CYCLOLAB

The Cyclodextrin Company

Cyclodextrin Derivatives
Syntheses and Applications

Milo Malanga

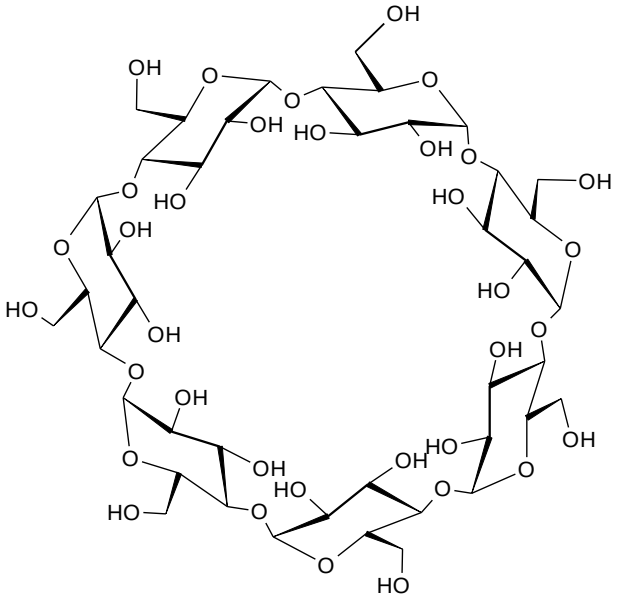
Why to modify Cyclodextrins?

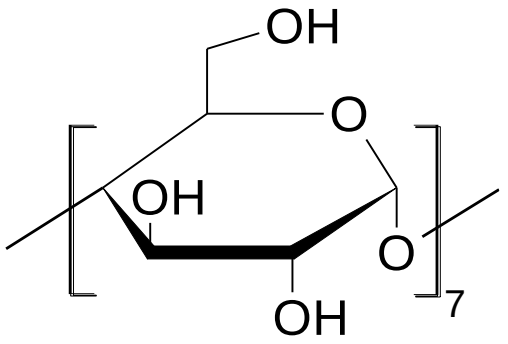
- Solubility improvement of the CD (and its complexes) in desired solvent, usually in water;

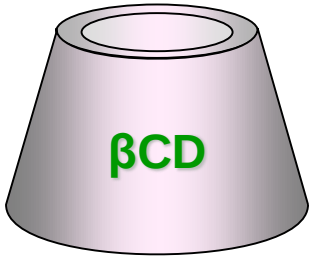
- better fit and/or association between the CD and its guest, with concomitant stabilization of the guest by changing its reactivity;

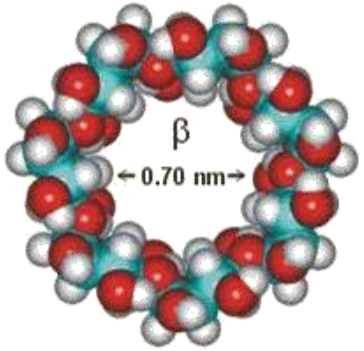
- more appropriate mimic of a binding site (e.g., in enzyme modeling) via attachment of specific groups; or

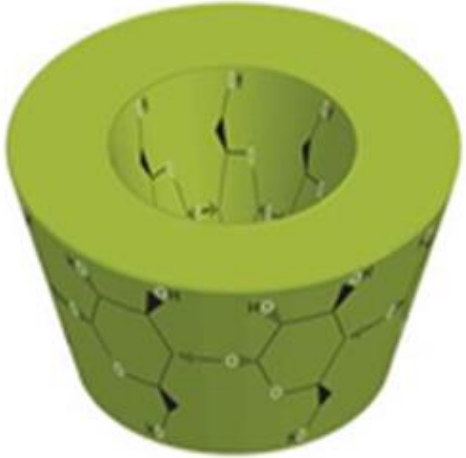
- formation of insoluble or immobilized CD-containing structures, polymers (e.g., for chromatographic purposes).

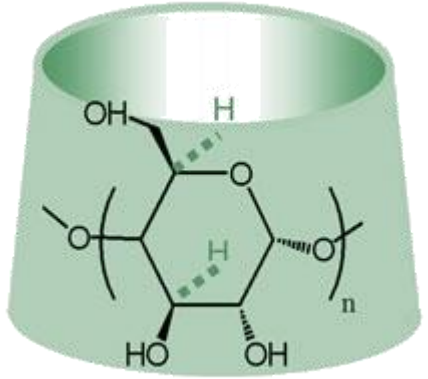






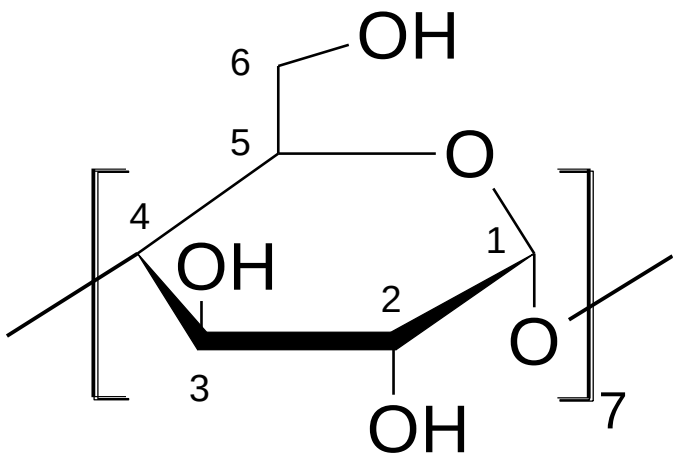






Characteristics of the Hydroxyl Groups

Less acidic, most nucleophilic

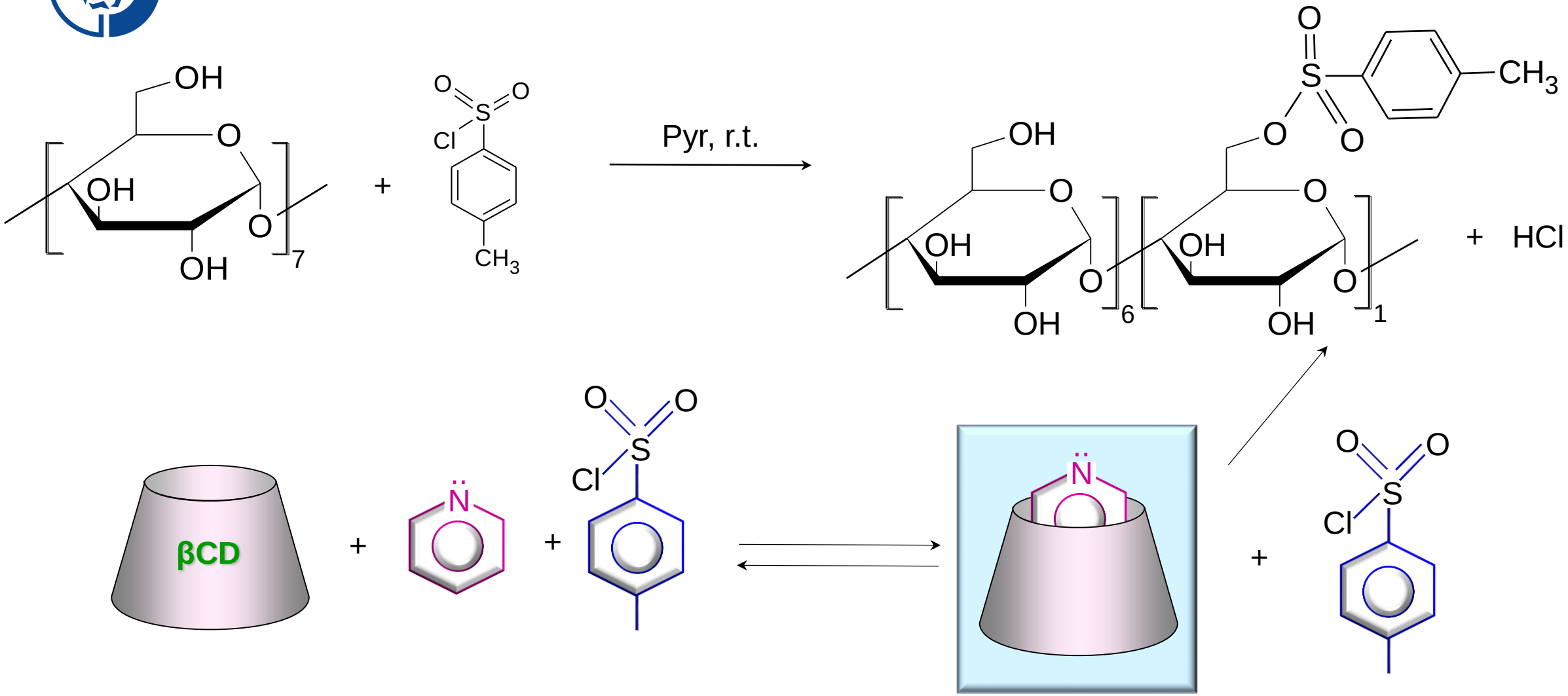


More acidic, less nucleophilic

Most acidic, more nucleophilic

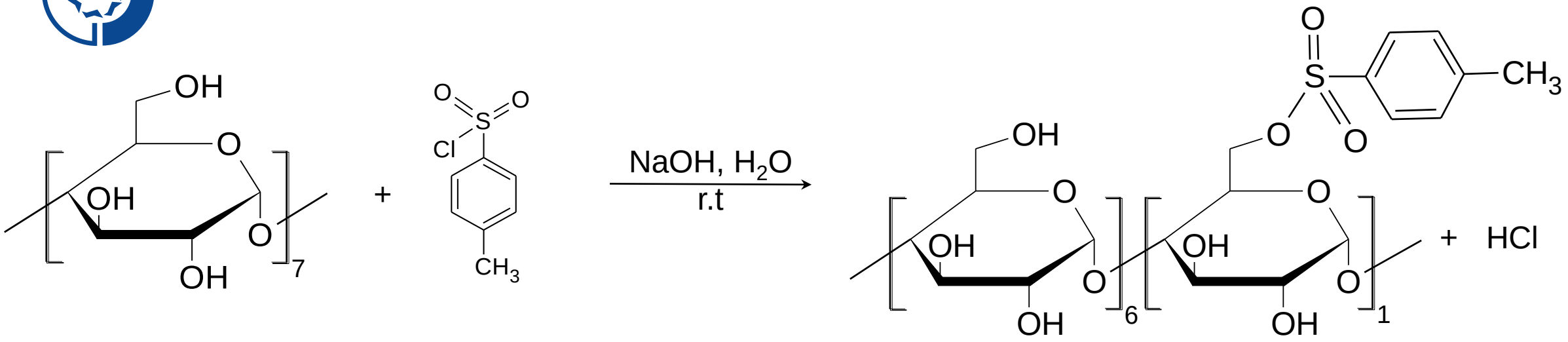
C1= anomeric carbon
C6= methylene unit (-CH_2-)
C2= easy accessible
C3= most hyndered, difficult to modify
C4, C5= not inolved in reaction

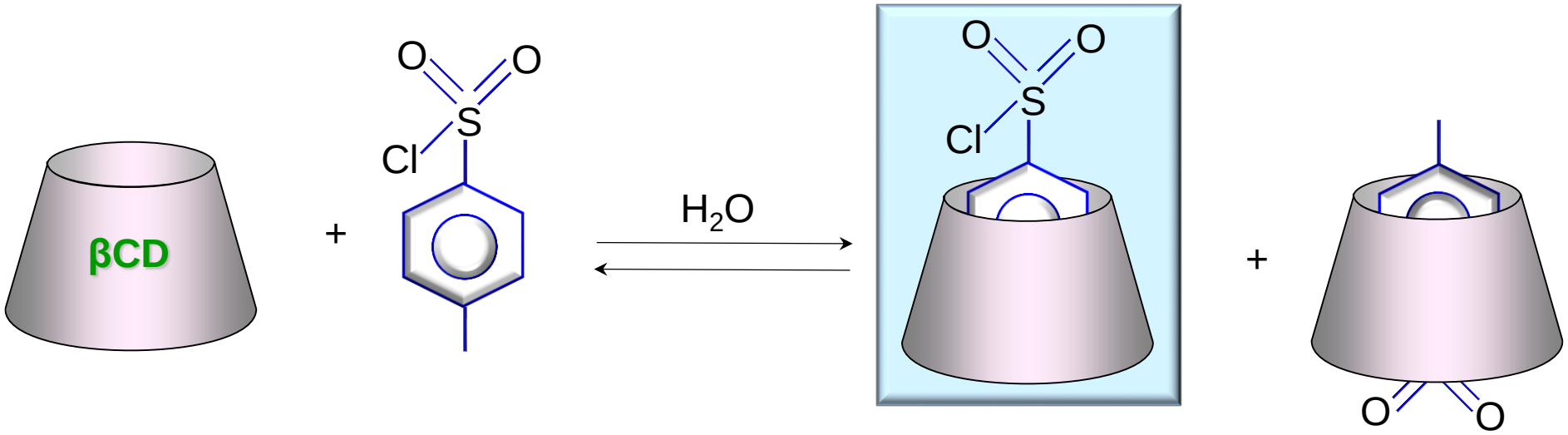
Mono-6-tosyl-βCD, Regioselective Synthesis



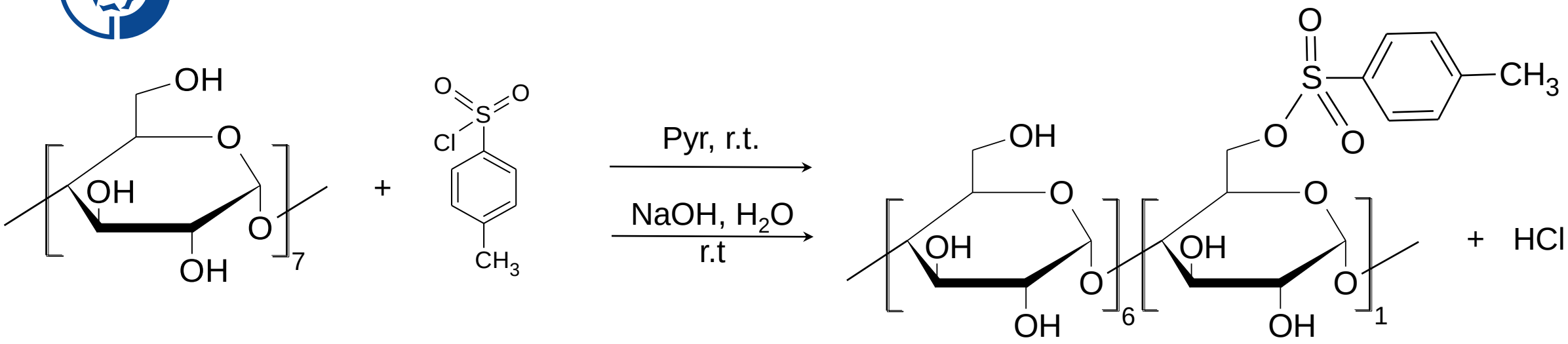
The most nucleophilic OH reacts with TsCl!

Mono-6-tosyl-βCD, Regioselective Synthesis





Mono-6-tosyl-βCD, Pyr *vs* H_2O

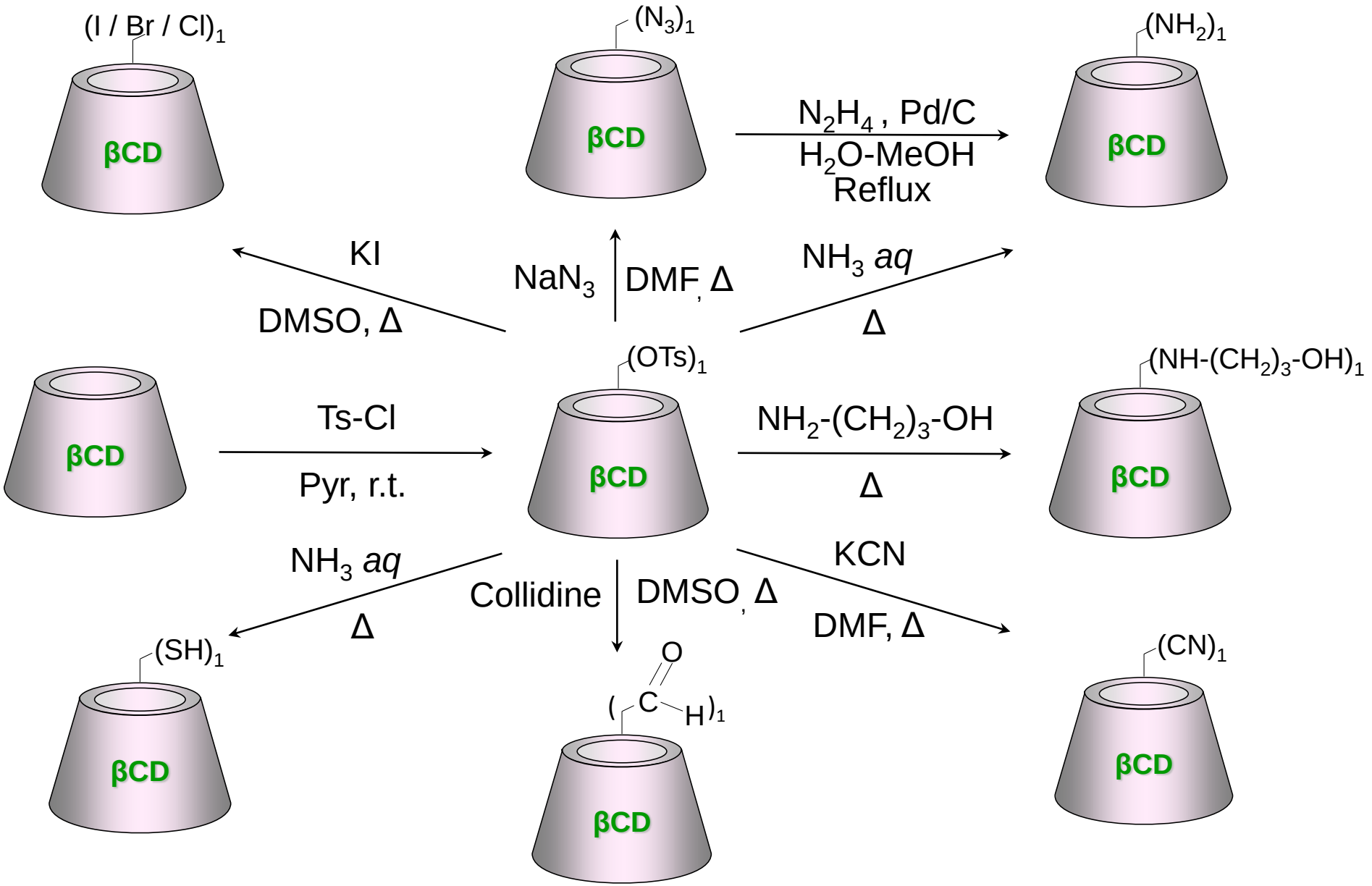


Pyridine
Over-tosylation
Hazardous solvent
Dry conditions
Difficult work-up:
CD-pyr gel, chlorination
Fast reaction

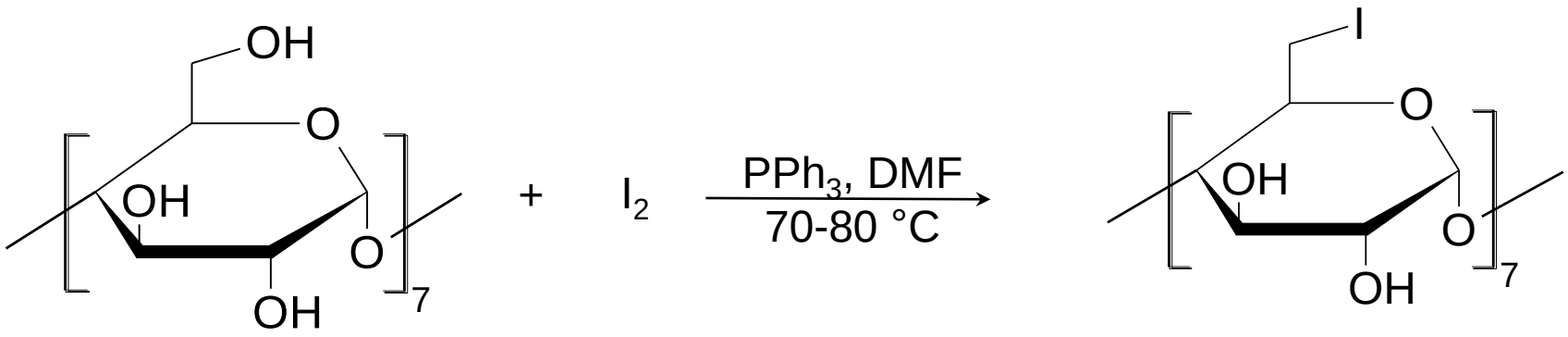
H_2O
Green solvent
Excess of TsCl
Hydrolysis of product
Low yield
Possible scale-up

Selective monotosylation for Ts_1α- and Ts_1γ-CD in aqueous solution is not possible.
Degree of Substitution difficult to control (exception Ts_1-βCD).
Ts_1-βCD production: 1 kg scale!

