Engineering Dendrimers for Biological Applications

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Outline

I: Dendrimer history, chemistry and structure

II: Properties of dendrimers in biological systems

III: Dendrimer host-guest complexes

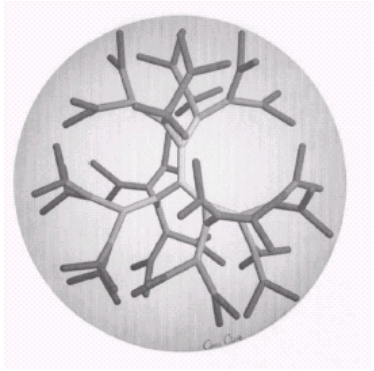
IV: Biological applications of dendrimers

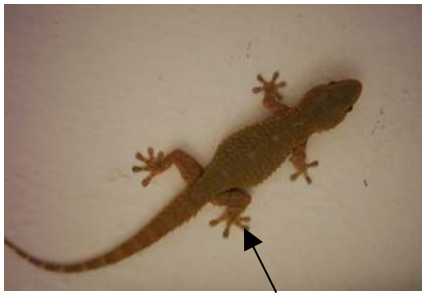
"Once considered little more than curiosities, these treelike molecules bear fruit with pharmaceutical, biotech, and polymer science applications."

(C&E News Cover Story, June 13, 2005)

History of Dendrimers

- Dendrimers are hyperbranched and symmetrical structures that are found widely in nature (i.e., tree branching, lightning patterns, snow flakes, bottom of Gecho's feet, etc.)
- Class of polymeric materials first reported by Vögtle[1] and others in the late 1970s and early 1980s (originally named 'cascade' molecules)
- Large molecular weights and sizes, yet dendrimers are well-defined structures with a low polydispersity in comparison with linear polymers
- Dendritic branching results in semi-globular to globular 3D structures that resemble biomolecules (i.e., proteins)

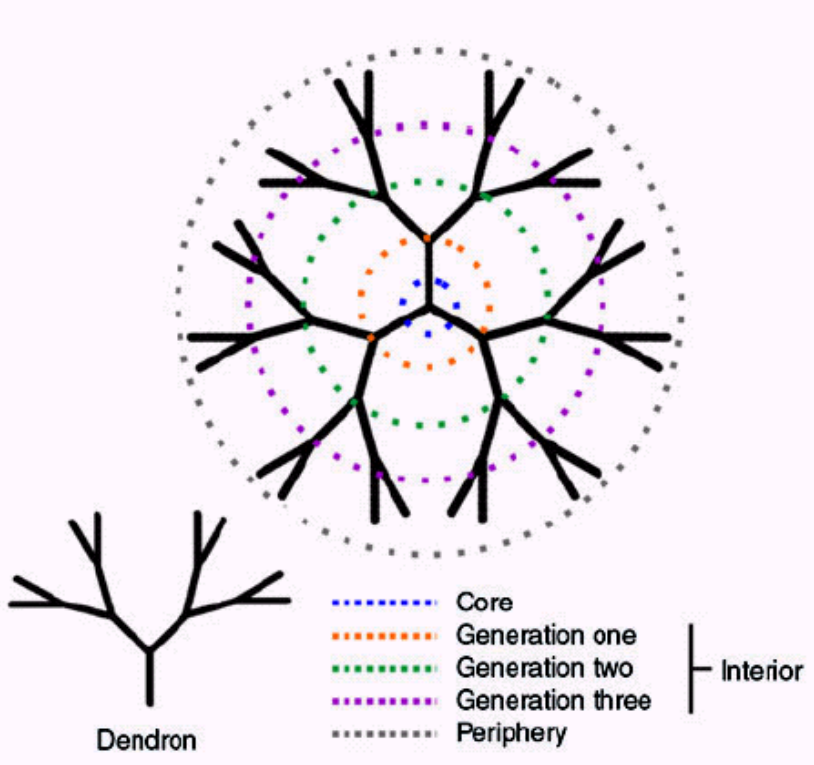


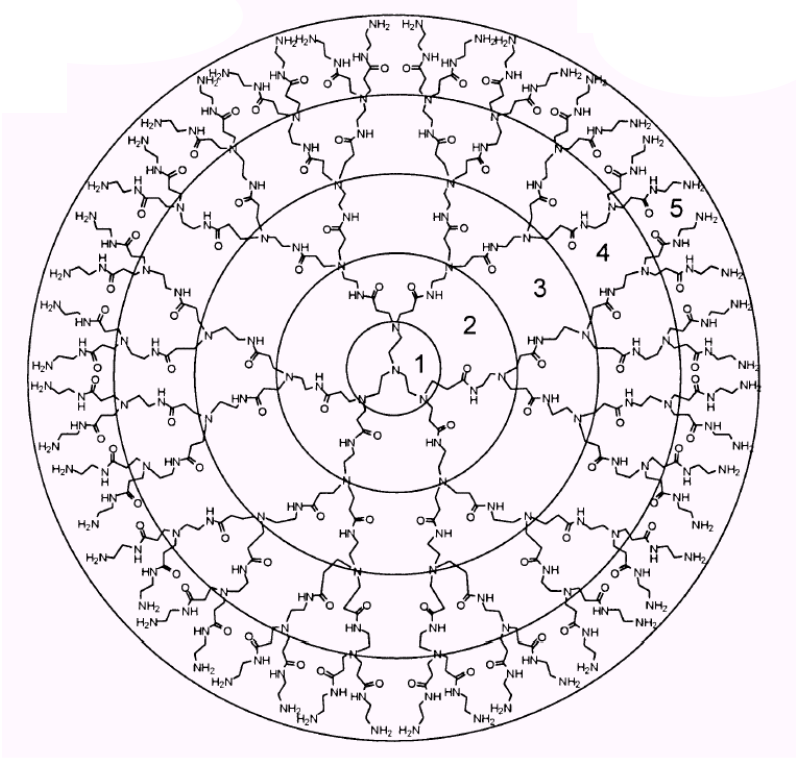


1. Vögtle et al. *Synthesis* **1978**, 155-158.

Dendrimer Nomenclature

- Structures are characterized by 'layers' between each focal point called generations (G)
  - generations are defined by the number of focal points (cascade points) from core

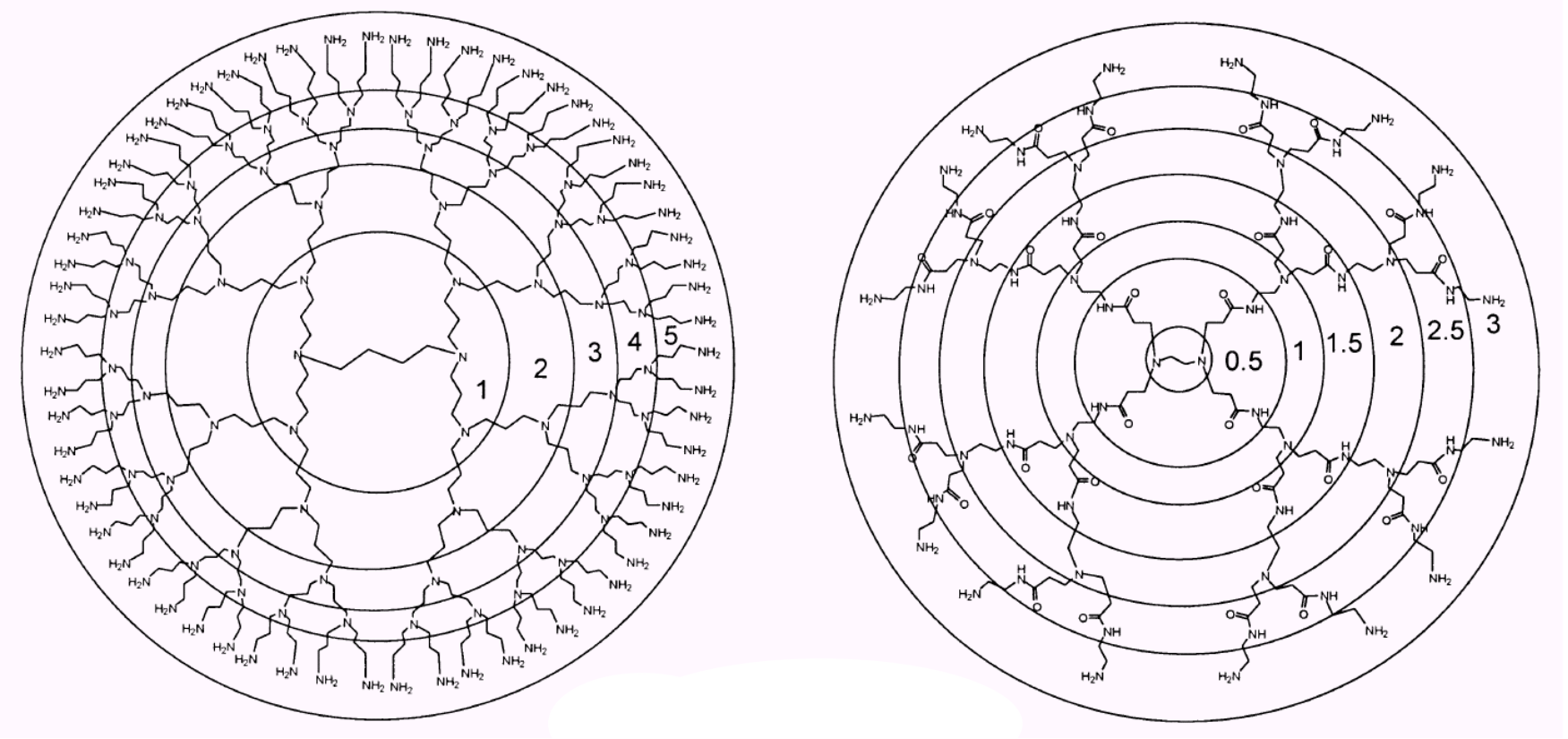




Poly(amidoamine) PAMAM (Starburst™) (G5)

Lee, et al. *Nature Biotechnology* **2005**, *23*, 1517

Full and half-generation dendrimers



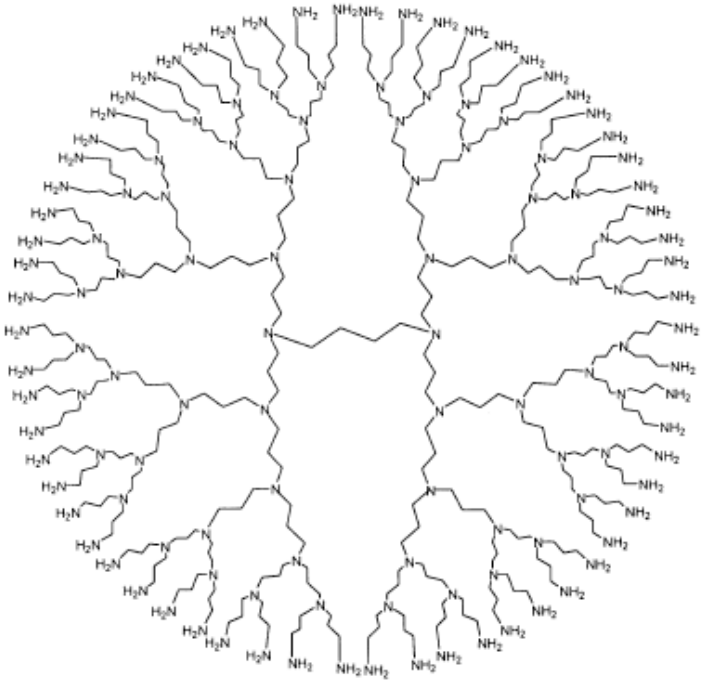
Poly(propyleneimine) (PPI) (G5)

Poly(amidoamine) PAMAM (G3)

