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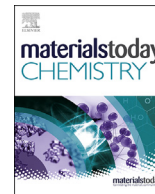
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Solid-phase dendrimer synthesis: a promising approach to transform dendrimer construction



A. Ya-Ting Huang^{a, b}, C.-L. Kao^{b, c, d, **}, A. Selvaraj^b, L. Peng^{a, *}

^a Aix-Marseille Université, CNRS, Centre Interdisciplinaire de Nanoscience de Marseille, UMR 7325, « Equipe Labellisée Ligue Contre le Cancer », 163 avenue de Luminy, 13288, Marseille, France

^b Department of Medicinal and Applied Chemistry, Drug Development and Value Creation Research Center, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, 80708, Kaohsiung, Taiwan

^c Department of Chemistry, National Sun Yat-sen University, 70 Lienhai Road, 80424, Kaohsiung, Taiwan

^d Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, 807, Taiwan

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ABSTRACT

Dendrimers, by virtue of their unique well-defined dendritic structure and multivalent cooperativity, hold great promise for a wide variety of applications, ranging from healthcare to energy production and environmental sustainability. However, dendrimer synthesis suffers from two inherent problems which greatly handicap their development and limit their application: the formation of structural defects caused by incomplete or side reactions, and difficulties associated with purification. Solid-phase synthesis may overcome both these problems, firstly by enabling complete chemical reactions through the use of a large excess of reagents, secondly by facilitating purification through simple washing and filtering steps. The end result is the speeded up reactions producing the desired product in high yield, with simultaneous suppression of by-products. In this review, we present the challenges and current state of research in solid-phase dendrimer synthesis, and provide our perspectives on its future development. We start with a short introduction to solid-phase synthesis and specific considerations for dendrimer construction. We then present exemplary studies to highlight the potential of, and challenges faced by, solid-phase dendrimer synthesis. Finally, we describe efforts to deliver more effective and reliable methods that will transform the synthesis of dendrimers and permit exploitation of their full potential.

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1. Introduction

Dendrimers are a special family of synthetic polymers with well-defined ramified molecular structures (Fig. 1A) [1]. The term “dendrimer” was coined by Tomalia et al. and is a portmanteau of the Greek words “dendron” (tree) and “meros” (part), which were chosen to emphasize the dendritic structures of the molecules [2]. The very first dendrimers were reported by Vögtle in 1978 when they were referred to as “cascade molecules” [3]. Later, Tomalia et al. synthesized the first family of dendrimers, the poly(amidoamine) (PAMAM) dendrimers, from generations 1 to 7 [2]. Structurally speaking, a dendrimer is composed of three main

components: (i) the central core from which the dendrimer emanates; (ii) the repetitive branching units, which allow dendrimer growth in geometrically organized radial layers called generations (G); and (iii) a large number of densely distributed terminal groups at the surface.

The distinctive molecular architecture makes dendrimers particularly appealing. They have a precisely controlled structure and cooperative multivalency, offering great potential for various applications such as sensors, molecular devices, catalysts, drugs, and drug delivery systems, which can contribute to healthcare, energy production, and environmental sustainability [4–9]. For example, a dendrimer against severe COVID-19 recently entered into Phase II clinical trial, and demonstrated reduced inflammation and neurological injury while improving patient outcomes [10]. Also, the dendrimer-based antiviral and antibacterial products VIRALEZE™ and VivaGel® have reached the market for medical use [11].

* Corresponding author.

** Corresponding author.

E-mail addresses: clkao@kmu.edu.tw (C.-L. Kao), ling.peng@univ-amu.fr (L. Peng).

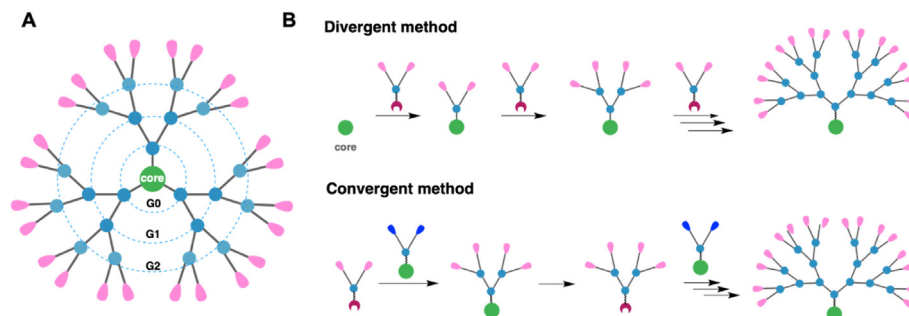


Fig. 1. Cartoon illustration of dendrimers and dendrimer synthesis. (A) General presentation of a dendrimer composed of a central core, radiating branch units defining the number of generations, and surface terminals. (B) Dendrimer synthesis via divergent and convergent approaches.

Many applications based on dendrimers, in particular the biomedical applications, have been hampered by the quality and consistency of dendrimer synthesis [6]. Unlike conventional polymer synthesis via a one-pot reaction, dendrimer construction requires a stepwise synthesis [1,12,13], which is usually achieved via two classic strategies, namely the divergent and convergent approaches (Fig. 1B). The divergent route proceeds “inside outward” by starting from the core and building the arms outwards through stepwise multiplication of the branching units [2,3], whereas the convergent route generates dendrimers via an “outside inward” construction of dendritic wedges (Fig. 1B) [14,15]. The combined divergent/convergent method, called the double-stage approach, has also been developed with the aim of accelerating dendrimer synthesis [16,17]. More recently, considerable attention has been focused on a strategy based on “click” chemistry [18,19], which offers the rapid and efficient synthesis of dendrimers under mild conditions.

However, all these synthesis strategies suffer from the inherent problem of structural defects caused by incomplete or side reactions. Notably, incomplete reactions are related to steric hindrance stemming from the dendritic structure and/or from the multiple reactions that are required for dendrimer construction [6]. Moreover, dendrimers with structural defects are usually extremely difficult to separate from intact dendrimers owing to their similar chemical composition and physical properties. The structural defects and purification problems worsen with higher-generation dendrimers. This constitutes the major obstacle to the reliable and consistent synthesis of various dendrimers.

Solid-phase synthesis can offer a solution to the above-mentioned problems associated with dendrimer construction (Fig. 2) [20]. It is a highly efficient synthesis driven by a large excess of reagents, that simultaneously reduces and/or suppresses side reactions. Moreover, it provides an extremely simple purification process comprising washing and filtration. In 1988, the first solid-

phase synthesis of dendrimers was reported by Tam, who prepared peptide dendrimers [21]. Since then, solid-phase synthesis has been used to generate a large array of dendrimers with various chemical compositions such as oligonucleotide, carbohydrate, polyester, polyamide, polyurea, polyether and triazine dendrimers, demonstrating its potential for accessing structurally diverse dendrimers.

In this short review, we present a general overview of the current state of solid-phase dendrimer synthesis (SPDS). First, we will provide an introduction to the technique challenges, and outline the specific considerations for solid-phase dendrimer synthesis. We will then showcase representative examples to highlight the progress, opportunities, and problems associated with solid-phase dendrimer synthesis. We will finish by presenting our view of the future challenges and perspectives.

2. Solid-phase synthesis

Solid-phase synthesis, which was pioneered by Merrifield for peptide synthesis, sparked a revolution in synthetic chemistry. In the early 1960s, Merrifield published a seminal work on the solid-phase synthesis of peptides, whereby amino acids were first attached to an insoluble resin, and subsequently assembled into peptides via a stepwise and iterative synthesis [22]. Compared with solution-phase synthesis, the solid-phase approach is very effective and can be performed conveniently in a short time with minimal side reactions. In addition, purification is readily achieved using washing and filtration. This inaugural solid-phase synthesis strategy revolutionized peptide synthesis, and Merrifield was awarded the Nobel Prize in Chemistry in 1984. Since then, solid-phase synthesis has grown rapidly in popularity. It has greatly simplified the synthesis of complex biological macromolecules, such as peptides [23,24], oligonucleotides [25], and oligosaccharides [26–28], otherwise difficult to accomplish in solution. Solid-phase synthesis

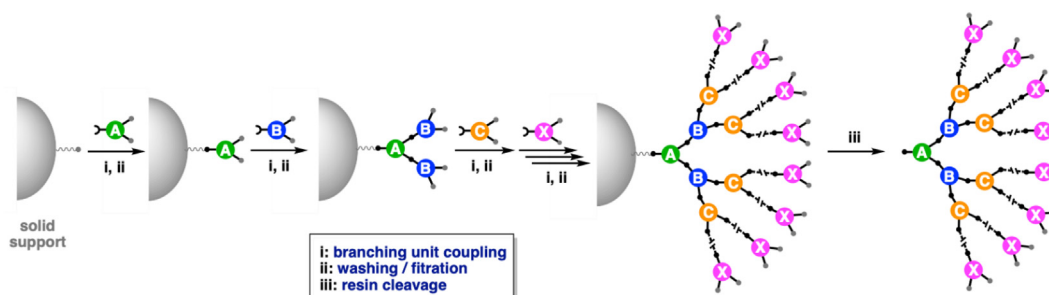


Fig. 2. Cartoon presentation of solid-phase dendrimer synthesis.

has also been implemented in the fabrication of structurally diverse small molecules [29] and polymers [30], as well as in combinatorial chemistry to construct new chemical entities and natural product-like libraries [31].

As mentioned above, the first solid-phase dendrimer synthesis was reported for the preparation of peptide dendrimers [21]. Since then, the technique has been used to generate various dendrimers including oligonucleotide dendrimers, carbohydrate dendrimers, PAMAM dendrimers, polyester dendrimers, polyurea dendrimers, polyether/polythioether dendrimers, and triazine dendrimers, demonstrating the remarkable structural and chemical diversity that can be achieved with the solid-phase synthesis approach. However, owing to their three-dimensional branched molecular architecture, special considerations are required for the solid-phase synthesis of dendrimers. Such considerations are different from those pertaining to the synthesis of linear molecules. We comment on these issues below before presenting exemplary studies on solid-phase dendrimer synthesis.

3. Challenges for solid-phase dendrimer synthesis

When performing solid-phase synthesis, several key issues, such as the resin, the linker, the loading capacity, and swelling should be considered carefully to ensure successful synthesis while minimizing side reactions [20]. These issues are of particular importance for dendrimer synthesis owing to the increase in the structural complexity and size of dendrimers at high generations [32–34]. Planning solid-phase synthesis starts with the judicious choice of a solid support, which comprises a resin bearing the appropriate linker attached to a reactive functionality for initiating the chemical reactions (Fig. 3). Scavenging and capping of the unreacted residual functionalities must also be carefully considered

to prevent the formation of undesired side products. Finally, it is also important to follow the progress of the reaction and precisely quantify the yield of each step using an appropriate monitoring method. We present below a short description of all these issues with a view to providing general information about solid-phase dendrimer synthesis.

3.1. Solid support

In solid-phase synthesis, the chemical stability, thermal resistance, mechanical strength, swelling property, and loading capacity of the solid support should be taken into account; all are important concerns for successful dendrimer synthesis [35]. Two categories of solid support are commonly used: (i) inorganic materials, such as silica, alumina, glass, and clay; and (ii) organic polymer materials. Inorganic materials often have relatively poor mechanical stability and low loading capacity, whereas organic polymeric supports are able to provide high loading capacity, and a variety of physical and chemical properties. Although inorganic materials allow easy diffusion of reagents and building blocks in and out of the matrix, their stiffness in solvents prohibits their use for the preparation of structurally cumbersome compounds, such as dendrimers. In contrast, organic resins comprising polymer networks are more flexible and can accommodate structurally bulky molecules. Therefore, organic resins are more appropriate for the solid-phase synthesis of dendrimers, branched peptides, and other bulky molecules.

