APPLICATIONS OF DENDRIMERS TO DRUG DELIVERY

Reported by: Catherine A. Brummond March 4, 2004

INTRODUCTION

The discovery and creation of new drugs is timely and costly process. It is estimated that every new drug takes 12 to 15 years to develop, at a cost of over $800 million.[1,2] A more efficient approach would be the devising of effective drug delivery systems for already developed experimental drugs that failed to make it to the market. Controlled release systems can improve the effectiveness of drug delivery by sustained release of the compound over time or by release at a specific target.[3] By controlling the time and location of delivery, side effects can be minimized and drug efficacy can be maximized, thus leading to a lower dosage for patients. Methods to achieve controlled release include chemical or enzymatic reaction, diffusion through a matrix, or solvent activation.

Currently the two common drug delivery systems are liposomes and polymeric systems. These both have limited applications, as liposome-based systems have poor stability and difficulty targeting specific tissues, and linear polymers are polydisperse.[3] Dendrimers offer advantages including a lower polydispersity index, multiple sites of attachment, and a controllable, well-defined size and structure that can be easily modified to change the chemical properties of the system.[4] In addition, macromolecules such as dendrimers have an enhanced permeability and retention effect that allows them to target tumor cells more effectively than small molecules.[5]

Dendrimers have applications in gene and antisense therapy, magnetic resonance imaging, and in boron neutron capture therapy.[6,7] Advances in dendrimer delivery systems, biodegradable dendrimers, and release from dendrimers can be applied to drug delivery in addition to other applications. This review will focus on the development of dendrimers into cancer drug carriers and the recent advances in the controlled degradation of dendrimers.

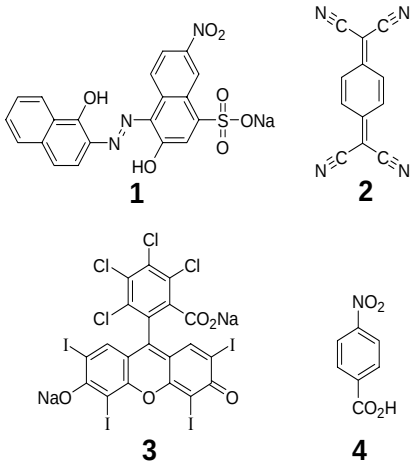
DENDRIMER DRUG CARRIERS

Two methods of dendrimer drug delivery are encapsulation of drugs and dendrimer-drug conjugates. Encapsulation of drugs uses the steric bulk of the exterior of the dendrimer or interactions between the dendrimer and the drug to trap the drug inside the dendrimer. Dendrimer-drug conjugates have the drug attached to the exterior of the dendrimer. Most of these conjugates are prodrugs and are inactive or have decreased activity relative to the free drug.

Encapsulation of Dyes in Dendrimers

In 1994, Meijer and coworkers reported the first encapsulation of a molecule inside a dendrimer, the so-called "dendritic box."[8] They were able to encapsulate three dyes (Chart 1): eriochrome black T (**1**), tetracyanoquinodimethane (**2**), and Rose Bengal (**3**) in a fifth generation (G5) diaminobutane-based-poly(propyleneimine) (PPI) dendrimer capped with N-*t*BOC-L-phenylalanine (Figure 1**)**. Encapsulation of each dye in PPI was supported by UV spectroscopy and differences in solubilities between the dye and the dye in the box. Free **1** was soluble in both water and methanol, but in the dendrimer **1** was insoluble in both. Attempts to liberate **1** by heat, dialysis, or standing for 3 months failed to release measurable amounts of the dye from the dendrimer.

Chart 1. Dyes encapsulated in the "dendritic box"



Later, Jansen and Meijer reported the shape-selective release of **3** and *p*-nitrobenzoic acid (**4**) from their "dendritic box" (Chart 1).[9] After encapsulating four molecules of **3** and 8-10 molecules of **4** per box, only the smaller *p*-nitrobenzoic acid was released upon treatment with 95% formic acid for 16 hours. Release of **3** required reflux in 12 N HCl for 2 hours. Small guests were released after deprotection of the bulky *t*-BOC or FMOC groups from the amino acids while larger guests required complete hydrolysis of the amino acid exterior of the dendrimer. Due to the harsh conditions required to release larger dyes, other encapsulations were constructed to function less by sterically locking the molecules in the dendrimer cavities and more through interactions inside the dendrimer.

