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Review

Peptide Multimerization as Leads for Therapeutic Development

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Abstract: Multimerization of peptide structures has been a logical evolution in their development as potential therapeutic molecules. The multivalent properties of these assemblies have attracted much attention from researchers in the past and the development of more complex branching dendrimeric structures, with a wide array of biocompatible building blocks is revealing previously unseen properties and activities. These branching multimer and dendrimer structures can induce greater effect on cellular targets than monomeric forms and act as potent antimicrobials, potential vaccine alternatives and promising candidates in biomedical imaging and drug delivery applications. This review aims to outline the chemical synthetic innovations for the development of these highly complex structures and highlight the extensive capabilities of these molecules to rival those of natural biomolecules.

Keywords: antimicrobial peptide; dendrimeric peptide; multimerization; therapeutics; biomedical applications

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1. Introduction

Oligomeric protein structures found in nature have inspired researchers to better design multimeric peptide therapeutics that are able to target these structures more efficiently. Developments that enable the mimicking of these complex assemblies include chemical peptide synthesis [1], bioconjugation [2,3] and dendritic polymer formation [4,5]. The sustained progress in protein structure determination [6] has not only led to the discovery of new therapeutic targets [7] but enabled a better understanding of the dynamic influence of these target proteins [8]. Advances in the development and utilization of biocompatible chemistries and methodologies can enable even an inexperienced bio/chemist to construct complex multimeric structures [9]. The high specificity and affinity of peptide drugs have been a strong driving force behind their expansion as therapeutics [10], such as the initial discovery of the native hormone peptides insulin [11] and glucagon [12] to the subsequent development and production of more active synthetic peptide analogues [13,14].

A recent review by our group outlined the chemical synthetic challenges over the last 100 years faced by researchers to undertake such a monumental task of synthetically producing such complex peptide structures and discussed the extensive chemical diversity of novel insulin analogues that have been achieved [15]. The same can be said for glucagon with the glucagon-like peptide 1 (GLP-1) [16] also having numerous promising synthetic analogues. For example, a modified GLP-1-albumin analogue that has been developed was shown to activate the GLP-1 receptor in vivo, of which the agonist, known as albiglutide, has now been officially approved by the FDA for clinical use under the tradenames Eperzan in Europe and Tanzeum in the US [17]. A number of other GLP-1 analogues are currently under review and look to also have promising activities and improved pharmacokinetics when compared with previous treatments [18]. The utility of peptides as potential

therapeutics has increased, as shown by the significant rate of FDA-approved peptide drugs [19]. However, peptidic drugs have widely acknowledged challenges and limitations because of their short plasma half-life, negligible oral bioavailability [20], unsuitability by standardised "Lipinski" rules [21], and degradation by numerous peptidases [22].

One approach to address several of the shortcomings of peptides is their covalent multimerization, which has shown broad benefits [23,24]. This can impart a multivalent nature to the molecule, where multiple surface structures are presented to receptors to achieve enhanced effect. This has attracted multiple groups from different research fields to develop novel methods that can produce not only poly-lysine- or peptide-based multimeric structures [25] but a wide range of biocompatible organic polymer-based analogues and combinations thereof [26]. Multimers can range from simple end-to-end or branched dimers (see Figure 1) to the more intricate multibranching dendrimer structures containing any number of biocompatible building blocks around a centralised core.

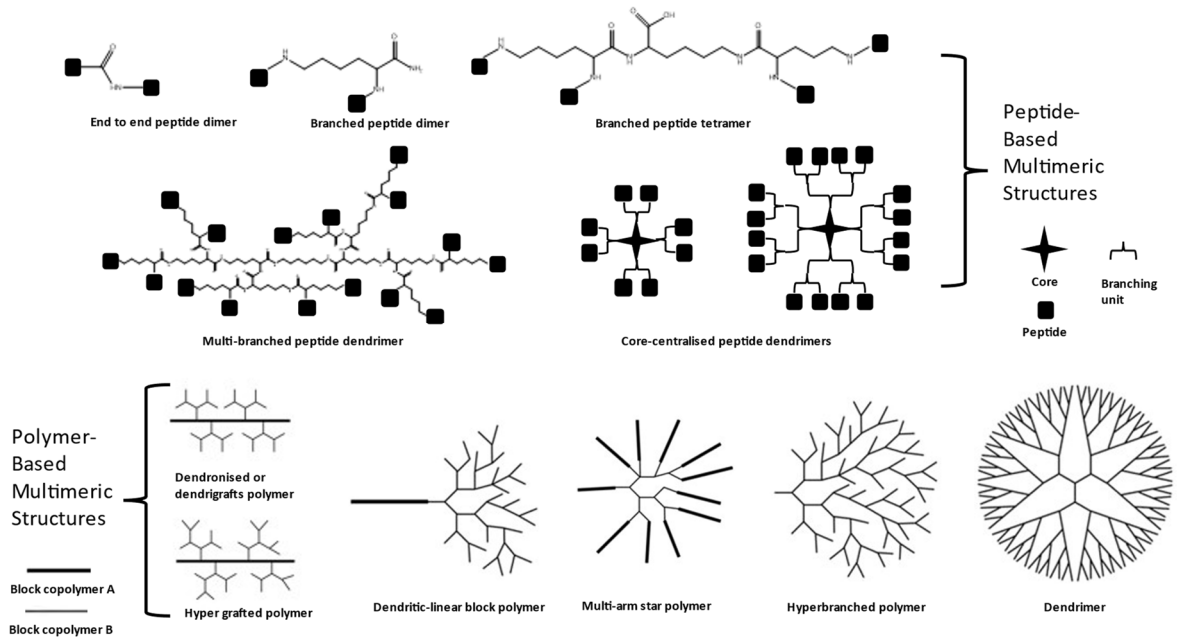

Figure 1. Peptide- and polymer-based multimeric structures adapted from [27]. The black star represents the core, the black square is a peptide, and the square bracket is a branching unit.

The current development of multimerization strategically positions researchers with an arsenal of tools to build and tailor these structures of increasing complexity and efficiency. These multimerized structures can target specific tissues and receptors, deliver drugs into a desired target site, release a drug molecule in a chosen physiological environment and even use particular moieties to cloak the therapeutic molecule to outsmart the pathogen and immune cells alike. Given the advanced development and application of peptide multimerization, in this review, we summarise the recent development of peptide-based multimerization therapeutics as inspiration for future drug development.

2. Overview of Multimerization and Peptide Dendrimers

Multimerization is a logically evolving strategy to mirror the native hetero and homo-multimeric protein structures [28]. To better distinguish between multimeric compounds and dendrimers, in this review, we will refer to multimers as analogues with two to four copies of presenting functionalities, whereas more complex dendrimer structures may contain any number of branches and >4 copies of these functionalities.

Many multimers have not only shown enhanced activity but displayed better in vivo stability over their monomeric counterparts. For instance, Gunasekera et al. showed the bioactivity enhancement of dimers Q5K and D9K, which led to the production of

a number of dimeric and backbone cyclized antimicrobial structures based on the minimum antibacterial region KR-12 (see Figure 2) [29]. Similarly, Carlucci et al. applied multimerized radio-pharmaceuticals to enhance their binding affinity, where they further explored the alteration of linker attributes such as length and flexibility and investigated ligand-receptor recognition and their effect on binding [30]. Bracci et al. demonstrated the high stability of several tetra-branched analogues of bioactive neuropeptides in plasma and serum to resist trypsin and chymotrypsin than the monomeric forms, including enkephalins, neurotensin and nociception [31].

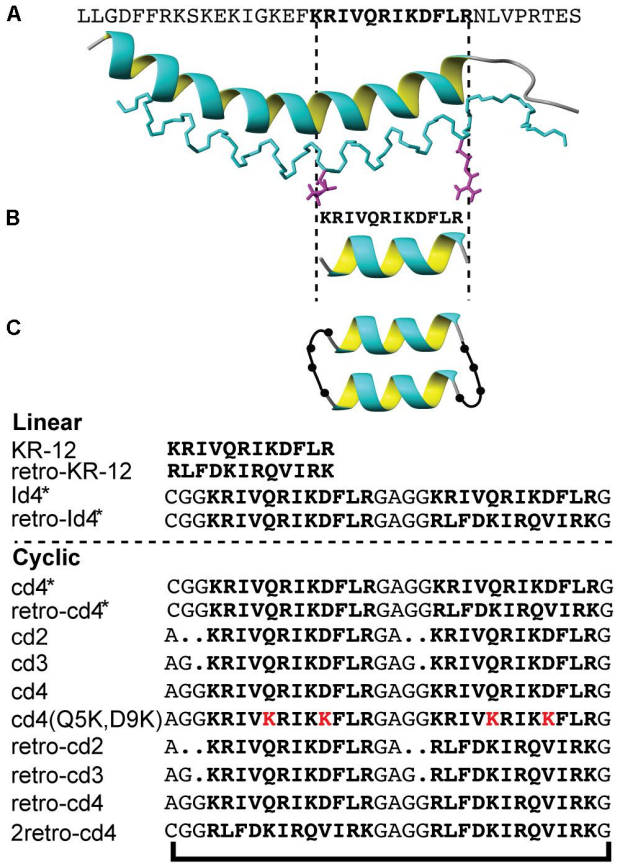

Figure 2. The design strategy of KR-12 cyclic analogues. (**A**) The three-dimensional structure of LL-37 (PDB code 2K60), (**B**) the minimum antibacterial region KR-12, and (**C**) a schematic representation of the backbone cyclized KR-12 dimer. Sequences of all analogues are provided. * indicates that the cysteine in the ligation-linker was not converted to an alanine in order to improve comparison with the non-ligated (linear dimer) peptide and the dot (.) is used to align the sequence. Reprinted with permission from [29] Copyright (2020), from Frontiers.

Currently, the covalent multimerization of these peptidic structures can be achieved in a number of ways, with one of the simplest being the utilization of a core [32] such as lysine which acts as a branching point where peptide sequences can be built using peptide synthesis and/or attached using chemical ligation methodologies to achieve the multimeric structure (Figure 3).