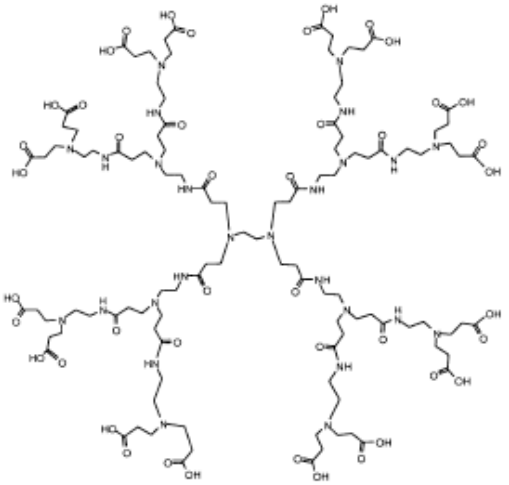
Chem. Soc. Rev. **2004**, *33*, 43-63

Depicting dendrimers in a simplified manner

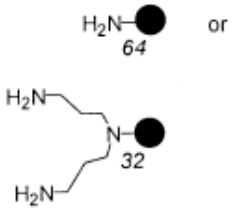
- 'Full picture' drawings of dendrimers can become crowded and confusing



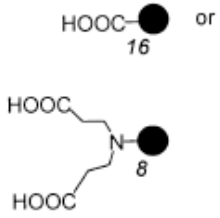
G5-PPI



G2.5-PAMAM



pincers & core



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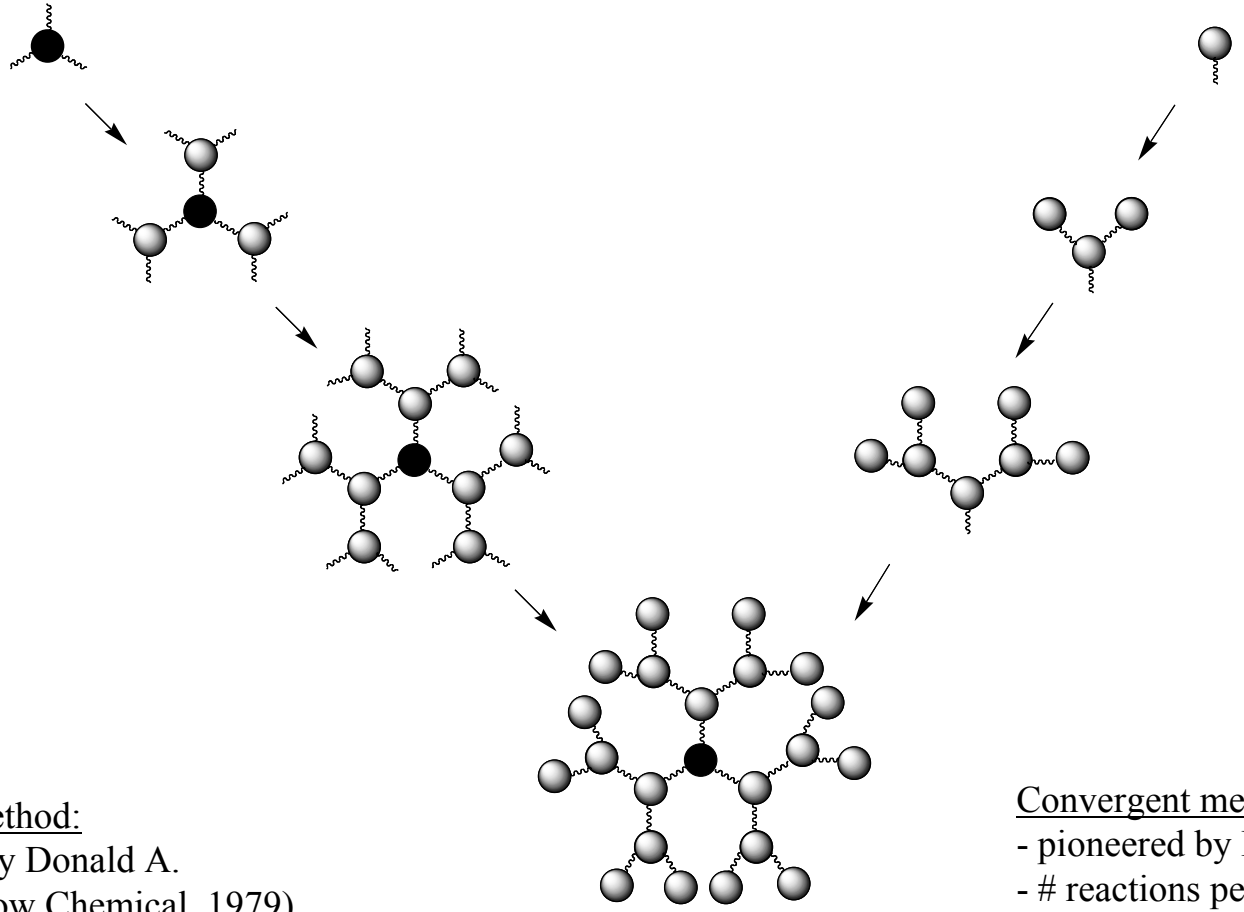
Dendrimer growth by divergent and convergent methods

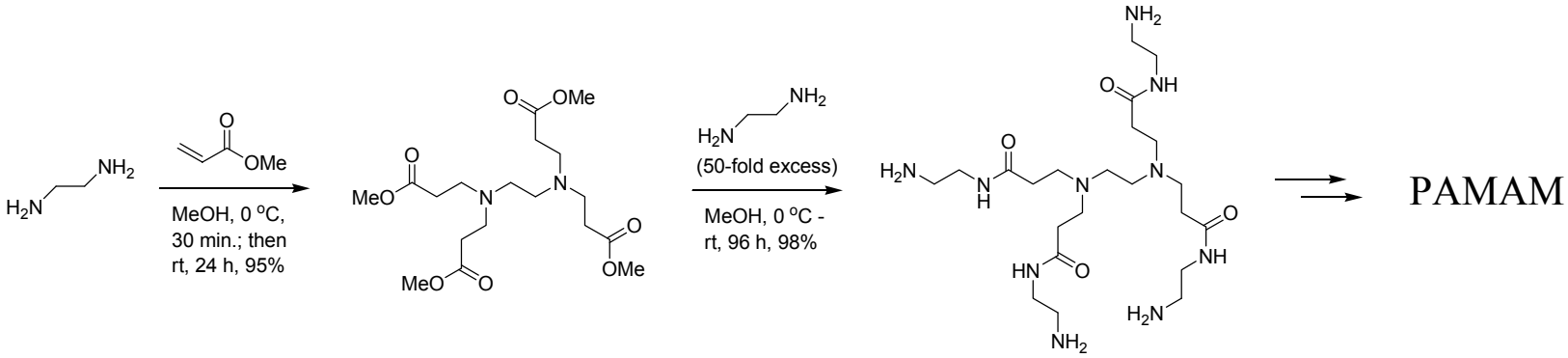


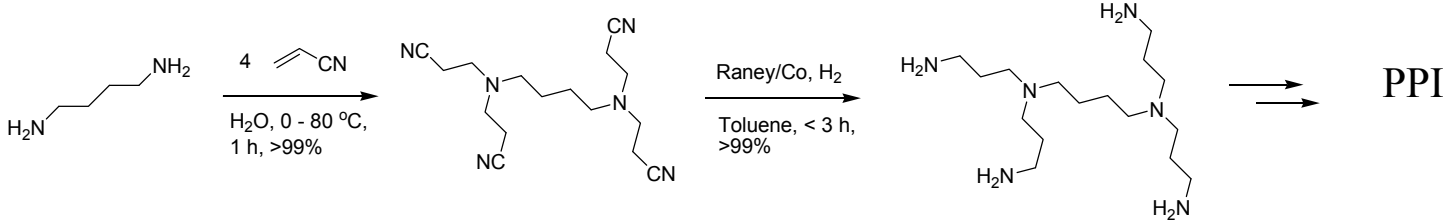
Divergent method:
- pioneered by Donald A. Tamalia (Dow Chemical, 1979)
- # reactions per growth step increases
- slight structural defects in large dendrimers

Convergent method:
- pioneered by Hawker & Fréchet
- # reactions per growth step remains constant
- defects easily separated

Tomalia, D. A. and Fréchet, J. M. Dendrimers and other Dendritic Polymers (2001)

Synthesis of PAMAM and PPI dendrimers (divergent)



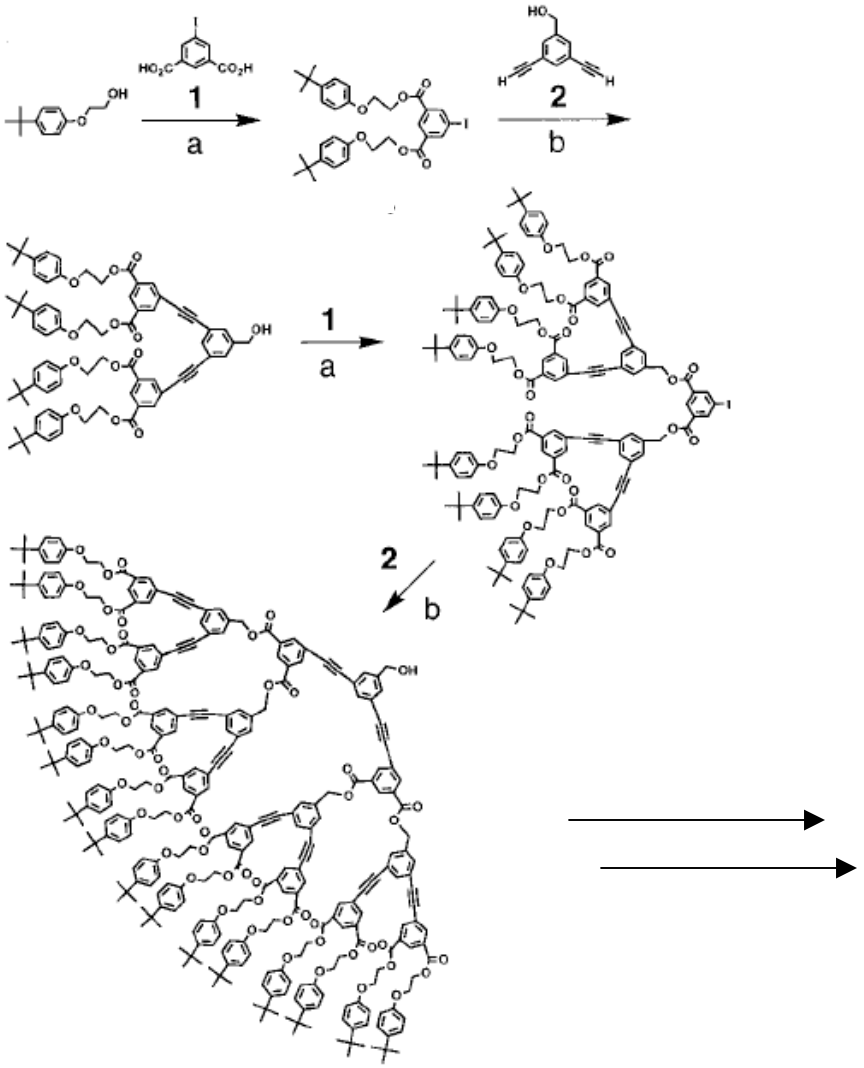


- both procedures used to prepare multi-kilogram scales of G = 0-12 dendrimers from cheap materials

Tomalia, D. A. and Fréchet, J. M. Dendrimers and other Dendritic Polymers (2001)

Orthogonal protection (convergent approach)

- Orthogonal coupling strategy (an 'outside-in' approach)



Reaction Conditions: a) PPh_3, DEAD, THF, 80%;
b) $Pd(PPh_3)_2Cl_2$, CuI, $PhCH_3$, 78-82%

Zeng, et al. *J. Am. Chem. Soc.* **1996**, *118*, 5326-5327

Commercially available dendrimers

- Various PAMAM dendrimers are available from Aldrich
  - Manufactured and licensed by Dendritech, Inc. (www.dendritech.com) and Dendritic NanoTechnologies, Inc. (www.dnanotech.com)

- COST???

