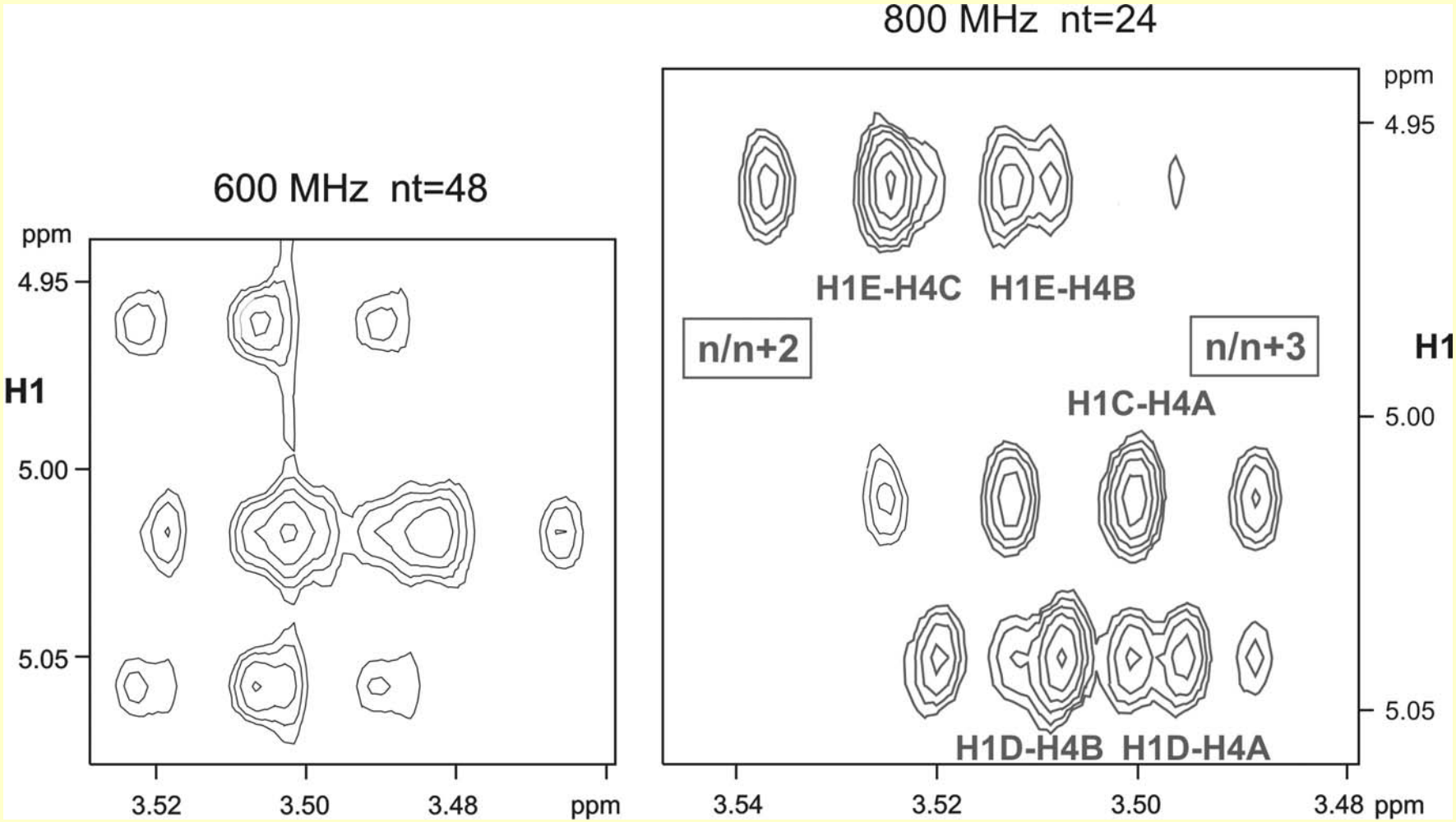
Nitz *et al*., *J. Biol. Chem.* **277,** 3440, 2002.