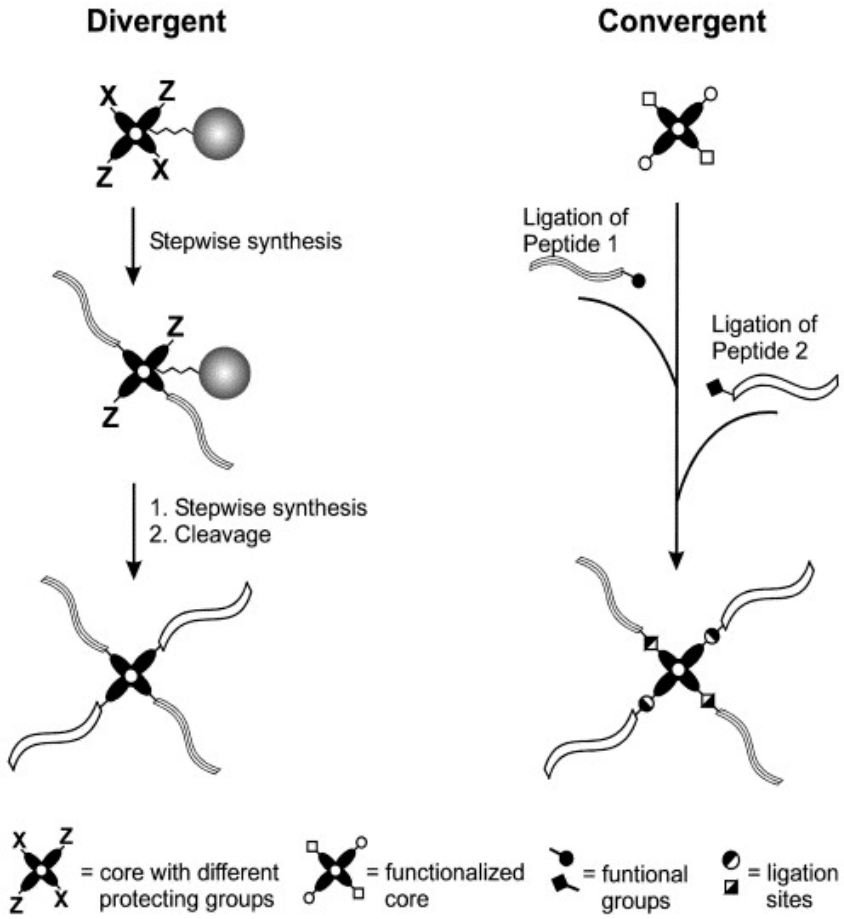

Figure 3. A schematic comparison of divergent and convergent synthesis methods. The grey sphere represents the solid support resin. Reprinted from [32] Copyright (2002), with permission from Elsevier.

Historically, these poly-lysine structures have also included the incorporation of spacing glycine residues to reduce any interchain aggregation that may occur [33]. Other more novel chemical spacers such as the use of a C12 long alkyl spacer in the synthesis of a glycodendrimer by Han et al. have now been applied to prevent issues such as crowding of surface structures and can even increase the flexibility of attached residues [34]. Recent developments in the use of arginine incorporated into the poly-lysine core were discussed by Sheveleva et al. which even allowed enhancements in transfection efficiency in these molecules [35], highlighting their practicality as efficient nanocarriers for drug and gene delivery.

The advanced development of multimeric core scaffold structures can not only build a wide range of multimer branching structures but form far more complex dendrimer structures which will be discussed in further detail below. The simpler poly-lysine-based peptide platforms can be synthesised entirely by standard solid-phase peptide synthesis (SPPS) as in the "direct" approach [36] or solution-phase peptide synthesis [37] and bioconjugation in solution of already purified segments in an "indirect" approach [38]. Given the well-developed SPPS methods [39], it allows for the step by step elongation of the multiple peptide chains with branching poly-lysine scaffolds on the solid phase. Solution-phase peptide synthesis involves a reaction that can be monitored to remove unwanted by-products at each step with better control over the target peptide purity, but it is time consuming and laborious of intermediate purification and characterisation of each step [40]. These approaches allow subsequent functionalisation with a well-equipped toolkit with a range of surface moieties to build a final decorated dendrimer structure (see Figure 4).

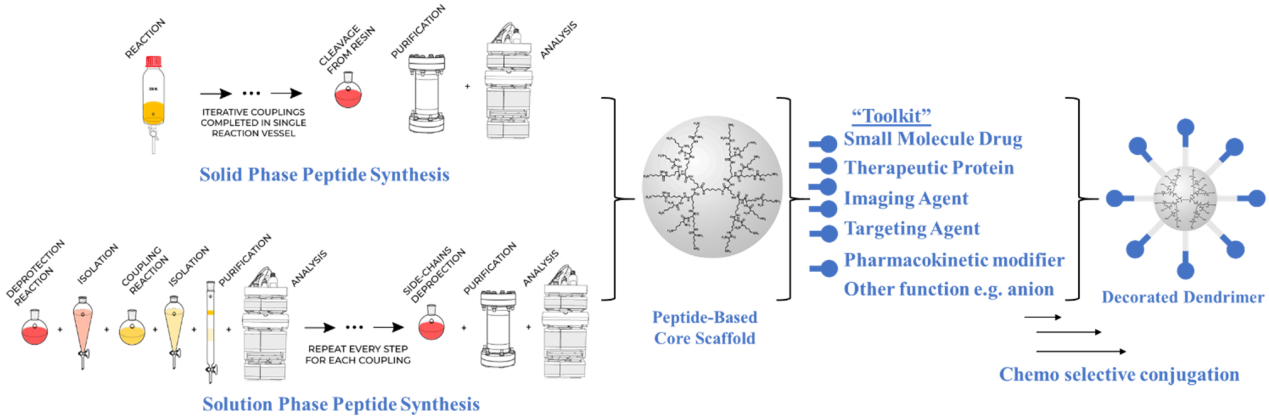

Figure 4. Solid/solution-phase synthesis schemes adapted from https://cpcscientific.com/why-solid-phase-synthesis/ (accessed on 23 December 2021), peptide core scaffold adapted from [41] and functionalisation toolkit/dendrimer structure adapted from https://starpharma.com/technology/advantages_of_dendrimers (accessed on 23 December).

Chemo-selective bioconjugation methodologies have also led the way in building these structures with a wide range of linker chemistries. Functionalisation of the N-terminal of peptide A and a C-terminal moiety of peptide B allows chemical conjugation to produce the desired multimeric structure. This was discussed by Kowalczyk et al. as a primary means to the building of poly-lysine-based multiple antigenic peptide (MAP) structures which exploits the thiol functionalisation of the antigen portion with an alkyl halide moiety on the tetravalent poly-lysine core to create the multimer conjugate [38] (Figure 5).

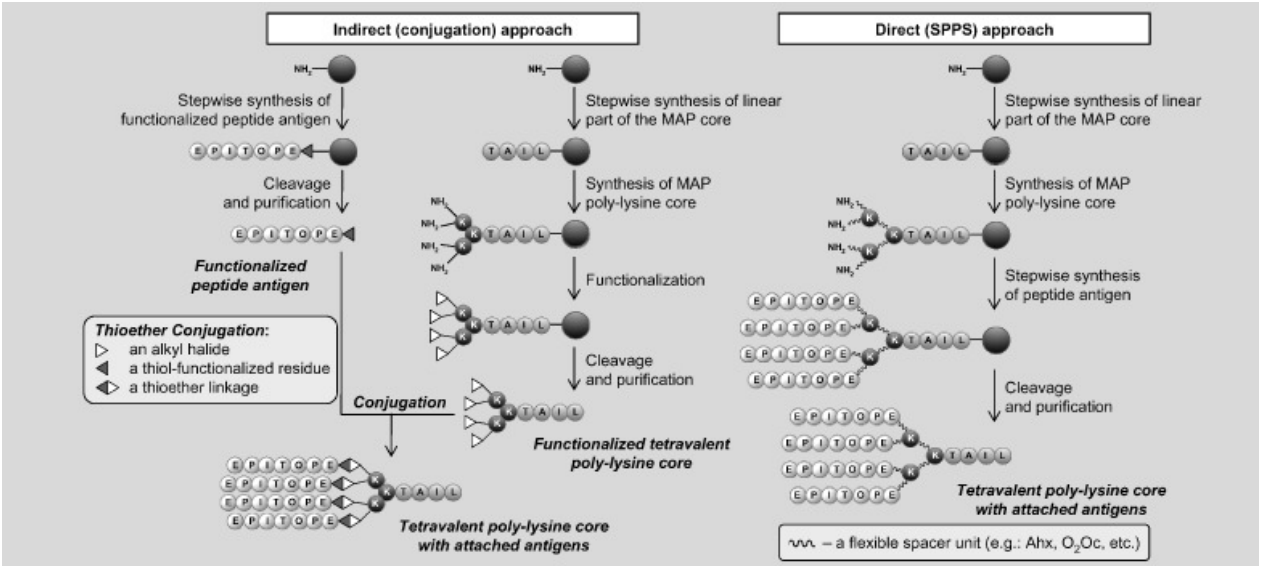

Figure 5. The synthesis of MAP structures via the direct SPPS approach and indirect chemical ligation approach. The grey sphere represents the solid support resin. Reprinted with permission from [38] Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd. (Oxford, UK).

The interest in creating larger constructs has seen a notable increase in papers exploring this idea, leading to a number of clinically developed examples [42]—these include multimeric ligands [43] and polymer–drug [44] and polymer–peptide conjugates [45]—and begins to bring to light the numerous benefits that multimerization offers [30]. An excellent review by Skwarczynski and Toth even gave insights into future of these structures such as the utilization of peptide-based synthetic vaccines, which can be chemically synthesised and could potentially mitigate a number of the disadvantages of traditional vaccines [46].

They further outline an exciting perspective of multimerized peptides for specific targeting, the introduction of modifications such as stapling, adjuvant usage and novel delivery systems for the therapeutic potential into the gastrointestinal tract (GT).

As these multimeric structures become more complex, other underlying factors come into consideration, including the flexibility of the core or branch subunits, which can have an effect on the conformational outcome of the final peptide structures [47]. Other more elaborate and specialized core architecture utilize specific folding of peptide/protein dendrimers into secondary structures by using poly proline-helices and 4-aminopyridine-2,4-dicarboxamide [48]. However, we will focus on the majority of cases in which cores are based on simpler small molecules.

Peptide dendrimers are an extension of multimers and fall under a type of classical amino-based polymer dendrimer which have a number of advantages over conventional linear polymer and peptide structures. Like the head-to-head dimers or multimers, the multivalent dendrimers have multiple surface structures presenting to receptors with expected high binding and affinity [49]. Other advantages of dendrimeric architecture include conveying unique properties to the molecule such as increased solubility for attached hydrophobic drug molecules [50], more desirable thermal stability [51], and the utilization of voids created in the macromolecular structure to hold therapeutic cargo for drug releasing [52]. Peptide dendrimers have also historically been built upon a poly-lysine core and will have functionalised peptides to achieve a desired effect [23]. To date, the variety of peptide dendrimers has grown to include a number of subtypes but can still be classified by the three main groups [32]. The first group includes grafted peptide consisting of an unnatural amino acid, organic group as the main branching core with peptide or protein surface group attachments. The second group involves polyamino acids with a natural amino acid core and terminal amino acids acting as surface functional groups. The last group is the classical peptide dendrimers where the core consists of amino acids usually poly-lysine and the surface functional groups are also peptide chains, these also include the subclass of MAP's and make up the majority of these dendrimer structures.

