Evaluation of Cholesterol Efflux Capacity of Isomers of Hydroxypropyl-β-Cyclodextrin

Poster # P3-12

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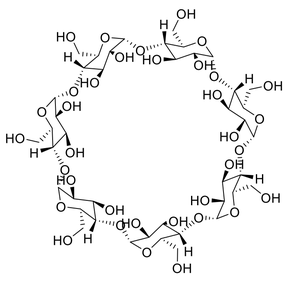
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Background

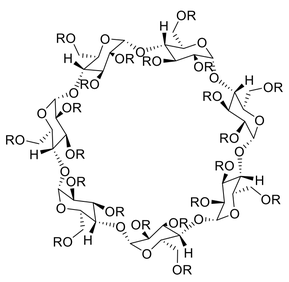
2-Hydroxypropyl-β-cyclodextrin (HPβCD) is produced as a complex mixture of regio-isomers, stereoisomers, and propylene glycol oligomers. The ability of HPβCD to bind and traffic intracellular cholesterol is central to its mechanism of action in Niemann-Pick disease type C and other neurological diseases.[1] Different average degrees of substitution (DS) of HPβCD mixtures affect solubilizing capacity, propensity to aggregate, cytotoxicity, hemolytic activity and *in vivo* toxicity.[2] Discrete isomers of HPβCD, however offer the opportunity to precisely define and optimize structural activity relationships (SAR). Selective hydroxypropyl group introduction was accomplished by alkylation of an appropriately protected cyclodextrin with a hydroxypropyl precursor that obviates oligomerization. The discrete isomers were evaluated in a cholesterol efflux capacity (CEC) assay to rank their cholesterol trafficking activity and compared to their activity in a hemolysis assay and binding energy to cholesterol measured by isothermal titration calorimetry (ITC).

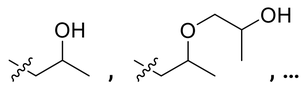
Preparation of Discrete Analogs of HPβCD

Production of HPβCD mixtures



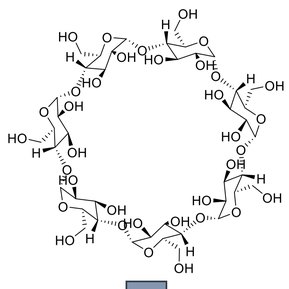
NaOH, H_2O



R= H,  , , ...