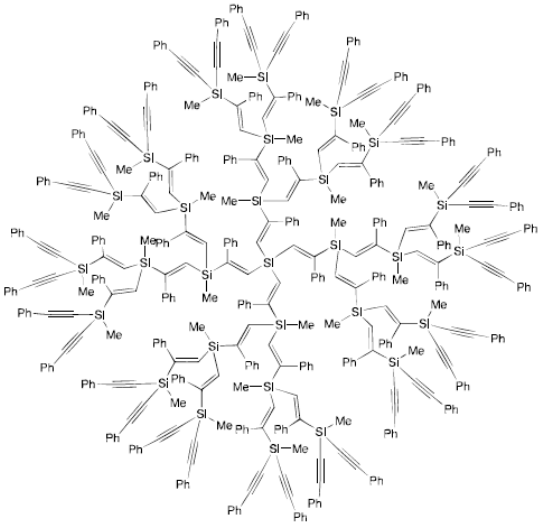
  - PAMAM-amidoethylethanolamine dendrimer, 1,4-diaminobutane core (G2) - $191.00 for 2g or $636.00 for 10g
  - PAMAM-amidoethylethanolamine dendrimer, 1,4-diaminobutane core (G6) - $484.50 for 2g

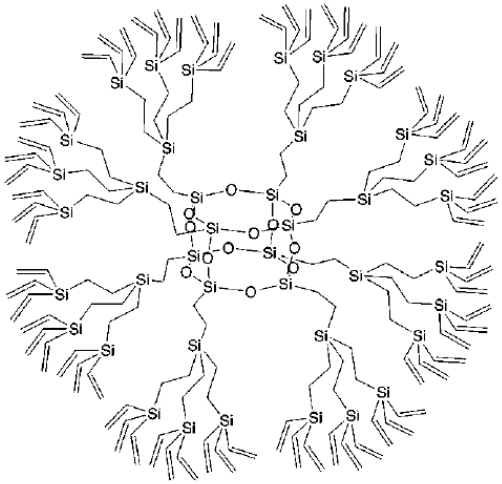
  *available as 10 wt% soln. in MeOH, but can purchase as solids directly from company

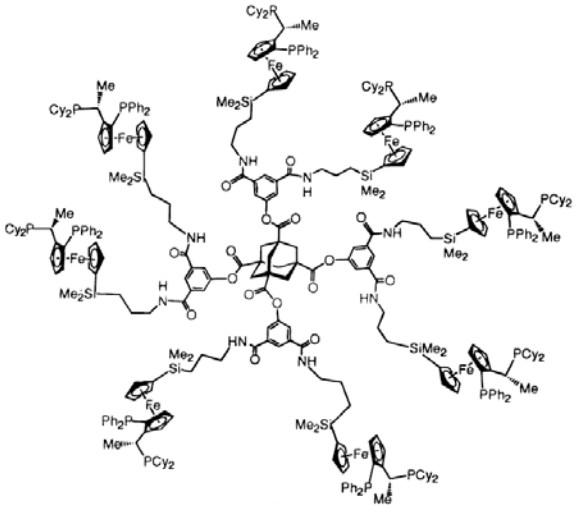
- Commercialized dendrimers:

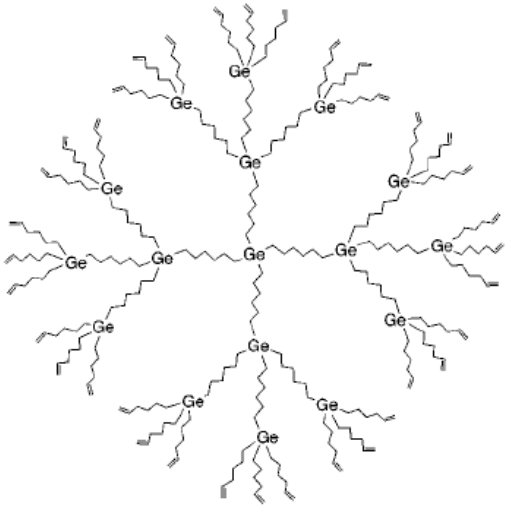
PRODUCT	PURPOSE	COMPANY
VivaGel™	Prevention of HIV	Starpharma
Stratus® CS	Cardiac marker diagnostic	Dade Behring
SuperFect®	Gene transfection	Qiagen
Alert Ticket™	Anthrax detection	U.S. Army Research Laboratory

Dendrimers with various designs and elements









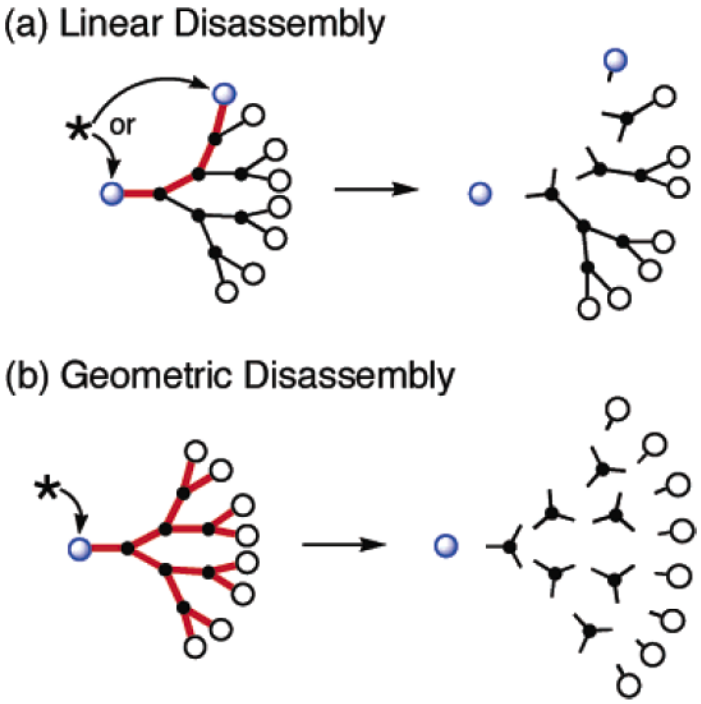
Togni, et al. *J. Am. Chem. Soc.*. **1998**, *120*, 10274

Majoral and Caminade. *Chem. Rev.* **1999**, *99*, 845

Dendrimer Disassembly

- Process that relies on a single triggering event to initiate multiple cleavages throughout a dendritic structure that result in release of individual subunits or larger dendrimer fragments

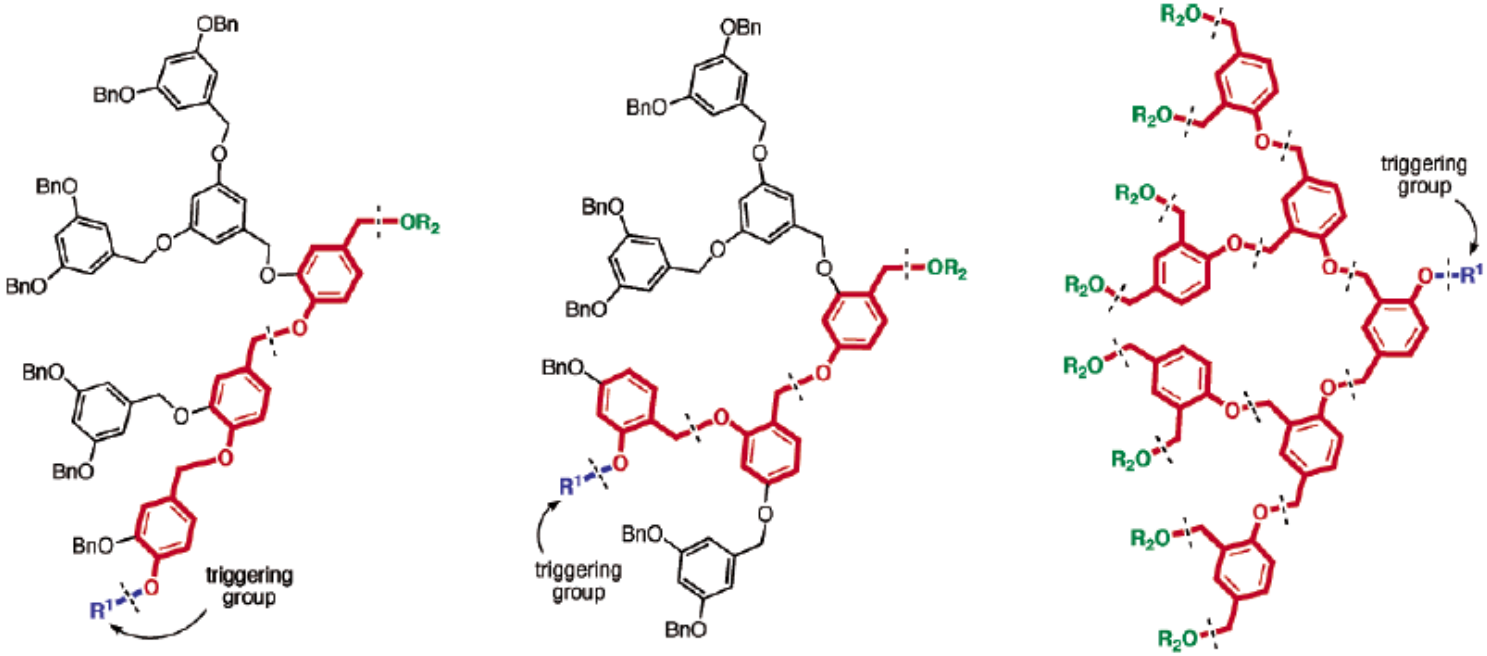
  - can design the trigger to respond to a chemical or physical stimulus
  - primarily designed for drug delivery systems



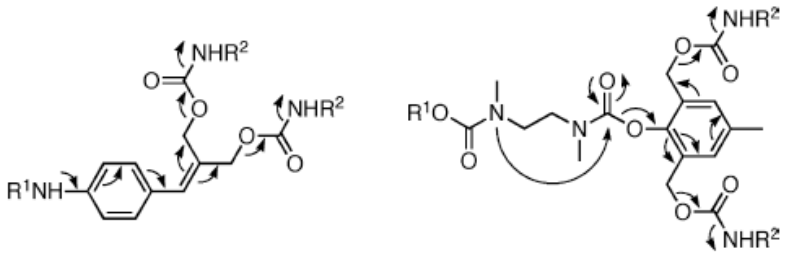
McGrath, et al. *Molecular Pharmaceutics* **2005**, *2*, 253-263.

Dendrimer Disassembly

- Dendrons capable of linear para, linear ortho and geometric disassembly

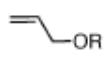
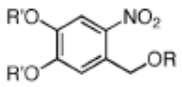
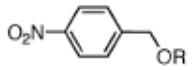
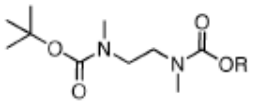
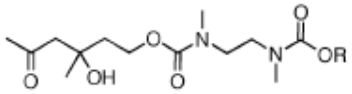
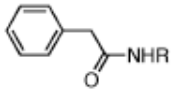


- Other geometric disassembly pathways



Dendrimer Disassembly

- Reported disassembly triggering groups and conditions

Allyl (Pd^0/BH_4^-):  Szalai, Kevwitch, McGrath *J. Am. Chem. Soc.* **2003**, *125*, 15688; Li, Szalai, Kevwitch, McGrath, *J. Am. Chem. Soc.* **2003**, *125*, 10516.	***o*-Nitrobenzyl (*hν*):**  Amir, Pessah, Shamis, Shabat, *Angew. Chem. Int. Ed.*, **2003**, *42*, 4494; Szalai, McGrath *Tetrahedron* **2004**, *60*, 7261.	***p*-Aminobenzyl (Zn^0/AcOH):**  de Groot, Albrecht, Koekkoek, Beusker, Scheeren, *Angew. Chem. Int. Ed.*, **2003**, *42*, 4490.
fBOC (1. TFA; 2. TEA):  Amir, Pessah, Shamis, Shabat, *Angew. Chem. Int. Ed.*, **2003**, *42*, 4494.	**Retro-aldol/retro-Michael (cat. antibody 38C2):**  Amir, Pessah, Shamis, Shabat, *J. Am. Chem. Soc.* **2004**, *126*, 1726.	**Phenylacetamide (penicillin-G-amidase):**  Amir, Shabat *Chem. Commun.* **2004**, 1614.