The trend to mimic the complexity of intricate natural structures with these synthetic designs has become more elaborate and included a range of variable components to induce desired effects. An interesting review by Sebestik et al. summarised the design of peptide and glycopeptide dendrimers structures and their biomedical applications [53]. It systemically outlined the fascinating design aspects available such as the use a compact core with more stability to trypsin cleavage, PEGylation to enhance solubility of attached molecules and the use of targeting sugars to reach particular areas of interest. As the dendrimeric structures have become quite intricate to achieve peptide-decorated molecules, it is important to understand the origins of classical polymeric dendrimer structures.

3. Overview of Dendrimers

Dendrimers are a class of compounds with a repeating branching structure which can be designed by use of different polymerization and synthetic chemical strategies. The name is derived from the Greek word dendron, which loosely translates to "tree" [54] and allows for an imagined branching structure from a central focal point. The evolution of these structures has become ever more intricate over time as the synthetic abilities to create complicated molecules expands. These compounds have ranged from the simpler homo and block copolymer star structures to multiple homopolymer stars, which are referred to as "miktoarm stars" [55] and the "hyperbranched" [56] crosslinked structures. A number of functionalities both on the surface and throughout the superstructure can be incorporated to achieve interesting and novel properties and abilities [57]. The highly branched polymers can achieve a globular structure [58] with internal voids [59] and multiple surface groups [60], which facilitate a range nano-based applications in pharmaceutical and biomedical fields.

The construction of these molecules can be achieved in a number of ways but most have a well-defined structure consisting of a central core, an inner shell and outer shell. The central core can consist of a central atom or group of atoms such as a benzene ring [61]

or an ethylene diamine [62]. This can branch to multiple layers within the inner shell structure, where each concentric layer is normally referred to as generations with the first being abbreviated as G1 and each subsequent layer G2, G3 and so forth [63] (see Figure 6). The final outer shell presents functional moieties that will vary as the to the desired effect of the dendrimer [64].

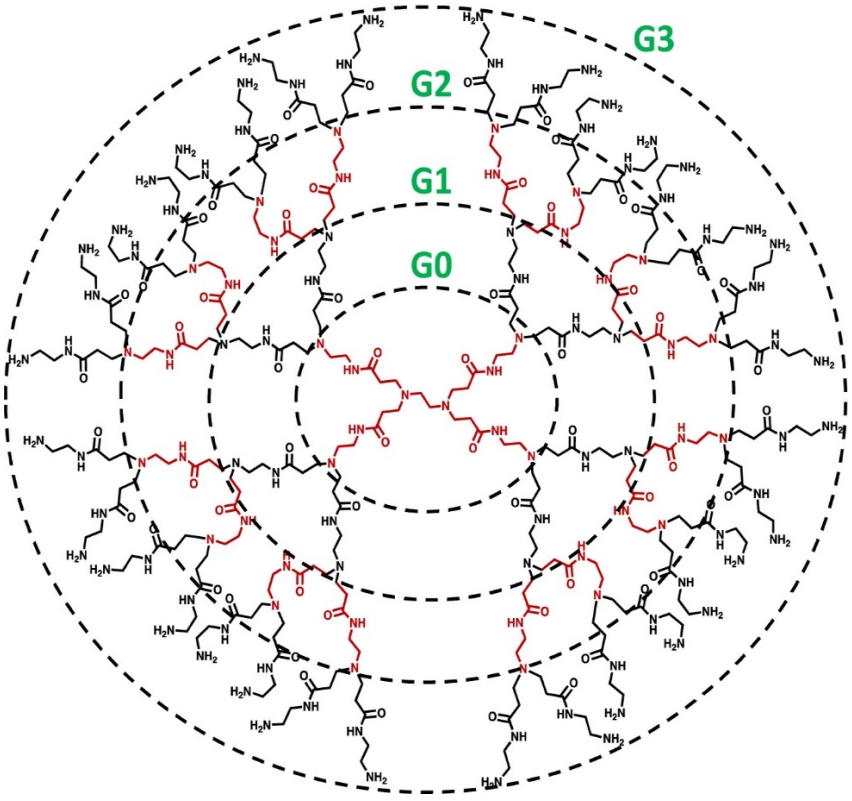

Figure 6. PAMAM (poly-amido amine) dendrimer structure, generations (G) denoted with dashed ovals. Reprinted with permission from [62] Copyright © 2020 John Wiley & Sons, Ltd. (Hoboken, NJ, USA).

As shown in Figure 7, the initial work reported by Vogtle et al. in 1978 described a "cascade" synthesis by repeatedly reacting mono and diamines [65], which was then built upon to generate the early dendrimers grounded on a polyamine-based polyamidoamine (PAMAM) structure, including the starburst type pioneered by Tomalia et al. [66–68] and poly(propyleneimine) (PPI) type by Meijer et al. [69]. This further expanded to incorporate aromatic core structures such as arborols by Newkome et al. [70] which utilized tris(hydroxymethyl)aminomethane and poly-lysine-based dendrimer structures [71]. The first macromolecular poly-lysine dendrimer was described by Denkewalter et al. at Allied Corporation [72] and was further developed by Tam et al. [73] to become another class as MAPs [74]. The synthesis of these structures has developed with the use of the Michael addition reaction as the most widely exploited synthetic method. PAMAM structures synthesised by Tomalia et al. consisted of alternating a double Michael addition of the primary amines of ethylediamine with methyl acrylate to achieve the desired branching architecture [75]. PPI was also grown in a similar fashion by a double Michael addition of acrylonitrile with a 1,4-diaminobutane core followed by a final hydrogenation with Raney cobalt [76].

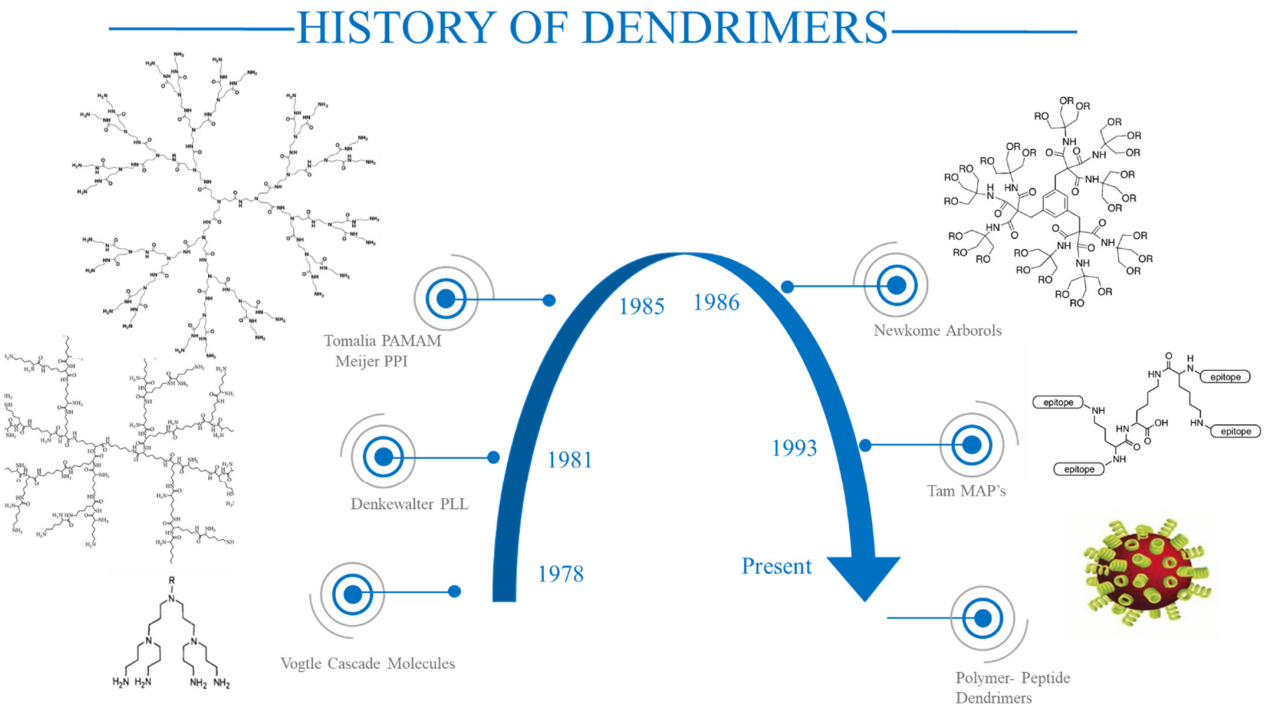

Figure 7. History of dendrimers. Structures adapted from [65,66,70,72,74,77].

The arborol-based dendrimers were originally created through a bromination reaction where the tris(hydroxymethyl)aminomethane was attached to a benzene ring core. Such benzoyl protected dendrimer were generated by a series of ester formation, hydrolysis of Tris in DMSO and final treatment with benzoyl chloride [70]. Further innovations not only facilitate these classical dendrimer structures but develop a subtype, named as metallodendrimers, which combined dendritic polymer structures with metallic complexes. A recent summary of the extensive work of Newkome highlighted the applications and potentials of metallodendrimers [78]. Poly-lysine-based dendrimer structures are built in a different manner by taking advantage of the bifurcate structure of lysine and utilize N^{α} and N^{ε} amino groups as reactive functionalities [79], where Boc-Lys-Boc and/or Fmoc-Lys(Fmoc) are used to create a branching structure and elongate the sequence via step-wise peptide synthesis or by chemical conjugation of peptide sequences [38]. As mentioned previously a range of polymer–peptide dendrimers is now possible with these classical dendrimer structures being utilized as a scaffold, whereby peptides are covalently linked not only on the surface but throughout the dendrimer structure and have even led to complex self-assembling nanoparticles [77] The incorporation of a range of other novel biocompatible building blocks also enables the ability of these molecules to be specifically tuned to a achieve a desired effect. Each component of the structure and selected dendrimer scaffold allows researchers to attain specific desired properties [80] and has expanded to many intricate biocompatible structures [81] and clinical applications [82].

One such success story that warrants mention is the poly-lysine-based antiviral dendrimer SPL7013 or astodrimer sodium developed by a Melbourne-based biopharmaceutical company, Starpharma [83]. This molecule which has shown clinical activity against simian/human immunodeficiency virus and Chlamydia trachomatis [84] is now available under the trade name VivaGel®. It has been applied for the prevention of sexually transmitted infections (STIs) on condoms [85] and treatment and prevention of bacterial vaginosis with mucoadhesive gel products [86]. Further novel antiviral properties of this molecule will be expanded on in a subsequent section below.

