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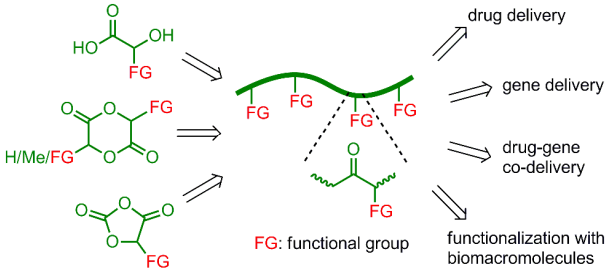
This review highlights the recent progress in the synthesis and biomedical applications of poly(α-hydroxyl acid)s with pendent functional groups.



Synthesis and Biomedical Applications of Functional Poly(α-hydroxyl acid)s

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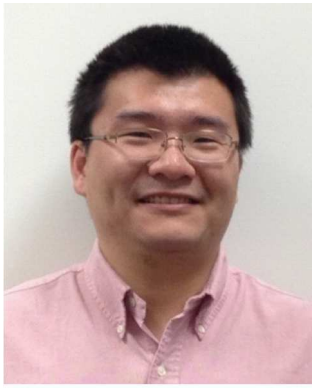
Abstract: Poly(α-hydroxyl acid)s (PAHAs) are an important class of biocompatible and biodegradable polymers. However, biomedical applications of conventional PAHAs have been restricted by the lack of functional groups. Therefore, recently there are significant research activities to design and synthesize functional PAHAs, and subsequently investigate their applications in biomedical areas. In this review, we present an overview on synthesis and biomedical applications of functional PAHAs. The synthetic approaches to incorporate functionalities with PAHAs as pendent groups are addressed. Applications of these functional PAHAs in drug delivery, gene delivery, co-delivery of drug and gene, and functionalization with biomolecules are highlighted.

Yun Yu obtained her B.S. and M.S. degrees in Polymer Science and Engineering in 2006 and 2009 from Beijing University of Chemical Technology. She earned her Ph.D. degree in Chemical Engineering in 2013 from University at Buffalo, the State University of New York with Professor Chong Cheng. Her dissertation research focused on the design and development of biodegradable polymeric biomaterials, conjugation/encapsulation strategies, and bio-conjugates for drug delivery. Currently as a formulation scientist at GrayBug, LLC, she is developing proprietary controlled release technology.



Jiong Zou obtained his B.S. degree in Chemistry in 2003, and Ph.D. degree in Organic Synthesis and Polymer Science with Professors Fenggang Tao and Ming Jiang in 2008 from Fudan University. He conducted his postdoctoral research with Professor Chong Cheng at University at Buffalo, the State University of New York during 2009-2011, and with Professor Karen L. Wooley at Texas A&M University during 2011-2014. He currently is a scientist at Dexcom.

Chong Cheng received a B.S. degree in Polymer Materials in 1993 from Hefei University of Technology, and a M.S. degree in Polymer Materials in 1996 from Beijing University of Chemical Technology with Professor Hongmin Zhang. He obtained a Ph.D. in Chemistry in 2003 with Professor Nan-Loh Yang from City University of New York, in which he also learned significantly from Professor George Odian. He conducted his postdoctoral research with Professor Karen L. Wooley at Washington University in Saint Louis during 2003-2007. He currently is an Associate Professor of Chemical Engineering at University at Buffalo, the State University of New York.

1. Introduction

Driven by the desire to attain materials with optimal properties for biomedical applications, the development of biodegradable polymers has attracted broad attention in the past few decades.[1, 2] Although biopolymers typically can undergo enzymatic degradation and have been used as biomaterials since antiquity,[3] their applicability has been limited due to their unfavorable immunogenic response, biosafety concern, and low versatility in properties. Moreover, depending on the availability and concentration of the enzymes, their degradation behavior can have site-to-site and patient-to-patient variations, which may also restrict them from general applications.[1] Accordingly, synthetic biodegradable polymers have been broadly studied for biomedical applications. With hydrolytically labile aliphatic ester linkages in backbone, aliphatic polyesters are important biodegradable polymers.[4, 5] Currently chemical synthetic approaches are of premier significance for the preparation of aliphatic polyesters.[6, 7] On the other hand, bacterial and plant production of aliphatic polyesters becomes increasingly important,[8] and the corresponding industrial applications have emerged. For instance, Metabolix has developed the technology for large-scale production of a broad range of polyhydroxyalkanoates through fermentation using renewable feedstocks. As the most extensively studied class of aliphatic polyesters, poly(α-hydroxyl acid)s (PAHAs; Table 1), such as polylactide (PLA), polyglycolide (PGA) and poly(lactide-*co*-glycolide) (PLGA), have been utilized for controlled drug delivery, gene therapy, tissue replacement, implantation devices and sutures, due to their desired degradability and biocompatibility, as well as excellent mechanical, physical and thermal properties.[1] The degradation of PAHAs generally takes place through hydrolysis in a moist environment and eventually forms monomers and oligomers that are soluble in aqueous media. PAHAs are generally considered both biodegradable and bioresorbable in the living body.[9]

Table 1. A list of abbreviations used in this review

AHA	α-hydroxyl acid
Bn	benzyl
BPDC	brush polymer-drug conjugate
CBz	carboxybenzyl
CCL	core-cross-linked
Cpt	camptothecin
DCC	dicyclohexylcarbodiimide
D_h	hydrodynamic diameter
DIEA	*N*,*N*-diisopropylethylamine
DL	dilactone
DMAP	4-dimethylaminopyridine
DMPA	2,2-dimethoxy-2-phenylacetophenone
Dox	doxorubicin
Dtxl	docetaxel
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
FGT	functional group transformation
HD	hexadecane
HOBt	1-hydroxybenzotriazole
LA	Lactide
lac-OCA	5-methyl-1,3-dioxolane-2,4-dione
MP	microparticle
MW	molecular weight
NP	nanoparticle
NC	nanocapsule
OCA	*O*-carboxyanhydride
OEG	oligo(ethylene glycol)
PAHA	poly(α-hydroxyl acid)
PDC	polymer-drug conjugate
pDNA	plasmid DNA
PEG	poly(ethylene glycol)
PEI	polyethylenimine
PGA	polyglycolide
PLA	polylactide
PLGA	poly(lactide-*co*-glycolide)
PLL	poly(L-lysine)
PLLA	poly(L-lactide)
PLR	poly(L-arginine)
PSMA	prostate-specific membrane antigen
Ptxl	paclitaxel
ROP	ring-opening polymerization
SDS	sodium dodecyl sulfate
siRNA	small interfering RNA
TBAI	tetrabutylammonium iodide
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene
TsOH	*p*-toluenesulfonic acid
TU	thiourea

PAHAs can be prepared by either condensation polymerization or ring-opening polymerization (ROP).[10] However, condensation polymerization typically cannot yield well-defined polymers with narrow molecular weight (MW) distribution.[11] Moreover, it is not easy to obtain high MW PAHAs by direct condensation polymerization (i.e. polyesterification) of α-hydroxyl acids (AHAs) such as lactic acid and glycolic acid,[12] because water as the byproduct of the condensation process can affect polymerization conversion and result in low MW polymers.[13] Thus, significant efforts have been made to remove water from condensation systems by various approaches, such as the addition of molecular sieve to reaction mixture and the utilization of high reaction temperature coupled with reduced pressure.[14, 15] Conventionally, PAHAs can also be prepared by ROP of cyclic dilactones (DLs) such as lactide and glycolide.[16] ROP of 1,3-dioxolane-2,4-diones, i.e. *O*-carboxyanhydrides (OCAs), recently has also been established as an effective approach for the preparation of PAHAs by Bourissou and co-workers.[17] Because ROP can possess characteristics of living polymerization under optimal conditions, it has been widely used to produce well-defined PAHAs with controlled MW and narrow MW distribution.[16, 18] Recently, organocatalyzed living ROP has attracted considerable interest, because it yields well-defined PAHAs with the absence of residual metal contaminants which may potentially compromise the polymer performance in biomedical applications.[18]

According to the structures of commonly used linear PAHAs, merely two chain-ends may be utilized to introduce functionalities. The applications of these conventional PAHAs in biomedical field typically rely on physical interaction between these PAHAs and biomedical ingredients. As reviewed elsewhere,[19] chain-end functionalities of PAHAs can allow regioselective terminal chemical modification and functionalization to tailor the polymers for enhanced biomedical applications. On the other hand, more functionalities of PAHAs are often required to meet the increasing desire to dramatically tune the structures and properties of the resulting biomaterials. Pendant functionalities incorporated as side groups of PAHAs would allow for greater extent of manipulation over the macromolecular structures, as well as material properties such as hydrophilicity, degradability and reactivity.[20] Over two decades ago, Kimura et al. and

Ouchi et al. reported respectively their studies on functional PAHAs with pendent carboxyl groups;[21-23] however, their seminal work received little attention at that time. Recently, along with the developments of polymer chemistry and biomedical science, a broad variety of functional PAHAs have been reported and their promising applications in biomedical areas have been demonstrated. Therefore, this review will describe the recent progress on the preparation and biomedical application of PAHAs with pendent functional groups. It should be noted that a variety of PAHAs with pendent alkyl or aromatic groups have been reported by Baker group and Möller group.[24-27] These PAHAs possess modified thermal and mechanical properties, but their significant biomedical applications have not been demonstrated except that hexyl-substituted PLA may potentially serve as an alternative to conventional PLA/PLGA for injectable drug delivery systems.[28] Because the synthesis and properties of these PAHAs with hydrophobic non-reactive pendent groups have been reviewed elsewhere,[29, 30] this review will focus on the synthesis and biomedical applications of PAHAs with pendent polar and/or reactive functionalities.

2. Synthesis of Functional PAHAs

2.1. General synthetic strategies

PAHAs with pendent functionalities can be prepared by two general strategies, including polymerization of functional monomers and post-polymerization functionalization. To employ the first strategy, functional monomers, including functional AHAs, DLs and OCAs, need to be synthesized and then polymerized by condensation polymerization or ROP. For functionalities that may interfere with polymerization process (such as carboxyl, hydroxyl and amine groups), protected functional monomers should be used in polymerization and deprotection is required after polymerization.

When post-polymerization functionalization strategy is adopted, either non-functionalized PAHAs (with only ester groups on polymer main chain) or PAHAs with pendent reactive groups can serve as

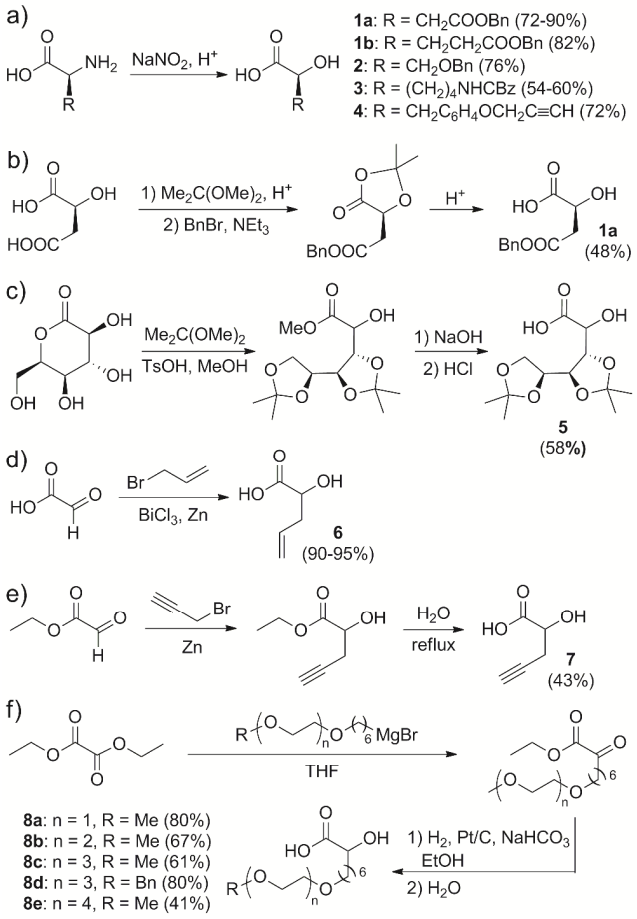
precursor polymers. However, non-functionalized PAHAs generally are less favorable precursor polymers regarding the structural control of the resulting functional polymers, because of their low reactivity in the functionalization reactions and the possible occurrence of degradation of polymer chains under the stringent reaction conditions.[31, 32] Thus, PAHAs with pendent reactive groups allowing for ready functional group transformation (FGT) reactions under mild reaction conditions can be the optimal precursor polymers. Because these reactive precursor polymers typically can only be prepared via polymerization of functional monomers, the synthetic strategy based on functional monomers is considered the primary option to access a broad variety of PAHAs with pendent functional groups. In this section, the synthesis of functional monomers will be reviewed at first, and then the synthetic approaches to functional PAHAs via functional monomers or by post-polymerization FGT reactions will be reviewed in details.

