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Synthesis of Hyperbranched Polymers and their Applications in Analytical Chemistry

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Hyperbranched polymers (HBPs) are increasingly attracting the attention of scientists due to their unique physical and chemical properties, as well as their potential applications in different fields. Many methods for the preparation of HBPs have hitherto been reported. This review focuses primarily on several novel synthesis methods for various HBPs. We present and discuss the advantages and disadvantages as well as recent progress relating to these synthesis methods, namely the slow monomer addition method, click chemistry, and the enzyme-catalyzed polymerization method. Besides, the applications of HBPs in analytical chemistry fields such as sample pretreatment and immunosensors are also briefly summarized.

1. Introduction

Over the past thirty years, highly branched polymers (especially dendritic polymers) have received considerable attention due to their remarkable properties and potential applications. Dendritic polymers, including dendrimers and hyperbranched polymers (HBPs), are the fourth major class of polymers after linear, branched, and cross-linked architectures. Dendrimers are three-dimensional monodisperse polymers with a perfectly tree-like globular ordered structure, and consist of branched units and terminal units. The synthesis of dendrimers requires multi-step approaches, including protection, deprotection, and complex purification steps. HBPs are three-dimensional polydisperse systems with an elliptic randomly branched structure, and consist of linear units, branched units, and terminal units. Compared to their dendrimeric analogues, HBPs have irregular shapes and are not perfectly symmetrical, but display comparable properties and can be easily synthesized through “one-pot” reactions.[1-5]

Although the theoretical possibility of the synthesis of HBPs was predicted by Flory in the middle of the 20th century, the first HBP (hyperbranched polyphenylene) was only intentionally synthesized by Kim and Webster in 1988. Hyperbranched polymers (HBPs) have since attracted much attention due to their unique physical and chemical properties, such as higher solubility, lower viscosity, non-/low entanglement, and large numbers of terminal functional groups. To date, numerous HBPs have been prepared by different methods, including the polycondensation of AB_n monomers, self-condensing vinyl polymerization, ring-opening multibranching polymerization, and so on.[6–11]

Most early studies on HBPs were focused on the synthetic methods, characterization, and industrial applications, such as in the fields of coatings, cross-linking or adhesive agents, rheology modifiers and processing aids, encapsulation nanomaterials, or the fabrication of organic–inorganic hybrids.[12,13] More recently, many delicate supramolecular structures, such as nano- or micro-scale vesicles, micelles, fibers, films, and tubules, have been prepared through the self-assembly of HBPs.[14-19] These characteristics make HBPs very useful and demonstrated excellent potential applications in biomedical fields, such as bioimaging, sensors, drug delivery, antifouling materials, tissue engineering, and cytomimetics, which has attracted considerable research interest and thereby promoted their rapid development. Details of these applications are provided in several recent papers and reviews.[20-23]

There have been some review papers on the synthesis and applications of HBPs. For example, in 2004, Gao and Yates wrote excellent reviews on these topics, respectively.[2,24] In 2012, Jin *et al.* wrote a tutorial review focused on the self-assembly of HBPs and their cytomimetic applications.[25] However, new reports appear quite frequently in this fast-moving field. In this review, we focus on recent developments concerning novel polymerization methods for HBPs, and their applications in analytical chemistry are also briefly summarized.

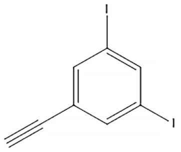
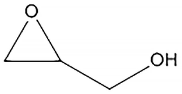
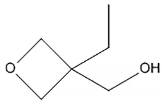
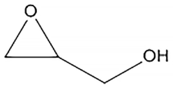
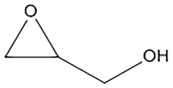
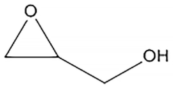
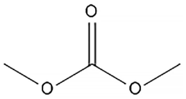
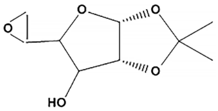
2. Synthesis of hyperbranched polymers

2.1 Slow monomer addition method

Since the first HBPs were synthesized from AB_2-type monomers, numerous HBPs have been prepared by various methods. The conventional approaches to HBPs, including the polycondensation of AB_n monomers, self-condensing vinyl polymerization, proton-transfer polymerization (PTP), and the so-called “A_2+B_3” methodology, each have certain merits, such as facile preparation or commercial availability of the monomers. However, these methods are still associated with some crucial problems, such as a low degree of branching (DB), very broad polydispersity (PDI), and uncontrollable gelation, which greatly limit the advanced application of HBPs.[26,27]

Hence, researchers have been searching for improved methods and a new strategy is eagerly anticipated. Polymerization under slow monomer addition (SMA) conditions offers a versatile and favorable method for the preparation of HBPs with controlled molecular weight, high DB, and narrow PDI. Thus, the SMA method has received significant attention. This synthetic method may be divided into two major categories

Table 1. The monomers used in SMA and the polymerization results

Monomer	Condition	Types of polymers	M*w*	PDI	Ref
	$Pd_2(dba)_3$,CuI, PPh_3, piperidine, rt	phenylacetylene	8000-90,000	1.3-8.5	[28]
	1,1,1-Tris(hydroxymethyl)propane, 95°C over 12h, under argon atmosphere	polyglycerols	1250-6500	1.13-1.47	[29]
	$BTSSbF_6$, 100°C in bulk	polyethers	594-5336	1.25-1.58	[30]
	trimethylolpropane (TMP), 95°C for 17h in dioxane	polyglycerols	4000-1475,000	1.1-1.4	[33]
	80°C for 12h in THF	polyglycerol	300-24,000	1.3-1.8	[34]
	TMP, 170°C over 12h	aliphatic polyether	830-1020	1.2-1.3	[35]
					