4. Innovations in Multimerization Synthetic Methodology

The efficiency and ease of researchers to form peptide bonds have come a long way from the early days of the pioneering of SPPS where Merrifield used HBr and AcOH to remove benzyloxycarbonyl (Cbz) from the N terminus of the peptide [87]. This led onto the initial development of tert-butyloxycarbonyl (Boc) and its subsequent utilization in early SPPS [88–90], which utilized deprotection steps of the N-protected amine by trifluoroacetic acid (TFA) and side-chain deprotection using the hazardous anhydrous hydrogen fluoride [91]. Our recent review of the 9-fluorenylmethoxycarbonyl (Fmoc) group in chemical peptide synthesis gave an excellent summary of the past contributions and current synthetic innovations and provided a detailed account of subsequent developments in biorthogonal protection chemistry to build peptide structures [92]. These have brought us to the modern and highly efficient automated synthesis of previously difficult sequences [93], including acyl carrier protein (ACP), β-amyloid(1–42) and even peptides up to 50 residues in length to be routinely performed [94]. This is now achieved with a range of tailored coupling reagents [95], milder piperidine as Fmoc deprotection agent and TFA as a global/side-chain deprotection of the peptide from the resin [96] (see Figure 8).

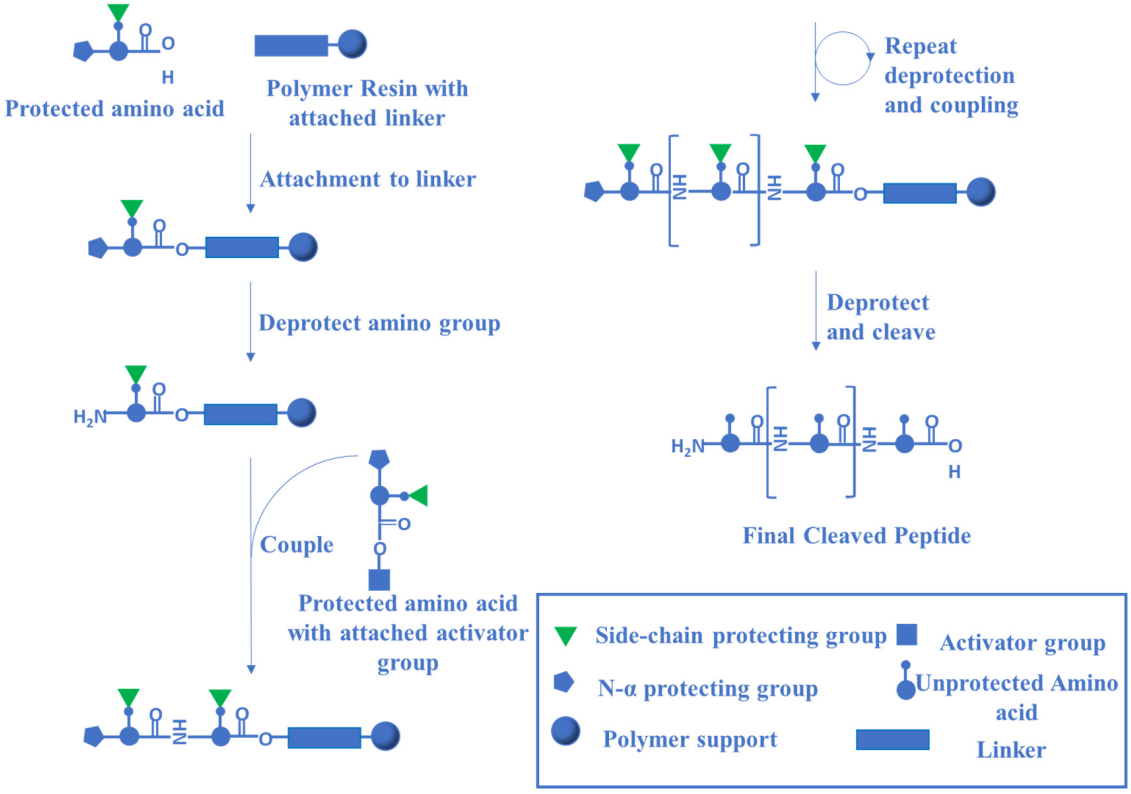

Figure 8. Solid-phase peptide synthesis (SPPS) synthetic scheme adapted from https://www.sigmaaldrich.com/AU/en/applications/chemistry-and-synthesis/peptide-synthesis (accessed on 23 December 2021).

More novel innovations by using four-dimensionally orthogonal protecting group strategies was recently discussed by Hiroaki Itoh and Masayuki Inoue, which outlined the fascinating SPPS routes by several research groups to produce a number of complex macrocyclic and tricyclic peptide natural products [97]. For example, one group utilized both the conventional Fmoc and Boc/t-Butyl protection, and Dde (N-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) as a fourth orthogonal protection group to introduce a branched chain into the structure, while an allyl group with subsequent removal by a Palladium reduction allowed a macrolactamization reaction to take place on the resin and final TFA cleavage to create the desired polymyxin analogue [98]. This drive to develop

more novel synthetic strategies and thus push the boundaries of the synthesis of complex biological structures. The opportunity now exists for the crossover of these methods from various scientific fields to build multimeric and dendrimeric-based structures. However, in order to build these complex branching structures, the development and utilization of chemical ligation methods to join two structures together is required. An excellent review by Gauthier and Klok outlined the vast array of chemo-selective reactions that can be now be utilized to undertake the convergent synthesis of peptide/protein–polymer conjugates [99]. This exhaustive list included Heck, Sonogashira and Suzuki coupling, Diels–Alder cycloaddition, click chemistry, Staudinger, Michael addition, reductive alkylation, oxime/hydrazone formation and Oxidative coupling (see Figure 9). These well-honed chemo-selective bioconjugation techniques have long been exploited to synthesise longer and more intricate native protein structures [100] and have naturally begun to be adopted by those attempting to build multimeric peptide structures.

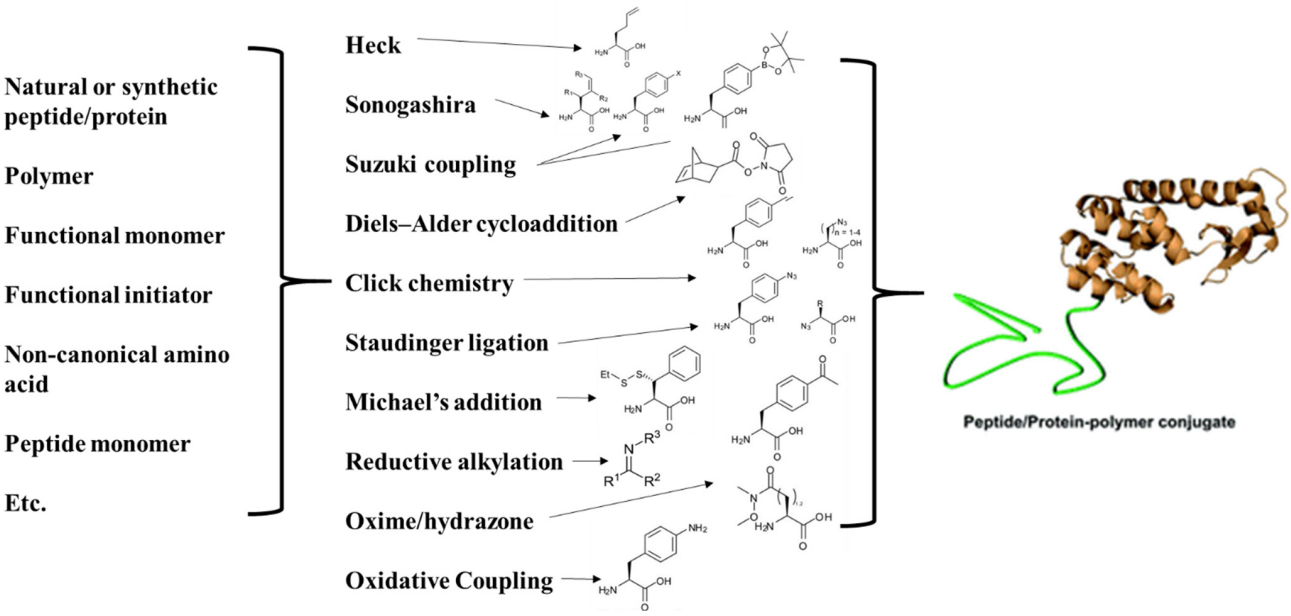

Figure 9. Summary of chemo-selective reactions, adapted from [99].

Specific chemical methodologies have matured over time since the functionalisation of the C- and N-terminals such as seen in native chemical ligation (NCL) described back in 1994 by Dawson et al. which involves the chemo selective conjugation of a C-terminal thioester and a N-terminal cysteine residue [101]. A recent review by Conibear et al. highlighted how this ground-breaking NCL protocol has further evolved and outlined the most recent innovations in thiol- and selenol-based chemistry which extends to a range of modern novel ligation chemistry built on the initial NCL methodologies [102]. This versatile method further allows crossover and integration with recombinant techniques to allow the utilization of expressed peptides which has led to so-called "Expressed Protein Ligation" (EPL) which circumvents the requirement of traditional chemical peptide synthesis [103]. The ligated products can now be desulfurised where a cysteine can be reduced to an alanine or deselenised within minutes [104].

Other conjugation strategies previously used in drug delivery molecules have been adopted by peptide chemists, including carbamate and carboxylic acid ester-based conjugation, hydrazone and thioether bond formation and specific enzymatically cleavable bonds. These methods were well summarised by Majumdar et al. of natural enzymes to release the drug "cargo" in a range of variable physiological environments [105]. This has also been a key strategy in the development of a range of chemotherapy drug delivery systems. It has led to a number of peptide-conjugates designed to release their cargo under specific enzymatic conditions in the vicinity of tumors, which will be discussed in more

detail below. Recent advancements in enzymatic technologies further facilitate solid-phase enzymatic peptide synthesis (SPEPS). For example, Shan et al. obtained an antioxidant tyrosine-alanine dipeptide (Tyr-Ala) by using recombinant carboxypeptidase Y (CPY) as the catalyst [106]. An excellent review by Nuijens et al. showed fascinating insights of the application of natural and engineered enzymes for enzyme-mediated ligation of peptide and protein sequences and cyclization [107]. These novel advancements give an exciting outlook to create peptide-based multimeric structures. An example demonstrated by Cao et al. used butelase-mediated ligation to create di-, tetra-, and octa-branching peptide dendrimers with potent activity against a range of antibiotic resistant bacterial strains [108].

The original concept of click chemistry by Sharpless et al. [109] and the subsequent expansion and utilization [110] can safely and selectively install reactive moieties on two distinct peptides/proteins [111]. The most well-known of these reactions was an extension of the azide–alkyne Huisgen cycloaddition by using an azide moiety and an appropriate propargylic group to create the so called "clicked" conjugates [112]. This led onto the original copper(I)-catalysed azide–alkyne cycloaddition (CuAAC) developed concurrently by Fokin and Sharpless et al. at Scripps Institutue [113] and Tornøe and Meldal at Carlsberg Laboratory [114]. Meldal et al. described a solid-phase reaction to produce 1,4-substituted [1–3]-triazoles in peptide backbones or side chains referred as peptidotriazoles. This reaction exploits the unique properties of these functionalities to allow relatively easy chemical conjugation of two separate peptide or protein structures via a 5-membered heteroatom ring linkage. The subsequent development of strain promoted azide–alkyne cycloaddition (SPAAC) [115] by the Bertozzi group utilizes a [3 + 2] cycloaddition in the same mechanism as the Huisgen 1,3-dipolar cycloaddition by taking advantage of a strained ring system such as difluorooctyne (DIFO) [116].