McGrath, et al. *Molecular Pharmaceutics* **2005**, *2*, 253-263.

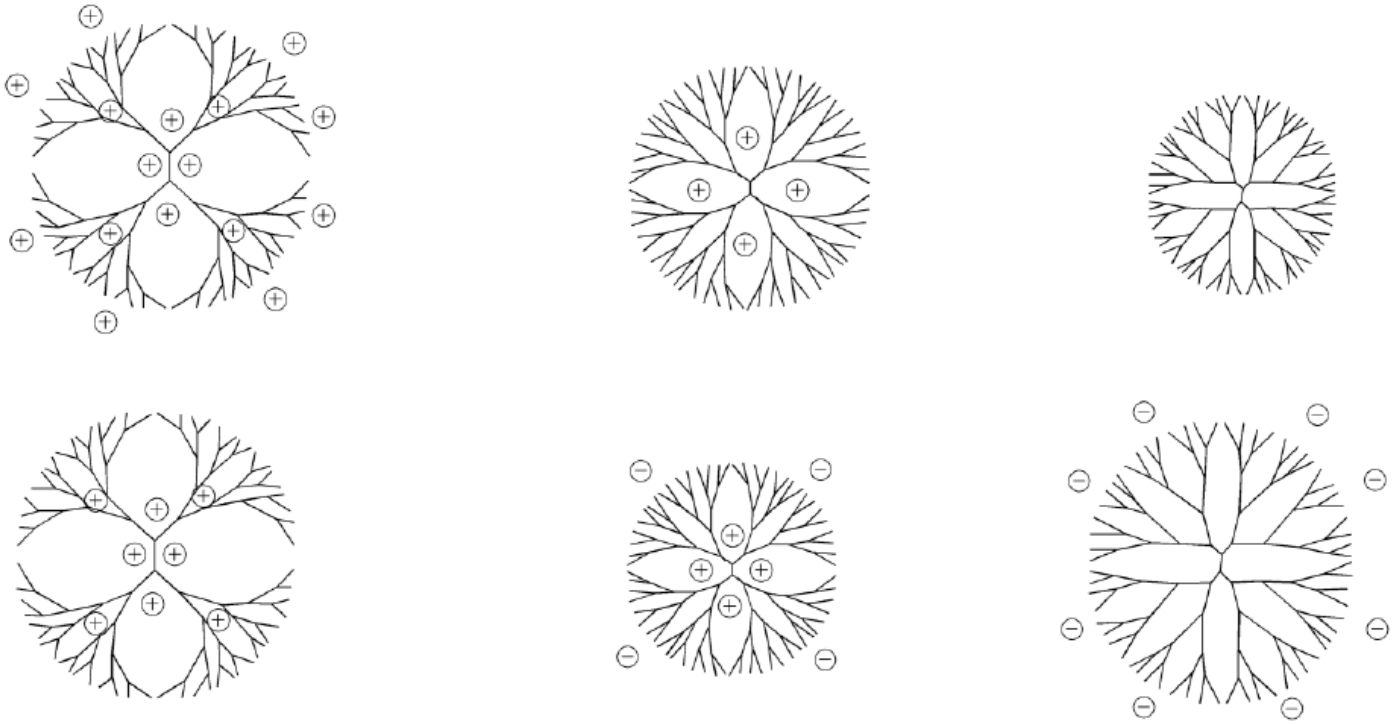
Part II

Properties of Dendrimers in Biological Systems

Physiochemical Properties of Dendrimers

- Dendrimers can be three-dimensional structures that are similar to proteins, though consisting of more covalent bonds
- Dendrimers can adopt 'native' (more tight) or 'denatured' (extended) conformations based on solvent pH

Amino-terminated PAMAM



Carboxy-terminated PPI

Increasing pH

Chem. Soc. Rev. **2004**, *33*, 43-63

Biocompatibility of Dendrimers

- Accumulation of low MW polymers (i.e., dendrimers) is not usually a problem due to excretion in the urine or in feces after metabolism

- Injected polymers of high MW are not easily excreted if they cannot be degraded or filtered via the kidneys

- To be useful in biological systems, dendrimers must be:

  - non-toxic
  - non-immunogenic (in not required - i.e., for vaccines)
  - able to cross biobarriers (i.e., intestine, blood-tissue barriers, cell membranes)
  - able to stay in circulation for the time needed to have a clinical effect
  - able to target specific structures

In vitro Toxicity of Dendrimers

- Dendrimers with cationic surface groups (i.e., amino-terminated dendrimers):

  - cause destabilization of the cell membranes and result in cell lysis
  - higher generations are more cytotoxic
  - amino-terminated dendrimers are less toxic than the more flexible linear amino functionalized polymers
  - dendrimers containing primary amines are more toxic than those with secondary and tertiary groups

- Dendrimers with anionic surface groups (i.e., carboxyl terminated)

  - anionic PAMAMs are slightly less toxic than cationic PAMAMs
  - lower generations show neither haematotoxicity or cytotoxicity at concentrations of 2 mg/ml
  - poly(aryl ether) dendrimers with anionic carboxyl groups are haemolytic to solutions of rat blood cells after 24 hours

- Surface derivation?

  - protection of PAMAM surface amines with PEGs or fatty acids reduces cytotoxicity (Caco-2 cells reduced from IC_{50} ~ 0.13 mM to >1 mM)

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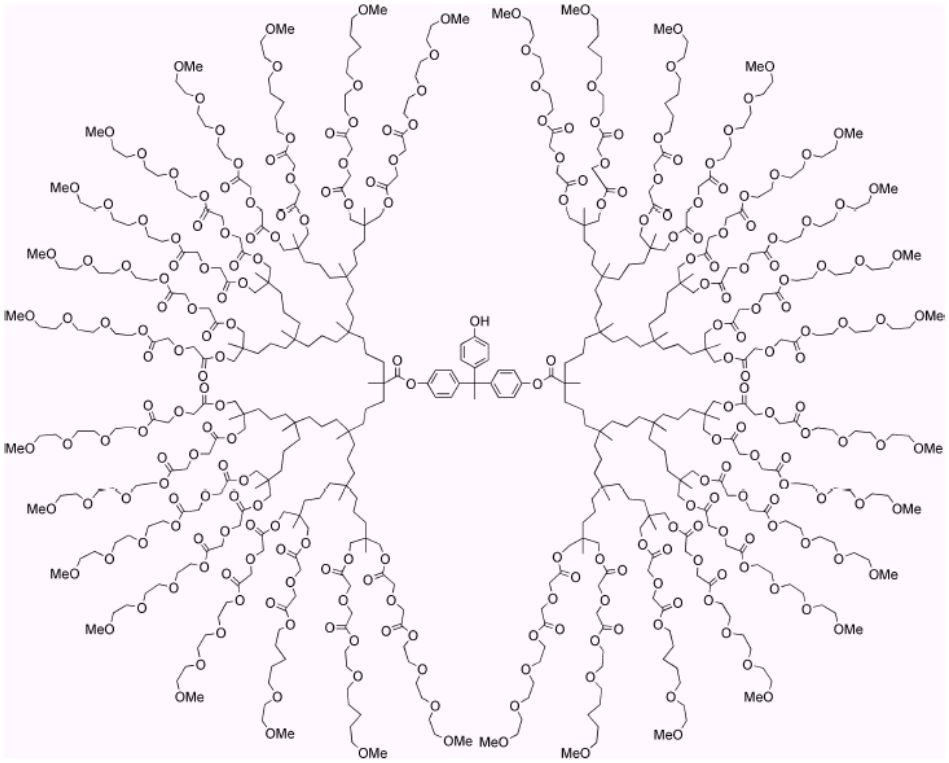
In vivo Toxicity of Dendrimers

- PAMAM dendrimers (up to G5) do not appear to be toxic when injected into mice at conc. of 10 mg/kg (modified or unmodified surface)

- Hydroxy- and methoxy-terminated polyester dendrimers (below) were not toxic *in vivo*
  - no acute or long-term toxicity when injected into mice



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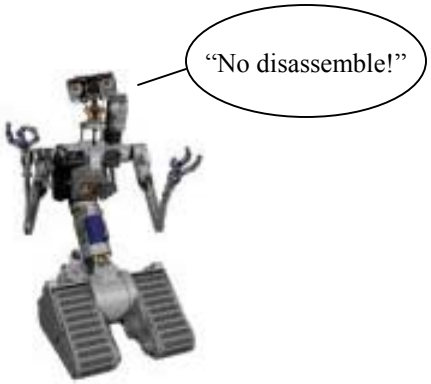
Degradation of Dendrimers?

- Large non-biodegradable dendrimers can cause bioaccumulation and possible toxic effects

- UV light decomposition is hampered by tissue permeability
  - lower frequency irradiation or X-rays???

- PAMAM and PPI dendrimers are difficult to degrade!!!

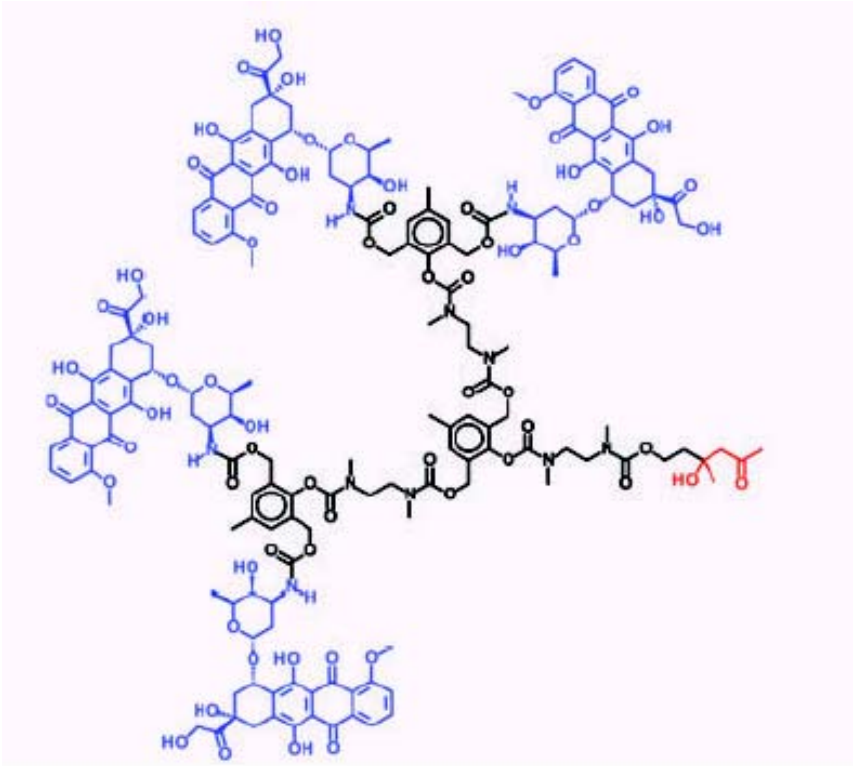


- Polyester dendrimers can be designed to produce nontoxic metabolites when hydrolyzed or degraded

- Disassembly by an *in vitro/ in vivo* chemical stimulus?