2.2. Synthesis of Functional Monomers

2.2.1. Synthesis of Functional AHAs

Functional AHAs are important chemicals for the preparation of functional PAHAs because they are not only the monomers for the synthetic routes via condensation polymerization but also the premier precursors of functional DLs and OCAs for the synthetic routes via ROP. They have been prepared by various synthetic approaches. Because of the broad availability of functional α-amino acids derived from natural sources (as L-isomers in general cases), diazotization of functional α-amino acids with sodium nitrite has become an important approach for the synthesis of functional AHAs (Scheme 1a).[21, 33] Following this approach, functional AHAs **1**-**4** were obtained in high yields (54-90%) relative to the functional α-amino acid precursors, with the retention of the stereochemistry.[33] Among them, AHAs **1**-**3** possess a benzyl (Bn) ester-protected carboxylic acid group, a Bn ether-protected hydroxyl group and a carboxybenzyl (CBz)-

protected amino group, respectively;[21, 33-38] while AHA **4** has a 4-(prop-2-yn-1-yoxyl)benzyl group which can be further converted to other functionalities or utilized for cross-linking.[39-41]



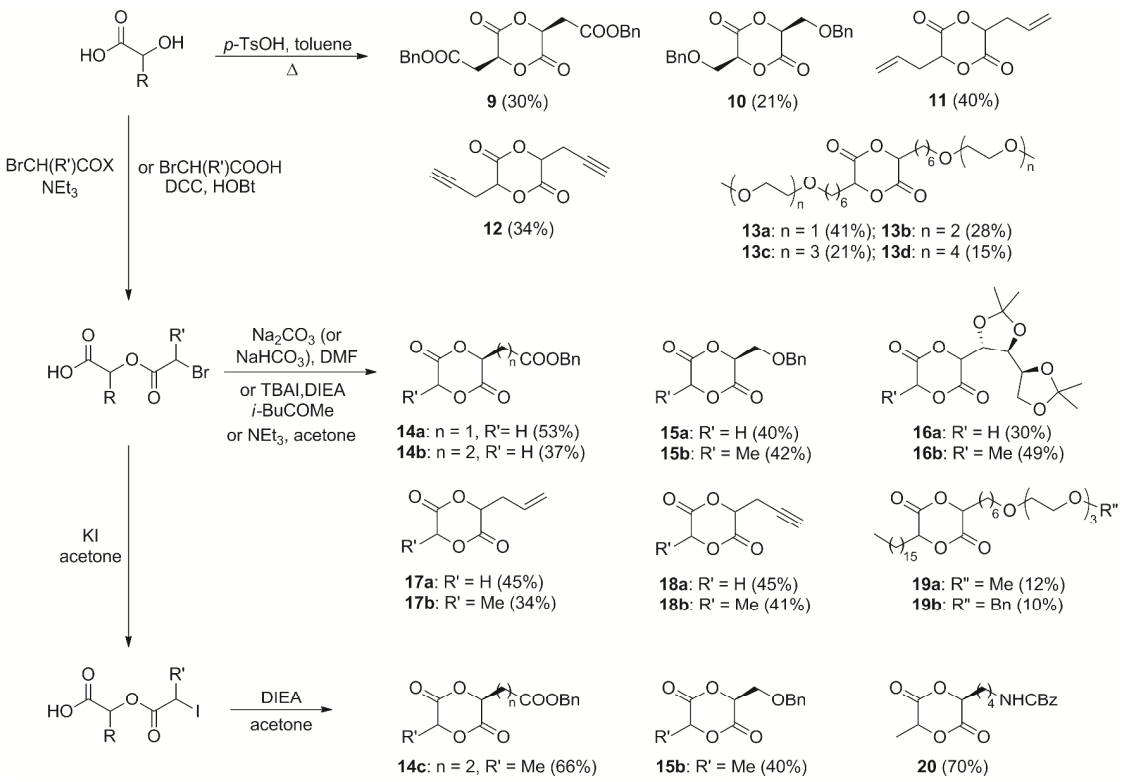
Scheme 1. Synthesis of functional AHAs

Moreover, functional AHAs may also be synthesized by FGT reactions using other precursors. Ouchi et al. prepared functional AHA **1a** by the reaction of L-malic acid with chloral hydrate to protect the AHA unit of the acid, subsequent benzylation of the remaining β-carboxylic acid group, followed by deprotection; but the overall yield of **1a** was very low (<10%).[23] By replacing chloral hydrate with 2,2-dimethoxypropane in the first protection step (Scheme 1b), Pounder and Dove modified the synthetic method and achieved an improved overall yield of **1a** (48%).[42, 43] Feng and co-workers obtained functional AHA **2** in racemic form in 25% overall yield by oxidation of 3-chloro-propanediol, followed by the reaction

of the resulting β-chlorolactide acid with sodium benzyl alcohol.[44, 45] Chittenden and co-workers prepared 3,4:5,6-di-*O*-isopropylidene gluconic acid, i.e. AHA **5**, in 58% overall yield by the reaction of *δ*-gluconolactone with 2,2-dimethoxypropane and subsequent deprotection of methyl ester (Scheme 1c).[46, 47] As reported by Leemhuis et al. and Rasano et al.,[48, 49] allylglycolic acid (**6**) was synthesized in 90-95% yield by the Barbier-type addition reaction of glyoxylic acid monohydrate with allyl bromide (Scheme 1d). Baker and co-workers further demonstrated the synthesis of 2-hydroxy-3-butynoic acid (**7**) in 43% overall yield by the Reformatsky-type reaction of ethyl glyoxylate with propargyl bromide, followed by hydrolysis of the resulting ester (Scheme 1e).[50] They also successfully obtained AHAs **8a-e** functionalized with exact length of oligo(ethylene glycol) (OEG) chains in 41-80% overall yields by the reactions of diethyl oxalate with the Grignard reagents generated from the corresponding OEG-functionalized hexyl bromides, subsequent catalytic hydrogenation of the formed keto esters, followed by hydrolysis of the resulting α-hydroxyl esters (Scheme 1f).[51, 52] It should be noted that AHA **8d** has a Bn ethyl-protected hydroxyl group which survived all of the synthetic steps, including the hydrogenation step catalyzed by Pt/C in the presence of $NaHCO_3$ in ethanol.[52]

2.2.2. Synthesis of Functional DLs

Using functional AHAs as precursors, functional DLs can be further prepared (Scheme 2). DLs with two identical functionalities, **9-13**, have been synthesized through the dimerization of functional AHAs catalyzed by *p*-toluenesulfonic acid (TsOH) in toluene under refluxing.[36, 42, 49-51] The yields of these DLs were relatively low (15-41%). Linear oligomers were the major byproducts of the dimerization reaction, and theoretically they may be thermally cracked to yield additional DLs. Relative to most other DLs, DL **11** was obtained in a little higher yield (40%),[49] and this might be ascribed to the workup process in which **11** was separated by distillation under reduced pressure in the presence of a transesterification catalyst (zinc oxide) to promote cracking of oligomers.[24]

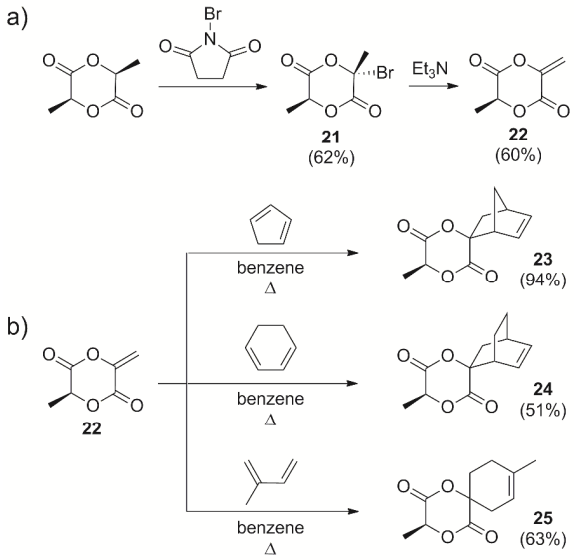


Scheme 2. Synthesis of functional DLs from AHAs

Functional DLs **14-20** have been prepared by condensation of functional AHAs with α-haloacyl halides or α-halocarboxylic acids to form functional esters, followed by ring-closure reaction of these esters.[33, 35, 42, 47, 48, 51-56] Most of these DLs are monofunctional, but DLs with two identical or different functionalities could be obtained when functional α-bromoacyl halides or α-bromocarboxylic acids are used in the reaction sequence.[24, 52] In the first condensation step of the DL synthesis, high yields (essentially quantitative yields in some cases) were typically achieved. AHAs can be directly used in the reaction, although pre-treatment of AHA **2** with dicyclohexylmime before the reaction has also been reported.[56] Generally α-haloacyl halides (such as bromoacetyl bromide and 2-bromopropionyl bromide or chloride) were used to react with AHAs, a base such as triethylamine (NEt_3) or *N*,*N*-diisopropylethylamine (DIEA) should be added to scavenge hydrogen halides generated and to shift the equilibrium to favor the condensation process; the use of any other catalyst, such as 4-dimethylaminopyridine (DMAP), is optional.[42] Weck and co-workers also employed (*S*) 2-bromopropionic acid, a type of α-bromocarboxylic

acid, to react with AHAs in presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt).[33] The DCC/HOBt method can retain stereochemistry of reaction precursors and lead to esters through clean conversions.

Traditionally, ring closure reactions of the functional esters via intermolecular nucleophilic substitution mechanism were performed in dry DMF in the presence of either $NaHCO_3$ or Na_2CO_3. Although the unimolecular cyclization process can be promoted by using low concentrations of the ester precursors in reaction systems, side reactions with the formation of oligomers and large cyclic compounds cannot be avoided. As a result, only up to moderate yields of DLs can typically be obtained by such direct ring closure reactions. Ring closure reactions of the functional esters in the presence of NEt_3 under refluxing in acetone were studied for the synthesis of amphiphilic DLs **19a-b**, but the overall yields were low (10-12%).[52] Weck and co-workers investigated ring closure of the functional esters by halide exchange using KI in acetone under refluxing to form the corresponding iodized esters, followed by ring closure in the presence of DIEA in acetone.[33] As demonstrated in the synthesis of DLs **14c** and **20**, ring closure of the functional esters obtained from the DCC/HOBt method led to relatively high overall yields (66-70%) via this modified cyclization approach. Recently, Bourissou and co-workers studied one-step ring closure of a functional ester with DIEA in methyl isobutyl ketone, in the presence of tetrabutylammonium iodide (TBAI) to in situ catalyze halide exchange, and reported 54% yield for the cyclization step to obtain DL **14b**.[56]

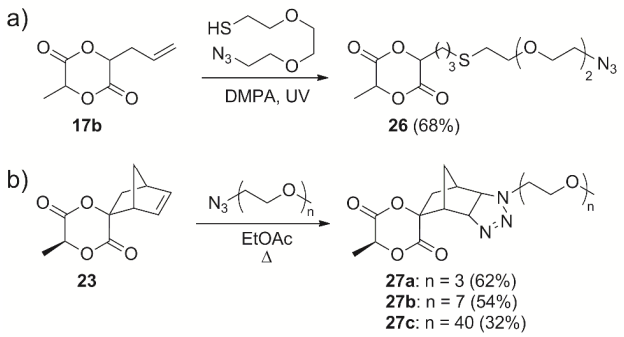


Scheme 3. Synthesis of functional DLs using L-LA as the starting material

Modification of non-functionalized DLs has also emerged as a valid synthetic approach for the preparation of functional DLs. As reported by Scheibelhoffer et al. in 1969,[57] monobrominated LA (**21**) can be prepared by bromination of L-LA with *N*-bromosuccinimide, and an exomethylenelactide (**22**) can be further obtained by dehydrobromination of **21** with NEt_3 (Scheme 3a). Although they only studied radical polymerization of **22**, in principle the C=C double bond of **22** can be utilized for a broad variety of reactions. As recently demonstrated by Hillmyer and co-workers,[58, 59] **22** can serve as a platform for Diels-Alder addition reactions with various dienes, including cyclopentadiene, 1,3-cyclohexadiene and isoprene, in benzene under refluxing (Scheme 3b). As a result, DLs **23**-**25** with unsaturated multicyclic substituents were obtained with moderate to high yields (51-94%).