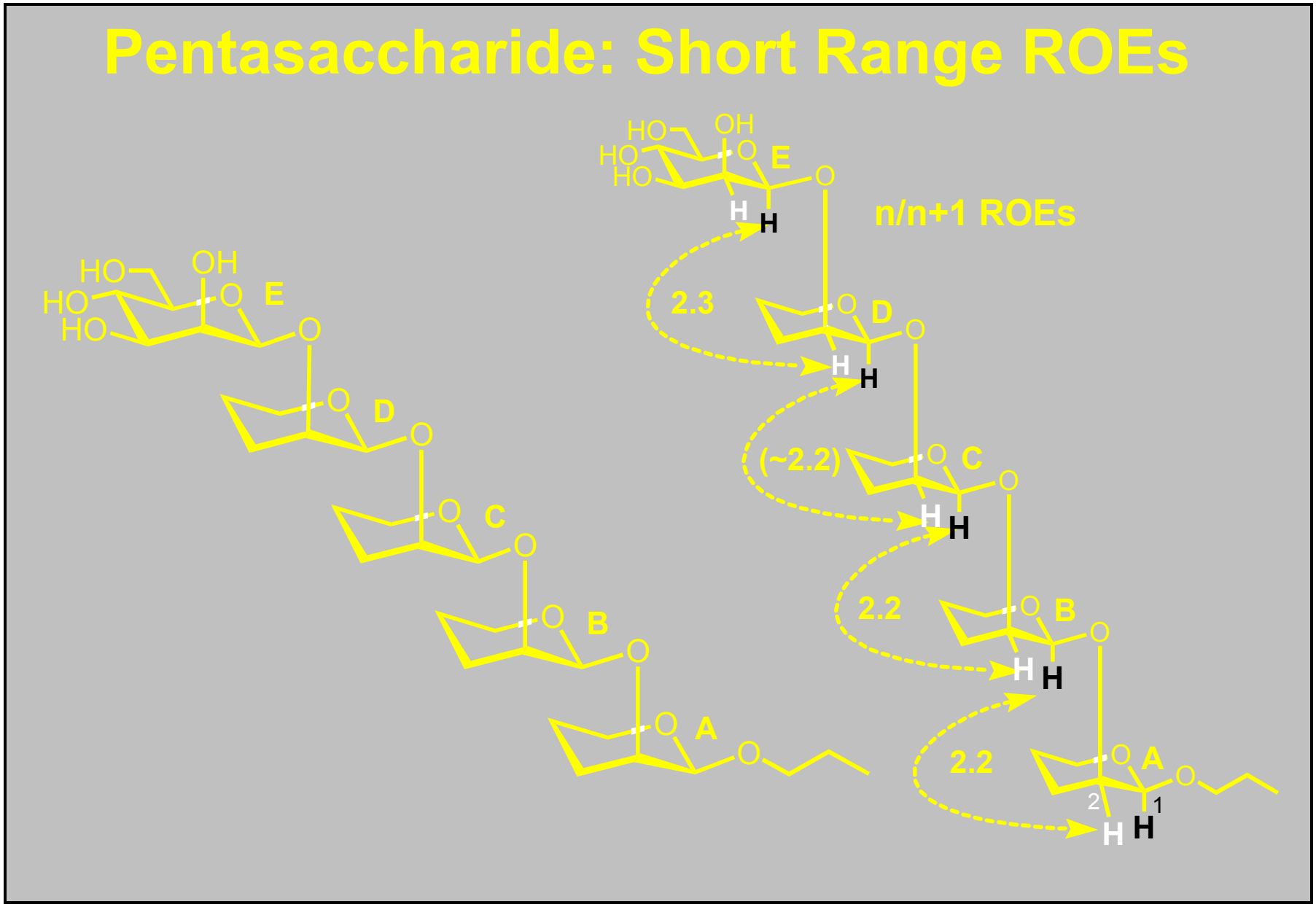
Pentasaccharide: 800 MHz TROESY

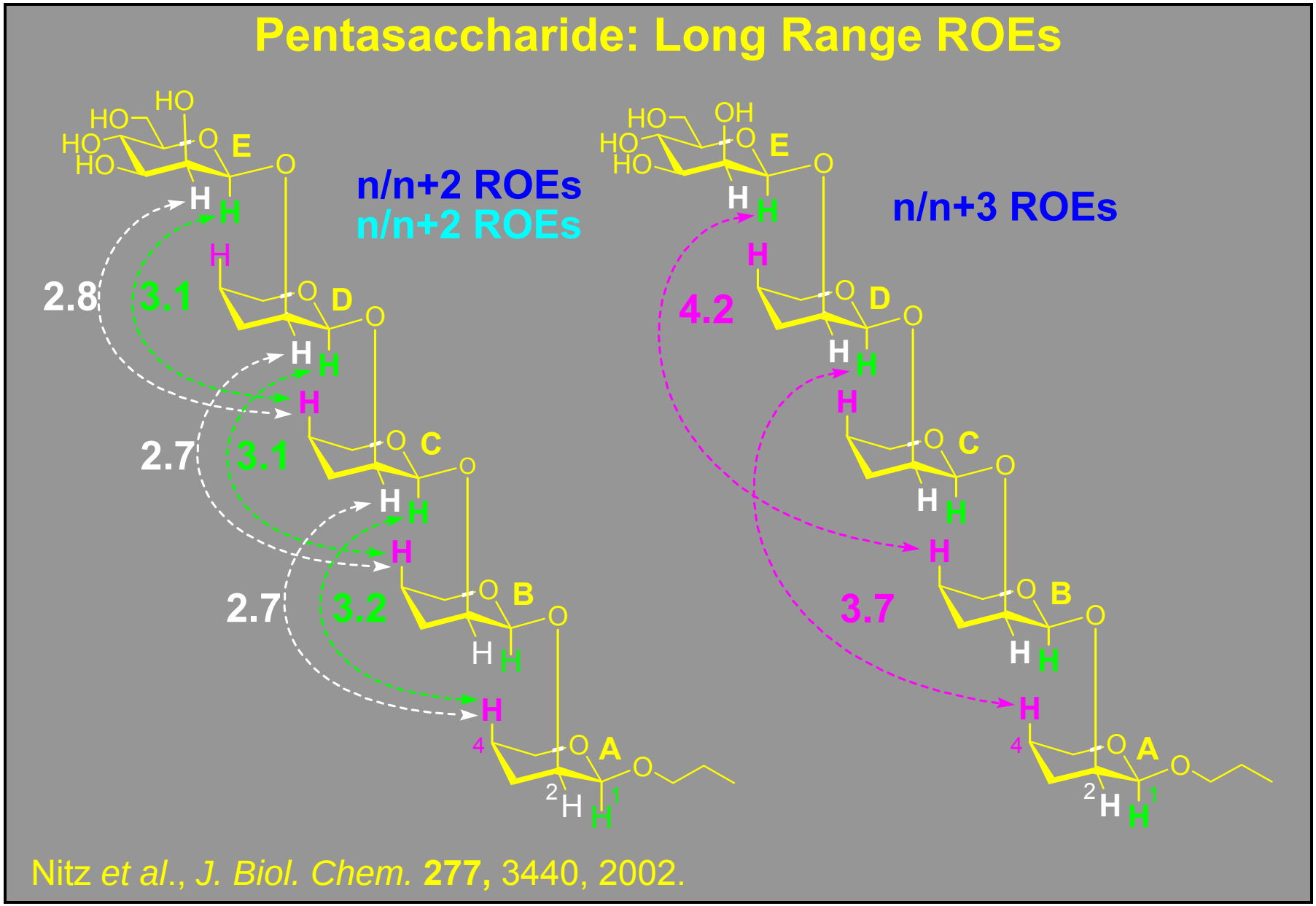


nt=24, spin lock: mix=400 ms, 4.2 kHz

Electronic Supplementary Material (ESI) for Chemical Society Reviews

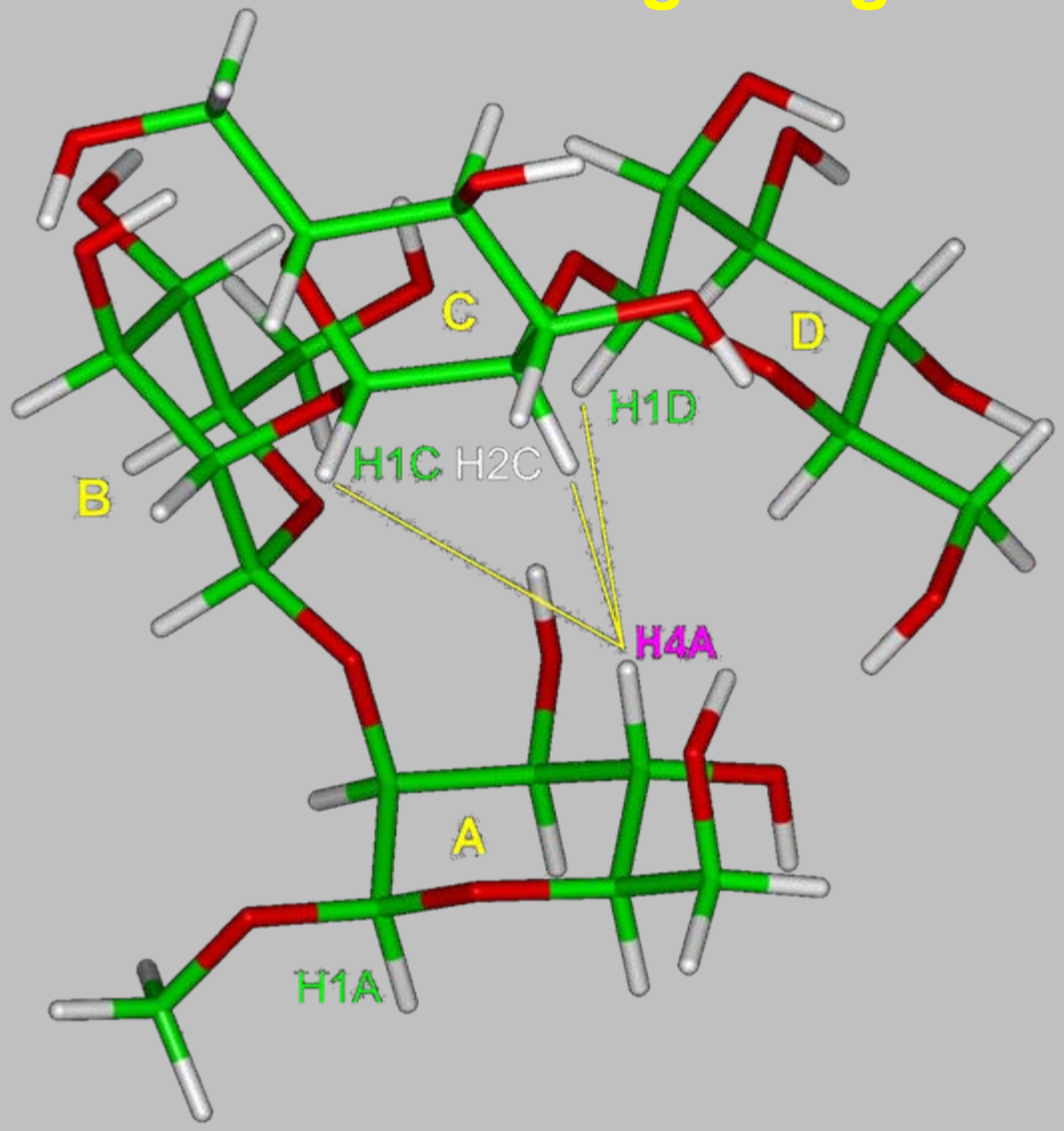




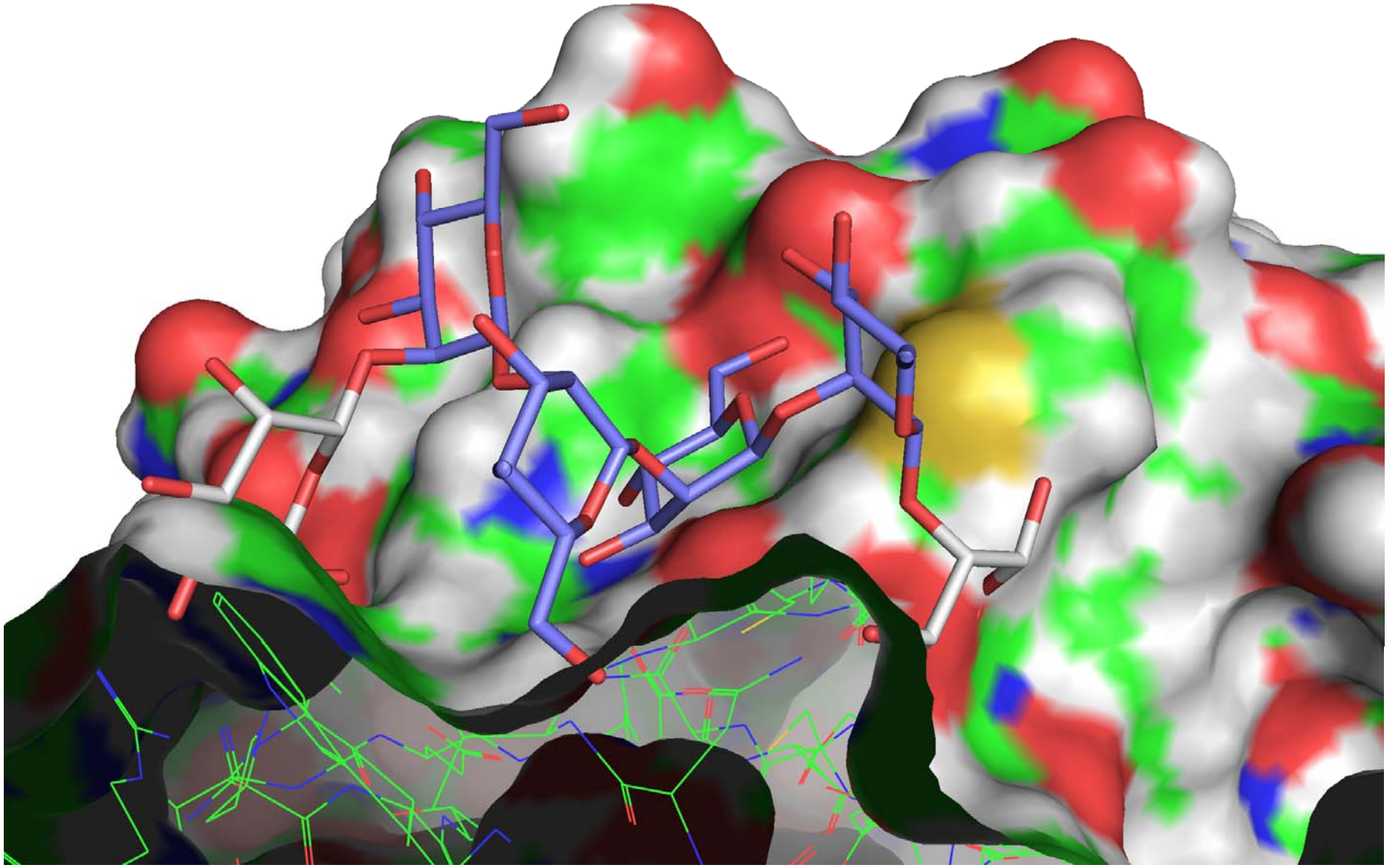


Electronic Supplementary Material (ESI) for Chemical Society Reviews

Tetrasaccharide: Long Range ROEs



Larger Oligosaccharides Clash with Antibody Site



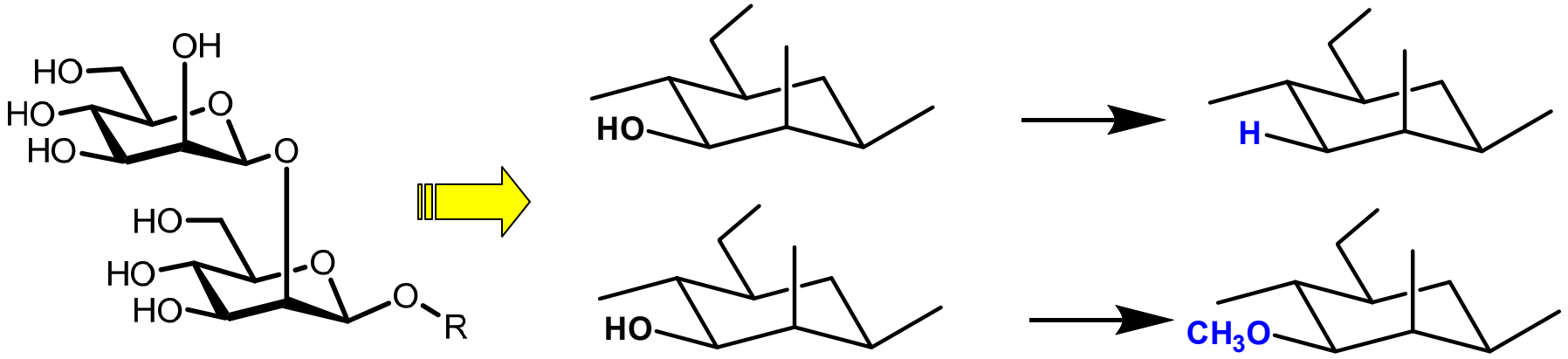
Johnson *et al., J. Biol. Chem.* **287**, 18078-18090, 2012.

Mapping the Carbohydrate Epitope of *Candida albicans* Protective Antibody C3.1

Functional Group Replacement

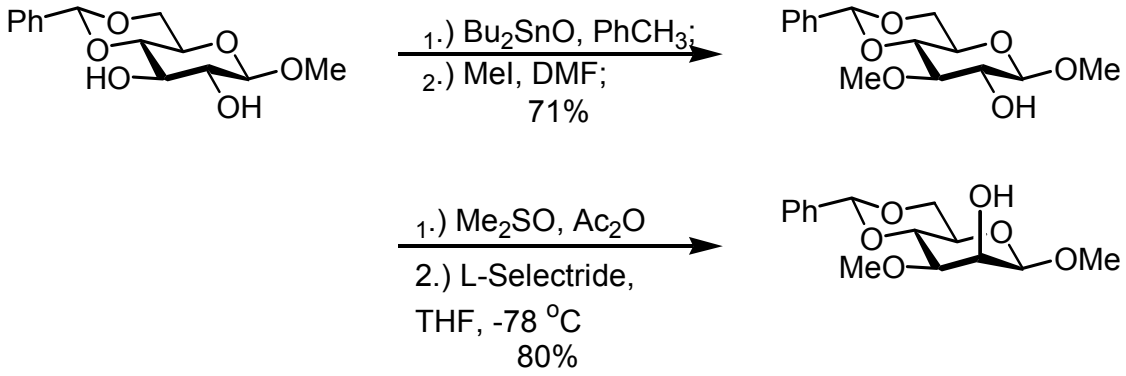
Sequential hydroxyl group substitution

Nikrad, Beierbeck and Lemieux. A Novel Procedure for the Detection of the Intermolecular Hydrogen Bonds Present in a Protein-Oligosaccharide Complex, *Can. J. Chem.,* **70**, 241-253, 1992.

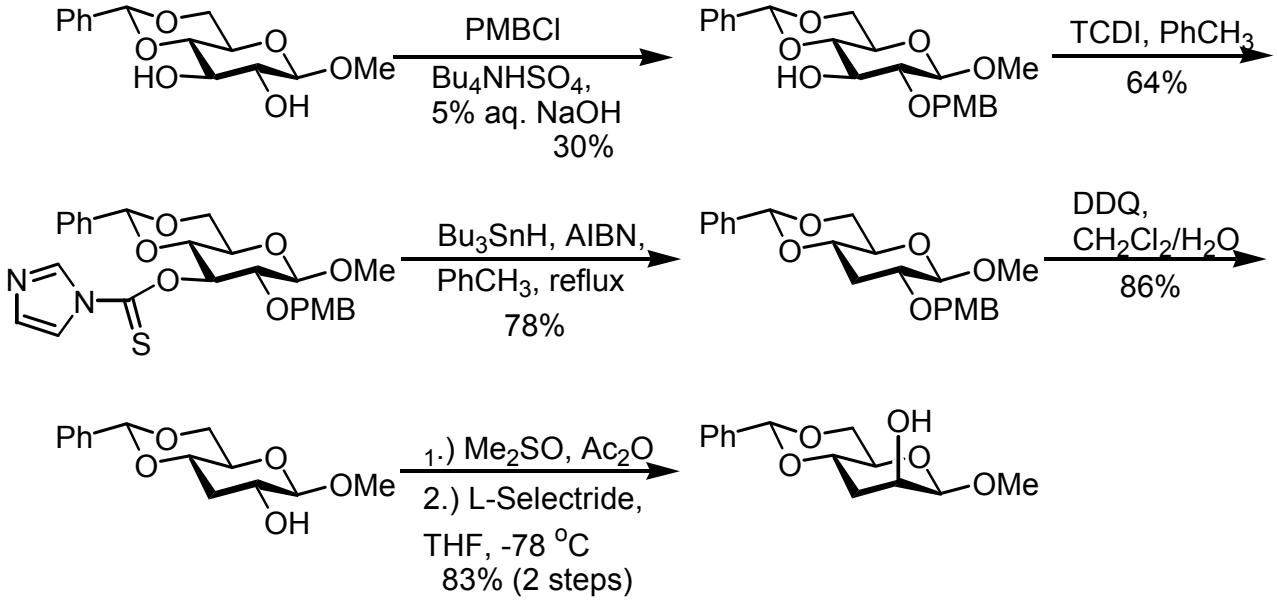


Nycholat and Bundle *Carbohydr. Res*., **344**, 555-569, 2009.
Bundle, et al., *ACS Chem. Biol.,* **7**, 1754-1763, 2012.