Compound Mixture Non-Tractable SAR

Beren Chemistry



Selective Protection

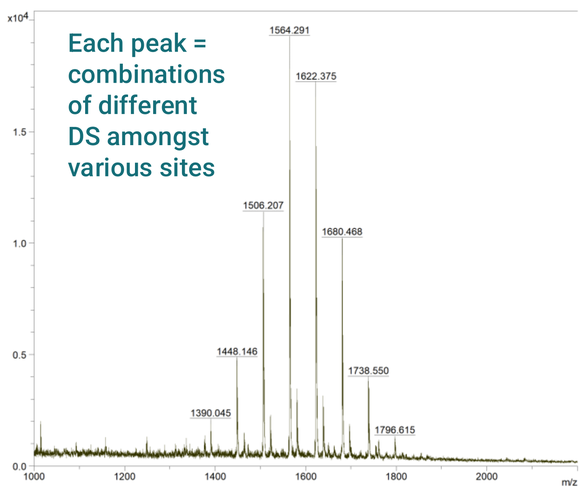
Protected Intermediate

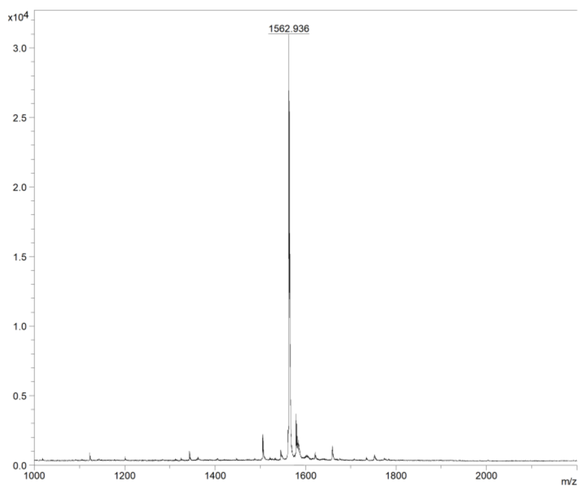
HP Precursor Introduction

HP-Functionalized Isomer

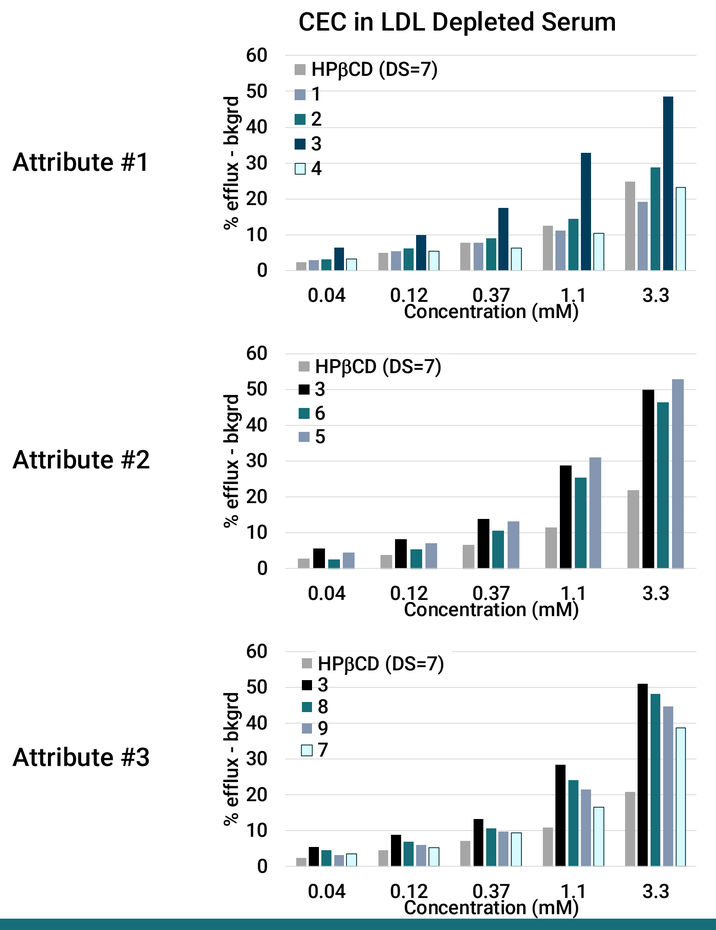
Reveal HP and PG Deprotection

Single Compound Tractable SAR

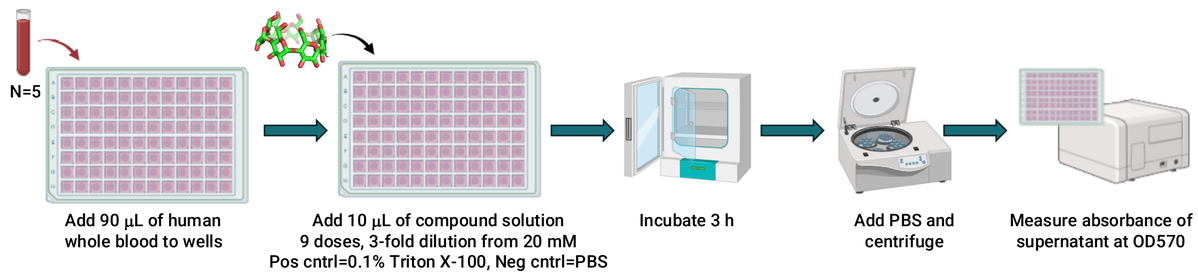


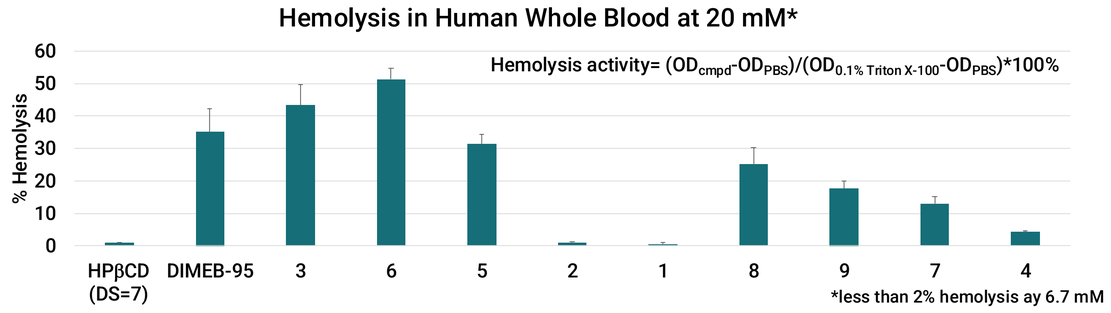


HPβCD Mixture Versus Discrete Isomers



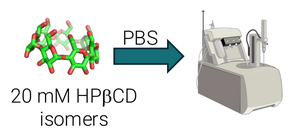
Hemolysis Assay



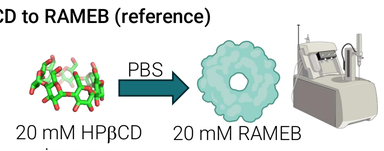


Isothermal Titration Calorimetry

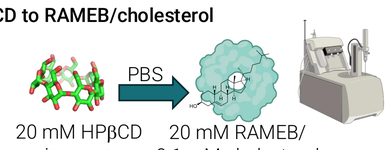
1. HPβCD to PBS (control)



