Self-Immolative Linkers in Polymeric Delivery Systems

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There has been significant interest in the methodologies of controlled release for a diverse range of applications spanning drug delivery, biological and chemical sensors, and diagnostics. The advancement in novel substrate-polymer coupling moieties has led to the discovery of self-immolative linkers. This new class of linker has gained popularity in recent years in polymeric release technology as a result of stable bond formation between protecting- and leaving groups, which becomes labile upon activation, leading to the rapid disassembly of the parent polymer. This ability has prompted numerous studies into the design and development of self-immolative linkers and the kinetics surrounding their disassembly. This review details the main concepts that underpin self-immolative linker technologies that feature in polymeric or dendritic conjugate systems and outlines the chemistries of amplified self-immolative elimination.

1. Introduction and Key Terminology

It has long been known that the reactivity or (biological) activity of an active compound (commonly a drug, referred to herein as a 'reporter' molecule) can be attenuated by the incorporation of a protecting group (also described as a 'trigger' group).[1-4] Traditional protecting group strategies involve the direct connection of a protecting moiety with a compound via a scissile bond (**Scheme 1a**). This linkage is cleaved using specific conditions, for instance by enzymatic catalysis (*e.g.* peptidyl amides) or by 'chemical modification' (*e.g.* (bio)reduction of a nitro trigger), where the cleavage mechanism and conditions are determined by the nature of the protecting group. This classical prodrug approach has been widely explored for small molecules[5, 6] as well as macromolecular conjugate systems.[7]

This strategy works very well when the linkage is easily accessible. However, when the trigger and/or the reporter moieties are sterically bulky, their spatial proximity can impair cleavage, thus preventing release.[8, 9] To overcome this problem, an alternative strategy has been suggested whereby an additional linker is incorporated between the trigger and the reporter moiety. In this approach, the linker forms a scissile bond with a protecting group and a stable bond to a reporter group, the latter of which becomes labile upon removal of the protecting group, resulting in rapid disassembly of the three components (**Scheme 1b**). This linker technology is becoming increasingly common in conjugate systems for drug delivery and is referred to as 'self immolative linkers'.[10-13]

Recent reviews have discussed self-immolative linkers for tumour-activated prodrug therapies and biological conjugates,[14-17] herein we systematically review the *mechanistic* aspects of self immolative technology focussing on their use in macromolecular systems.

Classical self-immolative linkers combine a protecting group with a single reporter moiety, whereby a *single* activation event leads to the release of a *single* reporter group. This release can be described as non-amplified. The evolution of self-immolative linker technology has led to self immolative systems capable of amplified release (**Scheme 1c**). In this case a *single* activation event leads to the release of multiple reporter groups.[18-20] More recently, systems capable of relayed amplified release whereby a *single* activation event facilitates *exponential* release have been developed (**Scheme 1d**). Examples of such system amplification are discussed in relevant sections.

For the purpose of clarity, a colour scheme has been adopted to highlight trigger (blue), self-immolative linker (green) and reporter (red) moieties.

Scheme 1 - a) (Bio)chemical cleavage of a two-component pro-moiety, b) self-immolative elimination of a three component pro-moiety, c) amplified disassembly of self-immolative polymeric promoieties, d) relayed amplified self-immolative elimination.

Scheme 2 - Lipase mediated self-immolative elimination of tetrahydro-*β*-carbolines from tentagel solid support **1**.[21]

2. Classes of Self-Immolative Elimination

2.1. Self-immolative elimination

Self-immolative elimination is the spontaneous and irreversible disassembly of a multicomponent compound into its constituent fragments through a cascade of electronic elimination processes. Self-immolative elimination is driven by two processes:- i) an increase in entropy coupled with ii) the irreversible formation of thermodynamically stable products (*e.g.* CO_2).

This phenomenon is most commonly observed for polysubstituted, electron-rich aromatic species that feature an electron-donating substituent (such as amino or hydroxyl residues) that is in conjugation (*ortho* or *para*) with a suitable leaving group based at a benzylic position. An electron-donating substituent is required commonly to lower the energy barrier of dearomatisation. However, for compounds of this type, the incorporation of a protecting group or trigger is often required to modulate the spontaneity of self-immolative elimination under physiological or basic conditions. For example, Sauerbrei and co-workers have demonstrated the self-immolative ability of a *p*-hydroxylbenzyl alcohol based linker with the controlled release of a series of tetrahydro-*β*-carbolines from a solid-phase polymer conjugate (**Scheme 2**).[21] In its 'pro' form, the phenolester of **1** is insufficiently electron-donating to facilitate self-immolative elimination. However, enzymatic cleavage of the scissile ester bond and enzyme assisted deprotonation of the corresponding phenol, afforded a strongly electron-donating phenoxide **2** that facilitates the formation of quinone methide intermediate **3** following an 1,6-elimination process. In the presence of solvent (*e.g.* H_2O or MeOH), solvolytic quenching of **3** restored the aromaticity to form *p*-hydroxybenzyl methyl ether **4**. The presence of a nucleophile or polar protic solvent is required to facilitate fragmentation and leads to concomitant quenching of the high energy, highly electrophilic intermediate. When the nucleophile is the solvent, self-immolative elimination is alternatively described as a process of solvolytic cleavage (or solvolysis). Polar protic solvents such as methanol are commonly employed in these studies and also serve as diagnostic probes in mechanistic degradation assays.

The elimination process may be described as a quinone (for self-immolative phenols) or an azaquinone (for self-immolative anilines) methide mediated elimination-addition reaction which describes the nature of the formally charged intermediate that is formed from disassembly.

A range of benzyl alcohols have been thoroughly investigated as a self-immolative linkers including 2- and 4- amino-,[10] hydroxy-[12, 13] and mercapto-[11, 22] functionalised benzyl alcohols (based on **5 – 7**, **Figure 1**). The reduced self-immolative ability of 2- and 4-hydroxybenzyl alcohol based linkers, as a result of the reduced nucleophilicity of oxygen with respect to nitrogen, can be enhanced by the presence of additional activating ring substituents or mildly basic conditions. The superior self-immolative ability of the aminobenzyl alcohol linker has favoured its use, prompting detailed kinetic studies regarding ring and methylene substituted analogues.[23-25] Hay and co-workers have reported that the rate of self-immolative elimination of the *p*-aminobenzyl alcohol linker is enhanced by the presence of electron-donating substituents or halides, and impaired by the presence of electron-withdrawing substituents. This effect is most strongly influenced when these substituents are in conjugation with the leaving group at a benzylic position and thus provide the greatest degree of (de)stabilisation of the azaquinone methide intermediate.

The most predominant enhancement in elimination rate was observed when additional substituents (*e.g.* methyl) were incorporated at the benzylic methylene position (the solvolytic site), which stabilises the charged intermediate species by hyperconjugation.

Despite the observation made by De Groot and co-workers of the elongated 1,8-self-immolative elimination of *p*-aminocinnamylalcohol **8**,[25] the distance over which self-immolative elimination can propagate has really restricted this phenomenon to monocyclic-aromatic systems. De Groot and co-workers have shown that naphthyl- **9** and biphenyl- **10** (bicyclic-aromatic) based self-immolative linkers are not susceptible to their corresponding 1,8- and 1,10-eliminations under aqueous conditions or even at elevated temperatures.[26]

Figure 1 Benzyl **5 – 7**, cinnamyl **8**, naphthyl **9** and biphenyl **10** based self-immolative linkers.[23, 25, 26]

Scheme 3 PGA mediated cyclisation elimination of 4-nitrophenol from first generation diethylenetriamine based dendron **14**.[27]

Despite the structural and electronic limitations of the self-immolative elimination process, the development of self-immolative linkers has led to a diverse range of homoaromatic[10, 11, 13, 28] coumarin,[29] furan,[30] thiophene,[30] thiazole,[30] oxazole,[30] isoxazole,[30] pyrrole,[30] pyridine,[31] pyrazole,[30] imidazole,[32, 33] and triazole[34] based heteroaromatic linkers that are self-immolative under both aqueous and physiological conditions.

