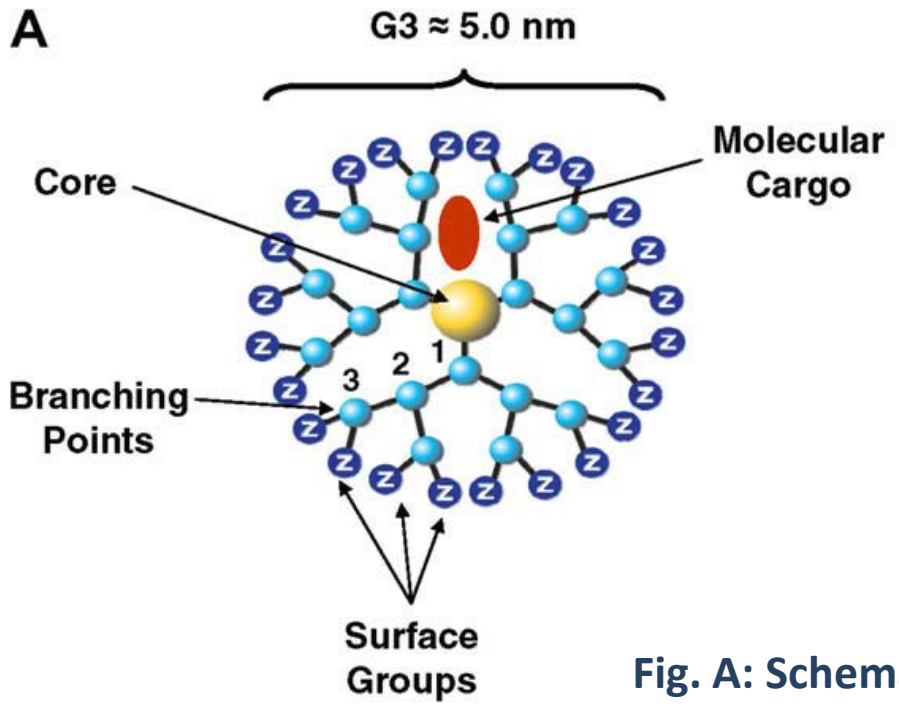
Medicinal Application of Dendrimers

Literature Seminar
Shogo HASHIZUME (M2)
‘11. 12. 6. (Tue.)

Prologue - *What is the Dendrimer??*

Dendrimer = a family of nanosized, 3D polymer
a class of macromolecules
having highly branched architecture



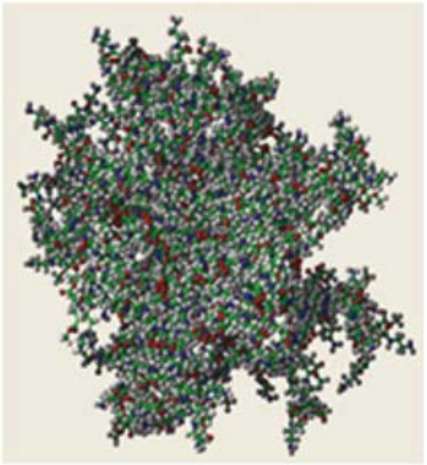
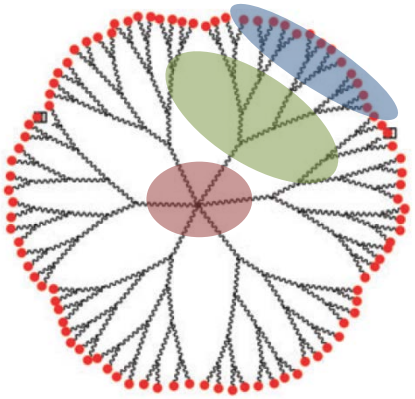


Fig. A: Schematic 2D presentation of dendrimer
Fig. B: 3D presentation of dendrimer

Prologue - *What is the Dendrimer??*

- Dendrimers consist of three main components…

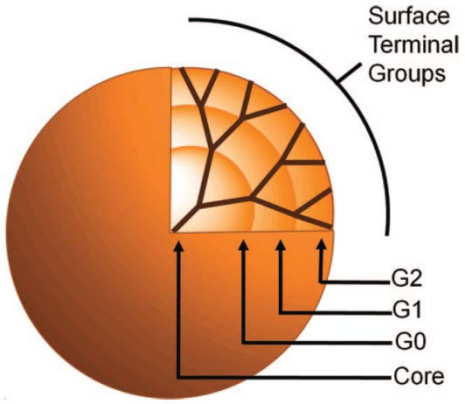


Surface: **functional peripheral group**

Interior: **affects host-guest properties**

Core: **affects 3D shape of dendrimer**

- Count branching points as the "generation"



➡ **This is a "G2 dendrimer."**

Higher generation dendrimers form more dense, dimensional shape.

Prologue - *Polymer vs Dendrimer*

What makes dendrimers great over simple linear/branched polymer ??

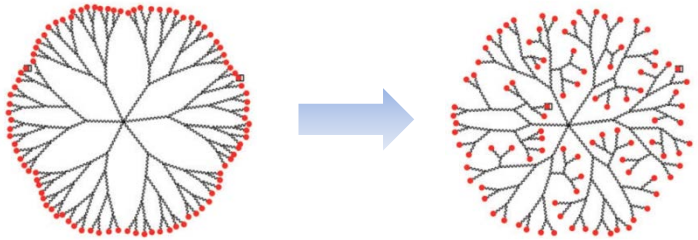
→ **It is "monodispersity"**

Polymer	**Dendrimer**
Simple chain growth procedure	**Stepwise growth**
↓	↓
Mixture of different products will be obtained…	***Nearly monodisperce product will be obtained !!***

Prologue - *Backholding of Dendrimers*

But…

Dendrimers always branch as simply extending outward…??



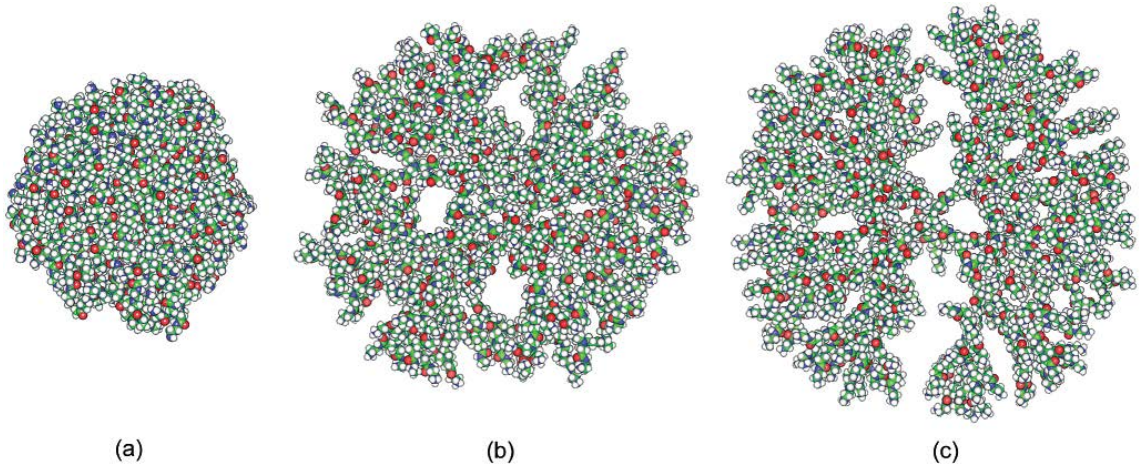
Doesn't occur ???

<u>Backholding actually occurs.</u>

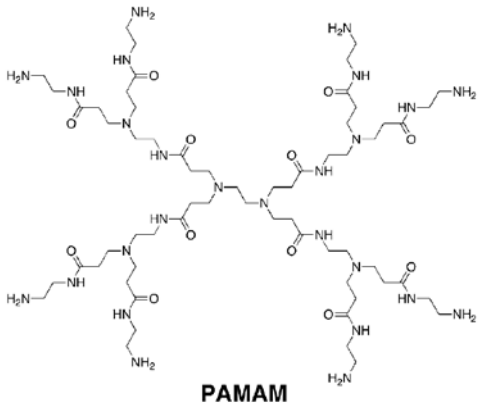
For example…

Conformational simulation of PAMAM at…

Baker, J. R. Jr. *et al.*
Macromolecules **2002**, *35*, 4510.

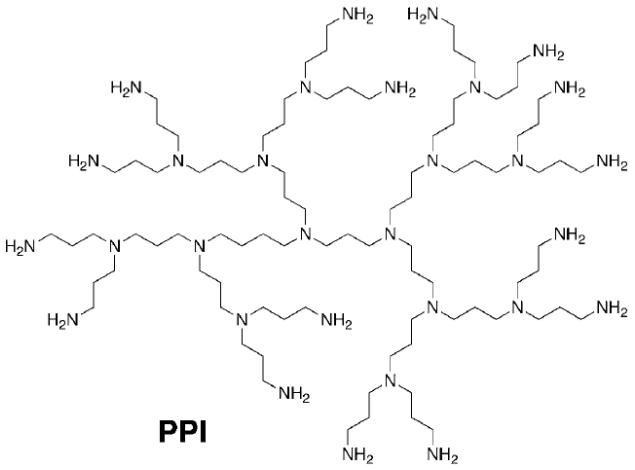


high pH **neutral pH** **low pH**



Prologue - *Early Development of Dendrimers*

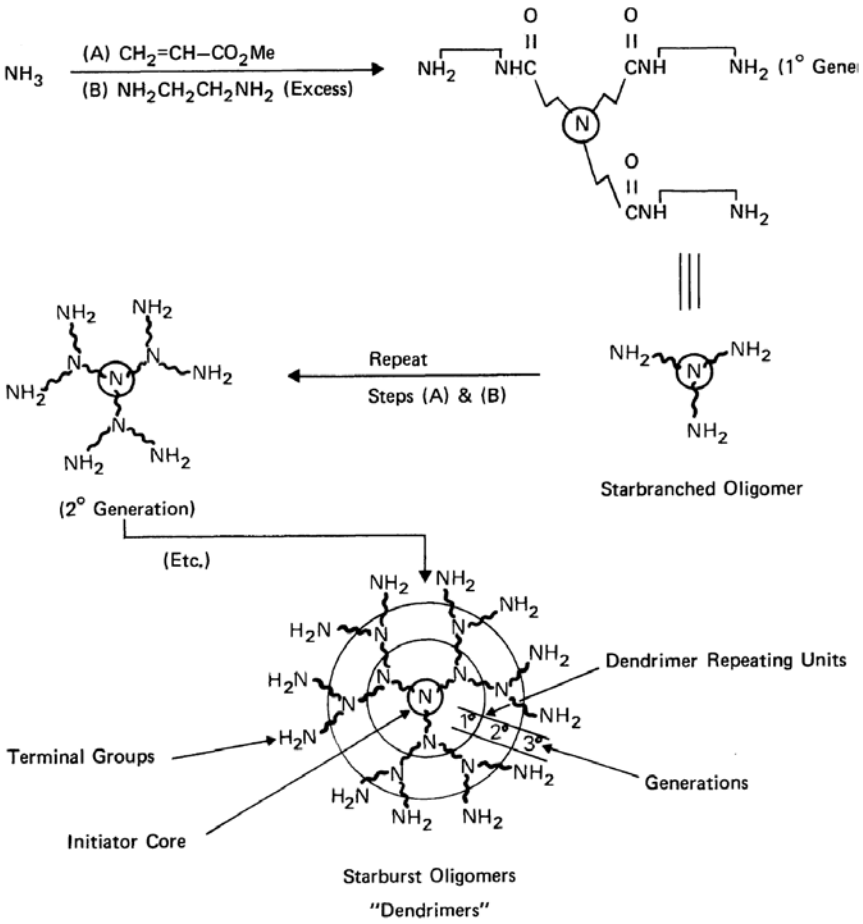
- First synthesis dendritic arms



Vogtle, F. *et al.*
Synthesis **1978**, *2*, 155.