At present, there are three major classes of solid polymer support: polystyrene (PS), polyacrylamide, and polyethylene glycol (PEG)-based resins. PS resins are highly stable under various reaction conditions, but their hydrophobic nature is not suitable for preparing compounds with high polarity. Polyamide resins have

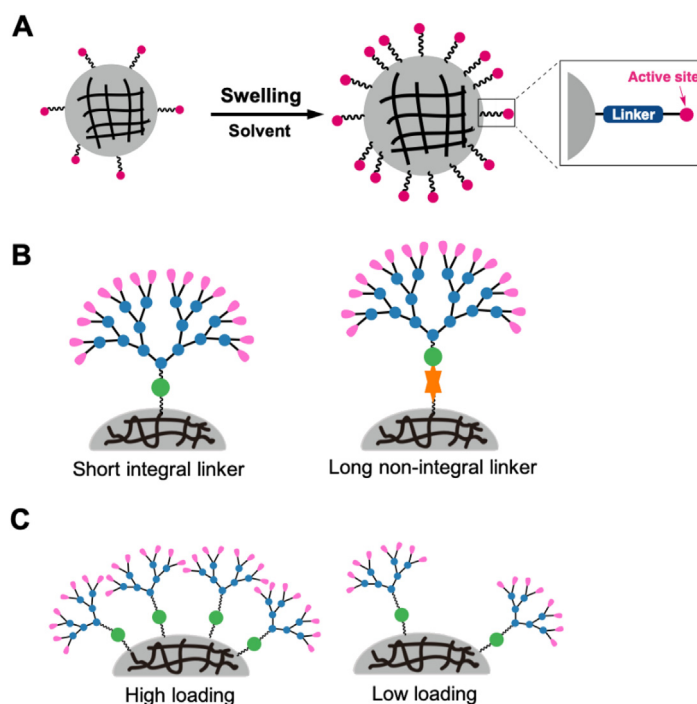


Fig. 3. Cartoon illustration of solid supports for solid-phase synthesis. (A) It is important to pre-rinse the polymeric resin and allow it to swell in order to release and expose the reactive sites for the initiation of the solid-phase synthesis. Dendrimer synthesis using: (B) solid supports with a short rigid integral linker (left) and a long flexible non-integral linker (right, the orange asterisk indicates the linker moiety); and (C) solid supports with a high loading capacity (left) and a low loading capacity (right). A resin with a long non-integral linker and a lower loading capacity is favorable for decreasing steric congestion during the synthesis of higher-generation dendrimers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

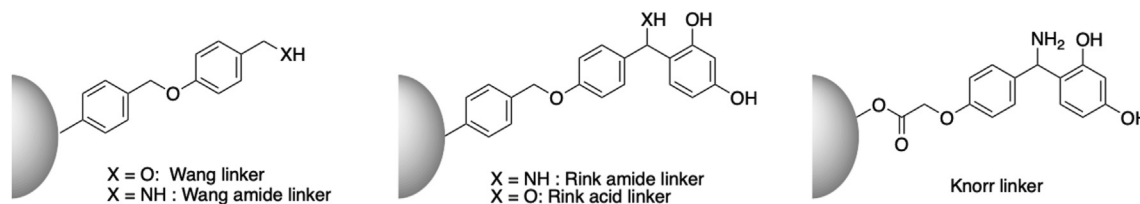


Fig. 4. Chemical structures of some non-integral linkers used in solid-phase synthesis.

the advantage of being compatible with polar solvents because they form strong hydrogen bonds, and are often used for peptide synthesis. The first PEG-based resin support was actually a PEG-PS resin, which was developed by combining hydrophilic PEG chains with a hydrophobic PS core. PEG-PS resins are therefore compatible with both polar and nonpolar solvents. Furthermore, the PEG chain not only has unique conformational flexibility and good compatibility with polar solvents, but also acts as a spacer to separate the starting point of the solid-phase synthesis from the hydrophobic PS core. For example, TentaGel® is a popular PEG-PS resin, and it was used for the first solid-phase synthesis of PAMAM dendrimers by Bradley. The long and flexible PEG chain of TentaGel® reduces the steric hindrance between the growing dendrimer and the resin.

The reactive functionalities of many polymeric solid supports are often buried within the matrix, hence having limited access to

reagents and impeded reactions. Therefore, it is essential to properly pre-rinse the resin and allow it to swell. The swelling step allows the polymeric chains to extend, exposing the reactive functionalities and making them accessible for the subsequent chemical synthesis (Fig. 3A). The properties of the solvent also affect the swelling of the solid support and hence the reaction efficiency. Consequently, the selection of solvents is also critical, especially for dendrimer synthesis.

3.2. Linker

The linker, which emanates from the solid support and harbors the reactive functionalities, is also important in solid-phase synthesis [36,37].

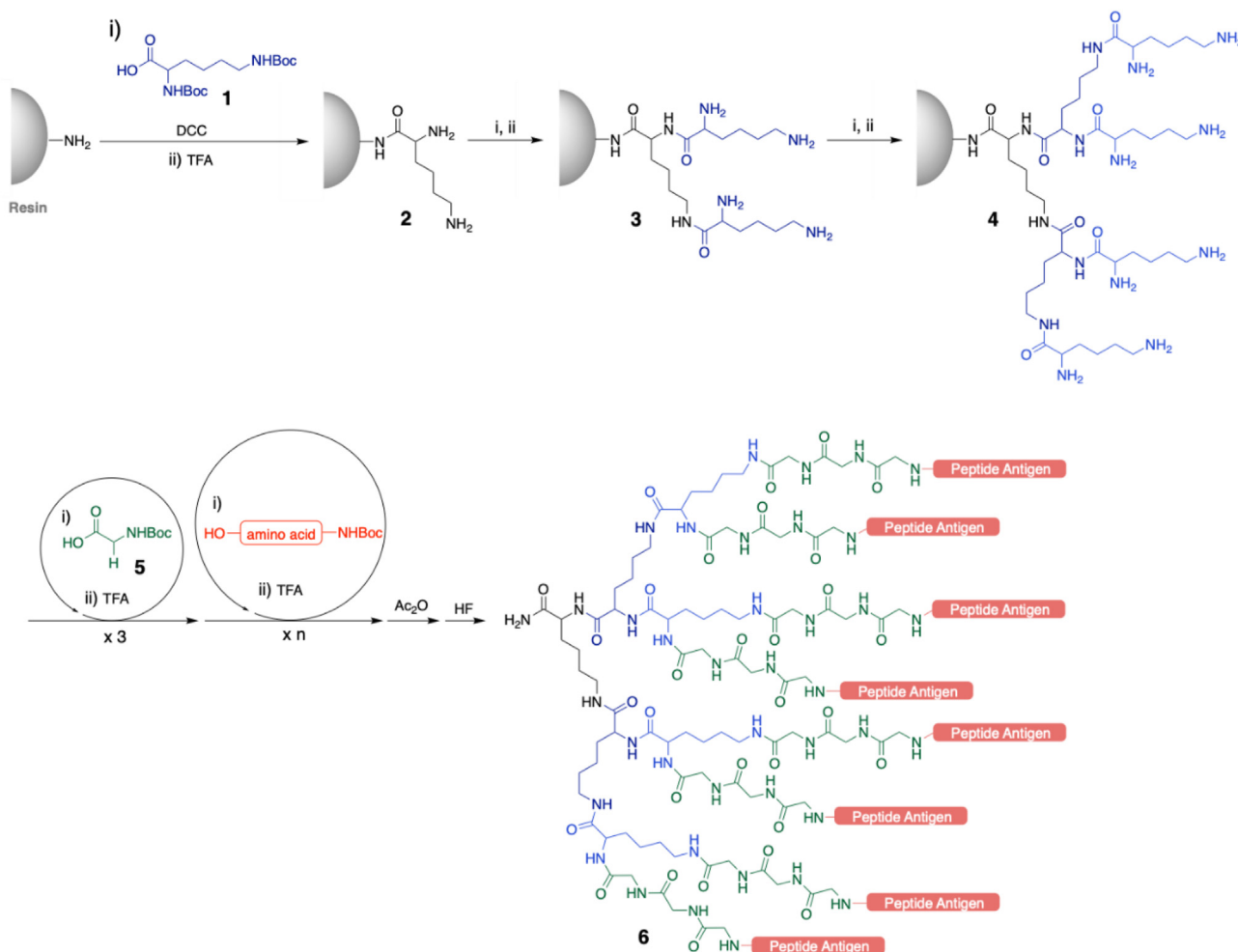


Fig. 5. Solid-phase synthesis of multiple antigen peptide systems, the first peptide dendrimers [21].

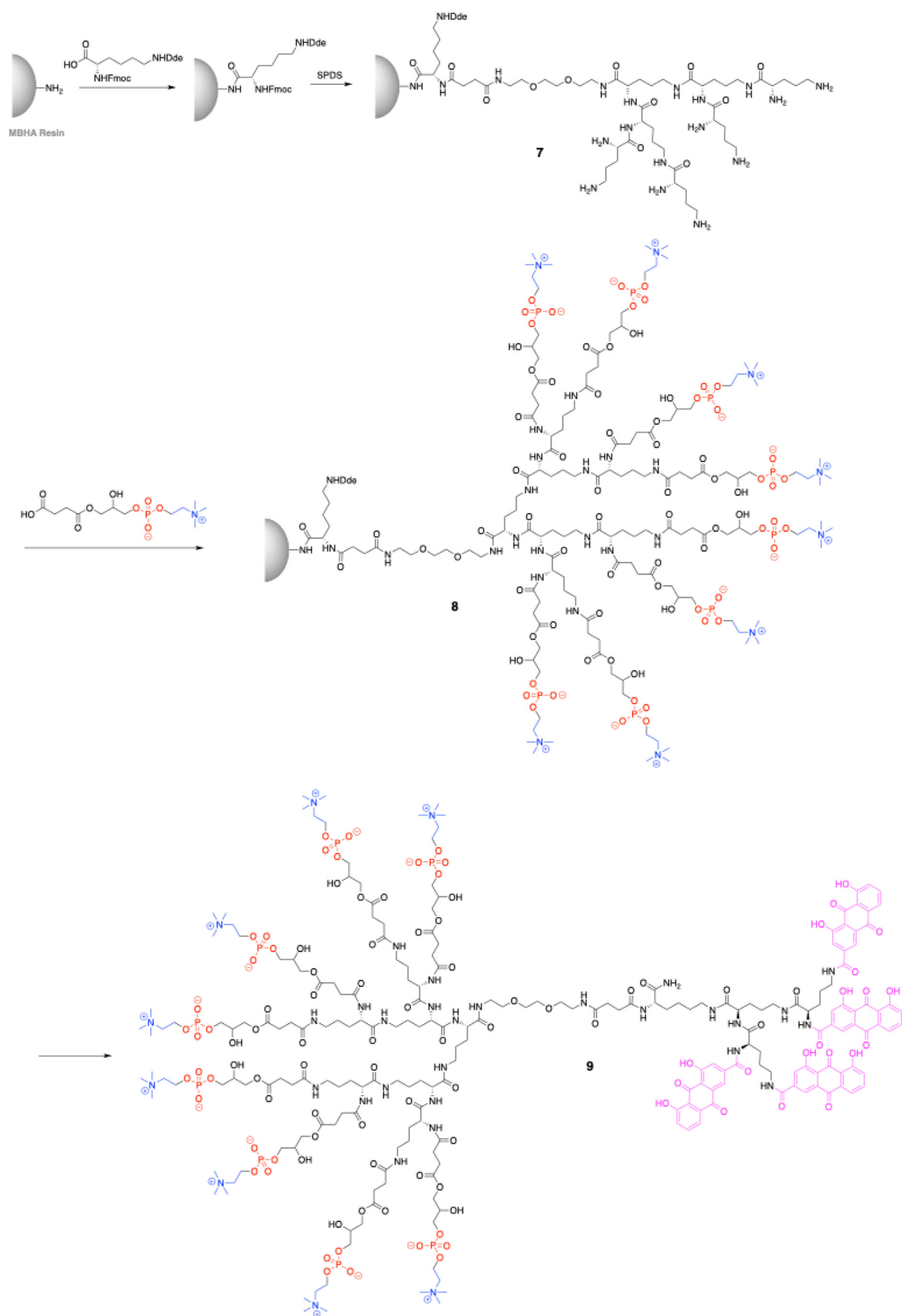


Fig. 6. Solid-phase synthesis of zwitterionic peptide dendrimer [52].