Synthesis of 3-*O*-Methyl β-Mannopyranoside

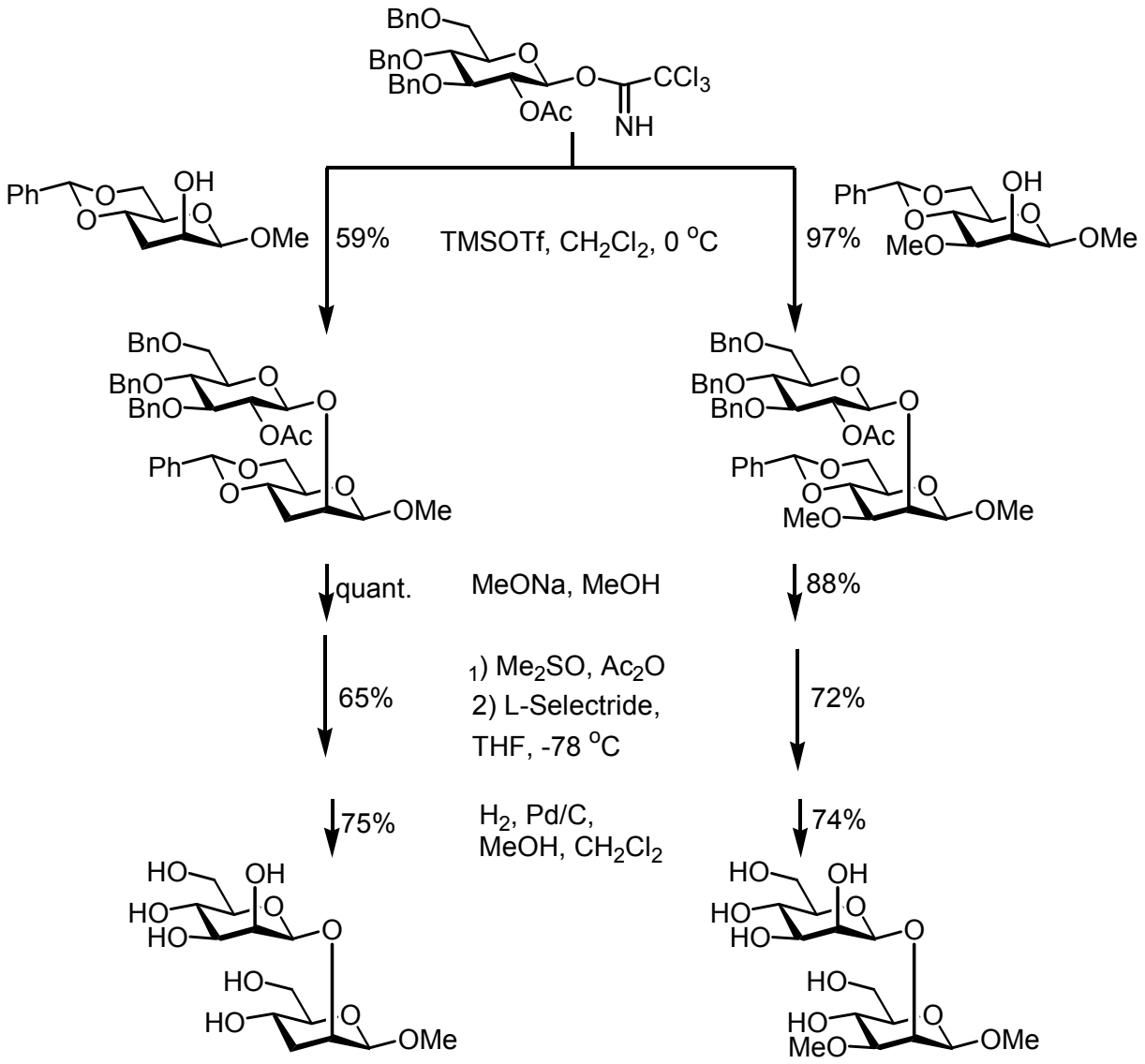


Synthesis of 3-*O*-Deoxy β-Mannopyranoside



Nycholat and Bundle *Carbohydr. Res.*, **344**, 555-569, 2009.

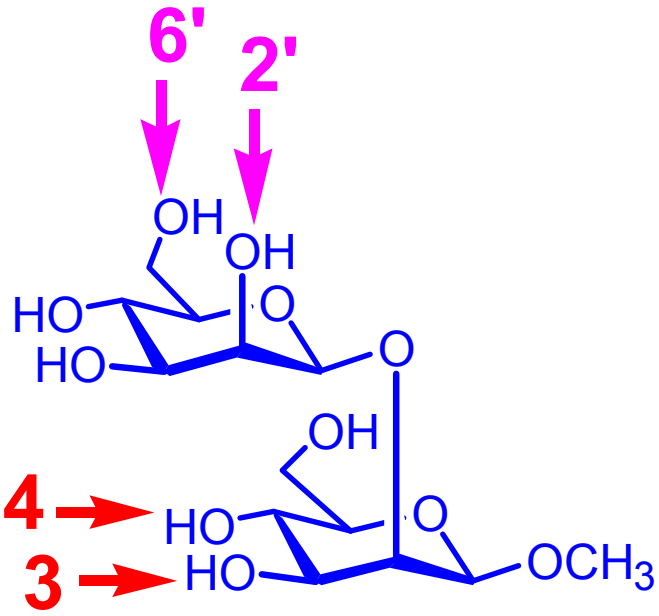
Synthesis of the 3-deoxy and 3-*O*-methyl β-Mannobiosides



Nycholat and Bundle *Carbohydr. Res.*, **344**, 555-569, 2009.

Inhibition by Modified Disaccharide Glycosides

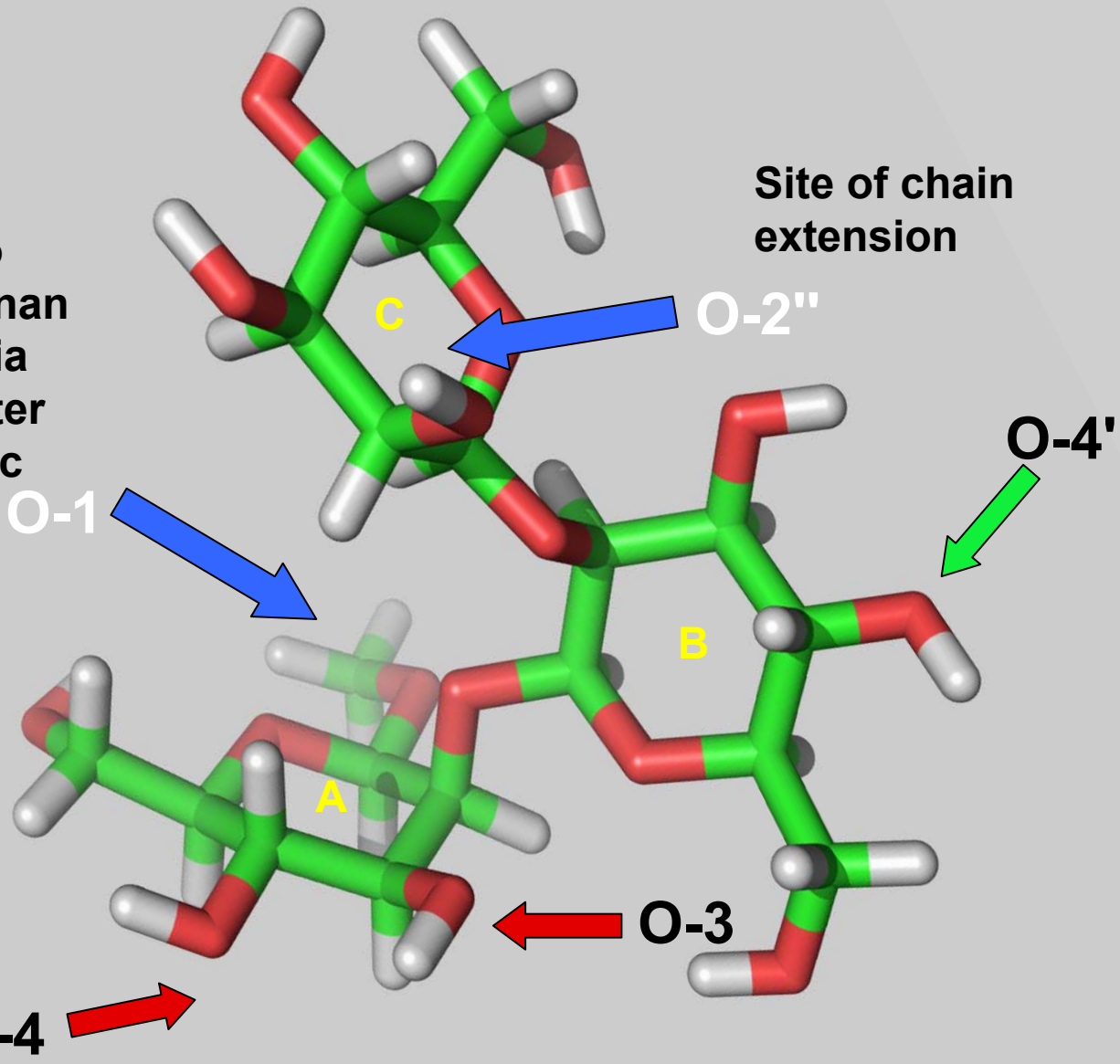
Functional Group Replacement	Deoxy Relative potency	*O*-Methyl Relative potency
3-OH	inactive	inactive
4-OH	inactive	inactive
6-OH	238	38
2′-OH	ND	94
3′-OH	26	50
4′-OH	inactive	4.6
6′-OH	7.3	5.3



Relative potency = 100

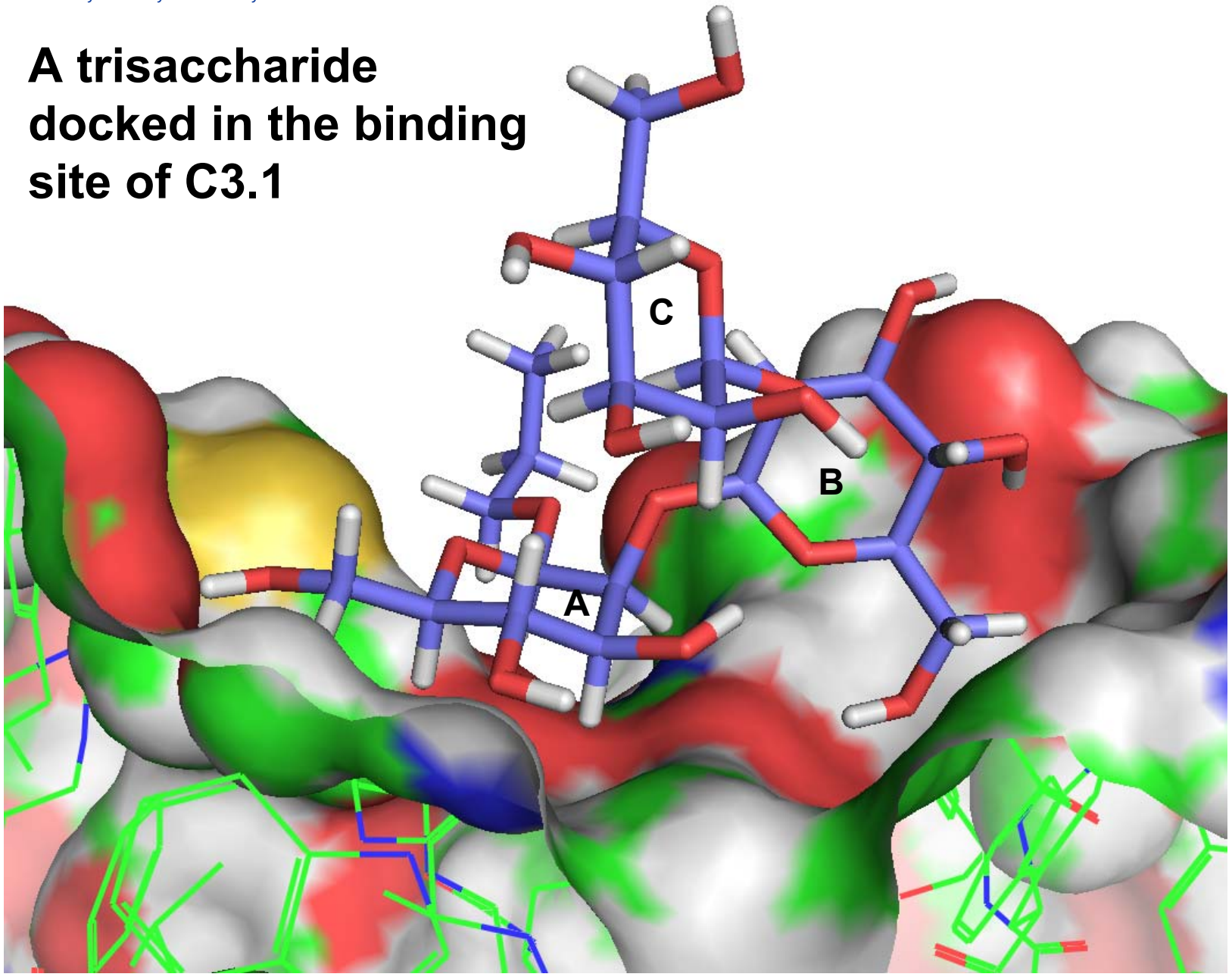
Bundle, et al., *ACS Chem. Biol.*, **7**, 1754-1763, 2012.