Degradation of dendrimers

- Degradation of β-hydroxy ketone (red) is initiated by a catalytic antibody, thereby releasing four equivalents of doxorubicin (blue)



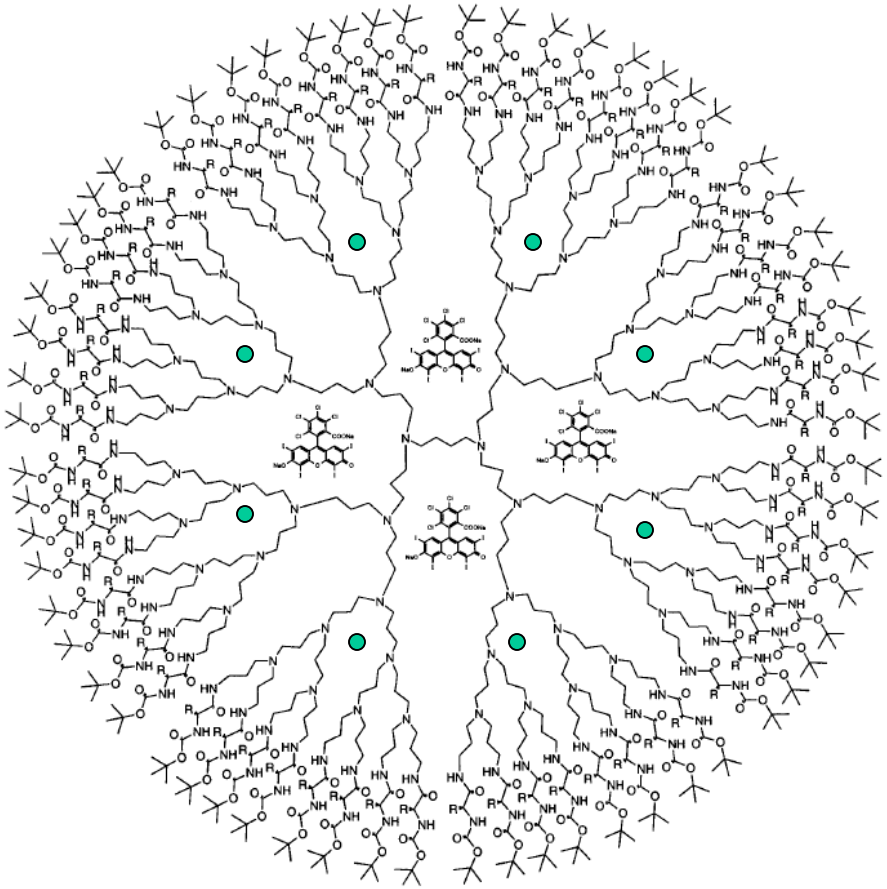
Lee, et al. *Nature Biotechnology* **2005**, *23*, 1517

Part III

Dendrimers as Host-Guest Complexes

Dendrimers as hosts

- PAMAM and PPI dendrimers serve as good hosts scaffolds due to their large size and surfaces
- Binding of hosts at core or cavities ('endo-receptor') or at outer shell ('exo-receptor')



R = Ph

'Dendritic box'

- Meijer's dendritic box with 4 Rose Bengal and 8-10 *p*-nitrobenzoic acid (●) molecules trapped inside

- cleavage of PGs with formic acid caused slow release of acid molecules

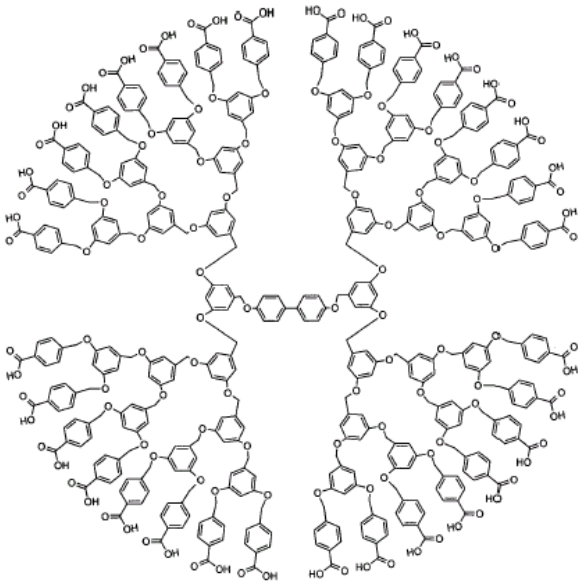
- reflux in 12 N HCl for 2 h to release Rose Bengal

Meijer, et al. *J. Am. Chem. Soc.* **1995**, *117*, 4417

Dendrimers as hosts

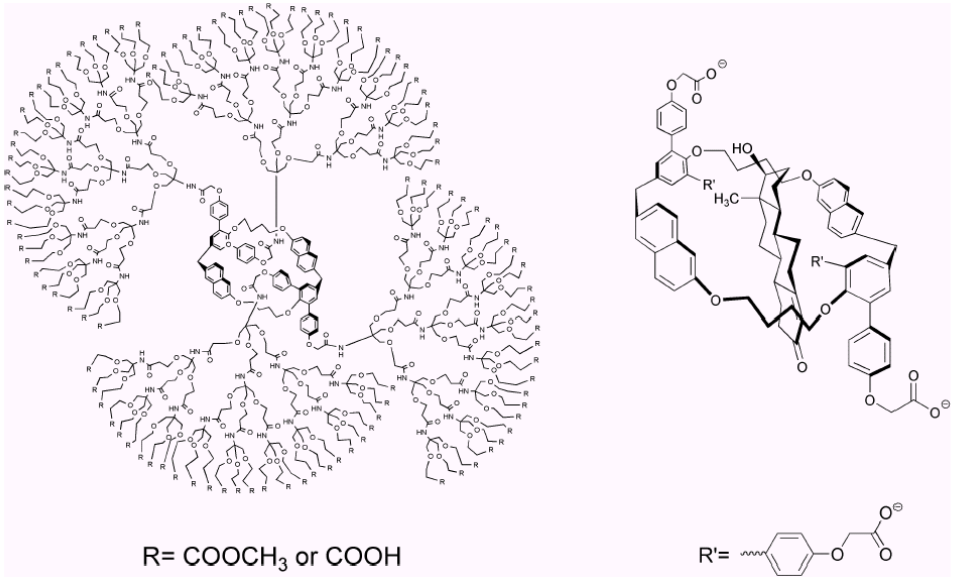
1) Binding to the dendrimer core ('endo-receptor') via hydrophobic interactions:

- poly(aryl ether) dendrimers with carboxylate surfaces can dissolve non-polar pyrene molecules in water
- good candidates for carrying hydrophobic bioactive compounds, i.e., steroids



Zimmerman, et al. *Chem. Rev*. **1997**, *97*, 1681

- Water-soluble 'dendrophanes' are excellent carriers of steroids!

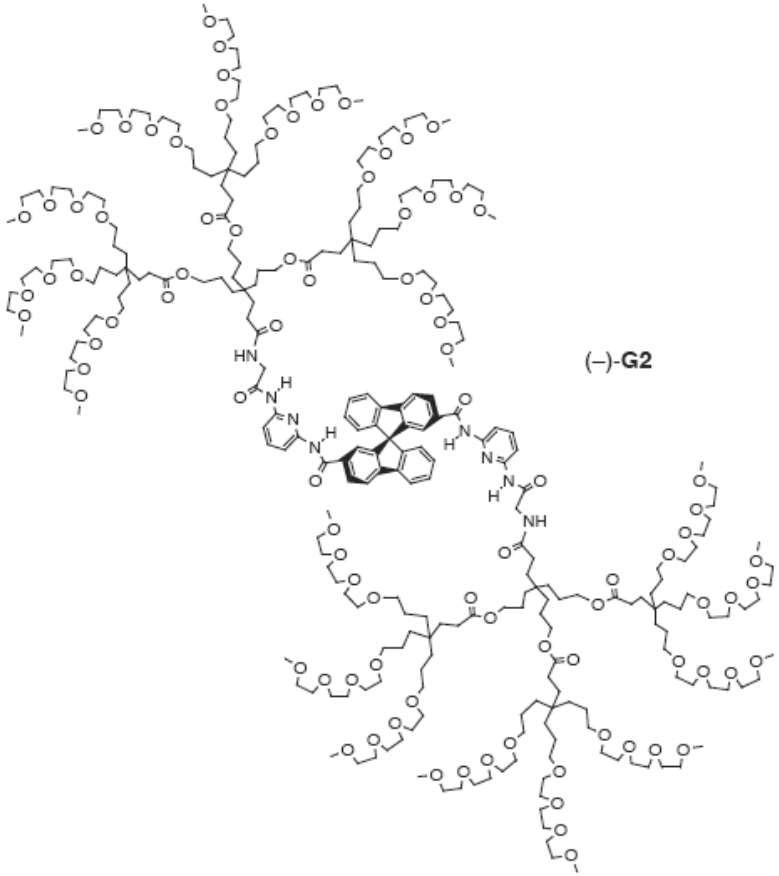


Diederich, et al. *Helv. Chim. Acta.* **1996**, *79*, 779

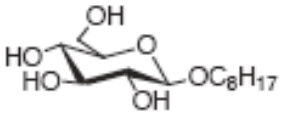
Dendrimers as hosts

2) Binding to the dendrimer core via polar interactions:

- Diederick and co-workers designed 'dendroclefts' for the chiral recognition of monosaccharide guests (built on an optically active (–)-9,9'-spirobi[9*H*-fluorene] core)



octyl β-D-glucoside

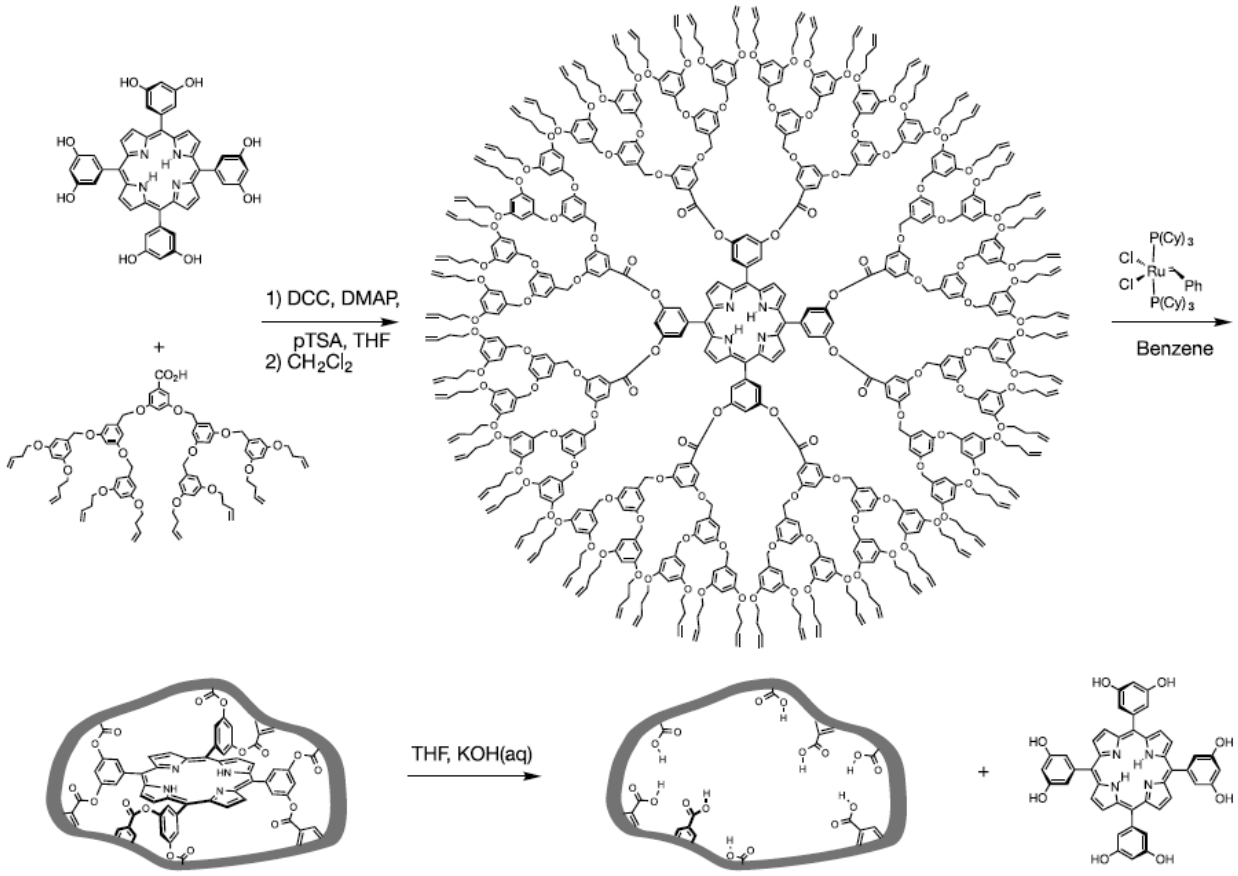


Diederich, et al. *Chem. Comm.* **1998**, 2501

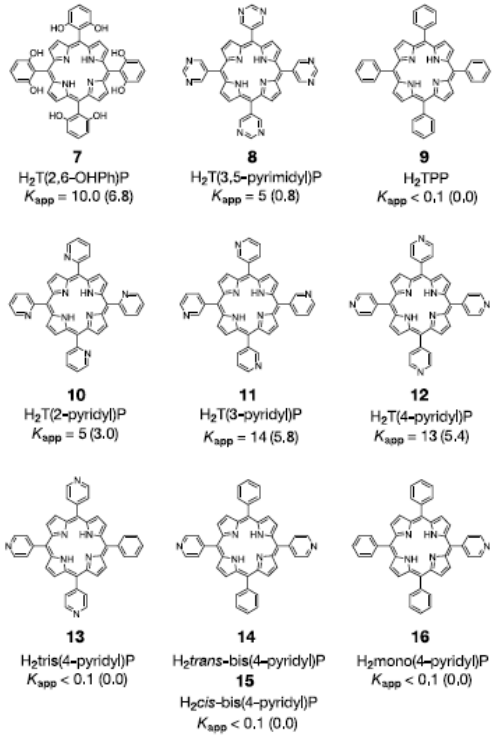
Dendrimers as hosts

3) Dendrimers as temporary scaffolds:

- 'Molecular imprinting' technology – a synthetic porphyrin-recognising host



Porphyrins



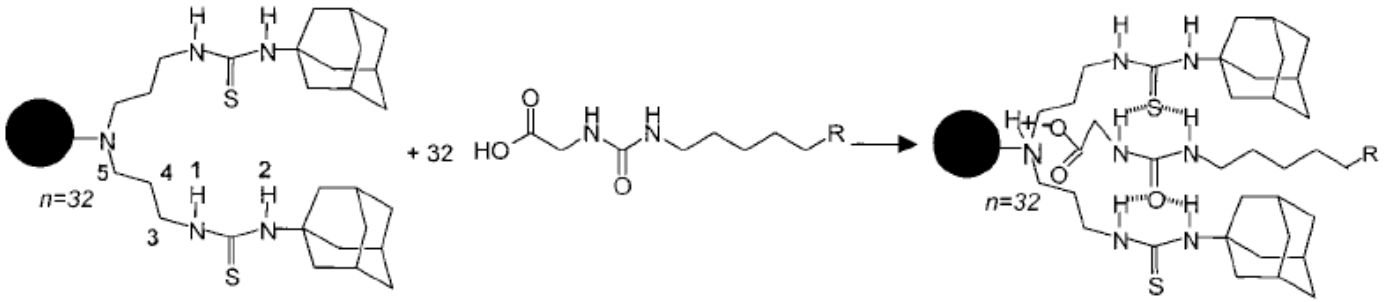
K_{app} (x 10^4 M^{-1})

Zimmerman, et al. *Nature* **2002**, *418*, 399

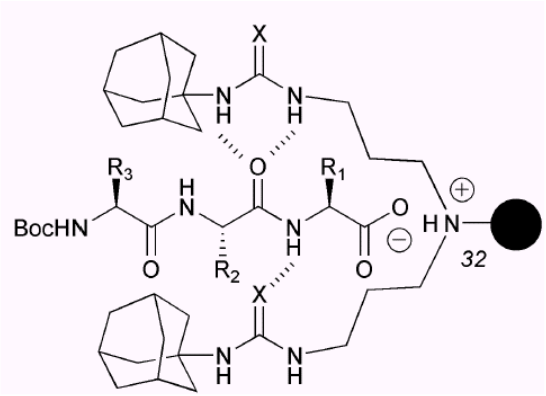
Dendrimers as hosts

- The 'click-in' design ('exo-receptors'):

  - thiourea-terminated PPI dendrimers were used for binding of polar molecules via H-bonds
  - possible host-guest carrier for peptides (drug delivery can be regulated by pH)



X = O,S (*N*-Boc-protected peptide)



Meijer, et al. *J. Org. Chem.* **2001**, *66*, 2136

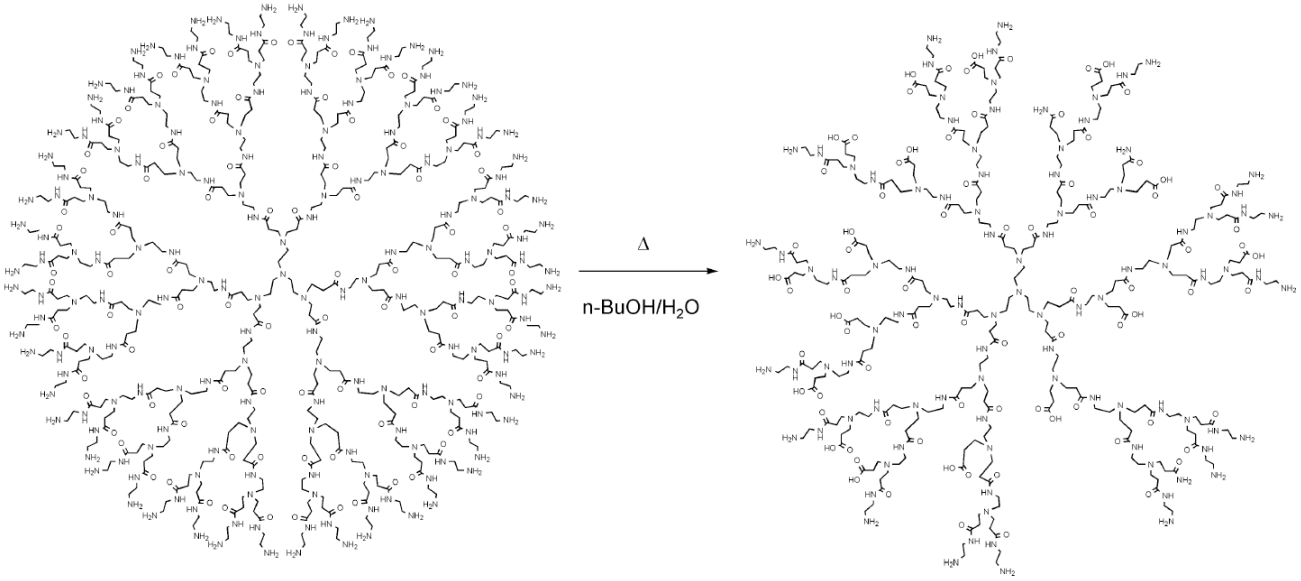
Part IV

Biological Applications of Dendrimers

Dendrimers as delivery vehicles

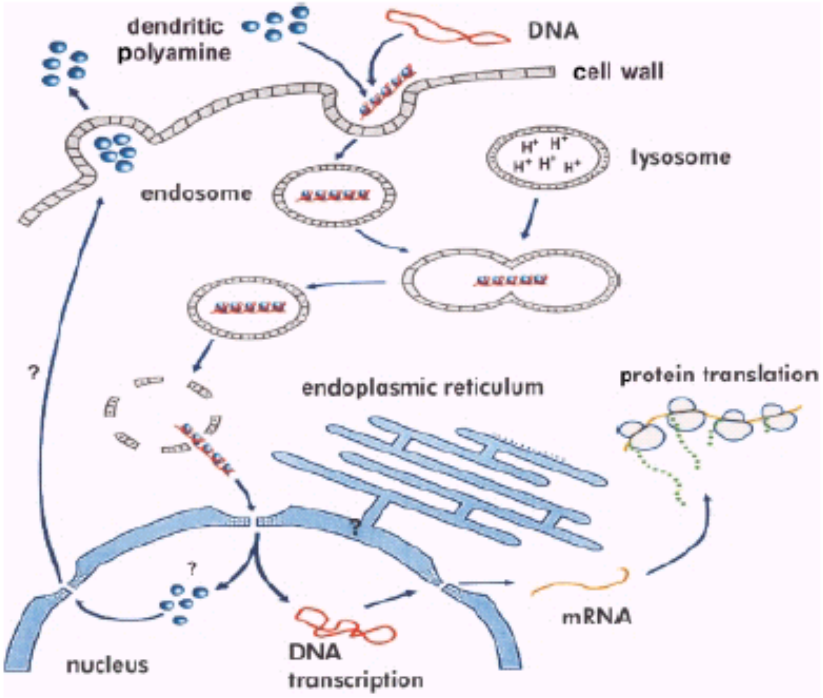
- Dendrimers are ideal for drug and gene delivery because they:
  - enhance aqueous solubility
  - increase circulation half-life
  - target certain tissues
  - improve transit across biological barriers
  - slow drug metabolism

- Dendrimers as gene transfection agents:
  - unmodified PAMAM and PPI dendrimers can form complexes with DNA
    - partially degraded or fragmented ('activated') dendrimers help transfection efficiency!
    - slight excess of primary amines is beneficial



Dendrimers as delivery vehicles

Hypothetical mechanism of DNA transfection with cationic 'activated' dendrimers



- PolyFect® is commercially available for *in vitro* transfection of DNA

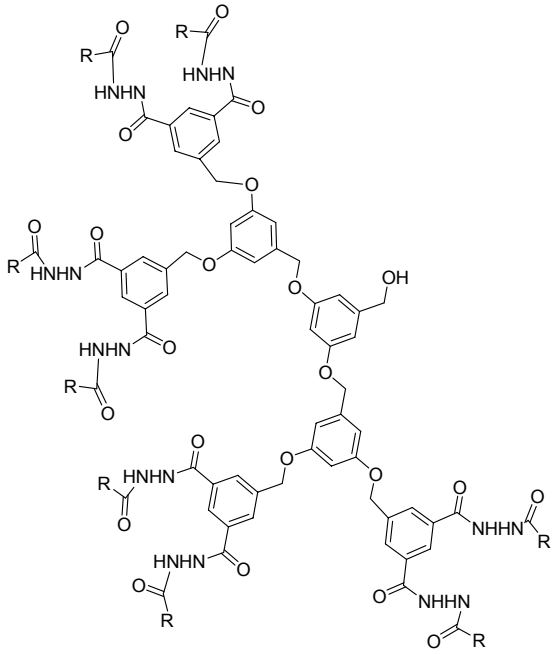
Haag, et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 1329

Dendrimers as delivery vehicles

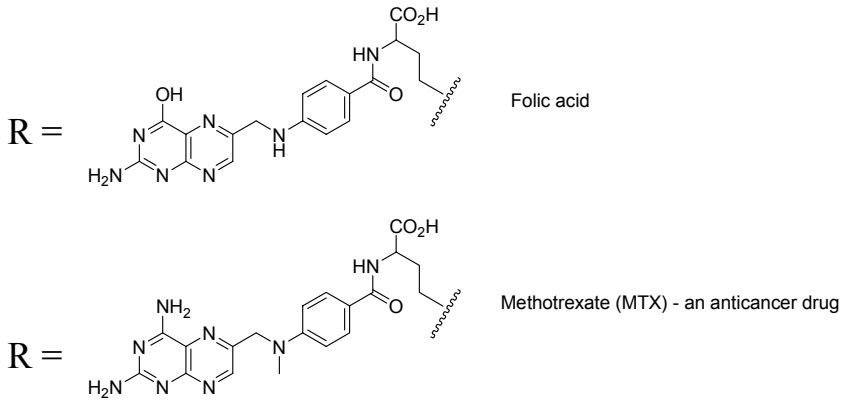
- Dendrimers as drug delivery agents:

  - cationic dendrimers with covalently attached drugs are used to deliver high concentrations of drugs close to a cell surface (‘extracellular matrix-targeted local drug delivery’)

  - ‘cell specific’ drug delivery?
    - folic acid derivatized dendrimers (folate receptors are overexpressed in cancer cells)
      - methotrexate (MTX) for treatment?