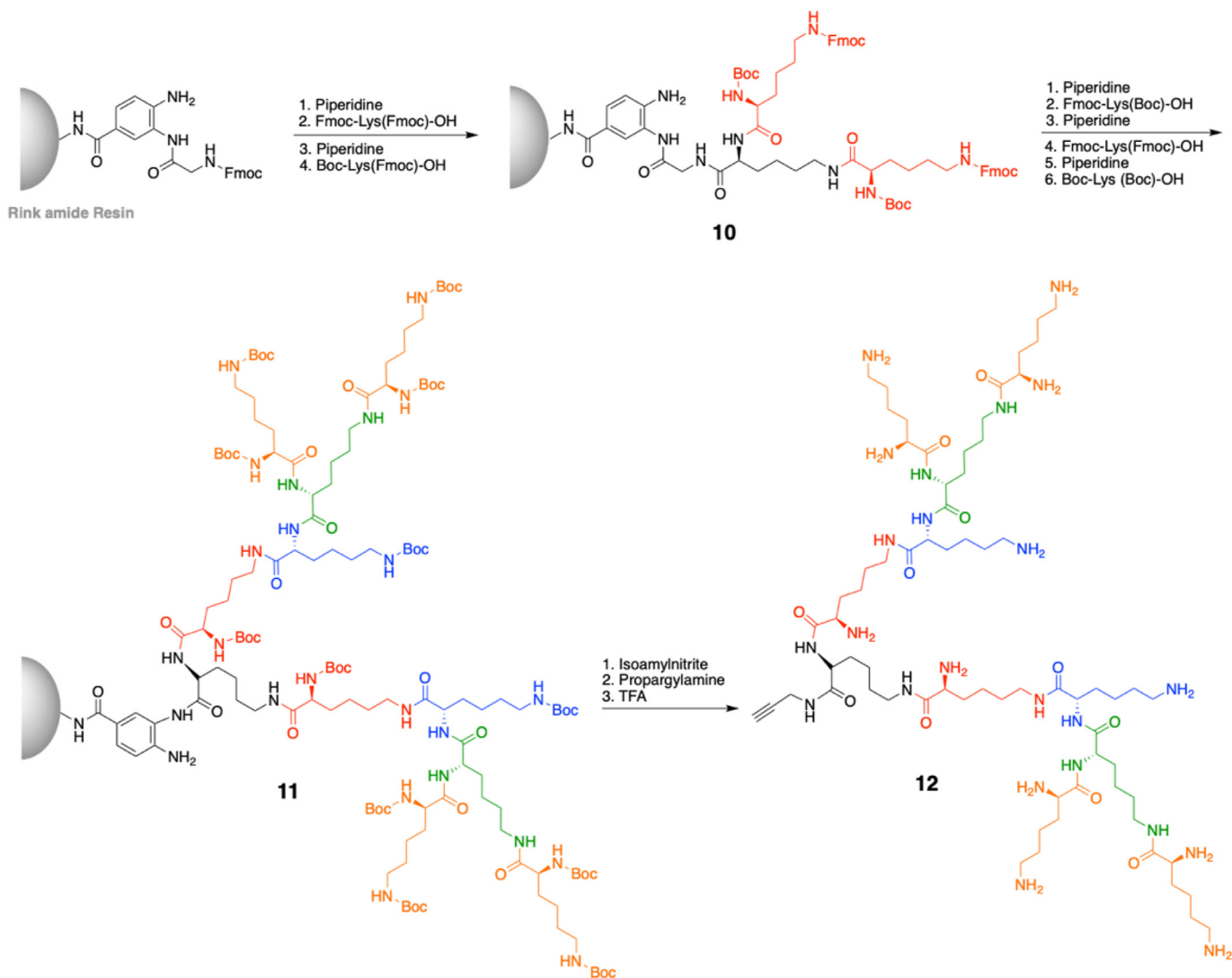


Fig. 7. Solid-phase synthesis of a fourth generation of defect lysine dendrimer [41].

A good linker should readily undergo functionalization with a variety of reagents allowing it to initiate synthesis on the solid support. It should also remain stable during the whole synthesis and permit efficient cleavage without affecting the final products.

Two types of linkers are commonly used for solid-phase synthesis, namely integral and non-integral linkers (Fig. 3B) [37]. An integral linker has the reactive functionality directly attached to the solid support for initiating solid-phase synthesis, whereas a non-integral linker is usually prepared by attaching an additional reactive chemical entity to the resin for solid-phase synthesis. Despite their widespread use, integral linkers have the disadvantage of little flexibility and limited diversity in synthesis. In contrast, non-integral linkers are able to provide more diverse functionalities for solid-phase synthesis. In most cases, solid supports with non-integral linkers are used for dendrimer synthesis because of the potential to not only introduce various functionalities at the core but also to avoid eventual steric hindrance when synthesizing high generation dendrimers.

The acid-labile Wang linker and the Rink amide linker are currently the two most widely-used non-integral linkers (Fig. 4). The Wang linker uses *p*-alkoxybenzyl alcohol to anchor synthetic product through an ester bond. The product with carboxylic acid

can be collected through acid hydrolysis and transesterification. The Rink amide linker includes a diphenylmethylamine to link the products. Because of the secondary amine, the Rink amide linker is more stable than the Wang linker, allowing more diverse reactions. In addition, the above-mentioned linkers can be further derivatized in order to introduce other functionalities into the final products. The *p*-alkoxybenzyl alcohol in the Wang linker can be replaced with *p*-alkoxybenzyl amine, giving the Wang amide linker, to obtain carboxamide products instead of carboxylic acid (Fig. 4). However, use of the Wang amide linker leads to a considerable amount of side products making it less popular than the Rink amide linker [38]. The Rink amide linker on the other hand has several derivatives, such as the Knorr linker and the Rink acid linker. Use of the Knorr linker also gives carboxamide product, while the Rink acid linker delivers carboxylic acid product instead of carboxamide.

3.3. Loading capacity

Loading capacity refers to the substitution capacity of the resin. Numerous solid supports with varying loading capacities of different linkers and functional groups are now commercially

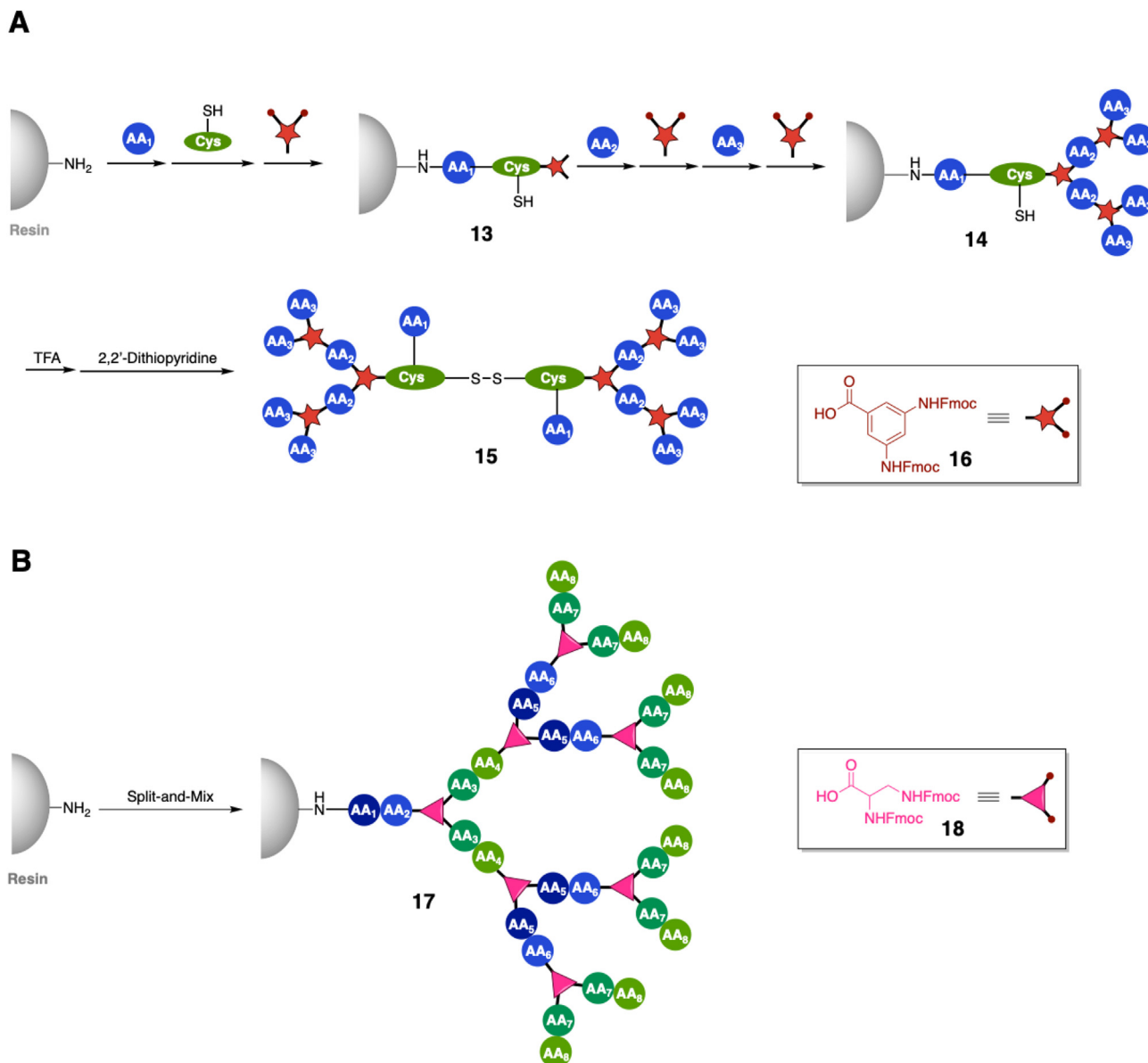


Fig. 8. (A) Solid-phase synthesis of peptide dendrimer libraries [47]; (B) Solid-phase synthesis of peptide dendrimer libraries using the split-and-mix approach. AA_n: amino acid residues; Cys: Cysteine [45].

available. Resins with high loading capacities harbor more reactive sites, thereby yielding more product in quantity than those with low loading capacities. High-loading resins are usually recommended for solid-phase peptide synthesis to achieve higher total production. However, during dendrimer synthesis, loading capacity needs to be carefully gauged in relation to the final dendrimer size and generation. Lower loading capacity is preferred when synthesizing higher generation dendrimers [34,39]. This is because higher generation dendrimers increase in size and occupy a greater space and volume as their branched structures grow. When using a solid support with a high loading capacity, steric hindrance may arise from two neighboring dendrimers on the same resin (Fig. 3C). This may affect or impede further reactions, resulting in incomplete reactions and hence defected dendrimers. Therefore, in addition to using flexible resin, reducing the loading capacity is another common strategy for preparing high-generation dendrimers using solid-phase synthesis.

3.4. Reactions

For solid-phase synthesis, the reactions employed should be highly efficient and devoid of possible side reactions and by-products. They should also be compatible with the solid support. Many reactions performed in solution can be implemented with high efficiency in solid-phase synthesis by using a large excess of reagents. However, success is not guaranteed every time. Notably, solid-phase reactions using bulky reagents should be avoided in dendrimer synthesis owing to steric shielding by the dendritic structure.

