CYCLOLAB

The Cyclodextrin Company

IECP 2020

Synthetic Strategies for the Preparation of Multifunctional Cyclodextrin Derivatives

Milo Malanga

Outlines

- Cyclodextrins Reactivity
- Most Important Cyclodextrin Intermediates
  - 6-Monotosyl-BCD
  - Per-6-halo-CD
    - Sugammadex
  - 2-*O*-propargyl-BCD
  - Per-2-*O*-propargyl-BCD
- Fluorescent CDs
- Folate-appended CDs
- Amphiphilic CDs
- CD polymers

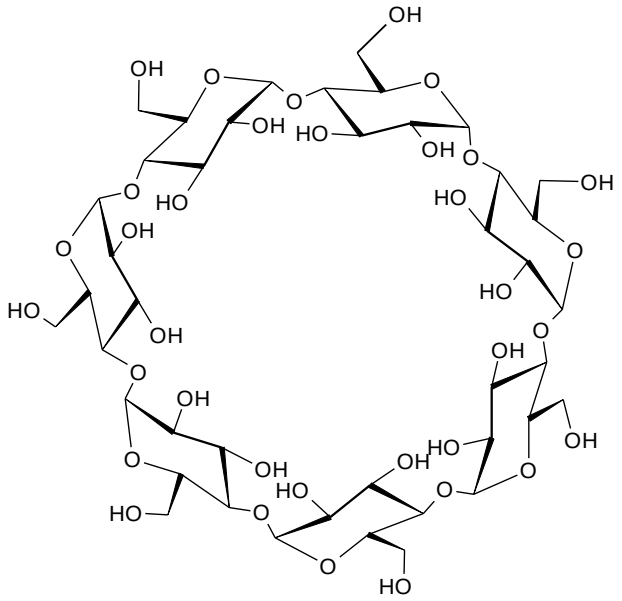
Why to modify Cyclodextrins?

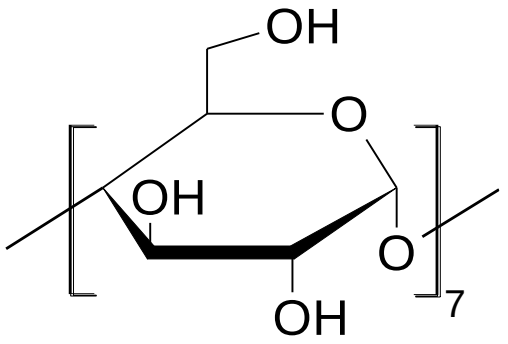
• Solubility improvement of the CD (and its complexes) in desired solvent, usually in water;

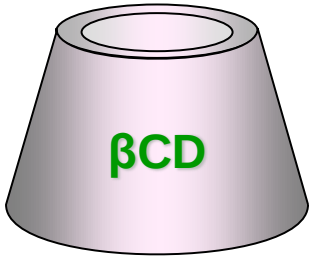
• better fit and/or association between the CD and its guest, with concomitant stabilization of the guest by changing its reactivity;

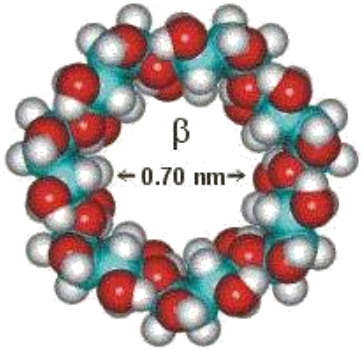
• more appropriate mimic of a binding site (e.g., in enzyme modeling) via attachment of specific groups; or

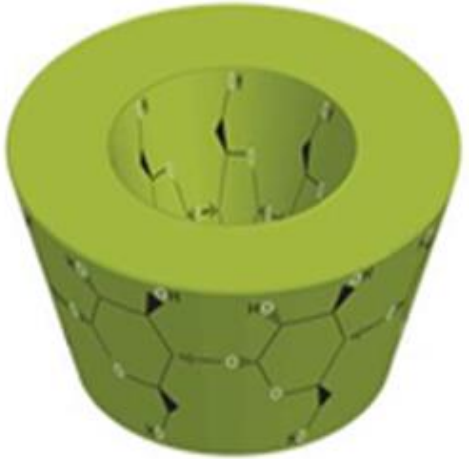
• formation of insoluble or immobilized CD-containing structures, polymers (e.g., for chromatographic purposes).

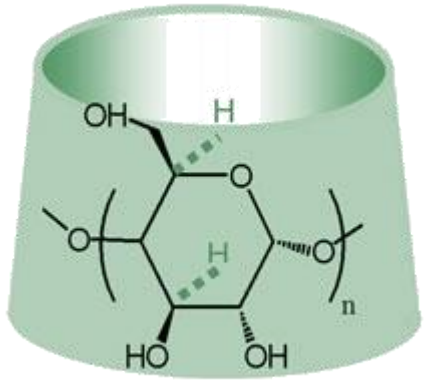






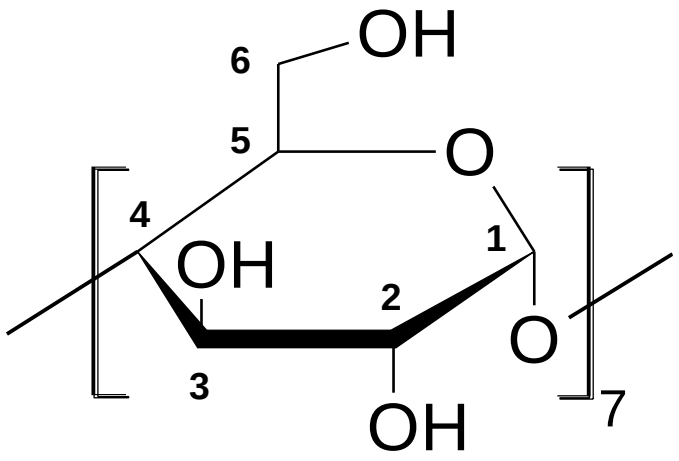






Characteristics of the Hydroxyl Groups

Less acidic, most nucleophilic

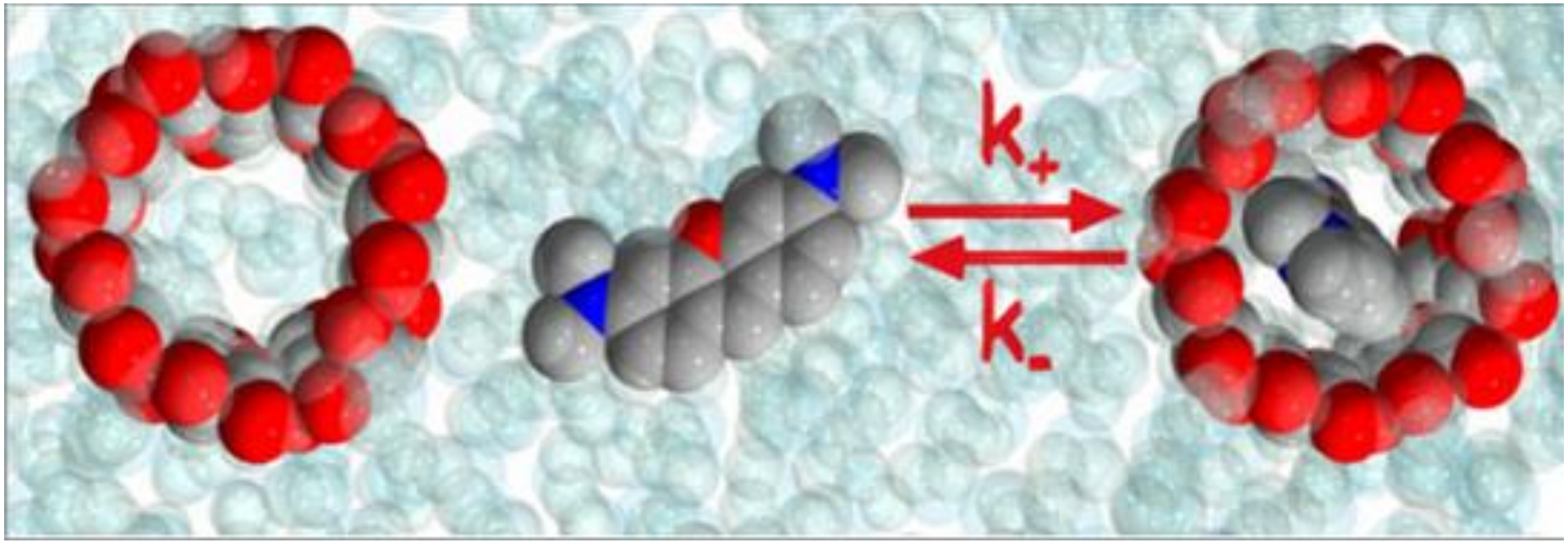


More acidic, less nucleophilic

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

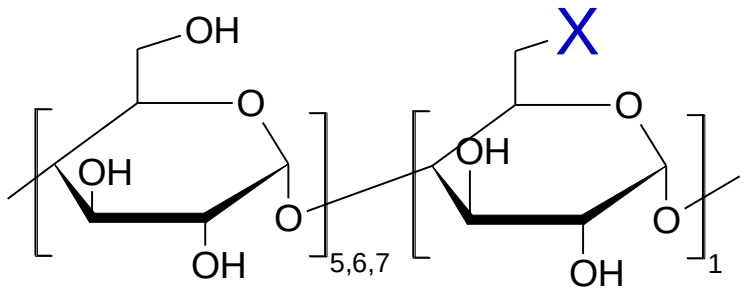
Cyclodextrins are CYCLIC Oligosaccharides



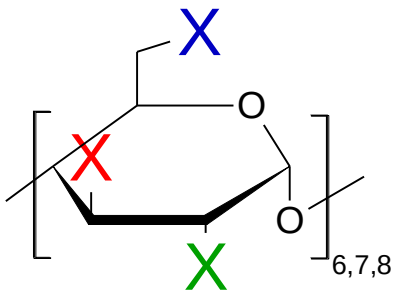
Reagents may interact with CDs in unexpected ways!

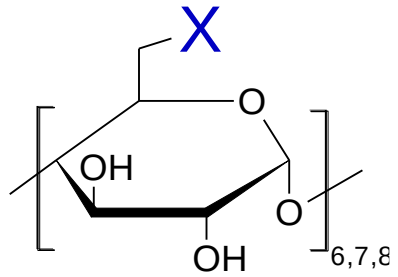
Most Important “Single Isomer” CD Intermediates

- **Mono-6-Substituted Cyclodextrin:**

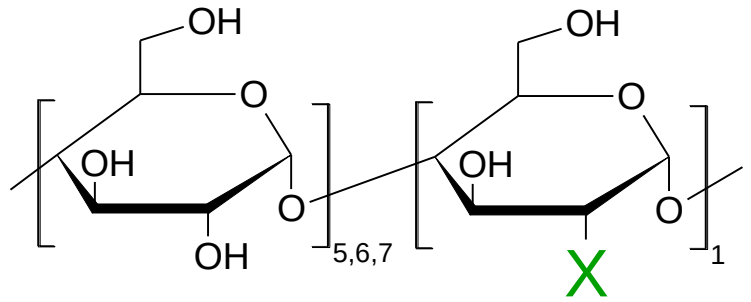


- **Per-6-Substituted Cyclodextrins:**

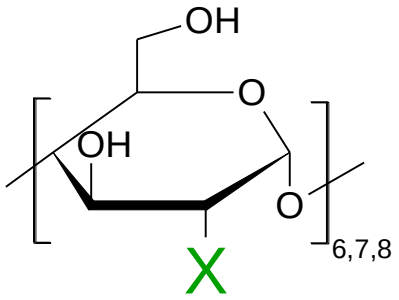




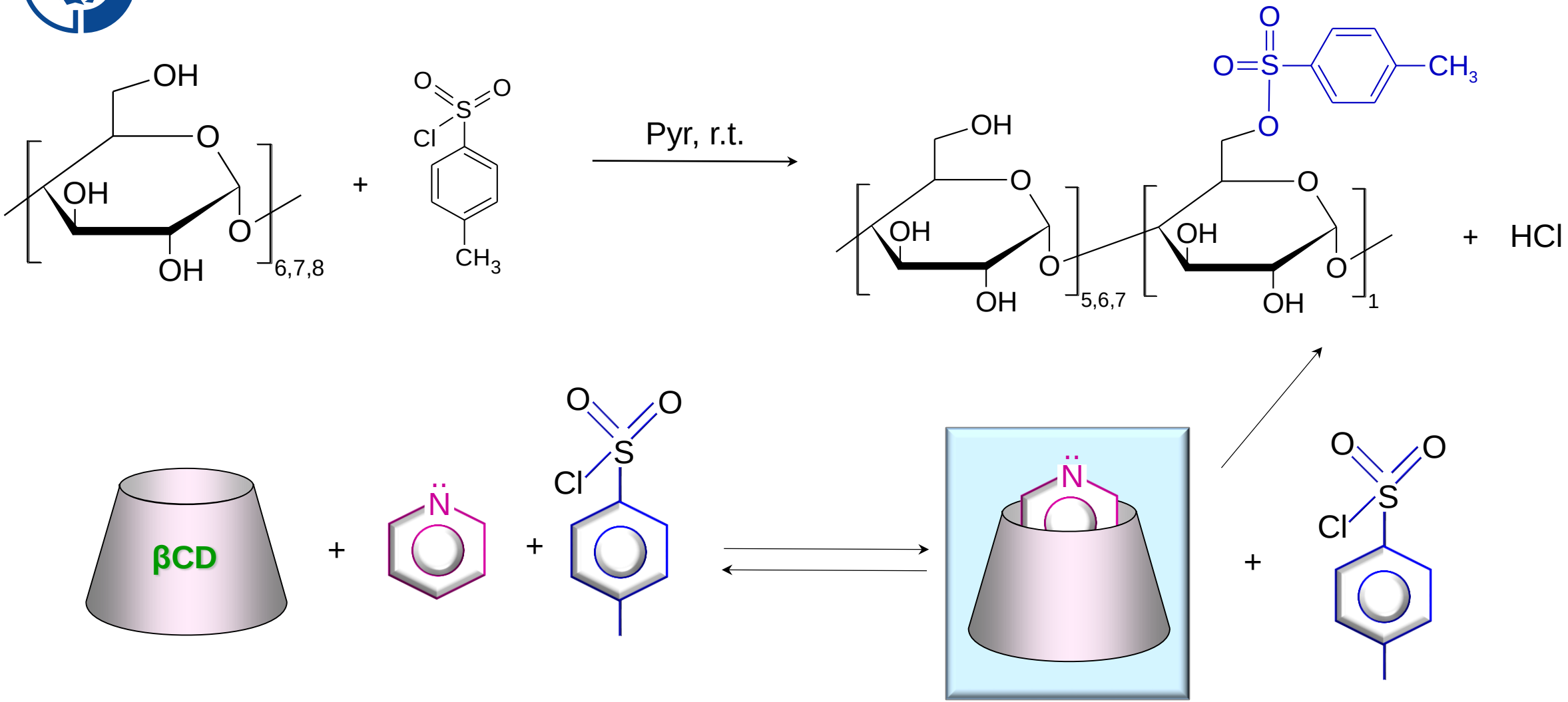
- **Mono-2-Substituted Cyclodextrin:**



- **Per-2-Substituted Cyclodextrin:**



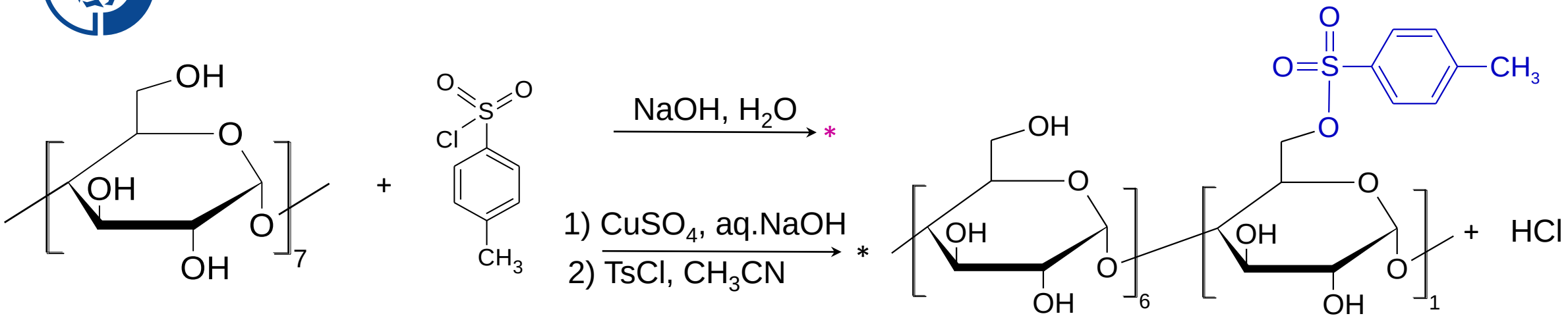
Mono-6-Tosyl-CDs (in Pyridine)

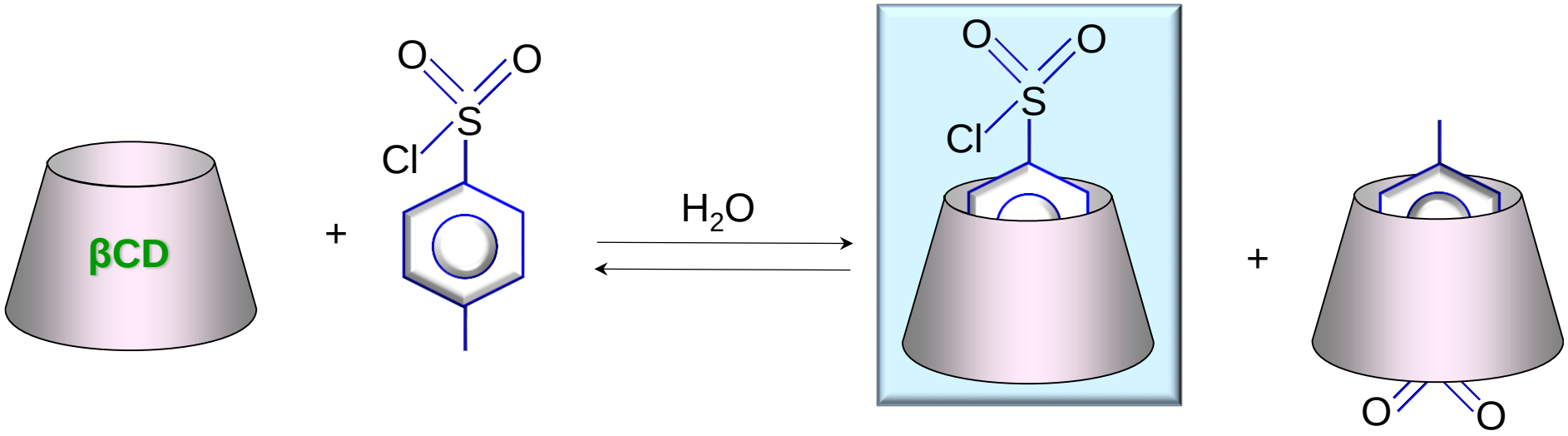


The most nucleophilic OH reacts with TsCl!

Tang W. et al., Nature protocols, 3, 4, 691-697, 2008

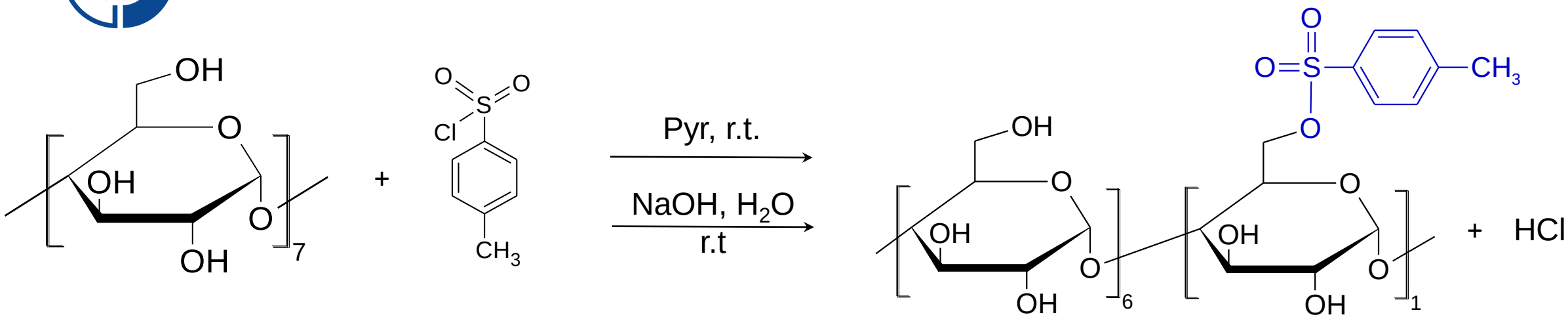
Mono-6-Tosyl-βCD (in H_2O)





Darcy R. et al., Organic Synthesis,10, 686, 2004
Defaye J. et al., Patent : US6570009 B1 (2003)

Mono-6-Tosyl-CDs (Pyridine *vs* H_2O)



Pyridine

α-, β-, γ-CD

Fast reaction

Over-tosylation

Dry conditions

Difficult work-up

Hazardous solvent

H_2O

Green solvent

Possible scale-up (β-CD)

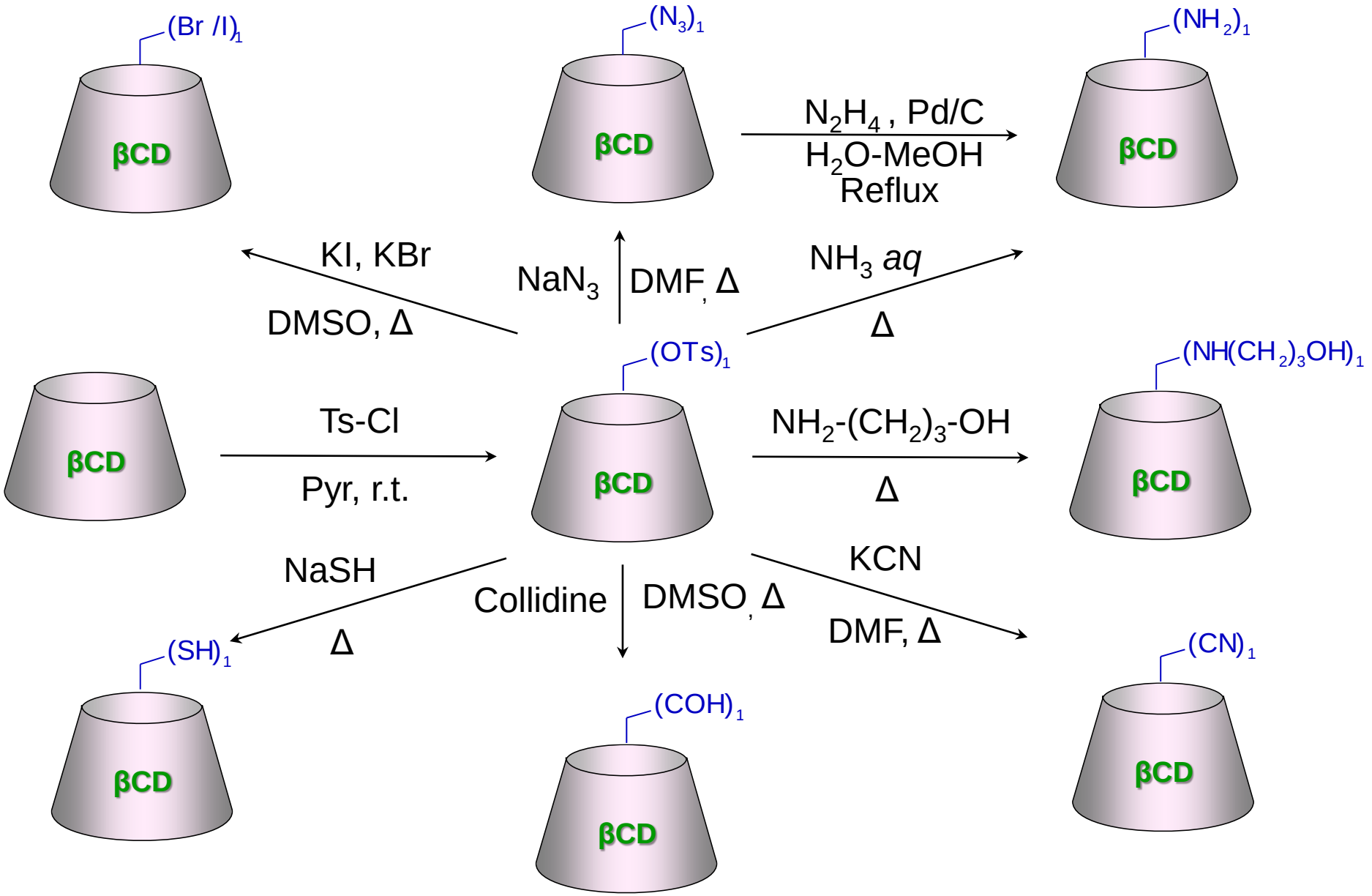
Only βCD

Low yield

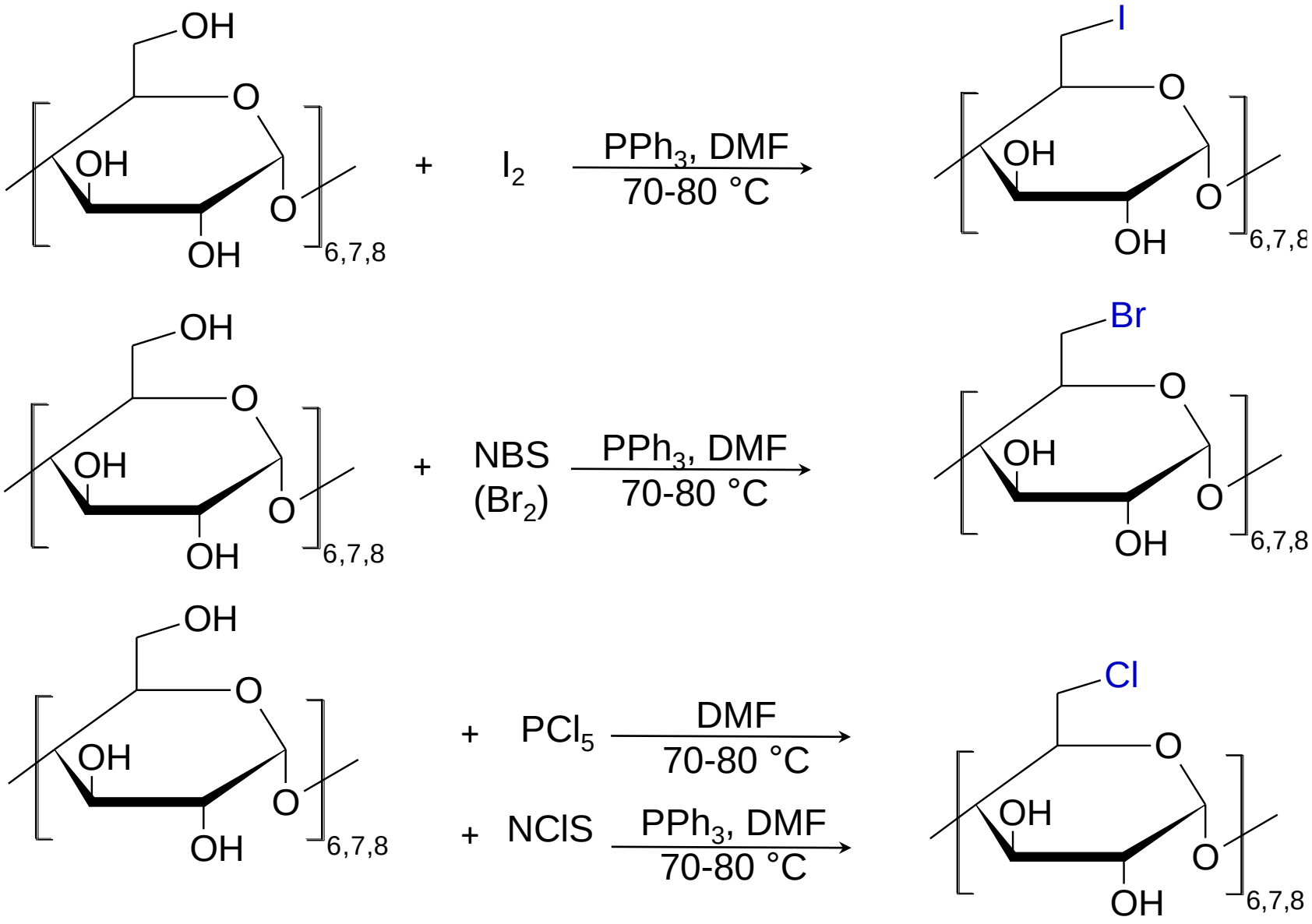
Hydrolysis of product

Excess of TsCl

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

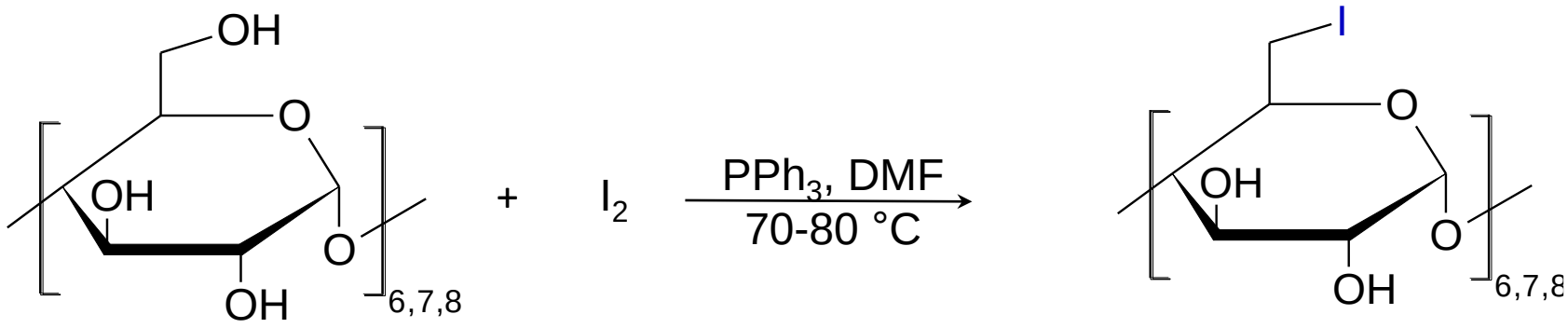


Per-6-Halogen-CDs

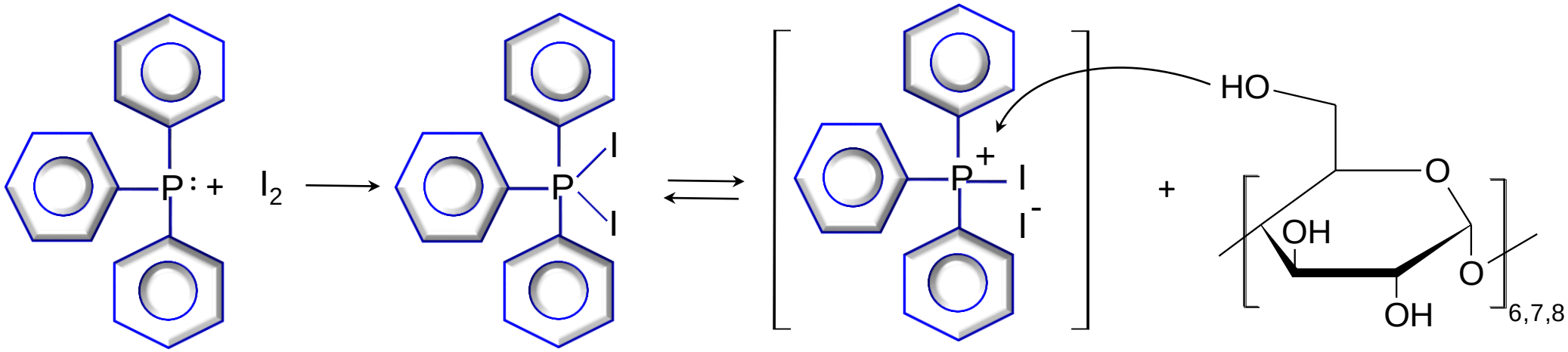


Gadelle A., Defaye J., Angew. Chem. Int. Ed. Engl. 30 (1991) No. 1, 78-80.