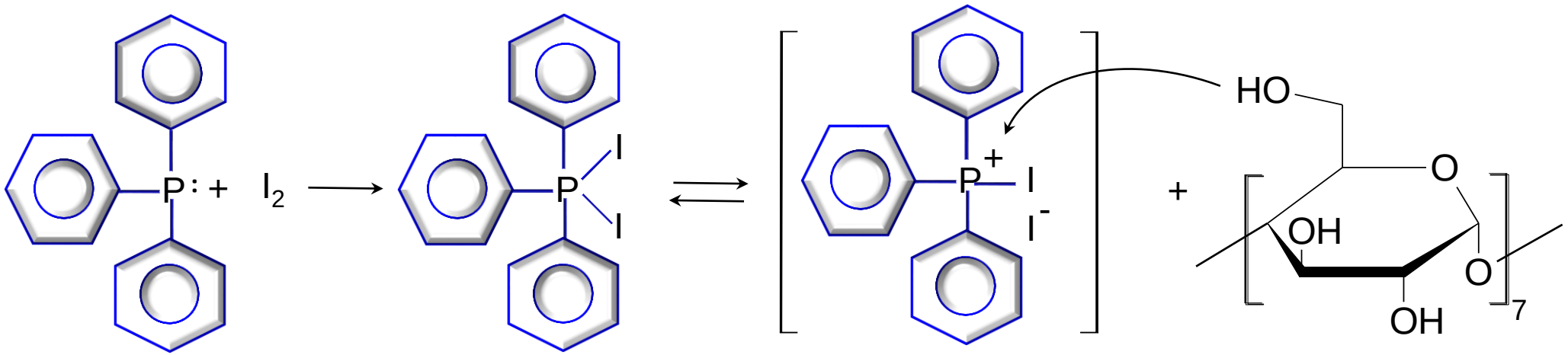
Mono-6-tosyl-βCD, the Key Intermediate!



Per-6-halogen-CD, Regioselective Synthesis



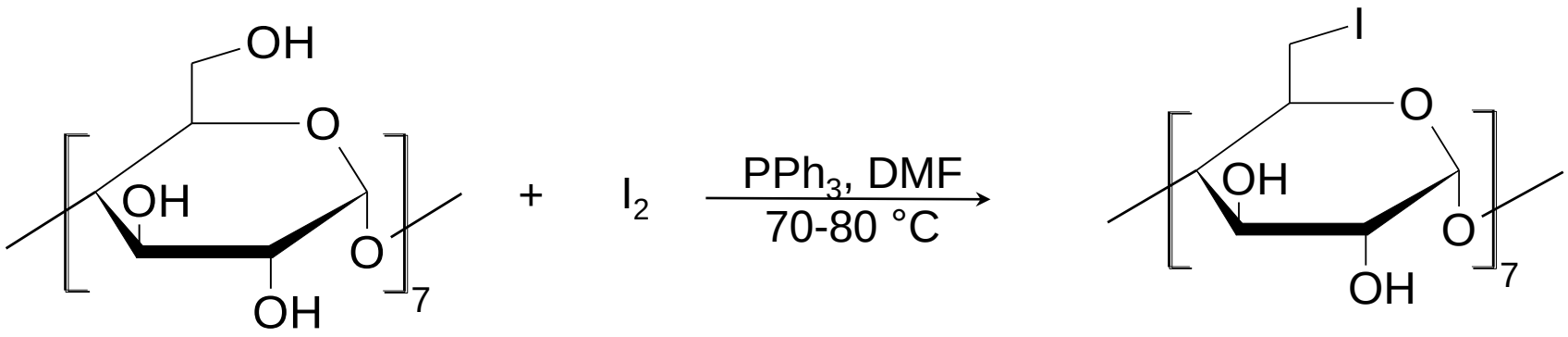
Appel type reaction



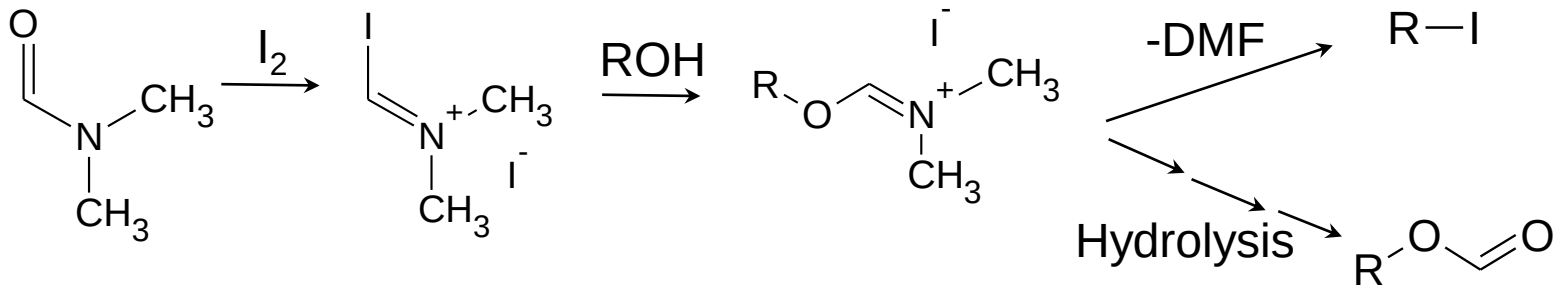
The less hindered OH reacts!

G. Hodosi *et al.*, Carbohydrate Research, 230, 327-342, **1992**

Per-6-halogen-CD, Regioselective Synthesis



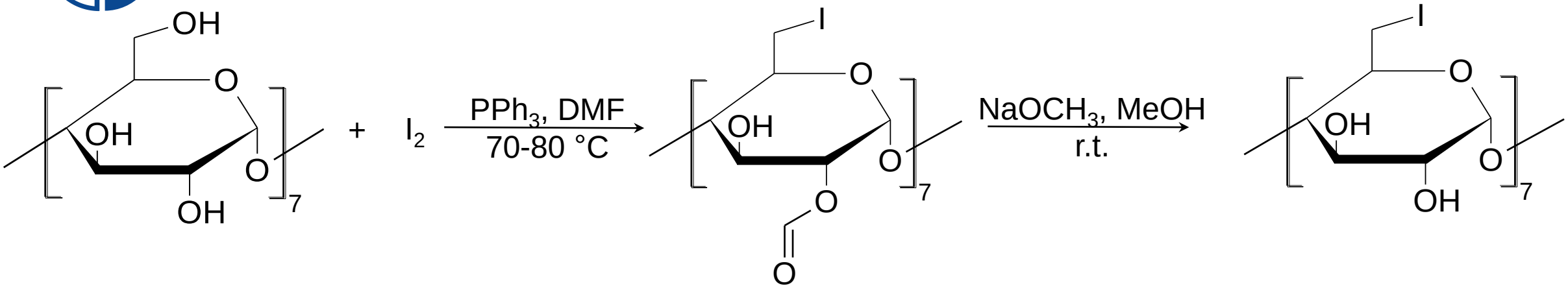
Vilsmeier-Haack type reaction

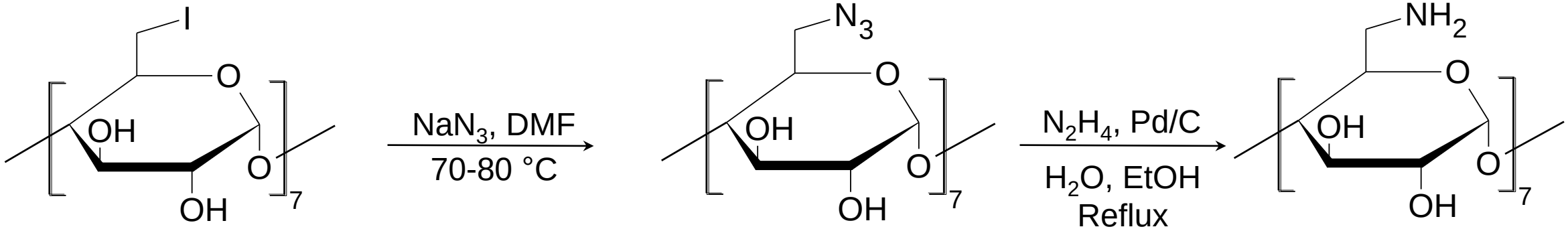


Both pathways occur!

G. Hodosi *et al.*, Carbohydrate Research, 230, 327-342, **1992**

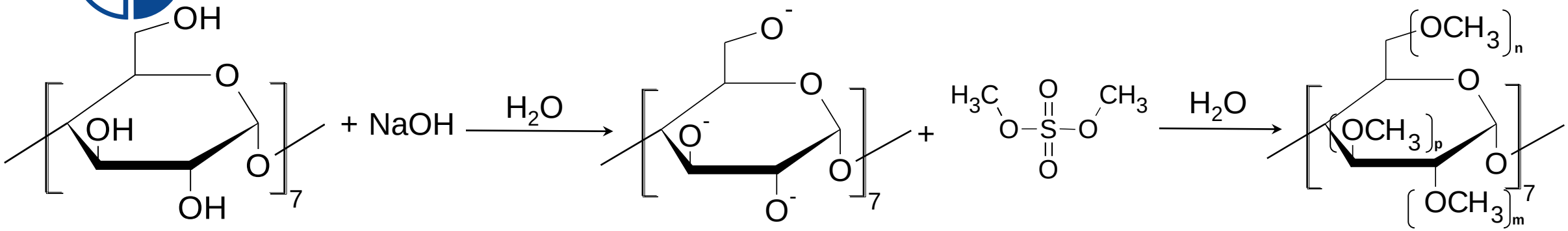
Per-6-halogen-CD, Versatile Compounds



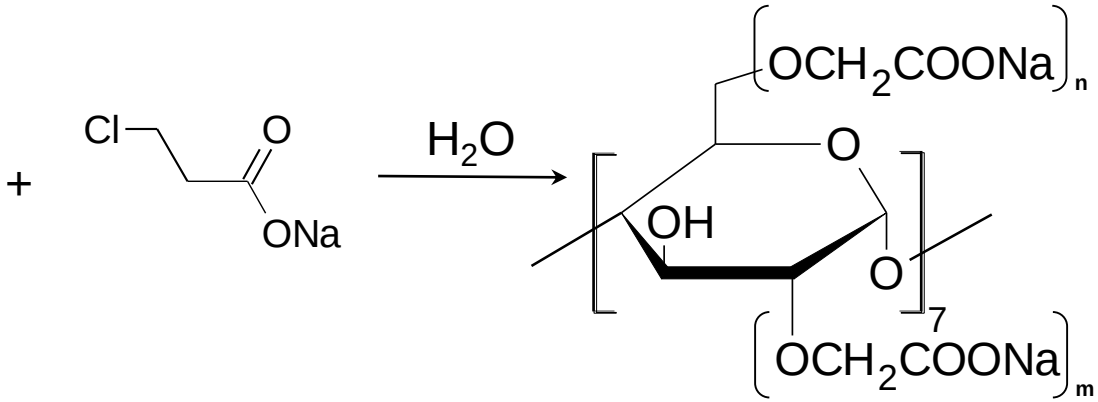


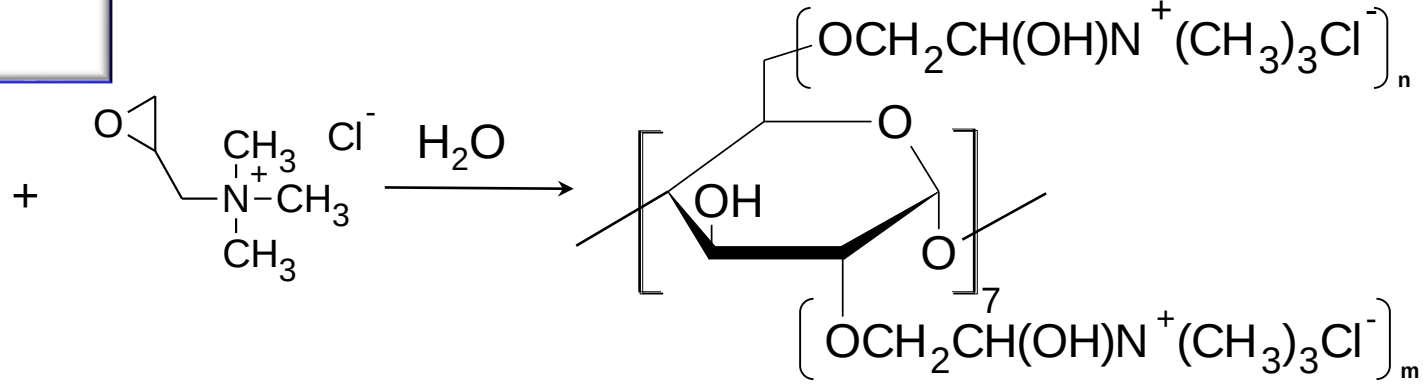
Selective per-6-halogenation also for α- and γ-CD.
Degree of Substitution difficult to control (exception I/Br_1-βCD).
Per-6-I/Br-CD production: 500 g scale.

Random Alkylation

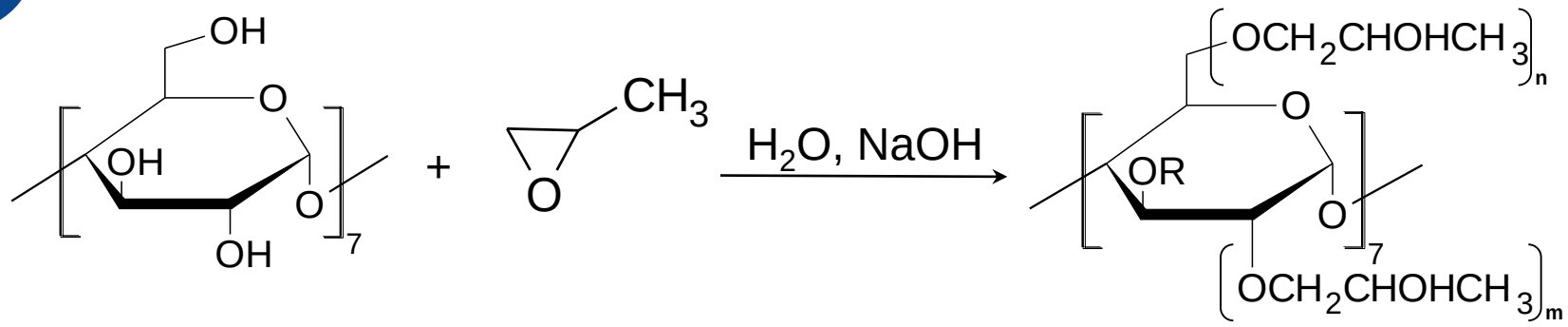


- Regioselective with appropriate base
- Degree of substitution can be controlled
- Substitution pattern: *O*(2)>>*O*(6)/*O*(3)
- The longer alkyl chain the more 2,6-*O*-selective.
- High solubility in water and organic solvents.
- Per-substituion difficult in water
- 5-1000 kg scale production.





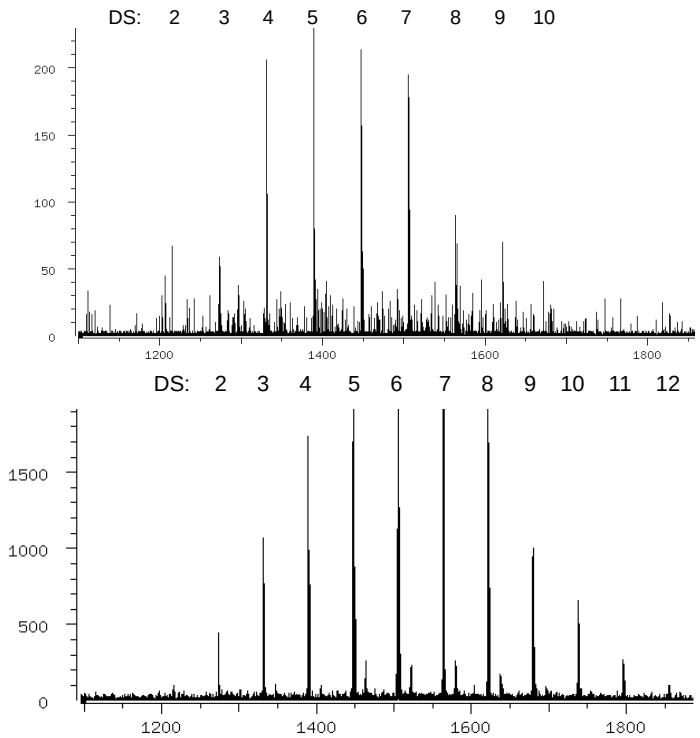
2010-FDA granted HPβCD Orphan Drug Status



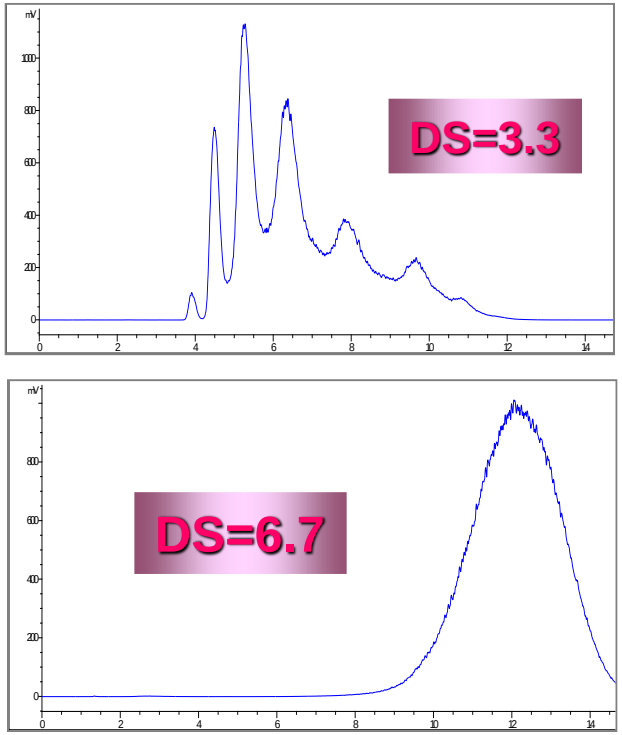
Ph. Eur.