	$BF_3{\cdot}OEt_2$, 10°C for 24h in dry CH_2Cl_2	glycopolymer	7400-122,400	1.70-1.45	[36]

according to the monomer used: step-growth polycondensation by SMA and ring-opening multibranching polymerization by SMA. The former route was first reported by Bharathi and Moore, who used 3,5-diiodophenylacetylene as an AB_2-type monomer, 1-(3,5-diiodophenyl)-3,3-diethyltriazene as a B_2 core molecule, and Pd_2(dibenzylideneacetone)$_3$ as a catalyst; a hyperbranched polyphenylacetylene of controlled molecular weight was thereby synthesized.[28] Sunder et al. first synthesized hyperbranched polyglycerols by anionic ring-opening multibranching polymerization of glycidol under SMA conditions.[29] In contrast, Magnusson and co-workers synthesized hyperbranched polyethers by cationic ring-opening multibranching polymerization under SMA conditions.[30,31]

To date, numerous HBPs have been synthesized by the SMA method, such as hyperbranched poly(siloxysilane),[32] hyperbranched polyglycerol,[33-35] and hyperbranched glycopolymer.[36] Monomers used in SAM and polymerization results such as molecular weight (M*w*), and polydisperity index (PDI, M*w*/M*n*) are summarized in Table 1.

In addition, Zhou and co-workers have studied the kinetics of the SMA method in the synthesis of hyperbranched polymers.[37] Both theory and experiment showed the SMA technique to be effective for increasing the molecular weight, reducing the degree of intramolecular cyclization, and controlling the polydispersity index of the final hyperbranched polymers at less than 2. Thus, well-defined HBPs synthesized by the SMA method should be a prospective future field of great interest.

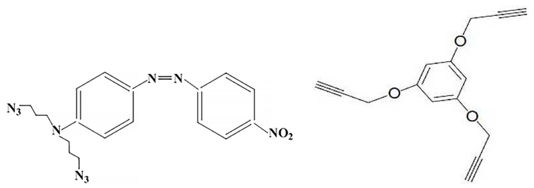
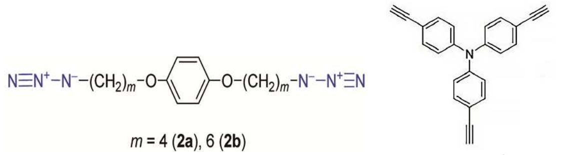
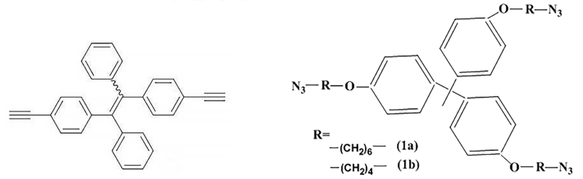
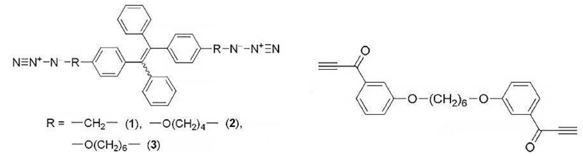
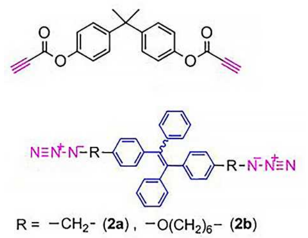
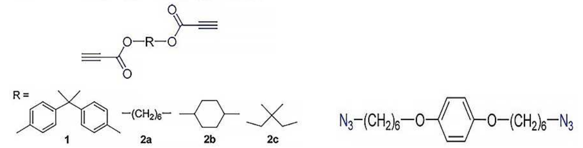
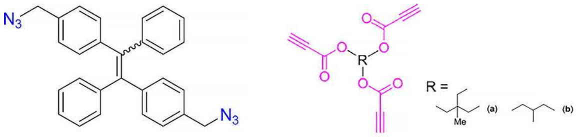
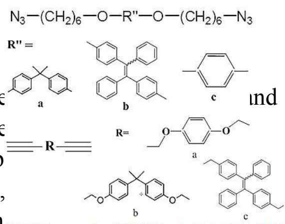
2.2 Click chemistry

The concept of click chemistry was first proposed by Sharpless and co-workers in 2001. Since then, this technique has been extensively used in polymer science due to its remarkable features, such as fast reaction rates, mild reaction conditions, regioselectivity, and simple product isolation procedures.[38-42] Click chemistry may be divided into two main types, that is, the azide–alkyne click reaction and the thiol–ene/yne click reaction.

2.2.1 Azide–alkyne click polymerization

Copper-catalyzed azide–alkyne cycloaddition (CuAAC) is the most widely employed type of click chemistry in the synthesis and post-polymerization functionalization of HBPs. Since Voit and co-workers first synthesized hyperbranched poly([1,2,3]triazole)s from AB_2 monomers by the CuAAC method, many polymer scientists have also carried out such experiments.[43] Table 2 lists the monomers used to synthesize

Table 2. The monomers used in synthesis polytriazoles by click polymerizations and the results.