Similarly, the general principle of protective group chemistry in solution-phase synthesis also applies in solid-phase synthesis [32,40]. Nevertheless, the choice of protecting groups and their removal methods requires additional and critical consideration when relating to solid-phase dendrimer synthesis. For example, the protection and deprotection reactions should be compatible with the reactivity and stability of the resin-bound products during

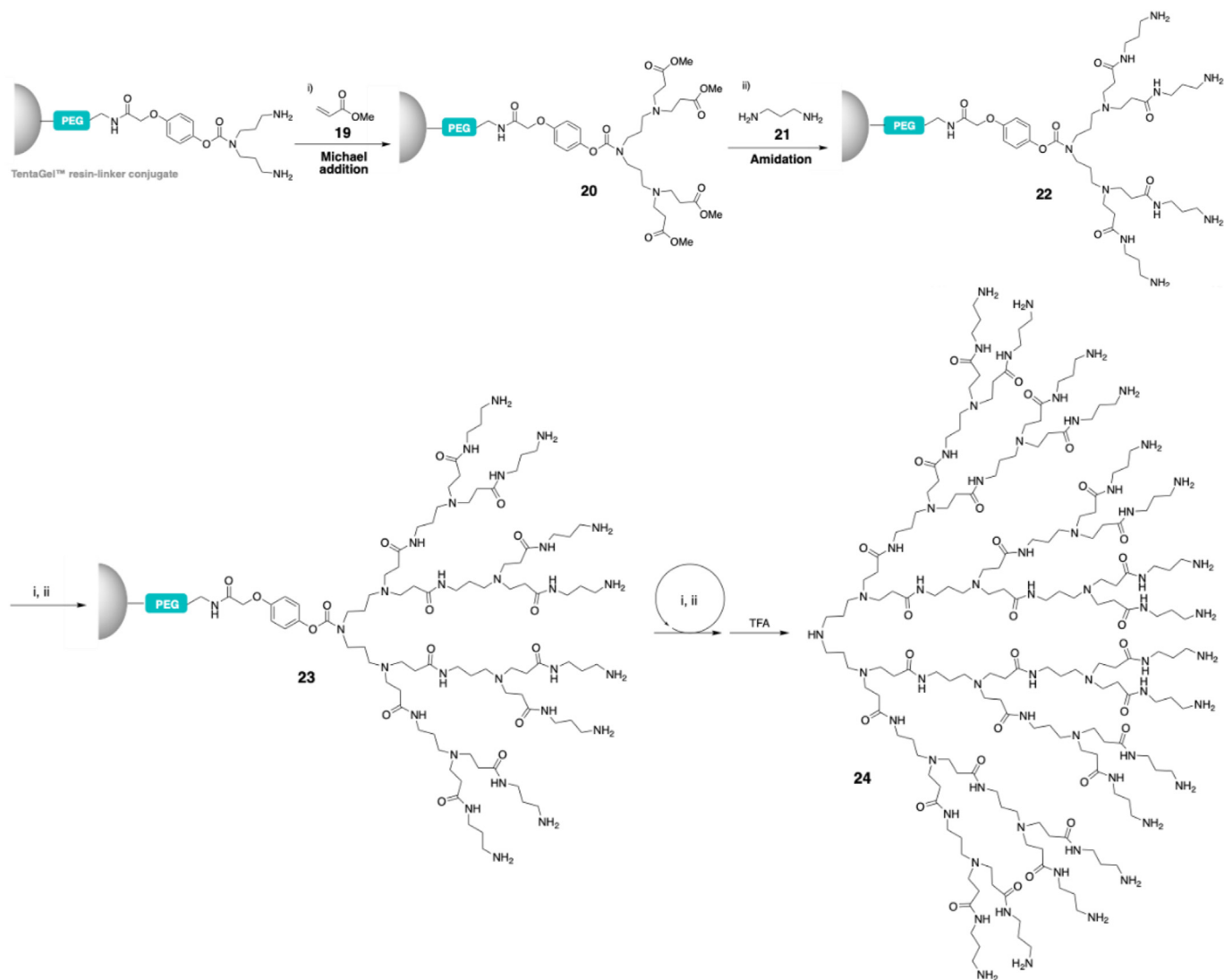


Fig. 9. Solid-phase synthesis of poly(amidoamine) (PAMAM) dendrimers via iterative Michael addition and amidation [39].

solid-phase synthesis. Also, unlike solution synthesis, highly efficient protecting and deprotecting reactions are required in solid-phase synthesis in order to avoid the formation of by-products of side reactions at each step which even at tiny levels can create a complex mixture of products and side-products by the end of the synthetic procedure. This is a very important consideration in dendrimer synthesis due to the difficulties in separating desired dendrimers from structurally similar defected analogs. It should be mentioned that bulky protecting groups constitute another major obstacle in dendrimer synthesis, especially for higher generation dendrimers. Indeed, the proximity of dendritic branches and terminals within the dendrimer structure generates steric hindrance, which reduces reactivity and product yield while increasing side products and impurities. Finally, the introduction of building units with orthogonal protecting groups constitutes an ongoing goal of scientists aiming to offer dendrimers with structural and functionality diversity [41]. Altogether, the appropriate arrangement of protection and deprotection reactions contributes not only to improving reaction efficiency and product purity but also to the successful construction of dendrimers in general.

In addition to the reactions that construct the desired products, scavenging and capping reactions are also unique and important

aspects of solid-phase synthesis. Scavengers can be used to neutralize active side-products to avoid their further reaction with the desired products on the resin. Capping refers to masking or inactivating the remaining unreacted functional sites on the solid support. Capping is important for the synthesis of linear products, such as peptides and oligonucleotides; however, it is rarely used in dendrimer synthesis because the resulting capping by-products have similar physical properties to dendrimers, and are also hard to separate from the reaction mixture.

3.5. Reaction monitoring

Monitoring reaction progress in solid-phase synthesis is crucial yet challenging. Conventional methods rely on analyzing protecting groups released from the solid support, or using a facile colorimetric test to track the appearance and disappearance of a chemical functionality or entity on the solid support [36]. Recently, emerging technological advances have made solid-state NMR, IR, and Raman spectroscopy suitable non-destructive methods for tracking the progress of the reaction *in situ* on the solid phase [42]. However, these methods are limited to the selective detection of certain special functional groups, and are not yet generally applicable to all

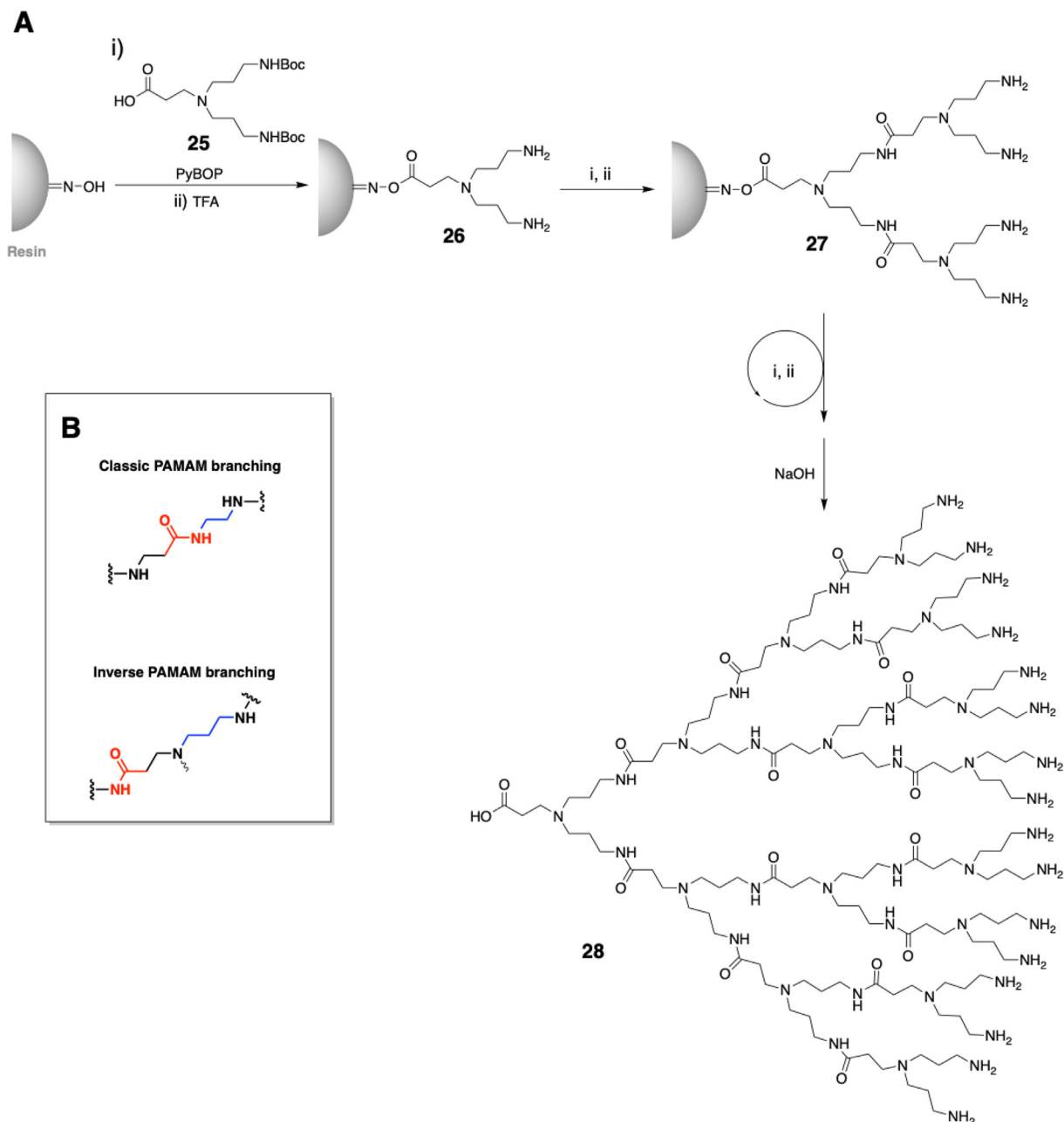


Fig. 10. Solid-phase synthesis of inverse poly(amidoamine) (PAMAM) dendrimers using peptide synthesis chemistry [55].

solid-phase synthesis methods, and in particular to the solid-phase synthesis of dendrimers. The development of online analytical methods enabling the monitoring of reaction progress will therefore greatly accelerate and facilitate solid-phase dendrimer synthesis.

Careful consideration of all the issues mentioned above has enabled successful solid-phase synthesis of a variety of dendrimers up to generation 7 [43]. We will comment on all these issues using the specific examples presented below.

4. Solid-phase dendrimer synthesis

In this section, we highlight pioneering and representative studies on the solid-phase synthesis of various dendrimers, and discuss the challenges and promises associated with specific

dendrimer synthesis. We start with peptide dendrimers, then progress to PAMAM, carbohydrate, oligonucleotide, polyester, polyurea, polyether/polythioether, and triazine dendrimers.