According to the reactivity of functional groups carried by DLs, more types of functional DLs can be synthesized through FGT reactions. Based on recent advances in click chemistries,[60] in principle, DLs carrying (cyclo)alkenyl groups and alkynyl groups may be employed as the ideal precursors to access a broad variety of functional DLs via thiol-ene, alkyne-azide and thiol-yne click reactions. As reported by Weck and co-workers,[61] an azido-functionalized DL **26** was synthesized in 68% yield by UV-induced thiol-ene reaction of allyl-functionalized DL **17b** with 2-(2-(2-azidoethoxy)ethoxy)ethanethiol in THF (Scheme

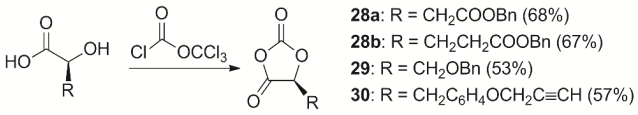
4a). They also investigated 1,3-dipolar cycloaddition reaction of the norbornene group of **23** with the azide group of monoazide end-functionalized OEG or poly(ethylene glycol) (PEG; Scheme 4b).[62] After refluxing the reaction mixtures in EtOAc for 3-4 days, OEG/PEG-functionalized DLs **27a-c** were formed in high reaction conversions (75-94%), although their yields after column separation (32-62%) were appreciably lower. The norbornenyl functionality of **23** has also been used for other reactions, such as ring-opening metathesis polymerization and hydrogenation.[58, 59]



Scheme 4. Synthesis of functional DLs from (cyclo)alkenyl-functionalized DLs via FGT reactions

2.2.3. Synthesis of Functional OCAs

Functional OCAs have emerged as an important class of monomers for the preparation of functional PAHAs. Functional OCAs **28-30** were converted from the corresponding functional AHAs by reactions with diphosgene (Scheme 5).[34, 37, 39, 43] Stereochemistry of the AHA precursors was retained in the resulting OCAs. Bourissou and co-workers conducted such a reaction by the treatment of AHA **1b** with dicyclohexylamine at first, followed by the addition of diphosgene into the suspension of the resulting salt with the presence of polystyrene-supported DIEA in diethyl ether.[34] They obtained OCA **28b** in 67% yield after 4 h of reaction. Dove group and Cheng group studied the direct reactions of functional AHAs **1a**, **2** and **4** with diphosgene in THF, respectively; with longer reaction times (16-18 h), the target OCAs were obtained in comparable yields (**28a**: 68%; **29**: 53%; **30**: 57%).[37, 39, 43]



Scheme 5. Synthesis of functional OCAs

2.3. Polymerization or FGT Approaches to Synthesize Functional PAHAs

A selective summary of PAHAs (**31-56**) with pendent functionalities that have been reported is shown in Figure 1. A broad variety of pendent functionalities can be incorporated with PAHAs, including carboxyl (**31-34**),[21-23, 33, 34, 42, 43, 56, 63] hydroxyl (**35-38**),[33, 35-37, 64] amine (**39-42**),[33, 38, 39, 65] OEG/PEG (**43** and **44**),[51, 52, 62] (cyclo)alkenyl (**45-48**),[48, 49, 53, 58, 59] alkynyl (**49-52**),[39, 50, 54, 55, 66] azido (**53**),[61] epoxy (**54**),[48] dihydropyrazine (DHP; **55**),[67] and aldehyde (**56**).[68] Most of these PAHAs are directly synthesized by polymerization of functional monomers (AHAs, DLs, or OCAs) with or without post-polymerization deprotection (Scheme 6). Functional PAHAs with high density of functional groups can be obtained through homopolymerization. When high density of functional groups is not needed, copolymerization of functional monomers with non-functionalized monomers is often employed. To introduce carboxyl, hydroxyl or amine groups, Bn ester, Bn ether or CBz-protected monomers are generally used, and deprotection is required for the resulting polymers. With few exceptions,[33] $Pd(OH)_2/C$ or Pd/C-catalyzed hydrogenation is typically used in the deprotection. Under optimized conditions, such deprotection reactions can be completed without inducing degradation reactions. On the other hand, (cyclo)alkenyl, alkynyl and azido functionalities can be directly introduced via functional monomers without protection. PAHAs **34**, **41**, **42**, **54-56** are eventually converted from other types of PAHAs through various FGT reactions.[39, 48, 63, 65, 68]

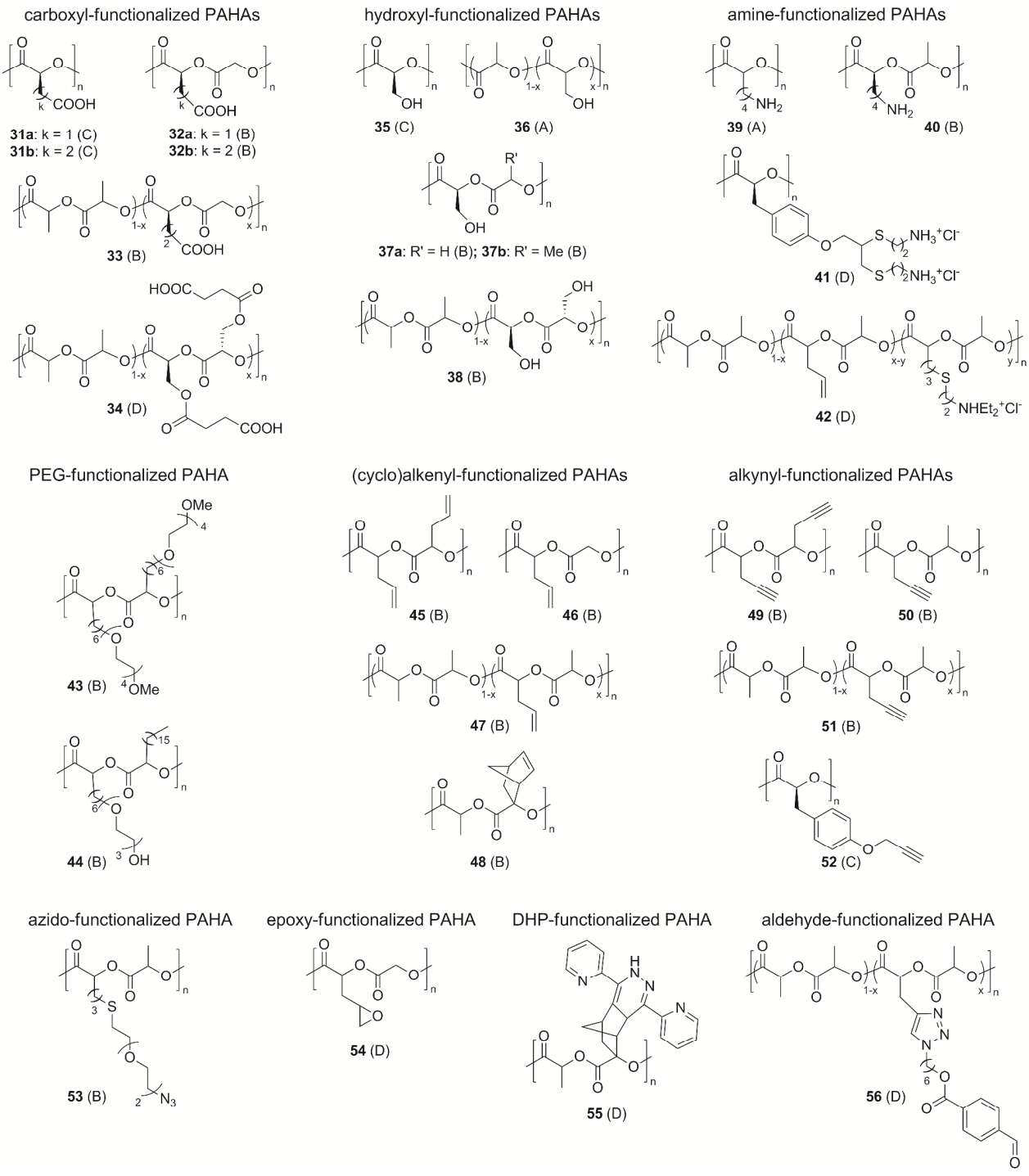
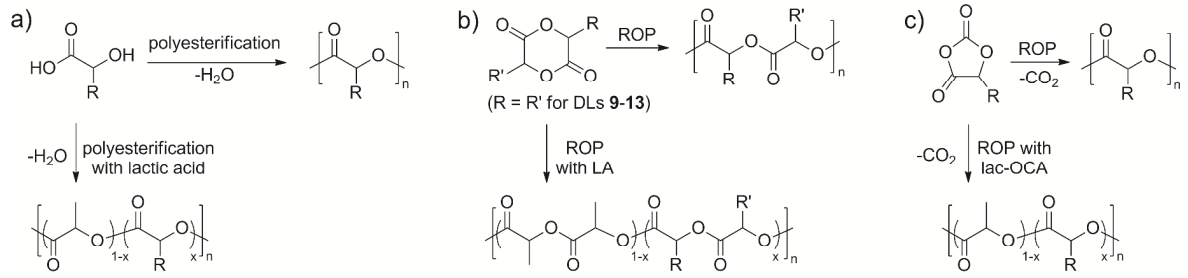


Figure 1. A selective summary of functional PAHAs (A: by polyesterification of AHAs; B: by ROP of DLs; C: by ROP of OCAs; D: by FGT via other types of functional PAHAs).



Scheme 6. Synthesis of functional PAHAs by polymerization of functional monomers

2.3.1. Functional PAHAs via Polyesterification of Functional AHAs

Condensation polymerization (i.e. polyesterification) of functional AHAs is a valid approach for the preparation of functional PAHAs. As demonstrated by Farokhzad, Langer and co-workers,[64] hydroxyl-functionalized PAHA **36** (M_n = 3.3 kDa, M_w/M_n = 2.83) was prepared by self-catalyzed polyesterification of AHA **2** with lactic acid, and subsequent deprotection of the Bn ether groups of the resulting copolymer. Park and co-workers also reported the synthesis of amine-functionalized PAHA **39** (peak MW (M_p) = 3.3 kDa, broad MW distribution) by polyesterification of the corresponding protected AHA monomer **3** with lactic acid catalyzed by TsOH, followed by the deprotection of CBz groups.[38] In each case, relatively high temperature (i.e. 150 ºC) was used in the polyesterification step and reduced pressure was applied at least in the late stage of reaction, in order to remove water from the reaction systems and to achieve very high conversions of AHAs required for the formation of polymers. Self-catalyzed polyesterification may take a few days,[64] whereas the use of an external acid (TsOH) as catalyst could significantly reduce the reaction time into several hours.[38] Although the polyesterification approach to synthesize functional AHAs is straightforward, it suffers from several drawbacks. Because of the inherent limitations of condensation polymerization,[11] it cannot yield well-defined PAHAs with high MW or narrow MW distribution. Moreover, due to the high reaction temperature, it may not be suitable for the synthesis of PAHAs with thermo-labile functionalities, such as (cyclo)alkenyl and alkynyl groups.