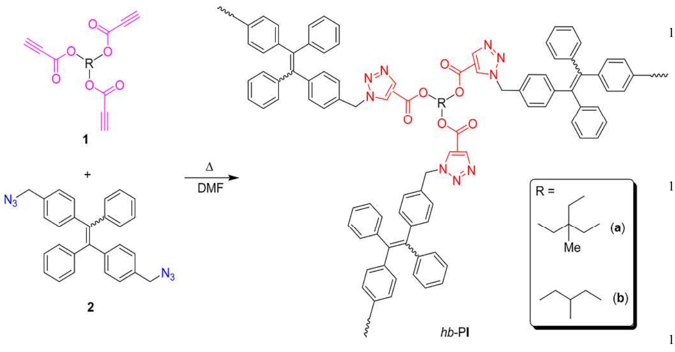
Monomer	Condition	M*w*	PDI	Ref
	SMA; CuSO$_4$, SA; under nitrogen for 24h in DMF	5600-6800	1.27-1.34	[48]
	Cp*Ru(PPh$_3$)$_2$Cl, THF, 60°C, 30-50min; [Cp*RuCl$_2$]$_n$, THF, 40°C, 2h	5400-9400 5000-7000	2.4-2.7 1.8-2.2	[49]
	Cu(PPh$_3$)$_3$Br, 60°C for 5-7h in DMF	9800-12,400	4.32-4.28	[50]
	metal-free, in DMF/toluene (1:1 volume ratio), 100 °C for 6 h	16,300-25,200	2.0-2.1	[54]
	metal-free, Monomers mol ratio 1:1; in DMF/toluene, 100 °C for 6 h	15,100-15,400	1.4-1.5	[55]
	metal-free, in DMF/toluene mixture (1:1, v/v) at 100 °C for 24 h; under nitrogen	10,500-18,700	1.27-1.53	[56]
	metal-free, in DMF, 60°C for 5h without protection from oxygen and moisture	4400-7200	2.21-2.65	[57]
	CuI@A-21, in THF, 60°C for 12h	17,900-69,600	1.86-3.25	[58]

hype nd the properties of the resulting mate and co-workers reported the prep branched poly([1,2,3]triazole-[1,3, polymerization from the AB$_2$ mono pynyloxy)-[1,3,5]triazine; the synthesized HBPs were characterized by IR, ^{1}H NMR, and size-exclusion liquid chromatography.[44] In 2008, Katritzky and co-workers tried to prepare HBPs from A$_2$+B$_3$-type monomers under CuSO$_4$/SA catalysis.[45] Unfortunately, however, the obtained HBPs showed poor solubility and were difficult to characterize.

Subsequently, researchers optimized the polymerization conditions of the CuAAC in terms of the catalytic system, solvents, and so on, and obtained a series of HBPs with higher solubilities.[46,47] For example, Xie and co-workers synthesized hyperbranched polytriazole by an A$_2$+B$_3$ route based on Cu-catalyzed click reaction and subsequently characterized the product. The obtained hyperbranched polytriazole showed high solubility in common organic solvents such as cyclopentanone, cyclohexanone, dimethyl formamide, and dimethyl sulfoxide, and also displayed nonlinear optical properties.[48] Qin and co-workers successfully prepared a series of functional hyperbranched polytriazoles with high molecular weight from A_2+B_3 monomer pairs by Cu-catalyzed click polymerization.[49,50] The obtained functional hyperbranched polytriazoles proved to be soluble in common organic solvents. In addition, Wu and co-workers reported the synthesis of a new series of hyperbranched polytriazoles containing perfluoroaromatic rings by click chemistry under copper catalysis.[51] By virtue of its great feasibility, the CuAAC reaction has proven effective for various material construction applications, involving small molecules, linear polymers, HBPs, and other nanoscale or macroscopic materials. The resulting products have been widely applied in different areas.[52] However, the copper catalyst could be used only once, and the removal of the copper residues from the final HBPs is very difficult. This limits the application of the products in optoelectronic devices and biomedical fields because even very small amounts of catalyst residues are toxic and may be detrimental to the photophysical properties or have fatal consequences for organisms. Thus, new metal catalyst systems that could greatly decrease the copper residues in the resulting HBPs, as well as metal-free click polymerization, need to be further explored.

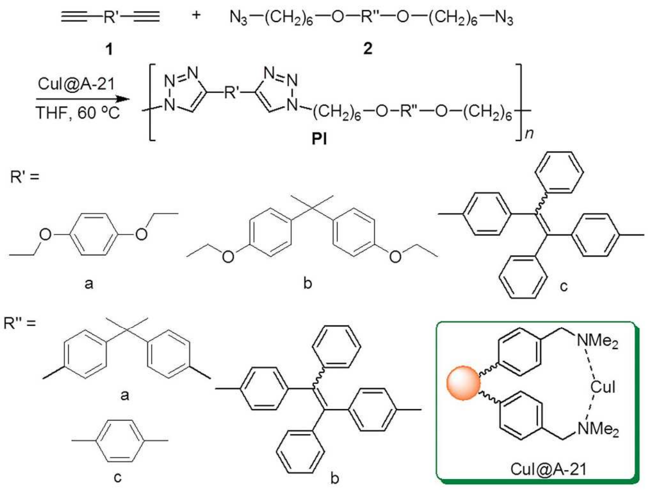
Indeed, metal-free click polymerization has attracted wide interest due to its excellent features, such as environmental friendliness and lower toxicity. For example, Tang's group has carried out extensive work, and developed an efficient metal-free click polymerization that was successfully applied for the preparation of functional poly(aroyltriazole)s.[53,54] Furthermore, they extended the range of alkyne monomers used for metal-free click polymerization from aroylacetylenes to propiolates.[55] The results showed that the metal-free click polymerization method proceeded readily under ambient atmosphere without protection from oxygen and moisture. Subsequently, these authors also efficiently synthesized a kind of polyester with high molecular weight and regioregularity from diazide monomers by the metal-free click polymerization.[56]

Scheme 1. Synthesis of hyperbranched polytriazoles via metal-free click polymerization (reproduced with permission of the American Chemical Society from ref. 57).