Per-6-Halogen-CDs, Regioselective Synthesis



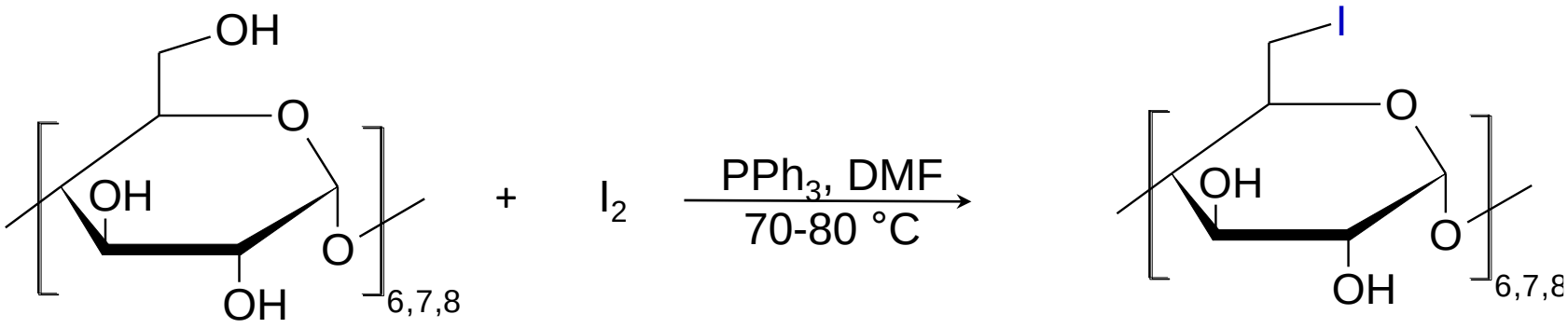
Appel type reaction



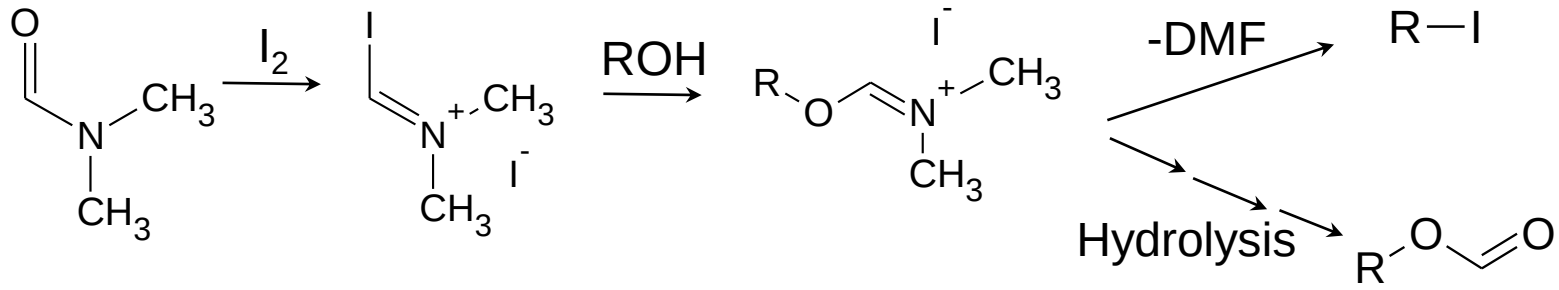
The less hindered OH reacts!

Nobel A. et al., Patent: US 6,670,340 (2003)

Per-6-Halogen-CDs, Side-Reactions



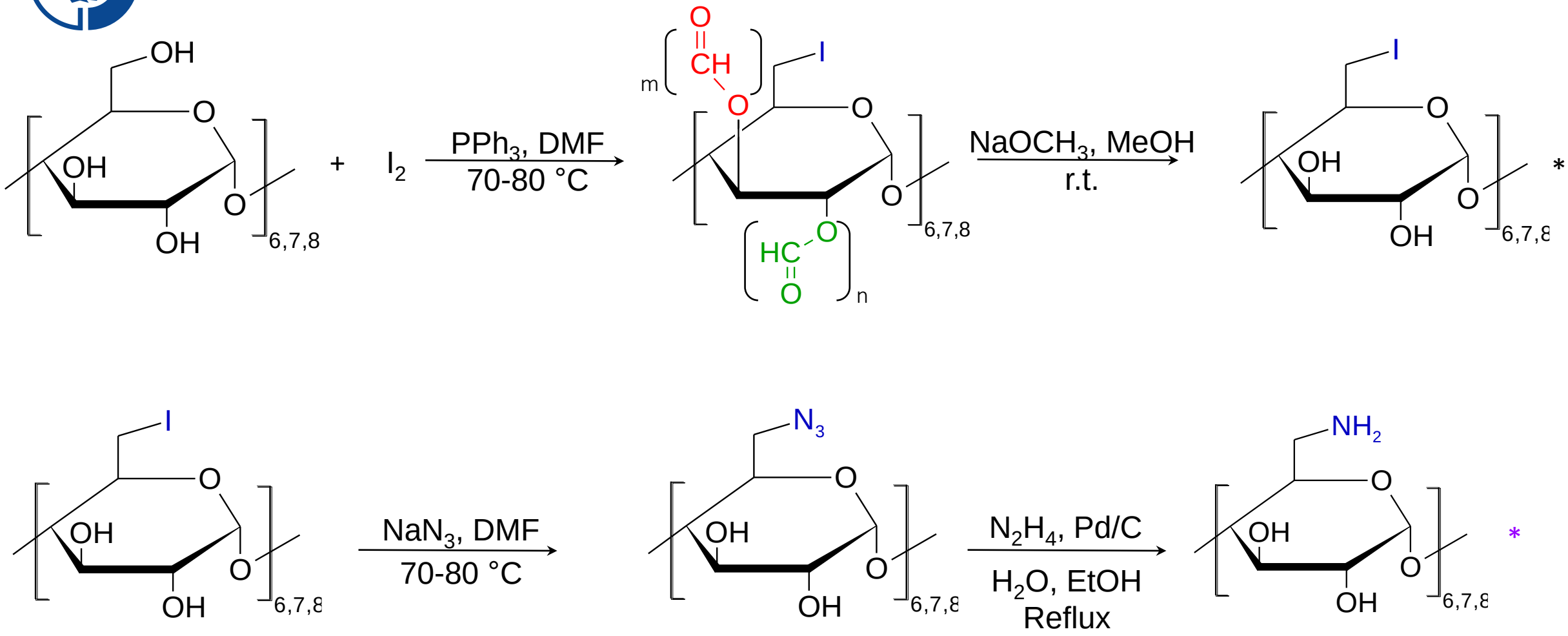
Vilsmeier-Haack type reaction



Both pathways occur!

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

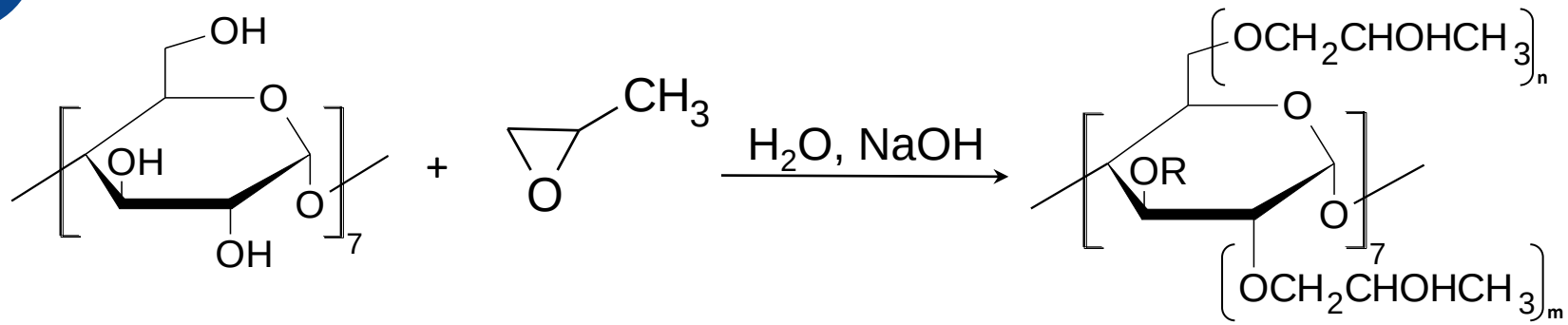
Per-6-Halogen-CDs, Versatile Compounds



∗ *Ashton P. R., Stoddart J. F. et al., J. Org. Chem., 60, 3898, 1995*

* *Jicsinszky L., Iványi R., Carbohydr. Polym., 45, 139-145, 2001*

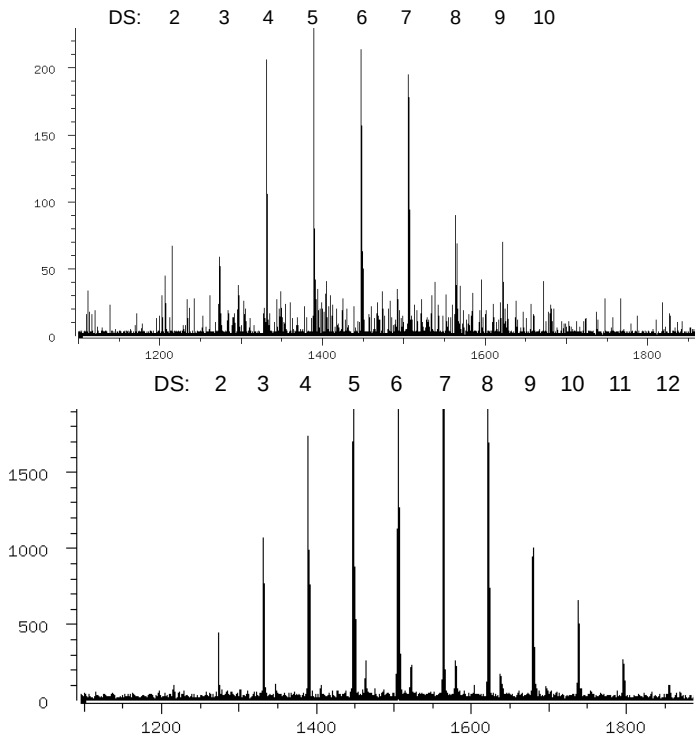
2010-FDA granted HPβCD Orphan Drug Status



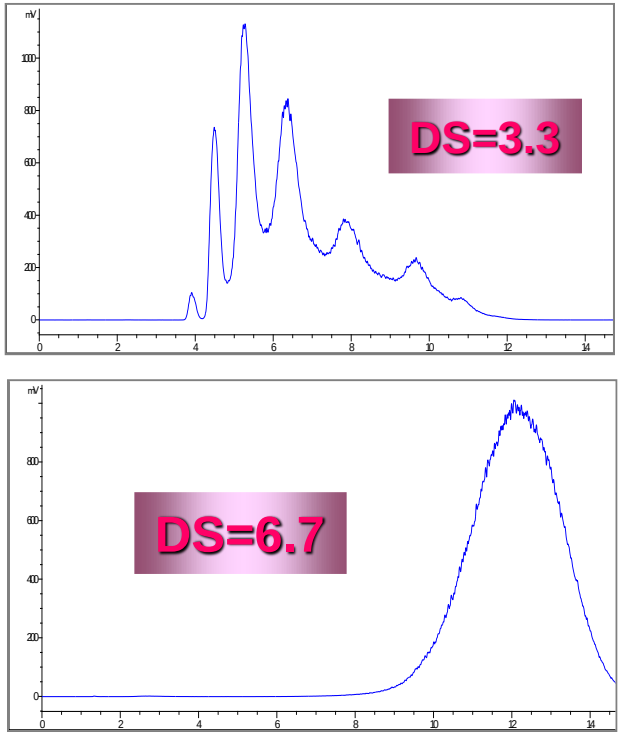
Ph. Eur.

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

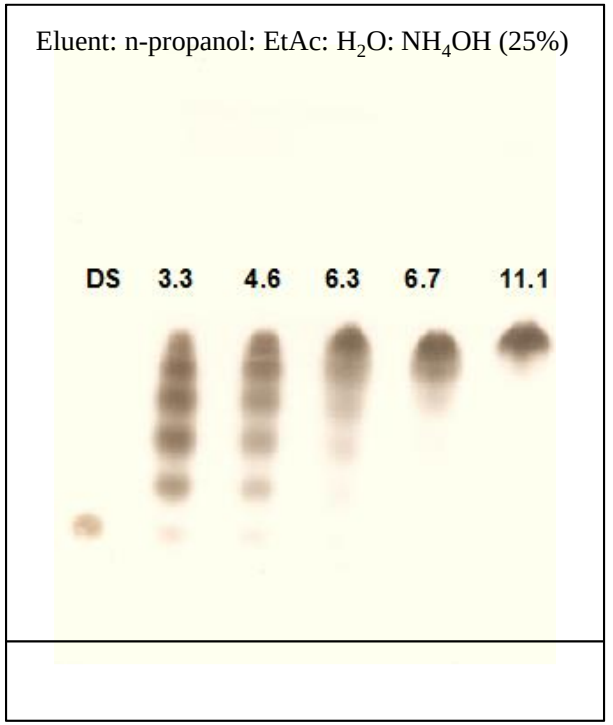
MALDI-TOF



HPLC

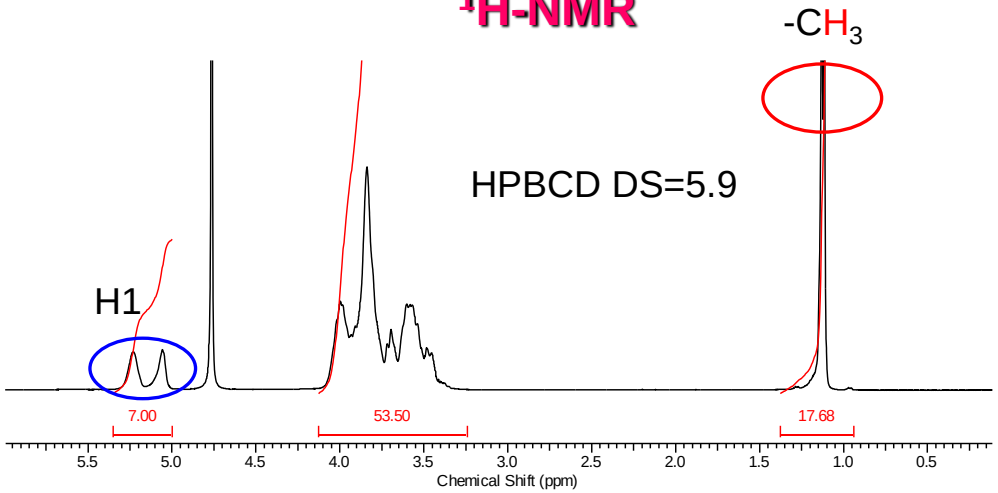


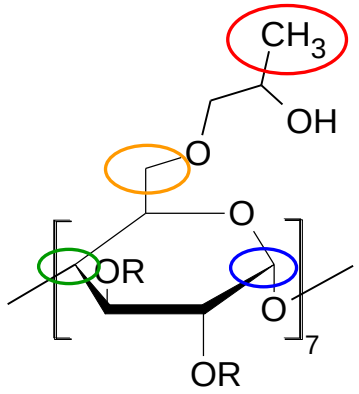
TLC



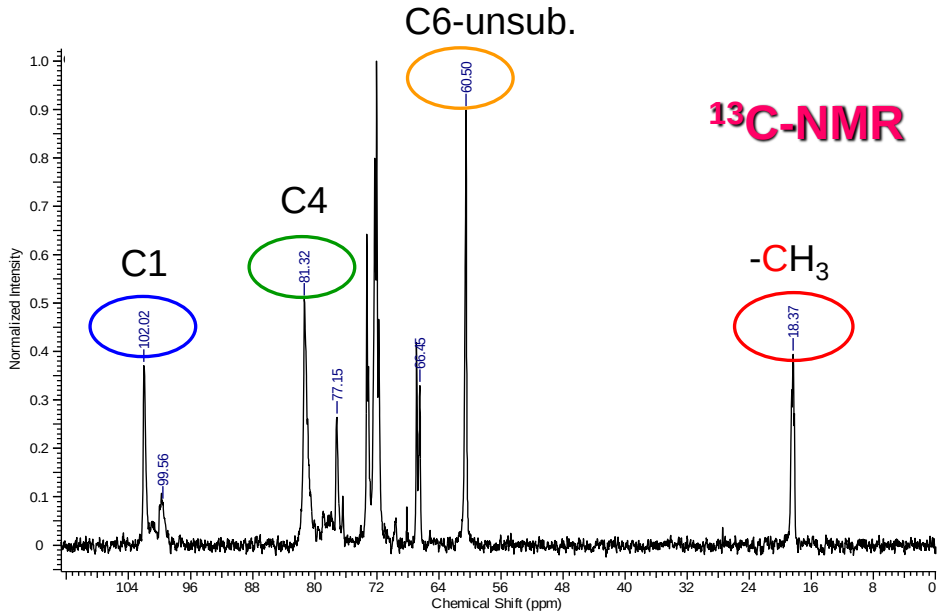
Information on the Positions of Substituents (NMR!)

^{1}H-NMR

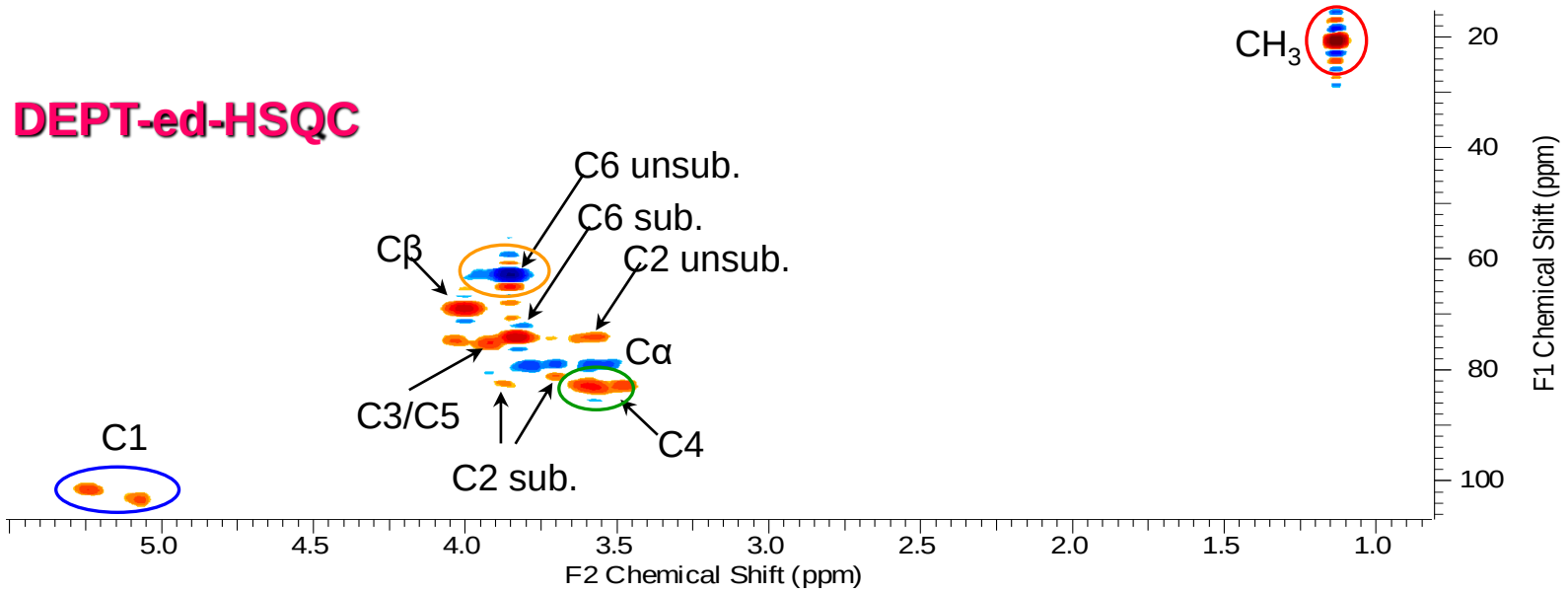




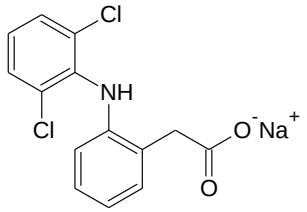
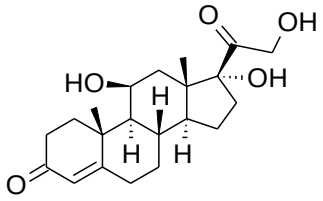
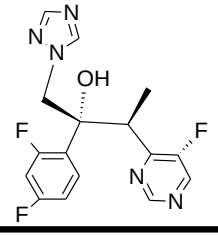
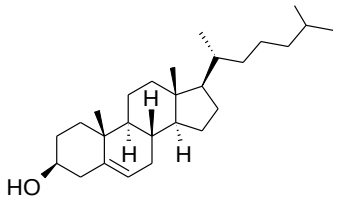
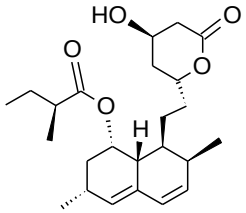
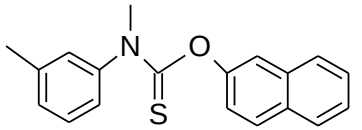
^{13}C-NMR