2.3.2. Functional PAHAs via ROP of Functional DLs

As compared with the polyesterification approach, ROP of functional DLs has been utilized for the synthesis of a much broader variety of functional PAHAs with more adjustable MW range and significantly enhanced structural control. ROP of a wide range of functional DL monomers (**9**-**20**, **23**-**27**) has been reported. In general, bulky substituents on DLs can reduce both polymerization rate and the exothermicity of the ROP process, thereby lowering the polymerizability of the cyclic monomers.[59] Both metal-containing catalysts and organocatalysts have been used for ROP of functional DLs. Catalyzing ROP through coordination-insertion mechanism, tin (II) octanoate ($SnOct_2$) is the most widely used metal-containing catalyst;[33, 50] while aluminum (III) isopropoxide ($Al(O\textit{i}\text{-}Pr)_3$) and zinc derivatives are also effective metal-containing catalysts for ROP of DLs.[21, 35] Among these metal-containing catalysts, $SnOct_2$ has been approved by the U.S. FDA for being used as a food additive, but the toxicity of organotin residues may still be concerned when the resulting polymers are considered for biomedical applications.[16] $SnOct_2$-catalyzed ROP of DLs typically is performed in bulk at relatively high temperature (≥ 110 ºC), with polymerization times ranging from minutes to a number of hours to yield high MW PAHAs. Alcohols are often used as co-initiators for $SnOct_2$-catalyzed ROP systems to achieve better control over MWs and α-terminals of the resulting functional PAHAs.[35] However, the occurrence of transesterification reaction is often appreciable in these systems, and MW distributions of the resulting PAHAs can become broad especially when long polymerization times are employed.[50] Recently, following the pioneering work of organocatalyzed-ROP by Hedrick, Waymouth and co-workers,[18] organocatalysts, such as DMAP, 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) and dual component catalytic systems composed of a thiourea (TU) catalyst with (-)-sparteine, have been utilized to catalyze ROP of functional DLs under mild temperatures (-20 – 40 ºC) in solutions (with CH_2Cl_2 as the most used solvent). These organocatalysts work in ROP systems through a nucleophilic polymerization mechanism with suppressed occurrence of unfavorable transesterification reactions, thereby exerting good structural control of the resulting functional PAHAs. Moreover, the functional PAHAs

obtained from organocatalyzed ROP systems may be utilized for biomedical applications without the concern of toxicity of residual metal contaminants. Relative to DMAP, TBD and TU/(-)-sparteine are much more reactive in catalyzing ROP systems.[42, 56, 58, 59] Alcohols are required as initiators for these organocatalyzed-ROP systems, and in general, the resulting well-defined PAHAs have quantitative alkyl α-terminals from the alcoholic initiators, as well as hydroxyl ω-terminals. Specific examples of the synthesis of functional PAHAs via ROP of functional DLs are described as follows.

Carboxyl-functionalized PAHAs have been obtained by ROP using Bn ester-protected DL monomers **9** and **14a-c**, followed by deprotection via catalytic hydrogenation.[21, 33, 42, 56] Relative to monofunctionalized DLs **14a-c**, bifunctionalized DL **9** with two bulky substituents has much lower reactivity in polymerization. The synthetic results from $SnOct_2$-catalyzed ROP of these monomers are not optimal regarding polymerization efficiency and control (M_w/M_n = 1.4-3.4).[21] Recently, TU/(-)-sparteine dual catalytic systems have been employed by Pounder et al. and Thillaye du Boullay et al. to polymerize DLs **14a** and **14b**, respectively.[42, 56] With the absence of undesirable transesterification reactions in these ROP systems and nearly quantitative monomer conversions within short polymerization times (6-60 min), well-defined poly(**14a**) (M_n up to 26 kDa, M_w/M_n = 1.11-1.19) and poly(**14b**) (M_n up to 36 kDa, M_w/M_n = 1.12-1.22) with controlled MWs and narrow MW distributions were obtained. Subsequent Pd/C-catalyzed hydrogenolysis of these polymers yielded well-defined carboxyl-functionalized PAHAs **32a** and **32b**. Similarly, well-defined PAHA **33** samples with lower densities of pendent carboxyl functionalities (M_n = 12-17 kDa, M_w/M_n = 1.10-1.11; 2-8 mol% of carboxyl group) were also prepared through TU/(-)-sparteine-catalyzed ROP of **14b** with D,L-LA, followed by catalytic hydrogenation of the resulting copolymers.[63]

Hydroxyl-functionalized PAHAs have been prepared by ROP of protected DLs **10**, **15a-b** and **16a-b** using metal-containing catalytic systems, followed by post-polymerization deprotection reactions.[33, 35, 36, 46, 47] As reported by Leemhuis et al.,[35] ethylzinc phenolate-catalyzed ROP of **15a** and **15b** yielded polymers (poly(**15a**): M_n up to 38 kDa, M_w/M_n = 1.4-1.7; poly(**15b**): M_n up to 11 kDa, M_w/M_n = 1.2-1.9) with better structural control as compared to the $SnOct_2$-catalyzed systems (poly(**15a**): M_n up to 10.5 kDa, M_w/M_n =

1.4-2.1; poly(**15b**): M_n up to 10.5 kDa, M_w/M_n = 1.3-2.1). As demonstrated by Noga et al.,[36] $SnOct_2$-catalyzed ROP of **10** with L-LA yielded a copolymer poly(**10**-*co*-L-LA) with M_n of 15 kDa and M_w/M_n of 1.5. With complete removal of Bn ether protecting groups, catalytic hydrogenation of poly(**15a**), poly(**15b**) and poly(**10**-*co*-L-LA) resulted in hydroxyl-functionalized PAHAs **37a**, **37b**, and **38**.[35, 36] On the other hand, although DL monomers **16a-b** with neighboring hydroxyl groups protected by isopropylidene acetal groups can be effectively polymerized using $SnOct_2$ catalyst, only partial removal of the cycloacetal protection groups of the resulting polymers could be accomplished through the post-polymerization deprotection reactions.[46, 47]

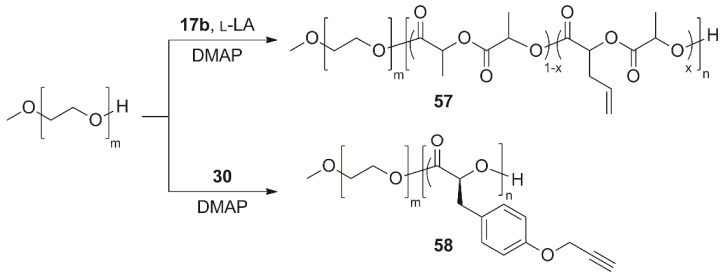
As reported by Weck and co-workers,[33] amine-functionalized PAHA **40** was also synthesized by $SnOct_2$-catalyzed ROP of DL **20** carrying CBz-protected amine groups, followed by deprotection of the resulting poly(**20**) (M_n = 8 kDa, M_w/M_n = 1.4). In their study, poly(**20**) was recalcitrant toward hydrogenolysis. Quantitative removal of the CBz groups was eventually achieved by the treatment of poly(**20**) with HBr (33%)/AcOH for 2 h.

OEG/PEG-functionalized PAHAs have been prepared by $SnOct_2$-catalyzed ROP of OEG-functionalized DLs **13a-d** and **19a-b**,[51, 52] and TBU-catalyzed ROP of OEG-functionalized DLs **27a-b** and PEG-functionalized DL **27c**.[62] For examples, water-soluble PAHA **43** with each AHA backbone unit carrying an OEG chain (M_n = 10.6 kDa, M_w/M_n = 1.12) was obtained by $SnOct_2$-catalyzed ROP of **13d**;[51] amphiphilic PAHA **44** with each AHA backbone unit carrying either a hydroxyl-terminated hydrophilic OEG_3 chain or a hydrophobic hexadecyl chain was synthesized by $SnOct_2$-catalyzed ROP of **19b**, followed by Pd/C-catalyzed hydrogenation in THF/methanol to quantitatively deprotect the Bn ether terminals of OEG_3 side chains.[52] However, in the studies of ROP of DLs **13a-d** and **27a-c**, the decrease of monomer conversions and the reduced level of polymer MW control were observed with the increase of ethylene glycol repeat units of DLs in each monomer series, indicating that polymerization efficiency and control were negatively affected by the increase of steric hindrance from the side chains of DLs.

The synthesis of PAHAs with functionalities compatible with ROP conditions is more straightforward, without the need of using protected monomers and post-polymerization deprotection. Therefore, PAHAs with clickable (cyclo)alkenyl, alkynyl or azido groups have been readily prepared by ROP of the corresponding DLs (**11-12**, **17-18**, **23-26**). As reported by Baker and co-workers,[50] $SnOct_2$-catalyzed ROP of diallyl-functionalized DL **11** and dipropynyl-functionalized DL **12** yielded allyl-functionalized PAHA **45** (M_n = 14.1 kDa, M_w/M_n = 1.32) and propynyl-functionalized PAHA **49** (M_n up to 56.5 kDa; M_w/M_n = 1.13-1.49), respectively. Hennink and co-workers studied $SnOct_2$-catalyzed ROP of monoallyl-functionalized DL **17a** and obtained allyl-functionalized PAHA **46** (poly(**17a**): M_n up to 17.4 kDa, M_w/M_n = 1.9-2.0).[48] Our group synthesized well-defined allyl-functionalized PAHA **47** (poly(**17b**-*b*-L-LA): M_n up to 21.9 kDa, M_w/M_n = 1.12-1.30, 26-52 mol% of allyl group) by DMAP-catalyzed ROP of monoallyl-functionalized DL **17b** with L-LA.[53, 65, 69] Moreover, we also prepared well-defined propynyl-functionalized homopolymer PAHA **50** (poly(**18b**): M_n = 14.4 kDa, M_w/M_n = 1.15) and random copolymer PAHA **51** (poly(**18b**-*b*-L-LA): M_n = 11.7 kDa, M_w/M_n = 1.23, 54 mol% of propynyl group) by DMAP-catalyzed ROP of monopropynyl-functionalized DL **18b** and L-LA/**18b** mixture, respectively.[55, 66] As reported by Hillmyer and co-workers,[58, 59] PAHAs with pendent monocycloalkenyl-functionalized DLs **23-25** can be converted into functional PAHAs through organocatalyzed ROP. Due to the bulkiness of the substituents, long reaction times (up to a number of days) are required even with the use of highly reactive organocatalyst TBD. Moreover, low polymerization temperature (such as -20 ºC) not only favors the equilibrium towards polymerization but also promotes the structural control of the resulting polymers. For instance, norbornenyl-functionalized PAHA **48** (M_n up to 30.1 kDa; M_w/M_n = 1.27-1.69) was obtained in high monomer conversion (≥ 94%) by TBD-catalyzed ROP of DL **23** at -20 ºC for 1-8 d.[58] As reported by Weck and co-workers, azido-functionalized PAHA **53** (M_n = 11 kDa; M_w/M_n = 1.6) was obtained by $SnOct_2$-catalyzed ROP of DL **26**.[61]

Because of the living characteristics of organocatalyzed-ROP of DLs, well-defined block copolymers with a functional PAHA block have been prepared by using hydroxyl-terminated

macroinitiators. Dove and co-workers investigated TU/(-)-sparteine-catalyzed ROP of DL **14a** using either hydroxyl-terminated PEG or in-situ prepared poly(L-LA) (PLLA)-based macroinitiators and obtained well-defined diblock copolymers having a PEG or PLLA first block and a poly(**14a**) second block.[42] Our group synthesized well-defined allyl-functionalized diblock copolymer **57** samples, including PEG_{45}-*b*-poly($\mathbf{17b}_{0.31}$-*co*-$LA_{0.69}$)$_{42}$ (M_n = 8.4 kDa, M_w/M_n = 1.3) and PEG_{45}-*b*-poly($\mathbf{17b}_{0.5}$-*co*-L-$LA_{0.5}$)$_{22}$ (M_n = 5.5 kDa, M_w/M_n = 1.05), by DMAP-catalyzed ROP of allyl-functionalized DL **17b** with L-LA using ω-hydroxyl PEG_{45} (PEG_{45}-OH) as the macroinitiator (Scheme 7a).[53, 70]



Scheme 7. Synthesis of diblock PEG-*b*-PAHA functional copolymers

2.3.3. Functional PAHAs via ROP of Functional OCAs

Following the pioneering work of Bourissou and co-workers on nucleophilically-activated ROP of 5-methyl-1,3-dioxolane-2,4-dione (lac-OCA),[17] ROP of functional OCAs has gradually emerged as an important approach for the synthesis of well-defined functional PAHAs.[34] Because the liberation of CO_2 promotes ROP of OCAs for both enthalpic and entropic reasons, ROP of OCAs is thermodynamically much more favorable than that of DLs.[17] Theoretically, both difunctional DLs and monofunctional OCAs can be converted via ROP into PAHAs with each AHA unit carrying a functional group. However, because of the low polymerizability of difunctional DLs with two bulky substituents,[23] ROP of functional OCAs is particularly useful for the preparation of high MW PAHAs with high densities of sizable functional groups.