2.2. Cyclisation Elimination

The term "self-immolative elimination" has also been used to describe cyclisation-elimination reactions that result in molecular fragmentation via activation of a latent nucleophile followed by displacement of a carbonyl linked reporter moiety.[35-37] In a manner analogous to self-immolative elimination, cyclisation-elimination is driven by i) the formation of thermodynamically stable fragments, which is combined with ii) an increase in entropy. This process has been observed most commonly for 4-aminobutanoyl esters and ethylenediamines. Cyclisation-elimination follows a series of equilibria and proton transfers which is strongly influenced by the environmental pH.[38, 39] This overall process is comparatively slow as reflected by prolonged half lives (typically 2 – 24 hours). In contrast, the cyclisation-elimination of 2-hydroxy[40] or 2-aminomethylbenzamides (**11** and **12**), 2-hydroxyhydrocinnamic esters[41] and 2-hydroxyphenyl acetyl ester (**13**) based self-immolative cyclisation linkers is enhanced through the incorporation of substituents (such as bulky aromatic or *gem*-dimethyl residues) which enforces a biased population of reactive conformations (**Figure 2**).[42]

Self-immolative cyclisation-elimination linkers have proven advantageous for the release of amino- or hydroxyl- based compounds from hydrolytically stable amide or carbamate linkages. For example, Shabat and Amir have demonstrated the release of *p*-nitrophenol from a first generation diethylene triamine based dendron (**Scheme 3**).[27] Amide **14** is highly stable under physiological conditions. However, the selection of the amide side chain of penicillin G affords an elegant solution to the need for mild cleavage of an otherwise stable functional group. Thus cleavage of either (or both) phenylacetamide protecting groups by penicillin-G-amidase enzymes affords amine **15**. This nucleophilic species then participates in a favourable 5-*exo*-trig (1,5-) cyclisation to liberate *p*-nitrophenol (the reporter unit in this model study) with concomitant formation of imidazolidinone **16**.

Figure 2 Amino- **11** or hydroxyl- **12** methylbenzamide and hydroxyl phenylacetyl ester **13** based self-immolative linkers.[40]

2.3. Amplified Self-Immolative Elimination

Amplified self-immolative elimination is defined by the release of *multiple* reporter groups via a series of electronic elimination processes upon a *single* activation event.[18-20] The incorporation of additional leaving groups into a linear self-immolative linker creates a branched self-immolative linker of type AB_n (where A represents an electron-donating group and B represents suitable leaving groups) capable of amplified release. For example, De Groot and co-workers have reported the self-immolative elimination of 2-phenylethanol from a first generation dendron featuring a 2,4,6-tris(hydroxymethyl)aniline AB_3 branched linker (**Scheme 4**).[18]

Scheme 4 Triple self-immolative elimination of 2-phenylethanol from first generation 2,4,6-tris(hydroxymethyl)aniline based AB_2 dendron **17**.[18]

Dendron **17**, when subjected to zinc in the presence of acetic acid, is reduced to afford aniline **18**, which, by dint of its low pK_a has a significant precentage in the non-protonated form and so, in turn undergoes a 1,6-elimination with concomitant decarboxylation to liberate 2-phenylethanol (the reporter molecule) and azaquinone methide **19**. Quenching with solvent reforms the electron-donating aniline which undergoes two sequential 1,4-elimination and concomitant decarboxylation reactions to liberate two additional 2-phenylethanol reporter molecules. Overall, three 2-phenylethanol molecules were released from a single reduction reaction. To prove the concept of amplified self-immolative elimination the reaction was conducted in MeOH. The use of an organic polar protic solvent as opposed to an aqueous system has proven to be an effective method to determine that self-immolative elimination and solvent quenching of the azaquinone methide intermediate does occur preferentially with respect to direct cleavage of the carbonate linkage. In the former case, solvent quenching with methanol affords benzylic methyl ether **23**, whilst direct cleavage of the carbamate linkage would yield the corresponding benzylic triol.

Only a few kinetic studies have been conducted upon branched self-immolative linkers, possibly as a result of the presumption that the differences in kinetic profiles between linear and branched linkers are insignificant. However, Erez and Shabat have conducted a systematic study on the relative rates of 1,4- and 1,6-elimination from 1,2- and 1,4- substituted linear- and 2,4-bis(hydroxymethyl)aniline AB_2 branched linkers.[43] The relative disassembly rates of phenylacetamide protected dendrons **24** and **25** (**Figure 3**), which featured *p*-nitroaniline and 5-amino-2-nitrobenzoic acid in alternating positions highlighted a small difference between rates of 1,4- and 1,6-elimination. A step-wise kinetic profile was observed, whereby release of the *ortho* substituent was slowed by competitive 1,6-elimination until release of the *para* substituent had proceeded to completion. This is indicative of a step-wise mechanism which follows preferential solvolysis of the *para* substituent (see **Scheme 4**). A larger discrepancy (two fold difference between 1,4- and 1,6-eliminations) was observed for the relative elimination rates of **26** and **27**, which cannot be attributed to their relative steric bulk acting to hinder enzymatic hydrolysis.

Figure 3 Linear aminobenzyl alcohol and AB_2 branched 2,4-bis(hydroxymethyl)aniline based self-immolative systems.[43]

Instead, the accumulation of the *ortho* substituted aniline intermediate, observed from the degradation of **26**, suggests that 1,4-elimination of the *ortho* substituent was the rate limiting process in this degradation study. Shabat and co-workers have also conducted a study regarding substituent effects upon the rates of the first order disassembly of 2,6-bis(hydroxymethyl)phenol based AB_2 dendrons.[44] It was shown that the incorporation of a *para* ethylester enhanced the rate of self-immolative elimination ($2.88 \pm 0.06\ h^{-1}$) through electron-withdrawing resonance stabilisation of the anionic charge which develops on the corresponding phenol prior to quinone methide rearrangement. The relative disassembly rate of the methyl substituted phenol **29** ($0.089 \pm 0.002\ h^{-1}$) was reduced approximately 30 fold, as a result of the destabilisation of the phenoxide intermediate via inductive electron donation (**Figure 4**). The difference in elimination rates (*ca.* 1.5 fold) between **28** and **29** correlated well with the pK_a values calculated for *p*-cresol and 4-hydroxybenzoic acid (10.26 and 8.34, respectively).