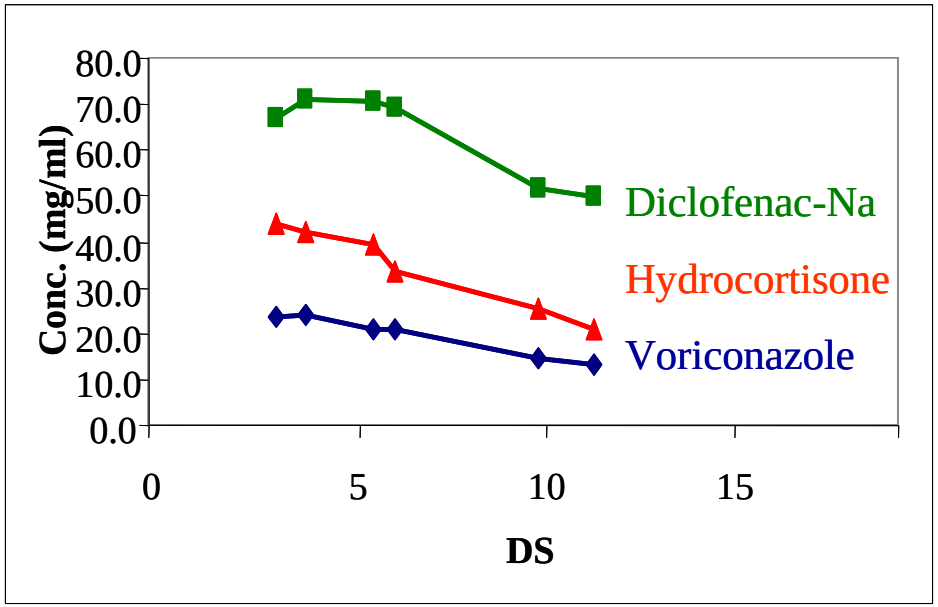


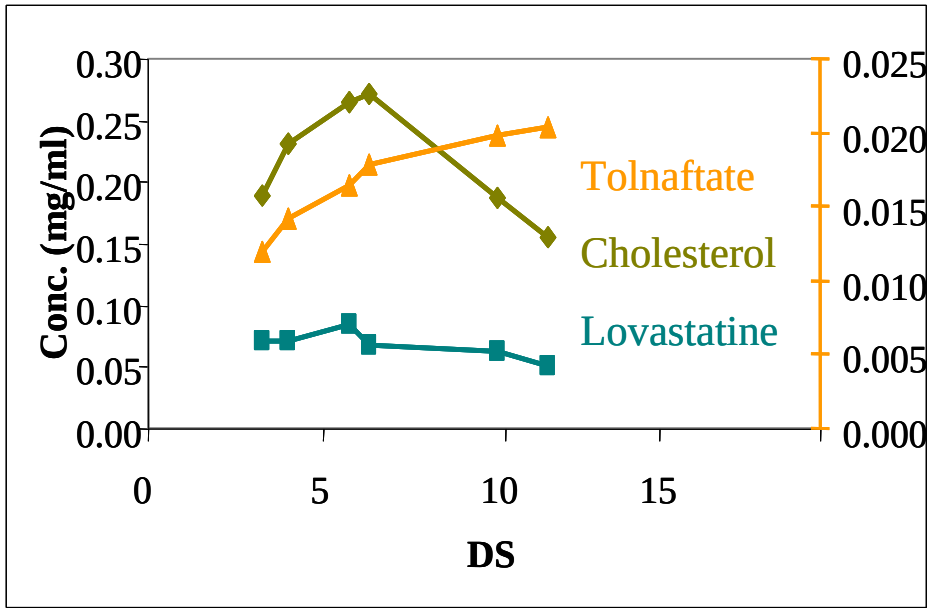
DEPT-ed-HSQC



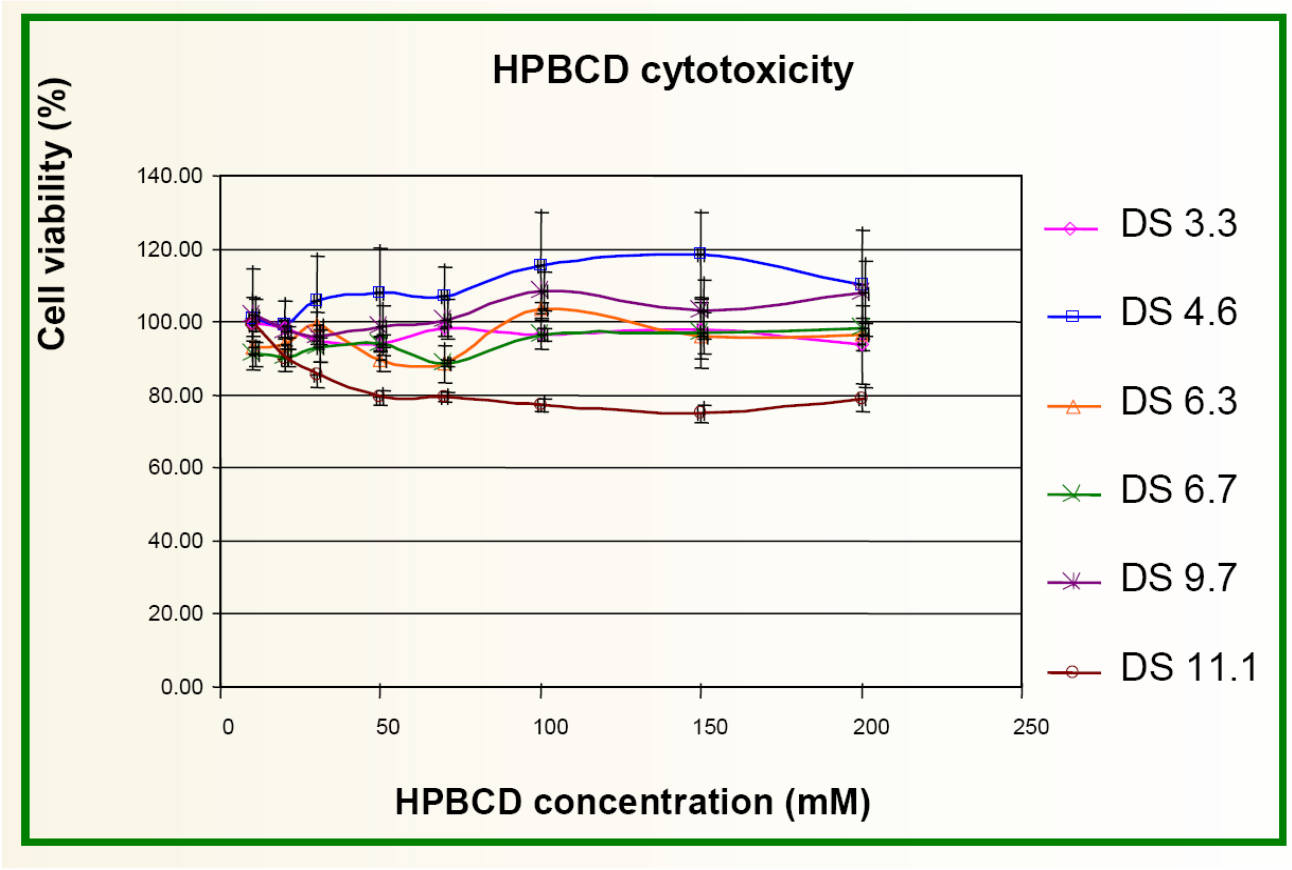
Solubilizing Capacity of HPBCDs with different DS

Diclofenac-Na	Hydrocortisone	Voriconazole	Cholesterol	Lovastatine	Tolnaftate
					



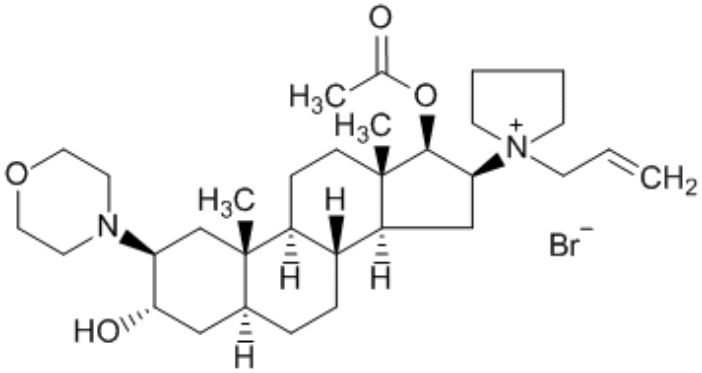


Cytotoxicity of HPBCDs with different DS

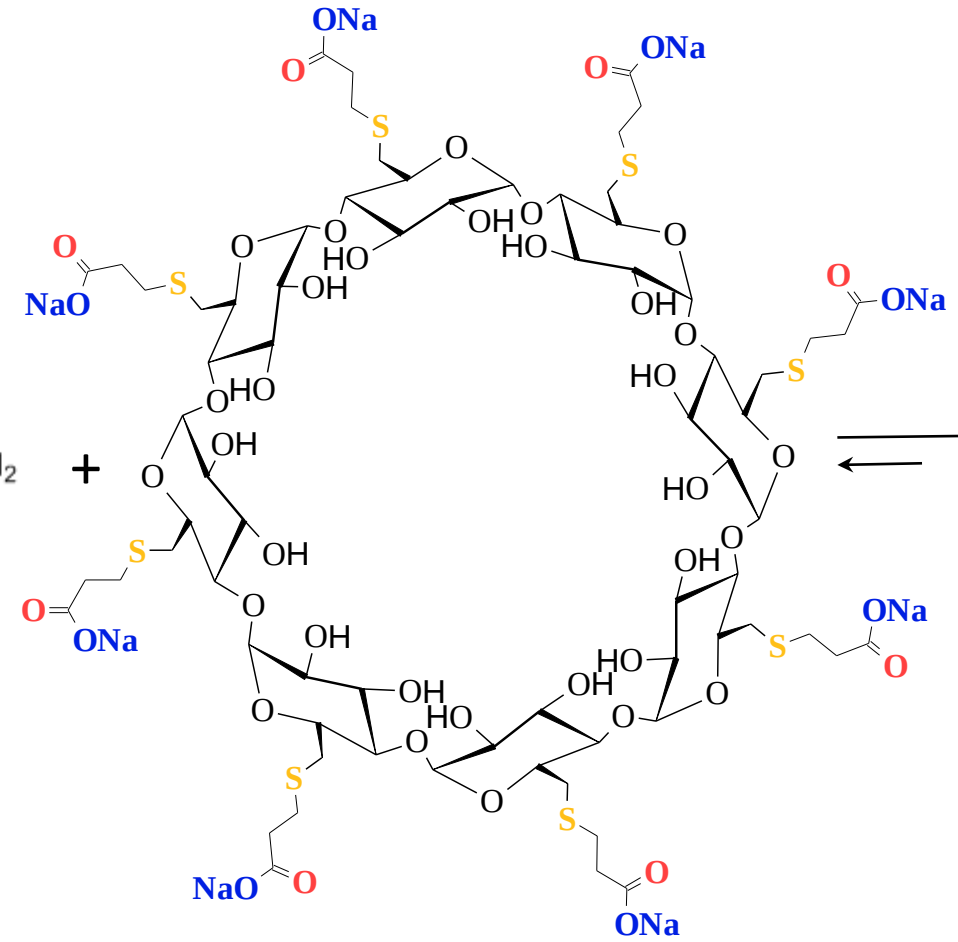


Regioselective with appropriate base and temperature.
Degree of Substitution can be controlled.
High solubility in water.
Challenging to characterize!
5-1000 kg scale production

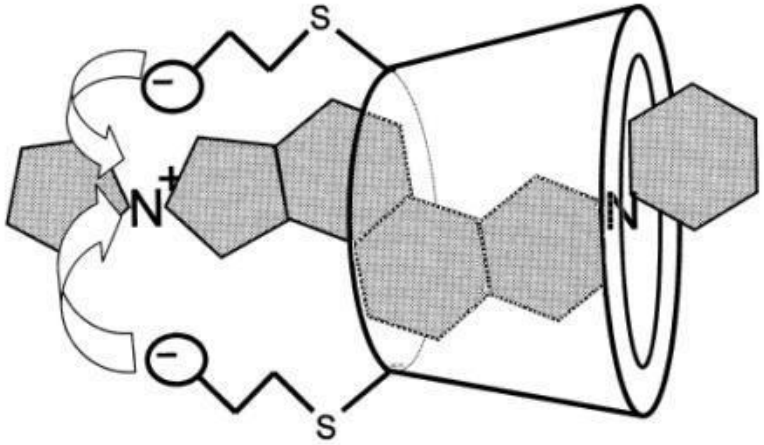
Sugammadex: Selective Relaxant Binding Agent!



Rocuronium Bromide
Aminosteroid



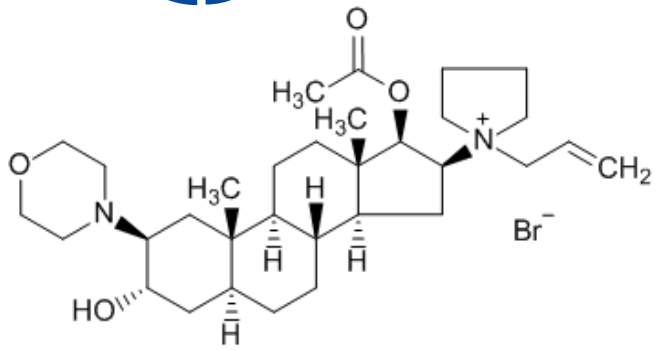
Sugammadex
(Bridion®)



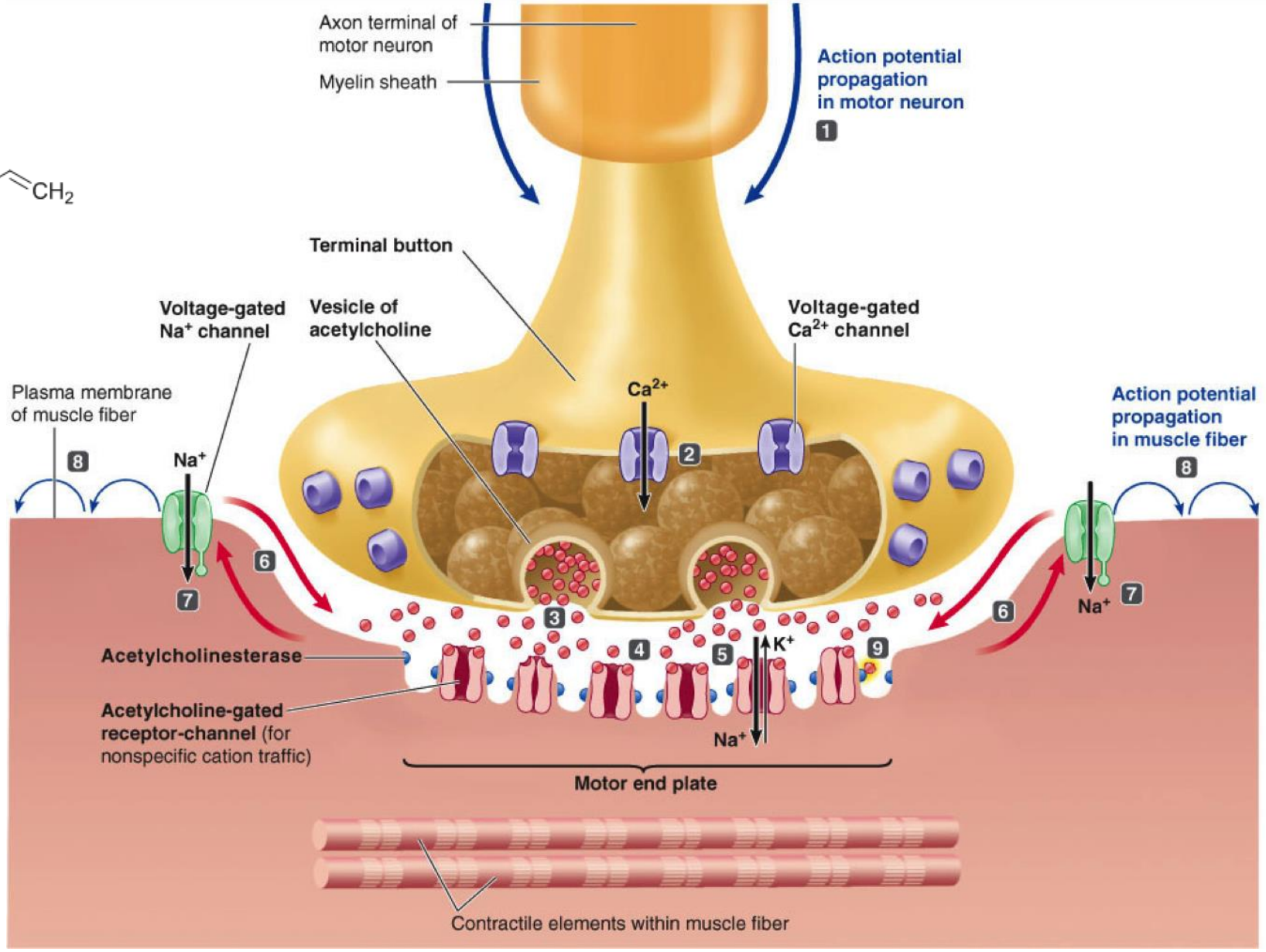
K_a=25.000.000 M*

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

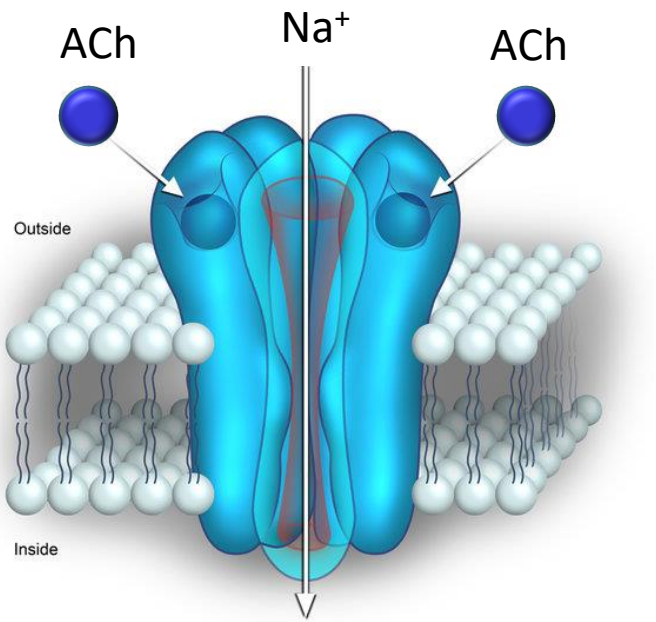
Detailed View of Neuromuscular Junction

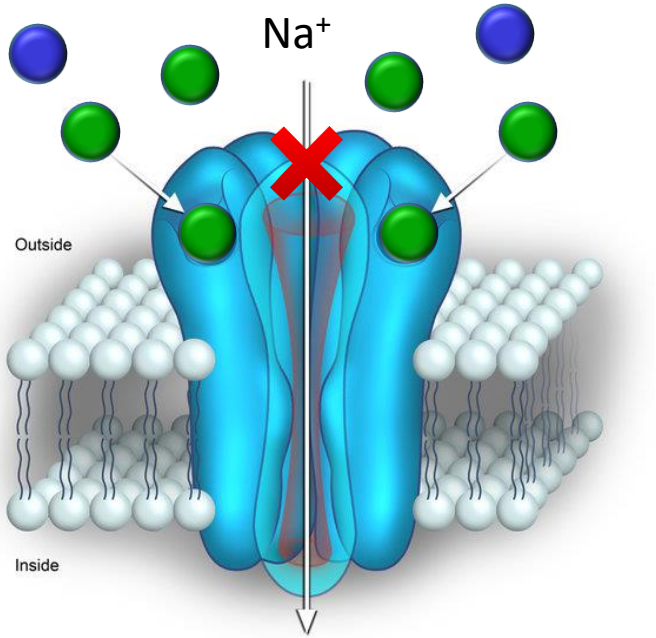


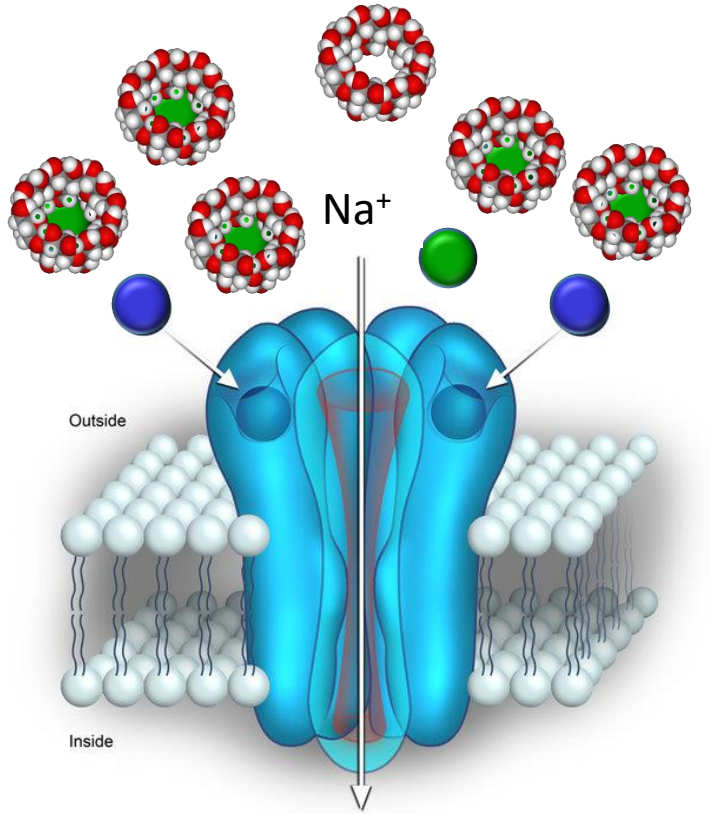
Rocuronium Bromide
Aminosteroid
Non-depolarizing blocking agent



Nicotinic Receptor, Rocuronium and Sugammadex







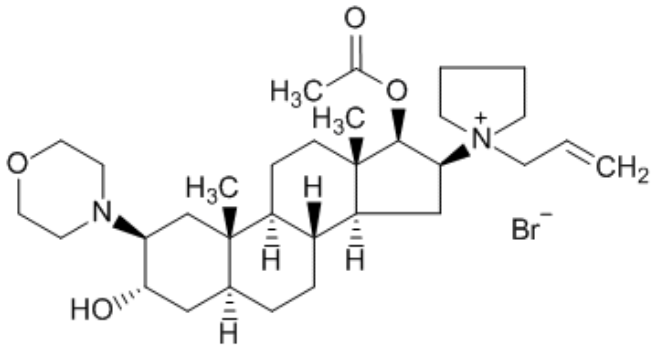
Acetylcholine

Rocuronium

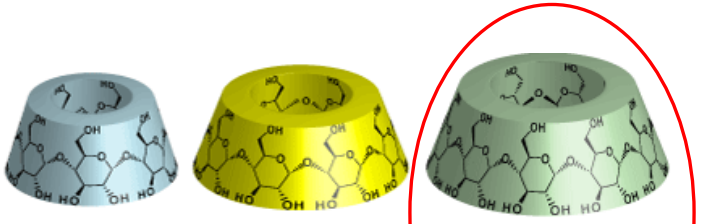
Sugammadex

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

Building-up of Sugammadex



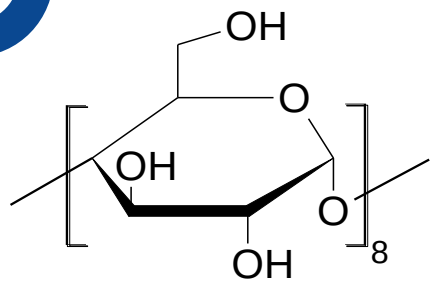
Rocuronium Bromide



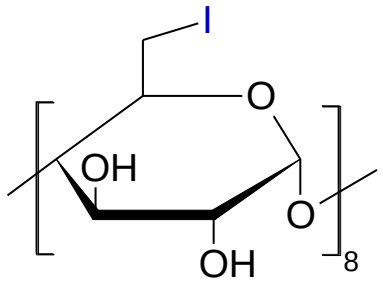
	α-CD	β-CD	γ-CD
No. of Glucose Units	6	7	8
Cavity Diameter (nm)	0.47	0.60	0.75
Height of Torus (nm)	0.79	0.79	0.79

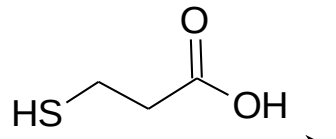
Compounds						In vitro reversal activity vs ~90% block by rocuronium [a] (isolated mouse hemi-diaphragm)		In vivo reversal activity vs ~90% block by rocuronium [b] (i.v., guinea-pigs)	
Compound No.	n	R	Prep. Method	MW	Purity [c]	EC_{50}, µM	max reversal, % (conc., µM)	ED_{50}, µmol/kg	max reversal, % (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)
18	8	*ortho*-S-Ph-CO_2Na	A	2562.4	>90%	226.7 ± 65.4	42.3 ± 13.8 (216)	n.t. [g]	n.t. [g]
19	8	*meta*-S-Ph-CO_2Na	A	2562.4	>80%	3.3 ± 0.5	95.7 ± 2.3 (7.2)	0.28 ± 0.05	102.0 ± 5.7 (1.3)