But, synthetical difficulties limited to only low generation compound.

- Synthesis of dendrimers at higher generations with well-defined structures



Tomalia, D. A. *et al.*
Polymer J. **1985**, *17*, 117.

Prologue - *Examples of Dendrimers*

Since then, over 100 dendrimer structure have been realized.

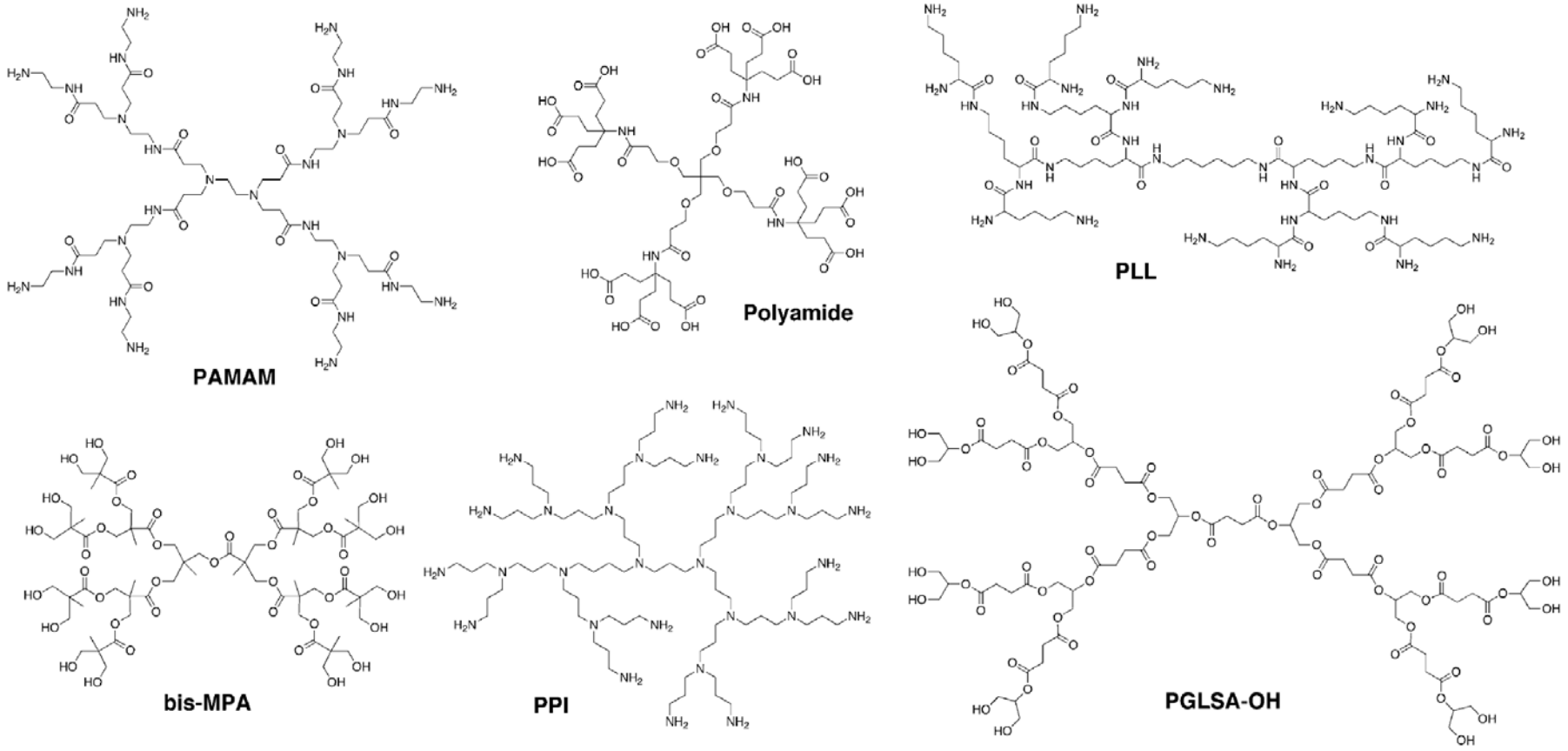


Fig. 1 Chemical structures of several commonly used, commercially available dendrimer structures.

Today's Contents

0. Prologue

1. How to Synthesize Dendrimers

2. Dendrimers as Drugs

3. Dendrimers as Drug Carriers

4. Perspectives

Other applications: Tissue engineering
Transfection
Magnetic resonance imaging (MRI) etc.

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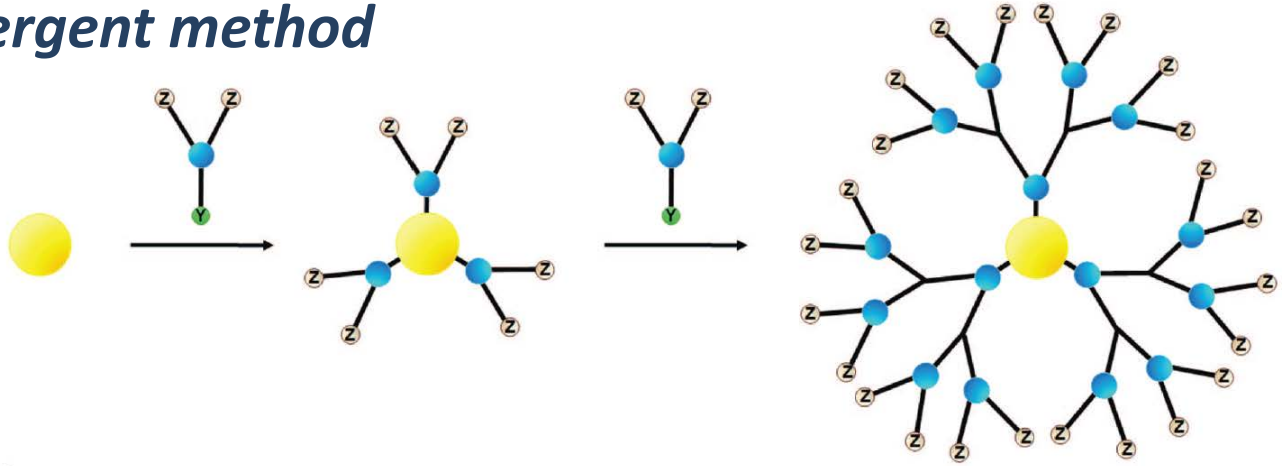
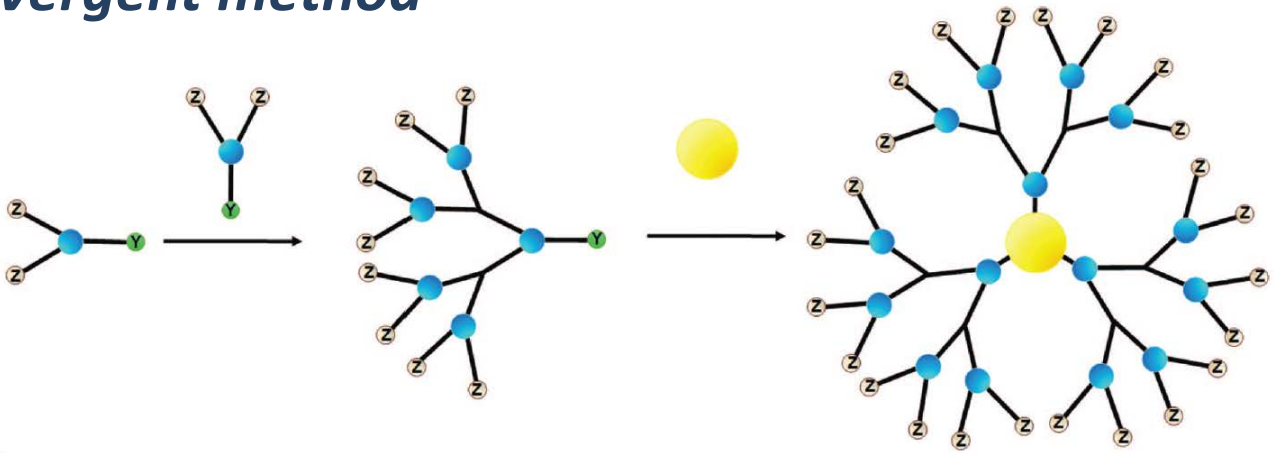
Divergent or Convergent ??