βCD ≤ 1.5 %
Propylene Glycol ≤ 2.5%
Degree of Substitution

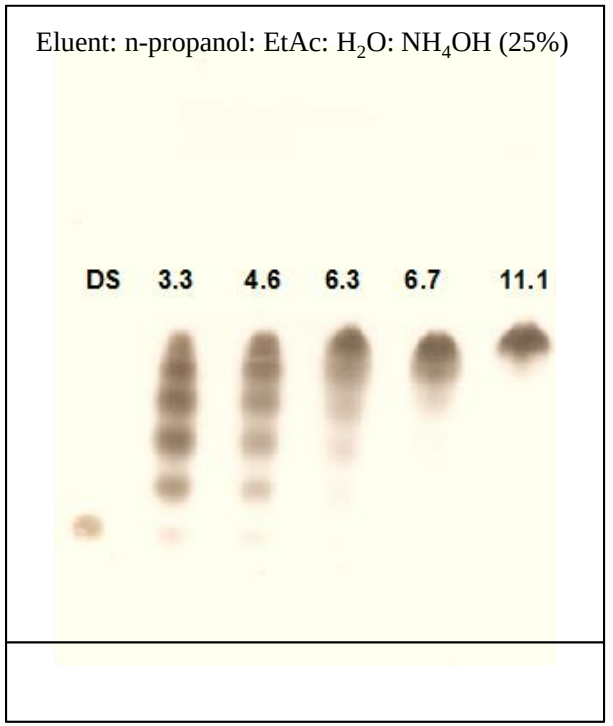
MALDI-TOF



HPLC

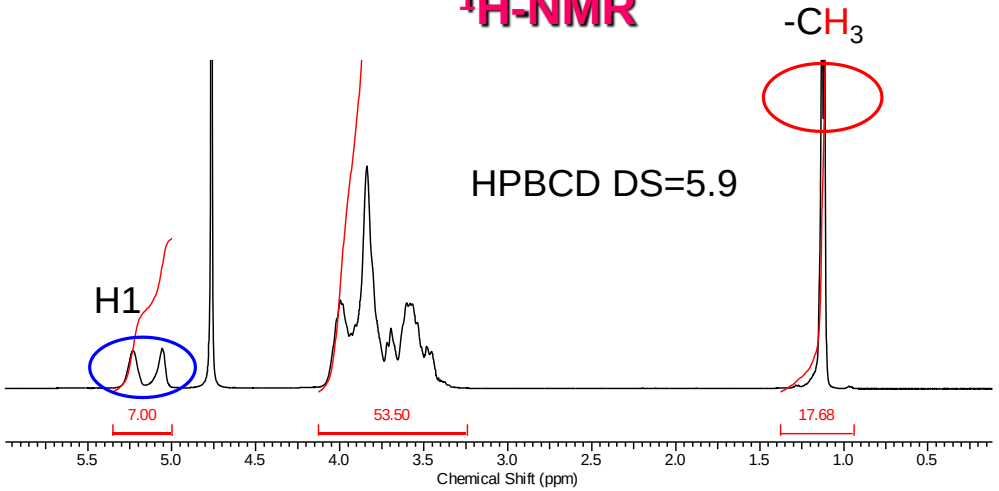


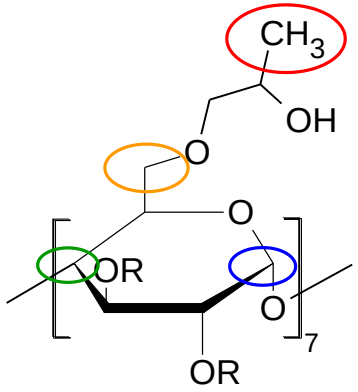
TLC



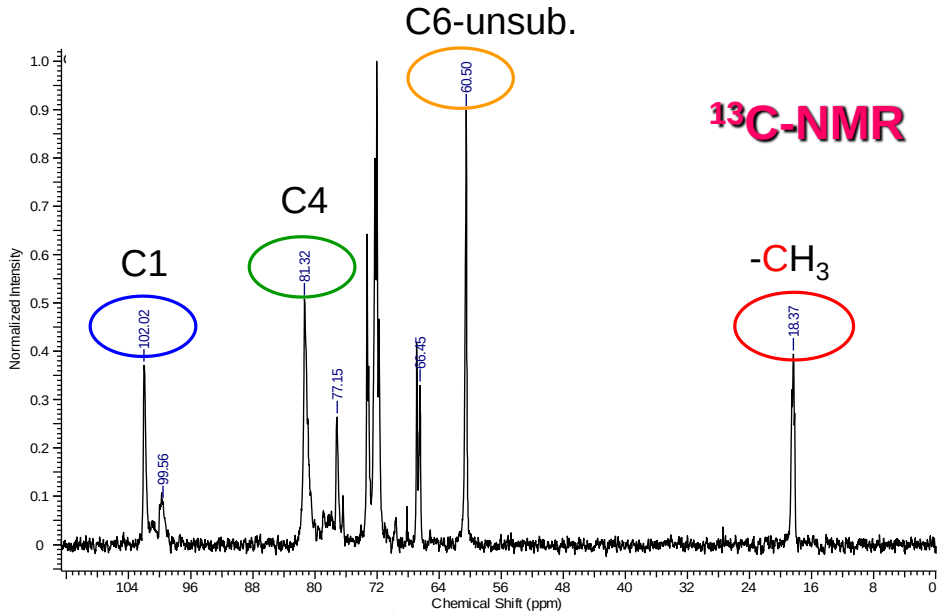
Information on the Positions of Substituents (NMR!)

^{1}H-NMR

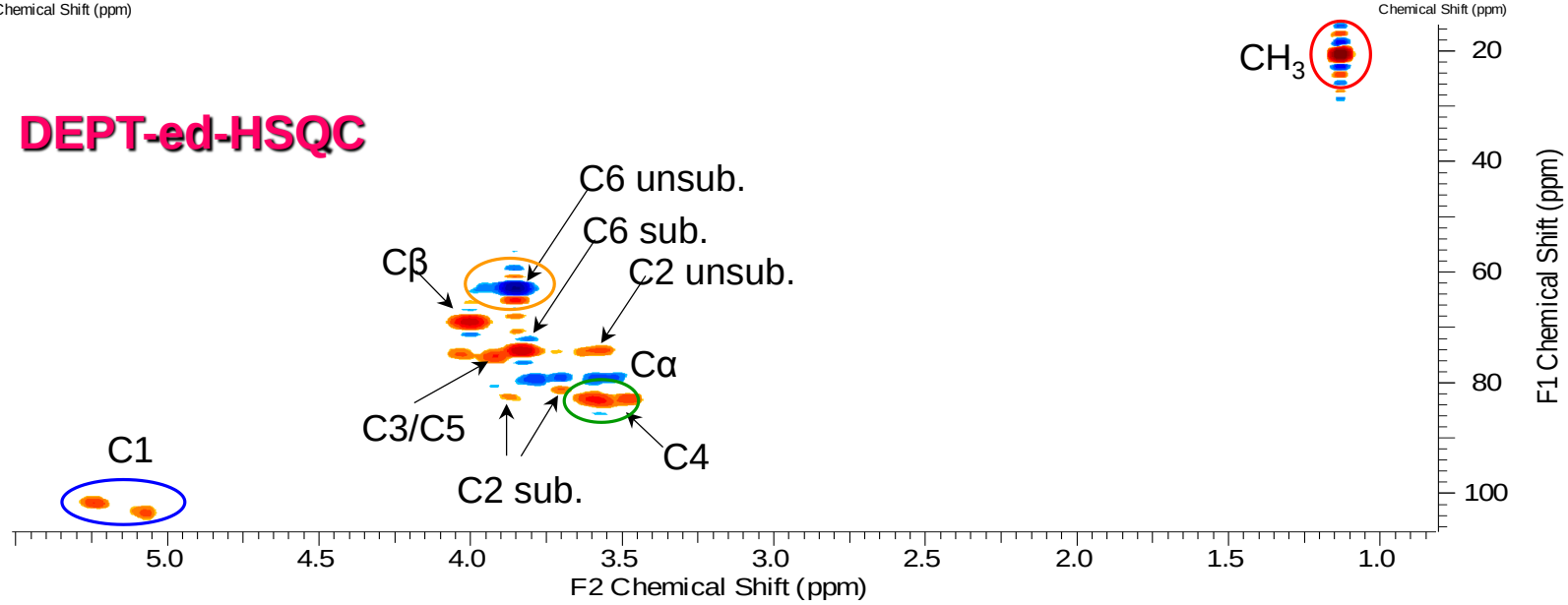




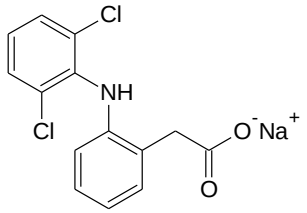
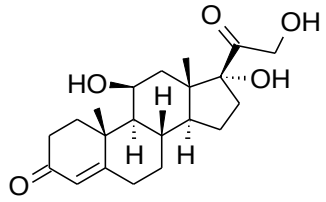
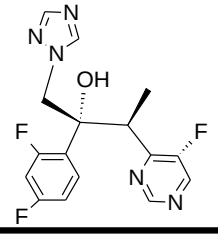
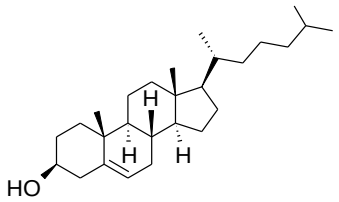
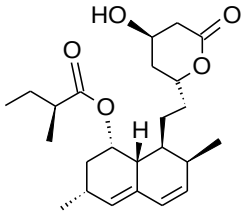
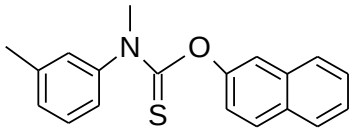
^{13}C-NMR

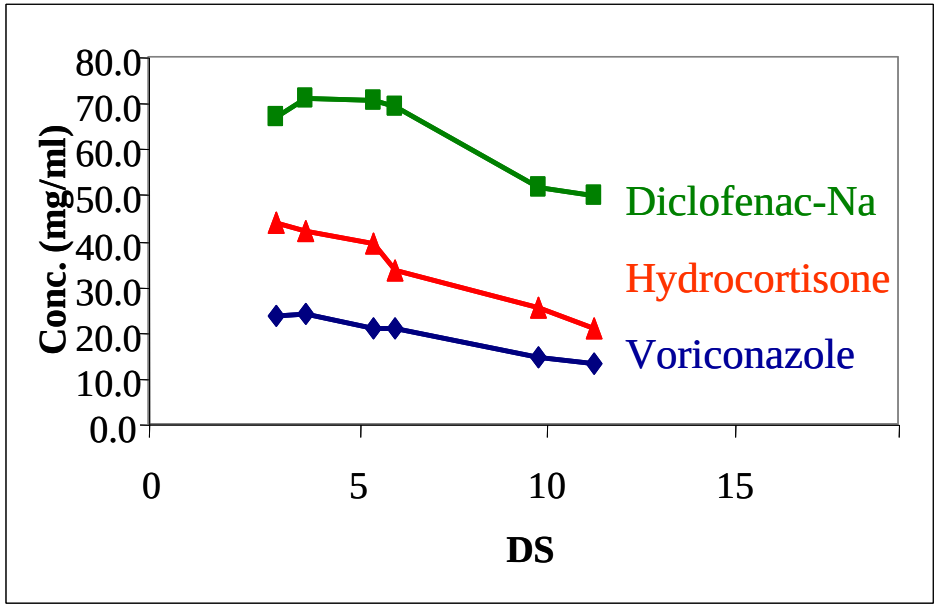


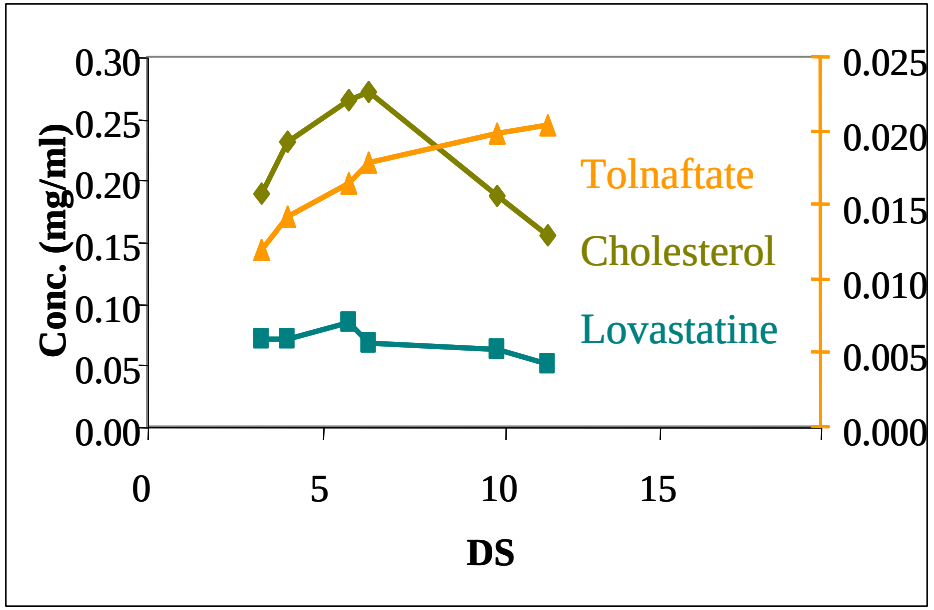
DEPT-ed-HSQC



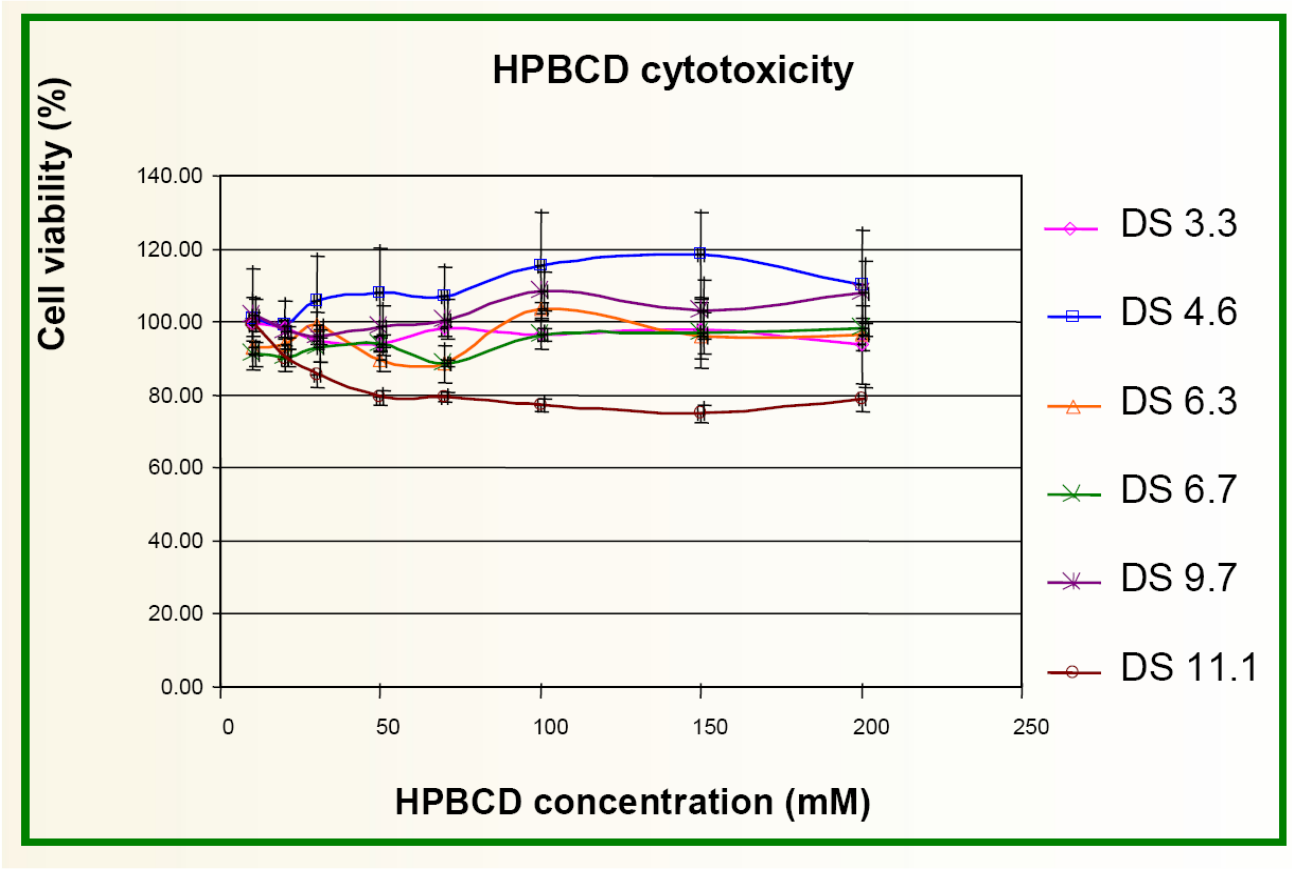
Solubilizing Capacity of HPBCDs with different DS

Diclofenac-Na	Hydrocortisone	Voriconazole	Cholesterol	Lovastatine	Tolnaftate
					



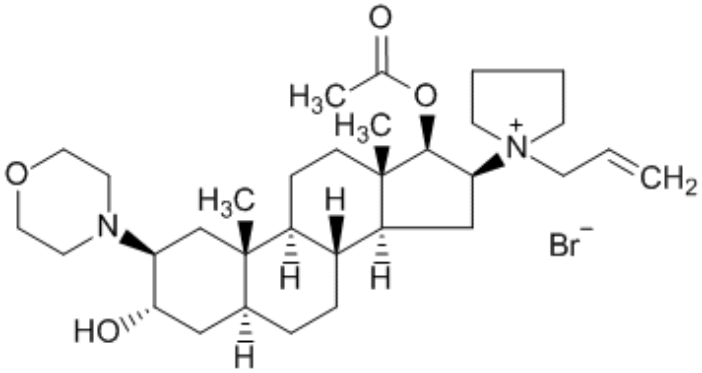


Cytotoxicity of HPBCDs with different DS

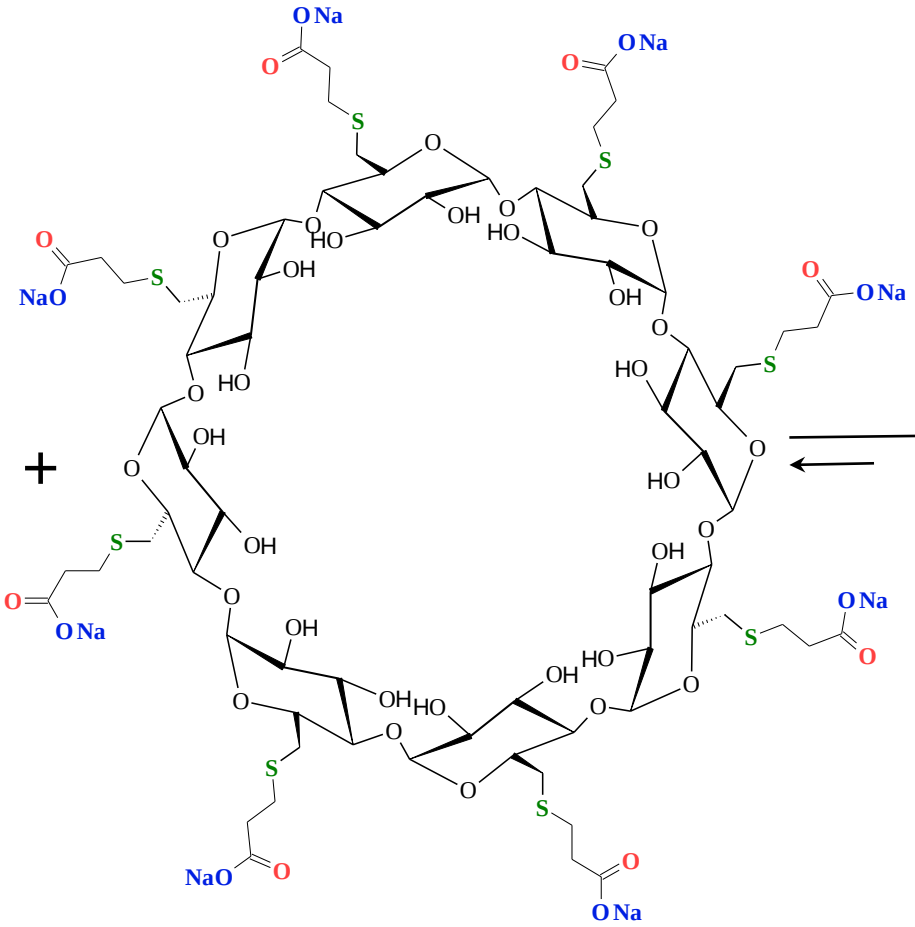


Regioselective with appropriate base and temperature.
Degree of Substitution can be controlled.
High solubility in water.
Challenging to characterize!
5-1000 kg scale production

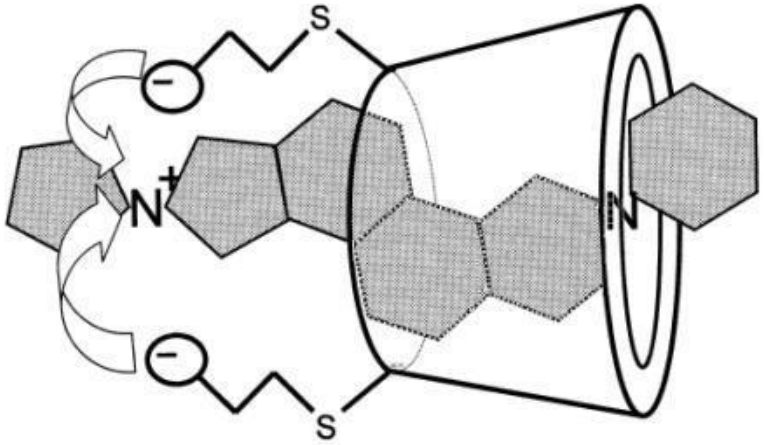
Sugammadex:Selective Relaxant Binding Agent!