Synthetic Strategies for Sugammadex

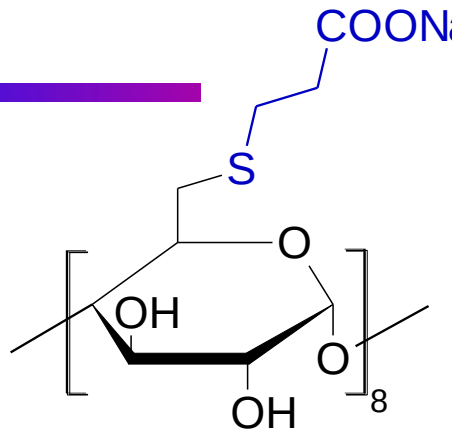


PPh_3, I_2, DMF
70-75 °C, 6 h

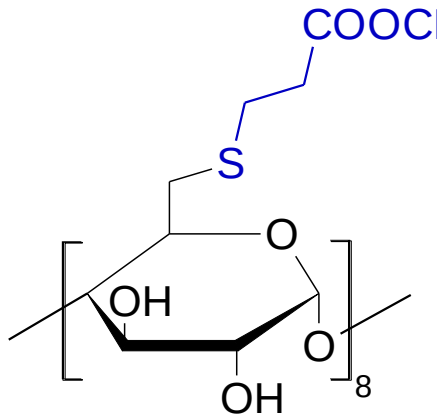




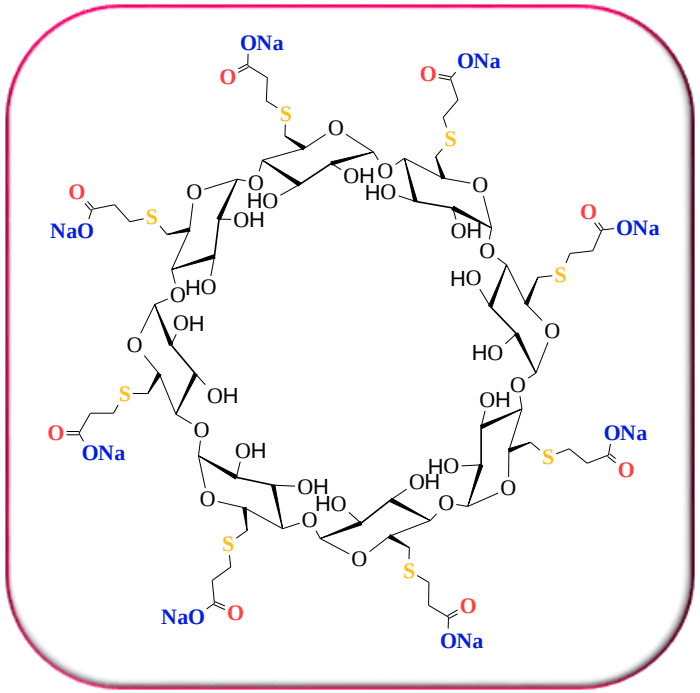
$NaOCH_3$/MeOH
DMSO, r.t., 2 h



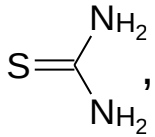
DMF, K_2CO_3
60-70 °C, 4 h

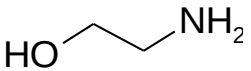


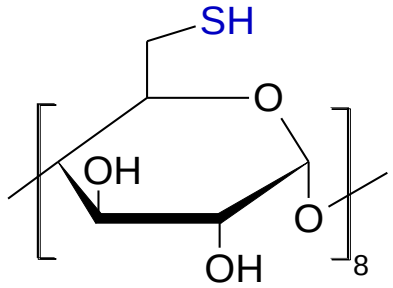
NaOH, H_2O
20-25 °C, 8 h



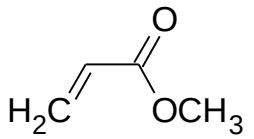
Sugammadex

1)  , DMF
65 °C, 8 h

2) 
65 °C, 2 h

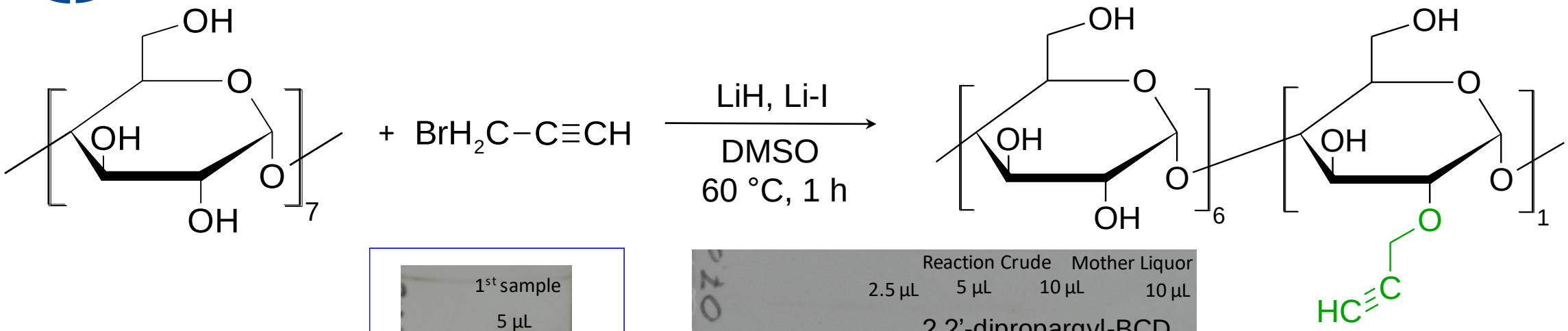


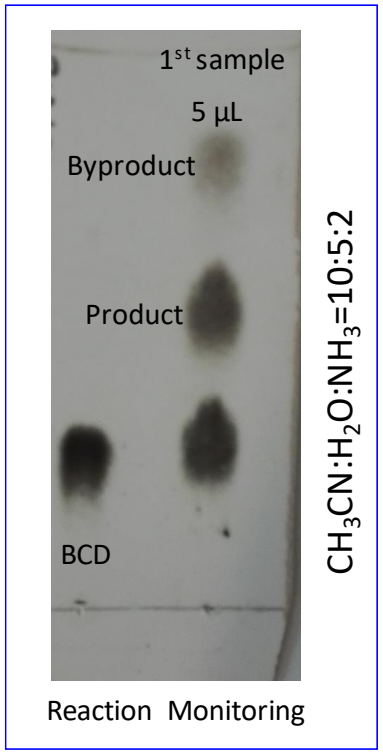
$NaOCH_3$, DMF
65 °C, 12 h

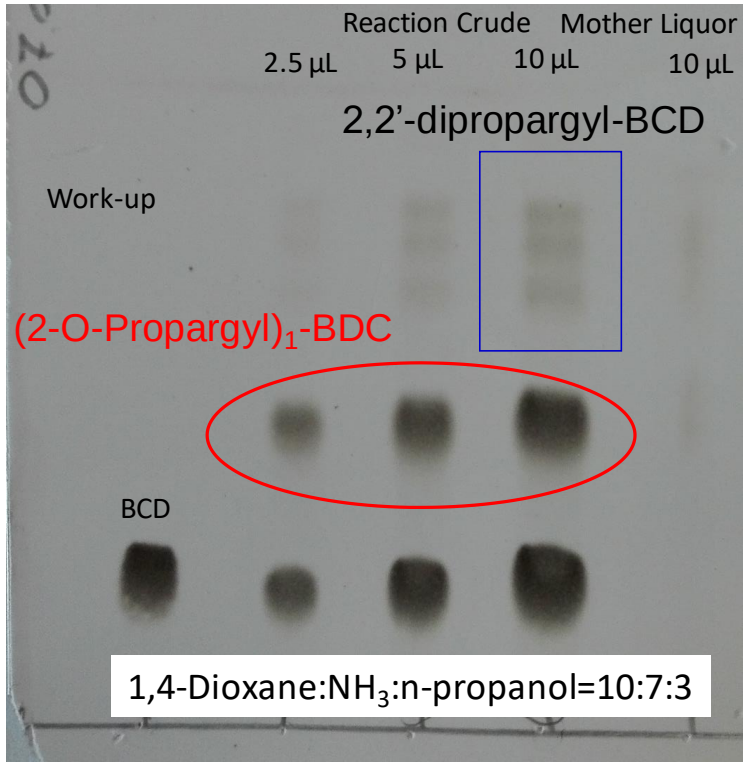
+ 

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

Mono-2-*O*-Propargyl-βCD

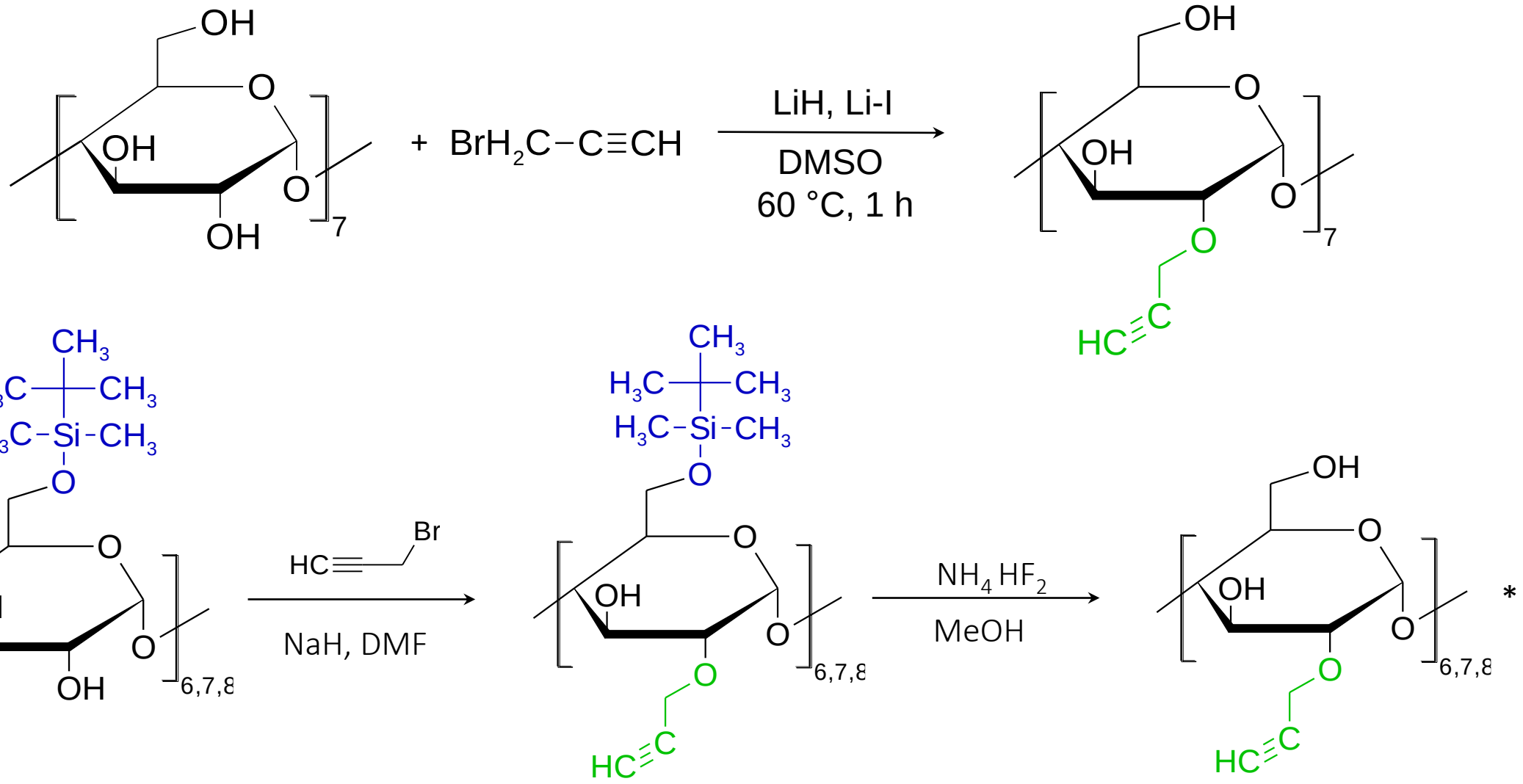






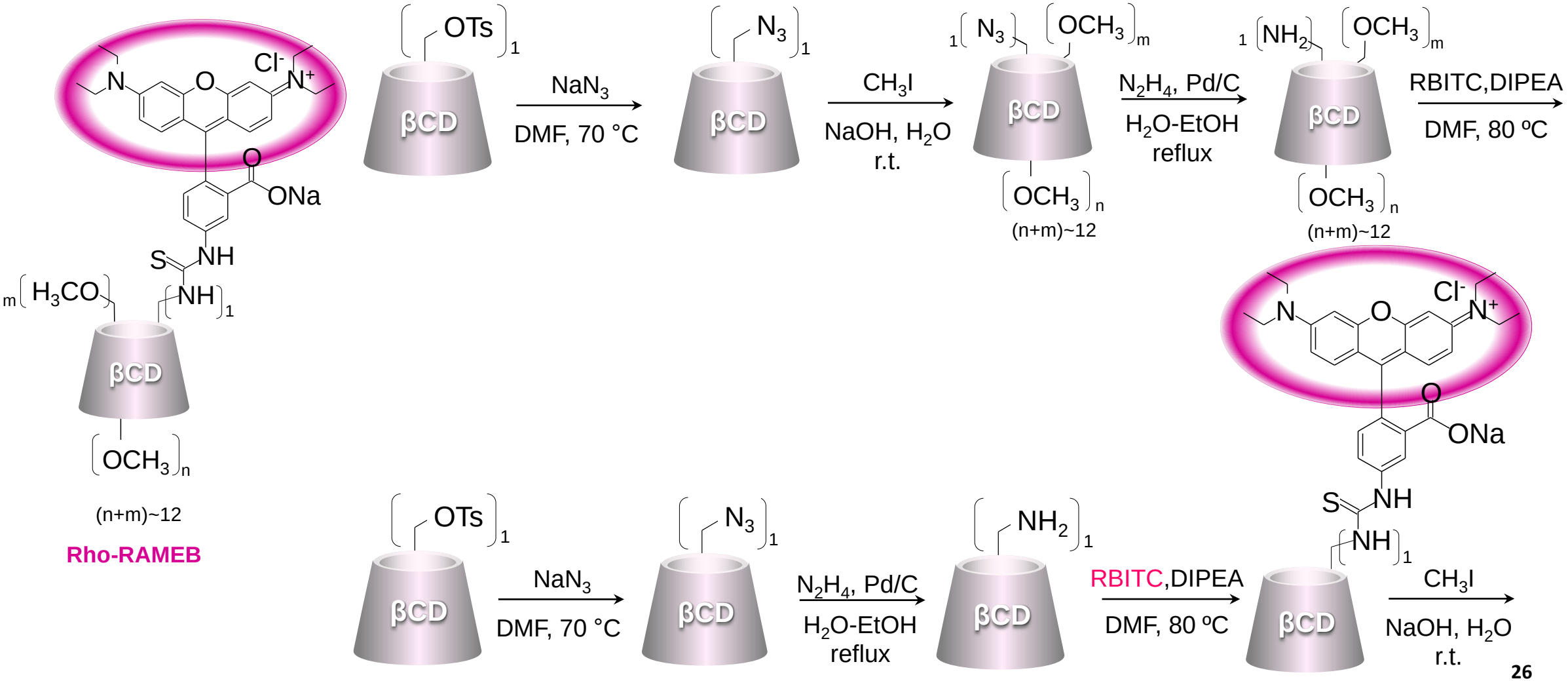
Casas-Solvas J. M. et al., Carbohydrate Chemistry: Proven Synthetic Methods, Vol. 5, 2019 (under publication)

Per-2-*O*-Propargyl CDs

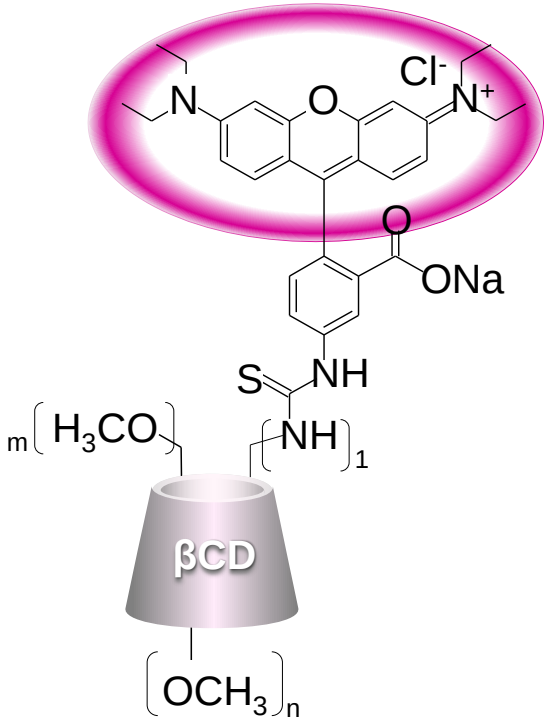


* *Darcy R. et al., Biomaterial, 33, 7775-7784, 2012*

Fluorescent CDs as Tools for Biological Investigation

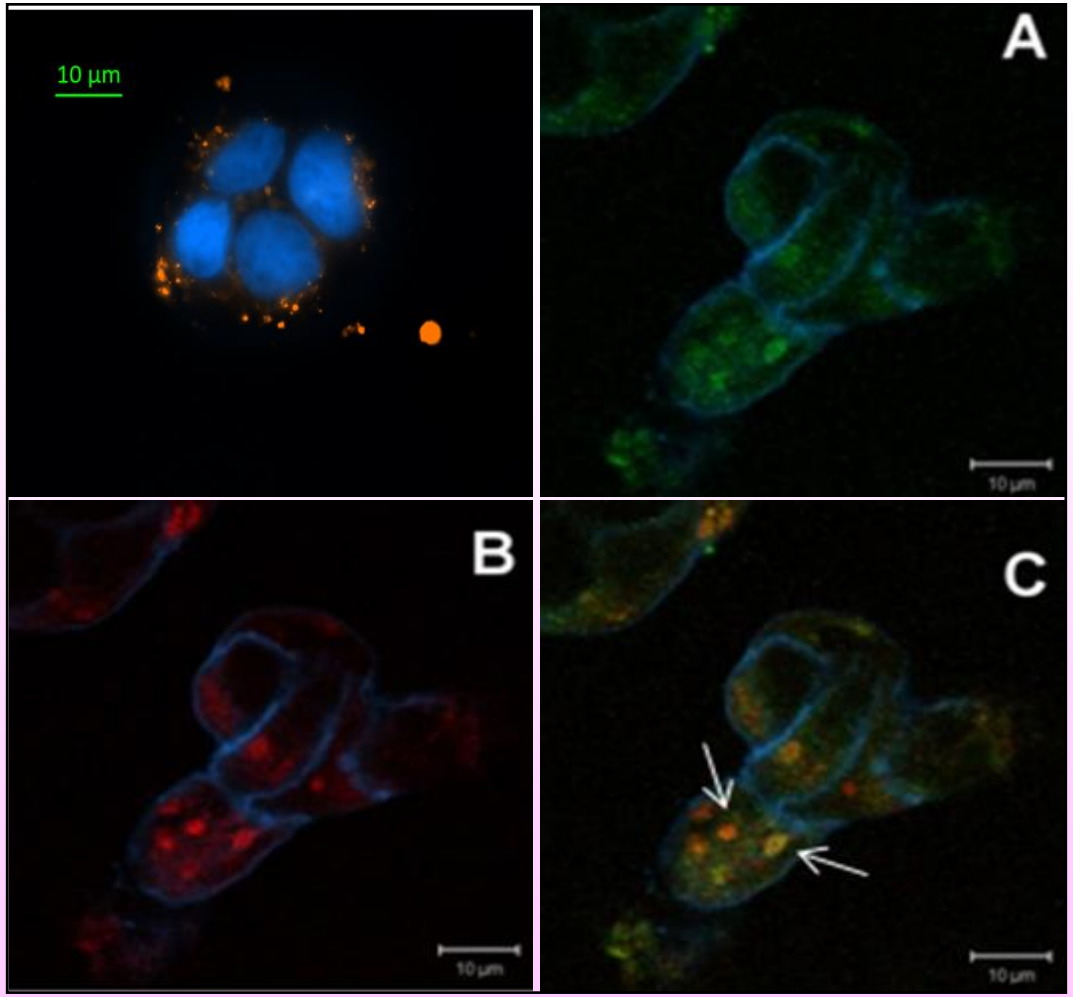


Application of Fluorescent CDs



(n+m)~12

Rho-RAMEB

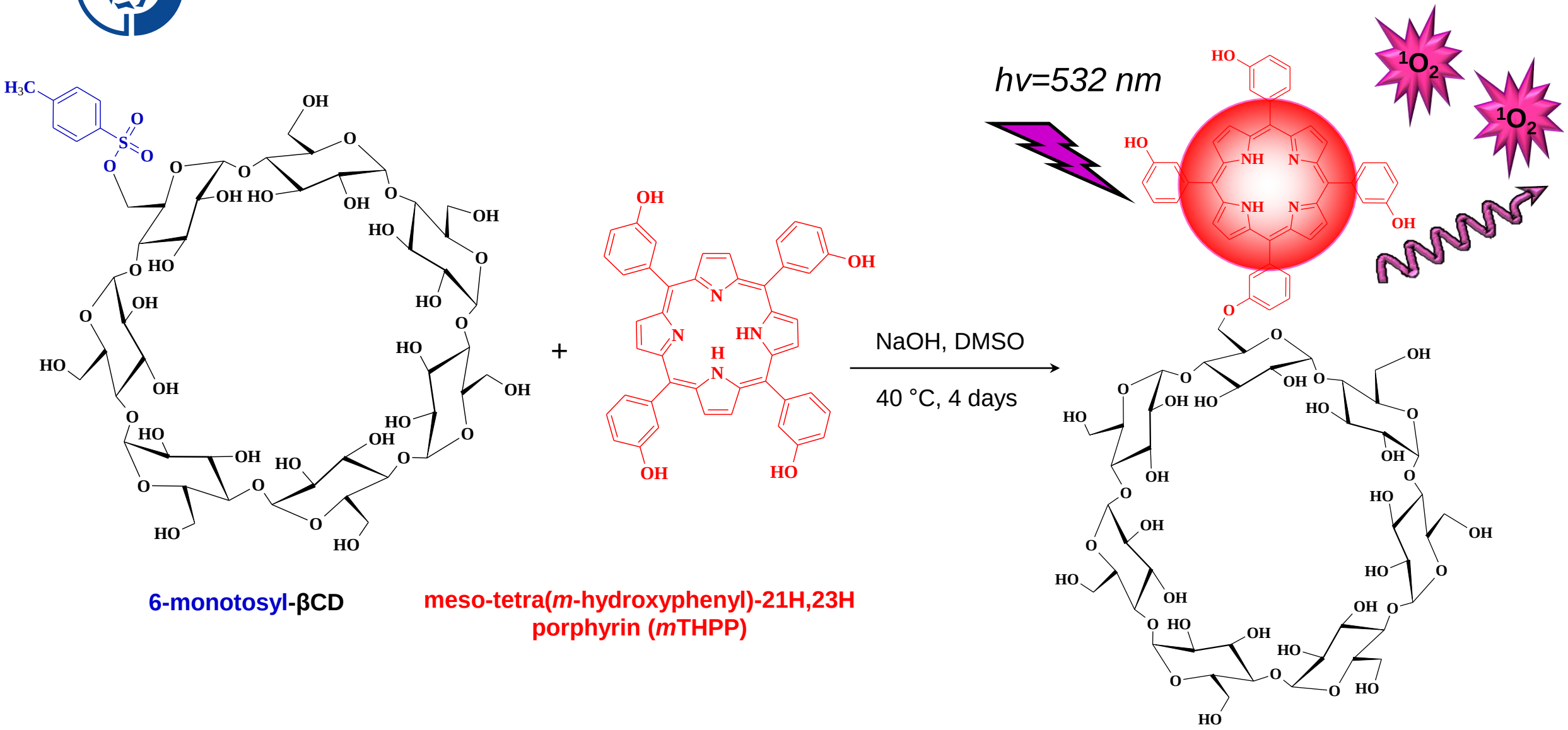


Fluorescent microscopic images of rhodamine-labeled random methylated β-cyclodextrin (Rho-RAMEB) in Caco-2 cells (top left). Rho-RAMEB is localized in large (red) vesicles. Cell nuclei are labeled with blue DAPI staining.

Confocal microscopic images of Flutax-Rho-RAMEB complexes in Caco-2 cells. Flutax (green, A) and Rho-RAMEB (red, B) containing vesicles can be detected alone, but together in the same vesicles (orange, C) as well. Cell membrane is stained in blue.

Réti-Nagy K. et al, Int J Pharm. 2015, 30, 496(2), 509-517

βCD-Porphyrin Conjugate



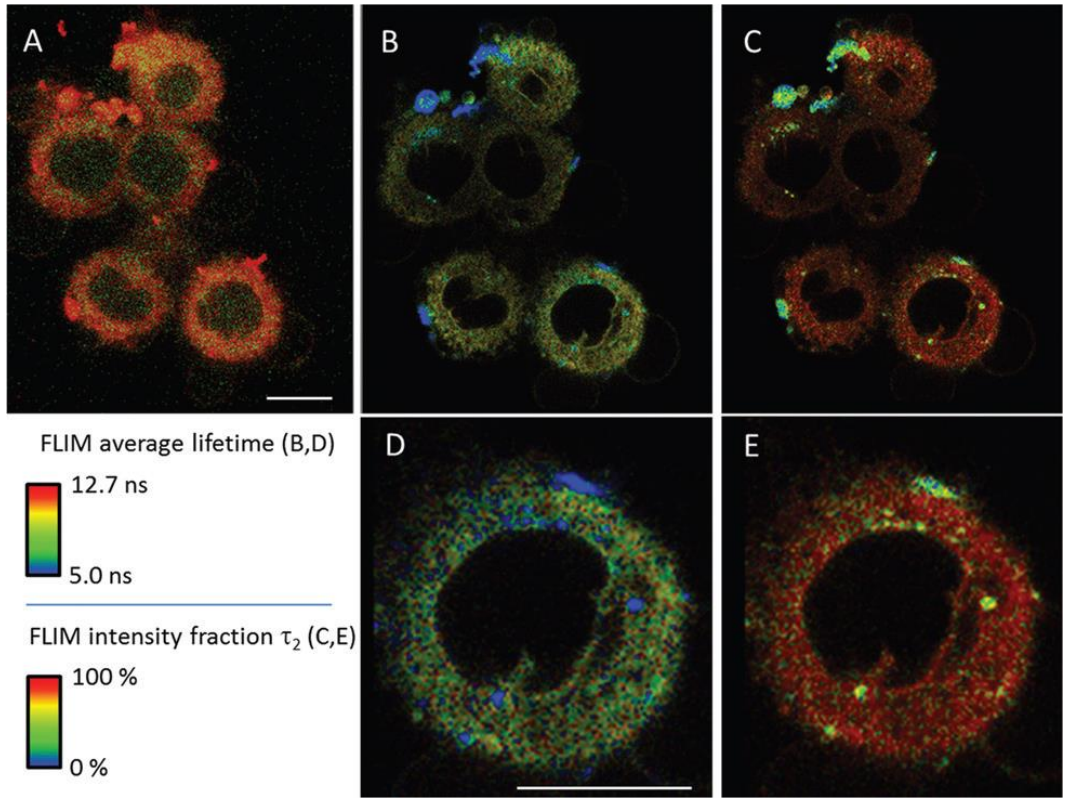
6-monotosyl-βCD

meso-tetra(*m*-hydroxyphenyl)-21H,23H porphyrin (*m*THPP)

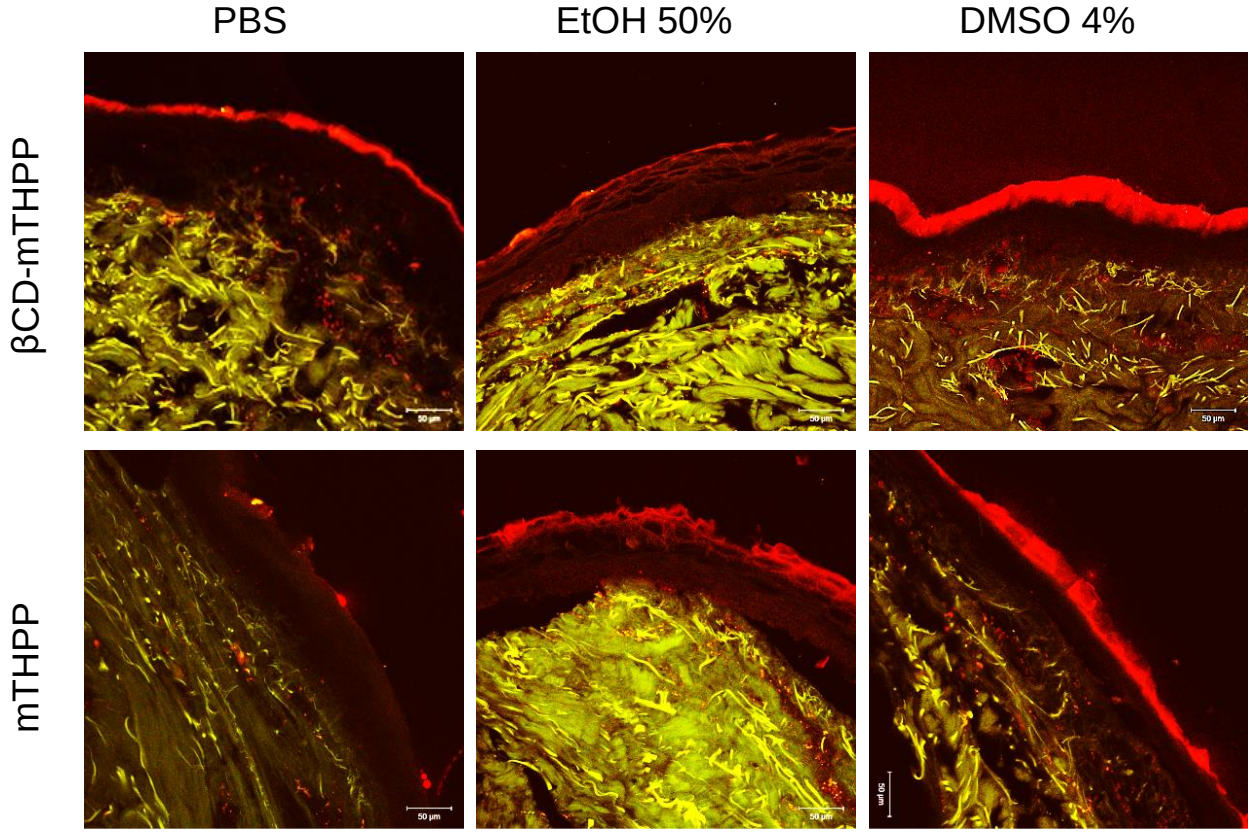
6-mono(*m*THPP)-βCD

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

βCD-Porphyrin Conjugate, Photosensitizers and Delivery System!



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).