Roughly, two synthetic strategies of dendrimer…

- Divergent method



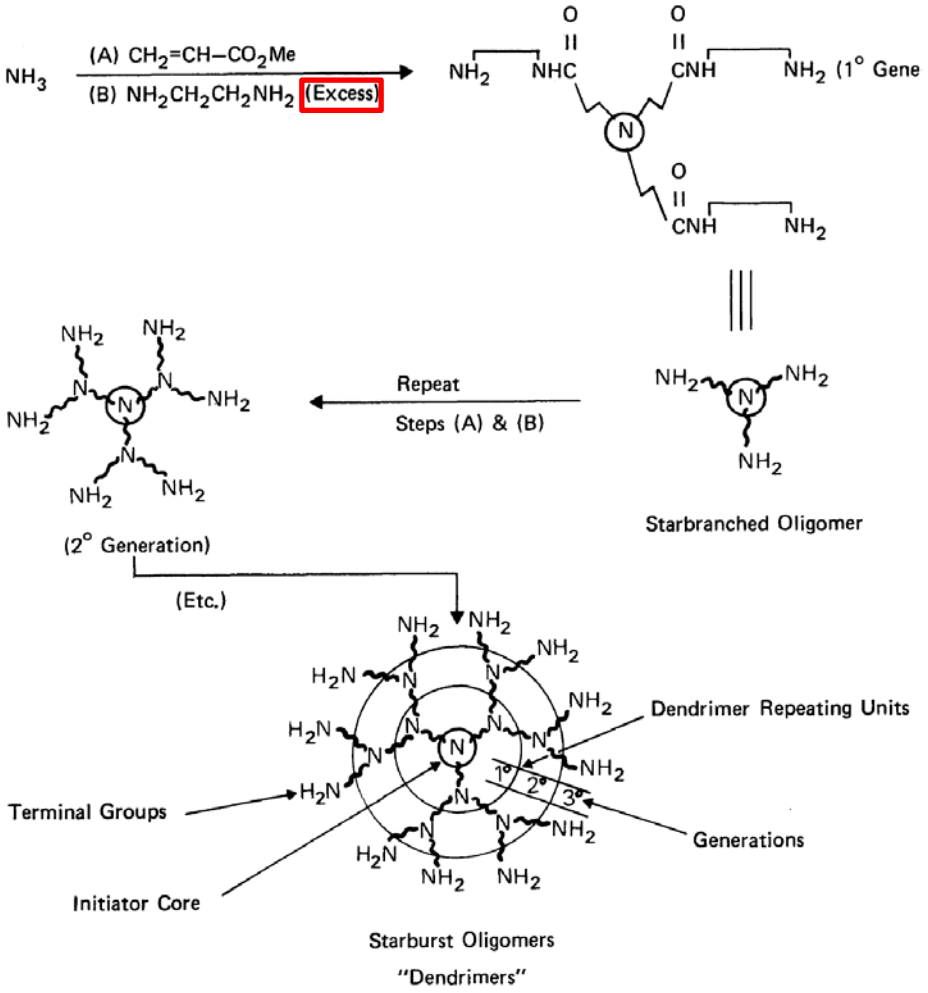
- Convergent method



Divergent Method

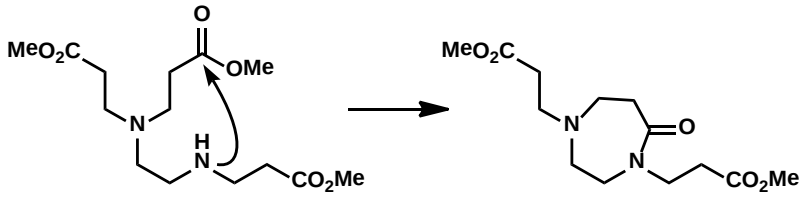
Tomalia's PAMAM synthesis

Tomalia, D. A. *et al.*
Polymer J. **1985**, *17*, 117.

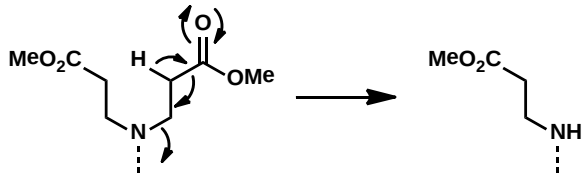


However, many side reactions…

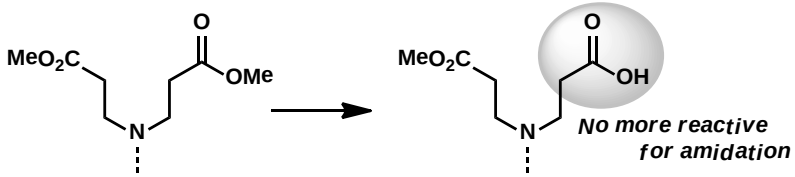
- Incomplete Michael addition



- Retro-Michael reaction @ high temp.



- Hydrolysis of methyl ester



Divergent Method

Divergent synthesis needs many steps

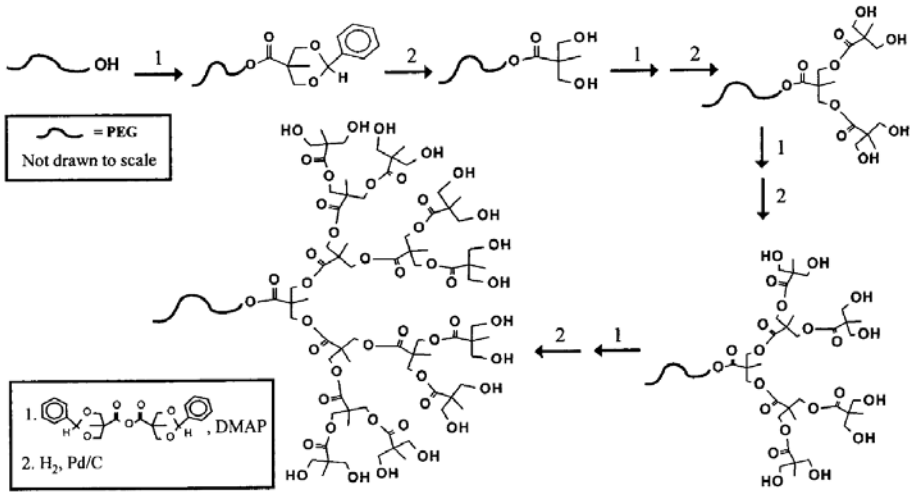
➡ **Improving yields & eliminating purif. steps are the keys.**

Need optimization of conditions & smart ligation system

Examples:

- Using anhydride coupling

Frechet, J. M. J. *et al. JACS* **2001**, *123*, 5908.

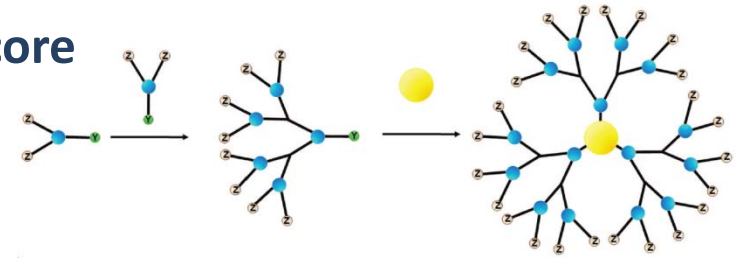


Up to *generation 6* dendrimer obtained **without chromatographic purification** !!

- Using click chemistry ➡ ***will be showed later***

Convergent Method

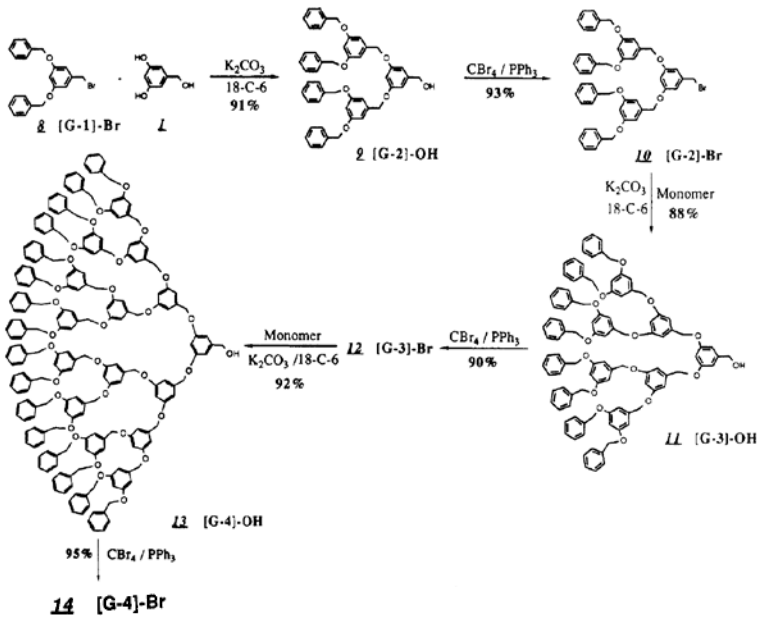
Constructing from periphery toward the core

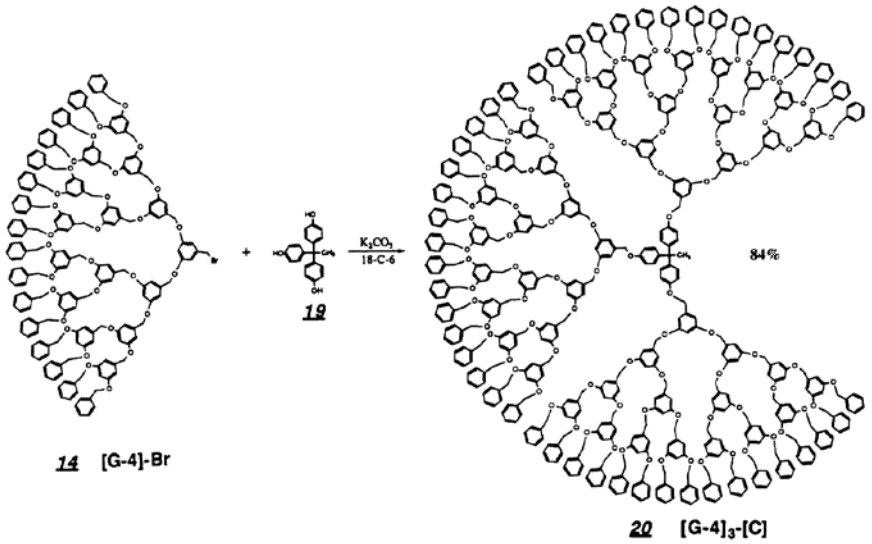


Only limited active sites per a reaction
(= more defect-free product)

First convergent synthesis:

Frechet, J. M. J. *et al. JACS* **1990**, *112*, 7638.