Rocuronium Bromide
Aminosteroid



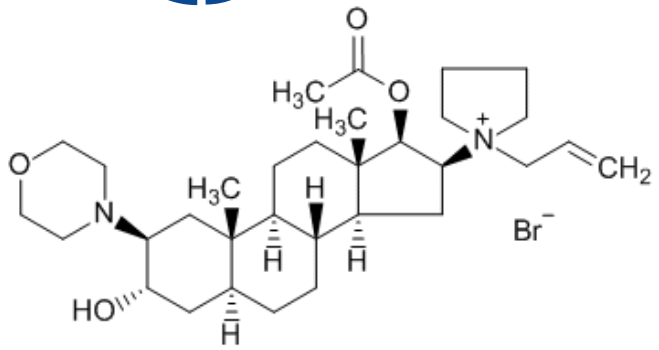
Sugammadex
(Bridion®)



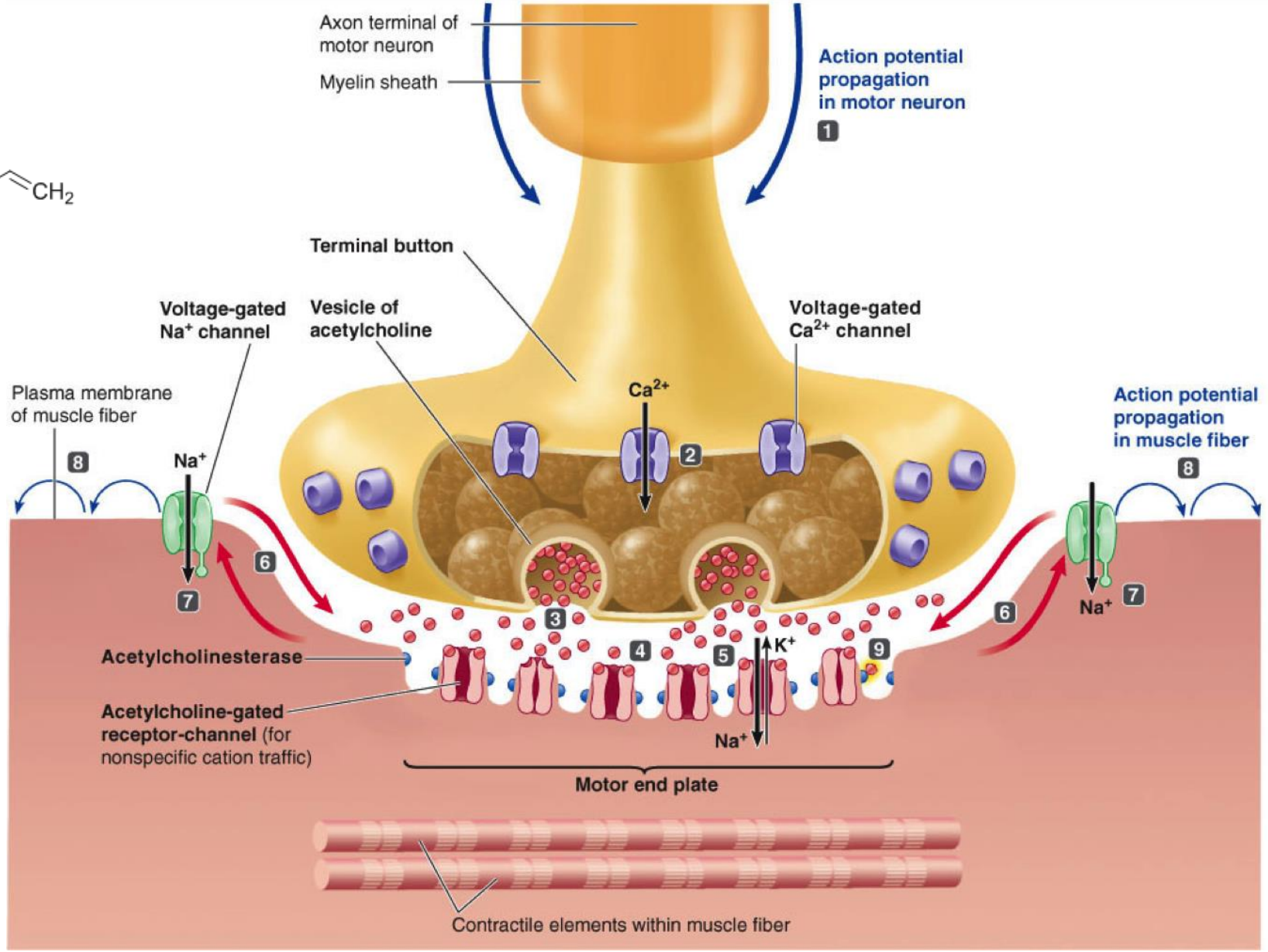
K_a=25.000.000 M*

* S. J. Schaller, H. Fink, Core Evidence, **2013**

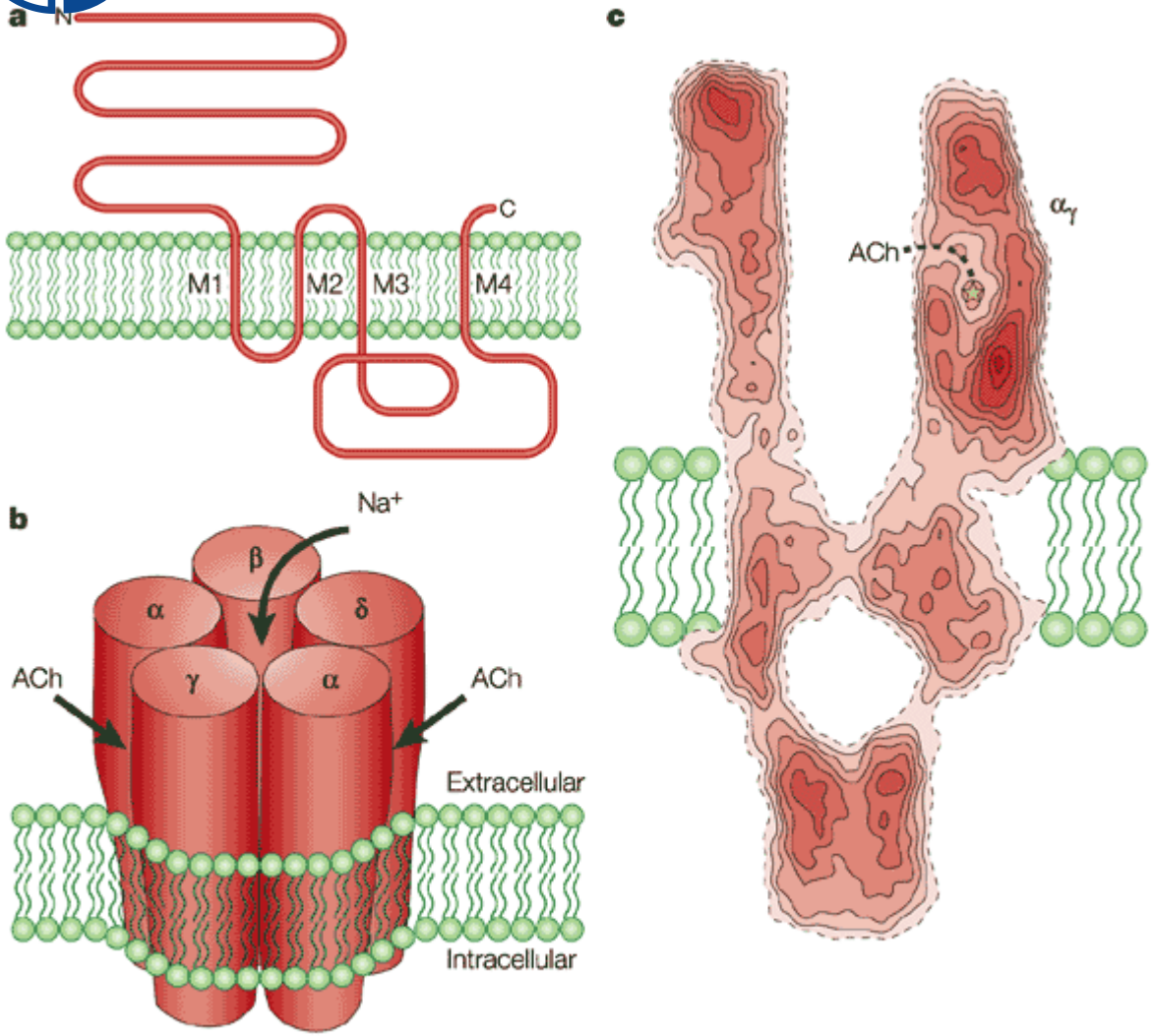
Detail View of Neuromuscolar Junction

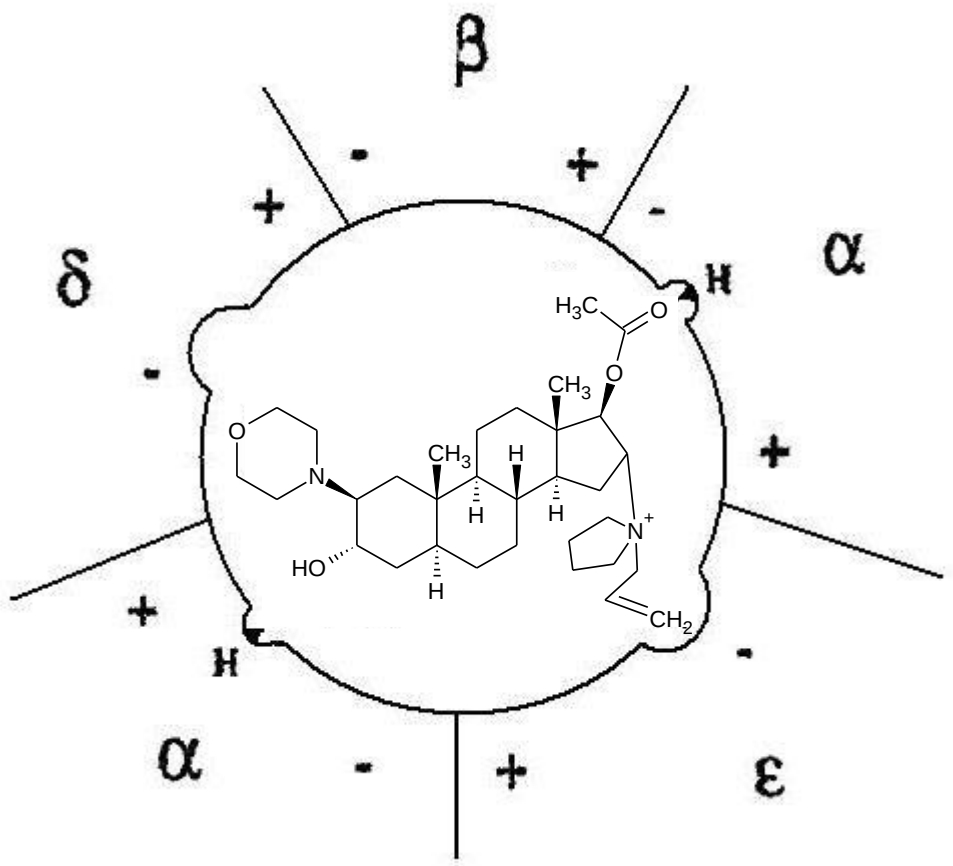


Rocuronium Bromide
Aminosteroid
Non-depolarizing
blocking agent



Nicotininc Receptor and Rocuronium





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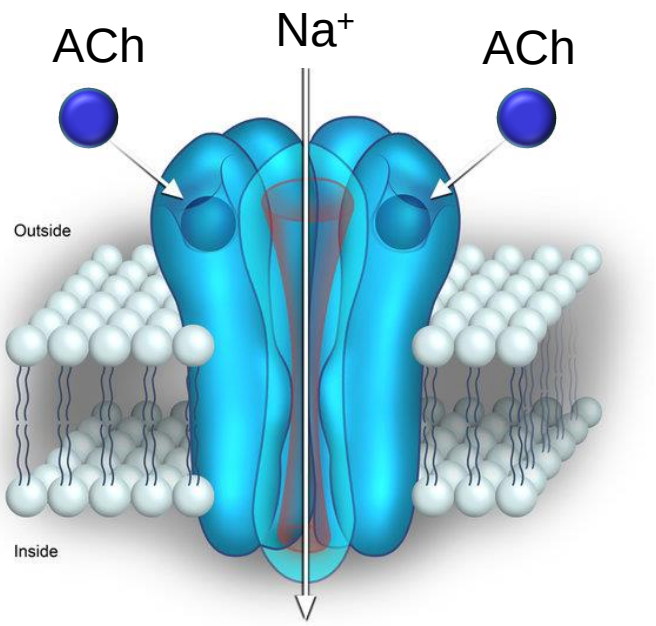
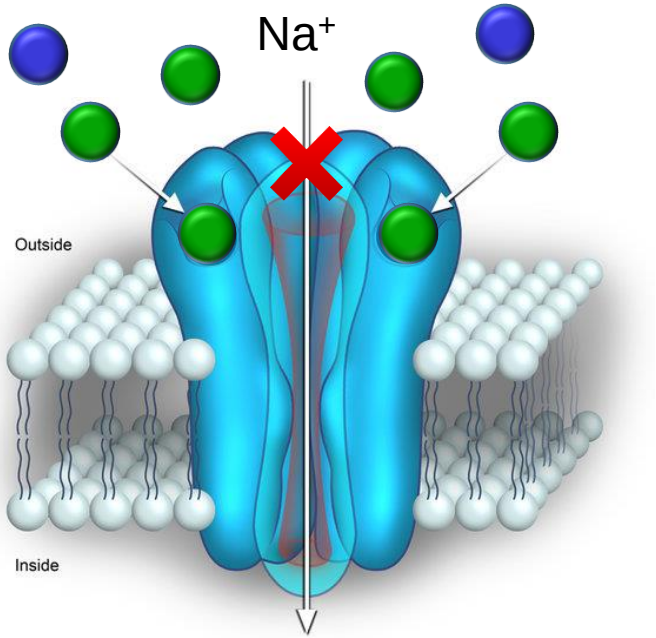
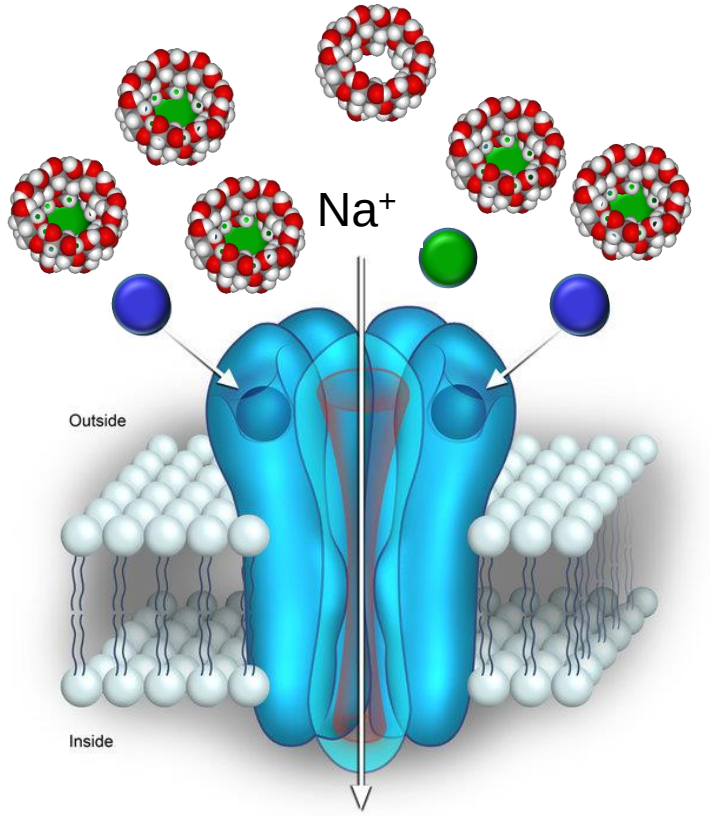
Nicotininc Receptor, Rocuronium and Sugammadex







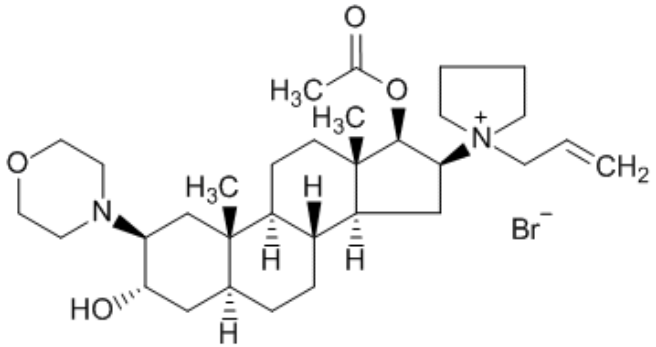
Acetylcholine

Rocuronium

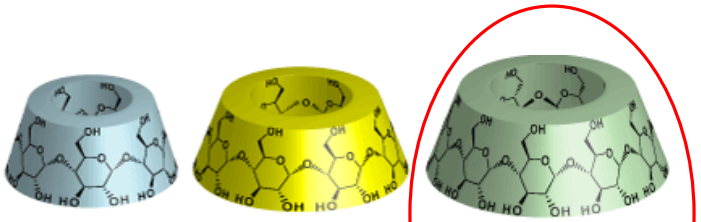
Sugammadex

No autonomic instability!
No need of co-administration of antimuscarinic agent (atropine) as for acetylcholinesterase inhibitor (neostigmine)

Building-up of Sugammadex

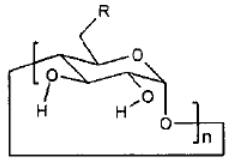


Rocuronium Bromide



	α-CD	β-CD	γ-CD
No. of Glucose Units	6	7	8
Cavity Diameter (nm)	0.47	0.60	0.75
Height of Torus (nm)	0.79	0.79	0.79