Metal-free click polymerization has become a versatile and powerful tool for the preparation of functional polytriazoles. However, hyperbranched polytriazoles have rarely been synthesized in the above-mentioned reports. On the basis of previous work, hyperbranched polytriazoles with high molecular weights and high regioregularities were synthesized from tripropiolates and tetraphenylethene-containing diazides by metal-free click polymerization under the optimized reaction conditions without protection from oxygen and moisture.[57] This synthesis is shown in Scheme 1. The obtained HBPs were soluble in common organic solvents and showed high thermal stability, and also showed a unique propensity for aggregation-induced emission, making them potentially useful as fluorescent chemosensors for the superamplified detection of explosives. Furthermore, the resultant polymers can be readily photo-cross-linked, yielding two-dimensional fluorescent patterns with high resolution.

Fig.1 Synthetic routes to polytriazoles by click polymerizations using recyclable and reusable supported Cu(I) catalyst (reproduced with permission of the Nature Publishing Group from ref. 58).



Besides using the approach of metal-free click polymerization, more recently, Tang's group developed a new alternative strategy to solve these difficulties.[58] In their study, hyperbranched polytriazoles were synthesized through click polymerizations using a recyclable and reusable supported Cu(I) catalyst (SCC) designated as CuI@A-21, in which the catalyst was immobilized on polystyrene spheres. This strategy is shown in Figure 1. Moreover, the advantage of CuI@A-21 over other copper catalysts was also studied. The results indicated that CuI@A-21 efficiently catalyzed azide–alkyne click polymerizations and could be reused over at least four cycles, and the final polytriazoles had copper residues of less than 116 ppm, which was 24 times lower than for other Cu(I) catalysts. More importantly, this method should broaden the application of azide–alkyne click polymerizations in optoelectronic and biomedical fields due to the lower copper residues, and also provide guidelines for other polymerizations with metal catalysts in polymer science.

In summary, with further research on new metal catalyst systems and metal-free click polymerization, the azide–alkyne click polymerization is expected to be at the forefront in the preparation and application of HBPs.

2.2.2 Thiol–ene/yne reaction

During the course of the development of azide–alkyne click polymerizations, the thiol–ene/yne reaction was also successfully developed and may be included in the catalogue of click polymerizations.[59-61] The thiol–ene click reaction has been used industrially since the 1970s, but has recently been used to prepare many types of dendrimers and hyperbranched polymers because of the rapid, robust, and efficient nature of the reaction.[62-64]

For instance, Ramakrishnan and co-workers reported a novel route for the synthesis of peripherally "clickable" hyperbranched polyethers by click reaction.[65] The method was based on self-condensation of a specifically designed AB_2-type monomer, bearing two propargylbenzyl ether groups and one hydroxyl group, and yielded a hyperbranched polyether bearing numerous peripheral allyl groups. It is noteworthy that these polymers can be readily transformed into interesting functional systems by clicking with a variety of thiols, and thus can be used as nano-dimensional clickable hyperscaffolds in various areas.

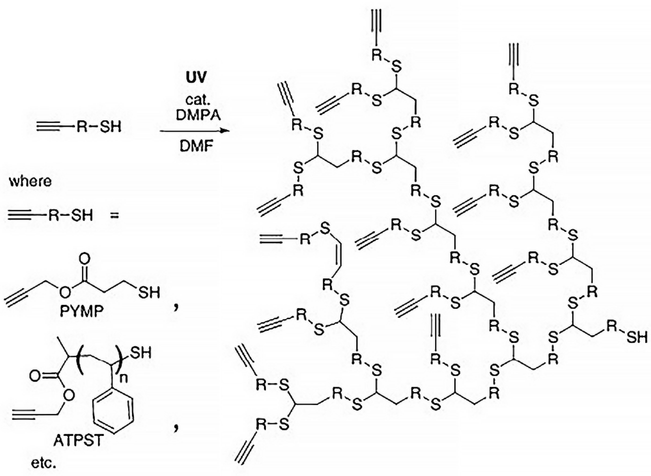
In 2013, Xue and co-workers successfully prepared a novel silicon-containing hyperbranched polymer by step-growth thiol–ene click polymerization under conditions of UV irradiation, using mercaptopropylmethyldiallylsilane as an AB_2-type monomer and mercaptopropyltriallylsilane as AB_3-type monomer, respectively.[66] The obtained Si-HBPs were characterized by NMR spectrometry, Fourier-transform infrared spectroscopy, gel-permeation chromatography, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The results showed that the obtained Si-HBPs could be employed as promising candidate materials for heavy metal adsorption due to the thioether bond.

Foix and co-workers synthesized a new multifunctional HBP with thioether and ester moieties in the structure and terminal hydroxyl groups by an iterative synthetic route, which consisted of esterification and thiol–ene click reaction.[67] The obtained HBP was used as a macroinitiator in the curing process of a commercially available cycloaliphatic epoxy resin, leading to a dual latent curing system promoted by UV irradiation. The study provided a latent curing agent, which showed no activity under normal conditions but became active in response to external stimuli, thus simplifying the curing process. However, the main drawback of this route was the tedious synthetic process for obtaining the thioether–ester HBP structure, which required a five-step iterative methodology.