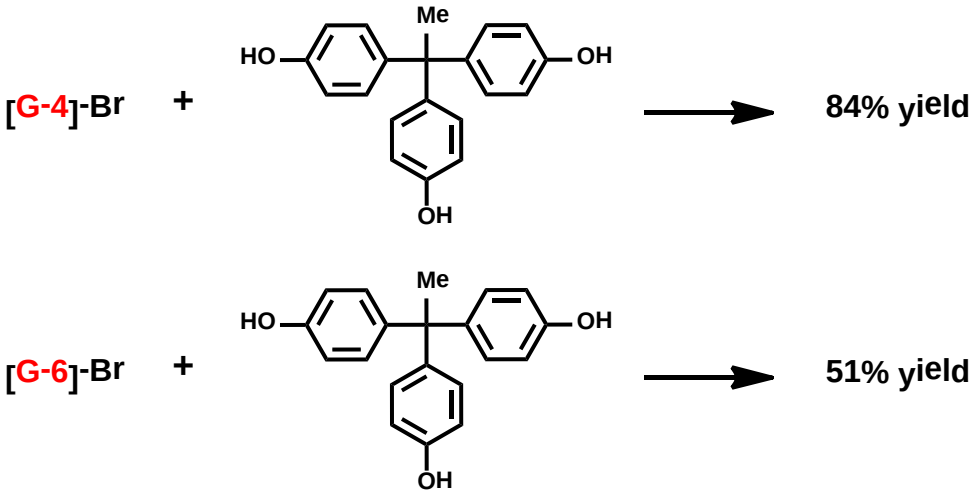




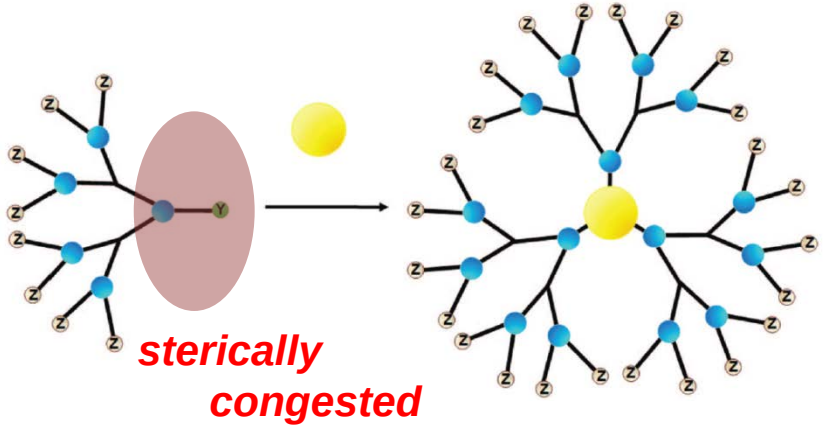
Convergent Method

However, high generation dendrimers are difficult by convergent method.

In the case of Frechet's synthesis (JACS,1990)…



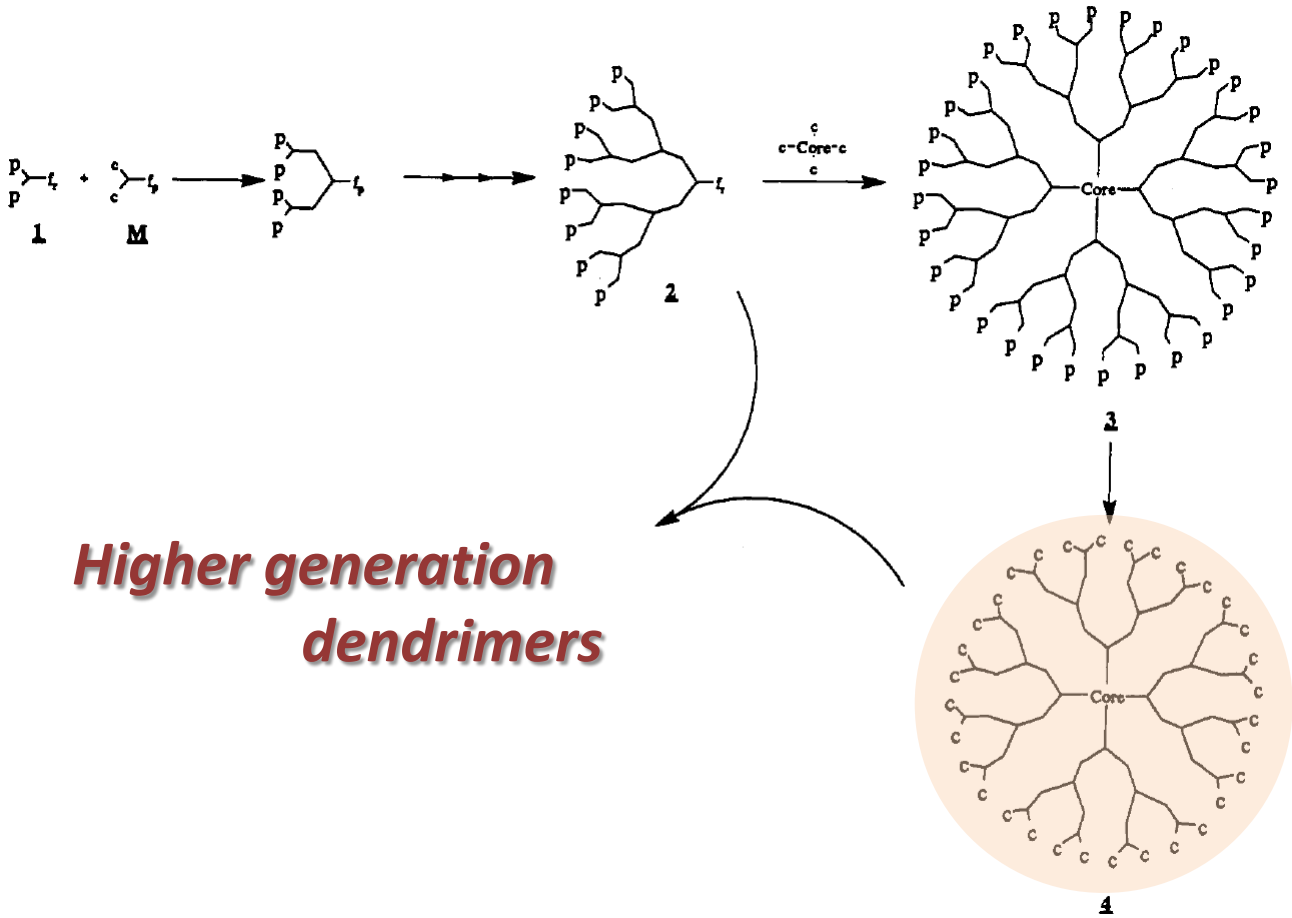
It's due to steric crowding at dendrons focal point.



Convergent Method

One solution is "double-staged" approach.

Frechet, J. M. J. *et al.*
JACS **1991**, *113*, 4252.



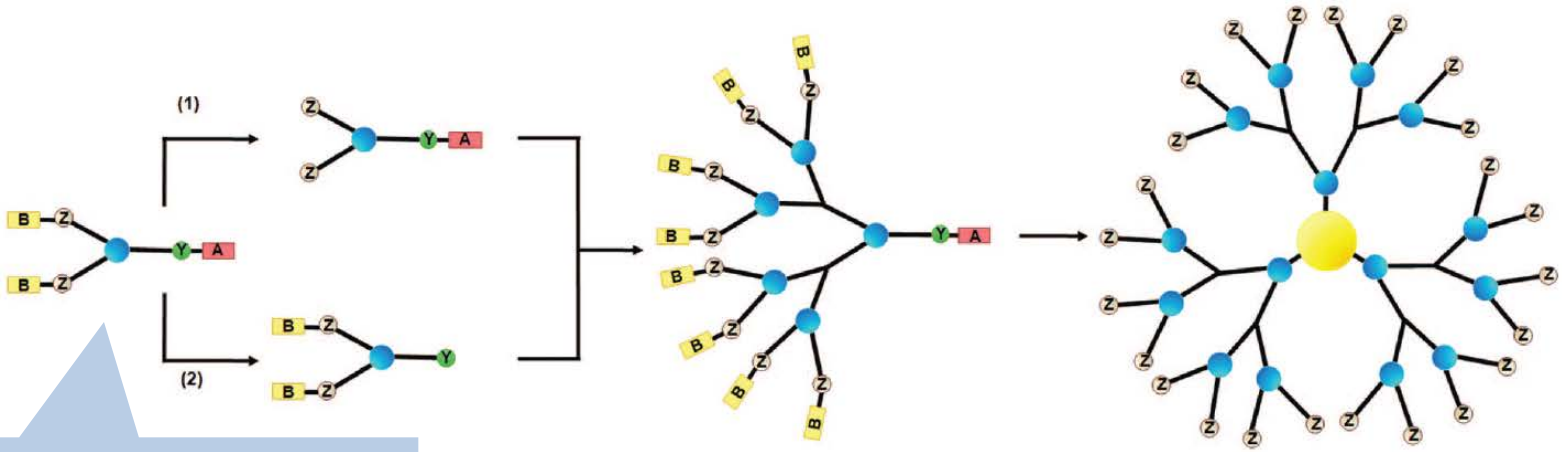
Higher generation dendrimers

Using as "flexible core" to reduce the steric hinderance

Combined Divergent-Convergent Method

"Double exponential" method

Wilkins, C. L.; Moore, J. S. *et al.*
JACS **1995**, *117*, 2159.



Orthogonally protected monomer

But this method has combined disadvantages of divergent/convergent method

- Higher generation dendrimers cannot be synthesized due to steric hinderance at the late stage.

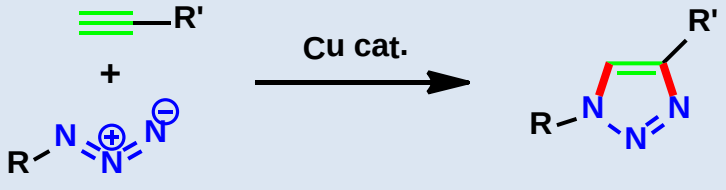
- Increasing of protection/activation chemistry with generation requires highly efficient reaction scheme.

Synthesis Using "Click Chemistry"

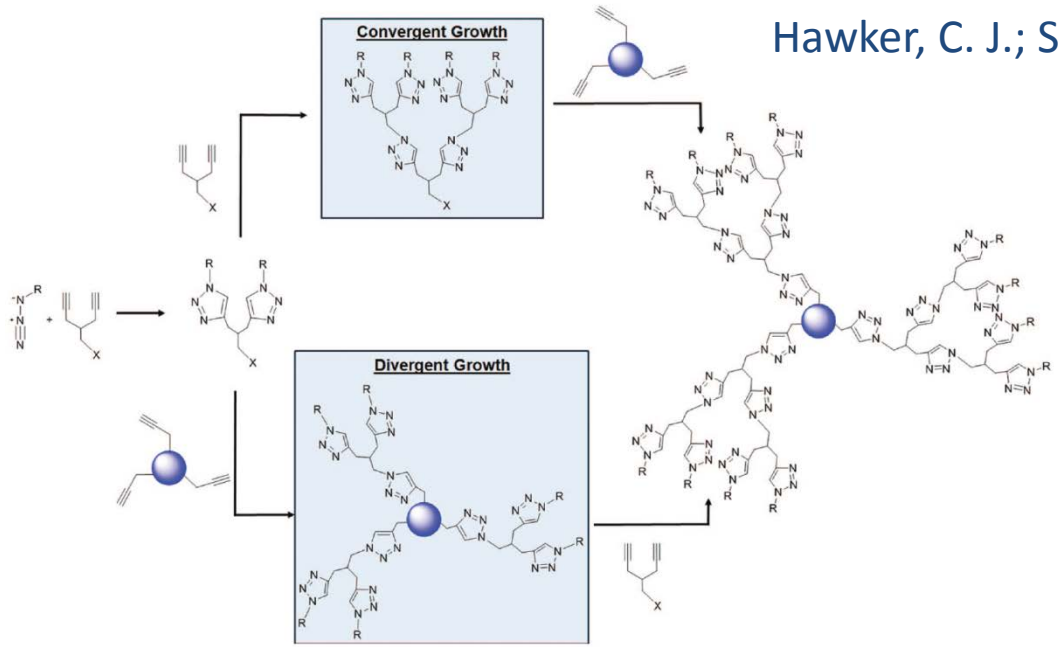
Improving yields of each step is the key to "monodispersity."