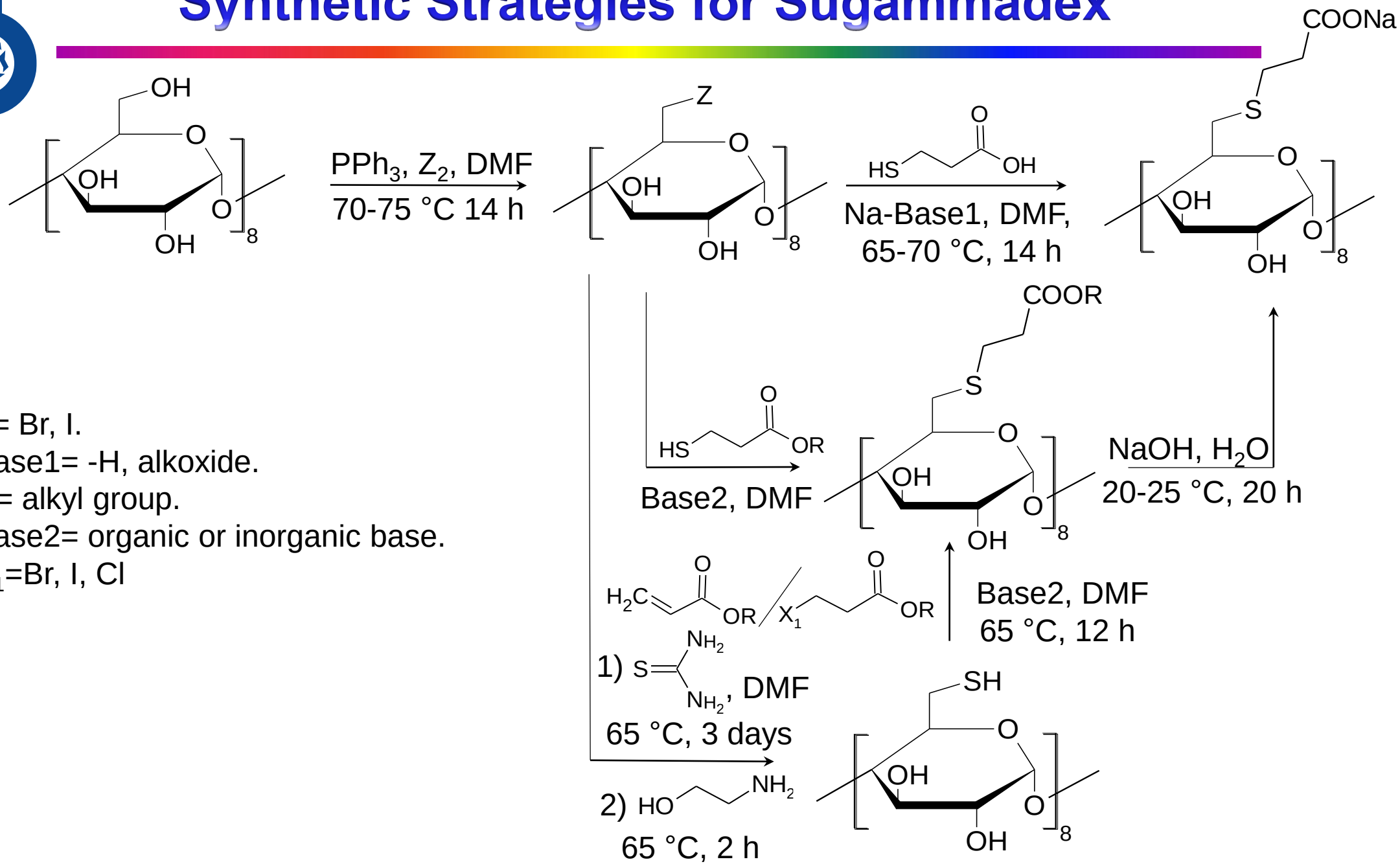
Table 1. Structures and Reversal Activities of Per-6-thiolated CDs against Rocuronium-Induced Neuromuscular Block *



Compounds						In vitro reversal activity vs ~90% block by rocuronium [a] (isolated mouse hemi-diaphragm)		In vivo reversal activity vs ~90% block by rocuronium [b] (i.v., guinea-pigs)	
Compound No.	n	R	Prep. Method	MW	Purity [c]	EC_{50}, µM	max reversal, % (conc., µM)	ED_{50}, µmol/kg	max reversal, % (dose, µmol/kg)
4	6	OH	n.a. [d]	972.9	>98% [e]	> 360.0	9.7 ± 3.0 (360)	1575.0 ± 1025.0	6.4 ± 3.9 (1018)
5	6	SCH_2CO_2Na	A	1549.3	>99%	> 18.0	22.8 ± 13.0 (18)	> 21	3.6 (21)
6	6	$SCH_2CH_2CO_2Na$	A	1633.5	>70%	> 360.0	0.0 ± 0.0 (360)	> 16	3.2 (16)
7	6	$SCH_2CH_2CH_2CO_2Na$	A	1717.7	>92%	> 18.0	5.3 ± 3.4 (18)	> 16	3.4 (16)
8	7	OH	n.a. [d]	1135.1	>98% [e]	> 360.0	29.0 ± 15.4 (360)	20.0 ± 7.0	92.9 ± 10.3 (113)
9	7	SCH_2CO_2Na	A	1807.6	>88% [f]	6.5 ± 1.5	97.3 ± 16.2 (16.2)	0.93 ± 0.26	89.6 ± 12.8 (9.6)
10	7	$SCH_2CH_2CO_2Na$	A	1905.8	>90%	3.3 ± 0.7	100.1 ± 2.8 (9)	0.75 ± 0.35	81.3 ± 9.4 (2.6)
11	7	$SCH_2CH_2CH_2CO_2Na$	A	2004.0	>90%	5.0 ± 0.7	95.3 ± 5.2 (14.4)	0.49 ± 0.10	99.6 ± 0.1 (3.2)
12	8	OH	n.a.	1297.2	>98% [e]	34.6 ± 10.4	94.1 ± 2.0 (144)	4.0 ± 0.0	104.7 ± 8.6 (47)
13	8	SCH_2CO_2Na	A	2065.8	>97%	1.2 ± 0.2	93.8 ± 2.7 (3.6)	0.10 ± 0.05	103.3 ± 4.3 (0.5)
14	8	$SCH_2CH_2CO_2Na$	A	2000.0	>97%	1.2 ± 0.8	95.1 ± 2.3 (3.6)	0.03 ± 0.00	92.5 ± 5.3 (0.3)
15	8	$SCH_2CH_2CH_2CO_2Na$	A	2290.2	>97%	1.4 ± 0.0	98.5 ± 4.5 (3.6)	0.06 ± 0.01	93.4 ± 10.6 (0.3)
16	8	$SCH_2CH_2CH_2CH_2CO_2Na$	A	2402.5	>97%	1.8 ± 0.1	98.9 ± 5.2 (5.4)	0.07 ± 0.00	99.0 ± 3.5 (0.3)
17	8	$SCH_2CH_2CH_2CH_2CH_2CO_2Na$	A	2514.7	>70%	7.0 ± 0.4	81.7 ± 12.6 (12.6)	0.74 ± 0.10	78.4 ± 7.7 (2.5)

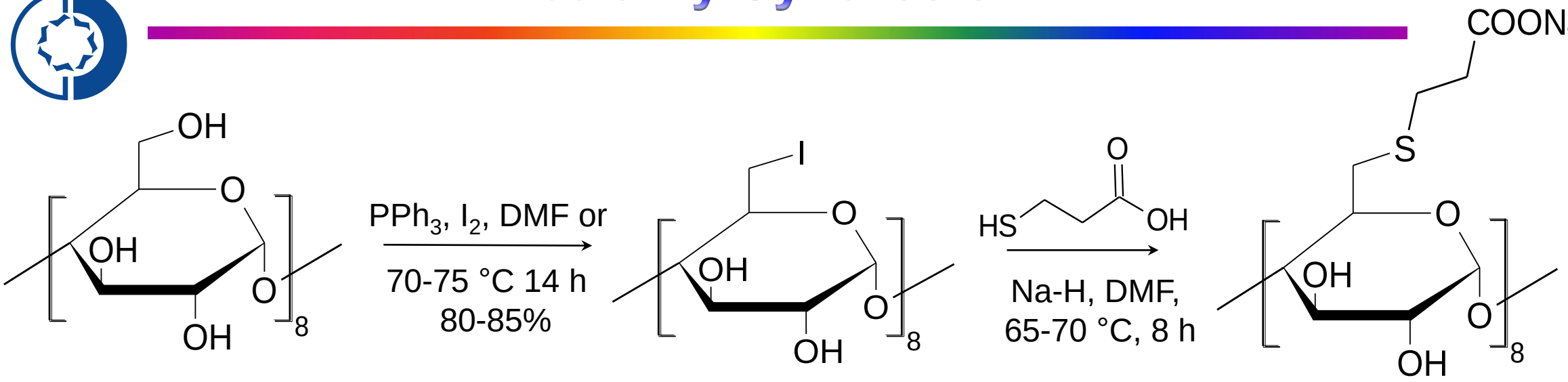
* J. M. Adam *et al.*, J. Med. Chem., 45, 1806-1816, **2002**

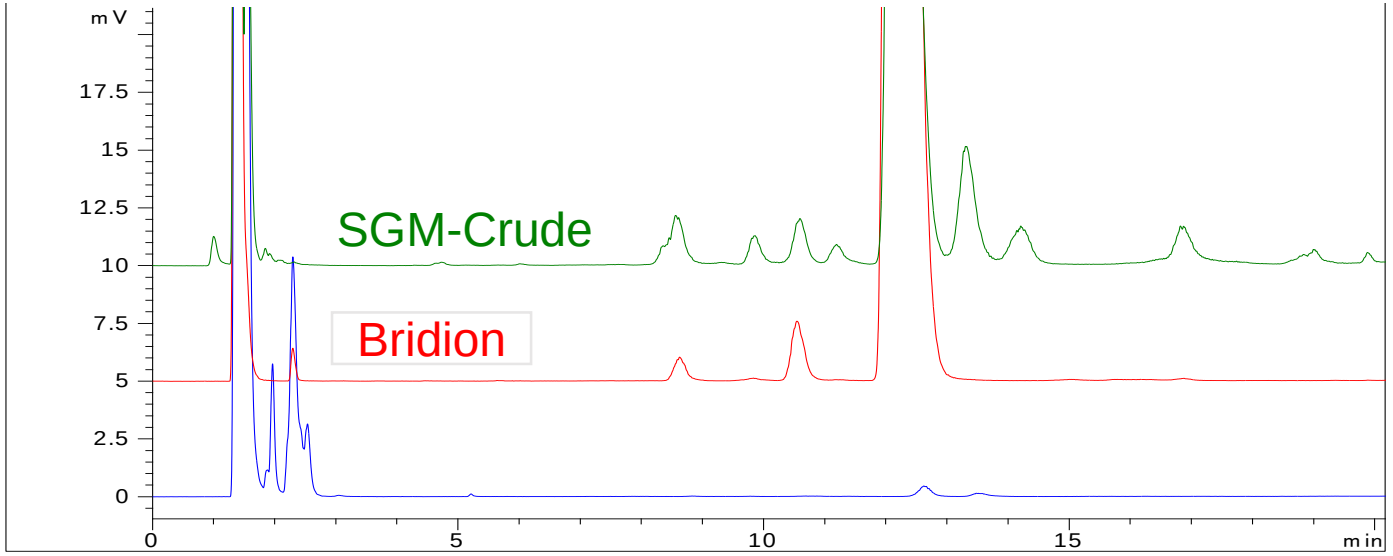
Synthetic Strategies for Sugammadex



Z= Br, I.
Base1= -H, alkoxide.
R= alkyl group.
Base2= organic or inorganic base.
X_1=Br, I, Cl

Not Only Synthesis!!

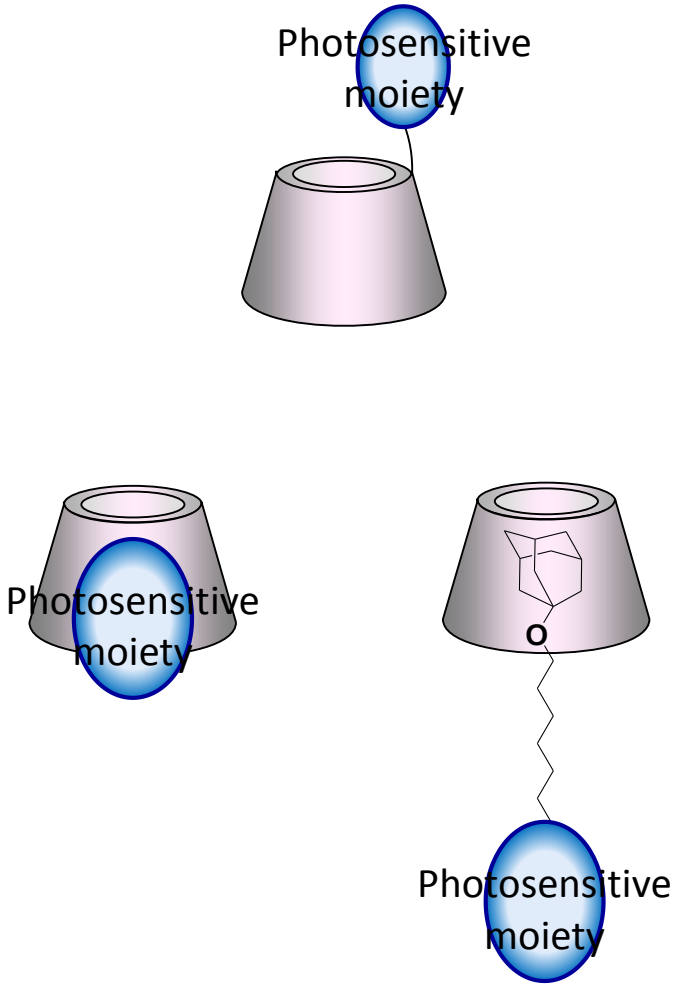




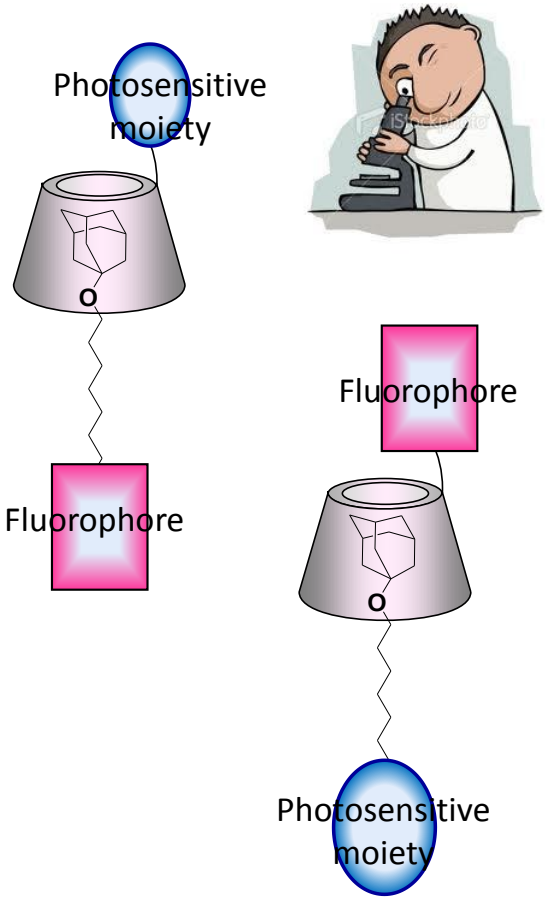
Purification is essential !!!
Characterization (HPLC/CE-MS, NMR, IR etc.)
Purity (HPLC, CE)
....
....

How to Use Cyclodextrins in PDT

Photodynamic therapy (PDT), is a form of phototherapy using nontoxic light-sensitive compounds (photosensitizers) that are exposed selectively to light, whereupon they release toxic species (reactive oxigen/nitrogen species, to targeted malignant and other diseased cells (phototoxicity).

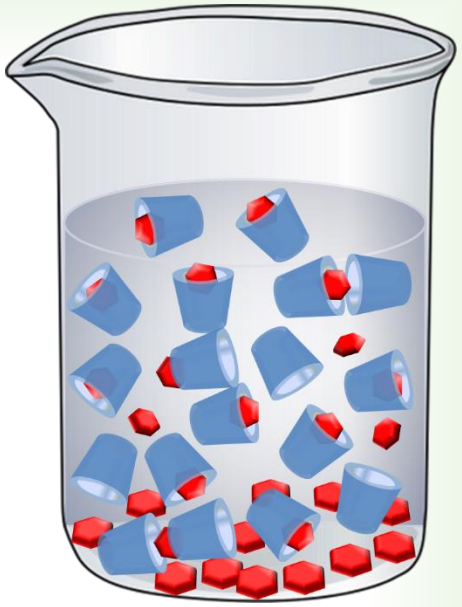


Visualization

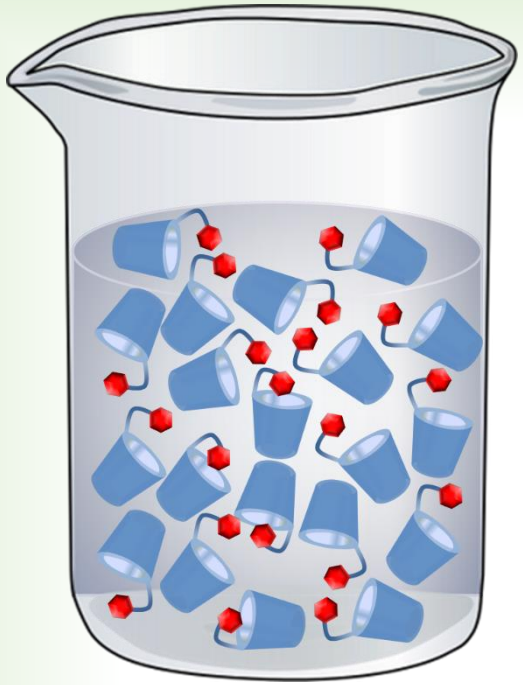


CDs for Improving Photophisycs of Photosensitiser

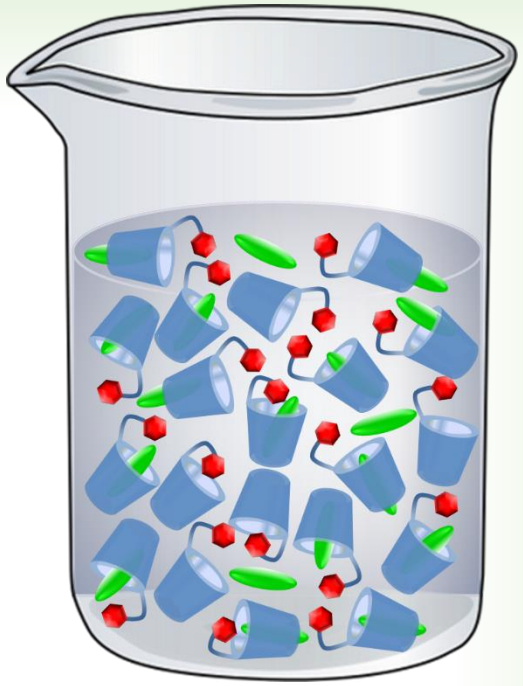
Insoluble
Photosensitizer



Photosensitizer
in solution



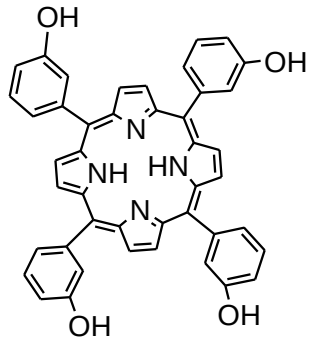
Water Soluble
Photosensitizer-CD
Conjugate



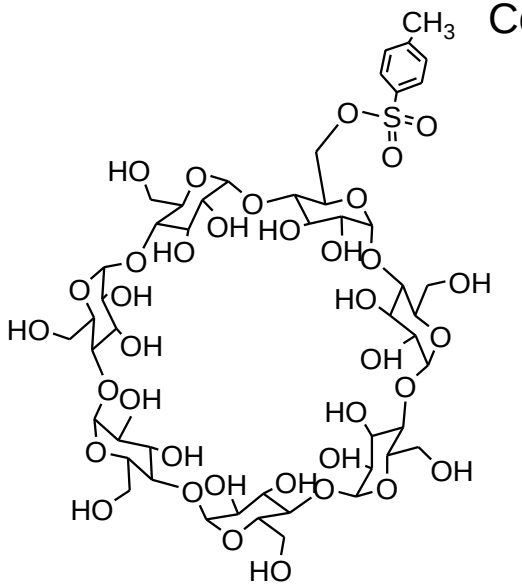
Water Soluble
Photosensitizer-CD
Conjugate
+
Drug (antibiotic, anticancer...)