To circumvent this drawback, the same research group sought to improve the method. Recently, they devised a new two-stage dual photoinitiated-thermal curing system for cycloaliphatic epoxy resins by the addition of an allyl-terminated hyperbranched polyester and a trithiol compound to the formulation. The allyl-terminated HBP obtained was used as a reactive modifier to achieve high glass transition temperature (T_g) thermosets and to enhance the mechanical properties.[68] Moreover, Imbesi and co-workers reported a heterogeneous, amphiphilic fluoropolymer bearing pendant alkene functionalities that were crosslinked to produce a polymeric network by photo-initiated thiol–ene click reaction to generate a benchmark, anti-biofouling surface.[69] More importantly, this study provided a more robust thiol–ene crosslinking chemistry to address the mechanical and processing drawbacks of previous systems, such as high coating deposition temperatures, long reaction times, and so on. To date, numerous multi-functional HBPs have been prepared by thiol–ene click reaction in the presence of different initiators, including hyperbranched poly(ester-amine), hyperbranched glycopolymers, and so forth.[70,71]

Similar to the thiol–ene click reaction, the thiol–yne reaction is also an important type of click reaction. In fact, thiol–yne click polymerization has been widely applied to synthesize functional polymers with novel structures, which have found application in various areas, such as photovoltaic materials, high refractive index optical materials, drug-delivery vehicles, and biomaterials.[72–75]

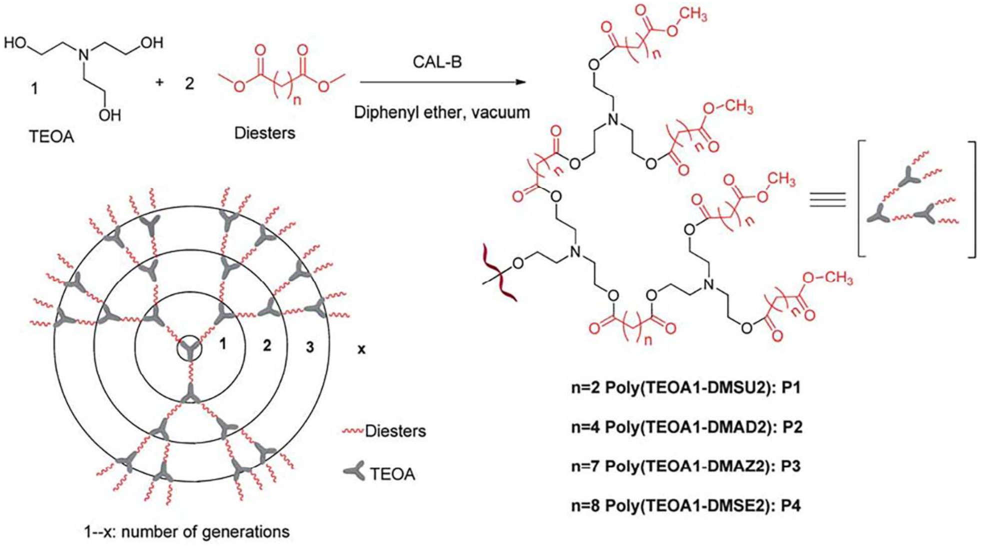
Scheme 2. Synthetic routes to hyperbranched polymers from small molecules by the thiol-yne click polymerizations (reproduced with permission of the American Chemical Society from ref. 76)



For example, Perrier and co-workers reported a versatile approach to the synthesis of functional hyperbranched polymers by photoinitiated thiol–yne click polymerization, employing 2,2-dimethoxy-2-phenylacetophenone as photoinitiator.[76] This strategy for synthesized process is shown in Scheme 2. In this study, two sulfur-containing HBPs were synthesized from small molecules and macromolecules, namely prop-2-ynyl-3-mercaptopropanoate (PYMP) and alkyne-terminated polystyrene thiol (ATPST), respectively. Compared to conventional polymerization methods, the degree of branching of the obtained HBPs was much higher by this method. The results indicated that various functional groups could be incorporated into the HBPs from various small molecular and macromonomers by the thiol–yne reaction. Alternating-block hyperbranched polymers were subsequently synthesized using the highly versatile thiol–yne reaction by the same authors.[77] Two linear block macromonomers were first designed and prepared from dimethyl acrylamide/*t*-butyl acrylate and styrene as monomers, respectively, by reversible addition–fragmentation chain-transfer polymerization. The prepared macromonomers were easily polymerized to form alternating-block hyperbranched polymers. The same authors also studied the self-assembly properties of the alternating-block hyperbranched polymers at various pH. The results showed that these products could potentially be used in drug delivery and other applications.

A disadvantage of this method was the very tedious procedure required for the synthesis of monomers containing both thiol and ethynyl groups. Recently, Gao and co-workers tried to solve this problem concerning the preparation of the monomers and hyperbranched polythioether-ynes by a new strategy, namely a combination of thiol–yne click reaction and thiol–halogen click-like reaction.[78] Two series of hyperbranched polythioether-ynes with high molecular weights and high degrees of branching were readily achieved. The main advantages of this strategy include its simplicity, rapidity, flexibility, and high efficiency for the preparation of multifunctional HBPs.

Scheme 3. Synthesis of hyperbranched poly(amine-ester)s with ester terminals (reproduced with permission of the Royal Society of Chemistry from ref. 91).



Compared to the developed azide–alkyne and thiol–ene click polymerizations, however, the application of the thiol–yne click polymerization to synthesize functional polymers is still limited.[79] Considering its advantages of readily available monomers and simple reaction conditions, it is anticipated that the thiol–yne click polymerization will be further developed for the preparation of functional polymers and find extensive applications in diverse areas.

In addition, thiol-Michael addition chemistry has also been studied as an example of click chemistry for the preparation of functional materials.[80,81] Although efficient and highly useful, thiol-based chemistry has their intrinsic drawbacks mainly derived from the manipulation of thiol functionalized compounds which are pungent, sensitive to oxidation and commercially limited to a relatively small range of simple molecules. Recently, in order to overcome these drawbacks, Rannard's group has carried out extensive research, and developed an efficient one-pot deprotection/functionalization thiol-Michael addition strategies in which xnthates have been utilized as protection groups of thiols that was successfully applied for the preparation of xanthate-functional linear-dendritic polymers hybrids and four generations of xanthate-functionalized dendron atom transfer radical polymerization macroinitiators.[82,83] This approach avoids the direct manipulation of these reactive and noxious thiol chemicals, and highly limits the potential of oxidative disulfide formation. Furthermore, the author also adopted this methodology to synthesize materials with site specific functionalization for potential drug delivery applications.