➡ **Using reliable reactions**

Cu-catalyzed cycloaddition of an alkyne and an azide.



Both of divergent/convergent approaches are possible.



Hawker, C. J.; Sharpless, K. B.; Fokin, V. V. *et al.*
ACIE **2004**, *43*, 3928.
Chem. Commun. **2005**, 5775.

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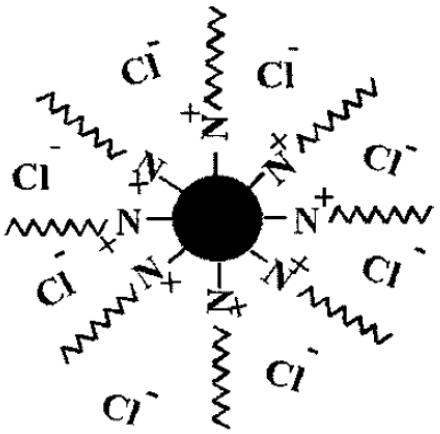
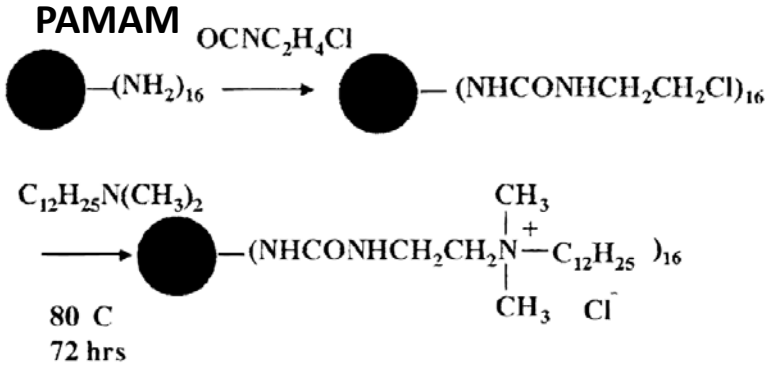
As Antimicrobial

- Cationic dendrimers with amphiphilic properties

Cooper, S. L. *et al. Biomacromolecules*, **2000**, *1*, 473.





Activities depend on generation number and alkyl chain length of ammonium.

***higher* generation = more surface groups**
***lower* generation = more potent for permeability**

However,

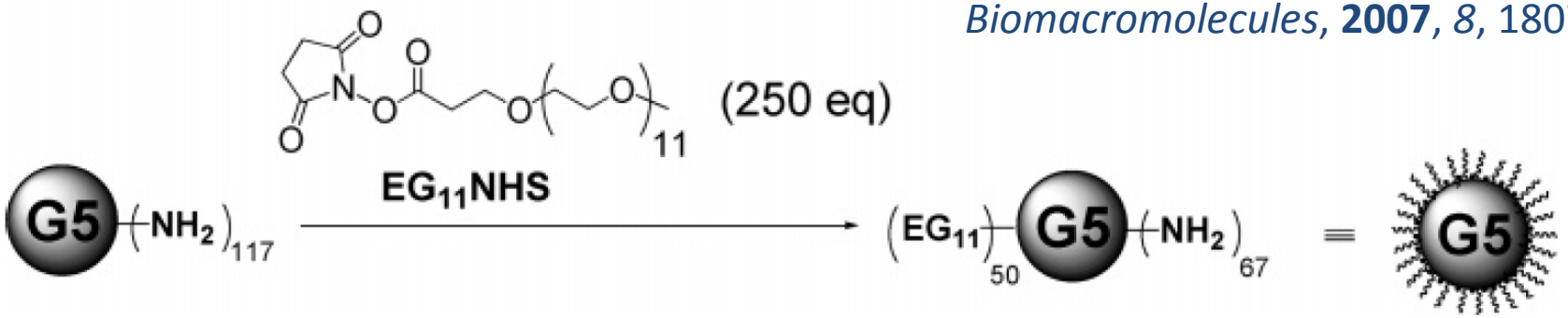
they have cytotoxicities against eukaryotic cells due to cationic nature.

Can we reduce the positive charge on surface ??

To Overcome The Drawbacks

- Partially PEGylated PAMAM dendrimer

Cai, C. *et al.*
Biomacromolecules, **2007**, *8*, 1807.



***ca.* 43% of amines PEGylated**

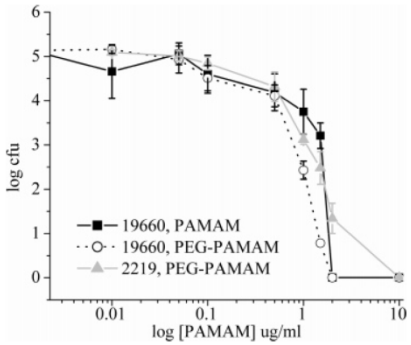


Figure 3. Concentration (cfu/mL) of PA, including PA19960 (lab strain) and PA2219 (clinical strain), upon incubation with PAMAM and PEG−PAMAM at various concentrations for 2 h. The data points are the mean of at least three separate experiments, and the error bar represents the standard deviation.

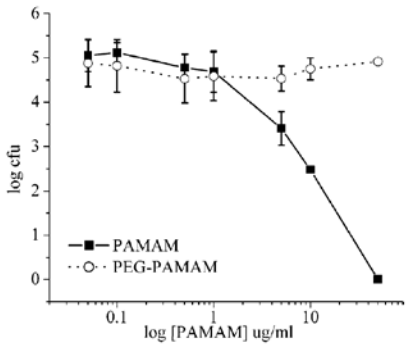


Figure 4. Concentration (cfu/mL) of bacteria SA ATCC 29213 upon incubation with G5 PAMAM and 43% PEG-coated PAMAM (PEG−PAMAM) at various concentrations for 2 h. The data points are the mean of at least three separate experiments, and the error bar represents the standard deviation.

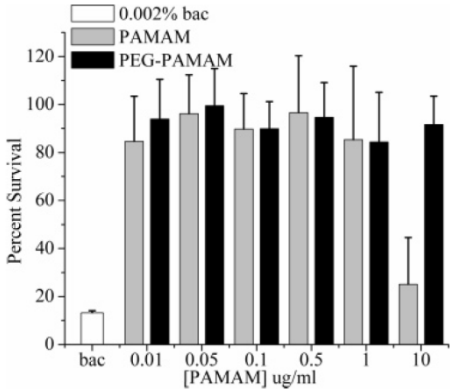
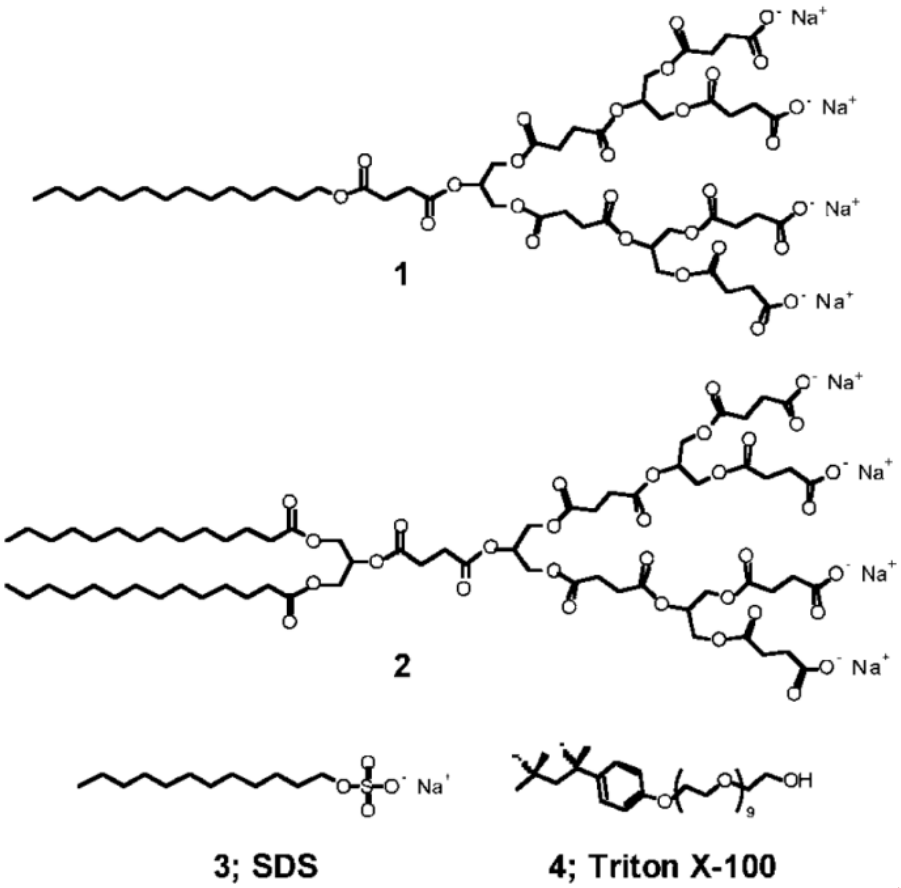


Figure 5. PAMAM cytotoxicity to HCECs measured by MTT survival assay with 0.002% benzalkonium chloride (bac) as the positive control. Percent survival of HCECs upon treatment with PAMAM and PEG−PAMAM at various concentrations is based on an untreated control. The data show the mean from two separate experiments with four replicates per condition, and the error bar represents a standard deviation.

Effective cytotoxicity against some species of bacteria without toxicity against eukaryotic cells.