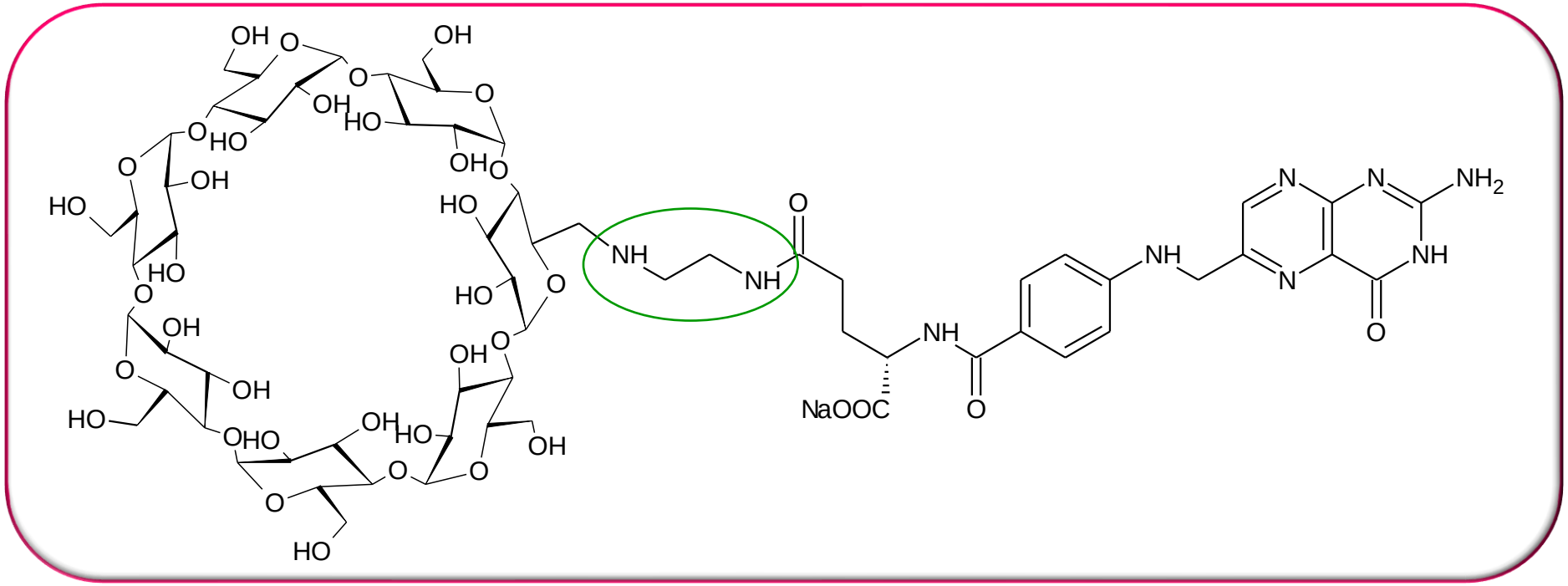


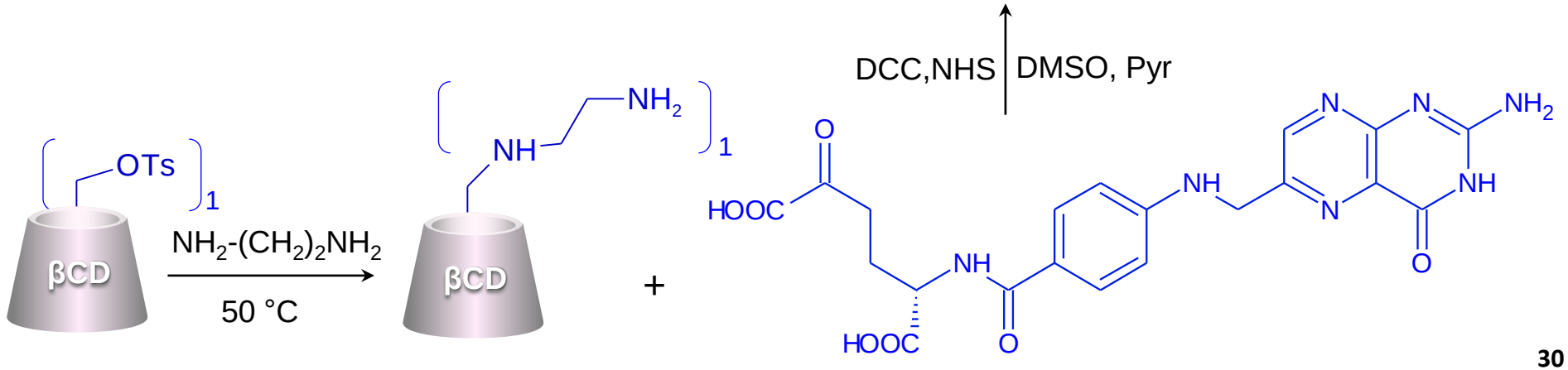
Two Photon Microscopy images of cryosectioned human skin

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**

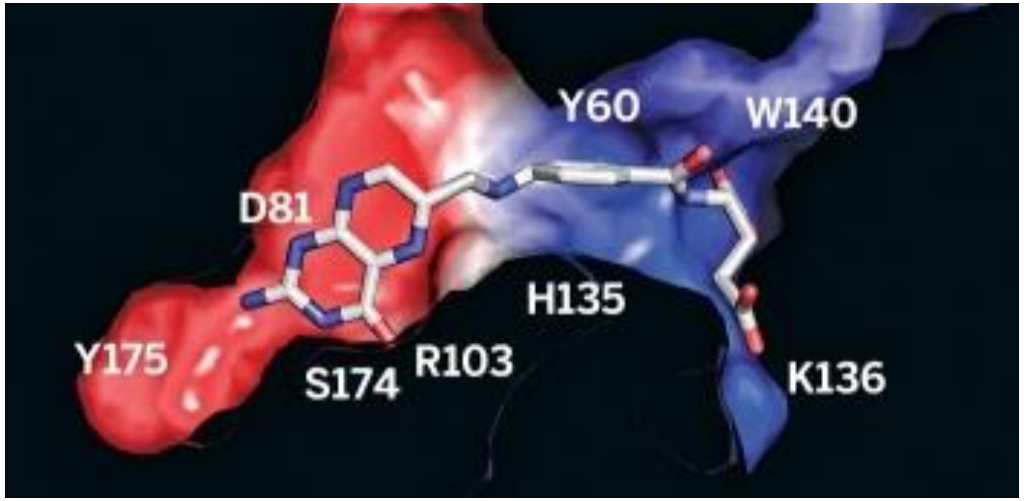
Folate appended-CDs



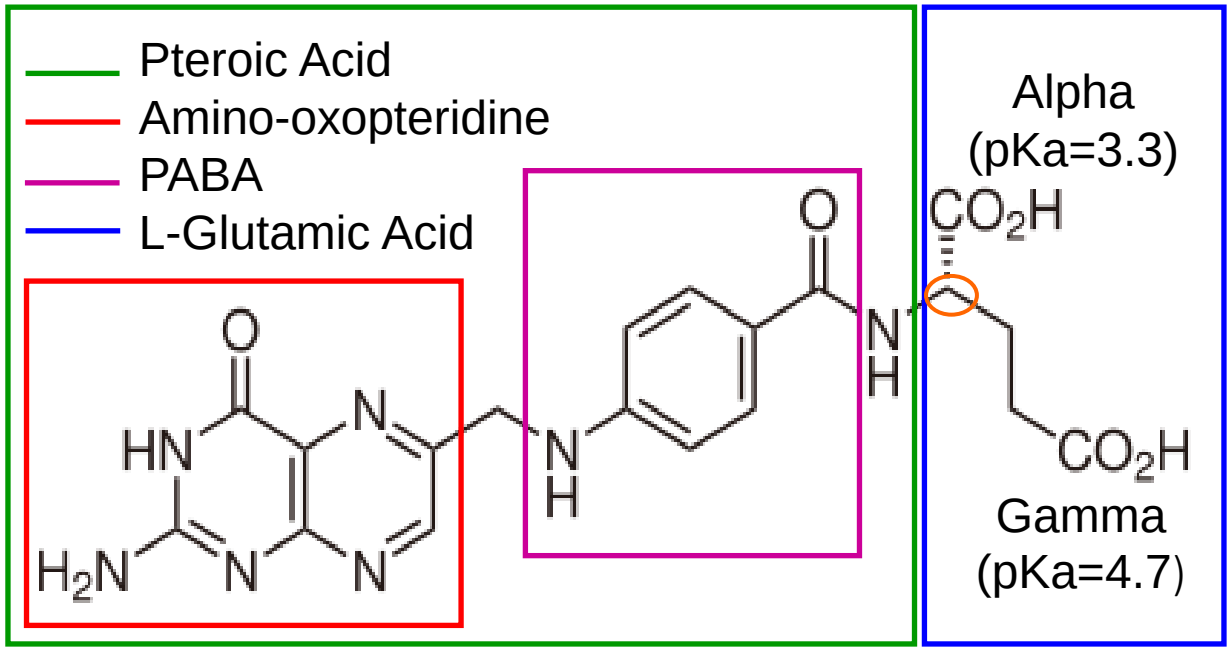


Caliceti P. et al., Bioconjugate Chem. 2003, 14, 899–908

Folate / Folate-Receptor Interactions



Pteridine ring of FA is perpendicular to PABA moiety

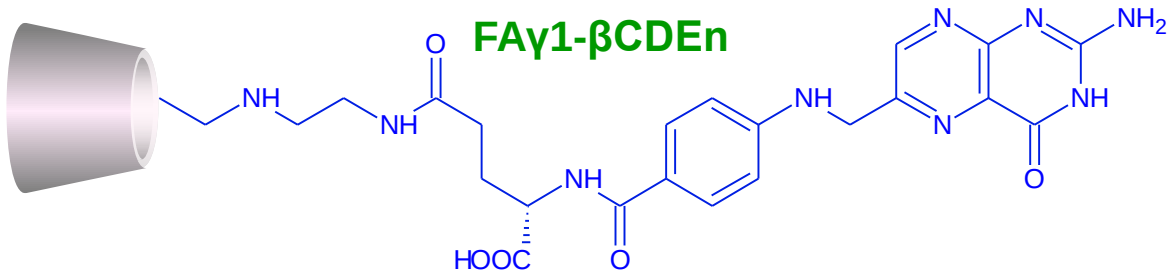


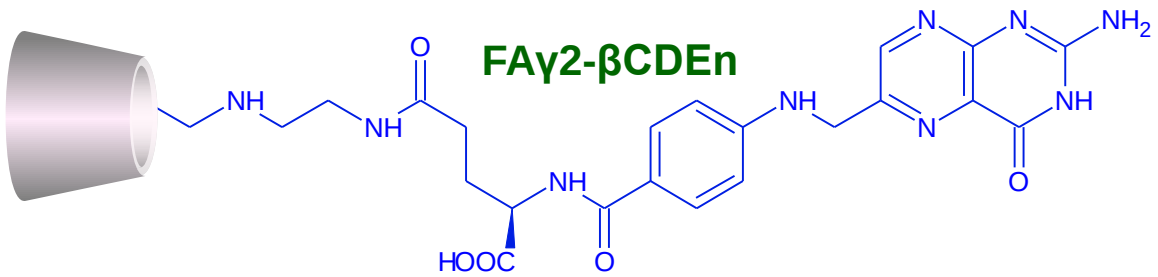
Folic acid drawbacks

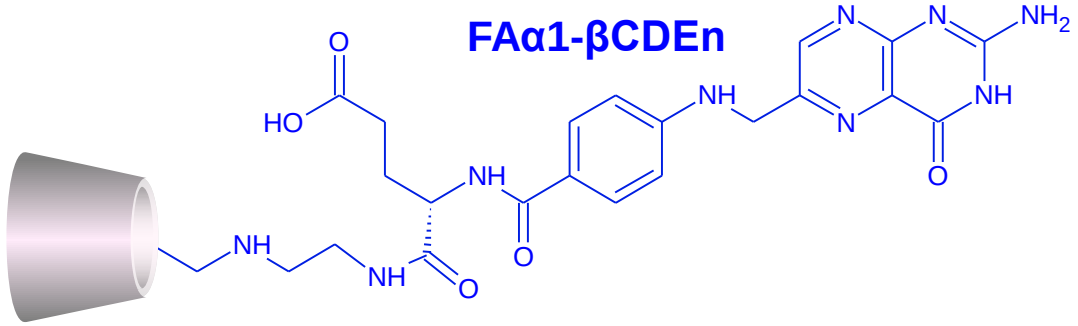
- Low solubility
- Dicarboxylic acid
- Only Gamma-modified-FA interacts with FR
- **Susceptible to racemization!**

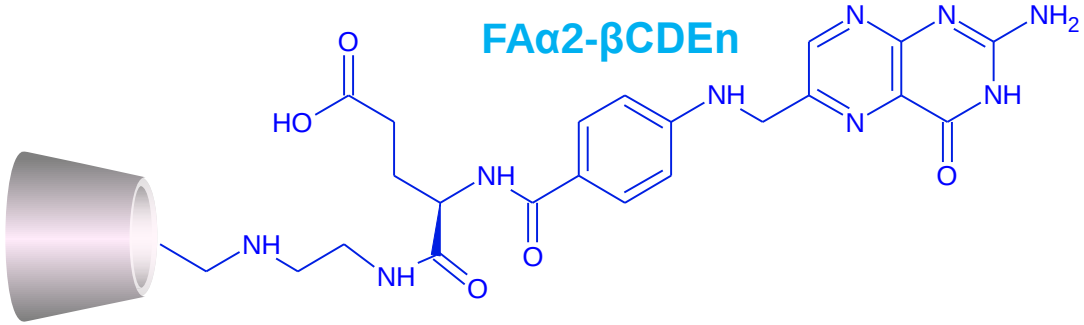
Chen C. et al., Nature, 500, 486–489 (22 August 2013)

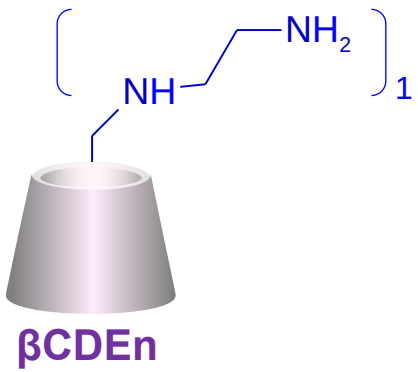
Folate appended-CDs Crude Composition

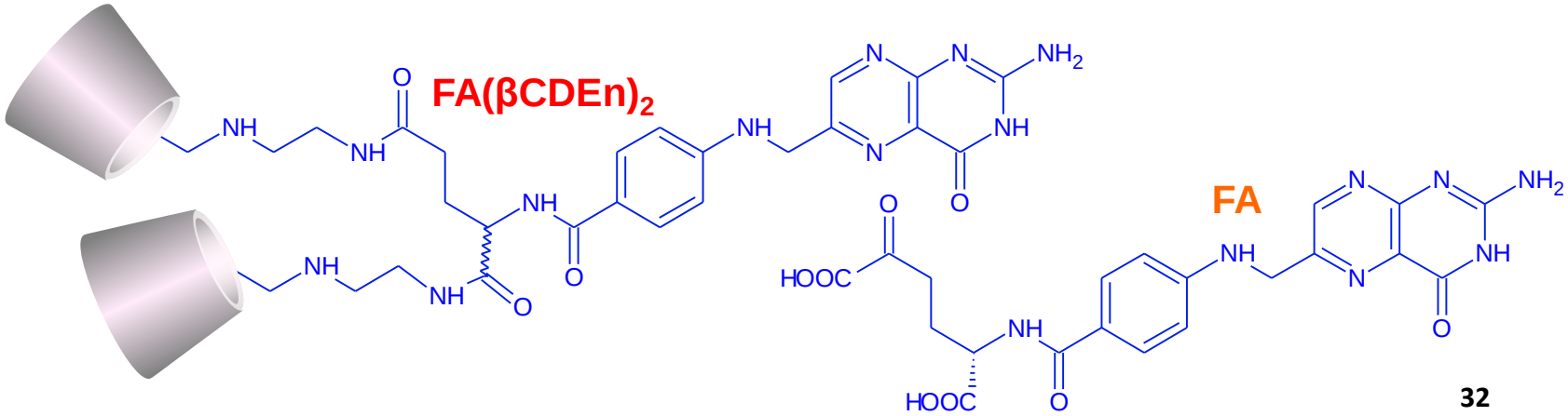




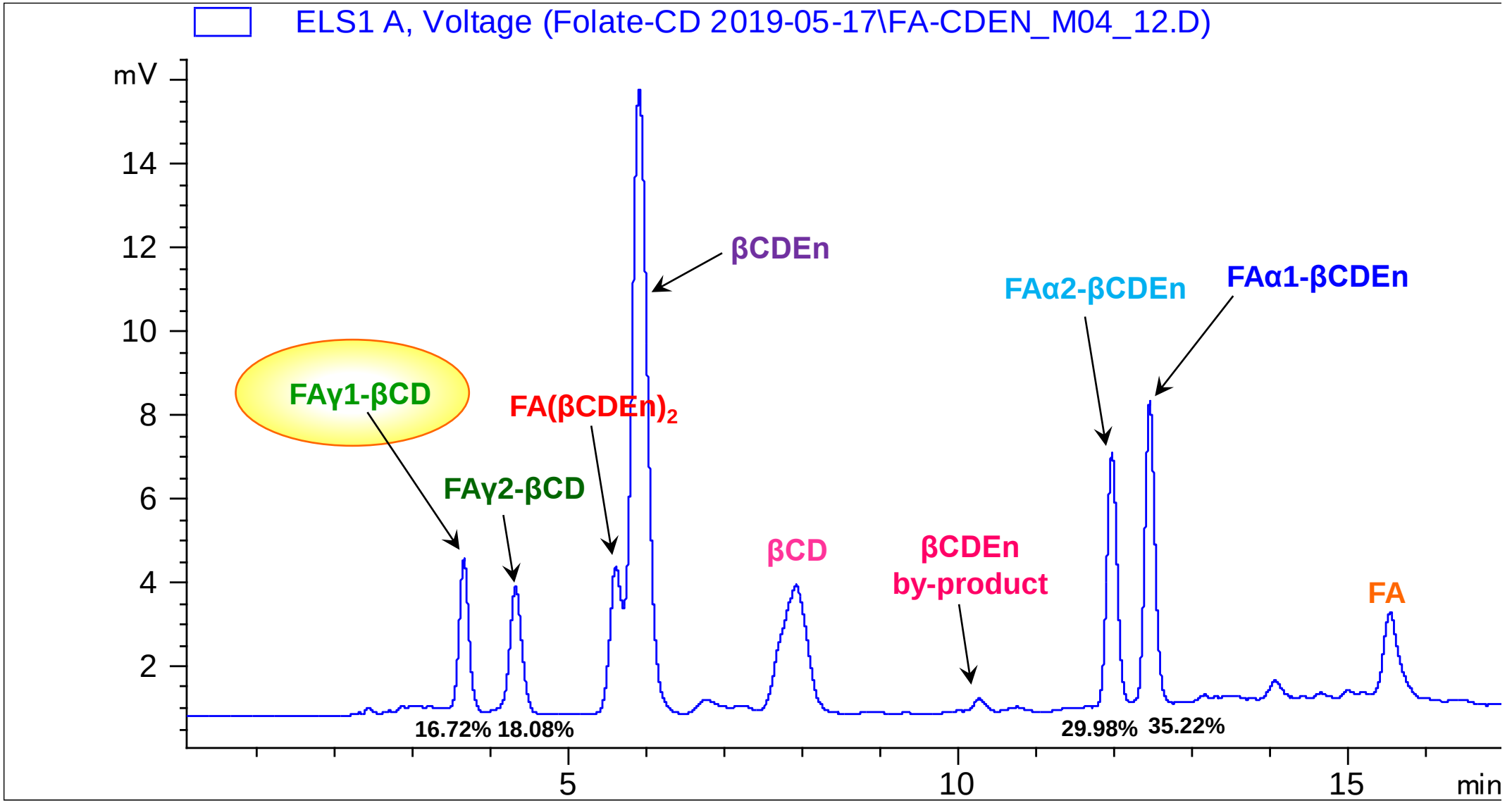




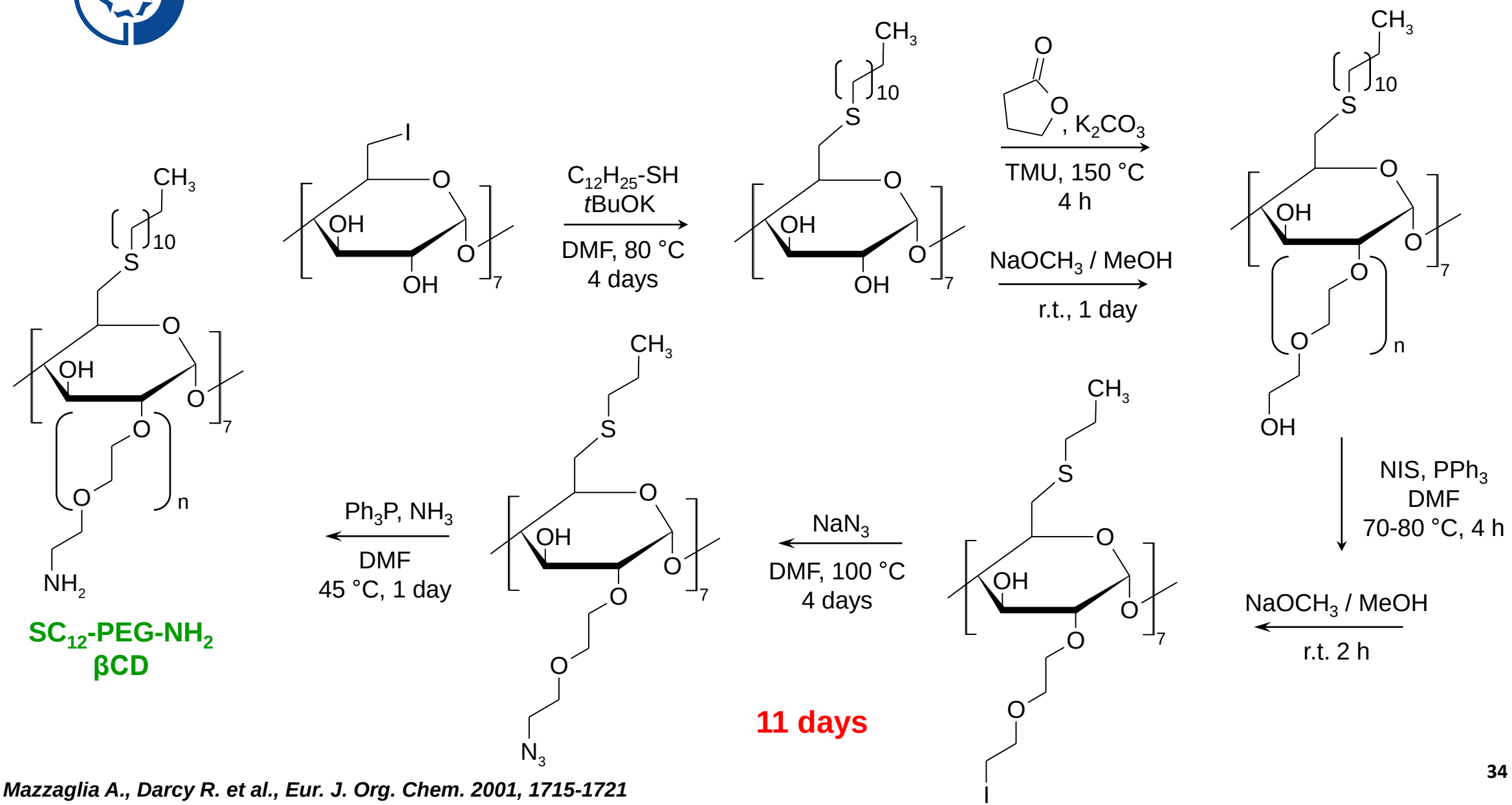




Folate appended-CDs Analytical method



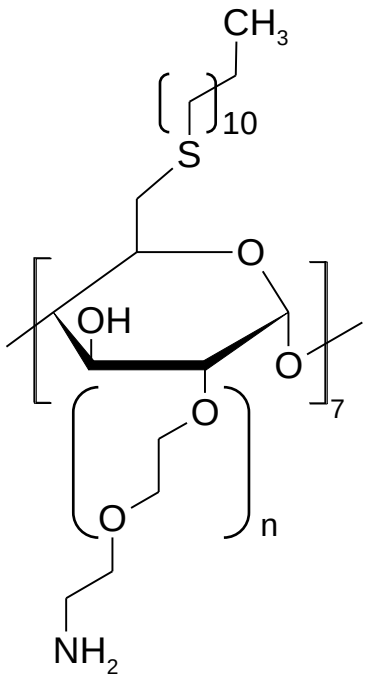
Amphiphilic Cyclodextrins (Original Version)



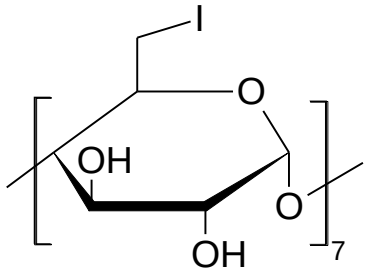
SC_{12}-PEG-NH_2
βCD

Mazzaglia A., Darcy R. et al., Eur. J. Org. Chem. 2001, 1715-1721

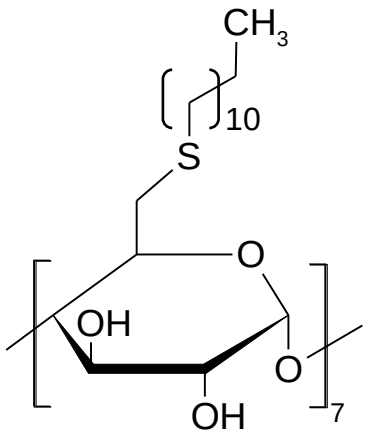
Amphiphilic Cyclodextrins (Updated)

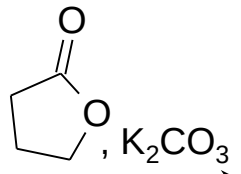


SC_{12}-PEG-NH_2
βCD



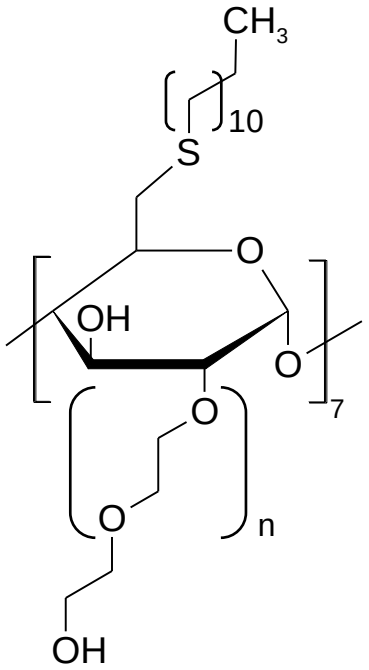
$C_{12}H_{25}$-SH
$NaOCH_3$/MeOH
DMF, 80 °C
2 h