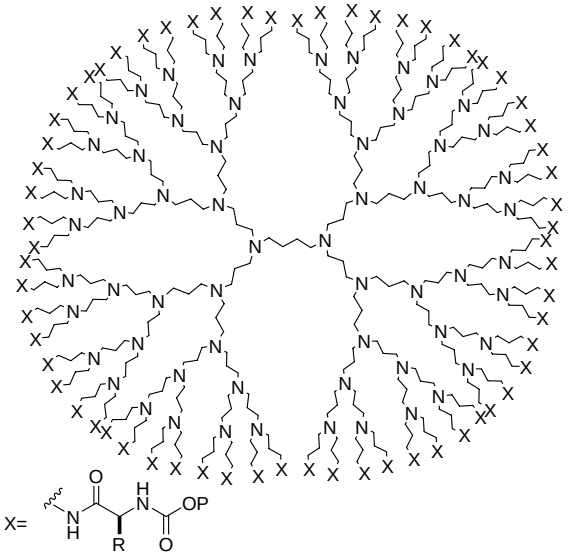


Figure 1. Capped fifth generation diaminobutane-based PPI dendrimer

Encapsulation of Drugs in Dendrimers

In addition to dyes, research began to focus on anti-cancer drugs for encapsulation. Kono and coworkers used G3 and G4 ethylenediamine based polyamidoamine (PAMAM) dendrimers with poly(ethyleneglycol) monomethyl ether (M-PEG) grafts (Figure 2) to encapsulate the anticancer drugs methotrexate (MTX, **5**) and doxorubicin (DOX, **6**) (Chart 2).[10] Using higher generations or longer PEG chains resulted in more encapsulated drug molecules per mole of dendrimer. Higher affinity for **5** was

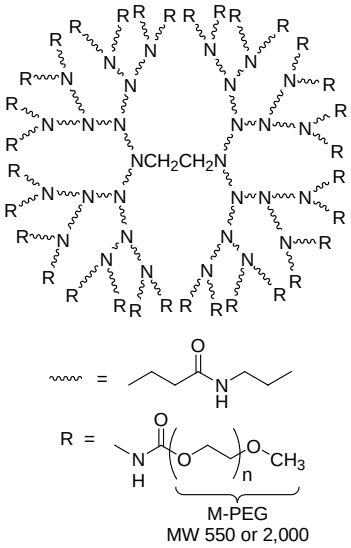
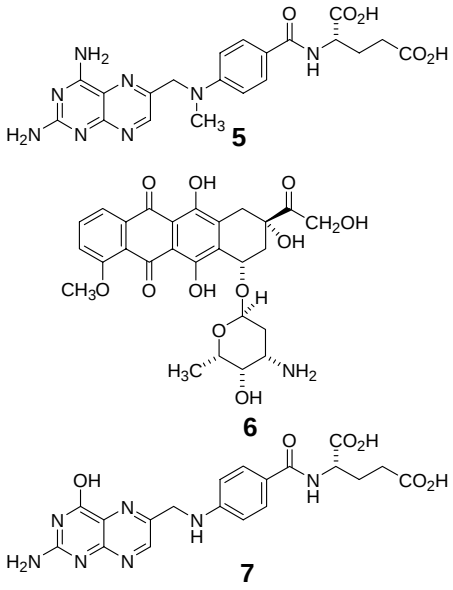


Figure 2. M-PEG capped G3 PAMAM

reported based on a greater number of encapsulated molecules by UV spectroscopy and thus suggested a more favorable acid-base interactions between the drug and the basic, hydrophobic interior of the dendrimer. In addition, there was a slower release of **5** than **6** from the dendrimer when measured by diffusion through a dialysis bag. Because drug release occured by dialysis, targeted delivery would be difficult to achieve, but sustained release would be easier to accomplish. Furthermore, because the encapsulation by the dendrimer varied significantly depending on the drug and the dendrimer structure, this method would be difficult to make universal for all drugs.

Chart 2. Anti-cancer Drugs and Folic Acid



Dendrimer-Drug Conjugates

Fréchet and coworkers attached MTX (**5)** or folic acid (FA, **7**) (Chart 2) to the exterior of the dendritic structure.[11] Delivery of FA conjugates were examined as a method to target tumor cells, as the folic acid receptor is overexpressed in various tumor cells, and folic acid conjugated to macromolecules can be brought into the cell by endocytosis.[12] The carboxylic acid(s) on **5** and **7** were attached to a hydrazide-terminated polyarylethyl dendritic structure (Figure 3). The conjugates were characterized by NMR and MALDI-TOF, but the targeting ability, the release of **4**, and the conjugate activity were not evaluated.