Key Polar Contacts (red)



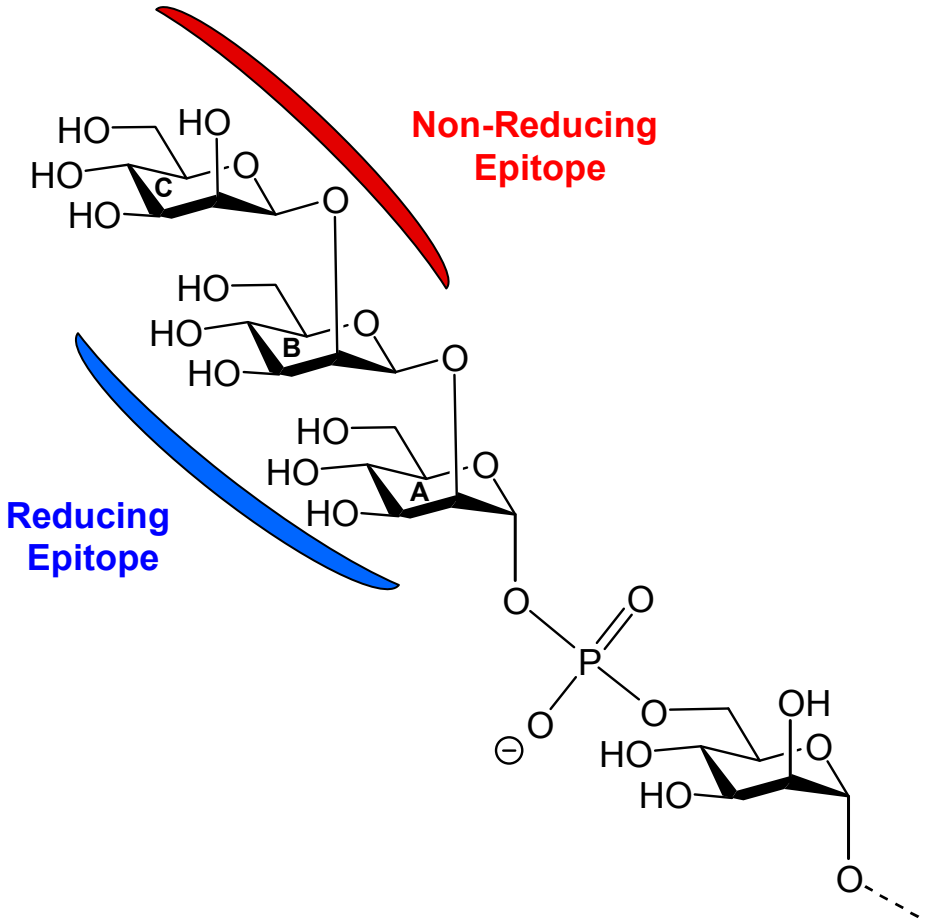
Bundle, et al., *ACS Chem. Biol.*, **7**, 1754-1763, 2012.

A trisaccharide docked in the binding site of C3.1

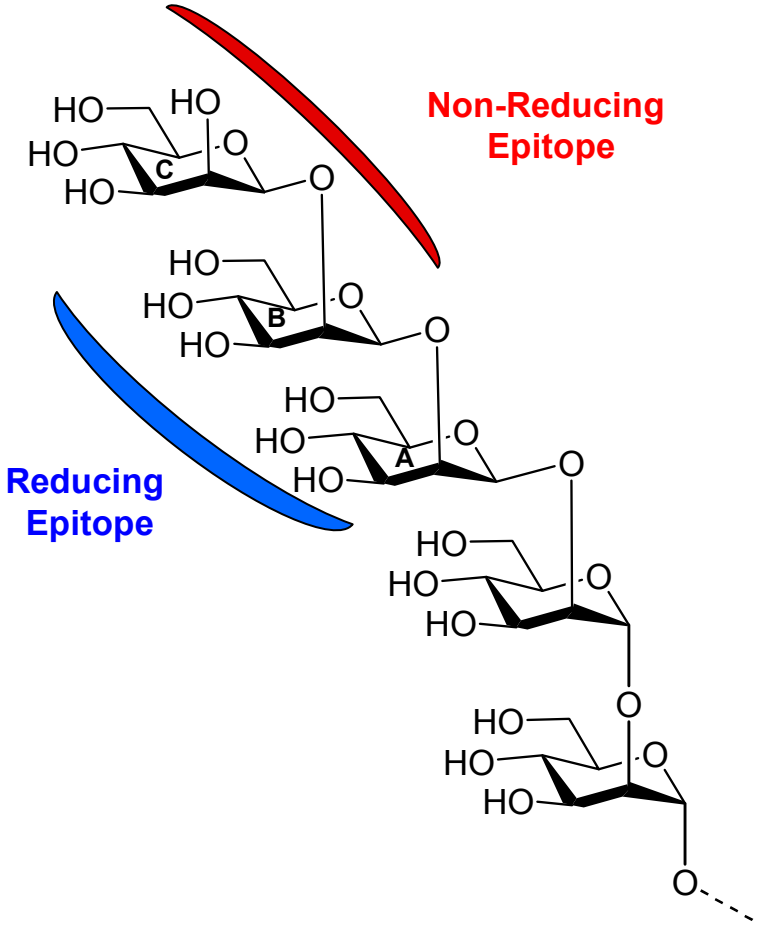


Johnson *et al., J. Biol. Chem.* **287**, 18078-18090, 2012.

Which β-Mannan is the Protective Epitope?