4.1. Peptide dendrimers

Peptide dendrimers have often been conceived as protein mimics, anticancer and antimicrobial agents, vaccines, drugs, and drug delivery systems [21,40,44–51]. In 1988, Tam described the first solid-phase synthesis of peptide dendrimers for the preparation of multiple antigen peptide systems (MAPs) on a polystyrene support (Fig. 5) [21]. These MAPs were composed of a polylysine dendrimer as the dendritic backbone, with peptide antigens appended on the polylysine dendrimer periphery to provide

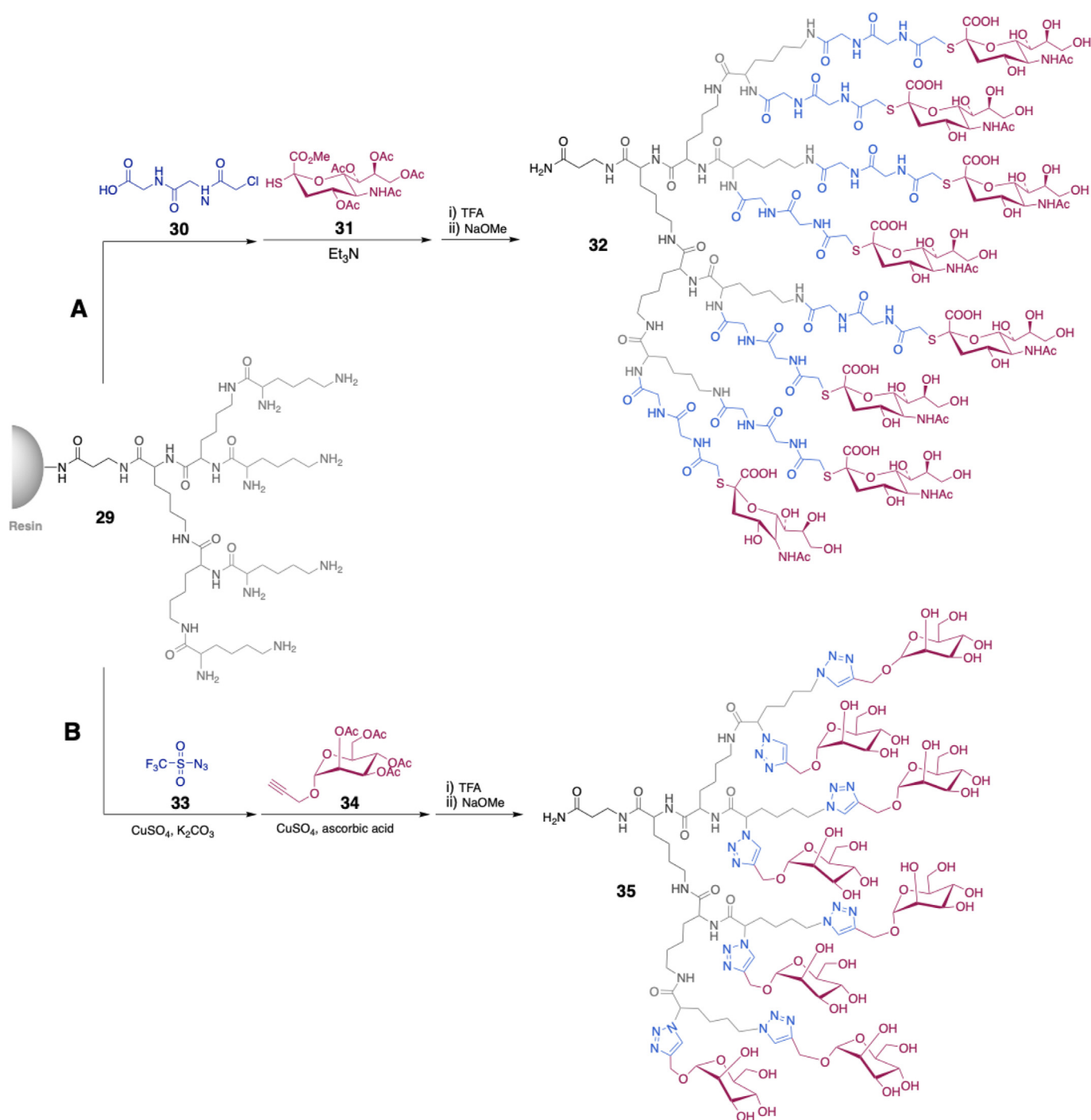


Fig. 11. Solid-phase synthesis of glyco-polylysine dendrimers via (A) thiolation and (B) click reaction [56,57].

multivalency. Both the polylysine dendrimer core and the peptide antigens at the terminals were achieved via solid-phase synthesis. The synthesis started with the assembly of the polylysine dendrimer on a solid support, which involved attaching protected lysine **1** via its free carboxylic acid terminal to the amine-bearing PS resin. Subsequent deprotection freed the two amine functions at the α -position and on the side chain for further coupling with the free C-terminal of **2**. Iterative synthesis allowed increasingly high generation of the peptide dendrimer to be made in this way, and the final product was cleaved from the resin once the desired

peptide antigen sequence had been achieved (Fig. 5). The dendritic MAPs thus obtained were used as immunogens for various immune response investigations as well as for antibody production.

Following the successful synthesis of MAPs, many peptide dendrimers, such as poly(glutamic acid), polyarginine, polyproline, and polyglycine as well as those composed of unnatural amino acid residues were also prepared by solid-phase synthesis on polystyrene resin [40]. Recently, Wang et al. applied solid-phase synthesis to prepare Janus dendrimer which bears polylysine backbone for dendrimer entities while carrying the hydrophilic zwitterionic

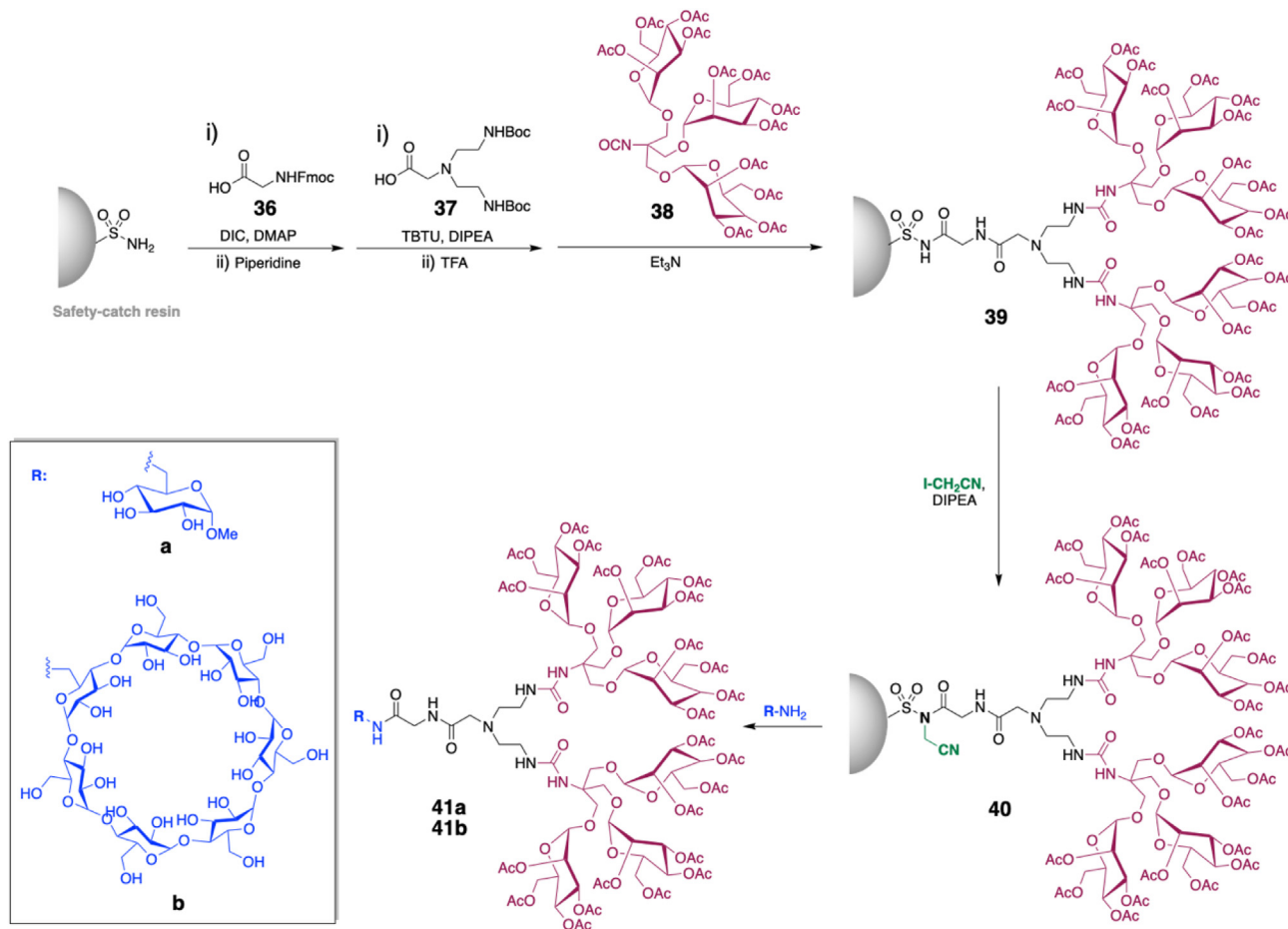


Fig. 12. Solid-phase synthesis of glycodendrimers using safety-catch resin for core diversification [58].

phosphocholine terminals at one face and the hydrophobic terminals at the other face (Fig. 6) [52]. Such a Janus dendrimer is otherwise difficult to prepare using solution synthesis. To achieve this, the authors first attached the protected lysine (Fmoc-Lys(Dde)-OH) to the Rink resin, and selectively removed Fmoc group to free the lysine α -amino group for subsequent conjugation with an extended chain. From this chain, a polylysine dendrimer backbone was built and the zwitterionic phosphocholine terminals were appended. After achieving this dendron entity, the ω -amino group of the first lysine residue attached to the resin (**7**) was then deprotected, allowing construction of the second polylysine dendron with the hydrophobic rhein as terminals. The obtained dendrimer **9** is amphiphilic and able to self-assemble into nanomicelles for encapsulation of the hydrophobic anticancer drug doxorubicin. The hydrophobic terminals further enabled strong binding with doxorubicin via π - π interaction and provided stable drug encapsulation, while the zwitterionic phosphocholine terminals avoided strong interaction with serum proteins, hence leading to a prolonged blood circulation time and significantly enhanced anticancer efficacy.

Also, Liao et al. prepared the “precisely defected” polylysine dendrimers using four distinctly protected lysine building units, which possess a different number and arrangement of Boc and Fmoc protecting groups on the α - and ω -amino groups of lysine (Fig. 7) [41]. By arranging the incorporation order of these four differently protected lysine units, polylysine dendrimers with precisely designed fracturing branches were constructed. It should

be mentioned that defect dendrimers were reported for their advantageous behavior in gene delivery more than 3 decades ago [53]. However, no well-defined defected dendrimers could be readily synthesized for detailed structure-activity relationship analysis. Precisely building the defected dendrimers using solid-phase dendrimer synthesis will allow such structure-activity relationship studies in the view to elaborating the most effective dendrimers for gene delivery.

Noteworthy, Reymond et al. pioneered the use of solid-phase synthesis to prepare peptide dendrimer libraries using Rink amide TentaGel® resin (Fig. 8) [46,47]. They inventively incorporated the catalytic triad serine-histidine-aspartate at variable positions while employing Fmoc-protected 3,5-diaminobenzoic acid (**15**) as the branching point to create combinatorial dendritic motifs that mimic esterases (Fig. 8A). By introducing cysteine, larger dendrimers were constructed via disulfide bridges.