Figure 4 First generation 2,4-bis(hydroxymethyl)phenol AB_2 based dendrons **28** and **29**.[44]

3. Self-Immolative Polymeric Systems

3.1. Self-Immolative Linear Polymers

Whilst there has been substantial progress on the synthesis, development and assembly of polymers that are becoming increasingly similar to their natural and biologically significant counterparts, in terms of architecture, structure and function, minimal attention has been paid to methodologies of polymer disassembly under physiological conditions.[45] This is surprising considering the ease with which natural polymers are assembled, modified and degraded. Thus, appropriately constructed polymers that are designed to undergo complete molecular disassembly upon a specific single activation event, to yield a high local concentration of an active species, would prove effective drug delivery vehicles and have already been shown to offer great promise as highly sensitive diagnostic tools.[46, 47]

Self-immolative polymers are constructed from the combination of multiple AB_n self-immolative linkers in a linear fashion. A single activation event facilitates a linear cascade of the polymeric backbone which affords multiple active reporter species, directly (active monomers), or by sequential elimination from the side chain of each monomer. Based upon the development of linear self-immolative linkers and the unsurpassed self-immolative ability of aniline based linkers, suitably modified branched linkers based on these

Scheme 5 Self-immolative disassembly of 2,4-bis(hydroxymethyl)aniline based AB_2 oligomer **30**, inset – monomers **34** and **35**.[48]

initial designs have been preferentially investigated and utilised. As demonstrated[48] by Warnecke and Kratz self-immolative oligomers can be obtained via a itrative activation-coupling pathway. For example dimer **30**, featuring a 2,4-bis(hydroxymethyl)aniline AB_2 branched linker, was synthesised via selective protection of one B functionality as a *t*-butyldimethylsilyl ether, followed by the sequential activation by phenylchloroformate and coupling of an additional 2,4-bis(hydroxymethyl)aniline linker. Desilylation in the presence of HCl/MeOH followed by activation by phenylchloroformate and coupling of tryptamine afforded the desired dimer **30** in 7 % overall yield via an eight step synthesis. Chemical reduction of the *p*-nitrobenzyloxycarbonyl trigger group, under neutral conditions, resulted in precipitation of the corresponding aniline **31**, which prohibited comparative studies of the rate of disassembly with varying pH. However, dimer disassembly (via **32**) and liberation of tryptamine was observed under acidic conditions (Zn/AcOH (0.3 %), H_2O:MeCN (9:1), pH = 3), albeit at a slow rate, as a result of partial protonation of the active aniline species at this pH. This reduction in elimination rate facilitated an in-depth kinetic study of dimer **30** and related monomers **34** and **35**, which highlighted three distinct steps to the fragmentation profile (**Scheme 5**). These were attributed to a rapid 1,6-depolymerisation to release the terminal tryptamine molecule prior to a phase of relative stagnation where little release was observed, followed by a constant but slower release of the side-chain tryptamine. This result was in agreement with previous studies[43] which revealed that 1,6-elimination led to depolymerisation and that this occurs preferentially to 1,4-elimination (see **Scheme 4** and **Figure 3**). The stagnant phase was attributed to the build up and solvent quenching of the azaquinone methide intermediate to form active monomer **32** and facilitate the steady further release of tryptamine. First order kinetics with respect to dimer **30** were obtained which indicated an E_1 type mechanism and the formation of a azaquinone methide intermediate.

This difference between the rate of reaction for 1,6- and 1,4-elimination pathways was also observed by Shabat and co-workers in the two-stage disassembly of *p*-nitroaniline functionalised 3-(2-amino-5-(hydroxymethyl)phenyl)prop-2-en-1-ol based polymer **38** and copolymer **39** (**Scheme 6**), which is exemplified by the reduced elimination rate observed for the elongated, vinylogous side chain of 2-amino-5-hydroxymethylcinnamyl alcohol.[49] Polymer **38**, which featured an average degree of polymerisation (d.p.) of 11 (determined by NMR spectroscopic analysis), was synthesised from the homo-polymerisation of the corresponding phenyl carbamate (a blocked isocyanate),[50] in the presence of dibutyltin dilaurate (DBTL) and was capped by the addition of 4-hydroxy-2-butanone. Polymer **38** was synthesised in 62 % yield in one synthetic step. The repetitive connectivity of monomers through the *p*-amino substituent with the benzylic alcohol substituent via urethane linkages, created a polymer that was capable of complete disassembly through repetitive sequential 1,6-elimination and decarboxylation reactions. Polymer disassembly was demonstrated by piperidine mediated β-elimination of the 4-hydroxy-2-butanone protecting group in MeOH/DMSO. As also demonstrated with monomer **40**, 1,6-elimination was comparably rapid (*ca.* 1 h) with respect to successive liberation of *p*-nitroaniline (*ca.* 20 h) via 1,6-elimination of the vinylogous side chain. The poor aqueous solubility of these polymers, as a result of their hydrophobic structure, prompted the synthesis of water-soluble copolymer **39**.

Scheme 6 Synthesis of monomeric, oligomeric and polymeric 2-amino-5-hydroxymethylcinnamyl alcohol based AB_2 polymers **38** – **40**.[49, 51]

Scheme 7 38C2 Antibody mediated self-immolative disassembly of linear (2-amino-5-hydroxymethyl)cinnamic acid based polymer **43**.[52]

Synthesised from the homopolymerisation of comonomer **37** in the presence of DBTL, copolymer **39** was prepared with an average degree of polymerisation of 7, and displayed enhanced solubility under physiological conditions. By preventing polymer aggregation, this facilitated the enhanced self-immolative elimination of *p*-nitroaniline (*ca.* 6 h) from its periphery upon incubation with a catalytic antibody.[49]

Linear self-immolative polymers of (2-amino-5-hydroxymethyl)cinnamic acid displayed both comparable aqueous solubility and rates of self-immolative elimination under physiological conditions.[45, 51] Weinstein and co-workers have demonstrated the potential of these polymers for enzyme detection. (2-Amino-5-hydroxymethyl)cinnamic acid exhibits a strong fluorescence which can be attenuated when the amino substituent is masked as an amide. In this study, oligomers **41** and **42** plus polymer such as **43**, in the presence of an antibody, disassemble via an initial retro-Michael reaction and decarboxylation to liberate the free aniline intermediate **44**. Subsequent depolymerisation affords a high local concentration of **45** which can be quenched by the nucleophilic addition of amino acid residues from the enzyme active site and surrounding surface to afford the aniline **46** (**Scheme 7**).[52] This results in the formation of a covalent linkage between the enzyme and liberated fluorescent reporter molecule. The ability of **43** to label the enzyme without significant loss in activity was attributed to the capability of much of the polymer to diffuse away from the active site and react with distant nucleophilic residues on the enzyme surface, resulting in allosteric labelling, at low polymer concentrations (100 μM). Monomer **41** and trimer **42** also displayed limited labelling capabilities as a result of their ability to deactivate the enzyme via alkylation of the ε-amino lysine residue of the active site with minimal additional tagging.

Esser-Kahn and co-workers have recently found that suitable side-chain modification of polymers based on the carbamate repeat unit (i.e. **41**) in conjunction with subsequent reaction with 2,4-toluene diisocyanate afforded cross-linked materials (M_n 15,600 – 17,300, PDI = 2.48 – 3.01) that were designed to rupture when exposed to appropriate chemical triggers.[53]

The sequential combination of multiple linear self-immolative linkers has also been demonstrated. In a seminal example reported by De Groot and co-workers, this approach has been employed as an effective method for maximising the enzymatic activity of prodrug conjugates through minimization of detrimental steric enzyme-prodrug interactions.[26] More recently, DeWit and co-workers have developed methodologies for the condensation polymerisation of *N*,*N*'-dimethylethylenediamine, and *p*-hydroxybenzyl alcohol[54] (d.p. 16, PDI = 1.58) or thioethanol[55] (d.p. 35, PDI = 1.6), to afford linear self-immolative polymers capable of cascade depolymerisation upon removal of a single trigger moiety installed as a capping agent.