βCD-Phorphyrin Conjugate

meso-tetra(m-hydroxyphenyl)-21,23Hporphyrin (mTHPP)

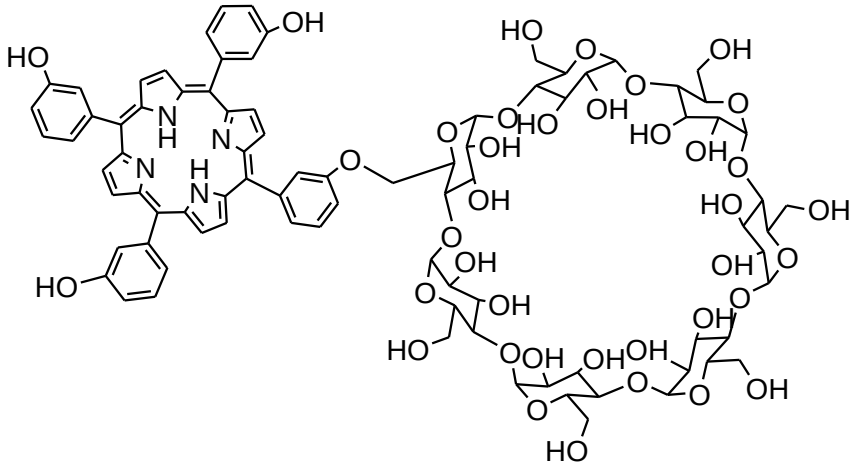


+



NaOH, DMSO, 40 °C, 4 days

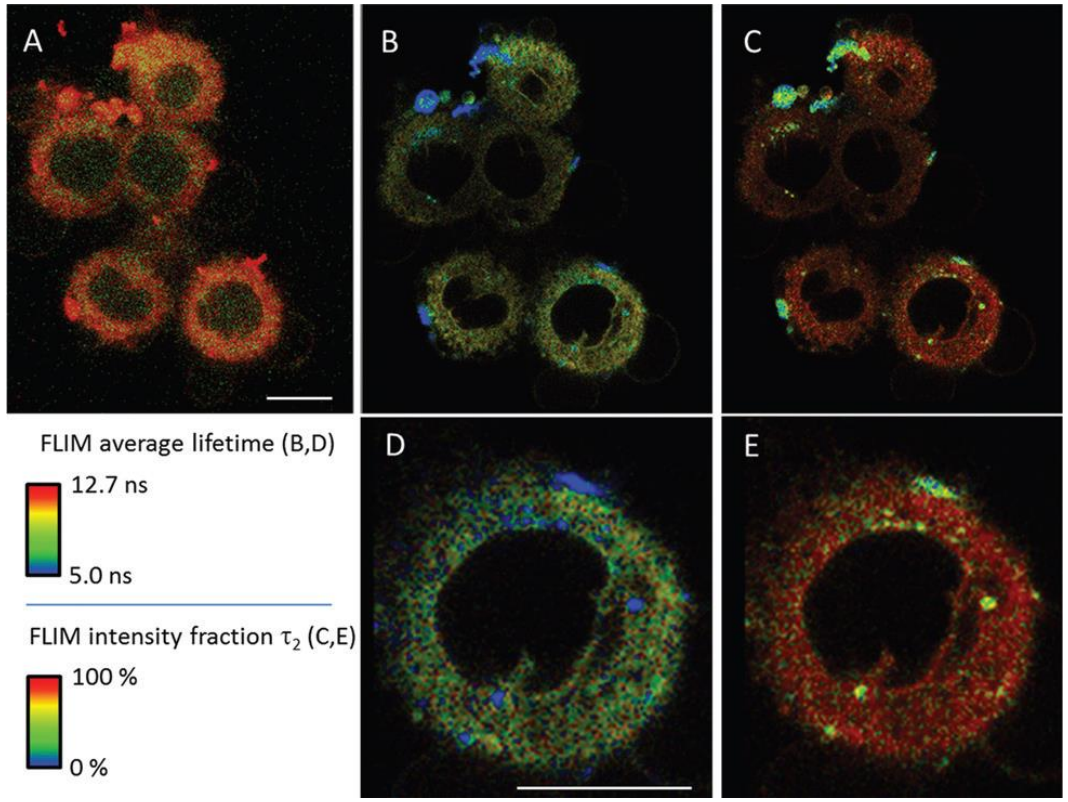
Column Chromatography



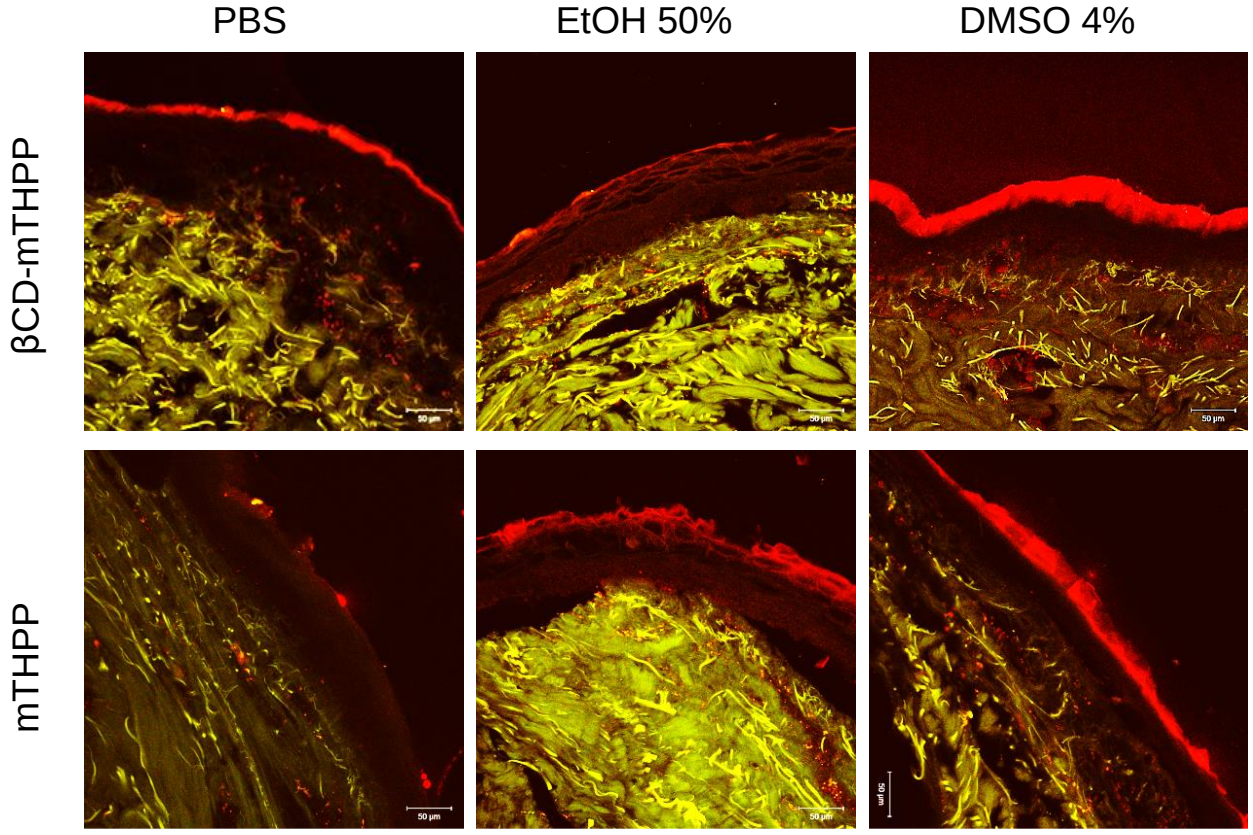
βCD-mTHPP

V. Kirejev, A. R. Conçalves *et al.*, Photochem. Photobiol. Sci., 13, 1185-1191, **2014**

βCD-Phorphyrin Conjugate, Photosensitizers and Delivery System!



(A) Confocal fluorescence image of a cluster of cancer cells incubated with βCD-mTHPP in 4% v/v DMSO–PBS showing overlay of cellular autofluorescence in green (500–550 nm) and βCD-mTHPP emission in red (660–740 nm).

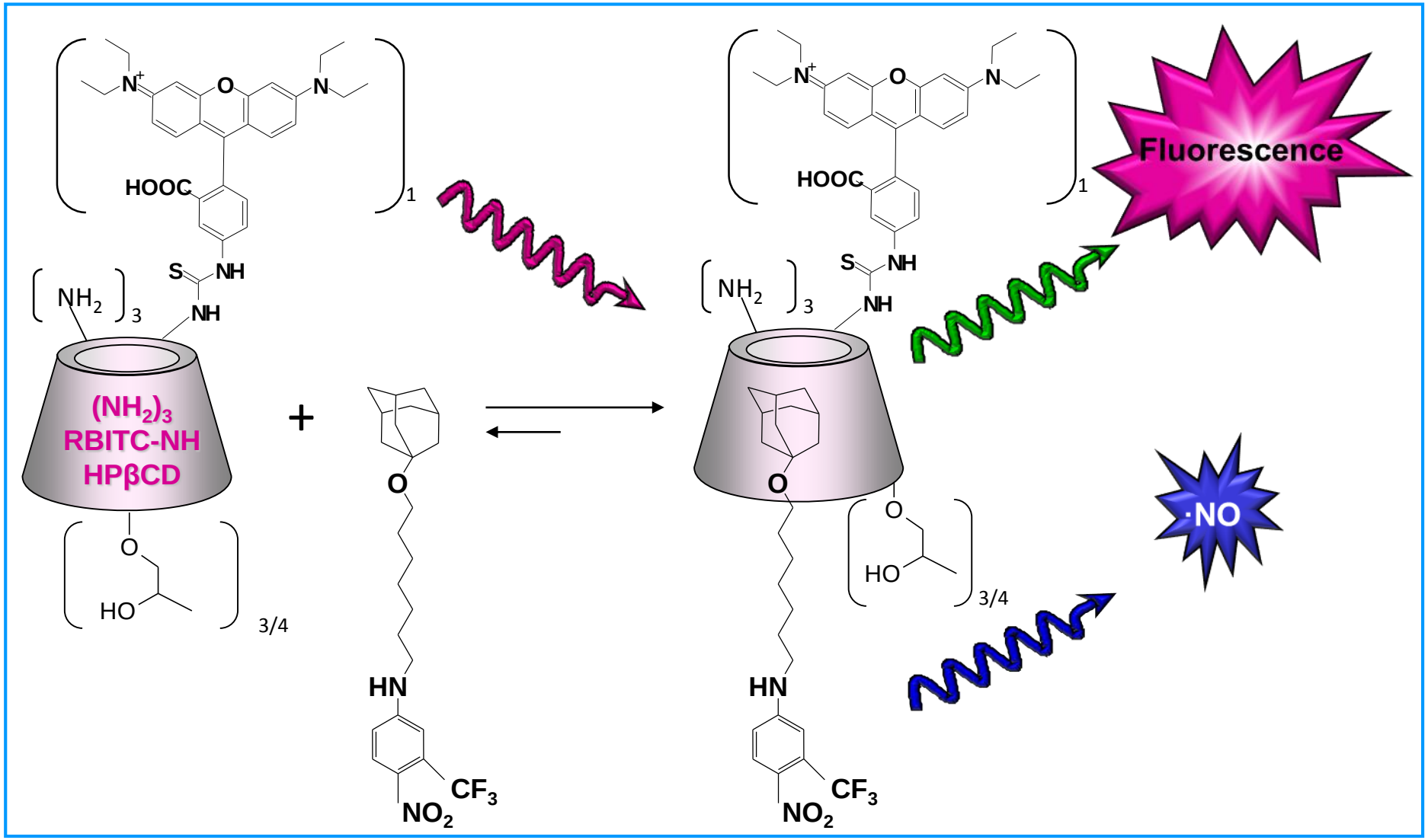


TPM images of cryosectioned human skin

Improved aqueous solubility.
Improved cytosolic uptake as monomer.
Improved biodistribution.
Multifunctional system upon complexation.

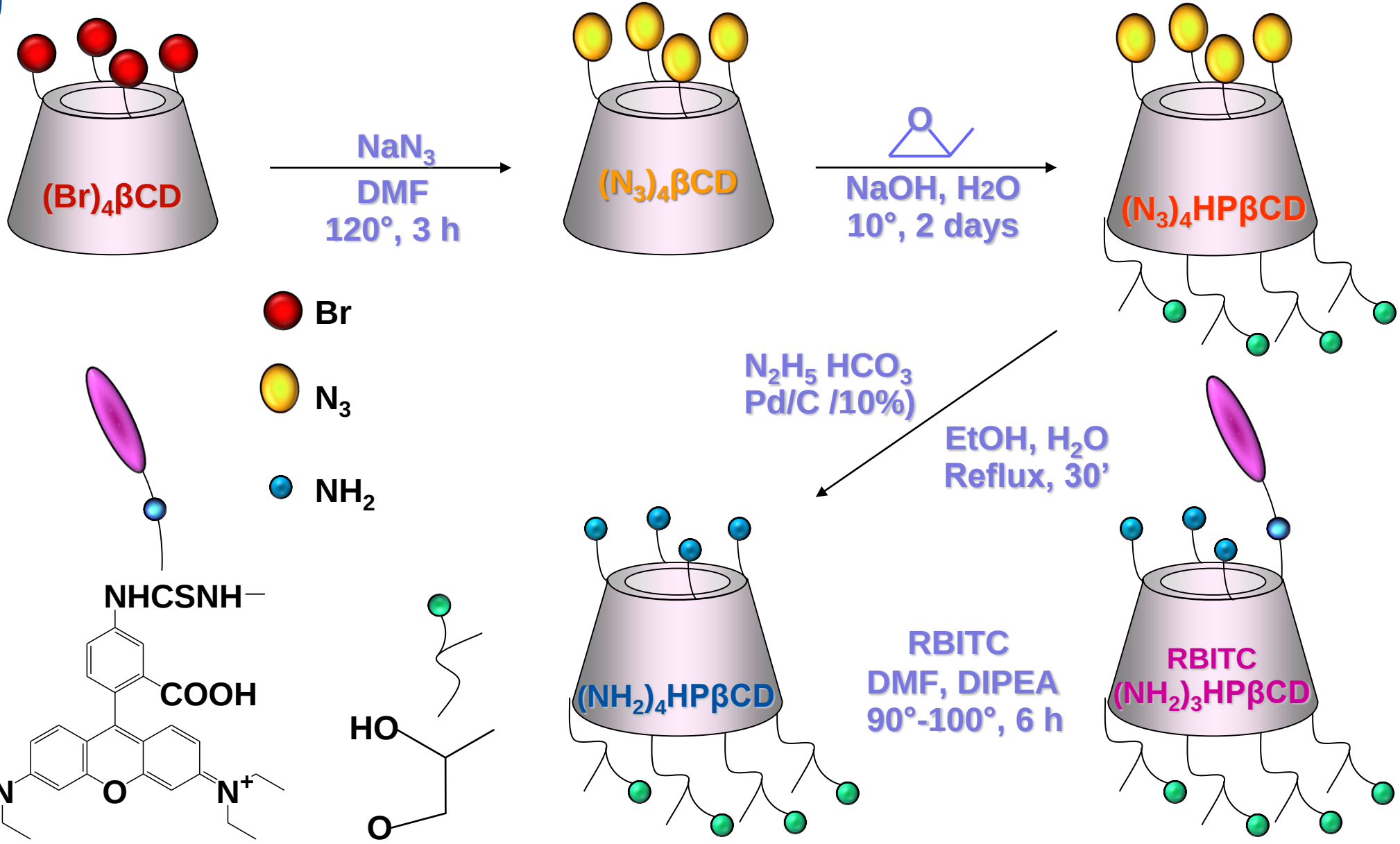
V. Kirejev, A. R. Conçalves *et al.*, Photochem. Photobiol. Sci., 13, 1185-1191, **2014**

A Host–Guest Supramolecular Complex with Photoregulated Delivery of Nitric Oxide and Fluorescence Imaging Capacity in Cancer Cells