For the first time, Reymond et al. also created peptide dendrimer libraries using a split-and-mix approach through solid-phase synthesis (Fig. 8B) [45]. These peptide dendrimers contain eight variable amino acid positions spanning outwards along three continuous branching units made of L-2,3-diaminopropanoic acid (**18**). A combinatorial library of peptide dendrimers was constructed with four different amino acids per variable position. The combinatorial construction of dendrimer libraries using a solid-phase strategy has since provided versatile and rapid access to peptide dendrimers with both sequence and structural diversity. These synthesized dendrimers are useful in various biological

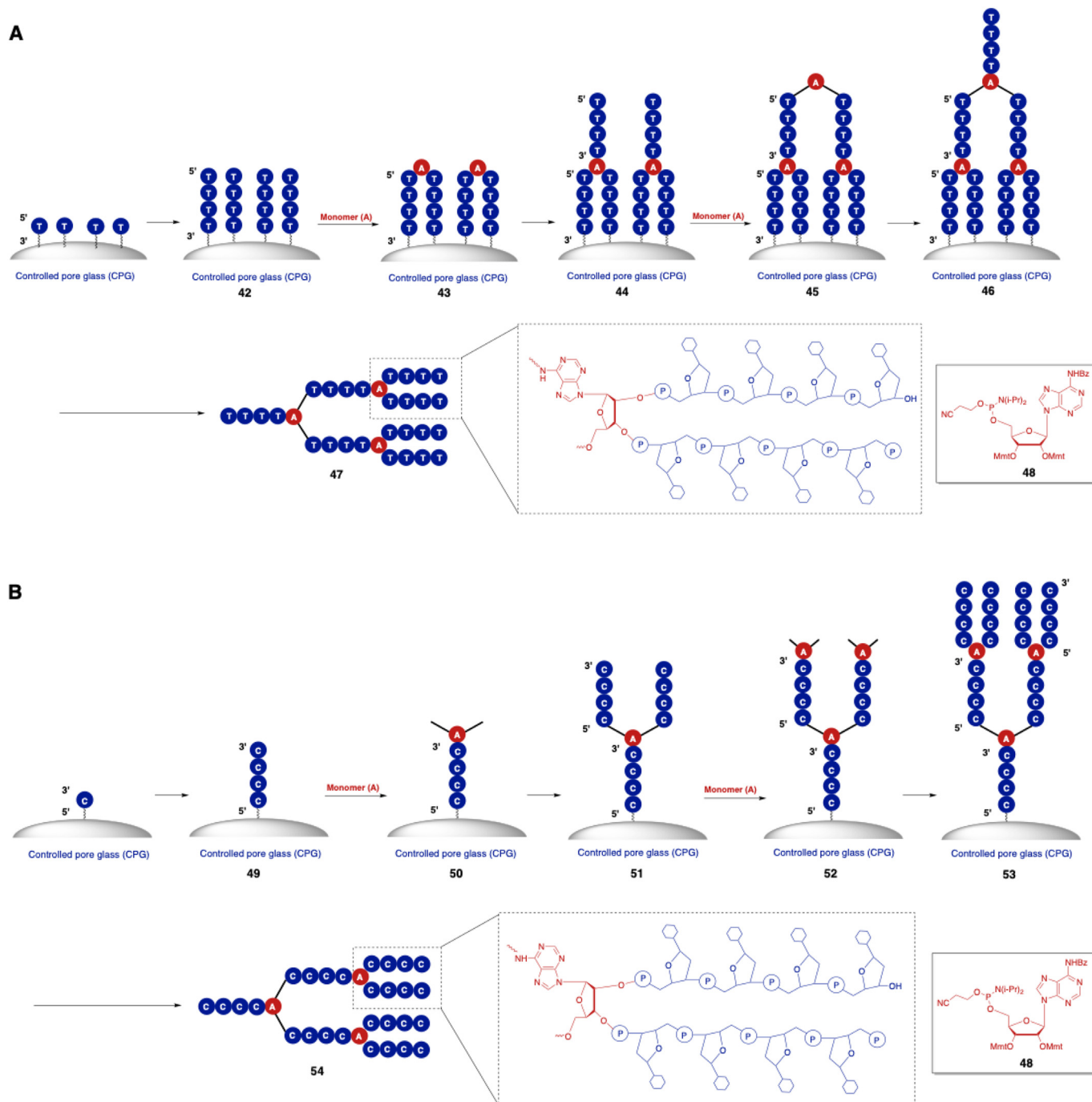


Fig. 13. Solid-phase synthesis of nucleotide dendrimers using (A) a convergent strategy [59] and (B) a divergent strategy [60].

applications, such as enzyme mimicry and drug screening, as well as drug and gene delivery [44–51].

4.2. PAMAM dendrimers

PAMAM dendrimers have become the most extensively investigated dendrimers since their first development and presentation in the seminal report by Tomalia [2,54]. The first solid-phase synthesis of PAMAM dendrimers was achieved by Bradley and co-workers in 1997 [39]. They used a TentaGel[®] resin-linker conjugate carrying a long and flexible PEG spacer, which was designed to make the reactive sites accessible to reagents while avoiding

steric hindrance during the preparation of high-generation dendrimers. The synthesis was carried out using an iterative two-reaction sequence consisting of a double Michael addition of the terminal amine to methyl acrylate (19), and subsequent amidation with diamine 21 (Fig. 9), similar to the traditional synthesis of PAMAM dendrimers in solution. The so-prepared TentaGel[®] resin-anchored PAMAM dendrimers capitalize on the dendrimer multiplicity and the resulting increase in the number of reactive sites, and can therefore be used as super high-loading reagents for the solid-phase synthesis of peptides, amidines, and aryl ethers [34].

Notably, the synthetic protocol for PAMAM dendrimers, which consists of iterative Michael addition and amidation, creates

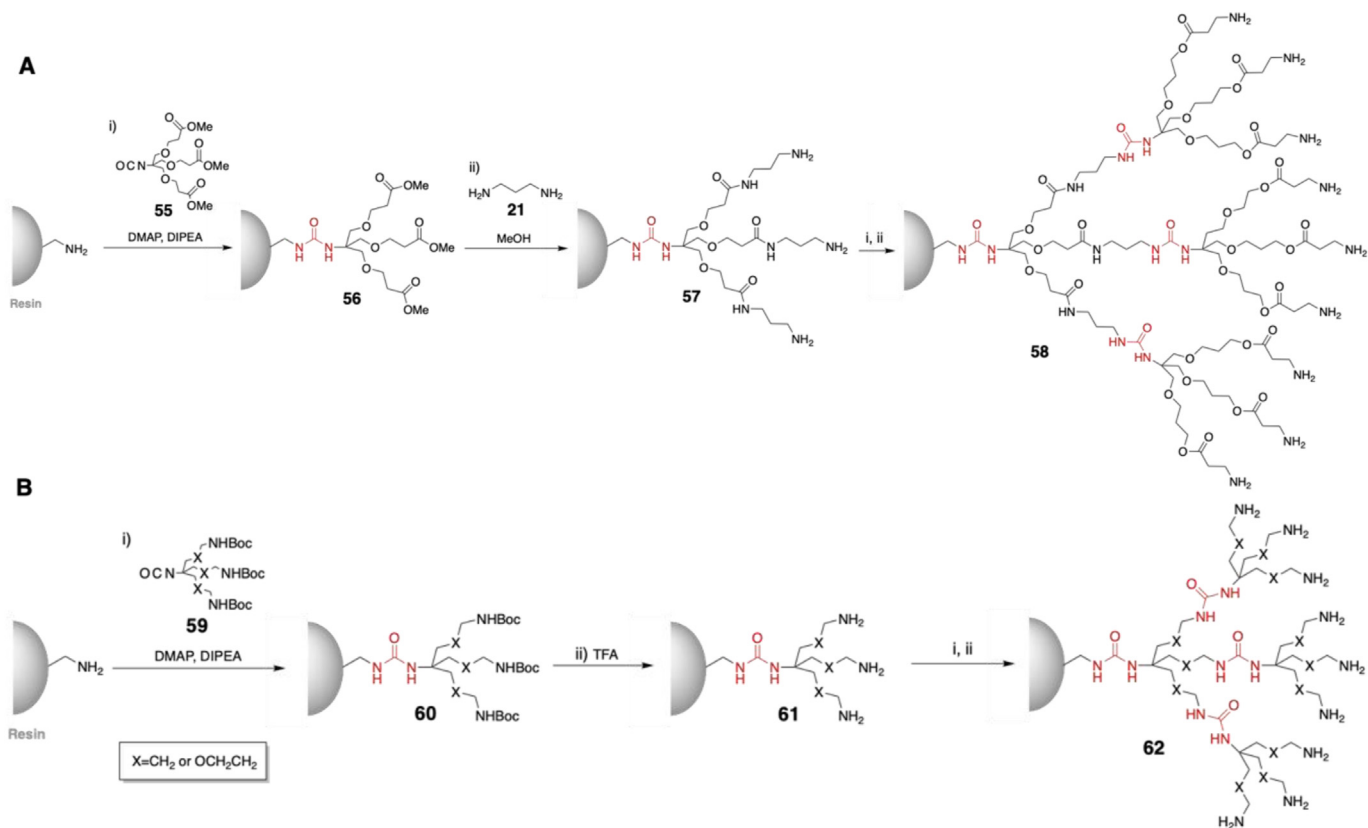


Fig. 14. Solid-phase synthesis of polyurea dendrimers via an iterative synthesis process consisting of (A) branching unit condensation and subsequent amidation [61] and (B) branching unit condensation and subsequent deprotection [62].

structural defects which are similar to those identified in dendrimer synthesis in solution, including cyclized peripheral groups, dimerized forms and retro-Michael products [2]. To circumvent this problem, Huang et al. developed a concise solid-phase synthesis for PAMAM dendrimers [55]. They ingeniously harnessed the solid-phase peptide synthesis (SPPS) approach to enable PAMAM dendrimer construction by a completely new synthetic route, namely, iterative amide bond formation using construction unit **25** harboring both a carboxylic acid function and amine terminals along with tertiary amine branching (Fig. 10A). In contrast to the conventional two-step iterative synthetic protocol for PAMAM synthesis, this approach significantly shortened the reaction steps, and simultaneously prevented the structural defects originating from Michael addition and amidation. This highly efficient protocol was carried out using a solid-phase peptide synthesizer, which enabled automatic dendrimer synthesis. Thus, the corresponding 2nd- to 5th-generation dendrimers were delivered with yields of up to 93% within 1 week at a 0.10 mmol scale. Furthermore, by reducing the loading capacity of the solid support, it was possible to prepare a 7th-generation dendrimer via the same chemistry [43]; at full loading capacity, only defective dendrimers were obtained. Compared with conventional PAMAM dendrimers, these dendrimers have the amide bond linkage in the opposite direction (Fig. 10B) as a result of the peptide synthesis chemistry. Therefore, they are also referred to as inverse PAMAM dendrimers. Also of note is that these inverse PAMAM dendrimers have one more methylene unit in the diamine connecting motif (Fig. 10B), which prevents the self-cyclization side reaction during

synthesis, and also ensures that the inverse PAMAM dendrimers are similar in size to the classic PAMAM dendrimers made by Tomalia et al.

4.3. Carbohydrate dendrimers

Carbohydrate dendrimers, also called glycodendrimers, are of particular interest for protein interaction and recognition by virtue of their glycomimetic properties. Their cooperative multivalency enhances their binding affinity to protein receptors. In 1993, Roy et al. applied solid-phase synthesis to construct the first glycodendrimer based on a polylysine peptide dendrimer core (Fig. 11) [56]. They decorated the periphery of the polylysine dendrimer with sialic acid via a diglycine linker through thiolation (Fig. 11, route A). More recently, they reported a highly efficient strategy for the synthesis of mannosylated dendrimers via click chemistry [57]. In this process, they transformed the amine terminals of polylysine to azido functions, then used a copper sulphate catalyst for azide-alkyne cycloaddition with propargyl- α -D-mannopyranoside (**34**) (Fig. 11, route B).