3.2. Self-Immolative Dendrimers

Dendrimers are perfectly branched and well-defined macromolecules which are characterised by high densities of terminal functional groups and compact, globular structures.[56] These branched macromolecules are obtained from a step-wise synthetic approach whereby selective combination of AB_n monomers via divergent[57, 58] or convergent[59] growth provide macromolecules which possess very few structural defects and high degrees of molecular uniformity. The unique nature of the dendritic architecture and potential for high peripheral loading or encapulation makes these branched macromolecules highly suitable candidates for applications including drug delivery, catalysis, sensors, imaging, gene therapy and solubilising agents.

The study of the release of molecular species by covalent dissociation of dendrimers has led to the concept of 'cleavable dendrimers'.[60] Several groups have investigated the controlled self-immolative elimination of peripheral groups from the surface of dendrimers for fragrance delivery or sensing applications.[61-63] Expansion of this concept has encompassed "self-immolative dendrimers".[18, 20, 64] Unlike conventional degradable dendrimers, where each molecule is released by an independent activation process, self-immolative dendrimers are able to release all peripheral molecular species (undergo complete fragmentation) in an amplified manner via a cascade of elimination reactions from a single activation event at the dendrimer core.

Self-immolative dendrimers are frequently synthesised via a divergent approach, which compliments the direction of the self-immolative cascade. Inclusion of a protecting group at the focal point facilitates the development of generations via a repetitive activation-coupling process. The size and thus

generation attainable for self-immolative dendrimers is dependent upon the steric bulk of the reporter group and linker multiplicity (i.e. the AB_n branched unit). Dendrimer solubility has also been found to be strongly influenced by the nature of the reporter group and linker hydrophobicity. Aniline based branched linkers have been utilised preferentially as a result of their unsurpassed self-immolative ability under physiological conditions and their ability to form carbamate linkages between the different generations. Phenol based linkers have also been employed as a consequence of their relative ease of synthesis, but require the incorporation of additional cyclisation elimination linkers such as ethylenediamine units to form stable carbamate linkages with trigger and reporter moieties.[36]

3.2.1. Self-Immolative Elimination Linker Types

3.2.1.1. Aniline Based AB_2 Linkers

De Groot and co-workers introduced the concept of branched self-immolative elimination with first and second generation dendrons featuring a 2-(4-aminobenzylidene)propane-1,3-diol AB_2 self-immolative linker that was capable of degrading via two 1,8-elimination processes from a single activation event.[18] The synthesis of dendron **47** (**Figure 5**), starting from 2-(4-nitrobenzylidene)propane-1,3-diol, facilitated the divergent growth of multiple generations via alternate acyl transfer with *p*-nitrophenylchloroformate followed by coupling of additional branched linker. Paclitaxel (Taxol), was utilised as the model reporter group and attached via the intermediate *p*-nitrophenylcarbonate. Dendron **47** was obtained in 12 % yield overall via a four step synthesis. The presence of an aniline based substituent resulted in the formation of stable carbamate linkages between dendron generations, and afforded a self-immolative dendron capable of disassembly under mildly acidic conditions (Zn/AcOH in MeOH). The validity of the self-immolative ability of **47** was examined by analogous reductive self-immolative elimination experiments conducted on 2'-*O*-(cinnamyloxycarbonyl) paclitaxel, which confirmed that the absence of an amino substituent inhibited drug release. The self-immolative ability of the 2-(4-aminobenzylidene)propane-1,3-diol branched linker was comparable with that of the linear *p*-aminocinnamyl alcohol linker capable of undergoing analogous 1,8-elimination in a manner similar to **38 – 40** (**Scheme 6**).[25]

Figure 5 Second generation 2-(4-aminobenzylidene)propane-1,3-diol AB_2 based dendron **47**.[18]

3.2.1.2. Aniline Based AB_3 Linkers

Shabat and co-workers have demonstrated the specific pencillin-G-amidase mediated hydrolysis of phenylacetamide protected, self-immolative, AB_3 functionalised, first generation dendrons to release tryptophan and melphalan under physiological conditions (based on **48**, **Figure 6**).[65] Furthermore, utilising the same 2,4,6-tris(hydroxymethyl)aniline AB_3 branched linker capable of undergoing triple elimination, Shabat and Sella have demonstrated the heightened sensitivity of fluorogenic probes designed to detect triacetone triperoxide explosives.[47] 2,4,6-Tris(hydroxymethyl)aniline AB_3 based dendron **48** (**Figure 6**) featuring the use of a borate ester as a masked hydroxyl group, was shown to undergo complete disassembly upon oxidation of the C-B bond and treatment with alkaline hydrogen peroxide. As a result of its dendritic platform, amplified release of three equivalents of peripheral (4-amino-1,3-phenylene)diacrylic acid via a single 1,6- and then two 1,4-elimination processes, facilitated detection of triacetone triperoxide at the microgram level by fluorescence spectroscopic analysis and upon unmasking of the acetylated aniline. As expected, self-immolative dendron **48** was reported to be 3-fold more sensitive than its monomeric analogue as well as the corresponding borate ester functionalised self-immolative sensors featuring peripheral *p*-nitroaniline and 7-amino-4-methylcoumarin studied by Lo and Chu.[66]

Figure 6 First generation 2,4,6-tris(hydroxymethyl)aniline AB_3 based dendron **48**.[47]

3.2.1.3. Phenol AB_2 Based Linkers

Szalai and co-workers have demonstrated that the self-immolative ability of the 2,4-bis(hydroxymethyl)phenol AB_2 linker under mildly basic conditions is sufficient to mediate the disassembly of ether linked first and second generation 2,4-bishydroxymethyl)phenol based dendrons.[20, 67] An independent observation reported by Senter and co-workers highlighted that replacement of a carbonate linkage with an ether moiety impacts significantly on the rate of self-immolative elimination of the incorporated linker.[68] This limits such linkers to the release of amino or hydroxyl substituted aromatic units, which have better abilities to act as leaving groups as a consequence of their reduced pK_a values (*c.f.* aliphatic amines and alcohols).[68, 69]

Szalai and co-workers devised an efficient synthetic methodology, which was applied to the divergent synthesis of *p*-nitrophenol functionalised dendrons based on **49** (**Figure 7**).[20, 67] By selective protection of dimethyl 4-hydroxyisophthalate or 2,4-diformylphenol, successive generations were compiled by repetitive reduction, chlorination and etherification. The self-immolative ability of dendron **49**, obtained in 23 % yield (over seven steps), was demonstrated by the release of *p*-nitrophenol via photolytic cleavage of the *o*-nitrobenzylether protecting group followed by disassembly of the corresponding phenol under mildly basic conditions. Degradation of dendron **49** was monitored by ^{1}H NMR spectroscopic analysis, which revealed the formation of 2,4-dimethylphenol from quenching of the quinone methide intermediate with sodium borohydride in DMSO-d_6.