At the same time, copolymerization of functional OCAs with non-functional OCA (i.e. lac-OCA) can be used for the preparation of well-defined PAHAs with lower densities of functionalities.[42] ROP of functional OCAs is typically conducted in mild temperatures in solutions. DMAP is the most used catalyst for ROP of functional OCAs,[34] while other types of *para*-substituted pyridines and (BDI-EI)ZnN(TMS) may also serve as effective catalysts.[41, 43] Alcoholic initiators are required as the initiators for these ROP systems, and the utilization of hydroxyl-terminated macroinitiators can readily enable the preparation of well-defined block copolymers having a PAHA block converted from functional OCAs.[41, 71]

Specific examples for the synthesis of well-defined functional PAHAs via ROP of functional OCAs are described as follows. Functional PAHAs with carboxyl or hydroxyl groups have been prepared by ROP of OCAs with Bn ester or Bn ether protection groups, followed by post-polymerization deprotection via Pd/C-catalyzed hydrogenation. Pounder and Dove studied organocatalyzed-ROP of OCA **28a** with a Bn ester group, and obtained well-defined poly(**28a**) (M_n up to 24.5 kDa; M_w/M_n = 1.03-1.28), which was further converted into carboxyl-functionalized PAHA **31a**.[43] In their investigation, 4-methoxypyridine was identified as the optimal ROP catalyst with the ideal balance of activity and polymerization control among a variety of *para*-substituted pyridines. As reported by Bourissou and co-workers,[34] well-defined poly(**28b**) with pendent Bn ester groups (M_n up to 18.3 kDa; M_w/M_n = 1.14-1.25) was prepared by DMAP-catalyzed ROP of OCA **28b**, and subsequently converted into carboxyl-functionalized PAHA **31b**. Cheng and co-workers obtained hydroxyl-functionalized PAHA **35** by the synthesis of well-defined poly(**29**) (M_n up to 141 kDa; M_w/M_n = 1.03-1.25) by DMAP-catalyzed ROP of Bn ether-functionalized OCA **29**, followed by Pd/C-catalyzed deprotection reaction.[37] They also prepared well-defined alkynyl-functionalized PAHA **52** (M_n up to 116 kDa; M_w/M_n = 1.02-1.15) by ROP of alkynyl-functionalized OCA **30**.[39] The ROP process can be catalyzed by either DMAP or (BDI-EI)ZnN(TMS).[41] By using camptothecin (Cpt) or its derivative as the alcoholic initiator, PAHA **52** with a Cpt-containing α-terminal (M_n up to 20.7 kDa; M_w/M_n = 1.06-1.09) was obtained.[41] Well-defined diblock copolymer **58** having a hydrophilic PEO block and an alkynyl-

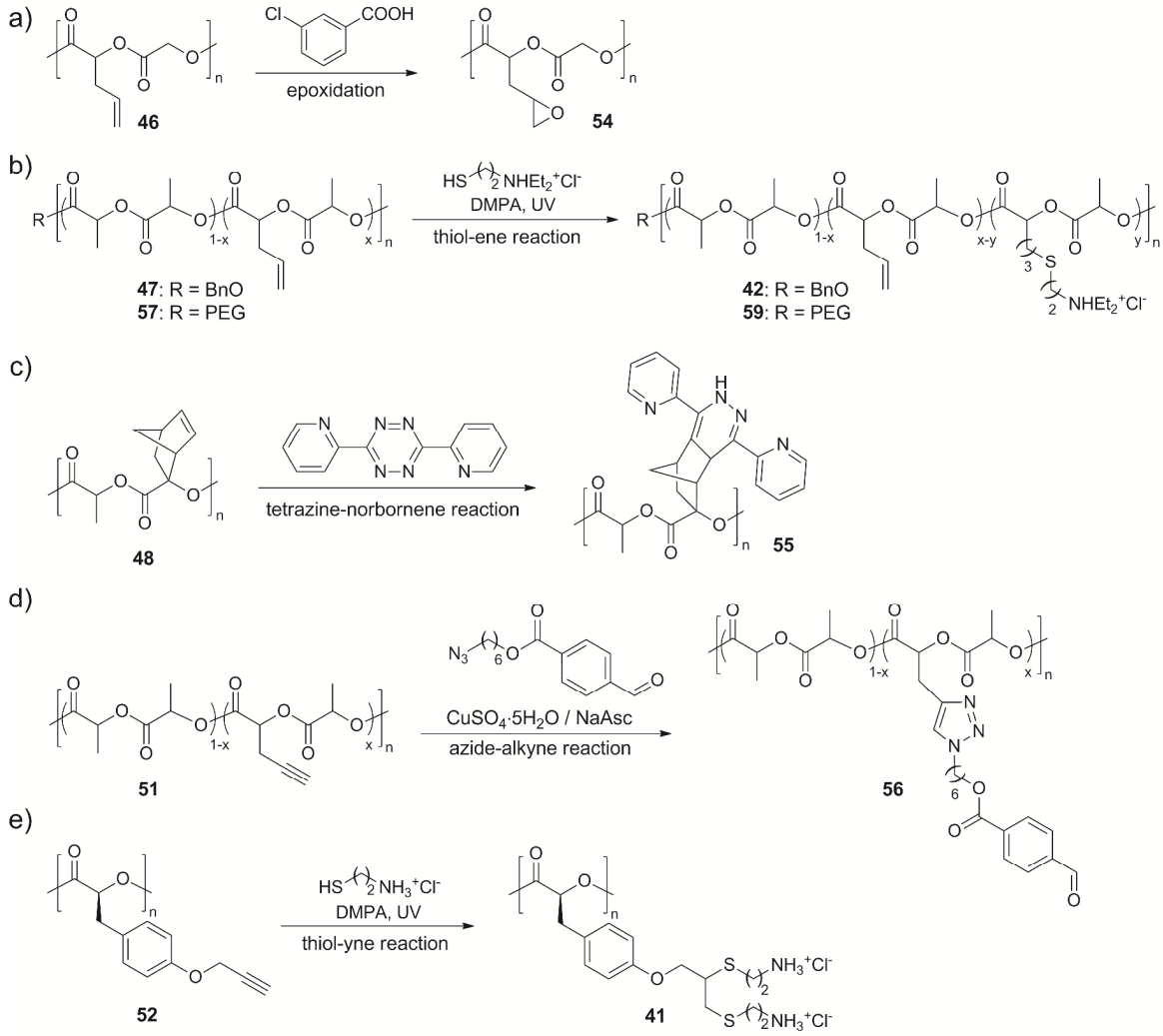
functionalized PAHA block was also synthesized by using PEG-OH as the macroinitiator for the ROP of OCA **30** (Scheme 7b).[40, 41]

2.3.4. Functional PAHAs via FGT Reactions

Functional PAHAs obtained through polymerization of functional monomers can be further converted into other types of functional PAHAs through FGT reactions. For instance, as reported by Noga et al.,[36] hydroxyl-functionalized PAHA **38** can be readily converted into carboxyl-functionalized PAHA **34** by modification with succinic anhydride. The discussion herein focuses on PAHAs with new kinds of functionalities achieved through FGT strategy, whereas biomedical functionalization of PAHAs by FGT reactions will be addressed in the next section of biomedical applications of functional PAHAs.

Because (cyclo)alkenyl groups can enable a series of highly efficient reactions, (cyclo)alkenyl-functionalized PAHAs are very useful FGT platforms to access new types of PAHAs. As reported by Hennink and co-workers,[48] allyl-functionalized PAHA **46** was readily oxidized using *m*-chloroperoxy benzoic acid to quantitatively convert the allyl groups into epoxide groups without considerable occurrence of chain scission of the substrate polymer, yielding epoxy-functionalized PAHA **54** (Scheme 8a); on the other hand, dihydroxylation of PAHA **46** only resulted in low MW products. As our group demonstrated recently, new functionalities can be incorporated with PAHA blocks by FGT via thiol-ene functionalization. By using allyl-functionalized PAHA **47** and diblock PEG-*b*-PAHA **57** as the precursor polymers and 2-(diethylamino)ethanethiol hydrochloride as the thiol agent, well-defined tertiary amine-functionalized PAHA **42** and PEG-*b*-PAHA **59** with tunable amine mol% were obtained in the presence of 2,2-dimethoxy-2-phenylacetophenone (DMPA; photoinitiator) after UV irradiation of the reaction systems for 1 h (Scheme 8b).[65, 70] Besides epoxidation and thiol-ene chemistries, metathesis reactions can also be used to prepare functional PAHAs using allyl-functionalized PAHAs as platforms.[49] With significant strain, norbornenyl groups can enable a variety of reactions. For instance, as illustrated by Dove and co-workers,[67] PAHA **48**

with pendent norbornenyl groups can readily undergo norbornene-tetrazine click reactions with 3,6-di-2-pyridyl-1,2,4,5-tetrazine to form DHP-containing PAHA **55** (Scheme 8c), and in principle, a variety of functionalities can be introduced to the macromolecular structures by using tetrazine-containing functionalization agents.



Scheme 8. Synthesis of functional PAHAs by FGT reactions

Alkynyl-functionalized PAHAs can also serve as very useful FGT platforms due to the high reactivity of alkynyl groups in azide-alkyne and thiol-yne reactions. Several groups have demonstrated that PEGylation of PAHAs can be readily accomplished by azide-alkyne reaction of alkyne-functionalized

PAHAs with azido-functionalized PEG.[50, 54, 55, 66] In contrast to ROP of OEG/PEG-functionalized DLs, the post-polymerization azide-alkyne modification approach may allow the synthesis of graft copolymers with both long PAHA-based backbones and sizable PEG side chains. Moreover, as we reported recently,[68] copper-catalyzed azide-alkyne reaction of 6-azidohexyl 4-formylbenzoate with alkynyl-functionalized PAHA **51** was highly efficient (>96% conversion of alkynyl groups), yielding PAHA **56** with pendent benzaldehyde functionalities (Scheme 8d). As demonstrated by Cheng and co-workers, the alkynyl groups of PAHA **52** can be quantitatively modified via thiol-yne chemistry.[39] By using 2-aminoethanethiol hydrochloride as the thiol agent in an excess amount, PAHA **41** with each AHA backbone repeat unit carrying two primary amine moieties was obtained in the presence of DMPA after UV irradiation of the reaction system for 40 min (Scheme 8e).

3. Biomedical Applications of Functional PAHAs

3.1. Functional PAHAs for Drug Delivery

The selection and design of polymer scaffolds for drug delivery systems is challenging and of great significance.[72] Generally, the ideal polymer scaffolds should: (i) be non-toxic, non-immunogenic and biocompatible; (ii) be biodegradable or have adequate MW (≤ 45 kDa) to allow elimination from the body; (iii) avoid non-specific binding and have long body residence time to allow distribution and accumulation in the desired body compartments.[73] With biocompatible and biodegradable properties, biodegradable polymers have becoming increasingly attractive as scaffolds in the design of polymeric nanomedicines. Among all kinds of biodegradable polymers, PAHAs have been widely employed for the preparation of drug delivery systems. Because PLA, PGA and PLGA are all approved by FDA for clinical use, they have been widely investigated for drug delivery. However, with low encapsulation capacity and limited conjugation sites, drug loading capacity of conventional PAHAs has been significantly restricted.