Most glycodendrimers have carbohydrate units at their peripheries. To introduce carbohydrate units to the dendrimer core, Fernandez et al. used an Ellman safety-catch resin to incorporate glucose or β -cyclodextrin in the dendrimer core during the resin cleavage step (Fig. 12) [58]. To achieve this, they first activated the dendrimer-anchored linker using iodoacetonitrile to produce **40**, which was subjected to chemo-selective treatment with the carbohydrate-bearing amine nucleophile to deliver the core-

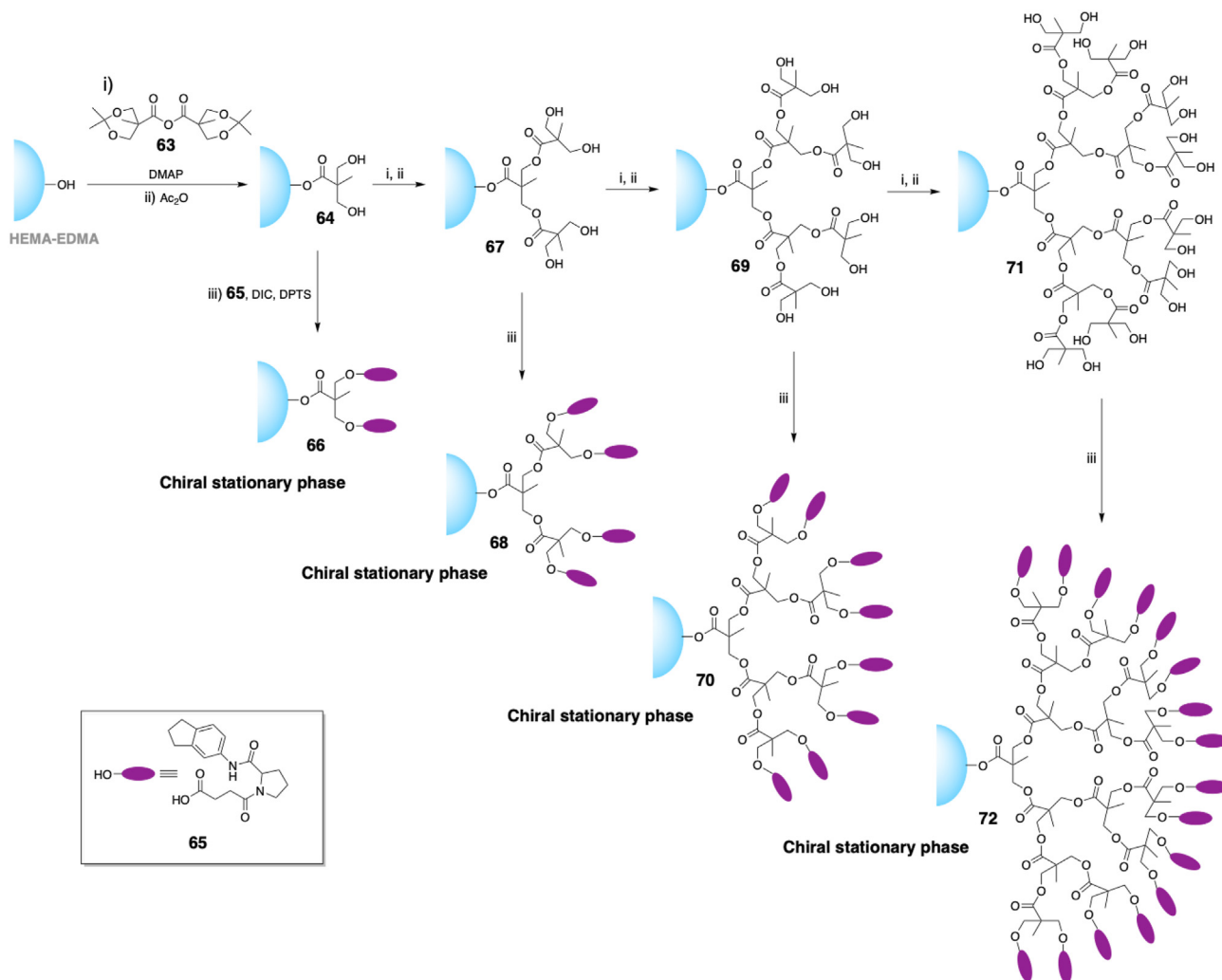


Fig. 15. Solid-phase synthesis of polyester dendrimer-bound polymer beads as a stationary phase for chiral HPLC separation [63].

substituted glycodendrimer **41** (Fig. 12). This method offers a practical and convenient means of diversifying the structure and chemistry of the glycodendrimer core.

4.4. Nucleic acid dendrimers

Nucleic acid dendrimers have been prepared by taking advantage of the solid-phase synthesis of oligonucleotides via phosphoramidite chemistry. Both convergent and divergent approaches have been applied to synthesize nucleic acid dendrimers in the solid phase (Fig. 13) [59,60]. For example, Hudson and Dhama prepared a second-generation nucleic acid dendrimer using the convergent strategy with the protected adenosine-2',3'-*O*-bis(phosphoramidite) **54** as the focal point to link neighboring branches together (Fig. 13A) [59]. Although this method has successfully delivered nucleic acid dendrimers, the reaction must be carefully and stringently controlled, which means it is less suitable for synthesizing high-generation dendrimers.

Divergent synthesis (Fig. 13B) was developed to overcome this limitation by implementing the chemical synthesis of oligonucleotides in the 5' → 3' ("backward") direction rather than the conventional 3' → 5' direction [60]. To achieve this, the oligonucleotides were first assembled onto a solid support of Controlled-Pore Glass (CPG) using an automated DNA synthesizer.

Branching unit **54** was then used in large excess to force the construction of branches to completion. This was followed by divergent growth to generate full-length dendrimers. These oligonucleotide dendrimers are potentially useful for the selective modulation of gene expression. They can also be employed as ligands for affinity purification of the branch recognition factors, which catalyze the maturation or splicing of precursor mRNAs in the cell.

4.5. Polyurea dendrimers

Polyurea dendrimers are a new family of biocompatible dendrimers that harbor urea functionalities. In 2000, Bradley reported the solid-phase synthesis of polyurea dendrimers using an AB₃-type branching unit **55** bearing an isocyanate moiety and three ester functions (Fig. 14A) [61]. The synthesis started by anchoring the isocyanate unit onto the amine-bearing solid support via the formation of urea, and was followed by subsequent amidation of the terminal ester functions with propylenediamine (**21**). The resulting amine-terminated resin was then subjected to iterative reaction with the isocyanate-bearing construction unit **55**, generating the corresponding polyurea dendrimers (Fig. 14A).

To avoid intra-molecular cyclization, Bradley et al. further devised new construction units **59** bearing protected amine terminals to replace the previous ester functions. In this way, they

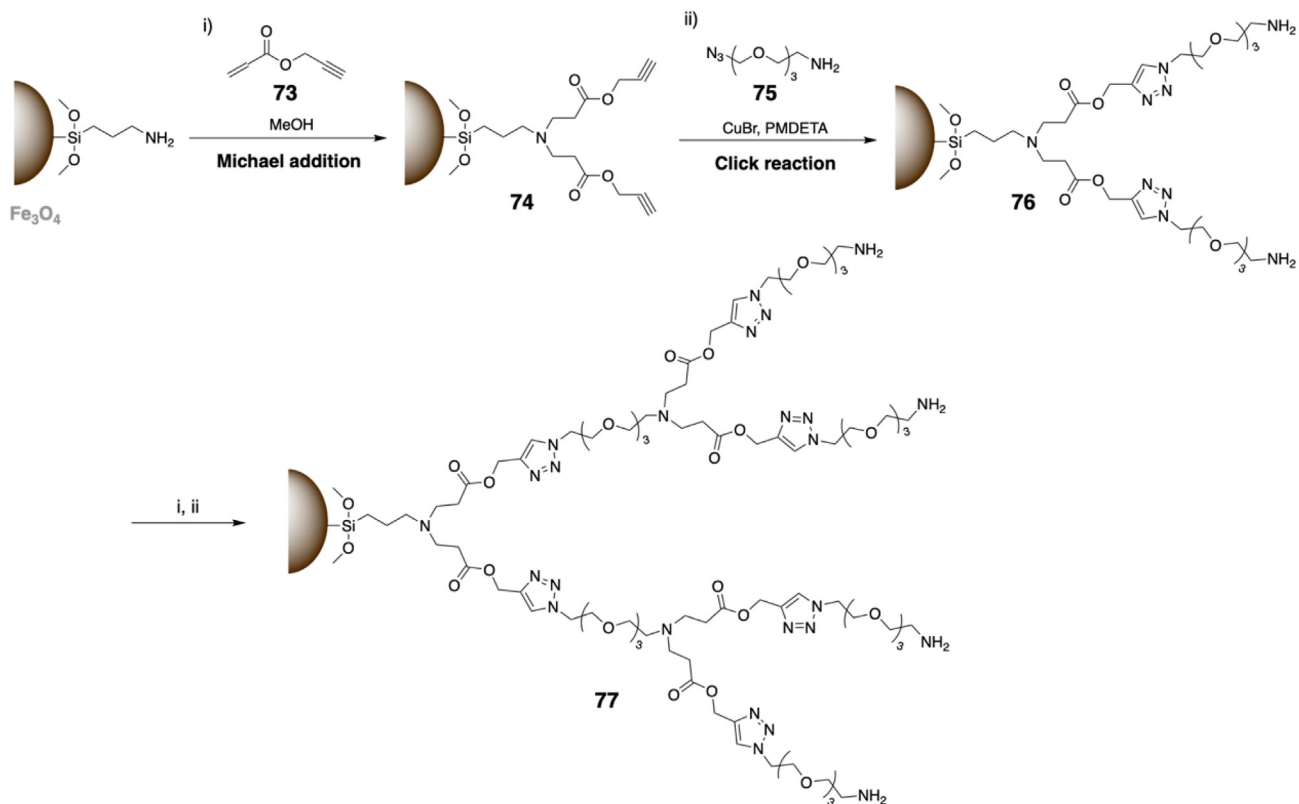


Fig. 16. Solid-phase synthesis of poly(amino)ester dendrimers to decorate Fe_3O_4 nanoparticles as magnetic resonance imaging agents [65].

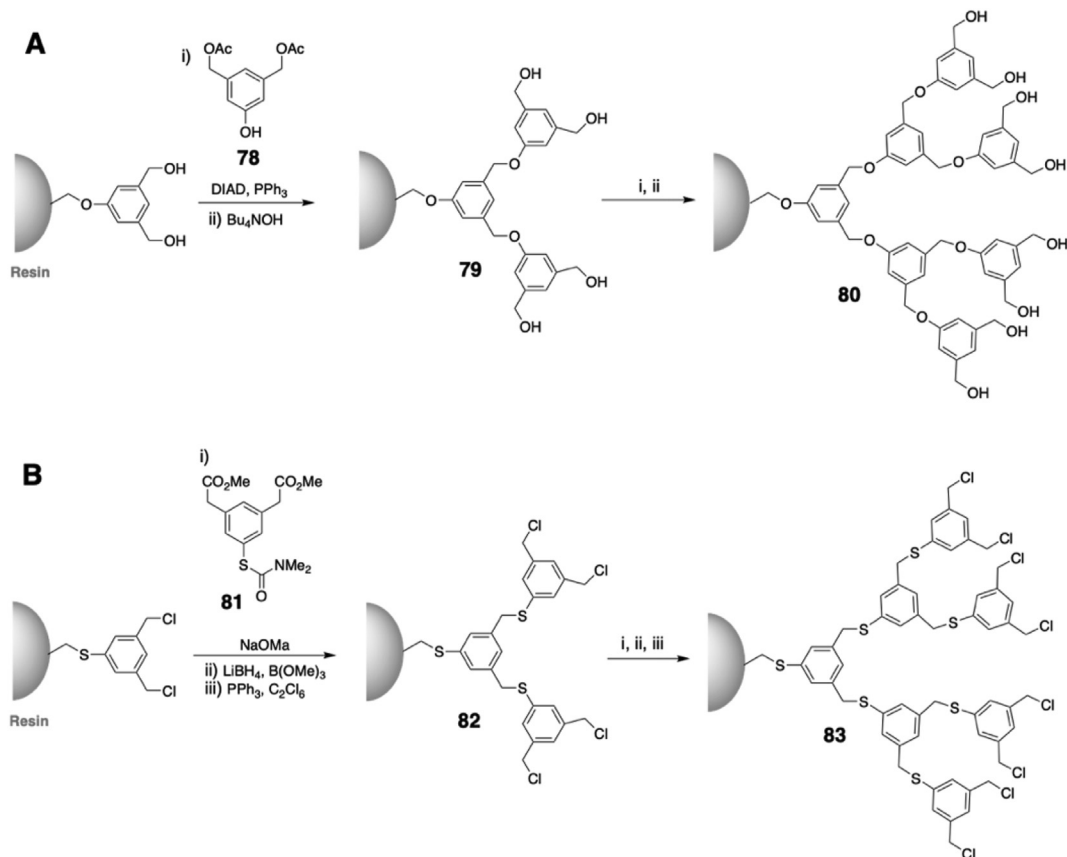


Fig. 17. (A) Solid-phase synthesis of polyether dendrimers via an iterative synthesis process involving Mitsunobu coupling and ester hydrolysis [66]; (B) Solid-phase synthesis of polythioether dendrimers using a chlorohydroxylation-nucleophilic substitution reaction [67].