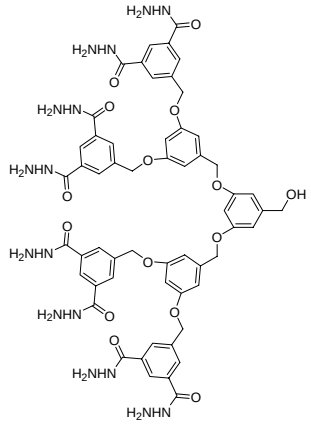


Figure 3. Hydrazide-terminated polyarylether dendritic structure

In 2002, Baker and coworkers conjugated both **4** by either an amide or ester bond, and **6** by an amide bond to a G5 capped ammonia based PAMAM dendrimer and tested the cytotoxic response towards human carcinoma cells.[13] Conjugate uptake by cells was measured by a fluorescein tag on the dendrimers. MTX-FA-dendrimer conjugates entered the cells with similar uptake to folic acid-dendrimer conjugates while the dendrimer alone showed less than 10% uptake. The conjugate with the ester linkage was hydrolyzed at the low pH of the endosome and was 4-times more effective in killing the tumor cells than free MTX. The amide conjugate was less toxic suggesting that the higher cytotoxicity for the ester conjugate resulted from intracellular drug delivery and release.

The ability of dendrimer drug carriers to show higher cytotoxicity towards carcinoma cells than free anti-cancer drugs shows promise for targeted drug delivery even though further examination of the targeted release is required. While other carriers were developed, additional research in the field focused on improving the dendrimer structure to make the drug carriers more biocompatible.

BIODEGRADABLE DENDRIMERS

The chemical and physical properties of a dendrimer can be optimized by systematically changing the monomer(s). By optimizing the monomer(s), dendrimers can be made to degrade into biodendrimers, which degrade to biocompatible building blocks *in vivo*.[14] Suitable monomers for biodendrimers include α-hydroxy acids, sugars, amino acids, fatty acids, poly(ethylene glycol) (PEG), poly(caproic acid) (PCL), and poly(trimethylene carbonate).[15,16] Factors affecting the degradation rate include: 1) the strength of the chemical bond between the monomers, 2) the hydrophobicity of the dendrimer, 3) the generation and molecular weight of the dendrimer, and 4) the chemical reactivity of the macromolecule.[15]

Seebach and coworkers reported the first enzymatically degradable dendrimer in 1996.[17] They examined polyester dendrimers based on the initial core in Figure 4. Their polyester dendrimers were degraded by the bacterial enzyme poly(3-hydroxybutyrate)-depolymerase to hydroxybutanoic acid and the triester of 1,3,5-benzenetricarboxylic acid with hydroxybutanoic acid. Because the dendrimer was degraded, by enzymes Seebach and coworkers termed their dendrimers "biodegradable."

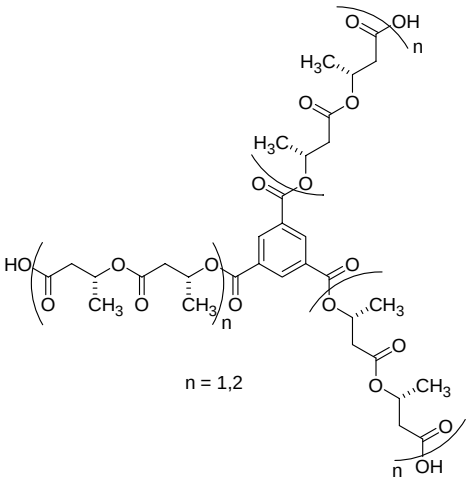


Figure 4. Polyester dendrimer core

Using known biocompatible monomers, Grinstaff and coworkers developed several polyether-ester dendrimers and used one composed of succinic acid and glycerol for the encapsulation of anti-cancer drug 10-hydroxycamptothecin (Figure 5).[14,16,18] ^{1}H NOESY spectroscopy indictated that the drug was encapsulated in the dendrimer. The encapsulated drug showed substantial cytotoxicity to a human breast cancer cell line.