Poly(aryl ether)-(G2)



Fréchet, et al. *Bioconjugate Chem.* **1999**, *10*, 1115

Dendrimers as delivery vehicles

'Multi-functionalized' PAMAM dendrimers as anticancer drug carriers:

- partially modified surface with acyl groups
- folate as a targeting ligand
- fluorophore (fluorescein) or radiolabeled
- ~9 wt% methotrexate

- intravenous administration to mice with subcutaneous tumors (human KB tumors overexpressing the folic acid receptor)

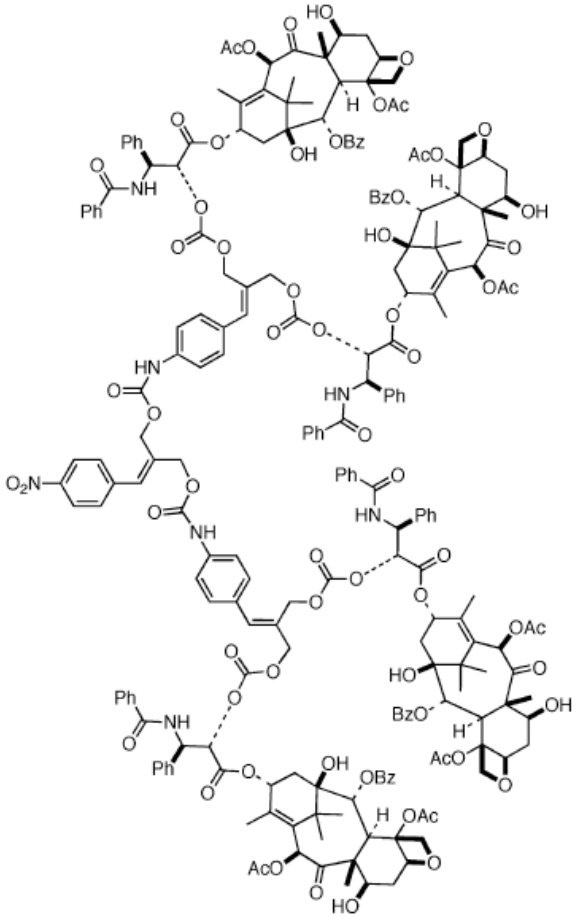
Results:

- conc. of dendrimer in tumor was 5 to 10 times greater than control lacking folate
- methotrexate conjugated to dendrimer had significantly lower toxicity and 10-fold higher efficacy than free methotrexate at an equivalent cumulative dose
- 15 biweekly injections of compound showed a significant decrease in the rate of tumor growth

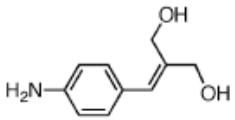
Kukowska-Latallo, et al. *Cancer Res.* **2005**, *65*, 5317

Dendrimers as delivery vehicles

- Paclitaxel releasing dendrimer via reduction of nitro group (trigger)



2-4 equiv.
of drug released



- aniline byproduct showed no cytotoxicity in several tumor cell lines

McGrath, D. V. *Molecular Pharm.* **2005**, *2*, 253

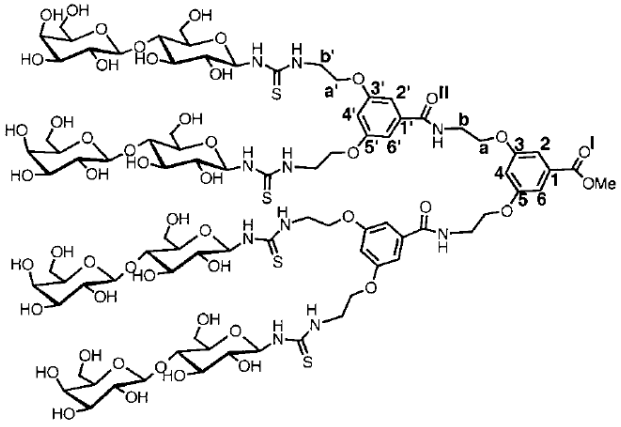
Dendrimers as delivery vehicles

➢ Dendrimers as glycocarriers:

- carbohydrate-based drugs are of interest as microbial anti-adhesins, microbial toxin antagonists, anti-inflammation drugs, antiviral and anticancer drugs
- glycosylated dendrimers are good mimics of natural glycoconjugates!

- M.O.? - Inhibit carbohydrate binding proteins (lectins) using a 'multivalency/cluster effect' of dendrimers

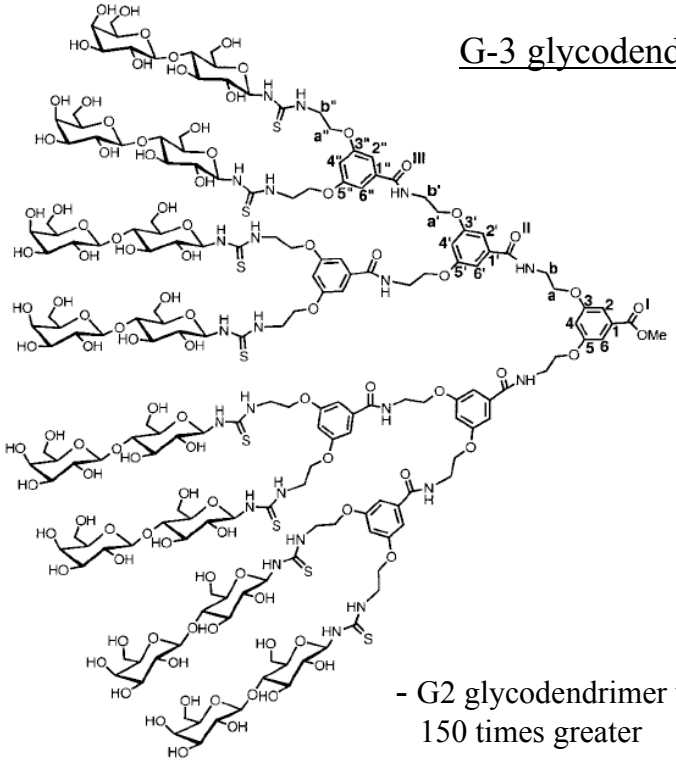
G-2 glycodendrimer



- G2 glycodendrimer was 1667 times greater at inhibiting mammalian galectin binding to immobilized glycoproteins relative to free lactose

Pieters, et al. *ChemBioChem.* **2001**, *2*, 822

G-3 glycodendrimer

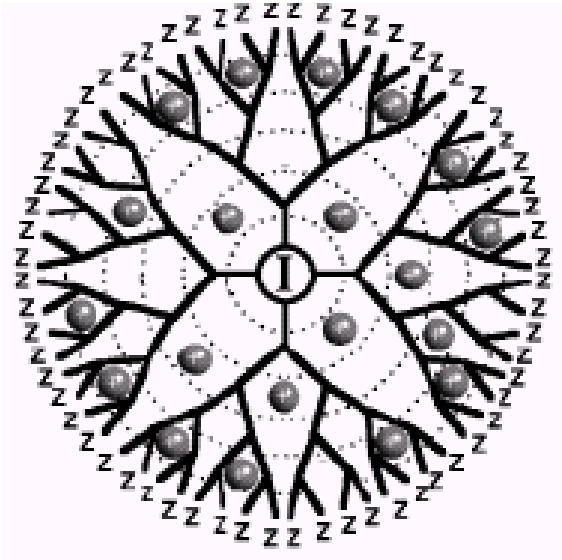


- G2 glycodendrimer was only 150 times greater

Dendrimers as delivery vehicles

- PAMAM dendrimer–Silver complexes:

  - silver ions complexed within dendrimer network
  - slowly releases silver into environment
  - displays anti-microbial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria without loss of solubility or activity

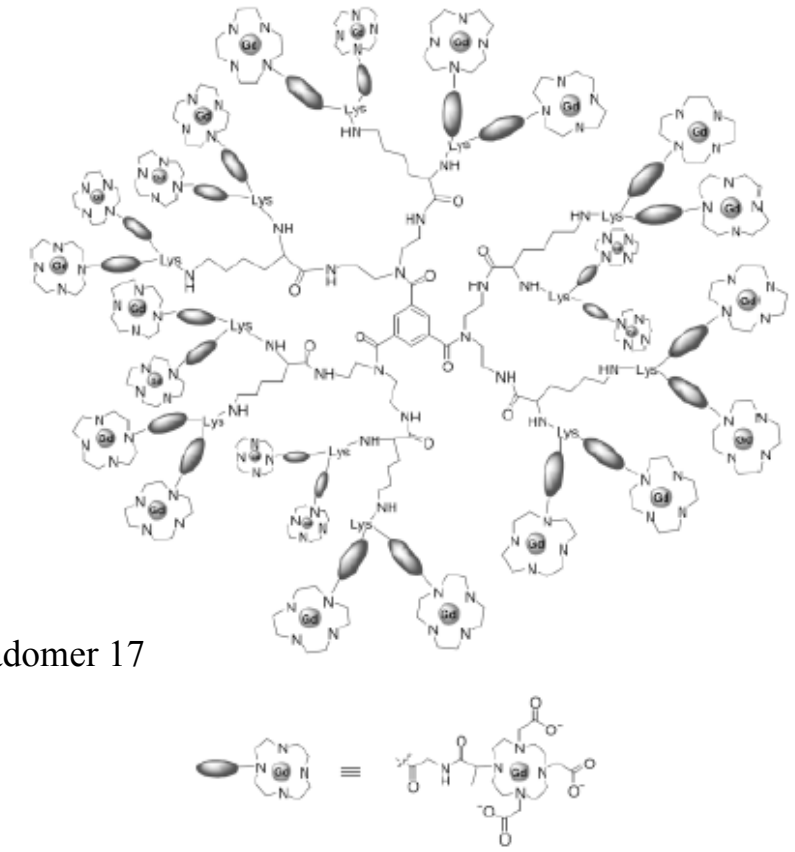


Balogh, et al. *Nano. Lett.* **2001**, *1*, 19

Dendrimers as imaging agents

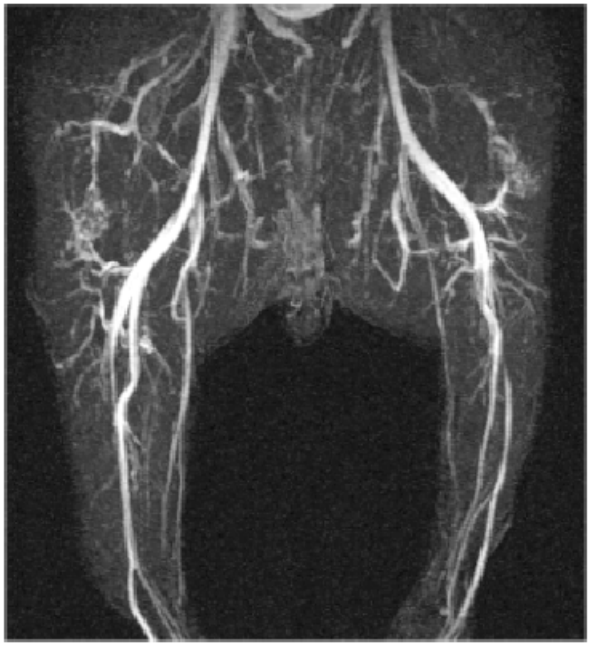
➢ Dendrimers as MRI contrast agents:

- PAMAM dendrimers containing Gd^{III}-chelates were initially synthesized by Wiener and co-workers. (Gadomer 17 is currently produced by Schering AG (Berlin, Germany))
- *In vivo* experiments show excellent imaging of blood vessels, long circulation times (>100 min.) and superior elimination rates



Gadomer 17

Image of peripheral blood vessels of a dog after injection with gadomer 17 (dose: 50 μmol/kg body weight)