Figure 7 Second generation 2,4-bis(hydroxymethyl)phenol AB_2 dendron **49**.[20, 67]

2,6-Bis(hydroxymethyl)-*p*-cresol is a commercially available AB_2 branched linker capable of double 1,4-elimination and is a structural varient of the 2,4-bis(hydroxymethyl)phenol AB_2 linker. Shabat and co-workers first demonstrated the self-immolative ability of this linker with the synthesis of first and second generation dendrons based on **50** (**Figure 8**), which featured a photolabile protecting group joined through an ethylenediamine cyclisation linker and peripheral aminomethylpyrene.[19] Photochemical cleavage of the protecting group initiated spontaneous cyclisation of the ethylenediamine spacer to afford 1,3-dimethylimidazolidin-2-one and the unprotected phenol, which under mildly basic conditions (10 % triethylamine (TEA) in MeOH), underwent 1,4-elimination and decarboxylation to liberate an aminomethylpyrene molecule via quinone methide formation. Solvolytic quenching and reformation of the phenol, facilitated a second 1,4-elimination and concomitant decarboxylation to liberate a second aminomethylpyrene molecule and 2,6-bis(hydroxymethyl)-*p*-cresol. The slightly basic conditions were necessary to facilitate self-immolative elimination via the formation of the more reactive phenoxide following deprotection. The equivalent third generation dendron could not be synthesised as a result of the limited loading capacity of higher generation dendrimers for sterically bulky peripheral substituents as a consequence of the compact structure of the dendron surface. However, the corresponding *p*-nitroaniline functionalised third generation dendron featuring a *t*-butyloxycarbonyl trigger, separated by a ethylenediamine based cyclisation linker, was synthesised and was shown to undergo complete disassembly to release eight molecules of *p*-nitroaniline under analogous conditions upon a single trifluoroacetic acid (TFA) mediated cleavage of the *t*-butyloxycarbonyl group followed by neutralisation with TEA.

Figure 8 First generation 2,4-bis(hydroxymethyl)-4-cresol based AB_2 dendron **50**.[19]

Fomina and co-workers have utilised 2,6-Bis(hydroxymethyl)-*p*-cresol featuring a UV and near-IR sensitive *ortho*-nitrobenzylether trigger for the synthesis of polymeric nanoparticles.[70] Copolymerisation with adipoyl chloride afforded polyesters (up to 65,000 Da, PDI = 1.54) capable of encapsulating Nile Red. Irradiation at 350 or 750 nm resulted in the self-immolative elimination of cresol units and the perforation of these nanoparticles.

3.2.1.4. Phenol AB_3 Based Linkers

Extending the results from the 2,6-bis(hydroxymethyl)-*p*-cresol AB_2 linker, 2,4,6-tris(hydroxymethyl)phenol is able to facilitate triple elimination of substituents via two 1,4-elimination processes and an additional 1,6-elimination pathway through the corresponding *p*-hydroxylmethyl group in a manner reminiscent of De Groot's 2,4,6-tris(hydroxymethyl)aniline **17** (see **Scheme 4**). Haba and co-workers have reported the synthesis and self-immolative elimination of a first generation 2,4,6-tris(hydroxymethyl)phenol based dendritic heterotrimeric prodrug (**Scheme 8**).[71] Employing a divergent synthetic approach facilitated the step-wise coupling of doxorubicin directly through its primary amine and etoposide and camptothecin via the bisamine cyclisation linkers. To account for the steric bulkiness of the peripheral substituents, the enzyme labile trigger moiety was joined through a series of linear *p*-hydroxybenzyl alcohol and bisamine cyclisation linkers. The self-immolative elimination of dendron **51** was demonstrated under physiological conditions in the presence of catalytic antibody 38C2 enzymes, which facilitated dendron disassembly through retro-aldol, retro-Michael cleavage of the trigger group. In this seminal tritherapy study, three different drugs were released from a single molecular scaffold, via a 1,6- and two 1,4-elimination processes, using only a single activation event and this approach proved more effective than the individual monomeric or heterodimeric[72] prodrugs when compared *in vitro*.[71]

Scheme 8 Triple self-immolative elimination of doxorubicin, etoposide and camptothecin from 2,4,6-tris(hydroxymethyl)phenol based AB_3 dendron **42**.[71]

3.2.1.5. Phenol AB_6 Based Linkers

Solubility studies conducted on higher generation AB_2 self-immolative dendrimers revealed that their high hydrophobicities tend to cause aggregation under aqueous conditions.[73, 74] This detrimental property poses a significant problem since the majority of systems developed to date have focussed around the area of controlled release for applications of biological importance. Shabat and Shamis have investigated the possibility of further increasing the multiplicity of branched linkers to afford lower generation self-immolative dendrimers with higher loading capabilities coupled with enhanced aqueous solubilities.[74] Based upon 2-(4-aminobenzylidene)propane-1,3-diol, modification at the *ortho* positions afforded an AB_6 branched linker (**Figure 9**). Starting from 2,4,6-triformylphenol, dendron **52** was obtained via attachment of an *N*-*t*-butyloxycarbonyl (Boc) protected ethylenediamine trigger group, followed by sequential condensation with diethyl malonate and diisobutylaluminium hydride mediated reduction. Acyl transfer utilising *p*-nitrophenylchloroformate followed by displacement of *p*-nitrophenol with aminomethylpyrene afforded the desired hexa-functionalised first generation dendron in 1 % yield overall. The self-immolative ability of this linker was demonstrated by the release of six molecules of pyrene (*ca.* 6 h), via four 1,6- and two 1,8-elimination processes mediated by TFA cleavage of the Boc protecting group, followed by neutralisation with tetrabutylammonium hydroxide.

Figure 9 First generation AB_6 dendron **52**.[74]

3.2.2. Relayed Amplified Self-Immolative Elimination

In amplified release systems described here the single activation of a self-immolative dendron or polymer leads to the enhanced liberation of a defined, finite number of reporter species. Shabat and Sella have developed a self-immolative dendron that is capable of undergoing a 'dendritic chain reaction' where the release of a reporter species facilitates the disassembly of additional dendrimer molecules.[46] This process may be expressed as a 'relayed amplified self-immolative elimination'. Shabat and Sella demonstrated the principal of relayed amplified self-immolative elimination with a first generation dendron capable of releasing a fluorogenic probe (for degradation analysis) and multiple reporter groups which could facilitate further dendron breakdown. Boronic acid protected 2,4,6-tris(hydroxymethyl)phenol based AB_3 dendron **53** featuring one molecule of *p*-nitroaniline and two molecules of choline was shown to degrade via relayed amplified elimination in the presence of hydrogen peroxide under mildly basic aqueous conditions (**Scheme 9**).

Scheme 9 Relayed amplified self-immolative elimination of first generation 2,4,6-tris(hydroxymethyl)phenol based AB_3 dendron **53**.[46]

Scheme 10 38C2 Antibody or PGA mediated self-immolative disassembly of 2,4-bis(hydroxymethyl)phenol based dendrimer **55**, inset – monomer **58**.[75]

The liberation of choline and its subsequent oxidation to hydrogen peroxide by the addition of choline oxidase enzyme facilitated exponential self-immolative elimination of all of the dendrons present, at hydrogen peroxide concentrations as low as 5 μM, as determined by the UV spectroscopic analysis of liberated *p*-nitroaniline. Unfortunately, the sensitivity of this dendritic probe was limited by hydrolysis of carbonate bound choline in the absence of activating enzymes. Shabat and Sella have extended this concept of relayed amplified self-immolative elimination to detect the presence of activating enzymes. Incubation of **54** in the presence of penicillin-G-amidase enzymes led to the release of choline, which in the presence of **53** and choline oxidase, facilitated the exponential release of *p*-nitroaniline. Instead, the conjugation of peripheral methanol which could be oxidised to hydrogen peroxide by alcohol oxidase enzymes to facilitate exponential self-immolative elimination, afforded a conjugate system that proved relatively stable to hydrolysis and thus provided enhanced sensitivity.[76] Sella and co-workers have extended this concept of relayed amplified self-immolative elimination to a two-component system, whereby the disassembly of the first component results in the exponential release of an analyte which facilitates the disassembly of a second component.[77] Furthermore, the incorporation of a reducible quinone trigger moiety and peripheral mercaptoacetic acid has been shown to be an effective sensor for the detection of sulfhydryl compounds.[78]

3.2.3 Receiver-Amplification Self-Immolative Elimination

Recevier-amplification self-immolative elimination may be defined as the release of a *single* reporter group or self-immolative dendron from the dendrimer core via a linear electronic cascade of an A_nB type self-immolative dendrimer upon a single activation event at the dendrimer periphery. The release of a self-immolative dendron results in the amplified release of multiple reporter groups.