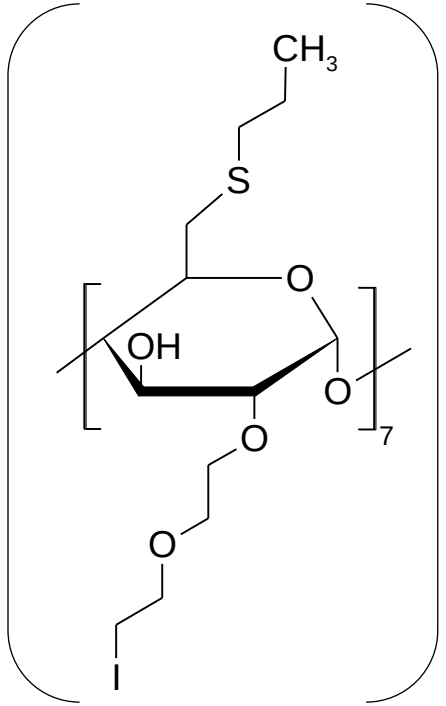


K_2CO_3
TMU, 150 °C
2 h

$NaOCH_3$ / MeOH
r.t., 1 day



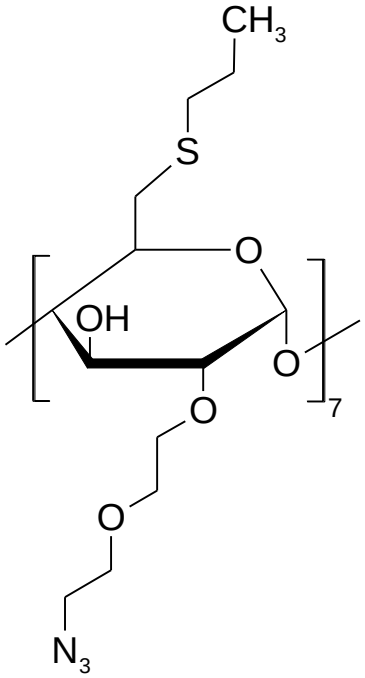
I_2, PPh_3
DMF
80 °C, 4 h



NaN_3
DMF, 80 °C
3 h

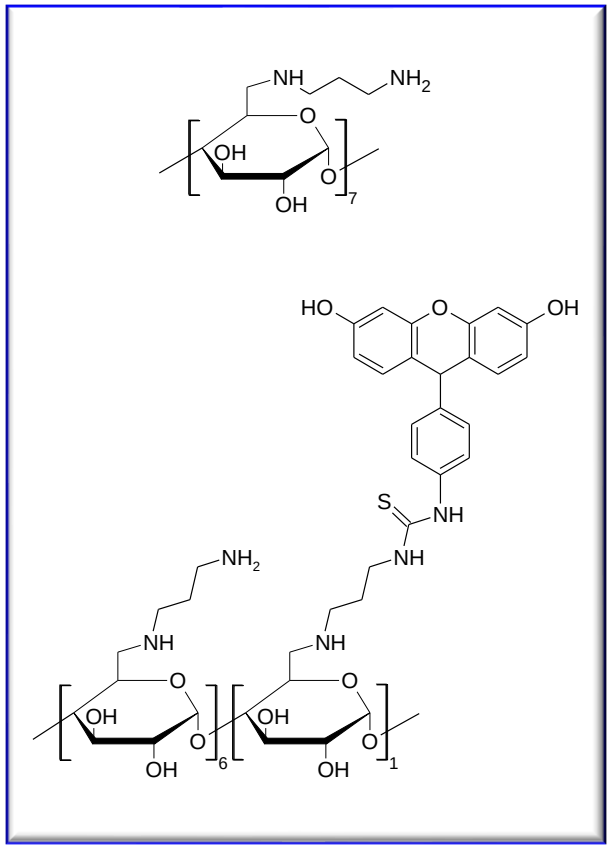
$NaOCH_3$ / MeOH
r.t. 2 h

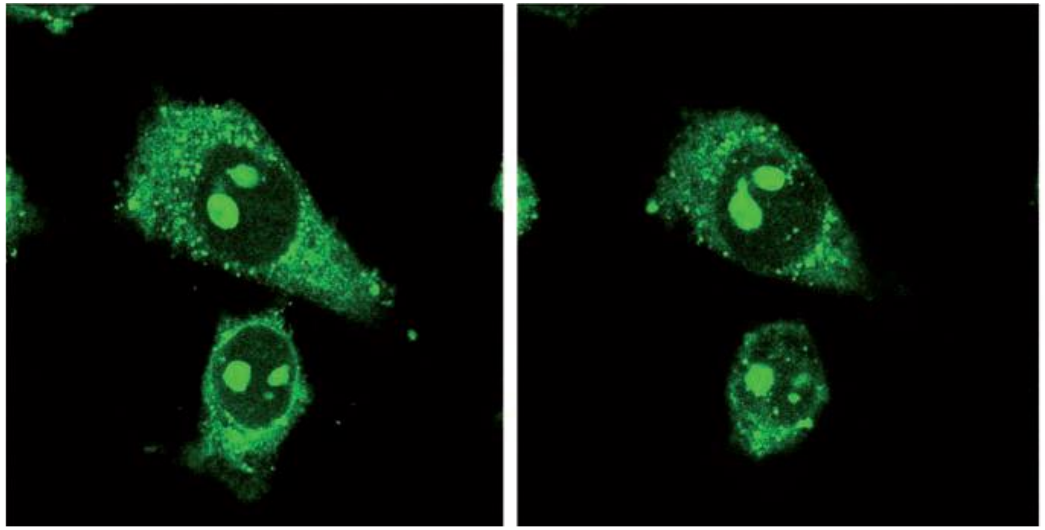
5 days



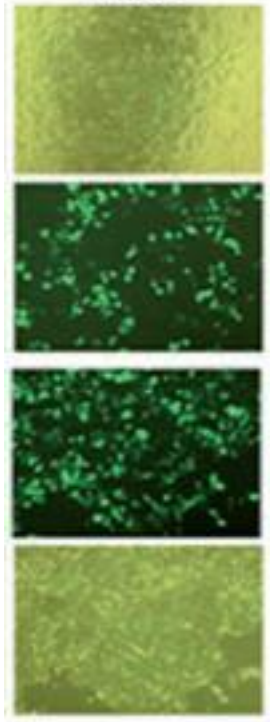
N_2H_4, Pd/C
H_2O-EtOH
Reflux, 2 h

Cell Penetrating CDs and DNA carriers





Confocal microscopy slices of immobilized HeLa cells



$\beta CD\text{-}(NH\text{-}(CH_2)_3\text{-}NH_2)_7$

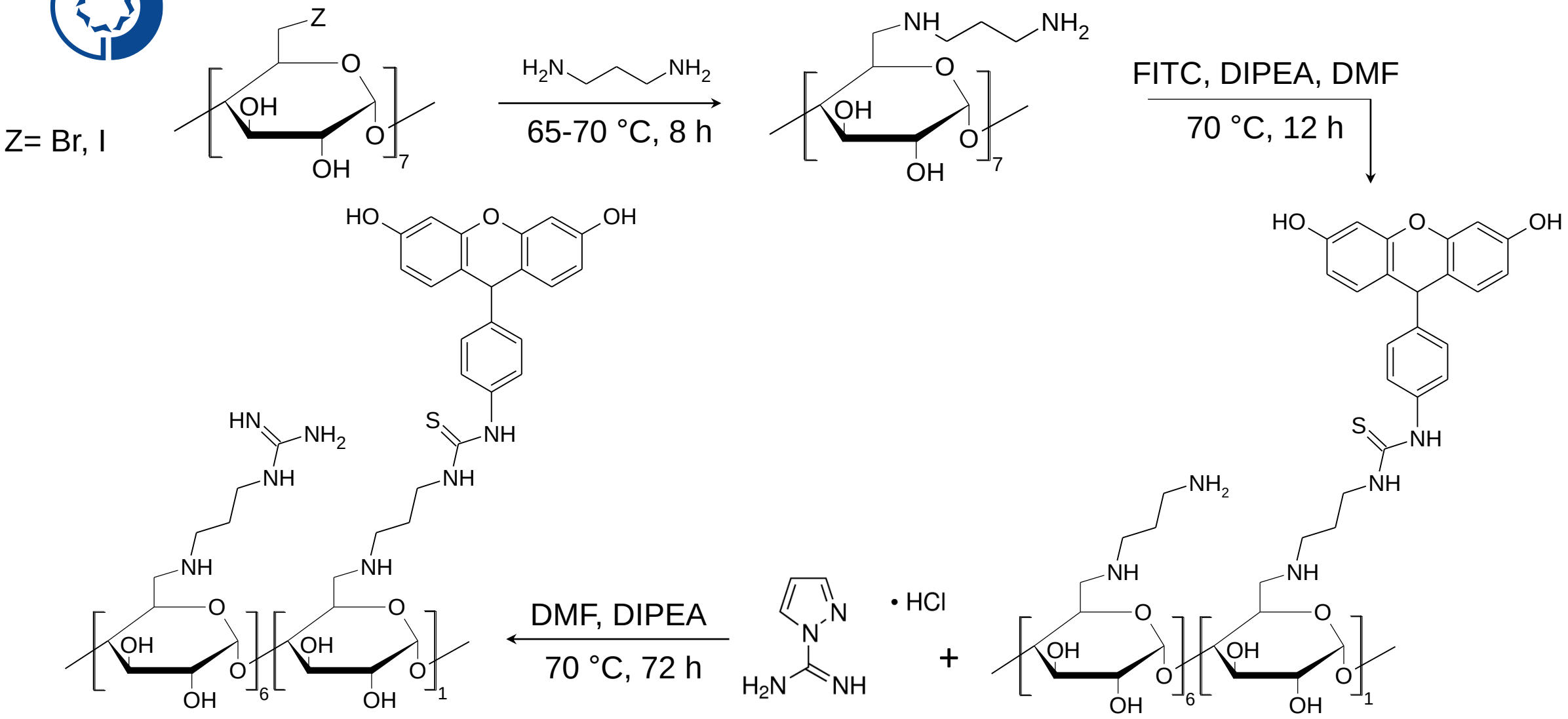
Lipofectamine 2000

Expression of eGFP protein

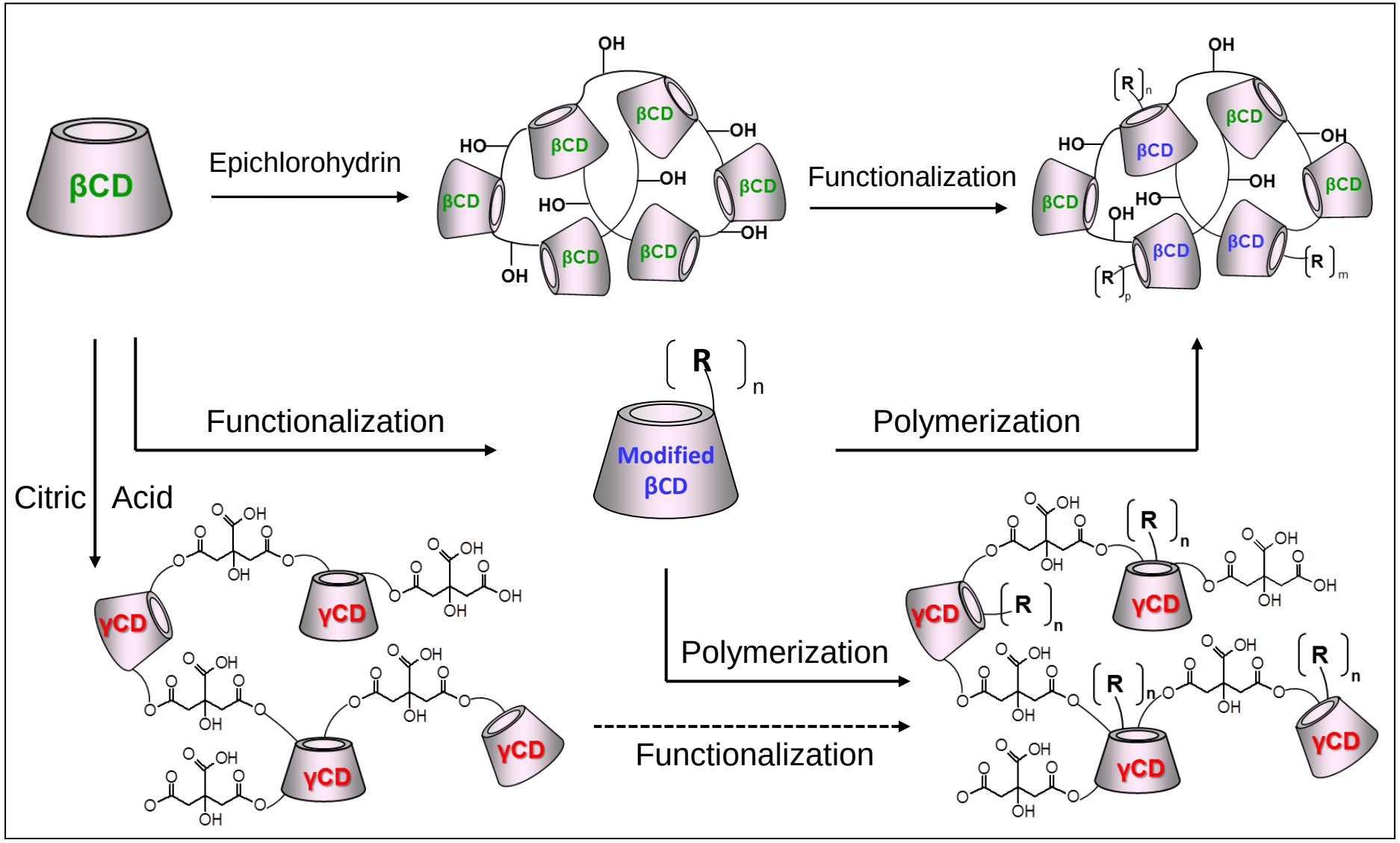
Optical and fluorescence microscopy images showing expression of eGFP protein in HEK 293T cells and comparison with Lipofectamine 2000 as control.

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

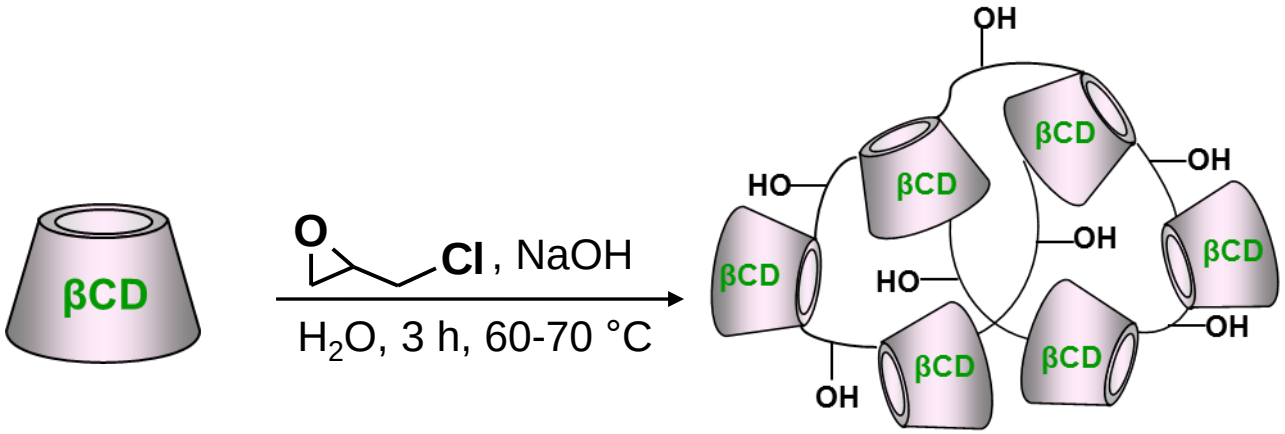
Cell Penetrating CDs and DNA carriers: Syntheses



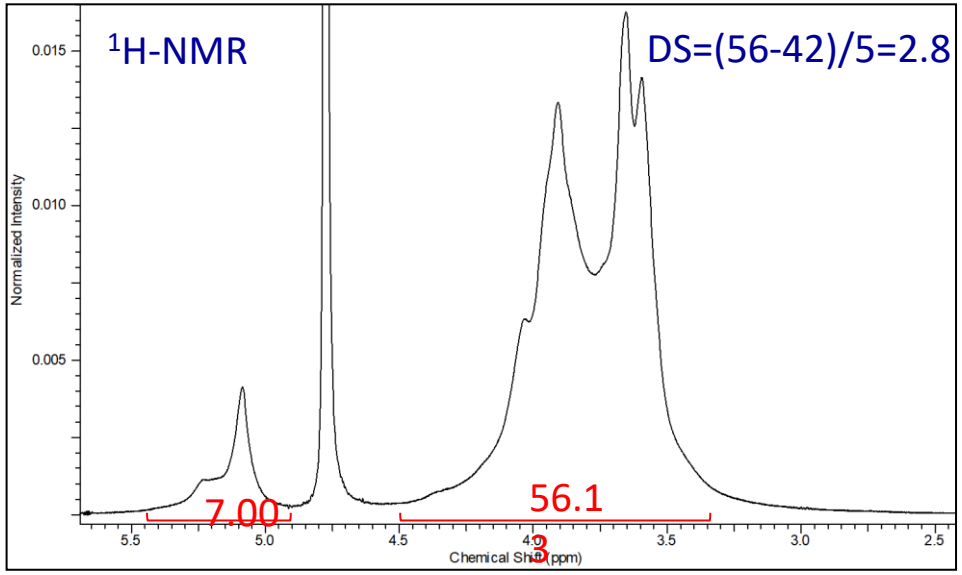
Cyclodextrin-Based Polymers

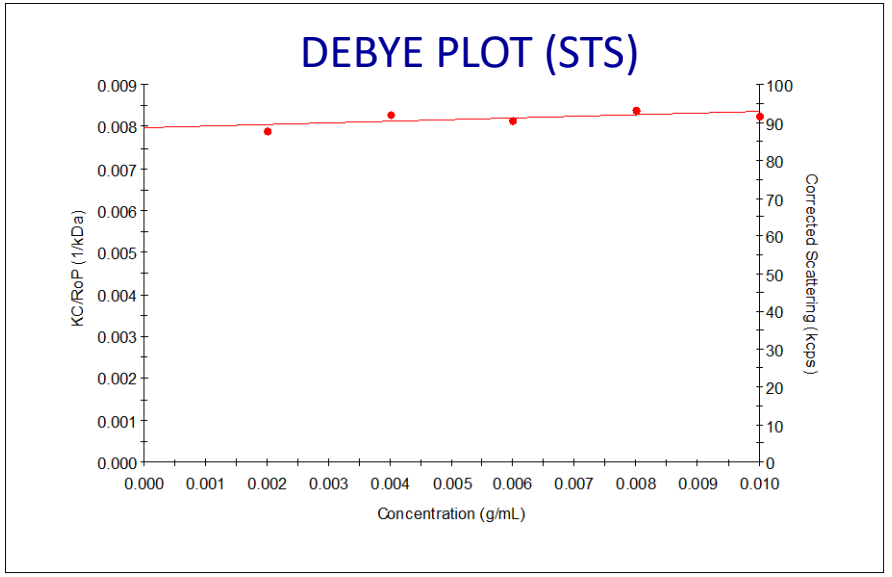


Epichlorohydrin Cross-linked CD Polymers

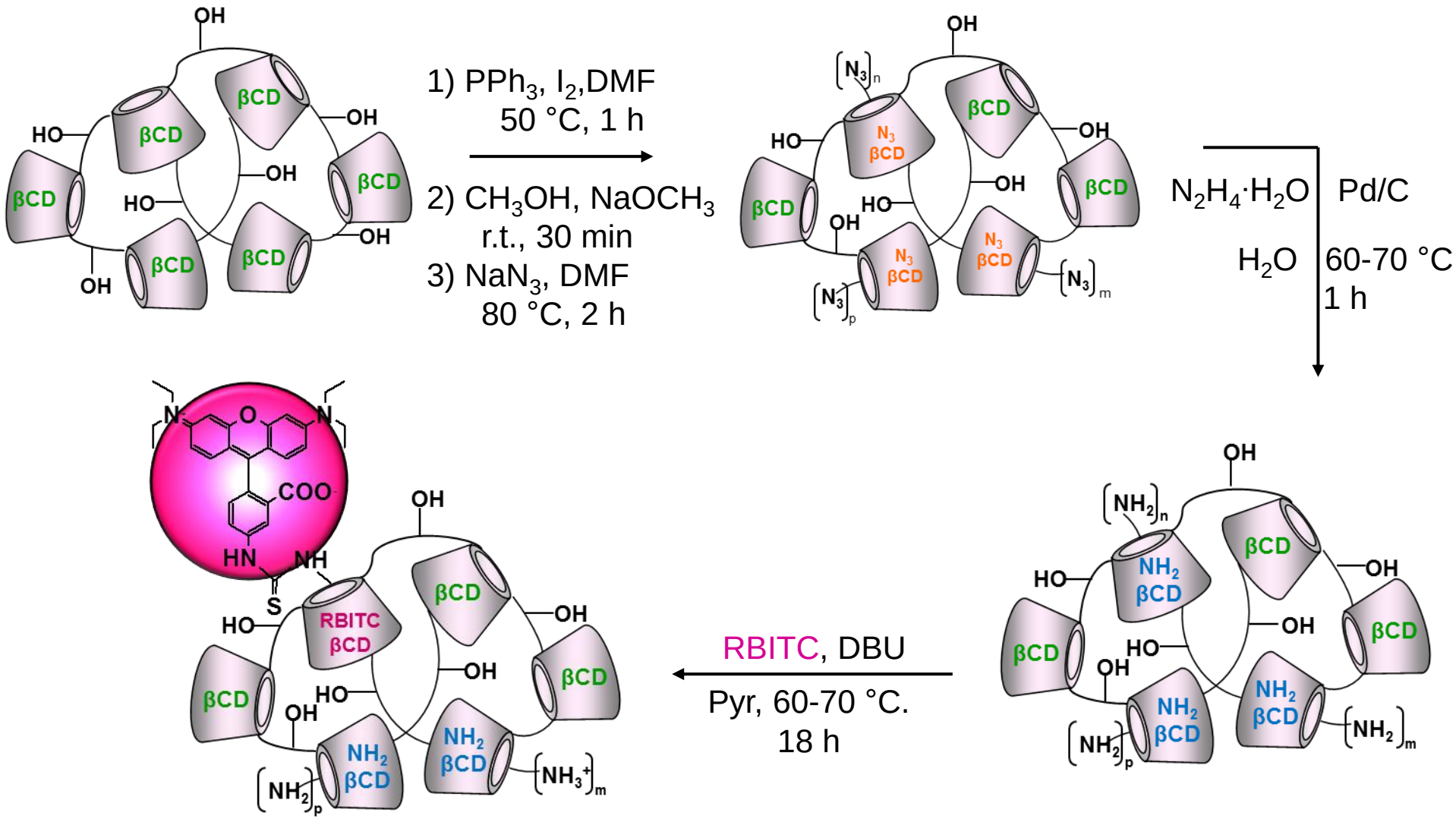


CD content=60%
Mw=100 kDa





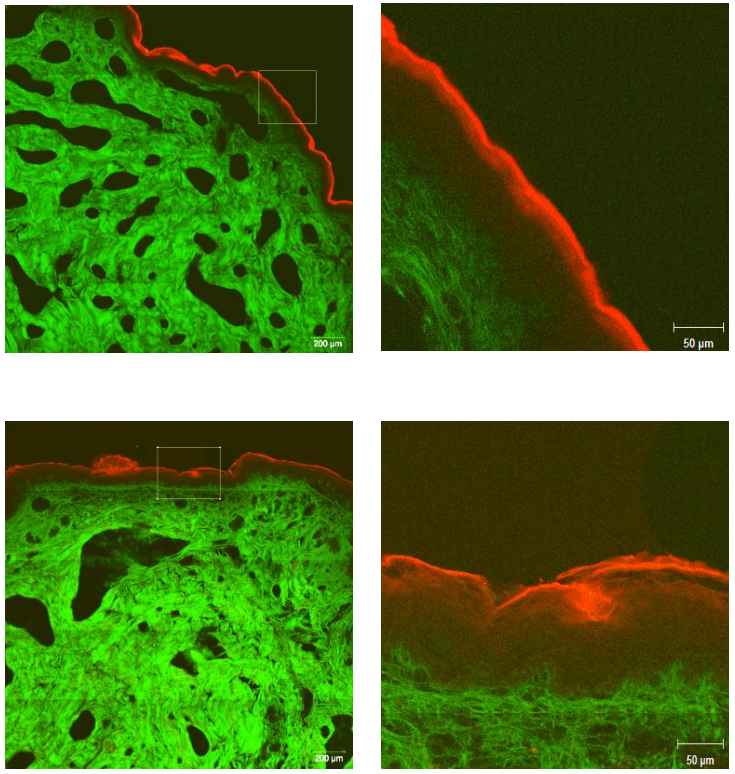
Modification Post-Polymerization



Application for Rhodamine-labelled βCD polymer

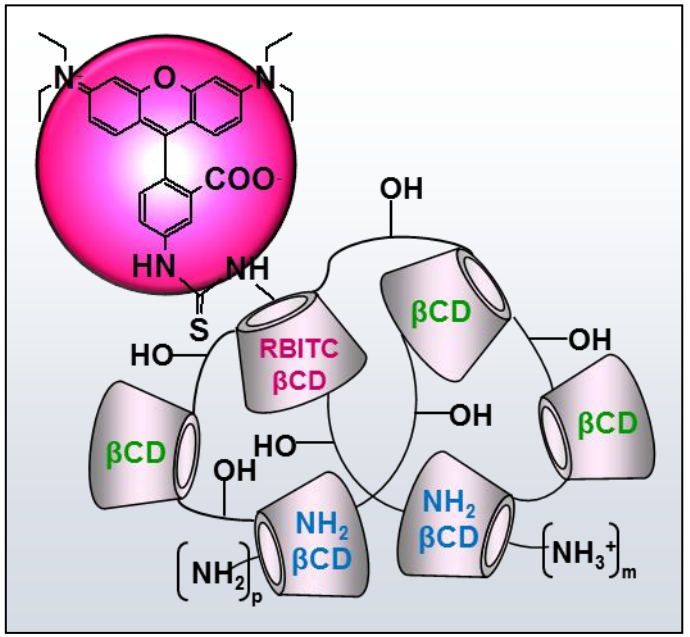
RBITC in MiliQ

pCD-RBITC in MiliQ



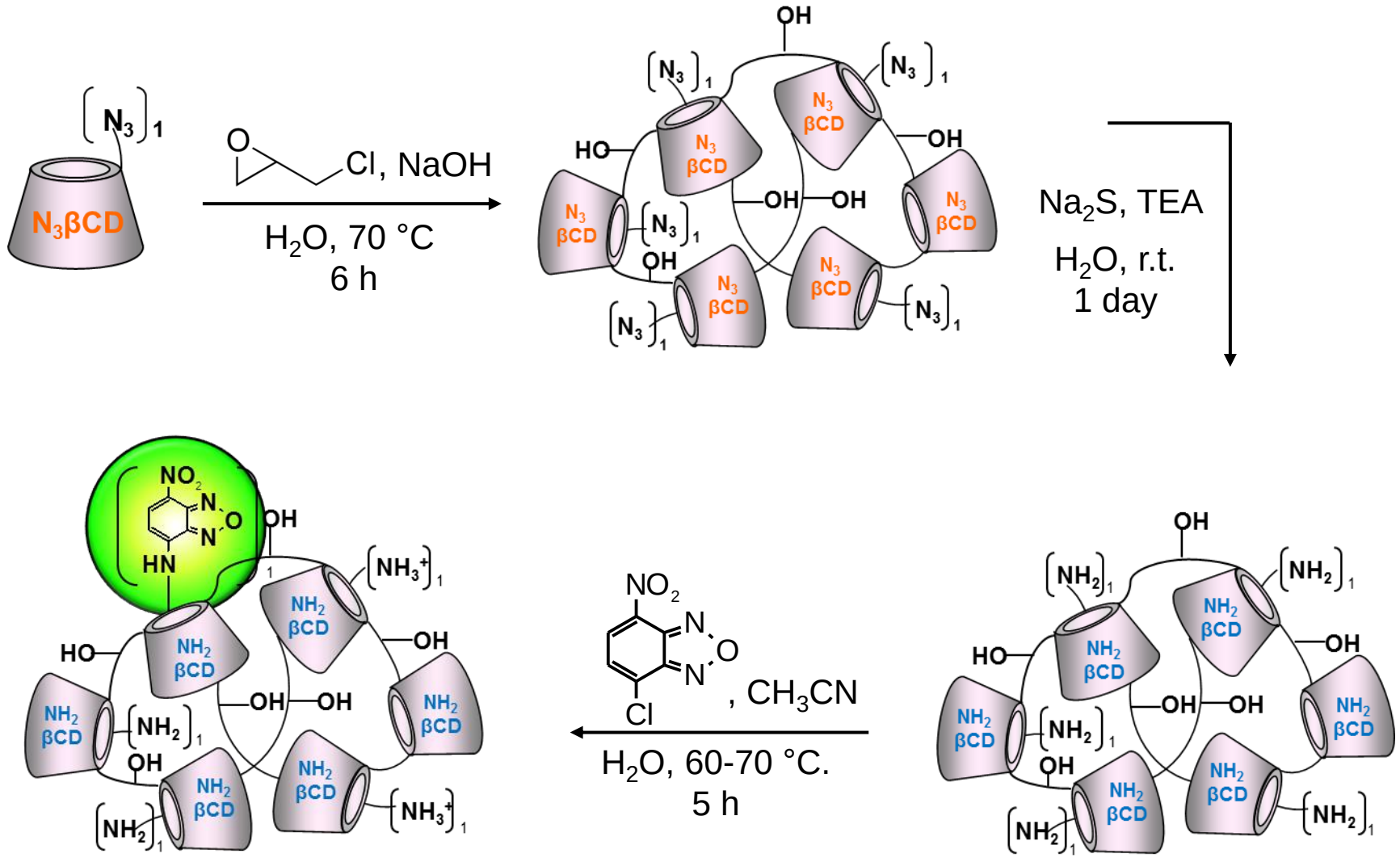
Cryosections

Topical delivery of β-cyclodextrin polymer labelled with RBITC



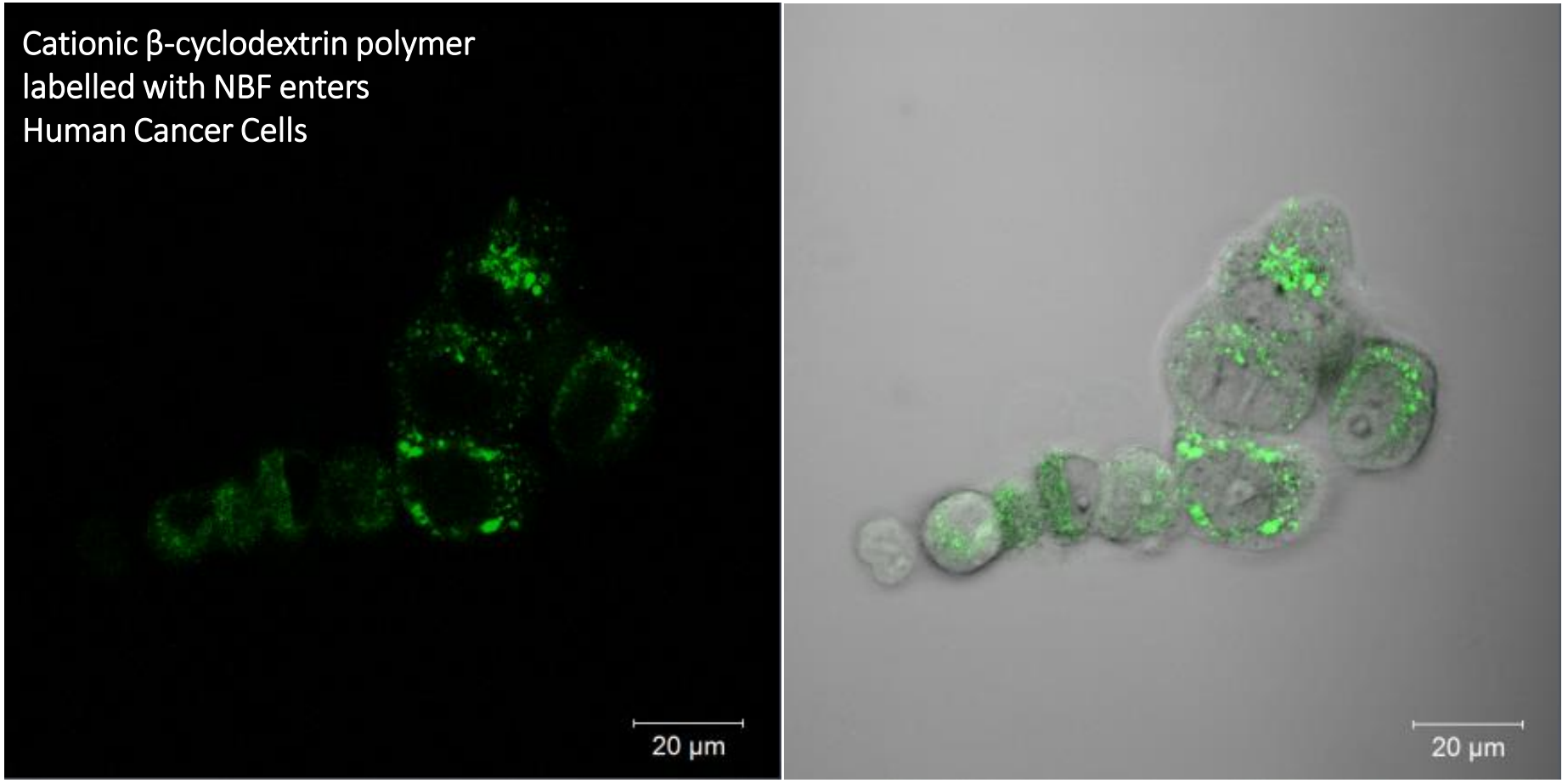
R. Gref, V. Agostoni, S. Daoud-Mahammed, V. Rodriguez-Ruiz, M. Malanga, L. Jicsinszky, P. Horcajada, C. Serre: French Patent Application, N°12/55065.