Fréchet, Szoka, and coworkers examined dendrimer-drug conjugates of polyester dendrimers composed of 2,2-bis(hydroxymethyl)propanoic acid monomers.[19,20] They evaluated a G4 dendrimer (**8**), a M-PEG capped G4-dendrimer (**9**), and a (polyethylene oxide) PEO star-G3-dendrimer conjugate (**10**) (Chart 3). A fourth compound with an acid-labile hydrazone linkage was used to connect **6** to their conjugate, allowing controlled release in the acidic environments of the cell. The pH of the endosomal and

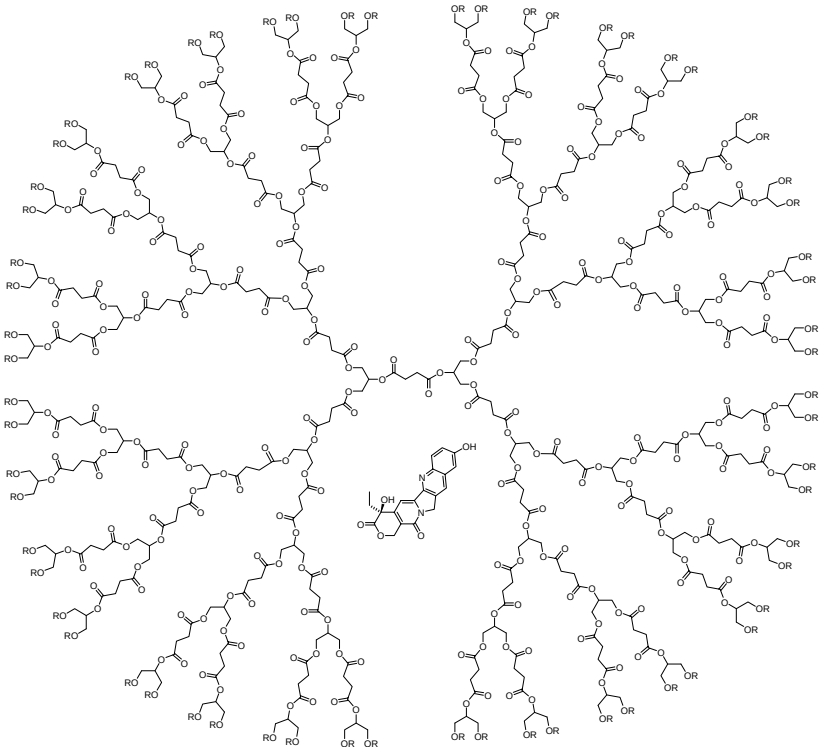
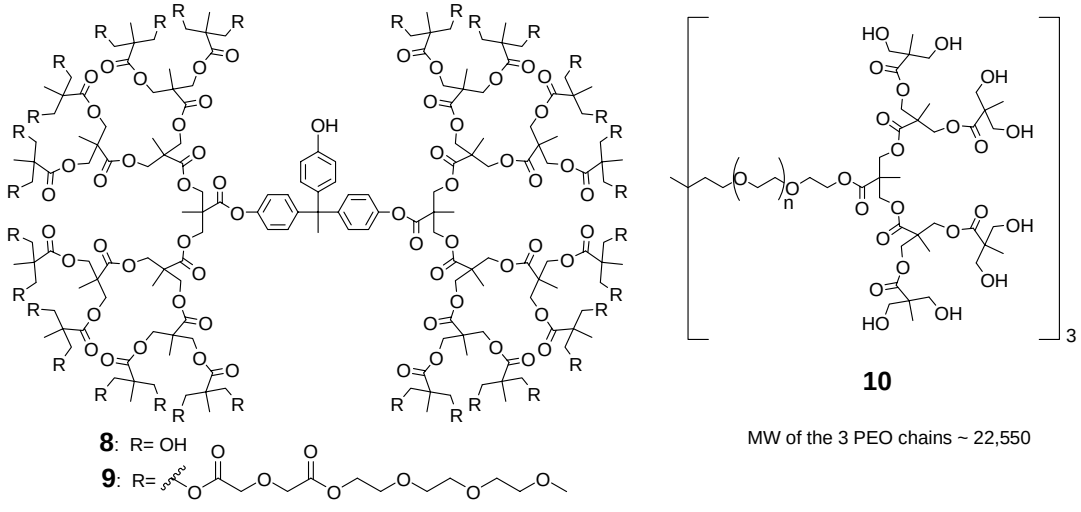