These innovations in conjugate chemistry have allowed a large range of novel compounds to be explored leading to many potential drug candidates and approved drugs. A recent article by Jiang et al. summarised the recent applications of click chemistry in drug development and provided an insight and importance of 1, 2, 3-triazole [117]. For example, it has been used to replace specific functional groups as a peptidomimetic, a linker for pharmacophores, drug delivery/targeting and probes for ligands. It further outlined the amazing developments of click chemistry, provided exciting possibilities at the further developments of Cu-free cycloaddition SPAAC and inverse-electron-demand Diels–Alder cycloaddition (IEDDA) reactions with tetrazines. They concluded with the exciting bioorthogonal reactions that are still in the initial phases of discovery such as that of thio acid/sulfonyl azide amidation ('sulfo-click' reaction), sulfur/fluoride exchange (SuFEx), metal-mediated amine-to-nitrile addition, thiol-ene click chemistry, and oxime-forming reaction. This extensive range of reactions looks to have promising capabilities to develop the next generation of multimerised molecules.

5. Multimeric and Dimeric Peptide Applications

5.1. Multiple Antigenic Peptide (MAP) Systems and Their Use as Immunogens and Vaccines

The multimerization of specific peptidic structural motifs is not a new concept, with the multiple antigenic peptides (MAP) system originally developed by Tam et al. [118], being a long-standing strategy for antigenic peptide synthesis [119] and vaccine design [120]. These MAPs consist of a small, immunogenically inert, two-fold bifurcating poly-lysine core linked with antigenic peptides which are capable of eliciting an antibody response [121]. It is well known that MAPs are efficient immunogens to study immunity mechanisms as multiple copies of the antigenic peptide structure are presented to elicit this immune response [122]. So, these multimeric MAP structures have been extensively studied for the design pitfalls needed to produce a successful multiple antigenic peptide candidate [123,124]. These multimer structures are designed to be recognised by the immune system components such as antigen-presenting cells or macrophages [125]. These compounds take advantage of the ability of these cells to recognise pathogen-associated molecular patterns via the so-called pattern recognition receptors (PRRs). They further lead to a cascade of pro-

cesses whereby these antigenic structures cause T-helper (CD4) cell activation, subsequent responses from cytotoxic T-lymphocytes and activation of T cells, B cells and dendritic cells in the lymphatic system, essentially activating the adaptive immunity mechanisms [126].

The simple designs and synthesis of MAPs provided an excellent and relatively cheap strategy to study these immune mechanisms, which further assist the development of peptide-based vaccines [127]. The journey to reach this point, however, has been an arduous task as researchers wrestle to ascertain the precise epitope designs required to achieve the desired strong and long lasting immunity against specific pathogens [128]. It is not only important for peptide/protein identification but also for understanding of native protein conformations [129] and their effects on antibodies-protein recognition [130]. Successful peptide vaccine candidates retain the required folding which is often achieved by flanking the epitope with specific sequences, this can induce folding [131] this ultimately alters antigenicity and immunogenicity [132]. Other techniques such as chemical modification [133] or peptide stapling [134] have also begun to unravel innovative strategies to produce the next generation of these molecules.

These peptide vaccine designs have developed alongside conventional vaccines to produce examples with comparable effects [135]. Due to the simplicity and lower cost of production of peptide vaccines, it highlights the enormous potential for vaccine development, especially with the current world contends with COVID-19 [136]. Traditional vaccines are fraught with difficulties in their production and use with complications such as shedding of the pathogen into the environment causing unintending consequences [137], such as the reactivated attenuated vaccines can become virulent again and cause infection of production workers [138] and the cold chain transport requirement for stability issues [139]. Peptide vaccines have been considered as a potential contender to mitigate these issues and the future of these molecules will be closely watched as the world contends with future pandemics.

5.2. Peptide Multimers as Antimicrobial and Antiviral Agents

The creation of multi-presenting peptide structures has been refined over the years and now expanded beyond the scope of the MAP system to develop multimeric peptide-decorated systems and target new therapeutic avenues. Such multivalent nature of these assemblies have been applied to specific target cellular surface structures such as bacteria [140] and viruses [141], for potential candidates of antimicrobial and antiviral therapeutics. An excellent review by Giuliani et al. highlighted a range of multimeric and peptidomimetic examples and gave fascinating insights into the capabilities of these and related antimicrobial compounds such as oligoacyllysines, ceragenins and peptoids [42].

As mentioned previously, multimerization can be employed in standard solid- and solution-phase peptide synthesis via an amino acid linkage, such as lysine [142]. With advanced development of bioconjugation, it has further expanded to create other novel amino linked analogues [143]. For example, the utilization of a 2,4-diaminobutyric acid (Dab) scaffold, by Otvos [144] and subsequently used by our group combined the functionalised monomeric peptide with several halide and maleimide-based linkers (see Figure 10), to obtain multimeric antimicrobial structures [145]. The dimer, "A3APO", and the tetramer of the rationally designed proline-rich antimicrobial peptide (PrAMP), Chex1-Arg20 (sequence in Figure 10), were found to have significantly lower MICs on a range of Gram-negative bacteria and increased membrane activity than that of the monomeric form [146]. The Chex1-Arg20 monomer is also known as ARV-1502 and is a lead compound aiming to target resistant bacteria. It is currently undergoing preclinical trials by the biotech startup, Arrevus Inc (Raleigh, NC, USA) [147] and it is expected that the multimer analogues will follow a similar path.

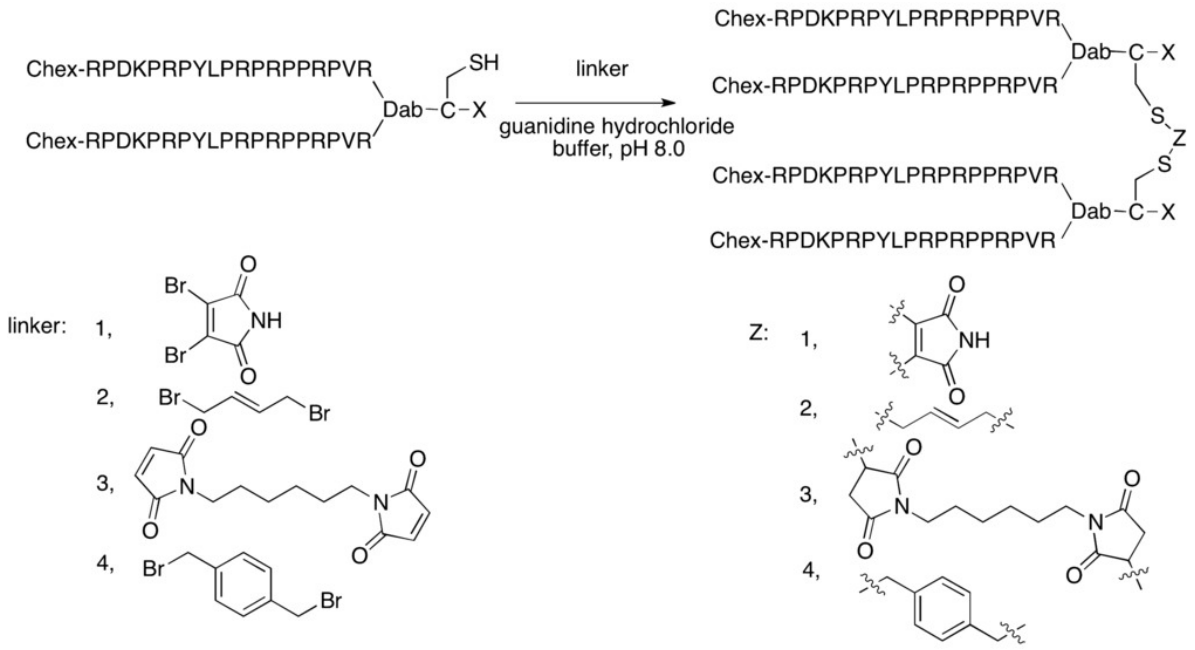

Figure 10. The preparation of dithiolmaleimide-linked tetrameric PrAMPs with C-terminal modifications as well as of the tetrameric PrAMP-hydrazide with different linkers. For linker 1, X represents the amide group, hydroxyl group, or hydrazide group and Z represents Z1. For linkers 2–4, X represents the hydrazide group and Z represents Z2–Z4. Chex1-Arg20 represent: Chex-RPDKPRPYLPRPRPPRPVR-NH_2. Chex is 1-aminocyclohexanecarboxylic acid. Reprinted from [148] Copyright (2015), with permission from Elsevier.

A thorough examination of chemical modifications and conjugated antimicrobial peptides was recently undertaken in a review article also by our group which highlighted the looming antimicrobial resistance (AMR) crisis, which lays in shadow of the current pandemic [3]. The authors summarised the recent synthetic efforts and outlined concise modification strategies such as lipidation, glycosylation and multimerization to circumvent biomedical challenges currently faced by superbugs, as current antibiotics struggle to keep up.

The multivalent nature of multimers bodes well for them as effective antimicrobial compounds as the mechanism of action of a range of AMPs is the insertion into bacterial membranes where the formation of pores occurs at the surface of the cell [149]. This eventually causes destabilisation of the membrane structure which ultimately leads to membrane permeability and cell lysis [150]. The pore-forming abilities are enhanced by the multiple presentation of peptide structures [151].

For example, Bai et al. demonstrated an enhanced antimicrobial effect with the dimer B2088 against the bacterial *P. aeruogenosa* ATCC27853 and *E. coli* ATCC 25922 over the monomeric peptide, V2 [152]. The use of a CuAAC reaction by Arnusch et al. produced a series of multimeric magainins, which found increased pore-forming capability of the tetravalent and octavalent peptides [153]. The authors go on to describe a tetravalent peptide analogue B4010, built on a poly-lysine core, even exhibited better antifungal properties than the small-molecule antibiotics, nanamycin and amphotericin B. All of these examples show exciting possibilities to the role these multivalent structures will play in the future of these class of drugs. As can be expected, the multimerization of drugs is a logical path forward in their design to reach ever-more novel targets and therapeutic applications.