Szalai and co-workers first demonstrated this concept with the disassembly of benzyl ether functionalised first and second generation dendrons that were capable of undergoing a linear electronic cascade upon allyl deprotection at the dendritic periphery to facilitate subsequent release of a single *p*-nitrophenol molecule from the dendron core.[79] Whilst at first this principal seems counter-effective, Shabat and co-workers have reported that the electronic cascade can propagate from the periphery of one dendron, through the dendrimer focal point where it is amplified and facilitates the liberation of multiple reporter groups from the periphery of a second dendron.[80,27]

First and second generation dendrimers based on **55**, have been shown to undergo complete disassembly under aqueous conditions upon a single triggering event at the dendron periphery. The suitably designed diethylenetriamine A_2B branched cyclisation linker propagates a linear self-immolative cascade towards the dendrimer core, via the formation of an imidazolidinone, whilst a 2,6-bis(hydroxymethyl)phenol based AB_2 branched linker facilitates amplified self-immolative elimination of peripheral 6-aminoquinoline (**Scheme 10**). The obvious advantage to this design is that multiple and different trigger moieties can be simultaneously incorporated to afford a self-immolative dendrimer that is sensitive to multiple orthogonally activating processes (*e.g.* different activating enzymes).[75]

3.3. Polymer-Dendron Conjugates

The tendency for higher generation self-immolative dendrimers to aggregate in aqueous solution as been an inherent problem with these systems and has prompted the development of strategies to improve dendrimer solubility. As a case in point, Shabat and co-workers have observed that enzymatic activation of higher generation dendrimers can be inefficient under physiological conditions.[73, 74] To negate this, simple modification of the leaving group (*e.g.* inclusion of ionizable side chains) can provide dramatic enhancements in dendrimer aqueous solubility.[73]

Unfortunately, this is not a suitable strategy for the controlled release of bulky, hydrophobic reporter groups that are not amenable to structural modification. As a consequence, Shabat and co-workers have studied the release of highly hydrophobic chemotherapic agents from water soluble *N*-(2-hydroxypropyl)methacrylamide (HPMA) based conjugates that feature a 2,4,6-tris(hydroxymethyl)aniline, AB_3 branched, linker that, in turn, is coupled through a Gly-Phe-Leu-Gly-Phe-Lys peptide and *p*-aminobenzyl alcohol self-immolative linker (**Scheme 11**).[81] Synthesised in a linear fashion, starting from L-Boc-Phe-OH, the corresponding peptide protected AB_3 prodrug was coupled to copolymeric HPMA (31,600 Da, PDI = 1.66) with an average loading of 7 taxol molecules per HPMA polymer (average of 2⅓ dendrons) as determined by UV spectroscopic analysis (self-immolative dendritic loading capacity is typically *ca.* 4).[18, 19] The incorporation of a linear

p-aminobenzyl alcohol self-immolative linker to maximise spatial separation of polymer and reporter moieties, was found necessary to relay enzymatic activity. As a consequence of its high loading self-immolative dendritic structure, polymer conjugate **59** exhibited superior antitumour activity with respect to traditional monomeric polymer-drug conjugates. To our knowledge this is the only example, to date, of a comb polymer conjugate which bears a self-immolative dendritic moiety that is capable of amplified release.

Scheme 11 Cathepsin B mediated self-immolative disassembly of HPMA – 2,4,6-tris(hydroxymethyl)aniline based AB_3 conjugate **59**.[81]

Alternative studies have focussed on the modification of self-immolative dendrimers with solubilising polymers based on **55** (**Scheme 10**). Modification of the branched linker to incorporate solubilising polymers was considered as a suitable point of conjugation, since linker multiplicity and self-immolative ability would not be compromised. Amidation of 4-hydroxybenzoic acid with propargylamine followed by formylation in the presence of formaldehdyde and sodium hydroxide afforded 4-hydroxy-3,5-bis(hydroxymethyl)-*N*-propargylbenzamide AB_2 linker **58** which facilitated the coupling of an azido functionalised polyethyleneglycol (PEG) chain via an efficient copper-catalysed cycloaddition process. Formation of the corresponding triazole, mediated by copper (II) sulfate and copper (0) in the presence of tris((1-benzyl-triazol-4-yl)methyl)amine in DMF, afforded a highly water soluble polymer-dendron conjugate.[82] Further work highlighted that the increased hydrophobicity of camptothecin functionalised dendrons, required extended PEG chains (up to 5000 Da) to provide adequate aqueous solubility and prevent aggregation *in vitro*.

The divergent synthetic approach undertaken with these conjugates facilitated the formation of 4-hydroxy-3,5-bis(hydroxymethyl)-*N*-propargylbenzamide based polymer-dendron conjugates bearing solubilising PEG (based on **55**, **Scheme 10**) and dual UV-vis and fluorescence sensing abilities at the periphery.[83] This route could enable the facile incorporation of alternative chromogenic reporter groups and thereby the type of signal output desired in the molecular probe. Furthermore, the nature of the trigger could be easily modified to allow for the highly sensitive detection of alternative enzymatic activities or chemical processes.

4. Non-Amplified Self-Immolative Polymeric Conjugates

4.1 Linear Self-Immolative Linkers in Polymer Conjugates

Self-immolative polymer conjugates may be defined as polymer conjugates which feature one or more non-amplified self-immolative linkers. The majority of research regarding self-immolative polymer conjugates has focussed on applications of biological relevance (*e.g.* drug delivery) and stems from pioneering studies on HPMA based conjugates conducted by Duncan and co-workers.[84] Synthetic polymers (*e.g.* HPMA and PEG) have been widely used in a biological targeting role because of their well-established biocompatibility, non-immunogenicity, non-toxicity and excellent aqueous solubility.[85,86] These polymers possess the added advantage of the versatility of synthetic chemistry that allows for molecular weight control and addition of biomimetic functions, in addition to the ability of passive tumour targetting via the enhanced permeability and retention effect.[87] Self-immolative linkers had been combined with these polymers to afford self-immolative copolymeric conjugates.

4.1.1 HPMA Polymer Conjugates

Kopecek and co-workers discovered that for the azoreductase enzyme mediated release of camptothecin from a HPMA polymeric conjugate, the inclusion of a self-immolative linker was necessary to obtain effective rates of cleavage.[88, 89] A *p*-aminobenzyl alcohol self-immolative linker was incorporated through an azo linkage, formed from the reaction of *p*-aminobenzyl alcohol and 2-chlorophenol in the presence of sodium nitrite under aqueous acidic conditions. An acrylate monomer capable of undergoing azo-di-isobutylnitrile initiated radical polymerisation was obtained from step-wise functionalisation of the phenol group with bromoacetic acid and 3-aminopropyl methacrylamide followed by incorporation of 9-aminocamptothecin at the benzylalcohol position via a

carbamate linkage using phosgene. Conjugate **60** (**Figure 10**) was obtained from HPMA copolymerisation and found to degrade rapidly in the presence of azoreductase enyzmes in a manner independent of the drug loading (2.4 – 6.8 wt. %).