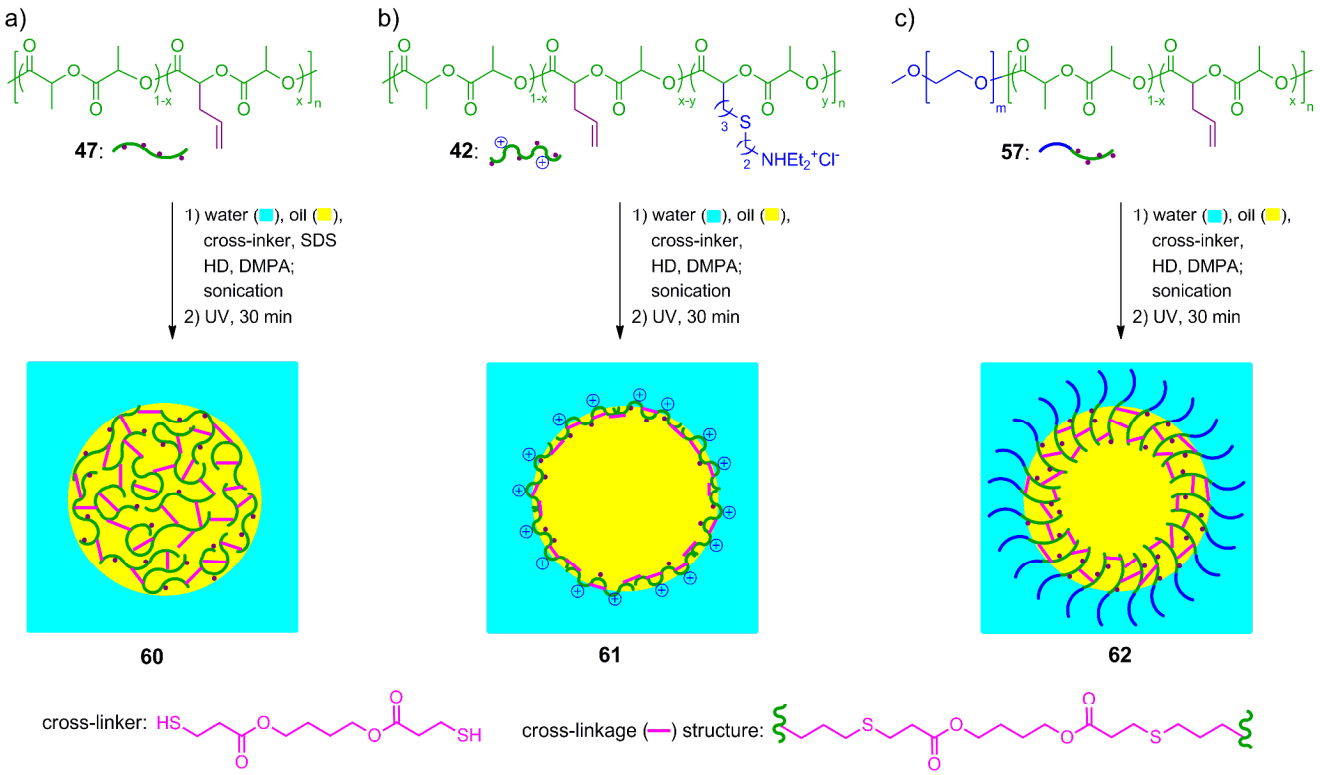
Recently, functional PAHA-derived carriers, with either microscopic or nanoscopic dimensions, have been prepared and investigated for drug delivery applications. Due to the presence of functionalities, these carriers have different hydrophilicity, degradability, and other physiochemical properties as compared to their non-functionalized analogues. Specifically, PAHAs carrying hydrophobic functionalities have enhanced swellability or solubility and degradability in aqueous media;[21-23, 74] PAHAs with OEG_3 or OEG_4 side chains have thermoresponsive properties in aqueous solutions, and are water-soluble at temperatures below the lower critical solution temperatures of the solutions;[51] functional PAHAs with amphiphilic structures may have interfacial activities and be able to form assemblies in selective solvents.[40, 53, 71, 75] Moreover, reactive groups of functional PAHAs can enable conjugation reactions to incorporate these carriers with biomedical functionalities for achieving enhanced biomedical functions. Drug moieties can be loaded into these carriers through either encapsulation or conjugation. Encapsulation approach is based on physical trapping of drug in the polymer matrix of carriers, and drug release is realized through diffusion of drug out of the matrix. Considerable burst release of encapsulated drugs is commonly observed in drug-encapsulated systems. Conjugation approach is based on covalent linking between drug moieties and polymer scaffolds, and drug release is realized by the cleavage of the conjugation linkage and subsequent drug diffusion out of the polymer-based domain.[76] Relative to drug-encapsulated systems, drug-conjugated systems typically require more challenging synthesis, but burst release can be effectively suppressed.

Drug encapsulation and release from functional PAHA-derived microparticles (MPs) have been investigated by Bourissou, Siepmann and co-workers.[63] These MPs were prepared from carboxyl-functionalized PAHA **33** to encapsulate the fragile drug apomorphine HCl through a solid-in-oil-in-water solvent extraction/evaporation method. With moderate encapsulation efficiencies (53-57%), ~11 wt% of drug was loaded into the MPs. Both polymer degradation rate and in vitro drug release rate increased with the increase of carboxyl mol% of **33**. As compared to PLGA MPs, these PAHA **33**-derived MPs possessed different drug release profiles and significantly reduced amounts of residue toxic solvents.

Because nanoscopic dimensions of carriers are required to avoid fast systemic clearance, nano-carriers typically are more relevant to in vivo drug delivery applications than micro-carriers.[77, 78] Specifically, nano-carriers with average hydrodynamic diameter (D_h) of 10-100 nm can not only avoid fast systemic clearance but also have enhanced permeability and retention effects to enable passive tumor-targeting.[78] A variety of functional PAHA-derived nano-carriers have been prepared. For instance, block copolymer micelles can be prepared from amphiphilic diblock copolymers having a hydrophilic PEG block and a hydrophobic functional PAHA block.[40, 71] As demonstrated by Cheng and co-workers,[40] core-cross-linked (CCL) micelles can be prepared from the preparation of micelles of diblock copolymer PEG-*b*-PAHA **58**, followed by azide-alkyne core-cross-linking reaction using a disulfide-containing diazido cross-linker. Core cross-linking resulted in enhanced structural stability of the polymeric micelles.[79] Moreover, with redox-responsive cross-linkage, these CCL micelles exhibited triggered release of cargo molecules in the presence of reducing reagent, indicating their potential applications in drug encapsulation and controlled release.[40]

Recently our group strategically integrated UV-induced thiol-ene cross-linking with transparent miniemulsions to enable highly efficient cross-linking of precursor polymers for the synthesis of well-defined biodegradable nanoparticles (NPs) and nanocapsules (NCs).[53, 75] By using 1,4-butanediol bis(3-mercapto-propionate) as the dithiol cross-linker, thiol-ene cross-linking reactions essentially were completed within 30 min in transparent miniemulsions in the presence of DMPA under UV irradiation, without significant template destabilization. Converting hydrophobic allyl-functionalized PAHA **47** into PAHA-based NPs **60**, the corresponding miniemulsion cross-linking system needs to be stabilized with hydrophobe hexadecane (HD) and external surfactant, such as sodium dodecyl sulfate (SDS).[53] On the other hand, amphiphilic allyl/amine-functionalized PAHA **42** and allyl-functionalized diblock copolymer **57** could serve as surfactants and be present at water-oil interfaces of the miniemulsion systems, and they were transformed into NCs **61** and **62** via thiol-ene miniemulsion cross-linking.[53, 75] The NCs **61** converted from PAHA **42** have positively charged shells because of the presence of tertiary amine groups,[75] while the NCs

converted from diblock copolymer **57** have PEGylated outer domains.[53] In principle, hydrophobic drugs are soluble in oil phase, and therefore, can be readily loaded at the miniemulsification stage during the in situ preparation of these NPs and NCs. Cationic NCs **61** derived from PAHA **42** were investigated for drug delivery application.[75] These NCs possess remarkable degradability without noticeable cytotoxicity, and can avoid fast renal clearance in a mouse model. With encapsulation efficiency of 74% and 11.6 wt% of doxorubicin (Dox) relative to the NCs, the Dox-encapsulated NCs (D_h = 20.0 nm, zeta-potential = 42 mV) can effectively bypass P-glycoprotein-mediated multidrug resistance of MCF7/ADR breast cancer cells, thereby resulting in significantly increased intracellular drug concentration and reduced cell viability.



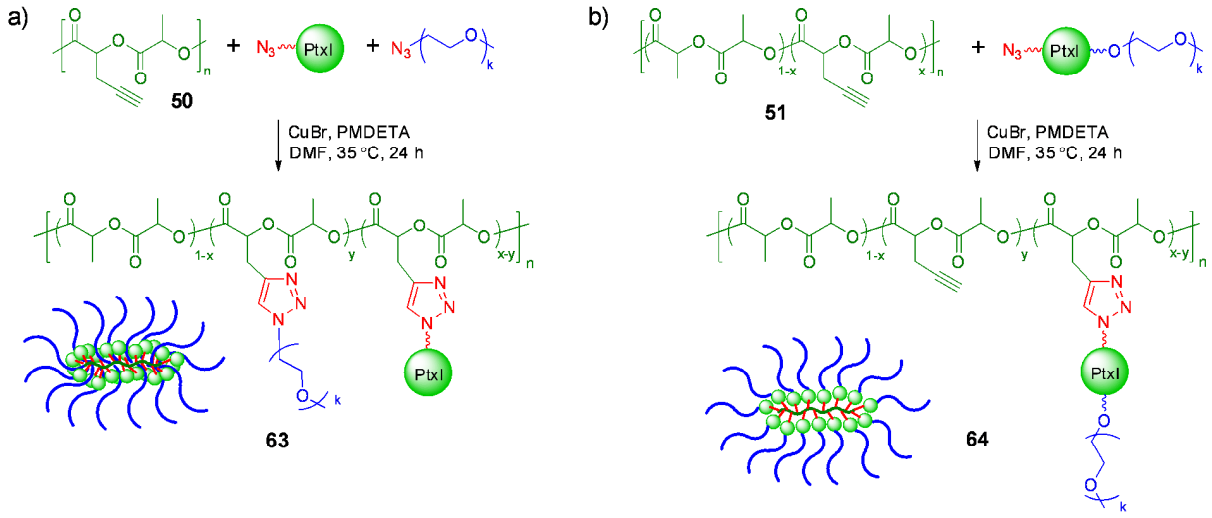
Scheme 9. Synthesis of PAHA-based NPs and NCs by UV-induced thiol-ene cross-linking of PAHA-based precursors in transparent miniemulsions

Based on polymer-drug conjugates (PDCs), conjugated drug delivery systems may possess well-defined structures with accurate drug loading and enable sustained drug release.[76] With remarkable

degradability and numerous pendent reactive functionalities, functional PAHAs may serve as excellent polymer scaffolds to conjugate drugs. Because the main chains of PAHAs, as well as most drugs, are hydrophobic, PAHA-based PDCs need to be either modified with hydrophilic groups or converted into NPs with hydrophilic coronas. With linear hydrophilic chain structures, PEG is typically used to modify PDCs and PDC-based NPs because PEG modification may help to remarkably enhance colloidal stability and circulation time but reduce cytotoxicity and immunogenicity of the PDCs and NPs.[80]

With dense PEG chains covalently linked to the PAHA-based backbones of PDCs, the corresponding modified PDCs can be termed as brush polymer-drug conjugates (BPDCs) because of the brush-like structure of the polymeric scaffolds.[55, 66, 81, 82] Two types of BPDCs **63** and **64** with PAHA backbones, PEG side chains and conjugated paclitaxel (Ptxl) moieties were designed and studied by our group.[55, 66] These BPDCs were different in grafting structures: both Ptxl and PEG grafts were linked directly to PLA backbone in BPDC **63** (i.e. PAHA-*g*-Ptxl/PEG),[66] whereas Ptxl moieties served as bridges to link PLA backbone and PEG chains in BPDC **64** (i.e. PAHA-*g*-Ptxl-PEG).[55] They were synthesized by copper-catalyzed azide-alkyne click reactions of alkyne-functionalized PAHAs **50** and **51** with azide-functionalized graft precursors in DMF (Scheme 10). Copper-based catalyst was readily removed from the BPDCs by passing the reaction solutions through a short alumina column during work-up. With well-controlled degradable structures and 23 wt% of Ptxl loadings, both types of BPDCs can be dissolved in water to form nano-objects (**63**: D_h = 15.5 nm; **64**: D_h = 18.4 nm). However, drug release from BPDC **64** cannot be easily controlled, because it requires the successive cleavages of both ester linkage at C-7 of Ptxl and cycloacetal-based conjugation linkage at C'-2 of Ptxl.[55] On the other hand, BPDC **63** with only hydrolysable cycloacetal-based conjugation linkage showed sustained drug release behavior, with 50 mol% of drug released within about 22 h under a physiological pH (7.4) at 37 ºC.[66] Moreover, BPDC **63** was more therapeutically effective towards MCF-7 breast cancer cells than free Ptxl at 0.1 and 1 μg/mL. It can be readily internalized into the cytoplasm via endocytosis. With hydrophobic inner domain and peripheral