Due to the aforementioned multivalent nature of multimers and peptide dendrimers, they are considered as promising candidates for synthetic vaccines, as well as antivirals. For example, Xiaoa and Tolbert demonstrated the dimeric HIV fusion inhibitor peptides, C37H6 and CP32M, can target trimeric gp41 protein and ultimately inhibit virus/host cell membrane formation [154]. They also describe several heterodimers created by a CuAAC

"click reaction" showed even more broader spectrum antiviral activities, thus lead to exciting insights to the future of multimeric peptides. An interesting report by Budge et al. highlighted the importance of multimer formation of RhoA-derived peptides to inhibit the replication of the respiratory syncytial virus (RSV) for the antiviral activity [155]. A further structure-activation study showed that replacement of cysteine with alanine demolished the peptide forming dimers and inhibition of RSV replication.

With the advent of the COVID-19 pandemic, researchers are scrambling to discover novel targets and peptides for promising effect. Chowdhury et al. recently found 2 lead candidates out of 50 peptides to inhibit SARS-CoV-1 through molecular simulation studies [156]. They also found that non-polar residues were significant contributing factors to activity and showed selective peptide inhibitors could target the spike protein of SARS-CoV-2, the virus causing COVID-19 [157].

The aforementioned potent antiviral poly-lysine dendrimer SPL7013 for the prevention of sexually transmitted infections (STIs) has also been applied as an antiviral to inactivate a broad spectrum of respiratory viruses including >99.9% of coronavirus SARS-CoV-2 [158]. This molecule has now shown activity against newly discovered variants of concern including the Alpha or UK (B.1.1.7) and the more recent Delta SARS-CoV-2 variant [159]. A recent article by Paull et al. found that astodrimer sodium (SPL7013) can inhibit the replication of SARS-CoV-2 in vivo, and such irreversibly blocked interaction leads SARS-CoV-2 to no longer infect cells [160]. Starpharma also announced the release of a nasal spray [161] under the tradename VIRALEZE™ as a promising weapon against COVID-19 that will aid in filling the gap during challenging global vaccine rollouts [162].

5.3. Multimers as Inhibitors and Mimetics

The use of natural multivalent inhibitors can be seen in lung tissue with viruses such as influenza. The fluid coating the inside the lungs of most mammals contains mucins which are proteins presenting oligosaccharides terminated in sialic acid, and these mucins are capable of binding to influenza, thereby inhibiting its ability to attach to target cells [163]. Our understanding of the interaction of bacteria and viruses with cellular structures has grown significantly over the years, which provides the guidance to develop potent inhibitor analogues by blocking these mechanisms. As shown in the antiviral dendrimer examples, the ability of these multimeric compounds to block cellular targets can also count toward to their potential as potent inhibitor candidates. These natural mechanisms have inspired the development of potent multimeric peptide inhibitors. For example, Yahi et al. demonstrated a dendrimeric peptide, based on the V3 loop of gp120 the HIV-1 surface envelop glycoprotein, for the inhibition of both CD4+ and CD4− cells during HIV-1 infection [164]. Several peptide dendrimers, namely SB105 and SB105_A10, made by Luganini et al. showed almost complete inhibition of human cytomegalovirus (HCMV) replication in fibroblasts and endothelial cells [165]. A further investigation of mechanism of action found these molecules bound to cellular heparan sulfate, giving an exciting starting point for further studies.

An excellent article by Wan et al. gave an extensive synthetic and structural analysis of a number of highly branched 2, 4, 8 and 16 mer [Lys8]-oxytocin or LVT peptide-decorated dendrimer designs via Fmoc SPPS and a CuAAC reaction which showed excellent binding affinity and have potent inhibition of colonic nociceptors [166] (see Figure 11). The group go on to demonstrate that the LVT ligands were freely available to interact with the human oxytocin receptor (OTR) and that analogues exhibited potent analgesic properties in an animal model of chronic abdominal pain. These exciting developments highlight the potential of peptide-decorated dendrimers and their abilities to reach therapeutic targets previously unreachable by linear peptides.

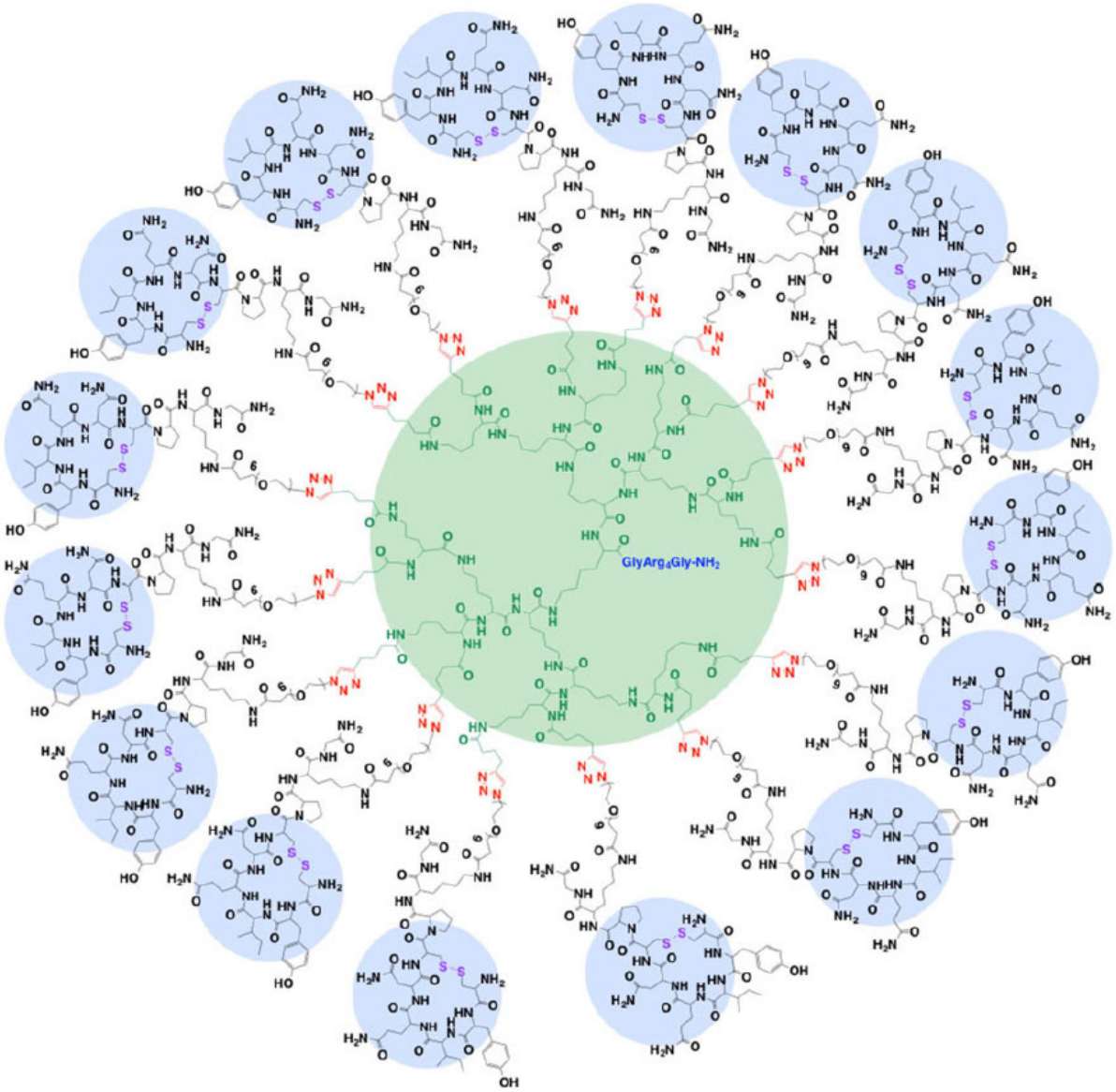

Figure 11. Schematic view of the dendrimeric peptide system consisting of sixteen cyclic disulfide LVT ligands conjugated via a pegylated triazole link to a lipidated core of (Lys)8(Lys)4Lys–(GlyArg4Gly)–NH2 (9). Each green ball represents a peptide unit, and the blue ball is a schematic dendrimer scaffold. The triazole is highlighted in red. Reproduced with permission from [166] Copyright (2016), CSIRO Publishing.

A similar pattern of multimeric peptides for the inhibition of intracellular oncotargets has now led to growth inhibition of tumors. For example, Nomizu et al. developed a peptide dendrimer, derived from the peptide laminin, to essentially block the laminin receptor on tumor cells, which prevented the malignant cells from being able to bind to walls in blood vesicles [167]. They also found that the inhibitory effect was increased as the number of copies of the peptide presented increased.

As mentioned previously, these multimeric and dendrimeric structures can reach large sizes for conferring a globular nature on the structure. For example, Fassina et al. discovered a peptide that mimics protein A which was able to recognise the Fc immunoglobulin portion of the protein [168]. This structure was discovered by screening a library of multimeric peptides with an enzyme-linked immunosorbent assay and found a useful candidate for affinity purification of antibodies. To overcome the previous bottlenecks in drug

development, current biological and synthetic chemical strategies have focused to design new multimeric ligands by unlocking new therapeutic pathways.

Quinlan et al. discussed an example such as this where they designed several multimeric peptides aimed at binding to the HIV-1 envelop glycoproteins [169], the design consisted of fused sulfopeptide mimetics of the CCR5 and CD-4 amino terminus of the target receptors. These molecules which were expressed as immunoadhesins were found to neutralise more HIV-1 than CD4-Fc or equimolar mixtures of immunoadhesin forms of each peptide, giving exciting insights into preventing the infection of HIV.

Putterman et al. designed a peptide mimetic of double stranded DNA, which was able to produce lupus-like autoimmunity against some other lupus autoantigens in immunised mice [170]. Tam et al. demonstrated the application of MAPs as protein mimetics a number of years ago [171], thus leading to the aforementioned novel examples in the extended application of the multimer and dendrimer structures.

5.4. Radio-Labelled Bioactive Multimeric Peptides

The deployment of a peptide attached to a radio-nuclide has been the focus of a great deal of research to image specific peptide receptors and targeted activity towards receptors overexpressed on tumors over the last two decades [172]. For example, Khoshbakht et al. developed a ^{18}F-FDG-Aoe-LIKKP-Pyr-F peptide with higher affinity for apoptotic cells and further hypothesised its potential in the diagnosis and therapy monitoring of apoptosis-related pathologies [173]. Aweda et al. [174] used a polycationic peptide conjugated with positron emission tomography (PET) probes, namely 1, 4, 7, 10-tetraazacyclododecane-N′,N″,N‴,N-tetraacetic acid (DOTA) and AB1-HLys-DOTA, to target bacterial membrane lipids. These PET peptides can provide fascinating insights into the imaging of live bacterial infections, of which the ^{64}Cu-AB1-HLys-DOTA analogue can even distinguish infected muscle caused *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vivo.