Figure 10 Azo protected HPMA self-immolative prodrug conjugate **60**.[88]

4.1.2 PEG Polymer Conjugates

The combination of multiple self-immolative linkers in a linear fashion has been demonstrated to be an effective method for enhancing the rate of enzymatic activation through minimising steric interactions between trigger and reporter moieties.[90] Ryu and co-workers have reported that the self-immolative elimination of paclitaxel from succinic acid based PEG conjugate **61**, is greatly enhanced ($t_{½}$ > 7 h and = 0.94 min, for **61** and **62**, respectively) by the inclusion of a second formalin based self-immolative linker capable of a 1,2-self-immolative elimination (deformylation) (**Figure 11**).

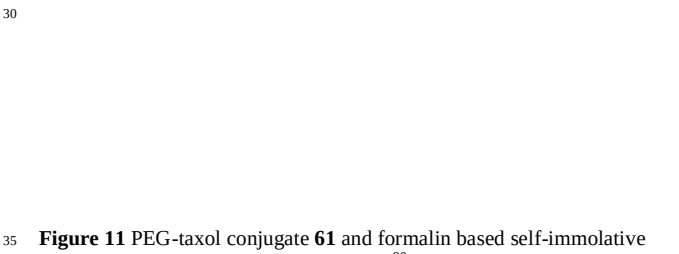

Figure 11 PEG-taxol conjugate **61** and formalin based self-immolative conjugate **62**.[90]

Greenwald and co-workers have conducted systematic studies on how the structures of amino- and hydroxy- substituted benzylalcohol based self-immolative linkers, and the nature of their conjugation with PEG prodrug conjugates of daunorubicin, effect the rate of enzyme mediated cleavage.[91, 92] The conjugation of PEG to *p*-aminobenzyl alcohol self-immolative linkers via hydrolytically stable amide or carbamate bonds (**Figure 12 – 63**, **64**) resulted in slow rates of hydrolysis. Carbamate linked conjugate **65**, featuring *p*-hydroxybenzyl alcohol, displayed comparable hydrolytic lability with respect to carbonate **66** as these linkages have reduced resonance into the carbonyl from the phenol oxygen. As expected, ester bound conjugates **67** – **69** were most rapidly hydrolysed and as a consequence, conveyed the highest activity *in vitro*. The incorporation of methyl or methoxy substituents at the *meta* positions of the *p*-hydroxybenzyl alcohol self-immolative linker resulted in reduced drug activity, in both cases, as a result of these substituents exerting a steric effect at the site of enzyme hydrolysis. This contributed to a *meta* destabilising effect of the methoxy substituents on the benzylic position (σ_m = +0.12), whilst negating the stabilising effect of *meta* methyl substituents (σ_m = -0.07).

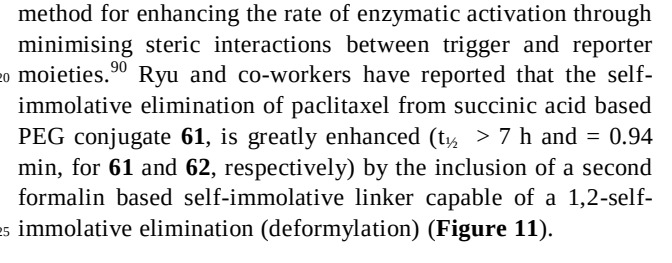

Figure 12 Self-immolative PEG-doxorubicin conjugates **63** – **69**.[91]

Further studies by Greenwald and co-workers have focussed on the structure-activity relationships of PEG-daunorubicin conjugates featuring *o*-hydroxyhydrocinnamic acid derived cyclisation linkers.[93] The incorporation of ring or *β*-substituents (*e.g. gem*-dimethyl) to this phenolic self-immolative linker has proven effective for increasing the population of the reactive conformer to facilitate 1,6-cyclisation.[41, 42] From the range of conjugates studied, *in vivo* evaluation highlighted that the arrangement of substituents about the self-immolative linker provided the most noticable enhancements in conjugate stability and drug efficacy. However, these enhancements were induced through steric interactions that prohibited enzymatic activation as opposed to facilitating enhancements in the cyclisation elimination process. Minimal variation of conjugate stability was observed from structural modification of the PEG chain or the nature of the polymer-self-immolative linker bond. Lee and co-workers have further applied these linker technologies to the selective self-immolative elimination of lysozyme enzymes from multi-PEGylated conjugates.[94]

Branched polymer conjugates have also been developed which feature a bicin cyclisation-elimination linker.[95, 96] This A_2B diethanolamine based branched linker facilitates the conjugation of two PEG polymer chains to a single reporter group which, in turn, has been shown to impart enhanced drug solubility and prolonged circulation times *in vivo*. PEG- drug and protein conjugates based on **70** have been shown to

Scheme 12 Cyclisation elimination of bicin based A_2B PEG-protein conjugate **70**.[95, 96]

fragment via formation of morpholinolactones **71** or **72** upon a single enzyme activation (**Scheme 12**). In line with the results reported earlier by Suggs,[97] following hydrolytic cleavage of the second PEG chain to form diol **73**, an enhanced rate of cyclisation-elimination to afford **72**, was observed. The reasons behind this rate enhancement are not completely clear. Reaction through the conformation of **73** proposed by Suggs allows approach of the alcohol on a Bürgi-Dunitz trajectory in a 6-*exo*-trig mode. In this conformation, with the second hydroxyethyl group equatorial, that hydroxyl group cannot reach the amide and its participation in the reaction appears limited to hydrogen bonding to the amine of the bicin linker. If this hydroxyethyl group is axial then an interaction with the amide carbonyl, through hydrogen bonding, is possible. Suggs's observation of reduction in hydrolysis rate, for the corresponding compound with a single hydroxyethyl group, by more than the statistical amount, suggests that both groups act cooperatively to enhance amide hydrolysis. To simplify the pharmacokinetic parameters, Filpula and co-workers modified the bicin linker to incorporate one PEG chain and an acetyl group, the latter being rapidly hydrolysed *in vivo*.[96]

4.1.3 Chemical Adapter Systems

Satchi-Fainaro and co-workers have reported the self-immolative elimination of a HPMA based copolymer conjugate of doxorubicin featuring a cephalosporin self-immolative linker based on **74**.[98] The self-immolative ability of this unique linker was demonstrated upon β-lactamase mediated hydrolysis of the β-lactam ring to form electron-donating enamine **75** capable of 1,4-elimination (**Scheme 13**). Liberation of doxorubicin was demonstrated *in vitro* by the co-administration of HPMA based polymers featuring the self-immolative prodrug and β-lactamase enzymes via a polymer-directed enzyme prodrug therapy strategy. Prior to this, Senter and co-workers reported the use of cephalosporin based self-immolative linkers in linear and branched PEG conjugates of doxorubicin.[99] The use of higher molecular weight branched PEG enhanced the conjugate hydrolytic stability, activity (IC_{50} = 8 μM, *c.f.* monomeric PEG conjugate = 80 μM) and circulation times *in vivo*.