Figure 5: 10-hydroxycampotothecin encapsulated in biodegradable polyether ester dendrimer

lysomomal environments is 4 to 6, and their goal was to have a linkage that could be cleaved in this pH while being stable at neutral pH.[20] All three of the compounds initially showed high cell viability and only in concentrations ≥ 5 mg/mL for the M-PEG capped dendrimer and ≥ 10 mg/mL for the other two compounds was there growth inhibition (no dead cells). The three compounds were tolerated well in mice after injections of 1.3 g/kg body weight. The G4-polymer and the M-PEG capped version had half-lives of about 8 minutes and were both "essentially completely excreted" after 4 and 5 hours, respectively. On the other hand, the DOX-PEO star-G3-dendrimer conjugate had a half-life of 72 minutes and no significant accumulation in any vital organ. In pH 7.0 buffered solution virtually no DOX was released, however at pH 4.5, 70% was released after 20 hours.

Chart 3. Polyester Dendritic Systems



CONTROLLED RELEASE

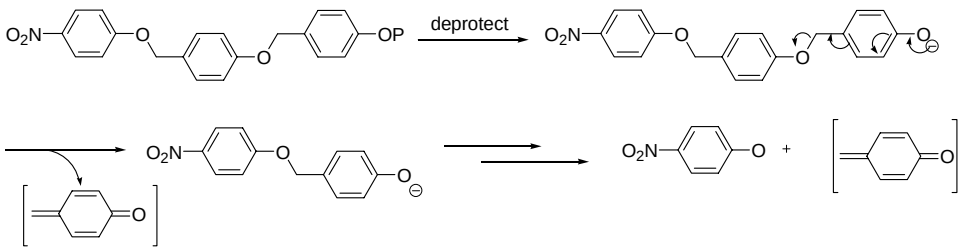
By combining the ideas of drug carriers and degradability, research has recently focused on controlled degradation of dendrimers and release of compounds. Some of the methods to initiate the

release include light, removal of protecting groups, and antibodies. McGrath and coworkers used light to cleave a dendrimer with a photolabile *o*-nitrobenzyl ether.[21] Irradiation at 350 nm produced the 1, 3, 5-benzenetricarboxylic acid core and each dendrimer fragment.

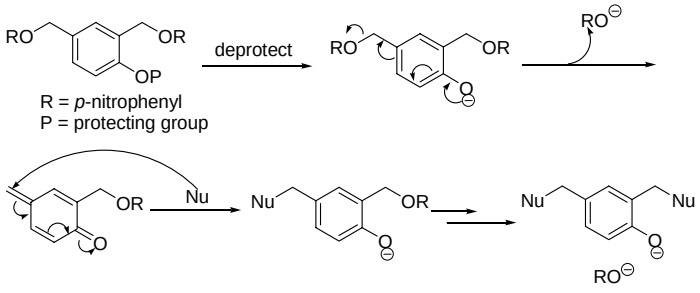
Cascade Release Dendrimers

In 2003, McGrath and coworkers[22] introduced a method to initiate release through deprotection of functional groups and resulted in an amplified degradation process. When several *p*-benzyl ether phenoxides were attached with the last one protected, the cleavage would continue in a linear cascade-like manner for each *p*-nitrophenol after deprotection (Scheme 1). When tested on zeroth through second generation dendrons, there was 85-100% yield of disassembly as measured by UV spectroscopy. McGrath and coworkers continued work in this area with the goal of exponential degradation. They called their method 'geometric dendrimer disassembly" and the one deprotection led to two additional fragmentations per subunit (Scheme 2).[23] Though only demonstrated with dendrons, McGrath and coworkers showed a way to generate an exponential number of fragments from one triggering event.