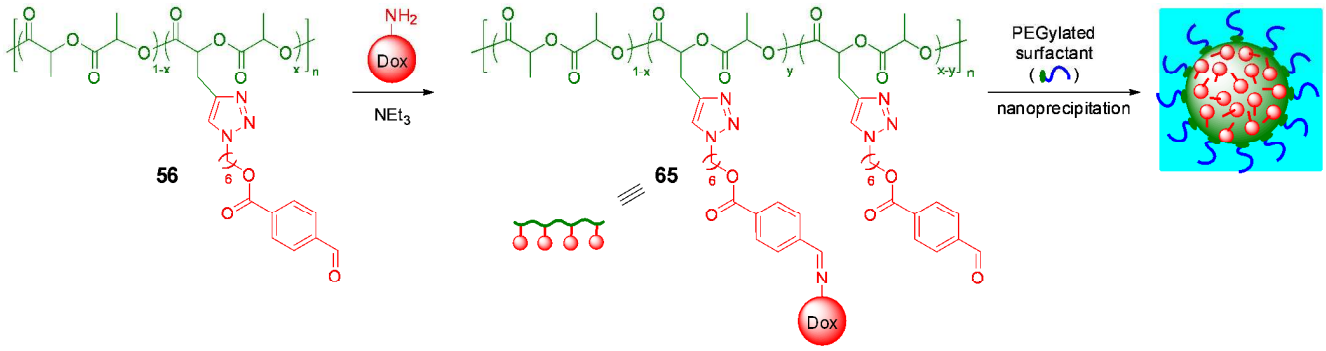
hydrophilicity, BPDC **63** can further effectively encapsulate nile red, a hydrophobic dye, for imaging purpose.



Scheme 10. Synthesis of PEGylated BPDCs with PAHA-based backbones

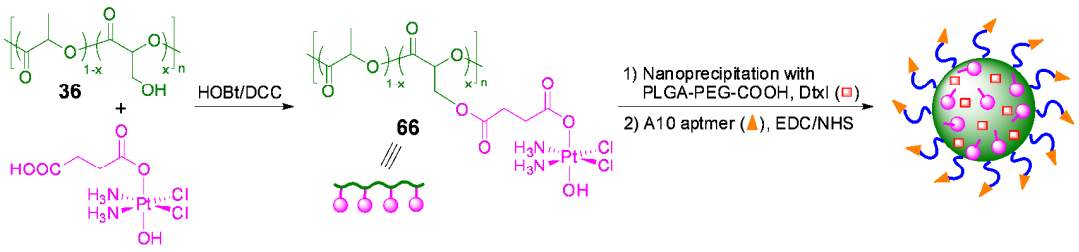
Functional PAHA-derived PDC-based NPs have been studied by several groups.[41, 64, 68, 83] Because drug release from NPs in which drug moieties are used as bivalent agents can be slow, typically drug is linked covalently with the polymer scaffolds of NPs as monovalent substituents. Because water-dispersible NPs can be readily prepared by nanoprecipitation approach, several types of functional PAHA-derived PDC-based NPs obtained via nanoprecipitation have been reported.[41, 64, 68] As we demonstrated recently,[68] well-defined PAHA-*g*-Dox PDC **65** with 32 wt% Dox loading was successfully synthesized through the conjugation reaction between Dox and aldehyde-functionalized PAHA **56** in the presence of NEt_3 (Scheme 11). PDC **65** was further formulated into PAHA-*g*-Dox-based NPs via a modified nanoprecipitation approach in the presence of a PEGylated surfactant. With 26 wt% of Dox, these NPs (D_h ~ 100 nm) had well-controlled sizes and exhibited remarkable colloidal stability in various aqueous solutions. Due to the acid-labile Shiff base linkage between Dox and polymer scaffold, Dox was much more effectively released from the NPs in pH 5.5 buffer than pH 7.4 buffer. In vitro study against MCF-7 breast cancer cells

illustrated that the NPs can be readily taken up and result in appreciably enhanced therapeutic efficiency than Dox·HCl.

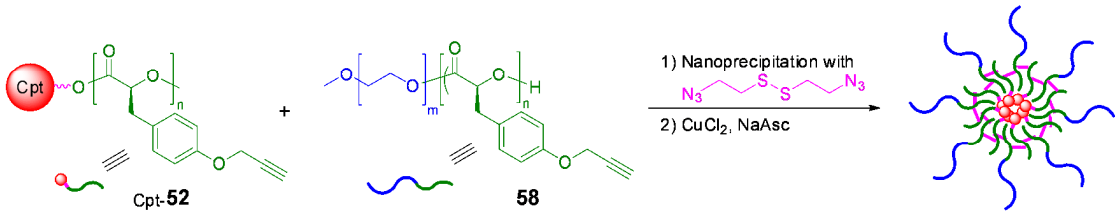


Scheme 11. Preparation of PAHA-*g*-Dox-based NPs

As reported by Farokhzad, Langer and co-workers,[64] functional PAHA-derived PDC-based NPs can serve as the platform of combination drug therapy to co-deliver multiple drugs with active targeting. HOBt/DCC-catalyzed esterification reaction between hydroxyl-functionalized PAHA **36** with a succinic acid-derivatized platinum (IV) prodrug was conducted, yielding PAHA-*g*-Pt(IV) PDC **66** with ~10 wt% of Pt(IV) (Scheme 12). PAHA-based NPs with conjugated Pt(IV) and encapsulated docetaxel (Dtxl) (D_h ~ 100 nm) were prepared by nanoprecipitation of PDC **66** in the presence of carboxyl-terminated PLGA-*b*-PEG (PLGA-*b*-PEG-COOH) and Dtxl using microfluidic devices. The surface of the NPs was further conjugated with the A10 aptamer, a targeting ligand that binds to the prostate-specific membrane antigen (PSMA), through amidation reaction. Controlled release of both drugs from the NPs having 5 wt% of Pt (IV) and up to 1 wt% of Dtxl over a period of 48-72 h was observed. Synergistic effects of the drugs of this NP-based combination drug therapy in killing PSMA-expressing LNCaP cells were verified by comparison studies.



Scheme 12. Preparation of aptamer-functionalized PAHA-based NPs with conjugated Pt(IV) and encapsulated Dtxl



Scheme 13. Preparation of CCL NPs with Cpt-conjugated PAHA-based cores

Conjugated drug moieties can also be incorporated with functional PAHA-based NPs through polymer chain-ends, with the pendent reactive groups to enable cross-linking for controlled drug release. As demonstrated by Cheng and co-workers,[41] alkynyl-functionalized PAHA **52** with α-terminal carrying a Cpt moiety via a disulfide linkage was prepared by DMAP-catalyzed ROP of OCA **30** using a Cpt-based disulfide-containing alcoholic initiator. CCL micellar NPs were prepared by nanoprecipitation of the Cpt-terminated PAHA **52** in the presence of diblock copolymer PEG-*b*-PAHA **58** and a disulfide-containing diazide, followed by azide-alkyne click cross-linking of the alkynyl-functionalized cores of the resulting micelles (Scheme 13). With the presence of disulfide-containing conjugation linkages and cross-linkages, the CCL NPs showed redox-responsive release of Cpt and exhibited enhanced in vitro cytotoxicity against MCF-7 breast cancer cells.

In addition, drug-conjugated NPs can also be prepared from functional PAHAs by using drug-containing cross-linkers. As our group demonstrated recently,[83] PAHA-based NPs with conjugated Ptxl

moieties were synthesized by azide-alkyne cross-linking reaction of alkyne-functionalized PAHA **51** with a Ptxl-based diazide in a miniemulsion. Because the release of one moiety of Ptxl from such NPs needs the cleavages of two hydrolysable cycloacetal groups, they might be suitable for drug delivery applications requiring very slow drug release.

3.2. Functional PAHAs for Gene Delivery

Because of the fragile nature of genetic materials and the biosafety concern of viral vectors, there is a significant demand for the development of polymeric vectors for safe and efficient gene delivery.[84-86] A variety of cationic polymers can lead to effective gene delivery.[87] However, conventional cationic polymers, such as polyethylenimine (PEI), poly(L-lysine) (PLL) and poly(L-arginine) (PLR), have considerable cytotoxicity possibly arising from their limited in vivo degradability.[88, 89] Non-functionalized PAHAs, such as PLGA and PLA, have also been used for gene delivery applications.[90, 91] Because non-functionalized PAHAs have no positively charged structures to adsorb negatively charged genes, sophisticated structural design and encapsulation process were required for the corresponding gene delivery systems, but encapsulation efficiency typically was low. Due to their slow hydrolysis process, the release of the encapsulated genetic payload is often too slow to reach high levels of target gene expression. Additionally, severely acidic gene-damaging environments (pH < 3) have been observed in the matrices of non-functionalized PAHAs as a result of the slow diffusion of acidic degradation products.[92]

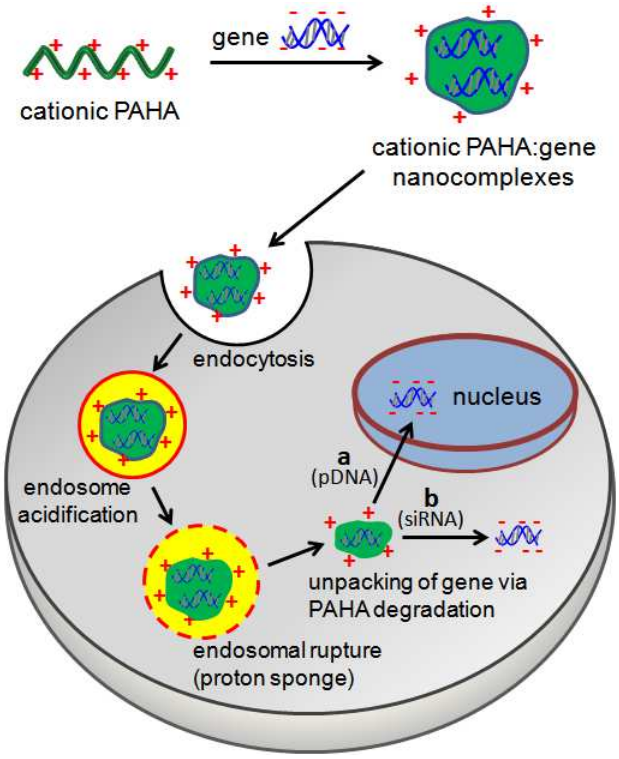


Figure 2. Conceptual diagram of gene delivery using amine-functionalized cationic PAHAs

The development of biodegradable cationic polymers as synthetic vectors for gene delivery has attracted significant interests.[93-95] Specifically, amine-functionalized cationic PAHAs have emerged as a novel class of PAHA-based materials to overcome the aforementioned limitations of conventional cationic polymers or PAHAs as scaffolds for gene delivery applications (Figure 2).[38, 39, 65, 70, 96] With positively charged macromolecular structures, they can readily form nanocomplexes with negatively charged genetic materials. The presence of protonable amine groups in the nanocomplexes can act as a weak base to absorb protons generated by ATPase, and such "proton sponge" effect can facilitate endosomal escape and improve transfection efficiency.[97] Unpacking of gene from the polymer-gene nanocomplexes can be promoted by the remarkable degradability of amine-functionalized PAHAs. Moreover, because the degradation residues of amine-functionalized PAHAs have both amine and carboxyl groups, the resulting polymeric matrices may possess mild acidity that is more compatible with fragile gene materials. Amine-functionalized PAHAs have been used for the delivery of plasmid DNA (pDNA) into cell nuclei and small interfering RNA (siRNA)

into cytoplasma, respectively. In the latter case, with the RNA-interference mechanism, the use of siRNA is to selectively silence specific molecular pathways to overcome critical barriers in disease treatment.[98]

As reported by Park and co-workers,[38, 96] PAHA **39** with quantitative one primary amine group per AHA unit is an effective synthetic vector without considerable cytotoxicity. The free PAHA **39** undergoes fast hydrolytic degradation with M_p to halve within 30 min incubation at 37 ºC and pH 7.3. However, the formation of nanocomplexes (size: 100-400 nm) between PAHA **39** and pDNA may effectively protect pDNA from nuclease degradation, and the nanocomplexes would not dissociate completely until 24 h. Comparison studies demonstrated that PAHA **39** led to about 3-fold higher transfection efficiency in 293 cells than PLL under optimized conditions, but PAHA **39** showed no cytotoxicity at concentration up to 300 μg/mL while PLL exhibited noticeable cytotoxicity in a concentration of 100 μg/mL.