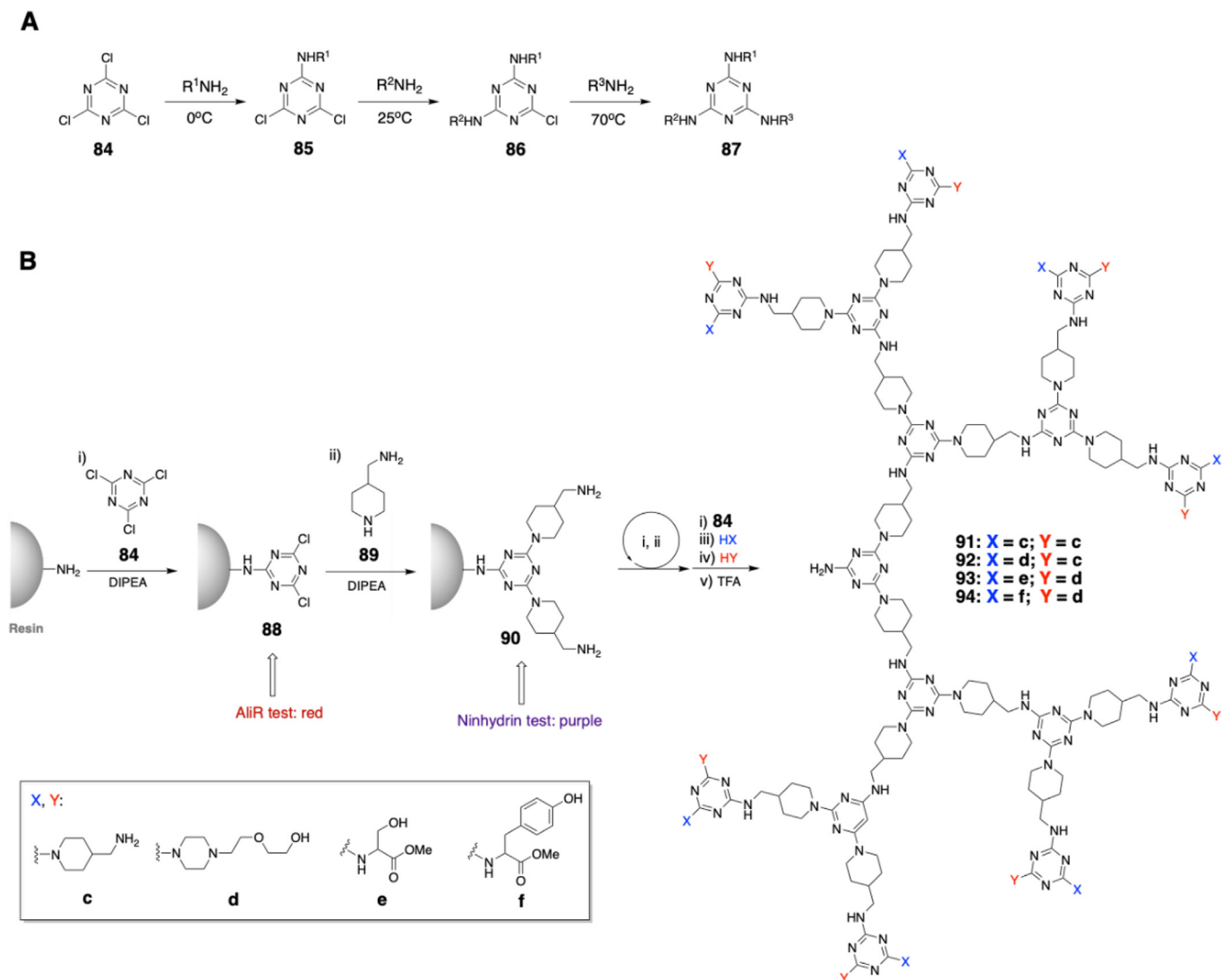


Fig. 18. (A) Temperature-dependent chemo-selective reactivity of cyanuric chloride [69]; (B) Solid-phase synthesis of triazine dendrimer libraries [71].

bypassed the amidation step, thereby obviating side reactions while significantly shortening and simplifying the synthesis (Fig. 14B) [62].

4.6. Polyester dendrimers

Fréchet and co-workers explored the solid-phase synthesis of polyester dendrimers on a polymer resin [63]. Their aim was to create stationary phases for HPLC-based chiral separation by capitalizing on the multivalent properties of the dendrimers to enhance the separation power. The dendrimer construction mainly relied on a 2,2-bis(hydroxymethyl)propanoic acid anhydride-derived branching unit **63** for ester bond formation, while an *L*-proline derivative **65** was appended to the dendrimer terminals to provide a chiral environment (Fig. 15). The so-obtained supported dendrimer resins were successfully used as chiral stationary phases for HPLC, enabling effective separation of enantiomers of amino acid derivatives.

Among the various polyester dendrimers, poly(aminoester) dendrimers have emerged as promising biodegradable and biocompatible materials for biomedical applications by virtue of

the presence of numerous ester and amine functions [64]. The ester backbones can be easily disassembled by enzymatic activity or in acidic/basic conditions, which imparts biodegradability. The numerous amine functionalities can also serve as buffers to neutralize the acids generated from ester hydrolysis, hence providing a neutral and benign environment during and after dendrimer degradation. With the aim of developing Fe_3O_4 as a magnetic resonance imaging agent for biomedical applications, Li et al. enclosed Fe_3O_4 nanoparticles within a poly(aminoester) dendrimer shell. They used consecutive iterations of Michael addition and a Cu(I)-mediated click reaction to construct poly(aminoester) dendrimers on the surface of Fe_3O_4 nanoparticles (Fig. 16). Their synthetic protocol was devoid of any protection/deprotection chemistry and provided the desired product in good yield [65].

4.7. Polyether/polythioether dendrimers

Polyether dendrimers are expected to exhibit better chemical inertness and stability compared with polyamide, polyester, and polyurea dendrimers mentioned above. This is by virtue of the

ether linkage, which is much more robust and resistant to reductive, hydrolytic, and nucleophilic reactions than amide, ester, and urea functionalities. Bradley et al. established the first solid-phase synthesis of polyether dendrimers based on a 3,5-bis(acetoxymethyl)phenol building block (**78**) using a solid polystyrene support [66]. They implemented a two-step synthetic sequence comprising a Mitsunobu reaction and ester hydrolysis to successfully construct polyether dendrimers (Fig. 17A).

Later, Portnoy et al. developed an improved strategy to synthesize polyether and poly(thioether) dendrimers. They replaced the Mitsunobu reaction with a chlorodehydroxylation-nucleophilic substitution reaction (Fig. 17B) employing the building block **81** which can be easily prepared from commercially available reagents. This new method avoided the Mitsunobu reaction, which requires expensive reagents with limited shelf-life. The polyether and polythioether dendrimers bearing phosphine ligands were also prepared and complexed to Pd and Co transition metals to create dendrimer-based multivalent catalysts for cross-coupling reactions [68]. Indeed, these resin-bound dendrimer catalysts significantly improved the selectivity and reactivity with a positive dendritic effect.

4.8. Triazine dendrimers

Triazine dendrimers (Fig. 18), composed of 1,3,5-triazine rings connected with diamine units [69], were first outlined in the patent filed in the 1990s by Meijer et al. The synthesis of triazine dendrimers is mainly based on nucleophilic aromatic substitutions of cyanuric chloride (**84**). By exploiting the different reactivities of the cyanuric chlorides with regard to amine nucleophiles, it is possible to obtain mono-, di-, or tri-substituted triazines by modulating the temperature and reaction time (Fig. 18A). This constitutes the foundation for the construction of triazine dendrimers. Today, a large variety of triazine dendrimers with different diamine linkers and surface functionalities has been established. Their potential for use in drug and gene delivery has been intensively investigated by Simanek et al. [70].

By harnessing the chemo-selective substitution of cyanuric chloride (**84**), Huang et al. developed solid-phase synthesis to prepare a library of triazine dendrimers with various combinations of terminal functionalities originating from the cyclic secondary amines and primary amines of amino acids (Fig. 18B) [71]. They also ingeniously established orthogonal staining methods using the Kaiser ninhydrin test and Alizarin R (AliR) to reveal, respectively, alkyl amines and aryl chlorides on the dendrimer terminals after each synthetic step. This allowed them to track the progress of the reaction, and hence easily and efficiently control the synthesis.

Notably, solid-supported triazine dendrimers have also been prepared to serve as supported reagents and devices. These dendrimers can be endowed with multivalent properties, yet can be easily recovered, regenerated, and reused for applications such as scavenging reagents and protein library screening [72].

Conclusions and perspectives

The solid-phase approach, with its simple operation and purification, as well as its potential for automation [20,22,73], offers a highly efficient and effective method for synthesizing dendrimers [33]. Most endeavors have been directed towards generating peptide and PAMAM dendrimers, as well as biologically relevant dendrimers of carbohydrates and their related conjugates. Efforts have also been devoted to synthesizing polyester, polyether/polythioether, polyurea, and triazine dendrimers using various solid

supports and diverse chemistry to construct dendrimers on the solid supports.

Nevertheless, it is to mention that heterogeneity is a major drawback of solid-phase synthesis in general, and for dendrimer synthesis in particular. In addition, substrates anchored to a solid support may have limited access to reagents owing to issues related to immobilization, steric hindrance, and size selectivity, which may slow down or even impede the reaction for dendrimer synthesis. As described in this review, using a solid support that bears a long and flexible PEG spacer may overcome this problem. It is also important to note that even a few side reactions with tiny levels of by-products in each step can create a complex mixture of products and side-products at the end of a synthetic procedure. Therefore, efficient protection and deprotection reactions are generally required in solid-phase dendrimer synthesis. Bulky protecting groups should be avoided because they may decrease the reactivity, hence reaction efficiency and yield, which is particularly important for the preparation of high generation dendrimer. It should also be mentioned that solid-phase dendrimer synthesis has not gone beyond generation 7. This is a limitation compared with solution-phase synthesis, where much higher generations can be achieved, such as generation 10 for PAMAM and polylysine, generation 12 for polyphosphorhydrazone, and generation 13 for triazine dendrimers.

Chemists continue to strive to find optimized chemical reactions involving the most appropriate building units, and use the convergent/divergent double strategy as well as click chemistry to construct higher-generation dendrimers more efficiently and in fewer steps [10]. However, the optimization of solid-phase dendrimer synthesis is laborious and tedious. One reason for this is that it is difficult to track the reactions without cleaving the products from their solid supports. Easy and convenient online and non-destructive analytical methods, such as solid-state NMR, IR, and Raman spectroscopy, which have been used in solid-phase peptide synthesis, would be extremely advantageous for solid-phase dendrimer synthesis.

Finally, solid-phase synthesis has the limitation of generating products in smaller quantities than solution-phase synthesis. Although the industrial production of peptides using solid-phase approaches can reach the kilogram scale, solid-phase dendrimer preparation in the laboratory is still limited to low-generation dendrimers at the milligram or gram scales. Therefore, continued efforts are needed to achieve the solid-phase synthesis of dendrimers with better quality, more diversity, and higher yields, at levels that are comparable with those currently obtained from solid-phase peptide synthesis.

Since their conception, dendrimers have become valuable chemical entities and functional materials, which have great potential for a wide range of applications by virtue of their unique structural properties and multivalent cooperativity [1,5]. Four decades on, their characteristic multi-step synthesis still requires tremendous effort, which greatly handicaps their development and limits their applications [6]. Although dendrimers have reached the market and been entered into clinical trials for anticancer, antibacterial, antiviral and anti-inflammation studies [10,11], continuing improvements in solid-phase dendrimer synthesis, alongside solution-phase synthesis, are crucial to obtain clean, pure and defect-free dendrimers. These improvements will transform dendrimer synthesis and enable the creation of structurally diverse dendrimers as new molecular paradigms and functional materials in a wide range of applications. We anticipate further research delivering more efficient methods for transforming dendrimer synthesis that will finally allow dendrimers to release their full potential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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