The benefits of multimerization in this field have also now come into sharp focus with a range of novel multimeric radio-labelled peptide compounds including anti-cancer targeted peptide therapies. Several radio-labelled peptides based on the RGD peptide, the binding motif of fibronectin, have been successfully modified in the past to produce glycosylated RGD peptides with enhanced biokinetics [175]. The tail-to-tail dimeric and tetrameric analogues of the cyclic peptide c(RGDfE)HEG combined with a N-(4- [^{18}F]fluorobenzylidene)oxime ([^{18}F]FBOA) radio label showed better in vivo imaging properties than the monomeric conterpart [176], while a PEGylated version of the dimeric RGD peptide [^{18}F]FPPRGD2 has been approved for clinical trials by the FDA [177].

Another example is the dimeric ^{68}Ga-labelled form of bombesin [178], a peptide with high binding to the bombesin receptor subtype (BB2) or gastrin-releasing peptide receptors (GRPr) that are commonly overexpressed in tumors. Meanwhile, the [^{64}Cu] chelated 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)-bombesin dimers are still currently under evaluation but the preliminary evidence shows promise to improve tumor retention over the monomer in vivo [179]. Several monomeric analogues, which originated from the octreotide peptide and utilize the traditional bifunctional coupling agent 6-hydrazinonicotinamide (HYNIC), i.e., HYNIC-TATE and HYNIC-TOC, have now have been clinically evaluated and are now commercially available in Europe [180]. The next generation of these molecules have been seen to combine HYNIC and EDDA (ethylenediamine-N,N′-diacetic acid) and to form dimeric and tetrameric cyclic RGDfK analogues which have significantly increased tumor uptake compared with the monomeric form highlighting their promise for future molecular imaging and cancer therapy [181].

The use of multimeric peptides to enhance the activity of radio imaging compounds for target receptors on tumors over the original monomeric compounds alone, is outlined in a review by Yan et al. [182]. They hypothesised an interesting notion that the improved affinity may be caused by the cooperative receptor–ligand interactions and receptor shielding from endogenous competition.

Recent developments give a glimpse into the exciting future of the multimeric radio compounds, such as the copper-based multimeric radio imaging compounds that showed high specific affinity for beta-amyloid (Aβ) aggregates [183] for PET imaging in the brains of 5xFAD Alzheimer's disease mice model. These mice have five AD-linked mutations and develop severe amyloid pathology, these exciting developments with these novel compounds give an insight into the possibilities into the imaging and early diagnosis of this disease. Given the advanced development of these methodologies, these increase the abilities of the multimeric radio compounds to expand into previously unreachable areas of diagnosis and therapy.

5.5. Peptide-Based Polymers: Dendrimer Systems

Apart from chemically synthesised multimeric peptides, peptide-based polymeric dendrimers have also been used for different biomedical applications. Structurally nanoengineered peptide polymers (SNAPPs) are star-shaped nanostructures which utilize ring opening polymerization (ROP) of α-aminoacid N-carboyanhydrides (NCAs) [184] and have led to a number of hybrid nanomaterials in which peptides can be grafted [185]. Zhou et al. produced the compound $P(K_{10}F_{7.5}L_{7.5})$, which showed MICs superior than those obtained by naturally occurring AMPs against *Escherichia coli*, *P. aeruginosa*, *S. marcescens*, *S. aureus*, and *C. albicans*. Examples of these compounds have even been seen to produce antimicrobials which with sub-μM activities in the nanomolar range (40–300 nm) against *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae* [186].

These nanostructures are easily and cheaply made and can rival the activities of the more costly and complex solid-phase multimeric peptide structures currently produced [187]. The SNAPPs made by Lam et al. [184] were seen to exhibit a multimodal mechanism that proceeds similarly to that of antimicrobial peptides (AMPs) where the outer membrane of target bacteria is destabilised. As these peptide structures are completely synthetic and not encountered in nature, they have also been seen to have no observable resistance over 600 generations with the tested strains. These structures have been observed in the field of polymer science for a number of years, with polymeric star-shaped nanostructures being produced which can have a range of properties in physiological environments and have been used as potential drug delivery vehicles and therapeutic alternatives [188].

Even complex water-soluble core cross-linked star polymers are showing interesting abilities such as low solution viscosities, encapsulation capabilities and compartmentalized functionalities [189]. These compounds are comprised entirely of amino acids via ring opening polymerization (ROP) of amino acid N-carboxyanhydrides (NCA) in a one-pot using sequential reagent addition and the arm-first approach and molecules can begin to have tunable properties since the peripheral groups, arm composition and core-functionality can be derived from separate chemistry utilized during the synthesis.

It is only natural that the evolution of these various nanostructure designs from different scientific fields will merge as they try to mimic the complex biological structures, that researchers sought to target. The complex dendritic structures (see Figure 12) that have evolved with advances in biocompatible polymer chemistry [190] have fostered the imagination of what intricate structures can be created utilizing bio-compatible building blocks. An interesting paper by Engler et al. looked at the effects side groups and the overall molecular weight of these molecules had on the antimicrobial activity and particular characteristics of the compounds [185]. They found a range of polypeptide analogues produced had similar characteristics to AMPs including effective MIC values, broad spectrum activity, and potential for preventing biofilm formation by mitigating microbial attachment such as analogues QC8 and QC10 which completely prevented *S. aureus* and *E. coli* from attaching making them useful in medical device coatings.

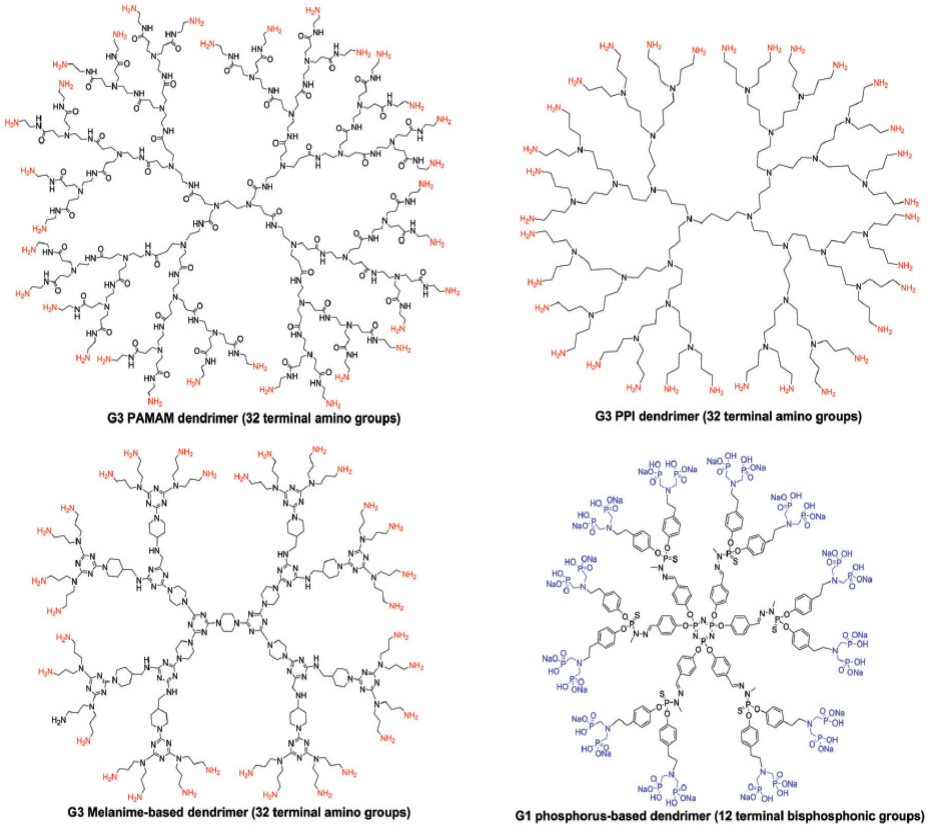

Figure 12. Examples of polymeric dendritic structures used for biological therapeutic applications as nanocarriers. Adapted from [191] with permission from Royal Society of Chemistry.

These polymer structures have evolved alongside the progression of peptide synthesis chemistry to create multibranched dendritic structures and/or components thereof which can be easily synthesised via techniques such as SPPS [192] and either built from the core out or pieced together via simple chemical manipulation such as that of "click chemistry" [193] and other bioconjugation techniques [102], previously described. The structures which are built in a step-wise manner where the structure is built from the core outwards adding each layer has been termed "divergent" and the ligation of several segments brought together onto a central core and built inwards termed "convergent" [194].

The concentric building of these branching structures around a centralised core has allowed the utilization of distinct chemistries to give rise to the possibility of an imagined multilayered cascade of metabolism of the nano construct in varying physiological conditions [195].

As mentioned previously, the use of a poly-lysine scaffold in the MAP system allowed the progression of these peptide-presenting dendritic structures. Larger and ever more complex peptide-decorated dendrimer structures have emerged which are built with not only poly-lysine-based core structures, but a range of highly branched organic polymer core dendritic scaffolds such as polyamidoamine-based (PAMAM) [196], polyphenylene dendrimers (PPDs) [197] and polystyrene [198].

The advancement of protein and peptide conjugate chemistry has also been a crucial element in the development in the complexity of these combined peptide–polymeric dendritic structures as a central core can be built upon utilizing diverse conjugate chemistries, growing each layer of the structure to achieve a highly branched multipeptide-decorated nanostructure similar to those polymeric forms [199]. A review by Wan and Alewood in 2016 [200] gave an excellent description of the chemical advancements which have enabled the creation of these structures and gave exciting possibilities in the range of potential bio-applications they may one day occupy. The paper also gave interesting insights into the developments that remain to incorporate cyclic peptides, the investigations into loading

capacity needed and also touched on the length and flexibility of spacers to allow successful multiple binding to oligomeric receptors.

The idea of utilizing targeting peptide motifs in therapeutic strategies was previously discussed and gives an insight into the possible combinations of incorporating biocompatible surface structures such as peptides and saccharides into a dendrimer structure. For example, Liu et al. discussed a range of peptide/saccharide-conjugated dendrimers which allowed specific targeting of drug cargoes [201] and Li who saw broad spectrum activity and high selectivity towards a range of bacteria with a cationic peptidopolysaccharide (chitosan-grafted-poylysine) [202]. This also now allowed researchers the possibility to tune the structures to target specific tissues and reach locations normally out of reach for many therapeutic molecules.

The use of glyco-conjugates which target specific tissues and therapeutic targets has now become a reality with researchers Ogura et al. showing a specificity to sinusoidal endothelial and stellate cells in the liver with the use of N-glycans which were trimmed from biantennary sialoglycans [203]. A group from University du Québec à Montréal has also utilized glycan residues incorporated into multilayered dendritic structure compiled through CuAAC "click chemistry" of azide and alkyne moieties based around either a poly-thioacetylated or a cyclotriphosphazene tricontapropargylated "hypercores". These structures show a complexity to rival nature with the so-called "onion peel" nonacontavalent derivative consisting of a 90-arm glycan-presenting dendrimer (see Figure 13) which shows fascinating properties such as an increase in activity observed as a function of the valency of the structure and programmable bioactivities [204].