Modification Pre-Polymerization

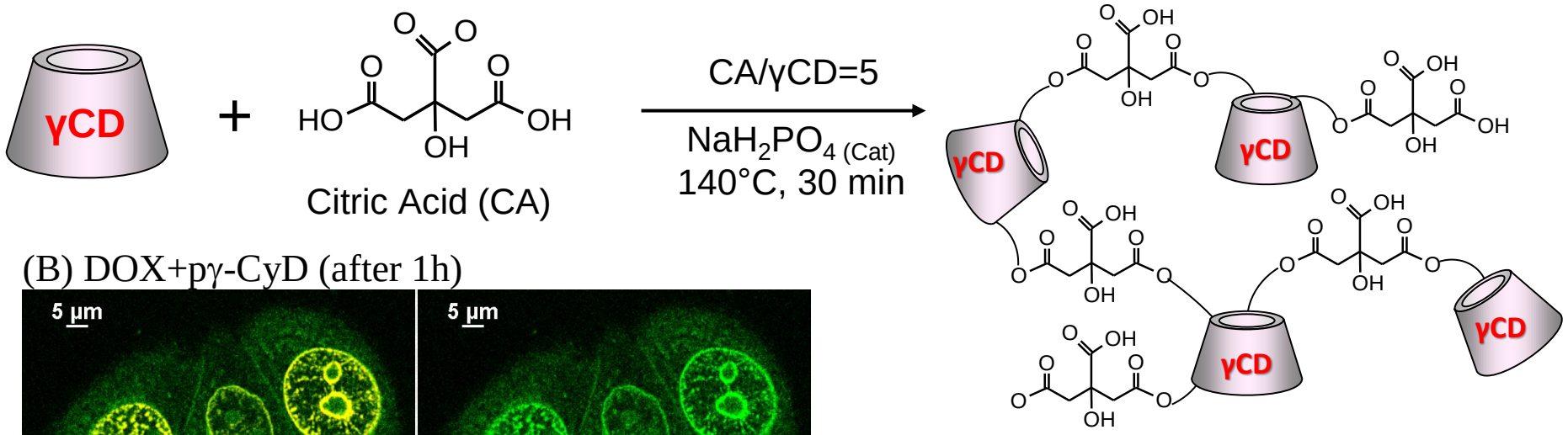


Malanga M. et al., Beilstein J. Org.Chem. 2014, 10, 3007-3018

Applications for NBF-labelled βCD polymer

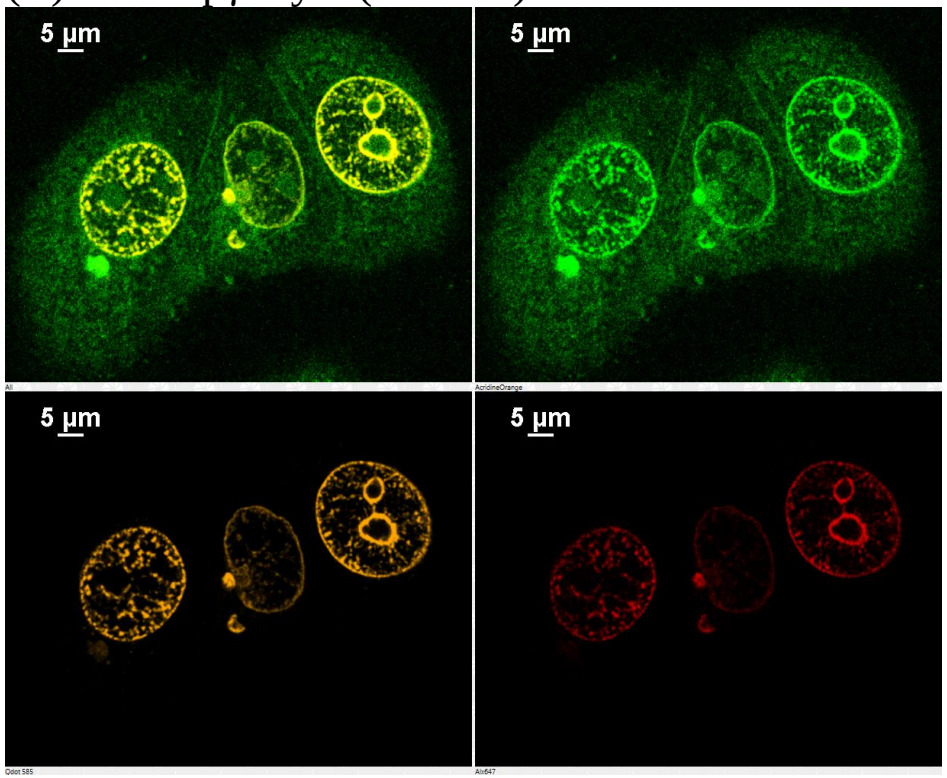


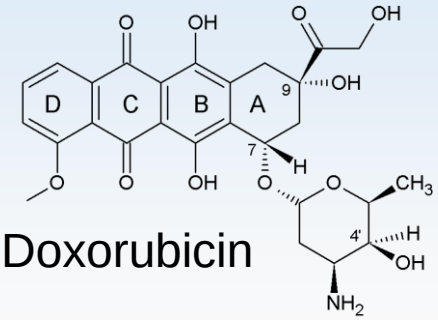
Citric acid Cross-Linked γCD polymer



CD content=60% (HPLC)
Mw= 50-70 kDa (SLS)

(B) DOX+pγ-CyD (after 1h)

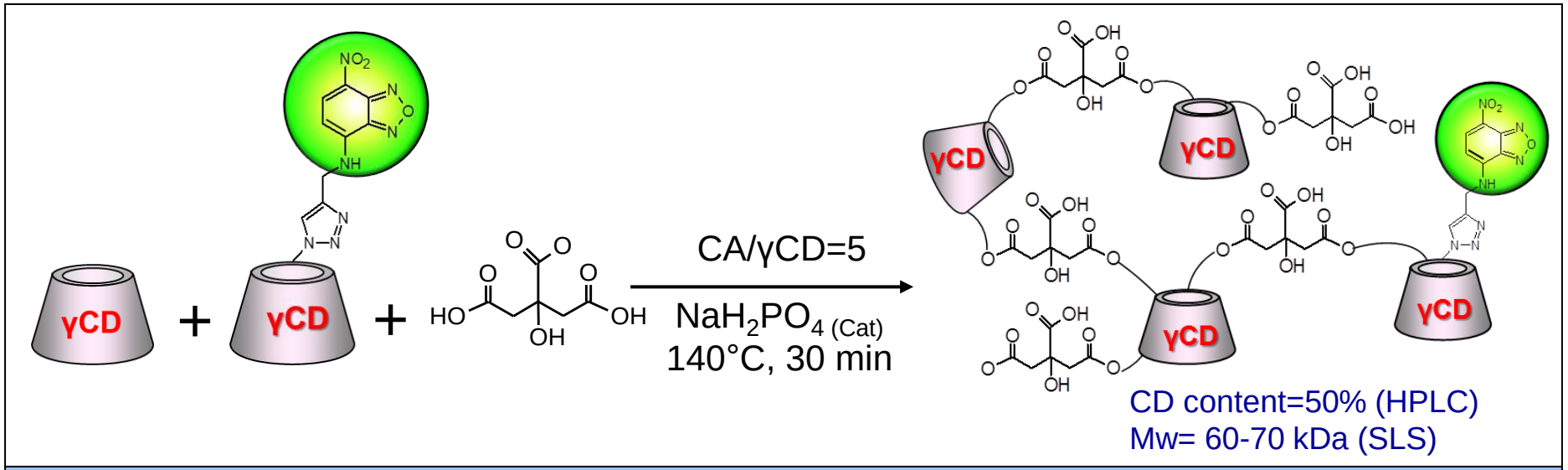


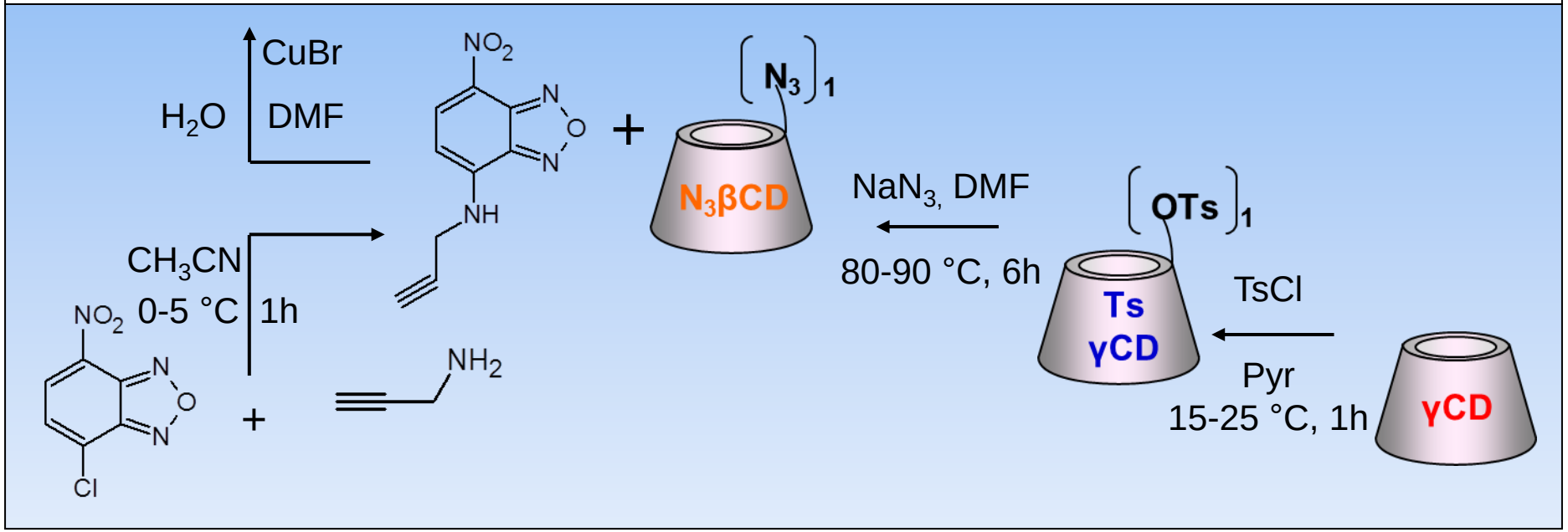


Doxorubicin

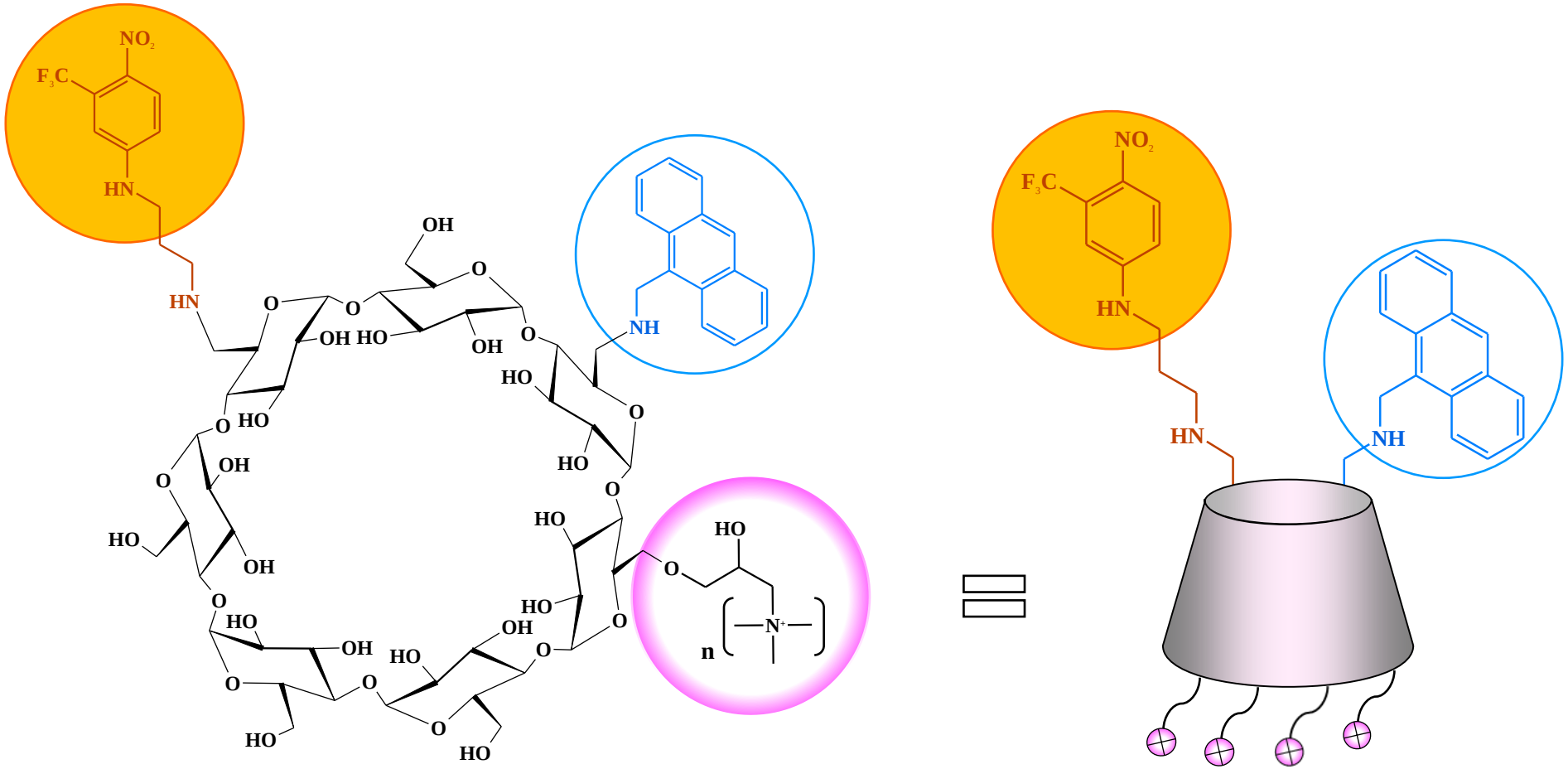
Anand R., Malanga M. et al., Photochem. Photobiol. Sci., 2013,12, 1841-1854

NBF-labelled Citric Acid Cross-Linked γCD Polymers



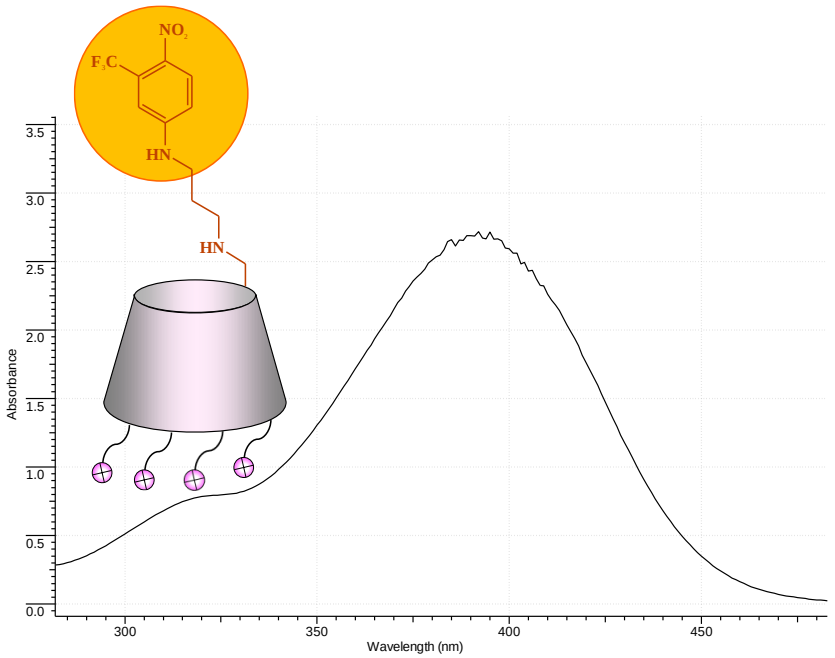


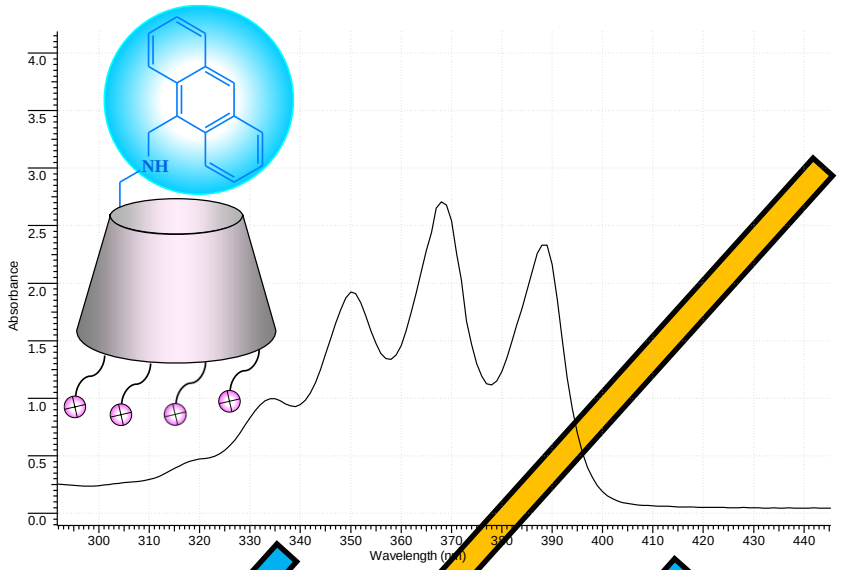
A Photoactivable Bichromophoric CD-system

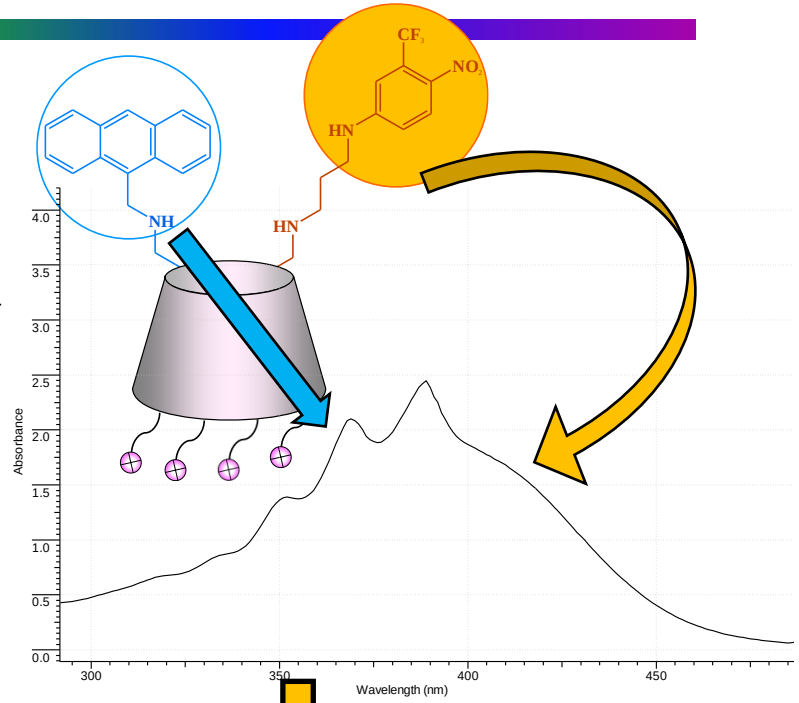


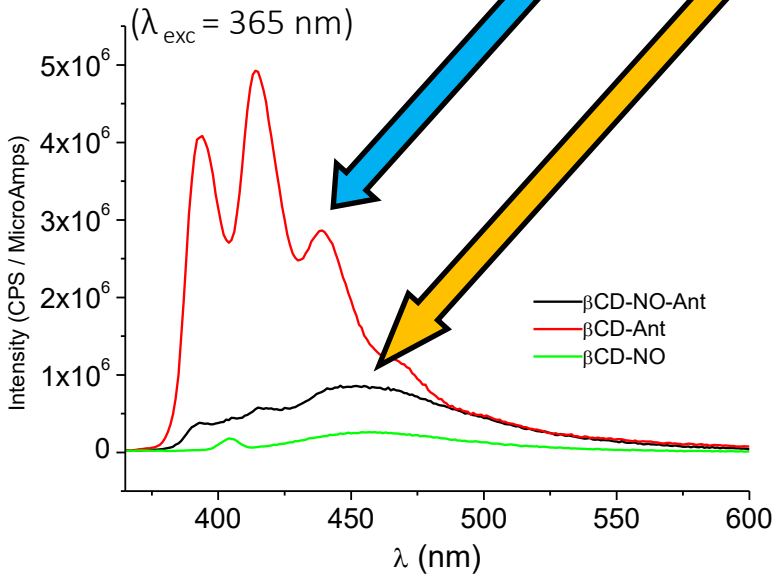
- Drug encapsulation – DNA targeted drug delivery
- Enhanced solubility
- Enhanced membrane penetration
- Antimicrobial activity of QA-CDs
- Potential interaction with the phosphate backbone of the DNA