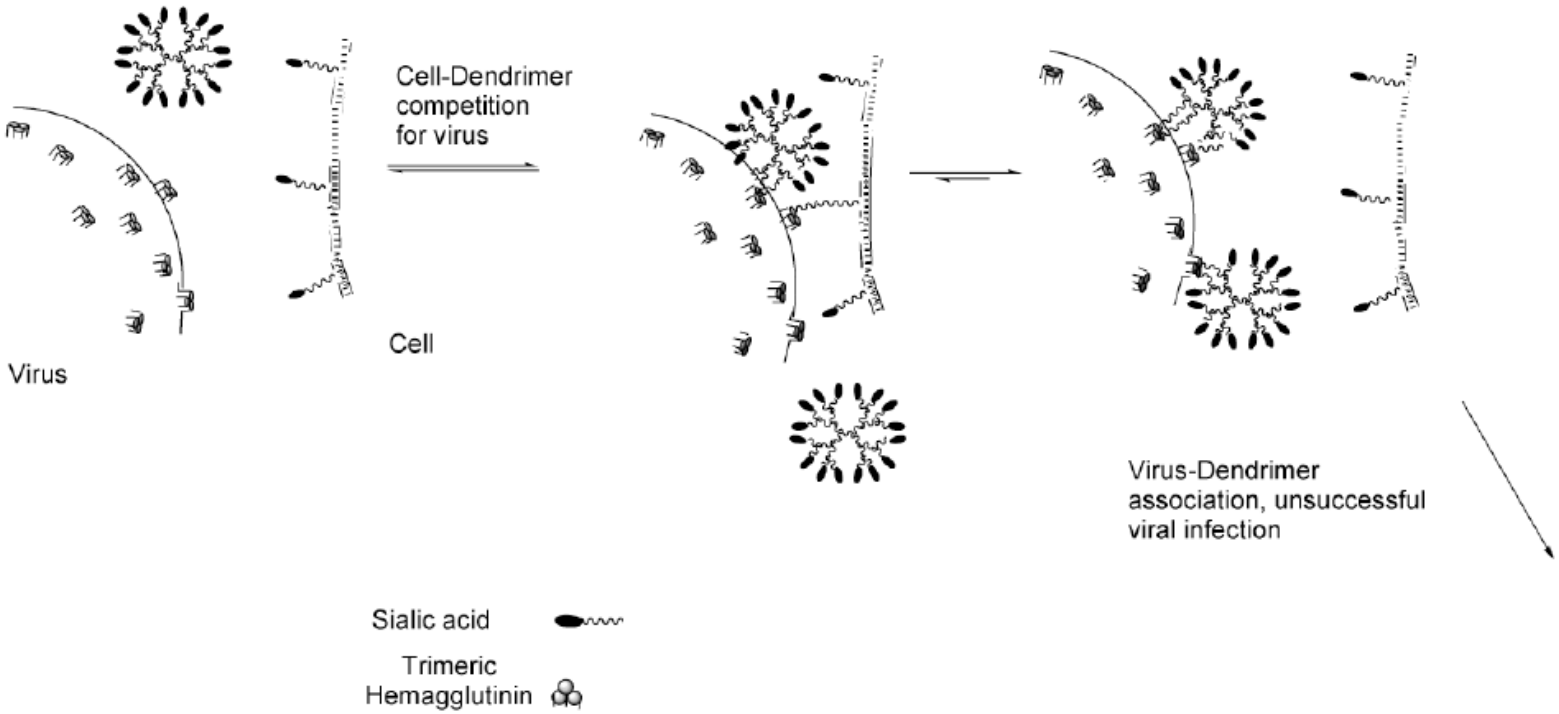


Haag, et al. *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 1329

Dendrimers as drugs

1) Dendrimers as antiviral drugs:

- antiviral dendrimers work as artificial mimics of anionic cell surfaces
- usually contain sulfonate or sialic acid residues at terminus



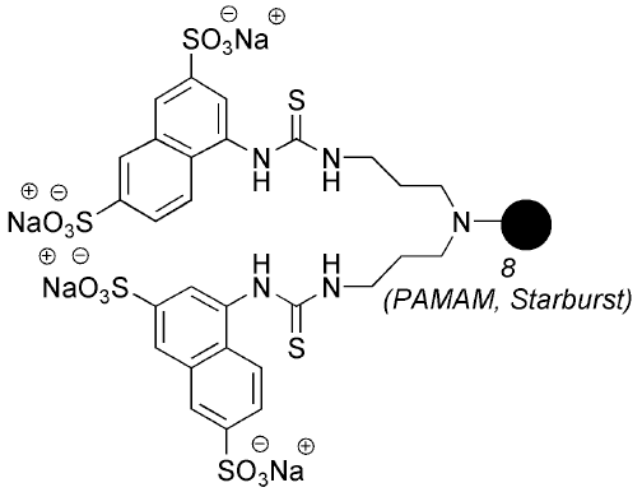
- penetration of cell wall by virus (endocytosis) is inhibited by binding with dendrimer

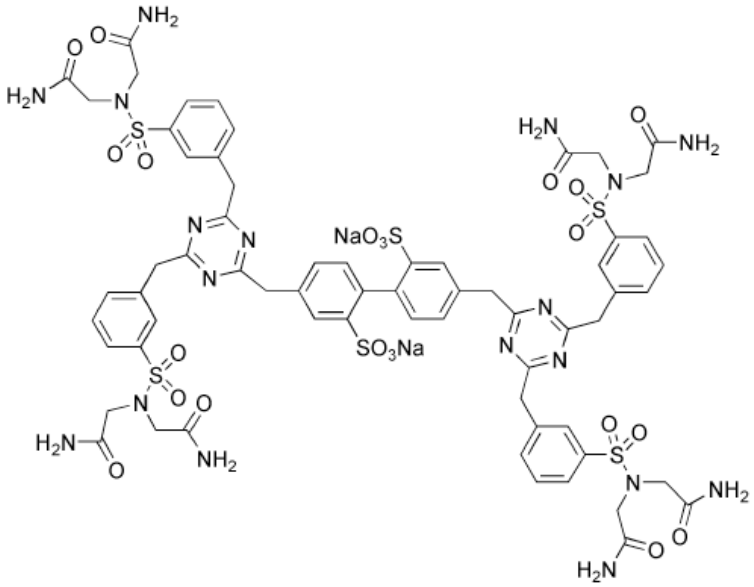
Chem. Soc. Rev. **2004**, *33*, 43-63

Examples of Antiviral Dendrimers

- PAMAM dendrimers modified with naphthyl sulfate show antiviral activity against HIV

Bernstein, et al. *Antimicrob. Agents Chemother.* **2000**, *44*, 2471





(Wyeth compd. RFI-641)

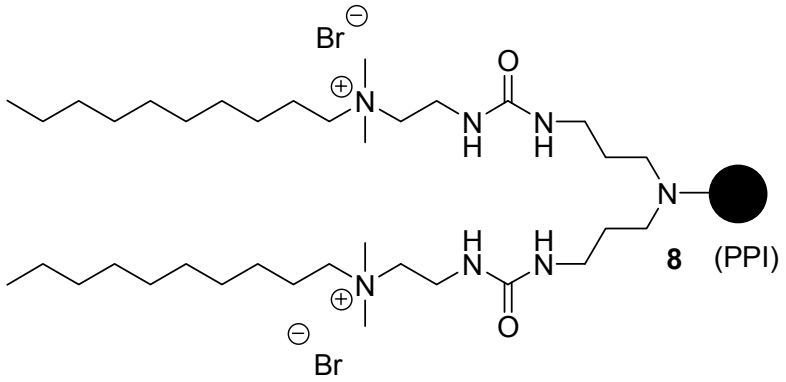
- Small dendrimer-like compound was found to inhibit respiratory syncytial virus (RSV) through possible binding with viral fusion protein
  - Inhibits RSV growth with an IC_{50} of 50nM

Krishnamurthy, et al. *Chem. Biol.* **2001**, *8*, 645

Dendrimers as drugs

2) Dendrimers as antibacterial drugs:

- dendrimers contain cationic surface functionalities
- adhere to and damage anionic bacterial membrane, causing lysis



Biocide for *Escherichia coli*

- PPI dendrimers with tertiary alkyl ammonium groups are potent biocides against Gram positive and Gram negative bacteria
  - antibacterial properties depended on dendrimer size, length of hydrophobic chain and counterion

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

Dendrimers as protein denaturants

➢ Dendrimers as **chaotropes** (water structure perturbing solutes)

- surface of dendrimers used to disorder the structure of water
  (analogous to disfavored interactions that cause protein denaturation)
- common chaotrope salts and solvents: $MgCl_2$, urea, sodium thiocyanate; acetonitrile, propanol and methanol

- prion proteins (PrP) are infectious agents that attain a pathogenic structure/conformation in the brain (form very insoluble aggregates) ⟶ transform normal proteins into deadly ones!
- prion diseases are a group of fatal neurodegenerative disorders that occur in hereditary, sporadic or infectious forms (i.e., *mad cow disease* and *Creutzfeldt-Jakobs disease (CJD)*)

* cationic (amine-terminated) PAMAM and PPI dendrimers (G0-G4) were used to <u>solubilize</u> and <u>remove</u> prion protein aggregates from infected neuroblastoma cells!!!

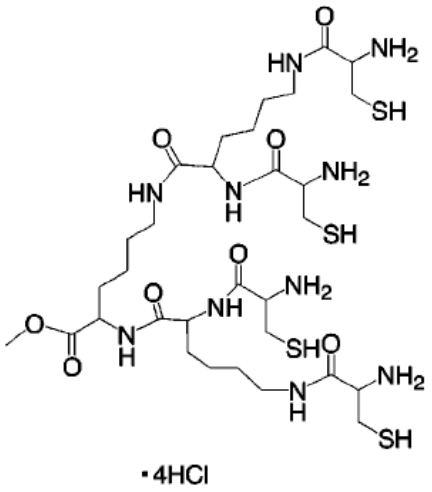
(Supattapone, et al. *PNAS* **1999**, *96*, 14529)

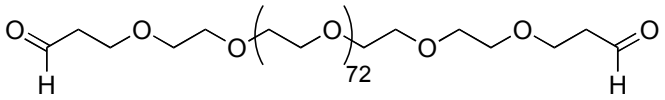
"The early onset of Creutzfeldt-Jakob Disease causes changes in bahavior and impairs the memory, where upon the disease rapidly progresses to involuntary movements, mental deterioration, and eventually coma. Although Creutzfeldt-Jakob disease is the most common human prion disease, it is still extremely rare and only occurs in about one out of every one million people." (www.medkuz.com)

"There is no treatment that can cure or control CJD." "About 90% of patients with CJD die within 1 year." (www.ninds.nih.gov)

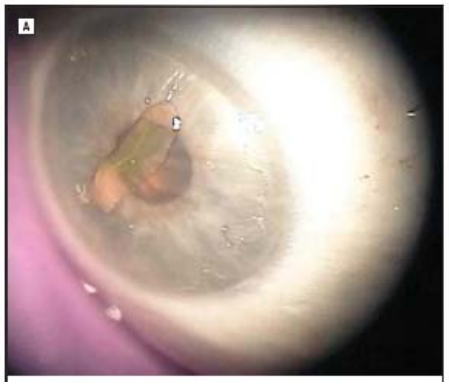
Dendrimers as scaffolds for tissue repair

- High functional-group densities and low solution-viscosities of dendrimers are useful as sealants for corneal incisions or wounds (i.e., after catarack surgery)





- Mixed to form a cross-linked hydrogel in a few min. at r.t.
- applied to incision



- In vitro study shows that hydrogel sealant withstands higher pressures and stress than 'self-seal' or nylon sutures

Grinstaff, et al. *J. Am. Chem. Soc.* **2004**, *126*, 12744

Velazquez, et al. *Arch. Ophthalmol.* **2004**, *122*, 867

Conclusions - Why Dendrimers?

- Dendrimers are extremely well-defined, globular, synthetic macromolecules with characteristics that make them useful for numerous biological applications
- Dendrimers are manufactured in high purities with few structural defects
- Easily characterized by standard methods (MS, IR, SEC, HPLC and NMR spectroscopy)
- Several dendrimers are commercially available as drug carriers, membrane permeability and targeting, diagnostic markers and biosensors

"'Is a dendrimer really a polymer?' 'Because of their dimensions, are dendrimers really hazardous?' 'Do we really need a polymer such as a dendrimer?' 'Do dendrimers really exist?'"

- questions to Donald Tomalia
from Professor Paul Flory
(Japan Intl. Polymer Conf., 1984)