2.3 Enzyme-catalyzed polymerization

In recent years, the application of enzyme-catalyzed polymerization for the synthesis of HBPs has received increasing attention because of its unique advantages. Polymer synthesis by enzyme-catalyzed polymerization was first reported in the mid-1980s, but only became established in 1999, after a breakthrough was achieved with the use of immobilized lipase B (Novozyme 435) as a catalyst to give polymers.[84] Various lipases from different sources have been used in enzyme-catalyzed polymer syntheses. Among these, lipase B, a hydrolysis enzyme from *C. antarctica* (CALB) immobilized on Lewatit by physical adsorption, has been the most widely employed enzyme for polymer synthesis due to its excellent activity towards many monomers.

Application of enzymatic methods for the synthesis of hyperbranched polymers was first reported by Frey and co-workers.[85] In their study, ε-caprolactone was used as a cyclic AB monomer and 2,2-bis(hydroxymethyl)butyric acid served as an AB_2 co-monomer, and the reaction was catalyzed by immobilized lipase B under mild conditions. A series of hyperbranched copolyesters with different DB was successfully prepared by the enzymatic route based on a novel combination of ring-opening AB polymerization and AB_2 polycondensation. The results showed this enzymatic route to be general and highly efficient, giving access to various hyperbranched aliphatic polyesters.

Utilization of enzyme-catalyzed polymerization for the synthesis of HBPs has since attracted more and more attention. In

2010, López-Luna and co-workers reported the CALB-catalyzed synthesis of hyperbranched polylactones.[86] In their work, 2,2-bis(hydroxymethyl)butyric acid was also used as an AB_2 co-monomer and 1,1,1,2-tetrafluoroethane was used as a green, non-toxic, relatively polar, and hydrophobic solvent. The main drawback was that the obtained hyperbranched polylactones had a low degree of branching, which may have been due to the limited solubility of 2,2-bis(hydroxymethyl)butyric acid in 1,1,1,2-tetrafluoroethane.

Subsequently, the same authors made improvements to the reaction medium.[87] In their later study, hyperbranched poly-l-lactides were synthesized by a novel CALB-catalyzed process in an ionic liquid, using bis(hydroxymethyl)butyric acid as the AB_2 core co-monomer and 1-butyl-3-methylimidazolium hexafluorophosphate $[C_4MIM][PF_6]$ as the medium. The results were consistent with those of other reported theoretical studies, showing imidazolium-based ionic liquids to be suitable for sustaining lipase activities with a high solvating ability for the substrates and products with high molecular weights.[88-90] However, the above studies were restricted to co-polymerization of ε-caprolactone or δ-valerolactone with 2,2-bis(hydroxymethyl)butyric acid as an AB_2-type core to prepare HBPs.

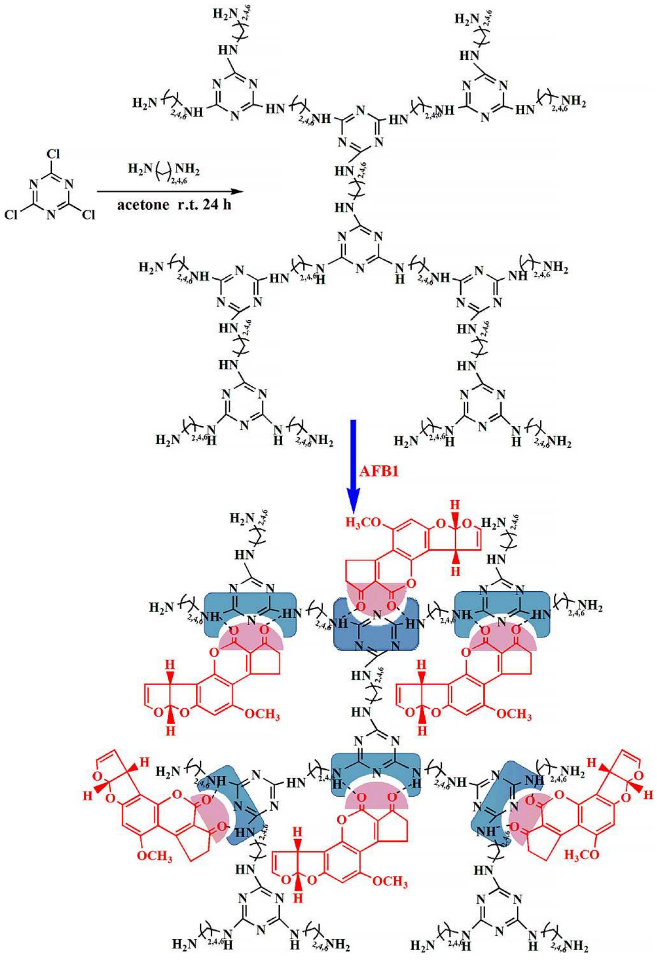
More recently, a series of novel multifunctional poly (amine-ester)-type HBPs has been successfully prepared by CALB-catalyzed polycondensation of triethanolamine (TEOA) and diesters by Xu and co-workers.[91] This synthesis is shown in Scheme 3. The reaction conditions were optimized, and the precise structure of the products, including the degree of branching, the percentages of the components, and outer surface groups, were confirmed by FTIR, NMR, GPC, and so on. Moreover, the biological properties of the multifunctional poly(amine-ester) HBPs were systematically investigated, including their biodegradability, biocompatibility, cytotoxicity, and amphiphilicity. The results indicated that the synthesis of multifunctional poly(amine-ester) HBPs can provide materials with potential applications in biomedical fields, such as drug delivery and therapeutics.