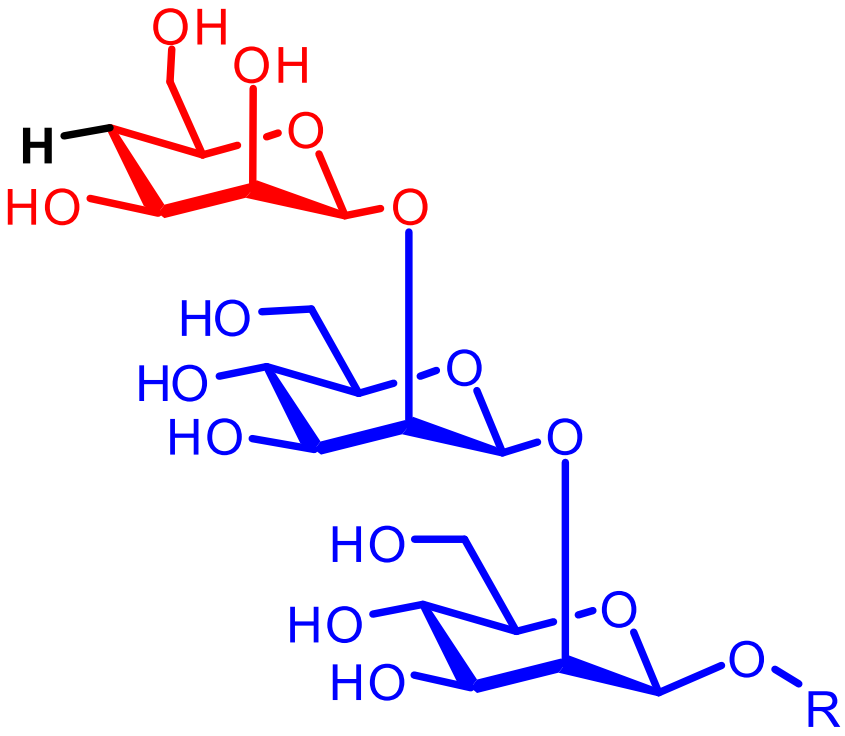


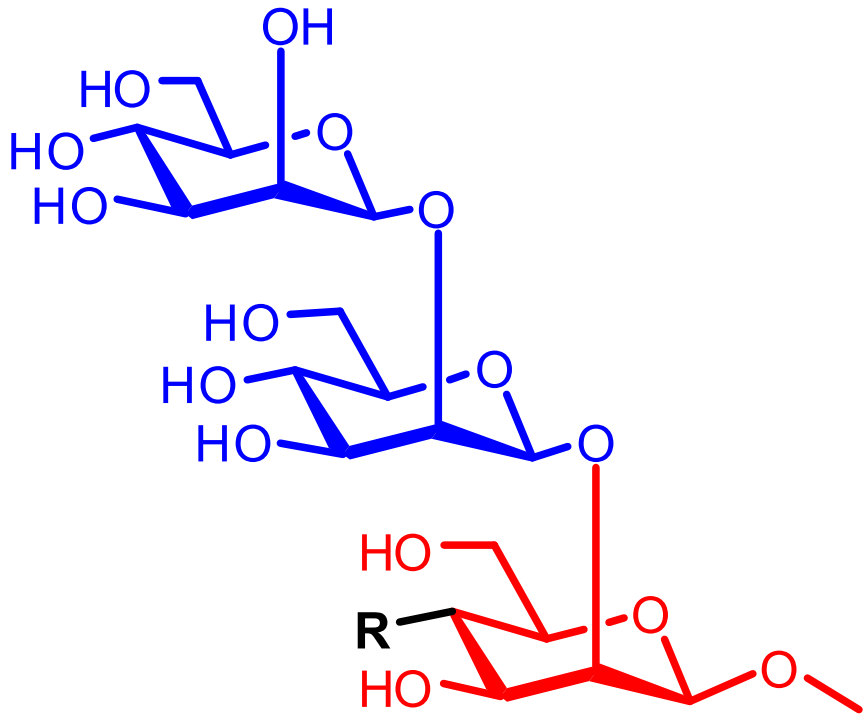
Phosphodiester linked



Glycosidically linked

Freezing Epitope Frame Shifting

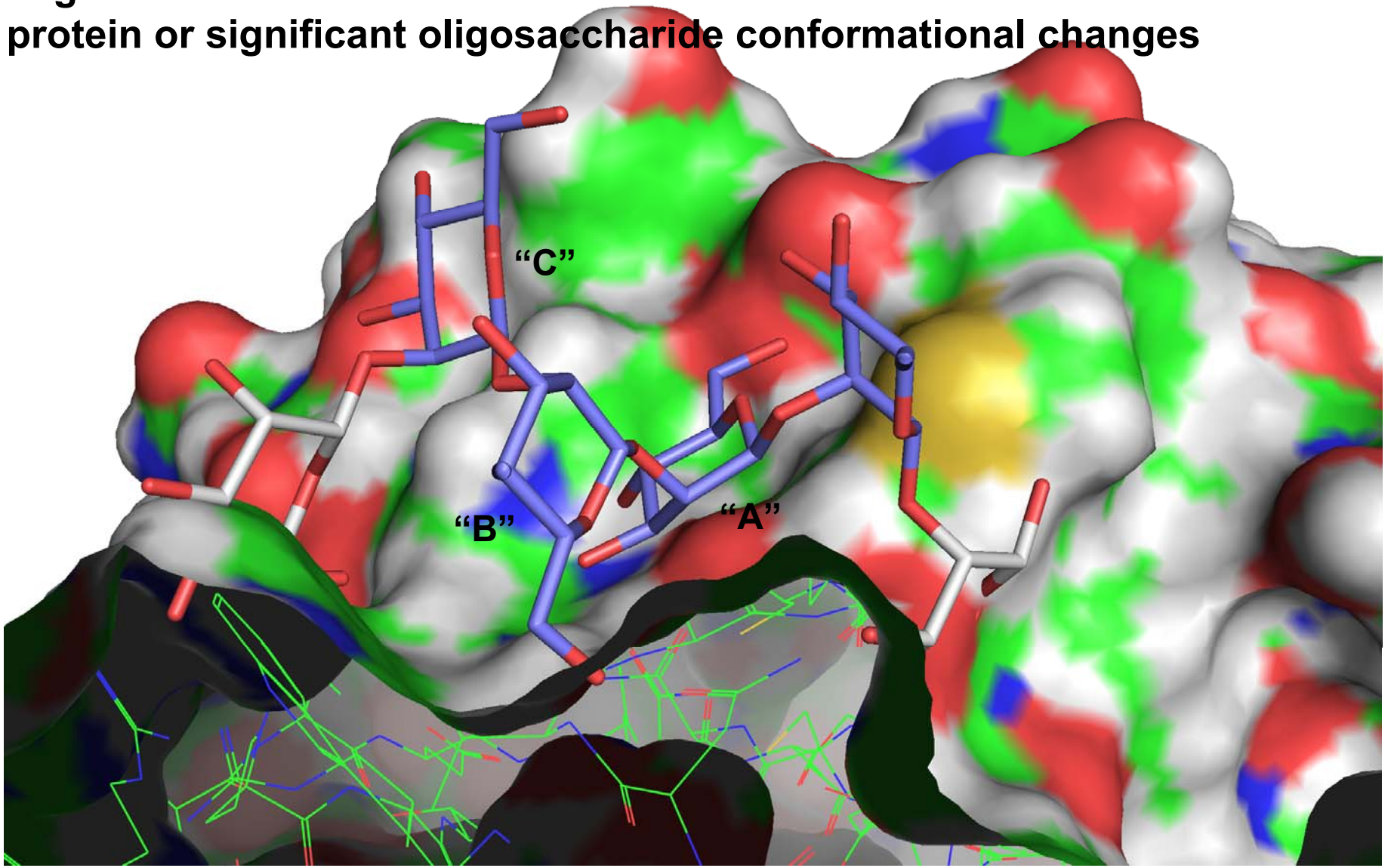




R = H
R = OMe

Costello and Bundle, *Carbohydr. Res*., 357, 7-15, 2012.
Bundle, *et al*., *ACS Chem. Biol.,* **7**, 1754-1763, 2012.

The Limits of Frame Shifting: Tetrasaccharide or larger oligosaccharide are unable to frame shift without steric clash with the protein or significant oligosaccharide conformational changes



Johnson *et al., J. Biol. Chem.* **287**, 18078-18090, 2012.

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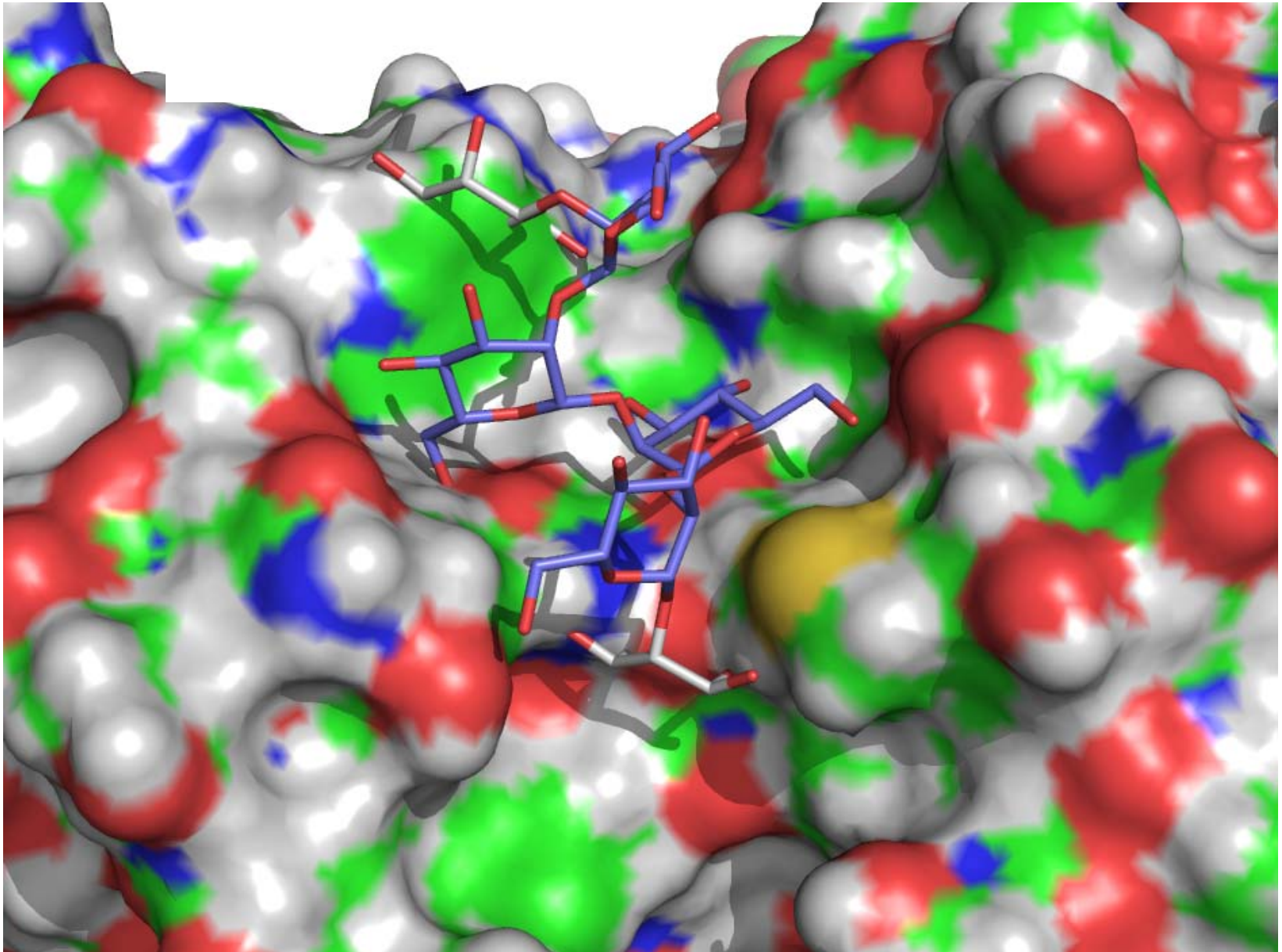
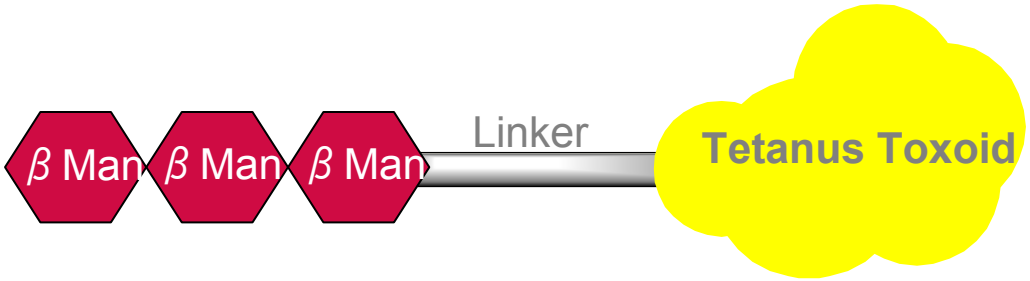
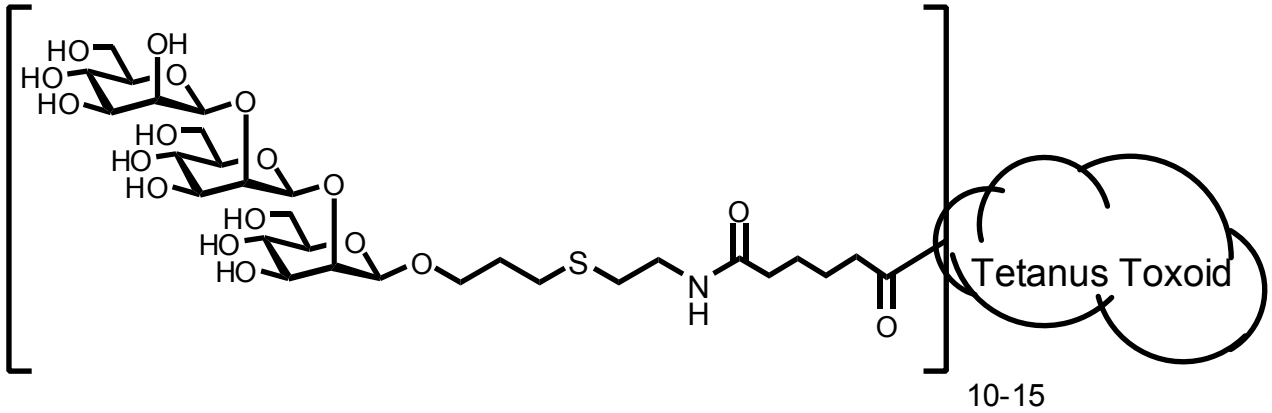
Allowed Binding Area for (1→2)-β-Mannans Accommodates Four Residues



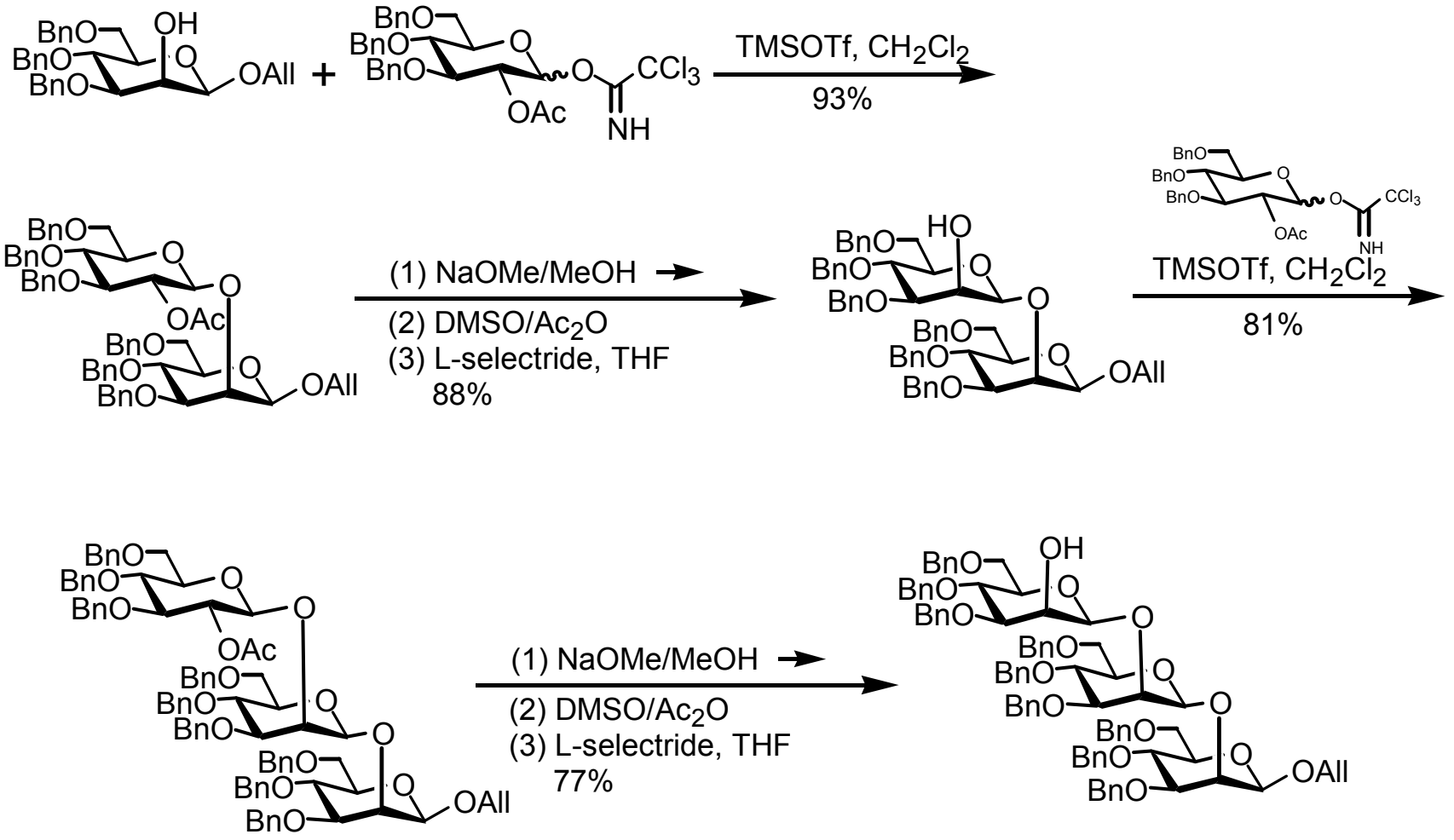
Synthesis, Conjugation and Evaluation of a Trisaccharide Conjugate Vaccine