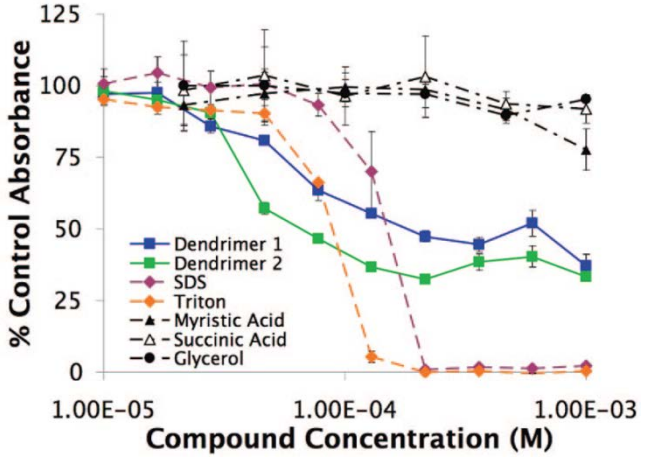
To Overcome The Drawbacks

- Anionic amphiphilic dendrimers

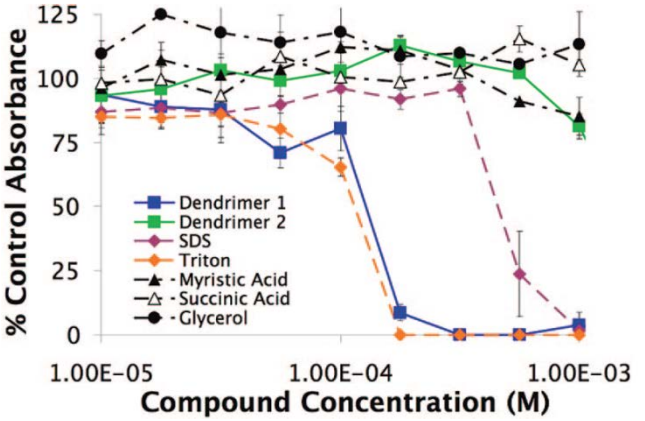


Grinstaff, M. W. *JACS* **2008**, *130*, 14444

Cytotoxicity against Gram-positive bacteria



Cytotoxicity against eukaryotic HUVEC cells



Selective cytotoxicity as antimicrobial

As Antiviral (Anti-HIV) - Anionic Dendrimers

Polyanions are known as anti-HIV by inhibiting gp120
(a glycoprotein on the surface of HIV envelope).
V3 loop in gp120 is a positively charged region.

- VivaGel (sulfonated polylysine dendrimer)

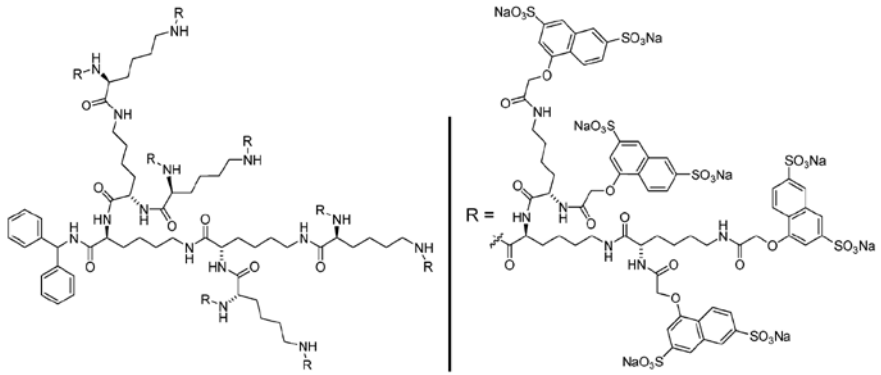
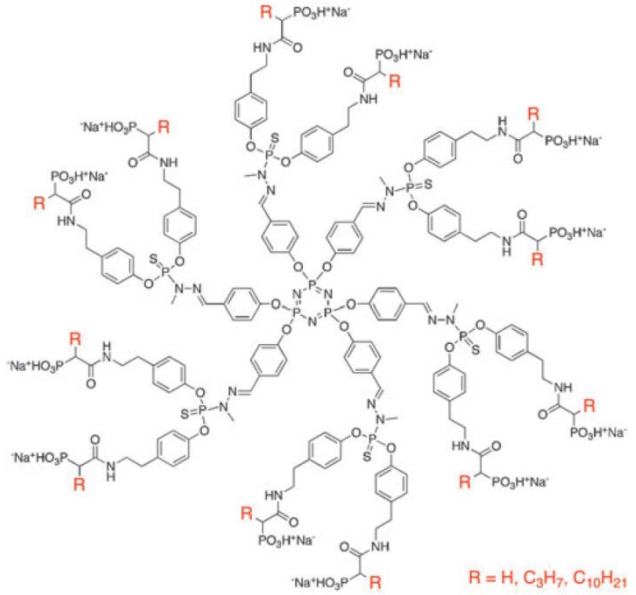


Figure 6. Chemical structure of SPL7013, the dendrimer antiviral in VivaGel.

McCarthy, T. D. *et al.*
Mol. Phrm. **2005**, *2*, 312

- Poly(phosphor-hydrazone) dendrimers with terminal phosphonic acid & alkyl chain



Blanzat, M.; Turrin, C.-O. *et al.*
Org. Biomol. Chem. **2009**, *7*, 3491

As Antiviral (Anti-HIV) - Mannose-Binding Dendrimer

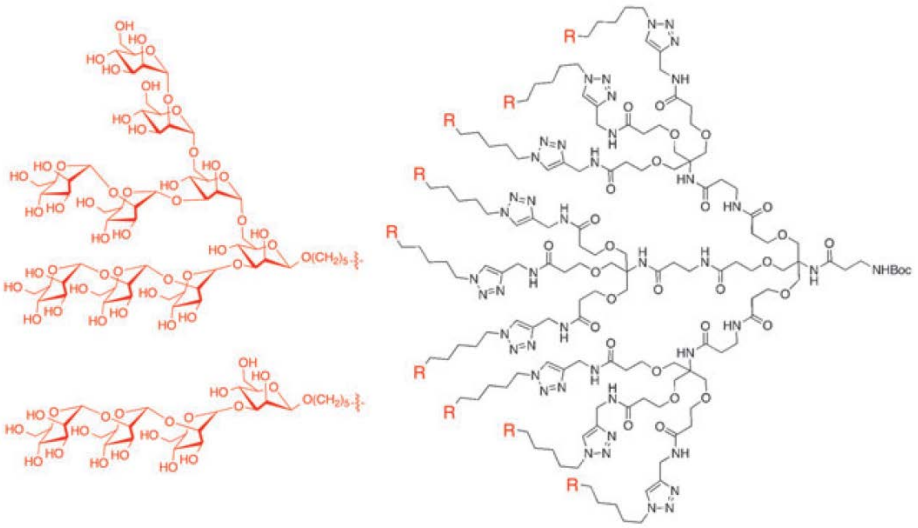


Fig. 5 Dendrimers synthesized with Man_4 and Man_9 groups on the periphery to function as HIV-1 inhibitors.

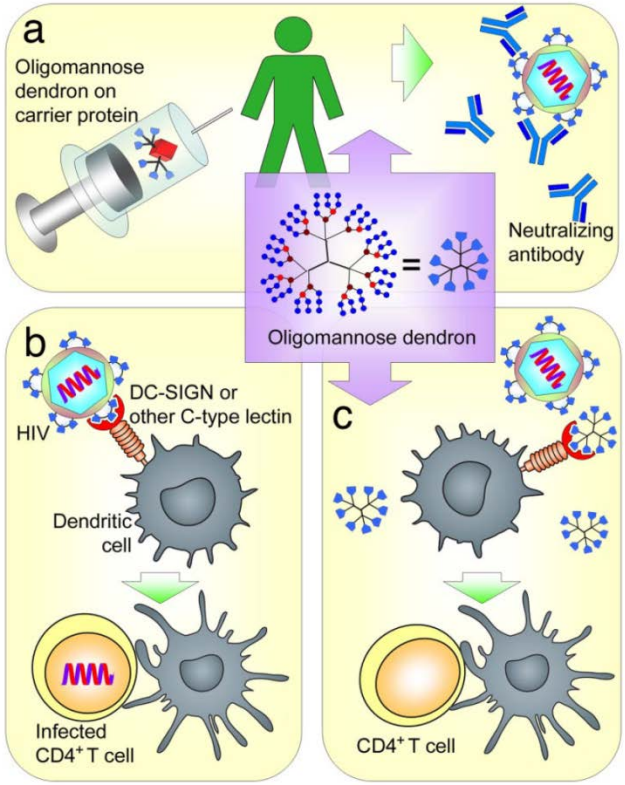


Fig. 2. Two strategies to target HIV-1 by oligomannose dendrons. (*a*) These glycodendrons can be conjugated to carrier proteins and serve as vaccines. (*b*) HIV-1 can bind dendritic cell-surface DC-SIGN or other mannose-binding proteins to enhance $CD4^+$ T cell infection. (*c*) Oligomannose dendrons can inhibit the binding of HIV-1 to dendritic cell-surface DC-SIGN or other mannose-binding proteins to prevent dendritic cell-enhanced $CD4^+$ T cell infection.

Inhibiting the interaction between HIV and dendric cells.

Wang, S.-K.; Liang, P.-H.; Wong, C.-H. *et al. PNAS* **2008**, *105*, 3690.

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1. How to Synthesize Dendrimers

2. Dendrimers as Drugs

3. Dendrimers as Drug Carriers

4. Perspectives

Advantages of Dendrimers as Drug Carriers

Chemotherapeutic drugs have some problems…

- Low solubitlity in water (due to hydrohobicity)
- Easily metabolized etc.

Dendrimers as one of the solutions…

- Solubilizing in water (Dendrimers' solubilities regulatable)
- Less filtered out of bloodstream (unable to exceed the renal threshold)
- Tumor selectivity (they uptake larger macromolecules)

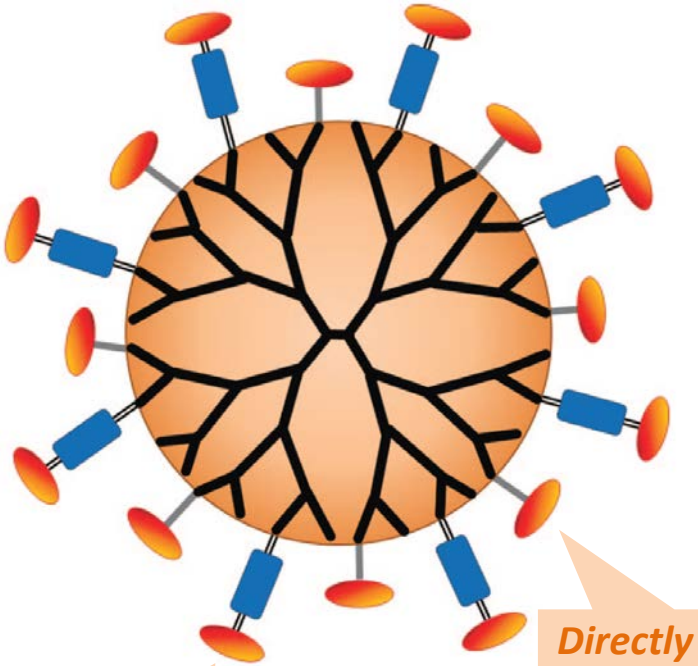
How Dendrimers Carry Drugs ??