2. HPβCD to RAMEB (reference)

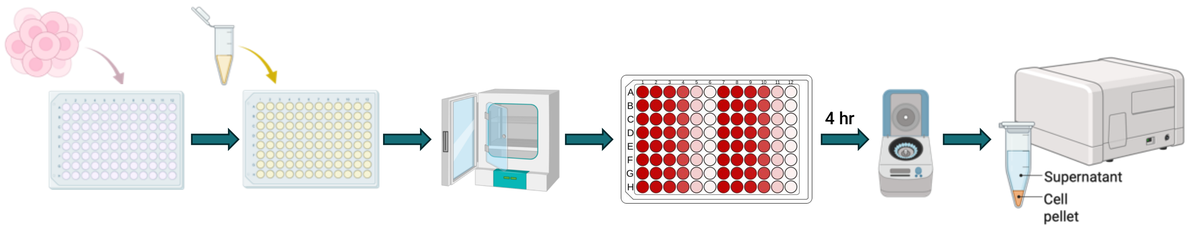


3. HPβCD to RAMEB/cholesterol



Mixture/Isomer	Kd (uM)	ΔH (kcal/mol)	TΔS (kcal/mol)
HPβCD (DS=7)	1535	-0.2	-3.7
3	60	0.1	-6.0
1	3290	2.2	-5.7
2	No binding observed		
6	43	-0.1	-6.0
5	27	0.1	-6.1
8	81	-0.1	-5.7
9	232	0.8	-5.8
7	No binding observed		
4	2230	0	-3.7

Cholesterol Efflux Capacity Assay



Percent cholesterol efflux= [Rm/(Rm+Rc)] X 100%

Conclusion

SAR between HP substitution and cholesterol trafficking was established by preparing HPβCD isomers and evaluating them in a set of *in vitro* assays. The following trends were identified:

- SAR relating DS, stereochemistry, and regio-chemistry was observed.
- A large decrease of cholesterol efflux was observed in a gamma versus a beta cyclodextrin construct.
- The hemolysis activity of the analogs positively correlated with CEC activity.
- Competitive ITC measurements determined dissociation constants, which corresponded to higher binding affinities with increased cholesterol efflux.

This initial SAR has contributed to the design of new molecular entities (NMEs) that are more effective at cholesterol trafficking, and further studies, including *in vivo* pharmacokinetic and efficacy studies will be presented in the future.

References

1. C. D. Davidson, N. F. Ali, M. C. Micsenyi, G. Stephney, S. Renault, K. Dobrenis, D. S. Ory, S. M. T. Vanier, S. U. Walkley, Chronic Cyclodextrin Treatment of Murine Niemann-Pick C Disease Ameliorates Neuronal Cholesterol and Glycosphingolipid Storage and Disease Progression, *PLoS One*, **2009**, *4*, e6951. S. S. Braga, Molecular Mind Games: The Medicinal Action of Cyclodextrins in Neurodegenerative Diseases, *Biomolecules* 2023, *13*, 666-78. T. Kavas, P. Nagy, G. Panyi, L. Szente, Z. Varga, F. Zakany. Cyclodextrins: Only Pharmaceutical Excipients or Full-Fledged Drug Candidates?, *Pharmaceutics*, **2022**, *14*, 2559-95. 2. M. Malanga, J. Szemán, É. Fenyvesi, I. Puskás, K. Csabai, G. Gyémánt, F. Fenyvesi, L. Szente, "Back to the Future": A New Look at Hydroxypropyl Beta-Cyclodextrins *J. Pharm. Sci.* 2016, *105*, 2921-31. P. Li, J. Song, X. Ni, Q. Guo, H. Wen, Q. Zhou, Y. Shen, Y. Huang, P. Qiu, S. Lin, H. Hu, Comparison in toxicity and solubilizing capacity of hydroxypropyl-β-cyclodextrin with different degree of substitution, *Int. J. Pharm.* 2016, *513*, 347-56.