Scheme 1. Linear Diassembly

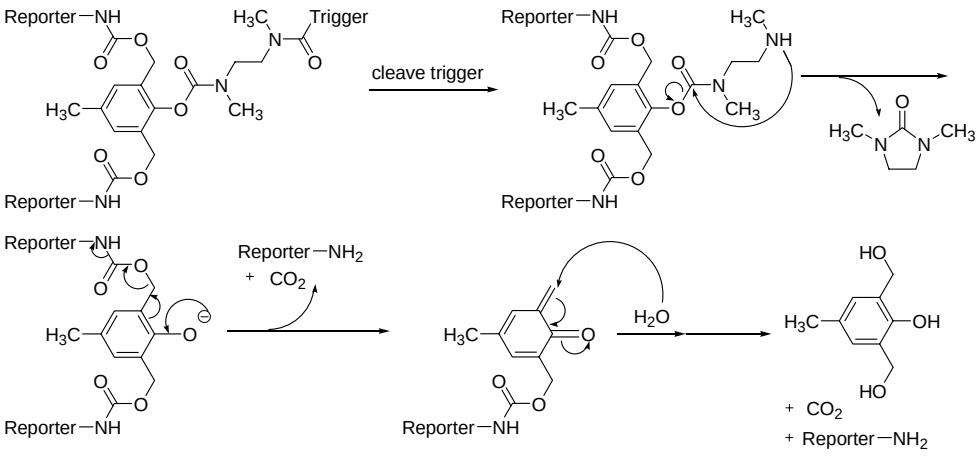


Scheme 2. "Geometric" Dissembly



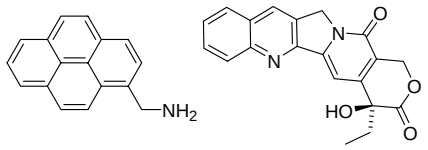
The ability to release an exponential number of fragments has obvious applications to drug delivery. Shabat and coworkers called their exponential release process "self-immolative."[24] The carbamate linkages throughout their structures are cleaved after the *t*-Boc or CBz "trigger" is deprotected and results in the exponential release of a colored reporter (Scheme 3). The rate determining step in the cascade was the cyclization to form a N,N'-dimethylurea derivative. Difficulties were encountered in the attachment of pyrene (11) as a reporter in higher generations dendrons because of limited space on the exterior of the dendrons (Chart 4). This led to limitation in the size of the reporter for higher generations of dendrons. Shabat and coworkers further expanded this concept by using anti-cancer drugs **5** and camptothecin (CPT, **12**) as the reporters and an enzymatic substrate as the trigger.[25] The enzymatic substrate was a linker that could be cleaved by the catalytic antibody 38C2 via a tandem retro-aldol-retro-Michael reaction.[26] The authors made DOX and CPT homodimeric and monomeric prodrugs, and a DOX-CPT heterodimeric drug and

Scheme 3. "Self-Immolative" Dissassembly



examined cytotoxicity towards human Molt-3 leukemia cell line.[25] The homodimeric prodrugs' IC_{50} values dropped about four to 40-fold, depending on structure when the antibody was added, but all had higher values than the corresponding free drugs. The DOX-CPT prodrug showed about a 550-fold decrease in IC_{50} to 0.17 nM. This was the lowest IC_{50} of the prodrugs and was lower than DOX, but still higher than CPT or the combination of DOX and CPT at 0.13 nM and 0.06 nM, respectively.

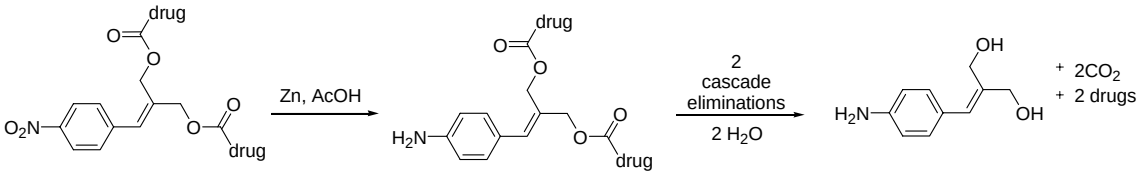
Chart 4. "Self-Immolative" Reporters



11 **12**

A third cascade release dendrimer was reported by de Groot et. al.[27] that is similar to that reported by Shabat and coworkers. The structure consists of a trigger, linkers and drugs. Once the trigger was initiated by reduction of a nitro group to an amine the additional 4-aminocinnamyl alcohol linkers and the drug, in this case Taxol, were released (Scheme 4). This linker could be used in a prodrug to reduce cytoxicity in seven human tumor cell lines by greater than 60 fold over the free drug.[28] Four Taxol molecules could be released from a second generation dendrimer, following zinc and acetic acid reductive conditions.[27]

Scheme 4. Cascade release



CONCLUSION AND FUTURE DIRECTIONS

Dendrimer carriers have progressed from merely carrying dyes, which are easily monitored by UV spectroscopy, to carrying anti-cancer drugs. Both encapsulation and dendrimer drug conjugates can be used as drug carriers; sustained release is favored by encapsulation, and targeted release is favored

by dendrimer-drug conjugates. The area of dendrimers for drug delivery is continuing to grow with the recent reports of cascade release dendrimers and additional ways to release drugs. Further testing needs to be done to determine the toxicity of the dendrimers and the ability to trigger drug release *in vivo.*

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