Roughly devided…

Physical Encapsulation

Encapsulating in hydrophobic interior

Chemical Conjugation



Directly coupled

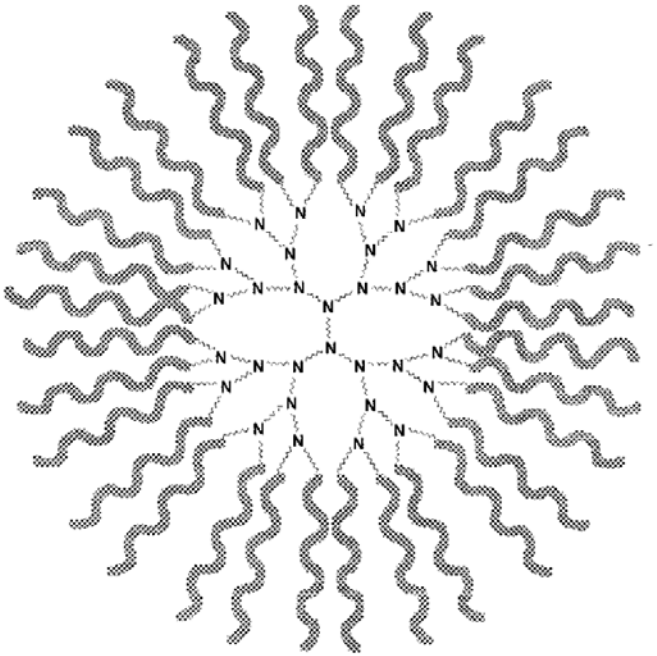
Coupled via linkage

Physical Encapsulation

Capture & release of drugs in dendrimers is effected by
hydrophobic forces, hydrogen bonding steric hinderance, electrostatic interactions

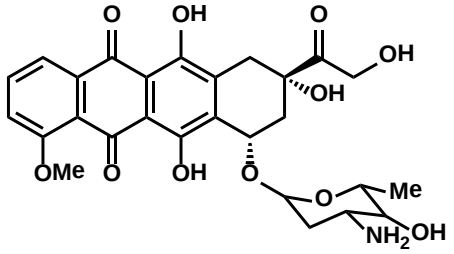
Several dendrimers are known to encapsulate drugs as guest molecules.

- DOX & MTX in PEGylated G3-NH_2 or G4-NH_2 PAMAM dendrimer

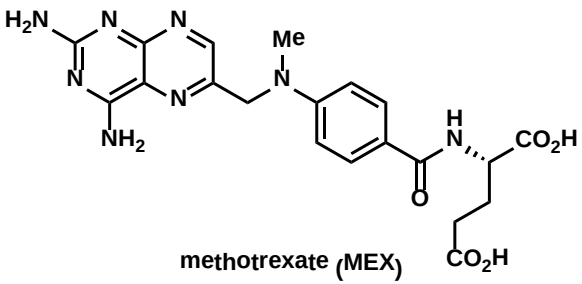


M-PEG-attached PAMAM dendrimer

Kono, K. *et al. Bioconj. Chem.* **2000**, *11*, 910.



doxorubicin (DOX)



methotrexate (MEX)

Release of Encapsulated Drugs

However, too rapid release of drugs from dendrimers is problematic.

Example:

Release of DOX from

PEGylated G4-PAMAM dendrimer

DOX was released 10 times faster in isotonic buffer than in non-isotonic buffer.

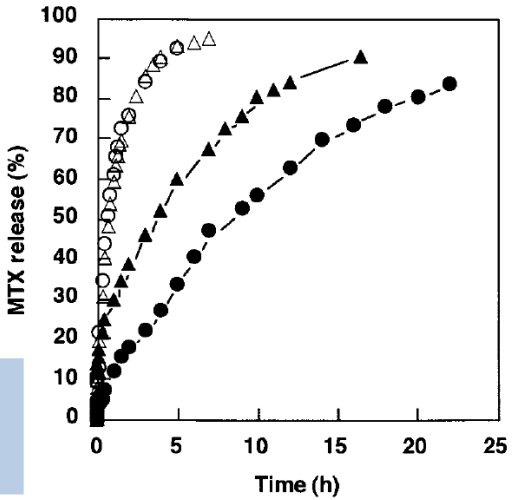


Figure 8. Release of MTX from the M-PEG(2000)-attached G4 dendrimer. The MTX-loaded M-PEG(2000)-G4 dendrimer (○, ●) or free MTX (△, ▲) dissolved in 1 mM Tris-HCl-buffered solution (pH 7.4) containing (open symbols) or not containing (closed symbols) 150 mM NaCl and dialyzed against the same solution. The time course of MTX concentration in the outer phase during the dialysis was shown in the figure.

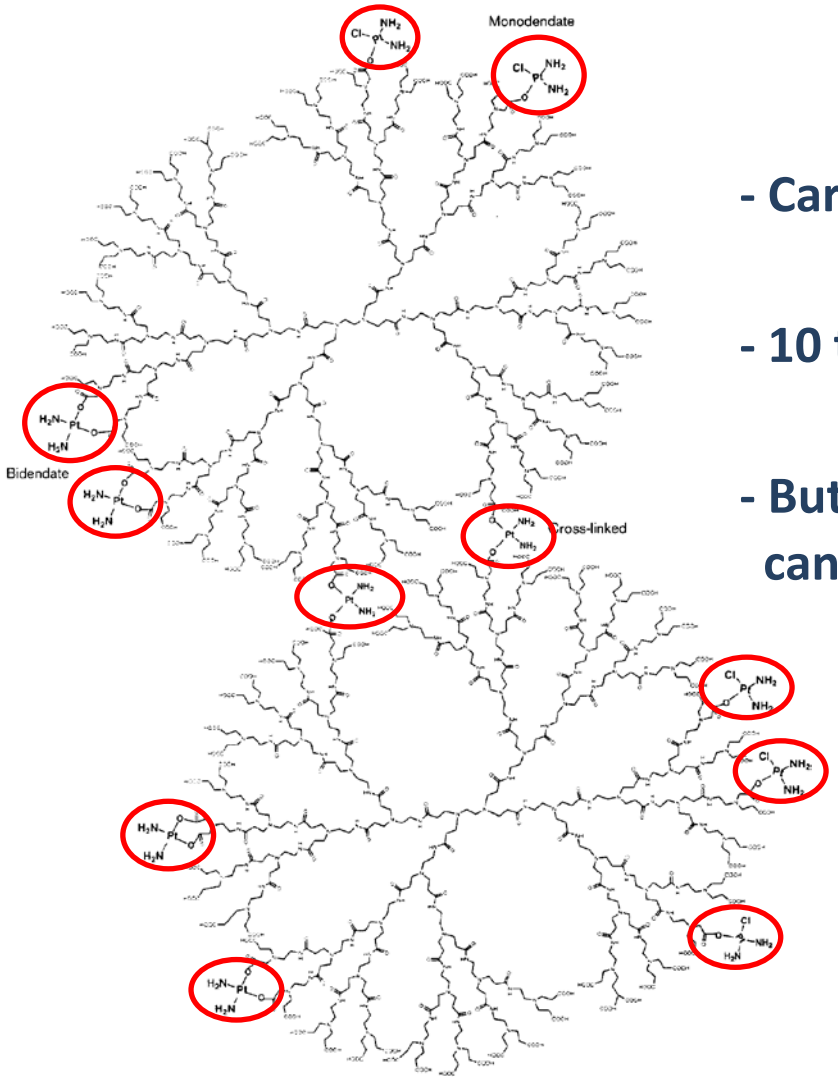
Kono, K. *et al. Bioconj. Chem.* **2000**, *11*, 910.

These premature drug release will cause non-selective toxicity *in vivo*.

Chemical Conjugation via Direct Coupling

Several conjugates between dendrimer and drug molecule have been reported…

- G3.5 PAMAM-cisplatin conjugate



- Carrying 20-25 wt% Pt

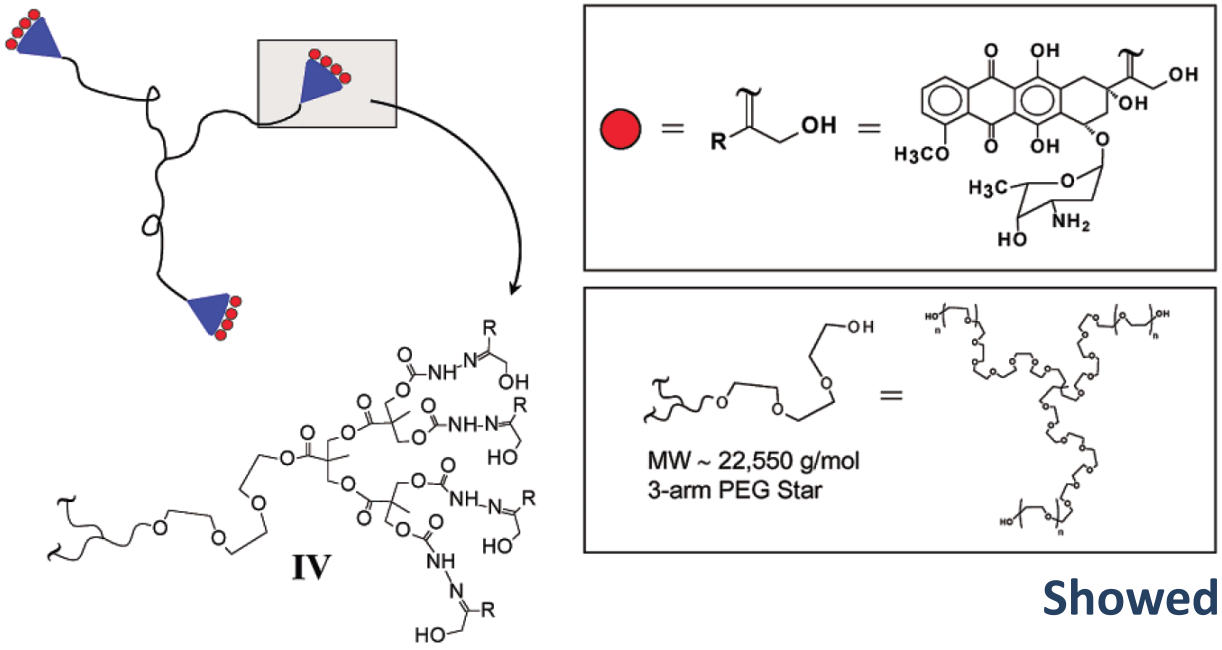
- 10 times aqueous solubility

- But <u>almost no toxicity</u> toward cancer cells (<u><1% Pt release</u>)

Duncan, R *et al.*
Anticancer Drugs **1999**, *10*, 767.