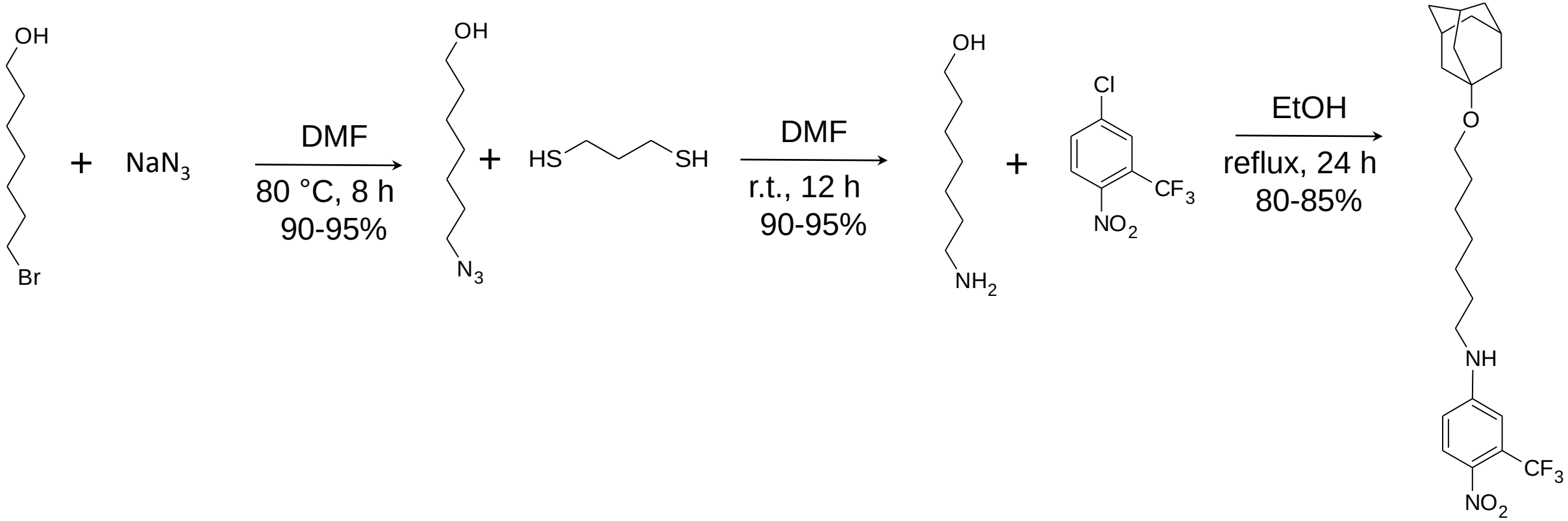


N. Kandoth, M. Malanga *et al.*, Chem. Asian J., 7, 2888-2894, **2012**

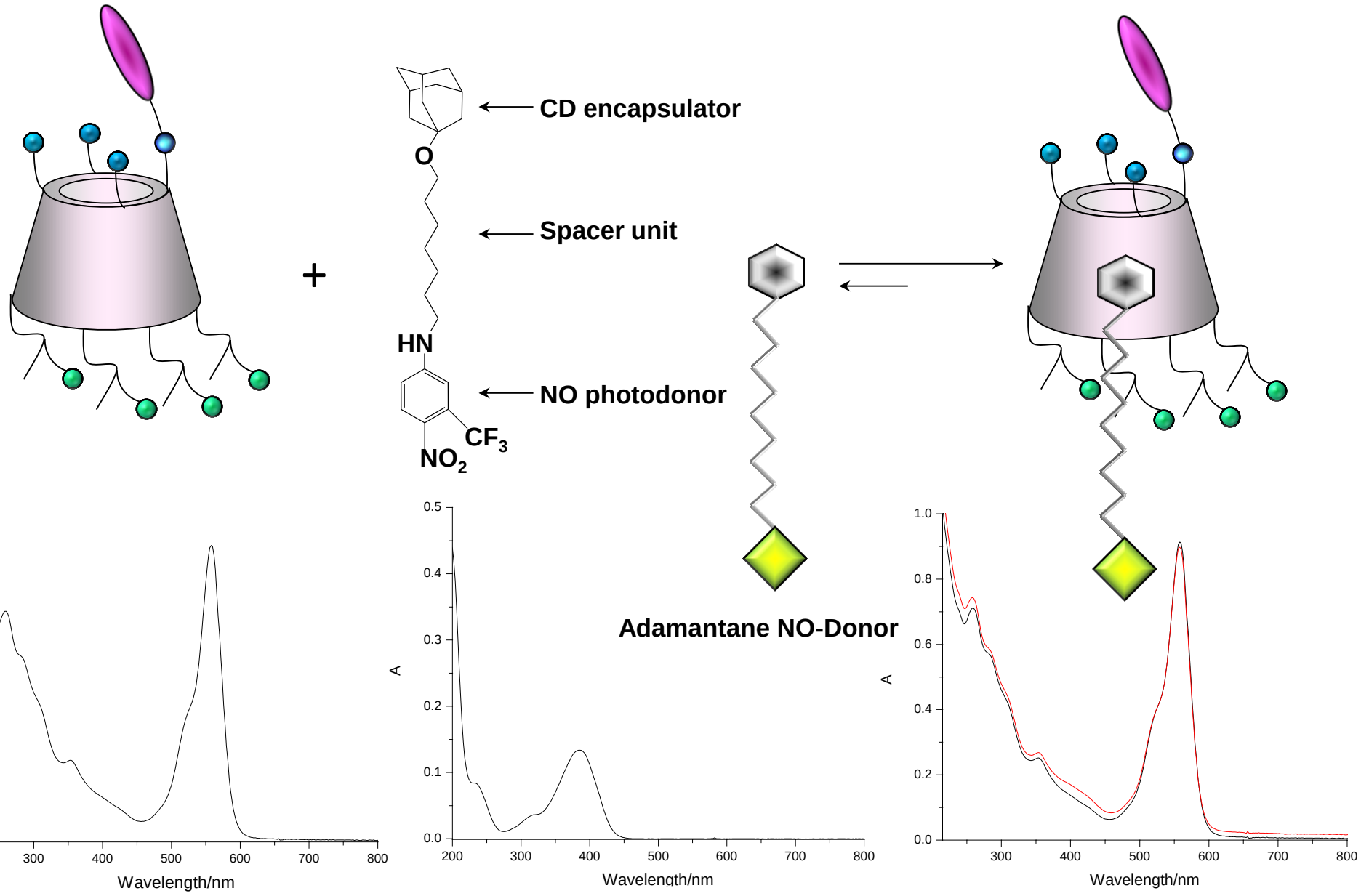
Synthesis of the Host



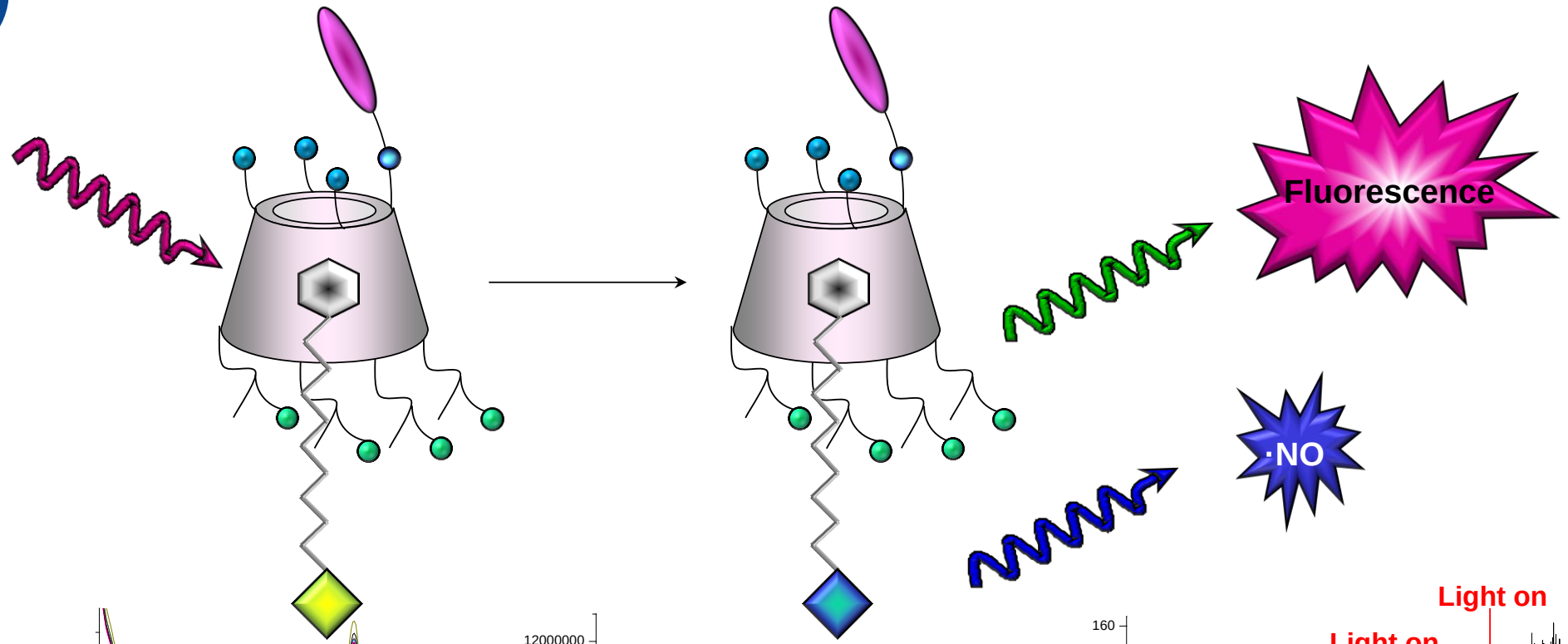
Synthesis of the Guest

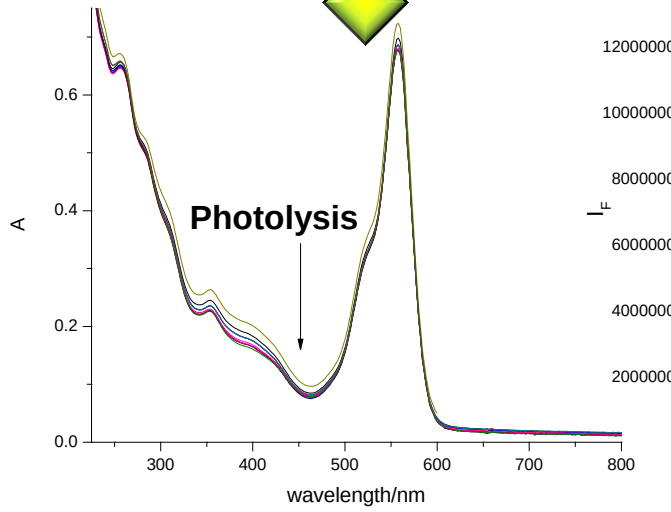


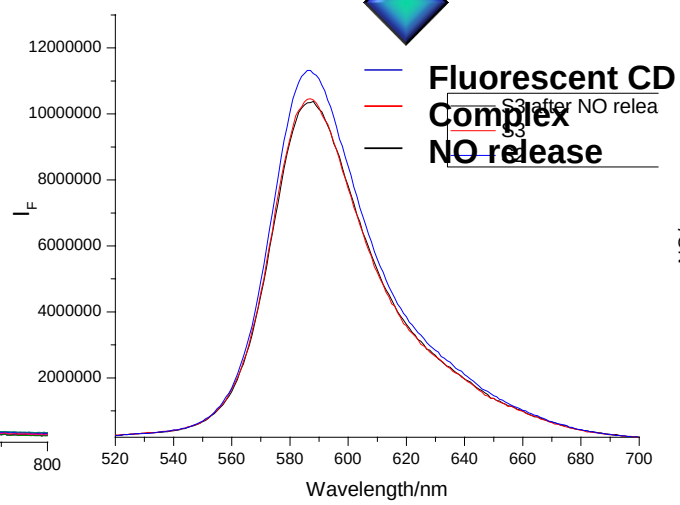
UV-vis Spectra of Host, Guest and Complex

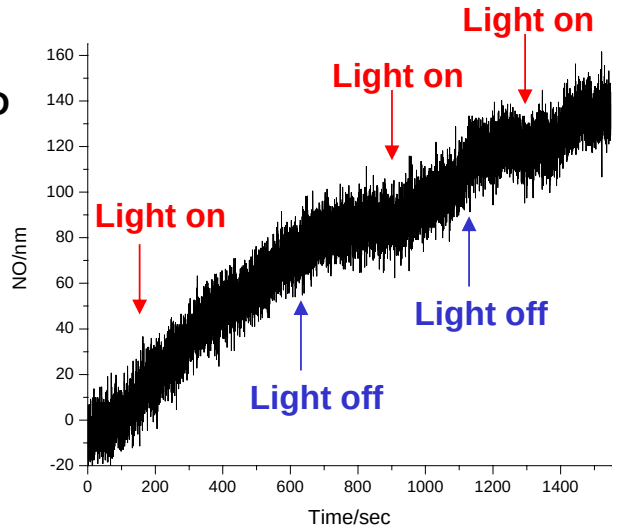


Photorealising of Nitric Oxide



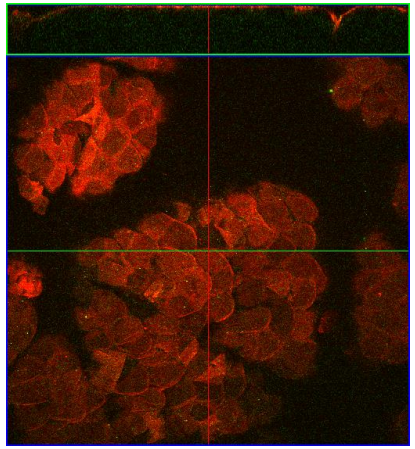


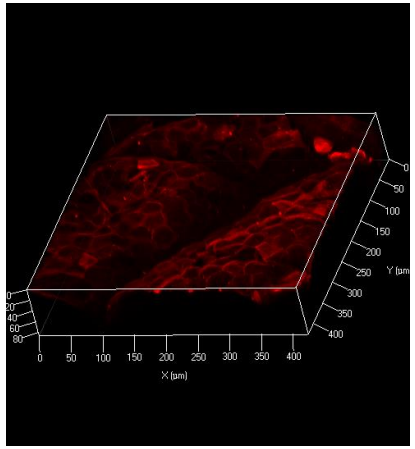




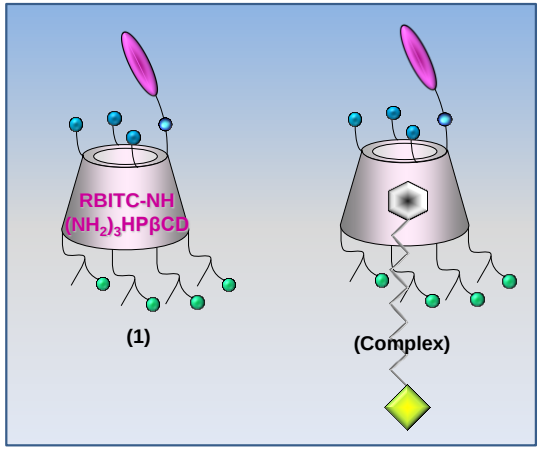
In-Vitro Activity of Fluorescent Nanostructures CD-based

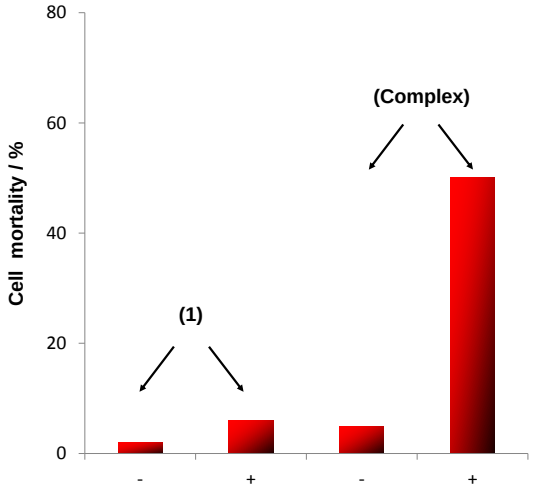
CD derivative and Human Skin

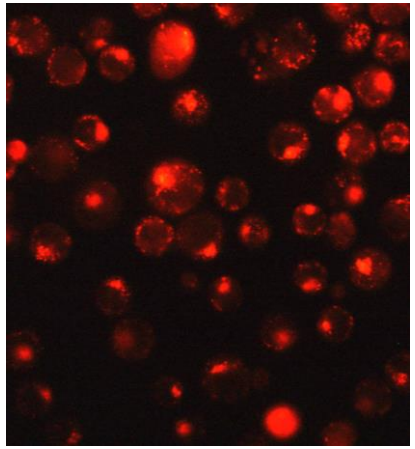




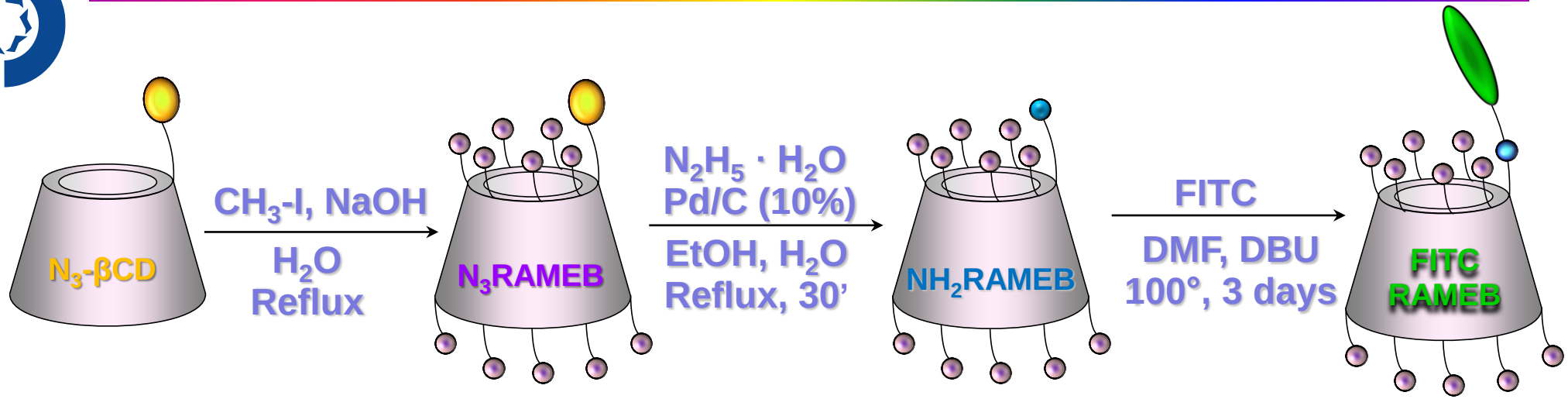
Complex and HeLa Cells ;