In addition, significant advances have been made by employing novel enzymes in polymer synthesis. For example, a new lipase from the yeast *Yarrowia lipolytica* was used in the enzyme-catalyzed ring-opening polymerization of lactones by Barrera-Rivera and co-workers.[92,93] The results showed that this enzyme could efficiently catalyze the ring-opening polymerization of ε-caprolactone in both organic solvents and various ionic liquids. The main advantages were that pre-culture steps were not needed, fast rate of the chemical reactions, and a reduction in the total reaction time.

Moreover, a novel cutinase from *Humicola insolens* showing activity for polyester synthesis was explored by Hunsen and co-workers.[94] In their study, the cutinase was immobilized on Lewatit and used as a catalyst for ring-opening polymerizations with ε-caprolactone and ω-pentadecalactone. The kinetics and mechanism of the reaction with the immobilized cutinase were subsequently explored by the same authors,[95] and the results indicated that the *Humicola insolens* cutinase was an excellent new biocatalyst with great promise for the synthesis of further HBPs.

Thermophilic enzymes have also been examined as potential catalysts in polymer synthesis due to their outstanding stability towards high temperatures, organic solvents, and chemical denaturants. For example, Yang and co-workers investigated the catalytic activities of thermophilic esterase from *Archaeoglobus fulgidus* and thermophilic lipase from *Fervidobacterium nodosum* in the ring-opening polymerization of ε-caprolactone.[96,97] The results showed that these thermophilic enzymes had stronger affinity for ε-caprolactone than the widely used CALB, which was confirmed by kinetic analysis and molecular docking studies.

Scheme 4. Synthesis protocol of hyperbranched polymers and its interaction with aflatoxins B1 (AFB1) (reproduced with permission of Elsevier Science Ltd from ref. 99).



To sum up, enzyme-catalyzed polymerization has undergone rapid development during the last decade by virtue of its high catalytic activity, good enantioselectivity, mild reaction conditions, and the biocompatibility and biodegradability of the catalytic products. It shows great potential for more environmentally friendly synthesis of polymers, making it particularly suitable for the synthesis of biomedical materials, drug intermediates, and so on.[98] Although this research has started only recently, with improvement of the reaction conditions and exploration of novel enzyme catalysts and reaction media, enzyme-catalyzed polymerization looks set to play an important role in future polymer science.

3. Application of HBPs in analytical chemistry

HBPs have been extensively applied in analytical chemistry areas such as immunosensors, extraction, heavy metal detection, early diagnosis, biotoxin detection, and so on. These applications rely on their large numbers of terminal functional groups, modification of which may provide a means of controlling the solubility, reactivity, and electrochemical and luminescence properties of HBPs.

3.1 Applications of HBPs in sample pretreatment

Sample pretreatment is of major importance in various analytical fields. The main objective is to achieve lower limits of detection by removing matrix constituents that may affect the detection or enrichment of analytes. Many methods, such as solid-phase microextraction, miniature clean-up columns with various extraction sorbents, and antibody-based clean-up columns, have hitherto been employed. However, because of the complexity of the matrices, a simpler, more rapid, and reliable sample preparation procedure still needs to be devised.

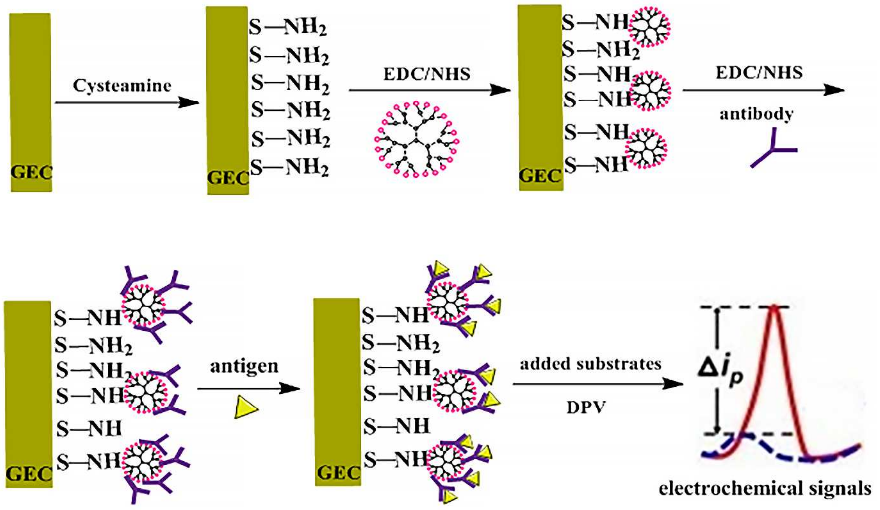
Liu and co-workers established a novel chemical clean-up procedure coupled to high-performance liquid chromatography for the determination of aflatoxins in cereal powder samples.[99] In their work, a new well-defined HBP containing hydrogen-bonding donor groups and π electrons was prepared and used as a solid-phase extraction sorbent to extract and enrich aflatoxins based on non-covalent interactions (Scheme 4). The prepared HBP showed higher adsorption efficiencies compared to a traditional C18 sorbent, indicating potential application as a sorbent in the rapid enrichment of trace analytes in real matrices. In addition, the same research group also synthesized a novel HBP containing stereocontortedcores for use as an on-line solid-phase microextraction adsorbent for highly efficient adsorption of polycyclic aromatic hydrocarbons (PAHs).[100] Compared to conventional adsorbent materials, the prepared porous polymer monolith exhibited higher enrichment efficiency and selectivity for PAHs.