Chemical Conjugation via Direct Coupling

- Polyester dendrimer-DOX conjugate



Showed decreased activity compared to free DOX

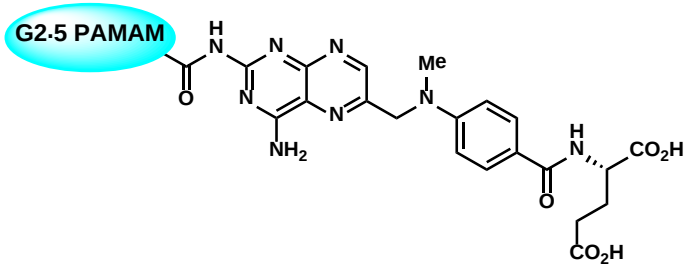
Frechet, J. M. J.; Szoka, F. C. Jr. *et al. Biocoj. Chem.* **2002**, *13*, 453.

Both *in vivo* and *in vitro* cancer activity are decreased due to limited release of the loaded drug.

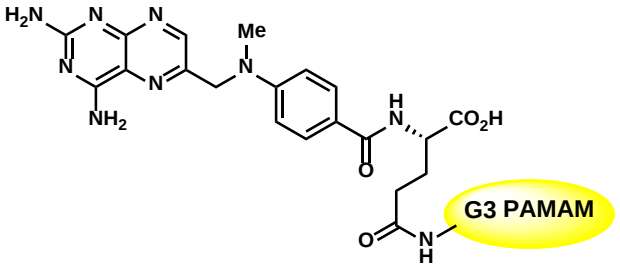
Conformation of Drugs on Dendrimer's Surface

Conformation of drug molecules on dendrimer's surface is critical to activity.

- **G2.5 or G3 PAMAM-MTX conjugate**



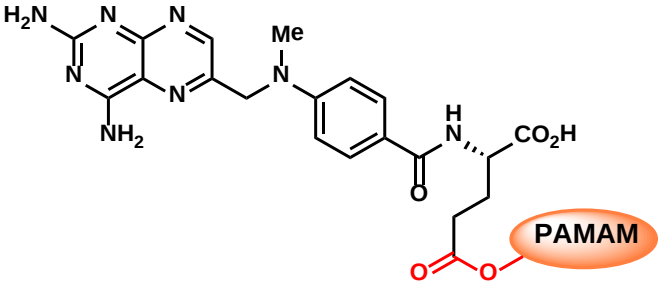
G2.5 PAMAM-MTX conjugate
3-fold more active

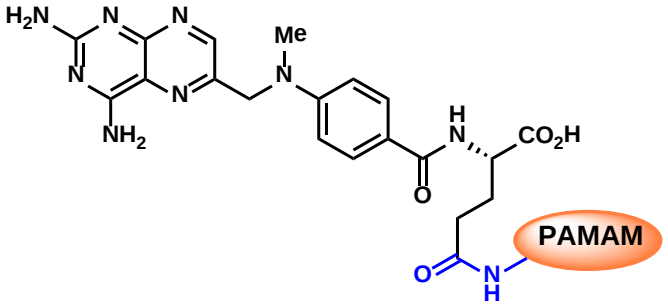


G3 PAMAM-MTX conjugate
10-fold less active

Kannan, R. M. *et al. Bioconjugate Chem.* **2006**, *17*, 275.

- **G5 PAMAM-NH_2 or -OH -MTX conjugate**





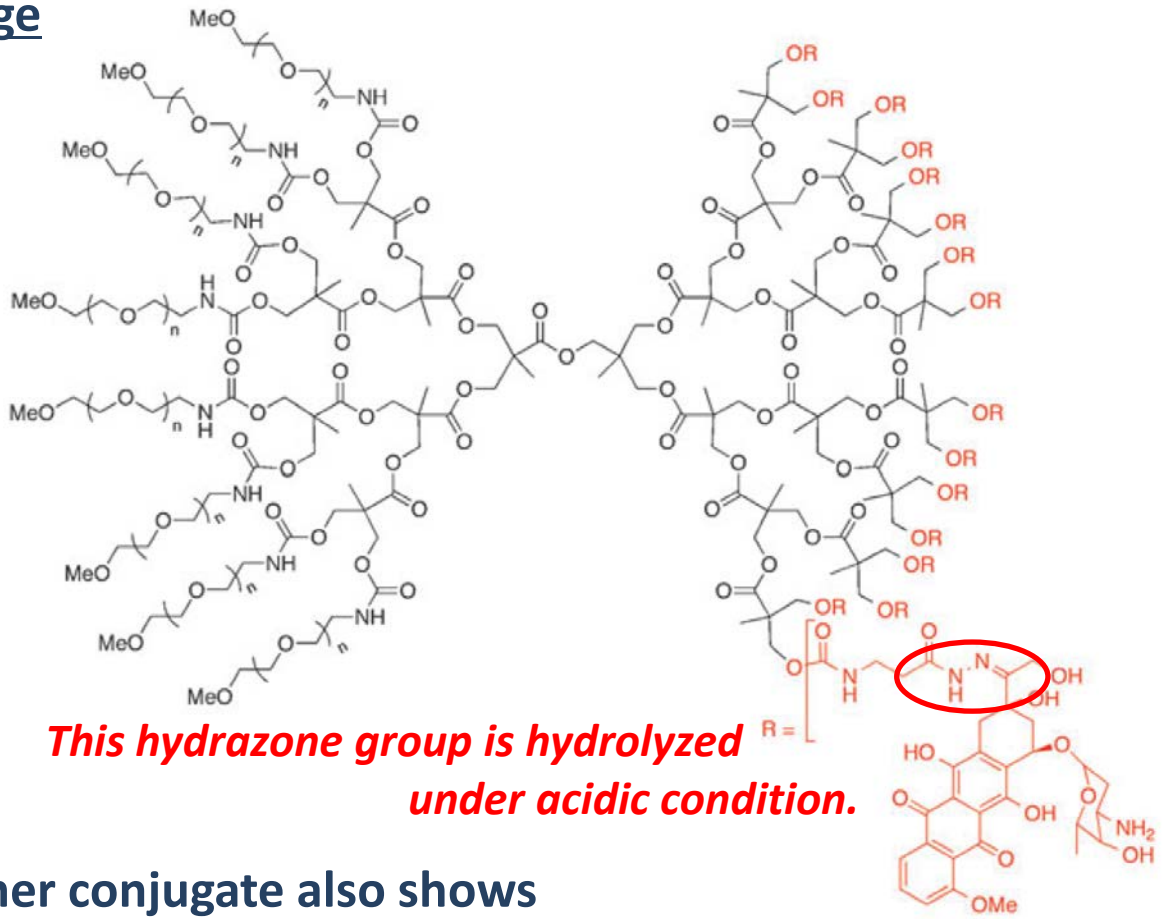
10-fold lower IC_{50}

Baker, J. R. Jr. *et al. Pharm. Res.* **2002**, *19*, 1310.

Chemical Conjugation via Linkages

Releasing drugs near the target is ideal…

pH-Sensitive hydrazone-linkage



at pH 7.4 : <10% release

at pH 5.0 : 100% release

This hydrazone group is hydrolyzed under acidic condition.

Hydrazone-linked DOX-dendrimer conjugate also shows improving activity in *in vivo* experiment.

Rihova, B. *et al. Controlled Release* **2001**, *74*, 225.

Next Challenge ??

Dendrimers as drug carriers work depending on pH environment.

Can we apply to more specific environment ??

such as cancer-specific intracellular enzymes…

Today's Contents

0. Prologue

1. How to Synthesize Dendrimers

2. Dendrimers as Drugs

3. Dendrimers as Drug Carriers

4. Perspectives

Perspectives

Dendrimers have potential to be prominent materials for medical application.

But, still not reach the success of linear polymers (I think) due to synthetic difficulties.

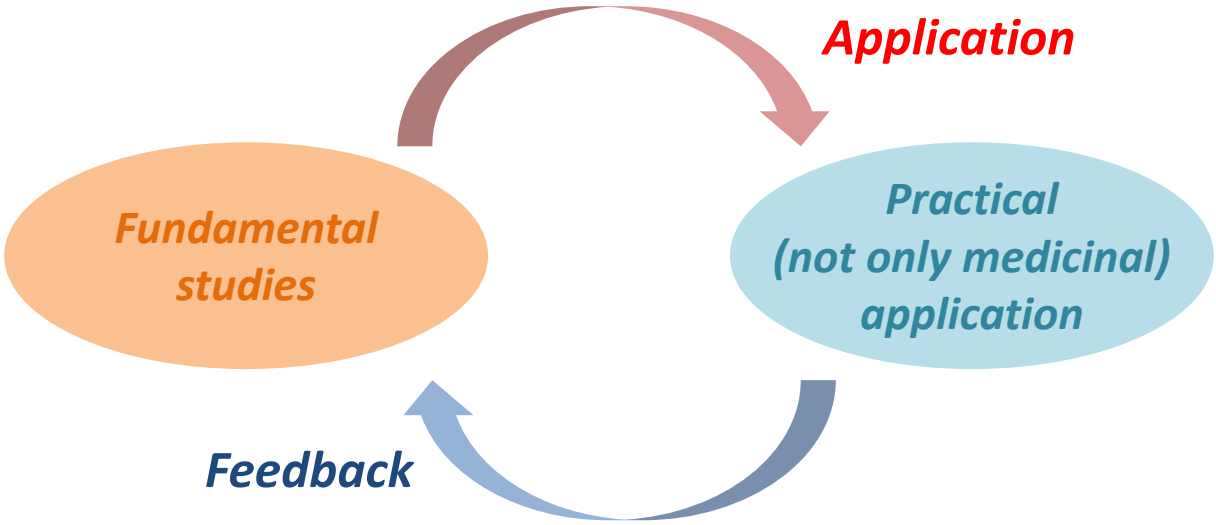
More fundamental, detailed studies are necessary…

Perspectives

Further studies about…

synthesis, physical properties, structural activity relationship

Correlative fundamental and application studies are necessary in this new, undeveloped area.



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- Turrin, C.-O. *et al. New J. Chem.* **2009**, *33*, 1809.

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- Grinstaff, M. W. *et al. Chem. Soc. Rev.* **2011**, *40*, 173.