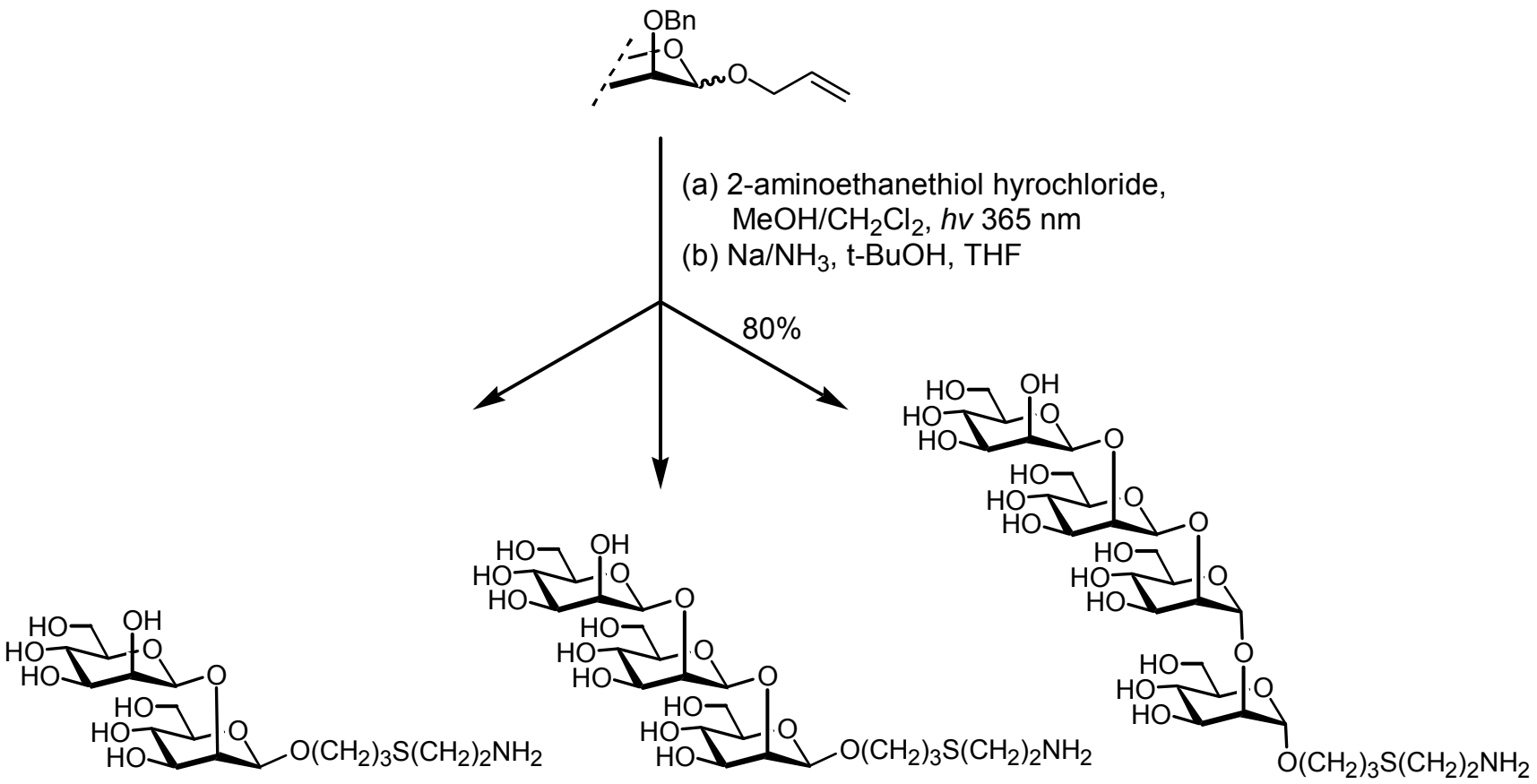
- Gives high antibody titres in rabbits
- Reduces fungal load in live challenge experiments
- Mediocre response in mice



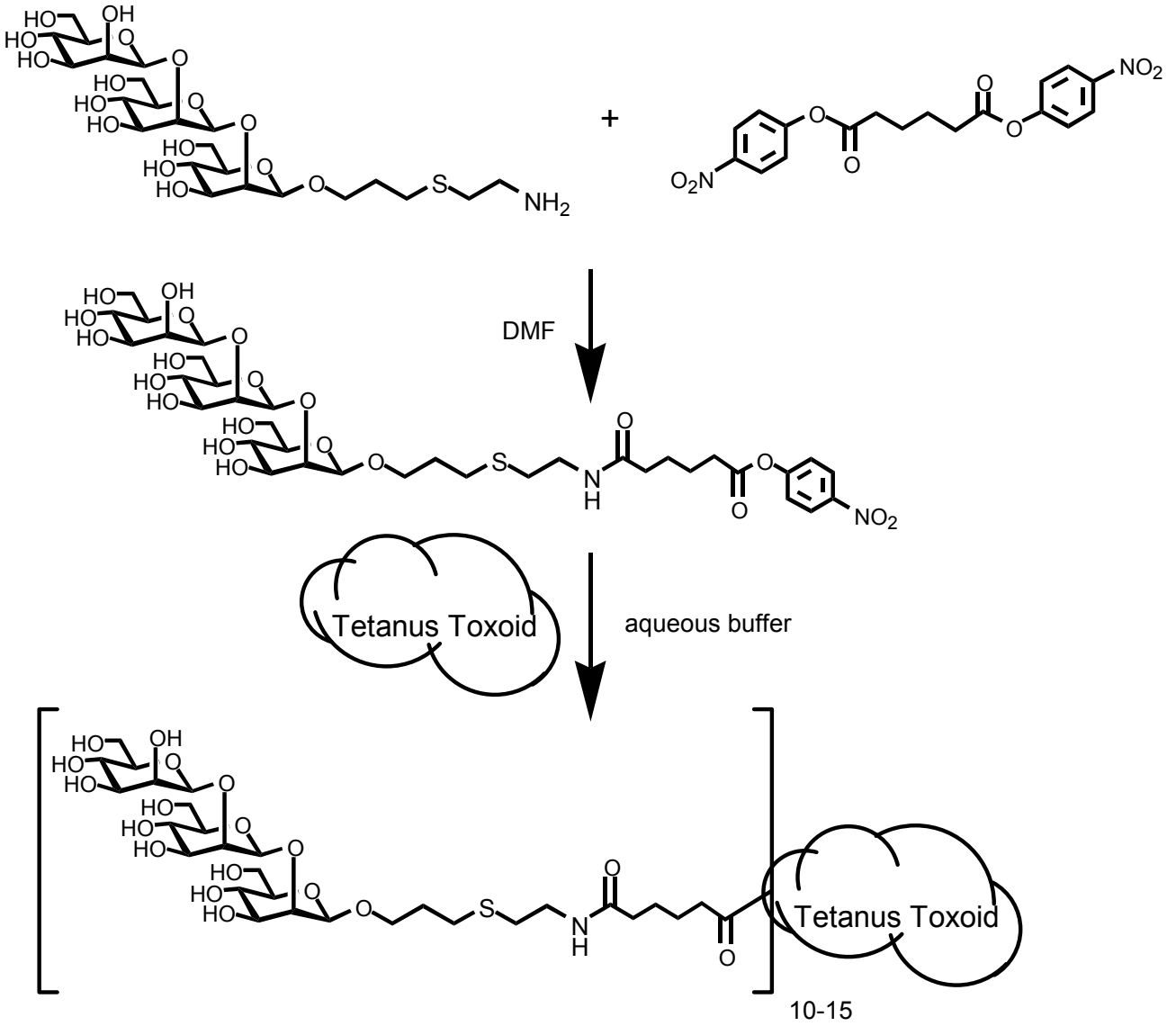
Wu, X.; Bundle, D. R. *J. Org. Chem.*, **70**, 7381-7388, 2005.



Kochetkov, N. K *et al*. *Carbohydr. Res*. **33**, C5, 1974.
Ekborg, G.; Lönngren, J.; Svensson, S. *Acta Chem. Scand*. **B29**, 1031, 1975.
Mathew, F.; Mach, M.; Hazen, K. C.; Fraser-Reid, B. *Synlett*, **9**, 1319, 2003.



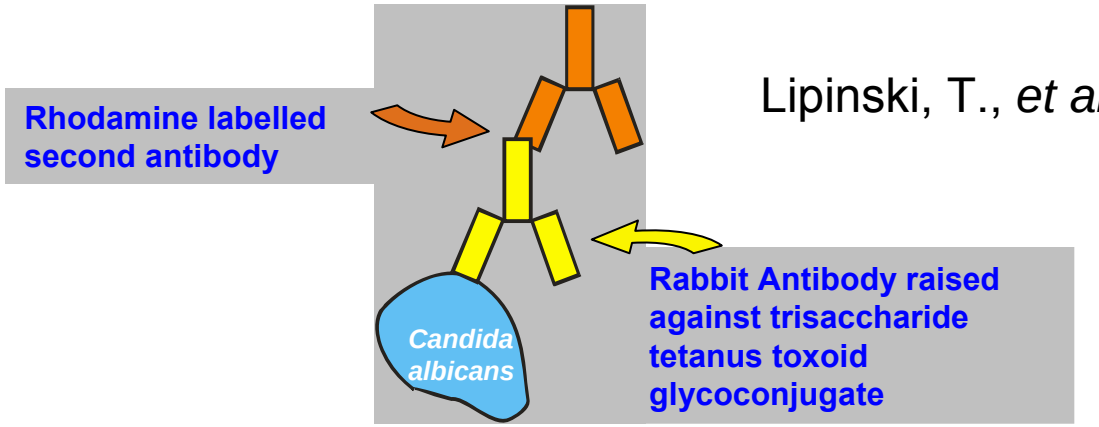
Wu, X.; Bundle, D. R. *J. Org. Chem.*, **70**, 7381-7388, 2005.



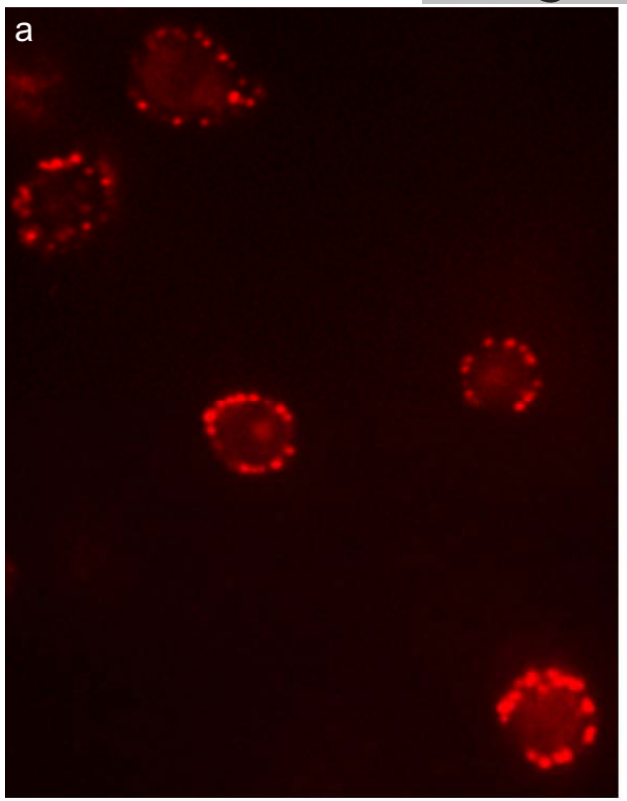
Wu, X.; Bundle, D. R. *J. Org. Chem.*, **70**, 7381-7388, 2005.