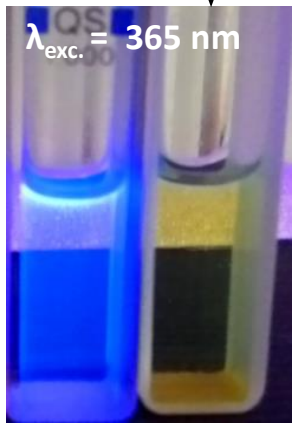
Absorption and Emission Spectra





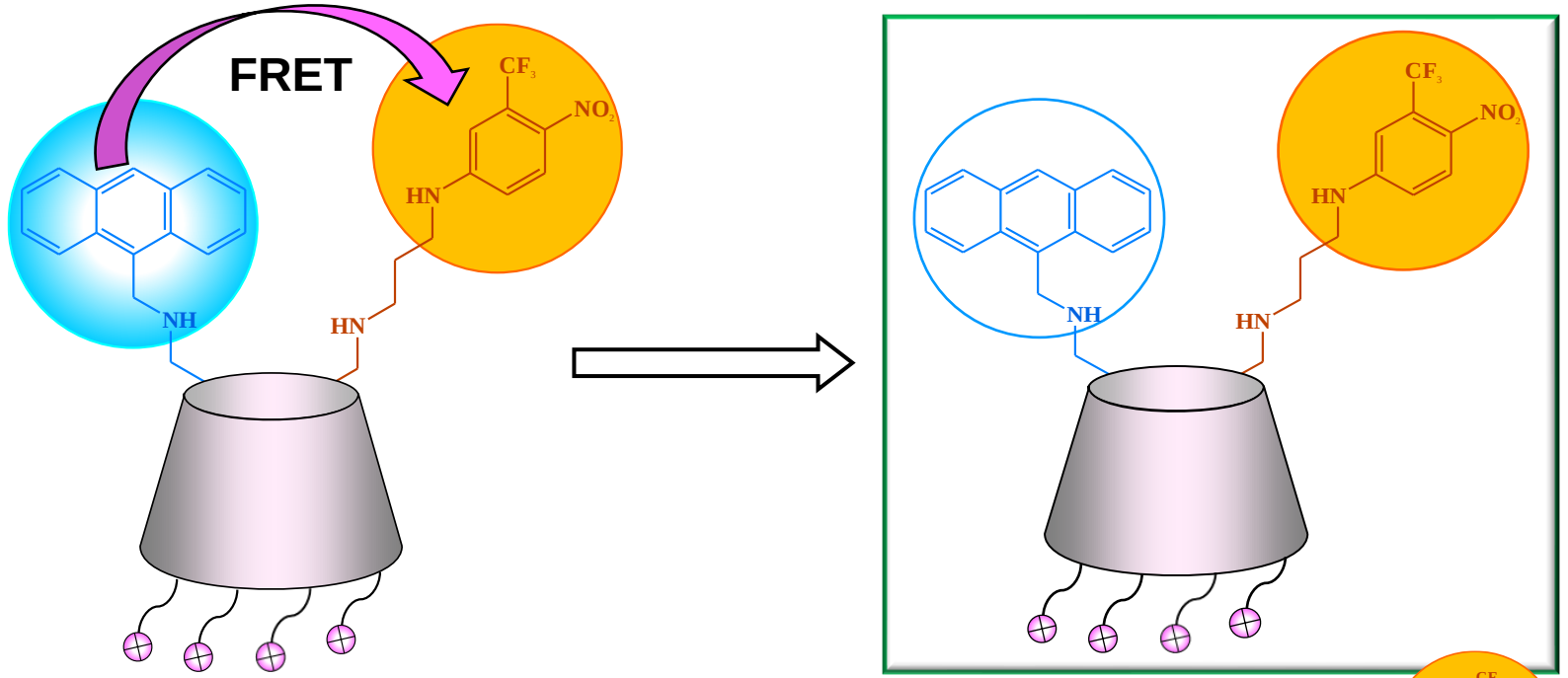


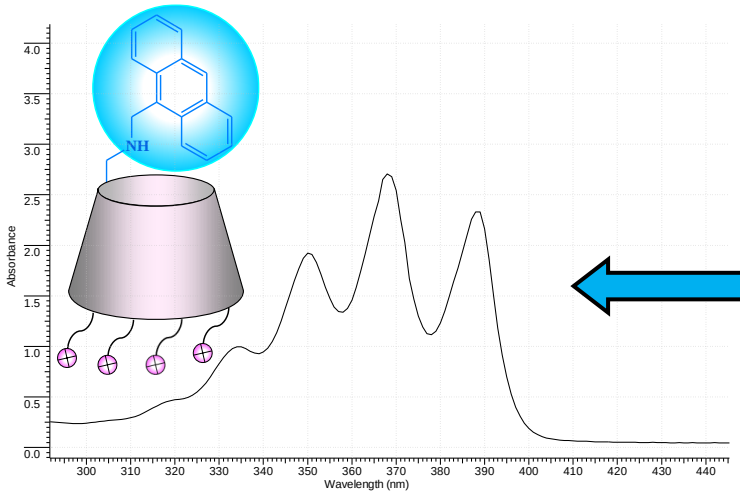


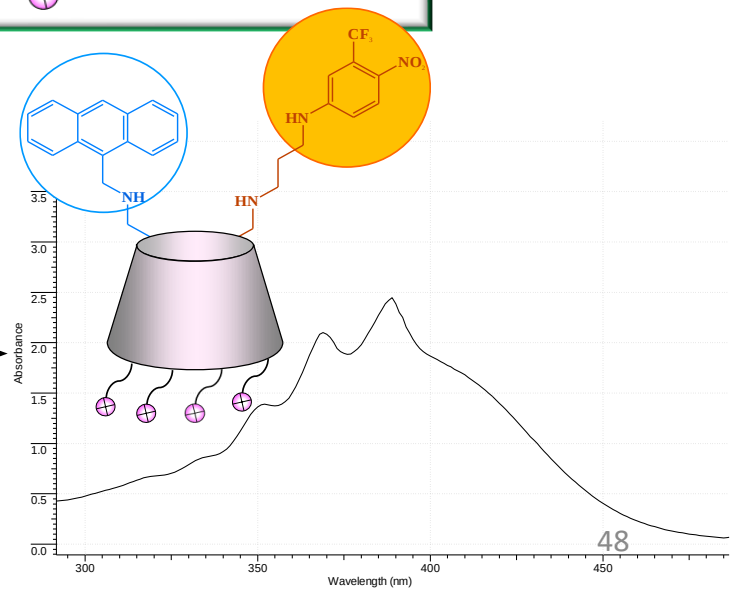


Anthracenyl-NO-donor FRET

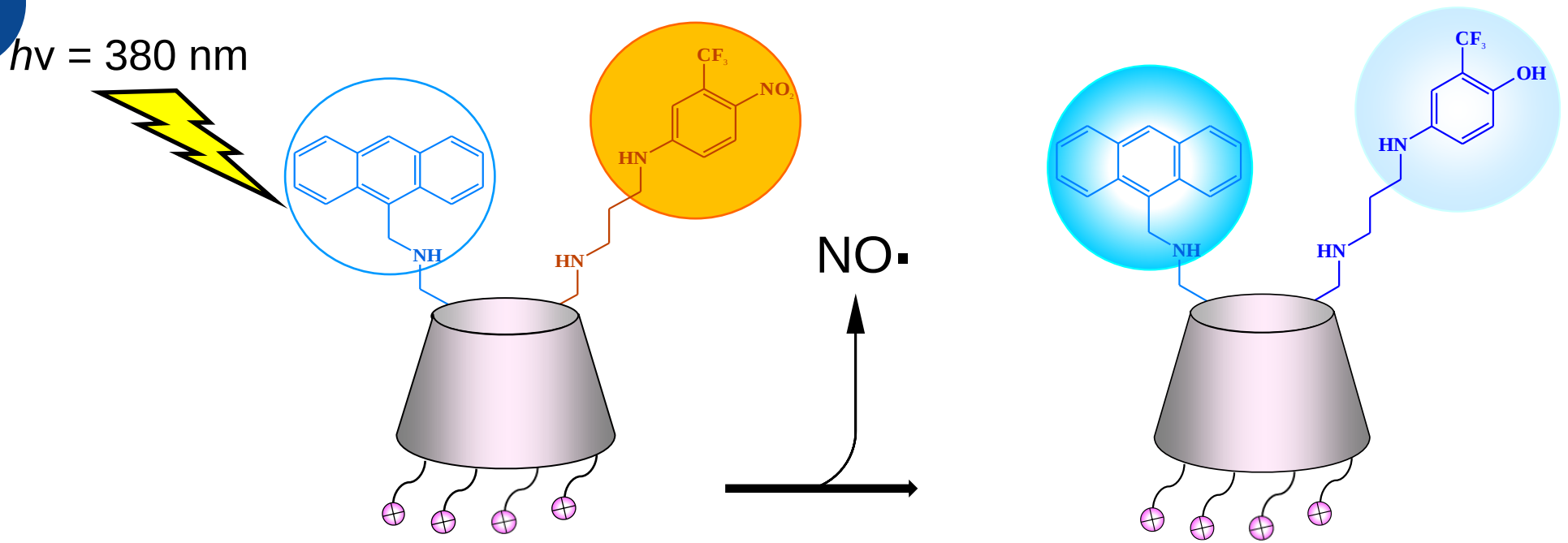
*h*v = 380 nm

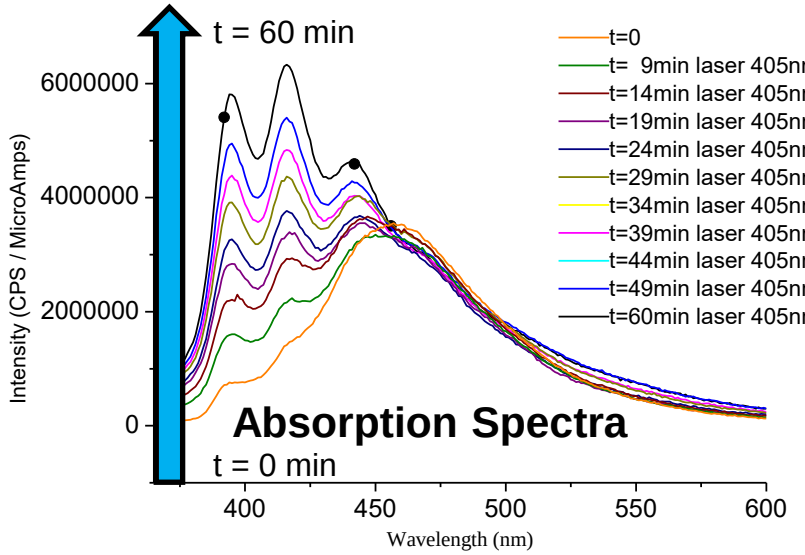


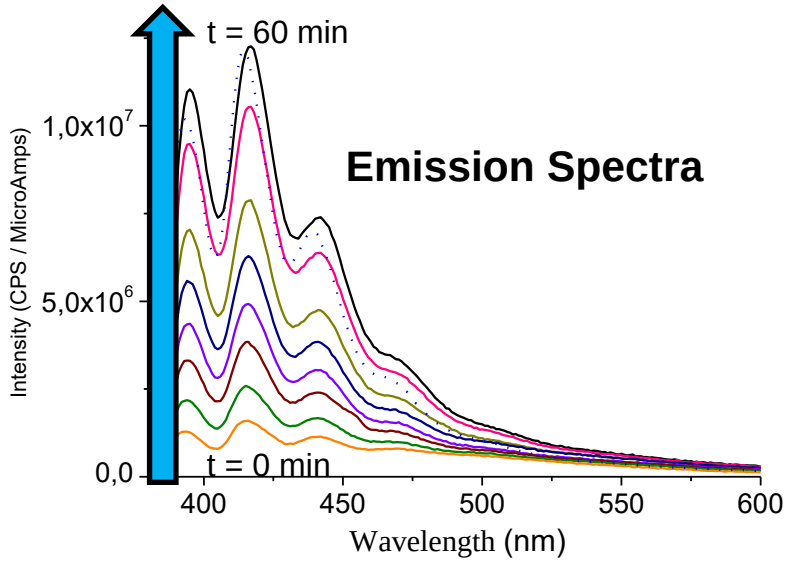




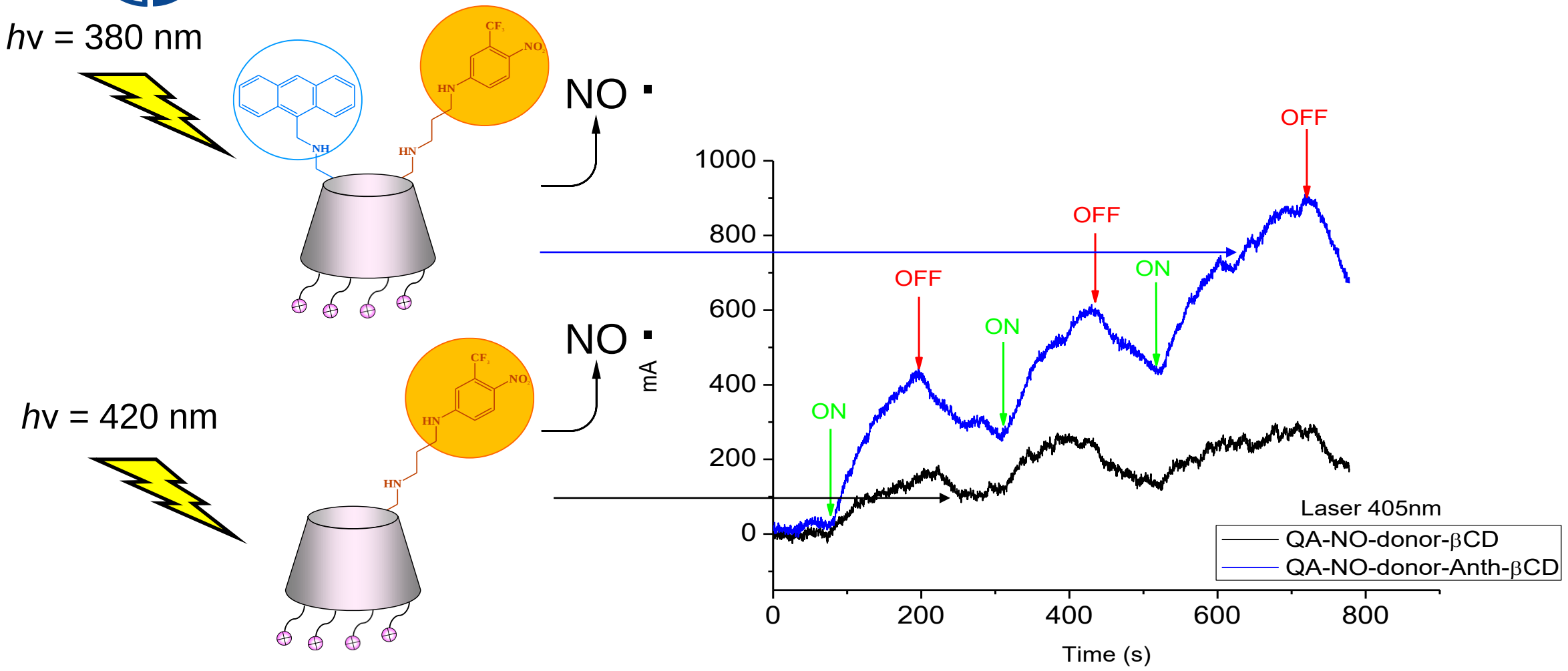
Light Irradition (Photolysis)







Nitric Oxide Photorelease

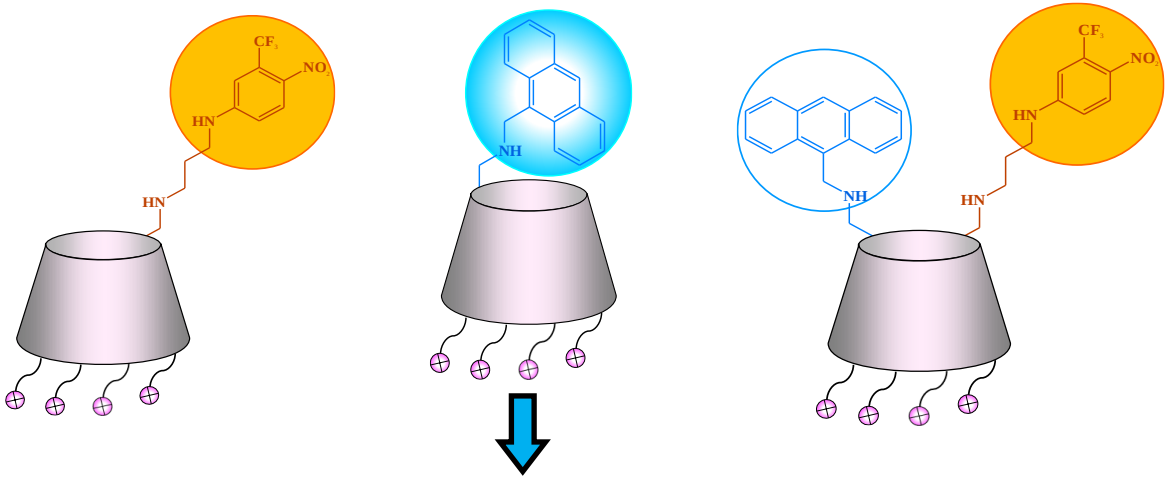


Ongoing Studies

Conclusions

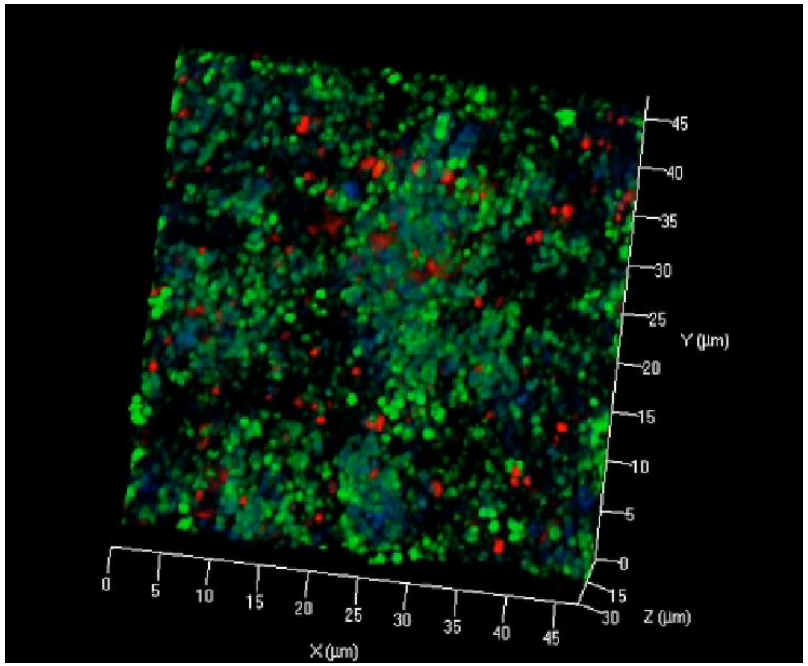
Synthesis and applications of:

- NO-photodonor appended βCD
- Anthracene appended βCD
- Bichromophoric βCD



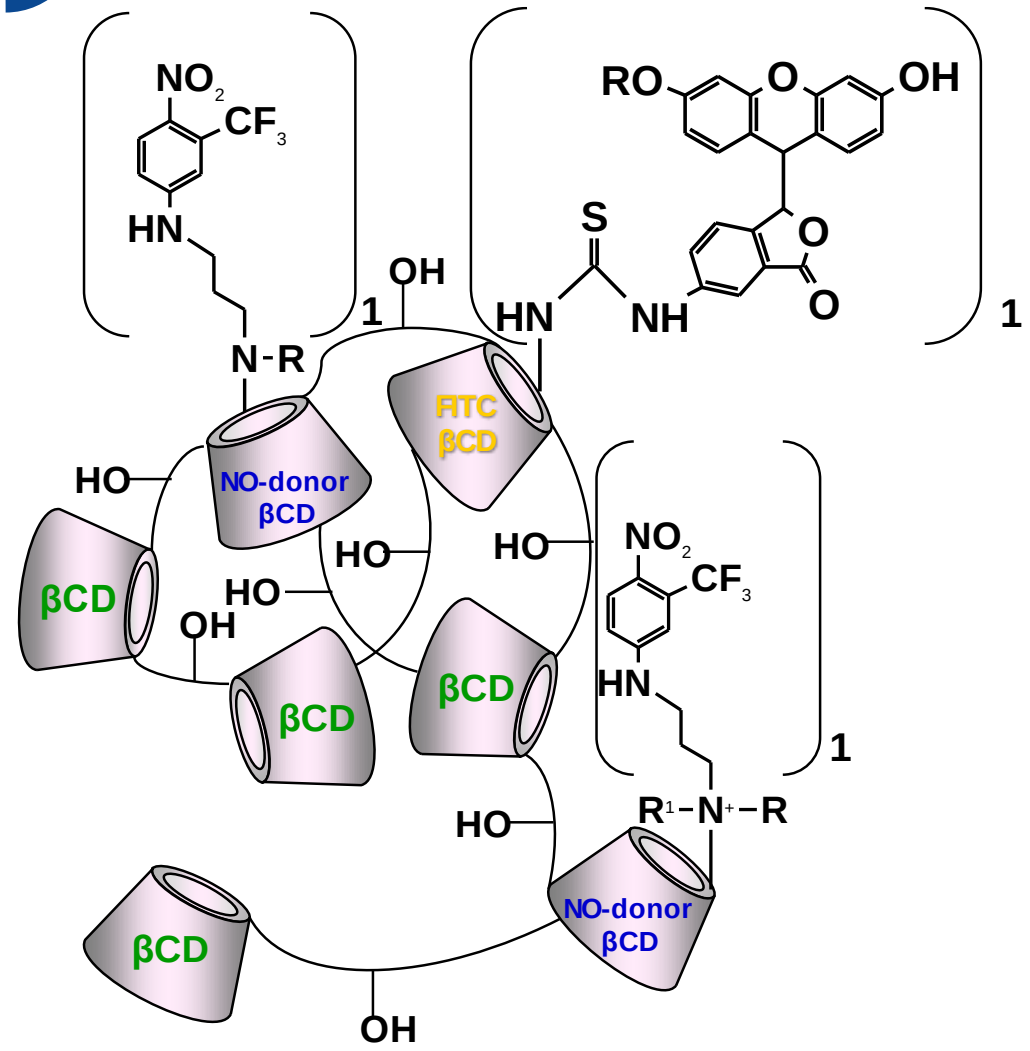
Future perspectives

- Two photon fluorescence microscopy for 3D visualization of bacterial biofilm (optical sectioning, irradiation in sections)
- Quantitative analysis of CD diffusion
- NO release trafficking on biofilms

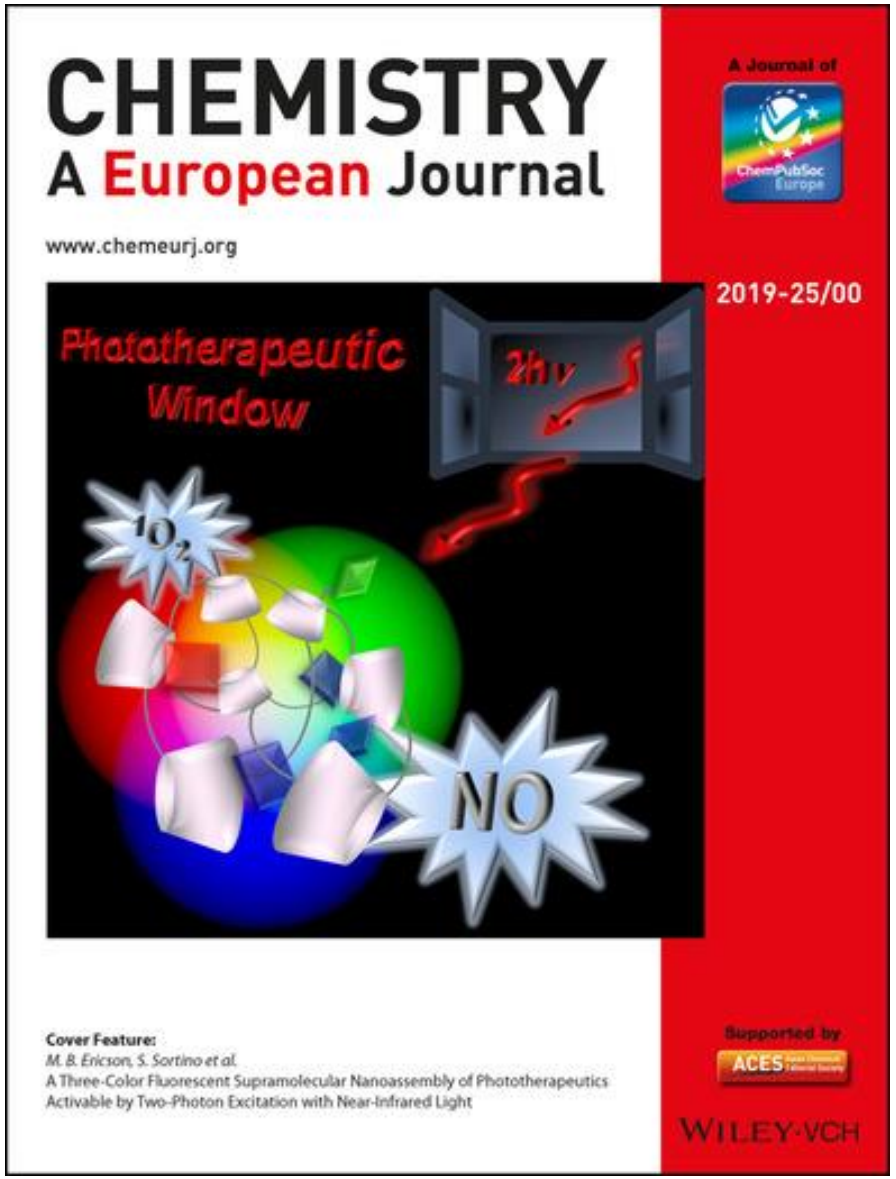


S. epidermidis culture 18hr growth. 2PM imaging, near IR excitation. LIVE/ DEAD stain + Quaternary ammonium-6-monoanthracenyl-β-Cyclodextrin

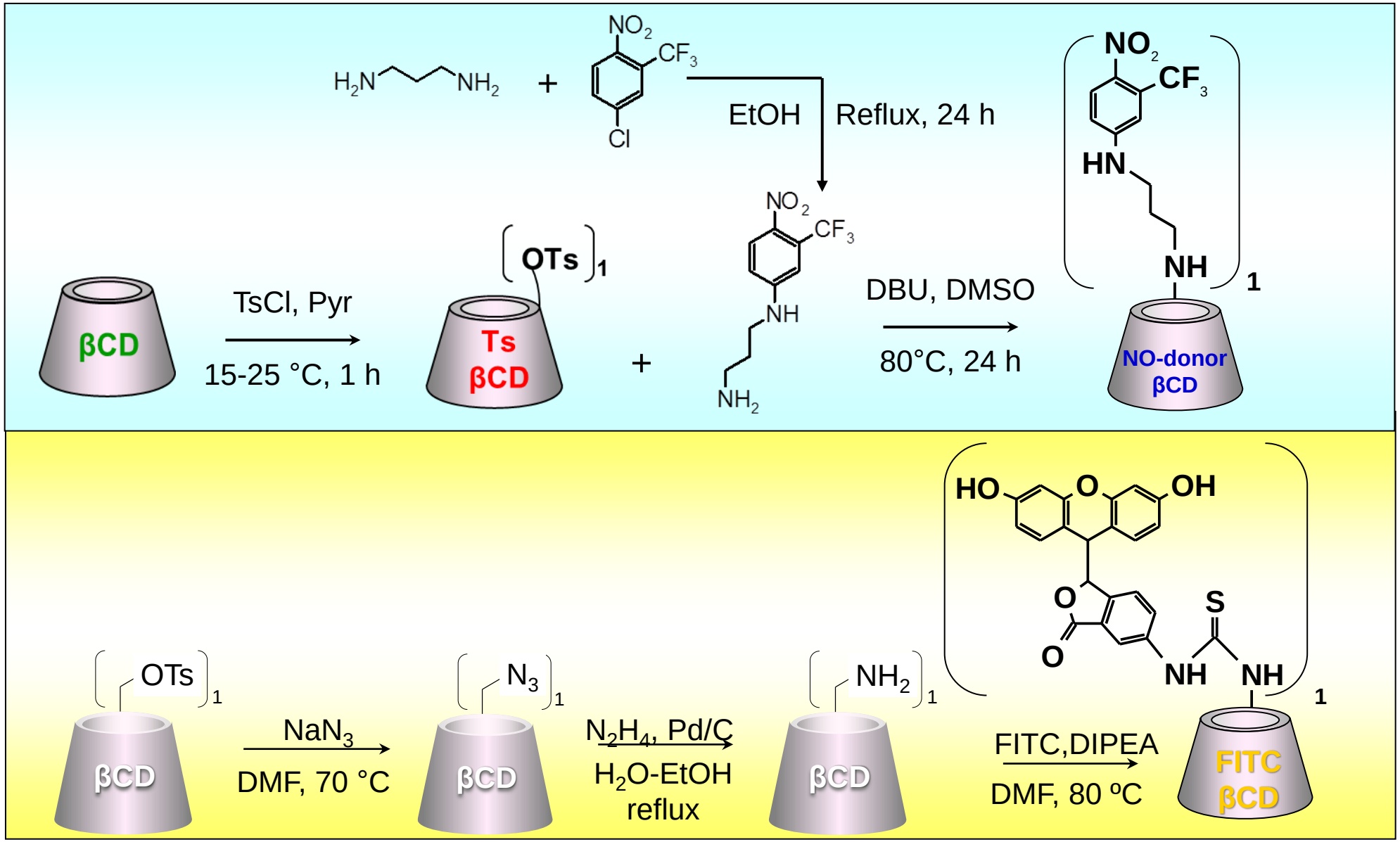
Photoactivable Fluorescent Cyclodextrin Polymer



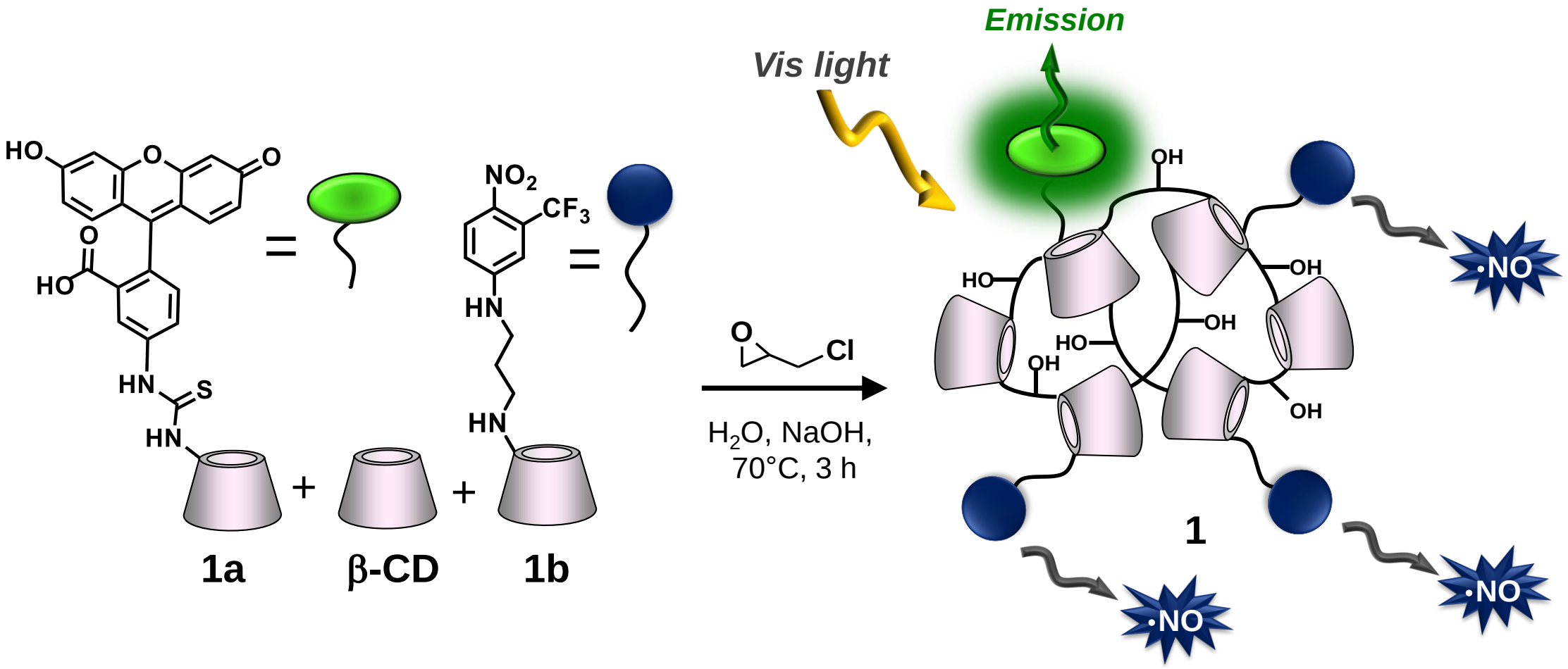
R= -H, $-CH_2CHOHCH_3$



Bichromophoric βCD-Polymer



Fluorescent Nitric Oxide-Releasing βCD-Polymer



Fraix A. et al., Chemistry, 2019, 23;25(29):7091-7095

Thank you for your kind attention!