The application of PAHA **41** with quantitative two primary amine groups per AHA unit as gene delivery scaffold was investigated by Cheng and co-workers.[39] PAHA **41** showed excellent cell penetration properties, and could be internalized to HeLa cells 3-5 times faster than PLL and PLR. PAHA **41** formed positively charged nanocomplexes (D_h: 90-100 nm; zeta potential: 20-30 mV) with plasmid DNA. PAHA **41** also exhibited higher transfection efficiency but lower cytotoxicity than PLL and PLR.

Recently functional PAHA **42** with tunable density of tertiary amine groups synthesized by our group has also been studied for gene delivery applications.[65] With remarkable degradability and showing no considerable cytotoxicity with concentrations up to 200-300 μg/mL, PAHA **42** can spontaneously form nanocomplexes with pDNA and IL-8 siRNA.[65, 69, 99] With the increase of amine mol%, PAHA **42** exhibited increased degradability, as well as higher in vitro efficiency in delivering pDNA and IL-8 siRNA via the nanocomplexes. Under optimized conditions, PAHA **42** with significantly high amine mol% (50-54 mol% relative to DL unit) led to high levels of pDNA transfection to two physiologically distinct cell lines (macrophage and fibroblast) with low levels of cytotoxicity when compared to a positive control, fugene-6; it also resulted in more effective IL-8 siRNA delivery to PC3 prostate cancer cells than a commercial transfection agent, Mirus TransIT. In addition, the applicability of cross-linked NCs **61** derived from PAHA

42 in the delivery of IL-8 siRNA has also been verified.[75, 100] Under optimized conditions, the gene silencing efficiency of IL-8 siRNA delivery to PC3 prostate cancer cells *via* NCs **61** was comparable to that *via* commercial transfection agents, including Mirus TransIT and lipofectamine.

Our recent studies have also demonstrated that tertiary amine-functionalized diblock copolymer PEG-*b*-PAHA **59** has excellent comprehensive properties to serve as synthetic vector for gene delivery.[70] Via its nanocomplexes with pDNAs, it led to successful pDNA delivery to four physiologically distinct cell lines (including macrophage, fibroblast, epithelial, and stem cell). It exhibited comparable transfection efficiency but minimal hemolytic activity and even lower cytotoxicity (noncytotoxic up to 3 mg/mL) than PAHA **42**. Moreover, PEG-*b*-PAHA **59** showed significantly improved resistance to serum inhibition in the delivery of pDNA than both fugene-6 and PAHA **42**.

3.3. Functional PAHAs for Drug-Gene Co-Delivery

Because combined therapies may enhance therapeutic efficacy through synergetic treatment effects, the co-delivery of drug and gene has become an emerging research area.[101, 102] Several types of co-delivery nano-carriers, including cationic assemblies (polymer nanoparticles,[103] liposomes,[104] and peptides[105]), dendrimers,[106] and inorganic nanoparticles,[107] have been reported. However, their applicability has been restricted by low therapeutic loadings, structural instability, or non-degradability. Thus, it remains a challenge to design and synthesize nanostructures with tailored properties for drug and gene co-delivery. Non-functionalized PAHAs have not been employed for the co-delivery of drug and gene. However, the recent development of functional PAHAs provides new promise for the co-delivery applications. Specifically, nanocarriers derived from amine-functionalized PAHAs may be utilized for such applications.

As we demonstrated very recently, with inner cavities and positively charged shells, cationic NCs **61** derived from amine-functionalized PAHA **42** can allow for significant drug loadings in cavities and remarkable gene loadings on shells, thereby enabling the co-delivery of drug and gene.[75] Using the NCs as

the scaffolds, the co-delivery of Dox and IL-8 siRNA to PC3 prostate cancer cells was probed (11.6 wt% Dox and 3.3 wt% IL-8 siRNA relative to NCs; Figure 3a). After the incubation of PC3 cells with the dually loaded NCs for 4 h, confocal images (Figure 3b) clearly showed co-localization of fluorescence from both Dox and FAM-labeled siRNA in the same cells, indicating the utility of such cationic NCs as scaffolds for co-delivery of drug and gene. The NCs loaded with both Dox and IL-8 siRNA exhibited noticeably higher cyctotoxicity than NCs loaded only with Dox towards PC3 cells at low Dox concentrations (≤ 1 μM), suggesting the synergic therapeutic effects of Dox and IL-8 siRNA.

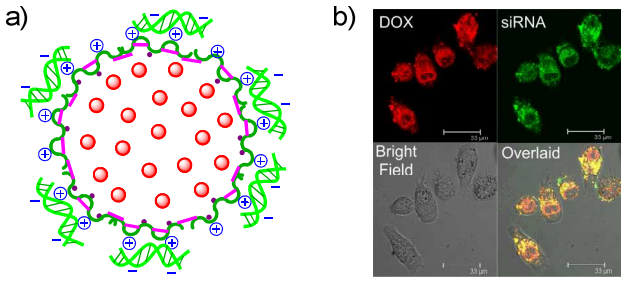


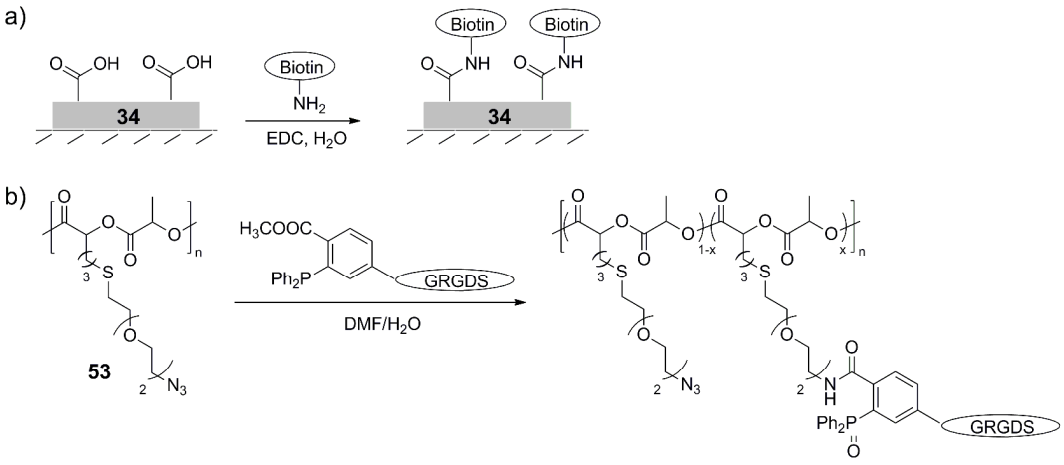
Figure 3. a) Schematic illustration of PAHA-based cationic NCs with encapsulated Dox and surface-adsorbed IL-8 siRNA; b) confocal images of PC3 cells treated by Dox/IL-8 siRNA-loaded cationic NCs. Adapted from Ref. 75.

3.4. Functionalization of PAHAs with Biomolecules

With versatile properties, polymer materials have also been employed for other biomedical applications besides therapeutic delivery. Specifically, because of their biocompatibility and biodegradability, conventional PAHAs have been widely investigated for applications as tissue engineering scaffolds.[108] However, biofunctionalization of the corresponding scaffolds is challenging due to their lack of functionalities. Thus, the utilization of functional PAHAs for tissue engineering applications may greatly facilitate biofunctionalization without sacrificing the biocompatible and biodegradable properties of

conventional PAHAs. Although currently the direct application of PAHAs with pendent functionalities as tissue engineering scaffolds has not been significantly explored, some pioneering studies on functionalization of functional PAHAs with biomolecules have been reported.

As demonstrated by Weck and co-workers,[36] surface modification of film of carboxyl-functionalized PAHA **34** with biotin was performed by the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-activated amidation reaction of **34** with an amine-substituted biotin derivative (Scheme 14a). The resulting biotin-modified polymer film exhibited enhanced ability to immobilize antibiotin antibody on surface. They also employed EDC-activated amidation chemistry to couple an RGD-containing peptide on the polymer film.[36] The RGD-functionalized polymer film showed enhanced adhesion of epithelial cells. Besides amidation, Staudinger ligation has also been verified as a valid strategy to incorporate RGD-containing peptide with PAHAs by the same group (Scheme 14b).[61] Using azido-functionalized PAHA **53** and triarylphosphine-modified GRGDS peptide as reactants, the Staudinger ligation reaction was conducted in DMF/water at room temperature and yielded (PAHA **53**)-*g*-(GRGDS peptide) with 14% functionalization.



Scheme 14. Functionalization of functional PAHAs with biomolecules

4. Conclusion and Outlook

In summary, a broad variety of PAHAs with pendent functional groups have been designed and synthesized in recent years. Polymerization of functional monomers, including functional AHAs, DLs and OCAs, is the premier method for the preparation of functional PAHAs. Protected monomers need to be used to introduce functionalities which may interfere with the polymerization process. Relative to polycondensation of functional AHAs, ROP of functional DLs and OCAs provides better control over the structures of functional PAHAs. Moreover, organocatalyzed-ROP allows the synthesis of well-defined functional PAHAs, as well as block copolymers having functional PAHA blocks. PAHAs with new functionalities can be accessed through post-polymerization FGT reactions. Relative to conventional PAHAs, functional PAHAs have modified physiochemical properties which can be utilized to achieve enhanced applicability in biomedical areas. Specifically, the reactivities of functionalities carried by these functional PAHAs can enable various types of biofunctionalization, as well as the formation of covalently-stabilized structures. In general, functional PAHA-based scaffolds have remarkable hydrolytic degradability and low cytotoxicity. The use of functional PAHAs as scaffolds for therapeutic delivery has been studied. For drug delivery applications, drug can be either encapsulated into or conjugated with functional PAHA-based scaffolds, and sustained drug release can be achieved through the conjugation approach. Cationic amine-functionalized PAHAs have emerged as a new class of synthetic biodegradable vectors for gene delivery. These cationic PAHAs and their derived materials may further be utilized for the co-delivery of drug and gene. Functionalization of functional PAHAs with biomolecules, such as biotin and RGD peptides, has also been demonstrated.

Because of their attractive physiochemical properties, the synthesis and biomedical application of functional PAHAs will remain a vivid research topic. At the current stage, the synthesis of well-defined functional PAHAs is relatively challenging, and it is important to develop new and facile synthetic approaches to allow for economical production of these materials. Utilization and further development of

highly efficient and bioorthogonal chemistries are demanded to facilitate the preparation of functional PAHA-based biomaterials with tailored structures and properties for enhanced biomedical applications. The preparation of well-defined and stimuli-responsive nanoscopic platforms from functional PAHAs and the integration of the scaffolds with therapeutic agents (drugs, targeting ligand, imaging elements, etc.) are generally required to construct functional PAHA-based systems for highly accurate and efficacious therapeutic delivery. Promising in vitro results have been obtained from functional PAHA-based therapeutic delivery systems, and the biomedical merits of these systems need to be further investigated through in vivo studies. It is also expected that functional PAHA-based biomaterials will have important applications in tissue engineering and other biomedical areas.

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TOC:

This review highlights the recent progress in the synthesis and biomedical applications of poly(α-hydroxyl acid)s with pendent functional groups.