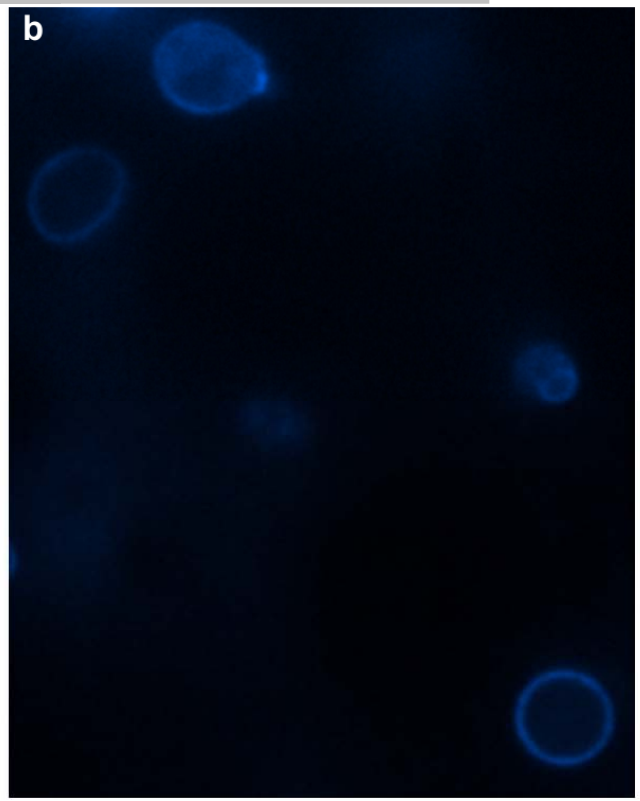
Electronic Supplementary Material (ESI) for Chemical Society Reviews

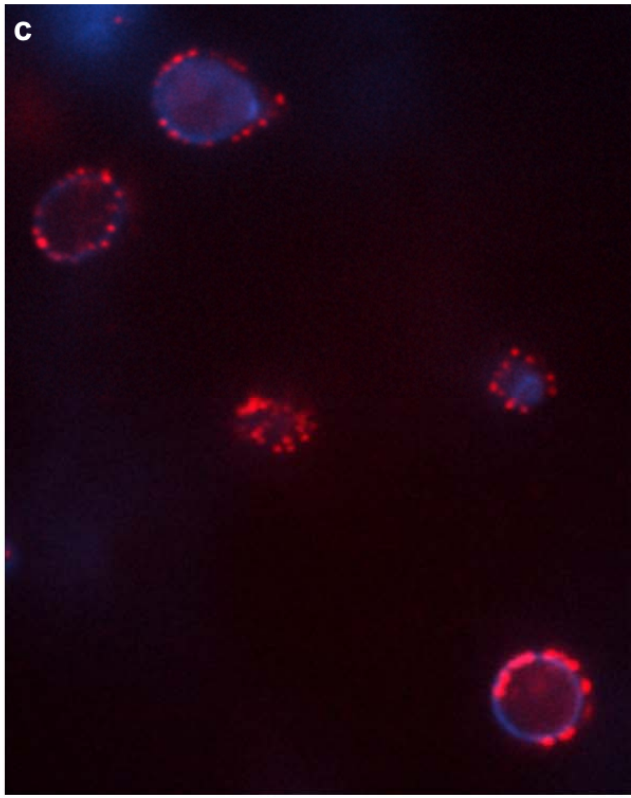
Trisaccharide Vaccine Induces Antibodies that bind to Native Antigen on Candida Cells



Lipinski, T., *et al*., *Vaccine*, **30**, 6263- 6269, 2012.



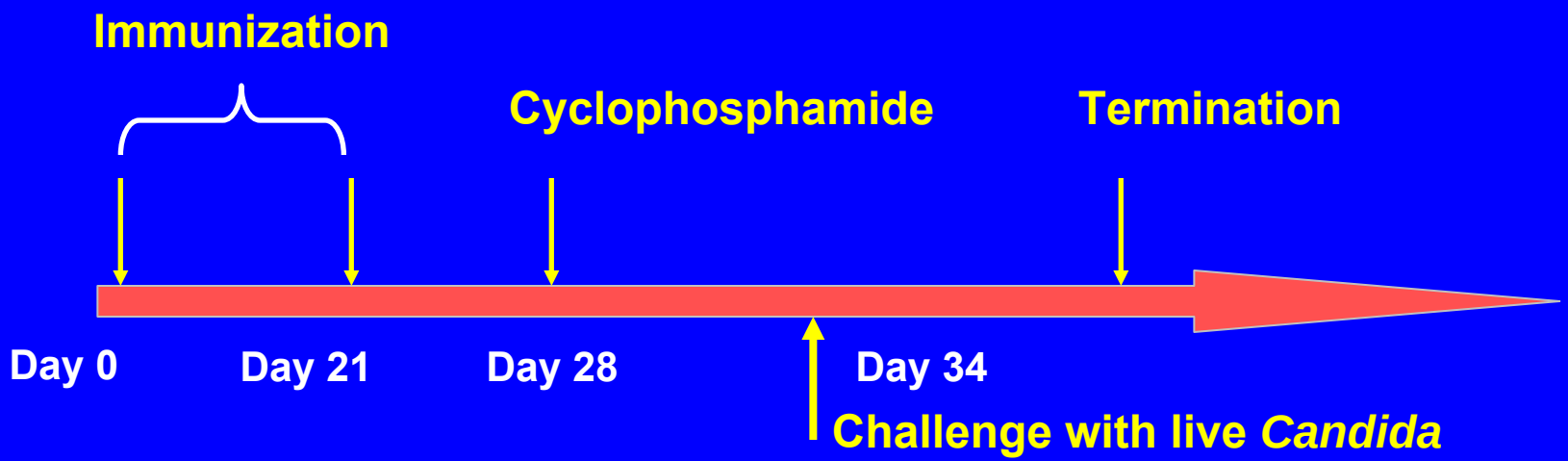




Rabbit Challenge

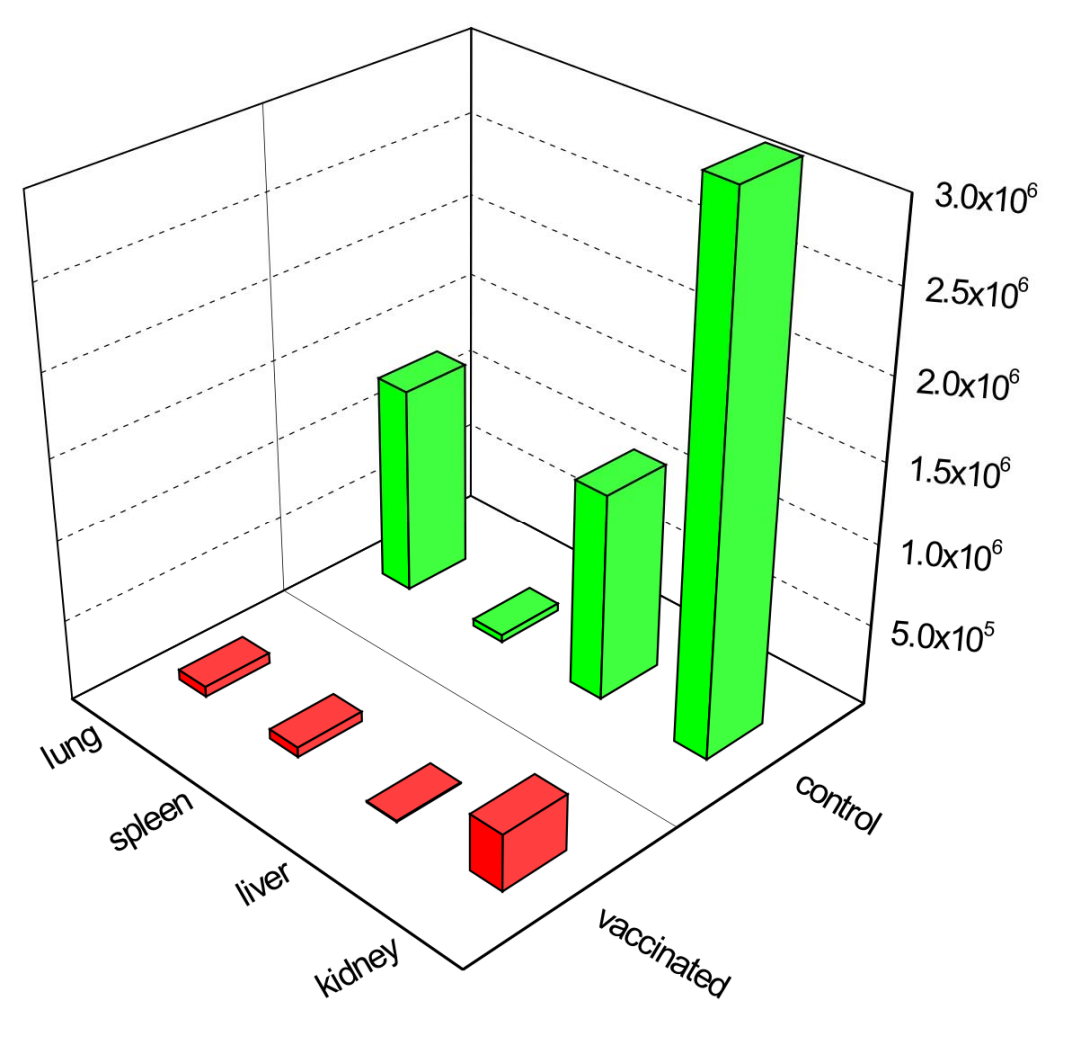
Protection experiments in neutropenic rabbits

Rabbits immunized twice with oligosaccharide-TT conjugates, rendered neutropenic and then challenged with live *C. albicans.*



Lipinski, T., *et al*., *Vaccine*, **30**, 6263-6269, 2012

Candida counts in New Zealand While Rabbits Immunized with Trimannose-TT and TT, and Challenged with *C. albicans* (3X10^3 cfu)



Statistical analysis by Generalized Estimating Equation:
significant reduction of *Candida* counts in kidney and liver
no statistically significant effect for spleen and lungs.

Control / Vaccinated ratio of *Candida* in examined organs:
Kidney: 9.3, p=0.016;
Liver: 193.6, p=0.035;
Spleen: 1.3, p=0.8;
Lungs: 20.4, p=0.99.
p-statistical significance value

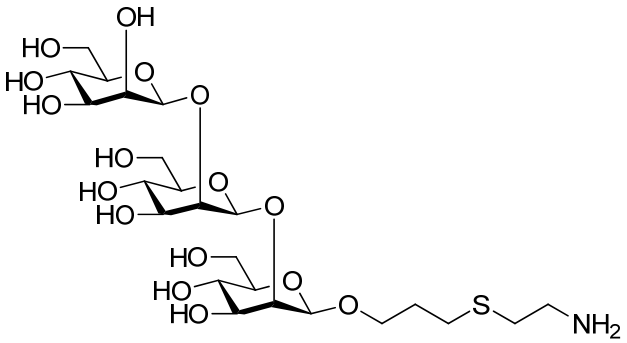
Lipinski, T., *et al*., *Vaccine*, **30**, 6263-6269, 2012.

Glycopeptide Vaccine

Components

β-mannan trisaccharide

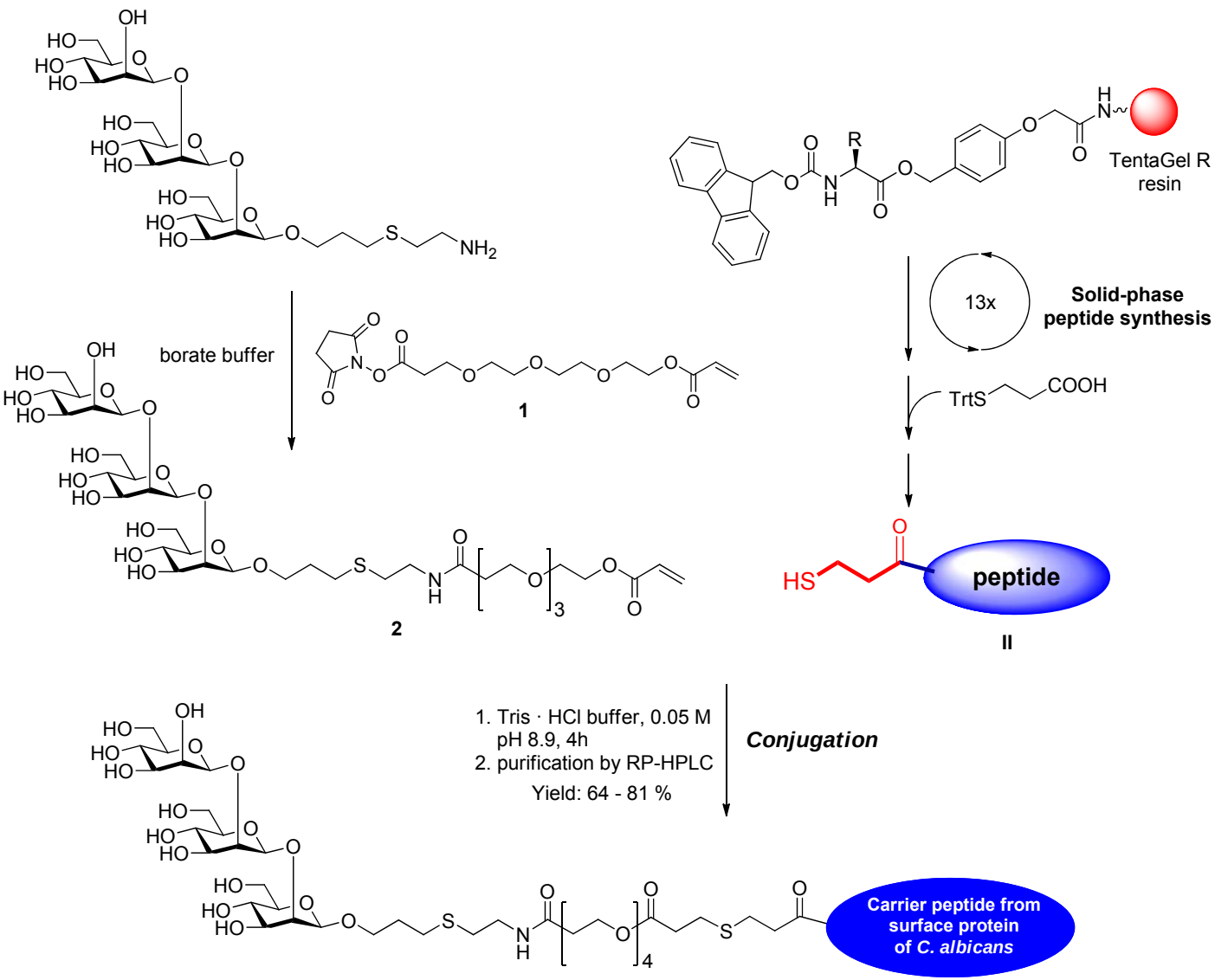
Predicted T-cell peptides from *C.albicans* cell wall proteins