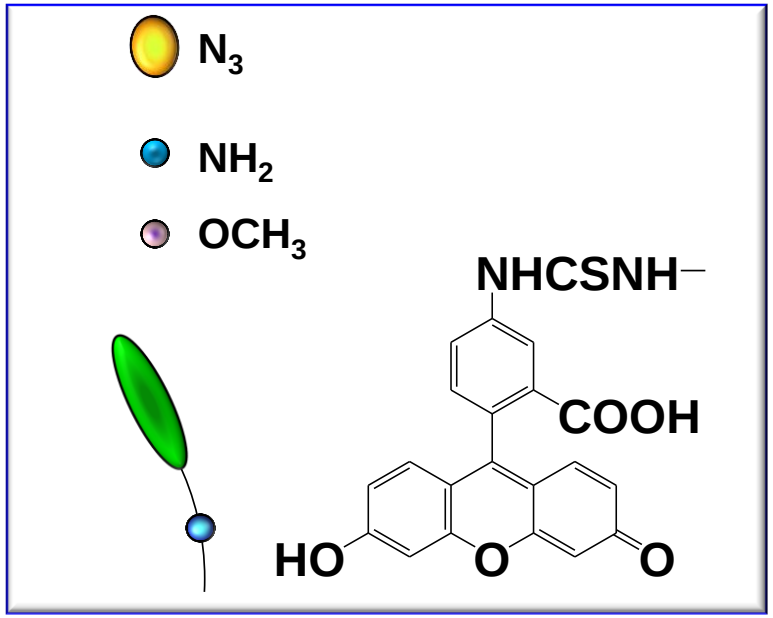


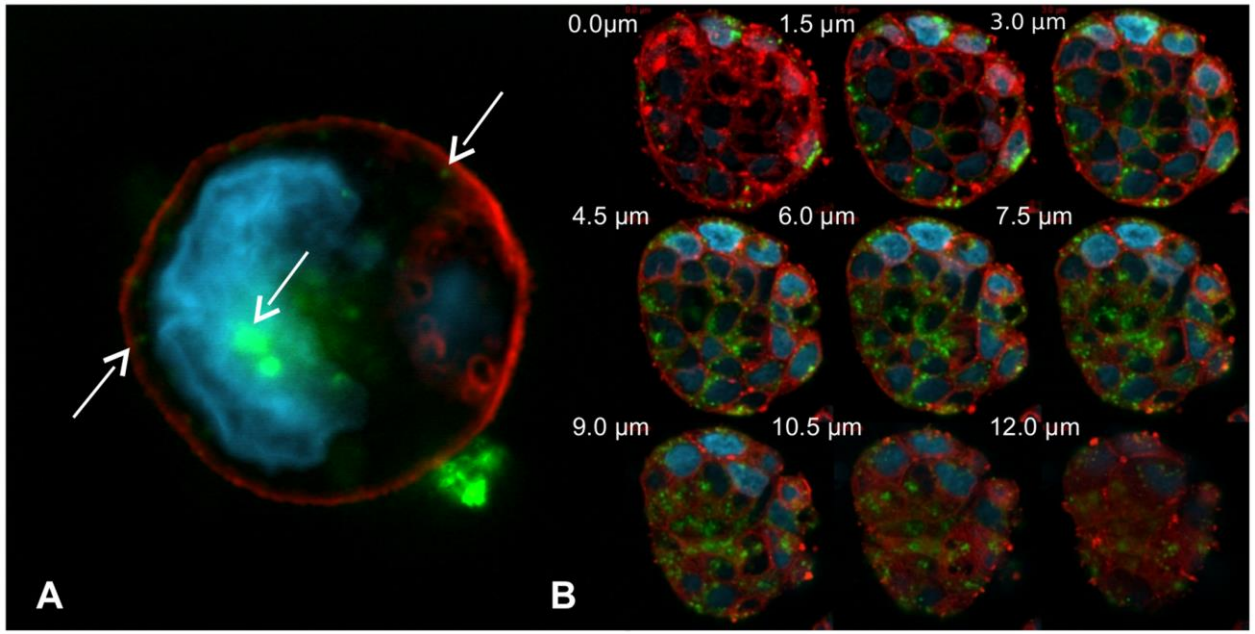




CDs for Visualization of Transmembrane Processes



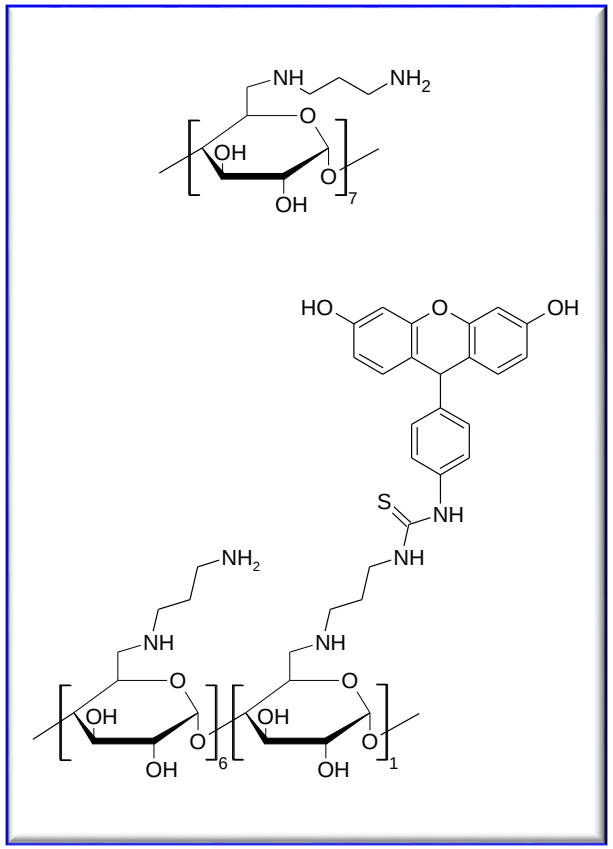


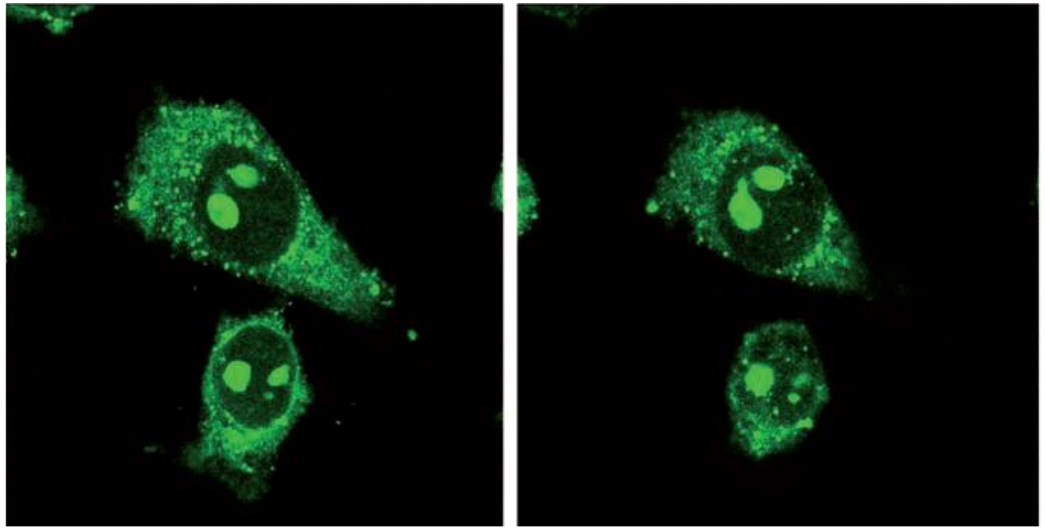


Confocal images of undifferentiated Caco-2 cells

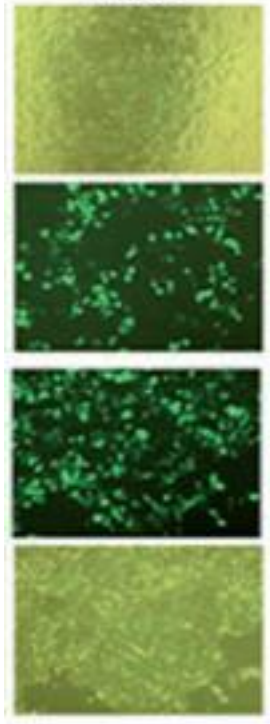
F. Fenyvesi, *et al.*,PLOS ONE, Volume 9, Issue 1, e84856, January **2014.**

Cell Penetrating CDs and DNA carriers





Confocal microscopy slices of immobilized HeLa cells



βCD-(NH-$(CH_2)_3$-NH_2)$_7$

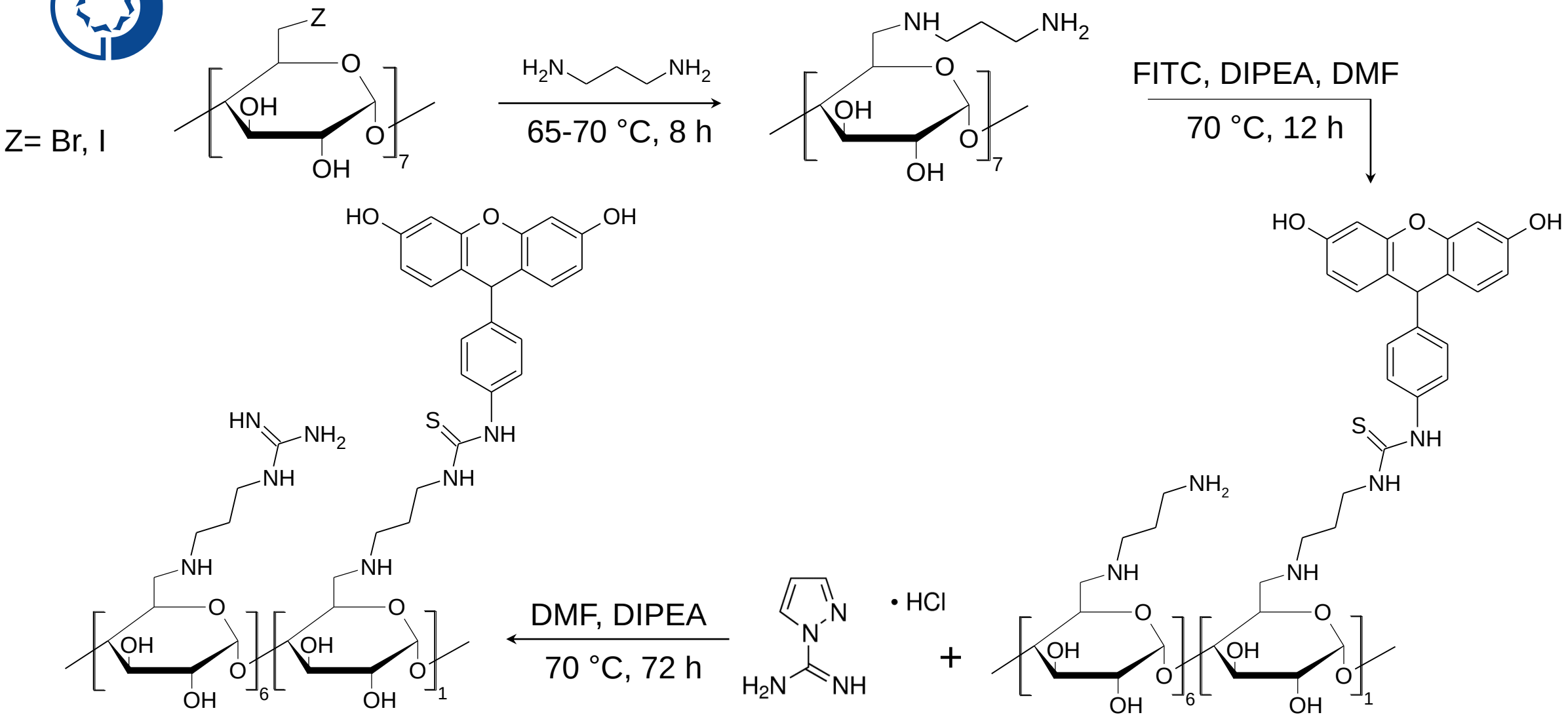
Lipofectamine 2000

Expression of eGFP protein

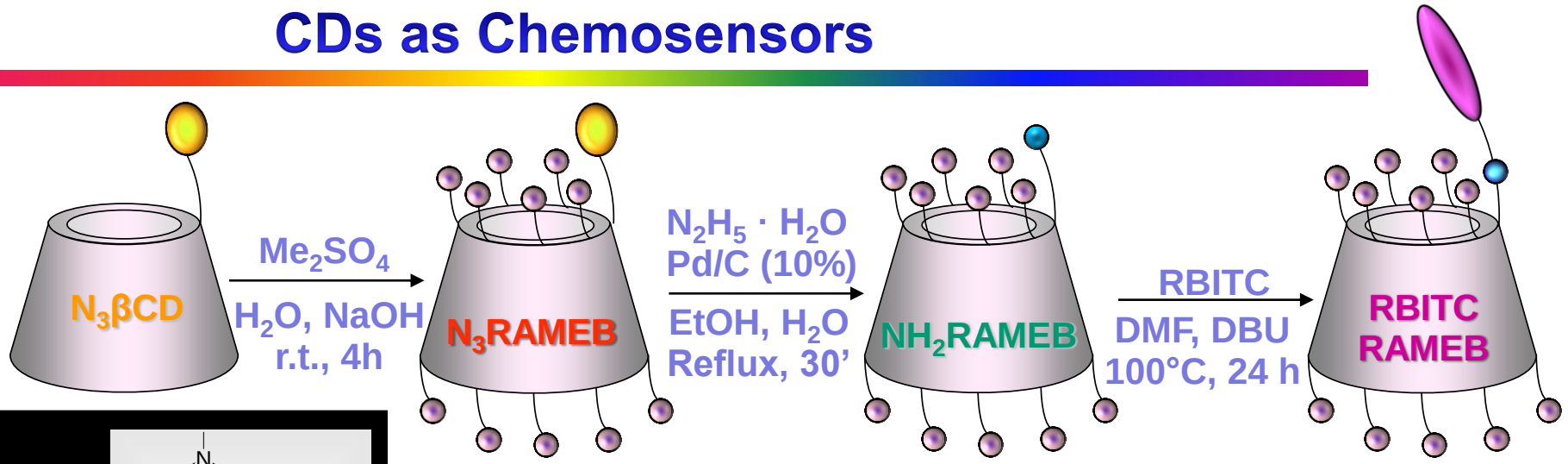
Optical and fluorescence microscopy images showing expression of eGFP protein in HEK 293T cells and comparison with Lipofectamine 2000 as control.

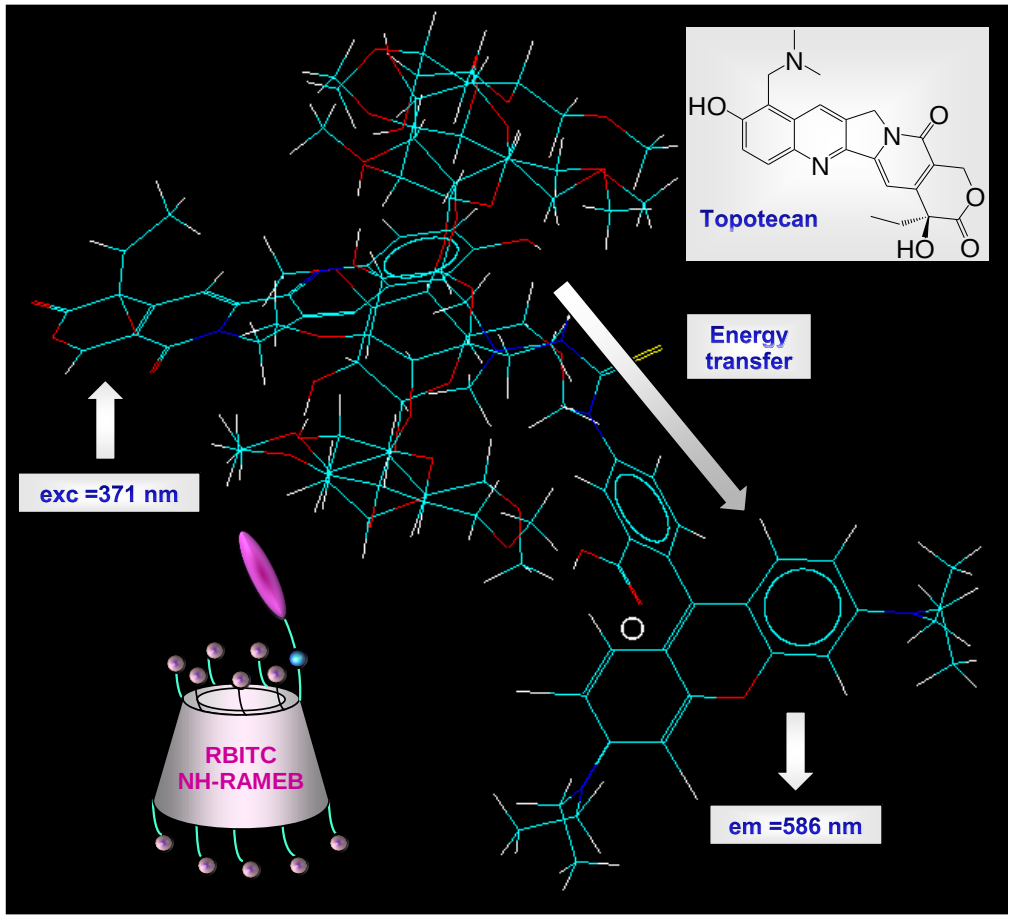
N. Mourtzis, *et al.*, Chem. Eur. J., 14, 4188 -4200, **2008**.

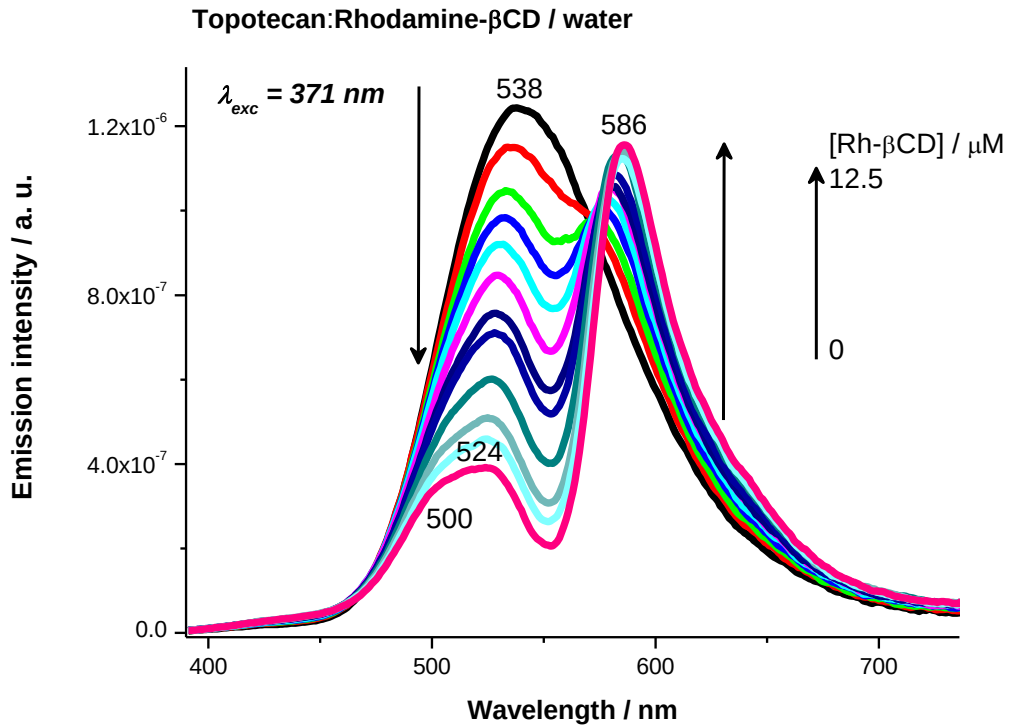
Cell Penetrating CDs and DNA carriers: Syntheses



CDs as Chemosensors



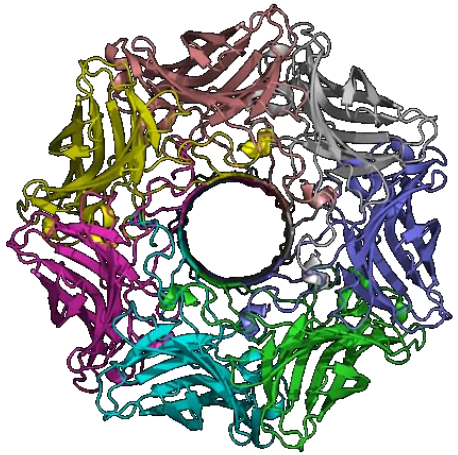




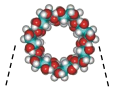
CDs as Anti-Infectives

Pore-forming proteins	
Pore-forming proteins	Pathogen
1. Protective antigen	*Bacillus anthracis*
2. α-Hemolysin	*Staphylococcus aures*
3. Ɛ-Toxin	*Clostridium perfringens*
4. Toxin C2	*Clostridium botulinum*
5. Toxins A, B and CDT	*Clostridium difficile*
6. Aerolysin	*Aeromonas hydrophilia*
7. VacA	*Helicobacter pylori*
8. p7 protein	Hepatitis C virus
9. Vpu	HIV
10. M2 protein	Influenza virus

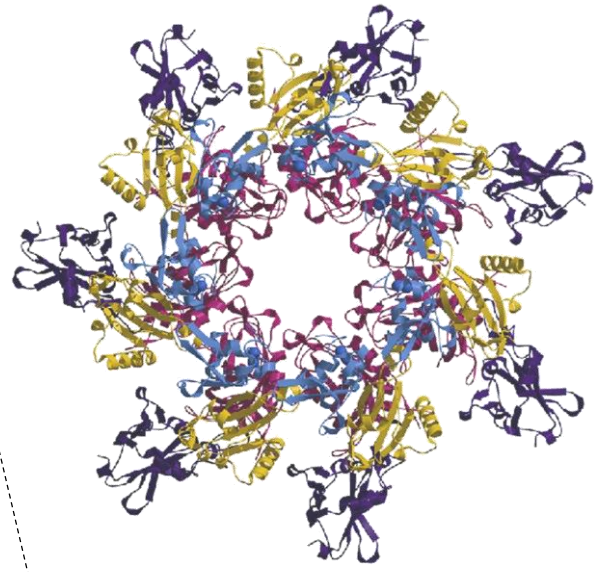
α-hemolisyn (α-Toxin)
S. aureus

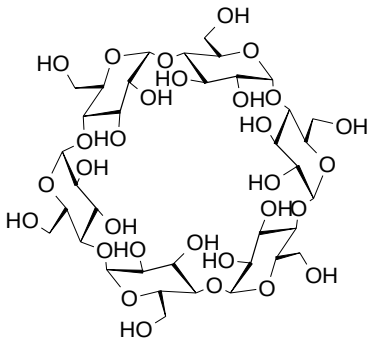


20 Å

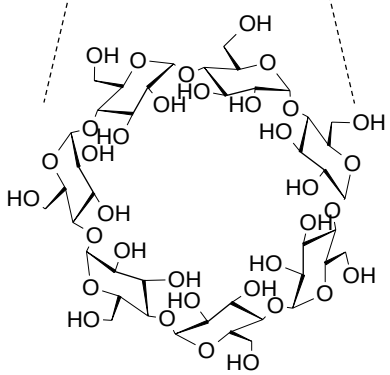


PA63
B. anthracis

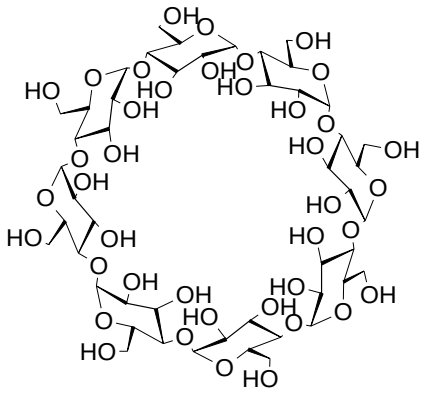




13.7 Å



15.3 Å



16.9 Å

V. A. Karginov, Current Opinion in Pharmacology, 13, 1-9, **2013**.