3.2 Applications of HBPs in immunosensors

Ge and co-workers synthesized a HBP by polycondensation between *p*-phenylenediamine and trimesic acid, and then conjugated it with lipase using carbodiimide as a coupling reagent.[101] The results showed that by using the HBP as an immobilization matrix the natural activity of the lipase was maintained, and that the conjugated lipase exhibited significantly enhanced stability at high temperature or in the presence of organic solvents compared to its native counterpart.

Following on from this experiment, there have been many researchers starting to investigate the applications of HBPs as carriers to immobilize bioactive compounds such as enzymes, antigens, antibodies, *etc*., on different activated substrates as sensitive or amplifying elements for the determination of various targets. Wherein, immunosensors based on HBPs as amplifying elements has received considerable attention in the fields of polymer science and chemical analysis. The assay principle of this immunosensor is shown in Scheme 5.

Scheme 5. Schematics illustration of immunosensor design based on HBPs as signal amplifying element.



For example, Shen and co-workers reported the use of a novel aromatic–aliphatic hyperbranched polyamide as a support for antibody immobilization in piezoelectric immunosensors for the detection of α-fetoprotein (AFP), a widely used tumor marker for the diagnosis of patients with germ cell tumors and hepatocellular carcinoma.[102] In this work, they first synthesized the HBP through direct polycondensation of the monomer 5-[3-(4-aminophenyl)propionylamino]isophthalic acid, then activated it with EDC/NHS, and subsequently covalently linked it to electrodes that had been modified with cystamine. The results showed that the aromatic–aliphatic hyperbranched polyamide could increase the amount of antibody immobilized and thus increased the sensitivity of the whole piezoelectric immunosensor.

The main disadvantage was the covalent attachment between the antibody and the HBP, which may have lowered the antibody activity.

Subsequently, the authors improved the immobilization of the antibody by the combined use of HBPs and protein A.[103] In this strategy, protein A was used as a binding material to immobilize antibodies onto an HBP that had been synthesized from *p*-phenylenediamine and trimesic acid and had already been covalently linked to the sensor. More importantly, the method provided a new strategy for highly sensitive clinical detection of target analytes and should also be applicable to the construction of other kinds of immunosensors.

Moreover, an ultra-sensitive, magnetic nanospheres-based electrochemical immunoassay amplified method using HBP conjugates to detect hepatitis B antigen (HBsAg) was developed by Ding and co-workers.[104] In their work, hepatitis B surface antibodies were covalently immobilized on the surface of magnetic nanospheres, while hepatitis B secondary antibody labeled with horseradish peroxidase (HRP) was covalently linked to the HBP. A sandwich-type immuno-complex was formed when HBsAg was present. The concentration of HBsAg was then determined by means of differential pulse voltammetry. The detection limit of this method was found to be 0.008 μg/L, which is lower than that of conventional ELISA. The results indicated that by using the HBP as an immobilization substrate, the activity of the immobilized bioactive compound was maintained, demonstrating good prospects for practical clinical analysis.

Other researchers have carried out a great deal of excellent work on the applications of HBPs in different types of sensors and have obtained many promising results.[105–110] These detection methods provide powerful candidates for sensor technologies, showing high sensitivity and rapid detection of various targets, and hence may find widespread applications.

Recently, in our lab, a successful attempt has been made in the synthesis of HBPs via polycondensation of AB_2 type monomer. The resulting HBPs contain functional end groups in which one sulfhydryl group and large number amino groups. Based on HBPs as signal amplifying element, we have successfully developed a simple, rapid and ultrasensitive immunochromatographic strip assay for the determination of bisphenol A (BPA) which is an estrogen mimic. The details were: 1) synthesis of HBPs; 2) preparation the hyperbranched immune complexes: the conjugation of antibody and gold nanoparticles with HBPs; 3) immunochromatographic strip assay. The signal enhancement method enabled us to achieve BPA detection with the visual sensitivity of 1 ng/mL by naked eyes, which is at least 10-fold improvement of the sensitivity of traditional lateral flow strip methods. The experiment demonstrated that the signal amplified method based on HBPs could be adopted for all lateral flow strip detection methods to analyze various environmental pollutants.

4. Conclusions and outlook

This short review has summarized some of the notable advances in the synthesis methods for HBPs, as well as the application of HBPs in analytical chemistry. These different synthesis methods each have certain advantages and disadvantages.

The synthesis of HBPs with both high molecular weight and a high degree of branching by the slow monomer addition method is a versatile and favorable method. It will continue to be widely used for the synthesis of HBPs, and it will certainly be combined with other techniques.

Click chemistry is an important and ideal method for the synthesis of HBPs. Although research on click polymerization has led to great progress being made, its further development is still full of challenges that await exploration. Opportunities mainly exist in areas such as the design of new monomers, the development of catalytic systems, exploration of applications, and so on. It is anticipated that through the collective efforts of researchers, click polymerization will be further developed to become a versatile, efficient, reliable, and powerful tool for the synthesis of various novel functional hyperbranched polymers.

Enzyme-catalyzed polymerization shows significant promise for the synthesis of HBPs. However, its research is still in the exploratory stage. Further research is required into problems such as the control of reaction conditions, exploration of novel reaction media and novel enzyme catalysts, and so forth. Besides, the application of HBPs demonstrated in this review only opens a narrow window.

In summary, rapid progress has been made in the application of different HBPs. However, the application of HBPs in analytical chemistry fields is still in its infancy. With deeper and broader research, HBPs are anticipated not only to make a great contribution to the development of polymer science, but also to be functionalized for practical applications.

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Notes and references

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