Linker

YGKDVKDLFDYAQE

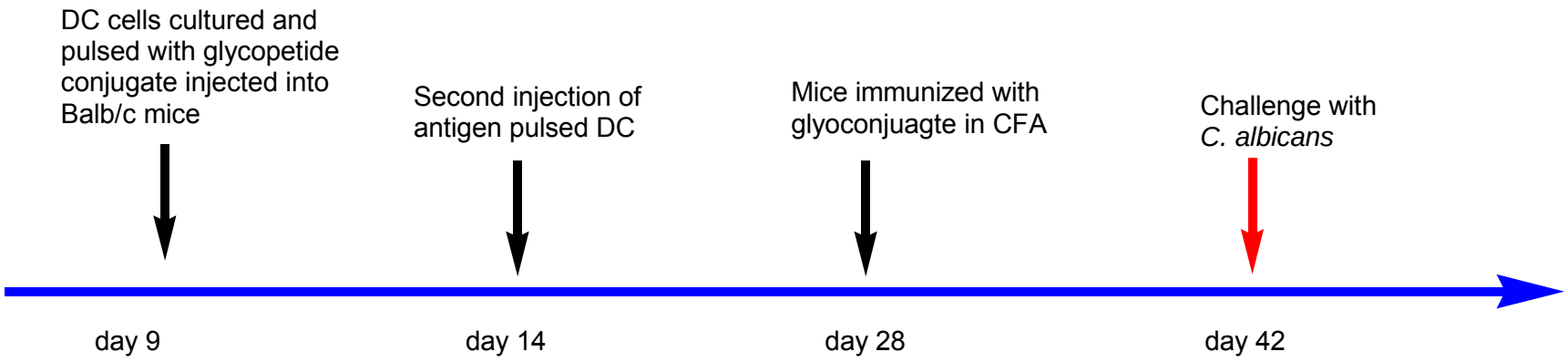
C. albicans Glycopeptide Vaccine (with Jim Cutler)



Dziadek; Jacques; Bundle. *Chem. Eur. J.*, **14**, 5908-5917, 2008.

Fungal Challenge

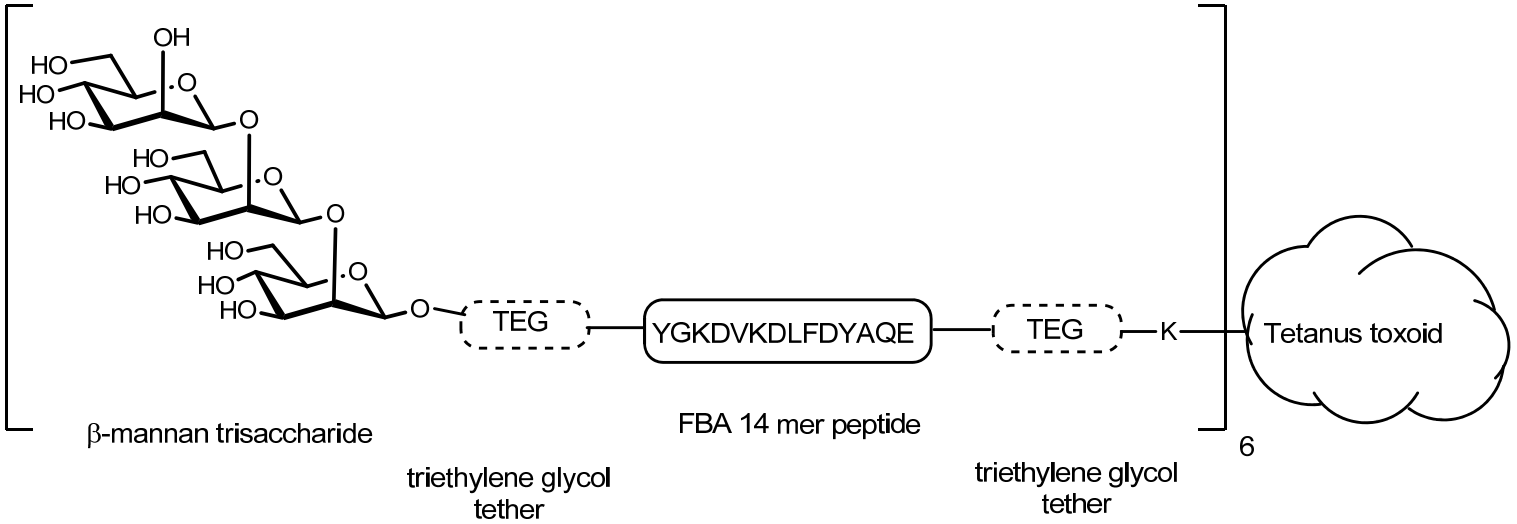
β-$(Man)_3$-Hwp1	β-$(Man)_3$-QGETEEALIQKRSY
β-$(Man)_3$-Eno1	β-$(Man)_3$-DSRGNPTVEVDFTT
β-$(Man)_3$-Pgk1	β-$(Man)_3$-VPLDGKTITNNQRI
β-$(Man)_3$-Gap1	β-$(Man)_3$-NRSPSTGEQKSSGI
β-$(Man)_3$-Fba	β-$(Man)_3$-YGKDVKDLFDYAQE
β-$(Man)_3$-Met6	β-$(Man)_3$-PRIGGQRELKKITE



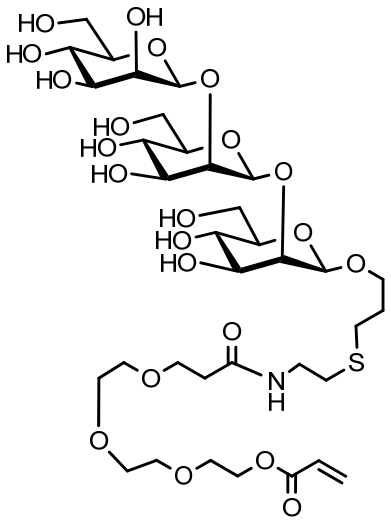
Hong, Dziadek, Bundle, Cutler *PNAS* **105**,*13*526-13531, 2008.

Electronic Supplementary Material (ESI) for Chemical Society Reviews

Avoiding Dendritic Cell Priming Glycopeptide Conjugated to Tetanus Toxoid



Building blocks



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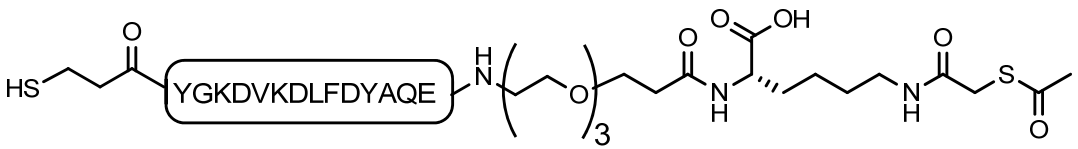
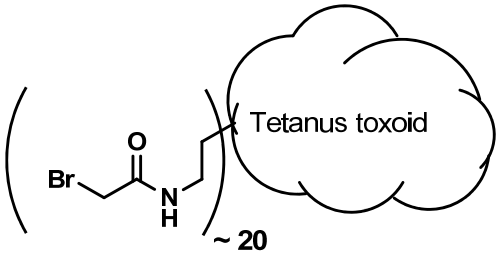


FBA

2



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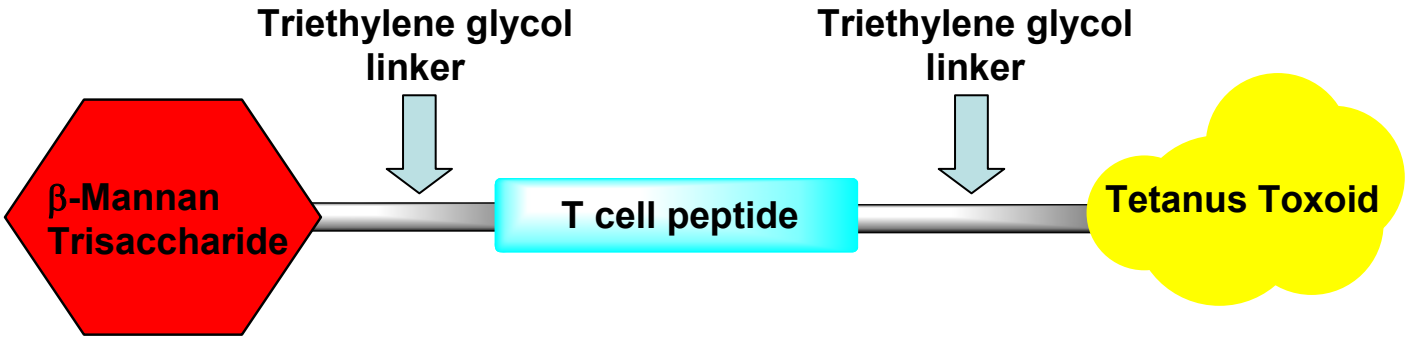

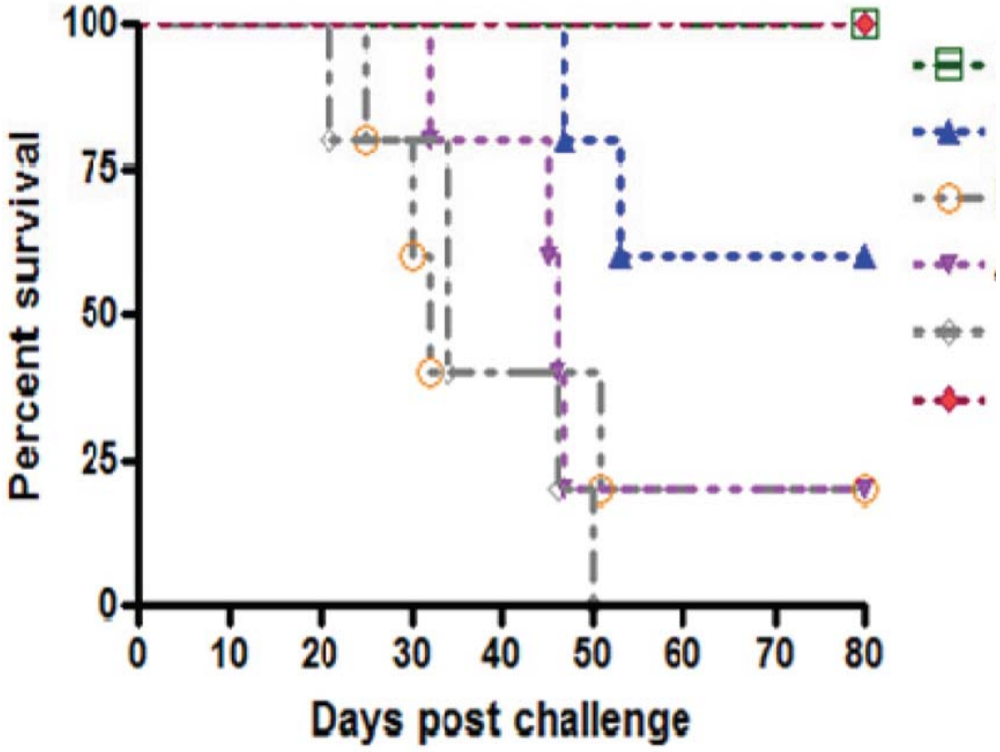

- 100% protection of mice
- Adjuvant not required

Xin *et* al., PLoS *One* **7**, e35106, 2012.