The cephalosporin self-immolative linkers are a rare example of a linker type which is connected to the polymeric support through a stable bond that is not cleaved concomitantly with activation. Shabat and co-workers have further developed this concept and have reported that simply modified linear self-immolative linkers can facilitate polymer conjugation via a chemically stable bond without compromising the reporter release (**Figure 13**).[100] Described as 'chemical adapter systems', these modified linkers commonly possess three different functionalities, the first acting as a handle to which a targeting or anchoring moiety is attached (*e.g.* polymer). The second functionality acts as a (bio)chemical trigger which facilitates the self-immolative elimination of a third functionality resulting in the liberation of a reporter group. In an initial study, Shabat and co-workers demonstrated the self-immolative ability of a HPMA based polymer conjugate of etoposide featuring 4-hydroxymandelic acid, a commercially available trifunctional linear self-immolative linker.[101] Starting from 4-hydroxymandelic acid, conjugate **76** (**Figure 13**) was synthesised in 13 % overall yield over 6 steps by incorporation of a Boc protected-*N,N'*-dimethyldiethylenediamine side chain, followed by the addition of a protecting group via the corresponding *p*-nitrophenylcarbonate intermediate, capable of retro-aldol, retro-Michael elimination. Etoposide was incorporated through a ethylenediamine based linker, whilst TFA mediated deprotection of the side chain facilitated conjugation to polymeric HPMA.

Scheme 13 β-lactamase mediated self-immolative elimination of cephalosporin based HPMA polymer conjugate **74**.[98]

Self-immolative elimination of etoposide from conjugate **76** was demonstrated under physiological conditions in the presence of antibody 38C2. Shabat and co-workers have also demonstrated the self-immolative ability of HPMA based conjugate **67** (**Figure 11**) featuring a 2-amino-3-methylamino propionic acid linker, capable of cyclisation-elimination upon enzyme mediated activation.[102] In both cases, UV spectroscopic analysis revealed low drug loadings (average of 3 per HPMA conjugate (30,000 Da)).[101, 102] This was attributed to an inefficient conjugation strategy which employed mild coupling conditions. However, it can be envisaged that the steric bulk of these chemical adaptor systems could hinder high loadings through congestion at the polymer surface.

Figure 13 Self-immolative 4-hydroxymandelic acid and 2-amino-3-methylamino propanoic acid based HPMA polymer conjugates **76** and **77**.[101, 102]

The fact that these self-immolative linkers can facilitate the release of a reporter group but are themselves bound to a polymeric support that is separable, makes them highly suitable for solid-phase synthesis applications. Zheng and co-workers have investigated the solid-phase synthesis of short-chain peptides utilising a styrenic polymer conjugate which featured a reducible quinone capable of conformationally enhanced lactonisation cyclisation-elimination.[103] Conjugate **78** facilitated the synthesis of *C*-terminal modified tetra- and penta-peptides in high yields (70 – 90 %), which were isolated via mild chemical reduction (*e.g.* sodium hydrosulfite) of the quinone moiety followed by 1,6-cyclisation-elimination (**Scheme 14**). Conjugate **79** featured a chain extended tether located between the quinone and peptide linker moieties as utilised in the synthesis of *N*-terminal modified peptides.[104] Zheng and co-workers have also shown that this self-immolative linker is highly stable to strong acidic and peptide coupling conditions, however, it undergoes facile cyclisation following mild chemical reduction ($NaBH_4$) and tetrabutylammoniumfluoride mediated $B_{Al}2$ type ester cleavage.

Scheme 14 Reductive cyclisation elimination of oligomeric peptides from dihydroquinone based styrenic solid supports **78** and **79**.[103, 104]

Biocatalysed linkers have opened up advantageous alternatives to classical chemical approaches since enzymatic cleavage often occurs under very mild conditions and with pronounced selectivity.[105] Waldmann *et al*. have studied the solid-phase synthesis and self-immolative abilities of polymer conjugates featuring a 2-(2-(aminomethyl)-4,5-dimethoxyphenyl)acetyl[106] based cyclisation linker modified through the *para* ether substituent.[107, 108] The utility of conjugate **80** was demonstrated with the synthesis of a series of hydroxyl and amino based compounds utilising carbon-carbon cross coupling, Mitsunobu esterification and Diels-Alder cycloaddition processes. High isolated yields (60 – 94 %) were obtained through facile pencillin-G-acylase mediated hydrolysis of the phenylacetamide protecting group, followed by cyclisation elimination of the reporter moiety from stable amide or ester bonds (**Scheme 15**).

Scheme 15 PGA mediated cyclisation-elimination of 2-(2-(aminomethyl)-4,5-dimethoxyphenyl)acetyl based solid support **80**.[107, 108]

Concluding Remarks

In this article we have reviewed a structurally diverse range of self-immolative linkers which undergo cyclisation or electronic cascade reactions driven by entropic and thermodynamic factors. (See Table 1 for a summary of references categorised by self-immolative linker type). These linkers form stable bonds between a macromolecular scaffold and its reporter groups and are able to facilitate enhanced conjugate disassembly upon a specific activation event. The ability of these linkers to spontaneously fragment, yielding a high local concentration of active monomeric species, has been effectively exploited with amplified self-immolative

polymer and dendrimer systems. Key studies in this area have highlighted the high loading capabilities of self-immolative dendrimers and the excellent solubility and stability characteristics of self-immolative polymers and self-immolative polymer conjugates. The blending of innovative strategies for release with skillful application of synthetic methods has driven the field forward. The ease with which these linkers can be modified and incorporated into macromolecular conjugates has made them applicable to a wide range applications spanning drug delivery, sensors and diagnostics. The development of relayed amplified self-immolative elimination, high multiplicity (*e.g.* AB_6) branched linkers and efficient homopolymerisation processes has raised new opportunities for improved controlled delivery systems and exciting avenues for further investigation.

Table 1 Classification of self-immolative linker technologies

Size	Polymer Type	Linker Multiplicity	Mechanism of Disassembly	Signal Amplification	References
Small Molecules	N/A	AB	SIE[a]	Non-amplified	9, 10, 11, 12, 13, 23, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 66, 68
		AB	CE[b]	Non-amplified	35, 38, 39, 40, 41, 42
		AB	SIE and CE	Non-amplified	36, 37
Polymeric systems	Linear	AB	SIE	Non-amplified	26
		AB	CE	Non-amplified	55
		AB	SIE and CE	Non-amplified	54
		AB_2	SIE	Amplified	45, 48, 49, 51, 52, 57, 53
	Dendrimers	AB	CE	Non-amplified	61, 62, 63
		AB and AB_2	SIE	Non-amplified and Amplified	43
		AB_2	SIE	Amplified	18, 20, 67, 73
		AB_2	SIE and CE	Amplified	19, 44, 72
		AB_2	SIE	Relayed amplified	46, 76, 77, 78
		AB_3	SIE	Amplified	47, 65
		AB_3	SIE and CE	Amplified	71
		AB_6	SIE	Amplified	74
		AB		Receiver amplified	79
		A_2B	CE	Receiver amplified	27, 75
(Mixed polymer types)	Polymer-dendron conjugates	AB_3	SIE	Amplified	81
		A_2B and AB_2	SIE and CE	Receiver amplified	80
	Polymer conjugates	AB	SIE	Non-amplified	21, 22, 88, 89, 91, 92, 94, 98, 99, 101
		AB	CE	Non-amplified	90, 93, 102, 103, 104, 106, 107, 108
		AB_2	SIE and CE	Amplified	82, 83, 70
		A_2B	CE	Receiver Amplified	95, 96
	Antibody conjugates	AB	SIE	Non-amplified	69

[a] Self-Immolative Elimination, [b] Cyclisation Elimination.

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Notes and References

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