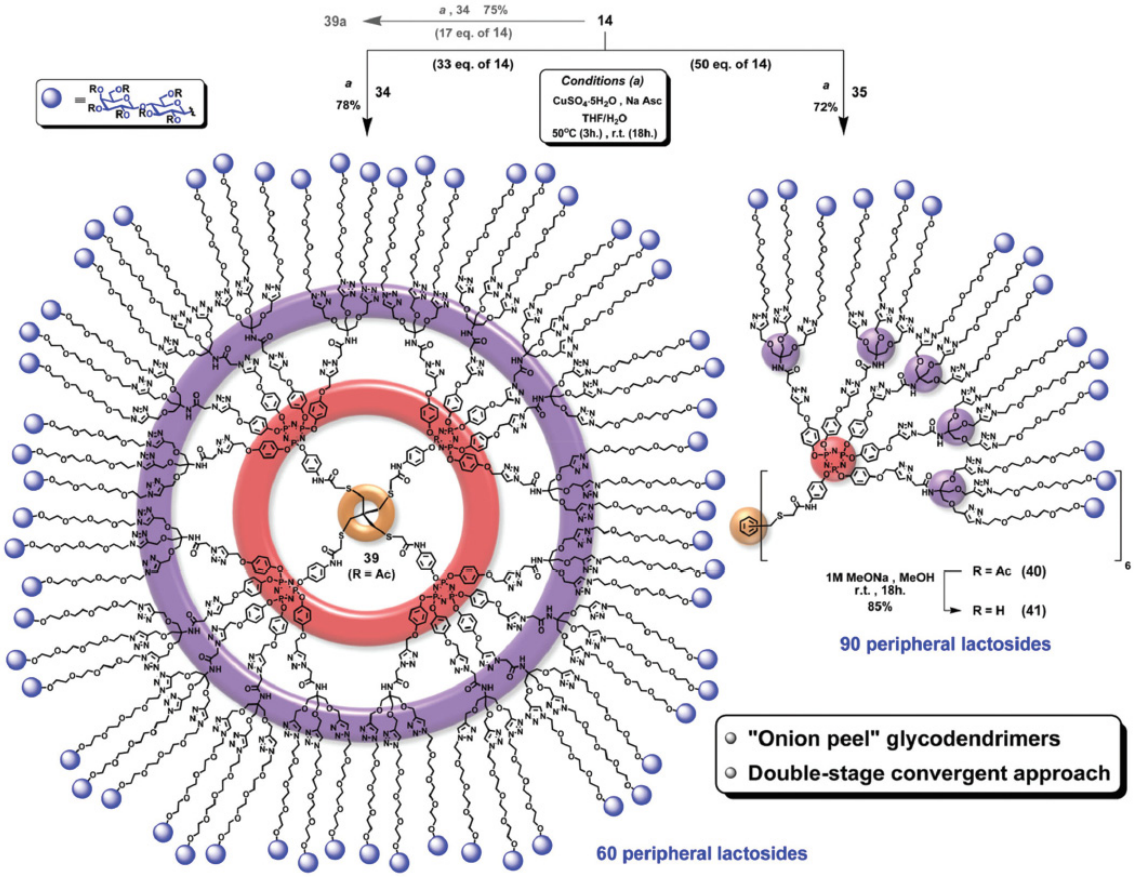

Figure 13. Synthesis of hexaconta-(39) and nonacontavalent (40 and 41) "onion peel" glycodendritic structures from hypercores with 60 and 90 peripheral lactosides, respectively. Reproduced from [204] with permission from the Royal Society of Chemistry.

These sorts of structures are becoming normalized in the dendrimer science community and give researchers the exciting ability to tune specific residues to target particular organs and tissues [205], an ability drug design chemists have long sought to find. Glycan residue reagents can now be purchased commercially depending on the application of the designed dendrimer. A company based in Japan called Gly-Tech has taken advantage of these latest developments to offer a broad range of Fmoc protected multisugar moiety derivatives [206] that even enable direct utilization in SPPS.

6. Perspective of Multimeric Delivery Systems

From the preceding examples, we can see that tools are available to utilize peptide sequences which can target an expressed structure on the surface of a cancer cell [207]. This now allows the selective delivery of therapeutic peptides [208], peptide–polymer conjugates and peptide conjugated small-molecule drugs to a desired target site [209]. For example, Liang et al. showed an elegant structure of a peptide–polymer conjugate capable of delivering the cytotoxic anticancer drug Doxil ® (Dox) using a cell penetrating peptide (CPP) which gave a tuneable toxicity and released the compounds at a specific pH [210].

Taking advantage of the overexpression of surface structures on cancer cells has been used to develop "Trojan horse" drug delivery vehicles based on the conjugation of cytotoxins with peptides. Christensen et al. used peptides that were selective substrates for the proteolytic enzymes, prostate-specific antigen (PSA or hk3) or human glandular kallikrein 2 (hK2) [211] which acted as prodrugs from which the active drug is only released in close vicinity to the cancer cells. Not only peptides but longer and more complex proteins, single-domain antibodies known as "nanobodies" and even complete antibody structures could be combined with dendrimer designs to target the unique antigens that are highly expressed in cancer cells.

A recent article in C&EN [212] magazine highlighted three approved antibody–drug conjugates (ADCs) that feature a peptide linker with the amino acid combination of valine and citrulline, which is cleaved by cathepsin enzymes in tumor cells [213]. Another example named "Enhertu" was also described as a "Trojan horse" as it is transported into the tumor cell (see Figure 14). Enhertu features a novel tetrapeptide linked with Trastuzumab deruxtecan (Dxd) [214], a potent chemotherapy drug metabolite and is combined with an antibody that also targets the HER2 protein [215].

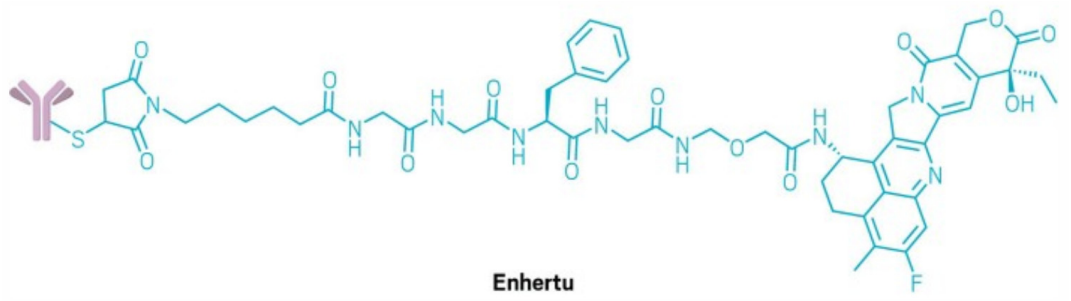

Figure 14. The structure of the ADC Enhertu, adapted from https://www.rxlist.com/enhertu-drug.htm#description (accessed on 23 December 2021).

The higher uptake and slower clearance of these monomeric structures from tumors are already showing enhanced effect [216] and the multimerization of these molecules is a logical step. The complex array of targeting biological structures that can be attached to dendrimer structures allow more well-defined and intricate targeting strategies to develop. The company Star Pharma has recently announced the HER2 nanobody conjugate known as DEP® HER2-lutetium, which it describes as a new "radiotheranostic", and utilizes the DEP technology which consists of a polylysine-based dendrimer architecture polydispersed with polyethylene glycol combined with Lutetium-177 and a HER nanobody [217]. They go on to describe promising preliminary data showing potent antitumor activity when

compared with a currently marketed treatment Herceptin labelled with lutetium. Preclinical research evaluation of a dendrimer-based ADC is underway with MSD, the tradename of Merck & Co. (Kenilworth, NJ, USA) [218]. This uses the DEP® technology and consists of a polylysine core structure with a specific covalent linkage with the antibody. These examples illustrate possibilities to use dendrimer structures to enhance the efficiency and specificity of these targeting molecules.

7. Future Directions/Conclusions

The idea of targeting therapeutics has been a long-standing and ever present hurdle that small-molecule drug design chemists desired to develop to reduce off-target effects [219]. This is a similar hurdle faced by peptide chemists for designing drug delivery compounds at the intended target site [220]. The rapid acceleration of automated peptide synthesis and ease of producing polymeric structures have pushed the boundaries of branching structures with multiple levels of complexity, high specific drug delivery targeting abilities [221], binding affinities [222] and reduced cytotoxicity [223]. This has even unravelled unknown abilities for therapeutics via the utilization of surface structures, such as the recent report of "coating" a poly-lysine dendrimer structure with galactose to reduce phagocytosis in comparing with the uncoated analogue [224]. The use of lipid chains has often been touted as an excellent means to reduce toxicity in peptides to reach unchartered territories, which can also applied in dendritic structures. For example, 2-amino tetra decanoic acid modified poly-lysine dendrimers were taken up into the mucosal surfaces [225], which cannot be achieved by many peptidic therapeutics [226]. Due to the hydrophobically collapsed conformation in water with the fatty acid chain backfolded onto the peptide dendrimer branches, the lipidated peptide dendrimers, TNS18, unfold to expose the lipid chain and hydrophobic residues while in contact with the bacterial membrane, thus leading to membrane disruption and cell death [227].

Because of the benefits of peptide-based therapeutics with larger constructs of multimer and dendrimer strategies, a number of key players in the pharmaceutical industry, including Novartis International [228] and Takeda Pharmaceutical Company [229], are directing their research into large constructed peptide and protein as new drug developments. Momenta Pharmaceuticals, Inc. (Cambridge, MA, USA), a Massachusetts biotechnology company collaborated with Melbourne-based biopharmaceutical company CSL, developed the Fc multimer proteins, M230 for the selective immunomodulator of Fc receptors [230]. Starpharma also now has a number of poly-lysine-based dendrimers based on their DEP® drug delivery platform by enhancing solubility, efficacy and pharmacokinetics, and the reductions in certain toxicities [231]. This further lead a number of DEP®-based partnered programs, including a collaboration with AstraZeneca for the development of AZD0466 to progress into the clinic [232]. Together with Sanofi Aventis, Starpharma also developed DEP® docetaxel [233] and DEP® cabazitaxel [234], which can be used as a combination therapy against pancreatic cancer in phase 2 clinical trials. However, it should be noted that large multimers/dendrimers could possibly increase cytotoxicity and production cost. Such limitations are a major research focus to resolve using chemical and recombinant strategies.

These developments give rise to a range of exciting possibilities in the future landscape of medicine with safer and more effective therapies by using large constructed multimeric peptides. By utilizing the multimeric strategies, the possibility to create intricate branching structures can inhibit and mimic the natural structures encountered on the surface of both human immune cells and the targeted pathogens. This will lead to exciting prospects for the next generation of these molecules creating safer and ever-more specific therapeutics.

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